Supplementary information for

Identify splenic IRF7 as nanotherapy target for tele-conditioning myocardial reperfusion injury

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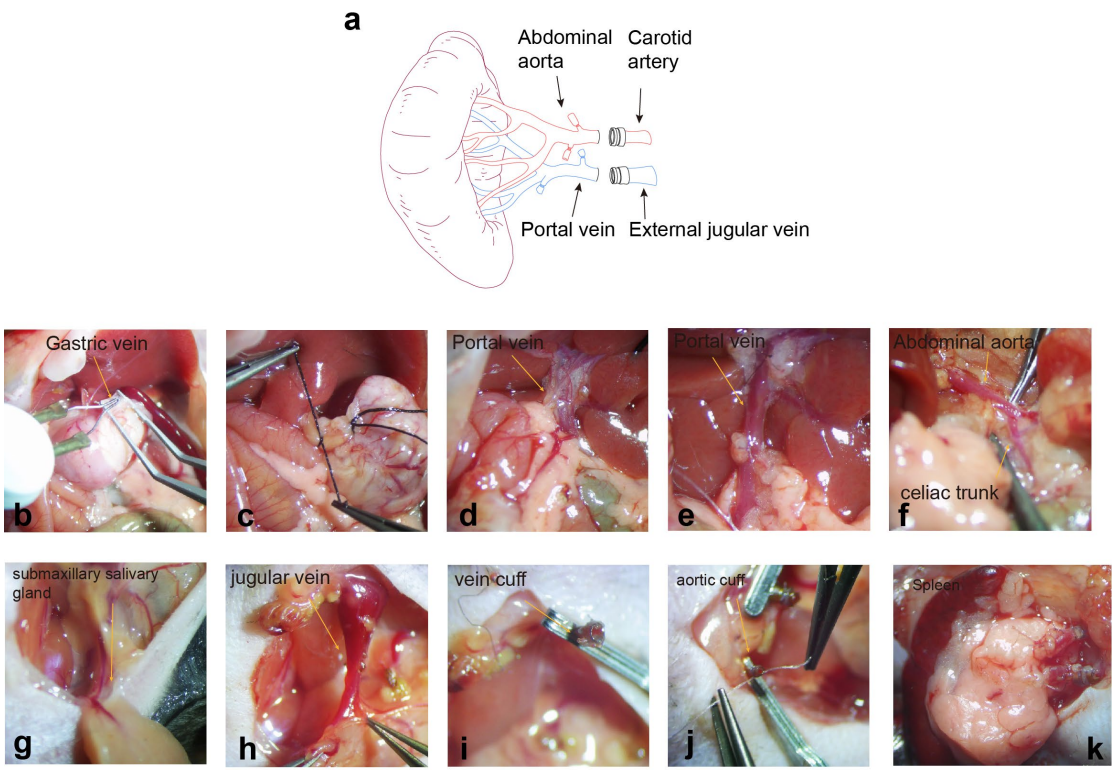
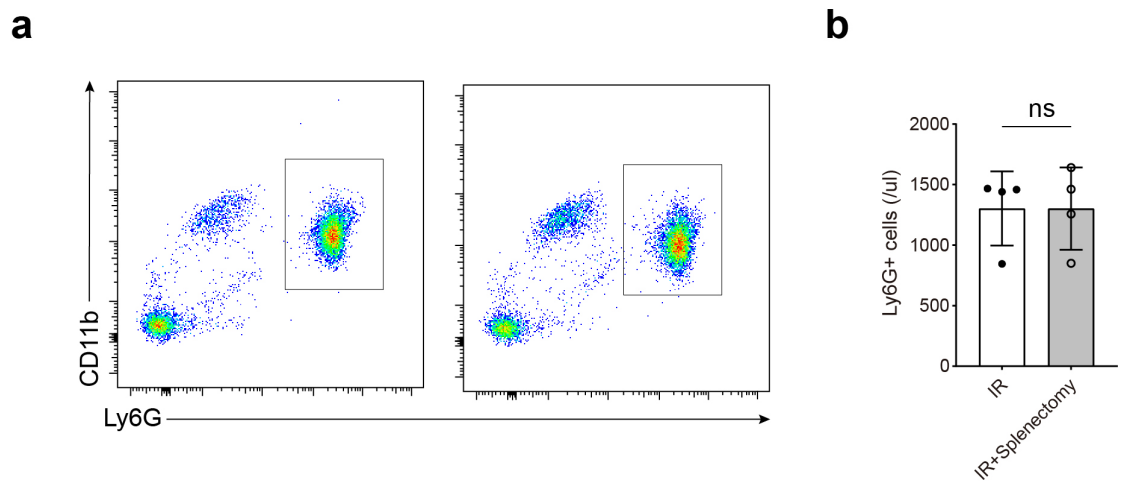
Supplementary Figures



