Expanded View Figures

Figure EV1. XIAP deficiency abrogates NOD2 signalling.

A Sanger-sequencing confirming XIAP frameshift (T933del-AGAAC) in patient 1 and a misssense mutation (c.595C>T:p.Q199X) in patient 2.

B Colonoscopy images of the XIAP-deficient patients at diagnosis.

C, D ELISA analysis of IL-6 and TNF in PBMC supernatants upon NOD2 ligand muramyl dipeptide (10 μg/ml) stimulation for 24 h. The mean of duplicate experiments is shown.

E Paraffin-embedded sections of colonic biopsies were stained with secondary Alexa fluor anti-rabbit 594 and or anti-mouse 488.

F, G BMDMs were seeded at a density of 4×10^5 cells per well, primed with 20 ng/ml of LPS for 6 h then treated, as indicated, with 500 nM of the indicated smac-mimetic compounds for 20 h. Cell viability (F) was determined by PI staining and flow cytometry and measured as a proportion of PI-negative (live) cells. Data represent the mean of three independent experiments (symbols). Error bars are the mean ± SD. Alternatively, cell lysates were analysed by western blot (G). Data representative of two independent experiments.

Source data are available online for this figure.

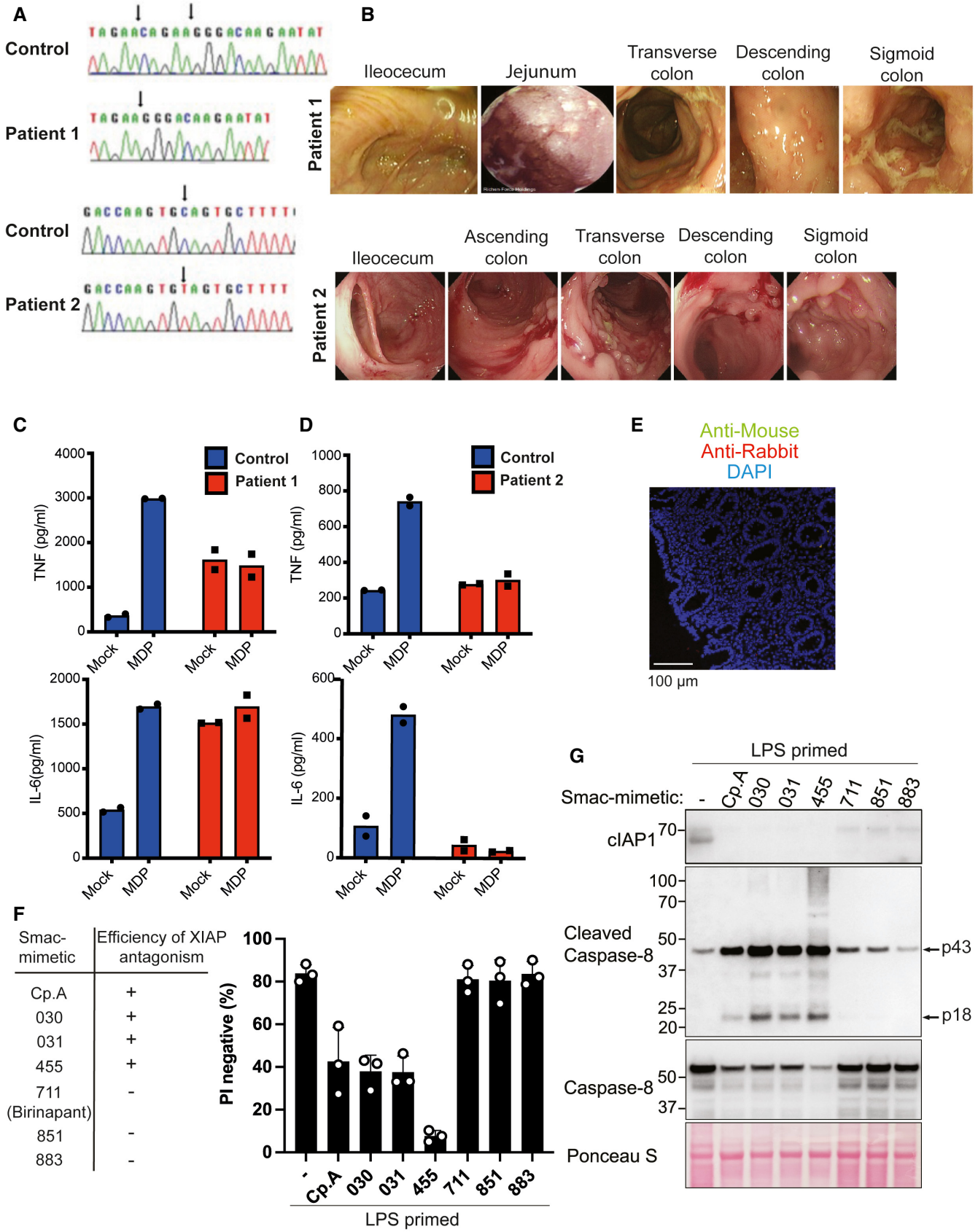
A
Control
Patient 1
Control
Patient 2
B
Ileocecum
Jejunum
Transverse colon
Descending colon
Sigmoid colon
Patient 1
Ileocecum
Ascending colon
Transverse colon
Descending colon
Sigmoid colon
Patient 2
C
Control
Patient 1
TNF (pg/ml)
IL-6(pg/ml)
Mock
MDP
D
Control
Patient 2
TNF (pg/ml)
IL-6 (pg/ml)
E
Anti-Mouse
Anti-Rabbit
DAPI
100 µm
F
Smac-mimetic
Efficiency of XIAP antagonism
Cp.A +
030 +
031 +
455 +
711 (Birinapant) -
851 -
883 -
PI negative (%)
LPS primed
G
LPS primed
Smac-mimetic: - Cp.A 030 031 455 711 851 883
cIAP1
Cleaved Caspase-8
p43
p18
Caspase-8
Ponceau S

