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Supplemental Information

Constitutive Interferon Maintains GBP Expression Required for Release of Bacterial Components Upstream of Pyroptosis and Anti-DNA Responses

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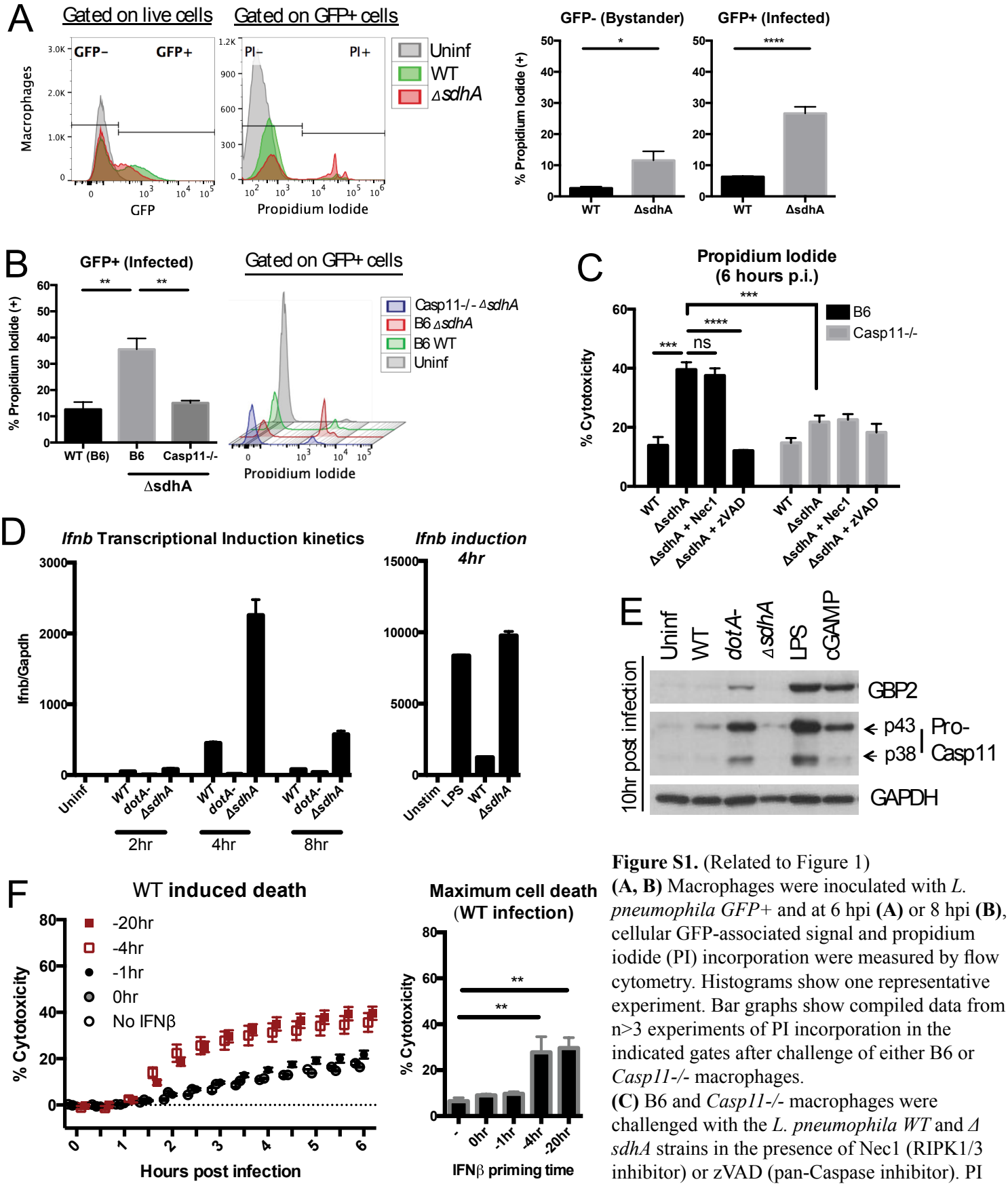


