SUPPLEMENTAL MATERIAL

Table S1 Primers used for quantitative real-time PCR

Gene	Forward Primer	Reverse Primer
CXCR2	5'- ACTACTGCAGGATTAAGTTTACCTC-3'	5'-TCTCTGAGTGGCATGGGACA-3'
CD68	5'-TGTCTGATCTTGCTAGGACCG-3'	5'-GAGAGTAACGGCCTTTTTGTGA-3'
VCAM	5'-GTTCCAGCGAGGGTCTACC-3'	5'-AACTCTTGGCAAACATTAGGTGT-3'
COX2	5'-TGAGCAACTATTCCAAACCAGC-3'	5'-GCACGTAGTCTTCGATCACTATC-3'
IL-1β	5'-CTTCCCCAGGGCATGTTAAG-3'	5'-ACCCTGAGCGACCTGTCTTG-3'
IL-6	5'-TTCCATCCAGTTGCCTTCTTG-3'	5'-TTGGGAGTGGTATCCTCTGTGA-3'
NOX1	5'-CCCATCCAGTCTCCAAACATGAC-3'	5'- ACCAAAGCTACAGTGGCAATCAC-3'
NOX2	5'-CTTCTTGGGTCAGCACTGGC-3'	5'-GCAGCAAGATCAGCATGCAG-3'
NOX4	5'-CTTGGTGAATGCCCTCAACT-3'	5'-TTCTGGGATCCTCATTCTGG-3'
TNF-α	5'-ATGGCCTCCCTCTCATCAGT-3'	5'-CTTGGTGGTTTGCTACGACG-3'
$P22^{phox}$	5'-CTCCTCTTCACCCTCACTCG-3'	5'- GTGGACTCCCATTGAGCCTA-3'
α-SMA	5'-TCCTGACGCTGAAGTATCCGATA-3'	5'-GGCCACACGAAGCTCGTTAT-3'
Collagen I	5'-GAGTACTGGATCGACCCTAACCA-3'	5'- GACGGCTGAGTAGGGAACACA-3'
Collagen III	5'-TCCCCTGGAATCTGTGAATC-3'	5'-TGAGTCGAATTGGGGAGAAT-3'
GAPDH	5'-GGTTGTCTCCTGCGACTTCA-3'	5'-GGTGGTCCAGGGTTTCTTACTC-3'

CXCR2, chemokine (C-X-C motif) receptor 2; VCAM, vascular cell adhesion molecule; COX2, cytochrome c oxidase subunit II; IL-1β, Interleukin-1β; IL-6, Interleukin-6; NOX1, NADPH oxidase 1; NOX2, NADPH oxidase 2; NOX4, NADPH oxidase 4; TNF-α, Tumor necrosis factor; $P22^{phox}$, cytochrome b-245, alpha polypeptide; α-SMA, α-smooth muscle actin; GAPDH, Glyceraldehyde 3-phosphate dehydrogenase.

1. Supplemental Methods

1.1 Animals and Treatment

Mice were anaesthetized with ketamine (200 mg/kg) and xylazine (10 mg/kg) by intra-peritoneal injection (i.p.). Anaesthesia was monitored by pinching the toe. Post-operative anaesthesia (buprenophine, 0.05 mg/kg/12h, i.p.) was administered for 48 h. On the last day of angiotensin II/DOCA infusion, all mice were anaesthetized by an overdose of pentobarbital (100 mg/kg, intra-peritoneal injection, Sigma-Aldrich). The aortas were removed and prepared for further histological and molecular analysis.

1.2 Telemetric Blood Pressure Recording

Mice were anesthetized with isoflurane. The left common carotid artery was isolated carefully, the catheter which was connected to the transducer was introduced into the carotid and advanced until the tip was just inside the thoracic aorta. One of the ECG transmitters was positioned along the right flank, the other was positioned close to the hindlimb. The transducer was implanted abdominal subcutaneously. Sutured the skin after all the above device was positioned appropriate. All the above operation procedure was completed under sterile conditions. Then mice were allowed to recover for 2 weeks, and blood pressure before and during angiotensin II treatment were recorded continuously in freely moving animals using receiver platforms (DSI), data was taken using the Data Quest system (DSI).

1.3 Histopathology

For the immunohistochemistry staining of CXCR2, F4/80 and rabbit Ig G in the aorta, the sections were deparafinated, antigen retrieved by citrate buffer, blocking antigen by 3% BSA, incubated CXCR2, F4/80-antibody (1:200, Abcam Cambridge, MA) in 4°C overnight, finally detected CXCR2, F4/80 in the aorta by HRP-DAB detection method. For DHE staining, the isolated aorta was cut into 3 mm rings and incubated in Krebs-Hepes-solution for 15 min at 37°C, then it was embedded in OCT and frozen in liquid nitrogen, sectioned (8 μm).

