

Supplementary Materials

Supplementary Fig. Legends

Fig. S1: Gene set enrichment analysis (GSEA) of transcriptional profiles in MICFs from *in vivo* and *in vitro* samples

A: Heat map analysis of cardiomyocyte and cardiac fibroblast related genes of *in vivo* and *in vitro* samples. **B** and **C:** GSEA analysis using hallmark gene sets for the differences pathways in *in vivo* samples versus *in vitro* samples at 1 week (B) and 2 weeks (C). **D** and **E:** Scatter plot of differentially expressed genes in MICFs from *in vivo* and *in vitro* samples at 1 week (D) or 2 weeks (E). FPKM, fragments per kilobase of transcript per million mapped reads.

Fig. S2: Silencing *Ifnar1* or *Ifnar2* enhances cardiac reprogramming efficiency in MICFs

A: qPCR analysis validated knockdown efficiency of shRNAs targeting *Ifnar1* or *Ifnar2*. n=3. **B** and **C:** Representative IF images for α -actinin (red) on MICFs infected with MGT or MGTMyoS and sh*Ifnar1/2* or shNT (B), with quantification of the absolute number (C). n=4. **D** and **E:** Representative whole-well images for α -actinin (green) and cTnI (red) on MICFs infected with MGT and sh*Ifnar1/2* or shNT after 4 weeks. **F:** Representative flow plots for α -actinin⁺ and cTnI⁺ cells reprogrammed from MICFs 28 days after lentiviruses infection.

All data are presented as the means \pm SD. The one-way ANOVA (A) or Two-way ANOVA (C) was used to determine the significance of differences between two groups. NS, not significant, *p < 0.05, **p < 0.01, *** p < 0.001, Scale bars, 100 μ m.

Fig. S3: Efficient cardiac reprogramming by silencing *Ifnar2* *in vivo*

A and **B:** Representative IF images for MYL2 (red), cTnI (grey), and GFP (green) after transplantation of MICFs expressing MGT and sh*Ifnar2* into MI hearts 4 weeks (A), with quantification of the percentage in (B). n=4. **C:** Schematic diagram showing the genetic fate mapping method to trace the lineage of resident MICFs using *Colla2*^{CreER}/R26-tdTomato mice. **D** and **E:** Representative IF images for tdTomato (red) and GFP (green) after injection of LV-GFP & LV-rtTA into *Colla2*^{CreER}/R26-tdTomato MI hearts 4 weeks (E), with quantification of the percentage GFP to tdTomato in (D). n=4. **F** and **G:** Representative IF images for α -actinin (green) and cTnI (red) on MICFs infected with MGT-HA and sh*Ifnar2*/NT-rtTA or sh*Ifnar2*/NT + rtTA (F), with quantification of the absolute number in (G). n=3. **H:** Representative IF images for tdTomato (red) and α -actinin (green) in **adjacent transverse** sections after injection of LV-MGT-HA and LV-shRNA-rtTA into *Colla2*^{CreER}/R26-tdTomato MI hearts for 4 weeks. **I:** Z stack images for HA tdTomato and cTnI (left panel) or tdTomato and α -actinin (right panel) positive iCMs. n=4. **J:** Schematic diagram shows the genetic fate mapping method to trace the lineage of resident CFs and fusion analyses using *Tcf21*^{CreER}/mTmG mice. mGFP+ cardiomyocytes indicate cardiac reprogramming, whereas mGFP+ mtdTomato+ cardiomyocytes indicate cell fusion between CFs and cardiomyocytes. **K** and **L:** Representative IF images on mTdtomato (red), mGFP (green) and cTnI (grey) after injection of LV-MGT-HA and LV-shRNA-rtTA into MI hearts after 4 weeks (K), with

quantification of the percentage in (L). n=4. **M** and **N**: Representative IF images on mTdtomato (red), mGFP (green) and α -actinin (grey) in **adjacent transverse** sections after injection of LV-MGT-HA and LV-shRNA-rtTA into MI hearts after 4 weeks (M), with quantification of the CM markers percentage (N). n=4. **O**: Representative IF images on iCMs exhibiting well sarcomeric organization after injection of LV-MGT-HA and LV-sh*Ifnar2*-rtTA into MI hearts after 4 weeks. mTdtomato (red), mGFP (green) and α -actinin (grey).

All data are presented as the means \pm SD. The unpaired t-test or Two-way ANOVA (G) was used to determine the significance of differences between two groups. NS, not significant, *p < 0.05, **p < 0.01, *** p < 0.001, Scale bars, 100 μ m.

Fig. S4: Segmental analysis of indicated mice strains

A: Quantification of the longitudinal peak strains as from base to middle of anterior and posterior for the mice injected with GFP+sh*NT*, GFP+sh*Ifnar2*, MGT+sh*NT* or MGT+sh*Ifnar2* lentiviruses at day 28 (A) after MI, results were presented as violin plots. n=14-15.

All data are presented as the means \pm SD. The Two-way ANOVA was used to determine the significance of differences between two groups. NS, not significant, *p < 0.05, **p < 0.01, *** p < 0.001.

