Figures and figure supplements

Reprogramming of bone marrow myeloid progenitor cells in patients with severe coronary artery disease

Marlies P Noz *et al*

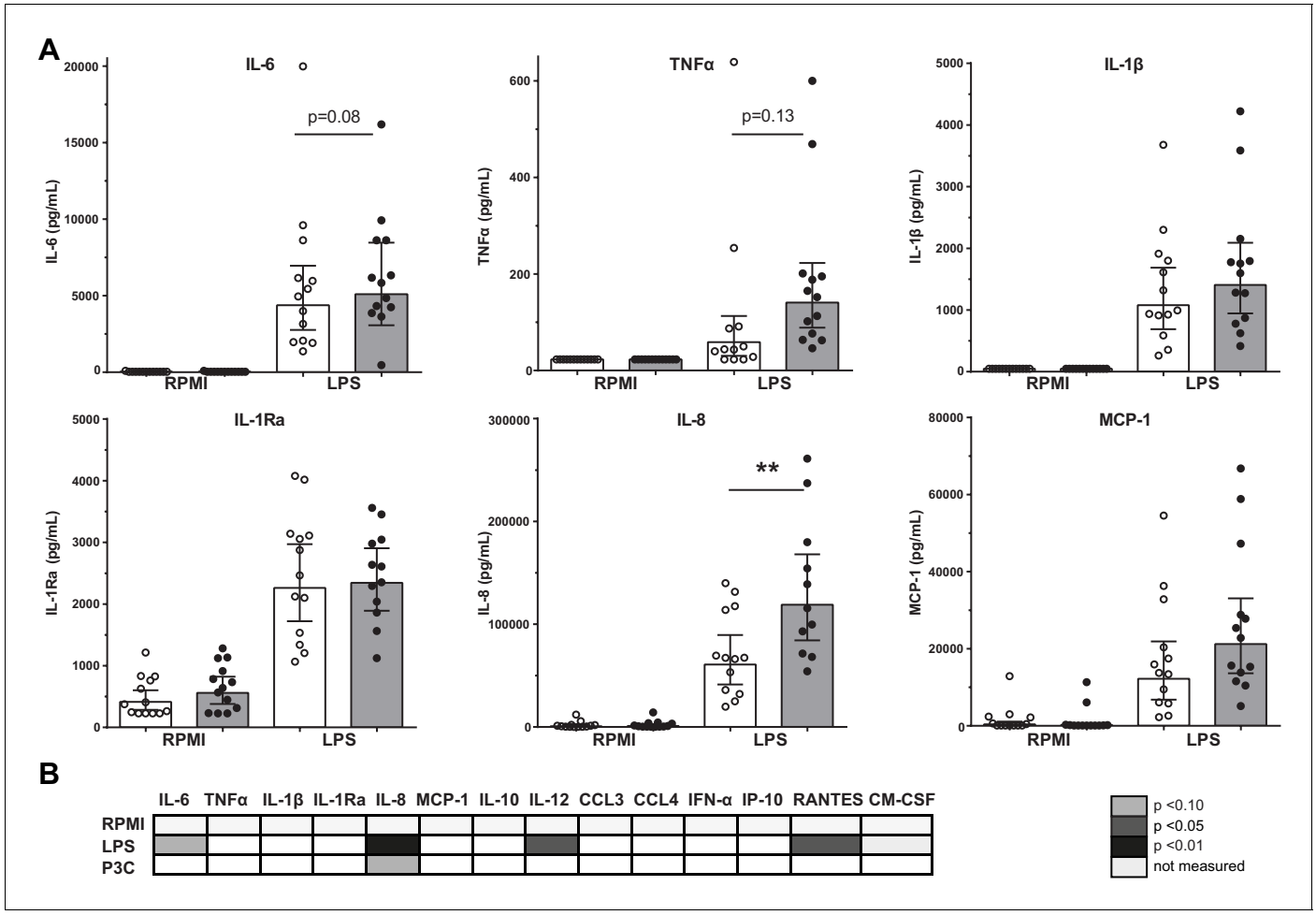


Figure 1. Cytokine production capacity of circulating PBMCs. (**A**) Cytokine production capacity of circulating PBMCs after LPS stimulation in control individuals (white bars, n = 13) and individuals with CAD (gray bars, n = 13). Geometric mean with 95% CI. (**B**) Table of cytokine/chemokine production (x-axis) after stimulation with LPS or P3C (y-axis) of PBMCs showing statistical differences between groups. The p-values are corrected for age and BMI with ANCOVA. Outliers were removed with an SD of >2.5 of Z-scores. * indicates p<0.05, **: p<0.01.

