Cell Reports, Volume *21*

Supplemental Information

Caspase-1 Engagement and TLR-Induced c-FLIP Expression Suppress ASC/Caspase-8-Dependent Apoptosis by Inflammasome Sensors NLRP1b and NLRC4

Nina Van Opdenbosch, Hanne Van Gorp, Maarten Verdonckt, Pedro H.V. Saavedra, Nathalia M. de Vasconcelos, Amanda Gonçalves, Lieselotte Vande Walle, Dieter Demon, Magdalena Matusiak, Filip Van Hauwermeiren, Jinke D'Hont, Tino Hochepied, Stefan Krautwald, Thirumala-Devi Kanneganti, and Mohamed Lamkanfi

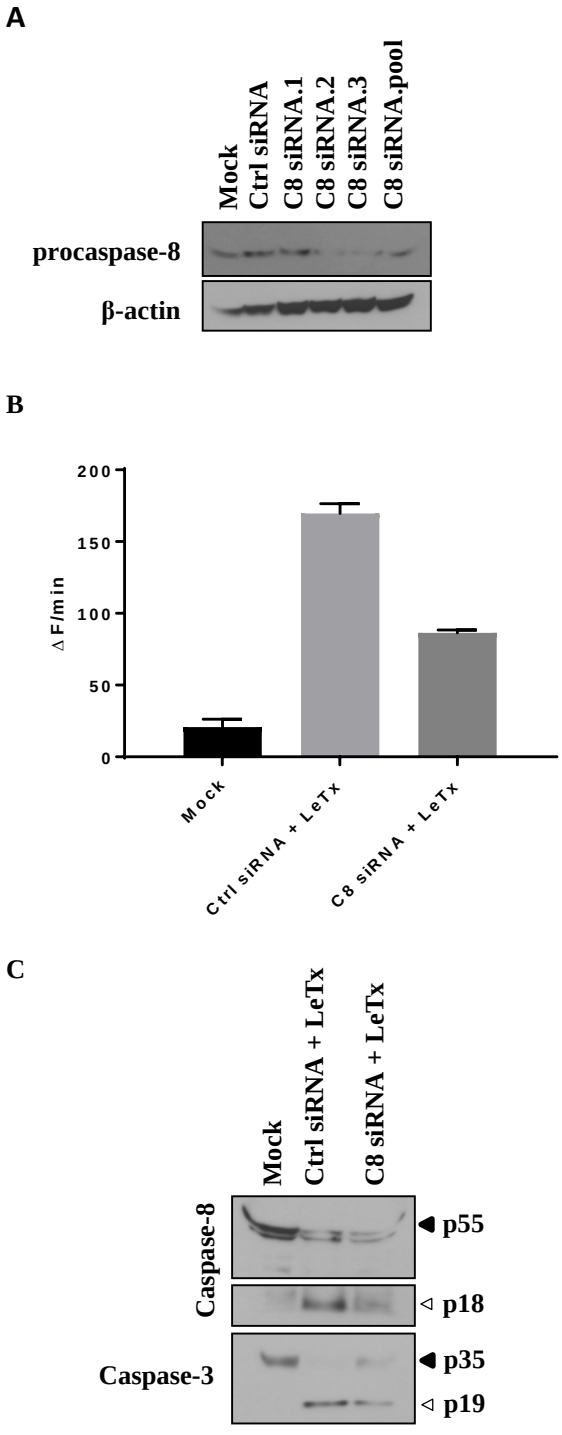

Figure S1 related to Figure 1. Caspase-8 mediates LeTx-induced apoptosis. A, *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$* BMDMs were transfected with scrambled and caspase-8 siRNA before lysates were immunoblotted for caspase-8 and β-actin. **B-C**, *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$* BMDMs were transfected with scrambled and caspase-8 siRNA, followed by LeTx treatment and analysis of SYTOX Green incorporation and DEVD-amc conversion (**B**). In parallel, lysates were prepared and immunoblotted for caspase-8 and caspase-3 (**C**).

