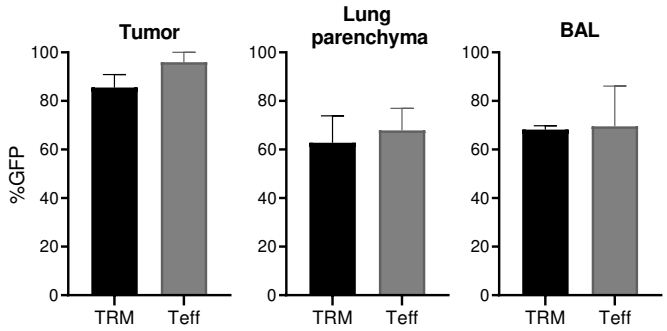

Fig S1: Percentage of GFP (surrogate of CXCR6 transcriptional expression) on T_{RM} and effector T cells in *Cxcr6* $^{gfp/+}$ after IN vaccination.
Cxcr6 $^{gfp/+}$ mice (n = 4) were grafted with TC-1 cells ($5x10^4$) in the tongue at D0, then vaccinated by IN route with STxB-E7 and poly-ICLC at D5 and D10, and sacrificed at D15. GFP expression on T_{RM} and effector $CD8^+$ T cells (Teff) was analyzed by flow cytometry in the tumor, lung parenchyma (CD8a iv-) and BAL (n=3 mice/group). Results are representative of two experiments. Mean ±sem is shown.*P < 0.05; ** P < 0.01

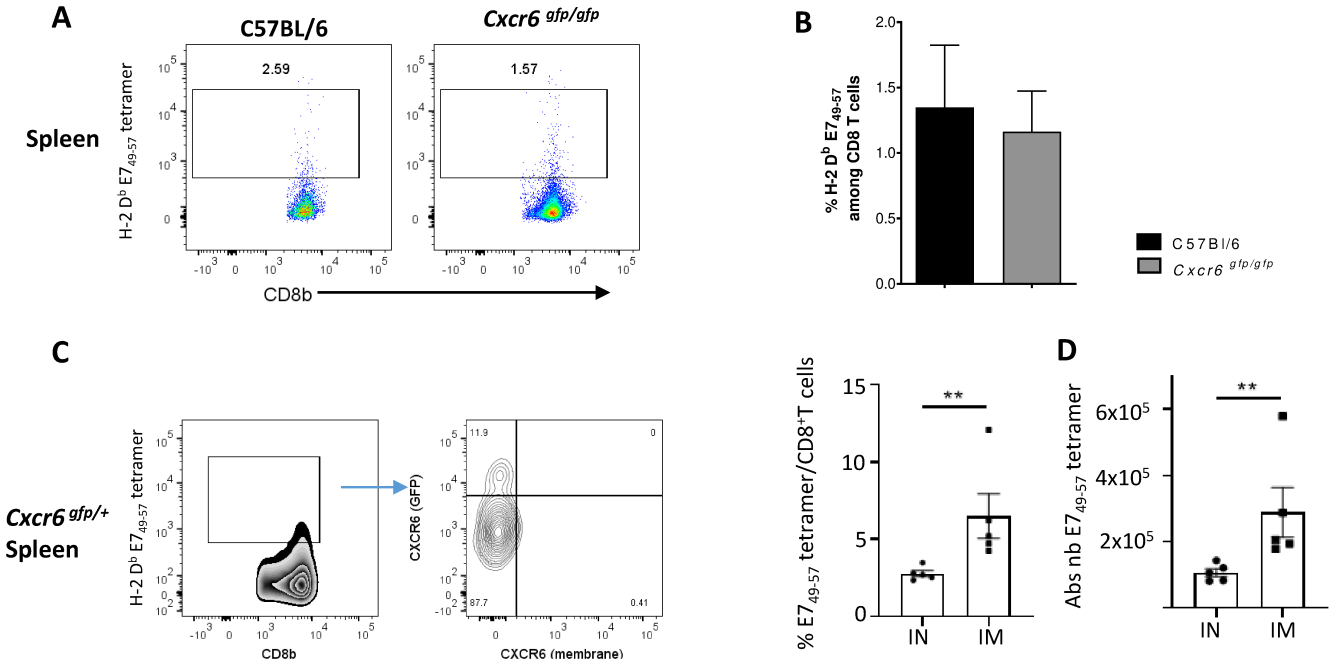

Fig S2*: Cxcr6*$^{gfp/gfp}$ and C57BL/6 mice have similar levels of H-2D^b E7 tetramer in the spleen after IN vaccination

(A-B) C57BL/6 or *Cxcr6*$^{gfg/gfp}$ mice were vaccinated with STxB-E7 by IN route at day 0 and 14, then sacrificed at day 21. Representative flow plots (A) and percentage of specific H-2D^b E7 tetramer gated on CD8$^+$ T cells (B) in the spleen. Mean ±sem. n=16 mice/group.

