Supplementary Materials and Methods:

MiR-34a-5p expression in human plasma

Blood samples from total knee replacement (TKR) patients and healthy controls were collected in ethylenediaminetetraacetic acid dipotassium salt (K2-EDTA) containing tubes and centrifuged at 4000rpm for ten minutes at 4°C to obtain a plasma supernatant. Plasma was aliquoted into cryotubes, flash frozen in liquid nitrogen, and stored in a vapour liquid nitrogen tank until use. Samples were excluded if patients self-reported the following pre-existing conditions: high blood pressure, high cholesterol, lung disease, diabetes, ulcer, stomach disease, kidney disease, liver disease, blood disease, cancer, rheumatoid arthritis (RA), heart attack, coronary artery disease, stroke, thyroid problems, dementia, chronic neck pain, or chronic pelvic pain. This ensured that our analysis was not confounded by these pre-existing conditions since healthy control volunteers did not report any of the above conditions.

Isolation and purification of RNA from plasma (n=37 TKR; n=34 healthy controls) was performed using the miRCURY RNA Isolation Kit– Biofluids (#300112, Exiqon) following manufacturer's instructions. From 200µl of human plasma, ten nanograms of RNA was used for reverse transcription using the miRCURY LNA Universal RT microRNA PCR (#203301, Exiqon) system for quantification of miR-34a-5p.

Total RNA extraction from human knee articular cartilage and human synovium

Late-stage radiographic knee OA articular cartilage (n=11) and synovium (n=7) used for RT-qPCR analysis were obtained from patients undergoing total knee replacement (TKR; KL III/IV), while early radiographic knee osteoarthritis (OA; KL 0/I) synovium (n=7) was obtained from patients undergoing joint arthroscopy. Normal articular cartilage samples used as controls (n=8) were

obtained from cadavers with no history of arthritis, joint injury, joint trauma or surgery, no prescription anti-inflammatory medications, no co-morbidities, ≥ 18 years old, and were harvested within 4 hours of death (University of Calgary; REB 15-0005). 200 mg of snap frozen tissue was crushed in liquid nitrogen using a Cellcrusher tissue pulverizer (Cellcrusher). The barrel and ball were precooled in liquid nitrogen immediately prior to crushing. 500 µl of TRIzol reagent (#15596026, Invitrogen) was added to further homogenize the cartilage on wet ice using the Vibra Cell sonicator (Model VC50, Sonics and Materials Inc.). Synovial tissue was homogenized on ice using a Polytron PT 2100 benchtop homogenizer (Kinematica AG). RNA was purified using a RNeasy Mini kit (#74104, Qiagen) according to manufacturer's instructions. RNA was quantified using Nano-Drop 1000 (Thermo Scientific). RNA was reverse-transcribed and miR-34a-5p expression was determined using RT-qPCR as described in "*Total RNA extraction from cells and Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR)*" (see below).

Destabilization of Medial Meniscus (DMM) surgery

C57BL/6J mice (Jackson Laboratories, USA) were bred and housed in a 21±1°C controlled room under a 12-hour light-dark cycle at the Krembil Research Institute animal facility. Animal access to food and water was ad-libitum. Ten-week-old C57BL/6J and 19-week-old high-fat diet (HFD)-fed C57BL/6J male mice were subjected to destabilization of the medial meniscus (DMM) surgery in the right knee as previously described [1]. All animal studies were performed according to relevant guidelines and regulations approved by the UHN's animal care committee (Animal Use Protocol #3729). As experimental controls, mice were subjected to sham surgery whereby the joint capsule was opened and the medial meniscotibial ligament was exposed but not transected.

In-situ hybridization

Mouse knee joints were harvested and fixed in 4% paraformaldehyde (n=3 four weeks, n=4 ten weeks post-Sham; n=3 four weeks, n=4 ten weeks post-DMM), while human cartilage from TKR patients (n=4 non-degenerated; n=4 degenerated) was fixed in neutral buffered formalin (NBF) for 48 h and decalcified in 10% EDTA (RNase-free) for two to three weeks at 4°C. Non-degenerated cartilage was obtained from the lateral femoral condyle of TKR patients that were microscopically determined as undamaged and used as controls. Joints sections were cut as described below for histology using RNase-free water. Slides were kept at 4°C until use. ISH was performed using the miRCURY LNA™ microRNA ISH Optimization Kit (FFPE) (#90000, Exiqon) according to manufacturer's instructions. Probes used were LNA microRNA-34a-5p double DIG-labeled (25 µ*M*; final probe concentration 160 n*M*), LNA Scramble-miR probe, double-DIG labeled (negative control; 25 µ*M*; final probe concentration 40nM), and LNA U6 snRNA probe, 5' DIG-labeled (positive control; 0.5 µ*M*; final probe concentration 1 n*M*). Nuclei were counterstained with Nuclear Fast Red (#H-3403; Vector laboratories). Cytoplasmic blue staining indicates positive probe binding. The average percentage of positive cells for mouse cartilage, synovium and meniscus, and human cartilage, was determined by two independent reviewers in a blinded fashion from three distinct regions of each section.

Chondrocyte and fibroblast-like synoviocyte (FLS) treatment with miR-34a-5p mimic or antisense oligonucleotide (ASO)

Human OA chondrocytes and FLS were were isolated by enzymatic digestion from cartilage and synovial tissue obtained from patients undergoing TKR at Toronto Western Hospital as previously described [2, 3]. Only first-passage chondrocytes were used for experiments while passage three

FLS were used. Cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin under 5% atmospheric CO_2. Cells were transfected in suspension with 100 n*M* hsa-miR-34a-5p *mir*Vana miRNA mimic (#4464066, Invitrogen), negative control mimic (cel-miR-39-3p; #479902-001, Exiqon), hsa-miR-34a-5p *mir*Vana miRNA inhibitor (ASO; #4464084, Invitrogen), or miRCURY LNA Power Inhibitor Control (Negative control A, #199006-101, Exiqon) for 24 h using Lipofectamine™ RNAiMAX transfection reagent (#13778075, Invitrogen, USA) according to manufacturer's instructions. The hsa-miR-34a-5p *mir*Vana miRNA mimic is a chemically modified double-stranded RNA that mimics endogenous, mature miR-34a-5p activity with high specificity due to inactivation of the passenger miRNA star strand, while the hsa-miR-34a-5p *mir*Vana miRNA inhibitor (ASO) is a chemically modified single-stranded RNA ASO that specifically binds to and reduces endogenous miR-34a-5p transcript levels [4]. The control oligonucleotide (*mir*Vana™ miRNA mimic Negative Control or miRCURY LNA Power Inhibitor Control) is uniquely designed to have no known human, mouse, or rat gene targets.

Total RNA extraction from cells and Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR)

Total RNA was extracted from human OA chondrocytes or FLS using TRIzol reagent, and GlycoBlue co-precipitant (15 mg/ml) (#AM9516, Invitrogen) for miRNA enrichment. After RNA quantification, 600 ng of mRNA was converted to cDNA using a QuantiTect Reverse Transcription PCR Kit (#205313, Qiagen) according to manufacturer's instructions. Diluted (5x) cDNA was used for RT-qPCR reactions in 96-well plates (#HSR9905, Biorad) using Sso Advanced Universal SYBR Green Supermix (#1725274, Biorad) according to manufacturer's

instructions. Primer specificity was confirmed by performing melting curve analysis on a Roche LightCycler 480 II real-time PCR system. GAPDH was used as an endogenous reference gene. OA phenotypic markers investigated were *ACAN, COL2A1, ADAMTS5, MMP13, COL1OA1, COL1A1, IL1B, ULK1, ATG3, ATG5, ACTA2, TGFB, TNF,* and *IL6*.

