Circulation Research

ORIGINAL RESEARCH

Dectin-1 Acts as a Non-Classical Receptor of Ang II to Induce Cardiac Remodeling

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BACKGROUND: Cardiac remodeling in heart failure involves macrophage-mediated immune responses. Recent studies have shown that a PRR (pattern recognition receptor) called dectin-1, expressed on macrophages, mediates proinflammatory responses. Whether dectin-1 plays a role in pathological cardiac remodeling is unknown. Here, we identified a potential role of dectin-1 in this disease.

METHODS: To model aberrant cardiac remodeling, we utilized mouse models of chronic Ang II (angiotensin II) infusion. In this model, we assessed the potential role of dectin-1 through using D1KO (dectin-1 knockout) mice and bone marrow transplantation chimeric mice. We then used cellular and molecular assays to discover the underlying mechanisms of dectin-1 function.

RESULTS: We found that macrophage dectin-1 is elevated in mouse heart tissues following chronic Ang II administration. D1KO mice were significantly protected against Ang II–induced cardiac dysfunction, hypertrophy, fibrosis, inflammatory responses, and macrophage infiltration. Further bone marrow transplantation studies showed that dectin-1 deficiency in bone marrow–derived cells prevented Ang II–induced cardiac inflammation and dysfunction. Through detailed molecular studies, we show that Ang II binds directly to dectin-1, causing dectin-1 homodimerization and activating the downstream Syk (spleen tyrosine kinase)/NF-κB (nuclear factor kappa B) signaling pathway to induce expression of inflammatory and chemoattractant factors. Mutagenesis studies identified R184 in the C-type lectin domain to interact with Ang II. Blocking dectin-1 in macrophages suppresses Ang II–induced inflammatory mediators and subsequent intercellular cross talk with cardiomyocytes and fibroblasts.

CONCLUSIONS: Our study has discovered dectin-1 as a new nonclassical receptor of Ang II and a key player in cardiac remolding and dysfunction. These studies suggest that dectin-1 may be a new target for treating hypertension-related heart failure.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: angiotensin II ■ dectin-1 ■ heart failure ■ inflammation ■ macrophages ■ mutagenesis

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Pathological cardiac remodeling is recognized as the critical process in the clinical course of heart failure (HF)—a leading cause of death worldwide.[1] Ang II (angiotensin II) is an important mediator of HF as it causes cardiomyocyte hypertrophy and cardiac fibrosis and arrhythmia.[2] Increasing evidence suggests the involvement of inflammatory responses downstream of Ang II.[3,4] Of the various immune cells, circulating monocytes and tissue-resident macrophages appear to be critical in cardiac disease.[5–7] For example, Ang II–induced hypertension and end organ damage are dependent on monocyte/macrophage inflammatory signaling.[8,9] Kriska et al[10] also found that macrophage 12-hydroxyeicosatetraenoic acid contributes to Ang II–mediated hypertension

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Novelty and Significance

What Is Known?

- Ang II (angiotensin II)–induced cardiac remodeling involves macrophage-mediated immune responses.
- A pattern recognition receptor called dectin-1, expressed on macrophages, mediates proinflammatory responses.
- Besides the classical β-glucan ligands, dectin-1 is also involved in non-pathogen–mediated sterile inflammation, and whether dectin-1 is involved in Ang II–induced cardiac inflammation and remodeling is largely unknown.

What New Information Does This Article Contribute?

- In conditions of Ang II–induced pathological cardiac remodeling, dectin-1 expression is increased in heart with macrophage infiltration.
- Macrophage dectin-1 deficiency prevents inflammatory cytokine induction and subsequent cardiac fibrosis and cellular hypertrophy in models of heart failure.
- Ang II activates the dectin-1/Syk (spleen tyrosine kinase) pathway by binding to dectin-1 directly. Key residues involved in this interaction include R184 in the C-type lectin domain.

Pathological cardiac remodeling is recognized as the critical process in the clinical course of heart failure—a leading the cause of death worldwide. Our study identified dectin-1 as a novel receptor of Ang II for inducing inflammation and cardiac remodeling, providing important insights into the potential therapeutic targets for the prevention of hypertension-related heart failure. Selective inhibition of dectin-1 may be a novel approach to prevent myocardial remodeling in patients.

Nonstandard Abbreviations and Acronyms

Ang II	angiotensin II
AT1R	angiotensin II receptor type 1
AT2R	angiotensin II receptor type 2
Bio-Ang II	biotinylated angiotensin II
BMDM	bone marrow–derived macrophage
CXCL1	C-X-C motif ligand 1
D1KO	dectin-1 knockout
HF	heart failure
NF-κB	nuclear factor kappa B
Syk	spleen tyrosine kinase
WT	wild type

and that CXCL1 (C-X-C motif ligand 1) and CXCR2 (CXC receptor 2) cause infiltration of monocytes in the heart, downstream of Ang II, to aggravate cardiac remodeling.[8] Similarly, Ang II–mediated cardiac remodeling is seen in pressure overload–driven HF.[11] These studies indicate an important role of Ang II and its signaling in monocytes/macrophages in the context of HF. However, the precise mechanism and direct receptors by which Ang II induces the genesis of inflammation in macrophages are not fully understood.

A class of receptors that play an important role in atypical functioning of macrophages are PRRs (pattern recognition receptors). Dectin-1—a member of the C-type lectin family of PRRs—is expressed selectively on myeloid-monocytic lineage cells including macrophages.[12] Dectin-1 possesses an immunoreceptor tyrosine-based activation motif (ITAM)-like motif in its cytoplasmic moiety and activates Syk (spleen tyrosine kinase), eventually activating NF-κB (nuclear factor kappa B) to induce proinflammatory molecules.[13] Originally, dectin-1 has been found to recognize β-glucans in fungal pathogens and then elicit antifungal proinflammatory immune responses. Recent studies have shown that besides the classical β-glucan ligands, dectin-1 is also involved in non–pathogen-mediated sterile inflammation.[14] For example, in models of intracerebral hemorrhage, dectin-1 was shown to cause macrophage polarization and the activated Syk/NF-κB pathway contributed to brain injury.[15] In a model of cardiac ischemia-reperfusion, Fan et al[16] found dectin-1 to be expressed on macrophages and be in macrophage polarization and neutrophil infiltration. It has been reported that ovalbumin-induced airway inflammation is ameliorated in dectin-1 knockout (D1KO) mice.[17] Despite these notable findings of dectin-1 in the heart, respiratory, and neurological disorders,[18] endogenous ligands activating dectin-1 in sterile inflammatory conditions have not been identified. In addition, whether dectin-1 is involved in Ang II–induced cardiac inflammation and remodeling is largely unknown.

In the present study, we investigated the expression and function of dectin-1 in mouse models of Ang II–induced cardiac remodeling. Our results show that dectin-1 levels are elevated in cardiac macrophages. Deficiency in dectin-1 protected mice from Ang II–induced cardiac dysfunction. Importantly, our detailed mechanistic studies in macrophages showed that Ang II binds directly to dectin-1 to initiate Syk/NF-κB signaling and the induction of inflammatory cytokines. These studies have identified dectin-1 as a

new nonclassical Ang II receptor and shown that Ang II/dectin-1 interaction plays a critical role in pathological cardiac remodeling.

METHODS

Data Availability

The data, analytical methods, and study materials are available from the corresponding author on reasonable request. Specifically, the statistical information is listed in Table S5.

A detailed description of the experimental methods, materials, experimental procedures, mouse models of cardiac dysfunction, and cell culture is provided in the Supplemental Material. Primers for quantitative real-time PCR (qPCR) assay are presented in Table S1.

RESULTS

Macrophage Dectin-1 Is Elevated in Heart Tissues Following Chronic Ang II Administration in Mice

To identify the potential involvement of dectin-1 in models of aberrant cardiac remodeling, we first measured the levels of dectin-1 in heart tissues. To do this, we infused Ang II in C57BL/6 mice for 4 weeks. Lysates prepared from heart tissues showed elevated levels of dectin-1 following Ang II administration (Figure 1A and 1B). mRNA levels of dectin-1 also showed increases (Figure 1C). We also performed analysis of dectin-1 expression from a previous study in which Ang II was infused for 2 weeks. Similar to our study, dectin-1 mRNA levels were found to be increased in heart tissues of mice challenged with Ang II (Figure S1A). We next performed immunofluorescence staining of mouse heart tissues from our study and confirmed dectin-1 increase following Ang II administration (Figure 1D and 1E).

Dectin-1 has been reported to be highly expressed by myeloid-lineage cells such as macrophages.[12] Therefore, we expected macrophages to be the primary source of increased dectin-1 in heart tissues. To confirm this, we performed double immunofluorescence staining of heart tissues for dectin-1 and markers of macrophages (CD68), cardiomyocytes (cTNT [cardiac troponin T]), and fibroblasts (vimentin). From this study, we found that Ang II induced increased infiltration of $CD68^+$ macrophages in cardiac tissue and confirmed macrophages as the cell types expressing dectin-1 in heart tissues (Figure 1F through 1H). These results also indicate that increased dectin-1 level in heart tissues may come from the increased cardiac macrophages in Ang II–challenged mice. We also performed double immunofluorescence staining of the heart tissues from a patient with HF. The data validated that dectin-1 was expressed on CD68-positive macrophages in the patient heart (Figure S1B). We then probed various primary cell types and lines for dectin-1 expression. Specifically, we prepared bone marrow–derived monocytes and differentiated the cells into macrophages. We also harvested primary cardiomyocytes and fibroblasts from mouse hearts. In addition, we examined the RAW 264.7 macrophage line and H9c2(2-1) cardiomyocyte-like cell line. Our results show that mouse primary bone marrow–derived macrophages (BMDMs) and RAW cells robustly express dectin-1 and downstream Syk protein (Figure 1I). Collectively, these results demonstrate that Ang II upregulates dectin-1 expression in the heart, primarily in CD68-positive macrophages.

Dectin-1 Deficiency Prevents Ang II–Induced Cardiac Dysfunction

To identify functional significance of dectin-1 in Ang II–induced cardiac remodeling, we utilized D1KO mice. These mice show no detectable dectin-1 protein in heart tissues (Figure S2A) and barely detectable mRNA (Figure S2B). D1KO mice were administrated Ang II for 4 weeks and compared with wild type (WT) mice. No changes to body weights were observed in the 4-week study (Figure S2C). However, 4 weeks of Ang II infusion caused increased systolic blood pressure in both WT and D1KO mice (Figure S2D). Levels of Ang II in heart tissues and serum were also increased in both WT and D1KO mice infused Ang II (Figure S2E and S2F). These data indicated that D1KO did not affect the hypertension and in vivo Ang II level.

