SUPPLEMENTAL MATERIAL

SUPPLEMENTARY MATERIALS AND METHODS

Cardiac surgery

10- 12-week-old mice were subjected to myocardial ischemia/reperfusion (I/R) injury and divided into 2 groups: I/R + III-11C and I/R + pUR4. Briefly, mice were anesthetized with isofluorane, intubated, and ventilated with a 50% air:oxygen mixture using a positive-pressure respirator (Minivent 845, Harvard; 160 strokes/min, 250 µl tidal volume). A left thoracotomy was performed via the fourth intercostal space and the lungs retracted to expose the heart. After opening the pericardium, the left anterior descending coronary artery was ligated with 8-0 silk suture approximately 2 mm below the edge of the left atrial appendage for 45 minutes, followed by the specified time of reperfusion (1 day, 7 days, 28 days or 8 weeks). Ligation was considered successful by the observation of ST wave elevation by electrocardiography and when the anterior wall of the left ventricle turned pale. Then, the lungs were inflated by increasing positive end-expiratory pressure and the thoracotomy site closed in layers with 6-0 suture. Animals were maintained on a 37 °C heating pad during the surgery and until recovery. Another group of mice underwent sham surgery, with a similar surgical procedure without tightening the suture around the coronary artery.

Peptide treatment

Mice received daily i.p. injections of pUR4 or III-11C (25 mg/kg/day) for 7 consecutive days starting the same day of I/R surgery or for 14 days starting 4 weeks after I/R injury. Cells received pUR4 or III-11C (500 nM) for 72h. Cell functional studies were performed in semiconfluent cell conditions (≈75% after 72h) to avoid contact inhibition.[1] Also, for ECM network assessment, we waited until cells were semiconfluent (≈75%) and then treated with peptides for 72 h to ensure a mature ECM meshwork formation.

Echocardiography analysis

Transthoracic echocardiography was performed at baseline and 4 weeks and 8 weeks after I/R to evaluate the development of HF symptoms under spontaneous respiration with isoflurane in an oxygen mix. Images were acquired from mice anesthetized with isoflurane using Visualsonics Vevo 2100 High-Resolution In Vivo Imaging System (Visual Sonics, Toronto, Canada). Echocardiographic measurements were taken as previously described[2] with some modifications. The challenge with I/R cardiac mouse models is that the part of the LV that is most affected by the ischemic event (mid to apical LV) is not what is measured by the shortening fraction (basal LV) and EF as supplied by the echocardiography machine (VEVO) is basically 2*SF, a minor regression

application. Therefore, we were not accurately quantifying cardiac function for this particular model of heart failure using the standard echocardiography approaches. We pursued a more accurate strategy to measure EF termed 5/6. 5/6 takes into account the distal wall motion abnormalities in I/R mice to measure ejection fraction of the left ventricle. We used a formula in common clinical use for LV volume (5/6 area*length) which accounts for the portions of the LV wall that is hypokinetic, akinetic, dyskinetic and/or aneurysmal. Two sonographers from the Cincinnati Children´s Hospital Medical Center echocardiography core analyzed the echocardiograms in a blinded manner and validated this measurement approach by qualitative global assessment of the images adquired.

Mouse adult cardiomyocytes isolation and culture

Adult CM were collected from 10- 12-week-old C57BL6/J WT mouse hearts by enzymatic digestion. Briefly, mice were given 100 µl heparin (100 U/ml) *via* intraperitoneal injection and anesthetized with isoflurane. The heart was quickly excised and retrograde-perfused using a Langendorff apparatus under constant pressure (60 mmHg; 37 °C, 4 min) in Ca^{2+}-free perfusion buffer containing 113 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 5.5 mM glucose, 0.6 mM KH_2PO_4, 0.6 mM Na_2HPO_4, 12 mM $NaHCO_3$, 10 mM $KHCO_3$, 10 mM Hepes, 10 mM 2,3-butanedione monoxime, and 30 mM taurine. Digestion was achieved by perfusing for 3 minutes with Ca^{2+}-free perfusion buffer containing collagenase II (units/ml) (600 units/mL of collagenase II in perfusion buffer; Worthington LS004177) followed by 8 minutes of perfusion with digestion buffer containing 12.5 µM $CaCl_2$. Subsequently, it was removed from the apparatus and gently teased into small pieces with fine forceps in the same enzyme solution. Heart tissue was further dissociated mechanically using 2, 1.5, and 1 mm-diameter pipettes, until all large heart tissue pieces were dispersed. The digestion buffer was neutralized with buffer containing 10 % FBS and 12.5 µM $CaCl_2$ and cell suspension was filtered through 200 µm mesh. Cardiomyocytes were pelleted by gravity (20 min) and the supernatant, containing CF, collected and stored on ice. CM were resuspended in perfusion solution containing 5 % FBS and 12.5 µM $CaCl_2$ and subsequently allowed to settle for 20 min.

The final CM pellet was resuspended in perfusion solution with 5% FBS and $CaCl_2$ was gradually increased from 62 µM to 1 mM final concentration. Finally, CM were resuspended in plating media (Medium 199 Hank's, 0.25 % BSA, 22 mM $NaHCO_3$, 0.05 % FBS, 0.001 % ITS (insulin-transferrin-selenium and 10 mM 2,3-butanedione monoxime) and plated onto a laminin-coated (0.5 mg/ml) glass chamber for further sarcomeric contractility studies.

Neonatal Rat Cardiomyocyte Isolation

1-2 day old Sprague-Dawley rat neonate hearts were utilized for neonatal rat ventricular myocytes (NRVMs) isolation. Hearts were enzymatically digested as previously described.[3] Isolated NRVMs were cultured in serum free DMEM (Gibco) and M199 (Gibco) media at 80:20 ratio supplemented with penicillin/streptomycin (100 U/mL). NRVMs were further treated with PBS, angiotensin II (AngII; 200 nM; Sigma), III-11C (500nM) or pUR4 (500 nM) for either 4 hours for RNA collection or 48 hours to induce hypertrophy for immunoflourescent staining.

Cardiomyocyte contractility

To assess CM contractility, sarcomere length (SL) was assessed in isolated cells using a commercially available bright field SL measurement system (Myocyte Contractility System; IonOptix). Cells were placed in a specialized glass slide chamber on a microscope stage (Olympus IX71) and aligned with the fast scan direction of the charge-coupled device (MyoPacer, IonOptix). CM were stimulated at 0.5 Hz, 10 V and visualized utilizing a field-rate camera (MYO100 MyoCam, Ionptix). Peak contraction, the percentage of peak cell shortening, and maximum rates of contraction and relaxation were determined. Data represented are an average of at least 10 CM for each group.

Immunofluorescence and cardiac morphology and histology

Hearts were collected at indicated time points (as described in figure legends), perfused with ice-cold PBS to remove red blood cells followed by perfusion with 50 mM KCl to arrest the heart in diastole and then fixed in 10% formalin solution at 4 °C overnight. Fixed hearts were embedded in paraffin and sectioned at 8 μm. Tissue sections were deparaffinized in xylene and rehydrated in decreasing concentrations of ethanol (100%, 95%, 75%, 50%). For fluorescent staining, deparaffinized sections were microwaved for 20’ with sodium citrate buffer (Vector H-3300) for epitope unmasking and permeabilized using 0.06% triton. Specimens were blocked using 4% goat serum in Tris-buffered Saline (TBS) and slides incubated with the following primary antibodies: fibronectin (1:100, Abcam ab2413); collagen type I (1:100, Abcam ab34710), wheat germ agglutinin-488 conjugated (1:100, Thermo Fisher W11261), CD45 (1:100, R&D Systems AF114) and sarcomeric alpha-actinin (1:100, Sigma, A7811) overnight at 4ºC in TBS + 1 % goat serum. Sections were washed three times for 5 min each in TBS + 4% goat serum and stained for 1 h at room temperature with 1:100 dilution of Alexa Fluor 488 and 1:500 dilution of Alexa Fluor 546 in 4ºC in TBS + 1 % goat serum. Slides were rinsed three times for 5 min each in TBS + 4% goat serum and mounted with DAPI prolong antifade reagent (Life Technologies P36962).

To evaluate cardiac fibrosis, picrosirius red staining was performed on deparaffinized and rehydrated sections. Specimens were incubated in 0.1% sirius red in saturated picric acid for 1 h, then washed in acidified water, cleared in ethanol and xylene, and mounted. The degree of fibrosis was quantified as the percentage of red (collagen) area to the total heart (excluding atria) using Image J software (National Institutes of Health).

For immunofluorescence, cells were plated on glass coverslips (Fisher Scientific) and fixed with 4% PFA. Preparations were incubated with blocking buffer (BB) containing PBS + 2% BSA (Sigma) for 10 min, followed by triton 0.1% permeabilization. Cells were then incubated for 1h at room temperature with the following primary antibodies used at 1:100 dilution except for p-FAK (1:50): fibronectin (Abcam ab2413), collagen type I (Abcam ab34710), phospho-histone 3 (Abcam ab5176), Tom 20 (Santa Cruz 11415), phospho-FAK (Abcam ab39967), F actin (life Technologies A12379), WGA and CD45 (R&D Systems AF114), α-SMA (Sigma A5228), vimentin (Abcam ab45939), washed three times for 5 min each in BB and incubated with a 1:100 dilution of Alexa Fluor 488 and 1:500 dilution of Alexa Fluor 546 in 2% BB for 1 h at room temperature. After washing the samples with BB three times for 5 min each, cells were mounted with DAPI prolong antifade reagent (Life Technologies P36962).

As negative controls, both for cultured cells and tissue sections, preparations were incubated with the corresponding secondary antibodies to determine the background fluorescence.

Second Harmonics Generation (SHG) imaging

SHG imaging was performed using the Nikon A1R Multiphoton Upright Confocal Microscope with the coherent chameleon II Ti-sapphire laser tuned to 840 nm. Emission spectrums for crystalline collagen I ranges from 450 to 550 nm. SHG signal was detected using the backward emission via non descanned detectors (NDDs). Autofluorescence was collected by the green filter cube and collagen by the red filter cube.

Infarct size assessment

Mice were euthanized 24 h post-I/R and peptide treatment. The heart was exposed and the prolene suture 8-0 was re-tied tightly. Using a syringe with 38 gauge needle, 2% Evans Blue (Sigma E2129) solution (in PBS) was injected into the apex carefully. The whole heart, except the area at risk should turn blue. The heart was remove from the chest cavity and rinsed extensively with PBS. The specimen was dried on a paper towel and placed into previously coated plates with 2% agarose (in PBS). Warm agarose (37℃) was used to completely cover the heart and let it solidify. Thin heart slices (1-2 mm) were cut with a scalpel and transferred to an ependorff containing 2.5% Triphenyltetrazolium chloride (TTC) solution (Sigma T8877) for 17 min. Samples were rinsed

in PBS, transferred to 15 ml conical tubes containing 10% formalin and stored in the dark at 4℃ overnight. Pictures were taken 24 h later. Blue areas indicate healthy tissue, red areas represent areas at risk and white areas indicate infarcted tissue.

Cell apoptosis

Primary MF were isolated 5 days after I/R injury, cultured and treated with pUR4 and III-11C for 72h. 10^6 cells were stained with FITC-conjugated Annexin V. Flow cytometry analysis was performed using a BD LSRFortessa and data analyzed using FlowJo. Additionally, the impact of FN polymerization inhibition on the rate of cardiac apoptosis was determined by deoxynucleotidyl transferase dUTP Nick-End Labeling (TUNEL) staining (Millipore QIA39-1EA) of heart sections per manufacturer's instructions.

Flow cytometry analysis of reactive oxygen species (ROS)

Primary MF were isolated 5 days after I/R injury and treated with pUR4 and III-11C for 72h. MF cultures were washed in PBS, trypsinized and resuspended in culture media without serum at 10^6 cells/ml. Samples were then incubated at 37°C in the dark with 5 mM dihydroethidium (DHE) for 30 min or with 1 mM MitoSox Red probe for 10 min (both from ThermoFisher Scientific). DHE and MitoSox Red are specific biomarkers of total and mitochondrial superoxide anion (O^{2-}), respectively. After incubation, cells were washed twice in PBS and dead cells excluded by incubation for 5 min with TO-PRO3 in HBSS/Ca/Mg phenol-red-free medium. ROS were detected by flow cytometry using a BD LSRFortessa and data analyzed using FlowJo.

Flow cytometry analysis of mitochondrial content

Primary MF were isolated 5 days after I/R injury and treated with pUR4 and III-11C for 72h. MF cultures were washed in PBS, trypsinized and incubated at 10^6 cells/ml in HBSS/Ca/Mg phenol-red-free medium (Sigma-Aldrich) containing 100 nM Mitotracker green (ThermoFisher) for 30 min in the dark. Dead cells were excluded by incubation for 5 min with TO-PRO3 in HBSS/Ca/Mg phenol-red-free medium. Fluorescent signal was detected by flow cytometry using a BD LSRFortessa and data analyzed using FlowJo.

ATP production, glycolysis and fatty acid oxidation studies

Primary MF were isolated 5 days after I/R injury and treated with pUR4 and III-11C for 72h. To determine the metabolic stage in pUR4-treated cells, ATP production, glycolysis and fatty acid oxidation were analyzed with a kit (Abcam, FLASC; Cayman Chemical, 600450 and Abcam, ab65341 respectively).

Oxygen Consumption Rate (OCR) measurement in isolated cells

OCR was measured using DW1 Oxygen Electrode Chamber from Hansatech Instruments Ltd. as previously reported. [4] Briefly, MF were treated with III-11C or pUR4 for 72 h and collected and re-suspended in respiration solution buffer (110 mM Mannitol, 60 mM KCl, 10 mM KH_2PO_4, 5 mM $MgCl_2$, 60 mM Tris-HCl and 0.5 mM Na_2EDTA) for OCR measurement. Temperature of the oxygen chamber was maintained at 37℃. Baseline OCR was measured with the introduction of 10 mM glutamate and 2.5 mM malate in the respiration solution buffer. Calcium-stimulated OCR was measured with the addition of 500 μM $CaCl_2$. Data was normalized versus total cell numbers.