For miRNA, ten nanograms of total RNA was reverse transcribed using a Universal cDNA synthesis kit II (#203301, Exiqon) and PCR reactions were run using ExiLENT SYBR Green master mix (#203421, Exiqon) according to manufacturer's instructions. MiR-24-3p was used as an endogenous reference gene since its expression remained consistent across different tissues, as well as plasma from humans and mice compared to other housekeeping genes that were tested. Relative gene/miRNA expressions were calculated by the $2^{-\Delta Ct}$ method. cDNA samples were excluded if GAPDH/miR-24 cycle threshold values (C_t) varied by more than 2 cycles.

Mouse intra-articular miR-34a-5p mimic knee injections

Ten-week-old C57BL/6J male mice were anesthetized with isofluorane and 5 µg (in 3 µl) of in-vivo ready miRCURY locked nucleic acid (LNA)^{TM} microRNA Mimic (mmu-miR-34a-5p; #479996-601, Exiqon) was injected intra-articularly using a 33G needle (#80308, Hamilton) into the right knees (n=8). Left knees were injected with 5 µg Negative Control miRCURY LNA miRNA Mimic (#YM00479902, Exiqon; n=8). Joints were collected eight weeks post-injection for histopathological assessments.

MiR-34a-5p expression in obese vs. non-obese total knee replacement (TKR) patients

According to their body mass index (BMI), TKR patients were stratified into obese (BMI ≥ 30; n=22) and non-obese (BMI = 18.9-29.9; n=29) groups. Relative expression levels of plasma miR-34a-5p, as quantified by qRT-PCR, were compared between both groups.

Mouse high-fat diet (HFD) regime

Male mice were fed a normal chow diet (5.8% fat by weight, Harlan, Teklad, LM-485) from birth until ten weeks of age (n=44). At ten weeks of age (baseline), mice were placed on a HFD (34.3% fat by weight, Harlan, TD 06414; n=19) for up to 18 weeks to induce obesity. This diet-induced model of obesity was previously characterized using dual-energy X-ray absorptiometry (DEXA) scanning to assess parameters such as body fat percentage and body area, fasting blood glucose levels, and insulin levels in HFD mice compared to LD mice [5]. Mice were placed on a lean diet (LD; 4.2% fat by weight, Harlan, TD 06416; n=25) for 18 weeks as controls. 18 week HFD-fed (n=3) and 18 week LD-fed (n=3) mouse knee joints were subjected to ISH as described above for miR-34a-5p detection and quantification. Mice were weighed at baseline and at the end of HFD or LD using a digital weighing machine (Ohaus Corporation, CS 200). DEXA scanning (tube peak potential: 80, 100, 140 kVp, Discovery A, Hologic) was used for whole body composition analysis as previously reported [5].

Mouse plasma collection and blood glucose measurement

Mouse cages were placed under a heat lamp for approximately 10 minutes to promote vasodilation and facilitate blood collection. Approximately 200 µl of blood was collected (baseline or end of HFD/LD) from the saphenous vein of non-anesthetized mice into EDTA K+ coated microvettes

(Sarstedt). Blood collection site was then treated topically with antibiotic ointment (Polysporin). Plasma was separated by centrifugation at 10,000 rcf at 4°C. For miR-34a-5p quantification in plasma, 10 ng of RNA from 50 μl of mouse plasma was reverse transcribed using the miRCURY LNA Universal RT microRNA PCR (#203301, Exiqon) system. Levels of miR-34a-5p were analyzed by miRNA qRT-PCR as described above ("*Total RNA extraction from cells and Quantitative Reverse Transcription Polymerase Chain Reaction [qRT-PCR]").* In order to measure fasting blood glucose levels, mice were fasted for six hours and blood glucose was measured from tail vein blood samples at ten weeks old [baseline; (n=5)] and at the end of nine weeks of HFD (n=5) with a one-touch glucose meter (Accu-chek Aviva, Roche).

High-fat diet (HFD) mice subjected to DMM surgery and intra-articular miR-34a-5p inhibitor (LNA-ASO) injections

Two experiments involving HFD-fed mice were performed. First, ten-week-old C57BL/6J male mice were placed on a HFD for 18 weeks. After 18 weeks of HFD (28 weeks of age), mice were subjected to DMM surgery followed by three bi-weekly intra-articular injections of 5 μg of miR-34a-5p LNA ASO (n=5) or control oligonucleotide (n=5) (i.e. weeks two, four, and six post-DMM surgery). The LNA modification of the ASO enables increased sensitivity, specificity, and in vivo stability **[6]**. Joints were collected ten weeks post-DMM surgery for histopathological assessments. Second, ten-week-old C57BL/6 mice were fed a HFD for nine weeks. 19-week-old mice were then subjected to DMM surgery followed by three weekly injections of 5 μg of miR-34a-5p LNA ASO (n=6) or control oligonucleotide (n=5) (i.e. weeks two, three, and four post-DMM surgery). Joints were then collected five weeks post-DMM surgery for histopathological assessments.

Histology and immunohistochemistry

Mouse knee joints were isolated and fixed for 48 h in 10% NBF at room temperature. Knee joints were decalcified in rapid decalcifier solution (RDO solution; Apex Engineering Products Corporation) for 1 h 45 min, washed three times with double distilled water and paraffin-embedded. Sections (5 μm) were dried at 40 °C for 24 h. Human knee OA cartilage was obtained from patients undergoing TKR surgery (KL III/IV), fixed in 10% NBF for 48 h, decalcified in RDO for approximately 3 h, and processed for histology as previously reported [7] . The sections were then de-paraffinized, rehydrated and stained with Safranin-O/Fast green (Sigma-Aldrich) according to the manufacturer's instructions. The OARSI scoring method for mice was used to analyze and score knee joint sections by two independent, blinded reviewers [8]. Average of scores from two independent reviewers is reported. To evaluate the degree of synovitis, sections were stained with Masson's trichrome (Sigma-Aldrich) as previously reported [9]. Sections were then scored by two scorers in a blinded fashion on a scale from 0 to 3 considering ECM deposition, cell number, and tissue thickness: 0, no synovitis; 1, mild synovitis; 2, moderate synovitis; and 3, severe synovitis. Chondrocyte cellularity above the tidemark was counted in three areas along the tibial plateau and the average cellularity per area was quantified by two independent, blinded reviewers.

Formalin-fixed, paraffin-embedded mouse knee joint sections from the medial aspect of the knee were deparaffinized in xylene and rehydrated in a graded series of alcohol washes (100%, 95%, 70%, and 50% ethanol). Slides were incubated in 10 m*M* sodium citrate buffer (pH 6.0) at 60°C for three hours for antigen retrieval. Sections were incubated with Dual Endogenous Enzyme Block (#S2003, Dako) for 30 minutes to block endogenous peroxidase activity. Sections were blocked with 1% BSA for 30 minutes and incubated overnight at 4°C with MMP-13 (1:50; #ab39012; Abcam), C1,2C (Col 2 3/4C$_{short}$; 1:250; #50-1035; IBEX Pharmaceuticals Inc.),

Cleaved Caspase-3 (Asp175) (1:100) (9661S; Cell signaling Technology), or anti-poly (ADP-ribose) polymerase (PARP) p85 fragment pAb (1:50; G734A; Promega). After two water washes, sections were incubated with rabbit or mouse biotinylated secondary antibodies for 30 min. Signal was amplified with horseradish peroxidase (HRP) conjugated to streptavidin using Vectastain Elite ABC kit (#PK-6100; Vector Laboratories) and developed with DAB peroxidase (HRP) Substrate Kit with nickel (#SK-4100; Vector Laboratories) according to manufacturer's instructions. Tissue sections were counterstained with eosin Y (#E511-25; Fisher Scientific), dehydrated using a graded series of alcohol washes and xylene, and mounted with Permount (#SP15-500; Fisher Scientific). Total cells and cells with positive staining were counted in three separate fields to determine the average percentage of chondrocytes positive for individual antigens in the tibial plateau and femoral condyle. The presence of extracellular matrix breakdown products was visualized by the degree of C1,2C staining in the extracellular matrix.