Next, we assessed cardiac function in mice through echocardiography (Figure 2A, top). Ang II reduced ejection fraction (EF%) and fractional shortening (FS%) and increased left ventricular internal diameter at end diastole in WT mice, while these Ang II–impaired functional parameters were significantly reversed by dectin-1 knockout in D1KO mice (Figure 2A through 2D; Table S2). We then examined the harvested heart tissues for histopathological alterations (Figure 2A, middle). Staining of heart tissues with hematoxylin and eosin, collagen dye picrosirus red, and Masson trichrome showed that Ang II–induced WT mice develop structural alterations, most notably as cardiac hypertrophy and fibrosis (Figure 2E through 2G). Cardiomyocytes in Ang II–induced WT mice also showed hypertrophy as assessed through WGA (wheat germ agglutinin) staining (Figure 2E and 2H). These structural alterations were significantly blunted in D1KO mice infused with Ang II, in agreement with the cardiac functional tests. Analysis of protein lysates from heart tissues confirmed these results and showed that key mediators of hypertrophy (MyH7) and fibrosis (Col-1 [collagen-1] and TGF-β1 [transforming growth factor beta 1]) in D1KO mice show no responses to Ang II, unlike WT mice

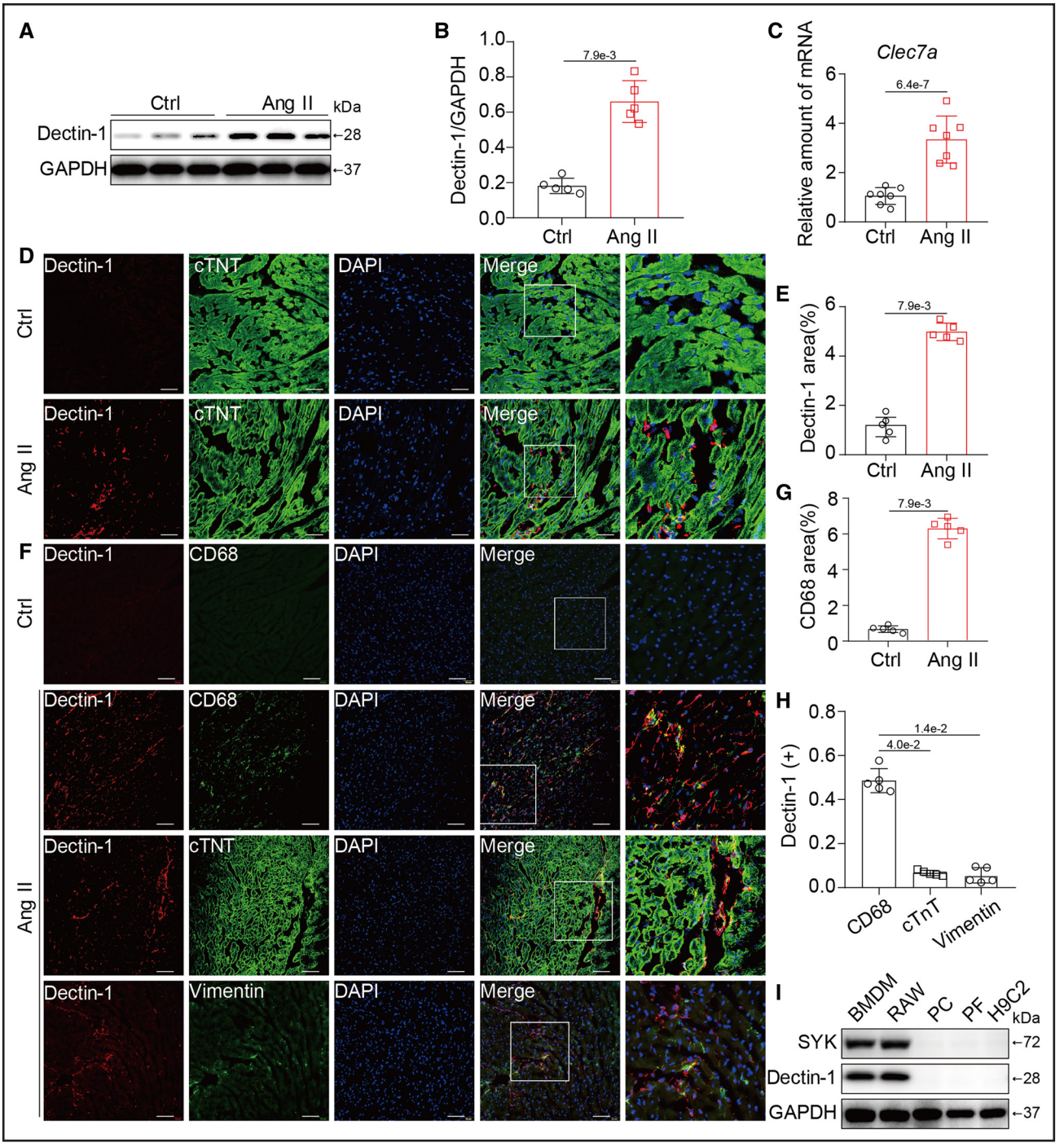


Figure 1. Dectin-1 levels are increased in the heart tissues following Ang II (angiotensin II) infusion.
A, C57B/L6 mice were administered Ang II for 4 weeks. Representative Western blot analysis of dectin-1 level in heart tissue of mice was performed with GAPDH as loading control (each lane is from an independent mouse heart). **B**, Densitometric quantification of blots in **A**. **C**, mRNA levels of *Clec7a* (dectin-1) in the heart tissues of mice. Transcripts were normalized to *Actb*. **D**, Representative immunofluorescence staining of dectin-1 (red) and cTNT (cardiac troponin T; green) in heart tissues. Sections were counterstained with DAPI (blue; scale bars, 50 μm). **E**, Quantification of dectin-1 immunoreactive area (%) in **D**. **F**, Representative immunofluorescence staining of dectin-1 (red) and CD68 (green) in heart tissues of control mice (first-line images). Representative immunofluorescence staining of dectin-1 (red) and CD68 (green, second-line images), cTNT (green, third-line images), and vimentin (green, fourth-line images) in heart tissues of Ang II–infused mice. Sections were counterstained with DAPI (blue; scale bars, 100 μm). **G**, Quantification of double immunoreactivity showing levels of CD68-positive area in the first- and second-line images of **F**. **H**, Quantification of double immunoreactivity showing levels of dectin-1–positive plus CD68–, cTNT–, and Vimentin-positive area in second-, third-, and fourth-line images of **F**. **I**, Representative Western blot analysis of Syk (spleen tyrosine kinase) and dectin-1 in mouse bone marrow–derived macrophages (BMDMs), RAW264.7 macrophages, primary cardiomyocytes (PCs), primary cardiac fibroblasts (PFs), and H9c2 cardiomyocyte-like cell line without Ang II challenge. GAPDH was used as control. **A**–**G**, n=5 to 7 mice per group; for **B**, **E**, and **G**, Mann-Whitney *U* test; for **C**, Student *t* test; for **H**, Kruskal-Wallis followed by Dunn post hoc multiple comparisons test; *P* values indicated.

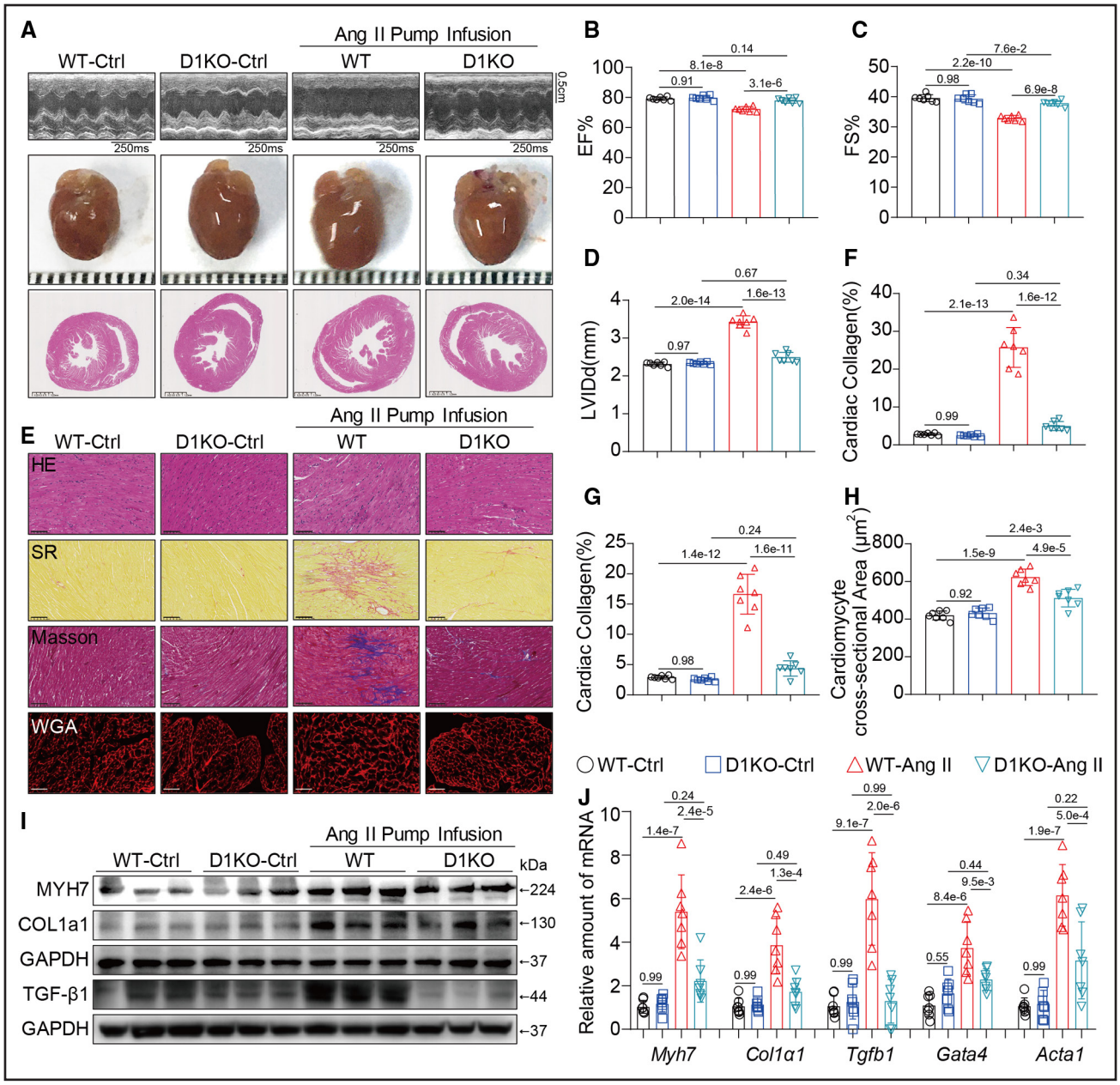


Figure 2. Dectin-1 deficiency protects against pathological cardiac remodeling induced by Ang II (angiotensin II). Wild type (WT) and dectin-1 knockout (D1KO) mice were administered with or without Ang II for 4 weeks. **A**, **Top**, Representative M-mode echocardiographic images. **Middle**, Harvested heart tissues from mice. **Bottom**, Representative hematoxylin and eosin (H&E)–stained heart sections. **B**–**D**, Echocardiographic analysis of LV ejection fraction (EF%), fractional shortening (FS%) value, and LV internal diameter at end diastole (LVIDd). **E**, Representative images of heart tissues from WT and D1KO mice stained with H&E, picrosirius red, Masson trichrome, and WGA (wheat germ agglutinin; scale bars, 100 μm). **F** and **G**, Quantification of interstitial fibrotic area (%) as determined by sirius red staining of heart sections (**F**) and Masson trichome staining (**G**). **H**, Quantitative analysis of myocyte area from WGA-stained sections in **E**. A minimum of 100 cells were measured from different visual fields of 4 samples per group. **I**, Representative Western blot analysis of MYH7 (myosin heavy chain 7), Col1a1 (collagen type I alpha 1), and TGF-β1 (transforming growth factor beta 1) in heart tissues. GAPDH was used as loading control. **J**, mRNA levels of *Myh7*, *Col1a1*, and *Tgfb1* in heart tissues of mice. Transcripts were normalized to *Actb*. **A**–**J**, n=7 per group, 1-way ANOVA followed by Tukey post hoc tests; number of comparisons, 10; *P* values indicated.

(Figure 2I; Figure S2G through S2I). Similarly, mRNA levels of genes involved in fibrosis and hypertrophy are induced by Ang II in WT mice but not in D1KO mice (Figure 2J). These results demonstrate that dectin-1 deficiency protects against Ang II–induced cardiac dysfunction and remodeling.

