Suppl. Figure 1: Surface **(A)** and total **(B)** CTLA-4 expression on primary $CD4^+/FoxP3^-$ T conventional and $CD4^+/FoxP3^+$ T regulatory cells comparing 2 month old wild-type and Y201V knock-in mice. CTLA-4 expression is displayed as fold change of the mean fluorescent intensity (MFI). Statistical analyses were performed using an unpaired Student's t test (***, $P < 0.001$).

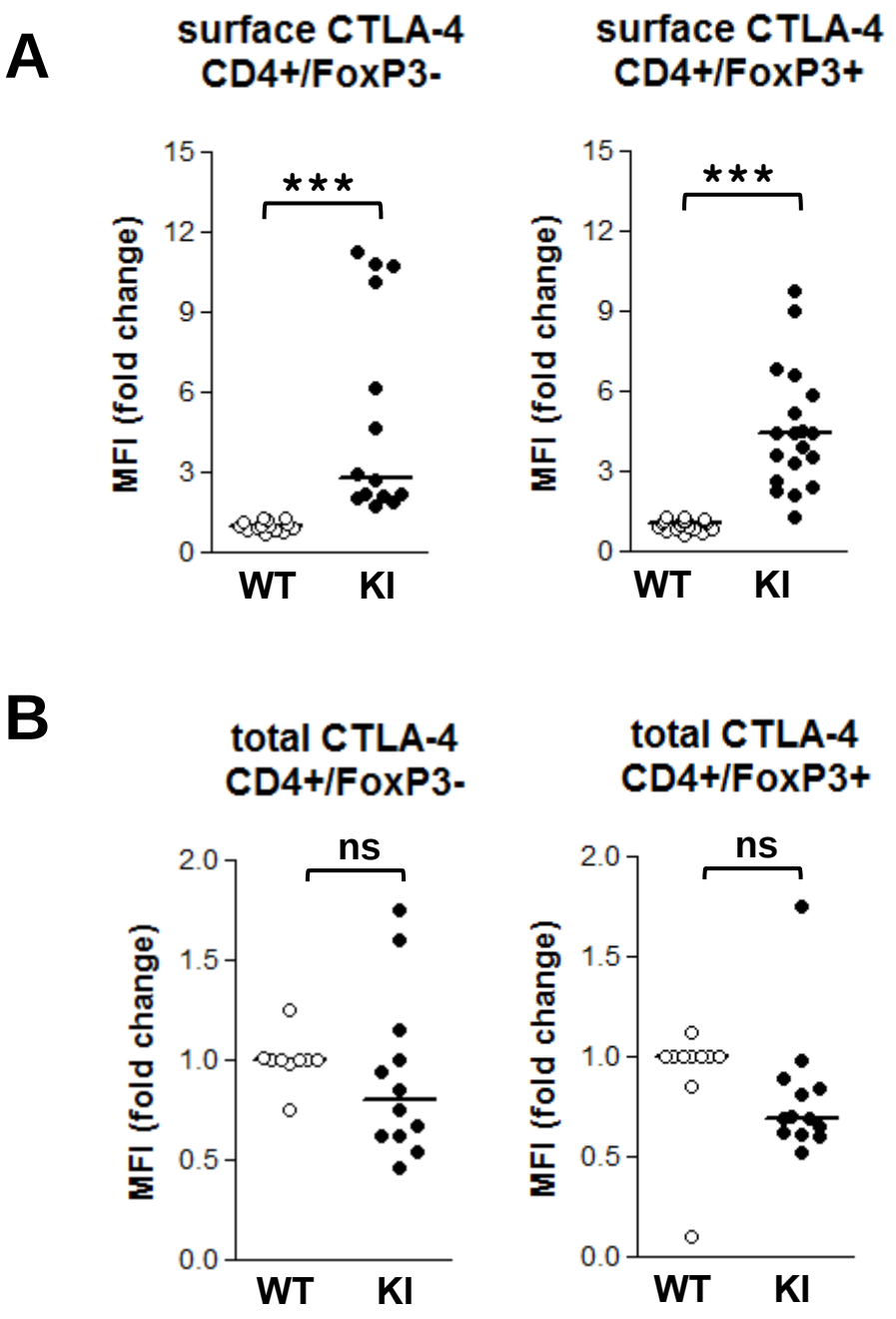
Suppl. Figure 2: (A) Total organ cellularity of axillary lymph node (axLN), mesenteric lymph node (mesLN) and spleen (SP) in 8 week old wild-type and Y201V knock-in mice. **(B)** Absolute numbers of conventional $CD4^+$ T cells and $CD4^+/FoxP3^+$ regulatory T cells in lymph nodes of Y201V knock-in mice and littermate controls. Numbers of activated T effectors and resting naïve T cells are displayed as ratio of $CD44^+$ over $CD62L^{hi}$ $CD4^+$ T cells. **(C)** Total cellularity of spleen and lymph nodes in 3 month old Y201V KI and wild-type mice. **(D)** Overall percentage of activated $CD4^+/CD44^+$, $CD4^+/CD69^+$ T cells as well as $CD4^+/FoxP3^+$ regulatory T cells in lymph nodes of 3 month old Y201V KI and wild-type mice. Statistical analyses were performed using an unpaired Student's t test (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Suppl. Figure 3: $CD4^+/CD44^-/CD62L^{hi}$ naïve T cells from lymph nodes of 8 week old WT and CTLA-4Y201V knock-in mice were labeled with CFSE and stimulated with soluble anti-CD3 (1ug/ml) and anti-CD28 (1ug/ml) in the presence of 200U/ml IL2 for 3 days. T cell proliferation was measured by flow cytometry based on CFSE dilution.