Supplementary Figure 1. Heterotopic spleen transplantation.

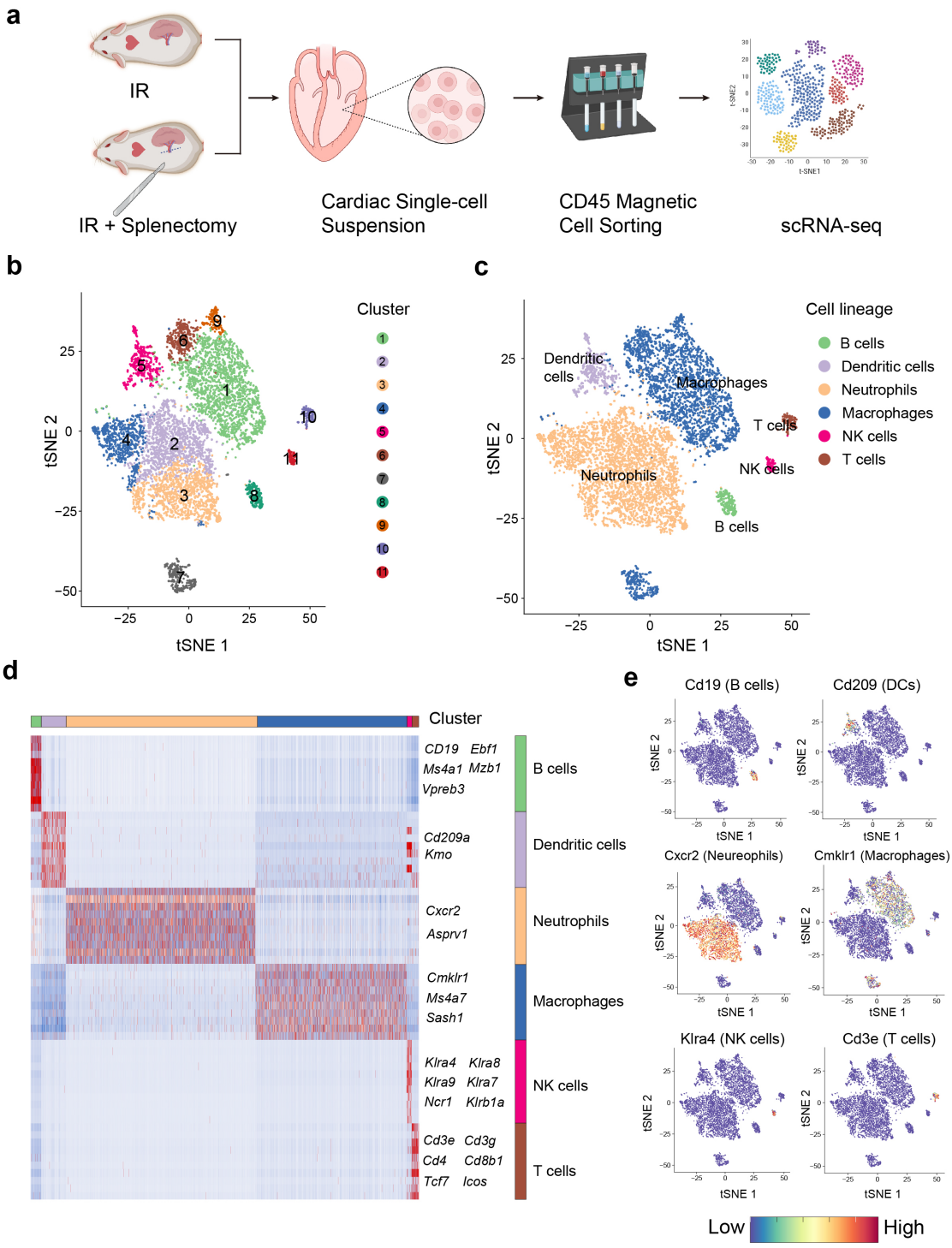
a, schematic representation of heterotopic spleen transplantation using cuff technique.

