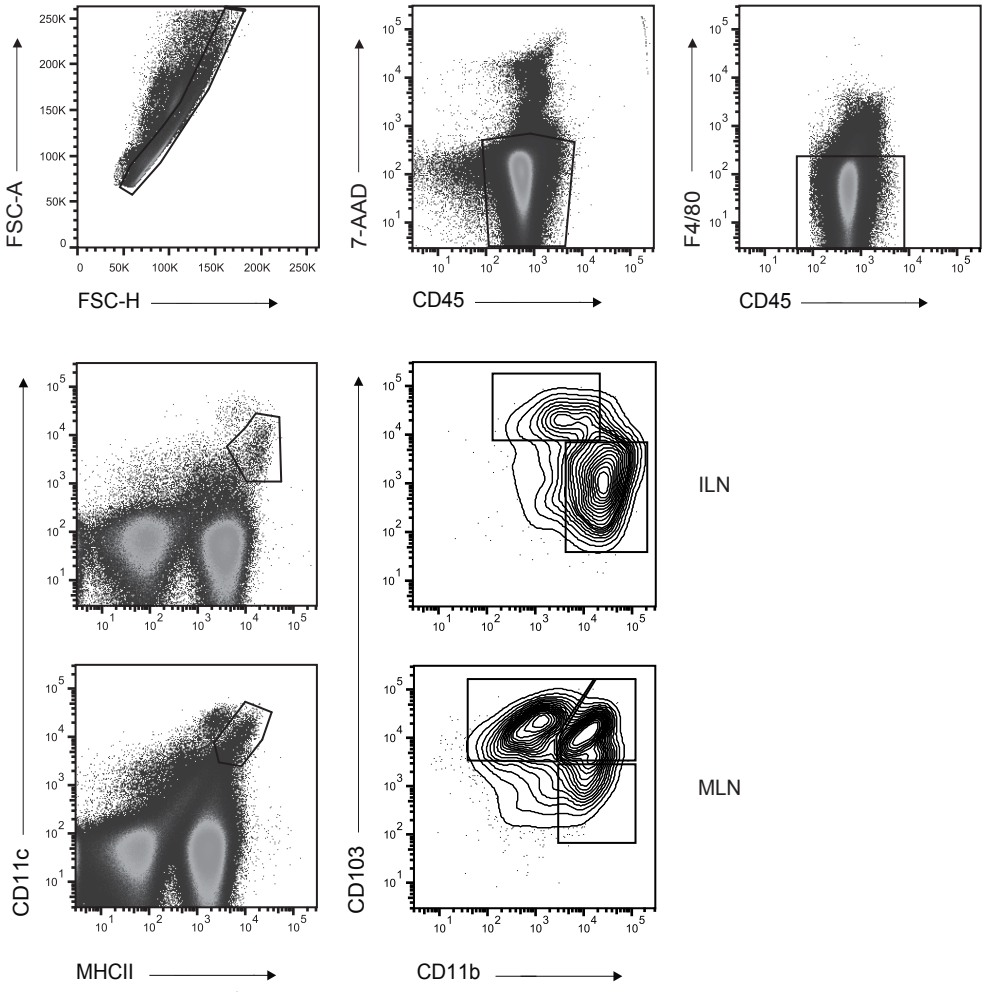
