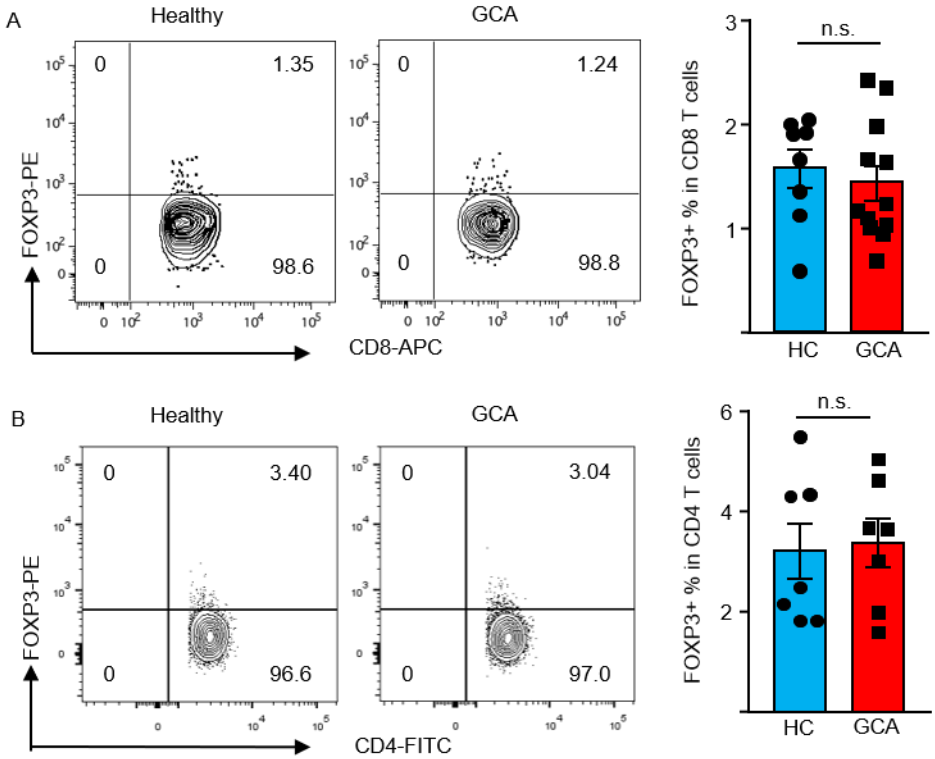
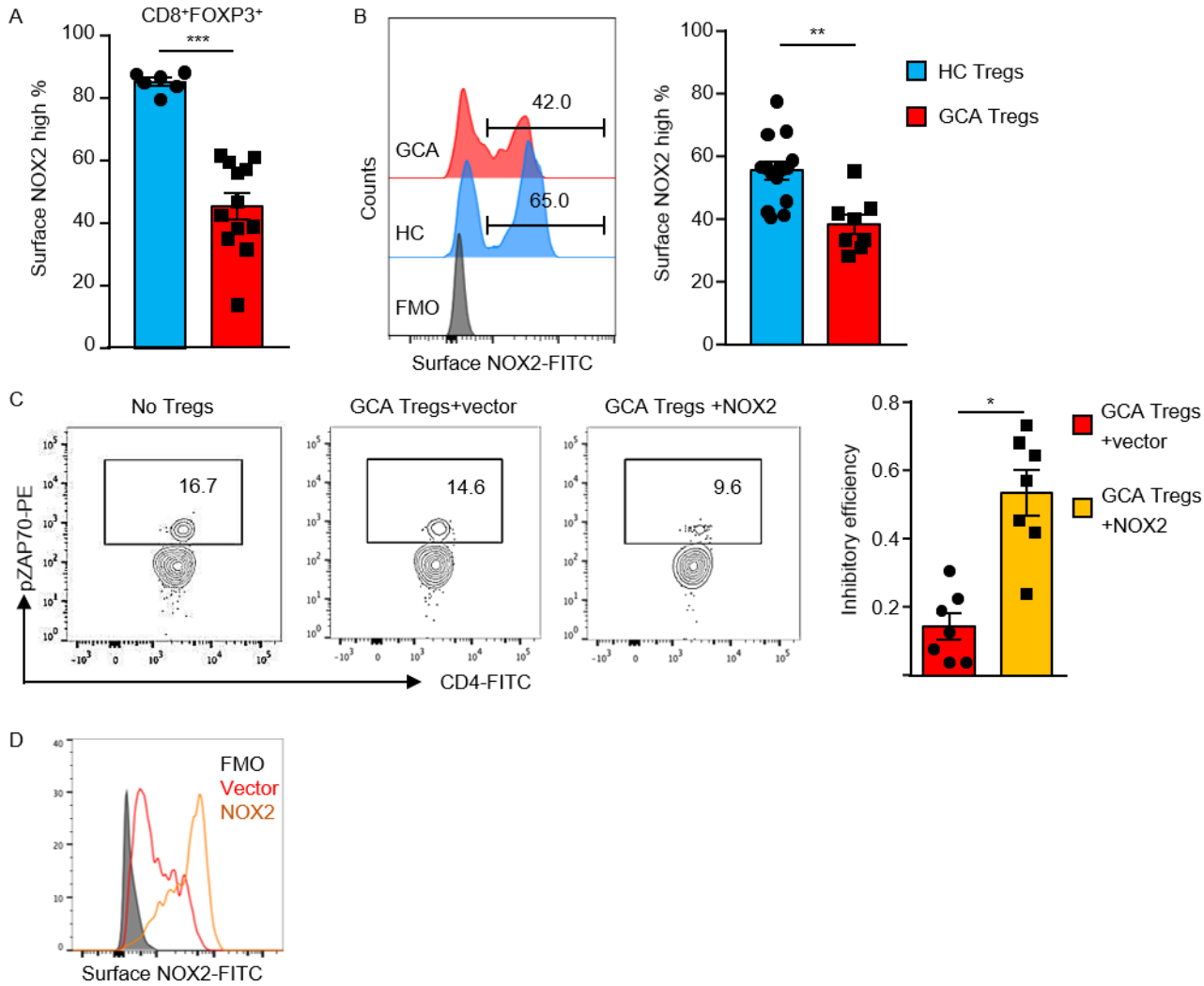