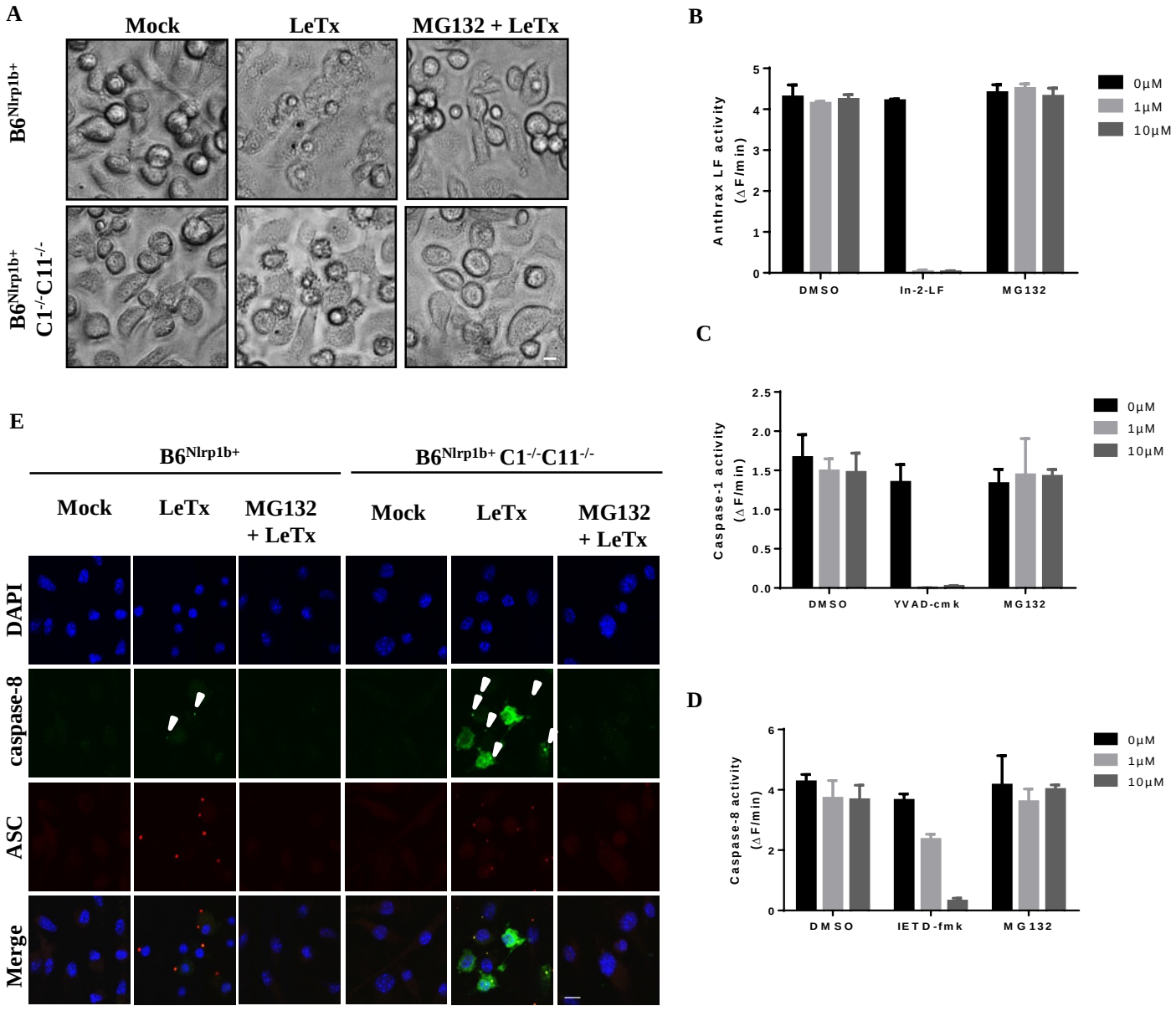

Figure S2 related to Figure 2. Proteasome inhibitor MG132 is able to inhibit both pyroptosis and apoptosis induced by LeTx, while it has no effect on the enzymatic activity of anthrax lethal factor, caspase-1 or caspase-8. **A**, *B6$^{Nlrp1b+}$* and *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$* BMDMs were treated or not with proteasome inhibitor MG132 for 30 minutes prior to stimulation with LeTx for 3 h before brightfield images (scale bar = 20μm) were acquired and cells were collected. Data are representative of results from three independent experiments (**A**). **B**, Recombinant LF was incubated with either DMSO, In-2-LF or MG132 with indicated concentrations and activity was measured against anthrax lethal factor substrate III (**B**). **C**, Recombinant caspase-1 was incubated with DMSO, Ac-YVAD-cmk and MG132 at indicated concentrations and activity was measured against the fluorogenic caspase-1 substrate peptide Ac-WEHD-amc (**C**). **D**, Recombinant caspase-8 was incubated with DMSO, z-IETD-fmk and MG132 at indicated concentrations and activity was measured against the fluorogenic caspase-8 substrate peptide Ac-IETD-amc (**D**). Data are shown as mean ± s.d. from a single representative experiment of two independent experiments, with each condition performed in triplicate. **E**, BMDMs from *B6$^{Nlrp1b+}$* and *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$* mice were either or not pretreated with the proteasome inhibitor MG132 for 30 minutes followed by LeTx stimulation for another 105 minutes. Cells were fixed with 4 % paraformaldehyde and stained for DAPI (blue), caspase-8 (green) and ASC (red). Confocal images were taken on an Olympus microscope using a x60 objective lens (scale bar = 10μm) (**E**). Data are representative of results from two independent experiments