Dectin-1 Knockout Reduces Inflammation in Hearts of Ang II–Infused Mice

Previous studies have shown that Ang II increases inflammation and monocyte infiltration in heart tissues.[19] We investigated whether dectin-1 deficiency protected

heart tissues by suppressing infiltration and associated inflammatory responses. First, we performed the transcriptome RNA sequencing using the heart tissues from WT and D1KO mice with or without Ang II challenge. As shown in Figure 3A and 3B and Figure S3A and S3B, the RNA sequencing and enrichment pathway analysis indicates that dectin-1 deficiency downregulated Ang II–induced expression of genes involved in inflammation and leukocyte recruitment in mouse hearts. Next, we measured the levels of chemoattractant factors CSF3 (colony stimulating factor-3; also known as G-CSF [granulocyte colony stimulating factor]),[20] CXCL1, and CXCR2[8] in the heart tissues of WT and D1KO mice infused with Ang II. Although Ang II induced the levels of these chemoattractants in WT mice, no increases were seen in D1KO mice (Figure 3B; Figure S4A through S4C). Furthermore, mRNA levels of these factors were induced by Ang II in WT mice but not in D1KO mice (Figure 3C). Macrophage number, as assessed by CD68 and F4/80 immunoreactivity, was also lower in heart tissues of D1KO mice compared with WT mice, when administered Ang II (Figure 3E and 3F; Figure S4D and S4E). We performed immunofluorescence staining of heart tissues to localize CXCL1 and G-CSF (granulocyte colony stimulating factor) to CD68-positive macrophages. Our results show that Ang II increases CD68-positive and CXCL1- and G-CSF–positive cells in WT mouse hearts (Figure S5A through S5D). As expected, D1KO mice did not show this increase in response to Ang II. Similar to macrophage increase, Ly-6G–positive neutrophils were also increased in WT heart tissues following Ang II infusion (Figure 3G; Figure S5E). The levels in D1KO hearts upon Ang II challenge, however, were indistinguishable from unchallenged WT and D1KO mice. This suppressed neutrophil recruitment in D1KO mice was associated with reduced levels of inflammatory cytokines in hearts. Immunohistochemical staining of heart tissues for TNF-α (tumor necrosis factor alpha) showed the same pattern (Figure 3H; Figure S4F). As expected, D1KO significantly inhibited Ang II–increased mRNA levels of proinflammatory cytokines (*Tnfa*), Th17-polarizing cytokines (*Il-23*, *Il-6*, and *Il-1*), and neutrophil-recruitment cytokines (*Il-17*) in mouse heart tissues (Figure 3I).

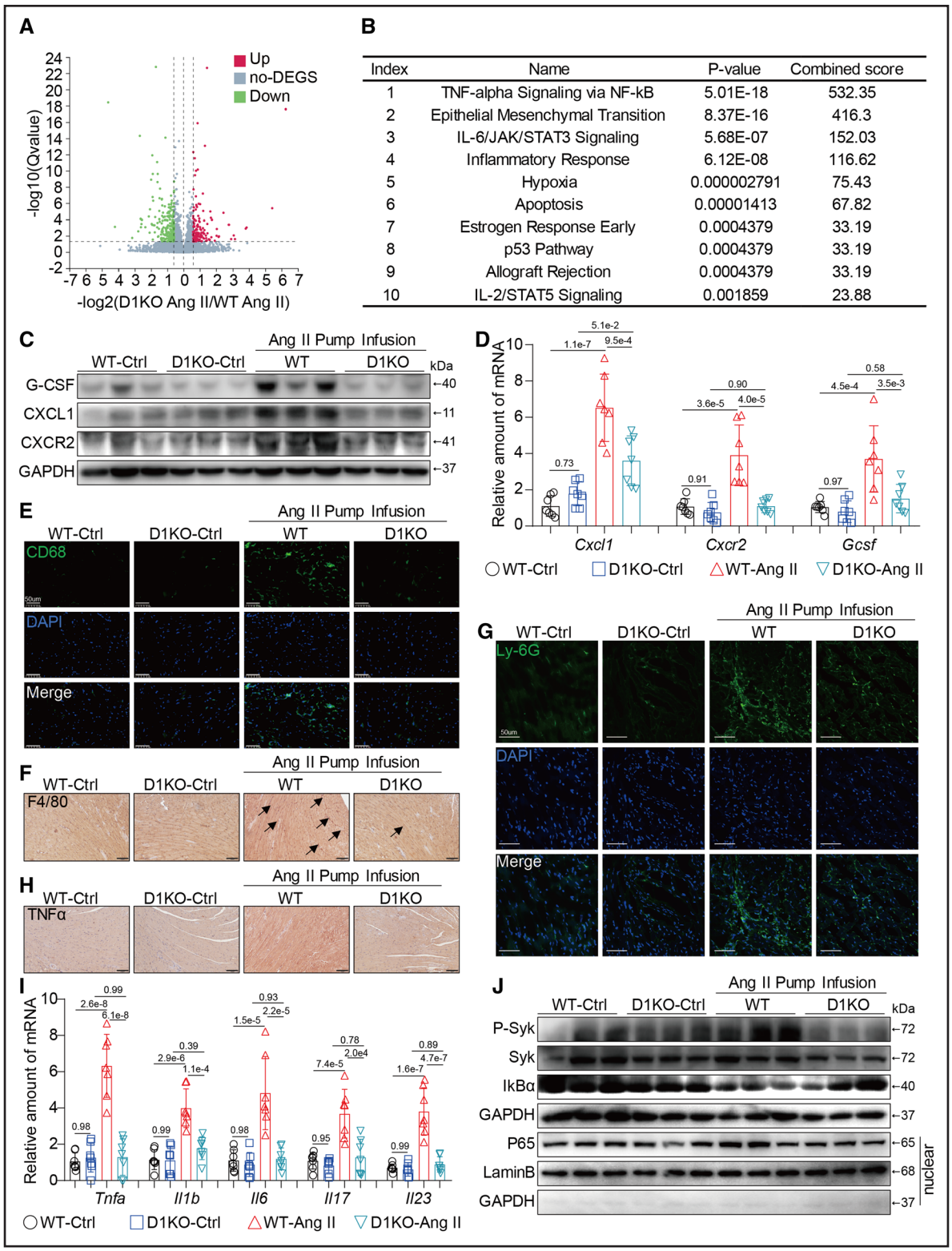
We next explored whether the canonical downstream Syk/NF-κB pathway[13] is activated by dectin-1 following Ang II infusion. Heart tissues from WT mice infused with Ang II showed increased phospho-Syk (p-Syk), reduced IκB-α (inhibitor of κB-α), and increased nuclear p65, indicating activation of Syk/NF-κB by Ang II (Figure 3J; Figure S5G through S5I). Unlike WT mice, D1KO mice did not show any changes in p-Syk levels or measures of NF-κB activation. Together, these results demonstrate that Ang II activates dectin-1 to induce Syk phosphorylation and NF-κB activation, leading to increased proinflammatory and chemoattractant proteins and immune cell recruitment.

Dectin-1 Expressed on Bone Marrow–Derived Cells Mediates Ang II–Induced Cardiac Inflammation and Remodeling

To further investigate the critical role of dectin-1 in macrophages, we conducted the bone marrow transplantation study. WT mice were given bone marrow from D1KO or WT mice to generate the WT mice with dectin-1–deficient bone marrow (D1KO BM→WT) and the control WT mice with WT bone marrow (WT BM→WT), respectively (Figure S6A and S6B). Echocardiography assessment showed that bone marrow transplantation did not affect the basal cardiac function in the chimeric mice (Figure S6C through S6E). Both WT BM→WT and D1KO BM→WT chimeric mice were infused with Ang II for 4 weeks. Analysis of body weight and systolic blood pressure showed no difference in both chimeric mice (Figure S7A and S7B). Assessment of cardiac function through echocardiography (Figure 4A, top) showed that D1KO BM→WT mice are resistant to Ang II–induced changes in EF%, FS%, and left ventricular internal diameter at end diastole (Figure 4A through 4C; Table S3). Transplantation of D1KO bone marrow cells to WT mice led to a considerable decrease in the heart size compared with the control chimeric mice (Figure S7C). Histologic staining showed that both cardiomyocyte hypertrophy and cardiac fibrosis are decreased in D1KO BM→WT mice with Ang II infusion (Figure 4D). Furthermore, mRNA and protein levels of cardiac remodeling factors and chemoattractants were significantly normalized in bone marrow–specific D1KO mice upon Ang II (Figure 4F through 4H; Figure S7D through S7F). Dectin-1 deficiency in macrophages also reduced inflammatory cell infiltration in Ang II–challenged mouse hearts (Figure 4I; Figure S7G and S7H). Similar signaling pathways confirmed that Ang II–induced inflammatory gene expression, Syk phosphorylation, and NF-κB activation in the heart tissues were significantly inhibited in D1KO BM→WT mice (Figure S7I through S7K). Collectively, these studies demonstrate that dectin-1 in myeloid cells critically contributes to Ang II–induced cardiac inflammation and injury.

Ang II Activates the Dectin-1/Syk Pathway in an AT1R-Independent Manner

Based on the apparent functional role of dectin-1 in cardiac remodeling and inflammation, we explored the potential molecular mechanisms underlying dectin-1 activation by Ang II. We exposed BMDMs to 1 μM Ang II and observed Syk phosphorylation peaking at 45 minutes (Figure 5A; Figure S8A). It is notable that Ang II stimulation directly activated dectin-1 but did not affect dectin-1 expression in macrophages (Figure 5A). Furthermore, we tested the effect of increasing Ang II concentrations and noted maximal response with 1 μM Ang II (Figure 5B; Figure S8B). We then treated BMDMs with



Index	Name	P-value	Combined score
1	TNF-alpha Signaling via NF-kB	5.01E-18	532.35
2	Epithelial Mesenchymal Transition	8.37E-16	416.3
3	IL-6/JAK/STAT3 Signaling	5.68E-07	152.03
4	Inflammatory Response	6.12E-08	116.62
5	Hypoxia	0.000002791	75.43
6	Apoptosis	0.00001413	67.82
7	Estrogen Response Early	0.0004379	33.19
8	p53 Pathway	0.0004379	33.19
9	Allograft Rejection	0.0004379	33.19
10	IL-2/STAT5 Signaling	0.001859	23.88

Figure 3. Dectin-1 knockout (D1KO) reduces inflammation in hearts of Ang II (angiotensin II)–infused mice.
Wild type (WT) and D1KO mice were administered with or without Ang II for 4 weeks. **A**, Volcano plots of RNA-seq FDR values versus $\log_2$ fold-change expression for individual genes in hearts from D1KO-Ang II vs WT-Ang II mice. **B**, The top 10 pathways obtained from the MSigDB Hallmark 2020 enrichment analysis of the changed genes in hearts from D1KO-Ang II vs WT-Ang II mice. **C**, Representative Western blot analysis of G-CSF (granulocyte colony stimulating factor), CXCL1 (C-X-C motif ligand 1), and CXCR2 (CXC receptor 2) in heart tissues. GAPDH was used as loading control. **D**, mRNA levels of *Cxcl1*, *Cxcr2*, and *Gcsf* (*Csf3*) in heart tissues of mice. Transcripts were normalized to *Actb*. **E**, Representative immunofluorescence staining of heart tissues for macrophage marker CD68 (green). Slides were counterstained with DAPI (blue; scale bars, 50 μm). **F**, Representative chromogen immunohistochemical staining of heart tissues for F4/80. (*Continued*)

D1Ab (dectin-1 neutralizing antibody) and then exposed the cells to Ang II. We observed that D1Ab prevented Ang II–induced p-Syk levels (Figure S8C and S8D). Similar effects were confirmed using BMDMs from WT or D1KO mice in response to Ang II (Figure S8E through S8H). As a PRR, dectin-1 homodimerization and Syk recruitment are 2 hallmarks of dectin-1 activation. Next, we transfected HEK-293T cells with Flag- and HA-tagged dectin-1 (Figure S8I) and exposed the cells to Ang II. Coimmunoprecipitation showed Ang II–induced Flag-HA interaction indicating dectin-1 homodimerization (Figure 5C; Figure S8J). Importantly, Ang II–induced dectin-1 dimerization appeared equivalent to the effects of a direct dectin-1 agonist curdlan[21] (Figure S8K and S8L). Downstream of dectin-1 dimerization, Ang II increased the interaction between dectin-1 and Syk in BMDMs in a time manner and reached peaking at 45 minutes (Figure 5D; Figure S8M). Pretreatment of macrophages with D1Ab reduced the interaction between dectin-1 and Syk induced by Ang II, as expected (Figure S8N and S8O). These results show that Ang II activates dectin-1 signaling by inducing dectin-1 dimerization and increases its interaction with downstream mediator Syk.

