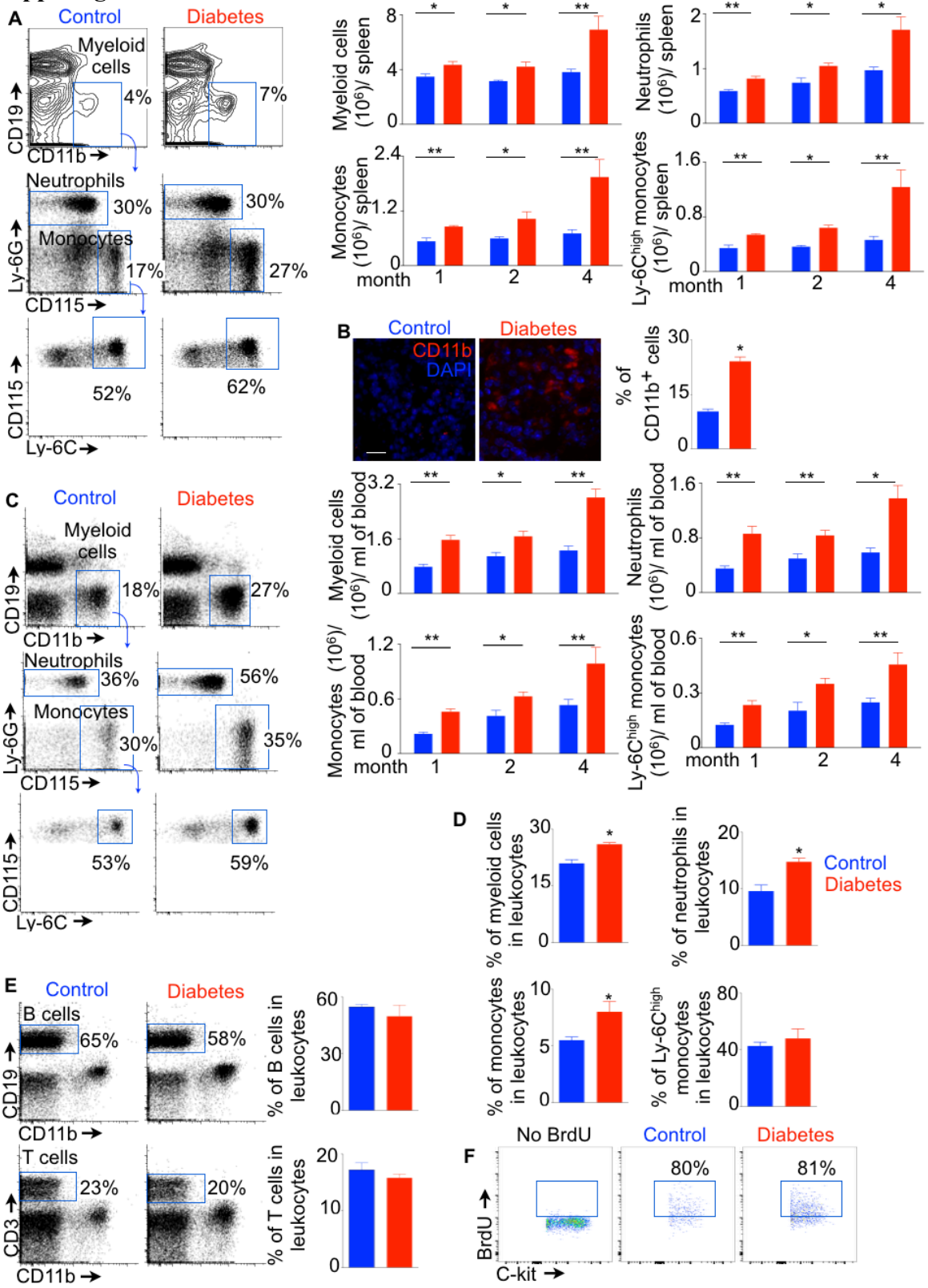
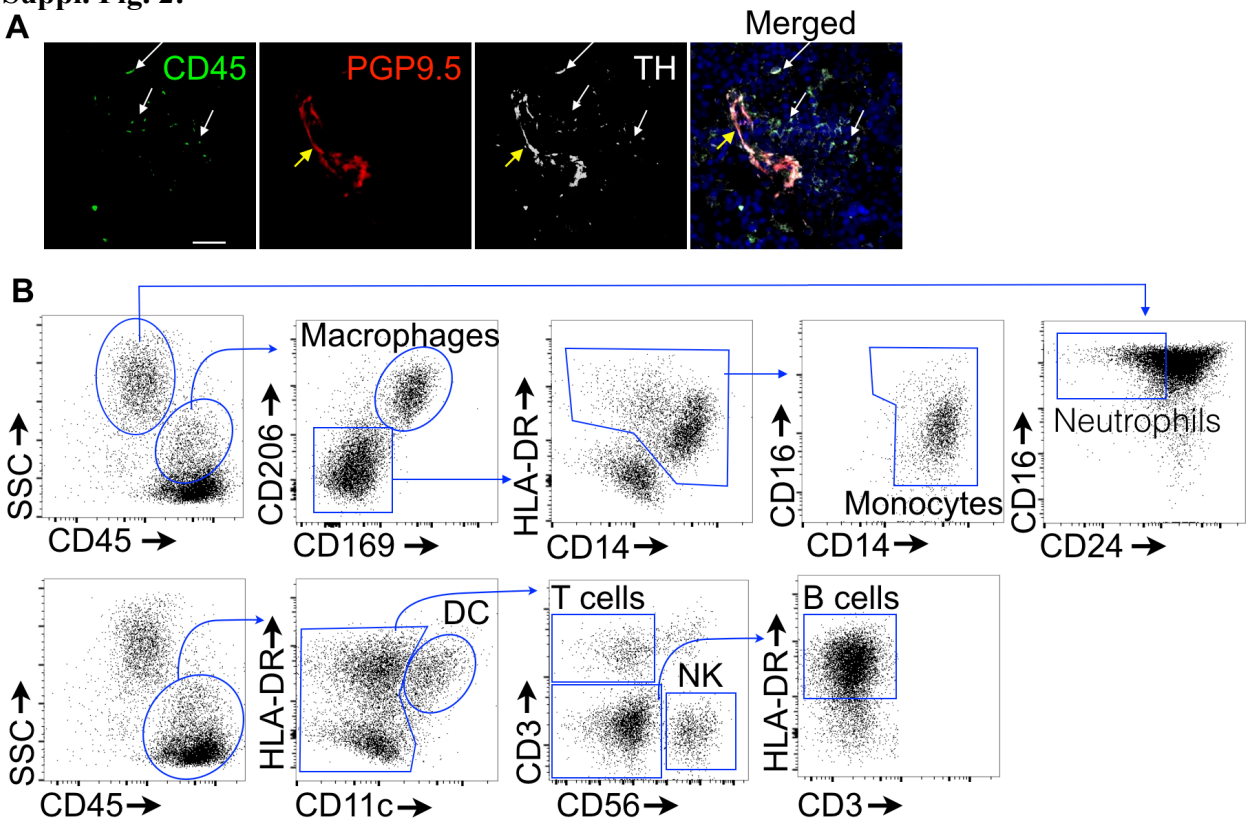
Supplemental Information:
SUPPLEMENTAL FIGURES
Suppl. Fig. 1:



