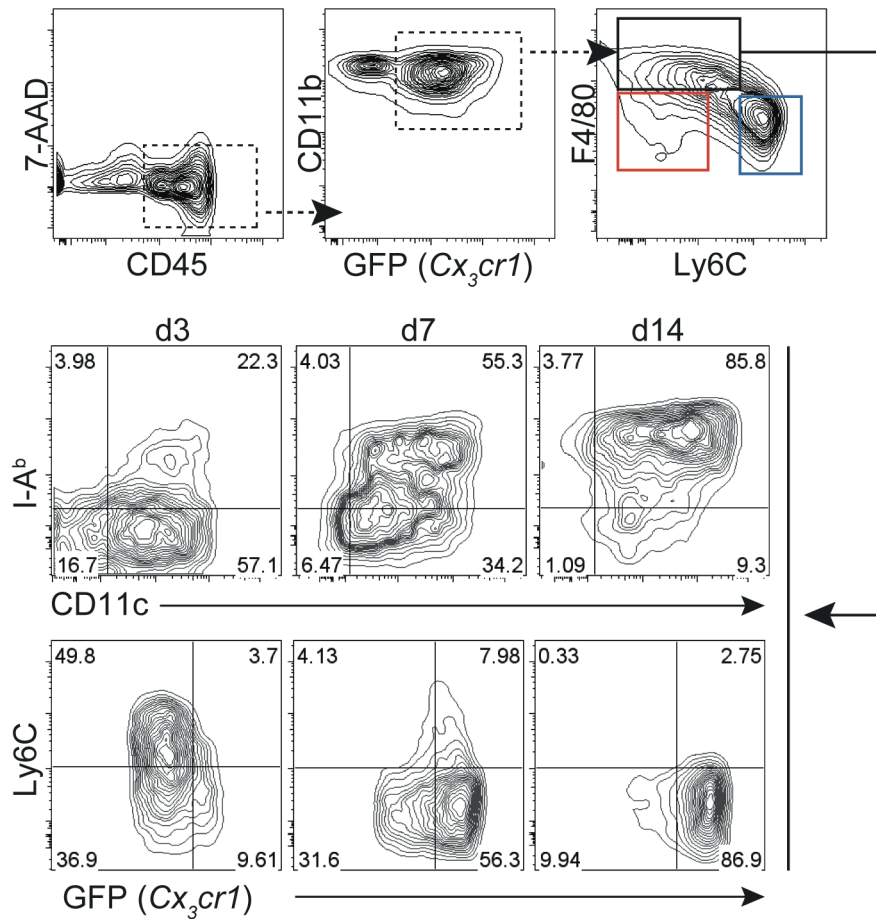
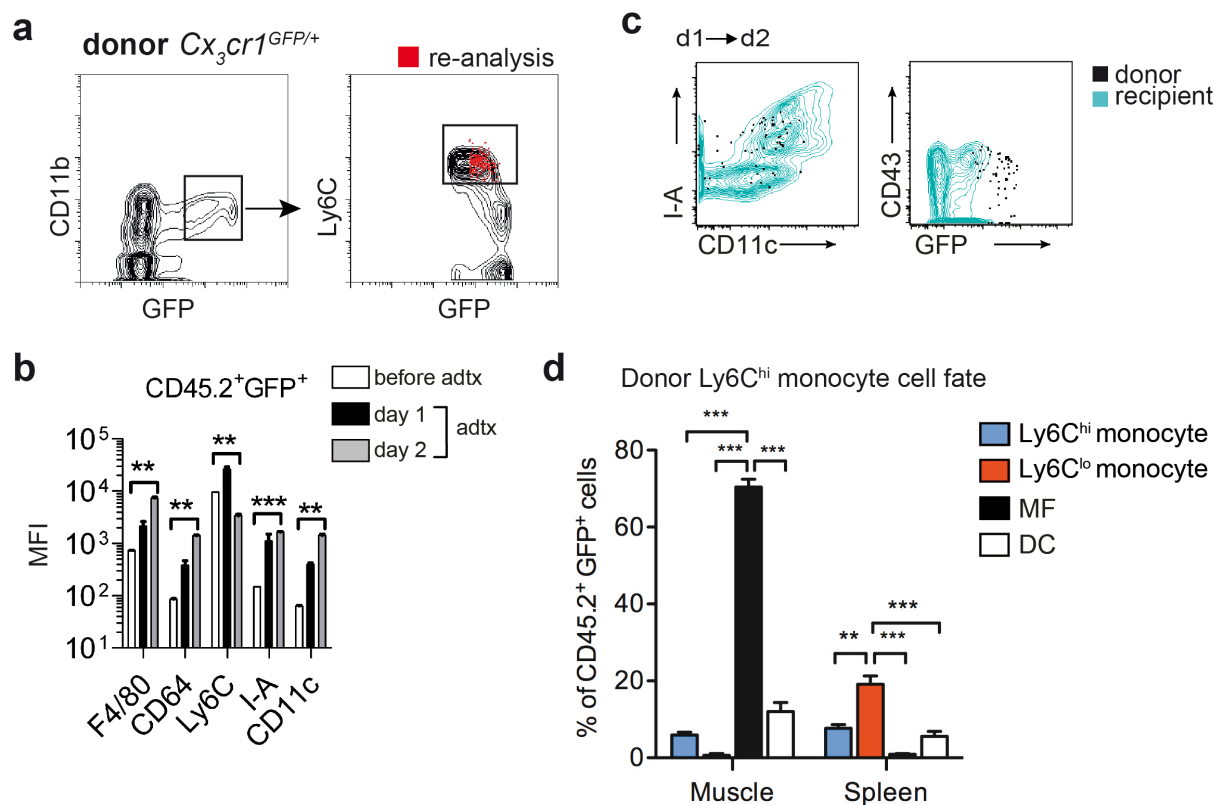




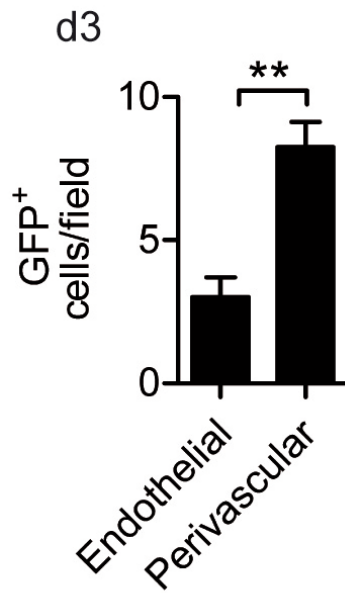
Supplementary Figure 1: Gating strategy and quantification of myeloid cells in ischemic muscle of *Cx3cr1*^{GFP/+} mice post HLI.

Representative flow cytometric analysis of ischemic *tibialis anterior* muscle of *Cx3cr1*^{GFP/+} mice. Macrophages (F4/80⁺Ly6C⁻, black), Ly6C^{hi} (blue), Ly6C^{lo} monocytes (red), n=3 independent experiments



