SUPPLEMENTAL MATERIAL

The short-chain fatty acid propionate protects from hypertensive cardiovascular damage

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Supplemental Methods

Echocardiography

Echocardiography was performed as described by Markó et al. In short, isoflurane anesthetized mice were examined on a VisualSonics Vevo 2100 system using a 30 MHz-Transducer (MS-400, VisualSonics). Left ventricular wall thickness was analyzed using M-mode images from the parasternal short-axis view.

In vivo electrophysiological studies

In vivo electrophysiological studies were performed as described by Markó et al. During isoflurane anesthesia (Univentor 400 anesthesia unit), body temperature was kept constant at 37 °C using a homeothermic blanket control unit with a rectal temperature control (Harvard Apparatus). After surgical preparation of the right jugular vein a 2 French octapolar electrophysiology catheter (CIB’ER mouse catheter, NuMed) was placed into the right ventricle. A standard limb lead surface ECG was recorded simultaneously using skin electrodes. Programmed stimulation was performed using a digital electrophysiology lab (EP Tracer; CardioTek) and a standardized protocol that included trains of 10 basal stimuli followed by up to 3 extra stimuli, delivered with a coupling interval decreasing in steps of 5 ms until ventricular refractory was reached. The stimulation procedures were repeated at three different basal cycle lengths (100, 90 and 80 ms). Occurrence and duration of inducible arrhythmias were digitally documented. Reproducible inducible protocols were calculated as the percentage of positive out of total protocols applied. Only reproducible ventricular arrhythmias longer than five consecutive beats were considered positive.

Histological analyses

Hearts from wild-type NMRI (WT) mice were immediately frozen in -40 °C isopentane and stored at -80 °C. Staining was performed on 5 µm cryosections fixed in -20 °C acetone for 30 min. For staining of a single antigens unspecific binding was blocked with 10 % normal donkey serum (NDS) for 2 hrs. Primary antibodies were diluted in 10 % NDS (anti-CD4 (H129.19), BD Pharmingen, 1:75; anti-CD8a (53-6.7), BD Pharmingen, 1:100; anti-collagen I (polyclonal), Southern Biotech, 1:100; anti-fibronectin (polyclonal), Abcam, 1:400; anti-FSP-1 (polyclonal), Abcam, 1:100) and incubated overnight at 4 °C in a humid chamber, followed by incubation with a Cy3-conjugated secondary antibody in PBS for 2 hrs at room temperature (RT) (anti-goat-IgG-Cy3 (polyclonal), Jackson ImmunoResearch, 1:300; anti-rabbit-IgG-Cy3 (polyclonal), Jackson ImmunoResearch, 1:400; anti-rat-IgG-Cy3 (polyclonal), Jackson ImmunoResearch, 1:200). Co-staining of connexin-43 (Cx43) and N-cadherin was performed using the M.O.M. immunodetection kit (Vector Laboratories). Unspecific binding was blocked with 10 % normal goat serum, 0.3 % Triton X-100 and 0.2 % bovine serum albumin (BSA) for 1 hr, followed by M.O.M. Mouse Ig Blocking reagent for 30 min. Both primary antibodies were diluted in serum blocking reagent (anti-connexin-43
(polyclonal), Cell Signaling Technology, 1:500; anti-N-cadherin (3B9), In vitrogen, 1:100) incubated overnight at 4°C in a humid chamber, followed by 1 hr incubation at RT with a biotin-conjugated goat anti-mouse IgG antibody in M.O.M. diluent (Vector Laboratories, 1:500). Secondary antibody (anti-rabbit IgG-Alexa 488 (polyclonal), life technologies, 1:1000) and the streptavidin conjugated Alexa 568 (1:100 dilution, Life technologies) were incubated for 1 hr at RT. Slides were covered in Vectashield mounting medium with DAPI (Vector Laboratories). Images were acquired using a Zeiss Axio Imager M2 microscope and AxioVision software (Zeiss).