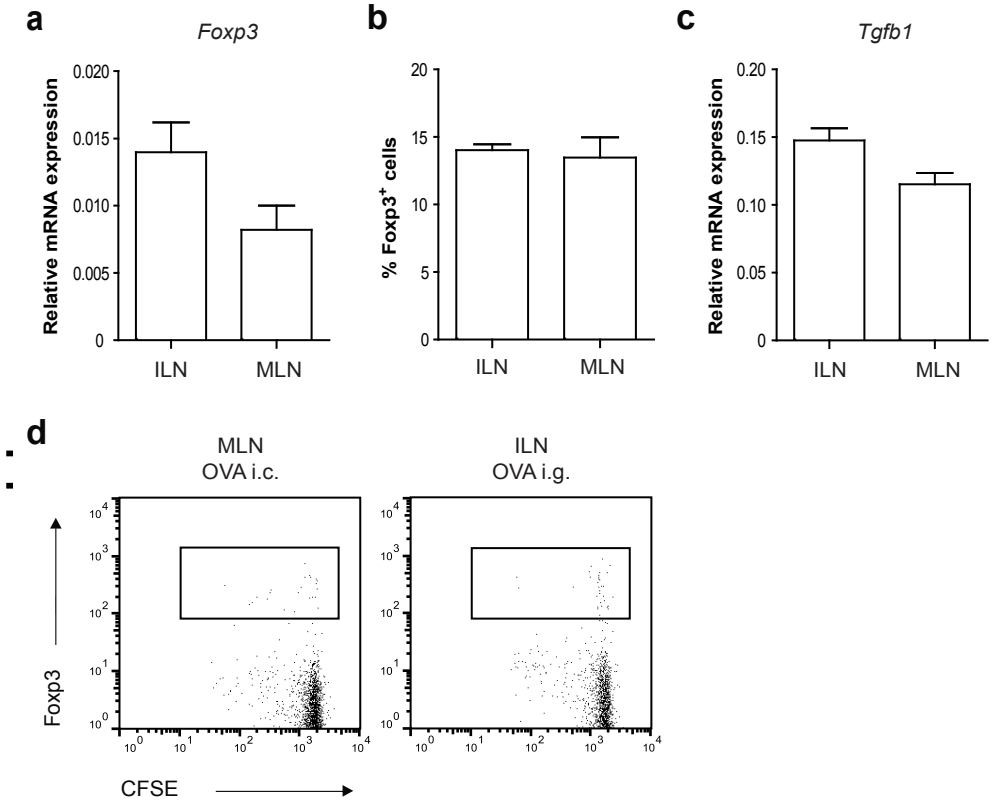