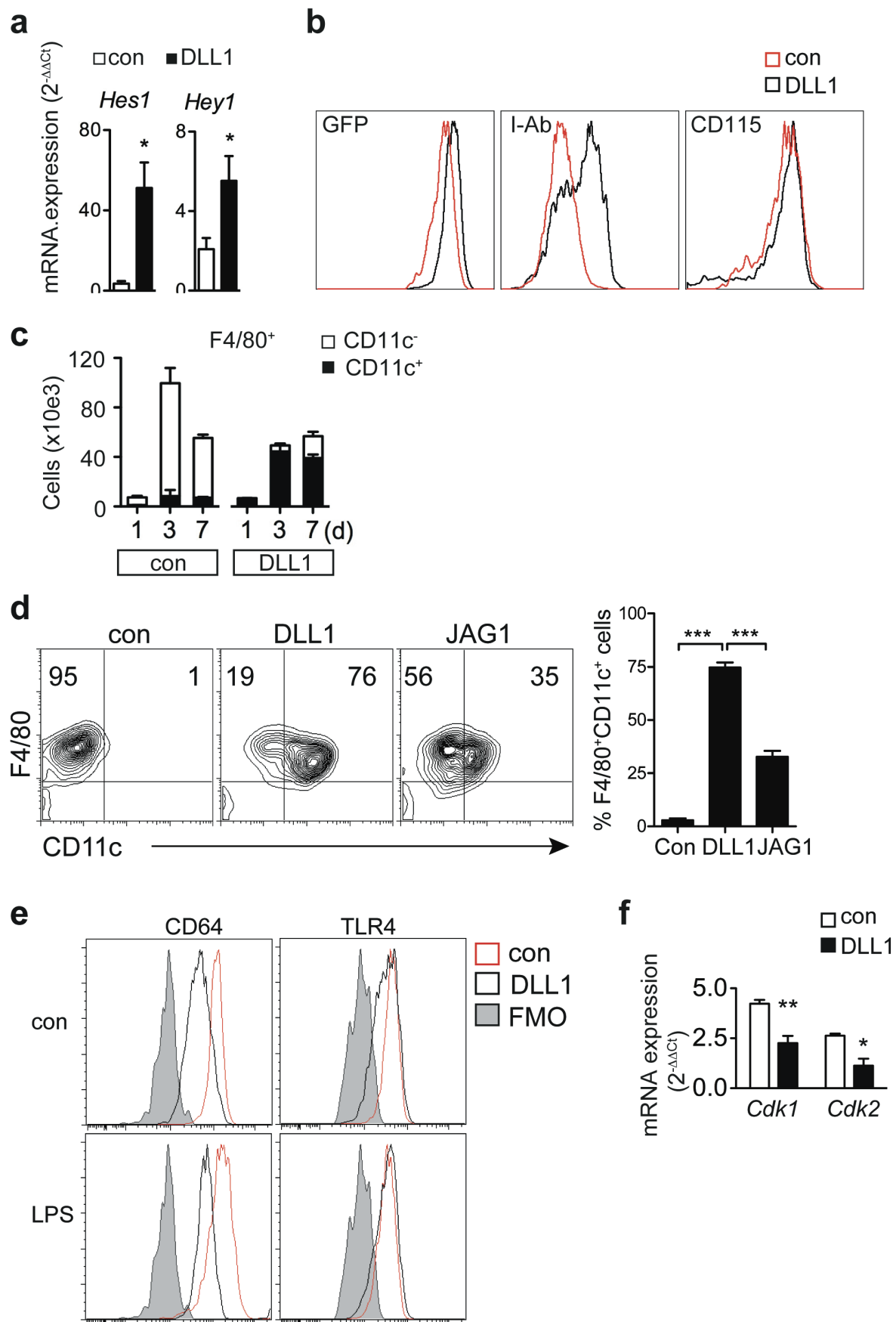
Supplementary Figure 2: Adoptive transfer of Ly6C^{hi} monocytes after HLI.

(a) Sorting strategy for Ly6C^{hi} monocytes from bone marrow of *Cx3cr1*^{GFP/+} donor mice. The sorted cells (red) were re-analyzed for purity and overlaid to initial sort file. (b) Change in mean fluorescent intensity (MFI) of surface markers on donor CD45.2⁺GFP⁺. Data cumulative of n=3 independent experiments, error bars represent s.e.m. **p<0.01, Student's unpaired t-test between naive (adtx) and d2 transferred cells. (c) Phenotype of donor cells in recipient muscle, representative of n=2 independent experiments. (d) Cell fate analysis at d2 of donor CD45.2⁺GFP⁺ monocytes recovered from ischemic muscle and spleen. n=3 independent experiments, error bars represent s.e.m. ***p<0.001, **p<0.01, Two-way ANOVA with Bonferroni post-test.



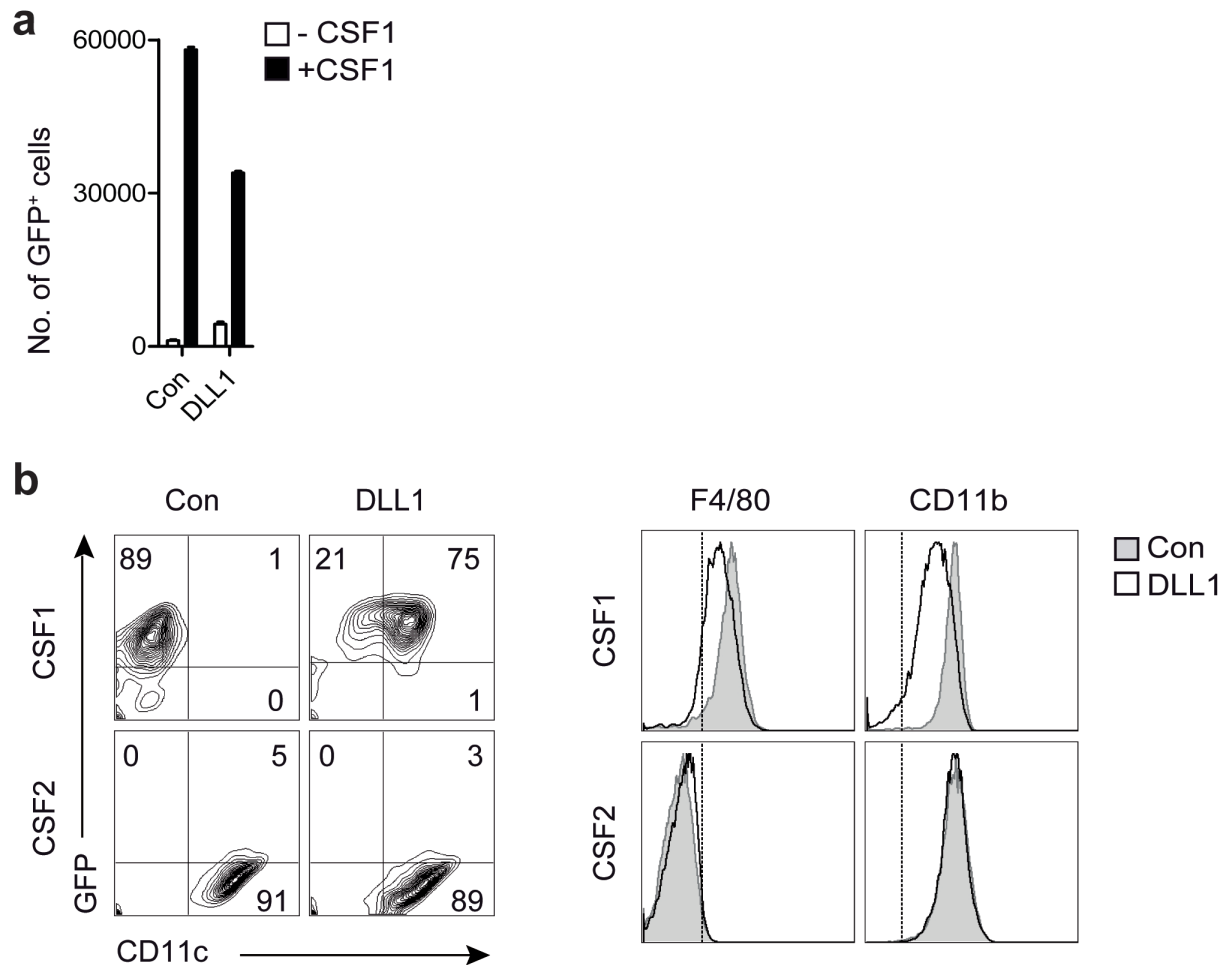
Supplementary Figure 3: GFP⁺ cell localization in collateral arteries in *Cx3cr1*^{GFP/+} mice after HLI.

(a) Quantification of GFP⁺ cells/field near collateral artery at d3. n=4 mice, error bars represent s.e.m. **p<0.01, Student's unpaired t test.



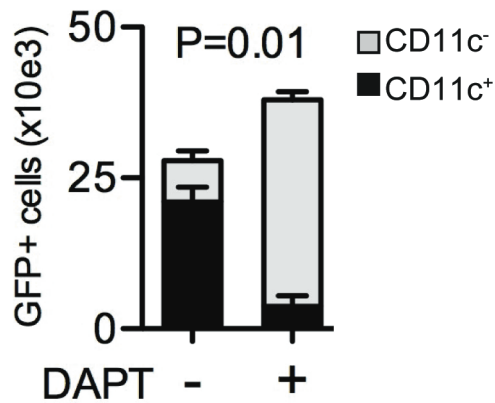
Supplementary Figure 4. Transcriptional and phenotypic changes induced by DLL1.

