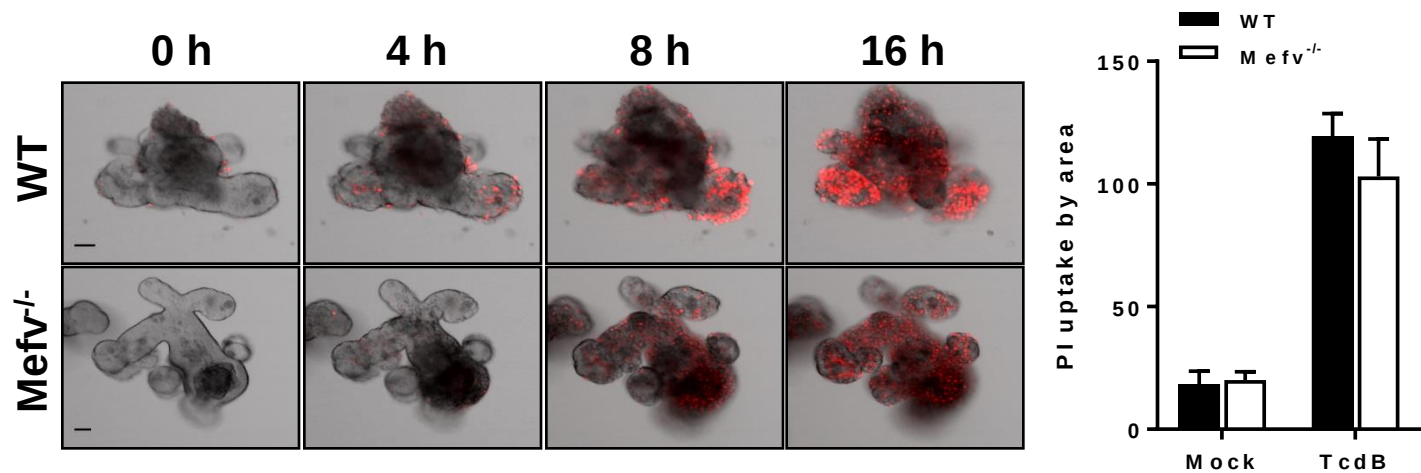


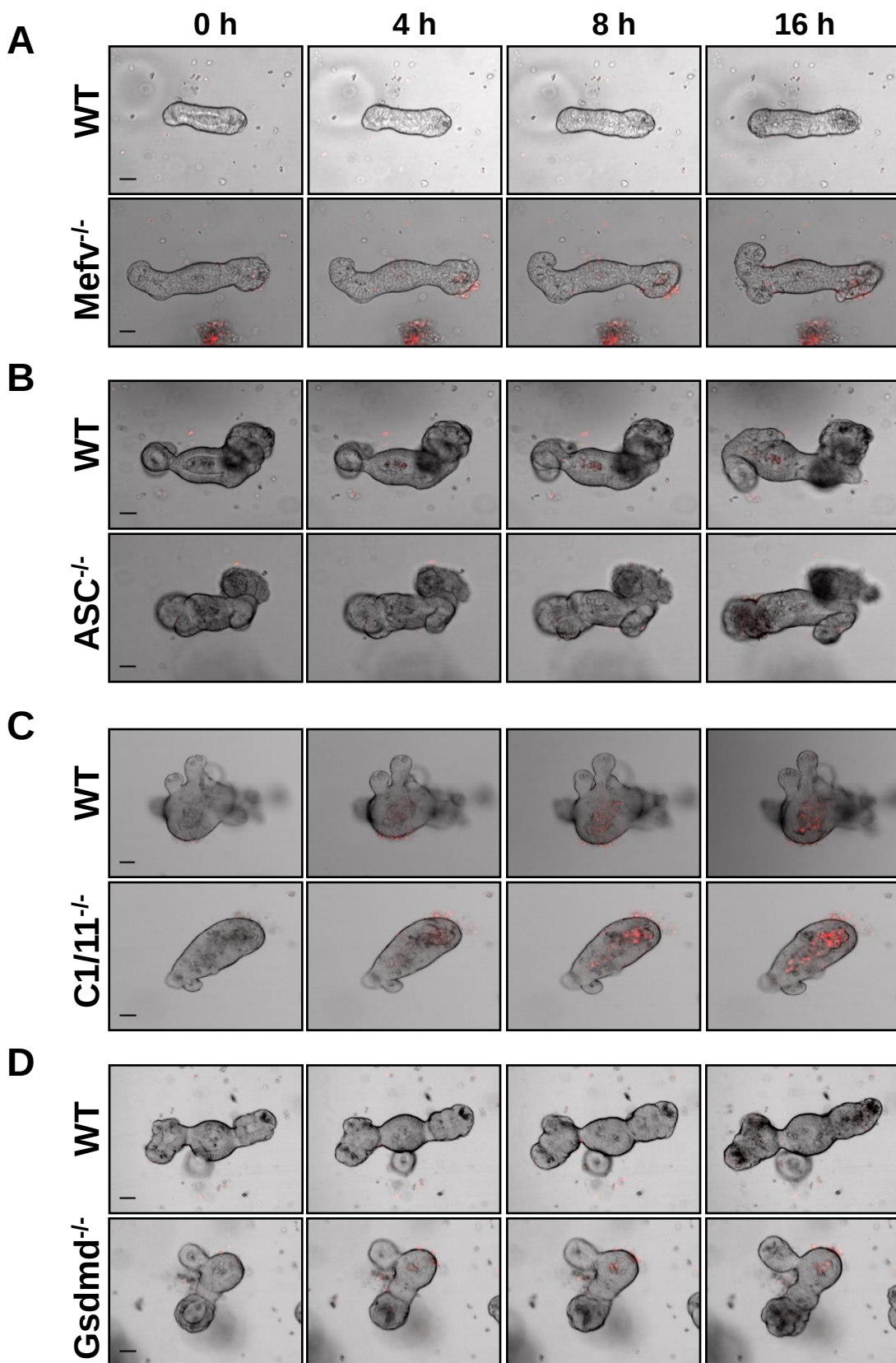
Supplementary Information for:

Apoptosis of intestinal epithelial cells restricts *Clostridium difficile* infection in a model of pseudomembranous colitis

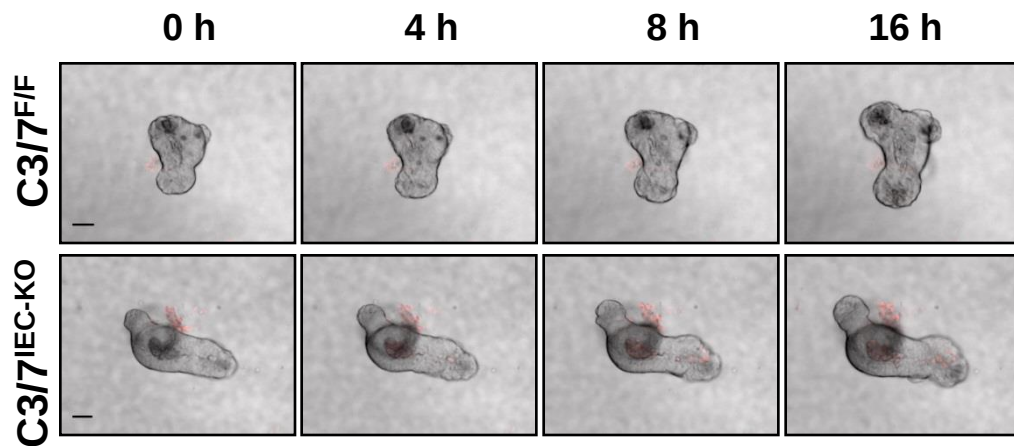
Saavedra et al.



