Supplementary Material

Regulatory T cells expressing granzyme B play a critical role in controlling lung inflammation during acute viral infection

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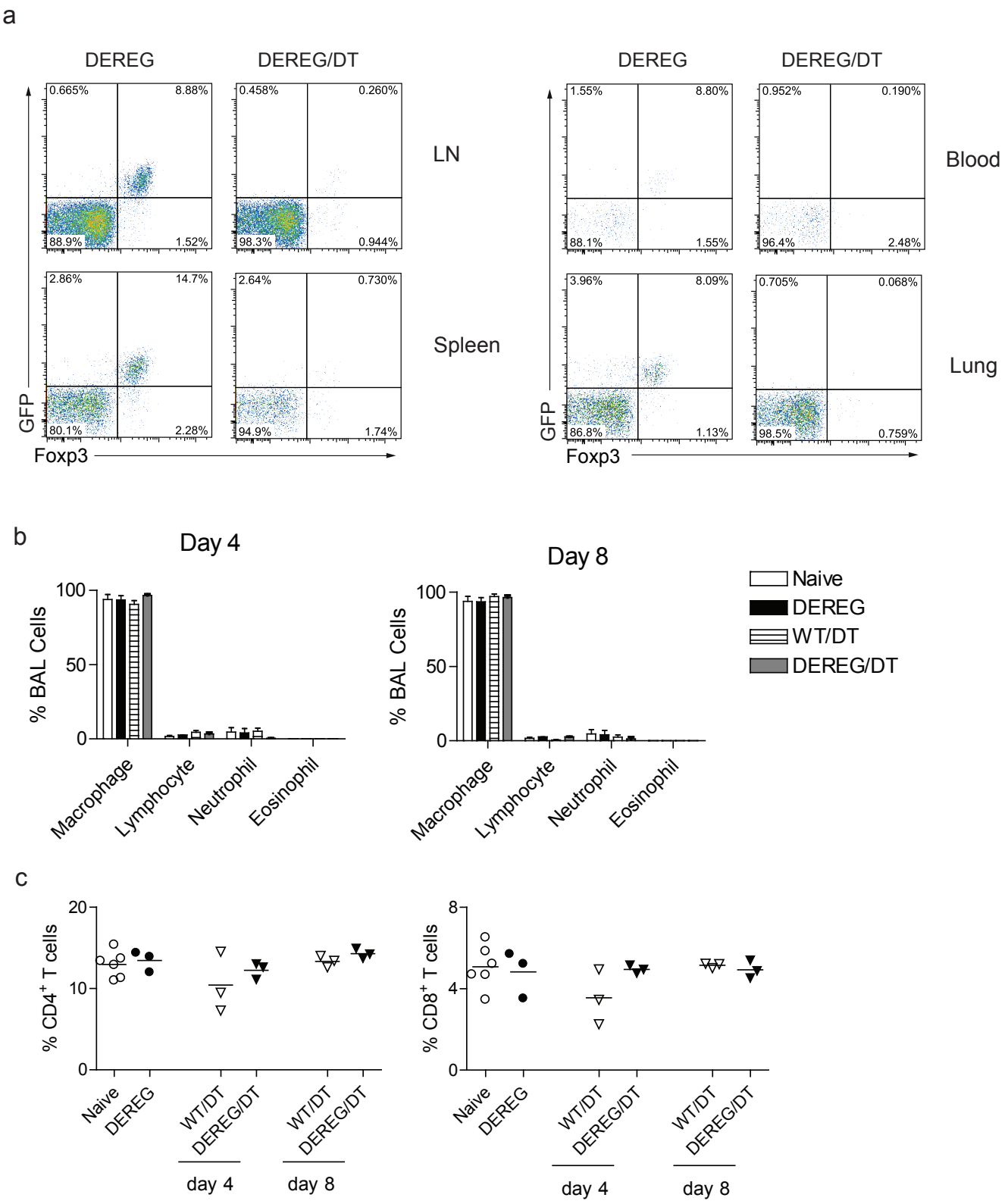
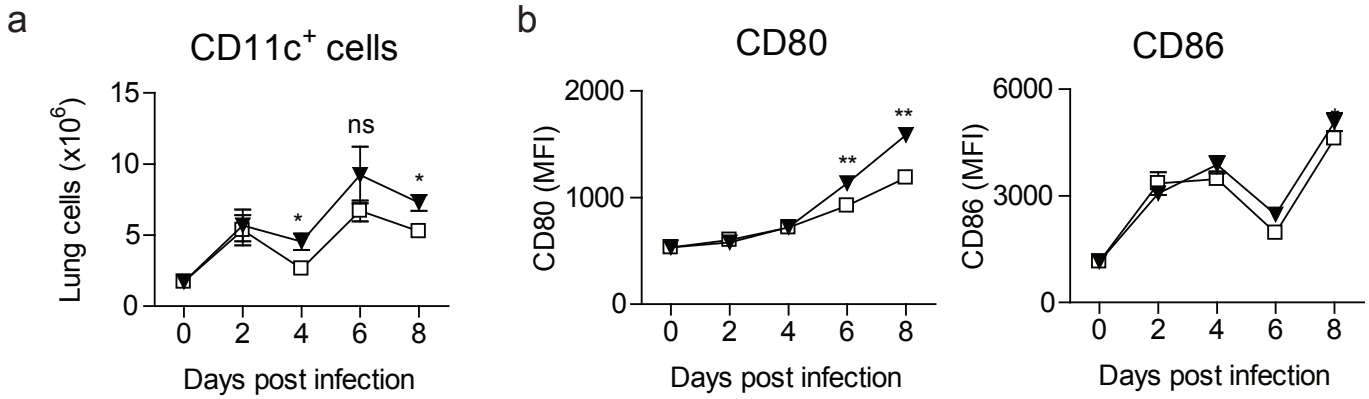
Supplementary Figure S1