Supplement Fig1. CD4⁺FOXP3⁺ and CD8⁺FOXP3⁺ T cells in GCA

A. PBMCs were isolated from GCA patients or age-matched healthy controls, stained for CD8 and FOXP3 and analyzed by flow cytometry. Representative contour plots and mean ± s.e.m from 8 controls and 12 GCA patients. Unpaired Mann–Whitney–Wilcoxon rank test. n.s. P>0.05

B. PBMCs were isolated from GCA patients and controls. PBMCs were stained for CD4 and FOXP3 and analyzed by flow cytometry. Representative contour plots and mean ± s.e.m from 7 controls and 7 GCA patients. Unpaired Mann–Whitney–Wilcoxon rank test. n.s.P>0.05



Supplement Fig 2. NOX2 surface expression and function in CD8⁺ Treg cells

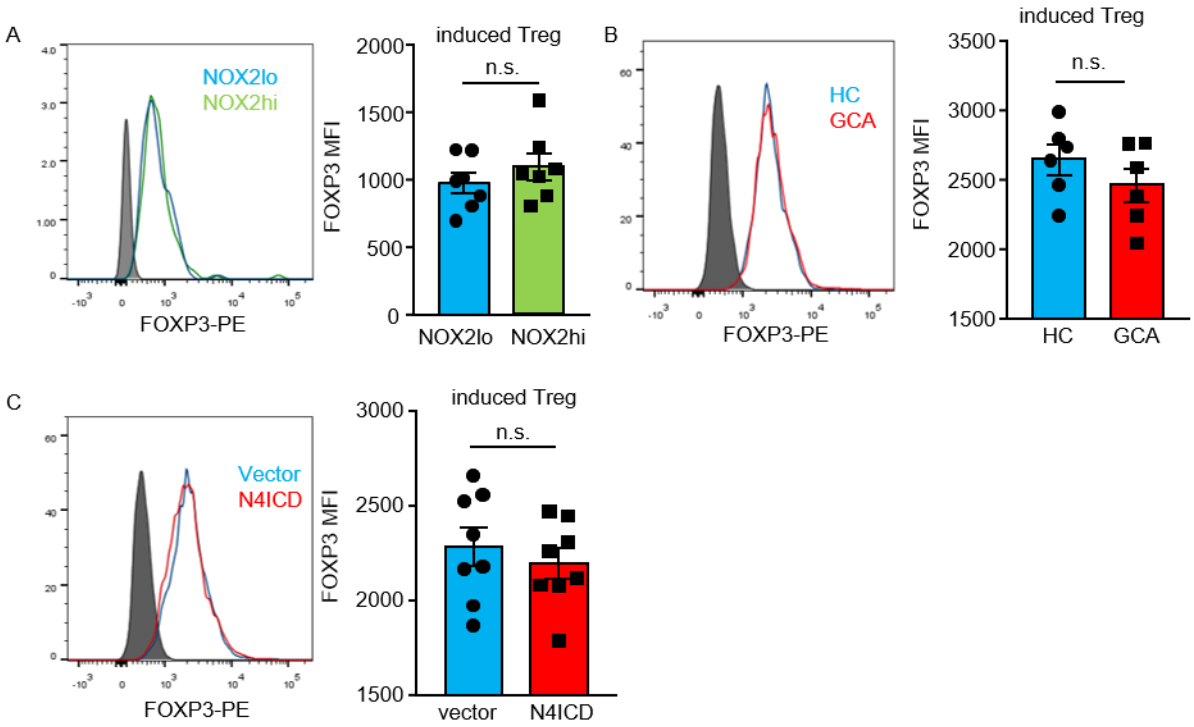
A.Cell surface NOX2 on CD8⁺FOXP3⁺ T cells from GCA patients and age-matched healthy individuals. Mean ± s.e.m from healthy (n=6) and GCA PBMCs (n=12) .

B. Cell surface NOX2 on induced CD8⁺ Tregs derived from GCA patients or age-matched healthy controls. Representative histogram and mean ± s.e.m from HC CD8⁺ Tregs (n=12) and GCA CD8⁺ Tregs (n=8).

C. CD8⁺ Tregs from GCA patients were transfected with empty vector (vector) or a NOX2-containing vector. Suppressive activity measured by mixing CD8⁺ Tregs with CD4⁺ T cells and determining pZAP70 by flow cytometry. Representative contour plots and mean ± s.e.m from 7 samples.

D. CD8⁺ Tregs from GCA patients were transfected with empty vector (vector) or a NOX2-containing vector. Cell surface NOX2 expression was analyzed by flow cytometry. Representative histogram from 3 samples.

Paired Mann–Whitney–Wilcoxon rank test (C). Unpaired Mann–Whitney–Wilcoxon rank test (A,B).*P<0.05, **P<0.01,***P<0.001