Figure EV1.

Figure EV2. Loss of GSDMD and GSDME does not prevent caspase-8-mediated cell death upon IAP loss or TAK1 inhibition.

A–C BMDMs of the indicated genotypes were seeded at a density of 7.5×10^4 cells per well and treated with LPS alone (A) or primed with LPS (100 ng/ml) for 3 h before treatment with either Cp. A (1 μM) (B) or with Cp. A and MCC950 (5 μM) (C). Cell viability was determined through IncuCyte analysis and measured as the proportion of Cytotox Green positive cells versus SPY620-DNA positive cells. Each graph is representative of three independent experiments, data points represent the mean of triplicate wells. Error bars are the mean ± SD.

D BMDMs were seeded at a density of 4×10^5 cells per well and pre-treated with TAK1i (250 nM) for 1 h prior to the addition of LPS (100 ng/nl) for 0, 10, 20, 40 and 60 min. Cell lysates were collected and analysed by western blot. Data represent one experiment.

E, F BMDMs of the indicated genotypes were seeded at a density of 7.5×10^4 cells per well and primed with LPS (100 ng/ml) for 3 h before treatment with either TAK1i (250 nM) (E) or with TAK1i and MCC950 (5 μM) (F). Cell viability was determined through IncuCyte analysis and measured as the proportion of Cytotox Green positive cells versus SPY620-DNA positive cells. Each graph is representative of three independent experiments, data points represent the mean of triplicate wells. Error bars are the mean ± SD.

Source data are available online for this figure.