b-k, major surgical procedures in heterotopic spleen transplantation.

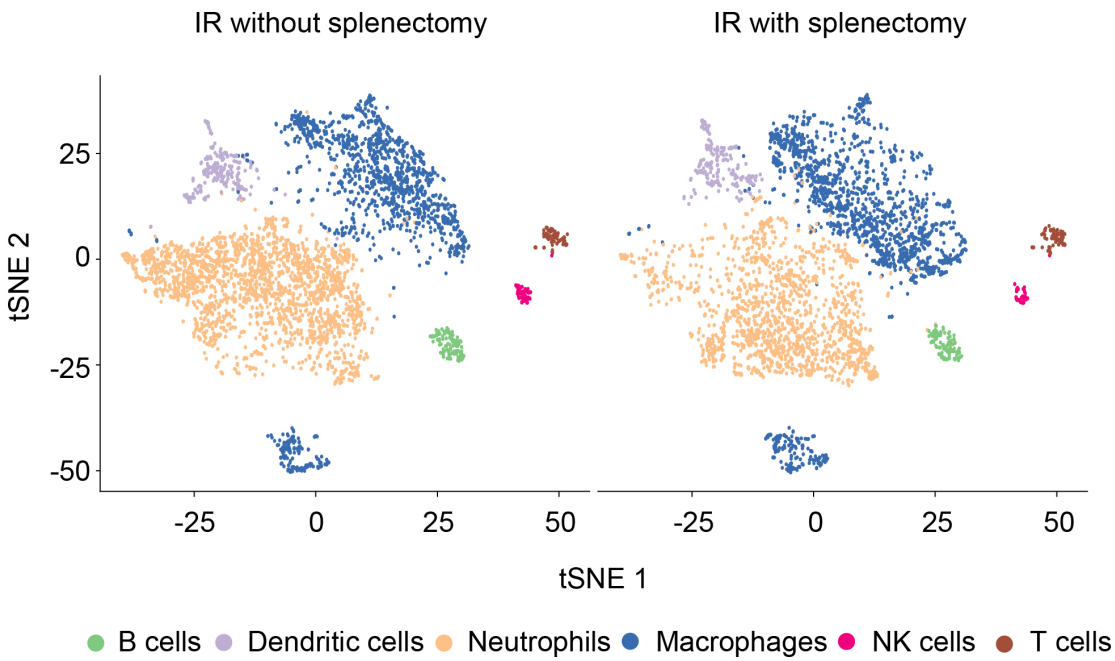


Supplementary Figure 2. Flow cytometric analyses of neutrophils.

