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ORIGINAL RESEARCH

Macrophage NCOR1 Deficiency Ameliorates Myocardial Infarction and Neointimal Hyperplasia in Mice

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BACKGROUND: NCOR1 (nuclear receptor corepressor 1) is an essential coregulator of gene transcription. It has been shown that NCOR1 in macrophages plays important roles in metabolic regulation. However, the function of macrophage NCOR1 in response to myocardial infarction (MI) or vascular wire injury has not been elucidated.

METHODS AND RESULTS: Here, using macrophage *Ncor1* knockout mouse in combination with a mouse model of MI, we demonstrated that macrophage NCOR1 deficiency significantly reduced infarct size and improved cardiac function after MI. In addition, macrophage NCOR1 deficiency markedly inhibited neointimal hyperplasia and vascular remodeling in a mouse model of arterial wire injury. Inflammation and macrophage proliferation were substantially attenuated in hearts and arteries of macrophage *Ncor1* knockout mice after MI and arterial wire injury, respectively. Cultured primary macrophages from macrophage *Ncor1* knockout mice manifested lower expression of inflammatory genes upon stimulation by interleukin-1β, interleukin-6, or lipopolysaccharide, together with much less activation of inflammatory signaling cascades including signal transducer and activator of transcription 1 and nuclear factor-κB. Furthermore, macrophage *Ncor1* knockout macrophages were much less proliferative in culture, with inhibited cell cycle progression compared with control cells.

CONCLUSIONS: Collectively, our data have demonstrated that NCOR1 is a critical regulator of macrophage inflammation and proliferation and that deficiency of NCOR1 in macrophages attenuates MI and neointimal hyperplasia. Therefore, macrophage NCOR1 may serve as a potential therapeutic target for MI and restenosis.

Key Words: inflammation ■ macrophage proliferation ■ myocardial infarction ■ neointimal hyperplasia ■ nuclear receptor corepressor 1

Cardiovascular diseases, particularly ischemic heart disease, remain the leading cause of mortality and morbidity worldwide.[1] Myocardial infarction (MI) resulting from sudden interruption of blood supply to the myocardium causes rapid death of cardiomyocytes. One of the most common treatments for MI is percutaneous coronary intervention. However, vascular injury caused by percutaneous coronary intervention may lead to restenosis, which may require revascularization.[2] A long-term study with a median of 5 years of follow-up has shown that even after the introduction of drug-eluting stents, the rate of revascularization remains 16.5%, comparing with 19.8% for bare-metal stents.[3] Furthermore, currently available drug-eluting stents does not improve a primary outcome, death from any cause and nonfatal spontaneous MI, compared with bare-metal stents.[3] Eventually, a large proportion of surviving MI patients develop heart failure, particularly after repeated interventional therapy. Therefore, new

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CLINICAL PERSPECTIVE

What Is New?

- Deletion of NCOR1 (nuclear receptor corepressor 1) in macrophages protects against myocardial injury after myocardial infarction and improves neointimal hyperplasia.
- NCOR1 deficiency inhibits macrophage proliferation.

What Are the Clinical Implications?

- Macrophage NCOR1 is a potential therapeutic target for myocardial infarction and restenosis.

Nonstandard Abbreviations and Acronyms

BrdU	bromodeoxyuridine
CM	conditioned medium
LC	littermate control
MI	myocardial infarction
MNKO	macrophage Ncor1 knockout
NCOR1	nuclear receptor corepressor 1
PI	propidium iodide
VSMCs	vascular smooth muscle cells

therapeutic strategies for both MI and restenosis are still in urgent need.

Inflammation plays important roles during MI and vascular injury. Anti-inflammatory strategy has been proved beneficial for patients with MI and high level of C-reactive protein.[4] Macrophages are a group of highly heterogeneous immune cells that are fundamental for inflammatory response.[5] Macrophage polarization has been considered a major factor that drives the inflammatory process in cardiovascular diseases, including MI and restenosis.[6–8] In recent years, more and more studies have demonstrated that macrophage proliferation also profoundly contributes to inflammation.[9–11] Over the pathophysiological course after MI, inflammatory macrophages such as CCR2$^+$ macrophages in the heart are originated from monocyte differentiation and maintained by local proliferation.[12,13] Similarly, accumulation of arterial macrophages comes from local proliferation rather than monocyte recruitment both in steady state and after severe depletion following sepsis.[14] Local proliferation of macrophages account for 87% of macrophage accumulation in established atherosclerotic plaques.[15] Proliferation of macrophages in vascular wall also substantially contribute to vascular inflammation and neointimal hyperplasia after vascular injury.[16] Thus, targeting macrophage inflammatory reaction and proliferation represents an attractive approach to treat MI and restenosis.

Transcriptional regulation is an essential mechanism for macrophage activation. NCOR1 (nuclear receptor corepressor 1) is a transcriptional coregulator and usually regulates gene expression by recruiting histone deacetylases to the binding sites of transcription factors and thus repressing their transcriptional activities.[17–19] NCOR1 has been reported to regulate different transcription factors in different cell types and to control different biological process.[20–26] Previous study has shown that macrophage NCOR1 deficiency induces an anti-inflammatory phenotype of macrophages by suppressing liver X receptor and thus improves insulin resistance in mice fed with a high fat diet.[22] However, it has not been explored whether macrophage NCOR1 plays a role in MI or restenosis and how NCOR1 affects macrophage phenotypes under these circumstances of cardiovascular injuries.

In this study, we set out to investigate the function of macrophage NCOR1 in MI and neointimal hyperplasia as well as effects of NCOR1 on macrophage phenotypes, particularly macrophage proliferation and inflammation. We first generated macrophage Ncor1 knock out mice (MNKO mice). We then examined the impacts of macrophage NCOR1 deficiency on cardiac function, remodeling, and inflammation, as well as macrophage proliferation after MI using a coronary artery ligation mouse model. Subsequently, we examined the impacts of macrophage NCOR1 deficiency on neointimal hyperplasia, vascular inflammation, and macrophage proliferation using a femoral artery wire injury mouse model. Finally, we studied how NCOR1 influenced inflammation and proliferation of macrophages in vitro and explored the cellular mechanisms.

METHODS

The authors declare that all supporting data are available within the article and its online supplementary files. Additional methods can be found in Data S1.

Animals and Treatments

Macrophage *Ncor1* knockout (MNKO) and littermate control (LC) mice were generated as previously described.[22] In brief, *Ncor1*$^{flox/flox}$ mice were mated with transgenic mice that express Cre recombinase under the control of the lysozyme M promoter to generate *Ncor1*$^{flox/flox}$;*LysM*^{cre} (MNKO) mice and *Ncor1*$^{flox/flox}$ (LC) mice. MNKO and LC mice have been kept in a C57BL6 background. Two-month-old age- and weight-matched male LC and MNKO mice were used for experiments.

All animal studies were approved by the Institutional Review and Ethics Board of Shanghai Ninth People's

Hospital, Shanghai Jiao Tong University School of Medicine. For in vivo labeling of DNA incorporation, mice were intraperitoneally injected with 1 mg bromodeoxyuridine (BrdU) (B5002, Sigma-Aldrich, St. Louis, MO, USA) daily for 7 days after surgeries.

Cell Culture

Peritoneal macrophages were obtained as previously described.[27] L929 cells were cultured in RPMI1640 media (Life Technologies/Thermo Fisher Scientific, Carlsbad, CA, USA) with 10% fetal bovine serum (Biological Industries, Cromwell, CT, USA) and 1% penicillin-streptomycin. L929 conditioned media were obtained as described before.[28] Interleukin (IL)-1β (10 ng/mL, PeproTech, Rocky Hill, NJ, USA) and IL-6 (20 ng/mL, Pepro Tech) were used to treat macrophages for different time periods.

Primary vascular smooth muscle cells (VSMCs) were isolated as previously described.[29] VSMCs were cultured in DMEM/F12 (Life Technologies/Thermo Fisher Scientific) with 20% fetal bovine serum (Gibco/Thermo Fisher Scientific) and 1% penicillin-streptomycin.

MI Mouse Model

MI was induced by permanent ligation of left main descending coronary artery as previously described.[30] In brief, mice were anesthetized with 2% isoflurane. The heart was pushed out through a small hole that was made at the fourth intercostal space with a mosquito clamp. The left descending coronary artery was located, sutured, and ligated at a site ≈3 mm from its origin using a 6-0 silk suture. After ligation, the heart was placed back into the intrathoracic space followed by closure of muscle and skin. The sham group underwent the same procedure except the ligation.

Echocardiography

Transthoracic echocardiography was performed using a Vevo2100 Ultrasound system (VisualSonics, Toronto, Canada) 1 week after MI surgery. The technician performed the measurements without knowing the experimental groups. Mice were anesthetized with isoflurane and placed on a warming plate to maintain body temperature. Hearts were imaged in 2-dimensional parasternal long axis view. Left ventricular internal diameters at end-diastole and end-systole were obtained on M-mode recording. Ejection fraction and fractional shortening were calculated.

Femoral Artery Wire Injury Mouse Model

Femoral artery wire injury model was performed as previously described.[31] Briefly, blunt dissection was used to isolate right femoral arteries of mice and a guide wire (0.38 mm in diameter, Cook Inc, Bloomington, IN, USA) was inserted into the arterial lumen for >5 mm toward to the iliac artery and then pulled out. The process was repeated for 3 times. After the last repeat, the guide wire was left in place for 2 minutes to denude the endothelium and dilate the artery. Mice were euthanized and femoral arteries were collected for histologic or biochemical analysis 4 weeks after operations.

Flow Cytometry

Mice were euthanized 1 week after MI surgery. Hearts were excised, minced, and placed in digestion solution containing 1.5 mg/mL Collagenase II (Worthington, Lakewood, NJ, USA), 1.5 mg/mL Collagenase IV (Worthington), and 60 U/mL DNase I (AppliChem, Lochem, Darmstadt, Germany) in hank's balanced salt solution. Heart tissues with digestion solution were first dissociated mechanically with gentle magnetic activated cell sorting Dissociator system (Miltenyi Biotec, Bergisch Gladbach, Germany) and then digested at 37℃ for 20 minutes. Single-cell suspensions were obtained by filtering digested tissues through 70-μm cell strainers (BD Biosciences, San Jose, CA, USA). Red blood cells were lysed before flow cytometry staining. Cardiac single-cell suspensions were centrifuged, blocked with Fc block for 10 minutes, and labeled with antibodies at 4℃ for 20 minutes. For intracellular staining, cells were fixed and permeabilized using a transcription factor staining buffer set (eBioscience, San Diego, CA, USA) after labeling for cell-surface markers, and then stained with anti-KI67 (nuclear proliferation antigen) antibodies. All samples were analyzed using LSR Fortessa (BD Biosciences).

The following antibodies were used: Fc block (101320, Biolegend, San Diego, CA, USA), CD45-PE-Cy7 (25-0451-81, eBioscience), CD11B-FITC (11-0112-82, eBioscience), F4/80-APC (17-4801-82, eBioscience), KI67-PE (12-5698-80, eBioscience), LY6G-APC (560593, BD Biosciences), and LY6C-PE (560599, BD Biosciences).

Histology

Hearts and femoral arteries were fixed in 4% paraformaldehyde for at least 24 hours. After dehydration in gradient ethanol, tissues were embedded in paraffin and cut into 5-μm-thick sections. Masson Trichrome staining were performed on the section of hearts for scar size evaluation. Serial cross-sections of femoral arteries were stained with hematoxylin and eosin. Scar area, intimal area, and medial area were measured using Image J software. All the measurements were performed in a blinded way.

Immunofluorescence

For immunofluorescence for tissues, paraffin sections of hearts and femoral arteries were deparaffinized and

rehydrated. After antigen retrieval treatment, the slides were incubated in blocking buffer containing 5% normal goat serum and 0.3% Triton X-100 in PBS at 37°C for 1 hour, and then incubated with primary antibodies at 4°C overnight and with fluorochrome-conjugated secondary antibodies (A11007 or A11008, Thermo Fisher Scientific) at room temperature for 2 hours the next day. The sections were finally counterstained with DAPI (P36931, Thermo Fisher Scientific) and fluorescent signals were captured using a fluorescence microscope.