(C) *Cxcr6* $^{gfp/+}$ mice were grafted with TC-1 cells (5x10^4) in the tongue at D0, then vaccinated via the IN route with STxB-E7 and poly-ICLC at D5 and D10, and mice were sacrificed at D15. Representative flow plots showing expression of CXCR6 (GFP and surface) among H-2D^b E7 tetramer in the spleen. D : C57BL/6 mice (n = 5) were immunized with STxB-E7 by IN or IM route at day 0 and 14, then sacrified at day 21. Percentage and absolute number are shown.

n=5 mice/group. Mean ±sem. Mann-Whitney t-test *p<0.05 **p<0.01

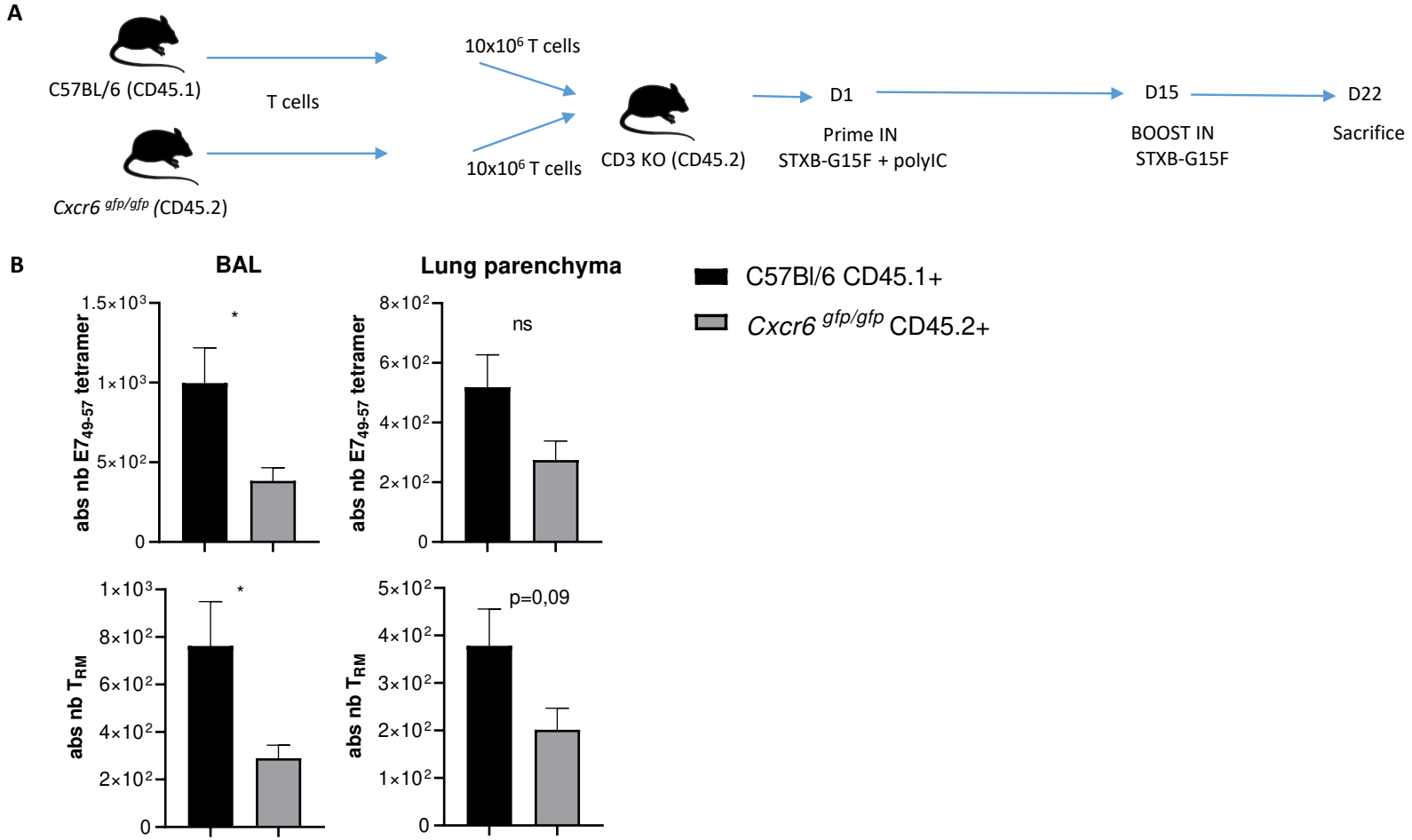

Fig S3 : Role of CXCR6 on T cells in the decrease number of specific CD8⁺T_{RM} in the lung.

A : Description of the experimental design of competitive adoptive transfer of T cells derived from CXCR6 deficient or non-deficient mice congenic for CD45

B : T cells isolated from spleen of naive C57BL/6 (CD45.1) and *Cxcr6* $^{gfp/gfp}$ (CD45.2) were expanded *in vitro* on anti-CD3 coated plates + IL-2 (4ng/ml) for 5 days in RPMI complete medium, then incubated an additional 2 days in the presence of IL-2 (2 ng/ml).