To understand how Ang II activates dectin-1, we explored whether AT1R (Ang II receptor type 1) and AT2R (Ang II receptor type 2) were involved in linking Ang II to dectin-1. Most of the Ang II functions in cardiac remodeling are believed to be mediated through classical ATR1.[22] However, recent studies suggest that Ang II may associate with signaling proteins independent of AT1R.[23] We knocked down the expression of AT1R in BMDMs (Figure S9A). Interestingly, exposure of these cells to Ang II showed even greater increase in dectin-1/Syk interaction and Syk phosphorylation (Figure 5E and 5F; Figure S9B and S9C). In addition, Ang 1-9 (angiotensin 1–9) and Ang 1-7 (angiotensin 1–7) have been reported to function through interacting and activating AT2R.[24] We also examined the possible involvement of ATR2 in Ang II–induced dectin-1 activation using a specific AT2R antagonist (PD123319, at 5 μmol/L) to fully block AT2R activity in BMDMs.[25] Similar to the AT1R knockdown, inhibition of AT2R by PD123319 slightly increased dectin-1/Syk interaction and Syk phosphorylation in Ang II–challenged BMDMs (Figure S10A through S10D). These results suggest that Ang II–induced dectin-1/Syk activation is independent of both AT1R and AT2R.

We further exclude the effect of AT1R—the most classical receptor of Ang II—in Ang II–infused mouse model treated with an AT1R blocker losartan. Our results showed that administration of losartan, as we expected, significantly reduced the Ang II–raised blood pressure (Figure S11A), which resulted in subsequent heart protection, including preserved cardiac function and reduced cardiac hypertrophy and fibrosis, in the Ang II–infused mouse heart (Figures S11B through S11M and S12A through S12D; Table S4). Losartan administration also reduced cardiac inflammation via reducing blood pressure (Figure S12E through S12H). Most importantly, administration of losartan failed to affect dectin-1/Syk signaling axle, as evidenced by the fact that losartan could not inhibit Syk phosphorylation and dectin-1/Syk complex formation in Ang II–infused mouse hearts (Figure S12I through S12N). These data have validated that Ang II–induced dectin-1/Syk activation is AT1R independent, indicating that Ang II causes heart injuries through 2 independent pathways, AT1R-mediated hemodynamic changes and dectin-1–mediated chronic inflammation.

Ang II Directly Binds to Dectin-1 Protein

Then, we hope to visualize the molecular relationship between Ang II and dectin-1 using a biotinylated Ang II (Bio-Ang II). The Bio-Ang II retains Ang II dectin-1–activating activity as is evident by increased Syk phosphorylation in BMDMs exposed to Bio-Ang II (Figure S13A and S13B). Following exposure of macrophages with Bio-Ang II or Biotin, we performed immunofluorescence costaining for Bio-Ang II, dectin-1, and AT1R, all of which were mainly located in the cell membrane (Figure 5G). Interestingly, a potential colocalization of dectin-1 and Bio-Ang II in cell surface (Figure 5G, middle) was observed, suggesting a potential interaction between Ang II and dectin-1.

We further utilized an ELISA-based system to identify direct Ang II/dectin-1 interaction. This ELISA-based system was used in which we captured rhDectin-1 (recombinant human Dectin-1) and added Bio-Ang II. Binding was then determined by streptavidin-HRP. Our results show that Bio-Ang II interacts with rhDectin-1, and this interaction can be competitively inhibited by label-free Ang II (Figure 5H). To strengthen this finding, we used biotinylated protein interaction pull-down assays. Bio-Ang II was added to streptavidin-agarose beads, and lysates from BMDMs (Figure 5I), HEK-293T cells expressing Flag-tagged dectin-1 (Figure 5J), and heart tissues of WT mice treated with or

Figure 3 Continued. Tissue sections were counterstained with hematoxylin (blue). Arrows indicate immunoreactivity (scale bars, 100 μm). **G**, Representative immunofluorescence staining of Ly-6G (green) in the heart tissue of mice. Sections were counterstained with DAPI (blue; scale bars, 50 μm). **H**, Representative chromogen immunohistochemical staining of heart tissues for TNF-α (tumor necrosis factor alpha). Tissue sections were counterstained with hematoxylin (blue; scale bars, 100 μm). **I**, mRNA levels of *Tnfa, Il1b, Il6, Il17*, and *Il23* in heart tissues of mice. Transcript levels normalized to *Actb*. **J**, Representative Western blot analysis of phospho-Syk (p-Syk), Syk (spleen tyrosine kinase), and IκB-α (inhibitor of κB-α) in mouse heart tissue lysates. GAPDH was used as loading control. Lower blots showing P65 in nuclear extracts prepared from mouse heart tissues, with lamin B as loading control. **A**–**J**, n=7 per group; 1-way ANOVA followed by Tukey post hoc tests; number of comparisons, 10; *P* values indicated.

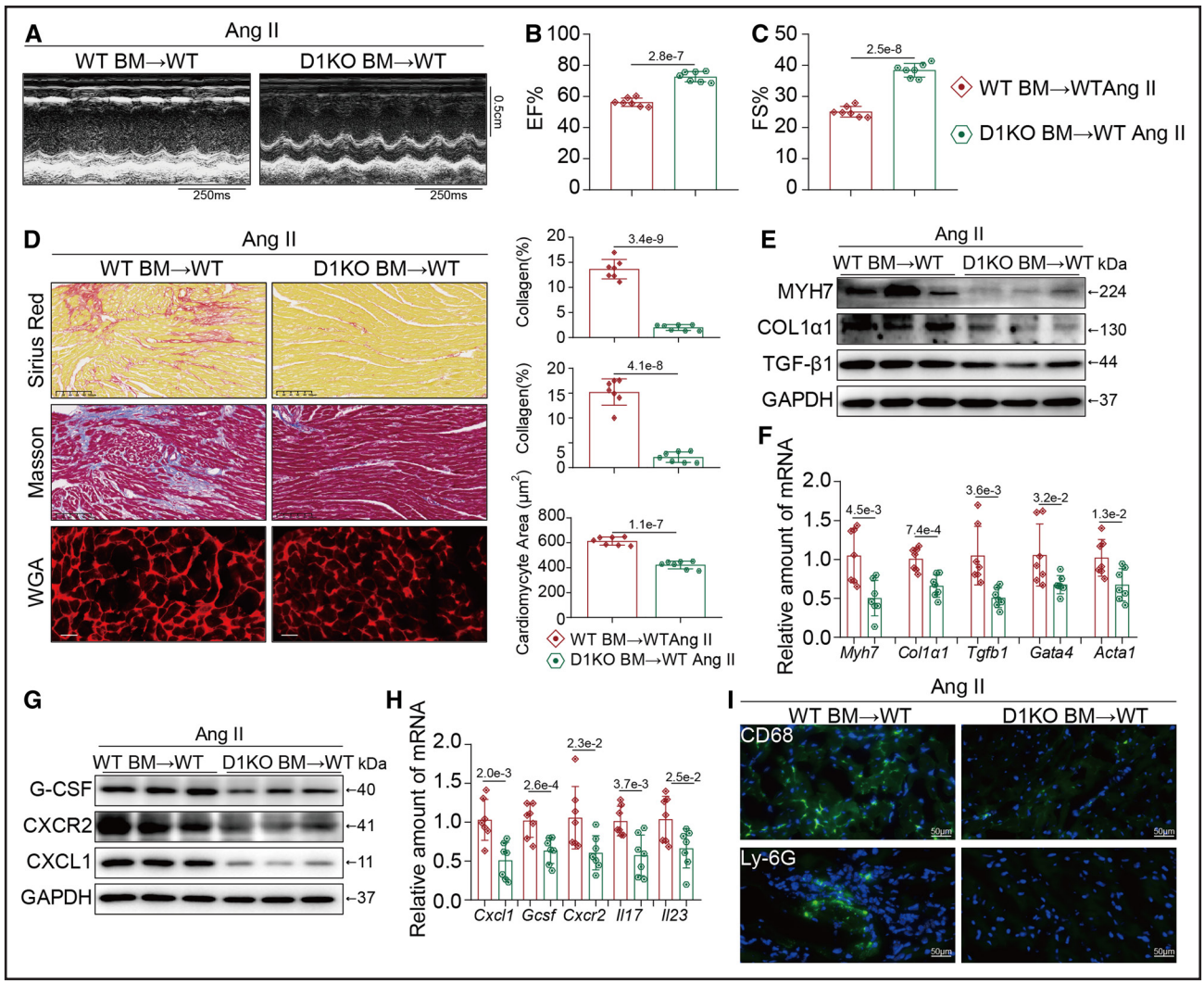


Figure 4. Dectin-1 expressed on bone marrow (BM)–derived cells mediates Ang II (angiotensin II)–induced cardiac remodeling. Bone marrow transplantation from dectin-1 knockout (D1KO) mice or wild type (WT) mice to WT mice was performed to establish the myeloid cell–specific dectin-1 KO mice (D1KO BM→WT) and control mice (WT BM→WT). Both WT BM→WT and D1KO BM→WT mice were infused with Ang II for 4 weeks. **A**, Representative M-mode echocardiographic images of mice from both groups. **B** and **C**, LV ejection fraction (EF%) and fractional shortening (FS%) value from echocardiographic analysis of mice. **D**, Representative staining images of mouse heart tissues for sirius red, Masson trichrome, and WGA (wheat germ agglutinin; scale bars, 100 μm). Quantification of interstitial fibrotic area (%) or myocyte area is shown in **right**, respectively. A minimum of 100 cells were measured from different visual fields of 7 samples per group. **E**, Representative Western blot analysis of MYH7, COL1α1 (collagen type I alpha 1), and TGF-β1 (transforming growth factor beta 1) in heart tissues. GAPDH was used as loading control. **F**, mRNA levels of *Myh7*, *Col1a1*, and *Tgfb1* in heart tissues of mice. Transcripts were normalized to *Actb*. **G**, Representative Western blot analysis of G-CSF (granulocyte colony stimulating factor), CXCL1 (C-X-C motif ligand 1), and CXCR2 (CXC receptor 2) in heart tissues. GAPDH was used as loading control. **H**, mRNA levels of *Cxcl1*, *Cxcr2*, *Gcsf*, *Il17*, and *Il23* in heart tissues of mice. Transcripts were normalized to *Actb*. **I**, Representative immunofluorescence staining for CD68 (upper) and Ly-6G (lower) in heart tissue of mice. Sections were counterstained with DAPI (blue; scale bar, 50 μm). **B**–**H**, n=7 per group, Student *t* test. **D**, **top**, n=7 per group, Mann-Whitney *U* test; *P* values indicated.

without Ang II (Figure 5K) were added. The interactions between Bio-Ang II and its classic receptor AT1R were used as positive controls. Our results show that Bio-Ang II binds to dectin-1 protein in these lysates (Figure 5I through 5K). Interestingly, lysates from Ang II–infused mice showed more dectin-1 binding with Bio-Ang II compared with lysates from control mice, possibly indicating increased dectin-1 levels from increased macrophage recruitment (Figure 5K). These results show that Ang II binds directly to dectin-1 to active the dectin-1/Syk signaling.

