SUPPLEMENTARY MATERIALS

A switch from cell-associated to soluble PDGF-B protects against atherosclerosis, despite driving extramedullary hematopoiesis

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Supplementary Materials and Methods

S1. Plasma cholesterol and triglyceride levels

Standard enzymatic techniques were used to assess plasma cholesterol (CHOD-PAP method – Cholesterol FS Ecoline no. 113009990314; DiaSys – Diagnostic Systems GmbH) and plasma triglycerides (FS5' Ecoline no. 157609990314; DiaSys – Diagnostic Systems GmbH) automated on the Cobas Fara centrifugal analyzer (Roche).

S2. Pro-inflammatory cytokines in plasma and BMDM conditioned medium

Cytokine levels in plasma and BMDM conditioned medium were assessed using a V-PLEX Proinflammatory Panel 1 Mouse Kit following manufacturer's protocol (K15048D-1, Meso Scale Diagnostics).

Supplementary Tables

Table S1: Immunohistochemical staining protocols of murine aortic root cryosections

	αSMA Double staining with CD31	**CD31** Double staining with αSMA	**PDGF-B**	**MOMA-2**
Fixation	Dry acetone		4% PFA in PBS	Dry acetone
Permeabilization	-		0.25% Triton-x100 in PBS	-
Blocking	0.3% H_2O_2 in methanol Serum-free protein block (X0909, DAKO)		0.3% H_2O_2 in methanol	0.3% H_2O_2 in methanol PBS 4% FCS and avidin block 1:5 (SP-2001, Vector)
Primary antibody Cat. no and company	F3777, Sigma FITC-conjugated	550274, BD	Ab23914, Abcam	Molecular Genetics department Maastricht University
Primary antibody dilution and buffer	1:300 TBS	1:25 TBS	1:700 TBT (TBS + 1% BSA + 0.1% Tween)	1:50 PBS, 4% FCS, biotin block 1:5 (SP-2001, Vector)
Secondary antibody Cat. no and company	Sheep anti-FITC-HRP, 11.426.346.910 Roche	Biotinylated rabbit anti-rat, BA-4001, Vector	Brightvision poly-HRP-anti-rabbit, DPVR-55-HRP, Immunologic	Biotinylated rabbit anti-rat, Molecular Genetics department Maastricht University
Secondary antibody dilution and buffer	1:300 TBS	1:200 TBS	-	1:300 PBS, 2% normal mouse serum (X0910, DAKO), 4% FCS
Tertiary step/antibody Cat. no and company	-	ABC-AP kit (AK-5000, Vector)	-	ABC-HRP kit (PK-4000, Vector)
Stain development	Diaminobenzidine kit (K346811-2, Agilent)	Vector Blue substrate kit, alkaline phosphatase (SK-5300, Vector)	Diaminobenzidine kit (K346811-2, Agilent)	AEC kit (2% buffer, 3% AEC, 2% H_2O_2 in milliQ, K3461, DAKO)

FCS; fetal calf serum, PBS; phosphate-buffered saline, PFA; paraformaldehyde, TBS; tris-buffered saline.

Table S2: Primer sets used for genotype confirmation through PCR and electrophoresis

Gene	Forward primer (5'- 3')	Reverse primer (5'- 3')
Pdgfb^{WT}	CATGCTGCCTTGTAATCCGTTC	CGGCGGATTCTCACCGT
Pdgfb^{ret}	CTCGGGTGACCATTCGGTAA	TCTAAGTCACAGCCAGGGAGT AGC

Table S3: Primer sets used for qPCR

Gene	**Forward primer (5'- 3')**	**Reverse primer (5'- 3')**
18s rRNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG
Pdgfb	CGGTCCAGGTGAGAAAGATTG	CGTCTTGGCTCGCTGCTC
Pdgfra	AGAGAGAATCGGCCCCAGTG	CCATAGCTCCTGAGACCCGC
Pdgrb	GGCCTTAGTGGTCCTTACCG	GCACAGGGTCCACGTAGATG

