

Epithelial derived TGF- β 1 acts as a pro-viral factor in the lung during influenza A infection

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Supplementary Figure legends

Supplementary Figure 1: Epithelial cell CCSP expression and BAL cell composition. (a)

Levels of bioactive TGF- β in the airways 12hrs post influenza infection in epTGF β KO and control mice. (b) CCSP mRNA expression from epithelial cells recovered from tracheal brushings from epTGF β KO and control mice at day 1 post influenza infection. (c) Cellular composition of the BAL including airway macrophages (AMs) (CD64⁺ CD11c⁺ SiglecF⁺), neutrophils (Ly6G⁺ CD11b⁺) and other CD45⁺ leukocytes at 12hrs and day 1 post infection. (d-e) *Tgfb1* mRNA levels in (d) epithelial cells and (e) airway leukocytes 12hrs post infection from control and epTGF β KO mice. (f) *Tgfb1* mRNA levels in epithelial cells and airway leukocytes 1 day post infection from control and epTGF β KO mice. Data shown are representative of two independent experiments with a total of N \geq 8 mice per group. Box and whisker plots depict the median and IQR and minimum and maximum values, Bar charts depict the median.

Supplementary Figure 2: Flow cytometry gating strategy for myeloid cells. FSC^{low} SSC^{low}

lymphocytes and CD45 negative cells were excluded before identifying airway macrophages as Siglec F⁺ CD11c⁺ and CD64⁺. Of the SiglecF negative cells, neutrophils were identified as

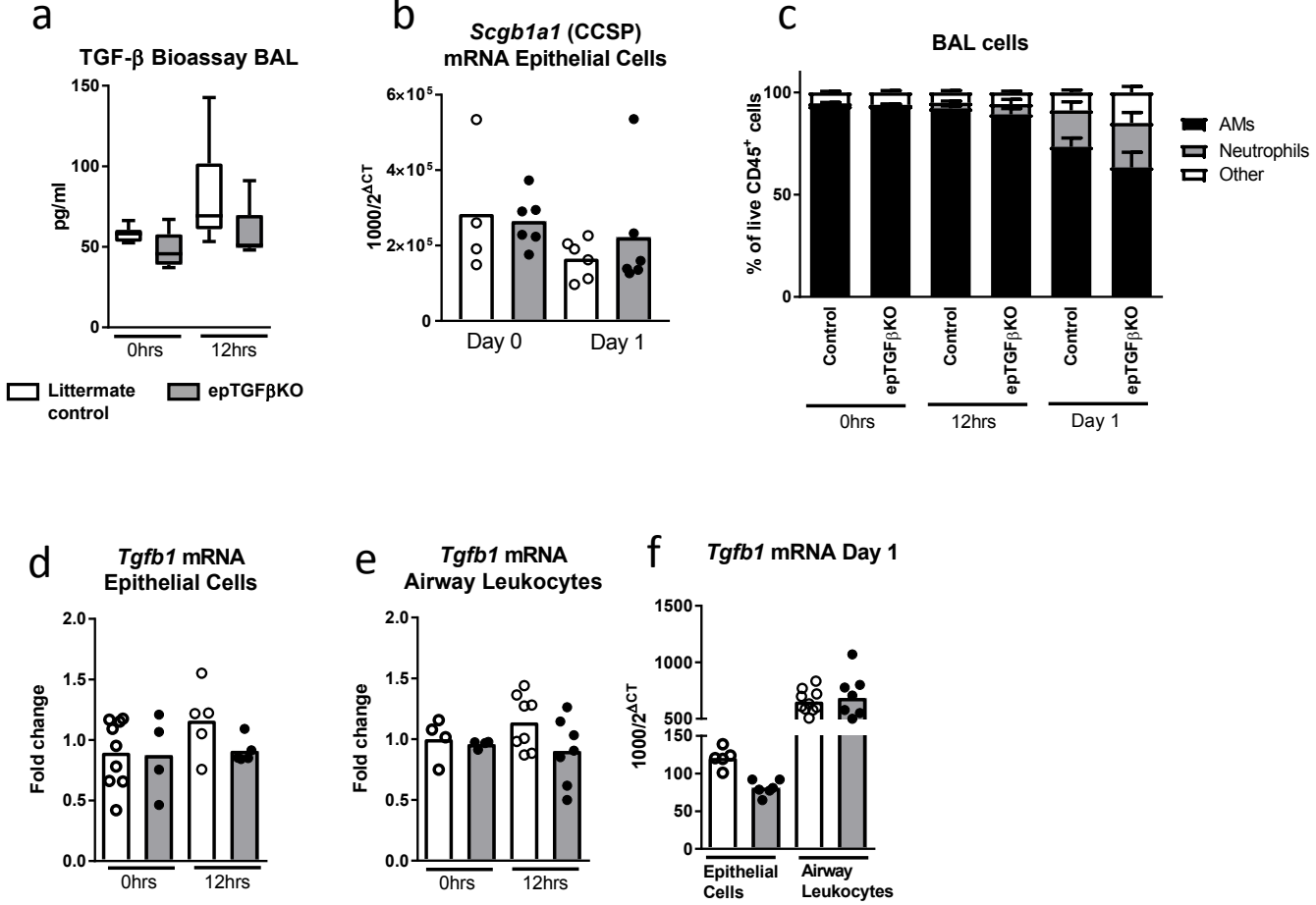
Ly6G⁺ CD11b⁺ and infiltrating monocytes and macrophages were identified as CD64⁺ CD11b⁺ Ly6G^{neg} and CD11c^{neg}. The inflammatory monocyte and macrophage gate contained cells with a range of Ly6C and MHCII expression. Representative plots shown from a control mouse BAL 3 days post influenza infection.

