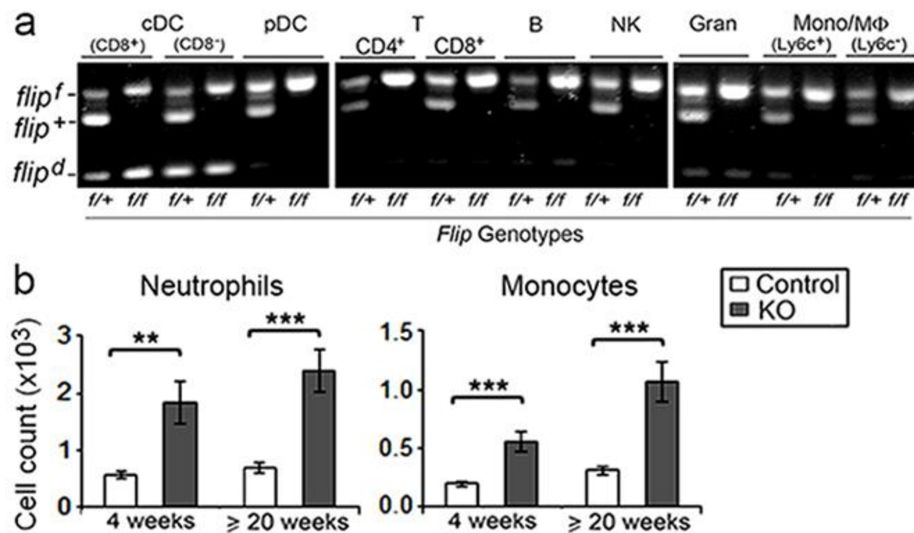
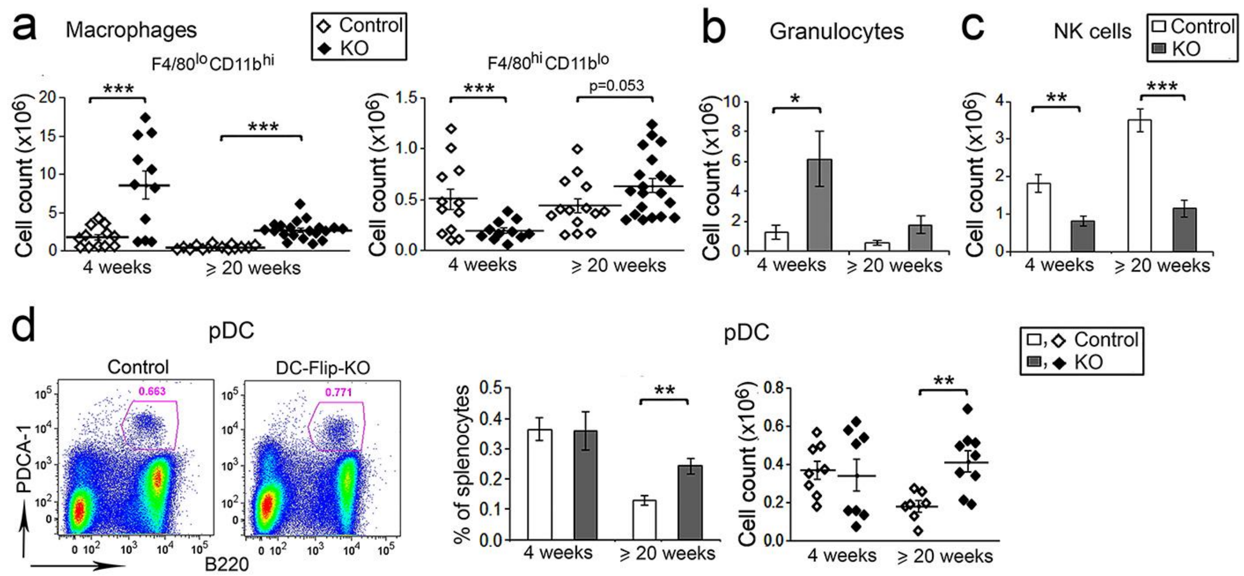
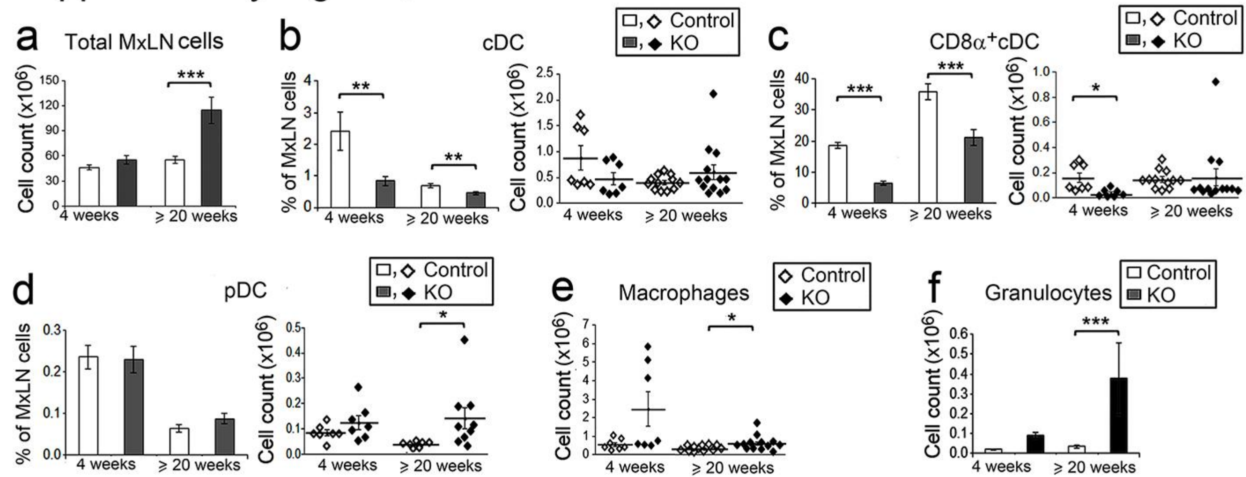