Supplement Fig 3. FOXP3 in induced CD8⁺ Treg cells

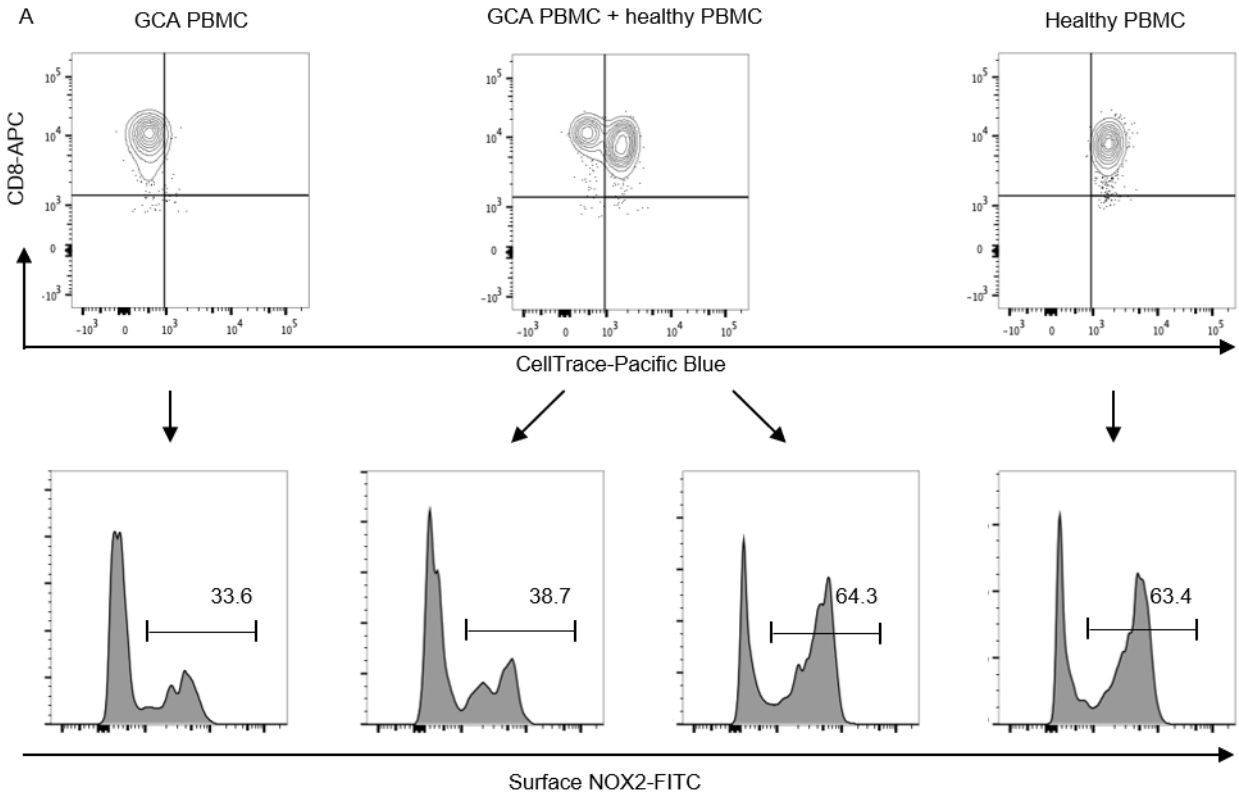
PBMCs were isolated from GCA patients and age-matched healthy individuals. CD8⁺ Tregs were induced by culturing PBMCs with low-dose anti-CD3 and IL-15.

A. Induced CD8⁺ Tregs were stained for cell surface NOX2 and FOXP3 and analyzed by flow cytometry. Cells with high surface NOX2 expression and low NOX2 expression were gated and analyzed for FOXP3 expression. Representative histograms and mean ± s.e.m from 7 samples.

B. Induced CD8⁺ Tregs were stained for FOXP3 and analyzed by flow cytometry. Representative histogram and mean ± s.e.m from 6 HC samples and 6 GCA samples.

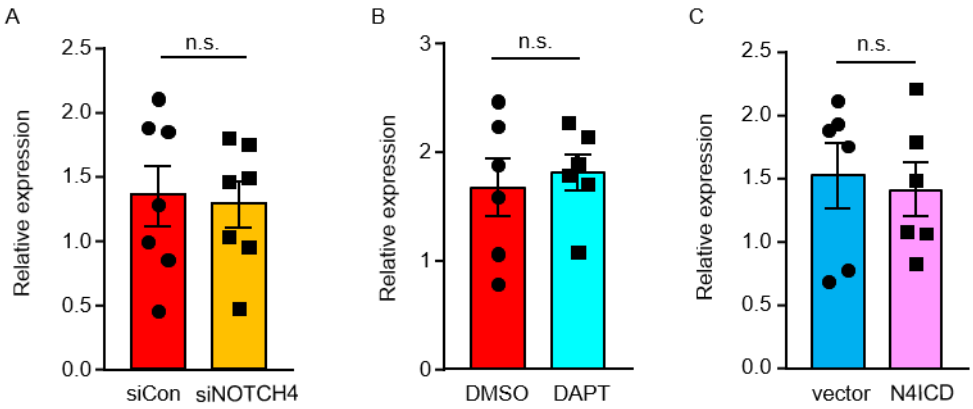
C. Induced CD8⁺ Tregs were transfected with a N4ICD-containing vector or control vector. FOXP3 expression was analyzed by flow cytometry. Representative histogram and mean ± s.e.m from 8 samples.

Paired Mann–Whitney–Wilcoxon rank test (A,C). Unpaired Mann–Whitney–Wilcoxon rank test (B). n.s. is $p>0.05$



Supplement Fig 4. CD8 Treg cell induction in mixed PBMC cultures

A. PBMCs from GCA patients and from healthy donors (labeled with CellTrace-Pacific Blue) were mixed and cultured with low-dose anti-CD3 and IL-15. After 6 days, $CD8^+$ Treg cells from HC and GCA were distinguished by CellTrace staining and surface NOX2 expression was analyzed by flow cytometry.