MiR-34a knock-out (KO) mice

Heterozygous constitutive miR-34a-KO mice were purchased from Taconic Biosciences (#9743; Material Transfer Agreement UHN Ref# 2016-0861). Briefly, the targeting vector was generated using C57BL/6J DNA and transfected into C57BL/6NTac embryonic stem cells. The miR-34a sequence is flanked by LoxP sites. The constitutive KO allele was generated after Cre-mediated recombination by crossing chimeras to a Cre-expressing mouse on a C57BL/6 background. The constitutive KO line was subsequently backcrossed to C57BL/6NTac animals for at least two generations and the Cre-transgene was removed by segregation (supplementary Fig. S5A).

Heterozygous mice ($Cre^{KO}/miR34a^{+}$) were bred in-house and offspring were genotyped to identify homozygous KO ($Cre^{KO}/miR34a^{-}$) and wild-types (WT) (Cre/ $miR34a^{+}$) mice. MiR-34a

homozygous KO mice were fertile and born in the expected Mendelian ratios without any overt developmental phenotypes. A more extensive characterization investigating approximately 300 physiological parameters of homozygous miR-34a-KO mice have been previously reported with no major phenotypic abnormalities compared to WT littermates [10]. Primers were designed to amplify CRE [deleted null (KO; 216 bp) or CRE WT (1824 bp)] or miR-34a alleles (222 bp). CRE primer sequence (5' to 3'): Forward TGCCCTGAAGTGAAGAGTACC; Reverse CCCAAACTCATTATGTAGTCAGG. MiR-34a primer sequence (5' to 3'): Forward CCGAGGACACAGGACATAGC; Reverse CCCAAACTCATTATGTAGTCAGG. Both primers are combined to determine zygosity.

Isolation and Culture of Mouse Articular Chondrocytes

WT and KO female mice were euthanized at five-weeks-old and articular cartilage was scraped from the knees (both tibial plateau and femoral condyle surfaces) and both hips (femoral head) using a scalpel and collected in sterile cold 1x PBS on ice. Primary chondrocytes were isolated according to protocol 3.3 in the text book Osteoporosis and Osteoarthritis [11]. Cartilage pieces were washed three times in cold 1x PBS. PBS was decanted and 15 ml of 3 mg/ml Collagenase *from Clostridium histolyticum* (#C0130-100MG, Sigma-Aldrich) solution was added to the cartilage pieces, poured into a sterile petri dish and incubated at 37°C in a cell culture incubator overnight. Cartilage pieces were pipetted up and down to release the cells, strained (70 µm cell strainer), and cells were centrifuged at 1500 x g for 5 minutes. Cells were washed with DMEM (supplemented with 10% FBS, 1% penicillin-streptomycin), pelleted and resuspended. Cells were counted then plated in 6-well plates and cultured for approximately ten days or until confluent.

Total RNA was extracted from first passage KO and WT mouse chondrocytes as described above, for qRT-PCR analysis of *Col2a1* and *Acan*.

Preparation of RNA sequencing Libraries

Total RNA extracted from cultured mouse chondrocytes was quantified using the Qubit RNA BR assay (ThermoFisher) on the Denovix DS-11 spectrophotometer (Denovix, Wilmington). RNA quality was assessed using the RNA nano chips on a Bioanalyzer instrument (Agilent, Santa Clara). RNA integrity numbers (RIN) for all samples were > 9.7. Sample libraries were prepared using TruSeq Stranded Total RNA with RiboZero following the low sample protocol as per manufacturer's recommendations (Illumina). Briefly, ribosomal RNA was first removed from 200 ng of total RNA. Remaining RNA was purified using a Agencourt RNAClean XP kit (Beckman Coulter), fragmented and reverse transcribed. The resulting cDNA was adenylated at the 3' ends, to which adapters were then ligated, which barcoded the samples enabling multiplexing and facilitated binding to the flow cell for sequencing. The modified cDNA was enriched using a Agencourt AMPure XP kit (Beckman Coulter), amplified by 15 cycles of PCR, and enriched once more. Library quality was assessed on a high sensitivity DNA chip on the Bioanalyzer (Agilent) and quantified using a KAPA Library Quantification Kit for Illumina platforms (Roche). Six libraries prepared from three biological samples for both WT and miR-34a-KO mice were diluted to 10 n*M* and volumetrically pooled. Pooled library sample was quantified using high sensitivity dsDNA assay (Denovix), diluted to 4 n*M*, denatured with 0.2N sodium hydroxide for 5 minutes and neutralized with 0.2 *M* Tris pH 7. Denatured libraries were then diluted down to 1.5 p*M*, 1% PhiX was added, and sequenced on an Illumina NextSeq 550 sequencer for 75 paired end read-cycles at the Arthritis Program's Diagnostic and Therapeutic Innovation Centre (Krembil Research

Institute, Toronto, ON, Canada). Sequencing data sets have been deposited in the gene expression omnibus (GEO) data repository with the accession code GSE133775.

Bioinformatics analysis on RNA sequencing data

Six paired-end samples belong to two groups: WT or miR-34a-KO. Conversion of bcl to Fastq files was performed for each sample using bcl2fastq conversion tool (v2.19.1.403). Initial quality assessment of each sample revealed that the reads were of high quality (> Q30). Cutadapt (v1.18) software was used to maintain minimum read length of 25 bp post trimming of adapters (very few reads being contaminated = 3%) as well as trimming of N's [12]. Splice-aware alignment of reads using a Hierarchical Graph FM index (HGFM) method was performed using HISAT2 software (v2.1.0) with parameters (--rna-strandness FR –dta) against mouse reference genome (mm10) [13]. To assemble the aligned reads into transcripts, StringTie software (v1.3.5) was used with parameters (--rf assuming it was a stranded library fr-firststrand) [14]. To obtain the abundance of each of these transcripts based only on the known reference genome and transcriptome, StringTie was run again using different parameters (-e -B -G referencetranscripts.gtf) to generate output of Ballgown software compatible table files. This script was provided by StringTie software named 'prepDE.py', which helps in retrieving raw counts for genes and transcripts separately based on the table files.

Differential expression analysis

Genes with at least ten counts per million (CPM) in at least two of the six samples were retained for the analysis, leaving 10195 of 24421. A list of differentially expressed genes was obtained using the negative binomial exact test with trended dispersion and a trimmed mean of M-values

(TMM) normalization. The resulting p-values were adjusted for false discovery rate (FDR) using the Benjamini-Hochberg method and significance was set at $p \leq 0.05$. The analysis was done in R (v3.6.0) using the edgeR (v3.25.8) package [15]. The heatmaps are the log fold change (FC) relative to the mean of the wild type group in each gene, logFC are calculated using TMM adjusted CPM+1 counts.

