Supplementary Information

Critical role for arginase II in osteoarthritis pathogenesis

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MATERIALS AND METHODS

Human OA cartilage

Human OA cartilage was sourced from individuals undergoing arthroplasty. The Institutional Review Board of Wonkwang University Hospital approved the use of these materials, and all participants provided written informed consent before the operative procedure. Details of the individual OA specimens are summarized in online supplementary table S1.

Mice and experimental OA

C57BL/6J mice were used for the experimental OA studies. *Arg2*$^{-/-}$ mice of the C57BL/6J background were purchased from Jackson Laboratory. All experiments were approved by the Gwangju Institute of Science and Technology Animal Care and Use Committee. Post-traumatic experimental OA was induced in 12-week-old male mice by DMM surgery using a standard protocol.[43] WT littermates were used as controls for *Arg2* KO mice. Experimental OA was also induced by IA injection (once weekly for 3 weeks) of adenovirus (1×10^9 plaque forming units [PFUs] in a total volume of 10 μl) expressing Arg-II (Ad-Arg-II), ZIP8 (Ad-ZIP8), or HIF-2α (Ad-HIF-2α).[9–12] The adenovirus expressing mouse HIF-2α was produced by Newgex. The adenoviruses Ad-C (ADV-1060), Ad-ZIP8 (ADV-272407), and Ad-Arg-II (ADV-278070) were purchased from Vector Biolabs. Mice were sacrificed at 8 weeks after DMM surgery or at 3 or 8 weeks after the first IA injection of adenovirus, and subjected to histological and biochemical analyses.

Histology and immunohistochemistry

Human OA cartilage was frozen, sectioned at 5 μm, and fixed in 4% paraformaldehyde. Sulfate proteoglycans were detected by Alcian blue staining. Arg-II in human cartilage sections was immunostained using a rabbit polyclonal antibody (PA5-27987, Thermo Fisher Scientific). Mouse knee joints were fixed in 4% paraformaldehyde, decalcified in 0.5 M EDTA, and embedded in paraffin. OA parameters in mice (cartilage destruction, osteophyte maturity, and thickening of the subchondral bone plate) were determined from frontal sections of joint tissues. The paraffin blocks were cut into 5-μm slices across each entire joint and 15 slides were harvested at intervals of approximately 80 μm. Six cartilage-lesion-containing areas sections were selected from the anterior to posterior of the knee compartment of each mouse. The sections were deparaffinized in xylene, hydrated with graded ethanol, and stained with Safranin-O/Fast Green. Cartilage destruction was scored by three observers under blinded conditions using the OARSI scoring system (grade 0-6).[44] Synovitis was determined by Safranin-O and hematoxylin staining, and synovial inflammation (grade 0-3) was scored as described previously.[45] Osteophyte formation was identified by Safranin-O staining, and osteophyte maturity (grade 0-3) was scored as described by Little et al.[6] All histological grading scores are presented as the mean grade of the six sections for each mouse, and a representative Safranin O-stained image was

presented. In an attempt to assess subchondral bone sclerosis, we measured the thickness of the subchondral bone plate as previously reported,[46] using an Aperio Scanscope CS2 (Leica Biosystems Image, Inc.). Arg-II, HIF-2α, ZIP8, MMP3, and MMP13 proteins and neoepitopes representing aggrecan breakdown (DIPEN and NITEGE[21]) were detected in joint sections by immunohistochemical staining of mouse cartilage sections, which was performed using the following antibodies: rabbit polyclonal antibody against Arg-II (PA5-27987, Thermo Fisher Scientific), mouse monoclonal antibody against HIF-2α (ab8365, Abcam), rabbit polyclonal antibody against ZIP8 (sc-133415, Santa Cruz), rabbit polyclonal antibody against MMP3 (ab52915; Abcam), rabbit polyclonal antibody against MMP13 (ab51072; Abcam), mouse monoclonal antibody against DIPEN (MBS442010, MyBioSource), and rabbit polyclonal antibody against NITEGE (NB100-74350, Novus).

Skeletal staining and histological analysis

Skeletons of E18.5 whole-mouse embryos were stained with Alcian blue and Alizarin red, as described previously.[47] Briefly, whole embryos were skinned, eviscerated, fixed with 95% ethanol for 4 days, and immersed in acetone for 3 days. Samples were stained with one volume of 0.3% Alcian blue 8GX in 70% ethanol, one volume of 0.1% Alizarin red S in 95% ethanol, one volume of 100% acetic acid, and 17 volumes of 100% ethanol. Bone histology was analyzed as described previously.[47] Metatarsal bones of 2-week-old mice were fixed in 4% paraformaldehyde, decalcified in 0.5 M EDTA (pH 7.4) for 7 days at 4°C, and embedded in paraffin. The deparaffinized sections were stained with 1% Alcian blue in 0.1N HCl for 10 minutes and washed with 0.1N HCl for 5 minutes. The lengths of the resting/proliferative and hypertrophic zones were measured using a microscope image-analysis program (AxioVision). Cartilage samples of 5-month-old mice were examined by Safranin-O staining.

Primary culture of articular chondrocytes and FLSs (fibroblast-like synoviocytes)

Human normal chondrocytes isolated from donor knee joints without any abnormality were obtained from Cell Applications, Inc. We used two different lots (donors) of human primary normal chondrocytes. The cells were provided at passage 1 (P1) and we used P2 cells for our experiments unless otherwise indicated. Human OA chondrocytes were sourced from individuals undergoing arthroplasty. Details of the individual OA specimens are summarized in online supplementary table S1. We used P1 OA chondrocytes for our experiments unless otherwise indicated. Mouse normal articular chondrocytes were isolated from the femoral condyles and tibial plateaus of postnatal day 5 WT or *Arg2*$^{-/-}$ mice by digesting cartilage tissue with 0.2% collagenease.[48] The cells were maintained as a monolayer in Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% fetal bovine serum and antibiotics (penicillin G and streptomycin). On culture day 2, cells were infected with 800 MOI (multiplicity of infection) of empty adenovirus (Ad-C); infected with Ad-Arg-II, Ad-HIF-2α, or Ad-ZIP8 at various MOIs; or treated with the inflammatory cytokines, IL-1β or TNF-α, as indicated in each

experiment. Mouse FLSs were isolated from joint tissues of WT mice, as described previously.[49] FLSs between passage 6 and 7 were used for further analysis. Pure FLSs (>90% $CD90^+$/<1% $CD14^+$) were identified by flow cytometry using antibodies against the fibroblast marker, CD90, and the macrophage marker, CD14 (Abcam). The *n* numbers in each experiment indicate the numbers of independent primary cultures.

Immunofluorescence microscopy

Primary-culture mouse chondrocytes infected with 800 MOI of Ad-C or Ad-Arg-II for 36 hours were subjected to double staining for mitochondria and Arg-II. Briefly, the infected cells were incubated at 37°C for 15 minutes with 25 nM of MitoTracker Red (M7512; Invitrogen). The cells were fixed in ice-cold 4% paraformaldehyde (pH 7.4) for 10 minutes at 4°C, permeabilized with 0.2% Triton X-100 in PBS for 10 minutes at room temperature, and blocked with 1% bovine serum albumin for 1 hour. Arg-II was detected by incubating cells for 1 hour at 37°C with a rabbit polyclonal antibody against Arg-II (ab203071, Abcam). The results were visualized with Alexa Fluor 488-conjugated anti-rabbit secondary antibodies (A11034; Invitrogen) and confocal imaging.

Microarray analyses

Microarray analysis of primary-culture passage 0 (P0) mouse chondrocytes stimulated with IL-1β treatment or overexpression of HIF-2α or ZIP8 was performed as described previously.[11,12] Briefly, total RNA was extracted from mouse articular chondrocytes using a Purelink RNA mini kit (Ambion). The concentration, purity, and integrity of the extracted RNA were determined by NanoDrop 2000 spectrophotometry (Thermo Fisher Scientific). RNA from mouse chondrocytes was analyzed using Affymetrix GeneChip Mouse Gene 2.0 ST Arrays (Macrogen Inc.) according to the provided protocol. The microarray data have been deposited to the Gene Expression Omnibus under accession codes GSE104794 (for HIF-2α), GSE104795 (for ZIP8), and GSE104793 (for IL-1β).

Transcription factor array analysis

Transcription factor array analyses were performed using a Cignal 45-Pathway Reporter Array (Qiagen), as previously described.[10] Briefly, mouse articular chondrocytes were plated to 96-well plates, treated with hyaluronidase for 4 hours to enhance transfection efficiency, and transfected with reporter constructs reconstituted from the kit using Attractene (Qiagen). Transfected cells were infected with Ad-C or Ad-Arg-II for 2 hours. After 24 hours, firefly luciferase and Renilla luciferase activities were measured using a Dual-Glo Luciferase Assay System (Promega). Firefly luciferase activity was expressed relative to Renilla luciferase activity for normalization of the transfection efficiency.

RT-PCR and qRT-PCR analyses

Total RNA was extracted from primary-culture chondrocytes using the TRI reagent (Molecular Research Center, Inc.). The RNA was reverse transcribed, and the resulting cDNA was PCR amplified using the primers and experimental conditions summarized in supplementary table S2. Transcript levels were quantified by qRT-PCR. siRNAs targeting mouse Smad 2, 3, 4 or human Arg-II were obtained from Dharmacon or Integrated Device Technology, respectively, and transfected using Lipofectamine^{TM} RNAiMAX (Invitrogen). Non-targeting (scrambled) siRNA was used as a negative control. The following siRNA sequences were used: 5′-UGCACUAUCACUUAGGCACTC-3′ for mouse Smad 2, 5′-UUCCGGUUGACAUUGGACAGT-3′ for mouse Smad 3, 5′-UGAGAAUGCACA AUCGCCGGA-3′ for mouse Smad 4, and 5′-CCAUGAGAGAUAUUGAUCGACUUGG-3′ for human Arg-II. Mouse chondrocytes were transfected by incubation for 6 hours with siRNA and Lipofectamine RNAiMAX and infected with adenoviruses as described above. Human Arg-II siRNA was used for reverse transfection at each passage during the serial-passage culture of human normal chondrocytes.

