**Supplemental materials**

**Full title. CXCR4-dependent macrophage-to-fibroblast signaling contributes to cardiac diastolic dysfunction in heart failure with preserved ejection fraction.**

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**Running title. Macrophagic CXCR4 and HFpEF**

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**Supplemental figures and figure legends**

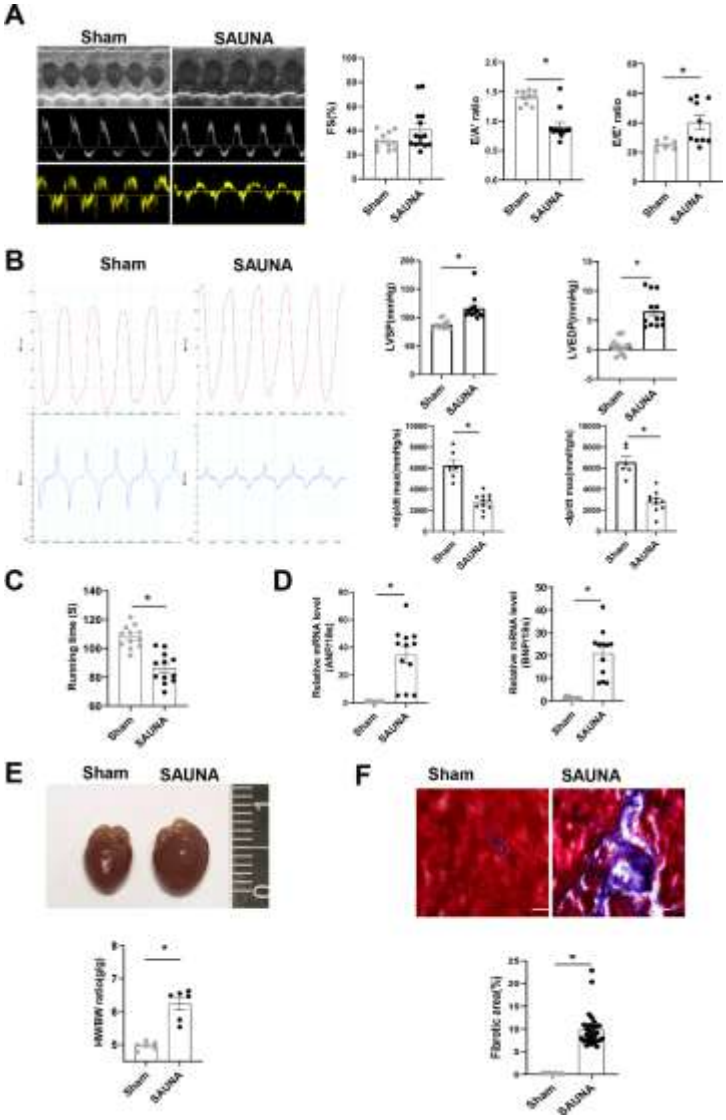


**Figure S1. SAUNA induced HFpEF**.

**(A)** Representative left ventricular M-mode echocardiographic, pulsed-wave Doppler and tissue Doppler tracings, measurement of FS%, E/A, and E/E'. **(B)** Graphic representation and quantification of LVSP and LVEDP, +dp/dt, and -dp/dt. **(C)** Recording of running times during exercise exhaustion test. **(D)** QPCR analysis of the mRNA levels of ANP, and BNP in the heart. **(E)** Representative heart size and HW/BW. **(F)** Masson's trichrome staining of heart tissues and quantification of fibrotic area. scale bars=50 μm. Sham, n = 10; SAUNA, n = 14. All data were analyzed using unpaired two-tailed student's t-test. *, $p<0.05$.

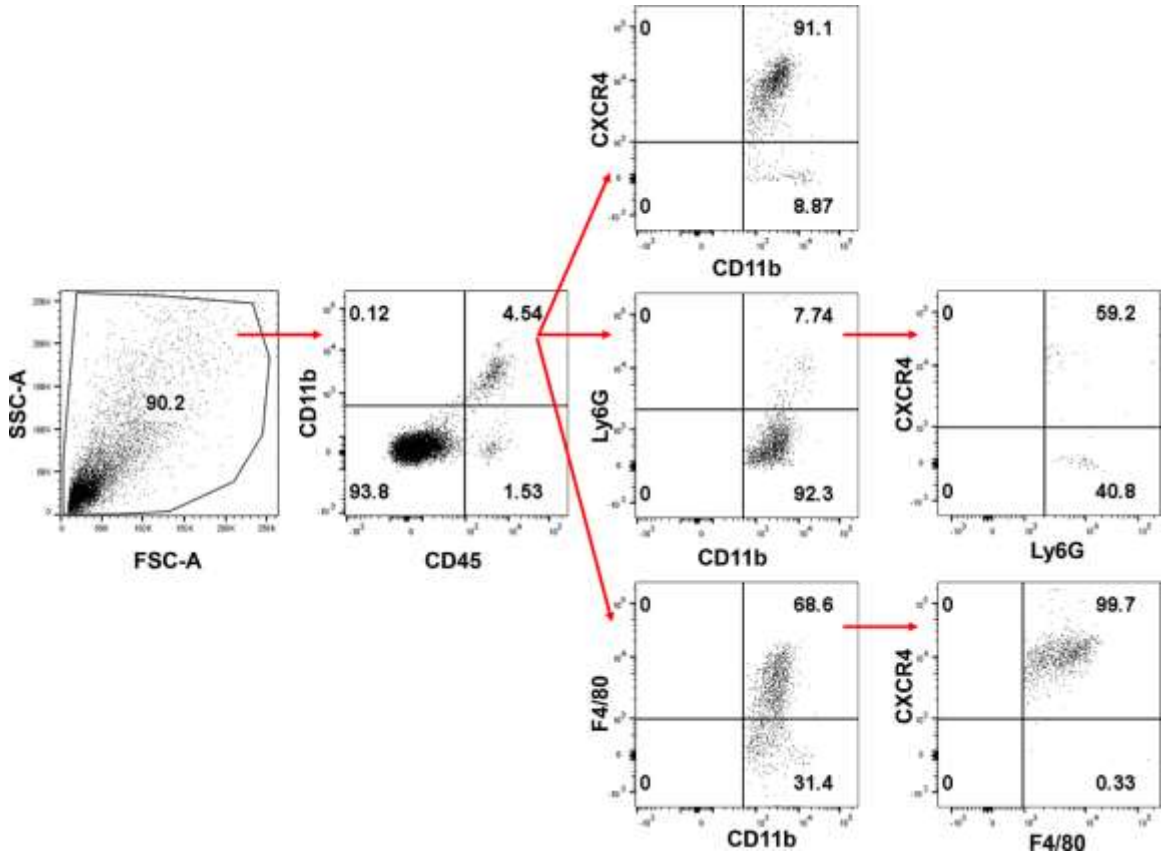


**Figure S2. Gating strategy for immune cells in heart post-SAUNA**

Gating strategy of CD45+ CD11b+ leukocytes and CD45+CD11b+ F4/80+ macrophages in the heart of sham and SAUNA group.