Integrative network of differentially expressed genes identified from RNA sequencing of wild-type and miR-34a knock-out mouse chondrocytes

Top 1% of predicted targets of hsa-miR-34a-5p and hsa-mir-34a-3p were independently identified using mirDIP (version 4.1) (http://ophid.utoronto.ca/mirDIP/). Transcription factor (TF) regulatory network for the nine genes of interest was obtained using data from 15 independent resources (see supplementary table S4), including human TF-target gene associations derived from Chromatin Immunoprecipitation (ChIP) high-throughput experiments, gene regulatory network inference and other predictive algorithms. Protein-protein interactions (PPI) for the resulting network were added by searching Integrated Interactions Network (http://ophid.utoronto.ca/iid) ver. 2018-11 [16]. Network was then visualized in NAViGaTOR ver. 3.0.10 [17, 18].

Supplementary Figures:

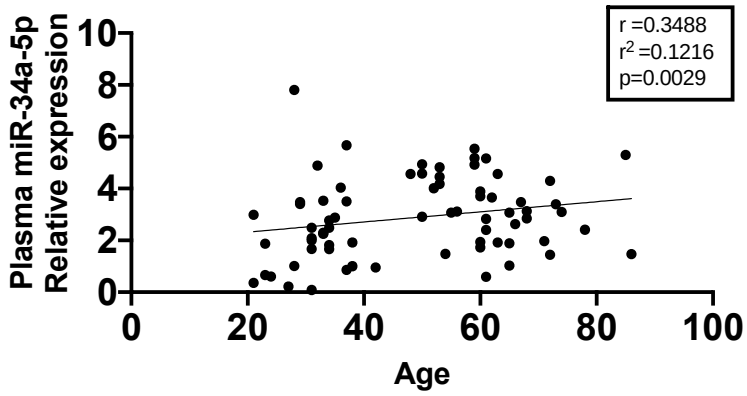

Fig. S1. Correlation between age and plasma miR-34a-5p expression. Two-tailed Pearson's correlation showing the relationship between age (TKR and healthy controls) and plasma miR-34a-5p. n = 71.

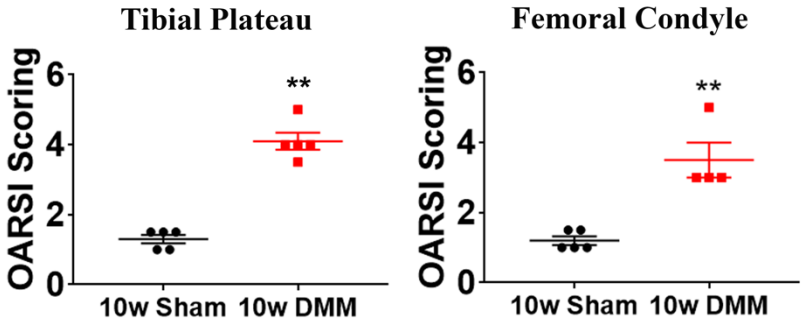

Fig. S2. Destabilization of the medial meniscus (DMM)-induced OA mouse model. OARSI scoring of tibial plateau and femoral condyle surfaces of the medial compartment of mouse knees ten weeks post-DMM (n = 4-5) or -sham (n = 5) surgery. Data is represented as mean ± SEM and analyzed using Mann-Whitney U tests. ** = $P < 0.01$.

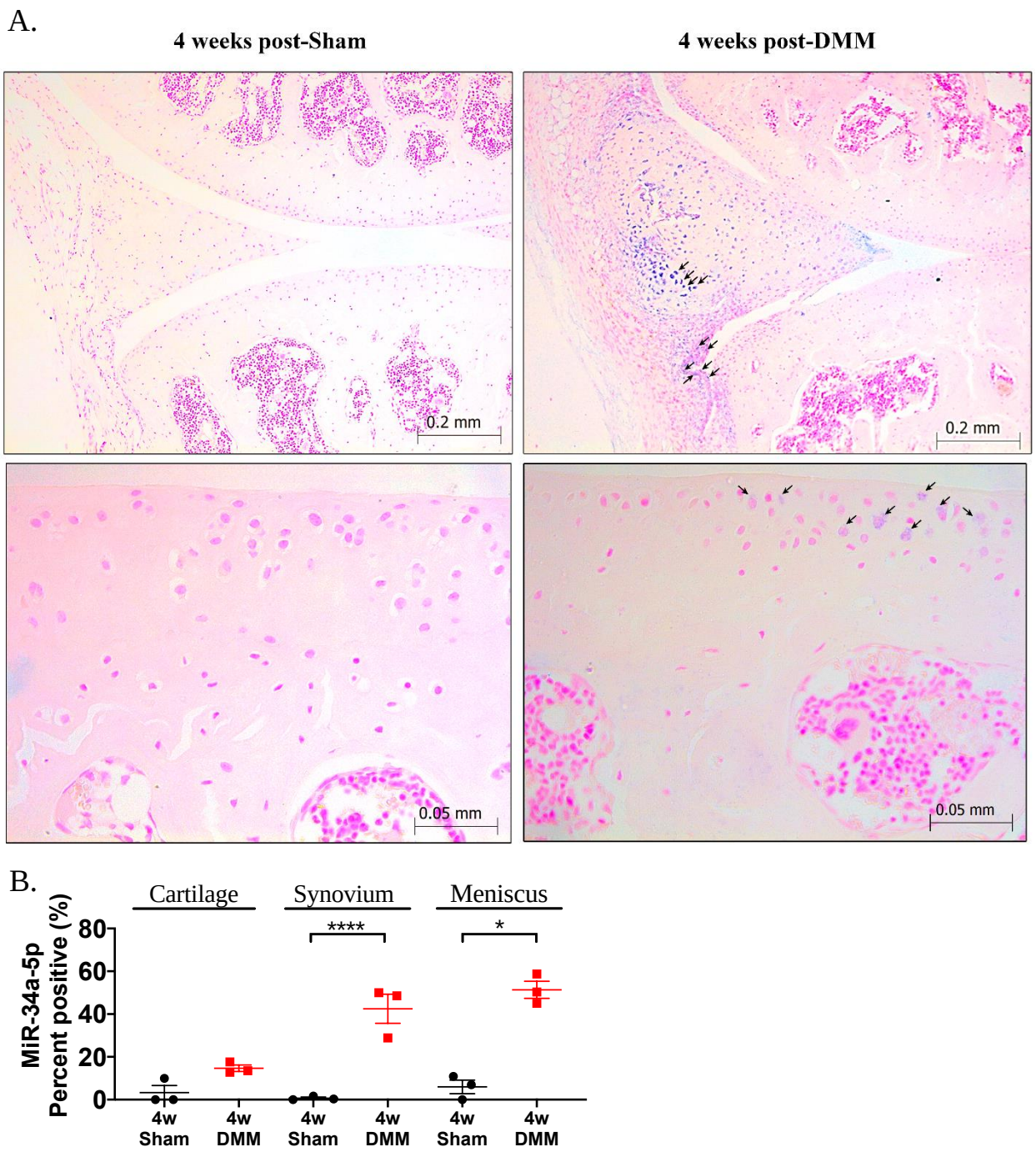

Fig. S3 In-situ hybridization of mouse knee sections four weeks post-DMM surgery. (A) Representative ISH images stained with miR-34a-5p probe in knee sections of mice four weeks post-sham or -DMM surgery (n = 3/group) showing the synovium and medial meniscus (top: 10x magnification) and articular cartilage (bottom: 40x magnification). Black arrows indicate miR-34a-5p positively stained cells. (B) ISH quantification of the percentage of miR-34a-5p positively stained cells in mouse knee articular cartilage, synovium and medial meniscus four weeks post-DMM or -sham surgery. Data are represented as mean ± SEM and were log-transformed prior to analysis by two-tailed Student's T-test. *= $P < 0.05$, *** = $P < 0.001$.

