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Supplementary Information for

Blocking CHOP-dependent TXNIP shuttling to mitochondria attenuates albuminuria and mitigates kidney injury in nephrotic syndrome

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Supplementary Methods

Adriamycin (ADR)-induced nephropathy

Male Balb/cJ mice (stock number 000651 from the Jackson Lab) at 10 weeks of age were injected with ADR in 0.9% saline at 12 mg/kg into the retro-orbital sinus to induce nephropathy.

Kidney mitochondrial fractionation

Kidneys were perfused with cold PBS first. Mitochondrial fractions were isolated from kidneys using Mitochondria Isolation Kit for Tissue (Thermo Fisher Scientific) according to the manufacturer's protocols.

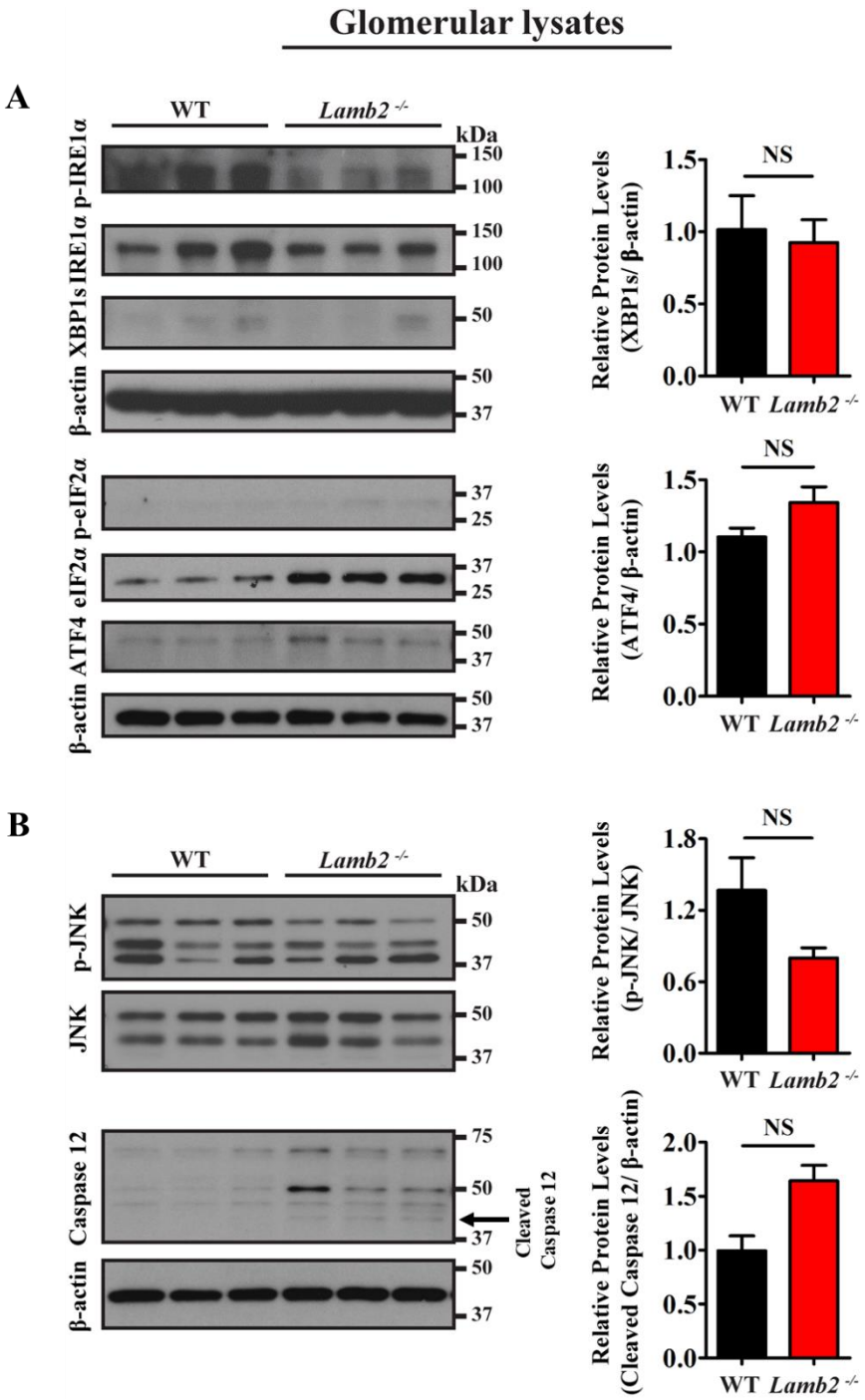

Fig. S1. **IRE1α/XBP1s and eIF2α/ATF4 pathways, as well as JNK and caspase 12 pathways are not activated in *Lamb2* $^{-/-}$ glomeruli.** *(A)* WB analysis of isolated glomerular lysates from WT and *Lamb2* $^{-/-}$ mice at P25 to detect p-IREα, IRE1α, XBP1s, p-eIF2α, eIF2α and ATF4. Densitometry analysis of XBP1s and ATF4 normalized to β-actin was performed. (B) WB analysis of glomerular lysates to detect p-JNK, JNK and cleaved caspase 12 (black arrow). Ratios of p-JNK to total JNK and cleaved caspase 12 to β-actin were quantified by densitometry analysis of three independent experiments. Mean ± SD; NS: not significant by t-test.