Figure S1. (Related to Figure 1)
(A, B) Macrophages were inoculated with *L. pneumophila GFP+* and at 6 hpi **(A)** or 8 hpi **(B)**, cellular GFP-associated signal and propidium iodide (PI) incorporation were measured by flow cytometry. Histograms show one representative experiment. Bar graphs show compiled data from n>3 experiments of PI incorporation in the indicated gates after challenge of either B6 or *Casp11-/-* macrophages.
(C) B6 and *Casp11-/-* macrophages were challenged with the *L. pneumophila WT* and *Δ sdhA* strains in the presence of Nec1 (RIPK1/3 inhibitor) or zVAD (pan-Caspase inhibitor). PI incorporation at 6 hpi is show.
(D) B6 macrophages were challenged with WT, *dotA-* mutant, or *ΔsdhA* mutants for the indicated times and *Ifnb* mRNA was measured by qRT-PCR.
(E) B6 macrophages were challenged with WT, *dotA-*, or *ΔsdhA* strains for 10 hours. Whole cell lysates were probed by Western blot for Gbp2, pro-Casp11 and GAPDH. 10 hour LPS incubation and cGAMP transfection are displayed.
(F) B6 macrophages were pre-treated with 100U/ml of IFNβ at the indicated times points prior to, or concurrent with *L. pneumophila* WT challenge (see timeline in Fig 1G). Displayed is PI incorporation as a function of time (left), and maximum cell death measured at 6 hpi (right).

