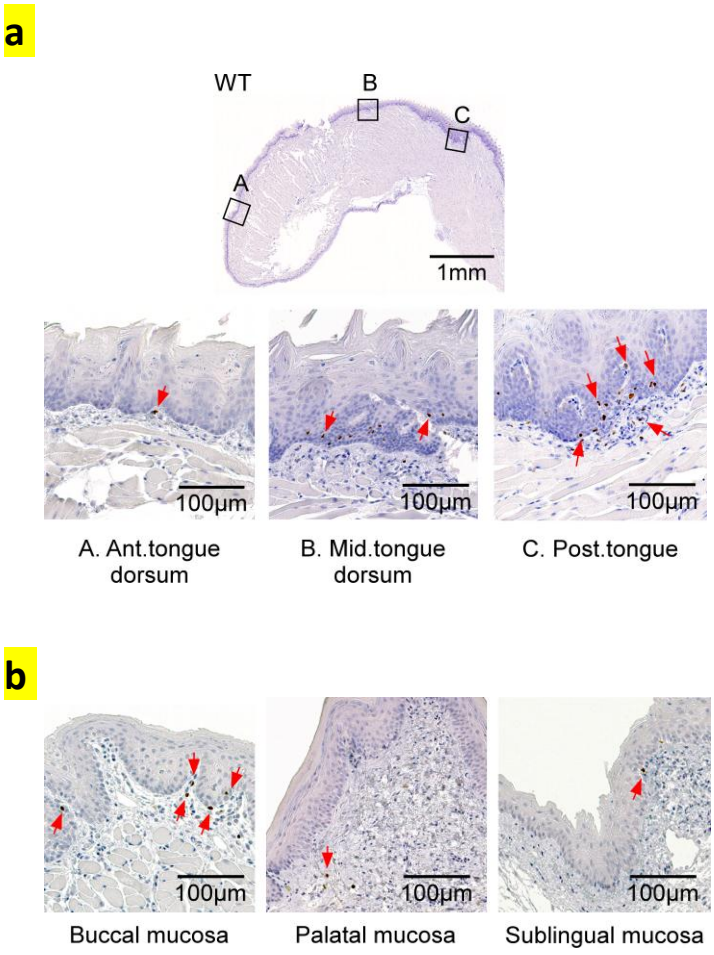
