cells

MDPI

Article

β2 integrins on dendritic cells modulate cytokine signaling and inflammation-associated gene expression, and are required for induction of autoimmune encephalomyelitis

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DEFINITION B044.2 rotated to position 10485

ACCESSION B044.2

REFERENCE 1 (bases 1 to 13010)

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12841 ACTTTATGCT TCCGGCTCGT ATGTTGTGTG GAATTGTGAG CGGATAACAA TTTCACACAG

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Figure S1. Structure and sequence of CD18 gene locus exon 3 targeting vector B044.2. B044.2 consists of the pGEM-T Easy vector (Promega, Madison, WI). The CD18 gene locus encompassing insert was derived from the BAC clone RP23-95M21 (222.chori.org/bacpac). Part of intron 2 and total exon 3 were cloned into the AgeI site of pGEM-T Easy. The FRT-neomycin resistance-SV40-FRT cassette was cloned downstream of exon 3 (AgeI and HindIII/SnaBI restriction sites). Sequences comprising exons 4-6 were cloned into SnaBI site. The thymidine kinase expression cassette was cloned into the AscI site at the 3′end of exon 6. The 5′ loxP site is located upstream of exon 3 and the 3′ loxP site adjacent to the FRT-neomycin resistance-SV40-FRT cassette upstream of exon 4.

Table S1. Antibodies used for flow cytometric analysis

Antibody specificity	Clone	Fluorescence label
CD3	145-2C11, 500A2	eFl506, PE-Cy5, APC
CD4	GK1.5, RM4-5	eFl450, FITC, AF700, APC-Cy7
CD8	07.06.53	eFl450, FITC, BV510, PE-Cy5, APC-Cy7
CD11a	M17/4	PE-Cy7
CD11b	M1/70	PE, PE-Cy5, PE-Cy7
CD11c	N418	APC, PE-Cy7
CD18	M18/2	FITC, APC
CD19	1D3	PE-Cy5, SB702, APC, APC-Cy7
CD25	7D4	FITC
CD40	1C10	APC
CD45	30-F11, A20	FITC, SB702
CD62L	MEL-14	APC
CD68	FA-11	FITC, PE
CD69	H1.2F3	PE
CD80	16-10A1	PE
CD86	GL1	FITC
CD103	2,00E+007	PE
CD172a	P84	PE-Cy7
CD207	eBioL31	PE
CD317	9,27E+002	BV711
F4/80	BM8, T45-2342	e-Flour450, BB790
Foxp3	FjK-16a	FITC
GATA-3	TWAJ	PE
GR-1	RB6-8C5	APC
Ly6-C	AL-21, HK1.4	FITC, BV570, APC
Ly6-G	1A8	e-Flour450
MHCII	M5/114.15.2	eFl450, PE-Cy5
NK1.1	PK136	PE-Cy5, APC
phospho-STAT1	A15158B	PE
phospho-STAT3	13A3-1	AF488
phospho-STAT5	12-9010-42	PE
ROR-γt	AFKJS-9	APC
T-bet	eBio4B1	PerCP-Cy5.5
XCR1	ZET	BV650

