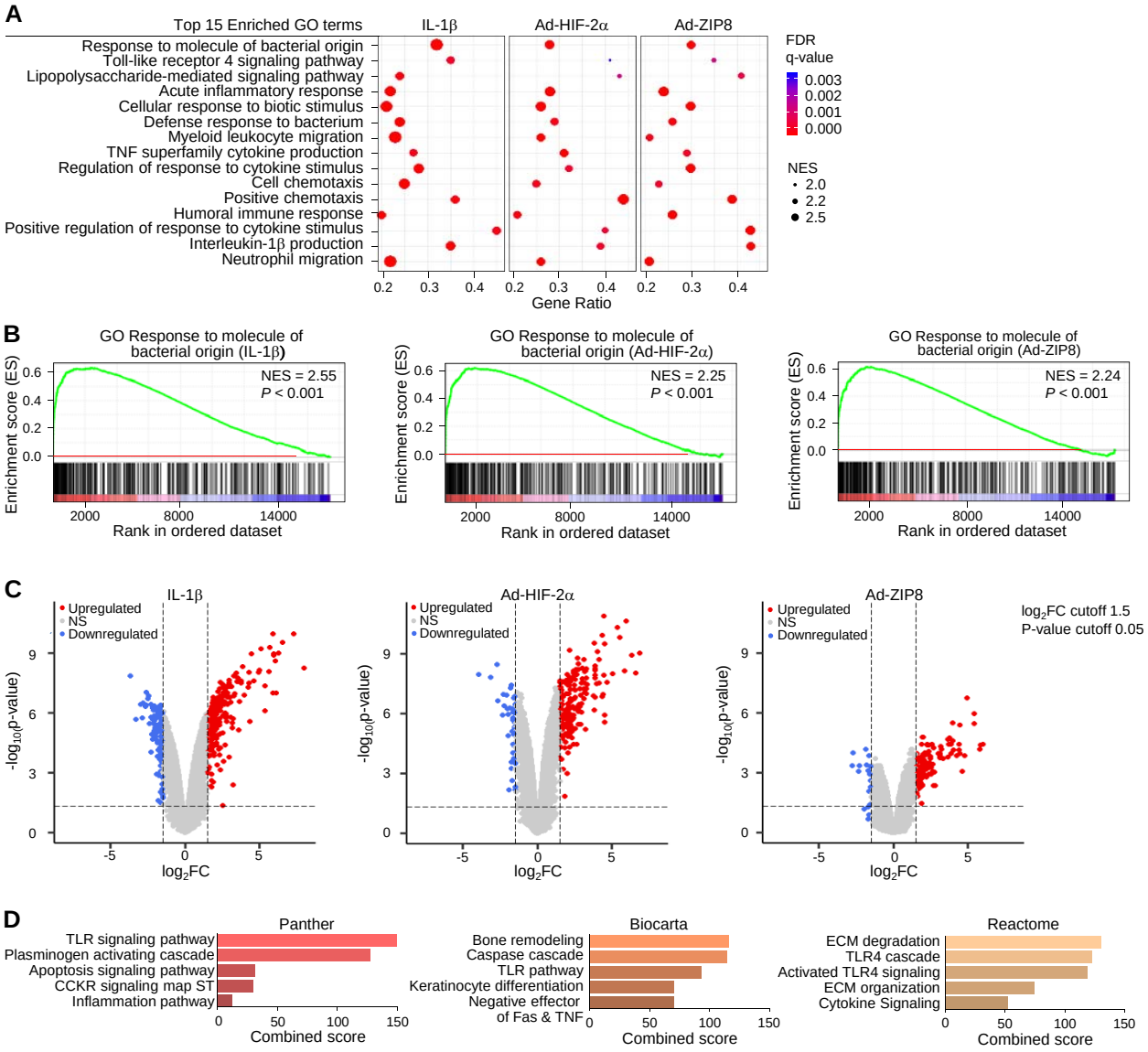
Supplementary Materials

LBP and CD14, cofactors of toll-like receptors, are essential for low-grade inflammation induced exacerbation of post-traumatic osteoarthritis

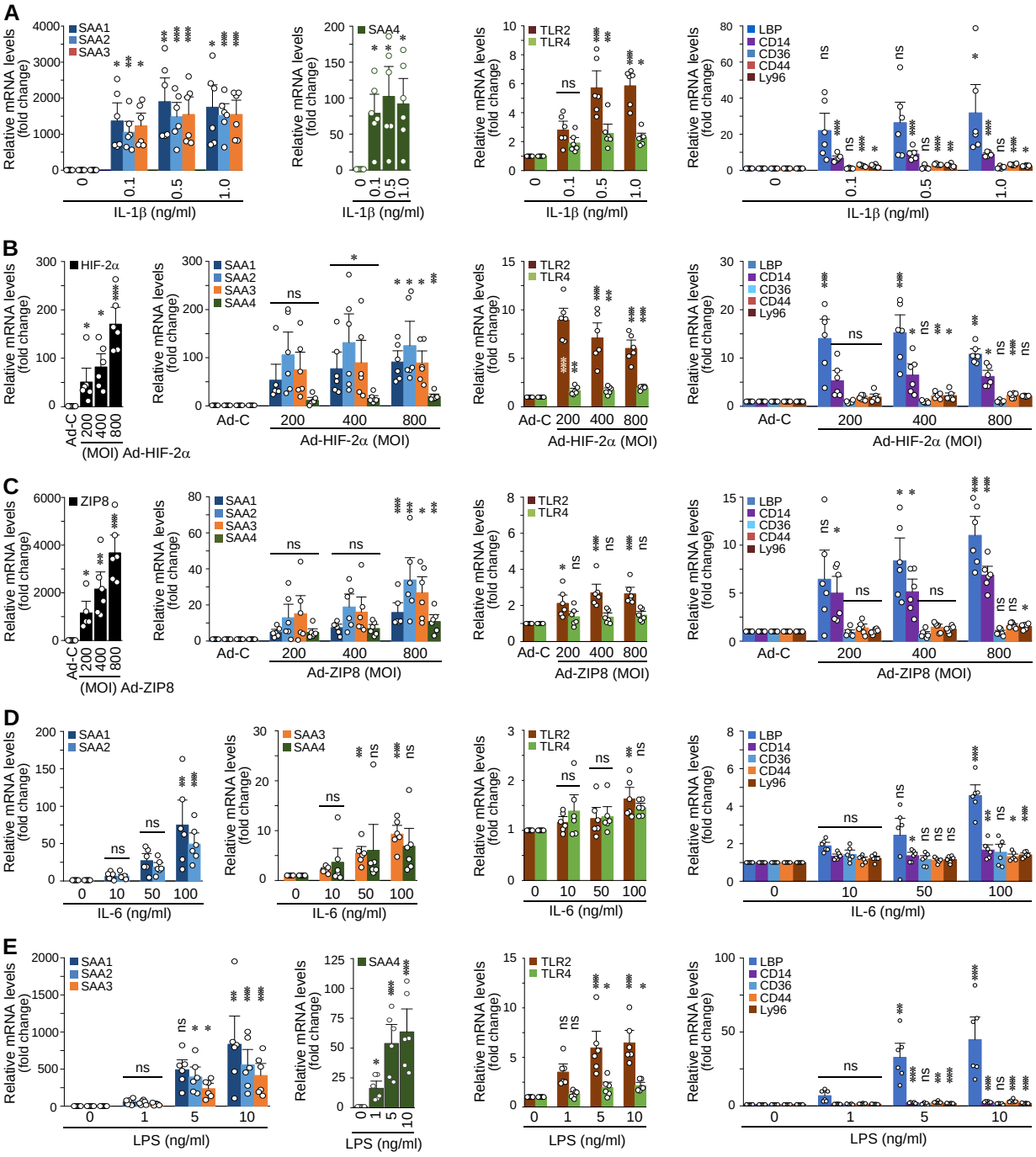
Yoonkyung Won, Jeong-In Yang, Seulki Park, Jang-Soo Chun