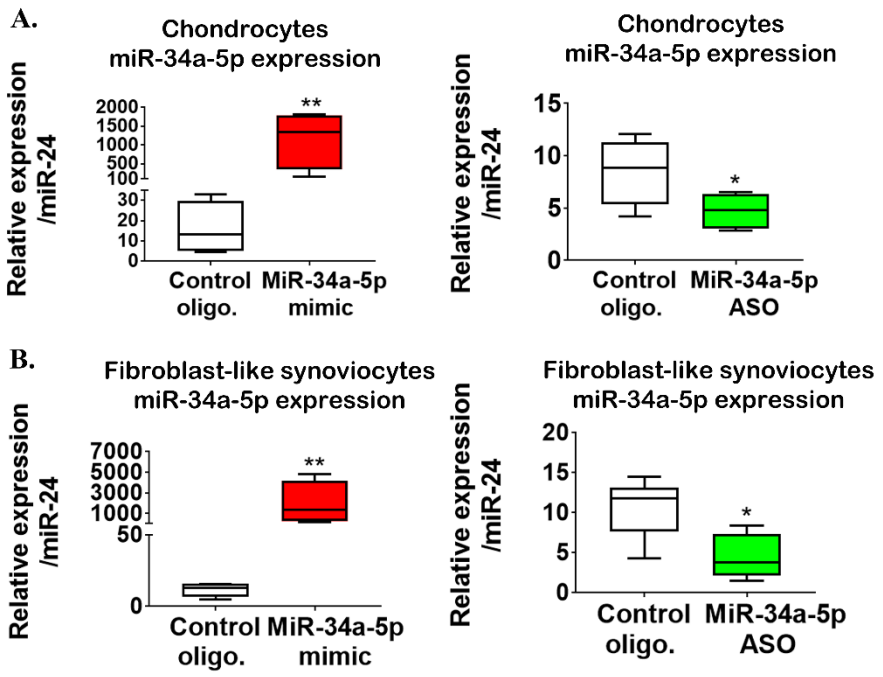

Fig. S4. MiR-34a-5p mimic and antisense oligonucleotide (ASO) treatment in human knee OA chondrocytes and fibroblast-like synoviocytes (FLS). (A) Relative expression of miR-34a-5p in human OA chondrocytes treated with 100 n*M* control oligonucleotide, miR-34a-5p mimic, and miR-34a-5p ASO for 24 h (n = 4). (B) Relative expression of miR-34a-5p in human OA FLS treated with 100n*M* control oligonucleotide, miR-34a-5p mimic, and miR-34a-5p ASO for 24 h (n = 4). Data are presented as box-and-whisker plots (2.5-97.5 percentile). Data was log-transformed prior to analysis by two-tailed, paired Student's t-test. * = $P < 0.05$, ** = $P < 0.01$.

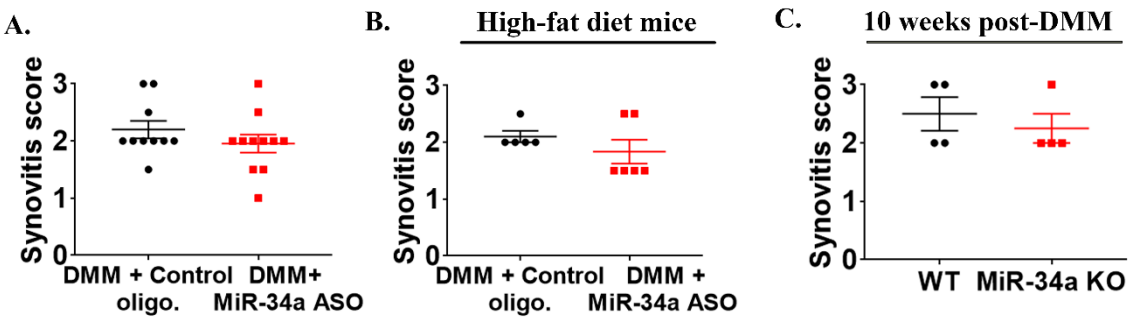

Fig. S5. Synovitis scores of mice subjected to DMM surgery with miR-34a-5p ASO or control oligonucleotide intra-articular injections. (A) Degree of synovitis was scored in knee sections from mice subjected to DMM surgery followed by control oligonucleotide (n = 10) or miR-34a-5p ASO (n = 11) intra-articular injections. (B) Synovitis scores in C57BL/6J mice fed HFD for nine weeks and subjected to DMM surgery followed by control oligonucleotide (n = 5) or miR-34a-5p ASO (n = 6) intra-articular injections. (A&B) oligo, oligonucleotide. (C) Synovitis scores of wild-type (WT; n = 4) or miR-34a knock-out (KO) mice (n = 4) subjected to DMM surgery. (A-C) Data are represented as mean ± SEM and analyzed using Mann-Whitney U tests.

