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Original Article

CD11b mediates hypertensive cardiac remodeling by regulating macrophage infiltration and polarization

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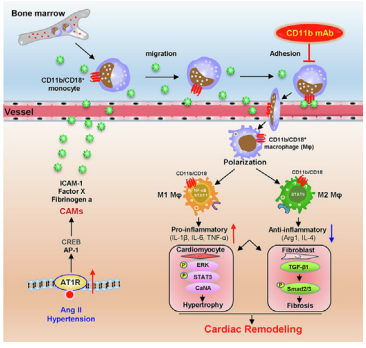
HIGHLIGHTS

- CD11b/CD18 is responsible for leukocyte adhesion to the endothelium and upregulated in Ang II-infused mouse heart.
- Ablation or pharmacological inhibition of CD11b prevents Ang II- or DOCA-salt-induced cardiac remodeling and dysfunction.
- Circulating $CD14^+CD11b^+CD18^+$ monocytes and the ligand levels are significantly higher in HF patients than in controls.
- Selectively inhibiting CD11b may represent a new therapeutic choice to suppress and treat hypertensive cardiac remodeling.

GRAPHICAL ABSTRACT



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ABSTRACT

Introduction: Leukocyte infiltration is an early event during cardiac remodeling frequently leading to heart failure (HF). Integrins mediate leukocyte infiltration during inflammation. However, the importance of specific integrins in hypertensive cardiac remodeling is still unclear.
Objectives: To elucidate the significance of CD11b in hypertensive cardiac remodeling.
Methods: Angiotensin (Ang II) or deoxycorticosterone acetate (DOCA)-salt was used to induce cardiac remodeling in mice of gene knockout (KO), bone marrow (BM) chimera, and the CD11b neutralizing antibody or agonist leukadherin-1 (LA1) treatment.
Results: Our microarray data showed that integrin subunits Itgam (CD11b) and Itgb2 (CD18) were the most highly upregulated in Ang II-infused hearts. CD11b expression and $CD11b/CD18^+$ myelomonocytes were also time-dependently increased. KO or pharmacological blockade of CD11b greatly attenuated cardiac remodeling and macrophage infiltration and M1 polarization induced by Ang II or DOCA-salt. This protection was verified in wild-type mice transplanted with CD11b-deficient BM cells. Conversely, administration of CD11b agonist LA1 showed the opposite effects. Further, CD11b KO reduced Ang II-induced macrophage adhesion and M1 polarization, leading to reduction of cardiomyocyte enlargement and fibroblast differentiation in vitro. The numbers of $CD14^+CD11b^+CD18^+$ monocytes and $CD15^+CD11b^+CD18^+$ granulocytes were obviously higher in HF patients than in normal controls.

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Conclusion: Our data demonstrate an important role of CD11b$^+$ myeloid cells in hypertensive cardiac remodeling, and suggest that HF may benefit from targeting CD11b.

Introduction

Sustaining hypertension is a leading cause of cardiac remodeling that ultimately leads to heart failure (HF). This process is driven by the complex interaction between infiltrated immune cells and resident myocardial cell and fibrocyte in the heart in response to hypertensive stimuli [1,2]. Emerging evidence indicates that leukocyte recruitment into the heart is an early event in cardiac disease, and these cells can produce excessive proinflammatory cytokines, thereby triggering cardiac hypertrophic and fibrotic responses [3–6]. The main sequential steps in leukocyte recruitment include intravascular migration, rolling, adhesion, crawling and transmigration, and these processes are predominantly controlled by a variety of chemokines and cell adhesion molecules (CAMs), including selectins, integrins and immunoglobulin superfamily (IgSF) [4]. Chemokines CXCL1 and CCL2 can direct the migration of monocytes/macrophages (MΦs), accelerating Ang II- or pressure overload-caused cardiac remodeling [7–9]. Moreover, increased levels of the IgSF members ICAM-1 and VCAM-1 promote MΦ adhere to endotheliocyte and are associated with hypertension and cardiac remodeling, as well as the incidence of HF in humans [10–13]. However, it is unclear whether specific integrins contribute to hypertensive cardiac remodeling.

Integrins are a major family of CAMs and type I heterodimeric transmembrane receptors consisting of 24 alpha (α) and 8 beta (β) subunits [14]. Among these receptors, β2-containing integrins are specifically expressed on leukocytes and are composed of a constant β (Itgb2, CD18) and a variable α subunit, including αL (Itgal, CD11a), αM (Itgam, CD11b), αx (Itgax, CD11c) and αD (Itgad, CD11d) [15]. CD11b is a 165 kDa adhesion subunit that binds noncovalently to CD18 to form the CD11b/CD18 heterodimeric complex (also known as αMβ2, Mac-1, and CR3), which functions as a receptor for several ligands, such as ICAM-1, fibronectin, fibrinogen, and factor X, thereby mediating the adhesion and transmigration of myeloid cells [15]. and playing an important role in some cardiovascular diseases [16]. However, its function in the pathogenesis of hypertensive cardiac remodeling is uncertain.

Here, our results clearly demonstrate a critical role of CD11b in exacerbating cardiac remodeling and recognize CD11b as the key regulator that mediates the adhesion and polarization of monocytes/macrophages after Ang II and high-salt treatment. We also confirmed that CD11b$^+$ macrophages induce cardiomyocyte hypertrophy and fibroblast differentiation, which cause cardiac dysfunction and adverse remodeling. Taken together, these results provide novel insight into the mechanism by which CD11b regulates hypertension-induced cardiac remodeling, and selective blockade of CD11b may represent an insightful therapeutic strategy for heart disease.

Methods

Animal models

Wild-type (WT) and CD11b knockout (KO) mice [CD11b$^{-/-}$, B6.129S4-*Itgam*$^{tm1Myd/J}$] were and purchased from the Jackson Laboratory. To establish hypertension-induced cardiac remodeling, male mice (eight to ten weeks) were subcutaneously treated with Ang II (1000 ng·kg^{-1}·min^{-1}) or DOCA and high-salt (DOCA-salt). anti-CD11b antibody (mAb, M1/70; 100 μg/mouse every-two days) or the CD11b agonist LA1 (2.5 mg/kg daily) were administered to mice.

Flow cytometry

Immune cells in blood and heart of WT or CD11b KO mice were analyzed by flow cytometry. Detailed methods are described in the Supplemental Material.

BM chimeric mice

BM chimera were generated with BM from WT or CD11b KO mice and was used to evaluate the function of CD11b$^+$ myeloid cells on cardiac remodeling. Detailed methods are described in the Supplemental Material.

Human study

65 HF subjects with decreased left ventricular ejection fraction (LVEF < 40 %) and 65 control groups were enrolled in two clinical cohorts during the June 2021 and February 2022. Patients with HF were diagnosed based on the 2021 European Society of Cardiology Guidelines. Blood from patients and controls was collected for flow cytometric analysis. Detailed methods are described in Supplemental file.

Ethics statement

Animal procedures were approved by the ethics committee of Dalian Medical University (Approval no. AEE21077). Human blood analysis was approved by the Ethics Committees of Dalian Medical University (No. LCKY2016-31) and Capital Medical University (2022-human-244).

Statistics

Results are showed by the mean ± SD and are statistically analyzed by GraphPad Prism 9.0. $P < 0.05$ was deemed to significant difference. Detailed methods are described in the Supplemental Material.

Results

CD11b/CD18 level and myelomonocytes are increased in Ang II-treated heart

To investigate which integrin family member participates in hypertensive cardiac remodeling, we first analyzed the cardiac gene expression profiles of integrins. Microarray analysis revealed that the α subunit CD11b (Itgam) and β subunit CD18 (Igtb2) were the most prominently upregulated genes on day one and then progressively decreased until day seven after infusion (Fig. 1**A and B**). Similarly, compared with those in the saline control group, several binding ligands for CD11b/CD18, including ICAM-1, VCAM-1, fibrinogen a/b, factor X and hmgb1, were highly expressed after Ang II infusion (Fig. 1**B**). The increased mRNA levels of CD11b, CD18, ICAM-1 and factor X were validated by qPCR analysis (Fig. 1**C**). Immunoblotting and immunohistochemistry further