Supplementary Figures 1–9

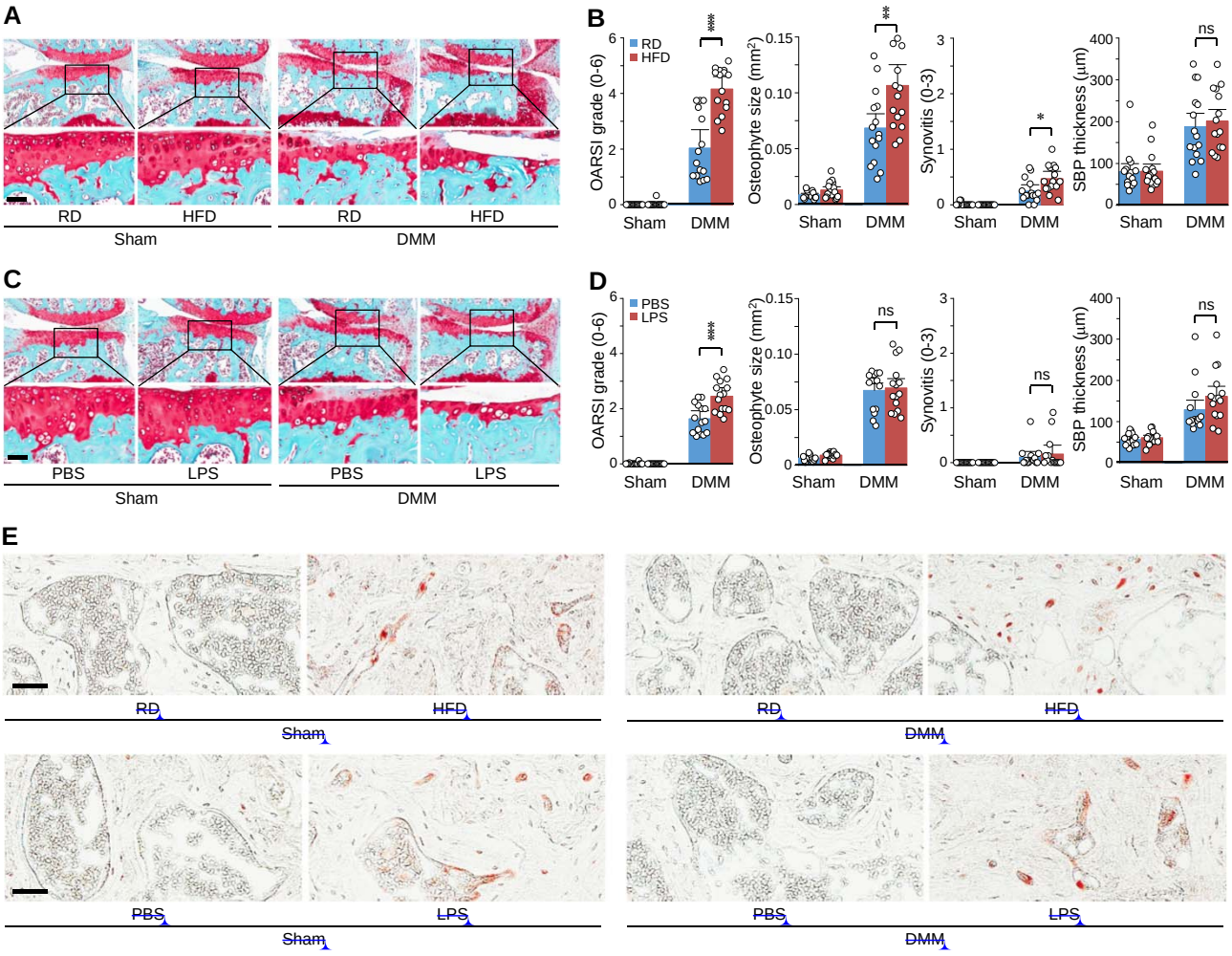
Supplementary Tables 1,2



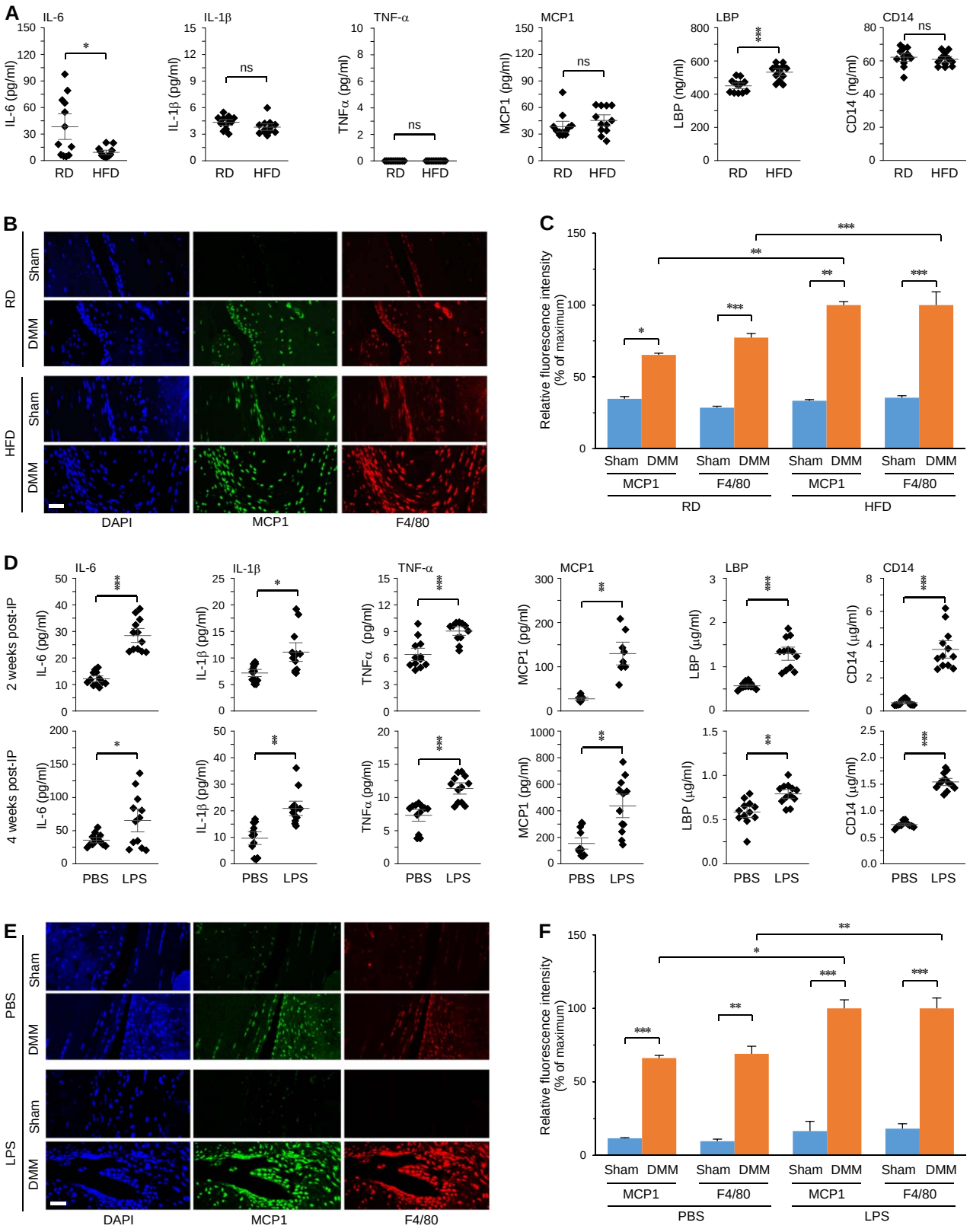
Supplementary Figure 1. Transcriptional profiling of mouse chondrocytes treated with OA-associated pathogenic catabolic mediators. Microarray data were obtained from primary-culture mouse articular chondrocytes that had been treated for 36 h with IL-1β (1 ng/ml) or infected with 800 MOI of Ad-C, Ad-HIF-2α, or Ad-ZIP8. **A**, Enrichment plot of the top 15 most highly enriched gene ontology (GO) biological process terms with normalized enrichment scores (NES). **B**, GSEA of microarray signals on GO biological processes. **C**, Volcano plots for upregulated and downregulated genes. $P < 0.05$ and absolute value of $\log_2$(fold change) > 1.5 were applied as threshold values. Upregulated gene transcripts are shown as red dots, downregulated genes are shown as blue dots, and genes with non-significant (NS) changes in expression levels are shown as gray dots. **D**, Five top-ranked annotations from Enrichr pathway analysis of the genes found to be upregulated in OA chondrocytes in the Panther, Biocarta, or Reactome pathway databases, and the corresponding combined scores are presented as bar graphs. The combined score is a representative parameter of the significance of the enrichment provided by Enrichr.