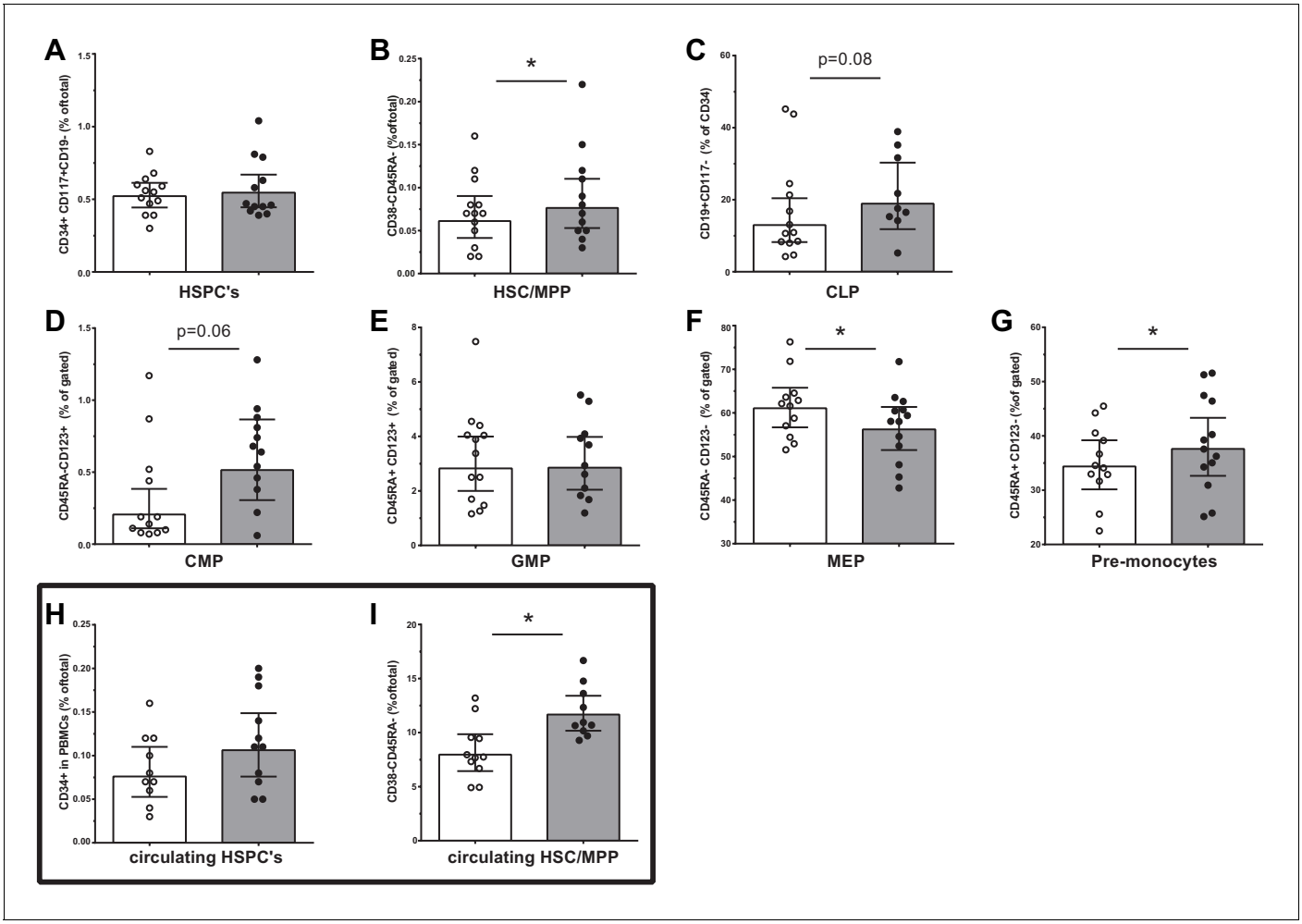
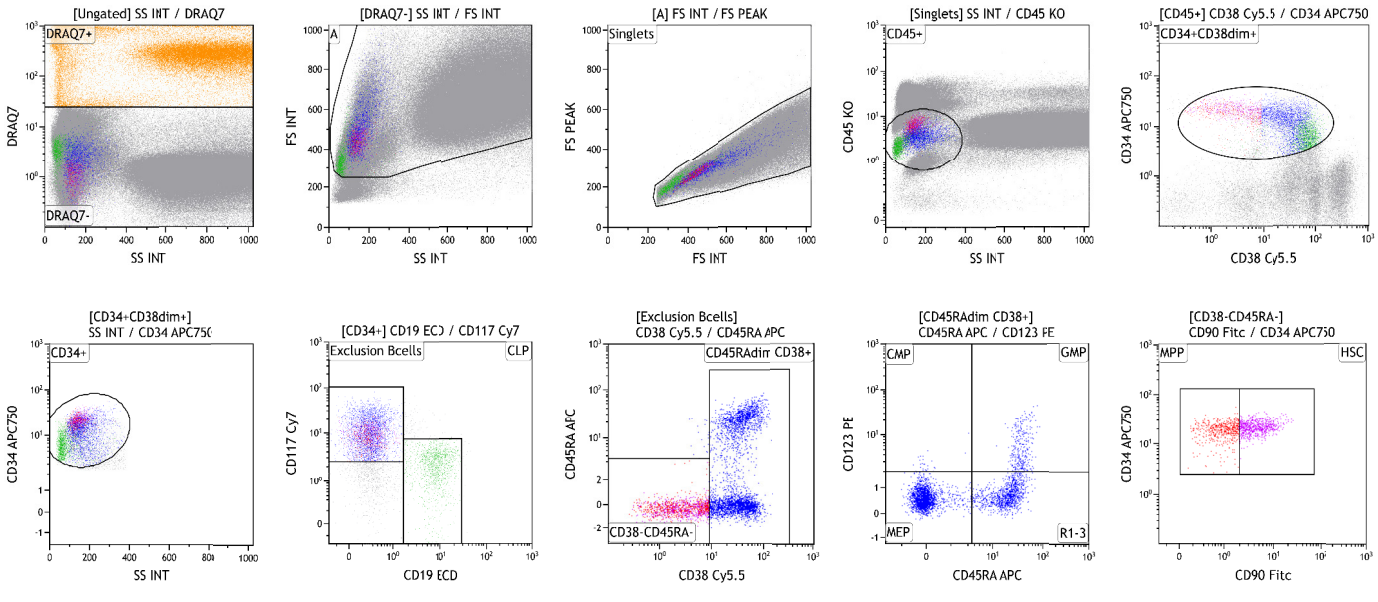


Figure 2. Progenitor cell populations in the bone marrow compartment (**A–G**) and in the circulation (**H and I**). Control individuals (white bars, n = 13) and individuals with CAD (gray bars, n = 13). HSC and MPP cell populations were combined as the CD90 expression marker was not available for n = 6 in each study group. Geometric mean with 95% CI. The p-values are corrected for age and BMI with ANCOVA. * indicates p<0.05, **: p<0.01. In top-down order: HSC indicates hematopoietic stem cell, MPP: multipotent progenitor, CLP: common lymphoid progenitor, CMP: common myeloid progenitor, GMP: granulocyte-macrophage progenitor, MEP: megakaryocyte erythrocyte progenitor.



