Supplementary material

Myeloid-specific deletion of Mcl-1 yields severely neutropenic mice which survive and breed in homozygous form

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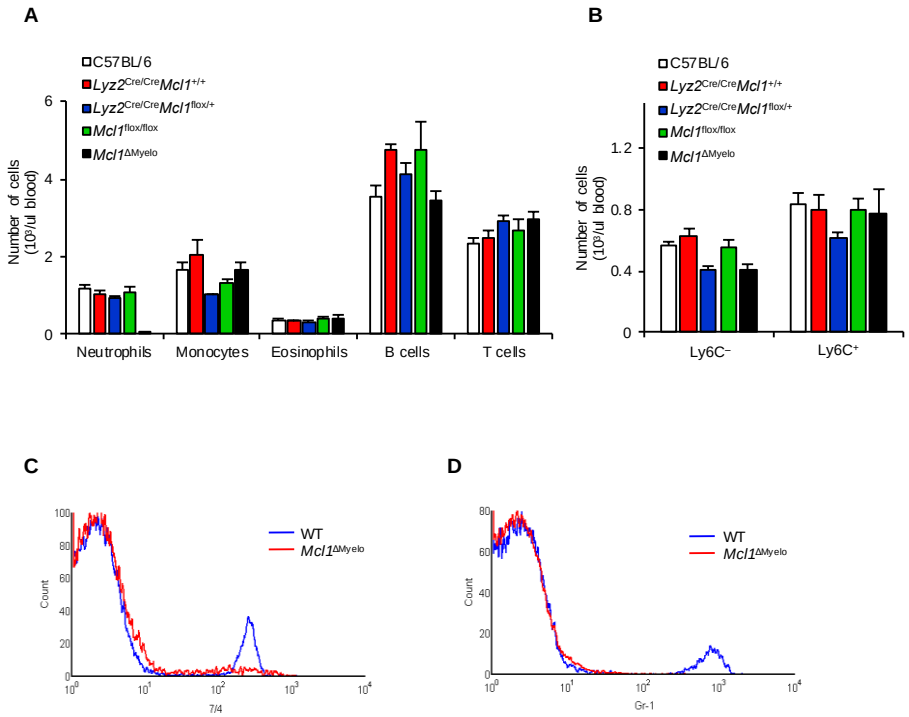

Figure S1

Analysis of peripheral leukocytes in various mutant mouse strains

Circulating neutrophils and other leukocytes were tested in C57BL/6, *Lyz2*$^{Cre/Cre}$*Mcl1*$^{+/+}$, *Lyz2*$^{Cre/Cre}$*Mcl1*$^{flox/+}$, *Mcl1*$^{flox/flox}$ and *Mcl1*$^{\Delta Myelo}$ mice by flow cytometry. (A) Quantitative analysis of the number of the indicated leukocyte subsets. (B) Quantitative analysis of the number of Ly6G^{+} and Ly6G^{-} monocyte subpopulations. (C) Flow cytometric analysis of circulating neutrophil counts in wild type (WT) or *Mcl1*$^{\Delta Myelo}$ mice stained with 7/4 (C) or RB6-8C5 (Gr-1; D) antibodies. Quantitative data show mean and SEM from, and flow cytometry histograms are representative of, 8-22 (A), 8-13 (B) or 2 (C, D) mice per group from 3 (A,B) or 2 (C, D) independent experiments.