Supplementary Figure 1. Generation of mouse line with conditional deletion of Flip in cDCs results in increased circulating neutrophils and monocytes. (a) PCR genotyping for *Flip*, employed 3 primers: (1) 5'-CTT TTG GCT TTT GGA TCA GTC ATT-3', (2) 5'- GGA ACC ACG AGA AGC CAA CAT-3' and (3) 5'-TCT CTA ACT CAT TCA GCT TCA ACC ACC A-3'. Alleles of *Flip* include the wild-type ($Flip^{+}$, 220bp, primers 1 and 2), the floxed ($Flip^{f}$, 270bp, primers 1 and 2) and the deleted ($Flip^{d}$, 150bp, primers 1 and 3). CD11c directed floxed *Flip* deletion in different cell types was examined employing cells purified from spleens of mice genotyped as $Flip^{flox/+}$ $CD11c^{cre}$ (control) or $Flip^{flox/flox}$ $CD11c^{cre}$ (KO). **(b)** Neutrophils and monocytes were identified from complete blood counts (n = 14-16 for 4 weeks and 8-11 for ≥20 weeks). The data are presented as the mean ±1 SE. (** $p < 0.01$ and *** $p < 0.001$, unpaired 2 sided *t*-test).

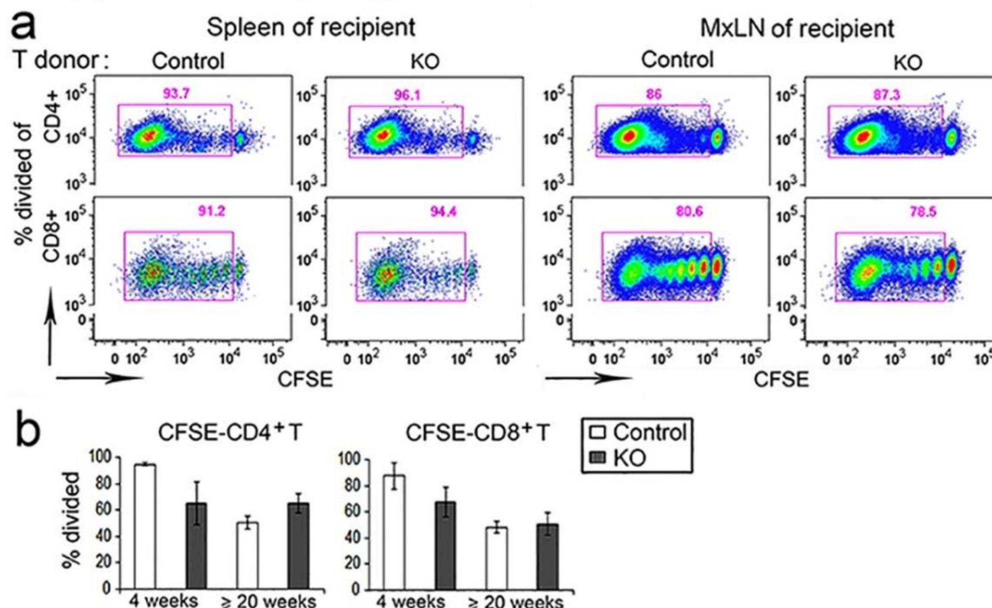
Supplementary Figure 2



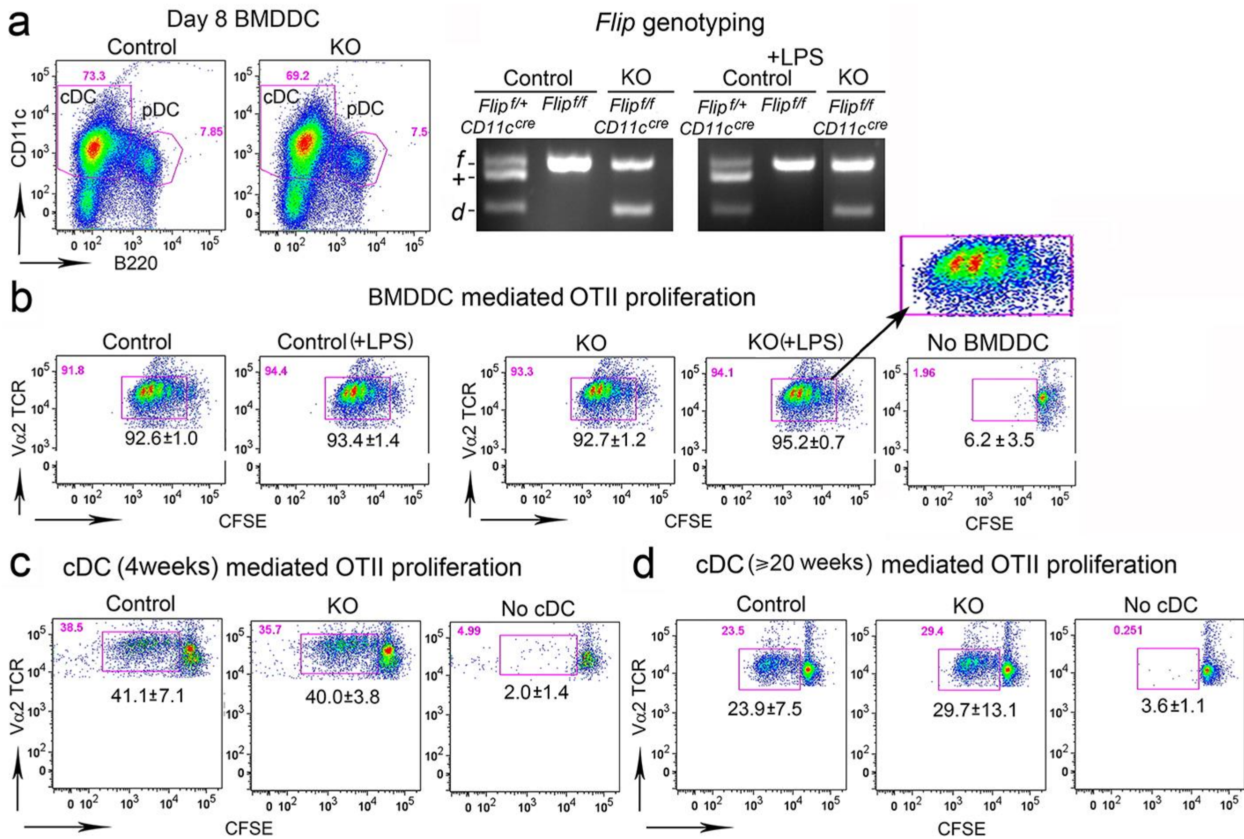
Supplementary Figure 2: Alteration of cells in the spleen of CD11c-Flip-KO mice. (a) Flow cytometry was employed to identify macrophages, defined as CD64^{+}F4/80^{lo}CD11b^{hi} or CD64^{+}F4/80^{hi}CD11b^{lo} ,**(b)** granulocytes (9-20 per group), defined as CD64^{-}CD11b^{hi}Ly6G^{+}, **(c)** NK cells (n = 5 per group), defined as TCRαβ^{-}CD11c^{lo}NK1.1^{+}DX5^{+} and **(d)** plasmacytoid DCs (pDC), defined as CD64^{-}PDCA-1^{+}B220^{+}CD11c^{lo}, employing CD11c-Flip-KO (KO) or control mice. The data are presented as the mean ±1 SE. (** represents $p < 0.01$ and *** $p < 0.001$, unpaired 2-sided *t*-test).



