Membrane-Tethered Metalloproteinase Expressed by Vascular Smooth Muscle Cells Limits the Progression of Proliferative Atherosclerotic Lesions

Richard H. Barnes, II, BS; Takeshi Akama, PhD; Miina K. Öhman, MD, PhD; Moon-Sook Woo, PhD; Julian Bahr, BS; Stephen J. Weiss, MD; Daniel T. Eitzman, MD; Tae-Hwa Chun, MD, PhD

Background—The MMP (matrix metalloproteinase) family plays diverse and critical roles in directing vascular wall remodeling in atherosclerosis. Unlike secreted-type MMPs, a member of the membrane-type MMP family, MT1-MMP (membrane-type 1 MMP; Mmp14), mediates pericellular extracellular matrix degradation that is indispensable for maintaining physiological extracellular matrix homeostasis. However, given the premature mortality exhibited by MT1-MMP-null mice, the potential role of the proteinase in atherogenesis remains elusive. We sought to determine the effects of both MT1-MMP heterozygosity and tissue-specific gene targeting on atherogenesis in APOE (apolipoprotein E)–null mice.

Methods and Results—MT1-MMP heterozygosity in the APOE-null background (Mmp14+/−/Apoε−/−) significantly promoted atherogenesis relative to Mmp14+/+/Apoε−/− mice. Furthermore, the tissue-specific deletion of MT1-MMP from vascular smooth muscle cells (VSMCs) in SM22a-Cre(+)Mmp14+/−/Apoε−/− (VSMC-knockout) mice likewise increased the severity of atherosclerotic lesions. Although VSMC-knockout mice also developed progressive atherosclerotic aneurysms in their iliac arteries, macrophage- and adipose-specific MT1-MMP-knockout mice did not display this sensitized phenotype. In VSMC-knockout mice, atherosclerotic lesions were populated by hyperproliferating VSMCs (smooth muscle actin– and Ki67–double-positive cells) that were characterized by a proinflammatory gene expression profile. Finally, MT1-MMP-null VSMCs cultured in a 3-dimensional spheroid model system designed to mimic in vivo–like cell–cell and cell–extracellular matrix interactions, likewise displayed markedly increased proliferative potential.

Conclusions—MT1-MMP expressed by VSMCs plays a key role in limiting the progression of atherosclerosis in APOE-null mice by regulating proliferative responses and inhibiting the deterioration of VSMC function in atherogenic vascular walls. (J Am Heart Assoc. 2017;6:e003693. DOI: 10.1161/JAHA.116.003693.)

Key Words: aneurysm • atherosclerosis • inflammation • matrix metalloproteinases • muscle • smooth

Vascular smooth muscle cells (VSMCs) constitute the major cellular component of the tunica media, where they play key roles in regulating vascular tone and blood flow. Under physiological conditions, the number of VSMCs within the arterial wall is tightly controlled, as is arterial wall thickness. In marked contrast, during the progression of atherosclerosis, VSMCs proliferate and transition from a contractile to a synthetic phenotype, thereby depositing excess extracellular matrix (ECM) molecules that lead to arterial wall thickening and stiffening, namely, arteriosclerosis. However, the molecular mechanisms that underlie VSMC proliferation and arteriosclerosis during the pathological process of atherosclerosis remain largely undefined.

ECM remodeling is mediated by members of the MMP (matrix metalloproteinase) gene family, a group of structurally related proteolytic enzymes that are broadly characterized as either secreted or membrane tethered. Consistent with their potential roles in vascular wall pathology, recent studies have characterized the roles of secreted MMPs, particularly MMP-2, MMP-8, and MMP-13, in promoting atherogenesis or plaque rupture. With regard to the membrane-tethered MMPs, MT1-MMP uniquely serves as a pericellular collagenase that...
VSMC MT1-MMP Limits Atherogenesis and Aneurysm

Barnes II et al

Clinical Perspective

What Is New?

- The molecular mechanisms underlying proliferative atherosclerosis have not been fully defined.
- This animal study suggests that a pericellular collagenase called MT1-MMP (membrane-type 1 matrix metalloproteinase), expressed by vascular smooth muscle cells, plays a critical role in limiting the progression of proliferative atherosclerotic lesions.
- The loss of vascular smooth muscle cell MT1-MMP leads to advanced proliferative atherosclerosis and atherosclerotic iliac artery aneurysm formation.

What Are the Clinical Implications?

- The functional impairment of vascular smooth muscle cells in regulating vascular wall extracellular matrix remodeling may contribute to the pathogenesis of proliferative atherosclerosis and atherosclerotic iliac artery aneurysm formation.

Material and Methods

Animals

Apoe<sup>−/−</sup> mice<sup>16</sup> were purchased from the Jackson Laboratory. MT1-MMP heterozygous Mmp14<sup>+/+</sup>/ Apoe<sup>−/−</sup> mice were maintained on a C57BL6/J background with >5 generations of backcrossing.<sup>14,17</sup> Mmp14<sup>−/+</sup>/ Apoe<sup>−/−</sup> mice were crossed to Apoe<sup>−/−</sup> mice to generate Mmp14<sup>−/+</sup>/ Apoe<sup>−/−</sup> breeders. Mmp14<sup>−/−</sup>/ Apoe<sup>−/−</sup> mice were used for breeding with Apoe<sup>−/−</sup> mice to generate Mmp14<sup>−/−</sup>/ Apoe<sup>−/−</sup> mice and their littermate Mmp14<sup>−/+</sup>/ Apoe<sup>−/−</sup> mice for this study. Mmp14<sup>−/−</sup>/ mice were generated as described previously.<sup>18</sup> These mice were crossed to SM22α-Cre (Tg[Tagln-cre]1Her/J),<sup>19</sup> Csf1r-Cre, or Fabp4-Cre-ERT2 transgenic mice (gift from Pierre Chambon, Institute of Genetics and Molecular and Cellular Biology, France),<sup>20</sup> and then further crossed to Apoe<sup>−/−</sup> mice.

Atherosclerosis Study

The atherosclerotic Western diet, composed of 17% (kcal/kcal) protein, 43% carbohydrate, and 41% fat with 1.5 g/kg cholesterol, was purchased from Research Diets. Male and female mice were fed a Western diet for 12 weeks beginning at 8 weeks of age. All procedures were approved by the University of Michigan committee on the use and care of animals, conforming to the guidelines of the International Association for Assessment and Accreditation of Laboratory Animal Care.

Morphometric Analysis of Atherosclerosis

After euthanasia by CO<sub>2</sub> asphyxiation, blood was collected through portal veins. Animals were perfused with PBS and then 10% formalin in PBS through their left ventricles at a rate...
of 1 mL/min, as described previously. Arterial trees were carefully dissected to include the brachiocephalic, left common carotid, and subclavian arteries as well as the descending thoracic and abdominal aortas with the bilateral iliac arteries. Adipose tissues attached to the arteries were carefully removed under a dissecting microscope. After Oil Red O staining and repeated washing in ethanol and water, aortic trees were pinned against a black background, and the aortic trees were digitally photographed under a dissecting microscope. Oil Red O-positive areas were quantified as the percentage of plaque area per total arterial tree or abdominal aorta.

**Collagen Staining and Histologic Analysis**

Sections were stained with hematoxylin and eosin and sirius red. The atheroma area and vascular wall area were quantified in cross-sections using ImageJ software (National Institutes of Health), and the average plaque and vascular wall areas were determined. Immunostaining was performed using the ABC system. The primary antibodies used were rabbit polyclonal anti-α-smooth muscle actin (anti-α-SMA) antibody, anti-F4/80 antibody, and anti-Ki67 antibody.

**Vascular Smooth Muscle Cells**

Mouse aortic smooth muscle cells were isolated with collagenase digestion, as described by others. Isolated primary mouse VSMCs were cultured in high-glucose DMEM with 10% FBS and passed twice before experiments. Mouse primary VSMCs immortalized with the SV40 large T antigen – often associated with obesity in humans. As such, we crossed MT1-MMP heterozygous (Mmp14+/−) mice with APOE-null mice to generate Mmp14+/−Apoe−/− mice and examined the effects of MT1-MMP heterozygosity on weight, fat mass, blood glucose, and insulin levels as well as cholesterol profiles. As expected, heterozygous (HT) Mmp14+/−Apoe−/− male mice on a Western diet were leaner than littermate wild-type (WT) Mmp14+/+Apoe−/− mice (WT 33.0±1.0 g, HT 29.0±0.9 g, n=9 each, P=0.009; Figure 1A). When fat mass was assessed at the end of the 12-week Western diet, the total and epididymal fat masses were significantly smaller in the Mmp14+/−Apoe−/− than Mmp14+/+Apoe−/− male mice (percentage of total fat mass per weight: WT 5.1±0.3%, HT 3.6±0.6%, n=9 each, P=0.03; percentage of epididymal fat per weight: WT 3.4±0.2%, HT 2.2±0.3%, n=9 each, P=0.007; Figure 1B). Fasting blood glucose levels were similar between the groups (WT 162±43 [n=8] versus HT 161±27 mg/dL [n=9], P=0.5); however, the fasting insulin concentration was substantially lower in the MT1-MMP heterozygous mice (WT 11.8±4.3 [n=8] versus HT 3.8±0.6 mU/L [n=9], P=0.04), suggesting an increased insulin sensitivity of Mmp14+/−Apoe−/− mice that occurs in parallel with their leaner phenotype. Of note, MT1-MMP heterozygosity did not change the blood cholesterol levels in APOE-null mice fed a Western diet (total cholesterol: WT 8±27 mg/dL [n=9], P>0.05; Figure 1C). We previously demonstrated that MT1-MMP heterozygosity renders mice resistant to obesity induced by a high fat diet. Based on this finding, we hypothesized that the allelic reduction of the MT1-MMP gene would protect mice from hypercholesterolemic atherosclerosis, a disease process that is often associated with obesity in humans.
1021±59 [n=8] versus HT 1012±52 mg/dL [n=9], P=0.9; direct low-density lipoprotein: WT 359±27 versus HT 349±23 mg/dL, P=0.8; Figure 1C). Consistent with their leaner phenotype and relative insulin sensitivity, the blood triglyceride content tended to be lower in HT mice, but this difference did not reach statistical significance (WT 142±10 mg/dL [n=8], HT 118±11 mg/dL [n=9], P=0.1).

Whole-Body MT1-MMP Heterozygosity Accelerates Atherogenesis

MT1-MMP WT and HT mice were fed a Western diet for 12 weeks beginning at 8 weeks of age. Contrary to our prediction, atherogenesis was more advanced in Mmp14+/−Apoe−/− mice compared with Mmp14+/+Apoe−/− littermate controls (Figure 1D). Particularly, advanced atherogenesis was notable in the abdominal aorta and iliac/femoral arteries of Mmp14+/−Apoe−/− mice (Figure 1D arrows). The percentage of total plaque area was increased by 21% in male mice (Mmp14+/−Apoe−/− 14.9±0.9% versus Mmp14+/+Apoe−/− 18.1±0.8%, n=7 each, P=0.04; Figure 1E, left), whereas the abdominal percentage of plaque area was increased by 200% (2.3±0.5% versus 6.9±0.5%, n=7 each, P<0.0001; Figure 1E, right). In female mice, the total plaque area in Mmp14+/−Apoe−/− animals was increased by 56% (15.1±1.3% [n=4] versus 23.6±1.6% [n=10], P=0.002; Figure 1F, left), with the

**Figure 1.** MT1-MMP (membrane-type 1 matrix metalloproteinase) heterozygosity promotes atherosclerosis. A, Mmp14+/+Apoe−/− mice and Mmp14+/−Apoe−/− mice were fed a Western diet for 12 weeks beginning at 8 weeks of age. Body weight (g) at the end of study. B, Percentage of fat mass (wt/wt) of total (epididymal plus inguinal) and epididymal fat pads. C, Fasting serum cholesterol (total and direct low-density lipoprotein) levels. Mean±SEM, n=8 and n=10, respectively; *P<0.05. D, Atherosclerotic lesions detected with Oil Red O staining in Mmp14+/−Apoe−/− and Mmp14+/+Apoe−/− male mice. Arrows point to the increased atherogenesis distributed in abdominal aorta and iliac arteries specifically, as found in Mmp14+/−Apoe−/− mice. E and F, Oil Red O–positive atherosclerotic plaque areas quantified in all aortic trees and abdominal aortas in male mice (n=7 each) and female mice (n=4 and n=10). Mean±SEM, *P<0.05, **P<0.005, ***P<0.0005. a. indicates artery.
abdominal plaque area increasing by 171% relative to the Mmp14+/−/Apoe−/− female controls (3.5±0.2% [n=4] versus 9.5±1.8% [n=10], P=0.002; Figure 1F, right).

We next examined left carotid artery sections to assess the structure of the atherosclerotic plaque and the associated level of vascular wall remodeling. Interestingly, the average plaque area of the Mmp14+/−/Apoe−/− mice was 3 times as large as that of the Mmp14+/+/Apoe−/− mice, but this was not statistically significant given the variable severity of atherosclerosis in the heterozygous group (WT 59±7×10³ μm² versus HT 189±84×10³ μm², n=6 each, P=0.15; Figure 2A). Concurrent with a trend of increased atherosclerosis, we observed a 1.7-fold increase in the vascular wall area, underscoring the presence of outward vascular wall remodeling in MT1-MMP heterozygous APOE-null mice (133±16×10³ μm² versus 234±19×10³ μm², n=6 each, P=0.004). Furthermore, both the atherosclerotic plaques and the vascular walls of MT1-MMP HT mice displayed an increased collagen fiber content relative to WT mice, as assessed by sirius red staining (sirius red–positive area: WT 26.6±3.3% versus HT 44.0±4.7%, n=6 each, P=0.009; Figure 2B), suggesting that MT1-MMP heterozygosity promoted vascular wall arteriosclerosis along with plaque formation in the APOE-null background. MT1-MMP HT mice also displayed an apparent loss of a contractile phenotype (ie, loss of SMA staining) coupled with disrupted elastic lamina,

Figure 2. MT1-MMP (membrane-type 1 matrix metalloproteinase) heterozygosity promotes plaque formation and outward vascular remodeling. A, Representative histology sections of left common carotid arteries. Hematoxylin and eosin (H&E) and sirius red staining. Scale=100 μm. B, Sirius red–positive area (%). C, Immunostaining of smooth muscle actin (SMA) in vascular walls and atherosclerotic plaques (orange). Nuclei were counterstained (blue). Lower panels are of higher magnification. Scale=100 μm. D, SMA-positive area (percentage). Mag indicates magnification. *P<0.05, **P<0.005

DOI: 10.1161/JAHA.116.003693
which in turn elicited more complex and advanced atherosclerotic lesions (Figure 2C and 2D).

MT1-MMP Gene Targeting in VSMCs Accelerates Atherosclerosis Progression

Although MT1-MMP heterozygosity was found to promote atherogenesis, the cellular mechanisms underlying the aggravated atherogenesis were unclear. In obesity, perivascular adipose tissues play a key role in atherogenesis.28 In contrast, dysfunctional VSMCs might also be responsible for inducing inflammatory atherogenesis.4 Because MT1-MMP is highly expressed in both adipocytes9,10 and VSMCs,15 we sought to use tissue-specific knockout mice in an effort to determine the cell type responsible for the MT1-MMP–dependent regulation of atherogenesis. When male mice in each group were fed a Western diet, we observed markedly augmented atherogenesis and aneurysm formation only with VSMC-specific MT1-MMP gene targeting (ie, SM22α-Cre(+)/Mmp14F/FApoel−/− mice), but not following adipocyte MT1-MMP gene targeting (ie, aP2-Cre-ERT2(+)Mmp14F/FApoel−/− mice; Figure 3A). Atherogenesis in SM22α-Cre(+)/Mmp14F/FApoel−/− mice.