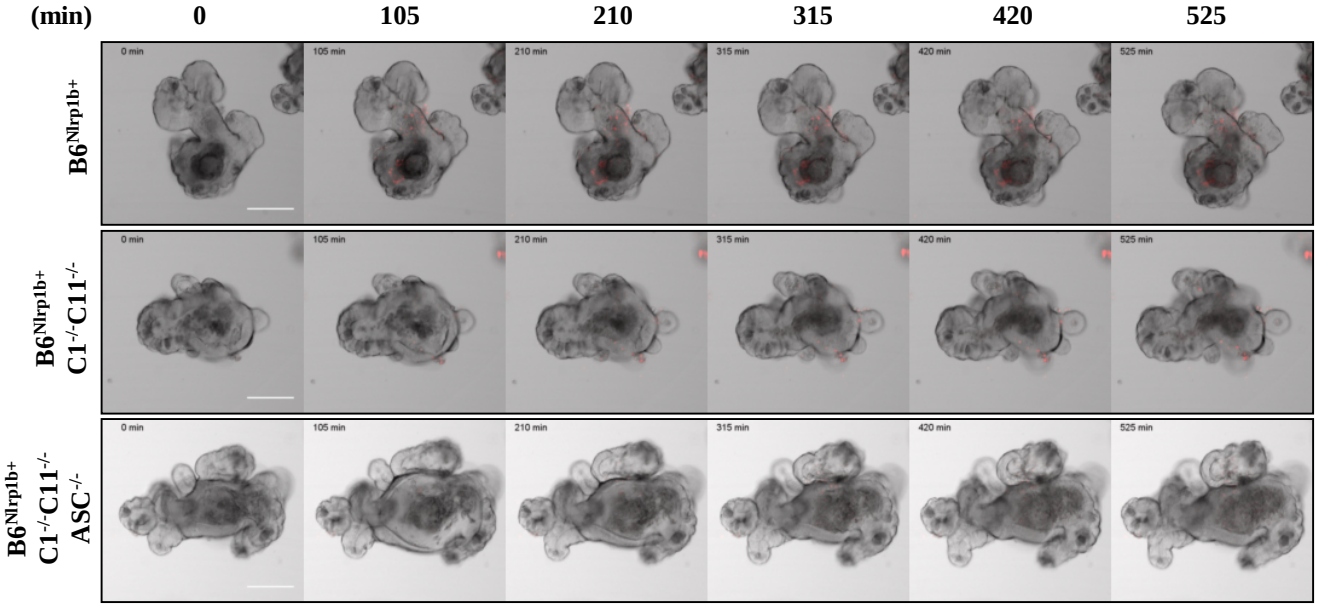

Figure S3 related to Figure 3. Culture of primary intestinal epithelial organoids do not undergo spontaneous cell death. Primary intestinal epithelial organoids from *B6$^{Nlrp1b+}$*, *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$* and *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$ ASC$^{-/-}$* were left unstimulated and followed in time for 16 h in the presence of propidium iodide (PI). All data are representative of results from three independent experiments (scale bar = 100µm).

