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Supplemental Information

Constitutive Interferon Attenuates RIPK1/3-Mediated Cytokine Translation

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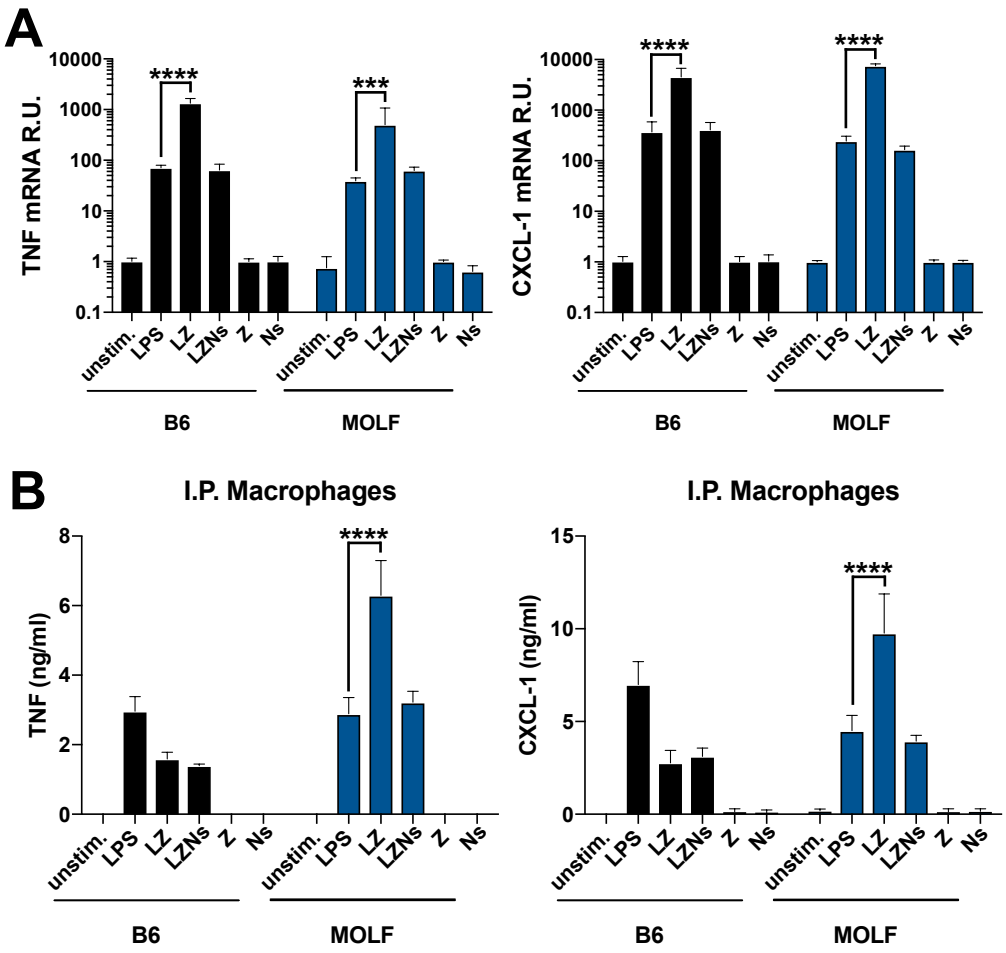

Figure S1, Related to Figure 1.
(A,B) TNF-α and CXCL-1 mRNA **(A)** and protein **(B)** levels in unstimulated, LPS, LPS/zVAD (LZ), LPS/zVAD/Nec-1s (LZNs), zVAD (Z), and Nec-1s (Ns) stimulated B6 and MOLF peritoneal macrophages. ELISA and qPCR data are shown as +/- SD from three independent experiments compared using a Two-way ANOVA: ***p<0.001, ****p<0.0001.