![Figure 3](image_url)

**Figure 3.** Vascular smooth muscle cell MT1-MMP (membrane-type 1 matrix metalloproteinase) gene targeting promotes atherosclerosis and aneurysm formation. A, Whole arterial trees assessed for Oil Red O–positive atherosclerotic plaque area (red). Arrows point to atherosclerotic aneurysms. Representative arterial trees from SM22α-Cre(−)/Mmp14F/FApoel−/− and SM22α-Cre(+)/Mmp14F/FApoel−/− mice are shown; n=13 and n=20, respectively. B, Higher magnification of aortic aneurysms found in SM22α-Cre(+)Mmp14F/FApoel−/− mice. C, Percentage of plaque area in Cre(−)/Mmp14F/FApoel−/−, SM22α-Cre(+)Mmp14F/FApoel−/−, aP2-Cre-ERT2(+)Mmp14F/FApoel−/− mice, males and females (n=8, n=13, and n=4 in male mice, and n=5, n=7, and n=7 in female mice, respectively). *P<0.05, ***P<0.0005.
mice frequently extended to femoral arteries with significant outward remodeling having been noted, in tandem with aneurysm formation in the iliac arteries (Figure 3A and 3B). Importantly, total plaque area was increased by 190% in SM22α-Cre(+)Mmp14+/−/ApoE−/− male mice compared with Cre(−)Mmp14+/−/ApoE−/− male mice (8.0±1.4% [n=8] versus 23.5±1.4% [n=13], P<0.0001) and 210% in female mice (4.9±0.9% [n=5] versus 15.5±3.5% [n=7], P<0.03). In contrast, significant differences were not observed between Cre-negative controls and the adipose-specific MT1-MMP deletion model (Figure 3C and Figure S1), suggesting that the VSMCs are the primary cell type that mediates MT1-MMP–dependent modulation of atherogenesis progression.

The Loss of VSMC MT1-MMP Leads to Proliferative Atherosclerotic Lesions and Aneurysm Formation

After 12-week Western diet feeding, SM22α-Cre(+) Mmp14+/−/ApoE−/− mice developed extensive atherosclerosis in their common iliac arteries followed by aneurysm formation (Figure 4A and 4B). No aneurysm formation was observed in the Cre(−)Mmp14+/−/ApoE−/− mice (8 male and 5 female mice), whereas 11 of 12 male and 6 of 7 female SM22α-Cre(+) Mmp14+/−/ApoE−/− mice developed strikingly enlarged aneurysms that were readily observable on dissection (Figure 3B). These dysmorphic lesions displayed significant vascular wall thickening as well as atheroma formation (Figure 4A). Masson’s Trichrome and Verhoeff–Van Gieson staining demonstrated the disruption and loss of elastic laminae (Figure 4A). SMA staining showed an increased number of SMA+ cells in vascular walls as well as within atheroma, where Ki67 staining confirmed that large numbers of SMA+ cells existed in a proliferative state (Figure 4A and 4D). Infiltration of F4/80-positive cells was observed in the atheroma and the vascular walls of affected vessels (Figure 4A, F4/80 staining) but to a lesser extent compared with VSMCs. Image quantification of the lesions confirmed an increased atheroma area (Figure 4B) and outward remodeling (Figure 4C), which were coupled with an increased number of proliferating VSMCs (Figure 4D). Because macrophages are potentially targeted by SM22α promoter-driven Cre expression,29 we specifically targeted these cells using Csf1r-Cre(+) Mmp14+/−/ApoE−/− mice and examined atherosclerosis and aneurysm formation. Neither littermate control Csf1r-Cre(−) Mmp14+/−/ApoE−/− mice nor Csf1r-Cre(+) Mmp14+/−/ApoE−/− mice developed atherosclerotic iliac aneurysm after 12 weeks of Western diet (n=7 for each group tested), and no significant differences in the sizes of atherosclerosis lesions were observed (n=4 each).

Proinflammatory and Metabolically Dysfunctional MT1-MMP–Null VSMCs

To assess the role of MT1-MMP in VSMC function, we isolated primary VSMCs from descending aortas. As expected, VSMCs isolated from SM22α-Cre(+) Mmp14+/−/ApoE−/− mice demonstrated specific Cre expression along with the suppression MT1-MMP gene expression (Figure S2). In vitro, MT1-MMP–null VSMCs displayed a flattened, spread shape with higher stress fiber formation (Figure 5A), whereas the expression of other MMPs (eg, MMP-2, MMP-8, MMP-9, MMP-13, and MT2-MMP [MMP15]), were not significantly different between control and MT1-MMP–deleted cells (Figure S2). The Acta2 gene, which encodes α-SMA, was expressed equally in primary VSMCs isolated from SM22α-Cre(−) Mmp14+/−/ApoE−/− and SM22α-Cre(+) Mmp14+/−/ApoE−/− mice (Figure S2). To gain further insight into the effects of MT1-MMP gene targeting in VSMCs, whole-genome transcriptome analysis was performed. Using a minimum 2-fold difference as a cutoff, 414 genes were found to be differentially expressed between the 2 groups (the top 70 genes are shown in Figure 5B, and all differentially expressed genes are listed in Table S1; the microarray data is available at NCBI Gene Expression Omnibus [GSE] as GSE100661). Interestingly, the genes upregulated in SM22α-Cre(+) Mmp14+/−/ApoE−/− mice (>2.3-fold change) were aggregated in the pathways of inflammation and cell killing (Figure 5C), suggesting the acquisition of a proinflammatory phenotype in MT1-MMP–null VSMCs.

MT1-MMP Gene Targeting Promotes VSMC Proliferation in 3-Dimensional Cell–Cell and Cell–ECM Contexts

To gain insights into the mechanism by which MT1-MMP–null VSMCs engage in the formation of proliferative atherosclerotic lesions, we developed an in vivo–like 3-dimensional (3-D) spheroid culture system in which in vivo–like cell–cell and cell–ECM interactions could be recapitulated. Using Ki67 as an index of proliferative responses, MT1-MMP–null primary VSMCs displayed markedly higher activity relative to WT cells (Figure 6A and 6B). To determine whether the observed differences in cell proliferation were a direct consequence of MT1-MMP gene targeting (ie, as opposed to a secondary response of VSMCs recovered from the advanced atherogenic lesions found in VSMC-knockout mice), we used immortalized mouse primary VSMCs to further define the role of MT1-MMP in regulating VSMC proliferation. In 3-D spheroid culture, similar to the findings obtained with freshly isolated VSMCs, small interfering RNA–mediated MT1-MMP gene silencing in mouse VSMCs induced a marked increase in the number of Ki67-positive VSMCs (Figure 6C and 6D). Of note, under 2-D culture...
conditions, MT1-MMP silencing exerted minimal effects on mouse VSMC proliferation (Figure 6E and 6F). Taken together, these results support a model in which MT1-MMP plays a required role in regulating VSMC proliferative activity but only under 3-D conditions that more closely recapitulate the in vivo environment.
Discussion

MMP family members play a key role in ECM turnover in a wide variety of developmental and disease processes. Among the collagen-degrading MMPs, secreted (MMP-3, MMP-8, MMP-13) and membrane-type MMPs (MT1- and MT2-MMP) display distinct temporospatial differences in their patterns of expression and activity. Unlike other MMPs, MT1-MMP (MMP14) is the only family member whose activity is indispensable for postnatal development. Because of the premature morbidity and mortality displayed by MT1-MMP–null mice, the role of the proteinase in cardiovascular disease has remained elusive. To date, the only MT1-MMP–expressing cellular compartment tested in a mouse atherogenesis model was bone marrow–derived myeloid cells; however, no substantial impact on atheroma size was observed following bone marrow reconstitution with Mmp14−/− cells. In our study, we identified the critical role played by VSMC-derived MT1-MMP in regulating the progression of atherosclerotic lesions and the associated formation of vascular aneurysms.

Previously, we demonstrated that MT1-MMP heterozygosity protects C57BL/6 mice from diet-induced adipose tissue expansion. Similarly, in this study, heterozygous Mmp14+/− mice displayed a leaner phenotype relative to WT mice. Given the metabolically improved status of Mmp14+/− mice, we initially hypothesized that MT1-MMP heterozygosity would protect mice from hypercholesterolemic atherosclerosis, which is often associated with increased adiposity. Contrary to our expectations, MT1-MMP heterozygous APOE-null mice developed more extensive atherosclerotic lesions than MT1-MMP–sufficient APOE-null mice, suggesting a potentially beneficial role for MT1-MMP in limiting disease progression. The results also

Figure 5. Proinflammatory gene expression in MT1-MMP (membrane-type 1 matrix metalloproteinase)–null vascular smooth muscle cells (VSMCs). A, Primary aortic VSMCs isolated from Cre(−)/Mmp14+/−Expressing APOE−/− and SM22α-Cre(+/−)/Mmp14+/−Expressing APOE−/− mice. F-actin (green), nuclei (blue). B, Representative genes differentially expressed in VSMCs from 2 independent pairs of Cre(−)/Mmp14+/−Expressing APOE−/− and SM22α-Cre(+/−)/Mmp14+/−Expressing APOE−/− mice. C, Gene Ontology (GO) biological processes represented by the genes upregulated in VSMCs isolated from the SM22α-Cre(+/−)/Mmp14+/−Expressing APOE−/− mice.
Figure 6. MT1-MMP (membrane-type 1 matrix metalloproteinase) limits vascular smooth muscle cell (VSMC) proliferation in 3-dimensional (3-D) organoids. A, Primary VSMCs isolated from Cre(−) Mmp14F/F Apoe−/− mice and Cre(+)Mmp14F/F Apoe−/− mice were cultured as a 3-D spheroids for 48 hours and stained for Ki67 (red), nuclei (DAPI, blue), and actin (phalloidin, green). B, Quantified intensity of Ki67 staining per spheroid. n=5 to 7. *P<0.05. C, Immortalized mouse VSMCs (MOVAS) transiently transfected with small interfering RNA (siRNA) control (siControl) and MT1-MMP siRNA (siMmp14). Ki67 (red), nuclei (blue), actin (green). D, Ki67 staining intensity per spheroid of MOVAS. **P<0.005. E, 2-D cultured MOVAS transfected with control and MT1-MMP siRNA. Ki67 (red), nucleus (blue), and actin (green). F, Ki67-positive nuclei per total nuclei count.
suggest that the inverse relationship observed between fat mass and atherosclerosis—the so-called obesity paradox in humans—may reflect the independent biological effects exerted by a cohort of modifier genes, including MT1-MMP, on adipose tissue and vascular function.

To define the cellular mechanisms by which MT1-MMP exerts its antiatherogenic effects, we embarked on a series of studies aimed at tissue-specific MT1-MMP gene targeting in APOE-null mice. In the early stages of our efforts, we focused on 2 cell types: adipocytes and VSMCs. We initially hypothesized that the loss of adipocyte MT1-MMP would modify atherogenesis via the potentially causal links that exist between adipose tissue and the vascular wall. In APOE-null mice, however, we were unable to observe a significant impact on atherogenesis following gene targeting of adipocyte-derived MT1-MMP. In contrast, the SM22α-Cre-mediated loss of VSMC MT1-MMP strikingly aggravated atherosclerosis progression in APOE-null mice. Whole-genome transcriptome analyses indicated that a series of proinflammatory genes were upregulated in MT1-MMP–null VSMCs, a finding consistent with the proatherogenic phenotype of SM22α-Cre(+)/Mmp14fl/fl/Apoel/− mice. Furthermore, our in vitro studies demonstrated that MT1-MMP targeting in either primary or immortalized VSMCs promotes cell proliferation under 3-D spheroid culture conditions. As such, 3-D spheroid culture appears to reflect a set of conditions that better reflect our in vivo findings and, as such, can be used to more faithfully address cellular behavior in tissue-like contexts by re-creating cell–cell and cell–ECM interactions ex vivo. Indeed, previous work has demonstrated that VSMC proliferative activity can be regulated by cell–cell adhesion and cell–ECM interactions. Of note, the enhanced proliferative responses displayed by MT1-MMP–null VSMCs were not observed under conventional 2-D culture conditions in which the cell–cell and cell–ECM interactions that are encountered in vivo are replaced by cell culture atop a nonphysiologic, planar, and rigid substratum. Finally, it is interesting to note that the proliferative effects of MT1-MMP gene silencing were not observed in other cell types, for example, 3T3-L1 preadipocytes (data not shown), reinforcing the unique role played by MT1-MMP in VSMC biology.

In considering the mechanisms by which MT1-MMP might control proliferative activity, efforts are complicated by the proteinase’s broad substrate repertoire, ranging from type I collagen to CD44 and cadherins. As such, MT1-MMP can potentially regulate VSMC proliferation by degrading any number of membrane-associated protein targets as well as pericellular ECM molecules, thereby modifying both cell–cell adhesion and cell–ECM interaction. In atherosclerosis, the expression of adhesion molecules and ECM proteins is highly upregulated; as such, a decrease in MT1-MMP activity would be predicted to trigger the excess accumulation of ECM macromolecules as well as cell-surface adhesion molecules. At this juncture, we posit that changes in the dynamics of ECM turnover and cell-surface molecule expression occurring within the vascular wall lead to the unregulated proliferation of VSMCs and the development of a proinflammatory phenotype. Interestingly, at least in terms of proliferative responses, the use of a 3-D spheroid model allowed us to recapitulate the MT1-MMP–dependent biological processes ex vivo. Nevertheless, it remains unclear how the cleavage of MT1-MMP substrate(s) accelerates VSMC proliferation in APOE-null mice. This caveat notwithstanding, our data clearly highlight the role played by VSMC-derived MT1-MMP in limiting the progression of proliferative atherosclerotic lesions. Because MT1-MMP is a membrane-bound proteinase, the physical proximity of substrates with the enzyme is likely critical for the protective effects exerted by VSMCs in limiting the expansion of atherosclerotic lesions. Our study also suggests that other atherosclerosis-associated MMPs (eg, MMP-2, MMP-8, MMP-13) do not compensate for the genetic loss of MT1-MMP and are unable to limit atherosclerosis progression. Although MT2-MMP (MMP15) was also expressed in mouse VSMCs (Figure S2), as reported previously in rat VSMCs, the biological phenotypes conferred by MT1-MMP gene loss were not rescued by the presence of MT2-MMP. Differences in the hemopexin domain structure, posttranslational modification, or protein trafficking may underlie the specific effects mediated by MT1-MMP versus MT2-MMP.

Although increased collagen content within atheroma might be predicted to play a protective role against plaque rupture, collagen accumulation in arterial walls could also accentuate vascular sclerosis and stiffening. In turn, stiff and sclerotic blood vessels could increase luminal shear stress, thereby increasing the chance of plaque rupture. In future studies, an assessment of hemodynamic changes and plaque instability in our model will be required to more accurately define the role of MT1-MMP in cardiovascular disease. Interestingly, in APOE-null mice, arterial wall stiffness is known to be increased through VSMC lysyl oxidase activity. In turn, increased tissue stiffness could control VSMC proliferation through the activation of a mechanotransduction pathway, for example, YAP/TAZ transcription activity. As such, we posit that the pathologic accumulation of ECM macromolecules secondary to the loss of MT1-MMP activity may further promote vascular wall rigidity in the APOE-null mice, leading to hyperproliferative vascular lesions and aneurysm formation. Given that we observed severe atherosclerosis and aneurysm formation in iliac and femoral arteries, site-specific increases in vascular wall thickening and arteriosclerosis are likely related to the distinct mechanical properties of vessel walls observed along the arterial tree. Indeed, femoral arteries as well as the abdominal aorta are known to display higher wall thickness with lower content of
elastic lamina compared with carotid arteries and thoracic aortas. Together, differences in mechanical stress and ECM composition may render VSMCs in abdominal aorta and femoral arteries more vulnerable to atherogenic proliferation. Finally, it remains to be determined whether the inflammatory gene expression profile observed in MT1-MMP–null VSMCs is restricted to atherogenic milieu encountered in vivo. Interestingly, in our hands, the increase in inflammatory gene expression of MT1-MMP–null VSMCs was coupled with decreased mitochondrial activity (T. Akama, PhD, unpublished data, 2016). Furthermore, recent studies have demonstrated a critical role played by a VSMC phenotypic switch to macrophage-like cells in atherogenesis. Because MT1-MMP also regulates inflammatory responses in macrophages, the proteinase may well play a key role in controlling a complex set of metabolic and phenotypic switching programs that are engaged in atherogenic VSMCs. Although further work is needed to delineate MT1-MMP function during atherogenesis, our work highlights the previously unsuspected vessel wall–protective effects exerted by this membrane-anchored metalloproteinase in the VSMC compartment.