Abv	Populations of interest	Immunofluorescent markers						
HSC	Hematopoietic stemcell	CD45+	CD34+	CD38-	CD117+	CD19-	CD45RA-	CD90+
MPP	Multipotent progenitor	CD45+	CD34+	CD38-	CD117+	CD19-	CD45RA-	CD90-
CLP	Common lymphoid progenitor	CD45+	CD34+	CD38+	CD117-	CD19+	CD45RA+	
CMP	Common myeloid progenitor	CD45+	CD34+	CD38+	CD117+	CD19-	CD45RA-	CD123+
GMP	Granulocyte macrophage progenitor	CD45+	CD34+	CD38+	CD117+	CD19-	CD45RA+	CD123+
MEP	Megakeratocyte erthrocyte progenitor	CD45+	CD34+	CD38+	CD117+	CD19-	CD45RA-	CD123-
R1-3	Pre-monocyte	CD45+	CD34-	CD38+	CD117+	CD19-	CD45RA+	CD123-

Figure 2—figure supplement 1. Gating strategy of hematopoietic stem and progenitor cells in the bone marrow. HSPCs were defined as CD45+CD34+CD38dim cells, after exclusion of dead cells and doublets. Next, the lymphoid lineage was excluded in CD19-CD117+ cells. In CD45RAdimCD38+ cells, CMP, GMP, MEP, and R1-3 progenitor populations were identified using CD123 and CD45RA expression, see Table for details. CD90 expression in CD38-CD45RA- cells determined MPP and HSC populations.

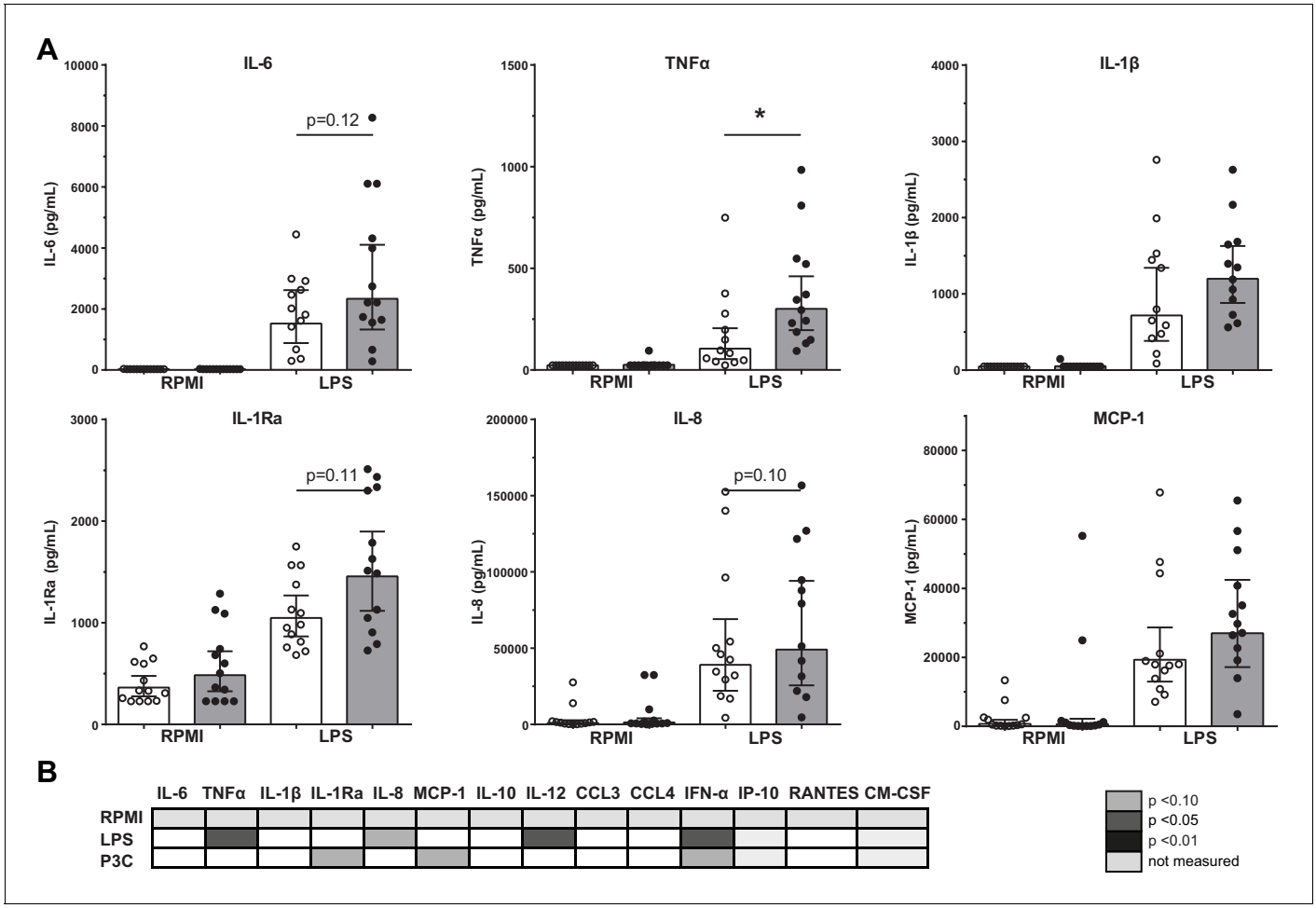


Figure 3. Cytokine production capacity of bone marrow MNCs. (**A**) Cytokine production capacity of BM-MNCs after LPS stimulation in control individuals (white bars, n = 13) and individuals with CAD (gray bars, n = 13). Geometric mean with 95% CI. (**B**) Table of cytokine/chemokine production (x-axis) after stimulation with LPS or P3C (y-axis) of BM-MNCs showing statistical differences between groups. The p-values are corrected for age and BMI with ANCOVA. Outliers were removed with an SD of >2.5 of Z-scores. * indicates p<0.05, **: p<0.01.

