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Supplemental information

Adipolin protects against renal injury via PPARα-dependent reduction of inflammasome activation

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Supplemental Table

Table S1. Mouse Primers used for quantitative RT-PCR, related to STAR Method

36B4:	Forward 5'- GCTCCAAGCAGATGCAGCA-3'
	Reverse 5'- CCGGATGTGAGGCAGCAG-3'
NLRP3:	Forward 5'-CGAGACCTCTGGGAAAAAGCT -3'
	Reverse 5'-GCATACCATAGAGGAATGTGATGTACA -3'
Caspase 1:	Forward 5'- GATGGCACATTTCCAGGACTGA -3'
	Reverse 5'-TGTTGCAGATAATGAGGGCAAGAC -3'
IL1β:	Forward 5'- AGTTGACGGACCCCAAAAG -3'
	Reverse 5'- AGCTGGATGCTCTCATCAGG -3'
IL18:	Forward 5'- CTGGCTGTGACCCTCTCTGT -3'
	Reverse 5'-CAAACTCCATCTTGTTGTGTCC -3'
TNFα:	Forward 5'- CGGAGTCCGGGCAGGT -3'
	Reverse 5'-GCTGGGTAGAGAATGGATGAACA -3'
IL6:	Forward 5'- GCTACCAAACTGGATATAATCAGG -3'
	Reverse 5'- CCAGGTAGCTATGGTACTCCAGAA -3'
MCP1:	Forward 5'- CCACTCACCTGCTGCTACTCAT -3'
	Reverse 5'- TGGTGATCCTCTTGTAGCTCTCC -3'
Collagen I:	Forward 5'-GTCCCAACCCCCAAAGAC-3'
	Reverse 5'-CAGCTTCTGAGTTTGGTGATA-3'
Collagen III:	Forward 5'-TGGTTTCTTCTCACCCTTCTT-3'
	Reverse 5'-TGCATCCCAATTCATCTACGT-3
TGFβ1:	Forward 5'- CACCGGAGAGCCCTGGATA -3'
	Reverse 5'- TTCCAACCCAGGTCCTTCCT -3'
LPL:	Forward 5'-GCCCAGCAACATTATCCAGT -3'
	Reverse 5'- GGTCAGACTTCCTGCTACGC-3'
HMGCS2:	Forward 5'-AAACTTCGCTCACACCTGCT -3'
	Reverse 5'- ACTTCCCTGCTTCCACATTG-3'
UCP2:	Forward 5' -TAGTGCGCACCGCAGCC -3'
	Reverse 5'-AGCTCATCTGGCGCTGCAG-3'
ACOX1:	Forward 5'-GCCAAGGCGACCTGAGTGAGC-3'
	Reverse 5'-ACCGCAAGCCATCCGACATTC-3'

gp91^{phox}:	Forward 5'- TTGGGTCAGCACTGGCTCTG-3'
	Reverse 5'- TGGCGGTGTGCAGTGCTATC-3'
p47^{phox}:	Forward 5'- GATGTTCCCCATTGAGGCCG-3'
	Reverse 5'- GTTTCAGGTCATCAGGCCGC-3'
p67^{phox}:	Forward 5'- CTGGCTGAGGCCATCAGACT-3'
	Reverse 5'- AGGCCACTGCAGAGTGCTTG-3'
p22^{phox}:	Forward 5'- GTCCACCATGGAGCGATGTG-3'
	Reverse 5'- CAATGGCCAAGCAGACGGTC-3'
F4/80:	Forward 5'-CTTTGGCTATGGGCTTCCAGTC-3'
	Reverse 5'-GCAAGGAGGACAGAGTTTATCGTG -3’
TLR2:	Forward 5'-AAGAAGCTGGCATTCCGAGGC-3'
	Reverse 5'-CGTCTGACTCCGAGGGGTTGA-3'
TLR4:	Forward 5'-AGTGGGTCAAGGAACAGAAGCA-3'
	Reverse 5'-CTTTACCAGCTCATTTCTCACC-3'

NLRP3: nucleotide-binding oligomerization domain-like receptor family, pyrin domain containing 3, IL: interleukin, TNFα: tumor necrosis factor α, MCP1: monocyte chemotactic protein 1, TGFβ1: transforming growth factor β1, LPL: lipoprotein lipase, HMGCS2: 3-hydroxy-3-methylglutaryl-CoA synthase 2, UCP2: uncoupling protein 2, ACOX1: acyl-CoA oxidase 1, TLR: toll like receptor

Table S2. Human Primers used for quantitative RT-PCR, related to STAR Method

36B4:	Forward 5'- TGCTCAACATCTCCCCCTTCTC -3'
	Reverse 5'- ACCAAATCCCATATCCTCGTCC -3'
NLRP3:	Forward 5'- TGAACAGCCACCTCACTT -3'
	Reverse 5'- CAACCACAATCTCCGAAT -3'
Caspase 1:	Forward 5'- TCCAATAATGGACAAGTCAAGCC -3'
	Reverse 5'-GCTGTACCCCAGATTTTGTAGCA -3'
IL1β:	Forward 5'- GCTGAGGAAGATGCTGGTTC -3'
	Reverse 5'- TGAAGGGAAAGAAGGTGCTC -3'
IL18:	Forward 5'-TCTTCATTGACCAAGGAAATCGG -3'
	Reverse 5'-TCCGGGGTGCATTATCTCTAC -3'
PPARα:	Forward 5'- CTGAAGCTGACAGCACTAC-3'
	Reverse 5'- TGAGATTAGCCACCTACCC-3'
LPL:	Forward 5'-CGCTCCATTCATCTCTTCATC -3'
	Reverse 5'-CAGCGGTTCTTTCTACAACTC -3'
HMGCS2:	Forward 5'-CAGCCATTCCCACACATGCTCA -3'
	Reverse 5'- GACTTTATAAAGCCCCAAGACT-3'
UCP2:	Forward 5'- GGCTGGAGGTGGTCGG -3'
	Reverse 5'- CAGCACAGTTGACAATGGC -3'
ACOX1:	Forward 5'- AGCGTTATGAGGTGG -3'
	Reverse 5'- CGGTGCACAAAATTTTTAA -3'
gp91^{phox}:	Forward 5'- TAGTGGGAGCAGGGATTG-3'
	Reverse 5'- TCAAAGGCATGTGTGTCC-3'
p47^{phox}:	Forward 5'- CCTGACGAGACGGAAGACC-3'
	Reverse 5'- CTTTCCTGATGACCCACCA-3'
p22^{phox}:	Forward 5'- ATTGTGGCGGGCGTGTT-3'
	Reverse 5'- GCACCGAGAGCAGGAGAT-3'
Adipo R1:	Forward 5'- AAACTGGCAACATCTGGACC-3'
	Reverse 5'-GCTGTGGGGAGCAGTAGAAG-3'
Adipo R2:	Forward 5'-ACAGGCAACATTTGGACACA -3'
	Reverse 5'- CCAAGGAACAAAACTTCCCA-3'