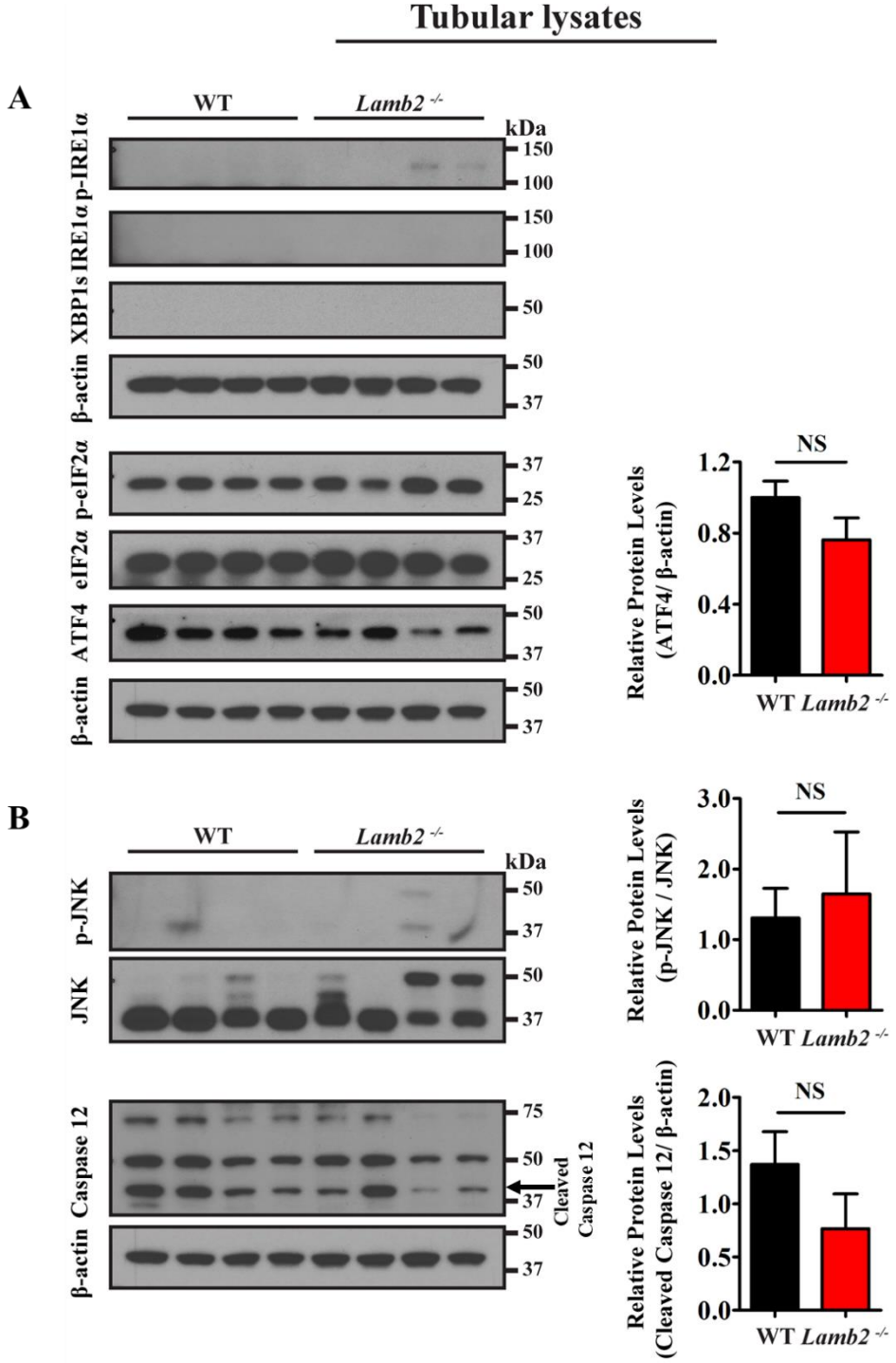

Fig. S2. IRE1α/XBP1s and eIF2α/ATF4 pathways, as well as JNK and caspase 12 pathways are not activated in *Lamb2* $^{-/-}$ tubules. *(A-B)* Isolated tubular lysates from WT and *Lamb2* $^{-/-}$ mice at P25 were analyzed by WB with the indicated antibodies. Ratios of ATF4 to β-actin, p-JNK to total JNK and cleaved caspase 12 (black arrow) to β-actin were quantified by densitometry analysis of three independent experiments. Mean ± SD; NS: not significant by t-test.

