Supplemental Material

Dectin-1 acts as a non-classical receptor of Angiotensin II to induce inflammation and cardiac remodeling

Short title: Dectin-1 mediates Ang II action in heart

Supplemental (Online) File: Materials and Methods, 5 Tables, and 16 Figures

Detailed Materials and Methods

General Reagents

Ang II peptide (A107852) was purchased from Aladdin Chemicals (Shanghai, China). Recombinant human Dectin-1 (rhDectin-1) was purchased from Sino Biological (10215-HNCH; Beijing, China). Antibodies against Syk (13198), NF-κB p65 (8242T), myeloid antigen Ly-6G (FITC-conjugated, 88876S), and Lamin B1 (13435) were purchased from Cell Signaling Technology (Beverly, MA, USA). Antibodies against Dectin-1 (ab140039, ab217331), phospho (p-) Syk (Y323, ab62338), and granulocyte colony stimulating factor (G-CSF, ab181053) were purchased from Abcam (Shanghai, China). Antibodies against angiotensin receptor AGTR1 (sc-515884) and Vimentin (sc-6260) were obtained from Santa Cruz Biotech (Texas, USA). Antibodies against cardiac myosin heavy chain-7 (MYH7, 22280-1-AP), CXCL1 (12335-1-AP), CXCR2 (20634-1-AP), collagen type I (COL1a1, 67288-1-Ig), HA-tag (51064-2-AP), AGTR1 (25343-1-AP), transforming growth factor-β1 (TGF β1, 21898-1-AP), Flag-tag (20543-1-AP), macrophage antigen F4/80 (28463-1-AP) and GAPDH (60004-1-Ig) were purchased from Proteintech (Shanghai, China). Macrophage CD68 antibody (MA5-13324) and Alexa Fluor 594-conjugated wheat-germ agglutinin (WGA, W11262) were purchased from Thermo Fisher (Waltham, MA, USA). Neutralizing monoclonal antibody against mouse Dectin-1 (R1-8g7) was obtained from InvivoGen (San Diego, CA, USA), and rat IgG2a Isotype (65209-1-Ig) was purchased from Proteintech (Shanghai, China). Rhodamine phalloidin (CA1610), Biotin (D8150) and Streptavidin/Alexa Fluor 488 (K0068R-AF488) were purchased from Solarbio (Beijing China). AT2R antagonist (PD123319) was purchased from selleck (Shanghai, China). Secondary anti-rabbit IgG HRP (7074), anti-mouse IgG HRP (7076) and anti-rat IgG HRP (7077) were purchased from Cell Signaling Technology. Goat anti-mouse IgG alexa fluor 488 (A-11001) and donkey anti-rabbit IgG alexa fluor 568 (A10042) were purchased from Thermo Fisher. Dectin-1 agonist (Curdlan AL; tlrl-curd) was purchased from InvivoGen. Biotinylated-Ang II (Bio-Ang II) was obtained from GL Biochem (Shanghai, China).

In vivo randomization and blinding procedures

Sample sizes were defined by a priori power calculation with G-Power 3.1.9 software (http://www.gpower.hhu.de/), with a statistical power of 80% and α=0.05. We used a random number table to perform randomization, as described previously[41]. Briefly, all animal experiments were performed and analyzed in a blinded manner. Each mouse was assigned a temporary random number within the weight range. When mice were randomly divided in each group, they were given their permanent numerical designation in the cages. For each group, a cage was selected randomly from the pool of all cages. All data were collected and analyzed by two observers who were not aware of the group assignment or treatment of the mice.

For all *in vivo* studies, we used male mice only. We do acknowledge that there may be sex differences in cardiac fibrosis and heart failure[42]. As the previous studies have shown that estrogen, as well as low testosterone levels, may be protective against cardiac fibrosis, we performed all mechanistic studies in male mice that generates robust cardiac fibrosis and tissue failure upon Ang II challenge.

Inclusion and exclusion criteria

Criteria for inclusions and exclusion were set before the study. Briefly, mice in the Ang II infusion group were included in the study if they underwent successful subcutaneous implantation of the Ang II mini-pump and had a significant increase in systolic blood pressure. Mice were excluded if systolic blood pressure did not rise after Ang II mini-pump implantation, or if the mini-pump was dislodged, or if the animal died prematurely, making it impossible to collect behavioral and histological data.

Mouse models of cardiac remodeling

All animal care and experimental procedures were approved by the Zhejiang Chinese Medical University Laboratory Animal Research Center and Welfare Committee (20201116-06). All animals received humane care according to the National Institutes of Health (USA) guidelines and ARRIVE guidelines[43]. The male *Clec7a*-KO mice (Dectin-1$^{-/-}$ mice, D1KO mice, Strain NO.T011442) on a C57BL/6 background and male C57BL/6 mice weight 18-22 g were obtained from GemPharmatech (Nanjing, China). The Dectin-1 knockout allele has the sequences corresponding to the cytoplasmic tail, transmembrane and stalk regions of the Dectin-1 locus (*Clec7a*) deleted. Mice were housed in a pathogen-free room under 22 ± 2 °C, 50–60% humidity, 12:12 h light-dark cycle, and fed a standard rodent diet in Zhejiang Chinese Medical University Laboratory Animal Research Center. Mice were acclimatized to the laboratory for at least 2 weeks before initiating the studies. We used two mouse models of cardiac remodeling and dysfunction: Ang II infusion and transverse aortic constriction (TAC).

For the Ang II infusion model, eight-week-old C57BL/6 mice (WT mice) and Dectin-1$^{-/-}$ mice (D1KO mice) were randomly divided into four groups: 1) WT mice controls (WT-Ctrl, n = 7); 2) Ang II-infused WT mice (WT-Ang II; n = 7); 3) D1KO mice controls (D1KO-Ctrl, n = 7); and 4) Ang II-infused D1KO mice (D1KO-Ang II; n = 7). Ang II was administered using osmotic mini pump that delivered 1000 ng/kg/min (Alzet MODEL 1004, CA, USA) for 4 weeks, as described previously[8]. At the end of treatment, mice were sacrificed under sodium pentobarbital for anesthesia and pain medication. Ang II levels were measured in heart tissues and blood samples by ELISA (cat: E-EL-H0326c, from Elabscience) at the conclusion of the study.

For the bone marrow transplantation model, we used bone marrow transplantation to produce chimeric mice to study the contribution of bone marrow-derived Dectin-1 using the method as previously described[16]. 14 WT mice fed with acidified water containing neomycin (1.1 mg/L) and polymyxin B sulphate (1000 U/L) for one week were subjected to total body irradiation (9 Gy) by cobalt one day before transplantation to obtain the recipient WT mice. The bone marrow cells were isolated from C57BL/6J (WT) mice and Dectin-1 KO mice by flushing femurs and tibiae with RPMI-1640 medium. Recipient WT mice received 1×10^6 bone marrow cells from WT or Dectin-1 KO mice, respectively. The chimeric mice were given acidified antibiotic water and sterilized food. After 4 weeks, the chimeric mice were sacrificed under sodium pentobarbital anesthesia and then confirmed by genotyping *Clea7a* gene in bone marrow and tail (*Clea7a*-WT Primer GCC AAT GCT GCC GAC TCC AG*; Clea7a*-KO Primer: GCC AAT GCT GCC GAC TCC AG;). Then the chimeric mice were then used to do experiments and then divided into two groups: (i) Donor WT mice → recipient WT mice (WT→WT, n=7); (ii) Donor D1KO mice→ recipient WT mice (D1KO→WT; n= 7). The chimeric mice were given acidified antibiotic water and sterilized food for 4 weeks and then proceeded to Ang II infusion. Mice in

both groups were infused with Ang II using osmotic mini pump that delivered 1000 ng/kg/min (Alzet MODEL 1004, CA, USA) for 4 weeks. At the end of treatment, mice were sacrificed under sodium pentobarbital for anesthesia and pain medication.

For the ARB intervention in Ang II infusion model, eight-week-old C57BL/6 mice (WT mice) were randomly divided into three groups: 1) WT mice controls (WT-Ctrl, n = 7); 2) Ang II-infused WT mice (WT-Ang II; n = 7); 3) Ang II-infused WT mice treated with Losartan potassium as previous reported (20mg/kg/d, oral)[44] (WT-Ang II-LOS; n = 7). Ang II was administered using osmotic mini pump that delivered 1000 ng/kg/min (Alzet MODEL 1004, CA, USA) for 4 weeks, as described previously[8]. Losartan potassium was administered 1 week after the Ang II infusion and followed-up after 3 weeks of treatment. At the end of treatment, mice were sacrificed under sodium pentobarbital for anesthesia and pain medication.

At the indicated time points, systolic blood pressure was measured by non-invasive tail-cuff Pressure Analysis System while mice were conscious (BP-98A; Softron, Tokyo, Japan). Measurements were performed during 1:00 pm to 5:00 pm with 5 days of previous training. When animals were sacrificed using sodium pentobarbital, hearts were harvested and weighed to compare heart weight/body weight (HW/BW, mg/kg) ratios, and tibia lengths were measured to determine heart weight:tibia length ratios. Heart tissues were snap-frozen in liquid nitrogen for gene and protein expression analyses or fixed with 4% paraformaldehyde for histological analysis.

Cardiac functional tests

Systolic and diastolic cardiac function was determined non-invasively by transthoracic echocardiography in anesthetized mice, one day before the sacrifice as described previously[45]. Mice was anesthetized using isoflurane and echocardiography was performed by SONOS 5500 ultrasound (Philips Electronics, Amsterdam, Netherland) with a 15-MHz linear array ultrasound transducer.

Heart tissue staining

Hearts were fixed in 4% paraformaldehyde and embedded in paraffin. Five μm thick sections were stained with hematoxylin and eosin (H&E) (Solarbio Life Sciences, Beijing, China), Picro Sirius Red, and Masson's Trichrome (Solarbio Life Sciences, Beijing, China) for routine histology and assessment of cardiac fibrosis. To quantify the level of fibrosis, 10 non-overlapping fields in each tissue (n = 7) were scored on a semiquantitative scale (<5%, 5–10%, 10–25%, 25–50%, 50–75%, and 75–100%), relative to total tissue area in the field. Sections were observed under a light microscope (Nikon, Japan).

For determination of cardiac myocyte cross-sectional areas, frozen heart sections were used. Briefly, portions of heart tissues were embedded in Optimal Cutting Temperature (OCT) media and sectioned at 5-μm thickness. Sections were stained with Alexa Fluor 594-conjugated wheat-germ agglutinin (1.0 mg/mL) and counterstained with DAPI. Images were captured using Nikon microscope (Nikon, Japan). Size of cells was measured.

For immunohistochemical staining, paraffin sections were deparaffinized and rehydration. Sections were treated with 3% H2O2 for 30 min to block endogenous peroxidase activity and

then blocked with 1% BSA in PBS for 30 min. Slides were incubated overnight at 4°C with primary antibodies against TNF-α (1:50, 60291-1-Ig), F4/80 (1:50, 28463-1-AP). Peroxidase-conjugated secondary antibodies were used for detection (1:500). Slides were counterstained hematoxylin for 5 min, dehydrated, and mounted. Images were viewed by a bright field microscope (Nikon).

For immunofluorescence staining, 5-μm thick OCT-embedded sections were fixed with 4% paraformaldehyde for 10 min and then blocked with 5% BSA in PBS for 30 min. Primary antibodies added, included anti-Dectin-1 (ab140039), anti-CD68 (MA5-13324), anti-Ly-6G/FITC (88876S), anti-G-CSF (ab181053), anti-Vimentin (sc-6260), anti-CXCL1 (12335-1-AP), and anti-CXCR2 (20634-1-AP). Antibody incubations were carried out overnight at 4°C. Fluorophore-conjugated secondary antibodies were added for 1 h at room temperature for detection. Sections were counterstained with DAPI and cover-slipped. Fluorescence images were captured with an Olympus microscope. Images were quantified in a blind manner using ImageJ (version 1.38×, National Institutes of Health, Bethseda, MD, USA). For each heart sample, at least five random fields were measured.

In all immune staining experiments, the normal rabbit IgG or normal mouse IgG staining followed by secondary antibody staining was utilized to validate antibody specificity and distinguish genuine target staining from the background.

Transcriptome sequencing

The heart tissues of mice in WT-Ang II group and D1KO-Ang II group were harvested. Total mRNA was isolated using TRIZOL (Takara, Kyoto, Japan), and the transcriptome sequencing was conducted by BGI (WuHan, China). The differentially expressed genes (DEGs) were defined by fold change > 1.5 or < 0.67, and $P < 0.05$. Further analysis, including PCA analysis, drawing of Volcano plot and Venn diagram were conducted in Dr. Tom in BGI (WuHan, China). Enrichment of intersecting genes were then conducted in the database of "MSigDB Hallmark 2020" in Enrichr software (https://maayanlab.cloud/Enrichr/)[46] using the raw values of RNA-seq data. The combined score was calculated as follows: $c = log(p) \cdot z$, where c is the combined score, p is the p-value computed using the Fisher exact test, and z is the z-score computed by assessing the deviation from the expected rank. Raw data of transcriptome sequencing were deposited in the Sequence Read Archive (SRA) in BioProject ID: PRJNA899459.

Transcriptome analyses

Levels of Dectin-1 in heart tissues of mice challenged with Ang II were determined from publicly available transcriptome data. GSE114116 was selected. The whole transcriptomic analysis of heart from Veh_H group (mice were treated with saline) and A_H group (mice were subcutaneously implanted Ang II pump (1000ng/kg/min)) were performed using RNA-seq in this database. Comparing with the heart of normal mice, a total of 1468 genes (987 upregulated and 481 down regulated) were differentially expressed (FDR<0.05) in heart of Ang II-induced mice. Dectin-1 levels were determined using data analysis tools in the expression profiling by array data or gene ID convert tools in expression profiling by high throughput sequencing data.

Human subject study

In this study, 1 patient (female, 68 years old, Han nationality) with heart failure who presented to Sir Run Run Shaw Hospital, Zhejiang University School of Medicine for heart replacement surgery was enrolled. A diagnosis was made if he fulfilled the following criteria of the 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure[1]: 1) A typical history with risk factors, symptoms and/or signs without the myocardial infarction history; 2) A typical Abnormal ECG; 3) Serum NT-proBNP ≥ 125 pg/mL or BNP ≥ 35 pg/mL; 4) Echocardiography findings of cardiac diastolic function failure. This study and all protocols used were approved by the Ethics Committee of Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University (Ethics number: 20211103-33). For immunofluorescence analyses, we collected LV heart tissue samples primarily from this patient.

Cell culture studies

Human embryonic kidney line HEK-293T was obtained from the Shanghai Cell Bank of Chinese Academy of Sciences (Shanghai, China). HEK-293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco/BRL life Technologies, Eggenstein, Germany) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/mL penicillin, and 100 U/mL streptomycin.

For bone marrow-derived macrophages (BMDMs) isolation, we harvested femurs of wildtype C57/B6 mice or Dectin-1 KO mice. Bones were notched to isolate cells. Cells were then passed through a 70-micron strainer to remove debris. Cells were differentiated into macrophages by culturing in RPMI-1640 media supplemented with 20% L929 conditioned media, 10% FBS and 1% penicillin-streptomycin for 7-10 days, as described previously[47]. BMDMs were plated at 2 x 10^5 density in 6-well culture plates. All cells were incubated in the serum-free medium for 12 h before the stimulation.

The isolation and culture of neonatal rat primary cardiomyocytes and fibroblasts was performed as described previously[48]. Briefly, heart tissues of neonatal Wistar rats were dissociated with trypsin and cells were plated for 1 hour into tissue culture dishes in DMEM with 10% FBS to reduce the number of nonmyocytes. Cells that did not attach to the plates were aspirated and plated into 6-well culture plates. Primary cardiomyocytes were cultured in the same growth medium in DMEM (Gibco/BRL life Technologies, Eggenstein, Germany) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/mL penicillin, and 100 U/mL streptomycin.

Following various treatments, levels of cytokines and other proteins in the culture medium were measured. Briefly, levels of interleukin-1β (IL-1β, MultiSciences, 70-EK201B/3), tumor necrosis factor (TNF-a, MultiSciences, 70-EK282/4), G-CSF (MultiSciences, 70-EK269/2), CXCL1 (MultiSciences, 70-EK296/2), and Ang II (Elabscience, E-EL-M2612c and Elabscience, E-EL-H0326c) were measured using ELISA.