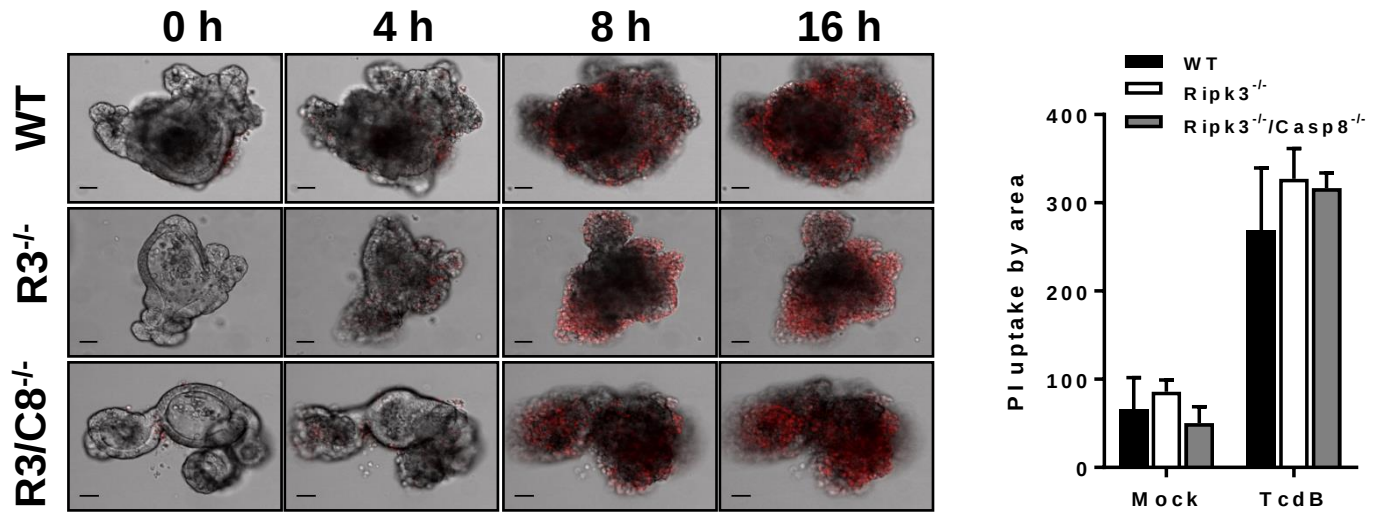
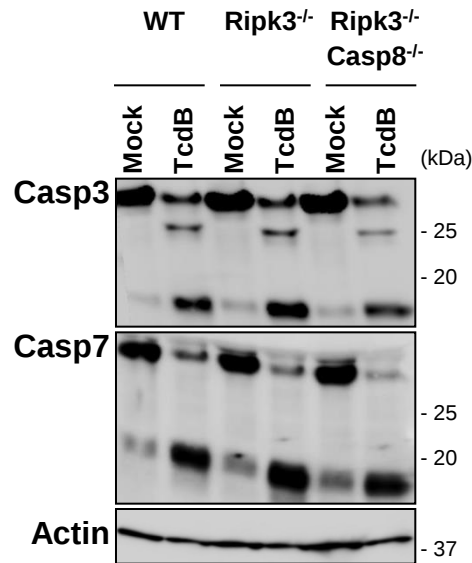
Supplementary Figure 1. Pyrin inflammasome activation and pyroptosis are dispensable for TcdB-induced IEC cytotoxicity. Primary intestinal organoids from wildtype (WT) and *Mefv*^{-/-} mice were stimulated with TcdB before PI incorporation was analyzed by live-imaging for 16 h. Graphs correspond to PI quantification plotted by organoid area. Scale bars: 30 μ m. Data are representative of at least 3 independent experiments.



