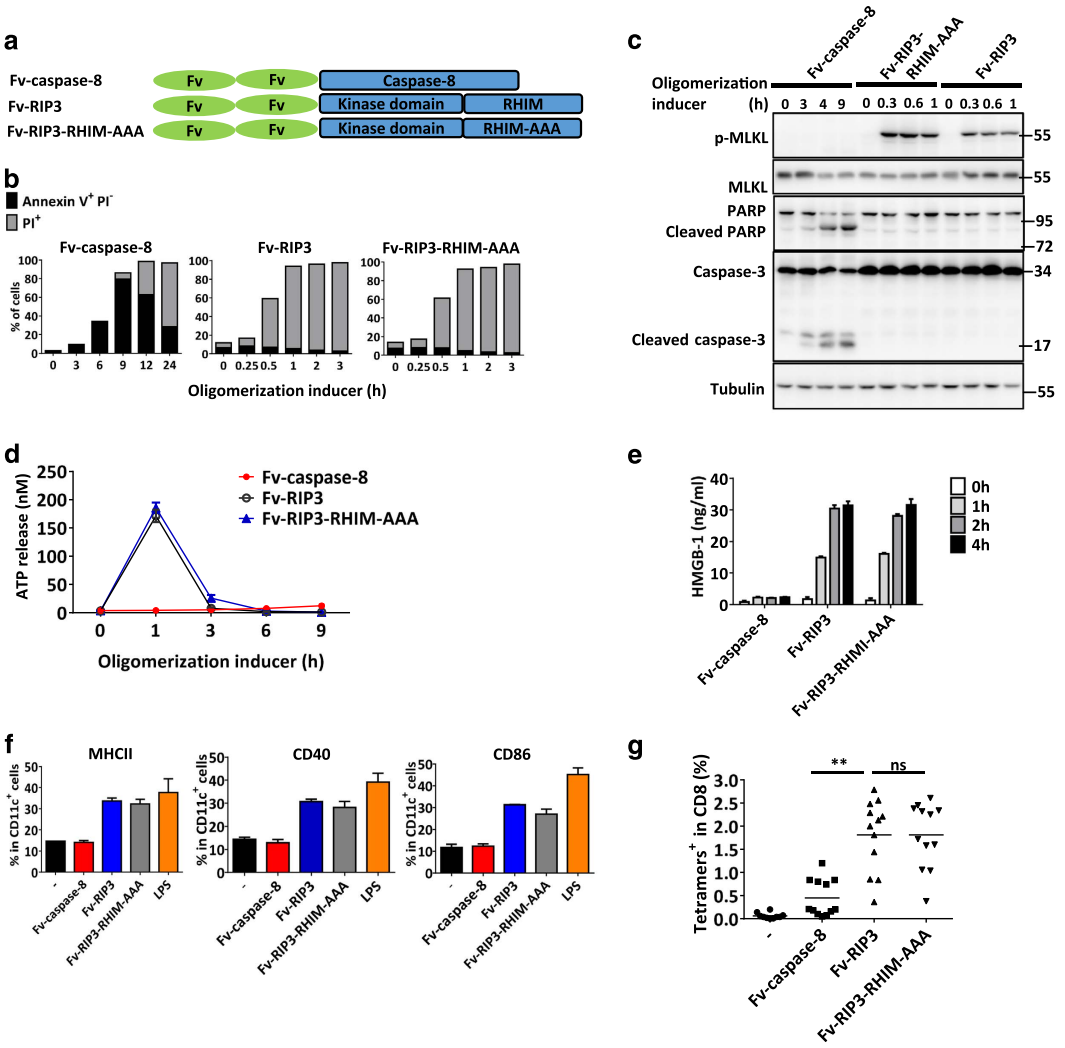
SUPPLEMENTARY INFORMATION

The RIP3-RIP1-NF-κB signaling axis is dispensable for necroptotic cells to elicit cross-priming of CD8+ T cells



