Supplementary Materials and Methods

Immunohistochemistry and immunofluorescence

Paraffin-embedded tissues were sectioned, deparaffinized, steamed in Target Retrieval Solution Ready to Use (Dako) for 40 minutes, blocked with Protein Block Serum-Free (Dako), and then incubated with primary antibodies overnight at 4°C. For IHC, primary antibodies were followed by secondary antibodies (Vector Laboratories) and then detected with 3,3'-Diaminobenzidine kit (Vector Laboratories). For IF, primary antibodies were followed by Alexa Fluor Dye secondary antibodies (Thermo Fisher Scientific) and mounted with Vectashield hard-set mounting medium with DAPI counterstain (Vector Laboratories). Images were captured at room temperature on a Nikon E800 fluorescence microscope with 40X Plan Apo objective and a Nikon DS-QiMc camera with Nikon Elements imaging software (vAR 3.0).

Immunoblotting

Cells were lysed in RIPA buffer (Sigma) supplemented with protease and phosphatase inhibitors (Sigma), and protein concentration of lysates was quantified using a BCA kit (Thermo Fisher Scientific). Lysates were fractionated on NuPAGE gels (Invitrogen). Proteins were transferred to poly-vinylidene difluoride membranes (Millipore), blocked, and then incubated with primary antibodies. Blots were developed using enhanced chemiluminescence (Thermo Fisher Scientific).

RNA isolation and quantitative Real-Time PCR

Total RNA was purified using RNeasy Plus Minikit (Qiagen). First-strand cDNA was synthesized using random hexamer primers (Promega) and Ready-To-Go You-Prime First-Strand Beads (GE Healthcare), according to the manufacturers' instructions. Quantitative PCR was performed on a StepOnePlus Real-Time PCR System (Applied Biosystems) using TaqMan Fast Advanced PCR

Master Mix (Applied Biosystems) with TaqMan primers. StepOne software (v2.3, Applied Biosystems) was used to calculate threshold cycle values (Applied Biosystems).

Colony forming unit assay

Adult prostate MSCs, expanded between passages 1 and 3, were plated at the following seeding densities per T25 flask: $5x10^4$, $1.5x10^5$, and $2.5x10^5$ cells. Fetal MSCs, expanded between passages 2 and 7, were plated at the following seeding densities per T25 flask: $5x10^3$, $1x10^4$, and $2x10^4$ cells. Three biological replicates were performed per experimental group and each seeding density was technically replicated two times. Cells were grown in DMEM supplemented with 10% FBS, GlutaMAX, and penicillin/streptomycin at 5% CO_2 and 37°C for 21 days. For exogenous ASPN, media contained either vehicle or 100ng/mL recombinant mouse ASPN (Origene). At 21 days, representative photographs were taken of each condition using a TE-200 inverted microscope (Nikon). Medium was aspirated from each flask, colonies were washed twice with PBS, and stained with 0.2% crystal violet in 50% methanol for 5 minutes. Flasks were destained by washing twice with distilled water. Flasks were air-dried and colonies counted.

Cell proliferation assay

Aspn$^{+/+}$ and *Aspn*$^{-/-}$ fetal MSCs, expanded between passages 1 and 3, were plated at 2500 cells/well of a 48-well plate and cultured for 1-7 days in DMEM supplemented with 10% FBS, GlutaMAX (1X), penicillin (100 U/mL), and streptomycin (100 µg/mL) at 5% CO_2 and 37°C. At each time point, media was removed, cells were incubated with 0.5 mg/mL Thiazolyl Blue Tetrazolium Bromide (Sigma) in complete media for one hour at 37°C and 5% CO_2. This solution was then removed, cells were incubated with DMSO and gently agitated for 5 minutes in the dark at room temperature, and the absorbance of the DMSO solution was read at 570 nm using an accuSkan FC Microplate Photometer (Thermo Fisher Scientific). Three biological replicates were

assessed per experimental group and each biological replicate had three technical replicates per time point.