Suppl. Figure 4: Wild-type and Y201V knock-in mice were immunized with MOG_{35-55} peptide emulsified in CFA and total cellularity of spleen and CNS **(A)** as well as absolute numbers of total $CD4^+$ T cells **(B)** were determined at peak disease. **(C)** CTLA-4 surface expression on $CD4^+$/$FoxP3^-$ T effector, $CD4^+$/$FoxP3^+$ T regulatory and $CD4^+$/$Foxp3^+$/MOG^+ antigen-specific T regulatory cells in the CNS of wild-type and Y201V knock-in mice at peak disease after induction of EAE. Statistical analyses were performed using an unpaired Student's t test (*, $P < 0.05$; **, $P < 0.01$**).**

Suppl. Figure 5: Thymocytes from 8 week old WT and CTLA-4 Y201V knock-in mice were isolated and analyzed by flow cytometry to evaluate the frequency of $CD4^+$/$FoxP3^+$T regulatory cells **(A)**. **(B)** Representative flow plot (left) and quantification (right) of FoxP3 protein expression levels in $CD4^+$ T regulatory cells.

Suppl. Figure 1:



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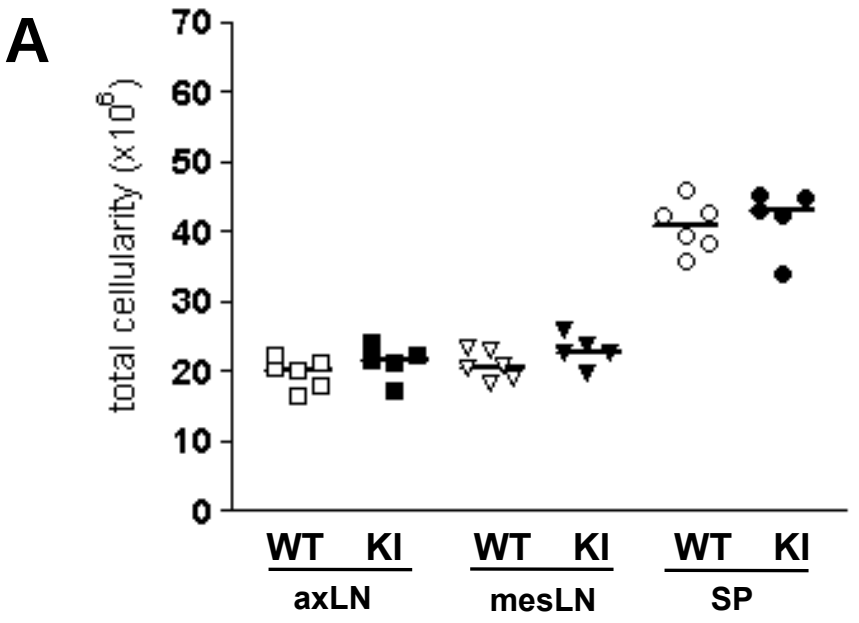