Western blotting

Total cell lysates were prepared in lysis buffer (150 mM NaCl, 1% NP-40, 50 mM Tris, 0.2% sodium dodecyl sulfate [SDS], 5 mM NaF) and used to detect Arg-II, p62, ATG5, ATG6, ERK, and Lamin B. Secreted proteins (ADAMTS5, MMP3, and MMP13) were detected after trichloroacetic acid (TCA) precipitation of proteins from 3 ml of serum-free conditioned medium. All lysis buffers contained a cocktail of protease inhibitors and phosphatase inhibitors (Roche). The following antibodies were used for Western blotting: rabbit polyclonal anti-Arg-II (PA5-27987, Thermo Fisher Scientific), rabbit polyclonal anti-ADAMTS5 (PA5-14350, Thermo Fisher Scientific), rabbit polyclonal anti-p62 (5114), rabbit monoclonal anti-ATG5 (12994), rabbit monoclonal anti-ATG6 (3495P, all from Cell Signaling Technology), mouse monoclonal anti-ERK1 (BD610408, BD Bioscience), rabbit monoclonal anti-MMP3 (ab52915), and anti-MMP13 (ab51072, both from Abcam).

Arginase activity assay

Primary-culture mouse chondrocytes were treated with the indicated concentrations of IL-1β for 36 hours and infected with 800 MOI of Ad-C or the indicated MOIs of Ad-Arg-II for 36 hours, or transfected with expression vectors for WT-Arg-II or Δ-Arg-II for 36 hours. Arginase activity was assessed with an Arginase Assay Kit (KA1609, Abnova). The method utilizes a chromogen that forms a colored complex specifically with urea produced in the arginase reaction, and the intensity of the color is directly proportional to the arginase activity in the sample.

Senescence and autophagy assay

Chondrocyte senescence was examined in P0 WT mouse chondrocytes, P2 human normal chondrocytes,

and P1 human OA chondrocytes infected with 800 MOI of Ad-C or Ad-Arg-II for 72 hours. Senescence was also examined in P6 WT and *Arg2*$^{-/-}$ mouse chondrocytes and P6 human normal and OA chondrocytes. Chondrocyte senescence was determined using a β-galactosidase (SA-β-gal) senescence staining kit (9860; Cell Signaling) and by detection of $P16^{INK4A}$ mRNA.[50] SA-β-gal-positive cells were quantified with the Image J software. Autophagy in chondrocytes was induced by nutrient and serum starvation in Hank's balanced salts solution (HBSS, Gibco).[51] Briefly, chondrocytes at culture day 2 were infected with 800 MOI of Ad-C or Ad-Arg-II for 36 hours and then incubated with HBSS for the indicated durations for serum/nutrient starvation. Autophagy was determined by detecting the expression levels of the marker proteins, p62, ATG5, and ATG6.[52]

Assays of ROS, JC-1, and NO

Mitochondrial and cytosolic ROS were detected with MitoSOX Red (M36008, Invitrogen) and dihydroethidium (DHE; D11347, Invitrogen), respectively. Briefly, mouse chondrocytes infected with Ad-C or Ad-Arg-II for 36 hours were incubated in PBS with 3 μM MitoSOX Red for 10 minutes or 5 μM DHE for 25 minutes. Fluorescence images were obtained using a Fluoview FV 1000 confocal laser-scanning microscope. Mitochondrial dysfunction in chondrocytes infected with Ad-C or Ad-Arg-II was assessed with a JC-1 assay kit (10009172, Cayman Chemical). The mitochondrial membrane potential uncoupler, valinomycin (V0627, Sigma), was used as a positive control. NO production in chondrocytes was determined by the Griess reaction using a colorimetric assay kit (STA-802, Cell Biolabs).

NF-κB reporter gene assay

The NF-κB reporter construct (pGL4.32[luc2P/NF-κB-RE/Hygro] vector) was purchased from Promega. Primary-culture mouse chondrocytes were pretreated with hyaluronidase type I-S (Sigma) for 6 hours in serum-free DMEM, and transfected with both a NF-κB reporter vector (1.0 μg) and a constitutive Renilla luciferase vector (0.1 μg). The cells were co-transfected with 1.0 μg of empty vector (Origene), WT-Arg-II expression vector (Origene), or Δ-Arg2 expression vector (H160F, Macrogen, Seoul, Korea). The reporter gene activity was measured using a Dual Luciferase Assay System (Promega).

References

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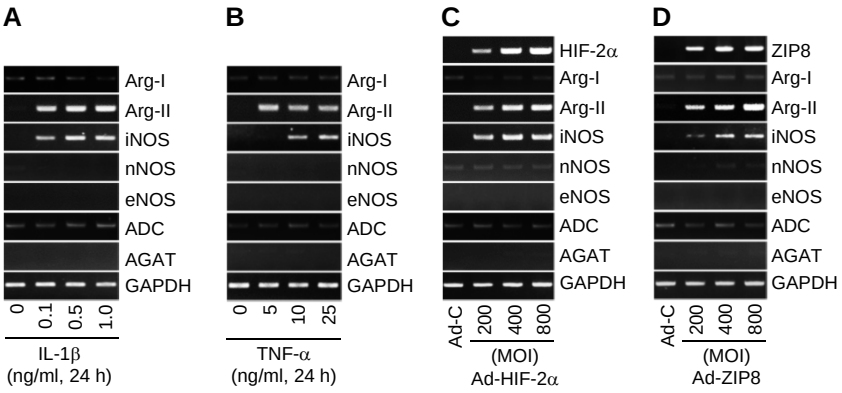

Figure S1. RT-PCR analyses of arginine-metabolizing enzymes in chondrocytes stimulated with OA-associated catabolic regulators. Primary-culture mouse chondrocytes were treated with the indicated concentrations of IL-1β (A) or TNF-α (B) or infected with Ad-C (800 MOI) or the indicated MOIs of Ad-HIF-2α (C) or Ad-ZIP8 (D). Images presented are representative of six independent primary cultures of chondrocytes.

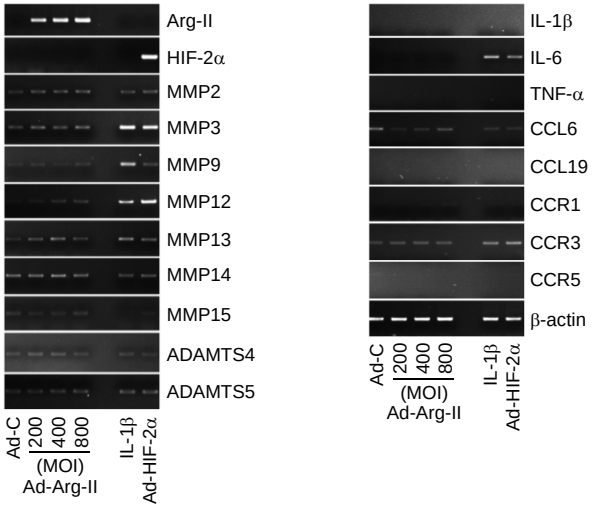

Figure S2. Arg-II overexpression in mouse FLSs (fibroblast-like synoviocytes) does not modulate the expression of OA-associated catabolic factors. Passage 6 (P6) or P7 FLSs were infected with Ad-C (800 MOI) or the indicated MOIs of Ad-Arg-II. Alternatively, FLSs were treated with IL-1β (1 ng/ml, 36 hours) or infected with Ad-HIF-2α (800 MOI, 36 hours) as positive controls. The presented RT-PCR images are representative of eight independent cell cultures.

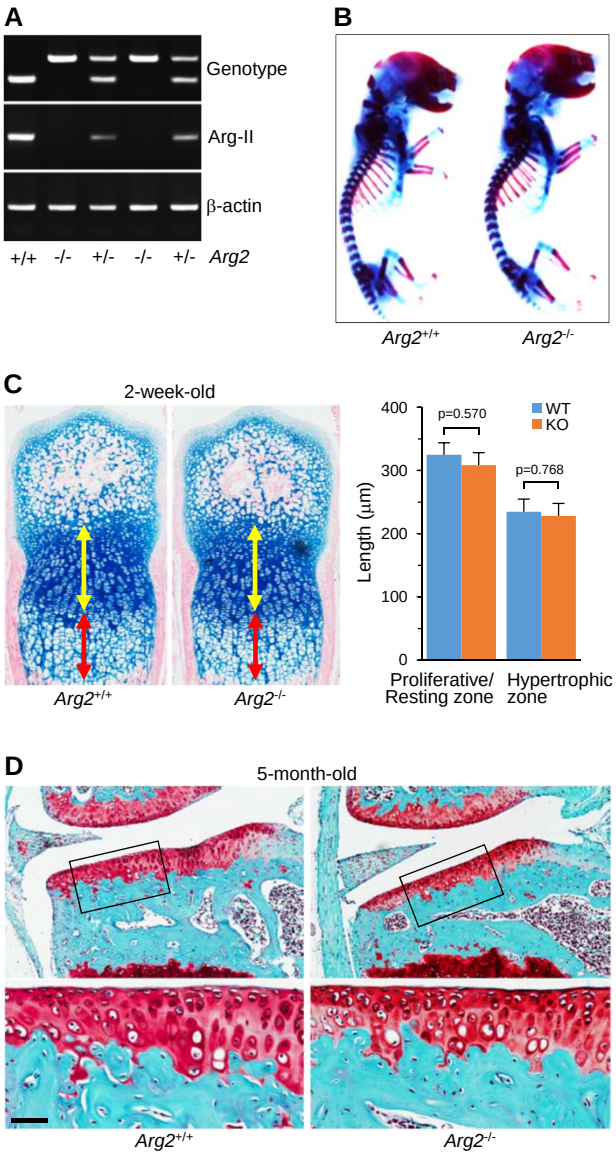

Figure S3. Characterization of *Arg2*$^{-/-}$ mice. (A) Genotypes and mRNA levels of Arg-II in *Arg2* homozygous (-/-) KO mice and heterozygous (+/-) and WT (+/+) littermates. (B**)** Representative skeletal staining images of E18.5 embryos of *Arg2*$^{-/-}$ mice and WT littermates (n = 5 mice/group). (C) Representative staining images of growth plates in metatarsal bones of 2-week-old *Arg2*$^{-/-}$ mice and WT littermates. Lengths of the resting/proliferative zone and hypertrophic zones were determined from five mice per group. Values are presented as means ± s.e.m. (two-tailed t-test). (D) Representative images of cartilage staining in 20-week-old *Arg2*$^{-/-}$ mice and WT littermates (n = 8 mice/group).