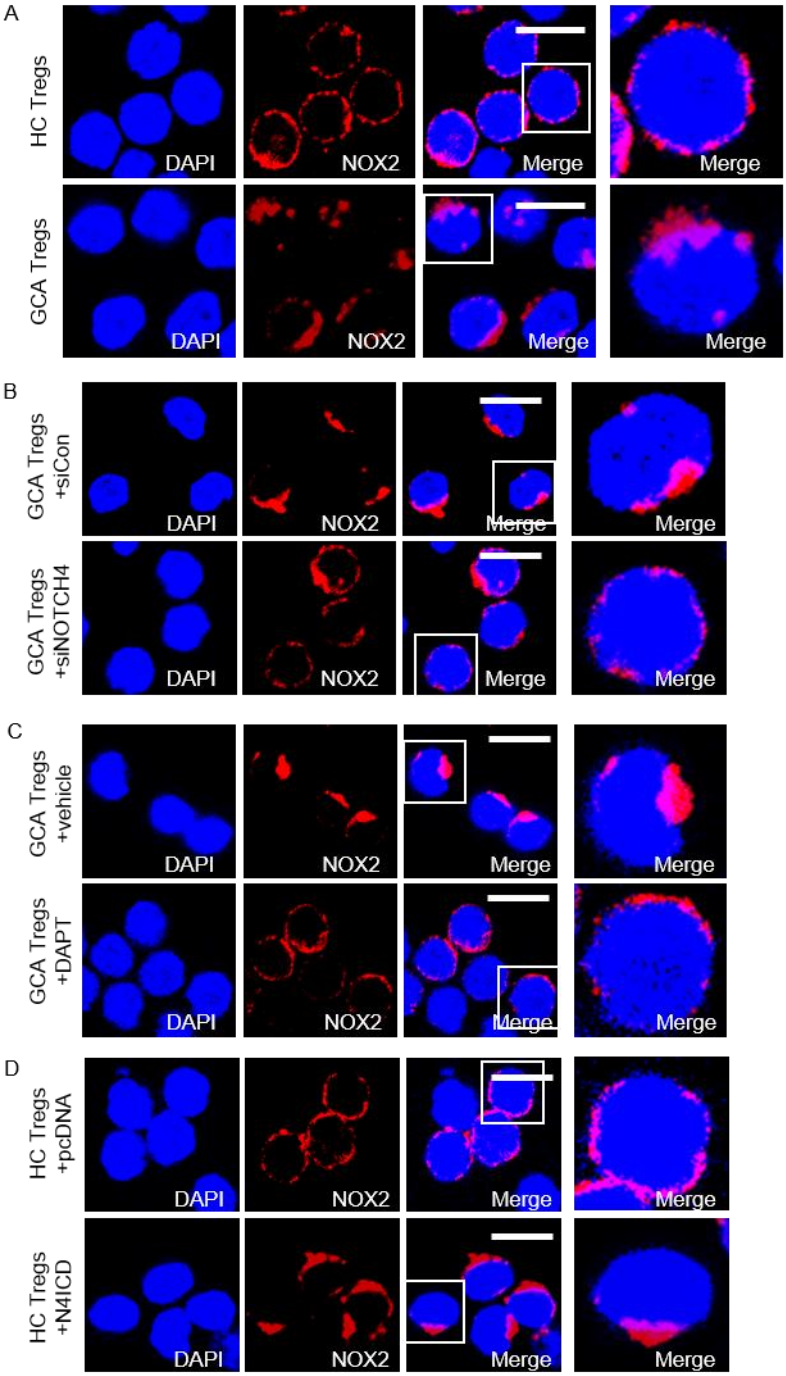


Supplement Fig5. NOX2 transcription is independent of NOTCH4 signaling

A-C. $CD8^+$ Tregs were induced with low-dose anti-CD3 and IL-15 and treated or transfected as indicated. NOX2 mRNA was quantified by q-PCR. Each dot represents one sample. Data are mean ± s.e.m.

Paired Mann–Whitney–Wilcoxon rank test. n.s. is $p>0.05$

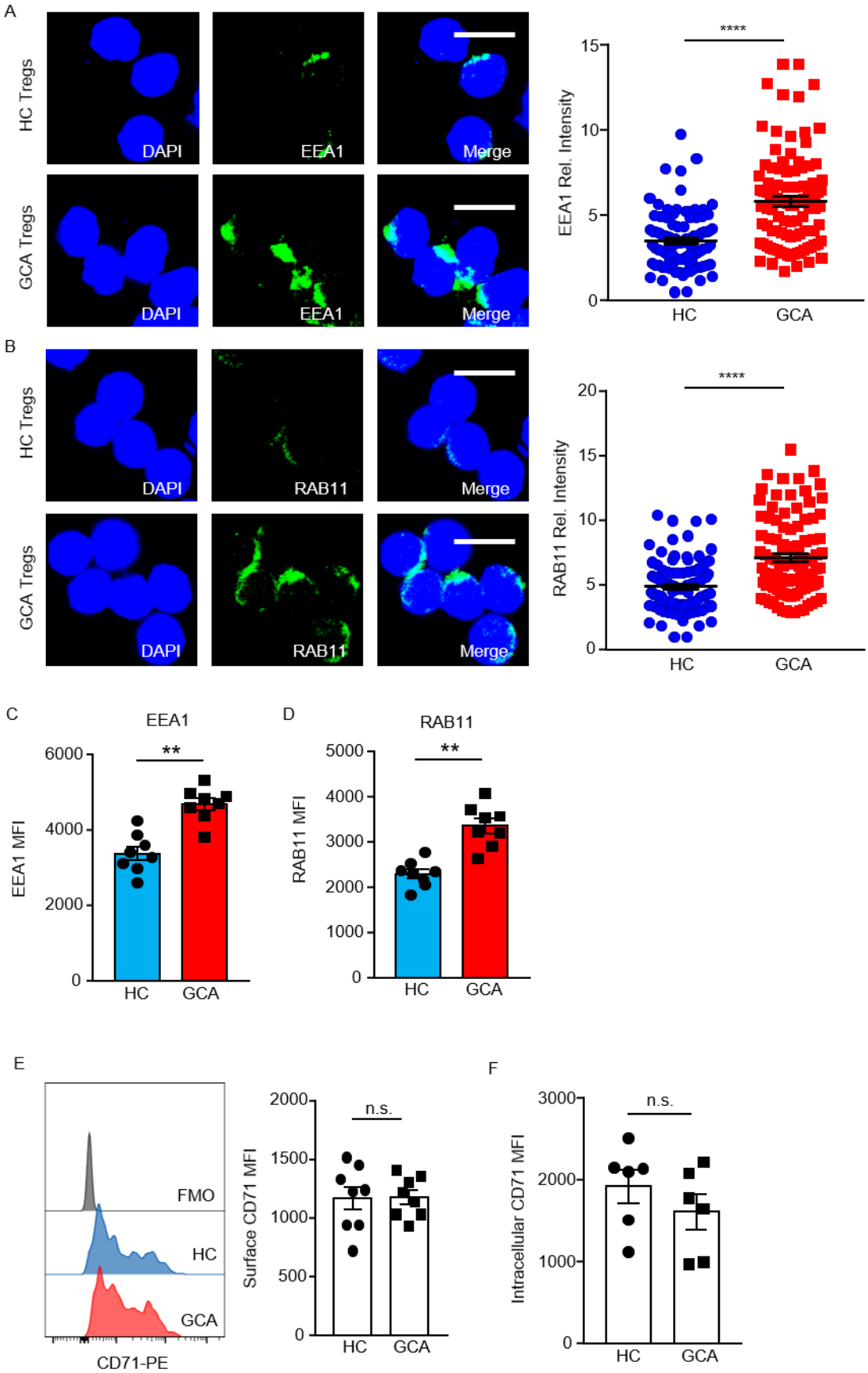
N



Supplement Fig 6. NOTCH4 regulates NOX2 subcellular localization

A. Confocal microscopy imaging of $CD8^+$ Tregs stained for DAPI (blue) and NOX2 (red). Scale bar; 10 μm.

B-D. $CD8^+$ Tregs were treated or transfected as indicated. Confocal microscopy imaging of $CD8^+$ Tregs stained for DAPI (blue) and NOX2 (red). Scale bar; 10 μm.