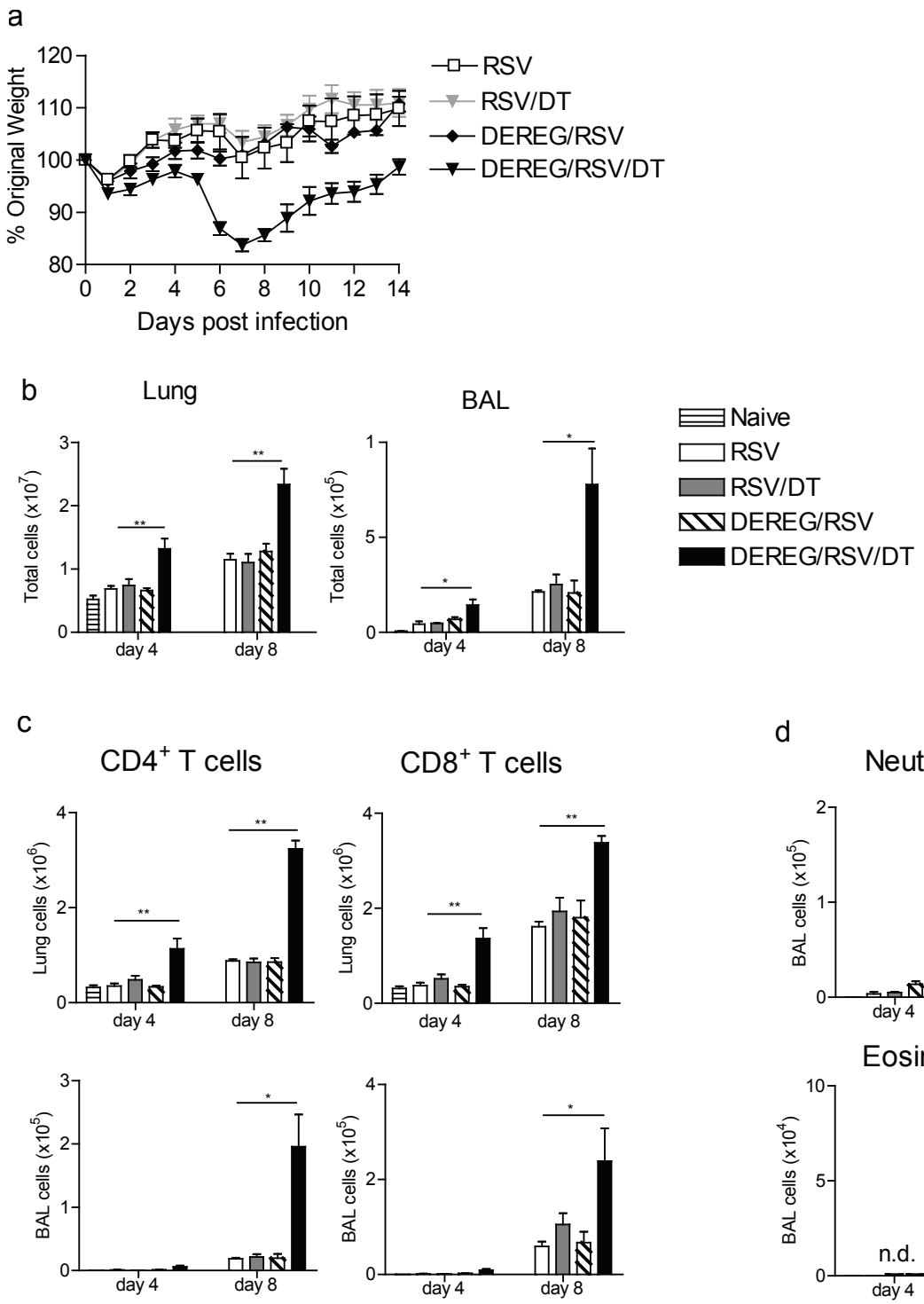
Supplementary Figure S1. Treg depletion in DEREG mice. DEREG BALB/c mice were injected with DT on day -2, -1, 2 and 5 when indicated (**a**) Mesenteric LN, spleen, blood and lung were analyzed for $Foxp3^{+}GFP^{+}$ cells by flow cytometry on day 0. Cells are previously gated on $CD3^{+}CD4^{+}$ cells. (**b**) DEREG and WT BALB/c mice were, when indicated, injected with DT on day -2, -1, 2 and 5 and harvested at different time points. Frequencies of macrophages, lymphocytes, neutrophils and eosinophils in the BAL were quantified using differential cell counting of H&E stained cytospins slides on day 4 (left) and 8 (right). (**c**) Frequencies of $CD4^{+}Foxp3^{-}$ T cells and $CD8^{+}$ T cells in the lung were quantified using flow cytometry on day 4 and 8. Error bars indicate the SEM. Graphs are representative of two independent experiments with 3-4 mice per group.