For mechanistic studies, we tested macrophage-derived condition media on primary cardiomyocytes and fibroblasts. For these studies, BMDMs from WT mice or D1KO mice were challenged with 1 μM Ang II for24 h. Condition media at 24 hours was collected and applied to cardiomyocytes and fibroblasts for varying time periods in a 1:1 ratio with normal media.

Gene knockdown and overexpression

Gene silencing was achieved by transfecting cells with siRNA (Gene Pharma). Custom siRNAs were synthesized for mouse AT1R (5'-GCGUCAUCCAUGACUGUAATT-3' and 5'-UUACAGUCAUGGAUGACGCTT -3'). Control cells were transfected with negative control siRNA. Silencing of AT1R in BMDMs was achieved by electroporation (Gene Pulser Xcell, BIO-RAD, USA) with specific siRNAs. Briefly, 5 x 10^6 BMDMs were resuspended in 750 µl of Opti-MEM and were electroporated (250V, 950µF, ∞Ω) with 120 pmol of total AT1R siRNA or NC sequences.

Dectin-1 expression in HEK-293T was achieved by transfecting cells with HA- and Flag-tag Dectin-1 plasmids (Dectin-1 cDNA ORF Clone, Human, N-HA tag, HG10215-NY; Dectin-1 cDNA ORF Clone, Human, N-DYKDDDDK (Flag) tag, HG10215-NF; Sino Biological lnc). Transfection of HEK-293T cells was carried out using Lipofectamine 3000 Transfection Reagent (Invitrogen, L3000008).

To identify key residues in Dectin-1 function, we transfected HEK-293T cells with wildtype Dectin-1 or mutant forms in which N176 (ASN-176-ALA, HA-D1^{N176A}), R184 (ARG-184-ALA, HA-D1^{R184A}), or S236 (SER-236-ALA, HA-D1^{S236A}) in the C-type lectin domain of Dectin-1 was changed to Ala. The wildtype and three mutant Dectin-1 plasmids were prepared by GeneChem lnc. Lysates from these cells were used in biotinylated-protein interaction pull-down assays.

WT and mutant Dectin-1 overexpression in D1KO BMDMs were achieved by transfecting cells with HA-tagged wildtype Dectin-1 (HA-D1^{wt}), mutant HA-tagged Dectin-1 with ASN-176-ALA (HA-D1^{N176A}), mutant HA-tagged Dectin-1 with ARG-184-ALA (HA-D1^{R184A}), or mutant HA-tagged Dectin-1 with SER-236-ALA) (HA-D1^{S236A}). Transfection of primary macrophage was carried out using jetOPTIMUS® according to the instruction (Polyplus, Ref#101000006).

Ang II-Dectin-1 binding assays

To assess binding of Ang II to rhDectin-1, we utilized an ELISA and protein-interaction pull-down assay. For the ELISA, we coated rhDectin-1 antibody on a 96-well plate overnight at 4 °C. The plates were washed and blocked with 3% bovine serum albumin for 1.5 h at room temperature. rhDectin-1 protein was added to the pre-coated wells at 6 µg/mL and incubated for 2 h at room temperature. After washing the plates, biotinylated Ang II was added at 0.5, 1, 50, or 100 µM, and the plates were incubated for 2h at room temperature. Unlabeled Ang II was added at 200 or 500 µM to determine whether it reduces labeled-Ang II binding. Streptavidin-conjugated horseradish peroxidase (Streptavidin-HRP) and TMB substrate were used for detection. Absorbance at 450 nm was measured by SpectraMax M5 microplate reader (Molecular Devices).

For the pull-down assays, we used Pierce Biotinylated Protein Interaction Pull-Down (Thermo Fisher, 21115). One hundred µL of 20 mM biotinylated-Ang II was added to 30 µL streptavidin-agarose beads and incubated at 4°C for 30 minutes. Biotin alone was used as a control. Lysates prepared from BMDMs and mouse heart tissues were then added to the streptavidin-agarose beads with biotinylated-Ang II. For some studies, lysates prepared from HEK-293T cells transfected with wildtype Dectin-1 or mutant Dectin-1 constructs were added. The mixture was

incubated at 4°C for 24 hours with gentle rocking. Samples were then spun and washed 3 times. Elution Buffer was added onto each spin column. Eluent was boiled with 5x loading buffer, and the samples were loaded on a 10% polyacrylamide gel for Western Blot analysis. Total lysates were used as an input control.

Computational docking and molecular simulation

The crystal structure of Dectin-1-DNA complex (SMR: Q9BXN2) was derived from SWISS-MODEL. Input files of ligand and receptor for docking were prepared using Graphical User Interface program AutoDock Tools 1.5.6 (The Scripps Research Institute, CA, USA)[49]. Molecular docking was performed by AutoDock Vina 1.0.2. Ang II was docked into the C-type lectin domain of Dectin-1 to generate 100 binding poses. The binding free energy between each docking pose and Dectin-1 was scored by the MM/GBSA method in AmberTools package after a structure minimization[50]. Finally, based on the per-residue decomposition energy calculations, the key residues for protein-ligand interaction were identified.

Immunofluorescence cell staining

For NF-κB (p65) translocation studies, cells were fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.1% Triton X-100 for 10min and then blocked with 5% BSA in PBS for 30 min. Next, fixed cells were incubated with anti-NF-κB (p65) antibody (1:200) overnight at 4°C. TRITC-conjugated secondary antibody (1:1000) was used for detection. Immunofluorescence was viewed and captured using Olympus fluorescence microscope. IgG staining was used as a primary antibody control.

BMDMs were stained for Dectin-1 and angiotensin receptor type 1 (AT1R). Cells were treated with biotinylated-Ang II at 1μM or free biotin at 1μM for 45 min. Cells were then fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.1% Triton X-100 for 10min and blocked with 5% BSA in PBS for 30 min. Staining was performed with anti-Dectin-1 (1:100) or anti-AT1R antibody (1:100) overnight at 4°C. Fluorophore-conjugated secondary antibodies (1:1000) and Streptavidin/SAlexa Fluor 488 (1:1000) were used for detection. Nuclei were stained with DAPI (Sigma, D6578). Immunofluorescence images were captured by confocal microscopy (FV3000, Olympus). IgG staining was used as a primary antibody control and Biotin was used to as a control for Bio-Ang II.

To detect cellular hypertrophy, primary cardiomyocytes were exposed to Ang II, with or without Dectin-1 antibody pretreatment. Cells were then fixed and permeabilized as indicated above. Cells were stained with rhodamine phalloidin (Solarbio, CA1610) at a concentration of 100 nM for 30 min. Nuclei were stained with the DAPI (Sigma, D6578). Immunofluorescence images were obtained.

Real-time quantitative PCR

RNA was isolated from cultured cells and heart tissues using TRIZOL (Thermo Fisher, 15596026) or Total RNA Purification Kit (RN001, EZ Bioscience). PrimeScript RT reagent Kit (Takara, RR037A) was used for cDNA synthesis. Quantitative real-time PCR was performed using ABI QuantStudio6 detection system (Applied Biosystems, Thermo Fisher). Primers for genes were obtained from Thermo Fisher. Sequences are presented in Supplementary Table S1. mRNA of target genes was normalized to *Actb* housekeeping gene.

Western blot analysis

Fifty micrograms of cell and tissue lysates were separated by 10% SDS-PAGE and electro-transferred to a PVDF membranes. Membranes were blocked in Tris-buffered saline containing 0.05% Tween20 and 5% non-fat milk for 1.5h. PVDF membranes were then incubated with specific primary antibodies. Immunoreactive bands were detected by incubating with secondary antibodies conjugated to horseradish peroxidase and enhanced chemiluminescence reagent (Bio-Rad). Densitometric quantification was performed using Image J analysis software version 1.38e. Proteins were normalized to their respective control (GAPDH for cytosolic proteins, Lamin B for nuclear fractions, and total protein for phosphorylated-form detection).

Statistical analysis

All representative images were selected as the best representation of the average seen in the particular condition/treatment group. Statistical analysis was performed with GraphPad Prism 8.0 software (San Diego, CA, USA). Data presented in in vitro study is representative of at least 3 independent experiments and is expressed as Mean ± SEM. The exact group size (n) for each experiment is provided and 'n' refers to independent biological replicates, not technical replicates. Shapiro-Wilk normality test ($P<0.05$) was used to determine the adherence to a normal (Gaussian) distribution of data, except for groups with small n ($n < 6$). Mann-Whitney U test were used to analyze two-group comparisons and Kruskal-Wallis followed by Dunn post hoc multiple comparisons test for multi-group comparisons when the data failed normality test or small sample size ($n<6$ per group). In in vitro experiments, we assumed that the data follows a Gaussian distribution by relying on the central limit theorem. An unpaired 2-tailed Student's t-test was applied for comparing two groups, and one-way ANOVA followed by Tukey post-hoc test was applied for comparing more than two groups. In in vivo experiments, for the comparisons of two groups, an unpaired 2-tailed Student's t-test was used for parametric data. For the comparisons of more than two groups, parametric data (one level are being compared) were analyzed by one-way ANOVA followed by Tukey post-hoc test and 2-way repeated-measures ANOVA analysis with a single pooled variance and a Tukey correction for pairwise comparisons within groups for each data set for parametric data (two levels are being compared). $P <0.05$ was considered statistically significant, while $P \geq 0.05$ was considered "not statistically different". Post-tests were run only if F achieved $P<0.05$ and there was no significant variance in homogeneity. Only within-test corrections were made in this study. Very specifically, the statistical information, including n for each group, normality test results (if applied), statistical methods applied, and p-values for each plot are listed in Supplementary Table S5.

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Supplementary Table S1: Primer sequences for real-time qPCR assay in this study.

Gene	Species	Sequence
Myh7	Mouse	ACTGTCAACACTAAGAGGGTCA TTGGATGATTTGATCTTCCAGGG
Col1a1	Mouse	GCTCCTCTTAGGGGCCACT CCACGTCTCACCATTGGGG
Tgfb	Mouse	CTCCCGTGGCTTCTAGTGC GCCTTAGTTTGGACAGGATCTG
Gata4	Mouse	CCCTACCCAGCCTACATGG ACATATCGAGATTGGGGTGTCT
Acta1	Mouse	CCCAAAGCTAACCGGGAGAAG CCAGAATCCAACACGATGCC
Clec7a (Dectin-1)	Mouse	GACTTCAGCACTCAAGACATCC TTGTGTCGCCAAAATGCTAGG
Nppa	Mouse	GCTTCCAGGCCATATTGGAG GGGGGCATGACCTCATCTT
Nppb	Mouse	GAGGTCACTCCTATCCTCTGG GCCATTTCCTCCGACTTTTCTC
Il17a	Mouse	TTTAACTCCCTTGGCGCAAAA CTTTCCCTCCGCATTGACAC
Il23	Mouse	ATGCTGGATTGCAGAGCAGTA ACGGGGCACATTATTTTTAGTCT
Cxcl1	Mouse	CTGGGATTCACCTCAAGAACATC CAGGGTCAAGGCAAGCCTC
Cxcr2	Mouse	ATGCCCTCTATTCTGCCAGAT GTGCTCCGGTTGTATAAGATGAC
Csf3	Mouse	ATGGCTCAACTTTCTGCCCAG CTGACAGTGACCAGGGGAAC
Actb	Mouse	GGCTGTATTCCCCTCCATCG CCAGTTGGTAACAATGCCATGT
Myh7	Rat	GAGGAGAGGGCGGACATT ACTCTTCATTCAGGCCCTTG
Col1a1	Rat	GACATCCCTGAAGTCAGCTGC TCCCTTGGGTCCCTCGAC
Tgfb	Rat	GCAACAACGCAATCTATGAC CCTGTATTCCGTCTCCTT
Gata4	Rat	GGTGTCTACTTGTGGACTAGAC TTTGGAGTCAGATCAGGTATGG
Acta1	Rat	AGCAGAAACTAGACACCATGTG TACTTCAGGGTCAGGATACCTC
Actb	Rat	AAGTCCCTCACCCTCCCAAAAG AAGCAATGCTGTCACCTTCCC

Supplementary Table S2: Biometric and echocardiographic measurements in mice challenged with Ang II.

			Ang II Infusion	
	WT-Ctrl	D1KO-Ctrl	WT	D1KO
	n=7	n=7	n=7	n=7
EF%	79.25±1.25	79.3±1.4$^{P=0.99}$	72.42±1.62$^{P=8.1e-8}$	78.08±1.71$^{P=3.1e-6}$
FS%	39.71±1.17	39.59±1.23$^{P=0.59}$	33.06±0.85$^{P=3.5e-11}$	37.81±0.97$^{P=1.3e-7}$
LVIDd,mm	2.31±0.12	2.34±0.12$^{P=0.99}$	3.41±0.27$^{P=7.9e-11}$	2.44±0.11$^{P=1.4e-10}$
LVIDs,mm	1.21±0.09	1.17±0.09$^{P=0.99}$	2.5±0.2$^{P=1.8e-11}$	1.48±0.14$^{P=1.3e-9}$
IVSD,mm	0.68±0.01	0.69±0.01$^{P=0.99}$	0.76±0.02$^{P=5.8e-9}$	0.7±0.02$^{P=4.6e-7}$
PWd,mm	0.69±0.01	0.67±0.02$^{P=0.054}$	0.73±0.02$^{P=4.1e-5}$	0.7±0.01$^{P=6.2e-5}$
HW/BW mg /g	5.48±0.5	5.32±0.41$^{P=0.71}$	6.84±0.39$^{P=7.4e-5}$	5.5±0.14$^{P=6.56e-5}$
HW/TL mg/mm	5.03±0.47	5.09±0.2$^{P=0.99}$	6.43±0.36$^{P=7.3e-6}$	5.17±0.11$^{P=1.90e-5}$

Transthoracic echocardiography was performed on wildtype (WT) and Dectin-1 knockout (D1KO) mice at the conclusion of the *in vivo* study. EF, ejection fraction %; FS, fractional shortening %; LVIDd, diastole left ventricle internal dimension; LVIDs, left ventricular internal dimension systole; IVSD, diastole interventricular septal thickness; PWd, diastole posterior wall thickness; HW/BW, heart weight/body weight; HW/TL, heart weight/tibia length. Data presented as Mean ± SEM [n = 7 per group].

Supplementary Table S3 Biometric and echocardiographic parameters of the chimeric mice with Ang II challenge.

	Ang II Pump Infusion	
	WT BM→WT	D1KO BM→WT
	n=7	n=7
EF%	56.27±2.46	72.73±3.32$^{2.8e-7}$
FS%	25.09±1.57	38.42±2.02$^{2.5e-8}$
LVIDd,mm	4.05±0.28	3.10±0.51$^{2.3e-3}$
LVIDs,mm	3.03±0.23	1.71±0.35$^{6.8e-6}$
IVSD,mm	0.63±0.10	0.98±0.11$^{1.3e-4}$
PWd,mm	0.67±0.28	1.09±0.06$^{3.9e-3}$
HW/BW mg /g	6. 54±0.45	5.97±0.36$^{3.71e-4}$
HW/TL mg/mm	6.35±0.48	6.01±0.26 $^{P=1.4e-2}$

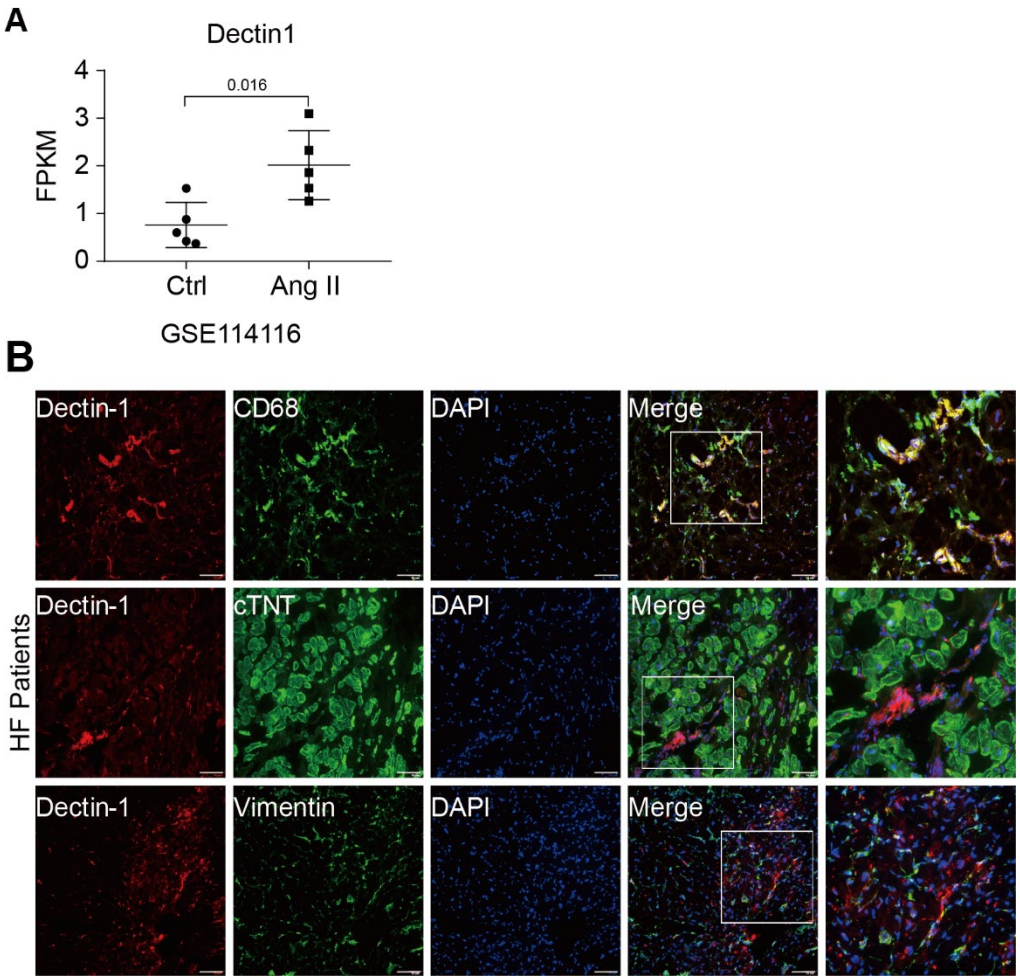
Transthoracic echocardiography was performed WT mice at the conclusion of the in vivo study. LOS, Losartan potassium; EF, ejection fraction %; FS, fractional shortening %; LVIDd, diastole left ventricle internal dimension; LVIDs, left ventricular internal dimension systole; IVSD, diastole interventricular septal thickness; PWd, diastole posterior wall thickness; HW/BW, heart weight/body weight; HW/TL, heart weight/ tibia length. Data presented as Mean ± SEM, [n=7 per group]

Supplementary Table S4: Biometric and echocardiographic parameters of the experimental mice.