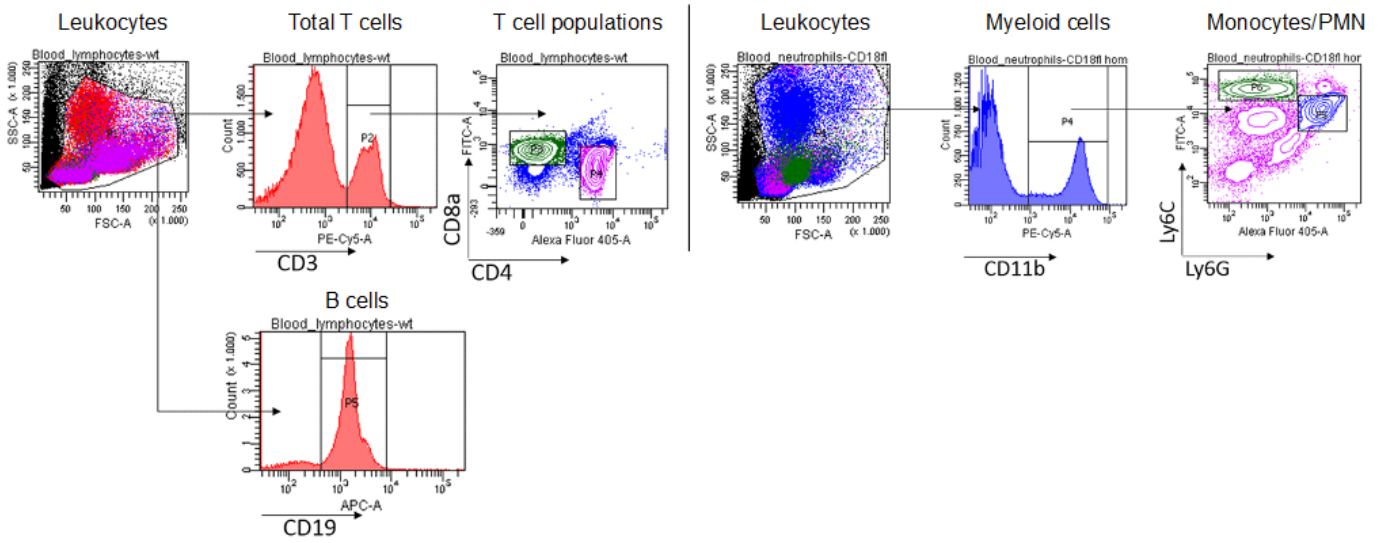

Figure S2. Gating strategy of leukocyte populations in blood (Attune NxT flow cytometer).