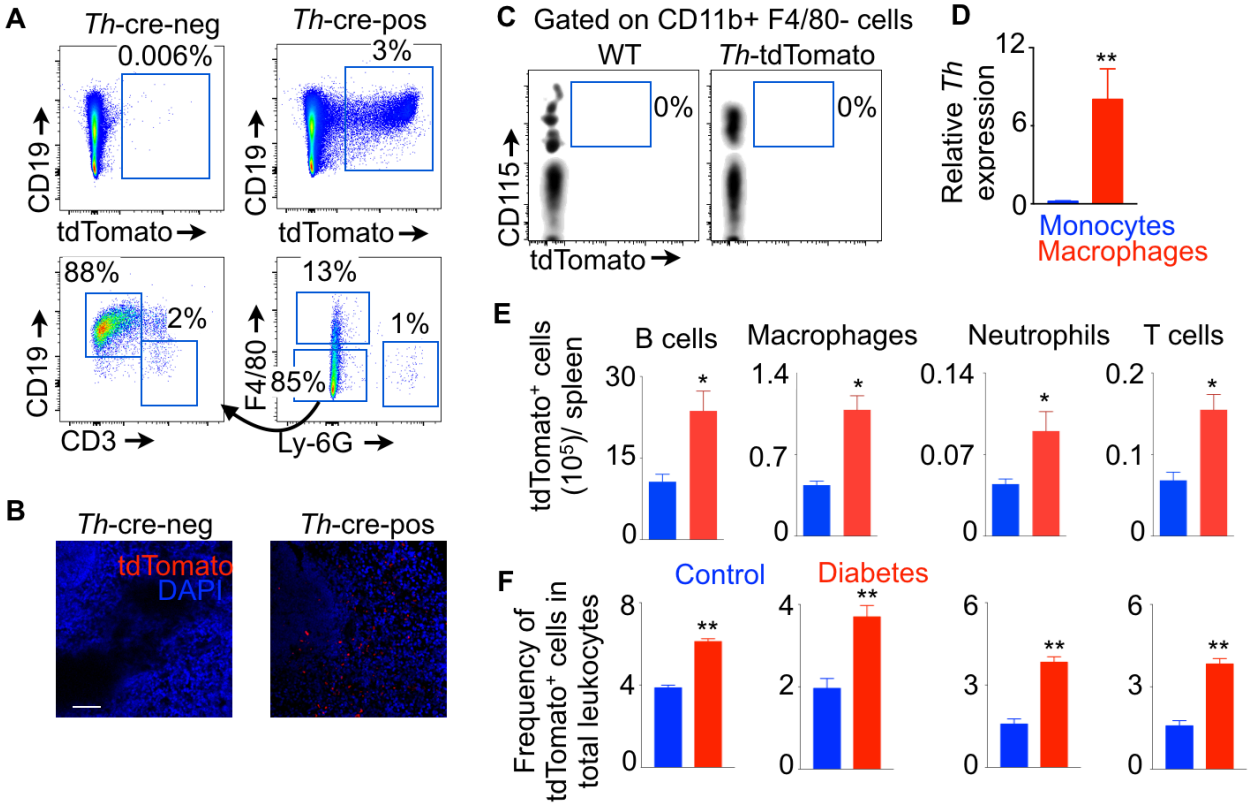
Suppl. Fig. 1: Diabetes increases splenic and blood myeloid cell content: Related to Figure 2. C57BL/6 mice fed on chow diet were injected with stz for 5 days to induce diabetes. One of three independent experiments is shown. **A**) Representative flow cytometry plots and quantification of different myeloid cells using flow cytometry. **B**) Representative confocal images showing splenic $CD11b^+$ cells. The bar graph represents quantification of splenic myeloid cells in C57BL/6 mice 8 weeks after stz treatment. (Scale bar = 20 μm). **C**) Representative flow cytometry plots and quantification of different myeloid cells in the blood. **D**) Frequency of different myeloid cells in the spleens of non-diabetic and diabetic mice. **E**) The flow cytometric plots show gating strategies for B and T lymphocytes. The bar graphs depict frequencies of the lymphocytes in the blood in diabetic and non-diabetic C57BL/6 mice. Data from one of three independent experiments are shown. **F**) $BrdU^+$ GMP in the spleen after 2 weeks of BrdU injection in C57BL/6 mice. n=5-6 mice/ time point/ group (A-C) and n=5 mice/ group (D-F). Mean ± s.e.m. * $P < 0.05$, ** $P < 0.01$.