Table S4: Antibodies used for flow cytometry

Antibody	Company	Catalog number	Dilution used
CD16/32	eBioscience	14-0161-82	1:100
CD45 PerCP	Biolegend	103130	1:100
CD3 eFluor 450	eBioscience	48-0032-82	1:100
NK-1.1 PE	BD Pharmingen	557391	1:100
Ly6G APC-Cy7	BD Pharmingen	560600	1:100
CD11b PE-Cy7	BD Pharmingen	552850	1:300
Ly-6C APC	Miltenyi Biotec	130-102-341	1:10
CD4 APC-H7	BD Pharmingen	560181	1:100
CD8 V500	BD Horizon	560776	1:200
CD8 FITC	eBioscience	11-0081-85	1:50
B220 V500	BD Horizon	561226	1:50
Sca-1 PerCP-Cyanine 5.5	eBioscience	45-5981-82	1:1000
c-Kit APC-eFluor780	eBioscience	47-1171-82	1:100
CD34 eFluor450	eBioscience	48-0341-82	1:50
CD16/32 PE-Cy7	eBioscience	25-0161-82	1:1000
B220 PE	BD Pharmingen	553089	1:100
CD3 PE	eBioscience	12-0031-82	1:800
CD11b PE	eBioscience	12-0112-82	1:800
Ly-6G PE	BD Pharmingen	551461	1:100
NK-1.1 PE	BD Pharmingen	557391	1:100
TER-119 PE	eBioscience	12-5921-82	1:200