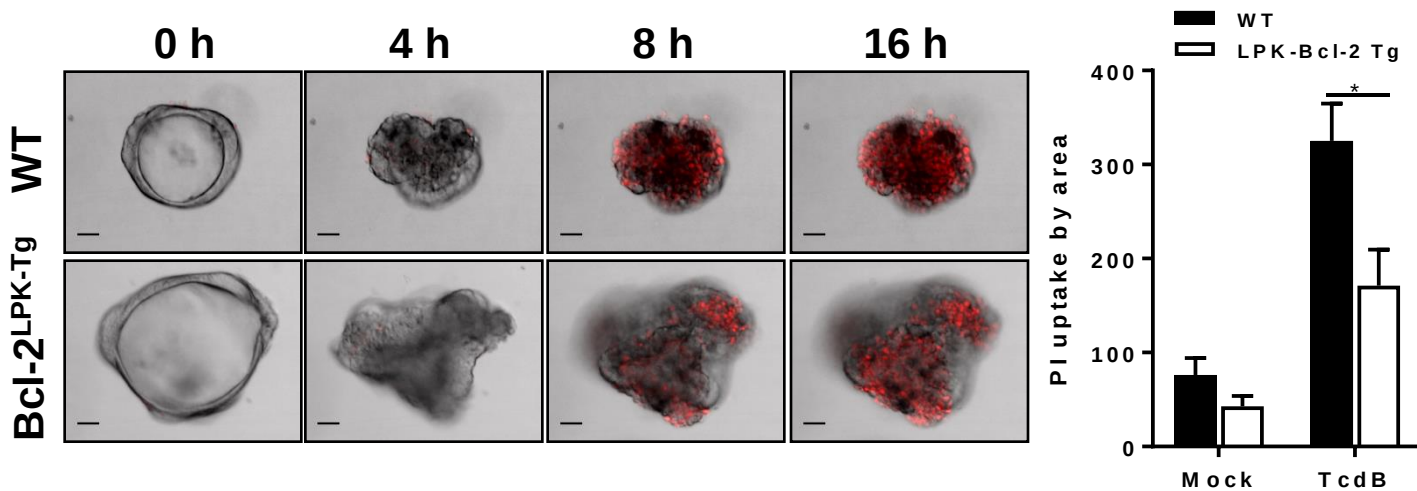
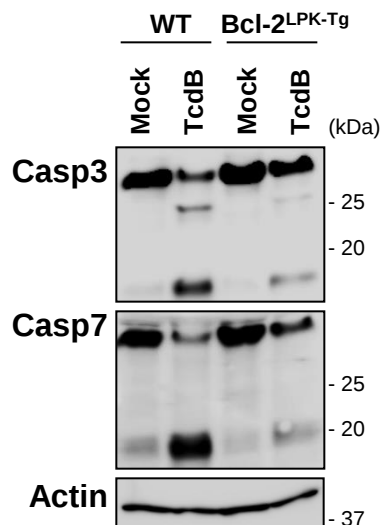
Supplementary Figure 2. Imaging control of *Mefv*^{-/-}, *Asc*^{-/-}, *Casp1/11*^{-/-} and *Gsdmd*^{-/-} organoids. A-D, Unstimulated primary intestinal organoids from (A) *Mefv*^{-/-}, (B) *Asc*^{-/-}, (C) *Casp1/11*^{-/-} or (D) *Gsdmd*^{-/-} mice analyzed by live-imaging for 16 h. Scale bars: 30 μ m. Data are representative of at least 3 independent experiments.



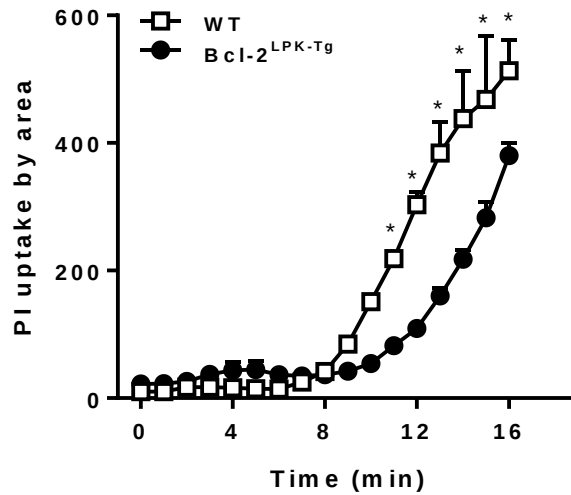
Supplementary Figure 3. Imaging of control *Casp3/7^{F/F}* and *Casp3/7^{IEC-KO}* IEC organoids. Unstimulated primary intestinal organoids from *Casp3/7^{F/F}* and *Casp3/7^{IEC-KO}* mice were analyzed by live-imaging for 16 h. Scale bars: 30 μ m. Data are representative of 3 independent experiments.

