Supporting Information

Croker et al. 10.1073/pnas.1110358108

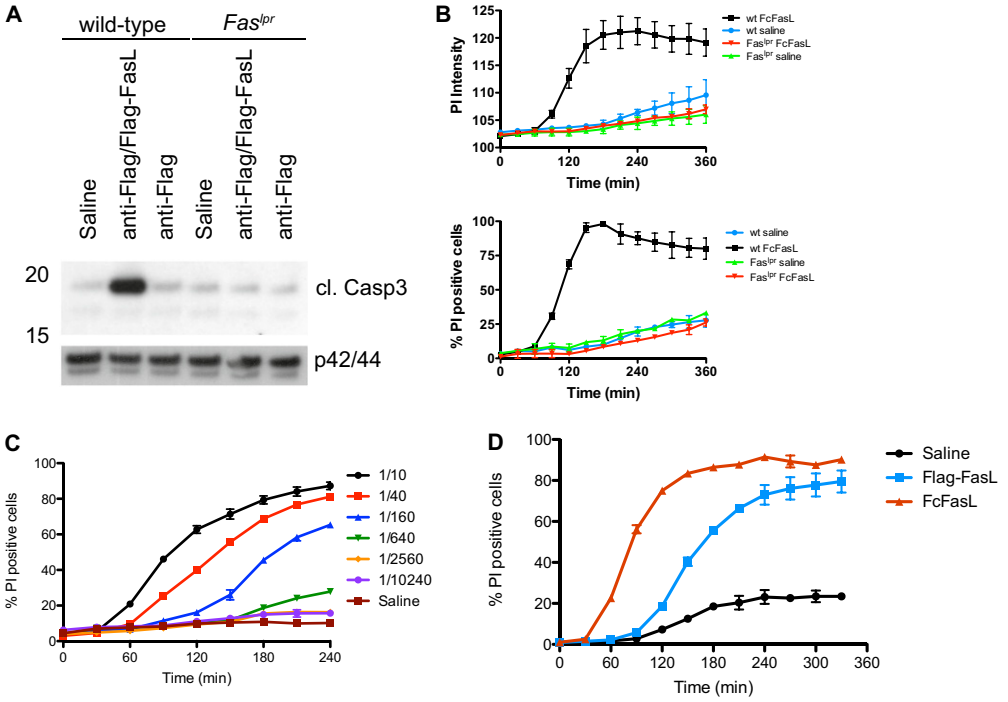


Fig. S1. FasL rapidly induces apoptosis of neutrophils. (*A*) Neutrophils from wild-type and *Fas*^{lpr} mutant mice were stimulated for 3 h with recombinant Flag-tagged FasL crosslinked with anti-Flag antibody, or anti-Flag antibody alone or saline. Apoptosis was assessed by immunoblot using antibodies to cleaved caspase-3 or ERK as a control. (*B*) Neutrophils from wild-type or *Fas*^{lpr} mice were treated with FcFasL and viability monitored by staining with propidium iodide. A custom MetaMorph journal suite (count nuclei and Integrated Morphometry Analysis) was used to identify all cells and count dead neutrophils to calculate the proportion of viable cells in the population. (*C*) The concentration of FcFasL determines the kinetics of apoptosis of wild-type neutrophils. (*D*) Comparison of FcFasL (1/5 dilution) and 100 ng/mL recombinant Flag-tagged FasL crosslinked with anti-Flag antibody on survival of wild-type neutrophils. Mean and SD of duplicate or triplicate fields of view shown. Data are representative of at least three independent experiments.

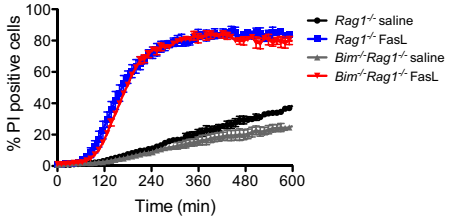


Fig. S2. Bim does not affect the kinetics of apoptosis in response to FasL. Neutrophils from mice reconstituted with *Rag1*$^{-/-}$ or *Bim*$^{-/-}$*Rag1*$^{-/-}$ bone marrow were incubated with different concentrations of FasL for the indicated time. Viability was monitored using propidium iodide and the proportion of dead cells was determined using MetaMorph. Data are mean ± SEM from three independent samples.

