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Supplemental information

A heart-brain-spleen axis controls cardiac remodeling to hypertensive stress

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Supplemental Figures

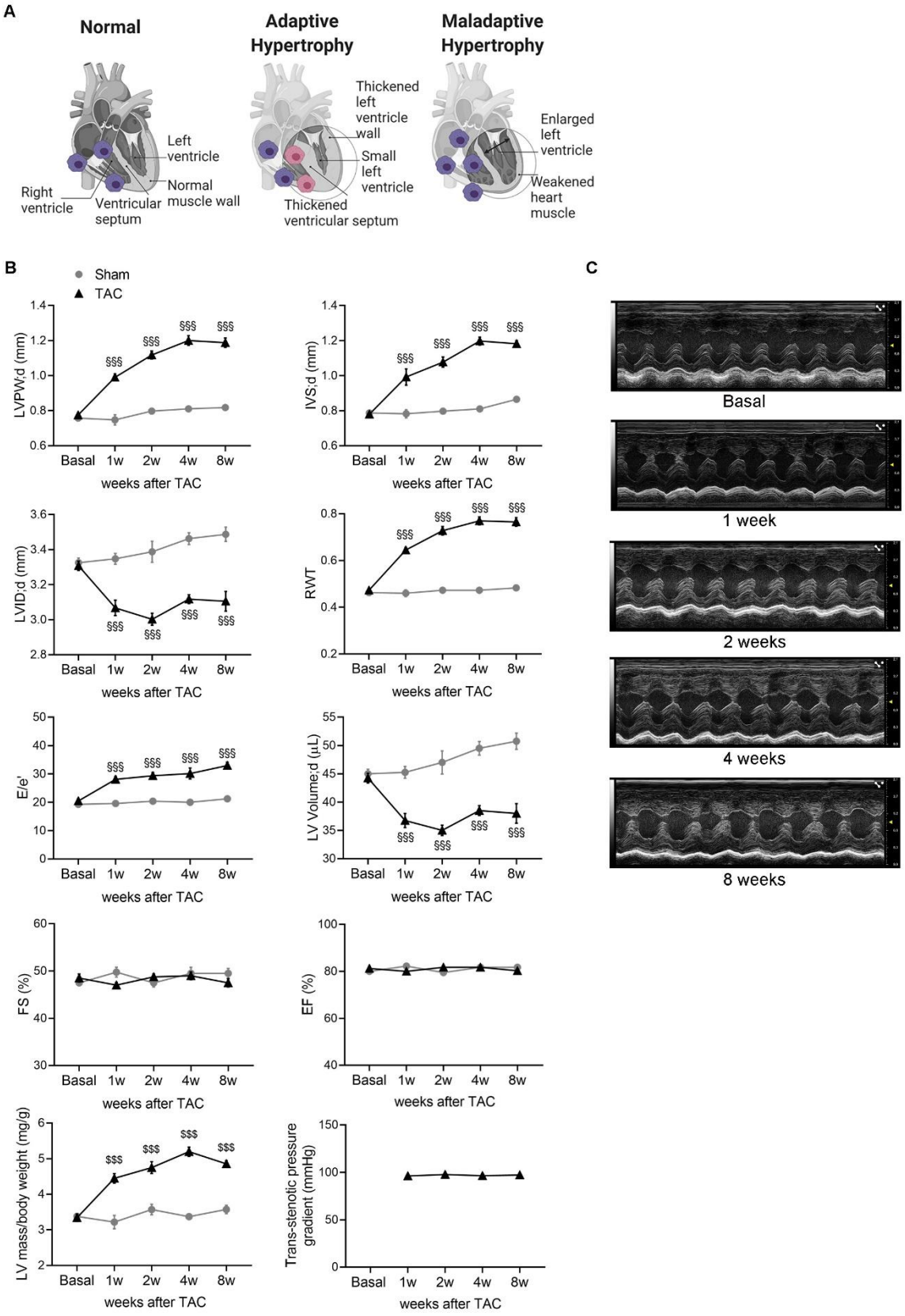

Figure S1

Figure S1. Pressure overload induces a longstanding adaptive LV concentric hypertrophy with preserved ejection fraction, before transitioning toward heart failure, related to Figures 1, 2, 3, 4 and 6.

A.) Schematic representation of the heart in normal conditions (left image), the heart developing adaptive hypertrophy (central image) or maladaptive hypertrophy (right image). B.) Serial longitudinal LV echocardiography in basal condition and at 1-2-4-8 weeks in C57Bl/6J mice after TAC or sham procedures. End-diastolic Left Ventricular Posterior Wall thickness (LVPW;d), end-diastolic Interventricular septal (IVS;d), end-diastolic Left Ventricular Internal Diameter (LVID;d), Relative Wall Thickness (RWT), E/e' ratio, end-diastolic Left Ventricle Volume (LV Volume;d), Fractional shortening (FS), Ejection Fraction (EF) and LV mass corrected for the body weight (LV mass/body weight) are shown. Trans-stenotic pressure gradient monitored in mice subjected to TAC during each session of echocardiographic follow up analysis is shown. C.) LV M-mode echocardiography at 1-2-4-8 weeks after TAC in C57Bl/6J mice. n=4 sham; n=4 TAC. Data as mean ± SEM and analyzed by two-way ANOVA and Sidak post hoc. §§§ $p<0.001$. Schematic was created with BioRender.com.

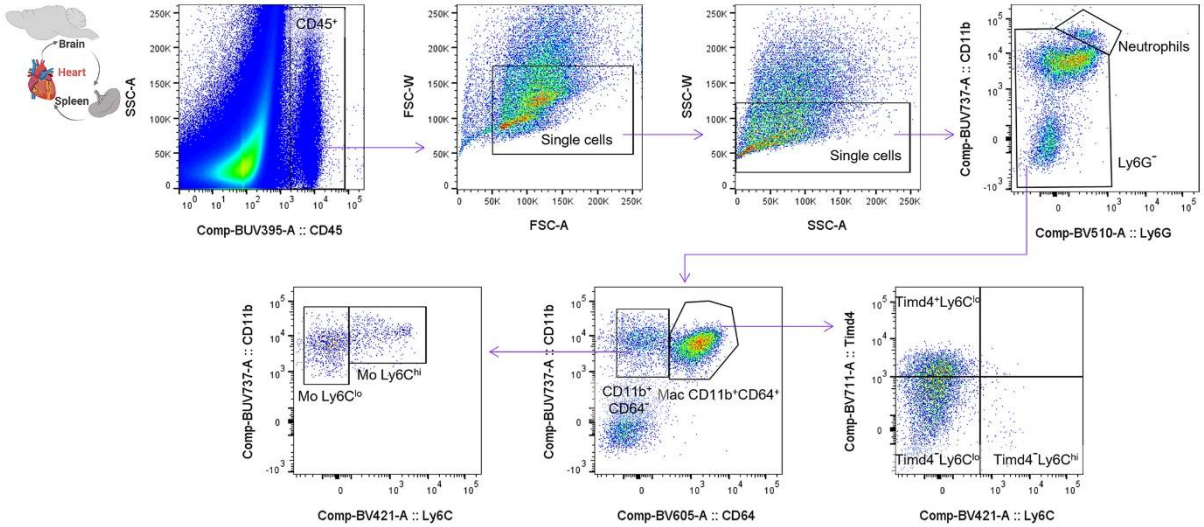
Brain
Heart
Spleen
SSC-A
CD45+
Comp-BUV395-A :: CD45
FSC-W
Single cells
FSC-A
SSC-W
Single cells
SSC-A
Comp-BUV737-A :: CD11b
Neutrophils
Ly6G-
Comp-BV510-A :: Ly6G
Comp-BUV737-A :: CD11b
Mo Ly6Chi
Mo Ly6Clo
Comp-BV421-A :: Ly6C
Comp-BUV737-A :: CD11b
CD11b+ CD64-
Mac CD11b+CD64+
Comp-BV605-A :: CD64
Comp-BV711-A :: Timd4
Timd4+Ly6Clo
Timd4-Ly6Clo
Timd4-Ly6Chi
Comp-BV421-A :: Ly6C

Figure S2

Figure S2. Gating strategy of the flow cytometry analysis used to identify cardiac monocyte and macrophage populations, related to Figures 1, 2, 3 and 6.
Cells were gated to identify total $CD45^+$ leukocytes and then doublets were excluded using forward scatter (FSC) and side scatter (SSC). Total leukocytes were parsed to exclude $CD11b^+Ly6G^+$ neutrophils and to identify $Ly6G^-CD11b^+$ monocytes and macrophages. $CD11b^+$ cells were further gated by CD64 expression to identify $CD11b^+CD64^-$ cells and $CD11b^+CD64^+$ macrophages. $CD11b^+CD64^-$ cells were then stratified into $Ly6C^{lo}$ and $Ly6C^{hi}$ monocytes. $CD11b^+CD64^+$ cardiac macrophages were parsed from the expression of Timd4 and Ly6C as a surrogate marker of recently recruited $CCR2^+$ macrophages. We identified $CD11b^+CD64^+Timd4^+Ly6C^{lo}$ resident macrophages expanding by local proliferation, the $CD11b^+CD64^+Timd4^-Ly6C^{lo}$ resident macrophages expanding by local proliferation and partially replenished by circulating monocytes and the recently recruited $CD11b^+CD64^+Timd4^-Ly6C^{hi}$ macrophages. Schematic was created with BioRender.com.

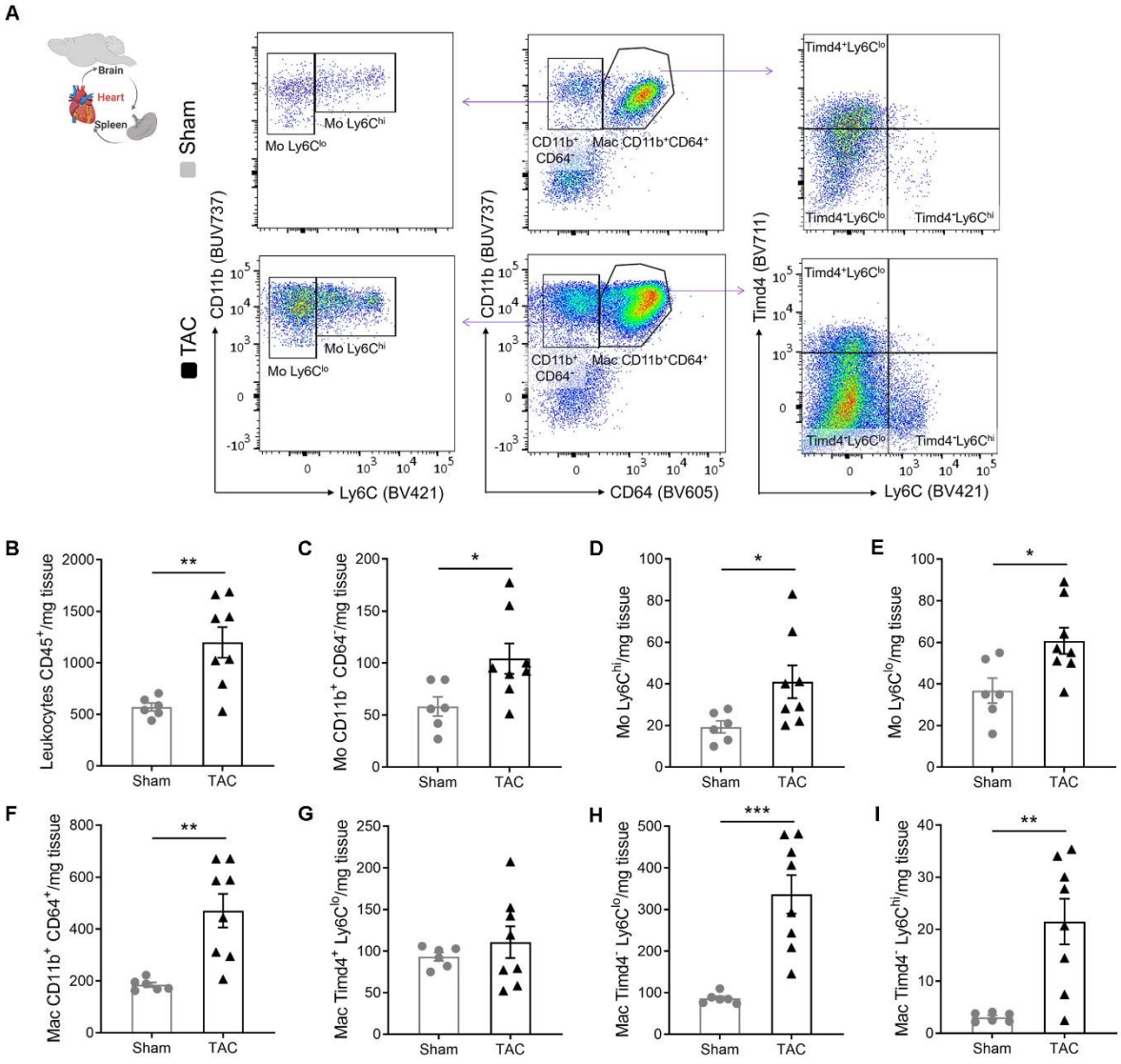
A
Brain
Heart
Spleen
Sham
TAC
Mo Ly6C^lo
Mo Ly6C^hi
CD11b^+ CD64^-
Mac CD11b^+CD64^+
Timd4^+Ly6C^lo
Timd4^-Ly6C^lo
Timd4^-Ly6C^hi
CD11b (BUV737)
Ly6C (BV421)
CD64 (BV605)
Timd4 (BV711)
B
Leukocytes CD45^+/mg tissue
C
Mo CD11b^+ CD64^-/mg tissue
D
Mo Ly6C^hi/mg tissue
E
Mo Ly6C^lo/mg tissue
F
Mac CD11b^+ CD64^+/mg tissue
G
Mac Timd4^+ Ly6C^lo/mg tissue
H
Mac Timd4^- Ly6C^lo/mg tissue
I
Mac Timd4^- Ly6C^hi/mg tissue
Sham
TAC

Figure S3

Figure S3. Pressure overload induces the expansion of monocyte and macrophage populations in the cardiac tissue, related to Figures 1, 2, 3 and 6.