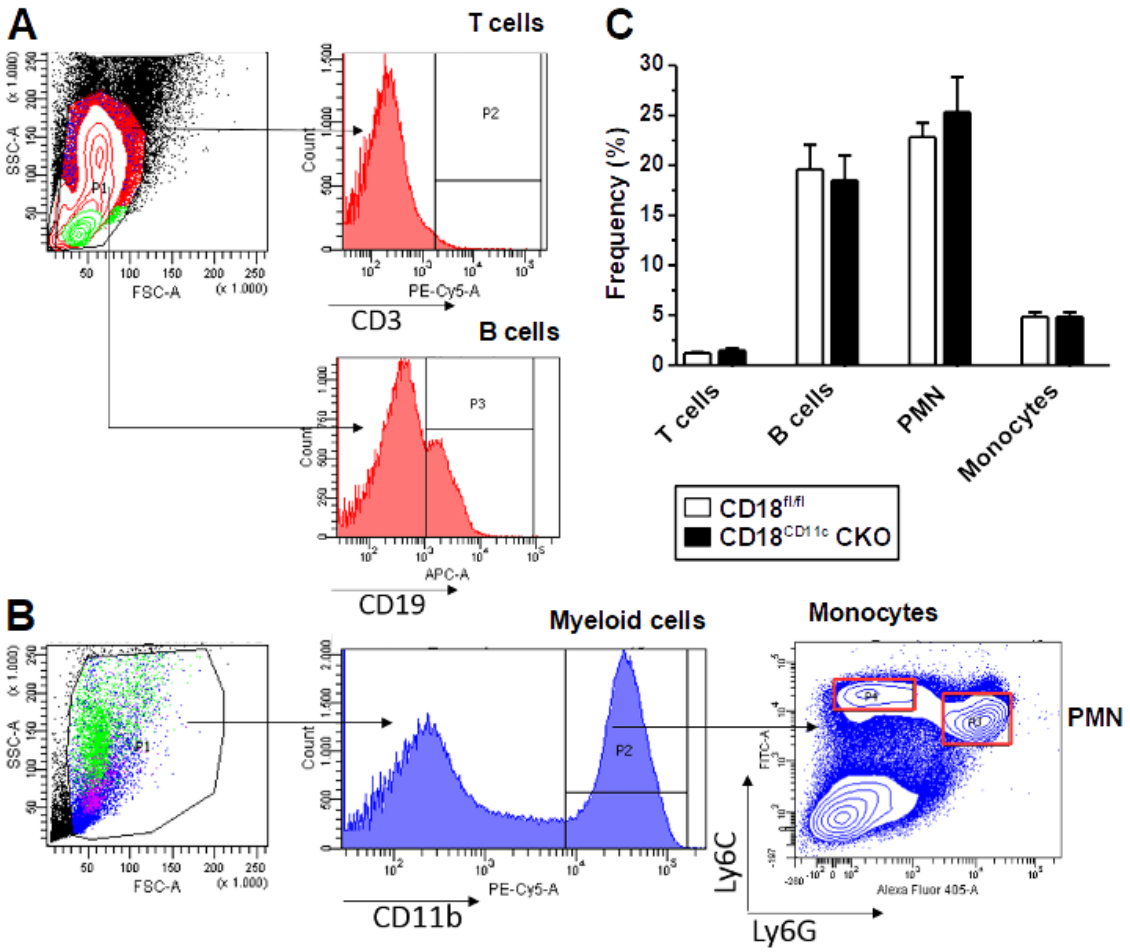

Figure S3. Frequencies of leukocyte populations in bone marrow are unaltered in $CD18^{CD11c}$ cKO mice. (**A**, **B**) Gating strategies of (**A**) B cells and T cells as well as of (**B**) polymorphonuclear granulocytes [PMN] and monocytes as assessed by flow cytometry are shown. (**C**) Frequencies of either cell type in bone marrow are shown (mean±SEM of 4 experiments).

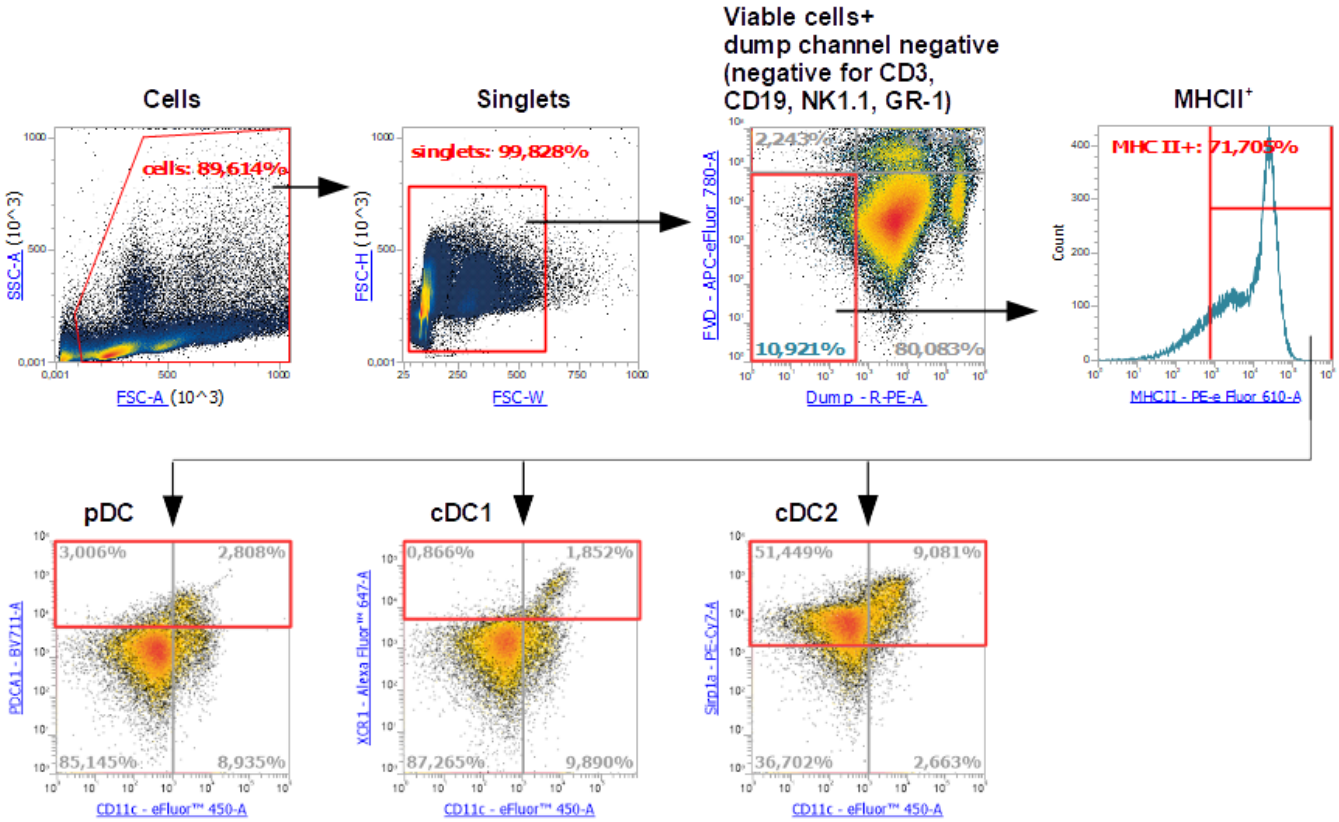

Figure S4. Gating strategy for splenic DC populations (Attune NxT flow cytometer).