For immunocytochemistry, peritoneal macrophages or VSMCs were cultured on coverslips. After treatment, the coverslips were fixed in 4% paraformaldehyde at room temperature for 15 minutes and washed with PBS for 3 times. The coverslips were then incubated in blocking buffer, primary antibodies, and secondary antibodies sequentially, and counterstained as described above. For quantification of the total number of cells, equal amounts of VSMCs or macrophages were seeded and DAPI-positive cells were counted at the end of the experiments.

For the BrdU incorporation assay, peritoneal macrophages were seeded on coverslips and cultured in L929 conditioned media and normal media for 48 hours. Next, 10 μmol/L BrdU was added to the media and macrophages were cultured for another 1 hour. Subsequently, the coverslips were washed with PBS twice, fixed in 4% paraformaldehyde at room temperature for 15 minutes, incubated with 1 mol/L HCl for 10 minutes and 2 mol/L HCl for 20 minutes, and then neutralized with 0.1 mol/L sodium borate buffer (pH=8.0). The coverslips were stained and counterstained as described above.

The following antibodies were used: anti-α-smooth muscle actin (BM0002, Boster bio-tech, Wuhan, Hubei, China), anti-BrdU (sc-32323, Santa Cruz Biotechnology, Dallas, Texas, USA), anti-KI67 (9129, Cell Signaling Technology, Danvers, MA, USA), and anti-MAC2 (14-5301-81, eBioscience), anti-CD68 (MCA1957GA, Bio-Rad). All cells were counted by independent investigators masked from experimental conditions.

Transwell Assay

Twenty-four well cell culture inserts with polycarbonate membrane filters containing 8-μm pores (Corning Inc. Corning, NY, USA) were used for transwell assay. VSMCs were seeded on the filters with serum-free media. Conditioned media from macrophages were added to the lower chamber. VSMCs were allowed to migrate at 37°C for 12 hours and then stained with 0.1% crystal violet (Sigma-Aldrich) at room temperature for 20 minutes. Five randomly selected fields of migrated cells were photographed. All cells were counted by independent investigators masked from experimental conditions.

Analysis of Cell Cycle

Cell-cycle was analyzed by propidium iodide (PI) staining and flow cytometry analysis. Peritoneal macrophages were harvested using 0.25% trypsin, washed with PBS containing 2 mmol/L EDTA, and then fixed in 70% ethanol at −80°C overnight. Subsequently, macrophages were stained with solution containing 0.05 mg/mL PI (Sigma-Aldrich), 0.1% Triton X-100, and 0.1 mg/mL ribonuclease A in PBS at room temperature for 1 hour in dark. Cell cycle distribution was analyzed by Quanta SC (Beckman, Fullerton, CA, USA). Data were analyzed using Flow Jo software.

Quantitative RT-PCR

Total RNA was isolated from tissues or cells using Trizol (Life Technologies/Thermo Fisher Scientific) and cDNA was synthesized using reverse transcription kits (Takara, Shiga, Japan) according to the manufacturer's instructions. Quantitative reverse transcription PCR was performed using SYBR Green Mix (Life Technologies/Thermo Fisher Scientific) on an ABI7900HI (Applied Biosystems/Thermo Fisher Scientific). Expression of target genes was normalized to that of reference genes including *l32* and *gapdh*. Primer sequences are listed in Table S1.

Western Blotting Analysis

Cells were lysed with lysis buffer containing protease inhibitors and total protein was extracted. Protein samples were separated by SDS-PAGE and analyzed by immunoblotting. The following antibodies were used: NCOR1 (5948, Cell Signaling Technology), p-STAT1$^{(Ser727)}$ (signal transducer and activator of transcription 1) (9177, Cell Signaling Technology), STAT1 (9172, Cell Signaling Technology), p-P38 (4511, Cell Signaling Technology), P38 (9212, Cell Signaling Technology), p-P65 (3031, Cell Signaling Technology), P65 (sc-8008, Santa Cruz Biotechnology), p-IKKβ (inhibitor of kappaB kinase beta) (2697, Cell Signaling Technology), IKKβ (2678, Cell Signaling Technology), PCNA (proliferating cell nuclear antigen) (ab29, Abcam, Cambridge, MA, USA), α-TUBULIN (T6199, Sigma-Aldrich), β-ACTIN (4967, Cell Signaling Technology), CYCLINB1 (CY3250, Abways, Shanghai, China), CYCLIND1 (CY5404, Abways), P21 (cyclin-dependent kinase inhibitor 1A) (CY5543, Abways), p-AKT (9271, Cell Signaling Technology), and AKT (9272, Cell Signaling Technology), p-ERK1/2 (4376, Cell Signaling Technology), and ERK1/2 (4695, Cell Signaling Technology).

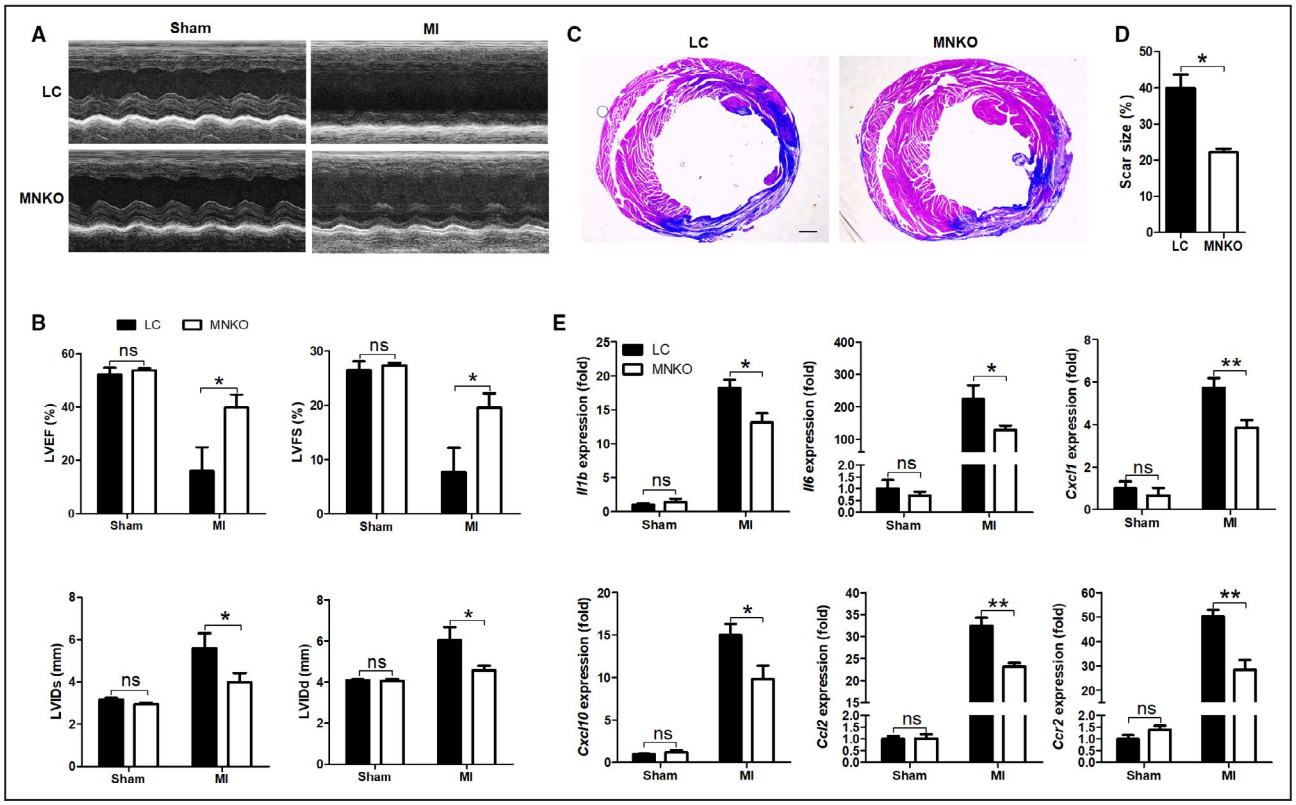

Figure 1. Macrophage NCOR1 (nuclear receptor corepressor 1) deficiency protects against myocardial infarction.
A, Representative echocardiographic images of littermate control mice and macrophage Ncor1 knockout mice 7 days after sham operation or myocardial infarction. **B**, Quantification of left ventricular ejection fraction, left ventricular fractional shortening, left ventricular end-systolic internal diameter and end-diastolic internal diameter. n=5:5:5:5. **C**, Representative Masson Trichrome staining of cardiac tissue sections of littermate control and macrophage Ncor1 knockout mice 7 days after myocardial infarction. Scale bar: 1 mm. **D**, Quantification of the scar size. n=5:4. **E**, Quantitative reverse transcription polymerase chain reaction analysis of inflammatory genes in ischemic region of hearts from littermate control and macrophage Ncor1 knockout mice 3 days after sham or myocardial infarction operation. n=3:3:6:6. Values are expressed as mean±SEM. *Ccl2* indicates C-C motif chemokine ligand 2; *Ccr2*, C-C motif chemokine receptor 2; *Cxcl1*, C-X-C motif chemokine ligand 1; *Cxcl10*, C-X-C motif chemokine ligand 10; *Il-1b*, interleukin 1 beta; *Il-6*, interleukin 6; LC, littermate control; LVEF, left ventricular ejection fraction; LVFS, left ventricular fractional shortening; LVIDd, left ventricular end-diastolic internal diameter; LVIDs, left ventricular end-systolic internal diameter; MI, myocardial infarction; MNKO, macrophage Ncor1 knockout; and ns, not significant. **P*<0.05, ***P*<0.01.

Statistical Analysis

All data were shown as mean±SEM. Statistical analysis was performed using Prism 5.0 (GraphPad Software, La Jolla, CA, USA). The differences between means of 2 experimental groups were analyzed by unpaired Student *t*-test or non-parametric test. Values of $P \leq 0.05$ were considered statistically significant.

RESULTS

Macrophage NCOR1 Deficiency Protects Against Acute MI

To study the role of macrophage NCOR1 in cardiovascular remodeling, we generated MNKO mice using cre-lox system. Peritoneal macrophages were isolated from LC or MNKO mice for detection of knockout efficiency. Results of quantitative reverse transcription polymerase chain reaction (qRT-PCR) and western blotting showed near non-detectable levels of mRNA and protein of NCOR1, respectively, in macrophages from MNKO mice compared with LC mice (Figure S1).

LC and MNKO mice were subjected to MI operation by ligation of left anterior descending artery. Cardiac function was measured by M-mode echocardiography 7 days later. MNKO mice showed significantly higher left ventricular ejection fraction and fractional shortening, as well as significantly lower left ventricular end-systolic inner chamber dimensions and left ventricular end-diastolic inner chamber dimensions (Figure 1A and 1B), suggesting improved cardiac function after MI. Although the infarct size is comparable between LC and MNKO mice at day 1 after MI (Figure S2), the scar area is much smaller in MNKO mice compared with LC mice at day 7 (Figure 1C and 1D). Consistently, less apoptotic cardiomyocytes were observed in peri-infarct region of hearts of MNKO mice (Figure S3A and S3B).

Inflammation is an important feature in cardiac remodeling after acute MI. We detected the expression of proinflammatory genes in the ischemic myocardium of LC and MNKO mice 3 days after MI. qRT-PCR results showed that expression of inflammatory genes, such as *Il1b*, *Il6*, *Cxc1*, *Cxc10*, *Ccl2* and *Ccr2*, was lower in MNKO mice compared with LC mice (Figure 1E), but the expression of *Tnfa* and *Nos2* was similar between the 2 strains (Figure S4). However, cardiac expression of inflammatory genes were comparable between LC and MNKO mice at day 7 after MI (Figure S5).

Macrophage NCOR1 Deficiency Inhibits Proliferation of Cardiac Macrophages After MI

Macrophages are critical players in cardiac inflammation. We first detected the content of resident macrophages in LC and MNKO mice. Flow cytometry analysis (gating strategy shown in Figure S6) demonstrated that NCOR1 deficiency did not affect the level of macrophages at baseline (Figure S7). Recruitment of circulating neutrophils and monocytes to the heart occurs soon after MI.[32] We next explored whether MNKO regulated the recruitments of neutrophils and monocytes. Flow cytometry analysis illustrated that the levels of neutrophils in blood were comparable between LC and MNKO mice both at baseline and after MI (Figure S8A and S8B). The levels of monocytes in blood were mostly comparable between the 2 genotypes except that MNKO mice had significantly more circulating LY6C^{low} monocytes than LC mice at baseline (Figure S8C and S8D). In addition, deletion of NCOR1 in macrophages did not affect the amount of neutrophils or monocytes in the heart after MI (Figure S9).

