SUPPLEMENTAL MATERIAL

Materials

RPMI 1640 and fetal calf serum (FCS) were from Gibco (Invitrogen, Karlsruhe, Germany), G-CSF was from Amgen (Gauting, Germany), MethoCult M3434 from Stem Cell Technologies (Köln, Germany) and collagenase II from Worthington (Troisdorf, Germany). All other materials were obtained from Sigma (Munich, Germany) or Applichem (Darmstadt, Germany).

Animals

Genetically modified mice lacking either the AMPKα1 or the AMPKα2 subunits, their respective wild-type littermates[1] and floxed AMPKα1 and α2 mice were kindly provided by Benoit Viollet (INSERM, U1016, Paris, France) and bred at the Goethe University Hospital animal facility. Floxed AMPKα2 mice were crossed with animals expressing the Cre-deleter under the control of the Tie-2 promoter (B6.Cg-Tg(Tek-cre)12Flv/J; Jackson Laboratories, Bar Harbor, USA) to generate mice lacking the α2 subunit in endothelial cells and some myeloid cells (Tie2-α2) or with VE-cadherin-Cre mice (B6-Tg(Cdh5-cre)/J; Polygene, Switzerland) to generate animals mice lacking the α2 subunit specifically in endothelial cells (AMPK$\alpha2^{\Delta EC}$). Floxed AMPKα1 and α2 mice were bred with LysM-Cre mice (B6.129P2-Lyz2tm1(cre)Ifo/J; Jackson Laboratories) to generate mice lacking the AMPKα1 or α2 subunit specifically in myeloid cells.

All animals were housed in conditions that conform to the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication No. 85-23). Both the University Animal Care Committee and the Federal Authority for Animal Research at the Regierungspräsidium Darmstadt (Hessen, Germany) approved the study protocol (#F28/25). For the isolation of organs, mice were sacrificed using 4% isoflurane in air and subsequent exsanguination or decapitation.

Blood Count

Blood was harvested (cardiac puncture) with a syringe containing 10% (vol/vol) EDTA buffer (10 mg/mL) to prevent clotting. Blood profiles were analyzed using a Vetscan HM5 (Abaxis, Griesheim, Germany) or by flow cytometric analysis on a BD FACSVerse flow cytometer (BD, Heidelberg, Germany). CD45 was used for total leukocyte number, myeloid cells were defined as CD45+ and CD11b+, neutrophils as CD45+ Ly6G+ cells, T-cells as CD45+ CD3+ and B-cells as CD45+ CD19+ cells. For the identification of leukocyte-platelet or neutrophil-platelet aggregates CD45 CD61 or Ly6G CD61 double positive cells were gated.

Stem and Progenitor Cell Populations

Mice were administered G-CSF (subcutaneous injection, 250 µg/kg/day, 4 days) and stem and progenitor cell populations in bone marrow were analyzed by flow cytometry (BD FACS Arial or FACS CantoII (BD Bioscience). Lineage staining: cKit (CD117; 2B8) BV421 (Biolegend, Munich , Germany, CD11b (M1/70) PE (Biolegend), Ly6G (1A8) A647 (eBioscience, Frankfurt, Germany), B220 (CD45R; RA3-6B2) BV510 (Biolegend), CD3 (145-2C11) PE-Cy7 (eBioscience), Ter119 (TER-119) APC-eF780 (eBioscience), CD71 (RI7217) FITC (Biolegend), Fc block (STEMCELL Technologies). Stem and Progenitor staining: CD3 (145-2C11), CD19 (1D3), CD41 (MWReg30), Ter119 (TER-119), B220 (RA3-6B2), CD11b (M1/70), Gr-1 (RB6-8C5) (all biotinylated, eBioscience); Streptavidin APC-eF780 (eBioscience), Sca-1 (D7) PB or PerCP-Cy5.5 (eBioscience), CD117 (2B8) PE-Cy7 (eBioscience), CD150 (TC15-12F12.2) PerCP-Cy5.5 or PE (eBioscience), CD48 (HM48.1) FITC (Biolegend), CD34 (RAM34) eF660 (eBioscience).

Colony Forming Unit Assay

After 4 days of G-CSF treatment 100µl of peripheral blood was obtained from the tail vein, transferred into tubes (buffered with 10% (vol/vol) EDTA (10 mg/mL) and erythrocytes were

lysed. The resulting cell suspension was carefully mixed with 3 mL of pre-warmed methylcellulose media (M3434, STEMCELL Technologies) supplemented with growth factors and plated on 35 mm dishes. After 6 days colonies formed were counted with an inverted microscope.

Hindlimb Ischemia

Arteriogenic and angiogenic capacity was investigated in a murine model of hindlimb ischemia using 6 to 8 week old wild-type and transgenic animals as described.[2] Briefly, the deep femoral artery was ligated using an electric coagulator (ERBOTOM ICC50, ERBE). Afterwards the superficial femoral artery and vein as well as the epigastric arteries were completely excised. The overlying skin was closed with 3 surgical staples. Relative blood flow was determined by laser Doppler imaging (Laser Doppler Perfusion Imager System, Wilmington, Germany) at regular intervals for up to 28 days post-ligation. The perfusion of the ischemic and non-ischemic limb was calculated on the basis of colored histogram pixels. To minimize variables including ambient light and temperature and to maintain a constant body temperature, mice were exposed to infrared light for 10 minutes before laser Doppler scans. Perfusion was expressed as the ratio of the ischemic to the non-ischemic hindlimb.

µ-CT Analyses

Mice were sacrificed and intravascular perfused through the aorta using a roller pump with 20 mL of a heparinized NaCl solution supplemented with sodium nitroprusside at a rate of 5 mL/minute. The perfusion was immediately followed by perfusion with a solution of 1% paraformaldehyde in 0.9% NaCl for prefixation of the vasculature. Mice were then perfused (5 mL in 2 minutes) with a contrast agent consisting to 20% of a $BaSO_4$ solution (Micropaque, Guerbet, Villepint, France) and 80% polyurethane (Dispercoll, Bayer, Leverkusen, Germany). Fixation was achieved with 50% formic acid for 5 minutes. Mice were stored overnight in a 4% PFA solution (4°C) and were subsequently scanned using a micro-CT (Skyscan 1176, Bruker micro-CT, Kontich, Belgium) at 50kV X-ray with a 0.5 mm aluminum filter, 9 µm isotopic resolution and 7 projection images per 0.3° rotation step. Data were reconstructed with the NRecon/ InstaRecon CBR Server software (Skyscan, Kontich, Belgium/ InstaRecon, Champaign, Illinois, USA). Image analysis and segmentation were performed with the Imalytics Preclinical Software (Gremse-IT, Aachen, Germany).

Immunohistochemistry

Semimembranosus and gastrocnemius muscles were embedded in TissueTek OCT Compound (Sakura, Staufen, Germany) and immediately frozen in dry ice. Transverse cryosections (10 µm) were cut at 3 different sites of the muscle (upper, middle and lower). Sections were dried at 37°C for 10 minutes and fixed in a solution of 4% PFA in PBS at room temperature for 15 minutes. After washing in PBS, samples were incubated with blocking buffer (PBS containing 3% BSA and 0.03% Triton X-100) at room temperature for 45 minutes, followed by incubation with anti-CD31 antibody, overnight at 4°C. After extensive washing and exposure to the secondary anti-rat antibody and anti-smooth muscle actin-Cy3 (1 hour at room temperature) sections washed and covered with fluorescent mounting medium (Dako) and analyzed using a confocal microscope (Zeiss, LSM 780). For each section, 5 non-overlapping pictures were taken and evaluated. For the visualization of infiltrating cells and ICAM expression, hindlimb muscles (M. semimembranosus and M. gastrocnemius) were placed in zinc fixative solution for 24 hours, dehydrated and cleared in ethanol and xylol and embedded in paraffin. Sections were cut in 10µm slices using a microtome and stretched by floating in 40°C warm water bath. Slices were dried on a heating plate at 37°C, deparaffinized and rehydrated in a series of xylol (5 minutes), 100 % ethanol (3 minutes), 96 % ethanol (3 minutes), 80 % ethanol (3 minutes), 70 % ethanol (3 minutes). After washing in PBS samples were incubated with blocking buffer (PBS containing 10% horse serum, 1% BSA and 0.3% Triton X-100) at room temperature for 2 hours before

being exposed to primary antibody (4°C, overnight). After extensive washing and exposure to the appropriate secondary antibody (1 hour at room temperature) samples were mounted in fluorescent mounting medium (Dako) and analyzed with a confocal microscope (Zeiss, LSM 780).