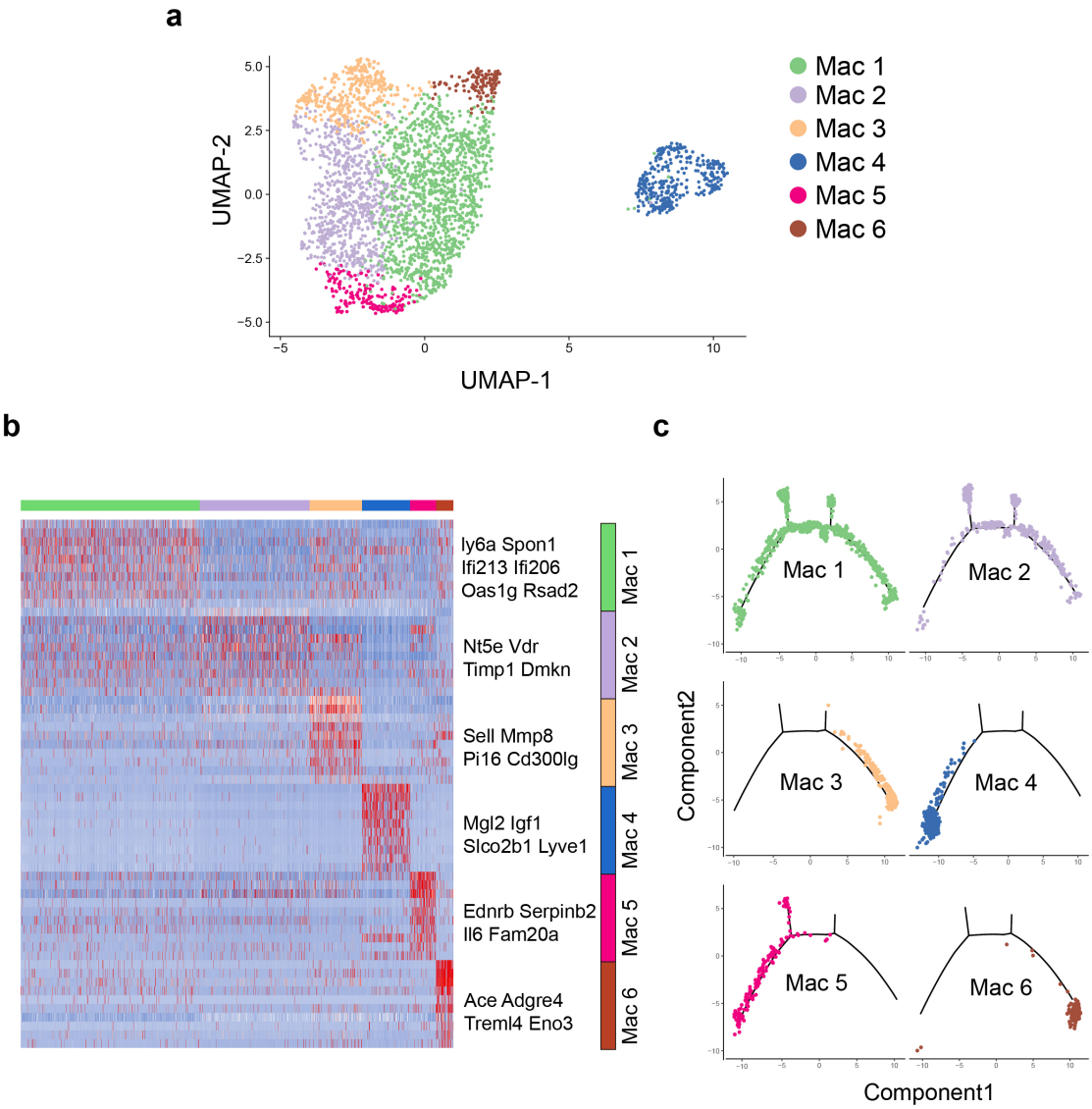
a, Flow cytometric analyses of immune cells in the blood of sham-operated mice and splenectomized mice subjected to myocardial ischemia-reperfusion at 1 day post the operation. **b**, Total number of monocytes in the blood were quantified. The data are expressed as mean ± s.d. Statistical analyses were based on Unpaired Students's *t*-test, ns = not significant.



