$CD4^+$ T cell help and innate-derived IL-27 induce Blimp-1 dependent IL-10 production by anti-viral CTL

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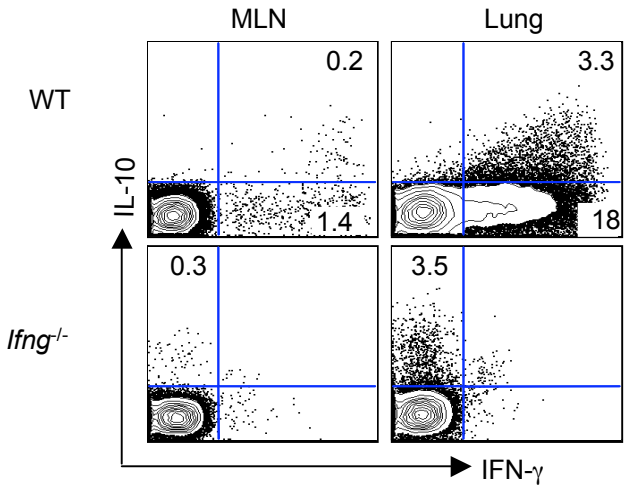
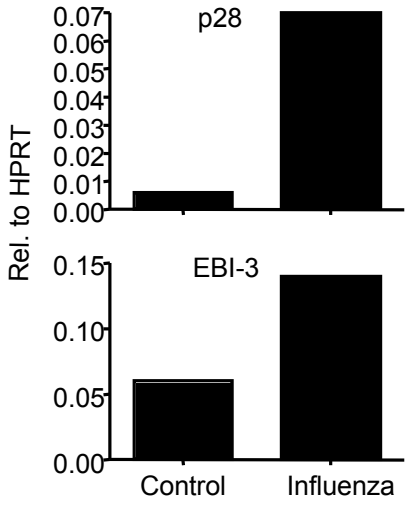
Supplementary Figure 1



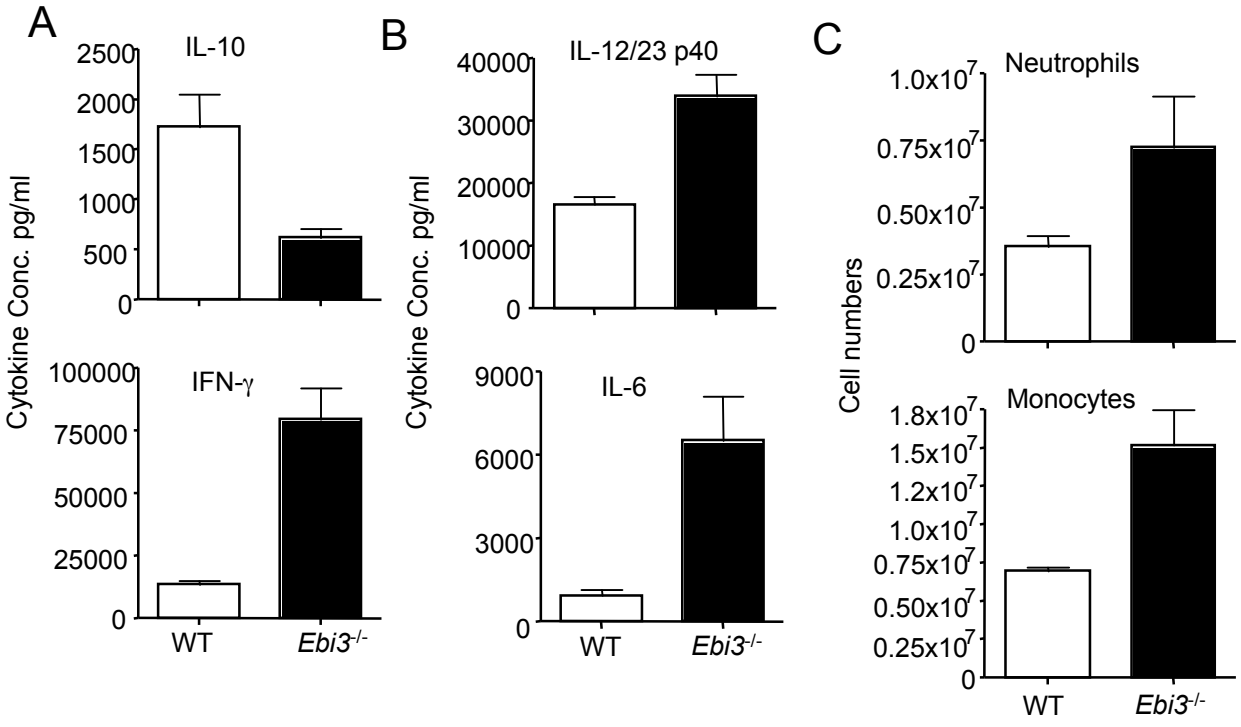
Supplementary Figure 1. IFN-γ is dispensable for the induction of IL-10 producing CTL during respiratory virus infection.
WT or *Ifng*$^{-/-}$ mice were infected with influenza. At d7 p.i., the production of IL-10 and IFN-γ by CTL was measured by ICS following flu-infected BMDC restimulation. Numbers are the percentages of cells in gated population.

