MicroRNA-301a and microRNA-450b in Canine Oral Melanoma

（犬のメラノーマにおけるマイクロ RNA450b と 301a に関する検討）

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DEDICATION

"My thesis is a tribute to the pioneers of my life—my beloved parents, my wife Jerin Sultana Jui, and my little son Umayr Hasan, who continue to inspire me every day. Their unwavering support and love have fueled my journey towards academic excellence, and I am forever grateful to have them in my life."

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Overview

MicroRNAs (miRNA) may offer a fruitful line of research on melanomas. Aberrantly expressed miRNAs reportedly act as oncogenes or tumor suppressor genes, can alter biological functions such as cell migration, proliferation, apoptosis, and metastasis, and modulate the immune response to several cancers. In a previous study, numerous dysregulated miRNAs were identified in COM through next-generation sequencing. Of these miRNAs, miR-301a and miR-450b show upregulation in COM and are believed to play a crucial role in the progression of melanoma tumors. The primary aim of this thesis is to investigate the expression patterns of miR-301a and miR-450b in a larger sample cohort of COM than in the previous study and to elucidate their functional roles using two COM-originating cell lines, KMeC and LMeC, which, respectively, represent a primary tumor originating cell line and a metastatic tumor originating cell line.

In the first chapter, experiments were designed to investigate the relative expression of miR-301a in COM using qRT-PCR, ascertain whether it could serve as a diagnostic biomarker, elucidate the functional roles of miR-301a in COM, and predict the possible pathways by which miR-301a exerts its effect on melanoma tumor progression. The relative expression of miR-301a was investigated in clinical oral tissue and plasma samples and COM cell (KMEC and LMEC) lines using qRT-PCR. Knockdown of miR-301a was also validated for KMEC and LMEC cells using qRT-PCR. I performed CCK-8 assays to assess cell proliferation, monolayer wound-healing, and transwell migration assays to assess cell migration, a colony-formation assay to assess clonogenicity, a TUNEL assay and flow cytometry to evaluate apoptosis-related effects, and enrichment and analyses to predict possible related pathways. miR-301a was markedly clinically upregulated in COM oral tissue and plasma, suggesting that it might be a biomarker for COM diagnosis. In vitro assays demonstrated that miR-301 significantly

inhibited apoptosis in COM cells while promoting cell migration, proliferation, and clonogenicity. I also found phenotypic variation between the metastatic LMeC cell line and the non-metastatic KMeC cell line. We also predicted that miR-301 exerts cancer-promoting effects through the Wnt signaling pathway for COM. The first study suggests that miR-301a is a COM oncomiR that regulates several oncogenic phenotypes and can potentially be a diagnostic biomarker.

In the second chapter, this study was designed to investigate the levels of miR-450b expression in COM using qRT-PCR. The goal was to determine if miR-450b could be used as a diagnostic biomarker and to understand its functional roles in COM. The study also aimed to investigate target mRNA genes in clinical (tumor tissue and plasma) samples and metastatic and primary COM cell lines and to predict the possible pathways by which miR-450b affects the progression of COM tumors. Knockdown and overexpression experiments were performed to determine the influence of miR-450b on cell proliferation, migration, colony formation, and apoptosis. miR-450b was significantly upregulated in COM and differentiated between metastatic and non-metastatic tumors, and its potential as a biomarker of metastatic and non-metastatic COM was further confirmed in ROC analysis. miR-450b knockdown promoted cell proliferation, migration, and clonogenicity and inhibited apoptosis, whereas its overexpression yielded the opposite effect. miR-450b directly binds 3′ UTR of PAX9 mRNA and modulates its function, leading to BMP4 downregulation and MMP9 upregulation at the transcript level. Furthermore, I surmised that miR-450b activates the Wnt signaling pathway based on gene ontology (GO) and enrichment analyses. The second study suggests that miR-450b has the potential as a diagnostic biomarker and could be a target candidate for COM treatment.

In final conclusion, my studies find that upregulation of miR-301a and miR-450b could be a potential diagnostic biomarker to discriminate metastatic from non-metastatic melanoma. miR-

301a and miR-450b may have oncogenic effects on melanoma formation and could be involved in the Wnt signaling pathway, which is critical in metastasis and melanoma progression.

General Introduction

miRNAs are short RNA molecules that are crucial in regulating gene expression. miRNA can be a diagnostic and prognostic biomarker for various diseases, including cancer. By analyzing miRNA expression in tissues or body fluids, medical professionals can identify specific disease states and predict a patient's response to treatment. This breakthrough discovery offers a promising alternative approach to traditional disease diagnosis and treatment methods. Canine oral melanoma is an aggressive disease that may cause death. Early diagnosis and treatment are urgent. miRNA can be a better option for diagnosing and treating the COM. In a previous study conducted by my lab, we investigated several miRNAs found to be dysregulated in COM. Among them, miR-301a and miR-450b show a higher expression value than the control sample. This led me to explore the expression patterns of miR-301a and miR-450b in both metastatic and non-metastatic melanoma to determine whether they could serve as potential diagnostic biomarkers. Furthermore, I aimed to uncover the functional roles of miR-301a and miR-450b in the primary tumor originating from COM, KMeC cell, and in the metastatic tumor originating from LMeC cell.

At the outset, I will delve into the fascinating history of studying dogs for the benefit of human diseases and the concept of "comparative oncology," a canine model of human cancer that has proven to be a fruitful avenue of research. In addition, I will provide a comprehensive overview of my study. My study is divided into two chapters to explore the complex mechanisms using two types of COM-originating cell lines. In Chapter I, I will delve into the elevated expression of miR-301a and its functional roles in COM. In Chapter II, I will discuss the upregulation of miR-450b and its functional roles in COM. Through my research, I hope to contribute to a deeper understanding of these diseases and pave the way for more effective treatments for dogs and humans.

G.1. Canine oral melanoma (COM)

Melanoma, a malignant tumor that arises in the skin cells that produce pigment, is the most prevalent form of oral cancer in dogs [1-4]. This type of cancer is commonly observed in certain dog breeds, such as Scottish terriers, golden retrievers, poodles, and dachshunds [2]. Although melanoma primarily affects older dogs, it may also develop in younger ones [5, 6]. The biological behavior of oral melanoma in dogs can vary significantly based on various factors such as the location, size, stage, and histologic parameters of the tumor [5-8]. These factors play a crucial role in determining the prognosis and appropriate treatment. Unfortunately, some melanomas exhibit unpredictable and unreliable biological behavior, making it challenging to treat them effectively. Therefore, there is a pressing need for further research to better understand this relatively common but often malignant tumor. Several previous studies have explored the molecular biological aspects of melanoma to better understand its behavior and identify potential treatment options [9, 10]. The biologic behavior of canine oral melanoma is extremely variable and best characterized based on anatomic site, size, stage, and histologic parameters. Oral and/or mucosal melanoma have been routinely considered an extremely malignant tumor with a high degree of local invasiveness and high metastatic propensity [2]. This biological behavior is extremely similar to human oral and/or mucosal melanoma [1, 11]. The location of melanoma is a strong indicator of how invasive and metastatic it may be. Melanomas located on haired skin away from mucosal margins typically behave in a benign manner [1]. For dogs with oral melanoma, primary tumor size is extremely prognostic. The World Health Organization staging scheme for dogs with oral melanoma is based on size, with stage I = <2-cm-diameter tumor, stage II = 2-cm- to <4-cm-diameter tumor, stage III = 4 cm or greater tumor and/or lymph node metastasis, and stage IV = distant metastasis (Table 1).

Table G- 1. Traditional World Health Organization TNM-based Staging Scheme for Dogs with Oral Melanoma

WHO size and stage		
T: Primary tumor		
	T1	Tumor ≤ 2 cm in diameter
	T2	Tumor 2-4 cm in diameter
	T3	Tumor > 4 cm in diameter
N: Regional lymph nodes		
	N0	No evidence of regional node involvement
	N1	Histologic/cytologic evidence of regional node involvement
	N2	Fixed nodes
M: Distant metastasis		
	M0	No evidence of distant metastasis
	M1	Evidence of distant metastasis
Stage I = T1 N0 M0		
Stage II = T2 N0 M0		
Stage III = T2/3 N0/1 M0		
Stage IV = Any T, any N and any M		

G.2. Epidemiology

COM is a common tumor that accounts for 30-40% of all malignant oral neoplasms in dogs. Due to its aggressive and invasive local behavior, it represents a significant clinical health issue and is highly prone to rapid metastasis [12]. Canine melanocytic neoplasms are commonly found in the oral cavity and mucus membranes of the lips (79%). Cutaneous tumors (11%), digital or subungual (8%), and other sites (2%) are less common. These tumors account for around 7% of all canine malignant neoplasms and are the leading malignancy (35%) of the oral cavity [1]. The location of the tumor affects its behavior, with oral melanomas showing metastasis in up to 97%, subungual melanomas in 100%, and digital tumors in 84% [13]. In contrast, cutaneous and ocular melanomas are generally benign [1].

The average survival times after surgical treatment for dogs with COM classified at stages I, II, and III are 17, 6, and 3 months, respectively [14]. According to a report by Bostock, 45% of dogs having malignant skin melanomas succumbed to their disease within a year, while only 8% of the dogs with "benign" skin melanomas died [7]. Additionally, if we compare the haired-skin melanoma dogs with a mitotic index of 2 or less, only 10% died two years after surgery. In contrast, more than 70% of dogs died due to tumors with a mitotic index of 3 or more. Williams and Packer reported in dogs with oral melanoma that, ~70% had metastasis when lymph adenomegaly was present, but more importantly, ~40% had metastasis when no lymph adenomegaly was present [15]. According to the report by Spangler and Kass, 38% and 12% of feet/mucosal surface of lips and cutaneous melanocytic tumors, respectively, exhibited malignant behavior. It has been observed that 4% and 27% of dogs that died due to a foot/lip and cutaneous melanoma, respectively, had a tumor score that would have predicted a benign behavior [8].

G.3.Treatment

G.3.1. Surgery

Surgery is currently considered the standard of care in treating canine COM [16]. Studies have shown that performing wide resections (unilateral mandibulectomies) does not lead to decreased local recurrence or metastatic disease compared to partial mandibulectomies [17]. One study found a correlation between clean surgical margins and increased MST [18]. However, other studies did not find such a correlation, possibly due to the presence of metastatic disease. Despite clean surgical margins, Sarowitz et al. reported that 11 out of 40 patients experienced local tumor regrowth in 2017 [19].

Four studies provided information about WHO classifications for the dogs enrolled in the research. The majority of the cases reported in these studies were either stage II or III. Only one study presented the median survival time (MST) for the dogs according to their stages. For stage I dogs, the MST was 559 days, while for stage II and III dogs [5, 20], it was 121 days. , For all stages combined, the MST was 228 days [5]. One study presented no data on the correlation between tumor stage and survival [17].

Only two of the four studies reported median disease-free intervals. The first study reported that the range was not reached, and the median disease-free interval was more than 567 days [20]. The second study reported a median disease-free interval of 152 days, ranging from 3 to 2360 days [19].

Most studies have observed local tumor regrowth and regional and distant metastasis. Both entities were commonly found in the research conducted [17, 19-28]. Metastasis was mainly observed in the regional lymph nodes and the thorax. However, it was also found to have spread to other abdominal organs, including the brain, heart, abdominal wall, and appendicular skeleton. This information has been reported in various studies [17, 19-28].

The complications that arise after wide and radical excisions are inherent to these procedures and are not directly related to the tumor unless the tumor remains in the surgical line. Among these complications, dehiscence is most commonly reported after caudal maxillary excisions [23]. Various studies have evaluated the effectiveness of surgical excision alone and found that it provides a good mean survival time (MST). However, the need for standardized outcomes in these studies makes comparisons difficult. Few studies have reported the World Health Organization (WHO) classification of tumors, and the starting point of reported MSTs needs to be clearly defined. Additionally, clean surgical margins and the location of tumors in the oral cavity are important variables with potential predictive value, but they were seldom reported in a standardized manner. Further studies are needed to understand the potential predictive value of surgical margins and COM location.

G.3.2. Chemotherapy

Chemotherapy (CT) is using drugs or chemical substances to treat rapidly dividing cancer cells. This treatment also includes tyrosine kinase inhibitors despite not being cytotoxic [29]. The perfect chemotherapeutic agent would possess selective toxicity, be highly distributed throughout the tumor burden, be free from resistance development, and be non-toxic to the patient [30]. Out of 12 studies, only 6 reported the response rate. Among them, dogs treated with intralesional cisplatin showed the highest overall response rate (60%) [31]. However, the median survival time (MST) in that study was only 116 days despite the good overall response rate. In all other studies, the response rate could have been better, being less than 20%. In a multivariate analysis, Boria et al. found that only the dose of cisplatin in mg/kg was significantly associated with the response [32]. On the other hand, mitoxantrone did not appear to be an effective chemotherapeutic, with only 1 out of 12 cases showing partial remission [33]. Median survival times should be evaluated based on whether the study considered survival time from the point of diagnosis, surgery, or at the point of the institution of adjunctive therapy.

Out of the 12 studies that reported MST, carboplatin provided the most extended survival times (440 and 389 days, respectively) [34, 35], but these were defined as survival from the point of diagnosis. Boston et al. reported a MST of 353 days from the point of surgery [36]. Mastinib, cisplatin in combination with piroxicam, and intralesional cisplatin resulted in poorer MSTs (119, 119, and 116, respectively) [31, 32, 37]. Still, the survival in these studies was taken from the initiation of CT, most commonly in dogs with non-resectable tumors or after recurrent disease.

In the majority of the studies that combined dogs with and without prior surgery, there needed to be more standardized reporting and specific outcomes of dogs, evaluating the effect of previous surgery and subsequent CT compared to only CT impossible. However, Brockley et al. and Boston et al. identified no significant differences in dogs that underwent only surgery (495 and 335 days, respectively) compared to dogs that underwent surgery and carboplatin therapy (389 and 352 days, respectively) [34, 36]. Interestingly, Brockley et al. showed that carboplatin makes no significant difference to survival if gross (macroscopic) disease is present (184 days) compared to palliative therapy alone (141 days).

Dank et al. found that stage of disease, treatment with RT therapy, and carboplatin dosage were not associated with a shorter progression-free survival or overall survival [35]. The lack of low MST in treated dogs may be related to dose reductions and subsequent lowered median dose delivered due to varied chemotherapeutic toxicities in three studies [34, 35, 38]. Tuohy et al. found that dogs receiving adjuvant therapy (14/29 receiving a form of adjunctive CT) after surgical excision had a higher hazard of disease progression but not death, compared with dogs that did not receive adjuvant therapy after adjustment for tumor size and presence of metastases at diagnosis [20].

Mastinib was evaluated in COM cases with advanced disease (stage III and IV only) that were progressive despite conventional treatment with surgery or RT. Despite the advanced disease,

survival was comparable to the combination of cisplatin and piroxicam and intralesional cisplatin implant, of which the majority were stage II and III [31, 32, 37]. The majority of adverse events for systemic CT were considered mild to moderate, and almost all were self-resolving. Carboplatin was associated with the highest grade of complication, all of which involved gastrointestinal toxicosis—vomiting or diarrhea (for which two dogs were euthanized) or hematological toxicities such as neutropenia and thrombocytopenia [34, 35, 38]. Local intralesional cisplatin was associated with local necrosis limited to the implant site or oral ulceration; three dogs developed oronasal fistulas and another two trismus that resolved after a few weeks [31].

In conclusion, the available studies lacked uniformity in design, control groups, and reporting of response and survival variables. From the limited studies available, it would appear that the inclusion of chemotherapeutics after surgery for non-resectable or progressive tumors does not offer a significant survival benefit beyond that of surgery [20, 34-36]. However, further studies with Mastinib, Toceranib, and other chemotherapeutics are required.

G.3.3. Radiotherapy with Adjunctive Therapy

Radiotherapy is a medical technique that uses ionizing radiation to kill cancer cells. It is usually administered with the help of a linear accelerator. It has long been used as an adjunctive therapy for sarcomas and carcinomas in veterinary medicine.

Several studies have evaluated the effect of combining radiotherapy with chemotherapy. Two of these studies included a control group receiving only radiotherapy, while the other were retrospective case series [39-41]. In one study, the melanoma vaccine was also used as an adjunctive therapy [42]. Overall, the outcome of these studies for oral malignant melanoma (OMM) was average. The number of dogs treated in these studies varied widely, with some studies including over 100 dogs and others having less than 40 [40, 41]. The majority of dogs were classified at stages II and III, and treatment regimens varied from 3 to 8 fractions, with

total Gys ranging from 24 to 50 Gy, depending on the study design and intention with therapy. Carboplatin was the most commonly used systemic chemotherapeutic, followed by cisplatin, and only one study used melphalan [39, 41, 43, 44]. Cisplatin was used as a local chemotherapeutic in one study [40].

It is important to note that radiotherapy is more effective in treating microscopic disease than macroscopic disease [45]. Only one study did not include surgery before the initiation of radiotherapy, meaning that only gross disease was present in that study [43].

In studies where multiple chemotherapeutics were administered to different dogs, the survival rate was reported for the group as a whole. As a result, it was not possible to identify individual treatment advantages [39-41]. Kawabe et al. compared orthovoltage X-ray (OVX), megavoltage X-ray (MVX), and electron beam radiotherapy, but the results were difficult to interpret, as 52/111 dogs received local or systemic therapy in addition to radiotherapy [40].

In the studies conducted, the median survival time for dogs who received radiation therapy along with an adjunctive chemotherapeutic was found to be between 134 [42] and 396 [20] days. However, a study by Proulx et al. showed that the administration of systemic CT (carboplatin or melphalan) did not have any effect on the time to the first event, development of pulmonary metastasis, or survival [41]. Similarly, a study by Murphy et al. found no evidence of a beneficial effect of carboplatin therapy when given in conjunction with radiation therapy [43]. The median dose of chemotherapeutic, particularly carboplatin, was below the recommended dose in some of the larger studies, which may be the reason for the lack of response. Higher doses of carboplatin may result in longer median survival times [40, 41, 43].

The studies indicate that the median survival time of dogs who received radiation therapy alone is comparable to the median survival time of dogs in other studies who also received radiation therapy despite the use of different radiotherapy protocols in these studies [46, 47]. Tuohy and colleagues conducted a study that showed that out of 29 dogs who had undergone surgical

excision and received a combination of adjuvant RT, CT, and/or immunotherapy, 14 dogs had a higher likelihood of disease progression compared to dogs who did not receive adjuvant therapy [20]. However, there was no significant difference in terms of death after adjusting for tumor size and the presence of metastases at the time of diagnosis. On the other hand, Cunha and colleagues reported that dogs who received surgery/CT/RT had the highest median survival time (380 days) followed by dogs treated with CT/RT therapy (150 days) [44]. Dogs treated with RT alone had the lowest median survival time (60 days), but the results were limited because only three dogs were in the RT-only group, and all were in stage IV, while the 4/5 dogs receiving surgery/CT/RT were in stage III, and one was in stage II [44]. Additionally, dogs in stage II had a significantly longer survival time when compared to dogs in stage IV [44]. Another study evaluated 107 dogs who received either OVX (68 dogs) or MVX (39 dogs) therapy [40]. The study revealed that dogs who received MVX had a significantly longer survival time (233 days) compared to dogs who received OVX therapy (121.5 days). The study evaluated the WHO classification and found that only dogs with stage III COM showed a significant difference in survival when OVX or MVX was used, with MVX resulting in longer survival. It was observed that dogs with stage I disease had a significant difference in median survival time compared to those with all other disease stages [40]. The most commonly reported side effects of radiation therapy (RT) and chemotherapy of Grade 1 and 2 in acute cases, only one study reported gastrointestinal and hematological toxicities caused by carboplatin, a chemotherapeutic drug [39]. Despite these adverse effects, they should not be a reason to avoid RT and chemotherapy. However, due to the varying study designs and RT and chemotherapy dosages, clear treatment guidelines cannot be provided. Although the overall response to RT with an adjunctive chemotherapeutic was good, the median survival time (MST) varied widely. While two studies identified no advantage of chemotherapeutics over RT alone, the only study that found a benefit over RT alone consisted of only three dogs in the

RT-only group. The available evidence does not support the use of adjunctive chemotherapeutics over RT alone to improve MST. Further studies are required with chemotherapeutics administered at higher dosages.

G.3.4. Immunotherapy

The immune system plays an active role in preventing tumor formation, which is called 'cancer immunosurveillance' [48]. This is the basis of immunotherapy [49], which focuses on either stimulating an immune response against cancer or reducing the immunosuppressive nature of the tumor microenvironment [50].

G.3.5. Vaccination

Therapeutic vaccination is a method used to train the immune system to identify antigens specific to tumors. The most commonly used vaccination strategies for treating canine OMM are whole-cell tumor vaccines and deoxyribonucleic acid (DNA) vaccines. Whole-cell tumor vaccines contain irradiated or lysed tumor cells, with or without immunostimulatory cytokines, and induce an immune response against many tumor antigens [51]. On the other hand, DNA vaccination, which is predominantly bacterial plasmid-based and occasionally dendritic cell-based, encodes tumor-specific xenoantigens and generates antigen-specific humoral and cellular immunity [52].

Oncept™ is a vaccine that encodes the huTYR pDNA, a human tyrosinase tumor-targeted antigen, through bacterial plasmid DNA. This vaccine is the first cancer vaccine to receive full approval from the US Department of Agriculture and has been extensively researched for its use in dogs with OMM [53-58].

Two studies have been conducted to evaluate the efficacy of Oncept™ as a surgery adjunctive, both of which have found it to provide no survival advantage. The mean survival times (MSTs) of dogs that received adjunctive vaccination ranged from 335 to 485 days, compared to surgical

controls with MSTs of 352 to 585 days [36, 56]. However, the presence of confounding adjunctive treatments, in addition to surgery, as well as the use of another DNA vaccine with unknown constituents (Wisconsin vaccine) in one of the studies, makes it difficult to draw a conclusive interpretation of these results [36, 56]. It should be noted that studies evaluating vaccination as the only surgical adjunctive did not include control groups and were largely limited to stage II OMM patients [54, 55]. In one such study, the median survival time of stage II patients who received vaccinations could not be determined by the end of the data analysis. However, it was reported that the lower 25th percentile of survival time in all vaccinated patients was 464 days [55]. Another study reported a median survival time of 806 days in six dogs that were still alive by the end of the study period, while the remaining sixteen dogs died due to progressive disease during the course of the study [54].

The use of dendritic cell-based DNA vaccines has only been reported in case studies [59]. In one study, autologous bone marrow-derived dendritic cells expressing a human melanoma antigen called gp100 (BM-DC Adhgp100) were administered to patients with stage I COM. The results were mixed, with one patient showing no signs of disease recurrence after 1440 days, and another having a survival time of 210 days. Another patient with stage III OMM who received the treatment survived until drowning at 660 days, but no post-mortem examination was conducted to evaluate the presence of the disease [59]. In a case series of dogs, combining vaccination strategies, including whole-cell and DNA, using an allogenic whole-cell tumor vaccine that expresses xenogeneic human glycoprotein 100 (Hgp100-ATCV), resulted in a response rate of only 16% (4 out of 25) with a median survival time of 153 days. Among the responders, the median survival time was 417 days while non-responders had a median survival time of 95 days. It is worth noting that most of these dogs had advanced-stage disease [60].

There is currently not enough high-quality evidence to support using vaccination as a surgical adjunctive treatment for dogs with OMM. The OEG rating for vaccination's effectiveness is a

C. The survival benefit provided by vaccination is difficult to determine due to a lack of consistent comparative control groups and other treatment modalities that may confound results [36, 53-58]. There is a need for randomized, double-blinded, controlled clinical studies to evaluate the usefulness of melanoma vaccines as both a surgical adjunctive and beyond that role.

G.3.6. Gene Therapy

Foreign DNA can be delivered into cells through a process called transfection, which uses either viral or non-viral vectors such as liposome delivery or DNA protein complexes [52]. This allows for the local delivery of different gene products, resulting in specific anti-tumoral responses while minimizing the potential toxicity that may arise from systemic exposure [52, 61]. In a clinical trial involving dogs with melanoma, it was found that the combined administration of suicide gene therapy and xenogeneic cells secreting cytokines significantly delayed or prevented distant metastasis and extended survival times [62]. The trial enrolled dogs with oral malignant melanoma (OMM), and 95% of the enrolled dogs had it [62]. The suicide gene therapy involved the infection of cells with the herpes simplex virus thymidine kinase gene, which facilitated the activation of ganciclovir. The xenogeneic cells secreted two cytokines, human granulocyte–macrophage colony-stimulating factor (hGM-CSF), and interleukin-2 (hIL-2).

The trial found that the percentage of metastasis-free patients at the study end in the combined treatment group (76%) was significantly higher than the untreated controls (29%), surgery-treated controls (48%), and the suicide gene-treated-only controls (56%) [62]. Also, the metastasis-free survival and median survival time (MST) were significantly extended in the combined treatment group (509 days and 160 days, respectively) as compared to untreated

controls (41 days and 69 days, respectively), surgery-treated controls (133 days and 82 days, respectively), and suicide gene-treated only controls (>159 days and 94 days, respectively) [62]. The combined treatment group had an overall response rate of 46%. One dog achieved complete remission of pulmonary metastasis but was later euthanized due to primary tumor progression after 123 days of treatment [62]. The study found that repeated injections of the suicide gene system and cytokine-secreting xenogeneic cells into the tumor bed can significantly control tumor growth, prevent distant metastasis, and increase the survival rate [62]. In a clinical trial, where patients with oral malignant melanoma (OMM) constituted 82% of the enrolled population, injecting xenogeneic Vero cells expressing human interleukin-2 around the tumor site during surgery or radiation therapy resulted in a median survival time (MST) of 270 days, compared to 72 days in those who only received the primary treatment [63]. However, other literature on gene therapy is limited to case reports and small case series, with low levels of evidence (LOE). In these reports, injecting immunogenes encoding T-cell activators (such as adenovector CD40 ligand or staphylococcal enterotoxin B and canine GM-CSF) or apoptosis promoter Fas-ligand have been found to be safe and have shown promising antitumoral effects, with reported overall response rates (OR) ranging from 55% to 100%. However, the studies largely involved dogs with oral melanoma and more targeted analysis of the oral subpopulation or larger-scale studies are needed to draw meaningful conclusions [64-67].

G.3.7. Checkpoint Inhibitors

T lymphocytes are the primary cells responsible for the immune response against tumors [52]. Immune checkpoints are receptors present on the surface of these T cells that provide regulatory feedback to limit the effector phase of T-cell expansion and function [52]. In healthy individuals, immune checkpoints help develop tolerance to self-antigens. However, their upregulation in many tumors is critical in tumor-associated immune suppression and evasion

[52]. Targeting inhibitory immune checkpoints using monoclonal antibodies can inhibit tumor-associated immunosuppression and enhance autoimmunity [52, 68]. A chimeric anti-programmed cell death ligand 1 (PD-L1) monoclonal antibody (c4G12) has been shown to improve survival in dogs with stage IV disease. In a prospective non-randomized clinical trial, the treatment group achieved a median survival time (MST) of 143 days, compared to 54 days in the institutional historical control group [69]. However, in another retrospective study, its antitumoral response was less apparent [69, 70]. The OR rate was just 14% (1/7), and no statistically significant change in MST in dogs with stage IV disease was achieved [69, 70].

In a non-randomized clinical trial, two types of anti-programmed cell death protein 1 (PD-1) monoclonal antibodies (chimeric rat-dog-ch-4F12-E6 and caninized-ca-4F12-E6) were evaluated in dogs with late-stage disease (91% stage IV) [69, 71]. The trial reported a mean survival time (MST) of 166 days, longer than institutional historical controls with a mean survival time of 55 days. However, adjunctive therapies like radiation therapy (RT) were used in some cases, making interpreting the findings challenging [69-71]. One study showed that RT improved the overall survival of the treatment group.

Another monoclonal antibody, chimeric mouse-dog anti-podoplanin (PDPN)-P388f, has been studied in a small case series involving three dogs [OBJ]. However, this study focused on the treatment's safety rather than its antitumoral effects. Well-designed studies with fewer confounding elements and investigation into the effectiveness of this treatment in less advanced stages of the disease are needed.

G.3.8. Nanotechnology

Limited evidence is available on the additional immunostimulatory benefits of 'in situ' vaccination with nanoparticles in conjunction with traditional RT and hyperthermia treatment. This evidence is in the form of low-quality case reports that do not consider the clinical stage of the patients [72, 73]. However, some studies have reported promising results. For instance,

dogs receiving magnetic iron oxide nanoparticle hyperthermia (mNPH) treatment alone had survival times of up to 780 and 1350 days. Dogs who received a combination of plant-based virus-like nanoparticles (VLP) treatment and RT had remission at 600 days, while those who combined all three therapies had remission at 300 and 540 days [72, 73].

G.4. miRNA

miRNAs are a type of small non-coding RNA molecules that are about 22 nucleotides long. They function as regulators of post-transcriptional regulation. The idea that miRNAs are involved in transcriptional regulation came from research conducted in the early 1980s, which discovered that a mutation in the lin-4 gene of *Caenorhabditis elegans* caused developmental abnormalities [74, 75]. Mutations in the *Caenorhabditis elegans* gene lin-14 can lead to developmental defects [76]. In 1987, Ferguson et al. discovered that a mutation in *lin-4* caused a negative regulation of *lin-14,* and a suppressor mutation in *lin-14* reversed the *lin-4* mutation phenotype [77]. In 1993, researchers found a regulatory mechanism mediated by non-coding RNA. Two small non-coding *lin-4* transcripts of 22 and 61 nt had sequence complementarity to the *lin-4* small RNAs and 3' untranslated region of *lin-14* [78, 79]. A second microRNA, let-7, was discovered in 2000. The function of let-7 is similar to lin-4, controlling the L4-to-adult stage transition of larval development [80]. Conserved among other species, the let-7 family suggests small RNA regulation is not specific to nematodes, unlike *lin-4* [80]. Almost all metazoan genomes, including worms, flies, plants, and mammals, have been identified to contain hundreds of miRNAs [81]. In 2002, Calin et al. discovered that chronic lymphocytic leukemia samples with deletions on 13q14 frequently exhibit down-regulation or deletion of miR-15 and miR-16 [82]. MiRNA has become a popular area of research for various diseases, including cancers and biomarkers, in recent years. In 2004, Takamizawa et al. demonstrated that miRNAs have prognostic value. They identified that the reduced expression of *let-7* in human lung cancers was associated with shortened postoperative survival [83]. In 2005, Zhao

et al. reported that miR-1 is specifically expressed in cardiac and skeletal muscle precursor cells. They also found that miR-1 regulates ventricular cardiomyocytes by affecting cardiac regulatory proteins to control differentiation and proliferation during cardiogenesis [84]. In 2007, Sonkoly et al. found that miR-203 expressing keratinocytes were upregulated in psoriasis-affected skin of humans with autoimmune disease compared to healthy skin or chronic inflammatory skin disease [85]. Stanczyk et al. observed increased expression of miR-155 and miR-146 in synovial fibroblasts and tissues affected by rheumatoid arthritis, another autoimmune disease [86]. In 2007, Schaefer and colleagues proposed that miRNAs may be involved in developing neurodegenerative disorders [87]. In 2010, Weber et al. explored the potential of miRNA profiling in various body fluids for detecting and monitoring physiopathological conditions [88]. Due to the significant implications of miRNA in cancer, miravirsen became the first to enter Phase I clinical trials in 2009. Miravirsen is a 15-nucleotide antisense RNA oligo that complements the 5' end of miR-122 and is used for the treatment of HCV [89].

G.5. miRNA biogenesis

miRNA biogenesis involves gene transcription, processing by Drosha, Exportin-5 exportation to the cytoplasm, Dicer processing, and loading onto AGO proteins. RNA polymerase II mainly transcribes miRNA genes [90]. The process of miRNA formation begins with the transcription of primary miRNA, which is then processed in the nucleus by an enzyme called Drosha. The resulting pre-miRNA molecule is transported out of the nucleus and into the cytoplasm by Exportin-5 (EXP5). Once in the cytoplasm, the pre-miRNA is cleaved by an enzyme called Dicer, located near the terminal loop, producing a small RNA duplex. This small RNA duplex is loaded onto an AGO protein and forms an RNA-induced silencing complex (RISC).

G.6. miRNA-mRNA binding: types of target sites

After being incorporated into the RISC, miRNA guides the complex to target the mRNA's 3' untranslated region (3' UTR), thereby leading to mRNA repression or degradation. The fate of mRNA depends on the degree of base pair matching. If the miRNA perfectly matches the mRNA, the target mRNA will be degraded. However, miRNA usually has mismatches to mRNA, which results in mRNA repression while it remains intact. Target degradation can also be triggered. Imperfect-centered miRNA binding sites are common and can mediate the repression of target mRNA. However, the functional importance relative to the seed region remains unknown [91]. The most effective binding site for miRNA-mRNA interaction is conserved Watson-Crick pairing in the miRNA seed region nucleotides 2-7 of the 5' end [92-96]. Listed in decreasing order of conservation and efficacy: 1) 8mer site. miRNA positions 2-8 match with an A in position 1 [96]. 2) 7mer-m8 site. miRNA position 2–8 match [94-96]. 3) 7mer-A1 site. miRNA position 2–7 matches with an A in position 1 [96]. 6mer sites have weaker preferential conservation and much lower efficacy, including 4) 6mer sites. miRNA position 2–7 match [96]. 5) Offset-6mer. miRNA position 3–8 match [97] and 6) 6mer-A1 site, in which the efficacy is difficult to distinguish from the background [97, 98]. The 3' end supplementary pairing can enhance canonical sites, but only up to 5% of seed-matched sites [94, 96-98].

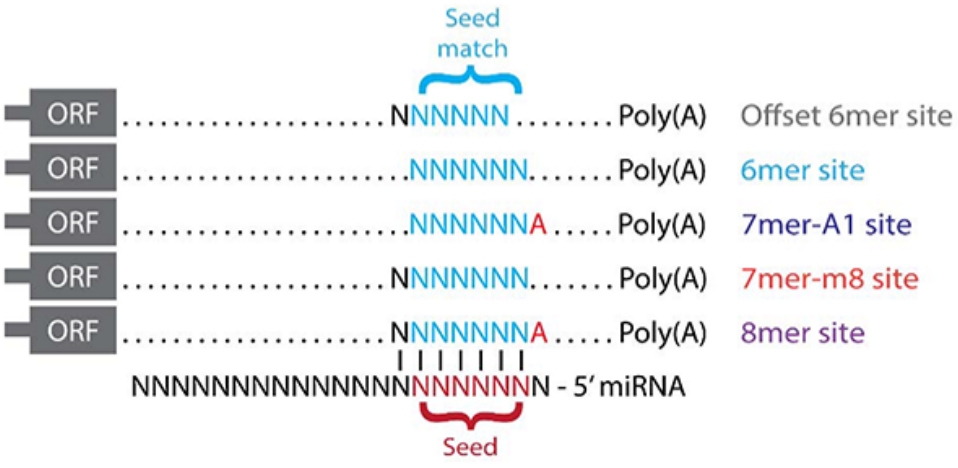


Figure G- 1. Canonical binding site types of miRNAs from Target scan website.

Computational methods can predict miRNA targets based on binding types and characteristics.

G.7. Study of Transcriptome

Most previous studies on comparative oncology have used a single or multiple gene approach to investigate similarities. However, as sequencing technologies have advanced, it has become far better to study comparative oncology at the whole transcriptomic level. While cancer transcriptome studies in veterinary research are rare, the trend of transcriptome studies has been increasing recently. Conversely, after the partial transcriptome from the human brain was first revealed, the study of the transcriptome in cancer has steadily increased year by year. For example, breast cancer transcriptome studies increased more than 200 times between 2006 and 2014, highlighting the need for studying the canine mammary gland tumor transcriptome in the field of comparative oncology. The transcriptome refers to the study of all RNA molecules in the cell, including mRNA and non-coding RNA transcripts produced by cells from the genome under specific circumstances. Studying the transcriptome under different circumstances allows the identification of genes or transcripts that are differentially expressed due to the condition. Some of these are drivers, while others change due to secondary effects. Therefore, comparing the transcriptome in various diseases or experimental conditions allows for the identification of differential as well as driver genes or transcripts.