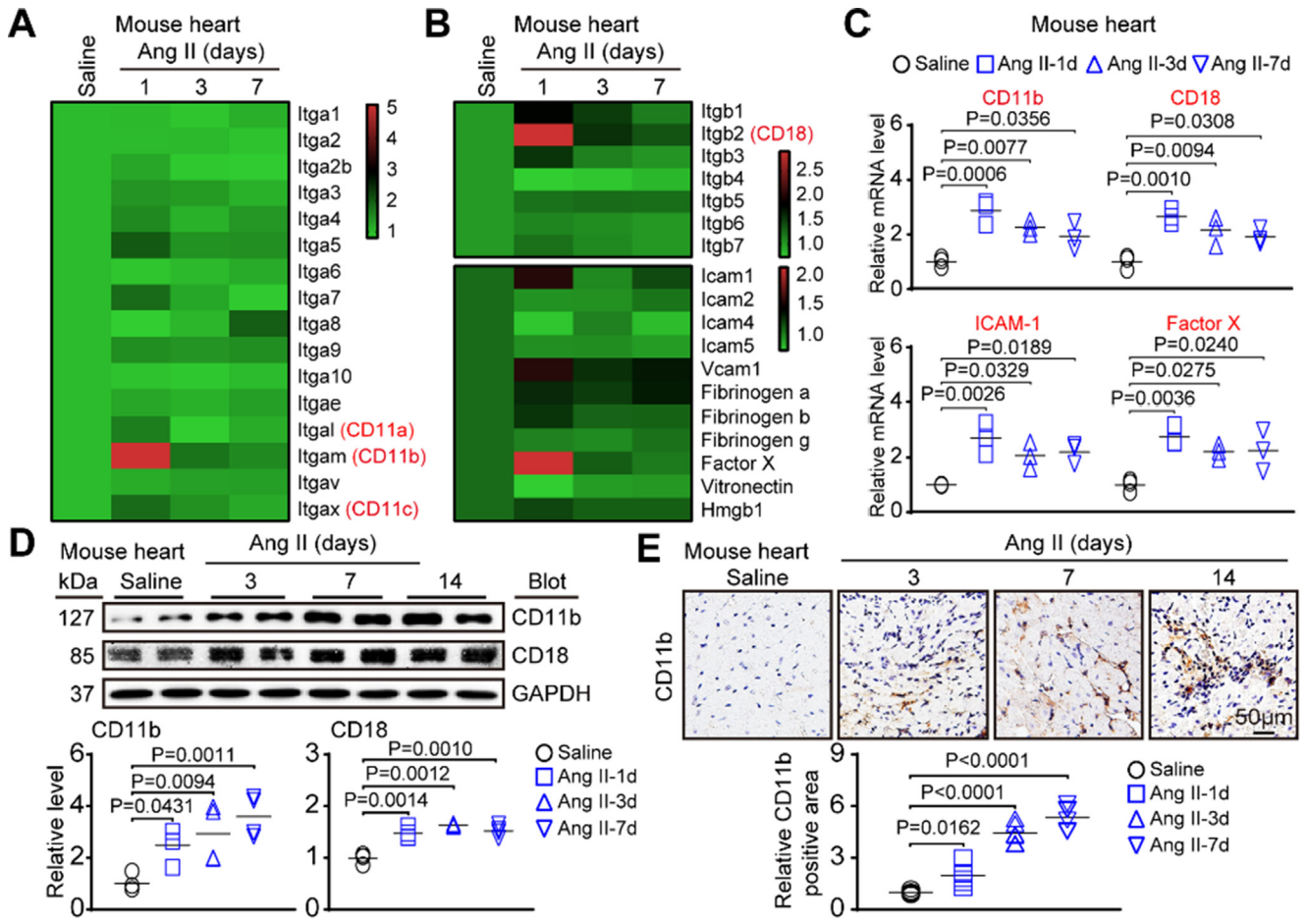


Fig. 1. Ang II increased the level of CD11b/CD18 and its ligands, as well as CD11b/CD18$^+$ myelomonocytes, in mouse hearts. A-B, WT mice were treated with Ang II for one, three and seven days. Heatmaps of cardiac α and β integrins and their ligands (n = 3). **C**, qPCR of CD11b, CD18, ICAM-1 and Factor X (n = 3). **D**, Western blot of CD11b and CD18 (top) and quantification (bottom, n = 4). **E**, Cardiac CD11b immunohistochemical staining (n = 6). **F**, Flow cytometric data of CD45$^+$, CD45$^+$CD11b$^+$, CD45$^+$CD18$^+$, and CD45$^+$CD11b$^+$CD18$^+$ myeloid cells, including Ly6C$^+$Ly6G$^-$F4/80$^+$ MΦs and Ly6G$^+$ neutrophils, in the aortas (left) and the percentage of each type of cell (n = 6). Data were presented as the mean ± SD and were analyzed with one-way ANOVA. Each *P* value is displayed in the image.

confirmed a time-dependent increase in CD11b protein levels in hearts infused of Ang II (Fig. 1**D and E**).

CD11b/CD18 is mainly expressed in myeloid leukocytes. We then examined whether Ang II affected the level of CD11b/CD18 on myelomonocyte infiltrates in the heart. Data indicated that compared with saline group, seven days of Ang II treatment time-dependently increased CD45$^+$ myeloid cells, including CD11b$^+$CD18$^+$Ly6C$^+$Ly6G$^-$F4/80$^+$ MΦs and CD11b$^+$CD18$^+$Ly6G$^+$ neutrophils, in the hearts (Fig. 1**F**). Taken together, our findings show that increased CD11b expression on myelomonocytes contributes to hypertensive cardiac remodeling.

Knockout of CD11b ameliorates Ang II-caused cardiac remodeling and dysfunction

We then evaluated the function of CD11b on WT and CD11b KO mice. We firstly confirmed the CD11b mRNA and protein levels in WT and CD11b KO mice (**Fig. S1A and B**). Systolic blood pressure (SBP) was elevated in WT mice compared with control after Ang II infusion, but the elevation was decreased in CD11b KO mice (**Fig. S1C**). Echocardiography showed that Ang II generated a compensatory augment in systolic function of the heart in WT mice, as showed by raised LVEF% and fractional shortening (FS%), whereas this compensatory effect was reversed in CD11b KO mice (Fig. 2**A**). Furthermore, CD11b KO dramatically attenuated LV hypertrophy, as showed by increases in LV interventricular septal (IVS), ratio of heart weight to body weight (HW/BW) and tibial length (HW/TL), myocyte cross sectional area (CSA) and the atrial natriuretic factor (ANF) mRNA expression compared with those in WT mice (**Table S1**; Fig. 2**B and C**). Accordingly, the cardiac fibrotic levels, the α-smooth muscle actin (α-SMA) positive areas and the mRNA levels of collagen I and α-SMA were significantly lower in CD11b KO group than in WT group after Ang II treatment (Fig. 2**D and E**). Upregulation of p-ERK1/2, p-STAT3, calcineurin A (CaNA), TGF-β1 and p-Smad2/3 protein levels in WT group were suppressed in CD11b KO group (Fig. 2**F**). However, the indices of cardiac function and remodeling in CD11b KO group were comparable to those of WT group treated with saline (Fig. 2**A-F**). Thus, CD11b acts as a pathogenic role cardiac remodeling and dysfunction.

Ablation of CD11b reduces Ang II-caused cardiac macrophage recruitment and polarization

To clarify the pathogenesis of CD11b KO ameliorates hypertensive cardiac remodeling, we evaluated the function of CD11b on cardiac proinflammatory cells recruitment. Flow cytometry indicated that Ang II induces an obvious infiltration in CD45$^+$ myeloid cells in WT mice, including CD11b$^+$Ly6G$^-$Ly6C$^+$F4/80$^+$ macrophages (MΦs) and CD11b$^+$Ly6G$^+$F4/80$^-$ neutrophils, which was decreased in CD11b KO mice (Fig. 3**A**). Moreover, the CD45$^+$ myeloid cells, including CD11b$^+$Ly6C$^+$Ly6G$^-$ monocytes and CD11b$^+$Ly6G$^+$ neu-

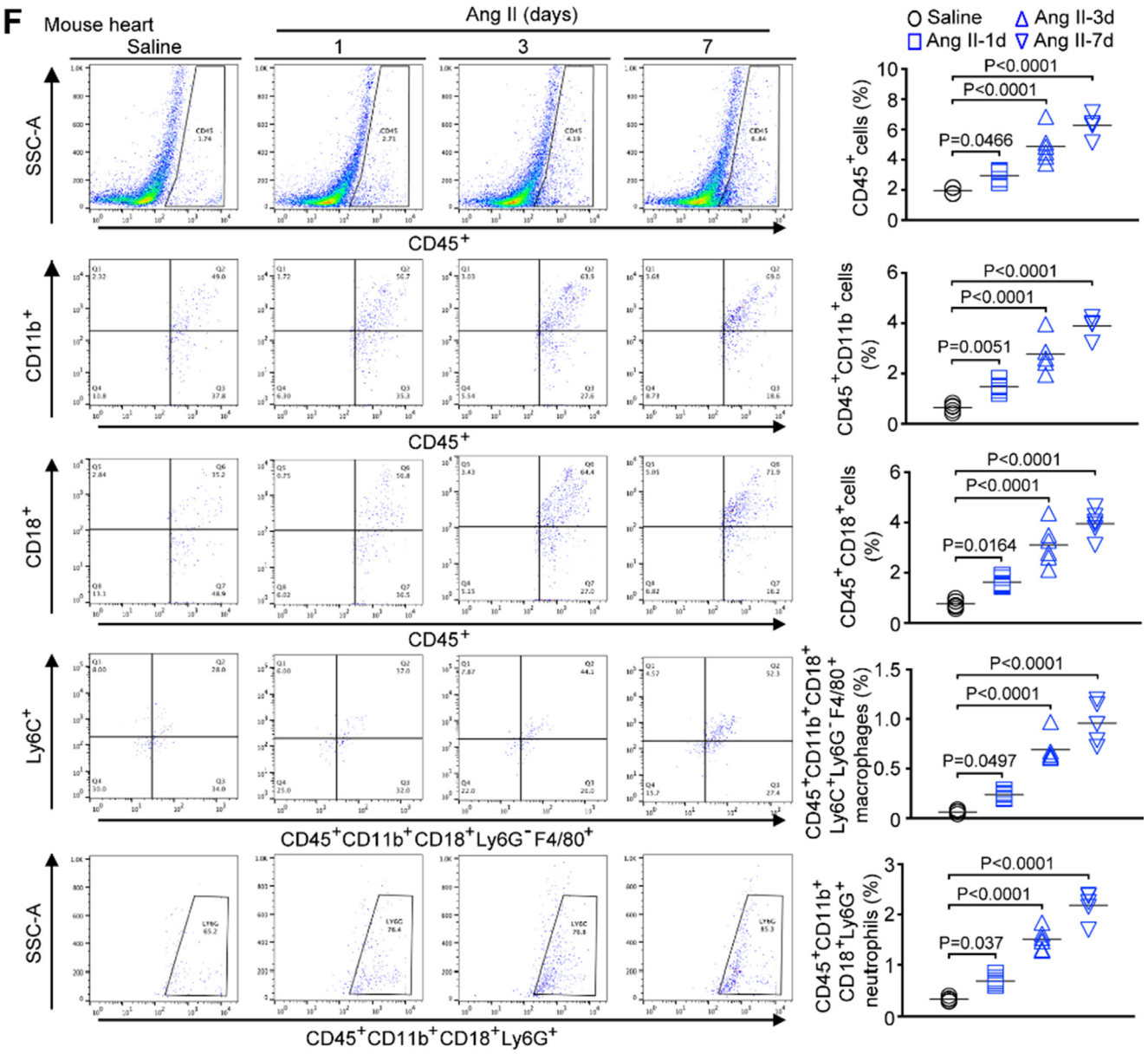


Fig. 1 (*continued*)

