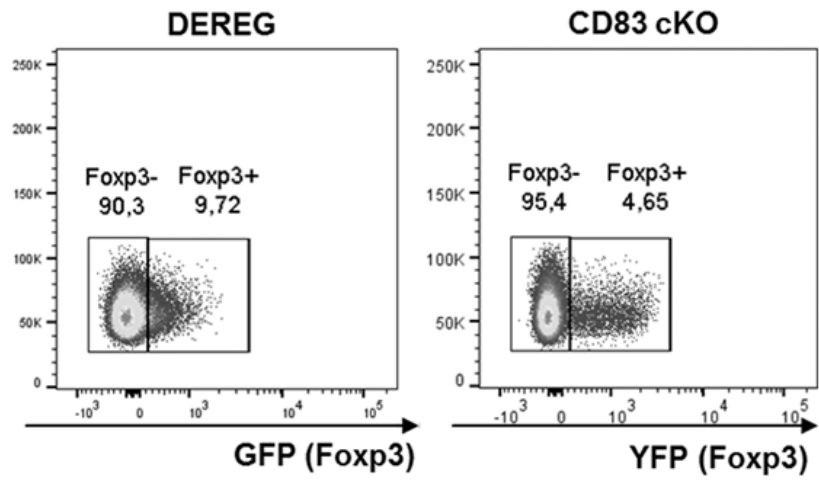
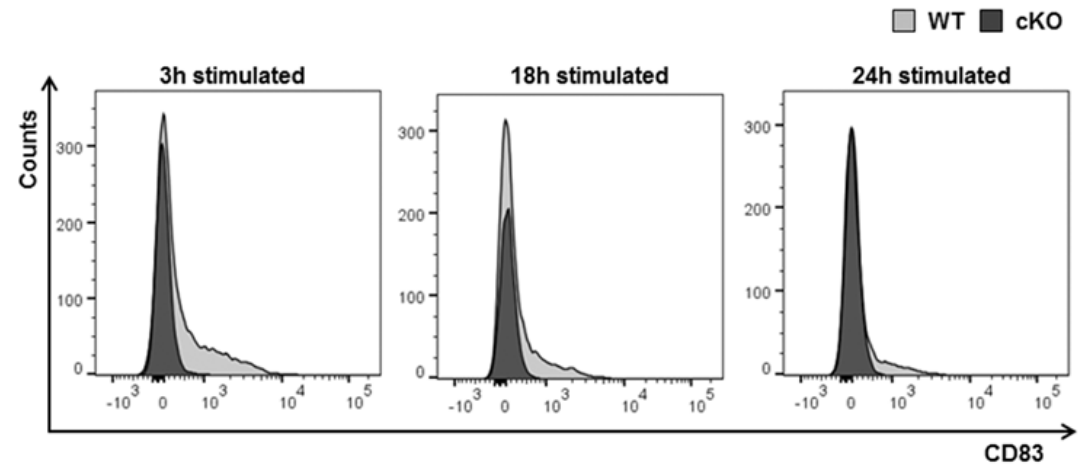


