Supplementary Fig 1 | CD4+ T cell phenotypes during the priming phase of EAE.

a, Percentages and activation status of CD4$^+$Foxp3$^-$ T cells in

dLN. **b,** and **c,** Cytokine production by CD4$^+$Foxp3$^-$ in dLN at various time points post immunization (N≥3, mean± SE).

d, Percentages of and IL-10 production by of CD4$^+$Foxp3$^+$ cells in dLN at various timepoints post immunization (N≥3, mean± SE).

e, Ly-6C expression by CD4$^+$Foxp3$^+$ T cells in dLN and **f,** spleen three days post EAE immunization (N≥7, mean± SE, *p < 0.05).

g, 2D2 cells (1X10^6) were transferred to WT or cKO mice at d -1. Mice were immunized with MOG-CFA on d 0 and injected with pertussis toxin on d 0 and 2. dLN were analysed on d4 for percentages of 2D2 cells

h, IFN-γ and IL-17A producing 2D2 cells **i,** IFN-γ and IL-17A production by recipient CD4$^+$ T cells.

(N=4, mean± SE, representative of two independent experiments).

j-k, FACS purified CD4$^+$Foxp3$^+$ cells from dLN three days post EAE induction were transferred into naïve CD45.1 mice.

Percentage of CD45.2$^+$ cells and CD45.2$^+$Foxp3$^+$cells in the spleen of recipient mice were determined 18 d.

Data are representative of two independent experiments each with at least 3 mice per group.

a

b
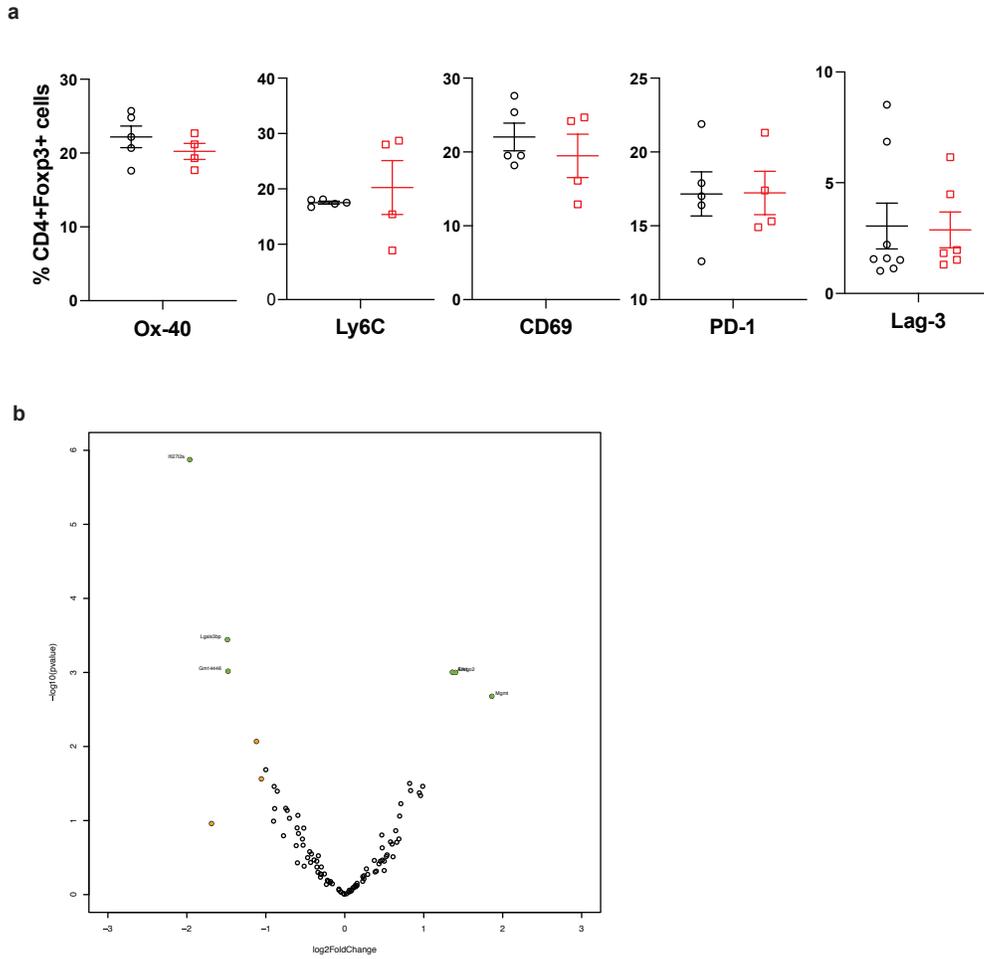
Supplementary Fig. 2 | Phenotypic and molecular characterization of cKO Tregs.
a, Cell surface antigen expression on CD4$^+$Foxp3$^+$ cells from spleen of
unimmunized Foxp3$^{YFP-Cre}$ (black) or IFNAR$^{fl/fl}$Foxp3$^{YFP-Cre}$ mice (red).
Data are representative of mean± SE, N≥4, *p < 0.05.
b, Volcano plots of RNA-seq analysis of Foxp3$^{YFP-Cre}$ or IFNAR$^{fl/fl}$Foxp3$^{YFP-Cre}$ mice LN from unimmunized mice. N=3 mice.
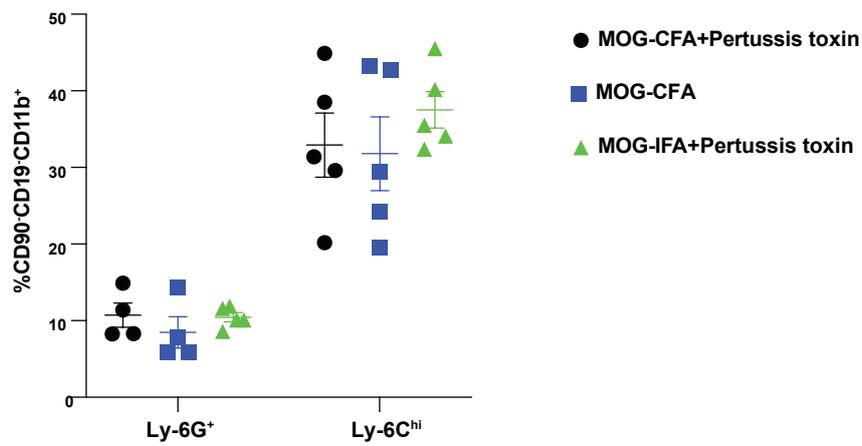
Supplementary Fig. 3 | Homing of MDSCs to dLN does not require Pertussis toxin or CFA.
Mice were divided into 3 groups.
One group was immunized with MOG-IFA s.c on d 0 and received Pertussis toxin i.p d 0 and d 2;
the second group received only MOG-CFA s.c on day 0 .
The control group received MOG-CFA s.c d 0 and pertussis toxin i.p on d 0 and 2.
dLN were analyzed for $CD11b^+$ cells at day 5. N=4, mean± SE.