Supplementary Figure 2



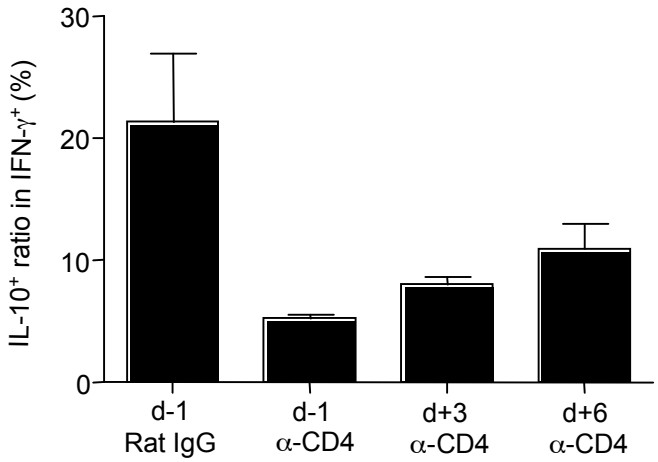
Supplementary Figure 2. Influenza infection induces IL-27 expression in the respiratory tract.
WT mice were infected with influenza. At d5 p.i., the expression of IL-27 subunits p28 and EBI-3 by lung cells were determined through quantitative RT-PCR. Lung cells from uninfected mice (Control). Lung cells from d5 infected mice (Influenza).

Supplementary Figure 3



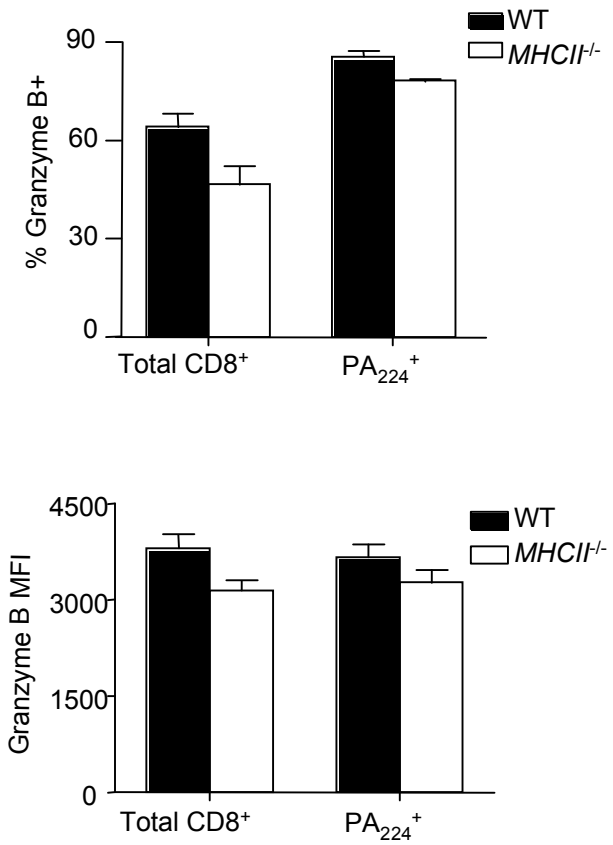
Supplementary Figure 3. *Ebi3*$^{-/-}$ mice exhibit diminished IL-10 production and enhanced pulmonary inflammation during influenza infection.
WT or *Ebi3*$^{-/-}$ mice were infected with influenza. At d7 p.i., the levels of IL-10 and IFN-γ (a) or IL-12/23 p40 and IL-6 (b) in BALF were determined through ELISA. (d) At d9 p.i., the numbers of lung monocytes/macrophages and neutrophils were measured through flow cytometry.

Supplementary Figure 4



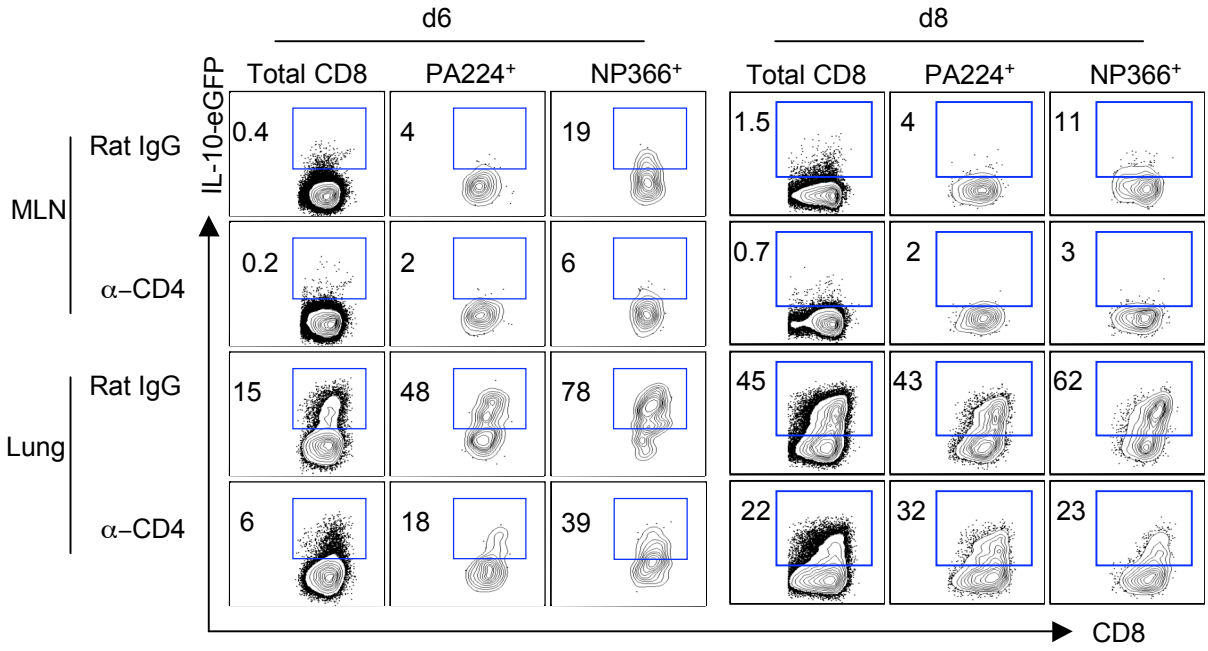
Supplementary Figure 4. Impact of $CD4^+$ T cell depletion at different days on the induction of IL-10 producing CTL.
WT mice were injected with Rat IgG control mAb or α-CD4 depleting mAb at various days p.i.. At d8 p.i., the production of IL-10 and IFN-γ by CTL was measured by ICS following flu-infected BMDC restimulation. The normalized percentages of $IL\text{-}10^+$ in influenza-specific $CD8^+$ T cells (reflected as $IFN\text{-}\gamma^+$) are depicted.