Macrophage proliferation plays an important role in myocardial injury.[11,33] During the acute phase after MI, recruited monocytes differentiate into macrophages and the number of macrophages are partially maintained by local proliferation.[12] Mice were injected with BrdU after MI to explore whether NCOR1 deficiency affected proliferation of cardiac macrophages. Immunofluorescence staining of sections of peri-infarct region of the heart illustrated that MNKO mice had fewer MAC2-positive macrophages and MAC2/BrdU double positive macrophages than LC mice (Figure 2A and 2B), indicating less proliferation of cardiac macrophages in MNKO mice after MI. Similar results were observed in the infarcted region when CD68 antibodies were used to detect macrophages (Figure S10A and S10B). Flow cytometry analysis revealed less accumulation of F4/80$^+$ macrophages in hearts of MNKO mice compared with LC mice 7 days after MI (Figure 2C and 2D). More importantly, using KI67 to stain for proliferative cells demonstrated significantly less F4/80$^+$KI67$^+$ macrophages in hearts of MNKO mice after MI (Figure 2E and 2F). These results together suggested that macrophage NCOR1 deficiency reduced accumulation and proliferation of macrophages in hearts after acute MI.

Macrophage NCOR1 Deficiency Improves Neointimal Hyperplasia and Inhibits Arterial Macrophage Proliferation After Femoral Artery Injury

To investigate the role of macrophage NCOR1 in vascular remodeling, we used a femoral artery wire injury mouse model to induce neointimal hyperplasia in LC and MNKO mice. Four weeks after wire injury, less intima area and smaller intima/media ratio were detected in femoral arteries of MNKO mice compared with those of LC mice (Figure 3A and 3B). Consistently, immunofluorescence illustrated significantly less staining of α-smooth muscle actin in MNKO mice, indicating reduced accumulation of smooth muscle cells (Figure 3C and 3D).

Inflammatory response also plays an important role in vascular remodeling.[2,34] qRT-PCR results showed that expression of inflammatory genes was significantly lower in injured arteries of MNKO mice than those of LC mice (Figure 3E). Consistently, results of immunofluorescence staining demonstrated that injured arteries of MNKO mice manifested much less MAC2 positive cells than those of LC mice, indicating reduced accumulation of macrophages (Figure 3F and 3G). Furthermore, the injured arteries of MNKO mice had markedly less MAC2 and BrdU double positive cells, suggesting that NCOR1 deficiency decreased macrophage proliferation in injured arteries (Figure 3F and 3G). Similar results were obtained when MAC2 and KI67 were used to detect proliferation of macrophages in injured arteries (Figure S11).

Mechanical injury-induced migration of VSMCs from arterial media to intima and subsequent proliferation of these cells are the main pathophysiological processes in neointimal hyperplasia.[35,36] To evaluate the effect of MNKO on migration and proliferation of VSMCs, conditioned medium were collected from LC and MNKO macrophages treated with IL-1β (designated as IL-1β conditioned medium [CM]) and used to stimulate VSMCs. Both KI67 staining and quantification of total VSMCs indicated that less proliferation of VSMCs was induced by IL-1β CM from MNKO macrophages compared with those from LC macrophages (Figure 4A and 4B). Transwell assay demonstrated that IL-1β CM derived from MNKO macrophages induced remarkably less migration of

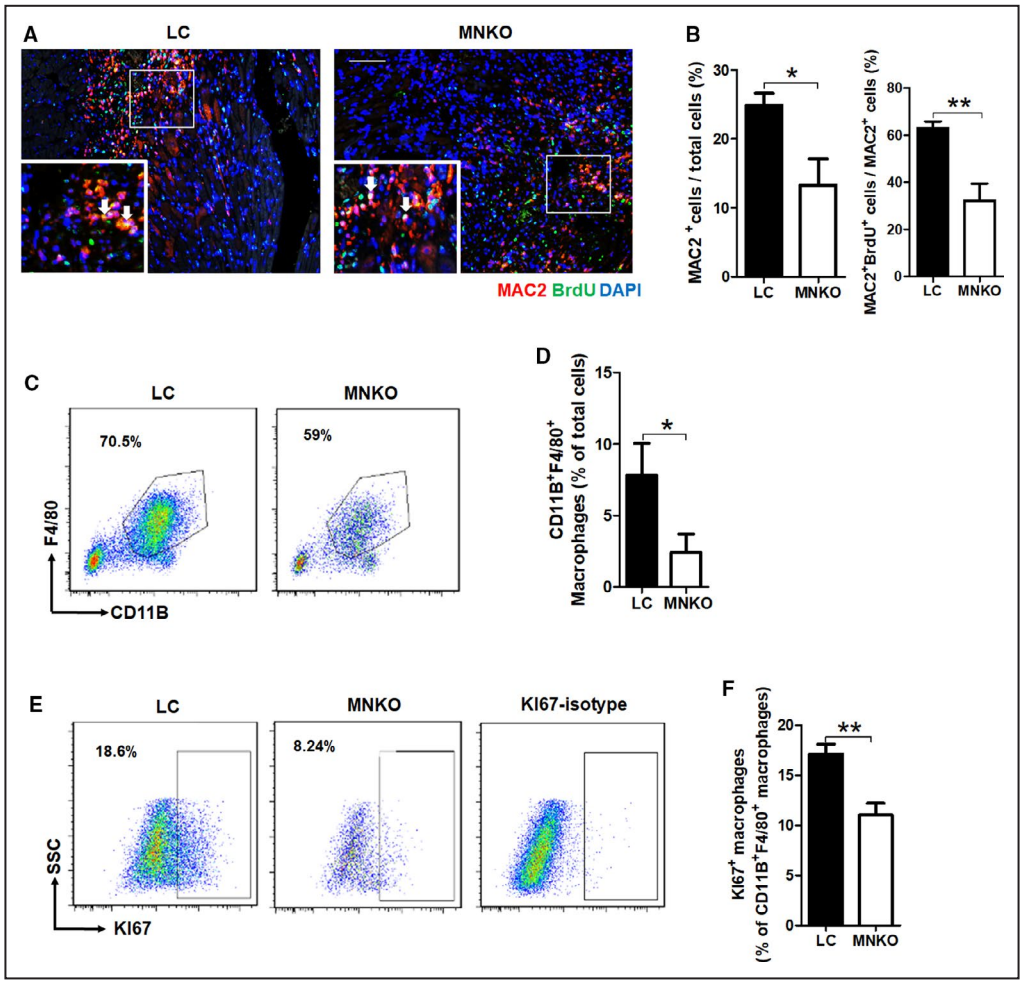

Figure 2. Macrophage NCOR1 (nuclear receptor corepressor 1) deficiency inhibits proliferation of cardiac macrophages after myocardial infarction.
A, Representative immunofluorescence staining of MAC2 and BrdU in peri-infarct region of hearts from littermate control and macrophage Ncor1 knockout mice 7 days after myocardial infarction. The arrows point to MAC2/BrdU double-positive cells. Scale bar: 200 μm. **B**, Quantification of MAC2-positve cells and MAC2/BrdU double-positive cells. n=3:3. **C**, Representative flow cytometry analysis of macrophages in whole hearts from littermate control and macrophage Ncor1 knockout mice 7 days after myocardial infarction. **D**, Quantification of $CD11B^+F4/80^+$ macrophages. n=5:4. **E**, Representative flow cytometry analysis of $KI67^+$ macrophages in whole hearts from littermate control and macrophage Ncor1 knockout mice 7 days after myocardial infarction. **F**, Quantification of $KI67^+$ macrophages. n=5:4. Values are expressed as mean±SEM. BrdU indicates bromodeoxyuridine; KI67, nuclear proliferation antigen; LC, littermate control; MAC2, galectin3; MNKO, macrophage Ncor1 knockout; and SSC, side scatter. $^*P<0.05$, $^{**}P<0.01$.

VSMCs than those from LC macrophages (Figure 4C and 4D).

NCOR1 Deficiency Suppresses Macrophage Inflammation

To gain mechanistic insight of the protective effect of macrophage NCOR1 deficiency on MI and neointimal hyperplasia, we treated LC and MNKO peritoneal macrophages with IL-1β or IL-6 to investigate the impacts of NCOR1 deficiency on macrophage inflammatory response. qRT-PCR results showed that macrophage NCOR1 deficiency strongly inhibited IL-1β-induced expression of inflammatory genes in macrophages (Figure 5A). We further examined the impact of NCOR1 deficiency on IL-1β-induced activation of classical pathways that control the release of inflammatory cytokines in macrophages. As expected, IL-1β-induced phosphorylation of STAT1 (signal transducer and activator of transcription 1), P38, P65, and IKKβ (inhibitor of kappaB kinase beta) was significantly attenuated in MNKO macrophages compared with LC macrophages (Figure 5B). Similar results were observed when macrophages were

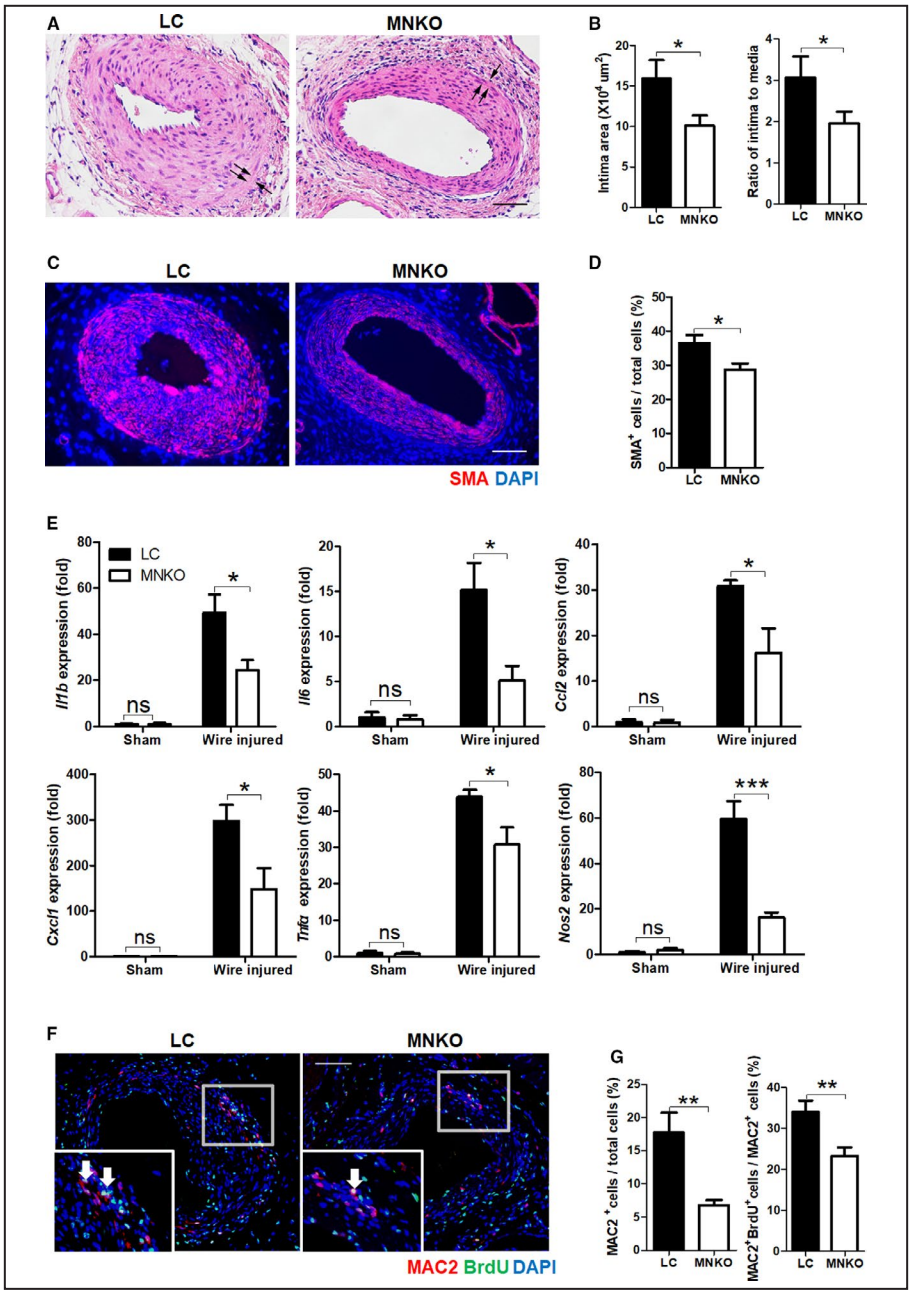

Figure 3. Macrophage NCOR1 (nuclear receptor corepressor 1) deficiency inhibits arterial injury-induced neointimal hyperplasia.

