SUPPLEMENTARY INFORMATION

Chronic variable stress activates hematopoietic stem cells

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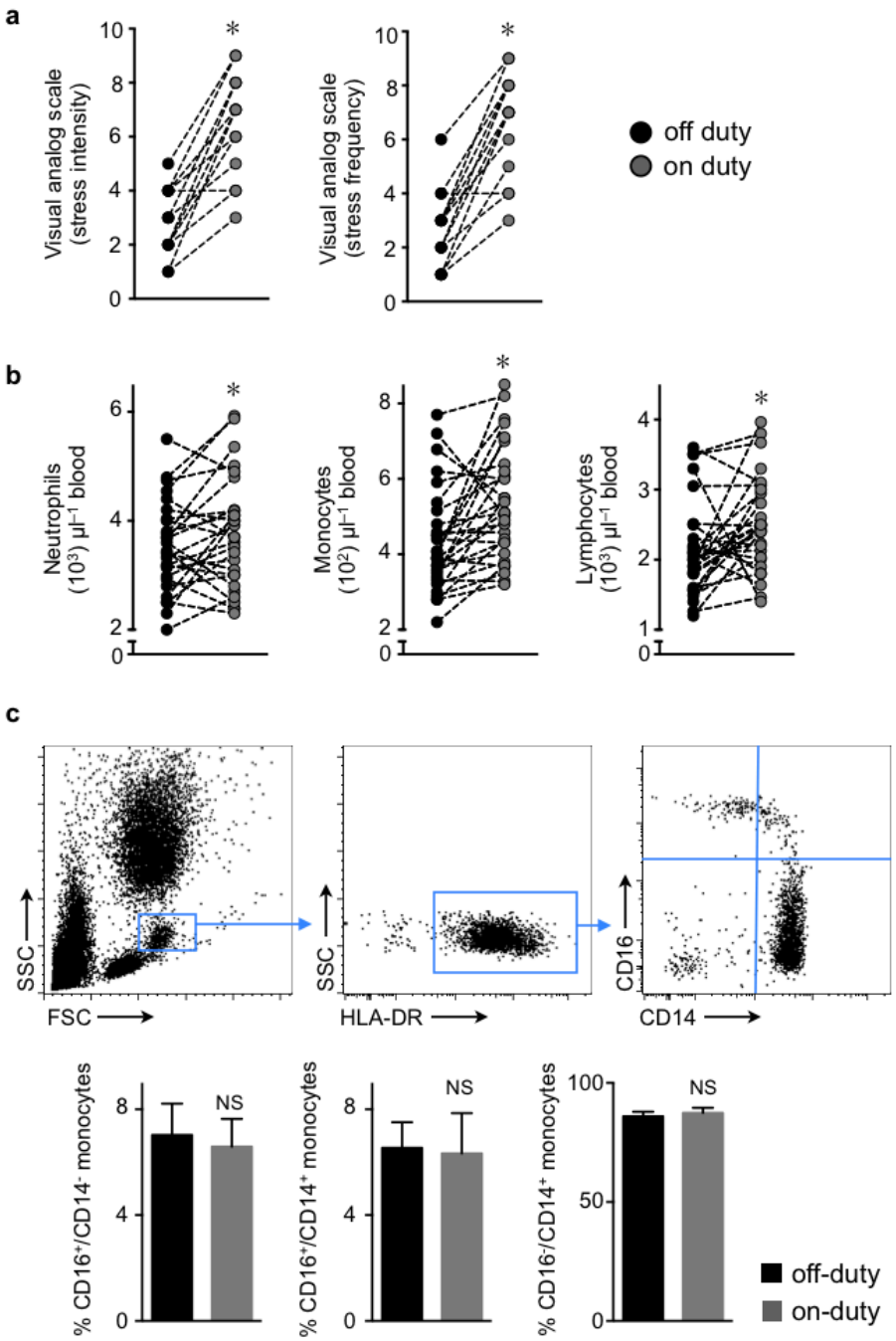
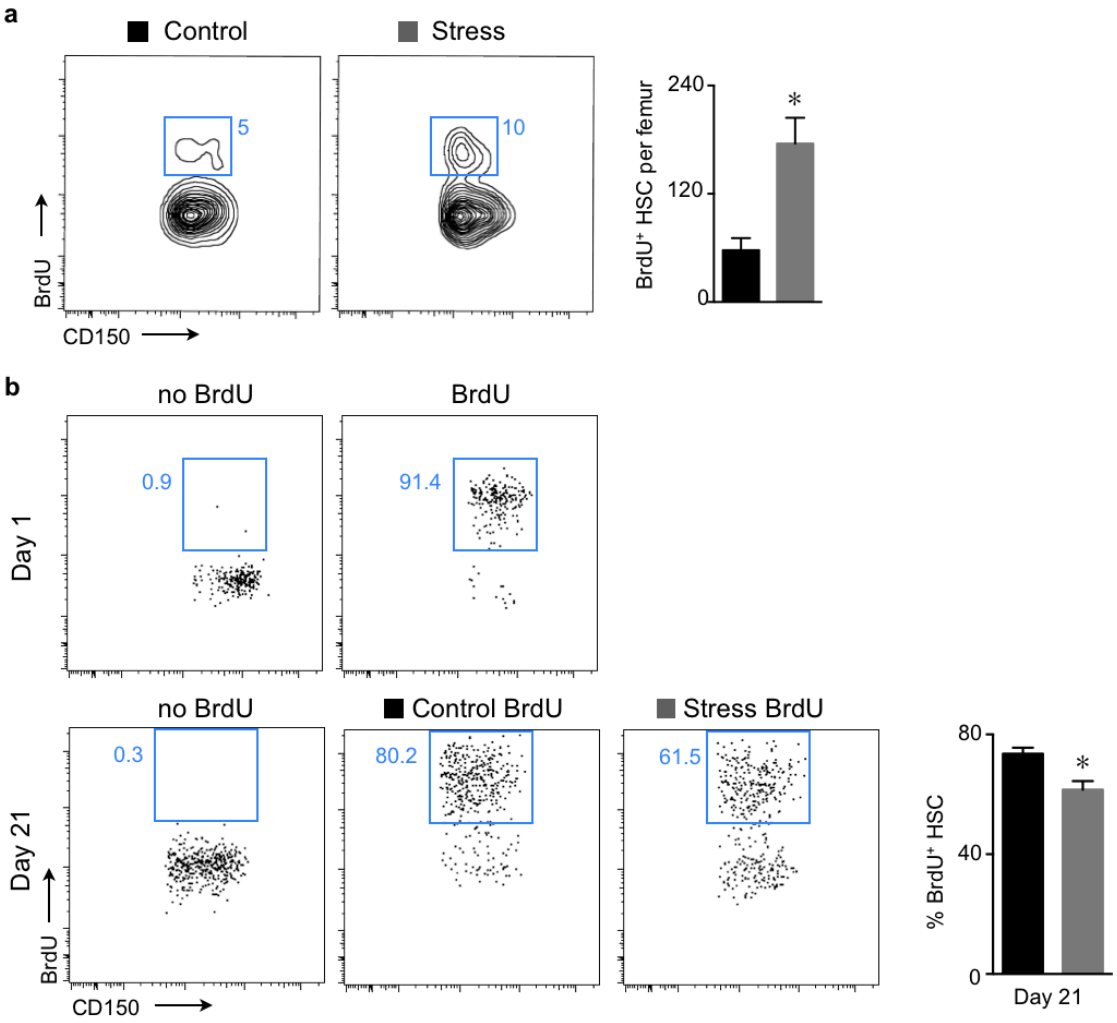
SUPPLEMENTARY FIGURES