Supplementary Figure 5



Supplementary Figure 5. CD4$^+$ T cell help has a minimal impact on Granzyme B expression by CTL.
WT or *MHCII*$^{-/-}$ mice were infected with influenza. At d7 p.i., the expression of Granzyme B (Gzmb) by total lung CD8$^+$ T cells or PA_{224} tetramer positive cells was determined through ICS. The percentages of Gzmb$^+$ cells and the MFI of Gzmb in Gzmb$^+$ cells are depicted.

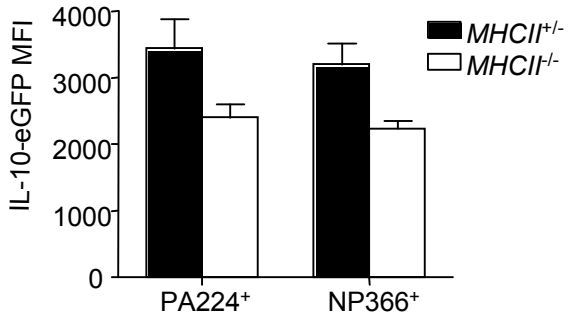
Supplementary Figure 6



Supplementary Figure 6. CD4$^+$ T cell depletion impairs the *in vivo* development of IL-10-expressing CTL

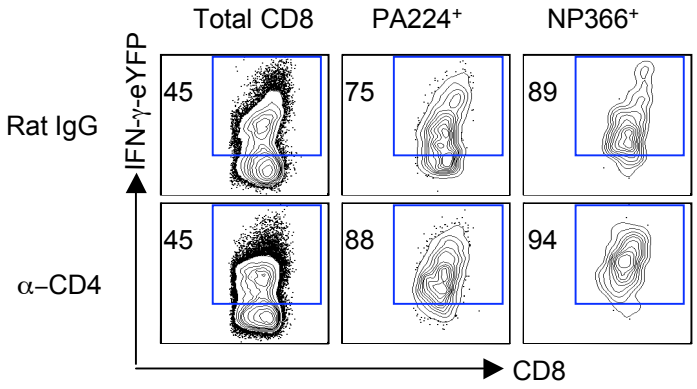
Vert-X mice were injected with either Rat IgG or α-CD4 Ab and infected with influenza. At d6 and 8 p.i., the percentages of IL-10-eGFP$^+$ in total CD8$^+$ T cells or influenza specific PA_{224} or NP_{366} tetramer positive cells were measured through flow cytometry. Numbers are the percentages of cells in gated population.

Supplementary Figure 7



Supplementary Figure 7. The absence of CD4$^+$ T cell impairs IL-10 expression at the level of individual cells
MHCII$^{+/-}$-Vert-X or *MHCII*$^{-/-}$-Vert-X mice were infected with influenza. At d7 p.i., the MFI of IL-10-eGFP in IL-10-eGFP$^+$ influenza specific CTL is depicted.

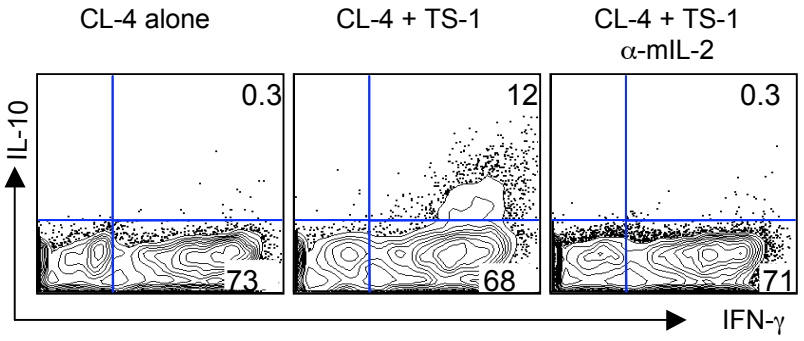
Supplementary Figure 8



Supplementary Figure 8. $CD4^+$ T cell depletion minimally affects the induction of IFN-γ producing cells *in vivo*

IFN-γ reporter (Yeti) mice were injected with either Rat IgG or α-CD4 Ab and infected with influenza. At d7 p.i., the expression of IFN-γ-eYFP in total $CD8^+$ cells or influenza specific PA_{224} or NP_{366} tetramer positive cells was measured by flow cytofluorometric analysis. Numbers are the percentages of cells in gated population.

