

Figure S1: Analysis of macrophage differentiation and CASP3, CASP8 and RIPK3 activation after deletion of $Z\alpha 2$ domain of ZBP1

A, Flow cytometry analysis of ex vivo BMDMs from WT and $Zbp1^{\Delta Z\alpha 2/\Delta Z\alpha 2}$ mice for expression of myeloid-specific markers, F4/80 and CD11b. **B and C**, Immunoblot analysis of caspase-3 (CASP3), caspase-8 (CASP8), RIPK3, phosphorylated RIPK3 (P-RIPK3), IAV-M1, IAV-NS1, and GAPDH in WT and $Zbp1^{\Delta Z\alpha 2/\Delta Z\alpha 2}$ BMDMs infected with IAV (**B**) or infected with IAV and subsequently treated with the pan-caspase inhibitor (zVAD, 30 μ M) (**C**).

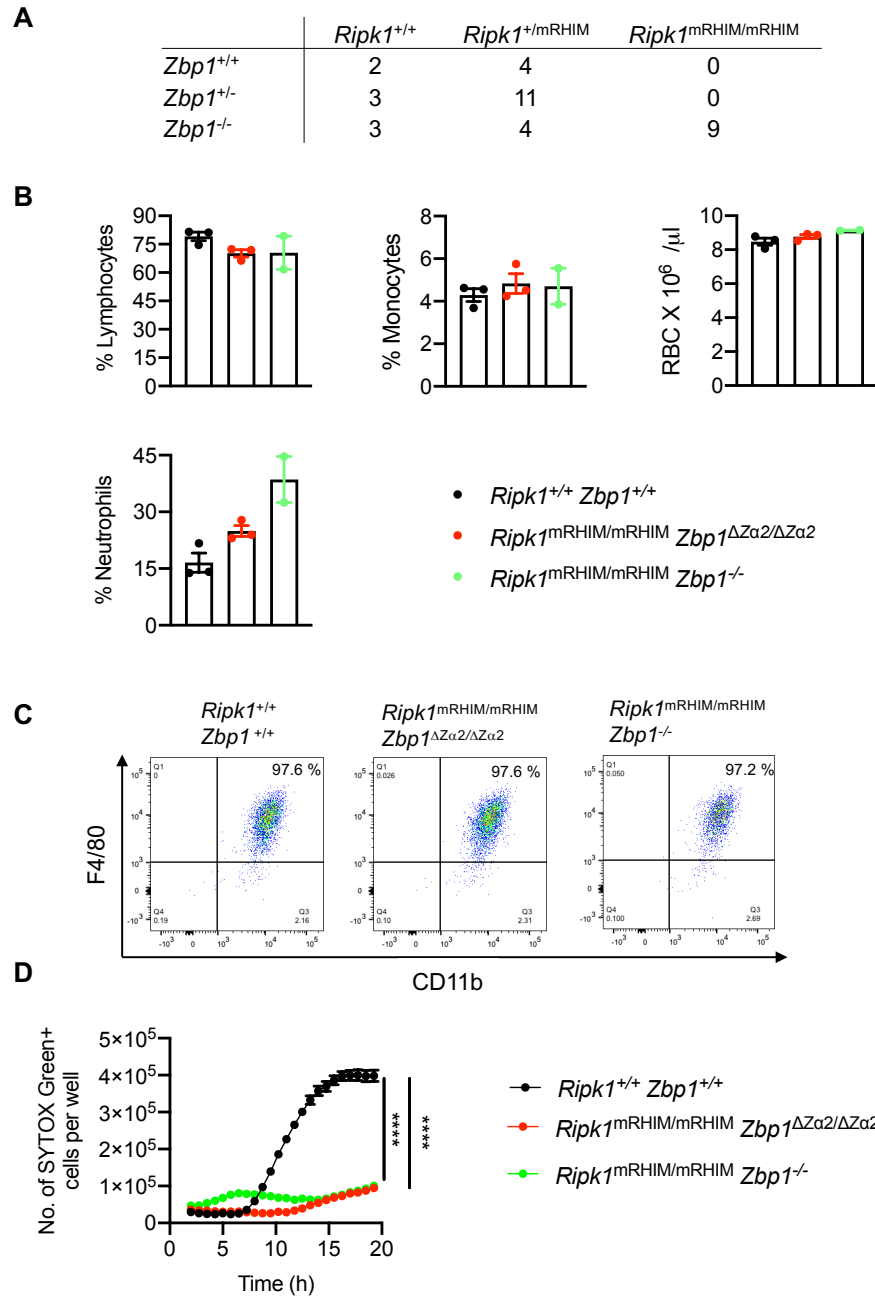


Figure S2: Immune cell population in *Ripk1*^{mRHIM/mRHIM}*Zbp1*^{ΔZα2/ΔZα2} and *Ripk1*^{mRHIM/mRHIM}*Zbp1*^{-/-} mice and IAV-induced cell death

A, Table representing the number of offspring of each genotype generated from intercrossing *Ripk1*^{+mRHIM}*Zbp1*^{+/-} (heterozygote) parents. **B**, Analysis of the number or percent of different immune cell types from the blood from indicated mouse strains ($n > 2$ in each genotype). Data are represented as mean \pm s.e.m. **C**, Flow cytometry analysis of ex vivo BMDMs from the indicated mouse strains for expression of myeloid-specific markers, F4/80 and CD11b. **D**, Cell death as measured by the number of SYTOX Green⁺ cells. BMDMs differentiated from the indicated mouse strains were infected with IAV, and cell death was monitored at regular intervals. **** $P < 0.0001$ (one-way ANOVA). Data are represented as mean \pm s.e.m. and are representative of two independent experiments.

Name	Sequence (5' to 3')
mZBP1.5'.sgRNA spacer	AGCAUAGGCGGGGCUUC
mZBP1.3'.sgRNA spacer	ATCTACCACTCACGTCAGGA
mZBP1 deletion ssODN donor Silent Blocking modification (upper case) *phosphorothioate linkages	a*c*ctgacccttgatccctgacctccccacactgactttgcctgtcatacctatgtcttgeccatctcc GCTagcagccccgcctatgctccatgtgcaggctctggggaggacact*c*t
mZBP1.deletion.NGS.F partial Illumina adaptors (upper case)	CACTCTTTCCCTACACGACGCTCTTCCGATCTaggtgccttctgctctgg ggact
mZBP1.deletion.NGS.R partial Illumina adaptors (upper case)	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTacaaacatctggcc ctacaccatct

Table S1: List of CRISPR/Cas9 gene editing construct sequences and relevant primers.