Acknowledgments
We like to thank Dr Pierre Chambon (Institute of Genetics and Molecular and Cellular Biology, France) for sharing Fabp4-Cre-ERT2 transgenic mice. Current affiliation of Ohman is Duke-Nus Medical School, Singapore.

Sources of Funding
Funding was provided by the McKay Research Grant from University of Michigan Cardiovascular Center, NIH R21HL106332, and R01DK102656 to Chun. NIH R01AI105068-01 to Weiss. NIH Cancer Biology Training Program Grant T32-CA009676 supported Bahr.

Disclosures
None.

References
1. Olivetti G, Anversa P, Mellisari M, Loud AV. Morphometric study of early postnatal development of the thoracic aorta in the rat. Circ Res. 1980;47:417–424.
2. Wagenknecht JE, Mecham RP. Vascular extracellular matrix and arterial mechan- ics. Physiol Rev. 2009;89:957–989.
3. Owens GK, Kumar MS, Wamhoff BR. Molecular regulation of vascular smooth muscle cell differentiation in development and disease. Physiol Rev. 2004;84:767–801.
4. Bennett MR, Sinha S, Owens GK. Vascular smooth muscle cells in atherosclerosis. Circ Res. 2016;118:692–702.
5. Page-McCaw A, Ewald AJ, Werb Z. Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol. 2007;8:221–233.
6. Kuzuya M, Nakamura K, Sasaki T, Wu Cheng X, Itohara S, Iuchi A. Effect of MMP–2 deficiency on atherosclerotic lesion formation in apoE-deficient mice. Arterioscler Thromb Vasc Biol. 2006;26:1120–1125.
7. Laxton RC, Hu Y, Duchene J, Zhang F, Zhang Z, Leung KY, Xiao Q, Scotland RS, Hodgkinson CP, Smith K, Willeit J, López-Otin C, Simpson IA, Kiechl S, Ahluwalia A, Xu Q, Ye S. A role of matrix metalloproteinase-8 in atherosclerosis. Circ Res. 2009;105:921–929.
8. Quillard T, Araujo HA, Franch G, Tesenitsky S, Libby P. Matrix metalloproteinase-13 predominates over matrix metalloproteinase-8 as the functional interstitial collagenase in mouse atheroma. Arterioscler Thromb Vasc Biol. 2014;34:1179–1186.
9. Sabeht F, Li XY, Saunders TL, Rowe RG, Weiss SJ. Secreted versus membrane-anchored collagenases: relative roles in fibroblast-dependent collagenolysis and invasion. J Biol Chem. 2009;284:23001–23011.
10. Chun TH, Holmbeck K, Liberf, Babiott GB, Allen ED, Weiss SJ. A pericellular collagenase directs the 3-dimensional development of white adipose tissue. Cell. 2006;125:577–591.
11. Holmbeck K, Blanco P, Caterina J, Yamada S, Kromer M, Kuznetsova NS, Manikani M, Robey PG, Poole AR, Piedbo P, Ward JM, Birkedal-Hansen H. MT1-MMP-deficient mice develop dwarfism, osteopenia, arthritis, and connective tissue disease due to inadequate collagen turnover. Cell. 1999;99:81–92.
12. Zhou Z, Apte SS, Soininen R, Cao R, Baaklini GY, Rauser RW, Wang J, Cao Y, Tryggvason K. Impaired endochondral ossification and angiogenesis in mice deficient in membrane-type matrix metalloproteinase I. Proc Natl Acad Sci USA. 2000;97:4052–4057.
13. Schneider F, Sukhova GK, Aikawa M, Canner J, Gerdes N, Shi GP, Apte SS, Libby P. Matrix metalloproteinase-14 deficiency in bone-marrow-derived cells promotes collagen accumulation in mouse atherosclerotic plaques. Circulation. 2008;117:931–939.
14. Chun TH, Inoue M, Morisaki H, Yamanaik A, Miyamoto Y, Okamura T, Sato- Kusubata K, Weiss SJ. Genetic link between obesity and MMP14-dependent adipogenic collagen turnover. Diabetes. 2010;59:2484–2494.
15. Filippov S, Koenig GC, Chun TH, Holtry KB, Ota I, Bugge TH, Roberts JD, Fay WP, Birkedal-Hansen H, Holmbeck K, Sabeht F, Allen ED, Weiss SJ. MT1-matrix metalloproteinase directs arterial wall invasion and neointima formation by vascular smooth muscle cells. J Exp Med. 2005;202:663–671.
16. Piedrahta JA, Zhang SH, Hamarajon JA, Oliver PM, Maeda N. Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. Proc Natl Acad Sci USA. 1992;89:4471–4475.
17. Yana I, Sagara H, Takaki S, Takatsu K, Nakamura K, Nakao K, Katsuki M, Taniguchi S, Aoki T, Sato H, Weiss SJ, Seiki M. Crosstalk between neovessels and mural cells directs the site-specific expression of MT1-MMP to endothelial tip cells. J Cell Sci. 2007;120:1607–1614.
18. Tang Y, Rowe RG, Botvnick EL, Kurup A, Putnam AJ, Seiki M, Weaver VM, Keller ET, Goldstein S, Dai J, Begun D, Saunders T, Weiss SJ. MT1-MMP-dependent control of skeletal stem cell commitment via a beta1-integrin/ YAP/TAZ signaling axis. Dev Cell. 2013;25:402–416.
19. Boucher P, Gotthardt M, Li WP, Anderson RG, Herz J. LRP: role in vascular wall integrity and protection from atherosclerosis. Science. 2003;300:329–332.
20. Imai T, Takakawa R, Marchand S, Dentsiz E, Bornert JM, Messaddeq N, Wenzel M, Mark M, Desvergne B, Wahl W, Chambon P, Metzger D. Peroxisome proliferator-activated receptor gamma is required in mature white adipocytes for their survival in the mouse. Proc Natl Acad Sci USA. 2004;101:4543–4547.
21. Ohman MK, Wright AP, Wikenheiser KJ, Luo W, Russo HM, Eitzman DT. Monocyte chemoattractant protein-1 deficiency protects against visceral fat-induced atherosclerosis. Arterioscler Thromb Vasc Biol. 2010;30:1151–1158.
22. Magnato-Garcia E, Tarrillo M, Lichtman AH. Mouse models of atherosclerosis. Curr Protoc Immunol. 2001;Chapter 15:Unit 15.2.1–1.23.
23. Chun TH, Inoue M. 3D adipocyte differentiation and peri-adipocyte collagen turnover. Methods Enzymol. 2014;538:15–34.
24. Lyon Ray J, Leach R, Herbert J-M, Benson M. Isolation of vascular smooth muscle cells from a single murine aorta. Methods Cell Sci. 2001;23:185–188.
25. Afroz T, Hussein M. C-Myc-binding sites mediate G1/S-associated repression of the plasma membrane Ca²⁺-ATPase 1–promoter. J Biol Chem. 2000;275:9062–9069.
26. Moreas G, Labuz JM, Leung BM, Inoue M, Chun TH, Takakawa S. On being the right size: scaling effects in designing a human-on-a-chip. Integr Biol (Camb). 2013;5:1149–1161.
27. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA. 2002;288:2466–2474.
28. Chang L, Villacorta L, Li R, Hamblin M, Xu W, Dou R, Zhang J, Wu J, Zeng R, Chen YE. Loss of perivascular adipose tissue on peroxisome proliferator-
activated receptor-gamma deletion in smooth muscle cells impairs intravascular thermoregulation and enhances atherosclerosis. Circulation. 2012;126:

1067–1078.

29. Shen Z, Li C, Frierl RA, Gerasimova AS, Lee SJ, Wu J, Wang MM, Lumeng CN, Brosius FC, Duan SZ, Mortensen RM. Smooth muscle protein 22 alpha-Cre is expressed in myeloid cells in mice. Biochem Biophys Res Commun. 2012;422:

639–642.

30. Mott JD, Werb Z. Regulation of matrix biology by matrix metalloproteinases. Curr Opin Cell Biol. 2004;16:

558–564.

31. Gustafson B. Adipose tissue, inflammation and atherosclerosis. J Atheroscler Thromb. 2010;17:

332–341.

32. Uglow EB, Slater S, Sala-Newby GB, Aguilera-Garcia CM, Angelini GD, Newby AC, George SJ. Dismantling of cadherin-mediated cell-cell contacts modulates smooth muscle cell proliferation. Circ Res. 2003;92:

1314–1321.

33. Koutsouki E, Beeching CA, Slater SC, Blaschuk OW, Sala-Newby GB, George SJ. N-cadherin-dependent cell-cell contacts promote human saphenous vein smooth muscle cell survival. Arterioscler Thromb Vasc Biol. 2005;25:

982–988.

34. Hou R, Liu L, Anees S, Hiroyasu S, Sibinga NES. The FAT1 cadherin integrates vascular smooth muscle cell growth and migration signals. J Cell Biol. 2006;173:

417–429.

35. Schlosser A, Pilecki B, Hemstra LE, Kejling K, Kristmannsdottir GB, Wulf-Katz S, Hansen PB, Stubbe J, Wrede C, Hegermann J, Ochs M, Rathkolb B, Schrewe A, Bekereldjian R, Wolf E, Gailus-Durner V, Fuchs H, Hrabe de Angelis M, Lindholt JS, Holmskov U, Sorensen GL. MFAP4 promotes vascular smooth muscle cell survival. Arterioscler Thromb Vasc Biol. 2016;36:

122–133.

36. Ikese M, Matsui Y, Ohta D, Danzaki K, Ito K, Kanayama M, Kurotaki D, Morimoto J, Kojima T, Tsutsui H, Uede T. Syndecan-4 deficiency limits neointimal formation after vascular injury by regulating vascular smooth muscle cell proliferation and vascular progenitor cell mobilization. Arterioscler Thromb Vasc Biol. 2011;31:

1066–1074.

37. Ohuchi E, Imai K, Fujii Y, Sato H, Seiki M, Okada Y. Membrane type 1 matrix metalloproteinase digests interstitial collagens and other extracellular matrix macromolecules. J Biol Chem. 1997;272:

2446–2451.

38. Kajita M, Itoh Y, Chiba T, Mori H, Okada A, Kino H, Seiki M. Membrane-type 1 matrix metalloproteinase cleaves CD44 and promotes cell migration. J Cell Biol. 2001;153:

893–904.

39. Covington MD, Burghardt RC, Parrish AR. Ischemia-induced cleavage of cadherins in NRK cells requires MT1-MMP (MMP-14). Am J Physiol Renal Physiol. 2006;290:

F43–F51.

40. Moiseeva EP. Adhesion receptors of vascular smooth muscle cells and their functions. Cardiovasc Res. 2001;52:

372–386.

41. Shofuda K, Yasumitsu H, Nishihashi A, Miki K, Miyazaki K. Expression of three membrane-type matrix metalloproteinases (MT-MMPs) in rat vascular smooth muscle cells and characterization of MT3-MMP with and without transmembrane domain. J Biol Chem. 1997;272:

9749–9754.
SUPPLEMENTAL MATERIAL
Table S1. List of genes differentially expressed in Cre(-) and SM22α-Cre(+)Mmp14^{F/F} VSMCs