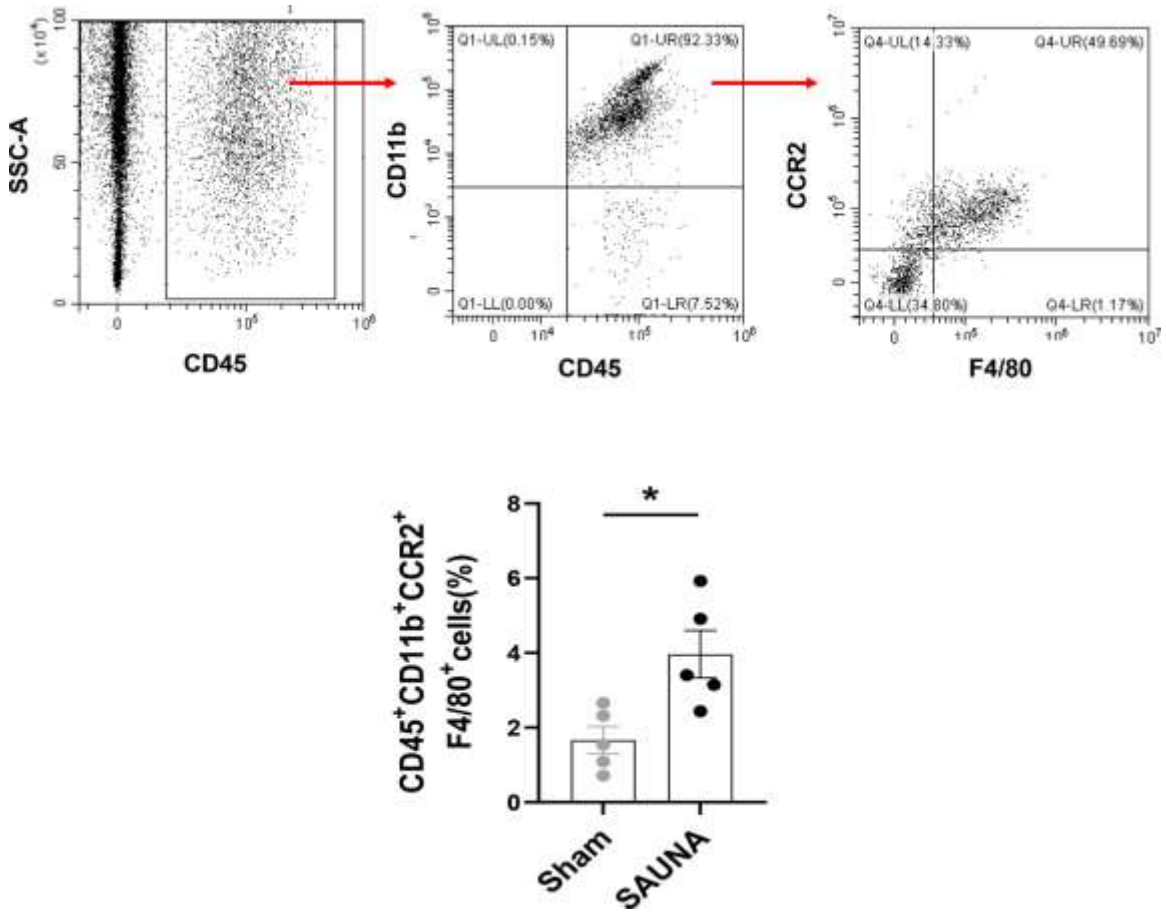


**Figure S3. SAUNA induced bone marrow-derived macrophages accumulating in the heart.**

Flow cytometry analysis the CD45+CD11b+CCR2+F4/80+ macrophages in the heart of sham and SAUNA group. Sham, n = 5; SAUNA, n = 5. Data were analyzed using two-way ANOVA with Bonferroni's multiple comparisons test. *, $p<0.05$.

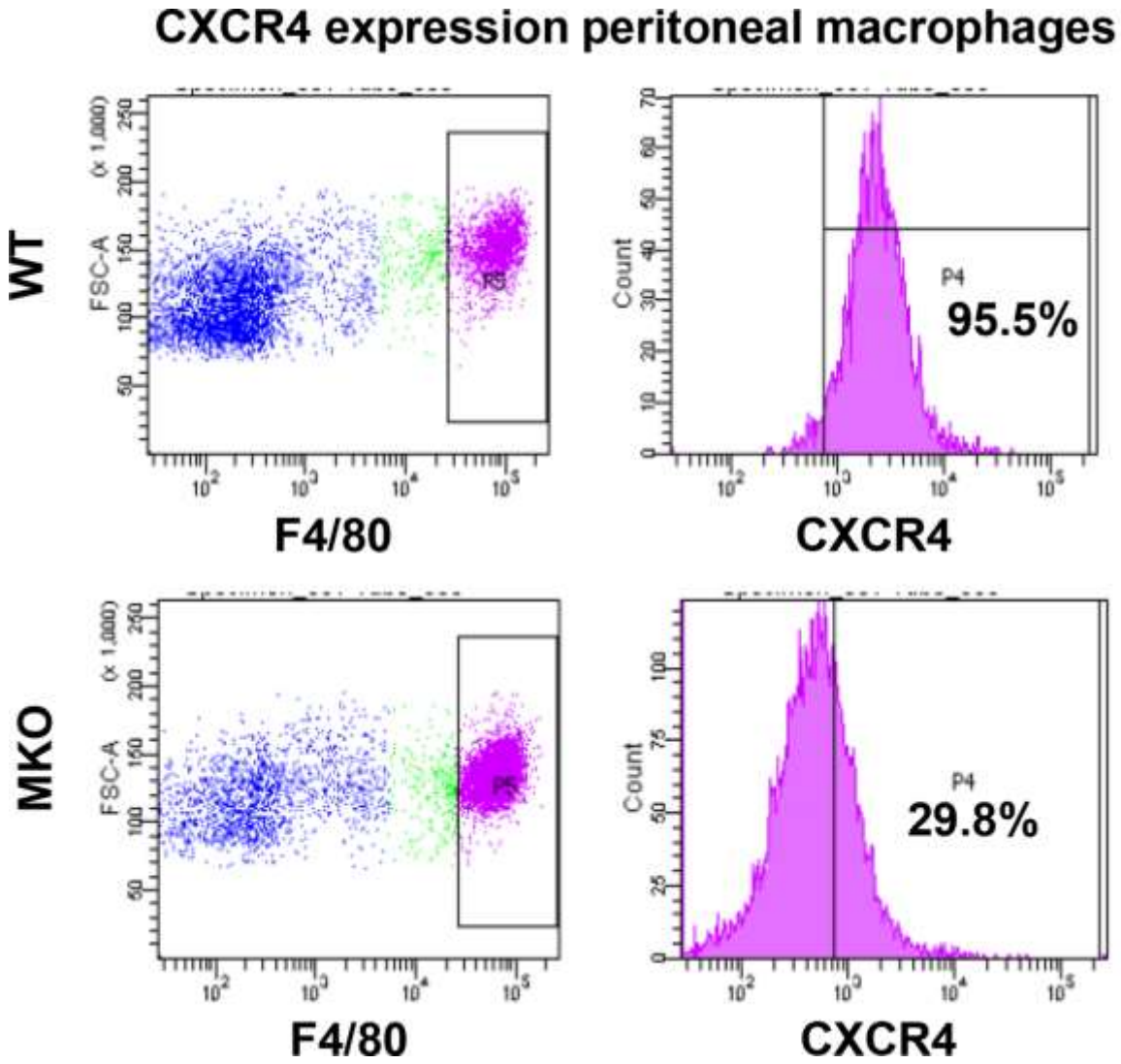


**Figure S4. CXCR4 was knockout in peritoneal macrophages of MKO mice.**

Flow cytometry analysis of CXCR4 expression in peritoneal macrophage of WT and MKO mice.

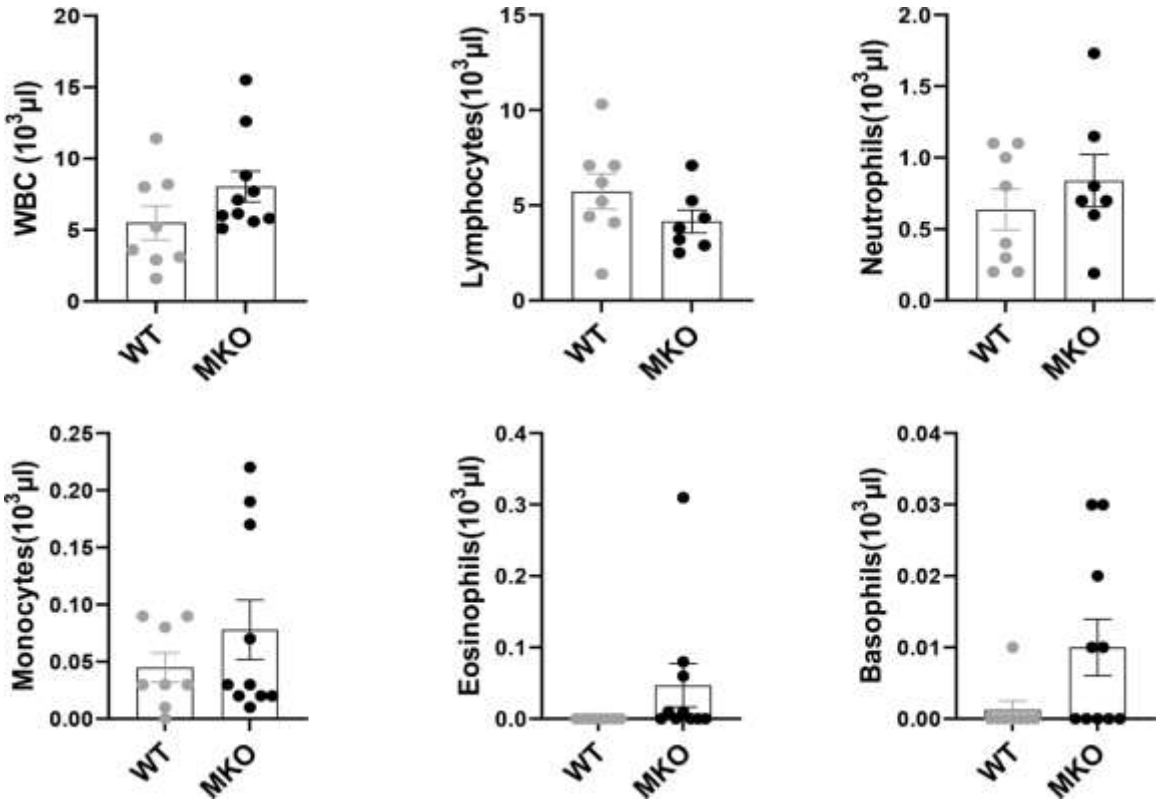


**Figure S5. Circulatory leukocytes did not differ at baseline among WT and MKO mice.**

Number of white blood cells (WBC), lymphocytes, neutrophils, monocytes, eosinophils and basophils in blood obtained from WT and MKO mice. *, $p<0.05$.