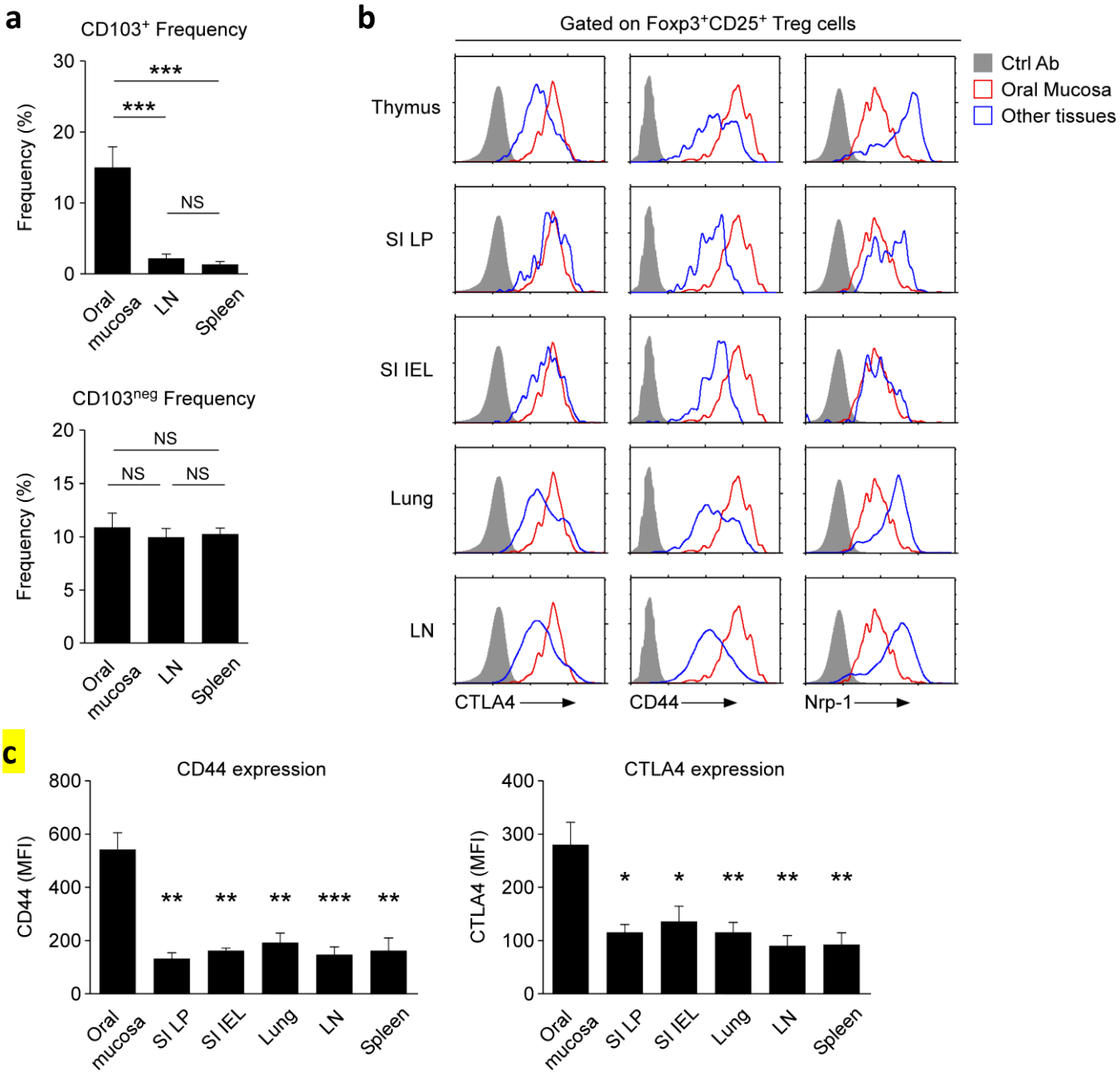
Supplementary Figure 1