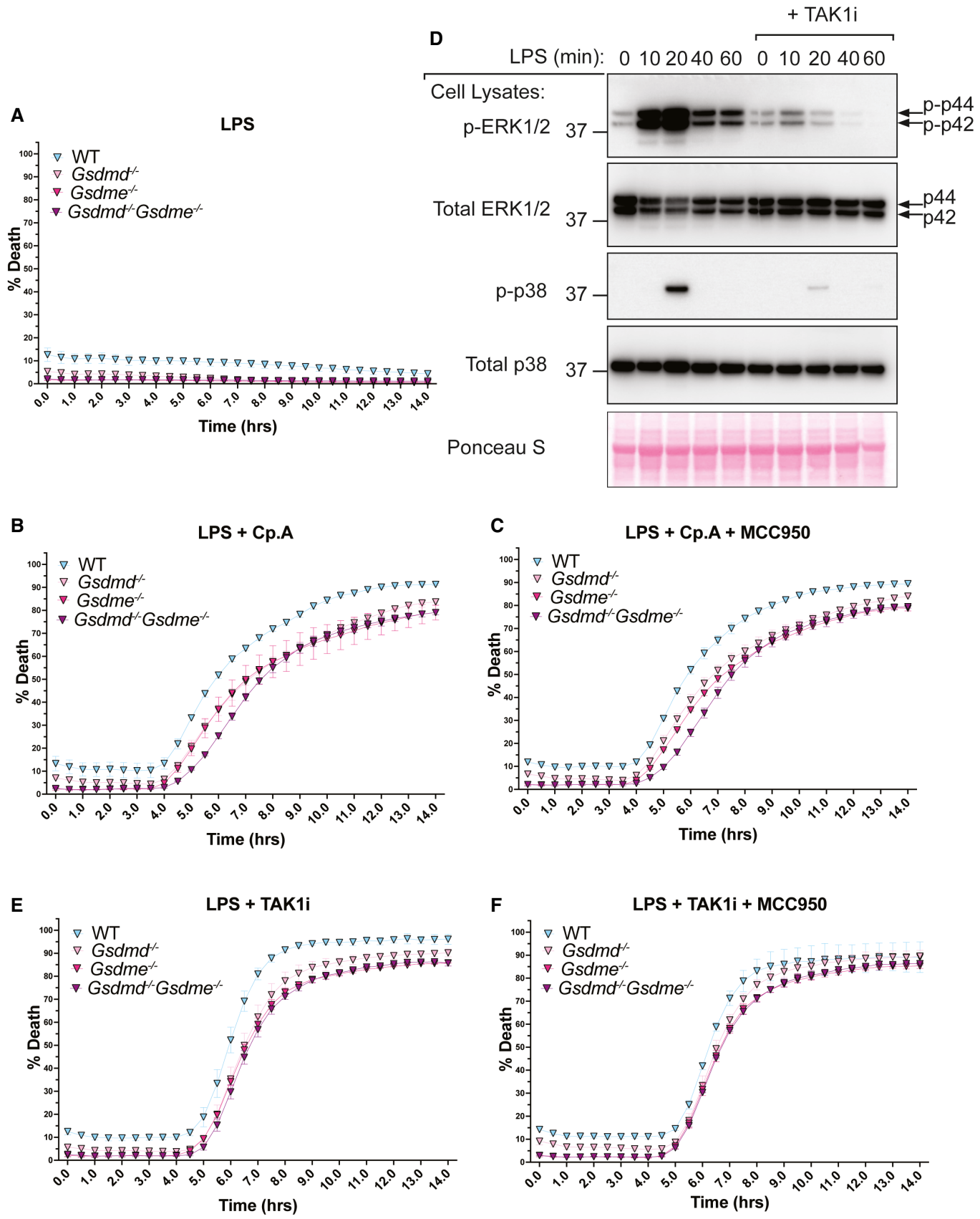
A
LPS
WT
Gsdmd-/-
Gsdme-/-
Gsdmd-/-Gsdme-/-
% Death
Time (hrs)
D
+ TAK1i
LPS (min): 0 10 20 40 60 0 10 20 40 60
Cell Lysates:
p-ERK1/2 37
p-p44
p-p42
Total ERK1/2 37
p44
p42
p-p38 37
Total p38 37
Ponceau S
B
LPS + Cp.A
C
LPS + Cp.A + MCC950
E
LPS + TAK1i
F
LPS + TAK1i + MCC950

Figure EV2.