| ENTREZ ID | SYMBOL | GENE NAME | Cre(-) 1 | Cre(-) 2 | Cre(+1) | Cre(+2) | Cre(AVG) | Cre(+AVG) | Log2 (pos-nega) | Fold-change |
|-----------|--------|-----------|----------|----------|---------|---------|----------|-----------|-----------------|-------------|
| 379043    | Raet1e | retinoic acid early transcript 1E | 3.4      | 3.2      | 5.6     | 6.7     | 3.3      | 6.2       | 2.8             | 7.1         |
| 57266     | Ccld14 | chemokine (C-X-C motif) ligand 14 | 5.4      | 4.7      | 8.6     | 6.4     | 5.0      | 7.5       | 2.5             | 5.7         |
| 20210     | Saa3   | serum amyloid A 3 | 3.4      | 6.0      | 7.3     | 7.0     | 4.7      | 7.1       | 2.5             | 5.5         |
| 14941     | G2md   | granzyme D | 2.0      | 2.2      | 6.8     | 2.1     | 2.1      | 4.4       | 2.4             | 5.1         |
| 57781     | Cd200r1| CD200 receptor 1 | 4.1      | 3.8      | 6.0     | 6.5     | 3.9      | 6.3       | 2.4             | 5.1         |
| 17533     | Mrc1   | mannose receptor, C type 1 | 4.9      | 5.7      | 7.0     | 7.7     | 5.3      | 7.4       | 2.1             | 4.2         |
| 80891     | Fcrls  | Fc receptor-like S, scavenger receptor | 6.0      | 3.7      | 8.2     | 5.6     | 4.8      | 6.9       | 2.1             | 4.2         |
| 232413    | Clec12a| C-type lectin domain family 12, member a | 4.8      | 5.3      | 7.3     | 6.8     | 5.0      | 7.0       | 2.0             | 4.1         |
| 56744     | Pf4    | platelet factor 4 | 4.3      | 5.2      | 5.5     | 8.1     | 4.8      | 6.8       | 2.0             | 4.1         |
| 21810     | Tgfbi  | transforming growth factor, beta induced | 6.0      | 6.7      | 8.2     | 8.5     | 6.3      | 8.3       | 2.0             | 4.0         |
| 72318     | Cyth4  | cytohesin 4 | 5.8      | 5.7      | 7.6     | 7.9     | 5.8      | 7.7       | 2.0             | 3.9         |
| 15951     | Ifi204 | interferon activated gene 204 | 3.2      | 4.3      | 6.1     | 5.3     | 3.8      | 5.7       | 2.0             | 3.9         |
| 320292    | Rasgef1b| RasGEF domain family, member 1B | 3.7      | 3.9      | 5.8     | 5.7     | 3.8      | 5.8       | 2.0             | 3.9         |
| 12774     | Ccr5   | chemokine (C-C motif) receptor 5 | 3.4      | 3.0      | 4.8     | 5.4     | 3.2      | 5.1       | 1.9             | 3.7         |
| 14276     | Folr2  | folate receptor 2 (fetal) | 5.0      | 4.5      | 8.0     | 5.4     | 4.8      | 6.7       | 1.9             | 3.7         |
| 20556     | Slfn2  | schlafen 2 | 6.2      | 5.9      | 7.9     | 8.0     | 6.0      | 7.9       | 1.9             | 3.7         |
| 15109     | Hal    | histidine ammonia lyase | 2.4      | 2.8      | 2.5     | 6.5     | 2.6      | 4.5       | 1.9             | 3.7         |
| 243277    | Gpr133 | G protein-coupled receptor 133 | 2.9      | 4.1      | 6.1     | 4.7     | 3.5      | 5.4       | 1.9             | 3.6         |
| 68774     | Ms4a6d | membrane-spanning 4-domains, subfamily A, member 6D | 5.7      | 5.6      | 7.5     | 7.5     | 5.6      | 7.5       | 1.8             | 3.6         |
| 17916     | Myo1f  | myosin IF | 5.6      | 5.7      | 7.3     | 7.7     | 5.7      | 7.5       | 1.8             | 3.6         |
| 107321    | Lpxn   | leupaxin | 3.2      | 3.7      | 5.5     | 5.0     | 3.4      | 5.2       | 1.8             | 3.5         |
| 246177    | Myo1g  | myosin IG | 3.3      | 3.3      | 4.4     | 5.8     | 3.3      | 5.1       | 1.8             | 3.5         |
| 20452     | St8sia4| ST8 alpha-N-acetylneuraminide alpha-2,8-sialyltransferase 4 | 4.0      | 4.5      | 5.7     | 6.3     | 4.3      | 6.0       | 1.8             | 3.4         |
| 109648    | Npy    | neuropeptide Y | 8.0      | 7.5      | 8.9     | 10.1    | 7.7      | 9.5       | 1.8             | 3.4         |
| 17084     | Ly86   | lymphocyte antigen 86 | 4.3      | 5.5      | 6.2     | 7.0     | 4.9      | 6.6       | 1.8             | 3.4         |
| 19264     | Ptprc  | protein tyrosine phosphatase, receptor type, C | 6.6      | 6.7      | 8.3     | 8.5     | 6.6      | 8.4       | 1.8             | 3.4         |
| 13733     | Emr1   | EGF-like module containing, mucin-like, hormone receptor-like sequence 1 | 6.1      | 6.0      | 7.9     | 7.6     | 6.0      | 7.8       | 1.7             | 3.3         |
| 14727     | Gp49a  | glycoprotein 49 A | 6.6      | 5.9      | 7.8     | 8.2     | 6.3      | 8.0       | 1.7             | 3.3         |
| 23845     | Clec5a | C-type lectin domain family 5, member a | 4.4      | 4.7      | 5.8     | 6.8     | 4.6      | 6.3       | 1.7             | 3.3         |
| 20568     | Slpi   | secretory leukocyte peptidase inhibitor | 5.0      | 4.7      | 6.2     | 6.9     | 4.9      | 6.6       | 1.7             | 3.3         |
| Gene Symbol | Description                                      | Value   |
|-------------|--------------------------------------------------|---------|
| Ccl9        | Chemokine (C-C motif) ligand 9                   | 5.2 6.0 6.9 7.7 5.6 7.3 1.7 3.2 |
| Pib         | Paired Ig-like receptor B                        | 5.5 5.9 7.1 7.7 5.7 7.4 1.7 3.2 |
| Bnk         | B cell linker                                    | 6.1 6.1 7.8 7.8 6.1 7.8 1.7 3.2 |
| AF357355    | snoRNA AF357355                                 | 3.2 3.2 6.1 3.6 3.2 4.9 1.7 3.2 |
| Msr1        | Macrophage scavenger receptor 1                 | 6.4 6.7 8.1 8.4 6.6 8.2 1.7 3.2 |
| Tspan13     | Tetraspanin 13                                  | 4.0 5.3 6.4 6.2 4.6 6.3 1.7 3.2 |
| Adam8       | A disintegrin and metallopeptidase domain 8     | 6.6 6.5 8.3 8.0 6.5 8.2 1.7 3.1 |
| Dpep2       | Dipeptidase 2                                   | 5.3 3.9 6.6 5.9 4.6 6.3 1.7 3.1 |
| Igap2       | IQ motif containing GTPase activating protein 2  | 5.4 4.9 6.3 7.3 5.2 6.8 1.6 3.1 |
| Tnfaip8l2   | Tumor necrosis factor, alpha-induced protein 8-like 2 | 4.6 4.9 6.1 6.8 4.8 6.4 1.6 3.1 |
| Cd48        | CD48 antigen                                    | 5.8 5.3 6.9 7.5 5.6 7.2 1.6 3.1 |
| AB124611    | cDNA sequence AB124611                         | 5.1 5.1 6.3 7.1 5.1 6.7 1.6 3.1 |
| Irf8        | Interferon regulatory factor 8                  | 5.5 5.2 6.6 7.3 5.4 7.0 1.6 3.1 |
| Ccl22       | Chemokine (C-C motif) ligand 22                 | 2.7 3.3 2.6 6.7 3.0 4.6 1.6 3.0 |
| Selplg      | Selectin, platelet (p-selectin) ligand          | 5.5 5.1 6.3 7.5 5.3 6.9 1.6 3.0 |
| Vav1        | Vav 1 oncogene                                  | 4.5 3.8 5.6 5.9 4.2 5.8 1.6 3.0 |
| Fabp5       | Fatty acid binding protein 5, epidermal         | 8.5 8.0 9.4 10.2 8.2 9.8 1.6 3.0 |
| Mpeg1       | Macrophage expressed gene 1                    | 6.9 6.6 8.0 8.7 6.8 8.3 1.6 3.0 |
| P2ry6       | Pyrimidinergic receptor P2Y, G-protein coupled, 6 | 6.9 6.6 8.5 8.2 6.7 8.3 1.6 3.0 |
| Ptpn6       | Protein tyrosine phosphatase, non-receptor type 6 | 5.0 5.3 6.4 7.1 5.2 6.7 1.6 3.0 |
| Trem2       | Triggering receptor expressed on myeloid cells 2 | 7.4 7.0 8.6 9.0 7.2 8.8 1.6 3.0 |
| Alox5ap     | Arachidonate 5-lipoxygenase activating protein   | 7.7 7.4 9.1 9.1 7.6 9.1 1.6 2.9 |
| C1qa        | Complement component 1, q subcomponent, alpha polypeptide | 6.6 6.9 8.2 8.4 6.7 8.3 1.6 2.9 |
| Ccl3        | Chemokine (C-C motif) ligand 3                  | 6.4 7.1 7.7 9.0 6.8 8.3 1.6 2.9 |
| Psm9        | Proteasome (prosome, macropain) subunit, beta type 9 (large multifunctional peptidase 2) | 4.6 4.8 5.8 6.7 4.7 6.3 1.6 2.9 |
| Fcgr3       | Fc receptor, IgG, low affinity III              | 6.3 6.7 7.8 8.3 6.5 8.1 1.5 2.9 |
| Ccr1        | Chemokine (C-C motif) receptor 1                | 3.3 3.4 4.0 5.7 3.3 4.9 1.5 2.9 |
| Cdt4        | CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated) | 3.9 3.4 3.7 6.6 3.6 5.1 1.5 2.9 |
| Ncf2        | Neutrophil cytosolic factor 2                  | 5.2 5.6 6.5 7.4 5.4 7.0 1.5 2.9 |
| Ncf4        | Neutrophil cytosolic factor 4                  | 3.6 4.3 5.2 5.8 4.0 5.5 1.5 2.9 |
| Fcgr1       | Fc receptor, IgG, high affinity I               | 4.6 4.6 6.4 5.8 4.6 6.1 1.5 2.9 |
| 1700054O19Rik| RIKEN cDNA 1700054O19 gene                     | 2.5 4.0 4.8 4.7 3.2 4.7 1.5 2.8 |
| gene   | description                                                                 | row_1 | row_2 | row_3 | row_4 | row_5 | row_6 | row_7 | row_8 | row_9 | row_10 |
|--------|------------------------------------------------------------------------------|-------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| 12767  | Cxcr4  | chemokine (C-X-C motif) receptor 4                                           | 6.8   | 6.6   | 8.1   | 8.3   | 6.7   | 8.2   | 1.5   | 2.8   |        |        |
| 23900  | Hcst   | hematopoietic cell signal transducer                                         | 5.1   | 5.7   | 6.2   | 7.6   | 5.4   | 6.9   | 1.5   | 2.8   |        |        |
| 54712  | Plxnc1 | plexin C1                                                                     | 6.5   | 5.6   | 7.4   | 7.7   | 6.0   | 7.5   | 1.5   | 2.8   |        |        |
| 19354  | Rac2   | RAS-related C3 botulinum substrate 2                                          | 6.9   | 6.9   | 8.1   | 8.7   | 6.9   | 8.4   | 1.5   | 2.8   |        |        |
| 668218 | Bin2   | bridging integrator 2                                                         | 3.0   | 2.9   | 3.9   | 5.0   | 2.9   | 4.4   | 1.5   | 2.8   |        |        |
| 217305 | Cd300ld| CD300 molecule-like family member d                                           | 4.8   | 5.2   | 6.2   | 6.7   | 5.0   | 6.5   | 1.5   | 2.8   |        |        |
| 12984  | Csf2rb2| colony stimulating factor 2 receptor, beta 2, low-affinity (granulocyte-macrophage) | 6.3   | 6.0   | 7.3   | 8.0   | 6.1   | 7.6   | 1.5   | 2.8   |        |        |
| 13618  | Ednrb  | endothelin receptor type B                                                    | 4.4   | 4.3   | 5.2   | 6.4   | 4.3   | 5.8   | 1.5   | 2.8   |        |        |
| 14130  | Fcgr2b | Fc receptor, IgG, low affinity IIb                                            | 5.4   | 5.4   | 6.4   | 7.5   | 5.4   | 6.9   | 1.5   | 2.8   |        |        |
| 14544  | Gda    | guanine deaminase                                                             | 4.8   | 5.9   | 6.3   | 7.4   | 5.4   | 6.8   | 1.5   | 2.8   |        |        |
| 14744  | Gpr65  | G-protein coupled receptor 65                                                 | 5.8   | 4.9   | 7.1   | 6.7   | 5.4   | 6.9   | 1.5   | 2.8   |        |        |
| 66857  | Plbd1  | phospholipase B domain containing 1                                            | 3.2   | 4.9   | 3.2   | 7.8   | 4.0   | 5.5   | 1.5   | 2.8   |        |        |
| 16534  | Kcnn4  | potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4 | 4.0   | 4.3   | 5.8   | 5.5   | 4.2   | 5.7   | 1.5   | 2.8   |        |        |
| 320207 | Pik3r5 | phosphoinositide-3-kinase, regulatory subunit 5, p101                         | 5.9   | 5.7   | 7.2   | 7.4   | 5.8   | 7.3   | 1.5   | 2.8   |        |        |
| 216869 | Arrb2  | arrestin, beta 2                                                              | 5.5   | 5.5   | 6.8   | 7.1   | 5.5   | 7.0   | 1.5   | 2.8   |        |        |
| 19735  | Rgs2   | regulator of G-protein signaling 2                                             | 5.3   | 4.4   | 6.2   | 6.5   | 4.9   | 6.3   | 1.5   | 2.8   |        |        |
| 217203 | Tmem106a| transmembrane protein 106A                                                   | 6.9   | 6.8   | 8.4   | 8.2   | 6.8   | 8.3   | 1.5   | 2.8   |        |        |
| 215632 | Psd4   | pleckstrin and Sec7 domain containing 4                                       | 4.1   | 2.9   | 4.7   | 5.1   | 3.5   | 4.9   | 1.5   | 2.7   |        |        |
| 226652 | Arhgap30| Rho GTPase activating protein 30                                               | 4.8   | 4.6   | 5.8   | 6.4   | 4.7   | 6.1   | 1.4   | 2.7   |        |        |
| 12047  | Bcl2a1d| B cell leukemia/lymphoma 2 related protein A1d                               | 7.6   | 7.5   | 8.9   | 9.1   | 7.5   | 9.0   | 1.4   | 2.7   |        |        |
| 76933  | Ifi27l2a| interferon, alpha-inducible protein 27 like 2A                               | 3.5   | 3.4   | 5.4   | 4.4   | 3.5   | 4.9   | 1.4   | 2.7   |        |        |
| 16197  | Il7r   | interleukin 7 receptor                                                        | 6.7   | 7.2   | 7.8   | 9.0   | 7.0   | 8.4   | 1.4   | 2.7   |        |        |
| 17085  | Ly9    | lymphocyte antigen 9                                                          | 4.1   | 4.3   | 5.2   | 6.1   | 4.2   | 5.7   | 1.4   | 2.7   |        |        |
| 105855 | Nckap1l| NCK associated protein 1 like                                                 | 6.9   | 6.8   | 8.2   | 8.4   | 6.8   | 8.3   | 1.4   | 2.7   |        |        |
| 239849 | Cd200r4| CD200 receptor 4                                                              | 6.6   | 6.2   | 7.7   | 8.0   | 6.4   | 7.9   | 1.4   | 2.7   |        |        |
| 16409  | Itgam  | integrin alpha M                                                             | 5.7   | 5.9   | 6.4   | 8.0   | 5.8   | 7.2   | 1.4   | 2.7   |        |        |
| 50934  | Slc7a8 | solute carrier family 7 (cationic amino acid transporter, y+ system), member 8 | 5.9   | 5.8   | 7.2   | 7.4   | 5.9   | 7.3   | 1.4   | 2.7   |        |        |
| 107769 | Tm6sf1 | transmembrane 6 superfamily member 1                                         | 5.4   | 5.5   | 6.6   | 7.2   | 5.5   | 6.9   | 1.4   | 2.7   |        |        |
| 94176  | Dock2  | dedicator of cyto-kinesis 2                                                   | 4.4   | 4.6   | 5.7   | 6.1   | 4.5   | 5.9   | 1.4   | 2.7   |        |        |
| 223433 | Fam105a| family with sequence similarity 105, member A                                | 5.1   | 5.0   | 6.1   | 6.8   | 5.1   | 6.5   | 1.4   | 2.7   |        |        |
| 279572 | Tlr13  | toll-like receptor 13                                                         | 6.6   | 6.2   | 7.5   | 8.1   | 6.4   | 7.8   | 1.4   | 2.7   |        |        |
| Symbol   | Description                                                                 | Gene Name          | Value1 | Value2 | Value3 | Value4 | Value5 | Value6 | Value7 | Value8 | Value9 | Value10 |
|----------|------------------------------------------------------------------------------|--------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|
| Hpgds    | hematopoietic prostaglandin D synthase                                       |                    | 5.0    | 4.9    | 6.3    | 6.4    | 4.9    | 6.3    | 1.4    | 2.6    |         |         |         |
| Htr2b    | 5-hydroxytryptamine (serotonin) receptor 2B                                  |                    | 3.8    | 4.2    | 5.3    | 5.6    | 4.0    | 5.4    | 1.4    | 2.6    |         |         |         |
| Apobec1  | apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1                |                    | 7.3    | 7.4    | 8.8    | 8.7    | 7.3    | 8.7    | 1.4    | 2.6    |         |         |         |
| Cd180    | CD180 antigen                                                                |                    | 6.9    | 6.7    | 8.3    | 8.0    | 6.8    | 8.2    | 1.4    | 2.6    |         |         |         |
| Emb      | embigin                                                                      |                    | 7.0    | 6.9    | 7.9    | 8.8    | 7.0    | 8.3    | 1.4    | 2.6    |         |         |         |
| Gatm     | glycine amidinotransferase (L-arginine:glycine amidinotransferase)           |                    | 4.8    | 4.9    | 5.7    | 6.8    | 4.9    | 6.2    | 1.4    | 2.6    |         |         |         |
| Sly      | Sycp3 like Y-linked                                                           |                    | 4.4    | 4.9    | 6.3    | 5.9    | 4.7    | 6.1    | 1.4    | 2.6    |         |         |         |