Cardiac interstitial fibrosis was analyzed using fibronectin immunofluorescence. The Cy-3 positive area was quantified in high-power fields (HPF, 400x) of crosscut cardiomyocytes using ImageJ software with a mean threshold for the Cy3-positive area. Cardiac perivascular fibrosis was quantified using collagen I immunofluorescence. Mean Cy3-positive fibrosis width was measured using AxioVision software (Zeiss) and normalized to the mean vessel width. Cardiac immune cells were stained using CD4 and CD8 specific antibodies and quantified per heart section. FSP-1 positive cells were assessed per HPF. The degree of Cx43 and N-cadherin colocalization was assessed using ImageJ software with the JACoP plugin. The amount of colocalization is expressed by an overlap coefficient with mean thresholds for both Cx43 and N-cadherin.

Hearts from apolipoprotein E knockout (ApoE<sup>−/−</sup>) mice were formalin-fixed and paraffin embedded. 10 µm transverse sections were stained with picrosirius red for detecting total collagen (Chondrex™, Redmond, Washington, USA) as described previously. For representative pictures, stained slides (2 µm thick) were rinsed with 0.5mL distilled water. Dried slides were mounted (Roti©-Mount HP68.1, Karlsruhe, Germany) and covered. Collagen content (%) was calculated using Adobe Photoshop CSS software by determining the number of red pixels (representing collagen) divided by the number to green pixels (representing non-collagen).

**Atherosclerosis quantification and plaque histology**

ApoE<sup>−/−</sup> mice were sacrificed and flushed with ice-cold PBS. Aortas were dissected from the aortic arch to the femoral bifurcation and fixed in 4 % PFA at 4 °C overnight. After removal of the adventitia, aortas were stained with Oil Red O solution (Sigma Aldrich) as described previously. To obtain photographs, aortas were pinned and photographed under a microscope (Leica MZ6, Wetzlar, Germany) and digital camera (Coolpix 4500, Nikon, Tokyo, Japan, Carl Zeiss, Jena, Germany).

The brachiocephalic artery (BCA) was dissected, fixed in formalin and embedded in paraffin. 7 µm thick cross sections of the BCA were prepared. The cross section with the maximum degree of stenosis was used for Movat staining. The adjacent sections in each direction were used for F4/80 and CD3 immunohistochemistry.

For Movat staining, slides were fixed in Bouin’s for 10 min at 50 °C, and then immersed in 5 % sodium thiosulfate for 5 min, 1 % alcian blue for 15 min, alkaline alcohol for 10 min at 60 °C. Movat Weigert’s solution was prepared out of 2 % alcohol hematoxylin, ferric chloride stock solution and iodine stock solution in ratio of 3:2:1. Tissues were stained in Weigert’s solution for 20 min. Crocein scarlet acid/ fuchsia working solution (in ratio of 3:1) was composed and used to stain the slides for 2 min. Slides then were put in 5 % phosphotungstic acid for 5 min and then transferred immediately to 1 % acetic acid for 5 min. Washing with tap and distilled water was done between each step. Dehydration was done in 95 % ethanol, then twice for 1 min in 100 % ethanol. Slides were immersed in alcohol saffron for 8 min, then twice in 100 % ethanol for 1 min, and then twice in xylol for 5 min. Finally, tissues were mounted in mounting medium (Roti-Mount HP68.1) and covered. Chemicals were purchased from Sigma, Chempur, Microm and Carl-Roth.

For CD3 staining, slides were incubated in antigen retrieval buffer pH 9 (52367, Dako, Carpinteria, CA, USA) for 20 min at 98 °C and then for 30 min at RT. For F4/80 staining, slides were incubated with Proteinase K (S3020, Dako, Glostrup, Denmark) for 2.5-3 min. Afterwards, all slides were loaded with 3 % H<sub>2</sub>O<sub>2</sub> for 10 min and then with horse serum (Vector, MP-7401 Burlingame, CA, USA) for 20 min. Without rinsing, slides were incubated with
rabbit anti-CD3 antibody (IS503, Dako, Glostrup, Denmark) or rat anti-F4/80 antibody (1:100, MCA497RT, Bio-Rad, Oxford, UK) overnight at 4 °C. After extensively washing with wash buffer (Dako, S0809, Glostrup, Denmark), slides were incubated either with ImmPRESS anti-rabbit IgG HRP (Vector, MP-7401, Burlington, CA, USA) or with ImmPRESS anti-rat IgG HRP (Vector, MP-7444, Burlington, CA, USA) for 30 min at RT. The staining was visualized using DAB stain (DM827, Agilent, Ratingen, Germany). Slides were immersed in Hematoxylin solution for 45 sec to stain the cell nuclei. After washing with tap water for 10 min, slides were dried, mounted (Roti®-Mount HP68.1, Karlsruhe, Germany) and covered.