A.) Flow cytometry analysis of monocytes and macrophages in the LV of mice subjected to TAC or sham for 4 days. B-I) Quantification of total $CD45^+$ leukocytes B.), total $CD11b^+CD64^-$ monocytes (Mo) C.), proinflammatory $CD11b^+CD64^-Ly6C^{hi}$ D.) and patrolling $CD11b^+CD64^-Ly6C^{lo}$ E.) monocyte subpopulations. By parsing $CD11b^+CD64^+$ total cardiac macrophages (Mac) F.) by the expression of Timd4 and Ly6C, $CD11b^+CD64^+Timd4^+Ly6C^{lo}$ resident macrophages G.), $CD11b^+CD64^+Timd4^-Ly6C^{lo}$ resident macrophages expanding by local proliferation and partially replenished by circulating cells H.) and $CD11b^+CD64^+Timd4^-Ly6C^{hi}$ recently recruited macrophages I.) were quantified. n=6 sham; n=8 TAC. Data as mean ± SEM and analyzed by unpaired T test *p<0.05, **p<0.01 and ***p<0.001. Schematic was created with BioRender.com.

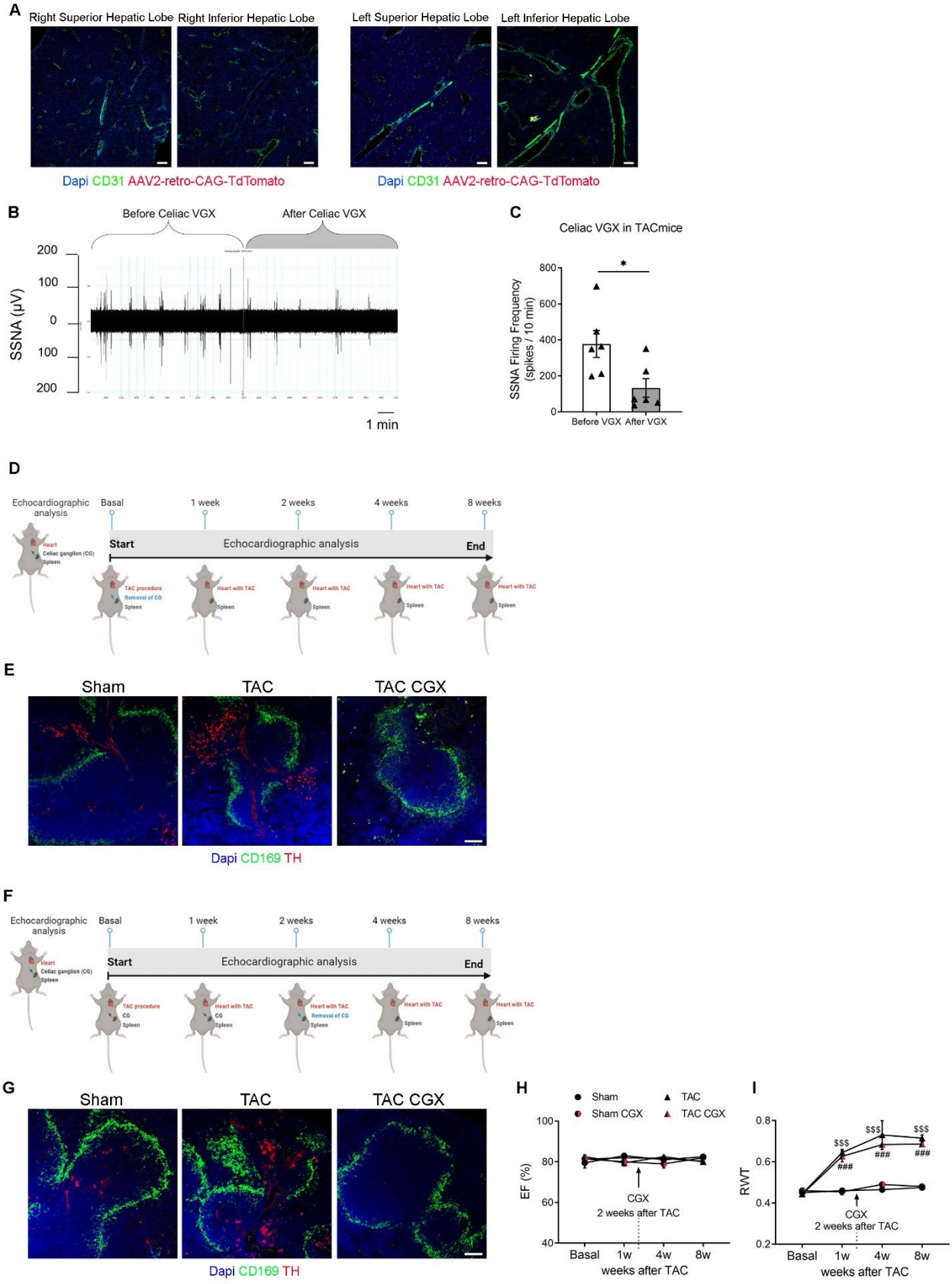
A
Right Superior Hepatic Lobe
Right Inferior Hepatic Lobe
Left Superior Hepatic Lobe
Left Inferior Hepatic Lobe
Dapi CD31 AAV2-retro-CAG-TdTomato
Dapi CD31 AAV2-retro-CAG-TdTomato
B
Before Celiac VGX
After Celiac VGX
SSNA (μV)
200
100
0
100
200
1 min
C
Celiac VGX in TACmice
SSNA Firing Frequency (spikes / 10 min)
800
600
400
200
0
*
Before VGX
After VGX
D
Echocardiographic analysis
Basal
1 week
2 weeks
4 weeks
8 weeks
Start
Echocardiographic analysis
End
Heart
Celiac ganglion (CG)
Spleen
TAC procedure
Removal of CG
Heart with TAC
E
Sham
TAC
TAC CGX
Dapi CD169 TH
F
Echocardiographic analysis
Basal
1 week
2 weeks
4 weeks
8 weeks
Start
Echocardiographic analysis
End
Heart
Celiac ganglion (CG)
Spleen
TAC procedure
CG
Heart with TAC
Removal of CG
G
Sham
TAC
TAC CGX
Dapi CD169 TH
H
Sham
Sham CGX
TAC
TAC CGX
EF (%)
100
80
60
40
CGX
2 weeks after TAC
Basal
1w
4w
8w
weeks after TAC
I
RWT
0.8
0.6
0.4
0.2
$$$
###
CGX
2 weeks after TAC
Basal
1w
4w
8w
weeks after TAC

Figure S4

Figure S4. Selective denervation of the spleen, related to Figure 1.
A.) Visualization in the right and left superior and inferior hepatic lobe of AAV2/retro-CAG-TdTomato injected in the LV (scale bar 100 μm). B. C.) SSNA raw signal in 1 day TAC mice before and after B.) celiac vagus nerve resection (VGX) and C.) firing frequency quantification. n=6 TAC. Data as mean ± SEM and analyzed by unpaired T-test. *p<0.05. D.) Schematics of celiac ganglionectomy (CGX) performed concomitantly to TAC or sham procedures. E.) Immunofluorescence analysis of $CD169^+$ macrophages (green) and Tyrosine Hydroxylase (TH) (red) expression in the spleen of sham or TAC mice concomitantly subjected to CGX or relative control (Scale bar 100 μm). F.) Schematics of CGX performed 2 weeks after TAC or sham. G.) Immunofluorescence analysis of $CD169^+$ macrophages (green) and TH (red) expression in the spleen of mice subjected to CGX or relative control procedure performed 2 weeks after sham or TAC (Scale bar 100 μm). H. I.) Serial longitudinal LV echocardiography in basal condition and at 1-4-8 weeks after TAC in mice subjected to CGX or relative control procedure, performed 2 weeks after TAC. H.) Ejection Fraction (EF) and I.) Relative Wall Thickness (RWT) are shown. n=3 sham; n=3 TAC; n=3 sham CGX; n=3 TAC CGX. Data as mean ± SEM and analyzed by two-way ANOVA and Tukey post hoc. §§§ p<0.001 sham vs TAC; ### p<0.001 sham CGX vs TAC CGX. Schematics were created with BioRender.com.

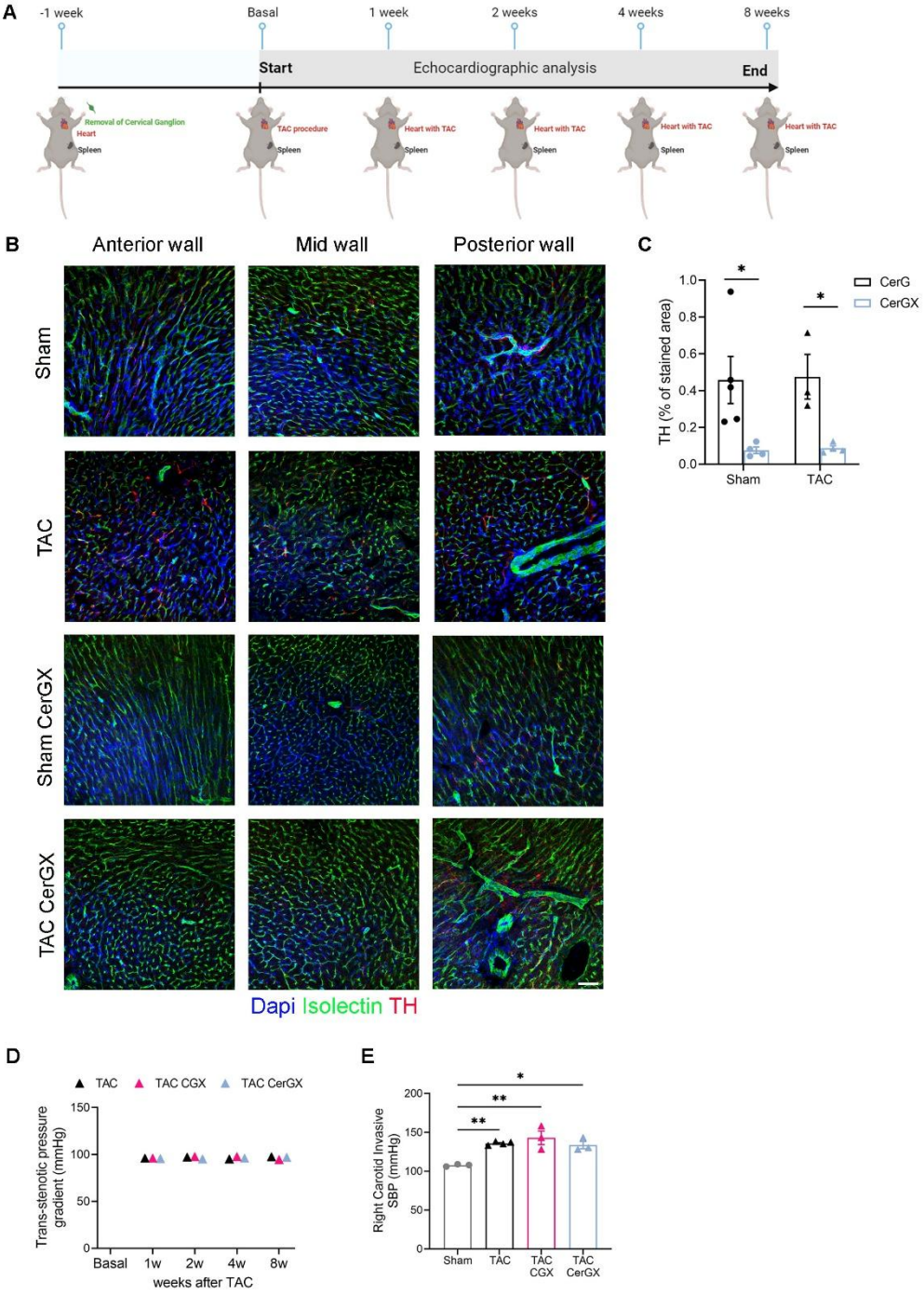
A
-1 week
Basal
1 week
2 weeks
4 weeks
8 weeks
Start
Echocardiographic analysis
End
Removal of Cervical Ganglion
Heart
Spleen
TAC procedure
Heart with TAC
B
Anterior wall
Mid wall
Posterior wall
Sham
TAC
Sham CerGX
TAC CerGX
Dapi Isolectin TH
C
CerG
CerGX
TH (% of stained area)
Sham
TAC
D
TAC
TAC CGX
TAC CerGX
Trans-stenolic pressure gradient (mmHg)
Basal
1w
2w
4w
8w
weeks after TAC
E
Right Carotid Invasive SBP (mmHg)
Sham
TAC
TAC CGX
TAC CerGX