Supplementary Figure S2



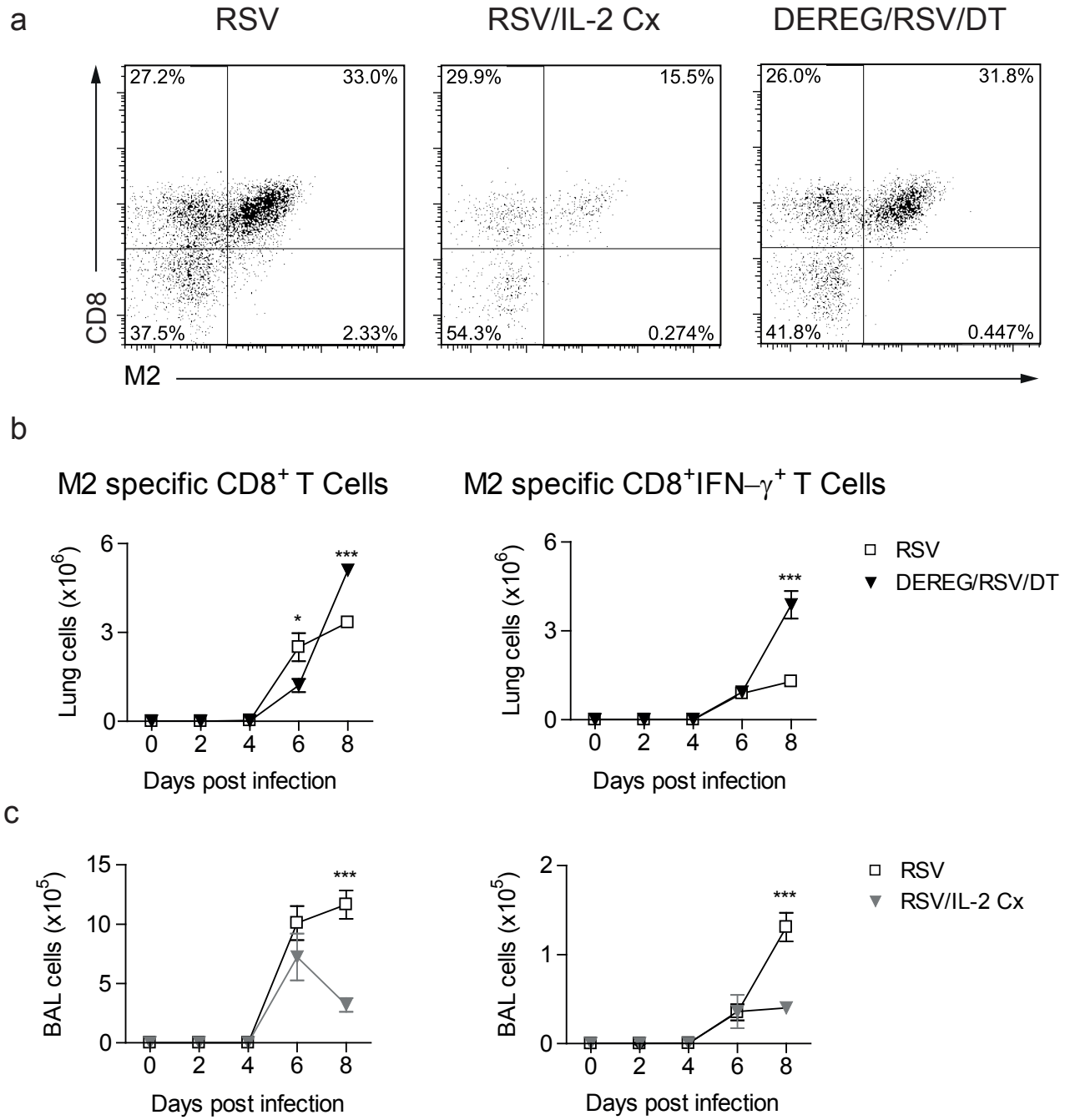
Supplementary Figure S2. Depletion of Foxp3$^+$ cells increases CD11c$^+$ cells during RSV infection of BALB/c mice. BALB/c mice and BALB/c DEREG mice were infected with RSV i.n. (day 0) (RSV and DEREG/RSV). When indicated, RSV infected DEREG or WT mice were injected i.p. (days -2,-1, 2, and 5) with 0.75 µg DT (DEREG/RSV/DT and RSV/DT). (**a**) Total numbers of CD11c$^+$ cells in the lung were enumerated on day 2, 4, 6 and 8 post infection. (**b**) Quantification of the MFI of CD80 and CD86 expression on CD11c$^+$ cells in the lung at different time points after RSV infection. Error bars indicate the SEM. Graphs are representative of two independent experiments with 4 mice per group in each.