S1A.1

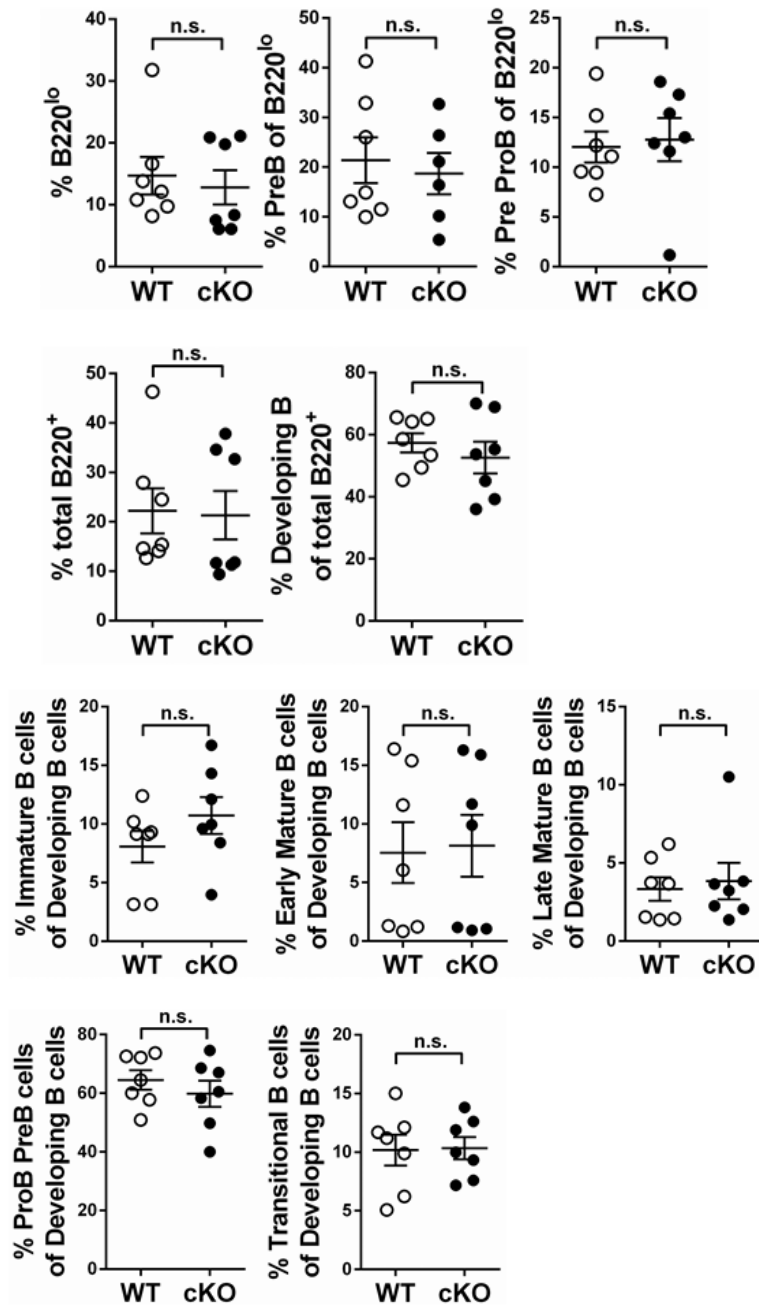
CD4<sup>+</sup> T cells



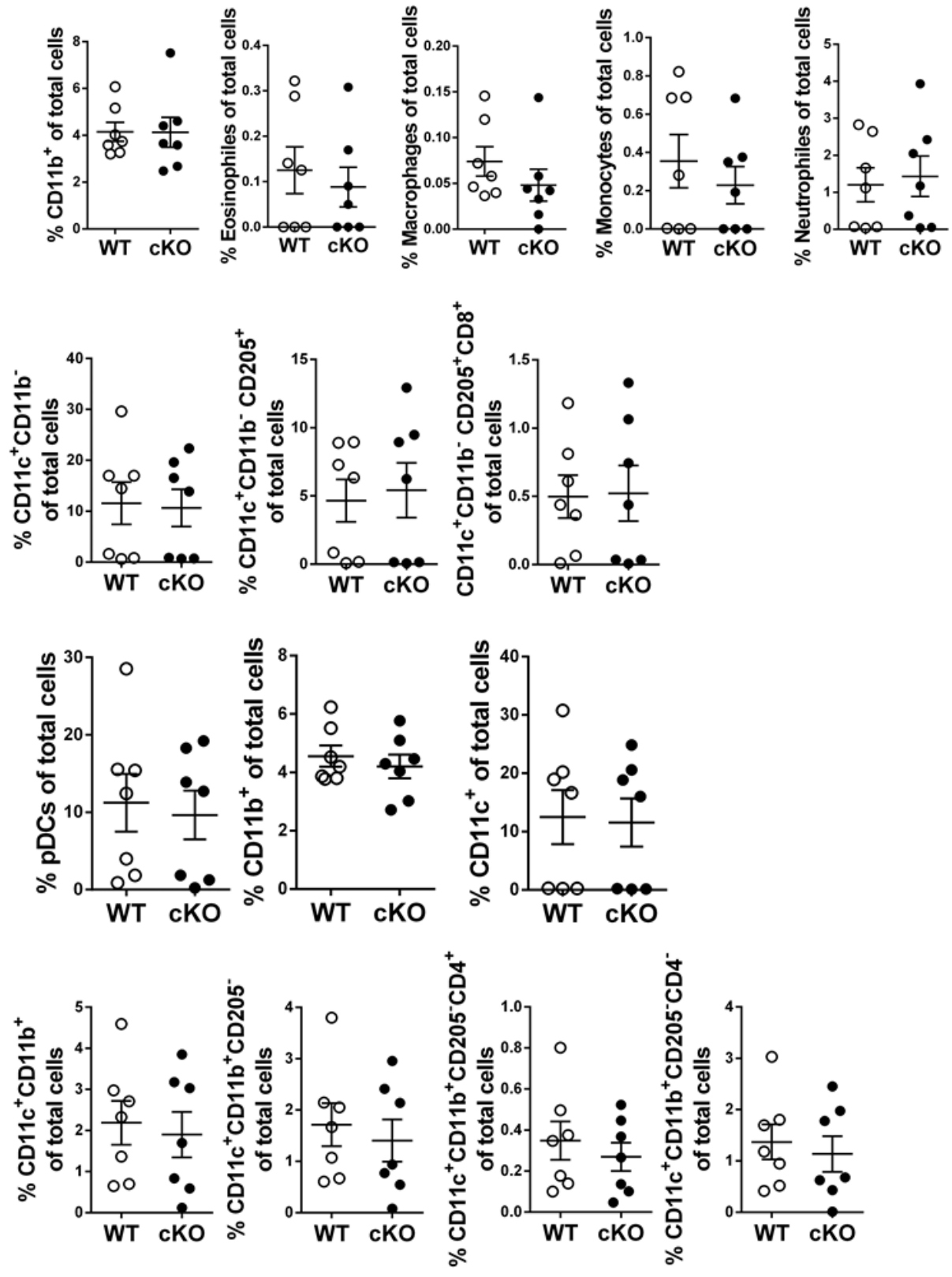
S1A.2



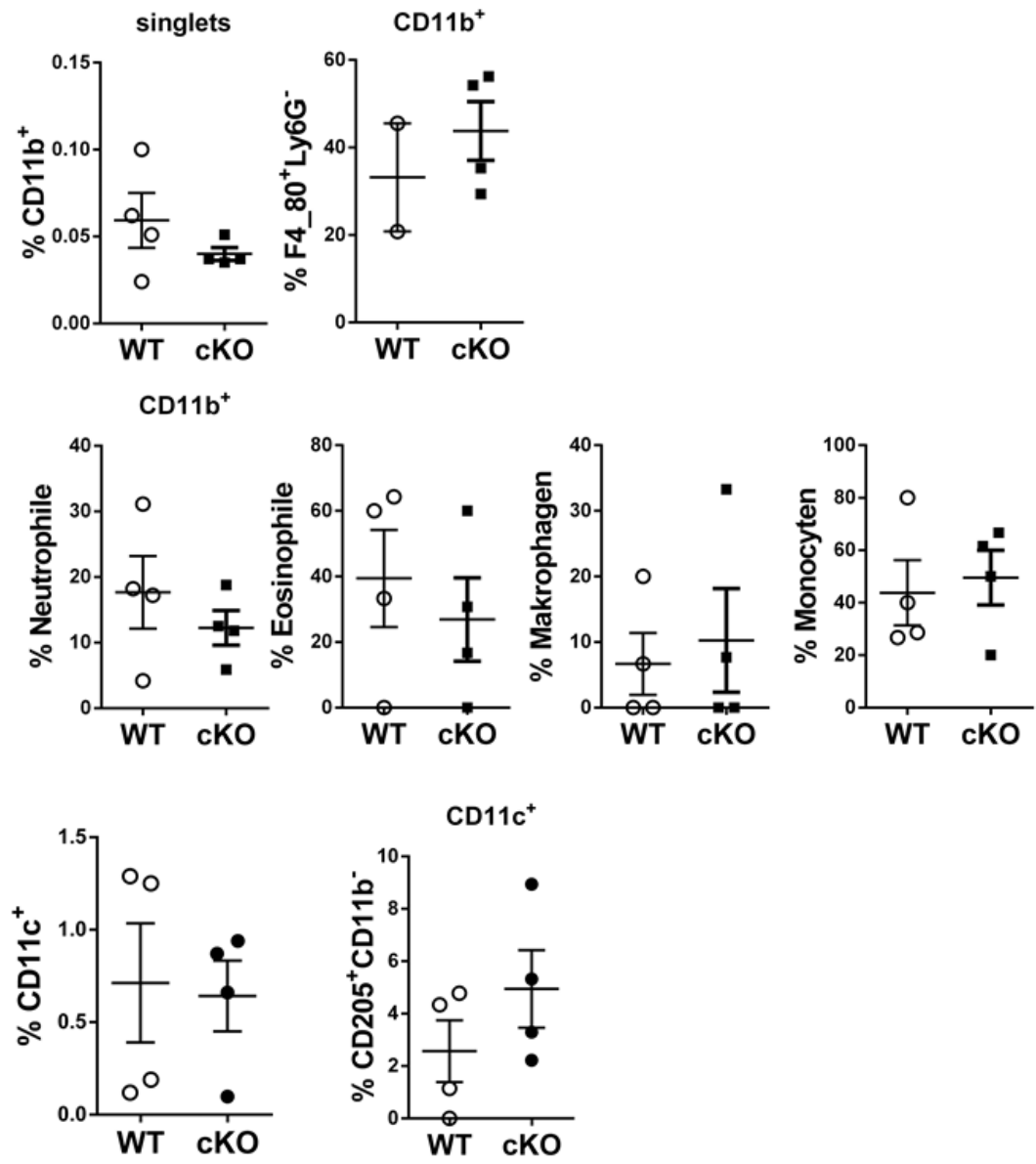
## S1B.1



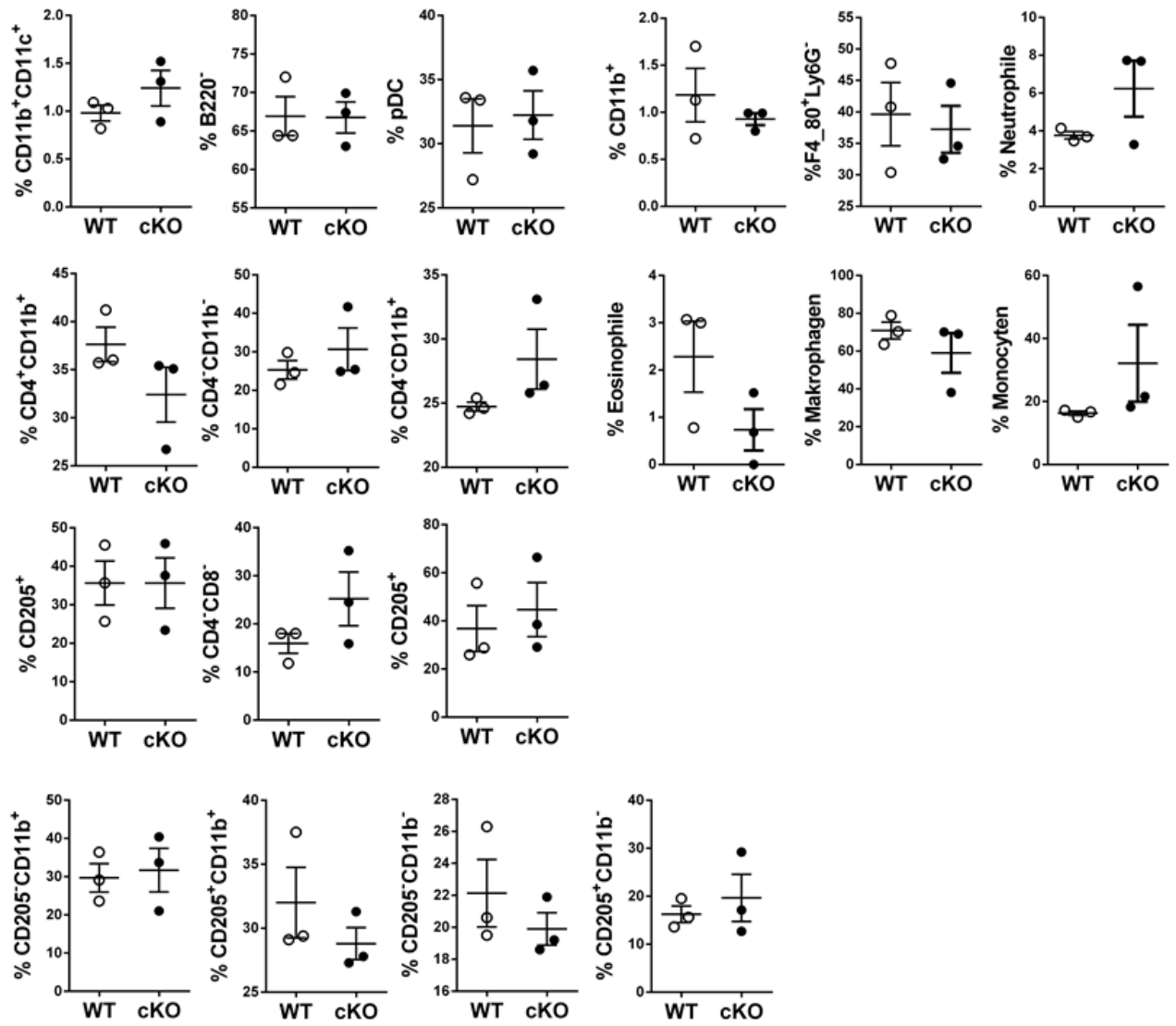