A
HC Tregs
GCA Tregs
DAPI
EEA1
Merge
EEA1 Rel. Intensity
****
HC
GCA
B
RAB11
RAB11 Rel. Intensity
C
EEA1
EEA1 MFI
**
D
RAB11
RAB11 MFI
E
FMO
HC
GCA
CD71-PE
Surface CD71 MFI
n.s.
F
Intracellular CD71 MFI

Supplement Fig 7. Endosomal function in $CD8^+$ Treg cells

A. $CD8^+$ Tregs were stained for the early endosome marker EEA1 (green) and DAPI (blue) and visualized by confocal microscopy. Scale bar; 10 µm. Mean ± s.e.m. of EEA1 staining intensities in 20 cells each from 5 GCA patients and 5 healthy controls.

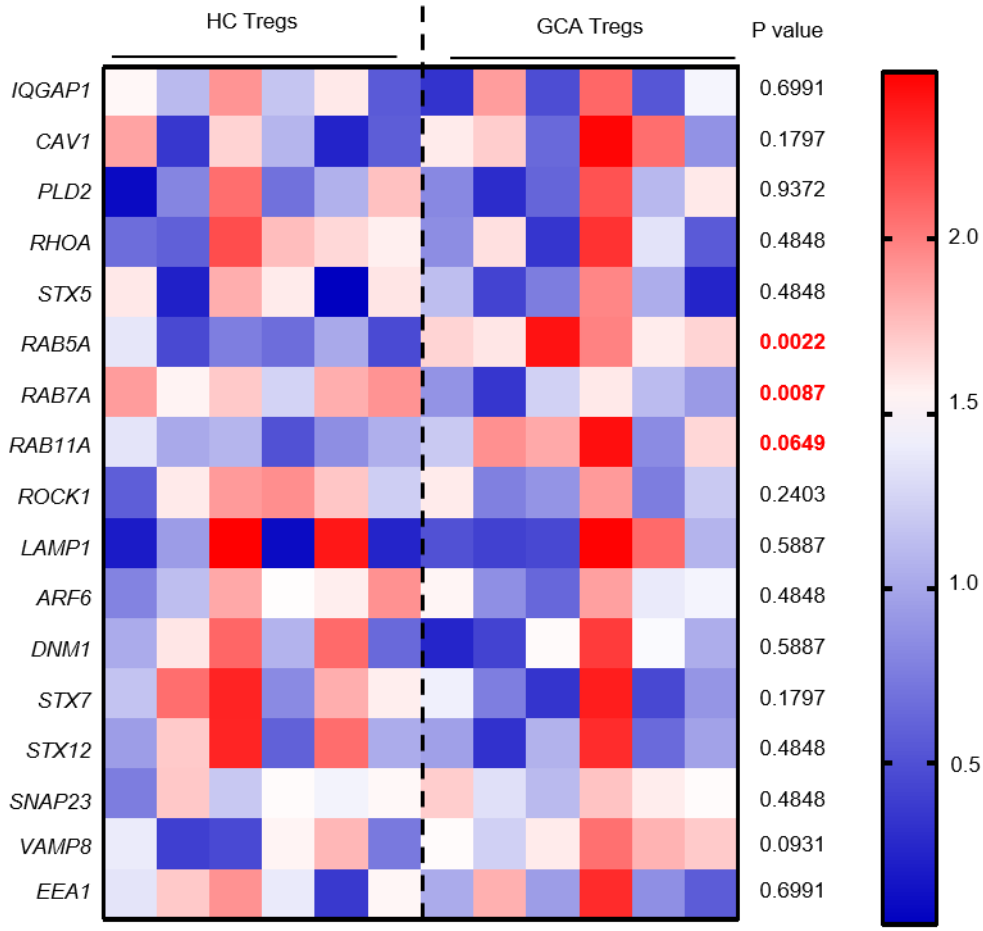
B. $CD8^+$ Tregs were stained for the recycling endosome marker RAB11 (green) and DAPI (blue) and visualized by confocal microscopy. Scale bar; 10 µm. Mean ± s.e.m. of EEA1 staining intensities in 20 cells each from 5 GCA patients and 5 healthy controls.

C-D. Expression of EEA1 and RAB11 protein was determined by flow cytometry in HC and GCA $CD8^+$ Treg cells. Mean ± s.e.m from n=8 samples each.