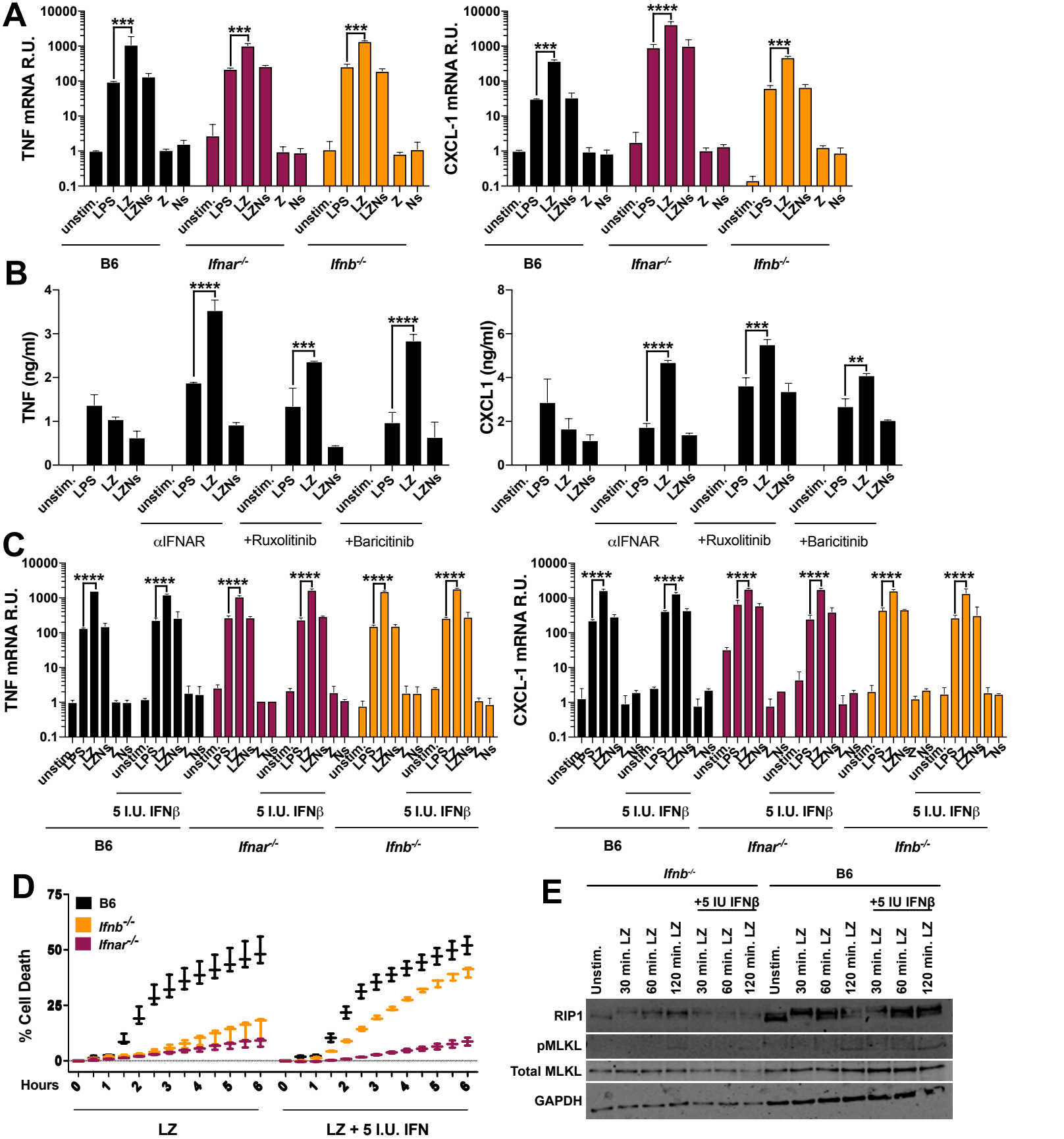

Figure S2, Related to Figure 2

(A) TNF-α and CXCL-1 mRNA levels after indicated stimulations of B6, *Ifnar*$^{-/-}$ and *Ifnb*$^{-/-}$ BMDMs. **(B)** TNF-α and CXCL-1 protein levels in B6 BMDMs after indicated stimulations +/- overnight treatment with IFNAR blocking antibody (αIFNAR), Ruxolitinib, or Baricitinib. **(C)** TNF-α and CXCL-1 mRNA levels after indicated stimulations +/- 5 I.U. IFNβ overnight priming in B6, *Ifnar*$^{-/-}$ and *Ifnb*$^{-/-}$ BMDMs. **(D)** Cell death as measured by propidium iodide incorporation over 6 hours in B6, *Ifnb*$^{-/-}$, and *Ifnar*$^{-/-}$ BMDMs stimulated with LZ +/- 5 I.U. IFNβ priming overnight.
(E) RIP1, Total and phospho-MLKL levels in B6 and *Ifnb*$^{-/-}$ BMDMs stimulated as indicated with LZ+/- 5 I.U. IFNβ priming overnight. In all panels, BMDMs were stimulated with LPS, LPS/zVAD (LZ), LPS/zVAD/Nec-1s (LZNs), zVAD (Z) or Nec-1s (Ns). ELISA and qPCR data are shown as +/- SD from three independent experiments compared using Two-way ANOVA: **p<0.01, ***p<0.001, ****p<0.0001. Kinetic cell death and western blot experiments are representative of three or more independent experiments.