S1B.2



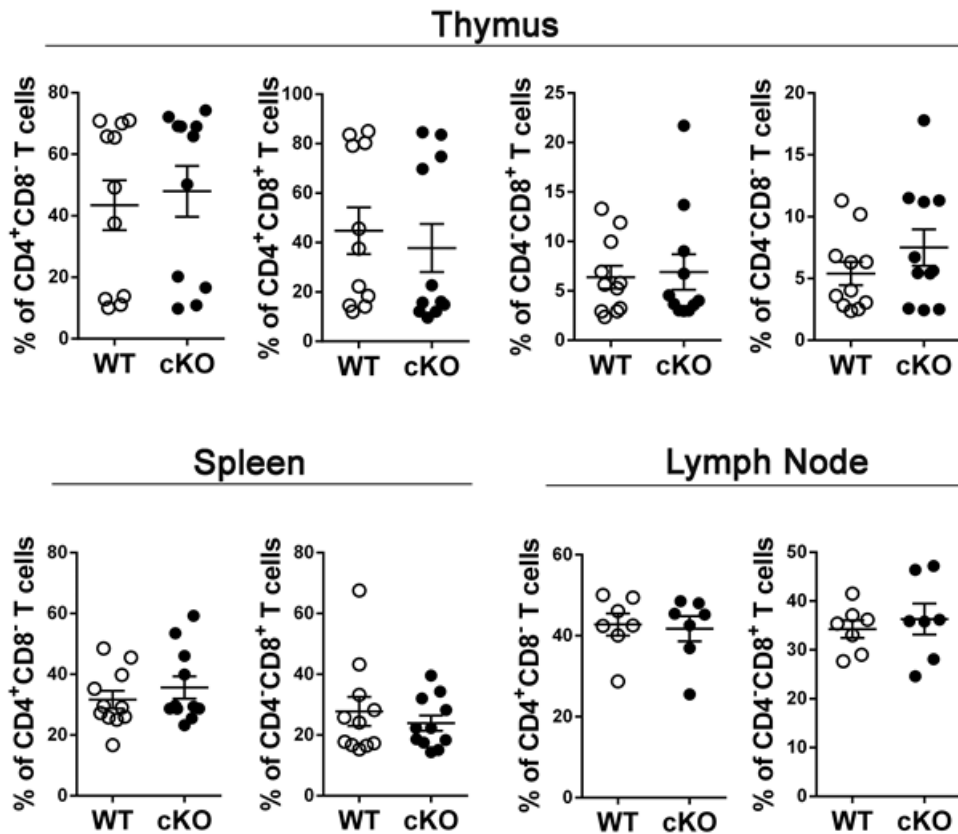
S1B.3



## S1B.4



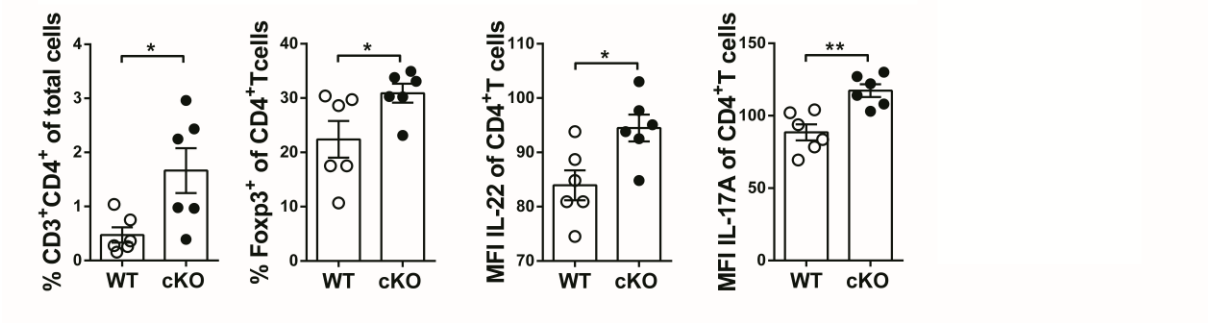
S1C



**Supplementary Figure 1. CD83cKO mice showed normal distribution of immune cells**