Fig. S5: Macrophages secrete IFN- β in the injured heart

A: Sc-RNA seq analysis of *Ifnb1* and *Ifna2* expression in injured hearts. **B**: A table summarizing the expression of type 1 interferons in the injured heart. **C**: RT-qPCR analyses for *Ifnb1* expression in hearts at 3, 5-, 14-, 21- and 28-days post MI. n=3. **D**: Public Sc-RNA seq analysis of *Ifnb1* expression for each cell types. CM: Cardiomyocytes, CF: Cardiac fibroblast; EC: Endothelial cells; Mp: Macrophage; GN: granulocyte; T: T cell. **E** and **F**: Representative IF images for CD31, CD68, CD8, Vimentini and cTnI on MICFs (E), with the quantification of the percentage in (F). n=3. **G**: Representative IF images for CD68 on MICFs with depleting macrophages or not (left panel), with the quantification of the percentage in (right panel) n=3. **H**: Representative IF images for cTnI (red) and α -actinin (green) on MGT+sh*Ifnar2/NT* transduced MICFs with depleting macrophages or not (left panel), with the quantification of the percentage in (right panel). n=4.

All data are presented as the means \pm SD. The unpaired t-test (G) or Two-way ANOVA (H) was used to determine the significance of differences between two groups. NS, not significant, *p < 0.05, **p < 0.01, *** p < 0.001, Scale bars, 100 μ m.

Fig. S6: Macrophages secrete IFN- β suppresses cardiac reprogramming

A: FACS identification of CD11b⁺ F4/80⁺ BMDM cells (left panel) and bright filed image of BMDM (right panel). **B**: Culture medium IFN- β concentration in WT or *Ifnb1*-KO BMDMs with the treatment of dsDNA for 24h. **C** and **D**: Representative IF images for cTnI (red) and α -actinin (green) on MGT+sh*Ifnar2/NT* transduced purified MICFs treated with KO-conditioned medium, conditioned medium or basal medium for 4 weeks (D),

with the quantification of the absolute number (C). n=4. **E**: Representative IF images for α -actinin (red), cTnI (grey), and GFP (green) on transplantation of MGT-transduced MICFs treated with CL2MDP into MI hearts 4 weeks, with CL2MDP injected time point at MI-1, MI-3. Red arrow indicates α -actinin⁺cTnI⁺ iCMs. **F**: Quantification of α -actinin⁺ or cTnI⁺ iCMs. n=4. **G**: Survival rate of mice injected with PBS or CL2MDP at MI D1, 3, 5. n=6. **H** and **I**:

Representative IF images for cTnI (red) and α -actinin (green) on MICFs infected with MGT and treated with IFNAR-neutralizing antibody or *shIfnar2* (H), with quantification of the absolute number and the time window of IFNAR-neutralizing antibody (I). n=3.

All data are presented as the means \pm SD. The Two-way ANOVA (C), One-way ANOVA (F, I) or unpaired t-test (B) was used to determine the significance of differences between two groups. NS, not significant, *p < 0.05, **p < 0.01, *** p < 0.001, Scale bars, 100 μ m.

Fig. S7: Efficient cardiac reprogramming with KD *Ifnar1/2* was not due to enhanced iCM proliferation.

A: Cell numbers difference between *shNT* and *shIfnar1/2*-infected MICFs after MGT transduction. Cell numbers were counted by nuclear staining at 5- 7- and 14- days after induction. n = 3. **B**: mRNA expression of proliferation-related marker genes in *shNT* or *shIfnar1/2* group in MICFs at 5-,10-,15- days after MGT transduction. n = 3. **C**: Representative IF images for Ki-67 (green) and cTnI (red) on MICFs infected with MGT and *shIfnar1/2* or *shNT* at day5, 10 and 15 after transduction. n=3.**D**: Schematic diagram of EdU treatment during cardiac reprogramming (upper panel), representative IF images for Edu (green) and cTnI (red) on MICFs infected with MGT and *shIfnar1/2* or *shNT* at 4 weeks after transduction (lower panel). n=3. **E**: Representative IF images for MGT-HA (red) on MICFs infected with MGT-HA and *shIfnar2* or *shNT* at day7 (left panel), with the quantification of percentage in (right panel). n=3. **F**: Representative IF images for STAT1-GFP (green), p-STAT1(grey) and GATA4 (red) on purified MICFs with the treatment of 100 IU/ml IFN- β for 1 hour. **G**: Quantification of the percentage of nuclear STAT1-GFP⁺ & p-STAT1⁺ cells/STAT1-GFP⁺ cells. n=8. **H**: Density plots showing signals of Cut-tag at GATA4 binding sites between control, STAT-i and *shIfnar2* group. **I**: CUT&Tag peak heatmap showing GATA4 binding loci enriched in control group but down-regulated with the treatment of *shIfnar2* or STAT-i. **J**: TRANSFAC and JASPAR PWMs analysis of the regions that enriched in infected MICFs with the treatment of *shNT*+DMSO compared to *shIfnar2* group (left panel) or STAT-i group (right panel). **K**: GSEA enrichment plots show that STAT1 targets are enriched in control group but down-regulated in *shIfnar2* or STAT1-I group. **L**: IGV tracks showing GATA4 CUT&Tag at *Oas2* and *Oas3* gene locus between control, STAT-i and *shIfnar2* group. **M**: Searching for Motif of STAT1::STAT2 in the control group.