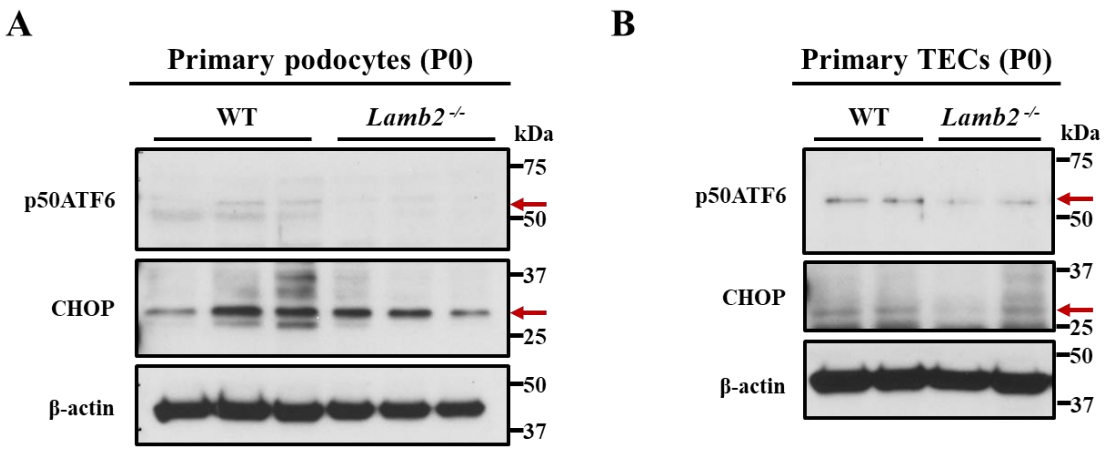

Fig. S3. The ATF6-CHOP signaling is not activated in both primary podocytes and primary TECs isolated from *Lamb2* $^{-/-}$ mice, which are not treated with albumin. Mouse primary podocytes and TECs were isolated and cultured. Representative immunoblots of p50ATF6 (red arrows) and CHOP (red arrows) expression in primary podocytes (P0) *(A)* and primary TECs *(B)*.