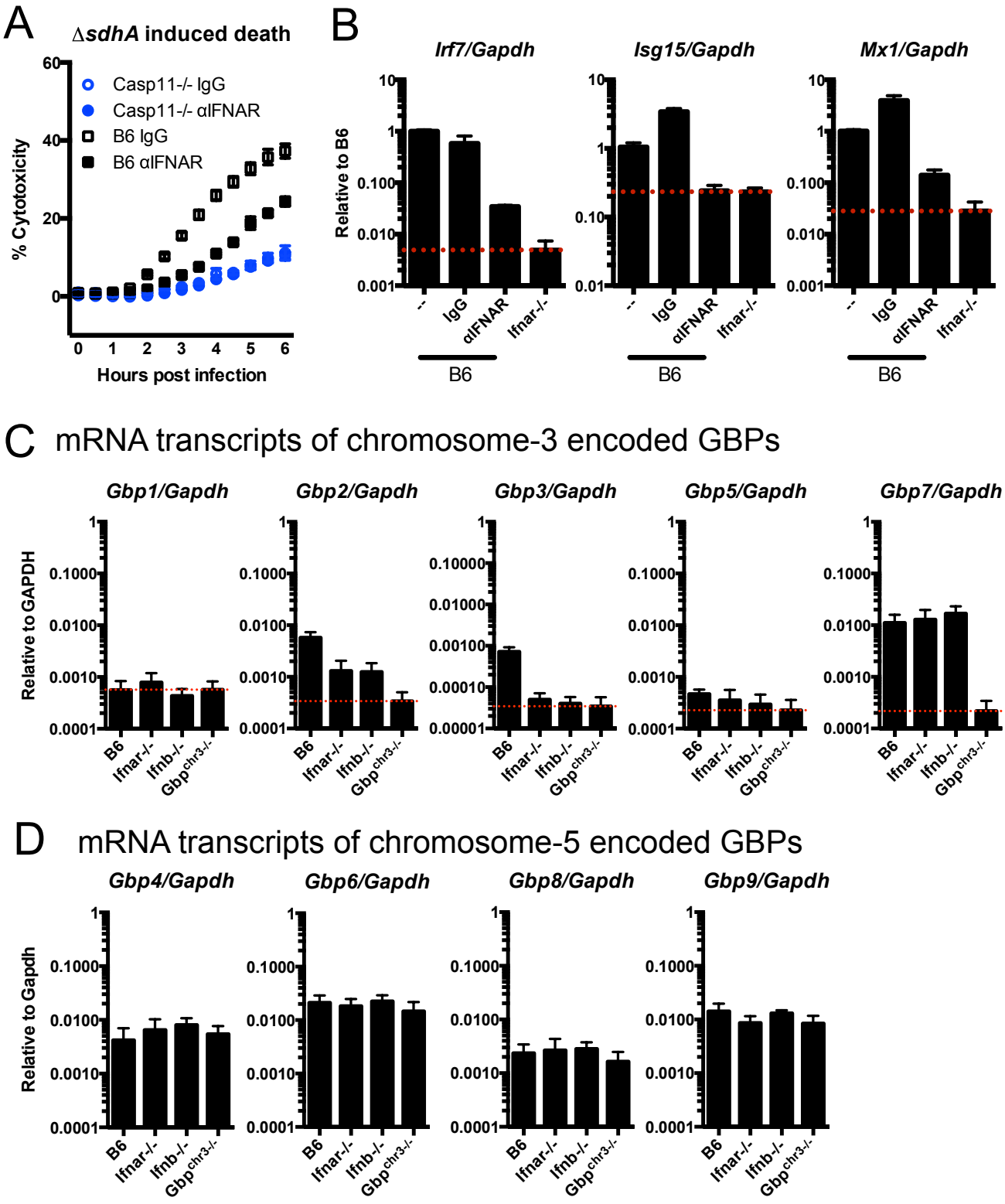


Figure S2. (Related to Figure 2)
(A) B6 and *Casp11-/-* Macrophages inoculated with *L. pneumophila ΔsdhA* after 20 hr treatment with noted antibodies. PI incorporation used to measure cell death.
(B) qRT-PCR of *Irf7, Isg15, Mx1* expression in the presence or absence of constitutive IFN signaling.
(C, D) qRT-PCR showing the baseline expression of noted genes in macrophages from various mouse strains. The CT values of genes of interest are normalized to sample-intrinsic *Gapdh* CT values. Dotted red line indicates background amplification from knock-out macrophages.

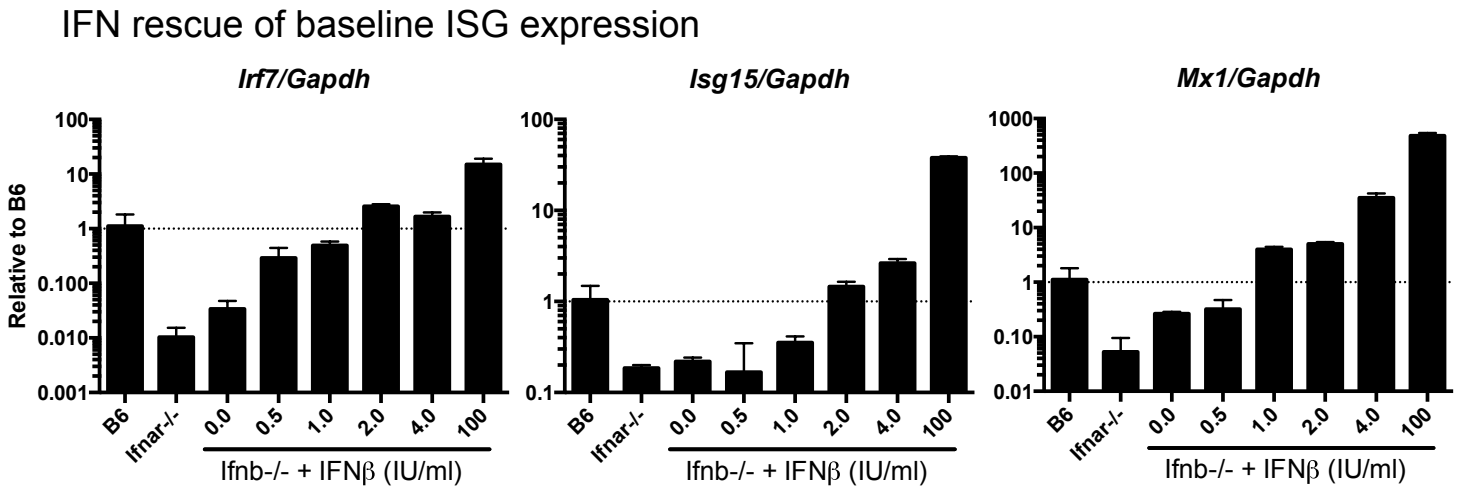


Figure S3. (Related to Figure 3)
Ifnb-/- macrophages were treated for 20 hours with various doses of recombinant IFNβ as in Fig 3B, C, D. Transcript levels were measured by qRT-PCR. Dotted black line indicates steady-state expression of genes of interest in B6 macrophages.