Suppl. Figure 1. Identification and tissue distribution of Foxp3$^+$ Treg cells in the oral mucosa

(a) Detection of Foxp3$^+$ cells at distinct sublocations of the dorsal mucosal area of the tongue by immunohistochemistry.

(b) Foxp3$^+$ cells in buccal, palatal and sublingual area of the oral mucosa. Foxp3$^+$ cells were visualized using anti-Foxp3 antibodies (red arrows).



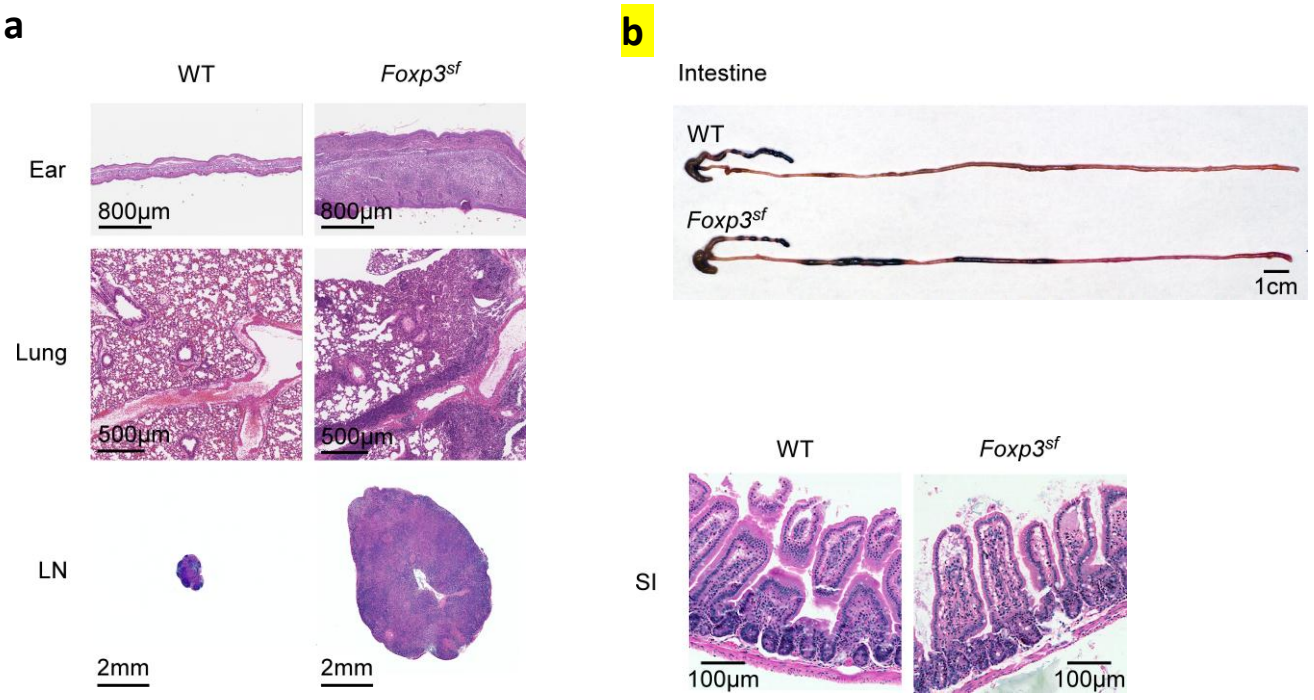
Suppl. Figure 2. Phenotype of oral mucosa Foxp3$^+$ Treg cells