Figure S5

Figure S5. Selective denervation of the heart, related to Figure 1.
A.) Schematics of cervical ganglionectomy (CerGX) performed 1 week before TAC or sham procedures. B.) Immunofluorescence analysis of Isolectin (green) and TH (red) expression in the anterior, mid and posterior wall of cardiac tissue of mice subjected to CerGX and 4 days of TAC or sham or to relative control procedure (Scale bar 50 μm). C.) Quantification of TH+ fibers in the LV of mice subjected to CerGX or to relative control procedure (CerG) and 4 days of TAC or sham. n=5 sham CerG; n=3 TAC CerG; n=4 sham CerGX; n=4 TAC CerGX. Data as mean ± SEM and analyzed by two-way ANOVA and Sidak post hoc. D.) Trans-stenotic aortic pressure gradient evaluated by ultrasonography at 1-2-4-8 weeks after TAC in mice subjected to TAC and to celiac ganglionectomy (CGX) or to CerGX. n=5 TAC; n=7 TAC CGX; n=6 TAC CerGX. Data as mean ± SEM and analyzed by two-way ANOVA and Tukey post hoc. E.) Right carotid invasive systolic blood pressure (SBP) measurement 4 days after TAC or sham in mice subjected to CGX or to CerGX. n=3 sham n=4 TAC; n=3 TAC CGX; n=3 TAC CerGX. Data as mean ± SEM and analyzed by one-way ANOVA and Tukey post hoc. *$p<0.05$ and **$p<0.01$. Schematics were created with BioRender.com.

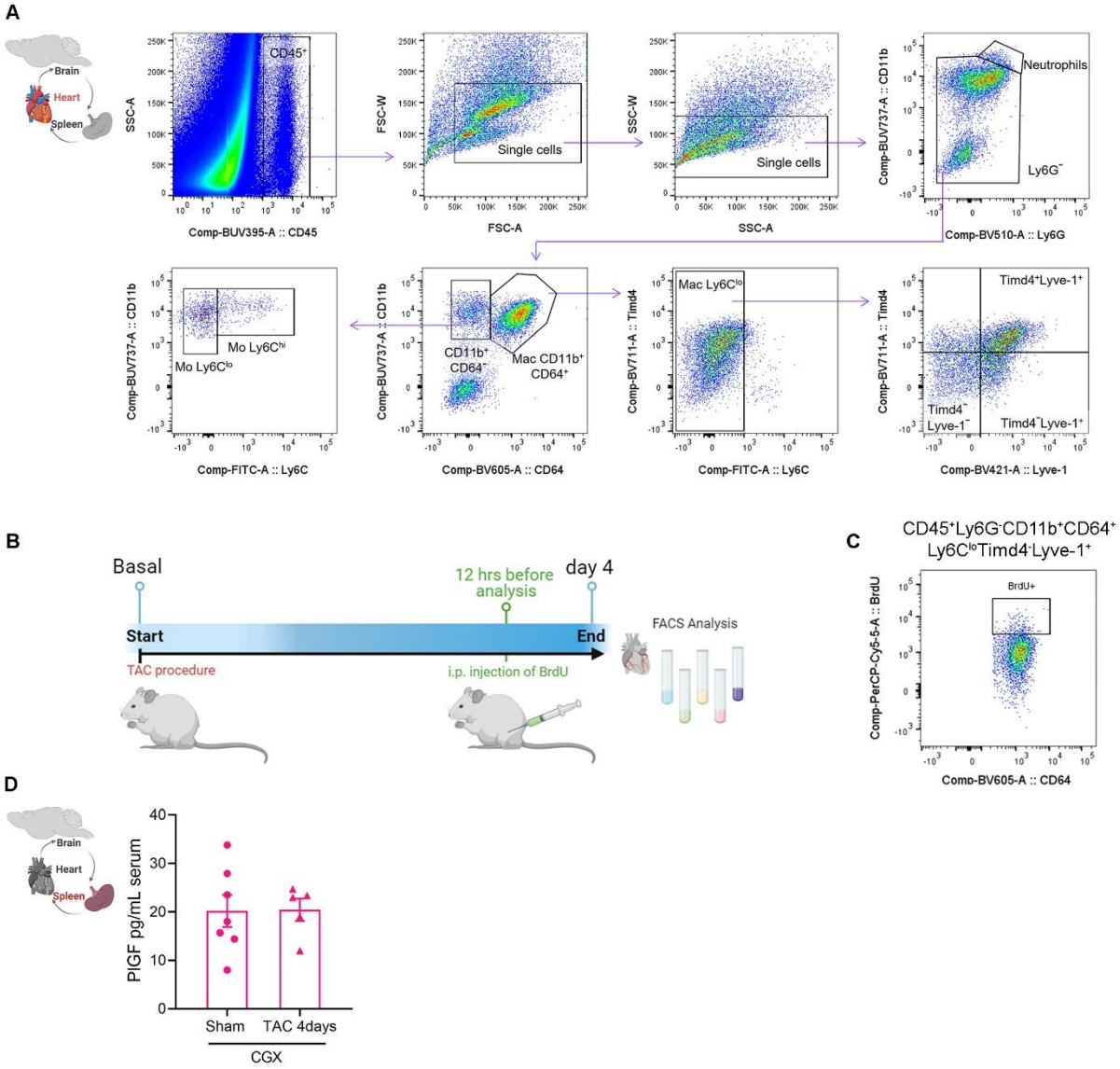
A
Brain
Heart
Spleen
CD45+
SSC-A
Comp-BUV395-A :: CD45
FSC-W
FSC-A
Single cells
SSC-W
SSC-A
Single cells
Comp-BUV737-A :: CD11b
Neutrophils
Ly6G-
Comp-BV510-A :: Ly6G
Comp-BUV737-A :: CD11b
Mo Ly6Chi
Mo Ly6Clo
Comp-FITC-A :: Ly6C
Comp-BUV737-A :: CD11b
CD11b+ CD64-
Mac CD11b+ CD64+
Comp-BV605-A :: CD64
Comp-BV711-A :: Timd4
Mac Ly6Clo
Comp-FITC-A :: Ly6C
Comp-BV711-A :: Timd4
Timd4+Lyve-1+
Timd4- Lyve-1-
Timd4-Lyve-1+
Comp-BV421-A :: Lyve-1
B
Basal
12 hrs before analysis
day 4
Start
End
TAC procedure
i.p. injection of BrdU
FACS Analysis
C
CD45+Ly6G-CD11b+CD64+
Ly6CloTimd4-Lyve-1+
BrdU+
Comp-PerCP-Cy5-5-A :: BrdU
Como-BV605-A :: CD64
D
Brain
Heart
Spleen
PlGF pg/mL serum
40
30
20
10
0
Sham
TAC 4days
CGX

Figure S6

Figure S6. Flow cytometry gating strategy used to identify resident cardiac macrophages expressing Lyve-1 marker and their proliferation rate, related to Figures 3, 4 and 6.
A.) Cells were gated to identify total $CD45^+$ leukocytes and then doublets were excluded using forward scatter (FSC) and side scatter (SSC). Total leukocytes were parsed to exclude $CD11b^+Ly6G^+$ neutrophils and $Ly6G^-CD11b^+$ monocyte and macrophage populations were identified. The cells were gated to identify $CD11b^+CD64^-Ly6C^{hi}$ monocytes and $CD11b^+CD64^+$ macrophages. $CD11b^+CD64^+$ cardiac macrophages were gated to identify $CD11b^+CD64^+Ly6C^{lo}$ resident macrophages and further gated by Timd4 and Lyve-1 to verify the expression of Lyve-1 marker in $Timd4^-$ population. B.) Schematics of BrdU administration in mice subjected to TAC or sham. C.) BrdU incorporation in $CD11b^+CD64^+Ly6C^{lo}Timd4^-Lyve\text{-}1^+$ resident macrophages. D.) Circulating PlGF levels in mice subjected to CGX and to TAC or sham procedure for 4 days. n=7 sham CGX; n=5 TAC CGX 4 days. Data as mean ± SEM and analyzed by unpaired T test. Schematics were created with BioRender.com.

