Innate lymphoid cells promote lung tissue homeostasis following acute influenza virus infection

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Supplementary Figures

Supplementary figure 1. Weight loss, lung function decline and early immunopathology are similar between influenza virus-infected wild-type and *Rag1*$^{-/-}$ mice

(**a-c**) WT C57BL/6 or *Rag1*$^{-/-}$ were infected with 0.5 LD_{50} PR8 i.n. and assessed for weight loss (**a**) and lung function by pulse oximetry (**b**). (**c**) Lung histological sections from naïve, WT or *Rag1*$^{-/-}$ mice at day 10 post infection (p.i.), stained with H&E. (**a-c**) Data shown is combination of 5 experiments, n = 3-5 mice per experiment. Scale bar = 100 μm.

Supplementary figure 2. Reduced eosinophilia and IL-5 levels in anti-CD90.2-treated mice during influenza virus infection

(**a**) Absolute cell numbers of neutrophils and eosinophils in the BAL fluid of isotype, anti-NK1.1 or anti-CD90.2 mAb-treated *Rag1*$^{-/-}$ mice at day 10 post PR8 influenza virus infection. (**b**) mRNA expression of IL-5 in lung tissue from naïve or antibody-treated influenza-infected *Rag1*$^{-/-}$ mice at day 10 post infection. mRNA expression levels normalized to β-actin and shown relative to expression levels in naïve mice. Data is representative of 3 experiments, n = 4 mice. * $p < 0.05$

Supplementary figure 3. CD90.2 expression on innate cell populations

Representative flow cytometry plots of CD90.2 expression on neutrophils ($Ly6G^+$), eosinophils (Siglec F^+), dendritic cells ($CD11c^+$), and macrophages ($CD11b^+$) in the lung at day 10 post PR8 influenza infection virus infection, gated on live cells. Data is representative of 3 independent experiments, n = 3-4 mice.

Supplementary figure 4. Administration of recombinant IL-13 fails to restore lung function in ILC-depleted mice

(**a-c**) *Rag1*$^{-/-}$ mice were infected with 0.5 LD_{50} PR8 influenza virus and treated with isotype mAb, anti-CD90.2 mAb or anti-CD90.2 + rIL-13 (5-10 μg i.p. every 2 days starting at D0). (**a**)

Measurement of IL-13 protein in the BAL fluid, N.D. not detected. (**b**) Histological analysis of lung tissue from antibody-treated mice at 10 days p.i. Black arrows denote regions of goblet cell hyperplasia. Scale bar = 50 μm. (**c**) Measurement of blood oxygen saturation levels by pulse oximetry. (**a-c**) Data is representative of 2 independent experiments, n = 4 mice.

Supplementary figure 5. IL-22 is not required for respiratory tissue remodeling following influenza virus infection

Weight loss (**a**) and pulse oximetry (**b**) of wild-type mice treated with isotype or anti-IL-22 mAb (200 μg) during PR8 influenza infection (0.5 LD_{50}). (**c**) H&E stained lung sections from antibody-treated mice at day 10 post influenza infection. (**a-c**) Data is representative of 2 independent experiments, n = 4 mice. Scale bar = 100 μm

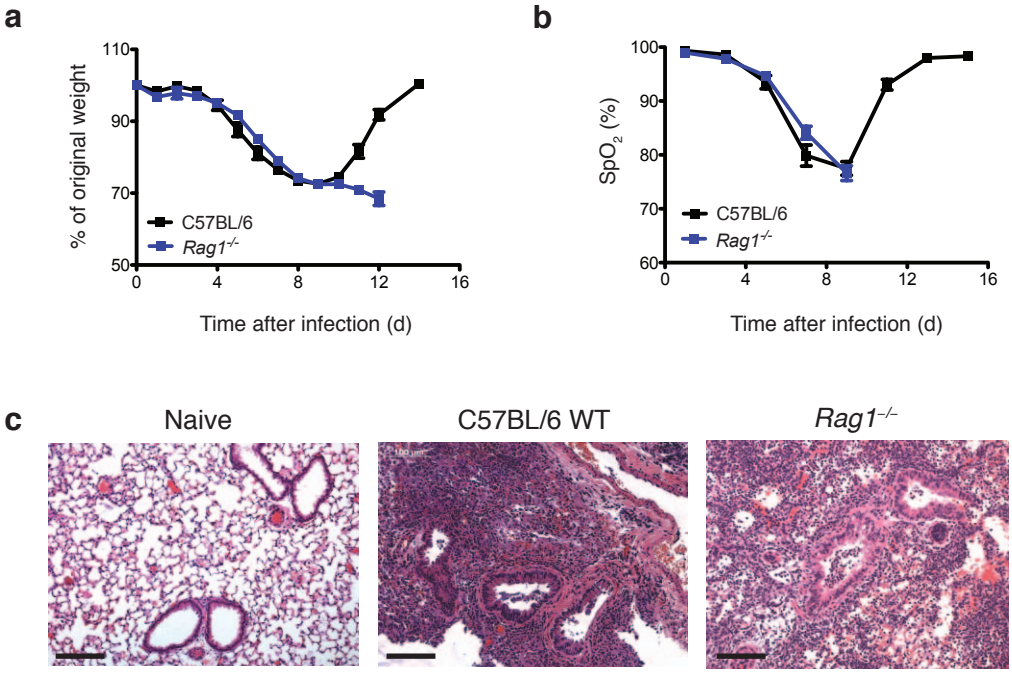
Supplementary figure 6. Treatment with amphiregulin protein does not affect IL-5 and IL-13 cytokine levels in ILC-depleted mice