trophils, were significantly elevated in the blood of WT mice after Ang II infusion, whereas these cells was substantially decreased in CD11b KO mice (**Fig. S1D**). Immunostaining confirmed that the infiltration of CD68$^+$ MΦs in CD11b KO mice was significantly decreased compared with Ang II-infused WT mice (Fig. 3**B**). To understand how CD11b regulates the cardiac inflammatory response, we evaluated the polarization of MΦs into M1 or M2 subsets in the heart. qPCR showed that CD11b KO mice had significantly lower expression of M1-type cytokines (IL-1β, IL-6, TNF-α and MCP-1) than WT mice following Ang II treatment (Fig. 3**C**). Moreover, Ang II-caused upregulation of p-p65 and p-STAT1 (the key regulators of M1 MΦ polarization) in WT mice were dramatically reversed in CD11b KO mice (Fig. 3**D**). There was no significant difference in the levels of M2-type cytokines (Arg1 and IL-4) and p-STAT6 between Ang II- and saline-infused WT mice, but these markers were highly upregulated in CD11b KO mice after Ang II infusion (Fig. 3**C and D**). Therefore, CD11b KO blocks MΦ infiltration and M1 polarization during cardiac remodeling.

CD11b deletion reduces Ang II-induced MΦ adhesion and polarization in vitro

Monocyte adhesion is pivotal early event in MΦ infiltration and polarization during inflammatory reactions. To determine whether CD11b affects these processes, we cultured BM-derived MΦs from WT or CD11b KO mice, and performed adhesion assay. Our results showed that WT MΦ adhesion to HUVECs was significantly enhanced after Ang II treatment, whereas it was substantially inhibited in CD11b KO MΦs (Fig. 4**A**). Furthermore, the Ang II-caused upregulation of IL-1β, IL-6, TNF-α and MCP-1 in WT MΦs were reversed in CD11b KO MΦs (Fig. 4**B**). Accordingly, the increases in p-p65 and p-STAT1 protein levels in WT MΦs after Ang II treatment were attenuated in CD11b KO MΦs (Fig. 4**C**). Although the mRNA levels of Arg1 and IL-4 and the protein levels of p-STAT6 in Ang II-treated WT MΦs were not significantly changed compared with saline-treated WT MΦs, CD11b KO led to upregulation of these markers in the Ang II groups (Fig. 4**B and C**).

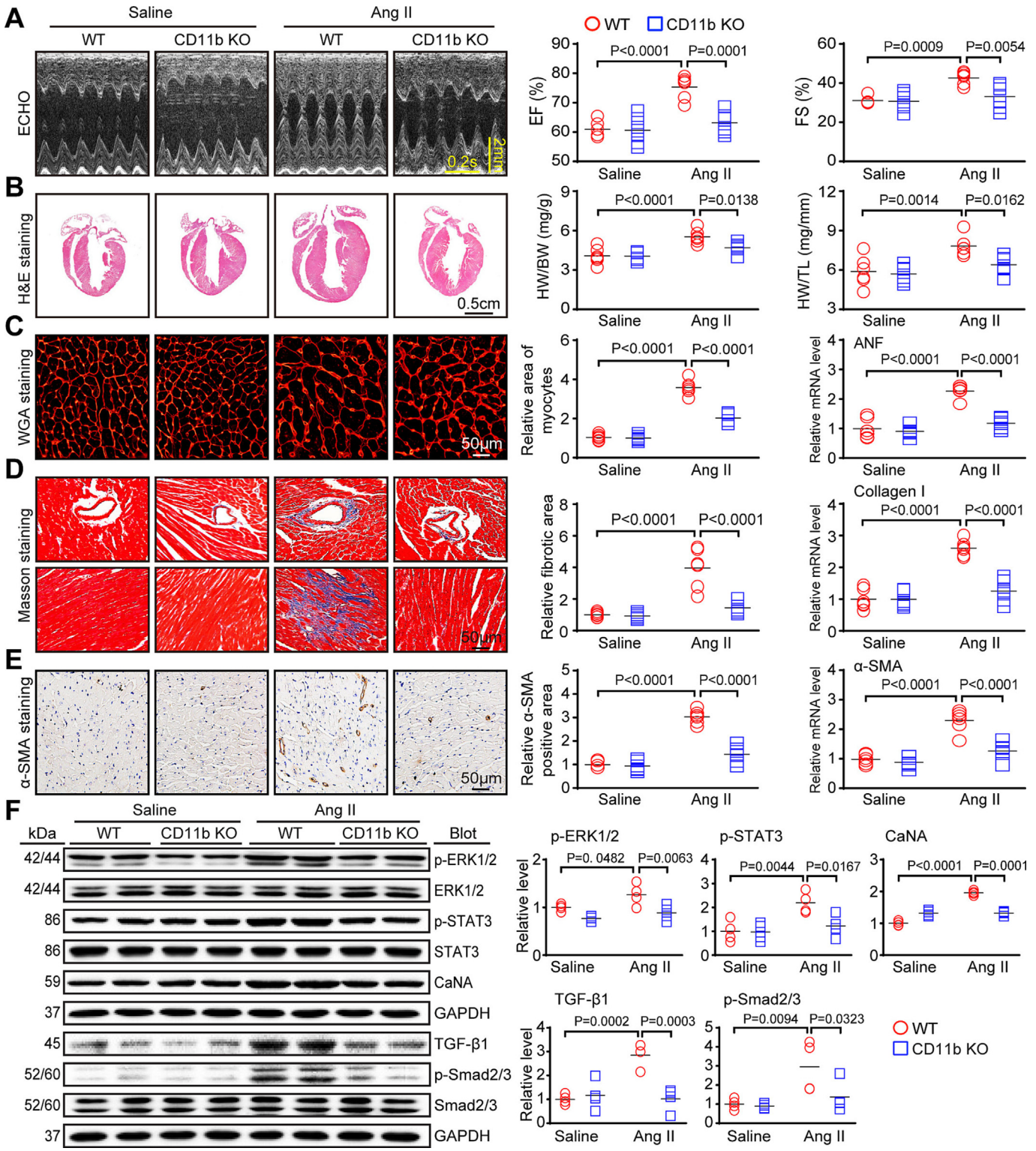


Fig. 2. CD11b deficiency weakens Ang II-caused cardiac hypertrophy and fibrosis. WT and CD11b KO mice were treated with saline or Ang II for 14 days. **A**, Echocardiography (left) and EF% and FS% (right, n = 6). **B**, H&E staining (left) and quantification of the HW/BW and HW/TL ratios (right, n = 6). **C**, WGA staining (left) and quantification (right, n = 6); qPCR of ANF (n = 6). **D**, Masson's trichrome staining (left) and quantification (right, n = 6); qPCR of collagen I (n = 6). **E**, α-SMA immunohistochemical staining (left) and quantification (right, n = 6); qPCR of α-SMA (n = 6). **F**, Immunoblot of p-ERK1/2, ERK1/2, p-STAT3, STAT3, CaNA, TGF-β1, p-Smad2/3 and Smad2/3 (left) and quantification (right, n = 4). Data were expressed as the mean ± SD and were analyzed with two-way ANOVA. Each *P* value is displayed in the image.

To verify the causal function of CD11b on MΦ adhesion and polarization in vitro, we pretreated BM-derived MΦs with the CD11b agonist LA1 and treated them with Ang II. Compared with vehicle treatment, LA1 treatment significantly increased MΦ adhesion and the levels of IL-1β, IL-6, TNF-α and MCP-1 mRNAs and p-p65 and p-STAT3 proteins, but markedly reduced the expression levels of Arg1, IL-4 and p-STAT6 in WT BM MΦs after saline or Ang II infusion**.** In contrast, LA1-induced effects were fully