Cell migration assays

To assess migration, cells were cultured in 6-well plates and grown to 100% confluency. Then, using a 1000 μL pipette tip, a scratch in the form of the letter "H" was made through the lawn of cells, the media was replaced, and a 0 time point picture was taken at spots with relatively equal scratch widths. At subsequent time points, pictures were taken at the same spots and percent closure of the scratch was calculated using the MRI Wound Healing Tool (ImageJ). Two biological replicates were performed per experimental group and each biological replicate was represented as the average of ≥ 9 technical replicates. For *Aspn*$^{+/+}$ and *Aspn*$^{-/-}$ fetal MSCs, which demonstrated a cell proliferation difference, we instead utilized a trans-well assay (Millipore) to assess cell migration. Fetal MSCs, expanded between passages 1 and 3, were serum-depleted for 24 hours, plated at 1.5×10^5 cells per well in serum-depleted media and were allowed to migrate toward the serum-replete (10% FBS) lower chamber via 8 μm pores. At four hours post-seeding, cell migration toward the lower chamber was assessed via cell stain-colorimetry according to the manufacturer's protocol. Three independent samples were assessed per experimental group. For exogenous ASPN, fetal MSCs were serum and calcium depleted for 24 hours, plated in serum-depleted and calcium low (80 mg/mL) media with either vehicle, 100 ng/mL recombinant mouse ASPN (Origene), 1 mM BAPTA, or 1 mM BAPTA plus ASPN.

Cytoskeletal remodeling

Cytoskeletal remodeling of cells was assessed as previously described (1). Briefly, serum-deprived cells adherent to collagen-coated plates were incubated for 20 minutes with ~5×10^5 ferrimagnetic RGD-coated microbeads. The synthetic RGD peptide binds avidly to cell surface integrin receptors and its subsequent movement couples that of the inner remodeling

cytoskeleton. Unbound beads were washed away and under microscopic observation, the spontaneous displacement of individual microbeads was recorded at set time intervals and graphed as mean square displacement (nm^2) over time (seconds). Potential confounders, such as microscope stage drift, were corrected for in the analysis. Two independent clones were assessed per experimental group.

PELICAN study

All studies using human tissue were approved by the Johns Hopkins Institutional Review Board. PELICAN (Project to ELIminate lethal CANcer) Rapid Autopsy Study of Prostate Cancer has been previously described (2). Patients were consented to participate in the Johns Hopkins Medicine IRB-approved study between 1995 and 2005. A study tissue microarray (TMA) was constructed from formalin fixed tissue taken at autopsy (n=16 patients).

At least three cores (0.6 mm) were sampled from each metastasis.

Four-micrometer-cut sections were stained for ASPN (Sigma Prestige) by IHC. Cases were scored by urologic pathologists for stromal ASPN expression. Using established scoring schemes (3), ASPN intensity was evaluated and assigned an incremental score of 0 (negative), 1 (weak), 2 (moderate), or 3 (strong). The extent of stromal staining was assigned a percentage from 0-100%. For each core, an ASPN score was calculated by multiplying the intensity score and the extent score (H-score). The mean H-score was recorded for each metastatic site. Fifteen of the 16 patients were evaluable, and the mean number of evaluable metastatic sites per patient was 4 (ranging from 2 to 6).

CP1 *E. coli* mouse model of prostate inflammation

All animal procedures were performed under JHU approved Institutional Animal Care and Use Committee (IACUC) protocol. Mice were purchased from The Jackson Laboratory and maintained under standard pathogen free conditions. CP1 bacteria was originally isolated from the expressed

prostatic secretions of a patient with chronic prostatitis (4). CP1 is an *E. coli* strain of the B1 clonal group. The CP1 bacteria mouse model of prostate inflammation was induced as described previously (5). In brief, a single dose of $1x10^8$ CFU CP1 or saline control in 10 µl of PBS was delivered to the urethra by catheterization of anaesthetized 8 week old male C57BL/6J mice.

MSC isolation

Human: MSCs were isolated from human tissue in accordance with IRB-approved protocols as described previously (6). Briefly, fetal prostate samples (~14-18 weeks gestation) were obtained in accordance with federal and state guidelines, dissociated into single cell suspensions, and cultured as described previously (7). Prostate tissue from young men (<25 years old) was obtained through a rapid organ donor program organized by the National Disease Research Interchange (NDRI). Prostate cancer tissue was obtained from patients undergoing a radical prostatectomy at the Johns Hopkins Brady Urological Institute via the Prostate Biospecimen Repository. Bone marrow-derived MSCs were obtained from healthy bone marrow donors through the Johns Hopkins Biospecimen Repository Core or purchased from RooserBio, Inc. Normal prostate fibroblasts were obtained as described previously (8). Tissues were dissociated and grown in RoosterNourish™-MSC (RoosterBio) to generate primary stromal cultures. RNA was isolated when cultures were at a confluence of approximately 80%.