G.8. Limitations in current cancer research

There are many theories on what causes and drives the development of various cancers. However, the traditional preclinical research methods that are commonly used to study cancer cells can be limiting [99]. These methods usually involve growing cancer cells in 2 or 3-dimensional cultures or using murine xenograft models to test cancer drugs. Unfortunately, this approach has led to high drug attrition rates. Additionally, there are several challenges

associated with overcoming cancer, which include: (1) difficulties related to targeting cancer stem cells (CSCs), (2) drug resistance of cancer stem cells, which can lead to anticancer drug immunity, (3) a lack of cancer epigenetic profiles and specificity of existing epi-drugs, (4) difficulties in diagnosing cancer, which can make treatment more challenging, (5) a lack of effective biomarkers to diagnose and predict cancer, (6) limitations of conventional chemotherapeutic agents, and (7) challenges in treating cancer metastasis (Fig 2) [100].

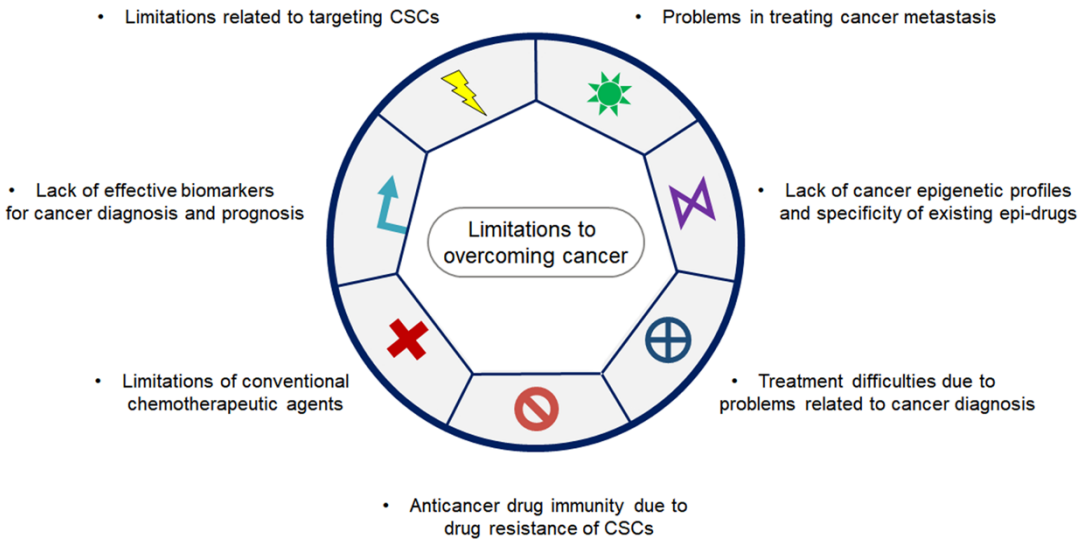


Figure G- 2. Limitations in the current cancer research [100].

G.9. Dog model for human diseases

The domestication of dogs is believed to have occurred between 12,000 to 40,000 years ago [101-104]. During dog domestication, they became loyal companions to humans. Unfortunately, they can also transmit diseases. The domestic dog, *Canis familiaris*, carries around 450 diseases. Around 360 of these diseases are similar to those found in the human field [105-107]. Due to their strong anatomical and physiological similarities, particularly in the cardiovascular, urogenital, nervous, and musculoskeletal systems, dogs have been a subject of early interest and research for scientists. They provide an excellent model for identifying and

studying disease loci, as they are often spontaneously afflicted by many heritable diseases [105, 106]. A well-known disorder in Briard's breed is congenital stationary night blindness. The counterpart of this disease in children is Leber amaurosis.

Canine counterparts of Duchenne muscular dystrophy exist in Golden Retriever, Beagle, and German short-haired pointer [108]. Alport syndrome (AS) is a genetic disorder that affects human kidneys. Defects in the glomerular basement membrane cause it. AS has also been found in several canine families. X-linked hereditary nephropathy (XLHN) was first identified in the Samoyed breed and later in a mixed-breed family [109]. A correlation has been discovered between the deposition of amyloid-β (Aβ) plaques in dogs with cognitive dysfunction (CCD) and Alzheimer's disease (AD) in humans. This discovery suggests that senile dogs suffering from CCD are a valuable non-transgenic model for studying the neurodegenerative processes associated with aging and early-stage AD [110]. Several studies indicate that dogs can be used as a model for human cancer.

G.10. Comparative Oncology: Human Vs Dog

Comparative oncology is a field of study that investigates cancers in companion animals to determine their relevance to human cancers [111]. Different types of cancers naturally occur in various companion animals, such as dogs, cats, rabbits, and horses [112, 113]. Since many dogs are diagnosed with cancer every year, canine cancers are extensively researched. Additionally, the availability of canine-specific resources, reagents, and scientific literature is increasing, making it possible to conduct comparative oncology research between humans and dogs (Figure 3 [100]). A lot of cancer research relies on mouse models because they are small in size and cost-effective [114]. However, these models have limitations in mimicking human cancers. In humans, tumors occur spontaneously while in mice, tumor formation must be induced. As a result, mouse models usually lack the gene networks and interactions that are

responsible for tumorigenesis in humans. Canines and humans share similar histological types of cancer, making dogs excellent models for comparative oncology.

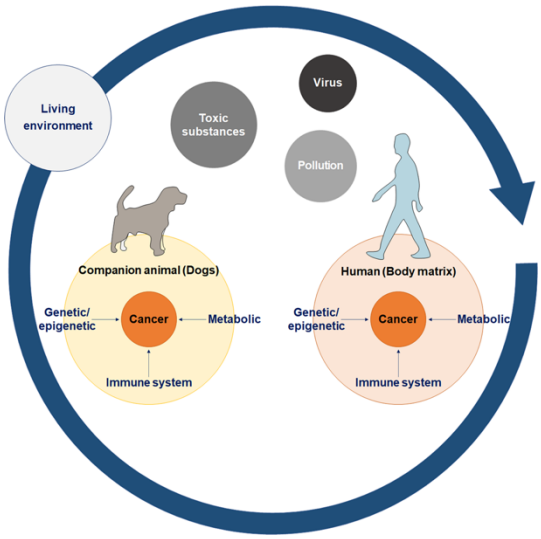


Fig 3. A schematic diagram of Human Vs dog as a companion animal [100]

There is strong evidence that canines and humans share similar genes and pathways involved in tumorigenesis. For instance, research has found a significant association between BRCA1 and BRCA2 SNP markers and mammary cancers in English Springer Spaniels as well as breast cancers in humans [111].

Around 1 million out of the 77 million dogs in the United States develop cancer each year. Half of these canine cancers are observed in dogs that are 10 years old or older, and a quarter of all dogs will have cancer at some point in their lifetime [115]. To conduct cancer research, the Canine Comparative Oncology Genomics Consortium (CCOGC) research project was launched at the National Cancer Center (NCI) in 2004. The project aims to establish a biorepository of canine cancer tissues and blood samples to decode their genes. However, the research work carried out in the past decade on canine cancers has mainly focused on tumor biology, pathology, genetics, and epigenetic pathways, but their thorough analysis is yet to be

conducted. Studies have shown that the noncoding regulatory regions of canine genomes are more similar to human genomes than mouse genomes [116]. Recent genomic and epigenomic comparisons across tissues of different species revealed that chromatin map overlaps more between canines and humans (about 40-50%) than between mice and humans (about 10-20%). Additionally, super-enhancers were more highly conserved between canines and humans (about 90% at a minimum mismatch ratio of 50%) than between humans and mice (about 30% at a minimum mismatch ratio of 50%) [117]. As a result, the epigenetic changes affected by the environment may be more similar between canines and humans as compared to between mice and humans. Therefore, researchers have focused on comparing genetic and epigenetic aspects of canine and human cancers.

G.11. miRNAs in Cancer

G.11.1. Mammary gland/breast cancer

It has been reported that in canine mammary gland tumors, miRNA-21 and miRNA-29b are expressed at higher levels compared to normal mammary gland tissues [118]. These miRNAs, known as oncomiRs, have also been found to be involved in regulating invasion, migration, and metastasis in human breast cancers [119]. However, in the case of canine tumors, miRNA-21 and miRNA-29b are involved in inhibiting tumor cell apoptosis. A study using a quantitative polymerase chain reaction array analyzed the expression of 277 miRNAs in tissues and cell lines from canine mammary gland tumors [120]. The results showed that miRNA-141 was overexpressed in the assessed cell lines and was experimentally validated to target the tumor suppressor INK4 mRNA, making it a potent oncomiR. The study found a direct correlation between the expression of miRNA-141 and its target mRNA (p16/INK4A). Two cell lines (CMT12 and CMT27) that overexpressed miRNA-141 did not express its target mRNA, while the cell line that was negative for the miRNA expressed the tumor suppressor gene. The study also revealed that miRNA-21, -155, and -9 were overexpressed in both human and canine

breast cancer tissues examined, while miRNA-31, -34a, and -143/145 were down-regulated in the tissues. Additionally, miRNA-429 and -200c were overexpressed by more than 1000- and 150-fold, respectively, in the canine mammary tumor cell lines studied. Both miRNA-429 and miRNA-200c were predicted to target the ERBB receptor feedback inhibitor 1 (ERRI1), which is another tumor suppressor gene, indicating that these overexpressed miRNAs are oncomiRs. In human breast cancer, miRNA-9 has been found to aid metastasis by targeting the mRNA that encodes E-cadherin [121]. This is evident as the expression levels of miRNA-9 are correlated with tumor grade and metastatic status. When E-cadherin is silenced, β-catenin signaling is activated, which enhances angiogenesis through vascular endothelial growth factor activation. In non-metastatic breast cancer cells, inducing miRNA-9 expression leads to the formation of lung micrometastasis in mice. Conversely, silencing miRNA-9 expression using miRNA sponges in highly malignant breast cancer cells prevents the establishment of metastases in the mouse model. This implies that targeted miRNA-9 could play a vital role in cancer therapy by preventing the formation of metastases in both humans and dogs affected by solid tumors.

G.11.2. Lymphoma

Canine lymphoma is a tumor that is commonly diagnosed and studied in dogs. It has been suggested that it could be used as a model for the study of human T-cell and B-cell lymphomas [122]. In a recent study, a miRNA microarray kit was used to identify several miRNAs that are dysregulated in canine T- and B-cell lymphomas as compared to peripheral blood mononuclear cells (PBMC). The study found that miRNA-19a, -19b, and -17-5p were up-regulated in their tumor samples and cell lines. Both miRNA-19a and miRNA-19b were overexpressed in B-cell and T-cell tumors as compared to PBMC. miRNA-19a + miRNA-19b belongs to the oncomiR-1 cluster (miRNA17-92), an oncogene that is known to effectively inhibit apoptosis. Hence, increased expression of miRNA-19a and miRNA-19b contributes to the oncogenesis of canine

T- and B-cell lymphomas [123]. miRNA-17-5p is similarly reported to be overexpressed in canine B-cell lymphomas, although the study suggested a dual role for the oncomiR as a tumor suppressor due to its interaction with several known promoters of cellular proliferation and other transcriptional regulators. This suggestion of a dual role for miRNA-17-5p has been previously made in human breast cancer. Another study reported the ability of miRNA-17-5p to differentiate between high- and low-grade samples of canine splenic lymphomas, with intermediate/high-grade samples expressing significantly higher levels of miRNA-17-5p. This example demonstrates that a miRNA can be a tumor suppressor or an oncomiR depending on the mRNA whose translation the miRNA is regulating. It has been reported that miRNA-203 has a tumor-suppressive effect in canine lymphoma [122]. Research has shown that miR-181a, miRNA-218, and miR-203 were expressed at lower levels in canine lymphoma cell lines and tissues. Similarly, miRNA-203 was also found to be down-regulated in human acute lymphoblastic leukemia and chronic myelogenous leukemia [124]. When miRNA-203 is expressed, it targets the ABL1 mRNA and inhibits the fusion of ABL1 and BCR which leads to the formation of the Philadelphia chromosome. This helps to prevent cell proliferation. The ability to inhibit tumor cell growth or invasion following ectopic expression of down-regulated miRNAs indicates that non-coding RNAs have significant value in the treatment of canine and human lymphoma and leukemia. Additionally, miRNA-155 was found to be down-regulated in canine splenic lymphomas [125] and in intermediate/high-grade samples compared to low-grade samples. In contrast, overexpression of miRNA-17-5p was correlated with the mitotic index of the tissues, suggesting that miRNA-17-5p (increased expression) and miRNA-155 (low to absent expression) could be used as potential markers for canine lymphomas.

G.11.3. Mast cell tumor

Canine mast cell tumors (MCT) are known to progress due to the overexpression of miRNA 9, as revealed in a study [126]. The study found that miRNA-9 is overexpressed in malignant cell

lines and high-grade tumors in canines, but not in normal canine bone marrow-derived mast cells (BMMCs) and low-grade tumors, respectively. The same study conducted an experiment on mouse BMMCs and discovered that forcing the overexpression of miRNA-9 in these cells significantly increased tissue invasion and metastasis by up-regulating CMA1 expression. This, in turn, activated matrix metalloproteinases, an enzyme that degrades the extracellular matrix and triggers matrix remodeling. Since miRNAs are involved in tumor progression and metastasis, they are potential candidates for targeted therapy, particularly in cases where resistance to conventional chemotherapeutic drugs has developed. Although mastocytomas (MCTs) are rare in humans , no reports regarding miRNA expression in human MCTs were found during literature searches.

G.11.4. Hemangiosarcoma

A recent study aimed to investigate the expression of miRNA-124 in clinical samples and cell lines of canine hemangiosarcoma [127]. The study concluded that miRNA-214 was down-regulated in all the samples and cell lines, indicating its role in the development of the disease. Furthermore, the study found that the ectopic expression of miRNA-214 led to growth inhibition in the cell lines in a dose-dependent manner. Additionally, miRNA-214 was found to increase apoptosis in the cell lines by elevating the expression of p53-regulated genes. The study also discovered that miRNA-214 regulates p53 by targeting the COP1 E3 ubiquitin-protein ligase, which is a negative regulator of p53 activity. These findings suggest that modulation of COP1/miR-214 could be an effective treatment for malignant endothelial proliferative diseases, including canine hemangiosarcoma [127].

G.11.5. Hepatocellular carcinoma

Lai et al. conducted a study comparing the tissue samples of clinical canine hepatocellular carcinoma (HCC) and canine HCC cell lines with normal canine liver samples [128]. The study

revealed that miR-10b and miR-21 were upregulated while the let-7 family, miR-1, and miR-122 were downregulated in canine HCC. These findings are consistent with studies in humans where miR-10b, miR-21, and miR-2116 are upregulated, and let-7a, let-7g, miR-1, and miR-12216 are downregulated in HCC tissues. Thus, it can be inferred that the regulation of miRNA in HCC is biologically similar between humans and dogs. This is the first study depicting the abnormal expression levels of miRNA in canine HCC tissues and cell lines. miRNAs can contribute to the development and progression of cancer by inhibiting translation or by promoting the degradation of target mRNAs. A study found that the MET gene, which is a direct target of miR-1, was significantly upregulated in both canine hepatocellular carcinoma (HCC) tissues and cell lines. The study also found a significant negative correlation between the expression levels of miR-1 and the MET gene, which suggests that miR-1:MET interactions are involved in canine HCC, similar to human HCC.

Chapter 1

Elevated expression of miR-301a and its functional roles in Canine Oral Melanoma

(Hasan, MD Nazmul, et al. *Veterinary and Comparative Oncology* 22.1 (2024): 78-88)

1.1 Abstracts

Aberrant expression of miRNAs is crucial for phenotypic change in different disease progressions. Numerous dysregulated miRNAs were explored in canine oral melanoma (COM). The upregulation of miR-301a in COM is one of them. The biological role of miR-301a in various human cancer types, including human malignant melanoma, has been articulated. However, the biological process of miR-301a in COM is unknown and requires exploration. This study aimed to investigate whether miR-301a could serve as early diagnostic biomarkers for COM, the functional roles of miR-301a in COM, and its possible pathways. The relative expression of miR-301a in tissues, plasma, and cell (KMEC and LMEC) lines was inspected using qRT-PCR. The knockdown of miR-301a in KMEC and LMEC cells was also validated using qRT-PCR. We performed CCK-8 assays for cell proliferation, monolayer wound healing, transwell migration assay for cell migration, colony formation assay to observe the clonogenicity, TUNEL assay, and flow cytometry for analyzing the percentage of apoptotic cells. Our study revealed that miR-301a was markedly upregulated in COM tissues and plasma, suggesting that it might be a biomarker for COM early diagnosis. An in vitro study demonstrated that miR-301 significantly inhibited apoptosis in COM cells while promoting cell migration, proliferation, and clonogenicity. Also, the phenotypic changes were different between the metastatic LMEC cell line and the primary KMEC cell line. Using KEGG and GO enrichment analysis, we also predicted that miR-301 could follow the Wnt signaling pathway in COM. Taken together, the findings suggest that miR-301a is one of the oncomiRs for COM via regulating several oncogenic phenotypes and could be a potential diagnostic biomarker.

Keywords: miR-301a, Canine oral melanoma, proliferation, migration, apoptosis.

1.2 Introduction

Oral melanoma is a highly aggressive oral cavity tumor in humans and dogs [129, 130]. Canine oral melanoma (COM) is a common tumor that accounts for 30-40% of all malignant oral neoplasia. Due to its violent and invasive local behavior, it represents significant clinical problems and is more prone to rapid metastasis [12]. For stage I, stage II, and stage III dogs, the average survival time after surgical treatment is 17, 6, and 3 months, respectively [14]. An adjuvant vaccine and radiation therapy are currently applied to treat COM patients along with surgical treatment [16, 73]. However, the prognosis of the therapies is still quite limited. Therefore, a new therapeutic approach is highly time demandable for the early diagnosis treatment of COM. To identify new therapeutic targets for COM patients and elucidate the functional aspect, molecular-based therapy may be an option. Therefore, identifying biomarkers that accurately detect COM at an initial stage and designing therapeutical approaches to exterminate tumor progression would significantly impact COM tumor consequences.

Recently, many studies have focused on the importance of miRNA-based therapy in the cancer progression [131]. miRNAs are a class of small non-coding RNA molecules with an average length of 20-24 nucleotides, which usually bind to 3'- untranslated regions of target mRNA genes, leading to gene silencing [132]. In tumor tissues, miRNAs either act as tumor suppressors by negatively regulating oncogenes or as oncogenes by silencing the tumor suppressor genes [133]. Numerous dysregulated miRNAs are crucial for migration, apoptosis, carcinogenesis, metastasis, cellular growth, and cell cycle regulation [134, 135]. Several dysregulated miRNAs are found in the COM tumor [14, 136, 137]. miR-301a is upregulated in many cancers and responsible for enhancing cell migration, proliferation, and inhibiting cell apoptosis [138-148]. Functional roles of miR-301a have been explored in human malignant melanoma [129]; however, it remains unclear and needs further exploration in COM.

This study aimed to investigate the bio function of miR-301a in COM and to predict the potential pathways involved in COM progression. Here, we validated expression profiles of the miRNA-301a in the COM tissues, plasma, and cell lines (KMEC and LMEC) using qRT-PCR. We further investigated the effects of the knockdown of miR-301a in KMEC and LMEC cell lines. Our data showed that miR-301a could be a potential biomarker for early diagnosis, enhanced cell proliferation, migration, and clonogenicity, and inhibits apoptosis. miR-301a may follow the Wnt signaling pathways in tumor progression. In short, our data elucidate the oncogenic bio function of miR-301a and suggest a new diagnostic approach by targeting miR-301a in COM.

1.3. Results

1.3.1. Samples Cohort

Forty clinical tissue samples were collected from Kagoshima University Veterinary Teaching Hospital (KUVTH). Ten samples were healthy canine oral tissues considered “control,” and 30 were COM tissue samples. Twenty-five plasma samples were included in this study (5 control, 20 melanoma). The patient's details are summarized in Table 1.

1.3.2 miR-301a can be a potential biomarker of canine oral melanoma

To know the relative expression pattern of miR-301a in COM, we performed a qRT-PCR. The results showed that miR-301a was upregulated in both COM (Fold change; FC=17, P<0.0001) tissues and plasma (FC=1.8, P=0.008) samples compared to control samples (Fig. 1 A-B). We further analyzed the ROC curve between the control and COM (Fig. 1 C). When we use miR-301a for COM diagnosis, the AUC value is 0.87 (P=0.011) in the control vs. melanoma group.

1.3.3. The knockdown of miR-301a in COM-originated cell lines

To elucidate the in vitro function of miR-301a in canine oral melanoma, two canine melanoma cell lines, KMEC and LMEC, were used. The KMEC cell line comes from the primary site, while the LMEC cell line is the metastatic site of the origin [149] . The expression of miR-301a was higher in LMEC (FC=2.6, P=0.03) than in KMEC (Fig. 2 A). Because miR-301a increased in COM, we focused on the functional change by inhibiting miR-301a. miR-301a and NC control inhibitors were transfected in KMEC and LMEC cell lines. The Knockdown of miR-301a and NC in the cell lines was verified using qRT-PCR. The relative expression of miR-301a was significantly decreased in KMEC (P=0.002) and LMEC (P=0.002) cell lines after transfection compared to the NC inhibitor (Fig. 2 B-C).

1.3.4. miR-301a promotes cell proliferation and colony formation

To understand the effects of miR-301a in cell proliferation and colony formation, we performed a CCK-8 assay and a colony formation. The CCK-8 results showed that after the knockdown of miR-301a, cell proliferation capacity was preferentially decreased in the KMEC cell line at 48h post-transfection and in the LMEC cell line at 72h post-transfection compared to the NC inhibitor (Fig. 3A). Moreover, in our study, the knockdown of miR-301a decreased the colony number (average 15 colonies) compared to the NC (average 31 colonies) in the LMEC cell line. However, colony numbers in the KMEC cell (average 6) were not significant compared to the NC inhibitor (average 8). The reason may be that KMEC cells are less aggressive in nature, suggesting that miR-301a might be involved in the metastatic condition of melanoma. Collectively, our results represent that miR-301a promoted cell proliferation in both the KMEC and LMEC and enhanced clonogenicity in the LMEC (Fig. 3B).

1.3.5. miR-301a enhanced cell migration

A monolayer wound-healing assay and a transwell migration assay were performed to evaluate and compare the influence of miR-301a on the migration of LMEC and KMEC cells. Our data revealed that the miR-301a inhibitor significantly inhibited the LMEC (0.39 mm in width, $P<0.0001$) cell for 24h and the KMEC (0.55 mm in width, $P<0.0001$) cell for 30h compared to the NC inhibitor (Fig. 4 A-B). Furthermore, in the transwell migration assay, the mir-301a inhibitor limits cell migration (from the upper chamber to the lower chamber) on LMEC (average 88 cells, $P=0.01$) and KMEC (average 40 cells, $P=0.03$) cell lines compared to the NC inhibitor (average 229, and 173 cells, respectively) (Fig. 4 C-D). Overall, miR-301a enhanced migration in KMEC and LMEC cells.

1.3.6. miR-301a inhibits apoptosis in the cells

To understand the apoptotic effects of miR-301a in the LMEC and KMEC cells, we conducted a TUNEL assay using a fluorescence microscope and an Annexin V-Biotin/PI assay using a flow cytometer. We observed that the mir-301a inhibitor significantly increased the percentage of TUNEL-positive cell numbers in LMEC (average 40%) and KMEC (average 23%) cells than NC inhibitors (17% and 13%, respectively) (Fig. 5 A-B). Furthermore, Flow cytometry results showed that miR-301a inhibitor significantly increased the percentage of early apoptosis in LMEC (average 7.86%) and KMEC (average 6.74%) cells compared to NC inhibitors (0.36% and 3.23%, respectively) (Fig. 5 C-D). Taken together, our data suggest that miR-301a has inhibitory effects on apoptosis.

1.4. Discussion

Dysregulation of microRNA (miRNA) can exert several functions in melanoma, such as acting as onco-miR, tumor suppressive miRNA, regulating the pathogenesis of melanoma by signaling and limiting cell death, migration, invasion, or metastasis [150]. Our previous study has screened many aberrantly expressed miRNAs in COM using a next-generation sequencing [136]. The present study focused on the molecular bio function of miR-301a in oral melanoma-originated KMEC and LMEC cell lines, which may elucidate the underlying molecular mechanisms of miR-301a in COM.

LMEC cells are more aggressive than KMEC cells due to the metastatic site of the origin [149]. miR-301a is a member of the miR-130 family, which also includes miR-130b, miR-301a, and miR-301b, and they all share the same seed sequence [151]. Here, we explored the relative expression of miR-301a was upregulated in COM tissues and plasma samples. ROC curve showed that highly expressed miR-301a in plasma could serve as diagnostic biomarkers for COM. The expression level of miR-301a increased in the LMEC cells more than in KMEC cells, suggesting miR-301a might be more associated with the metastatic progression of

melanoma. For example, interestingly, the colony formation was only suppressed in the LMEC in our results, and the effect of miR-301a inhibition of wound healing and apoptosis assay was more pronounced in the LMEC. Upregulation of miR-301a in human malignant melanoma correlated with metastasis and poor prognosis [129]. Also, miR-301a is upregulated in human ovarian cancer, prostate cancer, pancreatic cancer, gastric cancer, renal cell carcinoma, hepatocellular carcinoma, cervical cancer, esophageal cancer, breast cancer, and lung tumorigenesis [133, 138-141, 152-155]. However, no published report of the miR-301a functional study in canine melanoma exists.

Several studies showed that miR-301a promotes cell proliferation and migration and inhibits cell apoptosis. For example, mir-301a promotes cell growth or proliferation in malignant melanoma, colorectal, pancreatic, and gastric cancer Fields [129, 142-144]. miR-301a is associated with cell migration in malignant melanoma, hepatocellular carcinoma, gastric cancer, and bladder cancer [129, 145, 151, 156]. Some studies showed that miR-301a could inhibit cell apoptosis in different cancers, such as breast cancer, human ovarian cancer, and prostate cancer [146, 152, 157]. We also investigated the functional roles of miR-301a on KMEC and LMEC cells. The phenotypic changes differed between the metastatic LMEC cell line and primary KMEC cell lines. Cell proliferation was significantly inhibited at 48 hr. in KMEC and 72 hr. in the LMEC cell line after transfecting miR-301a, suggesting that miR-301a could initiate cell proliferation in the early stage of melanoma more than metastatic melanoma. One of the possible reasons is that metastatic conditions became more proliferative than the initial stage. Colonies were significantly formed in the LMEC cell line only, which suggested that miR-301a could accelerate the metastatic condition. Migration results revealed that wounds made by scratching were disjointed for 30 hr. in KMEC and 24 hr. in LMEC cells, which postulated that miR-301a could proliferate faster in metastatic condition than initial stage of melanoma. The apoptotic cell number was increased in the LMEC cell line than in the KMEC cell line after

transfecting the miR-301a inhibitor, proposing that miR-301a showed more impact on the apoptotic ability in metastatic melanoma. Overall, miR-301a could promote cell proliferation and migration and inhibit cell apoptosis in KMEC and LMEC cell lines with some unique characteristics.

It is strongly believed that miRNAs exert functional activity by targeting some genes using a unique signaling pathway. In human malignant melanoma, miR-301a might be involved in Akt and FAK signaling pathways via targeting PTEN [129]. miR-301a exerts its biological function through PTEN/PI3K/Akt signaling pathway in the human ovarian cancer [152]. In glioma, miR-301a is involved in the Wnt signal pathway via direct targeting Wnt1 mRNA [147, 148]. We also investigated possible target genes of miR-301a using the Target Scan database (https://www.targetscan.org/vert_80/). Common target genes of miR-301a in canines are screened in the DAVID bioinformatics database (https://david.ncifcrf.gov/). The KEGG and GO enrichment analysis showed that miR-301a might follow Wnt signaling pathways (Fig.6 A-B). The Wnt1 gene existed in the database query, a crucial target of Wnt signaling. Interestingly, the Wnt signaling pathway in canine malignant melanoma may support our prediction [158].

The present study has several limits. First, a large cohort of clinical samples is necessary to validate and strengthen our findings of the miR-301a expression to consider it a diagnostic biomarker. Second, we need to validate our possibly predicted target genes of mir-301a.

Overall, we propose a model in which the upregulation of miR-301a interacts with its target mRNA genes of 3' UTR regions, possibly by activating the Wnt signaling pathway in COM. miR-301a enhances cell proliferation, migration, and clonogenicity and inhibits cell apoptosis (Fig. 6 C).

In summary, to the author's knowledge, the current research revealed the biofunctional study of miR-301a in canine oral melanoma for the first time. miR-301a was upregulated in canine oral melanoma and might be a micro-invasive plasma-based biomarker. A biofunctional study revealed that miR-301a promotes cell proliferation, migration, and clonogenicity and inhibits apoptosis through the predicted Wnt signaling pathway. In addition, miR-301a may be more strongly associated with the metastatic condition. Understanding the molecular processes of miR-301a in controlling canine oral melanoma is essential for developing new testing methods and therapeutic interventions for this fatal disease.

1.5. Material and Methods

1.5.1. Clinical samples

Canine oral melanoma tissues and blood samples were received from the Veterinary Teaching Hospital (KUVTH, Kagoshima University, Japan) or collaborating veterinary clinics. The patient's owner gave their informed consent. The animal care ethics committee and the KUVTH authorities supported the study design and guidelines. (KVH220001). Tissue samples were placed in RNAlater and preserved at -80 °C freezer. The blood samples were drawn and immediately put in tubes treated with an anticoagulant (Terumo Venoject tubes containing 3.2% sodium citrate). Centrifugation was used to collect the plasma for 10 minutes at 3000*g. The plasma samples were then separated and centrifuged once more to remove debris at 16000*g and 4 °C. As previously mentioned, the upper part was collected without disturbing the pellet and kept at -80°C freezer [159, 160].

1.5.2. Cell lines and cell culture

In this study, two types of cell lines were used. Cell lines were cultured according to the previously published paper [149]. In brief, Cells were grown in Roswell Park Memorial Institute (RPMI) media-1640 (Gibco) with L-glutamine solution (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan), antibiotics (penicillin-streptomycin; Sigma) and 10%

fetal bovine serum (FBS) (BI, Biological Industries) and were kept at 37^0C in a controlled humid environment with 5% CO_2. KMEC and LMEC cells were maintained in liquid nitrogen using a freezing medium (CultureSure, Fujifilm Wako Pure Chemical Corporation, Osaka, Japan). 0.25% trypsin or 0.1% EDTA were frequently used to subculture the cells. Cell number was counted using LUNAII (Logos) instrument.

1.5.3. miR-301a inhibitor and Negative Control (NC) inhibitor transfection

KMEC and LMEC cells (1-5 Í 10^5) were transfected with mirVana miR-301a inhibitor (Ambion) or NC inhibitor control #1 (Ambion) at the 10nM concentration. Lipofectamine RNAiMAX Reagent (Invitrogen) and Opti-MEM media (Gibco) were used to transfect the cells and kept in the incubator for 24 to 48 hours after transfection. Fresh media was added after 24-48 hours of transfection.

1.5.4. RNA isolation

The mirVana^{TM} RNA Isolation Kit (Thermo Fisher Scientific) was used to isolate total RNA from tissues and cells, and the mirVana^{TM} Paris kit (Thermo Fisher Scientific) was used for Plasma samples as described (Husna et al., 2021) previously. In brief, miRNA homogenate additive was added in a 1:10 ratio of tissue and cell lysate (binding or lysis buffer) and kept on ice for 10 minutes. An equivalent volume of 2x denaturation solution for Plasma was added to 300 µL plasma. The exact amount of acid: phenol-chloroform (Ambion), was added to the cell lysate or plasma, vortexed, and centrifuged. The lysate's upper (aqueous part) phase was carefully separated and measured. The measured aqueous solution was mixed with 1.25 volumes of pure-grade ethanol and filtered. The filter was washed twice with wash solutions and repeatedly centrifuged [161]. In the final step, total RNA was collected using a 95°C pre-heated elution solution. NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific) was used to measure the RNA concentration, and RNA integrity was calculated using Bioanalyzer

2100 (Agilent). Cells had an RNA Integrity Number (RIN) greater than 9, whereas tissues had a RIN between 8.1 and 8.5.

1.5.5. CCK-8 Assay

In 96 well plates, KMEC and LMEC cells (2000–5000 cells/well) were plated and grown for 24 hours. Cells were transfected using a miR-301a and NC inhibitor at 10 nM concentration. A CCK-8 reagent was applied to measure the proliferation aptitude of KMEC and LMEC cells according to the manufacturer's protocol (Dojin Laboratories, Kumamoto, Japan). In brief, 10 µL of CCK-8 reagents were added into each well of the plates and kept in the incubator for 1-4 hours. The Optical density (OD) value was measured after 24, 48, and 72 hours at the absorbance of 450 nm using a MultiScan GO plate reader (Thermo Scientific).

1.5.6. Colony formation assay

KMEC and LMEC cells (1-5 Í 10^5) were transfected with miR-301a and NC in 24 well plates. Cells were trypsinized, counted, and seeded (2000- 2500 cells) into each well of the six-well plates after 24 hours of transfection and incubated for 8 -10 days. A standard protocol was followed. In brief, the media was washed out with cold PBS, 2-3 ml of a mixture of 6.0% glutaraldehyde and 0.5% crystal violet was added and left for 30 minutes. The mixture was washed out, and the plates were sunken in water carefully. Plates were dried at room temperature (20°C) [162]. The colony was observed under the stereomicroscope, and the photograph was captured using a digital camera. The colony was counted using Image J analysis software. In this study, three individual assays were performed.

1.5.7. Monolayer wound healing assay

Cells (1Í10^5) were suspended in 24 well plates, transfected with miR-301a and NC inhibitor, and kept in the incubator for 48 hours. Media were washed out using cold PBS from the Plates. After adding the media, the cells were kept at 37°C for an hour to settle properly. A wound

scratch was made in the cell well from the forward to backward position using sterilized pipette tips (200 µL). The photograph was taken under the microscope until it overlapped the wound areas. ToupView software was used to measure the width of the wound. Experiments were repeated three times separately.

1.5.8. Transwell migration assay

After seeding the KMEC and LMEC cells in 24 well plates for 24 hours, miR-301a and NC inhibitor were used to transfect them. Cells were trypsinized, counted, and 300 µL suspended cells were seeded (5Í 10^4) into the upper compartment of each transwell insert (6.5 mm insert, 8 µm pore, 24 well insert, Costar). 700 µl DMEM (Dulbecco's Modified Eagle Medium) media with 10% FBS was added into the wells of 24 well plates and kept in an incubator at 37°C for 24 hours. Following that, migrated cells were washed out with ice-cold PBS. The cells were fixed with 4% formaldehyde for 2 minutes and permeabilized with 100% methanol at room temperature for 20 minutes. Cells were stained for 15 minutes with a 0.5% crystal violet solution. A sterile dry swab removed the cells that did not migrate to the lower compartment. The transwell insert was observed Under the microscope, and images were captured randomly. Image J software was used to count the cells in each field. Three separate experiments were conducted in this study.

1.5.9. TUNEL Alexa Fluor Imaging Assay

An apoptosis assay was performed, followed by the manufacturing protocol (Invitrogen) to investigate cell apoptosis. In brief, KMEC and LMEC cells (5000 cells/well) were transfected with miR-301a inhibitor and NC inhibitor using Lipofectamine RNAi-MAX in the 96 well plates. Media was removed and washed out with PBS, fixed (in 4% paraformaldehyde for 15 minutes), and permeabilized (in 0.25% Triton X-100 for 20 minutes). TdT reaction buffer was added for 10 minutes, followed by the TdT reaction cocktail for 60 minutes of incubation at

37°C. The Click-iT reaction cocktail was added and held for 30 minutes. The Click-iT reaction cocktail was removed, and the DNA nuclei were stained with the Hoechst 33342 antibody. A KEYENCE fluorescence microscope was used for imaging (BZ-X series). This study included three separate experiments.

1.5.10. Flow Cytometry using Annexin V-Biotin/PI staining

The percentage of cell apoptosis of miR-301a inhibitor and NC inhibitor transfection was detected using an annexin V-Biotin and Propidium iodide (PI) Kit according to the manufacturer's guideline (Bio Vision). In brief, Cells were suspended (1-5 Í 10^5) with 200 µl of 1X binding buffer. 5 µl annexin V-Biotin and 5 µl PI were added and held for 5 minutes in the dark. Cells were centrifuged at 2300*g for 2 minutes to remove the binding buffer and washed again with 200 µl with the same binding buffer. 2% formaldehyde was used to fix the cells for 15 minutes. Following that, cells were stained with avidin-fluorescein and left for 15 minutes at room temperature. Finally, cells were analyzed for apoptosis using a Flow cytometer (BD Biosciences). Annexin V (+)/ PI (–) indicates early apoptosis of flow cytometry results.