Ly6C^{hi} monocytes were cultured in plates coated with DLL1 or control. **(a)** Quantitative RT-PCR analysis at d3 normalized to gene expression levels of input Ly6C^{hi} monocytes. n=3 independent experiments performed in duplicates, error bars represent s.e.m. *p <0.05, Student's paired t-test. **(b)** Representative histograms depicting expression of surface markers at d3. Image is a representative of n=2 independent experiments. **(c)** Quantification of absolute numbers of CD11b⁺GFP⁺ macrophages analysed for F4/80⁺CD11c⁺ expression at d1,3 and 7 of culture, error bars represent s.e.m. Data is representative of n=4 independent experiments performed in duplicates. **(d)** Flow cytometry of Ly6C^{hi} monocytes from *Cx3cr1*^{GFP/+} mice cultured for 3d on IgG-Fc (con), DLL1-Fc and JAG1-Fc chimeric proteins, in the presence of 10ng/mL CSF1. n=3 independent experiments, error bars represent s.e.m, ***p<0.001, one-way ANOVA with Bonferroni post-test. **(e)** Flow cytometry of cells cultured on control (IgG2A-Fc) or DLL1-Fc for 6d in presence of murine CSF1. FMO was used as staining controls. Data show representative histograms of n=3 independent experiments. **(f)** Expression of Cdk1 and Cdk2 by quantitative RT-PCR at d3 normalized to gene expression levels of input Ly6C^{hi} monocytes (d0). n=3 independent experiments, performed in duplicates, error bars represent s.e.m. **p<0.01 and *p <0.05, Student's paired t-test.



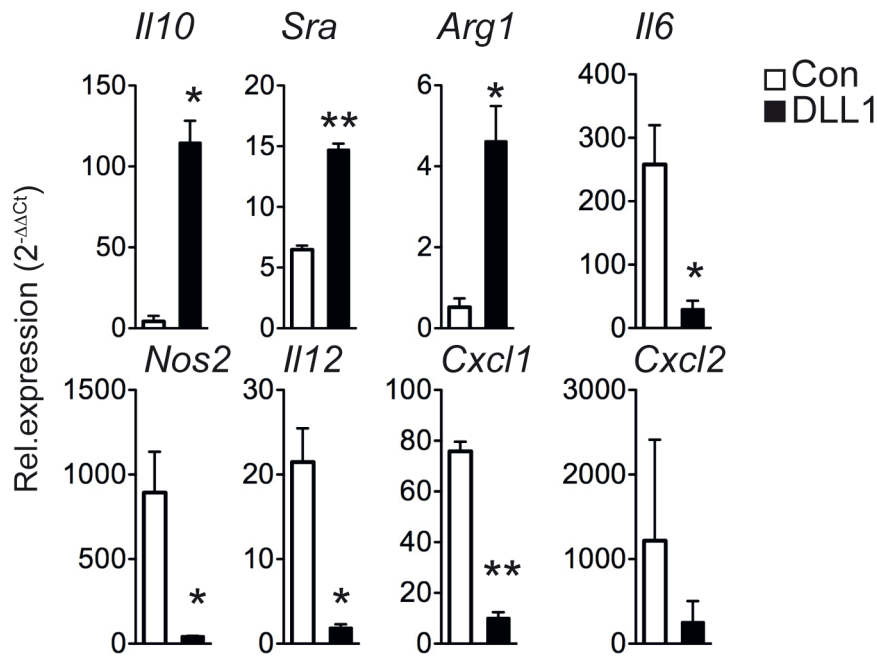
Supplementary Figure 5: Effects of CSF1 and CSF2 on macrophage phenotypes

Ly6C^{hi} monocytes were cultured in plates coated with DLL1 or control (IgG2A-Fc). **(a)** Quantification of absolute numbers of CD11b⁺GFP⁺ macrophages at d3 cultured in the presence (+CSF1) and without (-CSF1) from the day of seeding. Data is representative of n=2 independent experiments performed in duplicates, error bars represent s.e.m. **(b)** Ly6C^{hi} monocytes were cultured with CSF1 or CSF2 at 10ng/ml and analyzed for the expression of GFP, CD11b, CD11c and F4/80 at d3. Data show representative blots and histograms of flow cytometry, n=3 independent experiments.



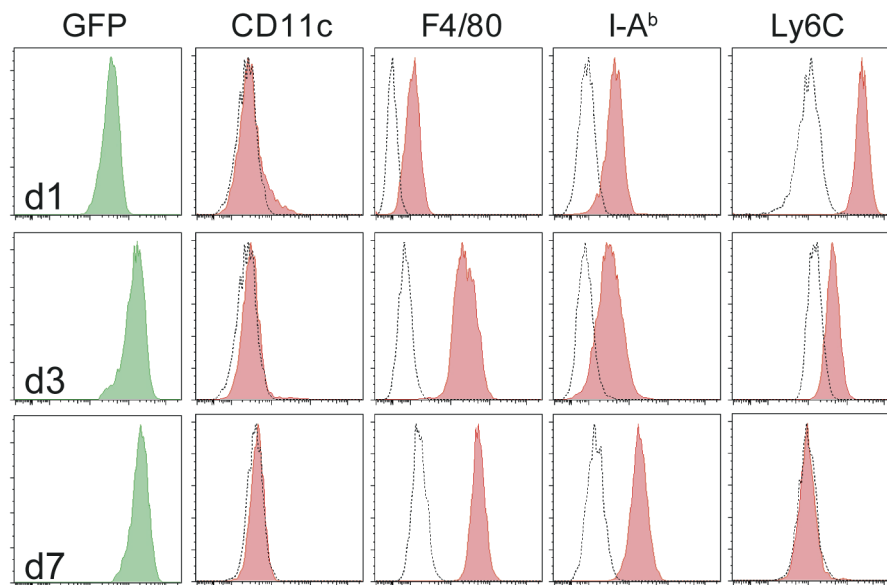
Supplementary Figure 6: Effect of Notch inhibition by DAPT on macrophage numbers and phenotype.

Ly6C^{hi} monocytes were cultured on DLL1 in the presence of either γ -secretase inhibitor DAPT (+) or carrier DMSO (-). Cells were gated for GFP and CD11c expression and absolute numbers in gate were quantified. n=3 independent experiments, error bars represent s.e.m., comparison for CD11c⁺ population by Student's paired t-test.



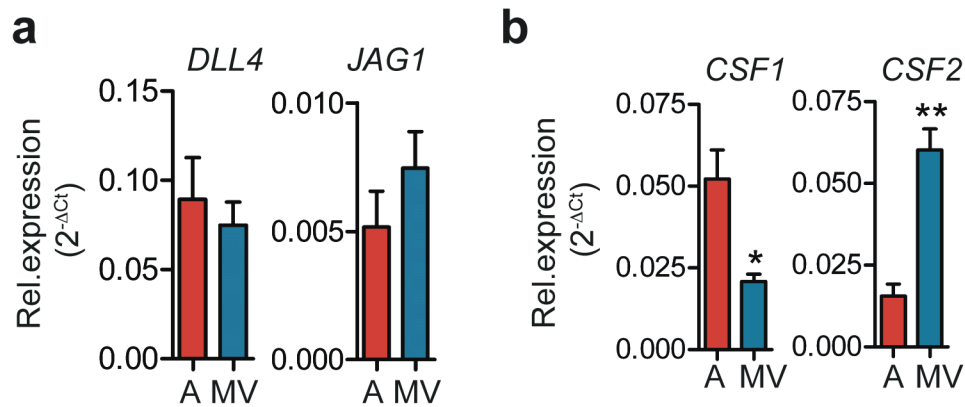
Supplementary Figure 7: Effect of DLL1 on macrophage activation profile after stimulation with IFN γ /LPS.

Ly6C^{hi} monocytes were cultured in plates coated with DLL1 or control (IgG2A-Fc), in presence of 10ng/ml murine CSF1. At day 5, medium was exchanged and cells were stimulated with 20ng/mL IFN- γ for 12hours and followed by LPS stimulation for 6 hours (final concentration of 100ng/mL). Transcriptional profiling of genes was performed with quantitative RT-PCR normalized to gene expression levels of unstimulated d6 macrophage. Data is cumulative pool of n=3 experiments performed in duplicates, error bars represent s.e.m. **p<0.01, * p<0.05, students paired t-test.