Quantitative real-time RT-PCR of cardiac tissue

Heart apexes were shock-frozen in liquid nitrogen and stored at -80 °C for later use. Total RNA was isolated using the RNeasy Mini Kit (QIAGEN) following the manufacturer’s protocol. RNA concentration and quality were assessed using the NanoDrop-1000 Spectrophotometer (Peqlab). 2 µg RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). TaqMan or SYBR Green assays were used to quantify target gene expression using the standard curve method on an Applied Biosystems 7500 Sequence Detector (Applied Biosystems). Expression of target mRNA was normalized to the 18S housekeeping gene. All primers and probes were designed with PrimerExpress 3.0 (Applied Biosystems) and synthesized by Biotez. The used primer and probe sequences are 18s forward (fwd) 5'-ACA TCC AAG GCA AGC AGC ATG TG-3', reverse (rev) 5'-TTT CCA CCA CCT ACC CCC GGA ATT TA-3', probe 5'-FAM-CGC GCA AAT TCA CTC CCG AC-TAMRA-3', Ctgf fwd 5'-CAC CGA GCC GTG GGA GTG TGC-3', rev 5'-CAC CGA GCC ACC GGA GAC-3', probe 5'-FAM-CAC TGC CAA AGA TGG TGC ACC CTG TTG TA-3', Il-10 fwd 5'-CAG GGA AGA CAA TAA CTA CGT-3', rev 5'-CGC AGC TCT AGG AGC ATG TG-3', Mhy7 fwd 5'-TGG CCA CCT ACC CCC TCC TCC CCG ACC ATG TG-5', rev 5'-CGT GCC TTA TGT AGC TCC TTG AG-3', Nppb fwd 5'-GGA ATG TCC AGT TGA GGA GGA TGG TGC TGC ACC CTG TTA-3', rev 5'-GGG CCA TTT CCT CCG ACT TTA-3', and Ngal fwd 5'-CAA TGC CAG GAT TCA ATG TCA CTA CGT-3', rev 5'-CGA ACT GAT CGC TCC GGA A-3', probe 5'-FAM-TCA CTG TCC CCC TGC AGC CAG A-TAMRA-3'.

Flow cytometry

To obtain single cell suspensions from splenic and cardiac tissue for flow cytometry, mice were euthanized, and the spleen was removed and kept at 4 °C. To remove remaining blood cells in the heart, mice were perfused for 5 min via a needle in the left ventricular apex (with the right atrium cut open) with 0.9% NaCl solution at 5 ml/min and 110 mmHg. After removal of both atria, the ventricular tissue was dissociated following a protocol provided by Miltenyi Biotech GmbH.

Splenocyte single-cell suspensions from WT mice were obtained using 70 µm strainers, followed by erythrocyte lysis and subsequent filtering using a 40 µm mesh. Cells were counted by trypan blue exclusion and stained for flow cytometric analysis. Single cell suspensions were either restimulated with 50 ng/mL phorbol 12-myristate 13-acetate (PMA, Sigma-Aldrich) and 750 ng/mL ionomycin (Sigma Aldrich) for 4 hrs at 37 °C and 5% CO2 in RPMI 1640 medium (Thermo Fisher) with 10 % FBS (Merck), 100 U/mL penicillin (Sigma Aldrich) and 100 U/mL streptomycin (Sigma Aldrich), followed by 1 hr with additional 0.75 µL/mL GolgiStop (BD Bioscience), or directly stained with antibodies (anti-CD3e-VioBlue, 17A2, Miltenyi; anti-CD3e-eFluor660, 17A2, ebioscience; anti-CD4-APC Vio770 (GK1.5), Miltenyi; anti-CD4-FITC (GK1.5), BD Pharmingen; anti-CD8a-PerCp-Cy5.5 (53-6.7), ebioscience; anti-CD25-VioBright FITC (7D4), Miltenyi; anti-CD44-FITC (IM7), BD Pharmingen; anti-CD62L-APC (MEL-14), BD Pharmingen, anti-F4/80-Pacific Blue (BM8), BioLegend). For all measurements, dead cell exclusion was performed using fixable viability dye for 405 nm (Thermo Fisher). Surface staining was performed for 30 min on ice in PBS supplemented with 2 mM EDTA and 0.5 % BSA together with FcR blocking reagent (Miltenyi). For intracellular staining, cells were permeabilized and fixed using the FoxP3 Staining Buffer Kit (ebioscience) and then stained on ice for 30 min using the respective antibodies (anti-Foxp3-PerCp-Cy5.5 (FJK-16s), ebioscience;
anti-IFN-γ-PE-Cy7 (XMG1.2), eBioscience; anti-IL-10-Pacific Blue (JES-16E3), BioLegend; anti-IL-17A-APC (eBio17B7), eBioscience; anti-RORγt-APC (RE2A278), Miltenyi; anti-Tbet-PE (RE102), Miltenyi). Cells were analyzed with the BD FACSCanto II flow cytometer and BD FACSDiva software (BD Bioscience). Data analysis was performed with FlowJo (TreeStar).