Suppl. Fig. 2:



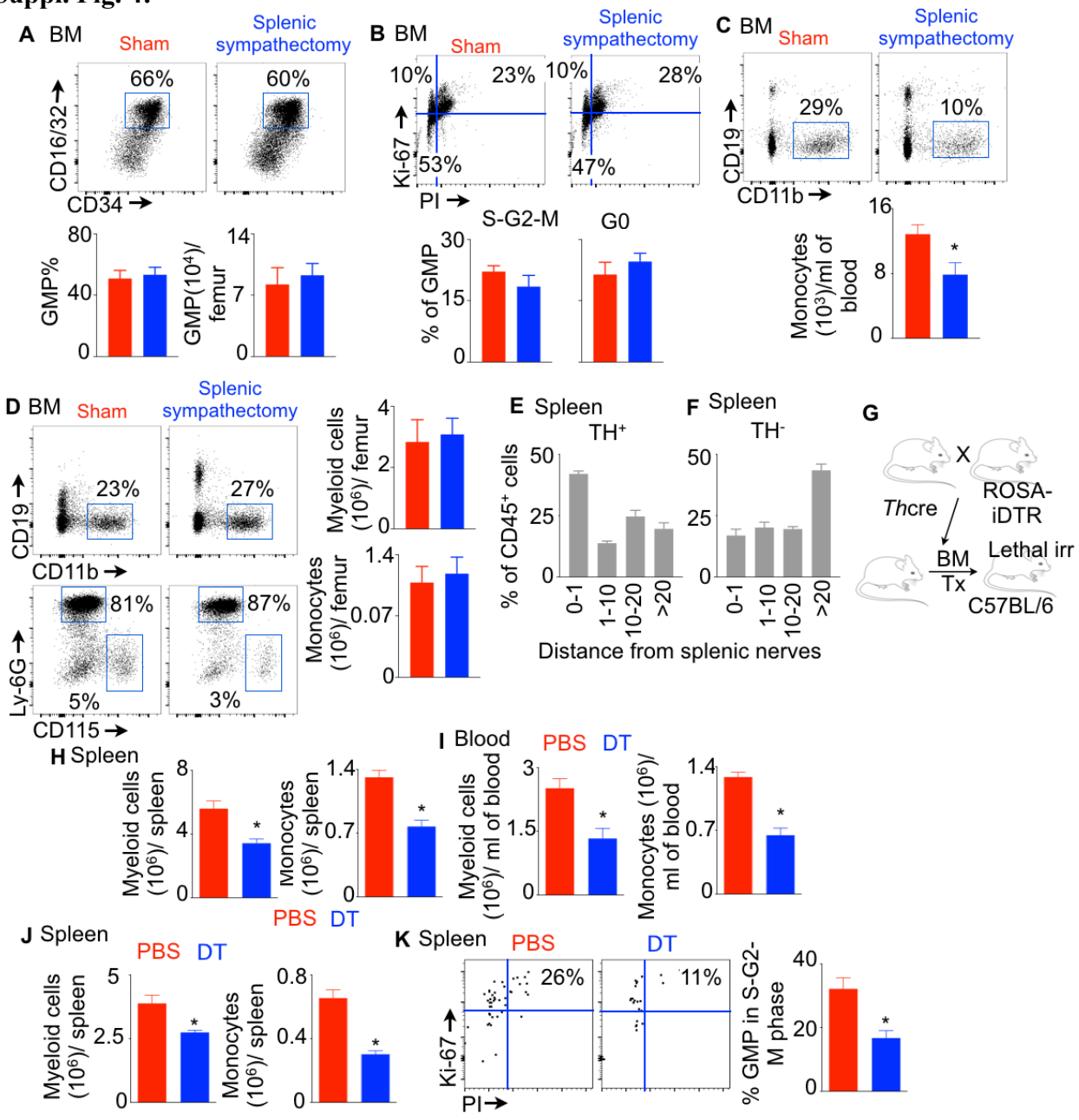
Suppl. Fig. 2: Splenic nerves and leukocytes express TH: Related to Figure 4. A) Confocal images showing TH staining in PGP9.5^{+} nerves and CD45^{+} leukocytes in the spleen of diabetic C57BL/6 mice. (Scale bar = 50 μm). **B**) Representative flow cytometric gating strategy for T cells, neutrophils, macrophages and dendritic cells in human spleen. n=4-5 samples/ group.

Suppl. Fig. 3:



Suppl. Fig. 3: Diabetes increases TH-expressing leukocytes in the spleens of *Th*-cre-ROSA-tdTomato mice: Related to Figure 4. Flow cytometric plots (**A**) and confocal images (**B**) show tdTomato$^+$ leukocytes. (Scale bar = 50 µm). **C**) Flow cytometric plots showing tdTomato expression in monocytes. **D**) The bar graph shows TH expression in THP-1 monocytes and macrophages derived from this cell line using quantitative RT-PCR. Enumeration of splenic tdTomato$^+$ leukocyte populations by flow cytometry (**E**) and confocal microscopy (**F**) n=7 per group. The experiments were independently repeated. n=5-6 mice (A,B,C,E&F) and n=6 samples/group (D). Mean ± s.e.m. * $P < 0.05$, ** $P < 0.01$.