NLRP3: nucleotide-binding oligomerization domain-like receptor family, pyrin domain containing 3, IL: interleukin, PPARα: peroxisome proliferator-activated receptor α, LPL:

lipoprotein lipase, HMGCS2: 3-hydroxy-3-methylglutaryl-CoA synthase 2, UCP2: uncoupling protein 2, ACOX1: acyl-CoA oxidase 1, Adipo R: adiponectin receptor

Supplemental Figure and Legends

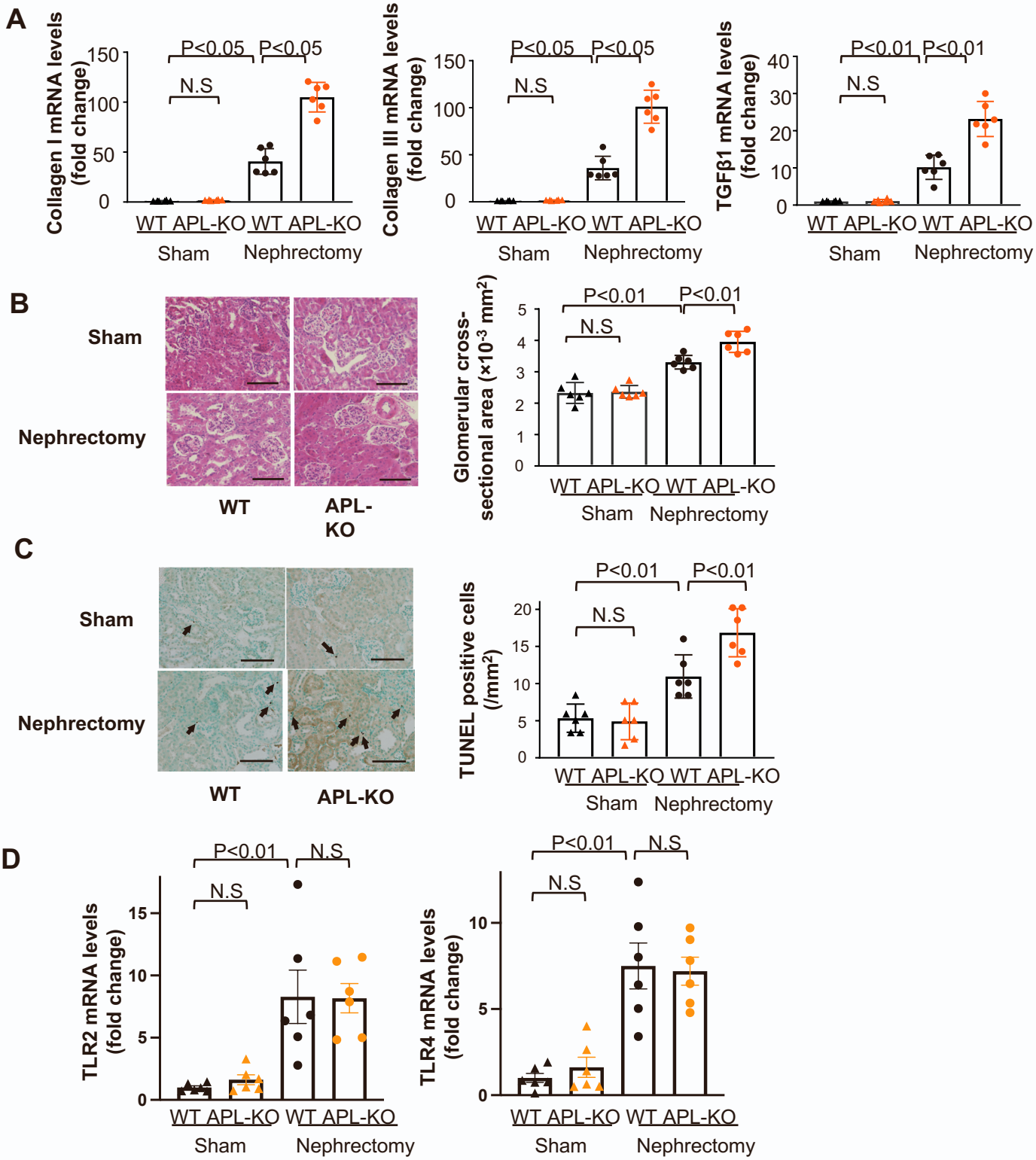

Figure S1. Adipolin-deficiency leads to increased expression levels of fibrosis-related genes, and exacerbation of glomerular hypertrophy and apoptosis in the remnant kidney after subtotal nephrectomy, related to Figure 1. A, Relative mRNA levels of Collagen I, Collagen III and transforming growth factor (TGF) β1 in the kidney in WT and APL-KO mice after subtotal nephrectomy or sham operation. **B,** Histological analysis of glomerular hypertrophy. Left panels show representative photos of the kidneys from WT and APL-KO mice after subtotal nephrectomy or sham operation as determined by H-E staining. Right panel shows quantitative analyses of glomerular cross-sectional area as measured by Image J. Scale bars show 100 μm. **C,** Histological analysis of renal apoptosis. Left panels show representative photos of the kidney from WT and APL-KO mice after subtotal nephrectomy or sham operation as determined by TUNEL staining. Right panel shows quantitative analysis of TUNEL-positive apoptotic cells. Black arrow indicates apoptotic nuclei (brown) Scale bars show 100 μm.