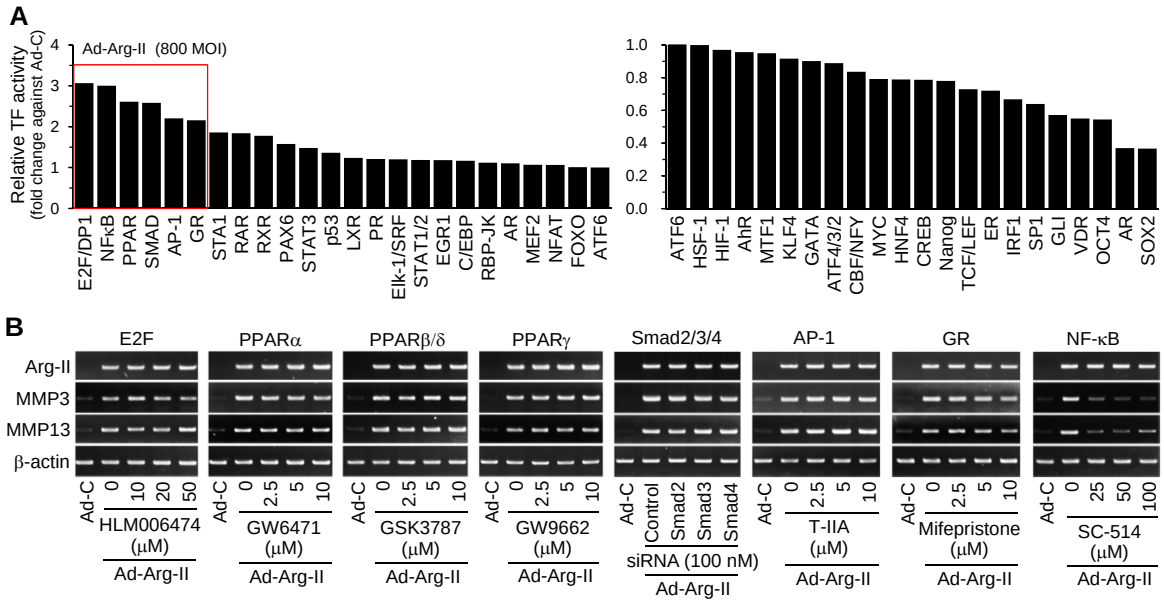

Figure S4. Transcription factor array analysis. (A**)** Primary-culture mouse articular chondrocytes were infected with Ad-C or Ad-Arg-II (800 MOI) for 36 hours. The transcriptional activities of the indicated transcription factors were determined using a transcription factor array kit. (B**)** Chondrocytes were infected with 800 MOI of Ad-C or Ad-Arg-II and incubated for 36 hours in the absence or presence of the indicated concentrations of HLM006474 to inhibit E2F (*n* = 5), GW6471 to inhibit PPARα (*n* = 8), GSK3787 to inhibit PPARβ (*n* = 6), GW9662 to inhibit PPARγ (*n* = 8), Tanshinone-IIA (T-IIA) to inhibit AP-1 (*n* = 8), Mifepristone to inhibit GR (*n* = 8), or SC-514 to inhibit NF-κB (*n* = 8). Alternatively, Smad 2, 3, and 4 were suppressed with specific siRNAs (*n* = 6). The indicated mRNAs were detected by RT-PCR analysis. The *n* numbers indicate the numbers of independent primary cultures of chondrocytes.

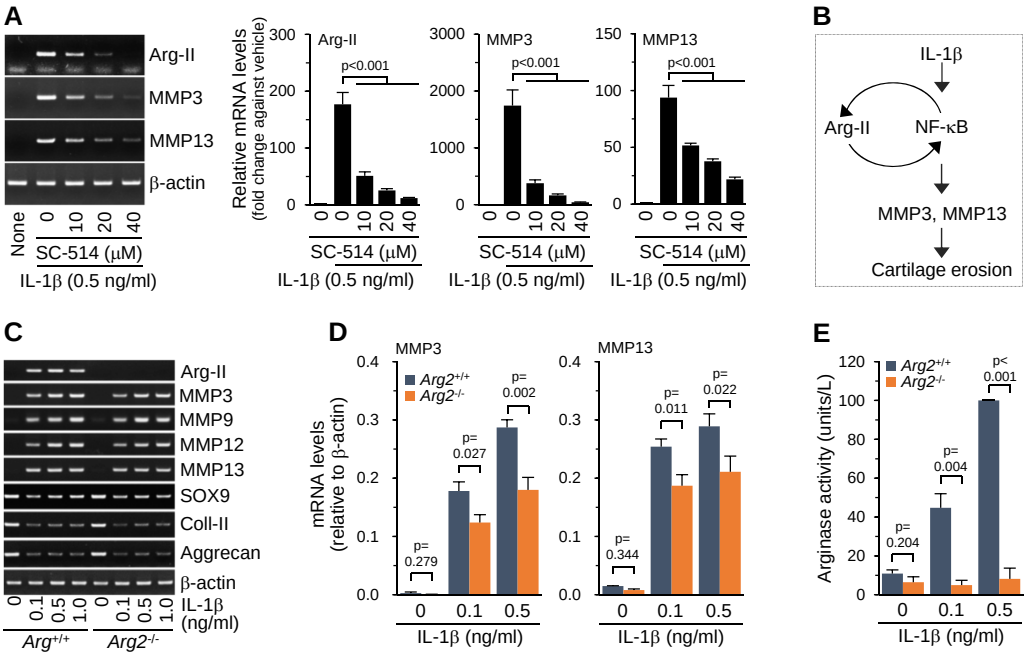

Figure S5. *Arg2*$^{-/-}$ chondrocytes exhibit partial inhibition of the IL-1β-induced upregulations of MMP3 and MMP13. (A**)** Chondrocytes were treated with IL-1β for 36 hours in the absence or presence of the indicated concentrations of SC-514 to inhibit NF-κB. Representative RT-PCR gel images and qRT-PCR analysis of Arg-II, MMP3, and MMP13 are shown (n = 5). (B) Proposed model of the IL-1β-, NF-κB-, Arg-II-, and MMP-related signaling mechanisms that lead to cartilage destruction. (C–E) WT (*Arg2*$^{+/+}$) and *Arg2*$^{-/-}$ chondrocytes were treated with the indicated concentrations of IL-1β for 36 hours and the indicated mRNAs were detected by RT-PCR analysis (C), the mRNA levels of MMP3 and MMP13 were assessed by qRT-PCR analysis (D; n = 4), and the arginase enzyme activity was assessed (E; n = 4). The n numbers indicate the numbers of independent primary cultures of chondrocytes. Values are means ± s.e.m.; one-way ANOVA with Bonferroni test (A) and two-tailed t-test (D and E).

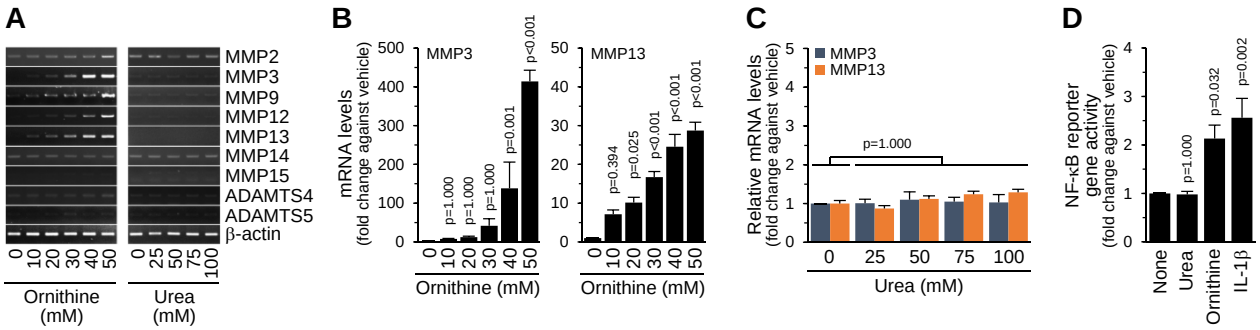

Figure S6. Ornithine, an Arg-II enzymatic product, causes NF-κB activation and expression of MMP3 and MMP13 in mouse chondrocytes. (A-D) Primary-culture chondrocytes were treated with the indicated concentrations of ornithine or urea for 36 hours under serum-free conditions. Presented are representative images of RT-PCR results (A), qRT-PCR results of dose sets for ornithine (B; n = 5) and urea (C; n = 8), and NF-κB reporter gene assay results (D; n = 5). The n numbers indicate the numbers of independent primary cultures of chondrocytes. Values are means ± s.e.m. (one-way ANOVA with Bonferroni test).

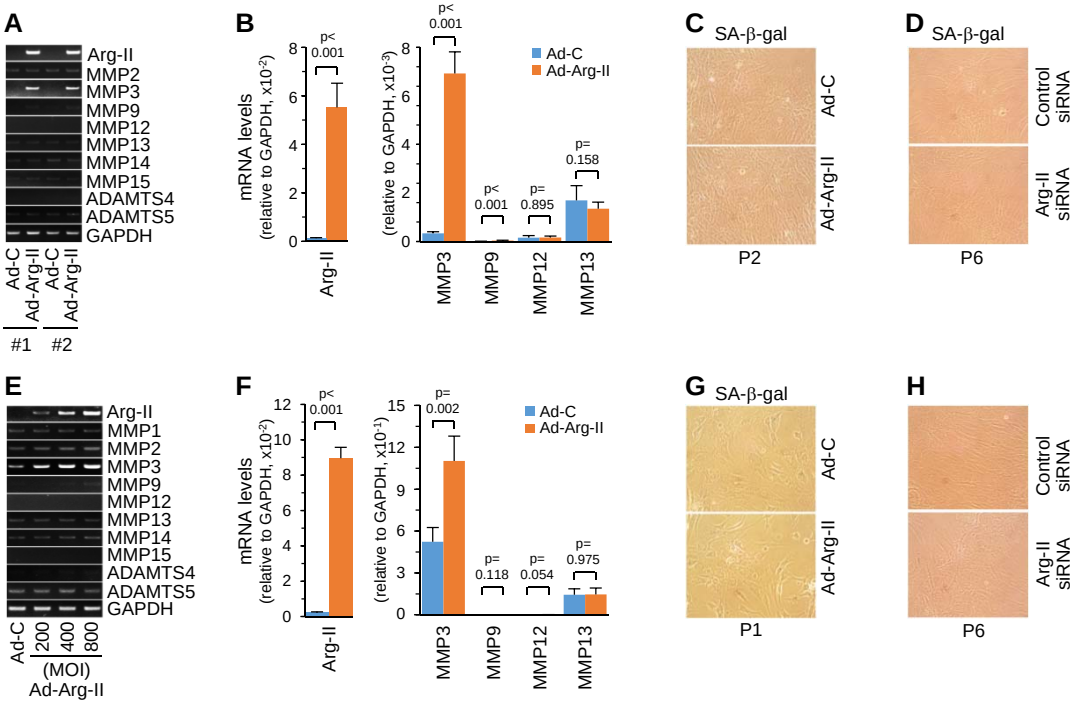

Figure S7. Arg-II functions in human chondrocytes. (A-D) Human normal chondrocytes (P2) were infected with 800 MOI of Ad-C or Ad-Arg-II. (A) Representative RT-PCR images of the indicated molecules at 36 hours post-infection from two different lots of cells. (B) mRNA levels of the indicated molecules quantified from eight replicates from two different lots of chondrocytes at 36 hours post-infection. (C) Representative negative SA-β-gal staining images at 72 hours post-infection. (D) Representative negative SA-β-gal staining images in P6 chondrocytes transfected with scramble or Arg-II-specific siRNA. (E-H) Human OA chondrocytes (P1) were infected with 800 MOI of Ad-C or Ad-Arg-II. (E) Representative RT-PCR images of the indicated molecules from three independent primary cultures of chondrocytes (three OA patients) at 36 hours post-infection. (F) mRNA levels of the indicated molecules quantified from eight replicates from three patients at 36 hours post-infection. (G) Representative negative SA-β-gal staining images at 72 hours post-infection. (H) Representative negative SA-β-gal staining images in P6 chondrocytes transfected with scramble or Arg-II-specific siRNA. Values are means ± s.e.m. (two-tailed *t*-test).