Figure EV3. Evaluation of Caspase-3, -7 and -9 processing in BMDMs in response to apoptotic and pyroptotic stimuli.

A–D BMDMs of the indicated genotypes were seeded at a density of 4 × 105 cells per well and primed with LPS (100 ng/ml) for 3 h before treatment with Cp. A (1 μM) or TAK1i (250 nM) for 6 h, ABT-737 (1 μM) and CHX (20 μg/ml) for 4 h, or nigericin (10 μM) for 45–60 min. Cell lysates were harvested and analysed by western blot. Data represent 3 (A and C) or 2 (B and D) independent experiments.

Source data are available online for this figure.

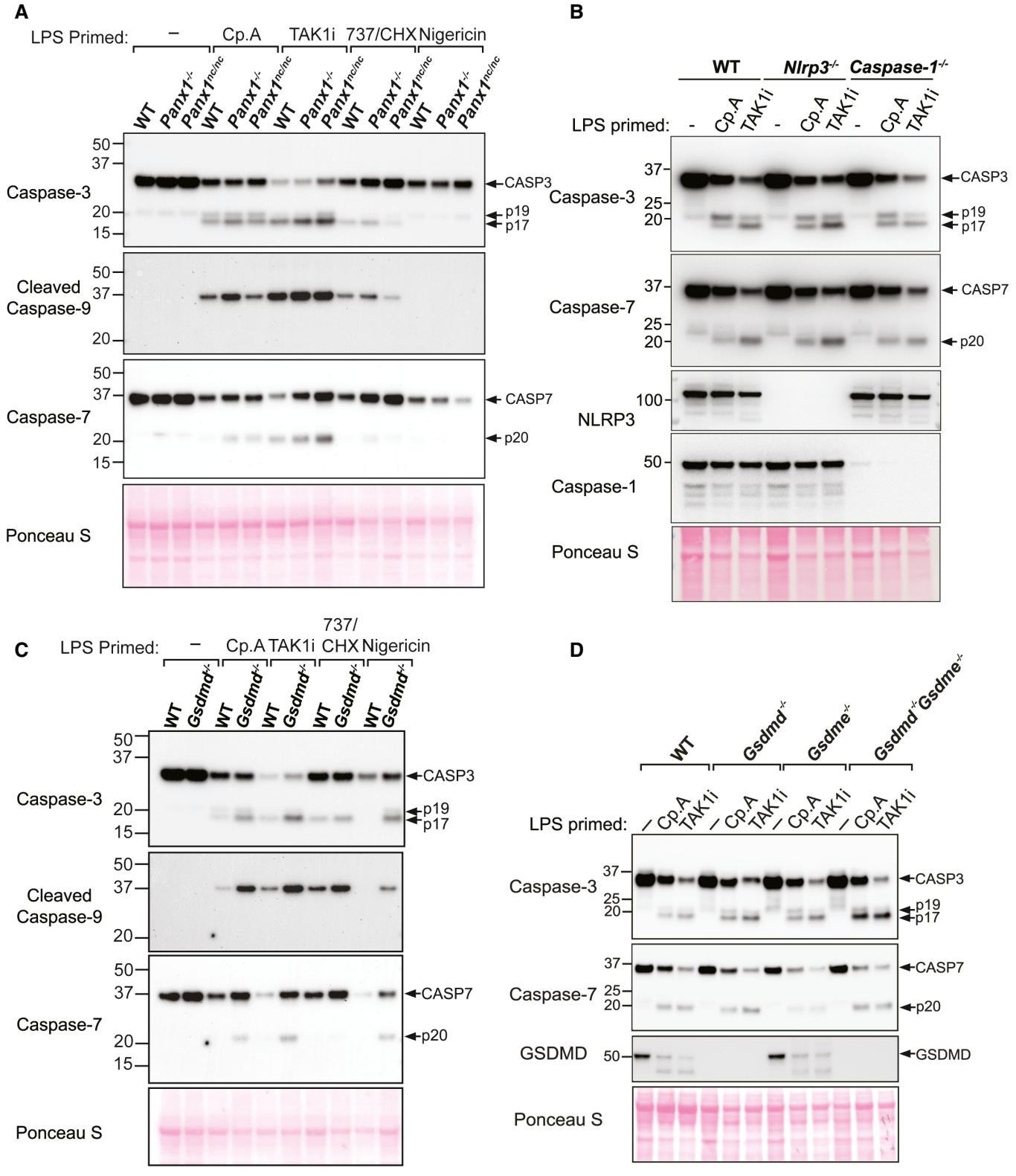
A
LPS Primed: – Cp.A TAK1i 737/CHX Nigericin
WT Panx1-/- Panx1inc/inc
Caspase-3
Cleaved Caspase-9
Caspase-7
Ponceau S
CASP3
p19
p17
CASP7
p20
B
WT Nlrp3-/- Caspase-1-/-
LPS primed: - Cp.A TAK1i
Caspase-3
Caspase-7
NLRP3
Caspase-1
Ponceau S
CASP3
p19
p17
CASP7
p20
C
LPS Primed: – Cp.A TAK1i 737/CHX Nigericin
WT Gsdmd-/-
Caspase-3
Cleaved Caspase-9
Caspase-7
Ponceau S
CASP3
p19
p17
CASP7
p20
D
WT Gsdmd-/- Gsdme-/- Gsdmd-/-Gsdme-/-
LPS primed: – Cp.A TAK1i
Caspase-3
Caspase-7
GSDMD
Ponceau S
CASP3
p19
p17
CASP7
p20
GSDMD