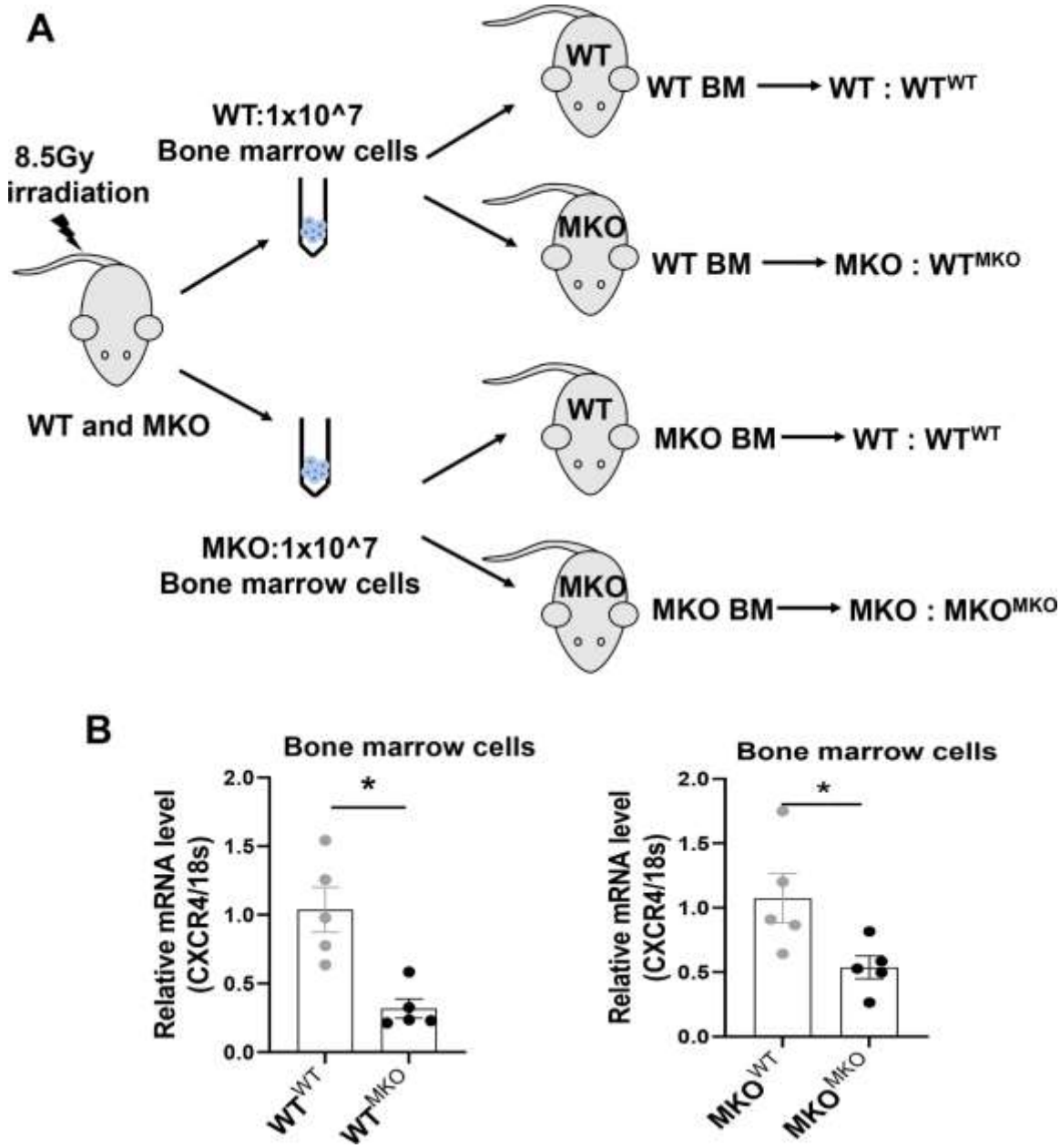


**Figure S6. Bone marrow transplantation experiment.**

**(A)** Schematic diagram of bone marrow transplantation experiment. **(B)** The mRNA levels of CXCR4 in the bone marrow cells of in $WT^{WT}$, $WT^{MKO}$, $MKO^{WT}$, and $MKO^{MKO}$ mice. All data were analyzed using unpaired two-tailed student's t-test. *, $p<0.05$.

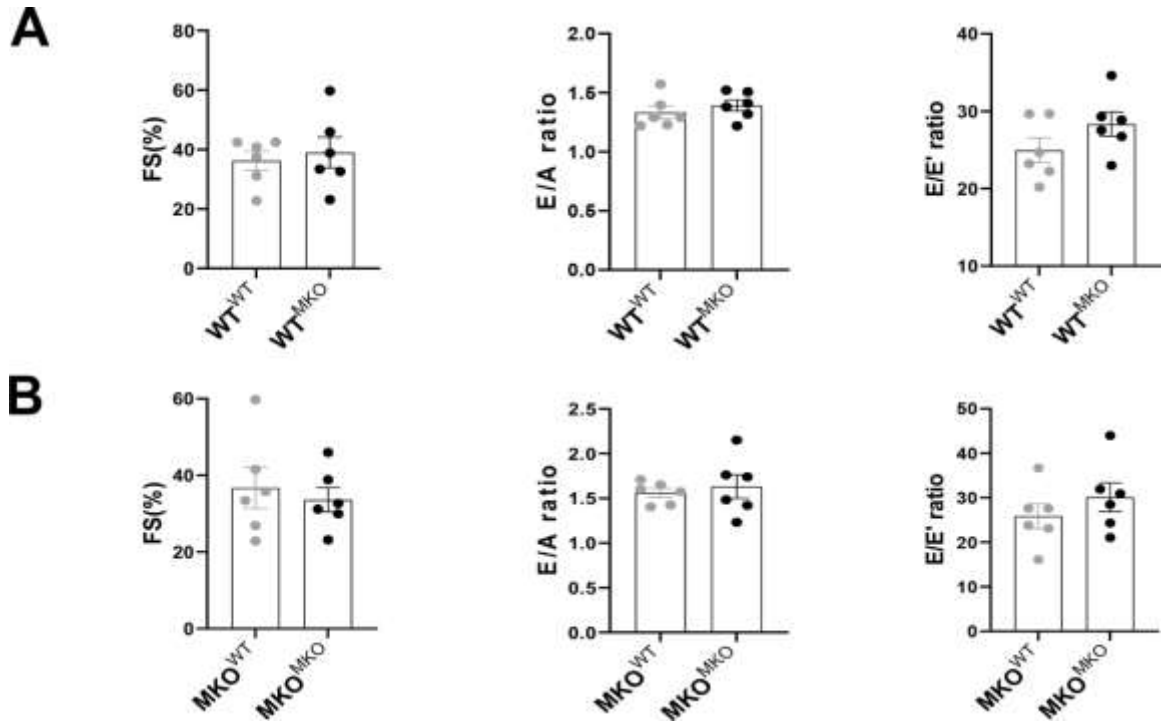


**Figure S7. Baseline cardiac function showed no difference between WT and MKO mice after bone marrow transplantation.**

**(A)** Measurement of FS%, E/A, and E/E' in baseline level of $WT^{WT}$ and $WT^{MKO}$. **(B)** Measurement of FS%, E/A, and E/E' in baseline level of $MKO^{WT}$ and $MKO^{MKO}$. $WT^{WT}$, n=6; $WT^{MKO}$, n=6; $MKO^{WT}$, n=6; $MKO^{MKO}$, n=6. Data were analyzed using unpaired two-tailed student's t-test *, $p<0.05$.

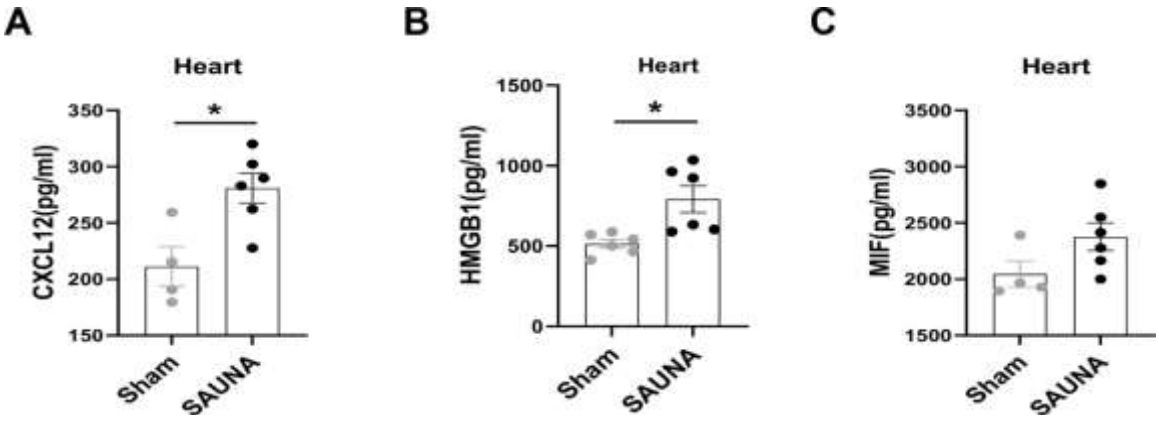


**Figure S8. Ligands for CXCR4 were detected in the SAUNA-induced heart.**

**(A)** Elisa analysis of the CXCL12 expression in the heart of sham and SAUNA group. Sham, n = 4; SAUNA, n = 6. **(B)** Elisa analysis of the HMGB1 expression in the heart of sham and SAUNA group. Sham, n = 4; SAUNA, n = 6. **(C)** Elisa analysis of the MIF expression in the heart of sham and SAUNA group. Sham, n = 4; SAUNA, n = 6.

