



## **Supplementary Information for**

### **ZBP1 Promotes Inflammatory Responses downstream of TLR3/TLR4 via Timely Delivery of RIPK1 to TRIF**

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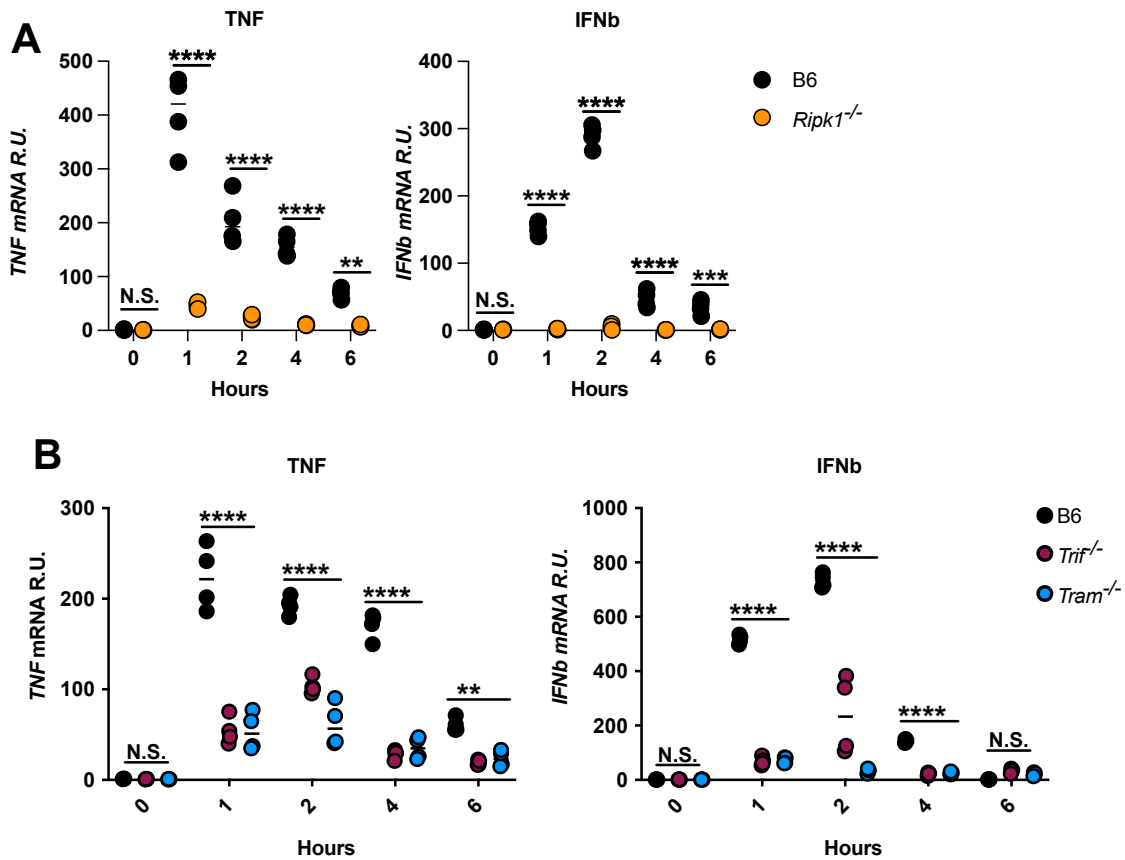
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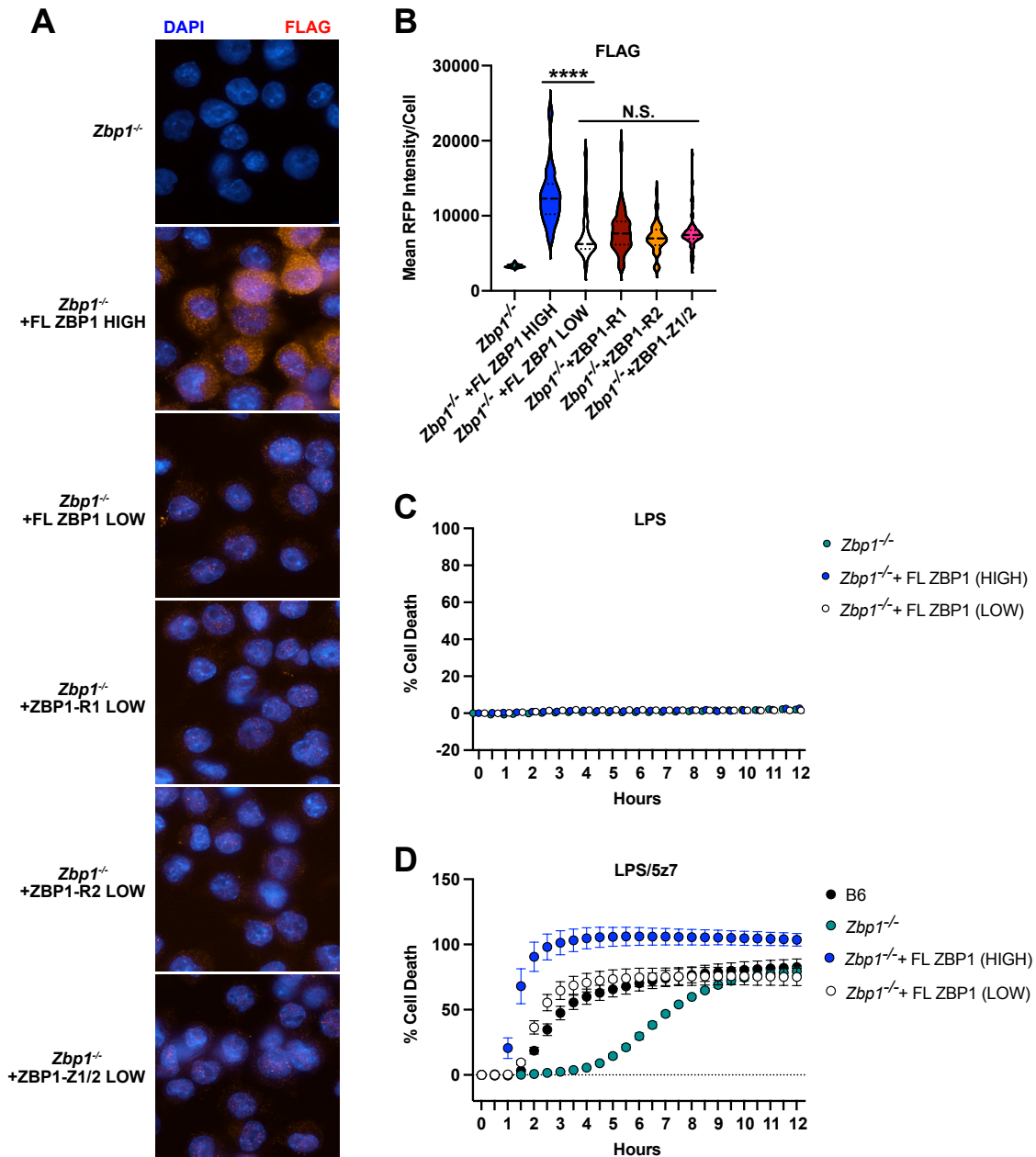
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#### **This PDF file includes:**

