Supplemental Data

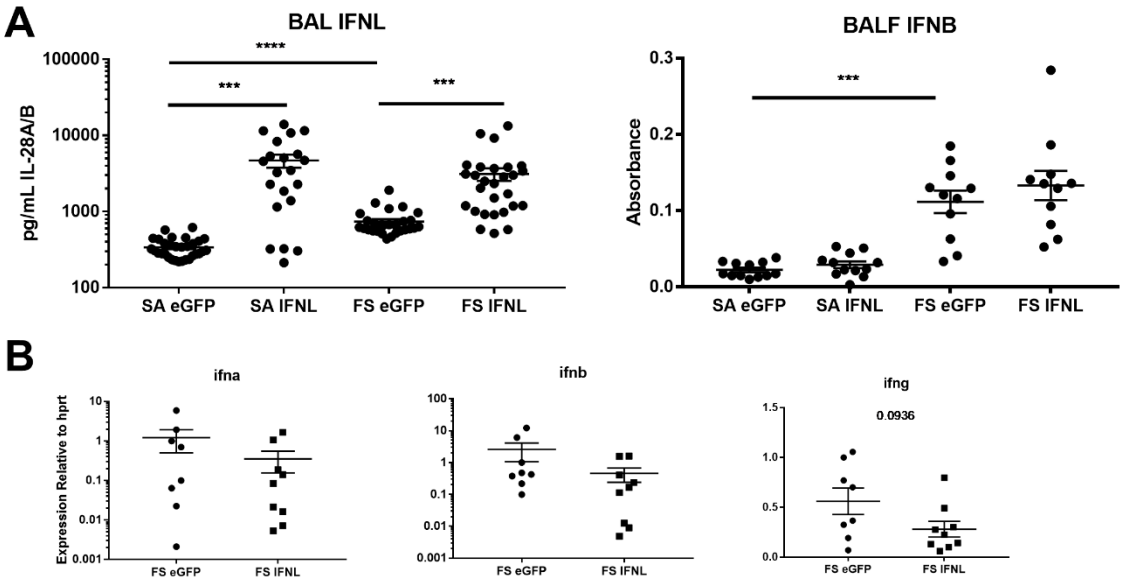

Figure S1.

IFNλ overexpression does not alter other interferons.

Mice were infected with influenza, five days later given adenovirus to overexpress IFNλ (Ad-IFNλ) or GFP (Ad-GFP), and twenty-four hours later challenged with 5×10^7 CFU MRSA as described in Figure 1. **(A)** ELISA for IFNλ or IFNβ in BALF (n = 4, three to eight independent experiments). **(B)** RT-PCR for interferon genes in lung RNA (n = 4, three independent experiments *$p < 0.05$, **$p < 0.01$, *** $p < 0.005$, ns = not significant.

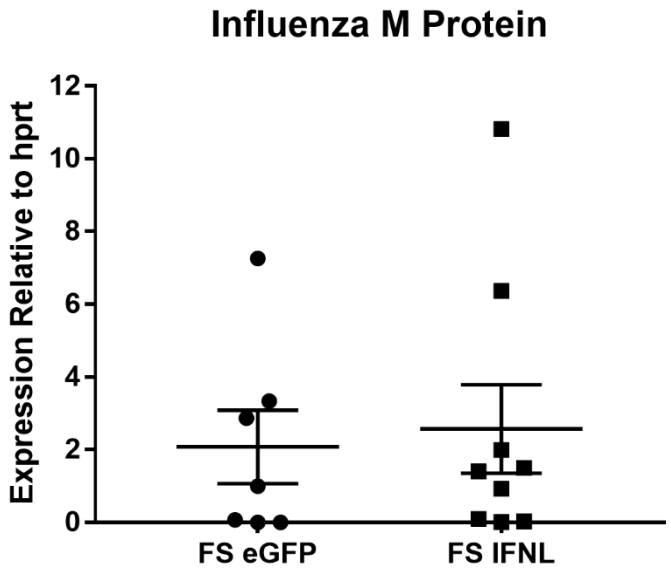

Figure S2.

IFNλ overexpression does not alter influenza viral burden.

Mice were infected with influenza, five days later given adenovirus to overexpress IFNλ (Ad-IFNλ) or GFP (Ad-GFP), and twenty-four hours later challenged with 5×10^7 CFU MRSA as described in Figure 1. **(A)** RT-PCR for influenza A/PR/8/34 H1N1 M protein in lung RNA (n = 4, two independent experiments). *p < 0.05, **p < 0.01, *** p < 0.005, ns = not significant.

CYTOKINE	FS EGFP	FS IFNL	SIGNIFICANCE	P-VALUE
EOTAXIN	2192 ± 602.9	2722 ± 780.4		0.5969
G-CSF	**1734 ± 233.7**	**3172 ± 350.7**	**	**0.0032**
GM-CSF	51.8 ± 4.484	54.31 ± 5.076		0.7153
IFNG	89.82 ± 15.33	87.61 ± 13.11		0.9139
IL-10	155.3 ± 36.67	100.6 ± 15.61		0.192
IL-12P40	129.2 ± 13.27	147 ± 21.05		0.4838
IL-12P70	186.4 ± 16.18	203.4 ± 16.14		0.4649
IL-13	52.41 ± 12.1	62.58 ± 13.65		0.5836
IL-17A	82.27 ± 15.97	82.54 ± 15.18		0.9901
IL-1A	**176.3 ± 24.06**	**248.4 ± 21.25**	*	**0.0363**
IL-1B	57.88 ± 4.573	74.5 ± 8.911		0.1181
IL-2	69.99 ± 13.24	76.65 ± 13.72		0.7305
IL-3	30 ± 6.312	29.3 ± 5.856		0.9367
IL-5	36.02 ± 6.619	32.38 ± 5.341		0.6733
IL-6	248.9 ± 30.36	285.5 ± 44.74		0.5069
IL-9	105.9 ± 17.06	111.5 ± 16.8		0.8198
KC	**380.9 ± 31.37**	**625.4 ± 53.82**	**	**0.0012**
MCP-1	4184 ± 893.5	3072 ± 563.6		0.3074
MIP-1A	2039 ± 277.9	2108 ± 338.6		0.8753
MIP-1B	1242 ± 146	1138 ± 158.6		0.6332
RANTES	2879 ± 423.5	2385 ± 517.1		0.4685
TNFA	207.1 ± 25.77	276.9 ± 36.97		0.1389

Figure S3.