| Aoah     | acyloxyacyl hydrolase                                                         |                    | 3.4    | 3.2    | 4.4    | 5.0    | 3.3    | 4.7    | 1.4    | 2.6    |         |         |         |
| Cc5      | chemokine (C-C motif) ligand 5                                                |                    | 4.7    | 5.3    | 5.1    | 7.6    | 5.0    | 6.4    | 1.4    | 2.6    |         |         |         |
| Cd14     | CD14 antigen                                                                  |                    | 5.6    | 5.8    | 7.1    | 7.0    | 5.7    | 7.1    | 1.4    | 2.6    |         |         |         |
| Myo1g    | myosin IG                                                                     |                    | 4.3    | 4.0    | 5.1    | 6.0    | 4.1    | 5.5    | 1.4    | 2.6    |         |         |         |
| Ncf1     | neutrophil cytosolic factor 1                                                |                    | 5.1    | 4.5    | 5.9    | 6.5    | 4.8    | 6.2    | 1.4    | 2.6    |         |         |         |
| H2-Aa    | histocompatibility 2, class II antigen A, alpha                               |                    | 2.8    | 2.7    | 2.6    | 5.6    | 2.8    | 4.1    | 1.4    | 2.6    |         |         |         |
| Pid1     | phosphotyrosine interaction domain containing 1                               |                    | 4.4    | 4.3    | 6.1    | 5.3    | 4.3    | 5.7    | 1.4    | 2.6    |         |         |         |
| AF251705 | cDNA sequence AF251705                                                        |                    | 6.2    | 6.0    | 7.1    | 7.9    | 6.1    | 7.5    | 1.4    | 2.6    |         |         |         |
| Mir24-1  | microRNA 24-1                                                                |                    | 3.3    | 4.0    | 5.6    | 4.5    | 3.7    | 5.0    | 1.4    | 2.6    |         |         |         |
| Rtp4     | receptor transporter protein 4                                               |                    | 3.1    | 2.7    | 5.4    | 3.0    | 2.9    | 4.2    | 1.4    | 2.6    |         |         |         |
| C3ar1    | complement component 3a receptor 1                                           |                    | 8.0    | 8.0    | 9.4    | 9.3    | 8.0    | 9.3    | 1.4    | 2.5    |         |         |         |
| Ctsc     | cathepsin C                                                                   |                    | 6.5    | 6.6    | 7.5    | 8.3    | 6.6    | 7.9    | 1.4    | 2.5    |         |         |         |
| Mir680-1 | microRNA 680-1                                                                |                    | 4.4    | 5.8    | 6.7    | 6.2    | 5.1    | 6.4    | 1.3    | 2.5    |         |         |         |
| Rgs1     | regulator of G-protein signaling 1                                           |                    | 4.2    | 4.6    | 6.1    | 5.4    | 4.4    | 5.7    | 1.3    | 2.5    |         |         |         |
| Hpse     | heparanase                                                                    |                    | 5.4    | 5.7    | 6.3    | 7.4    | 5.5    | 6.9    | 1.3    | 2.5    |         |         |         |
| Aldh1a3  | aldehyde dehydrogenase family 1, subfamily A3                                |                    | 4.6    | 4.0    | 6.2    | 5.0    | 4.3    | 5.6    | 1.3    | 2.5    |         |         |         |
| Bcl2a1b  | B cell leukemia/lymphoma 2 related protein A1b                              |                    | 7.4    | 7.1    | 8.8    | 8.4    | 7.3    | 8.6    | 1.3    | 2.5    |         |         |         |
| Mir5123  | microRNA 5123                                                                |                    | 3.2    | 3.5    | 4.5    | 4.8    | 3.3    | 4.7    | 1.3    | 2.5    |         |         |         |
| Pld4     | phospholipase D family, member 4                                             |                    | 6.0    | 4.4    | 5.7    | 7.3    | 5.2    | 6.5    | 1.3    | 2.5    |         |         |         |
| 1700040F15Rik | RIKEN cDNA 1700040F15 gene                                                    |                    | 4.4    | 5.6    | 6.5    | 6.1    | 5.0    | 6.3    | 1.3    | 2.5    |         |         |         |
| Acss1    | acyl-CoA synthetase short-chain family member 1                              |                    | 2.7    | 3.4    | 3.8    | 4.9    | 3.1    | 4.4    | 1.3    | 2.5    |         |         |         |
| Lair1    | leukocyte-associated Ig-like receptor 1                                      |                    | 5.5    | 5.2    | 6.9    | 6.4    | 5.3    | 6.7    | 1.3    | 2.5    |         |         |         |
| Naglu    | alpha-N-acetylgalcosaminidase (Sanfilippo disease IIIB)                       |                    | 5.5    | 4.9    | 6.0    | 7.0    | 5.2    | 6.5    | 1.3    | 2.5    |         |         |         |
| Nfam1    | Nfat activating molecule with ITAM motif 1                                   |                    | 4.4    | 4.0    | 5.2    | 5.9    | 4.2    | 5.5    | 1.3    | 2.5    |         |         |         |
| Pik3cg   | phosphoinositide-3-kinase, catalytic, gamma polypeptide                       |                    | 4.2    | 3.9    | 5.0    | 5.6    | 4.0    | 5.3    | 1.3    | 2.5    |         |         |         |
| Tlr8     | toll-like receptor 8                                                          |                    | 4.8    | 4.6    | 5.6    | 6.4    | 4.7    | 6.0    | 1.3    | 2.5    |         |         |         |
| Gene ID | Gene Name | Description                                                                 | Value1 | Value2 | Value3 | Value4 | Value5 | Value6 | Value7 |
|--------|-----------|------------------------------------------------------------------------------|--------|--------|--------|--------|--------|--------|--------|
| 210293 | Dock10    | dedicator of cytokinesis 10                                                  | 5.9    | 5.7    | 6.5    | 7.7    | 5.8    | 7.1    | 1.3    | 2.5    |
| 212937 | Tifab     | TRAF-interacting protein with forkhead-associated domain, family member B   | 3.6    | 3.3    | 4.2    | 5.4    | 3.5    | 4.8    | 1.3    | 2.5    |
| 16411  | Itgax     | integrin alpha X                                                             | 5.5    | 6.9    | 6.3    | 8.8    | 6.2    | 7.5    | 1.3    | 2.4    |
| 407790 | Ndufa4l2  | NADH dehydrogenase (ubiquinone) 1 alpha subcomplex, 4-like 2                | 3.6    | 6.2    | 6.3    | 6.1    | 4.9    | 6.2    | 1.3    | 2.4    |
| 16790  | Anpep     | alanyl (membrane) aminopeptidase                                              | 8.8    | 9.0    | 10.0   | 10.4   | 8.9    | 10.2   | 1.3    | 2.4    |
| 12044  | Bcl2a1a   | B cell leukemia/lymphoma 2 related protein A1a                               | 6.0    | 6.3    | 7.2    | 7.6    | 6.2    | 7.4    | 1.3    | 2.4    |
| 18187  | Nrp2      | neuropilin 2                                                                 | 6.7    | 6.2    | 7.6    | 7.9    | 6.5    | 7.7    | 1.3    | 2.4    |
| 71653  | 4930506M07Rik | RIKEN cDNA 4930506M07 gene                                                | 6.1    | 5.5    | 6.4    | 7.7    | 5.8    | 7.1    | 1.3    | 2.4    |
| 60533  | Cd274     | CD274 antigen                                                                | 4.7    | 4.5    | 4.6    | 7.1    | 4.6    | 5.8    | 1.3    | 2.4    |
| 18726  | Lilra6    | leukocyte immunoglobulin-like receptor, subfamily A (with TM domain), member 6 | 3.7    | 3.8    | 4.8    | 5.2    | 3.7    | 5.0    | 1.3    | 2.4    |
| 228026 | Pdk1      | pyruvate dehydrogenase kinase, isoenzyme 1                                   | 3.8    | 4.7    | 5.5    | 5.4    | 4.2    | 5.5    | 1.3    | 2.4    |
| 13034  | Ctse      | cathepsin E                                                                  | 3.0    | 4.3    | 3.2    | 6.7    | 3.7    | 4.9    | 1.3    | 2.4    |
| 15163  | Hcls1     | hematopoietic cell specific Lyn substrate 1                                   | 6.1    | 5.7    | 7.1    | 7.2    | 5.9    | 7.2    | 1.3    | 2.4    |
| 16822  | Lcp2      | lymphocyte cytosolic protein 2                                               | 5.3    | 5.3    | 6.2    | 6.9    | 5.3    | 6.5    | 1.3    | 2.4    |
| 237542 | Osbpl8    | oxysterol binding protein-like 8                                             | 7.6    | 7.1    | 8.6    | 8.7    | 7.4    | 8.6    | 1.3    | 2.4    |
| 12523  | Cd84      | CD84 antigen                                                                 | 6.8    | 7.1    | 7.8    | 8.7    | 7.0    | 8.2    | 1.3    | 2.4    |
| 56619  | Clec4e    | C-type lectin domain family 4, member e                                       | 5.4    | 3.8    | 5.5    | 6.2    | 4.6    | 5.8    | 1.3    | 2.4    |
| 211228 | Lrrc25    | leucine rich repeat containing 25                                            | 3.9    | 4.3    | 5.0    | 5.7    | 4.1    | 5.3    | 1.3    | 2.4    |
| 12508  | Cd53      | CD53 antigen                                                                 | 8.5    | 8.5    | 9.6    | 10.0   | 8.5    | 9.8    | 1.2    | 2.4    |
| 83490  | Pik3ap1   | phosphoinositide-3-kinase adaptor protein 1                                 | 5.5    | 5.5    | 6.8    | 6.6    | 5.5    | 6.7    | 1.2    | 2.4    |
| 56193  | Plek      | pleckstrin                                                                   | 7.7    | 7.6    | 8.6    | 9.3    | 7.7    | 8.9    | 1.2    | 2.4    |
| 78591  | A430104N18Rik | RIKEN cDNA A430104N18 gene                                                | 4.5    | 3.2    | 4.4    | 5.7    | 3.8    | 5.1    | 1.2    | 2.3    |
| 72042  | Cotl1     | coactosin-like 1 (Dictyostelium)                                             | 6.7    | 6.2    | 7.2    | 8.2    | 6.4    | 7.7    | 1.2    | 2.3    |
| 101056121 | LOC101056121 | Y-linked testis-specific protein 1-like                                       | 4.2    | 4.5    | 5.6    | 5.6    | 4.4    | 5.6    | 1.2    | 2.3    |
| 56857  | Slc37a2   | solute carrier family 37 (glycerol-3-phosphate transporter), member 2        | 5.2    | 4.7    | 6.2    | 6.2    | 5.0    | 6.2    | 1.2    | 2.3    |
| 170743 | Tlr7      | toll-like receptor 7                                                          | 4.9    | 4.8    | 5.7    | 6.4    | 4.8    | 6.1    | 1.2    | 2.3    |
| 436467 | Trav14-1  | T cell receptor alpha variable 14-1                                          | 2.9    | 3.9    | 4.7    | 4.5    | 3.4    | 4.6    | 1.2    | 2.3    |
| 53314  | Batf      | basic leucine zipper transcription factor, ATF-like                          | 3.8    | 3.7    | 4.5    | 5.4    | 3.7    | 4.9    | 1.2    | 2.3    |
| 12493  | Cd37      | CD37 antigen                                                                 | 4.9    | 4.7    | 5.9    | 6.2    | 4.8    | 6.1    | 1.2    | 2.3    |
| 16414  | Itgb2     | integrin beta 2                                                              | 7.3    | 7.1    | 7.8    | 9.1    | 7.2    | 8.4    | 1.2    | 2.3    |
| 56792  | Stap1     | signal transducing adaptor family member 1                                   | 3.4    | 3.7    | 5.0    | 4.6    | 3.6    | 4.8    | 1.2    | 2.3    |
| 239393 | Lrp12     | low density lipoprotein-related protein 12                                   | 6.6    | 6.4    | 7.2    | 8.2    | 6.5    | 7.7    | 1.2    | 2.3    |
| Gene ID | Gene Name | Description | Values |
|--------|-----------|-------------|--------|
| 22368  | Trpv2     | transient receptor potential channel, subfamily V, member 2 | 6.1  5.6  7.0  7.1  5.8  7.0  1.2  2.3 |
| 100504230 | AU020206 | expressed sequence AU020206 | 8.0  7.0  8.5  8.8  7.5  8.7  1.2  2.3 |
| 212032 | Hk3       | hexokinase 3 | 3.8  4.0  5.0  5.2  3.9  5.1  1.2  2.3 |
| 80719  | Igsf6     | immunoglobulin superfamily, member 6 | 6.0  6.3  6.8  8.0  6.2  7.4  1.2  2.3 |
| 78771  | Mctp1     | multiple C2 domains, transmembrane 1 | 5.4  4.9  6.1  6.6  5.2  6.3  1.2  2.3 |
| 20564  | Slit3     | slit homolog 3 (Drosophila) | 6.3  5.8  7.7  6.8  6.1  7.3  1.2  2.3 |
| 216991 | Adap2     | ArfGAP with dual PH domains 2 | 4.2  3.8  5.0  5.4  4.0  5.2  1.2  2.3 |
| 54725  | Cadm1     | cell adhesion molecule 1 | 6.9  5.7  7.4  7.6  6.3  7.5  1.2  2.3 |
| 18106  | Cd244     | CD244 natural killer cell receptor 2B4 | 3.0  2.8  3.3  4.9  2.9  4.1  1.2  2.3 |
| 16154  | Il10ra    | interleukin 10 receptor, alpha | 3.8  4.0  4.8  5.3  3.9  5.1  1.2  2.3 |
| 101056060 | LOC101056060 | Y-linked testis-specific protein 1-like | 4.8  5.5  6.4  6.2  5.1  6.3  1.2  2.3 |
| 23833  | Cd52      | CD52 antigen | 7.1  6.9  7.7  8.7  7.0  8.2  1.2  2.3 |
| 12825  | Col3a1    | collagen, type III, alpha 1 | 5.5  5.4  7.0  6.2  5.4  6.6  1.2  2.3 |
| 66102  | Cxcl16    | chemokine (C-X-C motif) ligand 16 | 6.8  6.4  7.7  7.9  6.6  7.8  1.2  2.3 |
| 16331  | Inpp5d    | inositol polyphosphate-5-phosphatase D | 5.8  5.3  6.6  6.9  5.6  6.7  1.2  2.3 |
| 68279  | Mcoln2    | mucolipin 2 | 5.3  4.4  6.0  6.1  4.9  6.0  1.2  2.3 |
| 109225 | Ms4a7     | membrane-spanning 4-domains, subfamily A, member 7 | 8.8  8.1  9.9  9.5  8.5  9.7  1.2  2.3 |
| 100034251 | Wfdc17     | WAP four-disulfide core domain 17 | 9.0  9.3  9.6  11.1  9.2  10.4  1.2  2.3 |
| 73149  | Clec4a3   | C-type lectin domain family 4, member a3 | 3.1  3.9  4.2  5.1  3.5  4.7  1.2  2.3 |
| 16658  | Mafb      | v-maf musculoaponeurotic fibrosarcoma oncogene family, protein B (avian) | 4.9  4.7  5.9  6.1  4.8  6.0  1.2  2.3 |
| 245945 | Rbm47     | RNA binding motif protein 47 | 4.1  3.9  5.0  5.3  4.0  5.1  1.2  2.3 |
| 21938  | Tnfrsf1b  | tumor necrosis factor receptor superfamily, member 1b | 6.8  6.5  7.3  8.4  6.7  7.8  1.2  2.3 |
| 57257  | Vav3      | vav 3 oncogene | 3.4  3.0  4.3  4.4  3.2  4.3  1.2  2.3 |
| 219144 | Arl11     | ADP-ribosylation factor-like 11 | 4.9  4.6  5.7  6.1  4.7  5.9  1.2  2.2 |
| 12260  | C1qb      | complement component 1, q subcomponent, beta polypeptide | 8.3  8.1  9.3  9.4  8.2  9.4  1.2  2.2 |
| 381654 | C87414    | expressed sequence C87414 | 2.3  4.2  4.3  4.6  3.3  4.4  1.2  2.2 |
| 108101 | Fermt3    | fermitin family homolog 3 (Drosophila) | 4.6  4.6  5.7  5.9  4.6  5.8  1.2  2.2 |
| 207839 | Galnt6    | UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminytransferase 6 | 4.9  4.5  5.4  6.3  4.7  5.9  1.2  2.2 |
| 18826  | Lcp1      | lymphocyte cytosolic protein 1 | 7.9  7.9  8.9  9.2  7.9  9.1  1.2  2.2 |
| 73656  | Ms4a6c    | membrane-spanning 4-domains, subfamily A, member 6C | 4.3  3.8  4.6  5.8  4.1  5.2  1.2  2.2 |
| ID    | Gene     | Description                                                                 | Score 1 | Score 2 | Score 3 | Score 4 | Score 5 | Score 6 | Score 7 | Score 8 | Score 9 | Score 10 |
|-------|----------|-----------------------------------------------------------------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|----------|
| 22368 | Trpv2    | transient receptor potential cation channel, subfamily V, member 2           | 6.3     | 6.1     | 7.5     | 7.3     | 6.2     | 7.4     | 1.2     | 2.2     |         |          |
| 54445 | Unc93b1  | unc-93 homolog B1 (C. elegans)                                              | 6.6     | 6.2     | 7.3     | 7.8     | 6.4     | 7.6     | 1.2     | 2.2     |         |          |
| 12721 | Cor1a    | corin, actin binding protein 1A                                              | 5.1     | 5.2     | 6.3     | 6.3     | 5.1     | 6.3     | 1.2     | 2.2     |         |          |
| 12978 | Csf1r    | colony stimulating factor 1 receptor                                         | 6.7     | 6.3     | 7.7     | 7.7     | 6.5     | 7.7     | 1.2     | 2.2     |         |          |
| 20311 | Cxcl5    | chemokine (C-X-C motif) ligand 5                                             | 3.5     | 4.3     | 4.6     | 5.5     | 3.9     | 5.1     | 1.2     | 2.2     |         |          |