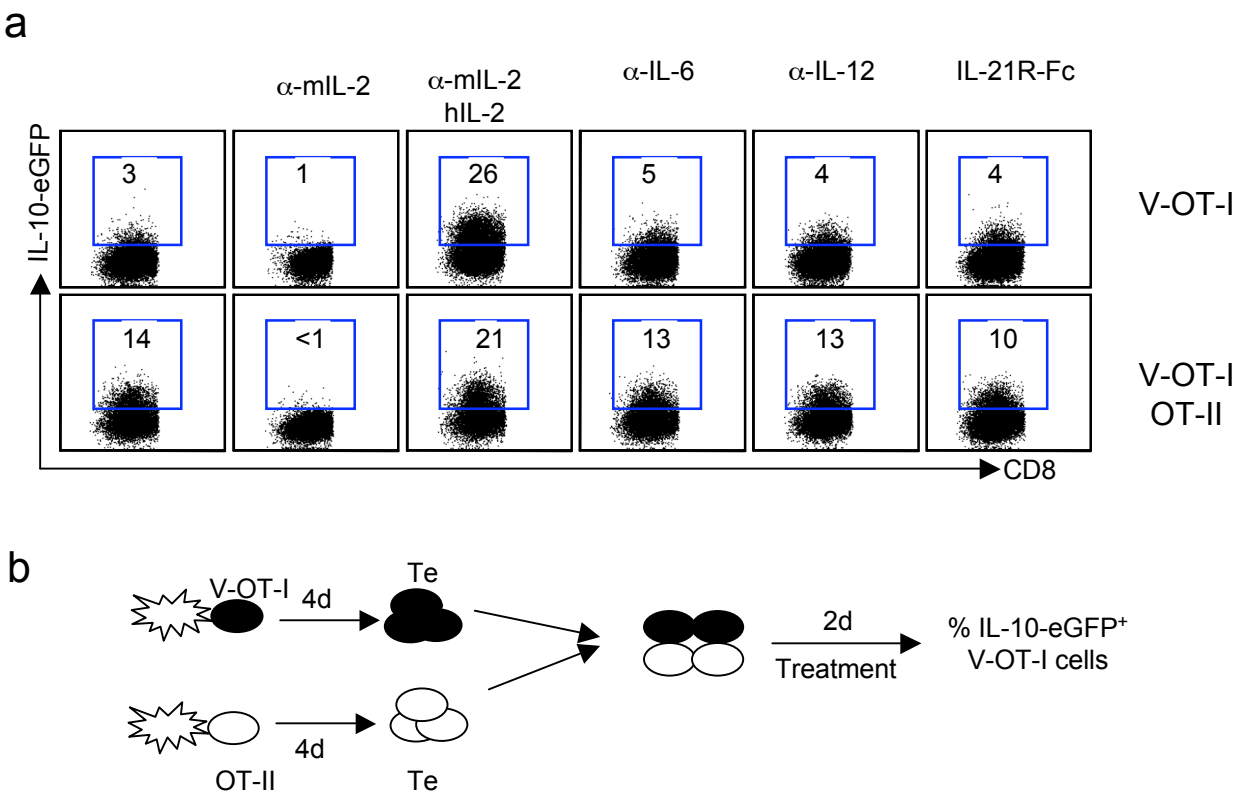
Supplementary Figure 9



Supplementary Figure 9. In vitro generated IL-10-producing cells are type 1 effectors

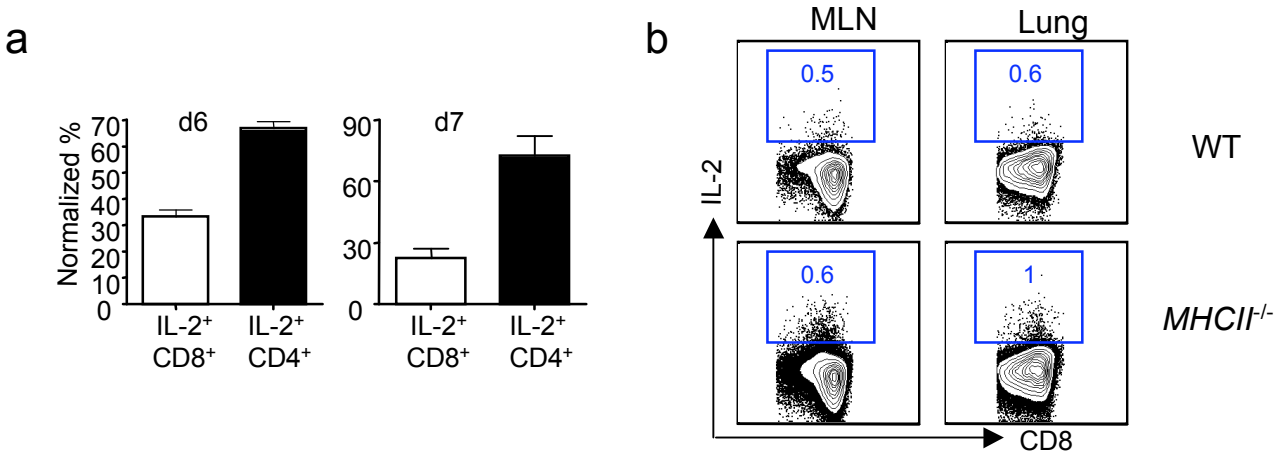
$CD8^+$ CL-4 TCR transgenic T cells were stimulated with influenza-infected DC in the presence or absence of $CD4^+$ TS-1 TCR transgenic T cells for 4d. The cultured cells were treated with various conditions as indicated. Then the production of IL-10 and IFN-γ by CL-4 cells were measured through ICS following restimulation with $HA_{533\text{-}541}$ peptide. Numbers are the percentages of cells in gated population.