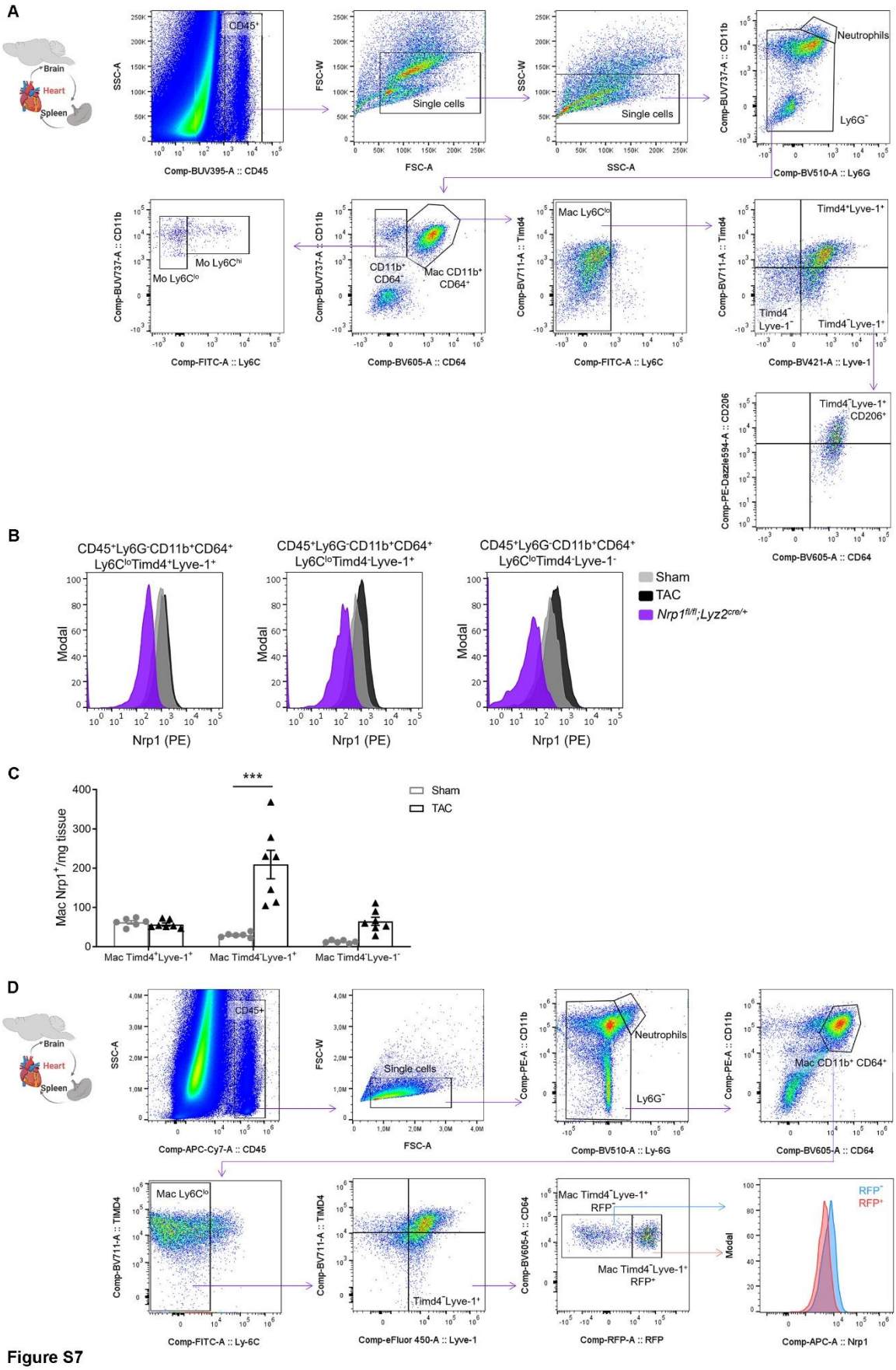
A
Brain
Heart
Spleen
CD45+
SSC-A
Comp-BUV395-A :: CD45
FSC-W
FSC-A
Single cells
SSC-W
SSC-A
Single cells
Neutrophils
Ly6G-
Comp-BUV737-A :: CD11b
Comp-BV510-A :: Ly6G
Mo Ly6Chi
Mo Ly6Clo
Comp-FITC-A :: Ly6C
CD11b+ CD64-
Mac CD11b+ CD64+
Comp-BV605-A :: CD64
Mac Ly6Clo
Comp-BV711-A :: Timd4
Timd4+Lyve-1+
Timd4- Lyve-1-
Timd4-Lyve-1+
Comp-BV421-A :: Lyve-1
Timd4-Lyve-1+ CD206+
Comp-PE-Dazzle594-A :: CD206
B
CD45+Ly6G-CD11b+CD64+ Ly6CloTimd4+Lyve-1+
CD45+Ly6G-CD11b+CD64+ Ly6CloTimd4-Lyve-1+
CD45+Ly6G-CD11b+CD64+ Ly6CloTimd4-Lyve-1-
Modal
Nrp1 (PE)
Sham
TAC
Nrp1fl/fl;Lyz2cre/+
C
***
Mac Nrp1+/mg tissue
Sham
TAC
Mac Timd4+Lyve-1+
Mac Timd4-Lyve-1+
Mac Timd4-Lyve-1-
D
Brain
Heart
Spleen
CD45+
Comp-APC-Cy7-A :: CD45
Single cells
FSC-A
Neutrophils
Ly6G-
Comp-PE-A :: CD11b
Comp-BV510-A :: Ly-6G
Mac CD11b+ CD64+
Comp-BV605-A :: CD64
Mac Ly6Clo
Comp-BV711-A :: TIMD4
Comp-FITC-A :: Ly-6C
Timd4-Lyve-1+
Comp-eFluor 450-A :: Lyve-1
Mac Timd4-Lyve-1+ RFP-
Mac Timd4-Lyve-1+ RFP+
Comp-BV605-A :: CD64
Comp-RFP-A :: RFP
RFP-
RFP+
Modal
Comp-APC-A :: Nrp1

Figure S7

Figure S7. Cardiac macrophages expressing NRP1, related to Figures 6 and 7.
A.) Flow cytometry gating strategy used to identify cardiac macrophages expressing NRP1. Cells were gated to identify total $CD45^+$ leukocytes and then doublets were excluded using forward scatter (FSC) and side scatter (SSC). Total leukocytes were parsed to exclude $CD11b^+Ly6G^+$ neutrophils and $Ly6G^-CD11b^+$ monocyte and macrophage populations were identified. The cells were gated to identify $CD11b^+CD64^-Ly6C^{hi}$ monocytes and $CD11b^+CD64^+$ macrophages (Mac). $CD11b^+CD64^+$ cardiac macrophages were gated to identify $CD11b^+CD64^+Ly6C^{lo}$ resident macrophages and further gated by Timd4 and Lyve-1 to distinguish $CD11b^+CD64^+Ly6C^{lo}Timd4^+Lyve\text{-}1^+$, $CD11b^+CD64^+Ly6C^{lo}Timd4^-Lyve\text{-}1^+$ and $CD11b^+CD64^+ Ly6C^{lo}Timd4^-Lyve\text{-}1^-$ macrophages. Total cardiac Mac $Ly6C^{lo}Timd4^-Lyve\text{-}1^+$ and Mac $Ly6C^{lo}Timd4^-Lyve\text{-}1^+Nrp1^+$ subpopulation was also parsed to evaluate CD206 expression. B.) Flow cytometry histogram of cardiac macrophages from sham or TAC mice and from *Nrp1*$^{fl/fl}$*;Lyz2*$^{cre/+}$ mice for the evaluation of NRP1 expression and selective deletion in myeloid lineage. C.) Quantification of NRP1 expression in $CD11b^+CD64^+Ly6C^{lo}Timd4^+Lyve\text{-}1^+$ (Mac $Timd4^+Lyve\text{-}1^+$), $CD11b^+CD64^+Ly6C^{lo}Timd4^-Lyve\text{-}1^+$ (Mac $Timd4^-Lyve\text{-}1^+$) and $CD11b^+CD64^+Ly6C^{lo}Timd4^-Lyve\text{-}1^-$ (Mac $Timd4^-Lyve\text{-}1^-$) macrophage subpopulations. Data as mean ± SEM and analyzed by two-way ANOVA and Tukey post hoc. ***$p<0.001$ sham vs TAC. D.) Flow cytometry gating strategy used to verify selective NRP1 deletion and BrdU incorporation in cardiac resident macrophages expressing red fluorescent protein (RFP) in *Nrp1*$^{fl/fl}$*;Rosa26*$^{LSL\text{-}RFP/+}$;*Cx3cr1*$^{Cre\text{-}ERT2/+}$ ($RM^{RFP\text{-}\Delta Nrp1}$) mice. Cells were gated to identify total $CD45^+$ leukocytes and then doublets were excluded using forward scatter (FSC). Total leukocytes were parsed to exclude $CD11b^+Ly6G^+$ neutrophils and $Ly6G^-CD11b^+CD64^+$ macrophages were identified. Cardiac macrophages were gated to identify $CD11b^+CD64^+Ly6C^{lo}$ resident macrophages and further gated by Timd4 and Lyve-1 to identify $CD11b^+CD64^+Ly6C^{lo}Timd4^-Lyve\text{-}1^+$ subpopulation. The cells were parsed to discriminate cardiac resident macrophages expressing RFP (Mac $Timd4^-$ $Lyve\text{-}1^+$ RFP^+) from non-resident fraction (Mac $Timd4^-$ $Lyve\text{-}1^+$ RFP^-), and their NRP1 expression. The last histogram shows NRP1 selective deletion in resident $Timd4^-$ $Lyve\text{-}1^+$ RFP^+ (red) macrophages compared to non-resident $Timd4^-$ $Lyve\text{-}1^+$ RFP^- (blu) fraction in $RM^{RFP\text{-}\Delta Nrp1}$ mice. For proliferation experiment, BrdU incorporation was evaluated in $Timd4^-$ $Lyve\text{-}1^+$ RFP^+ macrophages. Schematics were created with BioRender.com.

Supplemental Tables

Table S1. Serial longitudinal echocardiography of LV remodeling in basal condition and at 1-2-4-8 weeks after TAC in mice with CGX or control procedure (CG), and serial longitudinal echocardiography of LV remodeling in basal condition and at 1-2-4-8 weeks after TAC in mice with CerGX or control procedure (CerG), related to Figure 1.

		Sham		TAC	
Parameter	***Time point***	**CG**	**CGX**	**CG**	**CGX**
LVPW;d (mm) Mean ± SD	Basal	0.77 ± 0.01	0.80 ± 0.01	0.79 ± 0.02	0.78 ± 0.02
	1 week	0.79 ± 0.05	0.80 ± 0.05	**1.06 ± 0.08§§§**	**1.05 ± 0.06$^{###}$**
	2 weeks	0.81 ± 0.02	0.81 ± 0.01	**1.07 ± 0.08§§§**	**0.99 ± 0.08$^{###}$**
	4 weeks	0.82 ± 0.02	0.82 ± 0.02	**1.15 ± 0.02§§§**	**1.07 ± 0.08$^{###}$**
	8 weeks	0.77 ± 0.02	0.77 ± 0.03	**1.15 ± 0.06§§§**	**0.98 ± 0.14$^{###, ***}$**
IVS;d (mm) Mean ± SD	Basal	0.77 ± 0.02	0.80 ± 0.01	0.78 ± 0.02	0.78 ± 0.02
	1 week	0.79 ± 0.03	0.77 ± 0.01	**1.05 ± 0.09§§§**	**0.99 ± 0.07$^{###}$**
	2 weeks	0.78 ± 0.02	0.79 ± 0.01	**1.12 ± 0.06§§§**	**0.99 ± 0.06$^{###, **}$**
	4 weeks	0.83 ± 0.02	0.82 ± 0.06	**1.17 ± 0.04§§§**	**1.06 ± 0.12$^{###, *}$**
	8 weeks	0.77 ± 0.02	0.77 ± 0.03	**1.16 ± 0.07§§§**	**0.95 ± 0.09$^{###, ***}$**
LVID;d (mm) Mean ± SD	Basal	3.40 ± 0.09	3.44 ± 0.07	3.35 ± 0.11	3.42 ± 0.06
	1 week	3.39 ± 0.11	3.42 ± 0.10	**3.00 ± 0.17§§§**	**3.22 ± 0.09***
	2 weeks	3.48 ± 0.09	3.45 ± 0.27	**3.09 ± 0.17§§§**	3.24 ± 0.19
	4 weeks	3.50 ± 0.03	3.53 ± 0.03	**3.23 ± 0.18§**	**3.51 ± 0.20****
	8 weeks	3.48 ± 0.06	3.51 ± 0.02	**3.15 ± 0.11§§§**	**3.46 ± 0.08*****
RWT Mean ± SD	Basal	0.46 ± 0.02	0.47 ± 0.02	0.47 ± 0.02	0.46 ± 0.01
	1 week	0.47 ± 0.03	0.46 ± 0.01	**0.71 ± 0.08§§§**	**0.63 ± 0.05$^{###, *}$**
	2 weeks	0.46 ± 0.01	0.46 ± 0.04	**0.71 ± 0.06§§§**	**0.61 ± 0.06$^{###, **}$**
	4 weeks	0.47 ± 0.01	0.47 ± 0.02	**0.72 ± 0.04§§§**	**0.61 ± 0.06$^{###, ***}$**
	8 weeks	0.44 ± 0.01	0.44 ± 0.02	**0.74 ± 0.06§§§**	**0.56 ± 0.06$^{##, ***}$**
E/e' Mean ± SD	Basal	19.48 ± 0.95	20.73 ± 0.24	19.50 ± 1.34	20.09 ± 1.05
	1 week	20.19 ± 0.48	20.31 ± 0.53	**26.60 ± 1.65§§§**	**27.53 ± 2.82$^{###}$**
	2 weeks	20.04 ± 0.51	20.18 ± 0.81	**28.53 ± 1.70§§§**	**31.16 ± 3.96$^{###}$**
	4 weeks	19.38 ± 0.75	20.95 ± 0.85	**28.48 ± 0.97§§§**	**31.56 ± 1.76$^{###, *}$**
	8 weeks	20.54 ± 0.43	20.58 ± 0.34	**31.50 ± 0.98§§§**	**34.10 ± 2.35$^{###}$**
LV Volume;d (mm^3) Mean ± SD	Basal	47.38 ± 3.07	48.70 ± 2.48	45.75 ± 3.73	48.23 ± 2.10
	1 week	47.31 ± 3.68	48.02 ± 3.49	**35.27 ± 4.81§§§**	41.16 ± 4.13
	2 weeks	50.38 ± 3.28	49.41 ± 9.26	**37.70 ± 5.15§§§**	43.03 ± 6.45
	4 weeks	50.72 ± 0.96	52.03 ± 0.98	**42.16 ± 5.52§**	**51.27 ± 7.05****
	8 weeks	50.35 ± 1.94	51.07 ± 0.59	**39.34 ± 6.85§§§**	**49.59 ± 2.77*****
LV mass/	Basal	3.53 ± 0.22	3.42 ± 0.10	3.46 ± 0.38	3.44 ± 0.31
	1 week	3.56 ± 0.16	3.31 ± 0.17	**4.65 ± 0.64§§**	**4.80 ± 0.61$^{###}$**