Antibodies: Anti-CD144 (1:300, SC6458) was from Santa Cruz (Heidelberg, Germany), ICAM (1:100; Santa Cruz, sc-1511) anti-Ly6G (1:100 rat) and anti-CD31 (1:200, rat) were from BD Pharmingen and anti-alpha-smooth muscle actin-Cy3 (1/1000) was from Sigma.

Whole Mount Immunofluorescence

One day after femoral artery ligation mice were sacrificed using 4% isoflurane in air and animals were perfused with 5 mL NaCl (0.9%) and 5 mL formalin (4%). Semimembranosus and gastrocnemius muscles were taken and fixed for 24 hours in 4% formalin. Tissue was then permeabilized for 1 hour with 1% SDS in PBS, extensively washed in PBS, then incubated with glycine (100 mmol/L, 3 times for 15 minutes). After blocking for 3 hours (0.1% BSA, 0.2% Triton X-100, 0.05% Tween 20, 10% goat serum in PBS, 37°C) the tissue was incubated for 40 hours in primary Ly6G antibody (rat anti mouse BD 1:100, 37°C). After washing in PBS secondary antibodies (1% BSA in PBS) were applied together with smooth muscle actin (CY3 Sigma, 1:1000) and DAPI (20 µg/ml) and incubated for a further 16 hours (37°C). After washing 4 times with PBS the tissue was transferred into CUBIC2 solution.[3] Pictures were taken as Z-stack with a confocal microscope (Zeiss, LSM 780) with a 40x objective (EC-Plan-Neofluar 40x1.3 Oil DIC M27).

RNA Isolation and Quantitative Real Time PCR (RT-qPCR)

Total RNA was extracted using TriReagent (Sigma-Aldrich). For the generation of complementary DNA (cDNA) total RNA (1 µg) was reverse transcribed using the SuperScriptIII (Life Technologies GmbH, Darmstadt, Germany) and random hexamer primers according to the manufacturer's protocol. The amount of mRNA was quantified using the cycle threshold (cT) value using a SYBR green master mix (ABgene, Dreieich, Germany) with intron spanning primers in a Mx4000 multiplex qPCR system (Stratagene, Heidelberg, Germany). C_T values obtained were converted into relative amounts on the basis of a standard curve and mRNA levels were normalized to 18S rRNA. For the quantification of gene expression by RT-qPCR the following primers were used: 18S rRNA forward (5'-CTTTGGTCGCTCGCTCCTC-3'), 18S rRNA reverse (5'-CTGACCGGGTTGGTTTTGAT-3'), VEGF forward (5'-GCACTGGACCCTGGCTTTACTGCTGTA-3'), VEGF reverse (5'-GAACTTGATCACTTCATGGGACTTCTGCTC-3'), TNFα forward (5'-GGCCTTCCTACCTTCAGACC-3'), TNFα reverse (5'-CCGGCCTTCCAAATAAATAC-3'), Interleukin-1β forward (5'-CAGGCAGGCAGTATCACTCA-3'), Interleukin-1β reverse (5'-AGCTCATATGGGTCCGACAG-3'), MMP9 forward (5'-GAAGGCAAACCCTGTGTT-3') reverse (5'-AGAGTACTGCTTGCCCAGGA-3') and SDF1α primer set from Qiagen (PPM02965E, Heidelberg, Germany). For the mRNA expression profiling of the angiogenic markers total RNA was isolated from the M. semimembranosus or M. gastrocnemius of the control and ischemic limbs 3 days after the femoral artery ligation and qRT-PCR was performed using a custom array (Qiagen).

Immunoblotting

Cells were lysed in Triton X-100 buffer and detergent-soluble proteins were solubilized in SDS-PAGE sample buffer, separated by SDS-PAGE and subjected to Western blotting as described.[4] Proteins were visualized by enhanced chemiluminescence using a commercially available kit (Amersham, Freiburg, Germany). The antibodies against AMPKα2 (1:1000 A-20 goat), Bax (1:1000, sc-493), Api5 (1:1000) and phospho-serine (1:1000, AB1603) were from Santa Cruz Biotechnology (Heidelberg, Germany). The antibody against AMPKα1 (1:1000, 8056) was from Eurogentec (Seraing, Belgium), anti-β-actin (1:5000, A5541) was from Sigma, anti-non-muscle

myosin IIA (1:2000, ab24762) from ABCAM (Cambridge, UK), anti-Tim50 (1:500, ab23938, goat) was from ABCAM, anti-CRAMP (1:100, PA-CRPL-100) from Innovagen (Lund, Sweden), anti-IDH1 (1:1000, NBP2-32150) from Novus Biologicals (Abingdon, United Kingdom), anti-IDH3b (1:1000, ab121016) from ABCAM, anti-cleaved caspase-3 (1:800, 9662) from New England Biolabs GmbH (Frankfurt, Germany), anti-HIF-1α (1:800, 610959) from BD Pharmingen (Heidelberg, Germany), anti-hydroxy Pro564-HIF-1α (1:800, NB110-74679) from Novus Biologicals and anti-phospho-threonine (1:1000, AB1607) from Merck Millipore (Darmstadt, Germany).

Flow Cytometric Analysis of Infiltrated Immune Cells

One and three days after femoral artery ligation mice were sacrificed and perfused with 15 mL NaCl solution containing 5 U/mL heparin. Intact hindlimb muscles (M. semimembranosus) were removed and minced carefully. Single cell suspension was generated from the entire muscle by subsequent digestion in DMEM/F12 containing 300 U/mL Collagenase Type II at 37°C for 60 minutes. Cell suspension was passed through a 70 µm filter and washed in PBS. For flow cytometric analysis cells were incubated in Fc block solution (CD16/32 1/100 in PBS) to prevent non-specific antibody binding. Staining with corresponding antibodies were performed in PBS containing 2% FCS and 1 mmol/L EDTA and analyzed on a BD FACSVerse flow cytometer. Neutrophils were defined as CD45+, CD11b+ and Gr1+ cells, macrophages were defined as CD45+, CD11b+ and F4/80+ cells.

Neutrophil Isolation

Murine bone marrow cells were obtained from femurs and tibias and polymorphonuclear neutrophils (PMNs) were either isolated by using the EasySep™ Mouse Neutrophil Enrichment Kit (Stemcell Technologies, Köln, Germany) according to the manufacturer's protocol, or by discontinuous Percoll gradient (52%/64%/72%) centrifugation at 1000*g* for 30 minutes and 4°C. Neutrophils were harvested from the 64%/72% interface, washed in PBS, and maintained in RPMI 1640 medium supplemented with 10% FCS as indicated in the results section. Neutrophil viability was greater than 95% as assessed by the trypan blue exclusion test and purity was greater than 98% as analyzed by microscopy using Hemacolor staining (Merck, Darmstadt, Germany).

***In vitro* Transendothelial Migration**

Mouse lung endothelial cells were grown to a confluent monolayer on a fibronectin coated transwell filter (3µm pore size; BD Pharmingen). Endothelial cells were stimulated with 10 ng/mL tumor necrosis factor α (Peprotech, Hamburg, Germany) for 5 hours. After careful washing neutrophils ($5x10^5$ cells) were added on top of the endothelial cell monolayer and allowed to migrate towards a stromal cell-derived factor (SDF1) 1α gradient (100 ng/mL; Peprotech, Hamburg, Germany) for 2 hours. Cells that migrated to the lower chamber were collected and counted by flow cytometry (BD FACSVerse).