To assess aortic immune cell infiltration in ApoE<sup>−/−</sup>, the aorta was excised and freed from surrounding adipose tissue. A modified protocol of Butcher et al.<sup>5</sup> was used to obtain a single cell suspension. Briefly, the aorta was digested using a collagenase-containing digestion solution (600 U/ml Collagenase type II, 60 U/ml DNase I, in HBSS). After centrifugation, the pellet was resuspended in RPMI medium (Thermo Fischer Scientific) and cells were incubated at 37 °C for 30 min.

To analyze splenocytes from ApoE<sup>−/−</sup> mice, perfused spleen was dissected and cut into small portions and passed through a 100μm filter (Greiner Bio-One) using 0.5 HAS % PBS solution as a vehicle. Single cells were flushed with MACS buffer. MACS buffer was constituted from 1 mM EDTA (Titriplex, Merk, Darmstadt, Germany) and 2% fetal calf serum FCS (S0115, Biochrome, Berlin, Germany) in PBS. Afterwards, cells were centrifuged (300 g, 10 min, 4 °C), resuspended in RPMI 1640 (FG1415, Biochrome, Berlin, Germany) plus 5 % FCS, incubated for 30 min at 37 °C, and then washed twice with MACS buffer.

Finally, the single cells were resuspended in FACS buffer (PBS supplemented with 0.5 % BSA and 2 mM EDTA) and stained with the respective antibodies as described above (anti-CD3ε-APC-Cy7 (17A2), BioLegend; anti-CD4-FITC (RM4-5), BioLegend; anti-CD4-APC Vio770 (GK1.5), Miltenyi; anti-CD8a-PerCp-Cy5.5 (53-6.7), BioLegend; anti-CD25-VioBright FITC (7D4), Miltenyi; anti-CD44-PE-Cy7 (IM7), BioLegend; anti-CD62L-APC (MEL-14), BioLegend, anti-F4/80-Pacific Blue (BM8), BioLegend; anti-Foxp3-PerCp-Cy5.5 (FJK-16s), eBioscience; anti-RORγt-APC (RE2A278), Miltenyi; anti-Tbet-PE (RE102), Miltenyi). To exclude dead cells the LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit (Invitrogen) or DAPI was used. Flow cytometric measurements were performed on a BD LSRFortessa flow cytometer (BD Biosciences) and Kaluza Flow Analysis Software (Beckman Coulter Inc.) was used for data analysis.

**SCFA measurement**

Short chain fatty acids were analyzed as previously described<sup>6</sup> with minor modifications. 50 μl aliquots of mouse serum were mixed with 100 μl 200 mM crotonic acid (Sigma) as internal standard, 50 μl HCl (Sigma) and 200 μl ether (Sigma). Samples were shaken for 10 min at 1500 rpm at RT on a shaker (Eppendorf) and then centrifuged for 10 min at 1000×g (Eppendorf). 80 μL of the upper organic phase were transferred to a new glass vial containing 16 μl N-tert-butylmethylsilyl-N-methyltrifluoroacetamide (MTBSTFA, Sigma). Samples were shaken for 20 min at 80 °C at 500rpm on a shaker (Eppendorf) and subsequently incubated at RT for 24 hours for derivatization.