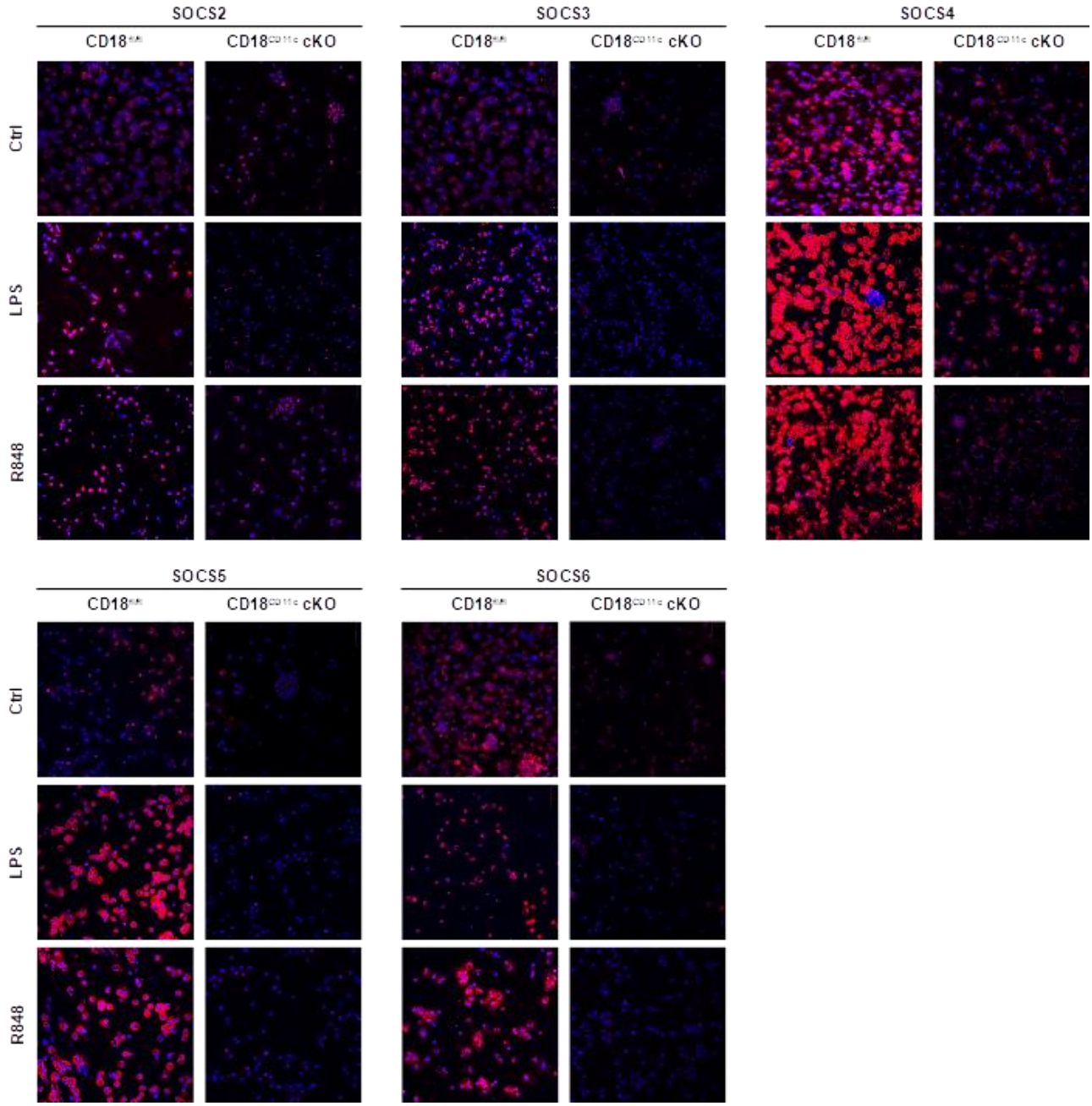

Figure S5. BMDC lacking β2 integrins display disturbed regulation of SOCS protein expression. BMDC ($CD18^{fl/fl}$, $CD18^{CD11c}$ cKO) were stimulated overnight in parallel with LPS and R848 or were left untreated (Ctrl). On the next day, SOCS protein expression was assessed on cytospins by immunofluorescence (AF647; red) and nuclei were stained with Hoechst dye (blue) (experimental details given in 2.10.). Pictures are representative of 3-4 experiments each.

