

Supp. Methods:

AAV-CM-Ang2 mice

C57BL/6 mice aged 12 weeks underwent echocardiographic imaging and non-invasive blood pressure measurements followed by tail vein injection of a recombinant adenoassociated virus 2/9 in low dosage (1×10^{12}) encoding for either Ang2 or the reporter gene LacZ. Echocardiography and blood pressure measurements were repeated at the age of 24 weeks followed by invasive left ventricular pressure measurements. Organs were harvested for histological analysis and PCR.

Pericyte coverage in X-LacZ-4 mice

X-LacZ-4 mice were transduced with either a rAAV.Ang2 or rAAV.GFP. After 14 days the diaphragms of these mice were harvested and X-Gal Stainings were performed. Pericytes were counted and displayed as number of pericytes per 50 μ m vessel length.

Quantification of Masson Trichrom Stainings

Masson Trichrom images were analysed utilizing ImageJ (W.S. Rasband, U. S. National Institutes of Health, Bethesda, Md, USA, <http://imagej.nih.gov/ij/>, 1997–2012). From multiple images of the complete circumference of the left ventricle background was subtracted and the blue area (fibrosis) was measured as a percentage of total muscle area.

Matrigel Assay:

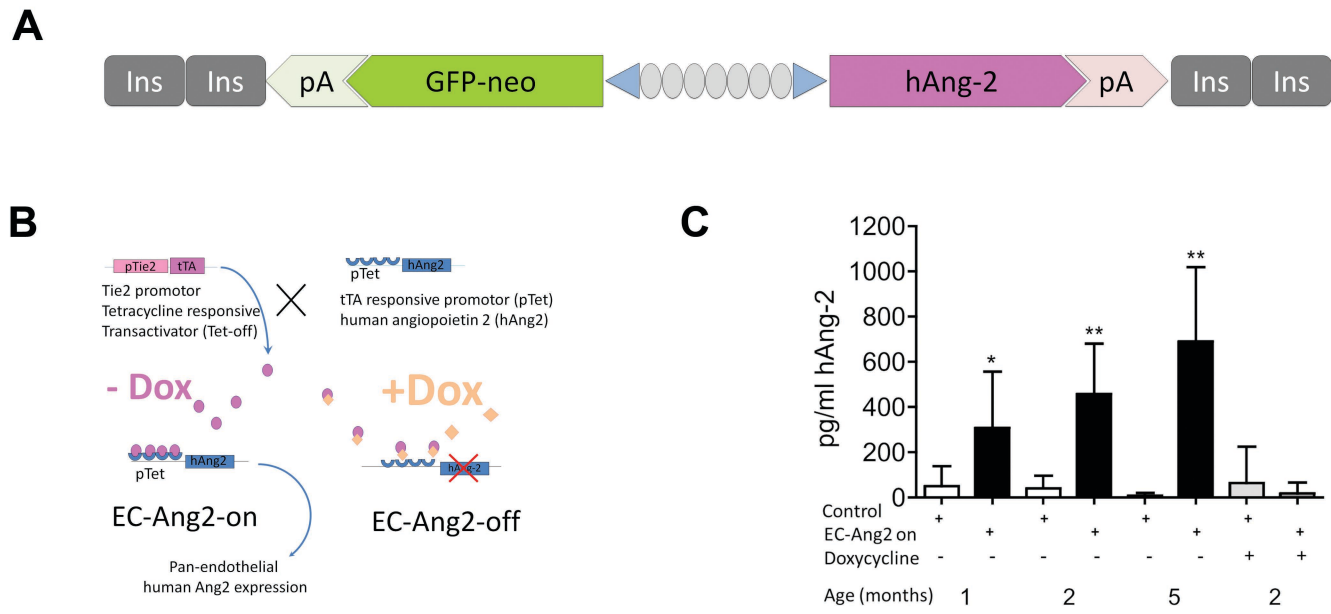
Mouse brain endothelial cells (bEnd3) were seeded into 6-well plates and transfected after 24 hours with Lipofectamine 2000 and 1 μ g of the according plasmid (Ang2, Ang1, PDGF-B, pcDNA). After 24 hours cells were labelled with DiI and seeded on Matrigel coated μ -slides (10000 cells per well). After completion of ring formation (approx.. 5 hours) ATCC-CCL-226

cells were labelled with DID and seeded on the preformed rings (5000 cells per well). Pictures were taken after 24 hours and CCL-226 cells per ring of bEnd3 cells were counted.