**In vitro cardiomyocyte experiments**

Neonatal rat cardiomyocytes (NRCM) were isolated from 1-3d old Wistar rats as described previously.<sup>7</sup> After decapitation, hearts were removed, atria excised and ventricles were minced and collected in Dulbecco’s Modified Eagle Medium (DMEM containing 4.5 mg/l glucose, Sigma Aldrich). Ventricular tissue was digested using digestion buffer (1xPBS, 0.5 mg/ml collagenase type II (Worthington), 0.6 mg/ml pancreatin (Sigma Aldrich)) and spun in a spinner bottle at low speed. After 20 min the digestion buffer containing cardiomyocytes and other cell types was removed, centrifuged (3 min, 1200 rpm, RT) and resuspended in plating medium (DMEM containing 10 % FBS (Biochrom), 1 % L-Glutamine (Sigma Aldrich) and 1 % penicillin/streptomycin (Sigma Aldrich)). New digestion buffer was then added to the ventricular tissue and the procedure was repeated 6-8 times. The collected cell suspensions were pooled and contaminating fibroblasts were removed by pre-plating
for 1 hr as described. After 12 hrs NRCM were set to serum-free medium for 24 hrs and then incubated for 30 min with 1 mM sodium chloride (NaCl, Sigma Aldrich), 250 nM Trichostatin A (Sigma Aldrich) or 1 mM sodium propionate (NaC3, Sigma Aldrich). To induce hypertrophy, NRCM were treated with angiotensin II (AngII, 500 nM, Sigma Aldrich). RNA isolation, cDNA synthesis and quantitative real-time RT-PCR was performed as recently described.8–10 Gene expression was determined using the SYBR Green PCR Master Mix (Applied Biosystems) on a Step-One Plus thermocycler (Applied Biosystems) using the standard curve method. Values were normalized to stably expressed glyceraldehyde-3-phosphate dehydrogenase (Gapdh). The primer sequences are Npaa fwd 5’- ACC TGC TAG ACC ACC TAG-3’, rev 5’-GCT GTT ATC TTC CGT ACC-3’ and Gapdh fwd 5’- CAA GGT CAT CCA CCA TGA CAA CTT TG-3’, rev 5’- GGG CCA TCC ACA GTC TTC TG-3’.

**Endothelial function in isolated mesenteric arteries**

To investigate endothelial function of in vivo treated WT mice we used the animal protocol described in the main text. To investigate endothelial function after in vitro induction of endothelial dysfunction we used wild-type C57Bl6/J mice. First order mesenteric arteries were removed immediately after sacrificing the mice under inhalation anesthesia with isoflurane and quickly transferred to cold (4 °C), oxygenated (95 % O2 / 5 % CO2) physiological salt solution (PSS) containing 119 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 25 mM NaHCO3, 1.2 mM MgSO4, 11.1 mM glucose, 1.6 mM CaCl2, and dissected into 2 mm rings whereby perivascular fat and connective tissue were removed without damaging the adventitia. For in vitro experiments artery rings were incubated for 24 hrs in DMEM (Gibco) containing 1 % FBS (Merck), 100 U/ml penicillin (Sigma Aldrich) and 100 U/ml streptomycin (Sigma Aldrich). To induce endothelial dysfunction, we used 10 nM murine IL-17A (Miltenyi) and 10 nM AngII (Calbiochem). To investigate the effects of propionate we added either 1 mM NaC3 (Sigma) or 1 mM NaCl (Sigma) as control. Each ring was positioned on two stainless steel wires (diameter 0.0394 mm) in a 5 ml organ bath of a Muralvany Small Vessel Myograph (DMT 610 M; Danish Myo Technology, Denmark). The organ bath was filled with PSS. The bath solution was continuously oxygenated with a gas mixture of 95 % O2 and 5 % CO2 and kept at 37 °C (pH 7.4). The mesenteric rings were placed under a tension equivalent to that generated at 0.9 times the diameter of the vessel at 100 mmHg by stepwise distending the vessel using LabChart DMT Normalization module. This normalization procedure was performed to obtain the passive diameter of the vessel at 100 mmHg11, 12 was used for data acquisition and display. After 60 min equilibration arteries were pre-contracted with 1 µM phenylephrine (PE) until a stable resting tension was acquired. Drugs were added to the bath solution if not indicated otherwise. Agonists were ranged: acetylcholine (ACh) from 0.01 to 10 µM. Tension is expressed as a percentage of the steady-state tension (100 %) obtained with PE.