Supplementary Figure 3: Immune response in the lung and airways after influenza infection in epTGFbKO and control mice. (a-b) Percentage of airway macrophages (CD64⁺ CD11c⁺ SiglecF⁺) expressing **(a)** MHC-II and **(b)** CD11b after influenza infection of epTGFbKO and control mice at day 1, 3, 6 post infection. **(c)** Number of airway IFN-g⁺ NK cells (NK1.1⁺CD3^{neg}) **(d)** Number of ILC1s in the lung (IFN-g⁺ICOS⁺CD45⁺Lin^{neg}) where lineage cocktail comprises (CD3, CD45R, CD11b, TER-119, Ly6G/GR1). **(e)** IFN-g⁺ CD8 T cells (CD8⁺CD3⁺) in the airways. **(f)** IFN-g⁺ CD4 T cells (CD4⁺CD3⁺) in the airways. **(g)** IL-17⁺ CD4 T cells (CD4⁺CD3⁺) in the airways. **(h-i)** Tregs (Foxp3⁺CD25⁺CD4⁺) in the **(h)** airways and **(i)** lung. **(j)** Levels of IL-1b in the BAL. **(k)** IL-10 levels in the lung tissue. Data shown is pooled from two independent experiments with a total of N≥11 mice per group. Box and whisker plots depict the median and IQR and minimum and maximum values. *p < 0.05, and **p < 0.01.