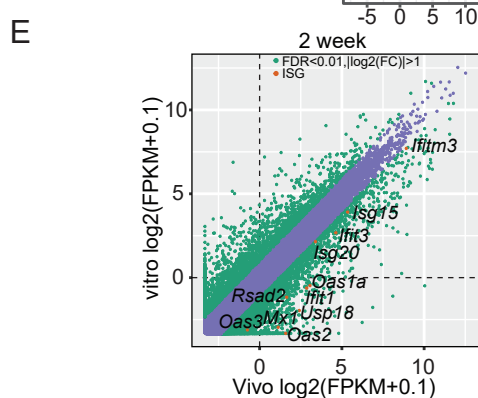
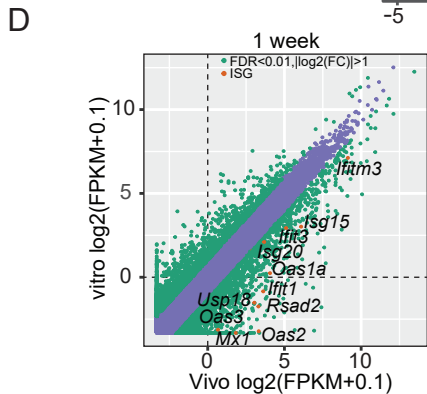
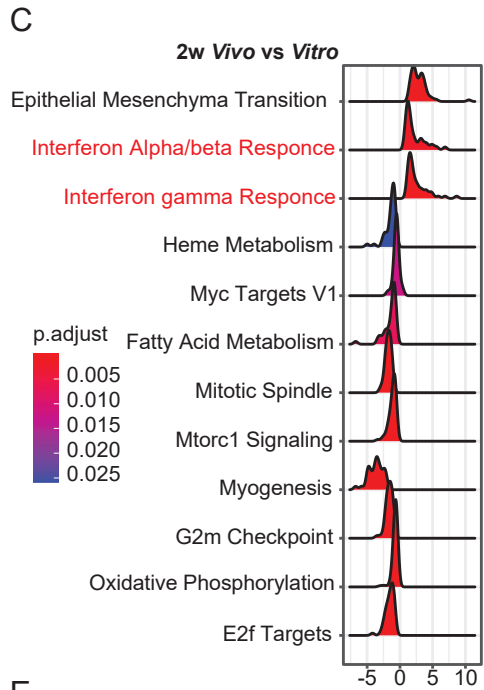
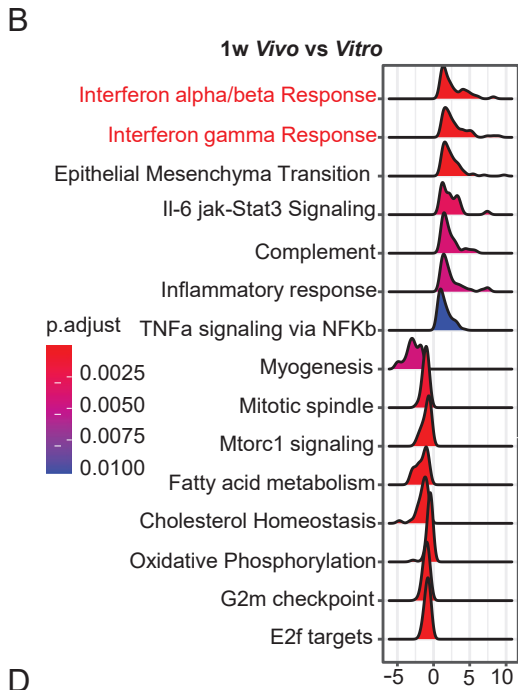
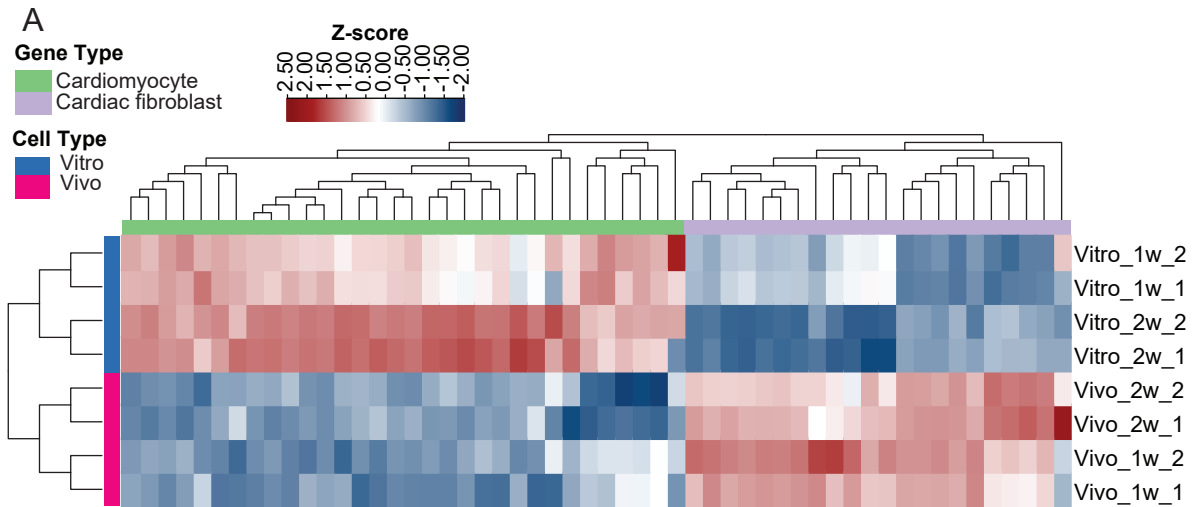
All data are presented as the means \pm SD. The One-way ANOVA (A,B) or unpaired t-test (E) was used to determine the significance of differences between two groups. NS, not significant, *p < 0.05, **p < 0.01, *** p < 0.001, Scale bars, 100 μ m (F=10 μ m).