1.4 Flow Cytometry

Aortic vessels were cleaned of fatty tissue, minced, and digested with aorta dissociation enzyme stock solution (125 U/ml collagenase type XI, 60 U/ml hyaluronidase type 1, 60 U/ml DNase I, and 450 U/ml collagenase type I, in 2.5 ml of PBS) to obtain the single cell suspensions. Blood was erythrocyte-depleted by lysing buffer (BD, New Jersey, USA).

1.5 Constitution of Bone Marrow Transplantation Chimeric Mice

Briefly, the bone marrow cells were extracted from C57BL/6J WT mice and CXCR2$^{-/-}$ mice by flushing femurs and tibiae with RPMI-1640 medium. Recipient mice were irradiated (8.5 Gy) by cobalt and then received 5X10^{6} bone marrow cells from C57BL/6J WT mice or CXCR2$^{-/-}$ mice. Then mice were kept in clean, individually ventilated cages. The chimeric mice were given acidified, antibiotic water and sterilized food. After 4 weeks, the chimeric mice were confirmed by phenotyping CXCR2 expression on bone marrow. Then the chimeric mice were then used to do experiments.

1.6 Vascular Relaxation Studies

Intact aortas were gently isolated from mice and stripped of adventitial fat. 4mm segments of the thoracic aorta were mounted on force transducers gently (Power lab, AD Instruments, Germany) in organ chambers filled with Krebs-Henseleit solution (37°C, pH 7.2-7.4, containing 120 mM NaCl, 5.5 mM KCl, 2.5 mM $CaCl_2$, 1.2 mM $MgCl_2 \cdot 6H_2O$, 1.2 mM Na_2HPO_4, 20 mM $NaHCO_3$, 0.03 mM EDTA-Na_2, 10 mM Glucose bubbled with Carbogen gas containing 95% O_2 and 5% CO_2). Vessels were then equilibrated for 1 hour before the stimulation of vaso-activity by 60% of the maximal KCl solution (37°C, pH 7.38-7.40, containing 5.248 g NaCl, 4.473 g KCl, 0.244 g $MgCl_2 \cdot 6H_2O$, 0.277 g $CaCl_2$, 2 g Glucose and 1.1915 g Hepes in 1000 ml H_2O). To test the relaxation ability of the aorta, we stimulated the aorta to contract with 1*10^{-6} M Noradrenalin Elisa (NE), and then applied a concentration gradient (1*10^{-9} M, 1*10^{-8} M, 1*10^{-7} M, 1*10^{-6} M, 1*10^{-5} M) acetylcholine (ACh) and sodium nitroprusside (SNP) to relax the aorta. Percent relaxation was calculated as described before[1].

1.7 Study Patients

Prior to blood collection, we carefully examined the medical history of control subjects and hypertensive patients. Any subjects with clear infectious diseases or immune diseases are excluded from our study. Namely, we would exclude any patients with potential diseases such as septicemia, anti-neutrophil cytoplasmic autoantibody-associated vasculitides, arthritis, inflammatory bowel disease, plasmodium vivax malaria, Helicobacter pylori-induced peptic ulceration and allergic[2-6]. Both control subjects and hypertensive patients had no apparent history using any drugs within 6 months prior to our study.