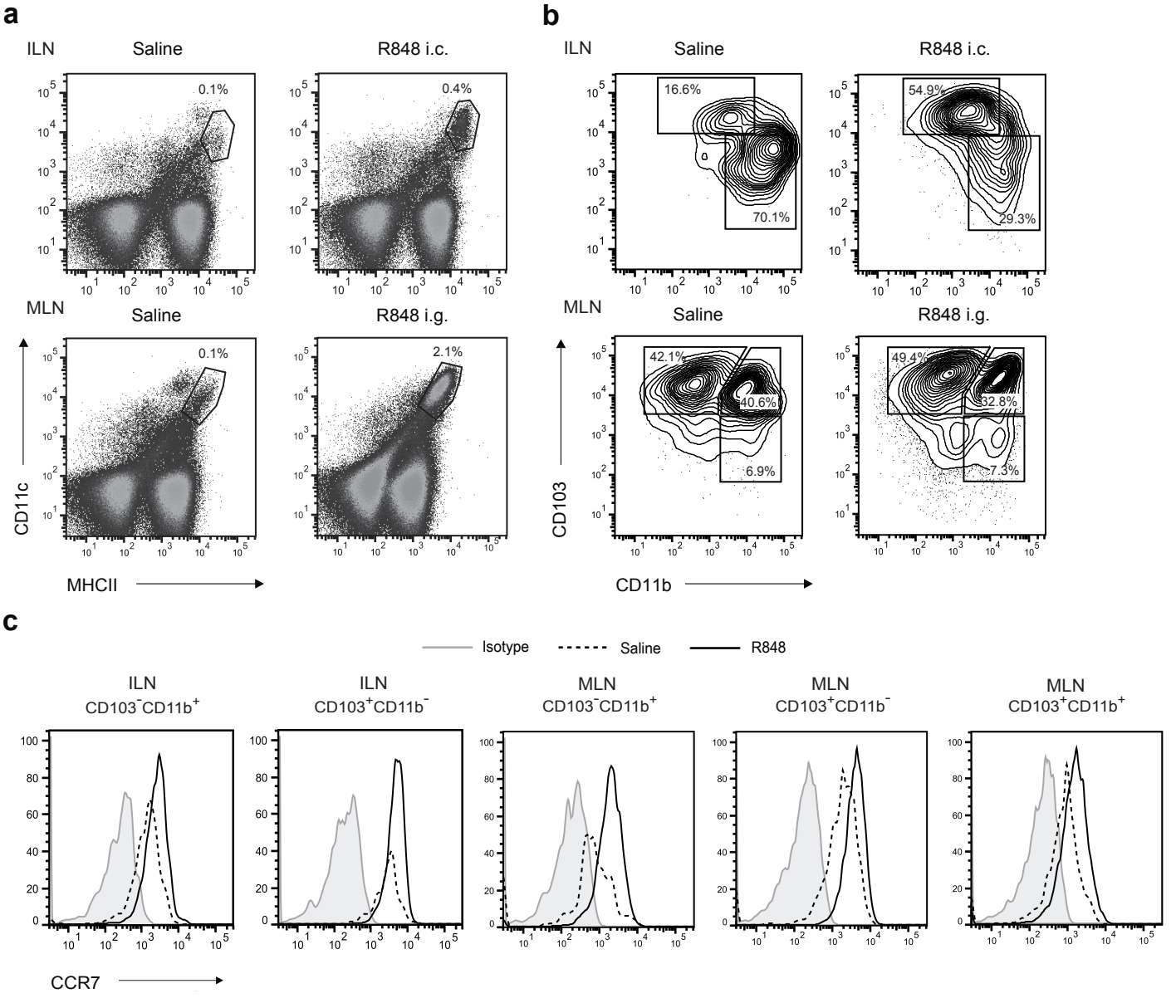
Supplementary Figure S1