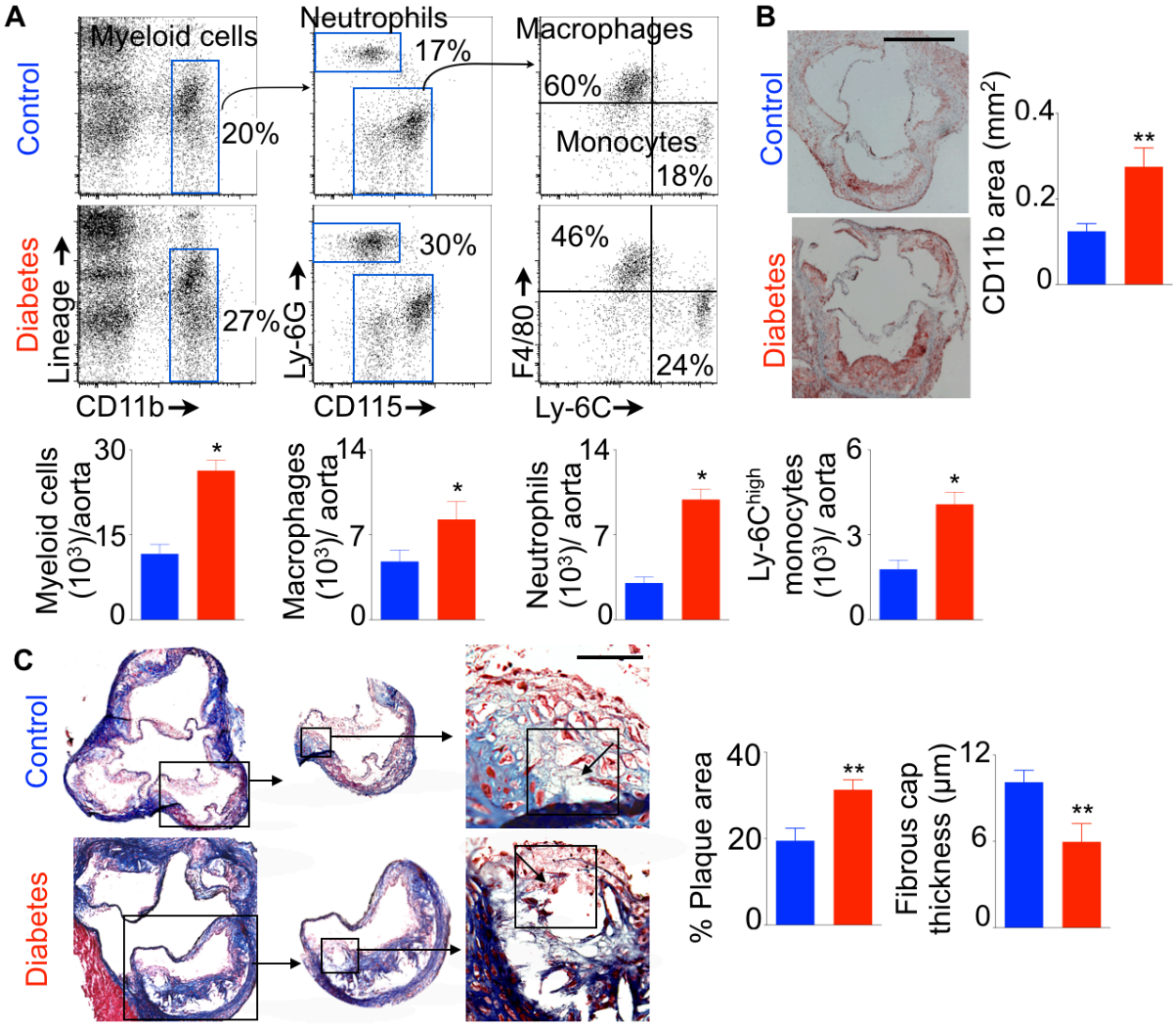
Suppl. Fig. 4:



Suppl. Fig. 4: Splenic sympathectomy does not affect the frequency, number and proliferation of GMP in the BM: Related to Figure 6. A) Quantification and (**B**) cell cycle analysis of GMP in the BM. Myeloid cell content in the blood (**C**) and femur (**D**) after surgical splenic sympathectomy. Distances between TH^+ (**E**) and TH^- (**F**) leukocytes and splenic nerves were quantified by confocal microscopy. **G**) Schematic diagram showing the experimental design. Flow cytometric quantification of myeloid cells and monocytes in the spleen (**H**) and blood (**I**) after TH^+ leukocyte depletion with DT in in wild type mice reconstituted with BM from *Th*-cre-ROSA-iDTR. Splenic myeloid cell number (**J**) and GMP proliferation (**K**) were determined by flow cytometry in *Th*-cre-ROSA-iDTR mice reconstituted with BM from wild type mice after DT-mediated depletion of splenic neurons. All the experiments with the