| 13058 | Cybb     | cytochrome b-245, beta polypeptide                                           | 7.6     | 7.2     | 7.9     | 9.2     | 7.4     | 8.6     | 1.2     | 2.2     |         |          |
| 17948 | Naip2    | NLR family, apoptosis inhibitory protein 2                                   | 5.1     | 4.8     | 5.8     | 6.4     | 5.0     | 6.1     | 1.2     | 2.2     |         |          |
| 11468 | Actg2    | actin, gamma 2, smooth muscle, enteric                                       | 8.1     | 7.6     | 9.4     | 8.5     | 7.8     | 9.0     | 1.1     | 2.2     |         |          |
| 12491 | Cd36     | CD36 antigen                                                                | 6.4     | 7.6     | 7.1     | 9.2     | 7.0     | 8.2     | 1.1     | 2.2     |         |          |
| 14017 | Evi2a    | ecotropic viral integration site 2a                                          | 5.7     | 5.7     | 6.8     | 6.8     | 5.7     | 6.8     | 1.1     | 2.2     |         |          |
| 23880 | Fyb      | FYN binding protein                                                          | 5.5     | 5.0     | 6.7     | 6.2     | 5.3     | 6.4     | 1.1     | 2.2     |         |          |
| 70719 | Hmha1    | histocompatibility (minor) HA-1                                              | 4.8     | 4.0     | 5.6     | 5.5     | 4.4     | 5.5     | 1.1     | 2.2     |         |          |
| 64099 | Parvg    | parvin, gamma                                                               | 3.5     | 3.9     | 3.7     | 5.8     | 3.7     | 4.8     | 1.1     | 2.2     |         |          |
| 23078 | Themis2  | thymocyte selection associated family member 2                              | 4.5     | 4.3     | 5.1     | 6.0     | 4.4     | 5.5     | 1.1     | 2.2     |         |          |
| 57425 | U90926   | cDNA sequence U90926                                                        | 3.9     | 5.3     | 4.8     | 6.7     | 4.6     | 5.7     | 1.1     | 2.2     |         |          |
| 12489 | Cd33     | CD33 antigen                                                                | 6.0     | 5.7     | 5.7     | 8.2     | 5.8     | 7.0     | 1.1     | 2.2     |         |          |
| 14191 | Fgr      | Gardner-Rasheed feline sarcoma viral (Fgr) oncogene homolog                 | 3.1     | 3.0     | 3.0     | 5.3     | 3.0     | 4.2     | 1.1     | 2.2     |         |          |
| 15117 | Has2     | hyaluronan synthase 2                                                        | 5.3     | 5.3     | 6.4     | 6.5     | 5.3     | 6.5     | 1.1     | 2.2     |         |          |
| 14728 | Lirlb4   | leukocyte immunoglobulin-like receptor, subfamily B, member 4               | 9.1     | 9.5     | 10.3    | 10.6    | 9.3     | 10.4    | 1.1     | 2.2     |         |          |
| 69189 | Memp1    | mast cell expressed membrane protein 1                                      | 3.4     | 3.1     | 3.2     | 5.6     | 3.3     | 4.4     | 1.1     | 2.2     |         |          |
| 257662| Olfr1290  | olfactory receptor 1290                                                      | 2.3     | 3.4     | 4.7     | 3.4     | 2.9     | 4.0     | 1.1     | 2.2     |         |          |
| 241452| Dhrs9    | dehydrogenase/reductase (SDR family) member 9                               | 4.0     | 4.2     | 5.2     | 5.3     | 4.1     | 5.3     | 1.1     | 2.2     |         |          |
| 56743 | Lat2     | linker for activation of T cells family, member 2                            | 6.3     | 6.3     | 7.4     | 7.4     | 6.3     | 7.4     | 1.1     | 2.2     |         |          |
| 11846 | Arg1     | arginase, liver                                                             | 3.8     | 3.4     | 4.3     | 5.2     | 3.6     | 4.7     | 1.1     | 2.2     |         |          |
| 665521| BC080696  | cDNA sequence BC080696                                                      | 3.5     | 4.0     | 4.7     | 5.0     | 3.7     | 4.8     | 1.1     | 2.2     |         |          |
| 18830 | Pltp     | phospholipid transfer protein                                                | 5.4     | 5.5     | 7.1     | 6.1     | 5.5     | 6.6     | 1.1     | 2.2     |         |          |
| 218442| Serinc5  | serine incorporator 5                                                        | 5.2     | 5.0     | 5.7     | 6.7     | 5.1     | 6.2     | 1.1     | 2.2     |         |          |
| 171209| Asic3    | acid-sensing (proton-gated) ion channel 3                                   | 3.1     | 3.3     | 5.1     | 3.5     | 3.2     | 4.3     | 1.1     | 2.1     |         |          |
| 232975| Atp1a3   | ATPase, Na+/K+ transporting, alpha 3 polypeptide                            | 5.6     | 5.3     | 6.0     | 7.2     | 5.5     | 6.6     | 1.1     | 2.1     |         |          |
| 20305 | Ccl6     | chemokine (C-C motif) ligand 6                                              | 6.6     | 7.8     | 6.5     | 10.1    | 7.2     | 8.3     | 1.1     | 2.1     |         |          |
| 329679| Fnip2    | folliculin interacting protein 2                                            | 6.2     | 6.5     | 7.1     | 7.7     | 6.3     | 7.4     | 1.1     | 2.1     |         |          |
| 83924 | Gpr137b  | G protein-coupled receptor 137B                                             | 6.4     | 5.8     | 6.8     | 7.6     | 6.1     | 7.2     | 1.1     | 2.1     |         |          |
| Gene ID | Description | Expression 1 | Expression 2 | Expression 3 | Expression 4 | Expression 5 | Expression 6 |
|--------|-------------|--------------|--------------|--------------|--------------|--------------|--------------|
| 15950  | IIf203      | interferon activated gene 203 | 3.4          | 3.1          | 5.0          | 3.7          | 3.2          | 4.3          | 1.1          | 2.1          |
| 21391  | Rasf4       | Ras association (RalGDS/AF-6) domain family member 4 | 4.6          | 3.4          | 4.9          | 5.3          | 4.0          | 5.1          | 1.1          | 2.1          |
| 76408  | Abcc3       | ATP-binding cassette, sub-family C (CFTR/MRP), member 3 | 5.0          | 4.2          | 5.9          | 5.5          | 4.6          | 5.7          | 1.1          | 2.1          |
| 620551 | LOC620551   | PRAME family member S-like | 3.3          | 3.9          | 4.8          | 4.5          | 3.6          | 4.7          | 1.1          | 2.1          |
| 20354  | Sema4d      | sema domain, immunoglobulin domain (lg), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4D | 4.3          | 4.1          | 5.1          | 5.4          | 4.2          | 5.3          | 1.1          | 2.1          |
| 20612  | Siglec1     | sialic acid binding Ig-like lectin 1, sialoadhesin | 4.6          | 4.0          | 5.0          | 5.8          | 4.3          | 5.4          | 1.1          | 2.1          |
| 65221  | Scl15a3     | solute carrier family 15, member 3 | 7.3          | 7.4          | 8.1          | 8.8          | 7.3          | 8.4          | 1.1          | 2.1          |
| 320148 | B430306N03Rik | RIKEN cDNA B430306N03 gene | 4.2          | 4.1          | 4.7          | 5.7          | 4.1          | 5.2          | 1.1          | 2.1          |
| 12229  | Btk         | Bruton agammaglobulinemia tyrosine kinase | 4.3          | 4.0          | 5.1          | 5.4          | 4.1          | 5.2          | 1.1          | 2.1          |
| 17474  | Clec4d      | C-type lectin domain family 4, member d | 8.4          | 8.2          | 9.1          | 9.6          | 8.3          | 9.3          | 1.1          | 2.1          |
| 100316820 | Mir1970   | microRNA 1970 | 3.8          | 3.7          | 4.9          | 4.8          | 3.8          | 4.9          | 1.1          | 2.1          |
| 100628621 | Mir3961   | microRNA 3961 | 4.9          | 4.0          | 5.6          | 5.4          | 4.4          | 5.5          | 1.1          | 2.1          |
| 20611  | Ssty1       | spermiogenesis specific transcript on the Y 1 | 5.0          | 5.9          | 6.6          | 6.4          | 5.4          | 6.5          | 1.1          | 2.1          |
| 226409 | Zranb3      | zinc finger, RAN-binding domain containing 3 | 4.3          | 4.6          | 5.2          | 5.9          | 4.5          | 5.6          | 1.1          | 2.1          |
| 232201 | Arhgap25    | Rho GTPase activating protein 25 | 4.8          | 5.4          | 5.7          | 6.7          | 5.1          | 6.2          | 1.1          | 2.1          |
| 380732 | Mir1        | mast cell immunoglobulin like receptor 1 | 4.5          | 4.4          | 5.5          | 5.4          | 4.4          | 5.5          | 1.1          | 2.1          |
| 320024 | Nceh1       | neutral cholesterol ester hydrolase 1 | 8.0          | 7.9          | 8.9          | 9.2          | 8.0          | 9.0          | 1.1          | 2.1          |
| 14051  | Eya4        | eyes absent 4 homolog (Drosophila) | 3.3          | 2.8          | 4.4          | 3.8          | 3.1          | 4.1          | 1.1          | 2.1          |
| 67731  | FbX32       | F-box protein 32 | 4.3          | 4.3          | 4.7          | 6.0          | 4.3          | 5.4          | 1.1          | 2.1          |
| 14176  | Fgf5        | fibroblast growth factor 5 | 4.1          | 3.8          | 4.4          | 5.6          | 3.9          | 5.0          | 1.1          | 2.1          |
| 237436 | Gas2i3      | growth arrest-specific 2 like 3 | 5.2          | 5.7          | 6.1          | 6.9          | 5.4          | 6.5          | 1.1          | 2.1          |
| 16114  | Igk-V28     | immunoglobulin kappa chain variable 28 (V28) | 4.9          | 4.3          | 5.8          | 5.5          | 4.6          | 5.7          | 1.1          | 2.1          |
| 319480 | Itga11      | integrin alpha 11 | 4.0          | 4.0          | 6.1          | 4.0          | 4.0          | 5.1          | 1.1          | 2.1          |
| 320024 | Nceh1       | neutral cholesterol ester hydrolase 1 | 8.0          | 7.9          | 9.0          | 9.1          | 8.0          | 9.1          | 1.1          | 2.1          |
| 20846  | Stat1       | signal transducer and activator of transcription 1 | 5.9          | 5.6          | 7.2          | 6.5          | 5.8          | 6.8          | 1.1          | 2.1          |
| 12053  | Bcl6        | B cell leukemia/lymphoma 6 | 6.4          | 6.1          | 7.6          | 7.0          | 6.2          | 7.3          | 1.1          | 2.1          |
| 12606  | Cebp        | CCAAT/enhancer binding protein (C/EBP), alpha | 4.6          | 4.3          | 5.3          | 5.7          | 4.4          | 5.5          | 1.1          | 2.1          |
| 19141  | Lgmn        | legumain | 8.4          | 8.3          | 9.3          | 9.4          | 8.3          | 9.4          | 1.1          | 2.1          |
| 241633 | Atp8b4      | ATPase, class I, type 8B, member 4 | 3.2          | 3.1          | 4.1          | 4.3          | 3.2          | 4.2          | 1.0          | 2.1          |
| 12362  | Casp1       | caspase 1 | 5.5          | 5.8          | 6.8          | 6.6          | 5.7          | 6.7          | 1.0          | 2.1          |
| 100628574 | Mir28b     | microRNA 28b | 4.0          | 5.3          | 6.1          | 5.2          | 4.6          | 5.7          | 1.0          | 2.1          |
| Gene ID | Gene Symbol | Gene Name | Fold Change |
|---------|-------------|-----------|-------------|
| 19200   | Pstpip1     | proline-serine-threonine phosphatase-interacting protein 1 | 5.0 5.3 5.9 6.4 5.1 6.2 1.0 2.1 |
| 18173   | Slc11a1     | solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1 | 5.0 4.9 5.5 6.4 4.9 6.0 1.0 2.1 |
| 18636   | Cfp         | complement factor properdin | 5.1 4.4 5.1 6.4 4.8 5.8 1.0 2.0 |
| 14127   | Fcer1g      | Fc receptor, IgE, high affinity I, gamma polypeptide | 9.6 9.2 10.4 10.5 9.4 10.5 1.0 2.0 |
| 16186   | Il2rg       | interleukin 2 receptor, gamma chain | 6.4 6.1 6.6 8.0 6.2 7.3 1.0 2.0 |
| 17101   | Lyst        | lysosomal trafficking regulator | 5.5 5.6 6.3 6.9 5.6 6.6 1.0 2.0 |
| 66742   | Piezo2      | piezo-type mechanosensitive ion channel component 2 | 3.6 4.2 5.4 4.4 3.9 4.9 1.0 2.0 |
| 19332   | Rab20       | RAB20, member RAS oncogene family | 4.4 4.1 5.4 5.2 4.3 5.3 1.0 2.0 |
| 11980   | Atp8a1      | ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1 | 3.6 3.4 4.2 4.8 3.5 4.5 1.0 2.0 |
| 14247   | Flt1        | Friend leukemia integration 1 | 5.4 5.4 6.2 6.7 5.4 6.4 1.0 2.0 |
| 211401  | Mtss1       | metastasis suppressor 1 | 6.6 6.5 7.7 7.4 6.5 7.6 1.0 2.0 |
| 17951   | Naip5       | NLR family, apoptosis inhibitory protein 5 | 4.1 3.5 4.1 5.5 3.8 4.8 1.0 2.0 |
| 241062  | Pgap1       | post-GPI attachment to proteins 1 | 5.2 5.4 5.6 7.1 5.3 6.3 1.0 2.0 |
| 19850   | Rnu3a       | U3A small nuclear RNA | 3.2 3.3 4.2 4.3 3.2 4.3 1.0 2.0 |
| 69583   | Tnfsf13     | tumor necrosis factor (ligand) superfamily, member 13 | 6.0 6.0 6.8 7.3 6.0 7.0 1.0 2.0 |
| 20303   | Ccl4        | chemokine (C-C motif) ligand 4 | 5.7 6.1 6.9 6.9 5.9 6.9 1.0 2.0 |
| 12483   | Cd22        | CD22 antigen | 3.7 3.8 4.1 5.5 3.8 4.8 1.0 2.0 |
| 234356  | Csgalnact1  | chondroitin sulfate N-acetylgalactosaminyltransferase 1 | 3.5 3.4 5.2 3.7 3.5 4.5 1.0 2.0 |
| 100041034 | LOC100041034 | Sp110 nuclear body protein-like | 5.8 5.3 6.4 6.7 5.5 6.5 1.0 2.0 |
| 320139  | Ptpn7       | protein tyrosine phosphatase, non-receptor type 7 | 4.2 4.1 5.1 5.3 4.2 5.2 1.0 2.0 |
| 57319   | Smpd3a      | sphingomyelin phosphodiesterase, acid-like 3A | 6.7 6.4 7.3 7.9 6.6 7.6 1.0 2.0 |
| 652925  | Tmem243     | transmembrane protein 243, mitochondrial | 3.8 4.2 4.8 5.3 4.0 5.0 1.0 2.0 |
| 12262   | C1qc        | complement component 1, q subcomponent, C chain | 7.1 7.2 8.1 8.1 7.1 8.1 1.0 2.0 |
| 101056308 | LOC101056308 | Y-linked testis-specific protein 1-like | 3.5 3.9 4.9 4.5 3.7 4.7 1.0 2.0 |
| 140795  | P2ry14      | purinergic receptor P2Y, G-protein coupled, 14 | 3.2 3.7 3.6 5.3 3.5 4.5 1.0 2.0 |
| 11835   | Ar          | androgen receptor | 5.1 4.9 3.8 4.1 5.0 4.0 -1.0 -2.0 |
| 19716   | Bex1        | brain expressed gene 1 | 5.7 6.4 3.2 6.9 6.1 5.1 -1.0 -2.0 |
| 241520  | Fam171b     | family with sequence similarity 171, member B | 4.3 4.8 3.5 3.6 4.5 3.5 -1.0 -2.0 |
| 18481   | Pak3        | p21 protein (Cdc42/Rac)-activated kinase 3 | 4.8 4.7 3.8 3.7 4.7 3.7 -1.0 -2.0 |
| Gene Symbol | Description                                      | Log2 Fold Change |
|------------|--------------------------------------------------|-----------------|
| Chst1      | carbohydrate (keratan sulfate Gal-6) sulfotransferase 1 | 4.6 4.6 3.9 3.2 4.6 3.6 -1.0 -2.0 |
| Pdgfd      | platelet-derived growth factor, D polypeptide      | 6.6 5.9 5.6 4.8 6.2 5.2 -1.0 -2.0 |
| Scx        | scleraxis                                         | 3.4 4.8 2.8 3.3 4.1 3.1 -1.0 -2.0 |
| Tjp2       | tight junction protein 2                          | 6.6 6.2 5.3 5.4 6.4 5.4 -1.0 -2.0 |
| Hid1       | H1D1 domain containing                            | 4.7 5.0 3.7 3.9 4.8 3.8 -1.0 -2.0 |
| Lrrc8c     | leucine rich repeat containing B family, member C | 8.2 8.1 6.6 7.7 8.2 7.1 -1.0 -2.0 |
| Mecom      | MDS1 and EVI1 complex locus                       | 4.1 4.1 3.1 3.0 4.1 3.1 -1.0 -2.0 |
| Cd109      | CD109 antigen                                      | 7.0 8.2 6.4 6.7 7.6 6.6 -1.0 -2.1 |
| Cdo1       | cysteine dioxygenase 1, cytosolic                 | 7.9 8.6 7.1 7.3 8.2 7.2 -1.0 -2.1 |
| Ereg       | epiregulin                                         | 8.7 8.3 7.2 7.8 8.5 7.5 -1.0 -2.1 |
| Zfpm2      | zinc finger protein, multitype 2                  | 5.7 5.4 4.7 4.3 5.5 4.5 -1.0 -2.1 |
| Ddr2       | discoidin domain receptor family, member 2        | 7.5 7.4 6.9 6.0 7.5 6.4 -1.1 -2.1 |
| Dhc24      | 24-dehydrocholesterol reductase                   | 7.4 7.5 6.7 6.1 7.5 6.4 -1.1 -2.1 |
| Lce1m      | late cornified envelope 1M                         | 5.3 4.6 3.9 3.9 4.9 3.9 -1.1 -2.1 |
| Ptgis      | prostaglandin I2 (prostacyclin) synthase           | 7.5 8.2 6.7 6.9 7.8 6.8 -1.1 -2.1 |
| Rab3ip     | RAB3A interacting protein                         | 7.4 7.5 6.6 6.1 7.4 6.4 -1.1 -2.1 |