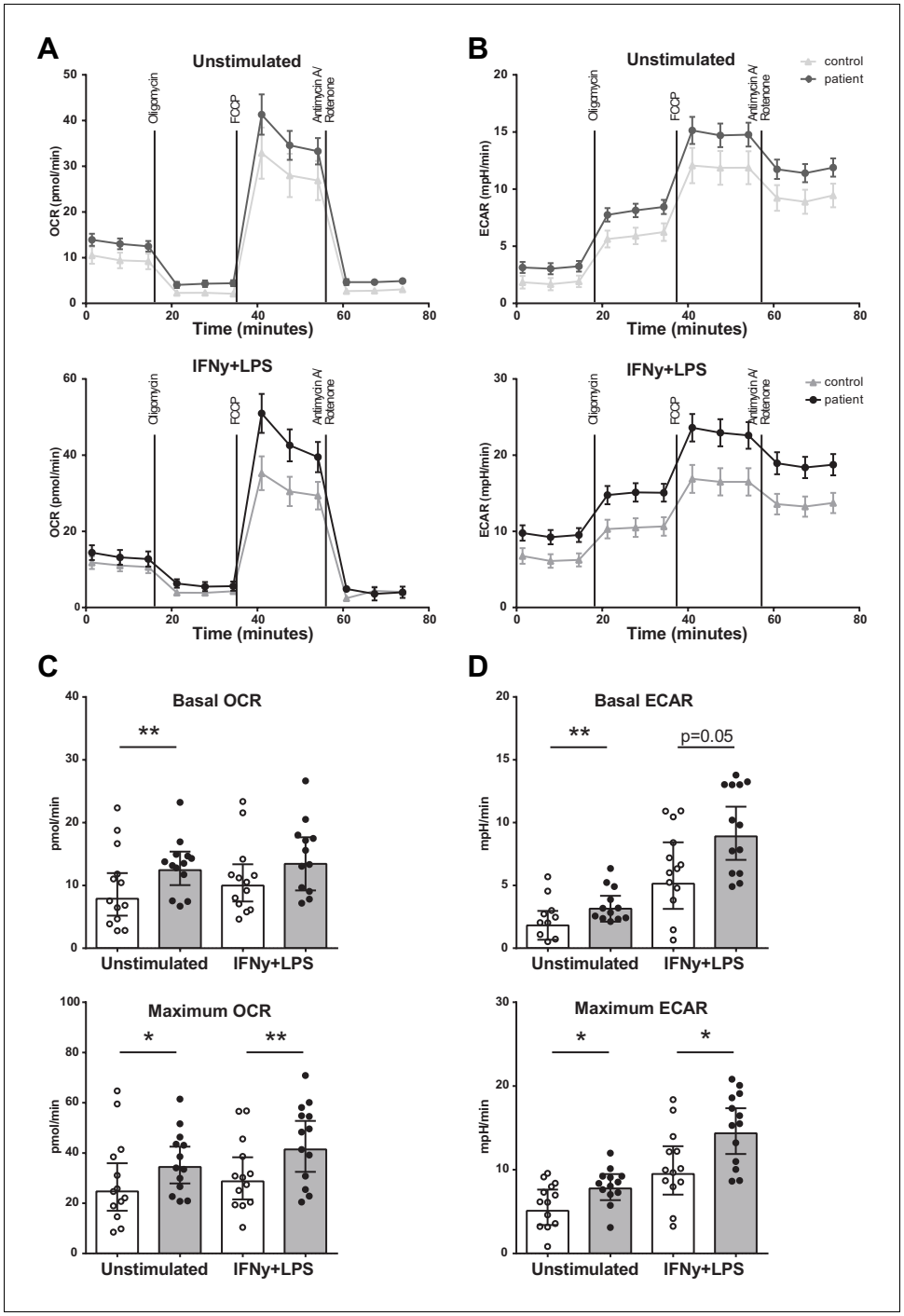


Figure 4. Metabolism of BM-MNCs assessed with Seahorse respirometry in unstimulated condition and 2 hours after IFN-γ+LPS stimulation. (**A, B**) Oxygen consumption and extracellular acidification rates over time using treatment with Oligomycin, FCCP, and Rotenone/Antimycin A. (**C, D**) Bar graphs of control individuals (white bars, n = 13) and individuals with CAD (gray bars, n = 13). Geometric mean with 95% CI. The p-values are corrected for age and BMI with ANCOVA. * indicates p<0.05, **: p<0.01. IFN-γ+LPS: 2 hr IFN-γ and LPS stimulation.

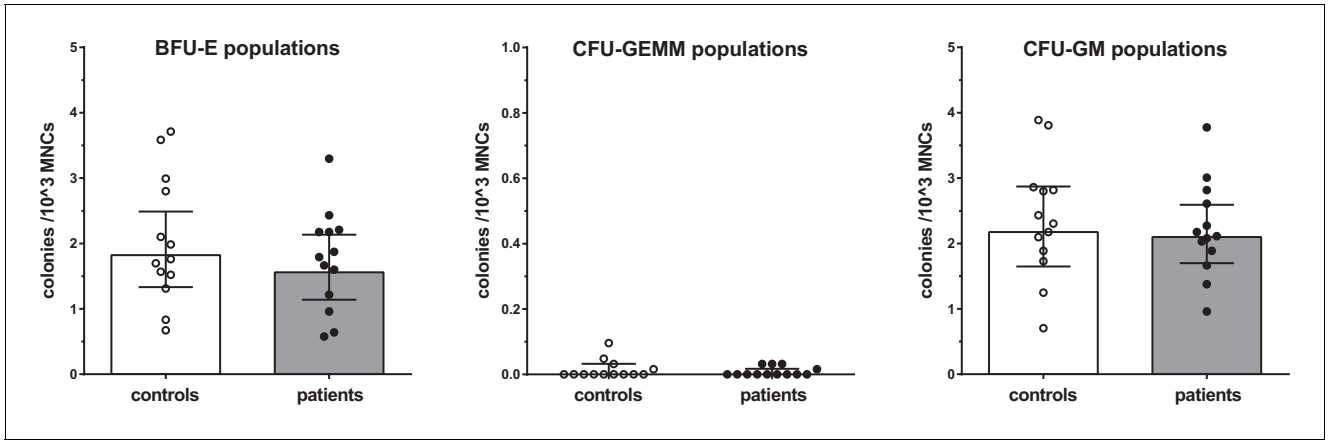


Figure 5. Proliferation capacity of bone marrow MNCs. Counted colonies per 10^3 cultured BM-MNCs of control individuals (white bars, n = 13) and individuals with CAD (gray bars, n = 13). Geometric mean with 95% CI. The p-values are corrected for age and BMI with ANCOVA. BFU-E indicates erythroid progenitor population, CFU-GEMM: myeloid progenitor population, CFU-GM: granulocyte-macrophage progenitor population.