body weight (mg/g) Mean ± SD	2 weeks	3.57 ± 0.16	3.23 ± 0.30	**5.04 ± 0.33§§§**	**4.77 ± 1.11$^{###}$**
	4 weeks	3.44 ± 0.09	3.55 ± 0.33	**5.48 ± 0.56§§§**	**5.14 ± 0.56$^{###}$**
	8 weeks	3.26 ± 0.29	2.85 ± 0.16	**5.03 ± 0.47§§§**	**4.37 ± 0.90$^{###}$**
FS % Mean ± SD	Basal	50 ± 2	47 ± 1	47 ± 3	48 ± 2
	1 week	48 ± 3	47 ± 1	48 ± 2	46 ± 4
	2 weeks	47 ± 2	48 ± 1	49 ± 2	**41 ± 5****
	4 weeks	47 ± 2	47 ± 1	49 ± 2	**42 ± 8****
	8 weeks	50 ± 2	49 ± 2	50 ± 1	**37 ± 5$^{###, ***}$**
EF % Mean ± SD	Basal	83 ± 2	80 ± 1	80 ± 3	81 ± 2
	1 week	81 ± 3	79 ± 1	80 ± 2	78 ± 4
	2 weeks	80 ± 2	80 ± 1	82 ± 2	**73 ± 5****
	4 weeks	80 ± 2	80 ± 1	81 ± 2	**73 ± 10****
	8 weeks	82 ± 2	81 ± 2	83 ± 1	**68 ± 7$^{###, ***}$**
Trans-stenotic pressure gradient (mmHg) Mean ± SD	Basal				
	1 week			96 ± 3	96 ± 3
	2 weeks			97 ± 3	97 ± 1
	4 weeks			95 ± 1	97 ± 2
	8 weeks			97 ± 2	94 ± 1

		Sham		**TAC**	
Parameter	***Time point***	**CerG**	**CerGX**	**CerG**	**CerGX**
LVPW;d (mm) Mean ± SD	Basal	0.78 ± 0.02	0.79 ± 0.05	0.76 ± 0.01	0.76 ± 0.01
	1 week	0.81 ± 0.02	0.79 ± 0.01	**1.05 ± 0.05§§§**	**1.01 ± 0.09$^{###}$**
	2 weeks	0.80 ± 0.05	0.78 ± 0.03	**1.02 ± 0.01§§§**	**1.08 ± 0.07$^{###}$**
	4 weeks	0.80 ± 0.02	0.79 ± 0.07	**1.06 ± 0.05§§§**	**1.08 ± 0.07$^{###}$**
	8 weeks	0.80 ± 0.02	0.79 ± 0.03	**1.10 ± 0.08§§§**	**1.16 ± 0.06$^{###}$**
IVS;d (mm) Mean ± SD	Basal	0.77 ± 0.02	0.76 ± 0.03	0.76 ± 0.02	0.77 ± 0.03
	1 week	0.80 ± 0.01	0.81 ± 0.03	**1.08 ± 0.06§§§**	**1.04 ± 0.08$^{###}$**
	2 weeks	0.80 ± 0.02	0.80 ± 0.01	**1.04 ± 0.03§§§**	**1.06 ± 0.08$^{###}$**
	4 weeks	0.84 ± 0.04	0.83 ± 0.03	**1.06 ± 0.05§§§**	**1.08 ± 0.08$^{###}$**
	8 weeks	0.82 ± 0.05	0.82 ± 0.02	**1.16 ± 0.04§§§**	**1.14 ± 0.04$^{###}$**
LVID;d (mm) Mean ± SD	Basal	3.44 ± 0.05	3.37 ± 0.21	3.30 ± 0.09	3.46 ± 0.08
	1 week	3.44 ± 0.07	3.34 ± 0.07	**2.95 ± 0.25§§§**	**3.06 ± 0.18$^{#}$**
	2 weeks	3.51 ± 0.15	3.42 ± 0.07	**3.14 ± 0.24§§**	**3.14 ± 0.23$^{#}$**
	4 weeks	3.51 ± 0.06	3.58 ± 0.06	**3.12 ± 0.19§§**	**3.16 ± 0.12$^{###}$**
	8 weeks	3.40 ± 0.18	3.42 ± 0.14	3.34 ± 0.15	3.27 ± 0.13
RWT Mean ± SD	Basal	0.45 ± 0.02	0.46 ± 0.01	0.46 ± 0.01	0.44 ± 0.02
	1 week	0.47 ± 0.01	0.48 ± 0.01	**0.73 ± 0.08§§§**	**0.67 ± 0.07$^{###}$**
	2 weeks	0.46 ± 0.01	0.46 ± 0.01	**0.66 ± 0.04§§§**	**0.68 ± 0.09$^{###}$**

	4 weeks	0.47 ± 0.01	0.45 ± 0.03	**0.68 ± 0.05§§§**	**0.69 ± 0.05###**
	8 weeks	0.48 ± 0.02	0.47 ± 0.02	**0.68 ± 0.06§§§**	**0.70 ± 0.02###**
E/e' Mean ± SD	Basal	20.20 ± 0.44	19.54 ± 1.14	19.34 ± 0.77	19.90 ± 0.64
	1 week	19.68 ± 1.10	20.37 ± 0.73	**24.94 ± 1.22§§§**	**25.50 ± 2.61###**
	2 weeks	20.13 ± 0.59	19.66 ± 1.04	**27.64 ± 0.41§§§**	**29.89 ± 1.84###**
	4 weeks	20.82 ± 1.67	21.09 ± 0.34	**29.04 ± 1.31§§§**	**29.13 ± 1.81###**
	8 weeks	20.97 ± 0.85	20.48 ± 1.02	**29.34 ± 0.72§§§**	**30.77 ± 1.83###**
LV Volume;d (mm^3) Mean ± SD	Basal	48.79 ± 1.78	46.70 ± 6.68	44.30 ± 2.79	49.64 ± 2.79
	1 week	48.80 ± 2.39	45.52 ± 2.31	**34.42 ± 8.31§§§**	37.54 ± 5.31
	2 weeks	51.23 ± 5.07	48.19 ± 2.26	**39.30 ± 7.20§§**	**39.54 ± 6.80#**
	4 weeks	51.30 ± 2.09	53.55 ± 2.29	**38.65 ± 5.53§§**	**39.80 ± 3.71###**
	8 weeks	47.70 ± 5.90	48.25 ± 4.70	45.55 ± 4.81	42.99 ± 3.80
LV mass/ body weight (mg/g) Mean ± SD	Basal	3.38 ± 0.12	3.46 ± 0.52	3.14 ± 0.28	3.58 ± 0.28
	1 week	3.54 ± 0.26	3.49 ± 0.13	**4.34 ± 0.29§**	**4.63 ± 0.60###**
	2 weeks	3.58 ± 0.52	3.46 ± 0.32	4.24 ± 0.59	**4.71 ± 0.24###**
	4 weeks	3.41 ± 0.17	3.53 ± 0.22	**4.17 ± 0.46§**	**4.66 ± 0.41###**
	8 weeks	3.10 ± 0.24	3.29 ± 0.33	**4.92 ± 0.49§§§**	**5.13 ± 0.67###**
FS % Mean ± SD	Basal	48 ± 3	48 ± 1	48 ± 3	49 ± 1
	1 week	47 ± 1	48 ± 2	47 ± 2	48 ± 2
	2 weeks	47 ± 1	48 ± 2	47 ± 1	48 ± 1
	4 weeks	49 ± 1	47 ± 2	50 ± 1	47 ± 1
	8 weeks	49 ± 3	50 ± 2	48 ± 1	48 ± 2
EF % Mean ± SD	Basal	80 ± 3	80 ± 1	81 ± 3	81 ± 1
	1 week	79 ± 2	81 ± 2	80 ± 2	81 ± 2
	2 weeks	79 ± 1	81 ± 2	80 ± 1	81 ± 1
	4 weeks	81 ± 1	79 ± 2	83 ± 1	80 ± 1
	8 weeks	81 ± 3	82 ± 2	80 ± 1	81 ± 2
Trans-stenotic pressure gradient (mmHg) Mean ± SD	Basal				
	1 week			96 ± 3	96 ± 4
	2 weeks			98 ± 2	93 ± 6
	4 weeks			95 ± 2	96 ± 3
	8 weeks			97 ± 3	97 ± 2

End-diastolic Left Ventricular Posterior Wall thickness (LVPW;d), end-diastolic Interventricular septal (IVS;d), end-diastolic Left Ventricular Internal Diameter (LVID;d), Relative Wall Thickness (RWT), E/e' ratio, end-diastolic Left Ventricle Volume (LV Volume;d), LV mass corrected for the body weight (LV mass/body weight), Fractional shortening (FS), Ejection Fraction (EF) and trans-stenotic pressure gradient.

Mice subjected to sham or TAC and to CGX or control procedure (CG): n=5 sham CG; n=5 TAC CG; n=3 sham CGX; n=7 TAC CGX. Data as mean ± SD and analyzed by two-way ANOVA and Tukey post hoc. § $p<0.05$ and §§§ $p<0.001$ sham CG vs TAC CG; ## $p<0.01$ and ### $p<0.001$ sham CGX vs TAC CGX; * $p<0.05$, ** $p<0.01$ and *** $p<0.001$ TAC CG vs TAC CGX.

Mice subjected to sham or TAC and to CerGX or control procedure (CerG): n=4 sham CerG; n=4 TAC CerG; n=4 sham CerGX; n=6 TAC CerGX. Data as mean ± SD and analyzed by two-way ANOVA and Tukey post hoc. § $p<0.05$ and §§§ $p<0.001$ sham CerG vs TAC CerG; # $p<0.05$ and ### $p<0.001$ sham CerGX vs TAC CerGX.

Table S2. Serial longitudinal echocardiography of LV remodeling in basal condition and at 1-2-4-8 weeks after TAC in mice subjected to splenectomy (-spleen) or control procedure (+spleen), related to Figure 2.