In vitro activated T cells were harvested, washed, mixed at equal numbers in PBS, then injected i.v into CD3 KO recipient mice which were then immunized with STxB-E7 by IN route at day 1 and 21 and sacrified at day 28. Absolute number of H-2D^b $E7_{49-57}$ tetramer and specific T_{RM} were analyzed in BAL and lung parenchyma.

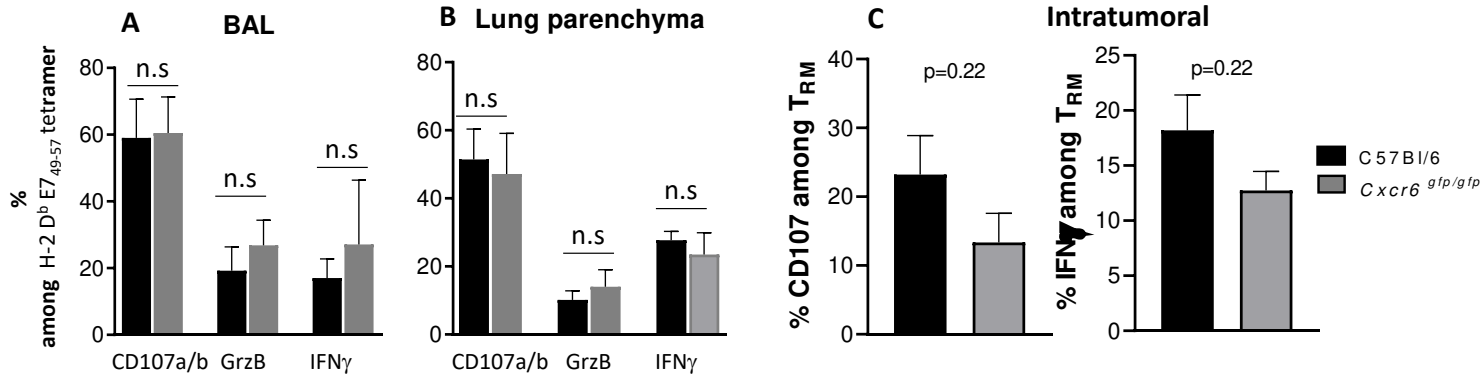

Figure S4: *Cxcr6* deficiency does not impair the functionality of antigen-specific CD8⁺ T cells

A-B : C57Bl/6 and *Cxcr6*$^{gfg/gfp}$ mice were vaccinated with STxB-E7 via the IN route at day 0 and 14, then sacrificed at day 21. C : C57BL/6 or *Cxcr6* $^{gfp/gfp}$ mice were grafted in the submucosal lining cheek (IC: intra-cheek) with TC-1 tumor cells, then vaccinated with STxB-E7 by IN route at day 7 and 14, and sacrified at day 20. Lymphocytes from BAL (A) , lung parenchyma (B) or from the tumor after cell dissociation (C) were stimulated with the $E7_{49-57}$ peptide (10 µg/mL)(A, B) or the TC1 tumor for 5h, in the presence of anti-CD107a/b antibody, Golgistop® (monensin) and Golgiplug® (Brefeldin A). Percentages of CD107a/b, GrzB and IFNγ among $E7_{49-57}$ tetramer in BAL (A) and lung parenchyma (B) or E7 spéoific T_{RM} (C) were analyzed by flow cytometry.

Mean ±sem. n=3-5 mice/group. Data are representative of 2 independent experiments. n.s = not significant

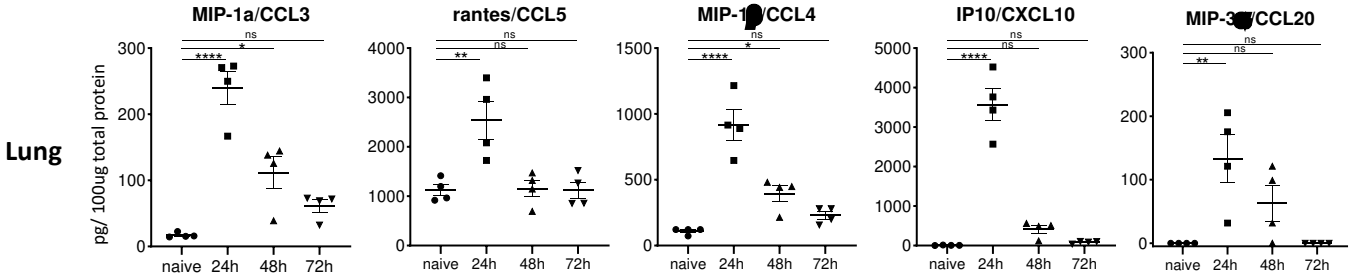

Figure S5 : Other chemokines are transiently modulated after IN vaccination.

C: C57BL/6 mice were vaccinated intranasally with STxB-E7 (20 µg)+poly-ICLC (10 µg). Chemokines CCL3, CCL5, CCL4, CXCL10 and CCL20 were measured by Luminex assay in lysate from lung at the indicated times after immunization. Data are representative of 2 independent experiments with 4 mice/group. Mean ±sem. Mann-Whitney t-test. *P < 0.05; ** P < 0.01; *** P < 0.001; ****P < 0.0001

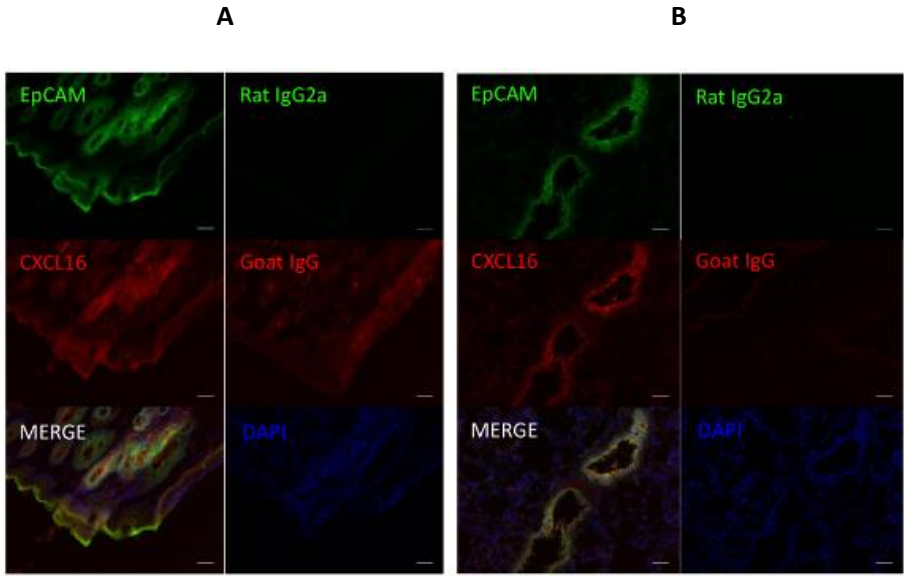

Fig S6 : CXCL16 is expressed by epithelial cells derived from the mucosal lining cheek and lung.

Frozen section from mucosal lining cheek (A) and lung (B) derived from mice previously injected with Poly:IC were stained with antibodies against epithelial cells (Epcam) or CXCL16. Isotype control antibodies were included in each experiment. Nuclei were highlighted using DAPI solution (1ug/mL, Sigma D9542), slides were mounted with mounting medium (DAKO, S3023). Images were acquired x20 on Vectra® 3 automated microscope and analyzed with inForm® Image Analysis Software. Scale bars are 50 μm.