Supplementary Figure 8: Dynamics of surface marker expression during differentiation of monocytes to macrophages.

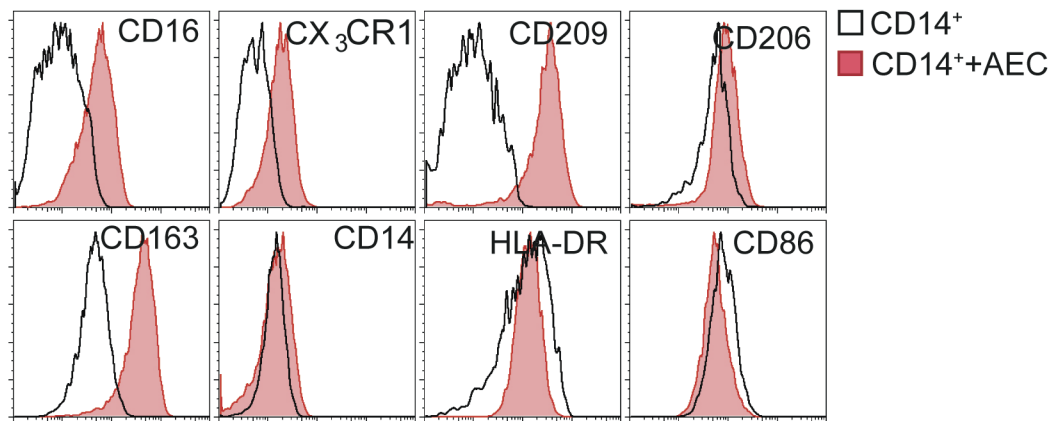
Ly6C^{hi} monocytes were cultured in plates coated with control (IgG2A-Fc), in presence of murine CSF. Expression of surface markers was determined at days 1, 3 and 7 of culture. Data show representative flow cytometric analysis of n=3 independent experiments.



Supplementary Figure 9: Comparison of arterial vs. microvascular EC.

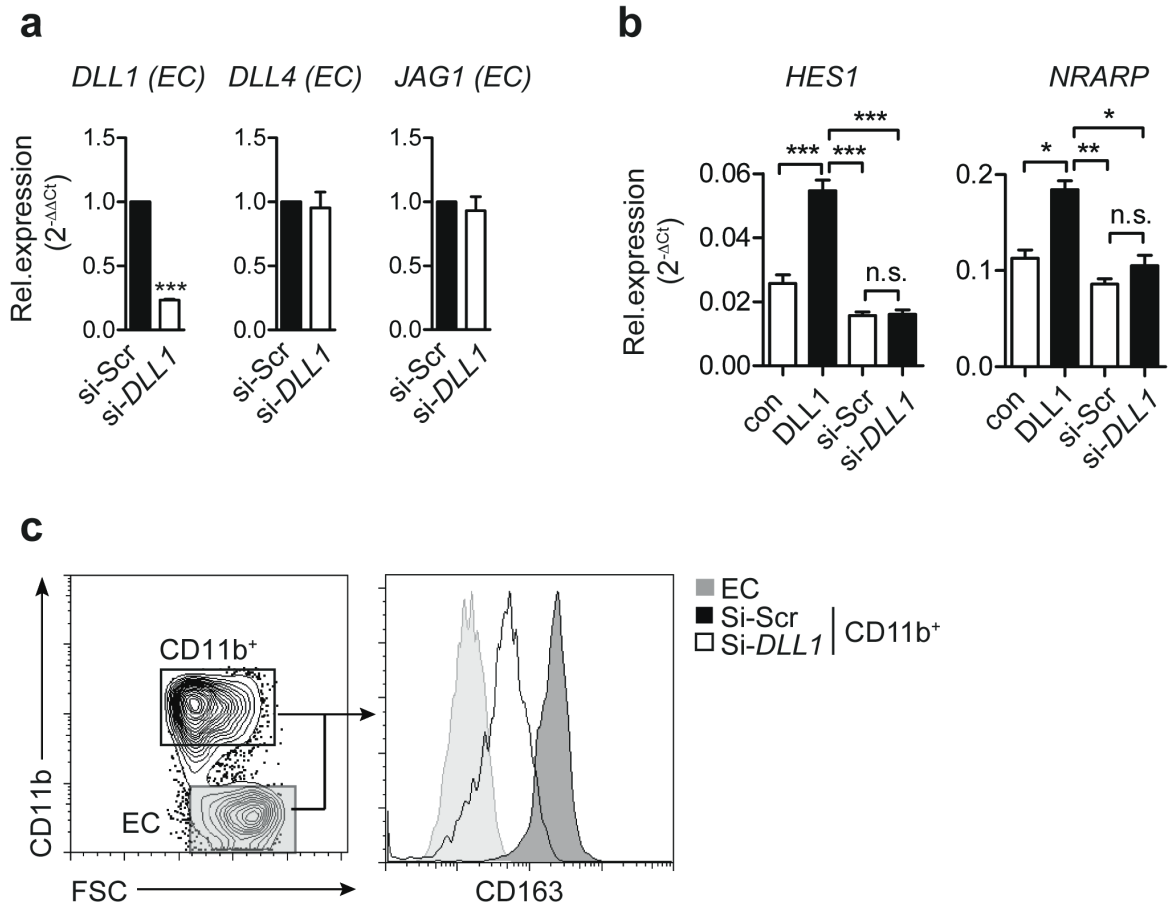
(a) Quantitative RT-PCR analysis of p3 primary human aortic EC (HAEC, A) or coronary microvascular (MV) EC. n=3 independent samples in duplicates/group, error bars represent s.e.m. **(b)** Quantitative RT-PCR analysis of co-cultured EC, isolated by CD31⁺/CD11b⁻ sorting. Data is cumulative of n=3 independent experiments, performed in duplicates, error bars represent s.e.m. *p<0.05, **p<0.01, student's unpaired t-test.

a



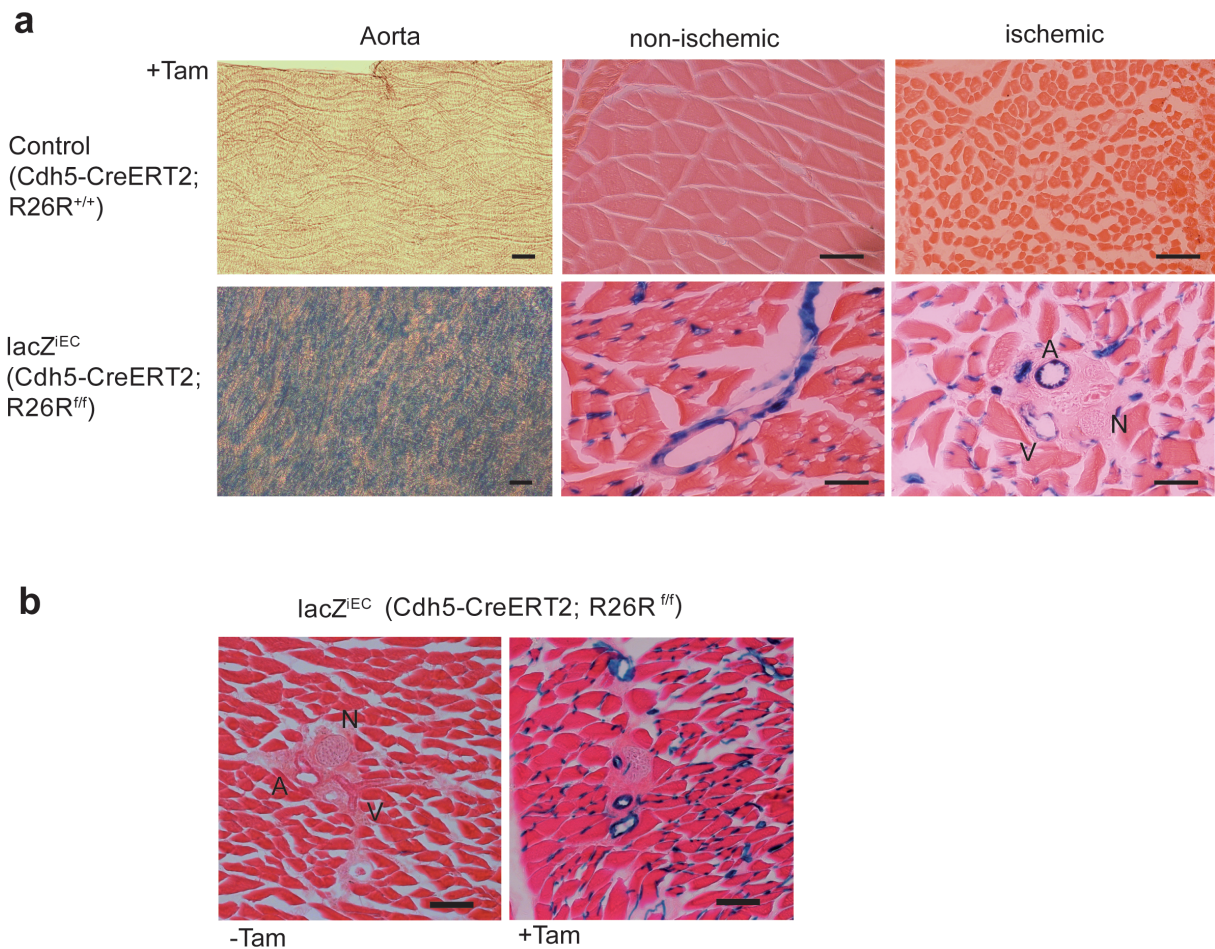
Supplementary Figure 10: Analysis of CD14⁺ monocytes co-cultured with arterial endothelial cells (AEC)