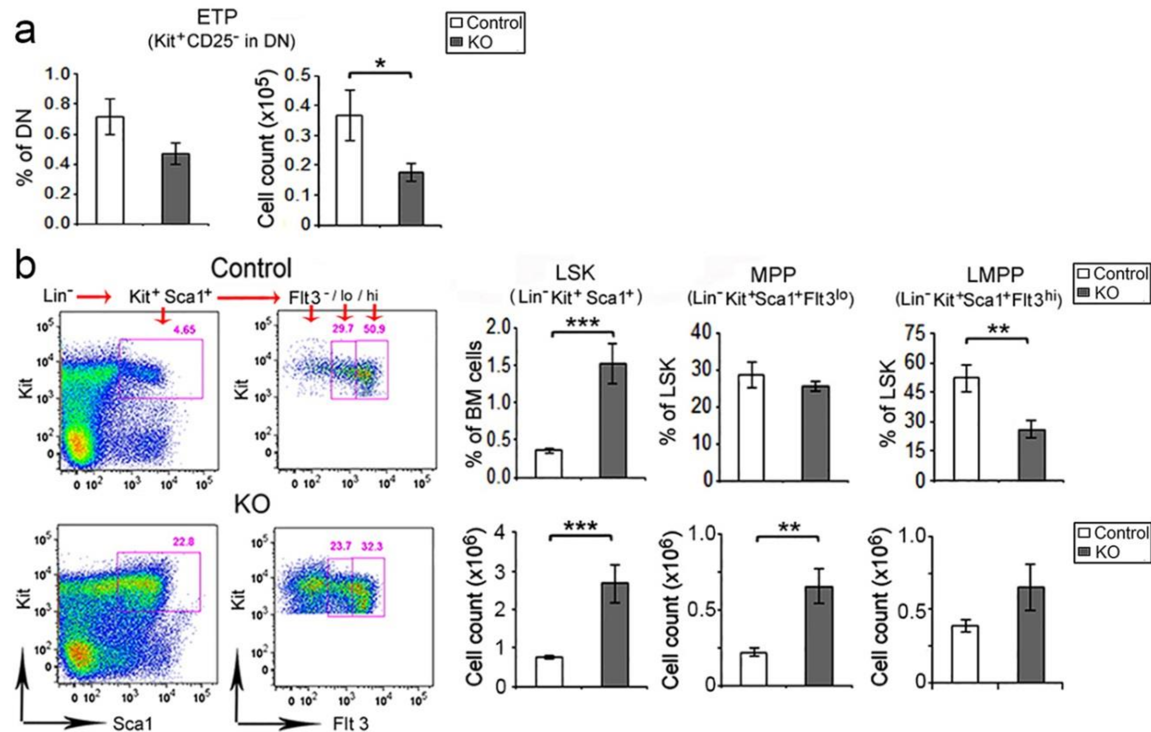
Supplementary Figure 3: Alteration of cells in the MxLN of CD11c-Flip-KO mice. (a) The number of cells in the mixed lymph nodes (MxLNs) (n = 24-29 per group), a mixture of cervical, brachial, axillary, and inguinal LNs, were increased at $\geq$ 20 weeks in the CD11c-Flip-KO (KO) mice. **(b-g)** MxLN cells analyzed by flow cytometry for **(b)** total CD11c$^+$ DC, **(c)** the CD8α$^+$ cDC subset, **(d)** pDC, **(e)** macrophages, defined as CD64$^+$F4/80$^+$CD11b$^+$, and **(f)** granulocytes (n = 4 at 4 weeks and 13-16 at $\geq$ 20 weeks). The data are presented as the mean ±1 SE. (* represents $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, unpaired 2-sided *t*-test).



Supplementary Figure 4: Normal CD11c-Flip-KO T cell expansion and proliferation. **(a)** The homeostatic proliferation of total T cells from DC-Flip-KO (KO) or littermate controls was examined *in vivo* by adoptive transfer into *Rag*$^{-/-}$ mice, employing CFSE-labelled T cells (10-20x10^6 200µl^{-1} PBS), supplemented with B cells (5x10^6). Data shown are representative for spleen (left) and MxLNs (right) from the recipients collected 6 days after transfer. The results are representative of 3 independent experiments. **(b)** *In vitro* anti-CD3/CD28 induced T cell proliferation. CFSE-labelled T cells were from the spleens of DC-Flip-KO or littermate control mice (n= 3-4 per group). They were cultured with plate bound anti-CD3 monoclonal antibody (1µg ml^{-1}) in the presence of anti-CD28 (1µg ml^{-1}) for 3 days. T cell activation was assessed by the percent reduction of CFSE in the $CD4^+$ or $CD8^+$ cell populations, determined by flow cytometry. The data are presented as the mean ±1 SE.