1.5.11. Quantitative real-time PCR (qRT-PCR)

We performed qRT-PCR as previously described [136, 161]. In short, 2ng total RNA from tissues and cells and an equivalent amount of 1.25 µL total RNA from plasma samples (spiked with miR-cel-39 to confirm equivalent RNA isolation) were used to make cDNA in a thermal cycler using the TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific) according to the manufacturer's protocol. For qRT-PCR, a TaqMan First Advanced Master Mix Kit and a Quant Studio 3 real-time PCR system (Thermo Fisher Scientific) were used. The $2^{-\Delta\Delta CT}$ method was applied to measure the relative expression of the miRNA-301a (Taqman ID: 000528) and normalized using internal controls; RNU6B for tissues and cells and miR-16 for plasma samples. In this study, the acceptable CT cycle was less than 36.

1.5.12. Statistical analysis

GraphPad Prism 9 was used for all statistical analyses. A one-way ANOVA test followed by the Kruskal-Wallis and Mann-Whitney U tests was used to show relative expression value. The results of time-dependent experiments were analyzed using a two-way ANOVA test, followed by Sidaq's multiple comparisons. ROC curves were plotted using Wilson and Brown method. The statistical analyses were considered significant when the $P<0.05$.

Table 1. COM tissue and plasma sample information

No	Age (Years)	Sex	Breed	WHO Stage	Tissues	Plasma
1	12.7	Male	Miniature Dachshund	IV	**P**	**P**
2	14.8	Male	Mongrel	IV	**P**	**P**
3	10	Male	Golden Retriever	IV	**P**	**P**
4	10.11	Male	Miniature Dachshund	I	**P**	—
5	7.11	Male	Miniature Dachshund	I	**P**	**P**
6	10.9	Male	Miniature Dachshund	IV	**P**	**P**
7	12	Male	Shiba	IV	**P**	—
8	13	Male	Pomerania	I	**P**	—
9	10.3	Male	Yorkshire	IV	**P**	**P**
10	10.2	Male	Chiwawa	IV	**P**	**P**
11	12.4	Female	Miniature Dachshund	IV	**P**	**P**
12	14.6	Female	Miniature Dachshund	II	**P**	—
13	15.2	Female	Mongrel	IV	**P**	—
14	12.11	Male	Miniature Dachshund	IV	**P**	—
15	12.4	Male	Shiba	IV	**P**	—
16	15.2	Female	Mongrel	IV	**P**	—
17	10.8	Male	Miniature Dachshund	IV	**P**	—
18	15.2	Male	Shiba	I	**P**	—
19	13.3	Male	Miniature Dachshund	I	**P**	**P**
20	8.2	Female	Miniature Dachshund	IV	**P**	**P**
21	12	Male	Mong	I	**P**	**P**
22	11.1	Male	Miniature Dachshund	IV	**P**	**P**
23	15.6	Male	Pomeranian	II	**P**	**P**
24	15.3	Female	Mong	I	**P**	**P**
25	11	Male	Miniature Dachshund	IV	**P**	**P**
26	15.3	Female	Mong	I	**P**	**P**

27	16.3	Male	Miniature Dachshund	IV	**P**	**P**
28	11.8	Female	Miniature Dachshund	I	**P**	**P**
29	14	Female	Dalmatian	II	**P**	**P**
30	12.1	Female	Toy poodle	IV	**P**	**P**

※ (**P**) indicates "Present," and (—) indicates "Absent."

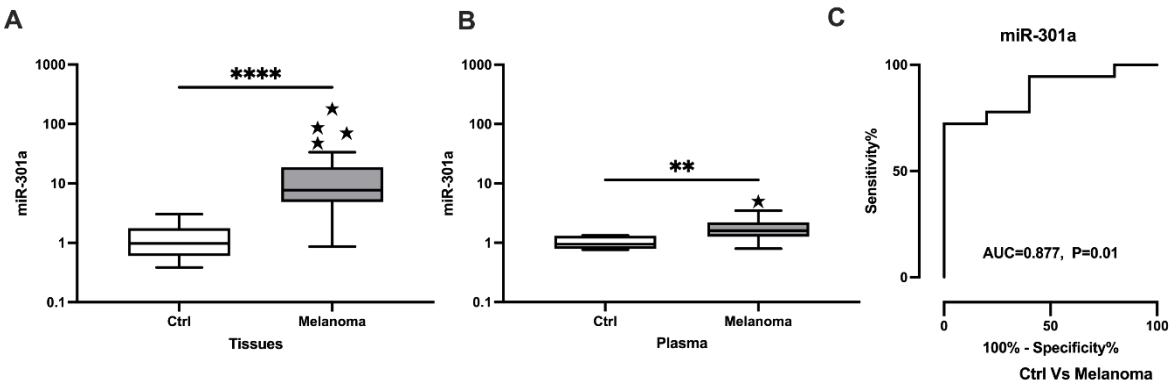


Figure 1- 1. Relative expression of miR-301a in clinical samples.

(A). Relative expression of miR-301a in healthy oral tissues (control, n=10) and canine oral melanoma tissues (n=30). (B). Relative expression of miR-301a in plasma samples (control, n=5, melanoma, n=20). (C). ROC curve analysis of miR-301a to measure the potentiality as a biomarker of canine oral melanoma. One-way ANOVA followed by Tukey's multiple comparisons and Mann-Whitney U test were used for statistical analysis. *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001.

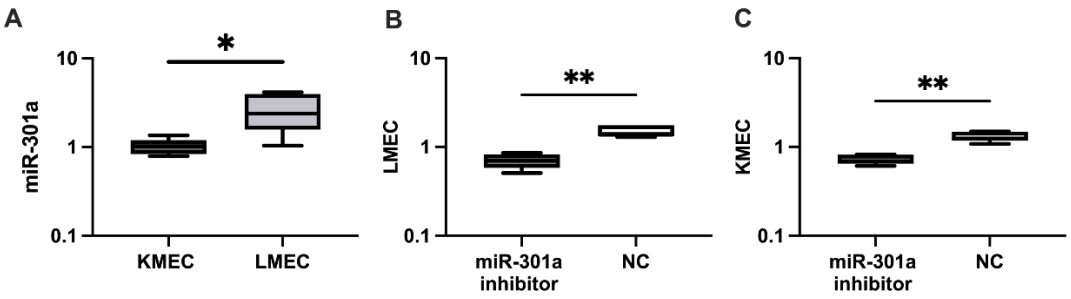


Figure 1- 2. The knockdown of miR-301a in KMEC and LMEC cell lines.

(A). Relative expression of miR-301a in KMEC and LMEC cell lines. (B, C). Knockdown of miR-301a inhibitor and NC inhibitor in KMEC and LMEC cell lines. Results are representative of three independent experiments. One-way ANOVA followed by Tukey's multiple comparisons and Mann-Whitney U test were used for statistical analysis. *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001. NC; negative control.

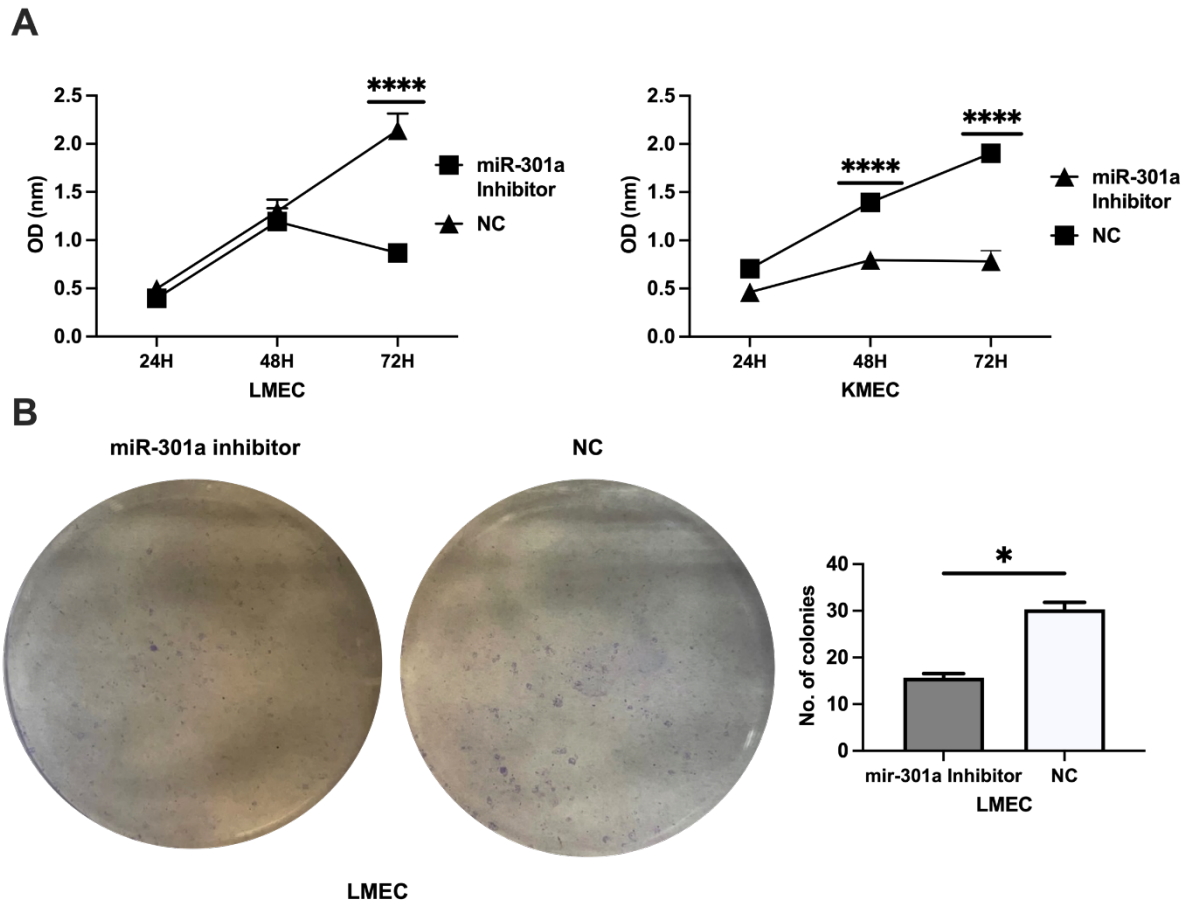


Figure 1- 3. Effects of miR-301a on cell proliferation and colony formation in canine oral melanoma cell lines.

(A). CCK8 assays were carried out in LMEC and KMEC cell lines in a time-dependent manner (24h, 48h, and 72h). (B). Colony formation assay performed in LMEC cell line. Cells>50 in number were scored. The number of colonies was measured by Image J software. The data represents the colony count ± SEM (right). Results are representative of three independent experiments. Two-way ANOVA followed by Sidaq's multiple comparisons was used for the CCK8 assay. *P<0.05, **P<0.01, ***P <0.001, ****<P,0.0001.

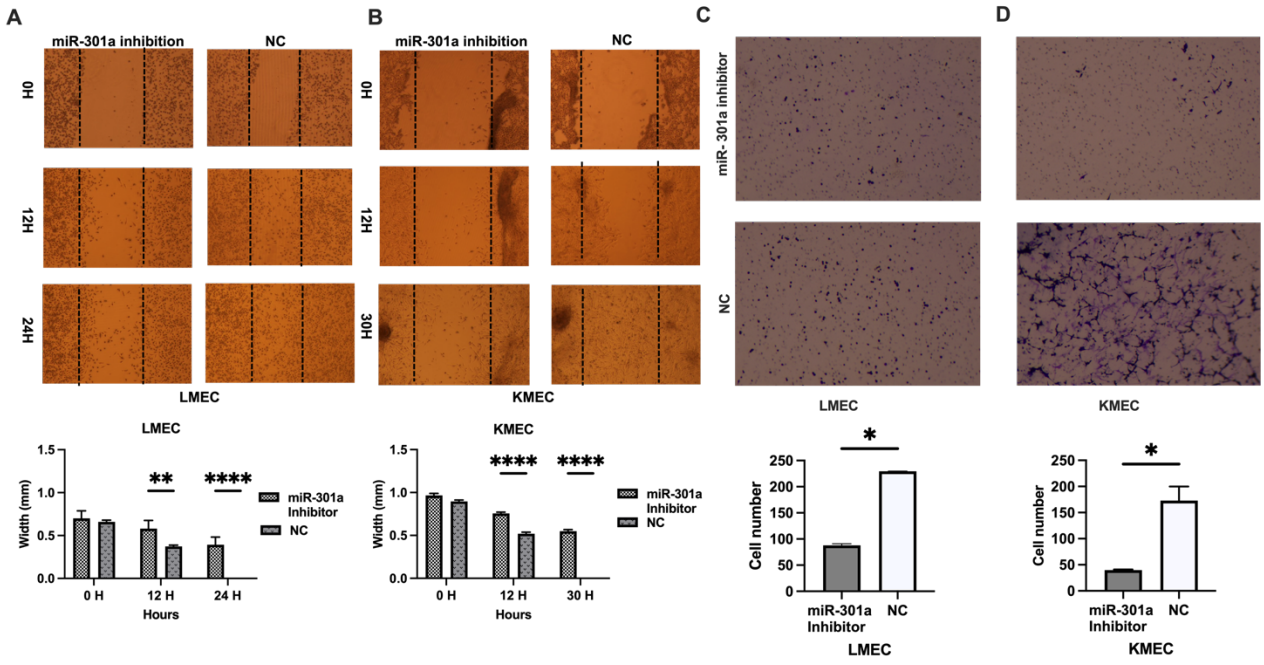


Figure 1- 4. Effects of miR-301a knockdown on cell migration.

(A, B). The effect of miR-301a inhibition on cell migration in LMEC and KMEC cell lines was analyzed using the wound healing assay. Representative images of the wound healing (Upper left) and calculated scratch area (Upper right) were illustrated. (C, D). Transwell migration

assay with miR-301a inhibitor in LMEC and KMEC cells. The number of migrated cells was measured by Image J software. Results are representative of three independent experiments. The data represents the cell count ± SEM (lower right). Two-way ANOVA followed by Sidaq's multiple comparisons for wound healing assay. *P<0.05, **P<0.01, ***P <0.001, ****P <0.0001.

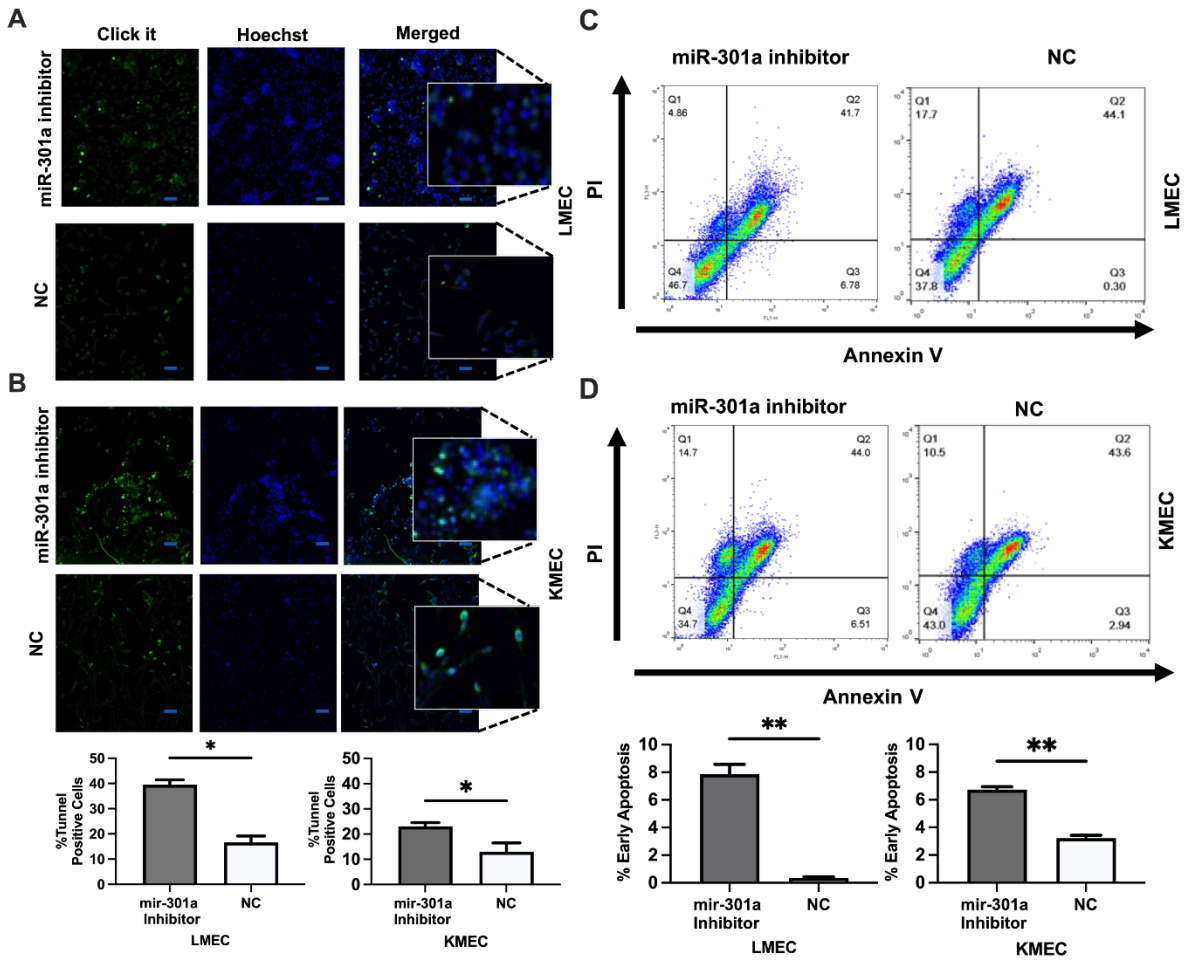


Figure 1- 5. Effects of miR-301a knockdown on cell Apoptosis.

(A, B). Tunel assay illustrated the percentage of Tunel-positive cells in LMEC and KMEC cell lines by miR-301a inhibitor and Inhibitor Negative control. (C, D). Annexin V/PI staining and flow cytometry showed the percentages of early apoptosis in LMEC and KMEC cell lines treated with miR-301a inhibitor and Inhibitor Negative control. Results are representative of three independent experiments. The squared box indicates TUNEL-positive (Cyan-blue colored) and TUNEL-negative cells (didn't produce cyan-blue color), and the images were captured with 10X (scale bar=50 µm) magnificent power. The data represents the cell count ± SEM (lower right). *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001.

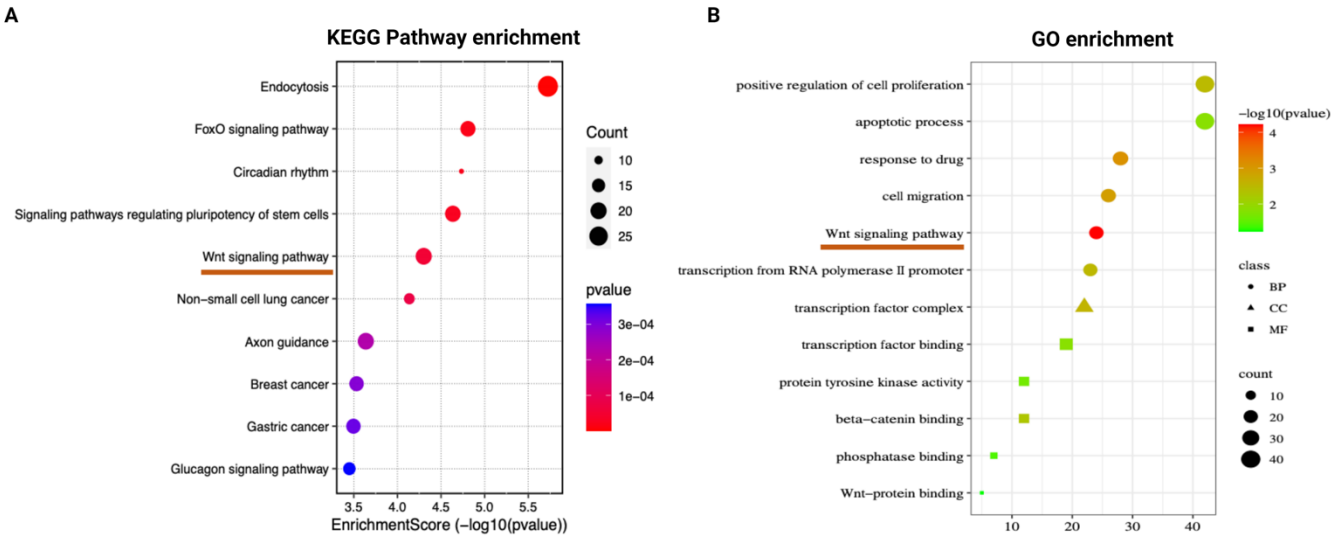


Figure 1- 6. miR-301a related possible pathway.

(A). KEGG enrichment and (B). GO enrichment of pathways involving predicted miR-301a targeting genes. P <0.05 was considered significant.

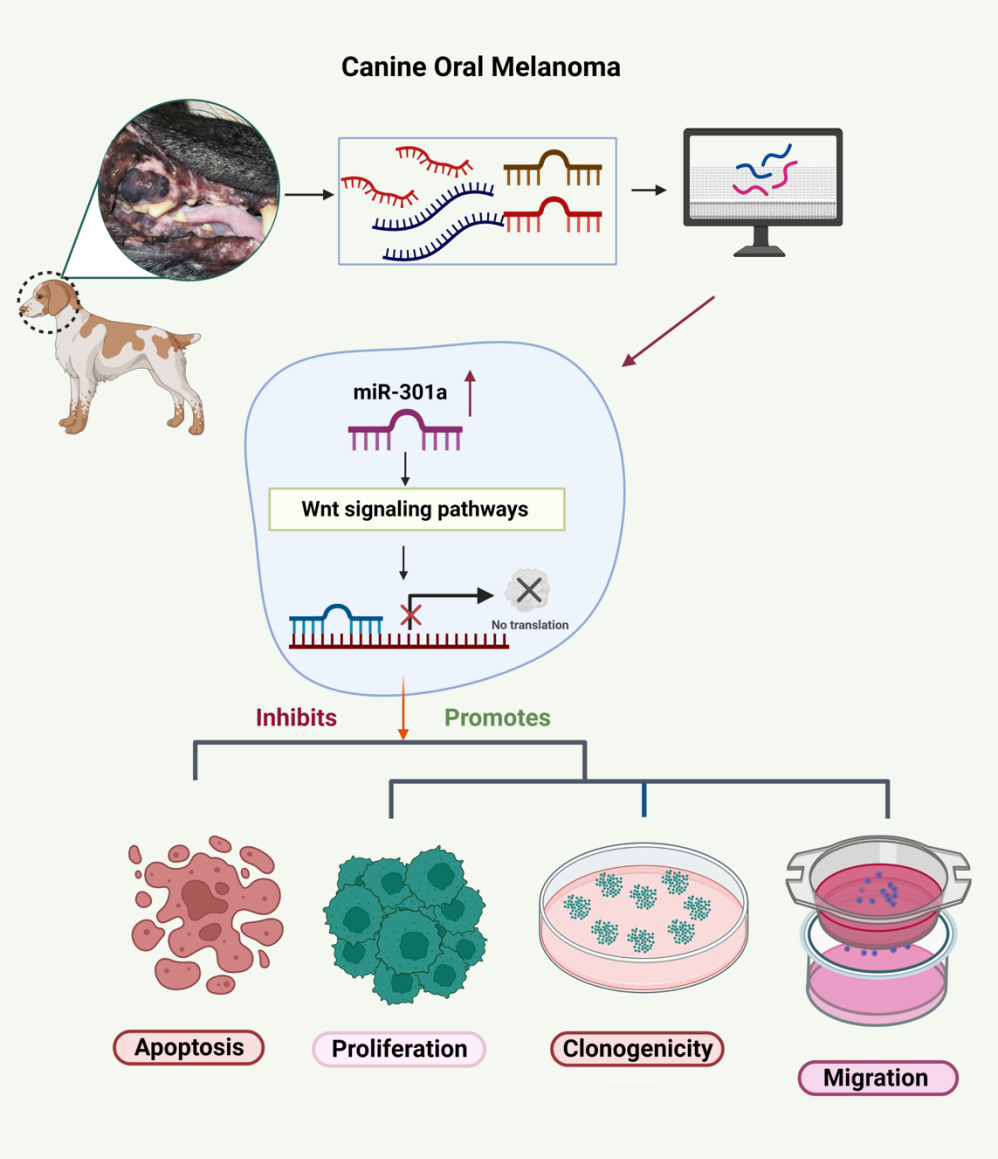
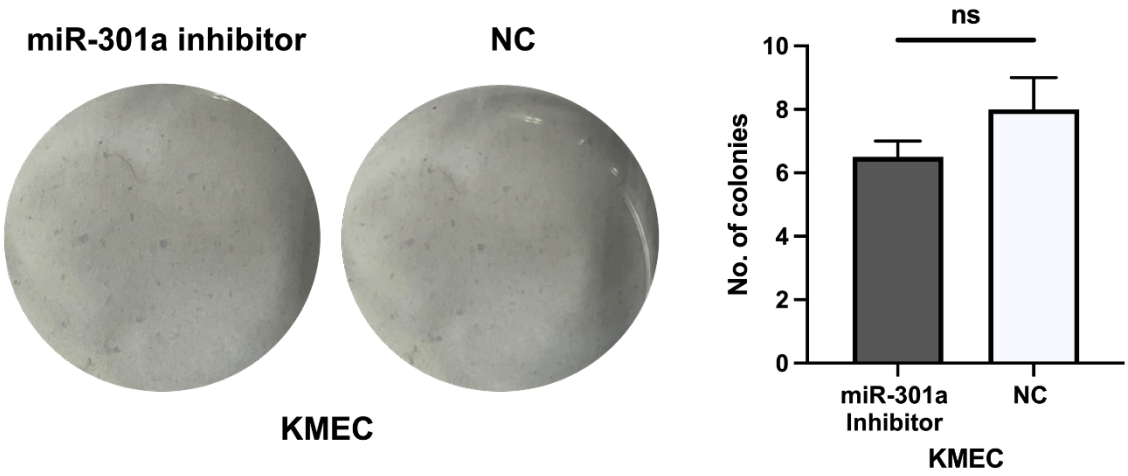


Figure 1- 7. A diagram of the proposed model for miR-301a in COM.

Upregulated miR-301a in COM and silences its target genes via the most likely Wnt-signaling pathway, which affects cell proliferation, migration, clonogenicity, and apoptosis.



Supplementary Figure 1- 1.Colony formation of KMEC cells.

The number of colonies was measured by Image J software. The data represents the colony count ± SEM (right). Results are representative of three independent experiments.

Chapter 2

Upregulation and functional roles of miR-450b in canine oral melanoma

(Hasan, MD Nazmul, et al. *Non-coding RNA Research*, 2024)

2.1. Abstract

Oral melanoma is dogs' most common and highly aggressive disease. Recent research revealed that dysregulated miRNAs are involved in melanoma disease progression. Though the underlying molecular mechanisms and functional roles of miR-450b were extensively investigated in multiple human cancers, they remained unclear in human and canine melanoma. This study aims to investigate the potentiality of miR-450b as a diagnostic biomarker and its functional roles in Canine oral melanoma progression. The qRT-PCR system was applied to analyze the expression levels of miR-450b and target mRNA genes in clinical samples and cell (KMEC and LMEC) lines. The knockdown and overexpression studies were performed to determine the impacts of miR-450b on cell proliferation, migration, colony formation, and cell apoptosis. We showed that miR-450b was significantly upregulated in COM, differentiated the metastatic group from the no-metastatic group compared to the control group. ROC analysis showed that miR-450b could be a potential biomarker of COM diagnosis. Inhibition of miR-450b enhanced cell proliferation, migration, and clonogenicity and inhibited cell apoptosis, whereas the overexpression of miR-450b showed the inverse effects on phenotypes of melanoma cells. miR-450b directly binds 3' UTR of PAX9 mRNA and modulates its function leading to BMP4 downregulation and MMP9 upregulation. Furthermore, we predicted that miR-450b could activate the Wnt signaling pathway using GO and KEGG enrichment analysis. Collectively, miR-450b could be implemented as a potential biomarker for COM diagnosis and treatment.

Keywords: miR-450b, knockdown, overexpression, proliferation, migration, colony formation, apoptosis, PAX9-BMP4-MMP9.

2.2. Introductions

Oral melanoma is a highly aggressive and the most common disease in humans and dogs due to its high rate of malignancy and poor prognosis behavior in nature [163-165]. Several genetic similarities are overlapped between humans and dogs, indicating that dogs might be the alternative model for the human melanoma study [130, 166, 167]. The fatality rate of canine malignant melanoma is 70% to 75% in phases II-III, and in most cases, dogs die within one year after surgery [165]. In human cancer, 1.7% of all cancer diagnoses worldwide are melanoma [168].

Surgical resections, radiation therapy, immunotherapy, and electrochemotherapy are conventional therapies for canine oral melanoma [169]. However, the success rate of conventional therapies is still challenging [170]. Therefore, it is time worth revealing the genetics and underlying molecular mechanism of melanoma disease progression to develop a molecular-based novel therapeutic approach. Researchers have focused on miRNA-based therapies because miRNAs act as potent genetic regulators; a single miRNA can modulate the cellular pathways targeting a diverse set of mRNA genes [171]. Recently, miRNA-based therapies have entered clinical trials, and siRNA-based therapeutics got approval from the Food and Drug Administration (FDA) [172]. Dysregulated miRNAs have been reported to play a vital role in cell proliferation, migration, metastasis, invasion, and angiogenesis in multiple cancers [134]. Several dysregulated miRNAs have been extensively studied in canine oral melanoma (COM) patients [14, 136, 137].

Aberrant expression of miR-450b has been explored in human hepatic ischemia, colorectal cancer, cervical cancer, breast cancer, gastric cancer, oral squamous cell carcinoma, hepatocellular carcinoma, lung squamous cell carcinoma, nasopharyngeal carcinoma including canine oral melanoma [136, 173-181]. Numerous research reports that miR-450b controls the target mRNA genes by interacting with critical signaling pathways; for example, miR-450b promotes hepatocellular carcinoma progression by activating PI3K/AKT and colorectal cancer

progression by the Wnt/β-Catenin signaling pathways [179, 182]. However, the molecular pathways and functional roles of miR-450b in human melanoma and canine oral melanoma are unknown and require further investigation.

Here, we validated the expression levels of miR-450b in clinical dog samples and analyzed the biomarker potentiality. The knockdown and overexpression studies were performed to determine the impacts of miR-450b on cell proliferation, migration, colony formation, and cell apoptosis. Furthermore, we explored the direct target mRNA gene of miR-450b using the target scan database and its potential signaling pathway using GO and KEGG enrichment analysis. Thus, focusing on miR-450b is a promising approach for COM diagnosis and therapy.

2. 3. Results

2.3.1. Sample characteristics

Forty clinical tissue samples of the dog were obtained from KUVTH. Among them, 30 samples of canine oral melanoma (15 metastatic and 15 without metastatic melanoma) and 10 samples of healthy canine oral tissues were used as "controls." This study included 25 plasma samples (5 control, 10 without metastatic melanoma, and 10 metastatic melanoma). Table 1 provides an overview of the patient's information.

2.3.2. Relative expression of miR-450b indicated that it could be a promising biomarker

We investigated the expression level of miR-450b using qRT-PCR. In clinical tissues, miR-450b was significantly upregulated in melanoma with metastasis (FC=495, P=0.0001) and melanoma without metastasis groups (FC=45, P=0.0001) compared to the control group (Fig. 1 A). The relative expression of miR-450b in plasma showed consistent results with clinical tissue results. miR-450b was preferentially upregulated in melanoma with metastasis (FC=9, P=0.0001) and melanoma without metastasis (FC=9, P=0.0001). Moreover, our data showed that miR-450b could differentiate between metastasis and without metastasis groups of melanomas in tissues (P=0.0001) and plasma (P=0.004). We further analyzed the ROC curve

of the miR-450b expression. Results revealed that the area under the curve (AUC) and p-value of control vs. melanoma was 0.93 (0.0037), control vs. no metastasis was 0.86 (0.027), and control vs. metastasis was 1.0 (0.002) (Fig. 1 C-E). Overall, the upregulation of miR-450b in COM can differentiate between metastasis and without metastasis groups and could be a potential biomarker.

2.3.3. Knockdown and overexpression of miR-450b in the cell lines

We validated the expression pattern of miR-450b in KMEC and LMEC cell lines using qRT-PCR. Results indicated that the mir-450b expression level was relatively higher in LMEC (FC=8, P=0.007) than KMEC cell line (Fig. 2 A). For the knockdown of miR-450b, cells were transfected using different concentrations (10nM, 15nM, 20nM) and validated using qRT-PCR. 15nM concentrations showed highly significant results in KMEC (P=0.002) and LMEC (P=0.002) cell lines compared to NC inhibitor (Fig. 2 B-C). 10nM miR-450b mimic showed that KMEC (P=0.002) and LMEC (P=0.002) cells were highly overexpressed compared to mimic NC (Fig. 2 D-E).

2.3.4. miR-450b enhanced cell proliferation and clonogenicity

CCK-8 and clonogenic assays were performed in KMEC and LMEC cells to assess the impact of miR-450b on proliferation and clonogenicity. CCK-8 results revealed that the knockdown of miR-450b significantly decreased the cell proliferation at 48h of post-transfection in LMEC (P=0.01) and KMEC (P=0.004) cells compared to the NC inhibitor (Fig. 3 A-B). Inversely, the overexpression of miR-450b significantly increased cell proliferation at 48h of post-transfection in LMEC (P=0.01) cells and 72h in KMEC (P=0.0001) cells compared to the mimic NC.

Clonogenic results showed that inhibition of miR-450b significantly decreased (P=0.005) the colony numbers (average 15) compared to NC inhibitor (average 39). In contrast,

overexpression of miR-450b increased (P=0.0002) the colony numbers (average 72) compared to mimic NC (average 14) in LMEC cells. However, no colony was observed in KMEC after transfection of miR-450b inhibitor and mimic compared to NC inhibitor (average 13) and mimic NC (average 14) (suppl. Fig. 1).

miR-450b may be involved in the metastatic progression of melanoma rather than the primary stage of melanoma. Taken together, miR-450b promotes cell proliferation in both cell lines and enhances clonogenicity in metastatic melanoma (LMEC).

2.3.5. miR-450b influences cell migration

We performed wound healing and transwell migration assay to investigate the effects of miR-450b inhibition and overexpression on LMEC and KMEC cells. Wound healing assay results indicated that the knockdown of miR-450b significantly inhibited cell migration. The wound scratch area became nonoverlapped at 24h post-transfection in LMEC (average 0.35mm in width, P<0.0001) and KMEC (average 0.19mm in width, P=0.04) cells compared to the NC inhibitor (Fig. 4 A-B). In contrast, the overexpression of miR-450b significantly increased the cell migration, and the wound scratch area overlapped at 18h in (P<0.0001) and 24h in KMEC (P<0.0001) cells compared to mimic NC (o.22mm, and 0.26mm in width, respectively) (Fig. 4 C-D).

Transwell migration results revealed that knockdown of miR-450b compared to the NC inhibitor, a smaller number of LMEC (average 55 cells migrated from the upper chamber of the transwell insert) compared to NC inhibitor (average 140 cells) (P=0.003) and KMEC (average 73 cells) compared to NC inhibitor (average 314 cells) (P=0.006) (Fig. E-F). Oppositely, the overexpression of miR-450b significantly increased migration ability in LMEC (average 331 cells, P=0.02) and KMEC (average 638 cells, P=0.04) cells compared to mimic NC (LMEC average 171 cells, KMEC average 176 cells, respectively) (Fig. 4 G-H). In

summary, the knockdown of miR-450b decreased cell migration, and the overexpression of miR-450b increased cell migration.