(**a-b**) *Rag1*$^{-/-}$ mice were infected with 0.5 LD_{50} PR8 influenza virus and treated with isotype mAb, anti-CD90.2 mAb or anti-CD90.2 mAb + recombinant murine amphiregulin (5-10 μg i.p. every 2 days starting at D0). Measurement of IL-5 (**a**) and IL-13 (**b**) mRNA expression levels in lung at day 10 p.i., normalized to β-actin and expressed relative to naïve expression levels. (**a-b**) Data is representative of 2 independent experiments, n = 4 mice.

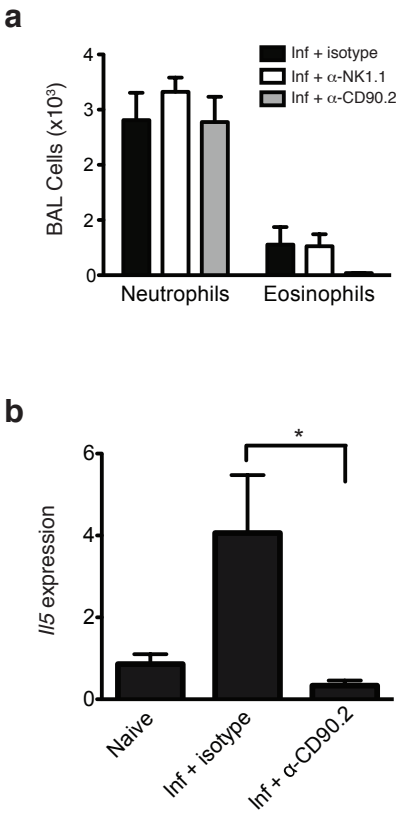
Supplementary Table 1. GO term gene list enriched in lung ILC and splenic LTi cell gene expression signatures

Supplementary Table 2. Top gene transcripts ("leading edge") in LPS-treated lung GSEA data set