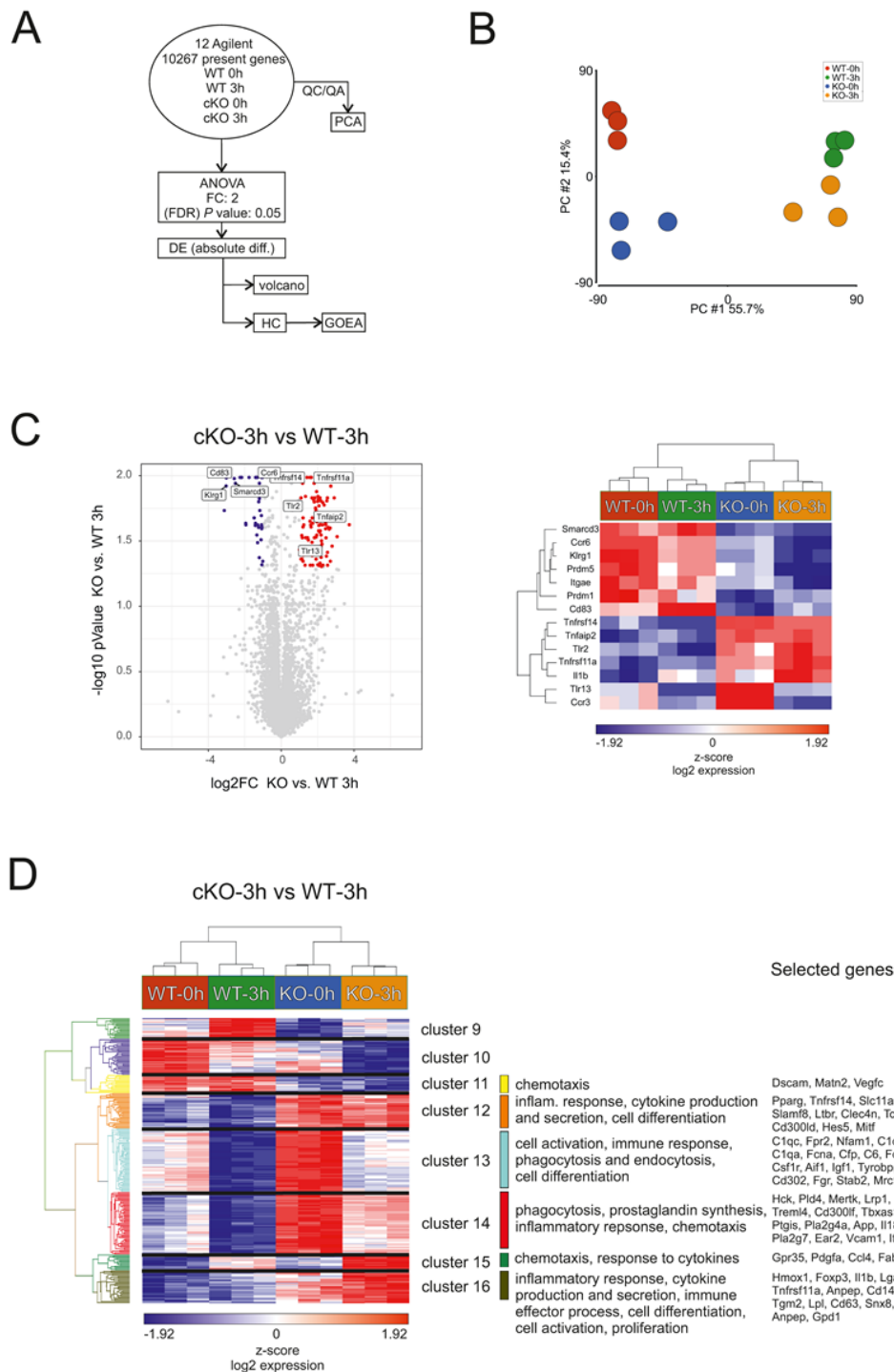
(A.1) Identification of Foxp3<sup>+</sup> Treg cells using GFP fluorescence in control (DEREG) animals and YFP fluorescence cKO mice (CD83cKO) (A.2) CD83 expression in stimulated splenic CD4<sup>+</sup> T cells (B.1) Flow cytometry analyses of bone marrow, staining for B cells (B.2) spleen, staining for dendritic cells, monocytes, eosinophils, neutrophils and macrophages (B.3) thymus and (B.4) lymph node cells staining for dendritic cell and monocyte numbers. (C) FACS analysis regarding the CD4<sup>+</sup>:CD8<sup>+</sup> T cell ratio in spleen, thymus and peripheral lymph nodes.