2.3.6 miR-450b exerted its effects to inhibit cell apoptosis

To understand the molecular mechanism underlying the functions of miR-450b on cell apoptosis, we performed a flow cytometry assay and TUNEL Alexa Fluor imaging assay. After transfection of miR-450b inhibitor, flow cytometry results revealed that the percentage of early cell apoptosis increased in LMEC (17.4%) and KMEC (5.0%) cells when compared to NC inhibitor (12.1% and 1.73%, respectively) (Fig. 5 A-B). In contrast, the overexpression of miR-450b drastically decreased the early cell apoptosis in LMEC (12.0%) and KMEC (1.74%) cells compared to mimic NC (14.64% and 4.21%, respectively) (Fig. 5 C-D). We further uncovered the TUNEL Alexa Fluor imaging results to know the apoptotic effects of miR-450b in the cells. Results showed that the knockdown of miR-450b dramatically increased the percentage of Tunel-positive cells in LMEC (38%) and KMEC (29%) cells when compared to NC inhibitor (27% and 13%, respectively) (Fig. 5 E-F), while the overexpression reduced the percentage of Tunel-positive cells in LMEC (9%) and KMEC (10%) compared to mimic NC (29% and 23%, respectively) (Fig.5 G-H). Considered together, miR-450b inhibits cell apoptosis.

2.3.7. Targets of miR-450b and the probable predictive pathways

We elucidated the target genes of miR-450b in COM using our next-generation sequencing (NGS) results of mRNA and target scan database (https://www.targetscan.org/vert_80/). Analyses uncovered that PAX9 was the potential binding target of miR-450b (Fig. 6 A). In addition, our previous study investigated that miR-450b correlated with BMP4 and MMP9 based on NGS results [136]. We further analyzed the GO enrichment and KEGG pathway to determine the probable pathways that miR-450b could follow. All genes related to miR-450b were applied to the DAVID bioinformatics database (https://david.ncifcrf.gov/). Database

results revealed that miR-450b is associated with many functions, such as cell proliferation, differentiation, aging, and response to hypoxia, and may follow the cAMP/calcium signaling/FoxO signaling pathways. KEGG and GO enrichment analysis showed it might follow the Wnt signaling pathway (Fig. 6 B-C).

2.3.8. Relative expression of PAX9, BMP4, and MMP9 in COM and cell lines

We extended the number of COM tissue samples (n=30) to validate the PAX9, BMP4, and MMP9 expression using qRT-PCR. Results suggested that PAX9 (FC=0.04, P<0.0001) and BMP4(FC=0.57, P=0.003) were significantly downregulated in melanoma tissues, whereas MMP9(FC=24.6, P<0.0001) was significantly upregulated in melanoma tissues compared to control samples (Fig. 7. A). We further validated the target mRNAs in KMEC and LMEC cell lines. Results showed that PAX9 (P=0.002) and BMP4 (P=0.008) increased in KMEC (FC= 3.80, FC=4.32, respectively) cells and decreased in LMEC (FC= 0.26, FC=0.23, respectively) cells. In addition, MMP9 (P=0.002) was decreased in KMEC (FC=0.26) and increased in LMEC cells (FC=3.81) (Fig. 7 B).

2.3.9. The knockdown and overexpression of miR-450b altered the expression of PAX9, BMP4 and MMP9

To understand whether miR-450b impacts PAX9, BMP4, and MMP9 expressions, we inhibited and overexpressed miR-450b in LMEC and KMEC cell lines. qRT-PCR results showed that the knockdown of miR-450b significantly increased the PAX9 (P=0.03, P=0.02, respectively) and BMP4 (P=0.004, P=0.002, respectively) expression levels, whereas it significantly decreased the MMP9 (P=0.004, P=0.009, respectively) expression level compared to NC inhibitor in LMEC and KMEC cells (Fig. 8 A-B). In contrast, overexpression results revealed that PAX9 (P=0.002, P=0.009, respectively), BMP4 (P=0.01, P=0.009, respectively), and MMP9 (P=0.004, P=0.01, respectively) were significantly decreased in LMEC and KMEC

cells (Fig. 8 C-D). Unfortunately, the overexpression study could not change the expression of MMP9 in LMEC and KMEC cells. Considered together, miR-450b changed the PAX9, BMP4, and MMP9 expressions in COM.

2.4. Discussion

Aberrantly expressed miRNAs involved in melanoma act as an oncogene or tumor suppressor gene and can alter biological functions such as cell migration, proliferation, apoptosis, and metastasis and modulate the immune response [183-185]. Several studies unveiled that dysregulated miRNAs involved in melanoma could serve as potential biomarkers to diagnose the initial stage of melanoma and response to therapy [186-189]. Therefore, revealing specific miRNAs' molecular mechanisms and biological functions is time-demandable research. Our previous study thoroughly investigated the miRNA differential expression profiles of COM [136]. Of them, miR-450b was selected to uncover whether it has the potentiality of early diagnosis, the underlying molecular mechanisms, and biological functions in the LMEC and KMEC cell lines. However, the biological functions of miR-450b are still unexposed in human melanoma and canine oral melanoma. Here, we studied the biological functions of miR-450b in COM for the first time.

Clinical samples and cell line results suggested that miR-450b might be responsible for the metastatic progression of COM. We elucidated that the relative expression of miR-450b was upregulated in COM tissues and plasma samples. The miR-450b expression level was higher in the metastatic group and could distinguish between metastatic and without metastatic group compared to the control group. ROC curves proved that miR-450b could be a potential biomarker for early disease diagnosis. Cell line results also revealed that miR-450b expression level was preferentially higher in LMEC than in KMEC cells. The difference in expression between the LMEC and KMEC cell lines could be their point of origin, which raises the possibility that miR-450b is responsible for the metastatic condition. The relative expression

of miR-450b was extensively studied in different cancers except for melanoma. Recent studies expose that elevated expression of miR-450b is involved in cancer progression in oral squamous cell carcinoma, colorectal cancer, lung cancer, and esophageal squamous cell carcinoma [173, 182, 190, 191].

Next, we performed the knockdown and the overexpression of miR-450b to explore the biological functions in the KMEC and LMEC cell lines. The knockdown of miR-450b inhibited cell proliferation, migration, and colony formation and promoted cell apoptosis. In contrast, the overexpression of miR-450b enhanced cell proliferation, migration, and colony formation and inhibited cell apoptosis. Overall data suggest that miR-450b could enhance cell proliferation, migration, and clonogenicity and inhibit cell apoptosis. Various research exposes that miR-450b can modulate cell proliferation, migration, invasion, colony formation, apoptosis, and metastasis in hepatic ischemia, colorectal cancer, cervical cancer, breast cancer, gastric cancer, oral squamous cell carcinoma, hepatocellular carcinoma, lung squamous cell carcinoma, and nasopharyngeal carcinoma [173-181].

miRNAs can alter cancer growth and progression by targeting the 3' untranslated region (UTR) of different mRNA genes and can control multiple signaling pathways involved in the cancer growth [192]. We have reported that miR-450b could follow the PAX9-BMP4-MMP9 axis in COM [136]. This study demonstrated that upregulated miR-450b expression was inversely related to PAX9 expression by directly targeting the 3' UTR of PAX9 mRNA in COM tissues and KMEC and LMEC cell lines. Our study validated that PAX9 and BMP4 expressions were downregulated, and MMP9 was upregulated in melanoma compared to the control. PAX9 and BMP4 were increased, and MMP9 was decreased in KMEC cells than in LMEC cells. This data suggested that PAX9, BMP4, and MMP9 might be involved in the progression of melanoma. Next, we investigated the expression of PAX9, BMP4, and MMP9 after the knockdown and overexpression of miR-450b in LMEC and KMEC cell lines. The knockdown

results revealed that PAX9 and BMP4 were upregulated, and MMP9 was downregulated, whereas PAX9 and BMP4 were downregulated after the overexpression.

PAX9 is involved in early developments, modulates the biological function of the cells, and may lead to carcinogenesis [193, 194]. The biological functions of PAX9 are less studied in both human and canine cancer fields. Some research shows that PAX9 is involved in cell proliferation, migration, and apoptosis in oral squamous cell carcinoma, cervical cancer, and oro-oesophageal epithelium [195-197]. Other studies report that PAX9 is an essential transcription factor in tooth development and palate morphogenesis that can modulate the expression of the BMP4 expression [198, 199]. Multiple research shows that BMP4 is involved in human malignant melanoma and alters biological functions [200-202]. A few research reveals that BMP4 expression inhibits the MMP9 expression levels in cancer cells [203, 204]. MMP9 is involved in the melanogenesis pathway and is considered a promising biomarker and therapeutic target for managing melanoma patients [205-207]. Overall, our data suggest that miR-450b regulates the PAX9 functions and that PAX9 interacts with BMP4 downregulation, as a result, MMP9 expression is increased.

In general, miR-450b regulates the target mRNA genes by activating critical signaling pathways [177, 179, 182, 208]. Our study further explored the probable predictive pathways of miR-450b in COM progression. KEGG pathway analysis revealed that miR-450b might be involved in cAMP/calcium signaling/FoxO signaling pathways. However, GO enrichment and KEGG pathway strengthen that miR-450b could follow the Wnt signaling pathway in our results. Ye et al. reported that miR-450b directly binds with the 3'-UTRs of SFRP2 and SIAH1 and activates Wnt/β-Catenin signaling pathways [182]. Multiple research reveals that melanoma progression occurs by activating the Wnt singling pathway [209-215]. Together with these previous studies and our results, the Wnt signal is a crucial pathway for melanoma development, via which it targets genes of miR-450b.

The present study has some limits. First, investigation of the ‘loss of function and gain in function’ of target genes of miR-450b in Vivo. Second, the exact validation of miR-450b targeting pathways and miR-450b targeting other mRNA genes in COM.

Altogether, we propose a model that the upregulation of miR-450b inversely regulates the PAX9 functions, and degradation of PAX9 function could interplay with BMP4 downregulation, resulting in MMP9 upregulation in COM by activating Wnt signaling pathways (Fig. 9). miR-450b exerts its function by promoting cell proliferation, migration, clonogenicity, and inhibiting cell apoptosis.

To the author's knowledge, miR-450b molecular and biological functions were investigated in oral melanoma of dogs for the first time, even though no miR-450b study has yet been published in human melanoma. Upregulated miR-450b could be a potential biomarker for diagnosing oral melanoma in dogs. The knockdown and overexpression of miR-450b suggested that miR-450b enhanced cell proliferation, migration, and colony formation and inhibited cell apoptosis. miR-450b directly binds 3’ UTR of PAX9 mRNA and modulates its function and leads to BMP4 downregulation and MMP9 upregulation. This study further explored that miR-450b regulates target genes by activating the Wnt singling pathway. The present study speculates a potential therapeutic strategy for COM diagnosis and treatment. Future research will include a feasibility test for applying miR-450b clinically as a potential biomarker of early disease diagnosis.

2.5. Material and methods

2.5.1. Clinical sample information

Canine oral melanoma tissues, blood samples, and healthy oral tissues were taken from the Kagoshima University Veterinary Teaching Hospital (KUVTH) or private veterinary clinics in and around Kagoshima City. All samples were received after getting the patient’s owner's consent. This study strictly followed the rules and regulations of the KUVTH and Kagoshima

University ethics committee during sample handling (KVH220001). Tissue samples were immersed in RNAlater instantly after the surgical operation and left at 4 °C for overnight incubation, finally kept at -80°C for long-time preservation. Blood samples were collected in tubes treated with 3.2% sodium citrate anticoagulant. Obtained blood samples were centrifuged at 3000*g for 10 minutes immediately after collection. The supernatant was transferred to the new Eppendorf tubes and centrifuged again to remove all existing cell debris at 16000*g for 10 minutes at 4 °C. Plasma was collected without touching the pellet and stored in a -80°C freezer [159, 160].

2.5.2. Cell lines and cell culture

This study used KMEC (primary site of origin) and LMEC (metastatic site of origin) cell lines. Cell culture methods and protocols were followed by a published paper on establishing the cell lines [149]. Briefly, Cells were cultured using Roswell Park Memorial Institute (RPMI) media-1640 (Gibco), L-glutamine solution (Fujifilm Wako Pure Chemical Corporation, Osaka, Japan), antibiotics (penicillin-streptomycin; Sigma) and 10% fetal bovine serum (FBS) (BI, Biological Industries) and maintained at 37 °C in a controlled humid environment with 5% CO_2. Freezing media (CultureSure, Fujifilm Wako Pure Chemical Corporation, Osaka, Japan) was used to store the cells in liquid nitrogen. Cold phosphate-buffered saline (PBS) and 0.25% trypsin or 0.1% EDTA were applied during the subculture. Cells were counted using the automated cell counter (LUNAII, Logos) instrument.

2.5.3. miR-450b and Negative Control (NC) inhibitor and mimic transfection

A mirVana miR-450b inhibitor (Ambion) and NC inhibitor#1 (Ambion) were used to knock down the cells (1-5 Í10^5) at 15 nM concentration. For the overexpression study, mirVana miR-450b mimic (Ambion) and Mimic NC (Ambion) were used at 10nM concentration.

Transfection was performed using Lipofectamine RNAiMAX reagent (Invitrogen) and Opti-MEM media (Gibco), and cells were grown for 24-48 hours according to the individual assay protocols. After 24 hours of transfection, new media was added.

2.5.4. RNA extraction

RNA was extracted from tissues and cells using the mirVana^{TM} RNA Isolation Kit (Thermo Fisher Scientific), and the mirVana^{TM} Paris kit (Thermo Fisher Scientific) was used for Plasma samples as described [160, 216] previously. Briefly, the tissue samples or cells received the required amount of lysis buffer, and the 300 µL plasma samples received the same amount of 2x denaturation solution. 1/10 of a miRNA homogenate additive was added to the lysate and left on ice for 10 minutes. Acid: Phenol-chloroform (Ambion) was added to the tissues, cell lysate, or plasma and vortexed thoroughly before centrifuging at 15000*g for 5 minutes at room temperature. The upper portion was transferred carefully to the Eppendorf tube and recorded the amount to add 1.25 times pure molecular grade ethanol and filtered using centrifugation. In the final step, the total RNA was settled down of the collection tube using an elution solution pre-heated at 95 °C. The total RNA level was calculated using a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific), and RNA integrity was evaluated using Bioanalyzer 2100 (Agilent). Tissues had an RNA Integrity Number (RIN) of 8.1-8.5, while cells had a RIN greater than 9.

2.5.5 CCK-8 Assay

KMEC and LMEC cells (2000–5000 cells/well) were seeded in 96 well plates and cultured for 24 hours. Knockdown of miR-450b was performed using miR-450b inhibitor at 15nm concentration, and for the overexpression of miR-450b, miR-450b mimic was used at 10nm concentration. NC control inhibitors and mimic NC were also transfected for comparison. The CCk-8 reagent (Dojin Laboratories, Kumamoto, Japan) was applied to evaluate the

proliferative ability of the KMEC and LMEC cells. According to the manufacturer's protocol, 10 µL of CCK-8 reagents were applied to each well of the plates and incubated for 1-4 hours. The optical density (OD) value was assessed at 450 nm absorbance using a MultiScan GO plate reader (Thermo Scientific) at the appropriate time intervals (0h, 24h, 48h, 72hr).

2.5.6 Colony Formation Assay

Cells (1-5 Í 10^5) were transfected with mir-450b inhibitor, NC inhibitor, miR-450b mimic, and mimic NC, respectively. Transfected cells were trypsinized, counted, and seeded (2000- 2500 cells/well) to the six-well plates and kept for 8-10 days at 37 °C. More than 50 cells were considered for a typical colony. According to the published protocol, cells were washed twice with cold phosphate-buffered saline (PBS). A mixture of 2-3 ml of 6.0% glutaraldehyde and 0.5% crystal violet was added and left for 30 minutes. The mixture was washed out and rinsed carefully with water. The colony plates were left at 20 °C for the drying [162]. The colony was observed under the stereomicroscope, and the photographs were captured using a digital camera. Image J analysis software was used to count the colonies. Three individual assays were carried out in this study.

2.5.7 Monolayer Wound Healing Assay

A wound-healing assay was performed to measure the effects of miR-450b inhibition and overexpression. Briefly, cells (1Í10^5) were seeded in 24 well plates and transfected with mir-450b inhibitor, NC inhibitor, miR-450b mimic, and mimic NC, respectively, and cultured for 48 hours. The media was removed and washed twice with cold PBS. A wound scratch line was drawn on the cells carefully from forward to backward position using sterilized pipette tips (200 µL). Cell debris was washed out using PBS, and prepared media was suspended. Wound healing time was recorded at the appropriate length of time. The wound's width was measured

using ToupView software. All analyses were conducted in triplicate, with three independent experiments.

2.5.8 Transwell migration assay

Cells were seeded in 24 well plates, transfected with mir-450b inhibitor, NC inhibitor, miR-450b mimic, and mimic NC, respectively, and cultured for 24 hours. Next, cells were trypsinized, counted, and seeded ($5Í10^4$) into the upper chamber of each transwell insert (6.5 mm insert, 8 µm pore, 24 well insert, Costar). 700 µl of DMEM (Dulbecco's Modified Eagle Medium) media containing 10% FBS was placed in the wells of 24 well plates and incubated at 37°C for 24 hours. The migrated cells were then washed with ice-cold PBS. The cells were fixed for 2 minutes in 4% formaldehyde and permeabilized for 20 minutes in 100% methanol at room temperature. Cells were stained with a 0.5% crystal violet solution and left for 15 minutes. Non-migrated cells were removed with a sterile dry swab. The transwell insert was observed under the microscope, and images were randomly captured. Image J software was used to count the cells in each field. This study included three separate experiments.

2.5.9 TUNEL Alexa Fluor Imaging Assay

An apoptosis assay was performed to investigate apoptotic effects on cells, followed by a manufacturing protocol (Invitrogen). In brief, cells (5000 cells/well) were transfected with mir-450b inhibitor, NC inhibitor, miR-450b mimic, and mimic NC, respectively, in 96 well plates. After removing the media and washing it with PBS, the cells were fixed (in 4% paraformaldehyde for 15 minutes) and permeabilized (in 0.25% Triton X-100 for 20 minutes). TdT reaction buffer was added for 10 minutes, and then the TdT reaction cocktail was placed for 60 minutes at 37°C. Next, the Click-iT reaction cocktail was added for 30 minutes. After removing the Click-iT reaction cocktail, the DNA nuclei were stained with the Hoechst 33342

antibody. Imaging was carried out using a KEYENCE fluorescence microscope (BZ-X series). There were three separate experiments in this study.

2.5.10 Flow Cytometry using Annexin V-Biotin/PI staining.

Flow Cytometry (BD Biosciences) was used to detect the percentage of cell apoptosis following mir-450b inhibitor, NC inhibitor, miR-450b mimic, and mimic NC transfection using an annexin V-Biotin and Propidium iodide (PI) Kit according to the manufacturer's instructions (Bio Vision). In short, cells (1-5Í10^5) were suspended in 200 µL of 1X binding buffer. Annexin V-Biotin and PI were added to the cells and allowed to stay in the dark for 5 minutes. The cells were centrifuged at 2300*g for 2 minutes to remove the binding buffer. Following washing with 200 µL of 1X binding buffer, the cells were fixed in 2% formaldehyde for 15 minutes, stained with avidin-fluorescein, and kept at room temperature for 15 minutes. Annexin V (+)/ PI (–) indicates early apoptosis of flow cytometry results. There were three separate experiments in this study.

2.5.11. Quantitative real-time PCR (qRT-PCR)

To assess the relative expression of miR-450b (Taqman ID: 006407) in tissues, plasma, and cell lines, a qRT-PCR was performed as previously described [160, 216]. First, 1.25 µL (2ng/µL for tissues or cells) total RNA was reverse transcribed to cDNA in T100 thermal cycler (Bio-Rad) using the TaqMan MicroRNA Reverse Transcription Kit (Thermo Fisher Scientific) according to the manufacturer’s protocol. miR-cel-39 was spiked in the plasma to ensure the same amount of RNA isolation. Next, a TaqMan First Advanced Master Mix Kit and a Quant Studio 3 real-time PCR system (Thermo Fisher Scientific) were applied for qRT PCR. Internal controls, RNU6B for tissues and cells and miR-16 for plasma samples, were used to normalize the expression value. To make cDNA, 250 ng total RNA was reverse transcribed for the target mRNA genes using ReverTra Ace qPCR RT master mix with gDNA Remover (Toyobo, Japan).

The qRT-PCR procedure was the same as explained above. GAPDH was used as an internal control for normalizing the mRNA expression level. This study included the Taqman gene assay IDs; GAPDH (ID: Cf04419463_gH), PAX9 (ID: Cf02705737_m1), MMP9 (ID: Cf02621845_m1), BMP4 (ID: Cf01041266). The expression levels were measured using the $2^{-\Delta\Delta CT}$ method. The acceptable Ct value was less than or equal to 36.

2.5.12 Statistical analysis

All statistical analyses were conducted using GraphPad Prism 9. A one-way ANOVA test was used to determine the relative expression value, followed by the Kruskal-Wallis and Mann-Whitney U tests. A two-way ANOVA test was used to analyze the results of time-dependent experiments, followed by Sidaq's multiple comparisons. Wilson and Brown's method was applied to analyze ROC curves. The statistical analyses were considered significant When the P value was less than 0.05.

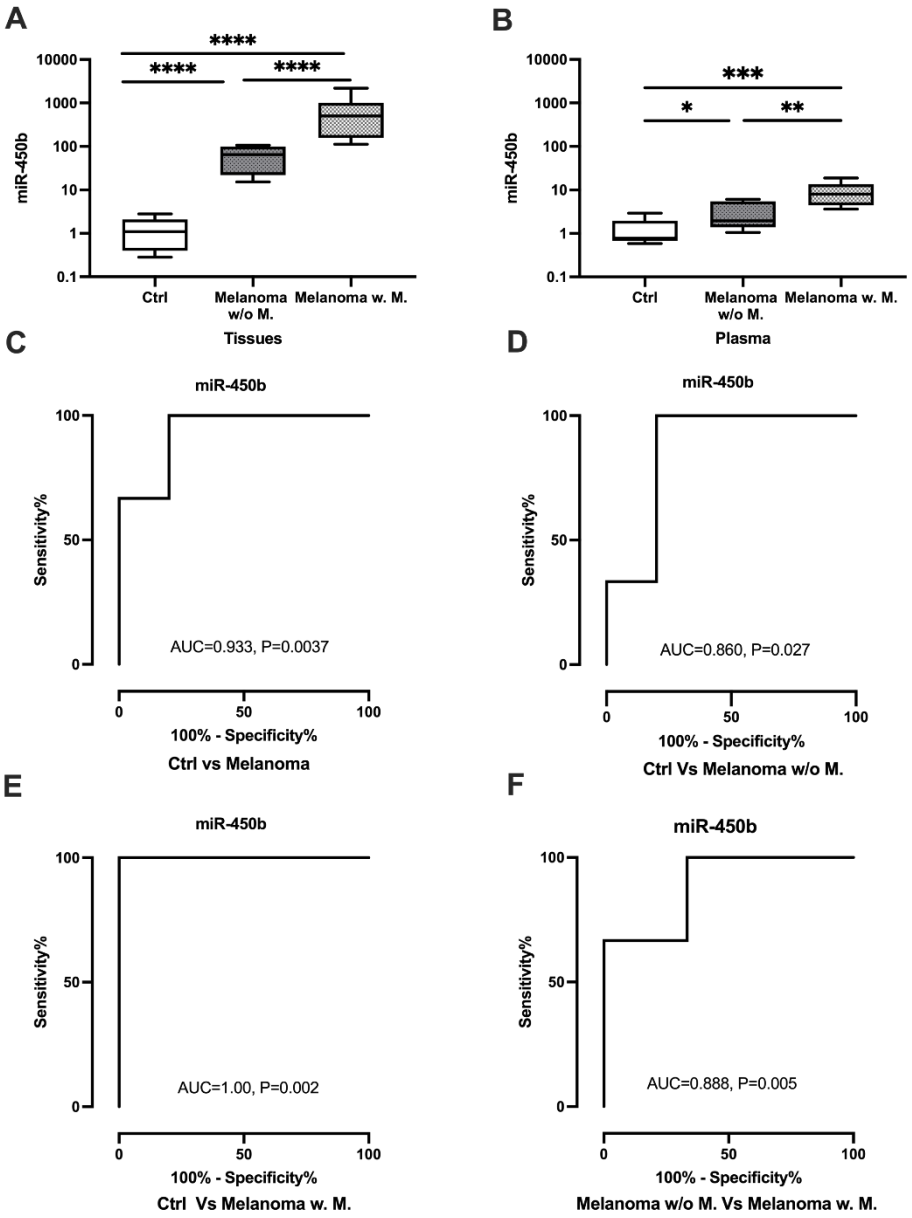


Figure 2- 1. Relative expression of miR-450b in clinical samples.

(A). Relative expression of miR-450b in healthy oral tissues (control, n=10) and canine oral melanoma tissues (Metastatic, n=15, no metastatic, n=15). (B). Expression of miR-450b in plasma samples. (C-F). ROC curve analysis of miR-450b to measure the potentiality as the biomarker. One-way ANOVA followed by Tukey's multiple comparisons and Mann-Whitney U test were used for statistical analysis. *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001.

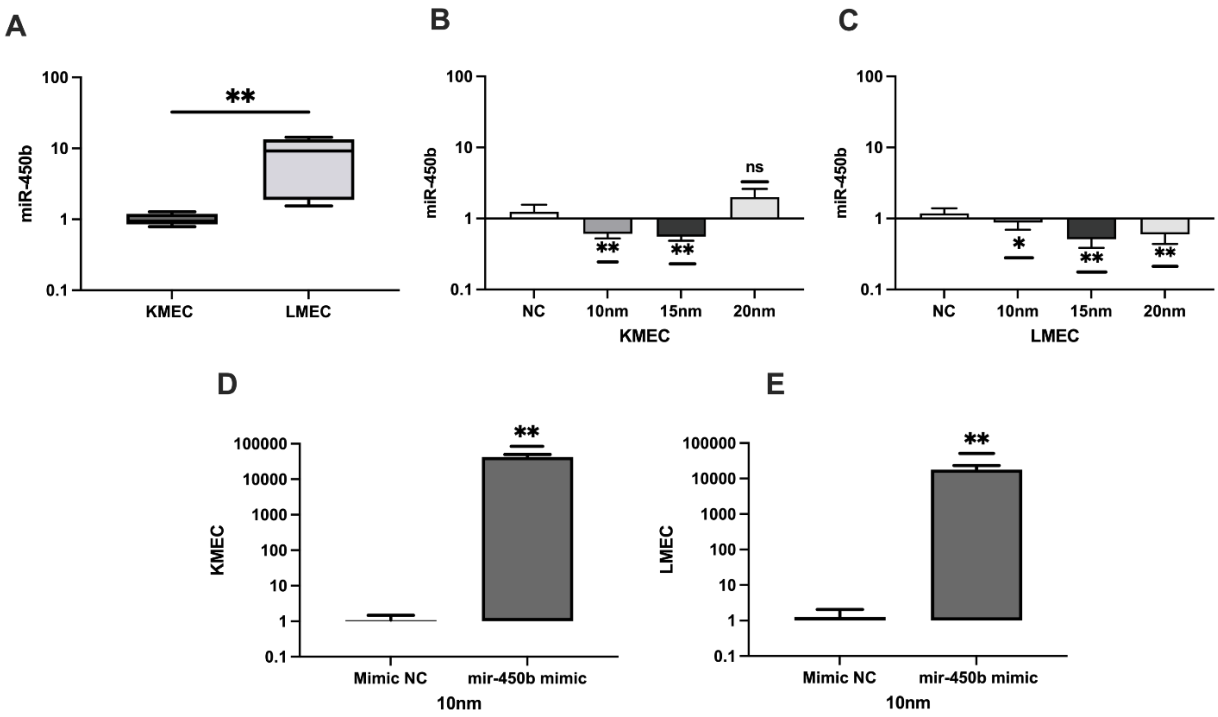


Figure 2- 2. Relative expression of miR-450b inhibitor and miR-450b mimic in KMEC and LMEC cell lines.

(A). Relative of expression of miR-450b in KMEC and LMEC cell lines. (B, C). Relative expression of miR-450b after transfected with miR-450b inhibitor for 24 h in KMEC and LMEC cell lines, respectively. (D, E). Relative expression of miR-450b after transfection with miR-450b mimics for 24 h in LMEC and KMEC cell lines, respectively. One-way ANOVA followed by Tukey's multiple comparisons and Mann-Whitney U test were used for statistical analysis. *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001.

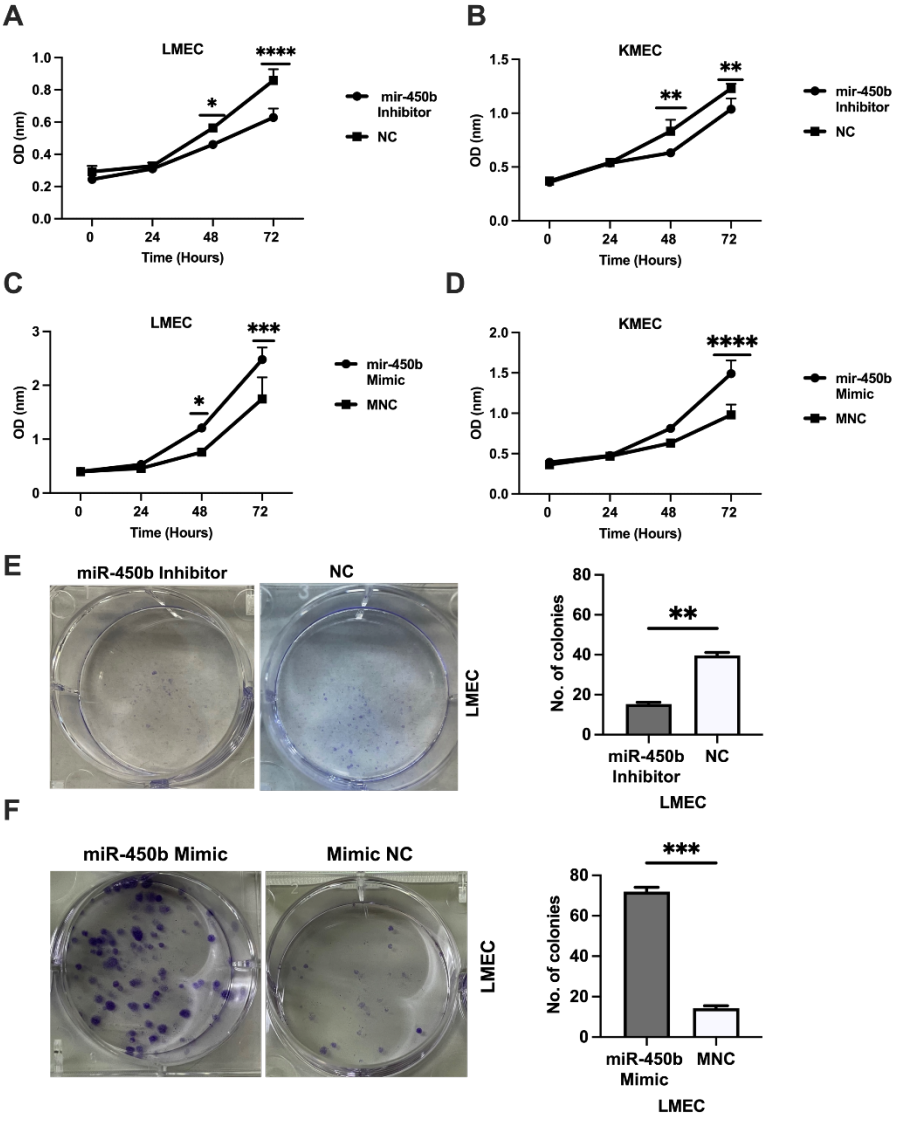


Figure 2- 3. Effects of miR450b on cell proliferation and colony formation in canine oral melanoma cell lines.

(A, B). CCK8 assay of miR-450b inhibitor (C, D). miR-450b mimic was carried out in LMEC and KMEC cell lines in a time-dependent manner. (E). Colony formation assay of miR-450b inhibitor and (F). miR-450b mimic performed in LMEC cell line. Cells>50 in number were scored. The number of colonies was measured by Image J software. The data represents the colony count ± SEM (right). Results are representative of three independent experiments. Two-way ANOVA followed by Sidaq's multiple comparisons was used for the CCK8 assay. *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001.

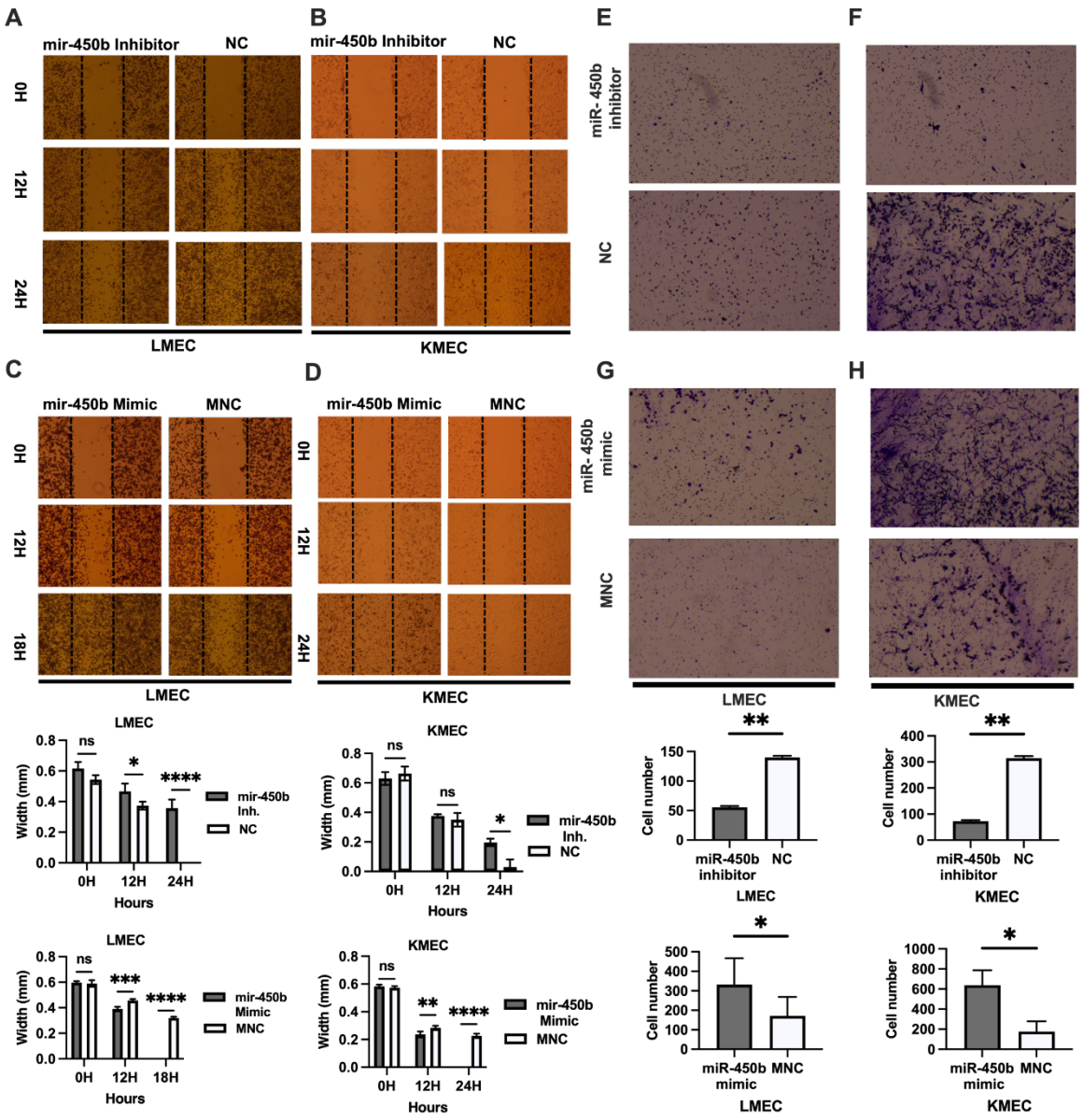


Figure 2- 4. Effects of miR-450b knockdown and overexpression on Canine oral melanoma cell migration.

(A-D). The effect of miR-450b inhibition and miR-450b mimic on cell migration in LMEC and KMEC cell lines was analyzed using the wound healing assay. Representative images of the wound healing (Upper left) and calculated scratch area (Upper right) were illustrated. (E-H). Transwell migration assay with miR-450b inhibitor and miR-450b mimic in LMEC and KMEC cells (lower panel). The number of migrated cells was measured by Image J software. Results

are representative of three independent experiments. The data represents the cell count ± SEM (lower right). Two-way ANOVA followed by Sidaq's multiple comparisons for wound healing assay. *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001.

