Supplementary Figures 1-3

Activation of the Nlrp3 inflammasome by islet amyloid polypeptide provides a mechanism for enhanced IL-1β in type 2 diabetes

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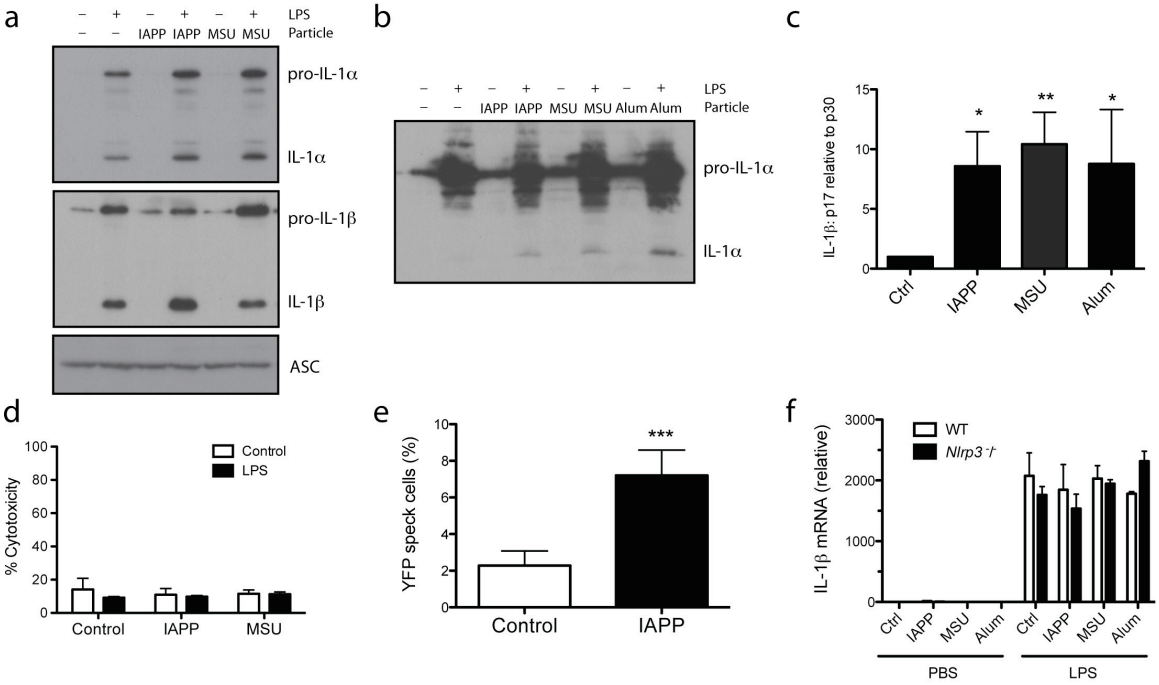

Supplementary Figure 1. IAPP activation of the inflammasome. a) Immunoblots of IL-1α (top panel), IL-1β (middle panel) and ASC (lower panel) from lysates of cells stimulated with LPS, followed by IAPP and MSU, as in figure 1b. b) Immunoblotting for IL-1α in supernatants from cells stimulated with LPS, followed by IAPP, MSU and Alum as in figure 1b. c) Quantification of three independent immunoblots for IL-1β in supernatants of BMDC stimulated as in figure 1b. The intensity of the p17 band relative to the p30 band was measured by densitometry. d) LDH assay for cytotoxicity of BMDC, treated with inflammasome activating agents as in figure 1b. e) Biological triplicate quantification for the formation of ASC specks, as in figure 1e. f) Q-PCR for IL-1β mRNA relative to GAPDH mRNA from cells treated as in figure 2a, either with or without LPS, and from *Nlrp3*$^{-/-}$ mice. Means ± SD, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. All data representative of three independent experiments.