		Ang II Infusion	
	WT-Ctrl	WT	WT-LOS
	n=7	n=7	n=7
EF%	81.31±2.30	63.76±4.39$^{P=4.9e-9}$	74.63±1.24$^{P=5.5e-6}$
FS%	44.29±2.62	29.83±2.89$^{P=4.6e-8}$	39.67±2.91$^{P=1.1e-5}$
LVIDd,mm	3.46±0.17	4.03±0.21$^{P=1.2e-5}$	3.74±0.09$^{P=9.6e-3}$
LVIDs,mm	1.83±0.06	2.83±0.20$^{P=9.8e-11}$	2.17±0.09$^{P=7.4e-8}$
IVSD,mm	0.81±0.05	0.93±0.07$^{P=2.4e-3}$	0.82±0.03$^{P=4.7e-3}$
PWd,mm	0.90±0.06	0.97±0.09$^{P=0.34}$	0.92±0.11$^{P=0.61}$
HW/BW mg /g	5.22±0.37	6.70±0.48$^{P=3.6e-5}$	5.39±0.43$^{P=1.4e-4}$
HW/TL mg/mm	4.70±0.25	5.79±0.26$^{P=4.7e-5}$	5.10±0.49$^{P=1.4e-5}$

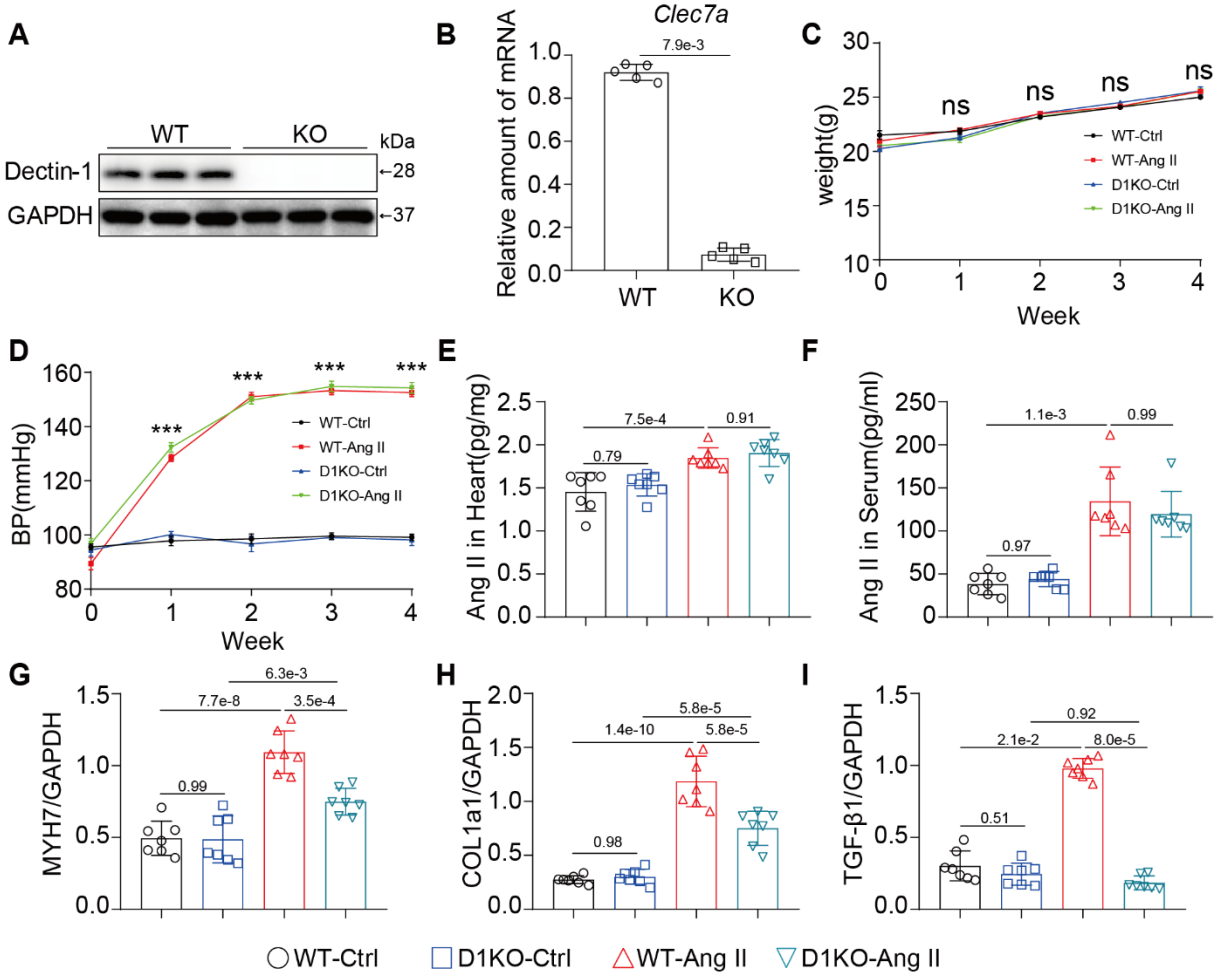
Transthoracic echocardiography was performed WT mice at the conclusion of the in vivo study. LOS, Losartan potassium; EF, ejection fraction %; FS, fractional shortening %; LVIDd, diastole left ventricle internal dimension; LVIDs, left ventricular internal dimension systole; IVSD, diastole interventricular septal thickness; PWd, diastole posterior wall thickness; HW/BW, heart weight/body weight; HW/TL, heart weight/ tibia length. Data presented as Mean ± SEM, [n=7 per group].

Supplementary Figure S1



Supplementary Figure S1: Dectin-1 levels are increased in the heart with heart failure.
(A) The relative levels of Dectin-1 in control (Ctrl) and Ang II-challenged mice, identified from a publicly available study: GSE114116. Data were analyzed to confirm findings of the present study [Mann-Whitney U-test, Two-tailed; P value indicated in figure].
(B) Representative dual-immunofluorescence staining of Dectin-1 (red) and macrophage antigen CD68 (green, upper panel), cardiomyocyte marker cTNT (green, middle panel), or fibroblast marker vimentin (green, lower panel) in the heart tissues of a patient with heart failure. Sections were counterstained with DAPI (blue) [scale bar = 50μm].

Supplementary Figure S2



Supplementary Figure S2: Dectin-1 deficiency protects against cardiac remodeling induced by Ang II.

(A) Representative western blot analysis of Dectin-1 protein in heart tissue from wildtype (WT) C57BL/6 and Dectin-1 knockout (KO) mice. GAPDH was used as loading control [n = 5 per group, Mann-Whitney U-test, Two-tailed].

(B) mRNA levels of *Clec7a* (Dectin-1) in the heart tissues from WT mice and Dectin-1 KO mice [n = 5 per group, Mann-Whitney U-test, Two-tailed].

(C) Wildtype (WT) and Dectin-1 knockout (D1KO) mice were challenged with Ang II for 4 weeks using osmotic pumps. Body weights were measured over the experimental period [n = 7 per group, two-way ANOVA followed by Tukey post-hoc tests].

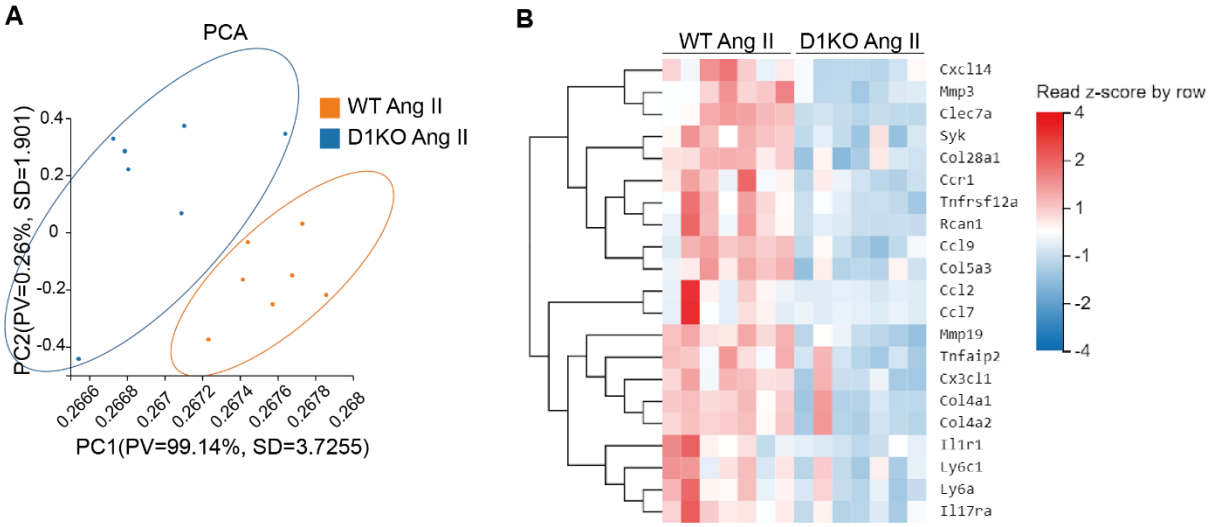
(D) Systolic blood pressure measurements were made weekly by non-invasive tail-cuff Pressure Analysis System in mice [n = 7 per group, two-way ANOVA followed by Tukey post-hoc tests].

(E) Ang II protein levels in mouse heart tissues as determined by ELISA [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

(F) Ang II levels in mouse serum as determined by ELISA [n = 7 per group, Kruskal-Wallis followed by Dunn post hoc multiple comparisons test, number of comparisons = 6].

(G-I) Densitometric quantification of immunoblots in Figure 2I. Levels of MYH7, COL1α1, and TGF-β1 were normalized to GAPDH [n = 7 per group, for panels G, H, one-way ANOVA followed by Tukey post-hoc tests; for panel I, Kruskal-Wallis followed by Dunn post hoc multiple comparisons test, number of comparisons = 6].

Supplementary Figure S3



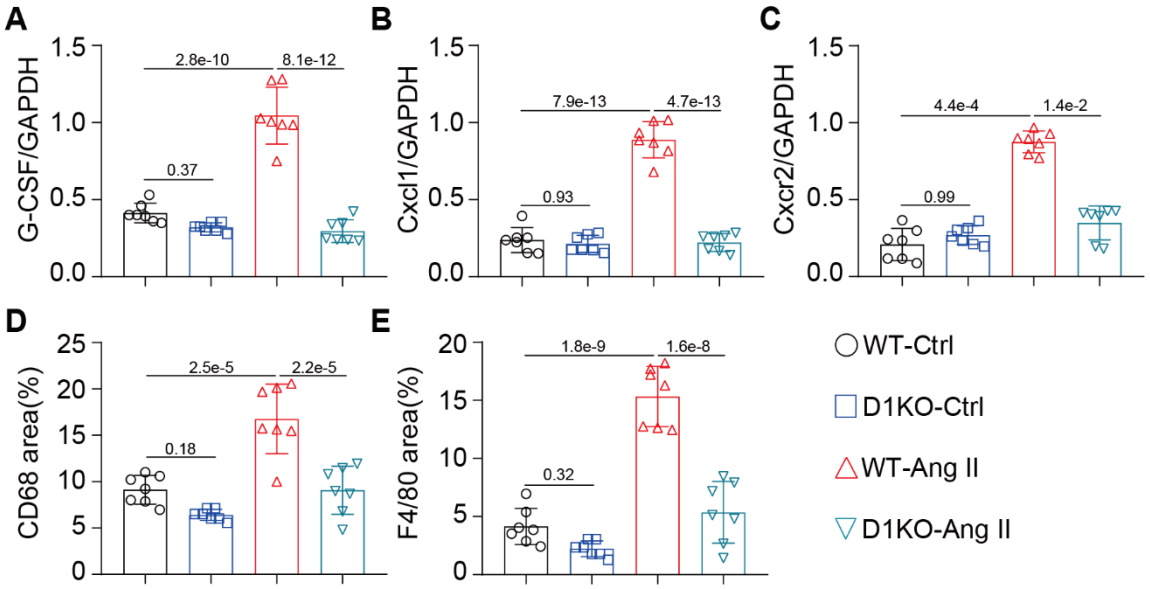
Supplementary Figure S3: RNA-sequencing analysis show that Dectin-1 knockout reduces the levels of pro-inflammatory and chemoattractant factors in heart tissues of Ang II-infused mice.

Heart tissues of Ang II-infused WT and D1KO mice were proceeded to RNA-sequencing analysis. The changed genes were analyzed.

(A) The PCA of two groups.

(B) Selected genes involved in leukocyte recruitment, pro-inflammatory, pro-fibrotic and pro-hypertrophic markers are shown as a heat map. Blue indicates low expression levels and red indicates high expression levels [n=7 biological replicates].