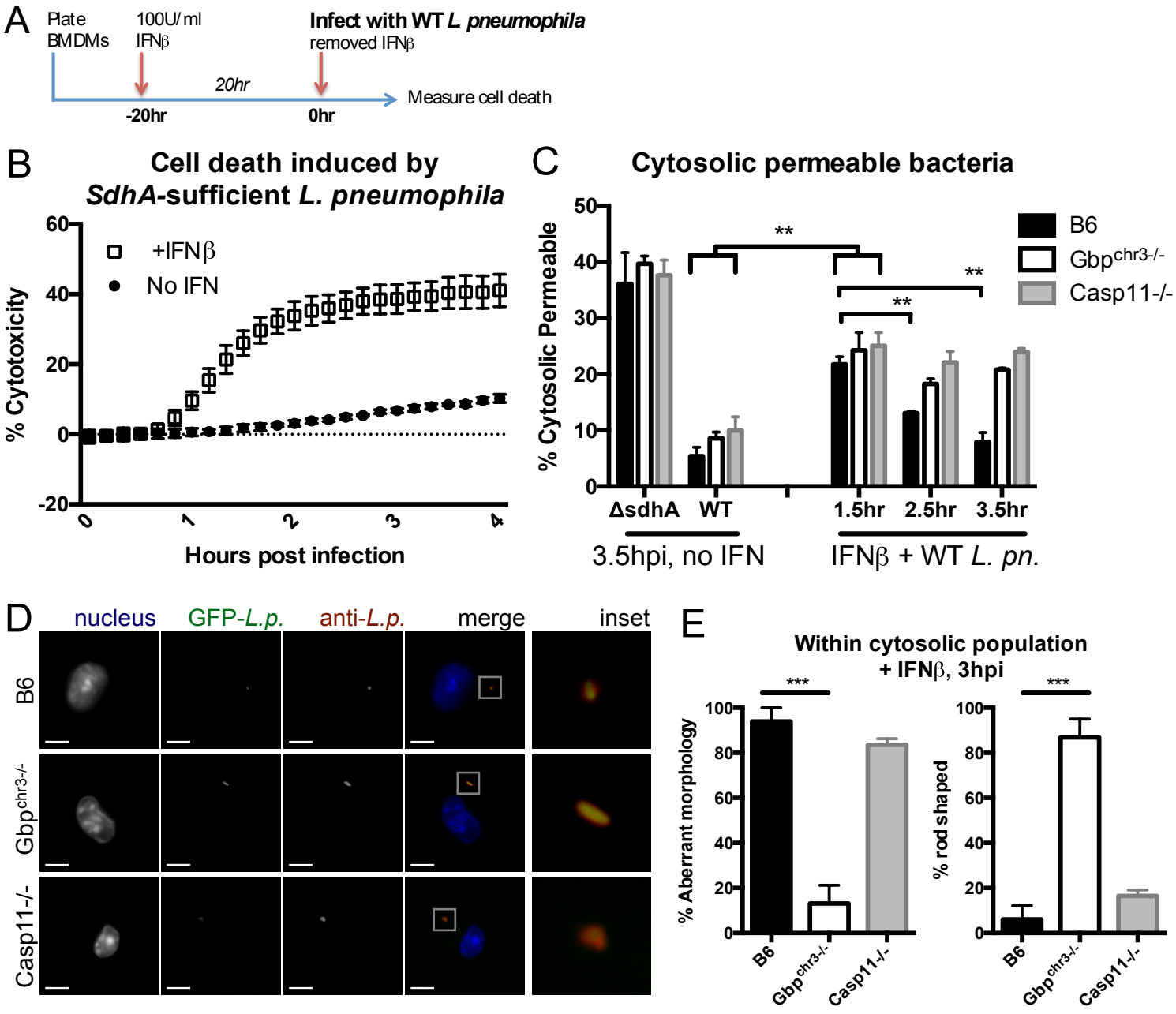


Figure S4. (Related to Figure 4)
(A) Macrophages were pre-treated for 20 hrs with 100U/ml of IFN prior to inoculation with WT L. pneumophila.
(B) Kinetics of WT L. pneumophila-induced cell death in the absence and presence of IFN pre-activation.
(C) Percentage of cytosolic-accessible L. pneumophila based on antibody staining without detergent permeabilization.
(D) Representative images of cytosolic WT bacteria in noted macrophages at 3 hpi. Images were taken using 63x lens, scale bar = 5m.
(E) Quantification of cytosolic accessible WT bacteria scored for aberrant (left) or rod-shaped (right) morphology at 3 hpi.