**Endothelial function in isolated perfused kidneys**

At the end of the experiment, kidneys of AngII infused ApoE/− mice treated with either C3 or control were isolated and perfused with Krebs–Henseleit buffer as described previously.13 Immediately after preparation and cannulation of the renal arteries, a bolus of 60 mM KCl was injected to test the viability of the preparation followed by a stabilization period of 30 min. To assess renal vasodilation, kidneys were pre-constricted with norepinephrine (1 µM; Sigma Aldrich). Changes in perfusion pressure reflected changes in vascular resistance of renal vessels. Endothelial dependent vasodilation was induced by carbachol (Sigma Aldrich). Concentration–response curves were recorded in presence of diclofenac (3 µM; Sigma Aldrich). Renal relaxation is expressed as a percentage of the actual pressor response to the pre-constricted kidney, which was set as 100 %.
Supplemental Tables

Table S1. Serum levels of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides were measured in AngII-infused ApoE⁻/⁻ mice treated with C3 (n=7) or control (n=5). P-values by unpaired t-test.

|                       | ApoE⁻/⁻ Ang II | ApoE⁻/⁻ Ang II + C3 | p-value |
|-----------------------|----------------|---------------------|---------|
| Total cholesterol (mg/dl) | 379.4 ± 44.97  | 432.6 ± 54.13       | 0.4938  |
| Triglycerides (mg/dl)    | 92.60 ± 14.94  | 136.7 ± 35.82       | 0.3473  |
| HDL (mg/dl)              | 80.60 ± 4.007  | 85.57 ± 5.246       | 0.5007  |
| LDL (mg/dl)              | 265.6 ± 45.62  | 299.7 ± 45.90       | 0.6206  |
Figure S1. **(A)** Serum propionate levels in AngII-infused WT mice treated with C3 or control. WT AngII n=6, WT AngII+C3 n=7, **p<0.05 by Mann-Whitney test.** *(B)* Body weights of sham or AngII infused WT mice treated with C3 or control. WT Sham n=5, WT AngII n=10, WT AngII+C3 n=11, ****p<0.0001 by one-way ANOVA and Tukey’s post-hoc. **(C)** Splenocytes isolated at day 14 of AngII infusion were analyzed for CD4⁺ central memory T cell (CD44⁺ CD62L⁺) frequencies (WT Sham n=8, WT AngII n=8, WT AngII+C3 n=9), **(D)** expression of Th1 transcription factor T-bet in CD4⁺ (WT Sham n=8, WT AngII n=10, WT AngII+C3 n=9), and **(E)** IFN-γ production in CD4⁺ after restimulation (WT Sham n=9, WT AngII n=6, WT AngII+C3 n=6). **(F)** Body weights of AngII-infused ApoE⁻/⁻ mice treated with C3 or control, ApoE⁻/⁻ AngII n=17, ApoE⁻/⁻ AngII+C3 n=21. **(G)** Splenocytes isolated from ApoE⁻/⁻ mice treated with C3 or control were analyzed for CD4⁺ central memory T cell (CD44⁺ CD62L⁺) frequencies (ApoE⁻/⁻ AngII n=15, ApoE⁻/⁻ AngII+C3 n=19) and **(H)** expression of Th1 transcription factor T-bet in CD4⁺ (ApoE⁻/⁻ AngII n=12, ApoE⁻/⁻ AngII+C3 n=15).
Figure S2. (A) Single cells were isolated from aortas of AngII-infused ApoE⁻/⁻ mice treated with C3 or control and analyzed by flow cytometry for cytotoxic T cells (CD3⁺ CD8⁺), T helper cells (CD3⁺ CD4⁺) and macrophages (F4/80⁺). Representative gating is shown. (B) T helper cells isolated from aortas were analyzed for CD44 and CD62L, representative flow cytometry plots for C3 and control treated mice are shown. (C) Quantification of aortic CD4⁺ central memory (CD44⁺ CD62L⁺) T cell frequencies, ApoE⁻/⁻ AngII n=6, ApoE⁻/⁻ AngII+C3 n=8.