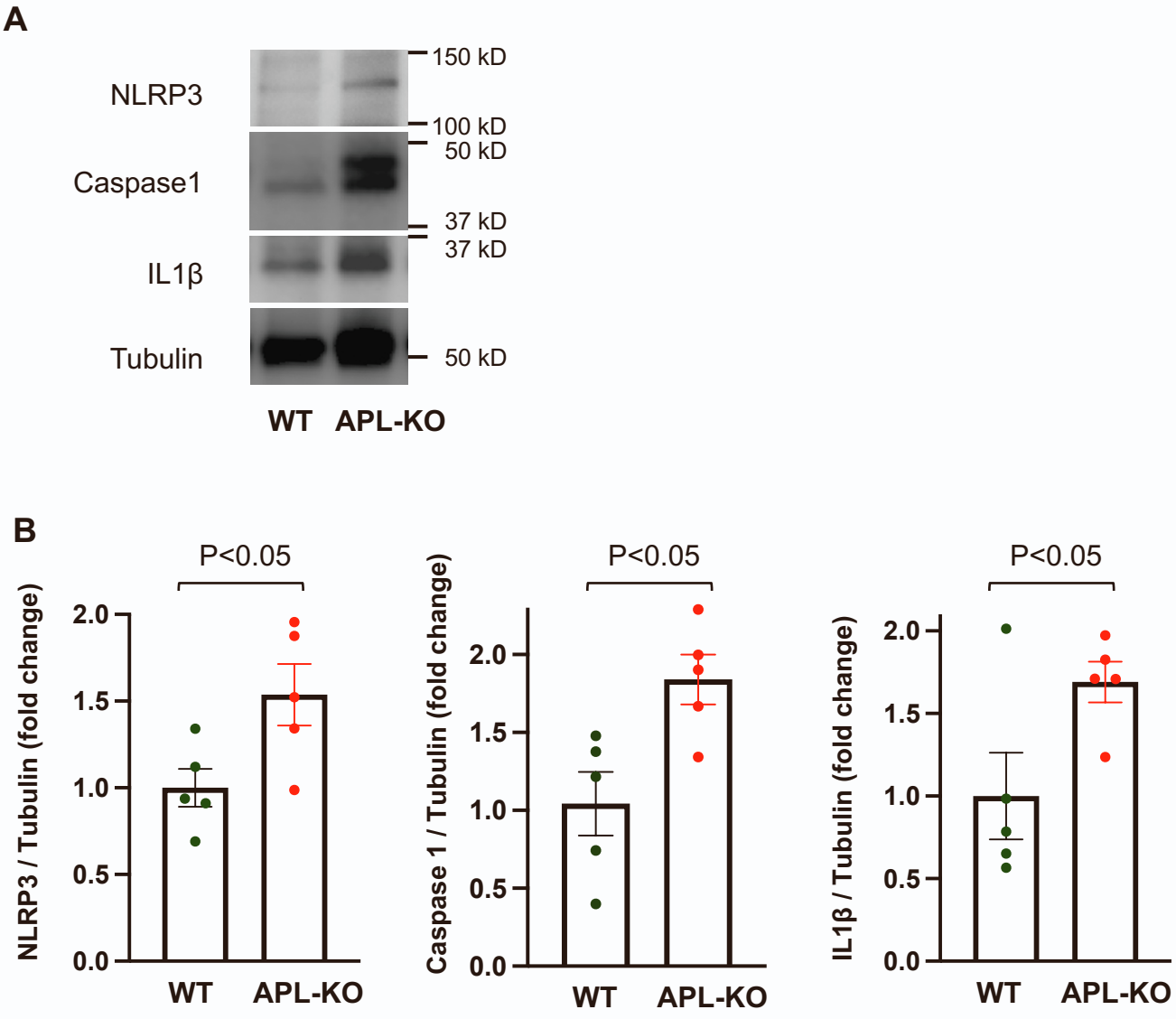

Figure S2. Adipolin deficiency enhances inflammasome activation in the remnant kidney , related to Figure 1.

A, The protein levels of NLRP3, Caspase 1, IL1β and α-tubulin (Tubulin) were evaluated by Western blot analysis in the remnant kidney of WT and APL-KO mice after subtotal nephrectomy operation.

B, Quantitative analyses of NLRP3/Tubulin, Caspase 1/Tubulin and IL1β/Tubulin.