Supplementary Figure 1



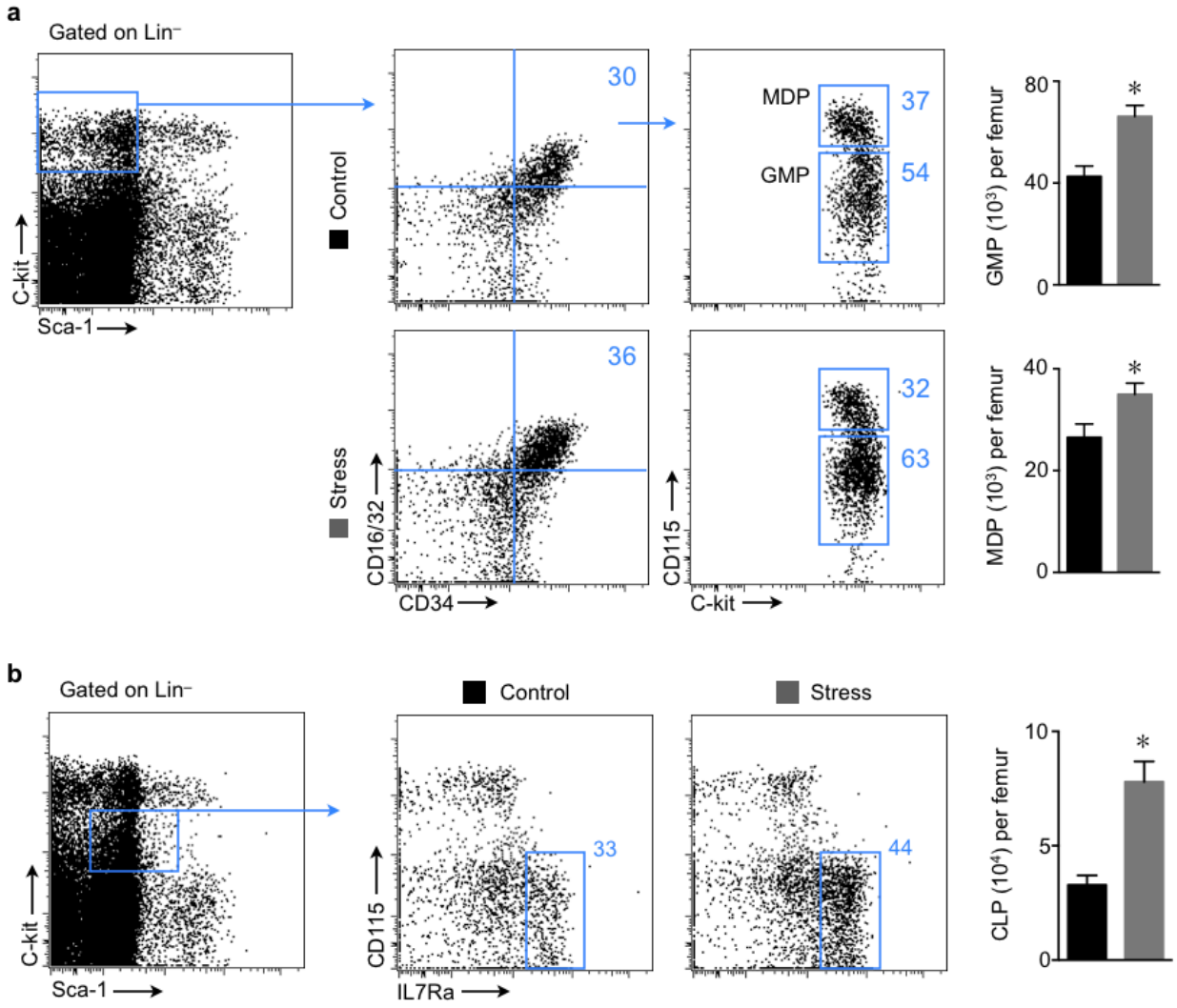
Supplementary Figure 1: ICU residents. a, Visual analog scales for stress intensity and frequency (n = 14, Wilcoxon test) and **b,** blood leukocyte subsets in medical residents (n = 29, Wilcoxon test). **c**, Gating strategy and analysis of monocyte subsets from medical residents (n = 14, Student's t–test, mean ± s.e.m.). * $P < 0.05$.