AMPK Downregulation

HEK293 cells were transiently transfected with either control oligonucleotides or small interfering RNA (siRNA) directed against the AMPKα1 or AMPKα2 subunits (Eurogentec) using Lipofectamin 2000 (Invitrogen, Göttingen, Germany) according to the manufacturer´s protocol.

***In vitro* Phosphorylation Assay of HIF-1α**

Human recombinant HIF-1α (100 ng per sample; ABCAM) was incubated with human recombinant AMPKα2 (150 µg per sample; Calbiochem, Darmstadt, Germany) in the presence of ATP for 30 minutes at 37°C. Samples without HIF-1α or AMPKα2 addition were used as controls. The kinase reaction was stopped and phosphorylation was assessed by SDS-PAGE.

Oxidative Phosphorylation and Glycolytic Flux Analyses

Neutrophils were seeded at $5x10^5$ cells per well on Seahorse XF96 polystyrene tissue culture plates (Seahorse Bioscience, Boston, USA)) in RPMI 1640 medium with 10% FCS. After three hours medium was changed to unbuffered DMEM assay medium for 1 hour before measuring in an XFe 96 extracellular flux analyzer (Seahorse Bioscience). Inhibitors and activators were used at the following concentrations: oligomycin (1 µmol/L), carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP; 5 µmol/L), rotenone (5 µmol/L), antimycin A (5 µmol/L), glucose (10 mmol/L) and 2-deoxy-D-glucose (100 mmol/L). Each measurement was averaged from triplicate values.

Assay of Reactive Oxygen Species production

Neutrophils ($1x10^6$cells/mL) were incubated with 2′,7′-dichlorofluorescein diacetate (Life Technologies, Darmstadt, Germany) and with or without phorbol 12-myristate 13-acetate (PMA, 100 ng/mL) for 20 minutes at 37°C. To stop the reaction cells were washed with ice-cold PBS and stored on ice until flow cytometric analysis. The production of reactive oxygen species was quantified by assessing the mean fluorescence intensity (excitation λ=492-495nm, emission λ =517-527nm).

Caspase-3/7 Activity Assay

Neutrophils ($1,5x10^5$ cells per well) were seeded onto a fibronectin coated 96 well plate in RPMI 1640 containing 10% FCS and caspase 3/7 reagent (5 µmol/L; Essen Bioscience, Michigan, USA). The pan caspase inhibitor Z-VAD-FMK (10 µmol/L; Enzo Life Sciences, Lörrach, Germany) was added to some wells as a control. Fluorescence was assessed using an automated imaging system (IncuCyte system 2010A; Essen Bioscience). Green fluorescence and phase contrast pictures were analyzed using ImageJ (version 1.47 National Institutes of Health). Each value was averaged from triplicates.

THP-1 cell culture

Monocytic THP-1 cells were differentiated to macrophages using PMA (10 nmol/L) in RPMI medium supplemented with heat-inactivated fetal calf serum (10%), sodium pyruvate (1 mmol/L), penicillin (100 U/mL) and streptomycin (100 µg/mL). After 5 days, medium was changed to PMA-free medium and the differentiated cells were then incubated with or without octyl-α-ketoglutarate (Cayman Europe, Hamburg, Germany) under normoxic or hypoxic (1% O_2) conditions for 4 hours.

Proteomics: Sample Preparation

Pellets of isolated neutrophils were solubilized in 10% SDS, 150 mmol/L NaCl, 100 mmol/L Tris/HCl pH 7.6, 100 mmol/L DTT. Samples were sonicated for 5 seconds and heated at 95°C for 5 minutes to facilitate protein solubilization. Samples were then incubated at 56°C for 30 min and centrifuged to remove insoluble material. Total protein (100 µg per sample) was diluted by adding 200 µL, 8 mol/L urea, 50 mmol/L Tris/HCl, pH 8.5 and loaded onto spin filters with a 30 kDa cut off (Microcon, Merck/Millipore, Darmstadt, Germany), and prepared as described.[5] Proteins were digested overnight with trypsin (sequencing grade, Promega, Mannheim, Germany) and eluted peptides were acidified by trifluoroacetic acid to a final concentration of 0.1 % and fractionated on multi-stop-and-go tips (StageTips) containing three strong cation exchange (SCX) disks and a stack of three C18-disks on top. SCX fractionation by StageTips was performed in four steps as described.[6] The first and second fractions were combined and all three fractions of each sample were eluted in wells of microtiter plates. Peptides were dried and resolved in 1% acetonitrile and 0.1 % formic acid.

Proteomics: Liquid Chromatography/Mass Spectrometry (LC/MS)

LC/MS was performed using a Q Exactive Plus (Thermo Scientific, Dreieich, Germany) equipped with an ultra-high performance LC unit (Dionex Ultimate 3000, Thermo Scientific) and a Nanospray Flex Ion-Source (Thermo Scientific). Peptides were loaded on a C18 reversed-phase precolumn followed by separation on a 2.4 µm reprosil C18 resin (Dr. Maisch GmbH, Ammerbuch, Germany) in-house packed picotip emitter tip (diameter 100 µm, 15 cm long from New Objectives, Woburn, USA) using a gradient from mobile phase A (4% acetonitrile, 0.1% formic acid) to 44 % mobile phase B (80% acetonitrile, 0.1% formic acid) for 105 minutes with a flow rate 500 nL/minute. Runs were finished by column washout with 99% mobile phase B for 5 minutes and re-equilibration in 1% mobile phase B.

MS data were recorded by data dependent acquisition using the Top10 method to select the most abundant precursor ions in positive mode for higher-energy collisional dissociation fragmentation. The lock mass option[7] was enabled to ensure high mass accuracy during many following runs. The full MS scan range was 300 to 2000 m/z with a resolution of 70000, and an automatic gain control value of $3x10^6$ total ion counts with a maximal ion injection time of 240 ms. Only higher charged ions (2+) were selected for MS/MS scans with a resolution of 17500, an isolation window of 2 m/z and an automatic gain control value set to 10^5 ions with a maximal ion injection time of 150 ms. Selected ions were excluded in a time frame of 30s following fragmentation event. Fullscan data were acquired in profile and fragments in centroid mode by Xcalibur software. The LC Unit was controlled by Chromeleon Xpress software (Thermo Scientific, Dreieich, Germany). The performance of both units LC and MS was integrated by DCMSLink.

Proteomics: MS Data Analysis

Xcalibur raw files were analyzed using the Max Quant (1.5.2.8; MPI of Biochemistry, Martinsried, Germany, http://141.61.102.17/maxquant_doku/doku.php?id=start) proteomics software.[8] The enzyme specificity was set to trypsin, missed cleavages were limited to 2. Acetylation of N-terminus (+42.01) and oxidation of methionine (+15.99) were selected for variable modification, carbamidomethylation (+57.02) on cysteines was set as fixed modification. The mouse reference proteome set from the UniProt Knowledgebase[9] (download June 2015, 76086 entries) was used to identify peptides and proteins and the false discovery rate was set to 5%. Label free quantification values were obtained from at least one identified peptide. **Online Table I** summarizes all protein identification, number of identified peptides, accession numbers and sequence coverage of each sample. For further analysis data were uploaded into Perseus software software (1.5.2.6, http://www.perseus-framework.org/; MPI of Biochemistry, Martinsried, Germany).[10] Identifications from reverse decoy database and known contaminants were excluded. For quantification, proteins were quality filtered according to a minimum of three valid values per group. For statistical comparison ANOVA and subsequent post hoc t-tests were used. Quantification results are summarized in **Online Table II**.

