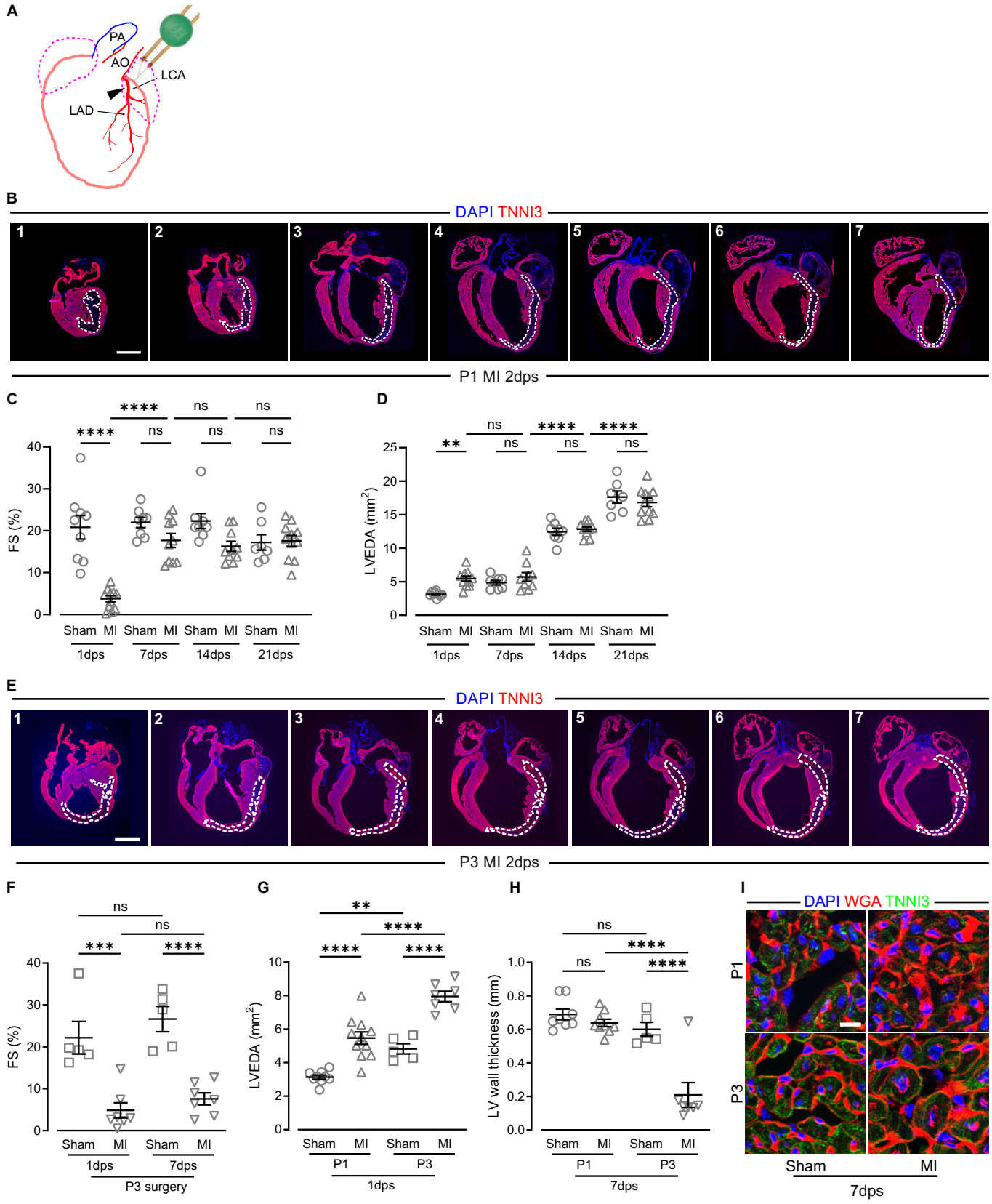
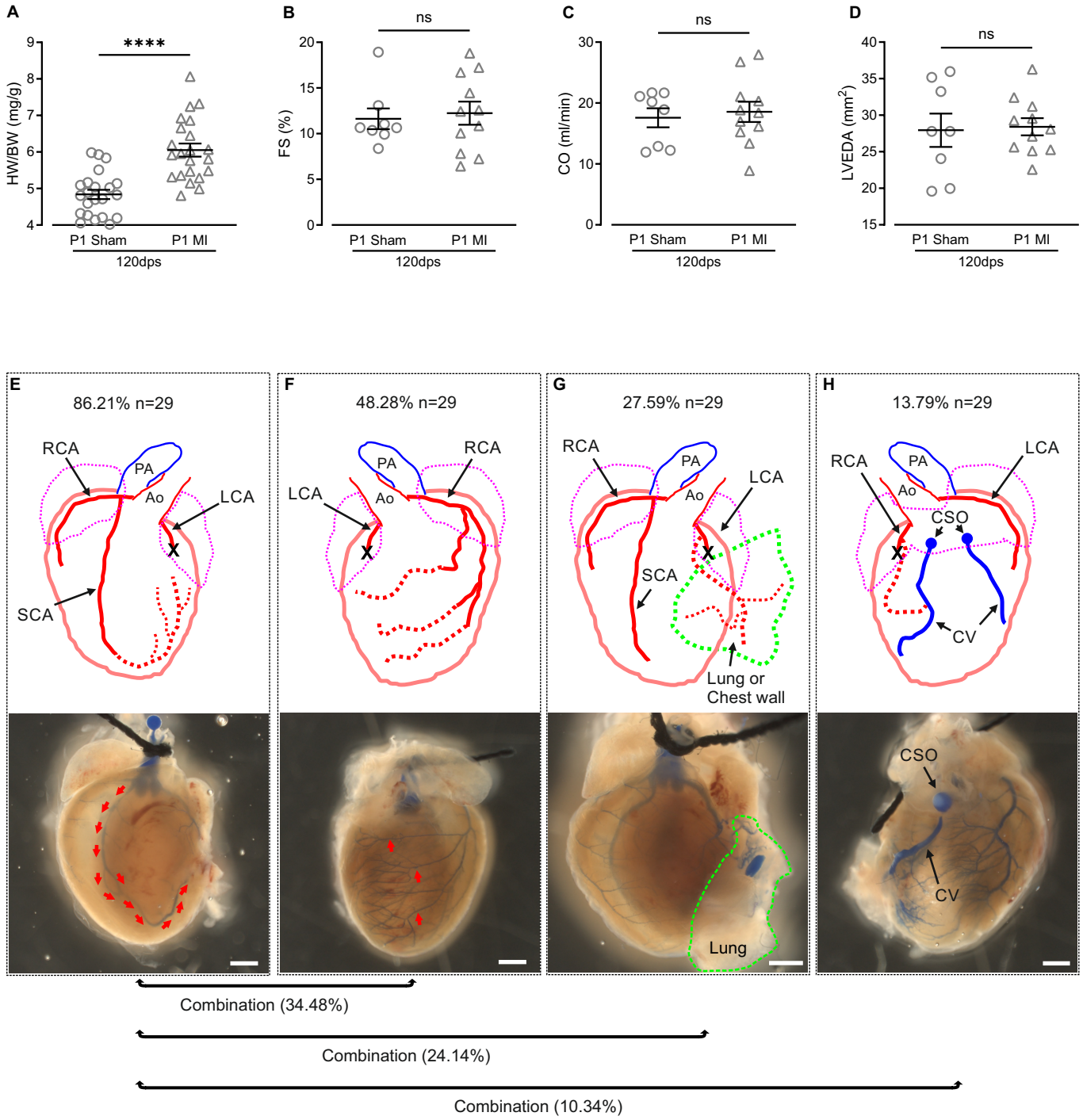