Supplementary Figure S4



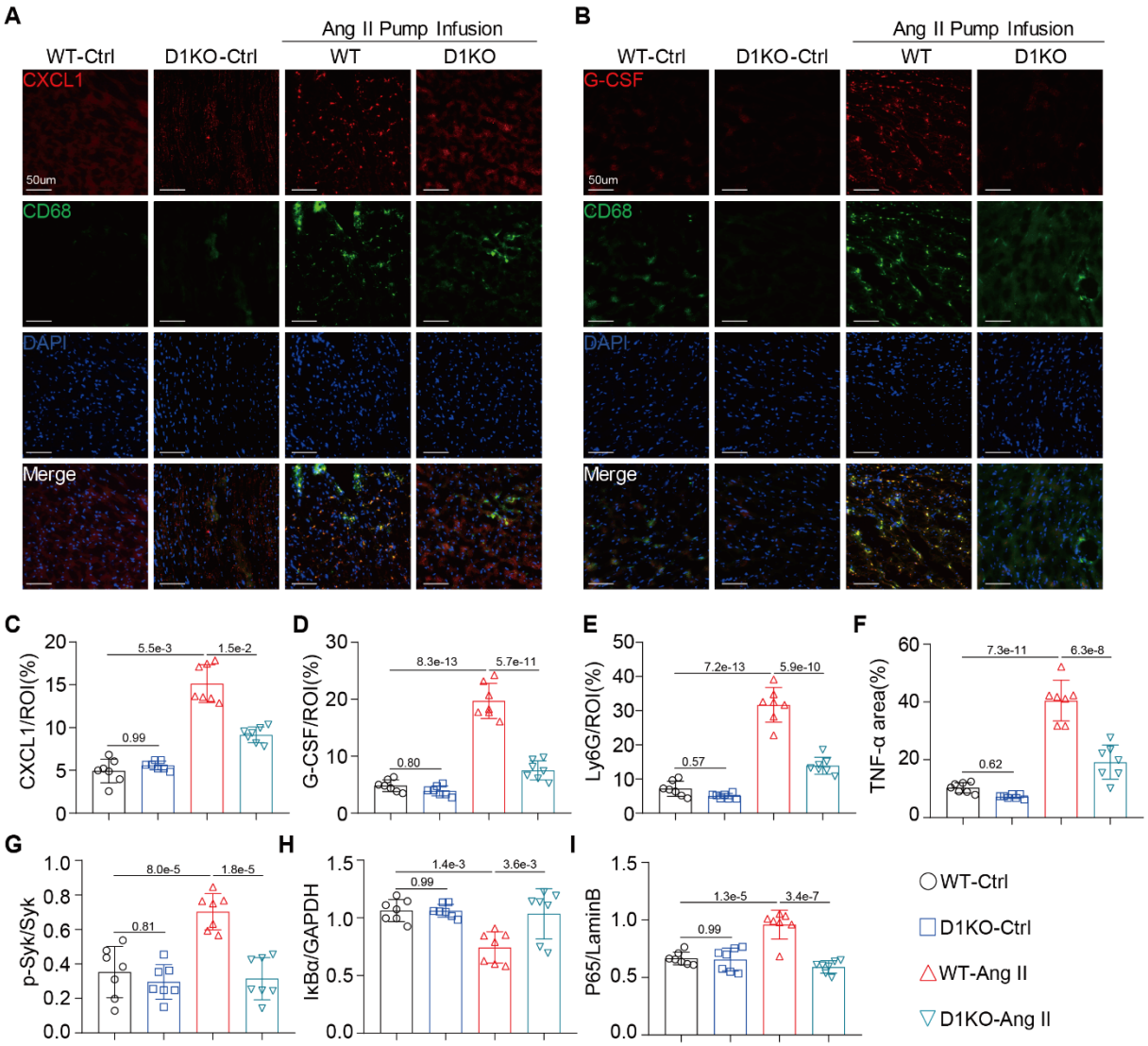
Supplementary Figure S4: Dectin-1 induces pro-inflammatory and chemoattractant factors in heart tissues following Ang II infusion.

(A-C) Densitometric quantification of immunoblots in Figure 3A. Levels of G-CSF, CXCL1, and CXCR2 were normalized to GAPDH [n = 7 per group; for panels A, B, one-way ANOVA followed by Tukey post-hoc tests; for panel C, Kruskal-Wallis followed by Dunn post hoc multiple comparisons test; number of comparisons = 6].

(D) Quantification of CD68 immunoreactivity in heart tissues in Figure 3C. Data shown as % positive area [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

(E) Quantification of F4/80 immunoreactivity in Figure 3D [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

Supplementary Figure S5



Supplementary Figure S5: Neutrophil infiltration in heart tissues following Ang II administration is mediated by Dectin-1.

(A) Representative dual-immunofluorescence staining of CXCL1 (red) and CD68 (green) in heart tissues of wildtype (WT) and Dectin-1 knockout (D1KO) mice. Mice were challenged with Ang II for 4 weeks. Sections were counterstained with DAPI (blue) [scale bar = 50 μm].

(B) Representative dual-immunofluorescence staining of G-CSF (red) and CD68 (green) in heart tissue of mice. Sections were counterstained with DAPI (blue) [scale bar = 50 μm].

(C) Quantification of CXCL1 immunoreactivity in panel A. Data shown as % positive area of region of interest (ROI) [n = 7 per group, Kruskal-Wallis followed by Dunn post hoc multiple comparisons test, number of comparisons = 6].

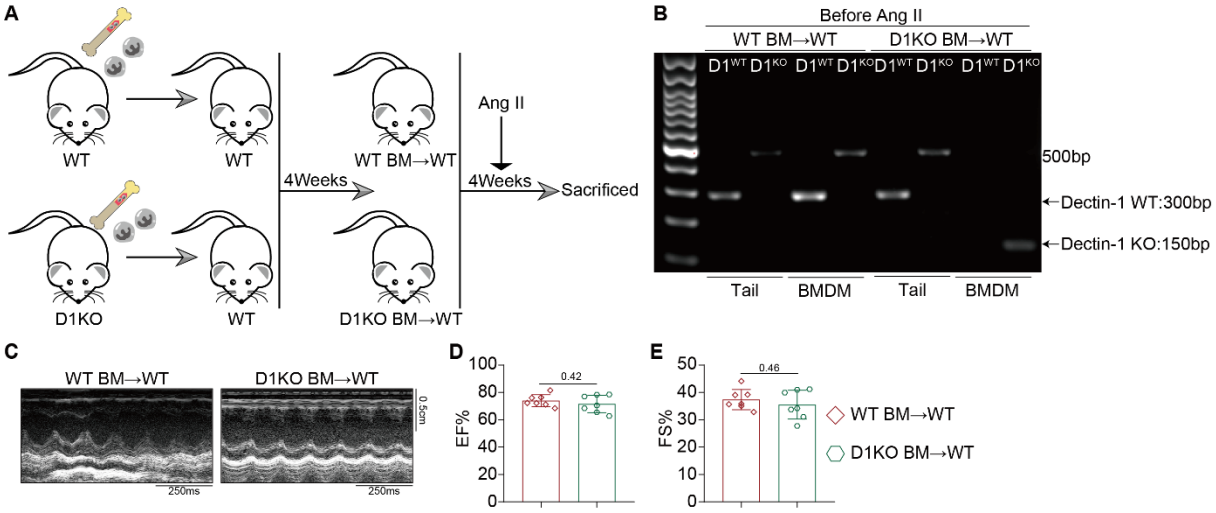
(D) Quantification of G-CSF immunoreactivity in panel B. Data shown as % positive area of region of interest (ROI) [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

(E) Quantification of Ly-6G immunoreactivity in Figure 3G. Data shown as % positive area of region of interest (ROI) [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

(F) Quantification of TNF-α immunoreactivity in Figure 3H [n = 7 per group,one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

(G-I) Densitometric quantification of immunoblots in Figure 3J. Phospho(p)- Syk levels were normalized to total Syk, IκBα to GAPDH, and nuclear p65 to Lamin B [n = 7 per group, for panel G, one-way ANOVA followed by Tukey post-hoc tests; for panels H, I, Kruskal-Wallis followed by Dunn post hoc multiple comparisons test, number of comparisons = 6].

Supplementary Figure S6



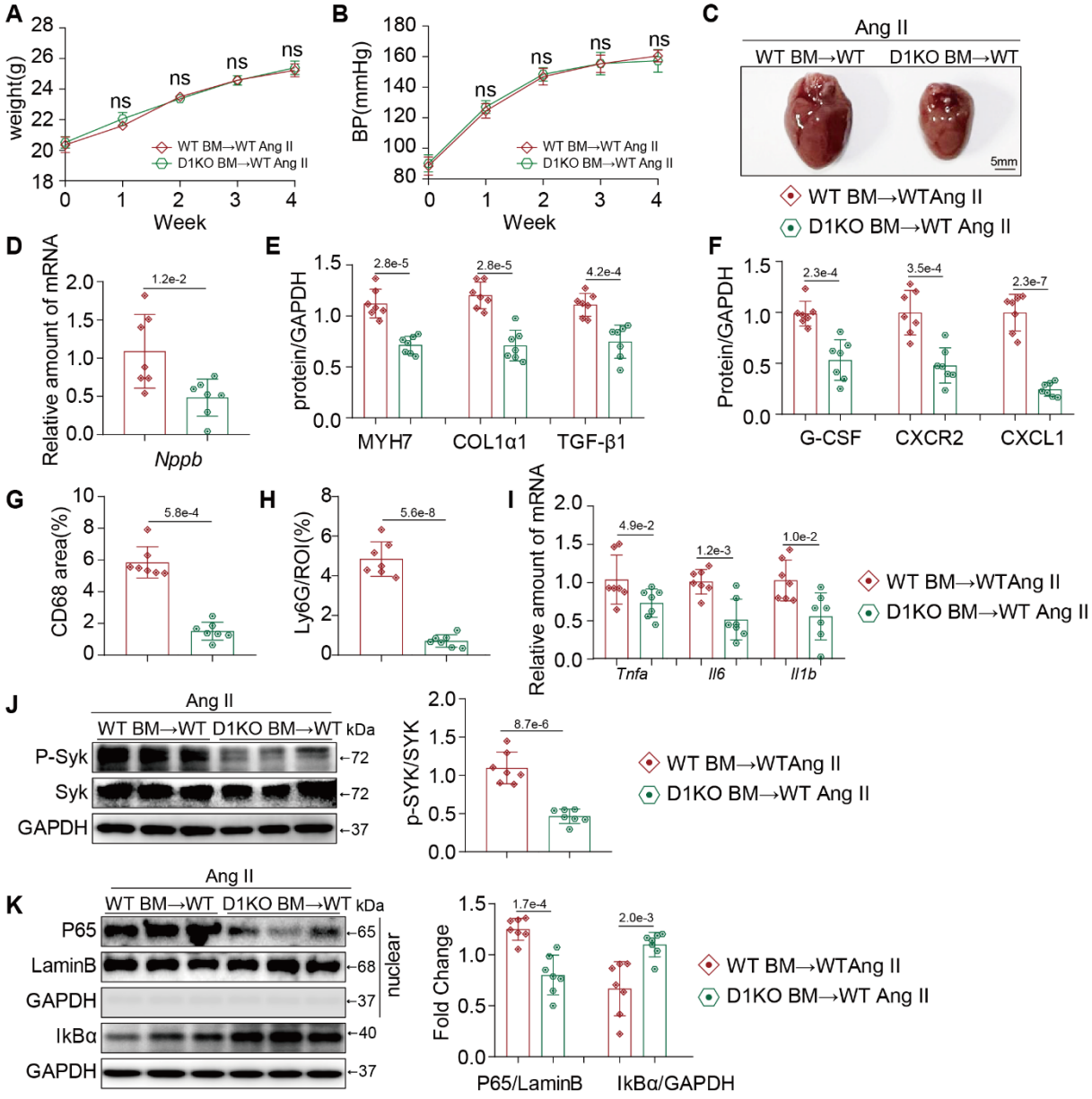
Supplementary Figure S6. WT BM→WT and D1KO BM→WT chimeric mice were generated by bone marrow transplantation.

(A) The schematic diagram showing the production and treatment of WT BM→WT and D1KO BM→WT chimeric mice.

(B)The representative DNA electrophoresis in chimeric mice show the success BMTs.

(C-E) Before Ang II infusion, mice from both groups were proceeded to echocardiographic analysis, which indicated that the bone marrow transplantation did not affect the basal heart function. Representative M-mode echocardiographic images (C), LV ejection fraction (EF%, D), and fractional shortening value (FS%, E) were shown [n = 7 per group, per group; Student's t-test;]

Supplementary Figure S7



Supplementary Figure S7. Bone marrow-derived Dectin-1 knockout alleviated Ang II-induced heart injuries in mice

WT BM→WT and D1KO BM→WT mice were challenged with Ang II for 4 weeks using osmotic pumps.

(A) Body weights were measured weekly over the experimental period [n = 7 per group, two-way ANOVA followed by Sidak's multiple comparisons].

(B) Systolic blood pressure measurements were made weekly by non-invasive tail-cuff Pressure Analysis System in mice [n = 7 per group, two-way ANOVA followed by Sidak's multiple comparisons].

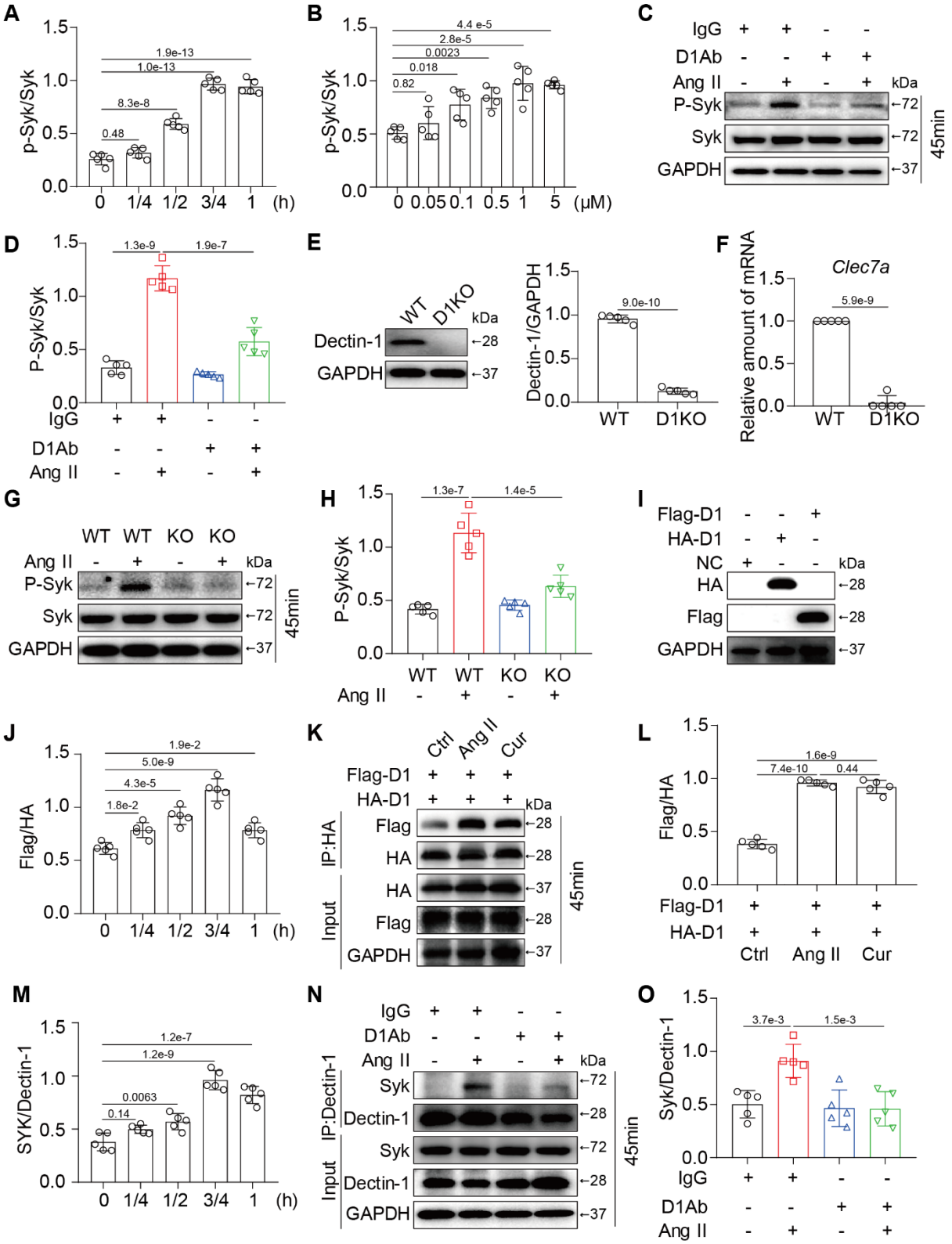
(C) Representative images of harvested heart tissues from WT BM→WT and D1KO BM→WT mice

(D) mRNA levels of *Nppb* in the heart tissues from WT BM→WT and D1KO BM→WT mice [n = 7 per group, Student's t-test].

(E-F) Representative western blot analysis of relevant proteins involved in cardiac fibrosis, hypertrophy, and macrophage infiltration in Figure4H and Figure4J. GAPDH was used as

loading control. [n = 7 per group, Student's t-test].
(G-H) Quantification of F4/80 and Ly6G immunoreactivity in Figure4L. [n = 7 per group, for panel G, Mann-Whitney U-test; for panel H, Student's t-test].
(I) mRNA levels of *Tnfa, Il6* and *IL1b* in the heart tissues from WT BM→WT and D1KO BM→WT mice [n = 7 per group, Student's t-test].
(J) Representative western blot analysis of relevant proteins (p-Syk and IκBα) involved in Dectin-1 signaling of WT BM→WT and D1KO BM→WT mice. GAPDH and total Syk were used as loading control. The densitometric quantification of proteins shown in right [n = 7 per group, Student's t-test].
(K) Representative western blot analysis of P65 in nuclear and cytoplasm of extracts prepared from mouse heart tissues, with Lamin B as nuclear loading control and GAPDH as cytoplasm loading control. The densitometric quantification of proteins shown in right [n = 7 per group, Student's t-test].