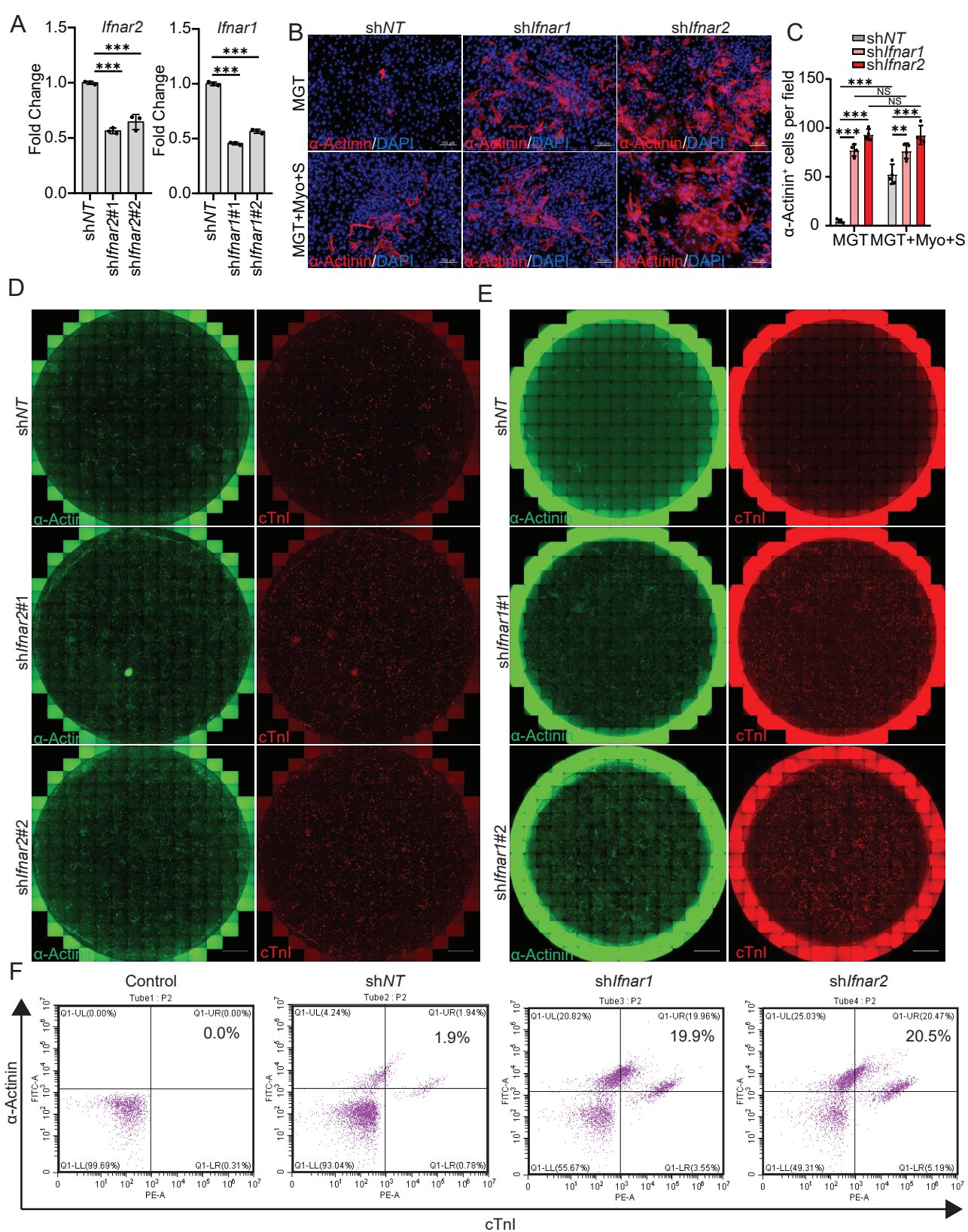
Fig. S8: The expression of *Ifnb1* after silencing of *Ifnar2* in MI hearts