Mouse: All animal protocols were approved by the Johns Hopkins University Animal Care and Use Committee. UGM and UGE were isolated from mice as described previously (9, 10). The UGS was removed from C57BL/6J embryonic day 15.5 males and then digested in 1% trypsin (Gibco Thermo Fisher Scientific) in Hank's Balanced Salt Solution (Gibco Thermo Fisher Scientific) at 4°C for 90 minutes. Following digestion, UGS were washed with DMEM (Gibco Thermo Fisher Scientific) containing 10% FCS (Gibco Thermo Fisher Scientific). UGM and UGE were then separated with a fine gauge needle. Fetal MSCs were isolated by euthanizing

C57BL/6J *Aspn*$^{+/+}$ or *Aspn*$^{-/-}$ pregnant females at embryonic day 13.5 via isoflurane inhalation followed by cervical dislocation. The uterine horns were dissected, and each embryo was separated from its placenta and embryonic sac. The head and red organs were removed and discarded, while the remaining tissue was minced with a sterile razor, and digested with trypsin + DNAse I (1 mg/mL) for single cell isolation. Adult prostate MSCs were isolated by euthanasia of ≥ 24-week-old male mice and *en bloc* removal of the genitourinary system (GU=prostate, seminal vesicles, bladder, vas deferens, urethra). The prostatic lobes were microdissected from the rest of the GU, finely minced with a sterile razor, digested in DMEM + 10% FBS + 1:10 dilution of collagenase/hyaluronidase for one hour at 37°C, triturated in pre-warmed 1X PBS + DNAse I (1 mg/mL), filtered through a 40 μm cell strainer, and plated on culture flasks. Fetal MSCs and adult prostate MSCs were cultured in DMEM supplemented with 10% FBS, GlutaMAX (1X), penicillin (100 U/mL), and streptomycin (100 μg/mL). Adult bone marrow-derived MSCs were isolated as described previously (11, 12). In brief, ≥ 24-week old male mice were euthanized and their hind legs removed. Femurs and tibias were isolated by removal of attached tendons and muscles, and bones were cracked using a sterile mortar and pestle into ice-cold 1X PBS supplemented with 2% FBS and EDTA (1 mM) to release the bone marrow. Bone marrow was plated on culture flasks and grown at 37°C in hypoxia (1% O_2, 5% CO_2) until fibroblast colonies were visible (~14 days), then hematopoietic cells were removed by CD45 and TER119 negative selection (EasySep Mouse Mesenchymal Stem/Progenitor Cell Enrichment Kit, STEMCELL Technologies). Bone marrow-derived murine MSCs were cultured in MesenCult Expansion Media (STEMCELL Technologies). Adult compact bone-derived MSCs were isolated as described previously (13). In brief, ≥ 24-week old male mice were euthanized and their hind legs removed. Femurs and tibias were isolated by removal of attached tendons and muscles, and bones were cracked using a sterile mortar and pestle into ice-cold PBS supplemented with 2% FBS and EDTA (1 mM) to release the bone marrow. Remaining bone fragments were washed by gentle agitation an additional 3-4 times with ice cold PBS until all bone marrow was released (indicated by loss of

red coloration). Bone fragments were then digested at 37°C shaking for 1 hour in complete media containing 1 mg/mL collagenase II (Gibco). Following digestion, bone fragments were washed several times with complete media, seeded onto culture flasks, and grown at 37°C in hypoxia (1% O_2, 5% CO_2) until adherent cells were detected.

MSC lineage specific differentiation assays

Osteogenic and adipogenic Ddifferentiation

Fetal MSCs, expanded between passages 1 and 3, were cultured under hypoxic conditions until reaching 80-90% confluency. Normal growth medium was then aspirated and replaced with either osteogenic or adipogenic differentiation-inducing medium (STEMCELL Technologies). Cells incubated at 37°C in hypoxia (1% O_2, 5% CO_2), and differentiation media was changed every 3-4 days until bone matrix formation or intracellular lipid droplets was observed (14-21 days). For BMP inhibition, osteogenic medium (STEMCELL Technologies) contained 250 nM LDN-193189 (SelleckChem) or vehicle control. For exogenous ASPN, osteogenic medium (STEMCELL Technologies) contained 100 ng/mL recombinant mouse ASPN (Origene) or vehicle control. Osteogenic and adipogenic differentiation was detected by Alizarin Red and Oil Red O staining, respectively. Alternatively, cells were harvested for RNA isolation and lineage-specific markers were detected by qRT-PCR. Two biological replicates and three technical replicates were performed per experimental group and condition.