A**B**

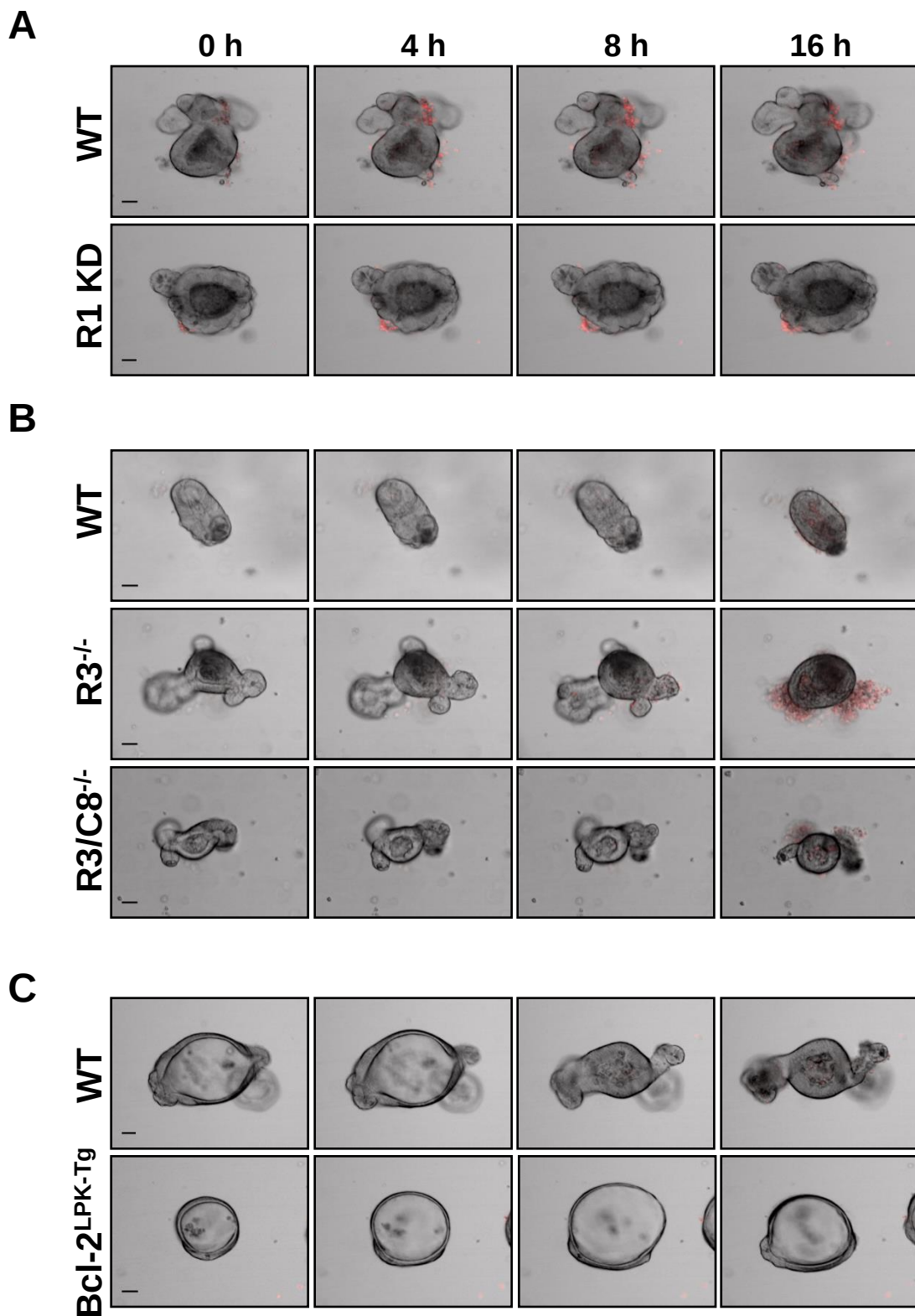
Supplementary Figure 4. RIP kinases and caspase-8 are dispensable for TcdB-induced IEC killing. (A) Primary intestinal organoids from wildtype (WT), *Ripk3*^{-/-} and *Ripk3*^{-/-}*Casp8*^{-/-} mice were stimulated with TcdB before PI incorporation was analyzed by live-imaging for 16 h. Graphs depict PI quantification plotted by organoid area. Scale bars: 30 μm. (B) Cell lysates from primary intestinal organoids of *Ripk3*^{-/-} and *Ripk3*^{-/-}*Casp8*^{-/-} or TcdB were immunoblotted for the indicated proteins. Data are representative of 3 independent experiments.

A**B**

Supplementary Figure 5. Bax/Bak pores contribute to TcdB-induced IEC killing. (A) Primary intestinal organoids from wildtype (WT) and *Bcl-2*^{LPK-Tg} mice were stimulated with TcdB before PI incorporation was analyzed by live-imaging for 16 h. Graphs depict PI quantification plotted by organoid area. Scale bars: 30 μ m. **(B)** Cell lysates from primary intestinal organoids of *Bcl-2*^{LPK-Tg} stimulated with TcdB were immunoblotted for the indicated proteins. Data are representative of 3 independent experiments. Data are shown as mean $\pm$ SD and were analyzed with 2-way ANOVA. *P < 0.05, **P < 0.01, and ***P < 0.001.