Cardiomyocyte cell size determination

To evaluate individual cardiomyocyte cross-sectional area, paraffin tissue sections were deparaffinized and labeled with sarcomeric α-actinin (1:500; Sigma-Aldrich #A7811) and wheat germ agglutinin (WGA, 1:300) (Alexa Fluor 488 conjugate; Invitrogen) to label the membranes followed by nuclear counterstaining with DAPI Prolong antifade. Confocal images were captured using a 20X objective, and the same fixed field dimensions were used for determination of CM cell size. CM were identified by α actinin staining. Cell surface areas of CM with centrally located nuclei were quantified using Image J software (National Institutes of Health) for a minimum of 200 CM/animal.

RNA and RT-qPCR

Total RNA was extracted from cells or left ventricular tissue using the GeneJet kit (Fisher Scientific K0732) according to manufacturer's protocol. Complementary DNA was synthetized by reverse transcription using iScript cDNA synthesis kit (BioRad 1708841). Quantitative real-time polymerase chain reaction (qRT-PCR) was performed using Taqman Master Mix (Applied Biosystems) on the StepOnePlus Real-Time PCR machine (Applied Biosystems) with the following Applied Biosystems verified primer-probe sets for mouse: Fn (Mm00692642), Collagen type I (Mm00801666), Acta 2 (Mm00725412), Periostin (Mm00450124), Collagen Type III (Mm01254476), Aurora b kinase (Mm01718146), C-myc (Mm01192721), Mmp-13 (mm00439491), Mmp-9 (Mm00442991), TIMP-1 (Mm00441825), TIMP-2 (Mm00441818), TLR-2 (Mm00442346), TLR-4 (Mm00445273), TNFα (Mm00443260), Ccl2 (Mm00441242), Ccl3 (Mm00441259), Il6 (Mm00446190), Il1β (Mm00434228), IFNγ (Mm01168137) and human: AUROBA B KINASE (Hs00177782), C-MYC (Hs00153408), TNFα (Hs01113624), IL6 (Hs00985639), IL1β (hs01555410), COL1α1 (Hs00164004), PPIA

(hs04194521), COL3α1 (Hs00943809), CCL2 (Hs00234140) and FN (Hs01549976). Results were normalized to 18S. Transcript level relative expression was determined using the comparative ΔΔCt method.[5] Following this analysis procedure along with a paired *t*-test, controls are set to one. Changes in mRNA expression were denoted as the *x*-fold change relative to the control.

Protein isolation and Western blotting

Left ventricular protein lysates were extracted using mPER lysis buffer (ThermoFisher Scientific 78501) containing protease/phosphatase inhibitors (ThermoFisher Scientific 1861280). LV samples were immediately frozen upon harvest and mechanically homogenized using a tissue homogenizer (OMNI International tissue master 125). Following homogenization, sample lysates were centrifuged at 14000 x g for 20 min at 4ºC and the supernatant was collected. For ECM separation, cells were treated as described[6]. The soluble fraction (DOC soluble) represented the cellular compartment, without matrix, and the insoluble fraction (pellet) represented mainly the cellular matrix (DOC insoluble). Protein quantification was performed by BCA assay (Pierce 23227) and equal amount of protein lysates were added to the NuPAGE 4-12 % Bis-Tris gels (Invitrogen). Briefly, 2 μg (DOC insoluble samples), 5 μg (DOC soluble) and 30 μg (rest of the experimental conditions) of protein were used for immunoblotting and transferred to PVDF membranes overnight at 10 V 4ºC. After blocking for 1.5h in Odyssey blocking buffer (LI-COR, P/N 927-40000) the blots were probed with the corresponding primary antibodies: Fn (1:1000; Abcam ab2413), Collagen type I (1:1000 Abcam ab34710), β1 integrin (1:1000 Millipore MAB1997), FAK (1:1000 Cell Signaing 3285S), phospho-FAK (1:1000 Abcam ab39967), total AKT (1:1000 Cell Signaling 2920S), p-AKT (1:1000 Cell Signaling 4060S), total ERK (1:1000 Cell Signaling 4696S) and p-ERK (1:1000 Cell Signaling 43775S). Primary antibodies were detected using fluorescent secondary antibodies (Li-COR Biosciences) and blots were imaged and quantified using ODYSSEY Infrared Imaging System (LI-COR). GAPDH (Millipore MAB374) was used as a loading control.

Cell proliferation

In vitro cell growth was monitored by counting cell number with a hemocytometer. Briefly, 50000 cells at passage 1 were seeded in p35 culture plates and treated with pUR4 or III-11C. After 72 h, cells were trypsinized and viable cells counted by tripan blue (Gibco 15250-061) exclusion.

***In vitro* scratch assay**

To evaluate the index of MF migratory capacity, we performed scratch assays as previously described[7]. Passage 1 cardiac MF were plated onto 6-well plates with pUR4 and III-11C peptides. Once the culture reached

confluence a scratch was made with a standard P-200 pipette tip. Cells were immediately washed with PBS and replaced with culture media with 1% FBS. To monitor the wound closure, images were taken in the same field at indicated time points post-scratch. Scratch wound closure was calculated as a percentage of baseline area using ImageJ (National Institutes of Health).

Integrin β1 internalization assay

Cardiac fibroblasts were grown in 15cm plates. Cells were lifted on ice and washed twice with ice cold PBS. Cell surface β1 integrin was labelled with 5 μg of anti-Integrin β1 antibody, clone MB1.2 (Millipore MAB1997) or 5 μg of normal rat IgG antibody as control(sc-2026 Santa Cruz) in 5 ml ice-cold DMEM/10%FBS at 4 °C for 60 minutes. Cells were washed twice with cold PBS and surface labeled control cells were lysed in IP buffer (50 mM Tris-HCl (pH=7.5), 150 mM NaCl, 1% Triton X100, 1% n-Octylglucoside). The cells used for the uptake experiment were incubated in DMEM/10%FBS containing 0.1 μM primaquine (Sigma) for 30 min at 37 °C. Plates were lifted on ice, washed with cold PBS followed by a 1 min acid wash at 4 °C (0.5% acetic acid (Fisher), 0.5 M NaCl, pH=3) followed by two washes with cold PBS. Cells were lysed in IP buffer, sonicated and cleared at 17000 *x g* for 10 min at 4 °C. Labeled β1 integrin proteins were precipitated with 50 μl Protein A/G Magnetic Beads (Thermo-Pierce 88803) for 60 min at 4 °C, washed 3 times in IP buffer and heated in 50ul loading dye for 10 min at 95°C. Proteins were separated on 6% polyacrylamide gels blotted to PVDF membranes and incubated with anti-Integrin β1 Antibody (Cell Signaling 4706). Pan-Cadherin Antibody (Cell Signaling 4068) was used as a loading control. Blots were imaged and quantified using ODYSSEY Infrared Imaging System (LI-COR).

Gelatin zymography

LV tissue was homogenized in 500 μl of lysis buffer (25 mM Tris-HCl, pH 7.5; 100 mM NaCl; and 1% Nonidet P-40 (NP-40)) containing protease and phosphatase inhibitors (ThermoFisher Scientific 1861280). Samples were centrifuged at 4°C at 15,000 x g to remove insoluble matter. Protein concentration was assessed using the Bradford assay (Biorad). Approximately 20 μg of protein was run on 10% Gelatin zymogram protein gels (Life Technologies). Gels were washed and incubated in zymography renaturing buffer (Life Technologies) for 30 minutes followed by incubation in developing buffer for 30 minutes at room temperature. Gels were then incubated at 37°C overnight in developing buffer. Next, gels were Coommassie stained for 1 hour at room temperature followed by destaining for approximately 6 hours. The gels were photographed and densitometry was performed using ImageJ software.

EdU staining

Mice underwent I/R cardiac injury and pUR4 or III-11C were administered for 7 consecutive days. To detect proliferative cells, 24 h after the last administration, mice were injected i.p. with EdU Alexa Fluor 488 (200 mg/kg) and hearts were harvested at 4 h post-injection. Fibroblasts were isolated following the protocol described above and cells were co-stained with FxCycle Violet to label DNA content. EdU visualization by flow cytometry was made by using Click-iT Plus EdU Alexa Fluor 488 Flow Cytometry Assay Kit per Manufacturer's protocol utilizing a BD LSRFortessa. Data were analyzed using FlowJo.

Peptide labeling and *in vivo* imaging using the In Vivo Imaging System (IVIS)

pUR4 and III-11C peptides were labeled with Tide Fluor™ 7WS, succinimidyl ester [TF7WS SE]*Superior replacement for Cy7* (AAT Bioquest 2333) per manufacture's instructions. WT mice were injected for 7 consecutive days with the labeled peptides and 24 h after the last administration animals were anesthetized with isoflurane. Animals were transferred to an IVIS 200 Series imaging system and infrared fluorescence images in whole animals were acquired using a charge-coupled device camera. The intensity of photon collected through IVIS was translated to false color images with strong fluorescence in yellow. The hearts were harvested immediately after body imaging and transferred to the IVIS and images were acquired using the same acquiring settings as above.

Bone marrow derived neutrophil isolation

Bone marrow was harvested from femurs and tibias of WT mice and mechanically disrupted with PBS. Polymorphonuclear leukocytes (PMN) were negatively selected using a PMN isolation kit (Miltenyi, Cambridge MA), as described.[8] Neutrophil purity was confirmed by FACS staining with anti-CD11b (clone M1/70) and anti-Ly6G (Clone 1A9) antibodies in each cell preparation (described later). For adhesion experiments, cells were used within 3h from the isolation. Purity was determined by CD11b and Ly6G staining and was >98% in all preparations.

Isolation of primary Mouse Heart Endothelial Cells

Murine coronary endothelial cells (MHECs) were isolated from hearts harvested from 5-8 mice at 2-3 weeks of age as previously described.[9] Briefly, hearts were digested in 2 mg/ml of collagenase shaking at 37°C for 30 minutes, and incubated with PECAM-1-coated sheep anti-rat-IgG Dynabeads (15µl/ml of cells) at room

temperature for 10 minutes with end-over-end rotation. Magnetically recovered PECAM-1 positive cells were resuspended in 10 ml of complete culture medium, (DMEM-20% supplemented with 100µg/ml heparin, 100µg/ml ECGS, NEAA, sodium pyruvate, L-glutamine, and Pen-Strep), and plated in gelatin-coated dishes. At 85% confluency cells were sorted for the second time with ICAM-2-coated sheep anti-rat-IgG Dynabeads beads (15µl/ml of cells). Bead-bound cells were washed and plated in complete DMEM culture medium. MHECs were used at passages 2 to 4 for experiments. For *in vitro* parallel flow chamber experiments, MHECs were plated on FN coated glass coverslips.

Measurement of interactions of bone marrow derived neutrophils with MHECs under defined shear flow conditions *in vitro*

Bone marrow derived neutrophil interactions with MHECs were observed by videomicroscopy (×20 objective) under defined laminar flow conditions in a parallel plate apparatus using a Nikon NIS-Elements software as described.[9] MHECs were freshly isolated, cultured and treated for 3 days with pUR4 (500 nM) or III-11C (500 nM) and treated with TNFα (125 ng/ml, 4h) to induce endothelial cell activation. A bolus of 1 million neutrophils were perfused across MHECs at shear 1 dyne/cm^2 and neutrophil interactions with MHECs were recorded for 10 minutes to monitor rolling and adhesion. Adhesion was also recorded in 6 additional fields of view (30 seconds/ field). The peptides were added to the flow buffer and was present for the duration of the experiment. Analysis was performed using the NIS Elements software. Firm arrest was defined by the number of neutrophils that remained arrested to MHECs from the initial contact and throughout the duration of the videos. Rolling was determined as the cells that rolled on MHECs during the duration of the videos. Accumulated cells were defined as the number of cells that were firmly arrested and the number rolling cells in the last 2 minutes of the video and in the 30 second videos of additional fields.

Also, after peptide administration and TNFα, MHECs were utilized for immunofluorescent staining as well as for FACS analysis.

MHECs immunocytochemistry

The cells were fixed in 4% paraformaldehyde (PFA) for 15 min, washed and incubated with blocking buffer (PBS + Triton-X100 0.2% + 5% Normal goat serum) for 2 h. Next, cells were incubated at 4^{o}C overnight with primary antibody: anti-mouse ICAM-1 (1:200; Biolegend, Dedham, MA, USA) or anti-mouse VCAM-1 (1:500; Biolegend, Dedham, MA, USA). Then, cells were washed and incubated with Alexa Fluor 488 (1:500;

Invitrogen, Life Technologies, Carlsbad, CA, USA) for 1 h at RT, and washed again. After that, mount medium with DAPI was used on the coverslips.

MHECs FACS staining

MHECs were detached using Trypsin TrypLE Express (Gibco, Waltham, MA, USA). MHECs or neutrophils were washed with FACS buffer (PBS + 2% FBS) and incubated with the appropriate antibodies (all of them from Biolegend, Dedham, MA, USA): CD31-APC (1:50; Cat. 102509), VCAM-1-PE (1:50; Cat. 105714), ICAM-1-FITC (1:50; Cat. 116105), VLA-4-FITC (1:50; Cat. 103605), LFA-1-APC (1:50; Cat. 101120). The data were acquired on a FACSCanto (Becton Dickinson) and analyzed using FlowJo software.

Cell cycle analysis by flow cytometry

Cardiac MF were treated with III-11C or pUR4 for 72 h, trypsinized and centrifuged at 200 x g for 5 min at RT with a subsequent wash with PBS without Ca^{+2} and Mg^{+2}. Cells were resuspended at 2 x 10^6 cells in 1 ml of ice-cold PBS. Next, they were vortexed gently, slowly adding to the cell suspension 9 ml of 70% ethanol dropwise. Preparation was stored at -20°C for at least 24 h before flow. Cells were centrifuged at 850 x g for 10 min at 4°C and resuspendend in PBS containing 0.1 mg/ml of RNA A (Sigma, R6513) for 45 min at 37°C. Next, cells were stained with 40 µm/ml of Propidium iodide (Invitrogen, P3566) for 5 min. Flow cytometry was performed on a BD Biosciences Fortessa II flow cytometer.