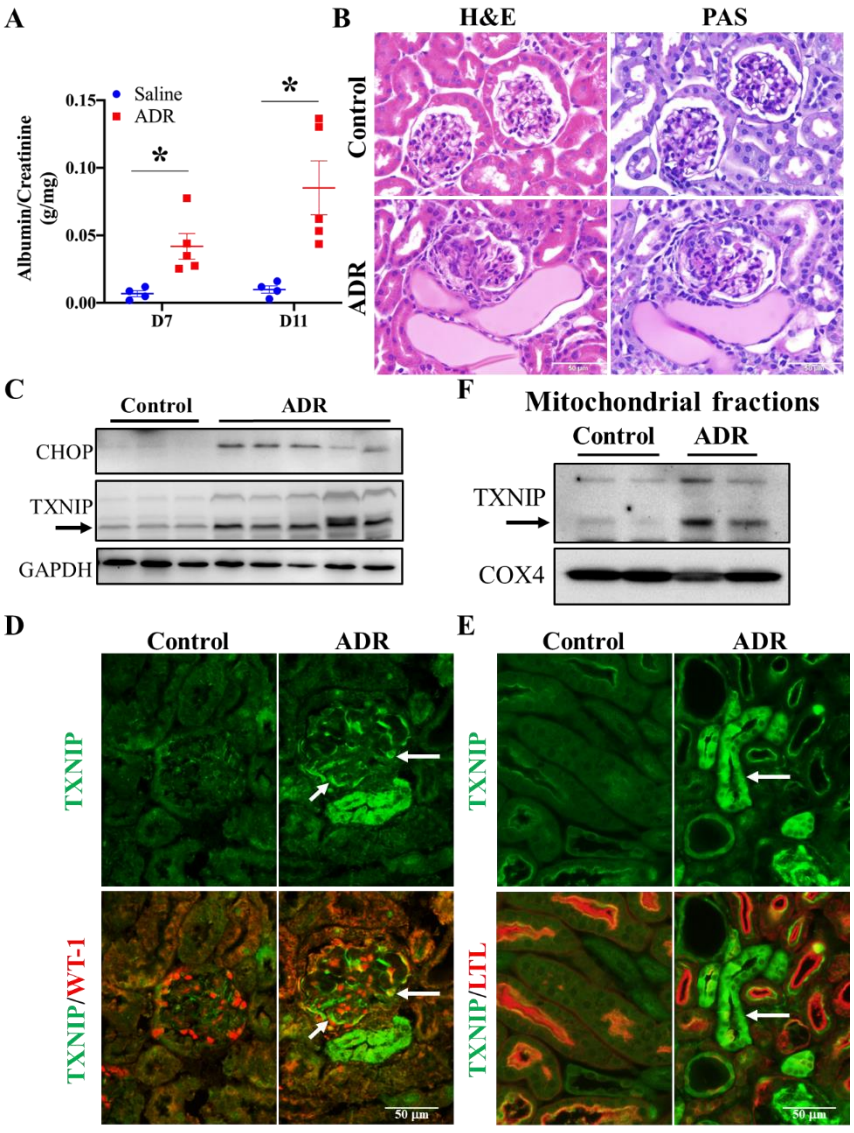

Fig. S4. ADR-induced nephropathy exhibited TXNIP upregulation in both podocytes and tubules, as well as increased mitochondrial accumulation. *(A)* Urinary albumin/creatinine ratios in saline- and ADR- treated male Balb/cJ mice on day 7 and 11 post-injection. Mean ± SD (n=4-5 mice/group). **P* < 0.05. *(B)* H&E and PAS staining of paraffin kidney sections from control and ADR-injected mice on day 11 post-injection. Scale bar: 50 µm. *(C)* Immunoblot to detect CHOP and TXNIP (black arrow) proteins from whole kidney lysates of saline- and ADR-injected mice on day 11 after injection. n=4-5 mice/group. *(D-E)* IF images of frozen kidney sections stained for TXNIP (green) with WT-1 (red) (*D*), as well as paraffin sections stained for TXNIP (green) with LTL (red), from saline- and ADR-injected mice on day 11 post-injection. White arrows indicate TXNIP staining in podocytes (*D*) or in tubules (*E*). Scale bar: 50 µm. (*F*) Representative immunoblots of TXNIP expression (black arrow) in the kidney mitochondrial fractions from control and ADR-injected mice on day 11 post-injection. COX IV was used as mitochondrial internal control.