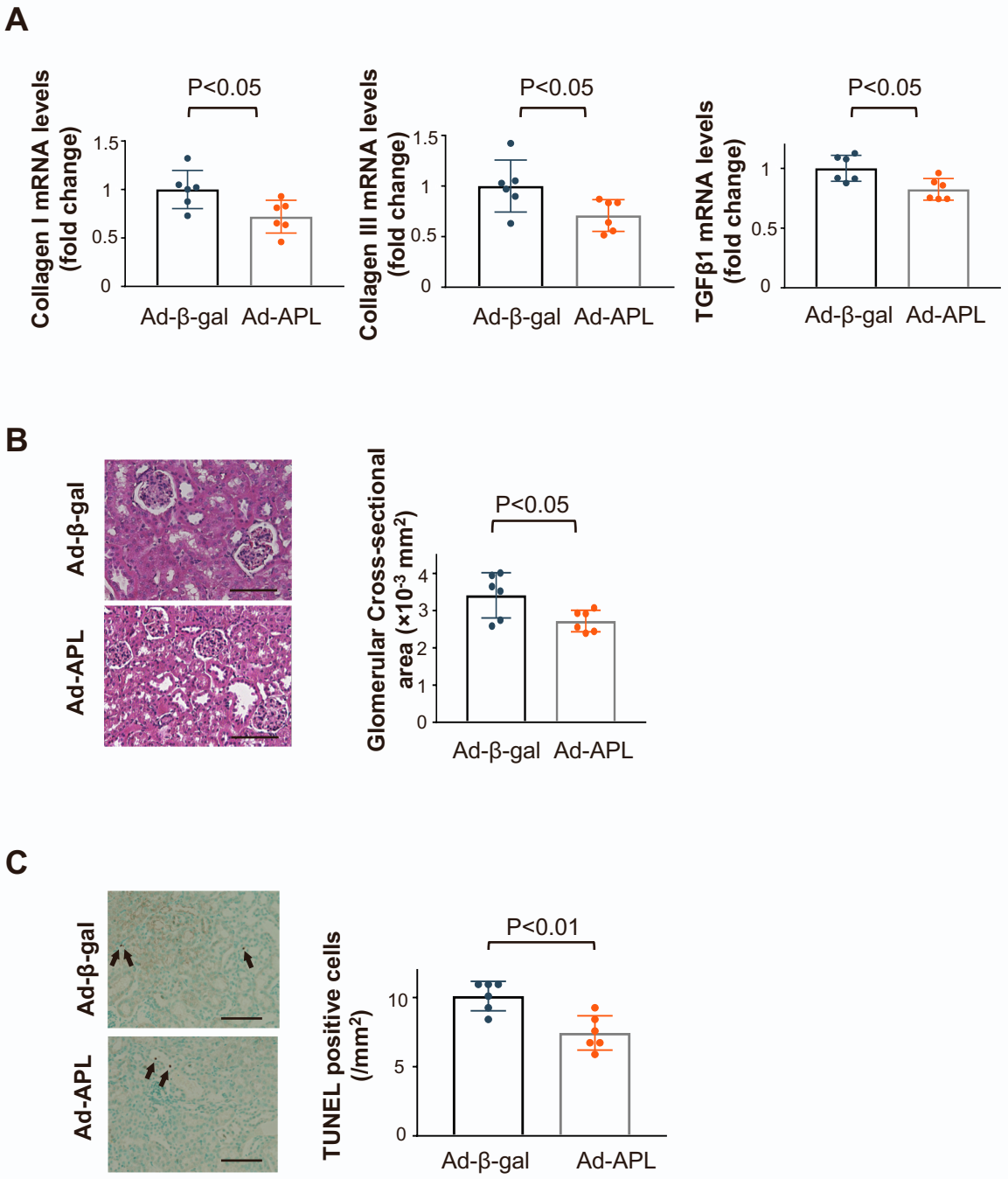

Figure S3. Systemic administration of adipolin to WT mice ameliorates renal injury after subtotal nephrectomy, related to Figure 2.

A, Relative mRNA levels of Collagen I, Collagen III and TGFβ1 in remnant kidneys in WT mice treated with Ad-β-gal or Ad-APL after subtotal nephrectomy.

B, Histological assessment of glomerular hypertrophy in the injured kidney.
Left panels show representative photos of the remnant kidneys from Ad-β-gal-treated or Ad-APL-treated WT mice as assessed by H-E staining. Right panel shows quantitative analysis of glomerular cross-sectional area as measured by Image J. Scale bars show 100 μm.

C, Histological analyses of renal apoptosis in the injured kidney. Left panels show representative photos of the remnant kidney from Ad-β-gal-treated or Ad-APL-treated WT mice as determined by TUNEL staining. Right panel shows quantitative analysis of TUNEL-positive apoptotic cells. Black arrow indicates apoptotic nuclei (brown). Scale bars show 100 μm.