A, Representative hematoxylin and eosin staining of cross section of femoral arteries from littermate control and macrophage Ncor1 knockout mice 28 days after wire injury. **B**, Quantification of intima area and intima to media ratio. n=7:7. **C**, Representative immunofluorescence staining of α-smooth muscle actin in injured arteries. **D**, Quantification of smooth muscle actin -positive cells. n=5:4. **E**, Quantitative reverse transcription polymerase chain reaction analysis of inflammatory genes in femoral arteries from littermate control and macrophage Ncor1 knockout mice 7 days after wire injury. Femoral arteries isolated from 3 to 4 mice were mixed together as 1 technical sample, and 3 technical samples were used in each group. **F**, Representative immunofluorescence staining of MAC2 and BrdU in injured femoral arteries from littermate control and macrophage Ncor1 knockout mice 21 days after wire injury. The arrows point to MAC2/BrdU double-positive cells. **G**, Quantification of MAC2-positive cells and MAC2/BrdU double-positive cells. n=5:5. All scale bars: 200 μm. Values are expressed as mean±SEM. *Ccl2* indicates C-C motif chemokine ligand 2; *Cxcl1*, C-X-C motif chemokine ligand 1; *Il-1b*, interleukin 1 beta; *Il-6*, interleukin 6; LC, littermate control; MNKO, macrophage Ncor1 knockout; *Nos2*, nitric oxide synthase 2; ns, not significant; and *Tnfa*, tumor necrosis factor. **P*<0.05, ***P*<0.01, ****P*<0.001.

challenged with IL-6 (Figure 5C and 5D). To further verify the anti-inflammatory signature of macrophage NCOR1 deficiency, we treated peritoneal macrophages with LPS, an agonist of toll-like receptor 4. qRT-PCR results revealed that deletion of NCOR1 significantly inhibited the expression of inflammatory cytokines induced by LPS in macrophages (Figure S12). Similar results were observed in bone marrow derived macrophages treated with LPS (Figure S13). In line with this, expression of anti-inflammatory M2-like genes was upregulated in MNKO macrophages (Figure S14).

NCOR1 Deficiency Inhibits Macrophage Proliferation In Vitro

We finally studied the role of NCOR1 in macrophage proliferation in vitro. Peritoneal macrophages isolated from LC or MNKO mice were cultured in normal media or L929 conditioned media (L929 CM) for 24 hours. Immunofluorescence staining demonstrated that the total cell number and percentage of KI67$^+$ cells were significantly reduced in MNKO macrophages compared with LC macrophages after stimulation with L929 CM (Figure 6A and 6B). Similarly, less BrdU$^+$ cells were observed in MNKO macrophages in BrdU incorporation assay (Figure 6C and 6D). Consistently, western blotting results illustrated significantly lower protein expression of PCNA in MNKO macrophages after stimulation with L929 CM (Figure 6E).

Cell cycle regulation is a critical mechanism to control cell proliferation.[37,38] We therefore tested whether NCOR1 deficiency affected cell cycle in macrophages. Flow cytometry was used to analyze cell cycle at different time points. The results demonstrated that percentages of cells in S and G2/M phases were markedly reduced in MNKO macrophages compared with LC macrophages (Figure 7A and 7B). CYCLINB1, CYCLIND1, CYCLIND2, and P21 are key regulators of cell cycle progression. qRT-PCR results showed that MNKO macrophages had significantly less expression of *Ccnb1*, *Ccnd1* and *Ccnd2* and more expression of *Cdkn1a* compared with LC macrophages (Figure 7C). Consistently, lower protein levels of CYCLINB1 and CYCLIND1 as well as higher protein level of P21 were detected in MNKO macrophages by western blotting (Figure 7D). Interestingly, analysis of ChIP-seq data

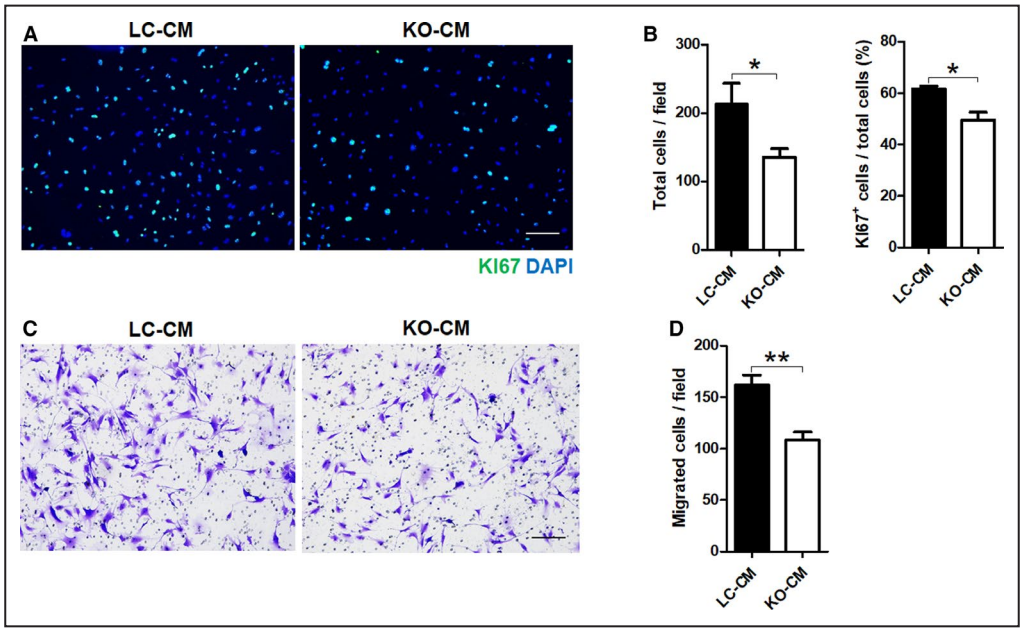

Figure 4. Conditioned media from NCOR1 (nuclear receptor corepressor 1)-deficient macrophages induce less proliferation and migration of vascular smooth muscle cells.
A, Representative immunofluorescence staining of KI67 in vascular smooth muscle cells (VSMCs) after incubation with conditioned media from littermate control macrophages or Ncor1 knockout macrophages for 48 hours. Littermate control and Ncor1 knockout macrophages were stimulated with interleukin-1β and respective conditioned media were collected. **B**, Quantification of total VSMCs and KI67$^+$ VSMCs. **C**, Representative staining of migrated VSMCs detected by transwell assay. Migration was induced by conditioned media collected from littermate control macrophages or Ncor1 knockout macrophages for 12 hours. **D**, Quantification of migrated VSMCs. Scale bars: 200 μm. Representative results of immunofluorescence staining and transwell assay from 3 independent experiments were shown. Values are expressed as mean±SEM. KO-CM indicates conditioned media from Ncor1 knockout macrophages; and LC-CM, conditioned media from littermate control macrophages. *P<0.05, **P<0.01.

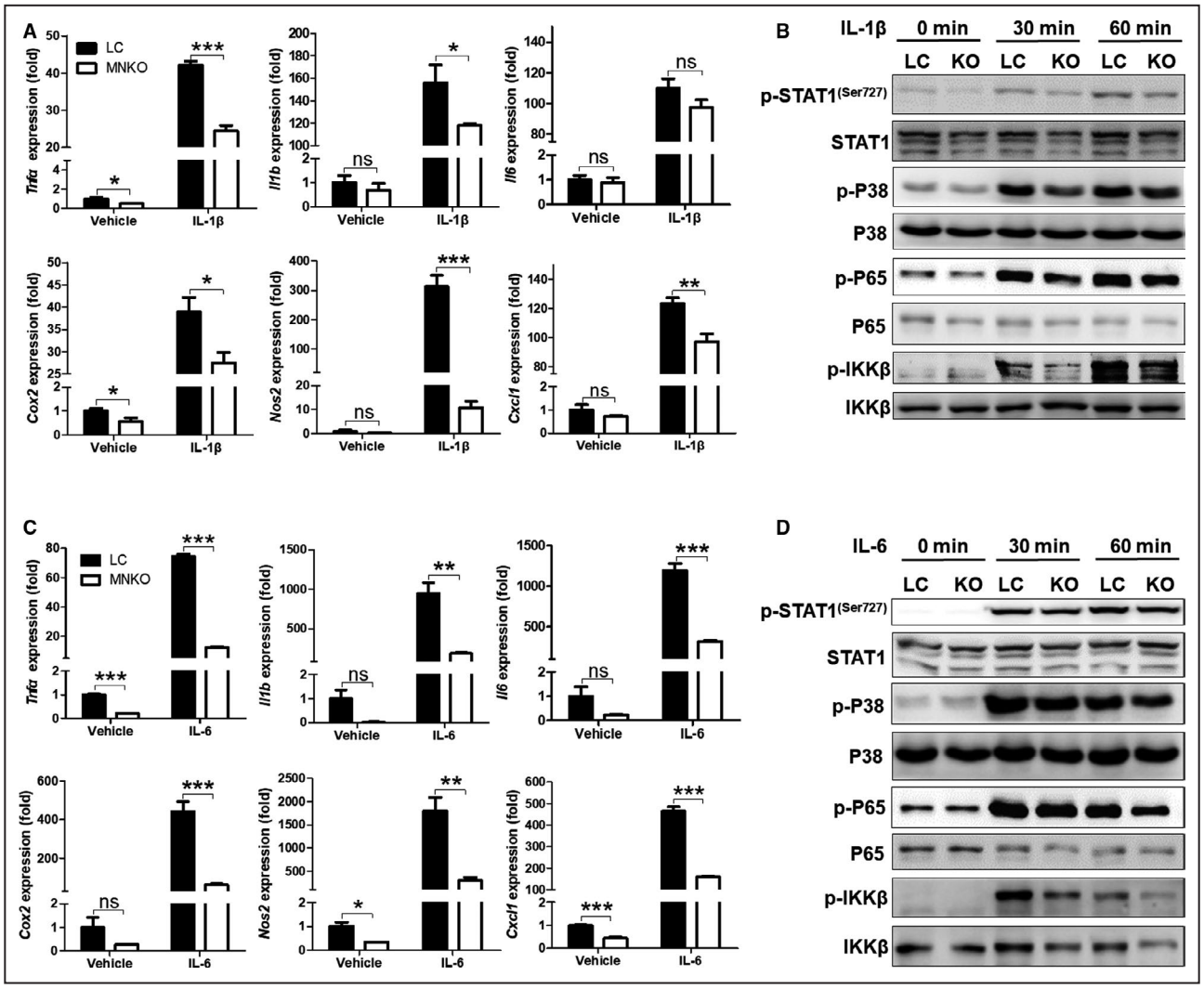

Figure 5. Macrophage NCOR1 (nuclear receptor corepressor 1) deficiency suppresses interleukin (IL)-1β- or IL-6-induced inflammation in macrophages.