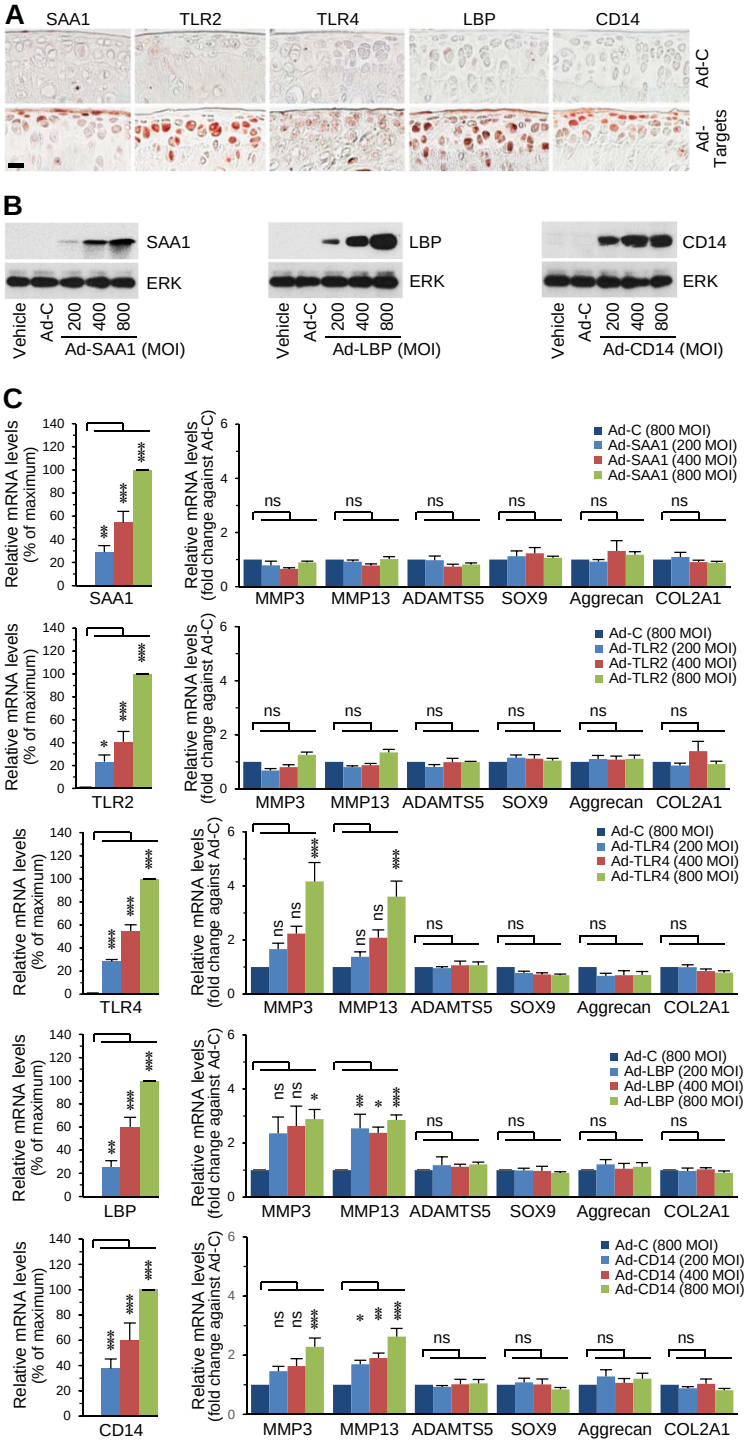
Supplementary Figure 2. Upregulation of TLR signaling components in chondrocytes stimulated with OA-associated catabolic signaling. **A**–**E**, Primary-culture mouse articular chondrocytes were treated for 36 h with the indicated concentrations of IL-1β (**A**), Ad-HIF2α (**B**), Ad-ZIP8 (**C**), IL-6 (**D**), or LPS (**E**). Relative mRNA levels (fold change against control) of the indicated molecules were determined by qRT-PCR analysis (n = 6). Means ± s.e.m.; assessed by one-way ANOVA with *post-hoc* Bonferroni test. * = $P < 0.05$, ** = $P < 0.005$, *** = $P < 0.001$. ns, not significant.



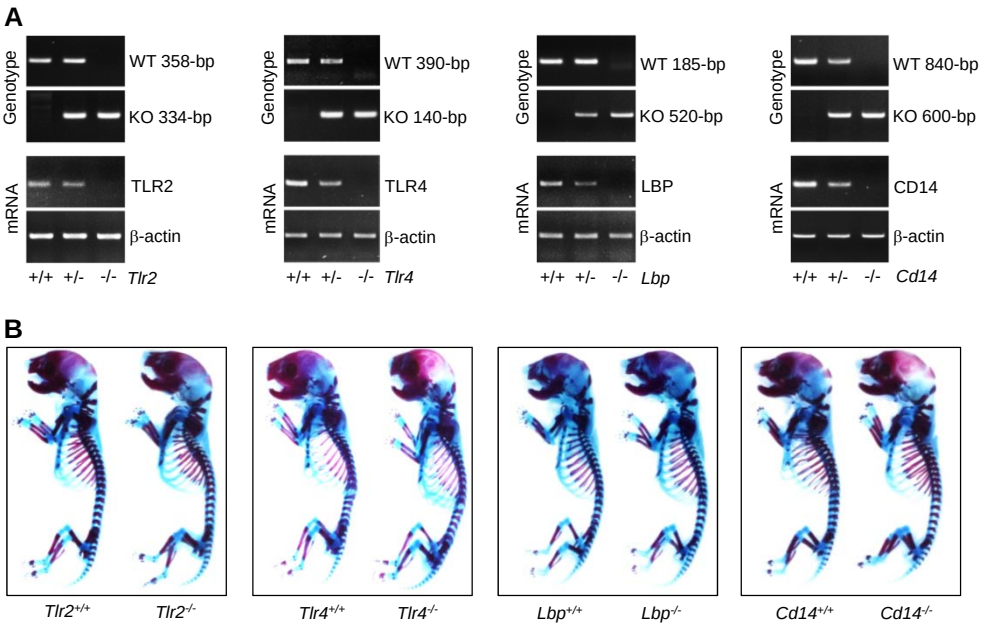
Supplementary Figure 3. HFD and metabolic endotoxemia enhance DMM-induced post-traumatic OA in mice. **A** and **B**, Mice were fed with RD or HFD, subject to DMM surgery or sham operation (n = 15 mice per group), and sacrificed at 6 weeks after the operation. Presented are representative Safranin-O staining images of joint sections (**A**) and scoring of OARSI grade, osteophyte size, synovitis, and SBP thickness (**B**). **C** and **D**, Sham- or DMM-operated mice were administrated with PBS or LPS (n = 15 mice per group) for 4 weeks to induce metabolic endotoxemia and sacrificed at 8 weeks post-surgery. Presented are representative Safranin-O staining images of joint sections (**C**) and scoring of OA manifestations (**D**). **E**, Representative immunostaining images of Osterix in subchondral bone plate of the indicated mice. Values are means ± 95% CI with Mann-Whitney *U* test for OARSI grade and synovitis; means ± s.e.m.; assessed by one-way ANOVA with *post-hoc* Bonferroni test for osteophyte size and SBP thickness. * = $P < 0.05$, ** = $P < 0.005$, *** = $P < 0.001$. ns, not significant. Scale bars, 50 µm.