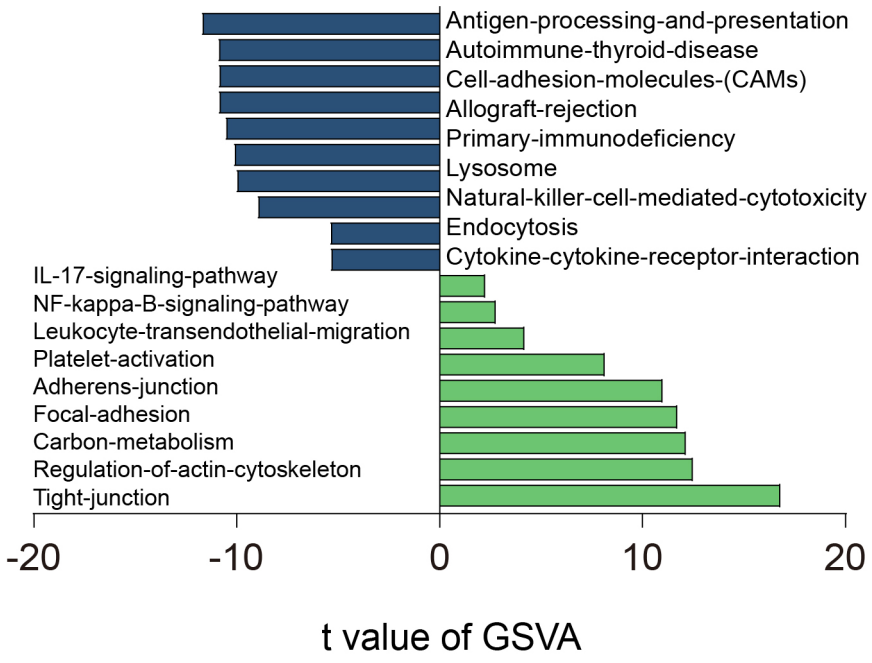
Supplementary Figure 3. Single-cell atlas of cardiac immune cells. a, Schematic diagram showing scRNA-seq pipeline of murine cardiac immune cells at 24-h after myocardial IR in sham-operated mice and splenectomized mice. **b**, tSNE plots of cardiac immune cells allocated into 11 clusters. **c**, tSNE plots of cardiac immune cells colored by cell type. **d**, Heat map showing selected cluster marker genes. **e**, feature plot representing the expression levels of the selected cell-type-specific marker genes.



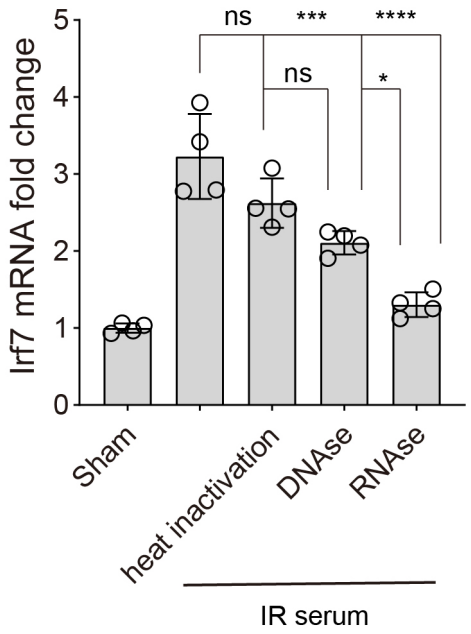
Supplementary Figure 4. tSNE plots of cardiac immune cells from sham-operated mice and splenectomized mice after myocardial IR. Cardiac immune cells were colored by cell type.



Supplementary Figure 5. Subclusters of cardiac macrophages and their developmental trajectory analysis. a, UMAP plots of cardiac immune cells allocated into 6 clusters. **b**, Heat map showing selected cluster marker genes. **c**, The Monocle prediction of monocyte-macrophage developmental trajectory with each Seurat-based cluster shown separately.