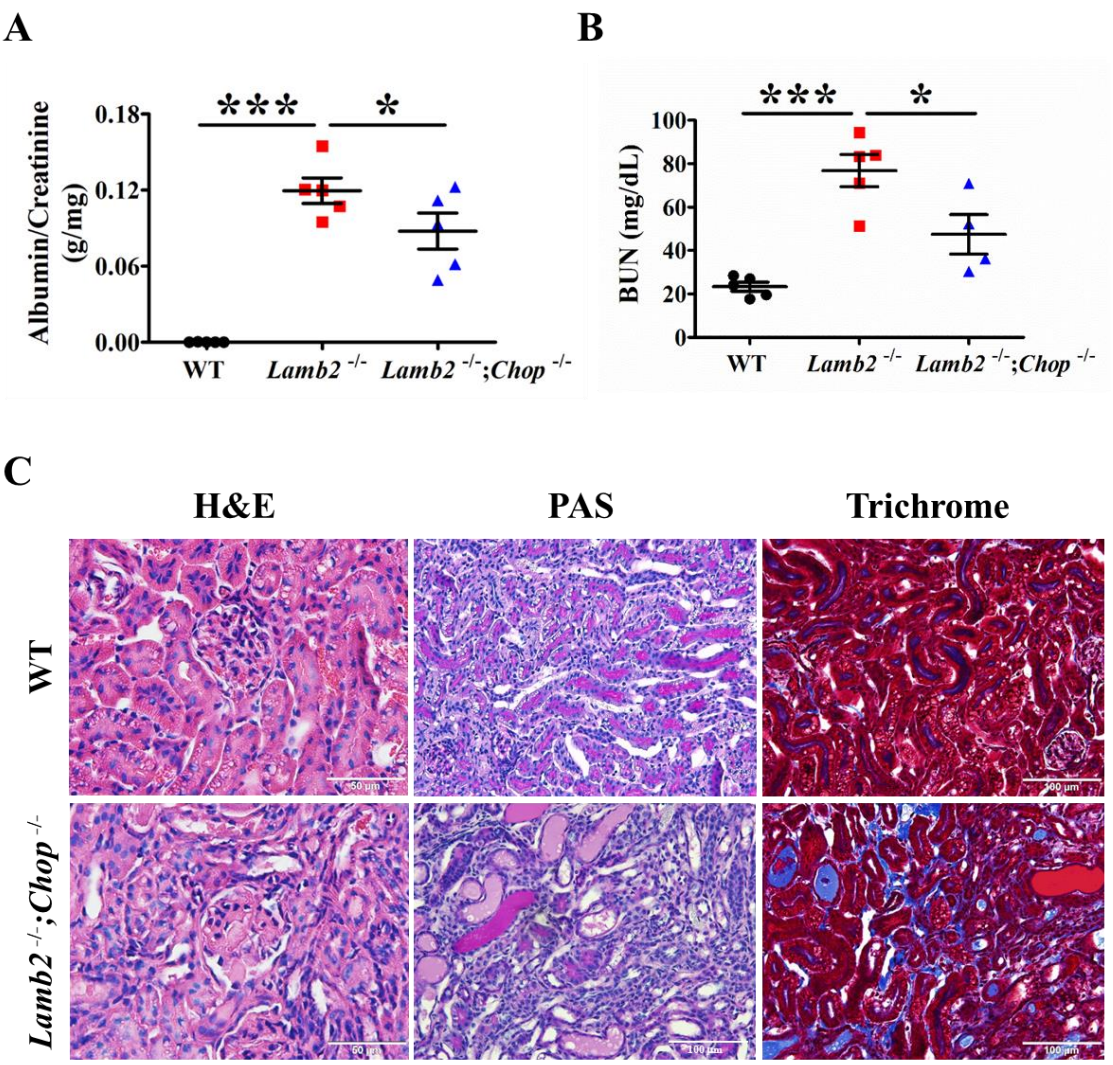

Fig. S5. Characterization of disease phenotype and renal histology in *Lamb2* $^{-/-}$*;Chop* $^{-/-}$ mice at a later stage of the disease (P35). *(A)* Urinary albumin/creatinine ratios in WT, *Lamb2* $^{-/-}$, and *Lamb2* $^{-/-}$*;Chop* $^{-/-}$ mice at P35. Mean ± SD (n=5 mice/genotype). **P* < 0.05, ****P* < 0.001. (*B*) BUN levels from mice of the indicated genotypes at P35. Mean ± SD (n=4-5 mice/genotype). **P* < 0.05, ****P* < 0.001. *(C)* H&E, PAS, and Gomori's Trichrome staining of paraffin kidney sections from WT and *Lamb2* $^{-/-}$*;Chop* $^{-/-}$ mice at P35. Scale bar: 50 µm for H&E or 100 µm for PAS and Trichrome staining.