| Sgcd       | sarcoglycan, delta (dystrophin-associated glycoprotein) | 5.7 5.7 4.5 4.8 5.7 4.7 -1.1 -2.1 |
| Syt4       | synaptotagmin IV                                  | 5.9 3.3 3.5 3.5 4.6 3.5 -1.1 -2.1 |
| Anxa8      | annexin A8                                        | 4.6 4.6 3.7 3.4 4.6 3.6 -1.1 -2.1 |
| Gas7       | growth arrest specific 7                          | 6.1 5.8 4.4 5.4 5.9 4.9 -1.1 -2.1 |
| Cxcl12     | chemokine (C-X-C motif) ligand 12                  | 9.1 10.1 8.6 8.5 9.6 8.5 -1.1 -2.1 |
| Igf11      | immunoglobulin superfamily, member 11             | 6.8 6.6 5.0 6.3 6.7 5.6 -1.1 -2.1 |
| Lrmt2      | leucine rich repeat transmembrane neuronal 2      | 4.2 4.6 3.2 3.5 4.4 3.3 -1.1 -2.1 |
| Ppp1r14a   | protein phosphatase 1, regulatory (inhibitor) subunit 14A | 4.6 3.4 3.2 2.8 4.0 3.0 -1.1 -2.1 |
| Sulf1      | sulfatase 1                                       | 9.3 9.5 8.7 8.0 9.4 8.4 -1.1 -2.1 |
| Cgref1     | cell growth regulator with EF hand domain 1       | 6.3 6.0 4.9 5.3 6.2 5.1 -1.1 -2.1 |
| Htr1b      | 5-hydroxytryptamine (serotonin) receptor 1B       | 6.2 5.6 4.7 5.0 5.9 4.8 -1.1 -2.1 |
| S100a7a    | S100 calcium binding protein A7A                  | 6.1 4.9 4.1 4.8 5.5 4.4 -1.1 -2.1 |
| Tsln6      | tetraspanin 6                                     | 8.3 7.6 7.1 6.6 7.9 6.8 -1.1 -2.1 |
| Tll7       | tubulin tyrosine ligase-like family, member 7     | 5.5 6.1 4.8 4.7 5.8 4.7 -1.1 -2.1 |
| Gene ID | Gene Name | Description                                                                 | Log2 Ratio |
|--------|-----------|------------------------------------------------------------------------------|------------|
| 14266  | **Aff2**  | S100 calcium receptor type, K protein tyrosine phosphatase, 5' nucleotidase, ecto | 5.5        |
| 10493  | **Fam110c** | family with sequence similarity 110, member C                               | 4.6        |
| 14205  | **Figf**  | c-fos induced growth factor                                                   | 6.3        |
| 20378  | **Frb2**  | frizzled-related protein                                                      | 6.6        |
| 16447  | **Ivl**   | involucrin                                                                   | 6.5        |
| 21956  | **Tnt2**  | troponin T2, cardiac                                                         | 5.7        |
| 12269  | **C4bp**  | complement component 4 binding protein                                       | 4.9        |
| 12389  | **Cav1**  | caveolin 1, caveolae protein                                                  | 8.1        |
| 73379  | **Dcbld2** | discoidin, CUB and LCCL domain containing 2                                 | 7.4        |
| 67350  | 1700084E18Rik | RIKEN cDNA 1700084E18 gene                                                 | 4.8        |
| 66175  | **Mustn1** | musculoskeletal, embryonic nuclear protein 1                                  | 9.8        |
| 269233 | **Fam171a1** | family with sequence similarity 171, member A1                             | 6.3        |
| 329628 | **Fat4**  | FAT tumor suppressor homolog 4 (Drosophila)                                  | 5.2        |
| 77446  | **Heg1**  | HEG homolog 1 (zebrafish)                                                    | 7.7        |
| 10053380 | **Sngh4** | small nuclear RNA host gene 4                                                | 4.7        |
| 23876  | **Fbln5** | fibulin 5                                                                     | 8.4        |
| 16008  | **Igfbp2** | insulin-like growth factor binding protein 2                                  | 6.5        |
| 233726 | **Ipo7**  | importin 7                                                                   | 7.9        |
| 13642  | **Efnb2** | ephrin B2                                                                    | 7.8        |
| 67374  | **Jam2**  | junction adhesion molecule 2                                                  | 8.8        |
| 68404  | **Nrn1**  | neuritin 1                                                                    | 7.1        |
| 71720  | **Osbp3** | oxysterol binding protein-like 3                                              | 4.8        |
| 19243  | **Ptp4a1** | protein tyrosine phosphatase 4a1                                              | 6.4        |
| 72780  | **Rspo3** | R-spondin 3 homolog (Xenopus laevis)                                         | 6.2        |
| 217410 | **Trib2** | tribbles homolog 2 (Drosophila)                                               | 5.9        |
| 12064  | **Bdnf**  | brain derived neurotrophic factor                                             | 5.6        |
| 14677  | **Gna1**  | guanine nucleotide binding protein (G protein), alpha inhibiting 1           | 6.3        |
| 22160  | **Twist1** | twist basic helix-loop-helix transcription factor 1                           | 6.1        |
| 73720  | **Cst6**  | cystatin E/M                                                                  | 5.4        |
| 319146 | **Ifnz**  | interferon zeta                                                               | 5.2        |
| 23959  | **Nt5e**  | 5' nucleotidase, ecto                                                        | 4.7        |
| 19272  | **Ptpkr** | protein tyrosine phosphatase, receptor type, K                                | 7.0        |
| 20197  | **S100a3** | S100 calcium binding protein A3                                              | 5.3        |
| ID       | Gene    | Description                                                                 | R1 | R2 | R3 | R4 | R5 | R6 | R7 | R8 |
|----------|---------|-----------------------------------------------------------------------------|----|----|----|----|----|----|----|----|
| 100217453 | Snord16a| small nucleolar RNA, C/D box 16A                                            |  7.0 |  6.5 |  5.3 |  5.8 |  6.7 |  5.6 | -1.2 | -2.3 |
| 11421    | Ace     | angiotensin I converting enzyme (peptidyl-dipeptidase A) 1                 |  3.4 |  4.8 |  2.9 |  2.9 |  4.1 |  2.9 | -1.2 | -2.3 |
| 170757   | Eltd1   | EGF, latrophilin seven transmembrane domain containing 1                    |  5.1 |  3.6 |  3.1 |  3.2 |  4.4 |  3.2 | -1.2 | -2.3 |
| 53614    | Reck    | reversion-inducing-cysteine-rich protein with kazal motifs                 |  6.9 |  7.4 |  6.1 |  5.8 |  7.2 |  6.0 | -1.2 | -2.3 |
| 58994    | Smpd3   | sphingomyelin phosphodiesterase 3, neutral                                  |  5.4 |  4.4 |  3.8 |  3.7 |  4.9 |  3.7 | -1.2 | -2.3 |
| 13797    | Emx2    | empty spiracles homeobox 2                                                 |  5.2 |  4.0 |  3.7 |  3.1 |  4.6 |  3.4 | -1.2 | -2.3 |
| 16773    | Lama2   | laminin, alpha 2                                                           |  6.2 |  6.3 |  5.0 |  5.1 |  6.3 |  5.1 | -1.2 | -2.3 |
| 17386    | Mmp13   | matrix metalloproteinase 13                                                |  8.3 |  9.1 |  6.8 |  8.1 |  8.7 |  7.5 | -1.2 | -2.3 |
| 22329    | Vcam1   | vascular cell adhesion molecule 1                                          |  8.2 |  8.3 |  7.2 |  6.9 |  8.2 |  7.0 | -1.2 | -2.3 |
| 217887   | BC022687| cDNA sequence BC022687                                                     |  6.7 |  6.6 |  5.4 |  5.4 |  6.6 |  5.4 | -1.2 | -2.3 |
| 19224    | Ptgs1   | prostaglandin-endoperoxide synthase 1                                      |  7.1 |  7.8 |  5.9 |  6.5 |  7.4 |  6.2 | -1.2 | -2.3 |
| 14611    | Gja3    | gap junction protein, alpha 3                                              |  5.1 |  3.5 |  3.2 |  2.8 |  4.3 |  3.0 | -1.2 | -2.3 |
| 13876    | Erg     | avian erythroleukemia virus E-26 (v-ets) oncogene related                 |  4.6 |  4.4 |  3.2 |  3.3 |  4.5 |  3.3 | -1.2 | -2.3 |
| 16669    | Krt19   | keratin 19                                                                 |  4.5 |  4.6 |  3.4 |  3.3 |  4.6 |  3.3 | -1.2 | -2.3 |
| 216459   | Myl6b   | myosin, light polypeptide 6B                                                |  5.6 |  5.5 |  4.5 |  4.2 |  5.6 |  4.3 | -1.2 | -2.3 |
| 18788    | Serpinb2| serine (or cysteine) peptidase inhibitor, clade B, member 2                |  7.8 |  6.1 |  4.1 |  7.3 |  6.9 |  5.7 | -1.2 | -2.3 |
| 20042    | Rps12   | ribosomal protein S12                                                      |  4.9 |  4.6 |  3.3 |  3.7 |  4.7 |  3.5 | -1.2 | -2.3 |
| 97848    | Serpinb6c| serine (or cysteine) peptidase inhibitor, clade B, member 6c             |  7.1 |  5.7 |  5.7 |  4.5 |  6.4 |  5.1 | -1.3 | -2.4 |
| 23967    | Osr1    | odd-skipped related 1 (Drosophila)                                         |  7.9 |  9.0 |  7.6 |  6.8 |  8.5 |  7.2 | -1.3 | -2.4 |
| 16367    | Irs1    | insulin receptor substrate 1                                               |  6.2 |  5.7 |  4.8 |  4.6 |  6.0 |  4.7 | -1.3 | -2.4 |
| 13395    | Dlx5    | distal-less homeobox 5                                                     |  3.0 |  5.2 |  3.0 |  2.6 |  4.1 |  2.8 | -1.3 | -2.4 |
| 110454   | Ly6a    | lymphocyte antigen 6 complex, locus A                                       |  8.1 | 10.1 |  8.1 |  7.6 |  9.1 |  7.9 | -1.3 | -2.4 |
| 73904    | 4833412C05Rik | RIKEN cDNA 4833412C05 gene                                                 |  5.9 |  4.9 |  3.9 |  4.3 |  5.4 |  4.1 | -1.3 | -2.4 |
| 14461    | Gata2   | GATA binding protein 2                                                     |  4.7 |  4.3 |  3.3 |  3.1 |  4.5 |  3.2 | -1.3 | -2.5 |
| 279653   | Pcdh19  | protocadherin 19                                                           |  7.3 |  6.7 |  5.9 |  5.6 |  7.0 |  5.7 | -1.3 | -2.5 |
| 109294   | Prex2   | phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2   |  5.5 |  5.5 |  4.3 |  4.1 |  5.5 |  4.2 | -1.3 | -2.5 |
| 229672   | Bcl2l15 | BCL2-like 15                                                               |  5.4 |  4.9 |  3.1 |  4.7 |  5.2 |  3.9 | -1.3 | -2.5 |
| 216616   | Efemp1  | epidermal growth factor-containing fibulin-like extracellular matrix protein 1 |  7.9 |  9.6 |  7.8 |  7.1 |  8.8 |  7.5 | -1.3 | -2.5 |
| 11668    | Aldh1a1 | aldehyde dehydrogenase family 1, subfamily A1                              |  7.8 |  7.0 |  6.6 |  5.5 |  7.4 |  6.1 | -1.3 | -2.5 |
| 407828   | BC023969| cDNA sequence BC023969                                                    |  3.8 |  5.7 |  3.3 |  3.5 |  4.7 |  3.4 | -1.3 | -2.5 |
| 216831   | Arhgap4 | Rho GTPase activating protein 44                                             |  6.4 |  6.4 |  5.3 |  4.9 |  6.4 |  5.1 | -1.3 | -2.5 |
| 211323   | Nrg1    | neuregulin 1                                                                |  6.6 |  5.6 |  4.8 |  4.7 |  6.1 |  4.8 | -1.3 | -2.5 |
| Gene      | Description                                      | Fold Change 1 | Fold Change 2 | Fold Change 3 | Fold Change 4 | Fold Change 5 | Fold Change 6 |
|-----------|--------------------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Itga2     | integrin alpha 2                                 | 6.5           | 6.7           | 4.6           | 5.8           | 6.6           | 5.2           |
| Kpp       | keratinocyte expressed, proline-rich             | 7.2           | 5.5           | 5.0           | 4.8           | 6.3           | 4.9           |
| Pdk4      | pyruvate dehydrogenase kinase, isoenzyme 4       | 7.5           | 6.6           | 5.5           | 5.8           | 7.1           | 5.6           |
| Tinagl1   | tubulointerstitial nephritis antigen-like 1      | 6.7           | 7.3           | 5.5           | 5.7           | 7.0           | 5.6           |
| Ghr       | growth hormone receptor                          | 7.7           | 7.4           | 6.3           | 6.1           | 7.6           | 6.2           |
| Haus7     | HAUS augmin-like complex, subunit 7              | 5.6           | 6.4           | 4.7           | 4.5           | 6.0           | 4.6           |
| Hsd11b1   | hydroxysteroid 11-beta dehydrogenase 1           | 5.3           | 7.2           | 5.0           | 4.6           | 6.2           | 4.8           |
| Hspb7     | heat shock protein family, member 7 (cardiovascular) | 7.6           | 8.1           | 6.4           | 6.4           | 7.9           | 6.4           |
| Ang2      | angiotensin, ribonuclease A family, member 2     | 7.4           | 7.7           | 5.9           | 6.2           | 7.5           | 6.0           |
| Bmp6      | bone morphogenetic protein 6                    | 5.0           | 4.8           | 3.6           | 3.2           | 4.9           | 3.4           |
| Pcdh18    | protocadherin 18                                 | 6.8           | 6.1           | 5.2           | 4.8           | 6.4           | 5.0           |
| Rnd1      | Rho family GTPase 1                             | 5.8           | 6.1           | 4.5           | 4.5           | 6.0           | 4.5           |
| Syt17     | synaptotagmin XVII                               | 7.0           | 7.0           | 5.2           | 5.9           | 7.0           | 5.5           |
| Gucy1b3   | guanylate cyclase 1, soluble, beta 3             | 6.2           | 5.3           | 4.1           | 4.4           | 5.8           | 4.3           |
| Procr     | protein C receptor, endothelial                  | 8.0           | 6.8           | 5.8           | 6.1           | 7.4           | 5.9           |
| Sdr39u1   | short chain dehydrogenase/reductase family 39U, member 1 | 7.1           | 4.8           | 4.1           | 4.9           | 6.0           | 4.5           |
| Dpt       | dermatopontin                                   | 6.9           | 5.8           | 4.4           | 5.3           | 6.4           | 4.8           |
| Gpr116    | G protein-coupled receptor 116                   | 4.9           | 4.3           | 3.0           | 3.1           | 4.6           | 3.1           |
| Mapk13    | mitogen-activated protein kinase 13              | 6.6           | 6.6           | 4.8           | 5.4           | 6.6           | 5.1           |
| Tnfrsf11b | tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin) | 7.6           | 7.9           | 6.5           | 6.0           | 7.8           | 6.2           |
| Ctl2a     | cytotoxic T lymphocyte-associated protein 2      | 9.5           | 8.7           | 7.9           | 7.2           | 9.1           | 7.6           |
| Pbp2      | phosphatidylethanolamine binding protein 2       | 5.7           | 4.6           | 4.0           | 3.2           | 5.1           | 3.6           |
| Sdcbp2    | syndecan binding protein (syntenin) 2            | 7.0           | 5.4           | 4.7           | 4.6           | 6.2           | 4.7           |
| Serpinb9b | serine (or cysteine) peptidase inhibitor, clade B, member 9b    | 9.4           | 9.1           | 8.0           | 7.4           | 9.2           | 7.7           |
| Cd24a     | CD24a antigen                                   | 9.4           | 8.5           | 6.3           | 8.6           | 9.0           | 7.4           |
| Gsta2     | glutathione S-transferase, alpha 2 (Yc2)        | 5.7           | 6.1           | 4.4           | 4.2           | 5.9           | 4.3           |
| Sox9      | SRY (sex determining region Y)-box 9             | 5.1           | 6.6           | 3.9           | 4.6           | 5.8           | 4.2           |
| Plagl1    | pleiomorphic adenoma gene-like 1                | 5.6           | 6.3           | 3.5           | 5.2           | 6.0           | 4.4           |
| Car2      | carbonic anhydrase 2                             | 6.1           | 5.2           | 3.8           | 4.3           | 5.7           | 4.0           |
| Ltbp1     | latent transforming growth factor beta binding protein 1 | 6.7           | 7.4           | 5.2           | 5.6           | 7.0           | 5.4           |
| Edn1      | endothelin 1                                    | 8.8           | 7.8           | 6.6           | 6.8           | 8.3           | 6.7           |