Supplementary Figure 10



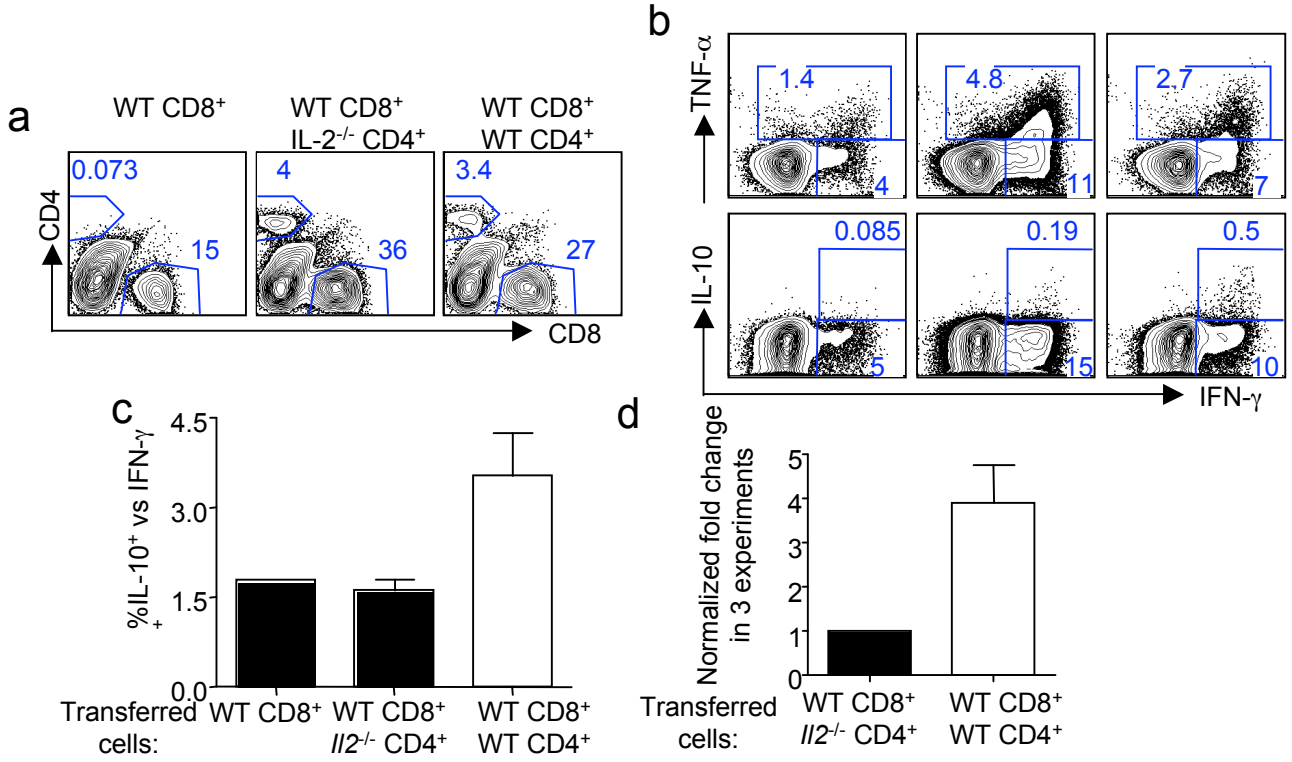
Supplementary Figure 10. $CD4^+$ T cell help for the induction of IL-10 producing CTL by is dependent on IL-2 and not IL-6, IL-12 or IL-21.
(a) $CD8^+$ Vert-X-OT-I (V-OT-I) double transgenic T cells were stimulated with influenza infected DC in the absence or presence of OT-II for 4d. The cultured cells were treated with various conditions as indicated. The expression of IL-10-eGFP by V-OT-I cells was measured by flow cytometry. Numbers are the percentages of cells in gated population. (b) Schematic depiction of the culture condition to assess the role of activated $CD4^+$ effector T cells (Te) providing the "help" to activated $CD8^+$ Te.

Supplementary Figure 11



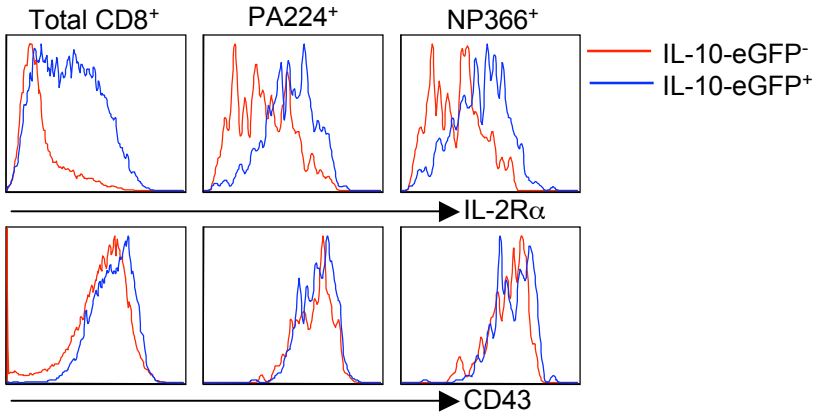
Supplementary Figure 11. $CD4^+$ T cells (and not $CD8^+$ T cells) are the predominant source of IL-2 necessary to support optimal IL-10 expression by CTL.
(a) WT mice were infected with influenza. At indicated days p.i., the production of IL-2 by gated $Thy1^+$ cells were measured by ICS following influenza infected BMDC restimulation. The normalized percentages of $CD4^+$ $IL\text{-}2^+$ cells or $CD8^+$ $IL\text{-}2^+$ cells in total $IL\text{-}2^+$ cells are depicted. (b) WT or *MHCII*$^{-/-}$ mice were infected with influenza. At d7 p.i., the production of IL-2 by gated $Thy1^+$ $CD8^+$ cells were measured by ICS following restimulation with influenza infected BMDC.