Supplementary table S1. Characteristics of the studied human specimens.

No	Age/ gender	ICRS grade	Joint	Weight (kg)	Height (m)	BMI (kg/m^2)	Other disease†				Medication/ disease	Use
							RA	DB	HT	CA		
1	69/F	4	Knee	57	1.56	23.42	-	-	-	-	None	IHC
2	69/F	4	Knee	67	1.56	27.53	-	-	+	-	Hypertension, Atherosclerosis	IHC
3	50/M	4	Knee	82	1.68	29.05	-	+	+	-	Hypertension, Diabetes	IHC
4	65/M	4	Knee	90	1.78	28.41	-	+	+	-	Gout	IHC
5	71/F	4	Knee	65	1.51	28.51	-	-	+	-	Hypertension	IHC
6	74/F	4	Knee	57	1.57	23.12	-	-	-	-	None	IHC
7	73/F	4	Knee	56	1.59	22.15	-	-	+	-	Hypertension, Atherosclerosis	IHC
8	69/F	4	Knee	60	157	24.34	-	-	-	-	None	Cell culture
9	79/F	4	Knee	50	160	19.53	-	-	+	-	Hypertension Hyperlipidemia	Cell culture
10	66/F	4	Knee	68	165	24.98	-	-	-	-	None	Cell culture

†RA, rheumatoid arthritis; DB, diabetes; HT, hypertension; CA, cancer; +, present; -, absent

Supplementary table S2. PCR primers and conditions.

Genes	Strand	Primer sequences	Size (bp)	AT (°C)	Origin
Acan	S AS	5′-CTGTCTTTGTCACCCACACATG-3′ 5′-GAAGACGACATCACCATCCAG-3′	581	58	Mouse
Adamts4	S AS	5′-CATCCGAAACCCTGTCAACTTG-3′ 5′-GCCCATCATCTTCCACAATAGC-3′	281	58	Mouse
ADAMTS4	S AS	5′-AGAAGAAGTTTGACAAGTGC-3′ 5′-GCGTGTATTCACCATTGAG-3′	225	60	Human
Adamts5	S AS	5′-GCCATTGTAATAACCCTGCACC-3′ 5′-TCAGTCCCATCCGTAACCTTTG-3′	292	58	Mouse
ADAMTS5	S AS	5′-ATCACCCAATGCCAAGG-3′ 5′-AGCAGAGTAGGAGACAAC-3′	246	60	Human
Agat	S AS	5′-TGAAGACAAGGCCACCCATC-3′ 5′-GCATTGCGCTGTACAAACCT-3	347	60	Mouse
Arg1	S AS	5′-GGGATTGGCAAGGTGATGGA-3′ 5′-CGGGAGGGTAACCATAAGCC-3′	323	55	Mouse
Arg2	S AS	5′-GAAGTGGTTAGTAGAGCTGTGT-3′ 5′-CCCAAGTCGATCAATCTCTCTC-3′	378	60	Mouse
Azin2	S AS	5’-CCAATGTGCTGGACCAA-3’ 5’-TGGTGGAGGAGGGCTTC-3’	274	60	Mouse
Actb (β-actin)	S AS	5′-ATATCGCTGCGCTGGTCGTC-3′ 5′-AGGATGGCGTGAGGGAGAGC-3	517	58	Mouse
Ccl19	S AS	5′-GCTAATGATGCGGAAGACTGCT-3′ 5′-AGCCCCTTAGTGTGGTGAAC-3′	136	60	Mouse
Ccl5	S AS	5′-CTCACCATCATCCTCACTG-3′ 5′-CTAGCTCATCTCCAAATAGTTG-3′	255	60	Mouse
Ccr1	S AS	5′-GAAGAAGGTCAAAGCCGTGC-3′ 5′-GGCAATCACCTCAGTCACCT-3′	175	60	Mouse
Ccr2	S AS	5′-CACCCTGTTTCGCTGTAGGA-3′ 5′-CATGGCCTGGTCTAAGTGCT-3′	181	60	Mouse
Ccr3	S AS	5′-GGAATGAGTGGGGTTTTGGC-3′ 5′-CACCCTCTGGATAGCGAGGA-3′	285	60	Mouse
Ccr5	S AS	5′-ACTGCTGCCTAAACCCTGTC-3′ 5′-ATGTTCTCCTGTGGATCGGG-3′	173	60	Mouse
Col2a1	S AS	5′-CACACTGGTAAGTGGGGCAAGACCG-3′ 5′-GGATTGTGTTGTTTCAGGGTTCGGG-3′	173	57	Mouse
Cox2	S AS	5′-GGTCTGGTGCCTGGTCTGATGATG-3′ 5′-GTCCTTTCAAGGAGAATGGTGC-3′	724	65	Mouse
Epas1 (HIF-2α)	S AS	5′-CGAGAAGAACGACGTGGTGTTC-3′ 5′-GTGAAGGCTGGCAGGCTCC-3′	333	64	Mouse
Gapdh	S AS	5′-TCACTGCCACCCAGAAGAC-3′ 5′-TGTAGGCCATGAGGTCCAC-3′	450	58	Mouse
GAPDH	S AS	5′-CGTCTTCACCACCATGGAGA-3′ 5′-CGGCCATCACGCCACAGTTT-3′	300	60	Human
Il1b (IL-1β)	S AS	5′-GTGTGACGTTCCCATTAGACAA-3′ 5′-CCGTCTTTCATTACACAGGACA-3′	234	60	Mouse
Il6 (IL-6)	S AS	5′-ACCACTCCCAACAGACCTGTCTATACC-3′ 5′-CTCCTTCTGTGACTCCAGCTTATCTGTTAG-3′	435	60	Mouse

MMP1	S AS	5′-GGAGGGGATGCTCATTTTGATG-3′ 5′-TAGGGAAGCCAAAGGAGCTGT-3′	541	60	Human
Mmp12	S AS	5′-CCCAGAGGTCAAGATGGATG-3′ 5′-GGCTCCATAGAGGGACTGAA-3′	482	60	Mouse
MMP12	S AS	5′-ATATGTTGACATCAACACAT-3′ 5′-ATAAGCAGCTTCAATGCCAG-3′	286	60	Human
Mmp13	S AS	5′-TGATGGACCTTCTGGTCTTCTGG-3′ 5′-CATCCACATGGTTGGGAAGTTCT-3′	473	58	Mouse
MMP13	S AS	5′-TTTGTCTGGCGTTTTTGGATGTTTA-3′ 5′-AGGAGCATGGCGACTTCTACCC-3′	341	60	Human
Mmp14	S AS	5′-GTGCCCTAGGCCTACATCCG-3′ 5′-TTGGGTATCCATCCATCACT-3′	580	55	Mouse
MMP14	S AS	5′-ATGAGGCGCCCCCGATGTGG-3′ 5′-TCCAATGTTGGGGCCTGGGAAGTAG-3′	369	60	Human
Mmp15	S AS	5′-GAGAGATGTTTGTGTTCAAGGG-3′ 5′-TGTGTCAATGCGGTCATAGGG-3′	260	62	Mouse
MMP15	S AS	5′-GTACTGGCGCTTCAACGAG-3′ 5′-CCACCTCCTCCATCTGCAC-3′	407	60	Human
Mmp2	S AS	5′-CCAACTACGATGATGAC-3′ 5′-ACCAGTGTCAGTATCAG-3′	233	60	Mouse
MMP2	S AS	5′-GCCTGAGCTCCCGGAAAAGATTGAT-3′ 5′-CAGCAGCCTAGCCAGTCGGATTTGA-3′	444	60	Human
Mmp3	S AS	5′-AGGGATGATGATGCTGGTATGG-3′ 5′-CCATGTTCTCCAACTGCAAAGG-3′	434	58	Mouse
MMP3	S AS	5′-GATGCGCAAGCCCAGGTGTG-3′ 5′-GCCAATTTCATGAGCAGCAACGAG-3′	406	60	Human
Mmp9	S AS	5′-TGCACTGGGCTTAGATCATTCC-3′ 5′-CGGTCCTTGAAGAAATGCAGAG-3′	450	58	Mouse
MMP9	S AS	5′-CGCCGCCACGAGGAACAAAC-3′ 5′-GGCCAACTACGACACCGACGAC-3′	424	60	Human
Nos1	S AS	5′-AACCCGACAGGCCAAAGAAA-3′ 5′-AGGAGCTGAAAACCTCATCTGT-3′	345	60	Mouse
Nos2	S AS	5′-TCACTGGGACAGCACAGAAT-3′ 5′-TGTGTCTGCAGATGTGCTGA-3′	510	60	Mouse
Nos3	S AS	5′-TTTAGGGCTGTGCGGCAA-3′ 5′-CTTGAGGTACAGGGCCCATC-3	222	60	Mouse
P16ink4a	S AS	5′-CATCAACACACCCCTTACCA-3′ 5′-GGAGCCAGTGTAGGGTCAAA-3′	342	60	Mouse
Slc39a8 (ZIP8)	S AS	5′-GAACAATTGCCTGGATGATCACGC-3′ 5′-AAGCCGGTTAACATCCCTGCATTC-3′	430	58	Mouse
Sox9	S AS	5′-CACTGGCAGTTACGGCATCAG-3′ 5′-CATGTAAGTGAAGGTGGAGTAGAGC-3′	457	61	Mouse
Tnfα (TNF-α)	S AS	5′-CTTGTCTACTCCCAGGTTCTCTTC-3′ 5′-ACAGAGCAATGACTCCAAAGTAGACC-3′	301	60	Mouse

AT, annealing temperature; S, sense; AS, antisense

Supplementary table S3. Additional information on statistical tests performed.