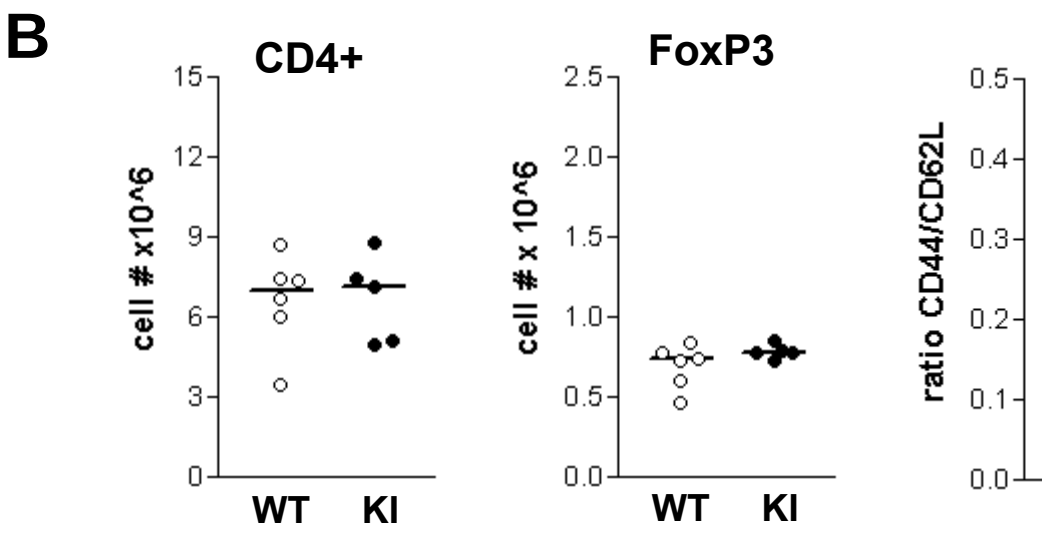

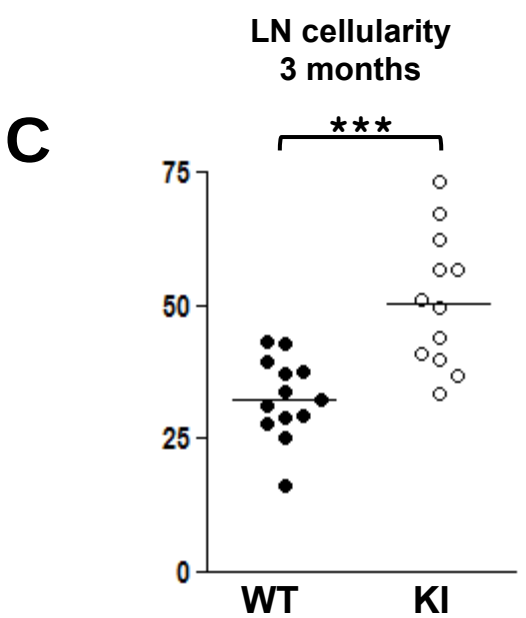

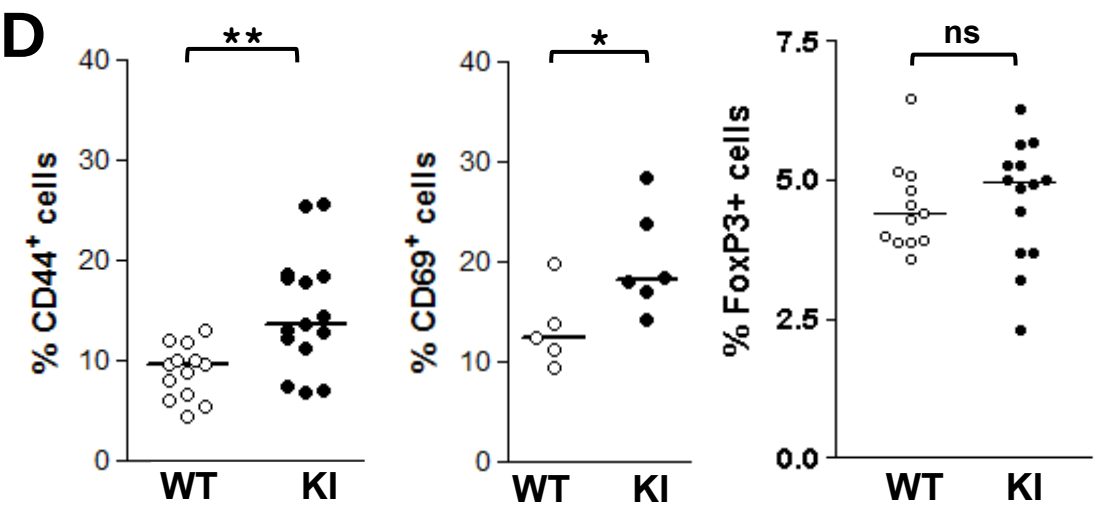