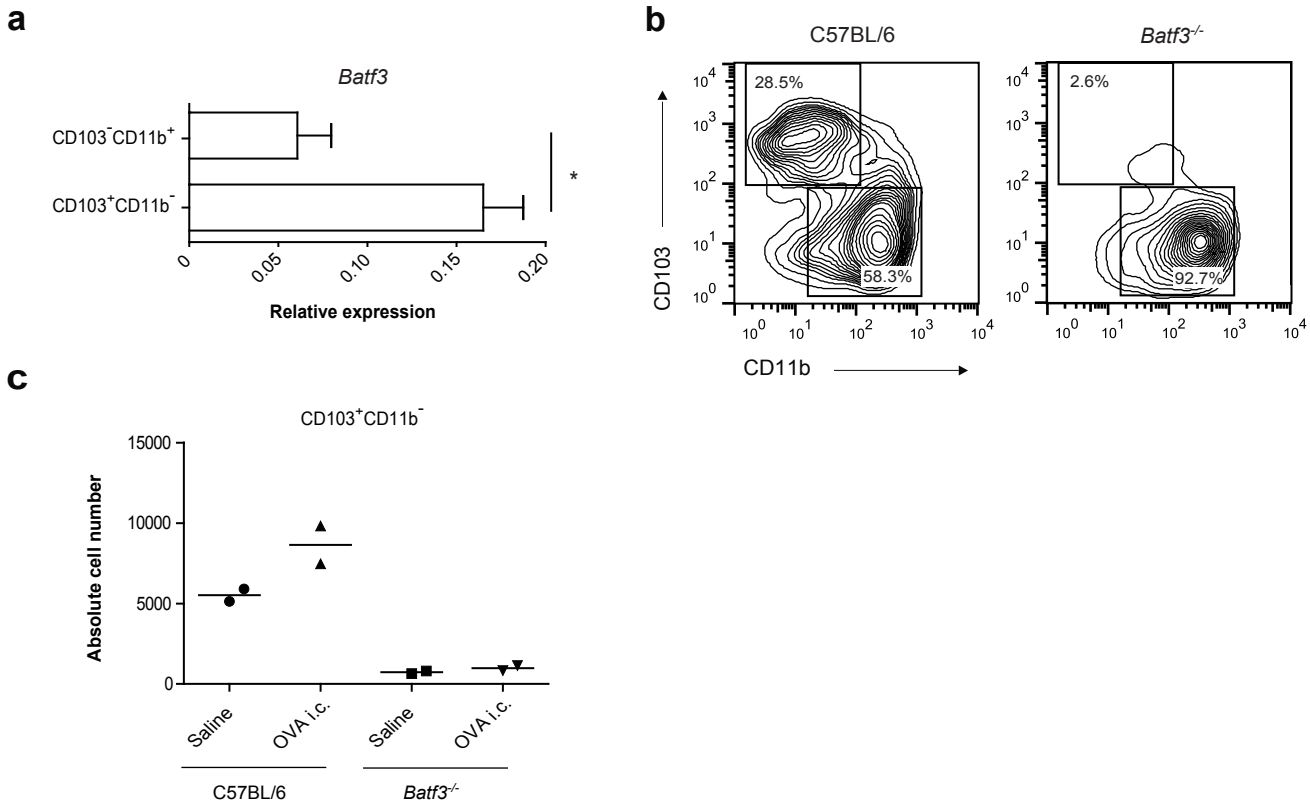
Supplementary Figure S2



Supplementary Figure S3



Supplementary Figure S4



Supplementary Figure S5

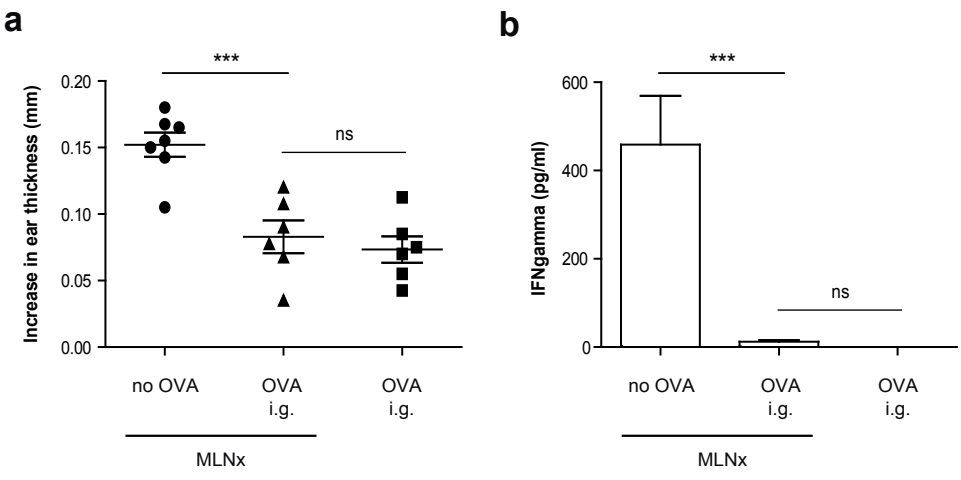


FIGURE LEGENDS

Supplementary Figure S1 Flow cytometric analysis of the DC composition in the lymph nodes. Gating strategy used to discriminate migratory DC subsets. MLN and ILN were digested using liberase/DNAse and single cell suspensions were stained for CD45, F4/80, CD11c, MHCII, CD103 and CD11b. Live $CD45^+F4/80^-$ single cells were gated and further analyzed for $CD11c^+MHCII^{high}$ migratory DCs that were subdivided on the basis of CD103 and CD11b expression.

Supplementary Figure S2 Colonic OVA administration does not induce antigen-specific T-cell proliferation and Foxp3+ Treg differentiation in the MLN. (**a-c**) Whole cell preparations of lymph nodes were analyzed for expression of mRNA for (**a**) *Foxp3* and (**c**) *Tgfb1* by quantitative PCR analysis, or (**b**) $Foxp3^+$ cells within the $CD4^+$ T cell gate by flow cytometry. Values are mean plus SEM for 3-7 mice per group. (**d**) $CD4^+KJ1.26^+$ OVA-specific T cells were purified from DO11.10 transgenic x *Rag*$^{-/-}$ mice and labeled with CFSE. Subsequently, BALB/c mice were given 6 x 10^6 CFSE-labeled T cells intravenously and one day later, received 70 mg OVA either i.g. or i.c. Three days after OVA administration, lymph nodes were isolated and analyzed for $CD4^+KJ1.26^+CFSE^+Foxp3^+$ T cells by flow cytometry. Data are representative of three independent experiments.

Supplementary Figure S3 The TLR7/8 ligand R848 stimulates DC migration and upregulates CCR7 cell-surface expression. Mice received 10 µg R848 i.g. or i.c. and 16-18h later, MLN and ILN were digested using liberase/DNAse and single cell suspensions were stained for CD45, F4/80, CD11c, MHCII, CD11b, CD103 and CCR7. (**a**) Live $CD45^+F4/80^-$ single cells were gated and further analyzed for $CD11c^+MHCII^{high}$ migratory DCs. Representative dot plots are shown. (**b**) The total $CD11c^+MHCII^{high}$ migratory DC population was further analyzed to identify DC subsets based on the expression of CD103 and CD11b. (**c**) Migratory DC subsets were stained for CCR7 after R848 i.g. (MLN) or i.c. (ILN). Data shown are from one of two experiments using cells pooled from 3 mice per experimental condition.

Supplementary Figure S4 $CD103^+$ DCs are absent in the colon-draining lymph nodes of *Batf3*$^{-/-}$ mice after colonic antigen administration. (**a**) ILN-derived $CD103^+$ and $CD103^-$ DC subsets from BALB/c mice were purified by flow cytometric cell sorting and expression of *Batf3* was determined by

quantitative PCR analysis. Results shown are mean plus SEM from 3 independent experiments using cells pooled from 20-30 mice per experiment. *P< 0.05 versus control, by Student's *t*-test. (**b, c**) C56BL/6 and *Batf3*$^{-/-}$ mice received 70 mg OVA i.c. and 16-18h later, ILN were digested using liberase/DNAse and single cell suspensions were stained for CD45, F4/80, CD11c, MHCII, CD11b and CD103. Live CD45^{+}F4/80^{-} single cells were initially gated and further analyzed for the expression of CD103 and CD11b within the CD11c^{+}MHCII^{high} migratory DCs. (**b**) Representative dot plots and (**c**) absolute cell numbers of CD103^{+}CD11b^{-} DCs are shown using cells pooled from 3 mice per experimental condition.

Supplementary Figure S5 Oral tolerance after antigen feeding is sustained in the absence of the MLN. MLNs were surgically excised (MLNx) and after 6 weeks mice were treated with 25 mg OVA i.g. Three days later, mice were sensitized subcutaneously in the tailbase with 100 µg OVA emulsified in Incomplete Freund's adjuvant. At day 8, mice were challenged with 10 µg OVA in the auricle of both ears. (**e**) After 24h, increases in ear thickness in both ears were determined and compared with values before challenge. (**f**) Cells purified from the inguinal lymph nodes after induction of the DTH reaction were restimulated for 48h with OVA protein. IFNγ levels were determined in supernatant by ELISA. Completeness of mesenteric lymphadenectomy was confirmed in all MLNx animals at autopsy. The results are depicted as mean plus SEM for 6-7 mice per experimental condition. ***P< 0.001 versus control, using one-way ANOVA.