(a) Frequencies of CD103$^+$ Foxp3$^+$ (top) or CD103^{neg} Foxp3$^+$ (bottom) cells were examined among CD4$^+$ T cells from the indicated organs and tissues. Graphs are summary of nine independent experiments.

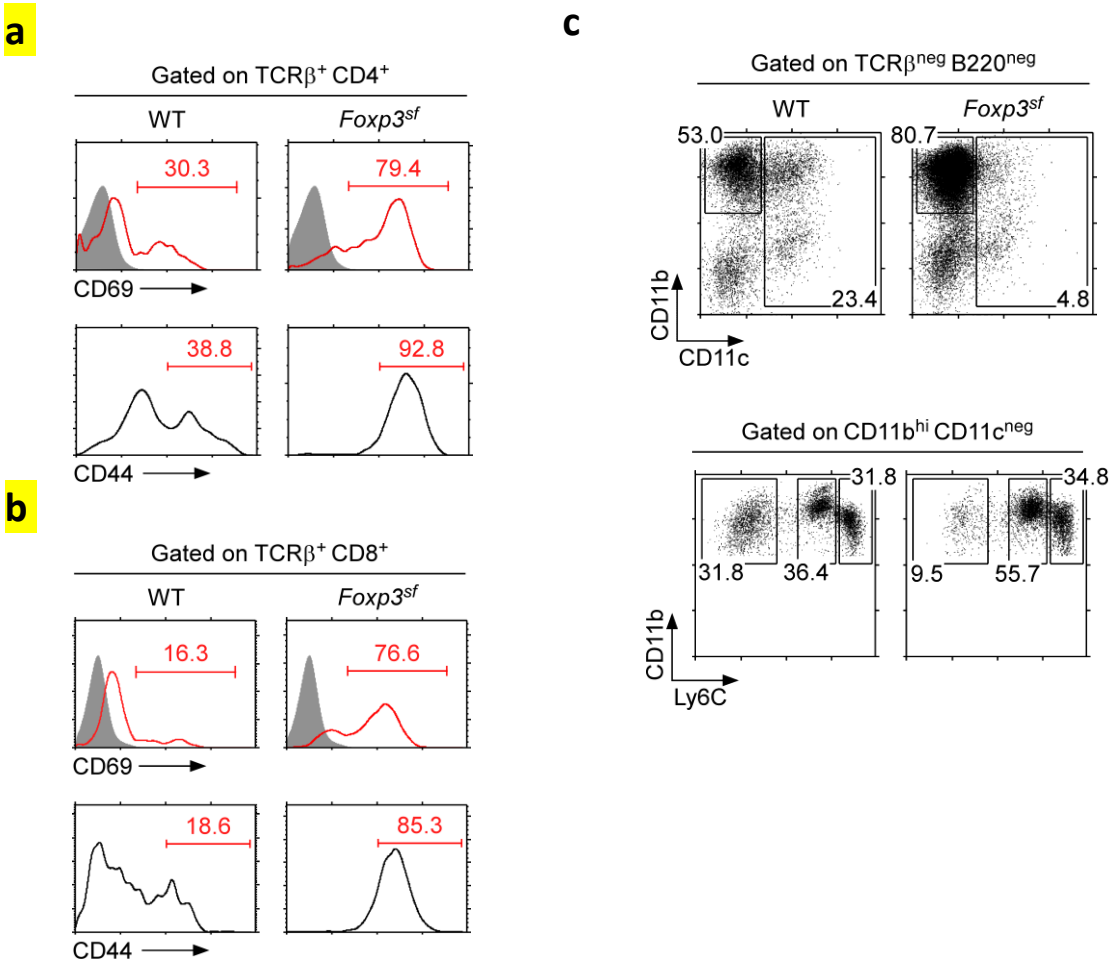
(b) Activation/differentiation marker expression on Foxp3$^+$CD25$^+$ Treg cells from thymus, small intestine lamina propria (SI LP), small intestine intraepithelial lymphocytes (SI IEL), lung, and lymph node (LN) are compared to those from the oral mucosa. Histograms are representative of five independent experiments and compared to control antibody (Ctrl Ab) staining.