Peritoneal macrophages were isolated from littermate control and macrophage Ncor1 knockout mice. **A**, Quantitative reverse transcription polymerase chain reaction analysis of inflammatory genes in macrophages stimulated with IL-1β for 6 hours. **B**, Western blotting analysis of phosphorylation and total protein levels of STAT1, P38, P65, and IKKβ in peritoneal macrophages treated with IL-1β for indicated time periods. **C**, Quantitative reverse transcription polymerase chain reaction analysis of inflammatory genes in macrophages stimulated with IL-6 for 6 hours. **D**, Western blotting analysis of phosphorylation and total protein levels of STAT1, P38, P65, and IKKβ in peritoneal macrophages treated with interleukin-6 for indicated time periods. Representative results of quantitative reverse transcription polymerase chain reaction and western blotting from 3 independent experiments were shown. Values are expressed as mean±SEM. *Cox2* indicates prostaglandin-endoperoxide synthase 2; *Cxcl1*, C-X-C motif chemokine ligand 1; IKKβ, inhibitor of kappaB kinase beta; *Il-1b or IL-1β*, interleukin 1 beta; *Il-6 or IL-6, interleukin 6*; LC, littermate control; MNKO or KO, macrophage Ncor1 knockout; *Nos2*, nitric oxide synthase 2; ns, not significant; STAT1, signal transducer and activator of transcription 1; and *Tnfa*, tumor necrosis factor. **P*<0.05, ***P*<0.01, ****P*<0.001.

from available data sets[39] identified NCOR1 recruitment to the promoter region of P21 (data not shown), further indicating the regulation of P21 by NCOR1. Furthermore, significant downregulation of p-AKT and p-ERK1/2 was observed in MNKO macrophages compared with LC macrophages at different time points after L929 CM stimulation (Figure S15). These results together suggested that NCOR1 deficiency inhibited macrophage proliferation.

DISCUSSION

Although it has been illustrated important in metabolic regulation, the role of macrophage NCOR1 in cardiovascular diseases such as MI and restenosis has not been elucidated. Through the current study, we demonstrated that deletion of NCOR1 in macrophages significantly improved myocardial healing after MI and attenuated neointimal hyperplasia after vascular injury

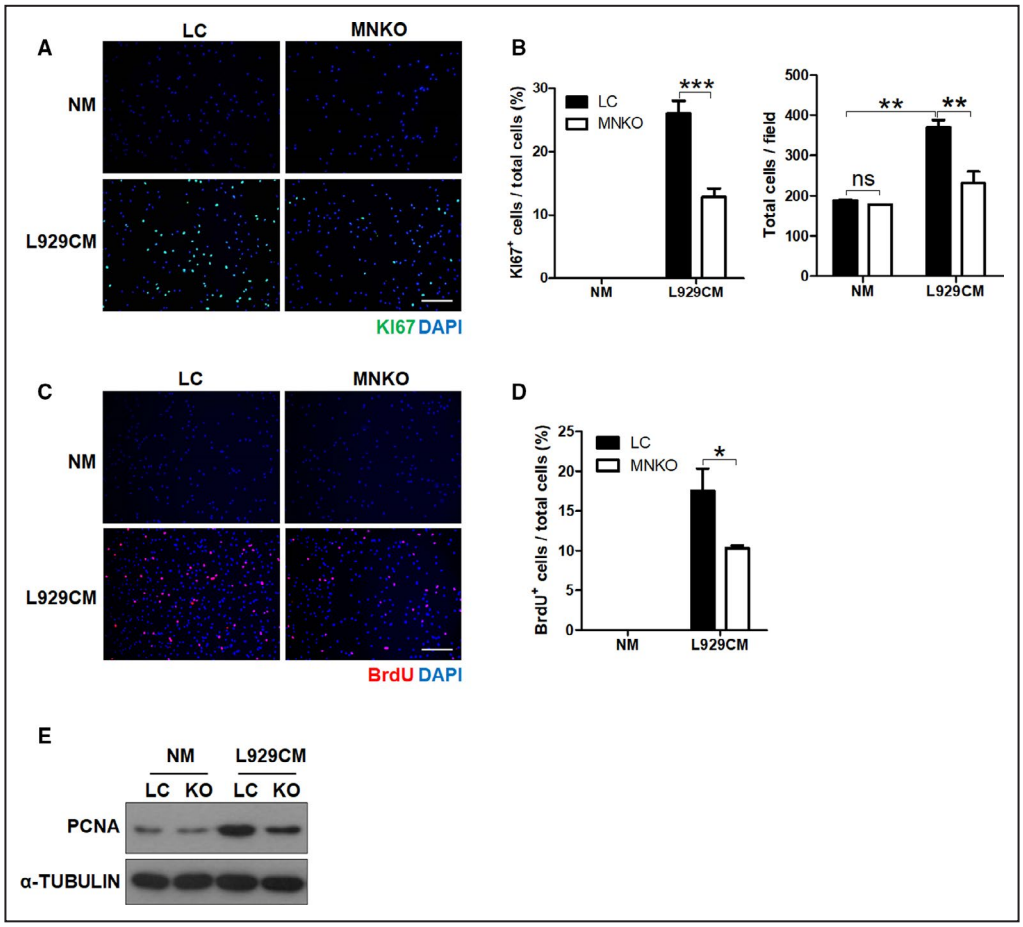

Figure 6. Macrophage NCOR1 (nuclear receptor corepressor 1) deficiency inhibits macrophages proliferation in vitro.

Peritoneal macrophages isolated from littermate control macrophages and macrophage Ncor1 knockout mice were treated with normal media or L929 conditioned media for 48 hours. **A**, Representative KI67 immunofluorescence staining of macrophages. **B**, Quantification of KI67+ and total cells. **C**, Representative BrdU immunofluorescence staining of macrophages. **D**, Quantification of BrdU-positive cells. **E**, Western blotting analysis of PCNA (proliferating cell nuclear antigen) in macrophages. α-TUBULIN was used as a loading control. Scale bars: 500 μm. Representative results of immunofluorescence staining and western blotting from 3 independent experiments were shown. Values are expressed as mean±SEM. BrdU indicates bromodeoxyuridine; KI67, nuclear proliferation antigen; L929 CM, L929 conditioned media; LC, littermate control; MNKO or KO, macrophage Ncor1 knockout; NM, normal media; ns, not significant; and PCNA, proliferating cell nuclear antigen. **P*<0.05, ***P*<0.01, ****P*<0.001.

in mice, likely attributable to suppressed macrophage proliferation and inflammation.

Our data have identified macrophage NCOR1 as a potential new target for treating MI and restenosis. Monocytes are recruited to the infarcted myocardium and differentiated into cardiac macrophages to remove dead cells and debris and facilitate the initial repair process.[32,40] However, monocytosis, exaggerated accumulation of macrophages and prolonged inflammation may impede healing, cause high secondary event rates, and eventually increase mortality.[41] In injured blood vessels, macrophages accumulate and secrete a lot of inflammatory cytokines and chemokines to stimulate the proliferation and migration of other vascular cells such as VSMCs and fibroblasts,[42] leading to the development of neointimal formation. NCOR1 is an essential regulator of gene transcription and previous studies have revealed its importance in physiological and pathophysiological processes. Muscle NCOR1 deficiency leads to enhanced exercise endurance in mice.[20] Deletion of adipocyte NCOR1 leads to reduced inflammation and enhanced systemic insulin sensitivity despite increased adipogenesis in a mouse model of obesity-induced type 2 diabetes mellitus.[21] Similarly, deletion of macrophage NCOR1 also inhibits inflammation and improves obesity-induced insulin resistance in mice, although different mechanisms are

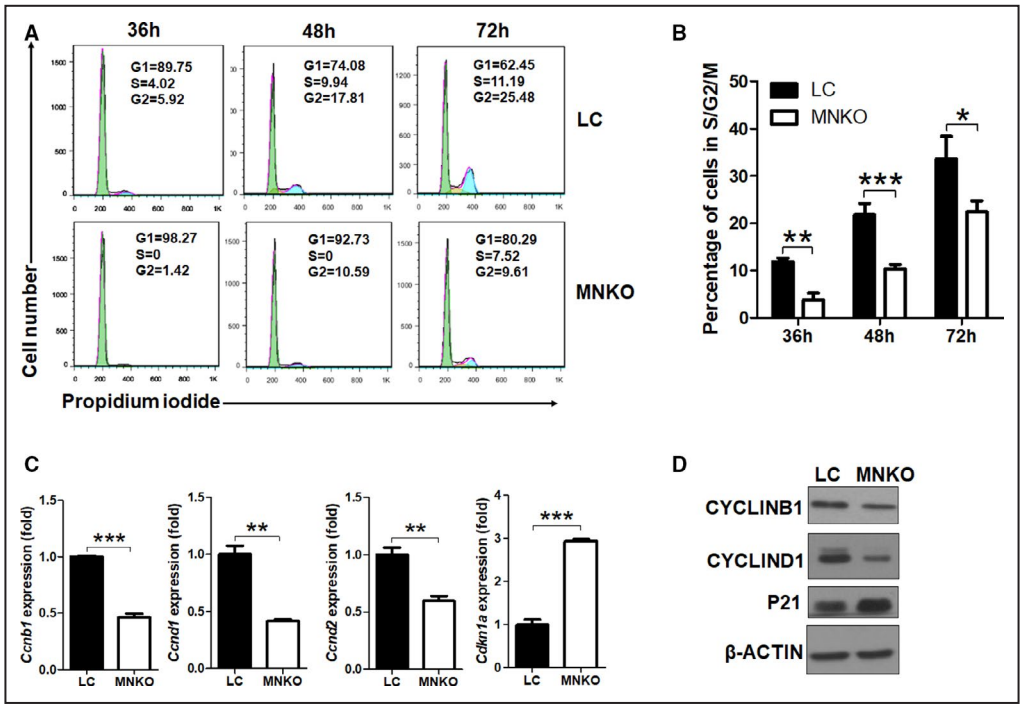

Figure 7. Impacts of macrophage NCOR1 (nuclear receptor corepressor 1) deficiency on cell cycle in macrophages.

A, Flow cytometry analysis of cell cycle phases of peritoneal macrophages isolated from littermate control or macrophage Ncor1 knockout mice and cultured in L929 conditioned media for indicated time periods. **B**, Quantification of percentages of cells in S/G2/M phase. Independent batches of cells were used for each time point. **C**, Quantitative reverse transcription polymerase chain reaction analysis of genes related to cell cycle regulation. Macrophages were cultured in L929 conditioned media for 24 hours. **D**, Western blotting analysis of proteins related to cell cycle regulation. β-ACTIN was used as a loading control. Representative results of flow cytometry, immunofluorescence staining, and western blotting from 3 independent experiments were shown. Values are expressed as mean±SEM. *Ccnb1* indicates cyclin B1; *Ccnd1*, cyclin D1; *Ccnd2*, cyclin D2; *Cdkn1a* or P21, cyclin-dependent kinase inhibitor 1A; LC, littermate control; and MNKO, macrophage Ncor1 knockout. **P*<0.05, ***P*<0.01, ****P*<0.001.

involved.[22] In this study, we demonstrated that macrophage NCOR1 deficiency markedly ameliorated MI and neointimal hyperplasia, with reduced inflammation and accumulation of macrophages in the heart and vascular wall, respectively, under pathological stress. These results suggest that targeting macrophage NCOR1 is a feasible approach to treat MI and restenosis.

Previously published data and our results together illustrated the complex roles of macrophage NCOR1 in atherosclerotic cardiovascular disease, with a protective function during atherosclerosis and a deleterious function during MI and vascular injury. Macrophage NCOR1 deficiency has been shown to aggravate atherosclerosis mostly by increase of oxidized low-density lipoprotein uptake via PPARγ (peroxisome proliferator-activated receptor gamma)-CD36 axis.[43] An increase of inflammatory cytokines was observed in NCOR1-deficient macrophages when stimulated by PPARγ agonists, which might have also contributed to the pro-atherosclerotic phenotype of macrophage NCOR1 knockout mice.[43] The increase of both oxidized low-density lipoprotein uptake and inflammatory cytokines required the activation of PPARγ, which resembled the environment of an atherosclerotic plaque.[43] Therefore, it is reasonable to assume that the proinflammatory signature of NCOR1-deficient macrophages is highly specific to the context of atherosclerosis. Our results revealed that macrophage NCOR1 deficiency manifested protective roles in MI and vascular injury by inhibiting inflammation in hearts and blood vessels. Different from the chronic inflammation in atherosclerosis, MI and vascular injury are both pathological process with acute and robust inflammation, especially during the early phase. Without agonists that exist in atherosclerotic plaques, PPARγ may no longer be the major player in modifying inflammatory reaction in macrophages during MI or vascular injury. Instead, under these circumstances significant inhibition of inflammatory cytokines in NCOR1-deficient macrophages may be because of other regulatory mechanisms such as the liver X receptors-omega 3 fatty acids-nuclear factor-κB axis.[22] Therefore, when considering

macrophage NCOR1 as a target for treating atherosclerotic cardiovascular disease, it needs to be taken into consideration that macrophage NCOR1 plays different or even opposite roles during different stages of this disease.