Supplementary Figure S3



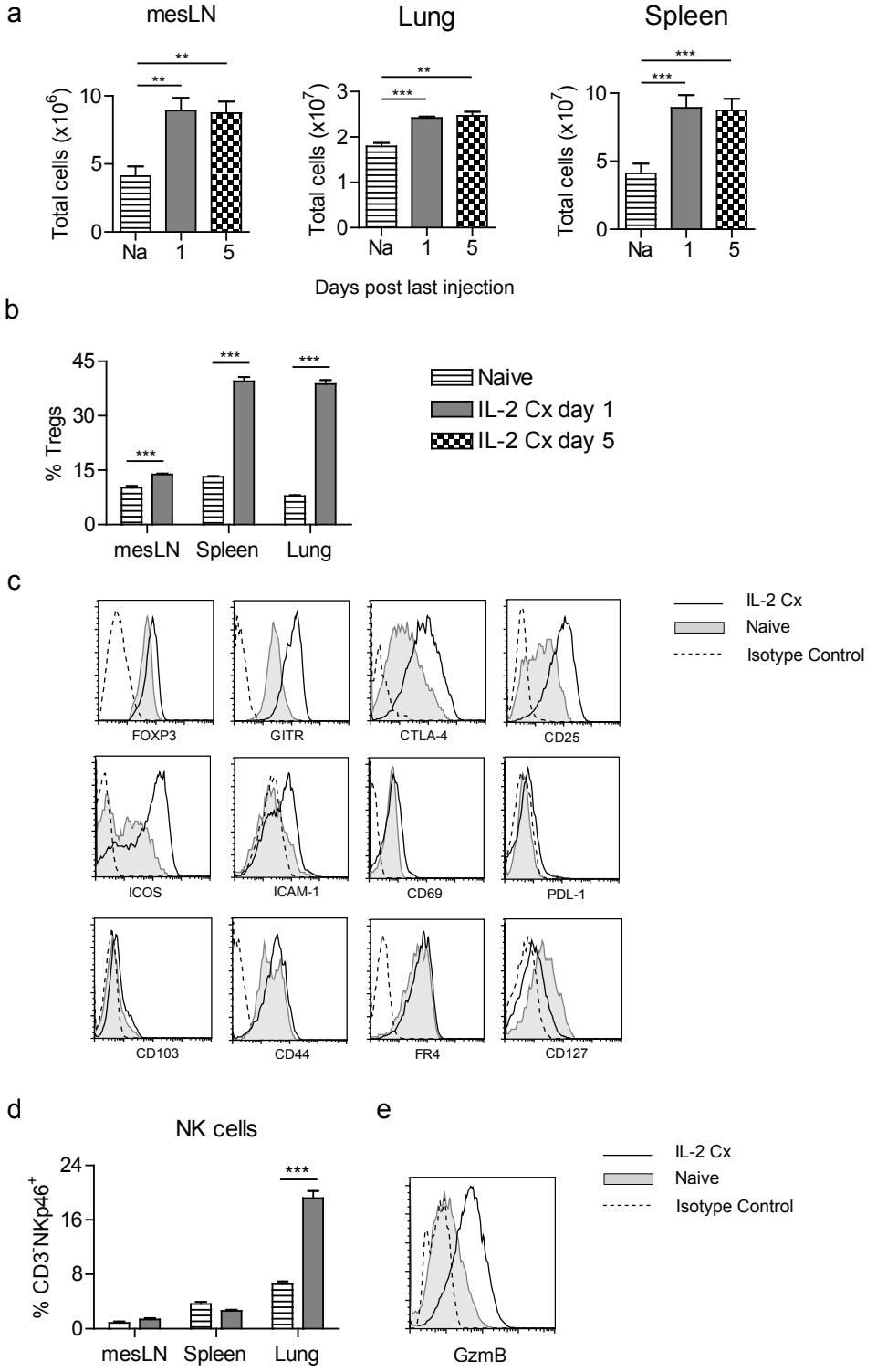
Supplementary Figure S3. Depletion of Foxp3$^+$ cells increases cellular influx in the lungs and weight loss during RSV infection of C57BL/6 mice. C57BL/6J (WT) mice and C57BL/6J DEREG mice were infected with RSV i.n. (day 0) (RSV and DEREG/RSV). When indicated, RSV infected DEREG or WT mice were injected i.p. (days -2,-1, 2, 5 and 8) with 0.75 µg DT (DEREG/RSV/DT and RSV/DT). (**a**) Illness was monitored daily by weight for 14 days after RSV infection; percentage of original weight is shown. (**b**) Total numbers of cells in the lung and BAL were enumerated on day 4 and 8 post infection. (**c**) Total numbers of CD3 gated CD4$^+$Foxp3$^-$ and CD8$^+$ T cells in the lung and BAL were quantified using flow cytometry on day 4 and 8 post RSV infection. (**d**) Total numbers of neutrophils and eosinophils in the BAL were quantified using differential cell counting on day 4 and 8 post infection. Error bars indicate the SEM. Graphs are representative of at least three independent experiments with 4 mice per group in each.