Sepsis Score:

A score-system to assess the clinical severity was used (cf. Suppl Table 1). In more detail, behavior was scored for normal behavior and provoked reaction. Normal behavior was scored as 0 when mice were agile in the cage and showed a normal interest on their surrounding and in the provoked behavior they show a normal escape reaction. Score of 5 equal's slower movements and more sitting periods, whereas provoked behavior of escape only occurred when approaching with fingers. A score of 10 equals a further reduced movement with unsteady walk and an escape reaction only after touching the animal. 20 was scored when the mice were lying in lateral position and did not show any flight reflex. Furthermore, assessment of pain was performed and scored using the following criteria: Normal group behavior, grooming and feeding behavior is scored as 0. In mice displaying a bent back, impaired cleaning and reduced feeding behavior score was 5. Whereas mild change in walking pattern, shivering, clearly reduced grooming and separation from the cagemates were classified as moderate pain (score of 10). Severe pain (score of 20) was classified by severe change in walking pattern, shivering, starving and grooming, separation and hiding within the cage. The other parameters of the scoring system were classified as displayed in Supplementary Table 1.

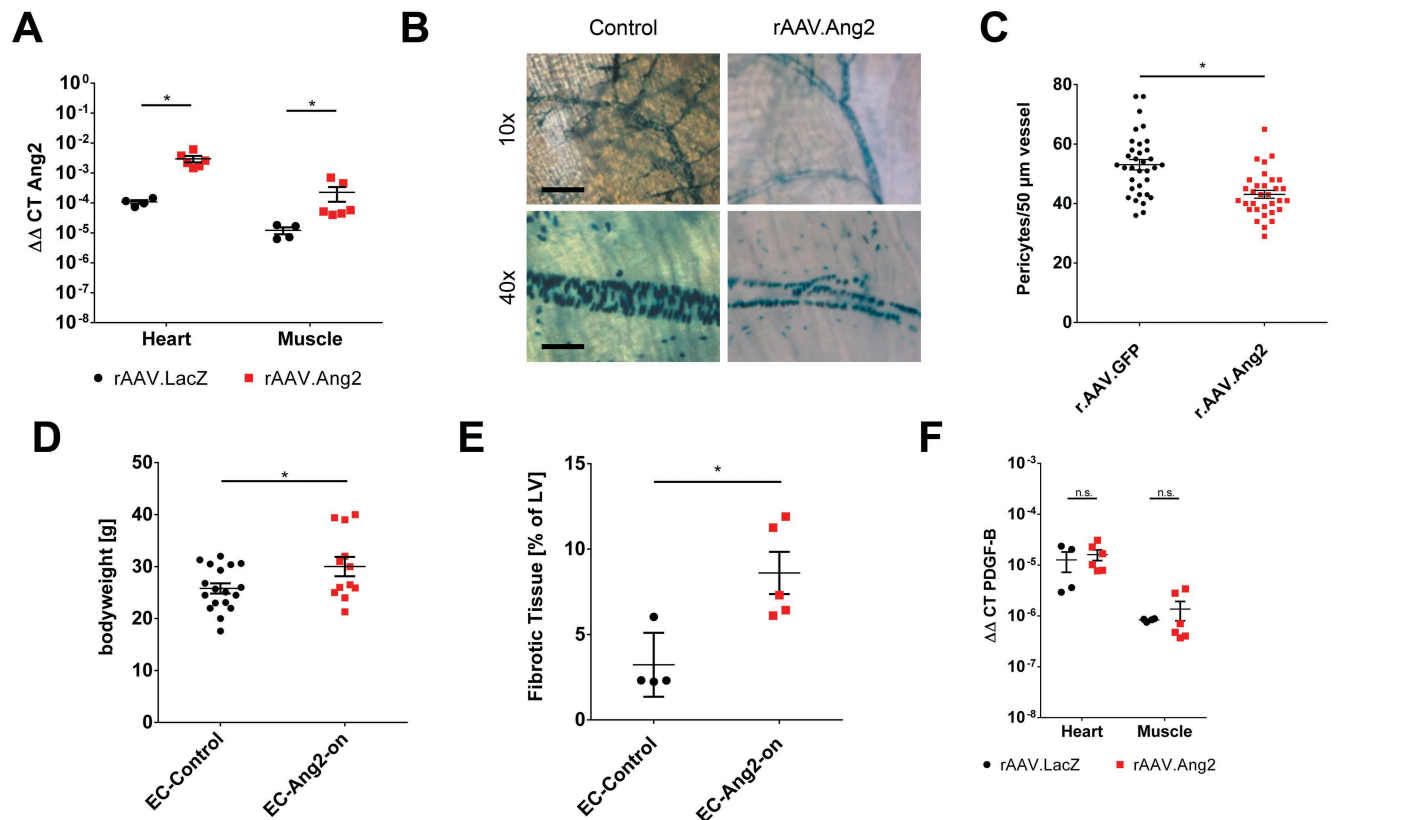
Suppl. Figure 1



Suppl. Figure 1:

(A) The tetracycline-response element (TRE) is constituted by seven repeats of the E. coli tet-operator sequence (light grey circles), which are flanked by 30 bp fragments of the CMV immediate early minimal promoter (blue triangles). The hAng-2 open reading frame (pink) was cloned into pUHD 10-3 (a gift from Hermann Bujard) as a Aval fragment. The corresponding transcription termination sequence is derived from SV40. The GFP open reading frame (green) is derived from GFPemd (Packard CytoGem) and fused with the ORF for the neomycin phosphotransferase (green) and the bovine growth hormone poly(A) signal from pSA-β-geo (a gift from Phil Soriano). The entire bi-directional expression cassette is flanked on either side by two repeats of HS4 insulator sequences (dark grey) of the chicken β-globin gene (a gift from Gary Felsenfeld). **(B)** Pan-endothelial human Ang2 overexpression is achieved by crossing an endothelial activator mouse (Tie2-tTA) with a responder mouse carrying a tetracyclin responsive promoter combined with the human Ang2 gene (TRE-hAng2). **(C)** EC-Ang2-on mice show a progressive increase of circulating human Ang-2 levels, unless Doxycycline is applied to abrogate Ang2 expression. * indicates p<0.05 vs. Control, ** indicates p<0.001 vs. Control.