Metabolomics: Sample Preparation

Isolated neutrophils (2.5×10^6 cells) stimulated as described, were recovered by centrifugation, washed with PBS and solubilized in ice-cold methanol/water (85/15, v/v). One 3 mm stainless steel bead was added to each tube and samples were vortexed for at least one minute. Samples were centrifuged (10000g, 4°C, 10 minutes), and the supernatants were immediately transferred to a fresh reaction tube and snap frozen in liquid nitrogen. Before further processing isotope labeled internal standards were added. Samples were evaporated in a vacuum concentrator plus (Eppendorf, Hamburg, Germany) at 45°C, resolved in 50 µl water and subsequently transferred to the LC-MS system.

Metabolomics: LC/MS

LC was performed on an Agilent 1290 Infinity pump system (Agilent, Waldbronn, Germany). Separation was performed on a Waters Acquity HSS T3 column (150 mm × 2.1 mm, 1.8 µm) at 40 C. Separation started with a 1.5 minute isocratic step using water/acetonitrile (98/2, v/v) and 0.15% formic acid followed by a 3 minute linear increase to 100% acetonitrile plus 0.15% formic acid at a flow rate of 400 µl/minute. Separation was followed by a cleaning and equilibration step, making 10 minutes total LC run time. Mass spectrometry was performed using a QTrap 5500 mass spectrometer (Sciex, Darmstadt, Germany) with electro spray ionization at 400°C with 4500 V in positive and -4500 V negative modes. MS parameters were set to CUR 30 psi, GS1 45 psi, and GS2 25 psi for both ionization modes. Data acquisition and instrument control were managed through the software Analyst 1.6.2. Peak integration, data processing, and analyte quantification were performed using MultiQuant 3.0 (Sciex, Darmstadt, Germany). Area under the peak was used as the quantitative measurement. The specific MRM transition for every compound was normalized to appropriated isotope labeled internal standards.

Statistical analysis

Data are expressed as mean ± SEM. Statistical evaluation was performed using Student's t test for unpaired data, one-way ANOVA followed by a Bonferroni t test or ANOVA for repeated measures where appropriate. Values of $P<0.05$ were considered statistically significant.

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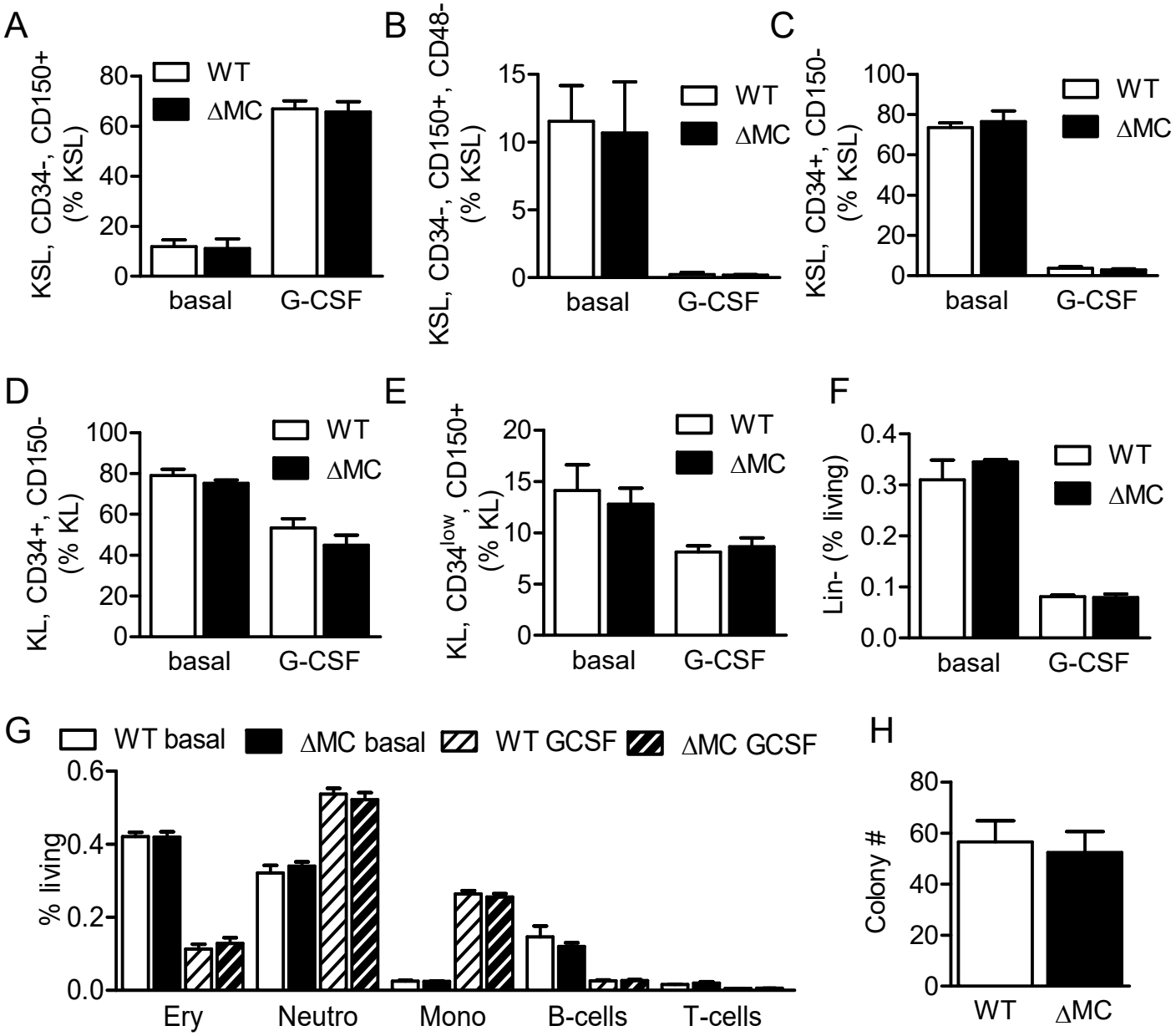
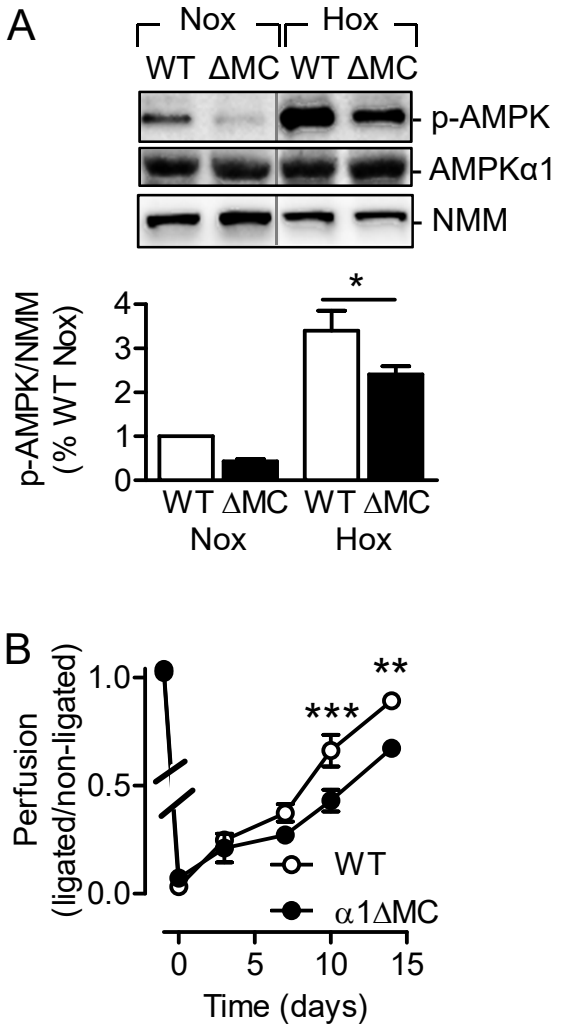
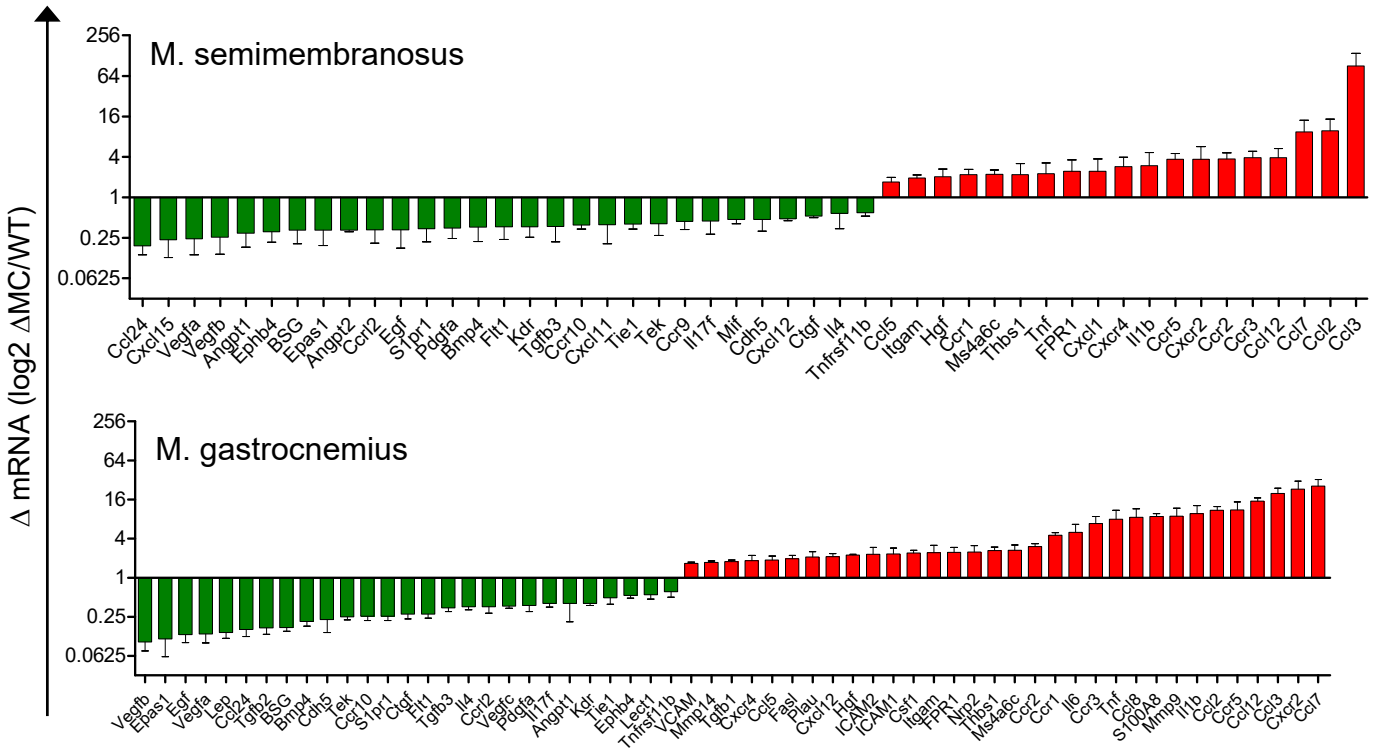