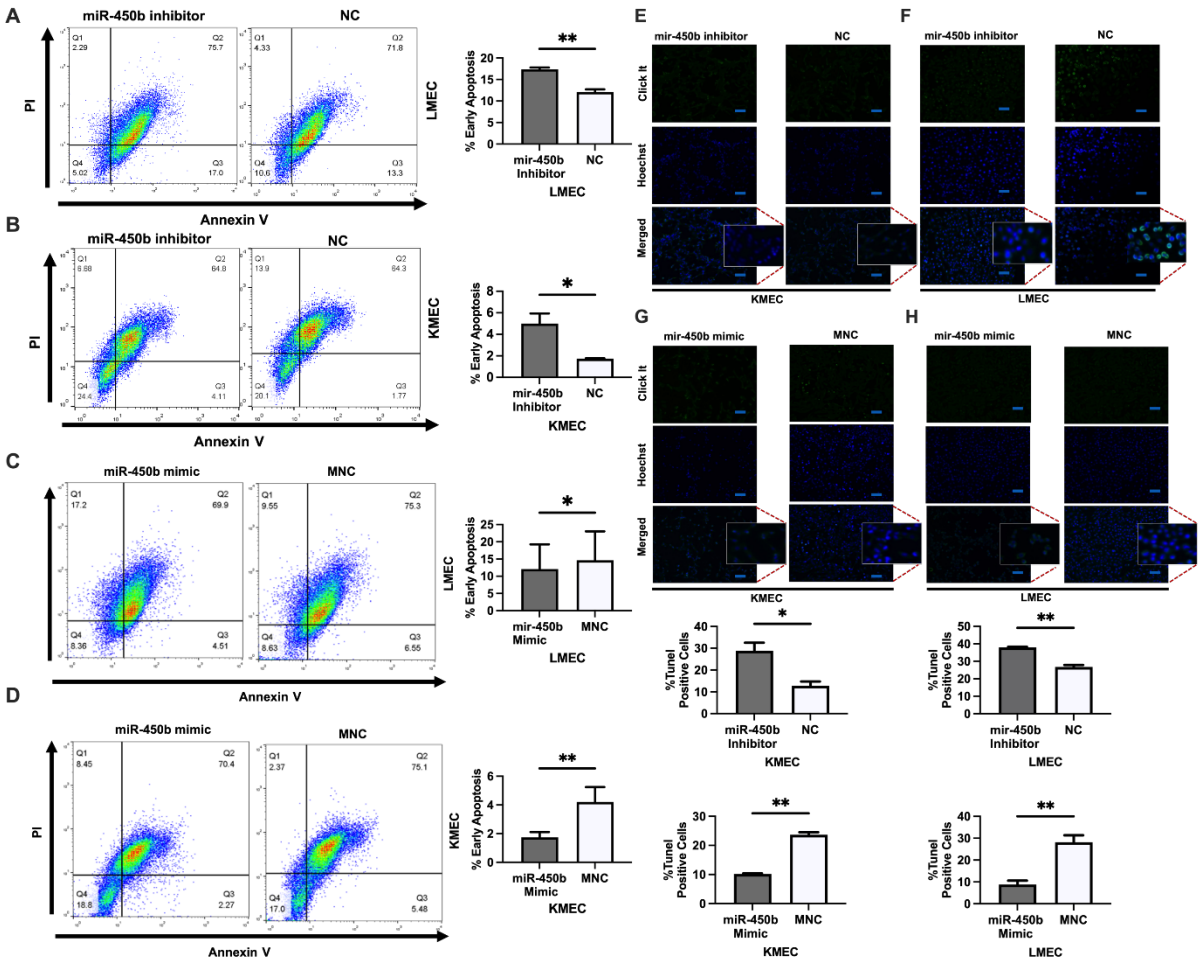


Figure 2- 5. Effects of miR-450b knockdown and overexpression on cell Apoptosis.

(A-D). Annexin V-Biotin/PI, staining, and flow cytometry showed the percentages of early apoptosis in LMEC and KMEC cell lines treated with miR-450b inhibitor and miR-450b mimic, respectively. (E-H). Tunel assay illustrated the percentage of Tunel-positive cells in LMEC and KMEC cell lines by miR-450b inhibitor and miR-450b mimic, respectively. The squared box indicates TUNEL-positive (Cyan-blue colored) and TUNEL-negative cells (didn't produce cyan-blue color), and the images were captured with 10X magnificent power (scale bar=50

μm). Results are representative of three independent experiments. The data represents the cell count ± SEM. *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001.

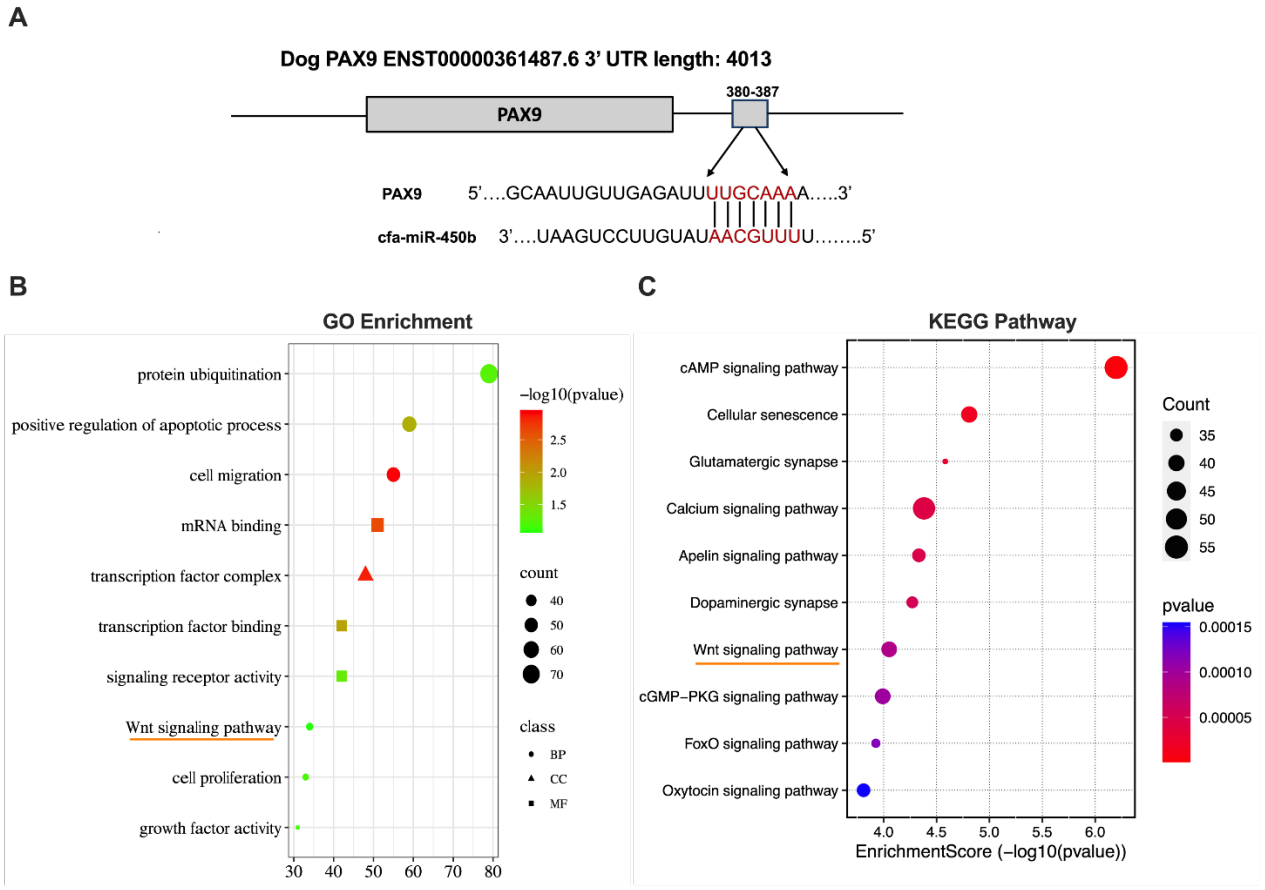


Figure 2- 6. Identification of miR-450b target genes in canine oral melanoma and their potential pathways.

(A). The schematic diagram illustrated the binding sites of miR-450b to PAX9 mRNA. (B, C). GO enrichment and KEGG enrichment of pathways involving predicted miR-450b targeting genes.

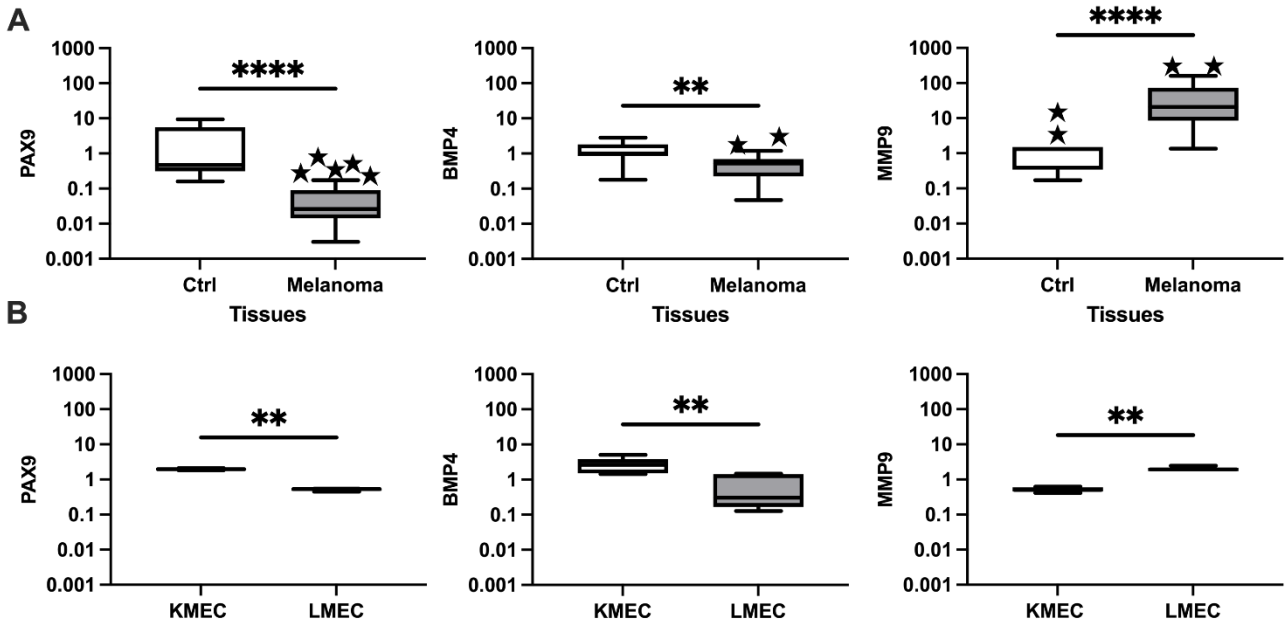


Figure 2- 7. The expression levels of PAX9-BMP4-MMP9 mRNA in clinical samples and cell lines.

(A). Relative expression of PAX9, BMP4, MMP9 in canine oral melanoma tissue samples (Control, n=10, Melanoma, n=30) and (B). KMEC and LMEC cell lines. Student t-test followed by the Mann-Whitney U test was used for statistical analysis. *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001.

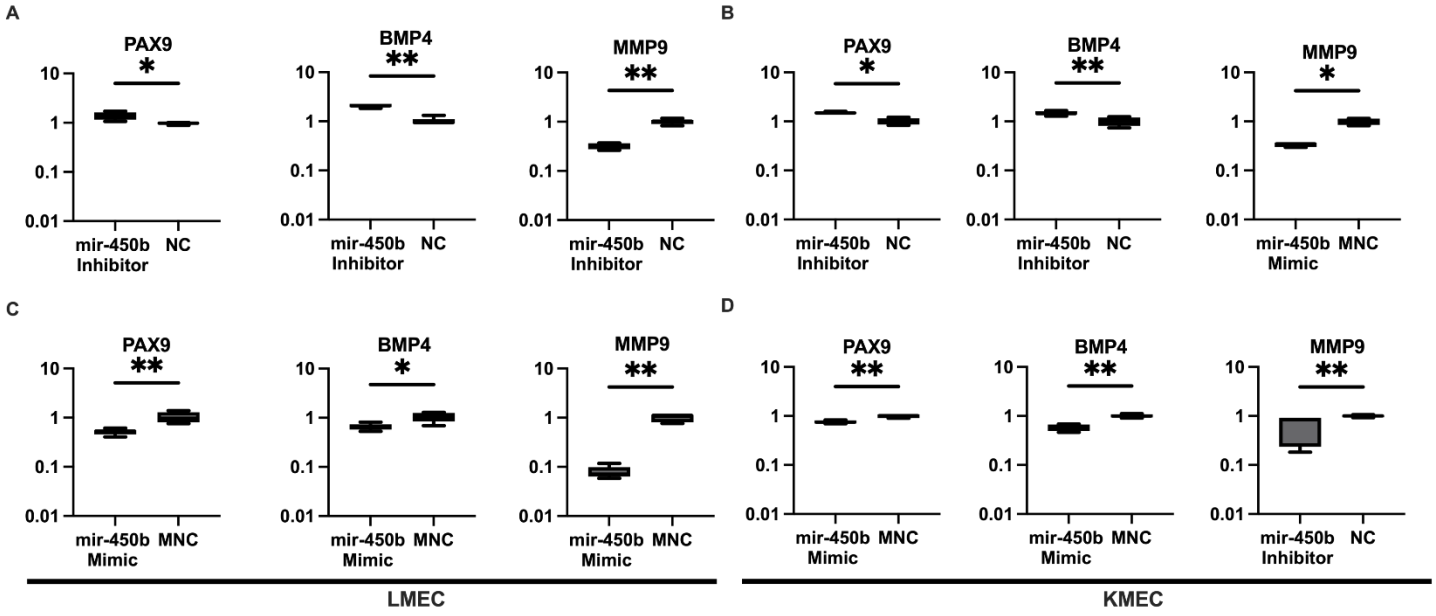


Figure 2- 8. The knockdown and overexpression of miR-450b affect the expression levels of PAX9-BMP4-MMP9 mRNA in KMEC and LMEC cell lines.

(A, B). The expression level of PAX9, BMP4, and MMP9 in indicated cells after the knockdown of miR-450b**.** (C, D). The expression level of PAX9, BMP4, and MMP9 in indicated cells after the overexpression of miR-450b. Student t-test followed by the Mann-Whitney U test was used for statistical analysis. *P<0.05, **P<0.01, ***P <0.001, ****P,0.0001.

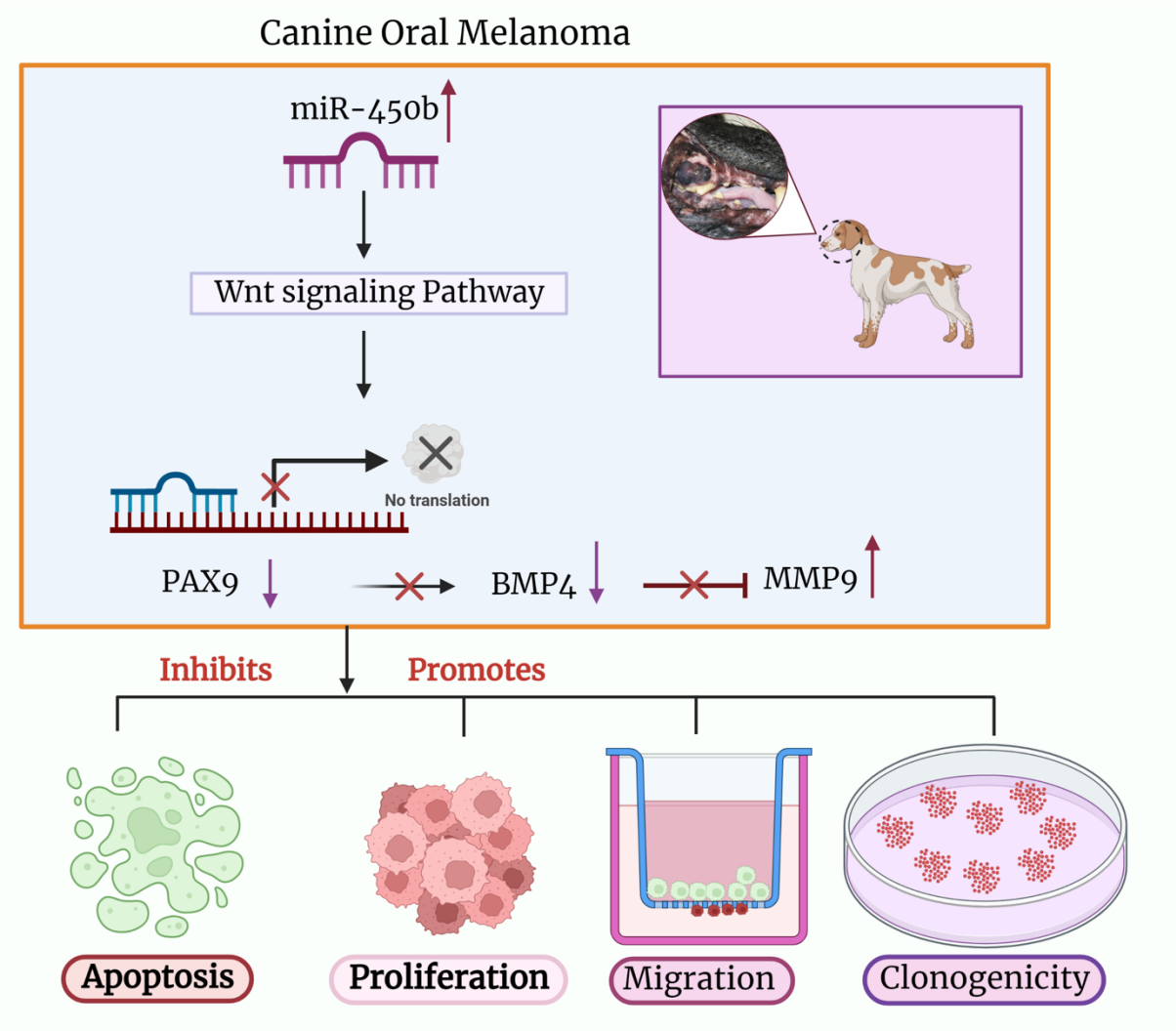
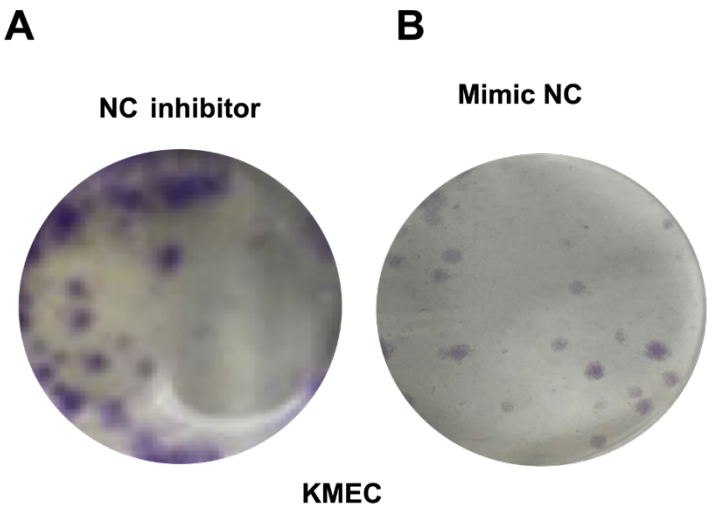


Figure 2- 9. An illustration of the proposed model for miR-450b and its target mRNA. Upregulation of miR-450b inversely regulates the PAX9 functions, and degradation of PAX9 function could interplay with BMP4 downregulation, resulting in MMP9 upregulation in COM by activating Wnt signaling pathways. miR-450b exerts its function by promoting cell proliferation, migration, clonogenicity, and inhibiting cell apoptosis.



Supplementary Figure 2- 1. Colony formation of KMEC cells.

NC inhibitor (A) and NC mimic (B). The number of colonies was measured by Image J software. The data represents the colony count ± SEM (right). Results are representative of three independent experiments.

Conclusion

To my knowledge, this study is the first portrait of comprehensively studied the potentiality of miR-301a and miR-450b to be a diagnostic biomarker and their functional roles in COM. Using qPCR, I successfully validated the expression pattern of miR-301a and miR-450b in COM clinical samples and two cell lines, namely KMeC and LMeC. This study revealed that miR-301a and miR-450b could be promising diagnostic biomarkers to differentiate melanoma patients from healthy ones. In addition, miR-450b can differentiate metastatic melanoma patients from nonmetastatic patients. Disease staging is essential to prevent or to provide early treatment. miR-450b has the advanced point of discriminating metastatic from non-metastatic melanoma, facilitating the diagnostic approach. Cell migration, proliferation, apoptosis, and extracellular vascularization are standard features of cancer progression. The miRNAs that affect the abovementioned features are oncogenic, and they have different activities in cancer progression and distant metastasis. Therefore, it is crucial to identify the functional roles of miRNA in cancer development. In this study, I have successfully revealed the functional roles of miR-301a and miR-450b in COM. I have applied several assays to identify their effects on melanoma.

The knockdown of miR-301a showed that miR-301a promoted cell migration, colony formation, and proliferation and inhibited cell apoptosis. This study proved that miR-301a has an oncogenic effect on COM. The knockdown and overexpression of miR-450b revealed that miR-450b promoted cell migration, proliferation, and colony formation and inhibited cell apoptosis.

Studying miRNA targets has a significant correlation, as in their human study. Important miR-301a and miR-450b targets were explored. These findings facilitate the knowledge of the role of miRNA in melanoma and contribute to further miRNA-based therapeutic development.

I have identified several target genes of miR-301a and miR-450b. NDRG2 is the direct target of miR-301a. However, unfortunately, inhibition of miR-301a didn't change the expression of NDRG2. Though the bioinformatic results showed the direct target, I posit that miR-301a may interact with other genes, which downregulates the expression value of NDRG2.

In case of miR-450b, we have identified three candidate gene which directly and indirectly interacts with miR-450b expression. PAX9 is the direct target gene of miR-450b. In this study, I revealed that upregulated miR-450b decreased the PAX9 expression, which has a concomitant effect on BMP4 downregulation and MMP9 upregulation.

Signaling pathways are important for the invention of cancer treatment. In this study, I have revealed several signaling pathways related to miR-301a and miR-450b using GO and KEGG enrichment analysis. The common signaling pathways between GO and KEGG were Wnt singling pathways in the case of both miR-301a and miR-450b. Wnt signaling pathways are important for melanoma tumor progression.

Overall, in this study, I hypothesize that upregulation of miR-301a and miR-450b in COM silences its target genes via the most likely Wnt-signaling pathway, which affects cell proliferation, migration, clonogenicity, and apoptosis.

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Appendix

Target genes of miR-301a using the Target scan database:

Ortholog of target gene	Gene Name	Total context++ score
SLAIN1	SLAIN Motif Family, Member 1	-1.2
KLF7	Kruppel-Like Factor 7 (Ubiquitous)	-0.98
PAN3	PAN3 Poly(A) Specific Ribonuclease Subunit Homolog (*S. Cerevisiae*)	-0.96
RTCA	RNA 3'-Terminal Phosphate Cyclase	-0.82
STX7	Syntaxin 7	-1
IRF1	Interferon Regulatory Factor 1	-0.73
SYBU	Syntabulin (Syntaxin-Interacting)	-0.71
NFIA	Nuclear Factor I/A	-0.69
FRZB	Frizzled-Related Protein	-0.7
WDR20	WD Repeat Domain 20	-0.66
BTG1	B-Cell Translocation Gene 1, Anti-Proliferative	-0.66
ABHD3	Abhydrolase Domain Containing 3	-0.65
TES	Testis Derived Transcript (3 LIM Domains)	-0.65
TSHZ1	Teashirt Zinc Finger Homeobox 1	-0.72
AK4	Adenylate Kinase 4	-0.62
CPEB2	Cytoplasmic Polyadenylation Element Binding Protein 2	-0.64
COX7A2L	Cytochrome C Oxidase Subunit Viia Polypeptide 2 Like	-0.81
FNDC4	Fibronectin Type III Domain Containing 4	-0.59
TMEM55A	Transmembrane Protein 55A	-0.57
SLC2A4RG	SLC2A4 Regulator	-0.56
APPL1	Adaptor Protein, Phosphotyrosine Interaction, PH Domain And Leucine Zipper Containing 1	-0.56
FOSL1	FOS-Like Antigen 1	-0.54
TRERF1	Transcriptional Regulating Factor 1	-0.53
OSBPL10	Oxysterol Binding Protein-Like 10	-0.53
LONRF1	LON Peptidase N-Terminal Domain And Ring Finger 1	-0.51
SNAP25	Synaptosomal-Associated Protein, 25kda	-0.59
SOS2	Son Of Sevenless Homolog 2 (Drosophila)	-0.51
HIVEP2	Human Immunodeficiency Virus Type I Enhancer Binding Protein 2	-0.49
IMPDH1	IMP (Inosine 5'-Monophosphate) Dehydrogenase 1	-0.47
ZNF3	Zinc Finger Protein 3	-0.47
CPEB3	Cytoplasmic Polyadenylation Element Binding Protein 3	-0.46
STK33	Serine/Threonine Kinase 33	-0.46
THOP1	Thimet Oligopeptidase 1	-0.46

NPNT	Nephronectin	-0.45
MAPK6	Mitogen-Activated Protein Kinase 6	-0.45
LMLN	Leishmanolysin-Like (Metallopeptidase M8 Family)	-0.44
MTMR10	Myotubularin Related Protein 10	-0.46
PAX6	Paired Box 6	-0.52
HSPA4L	Heat Shock 70kda Protein 4-Like	-0.44
SKP1	S-Phase Kinase-Associated Protein 1	-0.41
LCOR	Ligand Dependent Nuclear Receptor Corepressor	-0.43
TMOD3	Tropomodulin 3 (Ubiquitous)	-0.51
ACSL1	Acyl-Coa Synthetase Long-Chain Family Member 1	-0.46
FASTK	Fas-Activated Serine/Threonine Kinase	-0.4
NRBF2	Nuclear Receptor Binding Factor 2	-0.4
RUNX3	Runt-Related Transcription Factor 3	-0.39
UBE3B	Ubiquitin Protein Ligase E3B	-0.44
HOXB1	Homeobox B1	-0.39
CLOCK	Clock Circadian Regulator	-0.4
ZNF75A	Zinc Finger Protein 75a	-0.39
TGFBR2	Transforming Growth Factor, Beta Receptor II (70/80kda)	-0.41
ADAM12	ADAM Metallopeptidase Domain 12	-0.38
PTGES3	Prostaglandin E Synthase 3 (Cytosolic)	-0.38
STOX2	Storkhead Box 2	-0.39
ARHGEF4	Rho Guanine Nucleotide Exchange Factor (GEF) 4	-0.38
CNOT7	CCR4-NOT Transcription Complex, Subunit 7	-0.38
PDIK1L	PDLIM1 Interacting Kinase 1 Like	-0.38
SBSPON	Somatomedin B And Thrombospondin, Type 1 Domain Containing	-0.37
DENND1A	DENN/MADD Domain Containing 1A	-0.37
ANKRD10	Ankyrin Repeat Domain 10	-0.37
SNX2	Sorting Nexin 2	-0.49
CMPK2	Cytidine Monophosphate (UMP-CMP) Kinase 2, Mitochondrial	-0.45
RNF216	Ring Finger Protein 216	-0.54
MTMR9	Myotubularin Related Protein 9	-0.55
KIAA2022	Kiaa2022	-0.36
USP28	Ubiquitin Specific Peptidase 28	-0.36
BAI3	Brain-Specific Angiogenesis Inhibitor 3	-0.36
RAB30	RAB30, Member RAS Oncogene Family	-0.37
ZIC5	Zic Family Member 5	-0.4
NPTX1	Neuronal Pentraxin I	-0.38
SASH1	SAM And SH3 Domain Containing 1	-0.35
NEUROD1	Neuronal Differentiation 1	-0.35
SLC35F3	Solute Carrier Family 35, Member F3	-0.35
PHF20	PHD Finger Protein 20	-0.44
EGLN3	Egl-9 Family Hypoxia-Inducible Factor 3	-0.34

AGO4	Argonaute RISC Catalytic Component 4	-0.34
ATXN7L1	Ataxin 7-Like 1	-0.34
CALB1	Calbindin 1, 28kda	-0.34
SLC8A1	Solute Carrier Family 8 (Sodium/Calcium Exchanger), Member 1	-0.34
MAT2B	Methionine Adenosyltransferase II, Beta	-0.34
FUT9	Fucosyltransferase 9 (Alpha (1,3) Fucosyltransferase)	-0.33
LRP1B	Low Density Lipoprotein Receptor-Related Protein 1B	-0.35
CHMP4B	Charged Multivesicular Body Protein 4B	-0.35
ZFP91	ZFP91 Zinc Finger Protein	-0.32
CNIH1	Cornichon Family AMPA Receptor Auxiliary Protein 1	-0.32
TMEM63B	Transmembrane Protein 63B	-0.32
SPEN	Spen Homolog, Transcriptional Regulator (Drosophila)	-0.31
DLG2	Discs, Large Homolog 2 (Drosophila)	-0.31
TNFSF10	Tumor Necrosis Factor (Ligand) Superfamily, Member 10	-0.31
GAP43	Growth Associated Protein 43	-0.31
ACBD3	Acyl-Coa Binding Domain Containing 3	-0.32
TMEM87B	Transmembrane Protein 87B	-0.38
MCTP1	Multiple C2 Domains, Transmembrane 1	-0.34
MB21D2	Mab-21 Domain Containing 2	-0.54
GPR155	G Protein-Coupled Receptor 155	-0.3
HABP4	Hyaluronan Binding Protein 4	-0.35
SIX4	SIX Homeobox 4	-0.31
STT3B	STT3B, Subunit Of The Oligosaccharyltransferase Complex (Catalytic)	-0.35
GTF2H1	General Transcription Factor IIH, Polypeptide 1, 62kda	-0.36
HADHA	Hydroxyacyl-Coa Dehydrogenase/3-Ketoacyl-Coa Thiolase/Enoyl-Coa Hydratase (Trifunctional Protein), Alpha Subunit	-0.28
SEL1L3	Sel-1 Suppressor Of Lin-12-Like 3 (C. Elegans)	-0.65
ENPP6	Ectonucleotide Pyrophosphatase/Phosphodiesterase 6	-0.28
DAAM1	Dishevelled Associated Activator Of Morphogenesis 1	-0.39
SPRED1	Sprouty-Related, EVH1 Domain Containing 1	-0.39
EXOC5	Exocyst Complex Component 5	-0.34
RAB1A	RAB1A, Member RAS Oncogene Family	-0.33
KLF13	Kruppel-Like Factor 13	-0.27
ADAMTS20	ADAM Metallopeptidase With Thrombospondin Type 1 Motif, 20	-0.35
NOL4	Nucleolar Protein 4	-0.26

ITGA4	Integrin, Alpha 4 (Antigen CD49D, Alpha 4 Subunit Of VLA-4 Receptor)	-0.38
ARL4A	ADP-Ribosylation Factor-Like 4A	-0.36
EPHA7	EPH Receptor A7	-0.26
FAM13B	Family With Sequence Similarity 13, Member B	-0.31
GDA	Guanine Deaminase	-0.25
SLC25A32	Solute Carrier Family 25 (Mitochondrial Folate Carrier), Member 32	-0.3
WHSC1L1	Wolf-Hirschhorn Syndrome Candidate 1-Like 1	-0.27
DOCK3	Dedicator Of Cytokinesis 3	-0.24
KMT2C	Lysine (K)-Specific Methyltransferase 2C	-0.24
ESCO2	Establishment Of Sister Chromatid Cohesion N-Acetyltransferase 2	-0.46
ANKRD12	Ankyrin Repeat Domain 12	-0.31
RBM20	RNA Binding Motif Protein 20	-0.3
CRISPLD1	Cysteine-Rich Secretory Protein LCCL Domain Containing 1	-0.27
PIK3C2A	Phosphatidylinositol-4-Phosphate 3-Kinase, Catalytic Subunit Type 2 Alpha	-0.3
PLCB1	Phospholipase C, Beta 1 (Phosphoinositide-Specific)	-0.23
FAM53B	Family With Sequence Similarity 53, Member B	-0.23
RARB	Retinoic Acid Receptor, Beta	-0.23
MIER3	Mesoderm Induction Early Response 1, Family Member 3	-0.23
ZNF451	Zinc Finger Protein 451	-0.44
TRIM2	Tripartite Motif Containing 2	-0.22
TANC2	Tetratricopeptide Repeat, Ankyrin Repeat And Coiled-Coil Containing 2	-0.23
TOB2	Transducer Of ERBB2, 2	-0.28
ARHGEF12	Rho Guanine Nucleotide Exchange Factor (GEF) 12	-0.22
HOXA5	Homeobox A5	-0.21
HSPA8	Heat Shock 70kda Protein 8	-0.42
PELI1	Pellino E3 Ubiquitin Protein Ligase 1	-0.21
XKRX	XK, Kell Blood Group Complex Subunit-Related, X-Linked	-0.2
TBC1D19	TBC1 Domain Family, Member 19	-0.29
NUS1	Nuclear Undecaprenyl Pyrophosphate Synthase 1 Homolog (S. Cerevisiae)	-0.2
KATNBL1	Katanin P80 Subunit B-Like 1	-0.32
ZDHHC7	Zinc Finger, DHHC-Type Containing 7	-0.29
ST3GAL3	ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 3	-0.19
PTPN14	Protein Tyrosine Phosphatase, Non-Receptor Type 14	-0.21
NAV2	Neuron Navigator 2	-0.19
ZEB1	Zinc Finger E-Box Binding Homeobox 1	-0.18

DLC1	Deleted In Liver Cancer 1	-0.26
ZBTB16	Zinc Finger And BTB Domain Containing 16	-0.19
ACVR1C	Activin A Receptor, Type IC	-0.37
LIN28A	Lin-28 Homolog A (C. Elegans)	-0.17
ZNF275	Zinc Finger Protein 275	-0.18
SALL3	Sal-Like 3 (Drosophila)	-0.27
LRP6	Low Density Lipoprotein Receptor-Related Protein 6	-0.2
PXDN	Peroxidasin Homolog (Drosophila)	-0.23
RALGPS1	Ral GEF With PH Domain And SH3 Binding Motif 1	-0.17
KCNB1	Potassium Voltage-Gated Channel, Shab-Related Subfamily, Member 1	-0.15
ANKRD52	Ankyrin Repeat Domain 52	-0.14
TP63	Tumor Protein P63	-0.14
GAPVD1	Gtpase Activating Protein And VPS9 Domains 1	-0.14
LMBRD2	LMBR1 Domain Containing 2	-0.2
GOLT1B	Golgi Transport 1B	-0.5
MAP4K4	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 4	-0.3
ZC3H12C	Zinc Finger CCCH-Type Containing 12C	-0.13
HCFC2	Host Cell Factor C2	-0.63
KIAA1217	Kiaa1217	-0.13
ADAMTS19	ADAM Metallopeptidase With Thrombospondin Type 1 Motif, 19	-0.1
ANKIB1	Ankyrin Repeat And IBR Domain Containing 1	-0.31
LGALSL	Lectin, Galactoside-Binding-Like	-0.78
GGA2	Golgi-Associated, Gamma Adaptin Ear Containing, ARF Binding Protein 2	-0.11
FANCA	Fanconi Anemia, Complementation Group A	-0.29
ERBB2IP	Erbb2 Interacting Protein	-0.23
LAMC1	Laminin, Gamma 1 (Formerly LAMB2)	-0.09
FYN	FYN Oncogene Related To SRC, FGR, YES	-0.52
TEX2	Testis Expressed 2	-0.47
ETNK1	Ethanolamine Kinase 1	-0.26
RASD1	RAS, Dexamethasone-Induced 1	-0.39
NEK9	NIMA-Related Kinase 9	-0.28
SMURF2	SMAD Specific E3 Ubiquitin Protein Ligase 2	-0.45
C9orf69	Chromosome 9 Open Reading Frame 69	-0.51
GAN	Gigaxonin	-0.05
CDC73	Cell Division Cycle 73	-0.23
PPP2R5E	Protein Phosphatase 2, Regulatory Subunit B', Epsilon Isoform	-0.12
RMND5A	Required For Meiotic Nuclear Division 5 Homolog A (S. Cerevisiae)	-0.2
FBXO9	F-Box Protein 9	-0.2
MYO1E	Myosin IE	-0.24