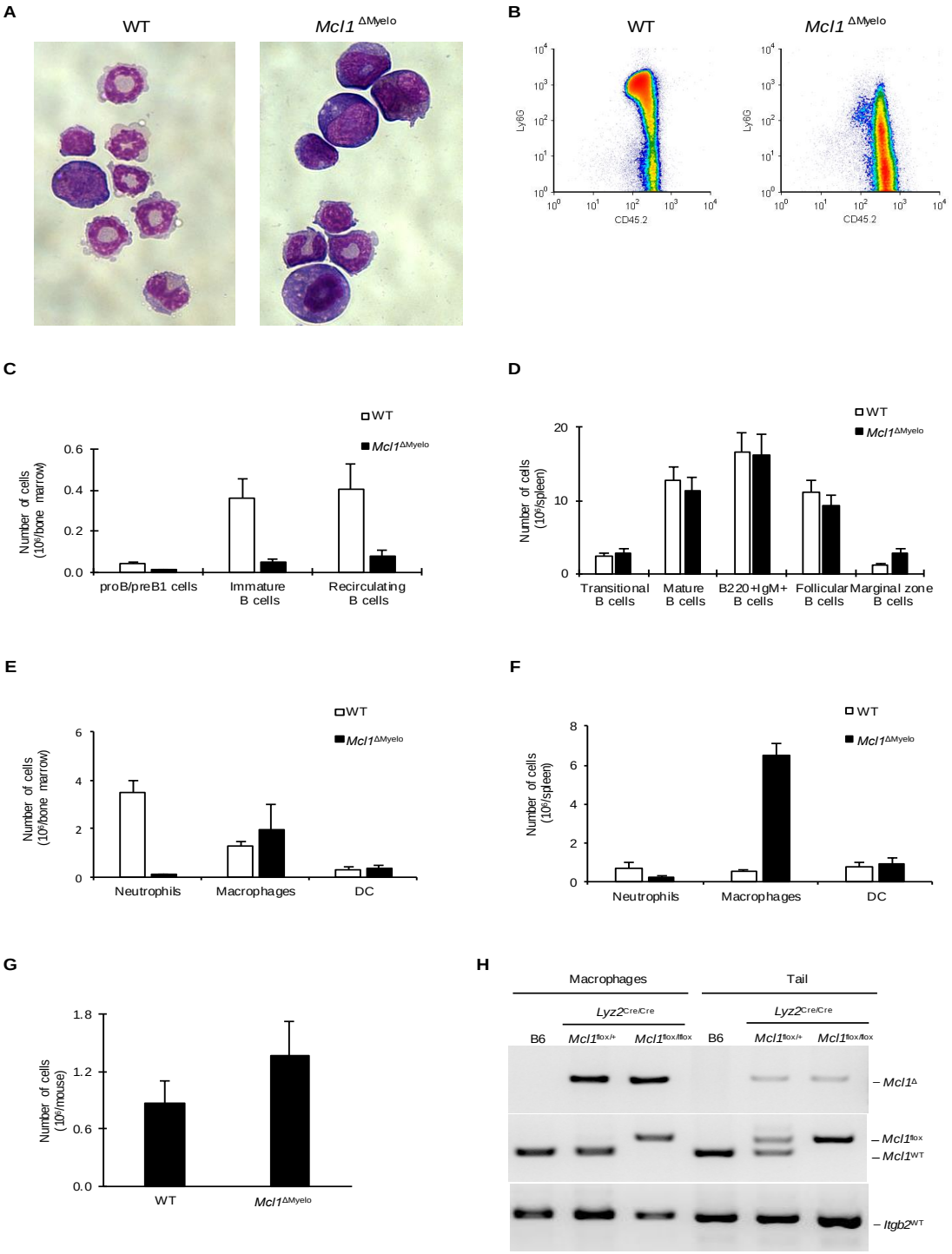

Figure S2

Analysis of tissue leukocytes and in vitro differentiated macrophages

A-F, bone marrow and splenic cells of wild type (WT) or *Mcl1*$^{\Delta Myelo}$ mice were analyzed. (A) Representative cell morphologies of WT and *Mcl1*$^{\Delta Myelo}$ mouse bone marrow cells analyzed by the cytospin approach. (B) Density plots of WT and *Mcl1*$^{\Delta Myelo}$ mouse bone marrow cells, characterized by Ly6G and CD45 (CD45.2) staining and flow cytometry. (C) B cell subpopulations of the bone marrow from WT and *Mcl1*$^{\Delta Myelo}$ mice, characterized by c-kit^{+}B220^{med} (proB/preB1 cells), IgM^{+}B220^{med} (immature B cells) and IgM^{+}B220^{+} (recirculating B cells) staining by flow

cytometry. (D) B cell subpopulations in the spleen of WT and *Mcl1*$^{\Delta Myelo}$ mice, characterized by IgM^{+}IgD^{+} (mature B cells), IgM^{+}IgD^{med} (transitional B cells), CD21^{+}CD23^{+} (follicular B cells) and CD21^{+}CD23^{med} (marginal zone B cells) staining by flow cytometry. (E) Macrophages and dendritic cells (DC) from the bone marrow and spleen (F) of WT and *Mcl1*$^{\Delta Myelo}$ mice. Macrophages were identified as CD45^{+}F4/80^{+} cells and DC were identified as CD45^{+}iA/B^{+}CD11c^{+} cells by flow cytometry. G-H, analysis of bone marrow-derived macrophages from C57BL/6 (B6), *Lyz2*$^{Cre/Cre}$*Mcl1*$^{flox/+}$ and *Mcl1*$^{\Delta Myelo}$ (*Lyz2*$^{Cre/Cre}$*Mcl1*$^{flox/flox}$) mice. (G) Macrophage cell counts were tested by flow cytometry. Mature macrophages were identified as F4/80^{+} cells. (H) PCR-based analysis of Cre mediated deletion of the *Mcl1* gene of in vitro cultured macrophages and tail samples. *Mcl1*$^{\Delta}$ indicates Cre-mediated deletion of the *Mcl1*^{flox} allele. The *Mcl1*^{flox} and *Mcl1*^{WT} PCR protocol used the same primer set with different product lengths. The *Itgb2*^{WT} PCR was used as an irrelevant control. Because of the saturating nature of PCR reactions, the deletion of the *Mcl1*^{flox} allele is best seen in the *Lyz2*$^{Cre/Cre}$*Mcl1*$^{flox/+}$ samples. Here, the practically complete lack of the *Mcl1*^{flox} PCR product indicates strong deletion of the floxed allele in macrophages, as compared to the normal presence of the *Mcl1*^{flox} allele in tail biopsies. The obvious presence of the *Mcl1*^{flox} PCR product in the *Mcl1*$^{\Delta Myelo}$ macrophage samples likely reflects efficient PCR-based amplification of a low amount of the *Mcl1*^{flox} allele still present in these samples in the absence of competing *Mcl1*^{WT} sequences, rather than the lack of deletion of the *Mcl1*^{flox} allele, as indicated by the strong *Mcl1*$^{\Delta}$ product present in those samples. Images are representatives of, and quantitative data show mean and SEM from, 1 (A) or 2-3 mice (B-F) per group from 2-3 independent experiments.