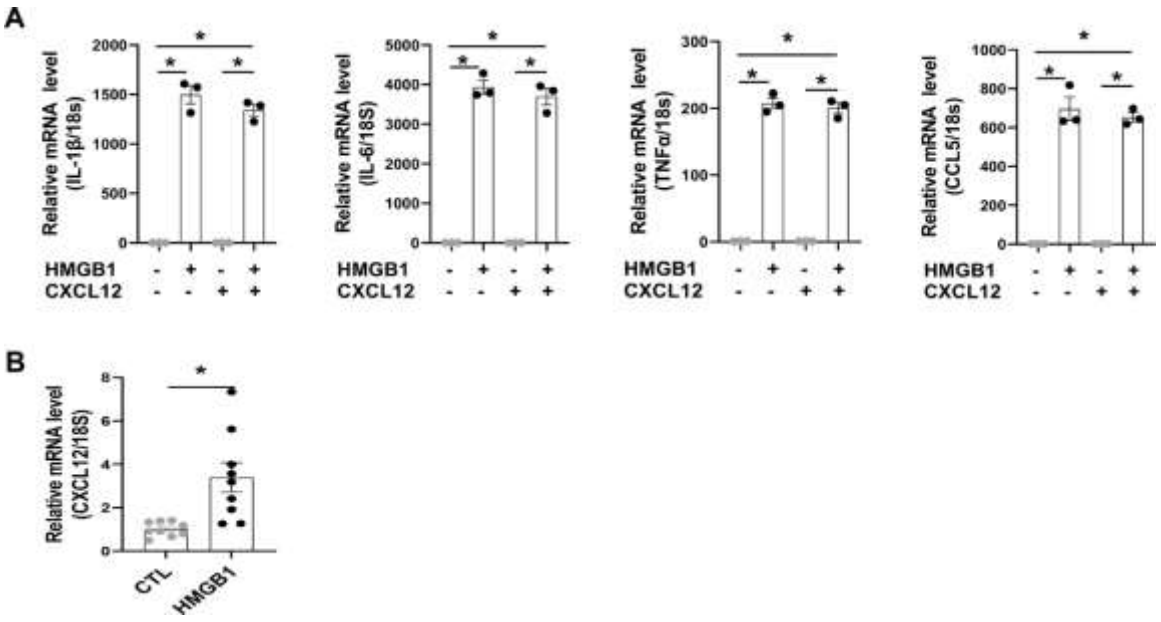


**Figure S9. HMGB1 alone or in complex with CXCL12 triggered the production of pro-inflammatory cytokines in macrophages.**

**(A)** The mRNA levels of IL-1β, IL-6, TNFα, and CCL5 in macrophages with or without CXCL12 after HMGB1 treatment. **(B)** The mRNA levels of CXCL12 in macrophages after HMGB1 treatment. Data were analyzed using unpaired two-tailed student's t-test *, $p<0.05$.

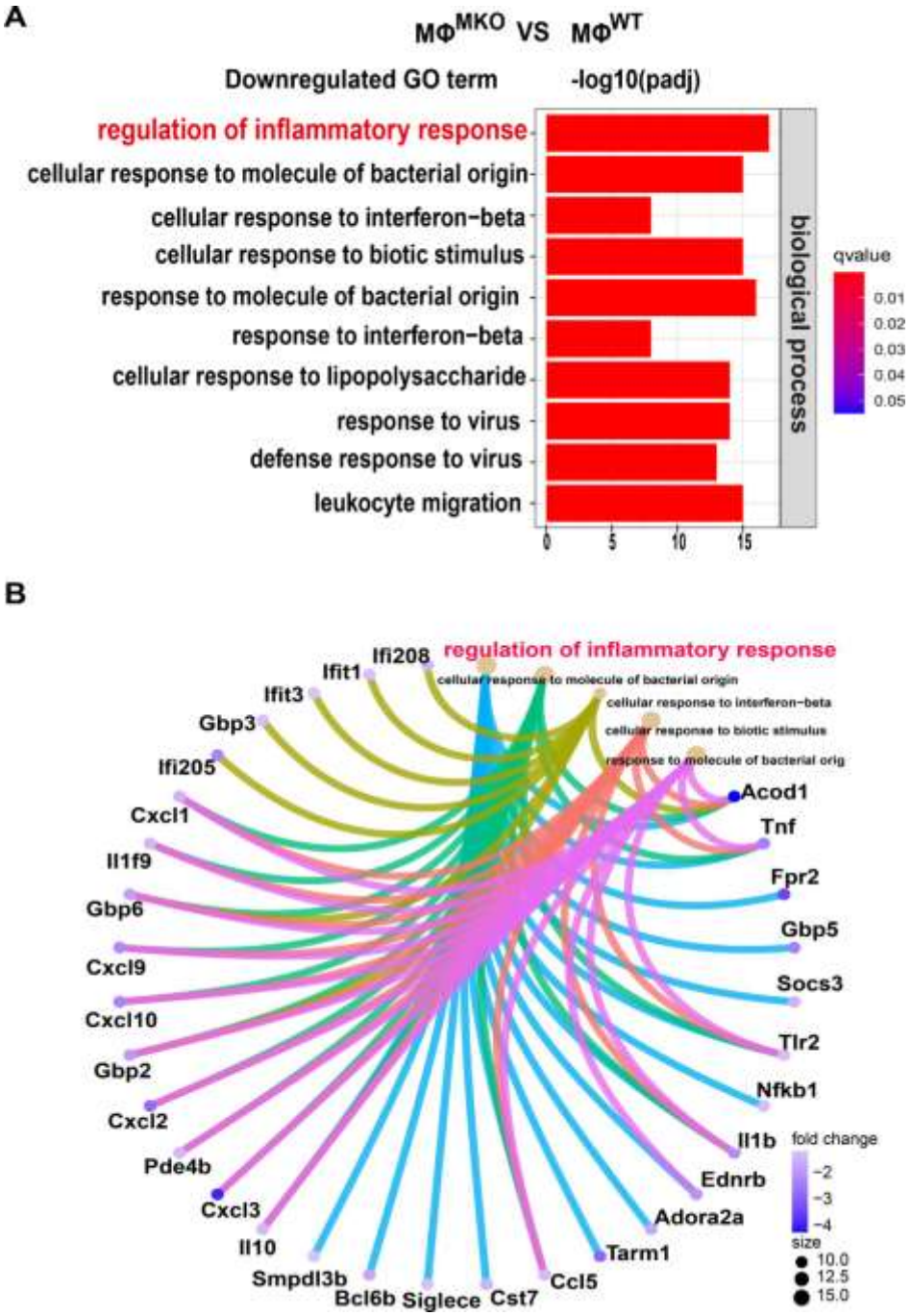


**Figure S10. CXCR4 governs a pro-inflammatory phenotype in macrophages.**

(A) GO enrichment analysis for biological process enriched in downregulated gene terms of $M\Phi^{WT}$ and $M\Phi^{MKO}$ after HMGB1 treatment. Top 10 of enriched GO term are listed. (B) Circos diagram of the indicated genes regulated in enriched biological processes in $M\Phi^{WT}$ and $M\Phi^{MKO}$ after HMGB1 treatment.