Fig.	Test for	Test used	Assumptions on normality	Test details ($\alpha = 0.05$)	
				F value or *t* value (degree of freedom)	Size of the effect
Fig. 1C	Arg-II mRNAs (IL-1β dose)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 44) = 25.454	η^2=0.63
	Arg-II mRNA (TNF-α dose)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 16) = 58.568	η^2=0.92
	HIF-2α mRNA (Ad-HIF-2α dose)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 36) = 51.905	η^2=0.81
	Arg-II mRNA (Ad-HIF-2α dose)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 36) = 78.917	η^2=0.87
	ZIP8 mRNA (Ad-ZIP8 dose)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 36) = 16.815	η^2=0.58
	Arg-II mRNA (Ad-ZIP8 dose)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 36) = 24.253	η^2=0.67
Fig. 2B	Synovitis	Non-parametric Mann-Whitney *U* test	Not normal and unequal variances		r=0.84
Fig. 2D	OARSI grade	Non-parametric Mann-Whitney *U* test	Not normal and unequal variances		r=0.63
	Osteophyte maturity	Non-parametric Mann-Whitney *U* test	Not normal and unequal variances		r=0.47
	Synovitis	Non-parametric Mann-Whitney *U* test	Not normal and unequal variances		r=0.40
	SBP thickness	Two tailed independent *t*-test	Normality and unequal variances	t (21.855) = 6.141	d=1.69
Fig. 3B	OARSI grade	Non-parametric Mann-Whitney *U* test	Not normal and unequal variances		r=0.61
	Osteophyte maturity	Non-parametric Mann-Whitney *U* test	Not normal and unequal variances		r=0.54
	SBP thickness	Two tailed independent *t*-test	Normality and equal variances	t (26) = 3.172	d=1.20
Fig. 4B	Relative mRNA (Arg-II)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 32)=17.436	η^2=0.62
	Relative mRNA (MMP3)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 32)=16.780	η^2=0.61
	Relative mRNA (MMP9)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 32)=11.950	η^2=0.53
	Relative mRNA (MMP12)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 32)=10.910	η^2=0.51
	Relative mRNA (MMP13)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 32)=7.183	η^2=0.40
	Relative mRNA (ADAMTS5)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3, 16)=1.872	η^2=0.26
Fig. 5B	Arginase activity (left)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3,12)=9.362	η^2=0.70
	Arginase activity (right)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3,12)=31.109	η^2=0.89
Fig. 5D	Arginase activity	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (2,12)=40.439	η^2=0.87
Fig. 5C	Relative mRNA (Arg-II)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (2,21)=9.042	η^2=0.46
	Relative mRNA (MMP3)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (2,21)=5.337	η^2=0.34
	Relative mRNA (MMP13)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (2,21)=3.807	η^2=0.27
Fig. 5F	NF-κB reporter gene assay (left)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (2,24)=69.260	η^2=0.85
	NF-κB reporter gene assay (right)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (2,24)=31.374	η^2=0.72

Fig. 5G	Relative mRNA (Arg-II)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (4,25) =8.014	η^2=0.56
	Relative mRNA (MMP3)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (4,25)=67.207	η^2=0.91
	Relative mRNA (MMP13)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (4,25)=52.971	η^2=0.89
Fig. 6D	NO levels (Ad-Arg-II)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (2,36)=56.190	η^2=0.76
	NO levels (WT vs KO)	Two tailed independent *t*-test	Normality and equal variances	t (6)=3.249	d=2.28
Fig. 6E	SA-β-gal positive cells	Two tailed independent *t*-test	Normality and equal variances	t (14)=4.781	d=2.39
	p16 mRNA levels	Two tailed independent *t*-test	Normality and equal variances	t (16)=6.118	d=0.47
Fig. S3C	Growth plate (PZ/RZ)	Two tailed independent *t*-test	Normality and equal variances	t (19)=0.578	d=0.26
	Growth plate (HZ)	Two tailed independent *t*-test	Normality and equal variances	t (19)=0.299	d=0.13
Fig. S5A	Relative mRNA (Arg-II)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (4,20)=51.007	η^2=0.91
	Relative mRNA (MMP3)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (4,20)=32.774	η^2=0.87
	Relative mRNA (MMP13)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (4,20)=48.241	η^2=0.91
Fig. S5D	Relative mRNA (MMP3)	Two tailed independent *t*-test	Normality and equal variances	t (10)=4.209	d=0.46
	Relative mRNA (MMP13)	Two tailed independent *t*-test	Normality and equal variances	t (10)=2.707	d=1.56
Fig. S5E	Arginase activity	Two tailed independent *t*-test	Normality and equal variances	t (4)=11.901	d=9.72
Fig. S6B	Relative mRNA (MMP3)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (5,24) =193.43	η^2=0.98
	Relative mRNA (MMP13)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (5,24)=33.79	η^2=0.88
	Relative mRNA (ADAMTS5)	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (5,24)=18.88	η^2=0.80
Fig. S6C	NF-κB reporter gene assay	One-way ANOVA with *post hoc* Bonferroni test	Normality and equal variances	F (3,16)=10.46	η^2=0.66
Fig. S7B	Relative mRNA (Arg-II)	Two tailed independent *t*-test	Normality and equal variances	t (7)=15.242	d= 7.62
	Relative mRNA (MMP3)	Two tailed independent *t*-test	Normality and equal variances	t (7)= 20.962	d= 10.48
	Relative mRNA (MMP9)	Two tailed independent *t*-test	Normality and equal variances	t (14)=8.481	d= 4.24
	Relative mRNA (MMP12)	Two tailed independent *t*-test	Normality and unequal variances	t (12.45)=0.134	d=0.07
	Relative mRNA (MMP13)	Two tailed independent *t*-test	Normality and equal variances	t (14)=1.490	d=-0.74
	Relative mRNA (ADAMTS5)	Two tailed independent *t*-test	Normality and unequal variances	t (8.18)=7.891	d=-3.95
Fig. S7F	Relative mRNA (Arg-II)	Two tailed independent *t*-test	Normality and equal variances	t (22)=14.189	d=5.79
	Relative mRNA (MMP3)	Two tailed independent *t*-test	Normality and equal variances	t (22)=2.817	d=1.15
	Relative mRNA (MMP9)	Two tailed independent *t*-test	Normality and equal variances	t (22)=1.626	d=0.66
	Relative mRNA (MMP12)	Two tailed independent *t*-test	Normality and equal variances	t (22)=2.162	d=0.88
	Relative mRNA (MMP13)	Two tailed independent *t*-test	Normality and equal variances	t (22)=0.032	d=0.01
	Relative mRNA (ADAMTS5)	Two tailed independent *t*-test	Normality and equal variances	t (22)=2.276	d=0.93

Supplementary table S4. Estimated mean changes and 95% CI of the difference between the means for evaluated parameters.

Fig.	Category	Group	Mean	Mean (95% CI)	95% CI of the difference between the means	*p* value
Fig. 1C.	IL-1β (ng/ml)	0	1.00			
		0.1	13.72	13.72 (9.85; 17.59)	-12.72 (-22.33; -3.11)	0.0041
		0.5	21.95	21.95 (16.73; 27.18)	-20.95 (-30.56; -11.34)	<0.0001
		1	30.08	30.08 (22.96; 37.20)	-29.08 (-38.69; -19.47)	<0.0001
	TNF-α (ng/ml)	0	1.00			
		5	3.77	3.77 (2.77; 4.76)	-2.77 (-5.09; -0.44)	0.0149
		10	6.31	6.31 (4.82; 7.81)	-5.31 (-7.64; -2.99)	<0.0001
		20	10.84	10.84 (9.68; 12.00)	-9.84 (-12.16; -7.51)	<0.0001
	Ad-HIF-2α (MOI)	Ad-C	1.00			
		200	10.04	10.04 (7.79; 12.30)	-9.04 (-15.45; -2.64)	0.0021
		400	17.51	17.51 (14.74; 20.28)	-16.51 (-22.91; -10.11)	<0.0001
		800	28.59	28.59 (23.33; 33.85)	-27.59 (-33.99; -21.18)	<0.0001
	Ad-ZIP8 (MOI)	Ad-C	1.00			
		200	12.74	12.74 (7.47; 18.01)	-11.74 (-25.19; 1.71)	0.1194
		400	20.28	20.28 (14.31; 26.25)	-19.28 (-32.73; -5.83)	0.0018
		800	34.34	34.34 (23.62; 45.06)	-33.34 (-46.79; -19.89)	<0.0001
Fig. 2B	Synovitis (0-3)	Ad-C	0.08	0.08 (0.01; 0.16)		
		Ad-Arg-II	2.29	2.29 (2.08; 2.51)		0.0023
Fig. 2D	OARSI grade (0-6)	Ad-C	0.00	0.00 (0.00; 0.00)		
		Ad-Arg-II	2.62	2.62 (1.57; 3.67)		0.0007
	Osteophyte maturity (0-3)	Ad-C	0.00	0.00 (0.00; 0.00)		
		Ad-Arg-II	0.57	0.57 (0.27; 0.88)		0.0122
	SBP thickness (μm)	Ad-C	44.41	44.41 (35.33; 53.49)		
		Ad-Arg-II	148.47	148.47 (116.52; 180.42)	-104.07 (-154.82; -53.32)	<0.0001
	Synovitis (0-3)	Ad-C	0.00	0.00 (0.00; 0.00)		
		Ad-Arg-II	0.38	0.38 (0.12; 0.63)		0.0312
Fig. 3B	OARSI grade (0-6)	WT Sham	0.02	0.02 (-0.01; 0.04)		
		WT DMM	3.84	3.84 (3.13; 4.54)		<0.0001
		KO Sham	0.06	0.06 (0.00; 0.12)		
		KO DMM	1.58	1.58 (0.88; 2.27)		vs WT DMM, 0.0013
	Osteophyte maturity (0-3)	WT Sham	0.00	0.00 (0.00; 0.00)		
		WT DMM	2.22	2.22 (1.89; 2.56)		<0.0001
		KO Sham	0.04	0.04 (-0.04; 0.12)		
		KO DMM	1.40	1.40 (1.05; 1.74)		vs WT DMM, 0.0042
	SBP thickness (μm)	WT Sham	46.78	46.78 (42.06; 51.50)		
		WT DMM	246.19	246.19 (176.57; 315.81)		<0.0001
		KO Sham	65.41	65.41 (47.27; 83.55)		
		KO DMM	144.55	144.55 (102.31; 186.79)	101.64 (35.78; 167.50)	vs WT DMM, 0.0039
Fig. 4B	Arg-II	Ad-C	1			
		Ad-Arg-II (200)	407.88	407.88 (224.48; 591.29)	-406.9 (-1175.5; 361.7)	0.8781
		Ad-Arg-II (400)	914.08	914.08 (559.06; 1269.10)	-506.2 (-1681.7; -144.4)	0.01281

