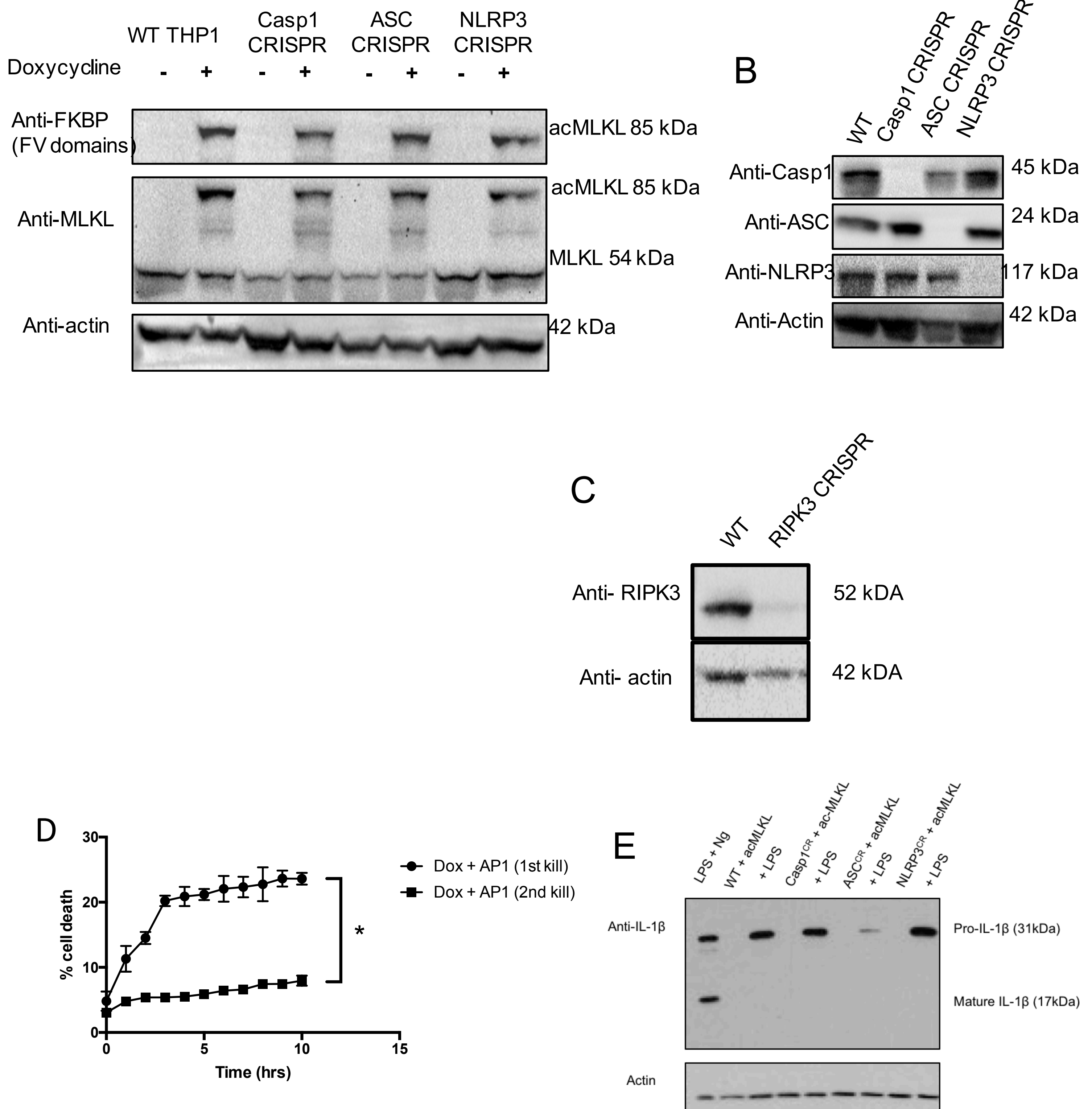

Fig S1: (A) Western blot of CRISPR edited acMLKL THP-1 cells treated with or without doxycycline for 24 hours in order to induce expression of MLKL. Antibodies against the FV domain (FKBP) and MLKL are shown and actin was used as a load control. (B) Western blot of CRISPR edited THP-1 cells. Actin was used as a load control. (C) Western blot of RIPK3 CRISPR edited THP-1 cells. Actin was used as a load control. (D) Cell death kinetics of THP-1 cells stably expressing doxycycline-inducible acMLKL, treated with dox+AP1 ("1st kill"), or cells surviving this treatment allowed to recover and re-treated with the same treatment 72h later ("2nd kill.") (E) WT and CRISPR edited THP-1 cells were primed with PMA and treated with or without LPS for 4 hours. WT acMLKL THP-1 cells were treated with LPS and Nigericin as a positive control. Cell lysates were analysed by western blot for the presence of intracellular mature IL-1β.