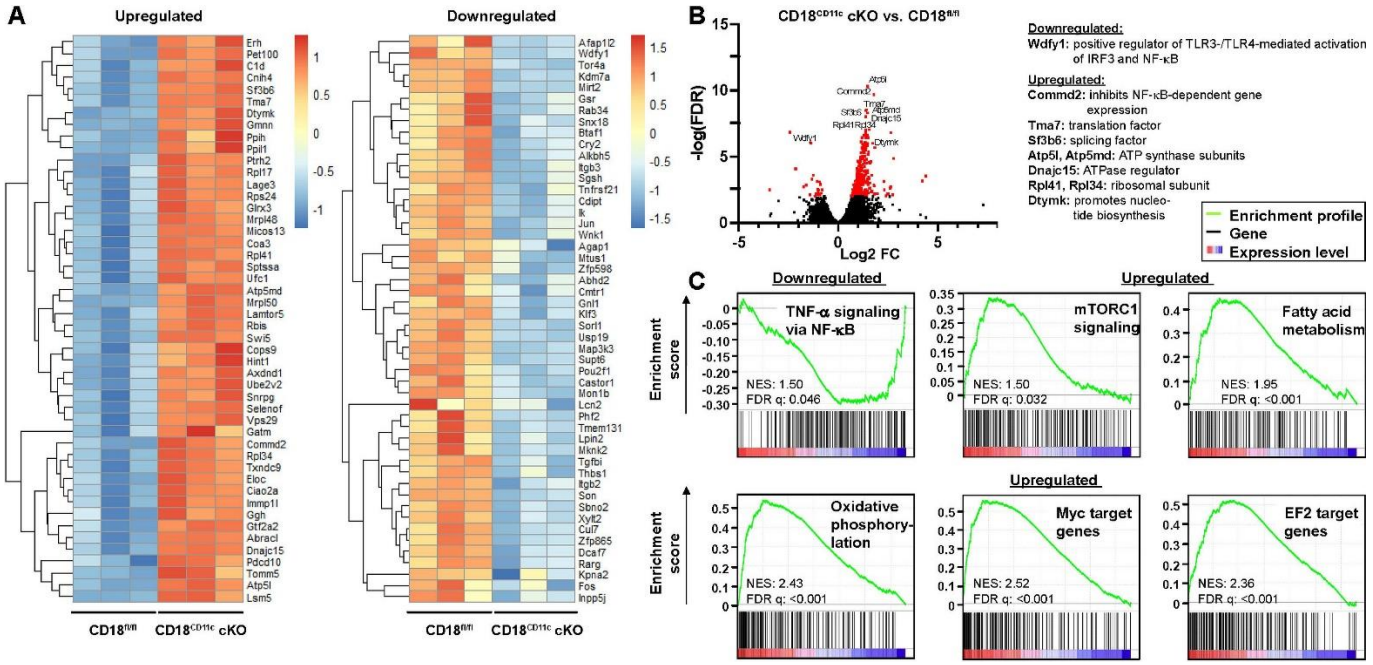

Figure S6. Downregulation of β2 integrins in BMDC results in attenuated expression of genes associated with inflammatory signaling and upregulation of genes involved in metabolic pathways, and transcription factor Myc/EF2 targets. Unstimulated BMDC ($CD18^{fl/fl}$, $CD18^{CD11c}$ cKO, each n=3) were subjected to RNA-seq analysis. (A) Heatmap representation of the Top 50 genes significantly upregulated (left panel) and downregulated (right panel) in CD18CD11c cKO versus $CD18^{fl/fl}$ BMDC clustered by hierarchical clustering. The color legend denotes the level of gene expression (low: blue, high: red) and represents z-scores. (B) Left panel: Volcano plot of all quantified mRNA species. Significantly regulated genes (t-test q-value < 0.05 and log2[fold-change] > 2) are given in red. Top 10 genes are named. Right panel: Functional role of Top 10 genes. (C) Gene set enrichment plots of significantly regulated pathways (FDR adjusted q-values <0.05). The normalized enrichment score (NES) and FDR q-values are given.