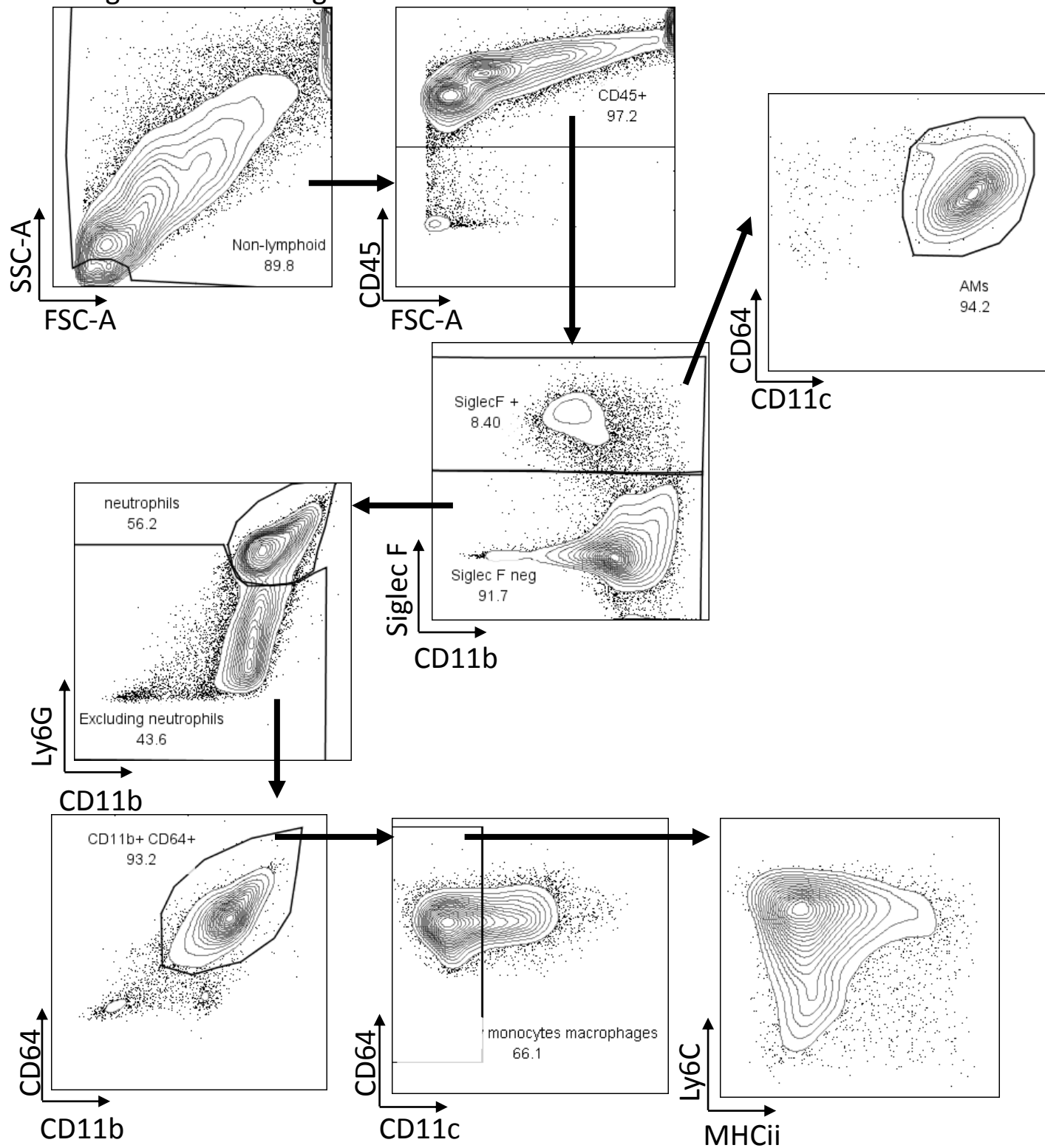
Supplementary Figure 4: Enhanced early IFN β response in epTGFbKO airway leukocytes. (a) X31 RNA levels in epithelial cells and airway leukocytes at 0, 12 and 24hrs post infection in epTGFbKO and control mice. **(b)** Graph shows correlation of IFN- β levels in the airways and lung viral titres at day 1 post infection. **(c-d)** mRNA levels of *Oas2* at **(c)** 12hrs and **(d)** 1 day post infection in epithelial cells and airway leukocytes from control and epTGFbKO mice. **(e-f)** mRNA levels of **(e)** *Irf7* and **(f)** *Ifitm3* in airway leukocytes 1 day post infection from

control and epTGFbKO mice. Data representative of two independent experiments total N \geq 8. Bar charts depict the median. Spearman correlation. **p < 0.01.

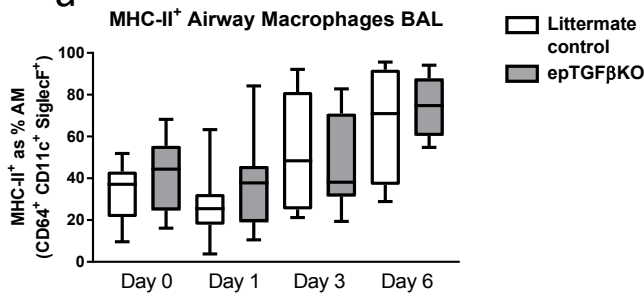
Supplementary Figure 5: IFN β response at day 1 post RSV infection. (a) Concentration of IFN- β in the airways of epTGFbKO mice and control mice after RSV infection at day 0, 1, 3 and 7 post infection. Limits of detection were 7.25 pg/ml. (b-c) mRNA levels of *Ifnb1* in (b) epithelial cells and (c) airway leukocytes 1 day after RSV infection. (d-e) mRNA levels of *Oas2* in (d) epithelial cells and (e) airway leukocytes 1 day after RSV infection. Data representative of two independent experiments N \geq 4. Bar charts depict the median. Box and whisker plots depict the median and IQR and minimum and maximum values and bar charts depict the median.