Supplementary Figure 6. Gene set variation analyses (GSVA) of Mac2 cells compared to Mac1 cells.



Supplementary Figure 7. Myocardial IR serum activated splenic IRF7. Mice were subjected to the I/R surgical procedures as outlined earlier. Following 1 hour of reperfusion, mice were euthanized, and sera were collected, and intravenously administered to healthy mice. One hour following administration, the spleens were harvested, and total mRNA was extracted. Statistical analyses were conducted by one-way ANOVA with Tukey's post hoc test, where *P < 0.05, **P< 0.01, ***P< 0.001, ****P< 0.0001, ns = no significancy.

a

PCR genotyping of IRF7 knockout mice

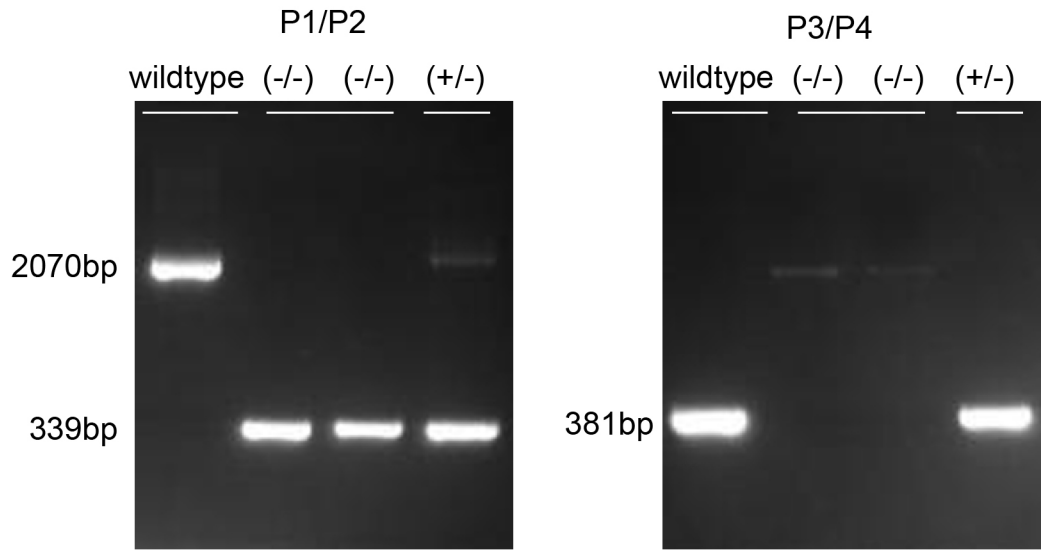
Primer Sequence
P1 5’-CACAGCACAGGGCGTTTTATCTTG-3’
P2 5’-ACTGGGGGTCACCTTCTTTCA-3’
P3 5’-ACTCCACAGCACAGGGCGTTTTAT-3’
P4 5’-GAGTGGGGAGGGGAGATTTGGTTA-3’