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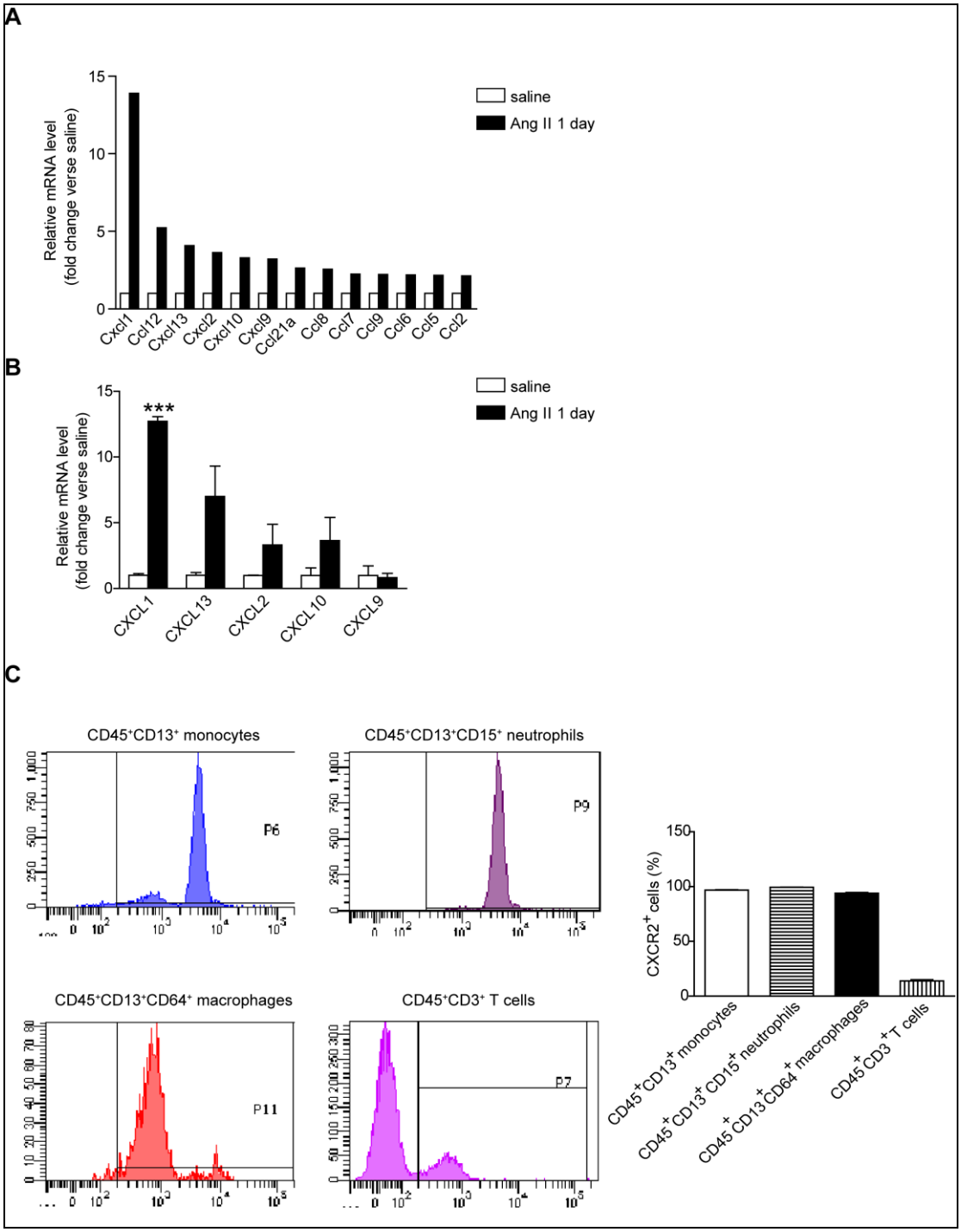
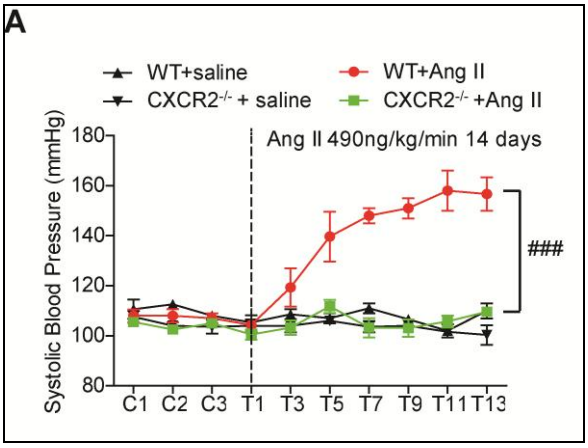
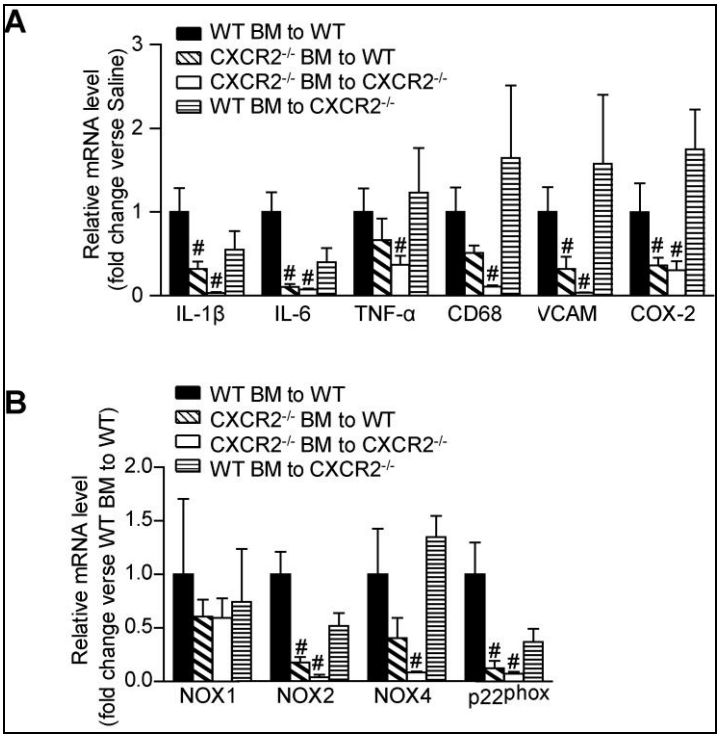
2. Supplemental Figure