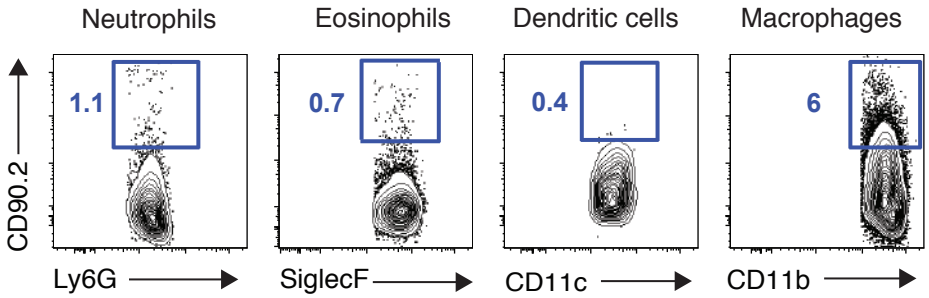
Supplementary figure 1



Supplementary figure 2

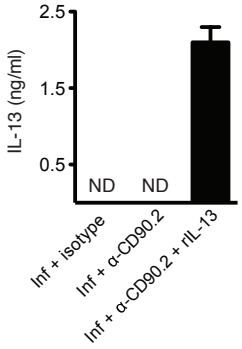


Supplementary figure 3



Supplementary figure 4

a



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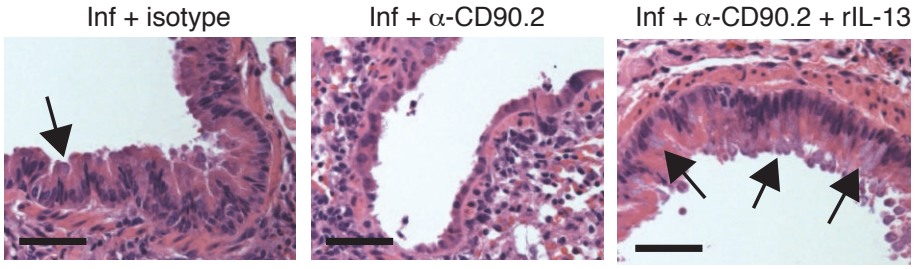

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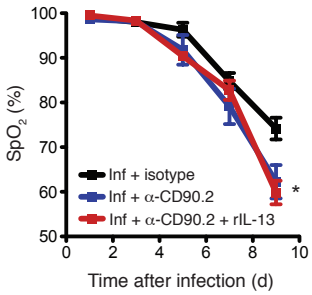
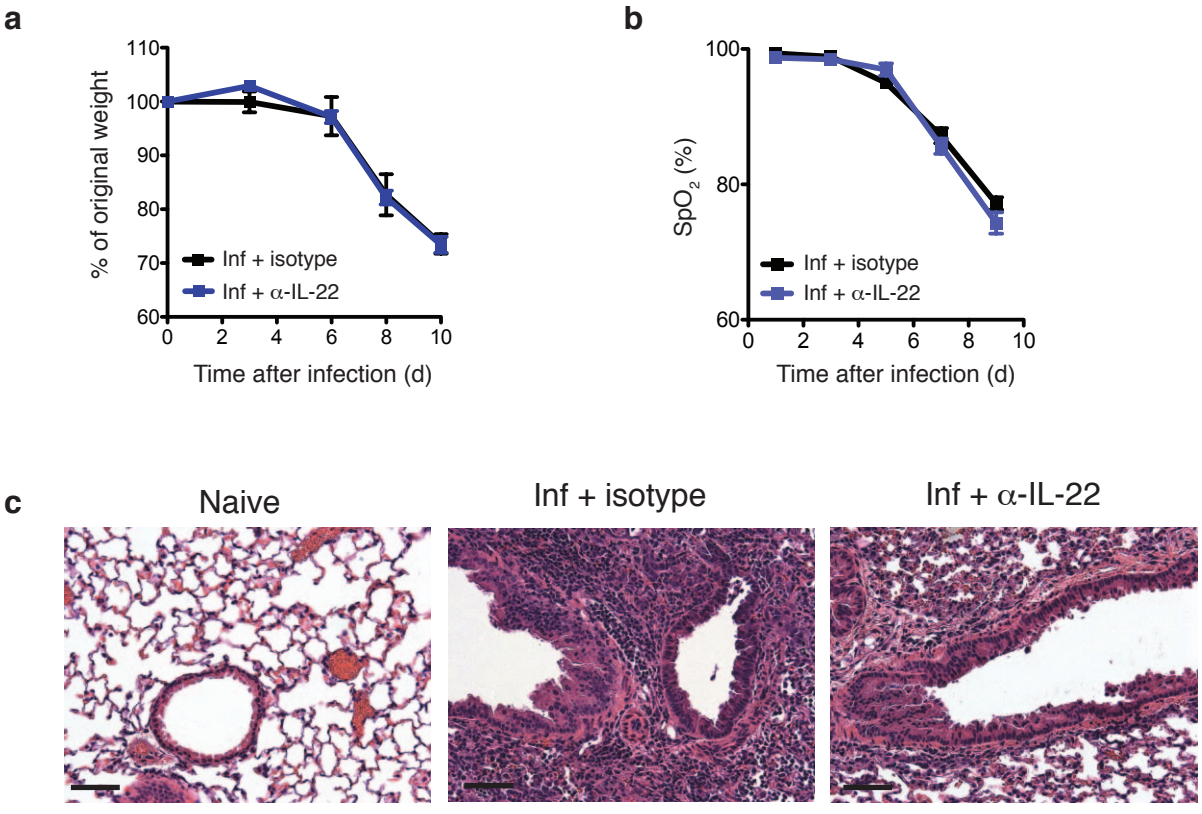
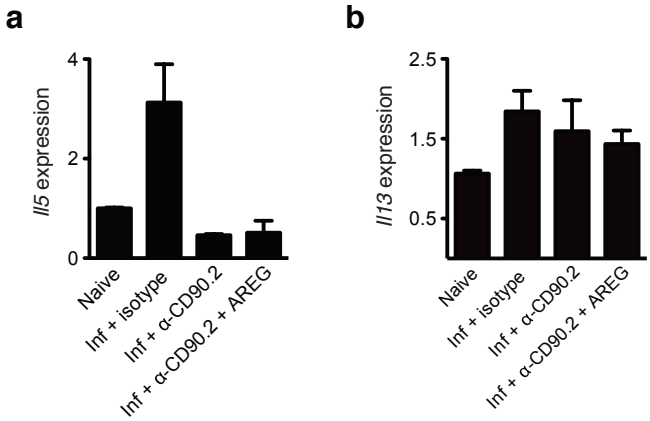


