Supplemental data for

Bastiaansen *et al*, “The lysine acetyltransferase PCAF is a key regulator of arteriogenesis”

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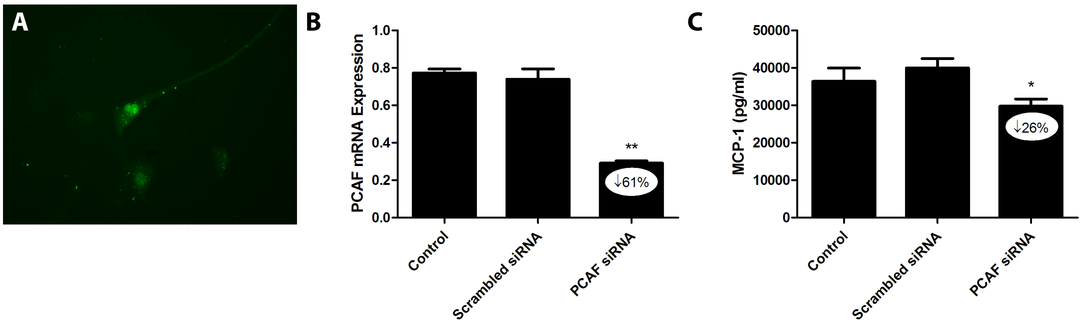
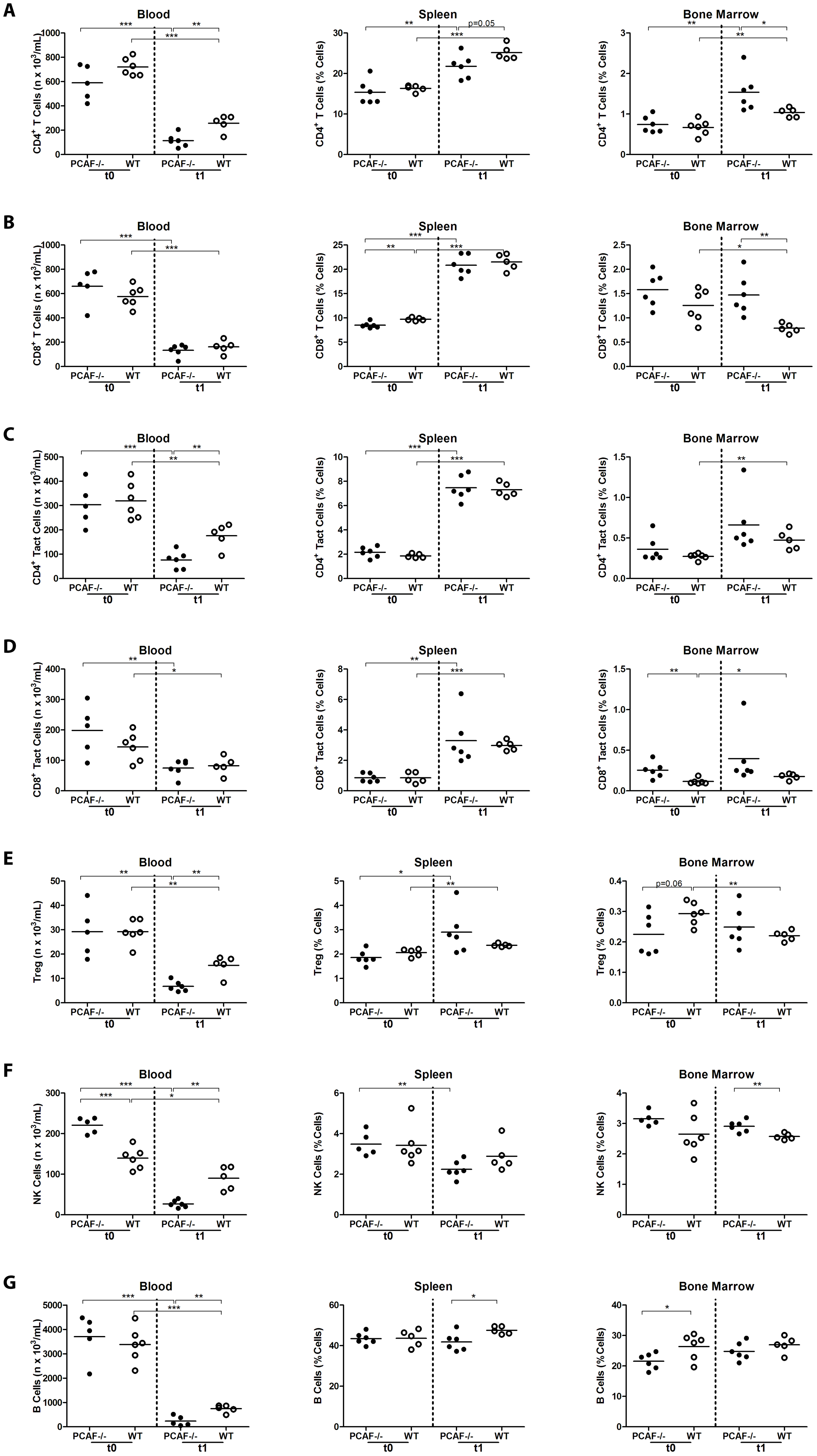
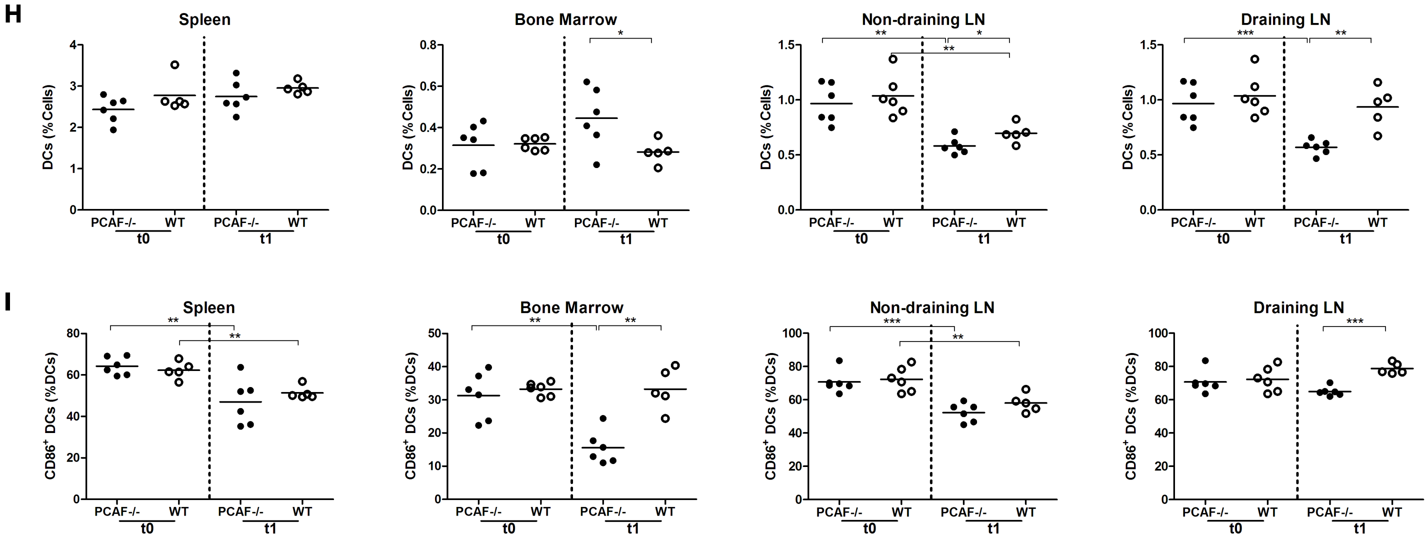