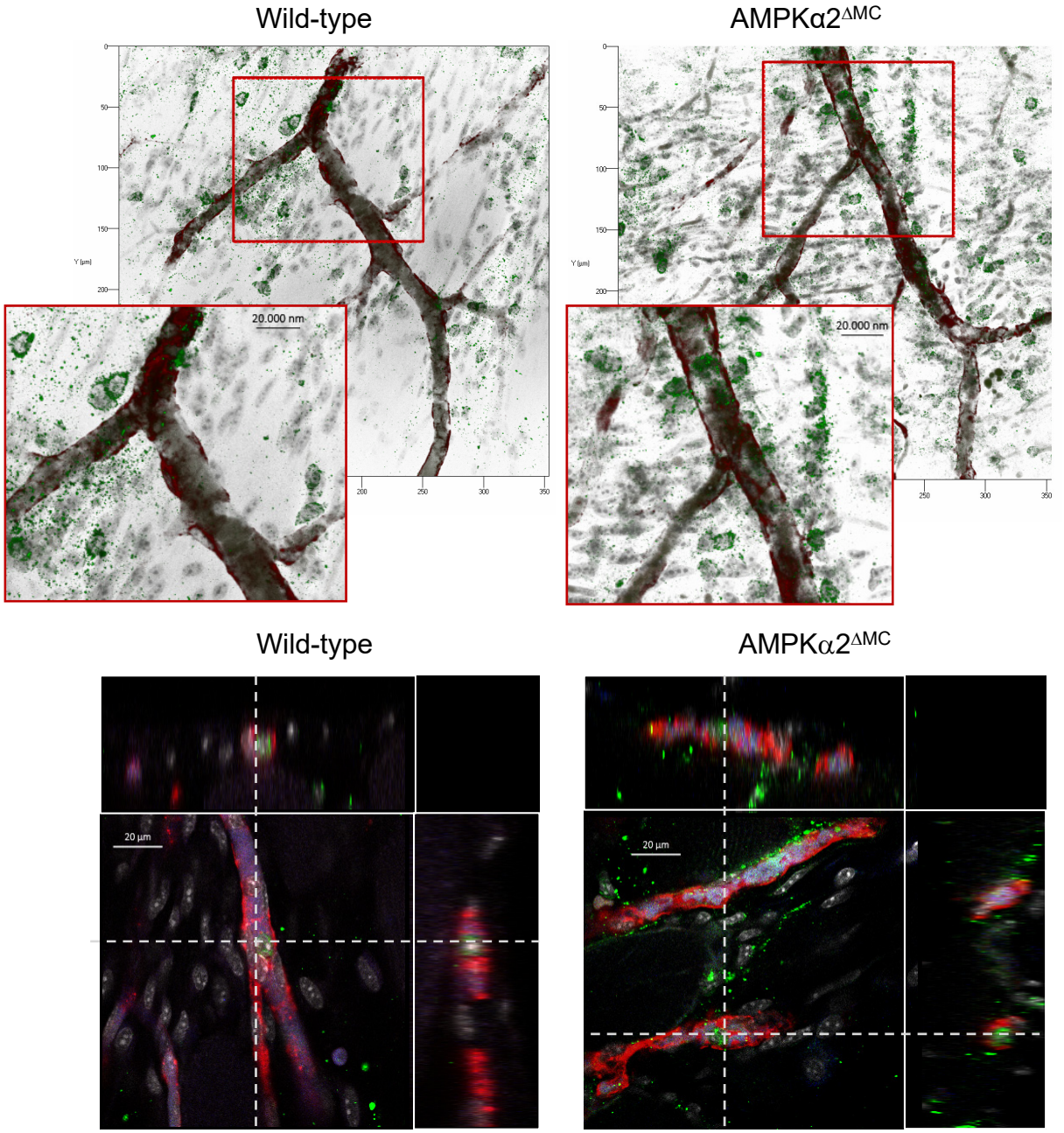
Online Figure I. Hematopoietic stem cell differentiation and mobilization. (**A-G**) Wild-type (WT) and AMPKα2$^{\Delta MC}$ (ΔMC) mice were administered G-CSF (250µg/kg/day, 5 days) and stem and progenitor cell populations, and differentiated cells in bone marrow as % of the cKit+Sca1+Lin- (%KSL), cKit+Sca1-Lin- (%KL) or living cell population (% living) were analyzed by flow cytometry. (**A**) Hematopoietic stem cells, (**B**) long-term hematopoietic stem cells, (**C**) multipotent progenitors, (**D**) granulocyte-macrophage progenitors, (**E**) megakaryocyte-erythrocyte progenitors, (**F**) lineage negative (Lin-) population, and (**G**) differentiated cell populations. (**H**) Proliferation and differentiation ability of mobilized progenitors as analyzed by using the colony-forming unit assay 5 days after G-CSF treatment. The graphs summarize data from 3-6 different animals per group (3 animals in control group; 6 animals in G-CSF treated group).