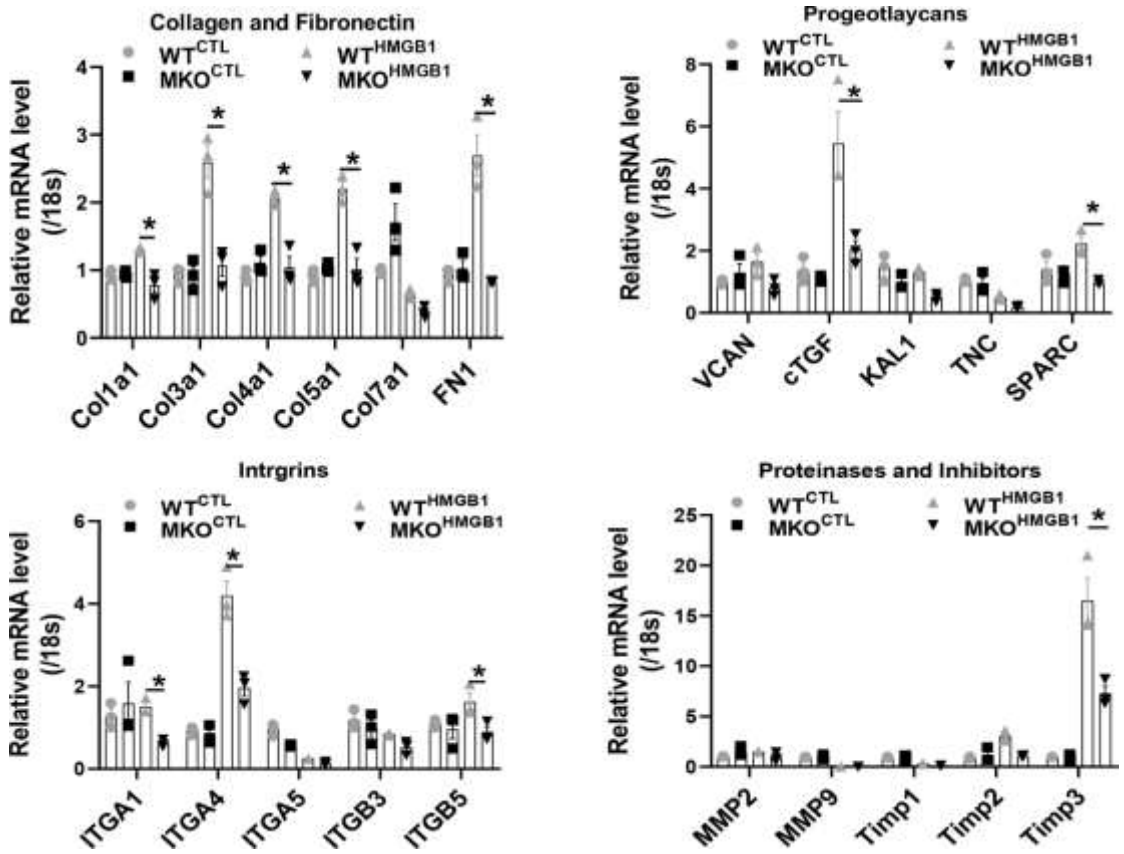


**Figure S11. Deletion of CXCR4 downregulated ECM expression in macrophages**

The mRNA levels of genes coding extracellular matrix proteins (collagen, fibronectin, proteoglycans, integrins, and matrix proteinases and inhibitors) in primary CFs co-cultured with $M\Phi^{WT}$ and $M\Phi^{MKO}$-CM with or without HMGB1 treatment. All data were analyzed using two-way ANOVA with Bonferroni's multiple comparisons test. *, $p<0.05$.

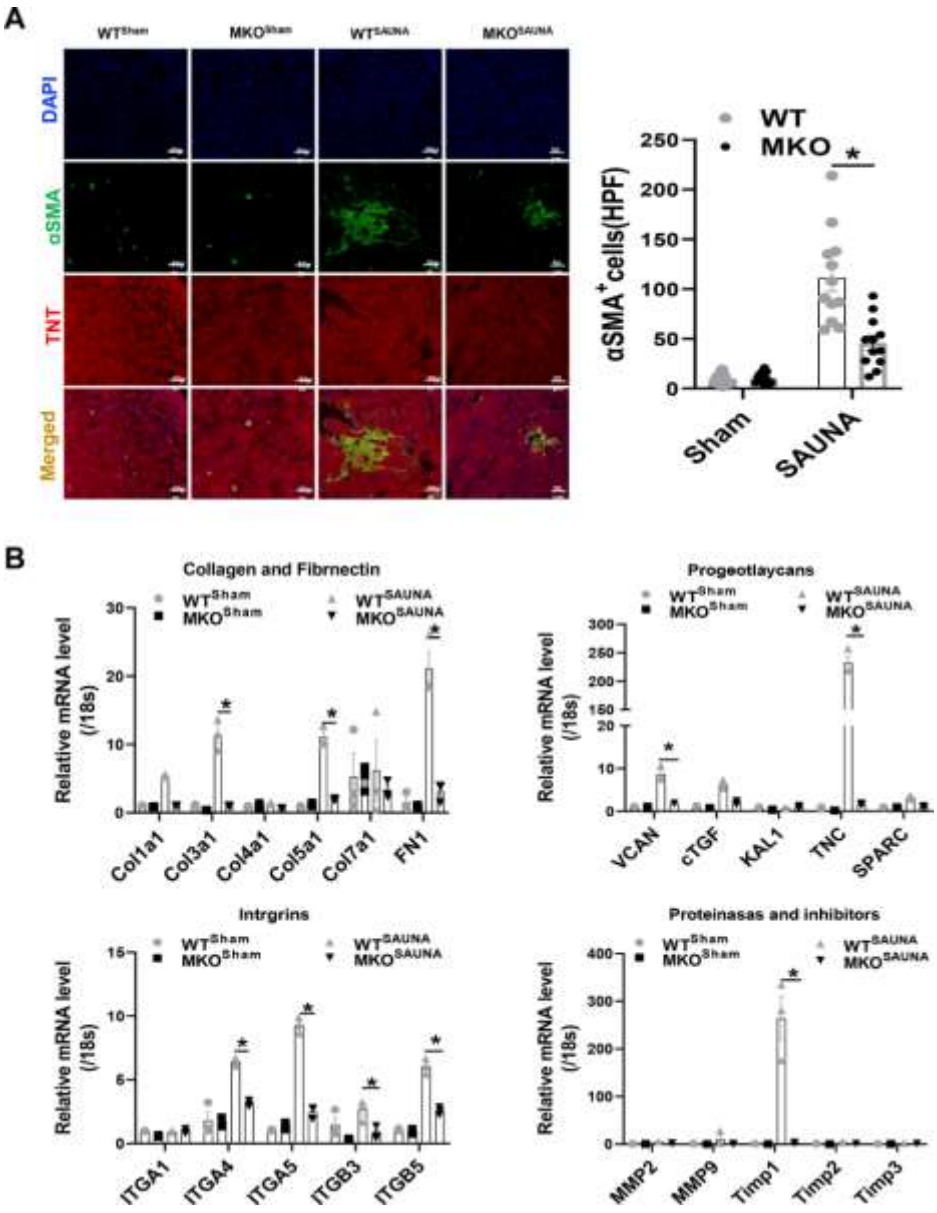


**Figure S12. Deletion of CXCR4 impaired activation of cardiac fibroblasts and downregulated ECM expression in the SAUNA-induced heart..**

**(A)**Immunofluorescence staining of anti-Troponin T (red) and anti-αSMA antibody (green) (DAPI, blue) in the heart of SAUNA induced WT and MKO mice. Scale bars=50 μm. **(B)**The mRNA levels of genes coding extracellular matrix proteins (collagen, fibronectin, proteoglycans, integrins, and matrix proteinases and inhibitors) in the heart of WT and MKO mice after sham or SAUNA operation.All data were analyzed using two-way ANOVA with Bonferroni's multiple comparisons test. *, $p<0.05$.

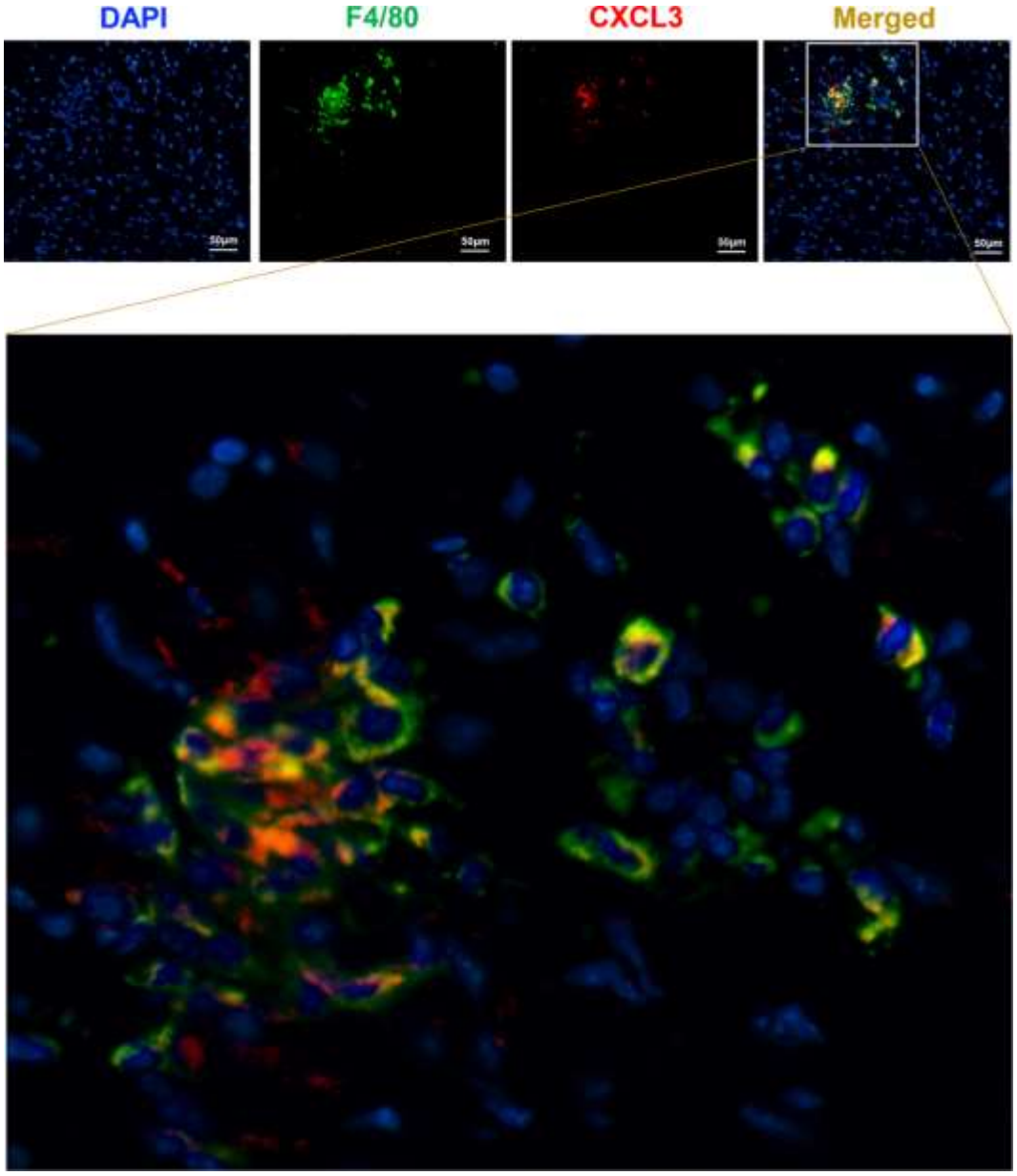


**Figure S13. CXCL3 localized predominately with macrophages in SAUNA-exposed heart**

Immunofluorescence staining of anti-CXCL3 (red) and anti-F4/80 antibody (green) (DAPI, blue) in SAUNA-exposed heart. Scale bars=50 μm.