Supplemental Figure I. PCAF knockdown by siRNA in vascular smooth muscle cells. (A) Vascular smooth muscle cells (VMSCs) were transfected with non-target fluorescent siGlow (Dharmacon) to test transfection efficiency, using Lipofectamine 2000 according to the manufacturer's instructions. VSMCs were visualized on a Leica fluorescence microscope. (B) VSMCs were plated and transfected with control short-interfering RNA (siRNA) or a combination of 4 siRNAs directed towards PCAF for 4 hours. Untransfected VMSCs were used as control. VSMCs were incubated with LPS (1 ng/ml) for 24 hours. To confirm PCAF knockdown, PCAF mRNA was measured by real-time quantitative PCR. Levels were normalized against the expression of HPRT1. PCAF specific siRNA reduced PCAF expression with 61% in comparison to scrambled siRNA. (C) Cell-free supernatant of LPS stimulated VSMCs was collected for MCP-1 quantification, measured by ELISA. Transfection with PCAF specific siRNA inhibited MCP-1 production of VSMCs in comparison to scrambled siRNA. All samples were performed in triplicates. *P < .05, **P < .01, scrambled siRNA versus PCAF siRNA.

Supplemental Figure II. Differential inflammatory gene expression in PCAF-/- and WT mice. Heatmap of differentially expressed inflammatory genes in adductor muscle group of PCAF-/- and WT mice, 1 day after HLI. Gene definitions containing any of these criteria (interleukin, chemokine, interferon, TGF, TNF, NFKB) were selected. Included are genes that were significantly different between PCAF-/- and WT mice (q-value < 5). Data are presented as the fold change in expression between day 1 and average preoperative baseline levels, generating t1/t0avg ratios. Red indicates increased and green indicates reduced expression relative to average baseline levels.


A
Blood
CD4+ T Cells (n x 10³/mL)
Spleen
CD4+ T Cells (% Cells)
p=0.05
Bone Marrow
CD4+ T Cells (% Cells)
B
Blood
CD8+ T Cells (n x 10³/mL)
Spleen
CD8+ T Cells (% Cells)
Bone Marrow
CD8+ T Cells (% Cells)
C
Blood
CD4+ Tact Cells (n x 10³/mL)
Spleen
CD4+ Tact Cells (% Cells)
Bone Marrow
CD4+ Tact Cells (% Cells)
D
Blood
CD8+ Tact Cells (n x 10³/mL)
Spleen
CD8+ Tact Cells (% Cells)
Bone Marrow
CD8+ Tact Cells (% Cells)
E
Blood
Treg (n x 10³/mL)
Spleen
Treg (% Cells)
Bone Marrow
Treg (% Cells)
p=0.06
F
Blood
NK Cells (n x 10³/mL)
Spleen
NK Cells (%Cells)
Bone Marrow
NK Cells (%Cells)
G
Blood
B Cells (n x 10³/mL)
Spleen
B Cells (%Cells)
Bone Marrow
B Cells (%Cells)
PCAF-/-
WT
t0
t1



Supplemental Figure III. Leukocyte subtypes in PCAF-/- and WT mice after HLI. Flow cytometry analysis of lymphocytes in blood (nx103/mL), spleen and bone marrow (% of total cells). In succession, values are presented for (A) CD4+ T helper cells, (B) CD8+ cytotoxic T cells, (C) activated CD4+ T helper cells, (D) activated CD8+ cytotoxic T cells, (E) regulatory T cells (Treg), (F) Natural Killer (NK) cells and (G) B lymphocytes. (H) Dendritic cells (DCs) in spleen, bone marrow, non-draining and draining (inguinal) lymph nodes (LN). (I) Activated DCs in spleen, bone marrow, non-draining and draining (inguinal) lymph nodes.