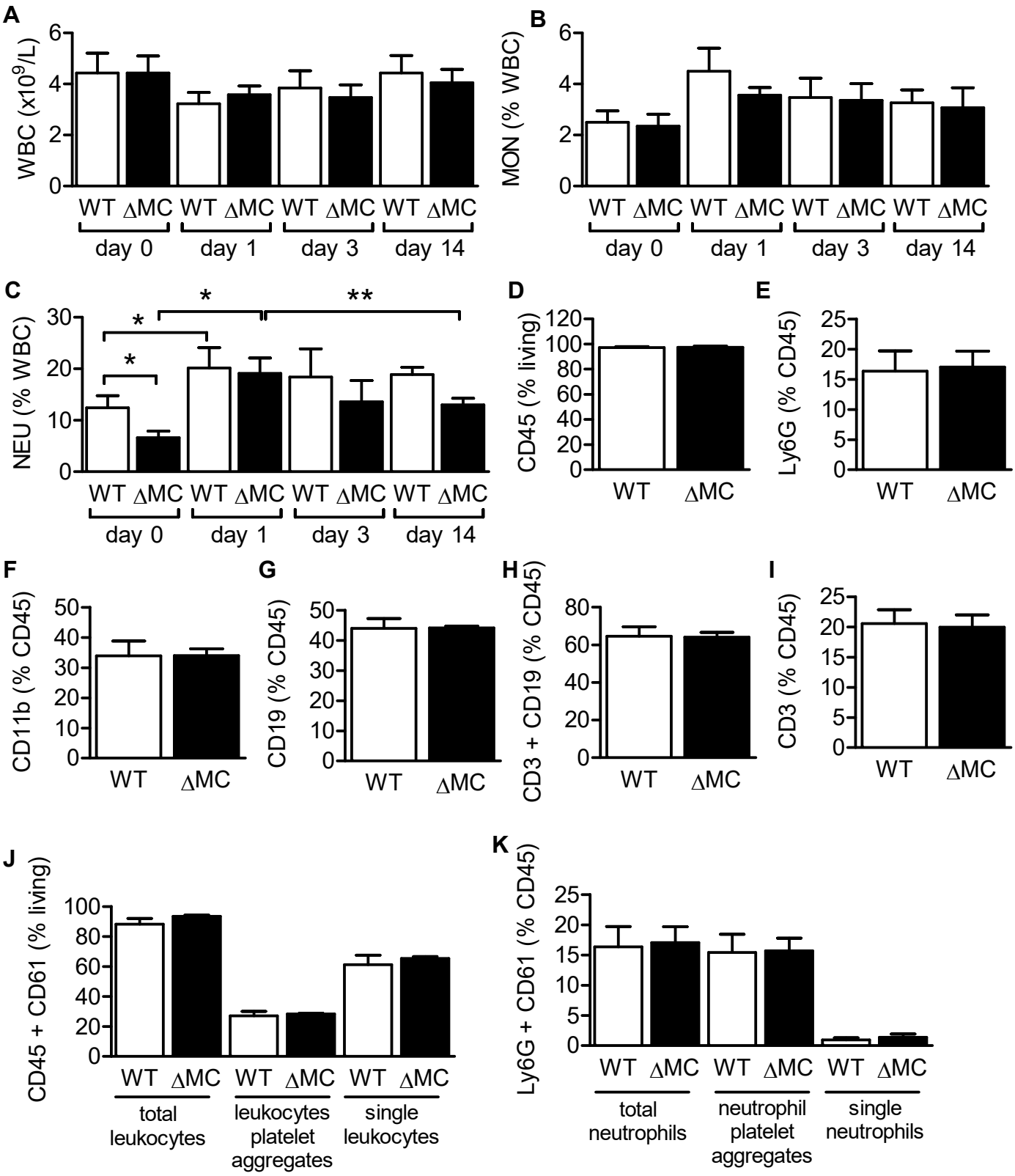
Online Figure II. **Consequence of myeloid cell-specific deletion of AMPKα1 on vascular repair after ischemia and AMPK phosphorylation in response to hypoxia**. (**A**) Neutrophils isolated from wild-type (WT) and AMPKα2$^{\Delta MC}$ mice (ΔMC) were incubated under normoxic (Nox) or hypoxic (Hox) conditions for 16 hours and AMPK phosphorylation (on Thr172) determined by Western blotting (n=3 different animals per group). Non muscle myosin (NMM) was used as an overlll loading control. The grey line indicates that non adjacent bands from the same Western blot are shown. (**B**) Wild-type (WT) and AMPKα1$^{\Delta MC}$ (α1ΔMC) mice were subjected to femoral artery ligation and the recovery of blood flow in the ligated limbs was monitored over 14 days by laser Doppler imaging and quantified relative to the non-ligated limb (n=7 different animals per group). **P<0.01, ***P<0.001 versus WT.



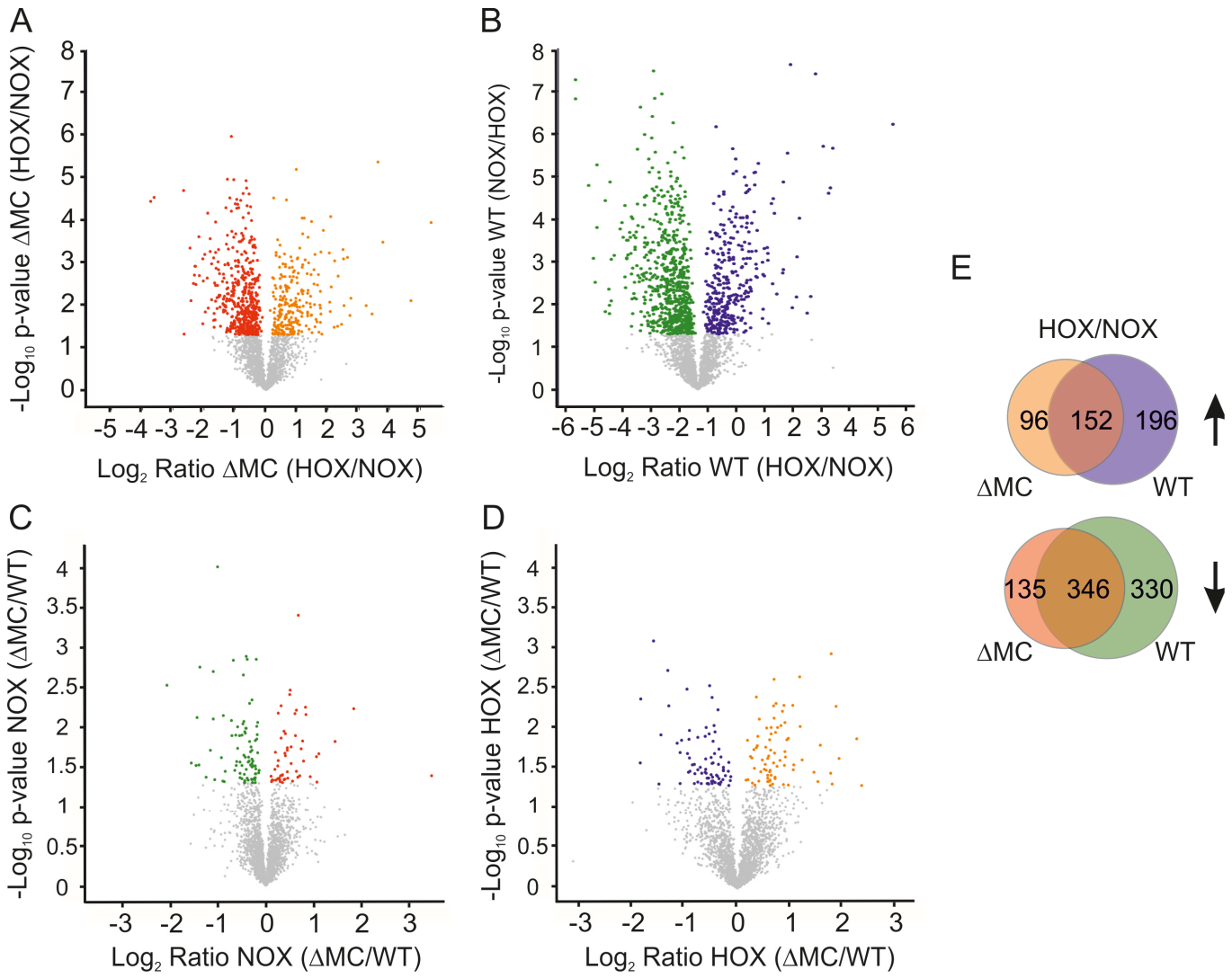
Online Figure III. **Altered gene expression profile in ischemic hindlimbs 72 hours post-surgery.** Chemokine and cytokine gene expression analysis in ischemic hindlimbs. Green bars show > 1.5 fold downregulated genes and red bars indicate significantly > 1.5 fold upregulated genes in AMPK$\alpha 2^{\Delta MC}$ compared to wild-type mice (n=4 animals in each group).