Supplementary Figure 12



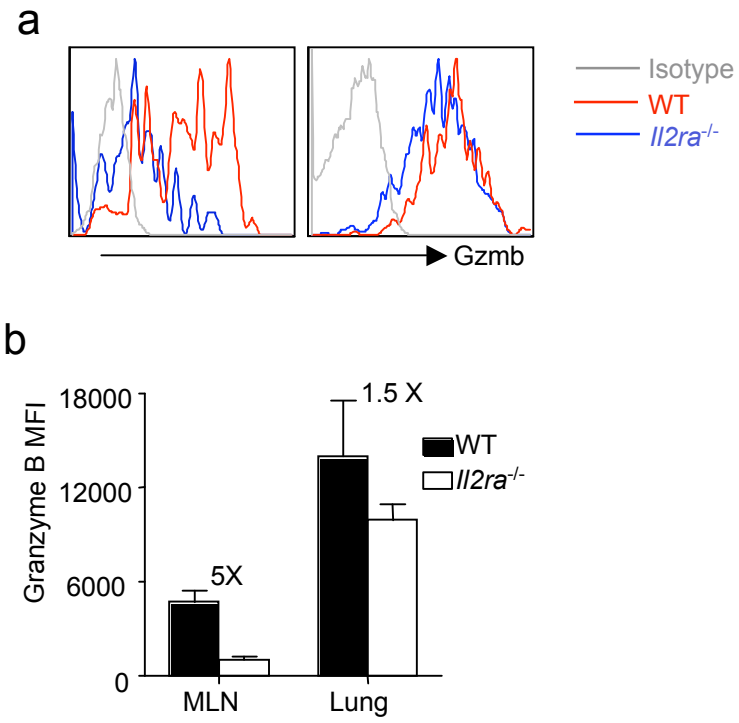
Supplementary Figure 12. IL-2 derived from CD4$^+$ T cells is required for the optimal induction of IL-10 producing CTLs *in vivo*.
WT CD8$^+$ T cells were co-transferred either with WT or *Il2*$^{-/-}$ CD4$^+$ T cells into *Rag2*$^{-/-}$ mice. The recipient mice were subsequently infected with influenza. (a) At d9 p.i., the percentages of CD4$^+$ T cells or CD8$^+$ T cells in the lung were determined by flow cytometry. (b) The production of TNF, IL-10 and IFN-γ by CTL was measured by ICS following restimulation with influenza-infected BMDC. (c) The normalized percentages of IL-10$^+$ cells among influenza-specific (IFN-γ$^+$) CD8$^+$ T cells from infected lungs of transferred mice are depicted. (d) The normalized fold changes of the percentages of IL-10$^+$ cells in antigen specific IFN-γ$^+$ secreting CTL detected in infected recipients of transferred WT or *Il2*$^{-/-}$ CD4$^+$ T cells from three individual experiments are depicted. Normalization method for panel d: % of IL-10$^+$ cells in antigen specific CTL from Rag2$^{-/-}$ recipient mice receiving *Il2*$^{-/-}$ CD4$^+$ T cells are designated as 1. The corresponding numbers in *Rag2*$^{-/-}$ mice receiving WT CD4$^+$ T cells are calculated as % of IL-10$^+$ cells in antigen specific CTL in these mice divided by the % of IL-10$^+$ cells in antigen specific CTL from *Rag2*$^{-/-}$ mice receiving *Il2*$^{-/-}$ CD4$^+$ T cells.

Supplementary Figure 13



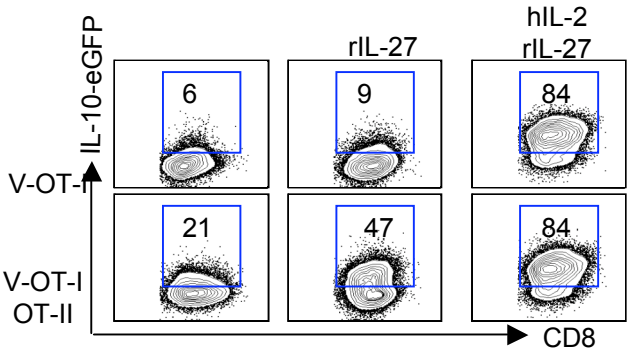
Supplementary Figure 13. IL-10-expressing cells express IL-2Rα
Vert-X mice were infected with influenza. At d7 p.i., the expression of IL-2Rα and CD43 by lung total $CD8^+$ cells or influenza specific PA_{224}^+ or NP_{366}^+ cells was measured by flow cytometry.

Supplementary Figure 14



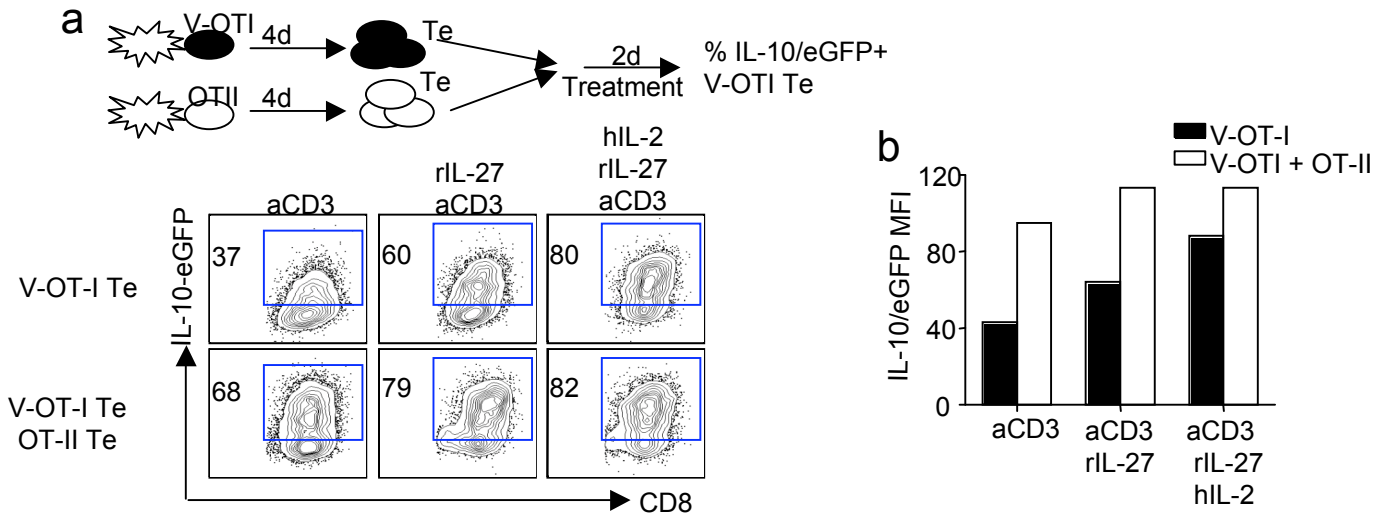
Supplementary Figure 14. IL-2 signaling is minimally required for Granzyme B expression by CTL in the lung
WT:*Il2ra*$^{-/-}$ mixed bone marrow chimera mice were infected with influenza. At d7 p.i., the expression of Granzyme B by influenza specific CD8^{+} PA$_{224}^{+}$ cells in MLN or lung was measured ICS.