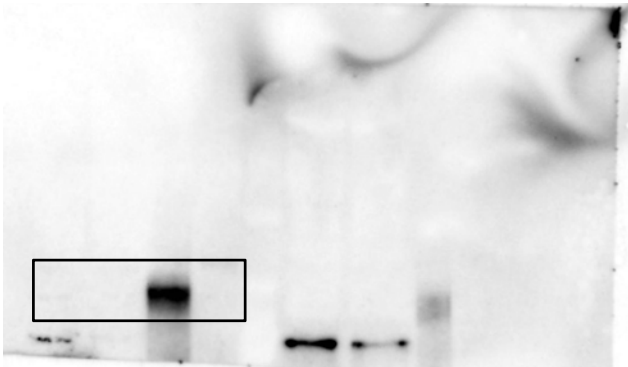


Supplementary Figure 6. RIP kinases and caspase-8 are dispensable, whereas Bax/Bak pores contribute to TcdA-induced IEC killing. Primary intestinal organoids from wildtype and *Bcl-2^{LPK-Tg}* mice were stimulated with TcdA before PI incorporation was analyzed by live-imaging for 16 h. Graphs depict PI quantification plotted by organoid area in intervals of 1 h. Data are representative of 3 independent experiments. Data are shown as mean $\pm$ SD and were analyzed with 2-way ANOVA. *P < 0.05, **P < 0.01, and ***P < 0.001.

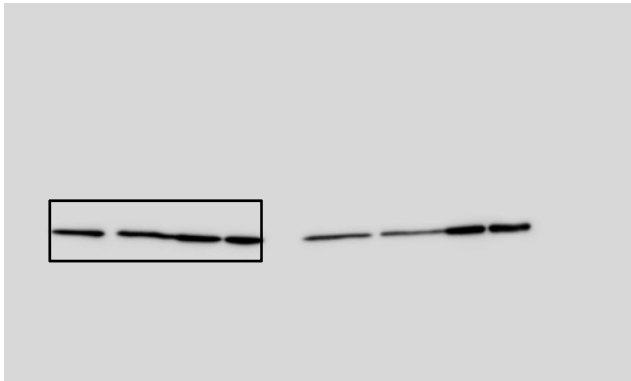


Supplementary Figure 7. Imaging control of IEC organoids. A-C, Unstimulated primary intestinal organoids from (A) *Ripk1*^{D138N} (Ripk1 kinase dead), (B) *Ripk3*^{-/-} and *Ripk3*^{-/-}*Casp8*^{-/-} or (C) *Bcl-2*^{LPK-Tg} mice were analyzed by live-imaging for 16 h. Scale bars: 30 μ m. Data are representative of 3 independent experiments.