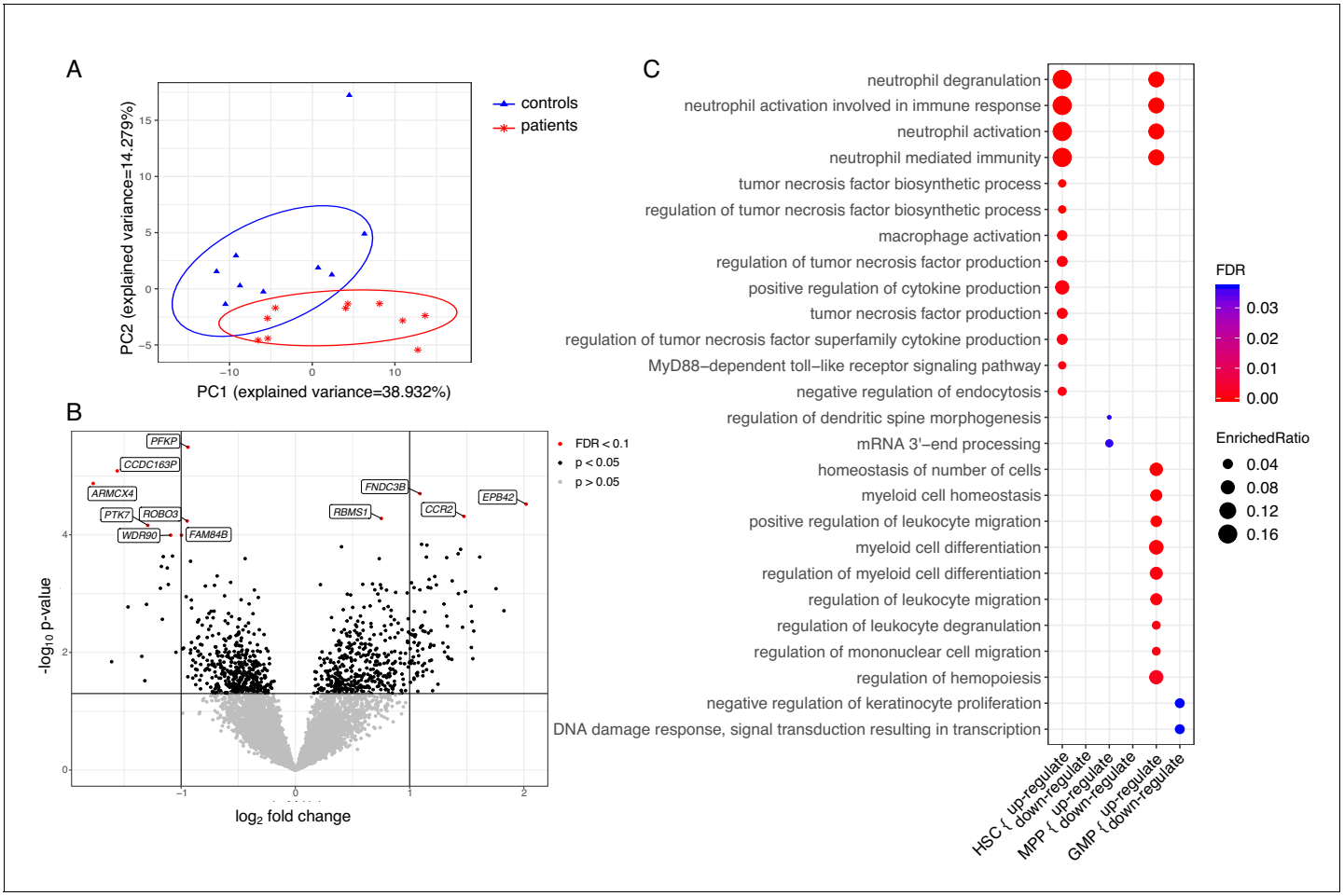


Figure 6. Transcriptome analyses of HSC, MPP, and GMP populations. Control individuals (n = 10) versus individuals with CAD (n = 10) for each cell population. (**A**) Principle component analysis (PCA) based on differentially expressed (DE) genes of the HSC population; (**B**) Volcano plot showing differential expressed genes between patients with CAD and individuals without atherosclerosis, controlled for age, in a combined analysis of HSC, MPP, and GMP population. Genes with an FDR <0.1 are named; (**C**) Gene ontology enrichment analysis of DE genes from HSCs, MPPs, and GMPs, depicting the FDR and enrichment ratio.