A
PA
AO
LCA
LAD
B
DAPI TNNI3
1
2
3
4
5
6
7
P1 MI 2dps
C
FS (%)
Sham MI Sham MI Sham MI Sham MI
1dps 7dps 14dps 21dps
D
LVEDA (mm2)
Sham MI Sham MI Sham MI Sham MI
1dps 7dps 14dps 21dps
E
DAPI TNNI3
P3 MI 2dps
F
FS (%)
Sham MI Sham MI
1dps 7dps
P3 surgery
G
LVEDA (mm2)
Sham MI Sham MI
P1 P3
1dps
H
LV wall thickness (mm)
Sham MI Sham MI
P1 P3
7dps
I
DAPI WGA TNNI3
P1
P3
Sham MI
7dps

Supplemental Figure 1: Histological and echocardiographic analysis of P1 and P3 MI, and sham hearts.

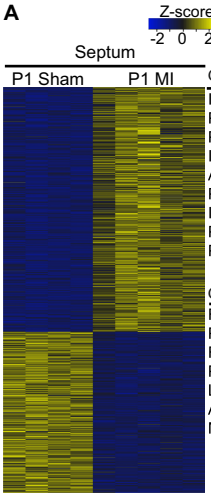
(**A**) Scheme of the cauterization injury of the LCA (black arrowhead) in the neonatal mouse heart; PA = Pulmonary Artery; Ao = Aorta; LAD = Left Anterior Descending coronary artery; LCA = Left Coronary Artery. (**B**) Serial sections taken at approximately equal distance through a P1 MI heart co-stained against TNNI3 and DAPI; dashed lines mark infarct areas; scale bar=1mm. (**C** and **D**) Echocardiographic assessment of fractional shortening (FS) (C) and LV end-diastolic area (LVEDA) (D) after P1 surgery. (**E**) Serial sections through a P3 MI heart co-stained as in A; scale bar=1mm. (**F-H**) Echocardiographic assessment of FS (F), LVEDA (G) and LV wall thickness (H). (**I**) CM cross-sectional area measured in heart sections co-stained against WGA, TNNI3 and DAPI. Scale bar=20µm; (**p<0.01; ****p<0.0001; ns = no significance; one-way ANOVA with Holm-Šídák post hoc test).