Fig. 2C



Pyrin



Actin

Fig. 3A

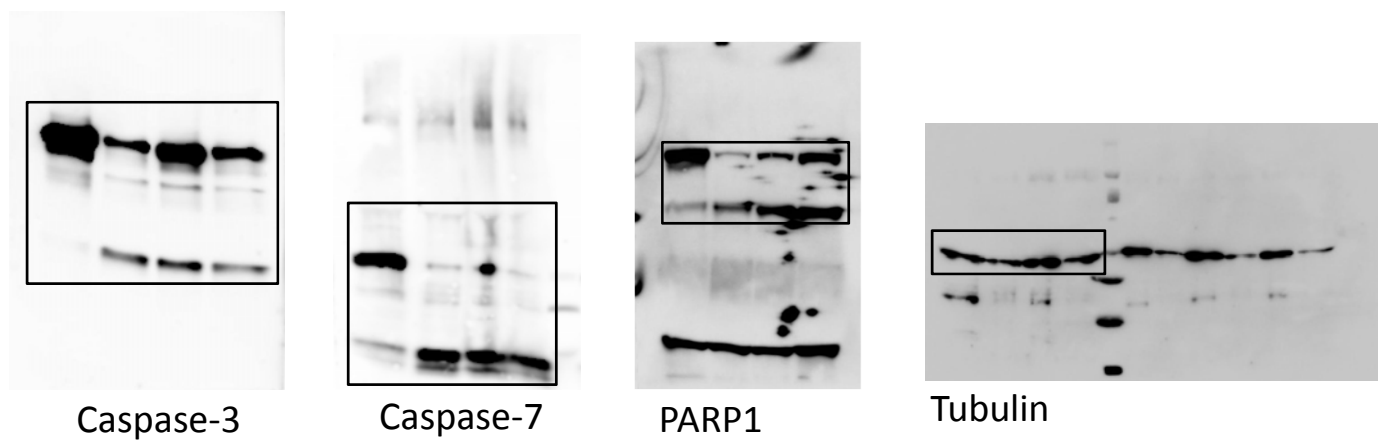


Fig. 3B

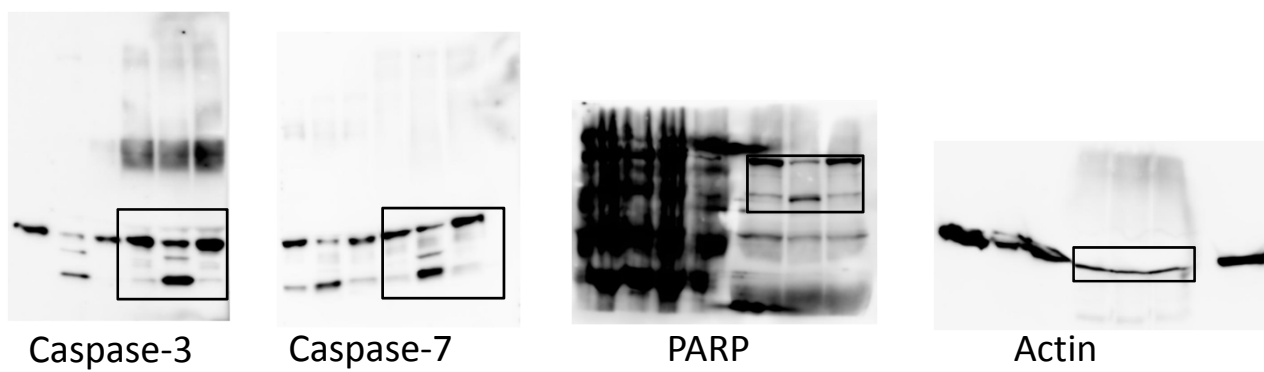


Fig. 3D

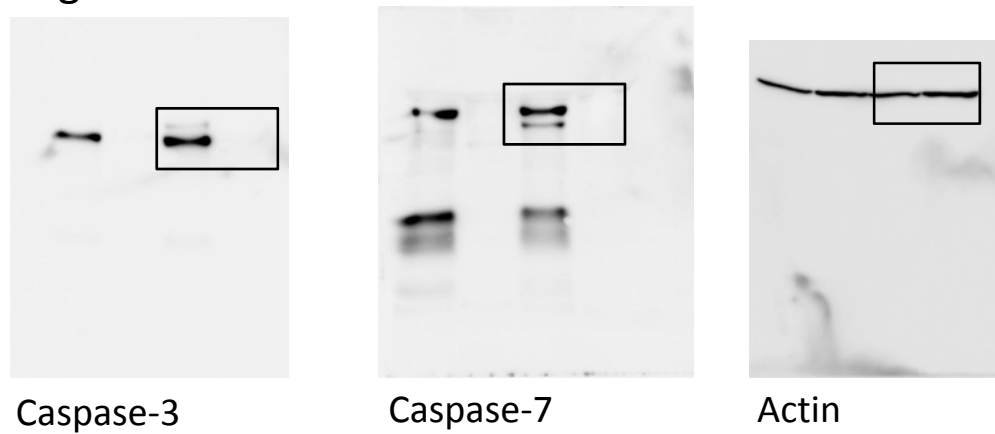


Fig. 4D

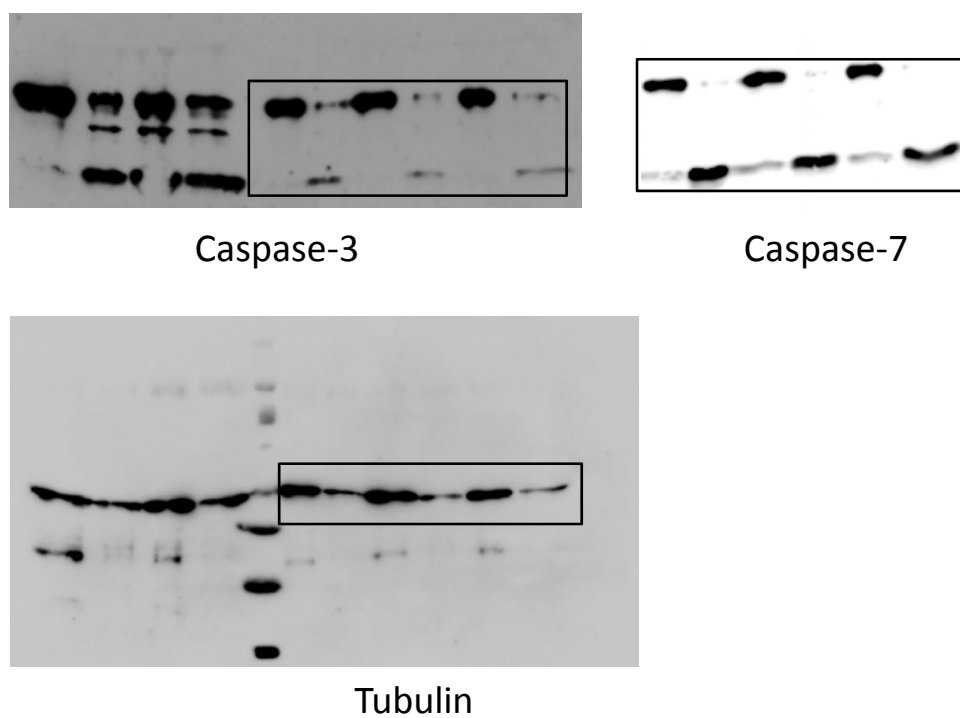


Fig. 4E

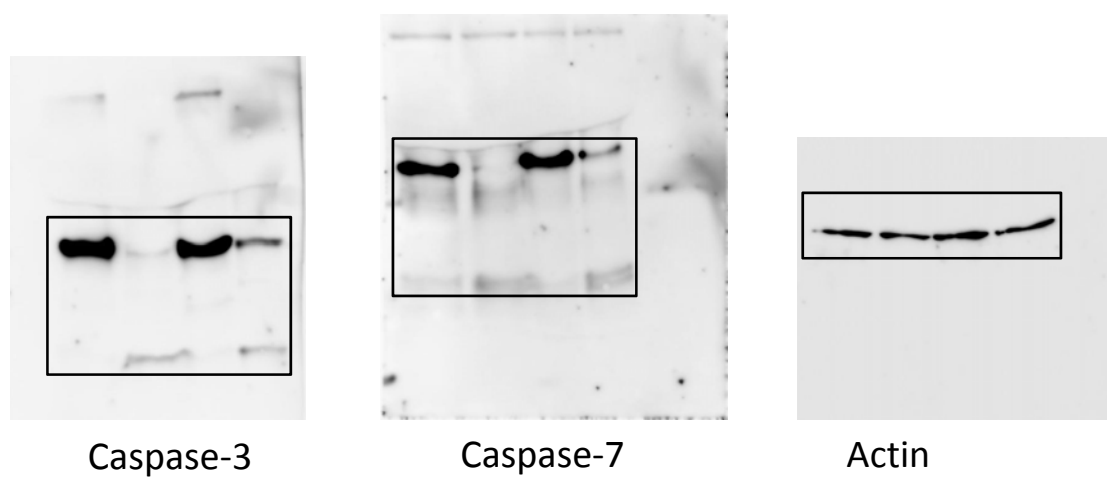
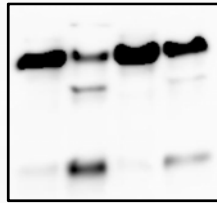
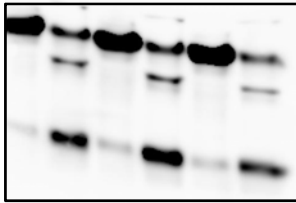


Fig. S4B

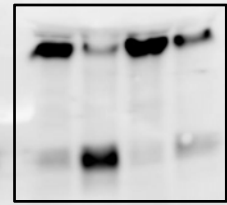
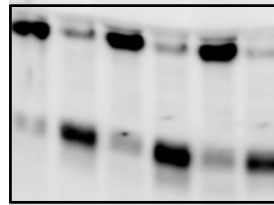
Fig. S5B



Caspase-3

Fig. S4B

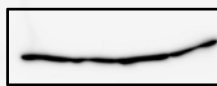
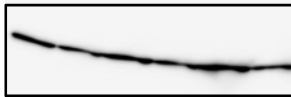
Fig. S5B



Caspase-7

Fig. S4B

Fig. S5B



Actin