Our results have demonstrated the importance of NCOR1 in regulating macrophage proliferation that may partially contribute to MI and neointimal hyperplasia. Although resident macrophages and the proliferation of these cells are cardioprotective in a long term, they play a neglectable role within 7 days after MI.[12] Instead, recruited monocyte-derived macrophages account for >95% of total macrophages in the infarct/peri-infarct zone during this early phase.[12] Moreover, these recruited macrophages are proliferative as early as 4 days after MI.[12] It is well known that recruited macrophages are proinflammatory and may promote injury after MI.[44] Therefore, recruited macrophages and the proliferation of them may play detrimental roles at early stage after MI. Our results demonstrated that NCOR1 deficiency decreased the amount and proliferation of these cells and therefore has a beneficial effect on cardiac remodeling at day 7 after MI. Similarly, NCOR1 deficiency decreased the amount and proliferation of macrophages in blood vessels after vascular injury. Our data implicated that the impacts of NCOR1 on macrophage proliferation at least partially contributed to the protective function of macrophage NCOR1 deficiency on myocardial healing after MI and neointimal hyperplasia after vascular injury.

Previous studies have illustrated the roles of NCOR1 in controlling the growth or proliferation of other cell types. Knockdown of *Ncor1* using siRNA promotes proliferation of SK-HEP-1, a cancer cell line of endothelial origin, and increases cancer growth and metastasis in mice,[45] whereas specific deletion of NCOR1 in hepatocytes inhibits diethylnitrosamine-induced hepatocarcinogenesis.[24] The discrepancies might be attributable to different strategies to deplete NCOR1 and different cancer models. Interestingly, specific deletion of NCOR1 in hepatocytes also increases proliferation of hepatocytes and enhances liver regeneration after partial hepatectomy.[24] Deletion of NCOR1 in cardiomyocytes promotes cardiac hypertrophy.[26] We for the first time revealed that NCOR1 deficiency decreased macrophage proliferation both in vivo and in vitro. As a transcriptional regulator, NCOR1 controlled expression of many genes related to cell cycle, such as *Ccnb1*, *Ccnd1,* and *Cdkn1a*. Along with this line, cell cycle progression of macrophages was inhibited by NCOR1 deficiency. In addition, downregulation of p-AKT and p-ERK1/2 in NCOR1-deficient macrophages implicated that PI3K-AKT and ERK1/2 pathways might be another part of the mechanisms of regulating macrophage proliferation by NCOR1. However, the detailed mechanisms remain to be further explored.

This study has added new evidence to support the mechanisms how NCOR1 controls macrophage inflammation. It has been revealed that NCOR1 deficiency in macrophages releases the depression of liver X receptors and thus increases biosynthesis of palmitoleic acid and ω3 fatty acids, both of which suppress expression of inflammatory genes through inhibition of nuclear factor-κB signaling pathway.[22] IL-1β and IL-6 are classic inflammatory cytokines that activate multiple inflammatory signaling pathways in macrophages. Both IL-1β and IL-6 were upregulated in hearts after MI and arteries after wire injury. Our in vitro results illustrated that NCOR1 deficiency impaired activation of p-STAT1, p-P38, p-P65, and p-IKKβ and inhibited inflammatory gene expression in macrophages upon stimulation by IL-1β or IL-6. These data not only substantiated the critical role of nuclear factor-κB signaling pathway, but also suggested the involvement of STAT1 signaling pathway in the hypo-inflammatory phenotype of NCOR1-deficient macrophages. Furthermore, NCOR1 deficiency also upregulated a subset of alternative macrophage-polarization markers, suggesting a macrophage phenotypic switch.

In summary, macrophage NCOR1 plays a pivotal role in MI and neointimal hyperplasia, likely through its regulation of macrophage inflammation and proliferation. These results have unveiled novel functions of macrophage NCOR1 in the setting of cardiovascular diseases. The findings support that selectively targeting NCOR1 in macrophages may provide an effective novel strategy to treat MI and restenosis.

ARTICLE INFORMATION

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Disclosures

None.

Supplementary Materials

Data S1
Table S1
Figures S1–S15

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Determination of infarct size

Mice were sacrificed 1 day after MI. Hearts were excised and blood was squeezed out. After frozen at -20℃ for 1 hour, hearts were transversely sliced into five 1mm-thick sections. The sections were then incubated with 2% triphenyltetrazolium chloride (TTC, Sigma-Aldrich) at 37℃ for 20 minutes. Each section was photographed and infarct size was calculated as a percentage of infarcted area to total left ventricle area. The measurement of infarct size was performed by independent investigators blinded from the experimental conditons.

TUNEL assay

For paraffin sections, after deparaffinized and antigen retrieval treatment, the slides were stained with anti-Troponin T-C antibodies (sc-20025, Santa Cruz Biotechnology, Dallas, Texas, USA) followed by flurochrome-conjugated secondary antibodies (A11001, Thermo Fisher Scientific). Subsequently, TdT-mediated dUTP nick end labeling (TUNEL) staining was done according to the manufacturer's protocol of In Situ Cell Death Detection Kit-TMR red (12156792910, Roche Diagnosticscs, Indianapolis, IN, USA). The sections were conterstained with DAPI (P36931, Thermo Fisher Scientific) and images were captured using a fluorecence microscope.

Cell culture

Mouse bone marrow derived macrophages (BMDMs) were isolated and cultured as previously described[28]. In brief, bone marrow cells were flushed out from femur and tibia of LC and MNKO mice, and then were cultured in L929 conditioned media for 7 days. BMDMs were used for experiment on day 8.

LPS (100ng/ml, L2880 Sigma-Aldrich) were used to treat peritoneal macrophages and BMDMs for different time periods.

Flow cytometry

Peripheral blood was collected and erythrocytes were lysed in RBC lysis buffer for twice. Cell suspensions were centrifuged, blocked with Fc block for 10 minutes, and

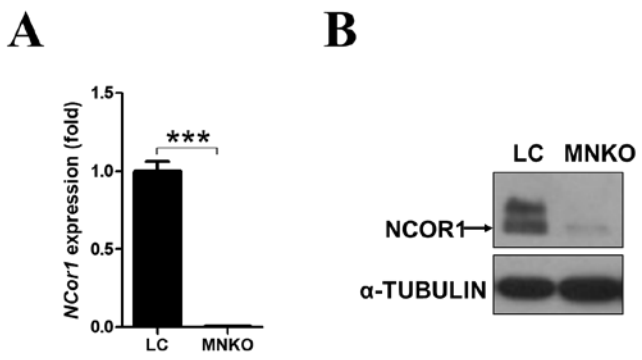
labeled with antibodies at 4℃ for 20 minutes. All samples were analyzed using LSR Fortessa (BD Biosciences).

The following antibodies were used: : Fc block (101320, Biolegend), CD45-PE-Cy7 (25-0451-81, eBioscience), CD11b-FITC (11-0112-82, eBioscience), Ly6G-APC (560593, BD Biosciences), Ly6C-PE (560599, BD Biosciences).

Table S1: List of primers for QRT-PCR

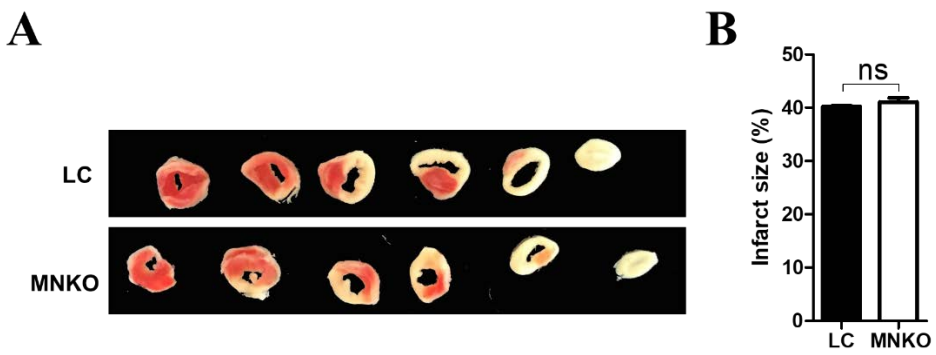
Gene	Forward	Reverse
l32	TTAAGCGAAACTGGCGGAAAC	TTGTTGCTCCCATAACCGATG
Ncor1	CTGGTCTTTCAGCCACCATT	CCTTCATTGGATCCTCCATC
Ccnb1	GTGCGCCTGCAGAAGAGTAT	TGCTCTTCCTCCAGTTGTCGG
Ccnd1	TGCTCTTCCTCCAGTTGTCGG	CCGGAGACTCAGAGCAAATCC
Ccnd2	CGCTACCGACTTCAAGTTTGC	TCATCATCCTGCTGAAGCCC
Cdkn1a	AGCAGAATAAAAGGTGCCACAG	CATGAGCGCATCGCAATCAC
Gapdh	ATGTTCCAGTATGACTCCACTCACG	GAAGACACCAGTAGACTCCACGACA
Il6	ACTGGGTGTACTCTGGCTCACAAA	ACTCTTCCCGTTGGTGGTGTTGAT
Nos2	CTGCTGGTGACAAGCACATTT	ATGTCATGAGCAAAGGCGCAGAAC
Il1β	AAGAGCTTCAGGCAGGCAGTATCA	TGCAGCTGTCTAATGGGAACGTCA
Cxcl1	TGGGATTCACCTCAAGAACA	TTTCTGAACCAAGGGAGCTT
Cxcl10	CCAAGTGCTGCCGTCATTTTC	GGCTCGCAGGGATGATTTCAA
Ccr2	GGAGCCATACCTGTAAATGCCATGC	CGGTGTGGTGGCCCCTTCAT
Tnfα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Cox2	CCCTGCTGCCCGACACCTTC	CCAGCAACCCGGCCAGCAAT
Ccl2	CAGCCAGATGCAGTTAACGC	GCCTACTCATTGGGATCATCTTG

Figure S1. Efficient deletion of NCOR1 in macrophages.



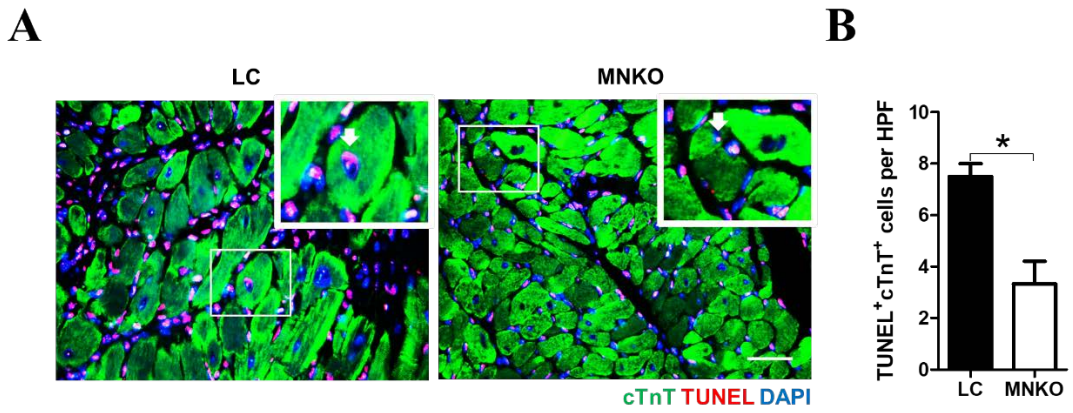
(A) QRT-PCR analysis of *Ncor1* gene expression in peritoneal macrophages isolated from littermate control (LC) or macrophage NCOR1 knockout (MNKO) mice. (B) Western blotting analysis of NCOR1 levels in peritoneal macrophages. α-TUBULIN was used as a loading control. Values are expressed as mean ± SEM. ***$p < 0.001$.

Figure S2. Macrophage NCOR1 deficiency does not affect the infarct size at day 1 after MI.