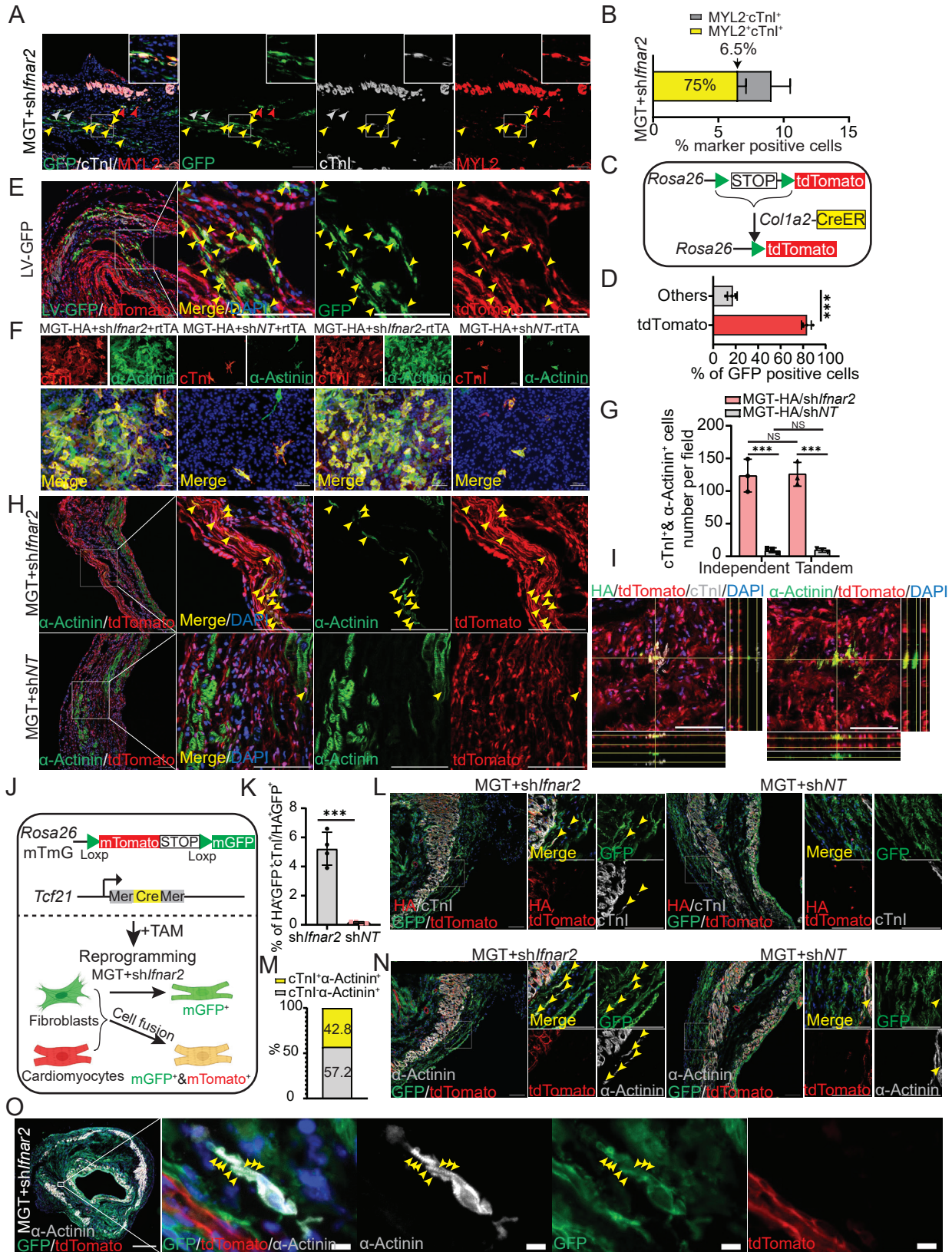
A: qPCR analysis validated knockdown efficiency of shRNAs targeting *Ccl2/7/12* compared to *shNT*. n=3. **B**: Schematic diagram of dual luciferase experiments. **C**: STAT1

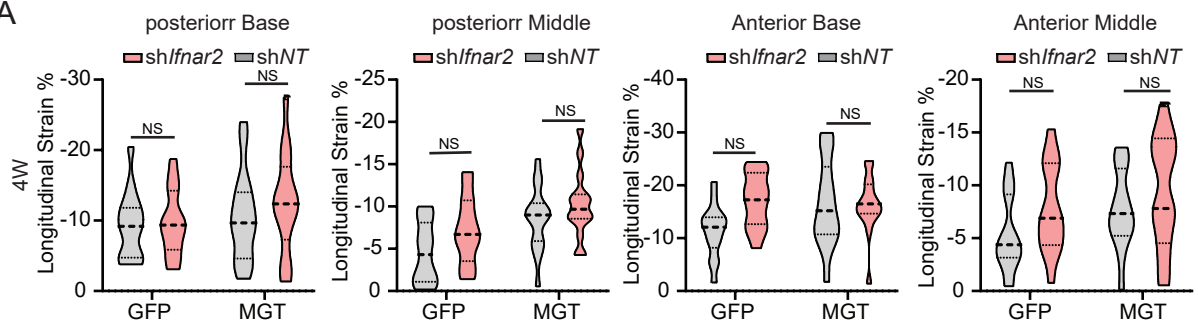
binding motifs from JASPAR database. **D**: Experimental workflow of RNA extraction from the isolated tissue of infarcted area (left panel) and LV-sh*Ifnar2*-mScarlet-H2B expression in the C56BL/6 mice infarct hearts (right panel). **E**: qPCR analysis of the *Ifnb1* expression from the infarcted area injected with LV- sh*Ifnar2*-mScarlet-H2B or sh*NT*-mScarlet-H2B at 7-, 14- days post MI. n=3. **F**: Representative IF images for CD68 (green) and CD3 (red) on *in vivo* knockdown experiment. n=3. **G**: Quantification of CD68 macrophages and CD3 T cells from *in vivo* knockdown experiment. **H**: qPCR analysis of the *Ifnb1* expression from the infarcted area administration of TMM/vehicle at 7- days post MI or sham.