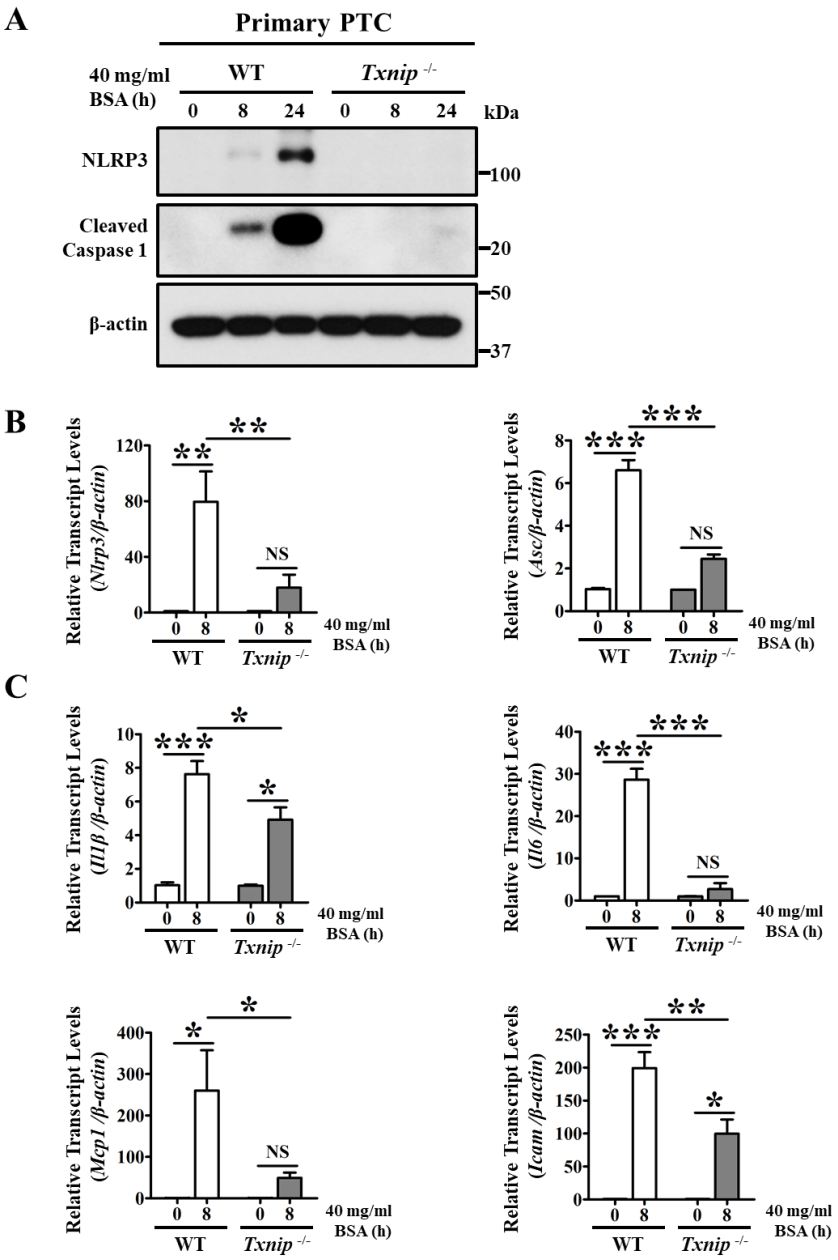

Fig. S6. TXNIP deletion suppresses activation of NLRP3 inflammasome and downstream proinflammatory cytokines in albuminuria. Primary PTCs were isolated and cultured from WT and *Txnip* $^{-/-}$ mice. Cultured PTCs were starved for 16h and then treated with 40 mg/ml BSA for 8 or 24 hours. *(A)* Representative WBs of NLRP3, cleaved caspase 1 and β-actin in whole cell lysates of primary PTCs in the absence or presence of 40 mg/ml BSA for 8 and 24 hours. The WB image shown is representative of at least three independent experiments. *(B-C)* Quantitative PCR analysis of primary PTCs treated without or with 40 mg/ml BSA for 8 hours for mRNA levels of NLRP3, ASC (*B*) and inflammatory cytokines IL1β, IL6, MCP-1 and ICAM (*C*). Gene expression was normalized to β-actin. Mean ± SD from three independent experiments. NS, not significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001 by ANOVA.