A
****
HW/BW (mg/g)
P1 Sham
P1 MI
120dps
B
ns
FS (%)
P1 Sham
P1 MI
120dps
C
ns
CO (ml/min)
P1 Sham
P1 MI
120dps
D
ns
LVEDA (mm2)
P1 Sham
P1 MI
120dps
E
86.21% n=29
RCA
PA
Ao
LCA
SCA
F
48.28% n=29
RCA
PA
LCA
Ao
G
27.59% n=29
RCA
PA
LCA
Ao
SCA
Lung or
Chest wall
H
13.79% n=29
RCA
PA
LCA
Ao
CSO
CV
Lung
Combination (34.48%)
Combination (24.14%)
Combination (10.34%)

Supplemental Figure 2: Histological and echocardiographic analysis of P1 MI and sham hearts at 120 dps, vessel casting in P1 MI hearts at 21 dps.

(**A**) Quantitation of heart- to body weight (HW/BW) ratio. (**B-D**) Echocardiographic assessment of FS (B), Cardiac Output (CO) (C) and LVEDA (D). (**E-H**) Schematics (upper panels) of collateral vessels connecting the LV coronary artery to septal coronary artery (E), right coronary artery (F), lung or other extra cardiac vessels (G) and coronary veins (H). Images of coronary artery vessel casting (lower panels) in P1 MI hearts; red arrows mark the extension of branches of septal (E) and right coronary artery (F), numbers indicate frequencies of the different variations. Scale bar=1mm. (CV = Coronary Vein; CSO = Coronary Sinus Orifice; ****p<0.0001; ns = no significance, unpaired t-test).

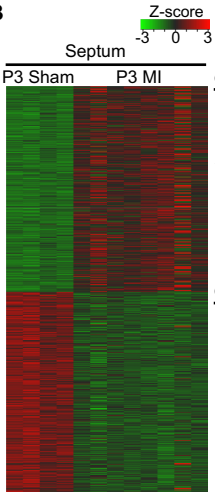
A



Gene ontology of upregulated genes	# of Genes	-log (P-value)
Inflammatory response	44	14.49321
Positive regulation of cell migration	31	12.15224
Positive regulation of inflammatory response	18	11.4507
Immune system process	38	9.228095
Angiogenesis	27	7.697353
Positive regulation of cell proliferation	41	6.624964
Innate immune response	33	6.162225
Positive regulation of angiogenesis	16	5.392967
Positive regulation of ERK1 and ERK2 cascade	20	5.311235

Gene ontology of downregulated genes	# of Genes	-log (P-value)
Fatty acid beta-oxidation	5	2.043763
Positive regulation of fatty acid oxidation	3	2.043125
Fatty acid metabolic process	9	2.038974
Positive regulation of fatty acid beta-oxidation	3	1.939344
Lipid metabolic process	17	1.887173
Angiogenesis	11	1.82281
Metabolic process	15	1.268561

B

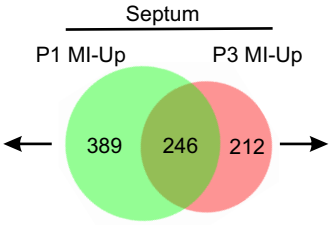


Gene ontology of upregulated genes	# of Genes	-log (P-value)
Inflammatory response	24	5.598298
Positive regulation of cell migration	20	6.903576
Positive regulation of inflammatory response	11	5.967721
Angiogenesis	29	12.26002
Positive regulation of cell proliferation	34	6.839406
Positive regulation of angiogenesis	13	4.82976
Positive regulation of ERK1 and ERK2 cascade	15	4.12601

Gene ontology of downregulated genes	# of Genes	-log (P-value)
Cell cycle	35	7.217229
DNA replication	15	6.849056
Cell division	20	3.773737
Metabolic process	22	3.440299
DNA replication initiation	5	2.93956
Mitotic nuclear division	13	2.040041
Positive regulation of fatty acid oxidation	3	1.99973
Negative regulation of cell growth	8	1.923943
Fatty acid metabolic process	9	1.912661
Oxidation-reduction process	23	1.826911
Regulation of mitotic cell cycle	4	1.444837