A
IL-6
IL-1β
TNF-α
MCP1
LBP
CD14
IL-6 (pg/ml)
IL-1β (pg/ml)
TNFα (pg/ml)
MCP1 (pg/ml)
LBP (ng/ml)
CD14 (ng/ml)
RD
HFD
ns
B
RD
HFD
Sham
DMM
DAPI
MCP1
F4/80
C
Relative fluorescence intensity (% of maximum)
Sham DMM Sham DMM Sham DMM Sham DMM
MCP1
F4/80
MCP1
F4/80
RD
HFD
D
2 weeks post-IP
4 weeks post-IP
IL-6 (pg/ml)
IL-1β (pg/ml)
TNFα (pg/ml)
MCP1 (pg/ml)
LBP (μg/ml)
CD14 (μg/ml)
PBS
LPS
E
PBS
LPS
Sham
DMM
DAPI
MCP1
F4/80
F
Relative fluorescence intensity (% of maximum)
Sham DMM Sham DMM Sham DMM Sham DMM
MCP1
F4/80
MCP1
F4/80
PBS
LPS

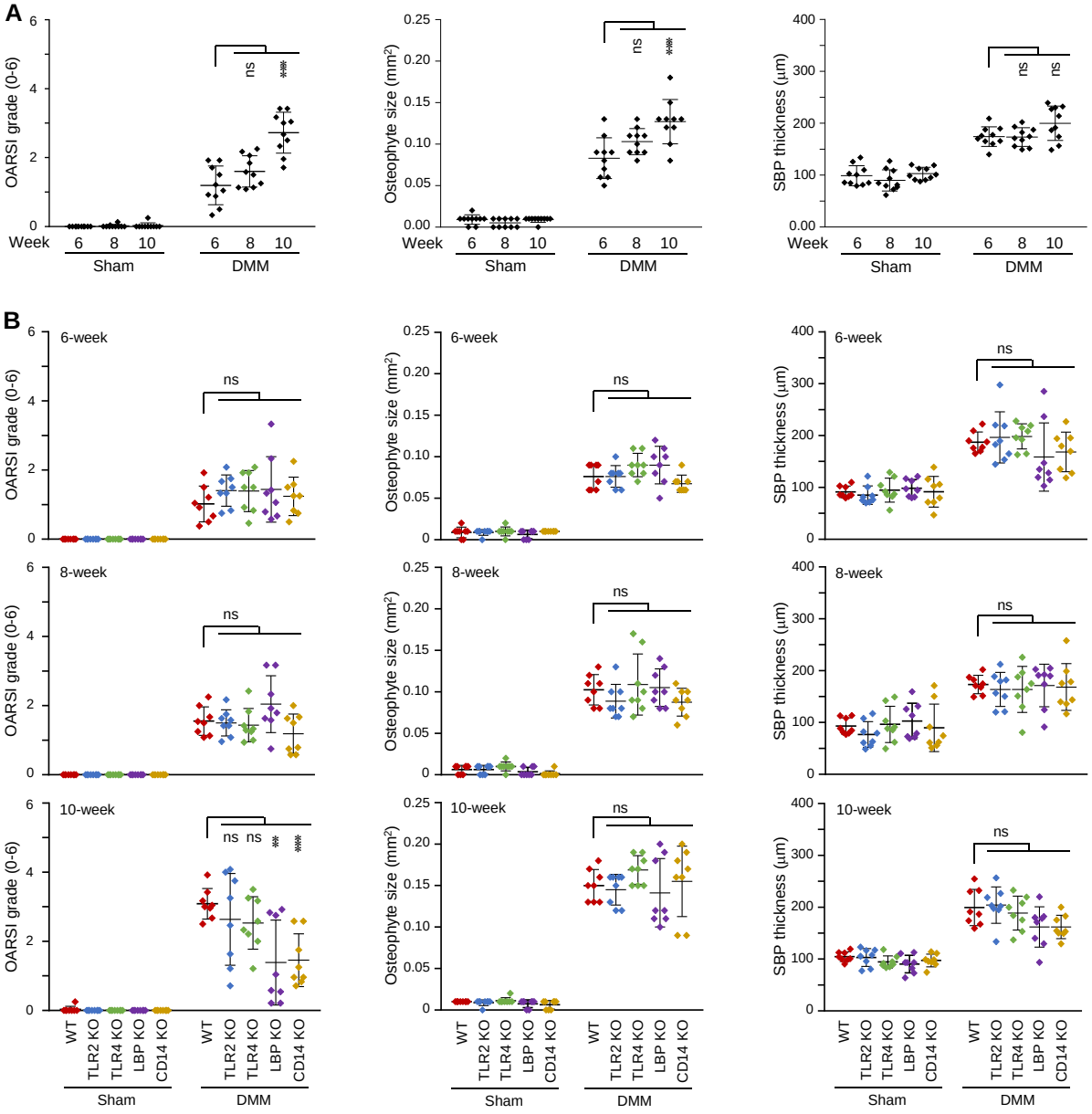
Supplementary Figure 4. Inflammatory responses in mice with HFD feeding or metabolic endotoxemia. **A**–**C**, Mice were fed with RD or HFD, subject to DMM surgery or sham operation, and sacrificed at 6 weeks after the operation. Concentrations of the indicated proteins in the sera were determined by ELISA (A, n = 12 mice per group). Synovial infiltration of MCP1- or F4/80-positive cells was determined by immunofluorescence microscopy (B, n = 4 mice per group) and quantified by using Image J software (C, n = 4 mice per group). **D**–**F**, Sham- or DMM-operated mice were administrated with PBS or LPS for 4 weeks and sacrificed at 8 weeks post-surgery. Serum concentrations of the indicated proteins at 2 or 4 weeks post-IP injection were determined by ELISA (D, n = 12 mice per group). Synovial infiltration of MCP1- or F4/80-positive cells was determined by immunofluorescence microscopy (B, n = 4 mice per group) and quantified by using Image J software (C, n = 4 mice per group) at 8 weeks post-DMM surgery. Values are presented as means ± s.e.m. and were assessed by two-tailed *t*-test. Scale bars, 50 μm.