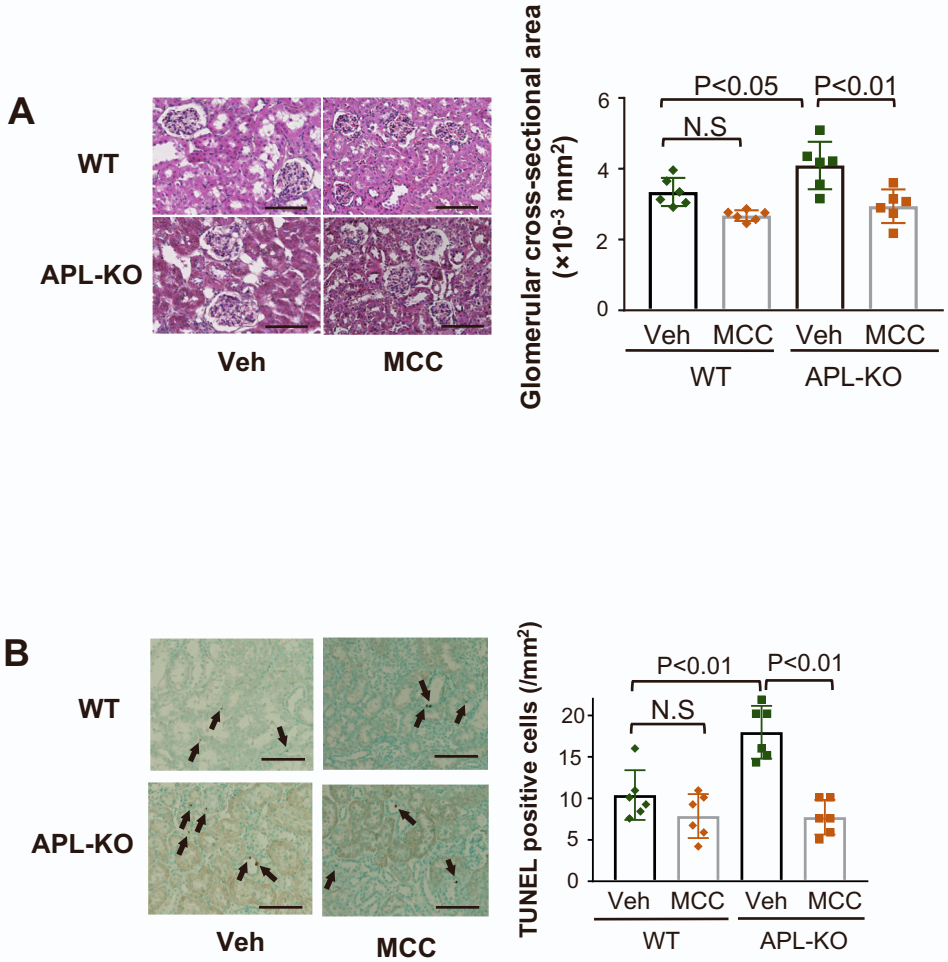

Figure S4. Inhibition of inflammasome activation ameliorates the exacerbation of glomerular hypertrophy and renal apoptosis in APL-KO mice after subtotal nephrectomy, related to Figure 3.

A, Histological analysis of glomerular hypertrophy in the injured kidneys. Left panels show representative photos of the remnant kidneys from WT and APL-KO mice treated with vehicle (Veh) or MCC950 (MCC) as determined by H-E staining. Right panel shows quantitative analysis of glomerular cross-sectional area as measured by Image J. Scale bars show 100μm.

B, Histological analysis of renal apoptosis in the injured kidneys. Left panels show representative photos of the remnant kidney from WT and APL-KO mice treated with vehicle (Veh) or MCC950 (MCC) as determined by TUNEL staining. Right panel shows quantitative analysis of TUNEL-positive apoptotic cells. Black arrow indicates apoptotic nuclei (brown). Scale bars show 100 μm.

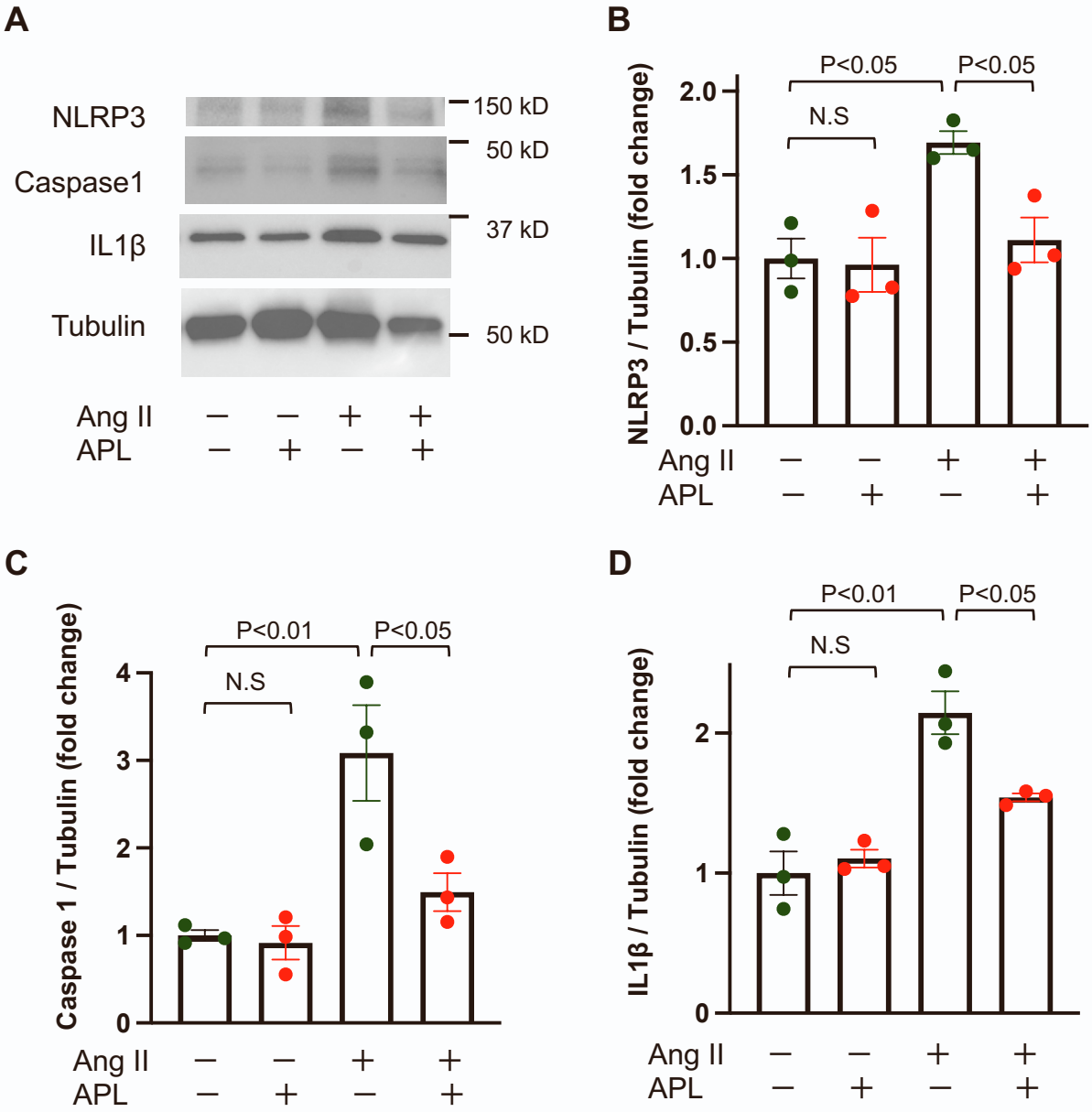

Figure S5. Adipolin treatment attenuates Ang II-stimulated inflammasome activation in HK-2 cells, related to Figure 4. HK-2 cells were pretreated with APL (300 ng/ml) or vehicle for 1 h followed by incubation in the presence or absence of Ang II (1 μmol/L) for 48 hours.