Identification of the Binding Positions of Ang II and Dectin-1

To gain an understanding of the nature of Ang II/dectin-1 interaction, we conducted a molecular docking and simulation study using the crystal structure of dectin-1 (SMR: Q9BXN2). Distributions of both docking scores and MM/GBSA scores show that Ang II may bind to the extracellular C-type lectin domain of dectin-1 (Figure 6A; Figure S14A). To predict the binding position of Ang II on C-type lectin domains, a

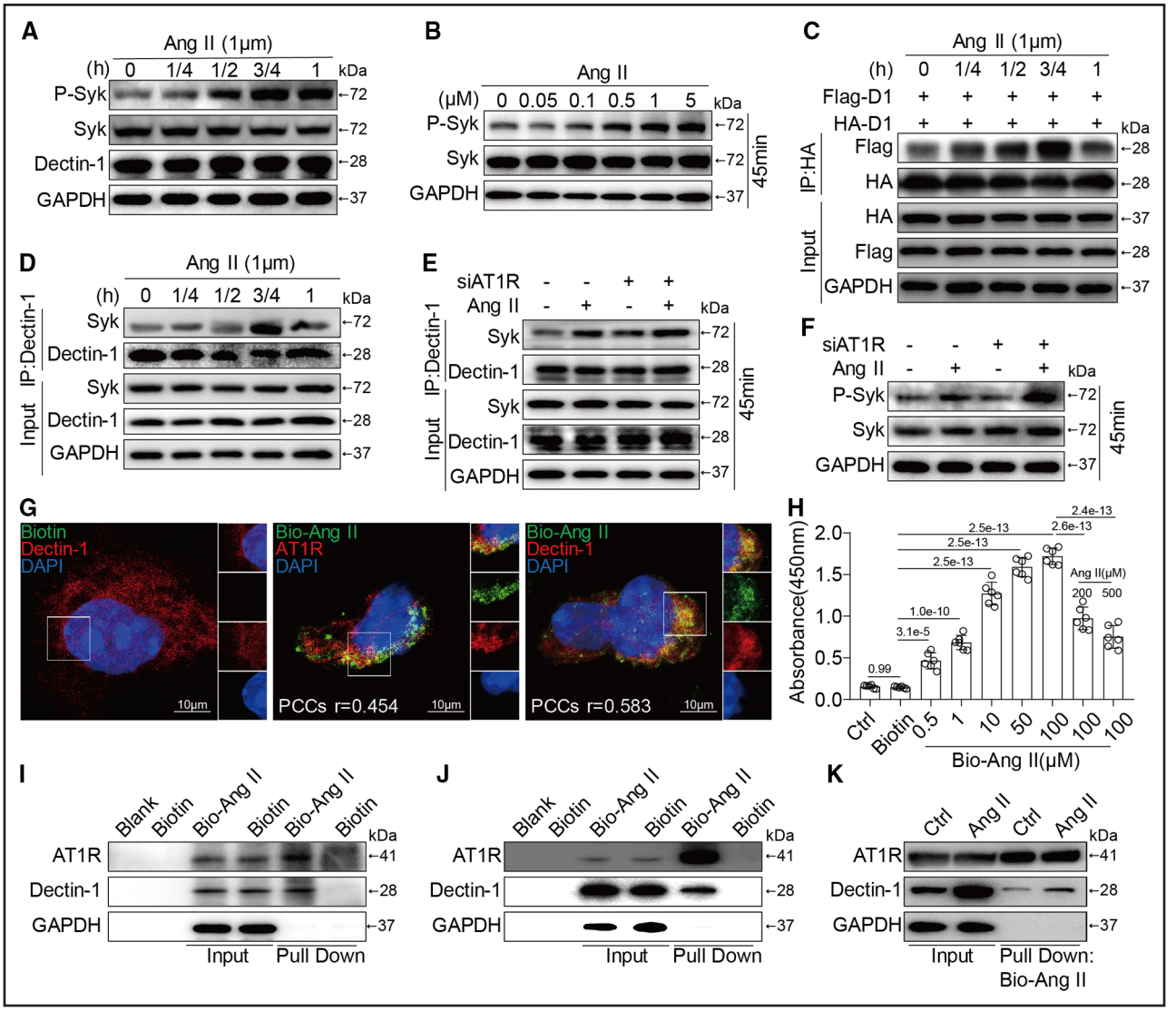


Figure 5. Ang II (angiotensin II) activates the dectin-1/Syk (spleen tyrosine kinase) pathway by directly binding to dectin-1 and inducing its homodimerization.

A, Timecourse of Syk phosphorylation. Bone marrow–derived macrophages (BMDMs) were exposed to 1 μM Ang II for indicated times. Total proteins were extracted and probed for p-Syk and Syk levels, with GAPDH as loading control. **B**, Dose course of Syk phosphorylation. BMDMs were exposed to increasing levels of Ang II for 45 minutes. Total proteins were used to measure phospho-Syk (p-Syk) and Syk levels, with GAPDH as loading control. **C**, HEK-293T (Human Embryonic Kidney-293T) cells were transfected with Flag- (Flag-D1) and HA-tagged dectin-1 (HA-D1). Time course of dectin-1 dimerization (Flag-HA interaction) was assessed following exposure of cells to 1 μM Ang II for indicated times. **D**, Timecourse of dectin-1/Syk interaction. BMDMs were exposed to 1 μM Ang II for indicated times, and coimmunoprecipitation using Syk antibody to probe for dectin-1 was performed. **E** and **F**, BMDMs were transfected with control siRNA (NC) or AT1R (angiotensin II receptor type 1) siRNA (siAT1R) and then exposed to 1 μM Ang II for 45 minutes. Dectin-1/Syk interaction was analyzed by coimmunoprecipitation (**E**), and Syk phosphorylation was analyzed with GAPDH as control (**F**). **G**, BMDMs were treated with 1 μM biotinylated angiotensin II (Bio-Ang II) or free biotin for 45 minutes, and cells were double-stained for Biotin (green) and dectin-1 (red) or ATR1 (red), respectively. Nuclei were counterstained with DAPI (blue); n=5 biological replicates. **H**, ELISA assay to determine Bio-Ang II and rhDectin-1 (recombinant human Dectin-1) interaction, as described in Methods. Free biotin was used as control and to determine whether it competes with Bio-Ang II (n=6 independent biological replicates; 1-way ANOVA followed by Tukey post hoc tests). **I**–**K**, Bio-Ang II was added to streptavidin-agarose beads and incubated. Biotin alone was used as a control. Lysates prepared from BMDMs (**I**), Flag-D1 expressing HEK-293 cells (**J**), and heart tissues from Ctrl and Ang II–challenged mice (**K**) were added to the streptavidin-agarose beads with Bio-Ang II. Eluent was then loaded on a polyacrylamide gel for Western blot analysis. Total lysates were used as an input control. AT1R was used as the positive control. **A**–**F** and **I**–**K**, n=5 biological replicates; the densitometric quantifications are shown in the Supplemental Material; *P* values indicated.

per-residue decomposition energy calculation was performed for the top 100 docking poses (Figure S14B). Five key residues in the C-type lectin domains with the lowest average energy values were identified as ASN176, ARG184, GLN230, THR234, and SER236 (Figure 6B). Among these, ASN176, ARG184, and SER236 showed the lowest energy. We then mutated ASN176, ARG184, and SER236 in the C-type lectin

domain to ALA, respectively, to explore the role of these 3 residues in Ang II/dectin-1 interaction.

Three separate mutation plasmids, HA-tagged dectin-1 with ASN176-ALA (HA-D1^{N176A}), HA-tagged dectin-1 with ARG184-ALA (HA-D1^{R184A}), and HA-tagged dectin-1 with SER236-ALA (HA-D1^{S236A}) were constructed and transfected into HEK-293T (Human Embryonic Kidney-293T) cells. Interestingly, we found that the interaction between Ang II and dectin-1 was remarkably reduced by ARG184 mutation, rather than N176A and S236A mutations, indicating that ARG184 mainly contributes to binding Ang II (Figure 6C). Consistently, IP assay showed that Ang II challenging failed to increase dimerization of wide-type dectin-1 with dectin-1^{R184A} mutation (Figure 6D and 6E). Interestingly, curdlan—a β-glucans–like dectin-1 agonist—still could induce dimerization of dectin-1^{R184A} mutation (Figure 6D and 6E). We then validated the role of ARG184 amino acid in mouse primary BMDMs. We transfected the HA-D1 and HA-D1^{R184A} plasmids into the dectin-1–deficient BMDMs from D1KO mice and then challenged the cells with Ang II or curdlan, respectively. As shown in Figure 6F through 6H, curdlan still activated the dectin-1/Syk pathway (dectin-1/Syk interaction and Syk phosphorylation) in macrophages with dectin-1^{R184A} mutation, whereas Ang II failed in dectin-1^{R184A} mutant macrophages. These results indicate that ARG184 amino acid is specifically responsible for Ang II/dectin-1 interaction and Ang II–induced dectin-1 activation.

Dectin-1 Deficiency Suppresses Ang II–Induced Inflammatory Responses in Macrophages and Remodeling in Cardiomyocytes and Fibroblasts

Our last objective was to utilize the cell culture system and assess whether dectin-1 mediates Ang II–induced inflammatory responses in macrophages and remodeling in other cardiac cells. BMDMs from WT or D1KO mice were exposed to Ang II. Ang II caused NF-κB activation in WT BMDMs as assessed by IκB levels and nuclear translocation of the p65 subunit of NF-κB (Figure 7A; Figure S15A through S15C). This activation was lacking in BMDMs from D1KO mice. Staining of cells for p65 confirmed reduced nuclear translocation of p65 by Ang II in D1KO DMBMs (Figure 7B; Figure S15D). Similar results were obtained when mRNA levels of inflammatory factors (*Tnfa*, *Il1b*, *Il6*, and *Il23*) and chemokines (*Cxcl1* and *Csf3*) were measured (Figure 7C and 7D). Furthermore, protein levels of selected factors in the culture medium were performed and observed to follow the same pattern (Figure 7E through 7G).

To assess the effect of macrophage-derived factors on other cardiac cells involved in remodeling and fibrosis, we prepared condition media from WT BMDMs or D1KO BMDMs, exposed to Ang II for 24 hours. Our results show that condition media from macrophages exposed to Ang II increase primary cardiomyocyte size and induce genes and proteins involved in cellular hypertrophy and fibrosis in rat primary cardiomyocytes (Figure 7H through 7J; Figure S15E through S15H). Media produced from D1KO BMDMs failed to cause hypertrophic and profibrotic changes in cardiomyocytes. Consistent with these results, primary fibroblasts from the neonatal rat heart increased fibrosis-associated factors upon exposure to condition media from Ang II–challenged WT BMDMs (Figure 7K and 7L; Figure S16A and S16B). Collectively, these results demonstrated that dectin-1 deficiency in macrophages suppresses Ang II–induced inflammatory mediators and subsequent intercellular cross talk, resulting in reduced cardiomyocyte and fibroblast remodeling.

DISCUSSION

In this study, we discovered an important role of dectin-1 in mediating Ang II–induced inflammation, cardiac remodeling, and dysfunction. A recent report has shown a crucial role of dectin-1 in cardiac inflammation and dysfunction in mouse myocardial IR injury.[16] However, the role of dectin-1 in hypertensive HF is unknown, and more importantly, it is nuclear how dectin-1 is activated at the molecular and cellular levels in heart diseases. We show that macrophages in the hypertensive heart robustly express dectin-1. Deficiency in dectin-1 prevents cardiac remodeling and dysfunction by suppressing the number of macrophages and neutrophils and the elaboration of inflammatory cytokines. Cardiomyocyte hypertrophy and cardiac fibrosis are also not evident in D1KO mice. Bone marrow transplantation study further validated that dectin-1 in myeloid cells contributes to Ang II–induced cardiac inflammation and remodeling. Interestingly, our in vitro studies showed that Ang II binds directly to the extracellular domain of dectin-1 to activate the dectin-1/Syk/NF-κB proinflammatory signaling pathway in macrophages. We identified ARG184 on dectin-1 as the key residue involved in Ang II binding. Following association with dectin-1 in macrophages, Ang II induces the expression of inflammatory factors and chemoattractants, which cross talk to cardiomyocytes and fibroblasts, causing hypertrophy and fibrosis in cardiac cells.