Figure EV3.

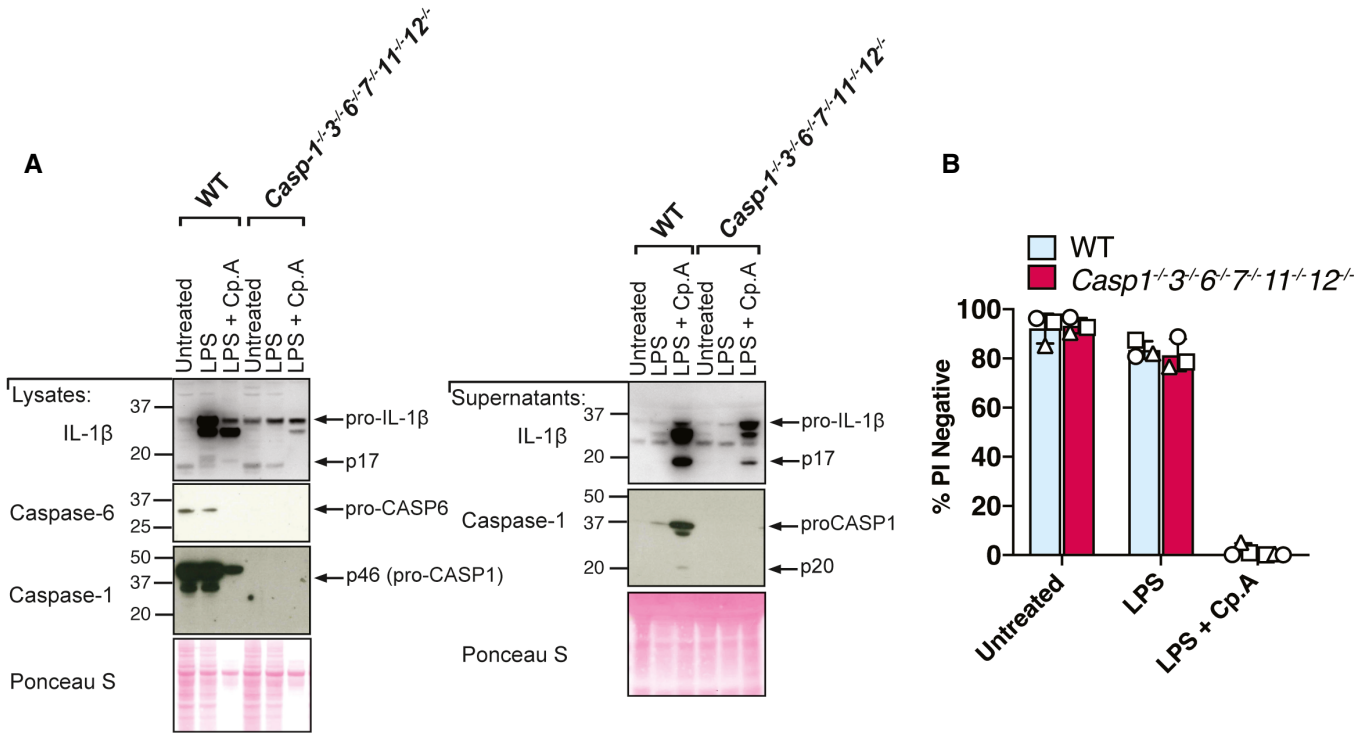

Figure EV4. In the absence of caspase-1, -3 and -7, cell death and IL-1β release upon IAP inhibition do not depend on caspase-6.

A, B iBMDMs of the indicated genotypes were seeded at a density of 3 × 105 cells per well and primed with 100 ng/ml of LPS for 3 h before treatment with Cp. A (2 μM) for 24 h. Total cell lysates and supernatants were analysed by western blot (A) or cell viability determined by propidium iodide (PI) uptake and flow cytometry (B). Data representative of three independent experiments (A), or three independent experiments (symbols) are shown (B). Error bars represent the mean ± SD.

Source data are available online for this figure.

Figure EV5. Caspase-3 and -7 deletion does not prevent fibroblast death upon IAP inhibition, and caspase-8 can process GSDMD into the p30 fore-forming fragment.

A Western blot showing CRISPR/Cas9 targeting of MCL-1 (polyclonal population) and caspase-8 processing in SV40T immortalised MEFs derived from two separate *caspase-3*$^{-/-}$*caspase-7*$^{-/-}$ (*C3*$^{-/-}$*C7*$^{-/-}$) embryos treated with or without ABT-737 (1 μM) or etoposide (34 μM) for 24 h. Data represent one experiment.

B, C SV40T immortalised MEFs of the indicated genotypes (*caspase-3*$^{-/-}$*caspase-7*$^{-/-}$ MEFs generated from two mice shown in C) were treated with ABT-737 (1 μM), etoposide (34 μM), TNF (100 ng/ml) or the IAP antagonist birinapant (1 μM) as depicted for 24 h and cell death analysed by PI uptake and flow cytometry. Three to five independent experiments (symbols) are shown. Error bars represent the mean ± SD.

D 293 T cells were transfected with the indicated cDNAs and caspase-1- or caspase-8-mediated processing of FLAG-tagged GSDMD evaluated by western blot. Ponceau staining depicts protein loading. One of two experiments.

Source data are available online for this figure.