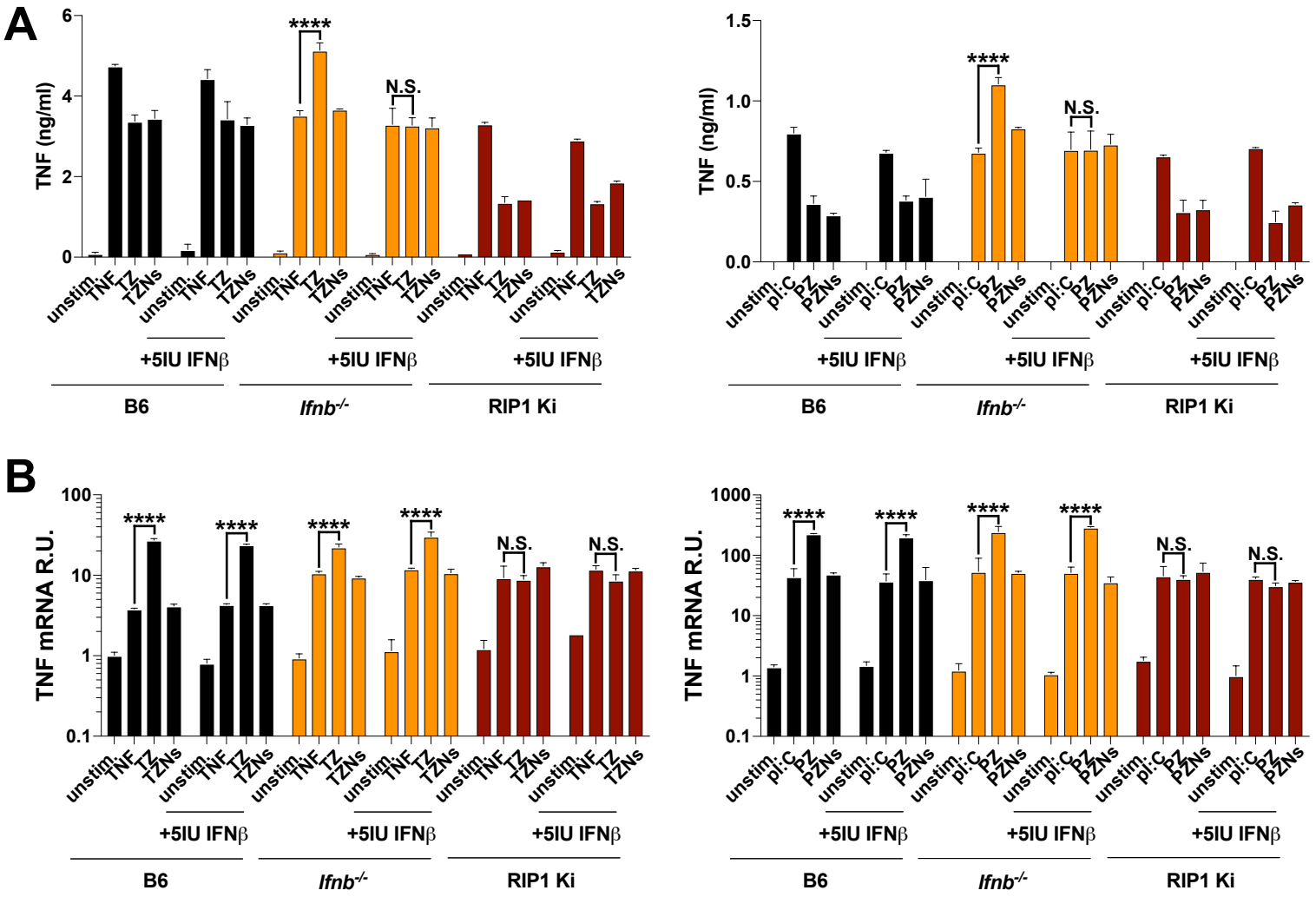

Figure S3, Related to Figure 2

(A,B) TNF-α and CXCL-1 protein **(A)** and mRNA **(B)** levels in unstimulated, TNF, TNF/zVAD (TZ), TNF/zVAD/Nec-1s (TZNs), or pI:C, pI:C/zVAD (PZ), pI:C/zVAD/Nec-1s (PZNs) stimulated B6, *Ifnb*$^{-/-}$ and RIP1 Ki BMDMs. ELISA and qPCR data are shown as +/- SD from three independent experiments compared using a Two-way ANOVA: n.s. ($p>0.05$), ****$p<0.0001$.