Flow cytometry of immune cell subsets

Peptide-treated hearts were dissected 7 days post-I/R as described above. Cells were spun (5 min x 6000 rpm) and washed with buffer (HBSS containing 0.5 mM EDTA and 25 mM Hepes) then incubated in 100 µL of ACK buffer (Life Technologies A10492-01) for 1 minute at room temperature and directly washed with buffer. Cells were spun down (5 min x 6000 rpm) and washed again with buffer. Cell suspensions were then filtered through a 35µm cell strainer cap (Falcon/Corning Life Sciences 352235). Cells were incubated with Zombie UV in buffer according to manufacturer's instructions for 15 min at 4°C followed by two subsequent washes with 1% BSA (Bioexpress 0332) in buffer. Cells were stained with the following antibodies: B220 (BD Pharmingen 552772), CD3 (Biolegend 100236), CD45 (BD Horizon 560501), CD11b (BD Pharmingen 557396), CD11c (Biolegend 117334), F4/80 (Biolegend 123118) and Ly6G (BD Pharmingen 561104) for 1 hour in 1% BSA in buffer. Cells were washed with 1% BSA in buffer, and flow cytometry was performed on a BD Biosciences Fortessa II flow cytometer. All data were analyzed using FlowJo.

Statistical Analysis

Data are reported as mean ± standard error of the mean (SEM). Sample size was estimated commensurate with prior *in vivo* work with pUR4 and III-11C in vascular studies. For single biochemical and physiological observations, student *t*-test was applied. Paired *t*-test was utilized when different treatments (pUR4 and III-11C) were applied to cells isolated from the same animal. Unpaired *t*-test was applied when pUR4 and III-11C were administered to different animals. Multiple responses were analyzed by one-way or two-way ANOVA. Post-hoc analysis was performed as indicated if statistical significance ($P \leq 0.05$) was achieved. Calculations were performed using Graphpad Prism 6.0.

Supplementary Table 1. Echocardiographic parameters of healthy mice treated with pUR4

	PBS	III-11C	pUR4
LVE;d area (mm^2)	12.93 ± 0.52	14.25 ± 0.53	13.48 ± 0.61
LVE;s (mm^2)	8.7 ± 0.6	9.52 ± 0.79	8.6 ± 0.45
LVL;d (mm)	4.88 ± 0.24	4.88 ± 0.27	4.75 ± 0.32
LVL;s (mm)	4.2 ± 0.22	4.37 ± 0.25	4.13 ± 0.23
EDV (mm^3)	52.3 ± 2.74	59.23 ± 3.3	52.78 ± 4.61
ESV (mm^3)	30.23 ± 2.02	35.27 ± 3.74	29.77 ± 2.4
EF (%)	42 ± 2.77	41.83 ± 3.3	43.33 ± 1.45
Heart rate (beats/min)	500 ± 17	460 ± 21	438 ± 23

Left Ventricular End diastole measured in the short axis (LVE;d); Left Ventricular End systolic measured in the short axis (LVE;s); Left Ventricular Length measured in the apical 4 chamber in diastole (LVL;d); Left Ventricular Length measured in the apical 4 chamber in systole (LVL;s); End-Diastolic Volume (EDV); End-Systolic Volume (ESV); Ejection Fraction (EF). Absence of statistical significance was determined with one-way ANOVA. n = 6 per group.

Supplementary Table 2. Echocardiographic parameters of mice treated with pUR4 post-I/R

	Sham	III-11C	pUR4
LVE;d area (mm^2)	13.45 ± 0.3	14.78 ± 0.95	12.78 ± 0.69
LVE;s (mm^2)	9.02 ± 0.26	18.68 ± 3.42**	10.42 ± 0.66##
LVL;d (mm)	5.47 ± 0.18	5.46 ± 0.24	4.94 ± 0.15
LVL;s (mm)	4.7 ± 0.18	5.2 ± 0.21	4.49 ± 0.18
EDV (mm^3)	61.48 ± 2.88	67.81 ± 6.53	52.43 ± 3.26#
ESV (mm^3)	35.48 ± 2.25	54.64 ± 4.96***	36.98 ± 1.42###
EF (%)	40.44 ± 1.84	18.75 ± 3.58****	28.4 ± 2.5††#
Heart rate (beats/min)	472 ± 15	484 ± 28	553 ± 21†

Left Ventricular End diastole measured in the short axis (LVE;d); Left Ventricular End systolic measured in the short axis (LVE;s); Left Ventricular Length measured in the apical 4 chamber in diastole (LVL;d); Left Ventricular Length measured in the apical 4 chamber in systole (LVL;s); End-Diastolic Volume (EDV); End-Systolic Volume (ESV); Ejection Fraction (EF).**P < 0.01 vs Sham, ***P < 0.001 vs Sham, ****P < 0.0001 vs Sham; †P < 0.01 vs Sham, ††P < 0.01 vs Sham; $^{\#}$P < 0.05 vs III-11C; $^{\#\#}$P < 0.01 vs III-11C, $^{\#\#\#}$P < 0.001 vs III-11C. Statistical significance was determined with one-way ANOVA with Tukey post-hoc analysis. n = 8-10 per group.

Supplementary Table 3. Echocardiographic parameters of mice treated with pUR4 4 weeks post-I/R

	Sham	III-11C	pUR4
LVE;d area (mm^2)	11.76 ± 0.97	17.03 ± 1.16*	17.73 ± 0.99††
LVE;s (mm^2)	6.57 ± 0.79	11.58 ± 0.95**	12.37 ± 0.75†††
LVL;d (mm)	5.16 ± 0.13	6 ± 0.11***	5.41 ± 0.09#
LVL;s (mm)	4.2 ± 0.19	5.25 ± 0.32**	4.93 ± 0.11†
EDV (mm^3)	50.42 ± 4.67	85.1 ± 5.86***	79.73 ± 3.85†††
ESV (mm^3)	22.68 ± 2.61	50.45 ± 4.28***	50.86 ± 3.44††††
EF (%)	54.88 ± 4.4	40.75 ± 1.03	36.14 ± 3.14††
Heart rate (beats/min)	453 ± 12	503 ± 27	458 ± 17

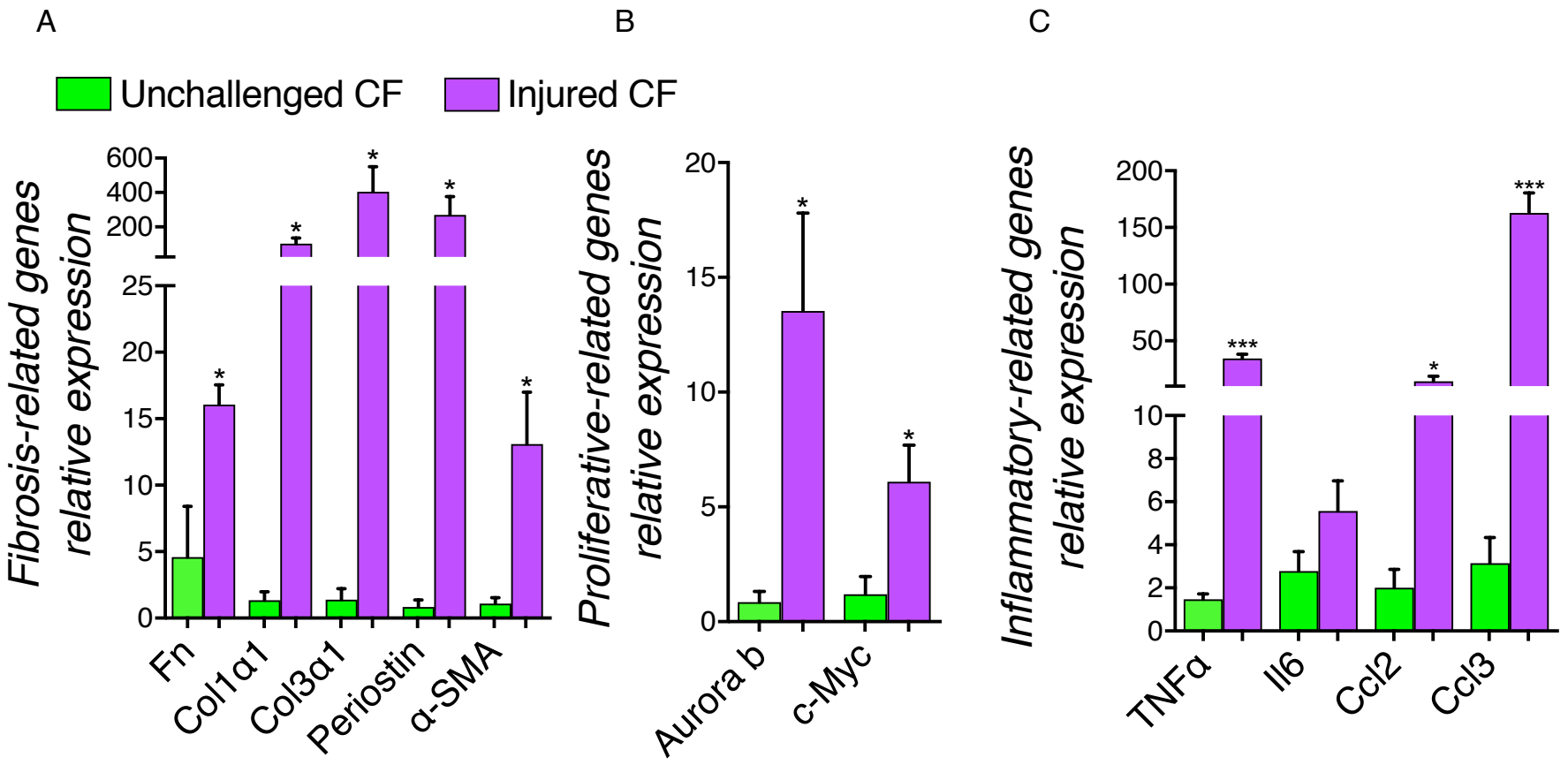
Left Ventricular End diastole measured in the short axis (LVE;d); Left Ventricular End systolic measured in the short axis (LVE;s); Left Ventricular Length measured in the apical 4 chamber in diastole (LVL;d); Left Ventricular Length measured in the apical 4 chamber in systole (LVL;s); End-Diastolic Volume (EDV); End-Systolic Volume (ESV); Ejection Fraction (EF). *$P < 0.05$ vs Sham, **$P < 0.01$ vs Sham, ***$P < 0.001$ vs Sham; †$P < 0.05$ vs Sham, ††$P < 0.01$ vs Sham, †††$P < 0.001$ vs Sham, ††††$P < 0.0001$ vs Sham; #$P < 0.05$ vs III-11C. Statistical significance was determined with one-way ANOVA with Tukey post-hoc analysis. n = 4-8 per group.

Supplementary Table 4. Echocardiographic parameters of Tcf21^{mERCremER};*Fn*$^{flox/flox}$ mice treated with III-11C or pUR4 4 weeks post-I/R

	Sham	Fn$^{+/+}$	CF-FN-/- + III-11C	CF-FN-/- + pUR4
LVE;d area (mm^2)	10.48 ± 0.72	17.34 ± 1.75**	13.7 ± 0.69	11.43 ± 0.44††
LVE;s (mm^2)	5.1 ± 0.39	13.44 ± 1.9***	7.7 ± 0.79#	5.5 ± 0.65†††
LVL;d (mm)	4.95 ± 0.19	5.06 ± 0.17	5.1 ± 0.21	4.73 ± 0.17
LVL;s (mm)	4.45 ± 0.26	4.56 ± 0.26	4.25 ± 0.20	3.9 ± 0.23
EDV (mm^3)	36.1 ± 7	74.36 ± 9.54**	57.98 ± 3.02	44.93 ± 1.51†
ESV (mm^3)	16.05 ± 1.38	53.37 ± 9.93**	27.53 ± 3.36#	18.57 ± 3.22††
EF (%)	61.5 ± 3.76	31.86 ± 5.18**	52.5 ± 5.13#	59 ± 6.41††
Heart rate (beats/min)	407 ± 22	450 ± 23	408 ± 13	385 ± 19

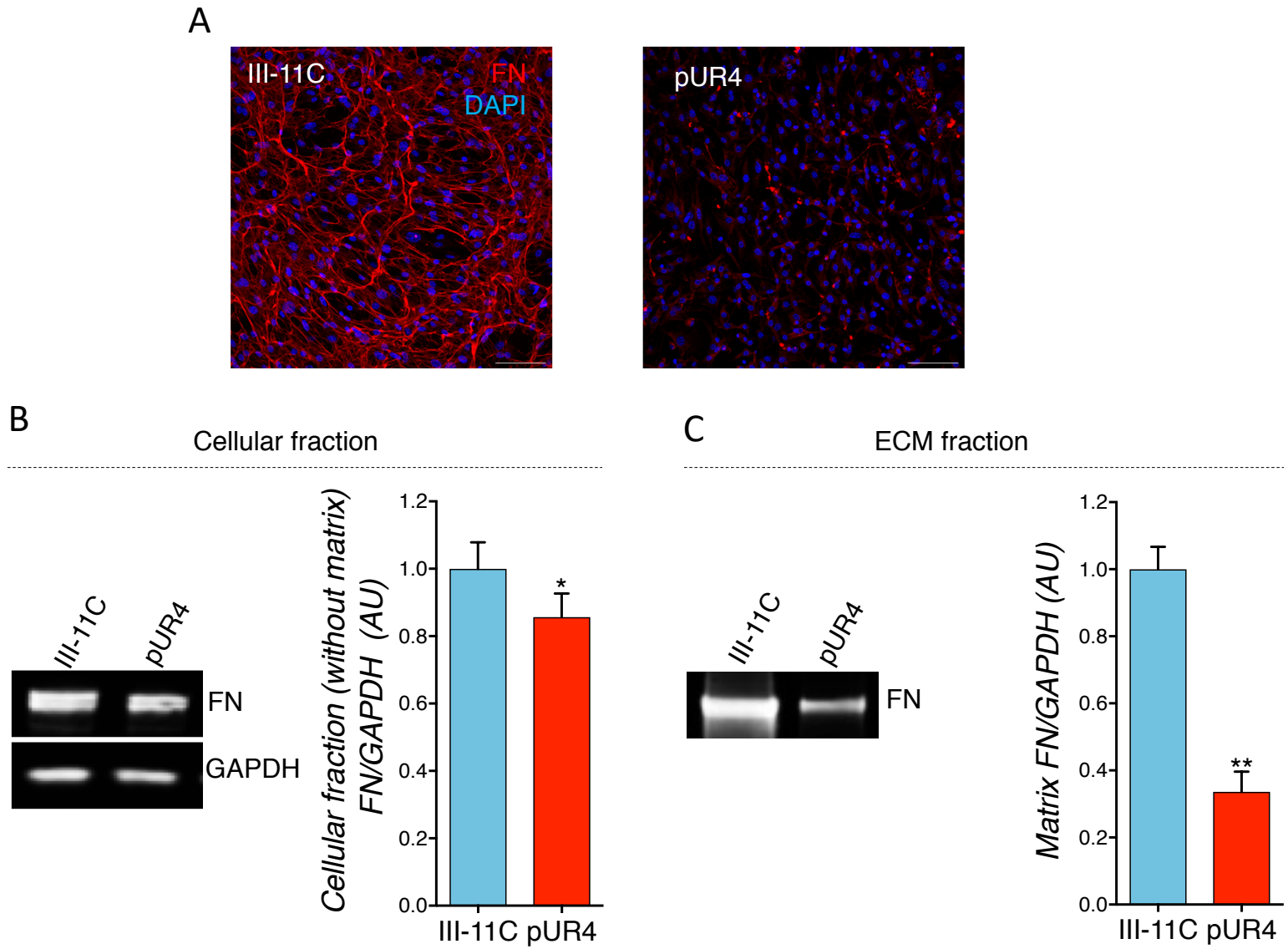
Left Ventricular End diastole measured in the short axis (LVE;d); Left Ventricular End systolic measured in the short axis (LVE;s); Left Ventricular Length measured in the apical 4 chamber in diastole (LVL;d); Left Ventricular Length measured in the apical 4 chamber in systole (LVL;s); End-Diastolic Volume (EDV); End-Systolic Volume (ESV); Ejection Fraction (EF). *Fn*+/+: *Fn*$^{flox/flox}$; CF- *Fn*$^{-/-}$: Tcf21^{mERCremER};*Fn*$^{flox/flox}$; **P < 0.01 vs Sham, ***P < 0.001 vs Sham; $^{\#}$P < 0.05 vs *Fn*$^{+/+}$; †P < 0.05 vs Sham; ††P < 0.01 vs *Fn*$^{+/+}$; †††P < 0.001 vs *Fn*$^{+/+}$. Statistical significance was determined with one-way ANOVA with Tukey post-hoc analysis. n = 6-7 per group.