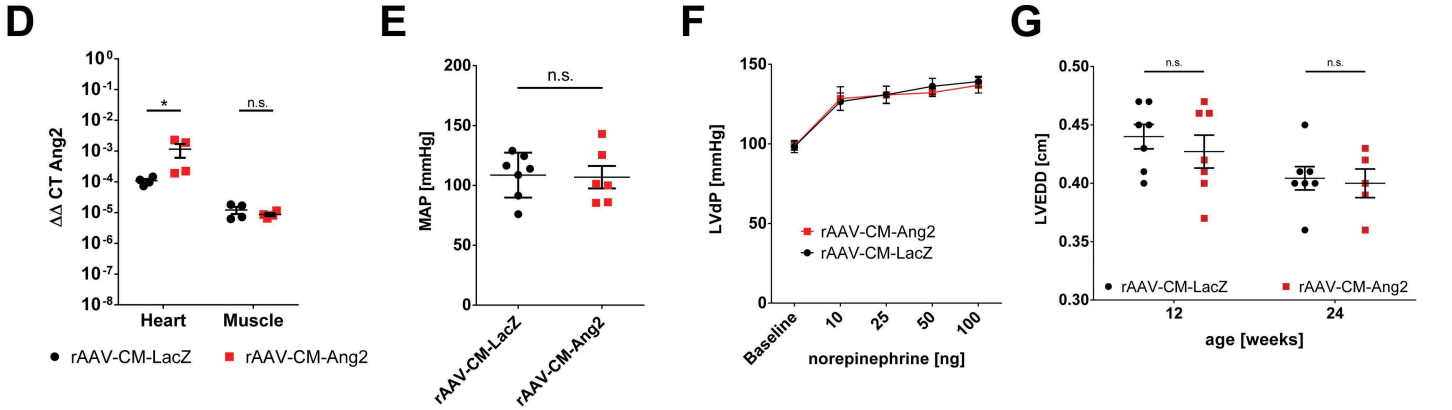
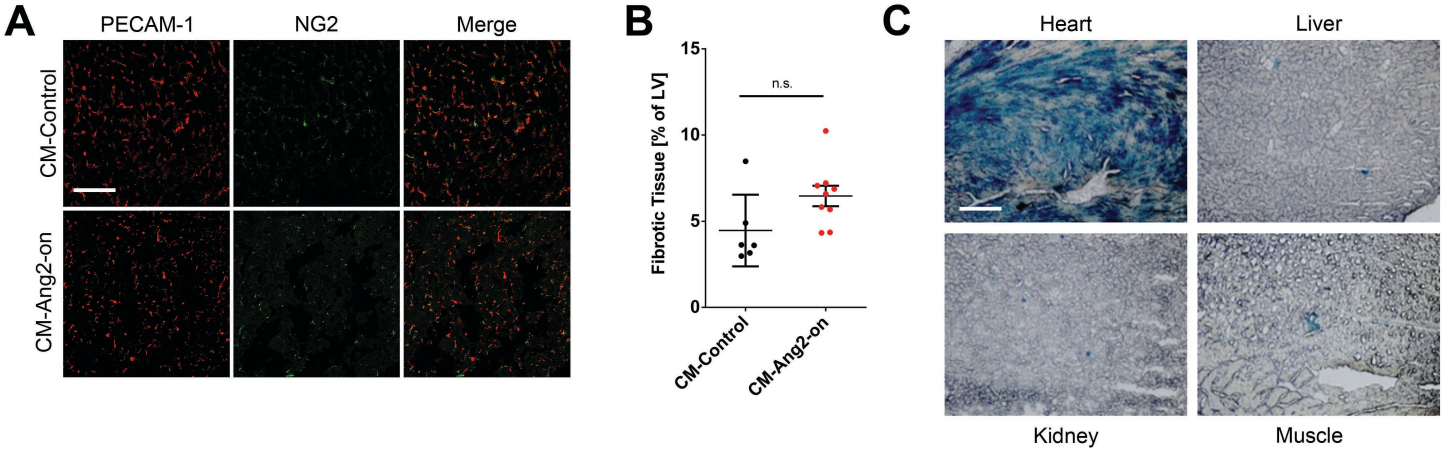
Suppl. Figure 2



Suppl. Figure 2:

(A) i.v. injection of a recombinant adeno-associated virus (myotropic rAAV2.9) coding for Ang2 in high dosage (5×10^{12}) increases mRNA levels in heart and muscle (B+C) X-LacZ-4 mice transduced with Ang2 show decreased pericyte coverage in whole mount stainings of the diaphragm ($n=33$ high power fields from $n=7$ diaphragms per group). (D) EC-Ang2-on mice display an increase in bodyweight compared to EC-Control mice ($n=18$ for EC-Control, 12 for EC-Ang2-on). (E) Quantification of fibrotic heart tissue (Masson Trichrom Staining) reveals increased myocardial fibrosis in EC-Ang2-on mice (F) AAV mediated Ang2 transduction through i.v. injection does not result in alterations of PDGF-B mRNA levels in isolates of the heart and peripheral muscle (M. quadriceps femoris). * indicates $p < 0.05$.