Figure S3. (A-B) Quantification of CD4$^+$ and CD8$^+$ cells in heart sections from sham or AngII-infused WT mice treated with C3 or control as measured by immunofluorescence, WT Sham n=5, WT AngII n=6, WT AngII + C3 n=6-7. (C) Single cell suspension from sham or AngII-infused WT mice treated with C3 or control were analyzed by flow cytometry for T-bet expression in CD4$^+$ T cells, WT Sham n=6, WT AngII n=6, WT AngII+C3 n=8. (D-F) Cardiac mRNA expression of Interleukin-10 (Il-10), connective tissue growth factor (Ctgf) and neutrophil gelatinase-associated lipocalin (Ngal), WT Sham n=7-9, WT AngII n=5-6, WT AngII+C3 n=5-6. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by one-way ANOVA and Tukey’s post hoc.
Figure S4. AngII-stimulated neonatal rat cardiomyocytes (NRCM) were treated with 1 mM sodium propionate (NaC3), 1 mM sodium chloride (NaCl) or 250 nM Trichostatin A (TSA). For hypertrophy induction, NRCM were stimulated with 500 nM AngII. mRNA was isolated and the expression of atrial natriuretic peptide (Nppa) quantified by qPCR. n=3 per group, *p<0.05, **p<0.01 by one-way ANOVA and Tukey’s post hoc.
Figure S5. (A) Body weights from C3 treated AngII-infused WT mice treated with anti-CD25 (PC61) or control IgG. (B-C) Splenic CD4$^+$ T cells harvested after 14 days of AngII infusion were analyzed for Tbet$^+$ and RORγt$^+$ Foxp3$^-$ subsets. P-value by Mann-Whitney test (D) Splenocytes were restimulated and analyzed for IFN-γ$^+$ production in CD4$^+$ by flow cytometry. (E-F) Splenocytes were analyzed for CD4$^+$ central memory (CD44$^+$ CD62L$^+$) and naïve (CD44$^-$ CD62L$^+$) subsets. (G) Cardiac hypertrophy index (heart weight [g]/tibia length [m]). (A-C, E-G) n=4 per group, (D) WT AngII+C3+IgG n=3, WT AngII+C3+PC61 n=4, p-values by one-tailed Mann-Whitney-test.
Figure S6. (A) Systolic blood pressure was measured by tail-cuff in week 4 of AngII infusion in ApoE⁻/⁻ treated with C3 or control. Mean systolic blood pressures are shown. ApoE⁻/⁻ AngII n=9, ApoE⁻/⁻ AngII+C3 n=14, *p<0.05 by unpaired t-test. (B) Endothelium-dependent vasorelaxation (acetylcholine) of isolated phenylephrine pre-contracted mesenteric arteries from AngII-infused WT mice treated with C3 or control. n=7 rings from n=3 animals per group, *p<0.05 by 2-way repeated measurement ANOVA. (C) Endothelium-dependent vasorelaxation of isolated perfused kidneys from AngII-infused ApoE⁻/⁻ mice treated with C3 or control. ApoE⁻/⁻ AngII n=5, ApoE⁻/⁻ AngII+C3 n=4, *p<0.05 by 2-way repeated measurement ANOVA. (D) To induce endothelial dysfunction ex vivo, isolated mesenteric arteries from untreated healthy mice were incubated with AngII and IL-17A for 24 hours in the presence or absence of C3. Following incubation, mesenteric arteries were pre-contracted with phenylephrine and tested for endothelium-dependent vasorelaxation. Control. Data from n=2 independent experiments, n=3-5 rings per group from n=6 animals. (E, F) Blood pressure was measured continuously using radiotelemetry in AngII infused WT mice treated with C3 and i.p. injections of anti-CD25 (PC61) or IgG control. Smoothened curves for systolic and diastolic blood pressure are shown left. Statistics by linear mixed model. Lower panels show systolic and diastolic pressures calculated as area under the curve in week 1 and week 2 of AngII infusion. n=4 per group.
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