Supplementary Figure 15



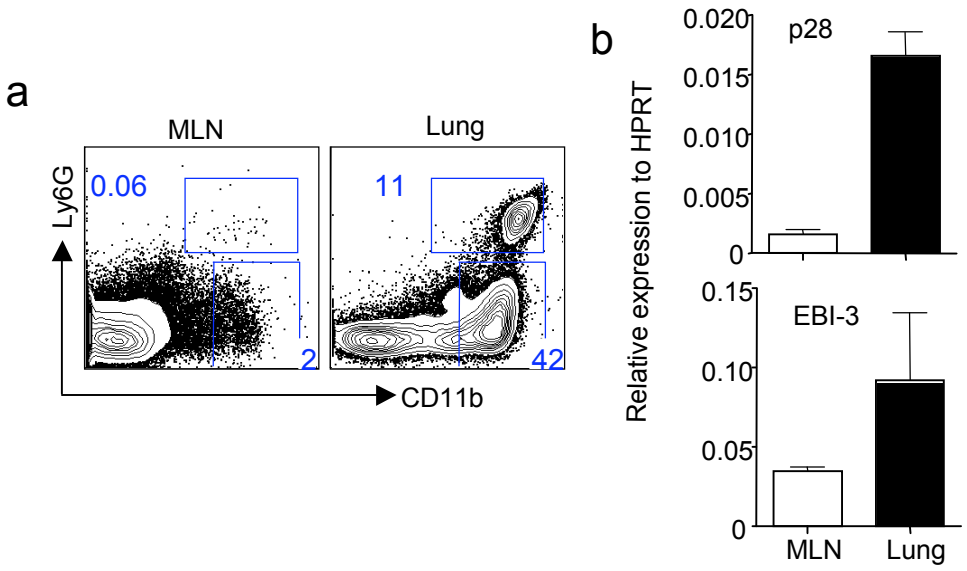
Supplementary Figure 15. IL-2 and IL-27 co-operatively induce IL-10 expressing CD8$^+$ CTL.
CD8$^+$ Vert-X-OT-I (V-OT-I) double transgenic T cells were stimulated with influenza-OVA infected DC in the absence or presence of OT-II for 4d. The expression of IL-10-eGFP by V-OT-I cells was measured by flow cytometry.

Supplementary Figure 16



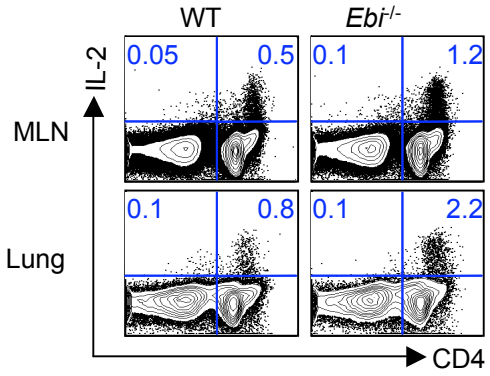
Supplementary Figure 16. IL-2 and IL-27 co-operatively induce IL-10 expression by CD8$^+$ CTL at the effector stage.
V-OT-I cells or OT-II cells were activated separately by influenza-OVA infected DC for 4d. The effector V-OT-I were left alone or mixed together with effector OT-II cells. The cells were then stimulated with plate-bound aCD3 and treated with various conditions as indicated. (a) The expression of IL-10-eGFP by V-OT-I was measured through flow cytometry. (b). The MFI of IL-10-eGFP by V-OT-I cells is depicted.

Supplementary Figure 17



Supplementary Figure 17. Neutrophils, monocytes/macrophages and IL-27 message are highly enriched in the influenza infected lungs.
WT mice were infected with influenza. (a) At d5 p.i., the percentages of neutrophils ($CD11b^+ Ly6G^+$) and monocytes/macrophages ($CD11b^+ Ly6G^-$) were evaluated by flow cytometry. (b) At d5 p.i., the expression of IL-27 subunits p28 and EBI-3 by lung cells were determined through quantitative RT-PCR.

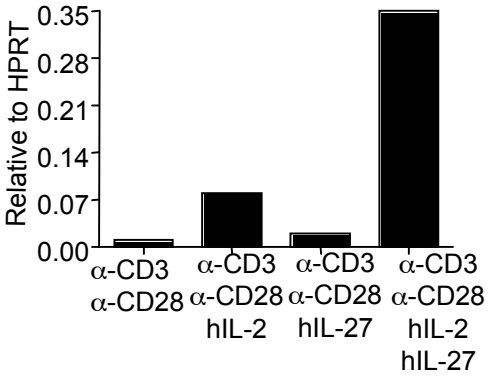
Supplementary Figure 18



Supplementary Figure 18. EBI-3 deficiency does not impair IL-2 production by T cells

WT or *Ebi3*$^{-/-}$ mice were infected with influenza. At d6 p.i., the production of IL-2 in gated Thy1^{+} cells was measured by ICS following restimulation with influenza-infected BMDC.