Symbol	Gene Name	PCAF-/-	WT		Ratio PCAF$^{-/-}$ vs WT
		logFC	logFC	q-value	FC
Arg1	arginase 1, liver	1.55	3.11	4.39	0.34
Pdk4	pyruvate dehydrogenase kinase, isoenzyme 4	0.17	1.29	3.00	0.46
Ptpn6	protein tyrosine phosphatase, non-receptor type 6, transcript variant 2	0.55	1.54	0.86	0.50
Lst1		0.37	1.36	0.86	0.50
Anxa2	annexin A2 (Anxa2), mRNA.	0.39	1.37	0.69	0.51
Serpinb1a	serine (or cysteine) peptidase inhibitor, clade B, member 1a	0.31	1.28	1.23	0.51
Slfn1	schlafen 1	0.79	1.74	1.66	0.52
Mmp9	matrix metallopeptidase 9	0.07	1.01	0.69	0.52
Cotl1	coactosin-like 1	0.66	1.60	1.23	0.52
Cd52	CD52 antigen	1.41	2.33	4.39	0.53
Tagln2	transgelin 2	0.23	1.15	0.86	0.53
Ccl9	chemokine (C-C motif) ligand 9	1.91	2.81	3.60	0.54
LOC100046120	PREDICTED: similar to clusterin	1.06	1.94	1.23	0.54
Angptl4	angiopoietin-like 4	0.43	1.31	3.60	0.54
Cfp	complement factor properdin	0.37	1.23	0.40	0.55
Cotl1	coactosin-like 1	0.42	1.25	0.59	0.56
Kcnab2	potassium voltage-gated channel, shaker-related subfamily, beta member 2	0.37	1.16	0.59	0.58
LOC100044439	PREDICTED: similar to cytochrome P450 CYP4F18	0.40	1.19	1.66	0.58
Sirpa	signal-regulatory protein alpha	0.55	1.33	1.94	0.58
Fbxo32	F-box protein 32	0.86	1.63	4.39	0.59
Alox5ap	arachidonate 5-lipoxygenase activating protein	1.13	1.87	1.94	0.60
Arhgdib	Rho, GDP dissociation inhibitor (GDI) beta	0.32	1.04	1.23	0.61
Emilin2	elastin microfibril interfacer 2	0.10	0.82	0.86	0.61
Lrrc33	leucine rich repeat containing 33	0.42	1.12	0.86	0.62
Fbxl22	F-box and leucine-rich repeat protein 22	0.26	0.96	2.42	0.62
Emilin2	elastin microfibril interfacer 2	0.12	0.80	1.94	0.62
Cyth4	cytohesin 4	0.74	1.41	4.39	0.63
Laptm5	lysosomal-associated protein transmembrane 5	0.92	1.59	0.98	0.63
Sdc3	syndecan 3	0.07	0.73	0.86	0.64
Ly6e	lymphocyte antigen 6 complex, locus E	0.01	0.66	0.33	0.64
Hdac4	histone deacetylase 4	0.02	0.66	0.00	0.64
Dok2	docking protein 2	0.50	1.14	1.94	0.64
Fcgr4	Fc receptor, IgG, low affinity IV	1.63	2.26	3.60	0.64
Alox5ap	arachidonate 5-lipoxygenase activating protein	0.85	1.48	2.42	0.65
Cap1	CAP, adenylate cyclase-associated protein 1	0.26	0.89	0.98	0.65
Tgfbi	transforming growth factor, beta induced	0.36	0.99	3.00	0.65
Aif1	allograft inflammatory factor 1	0.23	0.84	1.23	0.65
Mcm5	minichromosome maintenance deficient 5, cell division cycle 46	0.07	0.68	0.00	0.65
Fes	feline sarcoma oncogene	0.33	0.95	0.45	0.65
Gnb2	guanine nucleotide binding protein (G protein), beta 2	0.07	0.68	0.00	0.66
Cyba	cytochrome b-245, alpha polypeptide	0.56	1.17	1.23	0.66
Grn	granulin	0.21	0.81	0.00	0.66
Fxyd5	FXYD domain-containing ion transport regulator 5	0.78	1.38	3.60	0.66
Cdh15	cadherin 15	0.09	0.69	0.33	0.66
Rabgef1	RAB guanine nucleotide exchange factor	0.24	0.82	0.45	0.67
Oas1g	2'-5' oligoadenylate synthetase 1G	0.04	0.62	0.98	0.67
Arrb2	arrestin, beta 2	0.04	0.62	0.40	0.67
Emp3	epithelial membrane protein 3	0.33	0.90	3.60	0.67
Sh3bgrl3	SH3 domain binding glutamic acid-rich protein-like 3	0.60	1.16	1.23	0.68
S100a11	S100 calcium binding protein A11	0.54	1.11	4.39	0.68

Supplemental Table I. List of differentially expressed genes in de adductor muscle group of PCAF-/- and WT mice. Data are presented as the log fold change in expression between day 1 after HLI and average preoperative baseline levels, generating t1/t0avg ratios. Listed are the top 50 genes which showed an impaired upregulation in PCAF-/- mice compared to WT mice. Q-values less than 5% were considered significant. FC = fold change.