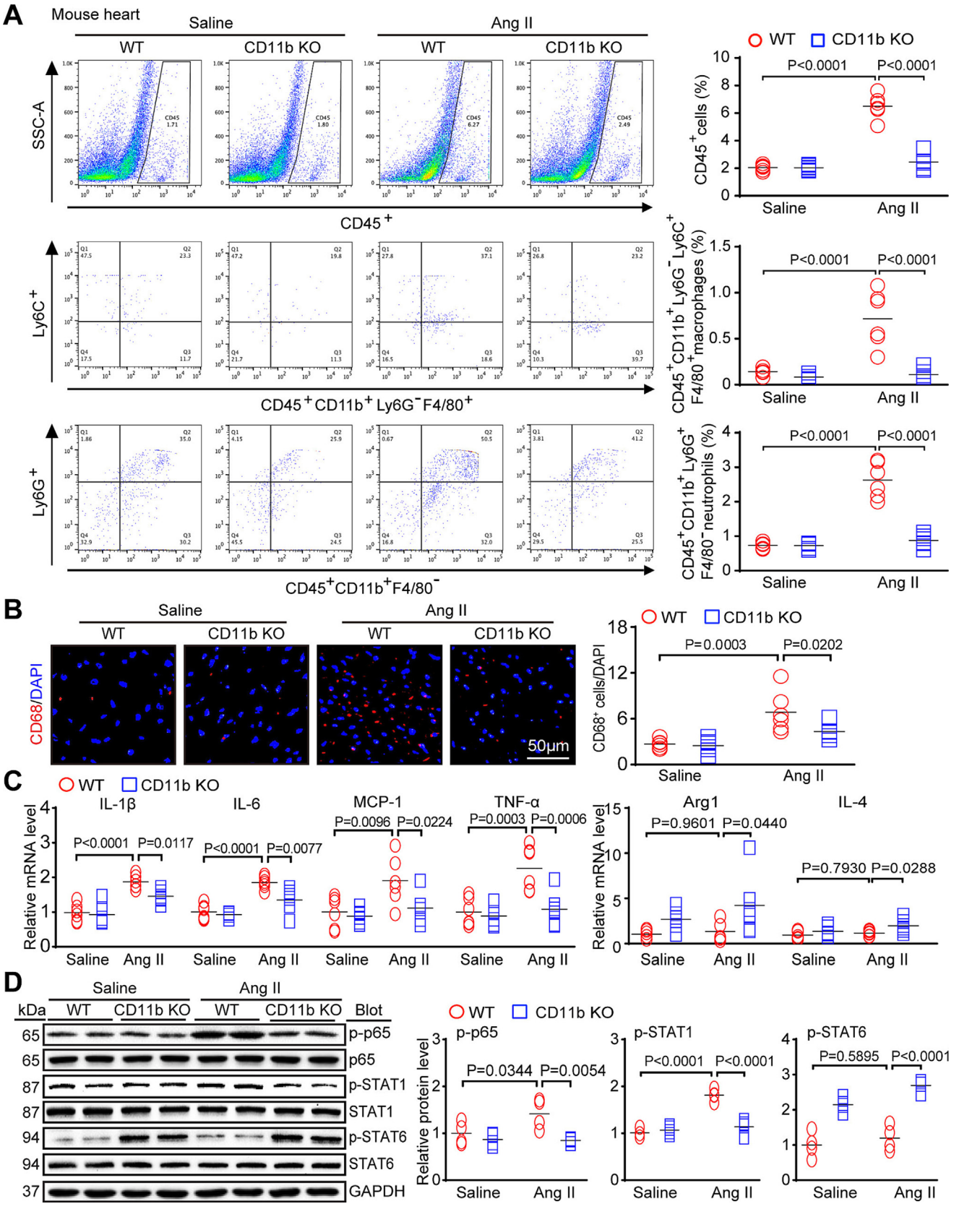
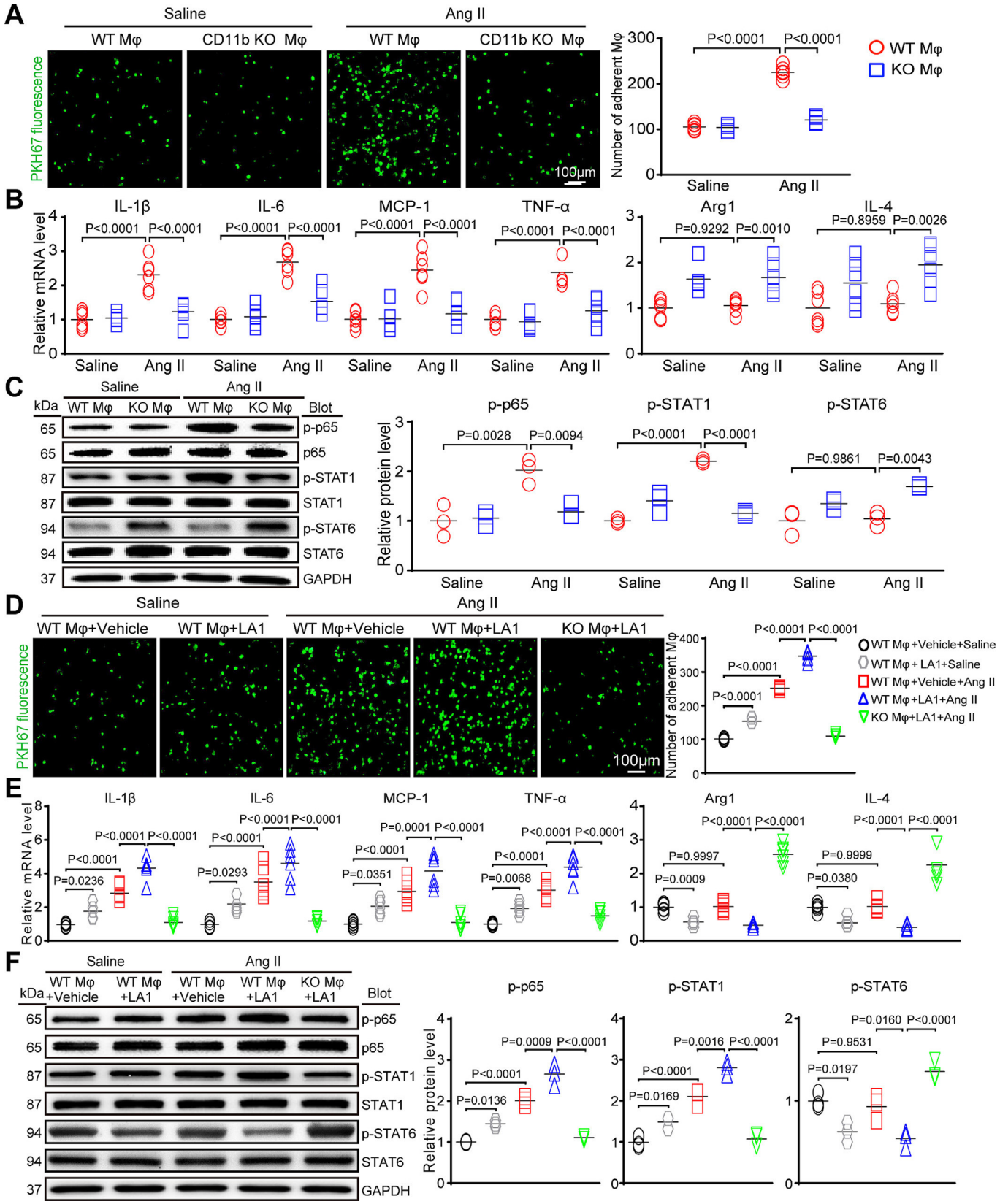


Fig. 3. CD11b KO ameliorates Ang II-induced CD11b-positive myelomonocyte infiltration and MΦ polarization. WT and CD11b KO mice were infused with saline or Ang II for 14 days. **A**, Flow cytometric data of CD45$^+$ myelomonocytes, including CD11b$^+$Ly6G$^-$Ly6C$^+$F4/80$^+$ MΦs and CD11b$^+$Ly6G$^+$F4/80$^-$ neutrophils (left), in the heart, and quantification (right, n = 6). **B**, CD68 immunofluorescent staining (red) and quantification (right, n = 6). **C**, qPCR of IL-1β, IL-6, MCP-1, TNF-α, Arg1 and IL-4 (n = 6). **D**, Immunoblot of p-p65, p65, p-STAT1, STAT1, p-STAT6 and STAT6 (left) and quantification (right, n = 4). Data were expressed as the mean ± SD and were analyzed with two-way ANOVA. Each *P* value is displayed in the image. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

abolished in CD11b KO MΦs (Fig. 4**D-F**). Interestingly, Ang II treatment highly upregulated M1 polarization related markers but did not affect M2 polarization related markers (Fig. 4**D-F**). Therefore, these results show that CD11b activation sufficiently promotes MΦ adhesion and M1 polarization in response to Ang II.

BM-derived CD11b-deficient cells ameliorate Ang II-caused cardiac remodeling and dysfunction

To directly ascertain whether CD11b-mediated recruitment of monocytes/MΦs is causally related to hypertensive cardiac remod-



eling, we constructed chimera via transplanting WT or CD11b KO BM into CD11b KO or WT mice. The genotypic analysis of chimeric mice indicated the success of bone marrow transplantation (BMT) (**Fig. S2A**). After six weeks, all chimeras were treated with Ang II. Comparing to WT mice reconstituted with WT BM, WT mice reconstituted with CD11b KO BM showed a marked reduction in SBP (**Fig. S2B**) and improvements in systolic dysfunction of heart (increased LVEF% and FS%) (Fig. 5**A; Table S2**), cardiac hypertrophy (reduced HW/BW and HW/TL ratios, CSA of myocytes and ANF mRNA level) (Fig. 5**B and C**), fibrosis (decreased fibrotic area, α-SMA$^+$ areas and collagen I and α-SMA mRNA levels) and CD68$^+$ MΦ infiltration (Fig. 5**D and E**), as well as the downregulation of M1-type cytokines (IL-1β, IL-6, TNF-α and MCP-1) and upregulation of M2-type cytokines (Arg1 and IL-4) (Fig. 5**F**). These beneficial functions were verified in CD11b KO mice reconstituted with CD11b KO BM (Fig. 5**A-F**), but were completely reversed in CD11b KO mice reconstituted with WT BM (Fig. 5**A-F**). Thus, these findings clearly demonstrate that CD11b$^+$ myeloid cells promote Ang II-induced cardiac remodeling.