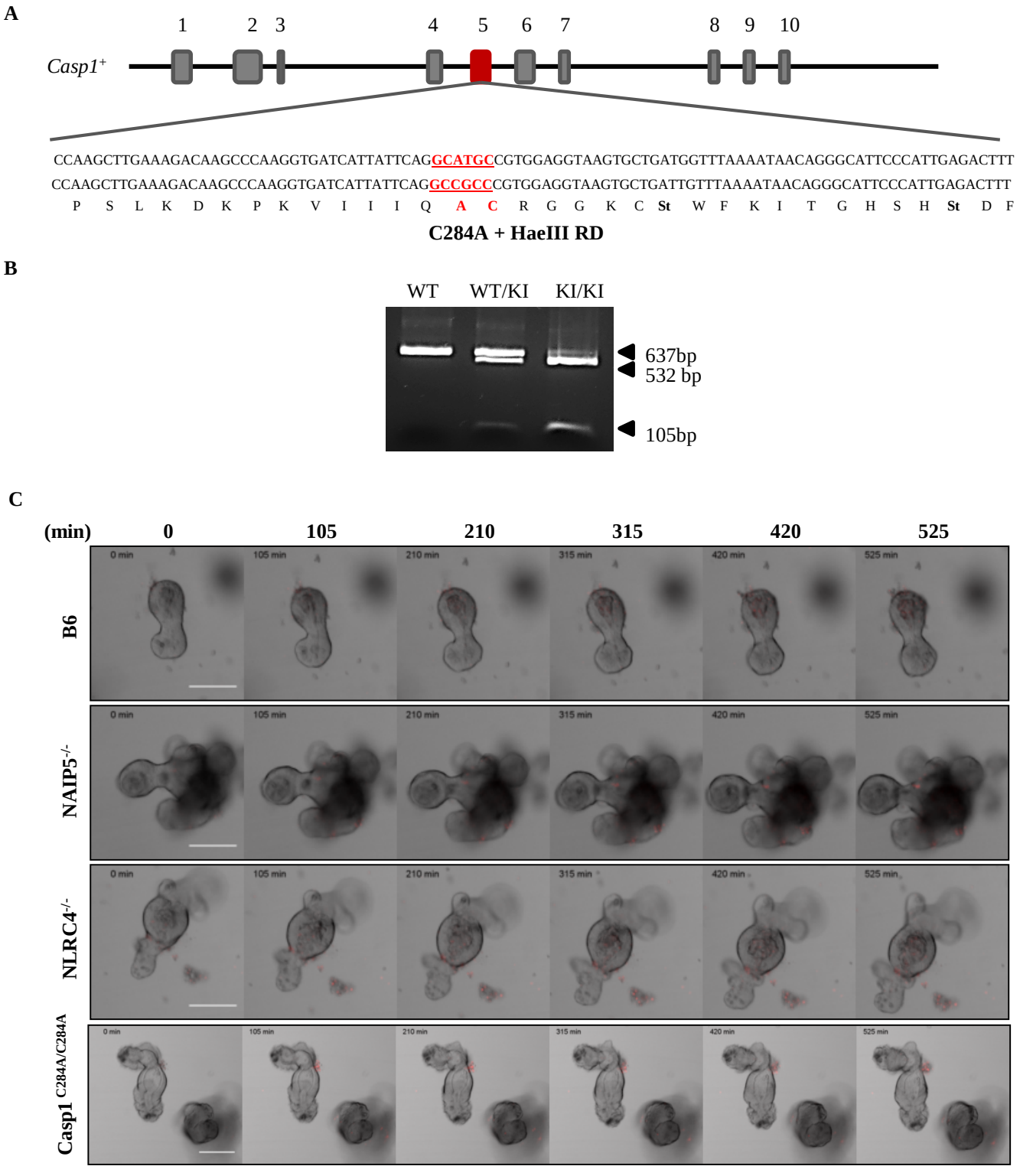

Figure S4 related to Figure 4. Caspase-1 catalytic dead mutant mice have the C284A mutantion in exon 5. A-B, Location of the knock-in mutation C284A in the caspase-1 gene with the generation of a new HaeIII restriction site (**A**). Genotyping results of wildtype, heterozygous and homozygous knock-in mice (**B**). **C,** Primary intestinal epithelial organoids from *B6*, *NAIP5*$^{-/-}$, *NLRC4*$^{-/-}$ and *C1*$^{C284A/C284A}$ were left unstimulated and followed in time for 16 h in the presence of propidium iodide (PI) (scale bar = 100µm) (**C**). All data are representative of results from three independent experiments.