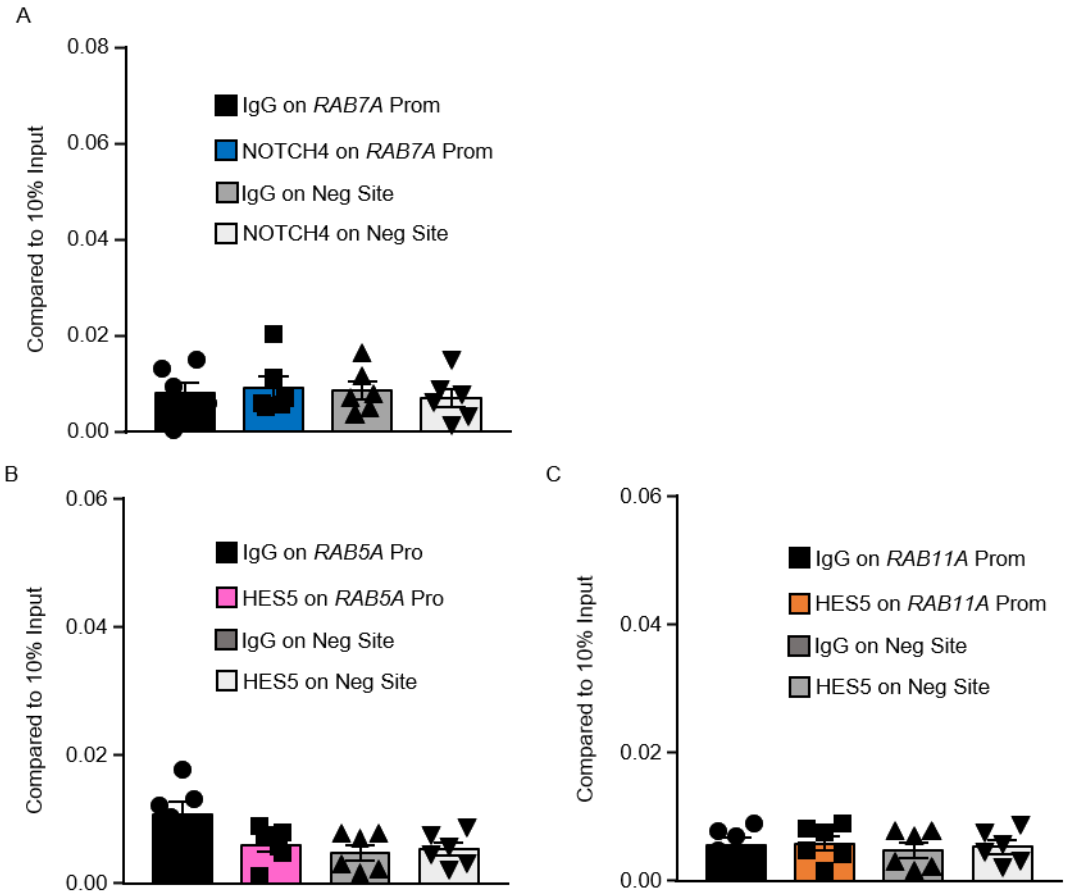
E. Surface expression of CD71 protein analyzed by flow cytometry on HC and GCA $CD8^+$ Treg cells. Representative histogram and mean ± s.e.m from n=8 samples each.

F. Intracellular expression of the CD71 protein analyzed by flow cytometry on HC and GCA $CD8^+$ Treg cells. Mean ± s.e.m from n=6 samples each.

Unpaired Mann–Whitney–Wilcoxon rank test (A,B,C,D,E,F). *P<0.05, **P<0.01,***P<0.001, ****P<0.0001.



Supplement Fig 8. Expression of endocytosis related gene in CD8⁺ Treg cells. CD8⁺ Tregs were induced from healthy or GCA PBMCs with low-dose anti-CD3 and IL-15. Gene expression of 17 endocytosis-related genes was assessed by q-PCR. Unpaired Mann–Whitney–Wilcoxon rank test was used.



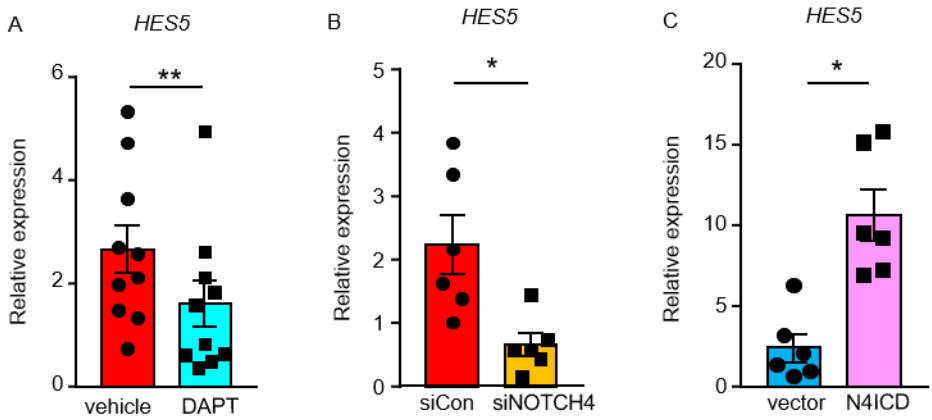
Supplement Fig 9. NOTCH4 occupancy on the *RAB7A* promotor and HES5 occupancy on the *RAB5A* and *RAB11A* promotor

A. The occupancy of NOTCH4 on the *RAB7A* promoter was determined by ChIP assay and normalized to 10% of Input.

B. The occupancy of HES5 on the *RAB5A* promoter was determined by ChIP assay and normalized to 10% of Input.

C. The occupancy of HES5 on the *RAB11A* promoter was determined by ChIP assay and normalized to 10% of Input.

All data are mean ± s.e.m from GCA $CD8^+$ Tregs (n=6).

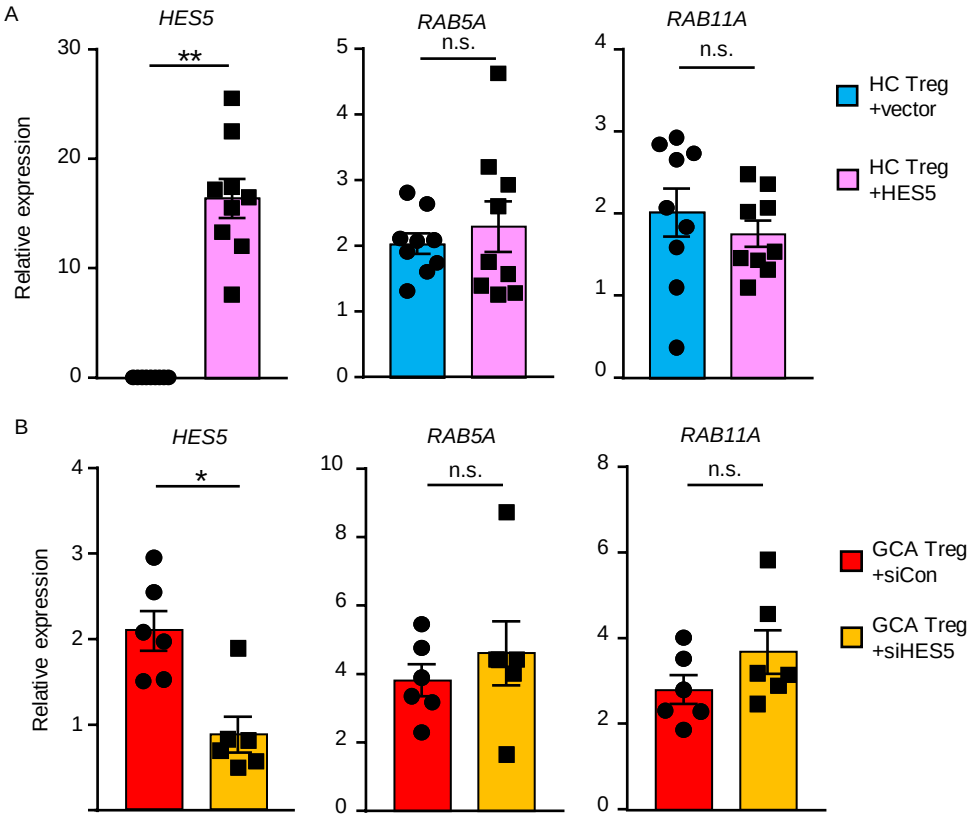


Supplement Fig 10. NOTCH4 signaling controls HES5 expression

A-C. To manipulate NOTCH4 signaling, $CD8^+$ Treg cells were treated or transfected as indicated and NOTCH signaling was assessed by q-PCR for HES5 transcripts. Each sample is indicated by a dot.