CD11b KO attenuates cardiac remodeling in mice induced by DOCA-salt challenge

To assess the general significance of our results, we examined whether CD11b KO could inhibit DOCA-salt-caused hypertensive cardiac remodeling. After three weeks of DOCA-salt treatment, promoted the SBP (**Fig. S3A**) and cardiac dysfunction in WT mice, as indicated by enhanced EF% and FS%, which was greatly attenuated in CD11b KO mice (**Fig. S3B; Table S3**). Furthermore, CD11b KO mice demonstrated observably decreased myocardial hypertrophy and fibrosis, as reflected by marked reductions in LV and IVS wall thickness, HW/BW and HW/TL ratios, myocyte CSA, collagen deposition, the areas of α-SMA$^+$ myofibroblasts and numbers of CD68$^+$ MΦs in the heart compared with those of WT controls after DOCA-salt treatment (**Fig. S3C-G**). Additionally, the upregulation of ANF, collagen I, α-SMA, IL-1β, IL-6, TNF-α and MCP-1 mRNA expression in WT group induced by DOCA-salt were observably reversed in CD11b KO group (**Fig. S3H-K**). There was no significant difference in M2-type cytokines (Arg1 and IL-4) in WT mice after DOCA-salt treatment compared with saline treatment, but these markers were remarkably upregulated in CD11b KO mice after DOCA-salt treatment (**Fig. S3K**). These results show that CD11b KO ameliorates cardiac remodeling induced by different neuroendocrine stimuli.

Pharmacological blockade of CD11b prevents Ang II-caused myocardial remodeling

To verify potential clinical translational value of our findings, WT mice were systemically administered anti-CD11b neutralizing monoclonal antibody (mAb) or control immunoglobulin G (IgG). After Ang II infusion, SBP was observably lower in anti-CD11b-treated mice than in IgG controls (**Fig. S4**). Moreover, IgG-administered mice developed a marked increase in myocardial hypertrophy, as reflected by enhanced LV and IVS, HW/BW and HW/TL ratios, myocyte CSA, ANF level (**Table S4; Fig. 6A and B**) and fibrosis, as showed by increased fibrotic areas, and α-SMA$^+$ myofibroblasts, and mRNA levels of collagen I and α-SMA (Fig. 6**C and D**), and these hypertrophic and fibrotic responses were substantially attenuated in anti-CD11b-treated mice (Fig. 6**A-D**). Accordingly, there was a significant decrease in the infiltration of CD68$^+$ MΦs and the IL-1β, IL-6, TNF-α and MCP-1 mRNA levels in anti-CD11b-treated group than in IgG controls after Ang II stimulation (Fig. 6**D and E**). There was no difference in Arg1 and IL-4 mRNA levels in IgG-administered mice following Ang II infusion, and anti-CD11b treatment significantly upregulated these markers (Fig. 6**E**). Finally, we measured cardiac function by echocardiography, and observed that the administration of anti-CD11b significantly improved Ang II-caused cardiac dysfunction compared with IgG-administrated mice (Fig. 6**F**). Collectively, current data demonstrate that selective blockade of CD11b may represent a novel policy for cardiac remodeling in vivo.

CD11b activation exacerbates myocardial hypertrophy, fibrosis and the inflammatory response after Ang II infusion

To confirm causative function of CD11b in regulating cardiac remodeling, WT mice or CD11b mice were systemically administered the CD11b agonist LA1 before continuous Ang II or saline treatment. Data indicated that LA1 resulted in an obvious increase in SBP compared with vehicle treatment after saline or Ang II administration (**Fig. S5A**). Echocardiography showed that LA1 treatment enhanced cardiac performance (increased EF% and FS%) after saline infusion, but greatly reduced cardiac contractile function (decreased EF% and FS%) after Ang II treatment (**Table S5; Fig. S5B**), suggesting that LA1 exhibits cardiac toxicity in mice. Accordingly, LA1-treated mice had increased hypertrophy (enhanced LV and IVS wall thickness, HW/BW and HW/TL ratios, enlarged myocytes, and ANF expression) (**Fig. S5C-E**), LV fibrosis (increased fibrotic area, α-SMA$^+$ areas and mRNA levels of collagen I and α-SMA) and infiltration of CD68$^+$ MΦs in the heart (**Fig. S5F-J**) compared with vehicle-administrated groups. Moreover, the IL-1β, IL-6, TNF-α and MCP-1 mRNA levels were upregulated, and the Arg1 and IL-4 mRNA levels were downregulated in LA1-treated group compared with vehicle-administrated mice after saline or Ang II treatment (**Fig. S5K**). Notably, the deleterious effects of LA1 alone or combined with Ang II were completely abrogated in CD11b KO mice (**Fig. S5A-K**), suggesting that hypertrophic effect of LA1 was mediated by CD11b activation.

CD11b-deficient macrophages abrogate Ang II-caused cardiomyocyte enlargement and myofibroblast activation in vitro

To determine whether CD11b$^+$ MΦs directly induce cardiomyocyte hypertrophy, we cocultured WT or CD11b KO MΦs with primary neonatal rat cardiac myocytes (CMs) and fibroblasts (CFs) in vitro. Immunostaining indicated that culturing CMs with CD11b KO MΦs observably suppressed Ang II-caused CM enlarge-

Fig. 4. CD11b deficiency inhibits Ang II-induced macrophage adhesion to ECs, as well as upregulation of M1 genes and downregulation of M2 genes in vitro, and CD11b activation with LA1 accelerates these effects. A, Macrophages were stained with PKH67 (green) and added to HUVECs that were prestimulated with Ang II for 24 h (left), and quantification of adherent cells (right, n = 3 independent experiments, 6 fields). **B**, qPCR of TNF-α, IL-1β, IL-6, MCP-1, Arg1 and IL-4 in macrophages (n = 3 independent experiments). **C**, Immunoblot of p65, STAT1 and STAT6 in macrophages (left) and quantification (right, n = 3 independent experiments). **D**, HUVECs were pretreated with Ang II for 24 h. Macrophages were stained with PKH67 (green), treated with the CD11b agonist LA1 for 30 min, and then added to the pretreated HUVECs. Representative images (left) and quantification of adherent cells on HUVECs (right, n = 3 independent experiments, 6 fields). **E**, qPCR of TNF-α, IL-1β, IL-6, MCP-1, Arg1 and IL-4 in macrophages (n = 3 independent experiments, 6 fields). **F**, Immunoblot of p65, STAT1 and STAT6 in macrophages (left) and quantification (right, n = 3 independent experiments). The data are expressed as m ± SD and were analyzed with two-way ANOVA followed by Sidak's multiple comparison test. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