Studies have established an important role of inflammatory cells in the development of cardiovascular diseases, particularly in hypertension-related cardiac remodeling.[26,27] Wong et al[28] found that macrophages interacted with neighboring cardiomyocytes by focal adhesion complexes, regulating myocardial inflammation and HF. Similar evidence also exists on the homing of monocytes/macrophages and dendritic cells into kidneys and aortas upon Ang II infusion.[29] Here, we analyzed CXCL1/CD68 and G-CSF/CD68 double-positive populations in the heart upon Ang II infusion. As expected, we found elevated levels

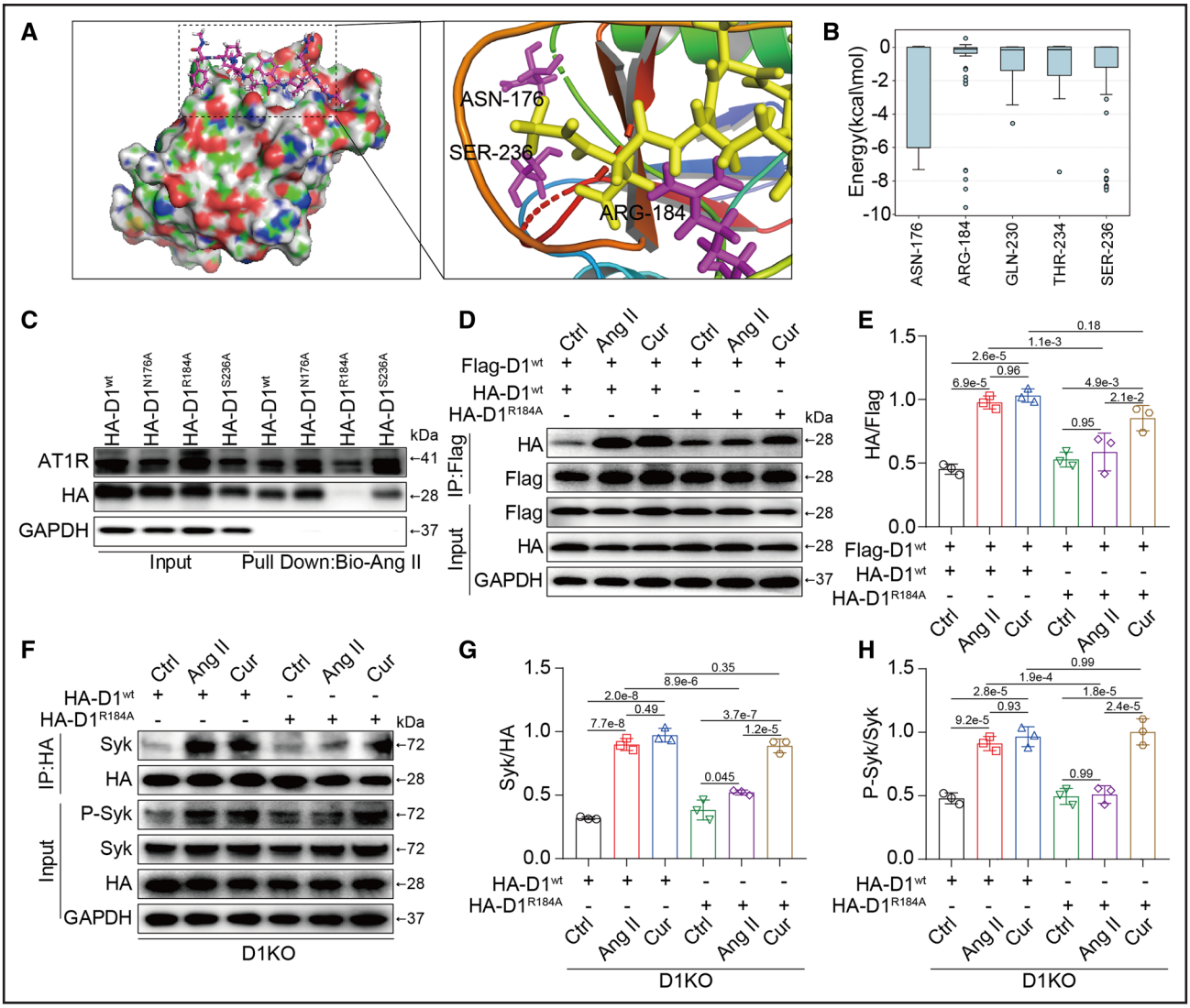


Figure 6. Identification of the binding positions of Ang II (angiotensin II) and dectin-1 protein.
A, Binding pose of Ang II to dectin-1 with the lowest binding energy is shown. The yellow sticks represent the structure of Ang II, and the purple sticks indicate the key amino acid residues binding with Ang II. **B**, Box plot of the per-residue decomposition energy of 5 key residues. **C**, HEK-293T (Human Embryonic Kidney-293T) cells were transfected with negative control (NC), HA-tagged wild-type dectin-1 (HA-D1^{wt}), HA-tagged dectin-1 with ASN176-ALA (HA-D1^{N176A}), HA-tagged dectin-1 with ARG184-ALA (HA-D1^{R184A}), or HA-tagged dectin-1 with SER236-ALA; HA-D1^{S236A}). Levels of proteins were detected by immunoblotting in input lysates, with GAPDH as loading control. Lysates were added to pull-down assays to detect the interaction with biotinylated angiotensin II (Bio-Ang II). **D**, HEK-293T cells were transfected with Flag-D1^{wt} and HA-D1^{wt} or HA-D1^{R184A}. Cells were then exposed to 1 μM Ang II or 100 μg/mL curdlan AL (Cur) for 45 minutes. Dectin-1 dimerization (Flag-HA interaction) was analyzed by coimmunoprecipitation using Flag antibody to probe for HA. **E**, Densitometric quantification of **E**. **F**, Bone marrow–derived macrophages (BMDMs) dectin-1 knockout (D1KO) mice were transfected with HA-D1^{wt} or HA-D1^{R184A}. Cells were then exposed to with 1 μM Ang II or 100 μg/mL Cur for 45 minutes. Dectin-1/Syk (spleen tyrosine kinase) interaction was analyzed by coimmunoprecipitation using the HA antibody to probe for Syk, and the Syk phosphorylation was analyzed by Western blot. **G**, Densitometric quantification of Syk-HA interaction in **G**. **H**, Densitometric quantification of Syk phosphorylation in **G**. **C**–**H**, n=3 biological replicates; 1-way ANOVA followed by Tukey post hoc tests; number of comparisons=10; *P* values indicated.

of proinflammatory and chemoattractant factors. These factors were likely responsible for the increased number of macrophages and neutrophils seen in heart tissues of mice. Importantly, more inflammatory cells result in more inflammatory cytokines in heart in a positive feedback, contributing to cardiac fibrosis and remodeling. Both macrophage infiltration and cytokine overproduction were blocked by dectin-1 knockout in the hearts of Ang II–infused mice. These results are consistent with research on dectin-1 in other systems. For example, dectin-1 has been reported to regulate the expression of CXCL1, CSF3, and other inflammatory factors through Syk/NF-κB pathway activation, leading to the recruitment of neutrophils.[30,31] We further show that dectin-1 mediates Ang II–induced overproduction of inflammatory cytokines in macrophages, which subsequently results in cardiomyocyte and fibroblast remodeling through intercellular cross talk.

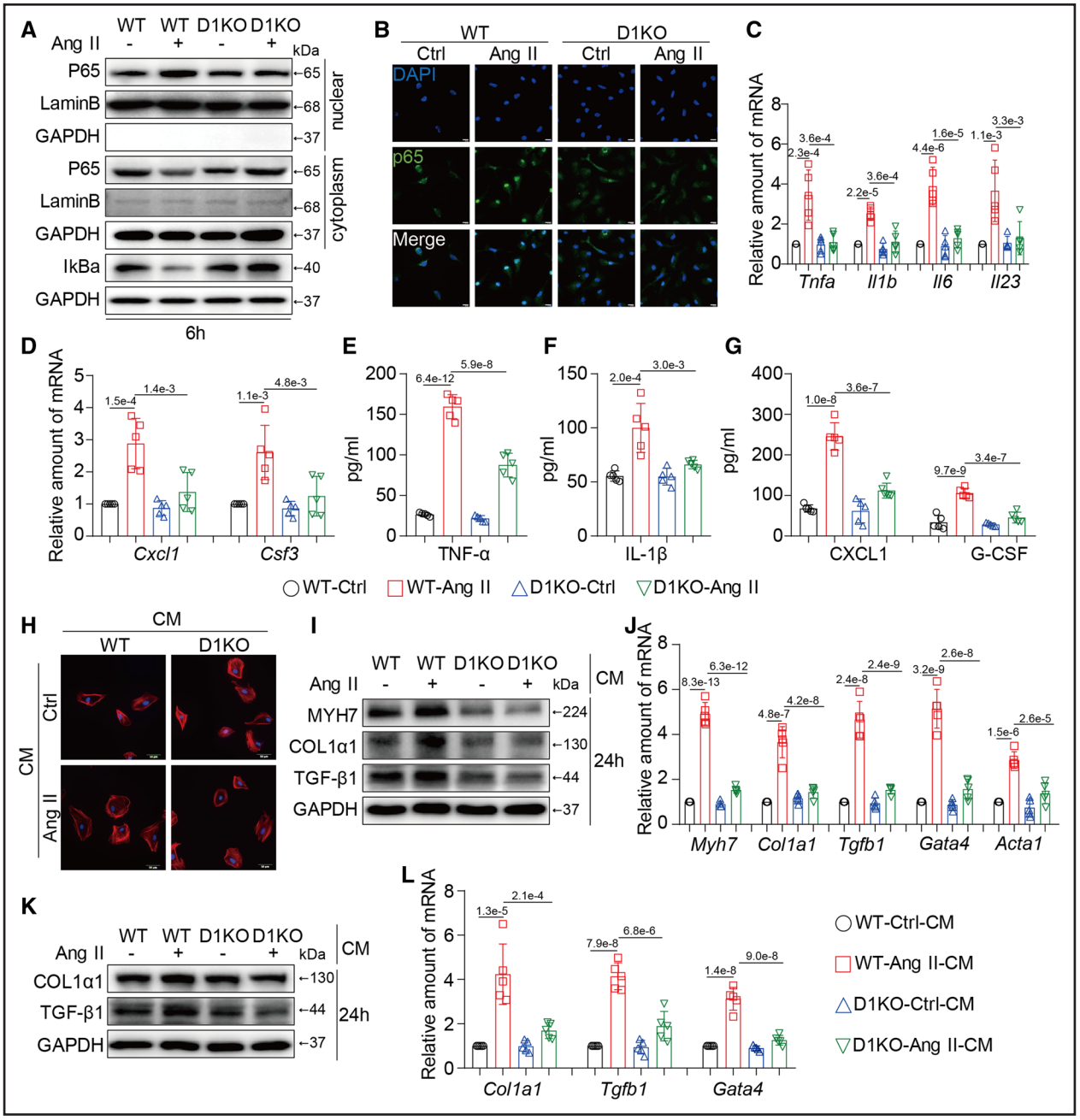


Figure 7. Blocking dectin-1 prevents Ang II (angiotensin II)–induced inflammatory responses in macrophages and remodeling-associated changes in cardiomyocytes and fibroblasts.

A–**D**, Bone marrow–derived macrophages (BMDMs) from the wild type (WT) and dectin-1 knockout (D1KO) mice were stimulated with 1 μM Ang II for 6 h. IκB-α (inhibitor of κB-α) in whole-cell lysates and p65 in cytosolic and nuclear sections were examined by Western blot, with lamin B and GAPDH as loading controls, respectively (**A**). Representative fluorescence staining images of p65 (red) was performed, with arrows indicating nuclear p65 reactivity (**B**). The mRNA levels of *Tnfa*, *Il1b*, *Il6,* and *Il23* in BMDMs were examined by quantitative real-time PCR (qPCR) and normalized to *Actb* (**C**). The mRNA levels of *Cxcl1*, *Cxcr2*, and *Gcsf* (*Csf3*) in BMDMs were examined and normalized to *Actb* (**D**). **E** through **G**, BMDMs from WT and D1KO mice were stimulated with or without 1 μM Ang II for 24 h. Protein levels of TNF-α (tumor necrosis factor alpha), IL (interleukin)-1β, CXCL1 (C-X-C motif ligand 1), and G-CSF (granulocyte colony stimulating factor) in cultural medium were measured by ELISA and expressed as pg/mL. **H**–**J**, BMDMs from WT and D1KO mice were challenged with or without 1 μM Ang II for 24 h. Condition media at 24 hours was collected and applied to primary cardiomyocytes for 24 h in a 1:1 ratio with normal media. Cardiomyocyte cell size was assessed by Rhodamine phalloidin staining (red); DAPI (blue) was used to counterstain (**H**). Representative Western blot analysis of MYH7, COL1A1 (collagen type I alpha 1), and TGFβ1 (transforming growth factor beta 1) in cardiomyocyte lysates, with GAPDH as loading control (**I**). mRNA levels of *Myh7*, *Col1a1*, *Tgfb1*, *Gata4*, and *Acta1* in cardiomyocytes were examined and normalized to *Actb* (**J**). **K** and **L**, BMDMs from WT and D1KO mice were exposed to 1 μM Ang II for 24 h. Condition media at 24 hours was collected and applied to primary cardiac fibroblasts for 24 h in a 1:1 ratio with normal media. Representative Western blot analysis of COL1A1 and TGF-β1 in fibroblasts, with GAPDH as loading control (**K**). mRNA levels of *Col1a1*, *Gata4*, and *Tgfb1* in primary fibroblasts were examined and normalized to *Actb* (**L**). **A**–**L**, n=5 biological replicates; 1-way ANOVA followed by Tukey post hoc tests; number of comparisons=6; *P* values indicated.