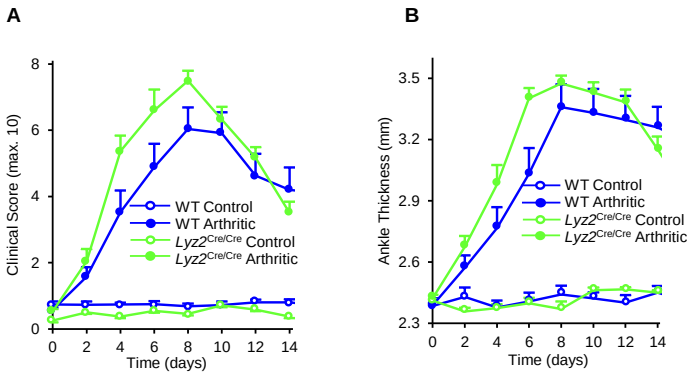

Figure S3

Autoantibody-induced arthritis in Lyz2$^{Cre/Cre}$ *mice*

Wild type (WT) or *Lyz2*$^{c/c}$ mice were injected with control (B×N) or arthritic (K/B×N) serum on Day 0. Arthritis development was followed by clinical scoring of the hind limbs (A) and ankle thickness measurement (B). Data show mean and SEM from 3-9 control and 5-15 arthritic serum-treated individual mice per group from 3 independent experiments.

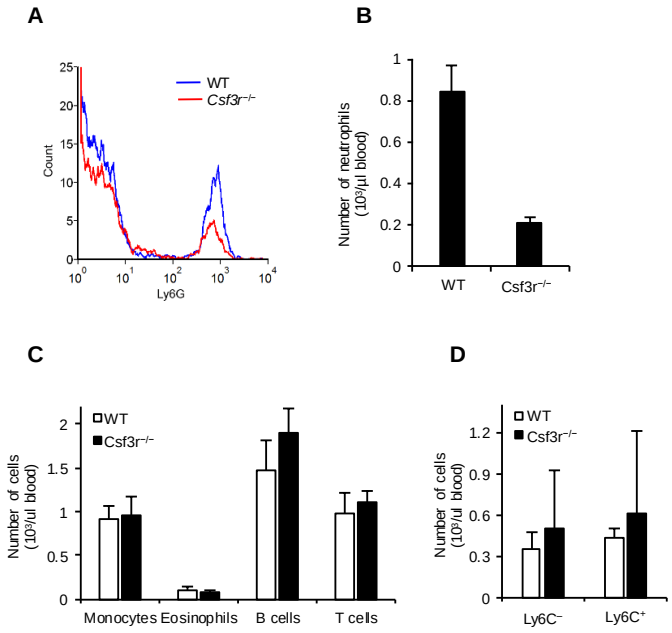

Figure S4

Moderate neutrophil deficiency in Csf3r$^{-/-}$ mice

(A) Flow cytometric histograms of Ly6G staining of peripheral blood leukocytes of wild type (WT) and *Csf3r*$^{-/-}$ mice. (B) Quantitative analysis of the number of mature neutrophils (CD11b^{+}Ly6G^{+}Siglec-F^{-}). (C, D) Quantitative analysis of the number of other leukocyte populations (C) and monocyte subpopulations (D). Flow cytometry histograms are representative of, and quantitative data show mean and SEM from, 8 (A, B, C) or 4 (D) mice per group from 3 (A, B, C) or 2 (D) independent experiments.