Supplementary Figure S4



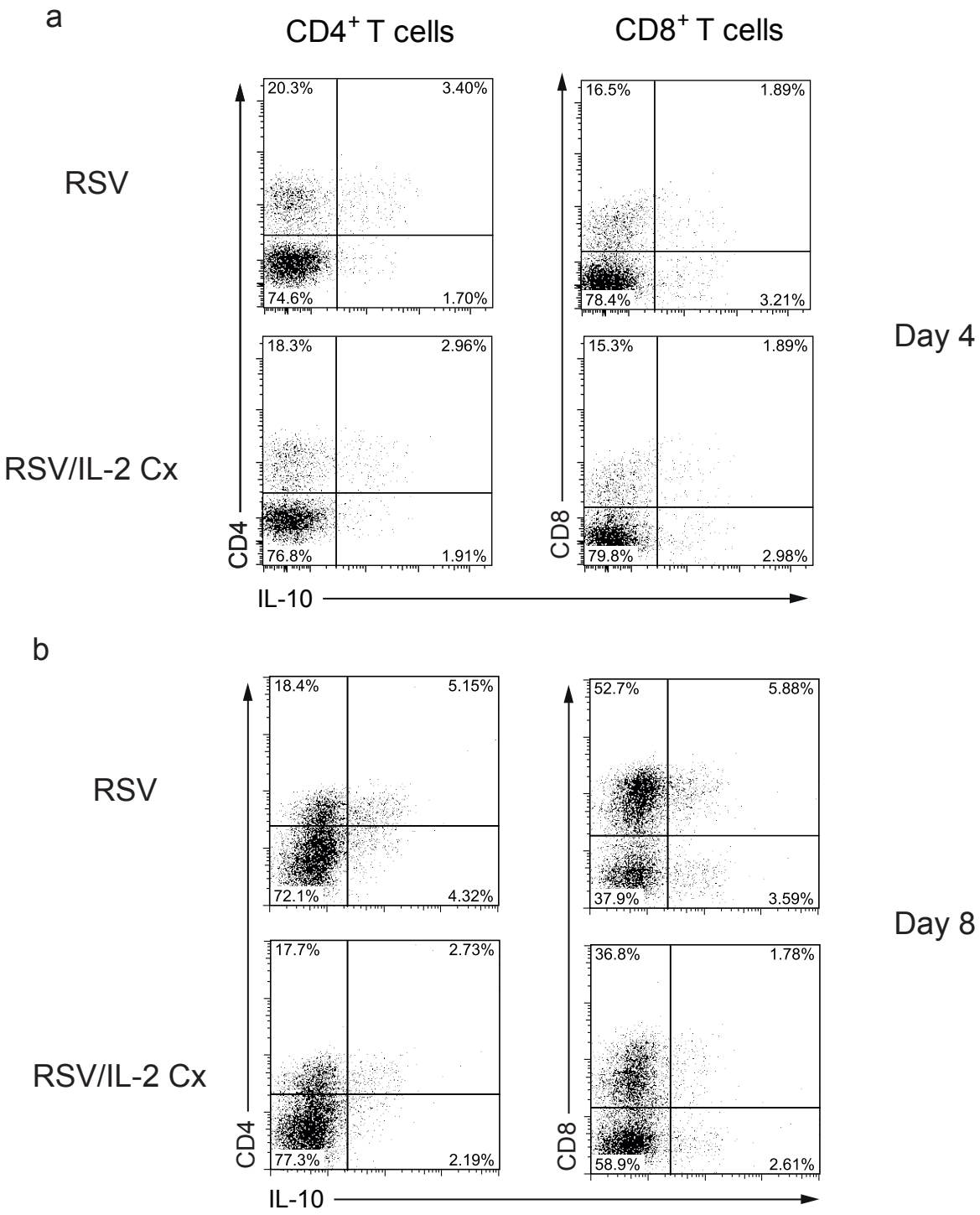
Supplementary Figure S4. Tregs control RSV antigen specific $CD8^+$ T cells in lung and BAL. BALB/c mice or BALB/c DEREG mice were infected with 10^6 FFU RSV i.n. (day 0). Where indicated, mice were injected i.p (days -2, -1, 2, 5 and 8) with 0.75 µg DT or IL-2 Cx (days 1, 2 and 3). (**a**) CD3 gated, M2 pentamer positive $CD8^+$ T cells in the BAL were analyzed by flow cytometry on day 8 post infection. (**b**) Total numbers of CD3 gated, M2 pentamer$^+$ $CD8^+$ T cells and M2 peptide restimulated $CD8^+$IFN-γ^+ T cells in the lung from Treg depleted mice were quantified using flow cytometry. (c) Total numbers of CD3 gated, M2 pentamer$^+$ $CD8^+$ T cells and M2 peptide restimulated $CD8^+$IFN-γ^+ T cells in the BAL from IL-2 Cx injected mice were quantified using flow cytometry. Error bars indicate the SEM. Graphs are representative of two independent experiments with 5 mice per group.