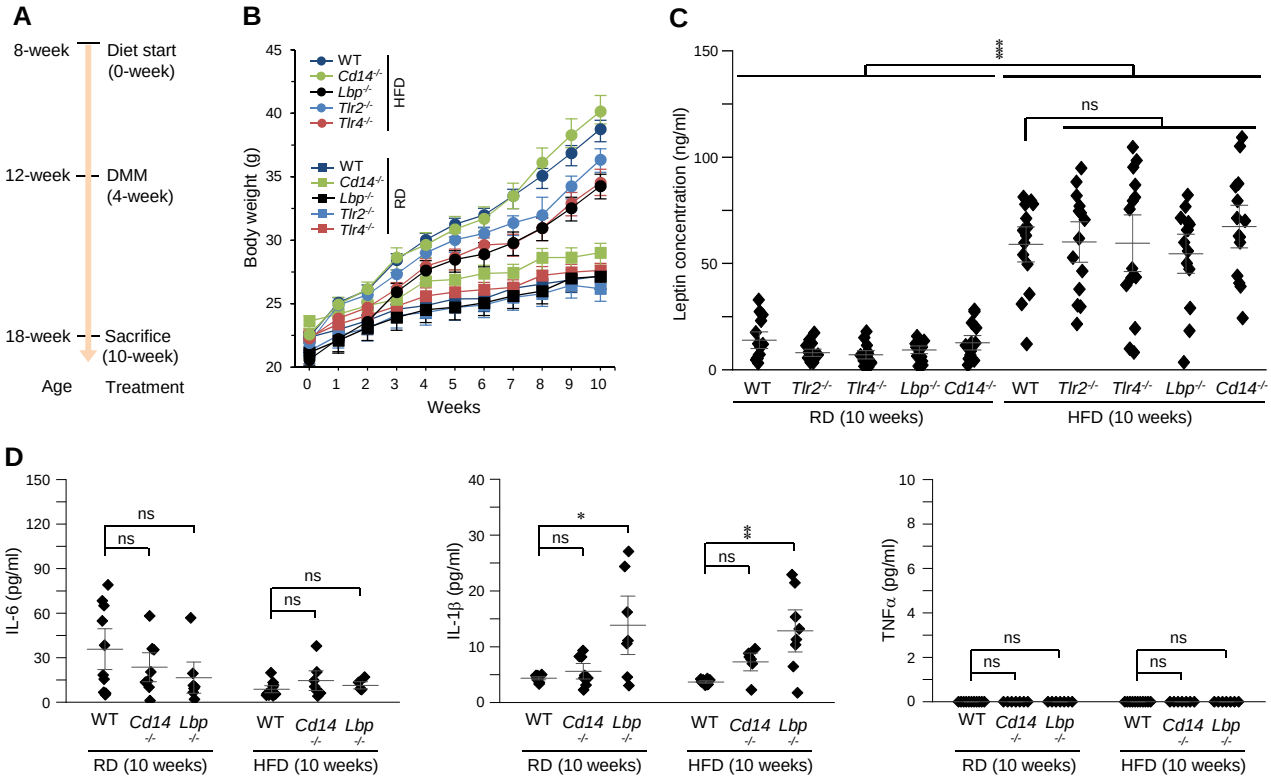
Supplementary Figure 5. Effect of overexpressing TLR signaling components in knee joint cartilage and primary-culture mouse articular chondrocytes. **A**, Representative immunostaining images of SAA1, TLR2, TLR4, LBP, and CD14 in knee-joint cartilage sections of mice that underwent IA injection (1×10^9 PFU in 10 μl) of Ad-C, Ad-SAA1, Ad-TLR2, Ad-TLR4, Ad-LBP, or Ad-CD14 once weekly for 3 weeks ($n \geq 5$ mice per group). **B**, Western blot analysis of SAA1, LBP, and CD14 secreted by primary-culture mouse articular chondrocytes infected with Ad-C (800 MOI) or the indicated MOIs of Ad-SAA1, Ad-LBP, or Ad-CD14 for 36 h ($n \geq 5$). **C**, Relative mRNA levels of matrix-degrading enzymes and cartilage ECM molecules in chondrocytes infected with the indicated MOI of Ad-C, Ad-SAA1, Ad-TLR2, Ad-TLR4, Ad-LBP, or Ad-CD14 for 36 h ($n = 7$). Means ± s.e.m..; assessed by one-way ANOVA with *post-hoc* Bonferroni test. * = $P < 0.05$, ** = $P < 0.005$, *** = $P < 0.001$. ns, not significant.



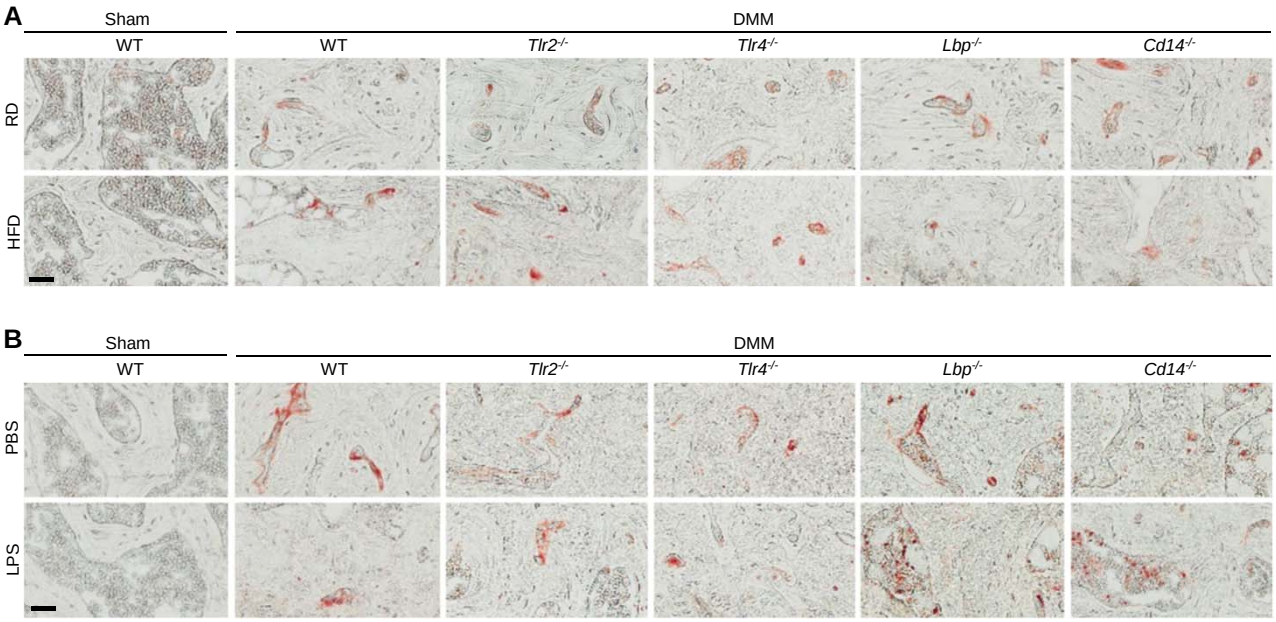
Supplementary Figure 6. Characterization of KO mice. **A**, Genotypes and mRNA levels of the KO-target genes in TLR2, TLR4, LBP, or CD14 heterozygous (+/-) and homozygous (-/-) KO mice and their WT (+/+) littermates ($n \geq 3$ mice per group). **B**, Representative skeletal staining images of E18.5 embryos of TLR2, TLR4, LBP, or CD14 homozygous (-/-) KO mice and their WT littermates ($n \geq 3$ mice per group).



Supplementary Figure 7. Characterization of DMM-induced post-traumatic OA in WT mice and KO mice of the indicated TLR signaling components. **A**, WT mice were subject to sham or DMM operation, and sacrificed at the indicated weeks after the operation. Presented are scoring of OARSI grade, osteophyte size, and SBP thickness (n = 10 mice per group). **B**, WT, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, *Lbp*$^{-/-}$, or *Cd14*$^{-/-}$ mice were subject to sham or DMM operation, and sacrificed at the indicated weeks after the operation. Presented are scoring of OARSI grade, osteophyte size, and SBP thickness (n = 8 mice per group). Means ± 95% CI; assessed by Mann-Whitney *U* test for OARSI grade or means ± s.e.m.; assessed by one-way ANOVA with *post-hoc* Bonferroni test for osteophyte size and SBP thickness. * = $P < 0.05$, ** = $P < 0.005$, *** = $P < 0.001$. ns, not significant.



Supplementary Figure 8. Characterization of body weight and serum levels of inflammatory mediators in DMM-operated WT and KO mice fed with RD or HFD. **A**, Schematic illustration of HFD feeding schedule in mice. **B**, Body weights of the indicated mice fed with RD or HFD (n = 15 mice per group). C, Serum concentrations of leptin in WT and the indicated KO mice (n = 14 mice per group). D. Serum concentrations of the indicated inflammatory mediators in WT, *Cd14*$^{-/-}$, and *Lbp*$^{-/-}$ mice (n = 6~8 mice per group). Values are means ± s.e.m.; assessed by one-way ANOVA with *post-hoc* Bonferroni test. * = $P < 0.05$, ** = $P < 0.005$, *** = $P < 0.001$. ns, not significant.