Supplementary Figure 2



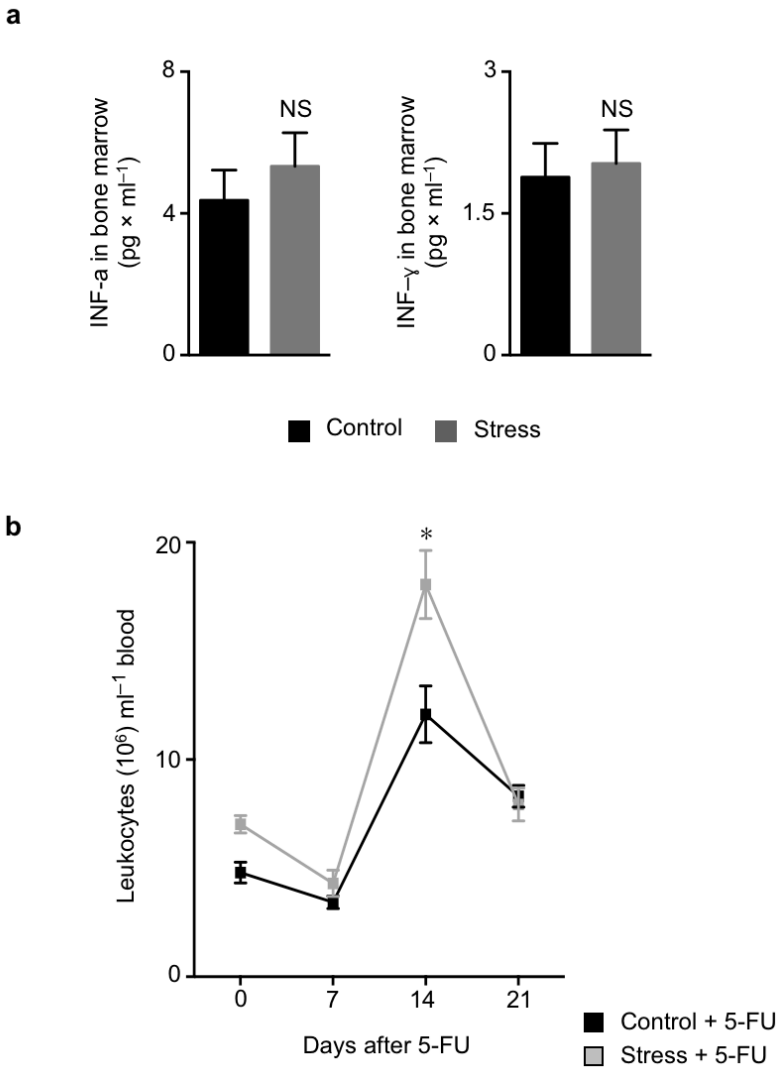
Supplementary Figure 2: HSC proliferation, a, Representative dot plots and analysis of HSC proliferation after 3 weeks of stress using an *in vivo* BrdU pulse 24 hours prior to the harvest (n = 4–5 per group, Mann–Whitney test). **b,** BrdU pulse–chase experiment. Mice were exposed to BrdU in drinking water for 2 weeks, which led to >90% BrdU labeling of HSC (Day 1, n = 3). Additional cohorts of mice were exposed to 3 weeks of stress or remained non–stressed after BrdU labeling. The lower panel shows representative dot plots and quantification of BrdU retention in HSC (Day 21, n = 10 per group, Student's t–test). Mean ± s.e.m., * $P < 0.05$.

Supplementary Figure 3



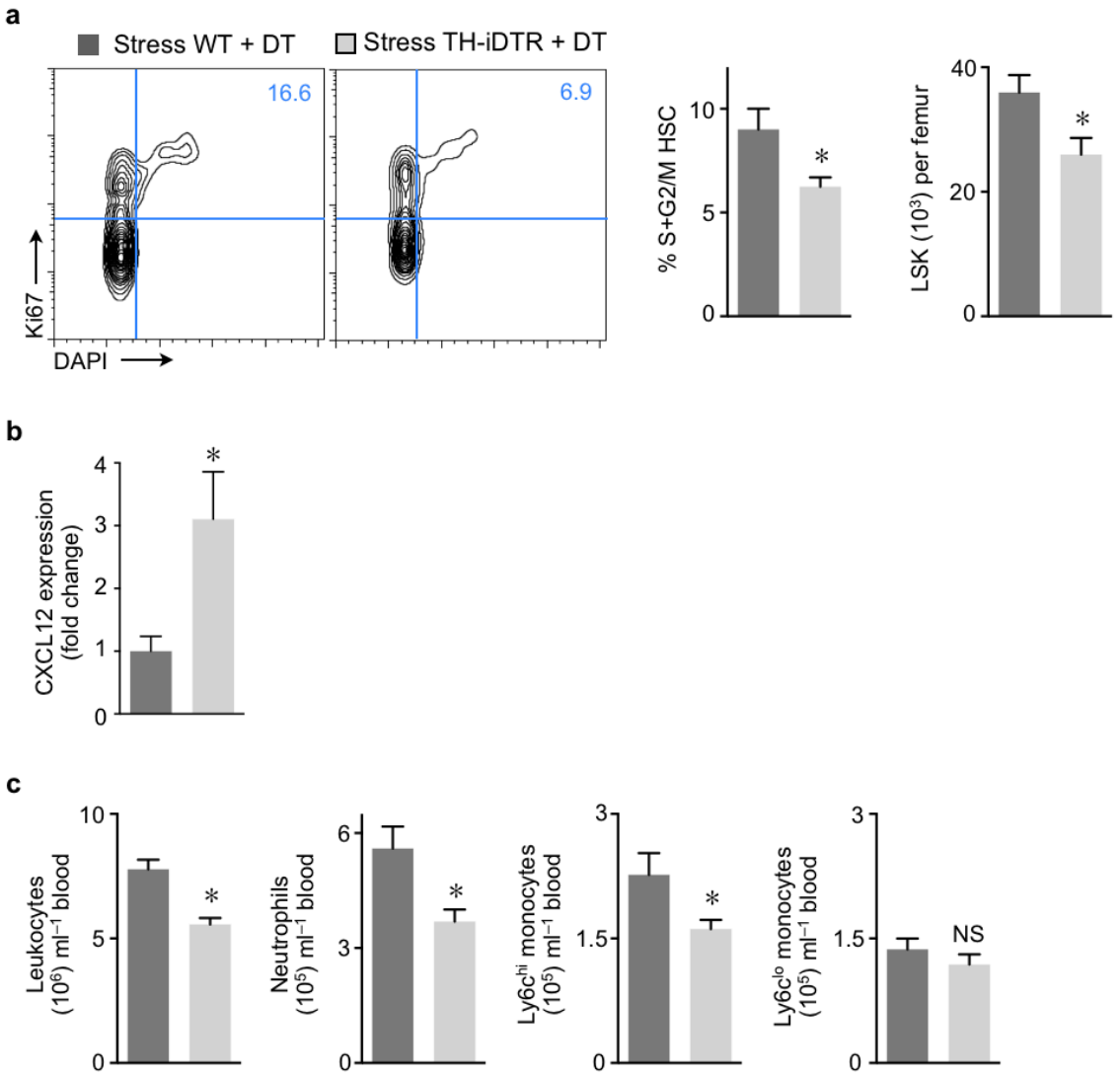
Supplementary Figure 3: Hematopoietic progenitor numbers. a, Gating strategy and quantification of GMP (Lin$^-$ c–kit$^+$ Sca–1$^-$ CD16/32$^+$ CD34$^+$ CD115$^-$) and MDP (Lin$^-$ c–kit$^{int/+}$ Sca–1$^-$ CD16/32$^+$ CD34$^+$ CD115$^+$) in the bone marrow of C57BL/6 mice after 3 weeks of stress (n = 5–7 per group, Mann–Whitney test). **b**, Gating strategy and quantification of common lymphoid progenitors (CLP, Lin$^-$ c–kit^{int} Sca–1^{int} CD115$^-$ IL7R$^+$) in the bone marrow of C57BL/6 mice after 3 weeks of stress compared to non–stressed controls (n = 5 per group, Mann–Whitney test). Mean ± s.e.m., * $P < 0.05$.