C

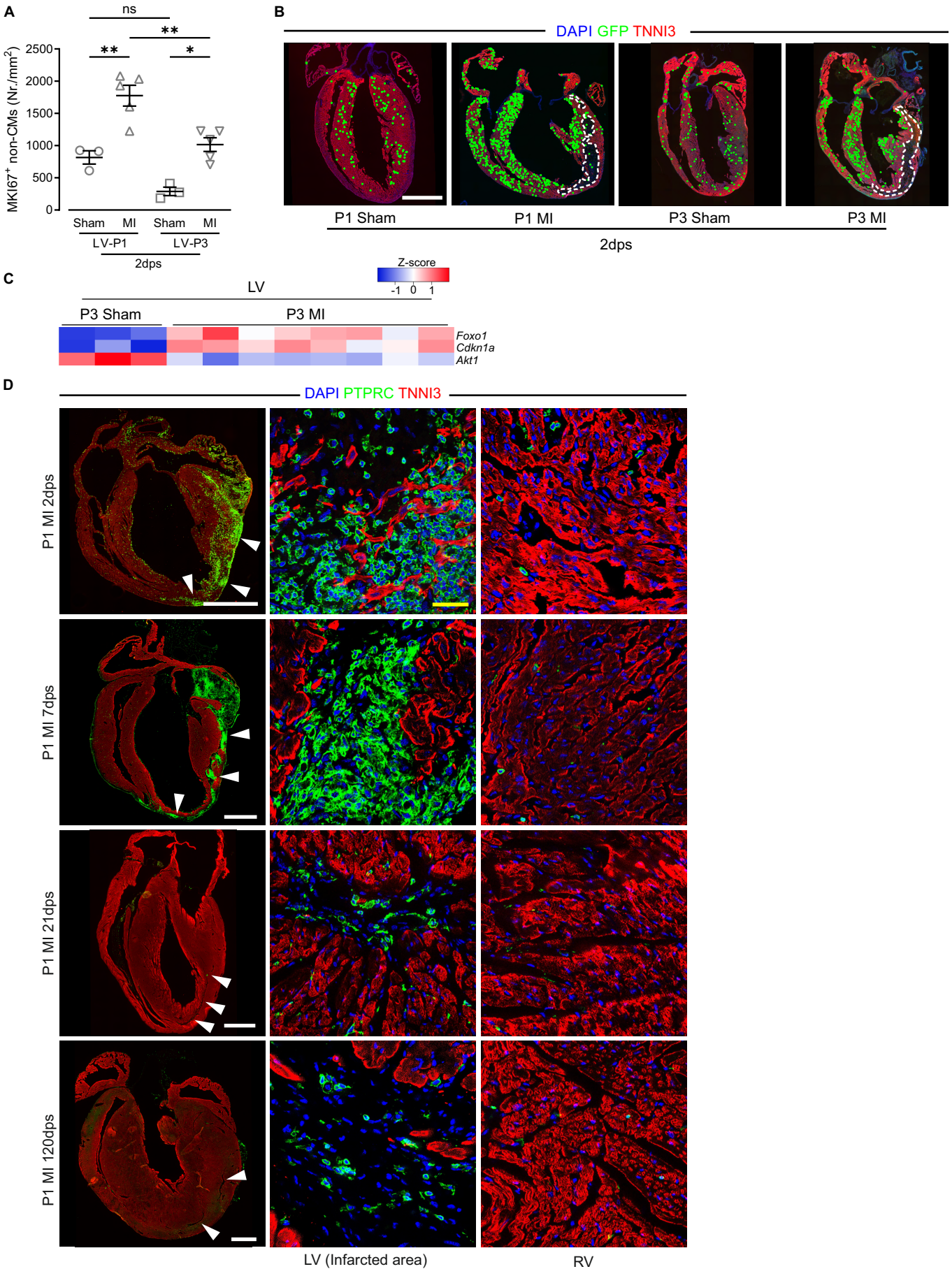
GO (Unique P1 upregulated genes)	#Gene
Immune system process	29
Inflammatory response	28
Innate immune response	24
Positive regulation of cell proliferation	19
Negative regulation of apoptotic process	19
Positive regulation of cell migration	18
Positive regulation of apoptotic process	18
Immune response	15
Regulation of cell proliferation	14
Regulation of apoptotic process	13
Positive regulation of ERK1 and ERK2 cascade	10
Cell proliferation	10



GO (Unique P3 upregulated genes)	#Gene
Positive regulation of cell proliferation	12
Angiogenesis	11
Negative regulation of cell proliferation	11
Negative regulation of apoptotic process	11
Response to hypoxia	10
Inflammatory response	8
Wnt signaling pathway	7

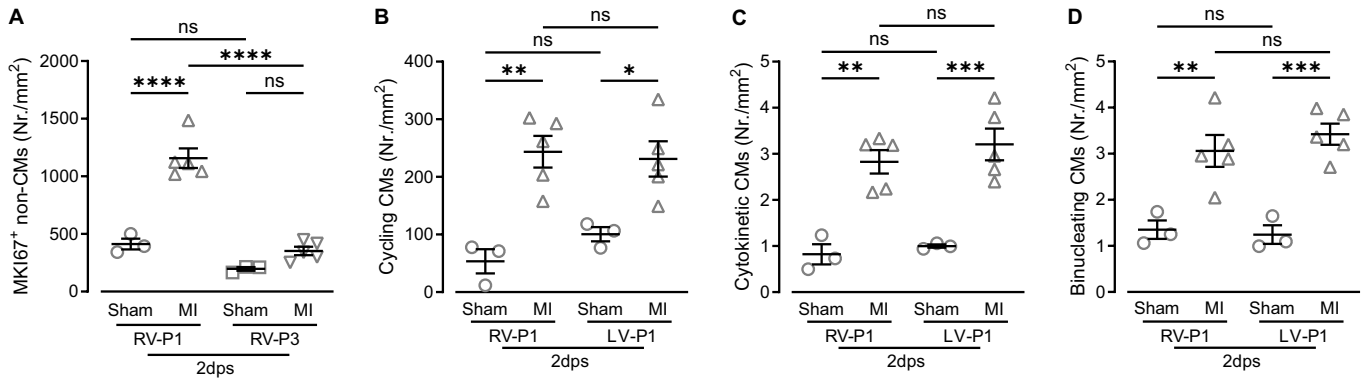
Supplemental Figure 3: Transcriptome analysis of the septum in P1 and P3 MI and sham hearts at 1 dps.