Supplementary Figure 1



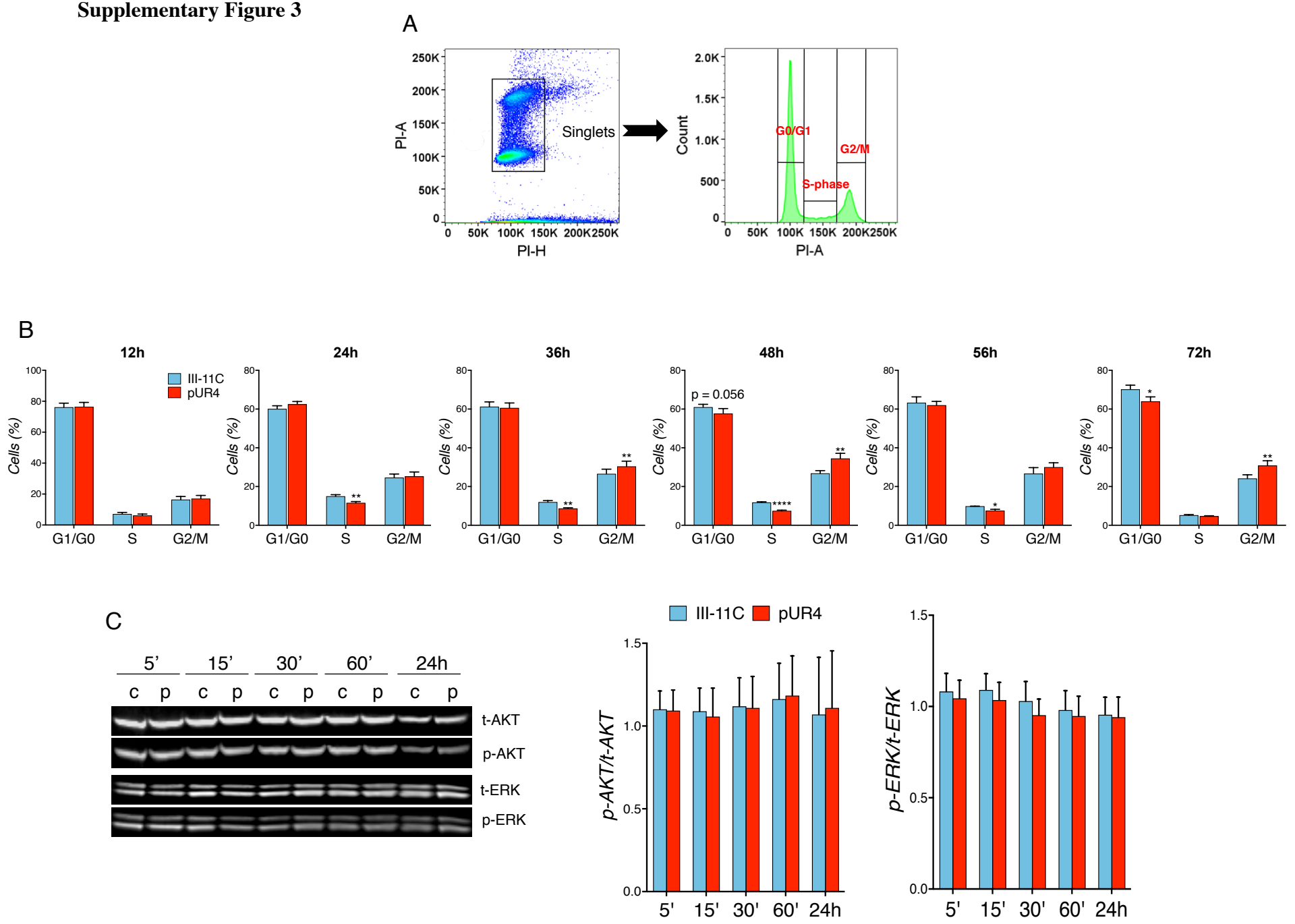
Supplementary Figure 1. **Activated gene profile in mouse MF. (A-C)** Fibrosis-related genes; fibronectin (Fn), collagen 1α1 (Col1α1), collagen 3α1 (Col3α1), alpha-smooth muscle actin (α-SMA). **(A)**, proliferation transcript expression profile **(B)** and pro-inflammatory cytokine transcript level expression; Tumor necrosis factor alpha (TNFα), Interleukin 6 (Il6), C-C motif chemokine ligand 2 (Ccl2), C-C motif chemokine ligand 3 (Ccl3) **(C)** displayed a significant increase in MF isolated 5 days post-I/R injury at passage 0 compared to healthy CF. Statistical significance was determined with unpaired *t*-test: *P<0.05, ***P<0.001. Data are represented as mean ± SEM. n=3-4.

Supplementary Figure 2



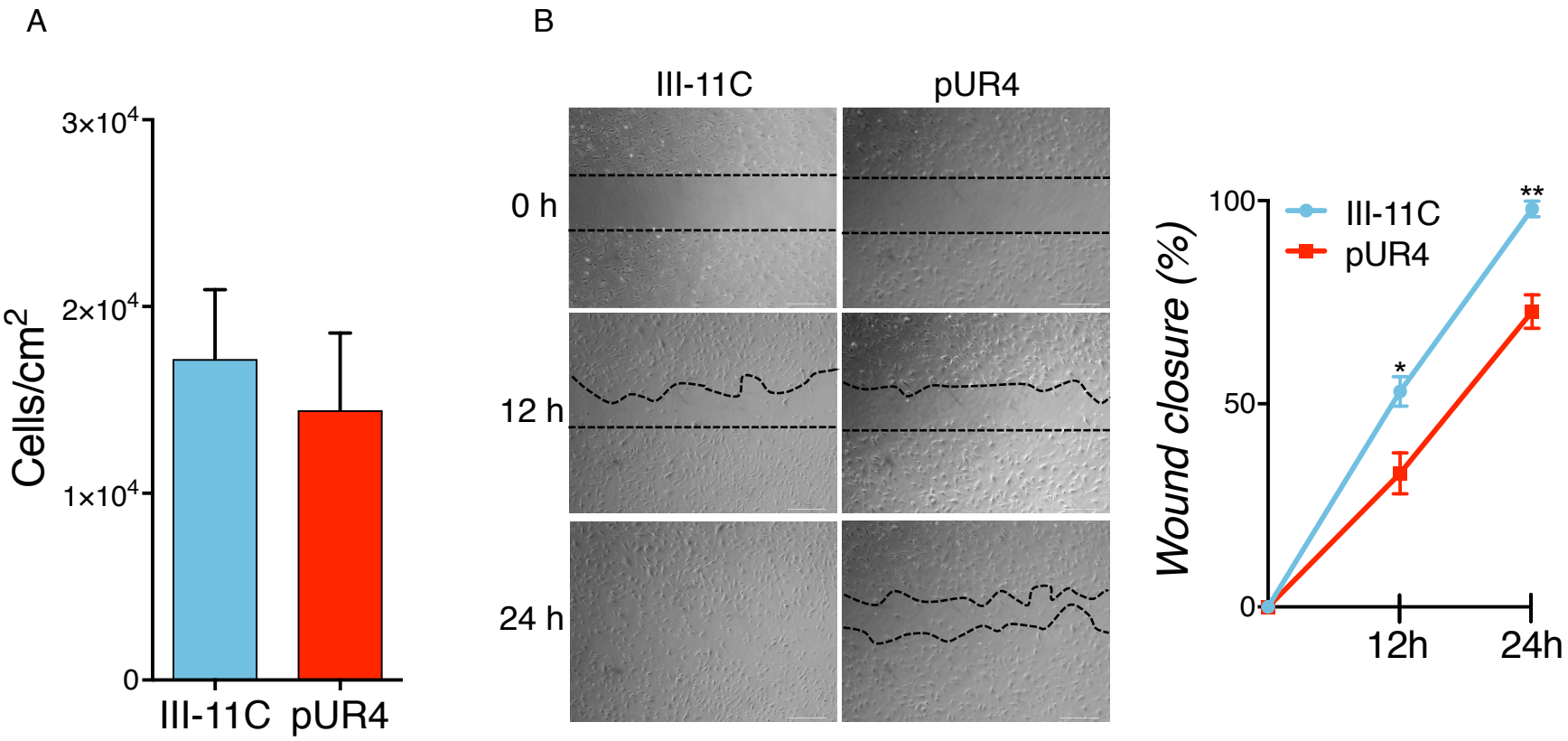
Supplementary Figure 2. **pUR4 reduces FN deposition into the ECM of mouse activated MF 72 h post-treatment. (A)** FN immunofluorescence staining in MF. Scale bars, 100 μm. **(B)** Cellular FN protein level expression (without matrix) (representative immunoblots, left, and densitometry, right). n=6. **(C)** Matrix FN deposition into the ECM of cardiac MF is decreased with pUR4 administration (representative immunoblots, left, and densitometry, right). n=6. Statistical significance was determined with paired *t*-test: *P<0.05, **P<0.01. Data are represented as mean ± SEM.

Supplementary Figure 3



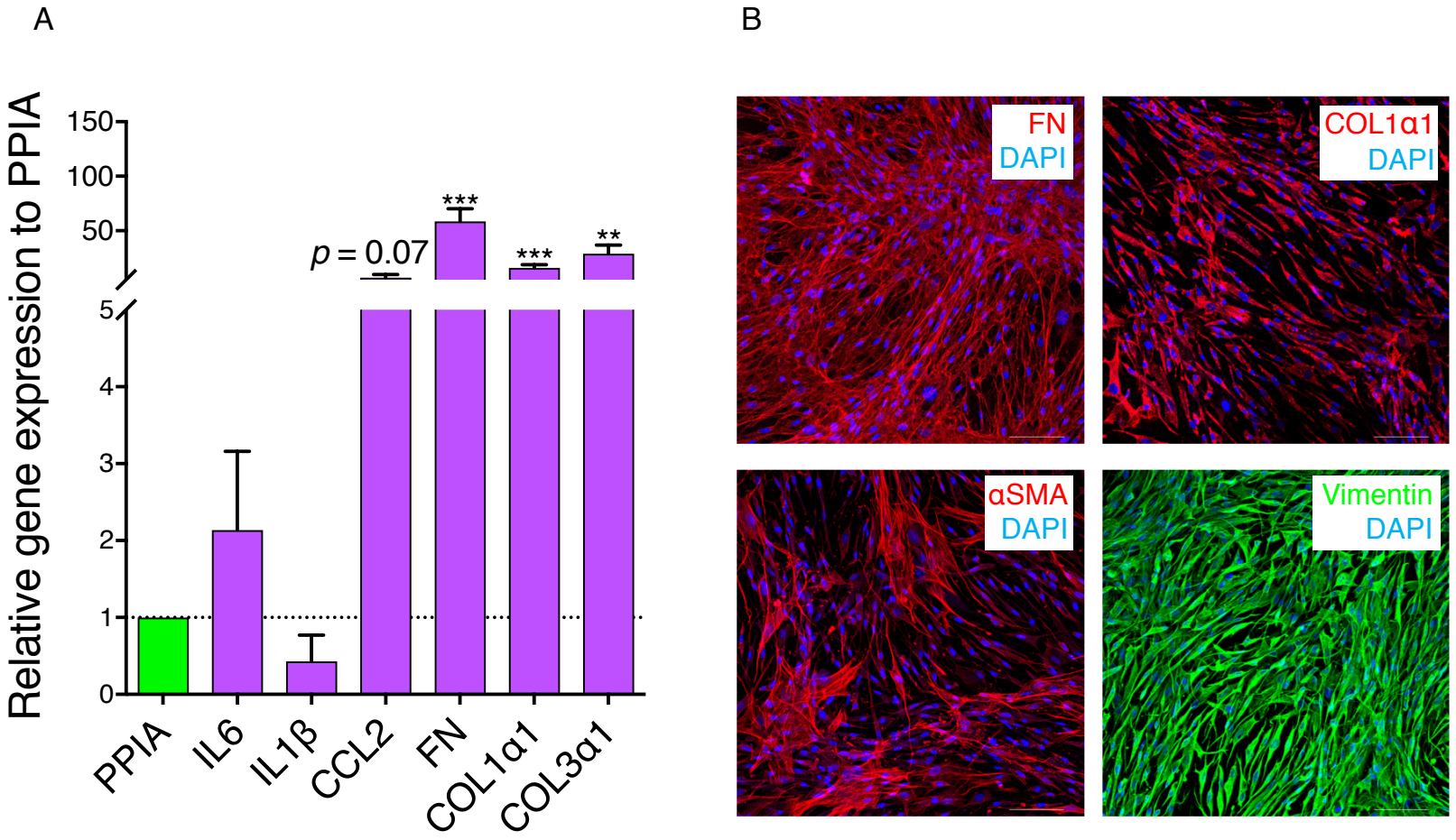
Supplementary Figure 3. **pUR4 induces cell cycle arrest at S phase in myofibroblasts. (A)** Flow cytometry strategy workflow to analyze the different phases of cell cycle. **(B)** Cell cycle distribution of myofibroblasts treated with III-11C or pUR4 for different time points was analyzed by flow cytometry. n=6. **(C)** Representative immunoblots using antibodies against phospho-Akt (Ser^{473}) and total Akt, phospho-ERK (Thr^{202}/Tyr^{204}) and total ERK (left) and quantification (right). Control peptide (III-11C) (c) and pUR4 (p). n=5. Propidium Iodide-Area (PI-A) and Propidium Iodide-Height (PI-H). Statistical significance was determined with paired *t*-test: *P<0.05, **P<0.01, ****P<0.001. Data are represented as mean ± SEM.