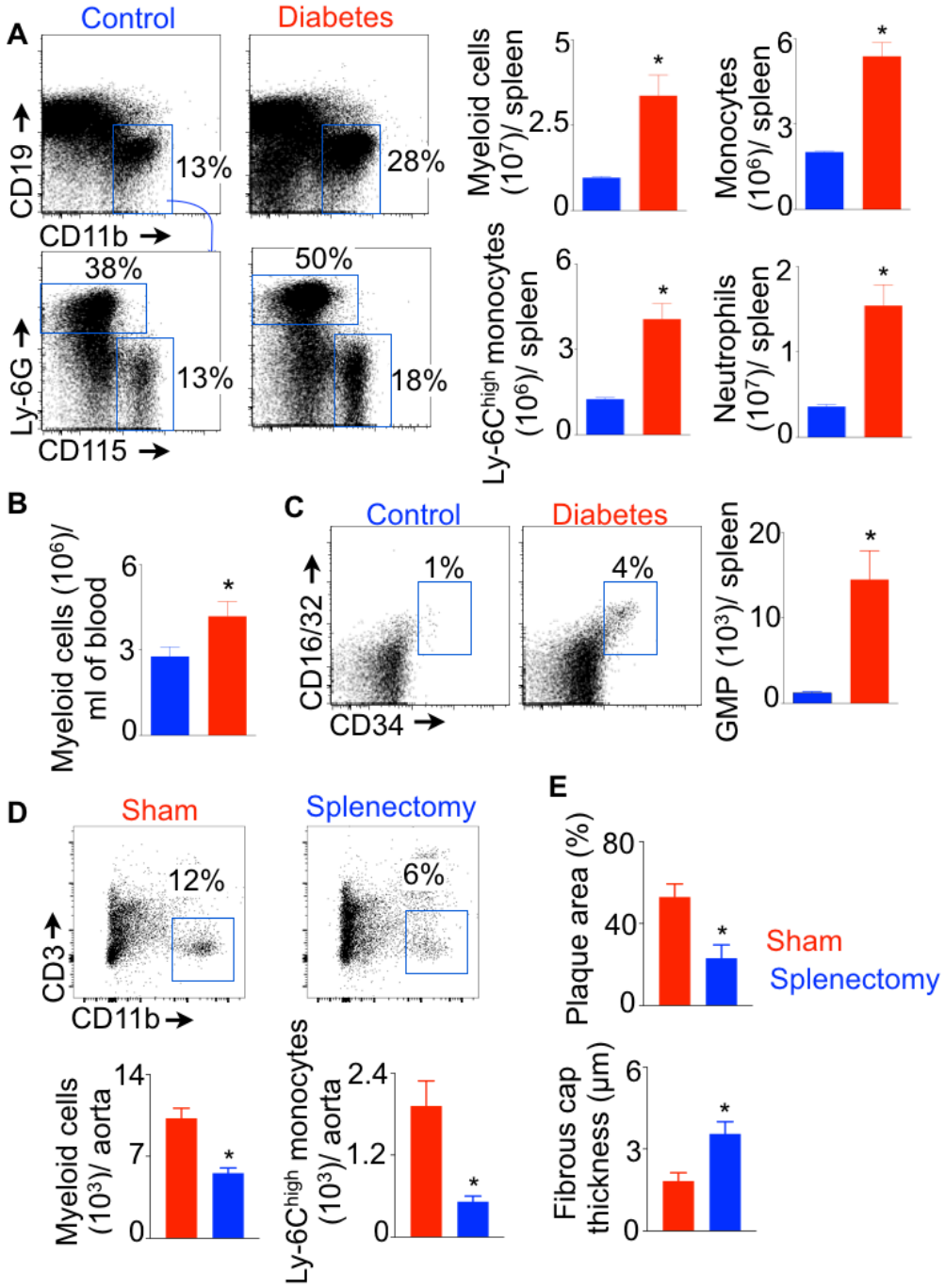
exception of splenic neuron depletion were performed at least twice. n=8-9 mice/ group (A-D), n=8/ group (E&F), n=6-7 mice/ group (H&I) and n=4 mice/ group (J&K). Mean ± s.e.m. * $P < 0.05$.

Suppl. Fig. 5:



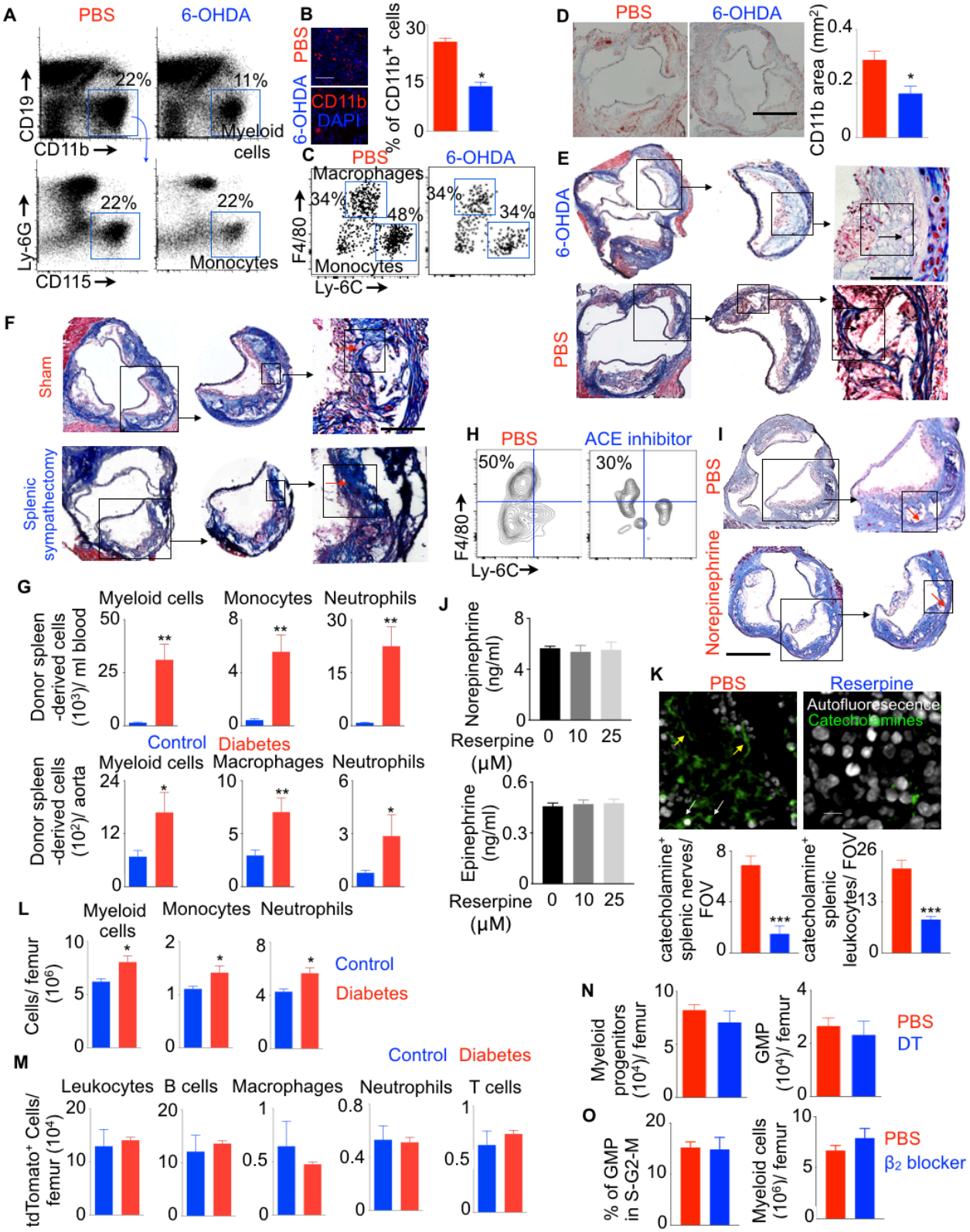
Suppl. Fig. 5: Accelerated atherosclerosis in diabetic mice: Related to Figure 7. *Apoe*$^{-/-}$ mice were fed on a high fat diet for 2 months and injected with stz for 5 days to induce type-1 diabetes. Control group received Na citrate buffer. **A**) Shows representative flow cytometric gating strategy for myeloid cells, neutrophils, monocytes and macrophages in the aorta at 8 weeks after stz injection. The bar graphs depict quantification of the myeloid cells. **B**) Immunological staining showing CD11b^{+} area in aortic roots. (Scale bar = 150 µm). **C**) Histo-pathological images of aortic roots stained with Masson's trichrome. The arrows point at the fibrous caps above the necrotic cores. (Scale bar = 50 µm). The bar graphs show quantification of plaque area and fibrous cap thickness. The data are pooled from two independent experiments. n=6 mice/group. Mean ± s.e.m. ** $P < 0.01$.

Suppl. Fig. 6:



Suppl. Fig. 6: Diabetes increases myeloid cell content in the spleen and blood of *Apoe*$^{-/-}$ mice: Related to Figure 7. A) Shows representative flow cytometric gating strategy and quantitation of splenic myeloid cells, neutrophils, monocytes and Ly-6C^{hi} monocytes. **B)** Quantification of myeloid cells in the blood. **C**) Enumeration of splenic GMP by flow cytometry. **D)** Flow cytometric enumeration of myeloid cells and inflammatory monocytes in the aortas of *Apoe*$^{-/-}$ mice 4 weeks after splenectomy. **E**) Quantification of atherosclerotic plaque area and fibrous cap thickness in aortic roots using Masson's trichrome staining. n=5 mice/ group. Mean ± s.e.m. * $P < 0.05$.