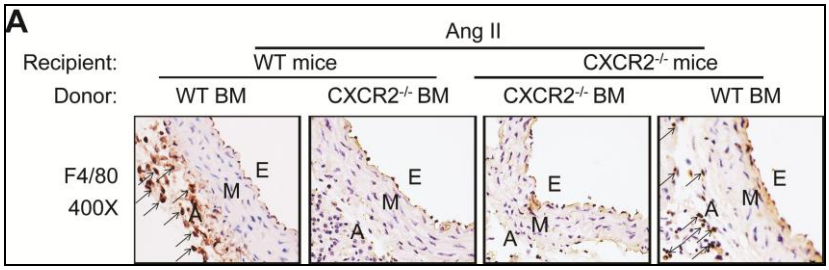
Supplemental Figure 1. Angiotensin II Infusion Significantly Increases the Expression of CXCL1 in The Aorta. **A,** Chemokine mRNA profiles in saline or angiotensin II-treated aortas on day 1 (n=3 per group). **B,** Validation of CXCL1, CXCL13, CXCL2, CXCL10 and CXCL9 mRNA expression levels by qPCR analysis. ***P<0.001 vs. saline, n=6 per group. **C,** Flow cytometry analysis of the percentage of $CXCR2^+$ cells in $CD45^+CD13^+$ monocytes, $CD45^+CD13^+CD15^+$ neutrophils, $CD45^+CD13^+CD64^+$ macrophages and $CD45^+CD3^+$ T cells in human blood.



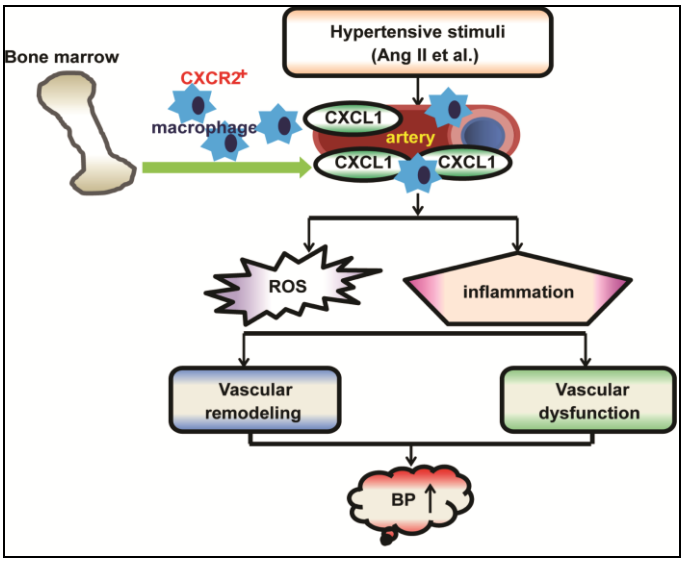
Supplemental Figure 2. Ablation of CXCR2 Inhibits Angiotensin II-Induced Hypertension. **A,** Systolic blood pressure was measured via the noninvasive tail-cuff method in WT and CXCR2-deficient ($CXCR2^{-/-}$) mice before (C) and after Ang II treatment (T) period. $^{###}P<0.001$ WT + Ang II vs. $CXCR2^{-/-}$ + Ang II, n=8 per group.



Supplemental Figure 3. CXCR2 Deficiency in Bone Marrow-Derived Cells Reduces Angiotensin II-Induced Vascular Inflammation and Oxidative Stress. **A,** mRNA levels of IL-1β, IL-6, TNF-α, CD68, VCAM-1 and COX-2 in the aorta were measured by qPCR analysis. **B,** qPCR analysis of NOX1, NOX2, NOX4 and $p22^{phox}$ mRNA expression in the aorta. $^{#}P<0.05$ vs. WT BM to WT; n=6 per group.



Supplemental Figure 4. CXCR2 Deficiency in Bone Marrow-Derived Cells Reduces Angiotensin II-Induced F4/80^{+} macrophages infiltration into the adventitia. **A,** Immunohistochemistry staining of the F4/80-positive cells in aortic sections. Arrows indicate the F4/80-positive cells. Scale bar, 50 μm.



Supplemental Figure 5. Central illustration of the study. Hypertensive stimuli (Angiotensin II etc.) induce chemokine release (such as CXCL1) from the vessel wall, stimulating migration and infiltration of bone marrow derived CXCR2^{+} macrophages. The infiltration of bone marrow derived-CXCR2^{+} macrophages drives vascular remodeling and dysfunction by causing vascular oxidative stress and inflammation, finally leading to hypertension.