IFNλ overexpression does not alter other inflammatory chemokines.

Mice were infected with influenza, five days later given adenovirus to overexpress IFNλ (Ad-IFNλ) or GFP (Ad-GFP), and twenty-four hours later challenged with 5×10^7 CFU MRSA as described in Figure 1. Lungs were snap-frozen, homogenized, and the supernatant assayed for inflammatory cytokines by multiplex (n=12, three independent experiments). Data are reported as mean +/- SEM, with significance and p-value calculated by Welch's t-test.

Figure S4

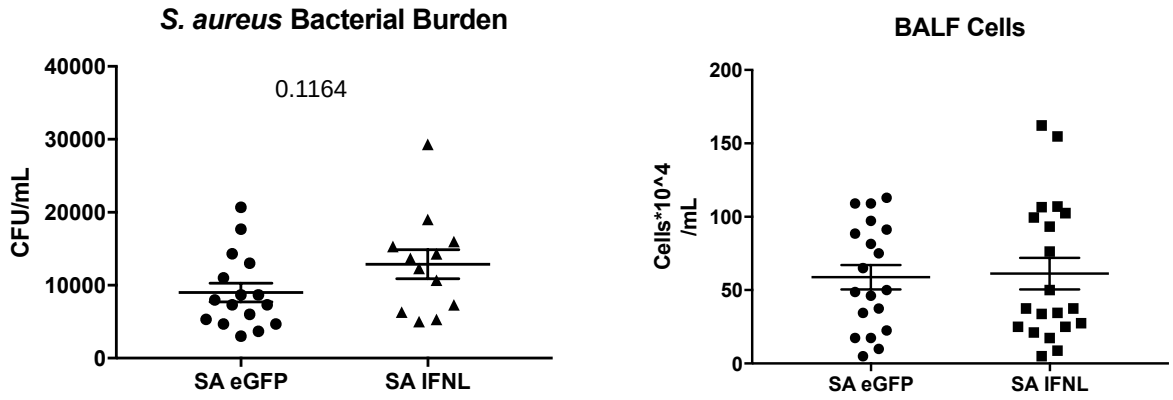

Figure S4.

IFNλ overexpression does not alter bacterial burden during MRSA lung infection alone.

Mice were infected with influenza, five days later given adenovirus to overexpress IFNλ (Ad-IFNλ) or GFP (Ad-GFP), and twenty-four hours later challenged with 5×10^7 CFU MRSA as described in Figure 1. Bacterial burden was determined by counting CFU from plated lung homogenate. BALF cell number was determined by lavaging mouse lungs at time of sacrifice with 1 mL sterile PBS, performing red blood cell lysis, and resuspending in PBS and counting total cells on a hemocytometer.

Figure S5

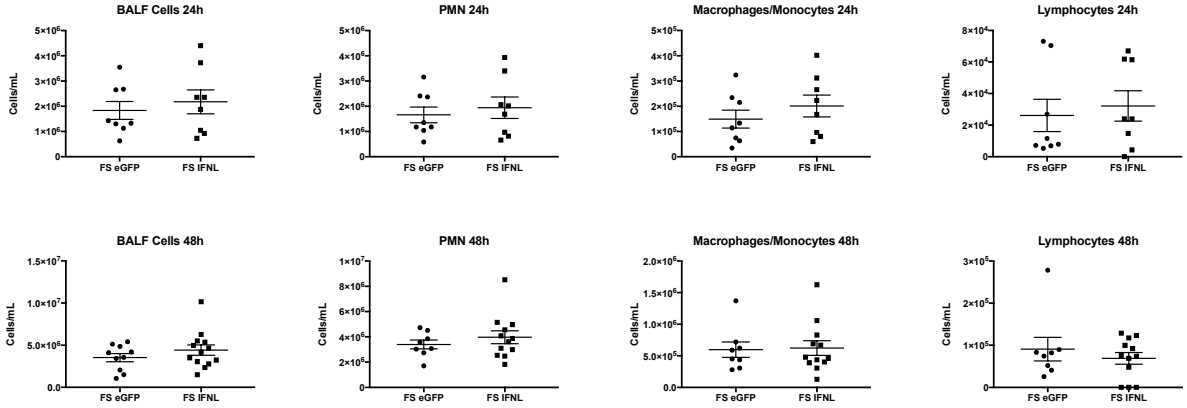

Figure S5.

IFNλ overexpression does not alter bronchoalveolar lavage cells during influenza/*S. pneumoniae* super-infection.

Mice were infected with influenza, five days later given adenovirus to overexpress IFNλ (Ad-IFNλ) or GFP (Ad-GFP), and twenty-four hours later challenged with 1000 CFU *S. pneumoniae*. Mice were sacrificed either 24 or 48 hours later. Lungs were lavaged with 1 mL sterile PBS and the resulting bronchoalveolar lavage fluid (BALF) was processed by cytospin and BALF cells were differentially counted after staining (Diff-Quik).