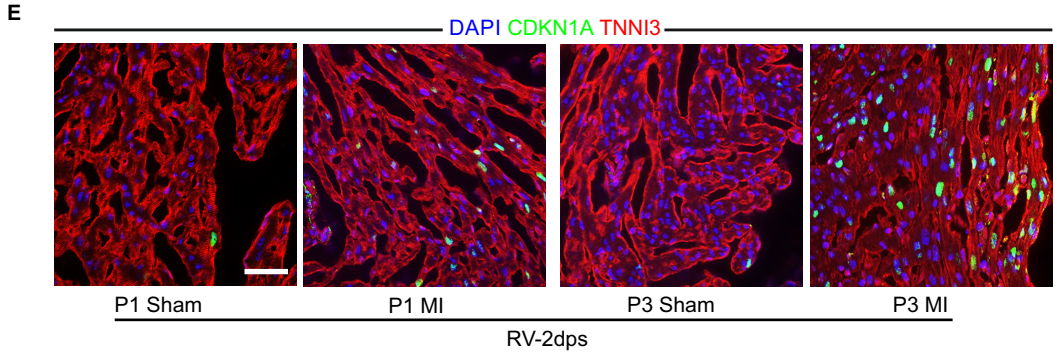
(**A** and **B**) Heatmap showing differentially up- (upper part of tables) and downregulated (lower part of tables) genes and selected GOs of biological processes in the septum of P1 (A) and P3 MI (B) hearts compared to sham hearts. (**C**) Venn diagram illustrating overlap of overexpressed genes in the septum. Left (P1) and right (P3) tables display selected GOs of biological processes of uniquely upregulated genes in MI hearts.

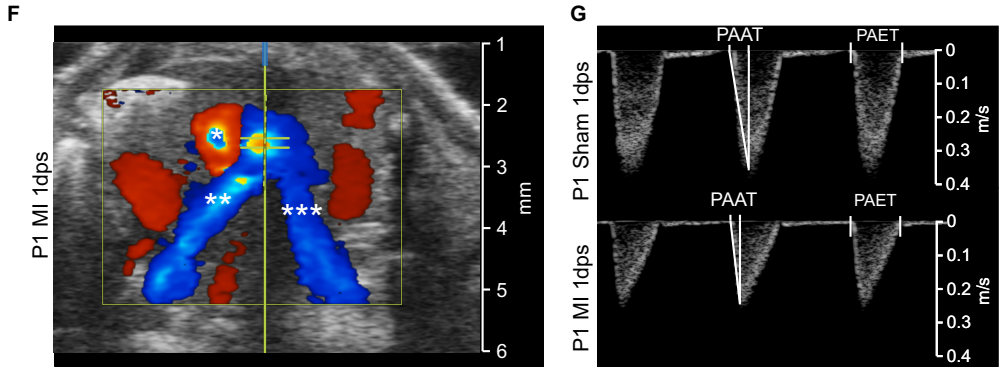

A
ns
**
**
*
MKI67+ non-CMs (Nr./mm2)
2500
2000
1500
1000
500
0
Sham
MI
Sham
MI
LV-P1
LV-P3
2dps
B
DAPI GFP TNNI3
P1 Sham
P1 MI
P3 Sham
P3 MI
2dps
C
LV
Z-score
-1 0 1
P3 Sham
P3 MI
Foxo1
Cdkn1a
Akt1
D
DAPI PTPRC TNNI3
P1 MI 2dps
P1 MI 7dps
P1 MI 21dps
P1 MI 120dps
LV (Infarcted area)
RV

Supplemental Figure 4: Increased proliferation of non-CMs and CMs, and strong inflammation in P1 and P3 MI, and sham hearts.

(**A**) Quantitation of MKI67^{+} non-CMs in LV heart sections. (**B**) Mosaic whole-heart images of *CAG-eGFP-anillin* hearts co-stained against eGFP and TNNI3. eGFP^{+} CMs are manually marked with green dots to visualize the distribution pattern of cycling CMs. (**C**) Heatmap showing the gene expression of *Foxo1, Cdkn1a* and *Akt1* in the LV of MI and sham hearts. (**D**) Mosaic (left panels) and magnified images (middle and right panels) of heart sections co-stained against PTPRC and TNNI3; arrowheads indicate infarcted or scarred areas; white scale bars=1mm; yellow scale bar=40μm. (*p<0.05; **p<0. 01; ns = no significance; one-way ANOVA with Holm-Šídák post hoc test).

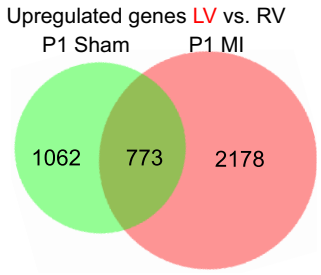






I

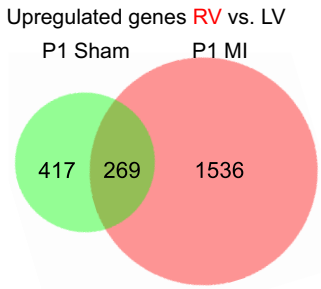
GO terms	Gene number
Oxidation-reduction process	97
Cell cycle	56
Cell division	47
Mitotic nuclear division	40
Apoptotic process	40
Lipid metabolic process	31
DNA repair	27
Fatty acid metabolic process	23
DNA replication	14
Fatty acid biosynthetic process	13
ATP metabolic process	12



GO terms	Gene number
Immune system process	144
Inflammatory response	132
Innate immune response	112
Immune response	83
Positive regulation of cell migration	62
Positive regulation of angiogenesis	45
Regulation of cell proliferation	54
Angiogenesis	55
Positive regulation of ERK1 and ERK2 cascade	47

J

GO terms	Gene number
Cell adhesion	26
Nervous system development	24
Positive regulation of cell proliferation	23
Protein phosphorylation	22
Positive regulation of gene expression	19
Negative regulation of apoptotic process	19
Heart development	18
Cell migration	17
Positive regulation of cell migration	17
Negative regulation of cell proliferation	15
Angiogenesis	14
Axon guidance	12



GO terms	Gene number
Oxidation-reduction process	97
Metabolic process	82
Lipid metabolic process	72
Cell cycle	65
DNA repair	40
Heart development	33
Fatty acid metabolic process	32
DNA replication	26
Mitochondrion organization	25
Fatty acid beta-oxidation	17
Lipid transport	15
Cardiac muscle contraction	14
Lipid catabolic process	14
Cardiac muscle tissue development	11

Supplemental Figure 5: Proliferation, CDKN1A protein expression, and transcriptome analysis (at 1 dps) in the RV and LV of P1, P3 MI and sham hearts; Doppler measurements of the blood flow in the pulmonary artery in P1 MI and sham hearts at 1 dps.

(**A**) Quantitation of MKI67^{+} non-CMs in RV. (**B-D**) Quantitation of cycling (B), cytokinetic (C) and binucleating (D) CMs in RV and LV of *CAG-eGFP-anillin* mice. (**E**) RV heart sections co-stained against CDKN1A, TNNI3 and DAPI; scale bar=40µm. (**F**) Colour doppler of the main pulmonary artery in a P1 MI heart: *ascending aorta; **right pulmonary artery; ***left pulmonary artery. (**G**) Flow velocity curve measured in the main pulmonary artery in a P1 MI and sham heart; PAAT: pulmonary artery acceleration time; PAET: pulmonary artery ejection time. (**H**) Doppler measurements of PAAT in the pulmonary artery. (**I** and **J**) Venn diagram comparing upregulated genes in the LV to RV (I) and in the RV to LV (J) in MI and sham hearts. Tables on the left and right show representative GO of biological processes, similar GO terms in sham LV (I) and MI RV (J) hearts are highlighted in red. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; ns = no significance; A-D: one-way ANOVA with Holm-Šídák post hoc test; H unpaired t-test).