wildtype: P1+P2 2070bp, P3+P4 381bp

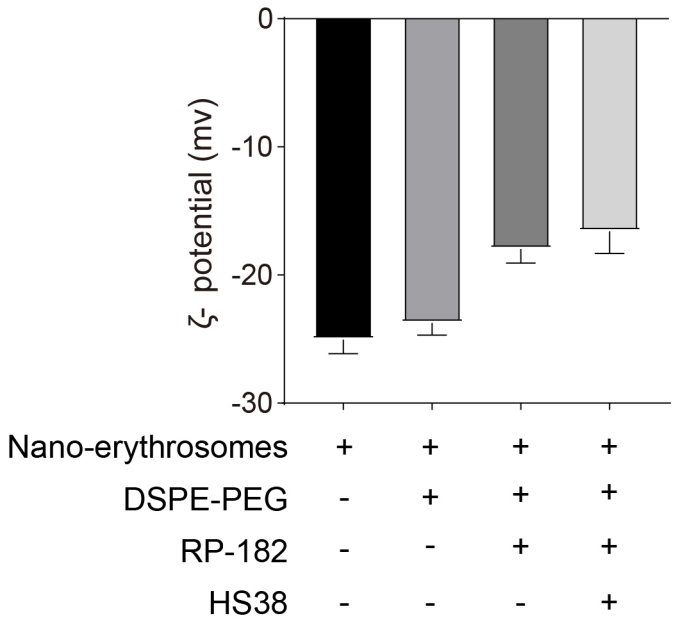
heterozygote (+/-): P1+P2 339bp+2070bp, P3+P4 381bp

homozygote (-/-): P1+P2 339bp, P3+P4 none

b



Supplementary Figure 8. Southern DNA blot analyses. a, PCR genotyping of IRF7 knock-out mice. **b**, Representative Southern DNA blot images.



Supplementary Figure 9. Zeta-potential of bare nano-erythrosomes, PEGylated erythrosomes, PEGylated erythrosomes/DSPE-PEG-RP182 and STEER-HS38.

Supplementary tables

Supplementary Table 1 Primer sequences used in this study

Mouse		
Gene	Forward primer	Reverse primer
GAPDH	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT
Irf7	GAGACTGGCTATTGGGGGAG	GACCGAAATGCTTCCAGGG
Nos2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC
Arg1	CTCCAAGCCAAAGTCCTTAGAG	AGGAGCTGTCATTAGGGACATC

Human		
Gene	Forward primer	Reverse primer
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG
Irf7	CCACGCTATACCATCTACCTGG	GCTGCTATCCAGGGAAGACACA

Supplementary Table 2 antibodies used in this study

Antibodies	Brand	Art. No.	Application
Zombie NIR	Biolegend	423105	FC
PE/Cyanine7 anti-mouse CD45 Antibody	Biolegend	103113	FC
Brilliant Violet 605™ anti-mouse/human CD11b Antibody	Biolegend	101237	FC
Alexa Fluor® 647 anti-mouse Ly-6C Antibody	Biolegend	128009	FC
PE Rat Anti-Mouse Ly-6G	BD Pharmingen	561104	FC
PE anti-mouse CD80 Antibody	Biolegend	104707	FC
Brilliant Violet 421™ anti-mouse CD206	Biolegend	141717	FC
Irf7	ABclonal	A22742	WB

GAPDH	Proteintech	60004-1-Ig	WB
Cd11b	abcam	Ab133357	IHC
TdTomato	Biorbyt	orb182397	IHC
Ly6G	Abmart	T61129m	IHC
F4/80	CST	70076	IHC

Supplementary Table 3 Peptides sequences in the play-off pool.

Peptides	Sequence
Lyp1	CGNKRTRGC
TT1	CKRGARSTC
linTT1	AKRGARSTA
UNO	CSPGAKVRC
mUNO	CSPGAK
CRV	CRVLRSGSC
M2pep	YEQDPWGVKWWY
RP182	KFRKAFKRFF
tLyp1	CGNKRTR
RPAR	GRPARPAR
iRGD	CRGDKGPDC
CSG	CSGRRSSKC
CAQK	CAQK
PL1	PPRRGLIKLKTS
PL2	TSKQNSR
PL3	AGRGRLVR
AT1	AGRGRLVRSAGGSVA
IP3	CKRDLSRRC
CAR	CARSKNKDC
CREKA	CREKA

CooP	CGLSGLGVA
CVG	CVGTNCY
Cerepep	GGSRRVISRAKLAAAL
Angiopep-2	TFFYGGSRGKRNNFKTEEY
MS1	CRGGKRSSC
VAG1	CRSMRSAKC
Insertless	SSVDKLAAALE
G7	CGGGGGGGC