- Fold Change indicates the change in expression relative to the reference sample.
| ID    | Gene      | Description                                      | Value1 | Value2 | Value3 | Value4 | Value5 | Value6 |
|-------|-----------|--------------------------------------------------|--------|--------|--------|--------|--------|--------|
| 13640 | Efna5     | ephrin A5                                        | 6.2    | 6.6    | 4.3    | 5.1    | 6.4    | 4.7    | -1.7   | -3.2   |
| 11459 | Acta1     | actin, alpha 1, skeletal muscle                   | 8.8    | 7.7    | 5.6    | 7.6    | 8.2    | 6.6    | -1.7   | -3.2   |
| 232431| Gprc5a    | G protein-coupled receptor, family C, group 5, member A | 4.6    | 4.8    | 2.6    | 3.4    | 4.7    | 3.0    | -1.7   | -3.2   |
| 68178 | Cgn1      | cingulin-like 1                                   | 7.7    | 7.3    | 5.6    | 6.1    | 7.5    | 5.8    | -1.7   | -3.2   |
| 68052 | Rps13     | ribosomal protein S13                             | 4.3    | 4.5    | 2.6    | 2.8    | 4.4    | 2.7    | -1.7   | -3.2   |
| 11435 | Chrna1    | cholinergic receptor, nicotinic, alpha polypeptide 1 (muscle) | 3.7    | 5.1    | 2.7    | 2.7    | 4.4    | 2.7    | -1.7   | -3.3   |
| 14368 | Fzd6      | frizzled homolog 6 (Drosophila)                   | 5.8    | 4.7    | 3.4    | 3.6    | 5.2    | 3.5    | -1.7   | -3.3   |
| 16400 | Itga3     | integrin alpha 3                                  | 5.4    | 5.1    | 3.3    | 3.7    | 5.2    | 3.5    | -1.7   | -3.3   |
| 64058 | Perp      | PERP, TPS3 apoptosis effector                     | 6.2    | 5.0    | 3.9    | 3.9    | 5.6    | 3.9    | -1.7   | -3.3   |
| 17389 | Mmp16     | matrix metallopeptidase 16                        | 4.7    | 5.1    | 3.3    | 3.1    | 4.9    | 3.2    | -1.7   | -3.3   |
| 320092| E030003E18Rik | RIKEN cDNA E030003E18 gene                  | 5.9    | 5.1    | 4.5    | 3.1    | 5.5    | 3.8    | -1.8   | -3.4   |
| 224093| Fam43a    | family with sequence similarity 43, member A      | 6.3    | 6.6    | 5.2    | 4.2    | 6.5    | 4.7    | -1.8   | -3.4   |
| 20324 | Sdpr      | serum deprivation response                       | 8.9    | 9.5    | 7.5    | 7.4    | 9.2    | 7.4    | -1.8   | -3.4   |
| 13717 | Eln       | elastin                                          | 5.1    | 6.6    | 4.0    | 4.1    | 5.8    | 4.0    | -1.8   | -3.5   |
| 67718 | Lce1h     | late cornified envelope 1H                        | 7.7    | 3.3    | 3.7    | 3.7    | 5.5    | 3.7    | -1.8   | -3.5   |
| 20708 | Serpinb6b | serine (or cysteine) peptidase inhibitor, clade B, member 6b | 8.3    | 7.9    | 6.7    | 5.9    | 8.1    | 6.3    | -1.8   | -3.5   |
| 14725 | Lrp2      | low density lipoprotein receptor-related protein 2 | 5.2    | 4.1    | 2.8    | 2.9    | 4.7    | 2.8    | -1.8   | -3.5   |
| 16891 | Lipg      | lipase, endothelial                              | 5.0    | 4.6    | 2.7    | 3.2    | 4.8    | 3.0    | -1.8   | -3.6   |
| 67828 | Lce1f     | late cornified envelope 1F                        | 7.2    | 3.1    | 3.3    | 3.2    | 5.1    | 3.3    | -1.9   | -3.6   |
| 18104 | Nqo1      | NAD(P)H dehydrogenase, quinone 1                  | 7.3    | 7.4    | 5.0    | 5.9    | 7.3    | 5.5    | -1.9   | -3.7   |
| 105450| Mmrn2     | multimerin 2                                      | 6.3    | 5.3    | 3.7    | 4.1    | 5.8    | 3.9    | -1.9   | -3.8   |
| 71690 | Esm1      | endothelial cell-specific molecule 1              | 8.1    | 8.2    | 5.9    | 6.5    | 8.2    | 6.2    | -1.9   | -3.8   |
| 94253 | Hecw1     | HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1 | 6.1    | 4.9    | 3.8    | 3.2    | 5.5    | 3.5    | -2.0   | -4.1   |
| 105349| Akr1c18   | aldo-keto reductase family 1, member C18          | 5.6    | 8.0    | 4.0    | 5.3    | 6.8    | 4.7    | -2.1   | -4.3   |
| 101772| Ano1      | anoctamin 1, calcium activated chloride channel   | 5.1    | 4.7    | 2.7    | 2.8    | 4.9    | 2.7    | -2.1   | -4.4   |
| 21892 | Tli1      | tolloid-like                                      | 8.4    | 8.0    | 5.6    | 6.5    | 8.2    | 6.1    | -2.1   | -4.4   |
| 69325 | 1700012B09Rik | RIKEN cDNA 1700012B09 gene                    | 5.8    | 6.9    | 3.7    | 4.5    | 6.4    | 4.1    | -2.3   | -4.9   |
| 12159 | Bmp4      | bone morphogenetic protein 4                     | 7.5    | 7.8    | 6.6    | 4.1    | 7.7    | 5.4    | -2.3   | -5.1   |
| 319154| Hist2h3b  | histone cluster 2, H3b                             | 6.2    | 4.5    | 2.5    | 3.5    | 5.4    | 3.0    | -2.4   | -5.2   |
| 22268 | Upk1b     | uroplakin 1B                                     | 9.2    | 5.8    | 4.3    | 5.9    | 7.5    | 5.1    | -2.4   | -5.2   |
| 12490 | Cd34      | CD34 antigen                                      | 7.0    | 5.8    | 3.5    | 4.5    | 6.4    | 4.0    | -2.4   | -5.3   |
| 114301| Palmd     | palmdelphin                                      | 6.1    | 5.2    | 3.3    | 3.1    | 5.6    | 3.2    | -2.4   | -5.4   |
| 20344 | Selp      | selectin, platelet                               | 6.3    | 6.5    | 3.9    | 4.1    | 6.4    | 4.0    | -2.5   | -5.5   |
| 59308 | Emcn      | endomucin                                        | 9.3    | 8.1    | 6.0    | 6.5    | 8.7    | 6.2    | -2.5   | -5.6   |
| 18613 | Pecam1    | platelet/endothelial cell adhesion molecule 1    | 6.7    | 6.3    | 3.9    | 4.1    | 6.5    | 4.0    | -2.5   | -5.6   |
| Gene ID  | Gene Name          | Description                                                   | FPKM 1  | FPKM 2  | FPKM 3  | FPKM 4  | FPKM 5  | FPKM 6  | FPKM 7  |
|---------|--------------------|---------------------------------------------------------------|---------|---------|---------|---------|---------|---------|---------|
| 12562   | Cdh5               | cadherin 5                                                    | 8.7     | 7.0     | 5.0     | 5.7     | 7.8     | 5.3     | -2.5    | -5.7    |
| 18812   | Prl2c3             | prolactin family 2, subfamily c, member 3                     | 9.5     | 9.0     | 6.4     | 6.6     | 9.2     | 6.5     | -2.7    | -6.6    |
| 18812   | Prl2c3             | prolactin family 2, subfamily c, member 3                     | 9.3     | 9.0     | 6.2     | 6.6     | 9.1     | 6.4     | -2.7    | -6.7    |
| 223272  | Itgb1I             | integrin, beta-like 1                                         | 7.6     | 7.7     | 4.5     | 5.2     | 7.6     | 4.9     | -2.8    | -6.7    |
| 76294   | Asb5               | ankyrin repeat and SOCs box-containing 5                     | 7.8     | 6.9     | 3.7     | 5.4     | 7.3     | 4.6     | -2.8    | -6.8    |
| 240873  | Tnfsf18            | tumor necrosis factor (ligand) superfamily, member 18         | 9.1     | 7.0     | 4.5     | 6.0     | 8.0     | 5.2     | -2.8    | -7.1    |
| 20753   | Sprr1a             | small proline-rich protein 1A                                 | 8.6     | 9.6     | 5.4     | 7.1     | 9.1     | 6.2     | -2.8    | -7.2    |
| 19263   | Ptprb              | protein tyrosine phosphatase, receptor type, B                | 6.5     | 6.4     | 3.7     | 3.5     | 6.4     | 3.6     | -2.9    | -7.4    |
| 228576  | Mall               | mal, T cell differentiation protein-like                     | 8.2     | 5.7     | 4.4     | 3.6     | 7.0     | 4.0     | -3.0    | -7.9    |
| 72381   | 2210409E12Rik      | transcription elongation factor B (SIII), polypeptide 2       | 6.4     | 6.0     | 4.0     | 2.3     | 6.2     | 3.2     | -3.0    | -8.1    |
| 12319   | Car8               | carbonic anhydrase 8                                          | 7.3     | 7.2     | 4.4     | 4.0     | 7.2     | 4.2     | -3.0    | -8.1    |
| 68632   | Myct1              | myc target 1                                                 | 7.4     | 6.7     | 3.9     | 4.2     | 7.1     | 4.0     | -3.0    | -8.1    |
| 74175   | Crtc1              | cysteine-rich C-terminal 1                                    | 6.6     | 6.4     | 3.1     | 3.8     | 6.5     | 3.4     | -3.1    | -8.3    |
| 224796  | Clic5              | chloride intracellular channel 5                              | 6.4     | 6.9     | 2.9     | 3.1     | 6.7     | 3.0     | -3.7    | -12.6   |
**Figure S1.** Arterial trees dissected from *Cre(-)*, *SM22α-Cre(+)*, and *aP2-Cre(+) MMP14F/F ApoE−/−* male and female mice after a 12-week Western diet.

|                | Male                      | Female                   |
|----------------|---------------------------|--------------------------|
| Cre(-)         | ![Arterial tree](cre_neg.png) | ![Arterial tree](cre_neg_female.png) |                |
| SM22α-Cre(+)   | ![Arterial tree](sm22a_cre_pos.png) | ![Arterial tree](sm22a_cre_pos_female.png) |                |
| aP2-Cre(+)     | ![Arterial tree](aP2_cre_pos.png) | ![Arterial tree](aP2_cre_pos_female.png) |                |
Figure S2. Gene expression of Acta2(α-SMA), Cre enzyme, MT1-MMP (MMP14), MMP2, MMP8, MMP9, MMP13, and MT2-MMP (MMP15) in primary VSMCs isolated from Cre(-) and SM22α-Cre(+) MMP14<sup>F/F</sup> Apoe<sup>−/−</sup> mice. Almost complete suppression of MT1-MMP expression in primary VSMCs was confirmed coupled with SM22-dependent Cre expression. Expression of other MMPs was not affected. *** P<0.001