S2



**Supplementary Figure 2. The migration to the CNS is not impaired in cKO Tregs.**

Flow cytometry analyses of brain, staining for CD4<sup>+</sup> T cells, Foxp3 T cells and the cytokines IL-22 and IL-17A.



**Supplementary Figure 3. CD83 deletion on Tregs leads to highly differentiated gene expression**

(A) Schematic description of the Microarray analysis workflow of cKO Tregs and DERE (WT) Tregs. (B) Principal component analysis (PCA) using all 10,267 present transcripts was performed in Partek Genomics Suite (PGS) using default settings, and principle component 1 (PC1) and PC2 are plotted in a two-dimensional graph. (C) Volcano plot of all present genes comparing stimulated cKO T cells versus stimulated WT Tregs. Interesting differentially



expressed genes has been highlighted. Clustering of differentially expressed genes. **(D)** Hierarchical clustering of genes differentially expressed ( $p\text{-value} \leq 0.05$  and fold change  $\geq 2$ ) comparing stimulated cKO T cells versus stimulated WT Tregs.

**Supplementary table 1. Oligonucleotide primer**

Name		Sequence	Annealing temperature
CD83	for	CGCAGCTCTCCTATGCAGTG	61°C
	rev	GTGTTTTGGATCGTCAGGGAATA	61°C
Foxp3	for	CCCAGGAAAGACAGCAACCTT	61°C
	rev	CCTTGCCTTTCTCATCCAGGA	61°C
GATA3	for	CCAAGCGAAGGCTGTCGGCA	61°C
	rev	TCCTCCAGCGCGTCATGCAC	61°C
HPRT	for	GTTGGATACAGGCCAGACTTTGTTG	61°C
	rev	GATTCAACTTGCGCTCATCTTAGGC	61°C
IFN $\gamma$	for	GCTTTGCAGCTCTTCCTCAT	61°C
	rev	GTCACCATCCTTTTGCCAGT	61°C
IL-1 $\beta$	for	TGCCACCTTTTGACAGTGATG	61°C
	rev	ATGTGCTGCTGCGAGATTTG	61°C
IL-6	for	ACAAAGCCAGAGTCCTTCAGAG	61°C
	rev	GAGCATTGGAAATTGGGGTAGG	61°C
IL-10	for	CCAAGCCTTATCGGAAATGA	61°C
	rev	TTTTCACAGGGGAGAAATCG	61°C
IL-17A	for	TTTAACTCCCTTGGCGCAAAA	61°C
	rev	CTTTCCTCCGCATTGACAC	61°C
Tbet	for	AGCAAGGACGGCGAATGTT	61°C
	rev	GGGTGGACATATAAGCGGTTC	61°C
TGF $\beta$	for	TGGAGCAACATGTGGA ACTCTA	61°C
	rev	AGACAGCCACTCAGGCGTATC	61°C

**Supplementary table 2. NFAT Expression Analysis**

Gene Name	Nfatc1	Nfatc2	Nfatc2ip	Nfatc3
Fold-Change (cKO vs. WT)	-1.11833	-1.18238	-1.05015	-1.25807
p-value (cKO vs. WT)	0.508406	0.0152669	0.736368	0.255363

Microarray analysis data. Differential expression analysis of Nfat genes by ANOVA showed significant downregulation of Nfatc2 in cKO Treg cells. The other Nfat members were not affected significantly.