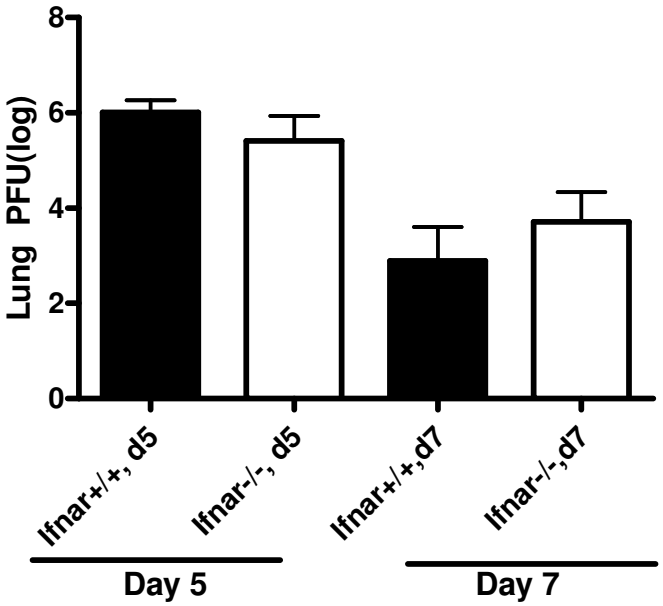
Supplemental Figures

Figure S1.

a.


8
6
4
2
0
Lung PFU(log)
Ifnar+/+, d5
Ifnar-/-, d5
Ifnar+/+,d7
Ifnar-/-,d7
Day 5
Day 7

b.

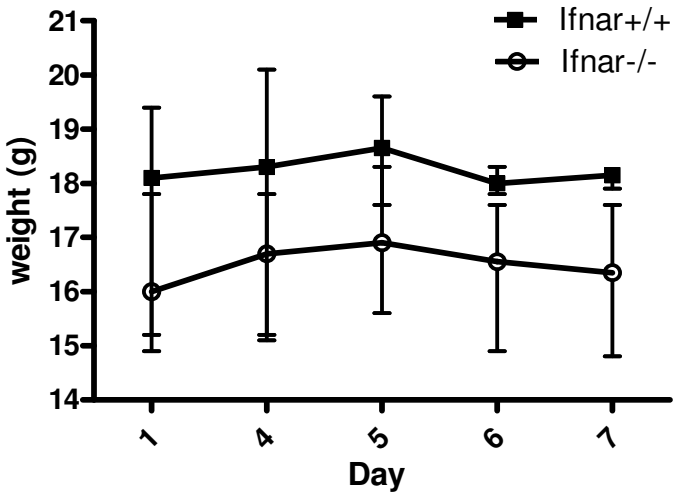

Ifnar+/+
Ifnar-/-
21
20
19
18
17
16
15
14
weight (g)
1
4
5
6
7
Day

Figure S2.

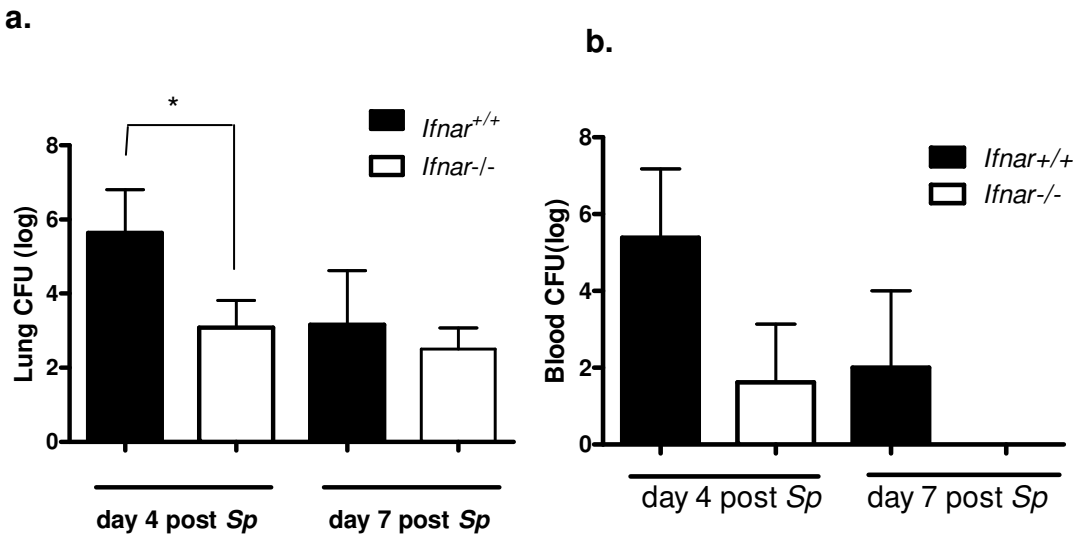
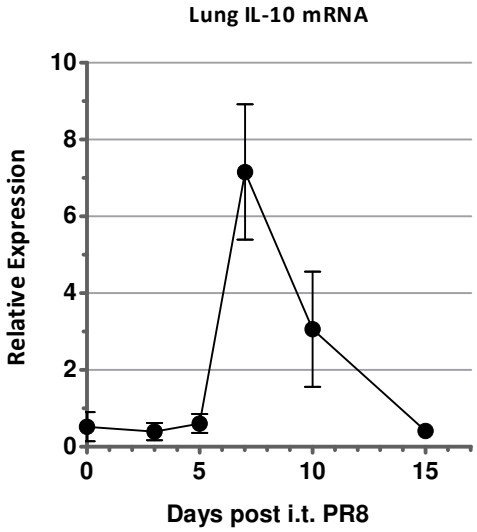

a.
b.
*
Ifnar+/+
Ifnar-/-
Lung CFU (log)
Blood CFU(log)
0
2
4
6
8
day 4 post Sp
day 7 post Sp

Figure S3.

a.


Lung IL-10 mRNA
Relative Expression
10
8
6
4
2
0
0
5
10
15
Days post i.t. PR8

b.

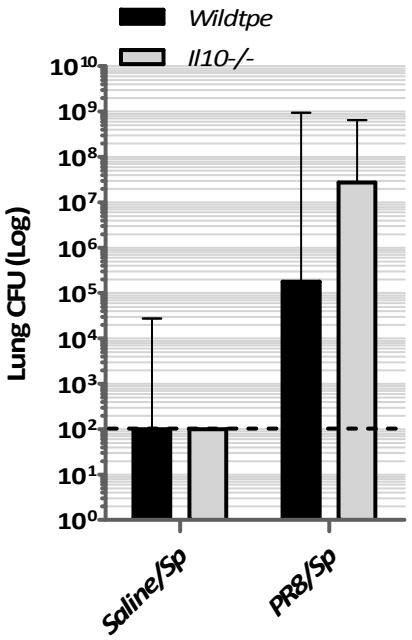

Wildtpe
Il10-/-
Lung CFU (Log)
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Saline/Sp
PR8/Sp

Figure S4

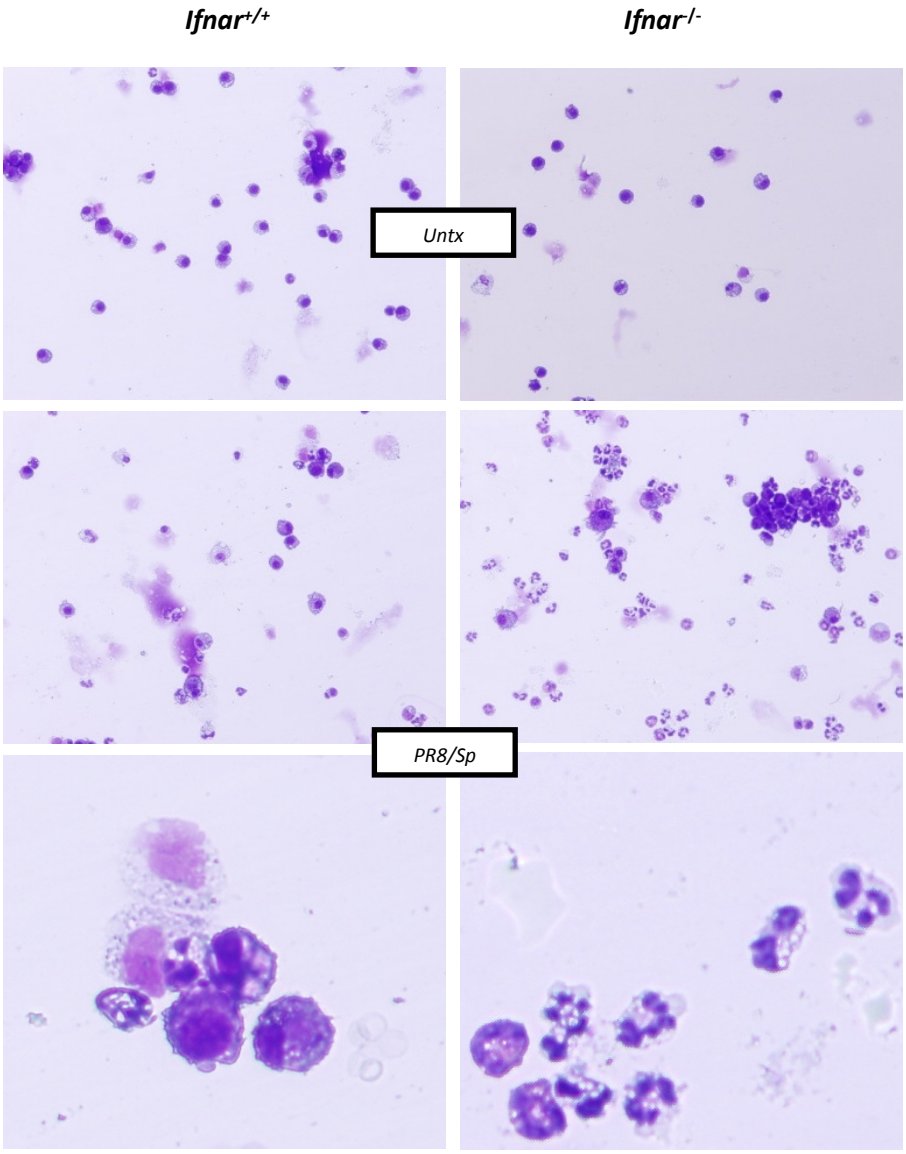


Figure S5

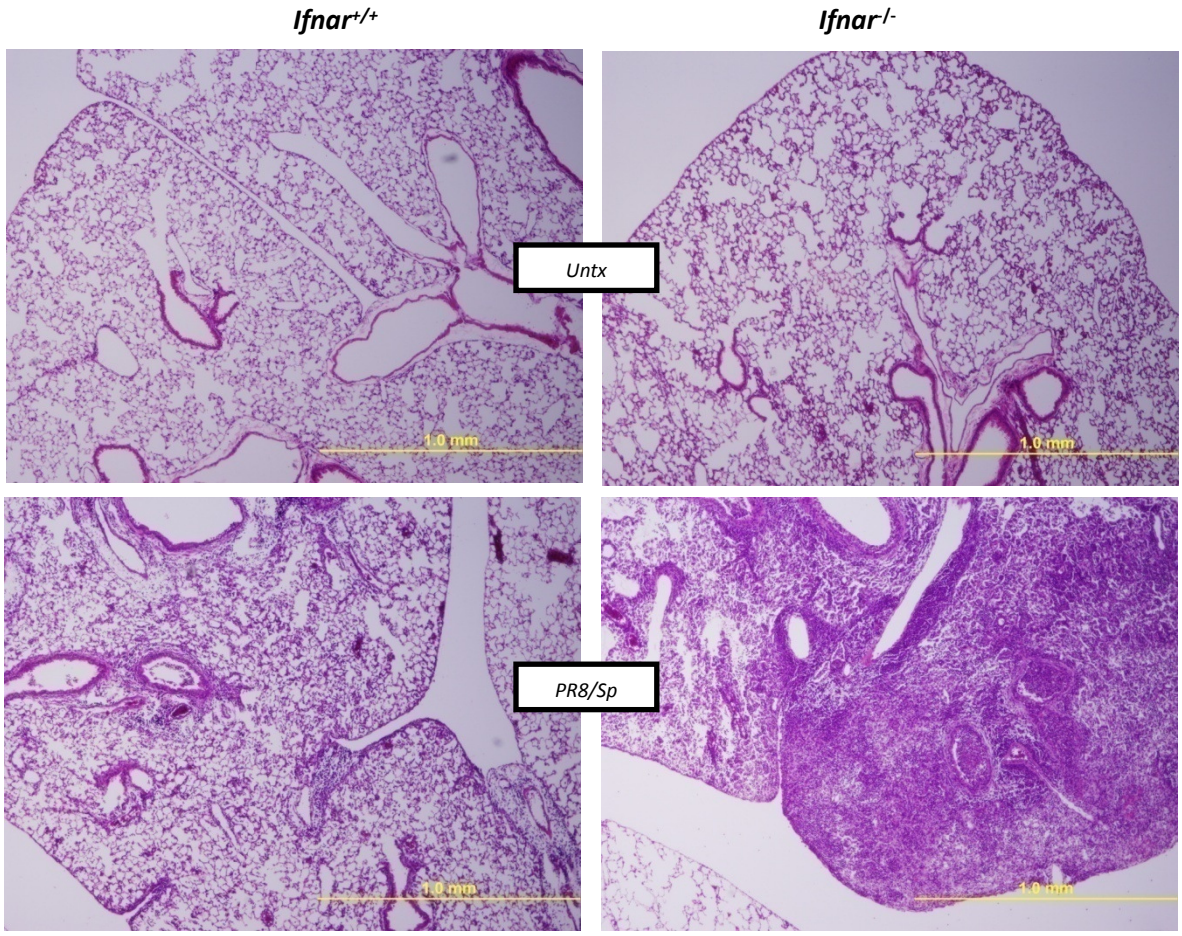


Figure S6.

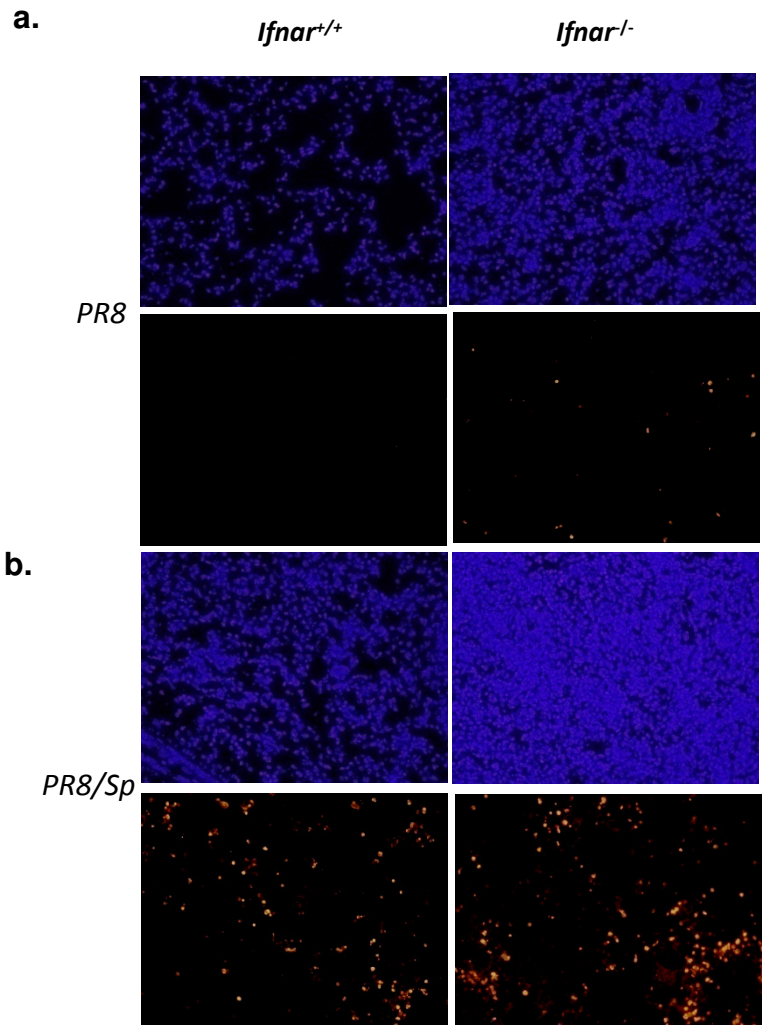
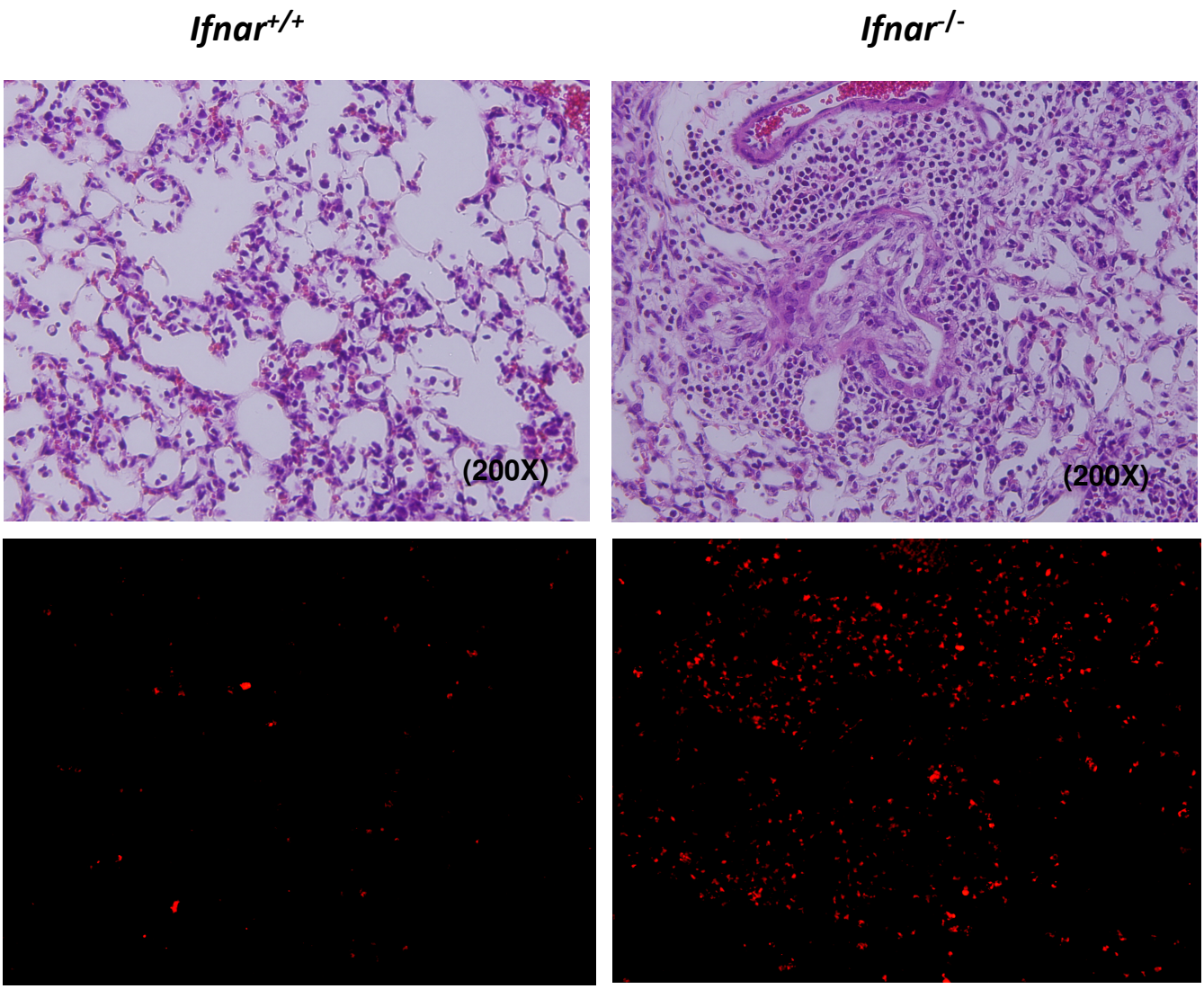


Figure S7.