Suppl. Fig. 7:


A
PBS
6-OHDA
22%
11%
CD19
CD11b
Myeloid cells
22%
22%
Ly-6G
CD115
Monocytes
B
PBS
6-OHDA
CD11b
DAPI
% of CD11b+ cells
C
PBS
6-OHDA
Macrophages
34%
48%
34%
34%
F4/80
Monocytes
Ly-6C
D
PBS
6-OHDA
CD11b area (mm²)
E
6-OHDA
PBS
F
Sham
Splenic sympathectomy
G
Myeloid cells
Monocytes
Neutrophils
Donor spleen -derived cells (10³)/ ml blood
Control
Diabetes
Myeloid cells
Macrophages
Neutrophils
Donor spleen -derived cells (10²)/ aorta
H
PBS
ACE inhibitor
50%
30%
F4/80
Ly-6C
I
PBS
Norepinephrine
J
Norepinephrine (ng/ml)
Reserpine (μM)
Epinephrine (ng/ml)
Reserpine (μM)
K
PBS
Reserpine
Autofluoresecence
Catecholamines
catecholamine+ splenic nerves/ FOV
catecholamine+ splenic leukocytes/ FOV
L
Myeloid cells
Monocytes
Neutrophils
Cells/ femur (10⁶)
Control
Diabetes
M
Control
Diabetes
Leukocytes
B cells
Macrophages
Neutrophils
T cells
tdTomato+ Cells/ femur (10⁴)
N
Myeloid progenitors (10⁴)/ femur
GMP (10⁴)/ femur
PBS
DT
O
% of GMP in S-G2-M
Myeloid cells (10⁶)/ femur
PBS
β2 blocker

Suppl. Fig. 7: Splenic myelopoiesis triggered by sympathetic activation accelerates atherosclerosis in diabetic *Apoe*$^{-/-}$ mice: Related to Figure 7. A) Flow cytometric plots showing splenic myeloid cells after 6-OHDA treatment**. B**) Confocal images show CD11b^{+} cells in the spleen. **C**) Representative flow cytometric plots for macrophages and monocytes in the aortas of *Apoe*$^{-/-}$ mice after 6-OHDA treatment. (Scale bar = 5 μm). **D**) Bright field microscopic images and quantification of CD11b^{+} cells in the aortic root. (Scale bar = 150 μm). Histopathological images of aortic roots stained with Masson's trichrome show plaque area and fibrous cap thickness in aortic roots after 6-OHDA treatment (**E**) (Scale bar = 50 μm) and splenic sympathectomy (**F**) (Scale bar = 50 μm). **G**) Enumeration of donor-derived myeloid cells in the blood and spleen of recipient non-diabetic (control) and diabetic *Apoe*$^{-/-}$ mice three weeks after spleen transplantation. n=4-6 per group. **H** Flow cytometric plots showing frequency of aortic macrophages in diabetic *Apoe*$^{-/-}$ mice injected with either PBS or ACE inhibitor. n=5 per group. **I**) Masson's trichrome staining to measure plaque area and fibrous cap thickness in diabetic *Apoe*$^{-/-}$ mice injected with either PBS or NE. n=5 per group. (Scale bar = 150 μm). **J**) Catecholamine secretion by macrophages in culture in presence or absence of reserpine. n=6 per group. **K**) Enumeration of catecholamine^{+} splenic nerve termini and leukocytes after reserpine treatment in diabetic *Apoe*$^{-/-}$ mice. The yellow and white arrows point at catecholamine^{+} splenic nerves and leukocytes, respectively. n=5 per group. (Scale bar = 3 μm). **L**) The numbers of myeloid subsets in the femur were quantified in non-diabetic and diabetic mice using flow cytometry. n=5 per group. Data from one of three independent experiments are shown. **M**) Enumeration of tdTomato^{+} leukocytes in the BM of *Th*-cre-ROSA-tdTomato mice. n=7 per group. **N**) The numbers of myeloid progenitors and GMP in the BM were determined by flow cytometry in diabetic mice expressing iDTR in hematopoietic cells after DT injection. n=6-7 per group. **O**) Myeloid cell number and GMP proliferation in the femur were determined by flow cytometry in diabetic mice after PBS or β_2 blocker treatment. n=9-10 per group. All of these experiments were repeated at least twice. n=6/ group (A-D), n=7-9/ group (E&F), n=4-6/ group (G-L), n=6-7/ group (M&N) and n=9-10 mice/ group (O). Mean ± s.e.m. * $P < 0.05$, ** $P < 0.01$.