Supplementary Figure 4



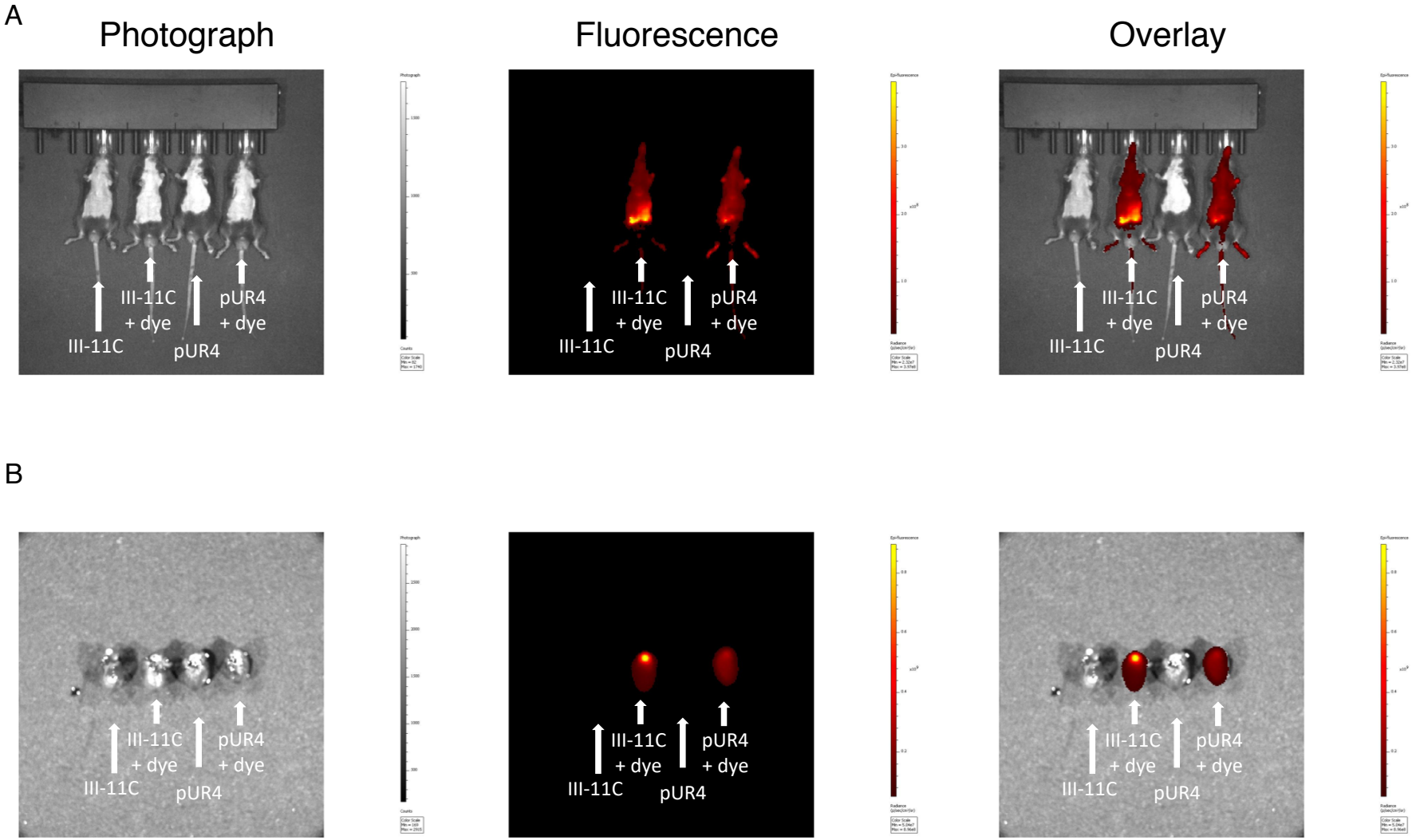
Supplementary Figure 4. **pUR4 decreases migration of healthy fibroblasts. (A)** pUR4 does not decrease cell proliferation monitored by counting cell number with a hemocytometer. n=4. **(B)** Scratch wound-healing assay in mouse CF cell migration; pictures were taken at 0 h, 12 h and 24 h post-scratch. Black dotted lines denote the wound borders (representative photographs, left, and mobility quantification, right). Scale bars, 1000 μm. n=4. Statistical significance was determined with paired *t*-test: *P<0.05, **P<0.01. Data are represented as mean ± SEM.

Supplementary Figure 5



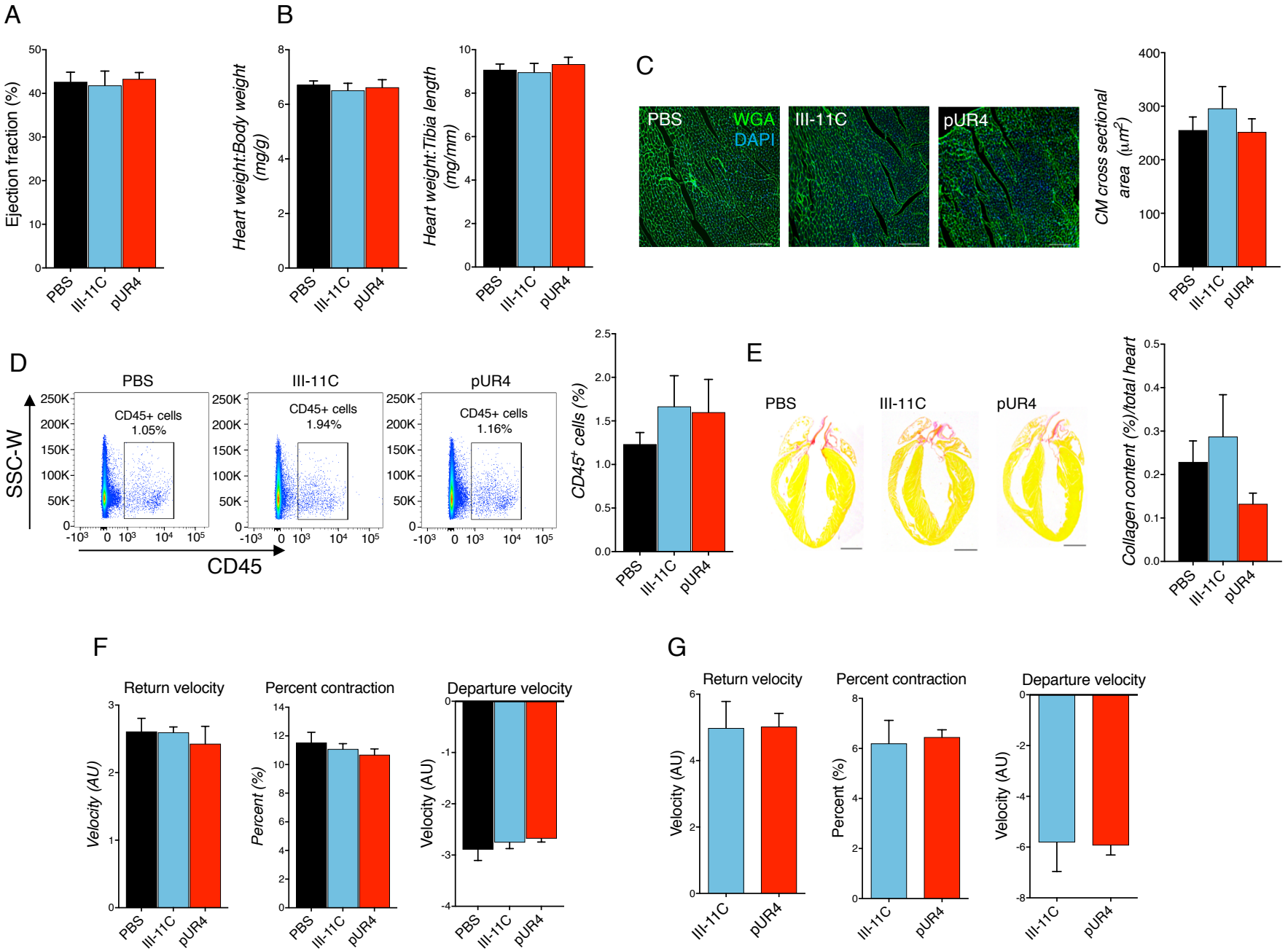
Supplementary Figure 5. **Failing human cardiac fibroblasts characterization. (A)** Pro-inflammatory cytokine and fibrosis-related transcript levels expression. Cyclophilin A (PPIA), interleukin 6 (IL6), Interleukin 1ß (IL1ß), C-C motif chemokine ligand 2 (CCL2), Fibronectin (FN), collagen 1α1 (COL1α1) and collagen 3α1 (COL3α1). n=6. **(B)** Fibronectin (FN), Collagen 1α1 (COL1α1), alpha-smooth muscle actin (α-SMA) and vimentin staining in failing human cardiac fibroblasts. Scale bars, 100 μm. Statistical significance was determined with unpaired *t*-test: **P<0.01, ***P<0.001. Data are represented as mean ± SEM.

Supplementary Figure 6



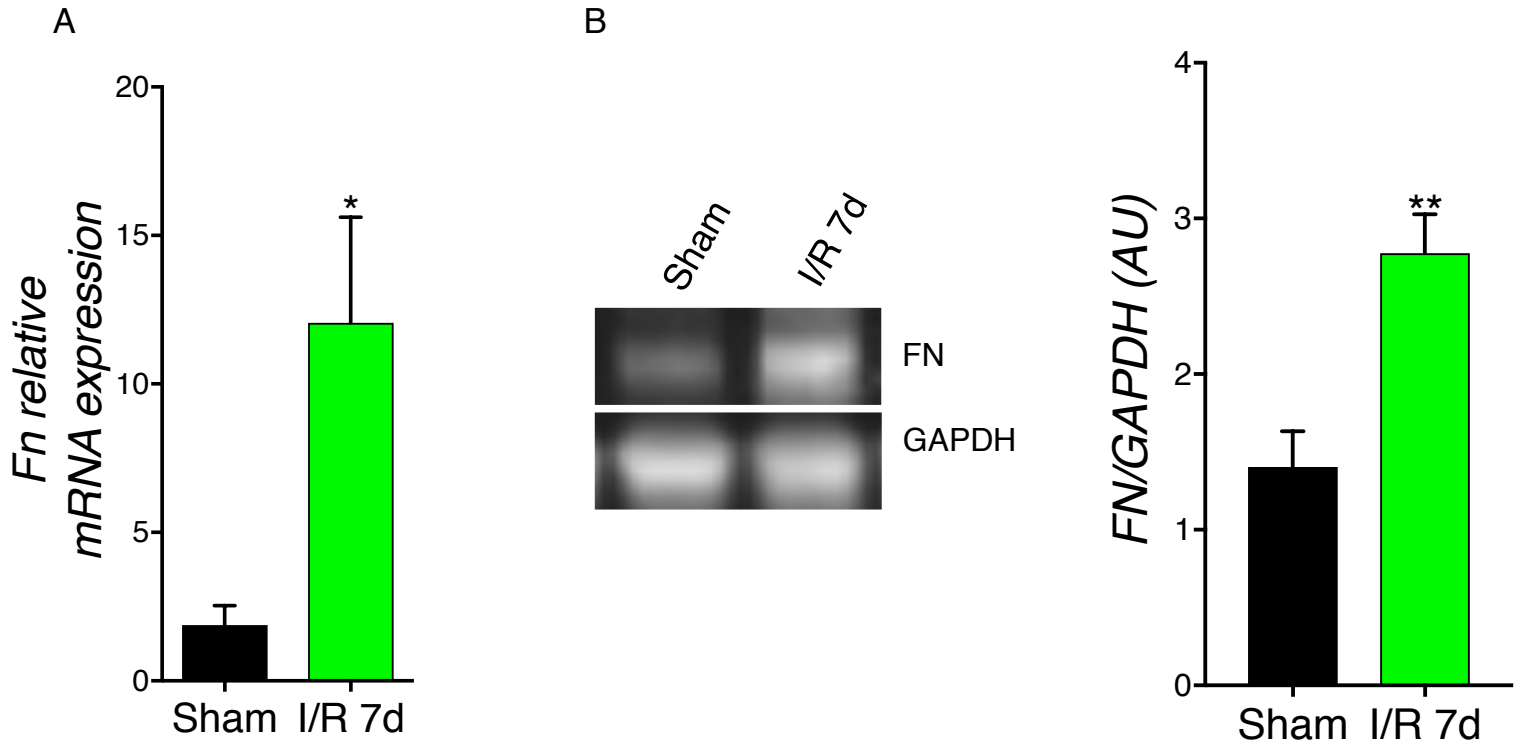
Supplementary Figure 6. **Peptide distribution in mice by noninvasive infrared fluorescence imaging. (A)** *In vivo* fluorescence IVIS imaging of intraperitoneal administration of labeled peptides with an infrared dye (750 nm) at 24 h after the last injection. Unlabeled peptides were injected as negative controls. Representative photograph and fluorescent images are shown. **(B)** Hearts from the same animals were immediately collected after the procedure and imaged with IVIS.