Supplementary Figure 9. Osterix expression in subchondral bone of WT and KO mice. **A**, WT and *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, *Lbp*$^{-/-}$, or *Cd14*$^{-/-}$ KO mice were fed with RD or HFD, subject to sham or DMM operation, and sacrificed 6 weeks after the operation. Presented are representative Osterix immunostaining images from subchondral bone of joint sections (n = 10 mice per group). **B**, Sham- or DMM-operated WT, *Tlr2*$^{-/-}$, *Tlr4*$^{-/-}$, *Lbp*$^{-/-}$, or *Cd14*$^{-/-}$ mice were treated with PBS or a subclinical low-dose of LPS for 4 weeks, and sacrificed 8 weeks after the surgery. Presented are representative Osterix staining images from subchondral bone of joint sections (n = 10 mice per group). Scale bars, 50 μm.

Supplementary Table 1. Sequences of primers.

Target (mouse)		Sequences	Purpose
ADAMTS5		S 5′-GCCATTGTAATAACCCTGCACC-3′ AS 5′-TCAGTCCCATCCGTAACCTTTG-3′	RT-PCR
Aggrecan		S 5′-CTGTCTTTGTCACCCACACAT-3′ AS 5′-GAAGACGACATCACCATCCAG-3′	RT-PCR
β-Actin		S 5′-ATATCGCTGCGCTGGTCGTC-3′ AS 5′-AGGATGGCGTGAGGGAGAGC-3′	RT-PCR
CD14	WT Mutant Common	S 5′-CCAAGTTTTAGCGCTGCGTAAC-3′ S 5′-CCGCTTCCATTGCTCAGCGG-3′ AS 5′-GCCAGCCAAGGATACATAGCC-3′	Genotyping
CD14		S 5′-AATTGGGCGAGAGAGGACTGAT-3′ AS 5′-AGCATCCCGCAGTGAATTGTGA-3′	RT-PCR
CD36		S 5′-TGTGGCTAAATGAGACTGGGAC-3′ AS 5′-CACACACCACCATTTCTTCTCC-3′	RT-PCR
CD44		S 5′-GAGGGCACAAGAAGAGTCATAC-3′ AS 5′-CCAAGGCAAAGAGAAGGAAAGC-3′	RT-PCR
COL2A1		S 5′-CACACTGGTAAGTGGGGCAAGACCG-3′ AS 5′-GGATTGTGTTGTTTCAGGGTTCGGG-3′	RT-PCR
HIF-2α (*Epas1*)		S 5′-CGAGAAGAACGACGTGGTGTTC-3′ AS 5′-GTGAAGGCTGGCAGGCTCC-3′	RT-PCR
LBP	WT Mutant Common	S 5′-AGAGGTTTCCAGGTCCCACT-3′ S 5′-CGTCCTGCAGTTCATTCAG-3′ AS 5′-CCTCAGTTTTTCCTTGTCTGC-3′	Genotyping
LBP		S 5′-CCCTTCACTCCCCAGATATACA-3′ AS 5′-GATCAGTCTCACTTGTGCCTTG-3′	RT-PCR
LBP		S 5′-GGACCTCTGCTCTCTACATTGC-3′ AS 5′-GCTGGTGGCTCTGCAGAGAGA-3′	RT-PCR (KO)
MD-2 (*Ly96*)		S 5′-GATGGTCTTCCTGGCGAGTTTA-3′ AS 5′-GAGGAGTTGCAGAACCACTGTT-3′	RT-PCR
MMP3		S 5′-TCCTGATGTTGGTGGCTTCAG-3′ AS 5′-TGTCTTGGCAAATCCGGTGTA-3′	RT-PCR
MMP13		S 5′-TGATGGACCTTCTGGTCTTCTGG-3′ AS 5′-CATCCACATGGTTGGGAAGTTCT-3′	RT-PCR
TLR2		S 5′-CAAACTGGAGACTCTGGAAGCA-3′ AS 5′-GCACCTACGAGCAAGATCAACA-3′	RT-PCR (mRNA/KO)
TLR2	WT Mutant Common	S 5′-ACGAGCAAGATCAACAGGAGA-3′ S 5′-GGGCCAGCTCATTCCTCCCAC-3′ AS 5′-CTTCCTGAATTTGTCCAGTACA-3′	Genotyping
TLR4		S 5′-TGAGGACTGGGTGAGAAATGAG-3′ AS 5′-ATACAATTCCACCTGCTGCCTC-3	RT-PCR (mRNA/KO)
TLR4	WT Mutant	S 5′-ATATGCATGATCAACACCACAG-3′ AS 5′-TTTCCATTGCTGCCCTATAG-3′ S 5′-GCAAGTTTCTATATGCATTCTC-3′ AS 5′-CCTCCATTTCCAATAGGTAG-3′	Genotyping

SAA1 (human)	S 5′-TGGAGAGCCTACTCTGACATGA-3′ AS 5′-CACCATGGCCAAAGAATCTC-3	RT-PCR
SAA1	S 5′-ATGAAGGAAGCTAACTGGAAAAACTC-3′ AS 5′-TCCTCCTCAAGCAGTTACTACTGCAA-3′	RT-PCR
SAA2	S 5′-ATGAAGGAAGCTGGCTGGAAAGATGG-3′ AS 5′-TCCTCCTCAAGCAGTTACTACTGCTC-3′	RT-PCR
SAA3	S 5′-GCCACCATGAAGCCTTCCATTGCCATCATT-3′ AS 5′-TCAGTATCTTTTAGGCAGGCCAGCAG-3′	RT-PCR
SAA4	S 5′-GTTCTTTGTTCCTGGGAGTCAG-3′ AS 5′-GTTTAGGAGGCCCTGGAAGTAT-3′	RT-PCR
SOX9	S 5′-ATGCTATCTTCAAGGCGCTG-3′ AS 5′-GACGTCGAAGGTCTCAATGT-3′	RT-PCR
ZIP8	S 5′-GAACAATTGCCTGGATGATCACGC-3′ AS 5′-AAGCCGGTTAACATCCCTGCATTC-3′	RT-PCR

S, sense; AS, antisense

Supplementary Table 2. mRNA levels of TLR signaling components in chondrocytes treated with IL-1β (1 ng/ml, 36 h) or infected with 800 MOI (36 h) of Ad-HIF-2α or Ad-ZIP8. Mean ± s.e.m. (n = 4).