(a) HAEC and CD14⁺ monocytes were co-cultured for 3 days. Co-cultured cells were gated for CD11b expression and analyzed by flow cytometry. Figure is a representative histogram comparing expression of macrophage surface markers on CD14⁺ cultured with M-CSF (open line) and CD14⁺ co-cultured with arterial endothelial cells (AEC, red tinted). Data is representative of n=3 experiments performed in duplicates.



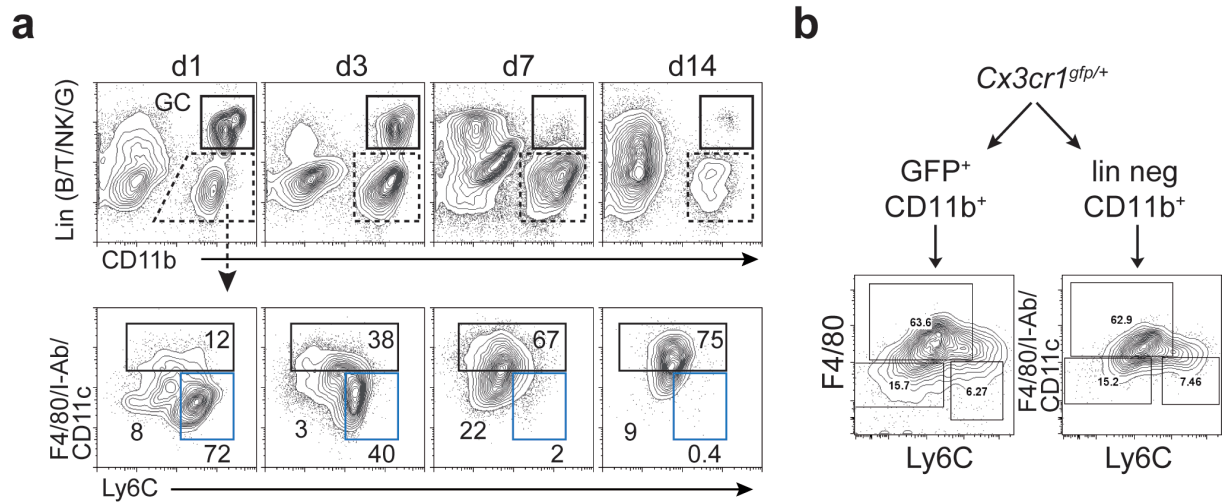
Supplementary Figure 11: Transfection of Arterial endothelial cells with si-DLL1 and analysis after co-culture with CD14⁺ monocytes

(a) Quantitative RT-PCR analysis of expression of *DLL1*, *DLL4* and *JAG1* in HAEC's after treatment with *DLL1* specific si-RNA (si-DLL1) or scrambled si-RNA (si-Scr). Data is cumulative of n=3 independent experiments, performed in duplicates, error bars represent s.e.m. ***p<0.001, student's paired t-test. **(b)** Quantitative RT-PCR analysis comparing expression of *HES1* and *NRARP* on Arterial endothelial cells cultured on DLL1 ligand to transfected endothelial cells (si-Scr and si-DLL1). Endothelial cells were cultured on IgG as control. n=3 independent experiments, error bars represent s.e.m. ***p<0.001, **p<0.01, *p<0.05, one way ANOVA with Bonferroni multiple comparison test. **(c)** Representative flow cytometry from d3 co-culture of human CD14⁺ monocytes with HAEC after treatment with *DLL1* specific si-RNA (si-DLL1) or scrambled si-RNA (si-Scr). n= 2 independent experiments.



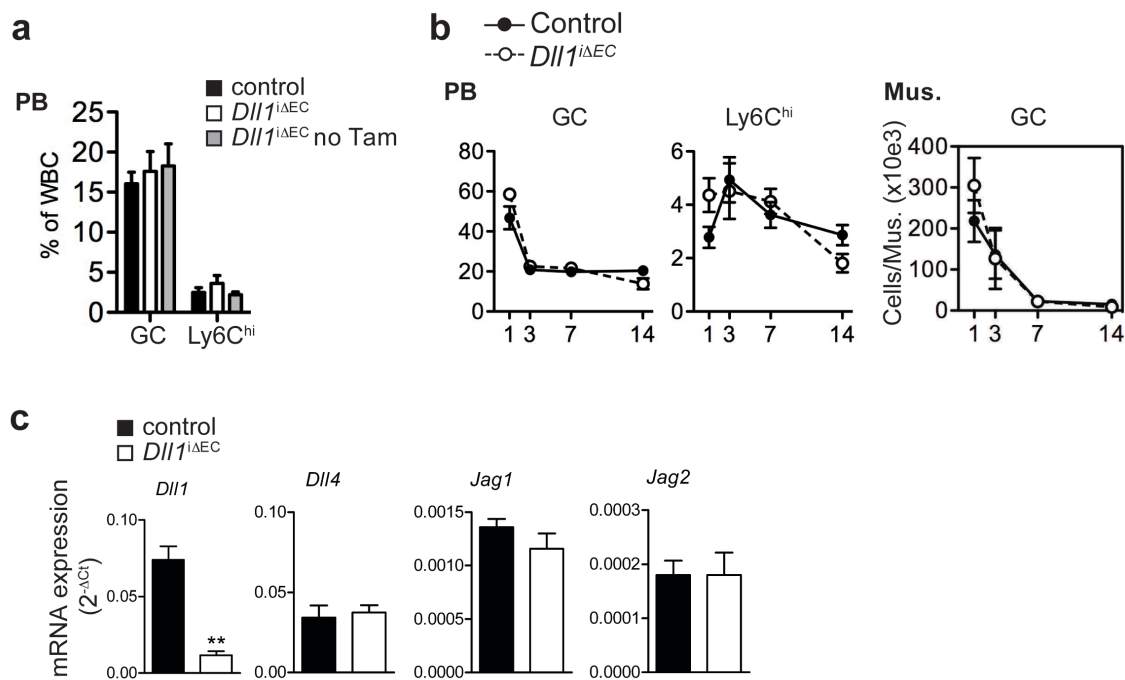
Supplementary Figure 12: Inducible *in vivo* EC targeting in lacZ^{iEC} Cre-reporter mice.

(a) β -galactosidase staining in lacZ^{iEC} and control mice after pulse of Tam in aorta (x10), baseline (non-ischemic) and ischemic muscle (x20). **(b)** Inducible β -galactosidase staining with and without Tam induction. Scale bar: 100 μ m.



Supplementary Figure 13: Non-GFP based gating strategy for myeloid subset analysis

(a) Gating and timecourse of cell populations in ischemic muscle. The Lineage cocktail (lin) used for analysis includes a combination of the markers, B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G (B/T/NK/G). After excluding granulocytes, (lin⁺CD11b⁺), the lin^{neg}CD11b⁺ population is gated for Ly6C^{hi} monocytes (blue box, Ly6C^{hi}F4/80/I-A^b/CD11c^{low/neg} and macrophages (black box, F4/80/I-A^b/CD11c⁺). **(b)** Representative comparison of two gating strategies for macrophages in the same *Cx3cr1*^{gfp/+} mouse showing identical population frequencies for macrophages and Ly6C^{hi} monocytes. n=3 independent experiments.