Supplementary Figure 7



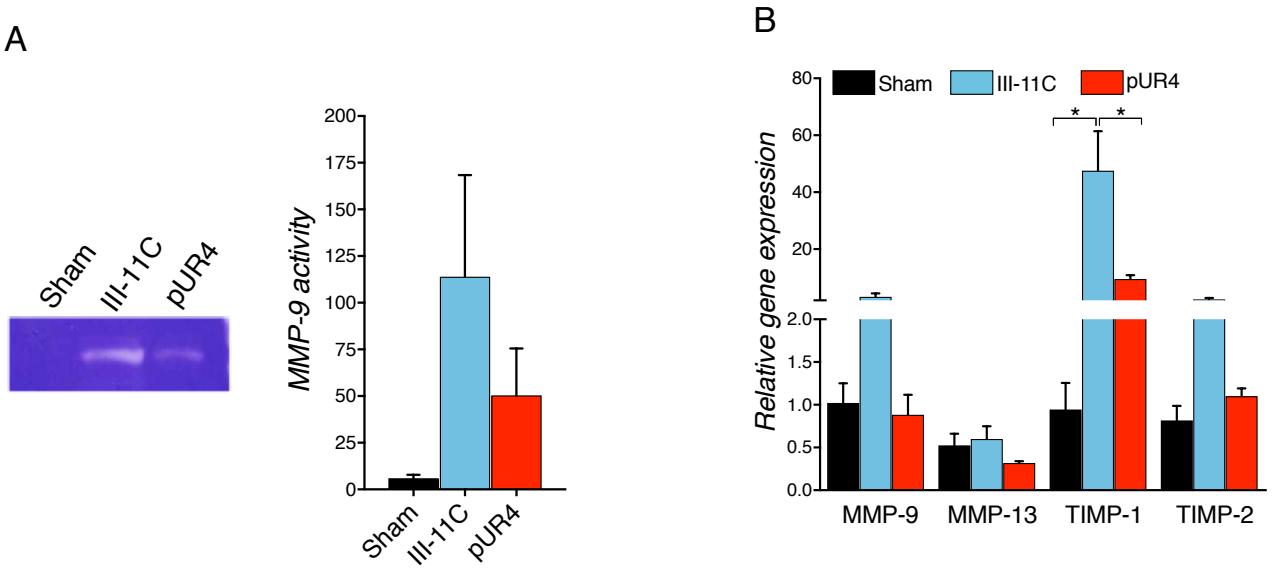
Supplementary Figure 7. **pUR4 administration in mice does not alter cardiac function, cardiomyocyte size, inflammatory cell infiltration or collagen content.** Healthy mice were injected with phosphate buffered saline (PBS), III-11C or pUR4 for 7 days. **(A)** Percent ejection fraction. n=6 per group. **(B)** Cardiac morphometry (heart weight to body weight (left) or heart weight to tibia length (right). n=6 per group. **(C)** Representative cardiomyocyte cross-sectional images of wheat germ agglutinin (WGA) staining (green) and nuclei (blue) in the left ventricle of the designated treatment (left) and quantification (right). Scale bars, 100 μm. n=3-4 mice per group. **(D)** Flow cytometry analysis of $CD45^+$ cells in the non-myocyte compartment reveals similar numbers in all three groups. n=3-4 per group. **(E)** Collagen content was evaluated by picrosirius red staining (representative pictures, left and quantification, right). Scale bars, 1000 μm. n=3-4 mice per group. **(F)** pUR4 does not affect CM contractility isolated form healthy animals (n=10 cells each from 3-4 mice). **(G)** Acute pUR4 treatment (1 h) on CM isolated 5 days after I/R does not affect cell contractility. (n=5-6 cells each from 4 mice). Absence of statistical significance was determined with one-way ANOVA (panels A, B, C, D, F and G) or pair *t*-test (panel E). Data are represented as mean ± SEM.

Supplementary Figure 8



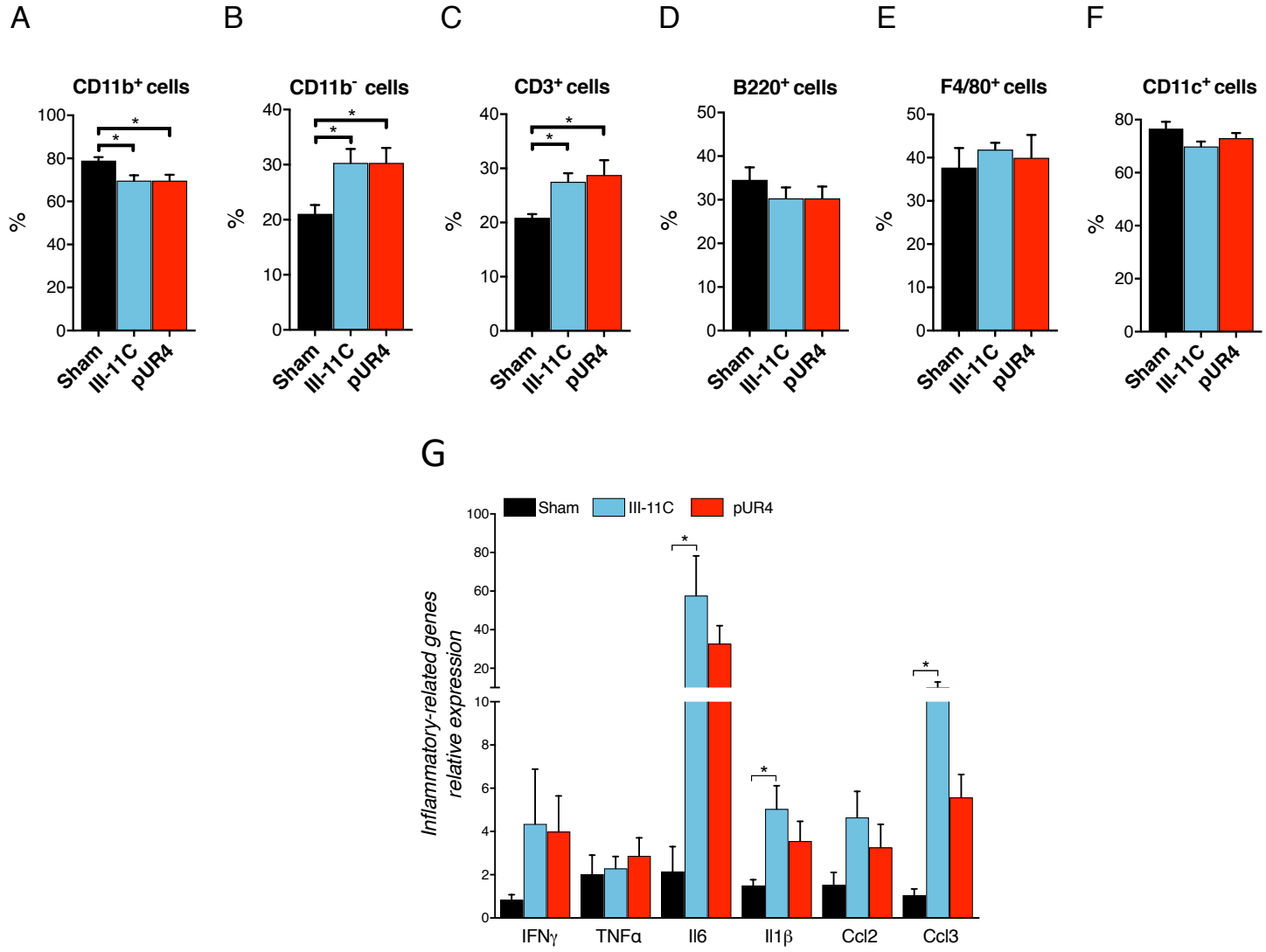
Supplementary Figure 8. **Fibronectin expression after I/R injury. (A)** RT-qPCR for mRNA levels of FN from left ventricles of pUR4 or III-11C-treated animals 7 d after I/R injury. n=6-8. **(B)** Western blotting for FN from left ventricles of pUR4 or III-11C-treated animals 7 d after I/R injury. n=5-6. Statistical significance was determined with unpaired *t*-test: *P<0.05, **P<0.01. Data are represented as mean ± SEM.

Supplementary Figure 9



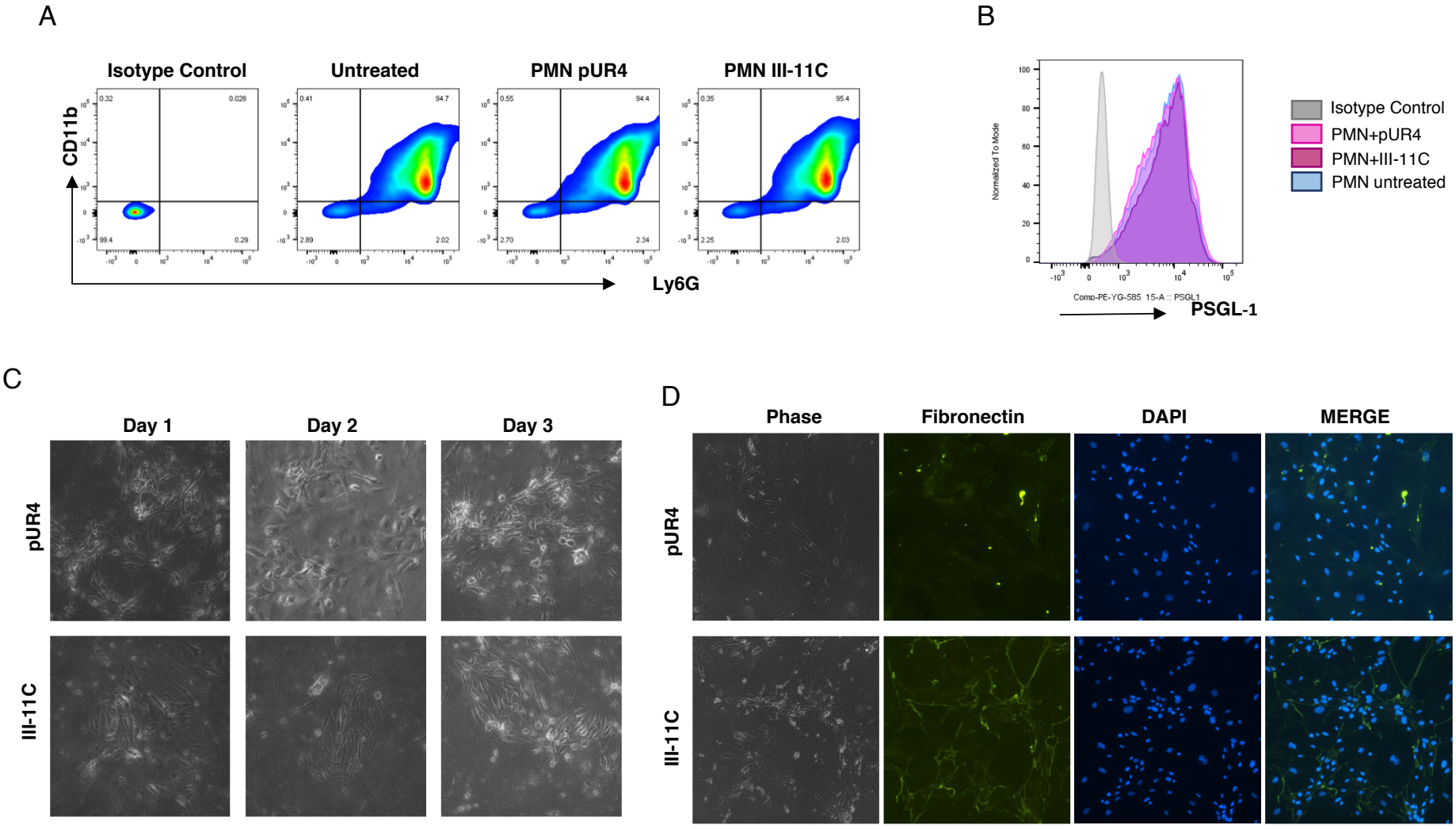
Supplementary Figure 9. **MMPs and TIMPs expression in peptide-treated hearts after cardiac injury. (A)** Matrix metalloproteinase-9 (MMP9) zymography (left) and quantification from the indicated treatment (right). n=6-8. **(B)** RT-qPCR for mRNA levels of the indicated MMPs and TIMPs from left ventricles of the indicated treatment. n=5-6. Statistical significance was determined with one-way ANOVA with Tukey post-hoc analysis: *P<0.05. Data are represented as mean ± SEM.

