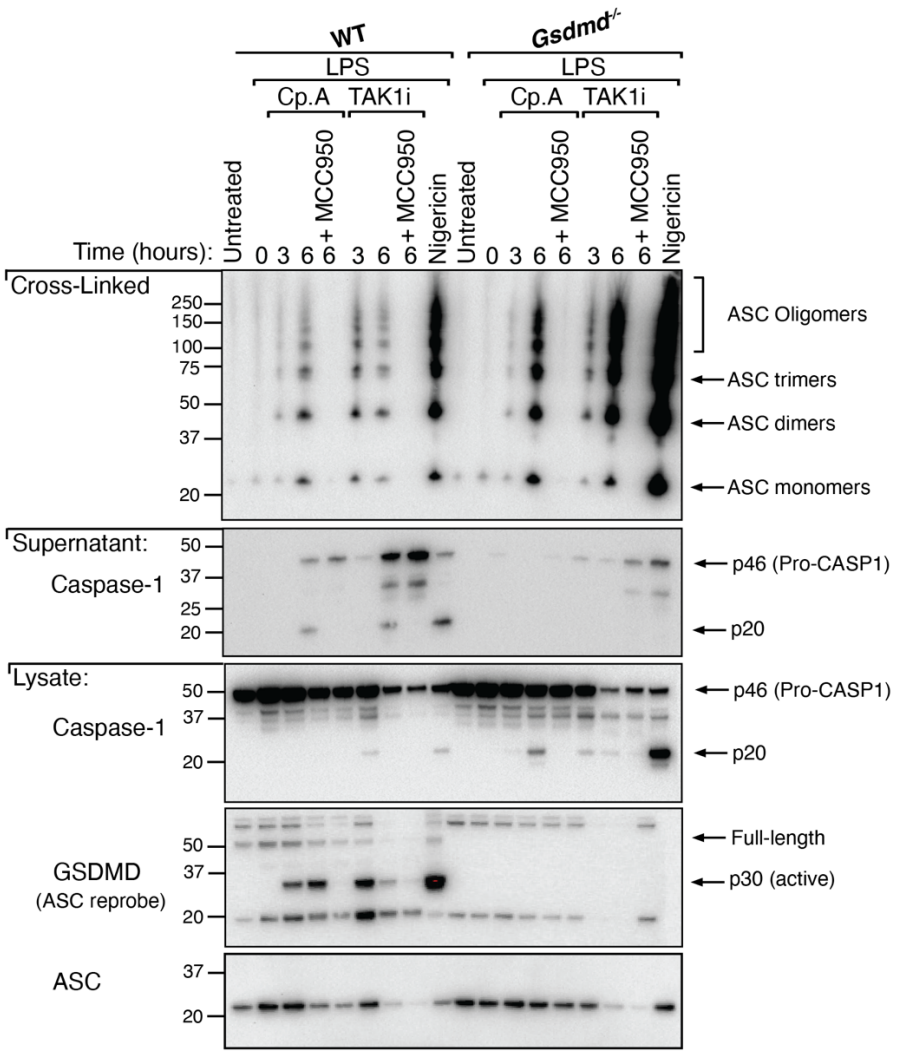
Appendix

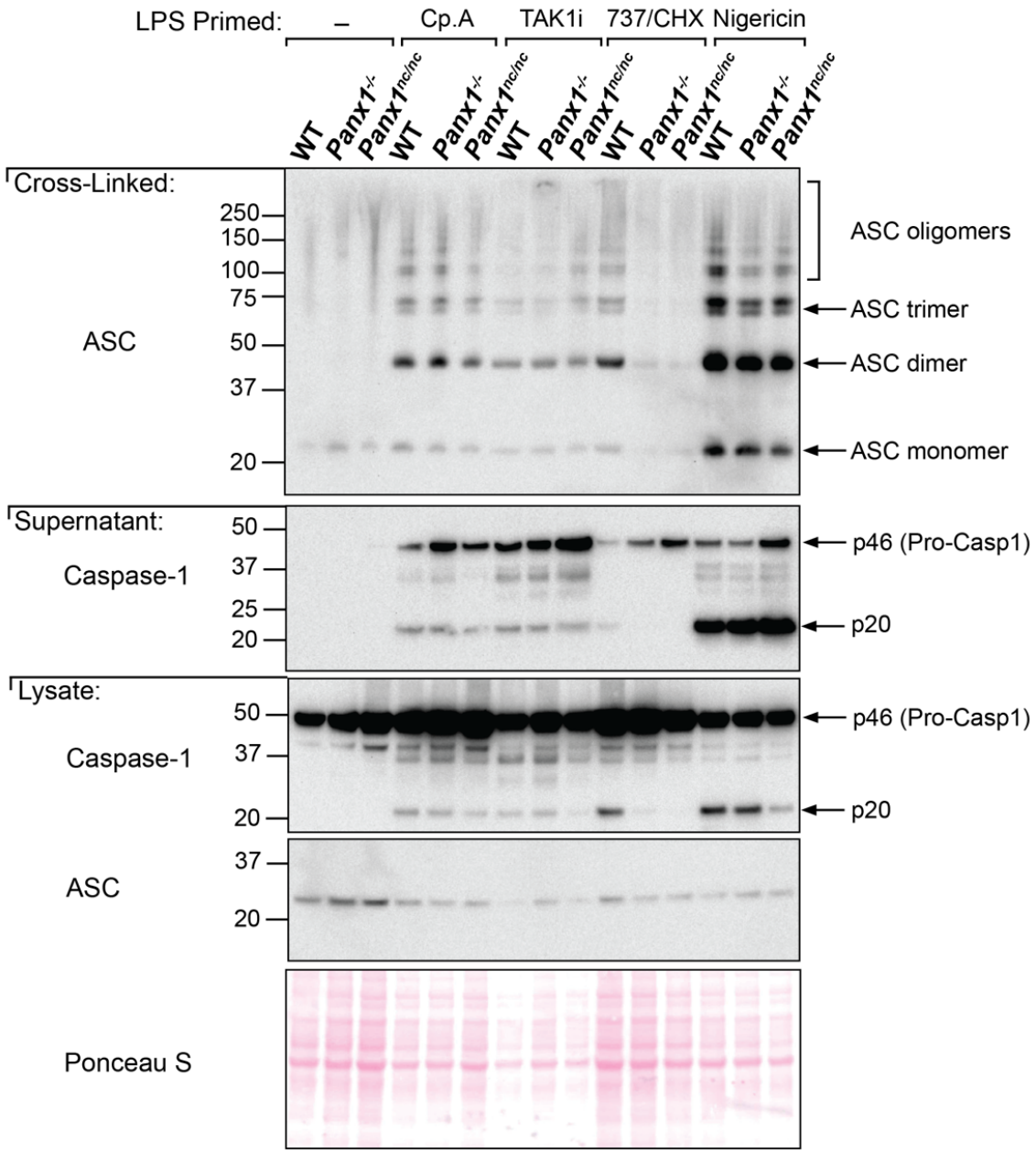
Caspase-8-driven apoptotic and pyroptotic crosstalk drives cell death and IL-1β release in X-linked inhibitor of apoptosis (XIAP) deficiency