		Ad-Arg-II (800)	1871.65	1871.6 (1228.0; 2515.2)	-957.6(-2639.2; -1102.0)	<0.0001
	MMP3	Ad-C	1			
		Ad-Arg-II (200)	1.56	1.56 (1.23; 1.88)	-0.56 (-2.67; 1.55)	1.0000
		Ad-Arg-II (400)	3.11	3.11 (2.40; 3.82)	-1.56 (-4.22; -0.01)	0.0491
		Ad-Arg-II (800)	5.85	5.85 (3.92; 7.77)	-2.73 (-6.96; -2.74)	<0.0001
	MMP9	Ad-C	1			
		Ad-Arg-II (200)	2.13	2.13 (1.06; 3.19)	-1.13 (-4.10; 1.85)	1.0000
		Ad-Arg-II (400)	3.09	3.09 (1.82; 4.37)	-0.96 (-5.07; 0.89)	0.3417
		Ad-Arg-II (800)	6.96	6.96 (4.54; 9.38)	-3.87 (-8.94; -2.98)	<0.0001
	MMP12	Ad-C	1			
		Ad-Arg-II (200)	1.87	1.87 (0.87; 2.87)	-0.87 (-3.03; 1.28)	1.0000
		Ad-Arg-II (400)	2.41	2.41 (1.55; 3.27)	-0.54 (-3.56; 0.74)	0.4485
		Ad-Arg-II (800)	5.14	5.14 (3.48; 6.81)	-2.73(-6.30; -1.99)	<0.0001
	MMP13	Ad-C	1			
		Ad-Arg-II (200)	1.31	1.32 (1.01; 1.63)	-0.32 (-1.36; 0.73)	1.0000
		Ad-Arg-II (400)	2.24	2.24 (1.49; 2.99)	-0.92 (-2.29; -0.19)	0.0130
		Ad-Arg-II (800)	2.46	2.46 (1.82; 3.10)	-0.22 (-2.50; -0.41)	0.0026
	ADAMTS5	Ad-C	1			
		Ad-Arg-II (200)	1.21	1.21 (1.04; 1.38)	-0.21 (-0.54; 0.12)	0.4237
		Ad-Arg-II (400)	1.02	1.02 (0.93; 1.11)	0.19 (-0.35; 0.30)	1.0000
		Ad-Arg-II (800)	1.17	1.17 (0.93; 1.40)	-0.15 (-0.50; 0.16)	0.8265
Fig. 5B	IL-1β (ng/ml)	0	1.64	1.64 (0.832; 2.45)		
		0.1	13.02	13.02 (7.44; 18.6)	-11.38 (-22.21; -0.56)	0.0369
		0.5	16.68	16.68 (11.53; 21.83)	-15.04 (-25.87; -4.22)	0.0053
		1.0	17.97	17.97 (12.29; 23.65)	-16.33 (-27.15; -5.51)	0.0028
	Ad-Arg-II (MOI)	Ad-C	1.29	1.29 (-0.19; 2.77)		
		Ad-Arg-II (200)	10.86	10.86 (7.3; 14.42)	-9.57 (-18.63; -0.51)	0.0358
		Ad-Arg-II (400)	21.72	21.72 (16.99; 26.45)	-20.42 (-29.48; -11.37)	<0.0001
		Ad-Arg-II (800)	26.62	26.62 (21.5; 31.74)	-25.33 (-34.38; -16.27)	<0.0001
Fig. 5C	Arg-II	EV	1.00			
		WT-Arg-II	6937.29	6937.2 (2031.4; 11843.1)	6936.2 (-568.7; 14441.3)	0.0765
		Δ-Arg-II	6853.22	6853.22 (1965; 11741.44)	84.07 (-7420.9; 7589.1)	1.0000
	MMP3	EV	1.00			
		WT-Arg-II	2.33	2.33 (1.46; 3.20)	1.33 (0.37; 2.29)	0.0050
		Δ-Arg-II	0.94	0.94 (0.80; 1.08)	1.39 (0.43; 2.35)	0.0034
	MMP13	EV	1.00			
		WT-Arg-II	1.74	1.74 (1.12; 2.36	0.74 (0.03; 1.45)	0.0386
		Δ-Arg-II	0.94	0.94 (0.73; 1.15)	0.80 (0.09; 1.50)	0.0241
Fig. 5D	Arg-II activity	EV	0.34	0.34 (0.19; 0.50)	13.04 (8.51; 17.57)	<0.0001
		WT-Arg-II	13.39	13.39 (9.49; 17.28)		
		Δ-Arg-II	1.08	1.08 (0.73; 1.42)	12.31 (7.78; 16.84)	<0.0001
Fig. 5F	NF-κB activity	EV	1.00			
		p65	10.26	10.26 (8.61; 11.91)	-9.27 (-11.54; -7.00)	<0.0001

		IL-1β	9.69	9.69 (8.36; 11.01)	-8.69 10.96; -6.42)	<0.0001
	NF-κB activity	EV	1.00		1.65 (1.11; 2.18)	<0.0001
		WT-Arg-II	2.65	2.65 (2.38; 2.92)		
		Δ-Arg-II	1.85	1.85 (1.43; 2.27)	0.79 (0.26; 1.33)	<0.0001
Fig. 5G	MMP3	Ad-C	0.31	0.31 (0.11; 0.52)	0.69 (0.30; 1.07)	<0.0001
		SC514 (0 μM)	1.00			
		SC514 (10 μM)	0.62	0.62 (0.40; 0.84)	0.38 (-0.01; 0.76)	0.0537
		SC514 (20 μM)	0.55	0.55 (0.39; 0.70)	0.46 (0.08; 0.84)	0.0112
		SC514 (40 μM)	0.55	0.55 (0.37; 0.73)	0.45 (0.06; 0.83)	0.0137
	MMP13	Ad-C	0.29	0.29 (0.24; 0.34)	0.71 (0.57; 0.86)	<0.0001
		SC514 (0 μM)	1.00			
		SC514 (10 μM)	0.89	0.89 (0.80; 0.98)	0.11 (-0.04; 0.25)	0.3043
		SC514 (20 μM)	0.77	0.77 (0.69; 0.86)	0.23 (0.08; 0.37)	0.0006
		SC514 (40 μM)	0.67	0.67 (0.61; 0.73)	0.33 (0.19; 0.48)	<0.0001
	Arg-II	Ad-C	0.00	0.00 (0.00; 0.00)	1.00 (0.77; 1.23)	<0.0001
		SC514 (0 μM)	1.00			
		SC514 (10 μM)	0.77	0.77 (0.63; 0.92)	0.23 (-0.003; 0.45)	0.0557
		SC514 (20 μM)	0.70	0.70 (0.59; 0.81)	0.30 (0.07; 0.53)	0.0041
		SC514 (40 μM)	0.79	0.79 (0.65; 0.94)	0.21 (-0.20; 0.44)	0.0951
Fig. 6D	None	None	4.41	4.41 (2.81; 6.01)	18.29 (13.82; 22.75)	<0.0001
	Ad-C vs Ad-Arg-II (w IL-1β)	Ad-C	22.70	22.36 (17.56; 27.16)		
		Ad-Arg-II	17.55	16.89 (13.94; 19.84)	5.15 (0.69; 9.62)	0.0192
	WT vs KO (w or wo IL-1β)	WT w IL-1β	2.03	2.03 (1.33; 2.72)		
		WT wo IL-1β	15.51	15.51 (12.10; 18.92)	-15.85 (-18.27; -13.41)	<0.0001
		KO w IL-1β	2.82	2.82 (1.08; 4.56)	vs KO (wo IL-1β) -24.28 (-31.77; -16.79)	
		KO wo IL-1β	22.63	22.63 (15.23; 30.02)	vs WT (w IL-1β) -9.70 (-18.29; -1.10)	0.0014
Fig. 6E	SA-β-gal	WT	50.27	50.27 (35.88; 64.67)		
		KO	11.89	11.89 (8.33; 15.46)	38.78 (21.16; 55.59)	0.0002
	p16	WT	1.00			
		KO	0.70	0.70 (0.60; 0.79)	0.29 (0.13; 0.45)	0.0028
Fig. S3C	Growth plate (PZ/RZ)	WT	324.83	324.83 (287.53; 362.14)		
		KO	308.55	308.55 (269.87; 347.23)	16.28 (-42.66; 75.23)	0.5699
	Growth plate (HZ)	WT	234.78	234.78 (195.32; 274.23)		
		KO	228.24	228.24 (205.65; 250.83)	6.54 (-39.22; 52.30)	0.7682
Fig. S5A	Arg-II	Ad-C	1.00			
		SC514 (0 μM)	176.87	176.87 (135.76; 217.99)	175.87 (131.18; 220.57)	<0.0001
		SC514 (10 μM)	50.97	50.97 (36.83; 65.11)	125.90 (81.21; 170.60)	<0.0001
		SC514 (20 μM)	25.46	25.46 (19.54; 31.37)	151.41 (106.72; 196.11)	<0.0001
		SC514 (40 μM)	11.97	11.97 (9.98; 13.97)	164.90 (120.21; 209.60)	<0.0001
	MMP3	Ad-C	1.00			