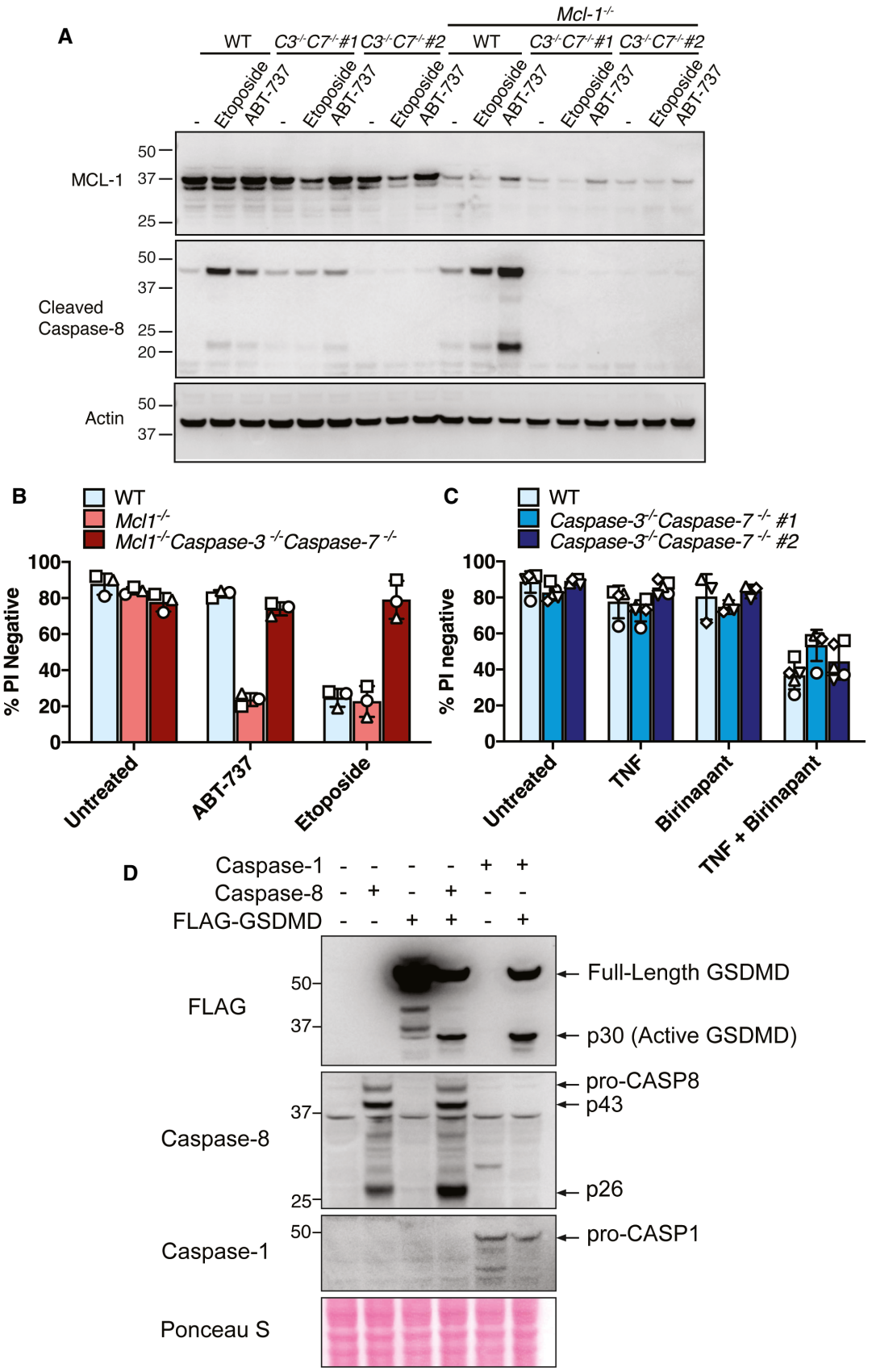
A
Mcl-1-/-
WT
C3-/-C7-/-#1
C3-/-C7-/-#2
WT
C3-/-C7-/-#1
C3-/-C7-/-#2
- Etoposide ABT-737
MCL-1
Cleaved Caspase-8
Actin
50
37
25
20
B
WT
Mcl1-/-
Mcl1-/-Caspase-3-/-Caspase-7-/-
% PI Negative
Untreated
ABT-737
Etoposide
C
WT
Caspase-3-/-Caspase-7-/- #1
Caspase-3-/-Caspase-7-/- #2
% PI negative
Untreated
TNF
Birinapant
TNF + Birinapant
D
Caspase-1
Caspase-8
FLAG-GSDMD
FLAG
Full-Length GSDMD
p30 (Active GSDMD)
Caspase-8
pro-CASP8
p43
p26
Caspase-1
pro-CASP1
Ponceau S

Figure EV5.