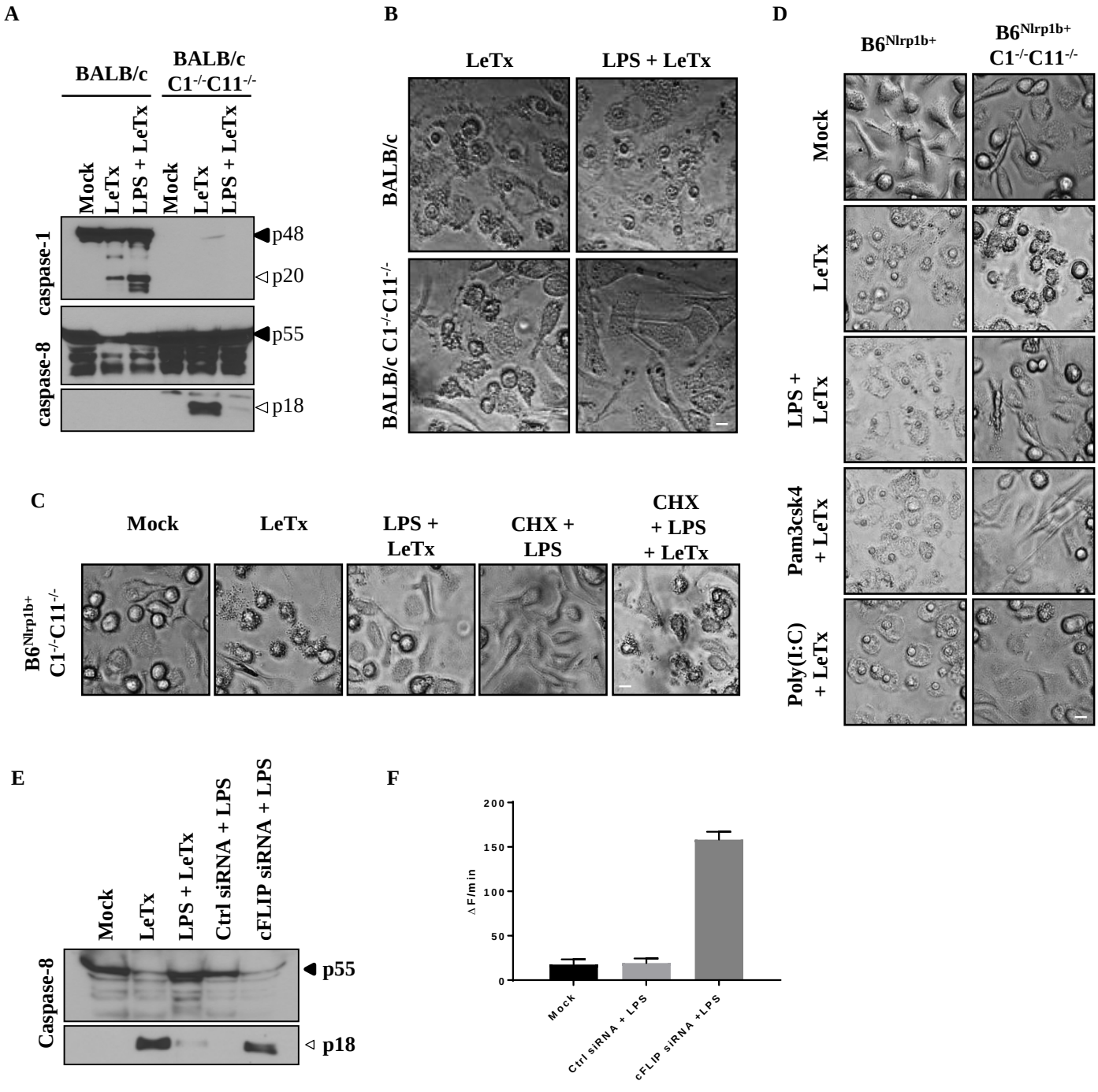

Figure S5 related to Figure 6. TLR-mediated inhibition of LeTx-induced apoptosis induction. A, B, BMDMs from wildtype mice or mice lacking caspases 1 and 11 in *BALB/c* genetic background were either or not primed with LPS for 3 h followed by stimulation with LeTx for another 3 h. Lysates were collected and immunoblotted for caspase-1 and -8 (**A**). Brightfield images were acquired from cells before collecting lysates (**B**). **C**, *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$* BMDMs were treated with cyclohexamide (CHX) prior to priming with LPS and LeTx-treatment for 3 h and brightfield images were acquired (**C**). **D**, *B6$^{Nlrp1b+}$* and *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$* BMDMs were left unprimed or primed with LPS, Pam3CSK4 or poly(I:C) for 3 h followed by LeTx stimulation. After 3 h of stimulation, brightfield images were taken before cells were collected (**D**). Data are representative of results from three independent experiments. **E-F**, Scrambled and c-FLIP siRNA transfection was performed on *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$* BMDMs followed by LPS. Subsequently, lysates were prepared and were immunoblotted for caspase-8 (**E**). Also a quantitative SYBR Green and DEVD-amc assay was performed (**F**). Scale bar is 20µm. All data are representative of results from three independent experiments.