Group		Gene symbol	Definition	Fold change		
				IL-1β	Ad-HIF-2α	Ad-ZIP8
TLRs		*Tlr1*	Toll-like receptor 1	0.93 ± 0.15	0.92 ± 0.14	0.84 ± 0.12
		Tlr2	Toll-like receptor 2	2.83 ± 0.49	2.62 ± 0.48	2.83 ± 0.47
		Tlr3	Toll-like receptor 3	2.20 ± 0.72	1.96 ± 0.56	1.75 ± 0.48
		Tlr4	Toll-like receptor 4	1.65 ± 0.17	2.00 ± 0.20	2.03 ± 0.26
		Tlr5	Toll-like receptor 5	1.66 ± 0.21	0.93 ± 0.14	0.76 ± 0.07
		Tlr6	Toll-like receptor 6	1.15 ± 0.23	0.87 ± 0.14	0.80 ± 0.14
		Tlr7	Toll-like receptor 7	1.03 ± 0.09	0.94 ± 0.24	0.91 ± 0.22
		Tlr8	Toll-like receptor 8	1.02 ± 0.12	1.11 ± 0.19	1.13 ± 0.22
		Tlr9	Toll-like receptor 9	0.95 ± 0.18	1.03 ± 0.12	0.85 ± 0.07
		Tlr10	Toll-like receptor 10	-	-	-
		Tlr11	Toll-like receptor 11	0.97 ± 0.12	1.04 ± 0.31	0.90 ± 0.14
		Tlr12	Toll-like receptor 12	1.10 ± 0.15	0.94 ± 0.07	0.91 ± 0.19
		Tlr13	Toll-like receptor 13	0.85 ± 0.15	1.13 ± 0.20	1.18 ± 0.23
Accessory molecules		*Lbp*	LPS binding protein	5.27 ± 2.14	23.06 ± 4.36	22.3 ± 4.37
		Cd14	CD14 antigen	3.19 ± 1.12	3.65 ± 0.76	2.69 ± 0.63
		Ly96	Lymphocyte antigen 96	2.35 ± 0.42	2.71 ± 0.73	1.97 ± 0.48
		Cd36	CD36 antigen	1.01 ± 0.15	1.01 ± 0.15	0.96 ± 0.14
		Cd44	CD44 antigen	2.59 ± 0.19	2.62 ± 0.29	2.36 ± 0.36
		Hmgb1	High mobility group box 1	0.91 ± 0.09	0.99 ± 0.05	1.06 ± 0.06
		Camp	Cathelicidin antimicrobial peptide	1.62 ± 0.24	1.72 ± 0.50	1.55 ± 0.36
		Tril	TLR4 interactor leucine-rich repeats	0.96 ± 0.27	0.86 ± 0.19	0.88 ± 0.18
		Grn	Granulin	1.11 ± 0.10	1.18 ± 0.15	1.16 ± 0.15
		Hsp90b1	Heat shock protein 90	1.04 ± 0.06	1.01 ± 0.03	0.98 ± 0.04
		Cnpy3	Canopy 3 homolog	1.00 ± 0.04	1.00 ± 0.06	1.08 ± 0.08
		Unc93b1	Unc-93 homolog B1	1.65 ± 0.26	1.10 ± 0.16	1.07 ± 0.15
TLR agonists: fatty acids, lipoproteins	Amyloid -β	*Apbb1ip*	~~Amyloid beta (A4) precursor protein binding, family A, member 1~~	0.80 ± 0.08	1.13 ± 0.11	0.90 ± 0.09
		Apba1	Amyloid beta (A4) precursor ~~protein-binding, family A, member 2~~	0.97 ± 0.08	0.99 ± 0.13	0.98 ± 0.12
		Apbb3	Amyloid beta (A4) precursor ~~protein-binding, family B, member 1~~	1.06 ± 0.08	0.84 ± 0.12	0.81 ± 0.08
		Apba2	Amyloid beta (A4) precursor ~~protein-binding, family B, member 2~~	1.03 ± 0.04	0.93 ± 0.09	0.96 ± 0.06
		Aplp2	Amyloid beta (A4) precursor ~~protein-binding, family B, member 3~~	1.02 ± 0.21	1.01 ± 0.07	0.93 ± 0.12
		Aplp1	~~Amyloid beta (A4) precursor protein~~	1.07 ± 0.03	0.85 ± 0.05	0.93 ± 0.05
		Apbb2	~~Amyloid beta (A4) precursor-like protein 1~~	1.21 ± 0.07	0.93 ± 0.06	0.92 ± 0.06
		Appbp2	~~Amyloid beta (A4) precursor-like protein 2~~	1.16 ± 0.04	0.96 ± 0.06	1.03 ± 0.09
		App	~~Amyloid beta (A4) precursor protein-binding, family B~~	0.82 ± 0.13	0.93 ± 0.17	1.00 ± 0.17
		Iapp	Islet amyloid polypeptide	0.94 ± 0.07	0.99 ± 0.14	1.03 ± 0.10

	Oxidized LDL	*Apob*	Apolipoprotein B	1.06 ± 0.16	1.05 ± 0.14	1.17 ± 0.26
		Apobec1	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 1	0.90 ± 0.17	0.99 ± 0.23	1.04 ± 0.19
		Apobec2	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 2	0.93 ± 0.15	0.83 ± 0.22	0.91 ± 0.23
		Apobec3	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide 3	1.73 ± 0.19	1.35 ± 0.22	1.23 ± 0.22
		Loxhd1	Lipoxygenase homology domains 1	1.01 ± 0.11	1.02 ± 0.18	1.01 ± 0.18
		Mpo	Myeloperoxidase	0.79 ± 0.16	1.15 ± 0.30	1.17 ± 0.24
	Serum amyloid A	*Saa1*	Serum amyloid A 1	49.92 ± 9.69	61.55 ± 22.96	29.49 ± 12.18
		Saa2	Serum amyloid A 2	60.63 ± 3.66	104.4 ± 45.45	60.92 ± 27.31
		Saa3	Serum amyloid A 3	44.84 ± 18.76	21.49 ± 7.88	20.66 ± 7.59
		Saa4	Serum amyloid A 4	19.82 ± 3.63	14.71 ± 3.71	13.05 ± 2.90
		Apcs	Serum amyloid P-component	0.95 ± 0.05	0.95 ± 0.14	0.86 ± 0.13
TLR agonists: peptides, proteins	Fibrinogen	*Fga*	Fibrinogen alpha chain	0.98 ± 0.17	1.00 ± 0.09	1.01 ± 0.13
		Fgb	Fibrinogen beta chain	0.98 ± 0.14	0.92 ± 0.11	1.01 ± 0.14
		Fgg	Fibrinogen gamma chain	1.03 ± 0.08	1.02 ± 0.13	0.91 ± 0.17
		Fibcd1	Fibrinogen C domain containing 1	0.92 ± 0.15	0.70 ± 0.13	0.84 ± 0.09
		Fgl1	Fibrinogen-like protein 1	1.50 ± 0.17	1.33 ± 0.16	1.45 ± 0.35
		Fsbp	fibrinogen silencer binding protein	1.00 ± 0.07	1.14 ± 0.11	1.15 ± 0.09
	S100	*S100a8*	S100 calcium binding protein A8	1.35 ± 0.50	0.94 ± 0.23	1.02 ± 0.16
		S100a9	S100 calcium binding protein A9	0.92 ± 0.24	1.09 ± 0.25	1.09 ± 0.21
TLR down-stream signaling molecules	TIRAP	*Tirap*	Toll-interleukin 1 receptor (TIR) domain-containing adaptor protein	1.01 ± 0.07	0.99 ± 0.11	0.96 ± 0.1
	MyD88	*Myd88*	Myeloid differentiation primary response gene 88	1.11 ± 0.23	1.61 ± 0.21	1.62 ± 0.25
	IRAK	*Irak1*	IL-1 receptor-associated kinase 1	1.26 ± 0.04	1.01 ± 0.06	1.02 ± 0.07
		Irak2	IL-1 receptor-associated kinase 2	1.68 ± 0.16	1.71 ± 0.33	1.30 ± 0.18
		Irak3	IL-1 receptor-associated kinase 3	6.45 ± 0.99	5.73 ± 0.96	4.04 ± 0.69
		Irak4	IL-1 receptor-associated kinase 4	1.14 ± 0.09	1.04 ± 0.11	1.02 ± 0.09
		Irak1bp1	IL-1 receptor-associated kinase 1 binding protein 1	1.46 ± 0.16	1.27 ± 0.12	1.23 ± 0.13
	TRAF family	*Traf1*	TNF receptor-associated factor 1	1.45 ± 0.18	1.34 ± 0.34	1.12 ± 0.26
		Traf2	TNF receptor-associated factor 2	1.51 ± 0.07	1.59 ± 0.13	1.56 ± 0.15
		Traf3	TNF receptor-associated factor 3	1.03 ± 0.11	1.09 ± 0.06	1.04 ± 0.13
		Traf4	TNF receptor-associated factor 4	1.12 ± 0.07	0.79 ± 0.11	0.87 ± 0.10
		Traf5	TNF receptor-associated factor 5	0.84 ± 0.11	1.01 ± 0.10	0.86 ± 0.05
		Traf6	TNF receptor-associated factor 6	1.10 ± 0.13	0.94 ± 0.12	0.88 ± 0.13
		Traf7	TNF receptor-associated factor 7	1.21 ± 0.05	1.06 ± 0.06	1.00 ± 0.06
		Trafd1	TRAF Zn finger domain containing 1	1.06 ± 0.08	0.85 ± 0.06	0.93 ± 0.06
		Traf3ip1	TRAF3 interacting protein 1	1.01 ± 0.06	1.11 ± 0.12	1.03 ± 0.08
		Traf3ip2	TRAF3 interacting protein 2	2.83 ± 0.25	2.76 ± 0.33	2.02 ± 0.24
	TAK1	*Map3k7*	Mitogen-activated protein kinase kinase kinase 7	1.09 ± 0.11	1.06 ± 0.03	1.03 ± 0.03
	TAB	*Tab1*	TGF-beta activated kinase 1/MAP3K7 binding protein 1	0.74 ± 0.11	0.87 ± 0.12	0.94 ± 0.09