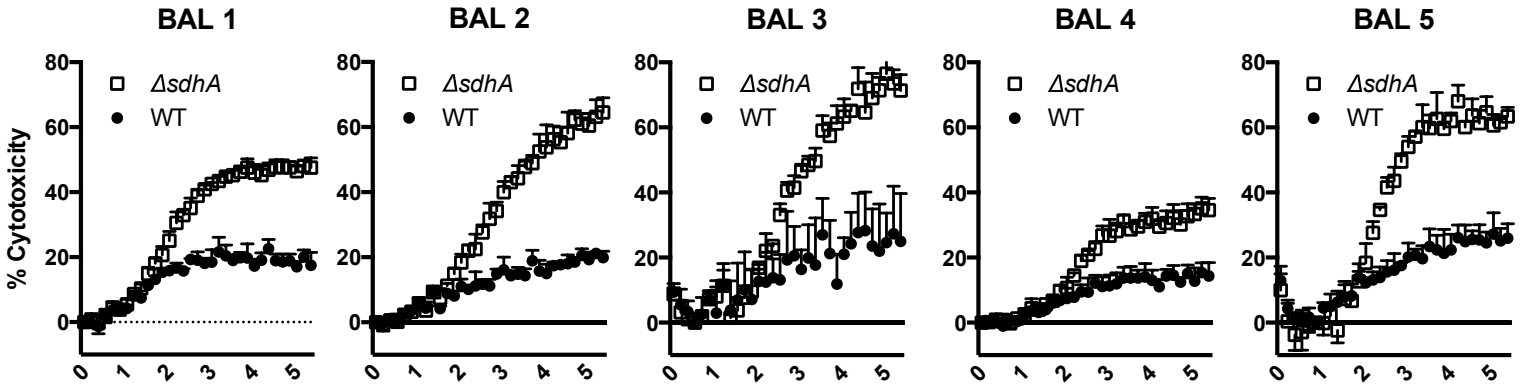


Figure S5 (Related to Figure 5)
Individual kinetics of *ex vivo* human bronchoalveolar lavage (BAL) cells infected with *L. pneumophila WT* or *ΔsdhA,* from 5 independent donors.