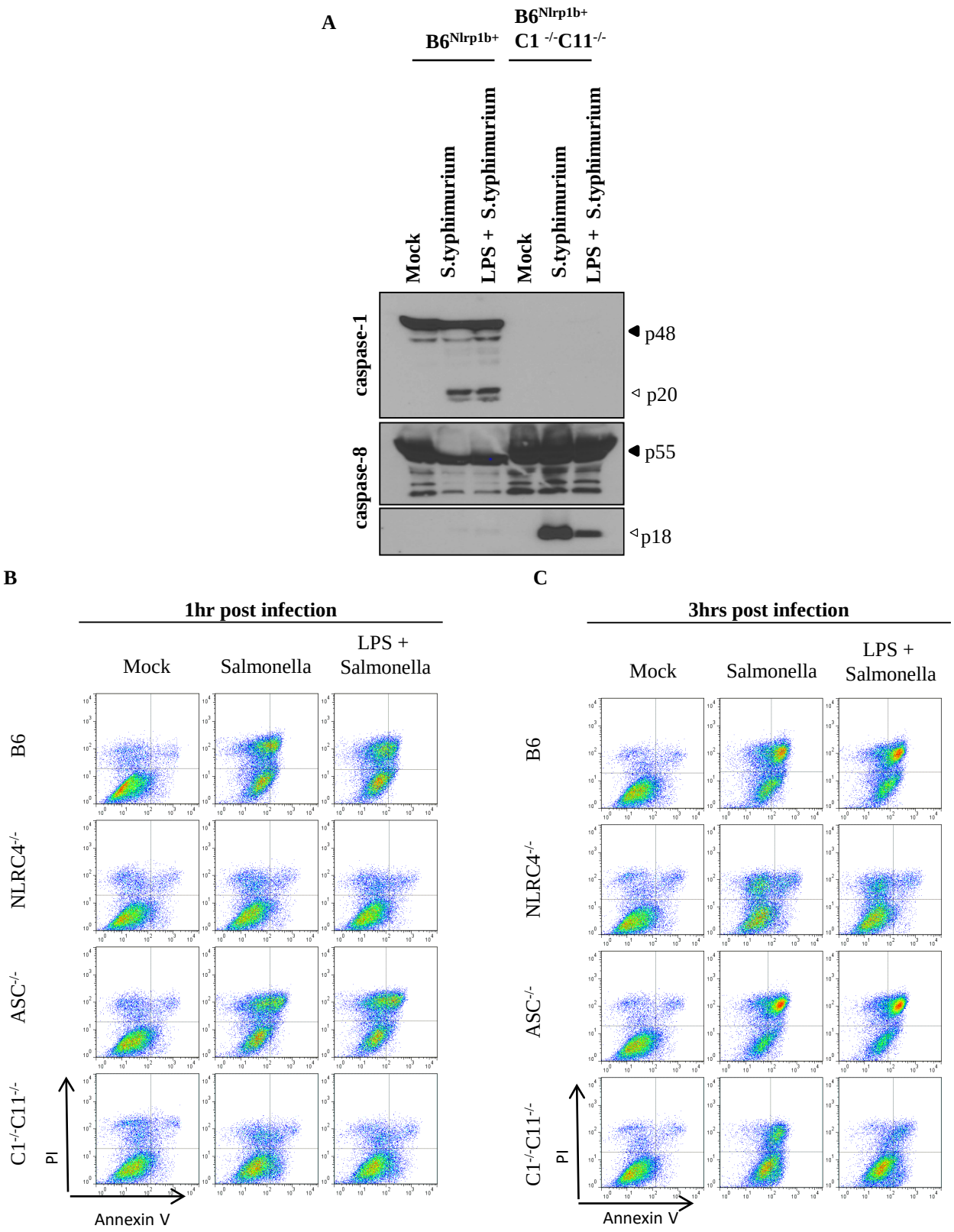

Figure S6 related to Figure 7. Priming-induced expression of c-FLIP prevents NLR-mediated apoptosis. A, LPS-primed *B6$^{Nlrp1b+}$* and *B6$^{Nlrp1b+}$C1$^{-/-}$C11$^{-/-}$* BMDMs were infected with *S.* typhimurium (M.O.I. 50) for 2 h and lysates were immunoblotted for caspases-1 and -8 (**A**). **B-C**, BMDMs from wildtype, *NLRC4$^{-/-}$*, *ASC$^{-/-}$* and *C1$^{-/-}$C11$^{-/-}$* mice were left unprimed or primed with LPS for 3 h and subsequently infected with *S.* typhimurium (M.O.I. 50) for 1 hr or 3 h. AnnexinV-PI staining was performed on stimulated cells and analysed by FACS (**B, C**). Data are representative of results from two independent experiments.