Supplementary Figure 10



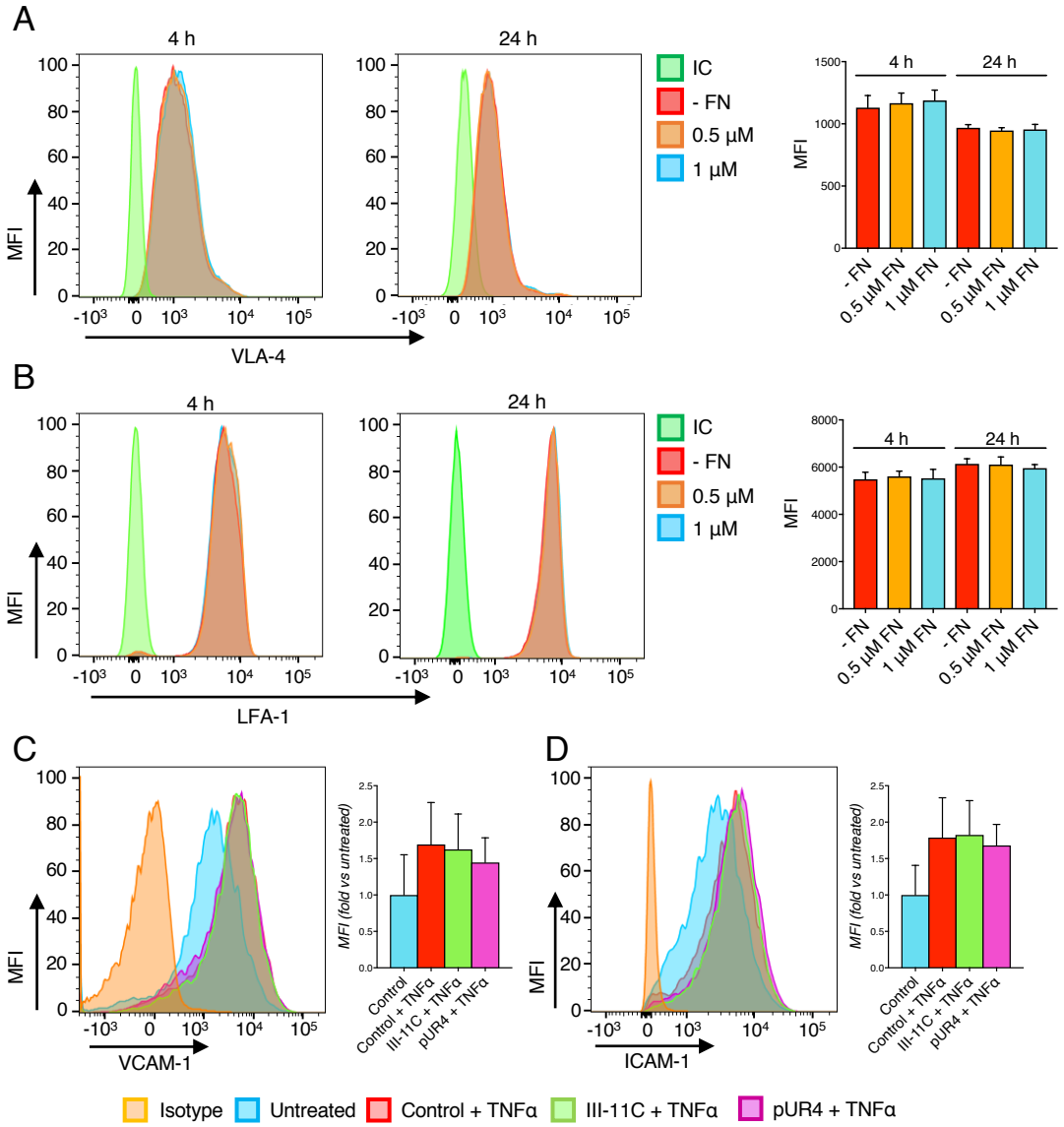
Supplementary Figure 10. **Flow cytometry analysis of the different immune cell compartments in the hearts of pUR4 or III-11C-treated animals 7 days post I/R.** Quantification using flow cytometry of myeloid compartment ($CD11b^+$) **(A)**, non-myeloid fraction ($CD11b^-$) **(B)**, T cells ($CD3^+$) **(C)**, B cells ($B220^+$) **(D)**, macrophages ($Cd11b^+$;$F4/80^+$) **(E)** and dendritic cells ($Cd11b^+$;$CD11c^+$) **(F)**. **(G)** Cytokine mRNA levels in hearts from sham, III-11C to pUR4-treated animals. n=5-6. Interferon gamma (IFγ), Tumor necrosis factor alpha (TNFα), Interleukin 6 (Il6), Interleukin 1ß (IL1ß), C-C motif chemokine ligand 2 (Ccl2) and C-C motif chemokine ligand 3 (Ccl3). Statistical significance was determined with one-way ANOVA with Tukey post-hoc analysis: *$P<0.05$. Data are represented as mean ± SEM. n=5-8.

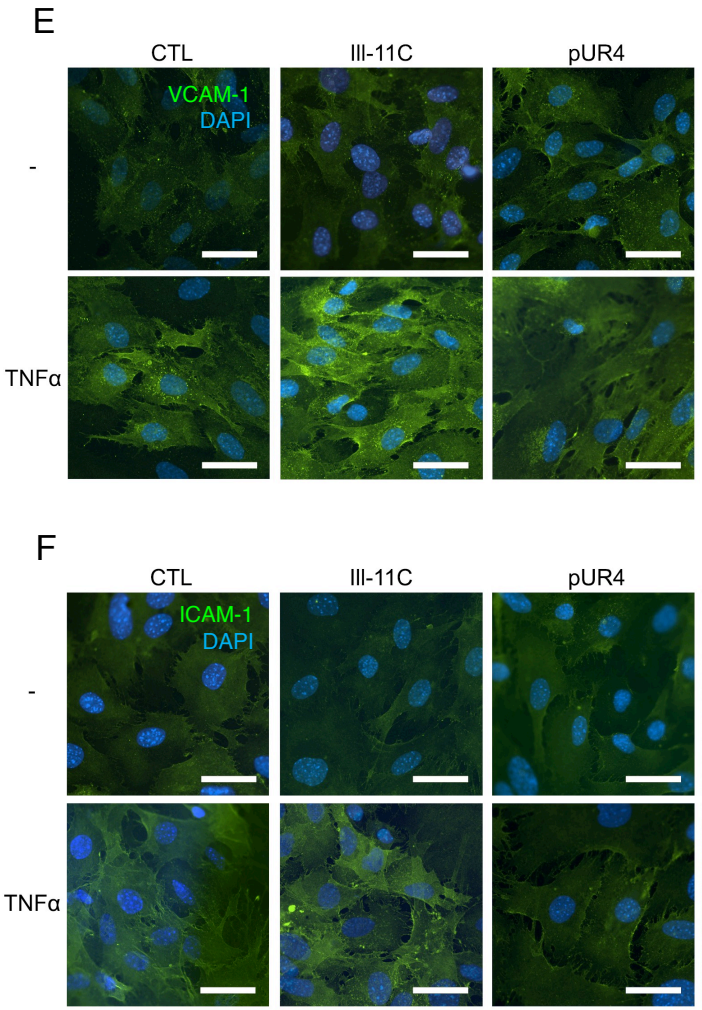
Supplementary Figure 11



Supplementary Figure 11. **pUR4 does not affect neutrophil and MHEC viability and phenotype.** Bone marrow (BM)-derived neutrophils and freshly isolated MHECs were treated with either pUR4 or III-11C. **(A)** Representative FACS plots of the neutrophil surface proteins Ly6G and CD11b after 4 h with the peptides. **(B)** Selectin P Ligand (PSGL-1) on BM neutrophils after 4 h treatment with pUR4 or III-11C. One representative experiment is shown from four different neutrophil preparations. **(C)** Representative 20x pictures showing MHEC monolayers at consecutive days after treatment with pUR4 or III-11C (day 0). **(D)** Representative 20x immunofluorescence pictures showing MHEC monolayers stained for fibronectin after 72 h treatment with pUR4 or III-11C.

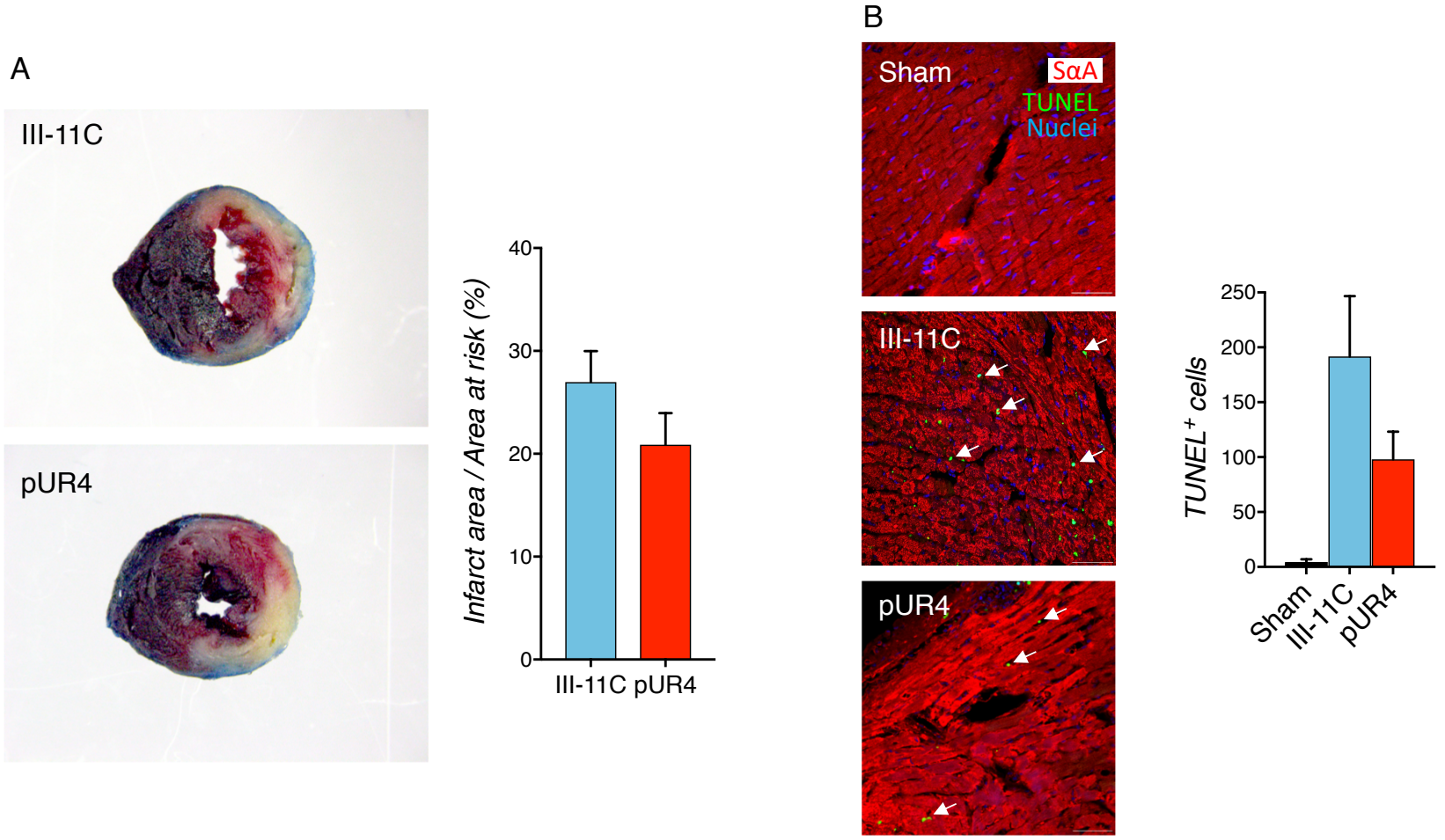
Supplementary Figure 12





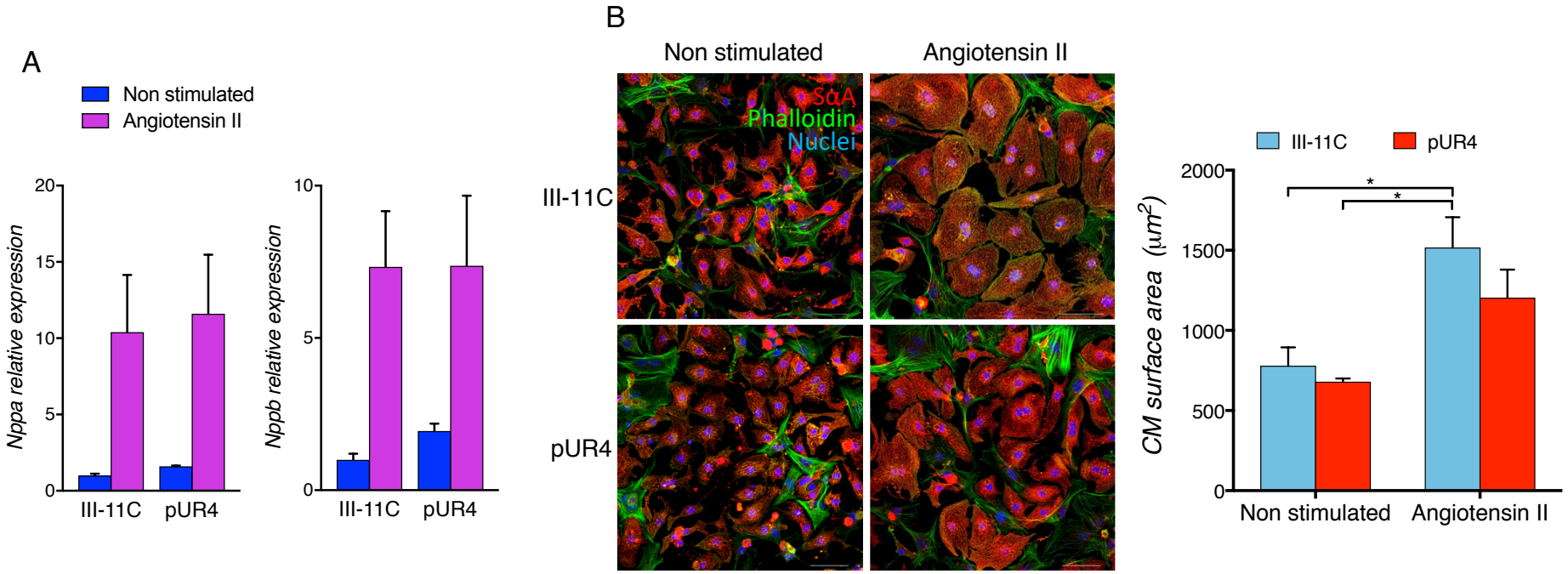
Supplementary Figure 12. **pUR4 does not affect neutrophil integrin expression but appears to reduce VCAM-1 and ICAM-1 in heart endothelial cells. (A and B)** Bone marrow derived neutrophils were isolated as indicated in the methods section, treated with the indicated doses of FN for 4 h and 24 h. FACS analysis for integrins VLA-4 **(A)** and LFA-1 **(B)** was performed after treatment. Representative flow charts (left) and quantification (right). n=3. **(C** and **D)** Heart endothelial cells were treated with media (control), III-11C or pUR4 for 72 h, then stimulated with TNFα for 4h, and harvested for FACS analysis of VCAM-1 **(C)** and ICAM-1. **(D)** Representative flow charts (left) and quantification (right). n=3. (**E** and **F**) Heart endothelial cells were treated with media (control, CTL), III-11C or pUR4 for 72 h, then stimulated with or without TNFα for 4h, fixed, permeabilized and stained for VCAM-1 **(E)** and ICAM-1 **(F)**. Scale bars, 50 μm. Mean fluorescence intensity (MFI). Absence of statistical significance was determined with one-way ANOVA. Data are represented as mean ± SEM.