		Sham		TAC	
Parameter	***Time point***	**+spleen**	**-spleen**	**+spleen**	**-spleen**
LVPW;d (mm) Mean ± SD	Basal	0.74 ± 0.02	0.77 ± 0.03	0.74 ± 0.05	0.77 ± 0.02
	1 week	0.75 ± 0.04	0.76 ± 0.03	**1.01 ± 0.06§§§**	**0.91 ± 0.03$^{###, ***}$**
	2 weeks	0.77 ± 0.03	0.77 ± 0.02	**1.02 ± 0.08§§§**	**0.90 ± 0.03$^{###, ***}$**
	4 weeks	0.78 ± 0.02	0.77 ± 0.02	**1.04 ± 0.06§§§**	**0.92 ± 0.03$^{###, ***}$**
	8 weeks	0.80 ± 0.00	0.78 ± 0.02	**1.06 ± 0.07§§§**	**0.92 ± 0.04$^{###, ***}$**
IVS;d (mm) Mean ± SD	Basal	0.74 ± 0.02	0.77 ± 0.03	0.74 ± 0.05	0.77 ± 0.02
	1 week	0.75 ± 0.04	0.76 ± 0.03	**1.01 ± 0.06§§§**	**0.91 ± 0.03$^{###, ***}$**
	2 weeks	0.77 ± 0.02	0.77 ± 0.02	**1.03 ± 0.07§§§**	**0.91 ± 0.02$^{###, ***}$**
	4 weeks	0.78 ± 0.02	0.77 ± 0.02	**1.05 ± 0.06§§§**	**0.91 ± 0.06$^{###, ***}$**
	8 weeks	0.80 ± 0.00	0.78 ± 0.02	**1.06 ± 0.07§§§**	**0.92 ± 0.04$^{###, ***}$**
LVID;d (mm) Mean ± SD	Basal	3.66 ± 0.09	3.60 ± 0.08	3.52 ± 0.05	3.57 ± 0.06
	1 week	3.65 ± 0.10	3.65 ± 0.06	**3.14 ± 0.30§§§**	**3.66 ± 0.11*****
	2 weeks	3.66 ± 0.10	3.66 ± 0.05	**3.19 ± 0.17§§§**	**3.62 ± 0.10*****
	4 weeks	3.68 ± 0.07	3.68 ± 0.04	**3.23 ± 0.13§§§**	**3.77 ± 0.16*****
	8 weeks	3.64 ± 0.02	3.67 ± 0.03	**3.38 ± 0.12§§§**	**3.94 ± 0.09$^{###, ***}$**
RWT Mean ± SD	Basal	0.43 ± 0.01	0.43 ± 0.01	0.42 ± 0.02	0.43 ± 0.01
	1 week	0.41 ± 0.03	0.42 ± 0.01	**0.64 ± 0.03§§§**	**0.50 ± 0.02$^{###, ***}$**
	2 weeks	0.42 ± 0.03	0.42 ± 0.01	**0.64 ± 0.02§§§**	**0.50 ± 0.02$^{###, ***}$**
	4 weeks	0.42 ± 0.02	0.42 ± 0.01	**0.65 ± 0.01§§§**	**0.49 ± 0.02$^{###, ***}$**
	8 weeks	0.44 ± 0.00	0.43 ± 0.01	**0.63 ± 0.04§§§**	**0.47 ± 0.02$^{###, ***}$**
E/e' Mean ± SD	Basal	19.62 ± 0.10	20.42 ± 0.61	20.76 ± 0.48	20.04 ± 0.17
	1 week	20.46 ± 0.44	20.78 ± 0.46	**25.29 ± 0.72§§§**	**30.79 ± 1.76$^{###, ***}$**
	2 weeks	20.63 ± 0.16	20.51 ± 0.46	**26.73 ± 1.54§§§**	**33.96 ± 1.66$^{###, ***}$**
	4 weeks	20.10 ± 0.39	20.48 ± 0.43	**28.87 ± 0.96§§§**	**36.00 ± 2.65$^{###, ***}$**
	8 weeks	20.45 ± 0.70	20.41 ± 0.45	**31.24 ± 3.65§§§**	**35.19 ± 2.43$^{###, ***}$**
LV Volume;d (mm^3) Mean ± SD	Basal	56.62 ± 3.40	54.53 ± 2.97	51.47 ± 1.89	53.51 ± 2.25
	1 week	56.19 ± 3.58	56.31 ± 2.36	**39.64 ± 8.92§§§**	**56.57 ± 4.10*****
	2 weeks	56.74 ± 3.70	56.82 ± 1.88	**40.67 ± 5.14§§§**	**55.27 ± 3.56*****
	4 weeks	57.47 ± 2.53	57.31 ± 1.56	**41.96 ± 3.89§§§**	**61.08 ± 6.02*****
	8 weeks	55.85 ± 0.69	57.08 ± 1.16	**46.76 ± 4.12§§**	**67.73 ± 3.85$^{###, ***}$**
LV mass/ body weight (mg/g) Mean ± SD	Basal	3.63 ± 0.17	4.23 ± 0.70	4.10 ± 0.22	4.15 ± 0.40
	1 week	3.67 ± 0.08	4.00 ± 0.49	**4.98 ± 1.09§§**	**5.09 ± 0.37$^{###}$**
	2 weeks	3.53 ± 0.08	4.18 ± 0.34	**4.75 ± 0.83§§**	**5.22 ± 0.33$^{###}$**
	4 weeks	3.60 ± 0.23	4.05 ± 0.33	**4.92 ± 0.64§§**	**5.29 ± 0.45$^{###}$**
	8 weeks	3.55 ± 0.16	3.85 ± 0.36	**5.46 ± 0.36§§§**	**5.33 ± 0.29$^{###}$**

FS % Mean ± SD	Basal	48 ± 1	48 ± 2	46 ± 2	46 ± 2
	1 week	47 ± 2	47 ± 1	**52 ± 2§**	**38 ± 2$^{\#\#\#, ***}$**
	2 weeks	47 ± 3	48 ± 2	**51 ± 1§**	**41 ± 1$^{\#\#\#, ***}$**
	4 weeks	47 ± 2	47 ± 2	50 ± 1	**36 ± 3$^{\#\#\#, ***}$**
	8 weeks	49 ± 1	47 ± 2	48 ± 3	**26 ± 4$^{\#\#\#, ***}$**
EF % Mean ± SD	Basal	80 ± 1	80 ± 2	78 ± 2	78 ± 2
	1 week	80 ± 2	80 ± 1	84 ± 2	**70 ± 2$^{\#\#\#, ***}$**
	2 weeks	79 ± 3	80 ± 2	83 ± 1	**73 ± 2$^{\#\#\#, ***}$**
	4 weeks	79 ± 2	80 ± 2	82 ± 1	**67 ± 4$^{\#\#\#, ***}$**
	8 weeks	81 ± 1	79 ± 2	80 ± 3	**51 ± 7$^{\#\#\#, ***}$**
Trans-stenotic pressure gradient (mmHg) Mean ± SD	Basal				
	1 week			92 ± 3	94 ± 3
	2 weeks			91 ± 3	92 ± 2
	4 weeks			94 ± 3	95 ± 3
	8 weeks			96 ± 1	97 ± 2

End-diastolic Left Ventricular Posterior Wall thickness (LVPW;d), end-diastolic Interventricular septal (IVS;d), end-diastolic Left Ventricular Internal Diameter (LVID;d), Relative Wall Thickness (RWT), E/e' ratio, end-diastolic Left Ventricle Volume (LV Volume;d), LV mass corrected for the body weight (LV mass/body weight), Fractional shortening (FS), Ejection Fraction (EF) and trans-stenotic pressure gradient. n=3 sham +spleen; n=4 TAC +spleen; n=8 sham -spleen; n=8 TAC -spleen. Data as mean ± SD and analyzed by two-way ANOVA and Tukey post hoc. § $p<0.05$, §§ $p<0.01$ and §§§ $p<0.001$ sham +spleen vs TAC +spleen; ### $p<0.001$ sham -spleen vs TAC -spleen; *** $p<0.001$ TAC +spleen vs TAC -spleen.

Table S3. Serial longitudinal echocardiography of LV remodeling in basal condition and at 1-2-4-8 weeks after TAC in *Nr4a1*$^{-/-}$ and WT mice, and serial longitudinal echocardiography of LV remodeling in basal condition and at 1-2-4-8 weeks after TAC in *Ccr2*$^{-/-}$ and WT mice, related to Figure 2.

Parameter	*Time point*	WT TAC	*Nr4a1*$^{-/-}$ TAC	*p-value*
LVPW;d (mm) Mean ± SD	Basal	0.74 ± 0.03	0.75 ± 0.02	0.99
	1 week	1.04 ± 0.06	1.08 ± 0.04	0.54
	2 weeks	1.14 ± 0.05	1.12 ± 0.03	0.97
	4 weeks	1.13 ± 0.05	1.16 ± 0.03	0.71
	8 weeks	1.12 ± 0.04	1.13 ± 0.07	0.99
IVS;d (mm) Mean ± SD	Basal	0.75 ± 0.03	0.75 ± 0.02	0.99
	1 week	1.04 ± 0.06	1.07 ± 0.05	0.93
	2 weeks	1.14 ± 0.04	1.11 ± 0.05	0.90
	4 weeks	1.13 ± 0.05	1.16 ± 0.03	0.69
	8 weeks	1.12 ± 0.04	1.13 ± 0.07	0.99
LVID;d (mm) Mean ± SD	Basal	3.33 ± 0.09	3.35 ± 0.11	0.99
	1 week	3.03 ± 0.10	2.99 ± 0.07	0.87
	2 weeks	3.00 ± 0.08	3.01 ± 0.06	0.99
	4 weeks	3.11 ± 0.06	3.19 ± 0.06	0.53
	8 weeks	3.20 ± 0.12	3.29 ± 0.08	0.40
RWT Mean ± SD	Basal	0.45 ± 0.01	0.45 ± 0.02	0.99
	1 week	0.69 ± 0.06	0.72 ± 0.04	0.54
	2 weeks	0.76 ± 0.05	0.74 ± 0.03	0.91
	4 weeks	0.73 ± 0.03	0.73 ± 0.03	0.99
	8 weeks	0.70 ± 0.03	0.69 ± 0.03	0.95
E/e' Mean ± SD	Basal	19.62 ± 0.29	20.00 ± 0.35	0.99
	1 week	27.26 ± 2.54	27.37 ± 1.10	0.99
	2 weeks	28.36 ± 1.94	27.90 ± 1.25	0.99
	4 weeks	30.02 ± 2.44	31.52 ± 2.00	0.56
	8 weeks	31.56 ± 1.92	31.84 ± 1.74	0.99
LV Volume;d (mm^3) Mean ± SD	Basal	45.12 ± 2.90	45.75 ± 3.62	0.99
	1 week	36.30 ± 2.64	35.21 ± 0.97	0.95
	2 weeks	34.96 ± 2.26	35.22 ± 1.77	0.99
	4 weeks	38.25 ± 1.70	40.52 ± 1.92	0.51
	8 weeks	41.12 ± 3.72	43.72 ± 2.68	0.35
LV mass/ body weight (mg/g) Mean ± SD	Basal	3.33 ± 0.18	3.63 ± 0.21	0.60
	1 week	4.75 ± 0.27	5.05 ± 0.37	0.60
	2 weeks	5.16 ± 0.28	5.12 ± 0.29	0.99
	4 weeks	5.11 ± 0.45	5.59 ± 0.25	0.15

	8 weeks	5.00 ± 0.48	5.37 ± 0.76	0.39
FS % Mean ± SD	Basal	48 ± 1	48 ± 1	0.99
	1 week	48 ± 2	49 ± 2	0.44
	2 weeks	48 ± 1	49 ± 1	0.80
	4 weeks	47 ± 2	49 ± 2	0.23
	8 weeks	49 ± 1	49 ± 2	0.95
EF % Mean ± SD	Basal	80 ± 1	80 ± 1	0.99
	1 week	80 ± 2	82 ± 2	0.40
	2 weeks	81 ± 1	82 ± 1	0.81
	4 weeks	80 ± 2	82 ± 2	0.28
	8 weeks	81 ± 1	82 ± 1	0.98
Trans-stenotic pressure gradient (mmHg) Mean ± SD	Basal			
	1 week	95 ± 5	95 ± 3	0.99
	2 weeks	95 ± 4	95 ± 2	0.99
	4 weeks	97 ± 3	97 ± 2	0.99
	8 weeks	95 ± 3	97 ± 4	0.76

Parameter	***Time point***	**WT TAC**	***Ccr2*$^{-/-}$ TAC**	***p-value***
LVPW;d (mm) Mean ± SD	Basal	0.75 ± 0.02	0.78 ± 0.01	0.98
	1 week	0.99 ± 0.05	0.96 ± 0.05	0.94
	2 weeks	1.03 ± 0.06	1.02 ± 0.06	0.99
	4 weeks	1.08 ± 0.05	1.04 ± 0.09	0.82
	8 weeks	1.14 ± 0.07	1.23 ± 0.01	0.09
IVS;d (mm) Mean ± SD	Basal	0.76 ± 0.02	0.78 ± 0.03	0.99
	1 week	1.01 ± 0.06	1.00 ± 0.05	0.99
	2 weeks	1.03 ± 0.05	1.02 ± 0.08	0.99
	4 weeks	1.09 ± 0.04	1.07 ± 0.09	0.99
	8 weeks	1.17 ± 0.04	1.21 ± 0.03	0.85
LVID;d (mm) Mean ± SD	Basal	3.38 ± 0.08	3.35 ± 0.12	0.99
	1 week	3.17 ± 0.08	3.14 ± 0.29	0.99
	2 weeks	3.10 ± 0.11	3.18 ± 0.09	0.90
	4 weeks	3.19 ± 0.12	3.20 ± 0.26	0.99
	8 weeks	3.22 ± 0.10	3.39 ± 0.02	0.43
RWT Mean ± SD	Basal	0.45 ± 0.02	0.46 ± 0.02	0.99
	1 week	0.63 ± 0.04	0.63 ± 0.09	0.99
	2 weeks	0.67 ± 0.05	0.64 ± 0.05	0.94
	4 weeks	0.68 ± 0.03	0.66 ± 0.09	0.99
	8 weeks	0.72 ± 0.03	0.72 ± 0.01	0.99