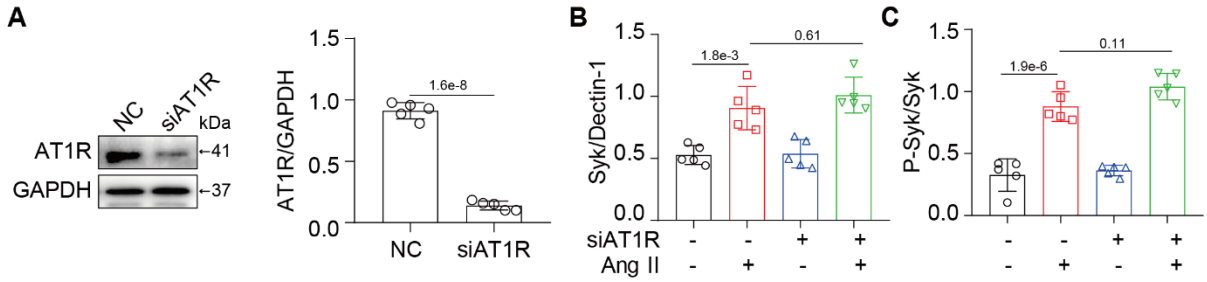
Supplementary Figure S8



Supplementary Figure S8: Ang II activates the Dectin-1-Syk pathway in mouse BMDMs.
(A) Densitometric quantification of immunoblots in Figure 5A [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 15].
(B) Densitometric quantification of immunoblots in Figure 5B [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 15].
(C) BMDMs from WT mice were treated with anti-Dectin-1 antibody (D1Ab) at 5 μg/mL or

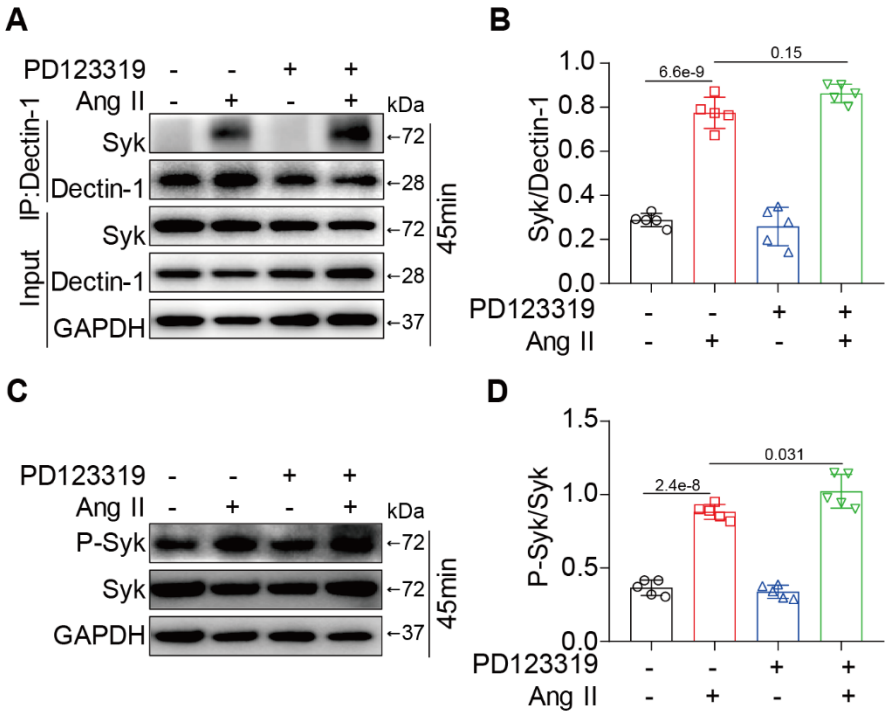
IgG for 30 min (rat IgG2a Isotype was used as negative control). Cells were then exposed to 1 μM Ang II for 45 min. Total proteins were extracted and subjected to analysis of p-Syk and Syk protein levels. GAPDH was used as loading control.
(D) Densitometric quantification of immunoblots in Supplementary Figure S8C. [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 15].
(E) BMDMs from WT mice or D1KO mice. Total proteins were extracted and subjected to analysis of Dectin-1 protein levels. GAPDH was used as loading control. The densitometric quantification was shown in right. [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 15].
(F) The mRNA level of *Clec7a* of the BMDMs from WT mice or D1KO mice. Data normalized to *Actb* levels. [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 15].
(G) BMDMs from WT mice or D1KO mice. Cells were then exposed to 1 μM Ang II for 45 min. Total proteins were extracted and subjected to analysis of p-Syk and Syk protein levels. GAPDH was used as loading control.
(H) The densitometric quantification of Supplementary Figure S8G. [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 15].
(I) HEK-293T cells were transfected with Flag- and HA-tagged Dectin-1 plasmids. Total lysates were probed for HA and Flag by immunoblotting. GAPDH was used as loading control.
(J) Densitometric quantification of immunoblots in Figure 5C [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 10].
(K) HEK-293T cells, transfected as indicated in Panels G, were exposed to 1 μM Ang II or 100 μg/mL Dectin-1 agonist Curdlan AL (Cur). Interaction between HA and Flag was detected by immunoblotting.
(L) Densitometric quantification of immunoblots in Panel L [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 3].
(M) Densitometric quantification of immunoblots in Figure 5D [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 10].
(N) BMDMs from WT mice were treated with anti-Dectin-1 antibody (D1Ab) at 5 μg/mL or IgG for 30 min (rat IgG2a Isotype was used as negative control). Cells were then exposed to 1 μM Ang II for 45 min. Total proteins were extracted and subjected to co-immunoprecipitation analysis of Dectin-1-Syk interaction.
(O) Densitometric quantification of immunoblots shown in Panel N [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

Supplementary Figure S9



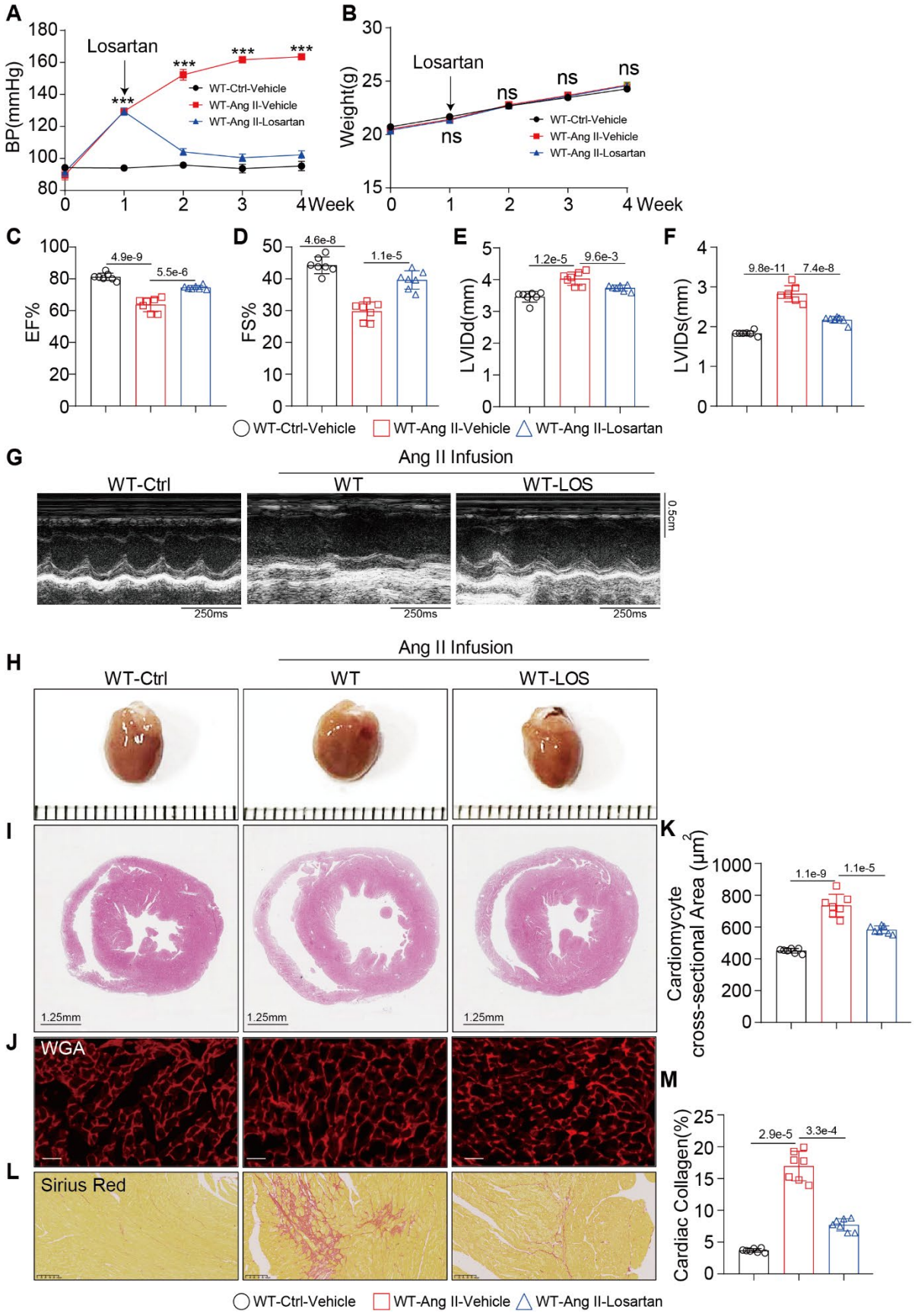
Supplementary Figure S9: Role of AT1R in Dectin-1 signaling activation in vitro.
(A) AT1R was knocked down in BMDMs from WT mice by AT1R siRNA (siAT1R). Control cells were transfected with negative control siRNA (NC). Lysates were used to probe for AT1R levels. GAPDH was used as loading control. Representative blots are shown on left and densitometric quantification on right [n = 5 biological replicates, Student's t test].
(B) Densitometric quantification of immunoblots in Figure 5E [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].
(C) Densitometric quantification of immunoblots in Figure 5F [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

Supplementary Figure S10



Supplementary Figure S10: Role of AT2R in Dectin-1 signaling activation.
BMDMs from WT mice were treated with vehicle or AT2R antagonist (PD123319, 5µmol/l) for 1h and then exposed to 1 µM Ang II for 45 minutes.
(A) Dectin-1-Syk interaction was analyzed by co-immunoprecipitation.
(B) Densitometric quantification of immunoblots shown in Panel A [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 3].
(C) BMDMs were challenged with 1 µM Ang II or biotinylated-Ang II (Bio-Ang II) for 45 min. Total proteins were extracted and subjected to analysis of P-Syk and Syk levels. GAPDH was used as loading control.
(D) Densitometric quantification of immunoblots shown in Panel C [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 3].

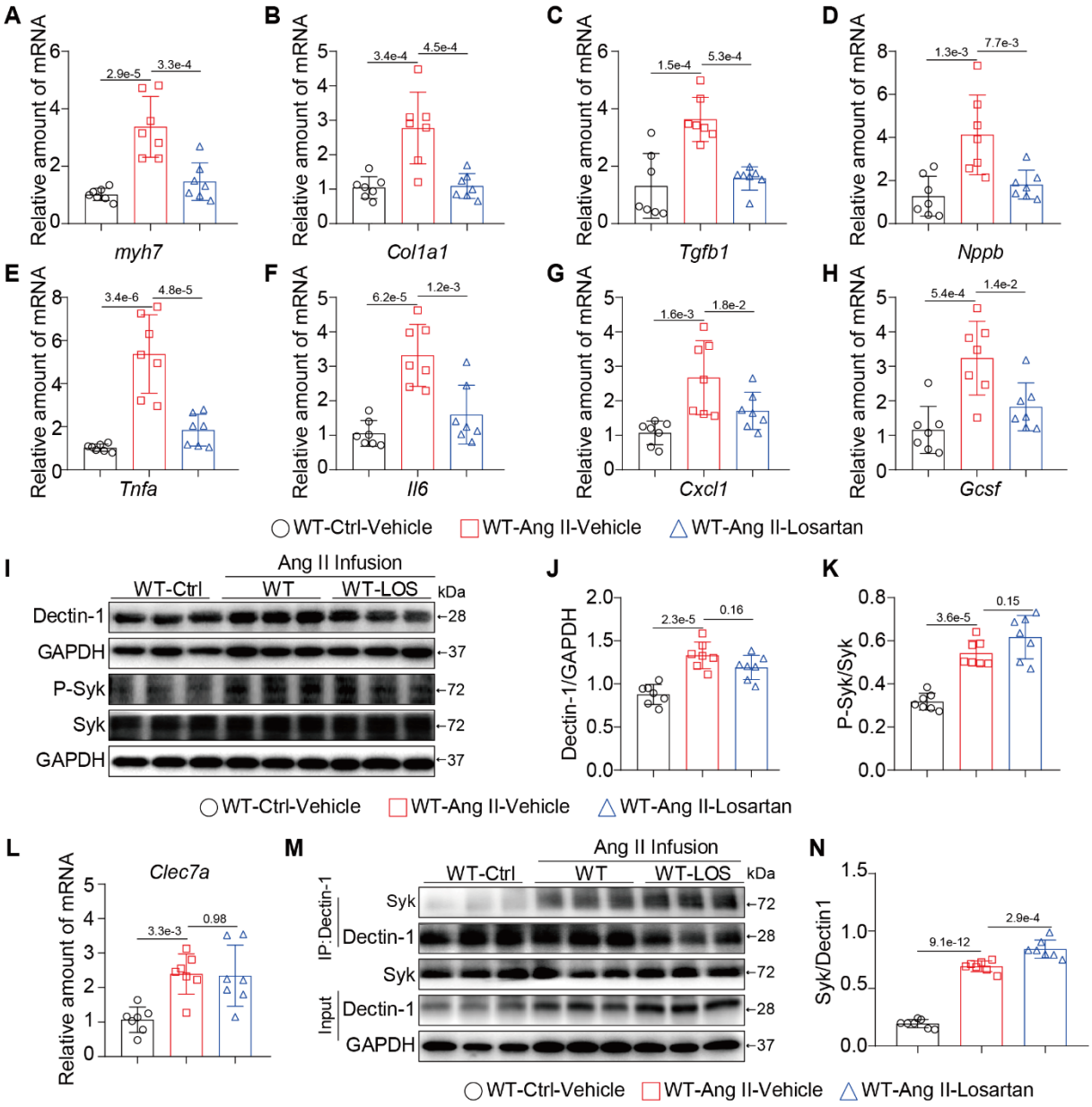
Supplementary Figure S11



Supplementary Figure S11: Role of AT1R in Dectin-1 signaling activation in vivo.
(A) Body weights of each group were measured over the experimental period [n = 7 per group, two-way ANOVA followed by Tukey post-hoc tests].

(B) Systolic blood pressure measurements of each group were made weekly by non-invasive tail-cuff Pressure Analysis System in mice [n = 7 per group, two-way ANOVA followed by Tukey post-hoc tests].
(C-F) Echocardiographic analysis of LV ejection fraction (C) (EF%), fractional shortening value (D) (FS%), LV internal diameter at end-diastole (E) (LVIDd) and LV internal dimension systole (F) (LVIDd). [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].
(G) Representative M-mode echocardiographic images are shown in the top panel.
(H) Representative images of harvested heart tissues from mice.
(I) Representative images of total heart tissues from each group with H&E staining [scale bar = 1.25 mm].
(J) Representative images of heart tissues from each group with WGA-stained sections [scale bar = 50 μm].
(K) Representative images of heart tissues from each group with Sirius Red staining [scale bar = 100 μm].
(L) The right panel shown the quantitative of myocyte area from WGA-stained sections in Supplementary Figures S11J. A minimum of 100 cells were measured from different visual fields of 4 samples per group
(M)The right panel shown the quantification of interstitial fibrotic area (%) as determined by Sirius Red staining of heart in Supplementary Figures S11L. [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

Supplementary Figure S12



Supplementary Figure S12: Role of AT1R in Dectin-1 signaling activation in vivo.

(A-H) The mRNA levels of *myh7, Cola1, Tgfb1, Nppb, Tnfa, Il6, Cxcl1* and *Gcsf (Csf3)* in heart lysates. GAPDH was used as loading control.