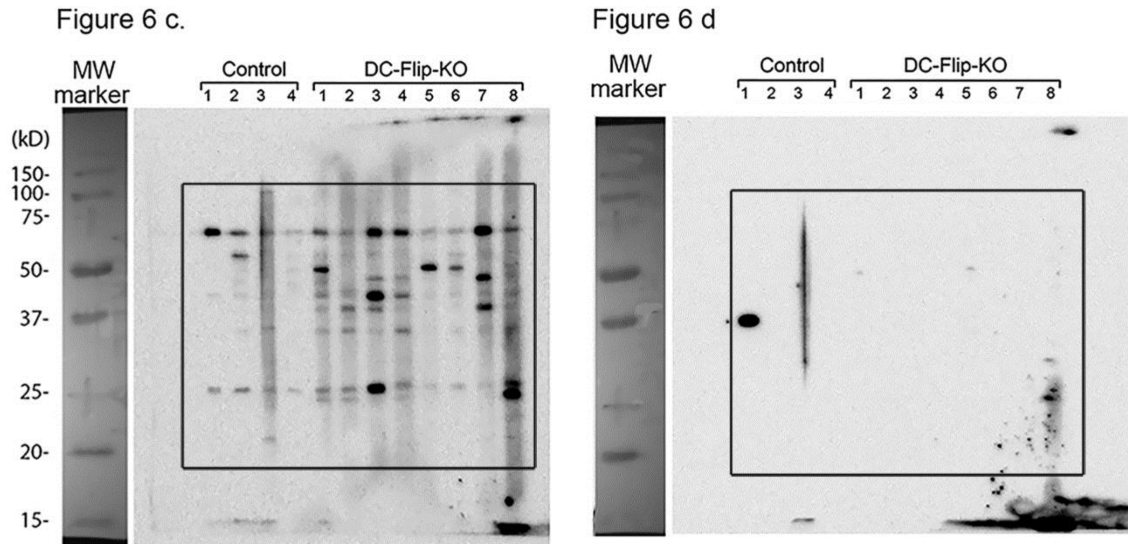


Supplementary Figure 5. No defect of antigen presentation by CD11c from DC-Flip-KO mice. (a) Bone marrow differentiated DCs (BMDDC) from control or CD11c-Flip-KO (KO) mice were gated as $CD64^{-}CD11c^{+}B220^{-}$ and pDCs are identified as $CD64^{-}CD11c^{lo}B220^{+}$ (representative of ≥ 5) The deletion of *Flip* is confirmed by PCR genotyping (right panel), as described in Supplementary Figure 1. **(b)** BMDDC were examined for their ability to present OVA peptide to $CD4^{+}$ OTII-T cells, utilizing $0.5x10^{4}$ BMDDC and $1x10^{5}$ CFSE labelled $CD4^{+}$OTII-T cells. The results are presented as mean ± SE in each representative histogram (n = 3). An example of divided cells in the gated box is enlarged. **(c-d)** $CD11c^{+}$ DCs were isolated from the spleens of 4 week **(c)** and ≥20 week **(d)** mice and employed as antigen presenting cells. Data presented are from experiments employing $2x10^{4}$ purified DCs and $1x10^{5}$ CFSE-$CD4^{+}$OTII-T cells. Proliferation of $CD4^{+}$OTII-T cells was determined by the dilution of CFSE, the % of divided cells in $CD4^{+}$ $V\alpha2^{+}$ population is presented as the mean ± SE for 3-4 mice in each age group.



Supplementary Figure 6. Progenitor cells in thymus and bone marrow. (a) Early thymic progenitors (ETP) are defined in CD4⁻CD8⁻ (DN) thymic cells and Kit⁺CD25⁻ (n = 10-11 per group) in the CD11c-Flip-KO (KO) and control mice. **(b)** Hematopoietic progenitors in the bone marrow are analyzed by Flow. The lineage antigens included CD3, CD11b, CD19, B220, Gr-1 and Ter119. The hematopoietic stem cells were identified as $Lin^-Kit^+Sca\ 1^+$ (LSK), and they give rise to downstream multipotent progenitor (MPP, $Lin^-Scal^+Kit^+Flt3^{lo}$), which in turn produce lymphoid-primed multipotent progenitor (LMPP, $Lin^-Scal^+Kit^+Flt3^{hi}$). The DC-Flip-KO and littermate control mice (n = 7 per group) were examined at 4 weeks of age. The data are presented as the mean ±1 SE. (* represents $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$, unpaired 2-sided *t*-test).

Supplementary Figure 7



Supplementary Figure 7. The full western blots in Figure 6c and 6d.

The black boxes represent the areas shown in Figure 6. The molecular weight (MW) images were taken from the same gel developed separately.