Figures S1 and S2



**Fig. S1.** (A) Relative TNF and IFN $\beta$  mRNA levels, normalized to GAPDH in B6 and *Ripk1*<sup>-/-</sup> mouse fetal liver macrophages stimulated with LPS for indicated time points. (B) Relative TNF and IFN $\beta$  mRNA levels, normalized to GAPDH in B6 and *Trif*<sup>-/-</sup> and *Tram*<sup>-/-</sup> macrophages stimulated with LPS for indicated time points. For experiments with mouse fetal liver macrophages, data points indicate the mean from triplicate wells of 4 technically independent experiments. For experiments with BMDMs, data points indicate the mean from triplicate wells of 4 biologically independent experiments. Analysis of variance (ANOVA) was used for comparison between groups: ns, nonsignificant ( $P > 0.05$ ); \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; \*\*\*\* $P < 0.0001$ .



**Fig. S2.** (A) Representative 60X images of FLAG-ZBP1 staining in B6, *Zbp1*<sup>-/-</sup>, and *Zbp1*<sup>-/-</sup> macrophages reconstituted with low or high levels of ZBP1 or with mutant ZBP1 constructs lacking RHIM1 (ZBP1-R1), RHIM2 (ZBP1-R2) or both Za domains. (B) Quantification of mean FLAG-ZBP1 signal intensity/cell as indicated. n=100 cells/group imaged across 4-5 fields of view. (C, D) Cell death as measured by propidium iodide incorporation in indicated cells stimulated with (C) LPS or (D) LPS/5z7. Data from cell death assays and imaging experiments are representative of 3 or more independent experiments. Analysis of variance (ANOVA) was used for comparison between groups: ns, nonsignificant (P>0.05); \*P<0.05; \*\*P<0.01; \*\*\*P<0.001; \*\*\*\*P<0.0001.