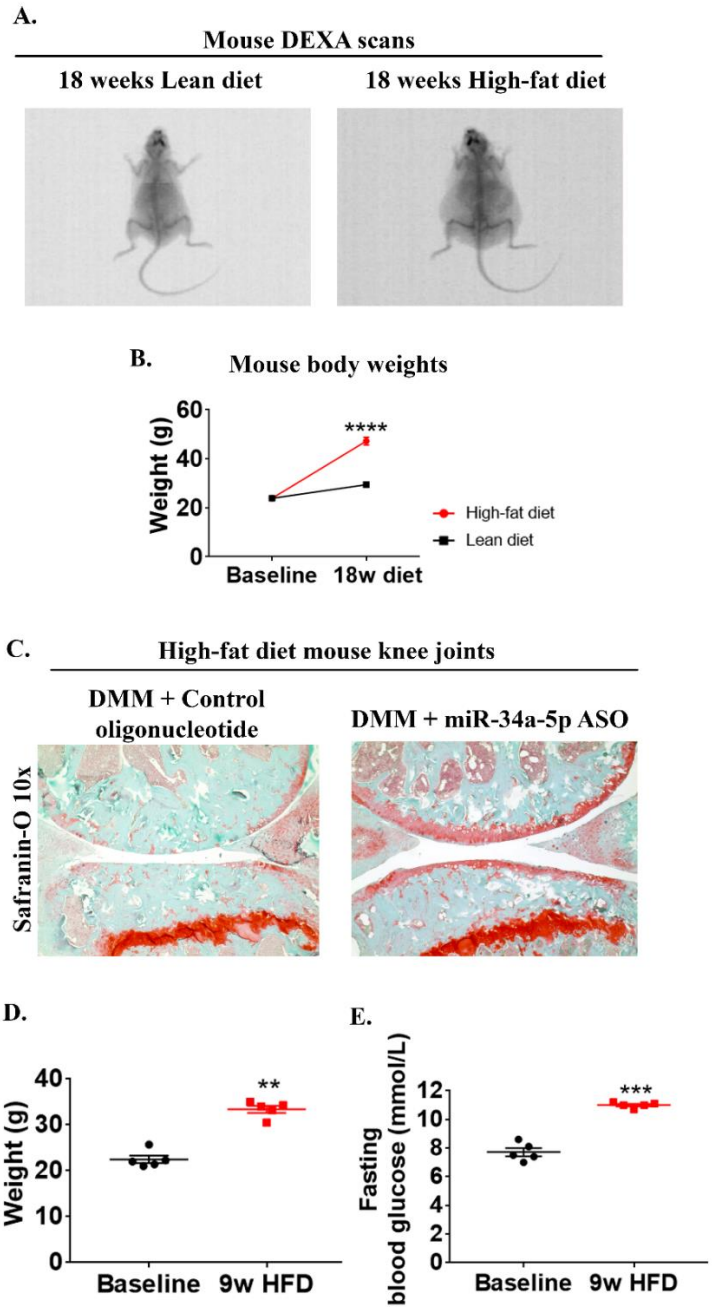

Fig. S6. Comparison of high-fat diet (HFD)- and lean diet (LD)-fed mice. (A) Dual-energy X-ray absorptiometry (DEXA) scan of mice fed a LD or HFD for 18 weeks. (B) Average weight of mice at baseline (ten-weeks-old; n = 35), and at the end of 18 weeks of HFD (n = 18) or LD (n = 14). Data represents mean ± SEM analyzed using two-way ANOVA and Tukey's multiple comparisons test. **** = $P < 0.0001$. (C) Ten-week-old mice were fed a HFD for 18 weeks then subjected to DMM surgery. Mice were injected with control oligonucleotide or miR-34a-5p antisense oligonucleotide (ASO) at two, four, and six weeks post-DMM surgery. Joints were collected ten weeks post-DMM surgery and stained with Safranin-O. (D) Average body weight and (E) fasting blood glucose levels of baseline (n = 5) and end of nine weeks HFD (n = 5). Data are represented as mean ± SEM and analyzed using two-tailed, paired Student's t-test. ** = $P < 0.01$, *** = $P < 0.001$.

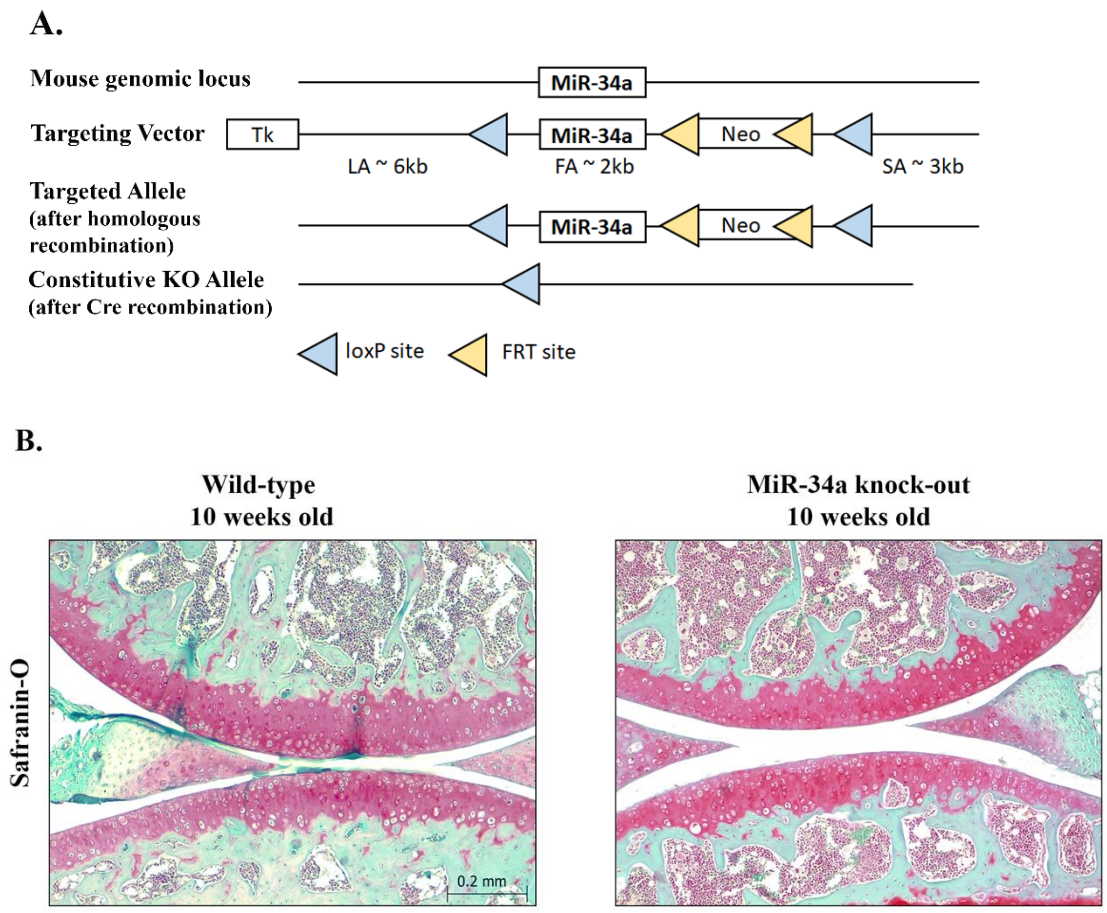

Fig. S7. Generation of constitutive miR-34a knock-out allele. (A) The targeting vector was generated using C57BL/6J DNA and transfected into C57BL/6NTac embryonic stem cells. The miR-34a sequence is flanked by LoxP sites. The constitutive knock-out (KO) allele was generated after Cre-mediated recombination by crossing chimeras to a Cre-Deleter on a C57BL/6 background. The constitutive KO line was subsequently backcrossed to C57BL/6NTac animals for at least two generations and the Cre-transgene was removed by segregation. SA, short arm of homology; FA, floxed arm of homology; LA, long arm of homology. (B) Representative images (10x magnification) showing Safranin-O staining of the medial knee compartment of ten-week-old wild-type (WT) and miR-34a-KO mice.

Supplementary Tables:

Table S1A. Demographics of healthy controls and TKR patients.

Subjects	Healthy controls	OA (TKR)
N	34	37
Mean BMI [95%CI]	23.25 [21.52, 24.97]	29.7 [27.7, 31.78]
Mean age [95%CI]	33.5 [30.46, 36.45]	62.3 [59.38, 65.22]
Age range	21-63	44-86
% Female (N)	52.9%	51.4%

TKR, Total Knee Replacement; BMI, body mass index

B. Demographics of TKR patients stratified according to BMI.

Subjects	Non-obese OA (TKR)	Obese OA (TKR)
BMI	$18.5 \leq BMI \leq 29.9\ kg/m^2$	$BMI \geq 30\ kg/m^2$
N	29	22
Mean age [95%CI]	66.9 [63.62, 70.17]	61.64 [57.36, 65.91]
Age range	53-87	48-86
% Female (N)	44.8%	50%

TKR, Total Knee Replacement; BMI, Body Mass Index

Table S2A. RT-qPCR human primer sequences

Gene	Primer sequence (5'-3')
GAPDH	Forward- GGAGCGAGATCCCTCCAAAAT Reverse- GGCTGTTGTCATACTTCTCATGG
ACAN	Forward – ACTCTGGGTTTTCGTGACTCT Reverse - ACACTCAGCGAGTTGTCATGG
COL2A1	Forward – ATGCCTGGTGAACGTGGT Reverse - AGGAGAGCCATCAGCACCT
COL10A1	Forward – TGCCTCTTGTCAGTGCTAACC Reverse - GCGTGCCGTTCTTATACAGG
IL1B	Forward – TCGCCAGTGAAATGATGGCTTA Reverse - GTCCATGGCCACAACAACTGA
ULK1	Forward – AGTCTCAGACGTGCTGGGGA Reverse - CGCATGGCGTGCAGGTAGTC
ATG3	Forward – GTGAAGGGAAAGGCACTGGA Reverse - CCACAAACTCTTCTGGGGT
ATG5	Forward – CTTCCTGGAGTCCTGCTACC Reverse - CAAAGCCAAACCTACTCCAAGC
MMP13	Forward – TGGAGTGCCTGATGTGGGTGAATA Reverse - TGGTGTCACATCAGACCAGACCTT
COL1A1	Forward- GCTCGTGGAAATGATGGTGC Reverse- ACCCTGGGGACCTTCAGAG
ACTA2	Forward- ACAGAGTATTTGCGCTCCGGA Reverse - CCGACCGAATGCAGAAGGA
TGFB	Forward – TGGAAGTGGATCCACGCGCCCAAGG Reverse- GCAGGAGCGCACGATCATGTTGGAC
TNFA	Forward – GTCTCCTACCAGACCAAG Reverse - CAAAGTAGACCTGCCCAGACTCT
IL6	Forward - TGACAAACAAATTCGGTACATCCT Reverse - TCTGCCAGTGCCTCTTTGCT
ADAMTS5	Forward – GAACATCGACCAACTCTACTCCG Reverse - CAATGCCCACCGAACCATCT