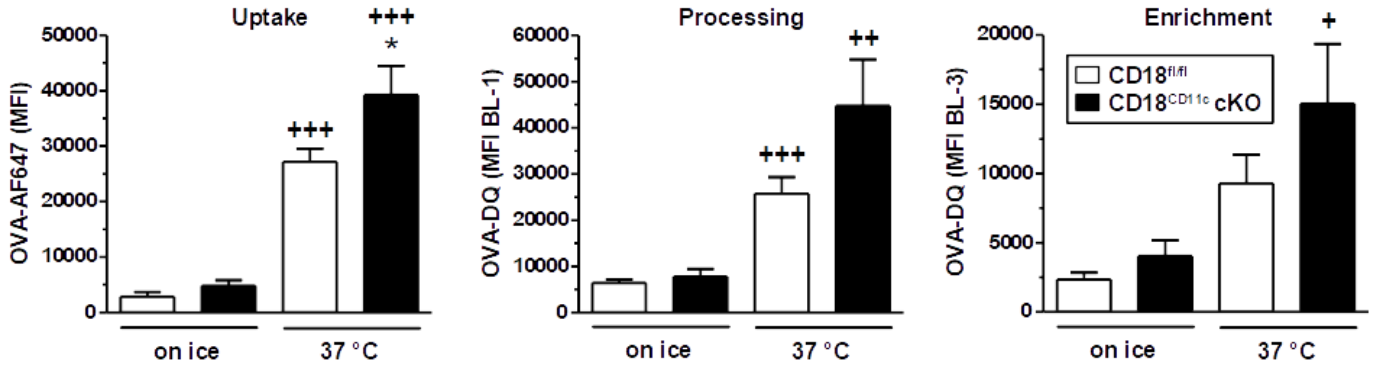

Figure S7. β2 integrin-deficient BMDC display higher antigen uptake and processing capacity. BMDC were kept on ice or at 37 °C, and OVA derivatives (OVA-AF647, OVA-DQ; each 25 μg/ml) were applied for 1h. OVA uptake (left panel), processing (middle panel) and endo-/lysosomal enrichment of processed OVA in MHCII-expressing cells were assessed by flow cytometry. Data denote the MFI (mean±SEM of 6-9 experiments). Statistical differences versus *$CD18^{fl/fl}$ and $^{+}$versus according control group (on ice) are indicated (one way ANOVA, Tukey test). $^{*,+}p<0.05$, $^{++}p<0.01$, $^{+++}p<0.001$.