Symbol	Gene Name	PCAF-/-	WT	
		logFC	logFC	q-value
Ccl9	chemokine (C-C motif) ligand 9	1.91	2.81	3.60
Tgfbi	transforming growth factor, beta induced	0.36	0.99	3.00
Tnfrsf19	tumor necrosis factor receptor superfamily, member 19	-0.64	-0.04	1.66
C1qtnf9	C1q and tumor necrosis factor related protein 9	-0.95	-0.50	3.00
LOC100041504	PREDICTED: similar to beta chemokine Exodus-2	-0.16	0.27	3.60
Cxcl12	chemokine (C-X-C motif) ligand 12, transcript variant 3	-0.58	-0.16	3.60
C1qtnf2	C1q and tumor necrosis factor related protein 2	-0.57	-0.16	0.00
Traf2	Tnf receptor-associated factor 2	-0.28	0.12	3.60
Ifitm3	interferon induced transmembrane protein 3	1.00	1.39	2.42
Tgfb3	transforming growth factor, beta 3	-0.40	-0.02	4.39
Il8ra	interleukin 8 receptor, alpha	0.07	0.43	0.98
Ifngr2	interferon gamma receptor 2	-0.48	-0.15	1.94
Il3ra	interleukin 3 receptor, alpha chain	-0.02	0.30	0.69
C1qtnf5	C1q and tumor necrosis factor related protein 5, transcript variant 1	-0.11	0.19	0.33
Traf3	Tnf receptor-associated factor 3, transcript variant 1	-0.26	0.04	4.39
Tnfaip8l2	tumor necrosis factor, alpha-induced protein 8-like 2	0.07	0.36	1.94
Ifit3	interferon-induced protein with tetratricopeptide repeats 3	-0.23	0.06	4.39
Irf7	interferon regulatory factor 7	0.00	0.24	1.94
Fadd	Fas (TNFRSF6)-associated via death domain	-0.04	0.19	4.39
Tnfrsf11a	tumor necrosis factor receptor superfamily, member 11a	0.14	0.37	2.42
Prkra	protein kinase, interferon inducible double stranded RNA dependent activator	-0.14	0.08	4.39
Il17rc	interleukin 17 receptor C	0.01	0.23	0.98
Trap1	TNF receptor-associated protein 1	-0.10	0.11	1.66
Tnf	tumor necrosis factor	-0.08	0.11	1.94
Il18	interleukin 18	-0.12	0.04	4.39

Symbol	Gene Name	PCAF-/-	WT	
		logFC	logFC	q-value
Irak1	interleukin-1 receptor-associated kinase 1	0.18	0.00	1.66
Ilf3	interleukin enhancer binding factor 3, transcript variant 3	0.19	0.00	0.98
Tgfbr2	transforming growth factor, beta receptor II, transcript variant 1	0.47	0.24	3.60
LOC100048583	PREDICTED: similar to interferon-inducible protein 203, transcript variant 1	0.16	-0.08	0.33
Ccrl1	chemokine (C-C motif) receptor-like 1	-0.15	-0.42	3.00
LOC545396	PREDICTED: similar to TGF beta-inducible nuclear protein 1	-0.06	-0.33	3.00
Nfkbia	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	0.36	0.06	3.60
Irf2bp2	PREDICTED: interferon regulatory factor 2 binding protein 2	0.09	-0.22	3.00
Nkiras1	NFKB inhibitor interacting Ras-like protein 1	0.15	-0.17	0.00
Ccl19	chemokine (C-C motif) ligand 19	1.02	0.68	0.40
Il15ra	interleukin 15 receptor, alpha chain, transcript variant 2	0.20	-0.15	2.42
Il6ra	interleukin 6 receptor, alpha	0.69	0.21	1.66
Il4i1	interleukin 4 induced 1	-0.39	-1.10	0.00
LOC100044430	PREDICTED: similar to Interferon activated gene 205	1.27	0.50	1.23
Cxcl1	chemokine (C-X-C motif) ligand 1	2.93	1.45	1.66

Supplemental Table II. List of significantly differentially expressed inflammatory genes in de adductor muscle group of PCAF-/- and WT mice. Data are presented as the log fold change in expression between day 1 after HLI and average preoperative baseline levels, generating t1/t0avg ratios. Gene definitions containing any of these criteria (interleukin, chemokine, interferon, TGF, TNF, NFKB) were selected. Q-values less than 5% were considered significant. FC = fold change.