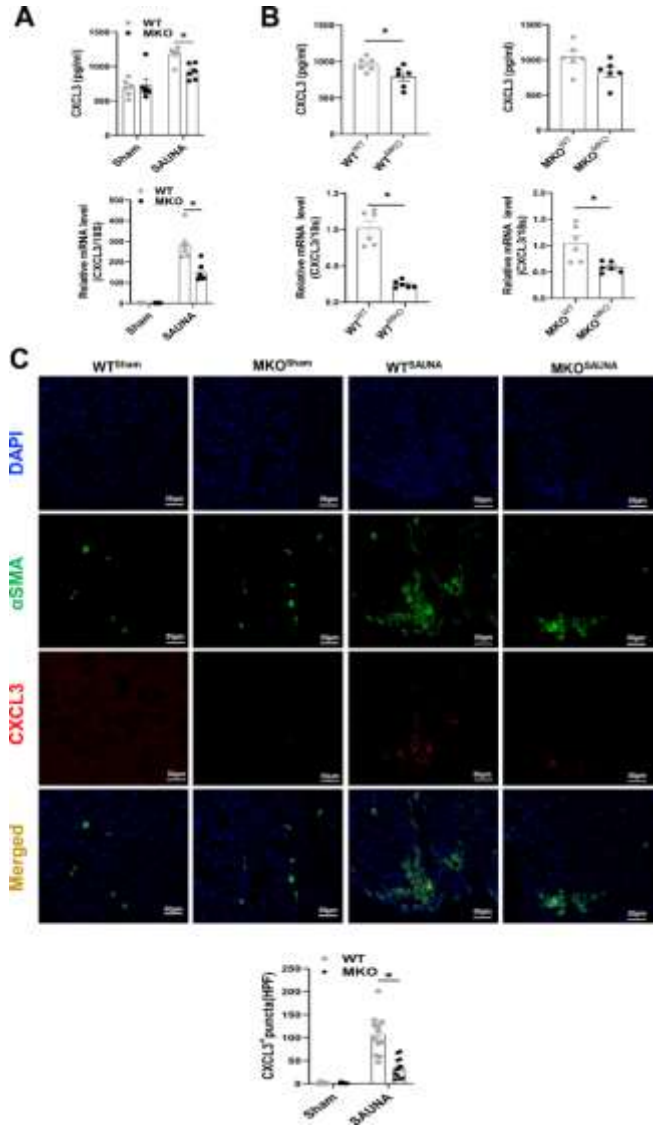


**Figure S14. CXCR4 blockade macrophages suppressed CXCL3 in the SAUNA-induced heart.**

**(A)** Elisa analysis of the CXCL3 expression and mRNA levels of CXCL3 in the heart of WT and MKO mice after sham or SAUNA operation. **(B)** Elisa analysis of the CXCL3 expression and mRNA levels of CXCL3 in SAUNA-induced heart of WT and MKO mice after bone marrow transplantation. **(C)** Immunofluorescence staining of anti-CXCL3 (red) and anti-αSMA antibody (green) (DAPI, blue) in the heart of SAUNA induced WT and MKO mice. Scale bars=50 μm. $WT^{sham}$, n=6; $MKO^{sham}$, n=6; $WT^{SAUNA}$, n=6; and $MKO^{SAUNA}$, n=6. Data were analyzed using unpaired two-tailed student's t-test (**A and B)** and two-way ANOVA with Bonferroni's multiple comparisons test (**C**). *, $p<0.05$.

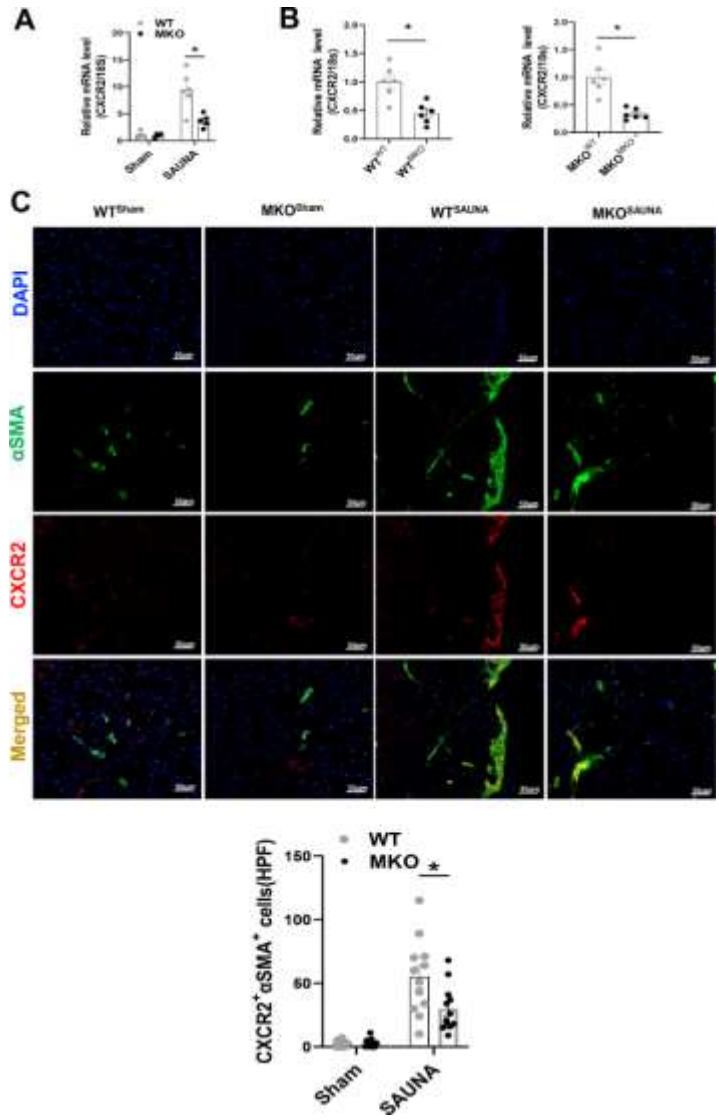


**Figure S15. CXCR4 blockade macrophages suppressed CXCR2 in fibroblasts of SAUNA-induced heart.**

**(A)** The mRNA level of the CXCR2 in the heart of WT and MKO mice after sham or SAUNA operation. **(B)** The mRNA level of the CXCR2 in SAUNA-induced heart of WT and MKO mice after bone marrow transplantation. **(C)** Immunofluorescence staining of anti-CXCR2 (red) and anti-αSMA antibody (green) (DAPI, blue) in the heart of SAUNA induced WT and MKO mice. Scale bars=50 μm. $WT^{sham}$, n=6; $MKO^{sham}$, n=6; $WT^{SAUNA}$, n=6; and $MKO^{SAUNA}$, n=6. Data were analyzed using unpaired two-tailed student's t-test (**A and B)** and two-way ANOVA with Bonferroni's multiple comparisons test (**C**). *, $p<0.05$.