Osteogenic-differentiated fetal MSCs were washed with PBS, fixed in ice cold 70% ethanol for one hour at room temperature, washed three times with distilled water, and stained with 2% Alizarin Red staining solution for one hour at room temperature. Cells were then washed twice with distilled water and mineralized nodules were present as bright red stained spots.

Adipogenic-differentiated fetal MSCs were washed with PBS, fixed in 10% formaldehyde for 30 minutes at room temperature, washed three times with distilled water, rinsed once with

60% isopropanol, and stained with 0.5% Oil Red O in isopropanol for 15 minutes at room temperature. Cells were then washed three times with distilled water and lipid droplets were present as bright red intracellular vesicles.

Chondrogenic differentiation

The method of chondrogenic differentiation was adapted from a prior report (14). Fetal MSCs ($5x10^5$ total cells) expanded between passages 1 and 3, were pelleted in a 14 mL polypropylene tube and incubated with chondrogenic differentiation-inducing media (STEMCELL Technologies). Cells incubated at 37°C in normoxia with the cap loosened to allow adequate airflow, and differentiation media was changed every 3-4 days for 21 days. Cartilage pellets were then harvested for paraffin-embedding and Alcian Blue staining or RNA isolation for detection of lineage-specific markers by qRT-PCR. Two biological replicates were performed per experimental group. For RNA isolation, more than 10 technical replicate pellets were pooled.

Chondrogenic pellets were fixed in 10% formalin for 30 minutes at room temperature, paraffin-embedded, cut into 6μm sections, deparaffinized, hydrated in distilled water, and stained with Alcian Blue staining solution for 30 minutes at room temperature. The slide was then washed in distilled water, dehydrated, cleared, and mounted. The cartilaginous matrix was present as a blue stain surrounding the cells.

Gene expression analysis

Gene expression was performed on WPMY-1-ASPN Neo, WPMY-1-ASPN D14, and WPMY-1-ASPN D13 clones using Affymetrix Human Clariom D arrays. Differential gene expression of *Aspn*$^{+/+}$ and *Aspn*$^{-/-}$ fetal MSCs and WPMY-1-ASPN Neo, WPMY-1-ASPN D14, and WPMY-1-ASPN D13 clones was analyzed using Transcriptome Analysis Console (Applied Biosystems).