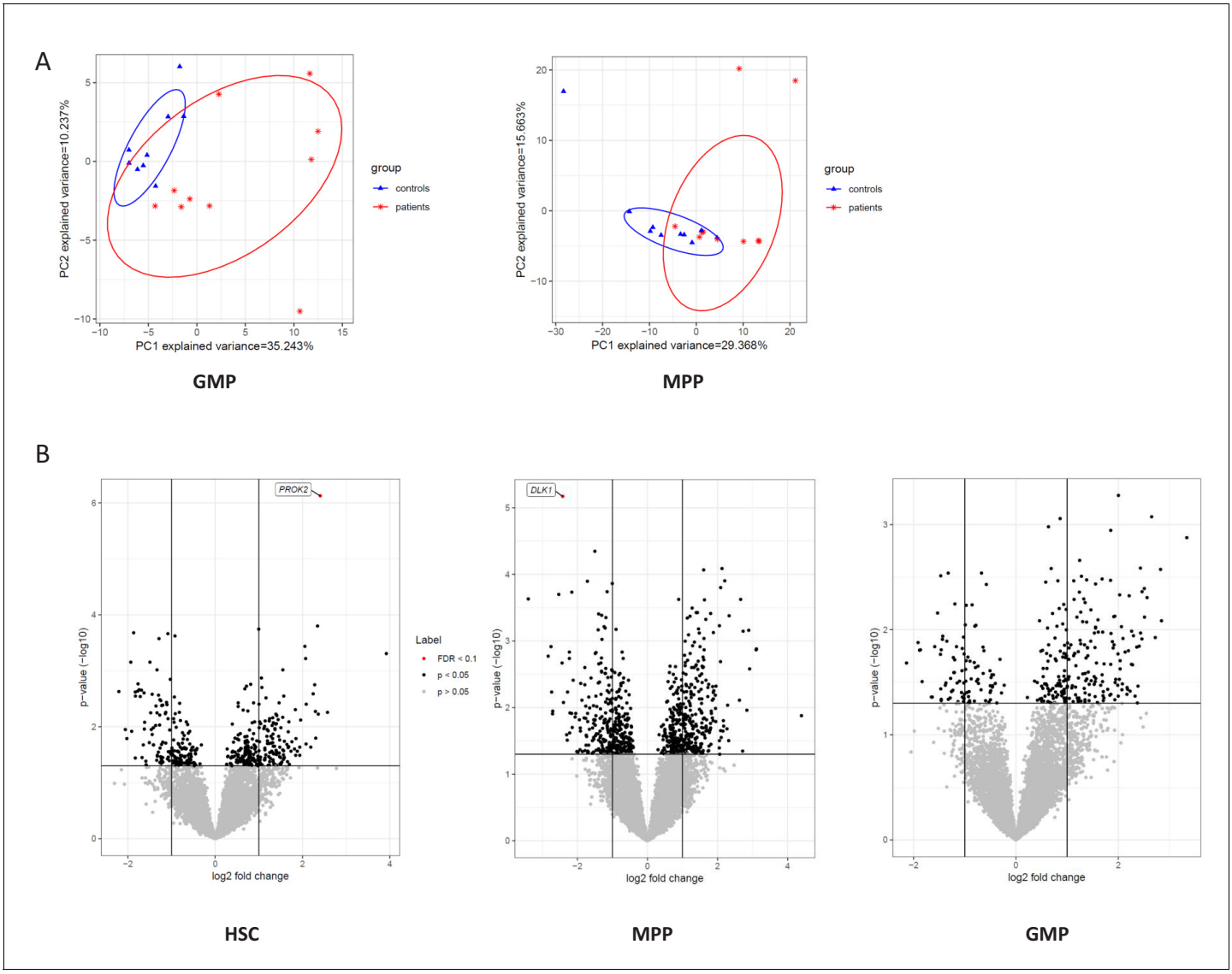


Figure 6—figure supplement 1. Transcriptome of HSC, MPP, and GMP populations. (**A**) PCA analysis based on DE genes of the GMP and MPP cell population; (**B**) Volcano plot showing differential expressed genes between CAD patients and controls, controlled for age, in the HSC, MPP, and GMP cell populations. Genes with an FDR <0.1 are called out.

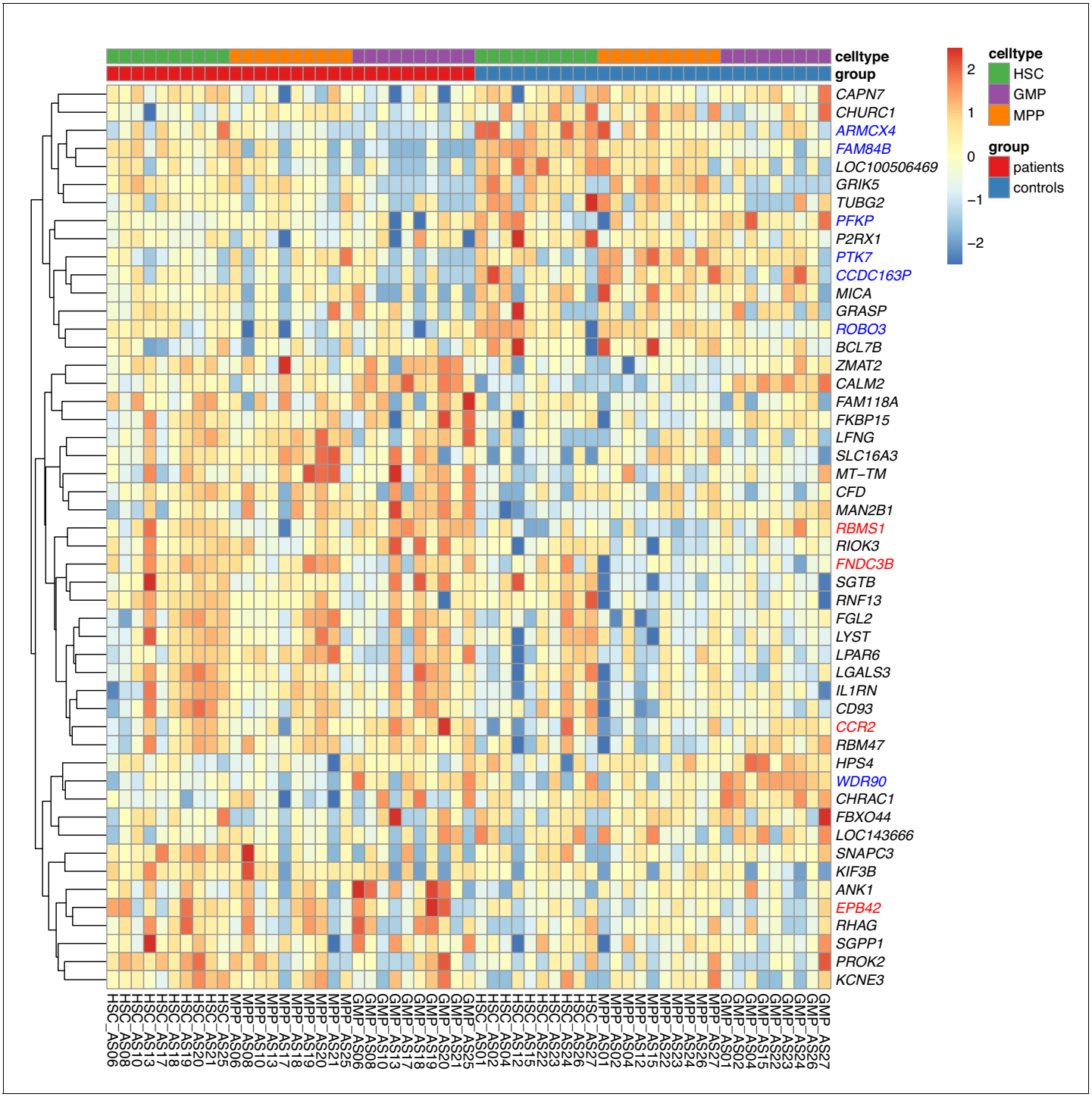


Figure 6—figure supplement 2. Combined heatmap showing the top 50 DE genes for the HSC, MPP, and GMP cell populations in the patients and the control subjects. Genes with an FDR <0.1 are colored in red and blue for up- and down-regulated, respectively.