Supplementary Figures

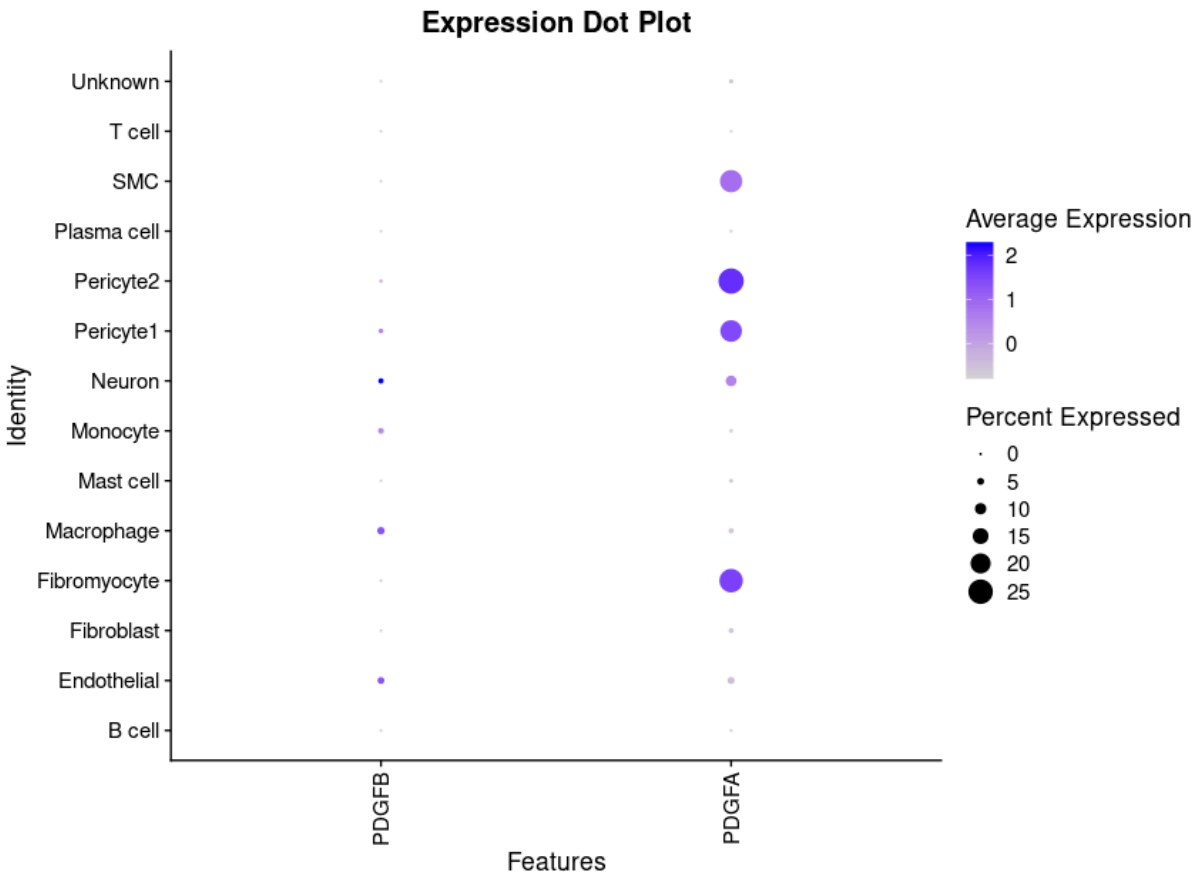

Figure S1: *PDGFB and PDGFA* expression in human atherosclerosis
Dot plot of *PDGFB* and *PDGFA* expression in single cell populations of human atherosclerotic coronary arteries (Wirka et al. [21]). SMC; smooth muscle cell.

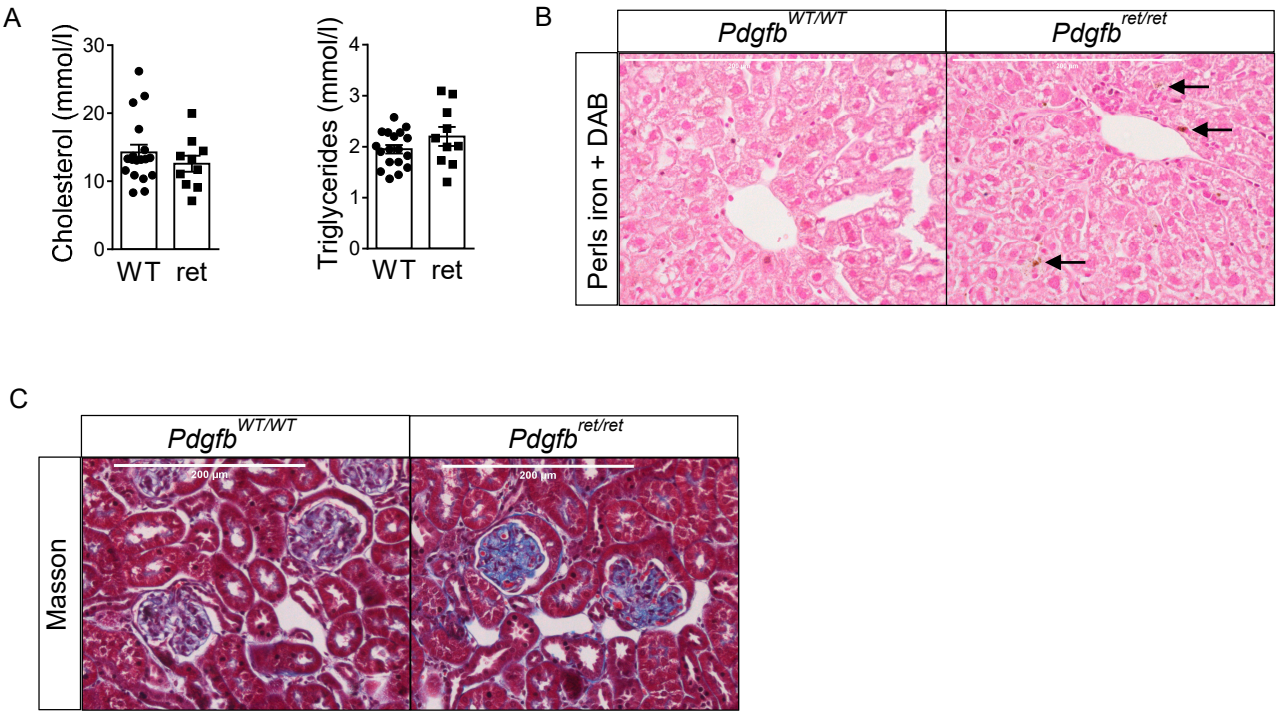

Figure S2: Similar cholesterol and triglyceride levels in *Pdgfb*$^{WT/WT}$ and *Pdgfb*$^{ret/ret}$ plasma and affected *Pdgfb*$^{ret/ret}$ liver and kidney

(**A**) Cholesterol and triglyceride levels in *Pdgfb*$^{WT/WT}$(*n*=18) and *Pdgfb*$^{ret/ret}$(*n*=10) plasma. (**B**) Representative photomicrographs of Perls iron staining combined with DAB in *Pdgfb*$^{WT/WT}$ and *Pdgfb*$^{ret/ret}$ liver. (**C**) Representative photomicrographs of Masson staining in *Pdgfb*$^{WT/WT}$ and *Pdgfb*$^{ret/ret}$ kidney, in which ECM is stained blue. Scale bars 200 µm. Data were tested for normality (Shapiro-Wilk) and equal variances (F-test). Variables that did not pass, were analyzed using Mann-Whitney U test. Variables that did pass, were analyzed using Student's t-test.