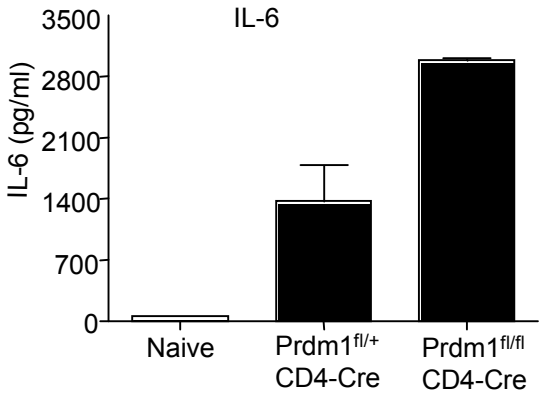
Supplementary Figure 19



Supplementary Figure 19. IL-2 and IL-27 synergistically induce IL-10 expression by human CTL

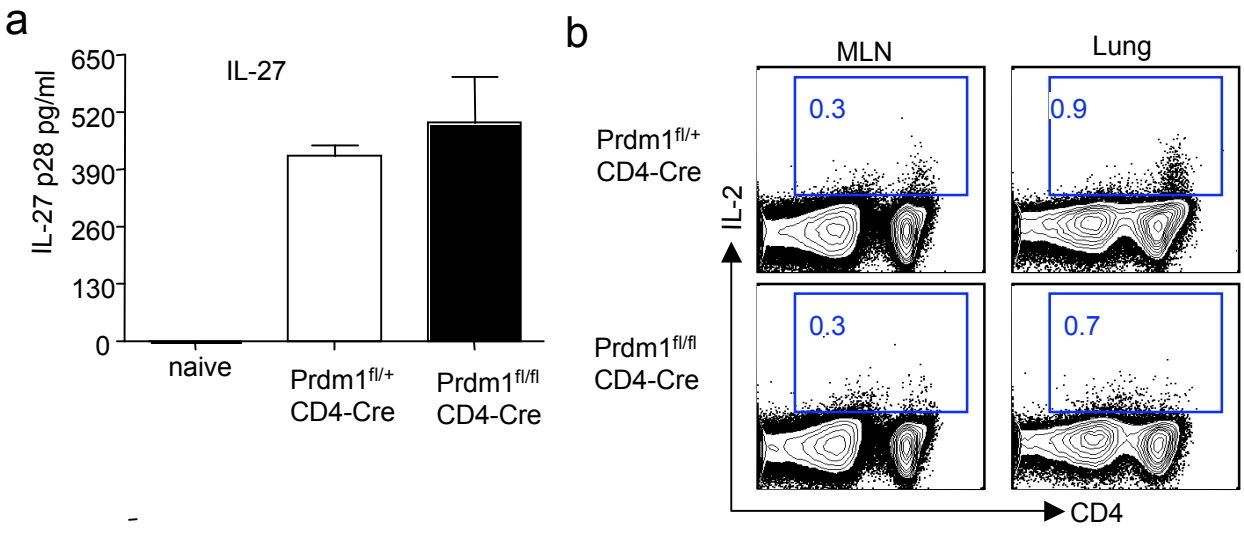
Purified human $CD8^+$ T cells were stimulated with α-CD3 plus α-CD28 with indicated conditions for 3d. The expression of IL-10 by $CD8^+$ T cells was subsequently determined by quantitative RT-PCR.

Supplementary Figure 20



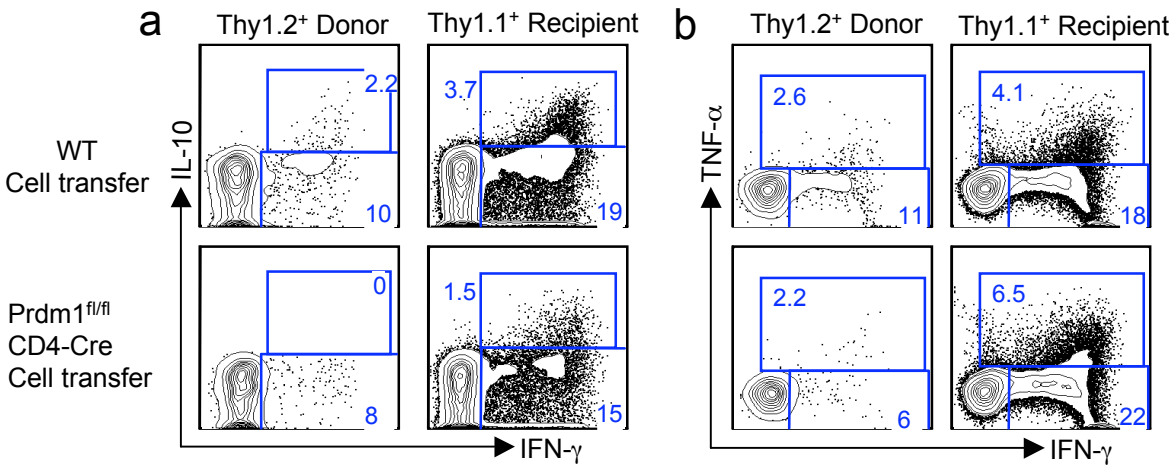
Supplementary Figure 20. Conditional deletion of Blimp-1 in T cells results in increased IL-6 release in the airway.
CD4-Cre *Prdm1*$^{fl/+}$ or CD4-Cre *Prdm1*$^{fl/fl}$ mice were infected with influenza. At d9 p.i., the levels of IL-6 in BALF were determined through ELISA.

Supplementary Figure 21



Supplementary Figure 21. Conditional deletion of Blimp-1 in T cells results in comparable IL-27 release in the airway and moderately diminished IL-2 production by T cells following influenza infection.
CD4-Cre *Prdm1*$^{fl/+}$ or CD4-Cre *Prdm1*$^{/fl}$ mice were infected with influenza. (a) At d7 p.i., the levels of IL-27 p28 in BALF were determined through ELISA. (b) The production of IL-2 by T cells (Thy1^{+}) was measured by ICS following restimulation with influenza-infected BMDC.

Supplementary Figure 22



Supplementary Figure 22. The requirement of Blimp-1 in IL-10 production by CTL is CD8$^+$ T cell intrinsic.
WT or CD4-Cre *Prdm1*$^{fl/fl}$ T cells were transferred into Thy1 mis-matched WT mice and infected with influenza. At d7 p.i., the production of IL-10 and IFN-γ (a) or TNF and IFN-γ (b) by CTL was measured by ICS following restimulation with influenza-infected BMDC.