A, The protein levels of NLRP3, Caspase 1, IL1β and α-tubulin (Tubulin) were evaluated by Western blot analysis.

B, Quantitative analyses of NLRP3/Tubulin, Caspase 1/Tubulin and IL1β/Tubulin.

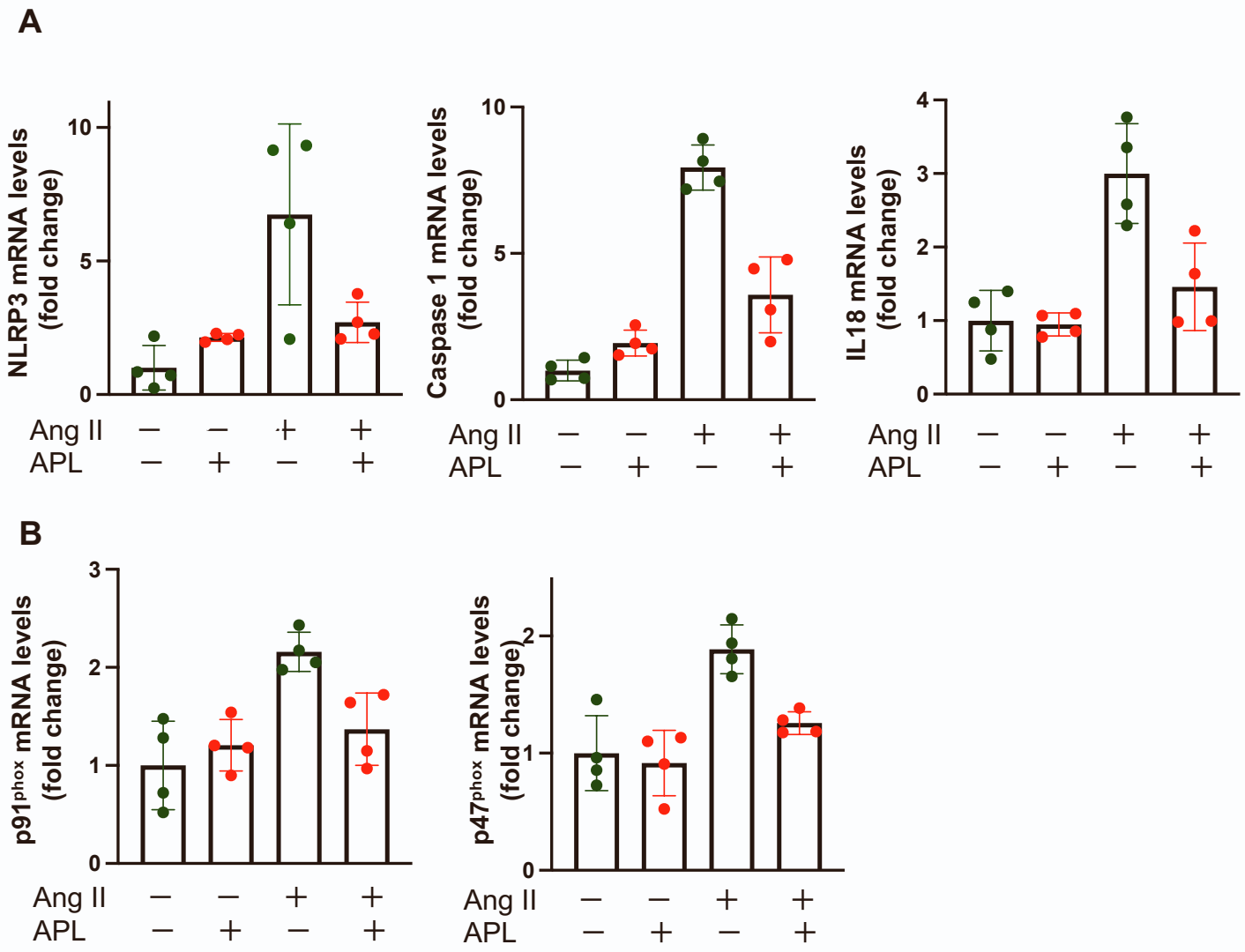

Figure S6. Adipolin treatment attenuates Ang II-stimulated inflammasome activation in MPC-5 podocytes, related to Figure 4.

A and B, Effect of APL on angiotensin (Ang) II-stimulated expression of inflammasome-related genes (NLRP3, Caspase 1 and IL18)(A) and oxidative stress markers (gp91^{phox} and p47^{phox})(B) in MPC-5 cells. Mouse podocyte cell lines, MPC-5 cells were pretreated with APL (300 ng/ml) or vehicle for 1 h followed by incubation in the presence or absence of Ang II (1 μmol/L) for 24 h.

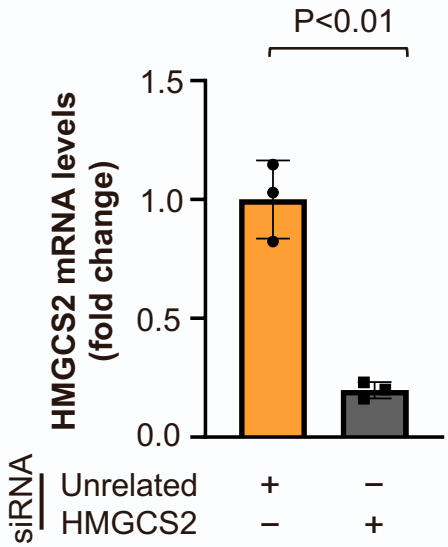

Figure S7. Effect of siRNA targeting HMGCS2 on expression of HMGCS2 in HK-2 cells, related to Figure 5.