Supplementary figure 5



Supplementary figure 6



Supplemental Table 1: GO term gene list enriched in lung ILC or splenic LTi gene expression signatures

ILC Enriched				LTi Enriched		
regulation of cell proliferation	**defense response**	**response to wounding**	**inflammatory response**	**immune response**	**hematopoetic or lymphoid organ development**	**cell activation**
CSF2	CXCL1	CXCL1	CXCL1	IL1F9	STAP1	FYB
CAV1	RARRES2	NFKBID	NFKBID	IL1R1	TGFBR1	KLRK1
FOSL2	NFKBID	C3	C3	SUSD2	G6PDX	MYO1F
FGF7	C3	CXCL3	CXCL3	PGLYRP1	RORC	RORC
PTGS2	CXCL3	PPARG	CXCL2	CD74	VAV1	SKAP2
CSF1	PPARG	TLR1	TLR1	TMEM173	CD74	VAV1
PPARG	TLR1	CXCL2	PPARG	FCER1G	HBA-A1	CD74
NFKBIA	CXCL2	GJA1	C1S	CD4	ZFP826	CXCR5
CD24A	C1S	C1S	CD24A	BCL6	HBA-A2	VAMP7
SCGB1A1	PRDX1	TIMP3	TGFB1	CLEC4D	CXCR5	PLCG2
TGFB1	CD24A	CD24A	CALCA	MPA2L	MFSD7B	FCER1G
ADA	TGFB1	TGFB1	CFH	LTB	PLCG2	CD4
CDH5	CALCA	CALCA	THBS1	LTA,	CD4	BCL6
LIF	SH2D1A	ARG1	NFKBIZ	CD7	BCL6	H2-DMA
SPRY2	CFH	CFH	IL6	RAB27A	HBB-B1	HDAC9
GPC3	THBS1	THBS1	IL5	IGH	H2-DMA	RAB27A
GATA3	NFKBIZ	NFKBIZ	CCL21A	IL18R1	HDAC9	LTB
BCL11B	IL6	IL6	SERPING1	IRGM1	LTB	LTA
CALCRL	IL5	IL5	CCL11	IL23R	LTA	HELLS
MYC	IL1RL1	CCL21A	HIF1A	LY96	HELLS	
DPT	CCL21A	SERPING1	CCR5	IGJ		
KLF5	SERPING1	PLAUR	CCR4	MYO1F		
IRS2	COTL1	CCL11	CXCL15	H2-AB1		
AR	CCL11	THBD	CCR2	VAV1		
IL6	HIF1A	HIF1A	ALOX5	H2-DMB2		
SPARC	PENK	CCR5	KDM6B	TNFSF8		
PROX1	CCR5	CCR4		PSMB9		
HES1	CCR4	CXCL15		BTLA,		
PRKCQ	CXCL15	CCR2		VAMP7		
ADRB2	CCR2	ALOX5		H2-EB1		
CDKN1A	ALOX5	PROS		PLCG2		
RBPJ	RBPJ	KDM6B		H2-AA		
KLF4	KDM6B			GBP4		
BMPR1A				H2-DMA		
NFIB						
IL2						

Supplementary Table 2: Top gene transcripts ("leading edge") in LPS-treated Lung GSEA data set

GSEA leading edge genes
Cxcl2
Tnfaip3
Myc
Csf2
Tiparp
Fgl2
Gadd45b
Cdkn1a
Chd7
Ctla2b
Aim1
Areg
Nr4a1
Ccr2
Ptgir
Skil
Tlr1
Calca
Fosl2