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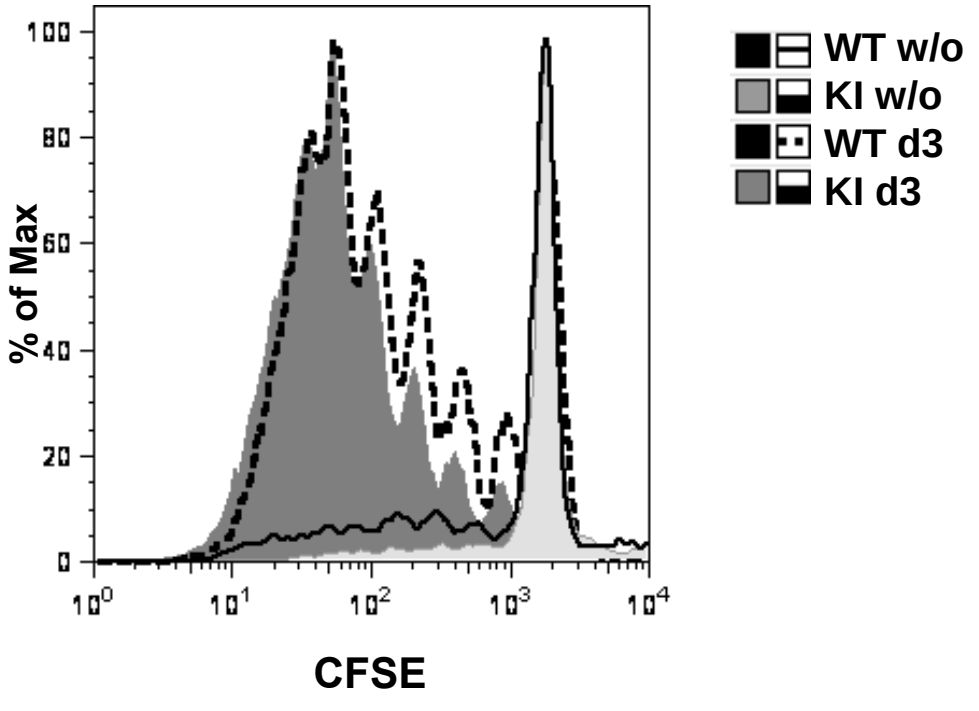
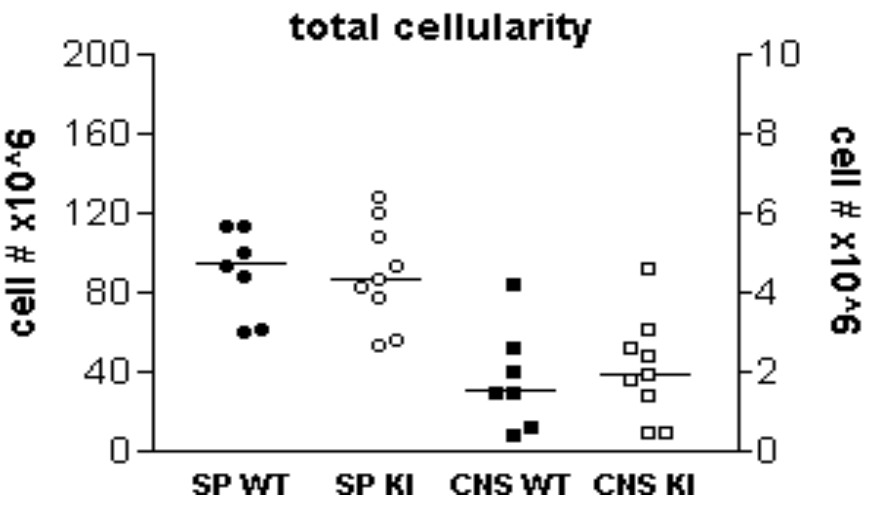