HK-2 cells were treated with siRNA targeting HMGCS2 (10 nmol/L) or control unrelated siRNA for 48 h. The mRNA levels of HMGCS2 were measured by quantitative real time-PCR methods.

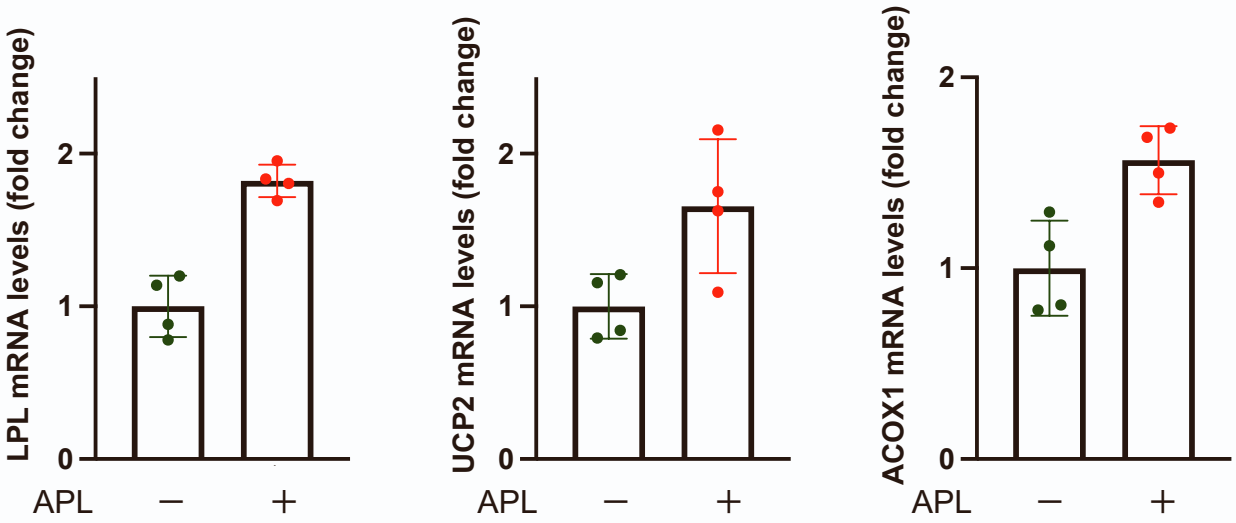

Figure S8. Adipolin increases expression of PPARα target genes in MPC-5 podocytes, related to Figure 6.

mRNA expression of lipoprotein lipase (LPL), uncoupling protein (UCP) 2 and acyl CoA oxidase (ACOX)-1, which are downstream molecules of PPARα, in HK-2 cells are determined after stimulation with APL protein (300 ng/mL) or vehicle for 6 h.