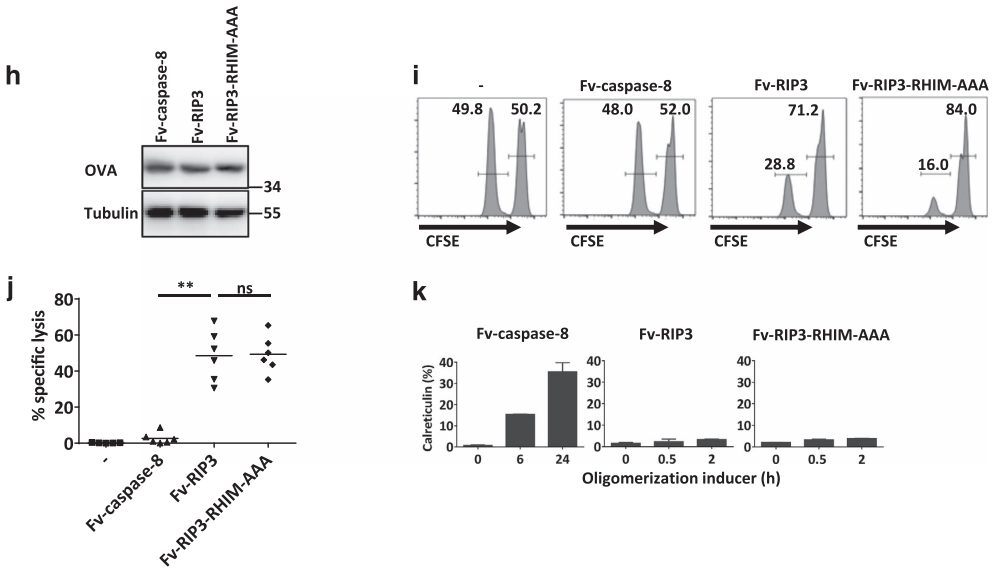


Figure S1 (**a**) Schematic representation of oligomerizable caspase-8 protease domain, wild-type RIP3 and RHIM-AAA mutant RIP3. Fv (FK506 binding domain F36V mutant) and HBD*(hormone-binding domain G521R mutant) are dimerization domains. (**b**) MCA205 cells expressing Fv-caspase-8, Fv-RIP3 or Fv-RIP3-RHIM-AAA were harvested after 50 nM AP20187 (oligomerization inducer) inducing oligomerization of corresponding Fv fusion protein in each cell line, stained with Annexin V and PI and analyzed by flow cytometry. (**c**) MCA205 cells expressing Fv-caspase-8, Fv-RIP3 or Fv-RIP3-RHIM-AAA were treated with AP20187 for indicated time points, lysates were collected and separated by SDS-PAGE and immunoblotted for detection of phospho-MLKL, MLKL, poly (ADP-ribose) polymerase (PARP) and caspase-3. Tubulin expression is the loading control. (**d** and **e**) The levels of ATP and HMGB-1 in the culture media of each MCA205 cell line were measured after oligomerization of indicated fusion protein. (**f**) BMDCs were co-cultured with AP20187 treated MCA205 cells expressing Fv-caspase-8, Fv-RIP3 or Fv-RIP3-RHIM-AAA for 24 h. Cell surface expression of MHCII, CD40 and CD86 on DCs were analyzed by FACS. BMDCs treated with LPS served as positive controls. (**g**) C57BL/6 mice were immunized with AP20187-treated MCA205-OVA cells expressing Fv-caspase-8, Fv-RIP3 or Fv-RIP3-RHIM-AAA, nine days later, the frequency of OVA specific (Tetramer$^+$) CD8$^+$ T cells was determined. Each dot corresponds to an individual mouse. (**h**) Western blotting analyses of OVA expression in MCA205-OVA cells expressing Fv-caspase-8, Fv-RIP3 or Fv-RIP3-RHIM-AAA. (**i** and **j**) The effect of dying MCA205-OVA cells expressing Fv-caspase-8, Fv-RIP3 or Fv-RIP3-RHIM-AAA on specific lytic activity of CD8$^+$ T cells. Representative FACS plots are shown in (**i**). The percentage of specific lysis is plotted in (**j**). (**k**) Exposure levels of calreticulin in MCA205 cells expressing Fv-caspase-8, Fv-RIP3 or Fv-RIP3-RHIM-AAA after AP20187 induced oligomerization. All data above were represented as mean ± s.e.m. of three independent experiments. For (**g**), statistical analysis by Kruskal-Wallis test and Dunn's post-test; for (**j**), statistical analysis done by Mann-Whitney test. $^{**}P<0.01$, ns, no significant difference.

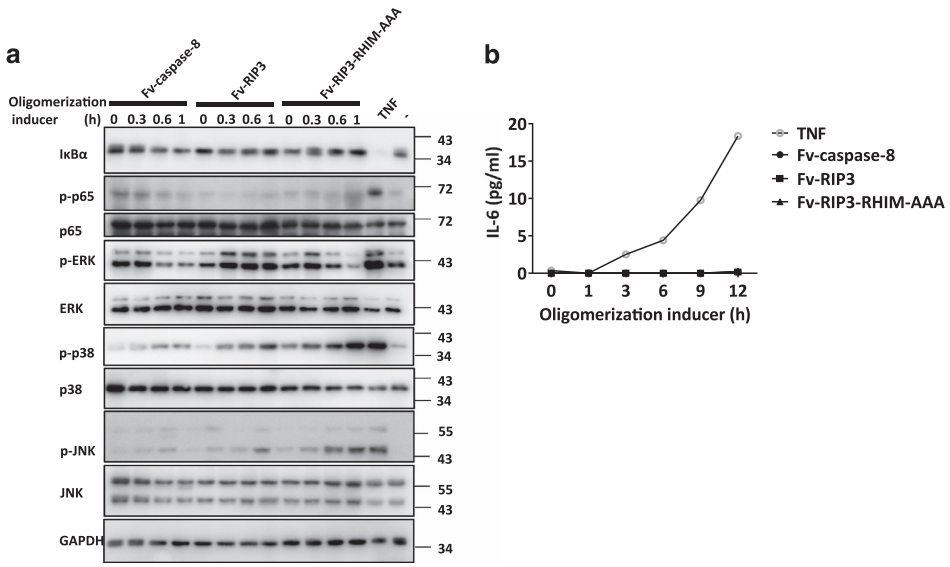


Figure S2 (**a**) Western blotting analyses of MAPKs and NF-κB activation in MCA205-OVA cells expressing Fv-caspase-8, Fv-RIP3 or Fv-RIP3-RHIM-AAA after oligomerization of the fusion protein in each line. MCA205-OVA cells treated with TNF served as a positive control. (**b**) Secretion of IL-6 was measured in the cells described in (**a**) using Enzyme Linked Immunosorbent Assay (ELISA) detection kit. MCA205-OVA cells treated with TNF served as a positive control.