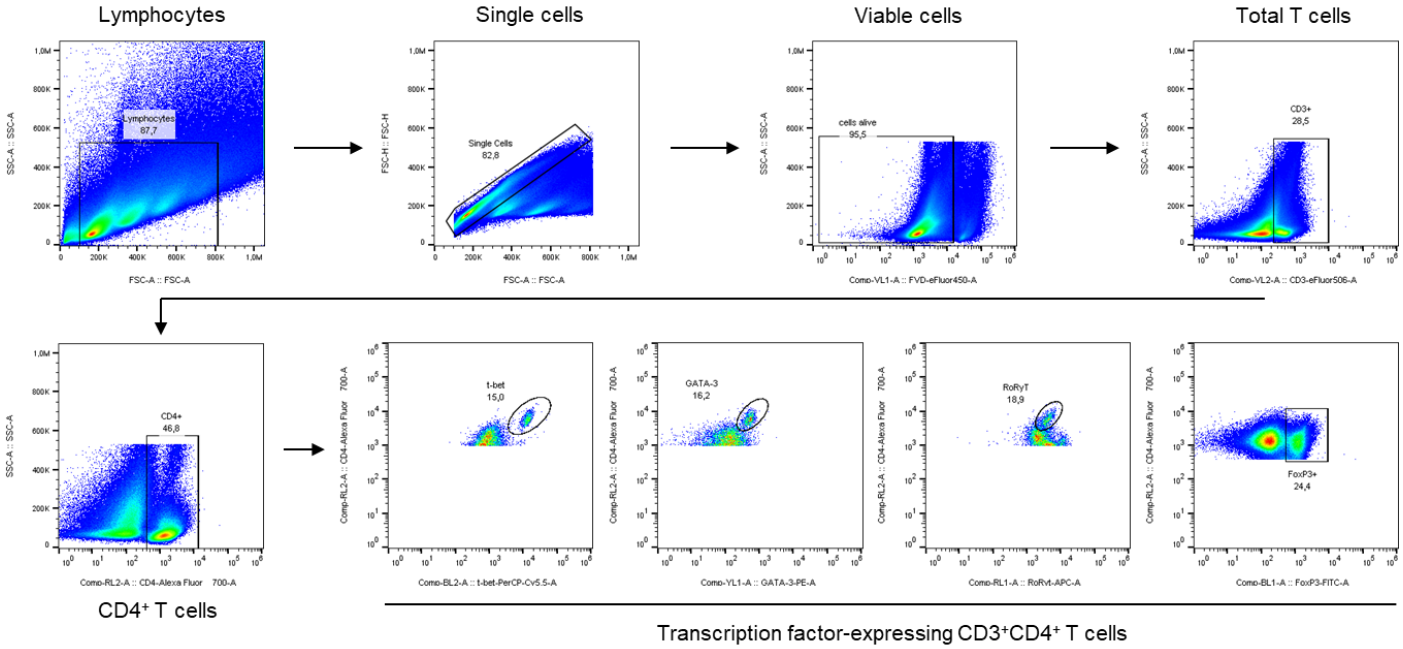

Figure S8. Gating strategy for intracellular detection of transcription factors in CD4+ T cells in spleen, lymph node and spinal cord (FACSymphony).

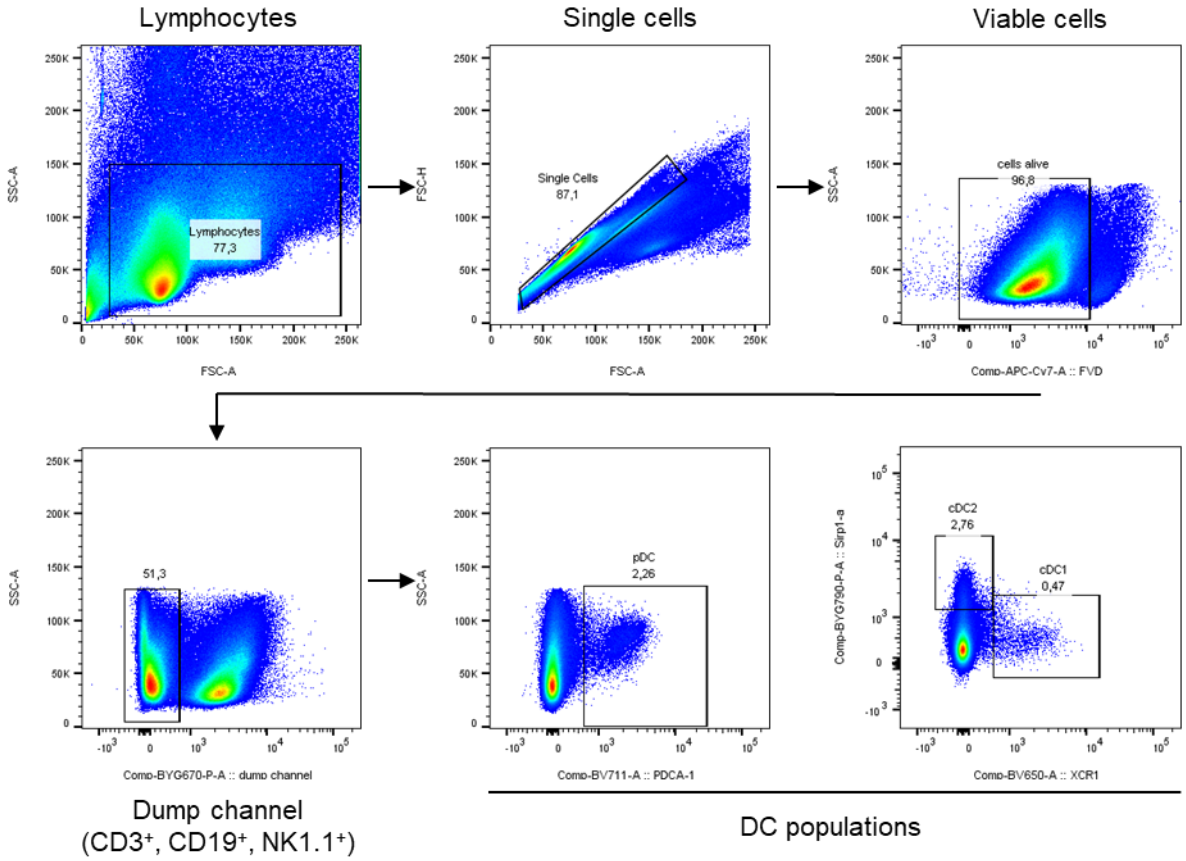

Figure S9. Gating strategy for detection of DC populations in spleen, lymph node and spinal cord (FACSymphony).