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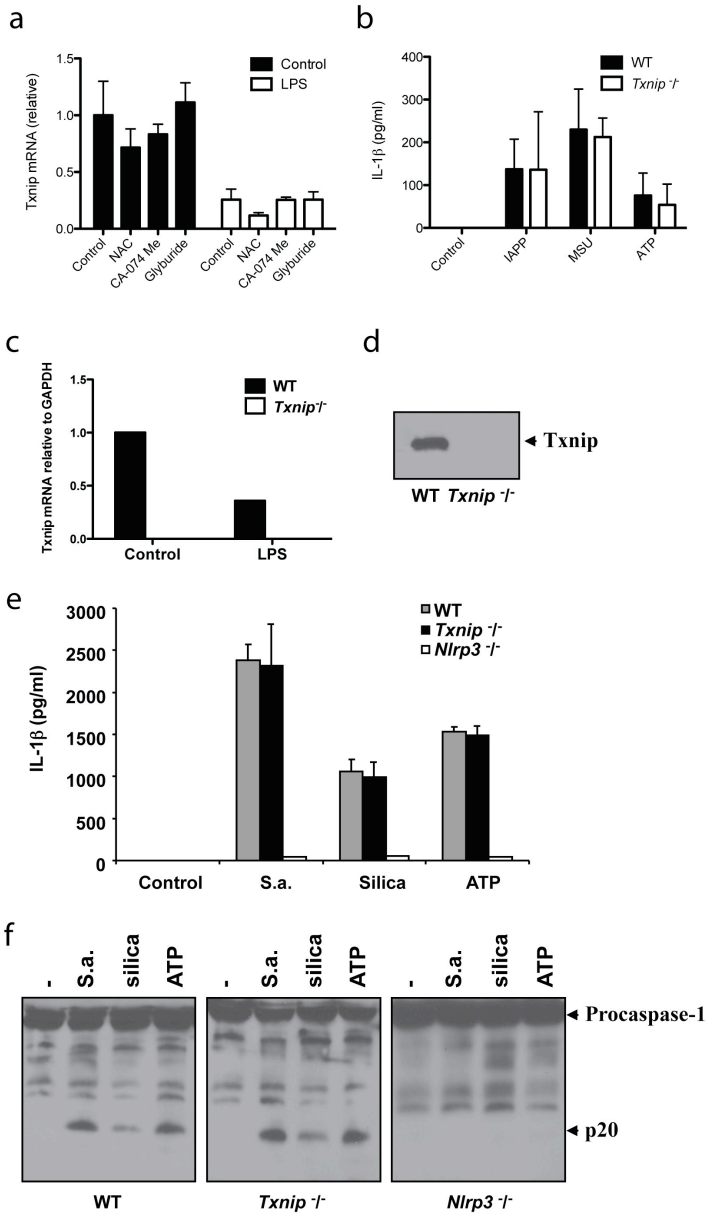

Supplementary Figure 2. Txnip is not involved in IL-1β activation by Nlrp3, or inhibition by glyburide. a) Txnip mRNA was measured relative to GAPDH in BMDM, before and after LPS priming with the addition of several inhibitors, as indicated. b) BMDM deficient for Txnip were primed with LPS then stimulated with IAPP or other inflammasome activators such as MSU and ATP. c) Deletion of the *Txnip* gene was confirmed by Q-PCR. d) Immunoblotting using anti-Txnip. e) IL-1β secretion from wild-type (WT), *Txnip*$^{-/-}$ and *Nlrp3*$^{-/-}$ BMDM primed with LPS for 4 hours and then stimulated with supernatant from *S. aureus* (S.a.) for 2 hours, silica (400 μg/ml) for 6 hours or ATP (5 mM) for 30 min. f) BMDM stimulated as in panel c, then immunoblotting cell extracts with an antibody that recognizes the p20 subunit of caspase-1. The 45 kDa procaspase-1 and mature p20 subunit are indicated by arrowheads. Means ± SD. All data representative of three independent experiments.

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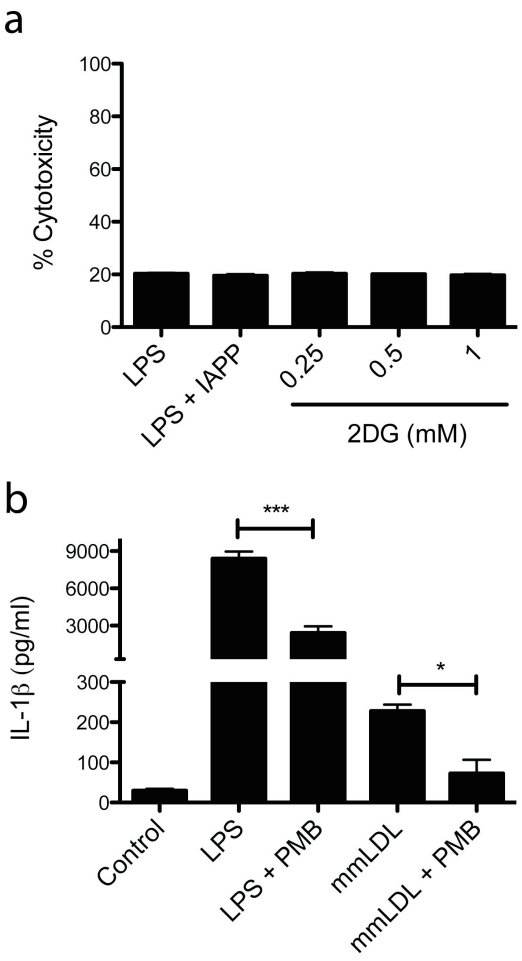

Supplementary Figure 3. Cytotoxicity of 2DG, and effect of polymyxin B on mmLDL. a) Supernatants from cells treated as in figure 3b were subjected to LDH assay as a measure of cytotoxicity. b) Polymyxin B (PMB) was added to preparations of LPS or mmLDL which were then used to prime BMDC, compared to untreated LPS or mmLDL. After 3 hours priming, cells were then activated with MSU crystals overnight and IL-1β production measured. Means ± SD, * $p<0.05$, *** $p<0.001$. All data representative of three independent experiments.

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