(J) Densitometric quantification of immunoblots in Supplementary Figures S12I. Dectin-1 levels were normalized to GAPDH [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

(K) Densitometric quantification of immunoblots in Supplementary Figures S12I. Phospho(p)-Syk levels were normalized to total Syk [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

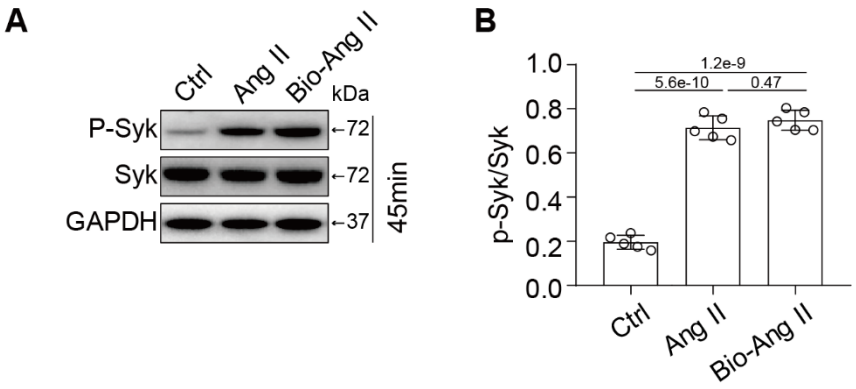
(L) The mRNA levels of Clec7a in heart tissues of mice. Transcripts were normalized to Actb.

(M)Representative western blot analysis of Dectin-1-Syk interaction was analyzed by co-immunoprecipitation in mouse heart tissue lysates, and in the below panels was representative western blot analysis of Syk and Dectin-1 in mouse heart tissue lysates as the input. GAPDH was used as loading control.

(N) Densitometric quantification of immunoblots in panel Supplementary Figures S12M. Syk

levels were normalized to total Dectin-1 [n = 7 per group, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

Supplementary Figure S13

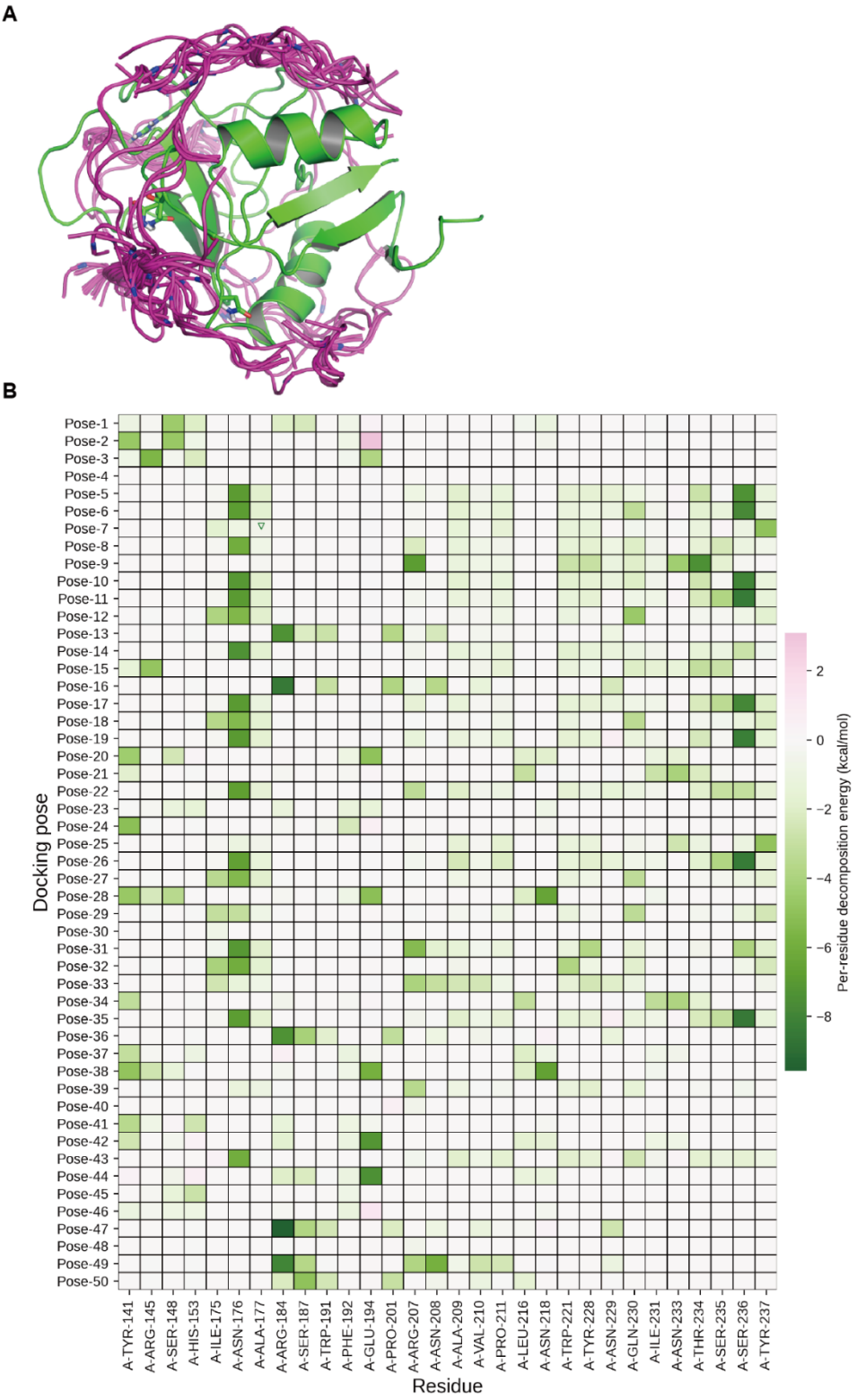


Supplementary Figure S13: Biotinylated-Ang II shown no difference to Ang II in activating the Dectin-1/Syk pathway**.**

(A) BMDMs were challenged with 1 μM Ang II or biotinylated-Ang II (Bio-Ang II) for 45 min. Total proteins were extracted and subjected to analysis of P-Syk and Syk levels. GAPDH was used as loading control.

(B) Densitometric quantification of immunoblots shown in Panel A [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 3].

Supplementary Figure S14

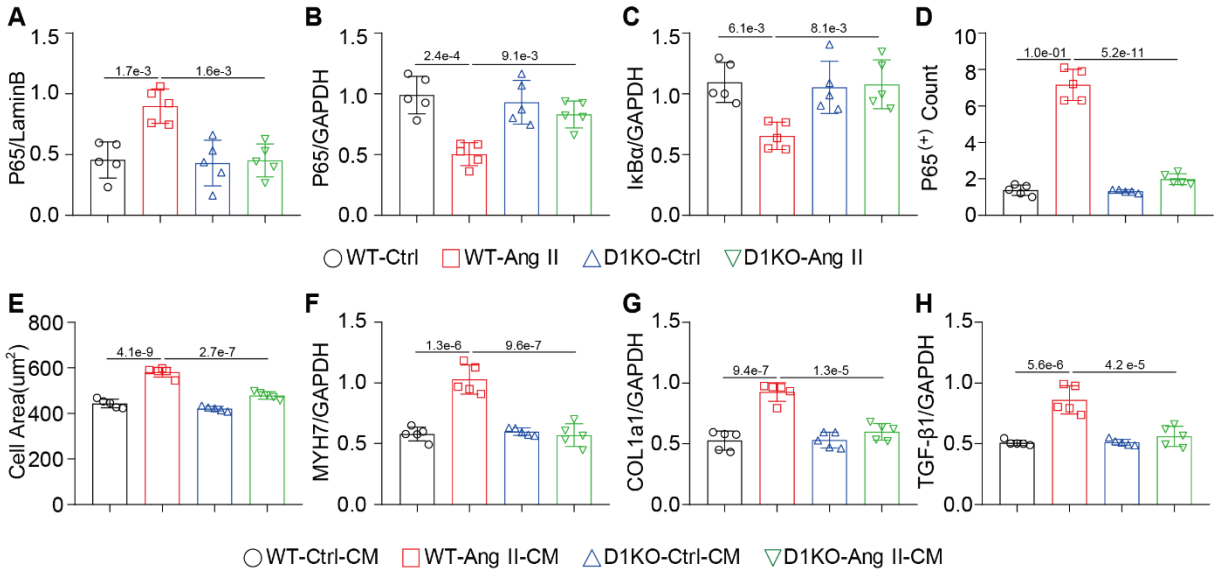


Supplementary Figure S14: Identification the binding positions of Ang II and Dectin-1 protein.

(A) A total of 100 Ang II-Dectin-1 binding conformations were produced by the docking program QVina-W. The Fuchsia stick showing the binding pose of Ang II to Dectin-1.

(B) Heat map showing residue decomposition energy (kcal/mol) from the 100 docking poses of Ang II with Dectin-1.

Supplementary Figure S15



Supplementary Figure S15: Blocking Dectin-1 signaling in BMDMs prevents Ang II responses in macrophages and cardiomyocytes.

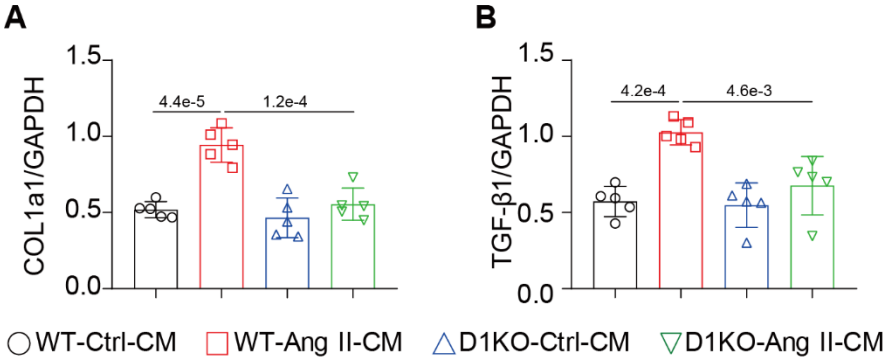
(A-C) Densitometric quantification of immunoblots in Figure 7A [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

(D) Quantification of p65 nuclear translocation as detected by immunofluorescence staining of BMDMs in Figure 7B [n=5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons =6].

(E) Quantification of cardiomyocyte cell size in Figure 7H [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

(F-H) Densitometric quantification of immunoblots in Figure 7I [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

Supplementary Figure S16



Supplementary Figure S16: Blocking Dectin-1 signaling in BMDMs prevents Ang II responses in cardiac fibrosis.

(A-B) Densitometric quantification of immunoblots shown in Figure 7K [n = 5 biological replicates, one-way ANOVA followed by Tukey post-hoc tests, number of comparisons = 6].

Supplementary Table S5: The detailed statistical information, methods, and results in each figure and table.

Figure#	Group	n	Shapiro-Wilk normality test P value (if applied)	Applied statistical test(s) and result(s)
1B	Ctrl Ang II	5 5	- -	Mann-Whitney U-test, Two-tailed P=7.9e-3
1C	Ctrl Ang II	7 7	0.6013 0.4875	Unpaired Student`s t-test, Two-tailed P=6.4e-7
1E	Ctrl Ang II	5 5	- -	Mann-Whitney U-test, Two-tailed P=7.9e-3
1G	Ctrl Ang II	5 5	- -	Mann-Whitney U-test, Two-tailed P=7.9e-3
1H	CD68 cTNT Vimentin	5 5 5	- - -	Kruskall-Wallis test: P=2.1e-3 Dunn's multiple comparisons CD68 vs cTnT, P=4.0e-2 CD68 vs Vimentin, P=1.4e-2
2B	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.9164 0.7392 0.5339 0.4048	One-way ANOVA: P=8.0e-9 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.91 WT-Ctrl vs WT-Ang II, P=8.1e-8 D1KO-Ctrl vs D1KO- Ang II, P=0.14 WT-Ang II vs D1KO- Ang II, P=3.1e-6
2C	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.5923 0.4271 0.4239 0.5466	One-way ANOVA: P=5.3e-11 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.98 WT-Ctrl vs WT-Ang II, P=2.2e-10 D1KO-Ctrl vs D1KO- Ang II, P=7.6e-2 WT-Ang II vs D1KO- Ang II, P=6.9e-8
2D	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.2745 0.6878 0.6997 0.1075	One-way ANOVA: P=3.3e-16 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.97 WT-Ctrl vs WT-Ang II, P=2.0e-14 D1KO-Ctrl vs D1KO- Ang II, P=0.67 WT-Ang II vs D1KO- Ang II, P=1.6e-13
2F	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.8562 0.7424 0.8508 0.1061	One-way ANOVA: P=1.3e-14 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II,

				P=2.1e-13 D1KO-Ctrl vs D1KO- Ang II, P=0.34 WT-Ang II vs D1KO- Ang II, P=1.6e-12
2G	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.8562 0.7424 0.9554 0.5313	One-way ANOVA: P=1.0e-13 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.98 WT-Ctrl vs WT-Ang II, P=1.4e-12 D1KO-Ctrl vs D1KO- Ang II, P=0.24 WT-Ang II vs D1KO- Ang II, P=1.6e-11
2H	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.5759 0.4574 0.9301 0.7870	One-way ANOVA: P=5.0e-10 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.92 WT-Ctrl vs WT-Ang II, P=1.5e-9 D1KO-Ctrl vs D1KO- Ang II, P=2.4e-3 WT-Ang II vs D1KO- Ang II, P=4.9e-5
2J-Myh7	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.0815 0.6957 0.6958 0.0456	One-way ANOVA: P=4.3e-8 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=1.4e-7 D1KO-Ctrl vs D1KO- Ang II, P=0.24 WT-Ang II vs D1KO- Ang II, P=2.4e-5
2J-Col1a1	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.2446 0.4565 0.6266 0.8231	One-way ANOVA: P=7.9e-7 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=2.4e-6 D1KO-Ctrl vs D1KO- Ang II, P=0.49 WT-Ang II vs D1KO- Ang II, P=1.3e-4
2J-Tgfb1	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.8231 0.8231 0.7218 0.3773	One-way ANOVA: P=1.4e-7 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=9.1e-7 D1KO-Ctrl vs D1KO- Ang II, P=0.99 WT-Ang II vs D1KO- Ang II, P=2.0e-6
2J-Gata4	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.6200 0.7613 0.5642 0.6207	One-way ANOVA: P=1.1e-5 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.55

				WT-Ctrl vs WT-Ang II, P=8.4e-6 D1KO-Ctrl vs D1KO- Ang II, P=0.44 WT-Ang II vs D1KO- Ang II, P=9.5e-3
2J-Acta1	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.1539 0.1539 0.1539 0.3839	One-way ANOVA: P=5.25e-8 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=1.9e-7 D1KO-Ctrl vs D1KO- Ang II, P=0.22 WT-Ang II vs D1KO- Ang II, P=5.0e-4
3D-Cxcl1	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.2567 0.3771 0.8100 0.2142	One-way ANOVA: P=7.4e-8 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.73 WT-Ctrl vs WT-Ang II, P=1.1e-7 D1KO-Ctrl vs D1KO- Ang II, P=5.1e-2 WT-Ang II vs D1KO- Ang II, P=9.5e-4
3D -Cxcl2	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.4382 0.4846 0.0611 0.2996	One-way ANOVA: P=2.6e-6 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.91 WT-Ctrl vs WT-Ang II, P=3.6e-5 D1KO-Ctrl vs D1KO- Ang II, P=0.90 WT-Ang II vs D1KO- Ang II, P=4.0e-5
3D-Gcsf	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.5548 0.3204 0.7849 0.4373	One-way ANOVA: P=8.9e-5 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.97 WT-Ctrl vs WT-Ang II, P=4.5e-4 D1KO-Ctrl vs D1KO- Ang II, P=0.58 WT-Ang II vs D1KO- Ang II, P=3.5e-3
3I-Tnfa	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.0915 0.7842 0.9045 0.3773	One-way ANOVA: P=3.2e-9 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.98 WT-Ctrl vs WT-Ang II, P=2.6e-8 D1KO-Ctrl vs D1KO- Ang II, P=0.99 WT-Ang II vs D1KO- ng II, P=6.1e-8
3I -Ilb1	WT-Ctrl D1KO-Ctrl	7 7	0.2892 0.1317	One-way ANOVA: P=6.7e-7 Tukey's multiple comparisons