		SC514 (0 μM)	277.47	277.47 (1199.0; 2286.7)	1741.87 (1173.8; 2309.9)	<0.0001
		SC514 (10 μM)	58.70	58.70 (262.42; 492.51)	1365.41 (797.3; 1933.5)	<0.0001
		SC514 (20 μM)	26.17	26.17 (110.39; 212.99)	1581.2 (1013.1; 2149.3)	<0.0001
		SC514 (40 μM)	4.27	4.27 (33.01; 49.77)	1701.5 (1133.4; 2269.6)	<0.0001
	MMP13	Ad-C	1.00			
		SC514 (0 μM)	93.78	93.78 (72.84; 114.71)	92.77 (70.35; 115.20)	<0.0001
		SC514 (10 μM)	51.68	51.68 (47.75; 55.60)	42.10 (19.67; 64.53)	<0.0001
		SC514 (20 μM)	37.73	37.73 (33.58; 41.88)	56.04 (33.61; 78.47)	<0.0001
		SC514 (40 μM)	21.74	21.74 (17.84; 25.63)	72.04 (49.61; 94.47)	<0.0001
Fig. S5D	MMP3	None	WT0.00 KO 0.00	0.00 (-0.00; 0.01) 0.00 (0.00; 0.00)	0.00 (-0.00; 0.01)	0.2792
		IL1β (0.1 ng/ml)	WT 0.18 KO 0.12	0.18 (0.15; 0.21) 0.12 (0.10; 0.15)	0.05 (0.01; 0.10)	0.0276
		IL1β (0.5 ng/ml)	WT 0.29 KO 0.18	0.29 (0.26; 0.31) 0.18 (0.14; 0.22)	0.11 (0.05; 0.16)	0.0018
	MMP13	None	WT 0.01 KO 0.01	0.01 (0.00; 0.03) 0.01 (0.00; 0.01)	0.01 (-0.01; 0.02)	0.3439
		IL1β (0.1 ng/ml)	WT 0.25 KO 0.19	0.25 (0.24; 0.27) 0.19 (0.15; 0.23)	0.07 (0.02; 0.12)	0.0110
		IL1β (0.5 ng/ml)	WT 0.29 KO 0.21	0.29 (0.27; 0.31) 0.21 (0.16; 0.27)	0.08 (0.01; 0.14)	0.0221
Fig. S5E	Arginase activity	None	WT 0.34 KO 0.20	0.34 (0.25; 0.44) 0.20 (0.04; 0.36)	0.14 (-0.12; 0.40)	0.2043
		IL1β (0.1 ng/ml)	WT 1.42 KO 0.15	1.42 (1.03; 1.82) 0.15 (-0.01; 0.31)	1.27 (0.67; 1.88)	0.0042
		IL1β (0.5 ng/ml)	WT 3.20 KO 0.20	3.20 (2.95; 3.45) 0.20 (-0.22; 0.63)	3.00 (2.30; 3.69)	<0.0003
Fig. S6B (Ornithine)	MMP3	None	1.00			
		10 mM	7.55	7.55 (3.25; 11.86)	-6.55 (-59.90; 46.80)	1.0000
		20 mM	12.12	12.12 (4.41; 19.83)	-11.12 (-64.47; 42.23)	1.0000
		30 mM	22.17	22.17 (7.03; 37.31)	-21.17 (-74.52; 32.18)	1.0000
		40 mM	78.82	78.82 (46.97; 110.68)	-77.82 (-131.17; -24.48)	0.0012
		50 mM	412.69	412.69 (342.95; 482.53)	-411.69 (-465.04; -358.34)	<0.0001
	MMP13	None	1.00			
		10 mM	7.11	7.11 (3.98; 10.25)	-6.11 (-14.53; 2.30)	0.3942
		20 mM	10.14	10.14 (6.38; 13.91)	-9.14 (-17.55; -0.73)	0.0250
		30 mM	16.72	16.72 (12.74; 20.70)	-15.72 (-24.13; -7.31)	<0.0001
		40 mM	24.54	24.54 (15.68; 33.39)	-23.54 (-31.95; -15.12)	<0.0001
		50 mM	28.71	28.71 (22.72; 34.71)	-27.71 (-36.12; -19.30)	<0.0001
Fig. S6B (Urea)	MMP3	None	1.00			
		25 mM	1.01	1.01 (0.82; 1.19)	-0.01 (-0.49; 0.48)	1.0000
		50 mM	1.10	1.10 (0.72; 1.48)	-0.10 (-0.58; 0.39)	1.0000
		75 mM	1.05	1.05 (0.84; 1.26)	-0.05 (-0.53; 0.44)	1.0000
		100 mM	1.03	1.03 (0.65; 1.41)	-0.03 (-0.51; 0.46)	1.0000
	MMP13	None	1.00			
		25 mM	0.87	0.87 (0.72; 1.02)	0.13 (-0.22; 0.47)	1.0000
		50 mM	1.12	1.12 (0.87; 1.38)	-0.12 (-0.47; 0.22)	1.0000
		75 mM	1.24	1.24 (1.01; 1.47)	-0.24 (-0.58; 0.10)	0.4515
		100 mM	1.28	1.28 (1.08; 1.49)	-0.28 (-0.63; 0.06)	0.1801

Fig. S6C	NF-κB activity	None	1.00			
		Urea	0.98	0.98 (0.80; 1.15)	0.02 (-1.04; 1.08)	1.0000
		Ornithine	2.13	2.13 (1.35; 2.91)	-1.13 (-2.19; -0.07)	0.0321
		IL-1β	2.56	2.56 (1.44; 3.68)	-1.56 (-2.62; -0.51)	0.0024
Fig. S7B	Arg-II	Ad-C	0.0006	0.0006 (0.0006; 0.0007)		
		Ad-Arg-II	0.0276	0.0276 (0.0242; 0.0311)	-0.027 (0.0312; 0.0223)	<0.0001
	MMP3	Ad-C	0.0004	0.0004 (0.0004; 0.0005)		
		Ad-Arg-II	0.0087	0.0087 (0.0079; 0.0094)	-0.008 (-0.009; -0.007)	<0.0001
	MMP9	Ad-C	0.0000	0.00001 (0.00001;0.00001)		
		Ad-Arg-II	0.0001	0.00005 (0.00004;0.00006)	-0.00004 (-0.0001;-0.00003)	<0.0001
	MMP12	Ad-C	0.0002	0.0002 (0.0001; 0.0003)		
		Ad-Arg-II	0.0002	0.0002 (0.00015; 0.0003)	-0.00001 (-0.0001; 0.0001)	0.8954
	MMP13	Ad-C	0.0021	0.0021 (0.0016; 0.0026)		
		Ad-Arg-II	0.0017	0.0017 (0.0014; 0.0019)	0.00044 (-0.00019; 0.0011)	0.1585
	ADAMTS5	Ad-C	0.0550	0.0550 (0.0453; 0.0647)		
		Ad-Arg-II	0.0144	0.0144 (0.0115; 0.0172)	0.0407 (0.0288; 0.0525)	<0.0001
Fig. S7F	Arg-II	Ad-C	0.23	0.23 (0.16; 0.30)		
		Ad-Arg-II	8.96	8.96 (7.75; 10.16)	-8.72 (-0.10; -0.07)	<0.0001
	MMP3	Ad-C	5.24	5.24 (3.23; 7.24)		
		Ad-Arg-II	11.02	11.02 (7.53; 14.51)	-5.78 (-1.00; -0.15)	0.0116
	MMP9	Ad-C	0.001	0.001 (0.0007; 0.0017)		
		Ad-Arg-II	0.002	0.002 (0.001; 0.003)	-0.0009 (-0.0002; 0.00002)	0.1182
	MMP12	Ad-C	0.0006	0.0006 (0.0003; 0.0008)		
		Ad-Arg-II	0.0087	0.0087 (0.0013; 0.0162)	-0.008 (-0.002; 0.00003)	0.0535
	MMP13	Ad-C	1.44	1.44 (0.61; 2.27)		
		Ad-Arg-II	1.46	1.46 (0.57; 2.35)	-0.02 (-0.13; 0.13)	0.9752
	ADAMTS5	Ad-C	4.71	4.71 (2.13; 7.28)		
		Ad-Arg-II	1.56	1.56 (0.72; 2.41)	3.14 (0.03; 0.60)	0.0399

Supplementary table S5. Microarray analysis of mRNAs in chondrocytes treated with IL-1β (1 ng/ml, 36 h) or infected with 800 MOI of Ad-HIF-2α or Ad-ZIP8 (36 h).

Amino acid	Gene symbol	Gene ID	Fold change		
			IL-1β	Ad-HIF-2α	Ad-ZIP8
Alanine	*Dpys*	64705	0.86 ± 0.06	0.98 ± 0.09	0.98 ± 0.12
	Gpt2	108682	0.44 ± 0.03	0.63 ± 0.08	0.72 ± 0.06
	Pah	18478	1.03 ± 0.10	0.93 ± 0.13	0.97 ± 0.15
	Spr	20751	1.02 ± 0.05	1.02 ± 0.10	1.03 ± 0.12
	Upb1	103149	1.01 ± 0.26	0.93 ± 0.22	1.06 ± 0.27
Arginine	*Agat*	67092	0.87 ± 0.15	1.03 ± 0.12	1.09 ± 0.08
	Arg1	11846	1.09 ± 0.12	0.93 ± 0.08	0.95 ± 0.08
	Arg2	11847	3.38 ± 0.55	3.00 ± 0.46	2.78 ± 0.85
	Azin2	242669	2.37 ± 0.19	1.44 ± 0.09	1.11 ± 0.07
	Nos1	18125	0.93 ± 0.06	0.75 ± 0.10	0.72 ± 0.10
	Nos2	18126	76.94 ± 12.11	34.64 ± 12.96	7.31 ± 3.13
	Nos3	18127	1.04 ± 0.14	0.83 ± 0.15	0.94 ± 0.21
Asparagine	*Aspg*	104816	1.01 ± 0.03	0.96 ± 0.19	1.04 ± 0.21
	Nit2	52633	0.97 ± 0.10	1.02 ± 0.21	1.08 ± 0.25
Aspartic acid	*Asph*	65973	1.00 ± 0.10	0.78 ± 0.04	0.78 ± 0.05
	Hif1an	319594	0.89 ± 0.11	0.88 ± 0.11	0.90 ± 0.11
Cysteine	*Cdo1*	12583	1.97 ± 0.32	2.45 ± 0.70	2.36 ± 0.81
	Cbs	12411	0.96 ± 0.09	0.80 ± 0.06	0.88 ± 0.07
	Comt	12846	1.07 ± 0.05	0.95 ± 0.05	0.91 ± 0.05
	Cps1	227231	0.96 ± 0.05	1.07 ± 0.05	1.20 ± 0.09
	Cth	107869	0.54 ± 0.06	0.87 ± 0.07	0.92 ± 0.06
	Dnmt1	13433	0.65 ± 0.04	1.31 ± 0.16	1.32 ± 0.15
	Dnmt3a	13435	0.96 ± 0.03	0.79 ± 0.05	0.84 ± 0.07
	Dnmt3b	13436	0.68 ± 0.04	0.88 ± 0.04	1.04 ± 0.05
	Dpep1	13479	0.95 ± 0.25	0.98 ± 0.13	0.98 ± 0.14
	Eif4a3	192170	0.85 ± 0.14	0.95 ± 0.13	0.96 ± 0.12
	Gamt	14431	0.67 ± 0.04	0.63 ± 0.08	0.66 ± 0.11
	Gclc	14629	1.76 ± 0.09	1.44 ± 0.30	1.36 ± 0.18
	Gclm	14630	0.89 ± 0.10	0.69 ± 0.09	0.82 ± 0.13
	Icmt	57295	1.02 ± 0.05	1.02 ± 0.10	0.98 ± 0.07
	Mthfr	17769	0.90 ± 0.05	0.91 ± 0.05	0.90 ± 0.08
	Mtr	238505	0.53 ± 0.08	0.67 ± 0.07	0.76 ± 0.10
	Mtrr	210009	0.71 ± 0.05	0.82 ± 0.08	0.90 ± 0.09
	Mut	17850	1.00 ± 0.05	0.93 ± 0.08	0.90 ± 0.05
	Nox4	50490	1.19 ± 0.21	1.11 ± 0.33	1.11 ± 0.38
	Ocln	18260	0.86 ± 0.12	0.94 ± 0.06	1.04 ± 0.11
	Pcmt1	18537	1.00 ± 0.10	1.11 ± 0.12	1.03 ± 0.12
	Pemt	18618	0.80 ± 0.06	0.94 ± 0.07	1.02 ± 0.10
	Txnrd1	50493	1.20 ± 0.03	1.21 ± 0.09	1.39 ± 0.13
Glutamic acid					
Glutamine	*Cdo1*	12583	1.97 ± 0.32	2.45 ± 0.70	2.36 ± 0.81
	Adssl1	11565	1.21 ± 0.26	1.29 ± 0.22	1.08 ± 0.19
	Aldh5a1	214579	0.98 ± 0.13	0.92 ± 0.06	0.78 ± 0.06
	Asns	27053	0.57 ± 0.05	0.80 ± 0.08	0.86 ± 0.04