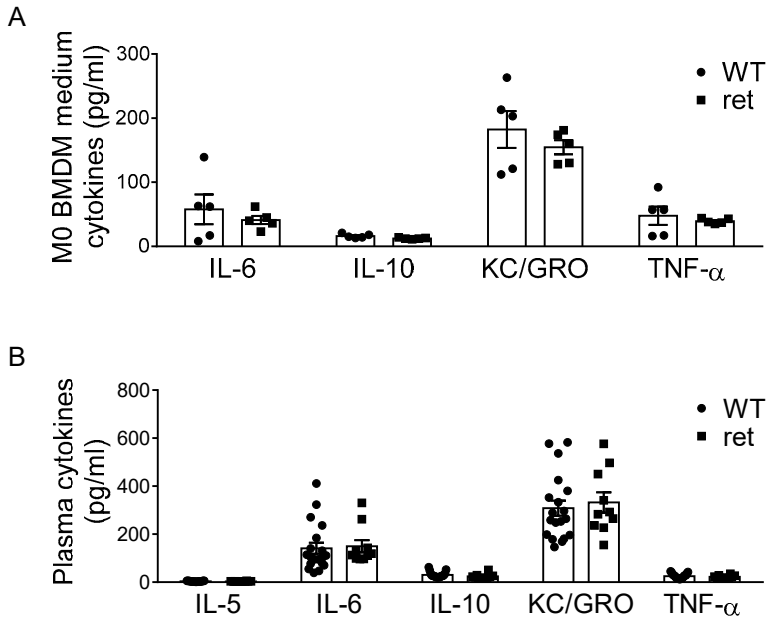

Figure S3: Pro-inflammatory cytokine levels in plasma and BMDM conditioned medium unaffected. (**A**) Levels of interleukin 6 (IL-6), IL-10, keratinocyte-derived chemokine/growth-related oncogene (KC/GRO or CXCL1) and tumor necrosis factor-α (TNFα) in BMDM-derived medium (*n*=5). IFN-γ, IL-1β, IL-5, IL-12 p70, IL-2 and IL-4 levels were undetectable. (**B**) Levels of IL-5, IL-6, IL-10, KC/GRO and TNF-α in *Pdgfb*$^{WT/WT}$(*n*=19) and *Pdgfb*$^{ret/ret}$(*n*=10) plasma. IFN-γ, IL-1β, IL-12 p70, IL-2 and IL-4 levels were undetectable. Graphs represent mean±SEM. Data were analyzed using two-way ANOVA.