CSMD1	CUB And Sushi Multiple Domains 1	-0.24
IQGAP2	IQ Motif Containing Gtpase Activating Protein 2	-0.22
TSPAN3	Tetraspanin 3	-0.32
MBD2	Methyl-CPG Binding Domain Protein 2	-0.6
INTS8	Integrator Complex Subunit 8	-0.33
KIAA1211	Kiaa1211	-0.4
NRD1	Nardilysin (N-Arginine Dibasic Convertase)	-0.3
GRIN2A	Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate 2A	-0.1

Target genes of miR-450b using the Target scan database:

Ortholog of target gene	Gene Name	Total context++ score
DCAF4L2	DDB1 And CUL4 Associated Factor 4-Like 2	-1.2
CAMK2N1	Calcium/Calmodulin-Dependent Protein Kinase II Inhibitor 1	-0.89
MACC1	Metastasis Associated In Colon Cancer 1	-0.85
FAM45A	Family With Sequence Similarity 45, Member A	-0.75
P2RY13	Purinergic Receptor P2Y, G-Protein Coupled, 13	-0.64
NFE2L2	Nuclear Factor, Erythroid 2-Like 2	-0.63
TFRC	Transferrin Receptor	-0.65
ZNF226	Zinc Finger Protein 226	-0.66
SPTSSB	Serine Palmitoyltransferase, Small Subunit B	-0.57
ZNF616	Zinc Finger Protein 616	-0.6
PPAT	Phosphoribosyl Pyrophosphate Amidotransferase	-0.72
C9orf66	Chromosome 9 Open Reading Frame 66	-0.55
CDKN2C	Cyclin-Dependent Kinase Inhibitor 2C (P18, Inhibits CDK4)	-0.65
TRO	Trophinin	-0.53
PI15	Peptidase Inhibitor 15	-0.53
FAM96A	Family With Sequence Similarity 96, Member A	-0.53
SLC17A6	Solute Carrier Family 17 (Vesicular Glutamate Transporter), Member 6	-0.52
TCEAL1	Transcription Elongation Factor A (SII)-Like 1	-0.51
ARL11	ADP-Ribosylation Factor-Like 11	-0.51
PAX9	Paired Box 9	-0.53
	Ac117834.1	-0.5
CAMTA1	Calmodulin Binding Transcription Activator 1	-0.56
C18orf42	Chromosome 18 Open Reading Frame 42	-0.58
TCEAL6	Transcription Elongation Factor A (SII)-Like 6	-0.49

TMEM178A	Transmembrane Protein 178A	-0.48
TCEAL2	Transcription Elongation Factor A (SII)-Like 2	-0.47
ART3	ADP-Ribosyltransferase 3	-0.47
KCNE3	Potassium Voltage-Gated Channel, Isk-Related Family, Member 3	-0.47
HRK	Harakiri, BCL2 Interacting Protein (Contains Only BH3 Domain)	-0.48
RAB40A	RAB40A, Member RAS Oncogene Family	-0.47
SNX9	Sorting Nexin 9	-0.47
RP11-861L17.3	Uncharacterized Protein	-0.47
GKAP1	G Kinase Anchoring Protein 1	-0.46
CHMP2B	Charged Multivesicular Body Protein 2B	-0.46
C1orf174	Chromosome 1 Open Reading Frame 174	-0.46
ADAM18	ADAM Metallopeptidase Domain 18	-0.45
CPA6	Carboxypeptidase A6	-0.45
TCEAL7	Transcription Elongation Factor A (SII)-Like 7	-0.45
PIN1	Peptidylprolyl Cis/Trans Isomerase, NIMA-Interacting 1	-0.57
CHCHD4	Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 4	-0.44
HERC4	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 4	-0.48
COA6	Cytochrome C Oxidase Assembly Factor 6 Homolog (S. Cerevisiae)	-0.43
RP11-295D22.1	Uncharacterized Protein	-0.43
MOSPD1	Motile Sperm Domain Containing 1	-0.49
TAF1D	TATA Box Binding Protein (TBP)-Associated Factor, RNA Polymerase I, D, 41kda	-0.48
PKIB	Protein Kinase (Camp-Dependent, Catalytic) Inhibitor Beta	-0.43
BCL2A1	BCL2-Related Protein A1	-0.43
SEPHS2	Selenophosphate Synthetase 2	-0.43
NUDCD2	Nudc Domain Containing 2	-0.45
IDO2	Indoleamine 2,3-Dioxygenase 2	-0.42
COQ2	Coenzyme Q2 4-Hydroxybenzoate Polyprenyltransferase	-0.42
	Rp11-215a19.2	-0.42
SMKR1	Small Lysine-Rich Protein 1	-0.42
N6AMT2	N-6 Adenine-Specific DNA Methyltransferase 2 (Putative)	-0.42
LUM	Lumican	-0.47
POC1B-GALNT4	POC1B-GALNT4 Readthrough	-0.42
UGT2B11	UDP Glucuronosyltransferase 2 Family, Polypeptide B11	-0.41
KIAA0040	Kiaa0040	-0.41
C4orf26	Chromosome 4 Open Reading Frame 26	-0.41

GNG2	Guanine Nucleotide Binding Protein (G Protein), Gamma 2	-0.64
TXNDC8	Thioredoxin Domain Containing 8 (Spermatozoa)	-0.41
C8B	Complement Component 8, Beta Polypeptide	-0.41
YIPF1	Yip1 Domain Family, Member 1	-0.53
RAD54B	RAD54 Homolog B (S. Cerevisiae)	-0.5
CAPZA2	Capping Protein (Actin Filament) Muscle Z-Line, Alpha 2	-0.4
SPIN4	Spindlin Family, Member 4	-0.4
KLKB1	Kallikrein B, Plasma (Fletcher Factor) 1	-0.39
VGLL3	Vestigial Like 3 (Drosophila)	-0.48
HPGDS	Hematopoietic Prostaglandin D Synthase	-0.39
HNRNPL	Heterogeneous Nuclear Ribonucleoprotein L	-0.39
STAT4	Signal Transducer And Activator Of Transcription 4	-0.39
PKIA	Protein Kinase (Camp-Dependent, Catalytic) Inhibitor Alpha	-0.53
ACTL6A	Actin-Like 6A	-0.39
UBE2W	Ubiquitin-Conjugating Enzyme E2W (Putative)	-0.42
KRT36	Keratin 36	-0.38
ZNF23	Zinc Finger Protein 23	-0.38
SLC26A4	Solute Carrier Family 26 (Anion Exchanger), Member 4	-0.38
FCGR2B	Fc Fragment Of Igg, Low Affinity Iib, Receptor (CD32)	-0.38
AC145676.2	Uncharacterized Protein	-0.38
TAX1BP1	Tax1 (Human T-Cell Leukemia Virus Type I) Binding Protein 1	-0.53
MYH2	Myosin, Heavy Chain 2, Skeletal Muscle, Adult	-0.37
PDPN	Podoplanin	-0.37
ORC5	Origin Recognition Complex, Subunit 5	-0.37
CLLU1	Chronic Lymphocytic Leukemia Up-Regulated 1	-0.37
AL357673.1	CDNA: FLJ21031 Fis, Clone CAE07336; HCG1780521; Uncharacterized Protein	-0.37
MYPN	Myopalladin	-0.63
MUCL1	Mucin-Like 1	-0.37
PLEKHF2	Pleckstrin Homology Domain Containing, Family F (With FYVE Domain) Member 2	-0.41
KCTD1	Potassium Channel Tetramerization Domain Containing 1	-0.51
XPO4	Exportin 4	-0.38
FOXD4	Forkhead Box D4	-0.36
RNASE8	Ribonuclease, Rnase A Family, 8	-0.36
FOXD4L1	Forkhead Box D4-Like 1	-0.36
ELAVL4	ELAV Like Neuron-Specific RNA Binding Protein 4	-0.36
HOXB2	Homeobox B2	-0.36
EDIL3	EGF-Like Repeats And Discoidin I-Like Domains 3	-0.36
C15orf61	Chromosome 15 Open Reading Frame 61	-0.37
RP11-625H11.1	Uncharacterized Protein	-0.36

CLDN1	Claudin 1	-0.38
TMEM260	Transmembrane Protein 260	-0.35
AC011897.1	Uncharacterized Protein	-0.35
PPP4R2	Protein Phosphatase 4, Regulatory Subunit 2	-0.38
DYNLT3	Dynein, Light Chain, Tctex-Type 3	-0.35
PDCD5	Programmed Cell Death 5	-0.36
SLC7A10	Solute Carrier Family 7 (Neutral Amino Acid Transporter Light Chain, Asc System), Member 10	-0.44
PABPC1	Poly(A) Binding Protein, Cytoplasmic 1	-0.35
IL17C	Interleukin 17C	-0.35
GSPT1	G1 To S Phase Transition 1	-0.47
CALM1	Calmodulin 1 (Phosphorylase Kinase, Delta)	-0.35
OLFM3	Olfactomedin 3	-0.37
RTN4	Reticulon 4	-0.35
GGNBP2	Gametogenetin Binding Protein 2	-0.49
IGSF1	Immunoglobulin Superfamily, Member 1	-0.34
APPBP2	Amyloid Beta Precursor Protein (Cytoplasmic Tail) Binding Protein 2	-0.46
FOXD4L6	Forkhead Box D4-Like 6	-0.34
CRP	C-Reactive Protein, Pentraxin-Related	-0.34
FOXD4L3	Forkhead Box D4-Like 3	-0.34
GSTA1	Glutathione S-Transferase Alpha 1	-0.34
SOX21	SRY (Sex Determining Region Y)-Box 21	-0.34
RP1-66C13.4	Uncharacterized Protein	-0.34
SLITRK4	SLIT And NTRK-Like Family, Member 4	-0.34
MED26	Mediator Complex Subunit 26	-0.34
PPP4R4	Protein Phosphatase 4, Regulatory Subunit 4	-0.33
BAIAP2L1	BAI1-Associated Protein 2-Like 1	-0.34
NPVF	Neuropeptide VF Precursor	-0.33
FAM111B	Family With Sequence Similarity 111, Member B	-0.33
ZNF845	Zinc Finger Protein 845	-0.43
GNG4	Guanine Nucleotide Binding Protein (G Protein), Gamma 4	-0.5
C1orf21	Chromosome 1 Open Reading Frame 21	-0.5
NT5DC4	5'-Nucleotidase Domain Containing 4	-0.32
CCL4L1	Chemokine (C-C Motif) Ligand 4-Like 1	-0.32
GNAQ	Guanine Nucleotide Binding Protein (G Protein), Q Polypeptide	-0.34
ZFYVE16	Zinc Finger, FYVE Domain Containing 16	-0.32
COLCA1	Colorectal Cancer Associated 1	-0.32
LDHAL6B	Lactate Dehydrogenase A-Like 6B	-0.32
PDS5A	PDS5, Regulator Of Cohesion Maintenance, Homolog A (S. Cerevisiae)	-0.32
CREG2	Cellular Repressor Of E1A-Stimulated Genes 2	-0.44
CREG1	Cellular Repressor Of E1A-Stimulated Genes 1	-0.35
SPRY2	Sprouty Homolog 2 (Drosophila)	-0.32
HAT1	Histone Acetyltransferase 1	-0.31

SLMO2	Slowmo Homolog 2 (Drosophila)	-0.36
SERTAD2	SERTA Domain Containing 2	-0.46
HDHD2	Haloacid Dehalogenase-Like Hydrolase Domain Containing 2	-0.31
MARCH11	Membrane-Associated Ring Finger (C3HC4) 11	-0.31
GPR158	G Protein-Coupled Receptor 158	-0.31
TMEM38B	Transmembrane Protein 38B	-0.36
ZNF83	Zinc Finger Protein 83	-0.39
CBFB	Core-Binding Factor, Beta Subunit	-0.32
GNG10	Guanine Nucleotide Binding Protein (G Protein), Gamma 10	-0.3
SOX2	SRY (Sex Determining Region Y)-Box 2	-0.3
AC016559.1	Uncharacterized Protein	-0.3
CPEB2	Cytoplasmic Polyadenylation Element Binding Protein 2	-0.32
SCOC	Short Coiled-Coil Protein	-0.39
KRT26	Keratin 26	-0.3
DSCAM	Down Syndrome Cell Adhesion Molecule	-0.3
ENTHD1	ENTH Domain Containing 1	-0.3
DMRT1	Doublesex And Mab-3 Related Transcription Factor 1	-0.3
SUPV3L1	Suppressor Of Var1, 3-Like 1 (S. Cerevisiae)	-0.3
CD58	CD58 Molecule	-0.37
FAM180A	Family With Sequence Similarity 180, Member A	-0.3
BEND6	BEN Domain Containing 6	-0.47
B3GNT5	UDP-Glcnac:Betagal Beta-1,3-N-Acetylglucosaminyltransferase 5	-0.3
CPB2	Carboxypeptidase B2 (Plasma)	-0.3
TMEM70	Transmembrane Protein 70	-0.3
PIP5K1B	Phosphatidylinositol-4-Phosphate 5-Kinase, Type I, Beta	-0.3
OR7D2	Olfactory Receptor, Family 7, Subfamily D, Member 2	-0.29
BAALC	Brain And Acute Leukemia, Cytoplasmic	-0.48
CCNC	Cyclin C	-0.3
TDGF1	Teratocarcinoma-Derived Growth Factor 1	-0.29
MOB4	MOB Family Member 4, Phocein	-0.33
LYZL6	Lysozyme-Like 6	-0.29
SQLE	Squalene Epoxidase	-0.29
CDKL4	Cyclin-Dependent Kinase-Like 4	-0.29
GEM	GTP Binding Protein Overexpressed In Skeletal Muscle	-0.29
RAB37	RAB37, Member RAS Oncogene Family	-0.29
CXCL3	Chemokine (C-X-C Motif) Ligand 3	-0.64
SP3	Sp3 Transcription Factor	-0.46
AC137056.1	Uncharacterized Protein; Cdna FLJ34659 Fis, Clone KIDNE2018863	-0.29
LAMP2	Lysosomal-Associated Membrane Protein 2	-0.29
GSTA2	Glutathione S-Transferase Alpha 2	-0.29
IL12B	Interleukin 12B (Natural Killer Cell Stimulatory Factor 2, Cytotoxic Lymphocyte Maturation Factor 2, P40)	-0.29
C10orf68	Chromosome 10 Open Reading Frame 68	-0.28

NDFIP1	Nedd4 Family Interacting Protein 1	-0.32
IARS2	Isoleucyl-Trna Synthetase 2, Mitochondrial	-0.31
TWIST1	Twist Basic Helix-Loop-Helix Transcription Factor 1	-0.62
RARG	Retinoic Acid Receptor, Gamma	-0.28
CR1	Complement Component (3b/4b) Receptor 1 (Knops Blood Group)	-0.28
DCLK1	Doublecortin-Like Kinase 1	-0.3
MRPL35	Mitochondrial Ribosomal Protein L35	-0.28
GDAP1	Ganglioside Induced Differentiation Associated Protein 1	-0.28
REXO1L1	REX1, RNA Exonuclease 1 Homolog (S. Cerevisiae)-Like 1	-0.28
GLS	Glutaminase	-0.28
AL161915.1	Uncharacterized Protein	-0.28
C19orf69	Chromosome 19 Open Reading Frame 69	-0.28
KLK12	Kallikrein-Related Peptidase 12	-0.28
CCDC179	Coiled-Coil Domain Containing 179	-0.28
ROR1	Receptor Tyrosine Kinase-Like Orphan Receptor 1	-0.28
FAM19A4	Family With Sequence Similarity 19 (Chemokine (C-C Motif)-Like), Member A4	-0.28
UBE2I	Ubiquitin-Conjugating Enzyme E2I	-0.28
HIST1H1D	Histone Cluster 1, H1d	-0.28
COX7B	Cytochrome C Oxidase Subunit Viib	-0.28
CYB5B	Cytochrome B5 Type B (Outer Mitochondrial Membrane)	-0.3
ARRDC4	Arrestin Domain Containing 4	-0.27
ASPN	Asporin	-0.27
OR8K1	Olfactory Receptor, Family 8, Subfamily K, Member 1	-0.27
FSBP	Fibrinogen Silencer Binding Protein	-0.34
CSTF2	Cleavage Stimulation Factor, 3' Pre-RNA, Subunit 2, 64kda	-0.28
POU5F1B	POU Class 5 Homeobox 1B	-0.27
ANKRD66	Ankyrin Repeat Domain 66	-0.27
RAB11FIP2	RAB11 Family Interacting Protein 2 (Class I)	-0.27
GLCCI1	Glucocorticoid Induced Transcript 1	-0.28
BANP	BTG3 Associated Nuclear Protein	-0.27
PPIL1	Peptidylprolyl Isomerase (Cyclophilin)-Like 1	-0.27
EZH2	Enhancer Of Zeste Homolog 2 (Drosophila)	-0.27
CCT3	Chaperonin Containing TCP1, Subunit 3 (Gamma)	-0.27
PLEKHS1	Pleckstrin Homology Domain Containing, Family S Member 1	-0.38
ZDHHC7	Zinc Finger, DHHC-Type Containing 7	-0.27
EOGT	EGF Domain-Specific O-Linked N-Acetylglucosamine (Glcnac) Transferase	-0.27
DSCR8	Down Syndrome Critical Region Gene 8	-0.27
RASSF5	Ras Association (Ralgds/AF-6) Domain Family Member 5	-0.33

VCP	Valosin Containing Protein	-0.27
PAX6	Paired Box 6	-0.26
TRIO	Trio Rho Guanine Nucleotide Exchange Factor	-0.26
CDRT4	CMT1A Duplicated Region Transcript 4	-0.26
AXDND1	Axonemal Dynein Light Chain Domain Containing 1	-0.26
LL22NC03-63E9.3	Uncharacterized Protein	-0.26
	Ac079602.1	-0.26
KHDC3L	KH Domain Containing 3-Like, Subcortical Maternal Complex Member	-0.26
TMEM218	Transmembrane Protein 218	-0.26
POMC	Proopiomelanocortin	-0.26
VMA21	VMA21 Vacuolar H+-Atpase Homolog (S. Cerevisiae)	-0.26
CDKN2AIP	CDKN2A Interacting Protein	-0.26
KRT13	Keratin 13	-0.26
DIAPH1	Diaphanous-Related Formin 1	-0.26
IFNE	Interferon, Epsilon	-0.32
KRTAP2-3	Keratin Associated Protein 2-3	-0.26
GKN1	Gastrokine 1	-0.26
RP11-676J12.7	Uncharacterized Protein	-0.26
RBM7	RNA Binding Motif Protein 7	-0.26
PPP1R12A	Protein Phosphatase 1, Regulatory Subunit 12A	-0.35
C2orf66	Chromosome 2 Open Reading Frame 66	-0.34
MAP7	Microtubule-Associated Protein 7	-0.29
FAM120AOS	Family With Sequence Similarity 120A Opposite Strand	-0.38
CENPK	Centromere Protein K	-0.26
FGF20	Fibroblast Growth Factor 20	-0.26
AL359195.1	Uncharacterized Protein; Cdna FLJ46261 Fis, Clone TESTI4025062	-0.26
OLIG3	Oligodendrocyte Transcription Factor 3	-0.26
ING3	Inhibitor Of Growth Family, Member 3	-0.26
HSPE1-MOB4	HSPE1-MOB4 Readthrough	-0.3
AC107021.1	HCG1786590; PRO2533; Uncharacterized Protein	-0.26
MRFAP1L1	Morf4 Family Associated Protein 1-Like 1	-0.26
C6orf195	Chromosome 6 Open Reading Frame 195	-0.25
RP11-65D24.2	HCG2045795; Uncharacterized Protein	-0.25
ECT2	Epithelial Cell Transforming Sequence 2 Oncogene	-0.28
BRF2	BRF2, RNA Polymerase III Transcription Initiation Factor 50 Kda Subunit	-0.27
HELLS	Helicase, Lymphoid-Specific	-0.31
SERP1	Stress-Associated Endoplasmic Reticulum Protein 1	-0.25
ZAR1	Zygote Arrest 1	-0.25
DCD	Dermcidin	-0.25
STARD4	Star-Related Lipid Transfer (START) Domain Containing 4	-0.35
MBLAC2	Metallo-Beta-Lactamase Domain Containing 2	-0.29

TPRG1L	Tumor Protein P63 Regulated 1-Like	-0.32
MRPS6	Mitochondrial Ribosomal Protein S6	-0.25
LCP1	Lymphocyte Cytosolic Protein 1 (L-Plastin)	-0.25
NAP1L6	Nucleosome Assembly Protein 1-Like 6	-0.25
DNAJC25	Dnaj (Hsp40) Homolog, Subfamily C , Member 25	-0.25
KRTAP6-3	Keratin Associated Protein 6-3	-0.25
DNAJC25-GNG10	DNAJC25-GNG10 Readthrough	-0.25
LHX8	LIM Homeobox 8	-0.3
EPT1	Ethanolaminephosphotransferase 1 (CDP-Ethanolamine-Specific)	-0.29
CMTM8	CKLF-Like MARVEL Transmembrane Domain Containing 8	-0.32
CAB39	Calcium Binding Protein 39	-0.44
RHBDL2	Rhomboid, Veinlet-Like 2 (Drosophila)	-0.25
CALM2	Calmodulin 2 (Phosphorylase Kinase, Delta)	-0.25
SPRED1	Sprouty-Related, EVH1 Domain Containing 1	-0.32
LRRC2	Leucine Rich Repeat Containing 2	-0.3
GPSM3	G-Protein Signaling Modulator 3	-0.25
CHODL	Chondrolectin	-0.28
CLEC1B	C-Type Lectin Domain Family 1, Member B	-0.24
EIF2D	Eukaryotic Translation Initiation Factor 2D	-0.24
C16orf80	Chromosome 16 Open Reading Frame 80	-0.24
TVP23C-CDRT4	TVP23C-CDRT4 Readthrough	-0.24
MZT1	Mitotic Spindle Organizing Protein 1	-0.38
RPP14	Ribonuclease P/MRP 14kda Subunit	-0.3
TMPO	Thymopoietin	-0.24
NRXN1	Neurexin 1	-0.24
PCDHB10	Protocadherin Beta 10	-0.24
BCL2	B-Cell CLL/Lymphoma 2	-0.24
CEP70	Centrosomal Protein 70kda	-0.27
HMGN4	High Mobility Group Nucleosomal Binding Domain 4	-0.24
ANKS1B	Ankyrin Repeat And Sterile Alpha Motif Domain Containing 1B	-0.24
PAX2	Paired Box 2	-0.24
COL17A1	Collagen, Type XVII, Alpha 1	-0.24
WWC2	WW And C2 Domain Containing 2	-0.28
PRTFDC1	Phosphoribosyl Transferase Domain Containing 1	-0.31
XIAP	X-Linked Inhibitor Of Apoptosis	-0.4
ZNF569	Zinc Finger Protein 569	-0.24
RPS6KB1	Ribosomal Protein S6 Kinase, 70kda, Polypeptide 1	-0.25
SF3B4	Splicing Factor 3b, Subunit 4, 49kda	-0.24
ESAM	Endothelial Cell Adhesion Molecule	-0.24
CCL28	Chemokine (C-C Motif) Ligand 28	-0.24
ARMC1	Armadillo Repeat Containing 1	-0.24
PEX10	Peroxisomal Biogenesis Factor 10	-0.27

SPPL2A	Signal Peptide Peptidase Like 2A	-0.32
TMEM257	Transmembrane Protein 257	-0.24
C20orf26	Chromosome 20 Open Reading Frame 26	-0.24
TDP2	Tyrosyl-DNA Phosphodiesterase 2	-0.24
CYYR1	Cysteine/Tyrosine-Rich 1	-0.24
HRG	Histidine-Rich Glycoprotein	-0.24
SPIDR	Scaffolding Protein Involved In DNA Repair	-0.24
ABCC1	ATP-Binding Cassette, Sub-Family C (CFTR/MRP), Member 1	-0.25
MSTN	Myostatin	-0.24
NXT2	Nuclear Transport Factor 2-Like Export Factor 2	-0.24
RAB3IP	RAB3A Interacting Protein	-0.56
LNP1	Leukemia NUP98 Fusion Partner 1	-0.3
SAMD11	Sterile Alpha Motif Domain Containing 11	-0.24
SLC25A32	Solute Carrier Family 25 (Mitochondrial Folate Carrier), Member 32	-0.31
NR4A2	Nuclear Receptor Subfamily 4, Group A, Member 2	-0.24
C9orf152	Chromosome 9 Open Reading Frame 152	-0.23
C11orf82	Chromosome 11 Open Reading Frame 82	-0.23
CXCL1	Chemokine (C-X-C Motif) Ligand 1 (Melanoma Growth Stimulating Activity, Alpha)	-0.23
ZNF28	Zinc Finger Protein 28	-0.23
NDNL2	Necdin-Like 2	-0.23
FHL5	Four And A Half LIM Domains 5	-0.37
TMTC4	Transmembrane And Tetratricopeptide Repeat Containing 4	-0.23
CXorf61	Chromosome X Open Reading Frame 61	-0.24
ITIH2	Inter-Alpha-Trypsin Inhibitor Heavy Chain 2	-0.3
DUSP6	Dual Specificity Phosphatase 6	-0.24
PDCD6	Programmed Cell Death 6	-0.23
PPP3CB	Protein Phosphatase 3, Catalytic Subunit, Beta Isozyme	-0.32
VEGFC	Vascular Endothelial Growth Factor C	-0.3
COPS2	COP9 Signalosome Subunit 2	-0.23
APOL4	Apolipoprotein L, 4	-0.23
ZNF440	Zinc Finger Protein 440	-0.23
KCNIP3	Kv Channel Interacting Protein 3, Calsenilin	-0.23
CASP7	Caspase 7, Apoptosis-Related Cysteine Peptidase	-0.23
METTL9	Methyltransferase Like 9	-0.26
TPD52	Tumor Protein D52	-0.38
CDKN1C	Cyclin-Dependent Kinase Inhibitor 1C (P57, Kip2)	-0.23
GIMAP2	Gtpase, IMAP Family Member 2	-0.23
MAF	V-Maf Avian Musculoaponeurotic Fibrosarcoma Oncogene Homolog	-0.24
RGS7BP	Regulator Of G-Protein Signaling 7 Binding Protein	-0.47
DUSP14	Dual Specificity Phosphatase 14	-0.23
CXCL2	Chemokine (C-X-C Motif) Ligand 2	-0.23
MAMDC2	MAM Domain Containing 2	-0.23

TUBD1	Tubulin, Delta 1	-0.22
CCDC113	Coiled-Coil Domain Containing 113	-0.22
PPAPDC1A	Phosphatidic Acid Phosphatase Type 2 Domain Containing 1A	-0.22
L2HGDH	L-2-Hydroxyglutarate Dehydrogenase	-0.22
HAPLN1	Hyaluronan And Proteoglycan Link Protein 1	-0.22
COCH	Cochlin	-0.37
AL136531.1	HCG2043693; Uncharacterized Protein	-0.22
TPST2	Tyrosylprotein Sulfotransferase 2	-0.24
WWOX	WW Domain Containing Oxidoreductase	-0.22
ISL1	ISL LIM Homeobox 1	-0.22
AC114783.1	Protein LOC339760	-0.22
ZNF80	Zinc Finger Protein 80	-0.22
SPIN2B	Spindlin Family, Member 2B	-0.22
C16orf72	Chromosome 16 Open Reading Frame 72	-0.3
TCTEX1D4	Tctex1 Domain Containing 4	-0.22
CYSLTR1	Cysteinyl Leukotriene Receptor 1	-0.22
NKAIN2	Na+/K+ Transporting Atpase Interacting 2	-0.22
DNAH10OS	Dynein, Axonemal, Heavy Chain 10 Opposite Strand	-0.22
TBPL1	TBP-Like 1	-0.22
SPOP	Speckle-Type POZ Protein	-0.22
EHF	Ets Homologous Factor	-0.22
ARPC5	Actin Related Protein 2/3 Complex, Subunit 5, 16kda	-0.26
MERTK	C-Mer Proto-Oncogene Tyrosine Kinase	-0.3
B3GNT2	UDP-Glcnac:Betagal Beta-1,3-N-Acetylglucosaminyltransferase 2	-0.22
LETM2	Leucine Zipper-EF-Hand Containing Transmembrane Protein 2	-0.22
PATE2	Prostate And Testis Expressed 2	-0.22
HSD17B4	Hydroxysteroid (17-Beta) Dehydrogenase 4	-0.22
PJA2	Praja Ring Finger 2, E3 Ubiquitin Protein Ligase	-0.23
UBE2U	Ubiquitin-Conjugating Enzyme E2U (Putative)	-0.22
POU3F1	POU Class 3 Homeobox 1	-0.22
FAM174A	Family With Sequence Similarity 174, Member A	-0.23
SPN	Sialophorin	-0.22
MAGED4	Melanoma Antigen Family D, 4	-0.22
EDN1	Endothelin 1	-0.22
GNAI3	Guanine Nucleotide Binding Protein (G Protein), Alpha Inhibiting Activity Polypeptide 3	-0.27
ACADSB	Acyl-Coa Dehydrogenase, Short/Branched Chain	-0.23
FAXDC2	Fatty Acid Hydroxylase Domain Containing 2	-0.22
ALG10	ALG10, Alpha-1,2-Glucosyltransferase	-0.22
TMOD2	Tropomodulin 2 (Neuronal)	-0.25
SLC33A1	Solute Carrier Family 33 (Acetyl-Coa Transporter), Member 1	-0.37
RAB39A	RAB39A, Member RAS Oncogene Family	-0.23
LMCD1	LIM And Cysteine-Rich Domains 1	-0.25

TTLL7	Tubulin Tyrosine Ligase-Like Family, Member 7	-0.25
PAIP1	Poly(A) Binding Protein Interacting Protein 1	-0.22
PHF20	PHD Finger Protein 20	-0.3
SEC63	SEC63 Homolog (S. Cerevisiae)	-0.36
DDX50	DEAD (Asp-Glu-Ala-Asp) Box Polypeptide 50	-0.41
SERPINI2	Serpin Peptidase Inhibitor, Clade I (Pancpin), Member 2	-0.21
RAB22A	RAB22A, Member RAS Oncogene Family	-0.31
RNFT1	Ring Finger Protein, Transmembrane 1	-0.21
UGT2A3	UDP Glucuronosyltransferase 2 Family, Polypeptide A3	-0.21
EHHADH	Enoyl-Coa, Hydratase/3-Hydroxyacyl Coa Dehydrogenase	-0.21
ACVR1	Activin A Receptor, Type I	-0.21
CALCB	Calcitonin-Related Polypeptide Beta	-0.21
LILRB4	Leukocyte Immunoglobulin-Like Receptor, Subfamily B (With TM And ITIM Domains), Member 4	-0.21
PDZD9	PDZ Domain Containing 9	-0.21
GRIK2	Glutamate Receptor, Ionotropic, Kainate 2	-0.21
RBMS3	RNA Binding Motif, Single Stranded Interacting Protein 3	-0.4
VPS26A	Vacuolar Protein Sorting 26 Homolog A (S. Pombe)	-0.21
IL36RN	Interleukin 36 Receptor Antagonist	-0.21
SPARCL1	SPARC-Like 1 (Hevin)	-0.21
CADM2	Cell Adhesion Molecule 2	-0.21
NUP62CL	Nucleoporin 62kda C-Terminal Like	-0.32
NAP1L2	Nucleosome Assembly Protein 1-Like 2	-0.21
FMO2	Flavin Containing Monooxygenase 2 (Non-Functional)	-0.21
PTP4A1	Protein Tyrosine Phosphatase Type IVA, Member 1	-0.21
DCAF13	DDB1 And CUL4 Associated Factor 13	-0.23
LIN54	Lin-54 Homolog (C. Elegans)	-0.22
CLRN1	Clarin 1	-0.21
CAV2	Caveolin 2	-0.23
HMGA2	High Mobility Group AT-Hook 2	-0.21
CARS	Cysteinyl-Trna Synthetase	-0.21
TMEM138	Transmembrane Protein 138	-0.21
PRDM5	PR Domain Containing 5	-0.21
GTF2H2	General Transcription Factor IIH, Polypeptide 2, 44kda	-0.21
LEPROT	Leptin Receptor Overlapping Transcript	-0.26
PTH	Parathyroid Hormone	-0.21
RASSF4	Ras Association (Ralgds/AF-6) Domain Family Member 4	-0.31
F13A1	Coagulation Factor XIII, A1 Polypeptide	-0.2
CHM	Choroideremia (Rab Escort Protein 1)	-0.2
SKI	V-Ski Avian Sarcoma Viral Oncogene Homolog	-0.2
ZFP69B	ZFP69 Zinc Finger Protein B	-0.21
PTER	Phosphotriesterase Related	-0.24
ONECUT1	One Cut Homeobox 1	-0.47
GTPBP3	GTP Binding Protein 3 (Mitochondrial)	-0.2

INSL5	Insulin-Like 5	-0.2
CD53	CD53 Molecule	-0.2
MGST2	Microsomal Glutathione S-Transferase 2	-0.2
FAM185A	Family With Sequence Similarity 185, Member A	-0.2
DNAJB9	Dnaj (Hsp40) Homolog, Subfamily B, Member 9	-0.2
IRAK2	Interleukin-1 Receptor-Associated Kinase 2	-0.22
C19orf57	Chromosome 19 Open Reading Frame 57	-0.2
SERPINA5	Serpin Peptidase Inhibitor, Clade A (Alpha-1 Antiproteinase, Antitrypsin), Member 5	-0.2
PCSK4	Proprotein Convertase Subtilisin/Kexin Type 4	-0.2
SLC34A3	Solute Carrier Family 34 (Type II Sodium/Phosphate Contransporter), Member 3	-0.2
PFDN1	Prefoldin Subunit 1	-0.21
MBP	Myelin Basic Protein	-0.22
PARK2	Parkin RBR E3 Ubiquitin Protein Ligase	-0.2
TMEM255A	Transmembrane Protein 255A	-0.2
TRMT13	Trna Methyltransferase 13 Homolog (S. Cerevisiae)	-0.21
CBLB	Cbl Proto-Oncogene B, E3 Ubiquitin Protein Ligase	-0.35
SMAD4	SMAD Family Member 4	-0.31
ALOX5AP	Arachidonate 5-Lipoxygenase-Activating Protein	-0.2
HOXC4	Homeobox Protein Hox-C4	-0.2
FAM57A	Family With Sequence Similarity 57, Member A	-0.26
CASC4	Cancer Susceptibility Candidate 4	-0.2
CYTIP	Cytohesin 1 Interacting Protein	-0.22
ACKR4	Atypical Chemokine Receptor 4	-0.2
BRI3BP	BRI3 Binding Protein	-0.24
CRISP2	Cysteine-Rich Secretory Protein 2	-0.2
TNFSF11	Tumor Necrosis Factor (Ligand) Superfamily, Member 11	-0.2
C15orf26	Chromosome 15 Open Reading Frame 26	-0.2
IMPG1	Interphotoreceptor Matrix Proteoglycan 1	-0.2
TCTN3	Tectonic Family Member 3	-0.23
CD46	CD46 Molecule, Complement Regulatory Protein	-0.2
AK7	Adenylate Kinase 7	-0.2
HNRNPK	Heterogeneous Nuclear Ribonucleoprotein K	-0.22
CLEC19A	C-Type Lectin Domain Family 19, Member A	-0.2
CXorf28	Chromosome X Open Reading Frame 28	-0.2
ADO	2-Aminoethanethiol (Cysteamine) Dioxygenase	-0.2
RRM1	Ribonucleotide Reductase M1	-0.2
KLF12	Kruppel-Like Factor 12	-0.22
CNIH1	Cornichon Family AMPA Receptor Auxiliary Protein 1	-0.21
KLF13	Kruppel-Like Factor 13	-0.2
ANKRA2	Ankyrin Repeat, Family A (RFXANK-Like), 2	-0.2
IRF2BPL	Interferon Regulatory Factor 2 Binding Protein-Like	-0.2
IKZF2	IKAROS Family Zinc Finger 2 (Helios)	-0.22
EBNA1BP2	EBNA1 Binding Protein 2	-0.2