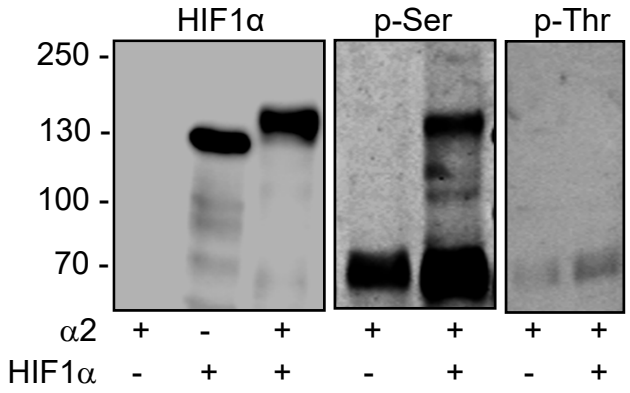
Online Figure IV. Neutrophil infiltration in the ischemic hindlimb 24 hours after ligation. (A) Overview of infitrated neutrophils in the murine M. gastrocnemius showing smooth muscle actin (red), Ly6G (green) and DAPI (grey) superimposed and inverted with a confocal laser scanning system (LSM 780); bar = 20 µm. (B) Intra- ad extra-vascular neutrophils in the ischemic M. gastrocnemius; VE-cadherin (blue), smooth muscle actin (red), Ly6G (green) and DAPI (white). Shown is a plane of a Z-stack (lower left), the dotted lines indicate the planes of the x and y axes; bar = 20 µm. Similar results were obtained in 4 different animals per genotype.



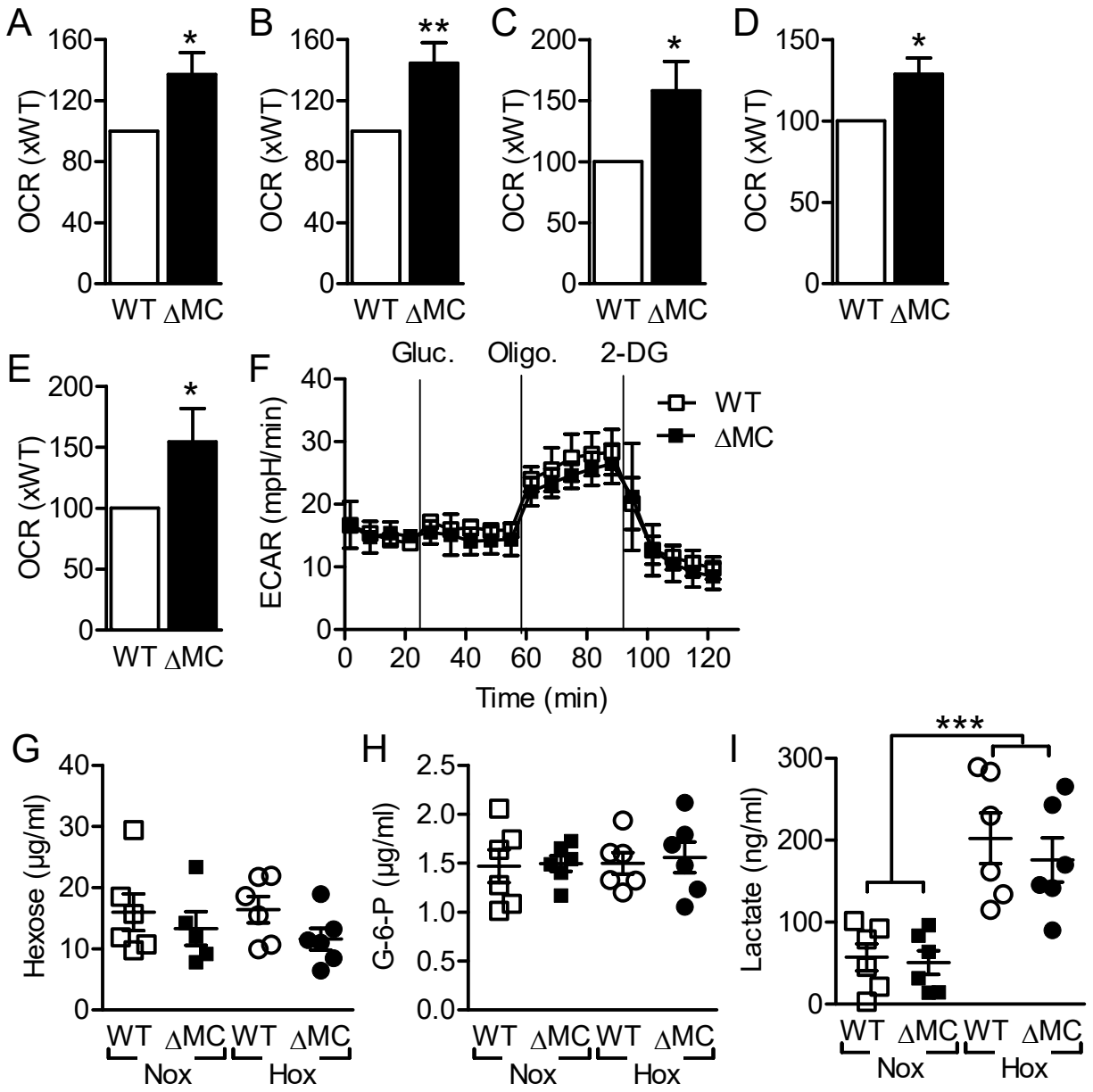
Online Figure V. **Time course of changes in circulating monocyte and neutrophil numbers after femoral artery ligation**. (**A-C**) Time course of changes in circulating white blood cells (WBC), monocytes (MON) and neutrophils (NEU) in blood from wild-type (WT) and AMPKα2$^{\Delta MC}$ littermates without ischemia (day 0) or 1, 3 or 14 days after femoral artery ligation. Blood was harvested by cardiac punction and analyzed using a cell counter; n=6-14 animals per group. (**D-I**) Composition of circulating leukocytes 24 hours after femoral artery ligation in AMPKα2$^{\Delta MC}$ mice versus wild-type littermates; n = 5 animals per group. (**J&K**) Circulating leukocyte-platelet (CD45+CD61) or neutrophil-platelet (Ly6G+CD61) aggregates as determined by flow cytometry 24 hours after femoral artery ligation; n = 5 animals per group. *P<0.05, **P<0.01 (ANOVA and Newman–Keuls Multiple Comparison Test).