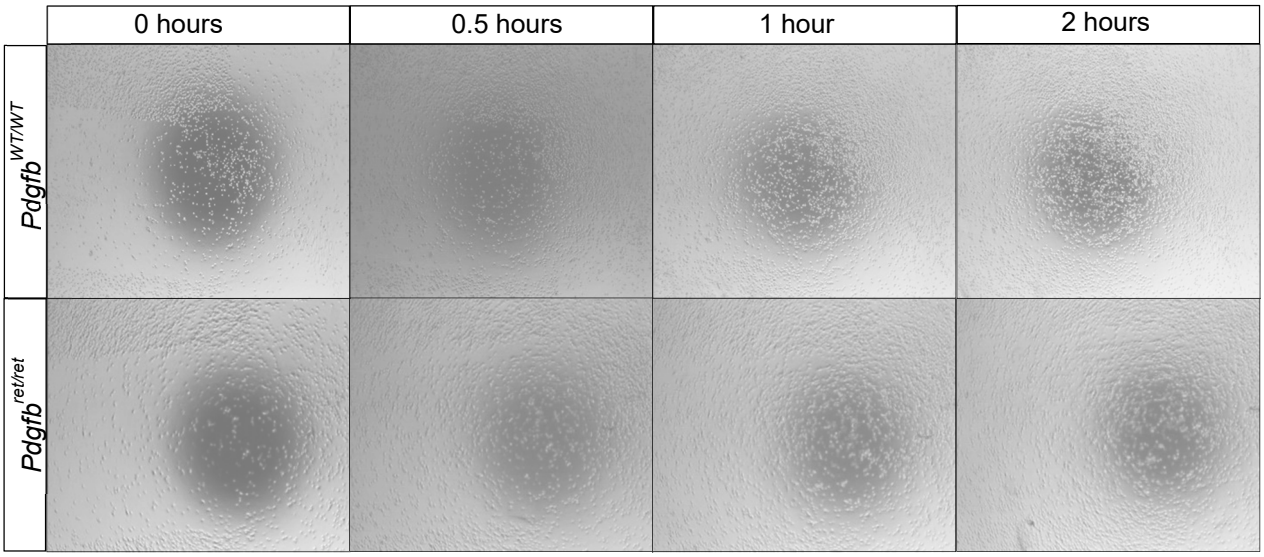

Figure S4: Pictures of BMDM scratch assay to assess migration *in vitro*.
Representative pictures of *Pdgfb*$^{WT/WT}$ and *Pdgfb*$^{ret/ret}$ BMDM migration over time (t=0, 0.5 hours, 1 hour and 2 hours) after scratch infliction.