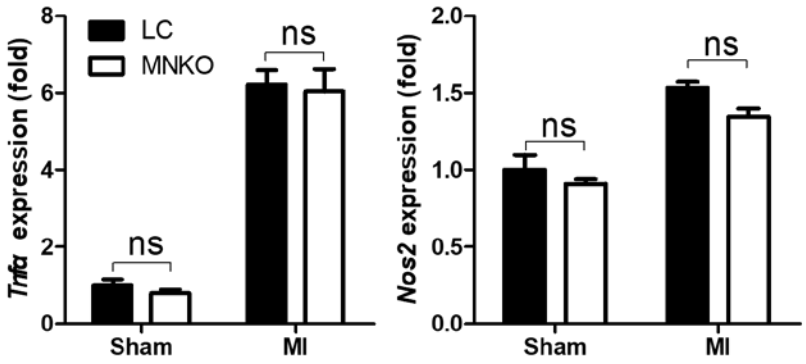
(A) Representative 2,3,5-triphenyltetrazolium chloride (TTC) staining of cardiac tissue sections of LC and MNKO mice 1 day after MI. (B) Quantification of the infarct size. n=3:3. Values are expressed as mean ± SEM. ns: not significant.

Figure S3. Macrophage NCOR1 deficiency decreases the apoptosis of cardiomyocytes in peri-infarct region 7 days after MI.



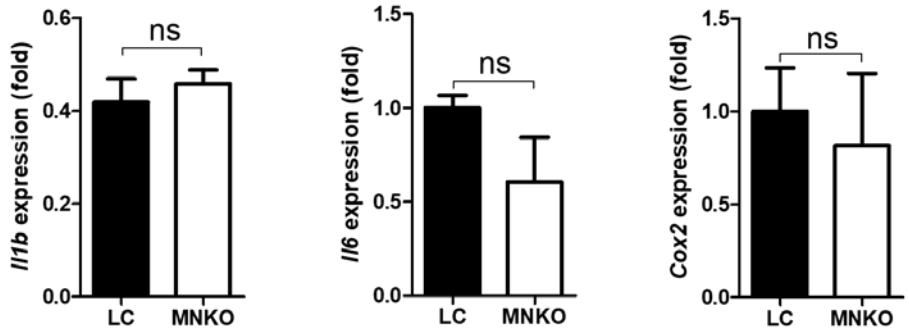
(A) Representative immunofluorescence staining of TUNEL and cTnT in hearts from LC and MNKO mice 7 days after MI. Arrows denote TUNEL/cTnT double-positive cells. Scale bar: 50 μm. (B) Quantification of apoptotic cardiomyocytes. n=3:3. Values are expressed as mean ± SEM. *P<0.05. HPF: high power field.

Figure S4. Macrophage NCOR1 deficiency does not affect the expression of *Tnfa* or *Nos2* in the heart.



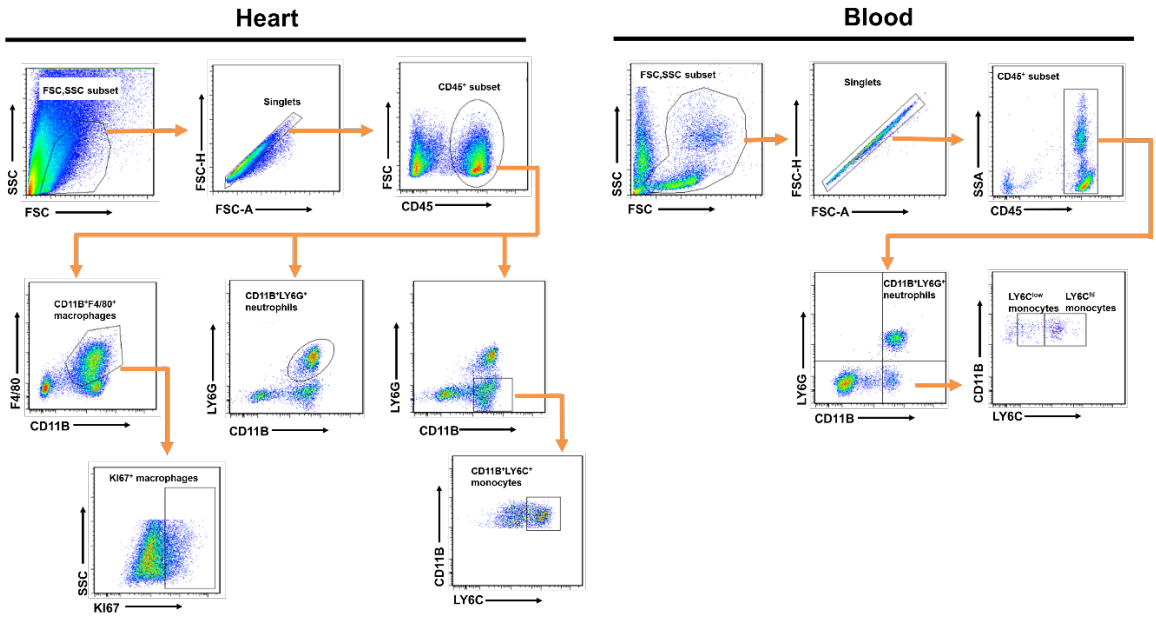
QRT-PCR analysis of *Tnfa* and *Nos2* in the ischemic region of hearts from LC and MNKO mice 3 days after MI. n=3:3:3:3. Values are expressed as mean±SEM. ns: not significant.

Figure S5. Comparable expression of inflammatory genes in heart samples from LC and MKNO mice 7 days after MI.



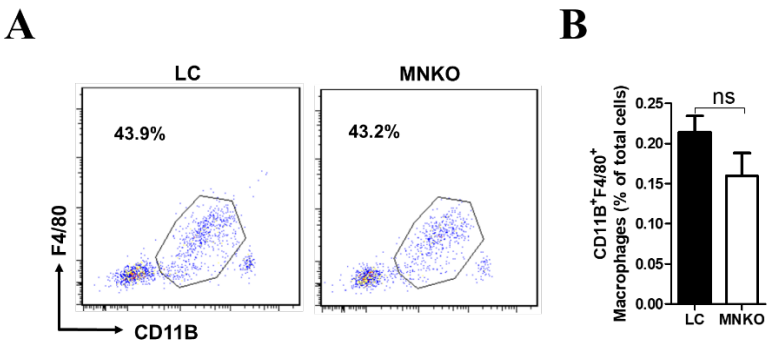
QRT-PCR analysis of inflammatory genes in ventricular samples from LC and MNKO mice 7 days after MI. n=3:3. Values are expressed as mean ± SEM. ns: not significant.

Figure S6. Gating strategy for flow cytometry analysis of myeloid cells in heart samples and blood.



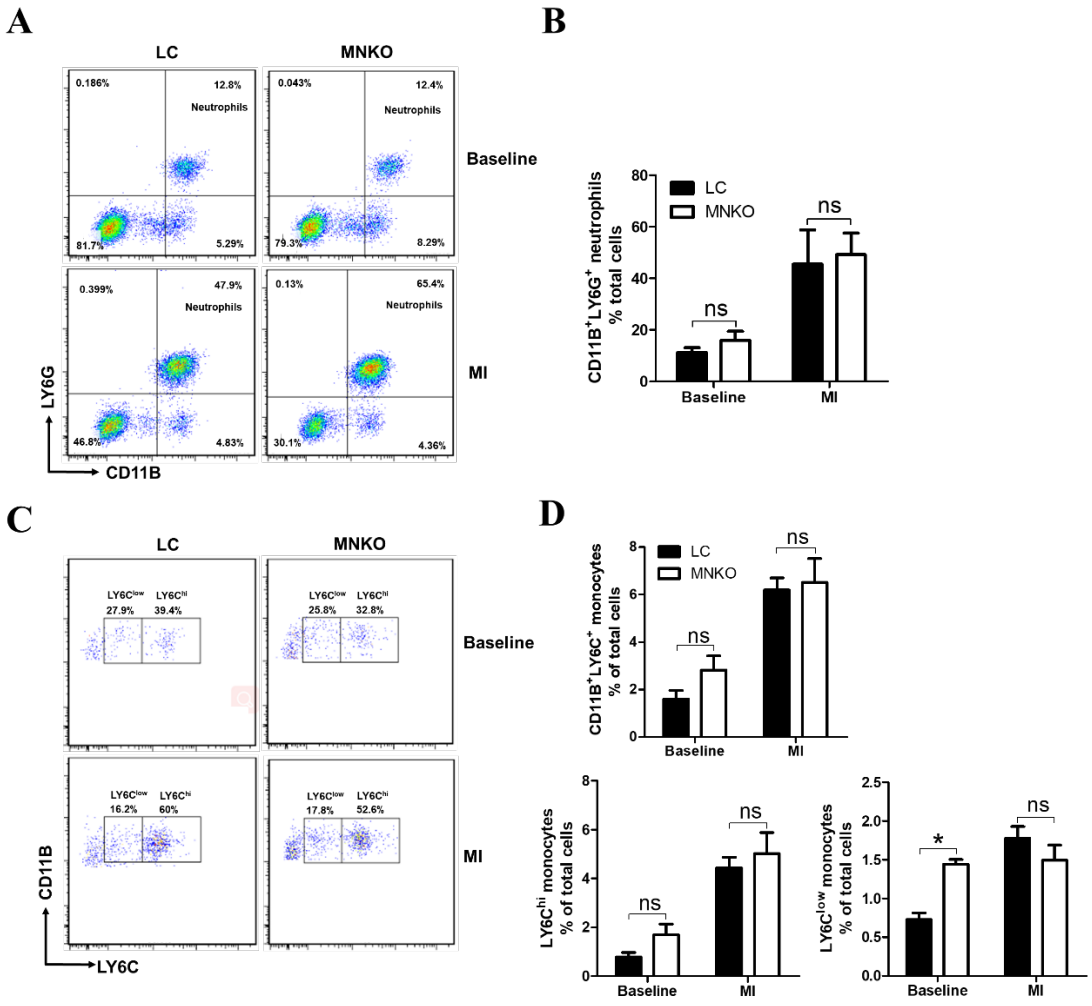
Single-cell suspension of heart samples or blood was prepared and labeled with various antibodies. Doublets were excluded and CD45 was used to identity $CD45^+$ immune cells, which included neutrophils, monocytes and macrophages. Neutrophils were selected by $CD11B^+LY6G^+$. Monocytes were stratified as $CD11B^+LY6G^-LY6C^+$. Macrophages were identified as $CD11B^+$ $F4/80^+$. Proliferated macrophages were further stratified as $KI67^+$.

Figure S7. Macrophage NCOR1 deficiency does not affect the content of resident macrophages in heart at baseline.



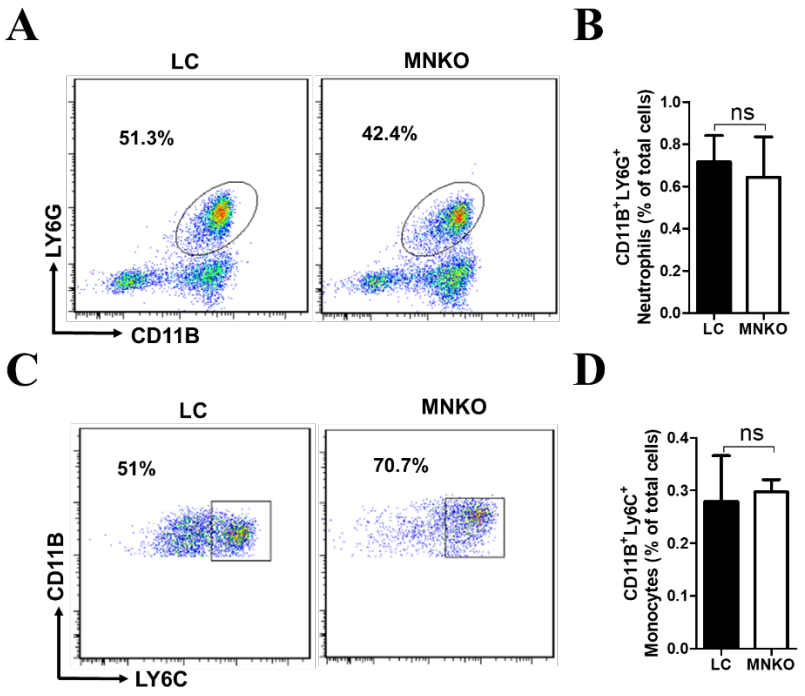
(A) Representative flow cytometry analysis of macrophages in hearts from LC and MNKO mice at baseline. (B) Quantification of $CD11B^{+}F4/80^{+}$ macrophages. n=3:3. Values are expressed as mean ± SEM. ns: not significant.