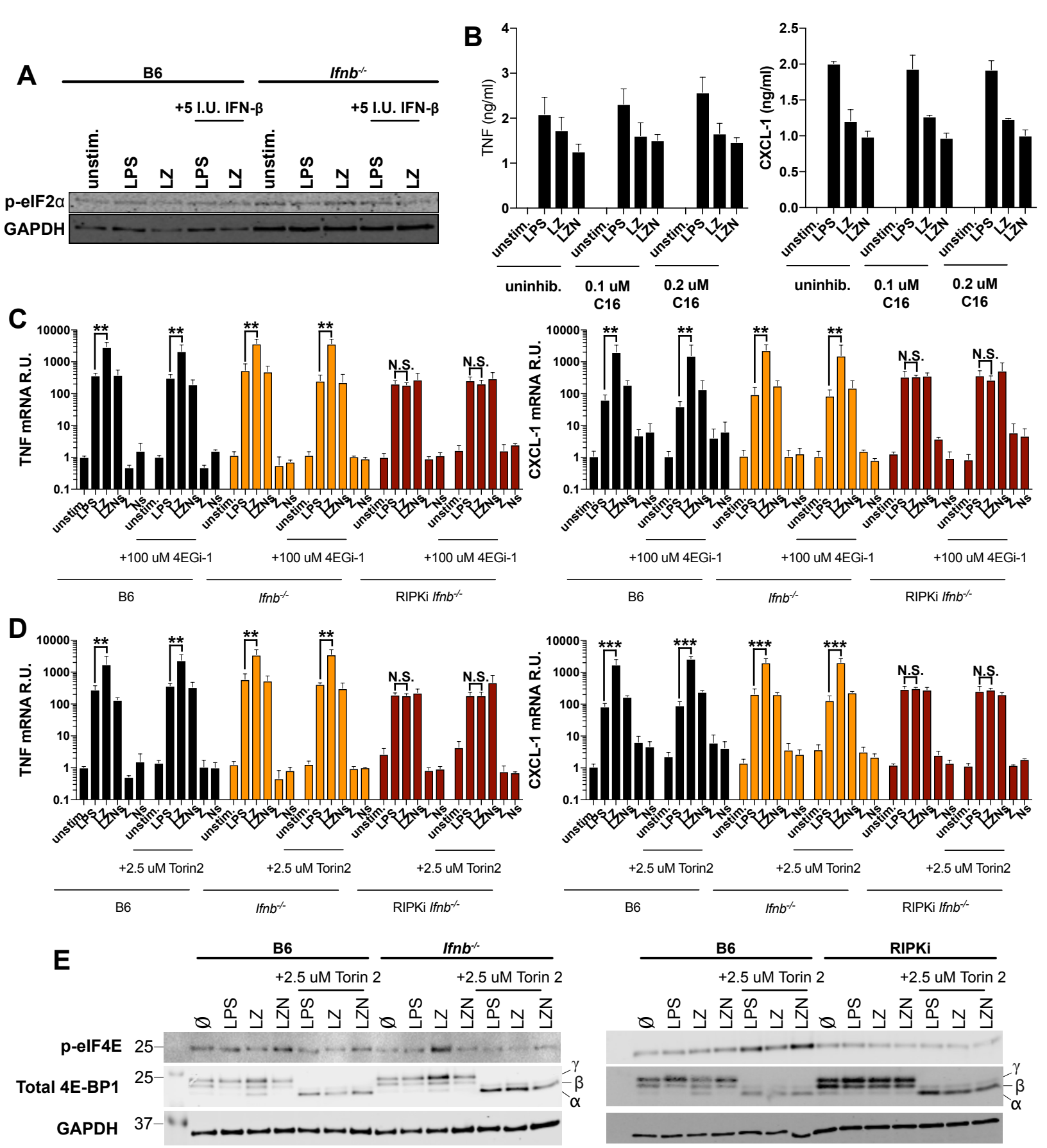

Figure S4, Related to Figure 4

(A) p-eIF2α levels in B6, and *Ifnb*$^{-/-}$ BMDMs stimulated as indicated +/- 5 I.U. IFNβ priming overnight. **(B)** TNF-α and CXCL-1 protein levels after indicated stimulations +/- treatment with PKR inhibitor C16 in B6 BMDMs. **(C, D)** TNF-α and CXCL-1 mRNA levels after indicated stimulations +/- treatment with 4EGi-1 **(C)** or Torin 2 **(D)** in B6, *Ifnb*$^{-/-}$, and RIP1 Ki *Ifnb*$^{-/-}$ BMDMs. **(E)** p-eIF4E and Total 4E-BP levels in B6, *Ifnb*$^{-/-}$ and RIP1 Ki BMDMs stimulated as indicated +/- Torin 2. In all panels, BMDMs were stimulated with LPS, LPS/zVAD (LZ), LPS/zVAD/Nec-1s (LZNs), zVAD (Z), Nec-1s (Ns), or LPS/zVAD/Nec-1 (LZN) as indicated. ELISA and qPCR data are shown as +/- SD from three independent experiments compared using a Two-way ANOVA: n.s. ($p>0.05$), **$p<0.01$, ***$p<0.001$. Western blot and kinetic cell death experiments are representative of three or more independent experiments.