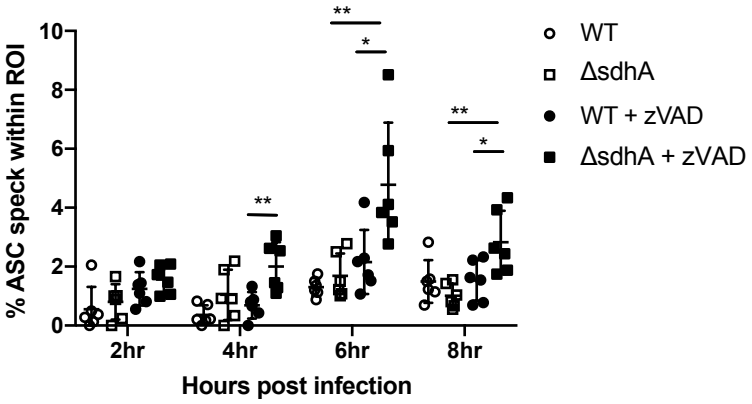
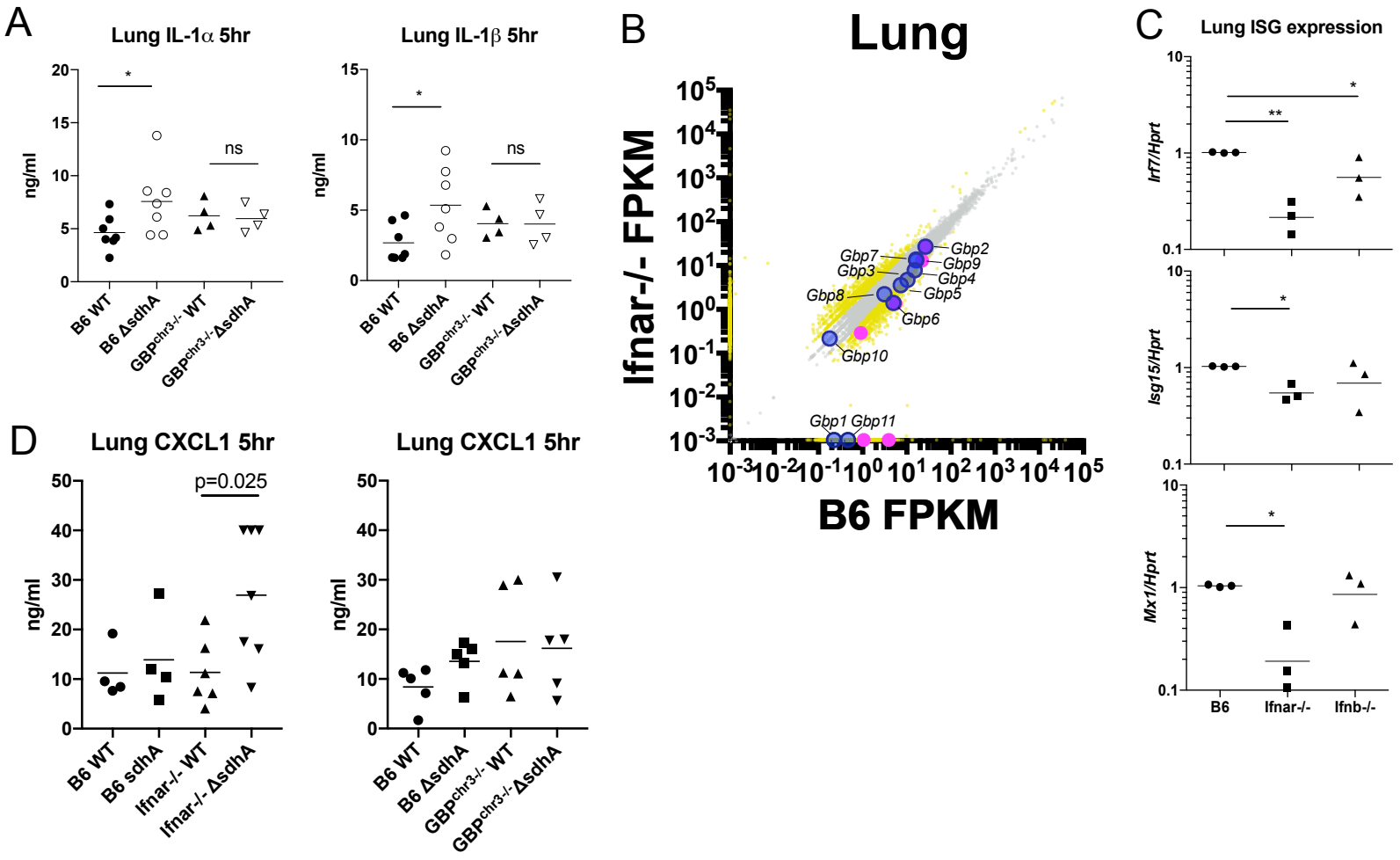


Figure S6. (Related to Figure 6)
B6 macrophages were challenged with the indicated strains of *L. pneumophila* in the presence of zVAD as indicated. ASC signal positivity is quantified within areas positive for anti-*Legionella* antibody (ROI).



Supporting Figure 7 (Related to Figure 7)
(A) Lung IL-1α and IL-1β were measured by ELISA. Each dot represents an animal, results are pooled from 2 experiments in which tissues were harvested between 12 and 18hrs post infection.
(B) Whole genome RNA-sequencing was performed on the indicated tissue and cellular populations. Fragments per kilobase mapped (FPKM) is plotted. Each dot is an individual gene. Gray dots represent genes where the expression difference between B6 and Ifnar-/- tissue is less than 2 fold. Yellow dots represent genes in which the expression difference between B6 and Ifnar-/- tissue is more than 2 fold. Blue dots represent the 11 Gbp genes encoded on the mouse genome, dots labeled. Magenta represent classical ISGs Stat1, Irf7, Isg15, Isg20, Mx1, Mx2.
(C) Various ISGs expression levels measured by qPCR from uninfected lungs from B6, Ifnar-/- and Ifnb-/- animals. Each dot represents an animal.
(D) Lung CXCL1 was measured by ELISA. Each dot represents an animal, results are pooled from 2 experiments in which tissues were harvested within a 4-6 hour window post infection.