Supplementary Figure 4



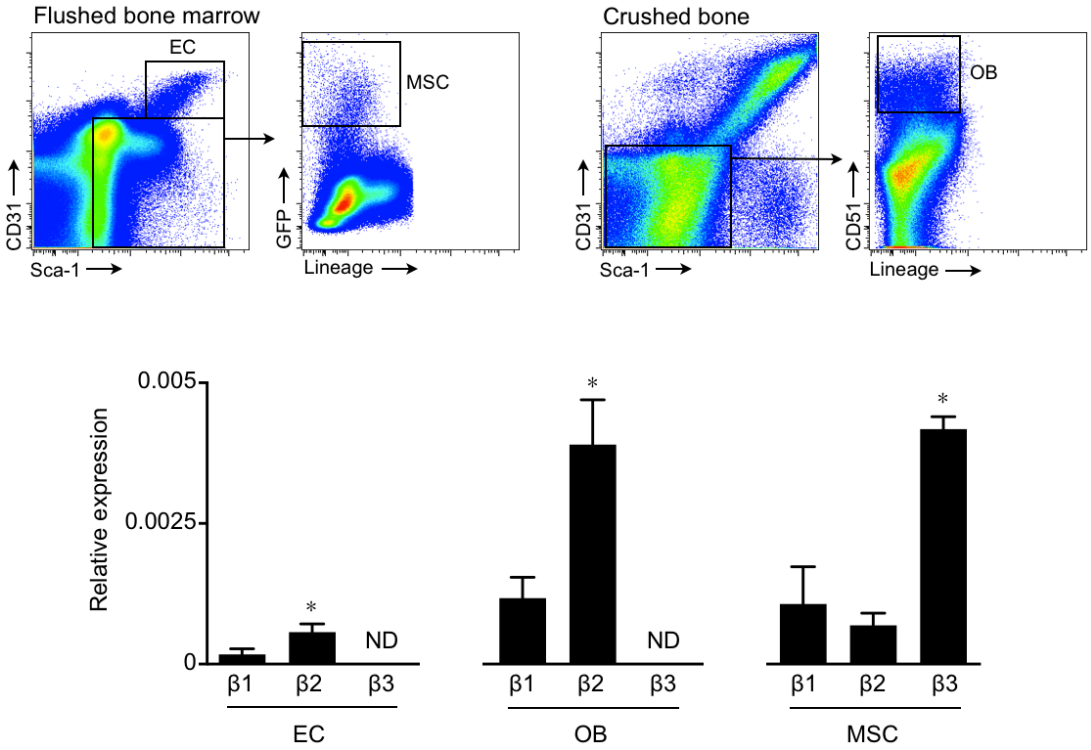
Supplementary Figure 4: Interferons and 5-FU challenge. a, ELISA for interferons α and ɣ in the bone marrow of C57BL/6 mice exposed to 3 weeks of stress (n = 7–8 per group, Student's t–test). **b,** 5–FU challenge in C57BL/6 mice. After 3 weeks of stress or in non–stressed controls, mice were injected with 5–FU (day 0, 150 mg/kg bodyweight). Blood leukocytes were analyzed on days 0, 7, 14 and 21 after injection (n = 5 per group, multiple t–tests corrected with Holm–Sidak method). Mean ± s.e.m., * $P < 0.05$.

Supplementary Figure 5



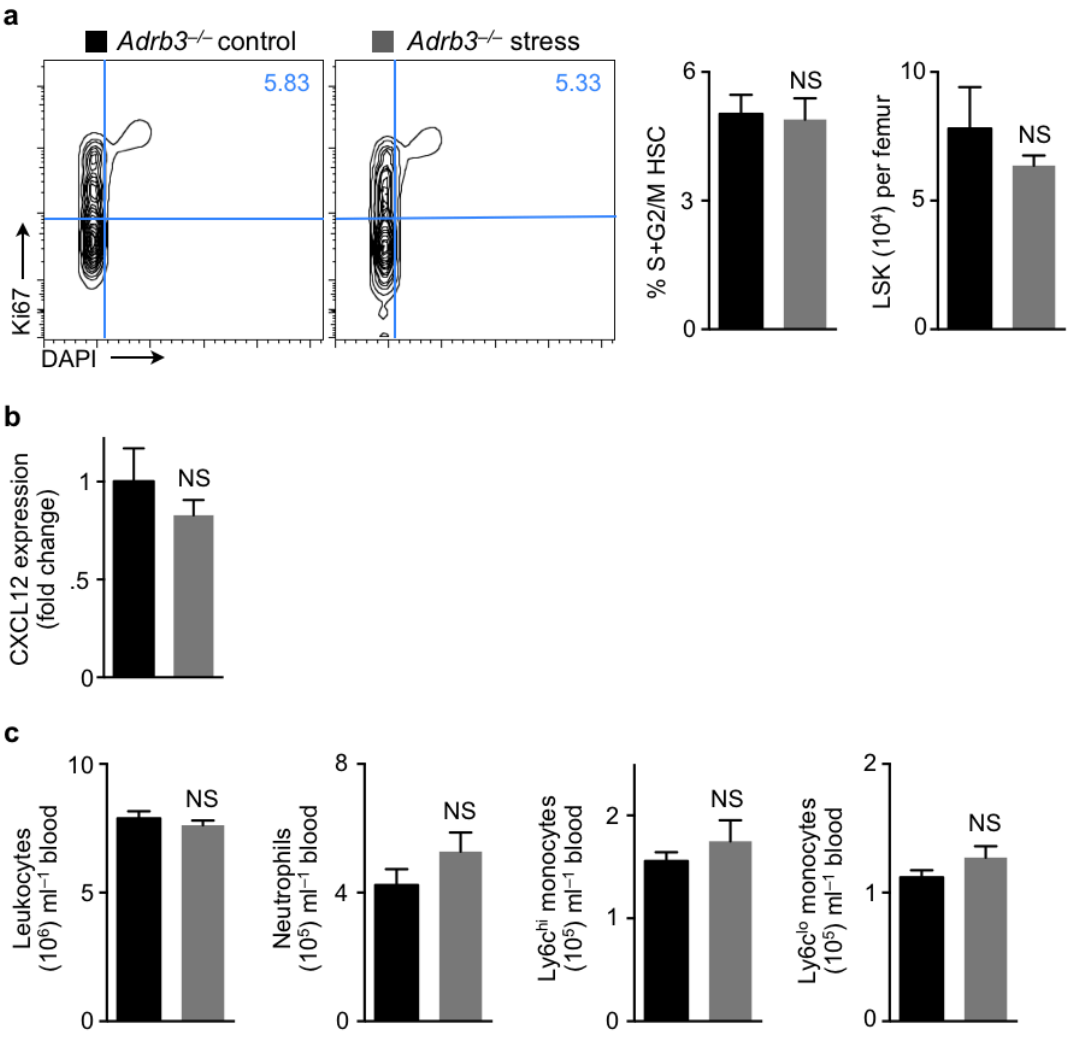
Supplementary Figure 5: Depletion of sympathetic nervous signaling. Tyrosine hydroxylase (TH) Cre–iDTR or controls were injected twice with diphtheria toxin (0.1 µg/kg bodyweight on days 0 and 2, DT). Mice were then exposed to 3 weeks of stress. **a,** Cell cycle analysis of bone marrow HSC (n = 7–8 per group, Mann–Whitney test). **b,** qPCR for relative expression of bone marrow CXCL12 (n = 7–8 per group, Mann–Whitney test). **c,** Quantification of total leukocytes, neutrophils and monocyte subsets in the blood (n = 7–8 per group, Student's t–test). Mean ± s.e.m., * $P < 0.05$.