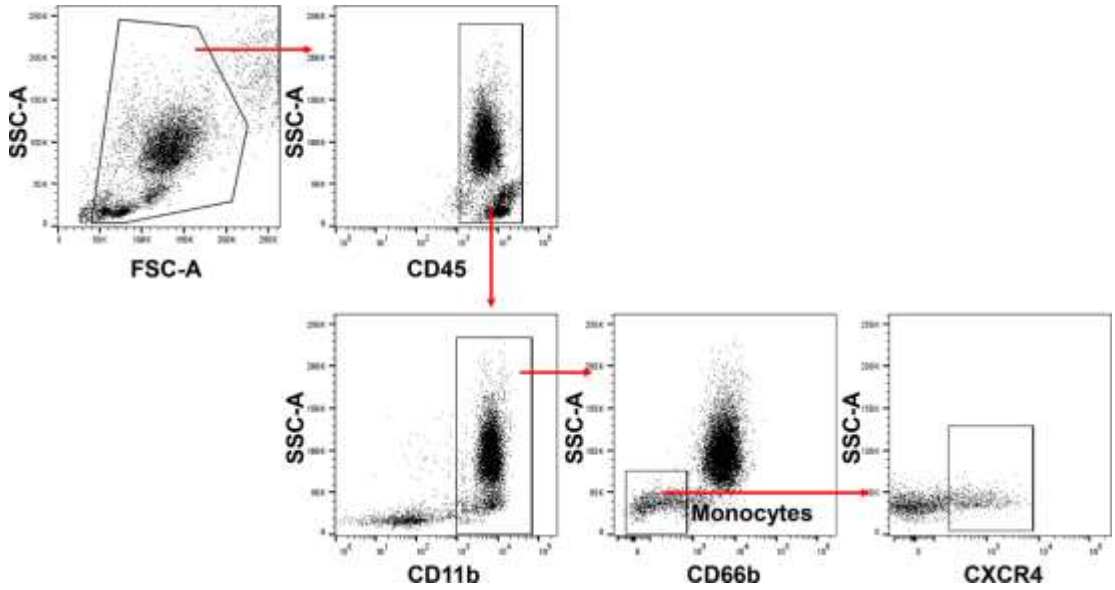


**Figure S16. The immune cells in peripheral blood of HFpEF patients.**

Gating strategy of CD45+ CD11b+ CD66B-CXCR4+ cells in peripheral blood of HFpEF patients.

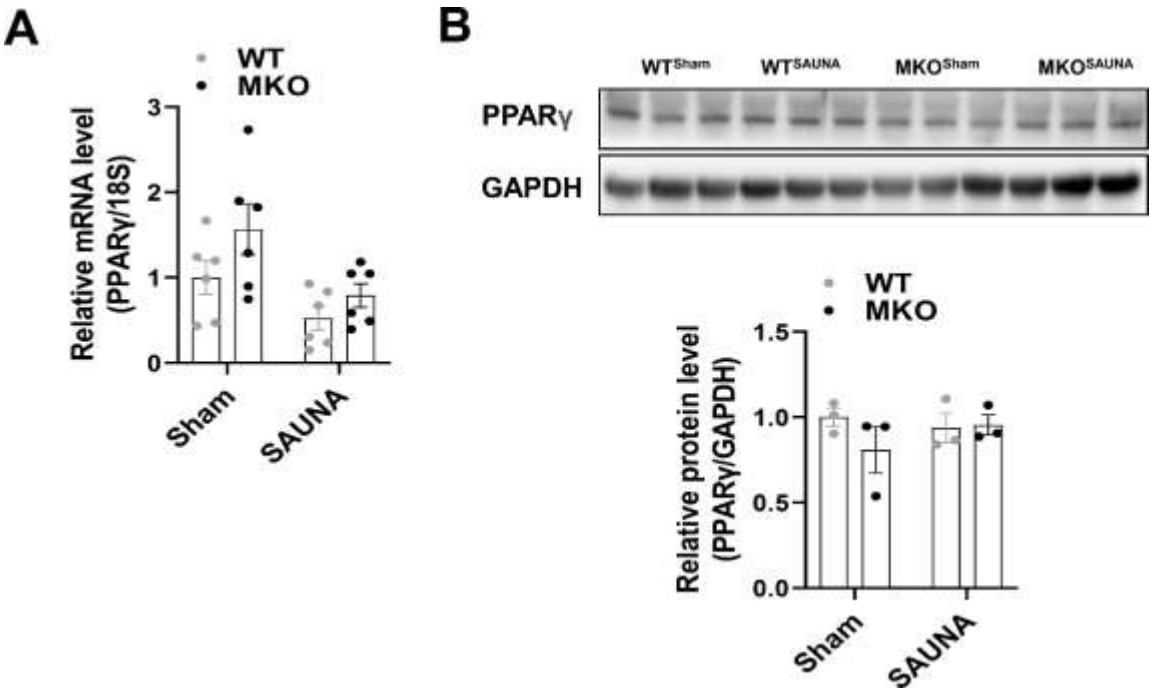


**Figure S17. There was no significant difference in the PPARγ activity between SAUNA treated-WT or MKO hearts.**

(A) The mRNA levels of PPARγ activity in SAUNA treated-WT or MKO hearts. (B) Western blot analysis of CXCR4 and PPARγ in SAUNA treated-WT or MKO hearts. Data were analyzed using unpaired two-tailed student's t-test (**A)** and two-way ANOVA with Bonferroni's multiple comparisons test (**B**).

**Table S1. Name and sequence of primers sets for real-time RT-PCR**

| Gene name | Primer sequence |
|---|---|
| F4/80 | Forward：GTGGAGGCAGTGATGCTCTT |
| | Reverse：TGGAAGCCCATAGCCAAAGG |
| CXCR4 | Forward： TGCAGCAGGTAGCAGTGAAA |
| | Reverse：TGTATATACTCACACTGATCGGTTC |
| CXCL12 | Forward：TGCATCAGTGACGGTAAACCA |
| | Reverse：TTCTTCAGCCGTGCAACAATC |
| 18s | Forward：CCCAGTAAGTGCGGGTCATAA |
| | Reverse：CCGAGGGCCTCACTAAACC |
| IL-1β | Forward: AGGCTCATCTGGGATCCTCT |
| | Reverse: AGGCTCATCTGGGATCCTCT |
| IL-6 | Forward: CCACTTCACAAGTCGGAGGCTTA |
| | Reverse: GCAAGTGCATCATCGTTGTTCATAC |
| TNFα | Forward: ACAAGATGCTGGGACAGTGA |
| | Reverse: ACCTGACCACTCTCCCTTTG |
| CCL5 | Forward：GCTGCTTTGCCTACCTCTCC |
| | Reverse：TCGAGTGACAAACACGACTGC |
| Col1a1 | Forward：GCTCCTCTTAGGGGCCACT |
| | Reverse：CCACGTCTCACCATTGGGG |
| Col3a1 | Forward：CTGTAACATGGAAACTGGGGAAA |
| | Reverse：CCATAGCTGAACTGAAAACCACC |
| Col4a1 | Forward：TCCGGGAGAGATTGGTTTCC |
| | Reverse：CTGGCCTATAAGCCCTGGT |
| Col5a1 | Forward：CTTCGCCGCTACTCCTGTTC |

| | |
|---|---|
| | Reverse：CCCTGAGGGCAAATTGTGAAAA |
| Col7a1 | Forward：GCCCAGAGATAGAGTGACCTG |
| | Reverse：CGCACTTCTCGAAAGTTGCTG |
| FN1 | Forward：ATGTGGACCCCTCCTGATAGT |
| | Reverse：GCCCAGTGATTTCAGCAAAGG |
| VCAN | Forward：TTTTACCCGAGTTACCAGACTCA |
| | Reverse：GGAGTAGTTGTTACATCCGTTGC |
| cTGF | Forward：CGCTGTGATGACGGTGGTTT |
| | Reverse：CCTGGCACCTGTATTCTCCTG |
| KAL1 | Forward：CAGCTAATGAATGGCGTTCTAGG |
| | Reverse：CTTAGGTTGATAACGAGGGCAG |
| TNC | Forward：ACGGCTACCACAGAAGCTG |
| | Reverse：ATGGCTGTTGTTGCTATGGCA |
| SPARC | Forward：GTGGAAATGGGAGAATTTGAGGA |
| | Reverse：CTCACACACCTTGCCATGTTT |
| ITGA1 | Forward：CCTTCCCTCGGATGTGAGTCA |
| | Reverse：AAGTTCTCCCCGTATGGTAAGA |
| ITGA4 | Forward：GATGCTGTTGTTGTACTTCGGG |
| | Reverse：ACCACTGAGGCATTAGAGAGC |
| ITGA5 | Forward：CTTCTCCGTGGAGTTTTACCG |
| | Reverse：GCTGTCAAATTGAATGGTGGTG |
| ITGB3 | Forward：CCACACGAGGCGTGAACTC |
| | Reverse：CTTCAGGTTACATCGGGGTGA |
| ITGB5 | Forward：GCTGCTGTCTGCAAGGAGAA |

| | |
|---|---|
| | Reverse：AAGCAAGGCAAGCGATGGA |
| TIMP1 | Forward：GCAACTCGGACCTGGTCATAA |
| | Reverse：CGGCCCGTGATGAGAAACT |
| TIMP2 | Forward：TCAGAGCCAAAGCAGTGAGC |
| | Reverse：GCCGTGTAGATAAACTCGATGTC |
| TIMP3 | Forward：CTTCTGCAACTCCGACATCGT |
| | Reverse：GGGGCATCTTACTGAAGCCTC |
| ACTA2 | Forward：GTCCCAGACATCAGGGAGTAA |
| | Reverse：TCGGATACTTCAGCGTCAGGA |
| POSTIN | Forward：CCTGCCCTTATATGCTCTGCT |
| | Reverse：AAACATGGTCAATAGGCATCACT |
| DDR | Forward：GCTCTCCAATCCGGCCTAC |
| | Reverse：CGGGCTCCATATAGTCCCCA |
| PDGFRA | Forward：TCCATGCTAGACTCAGAAGTCA |
| | Reverse：TCCCGGTGGACACAATTTTTC |
| TCF21 | Forward：CCCACTAAGAAAAGCCCGCTC |
| | Reverse：CCGTTCTCGTACTTGTCGTTG |
| CXCL3 | Forward：CAGTGCCTGAACACCCTACC |
| | Reverse：GGACTTGCCGCTCTTCAGTA |
| BNP | Forward：GGTGCTGTCCCAGATGATT |
| | Reverse：GCCATTTCCTCCGACTTT |
| ANP | Forward: GCCATTTCCTCCGACTTT |
| | Reverse：TCCAGGTGGTCTAGCAGGTT |
| PPARγ | Forward: GGAAGACCACTCGCATTCCTT |