Supplementary Figure 13



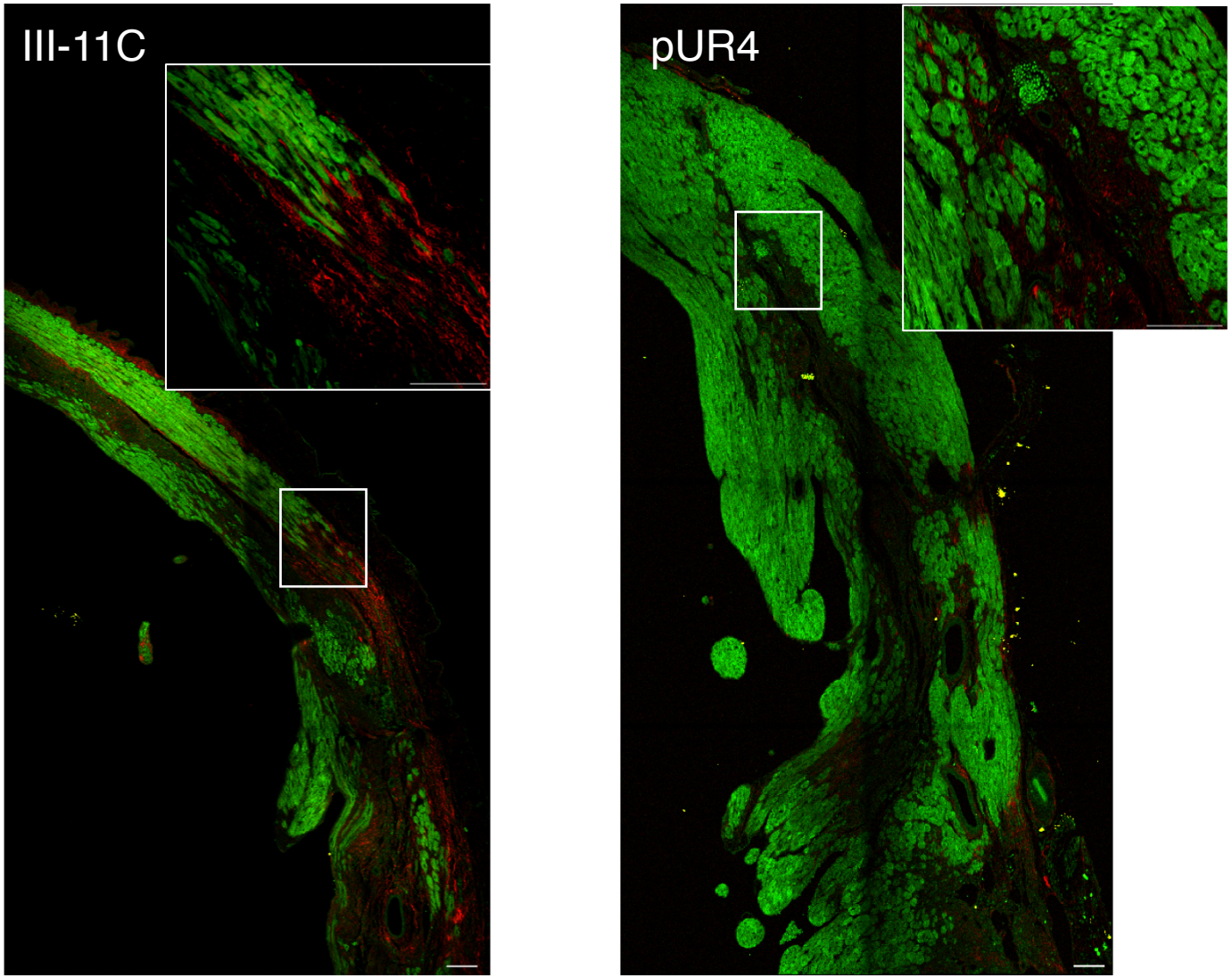
Supplementary Figure 13. **pUR4 does not affect infarct area size.** Animals underwent I/R and received one peptide injection (III-11C or pUR4). Hearts were harvested 24 hr later according to the experimental procedure. **(A)** Representative images of myocardial necrosis (infarct size) determined by Triphenyltetrazolium chloride (TTC) and Evans blue staining (left) and quantification of the area of infarct versus the area at risk (right). n=6. **(B)** Analysis of apoptosis by TUNEL staining in left ventricles of III-11C and pUR4-treated animals 24 h after cardiac injury and peptide administration. Representative images (left) and quantification of apoptotic cells (right). Sarcomeric α-actinin (SαA). Scale bars, 25 μm. n=4. Absence of statistical significance was determined with unpaired *t*-test (panel A) and one-way ANOVA (panel B). Data are represented as mean ± SEM.

Supplementary Figure 14



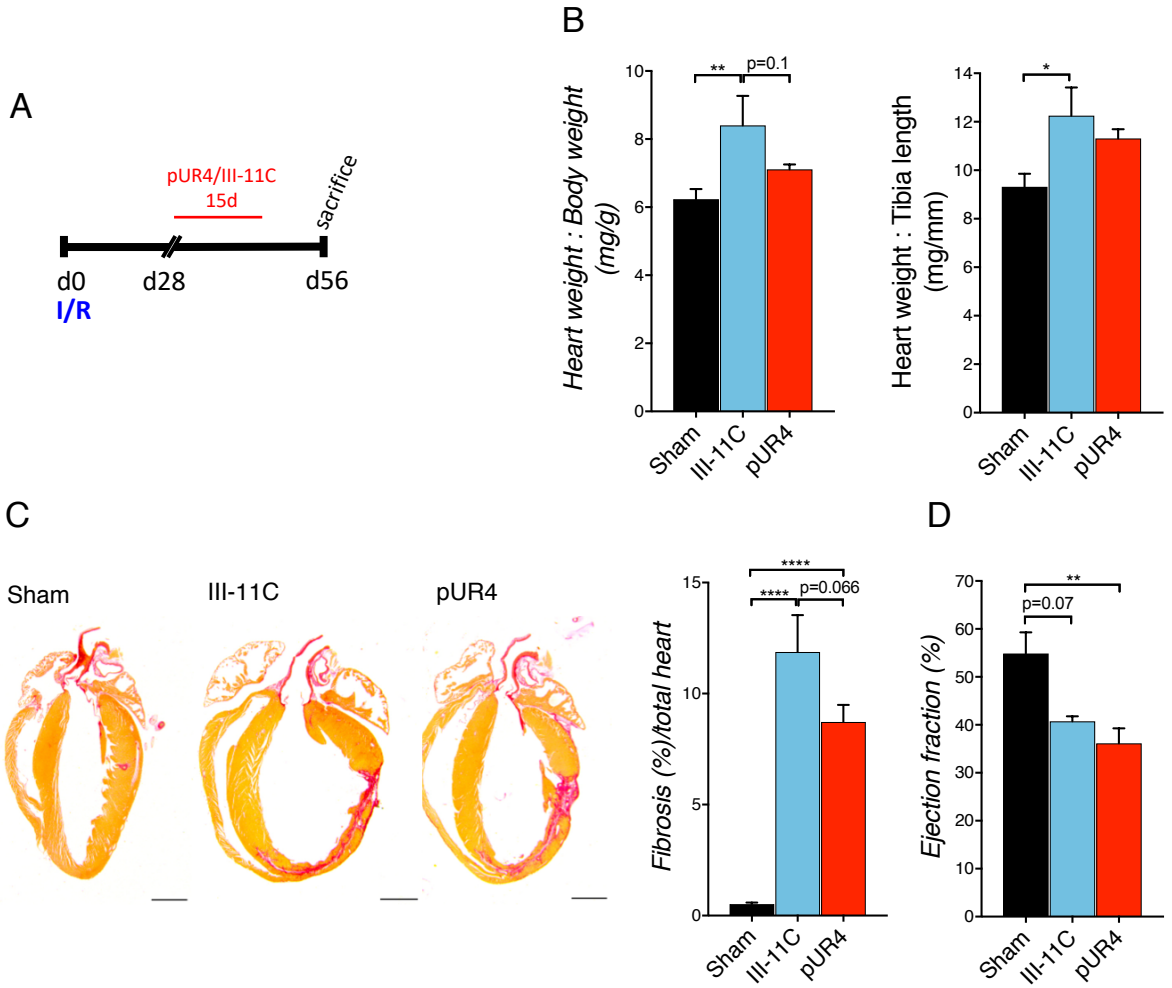
Supplementary Figure 14. **pUR4 does not affect angiotensin II (Ang II)-induced cardiomyocyte hypertrophy *in vitro*. (A)** Natriuretic peptide A (Nppa) and Natriuretic peptide B (Nppb) mRNA levels were measured in neonatal rat ventricular myocytes (NRVMs) 4 h post-Ang II treatment; no effect of pUR4 was observed. n=3. **(B)** Representative images of NRVMs treated with III-11C or pUR4 and treated with PBS or Ang II for 48 h (left); and quantitative results of the cell surface area of NRVMs treated with peptides and with PBS or Ang II for 48 h (right). Sarcomeric α-actinin (SαA, red); phalloidin (green); nuclei (blue). Scale bars, 100 μm. n=3. Statistical significance was determined using two-way ANOVA with Tukey post-hoc analysis: *P<0.05. Data are represented as mean ± SEM.

Supplementary Figure 15



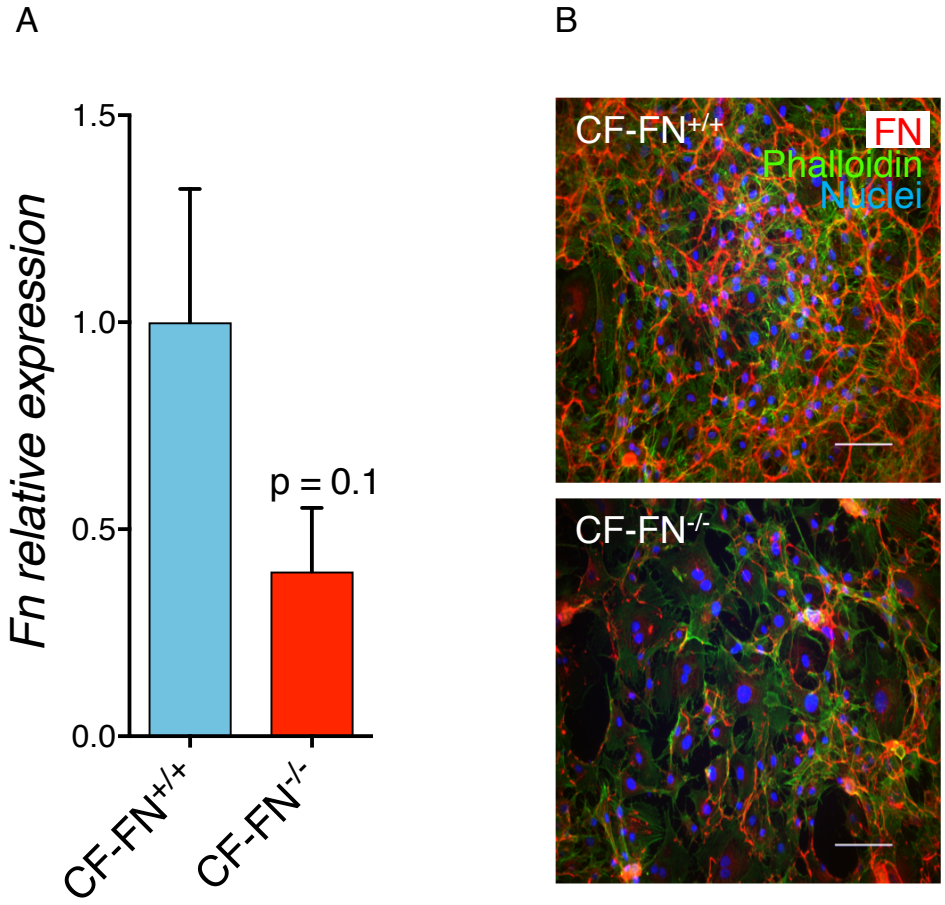
Supplementary Figure 15. **Second-harmonic generation (SHG) images of collagen.** Collagen deposition (red) in left ventricles of III-11C (left) or pUR4-treated animals (right) 4 weeks post-I/R was determined by SHG imaging. Green, autofluorescence and red, collagen. Scale bars, 100 μm.

Supplementary Figure 16



Supplementary Figure 16. **Role of pUR4 in reverting pre-established heart failure.** **(A)** Animals received daily intraperitoneal peptide injection for 15 days 4 weeks after I/R, and were followed for an additional 2 weeks post-treatment cessation. **(B)** Cardiac morphometry is shown as heart weight to body weight ratio (left) and heart weight to tibia length ratio (right). **(C)** Fibrotic scar formation was evaluated by picrosirius red staining (representative pictures, left and quantification, right). **(D)** Cardiac function evaluated by echocardiography shown by percent ejection fraction at 8 weeks post-I/R and 4 weeks post-treatment initiation. Statistical significance determined by one-way ANOVA with Tukey post-hoc analysis: *P<0.05, **P<0.01, ****P<0.0001. Data are represented as mean ± SEM. n=4-8 animals per group.

Supplementary Figure 17



Supplementary Figure 17. **FN expression in Tcf21^{mERCremER};*Fn*$^{flox/flox}$ mice.** Double transgenic mice were fed with regular chow (CF-FN$^{+/+}$) or with tamoxifen diet chow (CF-FN$^{-/-}$) for 15 days and healthy CF were isolated to evaluate FN expression. **(A)** FN transcript level expression from freshly isolated CF from CF-FN$^{+/+}$ and CF-FN$^{-/-}$. n=4-5. **(B)** FN immunofluorescence staining (red) in passage 0 CF. Scale bars, 100 μm. Lack of statistical significance was determined by unpaired *t*-test. Data are represented as mean ± SEM.

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