B. RT-qPCR mouse primer sequences

Gene	Primer sequence (5'-3')
Gapdh	Forward – AGGTCGGTGTGAACGGATTTG Reverse- GGGGTCGTTGATGGCAACA
Acan	Forward – GTGGAGCCGTGTTTCCAAG Reverse - AGATGCTGTTGACTCGAACCT
Col2a1	Forward – GGGTCACAGAGGTTACCCAG Reverse - ACCAGGGGAACCACTCTCAC

Table S3. Significantly upregulated or downregulated genes in miR-34a knock out (KO) mouse chondrocytes compared to wild-type (WT) as determined by RNA sequencing.

A. Upregulated genes

Genes	logFC	logCPM	PValue	FDR
Tgoln2	8.75949	4.059792	2.78E-15	2.84E-12
Hmga1-rs1	7.900169	3.261005	2.16E-09	1.16E-06
Gm12669	7.354835	2.852524	6.99E-07	0.000183
Bglap2	7.173949	2.674983	2.37E-06	0.000493
Fam102a	6.988022	2.511415	7.87E-06	0.001295
Ttc8	6.965935	2.499414	8.05E-06	0.001295
Bglap	6.890872	2.46113	1.28E-05	0.001888
2510003B16Rik	6.796461	2.352387	2E-05	0.002678
Zfp365	6.732009	2.329077	2.72E-05	0.003377
Clip4	6.676399	2.262446	3.89E-05	0.004264
Mrps11	6.549517	2.183688	6.61E-05	0.006605
Pparg	6.520048	2.170288	6.69E-05	0.006626
Rnf146	5.135692	7.640111	4.77E-36	4.86E-32
Prkg2	4.629543	3.400454	3.03E-07	9.52E-05
Gm5801	4.332627	3.168398	7.47E-06	0.00129
Gnal	4.035573	6.247594	8.82E-24	3E-20
Frzb	3.343638	3.832506	6.11E-07	0.000168
Galnt13	3.277318	4.633005	4.46E-10	2.84E-07
Smpd5	3.19463	4.204063	4.32E-08	1.63E-05
Col4a5	2.989052	3.503157	4.85E-05	0.005048
Slc8a3	2.851776	3.538447	8.68E-05	0.00819
Atp6v0c-ps2	2.739682	4.096678	1.76E-06	0.000397
Nrcam	2.723062	4.129882	1.29E-06	0.000312
Yipf1	2.578583	3.275651	0.00073	0.044054
Fbln7	2.419908	4.002421	3.24E-05	0.003796
Srp54c	2.416293	5.15828	1.82E-08	7.95E-06
Aldh1a1	2.401472	4.254395	8.06E-06	0.001295
Chkb	2.391868	3.895441	6.14E-05	0.006257
Pask	2.380647	3.954556	6.33E-05	0.006385
1700001G11Rik	2.378571	5.110302	6.06E-08	2.06E-05
Chadl	2.324164	4.096518	2.22E-05	0.002872
Vps11	2.320545	4.412448	7.47E-06	0.00129
Rtl1	2.318038	7.680827	3.21E-11	2.52E-08
Slc27a3	2.270183	3.54245	0.000661	0.040838
Slco4a1	2.255072	4.626828	3.01E-06	0.00059

Grem1	2.217332	6.516728	1.21E-10	8.78E-08
Ift81	2.200316	3.641892	0.000636	0.040513
Lgi4	2.18555	5.155609	5.3E-07	0.000154
Panx3	2.181675	4.972002	1.79E-06	0.000397
Epha2	2.161097	4.803085	3.61E-06	0.000657
Penk	2.13382	8.905443	5.93E-10	3.36E-07
Mfi2	2.129317	4.582262	1.3E-05	0.001888
Pcolce2	2.042024	4.492614	3.81E-05	0.00422
Cadm1	2.029615	4.011564	0.000307	0.022818
Ctsf	2.022202	4.165655	0.000139	0.011642
Abcc5	2.011536	4.29737	0.000112	0.009778
Reck	1.965368	5.361049	1.2E-06	0.000299
Col11a2	1.962159	5.829499	1.21E-07	3.97E-05
Gdf10	1.956151	5.214773	3.14E-06	0.000593
Chrdl1	1.934781	4.922994	1.67E-05	0.002306
C1rl	1.915842	3.988301	0.00063	0.040381
Enpp5	1.896527	4.231671	0.000304	0.022767
Clpb	1.854258	4.15826	0.000656	0.040838
Lrig3	1.828539	4.617036	0.000112	0.009778
1700047I17Rik2	1.791442	5.300303	2.02E-05	0.002678
Lect1	1.732757	6.569086	3.08E-07	9.52E-05
Col9a2	1.721836	5.018679	8.93E-05	0.008221
Ibsp	1.697349	11.07148	1.62E-06	0.000374
Trabd2b	1.685957	4.437571	0.000602	0.039113
Gclm	1.676706	4.680961	0.000315	0.023282
Itgbl1	1.674324	5.944261	3.39E-06	0.000629
Serpina3n	1.639572	7.658592	1.07E-06	0.000274
Hs6st2	1.58413	5.614498	3.15E-05	0.003777
Maoa	1.580225	4.957702	0.00035	0.025537
Smoc2	1.568052	5.602748	3.42E-05	0.003939
Adgrg2	1.557568	4.648699	0.000811	0.047791
Grb14	1.531473	4.842518	0.000613	0.039526
Tmem80	1.526997	5.421867	9.7E-05	0.008679
Col27a1	1.460956	5.752476	7.62E-05	0.007331
4931440P22Rik	1.446902	5.022847	0.000716	0.043476
Lmo7	1.416043	5.65477	0.000158	0.012812
Arvcf	1.400537	5.215053	0.000658	0.040838
Fmod	1.374914	8.41215	4E-05	0.004293
Pcdh7	1.346889	5.544323	0.000358	0.025537
Ephx1	1.331256	6.302652	9.44E-05	0.008518
Trp53bp1	1.309251	8.247309	8.76E-05	0.008195
Pi15	1.298719	6.687103	9.24E-05	0.008413
Acan	1.29392	7.203675	0.000117	0.010011

Inppl1	1.268429	5.780192	0.000481	0.032493
Edil3	1.257399	6.821626	0.000166	0.013204
4930539J05Rik	1.222059	5.728889	0.000789	0.047311
Htra4	1.193831	6.120367	0.000519	0.033895
Zwilch	1.192474	6.711074	0.000356	0.025537
Mme	1.13881	7.327858	0.000647	0.040838

N=84; P-values were adjusted for false discovery rate (FDR) using the Benjamini-Hochberg method and significance was set at 0.05, FC, fold change; CPM, counts per million.