Supplementary Figure S5



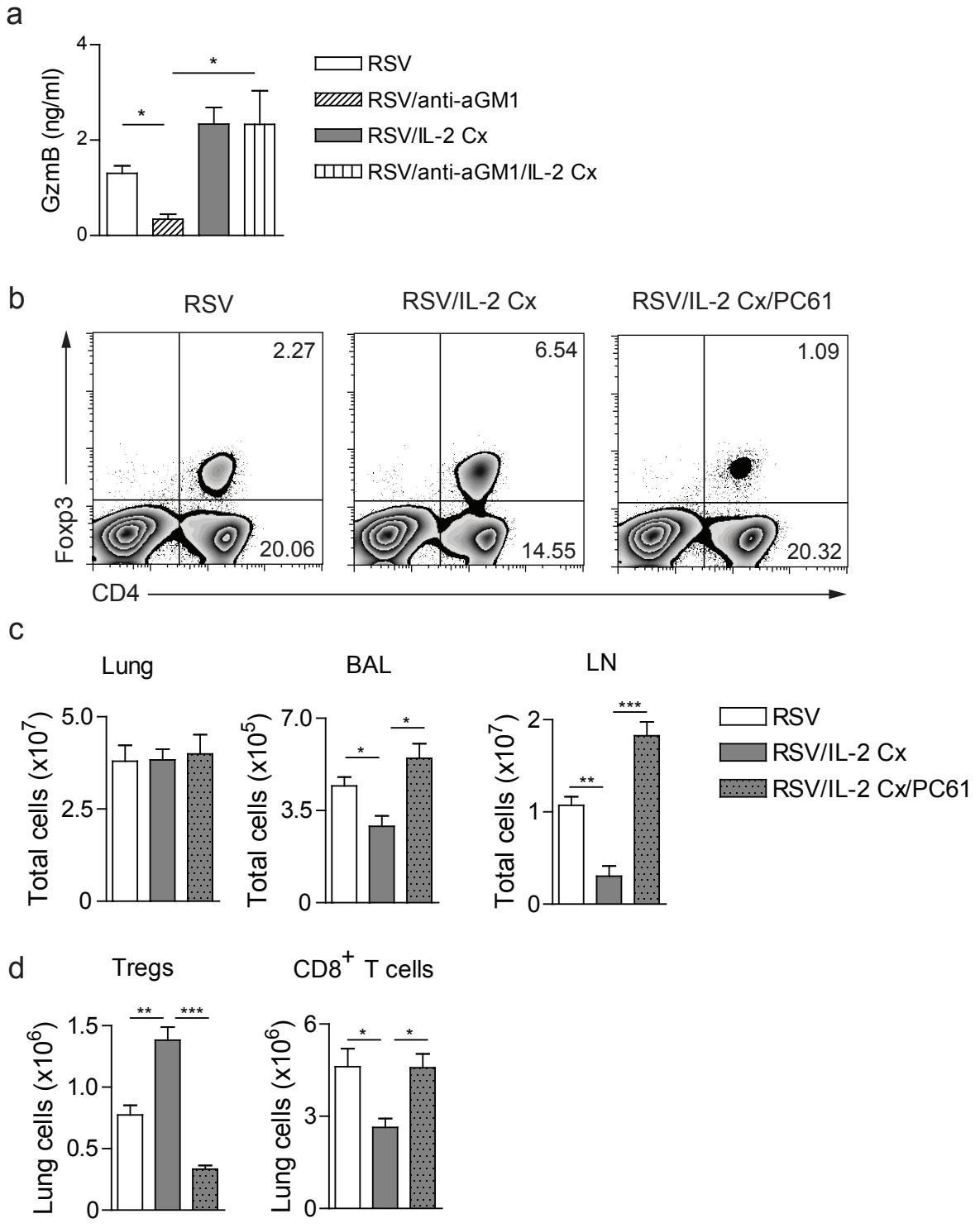
Supplementary Figure S5. IL-2 Cx injections expand and activate Tregs and lung NK cells**.** Lungs from mice injected i.p. daily for 3 d (days -2, -1 and 0) with IL-2 Cx were analyzed on day 1 and 5 after the last IL-2 Cx injection. (**a**) Total numbers of cells were enumerated in the mesenteric (mes) LNs, lung and spleen on day 1 and day 5 after the last IL-2 Cx injection. (**b**) Frequencies of Tregs ($CD4^+Foxp3^+$) were determined in the mesenteric LN, spleen and lung of naïve mice or day 1 after IL-2 Cx injections. (**c**) Lung Tregs ($CD4^+$ $Foxp3^+$) were analyzed for expression of cell surface molecules on day 1 after IL-2 Cx injections. (**d**) Frequency of NK cells ($CD3^-$ $NKp46^+$) in the mesLN, spleen and lung of naive mice or day 1 after IL-2 Cx injections was assessed by flow cytometry. (**e**) Lung NK cells were analyzed for intracellular GzmB expression by flow cytometry on day 1 after IL-2 Cx injections. Error bars indicate the SEM. Graphs are representative of two independent experiments with 4 mice per group in each.