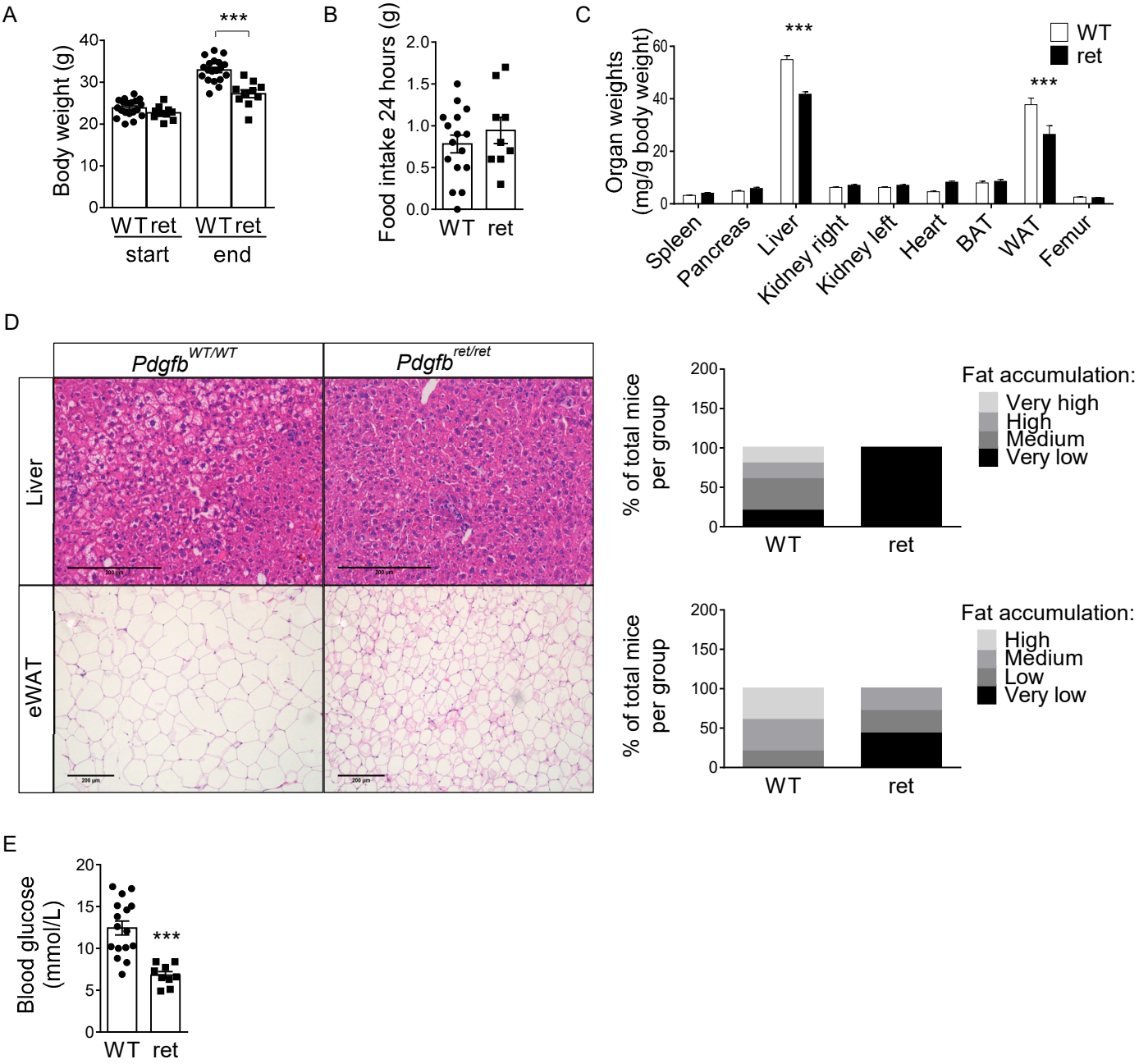

Figure S5: Decreased body weight gain and fat accumulation in *Pdgfb*$^{ret/ret}$ liver and epididymal white adipose tissue (eWAT). (**A**) *Pdgfb*$^{WT/WT}$(*n*=19) and *Pdgfb*$^{ret/ret}$(*n*=10) body weight before and after 10 weeks of high cholesterol diet (HCD) (**B**) 24-hour food intake as assessed in metabolic cages, in the 9th week of HCD. (**C**) Organ weights after 10 weeks of HCD, relative to body weight. (**D**) Fat accumulation as assessed by visual analogue scores ranging from very low to very high fat accumulation in HE-stained *Pdgfb*$^{WT/WT}$(*n*=5) and *Pdgfb*$^{ret/ret}$(*n*=7) liver and eWAT, with corresponding photomicrographs. (**E**) Blood glucose levels after 10 weeks of HCD. Results shown in B, C and E were obtained using 16 *Pdgfb*$^{WT/WT}$ and 9 *Pdgfb*$^{ret/ret}$ mice. Scale bars 200 µm. Graphs represent mean±SEM. ***p<0.001. Data were tested for normality (Shapiro-Wilk) and equal variances (F-test). Variables that did not pass, were analyzed using Mann-Whitney U test. Variables that did pass, were analyzed using Student's t-test. Data in A and C were analyzed using two-way ANOVA, including Bonferroni's multiple comparisons test.