B. Downregulated genes

Genes	logFC	logCPM	PValue	FDR
S100a9	-9.3302	6.308791	0.000803	0.047604
S100a8	-8.27036	9.485282	0.00068	0.041752
ChkbCpt1b	-7.29372	9.83279	0.000691	0.042209
Tctn1	-4.22237	8.973312	0.000508	0.033441
Rn45s	-4.15975	7.001892	0.000475	0.032295
Lars2	-3.67346	7.871267	0.000368	0.026044
Mir6236	-3.52075	6.446335	0.000409	0.028383
Foxp3	-3.5012	6.526689	0.000352	0.025537
Atg7	-3.36046	10.23306	0.000499	0.033236
Ccr1	-3.31266	6.756814	0.000252	0.019341
Rps27rt	-2.8383	5.99482	0.000419	0.028885
Gm32014	-2.82255	7.283598	0.000204	0.015891
Ms4a4b	-2.52191	9.184048	0.000204	0.015891
Mcrs1	-2.5175	5.863051	0.000489	0.032815
Slc4a5	-2.45438	6.664831	0.00015	0.012297
6430706D22Rik	-2.44715	5.761548	0.000504	0.033379
Gm10578	-2.42321	6.263641	0.000182	0.014418
Rab39b	-2.40486	7.127991	0.000107	0.009505
Plcxd1	-2.39449	5.397831	0.00083	0.048411
Aldh1a2	-2.36391	6.919169	8.95E-05	0.008221
Pf4	-2.35432	6.229226	0.00014	0.011642
Gm12191	-2.28939	6.156943	0.000131	0.011146
Ogg1	-2.20616	8.364701	7.12E-05	0.006912
Has2os	-2.16908	6.573381	5.64E-05	0.005809
Rasgrp4	-2.16775	5.654402	0.000292	0.022053
Sfrp1	-2.14844	5.642606	0.000267	0.020287
Invs	-2.13519	7.253673	4.17E-05	0.004426
Nxpe5	-2.03969	7.821402	3.44E-05	0.003939
Zcwpw1	-2.02496	5.234272	0.000656	0.040838

Ms4a6b	-1.9839	5.666381	0.00014	0.011642
Trmt2a	-1.95549	6.235121	3.07E-05	0.003729
H60b	-1.89362	6.913459	1.71E-05	0.002331
Sorbs2os	-1.87762	6.03557	3.58E-05	0.004038
Cnn1	-1.85707	7.706701	1.33E-05	0.001912
Fbn1	-1.8456	7.579989	1.28E-05	0.001888
E130310I04Rik	-1.82316	6.210745	2.23E-05	0.002872
Cxcl14	-1.78263	7.824215	1.10E-05	0.001677
Col4a2	-1.71915	5.466582	0.000164	0.013136
Zfp704	-1.71261	8.385137	8.42E-06	0.00132
Ifi27l2a	-1.70667	7.750156	8.13E-06	0.001295
Zranb3	-1.68066	7.62465	7.59E-06	0.00129
Ica1l	-1.66833	5.075756	0.000406	0.028322
Cenpe	-1.6503	6.74376	4.90E-06	0.000877
Bvht	-1.64531	5.621604	3.60E-05	0.004038
Hells	-1.62281	6.827928	3.11E-06	0.000593
Zdhhc17	-1.58642	6.385646	2.84E-06	0.000567
Il4ra	-1.56451	4.428661	0.000831	0.048411
Mylk	-1.53362	6.397104	1.34E-06	0.000318
5031434C07Rik	-1.5275	6.038993	2.83E-06	0.000567
Proz	-1.51026	4.955604	0.000154	0.012544
Postn	-1.49925	4.305467	0.000794	0.047311
Acta2	-1.49657	6.726456	5.80E-07	0.000164
Mrc1	-1.49605	6.795132	3.73E-07	0.000112
Eif3j1	-1.48715	4.887691	0.000116	0.010011
Nt5dc2	-1.47502	5.13343	2.38E-05	0.003028
Hist1h2bm	-1.46584	5.552964	2.00E-06	0.000426
1700071M16Rik	-1.46386	8.042863	4.95E-08	1.74E-05
Vcan	-1.45883	4.368721	0.000211	0.016266
Il1rn	-1.44033	6.884383	3.03E-08	1.19E-05
Ap3m1	-1.44004	4.162874	0.000357	0.025537
Col4a1	-1.43829	7.703557	9.26E-09	4.49E-06
Abi3	-1.42112	4.62415	3.20E-05	0.003796
Sgpp2	-1.396	6.071081	1.52E-08	7.04E-06
LOC100503496	-1.38988	4.361326	6.83E-05	0.006696
Gse1	-1.37393	7.085237	5.77E-10	3.36E-07
Gpr183	-1.36595	5.674382	2.14E-08	8.72E-06
4933400F21Rik	-1.34658	5.803115	3.00E-09	1.53E-06
Angpt2	-1.33489	7.649451	2.63E-10	1.79E-07
Gm10754	-1.32574	4.242579	1.62E-05	0.002258

Hist2h2bb	-1.31987	4.283601	1.02E-05	0.001571
Hist1h1b	-1.30403	4.114458	2.49E-05	0.00314
Gm15910	-1.30381	4.680464	6.87E-07	0.000183
Rspo2	-1.29705	5.246263	1.87E-08	7.95E-06
Ptx3	-1.29486	3.744968	7.88E-05	0.007505
Trim59	-1.26994	3.919743	2.76E-05	0.003385
Far1os	-1.26509	4.038744	1.45E-05	0.002055
Pgm3	-1.2534	3.880643	4.64E-05	0.004882
Akap12	-1.25243	3.428301	0.000442	0.030221
Exosc9	-1.25122	3.825967	4.00E-05	0.004293
Fam196b	-1.24751	3.219463	0.000405	0.028322
Myl9	-1.23682	4.394391	4.48E-08	1.63E-05
Fibin	-1.21086	5.113588	5.37E-13	4.98E-10
Cyfip1	-1.19913	6.538239	3.00E-20	5.11E-17
Mast4	-1.18759	6.786184	3.13E-21	6.39E-18
Hist1h1a	-1.18102	11.8353	5.64E-20	8.22E-17
Ccdc85b	-1.1775	16.94477	5.46E-16	6.19E-13
Hist1h1d	-1.17006	18.48052	5.53E-18	7.05E-15
Saa3	-1.16316	7.956529	8.56E-29	4.37E-25
Prune2	-1.15745	2.618022	1.89E-06	0.00041
Mmp3	-1.14874	3.483839	2.01E-11	1.71E-08
Iffo1	-1.13481	4.500962	1.28E-21	3.26E-18

N=91; *P*-values were adjusted for false discovery rate (FDR) using the Benjamini-Hochberg method and significance was set at $P \leq 0.05$, FC, fold change; CPM, counts per million.

Table S4. Bioinformatic Resources used for network integration and visualization

Data	First PMID	Date downloaded
AURA	22057158	May 2018
ChEA	20709693	May 2018
CR Cistrome	24253304	April 2018
CTCFBSDB_2.0	17981843	May 2018
ENCODE	21526222	May 2018
GTRD	27924024	June 2018
HmChiP	21450710	May 2018
ITFP	18713790	Aug 2014
JASPAR	14681366	June 2018
MSigDB	16199517	May 2018
Neph et al.	22959076	Feb 2015
PReMod	17148480	May 2018
ReMap2018	25477382	May 2018
TF2DNA	25428367	April 2018
TRED	15608156	August 2014

PMID, Pubmed unique identifier

Supplementary Materials and Methods References

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