Supplementary Figure S6



Supplementary Figure S6. IL-2 Cx injections do not induce IL-10 expression in T cells. BALB/c mice were infected with RSV i.n. (day 0). When indicated some mice were injected i.p. three times (days 1, 2 and 3 post infection) with IL-2 Cx. (**a** and **b**) BAL cells were restimulated with PMA and Ionomycin for 3 h. IL-10 expression of CD3 gated, $CD4^+$ and $CD8^+$ T cells was analyzed by flow cytometry in the BAL on day 4 (**a**) and day 8 (**b**) post RSV infection. Plots are representative of two independent experiments with 4 mice per group in each.

Supplementary Figure S7



Supplementary Figure S7. Tregs and not NK cells limit lung inflammation after IL-2 Cx injections. BALB/c mice were infected with RSV i.n. (day 0). When indicated some mice were injected i.p. three times (days 1, 2 and 3 post infection) with IL-2 Cx and with anti-asialo GM1 (anti-aGM1) given i.p. on days -2, 0 and 3 (a) or PC61 on days -1 and 3 (b, c and d). (**a**) The concentration of GzmB in the BAL on day 4 post RSV infection was measured by ELISA. (**b**) Frequencies of $CD4^+Foxp3^-$ and $CD4^+Foxp3^+$ T cells in the lung on day 8 post infection of RSV, RSV/IL-2 Cx or RSV/IL-2Cx/PC61 mice. (**c**) Total numbers of cells in the lung, BAL and LN were enumerated on day 8 post infection. (**d**) Total numbers of Tregs ($CD4^+Foxp3^+$), and $CD8^+$ T cells in the lung of RSV infected mice were quantified using flow cytometry on day 8 post RSV infection. T cells and Tregs are gated on $CD3^+$ cells. Error bars indicate the SEM. Graphs show two pooled independent experiments (**a**) or graphs are representative of two independent experiments with 4-5 mice per group.