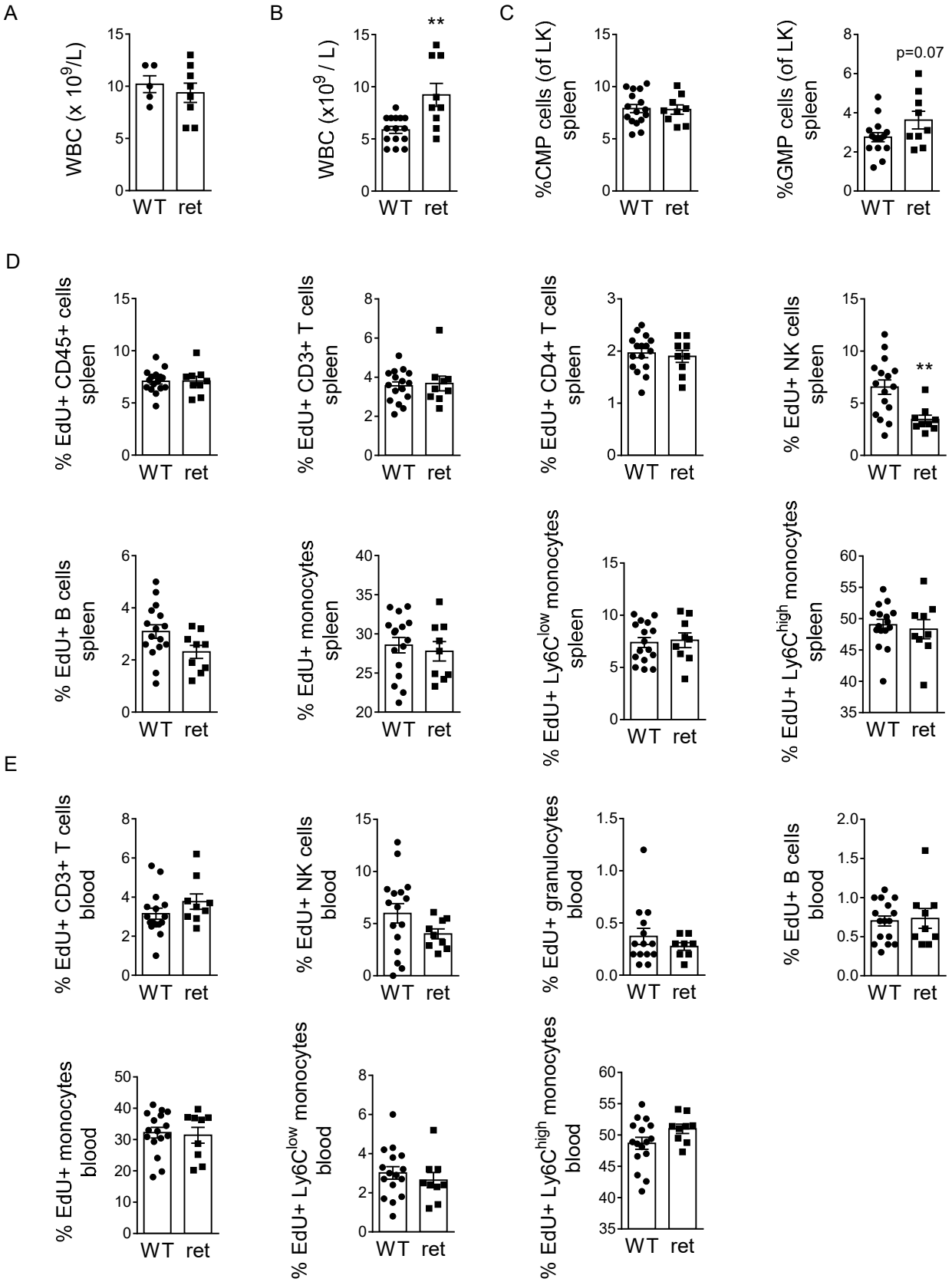
A
WBC (x 10^9/L)
WT ret
B
WBC (x10^9 / L)
**
C
%CMP cells (of LK) spleen
%GMP cells (of LK) spleen
p=0.07
D
% EdU+ CD45+ cells spleen
% EdU+ CD3+ T cells spleen
% EdU+ CD4+ T cells spleen
% EdU+ NK cells spleen
% EdU+ B cells spleen
% EdU+ monocytes spleen
% EdU+ Ly6C^low monocytes spleen
% EdU+ Ly6C^high monocytes spleen
E
% EdU+ CD3+ T cells blood
% EdU+ NK cells blood
% EdU+ granulocytes blood
% EdU+ B cells blood
% EdU+ monocytes blood
% EdU+ Ly6C^low monocytes blood
% EdU+ Ly6C^high monocytes blood

Figure S6: *Pdgfb*$^{ret/ret}$ leukocytosis confirmation in second mouse experiment after HCD and percentages of EdU positive leukocytes in blood and spleen. (**A**) White blood cell (WBC) counts in blood from *Pdgfb*$^{WT/WT}$(*n*=5) and *Pdgfb*$^{ret/ret}$(*n*=8) mice on standard laboratory diet. (**B**) WBC counts in *Pdgfb*$^{WT/WT}$(*n*=15) and *Pdgfb*$^{ret/ret}$(*n*=9) blood after 10 weeks HCD in the second mouse experiment. (**C**) Percentage progenitor cells of lineage^{-}c-Kit^{+} in *Pdgfb*$^{WT/WT}$(*n*=15-16) and *Pdgfb*$^{ret/ret}$(*n*=9) spleen. CMP = common myeloid progenitor, GMP = granulocyte monocyte progenitor. (**D**) Percentage of EdU positive leukocytes in *Pdgfb*$^{WT/WT}$(*n*=14-16) and *Pdgfb*$^{ret/ret}$(*n*=9) spleen and (**E**) blood. Graphs represent mean±SEM. **p<0.01. Data were tested for normality (Shapiro-Wilk) and equal variances (F-test). Variables that did not pass, were analyzed using Mann-Whitney U test. Variables that did pass, were analyzed using Student's t-test.