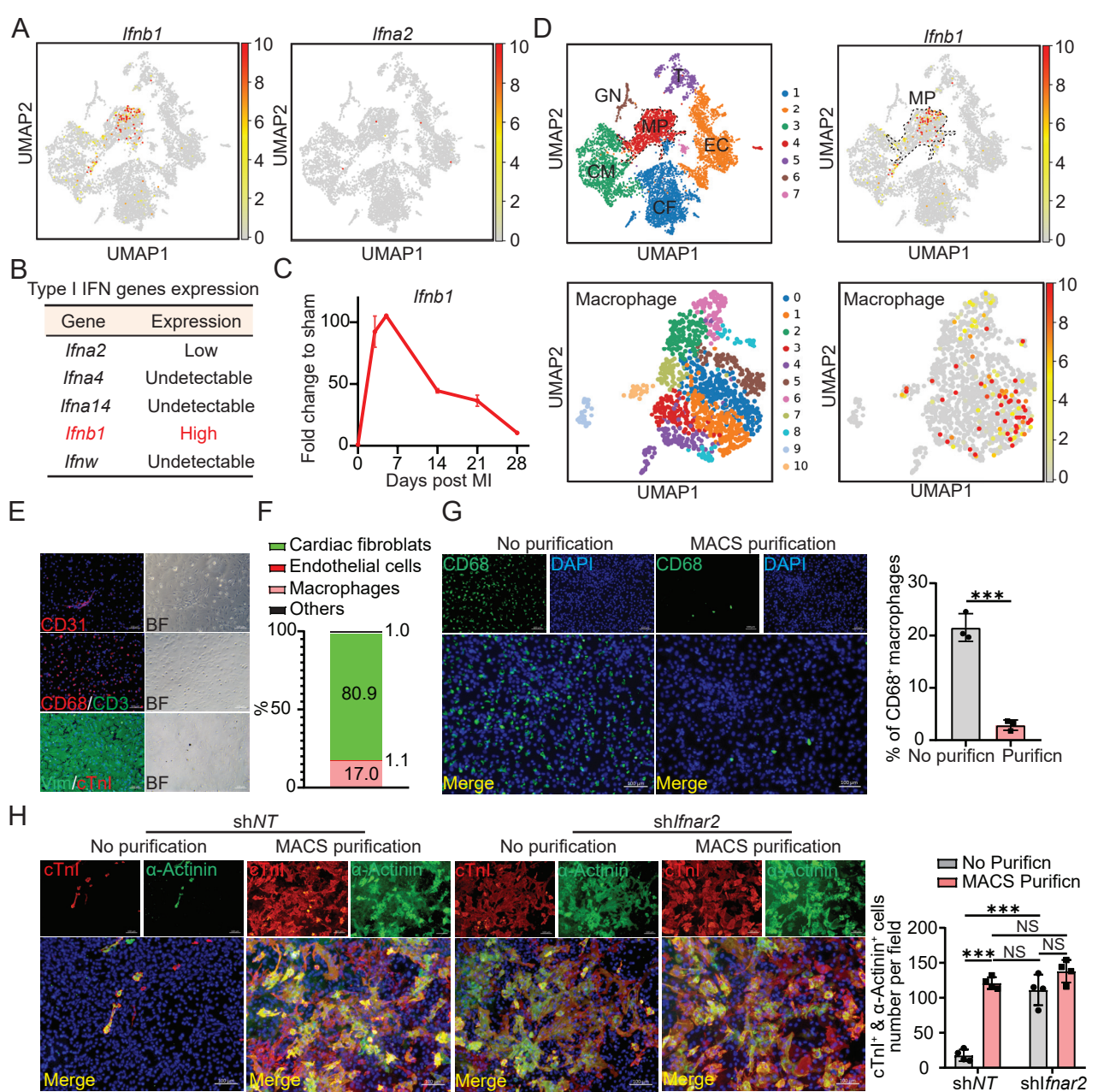
All data are presented as the means \pm SD. The unpaired t-test (A, E) or One-way ANOVA (G, H) was used to determine the significance of differences between two groups. NS, not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, Scale bars, 100 μ m (D:1000 μ m).