	Tab2	TGF-beta activated kinase 1/MAP3K7 binding protein 2	0.78 ± 0.11	0.76 ± 0.06	0.81 ± 0.05
	Tab3	TGF-beta activated kinase 1/MAP3K7 binding protein 3	1.06 ± 0.03	1.01 ± 0.11	0.90 ± 0.08
TOLLIP	*Tollip*	Toll interacting protein	0.94 ± 0.11	1.05 ± 0.15	1.00 ± 0.18
RIP	*Ripk1*	Receptor-interacting serine-threonine kinase 1	1.10 ± 0.10	1.06 ± 0.05	1.04 ± 0.06
	Ripk2	Receptor-interacting serine-threonine kinase 2	1.99 ± 0.28	1.39 ± 0.12	1.47 ± 0.15
	Ripk3	Receptor-interacting serine-threonine kinase 3	1.76 ± 0.13	2.15 ± 0.67	1.76 ± 0.51
	Ripk4	Receptor-interacting serine-threonine kinase 4	1.00 ± 0.14	1.00 ± 0.13	1.02 ± 0.11
PI3K	*Pik3ca*	PI3-kinase, catalytic, alpha polypeptide	1.01 ± 0.07	1.05 ± 0.06	0.97 ± 0.04
	Pik3cb	PI3-kinase, catalytic, beta polypeptide	0.67 ± 0.08	0.73 ± 0.08	0.80 ± 0.07
	Pik3r2	PI3-kinase, regulatory subunit, polypeptide 2 (p85 beta)	0.94 ± 0.08	0.95 ± 0.06	0.96 ± 0.06
	Pik3r1	PI3-kinase, regulatory subunit, polypeptide 1 (p85 alpha)	2.24 ± 0.30	1.44 ± 0.11	1.52 ± 0.16
	Pik3c2a	PI3-kinase, C2 domain containing, alpha polypeptide	1.25 ± 0.27	1.15 ± 0.12	1.04 ± 0.12
	Pik3c2g	PI3-kinase, C2 domain containing, gamma polypeptide	0.99 ± 0.24	0.93 ± 0.16	0.94 ± 0.09
	Smg1	SMG1 homolog, PI3-kinase-related kinase	1.12 ± 0.07	0.96 ± 0.17	0.96 ± 0.15
IKKs	*Chuk*	Conserved helix-loop-helix ubiquitous kinase	0.87 ± 0.12	0.78 ± 0.07	0.81 ± 0.11
	Ikbkb	Inhibitor of kappaB kinase beta	1.16 ± 0.16	1.03 ± 0.11	0.97 ± 0.12
	Ikbkg	Inhibitor of kappaB kinase gamma	1.07 ± 0.05	1.01 ± 0.13	1.02 ± 0.07
	Ikbke	Inhibitor of kappaB kinase epsilon	2.03 ± 0.19	1.66 ± 0.31	1.60 ± 0.36
NF-κB	*Rela*	v-rel reticuloendotheliosis viral oncogene homolog A (avian)	1.39 ± 0.14	1.17 ± 0.11	1.04 ± 0.10
	Relb	Avian reticuloendotheliosis viral (v-rel) oncogene related B	1.59 ± 0.18	1.43 ± 0.11	1.45 ± 0.13
	Rel	Reticuloendotheliosis oncogene	1.88 ± 0.43	1.51 ± 0.15	1.30 ± 0.10
	Nfkb1	NFκ light polypeptide gene enhancer in B cells 1, p105	1.66 ± 0.09	2.23 ± 0.16	1.97 ± 0.15
	Nfkb2	NFκ light polypeptide gene enhancer in B cells 2, p49/p100	1.51 ± 0.12	1.58 ± 0.08	1.50 ± 0.10
NF-κB inhibitor	*Nfkbia*	NFκ light polypeptide gene enhancer in B cells inhibitor, alpha	5.10 ± 0.13	2.44 ± 0.33	2.27 ± 0.33
	Nfkbib	NFκ light polypeptide gene enhancer in B cells inhibitor, beta	0.85 ± 0.10	1.01 ± 0.05	1.05 ± 0.08
	Nfkbid	NFκ light polypeptide gene enhancer in B cells inhibitor, delta	1.13 ± 0.11	1.44 ± 0.32	1.47 ± 0.37
	Nfkbiz	NFκ light polypeptide gene enhancer in B cells inhibitor, zeta	9.78 ± 0.88	6.00 ± 0.90	5.17 ± 0.82
	Nfkbil1	NFκ light polypeptide gene enhancer in B cells inhibitor like 1	0.83 ± 0.06	0.77 ± 0.10	0.85 ± 0.07
TRIF	*Ticam1*	TLR adaptor molecule 1	1.04 ± 0.09	0.98 ± 0.14	0.85 ± 0.10
TRAM	*Ticam2*	TLR adaptor molecule 2	1.00 ± 0.19	0.96 ± 0.12	1.22 ± 0.20
TBK1	*Tbk1*	TANK-binding kinase 1	1.33 ± 0.14	1.36 ± 0.13	1.22 ± 0.10
AP1	*Jun*	Jun proto-oncogene	2.37 ± 0.22	1.52 ± 0.29	1.10 ± 0.14
	Junb	Jun B proto-oncogene	1.79 ± 0.19	2.36 ± 0.23	2.25 ± 0.22
	Jund	Jun D proto-oncogene	1.07 ± 0.06	0.93 ± 0.10	0.94 ± 0.09
	Fos	FBJ osteosarcoma oncogene	1.40 ± 0.29	2.29 ± 0.59	1.84 ± 0.35
	Fra1	Fos-like antigen 1	8.13 ± 1.24	7.51 ± 2.46	3.36 ± 0.97

		Fra2	Fos-like antigen 2	2.78 ± 0.20	2.42 ± 0.23	1.89 ± 0.20
		Batf	Basic leucine zipper transcription factor, ATF-like	1.70 ± 0.26	3.03 ± 0.40	2.96 ± 0.41
		Atf1	Activating transcription factor 1	0.97 ± 0.18	1.20 ± 0.22	1.20 ± 0.22
		Atf3	Activating transcription factor 3	0.61 ± 0.04	0.59 ± 0.08	0.83 ± 0.10
		Atf4	Activating transcription factor 4	0.69 ± 0.06	0.80 ± 0.11	0.81 ± 0.06
		Atf6	Activating transcription factor 6	0.85 ± 0.10	0.75 ± 0.05	0.82 ± 0.06
		Atf6b	Activating transcription factor 6 beta	0.95 ± 0.10	0.99 ± 0.04	1.01 ± 0.03
		Atf7	Activating transcription factor 7	1.28 ± 0.03	0.97 ± 0.08	1.00 ± 0.06
		Atf7ip	Activating transcription factor 7 interacting protein	1.01 ± 0.08	1.10 ± 0.09	1.03 ± 0.08
		Atf7ip2	Activating transcription factor 7 interacting protein 2	0.85 ± 0.16	1.11 ± 0.33	1.13 ± 0.19
		Jdp2	Jun dimerization protein 2	0.49 ± 0.02	0.70 ± 0.06	0.78 ± 0.07