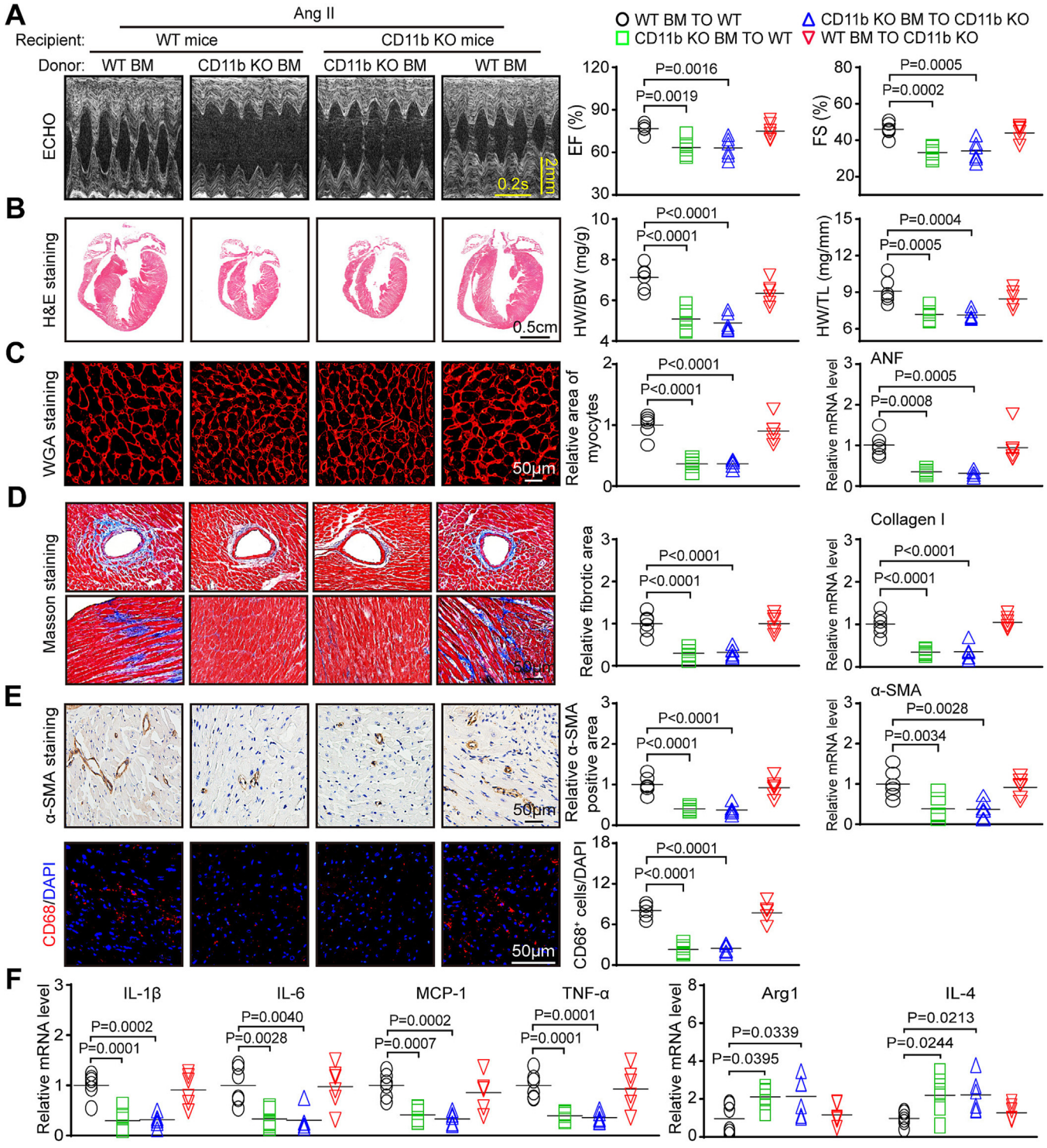


Fig. 5. BM-derived CD11b-deficient cells protect against Ang II-caused cardiac hypertrophy, fibrosis and inflammation. WT or CD11b KO mice were reconstituted with BM cells derived from WT or CD11b KO mice and treated with Ang II for 14 days. **A**, Echocardiography (left) and EF% and FS% (right, n = 6). **B**, H&E staining (left), and quantification of the HW/BW and HW/TL ratios (right, n = 6). **C**, WGA staining (left) and quantification (right, n = 6); qPCR of ANF (n = 6). **D**, Masson's trichrome staining (left) and quantification (right, n = 6); qPCR of collagen I (n = 6). **E**, α-SMA immunohistochemical staining (left) and quantification (right, n = 6); qPCR of α-SMA (n = 6); CD68 immunofluorescent staining (red) and quantification (right, n = 6). **F**, qPCR of IL-1β, IL-6, MCP-1, TNF-α, Arg1 and IL-4 (n = 6). Data were expressed as the mean ± SD and were analyzed with two-way ANOVA. Each *P* value is displayed in the image. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ment compared with culturing CMs with WT MΦs (Fig. S6**A**). Accordingly, Ang II-caused augment of the ANF and BNP mRNA levels and the p-ERK1/2, p-STAT3 and CaNA protein levels in CMs cocultured with WT MΦs were also markedly lower in CMs cocultured with CD11b KO MΦs (Fig. S6**B and C**). Furthermore, culturing CFs with CD11b KO MΦs observably suppressed Ang II-caused increases of collagen I and collagen III mRNA levels, and TGF-β1 and p-Smad2/3 protein levels compared with culturing

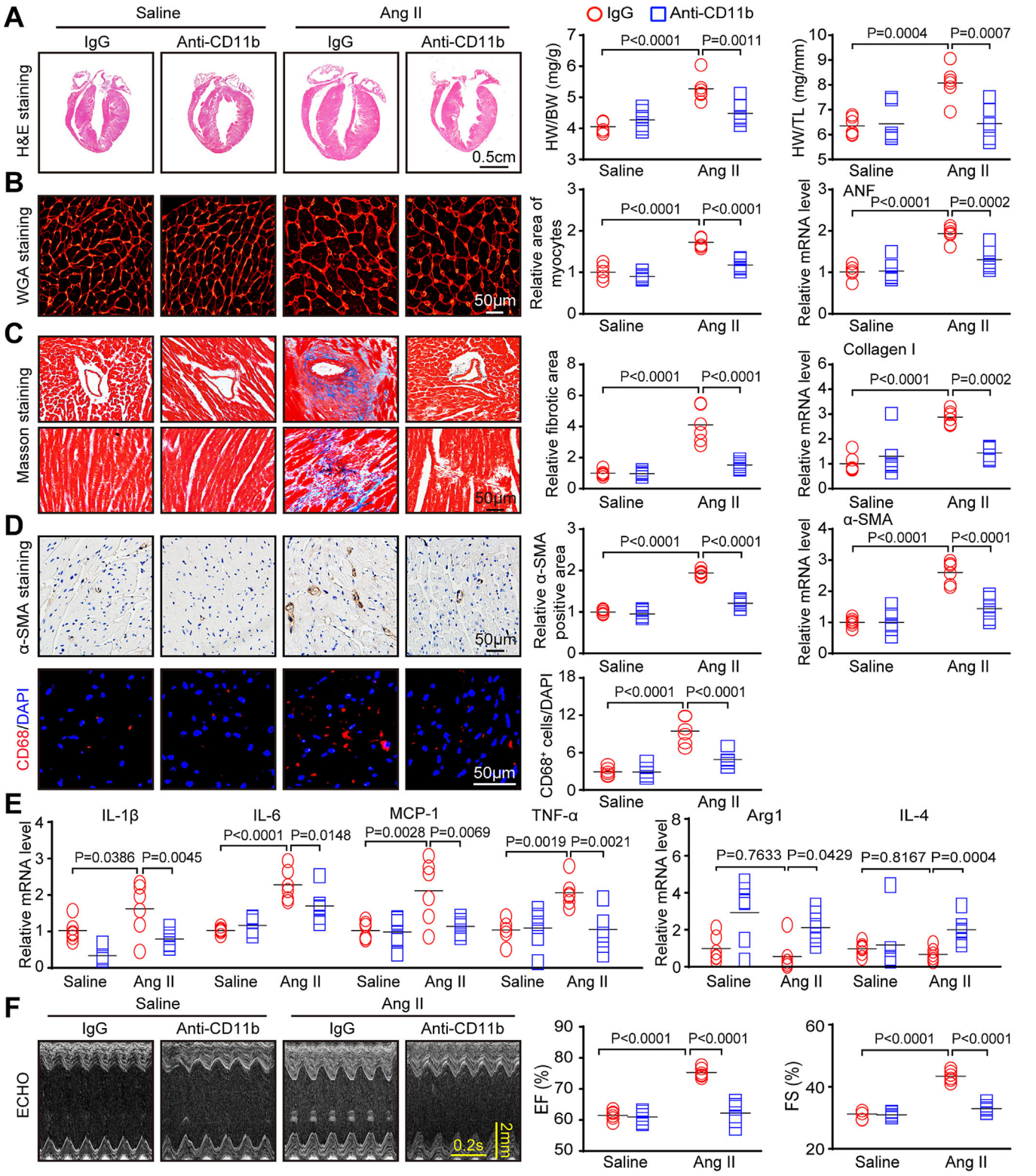


Fig. 6. Pharmacological blockade of CD11b prevents Ang II-caused cardiac remodeling. WT mice were administered CD11b mAb or IgG control and treated with Ang II for 14 days. **A**, H&E staining (left) and quantification of HW/BW and HW/TL ratios (right, n = 6). **B**, WGA staining (left) and quantitation (right, n = 6); qPCR of ANF (n = 6). **C**, Masson's trichrome staining (left) and quantification (right, n = 6); qPCR of collagen I (n = 6). **D**, α-SMA immunohistochemical staining (left) and quantification (right, n = 6); qPCR of α-SMA (n = 6); CD68 immunofluorescent staining (red) and quantification (right, n = 6). **E**, qPCR of IL-1β, IL-6, MCP-1, TNF-α, Arg1 and IL-4 (n = 6). **F**, Echocardiography (left) and EF% and FS% (right, n = 6). Data were expressed as the mean ± SD and were analyzed with two-way ANOVA. Each *P* value is displayed in the image. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

CFs with WT MΦs (**Fig. S6D and E**). Taken together, these results show that CD11b$^+$ MΦs have direct prohypertrophic and profibrotic effects.

Circulating CD11b/CD18$^+$ myelomonocytes and ligand levels are increased in patients with HF

To examine the association of circulating CD11b/CD18$^+$ immune cells and their ligand levels with HF in patients, we analyzed these parameters in 65 HF patients and 65 healthy subjects who enrolled between June 2021 and February 2022. HF patients were older, had significantly lower LVEF % and HDL cholesterol, and had higher SBP, heart rate, fasting blood glucose, white blood cell count and creatinemia than healthy subjects (**Table S6**). Moreover, in comparison with those of healthy subjects, the numbers of serum CD45$^+$CD11b$^+$, CD45$^+$CD18$^+$, and CD45$^+$CD11b$^+$CD18$^+$ myeloid cells, including CD14$^+$CD11b$^+$, CD14$^+$CD18$^+$ and CD14$^+$CD11b$^+$-CD18$^+$ monocytes, and CD15$^+$CD11b$^+$, CD15$^+$CD18$^+$ and CD15$^+$CD11b$^+$CD18$^+$ granulocytes were remarkably increased in HF patients (Fig. 7**A**). Similarly, serum levels of ligands such as ICAM-1, fibrinogen a and factor X were also higher in HF subjects than in healthy controls (Fig. 7**B**). Furthermore, we adjusted for sex, age, SBP, heart rate, cholesterol, leukocyte count, creatinemia and fasting blood glucose, and the multivariable logistic regression analysis showed that the amounts of CD45$^+$CD11b$^+$ cells [odds ratio (OR) 1.229], CD45$^+$CD18$^+$ cells (OR 1.112), CD45$^+$CD11b$^+$-CD18$^+$ cells (OR 3.569), CD45$^+$CD14$^+$CD11b$^+$ monocytes (OR 2.299), CD45$^+$CD14$^+$CD18$^+$ monocytes (OR 6.959), CD45$^+$CD14$^+$-CD11b$^+$CD18$^+$ monocytes (OR 2.847), CD45$^+$CD15$^+$CD11b$^+$ granulocytes (OR 3.809) and the levels of fibrinogen a (OR 1.211) were statistically associated with the presence of HF (**Table S7**). However, no relationship was found between CD45$^+$CD15$^+$CD11b$^+$-CD18$^+$ granulocytes, as well as serum levels of ICAM-1 and factor X, and HF.