Supplemental Figure 2

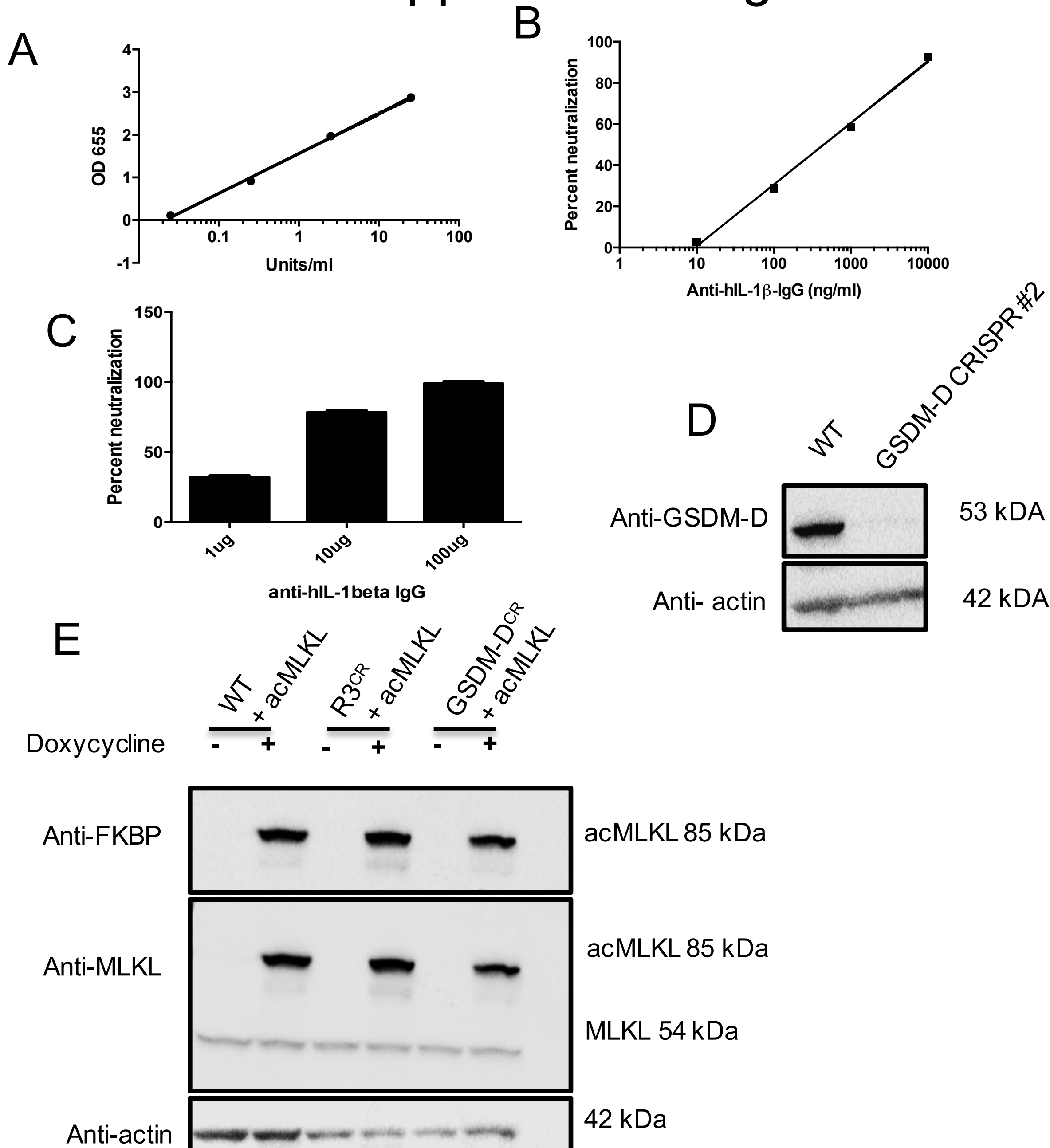

Fig S2: (A) Standard curve of recombinant human IL-1β used to calibrate the response to bioactive IL-1β in the HEK-blue™ IL-1β bioassay. Increasing amounts of recombinant human IL-1β were incubated in the presence of HEK-blue™ IL-1β cells for 24 hours to stimulate SEAP production. After incubation levels of SEAP in HEK-blue™ supernatants were assessed by incubation with the Quanti-blue colormetric dye for 2 hours. Absorbance at a wavelength of 655nm was read and a standard curve was generated. (B) Percent neutralization of recombinant human IL-1β by anti-human IL-1β-IgG neutralizing antibody. 2.5 Units/ml of recombinant human IL-1β was incubated with increasing amounts of anti-human IL-1β-IgG neutralizaing antibody for 24 hours in the presence of HEK-blue™ IL-1β cells for 24 hours, and SEAP reporter activity was assessed as in (A) above. Percent neutralization of recombinant human IL-1β was determined as compared to untreated control. (C) Percent neutralization of SEAP activity generated by THP-1 cells following MLKL activation by anti-human IL-1β-IgG. THP-1 cells were primed with LPS and treated with doxycycline for 24 hours. The next day cells were treated with AP1 for 5 hours after which supernatants were harvested and incubated with anti-human IL-1β-IgG neutralizing antibody in the presence of HEK-blue™ IL-1β reporter cells for 24 hours. After incubation levels of SEAP in HEK-blue™ supernatants were assessed as descrived above. (D) Western blot of GSDMD^{CR} THP-1 cells. Actin was used as a load control. (E) Western blot of CRISPR edited acMLKL THP-1 cells treated with or without doxycycline for 24 hours in order to induce expression of acMLKL. Antibodies against the FV domain (FKBP) and MLKL are shown and actin was used as a load control.