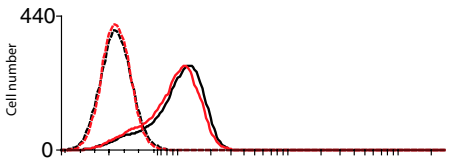


Fig. S3. Fas expression is equivalent on wild-type (black line) and *vav-Bcl-2* (red line) neutrophils. Neutrophils were stained with Fas antibodies (solid line) or an isotype control antibody (broken line).

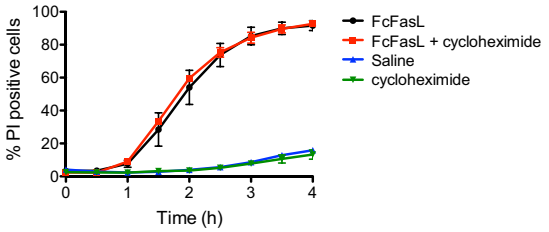
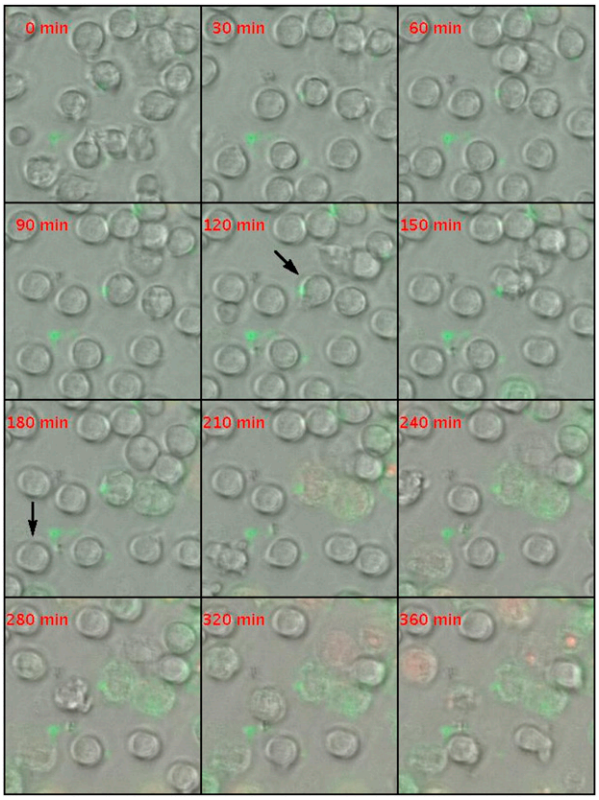
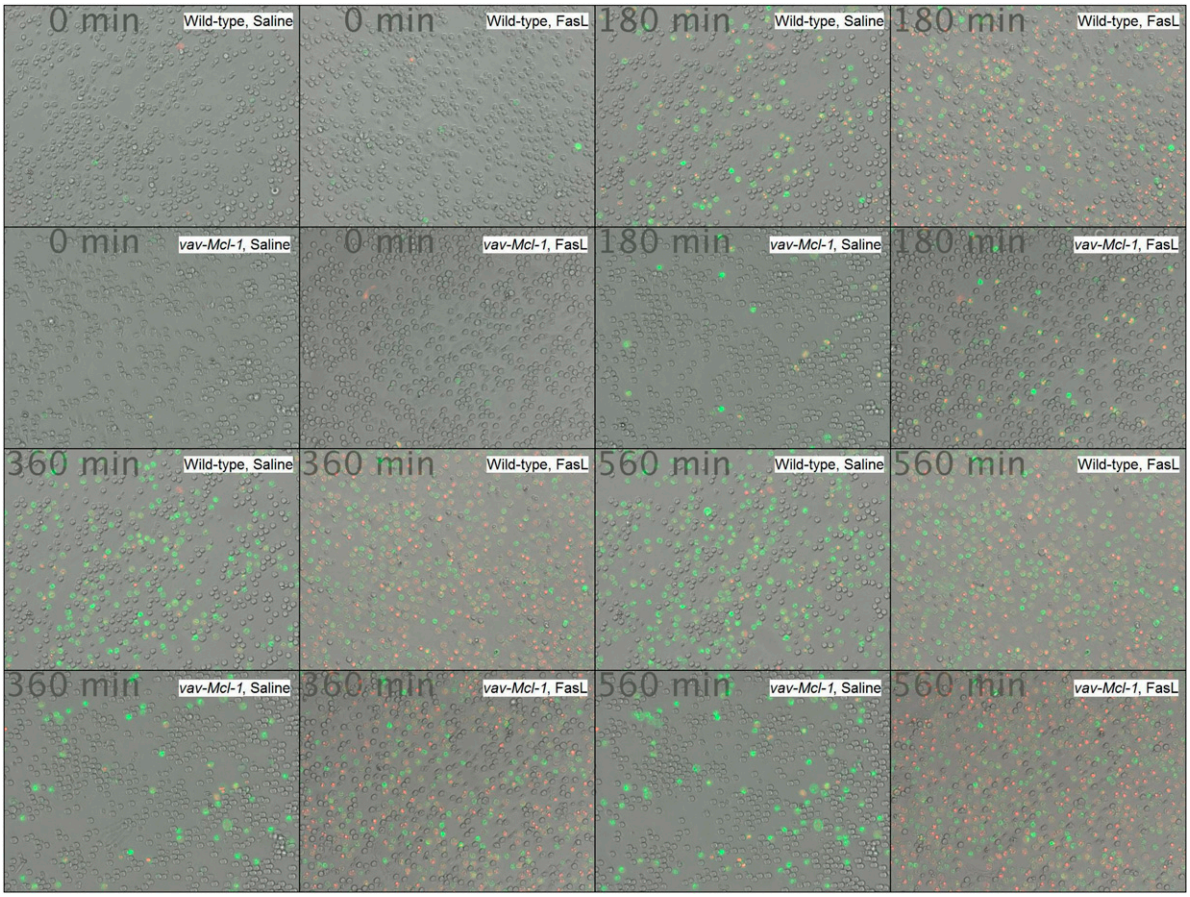