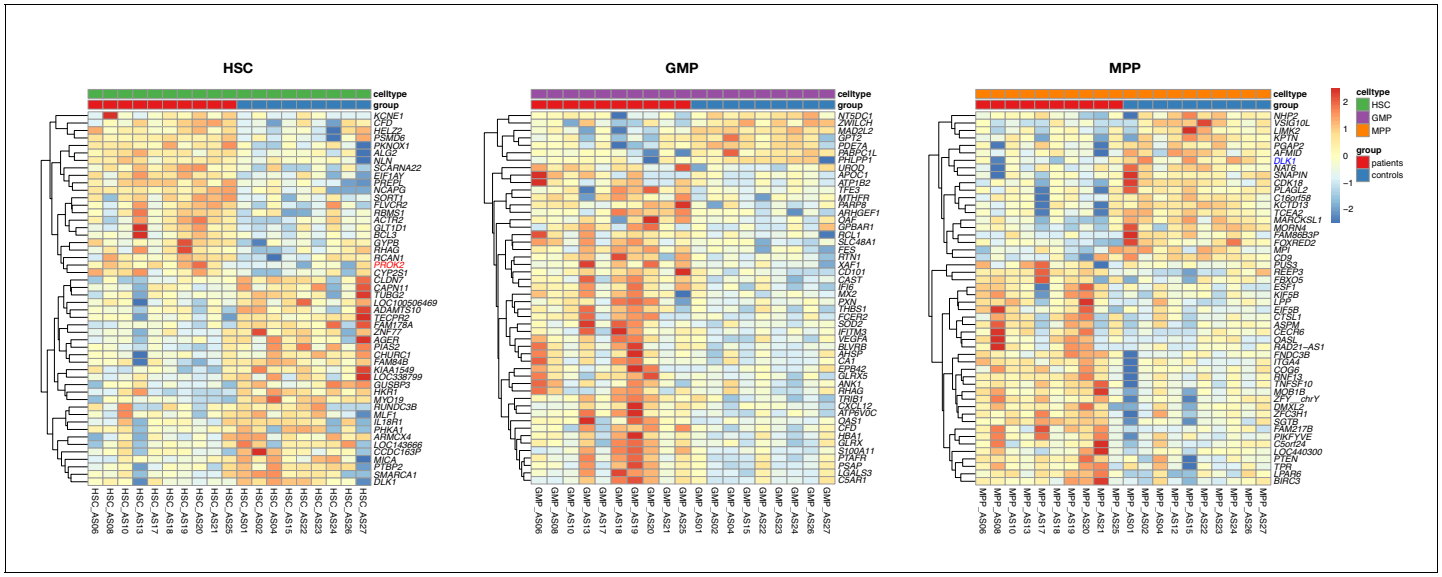


Figure 6—figure supplement 3. Separated heatmap showing the top 50 DE genes for each of the HSC, MPP, and GMP cell populations in the patients and the control subjects. Genes with an FDR <0.1 are colored in red and blue for up- and down-regulated, respectively.