Supplementary Figure 6



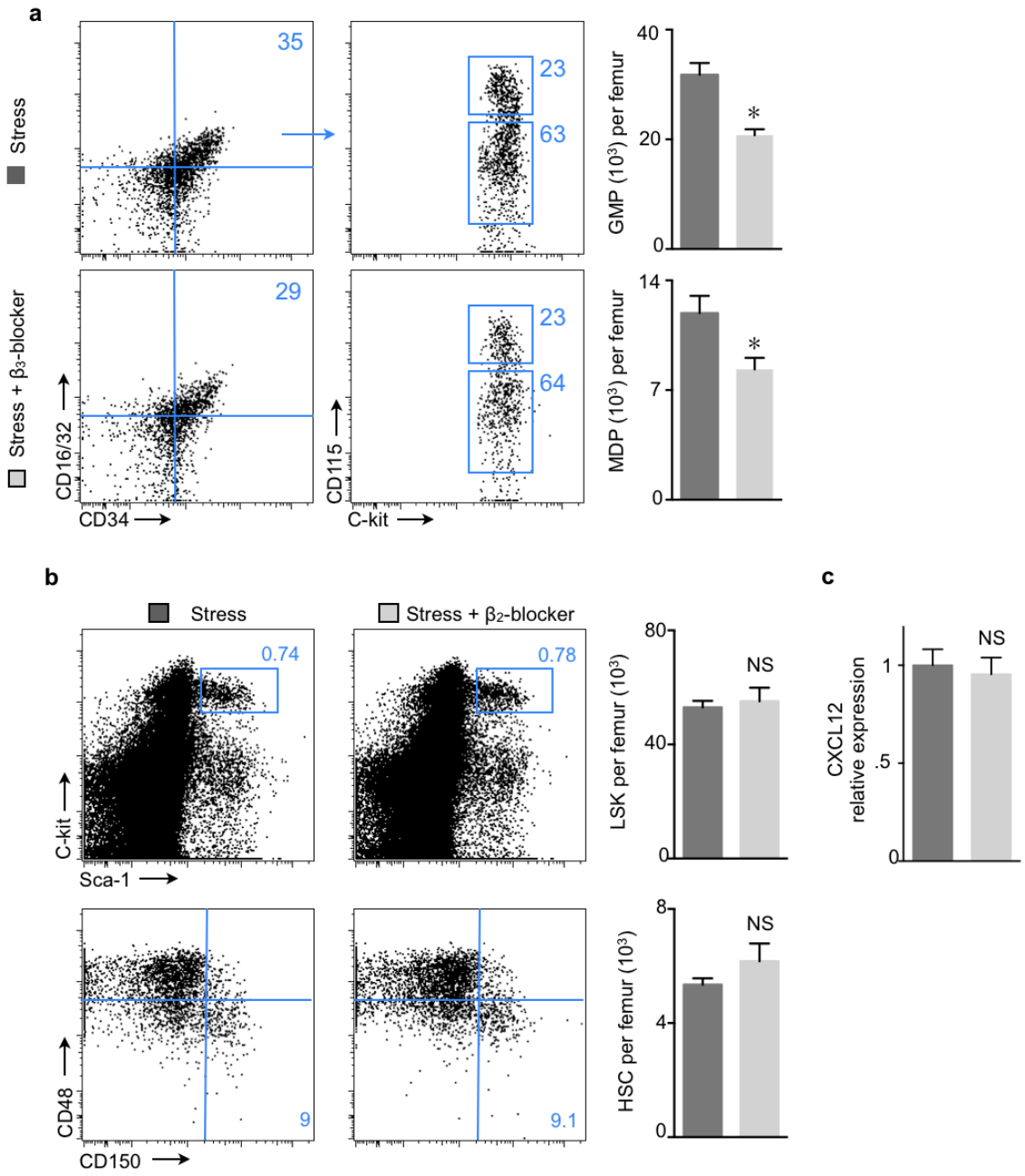
Supplementary Figure 6: Expression of β–adrenergic receptors in the bone marrow. Endothelial cells (EC), osteoblastic lineage cells (OB) and mesenchymal stromal cells (MSC) were FACS–sorted from nestin–GFP mice ($n = 3$). qPCR of sorted cells depicts the expression of β–adrenergic receptors (β_1, β_2, β_3) relative to *Gapdh* for each cell type ($n = 3$, one–way ANOVA). ND: not detectable. Mean ± s.e.m., * $P < 0.05$.