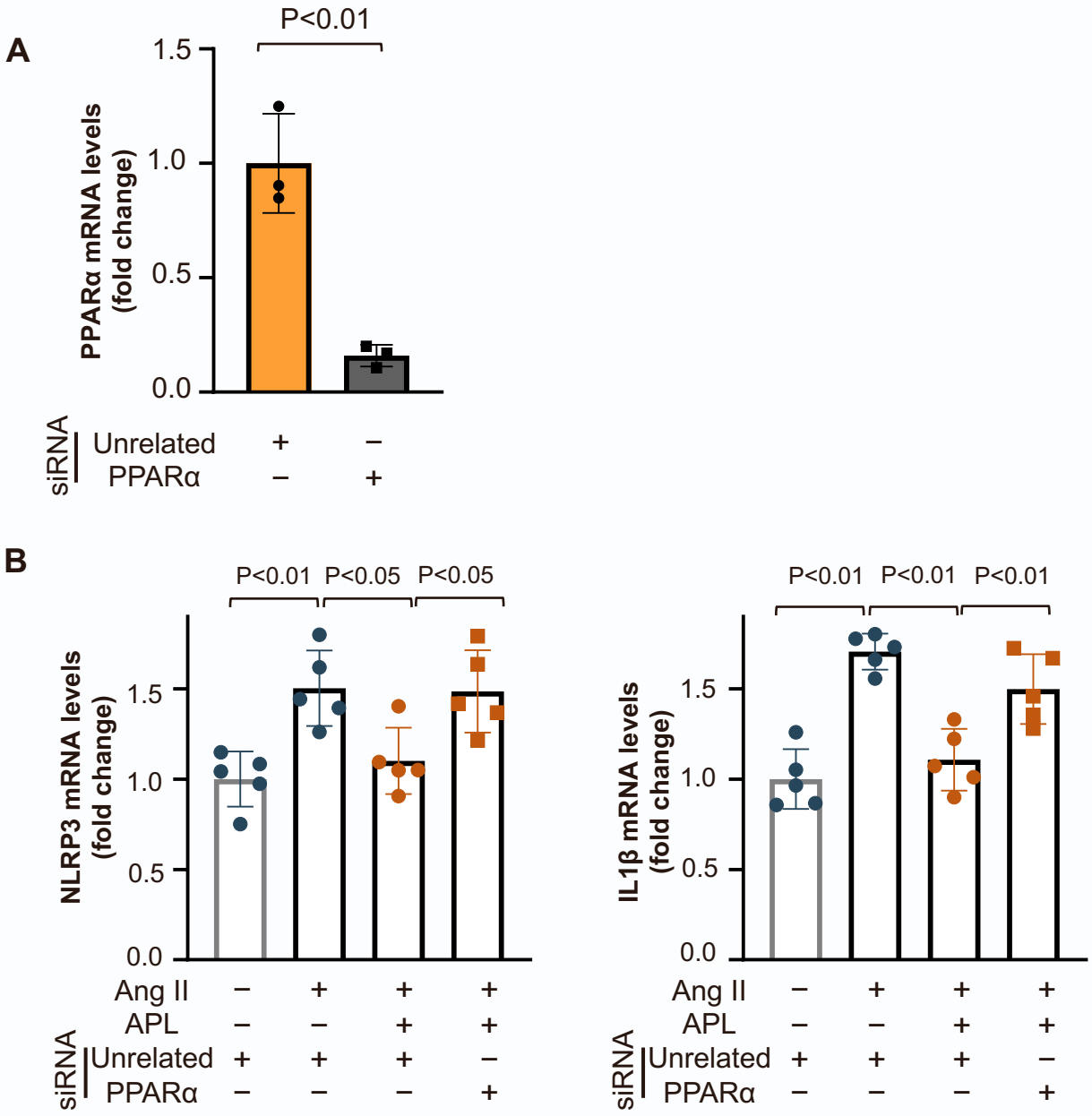

Figure S9. Ablation of PPARα reverses the suppressive effect of adipolin on Ang II-stimulated inflammasome activation in HK-2 cells, related to Figure 6.

A, The mRNA levels of PPARα were evaluated by quantitative real-time PCR methods in HK-2 cells treated with control unrelated siRNA and siRNA targeting PPARα.

B, Contribution of PPARα to the suppressive effect of adipolin on expression of inflammatory-associated genes, NLRP3 and IL1β, in HK-2 cells. HK-2 cells were pretreated with adipolin (APL) (300 ng/ml) or vehicle for 1 h followed by treatment with Ang (1 μmol/L) or vehicle for 24 h.

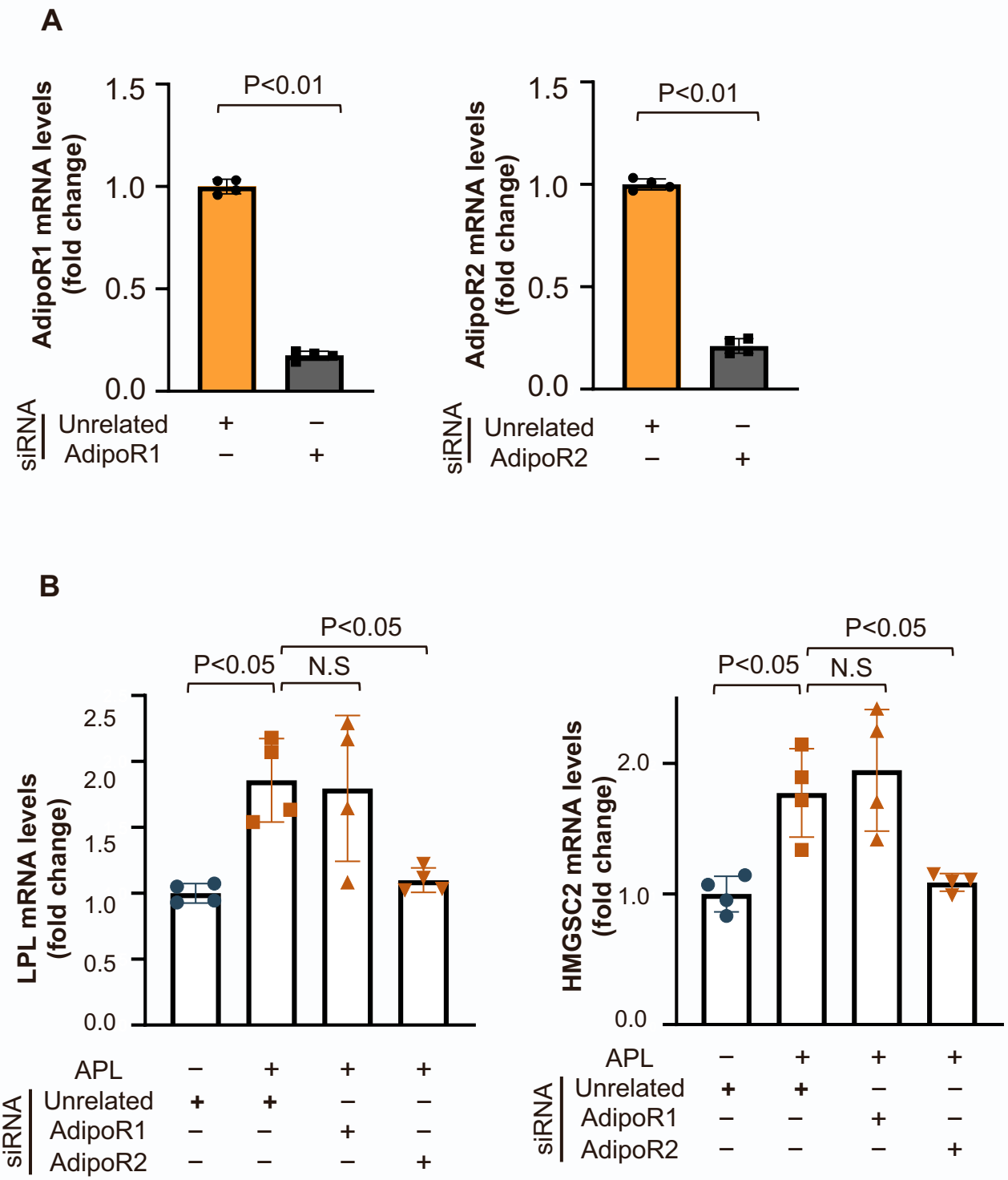

Figure S10. Adipolin increases expression of PPARα target genesin an AdipoR2-dependent manner, related to STSR Methods.

A, mRNA levels of AdipoR1 and AdipoR2 were evaluated by quantitative real-time PCR methods in HK-2 cells treated with control unrelated siRNA, or siRNAs targeting AdipoR1 or AdipoR2.

B, Contribution of AdipoR1 or AdipoR2 to the stimulatory effects of adipolin on expression of PPARα target gene, lipoprotein lipase (LPL), and HMGCS2 in HK-2 cells.