References

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Key Resources

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit Anti-ASPN antibody	Sigma Prestige	Cat# HPA008435 RRID:AB_1845112
Mouse Anti-Vimentin antibody	Sigma	Cat# V2258 RRID:AB_261856
Rabbit anti-phospho SMAD1/5/9 antibody	Cell Signaling Technology	Cat# 13820 RRID:AB_2493181
Rabbit anti-SMAD1 antibody	Cell Signaling Technology	Cat# 9743 RRID:AB_2107780
Mouse anti-GAPDH antibody	Santa Cruz Biotechnology	Cat# sc-32233 RRID:AB_627679
Mouse anti-CK8 antibody	Covance Research Products Inc	Cat# MMS-162P-250 RRID:AB_291334
Rabbit anti-CK14 antibody	Covance Research Products Inc	Cat# PRB-155P RRID:AB_292096
Rabbit anti-Ki67 antibody	Abcam	Cat# ab15580 RRID:AB_443209
Mouse anti-FLAG	Sigma	Cat# F1804
Rabbit anti-BMP4	R&D	Cat# MAB5020
Mouse anti-SMA	Dako	Cat# M085129
APC/Cy7 Rat anti-mouse CD45 antibody	BioLegend	Cat# 103115 RRID:AB_312980
APC Rat anti-mouse CD105 antibody	BioLegend	Cat# 120413 RRID:AB_2277915
PE/Cy7 Armenian Hamster anti-mouse/rat CD29 antibody	BioLegend	Cat# 102221 RRID:AB_528789
FITC Rat anti-mouse Ly-6A/E (Sca-1) antibody	BioLegend	Cat# 108105 RRID:AB_313342
Ly6G	BioLegend	Clone 1A8
Ly6C	BioLegend	Clone HK1.4
CD11b	BioLegend	Clone M1/70
CD11c	BioLegend	Clone N418
CCR2	BioLegend	Clone SA203G11
F4/80	BioLegend	Clone BM8
CD3	BD Pharmingen	Clone 145-2C11
CD4	BioLegend	Clone GK1.5
CD8a	BioLegend	Clone 53-6.7
FoxP3	BioLegend	Clone 150D
CD44	BioLegend	Clone IM7
ImmPRESS HRP anti-mouse IgG antibody, made in Horse	Vector Laboratories	Cat# MP-7402 RRID:AB_2336528
ImmPRESS HRP anti-rabbit IgG antibody, made in Horse	Vector Laboratories	Cat# MP-7401 RRID:AB_2336529
F(ab)2-Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	Thermo Fisher Scientific	Cat# A-11017 RRID:AB_2534084
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 594	Thermo Fisher Scientific	Cat# A-21207 RRID:AB_141637
IgG agarose	Sigma	Cat# A0919
anti-FLAG M2 magnetic beads	Sigma	Cat# M8823
3x FLAG peptide	Sigma	Cat# F4799
Bacterial and Virus Strains		
CP1 *E. coli* strain	Brian W. Simons (Simons et al., 2015)	
Biological Samples		
Human Primary Prostate Cancer	Mayo Clinic Progression cohort (Hurley et al., 2016)	
Metastatic Prostate Cancer TMA	PELICAN rapid autopsy study of prostate cancer	
Matched prostate inflammation & cancer tissue	Johns Hopkins School of Medicine	
Chemicals, Peptides, and Recombinant Proteins		
RGD-coated microbeads	Steven S. An (An et al., 2004)	
FuGENE HD Transfection Reagent	Promega	Cat# E2311
LIVE/DEAD Fixable Aqua Dead Cell Stain kit (405nm excitation)	Thermo Fisher Scientific	Cat# L34957
MesenCult Osteogenic Stimulatory Kit (Mouse)	STEMCELL Technologies	Cat# 05504
MesenCult Adipogenic Stimulatory Supplement (Mouse)	STEMCELL Technologies	Cat# 05503
MesenCult ACF Chondrogenic Differentiation Medium	STEMCELL Technologies	Cat# 05455
Alizarin Red Staining solution	Millipore	Cat# TMS-008-C
Oil Red O solution	Sigma	Cat# O1391
Alcian-Blue Staining solution	Millipore	Cat# TMS-010-C
Recombinant Human BMP4	Peprotech	Cat# 120-05
Recombinant Mouse BPM4	R & D	Cat#5020-BP
Recombinant Mouse ASPN generated in 293T cells	Origene	Custom
Matrigel, Growth Factor Reduced (GFR)	Corning	Cat# 356230
Recombinant Mouse EGF	BioLegend	Cat# 713108
Y-27632 (ROCK inhibitor)	STEMCELL Technologies	Cat# 72302
5α-Androstan-17β-ol-3-one (DHT)	Sigma	Cat# A8380
A83-01 (TGFb inhibitor)	Tocris	Cat# 2939
Advanced DMEM/F12	Thermo Fisher Scientific	Cat# 12634-010
50X B-27 Supplement	Life Technologies	Cat# 17504-044
GlutaMAX	Life Technologies	Cat# 35050-079
HEPES	Life Technologies	Cat# 15630-080
Penicillin/Streptomycin (10,000 U/mL)	Life Technologies	Cat# 15140-122
10X Collagenase/Hyaluronidase	STEMCELL Technologies	Cat# 07912
Dispase (5 U/mL)	STEMCELL Technologies	Cat# 07913
10X DNAse I	STEMCELL Technologies	Cat# 07900
Thiazolyl Blue Tetrazolium Bromide	Sigma	Cat# M2128
Target Retrieval Solution, Ready to Use	Dako	Cat# S1700
Protein Block Serum-Free	Dako	Cat# X0909
Antibody Diluent Reagent Solution, Ready-to-use	Invitrogen	Cat# 003218
ImmPACT DAB (3,3'-Diaminobenzidine) Peroxidase Substrate	Vector Laboratories	Cat# SK-4105
VECTASHIELD HardSet Antifade Mounting Medium with DAPI counterstain	Vector Laboratories	Cat# H-1500
FcR Blocking Reagent, mouse	Miltenyi	Cat# 130-092-575
TaqMan Fast Advanced Master Mix	Thermo Fisher Scientific	Cat# 4444557
SimplyBlue SafeStain	Invitrogen	Cat# LC6060
Critical Commercial Assays		
Pierce BCA Protein Assay Kit, Reducing Agent Compatible	Thermo Fisher Scientific	Cat# 23250
SuperSignal West Pico PLUS Chemiluminescent Substrate	Thermo Fisher Scientific	Cat# 34580
QCM 24-well Colorimetric Cell Migration Assay	Millipore	Cat# ECM508