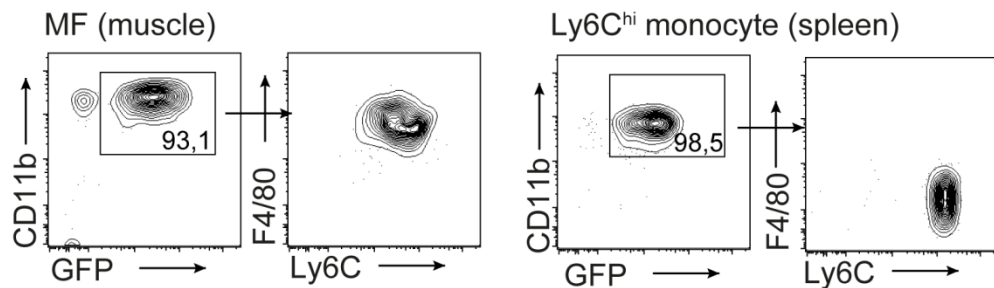
Supplementary Figure 14: Comparison of cell distribution kinetics and Notch ligand distribution between control and *Dll1*^{iΔEC} mice after HLI

(a) Distribution of leukocytes depicted as percent of WBC in littermate control, *Dll1*^{iΔEC} and *Dll1*^{iΔEC} without tamoxifen. n=5/10/10 mice/group, error bars represent s.e.m. **(b)** Analysis of cell populations by flow cytometry in peripheral blood (PB) and ischemic muscle (Mus) of induced endothelial *Dll1* mutant (*Dll1*^{iΔEC}) mice and control mice. n= 8 mice/group, error bars represent s.e.m. **(c)** Quantitative RT-PCR analysis of expression of Notch ligands from d3 ischemic muscle. Data is cumulative of n=3 mice/group, measurements performed in duplicates, error bars represent s.e.m.

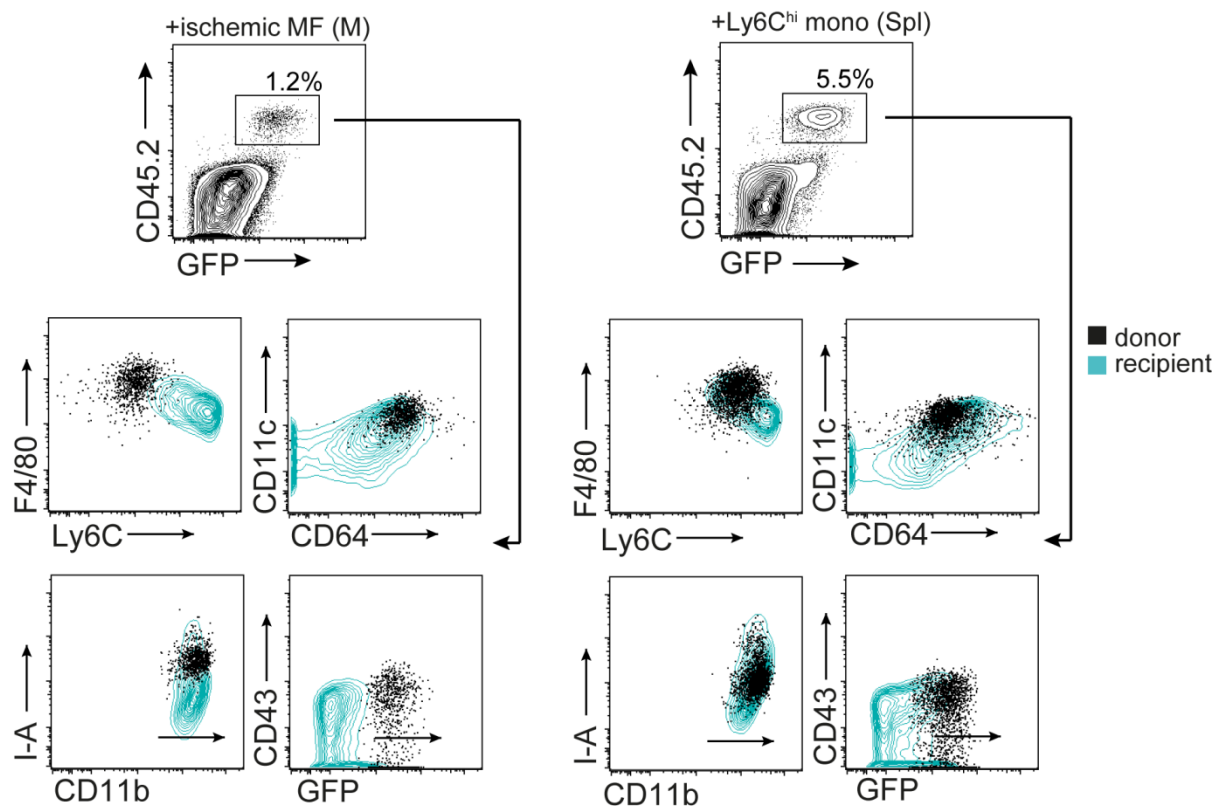
**p<0.01, student's unpaired t-test.