Dectin-1 has been reported to be highly expressed on various myeloid cells including monocyte/macrophage and neutrophil lineages.[32] Lower dectin-1 levels have been observed on dendritic cells and subpopulations of T cells.[33] Studies have also reported induction of dectin-1 expression following experimental autoimmune encephalomyelitis, indicating a role of this receptor in inflammatory process.[34] Largely in agreement with these studies, we show that dectin-1 is primarily expressed on CD68-positive macrophages in heart tissues, though we did find much lower immunoreactivity in cardiomyocytes and fibroblasts. Furthermore, we report that Ang II infusion increases the expression of dectin-1 in heart tissues of mice. There may be 2 possibilities for the apparent increase in dectin-1 expression in heart tissues of mice challenged with Ang II. One possibility is that infiltrating monocytes are the major contributors to the increased expression. Second possibility is that heart-resident macrophages increase dectin-1 expression. However, our in vitro data indicate that Ang II challenge did not induce dectin-1 overexpression in macrophages. Although not tested directly in our study, it is most likely that increased cell infiltration and accumulation is the main reason for increased dectin-1 levels. Support for this notion also comes from a recent study in myocardial ischemia/reperfusion injury that showed infiltrating macrophages following injury to increase dectin-1, and the cardiac resident macrophages were found to express much lower levels of dectin-1.[16] Furthermore, Using a bone marrow transplantation strategy, we confirmed that dectin-1 in myeloid cells mediated Ang II–induced cardiac inflammation and heart injuries. Based on these findings and our observations, we performed mechanistic studies in BMDMs. It would still be valuable to investigate whether dectin-1 conditional knockout on other immune cells has any functional relevance to cardiac remodeling. Since our experimental models are global or myeloid cell–specific D1KO mice as previous studies,[16] an interesting future study may be conducted to subtract the 2 inflammatory cell populations (macrophages and neutrophils) to see downstream effects in the model. These studies would certainly provide a finer assessment of the role these inflammatory cells play in cardiac remodeling.

Dectin-1 was first identified as a PRR of β-glucans in fungal pathogens.[35] Dectin-1 has a C-type lectin domain outside the cell membrane and an ITAM in its cytoplasm. This ITAM functions to mediate ligand-based activation of the signaling pathway through Syk.[36] Ligand (β-1,3-linked glucan) recognition by dectin-1 triggers intracellular signaling through a hem-ITAM in the cytoplasmic tail of the receptor that induces multiple downstream pathways, including the Raf-1 and Syk/p65 pathway.[37] In addition, 2 nonpathogen proteins, galectin-9 and secreted vimentin, have been reported to bind and activate dectin-1. Dectin-1 ligates the lectin galectin-9 to promote pancreatic carcinoma immune tolerance.[14] Thiagarajan et al[38] have reported that endogenous vimentin activates dectin-1 in the macrophage-rich artery wall to induce inflammation and contribute to atherogenesis. To our current knowledge, unfortunately, there is no definite involvement of galectin-9 and secreted vimentin in Ang II–induced HF. This promotes us to hypothesize if Ang II directly triggers dectin-1 activation by itself. Indeed, we saw that Ang II stimulation for a short time induced dectin-1 dimerization and dectin-1/Syk interaction in cultured macrophages. We further identified the direct interaction between Ang II and dectin-1 protein by pull-down assays and ELISA-based platforms. These data indicate that Ang II is a new activating ligand of dectin-1. The high-concentration, prolonged, and continuous Ang II, as an endogenous and long-standing ligand of dectin-1 in macrophages, affords a chronic inflammation in the pathological tissues. Our results also point to the key residue ARG184 in the C-type lectin domain, in participating in Ang II/dectin-1 interaction. Interestingly, our results indicate that the R184A mutation does not affect the curdlan/dectin-1 interaction and curdlan-induced dectin-1 activation. That is to say, the new agonist, Ang II, binds to dectin-1 at a new site/domain, which is different to the binding site of β-glucan–like agonist curdlan. Therefore, this finding may contribute to the design of an inhibitor that interferes with Ang II binding to dectin-1 but preserves β-glucan binding and dectin-1–mediated host defense to the fungal pathogen. Further studies are needed to dissect the binding mode of Ang II/dectin-1 using *x* ray diffraction-based cocrystal structural analysis.

It should be notable that circulating Ang II is a strong hypertensive peptide. AT1R is the best characterized Ang II receptor and mediates the major hemodynamic effects of Ang II. The Ang II–activated AT1R increases the cytosolic Ca^{2+} concentrations, which in turn triggers cellular responses such as stimulation of protein kinase C, contributing to the role of Ang II in arterial blood pressure regulation including vasoconstriction and retention of sodium and water.[22] Hypertension and hemodynamic changes resulting from increased circulating Ang II level are also important contributors to cardiac remodeling.[39] The role of dectin-1 in Ang II–induced hypertension is unknown. In this study, dectin-1 deficiency did not affect Ang II–induced change in blood pressure in mice in both Ang II–infused mice. Thus, the prevention of cardiac dysfunction and remodeling by dectin-1 deletion is independent on the hemodynamic mechanism. Few studies have addressed the role of macrophage AT1R in the proinflammatory activities of Ang II. Our previous study found that silencing or blocking the AT1 receptor was not able to attenuate Ang II–induced TNF-α production in H9c2 cells.[40] Here, we also show that Ang II activates dectin-1 signaling pathway independent of AT1R and AT2R in macrophage, and losartan treatment

showed no effect on dectin-1/Syk activation in the Ang II–infused mouse heart. Interestingly, it is observed that knockdown of AT1R seems to slightly increase Ang II–induced dectin-1 activation. We speculate that knocking down AT1R reduces AT1R-mediated Ang II consumption and then increases free Ang II to interact with dectin-1 in the cell membrane. These findings indicate that Ang II separately activates AT1R and dectin-1, responsible to its hemodynamic effects and proinflammatory actions, respectively. In current clinical practice, blood pressure-controlling drugs targeting the renin-angiotensin-aldosterone system alone could not completely improve cardiac function and other complications in hypertensive patients, especially in patients with HF with decreased EF%. Thus, dectin-1 inhibition may provide a potential strategy/target for hypertensive HF as a combination with angiotensin-converting enzyme inhibitors/Ang II receptor blockers.

In conclusion, this study identified, for the first time to our knowledge, dectin-1 as a new nonclassical Ang II receptor in inflammatory induction and cardiac remodeling, which is independent of AT1R. We demonstrate that macrophage dectin-1 mediates Ang II–induced Syk/NF-κB signaling pathway activation and inflammatory response and subsequently contributes to cardiac remodeling and dysfunction. This study provides (1) the evidence for a central role of dectin-1 in Ang II–induced inflammation and cardiac remodeling via directly binding to Ang II, (2) a deep understanding of the proinflammatory mechanism of Ang II, and (3) indication that dectin-1 may represent an attractive new strategy for treating hypertension-related HF.

ARTICLE INFORMATION

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G. Liang, G. Fu, and H. Huang contributed to the literature search and study design. G. Liang and S. Ye participated in the drafting of the article. S. Ye, X. Han, W. Luo, L. Wu, Y. Ye, and X. Zhao performed the experiments. Y. Gong collected the clinical samples. H. Huang, Y. Wang, W. Huang, and G. Liang revised the manuscript. S. Ye and X. Long contributed to data collection and analysis.

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Disclosures

None.

Supplemental Material

Supplemental Materials and Methods
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REFERENCES

1. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, Burri H, Butler J, Čelutkienė J, Chioncel O, et al; ESC Scientific Document Group. 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure. *Eur Heart J*. 2021;42:3599–3726. doi: 10.1093/eurheartj/ehab368
2. Forrester SJ, Booz GW, Sigmund CD, Coffman TM, Kawai T, Rizzo V, Scalia R, Eguchi S. Angiotensin II signal transduction: an update on mechanisms of physiology and pathophysiology. *Physiol Rev*. 2018;98:1627–1738. doi: 10.1152/physrev.00038.2017
3. Brasier AR, Recinos A 3rd, Eledrisi MS. Vascular inflammation and the renin-angiotensin system. *Arterioscler Thromb Vasc Biol*. 2002;22:1257–1266. doi: 10.1161/01.atv.0000021412.56621.a2
4. Rocha R, Martin-Berger CL, Yang P, Scherrer R, Delyani J, McMahon E. Selective aldosterone blockade prevents angiotensin II/salt-induced vascular inflammation in the rat heart. *Endocrinology*. 2002;143:4828–4836. doi: 10.1210/en.2002-220120
5. Laroumanie F, Douin-Echinard V, Pozzo J, Lairez O, Tortosa F, Vinel C, Delage C, Calise D, Dutaur M, Parini A, et al. CD4+ T cells promote the transition from hypertrophy to heart failure during chronic pressure overload. *Circulation*. 2014;129:2111–2124. doi: 10.1161/CIRCULATIONAHA.113.007101
6. Kallikourdis M, Martini E, Carullo P, Sardi C, Roselli G, Greco CM, Vignali D, Riva F, Ormbostad Berre AM, Stølen TO, et al. T cell costimulation blockade blunts pressure overload-induced heart failure. *Nat Commun*. 2017;8:14680. doi: 10.1038/ncomms14680
7. Patel B, Bansal SS, Ismahil MA, Hamid T, Rokosh G, Mack M, Prabhu SD. CCR2(+) monocyte-derived infiltrating macrophages are required for adverse cardiac remodeling during pressure overload. *JACC Basic Transl Sci*. 2018;3:230–244. doi: 10.1016/j.jacbts.2017.12.006
8. Wang L, Zhang YL, Lin QY, Liu Y, Guan XM, Ma XL, Cao HJ, Liu Y, Bai J, Xia YL, et al. CXCL1-CXCR2 axis mediates angiotensin II-induced cardiac hypertrophy and remodelling through regulation of monocyte infiltration. *Eur Heart J*. 2018;39:1818–1831. doi: 10.1093/eurheartj/ehy085
9. Barhoumi T, Fraulob-Aquino JC, Mian MOR, Ouerd S, Idris-Khodja N, Huo KG, Rehman A, Caillon A, Dancose-Giambattisto B, Ebrahimian T, et al. Matrix metalloproteinase-2 knockout prevents angiotensin II-induced vascular injury. *Cardiovasc Res*. 2017;113:1753–1762. doi: 10.1093/cvr/cvx115
10. Kriska T, Herrnreiter A, Pfister SL, Adebesin A, Falck JR, Campbell WB. Macrophage 12(S)-HETE enhances angiotensin II-induced contraction by a BLT2 (leukotriene B(4) type-2 receptor) and TP (thromboxane receptor)-mediated mechanism in murine arteries. *Hypertension*. 2022;79:104–114. doi: 10.1161/hypertensionaha.121.17824
11. Martini E, Kunderfranco P, Peano C, Carullo P, Cremonesi M, Schorn T, Carriero R, Termanini A, Colombo FS, Jachetti E, et al. Single-cell sequencing of mouse heart immune infiltrate in pressure overload-driven heart failure reveals extent of immune activation. *Circulation*. 2019;140:2089–2107. doi: 10.1161/CIRCULATIONAHA.119.041694
12. Goodridge HS, Reyes CN, Becker CA, Katsumoto TR, Ma J, Wolf AJ, Bose N, Chan AS, Magee AS, Danielson ME, et al. Activation of the innate immune receptor dectin-1 upon formation of a "phagocytic synapse". *Nature*. 2011;472:471–475. doi: 10.1038/nature10071
13. Wheeler ML, Limon JJ, Underhill DM. Immunity to commensal fungi: detente and disease. *Annu Rev Pathol*. 2017;12:359–385. doi: 10.1146/annurev-pathol-052016-100342
14. Daley D, Mani VR, Mohan N, Akkad N, Ochi A, Heindel DW, Lee KB, Zambirinis CP, Pandian GSB, Savadkar S, et al. Dectin 1 activation on macrophages by galectin 9 promotes pancreatic carcinoma and peritumoral immune tolerance. *Nat Med*. 2017;23:556–567. doi: 10.1038/nm.4314
15. Fu X, Zeng H, Zhao J, Zhou G, Zhou H, Zhuang J, Xu C, Li J, Peng Y, Cao Y, et al. Inhibition of dectin-1 ameliorates neuroinflammation by regulating microglia/macrophage phenotype after intracerebral hemorrhage in mice. *Transl Stroke Res*. 2021;12:1018–1034. doi: 10.1007/s12975-021-00889-2
16. Fan Q, Tao R, Zhang H, Xie H, Lu L, Wang T, Su M, Hu J, Zhang Q, Chen Q, et al. Dectin-1 contributes to myocardial ischemia/reperfusion injury by regulating macrophage polarization and neutrophil infiltration. *Circulation*. 2019;139:663–678. doi: 10.1161/CIRCULATIONAHA.118.036044