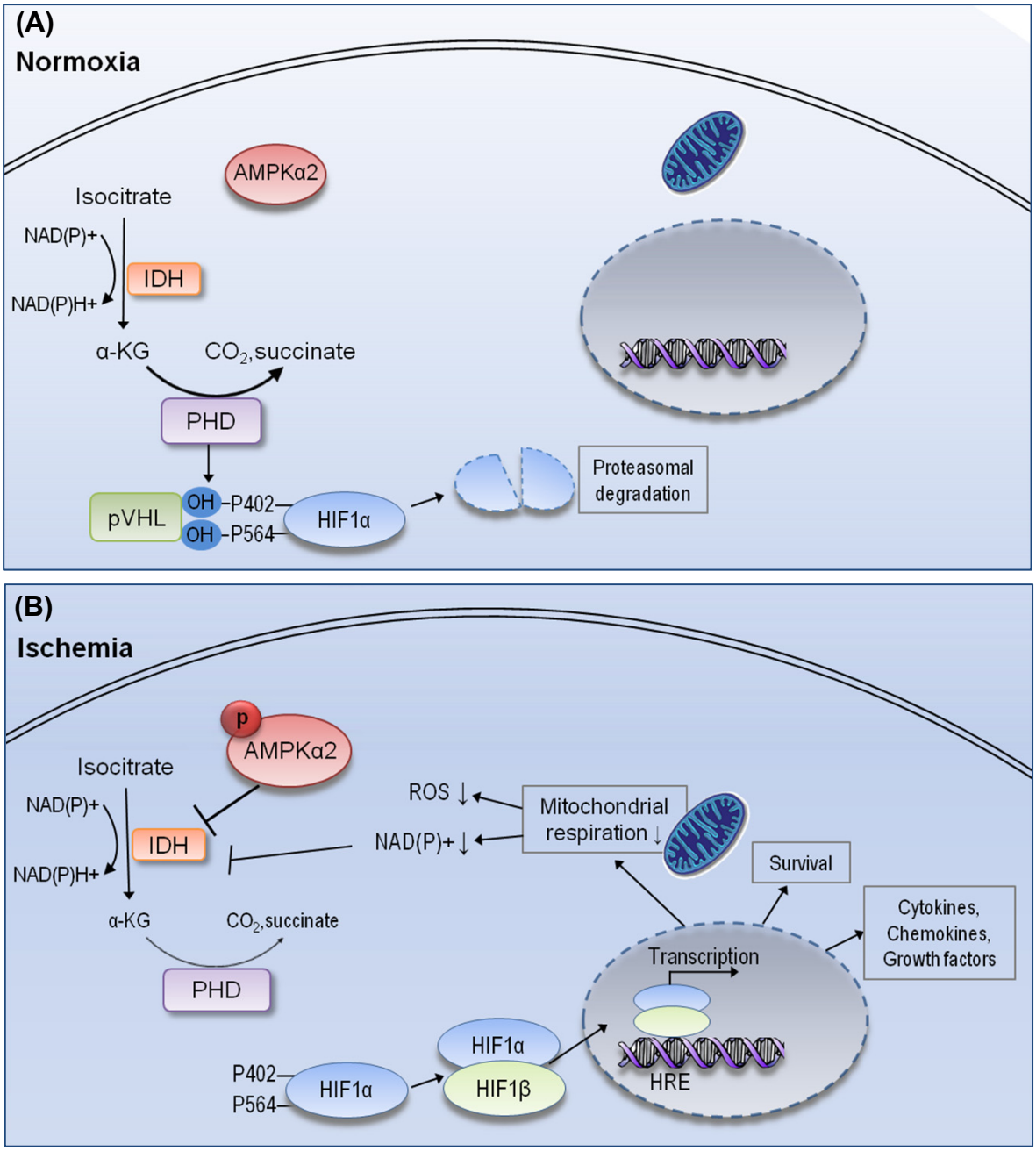
Online Figure VI. Differently expressed proteins in neutrophils from wild-type (WT) and AMPKα2$^{\Delta MC}$ (ΔMC) under conditions of normoxia (NOX) and hypoxia (HOX). Volcano plots showing p-values (-log$_{10}$) versus protein ratio (log$_2$) of (**A**) HOX/NOX in ΔMC, (**B**) HOX/NOX in WT, (**C**) ΔMC/WT in NOX, (**D**) ΔMC/WT in HOX. Significantly altered proteins are colored. Color code for upregulated proteins: ΔMC NOX (red); ΔMC HOX (orange); WT NOX (green); WT HOX (blue). (**E**) Two-way Venn diagram summaries of unique and overlapping proteins differentially expressed in ΔMC and WT cells under normoxia and hypoxia. Comparison of upregulated (upper panel) and downregulated (lower panel) proteins in ΔMC and WT neutrophils cultured under hypoxia.



Online Figure VII. Link between AMPKα2 and HIF-1α. In vitro phosphorylation of a HIF-1α peptide by recombinant human AMPKα2 protein. Phosphorylation was assessed by Western blotting with anti-serine or anti-threonine antibodies. Similar results were obtained in 2 additional experiments.



Online Figure VIII. **Effect of AMPKα2 deletion on metabolism in neutrophils.** Oxygen consumption rate (OCR) was assessed in bone marrow-derived neutrophils from wild-type (WT) and AMPKα2$^{\Delta MC}$ (ΔMC) mice. Quantification of mitochondrial respiration function parameters from **Fig 5C**: (**A**) basal respiration (**B**) non-mitochondrial respiration (**C**) maximal respiration. (**D**) spare respiratory capacity, and (**E**) proton leak. (**F**) Extracellular acidification rate (ECAR) profile showing glycolytic function in bone marrow derived neutrophils from WT and AMPKα2$^{\Delta MC}$ littermates. Vertical lines indicate the addition of glucose (Gluc; 10 mmol/L), oligomycin (Oligo; 1 μmol/L), and 2-deoxy-D glucose (2DG; 100 mmol/L). (G-I) Levels of hexose i.e. fructose + glucose (**G**), glucose-6-phosphate (G-6-P; **H**) and lactate (**I**) in neutrophils from wild-type (WT) and AMPKα2$^{\Delta MC}$ (ΔMC) mice maintained under normoxic (Nox) or hypoxic (Hox) conditions for 16 hours. Graphs summarize data from 8-9 (A-F) or 5-6 (G-J) different animals per group; *P<0.05, **P<0.01 versus wild-type, ***P<0.001 versus normoxia.



Online Figure IX. Scheme showing the consequences of ischemia-induced AMPKα2 activation in neutrophils. (**A**) Normoxia (AMPKα2 inactive). Isocitrate dehydrogenases (IDH) catalyze the oxidative decarboxylation of isocitrate to α-ketoglutarate (α-KG) by utilizing $NAD(P)^+$ as electron acceptor. α-KG is required as a cofactor for prolyl hydroxylases (PHD) and is thereby decarboxylated to succinate and CO_2. Active PHDs hydroxylate hypoxia-inducible factor-1α (HIF-1α) at proline residue 402 and 564. The hydroxylated proline residues are recognized and captured by the von-Hippel-Lindau protein (pVHL), leading to HIF-1α ubiquitylation and subsequent proteasomal degradation. (**B**) Hypoxia (AMPKα2 active). Phosphorylated and activated AMPKα2 leads to the inhibition of IDH and to a decrease in cellular α-KG levels which results in the inactivation of PHDs. Consequently, HIF-1α is not hydroxylated and can escape pVHL-mediated ubiquitylation and proteosomal degradation. Stabilized HIF-1α then translocates to the nucleus where, together with HIF-1β, it forms an active HIF complex that induces the expression of angiogenic cyto- an chemokines, and genes that support survival and decrease mitochondrial respiration. Less mitochondrial respiration reduces ROS formation and the the availability of $NAD(P)^+$. Reduced $NAD(P)^+$ availability concomitanly limits the activity of IDH to keep levels of α-KG and PHD activity low.