a

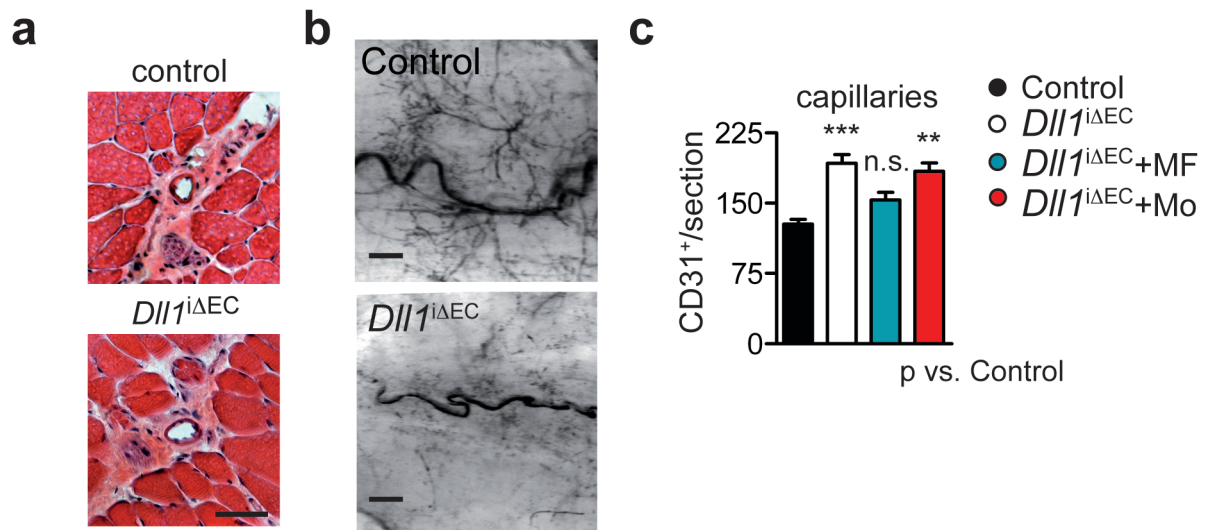
Sorted cells

**b**

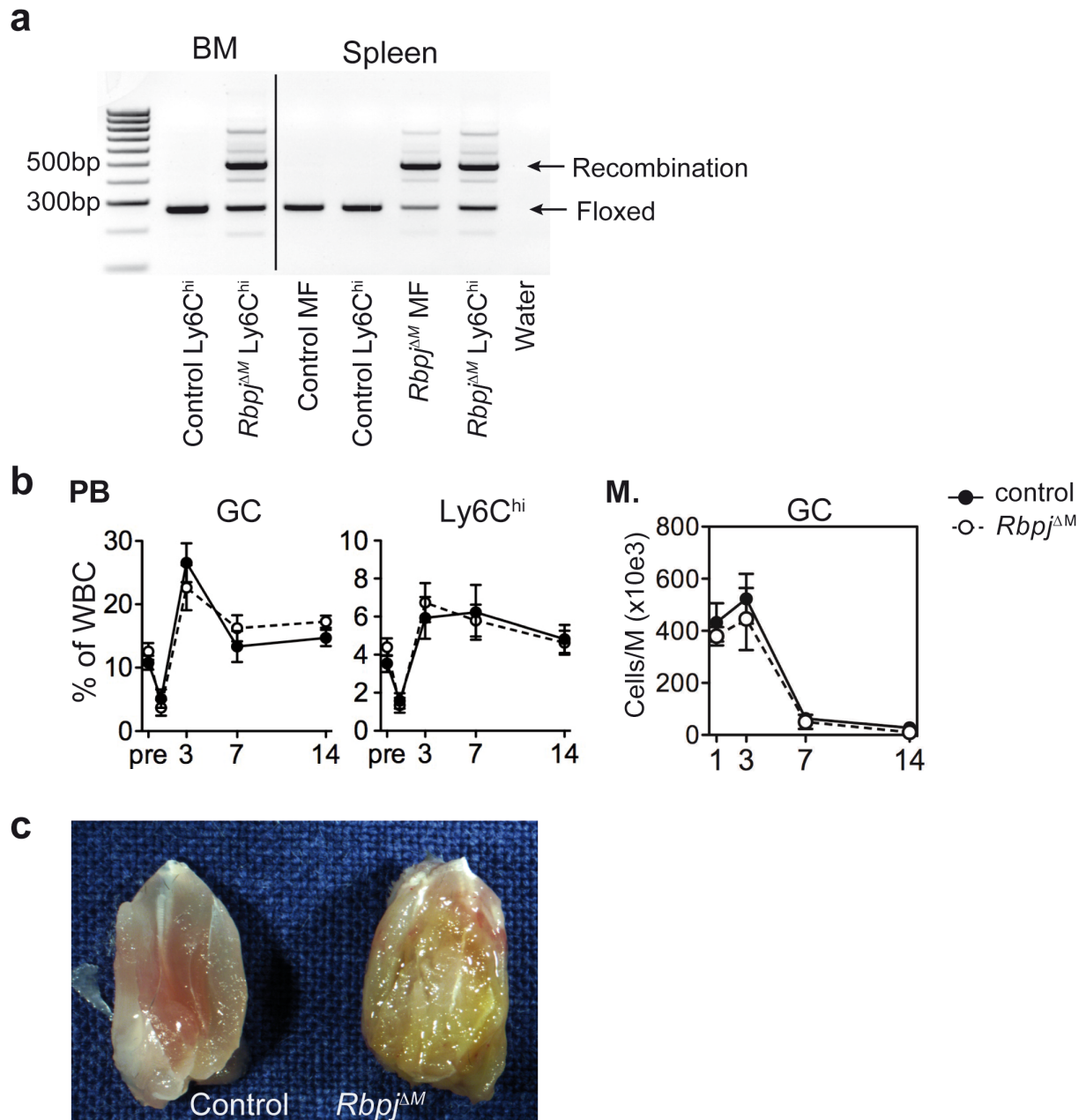
Muscle (24h after i.m. transfer)

**Supplementary Figure 15: Sorting strategy, analysis and recovery of donor**

cells after injection. Splenic monocytes or muscle macrophages (MF) were injected at a dose of 5×10^5 cells into muscle at day 1 after HLI. Flow cytometry analysis was performed 24h after injection. **(a)** Expression of surface markers on sorted donor cells before adoptive transfer. **(b)** Recovery and cell fate tracking of transferred donor cells in the ischemic muscle post adoptive transfer.



Supplementary Figure 16: Comparison of vessel architecture between control and *Dll1*^{iΔEC} mice after HLI. (a) Representative H&E stained muscle sections of baseline collateral arteries. Scale bar 100 μ m. (b) Representative visualization of hind limb arteries and collateral branches in the iTA muscle (M) by pigment particle perfusion. Scale bar 1mm. (c) Quantification of CD31⁺ vessels per section d14, n= 5 mice/group, error bars represent s.e.m. *** p<0.001 vs control by one way ANOVA and Dunnett's multiple comparison test



Supplementary Figure 17: Characterization of *Rbpj*^M mice

(a) Agarose gel-electrophoresis of PCR products from isolated Ly6C^{hi} monocytes or macrophages (MF) showing Cre-mediated recombination in monocytes and macrophages. (b) Kinetics of Granulocytes, Ly6C^{hi} monocytes in the peripheral blood (PB) and muscle (M) of *Rbpj*^{ΔM} mice and littermate controls. *n* = 7/9 mice/group, error bars represent s.e.m. (c) Representative micrograph of d14 isolated gastrocnemius muscle from ischemic limb. Magnification: 5x.

Supplementary Table 1: Phenotypic characterization by FACS

Cell type	Phenotype	
	Non-Cx3cr1-GFP strategy	Cx3cr1-GFP strategy
Granulocytes	(B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G) ⁺ CD11b ⁺	(B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G) ⁺ CD11b ⁺ GFP ^{neg}
Ly6C ^{hi} monocytes	(B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G) ^{neg} CD11b ⁺ Ly6C ^{hi} F4/80/I-A ^b /CD11c ^{lo/neg}	(B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G) ^{neg} CD11b ⁺ Ly6C ^{hi} GFP ⁺ F4/80 ^{lo/neg}
Ly6C ^{lo} monocytes	(B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G) ^{neg} CD11b ⁺ Ly6C ^{lo/neg} F4/80/I-A ^b /CD11c ^{lo/neg}	(B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G) ^{neg} CD11b ⁺ Ly6C ^{lo/-} GFP ^{hi} F4/80 ^{lo/neg} I-A ^b negCD11c ⁺ CD43 ⁺
Macrophages	(B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G) ^{neg} CD11b ⁺ F4/80/I-A ^b /CD11c ⁺	(B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G) ^{neg} CD11b ⁺ GFP ⁺ F4/80 ^{hi}
Dendritic cells (DC)		(B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G) ^{neg} CD11b ⁺ GFP ^{lo} F4/80 ^{lo} CD11c ⁺ I-A ^b hi

Lineage markers, Lin are B220/CD19/CD3 ϵ /CD90/NK1.1/Ly6G

Supplementary Table 2: Genetic strains used in the study

Name	Mouse description	Mouse background
GFP ⁺	<i>Cx3cr1</i> ^{GFP/+}	B6
Control	<i>LysM</i> ^{+/+} <i>Rbpj</i> ^{lox/lox} <i>Cx3cr1</i> ^{GFP/+}	B6
<i>Rbpj</i> ^{ΔM}	<i>LysM</i> ^{Cre/+} <i>Rbpj</i> ^{lox/lox} <i>Cx3cr1</i> ^{GFP/+}	B6
CD45.1 ⁺	B6.SJL- <i>Ptprc</i> ^a <i>Pepc</i> ^b /BoyJ	B6
Control	<i>Gt(ROSA)26Sor</i>	B6
<i>lacZ</i> ^{iEC}	<i>Cdh5(BAC)-CreERT2 Gt(ROSA)26Sor</i>	B6
Control	<i>Dll1</i> ^{lox/lox}	Mixed, B6;129
<i>Dll1</i> ^{iΔEC}	<i>Cdh5(BAC)-CreERT2 Dll1</i> ^{lox/lox}	Mixed, B6;129
<i>Dll1</i> ^{+/LacZ}	<i>Dll1</i> ^{+/LacZ}	129