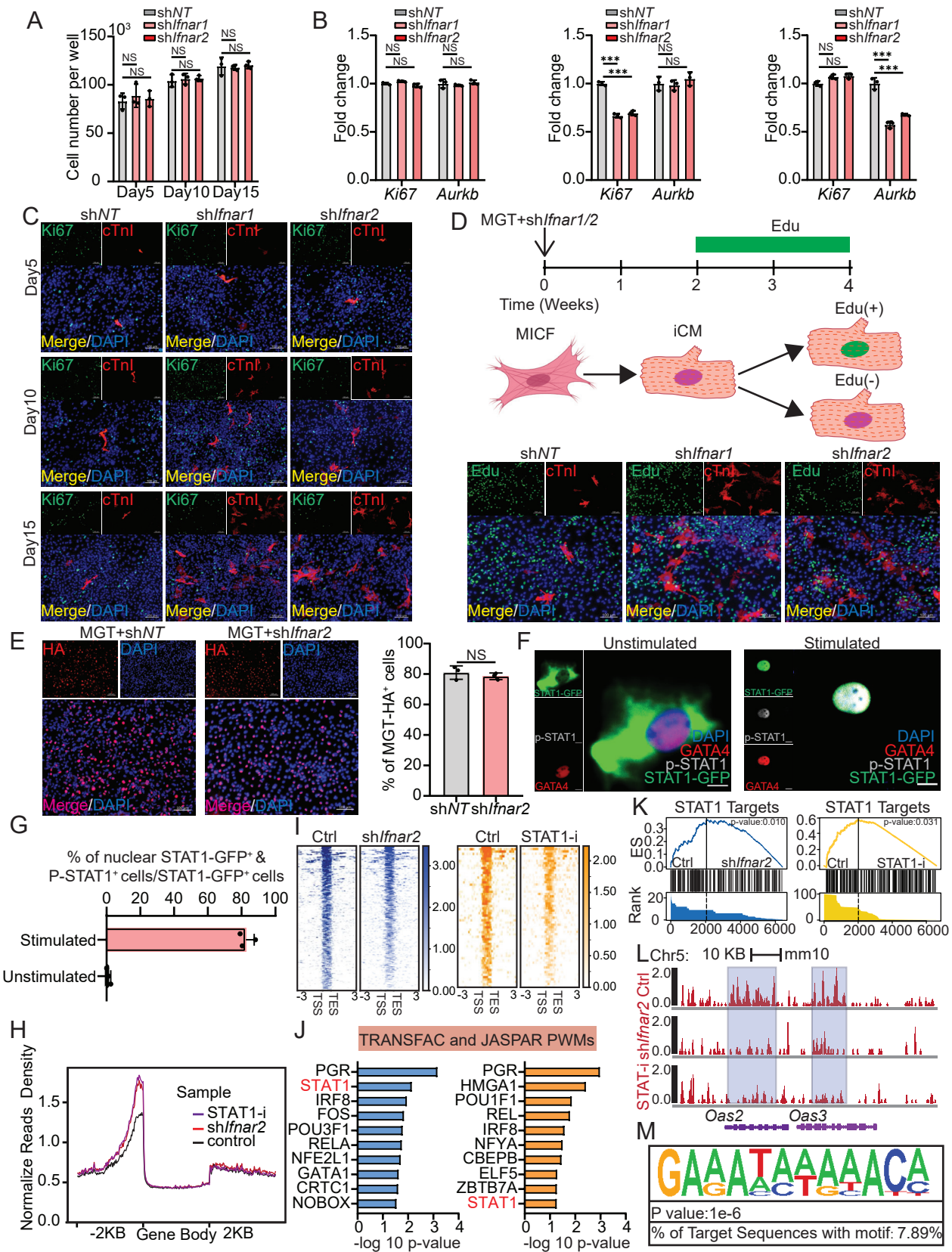






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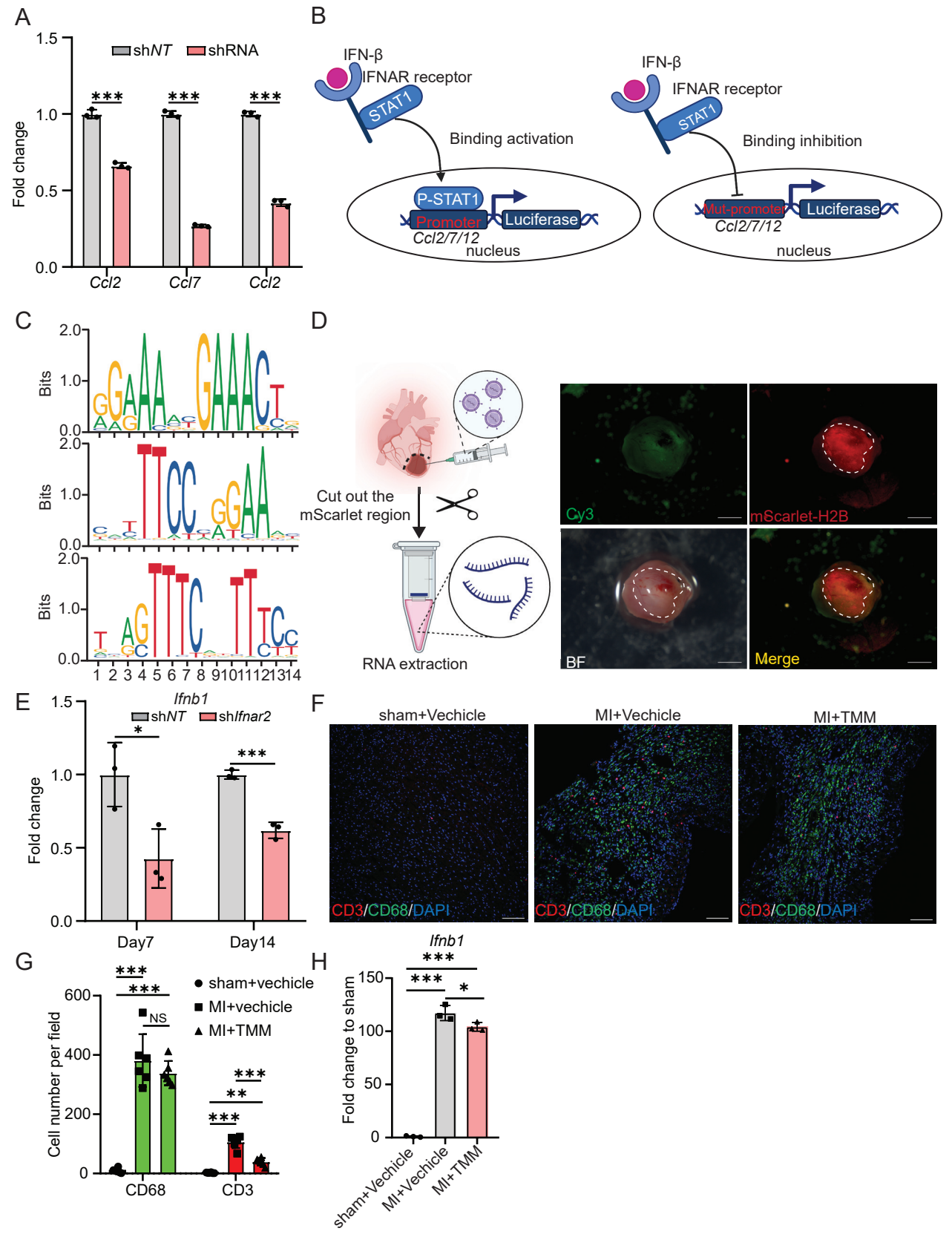


Table S1. Related to STAR Methods.