E/e' Mean ± SD	Basal	19.59 ± 1.23	20.59 ± 0.40	0.97
	1 week	27.00 ± 1.44	29.64 ± 3.09	0.36
	2 weeks	29.59 ± 1.48	28.96 ± 1.17	0.99
	4 weeks	30.96 ± 1.99	32.43 ± 3.54	0.87
	8 weeks	32.12 ± 2.42	30.98 ± 3.71	0.95
LV Volume;d (mm^3) Mean ± SD	Basal	46.66 ± 2.53	45.96 ± 3.91	0.99
	1 week	40.09 ± 2.35	39.58 ± 8.66	0.99
	2 weeks	37.89 ± 3.33	40.45 ± 2.78	0.91
	4 weeks	40.71 ± 3.65	41.38 ± 7.93	0.99
	8 weeks	41.78 ± 3.04	46.95 ± 0.78	0.36
LV mass/ body weight (mg/g) Mean ± SD	Basal	3.42 ± 0.21	3.74 ± 0.33	0.86
	1 week	4.54 ± 0.33	5.03 ± 0.29	0.50
	2 weeks	4.39 ± 0.32	4.97 ± 0.62	0.35
	4 weeks	4.92 ± 0.50	5.16 ± 0.87	0.94
	8 weeks	**5.17 ± 0.70**	**6.43 ± 0.08**	**0.002**
FS % Mean ± SD	Basal	49 ± 2	50 ± 1	0.99
	1 week	48 ± 2	47 ± 1	0.99
	2 weeks	48 ± 2	49 ± 4	0.99
	4 weeks	51 ± 2	48 ± 2	0.53
	8 weeks	52 ± 7	50 ± 2	0.90
EF % Mean ± SD	Basal	82 ± 2	82 ± 1	0.99
	1 week	81 ± 2	80 ± 1	0.99
	2 weeks	81 ± 2	82 ± 4	0.99
	4 weeks	83 ± 2	80 ± 2	0.44
	8 weeks	84 ± 6	83 ± 2	0.96
Trans-stenotic pressure gradient (mmHg) Mean ± SD	Basal			
	1 week	95 ± 4	99 ± 1	0.11
	2 weeks	95 ± 3	97 ± 2	0.73
	4 weeks	94 ± 3	96 ± 2	0.83
	8 weeks	96 ± 3	98 ± 2	0.74

End-diastolic Left Ventricular Posterior Wall thickness (LVPW;d), end-diastolic Interventricular septal (IVS;d), end-diastolic Left Ventricular Internal Diameter (LVID;d), Relative Wall Thickness (RWT), E/e' ratio, end-diastolic Left Ventricle Volume (LV Volume;d), LV mass corrected for the body weight (LV mass/body weight), Fractional shortening (FS), Ejection Fraction (EF) and trans-stenotic pressure gradient.

Nr4a1$^{-/-}$ and WT mice subjected to TAC: n=8 WT TAC; n=5 *Nr4a1*$^{-/-}$ TAC. Data as mean ± SD and analyzed by two-way ANOVA and Sidak post hoc.

Ccr2$^{-/-}$ and WT mice subjected to TAC: n=5 WT TAC; n=4 *Ccr2*$^{-/-}$ TAC. Data as mean ± SD and analyzed by two-way ANOVA and Sidak post hoc.

Table S4. Serial longitudinal echocardiography of LV remodeling in basal condition and at 1-2-4-8 weeks after TAC in *Cx3cr1*$^{-/-}$ and WT mice, related to Figure 3.

Parameter	*Time point*	WT TAC	*Cx3cr1*$^{-/-}$ TAC	*p-value*
LVPW;d (mm) Mean ± SD	Basal	0.79 ± 0.02	0.77 ± 0.02	0.98
	1 week	1.04 ± 0.09	0.90 ± 0.03	**0.0001**
	2 weeks	1.10 ± 0.03	0.91 ± 0.04	**<0.0001**
	4 weeks	1.15 ± 0.04	0.94 ± 0.05	**<0.0001**
	8 weeks	1.19 ± 0.02	0.87 ± 0.06	**<0.0001**
IVS;d (mm) Mean ± SD	Basal	0.79 ± 0.01	0.77 ± 0.02	0.95
	1 week	1.07 ± 0.08	0.88 ± 0.02	**<0.0001**
	2 weeks	1.12 ± 0.03	0.88 ± 0.02	**<0.0001**
	4 weeks	1.15 ± 0.03	0.90 ± 0.03	**<0.0001**
	8 weeks	1.19 ± 0.02	0.92 ± 0.09	**<0.0001**
LVID;d (mm) Mean ± SD	Basal	3.45 ± 0.06	3.28 ± 0.11	0.83
	1 week	3.20 ± 0.08	3.61 ± 0.20	0.07
	2 weeks	3.23 ± 0.09	3.81 ± 0.39	**0.0054**
	4 weeks	3.30 ± 0.11	3.77 ± 0.39	**0.0290**
	8 weeks	3.39 ± 0.09	3.76 ± 0.34	0.13
RWT Mean ± SD	Basal	0.46 ± 0.01	0.47 ± 0.01	0.99
	1 week	0.66 ± 0.05	0.49 ± 0.02	**<0.0001**
	2 weeks	0.69 ± 0.03	0.48 ± 0.06	**<0.0001**
	4 weeks	0.70 ± 0.02	0.49 ± 0.06	**<0.0001**
	8 weeks	0.70 ± 0.02	0.48 ± 0.08	**<0.0001**
E/e' Mean ± SD	Basal	19.96 ± 0.33	19.87 ± 0.75	0.99
	1 week	26.56 ± 1.89	28.72 ± 3.47	0.58
	2 weeks	31.67 ± 3.19	32.17 ± 1.85	0.99
	4 weeks	30.17 ± 1.51	33.74 ± 2.59	0.11
	8 weeks	32.40 ± 0.64	33.67 ± 3.07	0.92
LV Volume;d (mm^3) Mean ± SD	Basal	49.26 ± 2.17	43.63 ± 3.50	0.89
	1 week	41.14 ± 2.50	55.04 ± 7.21	0.14
	2 weeks	41.98 ± 2.83	63.00 ± 15.31	**0.0082**
	4 weeks	44.18 ± 3.75	61.75 ± 15.80	**0.0360**
	8 weeks	47.29 ± 3.17	61.10 ± 12.60	0.14
LV mass/ body weight (mg/g) Mean ± SD	Basal	3.80 ± 0.19	3.76 ± 0.29	0.99
	1 week	5.04 ± 0.47	4.91 ± 0.71	0.99
	2 weeks	5.42 ± 0.24	5.47 ± 0.78	0.99
	4 weeks	5.62 ± 0.51	5.27 ± 0.74	0.87
	8 weeks	5.84 ± 0.49	4.63 ± 0.48	**0.01**

FS % Mean ± SD	Basal	47 ± 2	48 ± 2	0.99
	1 week	48 ± 2	36 ± 4	**<0.0001**
	2 weeks	47 ± 1	34 ± 2	**<0.0001**
	4 weeks	48 ± 3	33 ± 3	**<0.0001**
	8 weeks	49 ± 3	34 ± 3	**<0.0001**
EF % Mean ± SD	Basal	80 ± 2	81 ± 2	0.99
	1 week	81 ± 2	67 ± 60	**<0.0001**
	2 weeks	79 ± 1	63 ± 3	**<0.0001**
	4 weeks	80 ± 3	63 ± 4	**<0.0001**
	8 weeks	81 ± 3	64 ± 4	**<0.0001**
Trans-stenotic pressure gradient (mmHg) Mean ± SD	Basal			
	1 week	95 ± 5	89 ± 2	0.05
	2 weeks	97 ± 2	96 ± 1	0.98
	4 weeks	97 ± 2	92 ± 1	**0.02**
	8 weeks	93 ± 4	88 ± 4	0.07

End-diastolic Left Ventricular Posterior Wall thickness (LVPW;d), end-diastolic Interventricular septal (IVS;d), end-diastolic Left Ventricular Internal Diameter (LVID;d), Relative Wall Thickness (RWT), E/e' ratio, end-diastolic Left Ventricle Volume (LV Volume;d), LV mass corrected for the body weight (LV mass/body weight), Fractional shortening (FS), Ejection Fraction (EF) and trans-stenotic pressure gradient. n=4 WT TAC; n=5 *Cx3cr1*$^{-/-}$ TAC. Data as mean ± SD and analyzed by two-way ANOVA and Sidak post hoc.

Table S5. Echocardiography of LV remodeling in basal condition and at the endpoint (5 weeks after TAC) in WT mice with a *Pgf*$^{-/-}$ spleen or *Pgf*$^{-/-}$ mice with a WT spleen, and serial longitudinal echocardiography of LV remodeling in basal condition and at the endpoint (5 weeks after TAC) in splenectomized mice (-spleen) treated with rPlGF or vehicle as control, related to Figure 4.

Parameter	*Time point*	*Pgf*$^{-/-}$ spleen in WT mice TAC	WT spleen in *Pgf*$^{-/-}$ mice TAC	*p-value*
LVPW;d (mm) Mean ± SD	Basal	0.77 ± 0.04	0.75 ± 0.02	0.92
	5 weeks	0.94 ± 0.05	1.06 ± 0.08	**0.0172**
IVS;d (mm) Mean ± SD	Basal	0.78 ± 0.03	0.75 ± 0.02	0.76
	5 weeks	0.95 ± 0.04	1.08 ± 0.07	**0.0045**
LVID;d (mm) Mean ± SD	Basal	3.37 ± 0.03	3.46 ± 0.07	0.56
	5 weeks	3.76 ± 0.14	3.46 ± 0.19	**0.0157**
RWT Mean ± SD	Basal	0.46 ± 0.02	0.44 ± 0.02	0.71
	5 weeks	0.50 ± 0.02	0.62 ± 0.08	**0.0044**
E/e' Mean ± SD	Basal	20.77 ± 0.66	20.43 ± 0.36	0.98
	5 weeks	38.00 ± 2.47	31.51 ± 4.99	**0.0181**
LV Volume;d (mm^3) Mean ± SD	Basal	46.39 ± 1.11	49.47 ± 2.48	0.60
	5 weeks	60.52 ± 5.32	49.49 ± 6.51	**0.0147**
LV mass/ body weight (mg/g) Mean ± SD	Basal	3.04 ± 0.32	3.38 ± 0.11	0.46
	5 weeks	4.79 ± 0.58	5.33 ± 0.26	0.15
FS % Mean ± SD	Basal	48 ± 2	46 ± 1	0.27
	5 weeks	37 ± 2	45 ± 1	**0.0004**
EF % Mean ± SD	Basal	81 ± 2	78 ± 2	0.35
	5 weeks	68 ± 3	78 ± 1	**0.0003**
Trans-stenotic pressure gradient (mmHg) Mean ± SD	Basal			
	5 weeks	95 ± 2	97 ± 3	0.46

Parameter	*Time point*	-spleen veh TAC	-spleen rPlGF TAC	*p-value*
LVPW;d (mm) Mean ± SD	Basal	0.80 ± 0.10	0.76 ± 0.02	0.79
	5 weeks	1.04 ± 0.19	1.17 ± 0.05	0.23
IVS;d (mm) Mean ± SD	Basal	0.80 ± 0.07	0.74 ± 0.03	0.69
	5 weeks	1.07 ± 0.13	1.12 ± 0.11	0.66
LVID;d (mm) Mean ± SD	Basal	3.15 ± 0.15	3.26 ± 0.15	0.58
	5 weeks	3.38 ± 0.15	2.99 ± 0.30	**0.0189**
RWT Mean ± SD	Basal	0.51 ± 0.07	0.46 ± 0.01	0.61
	5 weeks	0.63 ± 0.10	0.77 ± 0.12	**0.0423**

E/e' **Mean ± SD**	Basal	20.69 ± 2.14	20.98 ± 1.98	0.98
	5 weeks	23.88 ± 4.05	27.69 ± 2.34	0.12
LV Volume;d (mm^3) **Mean ± SD**	Basal	39.51 ± 4.62	43.15 ± 4.58	0.61
	5 weeks	46.84 ± 5.06	35.08 ± 8.84	**0.0192**
LV mass/ **body weight (mg/g)** **Mean ± SD**	Basal	3.60 ± 0.36	3.27 ± 0.38	0.67
	5 weeks	5.01 ± 0.96	4.80 ± 0.28	0.84
FS % **Mean ± SD**	Basal	45 ± 2	48 ± 2	0.76
	5 weeks	32 ± 8	46 ± 2	**0.0013**
EF % **Mean ± SD**	Basal	78 ± 3	80 ± 2	0.95
	5 weeks	61 ± 12	79 ± 2	**0.002**
Trans-stenotic pressure gradient (mmHg) **Mean ± SD**	Basal			
	5 weeks	93 ± 9	97 ± 7	0.63

End-diastolic Left Ventricular Posterior Wall thickness (LVPW;d), end-diastolic Interventricular septal (IVS;d), end-diastolic Left Ventricular Internal Diameter (LVID;d), Relative Wall Thickness (RWT), E/e' ratio, end-diastolic Left Ventricle Volume (LV Volume;d), LV mass corrected for the body weight (LV mass/body weight), Fractional shortening (FS), Ejection Fraction (EF) and trans-stenotic pressure gradient.