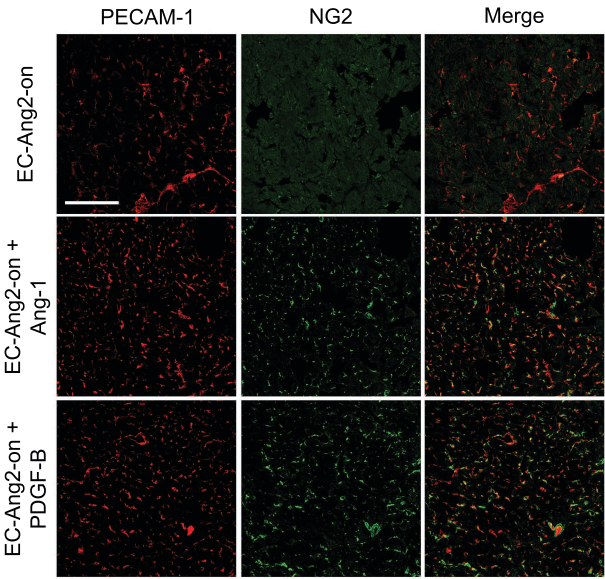
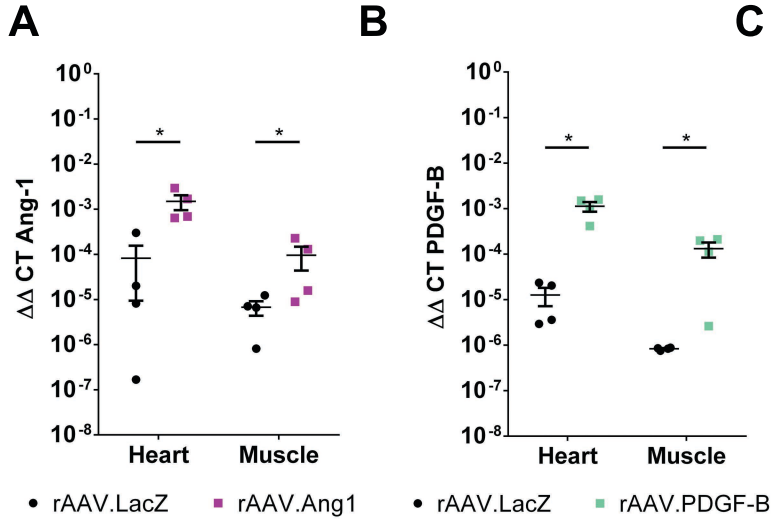
Suppl. Figure 3



Suppl. Figure 3:

(A) Low power images of PECAM-1 (red) / NG2 (green) stained heart tissue sections reveal a decreased pericyte coverage in CM-Ang2-on mice compared to control hearts (bar=100µm). **(B)** Quantification of myocardial fibrosis in CM-Ang2-on mice shows no significant increase compared to control mice **(C)** X-Gal stainings of tissue sections reveal a myocardial restricted reporter gene expression (LacZ) after tailvein injection of low doses of rAAV.LacZ (1 x 10¹² virus particles) and **(D)** increased Ang2-mRNA levels of Ang2 in the heart, but not in the peripheral muscle (M. quadriceps femoris) **(E)** rAAV-CM-Ang2 mice display no alterations in blood pressure (MAP) **(F)** left ventricular developed pressure (LVdP) and **(G)** left ventricular dilation (LVEDD). n.s. indicates non-significant, * indicates p<0.05.

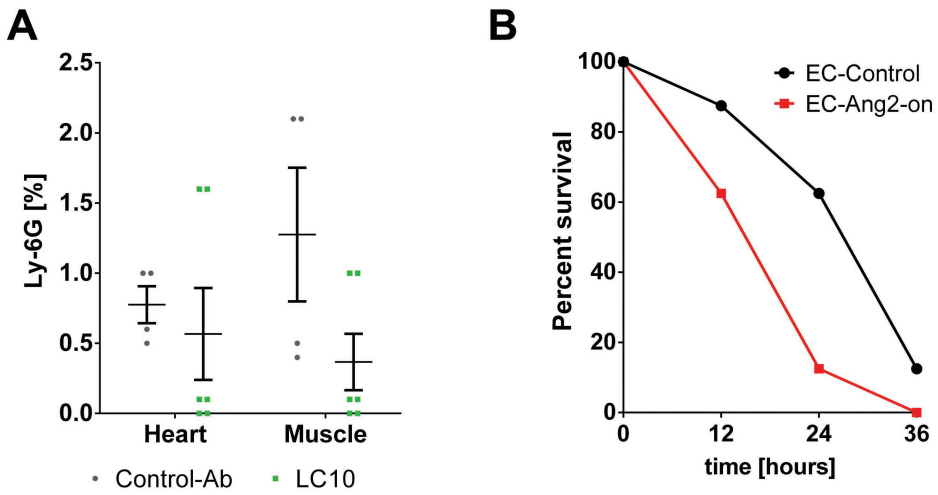
Suppl. Figure 4



Suppl. Figure 4:

(A + B) mRNA levels of Ang1 and PDGF-B after AAV mediated transduction of Ang1 **(A)** or PDGF-B **(B)**, respectively. (n=4 per group, tail-vein injection) **(C)** Low power images of PECAM-1 (red) / NG2 (green) stained heart tissue sections display prevention of decreased myocardial pericyte coverage in EC-Ang2-on mice following Ang1 or PDGF-B transduction at the age of 12 weeks (bar=100 μ m). * indicates p<0.05

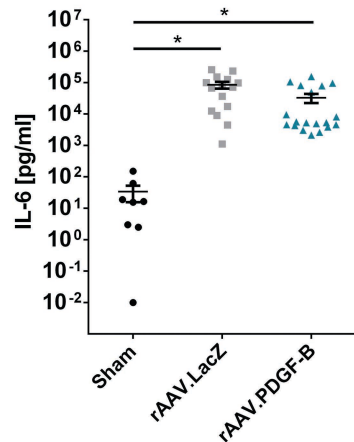
Suppl. Figure 5



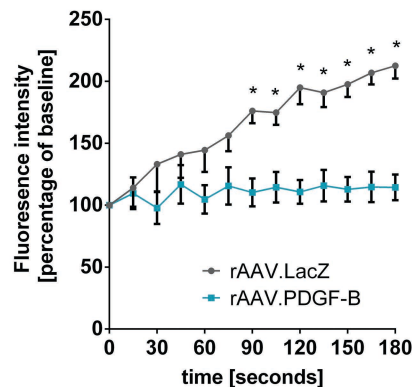
Suppl. Figure 5:
(A) FACS analysis of heart and muscle samples 24 hours after Sepsis induction displays no significant change in neutrophil recruitment after pre treatment with LC10 **(B)** EC-Ang2-on mice (20-24 weeks of age) displaying an overt edematous phenotype before LPS challenge, reveal an increased early mortality after injection of LPS (n=8 per group, Log-Rank p=0,043)

Suppl. Figure 6

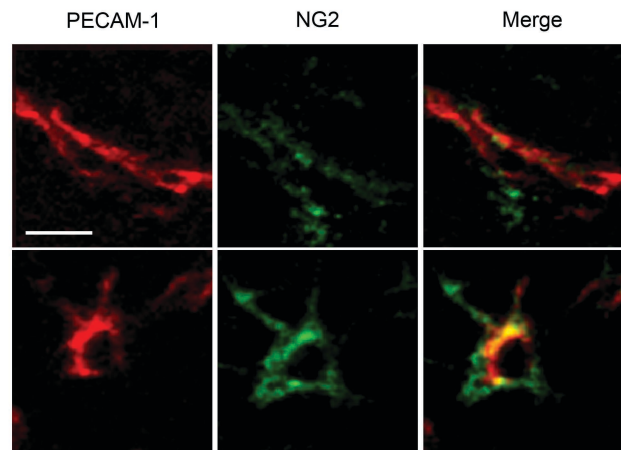
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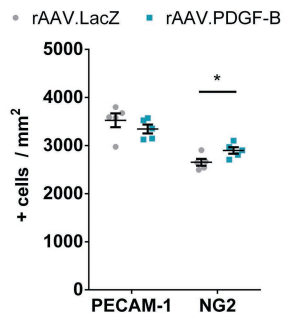
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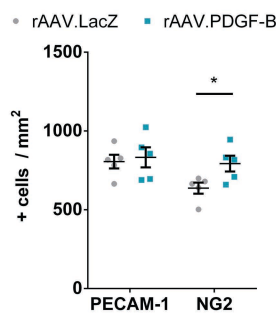
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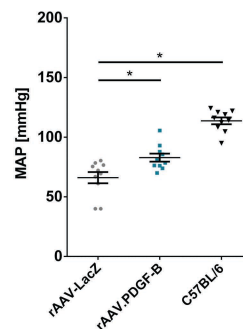
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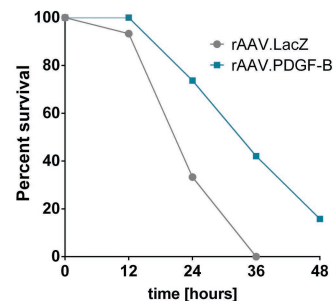
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F