17. Han W, Tang C, Baba S, Hamada T, Shimazu T, Iwakura Y. Ovalbumin-induced airway inflammation is ameliorated in dectin-1-deficient mice, in which pulmonary regulatory T cells are expanded through modification of intestinal commensal bacteria. *J Immunol*. 2021;206:1991–2000. doi: 10.4049/jimmunol.2001337
18. Kalia N, Singh J, Kaur M. The role of dectin-1 in health and disease. *Immunobiology*. 2021;226:152071. doi: 10.1016/j.imbio.2021.152071
19. He Z, Xu X, Chen C, Li H, Wang DW. Adenosine 2A receptor activation contributes to ang II-induced aortic remodeling by promoting macrophage retention. *Hypertension*. 2020;75:119–130. doi: 10.1161/hypertensionaha.119.13709
20. Jiang HM, Wang HX, Yang H, Zeng XJ, Tang CS, Du J, Li HH. Role for granulocyte colony stimulating factor in angiotensin II-induced neutrophil recruitment and cardiac fibrosis in mice. *Am J Hypertens*. 2013;26:1224–1233. doi: 10.1093/ajh/hpt095
21. Goodridge HS, Wolf AJ, Underhill DM. Beta-glucan recognition by the innate immune system. *Immunol Rev*. 2009;230:38–50. doi: 10.1111/j.1600-065X.2009.00793.x
22. Verma K, Pant M, Paliwal S, Dwivedi J, Sharma S. An insight on multicentric signaling of angiotensin II in cardiovascular system: a recent update. *Front Pharmacol*. 2021;12:734917. doi: 10.3389/fphar.2021.734917
23. Han J, Zou C, Mei L, Zhang Y, Qian Y, You S, Pan Y, Xu Z, Bai B, Huang W, et al. MD2 mediates angiotensin II-induced cardiac inflammation and remodeling via directly binding to Ang II and activating TLR4/NF-kappaB signaling pathway. *Basic Res Cardiol*. 2017;112:9. doi: 10.1007/s00395-016-0599-5
24. Carey RM. AT2 receptors: potential therapeutic targets for hypertension. *Am J Hypertens*. 2017;30:339–347. doi: 10.1093/ajh/hpw121
25. Chow BS, Koulis C, Krishnaswamy P, Steckelings UM, Unger T, Cooper ME, Jandeleit-Dahm KA, Allen TJ. The angiotensin II type 2 receptor agonist compound 21 is protective in experimental diabetes-associated atherosclerosis. *Diabetologia*. 2016;59:1778–1790. doi: 10.1007/s00125-016-3977-5
26. Mikolajczyk TP, Szczepaniak P, Vidler F, Maffia P, Graham GJ, Guzik TJ. Role of inflammatory chemokines in hypertension. *Pharmacol Ther*. 2021;223:107799. doi: 10.1016/j.pharmthera.2020.107799
27. Rhee AJ, Lavine KJ. New approaches to target inflammation in heart failure: harnessing insights from studies of immune cell diversity. *Annu Rev Physiol*. 2020;82:1–20. doi: 10.1146/annurev-physiol-021119-034412
28. Wong NR, Mohan J, Kopecky BJ, Guo S, Du L, Leid J, Feng G, Lokshina I, Dmytrenko O, Luehmann H, et al. Resident cardiac macrophages mediate adaptive myocardial remodeling. *Immunity*. 2021;54:2072–2088.e7. doi: 10.1016/j.immuni.2021.07.003
29. Loperena R, Van Beusecum JP, Itani HA, Engel N, Laroumanie F, Xiao L, Elijovich F, Laffer CL, Gnecco JS, Noonan J, et al. Hypertension and increased endothelial mechanical stretch promote monocyte differentiation and activation: roles of STAT3, interleukin 6 and hydrogen peroxide. *Cardiovasc Res*. 2018;114:1547–1563. doi: 10.1093/cvr/cvy112
30. Yadav M, Schorey JS. The beta-glucan receptor dectin-1 functions together with TLR2 to mediate macrophage activation by mycobacteria. *Blood*. 2006;108:3168–3175. doi: 10.1182/blood-2006-05-024406
31. Maruyama K, Takayama Y, Kondo T, Ishibashi KI, Sahoo BR, Kanemaru H, Kumagai Y, Martino MM, Tanaka H, Ohno N, et al. Nociceptors boost the resolution of fungal osteoinflammation via the TRP channel-CGRP-Jdp2 axis. *Cell Rep*. 2017;19:2730–2742. doi: 10.1016/j.celrep.2017.06.002
32. Deerhake ME, Shinohara ML. Emerging roles of dectin-1 in noninfectious settings and in the CNS. *Trends Immunol*. 2021;42:891–903. doi: 10.1016/j.it.2021.08.005
33. Taylor PR, Brown GD, Reid DM, Willment JA, Martinez-Pomares L, Gordon S, Wong SY. The beta-glucan receptor, dectin-1, is predominantly expressed on the surface of cells of the monocyte/macrophage and neutrophil lineages. *J Immunol*. 2002;169:3876–3882. doi: 10.4049/jimmunol.169.7.3876
34. Deerhake ME, Danzaki K, Inoue M, Cardakli ED, Nonaka T, Aggarwal N, Barclay WE, Ji RR, Shinohara ML. Dectin-1 limits autoimmune neuroinflammation and promotes myeloid cell-astrocyte crosstalk via Card9-independent expression of oncostatin M. *Immunity*. 2021;54:484–498.e8. doi: 10.1016/j.immuni.2021.01.004
35. Taylor PR, Tsoni SV, Willment JA, Dennehy KM, Rosas M, Findon H, Haynes K, Steele C, Botto M, Gordon S, et al. Dectin-1 is required for beta-glucan recognition and control of fungal infection. *Nat Immunol*. 2007;8:31–38. doi: 10.1038/ni1408
36. Brown GD. Dectin-1: a signalling non-TLR pattern-recognition receptor. *Nat Rev Immunol*. 2006;6:33–43. doi: 10.1038/nri1745
37. Tone K, Stappers MHT, Willment JA, Brown GD. C-type lectin receptors of the dectin-1 cluster: physiological roles and involvement in disease. *Eur J Immunol*. 2019;49:2127–2133. doi: 10.1002/eji.201847536
38. Thiagarajan PS, Yakubenko VP, Elsori DH, Yadav SP, Willard B, Tan CD, Rodriguez ER, Febbraio M, Cathcart MK. Vimentin is an endogenous ligand for the pattern recognition receptor dectin-1. *Cardiovasc Res*. 2013;99:494–504. doi: 10.1093/cvr/cvt117
39. Zablocki D, Sadoshima J. Solving the cardiac hypertrophy riddle: the angiotensin II-mechanical stress connection. *Circ Res*. 2013;113:1192–1195. doi: 10.1161/CIRCRESAHA.113.302501
40. Han J, Zou C, Mei L, Zhang Y, Qian Y, You S, Pan Y, Xu Z, Bai B, Huang W, et al. MD2 mediates angiotensin II-induced cardiac inflammation and remodeling via directly binding to Ang II and activating TLR4/NF-κB signaling pathway. *Basic Res Cardiol*. 2017;112:9. doi: 10.1007/s00395-016-0599-5
41. Chen J, Lai J, Yang L, Ruan G, Chaugai S, Ning Q, Chen C, Wang DW. Trimetazidine prevents macrophage-mediated septic myocardial dysfunction via activation of the histone deacetylase sirtuin 1. *Br J Pharmacol*. 2016;173:545–561. doi: 10.1111/bph.13386
42. Ortiz-Sanchez P, Villalba-Orero M, Lopez-Olaneta MM, Larrasa-Alonso J, Sanchez-Cabo F, Marti-Gomez C, Camafeita E, Gomez-Salinero JM, Ramos-Hernandez L, Nielsen PJ, et al. Loss of SRSF3 in cardiomyocytes leads to decapping of contraction-related mRNAs and severe systolic dysfunction. *Circ Res*. 2019;125:170–183. doi: 10.1161/CIRCRESAHA.118.314515
43. Percie du Sert N, Ahluwalia A, Alam S, Avey MT, Baker M, Browne WJ, Clark A, Cuthill IC, Dirnagl U, Emerson M, et al. Reporting animal research: explanation and elaboration for the ARRIVE guidelines 2.0. *PLoS Biol*. 2020;18:e3000411. doi: 10.1371/journal.pbio.3000411
44. Li XC, Zhu D, Chen X, Zheng X, Zhao C, Zhang J, Soleimani M, Rubera I, Tauc M, Zhou X, et al. Proximal tubule-specific deletion of the NHE3 (Na(+)/H(+) exchanger 3) in the kidney attenuates Ang II (angiotensin II)-induced hypertension in mice. *Hypertension*. 2019;74:526–535. doi: 10.1161/hypertensionaha.119.13094
45. Kandalam V, Basu R, Abraham T, Wang X, Soloway PD, Jaworski DM, Oudit GY, Kassiri Z. TIMP2 deficiency accelerates adverse post-myocardial infarction remodeling because of enhanced MT1-MMP activity despite lack of MMP2 activation. *Circ Res*. 2010;106:796–808. doi: 10.1161/CIRCRESAHA.109.209189
46. Roffel MP, Boudewijn IM, van Nijnatten JLL, Faiz A, Vermeulen CJ, van Oosterhout AJ, Affleck K, Timens W, Bracke KR, Maes T, et al. Identification of asthma-associated microRNAs in bronchial biopsies. *Eur Respir J*. 2022;59:2101294. doi: 10.1183/13993003.01294-2021
47. Trouplin V, Boucherit N, Gorvel L, Conti F, Mottola G, Ghigo E. Bone marrow-derived macrophage production. *J Vis Exp*. 2013;(81):e50966. doi: 10.3791/50966
48. Nakamura K, Fushimi K, Kouchi H, Mihara K, Miyazaki M, Ohe T, Namba M. Inhibitory effects of antioxidants on neonatal rat cardiac myocyte hypertrophy induced by tumor necrosis factor-alpha and angiotensin II. *Circulation*. 1998;98:794–799. doi: 10.1161/01.cir.98.8.794
49. Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem*. 2009;30:2785–2791. doi: 10.1002/jcc.21256
50. Salomon-Ferrer R, Case RA, Walker RC. An overview of the amber biomolecular simulation package. *WIREs Comput Mol Sci*. 2012;3:198–210. doi: 10.1002/wcms.1121