| | |
|---|---|
| | Reverse：GTAATCAGCAACCATTGGGTCA |
| PPARα | Forward: AGAGCCCCATCTGTCCTCTC |
| | Reverse：ACTGGTAGTCTGCAAAACCAAA |
| PPARβ | Forward: TCCATCGTCAACAAAGACGGG |
| | Reverse：ACTTGGGCTCAATGATGTCAC |
| Lysm-Cre | oIMR3066: CCC AGA AAT GCC AGA TTA CG |
| | oIMR3067：CTT GGG CTG CCA GAA TTT CTC |
| | oIMR3068：TTA CAG TCG GCC AGG CTG AC |
| CXCR4-lxop | 10378: CCA CCC AGG ACA GTG TGA CTC TAA |
| | 10379：GAT GGG ATT TCT GTA TGA GGA TTA GC |

**Table S2. RNA-seq result of the top 18 significant genes in $M\Phi^{WT}$ and $M\Phi^{MKO}$ under HMGB1 stimulation.**

| Gene_id | Gene_name | MKO | WT | log2Fold Change | padj |
|---|---|---|---|---|---|
| ENSMUSG00000095478 | Gm9824 | 180.5134 | 351.3764 | -0.96047 | 0.002101 |
| ENSMUSG00000061983 | Rps12 | 3255.999 | 4651.913 | -0.51488 | 0.002747 |
| ENSMUSG00000029379 | Cxcl3 | 2.424679 | 36.65793 | -3.9104 | 0.032014 |
| ENSMUSG00000021298 | Gpr132 | 232.3973 | 408.1813 | -0.81156 | 0.0391 |
| ENSMUSG00000062169 | Cnih4 | 1103.998 | 1406.751 | -0.34956 | 0.127725 |
| ENSMUSG00000014444 | Piezo1 | 8705.321 | 7426.549 | 0.229215 | 0.199229 |
| ENSMUSG00000021453 | Gadd45g | 2447.341 | 1923.054 | 0.347637 | 0.25559 |
| ENSMUSG00000050071 | Bex1 | 1546.675 | 1080.197 | 0.517379 | 0.25559 |
| ENSMUSG00000041453 | Rpl21 | 864.0283 | 1830.075 | -1.08283 | 0.25559 |
| ENSMUSG00000040253 | Gbp7 | 237.3416 | 414.9638 | -0.80507 | 0.25559 |
| ENSMUSG00000004891 | Nes | 1078.15 | 797.9487 | 0.43364 | 0.25559 |
| ENSMUSG00000027523 | Gnas | 917.1409 | 686.7162 | 0.417587 | 0.25559 |
| ENSMUSG00000032915 | Adgre4 | 123.6392 | 241.3621 | -0.9637 | 0.25559 |
| ENSMUSG00000029066 | Mrpl20 | 743.8891 | 948.4467 | -0.35012 | 0.25559 |
| ENSMUSG00000041992 | Rapgef5 | 174.4833 | 285.0491 | -0.7075 | 0.25559 |
| ENSMUSG00000022048 | Dpysl2 | 482.6345 | 681.1266 | -0.49636 | 0.25559 |
| ENSMUSG00000031604 | Msmo1 | 1726.224 | 2296.406 | -0.41153 | 0.284661 |
| ENSMUSG00000023367 | Tmem176a | 485.2247 | 687.2435 | -0.50149 | 0.284661 |
| ENSMUSG00000025934 | Gsta3 | 46.67984 | 116.6869 | -1.31995 | 0.284661 |
| ENSMUSG00000068396 | Rpl34-ps1 | 286.3335 | 424.6136 | -0.56772 | 0.284661 |

**Table S3. Patient clinical baseline characteristics**

| | **normal individuals (n=13)** | **HFpEF patients (n=23)** | **P valve (*, p<0.05)** |
|---|---|---|---|
| **Age (year)** | 32.31±11.96 | 59.78±9.46 | <0.0001* |
| **Gender（male/female）** | 6：7 | 15：8 | 0.3101 |
| **Comorbidity** | | | |
| Hypertension (n, %) | 0 (0%) | 23，100% | <0.0001* |
| Diabetes (n, %) | 0 (0%) | 0 (0%) | |
| Coronary heart disease (n, %) | 0 (0%) | 0 (0%) | |
| Peripheral arterial disease (n, %) | 0 (0%) | 0 (0%) | |
| **Doppler echocardiography** | | | |
| LVID. d (mm) | 4.40±0.32 | 4.81±0.64 | 0.0444* |
| LA (mm) | 3.00±0.30 | 3.62±0.48 | 0.0002* |
| LVEF (%) | 65.41±4.31 | 62.35±9.78 | 0.3057 |
| E/A | 1.60±0.35 | 0.76±0.15 | <0.0001* |
| E/E’ | 7.01±1.82 | 12.32±3.73 | <0.0001* |
| **Pro-BNP (pg/ml)** | 29.86±12.68 | 1435.76±1650.47 | 0.0421* |
| **Blood routine** | | | |
| Neutrophil absolute value(10^9/L) | 3.50±1.04 | 3.84±1.31 | 0.2911 |
| Lymphocyte absolute value(10^9/L) | 1.56±0.63 | 1.96±1.17 | 0.2129 |
| Monocyte absolute value(10^9/L) | 0.39±0.10 | 0.58±0.23 | 0.0093* |

**Table S4. The antibodies and cytokines used in the study**

| Name Company Item | Name Company Item No |
|---|---|
| ***For western blot:*** | |
| GAPDH | 1:3000, Cell Signaling Technology, Danvers, MA, USA, 5174 |
| aSMA | 1:1000, Cell Signaling Technology, Danvers, MA, USA, 48938 |
| Fibronectin | 1:1000, Abcam, Cambridge, MA, USA, ab2413 |
| PPARγ | 1:1000, Cell Signaling Technology, Danvers, MA, USA, 2435 |
| CXCR2 | 1:1000, Abcam, Cambridge, MA, USA, ab65968 |
| NF-κB p65 | 1:1000, 8242, Cell Signaling Technology, Danvers, MA, USA |
| pNF-κB p65 | 1:1000, 3033, Cell Signaling Technology, Danvers, MA, USA |
| MEK | 1:1000, ET1602-3, Huabio, China |
| pMEK | 1:1000, ET1612-40, Huabio, China |
| Erk | 1:1000, RT1484, Huabio, China |
| pErk | 1:1000, ET1603-22, Huabio, China |
| ***For immunofluorescence:*** | |
| F4/80 | 1:300, Abcam, Cambridge, MA, USA, 6640 |
| aSMA | 1:300, Cell Signaling Technology, Danvers, MA, USA, 48938 |
| CXCL3 | 1:1000, Abcam, Cambridge, MA, USA, ab220431 |
| CXCR4 | 1:1000, Abcam, Cambridge, MA, USA, ab181020 |
| ***For flow cytometry*** | |
| CD45 | 1:1000, BD Biosciences, San Jose, CA, USA, 557659, 555482 |
| CD11b | 1:1000, BD Biosciences, San Jose, CA, USA, 553312, 557657 |
| CD66B | 1:1000, BioLegend, San Diego, CA, USA, 305118 |
| F4/80 | 1:1000, BD Biosciences, San Jose, CA, USA,123108, 123114 |
| Ly6G | 1:1000, BD Biosciences, San Jose, CA, USA,560602 |
| CXCR4 | 1:1000, BD Biosciences, San Jose, CA, USA,551510, 551966 |
| CCR2 | 1:1000, BD Biosciences, San Jose, CA, USA, 150608 |
| ***Others*** | |

| | |
|---|---|
| Aldosterone | 0.3ug/h, Sigma-Aldrich Co., St. Louis, Missouri, 706035 |
| Recombinant Mouse CXCL3 | 10ng/ml, MCE, Shanghai, China, HY-P7153 |
| Recombinant Mouse HMGB1 | 1ug/ml, Abcam, Cambridge, MA, USA, ab181949 |
| Recombinant Mouse M-CSF | 10ng/ml, PEROTECH, New Jersey, USA, 315-02 |
| Anti-CXCL3 neutralizing antibody | 0.5ug/ml, R&D, Minneapolis, MN, USA, AF5568 |
| GW9662 | 10nM, MCE, Shanghai, China, HY-16578 |
| GW1929 | 10nM, MCE, Shanghai, China, HY-15655 |
| SB225002 | 10nM, MCE, Shanghai, China, HY-16711 |
| PD98059 | 10nM, MCE, Shanghai, China, HY-12028 |