RNeasy Plus Mini Kit	Qiagen	Cat# 74134
Ready-To-Go You-Prime-First-Strand Beads	GE Healthcare	Cat# 27-9264-01
Amicon Ultra-4 10K Centrifugal Filter Device	Millipore	Cat# UFC801008
EasySep Mouse Mesenchymal Stem/Progenitor Cell Enrichment Kit	STEMCELL Technologies	Cat# 19771
MesenCult Expansion Kit (Mouse)	STEMCELL Technologies	Cat# 05513
Deposited Data		
SuperSeries (GSE109646)	This study	GEO database
Experimental Models: Cell Lines		
Human: WPMY-1	ATCC	
Human: WPMY-1-ASPN variants	Paula J. Hurley (Hurley et al., 2016)	
Human: MSCs (various sources)	W. Nathaniel Brennen (Brennen et al., 2017)	
Human: PC-3	ATCC	
Human: DU-145	ATCC	
Mouse: MSCs (various sources)	this study	
Mouse: B6MycCaP	Leigh Ellis (Ellis et al., 2016)	
Mouse: TRAMP-C2	ATCC	
Mouse: B6CaP organoids	this study	
Experimental Models: Organisms/Strains		
C57BL/6J mice	Jackson Labs	
B6;129S5-*Aspn* ^{tm1Lex} /Mmucd mice	Mutant Mouse Regional Resource Center (UC Davis)	
C57BL/6-Tg(TRAMP)8247Ng/J mice	Jackson Labs	
Oligonucleotides		
murine *Aspn* wild-type specific forward primer	5’ AGTCTACTTTGCCACATTCACC 3’	
murine *Aspn* wild-type specific reverse primer	5’ GCTTTTGGCTGTGTGCTCTGC 3’	
murine *Aspn* mutation specific forward primer	5’CGAGAGTAACATTGGCACCCAAATG 3	
murine *Aspn* mutation specific reverse primer	5’ GCAGCGCATCGCCTTCTATC 3’	
Random hexamer primers	Promega	Cat# C118A
Human ASPN TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Hs01550903_m1
Human HPRT1 TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Hs02800695_m1
Human GAPDH TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Hs02758991_g1
Mouse Aspn TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm00445939_m1
Mouse Alpl TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm00475834_m1
Mouse Runx2 TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm00501584_m1
Mouse Sp7 TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm04209856_m1
Mouse Adipoq TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm00456425_m1
Mouse Pparg TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm00440940_m1
Mouse Tfap2a TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm00495574_m1
Mouse Col2a1 TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm01309565_m1
Mouse Col10a1 TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm00487041_m1
Mouse Acan TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm00545794_m1
Mouse Dcn TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm00514535_m1
mouse Bgn TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm01191753_m1
mouse Ecm2 TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm01349471_m1
mouse Hprt1 TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm03024075_m1
mouse Gapdh TaqMan qRT-PCR Gene Expression assay	Thermo Fisher Scientific	ID# Mm99999915_g1
Recombinant DNA		
pIRESneo3 vector	Clontech	Cat# 631621
ASPN (NM_017680) Human cDNA clone	OriGene	Cat# SC321136
Software and Algorithms		
ImageJ	NIH	https://fiji.sc
PRISM	Graphpad	https://ww.graphpad.com
RStudio	RStudio Team	http://www.rstudio.com
FlowJo	FLOWJO LLC	FlowJo
NIS Elements (vAR 3.0)	Nikon	https://www.nikoninstruments.com
StepOne Software (v2.3)	Applied Biosystems (Thermo Fisher Scientific)	https://www.thermofisher.com
Transcriptome Analysis Console (TAC, v4.0)	Thermo Fisher Scientific	https://www.thermofisher.com