(c) CD44 and CTLA4 expression among Foxp3$^+$ CD25$^+$ Treg cells of different tissues. MFI were determined from five independent experiments. Data are shown as mean ± SEM. Two-tailed Mann-Whitney U-test was used to calculate P-values between oral mucosa and one other tissue, where *, $P < 0.05$; **, $P<0.01$; ***, $P<0.001$ were considered statistically significant. NS = not significant.

Supplementary Figure 3

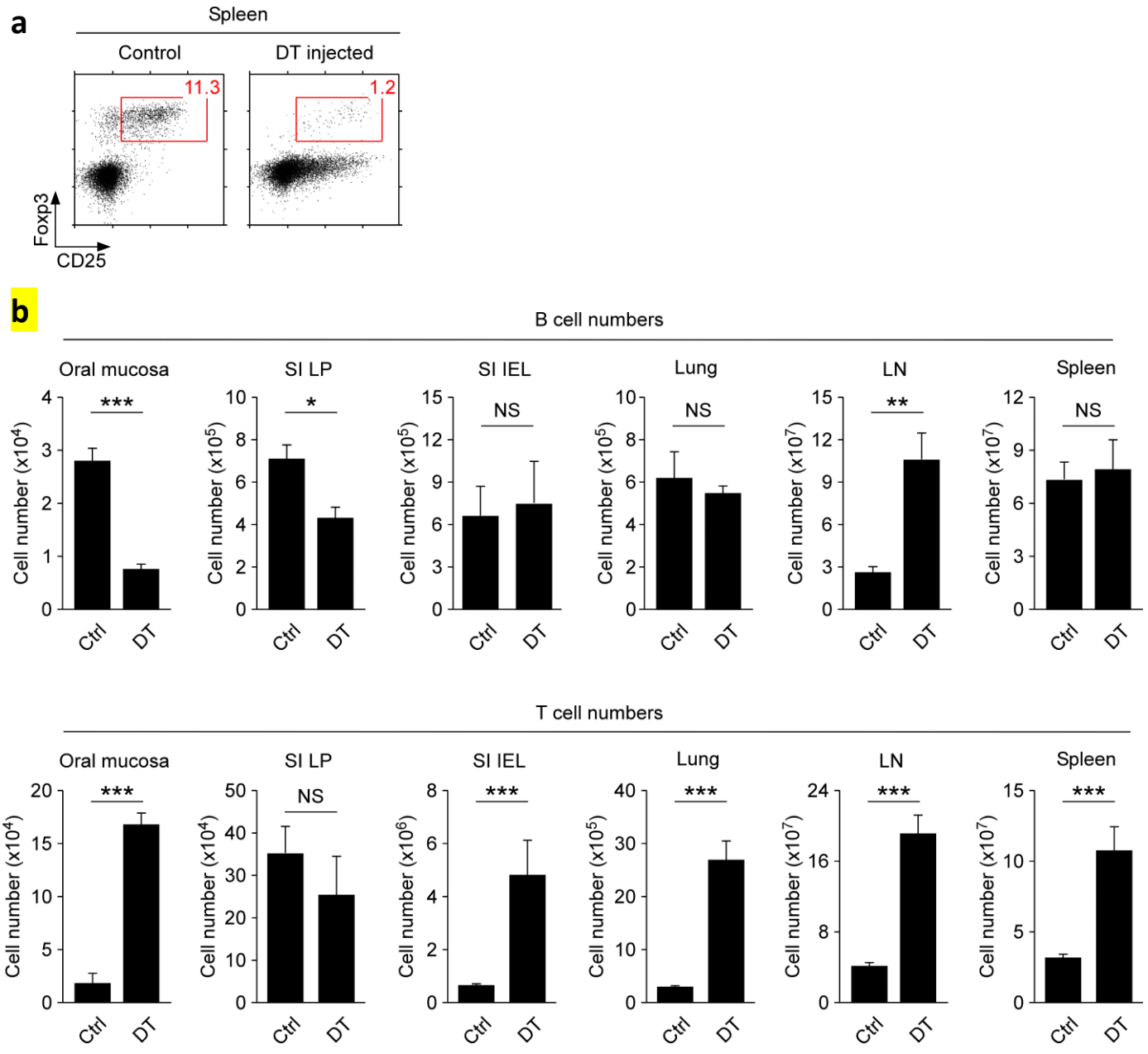


Suppl. Figure 3. Phenotypes of oral mucosal immune cells in Foxp3-deficient mice
(a) H&E staining of ear, lung, and lymph node of WT and *Foxp3*^{sf} mice.
(b) Length and gross anatomy comparison of intestine (top) and H&E staining of the small intestinal mucosa of WT and *Foxp3*^{sf} mice (bottom). Data are representative of two independent experiments.



Suppl. Figure 4. Phenotype of infiltrating cells of the oral mucosa of scurfy mice
(a, b) Surface CD44 and CD69 expressions on oral mucosal CD4 (a) and CD8 T cells (b) of WT and Foxp3^{sf} mice. Control antibody staining is shown in grey. Histograms are representative of five independent experiments.
(c) CD11c versus CD11b expression profiles of myeloid cells in the oral mucosa of WT and *Foxp3^{sf}* mice. CD45^{+} cell isolates were gated on TCRβ-negative and B220- negative cells (top), and CD11b^{hi} CD11c^{neg} macrophages/monocytes were further characterized by expression of Ly6C (bottom). Dot plots are representative of five independent experiments.