Supplementary Figure 7



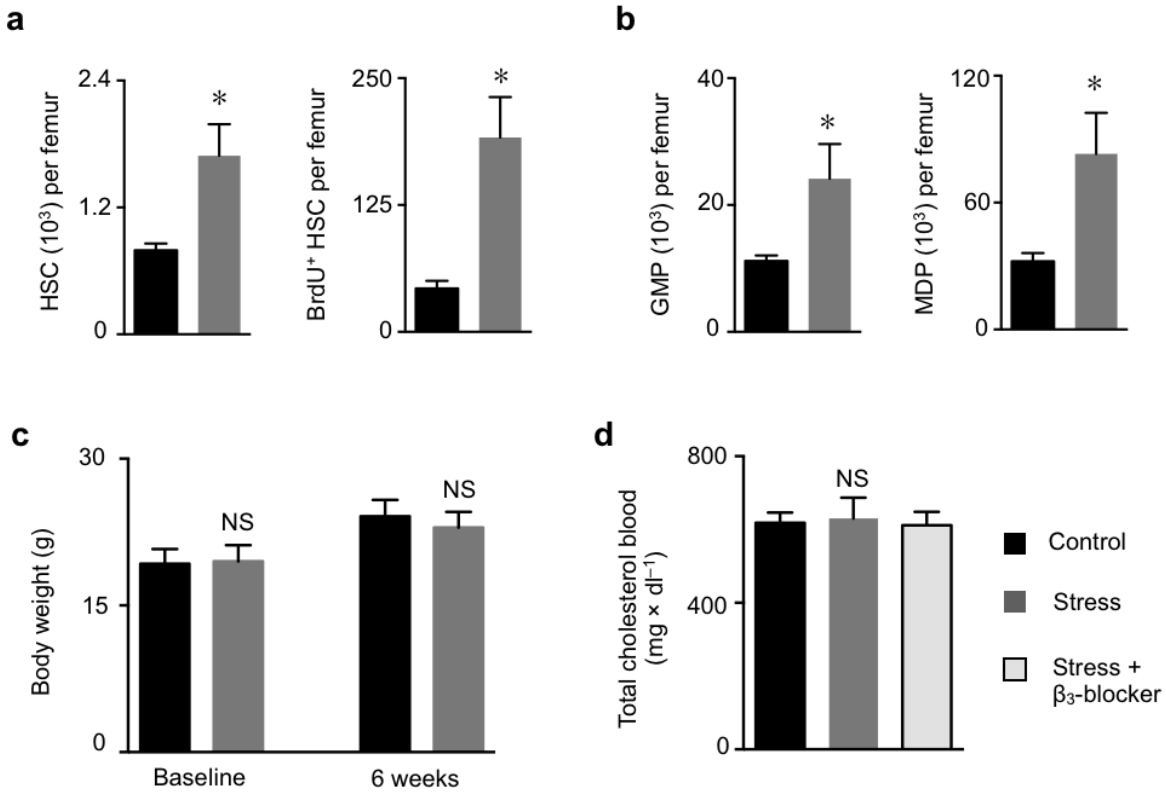
Supplementary Figure 7: β3–adrenoreceptor deficient mice (*Adrb3*$^{-/-}$) were stressed for 3 weeks. **a,** Cell cycle analysis of bone marrow HSC and quantification of LSK (n = 7–9 per group, Student's t–test). **b,** qPCR for relative expression of CXCL12 in the bone marrow (n = 7–9 per group, Student's t–test). **c,** blood leukocyte numbers. Mean ± s.e.m.

Supplementary Figure 8



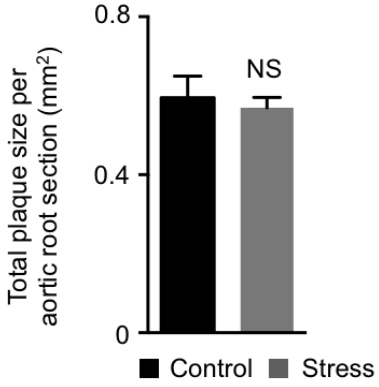
Supplementary Figure 8: β3 adrenoreceptor blocker treatment. a, Dot plots and quantification of GMP (Lin^- c–kit^+ $Sca–1^-$ $CD16/32^+$ $CD34^+$ $CD115^{low}$) and MDP (Lin^- c–$kit^{int/+}$ $Sca–1^-$ $CD16/32^+$ $CD34^+$ $CD115^+$) in the bone marrow of stressed mice after treatment with a β3 adrenoreceptor blocker (n = 5 per group, Mann–Whitney test). **b,** Representative dot plots and quantification of LSK and HSC from mice after 3 weeks of stress with and without β2 adrenoreceptor blocker treatment (*n* = 8 per group, Mann–Whitney test). **c,** qPCR for relative expression of CXCL12 in the bone marrow of stressed mice with or without β2 adrenoreceptor blocker treatment (*n* = 8 per group, Mann–Whitney test). Mean ± s.e.m., * $P < 0.05$.

Supplementary Figure 9



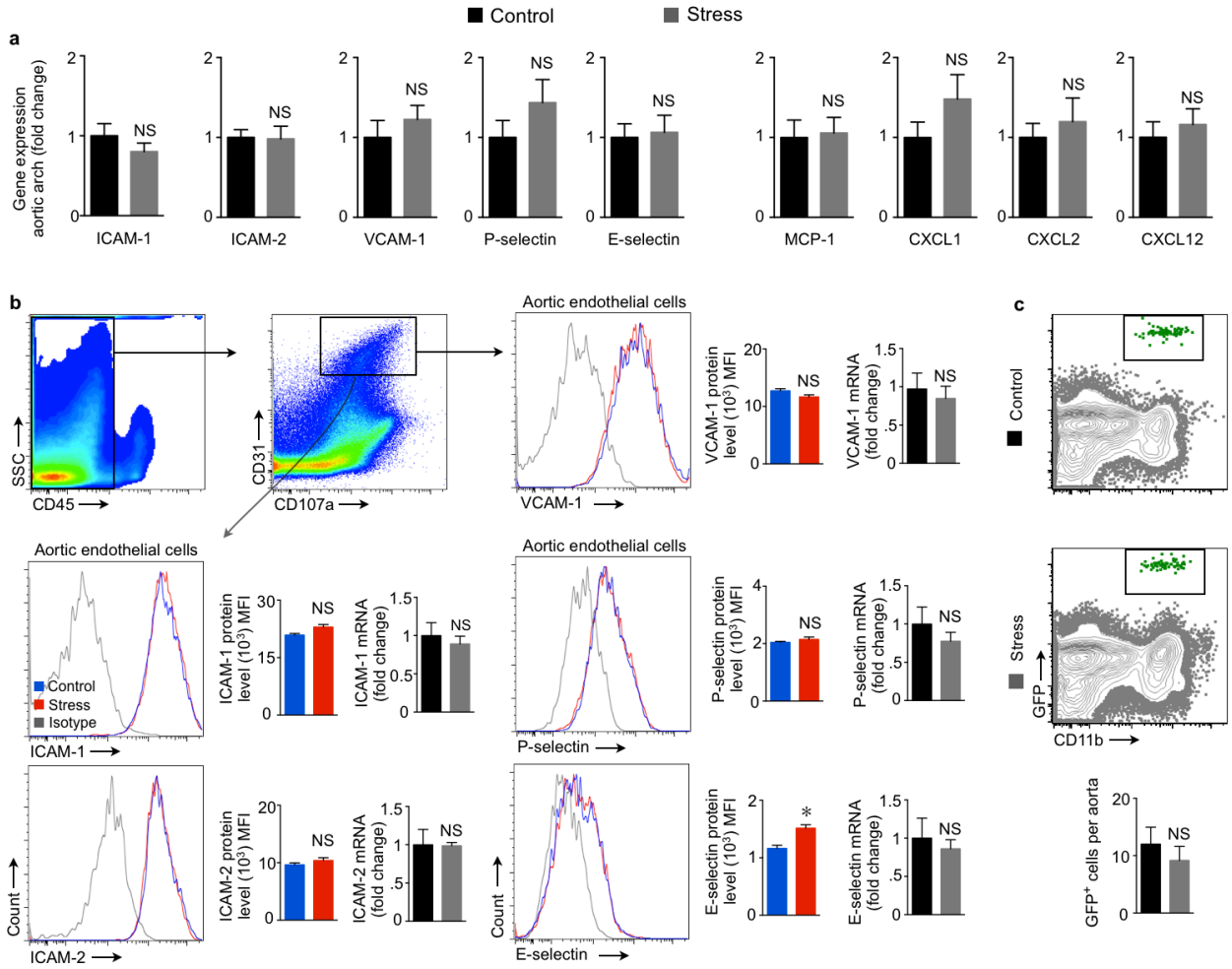
Supplementary Figure 9: Stress in atherosclerotic mice. a, Quantification of HSC numbers and BrdU incorporation 24 hours after BrdU pulse (n = 4–5 per group, Mann–Whitney test) in *ApoE*$^{-/-}$ mice on a high cholesterol diet after 6 weeks. **b**, Quantification of GMP and MDP numbers. **c,** Body weight in *ApoE*$^{-/-}$ mice was measured before and after 6 weeks of stress and high cholesterol diet (n = 15 per group, one–way ANOVA). **d,** Total cholesterol was measured in the blood of *ApoE*$^{-/-}$ mice with or without β_3 adrenoreceptor blocker treatment (n = 5 per group, one–way ANOVA). Mean ± s.e.m. * $P < 0.05$.