Suppl. Figure 4:

A



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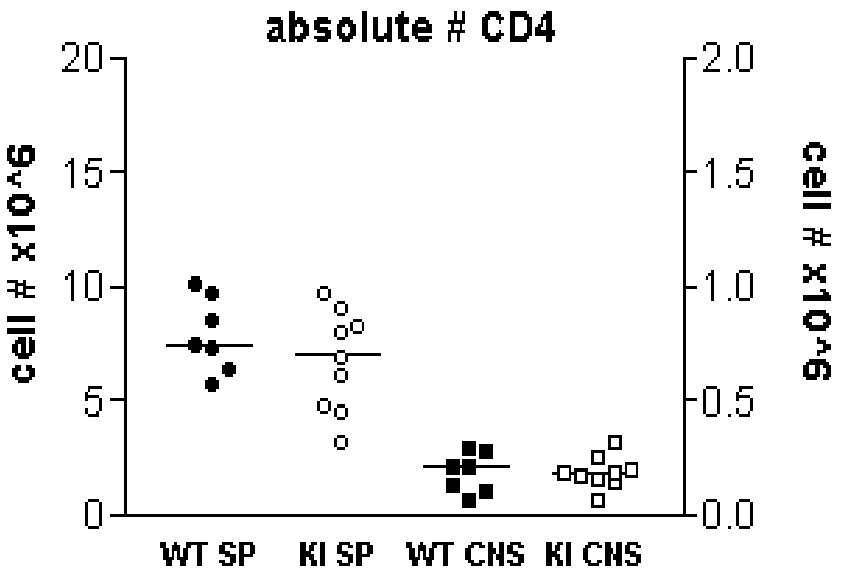

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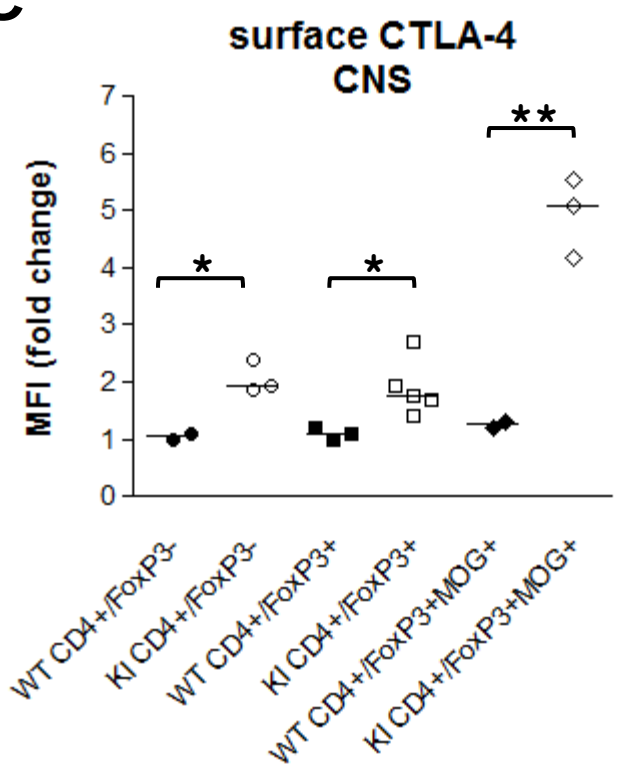

Suppl. Figure 5: