Supplementary Materials

Czepiel M. *et al.* **Angiotensin II receptor 1 controls profibrotic Wnt/β-catenin signalling in experimental autoimmune myocarditis**

Supplementary Methods

Mice. *Agtr1a*$^{-/-}$ mice[1] were originally obtained from the Jackson Laboratory and were back-crossed for at least 10 generations on BALB/c background. Mice were kept under standard laboratory conditions:12/12h light/dark cycle, room temperature 20-22ºC, humidity 45-55% with *ad libitum* access to food and water. All experiments were performed in accordance with Swiss and Polish law and were approved by local authorities (license number ZH49/2009 and ZH194/2012 for Switzerland and 206/2017 and 235/2019 for Poland). Animal experiment followed the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health.

EAM induction, generation of bone marrow chimera and pharmacological treatment. EAM was induced in 6-9 weeks old wild-type and *Agtr1a*$^{-/-}$ BALB/c mice by subcutaneous injection of 200 µg α-MyHC$_{614-634}$ peptide (Ac-RSLKLMATLFSTYASADR-OH, Caslo) emulsified 1:1 with Complete Freund's Adjuvant (CFA, BD Biosciences) on days 0 and 7. For generation of crisscross bone marrow chimeras, wild-type and *Agtr1a*$^{-/-}$ recipient mice were lethally irradiated (2 x 5.5 Gy) using a Gammatron (Co-60). 24h later, bone marrow from wild-type and *Agtr1a*$^{-/-}$ donor animals was isolated and crude bone marrow cells ($2x10^6$ in 200 µl) were intravenously injected to recipient mice. Recipient animals were kept on antibiotic regimen from the day of irradiation (amoxicillin + clavulanic acid: 1 mg/ml + 0.14 mg/ml in drinking water respectively). 6 weeks after immune system reconstitution EAM was induced in recipient mice as described above. In certain experiments, telmisartan (10 mg/kg/day, Cayman Chemical) or vehicle control were delivered continuously in drinking water to *Agtr1a*$^{-/-}$ mice from day 0 until day 21 of EAM.

[^{3}H]-thymidine proliferation assay CD4$^+$ T cell magnetic beads (Miltenyi) were used to purify CD4$^+$ T cells. CD4-negative population was used as antigen presenting cells. A total of $5x10^4$ CD4$^+$ T cells co-cultured with 10^5 irradiated (25 Gy) syngeneic APCs were re-stimulated for 48h in the presence of serial dilutions of the α-MyHC peptide. Proliferation was assessed by measuring [^{3}H]-thymidine incorporation during the last 16 hours.

Transthoracic echocardiography and Doppler imaging Transthoracic echocardiography was performed using a Vevo 2100 system equipped with 30-MHz transducer (VisualSonics). Anesthesia was induced by 5% isoflurane and confirmed by the absence of the withdrawal reflex of one of the hind paws. During echocardiogram acquisition isofluorane was reduced to 1.5-2%. Each animal was

placed in a supine position on a prewarmed platform. The limbs were taped over the metal ECG leads to enable continuous monitoring of the heart rate and respiration. Then, the prewarmed echo transmission gel was applied to the hairless chest. The heart was imaged in the bidimensional (2-D) mode, in the parasternal long-axis (PSLAX), short-axis (SAX) and apical 4-chamber views. For analysis of left ventricular end-diastolic volume (LV vol, d) and left ventricular end-systolic volume (LV vol, s) the endocardium of the left ventricle was traced at both diastole and systole. An integrated software tool (LV-Trace) was used for single-plane PSLAX analysis. Collected parameters include: heart rate (HR), ejection fraction (EF), stroke volume (SV), cardiac output (CO), left ventricular end-diastolic diameter (LVID, d), left ventricular end-systolic diameter (LVID, s), fractional shortening (FS), peak Doppler blood inflow velocity across the mitral valve during early diastole (E), peak Doppler blood inflow velocity across the mitral valve during late diastole (A).

Heart inflammatory cell isolation and cell cultures. 19-21 days after EAM induction, mice were euthanized and hearts were perfused with phosphate buffered saline (PBS) and isolated. 2-3 hearts with macroscopically visible myocarditis were pooled, transferred to pre-warmed Liberase (Roche) solution (20 ug/ml; 1-2 mL/heart), cut into small pieces and incubated at 37^{o}C for 45 min. Liberase was inactivated with fetal calf serum (FCS)-rich cell culture medium and the cell suspension was sequentially filtered through 70 µm and 40 µm cell strainers. Cells were centrifuged, resuspended in the growth medium Iscove`s modified Dulbecco`s medium (IMDM; Corning) supplemented with 20% inactivated FCS (ThermoFisher Scientific), 10 000 U/mL Penicillin/Streptomycin (Corning) and 0.1 mM 2-mercaptoethanol (Merck) and plated onto tissue culture multiple-well plates. Cell culture medium was changed 3 days after isolation to remove not attached cells and cell debris. Cells were used for experiments up to passage 3. For myofibroblast differentiation studies cultured inflammatory cells were transferred to differentiation medium Dulbecco's Modified Eagle Medium (DMEM, Corning) containing with 1 g/L glucose, L-glutamine, and sodium pyruvate, and supplemented with 10% inactivated FCS, 10 000 U/mL Penicillin/Streptomycin; 0.1 mM 2-mercaptoethanol and MEM Non-Essential Amino Acids Solution (Corning) and 10 ng/ml recombinant human TGF-β1 (Peprotech).

Immunoblotting. For protein expression analysis, at certain timepoints, *in vitro* cell cultures were harvested and lysed with RIPA lysis buffer (Cell signalling) containing Protease and Phosphatase Inhibitors cocktails (both ThermoFisher Scientific). Protein concentration in cell lysates was determined with Bradford reagent (Bio-Rad). Samples containing equal amount of proteins were diluted with 4x NuPAGE™ LDS Sample Buffer and 10x NuPAGE™ Sample Reducing Agent (both

ThermoFisher Scientific), boiled at 75ºC for 10 min and loaded onto 10% polyacrylamide gel. Proteins were separated at 140 V. For blotting a methanol-activated nitrocellulose membranes were used and the proteins were blotted at 30 V overnight. To prevent non-specific antibody binding membranes were blocked in TBST + 2% BSA (BioShop Canada) for 1h. Membranes were incubated with primary antibodies diluted in TBST + 5% non-fat dry milk (AppliChem) or TBST + 2% BSA for detection of phosphorylated proteins overnight at 4°C. Appropriate HRP-conjugated secondary antibodies diluted in TBST + 5% non-fat dry milk were applied for 1h at RT. Proteins were detected using the West Pico PLUS detection reagent (ThermoFisher Scientific) according to manufacturer's protocol. Chemiluminescent signal was acquired with ChemiDoc system (Bio-Rad). Signal densitometry analysis was performed with ImageJ software (LOCI, University of Wisconsin). The following antibodies were used in the study: anti-ACE (clone 2E2), anti-Renin (clone A-1), anti-Gα12 (clone E-12), anti-Gα13 (clone 6F6-B5, all Santa Cruz Biotechnology), anti-GAPDH (clone 14C10), anti-SMAD2 (clone D43B4), anti-pSMAD2 (clone 138D4), anti-TAK1 (polyclonal), anti-pTAK1 (polyclonal, all Cell Signalling) and anti-α-SMA (clone 1A4, Biolegend). Appropriate (anti-mouse IgG or anti-rabbit IgG) HPR-conjugated secondary antibodies were obtained from Santa Cruz Biotechnology.

Quantitative RT-PCR. For RNA isolation from the heart tissue, 25-30 mg of cardiac tissue was lysed in 500 µL of Qiazol (Qiagen) and homogenized using TissueLyserII (Qiagen). Homogenate was spun down for 2 min at 15 000 g and the supernatant was processed according to the manufacturer's instructions. RNA from *in vitro* cell cultures was isolated with Qiazol isolation following manufacturer's instructions. RNA concentration was measured using NanoDrop (TermoFischer) and 100-200 ng of RNA was used for cDNA synthesis with NG dART RT kit (EurX). Quantitative PCR was performed using SYBR Green qPCR Master Mix (EurX) with QuantStudio 6 Flex Real-Time PCR System (ThermoFisher Scientific). Transcript levels of *Gapdh* were used as endogenous reference and relative gene expression was determined using standard $2^{-\Delta\Delta Ct}$ method. Mouse specific primers used in the study:

Gapdh

F 5'-CTGCACCACCAACTGCTTAGC-3'

R 5'-GGCATGGACTGTGGTCATGAG-3'

Acta2

F 5'-CGCTGTCAGGAACCCTGAGA-3'

R 5'-ATCCCAGCCTCCGTTATCCT-3'

Col1a1

F 5’-GATGACGTGCAATGCAATGAA-3’

R 5’-CCCTCGACTCCTACATCTTCTGA-3’

Col3a1

F 5’-GAGGTCCTTCAGGTGAACCC-3’

R 5’-TCGCCCTTAGGTCCTGGAAT-3’

Vim

F 5’-CGAGAGAAATTGCAGGAGGAGA-3’

R 5’-CGTTCAAGGTCAAGACGTGC-3’

Fn1

F 5’-TACCAAGGTCAATCCACACCCC-3’

R 5’-GAGATGGCAAAAGAAAGCAGAGG-3’

Postn

F 5’-AGACTGCTTCAGGGAGACAC-3’

R 5’-ACGGCCTTCTCTTGATCGTC-3’

Fap

F 5’-ACAGTTTTCAGCCATGTCTTCATT-3’

R 5’-TGGAAGACAGACTTGCTTCTTTTC-3’

Il11

F 5’-TGCAGGTGGTCCTTCCCTA-3’

R 5’-GCCAGGCGAGACATCAAGAG-3’

Wnt1

F 5’-CTGTGCGAGAGTGCAAATGG-3’

R 5’-GATGAACGCTGTTTCTCGGC-3’

Wnt5a

F 5’-CAGCCCTGCTTTGGATTGTC-3’

R 5’-AGCCACTCCCGGGCTTAATA-3’

Wnt10b

F 5’-GGACATCCAGGCGAGAATGC-3’

R 5’-ATTTGCACTTCCGCTTCAGG-3’

Wnt11

F 5’-GGGCCAAGTTTTCCGATGC-3’

R 5’-TTCCAGGGAGGCACGTAGAG-3’

Mmp2

F 5'-CCCCCATGAAGCCTTGTTTAC-3'
R 5'-TACAGCTGTTGTAGGAGGTGC-3'
Mmp9
F 5'-AGCGCCAGCCGACTTTT-3'
R 5'-TAGCGGTACAAGTATGCCTCTG-3'
Nppa
F 5'- ATCTGATGGATTTCAAGAACCTGC -3'
R 5'- CTGCTTCCTCAGTCTGCTCAC -3'
Nppb
F 5'- GGTCCAGCAGAGACCTCAAAAT -3'
R 5'- CAACTTCAGTGCGTTACAGCC -3'
Acta1
F 5'- CTAACCGGGAGAAGATGACTCAA -3'
R 5'- CAGCACCGCCTGGATAGC -3'

Expression of genes involved in Ang II synthesis in unstimulated cells and treated with TGF-β for 24h (Fig. 3A) was obtained from the dataset of the whole genome transcriptome analysis published previously[2].

Flow Cytometry. Single cell suspensions of EAM hearts was performed as described above. *In vitro* expanded inflammatory cells were prepared by scraping cells from tissue culture plates and passing them through 70μm and 40μm cell strainers. Cells were stained with appropriate fluorochrome conjugated anti-mouse antibodies for 30 min at 4°C and analyzed with the FACSCanto 10 flow cytometer (BD Biosciences). Propidium iodide was used to exclude dead cells from analysis. Antibodies used in the study: anti-CD45-FITC (clone 30-F11, Invitrogen), anti-CD11b-APC (clone M1/70, Invitrogen), anti-CD11b-PE (clone M1/70, Invitrogen), anti-gp38-APC (clone 8.1.1, Invitrogen), anti-CD140a-PE (clone APA5, Invitrogen), anti-CD4-PECy7 (clone GK1.5, Invitrogen), anti-CD45-BV (clone 30-F11, Invitrogen), anti-CD133-PE/Cy7 (clone 315-2C11, BioLegend), anti-CD29-FITC (clone HMβ1-1, BioLegend), anti-CD3-FITC clone O17A2, BioLegend), anti-B220-APCCy7 (clone RA3-6B2, BioLegend), anti-CD49b-APC (clone DX5, BioLegend), anti-CD11c-PerCp (clone N418, BioLegend), anti-CD4-PECy7 (clone GK1.5, BioLegend), anti-CD3-FITC (clone 17A2, eBioscience), anti-B220-APCCy7 (clone RA3-6B2, BioLegend), anti-CD49b-APC (clone DX5, BioLegend), anti-CD11c-PerCp (clone N418, BioLegend), anti-CD45-BV (clone 30-F11, Invitrogen).

Histopathology and Immunohistochemistry. Mouse heart tissues were fixed in 4% formalin and embedded in paraffin. Cardiac inflammation and fibrosis were assessed using conventional Hematoxylin/Eosin and Masson's trichrome staining respectively. Myocarditis severity was scored on Hematoxylin/Eosin stained heart sections using a semi-quantitative 0-4 scale: 0 – no inflammatory infiltrates; 1 – small foci of inflammatory cells between myocytes; 2 – larger foci of >100 inflammatory cells; 3 – >10% of a cross-section involved; 4 – >30% of a cross-section involved. For immunohistochemistry antigen retrieval was performed using ER2 buffer (Leica) and sections were stained with anti-CD45 (clone A20, BD Biosciences), anti-CD3 (clone SP7, Neomarkers), anti-F4/80 (clone BM6, BMA Biomedicals), anti-periostin (polyclonal), anti-vimentin (clone EPR3776, both Abcam) and anti-β-catenin (clone 14, Transduction Laboratories) antibodies, followed by anti-mouse-HRP and chromogenic DAB staining. Counterstaining was performed using hematoxylin. Immunopositive cells and areas were quantified using Olympus BX51 microscope and cellSens (Olympus) or ImageJ software.

Immunocytochemistry. *In vitro* expanded and TGF-β stimulated inflammatory cells isolated from EAM hearts were fixed with 4% paraformaldehyde, followed by permeabilization with PBS + 0.1% Triton. Nonspecific antibody binding sites were blocked for 1 hour in blocking solution (PBS + 0.1% Triton and 2% BSA). Primary antibodies were diluted in blocking solution and applied overnight at 4°C. Fluorochrome-conjugated secondary antibodies were applied for 1 hour at room temperature in the dark. Hoechst was used to counterstain the cell nuclei. Coverslips were mounted with ProLong Diamond Antifade Mountant (ThermoFisher Scientific) and examined with the Olympus fluorescent microscope. Antibodies used in the study: α-SMA (clone 904601, Biolegend), Fibronectin (clone MA511981), β-catenin (clone MA1301, both ThermoFisher Scientific).

Nano-Liquid Chromatography (LC) with Mass Spectrometry (MS). Sample preparation and Mass spectrometry measurement were performed as described previously[3]. Briefly, supernatants of inflammatory cells isolated from EAM hearts and expanded *in vitro* were collected, spiked with [Asn1, Val5]-Ang II (Internal Standard – final concentration 200 pg/ml) and subjected to two stage cleanup procedure: (a) protein precipitation with HPLC-grade Acetonitrile followed by evaporation to dryness and (b) solid-phase extraction on C18 silica bonded SPE cartridges followed by elution into the Protein LoBind polypropylene tubes (Eppendorf), concentration with vacuum centrifuge and O/N lyophilization. Cell lysates were harvested with Protein Extraction Solution (0.9% saline, 0.1 M HCl) containing 10% (v/v) Protease Inhibitor Cocktail (10 mM ethylenediaminetetraacetic acid

(EDTA) disodium salt, 5 mM 1,10-phenanthroline, 20 mM 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), 400 μM amastatin, 150 μM pepstatin A, 100 μM thiorphan, and 500 μM aliskiren) and subjected to sample clean up procedure described above. Prior measurements, samples were reconstituted in 1% (v/v) acetic acid in water and analyzed using LC/MS system.

Expression and purification of recombinant GST- Rhotekin Rho-Binding Domain (RBD). GST-fusion proteins of the Rho-binding domain (RBD) of Rhotekin and RhoA were expressed using the bacterial expression vector pGEX4T1 in the BL21DE3 strain of *Escherichia coli* and purified as indicated previously[4]. Bacterial lysates expressing GST-RBD were incubated with the glutathione sepharose beads (Amersham Pharmacia Biotech) for 1h at 4°C, the resin was washed five times with ten volumes of buffer A (50 mM Tris pH 7.4, 500 mM NaCl, 10 mM $MgCl_2$, 0.1% SDS, 0.5% sodium deoxycholate, 1% (w/v) triton X-100, 1 mM PMSF, 1 mM benzamidine, 2 μg/ml leupeptin, 2 μg/ml pepstatin). The protein content of the beads was assessed by coomassie blue staining of SDS-PAGE gels. Beads were used immediately for Rhotekin RBD pulldown assay.

Rhotekin RBD pulldown assay. Cells were lysed in RBD lysis buffer (20 mM Tris, pH 7.4, 150 mM NaCl, 30 mM $MgCl_2$, 1 mM dithiothreitol, 0.5% (w/v) Triton X-100, 4μg/ml Leupeptin, 2μg/ml Aprotinin, 2μg/ml Pepstatin, 0.1 mM phenylmethylsulfonyl fluoride). Lysates were subjected to centrifugation at 20,600 × *g* for 10 min at 4 °C and incubated with 30 μg of RDB beads for 1h at 4 °C. Beads were then washed three times with RBD buffer, resuspended in SDS-PAGE sample buffer (100 mM Tris, pH6.8, 2% SDS, 10% glycerol, 5% β-mercaptoethanol). Denatured sampled were separated on acrylamide gels and electroblotted onto nitrocellulose membranes. Active RhoA was detected western blot using a monoclonal anti-RhoA antibody (Santa Cruz Biotechnology) and quantified by densitometry using Image J. The amounts of active GTP-bound RhoA were normalized to the total RhoA content of cell lysates.

AKAP-Lbc immunoprecipitation. Cells were lysed in buffer A (20 mM Tris pH7.4, 150 mM NaCl, 1% (w/v) Triton-X-100, 0.1% sodium deoxycholate, 5μg/ml aprotinin, 10μg/ml leupeptin and 1 mM PMSF) during 4h at 4°C on a rotating wheel. The solubilized material was centrifuged at 34,000 x g for 30 min at 4°C and the supernatants were dialysed overnight against buffer B (20 mM Tris pH 7.4, 150mM NaCl, 1% (w/v) Triton-X-100, 5μg/ml aprotinin, 10 μg/ml leupeptin and 1 mM PMSF). 2mgs of dialysed lysates were then incubated 4h at 4°C with 4μg of rabbit polyclonal anti-AKAP-Lbc antibodies (Covance) and 20μl of Protein-A-sepharose beads to immunoprecipitate endogenous AKAP-Lbc proteins. Following centrifugation on a bench-top centrifuge, the pelleted beads were

washed five times with buffer B, twice with PBS and proteins were eluted in SDS-PAGE sample buffer by boiling samples for 3 min at 95°C. Eluted proteins were analysed by SDS-PAGE and immunoblotting.

Immunoprecipitation and GSK3β activity measurement. Immunoprecipitation and GSK3β activity measurement was performed as described previously[5] with modifications. Cells were harvested and lysed on ice with Cell Lysis Buffer (CLB) (50 mM Tris-HCl pH 7.5, 1% (v/v) Triton X-100, 0.27 M sucrose, 1 mM EDTA, 0.1 mM EGTA, 0.1% (v/v) β-mercaptoethanol, complete protease and phosphatase inhibitor cocktail (ThermoFisher Scientific). Lysates were centrifuged and the supernatant was immediately processed for protein concentration measurement (Bradford method) and for immunoprecipitation assay. For immunoprecipitation, anti-GSK3β monoclonal antibody (12 μg/mL; clone 7/GSK-3b, BD Biosciences) was incubated with freshly prepared 50% mixture of protein G-Sepharose beads (Sigma) and kinase buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.03% (v/v) Brij-35, and 0.1% (v/v) β –mercaptoethanol, 10 mM $MgCl_2$) for 1h at 4°C with shaking. The mixture was washed 2 times with ice-cold PBS to remove unbound antibody and resuspended in ice-cold CLB. Cell lysates were incubated with antibody-conjugated G-Sepharose beads for 2h at 4°C with shaking. Beads were then washed once with CLB containing 0.5M NaCl, resuspended in kinase buffer and processed directly to GSK3β activity measurement with the ADP-GLO reagent (Promega) according to manufacturer's protocol. Briefly, kinase reactions containing immunoprecipitated sample (from above), ATP (100 μM) and kinase substrate (200 μg/ml phospho-glycogen synthase 2 (pGS2) peptide) were prepared in 96-well plate and incubated at 30°C for 20 min with gentle shaking. Next, ADP-GLO reagent was added and plates were incubated for 40 min at RT followed by 40 min incubation with the Kinase Detection Reagent (Promega). Luminescence signal (corresponding to GSK3β activity) was recorded with a plate reader (Tecan).

Statistical analysis. For normally distributed data unpaired Student's t-test or one-way ANOVA followed by Fisher's LSD tests were used. For nonparametric data Mann-Whitney test or Kruskal-Wallis followed by Dunn's test were used. Differences were considered statistically significant for $p < 0.05$. All analyses were performed with the Prism 8 software (GraphPad).

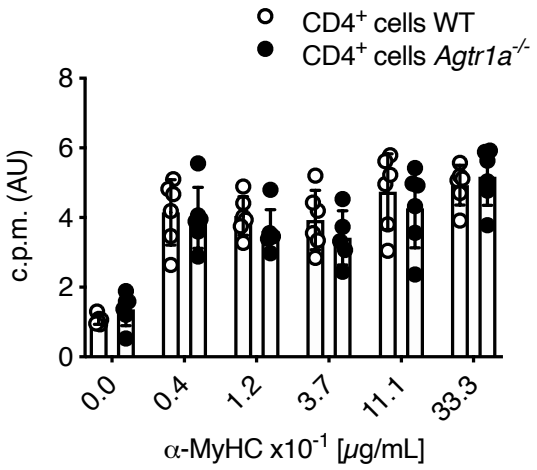
References:

1. Ito M, Oliverio MI, Mannon PJ, Best CF, Maeda N, Smithies O, Coffman TM. Regulation of blood pressure by the type 1A angiotensin II receptor gene. *Proc Natl Acad Sci* 1995;**92**:3521–3525.
2. Blyszczuk P, Müller-Edenborn B, Valenta T, Osto E, Stellato M, Behnke S, Glatz K, Basler K, Lüscher TF, Distler O, Eriksson U, Kania G. Transforming growth factor-β-dependent Wnt secretion controls myofibroblast formation and myocardial fibrosis progression in experimental autoimmune myocarditis. *Eur Heart J* 2017;**38**:1413–1425.
3. Olkowicz M, Chlopicki S, Smolenski RT. A Primer to Angiotensin Peptide Isolation, Stability, and Analysis by Nano-Liquid Chromatography with Mass Detection. *Methods Mol Biol* 2017;**1614**:175–187.
4. Cavin S, Maric D, Diviani D. A-kinase anchoring protein-Lbc promotes pro-fibrotic signaling in cardiac fibroblasts. *Biochim Biophys Acta* 2014;**1843**:335–345.
5. Cole AR, Sutherland C. Measuring GSK3 Expression and Activity in Cells. *Methods in Molecular Biology* 2008. p. 45–65.

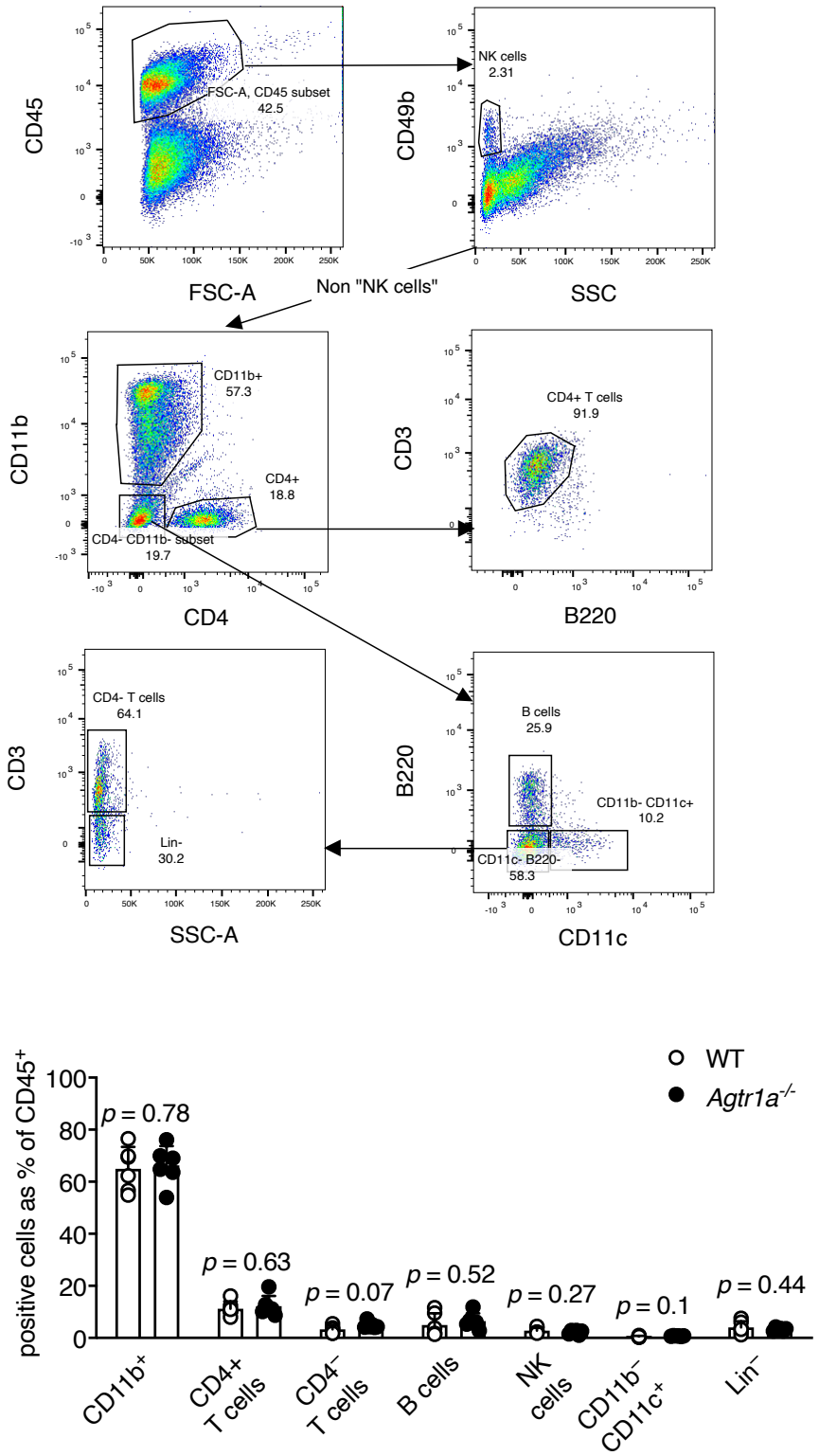
Supplementary Table 1 Echocardiography analyses of cardiac function of wild-type (WT) and *Agtr1a*$^{-/-}$ mice before induction of EAM (d0) and 40 days after EAM induction. Abbreviations: bpm: beat per minute; LV vol, d: left ventricular end diastolic volume; LV vol, s: left ventricular end systolic volume; EF: ejection fraction; SV: stroke volume; CO: cardiac output; LVID, d: left ventricular end diastolic internal diameter; LVID, s: left ventricular end systolic internal diameter; FS: fractional shortening; MV E/A: peak Doppler blood inflow velocity across the mitral valve during early diastole (E) to peak Doppler blood inflow velocity across the mitral valve during late diastole (A) ratio.

Parameter	Day 0			Day 40		
	WT (n=7)	*Agtr1a*$^{-/-}$ (n=7)	*p* value	WT (n=7)	*Agtr1a*$^{-/-}$ (n=7)	*p* value
	Mean ± SD	Mean ± SD		Mean ± SD	Mean ± SD	
Heart Rate [BPM]	408 ± 33	371 ± 54	0.136	400 ± 29	398 ± 67	0.959
LV Area; s [mm^2]	15.0 ± 1.4	13.2 ± 1.3	0.026	17.1 ± 1.1	14.7 ± 1.9	0.013
LV Area; d [mm^2]	23.4 ± 1.9	21.4 ± 1.5	0.050	24.3 ± 1.0	23.0 ± 1.6	0.097
LV volume; s [µl]	33.3 ± 4.6	27.4 ± 4.0	0.027	41.3 ± 4.3	32.8 ± 5.5	0.008
LV volume; d [µl]	68.2 ± 9.4	58.5 ± 7.4	0.054	71.4 ± 4.3	65.5 ± 7.5	0.101
Stroke Volume [µl]	35.0 ± 5.1	31.1 ± 4.1	0.141	30.1 ± 3.3	32.8 ± 3.1	0.143
Ejection Fraction [%]	51.2 ± 1.9	53.2 ± 2.7	0.143	42.2 ± 4.3	50.2 ± 3.8	0.003
Fractional Shortening [%]	15.3 ± 2.1	18.3 ± 5.2	0.185	13.9 ± 3.0	17.3 ± 5.6	0.173
Cardiac Output [ml/min]	14.3 ± 2.5	11.4 ± 1.4	0.021	12.1 ± 2.0	13.1 ± 3.0	0.455
LV Mass [mg]	85.6 ± 10.7	84.9 ± 13.6	0.918	107.9 ± 21.2	95.7 ± 21.0	0.321
MV E/A	2.1 ± 0.5	1.7 ± 0.3	0.080	2.2 ± 0.5	1.7 ± 0.1	0.026

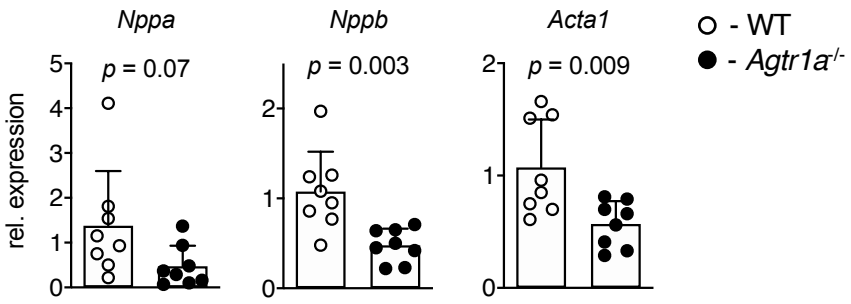
Supplementary Figures



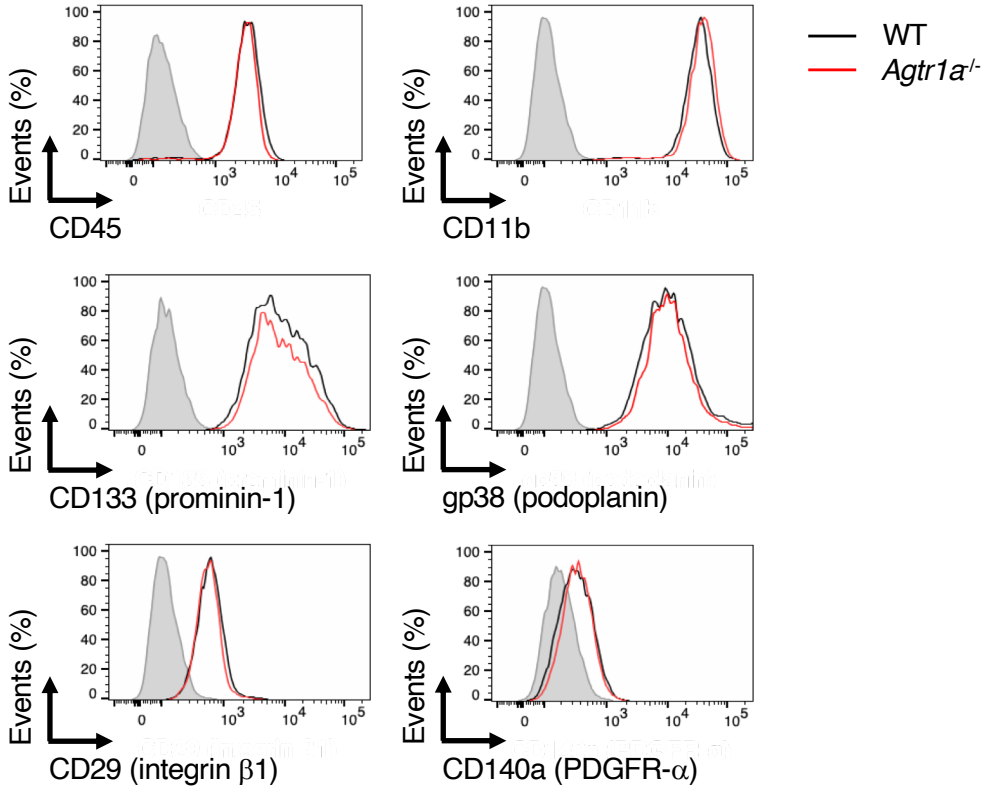
Suppl. Figure 1. EAM was induced in wild-type (WT) and in *Agtr1a*$^{-/-}$ mice by αMyHC/CFA immunisation at day 0 and 7. Graph shows proliferation of wild-type (n=6) and *Agtr1a*$^{-/-}$ (n=6) splenic CD4^{+} T cells isolated from EAM mice (21d) and cultured for 72h in the presence of wild-type antigen presenting cells and indicated concentration of αMyHC peptide. Data are representative of two independent experiments. c.p.m. – counts per minute.



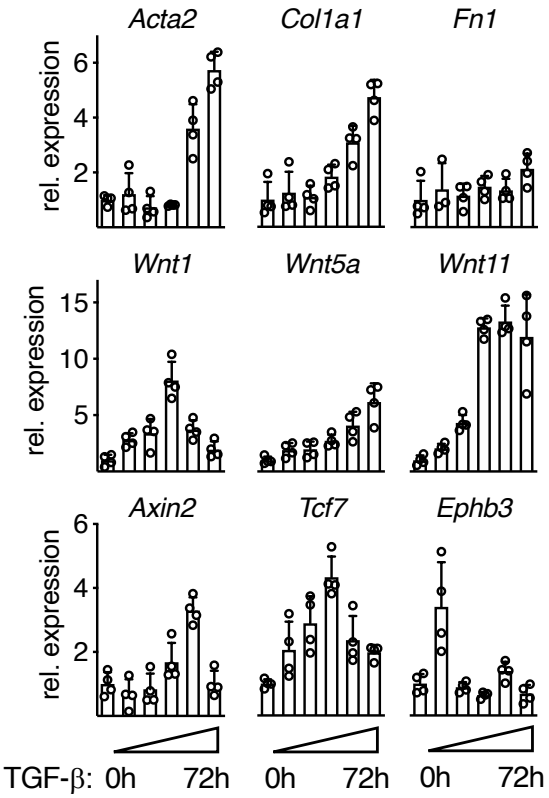
Suppl. Figure 2. Panel (A) shows gating strategy of pre-gated single cell suspension of cardiac cells obtained from mice at day 21 of EAM. Quantifications of indicated cell subsets of percentage of $CD45^+$-gated cells in hearts of wild-type (WT, n=6) and *Agtr1a*$^{-/-}$ (n=6) mice at day 21 of EAM are shown in (B). *p* values were calculated with the unpaired Student's t-test.



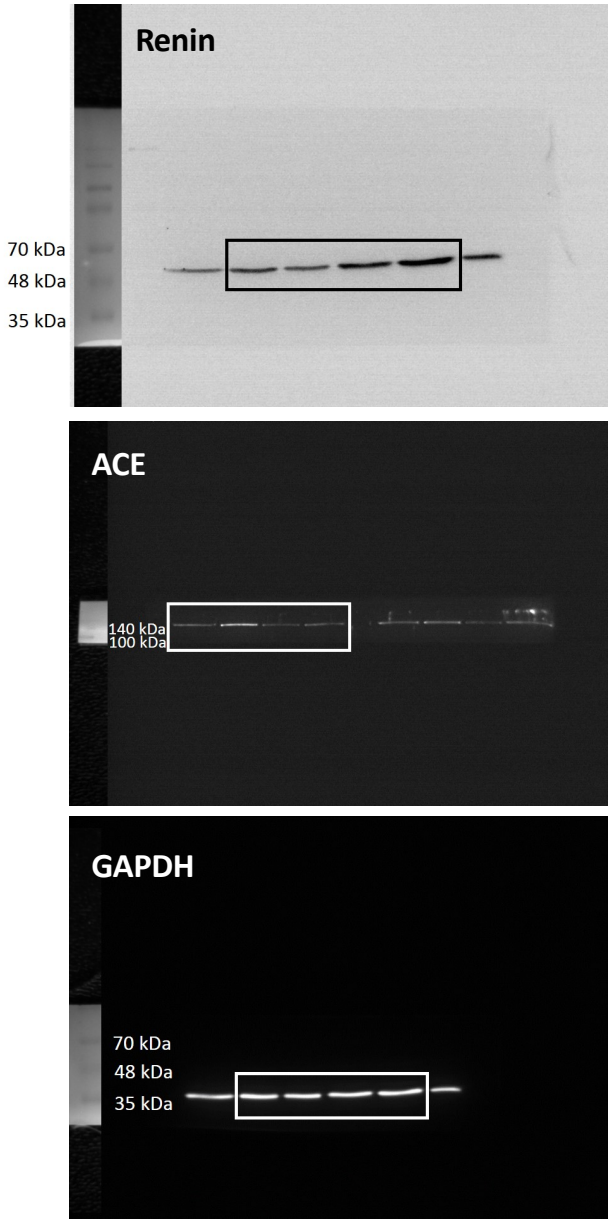
Suppl. Figure 3. RNA was isolated from heart tissue of wild-type (WT, n=8) and *Agtr1a*$^{-/-}$ (n=8) at day 40 of EAM. Graphs show expression of cardiac hypertrophy/heart failure markers *Nppa* (encoding natriuretic peptide A), *Nppb* (encoding natriuretic peptides B) and *Acta1* (encoding actin, alpha skeletal muscle). *p* values were calculated with the unpaired Student's t-test.



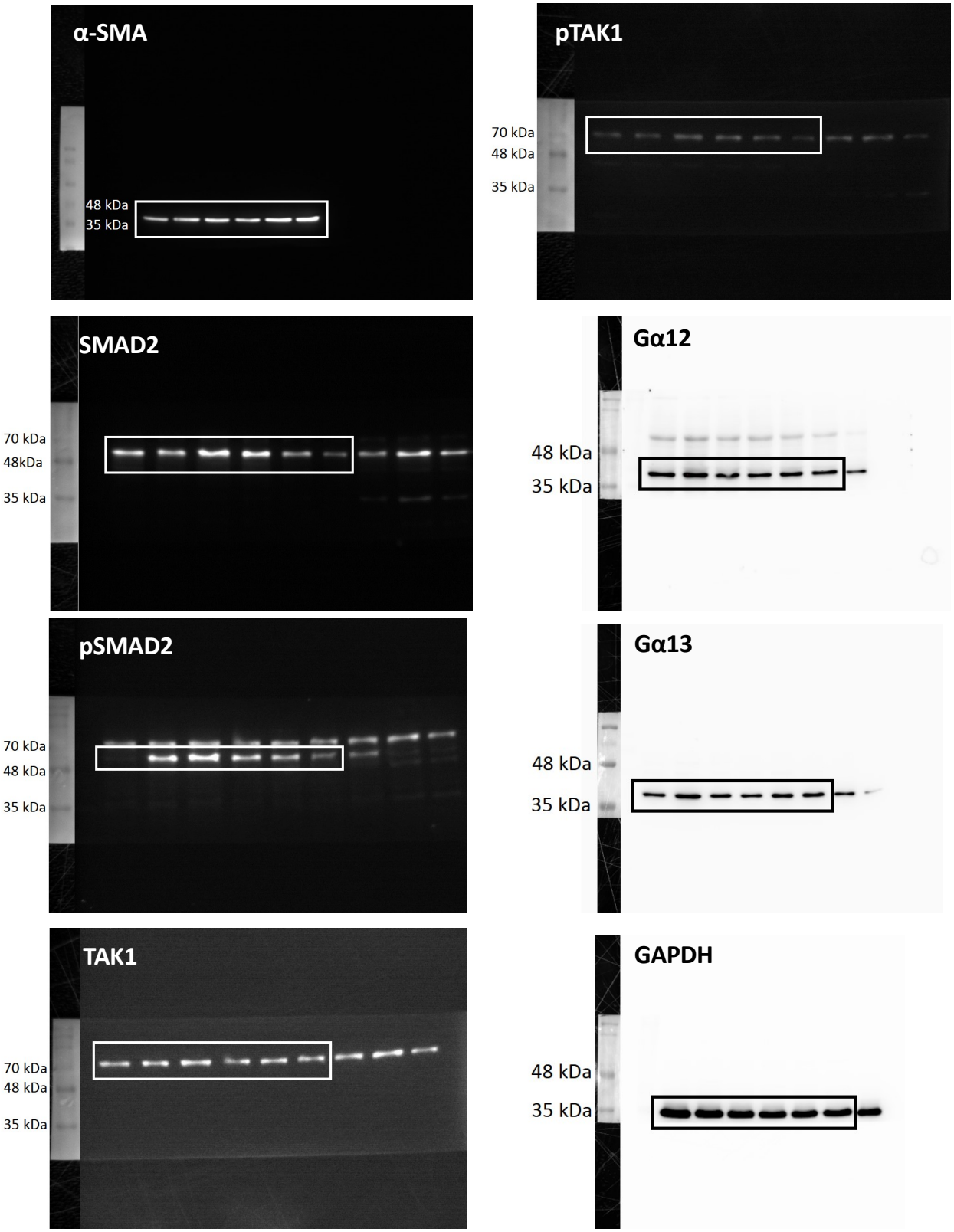
Suppl. Figure 4. Inflammatory cells were isolated from hearts of wild-type (WT) and *Agtr1a*$^{-/-}$ mice at day 17-21 of EAM and expanded. Representative flow cytometry analyses of indicated markers are shown for WT (black) and *Agtr1a*$^{-/-}$ (red) cells. Data are representative of two independent experiments. Unstained cells are shown in grey.



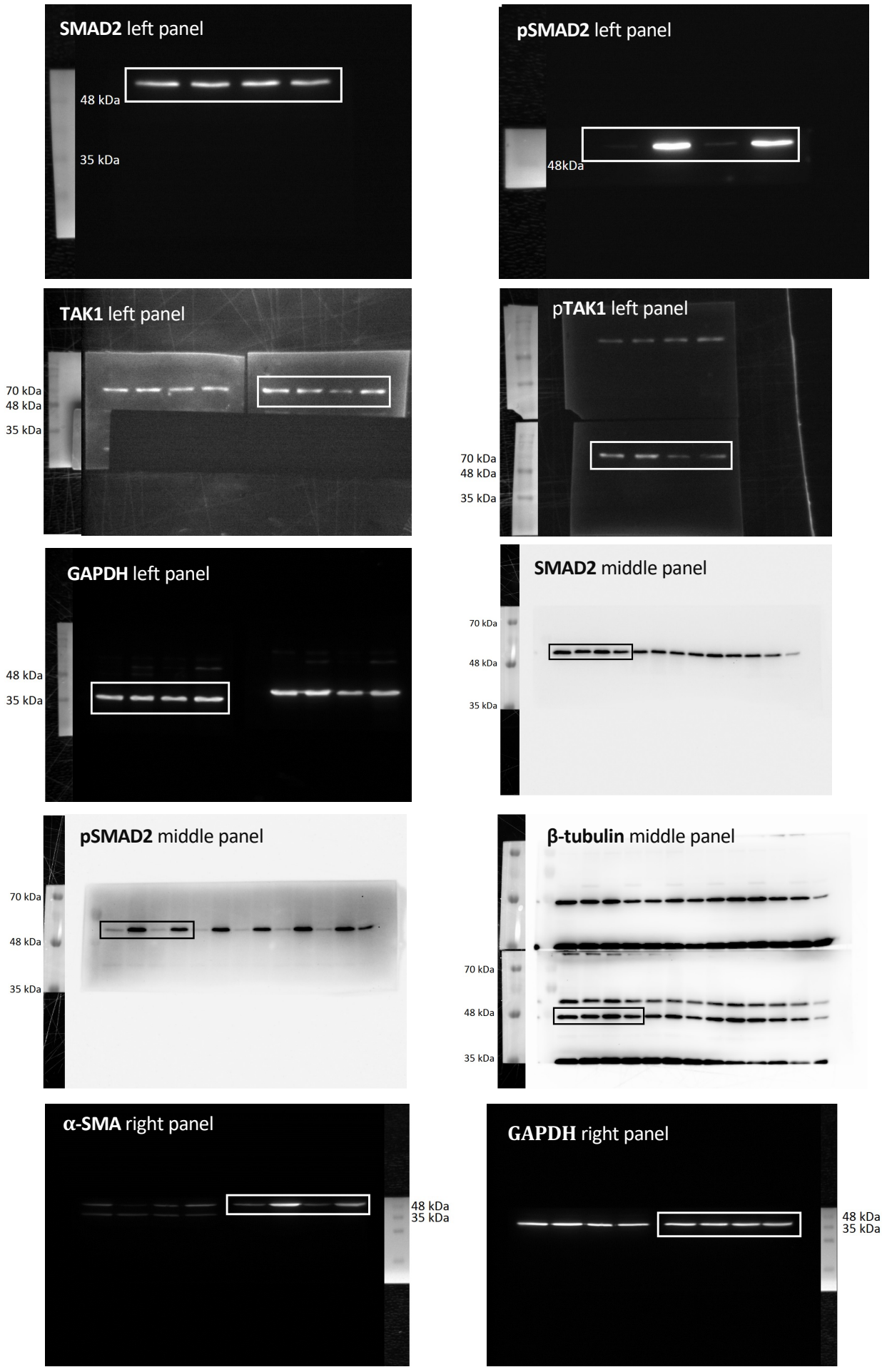
Suppl. Figure 5. Inflammatory cells were isolated from hearts of wild-type mice at day 17-21 of EAM, expanded and stimulated with TGF-β1 (10 ng/mL) for up to 72h. Graphs show expression of profibrotic genes (*Acta2*, *Col1a1* and *Fn1*), selected *Wnt1, Wnt5a, Wnt11* and Wnt/β-catenin target genes (*Axin2*, *Tcf7* and *Ephb3*), n=4.



Suppl. Figure 6. Full original immunoblots presented in the Figure 3C. Frames indicate cropped area.

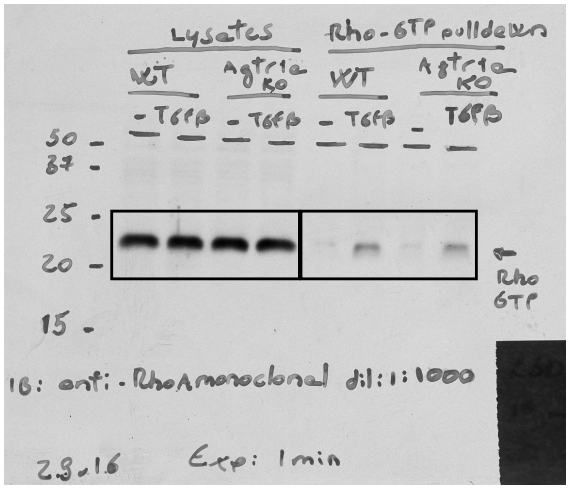


Suppl. Figure 7. Full original immunoblots presented in the Figure 4A. Frames indicate cropped area.

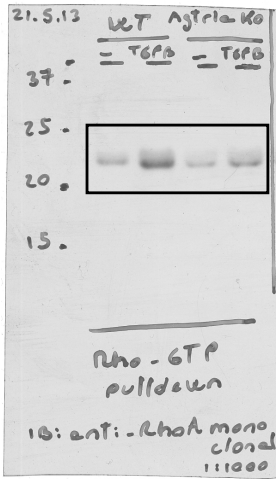


Suppl. Figure 8. Full original immunoblots presented in the Figure 5C. Frames indicate cropped area. Additional bands on β-tubulin immunoblots come from previous stainings with SMAD2 (higher band) and GAPDH (lower band).

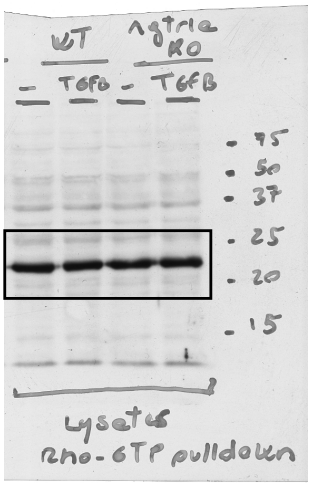
Rho-GTP and total RhoA, Fig. 6A left panel



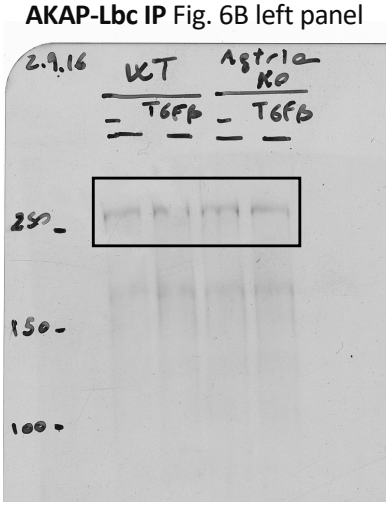
Rho-GTP Fig. 6A right panel



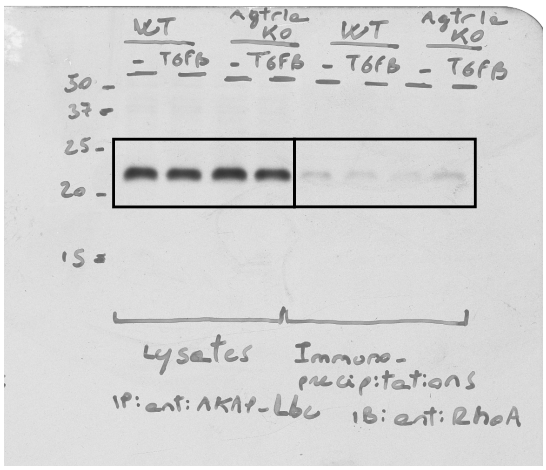
Total RhoA Fig. 6A right panel



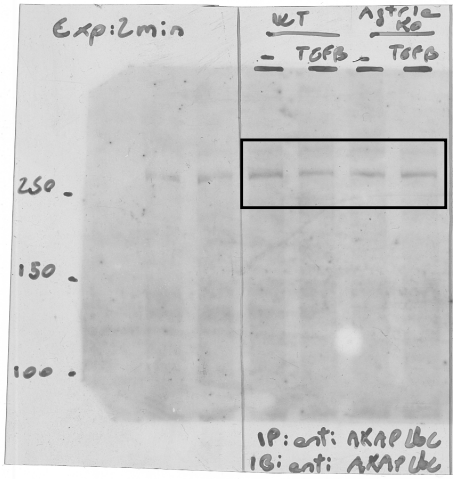
AKAP-Lbc IP Fig. 6B left panel



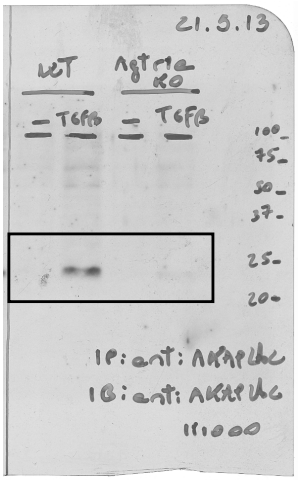
RhoA cell lysates and RhoA Co-IP Fig. 6B left panel



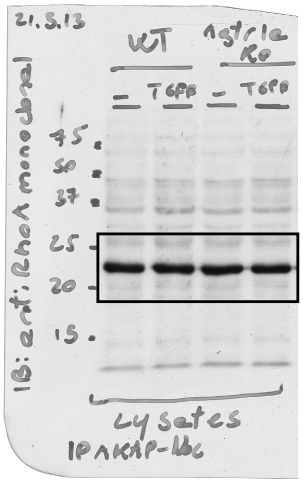
AKAP-Lbc IP Fig. 6B right panel



RhoA Co-IP Fig. 6B right panel



RhoA cell lysates Fig. 6B right panel



Suppl. Figure 9. Full original immunoblots presented in the Figure 6. Frames indicate cropped area.