Fig. S4. Protein synthesis is not required for FasL to induce the death of neutrophils. Purified bone marrow neutrophils were incubated with 0.1 ng/mL cycloheximide with or without FcFasL for the indicated time. Viability was monitored using propidium iodide and the proportion of dead cells was determined using MetaMorph. Data are mean ± SEM from two independent samples. Two fields of view per condition were monitored.



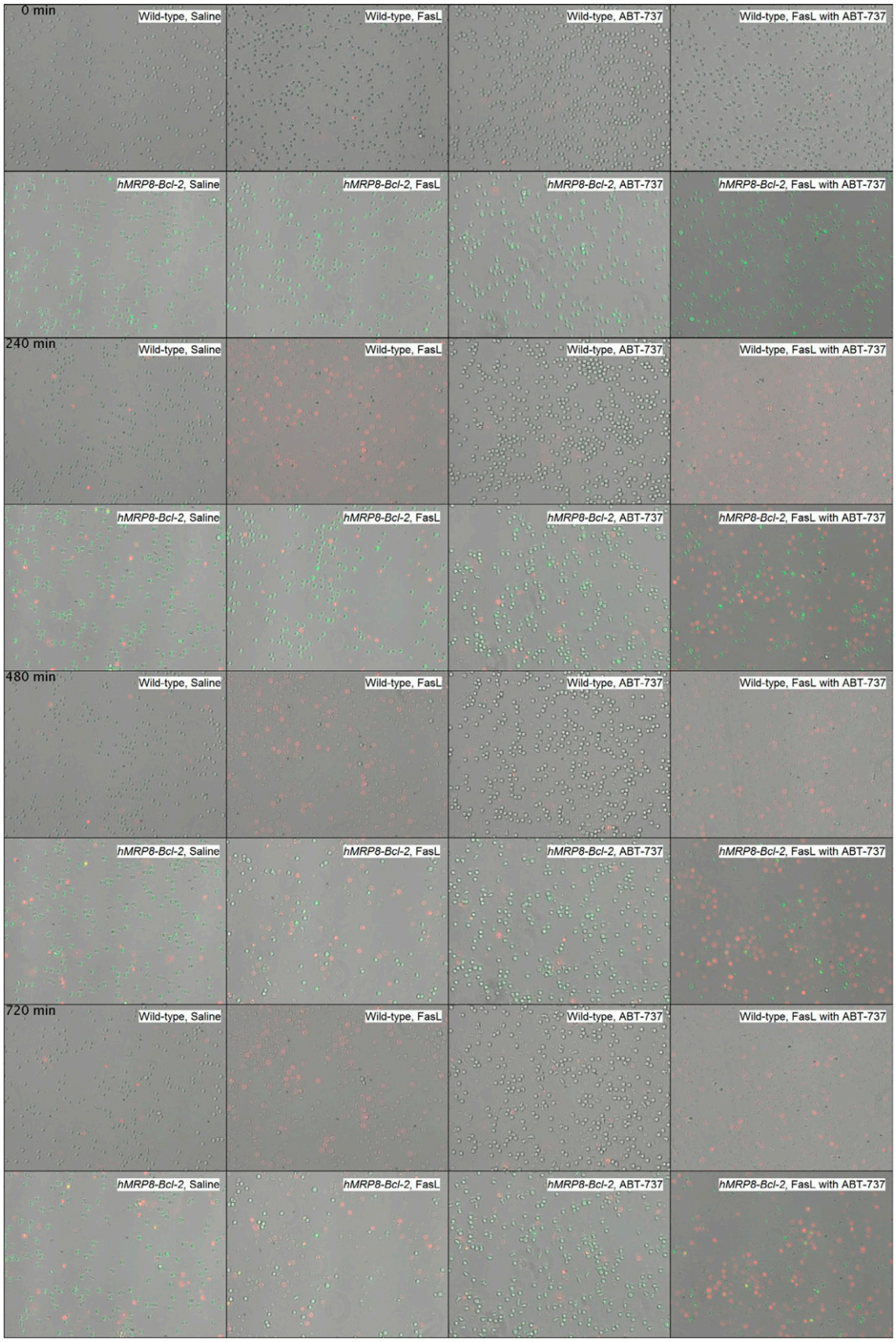
Movie S1. Wild-type neutrophils were stimulated with FasL in the presence of Annexin V (green) and PI (red). The arrows highlight neutrophils that will undergo apoptosis by blebbing before externalizing phosphatidylserine to become Annexin V-positive and then losing membrane integrity to become PI positive. Note the rapid transition from Annexin V-positive to PI-positive.

Movie S1



Movie S2. Mcl-1 transgenic neutrophils are resistant to FasL. Wild-type (*Upper*) or *vav-Mcl-1* transgenic (*Lower*) bone marrow neutrophils were incubated without growth factor (*Left*) or with FasL (*Right*). Neutrophils were incubated in the presence of Annexin V (green) and PI (red). The time is indicated in the top left corner of each panel.

Movie S2



Movie S3. Inhibition of Bcl-2 with ABT-737 restores sensitivity of *hMRP8-Bcl-2* neutrophils to FasL. Wild-type (*Upper*) or *hMRP8-Bcl-2* transgenic (*Lower*) neutrophils were incubated without growth factor (*Left*) or with FasL (*Center Left*), ABT-737 (*Center Right*), or FasL with ABT-737 (*Right*). Neutrophils were labeled with Cell Tracker Green (green) and incubated in the presence of PI (red) as a marker of cell viability.

Movie S3