	WT-Ang II D1KO- Ang II	7 7	0.1622 0.7161	WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=2.9e-6 D1KO-Ctrl vs D1KO- Ang II, P=0.39 WT-Ang II vs D1KO- Ang II, P=1.1e-4
3I-Il6	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.5394 0.4247 0.3207 0.6492	One-way ANOVA: P=1.5e-6 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.98 WT-Ctrl vs WT-Ang II, P=1.5e-8 D1KO-Ctrl vs D1KO- Ang II, P=0.93 WT-Ang II vs D1KO- Ang II, P=2.2e-5
3I -Il17	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.5400 0.0914 0.6021 0.2199	One-way ANOVA: P=8.3e-6 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.95 WT-Ctrl vs WT-Ang II, P=7.4e-5 D1KO-Ctrl vs D1KO- Ang II, P=0.78 WT-Ang II vs D1KO- Ang II, P=2.0e-4
3I -Il23	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.5498 0.5919 0.5730 0.1689	One-way ANOVA: P=1.5e-8 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=1.6e-7 D1KO-Ctrl vs D1KO- Ang II, P=0.89 WT-Ang II vs D1KO- Ang II, P=4.7e-7
4B	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.5073 0.1140	Unpaired Student`s t-test, Two-tailed P=2.8e-7
4C	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.3704 0.5970	Unpaired Student`s t-test, Two-tailed P=2.5e-8
4D-upper	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.8456 0.0281	Mann-Whitney U-test, Two-tailed P= 5.8e-4
4D-mid	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.1532 0.1759	Unpaired Student`s t-test, Two-tailed P=4.1e-8
4D-lower	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.1140 0.0932	Unpaired Student`s t-test, Two-tailed P=1.1e-7
4F-Myh7	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.0945 0.6618	Unpaired Student`s t-test, Two-tailed P=4.5e-3
4F-Col1a1	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.2292 0.6718	Unpaired Student`s t-test, Two-tailed P=7.4e-4

4F-Tgfb1	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.0748 0.9163	Unpaired Student`s t-test, Two-tailed P=3.6e-3
4F-Gata4	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.0798 0.5222	Unpaired Student`s t-test, Two-tailed P=3.2e-2
4F-Acta1	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.3839 0.5002	Unpaired Student`s t-test, Two-tailed P=1.3e-2
4H-Cxcl1	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.7987 0.2473	Unpaired Student`s t-test, Two-tailed P=2.0e-3
4H -Cxcl2	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.1917 0.9484	Unpaired Student`s t-test, Two-tailed P=2.3e-2`
4H-Gcsf	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.3656 0.3249	Unpaired Student`s t-test, Two-tailed P=2.6e-3
4H -Il17	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.1142 0.4979	Unpaired Student`s t-test, Two-tailed P=3.7e-3
4H-Il23	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.1864 0.3633	Unpaired Student`s t-test, Two-tailed P=2.5e-2
5H	Ctrl Biotin Bio-Ang II-0.5 Bio-Ang II-1 Bio-Ang II-10 Bio-Ang II-50 Bio-Ang II-100 Bio-Ang II-100/Ang II (200uM) Bio-Ang II-100/Ang II (500uM)	6 6 6 6 6 6 6 6 6	0.1242 0.5244 0.7102 0.1778 0.0782 0.7742 0.7581 0.1872 0.6048	One-way ANOVA: P=9.2e-12 Tukey's multiple comparisons Ctrl vs. Biotin, P=0.99 Biotin vs. Bio-Ang II-0.5, P=3.1e-5 Biotin vs. Bio-Ang II-1, P=1.0e-10 Biotin vs. Bio-Ang II-10, P=2.5e-13 Biotin vs. Bio-Ang II-50, P=2.5e-13 Biotin vs. Bio-Ang II-100, P=2.5e-13 Bio-Ang II-100 vs. Bio-Ang II-100/Ang II (200uM) , P=2.6-13 Bio-Ang II-100 vs. Bio-Ang II-100/Ang II (500uM), P=2.4e-13
6E	Panel1 Panel2 Panel3 Panel4 Panel5 Panel6	3 3 3 3 3 3	- - - - - -	One-way ANOVA: P=2.1e-9 Tukey's multiple comparisons Panel1 vs. Panel2, P=6.9e-5 Panel1 vs. Panel3, P=2.6e-5 Panel2 vs. Panel3, P=0.96 Panel2 vs. Panel5, P=1.1e-3 Panel3 vs. Panel6, P=0.18 Panel4 vs. Panel5, P=0.95 Panel4 vs. Panel6, P=4.9e-3 Panel5 vs. Panel6, P=2.1e-2
6G	Panel1 Panel2 Panel3 Panel4 Panel5 Panel6	3 3 3 3 3 3	- - - - - -	One-way ANOVA: P=2.1e-9 Tukey's multiple comparisons Panel1 vs. Panel2, P=7.7e-8 Panel1 vs. Panel3, P=2.0e-8 Panel2 vs. Panel3, P=0.49 Panel2 vs. Panel5, P=8.9e-6

				Panel3 vs. Panel6, P=0.35 Panel4 vs. Panel5, P=0.045 Panel4 vs. Panel6, P=3.7e-7 Panel5 vs. Panel6, P=1.2e-5
6H	Panel1 Panel2 Panel3 Panel4 Panel5 Panel6	3 3 3 3 3 3	- - - - - -	One-way ANOVA: P=5.2e-7 Tukey's multiple comparisons Panel1 vs. Panel2, P=9.2e-5 Panel1 vs. Panel3, P=2.8e-5 Panel2 vs. Panel3, P=0.93 Panel2 vs. Panel5, P=1.9e-4 Panel3 vs. Panel6, P=0.98 Panel4 vs. Panel5, P=0.99 Panel4 vs. Panel6, P=1.8e-5 Panel5 vs. Panel6, P=2.4e-5
7C-Tnfa	WT-Ctrl WT-Ang II D1KO-Ctrl D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=5.4e-5 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=2.3e-4 WT-Ang II vs D1KO- Ang II, P=3.6e-4
7C-Il1b	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=2.3e-6 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=2.2e-5 WT-Ang II vs D1KO- Ang II, P=3.6e-4
7C-Il6	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=9.7e-7 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=4.4e-6 WT-Ang II vs D1KO- Ang II, P=1.6e-5
7C-Il23	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=4.9e-4 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=1.1e-3 WT-Ang II vs D1KO- Ang II, P=3.3e-3
7D-Cxcl1	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=3.7e-5 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=1.5e-4 WT-Ang II vs D1KO- Ang II, P=1.4e-3
7D-Gcsf	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=3.2e-4 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=1.1e-3 WT-Ang II vs D1KO- Ang II, P=4.8e-3
7E-TNFa	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=1.2e-12 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=6.4e-12 WT-Ang II vs D1KO- Ang II, P=5.9e-8
7F-IL-1β	WT-Ctrl D1KO-Ctrl WT-Ang II	5 5 5	- - -	One-way ANOVA: P=7.2e-5 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II,

	D1KO- Ang II	5	-	P=2.0e-4 WT-Ang II vs D1KO- Ang II, P=3.0e-3
7G-CXCL1	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=2.3e-9 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=1.0e-8 WT-Ang II vs D1KO- Ang II, P=3.6e-7
7G-G-CSF	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=2.3e-9 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=9.7e-9 WT-Ang II vs D1KO- Ang II, P=3.4e-7
7J-Myh7	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=1.2e-13 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=8.3e-13 WT-Ang II-CM vs D1KO-Ang II-CM, P=6.3e-12
7J-Col1a1	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=1.6e-8 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=4.8e-7 WT-Ang II-CM vs D1KO-Ang II-CM, P=4.2e-8
7J-Tgfb1	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=5.5e-10 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=2.4e-8 WT-Ang II-CM vs D1KO-Ang II-CM, P=2.4e-9
7J-Gata4	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=6.3e-10 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=3.2e-9 WT-Ang II-CM vs D1KO-Ang II-CM, P=2.6e-8
7J-Acta1	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=2.0e-7 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=1.5e-6 WT-Ang II-CM vs D1KO-Ang II-CM, P=2.6e-5
7L-Col1a1	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=4.6e-6 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=1.3e-5 WT-Ang II-CM vs D1KO-Ang II-CM, P=2.1e-4
7J-Tgfb1	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=2.4e-8 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=7.9e-8 WT-Ang II-CM vs D1KO-Ang II-CM, P=6.8e-6
7J-Gata4	WT-Ctrl-CM D1KO-Ctrl-CM	5 5	- -	One-way ANOVA: P=2.5e-9 Tukey's multiple comparisons

	WT-Ang II-CM D1KO- Ang II-CM	5 5	- -	WT-Ctrl-CM vs WT-Ang II-CM, P=1.4e-8 WT-Ang II-CM vs D1KO-Ang II-CM, P=9.0e-8
S1A	Ctrl Ang II	5 5	- -	Mann-Whitney U-test, Two-tailed P=0.016
S2B	WT KO	5 5	- -	Mann-Whitney U-test, Two-tailed P=7.9e-3
S2C	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.6863 0.7093 0.9471 0.7074	two-way ANOVA: Interaction: P=0.02 Row Factor: P<0.0001 Column Factor: P=0.23 Tukey's multiple comparisons 0week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II, P=0.43 WT-Ang II vs D1KO-Ang II, P=0.60 1week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II, P=0.98 WT-Ang II vs D1KO-Ang II, P=0.71 2week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II, P=0.83 WT-Ang II vs D1KO-Ang II, P=0.92 3week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II, P=0.99 WT-Ang II vs D1KO-Ang II, P=0.99 4week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II, P=0.43 WT-Ang II vs D1KO-Ang II, P=0.99
S2D	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.7074 0.8681 0.0567 0.0669	two-way ANOVA: Interaction: P<0.0001 Row Factor: P<0.0001 Column Factor: P<0.0001 Sidak's multiple comparisons 0week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II, P=0.073 WT-Ang II vs D1KO-Ang II, P=0.26 1week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II,

				P=2.7e-14 WT-Ang II vs D1KO-Ang II, P=0.42 2week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II, P=2.4e-14 WT-Ang II vs D1KO-Ang II, P=0.96 3week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II, P=2.4e-14 WT-Ang II vs D1KO-Ang II, P=0.92 4week: Ctrl vs Ang II WT-Ctrl vs. WT-Ang II, P=2.4e-14 WT-Ang II vs D1KO-Ang II, P=0.90
S2E	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.0736 0.1731 0.0574 0.4489	One-way ANOVA: P=2.5e-5 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.79 WT-Ctrl vs WT-Ang II, P=7.5e-4 WT-Ang II vs D1KO- Ang II, P=0.91
S2F	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.8326 0.1343 0.0244 0.0004	Kruskall-Wallis test: P=1.0e-4 Dunn's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=1.1e-3 WT-Ang II vs D1KO- Ang II, P=0.99
S2G	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.5340 0.1285 0.4612 0.4557	One-way ANOVA: P=1.e-8 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=7.7e-8 D1KO-Ctrl vs D1KO- Ang II, P=6.3e-3 WT-Ang II vs D1KO- Ang II, P=3.5e-4
S2H	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.9564 0.9536 0.3123 0.1707	One-way ANOVA: P=2.4e-11 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.98 WT-Ctrl vs WT-Ang II, P=1.4e-10 D1KO-Ctrl vs D1KO- Ang II, P=3.6e-5 WT-Ang II vs D1KO- Ang II, P=5.8e-5
S2I	WT-Ctrl D1KO-Ctrl	7 7	0.2081 0.2222	Kruskall-Wallis test: P=1.9e-4 Dunn's multiple comparisons

	WT-Ang II	7	0.9383	WT-Ctrl vs D1KO-Ctrl,
	D1KO- Ang II	7	0.0163	P=0.99
				WT-Ctrl vs WT-Ang II, P=2.1e-2
				D1KO-Ctrl vs D1KO- Ang II, P=0.92
				WT-Ang II vs D1KO- Ang II, P=8.0e-5
S4A	WT-Ctrl	7	0.25419	One-way ANOVA: P=1.3e-12
	D1KO-Ctrl	7	0.46314	Tukey's multiple comparisons
	WT-Ang II	7	0.24196	WT-Ctrl vs D1KO-Ctrl,
	D1KO- Ang II	7	0.06058	P=0.37
				WT-Ctrl vs WT-Ang II, P=2.8e-10
				WT-Ang II vs D1KO- Ang II, P=8.1e-12
S4B	WT-Ctrl	7	0.1758	One-way ANOVA: P=2.2e-14
	D1KO-Ctrl	7	0.0663	Tukey's multiple comparisons
	WT-Ang II	7	0.5960	WT-Ctrl vs D1KO-Ctrl,
	D1KO- Ang II	7	0.1240	P=0.93
				WT-Ctrl vs WT-Ang II, P=7.9e-13
				WT-Ang II vs D1KO- Ang II, P=4.7e-13
S4C	WT-Ctrl	7	0.4564	Kruskall-Wallis test: P=4.2e-4
	D1KO-Ctrl	7	0.7740	Dunn's multiple comparisons
	WT-Ang II	7	0.7083	WT-Ctrl vs D1KO-Ctrl,
	D1KO- Ang II	7	0.0037	P=0.99
				WT-Ctrl vs WT-Ang II, P=4.4e-4
				WT-Ang II vs D1KO- Ang II, P=1.4-2
S4D	WT-Ctrl	7	0.5422	One-way ANOVA: P=2.4e-7
	D1KO-Ctrl	7	0.4632	Tukey's multiple comparisons
	WT-Ang II	7	0.1946	WT-Ctrl vs D1KO-Ctrl,
	D1KO- Ang II	7	0.6087	P=0.18
				WT-Ctrl vs WT-Ang II, P=2.5e-5
				WT-Ang II vs D1KO- Ang II, P=2.2e-5
S4F	WT-Ctrl	7	0.4940	One-way ANOVA: P=4.3e-11
	D1KO-Ctrl	7	0.4632	Tukey's multiple comparisons
	WT-Ang II	7	0.0568	WT-Ctrl vs D1KO-Ctrl,
	D1KO- Ang II	7	0.5960	P=0.32
				WT-Ctrl vs WT-Ang II, P=1.8e-9
				WT-Ang II vs D1KO- Ang II, P=1.6e-8
S5C	WT-Ctrl	7	0.8945	Kruskall-Wallis test: P=1.6e-3
	D1KO-Ctrl	7	0.4632	Dunn's multiple comparisons
	WT-Ang II	7	0.0357	WT-Ctrl vs D1KO-Ctrl,
	D1KO- Ang II	7	0.8323	P=0.99
				WT-Ctrl vs WT-Ang II, P=5.5e-3
				WT-Ang II vs D1KO- Ang II, P=1.5e-2
S5D	WT-Ctrl	7	0.8219	One-way ANOVA: P=5.5e-14
	D1KO-Ctrl	7	0.7713	Tukey's multiple comparisons
	WT-Ang II	7	0.3569	WT-Ctrl vs D1KO-Ctrl,

	D1KO- Ang II	7	0.7786	P=0.80 WT-Ctrl vs WT-Ang II, P=8.3e-13 WT-Ang II vs D1KO- Ang II, P=5.7e-11
S5E	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.6616 0.1731 0.8138 0.5168	One-way ANOVA: P=4.3e-14 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.57 WT-Ctrl vs WT-Ang II, P=7.2e-13 WT-Ang II vs D1KO- Ang II, P=5.9e-10
S5F	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.5421 0.4632 0.1847 0.9462	One-way ANOVA: P=4.3e-12 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.62 WT-Ctrl vs WT-Ang II, P=7.3e-11 WT-Ang II vs D1KO- Ang II, P=6.3e-8
S5G	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.7748 0.7146 0.3985 0.1562	One-way ANOVA: P=2.7e-6 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.81 WT-Ctrl vs WT-Ang II, P=8.0e-5 WT-Ang II vs D1KO- Ang II, P=1.8e-5
S5H	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.8794 0.4252 0.1637 0.0207	Kruskall-Wallis test: P=6.5e-3 Dunn's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=2.3e-2 WT-Ang II vs D1KO- Ang II, P=1.5e-2
S5I	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.3970 0.1489 0.0037 0.4463	Kruskall-Wallis test: P=1.5e-3 Dunn's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=3.2e-2 WT-Ang II vs D1KO- Ang II, P=7.6e-4
S6D	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.6941 0.2840	Unpaired Student`s t-test, Two-tailed P=0.42
S6E	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.5162 0.3391	Unpaired Student`s t-test, Two-tailed P=0.46
S7A	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.6691 0.8934	two-way ANOVA: Interaction: P=0.2823 Row Factor: P<0.0001 Column Factor: P=0.1458 Sidak's multiple comparisons 0week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P=