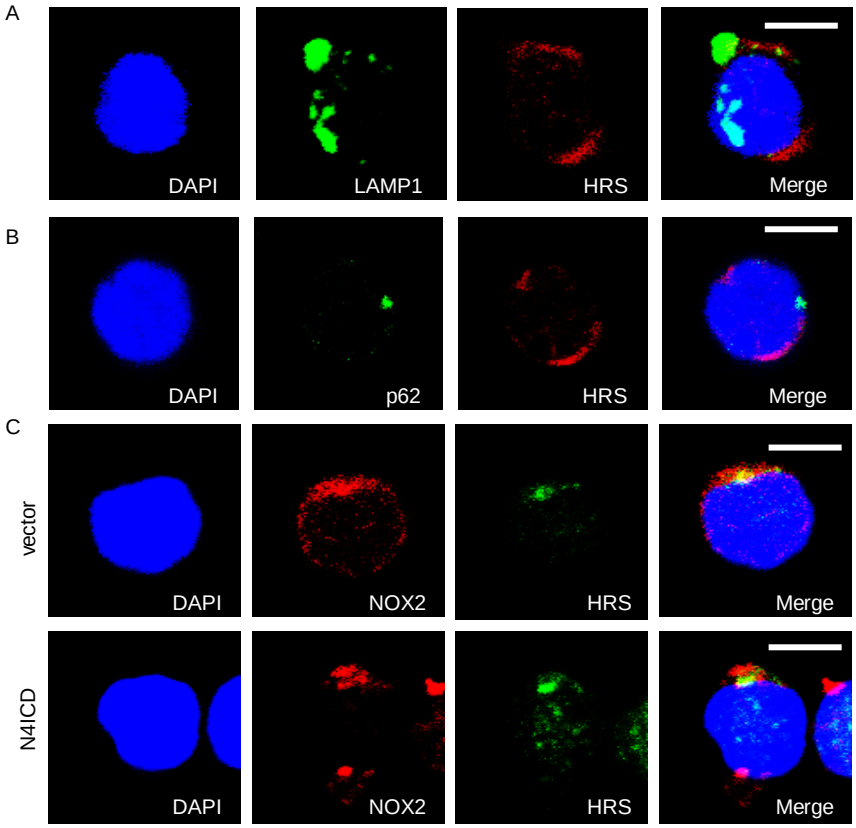
Data are mean ± s.e.m. Paired Mann–Whitney–Wilcoxon rank test. *P<0.05, **P<0.01

Supplement Fig 11. HES5 does not affect the expression of *RAB5A* or *RAB11A*



Supplement Fig 11. HES5 does not affect the expression of *RAB5A* or *RAB11A*

HC $CD8^+$ Tregs (A) and GCA $CD8^+$ Tregs (B) were induced as in Fig.1 and treated or transfected as indicated. The mRNA level of *HES5*, *RAB5A* and *RAB11A* was determined by q-PCR. Data are mean ± s.e.m. Paired Mann–Whitney–Wilcoxon rank test. *P<0.05, **P<0.01.



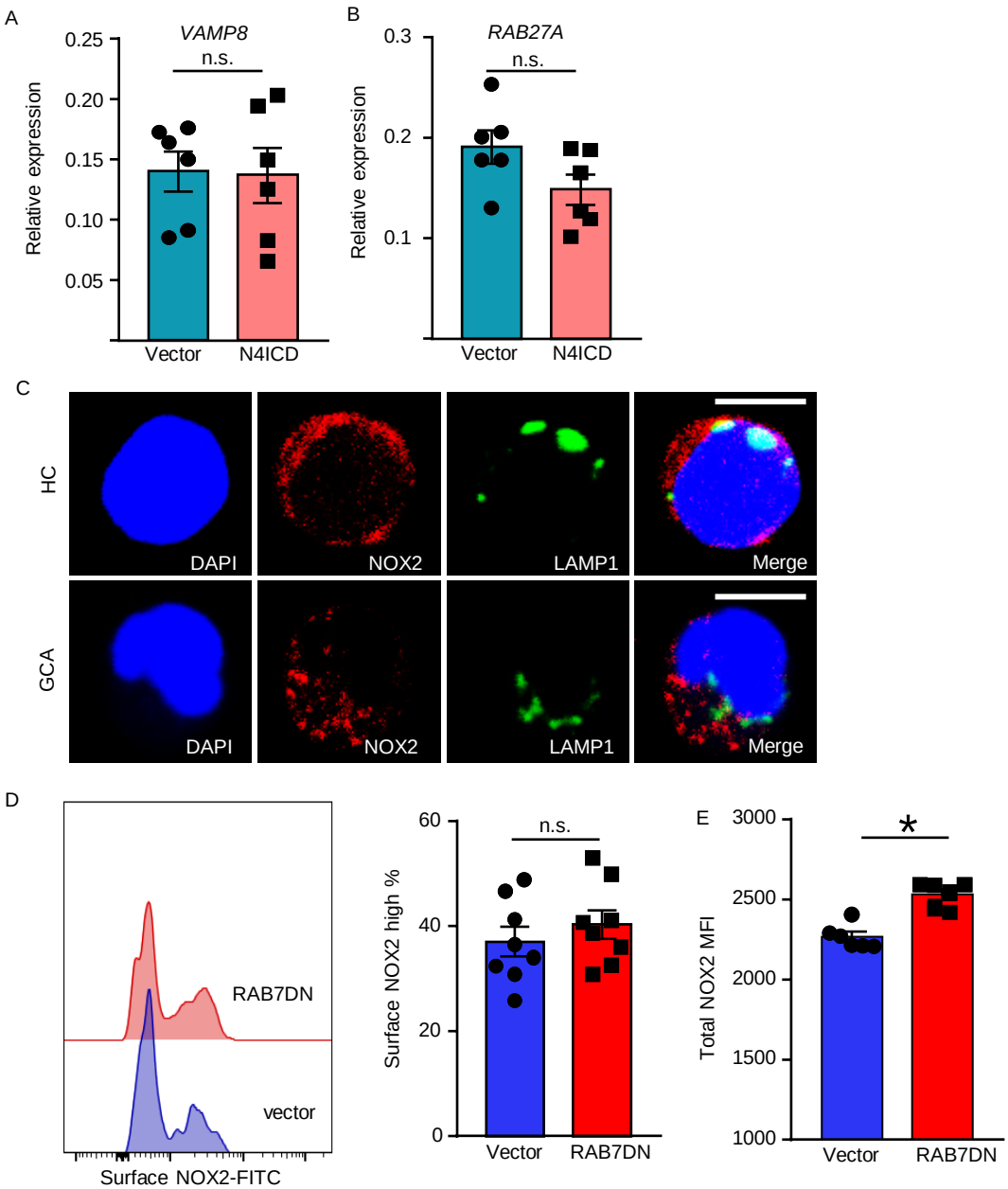
Supplement Figure 12. Subcellular distribution of HRS and NOX2 in CD8⁺ Treg cells.