Sequence of shRNA and sgRNA

| Gene | Forward Sequence& Reverse Sequence | Target sequence |
|----------------------------|--|-------------------------------|
| <i>Ifnar1</i> -shRN A-1 | CCGGGGGAAATCACACATCCTTTCTCGAGA AAGGATGTGTGATTTCCCTTTTTG AATTCAAAAAGGGAAATCACACATCCTTTC TCGAGAAAGGATGTGTGATTTCCC | GGGAAATCA CACATCCTTT |
| <i>Ifnar1</i> -shRN A-2 | CCGGGCAAAGACATGTAAGCAAACCTCGAGT TTGCTTACATGTCTTTGCTTTTTG AATTCAAAAAGCAAAGACATGTAAGCAAAC TCGAGTTTGCTTACATGTCTTTGC | GCAAAGACA TGTAAGCAA A |
| <i>Ifnar2</i> -shRN A-1 | CCGGGTCTTGAACTTCCGCCACTTTCTCGAG AAAGTGGCGGAAGTTCAAGACTTTTTG AATTCAAAAAGTCTTGAACTTCCGCCACTTT CTCGAGAAAGTGGCGGAAGTTCAAGAC | GTCTTGAACT TCCGCCACTT T |
| <i>Ifnar2</i> -shRN A-2 | CCGGGGGAGAGAAAGGGAAAGAACTCGAG TTCTTTCCCTTTCTCTCCCTTTTTG AATTCAAAAAGGGAGAGAAAGGGAAAGAA CTCGAGTTCTTTCCCTTTCTCTCCC | GGGAGAGAA AGGGAAAGA A |
| <i>Ccl2</i> -shRNA | CCGGGCAAGATGATCCCAATGAGTACTCGA GTACTCATTGGGATCATCTTGCTTTTTG | GCAAGATGA TCCCAATGA GTA |
| <i>Ccl7</i> -shRNA | CCGGCGAGGAGGCTATAGCATACTTCTCGA GAAGTATGCTATAGCCTCCTCGTTTTG | CGAGGAGGC TATAGCATA CTT |
| <i>Ccl12</i> -shRNA | CCGGGCTGTGATCTTCAGGACCATACTCGA GTATGGTCCTGAAGATCACAGCTTTTTG | GCTGTGATCT TCAGGACCA TA |

| | | |
|-----------|--|------------------------------|
| shNT | CCGGCCTAAGGTAAAGTCGCCCTCGCTCGA GCGAGGGGCGACTTAACCTTAGGTTTTTG AATTCAAAAACCTAAGGTAAAGTCGCCCTC GCTCGAGCGAGGGGCGACTTAACCTTAGG | CCTAAGGTT AAGTCGCC TCG |
| sgIfnb1-1 | ATCTTGAAGTCCGCCCTGTAGG | ATCTTGAAGT CCGCCCTGT |
| sgIfnb1-2 | GAAGTACAACAGCTACGCCTGG | GAAGTACAA CAGCTACGC C |

Table S2. Sequences for Primer. Related to STAR Methods

1. Primer sequence of qPCR

| Gene | Forward | Reverse |
|---------------|-------------------------|-------------------------|
| <i>Myh6</i> | GCCCAGTACCTCCGAAAGTC | GCCTTAACATACTCCTCCTTGTC |
| <i>Actc1</i> | CTGGATTCTGGCGATGGTGTA | CGGACAATTCACGTTACAGCA |
| <i>Tnnt2</i> | CAGAGGAGGCCAACGTAGAAG | CTCCATCGGGGATCTTGGGT |
| <i>Ryr2</i> | ACGGCGACCATCCACAAAG | AAAGTCTGTTGCCAAATCCTTCT |
| <i>Ccl2</i> | TTAAAAACCTGGATCGGAACCAA | GCATTAGCTTCAGATTTACGGGT |
| <i>Ccl7</i> | GCTGCTTTCAGCATCCAAGTG | CCAGGGACACCGACTACTG |
| <i>Ccl12</i> | ATTTCCACACTTCTATGCCTCCT | ATCCAGTATGGTCCTGAAGATCA |
| <i>Ifnar1</i> | AGCCACGGAGAGTCAATGG | GCTCTGACACGAACTGTGTTTT |
| <i>Ifnar2</i> | CTTCGTGTTTGGTAGTGATGGT | GGGGATGATTTCCAGCCGA |
| <i>Ifnb1</i> | CAGCTCCAAGAAAGGACGAAC | GGCAGTGTAACCTTCTGCAT |
| <i>Ki67</i> | ATCATTGACCGCTCCTTTAGGT | GCTCGCCTTGATGGTTCCT |
| <i>Aurkb</i> | CAGAAGGAGAACGCCTACCC | GAGAGCAAGCGCAGATGTC |
| <i>Gapdh</i> | AGGTCGGTGTGAACGGATTTG | TGTAGACCATGTAGTTGAGGTCA |

2. Mouse Genotyping Primer Sequences

| Mouse line | Forward | Reverse |
|------------|---------|---------|
|------------|---------|---------|

| | | |
|----------------------|---|---|
| <i>Colla2-CreER</i> | AATAAGCCATCTCGCCTGCCG TTC | GGATAGTTTTTACTGCCAGAC CGC (Mut) TCGTGTCAGCCTTGGTCAACT CTC (WT) |
| <i>Tcf21-CreER</i> | TCAATGGGCGGGGGTCGTT | TCTTGCGGGGTGGGATAGGG AG |
| <i>Rosa-mTmG</i> | CTCTGCTGCCTCCTGGCTTCT | CGAGGCGGATCACAAGCAAT A (WT) TCAATGGGCGGGGGTCGTT (Mut) |
| <i>Rosa-tdTomato</i> | AAGGGAGCTGCAGTGGAGTA(WT) CTGTTCCCTGTACGGCATGG(M ut) | CCGAAAATCTGTGGGAAGTC (WT) GGCATTAAAGCAGCGTATCC (Mut) |