Table S1 List of q-PCR primers

Target gene	Species	Direction	Sequence
Chop	Mouse	Forward	CTGGAAGCCTGGTATGAGGAT
		Reverse	CAGGGTCAAGAGTAGTGAAGGT
Txnip	Mouse	Forward	TATGTACGCCCCTGAGTTCC
		Reverse	GCTCACTGCACGTTGTTGTT
Nlrp3	Mouse	Forward	CCTTCCAGGATCCTCTTCCT
		Reverse	CTTGGGCAGCAGTTTCTTTC
Asc	Mouse	Forward	TGAGCAGCTGCAAACGACTA
		Reverse	ACTTCTGTGACCCTGGCAAT
Il1β	Mouse	Forward	GCACTACAGGCTCCGAGATGAAC
		Reverse	TTGTCGTTGCTTGGTTCTCCTTGT
Il6	Mouse	Forward	TAGTCCTTCCTACCCCAATTTCC
		Reverse	TTGGTCCTTAGCCACTCCTTC
Mcp1	Mouse	Forward	TTAAAAACCTGGATCGGAACCAA
		Reverse	GCATTAGCTTCAGATTTACGGGT
Icam	Mouse	Forward	AGCACCTCCCCACCTACTTT
		Reverse	AGCTTGCACGACCCTTCTAA
Tgfβ	Mouse	Forward	TGGAGCAACATGTGGAACTC
		Reverse	CAGCAGCCGGTTACCAAG