Supplementary Table 3: Murine RT-PCR primers used in the study

Gene	Primer pair
<i>Rps9</i>	Forward: GGA TTT CTT GGA GAG GCG GC Reverse: ACC TGC TTG CGG ACC CTA AT
<i>Etv3</i>	Forward: TCG CTC GAG TGG TGT GGT TC Reverse: GTG AGC TGG CAG AGA AGCG A
<i>MafB</i>	Forward: CGC GAG AGA GAC GCC TAC AA Reverse: CCC GCC AGG ACT CAC AGA AA
<i>Mrc1</i>	Forward: CTC GTG GAT CTC CGT CAC AC Reverse: GCA AAT GGA GCC GTC TGT GC
<i>Myc</i>	Forward: GCT GTT TGA AGG CTG GAT TTC Reverse: GAG TCG TAG TCG AGG TCA TAG T
<i>Sra</i>	Forward: GCA AAG CAA CAG GAG GAC ATC A Reverse: CTG TTC CAC GTG CGC TTG TT
<i>Cxcl1</i>	Forward: TAA CCA GTT CCA GCA CTC CAG ACT Reverse: TGT TCT TGA GGT GAA TCC CAG CCA
<i>Cxcl2</i>	Forward: ACA TCC CAC CCA CAC AGT GAA AGA Reverse: TCC TTC CAT GAA AGC CAT CCG ACT
<i>Il12</i>	Forward: ACC TGC TGA AGA CCA CAG ATG ACA Reverse: TAG CCA GGC AAC TCT CGT TCT TGT
<i>Il10</i>	Forward: GGG TTG CCA AGC CTT ATC GGA AAT Reverse: TCT TCA GCT TCT CAC CCA GGG AAT
<i>Il6</i>	Forward: ATC CAG TTG CCT TCT TGG GAC TGA Reverse: TAA GCC TCC GAC TTG TGA AGT GGT
<i>Hes1</i>	Forward: CCG GAC AAA CCA AAG ACG GC Reverse: GGA ATG CCG GGA GCT ATC TTT CT
<i>Hey1</i>	Forward: GCG CGG ACG AGA ATG GAA AC Reverse: GGC GCT TCT CGA TGA TGC CT
<i>Hey2</i>	Forward: TGA AGC GCC CTT GTG AGG AA Reverse: TTG TAG CGT GCC CAG GGT AA
<i>Dll1</i>	Forward: TCC GAT TCC CCT TCG GCT TC Reverse: TGG GTT TTC TGT TGC GAG GT
<i>Ets2</i>	Forward: TGG CAT CCC CAA AAA CCC CT Reverse: GCC GTT CAT GCC AAA CTG GT
<i>Mertk</i>	Forward: CGC TCT GGA GTG GAG GCA C Reverse: GCC TGT GGT TGA CTG GGA GT
<i>Spi1</i>	Forward: TGA TGG AGA AGC TGA TGG CTT GGA Reverse: TGC TTG GAC GAG AAC TGG AAG GTA
<i>Cdk1</i>	Forward: CCC GGC GAG TTC TTC ACA GA Reverse: CCA CAG CGT CAC TAC CTC GT
<i>Cdk2</i>	Forward: TTG GAG AGG GCA CGT ACG GA Reverse: TTC AGT CTC AGT GTC GAG CCG

<i>Arg1</i>	Forward: GTC CCT AAT GAC AGC TCC TTT C Reverse: CCA CAC TGA CTC TTC CAT TCT T
<i>Nos2</i>	Forward: TGG TGG TGA CAA GCA CAT TTG GGA Reverse: TGT CAT GAG CAA AGG CGC AGA ACT
<i>Zbtb46</i>	Forward: AGA GAG CAC ATG AAG CGA CA Reverse: CTG GCT GCA GAC ATG AAC AC
<i>Dll4</i>	Forward: GGC CGG GAA CCT TCT CAC TC Reverse: TTT CCT GGC GAA GTC TCT GGC
<i>Jag1</i>	Forward: CAA ATG AGT GCG AGG CCA AAC CTT Reverse: AGC CAG GAA GGC AAT CAC AGT AGT
<i>Jag2</i>	Forward: CAA TGA CAC CAC TCC AGA TGA G Reverse: GGC CAA AGA AGT CGT TGC G

Supplementary Table 4: Human RT-PCR primers used in the study

Gene	Primer pair
<i>RPS9</i>	Forward: TGG TTT GCT TAG GCG CAG AC Reverse: CCG CGG GGT CAC ATA AGT TT
<i>ETV3</i>	Forward: CAG AAG GAG GTG GAG GGT ATC A Reverse: CGA TGA CAT GGC GGA ACT CT
<i>MAFB</i>	Forward: CAA CGA CTT CGA CCT GCT CAA Reverse: CGG AGC TAC ACG GAG TGC TG
<i>MYC</i>	Forward: GTA GTG GAA AAC CAG CAG CCT C Reverse: GTT CTC CTC CTC GTC GCA GTA
<i>HES1</i>	Forward: CAC GAC ACC GGA TAA ACC AAA G Reverse: CGC GAG CTA TCT TTC TTC AGA G
<i>NRARP</i>	Forward: ACA CTG CGT GGT CAA TGT GG Reverse: CAG GCT GGG CGG TAT TTT CA
<i>IL10</i>	Forward: TCC TTG CTG GAG GAC TTT AAG GGT Reverse: TGT CTG GGT CTT GGT TCT CAG CTT
<i>IL8</i>	Forward: AGC CTT CCT GAT TTC TGC AGC TCT Reverse: AAT TTC TGT GTT GGC GCA GTG TGG
<i>CSF1</i>	Forward: TAC TGT AGC CAC ATG ATT GGG A Reverse: CCT GTG TCA GTC AAA GGA AC
<i>CSF2</i>	Forward: CAC TGC TGC TGA GAT GAA TGA A Reverse: GTC TGT AGG CAG GTC GGC TC
<i>DLL1</i>	Forward: GAG CGT GGG GAG AAA GTG TG Reverse: TCT GCA CTT GCA TTC CCC TG
<i>DLL4</i>	Forward: ATC AGC GAT ATG CTC CCC CA Reverse: TGC CTT ATA CCT CCG TGG CA
<i>JAG1</i>	Forward: GTA GCA ACA CAG GCC CTG AC Reverse: AGG CGT GCT CAG CAA TTT CA
<i>HEY1</i>	Forward: AGG CTG GTA CCC AGT GCT TT Reverse: GCG CGT CAA AGT AAC CTT TCC

Supplementary Table 5: Antibodies and fluorescence dyes for flow cytometry and immunostaining

Antibody	Clone	Dilution	Company
Anti-mouse CD3 ϵ	145-2C11	1:100	BioLegend
Anti-mouse CD45R/B220	RA3-6B2	1:400	BioLegend
Anti-mouse Ly6G	1A8	1:400	eBioscience
Anti-mouse CD19	1D3	1:400	BD Pharmingen
Anti-mouse CD90	53-2.1	1:500	BD Pharmingen
Anti-mouse NK1.1	PK136	1:400	BioLegend
Anti-mouse CD115	AFS98	1:100	Biolegend
Anti-mouse/human CD11b	M1/70	1:400	BioLegend
Anti-mouse Ly6C	HK1.4	1:2800	BioLegend
Anti-mouse F4/80	BM8	1:100	BioLegend
Anti-mouse CD11c	N418	1:400	BioLegend
Anti-mouse CD45.2	104	1:200	BioLegend
Anti-mouse CD64	X54-5/7.1	1:400	BioLegend
Anti-mouse I-Ab	AF6-120.1	1:100	eBioscience
Anti-mouse CD45	30-F11	1:100	BD Pharmingen
Anti-mouse CD31	MEC 13.3	1:100	BD Pharmingen
Rabbit anti-GFP	SP3005P	1:200	Acris GmbH
Anti-mouse CD284 (TLR4)	SA 15-21	1:100	Biolegend
Anti-mouse CD43	S7	1:400	BD Pharmingen
Anti-human CD14	M ϕ P9	1:400	BD Pharmingen
Anti-human CD16	B73.1	1:100	BD Pharmingen
Anti-human CD163	GHI/61	1:100	BD Pharmingen
Anti-human CD206	19.2	1:100	BD Pharmingen
Anti-human CD11b	ICRF44	1:400	BD Pharmingen
Anti-human CD209	DCN46	1:100	BD Pharmingen
Anti-human CD86	2331	1:100	BD Pharmingen
Anti-human HLA-DR	L243	1:400	BioLegend
Streptavidin PerCP		1:100	BD Pharmingen
Anti-mouse SMA-Cy3 TM	1A4	1:100	Sigma Aldrich
Alkaline phosphatase Anti-Rat IgG (H+L)	Polyclonal	1:200	AbCam
FITC Affinipure Goat Anti-Rabbit IgG (H+L)	Polyclonal	1:200	Jackson ImmunoResearch
Cy3 TM Affinipure Donkey Anti-Rat IgG (H+L)	Polyclonal	1:100	Jackson ImmunoResearch
AnnexinV		1:50	BioLegend
7AAD		1:100	BioLegend

DAPI		1:5000	Invitrogen
Propidium Iodide		1:12000	Sigma