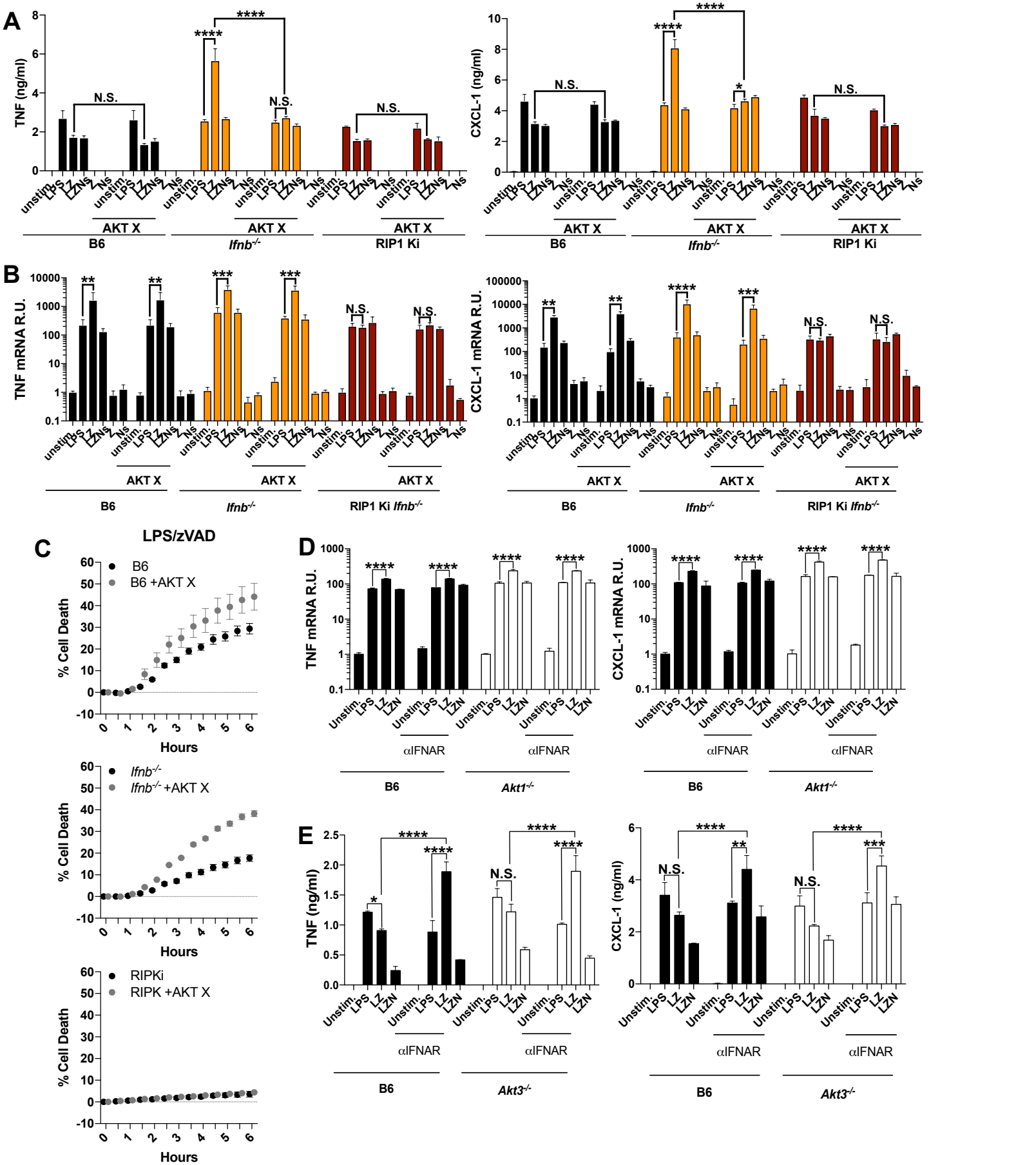

Figure S5, Related to Figure 4

(A,B) TNF-α and CXCL-1 protein **(A)** and mRNA **(B)** levels in B6, *Ifnb*$^{-/-}$, and RIP1 Ki *Ifnb*$^{-/-}$ BMDMs stimulated as indicated +/- treatment with AKT X. **(C)** Cell death as measured by propidium iodide incorporation over 6 hours in B6, *Ifnb*$^{-/-}$, and RIP1 Ki *Ifnb*$^{-/-}$ BMDMs stimulated with LZ +/- treatment with AKT X. **(D)** TNF-α and CXCL-1 mRNA levels in B6 and *Akt1*$^{-/-}$ BMDMs stimulated as indicated +/- αIFNAR antibody. **(E)** TNF-α and CXCL-1 protein levels in B6 and *Akt3*$^{-/-}$ BMDMs stimulated as indicated +/- αIFNAR antibody. In all panels, BMDMs were stimulated with LPS, LPS/zVAD (LZ), LPS/zVAD/Nec-1s (LZNs), zVAD (Z), Nec-1s (Ns), or LPS/zVAD/Nec-1 (LZN) as indicated. ELISA and qPCR data are shown as +/- SD from three independent experiments compared using a Two-way ANOVA: n.s. ($p>0.05$), *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$. Kinetic cell death experiments are representative of three or more independent experiments.