				0.93 1week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P= 0.10 2week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P= 0.97 3week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P=0.99 4week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P= 0.91
S7B	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.3047 0.1560	two-way ANOVA: Interaction: P=0.6869 Row Factor: P<0.0001 Column Factor: P= 0.6705 Sidak's multiple comparisons 0week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P= 0.98 1week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P=0.92 2week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P=0.98 3week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P=0.99 4week: WT-BM→WT Ang II vs D1KO BM→WT Ang II, P=0.83
S7D	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.3615 0.4681	Unpaired Student`s t-test, Two-tailed P=0.012
S7E-MYH7	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.5351 0.4453	Unpaired Student`s t-test, Two-tailed P=2.8e-5
S7E-COL1A1	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.5136 0.4889	Unpaired Student`s t-test, Two-tailed P=2.8e-5
S7E-TGFB1	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.6836 0.2278	Unpaired Student`s t-test, Two-tailed P=4.2e-4
S7F-G-CSF	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.1736 0.8039	Unpaired Student`s t-test, Two-tailed P=2.3e-4
S7F-CXCR2	WT-BM→WT Ang II D1KO BM→WT Ang	7 7	0.7369 0.8052	Unpaired Student`s t-test, Two-tailed

	II			P=3.5e-4
S7F-CXCL1	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.0980 0.4562	Unpaired Student`s t-test, Two-tailed P=2.3e-7
S7G	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.0097 0.8993	Mann-Whitney U-test, Two-tailed P= 5.8e-4
S7H	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.4643 0.6655	Unpaired Student`s t-test, Two-tailed P=5.6e-8
S7I-Tnfa	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.0704 0.4225	Unpaired Student`s t-test, Two-tailed P=4.9e-2
S7I-Il6	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.7882 0.2855	Unpaired Student`s t-test, Two-tailed P=1.2e-3
S7I-Il1b	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.2196 0.8106	Unpaired Student`s t-test, Two-tailed P=1.0e-2
S7J	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.4030 0.2432	Unpaired Student`s t-test, Two-tailed P=8.7e-6
S7K-P65/LaminB	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.3168 0.9769	Unpaired Student`s t-test, Two-tailed P=1.7e-4
S7K-P65/GAPDH	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.2639 0.1033	Unpaired Student`s t-test, Two-tailed P=2.0e-3
S8A	0 1/4 1/2 3/4 1	5 5 5 5 5	- - - - -	One-way ANOVA: P=2.2e-15 Tukey's multiple comparisons 0 vs. 1/4, P=0.48 0 vs. 1/2, P=8.3e-8 0 vs. 3/4, P=1.0e-13 0 vs. 1, P=1.9e-13
S8B	0 0.05 0.1 0.5 1 5	5 5 5 5 5 5	- - - - - -	One-way ANOVA: P=4.2e-6 Tukey's multiple comparisons 0 vs. 0.05, P=0.82 0 vs. 0.1, P=0.019 0 vs. 0.5, P=2.4e-3 0 vs. 1, P=2.8e-5 0 vs. 5, P=4.4e-5
S8D	IgG-Ctrl IgG-Ang II D1Ab-Ctrl D1Ab - Ang II	5 5 5 5	- - - -	One-way ANOVA: P=2.4e-10 Tukey's multiple comparisons IgG-Ctrl vs IgG-Ang II, P=1.3e-9 IgG-Ang II vs D1Ab-Ang II, P=1.9e-7
S8E	WT D1KO	5 5	- -	Unpaired Student`s t-test, Two-tailed P=9.0e-10
S8F	WT D1KO	5 5	- -	Unpaired Student`s t-test, Two-tailed P=5.9e-9
S8H	WT-Ctrl WT-Ang II D1KO-Ctrl D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=6.4e-8 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=1.3e-7 WT-Ang II vs D1KO- Ang II,

				P=1.4e-5
S8J	0 1/4 1/2 3/4 1	5 5 5 5 5	- - - - -	One-way ANOVA: P=1.1e-8 Tukey's multiple comparisons 0 vs. 1/4, P=1.8e-2 0 vs. 1/2, P=4.3e-5 0 vs. 3/4, P=5.0e-9 0 vs. 1, P=1.9e-2
S8L	Ctrl Ang II Cur	5 5 5	- - -	One-way ANOVA: P=4.4e-12 Tukey's multiple comparisons Ctrl vs. AngII, P=7.4e-10 Ctrl vs. Cur, P=1.9e-9 AngII vs. Cur, P=0.44
S8M	0 1/4 1/2 3/4 1	5 5 5 5 5	- - - - -	One-way ANOVA: P=4.2e-6 Tukey's multiple comparisons 0 vs. 1/4, P=0.14 0 vs. 1/2, P=6.3e-3 0 vs. 3/4, P=1.2e-9 0 vs. 1, P=1.3e-7
S8O	IgG-Ctrl IgG--Ang II D1Ab-Ctrl D1Ab - Ang II	5 5 5 5	- - - -	One-way ANOVA: P=6.3e-3 Tukey's multiple comparisons IgG-Ctrl vs IgG-Ang II, P=3.7e-3 IgG-Ang II vs D1Ab-Ang II, P=1.5e-3
S9A	NC siAT1R	5 5	- -	Unpaired Student`s t-test, Two-tailed P=1.6e-8
S9B	NC-Ctrl NC-Ang II siAT1R-Ctrl siAT1R- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=2.5e-5 Tukey's multiple comparisons NC-Ctrl vs. NC-AngII, P=1.8e-3 NC-AngII vs. siAT1R-AngII, P=0.61
S9C	NC-Ctrl NC-Ang II siAT1R-Ctrl siAT1R- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=7.2e-9 Tukey's multiple comparisons NC-Ctrl vs. NC-AngII, P=1.9e-6 NC-AngII vs. siAT1R-AngII, P=0.11
S10B	Vehicle-Ctrl Vehicle-Ang II PD12331-Ctrl PD12331-Ang II	5 5 5 5	- - - -	One-way ANOVA: P=1.7e-11 Tukey's multiple comparisons Vehicle-Ctrl vs. Vehicle-AngII, P=6.6e-9 Vehicle-AngII vs. PD12331-AngII, P=0.15
S10D	Vehicle-Ctrl Vehicle-Ang II PD12331-Ctrl PD12331-Ang II	5 5 5 5	- - - -	One-way ANOVA: P=3.4e-11 Tukey's multiple comparisons Vehicle-Ctrl vs. Vehicle-Ang II, P=2.4e-8 Vehicle-Ang II vs. PD12331-AngII, P=0.031
S11A	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.9509 0.8966 0.9090	two-way ANOVA: Interaction: P=0.8544 Row Factor: P<0.0001 Column Factor: P=0.7906 Tukey's multiple comparisons 0week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P= 0.9052

				WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P= 0.9801 1week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P= 0.8193 WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P= 0.9950 2week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P= 0.9865 WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P= 0.9914 3week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P= 0.9052 WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P= 0.9950 4week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P= 0.6211 WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P= 0.9974
S11B	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.4954 0.1864 0.1737	two-way ANOVA: Interaction: P<0.0001 Row Factor: P<0.0001 Column Factor: P<0.0001 Tukey's multiple comparisons 0week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P=0.3432 WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P=0.8127 1week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P=4.3e-10 WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P=0.99576 2week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P=4.3e-10 WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P=4. 4e-10 3week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P=4.3e-10 WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P=4.4e-10 4week: WT-Ctrl-Vehicle vs. WT-Ang II—Vehicle, P=4.4e-10 WT-Ang II--Vehicle vs. WT-Ang II-Losartan, P=4.4e-10
S11C	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.8199 0.1513 0.2228	One-way ANOVA: P=7.4e-9 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=4.9e-9 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=5.5e-6
S11D	WT-Ctrl-Vehicle	7	0.7889	One-way ANOVA: P=5.7e-8

	WT-Ang II-Vehicle WT-Ang II-Losartan	7 7	0.1969 0.8506	Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=4.6e-8 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=1.1e-5
S11E	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.0666 0.4579 0.4864	One-way ANOVA: P=1.8e-5 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=1.2e-5 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=9.6e-3
S11F	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.0983 0.7936 0.0624	One-way ANOVA: P=1.3e-10 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=9.8e-11 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=7.4e-8
S11K	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.2995 0.9463 0.4752	One-way ANOVA: P=2.0e-9 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=1.1e-9 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=1.1e-5
S11M	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.8562 0.4836 0.2570	One-way ANOVA: P=5.6e-12 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=4.6e-12 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=1.9e-9
S12A	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.9968 0.2308 0.3410	One-way ANOVA: P=2.2e-5 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=2.9e-5 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=3.3e-4
S12B	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.9050 0.6303 0.7310	One-way ANOVA: P=1.2e-4 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=3.4e-4 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=4.5e-4
S12C	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.0794 0.3311 0.0157	One-way ANOVA: P=8.1e-5 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=1.5e-4 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=5.3e-4
S12D	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.2327 0.5774 0.2956	One-way ANOVA: P=1.1e-3 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II- Vehicle, P=1.3e-3 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=7.7e-3
S12E	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.8866 0.4947 0.1660	One-way ANOVA: P=2.4e-6 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=3.4e-6 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=4.8e-5

S12F	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.1501 0.4475 0.0898	One-way ANOVA: P=5.8e-5 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=6.2e-5 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=1.2e-3
S12G	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.2118 0.1825 0.9355	One-way ANOVA: P=2.2e-3 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=1.6e-3 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=1.8e-2
S12H	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.1564 0.9352 0.1426	One-way ANOVA: P= 6.5e-4 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II- Vehicle, P= 5.4e-4 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=1.4e-2
S12J	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.9588 0.9653 0.7328	One-way ANOVA: P= 2.8e-5 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P= 2.3e-5 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=0.16
S12K	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.6105 0.0535 0.4679	One-way ANOVA: P= 7.9e-7 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P= 3.6e-5 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P= 0.15
S12L	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.9117 0.4487 0.2932	One-way ANOVA: P=1.6e-3 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=3.3e-3 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P= 0.98
S12N	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.4088 0.8115 0.4587	One-way ANOVA: P= 8.1e-14 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P= 9.1e-12 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P= 2.9e-4
S13B	Ctrl Ang II Bio-Ang II	5 5 5	- - -	One-way ANOVA: P=1.0e-8 Tukey's multiple comparisons Ctrl vs Ang II, P=5.6e-10 Ctrl vs Bio-Ang II, P=1.2e-9 Ang II vs Bio-Ang II, P=0.47
S15A	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=4.2e-2 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=1.7e-3 WT-Ang II vs D1KO- Ang II, P=1.6e-3
S15B	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=2.1e-4 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=2.4e-4 WT-Ang II vs D1KO- Ang II, P=9.1e-3

S15C	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=3.1e-3 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=6.1e-3 WT-Ang II vs D1KO- Ang II, P=8.1e-3
S15D	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	5 5 5 5	- - - -	One-way ANOVA: P=1.9e-12 Tukey's multiple comparisons WT-Ctrl vs WT-Ang II, P=1.0e-10 WT-Ang II vs D1KO- Ang II, P=5.2e-11
S15E	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=3.8e-10 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=4.1e-9 WT-Ang II-CM vs D1KO-Ang II-CM, P=2.7e-7
S15F	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=2.3e-7 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=1.3e-6 WT-Ang II-CM vs D1KO-Ang II-CM, P=9.6e-7
S15G	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=3.3e-7 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=9.4e-7 WT-Ang II-CM vs D1KO-Ang II-CM, P=1.3e-5
S15H	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=1.9e-6 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=5.6e-6 WT-Ang II-CM vs D1KO-Ang II-CM, P=4.2e-5
S16A	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=6.6e-6 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=4.4e-5 WT-Ang II-CM vs D1KO-Ang II-CM, P=1.2e-4
S16B	WT-Ctrl-CM D1KO-Ctrl-CM WT-Ang II-CM D1KO- Ang II-CM	5 5 5 5	- - - -	One-way ANOVA: P=1.4e-4 Tukey's multiple comparisons WT-Ctrl-CM vs WT-Ang II-CM, P=4.2e-4 WT-Ang II-CM vs D1KO-Ang II-CM, P=4.6e-3

Table#

S2-EF%	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.9164 0.7392 0.5339 0.4048	One-way ANOVA: P= 8.0e-9 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P= 8.1e-8 WT-Ang II vs D1KO- Ang II,

				P= 3.1e-6
S2-FS%	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.2402 0.4271 0.4239 0.5466	One-way ANOVA: P= 1.7e-11 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.59 WT-Ctrl vs WT-Ang II, P= 3.5e-11 WT-Ang II vs D1KO- Ang II, P= 1.3e-7
S2-LVIDd	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.9740 0.6824 0.3033 0.8833	One-way ANOVA: P= 4.7e-12 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P= 7.9e-11 WT-Ang II vs D1KO- Ang II, P= 1.4e-10
S2-LVIDs	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.2260 0.4420 0.3036 0.6935	One-way ANOVA: P=1.6e-12 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P= 0.99 WT-Ctrl vs WT-Ang II, P=1.8e-11 WT-Ang II vs D1KO- Ang II, P= 1.3e-9
S2-IVSD	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.0767 0.8289 0.3972 0.1179	One-way ANOVA: P=1.1e-9 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P=5.8e-9 WT-Ang II vs D1KO- Ang II, P=4.6e-7
S2-PWd	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.6276 0.4512 0.0576 0.6380	One-way ANOVA: P=1.5e-7 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.054 WT-Ctrl vs WT-Ang II, P=4.1e-5 WT-Ang II vs D1KO- Ang II, P=6.2e-5
S2-HW/BW	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.5118 0.4219 0.7169 0.4386	One-way ANOVA: P= 3.2e-6 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P= 0.71 WT-Ctrl vs WT-Ang II, P= 7.4e-5 WT-Ang II vs D1KO- Ang II, P= 6.56e-5
S2-HW/TL	WT-Ctrl D1KO-Ctrl WT-Ang II D1KO- Ang II	7 7 7 7	0.1634 0.8416 0.6222 0.3991	One-way ANOVA: P= 1.1e-6 Tukey's multiple comparisons WT-Ctrl vs D1KO-Ctrl, P=0.99 WT-Ctrl vs WT-Ang II, P= 7.3e-6 WT-Ang II vs D1KO- Ang II, P=1.90e-5
S3-EF%	WT-BM→WT Ang II D1KO BM→WT Ang	7 7	0.5073 0.1140	Unpaired Student`s t-test, Two-tailed

	II			P=2.8e-7
S3-FS%	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.3704 0.5970	Unpaired Student`s t-test, Two-tailed P=2.5e-8
S3-LVIDd	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.0315 0.0307	Mann-Whitney U-test, Two-tailed P=2.3e-3
S3-LVIDs	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.4391 0.1038	Unpaired Student`s t-test, Two-tailed P= 6.8e-6
S3-IVSD	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.8797 0.3190	Unpaired Student`s t-test, Two-tailed P= 1.3e-4
S3-PWd	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.7848 0.4824	Unpaired Student`s t-test, Two-tailed P=3.9e-3
S3-HW/BW	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.2070 0.6723	Unpaired Student`s t-test, Two-tailed P= 3.71e-4
S3-HW/TL	WT-BM→WT Ang II D1KO BM→WT Ang II	7 7	0.6293 0.2335	Unpaired Student`s t-test, Two-tailed P=1.4e-2
S4-EF%	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.8199 0.1513 0.2228	One-way ANOVA: P= 7.4e-9 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=4.9e-9 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=5.5e-6
S4-FS%	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.7889 0.1969 0.8506	One-way ANOVA: P= 5.8e-8 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=4.6e-8 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=1.1e-5
S4-LVIDd	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.0666 0.4579 0.4864	One-way ANOVA: P= 1.9e-5 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=1.2e-5 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=9.6e-3
S4-LVIDs	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.0983 0.7936 0.0624	One-way ANOVA: P=1.9e-5 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=9.8e-11 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=7.4e-8
S4-IVSD	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.0768 0.4677 0.7688	One-way ANOVA: P=1.3e-3 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=2.4e-3 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=4.7e-3
S4-PWd	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.4260 0.4530 0.0413	One-way ANOVA: P=0.36 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=0.34 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=0.61

S4-HW/BW	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.4259 0.4437 0.6288	One-way ANOVA: P=1.9e-5 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=3.6e-5 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=1.4e-4
S4-HW/TL	WT-Ctrl-Vehicle WT-Ang II-Vehicle WT-Ang II-Losartan	7 7 7	0.3942 0.2379 0.1185	One-way ANOVA: P= 6.3e-6 Tukey's multiple comparisons WT-Ctrl-Vehicle vs. WT-Ang II-Vehicle, P=4.7e-5 WT- Ang II-Vehicle vs. WT-Ang II- Losartan, P=1.4e-5