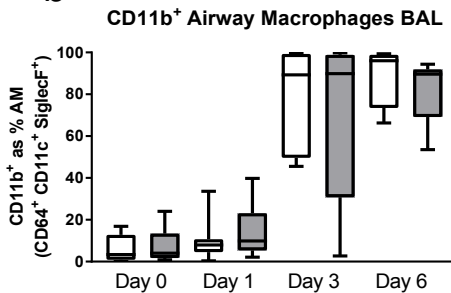
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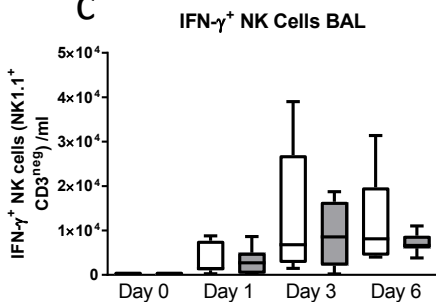
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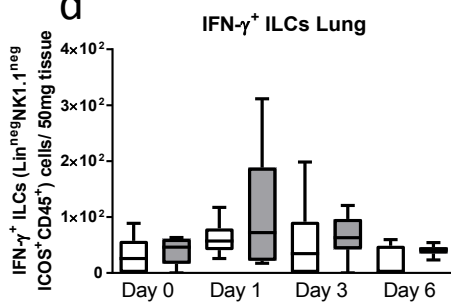
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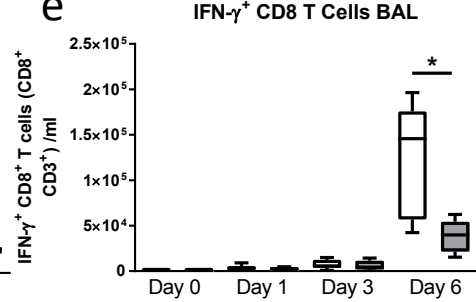
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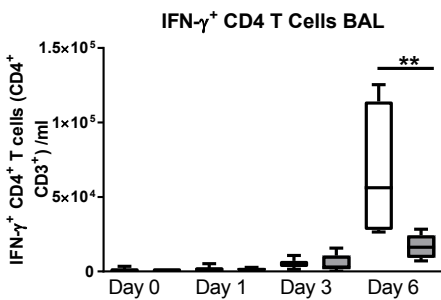
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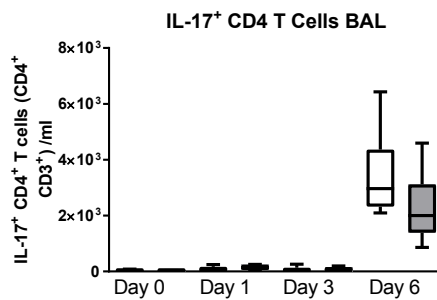
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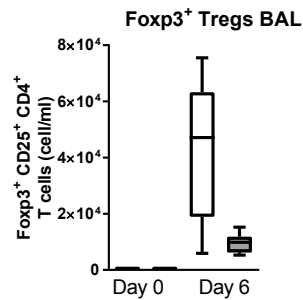
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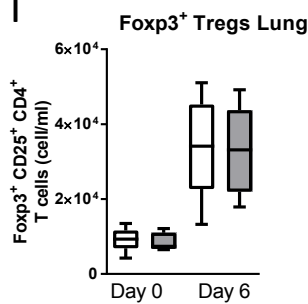
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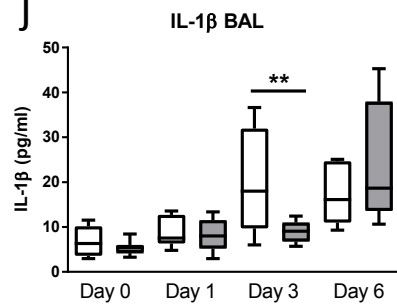
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