ZFP2	ZFP2 Zinc Finger Protein	-0.2
EVI5	Ecotropic Viral Integration Site 5	-0.22
RAB3D	RAB3D, Member RAS Oncogene Family	-0.2
LYVE1	Lymphatic Vessel Endothelial Hyaluronan Receptor 1	-0.26
MAT2B	Methionine Adenosyltransferase II, Beta	-0.2
ERAS	ES Cell Expressed Ras	-0.2
KRTAP1-1	Keratin Associated Protein 1-1	-0.2
IFT43	Intraflagellar Transport 43 Homolog (Chlamydomonas)	-0.2
OVCH1-AS1	OVCH1 Antisense RNA 1	-0.2
LOH12CR1	Loss Of Heterozygosity, 12, Chromosomal Region 1	-0.2
MAGED4B	Melanoma Antigen Family D, 4B	-0.2
JAM2	Junctional Adhesion Molecule 2	-0.26
PNRC1	Proline-Rich Nuclear Receptor Coactivator 1	-0.19
AC093157.1	Uncharacterized Protein	-0.19
DKK2	Dickkopf WNT Signaling Pathway Inhibitor 2	-0.19
NR5A2	Nuclear Receptor Subfamily 5, Group A, Member 2	-0.2
MRO	Maestro	-0.26
DERL2	Derlin 2	-0.19
GJB7	Gap Junction Protein, Beta 7, 25kda	-0.19
ZNF367	Zinc Finger Protein 367	-0.19
TNFSF13B	Tumor Necrosis Factor (Ligand) Superfamily, Member 13b	-0.19
HIST1H2BF	Histone Cluster 1, H2bf	-0.19
ZDHHC17	Zinc Finger, DHHC-Type Containing 17	-0.24
NECAP2	NECAP Endocytosis Associated 2	-0.2
ZNF33B	Zinc Finger Protein 33B	-0.21
FERMT1	Fermitin Family Member 1	-0.2
ZNF286B	Zinc Finger Protein 286B	-0.19
SLC47A2	Solute Carrier Family 47 (Multidrug And Toxin Extrusion), Member 2	-0.19
RP1	Retinitis Pigmentosa 1 (Autosomal Dominant)	-0.19
LRRC57	Leucine Rich Repeat Containing 57	-0.19
IGFBP1	Insulin-Like Growth Factor Binding Protein 1	-0.19
MUC7	Mucin 7, Secreted	-0.19
PAK3	P21 Protein (Cdc42/Rac)-Activated Kinase 3	-0.26
MS4A13	Membrane-Spanning 4-Domains, Subfamily A, Member 13	-0.19
HUS1B	HUS1 Checkpoint Homolog B (S. Pombe)	-0.19
LYPLA1	Lysophospholipase I	-0.21
TRAPPC13	Trafficking Protein Particle Complex 13	-0.24
NUTF2	Nuclear Transport Factor 2	-0.2
GREM2	Gremlin 2, DAN Family BMP Antagonist	-0.22
RIMKLB	Ribosomal Modification Protein Rimk-Like Family Member B	-0.19
TCF7L2	Transcription Factor 7-Like 2 (T-Cell Specific, HMG-Box)	-0.19
OOEP	Oocyte Expressed Protein	-0.19

VIP	Vasoactive Intestinal Peptide	-0.19
ORC6	Origin Recognition Complex, Subunit 6	-0.19
CST3	Cystatin C	-0.19
RGS5	Regulator Of G-Protein Signaling 5	-0.33
TRMT12	Trna Methyltransferase 12 Homolog (S. Cerevisiae)	-0.19
KCTD9	Potassium Channel Tetramerization Domain Containing 9	-0.19
ZIK1	Zinc Finger Protein Interacting With K Protein 1	-0.19
STK31	Serine/Threonine Kinase 31	-0.19
USP9Y	Ubiquitin Specific Peptidase 9, Y-Linked	-0.19
AL136115.1	HCG2032337; PRO1848; Uncharacterized Protein	-0.19
FUT9	Fucosyltransferase 9 (Alpha (1,3) Fucosyltransferase)	-0.19
PRDM4	PR Domain Containing 4	-0.19
PLSCR4	Phospholipid Scramblase 4	-0.19
PITPNB	Phosphatidylinositol Transfer Protein, Beta	-0.2
EBF1	Early B-Cell Factor 1	-0.28
TMEM170A	Transmembrane Protein 170A	-0.3
CCL13	Chemokine (C-C Motif) Ligand 13	-0.19
CCNJ	Cyclin J	-0.19
PTGER3	Prostaglandin E Receptor 3 (Subtype EP3)	-0.19
SHROOM1	Shroom Family Member 1	-0.22
USO1	USO1 Vesicle Transport Factor	-0.19
NXF3	Nuclear RNA Export Factor 3	-0.19
NENF	Neudesin Neurotrophic Factor	-0.33
FAM189A2	Family With Sequence Similarity 189, Member A2	-0.19
ZBTB5	Zinc Finger And BTB Domain Containing 5	-0.19
GPR37	G Protein-Coupled Receptor 37 (Endothelin Receptor Type B-Like)	-0.2
ALG10B	ALG10B, Alpha-1,2-Glucosyltransferase	-0.19
RNF125	Ring Finger Protein 125, E3 Ubiquitin Protein Ligase	-0.18
AP001652.1	Uncharacterized Protein; Cdna FLJ60524	-0.18
MTFR1	Mitochondrial Fission Regulator 1	-0.18
NSG1	Neuron-Specific Protein Family Member 1	-0.26
CUL5	Cullin 5	-0.2
HSPA13	Heat Shock Protein 70kda Family, Member 13	-0.37
TMEM154	Transmembrane Protein 154	-0.18
AAED1	Ahpc/TSA Antioxidant Enzyme Domain Containing 1	-0.18
TRIM58	Tripartite Motif Containing 58	-0.2
MROH8	Maestro Heat-Like Repeat Family Member 8	-0.18
LUZP6	Leucine Zipper Protein 6	-0.18
PMS2	PMS2 Postmeiotic Segregation Increased 2 (S. Cerevisiae)	-0.18
THSD7A	Thrombospondin, Type I, Domain Containing 7A	-0.18
IGF1	Insulin-Like Growth Factor 1 (Somatomedin C)	-0.41
PURA	Purine-Rich Element Binding Protein A	-0.23
TAL2	T-Cell Acute Lymphocytic Leukemia 2	-0.24

NMNAT2	Nicotinamide Nucleotide Adenylyltransferase 2	-0.18
UNG	Uracil-DNA Glycosylase	-0.18
TMEM144	Transmembrane Protein 144	-0.37
PPA1	Pyrophosphatase (Inorganic) 1	-0.18
ZNF347	Zinc Finger Protein 347	-0.18
BTG3	BTG Family, Member 3	-0.18
PBX3	Pre-B-Cell Leukemia Homeobox 3	-0.19
BCL6	B-Cell CLL/Lymphoma 6	-0.18
MAPK6	Mitogen-Activated Protein Kinase 6	-0.18
ARL5A	ADP-Ribosylation Factor-Like 5A	-0.19
HADHB	Hydroxyacyl-Coa Dehydrogenase/3-Ketoacyl-Coa Thiolase/Enoyl-Coa Hydratase (Trifunctional Protein), Beta Subunit	-0.26
PAXIP1-AS2	PAXIP1 Antisense RNA 2	-0.18
CLEC3A	C-Type Lectin Domain Family 3, Member A	-0.18
GNA13	Guanine Nucleotide Binding Protein (G Protein), Alpha 13	-0.24
HTR7	5-Hydroxytryptamine (Serotonin) Receptor 7, Adenylate Cyclase-Coupled	-0.18
AMPH	Amphiphysin	-0.18
RP11-650K20.3	Uncharacterized Protein	-0.18
USP33	Ubiquitin Specific Peptidase 33	-0.18
USP50	Ubiquitin Specific Peptidase 50	-0.18
NANP	N-Acetylneuraminic Acid Phosphatase	-0.21
AZI2	5-Azacytidine Induced 2	-0.23
HIST1H2BD	Histone Cluster 1, H2bd	-0.24
ATP6V1D	Atpase, H+ Transporting, Lysosomal 34kda, V1 Subunit D	-0.2
TNFSF18	Tumor Necrosis Factor (Ligand) Superfamily, Member 18	-0.18
TTC6	Tetratricopeptide Repeat Domain 6	-0.18
ZNF365	Zinc Finger Protein 365	-0.18
PWP1	PWP1 Homolog (S. Cerevisiae)	-0.22
WWP1	WW Domain Containing E3 Ubiquitin Protein Ligase 1	-0.18
CDC5L	Cell Division Cycle 5-Like	-0.18
KIAA2022	Kiaa2022	-0.18
SHISA2	Shisa Family Member 2	-0.18
PXDNL	Peroxidasin Homolog (Drosophila)-Like	-0.18
IGSF10	Immunoglobulin Superfamily, Member 10	-0.28
HLCS	Holocarboxylase Synthetase (Biotin-(Proprionyl-Coa-Carboxylase (ATP-Hydrolysing)) Ligase)	-0.22
SOWAHC	Sosondowah Ankyrin Repeat Domain Family Member C	-0.19
CCDC147	Coiled-Coil Domain Containing 147	-0.18
VAMP1	Vesicle-Associated Membrane Protein 1 (Synaptobrevin 1)	-0.18
GAR1	GAR1 Ribonucleoprotein	-0.18

UNCX	UNC Homeobox	-0.18
RP11-122A3.2	Uncharacterized Protein LOC100127983	-0.57
PPP6R1	Protein Phosphatase 6, Regulatory Subunit 1	-0.21
ZBTB34	Zinc Finger And BTB Domain Containing 34	-0.18
MSR1	Macrophage Scavenger Receptor 1	-0.18
ST6GALNAC 5	ST6 (Alpha-N-Acetyl-Neuraminyl-2,3-Beta-Galactosyl-1,3)-N-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 5	-0.18
CHMP4B	Charged Multivesicular Body Protein 4B	-0.19
SMIM19	Small Integral Membrane Protein 19	-0.38
FMN2	Formin 2	-0.28
TMEM169	Transmembrane Protein 169	-0.17
MICU3	Mitochondrial Calcium Uptake Family, Member 3	-0.17
PSMA8	Proteasome (Prosome, Macropain) Subunit, Alpha Type, 8	-0.17
GABRB2	Gamma-Aminobutyric Acid (GABA) A Receptor, Beta 2	-0.17
GPR135	G Protein-Coupled Receptor 135	-0.25
GALNT7	UDP-N-Acetyl-Alpha-D-Galactosamine:Polypeptide N-Acetylgalactosaminyltransferase 7 (Galnac-T7)	-0.18
CA8	Carbonic Anhydrase VIII	-0.28
GCNT1	Glucosaminyl (N-Acetyl) Transferase 1, Core 2	-0.27
MFF	Mitochondrial Fission Factor	-0.17
ATP6V0E1	Atpase, H+ Transporting, Lysosomal 9kda, V0 Subunit E1	-0.22
DNASE2	Deoxyribonuclease II, Lysosomal	-0.19
	Ac008964.1	-0.17
SLC35F5	Solute Carrier Family 35, Member F5	-0.25
ZBTB37	Zinc Finger And BTB Domain Containing 37	-0.19
PCDHB14	Protocadherin Beta 14	-0.17
FGFR1OP2	FGFR1 Oncogene Partner 2	-0.2
TMEM75	Transmembrane Protein 75	-0.17
UNC80	Unc-80 Homolog (C. Elegans)	-0.17
PLIN1	Perilipin 1	-0.17
GATC	Glutamyl-Trna(Gln) Amidotransferase, Subunit C	-0.26
PYGO1	Pygopus Homolog 1 (Drosophila)	-0.17
SOX9	SRY (Sex Determining Region Y)-Box 9	-0.18
C14orf28	Chromosome 14 Open Reading Frame 28	-0.21
PPP1CC	Protein Phosphatase 1, Catalytic Subunit, Gamma Isozyme	-0.23
DMRTC1	DMRT-Like Family C1	-0.17
HNRNPA3	Heterogeneous Nuclear Ribonucleoprotein A3	-0.17
GRB10	Growth Factor Receptor-Bound Protein 10	-0.17
HERC3	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 3	-0.17
TMEM132C	Transmembrane Protein 132C	-0.17
FGF7	Fibroblast Growth Factor 7	-0.17
ZNF235	Zinc Finger Protein 235	-0.17

LRRC23	Leucine Rich Repeat Containing 23	-0.17
C18orf63	Chromosome 18 Open Reading Frame 63	-0.17
KRT40	Keratin 40	-0.17
PTPRR	Protein Tyrosine Phosphatase, Receptor Type, R	-0.17
CDC42EP3	CDC42 Effector Protein (Rho Gtpase Binding) 3	-0.2
ZNF415	Zinc Finger Protein 415	-0.18
SMCHD1	Structural Maintenance Of Chromosomes Flexible Hinge Domain Containing 1	-0.2
GIMAP4	Gtpase, IMAP Family Member 4	-0.2
SHB	Src Homology 2 Domain Containing Adaptor Protein B	-0.17
TMEM74	Transmembrane Protein 74	-0.17
CTSE	Cathepsin E	-0.21
SMIM14	Small Integral Membrane Protein 14	-0.19
SEMA3D	Sema Domain, Immunoglobulin Domain (Ig), Short Basic Domain, Secreted, (Semaphorin) 3D	-0.18
CXorf57	Chromosome X Open Reading Frame 57	-0.17
KCNJ11	Potassium Inwardly-Rectifying Channel, Subfamily J, Member 11	-0.17
MEMO1	Mediator Of Cell Motility 1	-0.17
RPL22	Ribosomal Protein L22	-0.17
RGN	Regucalcin	-0.17
MDGA2	MAM Domain-Containing Glycosylphosphatidylinositol Anchor Protein 2	-0.17
EIF2S2	Eukaryotic Translation Initiation Factor 2, Subunit 2 Beta, 38kda	-0.17
HIST1H4I	Histone Cluster 1, H4i	-0.17
TFAM	Transcription Factor A, Mitochondrial	-0.27
KBTBD7	Kelch Repeat And BTB (POZ) Domain Containing 7	-0.17
C1orf52	Chromosome 1 Open Reading Frame 52	-0.17
TBC1D31	TBC1 Domain Family, Member 31	-0.17
FAM21D	Family With Sequence Similarity 21, Member D	-0.17
UBL3	Ubiquitin-Like 3	-0.37
PMEPA1	Prostate Transmembrane Protein, Androgen Induced 1	-0.19
NLN	Neurolysin (Metallopeptidase M3 Family)	-0.19
GLRA3	Glycine Receptor, Alpha 3	-0.17
CCR1	Chemokine (C-C Motif) Receptor 1	-0.17
IQSEC1	IQ Motif And Sec7 Domain 1	-0.17
RANBP9	RAN Binding Protein 9	-0.17
SSR3	Signal Sequence Receptor, Gamma (Translocon-Associated Protein Gamma)	-0.2
ZDHHC2	Zinc Finger, DHHC-Type Containing 2	-0.22
SAMD5	Sterile Alpha Motif Domain Containing 5	-0.17
C9orf57	Chromosome 9 Open Reading Frame 57	-0.16
ITGB1	Integrin, Beta 1 (Fibronectin Receptor, Beta Polypeptide, Antigen CD29 Includes MDF2, MSK12)	-0.16
FUBP3	Far Upstream Element (FUSE) Binding Protein 3	-0.16
ADCY10	Adenylate Cyclase 10 (Soluble)	-0.16

LGI1	Leucine-Rich, Glioma Inactivated 1	-0.16
AKIRIN1	Akirin 1	-0.16
BCL7A	B-Cell CLL/Lymphoma 7A	-0.19
NUDT5	Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 5	-0.17
MARCH5	Membrane-Associated Ring Finger (C3HC4) 5	-0.34
LIPA	Lipase A, Lysosomal Acid, Cholesterol Esterase	-0.16
SOST	Sclerostin	-0.16
EIF4G2	Eukaryotic Translation Initiation Factor 4 Gamma, 2	-0.16
OGN	Osteoglycin	-0.16
TMEM17	Transmembrane Protein 17	-0.16
AC007461.1	Uncharacterized Protein	-0.61
CLINT1	Clathrin Interactor 1	-0.17
GABBR2	Gamma-Aminobutyric Acid (GABA) B Receptor, 2	-0.16
KLF7	Kruppel-Like Factor 7 (Ubiquitous)	-0.18
H3F3B	H3 Histone, Family 3B (H3.3B)	-0.2
CREBL2	Camp Responsive Element Binding Protein-Like 2	-0.35
ZNF7	Zinc Finger Protein 7	-0.2
POLR2D	Polymerase (RNA) II (DNA Directed) Polypeptide D	-0.16
MED17	Mediator Complex Subunit 17	-0.28
PIGY	Phosphatidylinositol Glycan Anchor Biosynthesis, Class Y	-0.16
TMEM123	Transmembrane Protein 123	-0.17
USP47	Ubiquitin Specific Peptidase 47	-0.26
RSPO2	R-Spondin 2	-0.16
VSTM2A	V-Set And Transmembrane Domain Containing 2A	-0.16
SRSF2	Serine/Arginine-Rich Splicing Factor 2	-0.16
HIST1H3G	Histone Cluster 1, H3g	-0.29
SLC6A15	Solute Carrier Family 6 (Neutral Amino Acid Transporter), Member 15	-0.17
TMEM232	Transmembrane Protein 232	-0.16
AOX1	Aldehyde Oxidase 1	-0.18
SLC12A2	Solute Carrier Family 12 (Sodium/Potassium/Chloride Transporter), Member 2	-0.17
NTM	Neurotrimin	-0.19
TUBA4A	Tubulin, Alpha 4a	-0.33
CTSA	Cathepsin A	-0.16
SH3GL3	SH3-Domain GRB2-Like 3	-0.16
LAIR1	Leukocyte-Associated Immunoglobulin-Like Receptor 1	-0.16
ADRB1	Adrenoceptor Beta 1	-0.16
IL23R	Interleukin 23 Receptor	-0.16
KRTAP9-2	Keratin Associated Protein 9-2	-0.16
CCNE2	Cyclin E2	-0.17
SLC30A4	Solute Carrier Family 30 (Zinc Transporter), Member 4	-0.2
AC012215.1	Uncharacterized Protein	-0.17
C10orf82	Chromosome 10 Open Reading Frame 82	-0.16
MATN2	Matrilin 2	-0.16

ZC3HC1	Zinc Finger, C3HC-Type Containing 1	-0.16
GRP	Gastrin-Releasing Peptide	-0.16
TLX1NB	TLX1 Neighbor	-0.16
GLRB	Glycine Receptor, Beta	-0.17
NFKBIA	Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-Cells Inhibitor, Alpha	-0.16
G3BP2	Gtpase Activating Protein (SH3 Domain) Binding Protein 2	-0.27
MRPS34	Mitochondrial Ribosomal Protein S34	-0.16
RP11-796G6.2	Uncharacterized Protein	-0.16
PLP1	Proteolipid Protein 1	-0.16
SRRM1	Serine/Arginine Repetitive Matrix 1	-0.16
ZCCHC16	Zinc Finger, CCHC Domain Containing 16	-0.16
MAGEB18	Melanoma Antigen Family B, 18	-0.16
PAPOLA	Poly(A) Polymerase Alpha	-0.2
NEDD1	Neural Precursor Cell Expressed, Developmentally Down-Regulated 1	-0.16
GPM6A	Glycoprotein M6A	-0.17
UPF3B	UPF3 Regulator Of Nonsense Transcripts Homolog B (Yeast)	-0.21
GPC3	Glypican 3	-0.16
MYH3	Myosin, Heavy Chain 3, Skeletal Muscle, Embryonic	-0.16
KCNH7	Potassium Voltage-Gated Channel, Subfamily H (Eag-Related), Member 7	-0.16
EPB42	Erythrocyte Membrane Protein Band 4.2	-0.16
AL021546.6	Glutamyl-Trna(Gln) Amidotransferase Subunit C, Mitochondrial	-0.23
ZBTB10	Zinc Finger And BTB Domain Containing 10	-0.22
GAS1	Growth Arrest-Specific 1	-0.15
ELOVL6	ELOVL Fatty Acid Elongase 6	-0.18
SLC38A4	Solute Carrier Family 38, Member 4	-0.15
UBQLN1	Ubiquilin 1	-0.17
MCF2	MCF.2 Cell Line Derived Transforming Sequence	-0.15
DMRTC1B	DMRT-Like Family C1B	-0.15
THAP9	THAP Domain Containing 9	-0.19
RASSF6	Ras Association (Ralgds/AF-6) Domain Family Member 6	-0.2
PLCB1	Phospholipase C, Beta 1 (Phosphoinositide-Specific)	-0.15
KRTAP17-1	Keratin Associated Protein 17-1	-0.15
C5AR1	Complement Component 5a Receptor 1	-0.15
GRIA4	Glutamate Receptor, Ionotropic, AMPA 4	-0.15
DLST	Dihydrolipoamide S-Succinyltransferase (E2 Component Of 2-Oxo-Glutarate Complex)	-0.15
CTBP2	C-Terminal Binding Protein 2	-0.24
ABHD17B	Abhydrolase Domain Containing 17B	-0.15
KDM1B	Lysine (K)-Specific Demethylase 1B	-0.15
C20orf85	Chromosome 20 Open Reading Frame 85	-0.15

AKAP10	A Kinase (PRKA) Anchor Protein 10	-0.15
ABHD3	Abhydrolase Domain Containing 3	-0.15
XPNPEP1	X-Prolyl Aminopeptidase (Aminopeptidase P) 1, Soluble	-0.15
ZBTB46	Zinc Finger And BTB Domain Containing 46	-0.15
IL1F10	Interleukin 1 Family, Member 10 (Theta)	-0.15
HSPA1A	Heat Shock 70kda Protein 1A	-0.15
PLEKHA8	Pleckstrin Homology Domain Containing, Family A (Phosphoinositide Binding Specific) Member 8	-0.16
FAM168A	Family With Sequence Similarity 168, Member A	-0.22
ZNF597	Zinc Finger Protein 597	-0.2
JMY	Junction Mediating And Regulatory Protein, P53 Cofactor	-0.21
LEPROTL1	Leptin Receptor Overlapping Transcript-Like 1	-0.55
SLC5A12	Solute Carrier Family 5 (Sodium/Monocarboxylate Cotransporter), Member 12	-0.38
DUSP5	Dual Specificity Phosphatase 5	-0.16
TLDC2	TBC/Lysm-Associated Domain Containing 2	-0.15
RP1-228P16.5	Uncharacterized Protein	-0.15
CXXC5	CXXC Finger Protein 5	-0.25
HSP90AB1	Heat Shock Protein 90kda Alpha (Cytosolic), Class B Member 1	-0.15
SLC35D2	Solute Carrier Family 35 (UDP-Glcnac/UDP-Glucose Transporter), Member D2	-0.23
MAN1A1	Mannosidase, Alpha, Class 1A, Member 1	-0.19
APOD	Apolipoprotein D	-0.15
GTF2H2C	General Transcription Factor IIH, Polypeptide 2C	-0.15
RALB	V-Ral Simian Leukemia Viral Oncogene Homolog B	-0.15
TRIQK	Triple Qxxk/R Motif Containing	-0.24
FUT11	Fucosyltransferase 11 (Alpha (1,3) Fucosyltransferase)	-0.18
GOLPH3L	Golgi Phosphoprotein 3-Like	-0.24
TNNI1	Troponin I Type 1 (Skeletal, Slow)	-0.15
SRP54	Signal Recognition Particle 54kda	-0.15
TNRC6B	Trinucleotide Repeat Containing 6B	-0.2
ST6GAL2	ST6 Beta-Galactosamide Alpha-2,6-Sialyltranferase 2	-0.15
RRAGA	Ras-Related GTP Binding A	-0.15
ZNF525	Zinc Finger Protein 525	-0.15
LHFPL3	Lipoma HMGIC Fusion Partner-Like 3	-0.15
KLHL38	Kelch-Like Family Member 38	-0.25
GADD45B	Growth Arrest And DNA-Damage-Inducible, Beta	-0.15
RAB33B	RAB33B, Member RAS Oncogene Family	-0.15
PDE4B	Phosphodiesterase 4B, Camp-Specific	-0.16
CS	Citrate Synthase	-0.15
XRCC4	X-Ray Repair Complementing Defective Repair In Chinese Hamster Cells 4	-0.15
GLRX	Glutaredoxin (Thioltransferase)	-0.15
HMGN3	High Mobility Group Nucleosomal Binding Domain 3	-0.32

UXS1	UDP-Glucuronate Decarboxylase 1	-0.16
ACER3	Alkaline Ceramidase 3	-0.17
TPP2	Tripeptidyl Peptidase II	-0.3
DCP1A	Decapping Mrna 1A	-0.17
CFL2	Cofilin 2 (Muscle)	-0.73
CSNK2A2	Casein Kinase 2, Alpha Prime Polypeptide	-0.15
ANXA13	Annexin A13	-0.15
KL	Klotho	-0.15
LONRF1	LON Peptidase N-Terminal Domain And Ring Finger 1	-0.15
ATP2C1	Atpase, Ca++ Transporting, Type 2C, Member 1	-0.15
STOML3	Stomatin (EPB72)-Like 3	-0.15
ATF3	Activating Transcription Factor 3	-0.15
C2orf74	Chromosome 2 Open Reading Frame 74	-0.19
EFR3A	EFR3 Homolog A (S. Cerevisiae)	-0.15
C21orf59	Chromosome 21 Open Reading Frame 59	-0.28
CYP7B1	Cytochrome P450, Family 7, Subfamily B, Polypeptide 1	-0.16
C1QTNF3	C1q And Tumor Necrosis Factor Related Protein 3	-0.15
COIL	Coilin	-0.15
NRBF2	Nuclear Receptor Binding Factor 2	-0.15
	Dkfzp779l1853	-0.15
RAB10	RAB10, Member RAS Oncogene Family	-0.18
ZNF382	Zinc Finger Protein 382	-0.19
MYBL1	V-Myb Avian Myeloblastosis Viral Oncogene Homolog-Like 1	-0.15
KLHL15	Kelch-Like Family Member 15	-0.15
HGF	Hepatocyte Growth Factor (Hepapoietin A; Scatter Factor)	-0.15
EPC2	Enhancer Of Polycomb Homolog 2 (Drosophila)	-0.18
GLE1	GLE1 RNA Export Mediator	-0.2
NELL2	NEL-Like 2 (Chicken)	-0.14
PEX12	Peroxisomal Biogenesis Factor 12	-0.14
ZNF658	Zinc Finger Protein 658	-0.14
EPB41L4B	Erythrocyte Membrane Protein Band 4.1 Like 4B	-0.22
FILIP1	Filamin A Interacting Protein 1	-0.19
CCR5	Chemokine (C-C Motif) Receptor 5 (Gene/Pseudogene)	-0.14
CTCF	CCCTC-Binding Factor (Zinc Finger Protein)	-0.14
C8orf58	Chromosome 8 Open Reading Frame 58	-0.14
DTX4	Deltex Homolog 4 (Drosophila)	-0.14
LEF1	Lymphoid Enhancer-Binding Factor 1	-0.14
SLC25A25	Solute Carrier Family 25 (Mitochondrial Carrier; Phosphate Carrier), Member 25	-0.14
MBNL3	Muscleblind-Like Splicing Regulator 3	-0.2
CAPRIN1	Cell Cycle Associated Protein 1	-0.19
PLEKHA7	Pleckstrin Homology Domain Containing, Family A Member 7	-0.17
KPNA5	Karyopherin Alpha 5 (Importin Alpha 6)	-0.14

TET2	Tet Methylcytosine Dioxygenase 2	-0.14
LHX1	LIM Homeobox 1	-0.14
TMEM47	Transmembrane Protein 47	-0.18
FAM115A	Family With Sequence Similarity 115, Member A	-0.14
EMX2	Empty Spiracles Homeobox 2	-0.16
ZFP36L1	ZFP36 Ring Finger Protein-Like 1	-0.2
DSE	Dermatan Sulfate Epimerase	-0.14
SELE	Selectin E	-0.14
INHBA	Inhibin, Beta A	-0.16
CYS1	Cystin 1	-0.16
ARID4A	AT Rich Interactive Domain 4A (RBP1-Like)	-0.16
PPP3CA	Protein Phosphatase 3, Catalytic Subunit, Alpha Isozyme	-0.14
NCOA2	Nuclear Receptor Coactivator 2	-0.14
EMR3	Egf-Like Module Containing, Mucin-Like, Hormone Receptor-Like 3	-0.14
C9	Complement Component 9	-0.14
INSL3	Insulin-Like 3 (Leydig Cell)	-0.14
FAM89B	Family With Sequence Similarity 89, Member B	-0.14
ZNF286A	Zinc Finger Protein 286A	-0.14
AL031663.2	CDNA FLJ26875 Fis, Clone PRS08969; Uncharacterized Protein	-0.14
AP3M1	Adaptor-Related Protein Complex 3, Mu 1 Subunit	-0.17
FITM2	Fat Storage-Inducing Transmembrane Protein 2	-0.19
ATP13A5	Atpase Type 13A5	-0.14
SLC35E2B	Solute Carrier Family 35, Member E2B	-0.14
CLEC1A	C-Type Lectin Domain Family 1, Member A	-0.14
UNK	Unkempt Homolog (Drosophila)	-0.14
PIK3R1	Phosphoinositide-3-Kinase, Regulatory Subunit 1 (Alpha)	-0.14
LMBR1	Limb Development Membrane Protein 1	-0.22
VPS53	Vacuolar Protein Sorting 53 Homolog (S. Cerevisiae)	-0.16
KRT33B	Keratin 33B	-0.14
RD3L	Retinal Degeneration 3-Like	-0.14
KERA	Keratocan	-0.14
ACRC	Acidic Repeat Containing	-0.14
UGT3A2	UDP Glycosyltransferase 3 Family, Polypeptide A2	-0.14
KCNA3	Potassium Voltage-Gated Channel, Shaker-Related Subfamily, Member 3	-0.14
SLC25A21-AS1	SLC25A21 Antisense RNA 1	-0.16
YIPF5	Yip1 Domain Family, Member 5	-0.28
C19orf40	Chromosome 19 Open Reading Frame 40	-0.23
CELF1	CUGBP, Elav-Like Family Member 1	-0.17
ST6GALNAC3	ST6 (Alpha-N-Acetyl-Neuraminyl-2,3-Beta-Galactosyl-1,3)-N-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 3	-0.14

CPEB4	Cytoplasmic Polyadenylation Element Binding Protein 4	-0.16
SERPINB2	Serpin Peptidase Inhibitor, Clade B (Ovalbumin), Member 2	-0.24
ERAP2	Endoplasmic Reticulum Aminopeptidase 2	-0.14
ZNF528	Zinc Finger Protein 528	-0.14
EMB	Embigin	-0.14
LHFPL2	Lipoma HMGIC Fusion Partner-Like 2	-0.14
HOXC13	Homeobox C13	-0.14
KCNK2	Potassium Channel, Subfamily K, Member 2	-0.15
ICT1	Immature Colon Carcinoma Transcript 1	-0.14
XKR4	XK, Kell Blood Group Complex Subunit-Related Family, Member 4	-0.14
CSDE1	Cold Shock Domain Containing E1, RNA-Binding	-0.14
GBP6	Guanylate Binding Protein Family, Member 6	-0.14
SEC24D	SEC24 Family, Member D (S. Cerevisiae)	-0.14
LIX1	Lix1 Homolog (Chicken)	-0.14
CXorf23	Chromosome X Open Reading Frame 23	-0.15
CXXC4	CXXC Finger Protein 4	-0.14
GAS2L3	Growth Arrest-Specific 2 Like 3	-0.14
AGTPBP1	ATP/GTP Binding Protein 1	-0.15
GNB1	Guanine Nucleotide Binding Protein (G Protein), Beta Polypeptide 1	-0.19
FBXO17	F-Box Protein 17	-0.14
EPS15L1	Epidermal Growth Factor Receptor Pathway Substrate 15-Like 1	-0.14
TIMM17A	Translocase Of Inner Mitochondrial Membrane 17 Homolog A (Yeast)	-0.2
PRKAA1	Protein Kinase, AMP-Activated, Alpha 1 Catalytic Subunit	-0.14
FAM81A	Family With Sequence Similarity 81, Member A	-0.14
C2ORF15	Uncharacterized Protein C2orf15	-0.14
CNEP1R1	CTD Nuclear Envelope Phosphatase 1 Regulatory Subunit 1	-0.14
TXK	TXK Tyrosine Kinase	-0.21
KCNJ6	Potassium Inwardly-Rectifying Channel, Subfamily J, Member 6	-0.13
ZNF502	Zinc Finger Protein 502	-0.13
HEMGN	Hemogen	-0.13
LUZP2	Leucine Zipper Protein 2	-0.13
ZNF800	Zinc Finger Protein 800	-0.13
STAM	Signal Transducing Adaptor Molecule (SH3 Domain And ITAM Motif) 1	-0.13
GIT2	G Protein-Coupled Receptor Kinase Interacting Arfgap 2	-0.14
LPAR1	Lysophosphatidic Acid Receptor 1	-0.14
UBXN2A	UBX Domain Protein 2A	-0.17
TRIP11	Thyroid Hormone Receptor Interactor 11	-0.16

TNKS	Tankyrase, TRF1-Interacting Ankyrin-Related ADP-Ribose Polymerase	-0.14
CRK	V-Crk Avian Sarcoma Virus CT10 Oncogene Homolog	-0.14
AADAT	Aminoadipate Aminotransferase	-0.14
CLEC5A	C-Type Lectin Domain Family 5, Member A	-0.13
RAB23	RAB23, Member RAS Oncogene Family	-0.21
C2orf15	Chromosome 2 Open Reading Frame 15	-0.19
MRPL30	Mitochondrial Ribosomal Protein L30	-0.19
TMEM185B	Transmembrane Protein 185B	-0.14
PGAM5	Phosphoglycerate Mutase Family Member 5	-0.22
DTWD2	DTW Domain Containing 2	-0.17
C1orf200	Chromosome 1 Open Reading Frame 200	-0.13
AGBL4	ATP/GTP Binding Protein-Like 4	-0.13
NECAB1	N-Terminal EF-Hand Calcium Binding Protein 1	-0.13
PROCR	Protein C Receptor, Endothelial	-0.13
PRPS2	Phosphoribosyl Pyrophosphate Synthetase 2	-0.13
MAGEH1	Melanoma Antigen Family H, 1	-0.13
LRIG3	Leucine-Rich Repeats And Immunoglobulin-Like Domains 3	-0.13
PSENEN	Presenilin Enhancer Gamma Secretase Subunit	-0.17
TMEM66	Transmembrane Protein 66	-0.14
MED13L	Mediator Complex Subunit 13-Like	-0.17
CAMK1D	Calcium/Calmodulin-Dependent Protein Kinase ID	-0.18
COA1	Cytochrome C Oxidase Assembly Factor 1 Homolog (S. Cerevisiae)	-0.38
FCHSD1	FCH And Double SH3 Domains 1	-0.13
B4GALT5	UDP-Gal:Betaglcnac Beta 1,4- Galactosyltransferase, Polypeptide 5	-0.13
SLC38A11	Solute Carrier Family 38, Member 11	-0.13
NBEA	Neurobeachin	-0.13
TMCC1	Transmembrane And Coiled-Coil Domain Family 1	-0.13
MSL3	Male-Specific Lethal 3 Homolog (Drosophila)	-0.13
KCNMB2	Potassium Large Conductance Calcium-Activated Channel, Subfamily M, Beta Member 2	-0.13
MTL5	Metallothionein-Like 5, Testis-Specific (Tesmin)	-0.2
ERI1	Exoribonuclease 1	-0.2
HOXD4	Homeobox D4	-0.14
NAA30	N(Alpha)-Acetyltransferase 30, Natc Catalytic Subunit	-0.2
NUDT7	Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 7	-0.17
ZNF510	Zinc Finger Protein 510	-0.18
TMOD3	Tropomodulin 3 (Ubiquitous)	-0.24
HADH	Hydroxyacyl-Coa Dehydrogenase	-0.16
PIGK	Phosphatidylinositol Glycan Anchor Biosynthesis, Class K	-0.21
MOV10	Mov10, Moloney Leukemia Virus 10, Homolog (Mouse)	-0.15
KIAA1841	Kiaa1841	-0.23