WT mice with a $Pgf^{-/-}$ spleen and $Pgf^{-/-}$ mice with a WT spleen subjected to TAC: n=4 WT transplanted with $Pgf^{-/-}$ spleen; n=3 $Pgf^{-/-}$ transplanted with WT spleen. Data as mean ± SD and analyzed by two-way ANOVA and Sidak post hoc. Trans-stenotic pressure gradient was analyzed by unpaired T test

Splenectomized mice (-spleen) treated with rPlGF or vehicle as control subjected to TAC: n=5 TAC -spleen veh; n=4 TAC -spleen rPlGF. Data as mean ± SD and analyzed by two-way ANOVA and Sidak post hoc. Trans-stenotic pressure gradient was analyzed by unpaired T test.

Table S6. Serial longitudinal echocardiography of LV remodeling in basal condition and at 1-2-4-8 weeks after TAC in *Nrp1$^{fl/fl}$;Lyz2$^{cre/+}$* and *Nrp1$^{fl/fl}$;Lyz2$^{+/+}$* mice, and serial longitudinal echocardiography of LV remodeling in basal condition and at 2-4 weeks after TAC in RM$^{RFP-\Delta Nrp1}$ and RM^{RFP} mice, related to Figure 6.

Parameter	*Time point*	*Nrp1$^{fl/fl}$;Lyz2$^{+/+}$* TAC	*Nrp1$^{fl/fl}$;Lyz2$^{cre/+}$* TAC	*p-value*
LVPW;d (mm) Mean ± SD	Basal	0.75 ± 0.03	0.74 ± 0.02	0.99
	1 week	1.02 ± 0.02	0.94 ± 0.03	**<0.0001**
	2 weeks	1.08 ± 0.02	0.97 ± 0.02	**<0.0001**
	4 weeks	1.10 ± 0.02	0.97 ± 0.05	**<0.0001**
	8 weeks	1.11 ± 0.03	0.96 ± 0.04	**<0.0001**
IVS;d (mm) Mean ± SD	Basal	0.75 ± 0.03	0.74 ± 0.02	0.99
	1 week	1.03 ± 0.02	0.94 ± 0.03	**<0.0001**
	2 weeks	1.08 ± 0.02	0.97 ± 0.02	**<0.0001**
	4 weeks	1.10 ± 0.02	0.97 ± 0.05	**<0.0001**
	8 weeks	1.11 ± 0.03	0.96 ± 0.04	**<0.0001**
LVID;d (mm) Mean ± SD	Basal	3.43 ± 0.09	3.45 ± 0.06	0.99
	1 week	3.11 ± 0.10	3.34 ± 0.08	**0.0001**
	2 weeks	3.12 ± 0.07	3.35 ± 0.04	**<0.0001**
	4 weeks	3.11 ± 0.11	3.41 ± 0.07	**<0.0001**
	8 weeks	3.12 ± 0.13	3.49 ± 0.10	**<0.0001**
RWT Mean ± SD	Basal	0.44 ± 0.02	0.43 ± 0.01	0.99
	1 week	0.66 ± 0.03	0.56 ± 0.02	**<0.0001**
	2 weeks	0.69 ± 0.02	0.58 ± 0.01	**<0.0001**
	4 weeks	0.71 ± 0.04	0.57 ± 0.04	**<0.0001**
	8 weeks	0.71 ± 0.05	0.55 ± 0.04	**<0.0001**
E/e' Mean ± SD	Basal	20.66 ± 0.54	20.69 ± 0.51	0.99
	1 week	27.94 ± 1.75	27.13 ± 1.67	0.98
	2 weeks	31.07 ± 4.23	31.98 ± 2.82	0.96
	4 weeks	33.21 ± 2.14	33.52 ± 2.96	0.99
	8 weeks	35.46 ± 0.78	35.70 ± 3.34	0.99
LV Volume;d (mm^3) Mean ± SD	Basal	48.65 ± 2.98	49.16 ± 2.07	0.99
	1 week	38.39 ± 2.97	45.50 ± 2.65	**0.0001**
	2 weeks	38.68 ± 2.23	45.85 ± 1.32	**0.0001**
	4 weeks	38.22 ± 3.28	47.66 ± 2.47	**<0.0001**
	8 weeks	38.67 ± 4.06	50.51 ± 3.41	**<0.0001**
LV mass/ body weight (mg/g) Mean ± SD	Basal	3.52 ± 0.30	3.80 ± 0.33	0.76
	1 week	4.91 ± 0.60	5.46 ± 0.58	0.12
	2 weeks	5.04 ± 0.39	5.07 ± 0.30	0.99
	4 weeks	4.98 ± 0.48	4.73 ± 0.42	0.84

	8 weeks	4.98 ± 0.45	4.59 ± 0.38	0.43
FS % Mean ± SD	Basal	48 ± 2	48 ± 1	0.99
	1 week	51 ± 1	44 ± 3	**<0.0001**
	2 weeks	51 ± 1	44 ± 2	**<0.0001**
	4 weeks	52 ± 2	43 ± 3	**<0.0001**
	8 weeks	50 ± 1	41 ± 1	**<0.0001**
EF % Mean ± SD	Basal	80 ± 2	80 ± 1	0.99
	1 week	83 ± 1	77 ± 3	**<0.0001**
	2 weeks	83 ± 1	76 ± 3	**<0.0001**
	4 weeks	84 ± 2	75 ± 3	**<0.0001**
	8 weeks	83 ± 1	73 ± 1	**<0.0001**
Trans-stenotic pressure gradient (mmHg) Mean ± SD	Basal			
	1 week	95 ± 3	94 ± 2	0.85
	2 weeks	94 ± 2	94 ± 3	0.99
	4 weeks	95 ± 3	97 ± 3	0.69
	8 weeks	96 ± 2	96 ± 3	0.99

Parameter	*Time point*	**RM^{RFP} TAC**	**$RM^{RFP-\Delta Nrp1}$ TAC**	*p-value*
LVPW;d (mm) Mean ± SD	Basal	0.77 ± 0.03	0.76 ± 0.07	0.76
	2 weeks	0.98 ± 0.14	1.01 ± 0.20	0.98
	4 weeks	1.03 ± 0.05	0.97 ± 0.08	0.30
IVS;d (mm) Mean ± SD	Basal	0.77 ± 0.04	0.77 ± 0.07	0.99
	2 weeks	1.01 ± 0.08	0.97 ± 0.07	0.66
	4 weeks	1.05 ± 0.07	0.94 ± 0.08	**0.0351**
LVID;d (mm) Mean ± SD	Basal	3.26 ± 0.18	3.30 ± 0.21	0.74
	2 weeks	3.19 ± 0.21	3.23 ± 0.22	0.73
	4 weeks	3.2 ± 0.20	3.44 ± 0.55	0.27
RWT Mean ± SD	Basal	0.48 ± 0.04	0.47 ± 0.06	0.96
	2 weeks	0.63 ± 0.11	0.62 ± 0.06	0.98
	4 weeks	0.65 ± 0.06	0.56 ± 0.07	**0.0427**
E/e' Mean ± SD	Basal	20.42 ± 1.01	20.89 ± 1.77	0.90
	2 weeks	25.56 ± 3.19	26.29 ± 3.74	0.97
	4 weeks	24.78 ± 3.69	25.45 ± 4.68	0.99
LV Volume;d (mm^3) Mean ± SD	Basal	43.08 ± 5.47	44.28 ± 6.47	0.98
	2 weeks	40.81 ± 6.55	42.11 ± 7.13	0.98
	4 weeks	41.18 ± 6.28	50.61 ± 20.42	0.58
LV mass/	Basal	3.37 ± 0.60	3.51 ± 0.38	0.95
	2 weeks	4.71 ± 0.58	5.17 ± 0.92	0.60

body weight (mg/g) Mean ± SD	4 weeks	4.76 ± 0.20	5.04 ± 1.56	0.95
FS % Mean ± SD	Basal	44 ± 3	44 ± 4	0.99
	2 weeks	46 ± 3	39 ± 6	0.15
	4 weeks	45 ± 4	33 ± 10	**0.0471**
EF % Mean ± SD	Basal	76 ± 3	77 ± 5	0.88
	2 weeks	78 ± 4	71 ± 8	0.05
	4 weeks	77 ± 4	61 ± 18	**0.0424**
Trans-stenotic pressure gradient (mmHg) Mean ± SD	Basal			
	2 weeks	94 ± 7	97 ± 6	0.59
	4 weeks	92 ± 6	97 ± 5	0.17

End-diastolic Left Ventricular Posterior Wall thickness (LVPW;d), end-diastolic Interventricular septal (IVS;d), end-diastolic Left Ventricular Internal Diameter (LVID;d), Relative Wall Thickness (RWT), E/e' ratio, end-diastolic Left Ventricle Volume (LV Volume;d), LV mass corrected for the body weight (LV mass/body weight), Fractional shortening (FS), Ejection Fraction (EF) and trans-stenotic pressure gradient.

Nrp1$^{fl/fl}$*;Lyz2*$^{cre/+}$ and *Nrp1*$^{fl/fl}$*;Lyz2*$^{+/+}$ mice subjected to TAC: n=6 *Nrp1*$^{fl/fl}$*;Lyz2*$^{+/+}$ TAC; n=7 *Nrp1*$^{fl/fl}$*;Lyz2*$^{cre/+}$ TAC. Data as mean ± SD and analyzed by two-way ANOVA and Sidak post hoc.

RM$^{RFP\text{-}\Delta Nrp1}$ and RM^{RFP} mice subjected to TAC: n=6 RM^{RFP} TAC; n=8 RM$^{RFP\text{-}\Delta Nrp1}$ TAC. Data as mean ± SD and analyzed by two-way ANOVA and Sidak post hoc.

Table S7. Clinical characteristics of hypertensive heart disease (HTN-HD) and controls (Ctrl), related to Figure 7.

Variables	**Ctrl (n=16)**	**HTN-HD (n=14)**
Age, years	47.8±10.5	56.9±5.3 **
Male, n (%)	14 (87.5)	12 (85.7)
Risk factors		
History of Hypertension, n (%)	0 (0%)	14 (100%)
Hypercholesterolaemia, n (%)	5 (31.3)	9 (64.3)
Diabetes mellitus, n (%)	2 (12.5)	5 (35.7)
Kidney disease, n (%)	1 (6.3)	3 (21.4)
CAD, n (%)	0 (0)	11 (78.6) **
BMI, kg/m^2	24.6±3	25.3±4.6
Smokers, n (%)	2 (12.5)	1 (7.1)
DCM, n (%)	5 (31.3)	12 (85.7) **
HCM, n (%)	1 (6.3)	1 (7.1)
ARVCM, n (%)	2 (12.5)	0 (0)
Echocardiography		
LVEDD (mm)	51.5 (41.8; 75.8)	66 (60; 79.5)
LVESD (mm)	34 (25.5; 64.5)	55.5 (51.3; 68.5)
EF (%)	30 (15; 64)	17 (14.3; 20.8)
LA area (cm^2)	27.65±10.13	27.08±6.99
RA area (cm^2)	22.5 (15.8; 37.3)	20 (15; 21)

The continuous variables are presented as mean (SD) or as median (Q1; Q3), whereas the categorical variables are presented as numbers and percentages. Hypertension was defined as the history of the previous diagnosis of hypertension. *****$p<0.05$ vs Ctrl, ******$p<0.01$ vs Ctrl.

Abbreviations: ARVCM - Arrhythmogenic right ventricular cardiomyopathy; BMI – Body mass index; CAD – coronary artery disease; DCM - Dilated cardiomyopathy; EF - Ejection fraction; HCM - Hypertrophic cardiomyopathy; HTN-HD - hypertensive heart disease; LA - Left atrium; LVEDD - Left ventricular end-diastolic diameter; LVESD - Left ventricular end-systolic diameter; RA - Right atrium.