Sebastian A. Hughes *et al.*

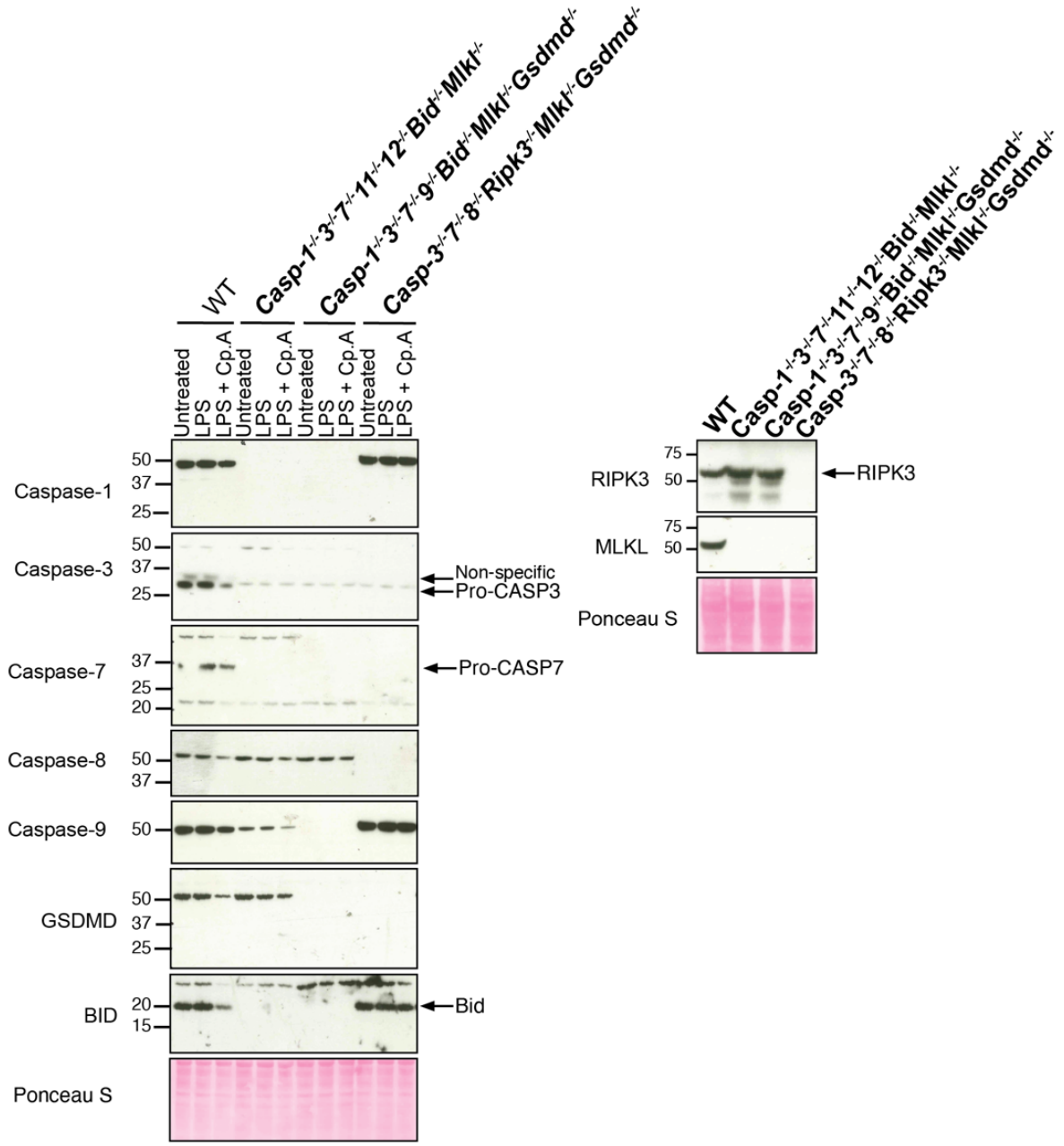


Appendix Figure S1. The deletion of GSDMD does not delay NLRP3-dependent ASC-oligomerisation upon caspase-8 activation.