Supplemental Table 1: Echocardiography parameters

Condition	Time point	EF ± SEM (%)	FS ± SEM (%)	CO ± SEM (ml/min)	LVEDA ± SEM (mm^2)	Wall thickness ± SEM (mm)	RV FAC ± SEM (%)	RV length ± SEM (mm)	RVEDA ± SEM (mm^2)	PAAT ± SEM (ms)	PAAT/PAET ± SEM (ratio)
P1 Sham	1dps	86.02 ± 2.39 n=9	20.82 ± 2.81 n=9		3.14 ± 0.13 n=9	0.40 ± 0.03 n=9	57.07 ± 6.13 n=9	4.00 ± 0.06 n=9	0.77 ± 0.07 n=9	12.65 ± 0.38 n=8	0.23 ± 0.01 n=8
	7dps	79.10 ± 2.02 n=8	21.97 ± 1.21 n=8		4.86 ± 0.33 n=8	0.69 ± 0.03 n=8	46.46 ± 3.68 n=8	6.09 ± 0.31 n=7	1.57 ± 0.17 n=8		
	14dps	70.48 ± 3.65 n=8	22.31 ± 1.81 n=8		12.45 ± 0.52 n=8			7.90 ± 0.30 n=7			
	21dps	67.34 ± 2.41 n=7	17.22 ± 1.83 n=7		17.63 ± 0.90 n=7			9.75 ± 0.15 n=7			
	120dps	43.79 ± 3.34 n=8	11.62 ± 1.14 n=8	17.58 ± 1.56 n=8	27.94 ± 2.29 n=8			12.48 ± 0.33 n=7			
P1 MI	1dps	15.92 ± 2.87 n=11	3.78 ± 0.74 n=11		5.47 ± 0.32 n=11	0.31 ± 0.02 n=11	48.44 ± 5.61 n=11	4.55 ± 0.11 n=11	0.70 ± 0.07 n=11	10.88 ± 0.51 n=6	0.17 ± 0.01 n=6
	7dps	60.63 ± 4.37 n=10	17.67 ± 1.66 n=10		5.71 ± 0.66 n=9	0.64 ± 0.02 n=9	46.23 ± 3.28 n=10	7.65 ± 0.24 n=11	1.50 ± 0.19 n=10		
	14dps	58.40 ± 2.14 n=10	16.29 ± 1.19 n=10		12.85 ± 0.33 n=10			11.22 ± 0.25 n=11			
	21dps	59.31 ± 2.23 n=11	17.57 ± 1.31 n=11		16.82 ± 0.64 n=11			13.06 ± 0.31 n=11			
	120dps	39.87 ± 3.80 n=11	12.24 ± 1.27 n=11	18.56 ± 1.66 n=11	28.41 ± 1.18 n=11			16.72 ± 0.37 n=11			
P3 Sham	1dps	79.57 ± 2.72 n=5	22.19 ± 3.88 n=5		4.82 ± 0.31 n=5	0.41 ± 0.02 n=5	52.66 ± 5.89 n=5		0.91 ± 0.15 n=5		
	7dps	83.91 ± 3.61 n=5	26.64 ± 3.01 n=5		6.37 ± 0.66 n=5	0.60 ± 0.04 n=5	58.55 ± 3.74 n=5		2.14 ± 0.43 n=5		
P3 MI	1dps	8.79 ± 1.00 n=7	4.83 ± 1.83 n=7		7.95 ± 0.31 n=7	0.23 ± 0.02 n=7	23.70 ± 4.20 n=8		1.72 ± 0.11 n=8		
	7dps	10.02 ± 1.07 n=7	7.59 ± 1.44 n=7		16.50 ± 1.73 n=7	0.21 ± 0.07 n=7	15.88 ± 3.80 n=7		3.43 ± 0.45 n=7		

Supplemental Table 2: Descriptive statistics

Parameter	Condition	n=	Average ± S.E.M	Related figures
Injury/scar size (%)	P1 MI 2dps	5	38.99 ± 1.18	Figure 1B
	P1 MI 7dps	5	19.40 ± 2.88	
	P1 MI 21dps	5	6.59 ± 0.33	
	P1 MI 120dps	4	2.92 ± 0.10	
	P3 MI 2dps	6	42.74 ± 1.64	Figure 1E
	P3 MI 7dps	6	70.41 ± 2.99	
CM cross-sectional area (μm^2)	P1 Sham 7dps LV	4	63.23 ± 3.59	Figure 1L
	P1 MI 7dps LV	8	82.10 ± 3.55	
	P3 Sham 7dps LV	3	63.21 ± 6.01	
	P3 MI 7dps LV	6	110.11 ± 1.06	
	P1 Sham 120dps RV	7	259.45 ± 22.43	Figure 2N
	P1 MI 120dps RV	8	371.70 ± 23.11	
	P3 Sham 120dps LV	7	271.17 ± 9.75	
	P3 MI 120dps LV	8	346.33 ± 23.11	
	P1 Sham 4dps RV	3	34.52 ± 0.24	Figure 8D
	P1 MI 4dps RV	3	39.44 ± 1.04	
	P3 Sham 4dps RV	3	41.37 ± 0.88	
	P3 MI 4dps RV	3	84.88 ± 2.11	
CM length/width (ratio) in RV	P1 Sham 120dps	7	6.55 ± 0.25	Figure 2J
	P1 MI 120dps	9	6.98 ± 0.21	
HW/TL (g/mm)	P1 Sham 120dps	22	0.0093 ± 0.00043	Figure 2L
	P1 MI 120dps	22	0.012 ± 0.00048	
HW/BW (mg/g)	P1 Sham 120dps	22	4.84 ± 0.13	Supplemental Figure 2A
	P1 MI 120dps	22	6.05 ± 0.18	
$MKI67^+$ CMs ($Nr./mm^2$)	P1 Sham 2dps LV	3	329.40 ± 26.65	Figure 4B Figure 7B-C
	P1 MI 2dps LV	5	506.17 ± 31.93	
	P3 Sham 2dps LV	3	162.62 ± 6.55	
	P3 MI 2dps LV	5	274.28 ± 23.12	
	P1 Sham 2dps RV	3	248.79 ± 28.46	Figure 7B
	P1 MI 2dps RV	5	657.32 ± 30.30	
	P3 Sham 2dps RV	3	125.84 ± 22.00	Figure 7C
	P3 MI 2dps RV	5	233.24 ± 17.76	
$MKI67^+$ non-CMs ($Nr./mm^2$)	P1 Sham 2dps LV	3	816.47 ± 102.47	Supplemental Figure 4A
	P1 MI 2dps LV	5	1775.05 ± 160.65	
	P3 Sham 2dps LV	3	289.51 ± 66.04	
	P3 MI 2dps LV	5	1015.25 ± 106.29	
	P1 Sham 2dps RV	3	412.32 ± 46.57	Supplemental Figure 5A
	P1 MI 2dps RV	5	1157.28 ± 84.57	
	P3 Sham 2dps RV	3	196.72 ± 15.80	
	P3 MI 2dps RV	5	352.37 ± 36.18	