Discussion

Current data demonstrated the causal significance of CD11b in accelerating hypertensive cardiac remodeling. Genetic deletion or blockade of CD11b significantly attenuated hypertension-induced cardiac remodeling and dysfunction by decreasing MΦ adhesion and M1 polarization in vivo and in vitro. In contrast, LA1-mediated activation of CD11b exerted the opposite effect. Consequently, these data reveal unequivocal evidence that hypertension-activated CD11b critically contributes to the progression of cardiac remodeling, and blocking CD11b may represent a novel policy for HF. A schematic diagram is shown in Fig. 8.

Inflammation is a hallmark of cardiac remodeling and HF. When faced with heart injury, resident immune cells are rapidly activated and release a myriad of cytokines, chemokines and CAMs, which are responsible for the recruitment of circulating leukocytes into the injured myocardium [4,17]. Previous studies have demonstrated that chemokines such as CXCL1 and CCL2 can promote the infiltration of CXCR2$^+$ and CCR2$^+$ monocytes/MΦs in the heart, which triggers cardiac remodeling after Ang II infusion or pressure overload [7–9]. Increasing evidence indicates that CD11b/CD18 binding to its ligands (ICAM-1 and VCAM-1) predominantly mediates leukocyte adhesion to the endothelium and plays an important role in some cardiovascular diseases [18]. Deficiency or blockade of ICAM-1 and VCAM-1 markedly decreased the infiltration of monocytes and T cells of heart and alleviated pressure overload- or Ang II-caused cardiac remodeling [10,11,13,19,20]. In addition to CD11b/CD18-ICAM-1 signaling, integrin-α4β1/VLA-4-VCAM interactions also promotes adhesion and infiltration of CD11b$^+$ monocytes/MΦs in tumors [21]. Moreover, α4β1 collaborates with SDF-1α and IL-1β to enhance infiltration of CD11b$^+$ monocytes and granulocytes in tumors, whereas blockage of α4β1, SDF-1α, or IL-1β reduces these effects [22], suggesting another mechanism to regulate adhesion and infiltration of CD11b$^+$ myeloid cells. Several studies have indicated that CD11b KO inhibits the adhesion and infiltration of monocytes/MΦs and neutrophils, thereby delaying the progression of atherosclerosis, thrombosis, neointimal hyperplasia, and ischemic cardiac/cerebral diseases in different animal models [23–27]. More recently, our data reveal that CD11b promotes macrophage adhesion and migration, thereby contributing to Ang II or DOCA-salt-induced hypertension and vascular dysfunction [18]. Consistent with this report**,** our present study confirmed the effect of CD11b on blood pressure, and further demonstrated that ablation or blockade of CD11b suppressed Ang II-induced MΦ adhesion and migration, leading to reduced MΦ infiltration and subsequently cardiac remodeling (Figs. 2-6). Conversely, LA1-induced activation of CD11b accelerated the Ang II-induced response (**Fig. S5**), Thus, the results suggest that CD11b-mediated adhesion and migration of MΦs contributed to hypertension-dependent cardiac remodeling.

Cardiac remodeling and HF, as evidenced by hypertrophy and fibrosis [28–31], are characterized by abundant myeloid cell infiltration, which is essential for inflammation and subsequent pathological remodeling [17]. Murine monocytes are vital innate immune cells and are classified into Ly-6C^{high} and Ly-6C^{low} subsets. Ly6C^{high} monocytes are equivalent to classic CD14^{high} monocytes in humans and preferentially differentiate into inflammatory M1 MΦs, whereas Ly6C^{low} monocytes differentiate into reparative M2 MΦs in the heart [17,32]. Several transcription factors are known to regulate MΦ polarization. STAT1 and NF-κB upregulate the target genes (IL-1β, TNF-α and iNOS) and drive M1 MΦ differentiation. Conversely**,** the IL-4/KLF4-STAT6 signaling pathway can induce PPAR-δ or Arg-1 expression to promote M2 polarization [32]. Importantly, dysregulation of MΦ phenotypes induces excessive inflammation leading to cardiac damage during hypertension [17,32]. Ang II infusion can skew MΦs toward the M1 phenotype, which produce proinflammatory cytokines that contribute to development of hypertension and cardiac remodeling [7,8,33,34]. Interestingly, several integrins are reported to play a role in MΦ polarization. VLA-2 (also known as α2β1) mediates IL-4 expression and induces the M2 MΦ phenotype via STAT6 activation [35]. Conversely, vitronectin-ITGAV signaling can activate NF-κB to release proinflammatory cytokines and promote M1 MΦs [36]. Moreover, ICAM-1 can reduce PI3K-AKT-mediated efferocytosis and inhibit M2 MΦs [37]. CD11b activation results in M1 MΦ polarization by increasing the expression of miRNA-Let7a in mice and human cancer [38]. CD11b/CD18 activation inhibits M2 MΦ polarization and foam cell formation during atherosclerosis [39]. However, it is unclear how CD11b/CD18 regulates MΦ polarization during hypertensive cardiac remodeling. Our results showed that ablation or blockade of CD11b in mice significantly inhibited Ang II-caused NF-κB and STAT1 activation, but activated STAT6 activity, which suppressed MΦ polarization toward the M1 phenotype, thereby attenuating cardiac myocyte hypertrophy and fibrosis via multiple signaling pathways (Figs. 3-6), whereas LA1-mediated activation of CD11b accelerated M1 MΦ polarization (**Fig. S5**). Taken together, these data reveal that CD11b decreases MΦ polarization through the NF-κB/STAT1 and STAT6 signaling pathways during cardiac remodeling.

CD11b/CD18 is specifically expressed on leukocytes and is significantly upregulated during various stresses. Ischemia/reperfusion injury markedly increases CD11b/CD18 expression on neutrophils and monocytes in the ischemic heart [40]. Moreover, CD11b expression on coronary sinus leukocytes was highly increased, and the level of CD11b/CD18 was positively associated

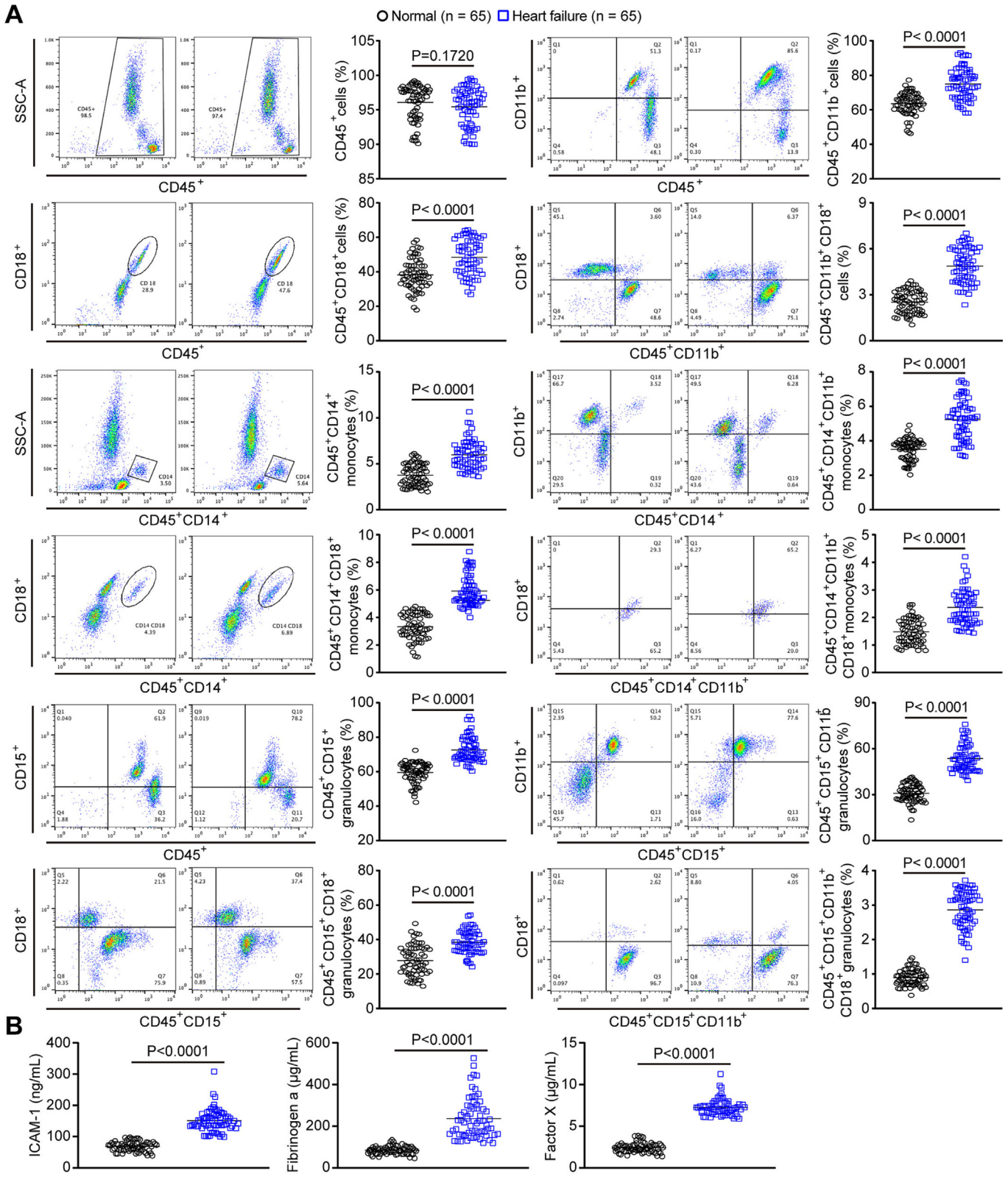


Fig. 7. CD11b/CD18 positive myelomonocytes and the levels of ligands are increased in the blood of patients with HF. A, Flow cytometric analysis of $CD45^+$ myelomonocytes, including $CD11b^+$, $CD18^+$, and $CD11b^+CD18^+$ cells; $CD14^+CD11b^+$, $CD14^+CD18^+$ and $CD14^+CD11b^+CD18^+$ monocytes; and $CD15^+CD11b^+$, $CD15^+CD18^+$ and $CD15^+CD11b^+CD18^+$ granulocytes, in the blood of HF subjects (n = 65) and controls (n = 65). **B**, ELISA of blood ICAM-1, fibrinogen a and factor X levels in the blood of HF subjects (n = 65) and controls (n = 65). Data were expressed as the mean ± SD and were analyzed with a sample *t* test. Each *P* value is displayed in the image.