Supplementary Figure 10



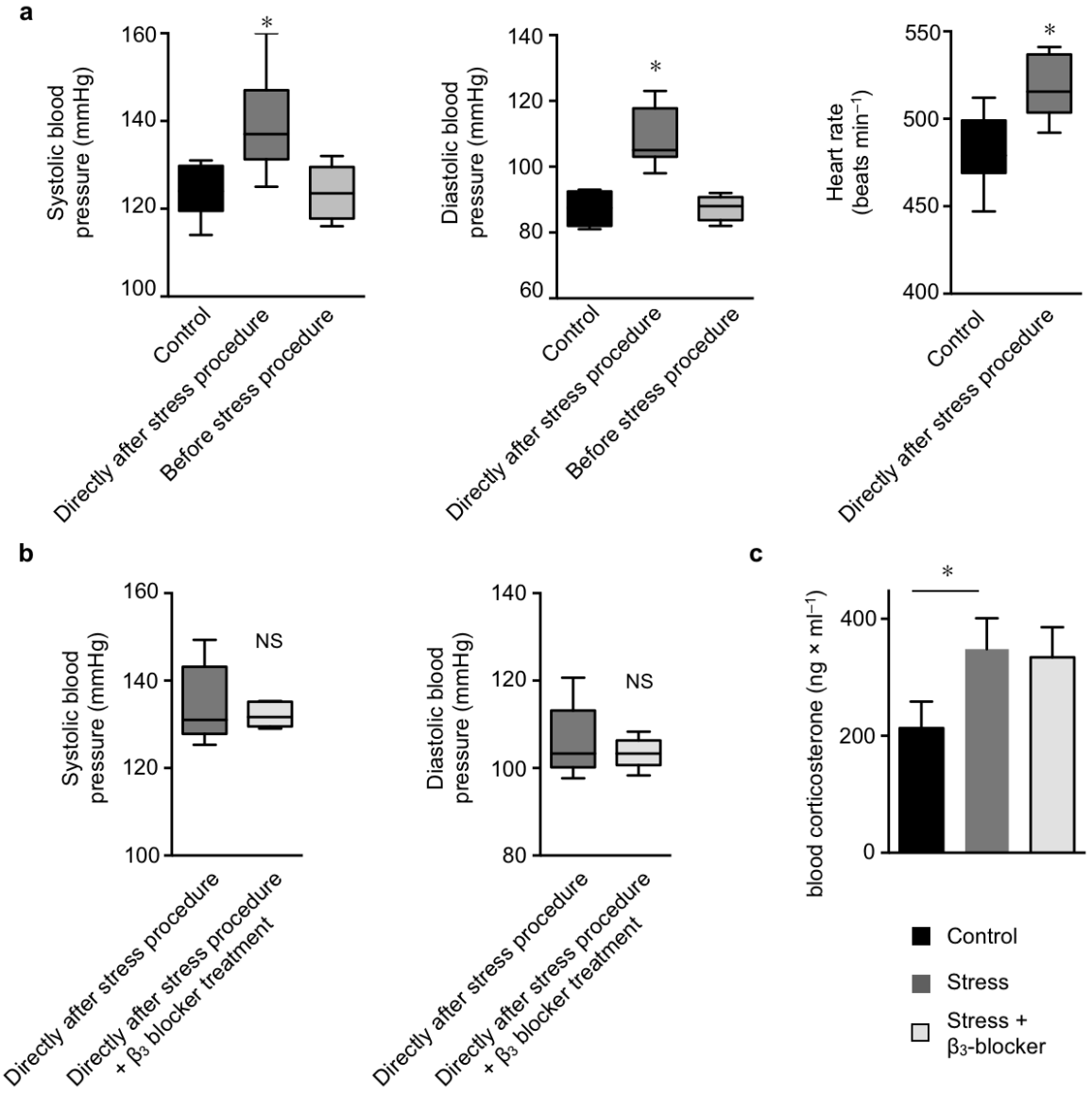
Supplementary Figure 10: Plaque size in aortic root sections of *ApoE*$^{-/-}$ mice after 6 weeks of stress (n = 10 per group, Student's t–test). Mean ± s.e.m.