SUPPLEMENTAL FIGURE LEGENDS

Figure S1: *Ifnar*$^{-/-}$ are similarly sensitive to PR8 as their wildtype counterparts. *Ifnar*$^{+/+}$ and *Ifnar*$^{-/-}$ animals were infected with 200 PFUs of PR8 influenza. (**a**) At day 5 and day 7 post infection, animals were sacrificed for assessment of viral PFUs in lung homogenates. (n=4/group) (**b**) Weights were obtained in *Ifnar*$^{+/+}$ and *Ifnar*$^{-/-}$ animals following i.t. PR8.

Figure S2: Lung and blood bacterial burden at day 4 and 7 following secondary *S. pneumoniae* challenge. *Ifnar*$^{+/+}$ and *Ifnar*$^{-/-}$ animals were administered i.t. PR8, followed 5 days later by i.t. *S. pneumoniae*. On day 4 and 7 following i.t. *S. pneumoniae*, (**a**) lung homogenates and (**b**) blood were collected for assessment of CFU(*, p=0.05, Mann-Whitney test; n=4/group)

Figure S3. IL-10 does not explain the enhanced sensitivity observed in *Ifnar*$^{+/+}$ mice. (**a**) Levels of IL-10 gene expression in lung homogenates were assessed at various timepoints following i.t. PR8 in wildtype animals. *Il10* transcript is not upregulated until day 7 post PR8 infection. (**b**) *IL-10*$^{-/-}$ and wildtype controls were administered saline or PR8, followed 5 days later by i.t. *S. pneumoniae* (2000 CFU). Influenza-infected wildtype and *Il10*$^{-/-}$ animals have comparable *S. pneumoniae* lung burdens. Data are representative of three independently performed experiments, n=4/group.

Figure S4: BAL cytospins from doubly infected mice of both genotypes show an apparent increase in the number of infiltrating PMNs in *Ifnar*$^{-/-}$ mice. Bronchoalveolar lavage was performed 14 hours post secondary challenge with *Sp,* in doubly infected *Ifnar*+/+ and *Ifnar-/-* animals. Cytospins were performed for Diff-Quik staining to assess cell counts and differentials. Panels depict representative sections of the cytospin slides made from *Ifnar*$^{+/+}$ and *Ifnar*$^{-/-}$ animals.

Figure S5: Doubly infected *Ifnar*$^{-/-}$ mice demonstrate a grossly apparent increase in inflammation in infected lobes. H&E stains of paraffin-embedded lung sections were examined at baseline (top panels), and at 48 hours post secondary infection in PR8/*S. pneumoniae*-infected *Ifnar*$^{+/+}$ *and Ifnar*$^{-/-}$ animals (bottom panels). No apparent differences were appreciated in animals of either genotype in singly infected groups (*PR8* or *S. pneumoniae* alone, data not shown). Lung sections are representative of n=3/group (n= 2/group for untreated).

Figure S6: Absence of type I IFN signaling alters neither apoptosis in *PR8/S. pneumoniae* infected animals. DAPI (upper panels) and TUNEL (lower panels) staining of lung sections from *PR8/Saline* (**a**) infected or *PR8/S. pneumoniae* (**b**) infected animals. TUNEL staining revealed no discernible differences in apoptosis between comparably infected *Ifnar*$^{+/+}$ or *Ifnar*$^{-/-}$ groups. Representative sections from n=3/group.

Figure S7. *Ifnar-/-* animals have enhanced inflammatory cells that are MPO-positive on histology. H&E staining (upper panels) and myeloperoxidase (MPO, lower panels) staining of lung sections obtained from doubly infected *Ifnar*$^{+/+}$ and *Ifnar*$^{-/-}$ mice. Sections are representative of n=3/group.