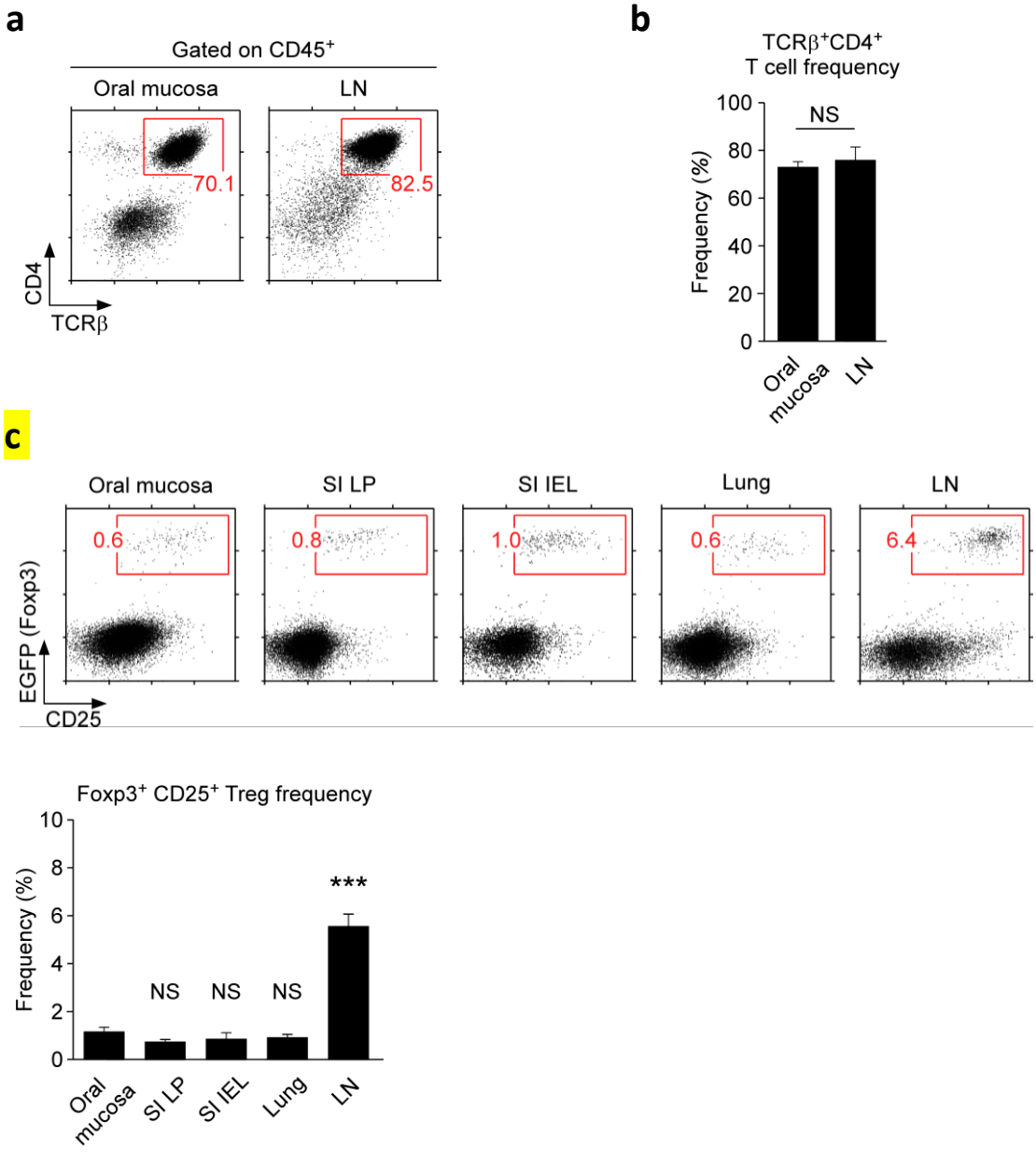
Supplementary Figure 5



Suppl. Figure 5. Foxp3⁺ Treg cell ablation by DT injection

(a) Frequency of Foxp3⁺CD25⁺ T cells among spleen CD4⁺ T cells of *Foxp3^{DTR}* mice after two weeks of injection with either vehicle control or DT. Dot plots are representative of three independent experiments.

(b) B220⁺ B cell (top) and TCRβ⁺ T cell (bottom) numbers of control (Ctrl) or DT-injected Foxp3-DTR mice in the indicated tissues. Bar graph shows summary of five independent experiments. Data are shown as mean ± SEM. Two-tailed Mann-Whitney U-test was used to calculate P-values between DT- and control vehicle (Ctrl)-injected Foxp3DTR mice, where *, $P < 0.05$; **, $P<0.01$; ***, $P<0.001$ were considered statistically significant. NS = not significant.



Suppl. Figure 6. Adoptive transfer of CD25$^-$Foxp3$^-$ naïve CD4 T cells into *Rag2^{KO}* mice.
(a) TCRβ and CD4 expression profiles of CD45$^+$ cells in LN and oral mucosa of *Rag2^{KO}* host mice. CD25$^-$ Foxp3$^-$ naïve CD4 T cells were electronically sorted and transferred into *Rag2^{KO}* host mice. Five ~ six weeks after transfer, donor T cells were recovered from the oral mucosa and LN of host mice. Dot plots are representative of five independent experiments.
(b) Frequency of TCRβ^{hi} CD4$^+$ T cells among CD45$^+$ cells in the oral mucosa and LN of host mice. Data are summary of five independent experiments.
(c) Frequency of Foxp3$^+$ CD25$^+$ Treg cells among donor CD4$^+$ T cells in the indicated tissues of *Rag2^{KO}* host mice. Dot plots are representative (top) and bar graphs show summary of five independent experiments (bottom). Bar graph are shown as mean ± SEM. Two-tailed Mann-Whitney U-test was used to calculate P-values between oral mucosa and one other tissue, where *, P < 0.05; **, P<0.01; ***, P<0.001 were considered statistically significant. NS = not significant.