DEFB132	Defensin, Beta 132	-0.13
ABCA4	ATP-Binding Cassette, Sub-Family A (ABC1), Member 4	-0.13
CCDC148	Coiled-Coil Domain Containing 148	-0.13
SLC25A23	Solute Carrier Family 25 (Mitochondrial Carrier; Phosphate Carrier), Member 23	-0.13
RAB9B	RAB9B, Member RAS Oncogene Family	-0.13
PPM1K	Protein Phosphatase, Mg2+/Mn2+ Dependent, 1K	-0.13
LRCH2	Leucine-Rich Repeats And Calponin Homology (CH) Domain Containing 2	-0.15
CAPN13	Calpain 13	-0.13
KCNQ5	Potassium Voltage-Gated Channel, KQT-Like Subfamily, Member 5	-0.28
RSRC1	Arginine/Serine-Rich Coiled-Coil 1	-0.18
ACAD8	Acyl-Coa Dehydrogenase Family, Member 8	-0.26
RPL37	Ribosomal Protein L37	-0.15
SRSF1	Serine/Arginine-Rich Splicing Factor 1	-0.27
ATF1	Activating Transcription Factor 1	-0.15
PTBP1	Polypyrimidine Tract Binding Protein 1	-0.13
KLHL23	Kelch-Like Family Member 23	-0.2
CLIP4	CAP-GLY Domain Containing Linker Protein Family, Member 4	-0.15
LPP	LIM Domain Containing Preferred Translocation Partner In Lipoma	-0.13
ELOVL5	ELOVL Fatty Acid Elongase 5	-0.13
FZD10	Frizzled Family Receptor 10	-0.13
NFKBID	Nuclear Factor Of Kappa Light Polypeptide Gene Enhancer In B-Cells Inhibitor, Delta	-0.13
MEF2C	Myocyte Enhancer Factor 2C	-0.17
B4GALT6	UDP-Gal:Betaglcnac Beta 1,4- Galactosyltransferase, Polypeptide 6	-0.19
FAM21C	Family With Sequence Similarity 21, Member C	-0.13
MYO3B	Myosin IIIB	-0.13
C1orf216	Chromosome 1 Open Reading Frame 216	-0.13
FAM21B	Family With Sequence Similarity 21, Member B	-0.13
REEP3	Receptor Accessory Protein 3	-0.16
FAM118A	Family With Sequence Similarity 118, Member A	-0.14
FYTTD1	Forty-Two-Three Domain Containing 1	-0.18
C20orf196	Chromosome 20 Open Reading Frame 196	-0.18
LPCAT2	Lysophosphatidylcholine Acyltransferase 2	-0.13
VPS36	Vacuolar Protein Sorting 36 Homolog (S. Cerevisiae)	-0.21
PIK3CG	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase, Catalytic Subunit Gamma	-0.17
SOCS2	Suppressor Of Cytokine Signaling 2	-0.13
CCDC91	Coiled-Coil Domain Containing 91	-0.13
AKAP7	A Kinase (PRKA) Anchor Protein 7	-0.13
PERP	PERP, TP53 Apoptosis Effector	-0.16

FAM21A	Family With Sequence Similarity 21, Member A	-0.13
APPL1	Adaptor Protein, Phosphotyrosine Interaction, PH Domain And Leucine Zipper Containing 1	-0.13
CYBB	Cytochrome B-245, Beta Polypeptide	-0.13
FP15737		-0.13
RGMB	RGM Domain Family, Member B	-0.16
KANK4	KN Motif And Ankyrin Repeat Domains 4	-0.17
PSD3	Pleckstrin And Sec7 Domain Containing 3	-0.41
TMX3	Thioredoxin-Related Transmembrane Protein 3	-0.14
RNF150	Ring Finger Protein 150	-0.18
CA10	Carbonic Anhydrase X	-0.12
RUNX2	Runt-Related Transcription Factor 2	-0.12
PPP1R2	Protein Phosphatase 1, Regulatory (Inhibitor) Subunit 2	-0.12
C10orf88	Chromosome 10 Open Reading Frame 88	-0.12
RBMS1	RNA Binding Motif, Single Stranded Interacting Protein 1	-0.12
STEAP4	STEAP Family Member 4	-0.19
TMEM64	Transmembrane Protein 64	-0.21
WAPAL	Wings Apart-Like Homolog (Drosophila)	-0.15
UGT8	UDP Glycosyltransferase 8	-0.13
DLGAP2	Discs, Large (Drosophila) Homolog-Associated Protein 2	-0.12
TRIM16L	Tripartite Motif Containing 16-Like	-0.12
CCDC68	Coiled-Coil Domain Containing 68	-0.12
TRIM16	Tripartite Motif Containing 16	-0.12
LHFPL1	Lipoma HMGIC Fusion Partner-Like 1	-0.12
FAM129C	Family With Sequence Similarity 129, Member C	-0.12
NBPF6	Neuroblastoma Breakpoint Family, Member 6	-0.12
PECR	Peroxisomal Trans-2-Enoyl-Coa Reductase	-0.15
CLDN11	Claudin 11	-0.12
CDK6	Cyclin-Dependent Kinase 6	-0.14
LPGAT1	Lysophosphatidylglycerol Acyltransferase 1	-0.16
SLC39A14	Solute Carrier Family 39 (Zinc Transporter), Member 14	-0.12
SRSF4	Serine/Arginine-Rich Splicing Factor 4	-0.12
LACC1	Laccase (Multicopper Oxidoreductase) Domain Containing 1	-0.12
OTOGL	Otogelin-Like	-0.12
TERF1	Telomeric Repeat Binding Factor (NIMA-Interacting) 1	-0.12
IL10	Interleukin 10	-0.12
GALNT3	UDP-N-Acetyl-Alpha-D-Galactosamine:Polypeptide N-Acetylgalactosaminyltransferase 3 (Galnac-T3)	-0.12
FAM196A	Family With Sequence Similarity 196, Member A	-0.12
ZNF366	Zinc Finger Protein 366	-0.12
MUC3A	Mucin 3A, Cell Surface Associated	-0.12
PNRC2	Proline-Rich Nuclear Receptor Coactivator 2	-0.12
RAP2B	RAP2B, Member Of RAS Oncogene Family	-0.46
ZPLD1	Zona Pellucida-Like Domain Containing 1	-0.12

RFXAP	Regulatory Factor X-Associated Protein	-0.19
KMT2E	Lysine (K)-Specific Methyltransferase 2E	-0.12
DYM	Dymeclin	-0.13
SH3BGRL2	SH3 Domain Binding Glutamic Acid-Rich Protein Like 2	-0.16
ENOX2	Ecto-NOX Disulfide-Thiol Exchanger 2	-0.15
GPR50	G Protein-Coupled Receptor 50	-0.13
ZNF160	Zinc Finger Protein 160	-0.13
BUB3	BUB3 Mitotic Checkpoint Protein	-0.14
IL6ST	Interleukin 6 Signal Transducer (Gp130, Oncostatin M Receptor)	-0.12
PAK2	P21 Protein (Cdc42/Rac)-Activated Kinase 2	-0.12
CLCN3	Chloride Channel, Voltage-Sensitive 3	-0.14
GINS3	GINS Complex Subunit 3 (Psf3 Homolog)	-0.12
ZNF841	Zinc Finger Protein 841	-0.25
TMEM43	Transmembrane Protein 43	-0.17
PRR5L	Proline Rich 5 Like	-0.12
PLXDC2	Plexin Domain Containing 2	-0.26
TLR10	Toll-Like Receptor 10	-0.12
HMGCS2	3-Hydroxy-3-Methylglutaryl-Coa Synthase 2 (Mitochondrial)	-0.13
NUS1	Nuclear Undecaprenyl Pyrophosphate Synthase 1 Homolog (S. Cerevisiae)	-0.12
EFNB2	Ephrin-B2	-0.12
PYROXD1	Pyridine Nucleotide-Disulphide Oxidoreductase Domain 1	-0.12
CCDC43	Coiled-Coil Domain Containing 43	-0.12
JAK1	Janus Kinase 1	-0.12
CD300LF	CD300 Molecule-Like Family Member F	-0.12
IFIT5	Interferon-Induced Protein With Tetratricopeptide Repeats 5	-0.18
KRT15	Keratin 15	-0.21
FAM89A	Family With Sequence Similarity 89, Member A	-0.18
DIAPH2	Diaphanous-Related Formin 2	-0.14
RECK	Reversion-Inducing-Cysteine-Rich Protein With Kazal Motifs	-0.12
KCNH5	Potassium Voltage-Gated Channel, Subfamily H (Eag-Related), Member 5	-0.16
MAATS1	MYCBP-Associated, Testis Expressed 1	-0.12
CLMP	CXADR-Like Membrane Protein	-0.14
PLEKHA3	Pleckstrin Homology Domain Containing, Family A (Phosphoinositide Binding Specific) Member 3	-0.16
TMTC3	Transmembrane And Tetratricopeptide Repeat Containing 3	-0.12
ATP2B2	Atpase, Ca++ Transporting, Plasma Membrane 2	-0.12
SPOPL	Speckle-Type POZ Protein-Like	-0.17
GRIN2A	Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate 2A	-0.19

NADK2	NAD Kinase 2, Mitochondrial	-0.21
UCP3	Uncoupling Protein 3 (Mitochondrial, Proton Carrier)	-0.12
DOK6	Docking Protein 6	-0.12
DDAH1	Dimethylarginine Dimethylaminohydrolase 1	-0.12
PAICS	Phosphoribosylaminoimidazole Carboxylase, Phosphoribosylaminoimidazole Succinocarboxamide Synthetase	-0.16
NRG1	Neuregulin 1	-0.12
AC068987.1	HCG1997999; Cdna FLJ33996 Fis, Clone DFNES2008881	-0.12
OPRK1	Opioid Receptor, Kappa 1	-0.12
EXOC8	Exocyst Complex Component 8	-0.12
PCDHB16	Protocadherin Beta 16	-0.12
BECN1	Beclin 1, Autophagy Related	-0.12
TMPRSS11D	Transmembrane Protease, Serine 11D	-0.12
SERINC5	Serine Incorporator 5	-0.13
NDRG3	NDRG Family Member 3	-0.12
NMU	Neuromedin U	-0.12
ONECUT2	One Cut Homeobox 2	-0.12
MTDH	Metadherin	-0.17
STEAP2	STEAP Family Member 2, Metalloreductase	-0.12
KIAA0087	Kiaa0087	-0.12
MYH4	Myosin, Heavy Chain 4, Skeletal Muscle	-0.12
ZNF283	Zinc Finger Protein 283	-0.12
GBP3	Guanylate Binding Protein 3	-0.12
FLRT3	Fibronectin Leucine Rich Transmembrane Protein 3	-0.12
RBMXL2	RNA Binding Motif Protein, X-Linked-Like 2	-0.12
CD2AP	CD2-Associated Protein	-0.12
ZNF621	Zinc Finger Protein 621	-0.15
FAM103A1	Family With Sequence Similarity 103, Member A1	-0.12
SLC25A6	Solute Carrier Family 25 (Mitochondrial Carrier; Adenine Nucleotide Translocator), Member 6	-0.12
WDR7	WD Repeat Domain 7	-0.12
DTD1	D-Tyrosyl-Trna Deacylase 1	-0.12
VPS33A	Vacuolar Protein Sorting 33 Homolog A (S. Cerevisiae)	-0.14
HIPK3	Homeodomain Interacting Protein Kinase 3	-0.12
THPO	Thrombopoietin	-0.18
TEX15	Testis Expressed 15	-0.16
SFT2D2	SFT2 Domain Containing 2	-0.15
ELL2	Elongation Factor, RNA Polymerase II, 2	-0.12
ST20-MTHFS	ST20-MTHFS Readthrough	-0.12
EFCAB6	EF-Hand Calcium Binding Domain 6	-0.15
CNTNAP3	Contactin Associated Protein-Like 3	-0.22
TIMP3	TIMP Metallopeptidase Inhibitor 3	-0.14
VAT1L	Vesicle Amine Transport 1-Like	-0.12
SPIN2A	Spindlin Family, Member 2A	-0.12
UNC50	Unc-50 Homolog (C. Elegans)	-0.31

ADAM9	ADAM Metallopeptidase Domain 9	-0.12
UBE2K	Ubiquitin-Conjugating Enzyme E2K	-0.29
TMEM203	Transmembrane Protein 203	-0.15
ZBTB43	Zinc Finger And BTB Domain Containing 43	-0.12
SELL	Selectin L	-0.12
POLR2C	Polymerase (RNA) II (DNA Directed) Polypeptide C, 33kda	-0.12
C17orf78	Chromosome 17 Open Reading Frame 78	-0.12
NSUN7	NOP2/Sun Domain Family, Member 7	-0.12
NTNG1	Netrin G1	-0.12
LYST	Lysosomal Trafficking Regulator	-0.12
THRAP3	Thyroid Hormone Receptor Associated Protein 3	-0.12
SPIRE1	Spire-Type Actin Nucleation Factor 1	-0.22
TMEM200C	Transmembrane Protein 200C	-0.44
ERBB4	V-Erb-B2 Avian Erythroblastic Leukemia Viral Oncogene Homolog 4	-0.12
POLR3G	Polymerase (RNA) III (DNA Directed) Polypeptide G (32kd)	-0.14
ITCH	Itchy E3 Ubiquitin Protein Ligase	-0.13
MCL1	Myeloid Cell Leukemia Sequence 1 (BCL2-Related)	-0.12
PELI2	Pellino E3 Ubiquitin Protein Ligase Family Member 2	-0.12
UBE3A	Ubiquitin Protein Ligase E3A	-0.12
GDA	Guanine Deaminase	-0.11
FAM110C	Family With Sequence Similarity 110, Member C	-0.11
USP25	Ubiquitin Specific Peptidase 25	-0.11
ZNF558	Zinc Finger Protein 558	-0.11
ABLIM1	Actin Binding LIM Protein 1	-0.11
GFRA2	GDNF Family Receptor Alpha 2	-0.11
ASB5	Ankyrin Repeat And SOCS Box Containing 5	-0.11
SI	Sucrase-Isomaltase (Alpha-Glucosidase)	-0.11
IQCK	IQ Motif Containing K	-0.11
GBP1	Guanylate Binding Protein 1, Interferon-Inducible	-0.11
VPS37A	Vacuolar Protein Sorting 37 Homolog A (S. Cerevisiae)	-0.21
CNOT7	CCR4-NOT Transcription Complex, Subunit 7	-0.21
FAM168B	Family With Sequence Similarity 168, Member B	-0.12
PCDH7	Protocadherin 7	-0.12
ATP11C	Atpase, Class VI, Type 11C	-0.11
CXADR	Coxsackie Virus And Adenovirus Receptor	-0.11
OTX1	Orthodenticle Homeobox 1	-0.11
YEATS2	YEATS Domain Containing 2	-0.11
TSPYL4	TSPY-Like 4	-0.11
RCOR1	REST Corepressor 1	-0.17
SLC16A1	Solute Carrier Family 16 (Monocarboxylate Transporter), Member 1	-0.11
GTF2A1	General Transcription Factor IIA, 1, 19/37kda	-0.19
ZNF557	Zinc Finger Protein 557	-0.11
RAB30	RAB30, Member RAS Oncogene Family	-0.17

PIGA	Phosphatidylinositol Glycan Anchor Biosynthesis, Class A	-0.11
METTL8	Methyltransferase Like 8	-0.13
CYTH3	Cytohesin 3	-0.12
ABI1	Abl-Interactor 1	-0.18
TFEC	Transcription Factor EC	-0.11
LINGO2	Leucine Rich Repeat And Ig Domain Containing 2	-0.11
SGMS1	Sphingomyelin Synthase 1	-0.11
MRC1L1	Cdna FLJ56855, Highly Similar To Macrophage Mannose Receptor 1	-0.11
KIF15	Kinesin Family Member 15	-0.11
MOG	Myelin Oligodendrocyte Glycoprotein	-0.11
MRC1	Mannose Receptor, C Type 1	-0.11
GLIS2	GLIS Family Zinc Finger 2	-0.11
CCNK	Cyclin K	-0.2
DUSP7	Dual Specificity Phosphatase 7	-0.18
SETDB2	SET Domain, Bifurcated 2	-0.12
RNF180	Ring Finger Protein 180	-0.12
XK	X-Linked Kx Blood Group (Mcleod Syndrome)	-0.11
DNAJC13	Dnaj (Hsp40) Homolog, Subfamily C, Member 13	-0.11
EGLN3	Egl-9 Family Hypoxia-Inducible Factor 3	-0.11
ZNF532	Zinc Finger Protein 532	-0.11
EREG	Epiregulin	-0.11
STARD3NL	STARD3 N-Terminal Like	-0.11
METTL4	Methyltransferase Like 4	-0.12
SLC17A1	Solute Carrier Family 17 (Organic Anion Transporter), Member 1	-0.11
PYURF	PIGY Upstream Reading Frame	-0.12
VASH2	Vasohibin 2	-0.12
MMP19	Matrix Metallopeptidase 19	-0.12
MTX2	Metaxin 2	-0.18
ZNF483	Zinc Finger Protein 483	-0.26
TCEB1	Transcription Elongation Factor B (SIII), Polypeptide 1 (15kda, Elongin C)	-0.16
METTL15	Methyltransferase Like 15	-0.11
BLMH	Bleomycin Hydrolase	-0.11
ASH2L	Ash2 (Absent, Small, Or Homeotic)-Like (Drosophila)	-0.11
TNFAIP6	Tumor Necrosis Factor, Alpha-Induced Protein 6	-0.11
DST	Dystonin	-0.11
CEP44	Centrosomal Protein 44kda	-0.12
MTHFS	5,10-Methenyltetrahydrofolate Synthetase (5-Formyltetrahydrofolate Cyclo-Ligase)	-0.11
EIF2AK4	Eukaryotic Translation Initiation Factor 2 Alpha Kinase 4	-0.11
MIA	Melanoma Inhibitory Activity	-0.34
S1PR1	Sphingosine-1-Phosphate Receptor 1	-0.26
RCOR3	REST Corepressor 3	-0.11

CACNG6	Calcium Channel, Voltage-Dependent, Gamma Subunit 6	-0.11
SLC46A3	Solute Carrier Family 46, Member 3	-0.11
RNF114	Ring Finger Protein 114	-0.15
AICDA	Activation-Induced Cytidine Deaminase	-0.11
CEP170	Centrosomal Protein 170kda	-0.11
ABCC12	ATP-Binding Cassette, Sub-Family C (CFTR/MRP), Member 12	-0.11
CTC-534A2.2	CDNA FLJ26957 Fis, Clone SLV00486; Uncharacterized Protein	-0.21
SLC39A8	Solute Carrier Family 39 (Zinc Transporter), Member 8	-0.12
NR3C2	Nuclear Receptor Subfamily 3, Group C, Member 2	-0.11
SLC5A3	Sodium/Myo-Inositol Cotransporter	-0.2
FLVCR1	Feline Leukemia Virus Subgroup C Cellular Receptor 1	-0.15
KITLG	KIT Ligand	-0.11
P2RY1	Purinergic Receptor P2Y, G-Protein Coupled, 1	-0.13
ZFAND5	Zinc Finger, AN1-Type Domain 5	-0.17
TMPRSS15	Transmembrane Protease, Serine 15	-0.11
CNTNAP3B	Contactin Associated Protein-Like 3B	-0.11
GRID2	Glutamate Receptor, Ionotropic, Delta 2	-0.11
GPR110	G Protein-Coupled Receptor 110	-0.11
RP11-422N16.3	Uncharacterized Protein	-0.11
CSF2RB	Colony Stimulating Factor 2 Receptor, Beta, Low-Affinity (Granulocyte-Macrophage)	-0.11
PDGFRA	Platelet-Derived Growth Factor Receptor, Alpha Polypeptide	-0.11
F2R	Coagulation Factor II (Thrombin) Receptor	-0.11
RUNX1	Runt-Related Transcription Factor 1	-0.21
SEPT10	Septin 10	-0.14
MARVELD1	MARVEL Domain Containing 1	-0.11
ZNF654	Zinc Finger Protein 654	-0.11
PVRL3	Poliovirus Receptor-Related 3	-0.11
TBX20	T-Box 20	-0.11
NR5A1	Nuclear Receptor Subfamily 5, Group A, Member 1	-0.11
KCMF1	Potassium Channel Modulatory Factor 1	-0.12
ARSF	Arylsulfatase F	-0.11
BRD3	Bromodomain Containing 3	-0.11
FANCG	Fanconi Anemia, Complementation Group G	-0.11
XPO1	Exportin 1 (CRM1 Homolog, Yeast)	-0.13
MAP3K7	Mitogen-Activated Protein Kinase Kinase Kinase 7	-0.16
RP11-644F5.10	Uncharacterized Protein	-0.11
AGMAT	Agmatine Ureohydrolase (Agmatinase)	-0.16
MARCH7	Membrane-Associated Ring Finger (C3HC4) 7, E3 Ubiquitin Protein Ligase	-0.11
DLX2	Distal-Less Homeobox 2	-0.1

RUNDC3B	RUN Domain Containing 3B	-0.1
RASA3	RAS P21 Protein Activator 3	-0.1
VSNL1	Visinin-Like 1	-0.1
IGSF11	Immunoglobulin Superfamily, Member 11	-0.1
GBX2	Gastrulation Brain Homeobox 2	-0.2
PGRMC2	Progesterone Receptor Membrane Component 2	-0.1
SNTB2	Syntrophin, Beta 2 (Dystrophin-Associated Protein A1, 59kda, Basic Component 2)	-0.12
SPIB	Spi-B Transcription Factor (Spi-1/PU.1 Related)	-0.2
SOX30	SRY (Sex Determining Region Y)-Box 30	-0.14
PCDH20	Protocadherin 20	-0.27
SLC13A1	Solute Carrier Family 13 (Sodium/Sulfate Symporter), Member 1	-0.1
TRAPPC8	Trafficking Protein Particle Complex 8	-0.1
RSAD2	Radical S-Adenosyl Methionine Domain Containing 2	-0.1
NFATC2	Nuclear Factor Of Activated T-Cells, Cytoplasmic, Calcineurin-Dependent 2	-0.1
BZW1	Basic Leucine Zipper And W2 Domains 1	-0.15
DNAJB1	Dnaj (Hsp40) Homolog, Subfamily B, Member 1	-0.17
MFHAS1	Malignant Fibrous Histiocytoma Amplified Sequence 1	-0.12
PPL	Periplakin	-0.13
NFIX	Nuclear Factor I/X (CCAAT-Binding Transcription Factor)	-0.1
AWAT1	Acyl-Coa Wax Alcohol Acyltransferase 1	-0.1
PAQR9	Progestin And Adipoq Receptor Family Member IX	-0.1
KDELR2	KDEL (Lys-Asp-Glu-Leu) Endoplasmic Reticulum Protein Retention Receptor 2	-0.17
RBM8A	RNA Binding Motif Protein 8A	-0.11
MPP6	Membrane Protein, Palmitoylated 6 (MAGUK P55 Subfamily Member 6)	-0.12
HIPK1	Homeodomain Interacting Protein Kinase 1	-0.12
FAXC	Failed Axon Connections Homolog (Drosophila)	-0.19
LARP1B	La Ribonucleoprotein Domain Family, Member 1B	-0.13
SEPT7	Septin 7	-0.12
SLC30A7	Solute Carrier Family 30 (Zinc Transporter), Member 7	-0.1
ESRRG	Estrogen-Related Receptor Gamma	-0.1
C1QL2	Complement Component 1, Q Subcomponent-Like 2	-0.1
TSHR	Thyroid Stimulating Hormone Receptor	-0.1
ITGA6	Integrin, Alpha 6	-0.1
RIIAD1	Regulatory Subunit Of Type II PKA R-Subunit (Riia) Domain Containing 1	-0.1
OXTR	Oxytocin Receptor	-0.14
KCNC3	Potassium Voltage-Gated Channel, Shaw-Related Subfamily, Member 3	-0.1
POLH	Polymerase (DNA Directed), Eta	-0.13
TMEM168	Transmembrane Protein 168	-0.14

RALGAPB	Ral Gtpase Activating Protein, Beta Subunit (Non-Catalytic)	-0.11
DCX	Doublecortin	-0.1
GALNT15	UDP-N-Acetyl-Alpha-D-Galactosamine:Polypeptide N-Acetylgalactosaminyltransferase 15	-0.14
YWHAH	Tyrosine 3-Monooxygenase/Tryptophan 5-Monooxygenase Activation Protein, Eta Polypeptide	-0.1
HNF4G	Hepatocyte Nuclear Factor 4, Gamma	-0.1
DNAJC11	Dnaj (Hsp40) Homolog, Subfamily C, Member 11	-0.1
PCSK2	Proprotein Convertase Subtilisin/Kexin Type 2	-0.1
AASDH	Aminoadipate-Semialdehyde Dehydrogenase	-0.1
C1GALT1C1	C1GALT1-Specific Chaperone 1	-0.41
EEF1E1	Eukaryotic Translation Elongation Factor 1 Epsilon 1	-0.28
MBOAT1	Membrane Bound O-Acyltransferase Domain Containing 1	-0.28
GOLGA5	Golgin A5	-0.17
PRELID2	PRELI Domain Containing 2	-0.13
SNAP29	Synaptosomal-Associated Protein, 29kda	-0.17
NEK10	NIMA-Related Kinase 10	-0.13
BAG5	BCL2-Associated Athanogene 5	-0.1
COL4A5	Collagen, Type IV, Alpha 5	-0.12
ZNF300	Zinc Finger Protein 300	-0.1
PDE12	Phosphodiesterase 12	-0.21
CNKSR2	Connector Enhancer Of Kinase Suppressor Of Ras 2	-0.1
METTL23	Methyltransferase Like 23	-0.1
MARC2	Mitochondrial Amidoxime Reducing Component 2	-0.18
BACH2	BTB And CNC Homology 1, Basic Leucine Zipper Transcription Factor 2	-0.11
TSLP	Thymic Stromal Lymphopoietin	-0.28
IGF2BP3	Insulin-Like Growth Factor 2 Mrna Binding Protein 3	-0.14
FAM120A	Family With Sequence Similarity 120A	-0.13
SFRP2	Secreted Frizzled-Related Protein 2	-0.26
PARP8	Poly (ADP-Ribose) Polymerase Family, Member 8	-0.15
PRKAR2B	Protein Kinase, Camp-Dependent, Regulatory, Type II, Beta	-0.11
CERS6	Ceramide Synthase 6	-0.11
TBX18	T-Box 18	-0.1
LAMP1	Lysosomal-Associated Membrane Protein 1	-0.1
CPNE4	Copine IV	-0.1
RPE65	Retinal Pigment Epithelium-Specific Protein 65kda	-0.1
ANGPTL2	Angiopoietin-Like 2	-0.1
TUSC5	Tumor Suppressor Candidate 5	-0.1
VTI1B	Vesicle Transport Through Interaction With T-Snares 1B	-0.11
MRPL50	Mitochondrial Ribosomal Protein L50	-0.13
LIMS1	LIM And Senescent Cell Antigen-Like Domains 1	-0.18
PHIP	Pleckstrin Homology Domain Interacting Protein	-0.11
UBASH3B	Ubiquitin Associated And SH3 Domain Containing B	-0.11

AARD	Alanine And Arginine Rich Domain Containing Protein	-0.14
PRKCE	Protein Kinase C, Epsilon	-0.1
AGAP2	Arfgap With Gtpase Domain, Ankyrin Repeat And PH Domain 2	-0.1
FOXR2	Forkhead Box R2	-0.1
TENM1	Teneurin Transmembrane Protein 1	-0.1
TMEFF1	Transmembrane Protein With EGF-Like And Two Follistatin-Like Domains 1	-0.1
SEC24C	SEC24 Family, Member C (S. Cerevisiae)	-0.1
HAVCR1	Hepatitis A Virus Cellular Receptor 1	-0.1
ZFX	Zinc Finger Protein, X-Linked	-0.1
PLEKHG1	Pleckstrin Homology Domain Containing, Family G (With Rhogef Domain) Member 1	-0.2
OLIG2	Oligodendrocyte Lineage Transcription Factor 2	-0.15
POU2F1	POU Class 2 Homeobox 1	-0.1
TAF13	TAF13 RNA Polymerase II, TATA Box Binding Protein (TBP)-Associated Factor, 18kda	-0.1
MTX3	Metaxin 3	-0.1
ADAMTS20	ADAM Metallopeptidase With Thrombospondin Type 1 Motif, 20	-0.11
EGR2	Early Growth Response 2	-0.1
TANGO6	Transport And Golgi Organization 6 Homolog (Drosophila)	-0.1
FGF14	Fibroblast Growth Factor 14	-0.1
WBP1L	WW Domain Binding Protein 1-Like	-0.1
GGT7	Gamma-Glutamyltransferase 7	-0.1
GDNF	Glial Cell Derived Neurotrophic Factor	-0.1
POPDC2	Popeye Domain Containing 2	-0.1
KBTBD6	Kelch Repeat And BTB (POZ) Domain Containing 6	-0.1
LINS	Lines Homolog (Drosophila)	-0.14
C8orf44-SGK3	C8orf44-SGK3 Readthrough	-0.11
HMGB1	High Mobility Group Box 1	-0.22
MEIS2	Meis Homeobox 2	-0.15
MGEA5	Meningioma Expressed Antigen 5 (Hyaluronidase)	-0.1
IQCG	IQ Motif Containing G	-0.12
NKAP	NFKB Activating Protein	-0.18
ZNF711	Zinc Finger Protein 711	-0.1
MEX3B	Mex-3 RNA Binding Family Member B	-0.1
GRIP1	Glutamate Receptor Interacting Protein 1	-0.1
KRT74	Keratin 74	-0.1
SOGA2	SOGA Family Member 2	-0.1
IL1A	Interleukin 1, Alpha	-0.1
SETD7	SET Domain Containing (Lysine Methyltransferase) 7	-0.1
RNF4	Ring Finger Protein 4	-0.14
NEDD4	Neural Precursor Cell Expressed, Developmentally Down-Regulated 4, E3 Ubiquitin Protein Ligase	-0.1

DCUN1D4	DCN1, Defective In Cullin Neddylation 1, Domain Containing 4	-0.1
EDA	Ectodysplasin A	-0.1
CASP3	Caspase 3, Apoptosis-Related Cysteine Peptidase	-0.1
TRAF6	TNF Receptor-Associated Factor 6, E3 Ubiquitin Protein Ligase	-0.17
ZBTB4	Zinc Finger And BTB Domain Containing 4	-0.1
EMCN	Endomucin	-0.1
HSF2	Heat Shock Transcription Factor 2	-0.1
BET1L	Bet1 Golgi Vesicular Membrane Trafficking Protein-Like	-0.1
PRKAB2	Protein Kinase, AMP-Activated, Beta 2 Non-Catalytic Subunit	-0.1
UBFD1	Ubiquitin Family Domain Containing 1	-0.1
MFAP3L	Microfibrillar-Associated Protein 3-Like	-0.1
ITPRIPL2	Inositol 1,4,5-Trisphosphate Receptor Interacting Protein-Like 2	-0.1
ESCO1	Establishment Of Sister Chromatid Cohesion N-Acetyltransferase 1	-0.1
ZNF703	Zinc Finger Protein 703	-0.33
SUN1	Sad1 And UNC84 Domain Containing 1	-0.1
MSANTD3-TMEFF1	MSANTD3-TMEFF1 Readthrough	-0.09
CCDC39	Coiled-Coil Domain Containing 39	-0.09
DMC1	DNA Meiotic Recombinase 1	-0.09
PAPD5	PAP Associated Domain Containing 5	-0.09
ALKBH8	Alkb, Alkylation Repair Homolog 8 (E. Coli)	-0.09
KBTBD12	Kelch Repeat And BTB (POZ) Domain Containing 12	-0.09
RBM41	RNA Binding Motif Protein 41	-0.09
ACTN1	Actinin, Alpha 1	-0.09
BCL2L13	BCL2-Like 13 (Apoptosis Facilitator)	-0.09
RAD51AP1	RAD51 Associated Protein 1	-0.14
PDCL3	Phosducin-Like 3	-0.24
MTMR2	Myotubularin Related Protein 2	-0.13
ALDH18A1	Aldehyde Dehydrogenase 18 Family, Member A1	-0.09
GPRC5B	G Protein-Coupled Receptor, Family C, Group 5, Member B	-0.09
C17orf102	Chromosome 17 Open Reading Frame 102	-0.09
ZNF705G	Zinc Finger Protein 705G	-0.09
ZNF705B	Zinc Finger Protein 705B	-0.09
MTPN	Myotrophin	-0.09
ALDH3A2	Aldehyde Dehydrogenase 3 Family, Member A2	-0.11
ZNF451	Zinc Finger Protein 451	-0.12
ATP6V0E2	Atpase, H+ Transporting V0 Subunit E2	-0.1
MGLL	Monoglyceride Lipase	-0.1
NCBP2	Nuclear Cap Binding Protein Subunit 2, 20kda	-0.1
CDK14	Cyclin-Dependent Kinase 14	-0.1

CNTF	Ciliary Neurotrophic Factor	-0.1
RASGRF2	Ras Protein-Specific Guanine Nucleotide-Releasing Factor 2	-0.11
SCRN3	Secernin 3	-0.12
ZNF705D	Zinc Finger Protein 705D	-0.09
RIMS2	Regulating Synaptic Membrane Exocytosis 2	-0.09
RAPGEF5	Rap Guanine Nucleotide Exchange Factor (GEF) 5	-0.09
CEMP1	Cementum Protein 1	-0.09
HDX	Highly Divergent Homeobox	-0.09
SALL4	Sal-Like 4 (Drosophila)	-0.09
KAT2B	K(Lysine) Acetyltransferase 2B	-0.1
LSM14B	LSM14B, SCD6 Homolog B (S. Cerevisiae)	-0.12
C1QTNF2	C1q And Tumor Necrosis Factor Related Protein 2	-0.17
CAST	Calpastatin	-0.2
UPB1	Ureidopropionase, Beta	-0.1
UBAC1	UBA Domain Containing 1	-0.15
SUN2	Sad1 And UNC84 Domain Containing 2	-0.09
HEY2	Hairy/Enhancer-Of-Split Related With YRPW Motif 2	-0.45
ST3GAL5	ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 5	-0.09
RDH10	Retinol Dehydrogenase 10 (All-Trans)	-0.09
C15orf52	Chromosome 15 Open Reading Frame 52	-0.09
PTBP2	Polypyrimidine Tract Binding Protein 2	-0.22
AKAP5	A Kinase (PRKA) Anchor Protein 5	-0.09
ATP13A3	Atpase Type 13A3	-0.12
ZIC3	Zic Family Member 3	-0.2
PCDH10	Protocadherin 10	-0.11
SGK3	Serum/Glucocorticoid Regulated Kinase Family, Member 3	-0.1
NMT2	N-Myristoyltransferase 2	-0.11
C18orf54	Chromosome 18 Open Reading Frame 54	-0.09
POLE3	Polymerase (DNA Directed), Epsilon 3, Accessory Subunit	-0.09
MEOX2	Mesenchyme Homeobox 2	-0.09
SLC18B1	Solute Carrier Family 18, Subfamily B, Member 1	-0.09
ZFC3H1	Zinc Finger, C3H1-Type Containing	-0.09
FLVCR2	Feline Leukemia Virus Subgroup C Cellular Receptor Family, Member 2	-0.09
ASB14	Ankyrin Repeat And SOCS Box Containing 14	-0.09
ISY1-RAB43	ISY1-RAB43 Readthrough	-0.09
CHIC1	Cysteine-Rich Hydrophobic Domain 1	-0.25
BVES	Blood Vessel Epicardial Substance	-0.12
C2orf88	Chromosome 2 Open Reading Frame 88	-0.09
CSNK1A1	Casein Kinase 1, Alpha 1	-0.11
FBXO48	F-Box Protein 48	-0.24
ZNF106	Zinc Finger Protein 106	-0.09
LRRC55	Leucine Rich Repeat Containing 55	-0.09
ELMOD1	ELMO/CED-12 Domain Containing 1	-0.09

GNA15	Guanine Nucleotide Binding Protein (G Protein), Alpha 15 (Gq Class)	-0.09
CLDN12	Claudin 12	-0.09
ZNF697	Zinc Finger Protein 697	-0.09
TRIM63	Tripartite Motif Containing 63, E3 Ubiquitin Protein Ligase	-0.09
ZNF287	Zinc Finger Protein 287	-0.15
TMC6	Transmembrane Channel-Like 6	-0.2
ZNF281	Zinc Finger Protein 281	-0.1
PATZ1	POZ (BTB) And AT Hook Containing Zinc Finger 1	-0.12
CEP57L1	Centrosomal Protein 57kda-Like 1	-0.1
FOXG1	Forkhead Box G1	-0.11
COL9A1	Collagen, Type IX, Alpha 1	-0.14
ME1	Malic Enzyme 1, NADP(+)-Dependent, Cytosolic	-0.1

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