with restenosis in patients after coronary stenting [41]. In addition, ICAM1 expression in intramyocardial endothelial cells is markedly enhanced within 48 h of pressure overload and remains increased during HF progression [19], suggesting that the upregulation of CD11b/CD18 and its ligands is crucial for leukocyte infiltration and these diseases [13,23–27]. To further prove the clinical rele-

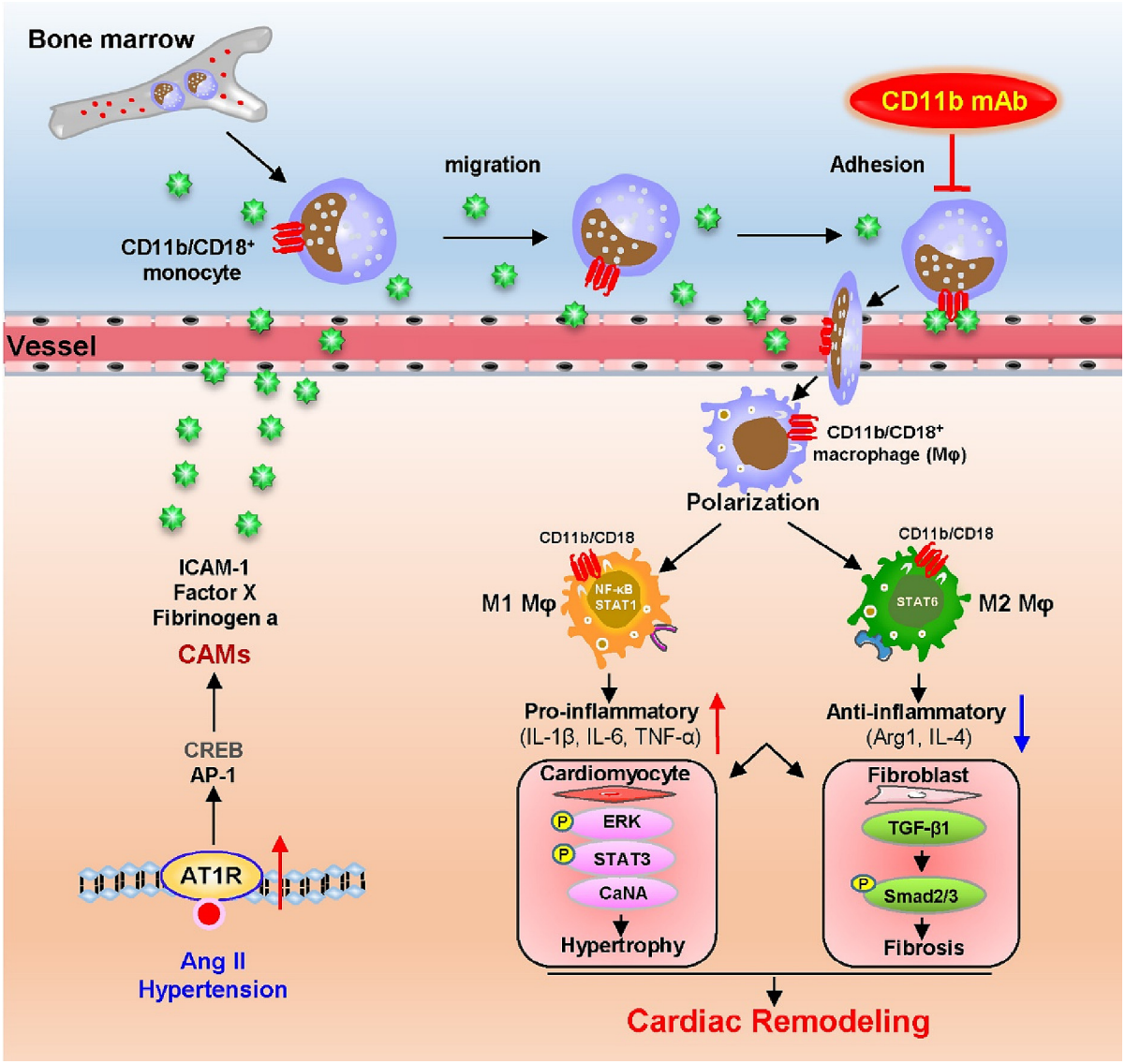


Fig. 8. A graphical abstract for CD11b contributes to cardiac hypertensive remodeling. Ang II stimulates the endothelial cells to release abundant CAMs, including ICAM-1, factor X and fibrinogen a, which recruit bone marrow-derived CD11b/CD18$^+$ monocytes. These cells migrate and adhere to the vascular endothelium, then infiltrate into heart and differentiate into CD11b/CD18$^+$ M1 macrophages that produce large amount of proinflammatory cytokines (IL-1β, IL-6 and TNF-α), thereby promoting cardiac remodeling and dysfunction. Ablation or inhibition of CD11b with neutralizing antibody significantly attenuates these effects.

vance of current findings in animals and cells, we analyzed a number of ligands and CD11b/CD18$^+$ immune cells. Consistent with the data in Ang II-infused heart (Fig. 1), the numbers of CD45$^+$CD14$^+$-CD11b$^+$CD18$^+$ monocytes and CD45$^+$CD15$^+$CD11b$^+$CD18$^+$ granulocytes, as well as the serum levels of ICAM-1, fibrinogen a and factor X, were observably magnified in HF subjects compared with controls. Interestingly, the number of CD45$^+$CD14$^+$CD11b$^+$CD18$^+$ cells and the level of fibrinogen a were markedly associated with the incidence of HF in patients (Fig. 7). Thus, these results indicate the translational relevance of CD11b$^+$CD18$^+$ monocytes and the ligands in patients with HF.

CD11b/CD18 and its ligands have important roles in various inflammatory diseases, allowing for the identification of novel therapeutic candidates for treating these diseases. Indeed, some mAbs or small molecular drugs against CD11b/CD18 or its ligands (ICAM-1 or VCAM-1) have been discovered or developed for clinical trials or preclinical animal studies. BI-505 (a human anti-ICAM-1 mAb) has been safely administered to multiple myeloma patients (ClinicalTrials Identifier: NCT01025206). The administration of ICAM-1 or VCAM-1 mAb observably prevents Ang II-caused mouse hypertension and HF [10,11,42]. Inhibiting CD11b/CD18 in mice with anti-M7 mAb selectively reduces leukocyte recruitment and prevents bacterial sepsis and atherogenic lesion formation [43,44]. Moreover, blocking CD11b with M1/70 rat mAb or CD18 with anti-human CD18 mAb attenuates balloon or stent-induced neointimal thickening in rabbits or primates [45,46]. In addition, blockade of CD11b/CD18 with the inhibitor NPC15669 dramatically improves ischemia-induced cardiac dysfunction in piglets [47]. Here, our data extend the applications of these targets, and demonstrate that targeting CD11b is an effective therapy for hypertensive cardiac remodeling in mice (Fig. 6).

Study limitations: We did not specifically examine the role of CD11b$^+$ MΦ ablation in the development of HF. Additionally, we did not examine the effects of CD18 inhibition on MΦ adhesion and polarization during hypertensive cardiac remodeling. Moreover, we did not study how Ang II upregulates ICAM-1, factor X and

fibrinogen a on endothelial cells and CD11b/CD18 expression on monocytes/MΦs, and whether these ligands recruit BM-derived CD11b/CD18^{+} monocytes in the heart.

Conclusion

This study demonstrates that CD11b/CD18-mediated early monocyte adhesion and MΦ polarization can contribute to pathological cardiac remodeling in hypertension. These results also identify the importance of targeting CD11b/CD18 on monocyte/MΦ populations, as opposed to systemic anti-inflammatory therapy, to achieve optimal therapeutic benefits for hypertensive cardiac remodeling and highlight the translation of mouse experiments to human patients with HF, showing novel insights into the significance of CD11b/CD18 in cardiac remodeling.

Availability of data and material

Data supporting current study are available from authors on reasonable demand. Microarray data is available in the Gene Expression Omnibus website under accession number GSE59437 (https://www.ncbi.nlm.nih.gov/geo/).

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Compliance with Ethics Requirements

Animal procedures were approved by the ethics committee of Dalian Medical University (Approval no. AEE21077). Human blood analysis was approved by the Ethics Committees of Dalian Medical University (No. LCKY2016-31) and Capital Medical University (2022-human-244).

All experiments involving animals were conducted according to the ethical policies and procedures approved by the ethics committee of Dalian Medical University (Approval no. AEE21077).

Human blood analysis was approved by the Ethics Committees of First Affiliated Hospital of Dalian Medical University (No. LCKY2016-31) and Beijing Chao-Yang Hospital of Capital Medical University (2022-human-244).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jare.2023.02.010.

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