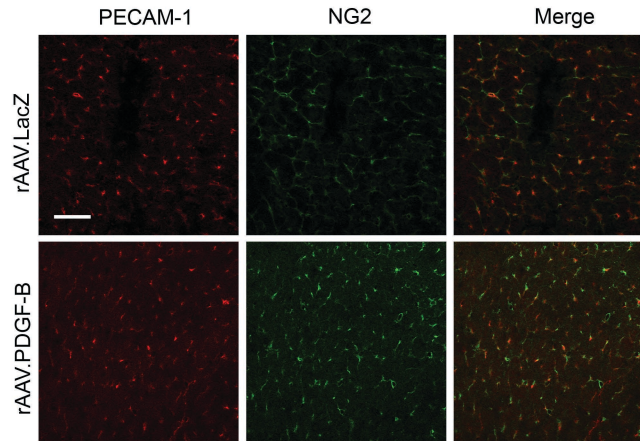
G



Suppl. Figure 6: PDGF-B reduces pericyte loss and mortality in a sepsis model

(A) Interleukin 6 levels increase 12h after intraperitoneal injection of LPS in control and rAAV.PDGF treated mice. (B) PDGF-B overexpression via rAAV i.v. injection prevents increased microvascular permeability 12h after LPS injection (n=4). (C) rAAV.PDGF-B treatment prevents pericyte loss and capillary rarefaction in the coronary microcirculation (bar = 5µm). (D) Myocardial and (E) peripheral microcirculation of quadriceps muscle display increased capillary density and pericyte coverage (n=5). (F) Mean arterial pressure (MAP) and (G) survival were increased following sepsis induction in mice pretreated with rAAV.PDGF-B (n=15 for rAAV.LacZ, 20 for rAAV.PDGF-B, Log-Rank p=0.001). * indicates p<0.05

H

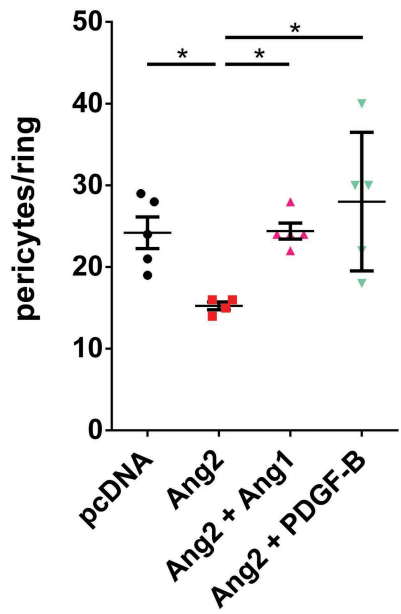


Suppl. Figure 6 continued:

(H) Low power images of PECAM-1 (red) / NG2 (green) stainings in mice with LPS induced sepsis after treatment with rAAV.LacZ or rAAV.PDGF-B

Suppl. Figure 7

A



Suppl. Figure 7:

(A) Matrigel co-culture of endothelial cells (bEnd3) and pericytes (ATCC-CCL-226) reveals a decreased recruitment of pericytes to Ang2-transfected endothelial tubes than in pcDNA transfected or Ang2 + Ang1 or Ang2 + PDGF-B transfected endothelial tubes. * indicates p<0.05.

Suppl. Table 1

points	0	5	10	20
behaviour	normal movement of the mouse in the cage, regular escape reflex	movement slowed down, mouse still moving on it's own	no movement on it's own, tipping on mouse results on movement	mouse comatose / apathetic
weight loss	0 - 5 %	5 - 10%	10 - 15%	> 15%
ascites	none	mild	moderate	severe
dyspnoea	none	mild	moderate	severe
pain	no signs of pain	mouse with bent back, impaired cleaning behaviour	mild change in walking pattern, shivering	severe change in walking pattern, shivering

Suppl. Table 1:

The scoring system to assess severity of the systemic inflammation includes five categories (behaviour, weight loss, ascites, dyspnoea, pain). At every timepoint of assessment a score is given for the five categories, ranging from 0 (no impairment) to 20 (severe impairment). The experiment was terminated, when a mouse reached a combined score of 20 or more. The termination was equated with the death of the animal.