Figure S8. The effects of macrophage NCOR1 deficiency on the levels of neutrophils and monocytes in blood at baseline or after MI.



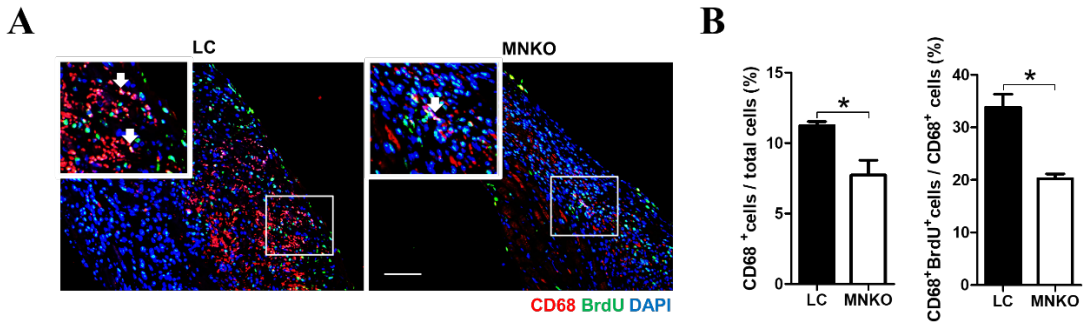
(A) Representative flow cytometry analysis of neutrophils in blood from LC and MNKO mice at baseline or 1 day after MI. (B) Quantification of neutrophils. n=3:3:3:3. (C) Representative flow cytometry analysis of LY6C^{hi} and LY6C^{low} monocytes in blood from LC and MNKO mice at baseline or 3 days after MI. (D) Quantification of total monocytes, LY6C^{hi} and LY6C^{low} monocytes. n=3:3:3:3. Values are expressed as mean ± SEM. *P<0.05, ns: not significant.

Figure S9. Macrophage NCOR1 deficiency does not affect the recruitment of neutrophils or monocytes to the heart after MI.



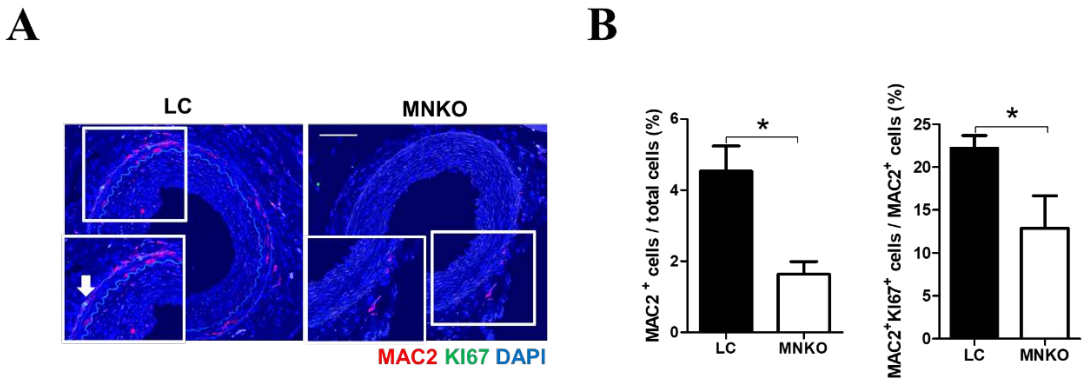
(A) Representative flow cytometry analysis of neutrophils in hearts from LC and MNKO mice 1 day after MI. (B) Quantification of $CD11B^{+}LY6G^{+}$ neutrophils. n=3:3. (C) Representative flow cytometry analysis of monocytes in hearts from LC and MNKO mice 1 day after MI. (D) Quantification of $CD11B^{+}LY6G^{-}LY6C^{+}$ monocytes. n=3:3. Values are expressed as mean ± SEM. ns: not significant.

Figure S10. Macrophage NCOR1 deficiency inhibits proliferation of cardiac macrophages in infarcted region after MI.



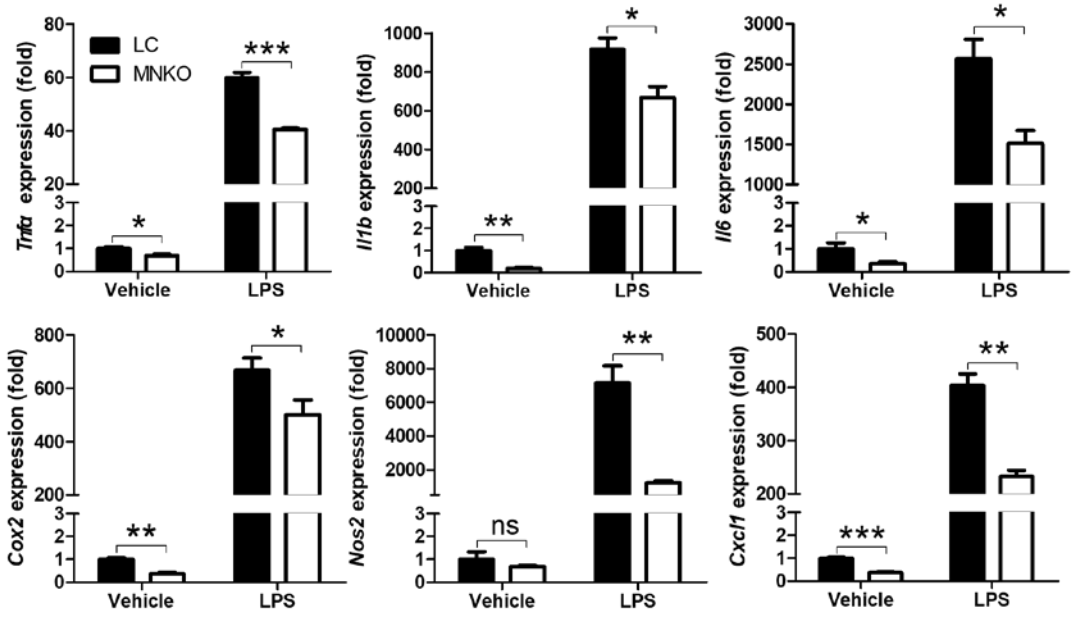
(A) Representative immunofluorescence staining of CD68 and BrdU in infarcted region of hearts from LC and MNKO mice 7 days after MI. The arrows point to CD68/BrdU double-positive cells. Scale bar: 200μm. (B) Quantification of CD68-positve cells and CD68/BrdU double-positive cells. n=3:3. Values are expressed as mean ± SEM. *P < 0.05.

Figure S11. Macrophage NCOR1 deficiency inhibits macrophages proliferation in femoral arteries after wire injury.



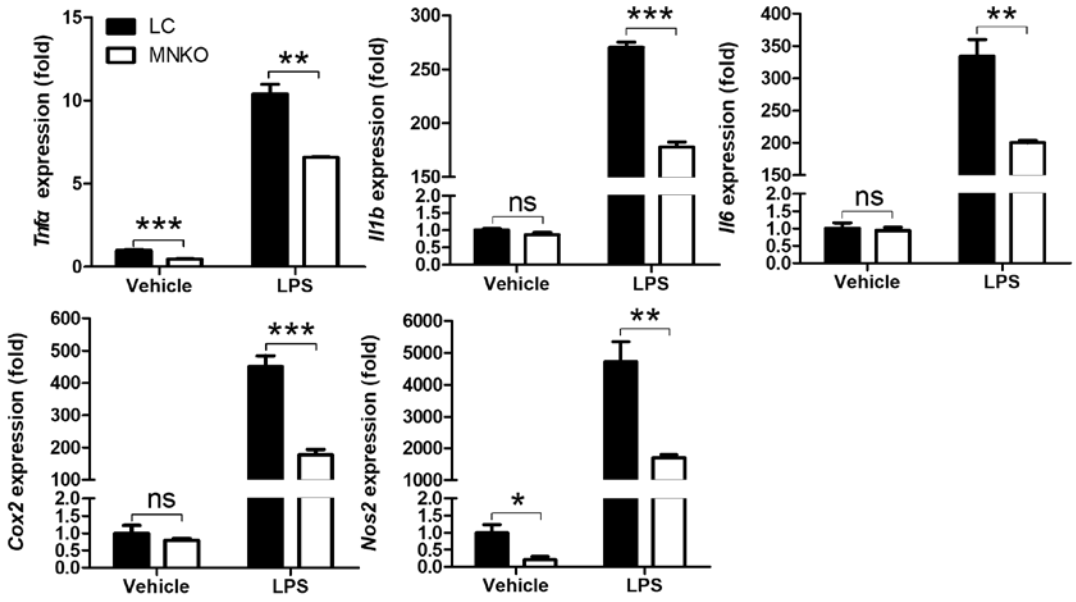
(A) Representative immunofluorescence staining of macrophages in femoral arteries of LC and MNKO mice 4 weeks after wire injury. The arrow points to MAC2/KI67 double-positive cells. (B) Quantification of MAC2-positive macrophages and MAC2/KI67 double-positive cells. Scale bar: 200μm. n=4:4. Values are expressed as mean ± SEM. *P < 0.05.

Figure S12. Macrophage NCOR1 deficiency suppresses LPS-induced inflammation in macrophages.



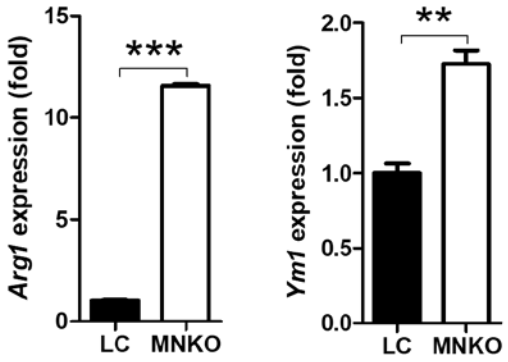
Peritoneal macrophages were isolated from LC and MNKO mice. Inflammatory genes were analyzed using QRT-PCR in macrophages stimulated with LPS for 6 hours. Values are expressed as mean ± SEM. *P < 0.05, **P < 0.01, ***p < 0.001, ns: not significant.

Figure S13. Macrophage NCOR1 deficiency suppresses LPS-induced inflammation in bone marrow derived macrophages (BMDMs).



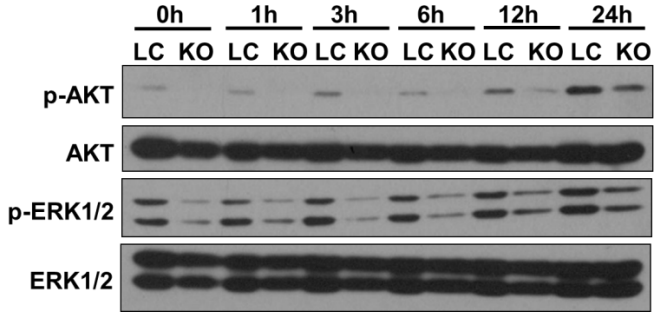
BMDMs were isolated from LC and MNKO mice. QRT-PCR was used to analyze inflammatory genes in BMDMs stimulated with LPS for 12 hours. Values are expressed as mean ± SEM. *P < 0.05, **P < 0.01, ***p < 0.001, ns: not significant.

Figure S14. Macrophage NCOR1 deficiency upregulates expression of anti-inflammatory genes in macrophages.



QRT-PCR analysis of M2-like genes in macrophages isolated from LC and MNKO mice. Values are expressed as mean ± SEM. **P < 0.01, ***p < 0.001.

Figure S15. Macrophage NCOR1 deficiency downregulates p-AKT and p-ERK1/2 in macrophages.



Western blotting analysis of phosphorylation and total protein levels of AKT and ERK1/2 in peritoneal macrophages isolated from LC or MNKO (KO) mice and cultured in L929 CM for indicated time periods.