Supplemental Table 2 (continued)

Parameter	Condition	n=	Average ± S.E.M	Related figures
Cycling CMs (Nr./mm^2)	P1 Sham 2dps LV	3	100.42 ± 12.32	Figure 4D Supplemental Figure 5B
	P1 MI 2dps LV	5	231.04 ± 30.64	
	P3 Sham 2dps LV	7	78.92 ± 17.85	
	P3 MI 2dps LV	7	78.67 ± 21.16	
	P1 Sham 2dps RV	3	53.66 ± 21.00	Figure 7D Supplemental Figure 5B
	P1 MI 2dps RV	5	243.60 ± 27.55	
	P3 Sham 2dps RV	7	41.69 ± 7.88	
	P3 MI 2dps RV	7	132.58 ± 23.45	
Cytokinetic CMs (Nr./mm^2)	P1 Sham 2dps LV	3	1.00 ± 0.04	Figure 4F Supplemental Figure 5C
	P1 MI 2dps LV	5	3.20 ± 0.34	
	P3 Sham 2dps LV	3	0.19 ± 0.05	
	P3 MI 2dps LV	4	0.40 ± 0.06	
	P1 Sham 2dps RV	3	0.82 ± 0.22	Figure 7E Supplemental Figure 5C
	P1 MI 2dps RV	5	2.83 ± 0.26	
	P3 Sham 2dps RV	3	0.23 ± 0.02	
	P3 MI 2dps RV	4	0.69 ± 0.11	
Binucleating CMs (Nr./mm^2)	P1 Sham 2dps LV	3	1.35 ± 0.20	Figure 4G Supplemental Figure 5D
	P1 MI 2dps LV	5	3.06 ± 0.35	
	P3 Sham 2dps LV	3	0.70 ± 0.03	
	P3 MI 2dps LV	4	1.38 ± 0.28	
	P1 Sham 2dps RV	3	1.25 ± 0.20	Figure 7F Supplemental Figure 5D
	P1 MI 2dps RV	5	3.42 ± 0.23	
	P3 Sham 2dps RV	3	0.68 ± 0.11	
	P3 MI 2dps RV	4	1.51 ± 0.18	
CM binucleation rate at P5 (%)	P1 Sham	3	12.12 ± 0.96	Figure 4I
	P1 MI	9	16.98 ± 0.90	
	P3 Sham	6	10.51 ± 0.54	
	P3 MI	6	12.47 ± 0.84	
CDKN1A$^+$ CMs (Nr./mm^2)	P1 Sham 2dps LV	4	102.97 ± 37.79	Figure 5B
	P1 MI 2dps LV	6	322.90 ± 46.25	
	P3 Sham 2dps LV	6	94.73 ± 13.68	
	P3 MI 2dps LV	5	788.32 ± 160.20	
	P1 Sham 2dps RV	4	76.68 ± 16.73	Figure 7G
	P1 MI 2dps RV	6	345.39 ± 15.98	
	P3 Sham 2dps RV	6	79.23 ± 8.83	
	P3 MI 2dps RV	5	709.35 ± 86.90	

Supplemental Table 2 (continued)

Parameter	Condition	n=	Average ± S.E.M	Related figures
cCASP3$^+$/DAPI$^+$ CMs (Nr./mm^2)	P1 Sham 2dps	3	512.95 ± 111.00	Figure 5E
	P3 MI 2dps	3	1208.80 ± 85.06	
TUNEL$^+$/DAPI$^+$ CMs (Nr./mm^2)	P1 Sham 2dps	5	235.37 ± 30.05	Figure 5G
	P3 MI 2dps	5	1252.18 ± 165.46	
PTPRC$^+$/DAPI$^+$ CMs (Nr./mm^2)	P1 Sham 2dps	5	2190.26 ± 397.03	Figure 5I
	P3 MI 2dps	5	509.07 ± 44.28	
Capillary density (EC/CM)	P1 Sham 7dps LV	4	0.92 ± 0.03	Figure 5K
	P1 MI 7dps LV	8	0.96 ± 0.04	
	P3 Sham 7dps LV	3	1.16 ± 0.04	
	P3 MI 7dps LV	6	0.91 ± 0.05	
	P1 Sham 7dps RV	4	0.92 ± 0.05	Figure 8F
	P1 MI 7dps RV	8	1.16 ± 0.03	
	P3 Sham 7dps RV	3	1.00 ± 0.07	
	P3 MI 7dps RV	6	0.80 ± 0.02	
RV thickness (µm)	P1 Sham 4dps	3	256.40 ± 4.43	Figure 8B
	P1 MI 4dps	3	369.36 ± 20.79	
	P3 Sham 4dps	3	403.40 ± 3.23	
	P3 MI 4dps	3	324.14 ± 9.83	