Supplementary Figure 11



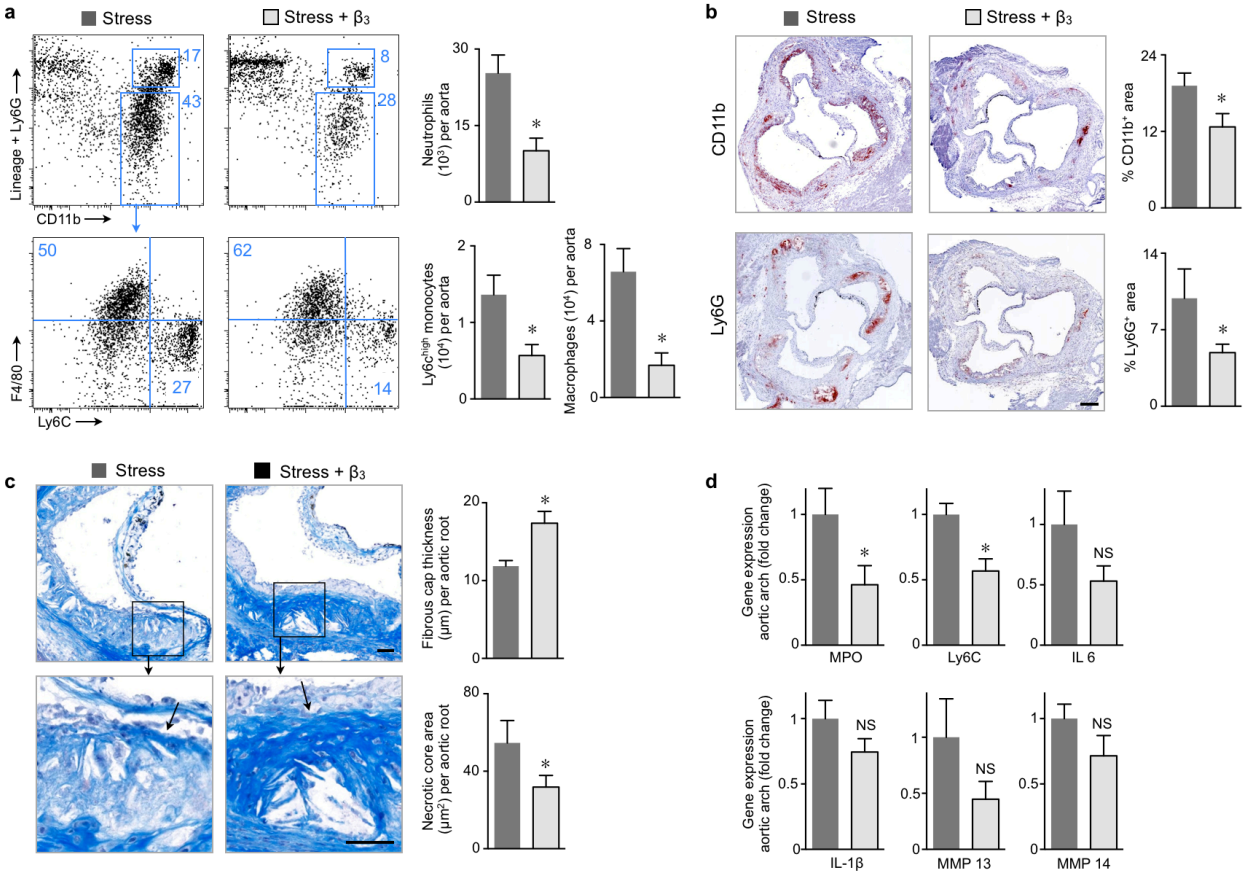
Supplementary Figure 11: Adhesion molecules. a, qPCR for expression of leukocyte adhesion molecules and chemokines in aortae of *ApoE*$^{-/-}$ mice after 6 weeks of stress (n = 9–10 per group, Student's t–test). **b,** Gating endothelial cells and quantification of mean fluorescence intensity (MFI) for leukocyte adhesion molecules in *ApoE*$^{-/-}$ mice after 6 weeks of stress (left bar graphs, n = 8 per group, Mann–Whitney test). Additionally, endothelial cells FACS–sorted from aortae were assessed with qPCR for leukocyte adhesion molecule mRNA (right bar graphs, n = 7 per group, Student's t–test). **c,** Quantification of GFP^{+} myeloid cells in the aorta after adoptive transfer of GFP^{+} Ly6C^{high} monocytes and neutrophils in *ApoE*$^{-/-}$ mice after 6 weeks of stress compared to non–stressed *ApoE*$^{-/-}$ (n = 6 per group, Mann–Whitney test). Mean ± s.e.m., * P < 0.05.

Supplementary Figure 12



Supplementary Figure 12: Stress physiology. a, Blood pressure was measured in non–stressed control mice and in mice after exposure to stress for 6 weeks. In stressed mice, blood pressure was measured before the stress procedure and directly thereafter (n = 8 per group, one–way ANOVA). Heart rate was measured directly after stress (n = 7–8 per group, Mann–Whitney test). Mean ± 95% confidence interval. **b,** Systolic and diastolic blood pressure in *ApoE*$^{-/-}$ mice on a high cholesterol diet after 6 weeks of stress with and without β_3 adrenoreceptor blocker treatment (n = 5 per group, Mann–Whitney test). **c,** ELISA for blood corticosterone in mice after one week of stress with and without β_3 adrenoreceptor blocker treatment (n = 10 per group, one–way ANOVA). Mean ± 95% confidence interval or ± s.e.m. * $P < 0.05$.

Supplementary Figure 13



Supplementary Figure 13: β3 adrenoreceptor blocker treatment dampens stress impact. a, Dot plots and quantification of myeloid cells in aortae of *ApoE*$^{-/-}$ mice after 6 weeks of stress with or without β3 adrenoreceptor blocker treatment (n = 5 per group, Mann–Whitney test). **b,** Histology for CD11b and Ly6G, scale bar indicates 200μm (n = 5 per group, Mann–Whitney test). **c,** Masson Trichrome staining (arrows point at fibrous cap covering a necrotic core). Scale bars depict 50 μm (n = 5 per group, Mann–Whitney). **d,** qPCR for aortic expression of inflammatory genes in *ApoE*$^{-/-}$ mice after 6 weeks of stress with and without β3 adrenoreceptor blocker treatment (n = 5 per group, Mann–Whitney test). Mean ± s.e.m., * $P < 0.05$.

Supplementary Table 1

Day	Procedure	Duration
Day 1	cage tilt	6 hours
Day 2	isolation/ crowding	4 hours / 2 hours
Day 3	damp bedding	6 hours
Day 4	removal of bedding	6 hours
Day 4/5	overnight illumination	12 hours
Day 5	cage tilt	6 hours
Day 6	rapid light-dark changes	2 hours
Day 7	rapid light-dark changes	2 hours

Supplementary Table 1: Mouse stress protocol. Different stressors were used to avoid habituation. The order of stressors changed randomly in consecutive weeks.

Supplementary Table 2

Group	Dose	Response	Tested
Control	15,000	1	7
Control	62,000	7	10
Control	125,000	6	7
Control	500,000	10	10
Stress	15,000	1	10
Stress	62,000	4	8
Stress	125,000	3	5
Stress	500,000	10	10

Supplementary Table 2: Long-term competitive repopulation assay using limiting dilutions of whole bone marrow from stressed or non-stressed CD45.1 donor mice, co–transferred with 5 × 10^5 CD45.2 competitor cells into lethally irradiated CD45.2 recipients. Table lists mice per dilution step and response (> 0.1% multi-lineage blood chimerism).