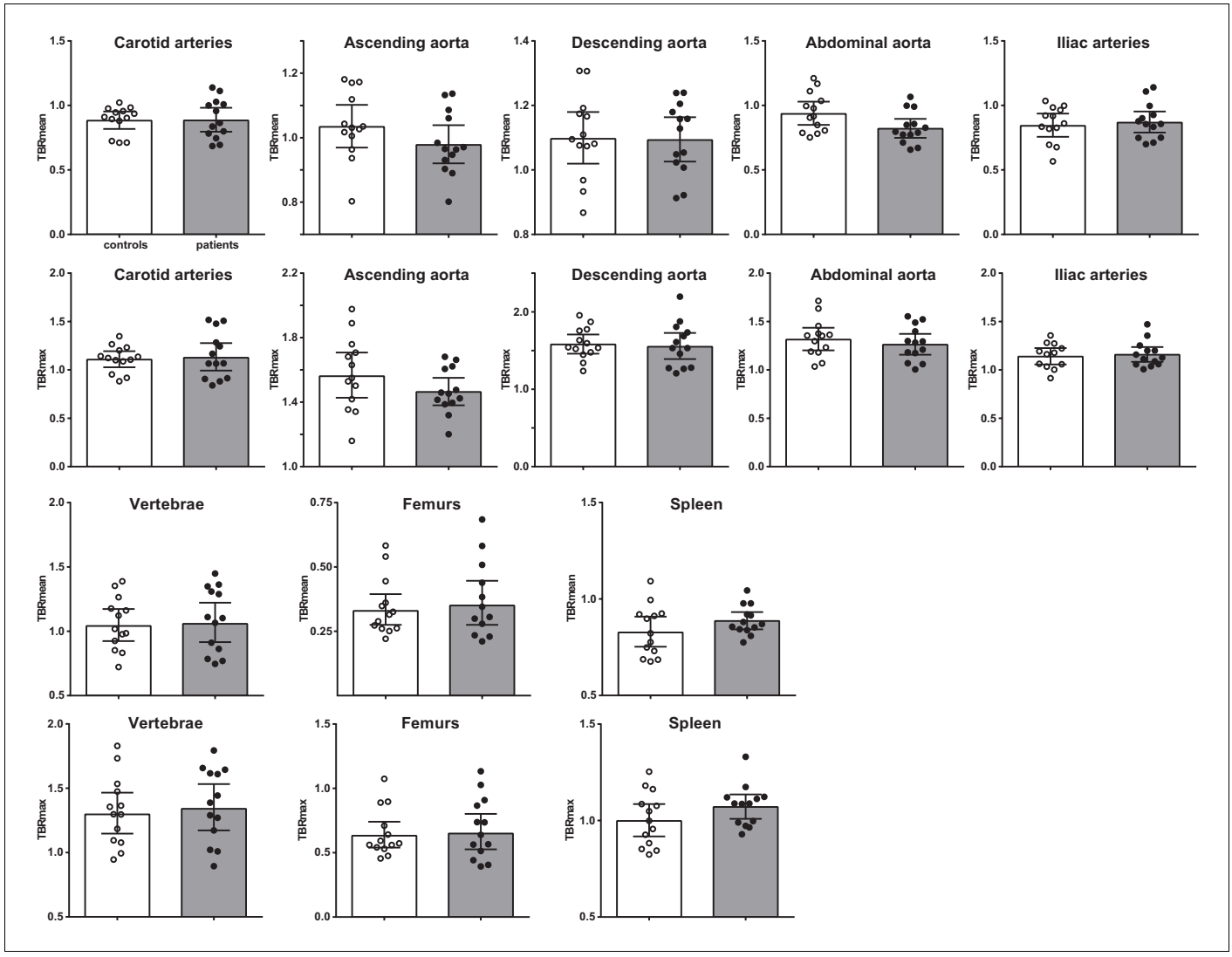


Figure 7. Vascular wall inflammation and hematopoietic tissue activation on [^{18}F]FDG PET/CT scan. Standard uptake value of each region in control individuals (white bars, n = 13) and individuals with CAD (gray bars, n = 13). Geometric mean with 95% CI. The p-values are corrected for age and BMI with ANCOVA.* indicates p<0.05, **: p<0.01. TBR: target SUV/mean blood pool SUV or mean liver SUV as background.

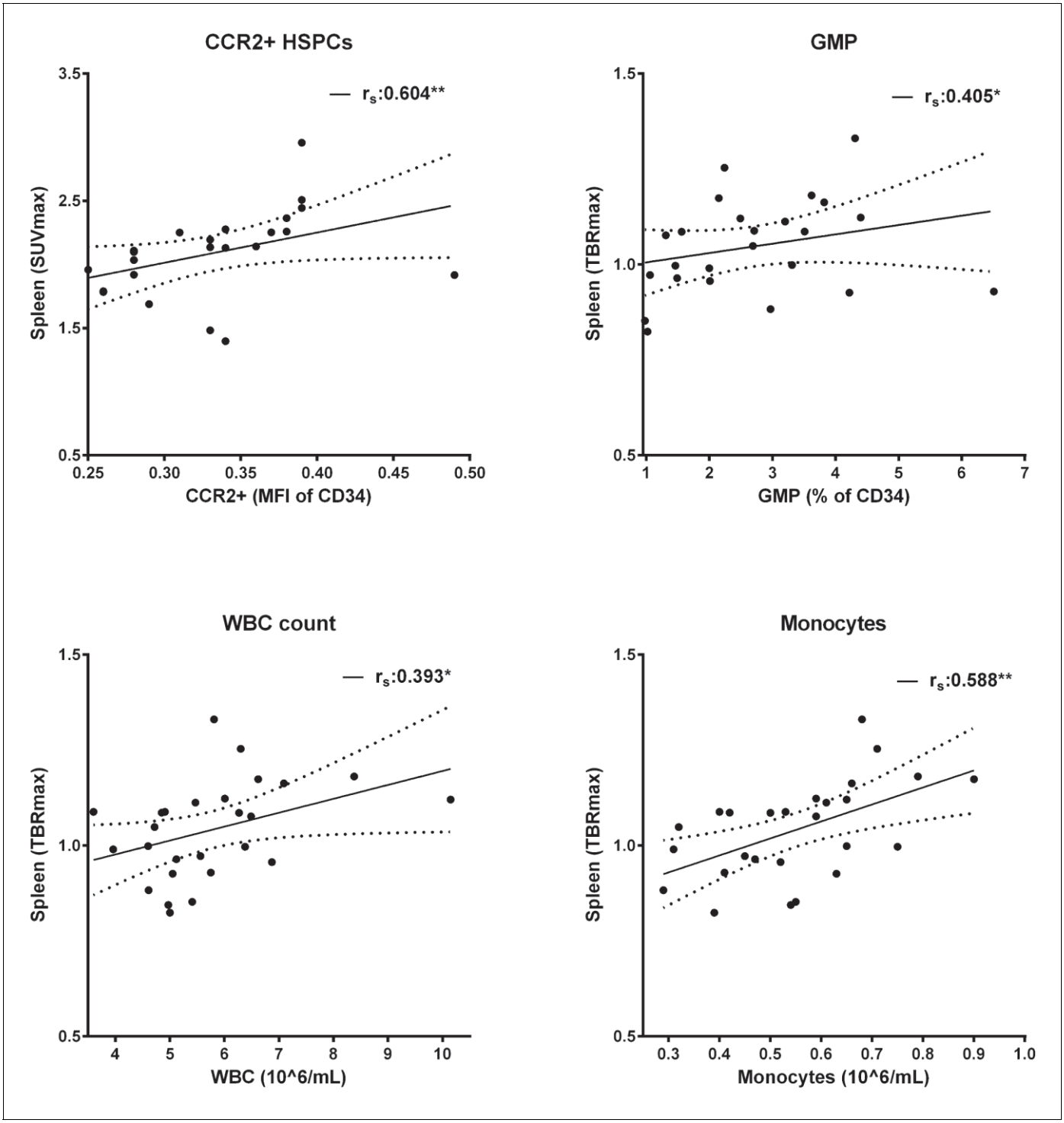


Figure 7—figure supplement 1. Splenic activity correlates with progenitor cells and circulating immune cells. Linear regression with 95% CI (n = 26). Spearman correlation coefficient (r_s). * indicates p<0.05, **: p<0.01. HSPCs: hematopoietic stem and progenitor cells, GMP: granulocyte macrophage progenitor cells, WBC: white blood cells.