	Asnsd1	70396	0.74 ± 0.08	0.82 ± 0.09	0.90 ± 0.07
	Cad	69719	0.57 ± 0.04	0.83 ± 0.06	0.87 ± 0.04
	Ctps	51797	1.74 ± 0.15	1.57 ± 0.22	1.69 ± 0.19
	Ctps2	55936	1.93 ± 0.06	1.68 ± 0.17	1.66 ± 0.12
	Gfpt1	14583	0.70 ± 0.05	0.78 ± 0.06	0.75 ± 0.06
	Gfpt2	14584	0.47 ± 0.04	0.74 ± 0.04	0.73 ± 0.06
	Ggh	14590	1.53 ± 0.41	1.19 ± 0.11	1.18 ± 0.06
	Gldc	104174	0.97 ± 0.10	1.02 ± 0.21	1.08 ± 0.25
	Gls	14660	0.60 ± 0.03	0.70 ± 0.04	0.73 ± 0.03
	Gls2	216456	1.31 ± 0.21	1.28 ± 0.19	1.41 ± 0.20
	Glud1	14661	0.85 ± 0.03	0.99 ± 0.06	0.96 ± 0.05
	Gmps	229363	0.95 ± 0.12	1.13 ± 0.20	1.05 ± 0.17
	Gmps	229363	0.76 ± 0.09	0.96 ± 0.06	0.99 ± 0.08
	Mecp2	17257	1.10 ± 0.18	0.97 ± 0.09	0.97 ± 0.07
	Nit2	52633	0.85 ± 0.04	0.91 ± 0.08	0.98 ± 0.09
	Pfas	237823	0.86 ± 0.07	0.91 ± 0.03	0.93 ± 0.07
	Sirt4	75387	1.02 ± 0.09	0.90 ± 0.12	0.98 ± 0.15
Glycine	*Baat*	12012	1.18 ± 0.12	0.93 ± 0 .04	0.90 ± 0.05
	Dmgdh	74129	0.89 ± 0.14	0.68 ± 0.05	0.82 ± 0.05
	Gart	14450	0.57 ± 0.03	0.79 ± 0.06	0.82 ± 0.07
	Gldc	104174	1.18 ± 0.17	1.16 ± 0.14	0.96 ± 0.08
	Glyat	107146	0.82 ± 0.09	1.05 ± 0.17	1.06 ± 0.15
	Phgdh	236539	0.52 ± 0.05	0.85 ± 0.12	0.88 ± 0.15
	Shmt1	20425	0.71 ± 0.10	0.81 ± 0.07	0.93 ± 0.05
	Shmt2	108037	0.85 ± 0.04	0.91 ± 0.08	0.98 ± 0.09
	Slc25a32	69906	0.60 ± 0.07	0.78 ± 0.08	0.83 ± 0.07
Histidine	*Amdhd1*	71761	0.95 ± 0.06	0.96 ± 0.10	0.92 ± 0.08
	Ftcd	14317	0.95 ± 0.18	1.01 ± 0.17	1.01 ± 0.16
	Hal	15109	1.49 ± 0.26	1.70 ± 0.43	1.13 ± 0.17
	Hdc	15186	1.07 ± 0.17	1.15 ± 0.18	0.95 ± 0.14
	Uroc1	243537	0.98 ± 0.09	1.08 ± 0.20	0.92 ± 0.17
Isoleucine	*Aldh6a1*	104776	1.29 ± 0.18	0.89 ± 0.12	1.04 ± 0.11
	Ghr	14600	0.77 ± 0.09	0.76 ± 0.03	0.67 ± 0.03
	Stat5a	20850	1.31 ± 0.04	1.52 ± 0.23	1.34 ± 0.18
	Stat5b	20851	0.90 ± 0.09	1.29 ± 0.15	1.14 ± 0.16
Leucine	*Aldh6a1*	104776	1.29 ± 0.18	0.89 ± 0.12	1.04 ± 0.11
	Ghr	14600	0.77 ± 0.09	0.76 ± 0.03	0.67 ± 0.03
	Shmt2	108037	0.56 ± 0.06	0.99 ± 0.08	0.93 ± 0.07
	Stat5a	20850	1.31 ± 0.04	1.52 ± 0.23	1.34 ± 0.18
	Stat5b	20851	0.90 ± 0.09	1.29 ± 0.15	1.14 ± 0.16
Lysine					
Methionine	*Adi1*	104923	0.90 ± 0.11	0.95 ± 0.07	0.95 ± 0.08
	Bhmt2	64918	1.00 ± 0.13	0.82 ± 0.16	0.93 ± 0.14
	Comt	12846	1.07 ± 0.05	0.95 ± 0.05	0.91 ± 0.05
	Cps1	227231	1.00 ± 0.13	0.92 ± 0.07	0.91 ± 0.06
	Dnmt3b	13436	0.80 ± 0.11	1.13 ± 0.08	1.27 ± 0.19
	Gclm	14630	0.96 ± 0.05	1.07 ± 0.05	1.20 ± 0.09
	Gnmt	14711	0.89 ± 0.10	0.69 ± 0.09	0.82 ± 0.13
	Mthfd1	108156	0.68 ± 0.04	0.88 ± 0.04	1.04 ± 0.05

	Mtr	238505	0.53 ± 0.08	0.67 ± 0.07	0.76 ± 0.10
	Nox4	50490	1.19 ± 0.21	1.11 ± 0.33	1.11 ± 0.38
	Txnrd1	50493	1.20 ± 0.03	1.21 ± 0.09	1.39 ± 0.13
Phenylalanine	*Dpys*	64705	0.86 ± 0.06	0.98 ± 0.09	0.98 ± 0.12
	Spr	20751	1.02 ± 0.05	1.02 ± 0.10	1.03 ± 0.12
Proline	*Aldh4a1*	212647	2.04 ± 0.30	1.60 ± 0.24	1.50 ± 0.25
	Ero1l	50527	1.37 ± 0.12	2.42 ± 0.29	1.00 ± 0.07
	Ero1lb	67475	1.21 ± 0.17	1.15 ± 0.18	0.95 ± 0.17
	Prdx4	53381	0.87 ± 0.17	0.93 ± 0.05	0.94 ± 0.03
	Prodh	19125	0.94 ± 0.14	0.92 ± 0.10	0.98 ± 0.15
	Prodh2	56189	1.03 ± 0.09	0.97 ± 0.14	0.92 ± 0.16
Serine	*Baat*	12012	1.00 ± 0.16	1.06 ± 0.16	1.04 ± 0.15
	Dao	13142	1.00 ± 0.10	1.11 ± 0.12	1.03 ± 0.12
	Gldc	104174	0.85 ± 0.04	0.91 ± 0.08	0.98 ± 0.09
	Lipc	15450	1.07 ± 0.10	1.06 ± 0.13	1.04 ± 0.13
	Pcmt1	18537	0.93 ± 0.12	0.94 ± 0.05	0.99 ± 0.05
	Psph	100678	0.42 ± 0.03	0.61 ± 0.07	0.71 ± 0.06
	Serinc1	56442	1.18 ± 0.12	0.93 ± 0.04	0.90 ± 0.05
	Serinc2	230779	1.18 ± 0.17	1.16 ± 0.14	0.96 ± 0.08
	Serinc3	26943	1.18 ± 0.01	0.96 ± 0.04	0.93 ± 0.02
	Serinc5	218442	0.46 ± 0.03	0.59 ± 0.06	0.74 ± 0.07
	Shmt2	108037	0.56 ± 0.06	0.99 ± 0.08	0.93 ± 0.07
	Srr	27364	1.06 ± 0.15	0.77 ± 0.10	0.87 ± 0.07
Threonine	*Nit2*	52633	0.85 ± 0.04	0.91 ± 0.08	0.98 ± 0.09
Tryptophan	*Acmsd*	266645	1.14 ± 0.11	0.72 ± 0.12	0.85 ± 0.14
	Atp7a	11977	1.21 ± 0.19	0.96 ± 0.13	0.94 ± 0.10
	Gcdh	270076	1.08 ± 0.04	0.94 ± 0.09	1.06 ± 0.11
	Hgd	15233	0.98 ± 0.13	0.90 ± 0.19	0.99 ± 0.22
Tyrosine	*Hgd*	15233	0.98 ± 0.13	0.90 ± 0.19	0.99 ± 0.22
	Tdo2	56720	1.07 ± 0.05	1.04 ± 0.16	0.99 ± 0.13
Valine	*Aldh6a1*	104776	1.29 ± 0.18	0.89 ± 0.12	1.04 ± 0.11
	Bcat2	12036	0.94 ± 0.10	1.06 ± 0.11	1.09 ± 0.09
	Ghr	14600	0.77 ± 0.09	0.76 ± 0.03	0.67 ± 0.03
	Stat5a	20850	1.31 ± 0.04	1.52 ± 0.23	1.34 ± 0.18
	Stat5b	20851	0.90 ± 0.09	1.29 ± 0.15	1.14 ± 0.16