CD8⁺ Treg cells were induced ex vivo from healthy individuals, stained by dual-color immunofluorescence and visualized by confocal microscopy. Nuclei were marked with DAPI (blue). Scale bar; 5 µm.

A. Co-staining for the lysosomal marker LAMP1 (green) and HRS (red).

B. Co-staining for the autophagy marker p62 (green) and HRS (red).

C. To activate NOTCH signaling, CD8⁺ Treg cells were transfected with an empty vector (upper row) or an N4ICD-containing vector (lower row). Transfected cells were stained for HRS (green) and NOX2 (red).



Supplement Fig13. Late endosomes and lysosomes in CD8⁺ Treg cells

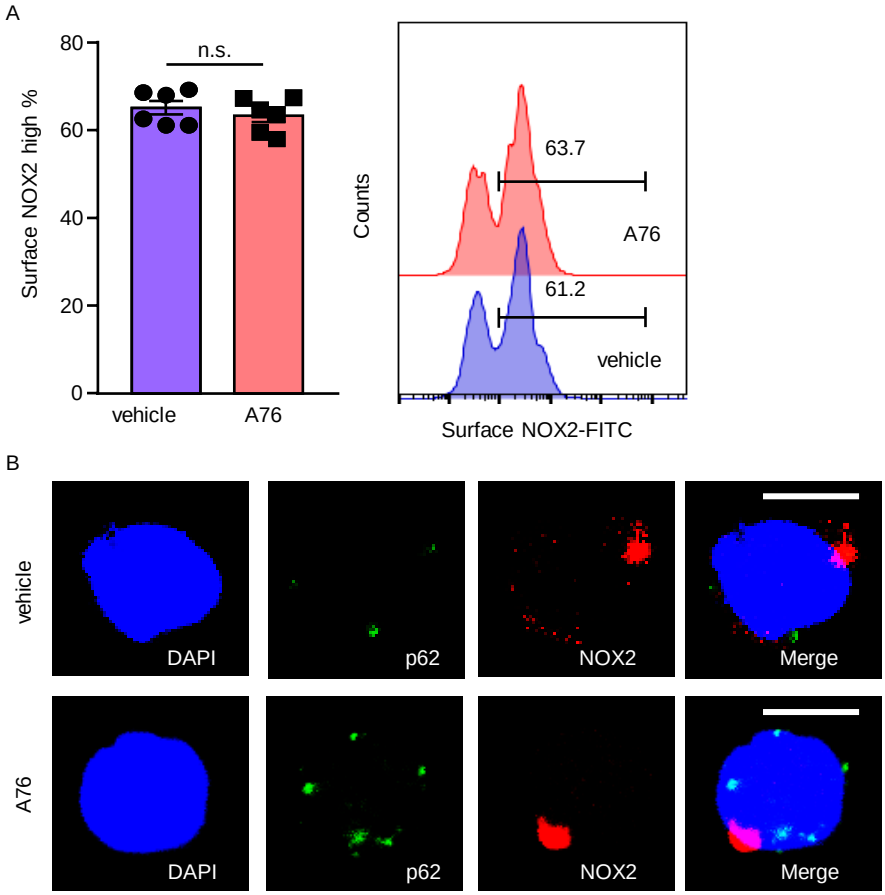
A,B. Healthy CD8⁺ Treg cells were transfected with a N4ICD-containing or empty vector. mRNA levels of *VAMP8* and *RAB27A* were determined by q-PCR. Mean ± s.e.m. from 6 samples.

C. Patient-derived and control CD8⁺ Treg cells were stained for the lysosomal marker LAMP1 (green), NOX2 (red) and DAPI (blue), and visualized by confocal microscopy. Scale bar; 5 µm.

D. GCA CD8⁺ Treg cells were transfected with RAB7DN-containing or control vector. Cell surface NOX2 was analysed by flow cytometry. Representative histograms and mean ± s.e.m from 8 samples.

E. CD8⁺ Treg cells were transfected with RAB7DN-containing or control vector. Total cellular NOX2 was quantified by flow cytometry. Mean ± s.e.m from 6 samples.

Paired Mann–Whitney–Wilcoxon rank test (A,B,D). n.s. P>0.05, *P<0.05



Supplement Fig 14. Autophagosomes in CD8$^+$ Treg cells

A. CD8$^+$ Tregs were treated with the autophagy activator A769662 or vehicle. Cell surface NOX2 expression determined by flow cytometry. Representative histograms and percentages of NOX2^{high} cells from 6 samples each.

B. CD8$^+$ Tregs were treated with the autophagy activator A769662 or vehicle and stained for the autophagosome marker p62 (green), NOX2 (red) and DAPI (blue), and visualized by confocal microscopy. Scale bar; 5 μm.