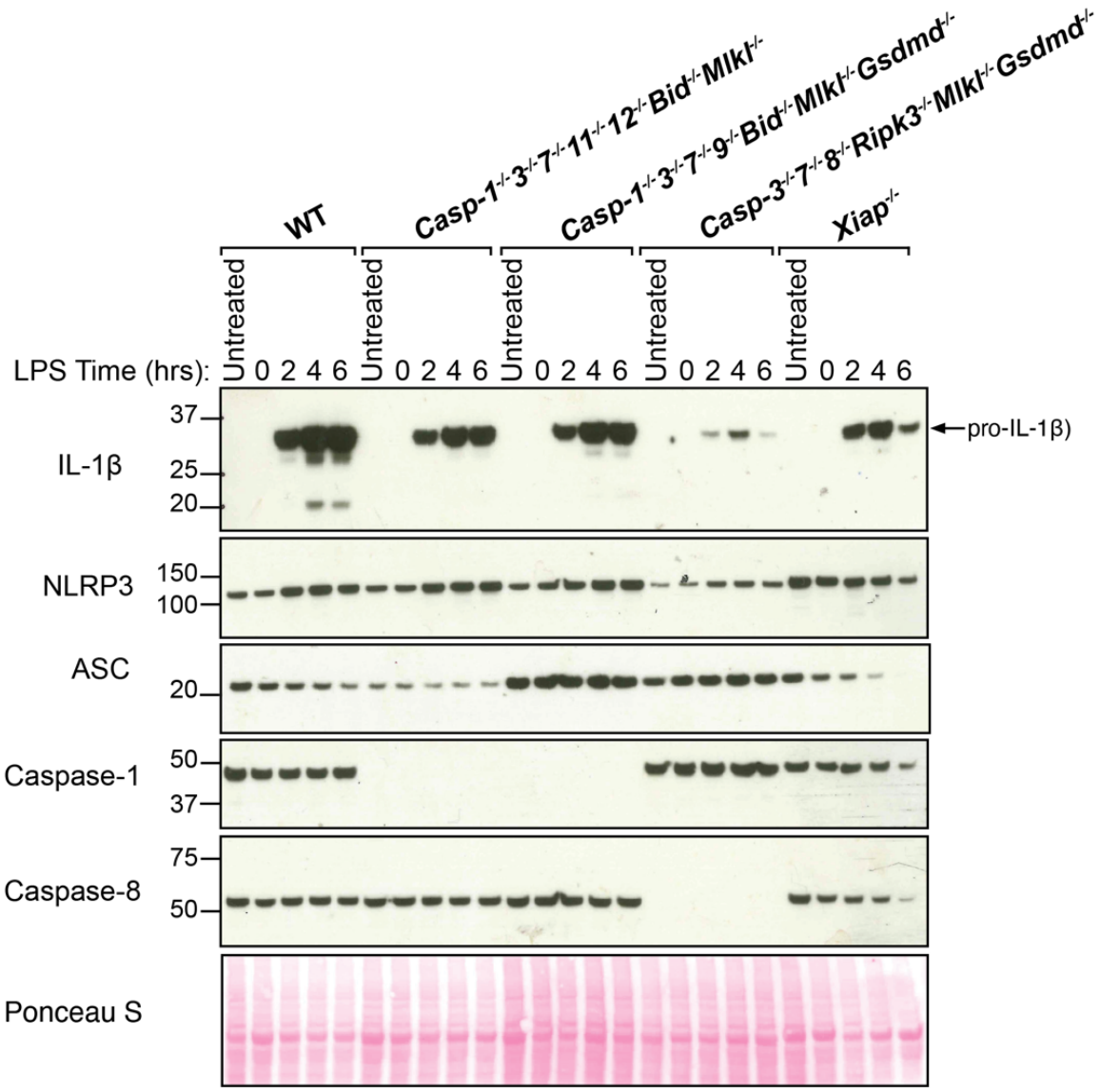
BMDMs of the indicated genotypes were seeded at a density of 2 x 10^6 cell per well and primed with 100 ng/ml LPS for three hours before treatment with Cp. A (1 μM) or TAK1i (250 nM) for three and six hours. Cells were additionally treated with MCC950 (5 μM) for 30 minutes prior to treatment with Cp. A or TAK1i for six hours. PBS insoluble fractions of cell lysates were crosslinked to assess ASC-oligomerisation and these, alongside cell lysates and supernatants, were analysed by western blot. Data represents two independent experiments.



Appendix Figure S2. Pannexin-1 is required for efficient intrinsic apoptotic activation of NLRP3 but is dispensable for caspase-8-driven NLRP3 activity.
BMDMs of the indicated genotypes were seeded at a density of 2 x 10^6 cell per well and primed with 100 ng/ml LPS for three hours before treatment with Cp. A (1 μM), TAK1i (250 nM) for six hours, ABT-737 (1 μM) and CHX (20 μg/ml) for four hours, or nigericin (10 μM) for 45 minutes. Cell lysates and supernatants were then analysed by western blot. PBS insoluble fractions of cell lysates were crosslinked to assess ASC-oligomerisation and these, alongside cell lysates and supernatants, were analysed by western blot. Ponceau staining depicts protein loading. Data represents one of four independent experiments.



Appendix Figure S3. Validation of CRISPR/Cas9 genetic targeting of the cell death machinery in immortalised BMDMs.
iBMDMs of the indicated genotypes were primed with 50 ng/ml LPS for three hours before treatment with Cp. A (1 μM) for 24 hours as indicated and total cell lysates were then analysed by western blot. Ponceau staining depicts protein loading. One of two experiments.



Appendix Figure S4. Evaluation of inflammasome priming in gene targeted iBMDMs. iBMDMs of the indicated genotypes were untreated or primed with LPS (100 ng/ml) for 0, 2, 4 or 6 hours and total cell lysates analysed by western blot. Ponceau staining depicts protein loading. Data represents one experiment.