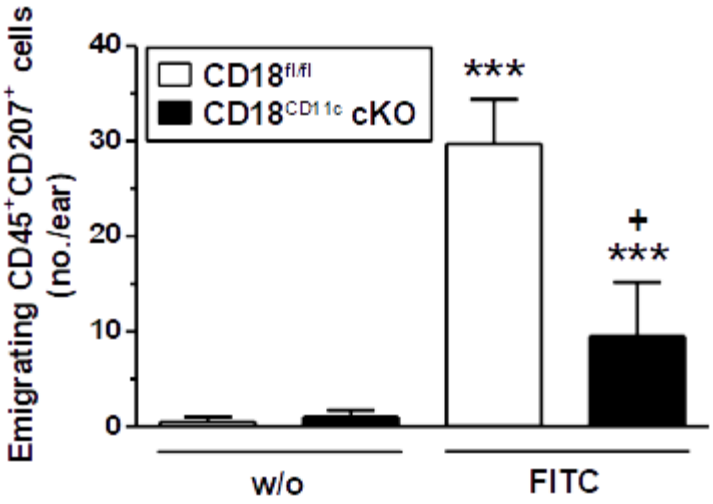

Figure S10. CD207^{+} DC of CD18^{CD11c} cKO mice emigrate at lower extent from ears after FITC application. Mouse ears were split, both halves were placed into 12-well plates (1 ml of IMDM-based culture media) with the outside upwards. Then, 5 µl of FITC solution (fluorescein isothiocyanate, 5 mg/ml) dissolved in 1:1 (v/v) acetone:dibutylphalate (all from sigma-Aldrich) were applied to half of all samples. On the next day, frequencies of CD45^{+}CD207^{+} DC in culture media was assessed by flow cytometry (mean ±SEM, n=4). Statistical differences versus *untreated (w/o) and $^{+}$CD18$^{fl/fl}$ are indicated (one way ANOVA, Tukey test). $^{+}p<0.05$, ***$p<0.001$.

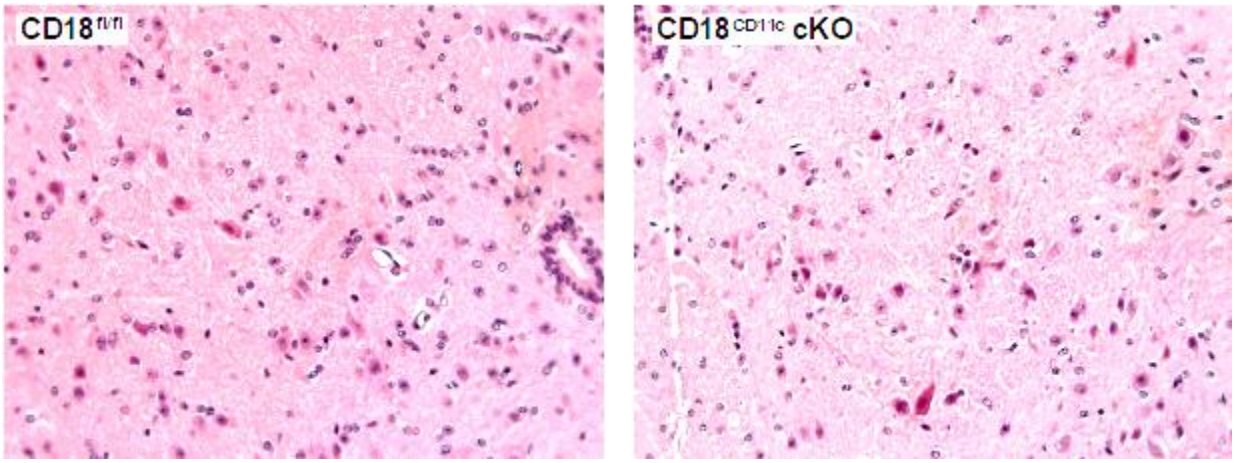

Figure S11. Spinal cord of CD18^{CD11c} cKO mice displays somewhat lower leukocyte infiltration. Spines were retrieved on d38 after the onset of EAE and stored in 10% paraformaldehyde for histopathological analysis. Paraffin-embedded blocks were prepared, and derived sections (5 µm) were stained with hematoxylin and eosin (H&E) to assess infiltration of leukocytes. For this, H&E-stained sections were examined microscopically using a BX40 microscope equipped with a CCD camera (both from Olympus, Hamburg, Germany) for total cells per field of view (CD18$^{fl/fl}$: 338; CD18^{CD11c} cKO: 264). Pictures are representative of samples obtained from two animals each.