

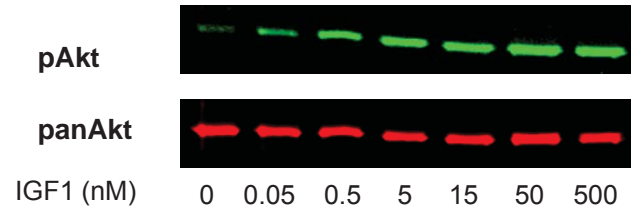
Supplemental Information

IGF1 Treatment Improves Cardiac Remodeling after Infarction by Targeting Myeloid Cells

Andre Heinen, Rianne Nederlof, Priyadarshini Panjwani, André Spsychala, Tengis Tschaidse, Heiko Reffelt, Johannes Boy, Annika Raupach, Stefanie Gödecke, Patrick Petzsch, Karl Köhrer, Maria Grandoch, Anne Petz, Jens W. Fischer, Christina Alter, Jelena Vasilevska, Philipp Lang, and Axel Gödecke

Figure S1

A



B

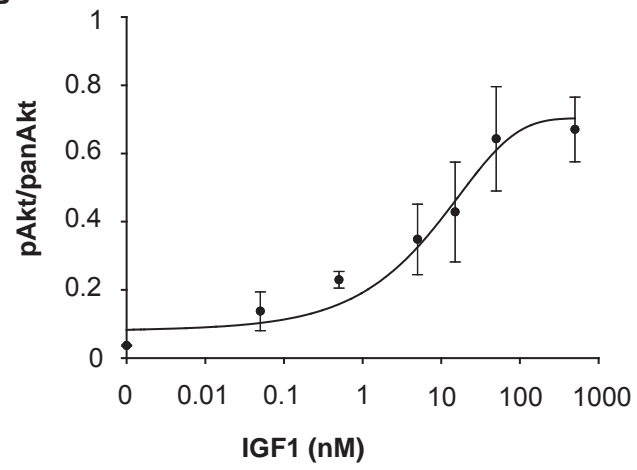


Figure S2

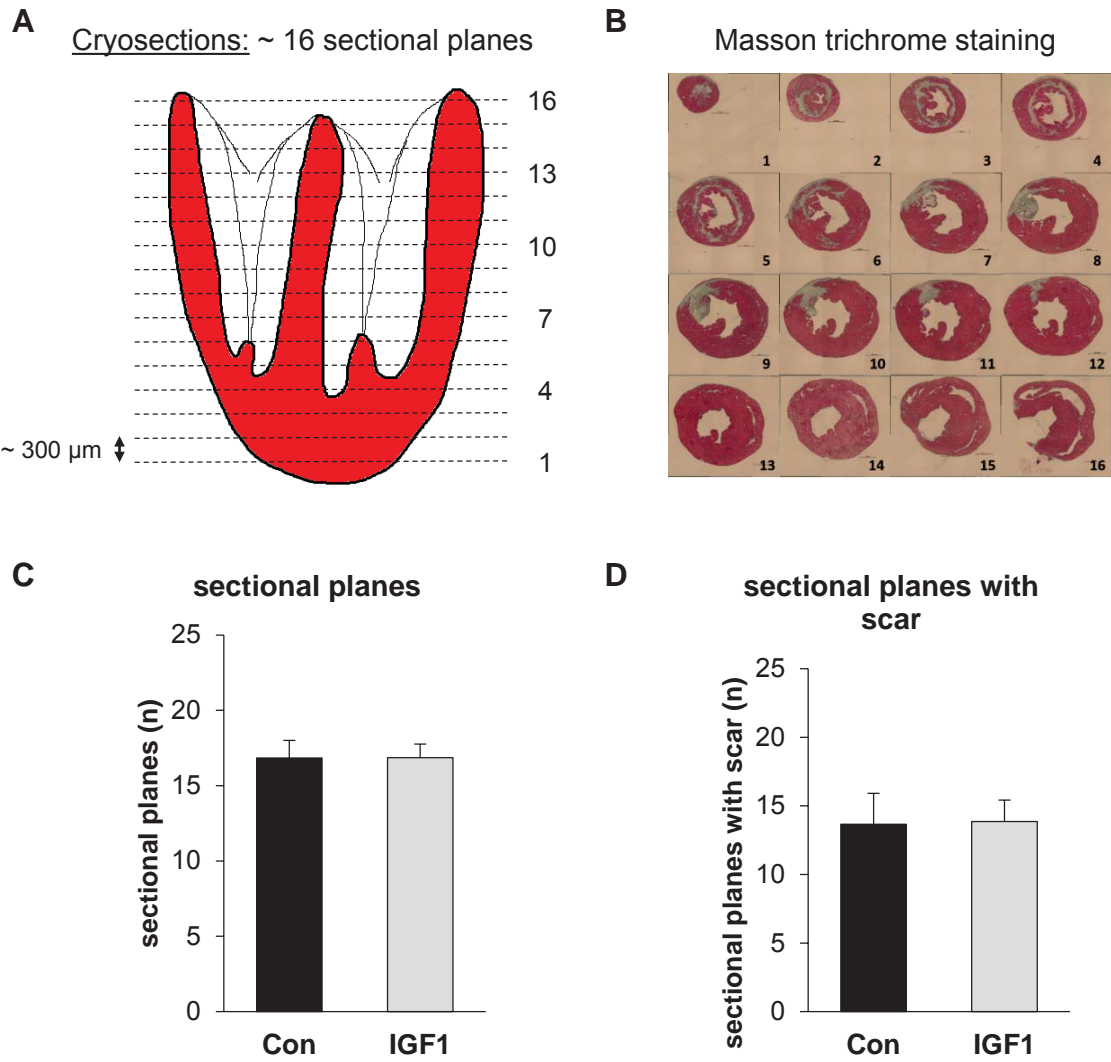


Figure S3A

Leukocyte migration day 1

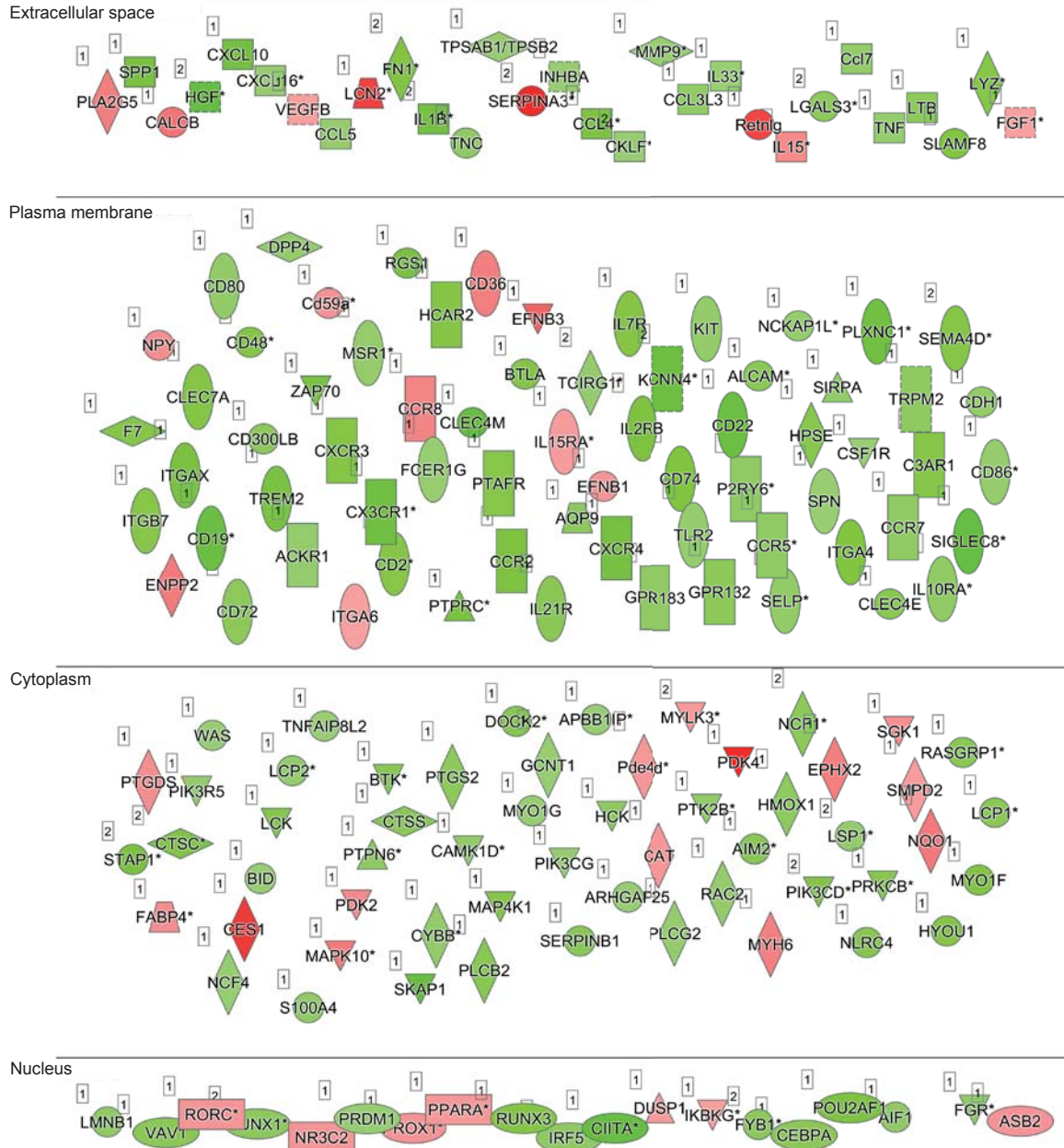
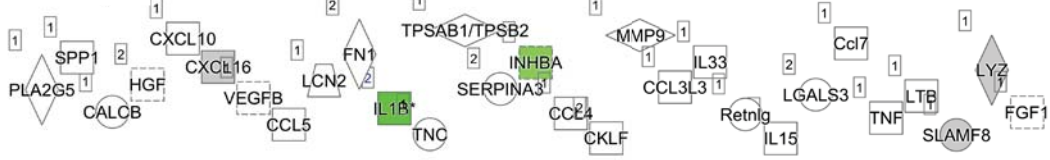


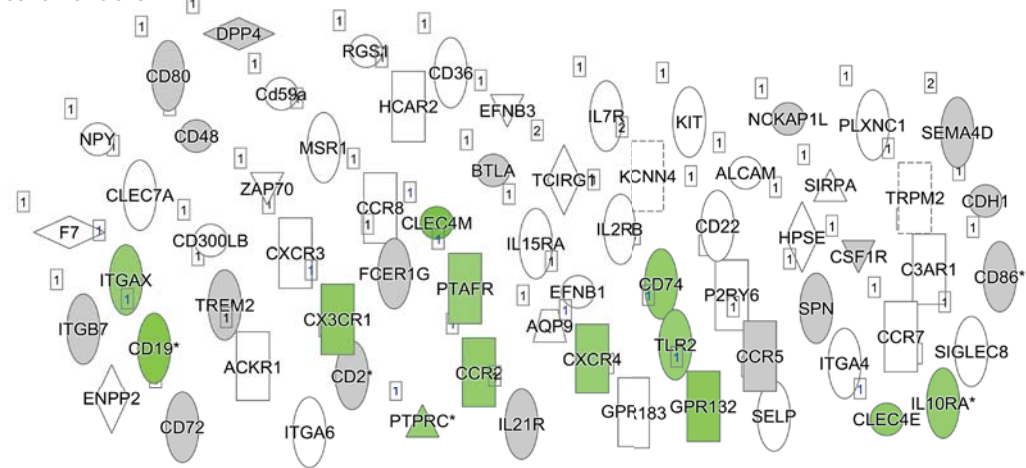
Figure S3B

Leukocyte migration day 2

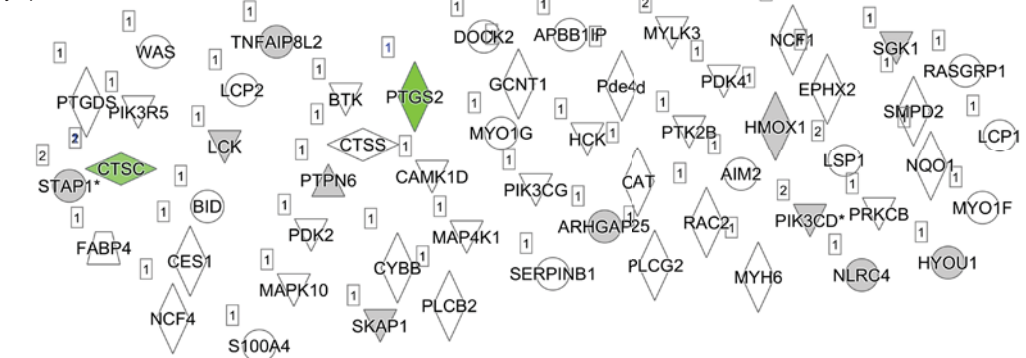
Extracellular space



Plasma membrane



Cytoplasm



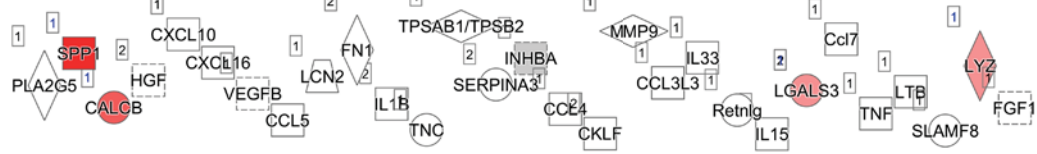
Nucleus



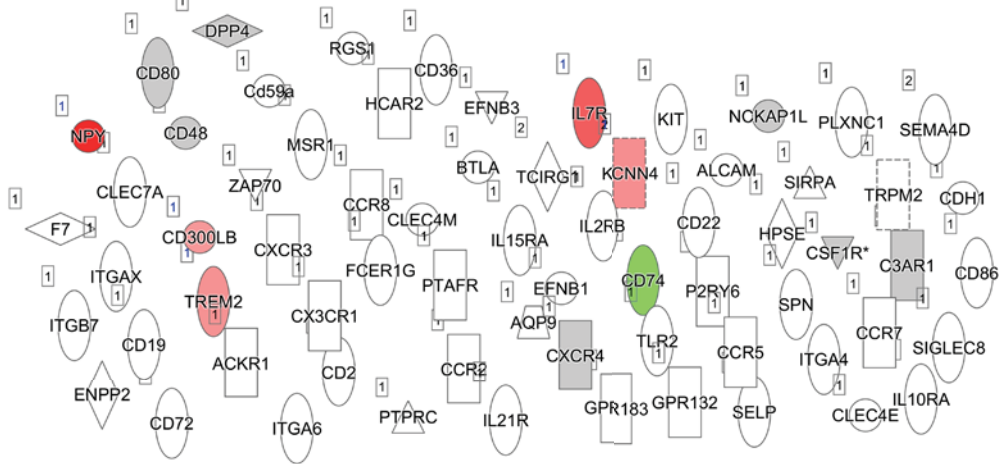
Figure S3C

Leukocyte migration day 7

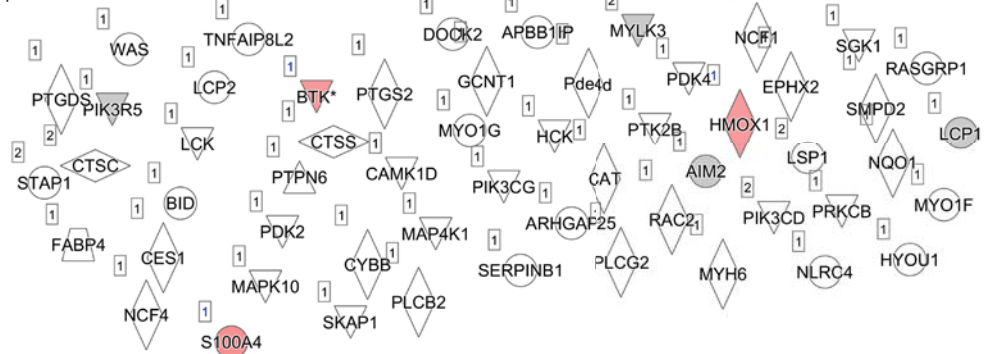
Extracellular space



Plasma membrane



Cytoplasm



Nucleus

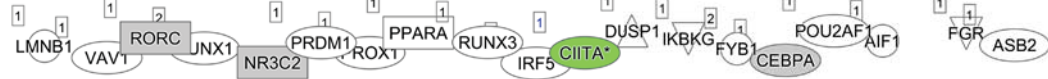
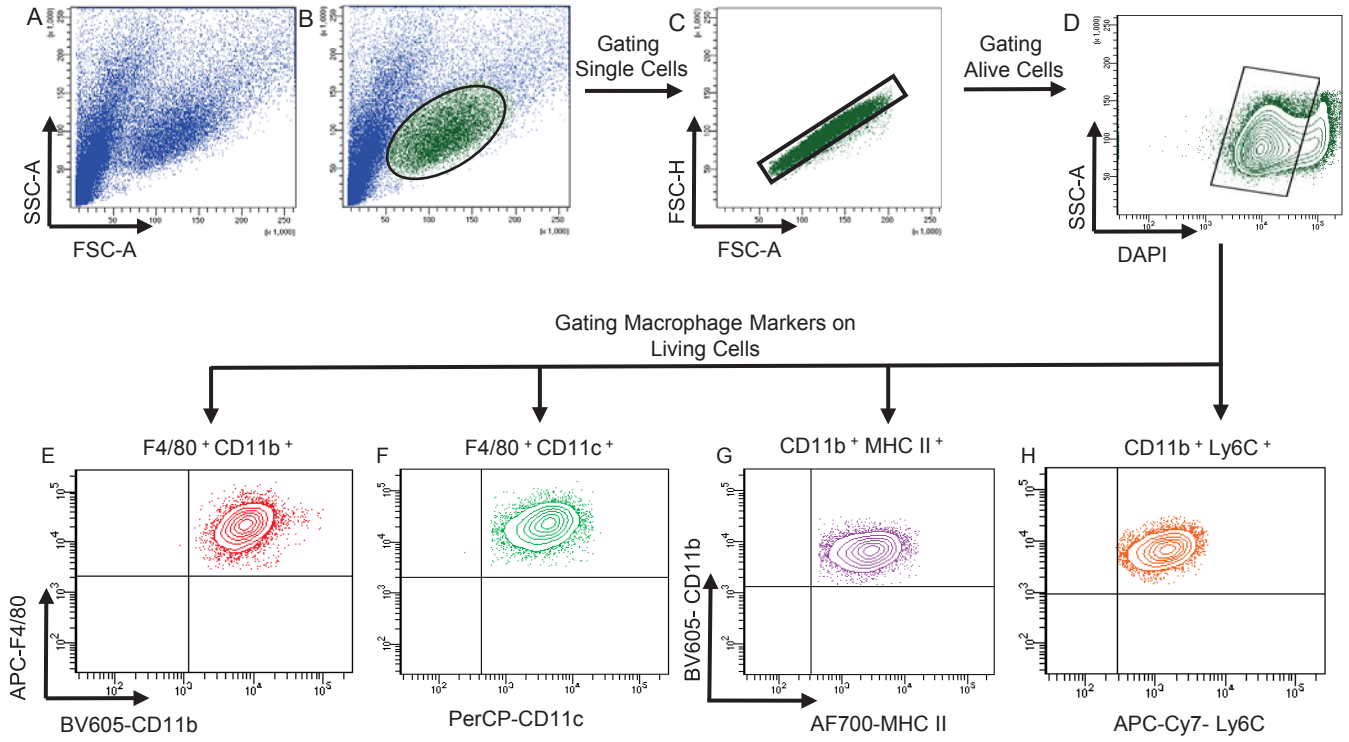


Figure S4



Supplemental figure legends

Figure S1 - IGF1 dose response curve

Isolated hearts of mice were perfused for 10 min with indicated concentrations of IGF1.

A IGF1 treatment dose-dependently increases Akt phosphorylation (Ser 473) in isolated hearts of C57Bl/6J mice. Example western blot of phosphoAkt and panAkt.

B Summarized dose response curve of pAkt/panAkt (n = 3 hearts for each concentration).

Data information: Data are presented as mean \pm SD.

Figure S2 - Heart cryosection protocol and scar staining

C57Bl/6J mice were subjected to 45 min left anterior descending coronary artery occlusion and 1 week of reperfusion. At the start of reperfusion, mice were treated with IGF1 (IGF1) or vehicle (Con) over three days.

Hearts were harvested 1 week after myocardial infarction.

A-B For histological analysis, cryosections (4 μ m) were taken from short axis sectional planes (distance 300 μ m), and Masson trichrome staining was used to assess scar size.

C-D Summarized data of number of sectional planes (left) and sectional planes with scar (right) (n = 7-8 hearts for each group).

Data information: In (C-D), data are presented as mean \pm SD.

Figure S3 - Microarray analysis of the infarct region on days 1, 2 and 7

C57Bl/6J mice were subjected to 45 min left anterior descending coronary artery occlusion, followed by reperfusion. At the start of reperfusion, mice were treated with IGF1 over three days. Agilent microarray analysis of the infarct region was performed on days 1, 2 and 7 after MI. Genes altered in the leukocyte migration pathway (the pathway with the highest change in activity score) are shown on day 1 (Fig S3A), 2 (Fig S3B) and 7 (Fig S3C). Genes downregulated in IGF1 treated animals are shown in green, upregulated genes are shown in red.

Figure S4 - Gating and cell identification using flow cytometer

- A Forward and sideward scatter of all the events separated by size and granularity.
- B Gating for identification of macrophages separated by size and granularity
- C Pulse geometric gate (height and area occupied by cells) for identification of single cells.
- D Viability gate for living cells using DAPI.
- E-F Living cells positive for F4/80, CD11b and CD11c.
- G-H Living cells positive for CD11b, MHCII and Ly6C (low).

Supplemental tables

Table S1: Echocardiographic data of C57Bl/6J mice

pre-OP				
	Con	p-value	IGF1	p-value
CO (ml/min)	24.3±3.5		24.2±3.4	
EDV (μl)	68.9±10.0		66.9±9.1	
ESV (μl)	24.6±4.7		23.0±3.7	
LVAW,d (mm)	0.88±0.04		0.78±0.05	
LVAW,s (mm)	1.37±0.03		1.27±0.09	
LVID,d (mm)	3.70±0.22		3.85±0.28	
LVID,s (mm)	2.30±0.27		2.33±0.20	
LVPW,d (mm)	0.74±0.05		0.80±0.16	
LVPW,s (mm)	1.24±0.12		1.27±0.18	
LV mass (mg)	84.7±6.6		86.4±10.3	
Week 1				
	Con	p-value	IGF1	p-value
CO (ml/min)	14.9±2.8	< 0.001 *	21.9±3.8	0.005 #
EDV (μl)	79.7±8.7		72.7±9.7	
ESV (μl)	53.2±8.4	< 0.001 *	33.4±6.8	0.003 #
LVAW,d (mm)	1.06±0.09	0.004 *	0.89±0.12	0.004 #
LVAW,s (mm)	1.26±0.18		1.32±0.17	
LVID,d (mm)	4.06±0.26		3.88±0.31	
LVID,s (mm)	3.42±0.30		2.66±0.47	0.010 #
LVPW,d (mm)	0.75±0.12		0.76±0.08	
LVPW,s (mm)	0.93±0.16	0.016 *	1.16±0.13	0.041 #
LV mass (mg)	117.5±25.8	0.016 *	93.0±14.7	0.013 *
Week 4				
	Con	p-value	IGF1	p-value
CO (ml/min)	16.4±1.4	0.005 *	21.1±5.2	
EDV (μl)	91.6±16.9	0.015 *	78.7±12.9	
ESV (μl)	61.5±14.6	< 0.001 *	41.2±13.5	< 0.001 * 0.003 #
LVAW,d (mm)	0.85±0.09		0.97±0.09	0.003 *
LVAW,s (mm)	1.08±0.26		1.40±0.16	
LVID,d (mm)	4.46±0.41	< 0.001 *	4.36±0.38	0.013 *
LVID,s (mm)	3.58±0.60	< 0.001 *	3.09±0.58	0.014 *
LVPW,d (mm)	0.79±0.11		0.77±0.08	
LVPW,s (mm)	1.10±0.23		1.20±0.20	
LV mass (mg)	120.8±25.7	0.006 *	126.9±14.2	0.003 *

* = vs. pre-OP; # = vs. Con

Table S2: Regional wall motion analysis

Figure 2C		Displacement - radial		
Segment	group	pre-OP	week 1	p-value
Lateral wall	Con	0.45±0.09	0.27±0.10	0.002 *
	IGF1	0.41±0.07	0.30±0.11	
Posterior wall	Con	0.47±0.09	0.30±0.12	0.010 *
	IGF1	0.44±0.12	0.30±0.11	0.035 *
Inferior free wall	Con	0.58±0.08	0.44±0.14	0.032 *
	IGF1	0.52±0.07	0.40±0.13	
Ant. septal wall	Con	0.54±0.07	0.46±0.14	
	IGF1	0.51±0.07	0.50±0.15	
Average	Con	0.49±0.06	0.33±0.09	< 0.001 *
	IGF1	0.47±0.06	0.36±0.08	0.019 *

Figure 2D		Strain - radial		
Segment	group	pre-OP	week 1	p-value
Lateral wall	Con	23.3±9.8	13.2±14.0	
	IGF1	23.6±6.5	16.2±8.2	
Posterior wall	Con	26.9±10.0	13.7±8.8	0.024 *
	IGF1	28.6±10.4	12.7±10.0	0.006 *
Inferior free wall	Con	31.7±10.7	24.1±10.3	
	IGF1	30.5±9.6	23.0±12.9	
Ant. septal wall	Con	29.2±7.0	25.5±7.9	
	IGF1	28.1±5.3	28.9±10.9	
Average	Con	26.9±6.8	15.3±7.8	0.003 *
	IGF1	25.3±4.0	18.2±6.1	

Figure 2E		Strain - circumferential		
Segment	group	pre-OP	week 1	p-value
Lateral wall	Con	-22.3±7.7	-10.8±6.5	0.009 *
	IGF1	-22.0±3.9	-13.1±9.2	0.045 *
Posterior wall	Con	-26.4±12.3	-15.4±11.0	
	IGF1	-25.1±10.7	-13.9±4.1	
Inferior free wall	Con	-28.7±5.6	-26.6±10.3	
	IGF1	-23.7±7.7	-21.6±10.4	
Ant. septal wall	Con	-26.2±6.2	-21.4±9.1	
	IGF1	-28.2±7.2	-23.7±9.3	
Average	Con	-25.6±3.4	-16.6±5.3	0.001 *
	IGF1	-24.6±2.7	-17.4±5.1	0.006 *

* = vs. pre-OP

Table S3: Echocardiographic data of iCM-IG1RKO mice

pre-OP					
	WT - Con	WT - IGF1	KO - Con	KO - IGF1	p-value
CO (ml/min)	31.2±3.9	28.3±4.8	28.8±4.8	29.6±1.9	
EDV (μl)	87.7±10.2	84.6±13.3	86.2±14.3	91.3±10.4	
ESV (μl)	34.8±5.9	34.1±6.2	35.2±9.4	38.4±5.1	
LVAW,d (mm)	0.81±0.11	1.06±0.15	0.91±0.09	0.96±0.11	
LVAW,s (mm)	1.24±0.12	1.54±0.15	1.35±0.05	1.38±0.15	
LVID,d (mm)	4.30±0.16	4.08±0.37	4.12±0.27	4.19±0.30	
LVID,s (mm)	2.97±0.20	2.54±0.50	2.74±0.37	2.74±0.29	
LVPW,d (mm)	0.77±0.05	0.84±0.09	0.73±0.07	0.72±0.12	
LVPW,s (mm)	1.25±0.09	1.33±0.14	1.19±0.14	1.25±0.13	
LV mass (mg)	106.1±8.8	108.8±12.9	101.7±16.2	110.8±19.3	
Week 1					
	WT - Con	WT - IGF1	KO - Con	KO - IGF1	p-value
CO (ml/min)	19.9±6.5 *	23.7±5.1	20.9±2.8	22.3±3.9	WT-Con: < 0.001 * KO-Con: 0.002 * KO-IGF1: 0.009 *
EDV (μl)	89.7±28.8	85.9±7.5	100.9±5.7	73.0±8.9	
ESV (μl)	53.0±16.4	42.4±6.6	61.5±9.3	32.4±2.4	WT-Con: 0.022 * KO-Con: < 0.001 * KO-IGF1: 0.006 #
LVAW,d (mm)	0.93±0.15	1.03±0.13	0.88±0.14	0.93±0.03	
LVAW,s (mm)	1.37±0.23	1.40±0.14	1.15±0.26	1.32±0.09	
LVID,d (mm)	4.48±0.41	3.95±0.33	4.68±0.28	3.72±0.27	KO-Con: 0.015 * KO-IGF1: < 0.001 #
LVID,s (mm)	3.33±0.52	2.72±0.46	3.64±0.49	2.46±0.25	KO-Con: 0.002 * KO-IGF1: 0.001 #
LVPW,d (mm)	0.78±0.15	0.91±0.13	0.77±0.14	0.71±0.09	
LVPW,s (mm)	1.06±0.18	1.34±0.16	1.09±0.17	1.17±0.11	WT-Con: 0.041 * WT-IGF1: 0.016 #
LV mass (mg)	128.9±24.9	126.4±28.4	130.7±26.1	87.7±5.8	KO-Con: 0.047 * KO-IGF1: 0.021 #
Week 4					
	WT - Con	WT - IGF1	KO - Con	KO - IGF1	p-value
CO (ml/min)	17.5±2.4 *	23.4±2.5	20.9±3.5	21.1±3.4	WT-Con: 0.001 * WT-IGF1: 0.040 * KO-Con: 0.002 * KO-IGF1: 0.003*
EDV (μl)	94.9±34.1	84.1±9.9	116.4±17.3	82.9±10.1	KO-Con: < 0.001 *
ESV (μl)	61.7±31.6	41.7±8.1	76.2±15.0	42.9±7.4	WT-Con: 0.003 * KO-Con: < 0.001 * KO-IGF1: 0.001 #
LVAW,d (mm)	0.90±0.14	1.08±0.22	0.86±0.17	0.99±0.10	
LVAW,s (mm)	1.21±0.17	1.46±0.26	1.09±0.28	1.35±0.06	
LVID,d (mm)	4.43±0.54	4.16±0.34	4.84±0.25	4.13±0.32	KO-Con: 0.002 * KO-IGF1: 0.011 #
LVID,s (mm)	3.39±0.68	2.94±0.43	3.87±0.45	3.02±0.28	KO-Con: < 0.001 * KO-IGF1: 0.024 #
LVPW,d (mm)	0.79±0.11	0.85±0.07	0.74±0.17	0.80±0.13	
LVPW,s (mm)	1.11±0.15	1.20±0.09	1.01±0.21	1.16±0.09	
LV mass (mg)	125.6±34.8	125.7±21.5	124.4±27.0	116.0±5.8	

* = vs. pre-OP; # = vs. Con

Table S4: Echocardiographic data of My-IG1RKO mice

pre-OP					
	WT - Con	WT - IGF1	KO - Con	KO - IGF1	p-value
CO (ml/min)	25.9±6.7	23.5±5.7	20.2±5.3	24.7±6.4	
EDV (μl)	79.2±17.3	71.1±14.7	66.0±15.0	77.7±18.0	
ESV (μl)	31.8±39.8	27.5±6.0	26.3±7.0	31.6±9.3	
LVAW,d (mm)	0.91±0.05	0.82±0.07	0.83±0.07	0.88±0.07	
LVAW,s (mm)	1.31±0.16	1.24±0.05	1.26±0.15	1.24±0.06	
LVID,d (mm)	3.82±0.39	3.85±0.29	3.71±0.35	3.94±0.57	
LVID,s (mm)	2.48±0.41	2.54±0.35	2.46±0.38	2.76±0.86	
LVPW,d (mm)	0.82±0.08	0.75±0.03	0.81±0.08	0.76±0.09	
LVPW,s (mm)	1.22±0.05	1.19±0.15	1.13±0.11	1.14±0.18	
LV mass (mg)	93.1±17.2	86.8±13.7	84.7±17.6	90.2±15.8	
Week 1					
	WT - Con	WT - IGF1	KO - Con	KO - IGF1	p-value
CO (ml/min)	14.4±3.6	22.5±5.5	15.4±4.5	16.7±4.9	WT-Con: < 0.001 * KO-Con: 0.042 * KO-IGF1: < 0.001 *
EDV (μl)	70.8±17.3	81.1±17.1	75.3±16.0	89.9±38.1	
ESV (μl)	44.1±12.0	41.9±8.6	46.1±8.7	59.2±30.9	WT-IGF1: 0.025 * KO-Con: < 0.001 * KO-IGF1: < 0.001 *
LVAW,d (mm)	1.05±0.14	0.97±0.10	0.89±0.11	1.01±0.11	WT-Con: 0.045 * WT-IGF1: 0.023 * KO-IGF1: 0.043 *
LVAW,s (mm)	1.36±0.19	1.29±0.11	1.24±0.13	1.25±0.13	
LVID,d (mm)	3.82±0.60	4.05±0.49	4.09±0.50	4.09±0.68	KO-Con: 0.041 *
LVID,s (mm)	2.80±0.56	2.97±0.61	3.08±0.62	3.28±0.89	KO-Con: 0.007 * KO-IGF1: 0.024 *
LVPW,d (mm)	0.83±0.07	0.82±0.08	0.78±0.10	0.79±0.11	
LVPW,s (mm)	1.09±0.14	1.15±0.09	1.06±0.16	1.03±0.15	
LV mass (mg)	108.2±26.1	113.3±24.8	108.6±36.6	124.4±37.6	WT-IGF1: 0.036 * KO-Con: 0.042 * KO-IGF1: 0.003 *

continued

continuation **Table S4**

Week 4					
	WT - Con	WT - IGF1	KO - Con	KO - IGF1	p-value
CO (ml/min)	14.7±3.3	22.8±4.2	14.5±2.7	18.8±5.5	WT-Con: < 0.001 * KO-Con: 0.014 * KO-IGF1: 0.018 *
EDV (μl)	79.8±11.3	88.6±16.0	73.7±16.2	95.8±33.1	WT-IGF1: 0.018 * KO-IGF1: 0.002 *
ESV (μl)	51.2±7.3	47.5±7.8	46.1±14.6	63.1±28.4	WT-Con: 0.002 * WT-IGF1: 0.002* KO-Con: < 0.001 * KO-IGF1: < 0.001 *
LVAW,d (mm)	0.92±0.06	0.92±0.09	0.93±0.07	1.04±0.14	KO-IGF1: 0.030 *
LVAW,s (mm)	1.12±0.11	1.22±0.14	1.17±0.15	1.29±0.17	
LVID,d (mm)	4.25±0.33	4.30±0.33	4.06±0.53	4.26±0.63	WT-Con: 0.029 * WT-IGF1: 0.020*
LVID,s (mm)	3.32±0.36	3.26±0.32	3.16±0.66	3.34±0.94	WT-Con: < 0.001 * WT-IGF1: 0.003 * KO-Con: 0.002 * KO-IGF1: 0.005 *
LVPW,d (mm)	0.83±0.07	0.82±0.06	0.85±0.12	0.97±0.17	KO-IGF1: 0.002 *
LVPW,s (mm)	1.08±0.06	1.20±0.10	1.16±0.07	1.27±0.22	
LV mass (mg)	112.3±10.6	120.9±15.4	116.0±40.8	143.3±30.2	WT-IGF1: 0.006 * KO-Con: 0.006 * KO-IGF1: < 0.001 *

* p<0.05 vs pre-OP, # p<0.05 vs WT-Con, § p<0.05 vs WT-IGF1

Table S5: P-values from statistical tests (Fig. 1-6).

Figure	parameter	week 1	p-value	week 4	p-value
Fig. 1C	EF	Con	< 0.001 (vs. pre-OP)	Con	< 0.001 (vs. pre-OP)
		IGF1	0.009 (vs. pre-OP) < 0.001 (vs. Con)	IGF1	< 0.001 (vs. pre-OP) 0.006 (vs. Con)
Fig. 1D	FAC	Con	< 0.001 (vs. pre-OP)	Con	< 0.001 (vs. pre-OP)
		IGF1	0.016 (vs. pre-OP) < 0.001 (vs. Con)	IGF1	0.012 (vs. pre-OP) 0.002 (vs. Con)
Fig. 1E	SV	Con	< 0.001 (vs. pre-OP)	Con	< 0.001 (vs. pre-OP)
		IGF1	0.009 (vs. Con)		
Fig. 2A	EF	Con	< 0.001 (vs. pre-OP)		
		IGF1	0.005 (vs. pre-OP) < 0.001 (vs. Con)		
Fig. 2C	Displ.- radial	Ant. free wall	Con	< 0.001 (vs. pre-OP)	
			IGF1	0.006 (vs. pre-OP) 0.006 (vs. Con)	
Fig. 2D	Strain - radial	Ant. free wall	Con	< 0.001 (vs. pre-OP)	
			IGF1	0.005 (vs. pre-OP) 0.041 (vs. Con)	
Fig. 2E	Strain - circumf.	Ant. free wall	Con	< 0.001 (vs. pre-OP)	
			IGF1	0.006 (vs. Con)	
Fig. 2F	Scar size		0.03 (vs. Con)		
Fig. 2H	Capillary density	Borderzone	0.003 (vs. Con)		
		Scar area	0.037 (vs. Con)		
Fig. 3B	EF	WT-Con	< 0.001 (vs. pre-OP)	WT-Con	< 0.001 (vs. pre-OP)
		WT-IGF1	0.028 (vs. pre-OP) 0.041 (vs. WT-Con)	WT-IGF1	0.034 (vs. pre-OP) 0.013 (vs. WT-Con)
		KO-Con	< 0.001 (vs. pre-OP)	KO-Con	< 0.001 (vs. pre-OP)
		KO-IGF1	< 0.001 (vs. KO-Con)	KO-IGF1	0.05 (vs. pre-OP) 0.003 (vs. KO-Con)
Fig. 3C	FAC	WT-Con	< 0.001 (vs. pre-OP)	WT-Con	< 0.001 (vs. pre-OP)
		WT-IGF1	0.026 (vs. WT-Con)	WT-IGF1	0.036 (vs. WT-Con)
		KO-Con	< 0.001 (vs. pre-OP)	KO-Con	< 0.001 (vs. pre-OP)
		KO-IGF1	< 0.001 (vs. KO-Con)		
Fig. 3D	SV	WT-Con	< 0.001 (vs. pre-OP)	WT-Con	< 0.001 (vs. pre-OP)
		KO-Con	0.015 (vs. pre-OP)	KO-Con	0.025 (vs. pre-OP)
		KO-IGF1	0.019 (vs. pre-OP)	KO-IGF1	0.013 (vs. pre-OP)

continued

continuation **Table S5**

		<u>week 1</u>	<u>p-value</u>	<u>week 4</u>	<u>p-value</u>
Fig. 4D	EF	WT-Con	< 0.001 (vs. pre-OP)	WT-Con	< 0.001 (vs. pre-OP)
		WT-IGF1	< 0.001 (vs. pre-OP) 0.012 (vs. WT-Con)	WT-IGF1	< 0.001 (vs. pre-OP) 0.009 (vs. WT-Con)
		KO-Con	< 0.001 (vs. pre-OP)	KO-Con	< 0.001 (vs. pre-OP)
		KO-IGF1	< 0.001 (vs. pre-OP) 0.002 (vs. WT-IGF1)	KO-IGF1	< 0.001 (vs. pre-OP) 0.005 (vs. WT-IGF1)
Fig. 4E	FAC	WT-Con	< 0.001 (vs. pre-OP)	WT-Con	< 0.001 (vs. pre-OP)
		WT-IGF1	0.001 (vs. pre-OP) 0.016 (vs. WT-Con)	WT-IGF1	< 0.001 (vs. pre-OP) 0.007 (vs. WT-Con)
		KO-Con	< 0.001 (vs. pre-OP)	KO-Con	< 0.001 (vs. pre-OP)
		KO-IGF1	< 0.001 (vs. pre-OP) < 0.001 (vs. WT-IGF1)	KO-IGF1	< 0.001 (vs. pre-OP)
Fig. 4F	SV	WT-Con	< 0.001 (vs. pre-OP)	WT-Con	< 0.001 (vs. pre-OP)
		KO-Con	0.008 (vs. pre-OP)	KO-Con	0.002 (vs. pre-OP)
		KO-IGF1	< 0.001 (vs. pre-OP)	KO-IGF1	0.001 (vs. pre-OP)
Fig. 5D	CD206	IGF1 (10 ng/ml)	0.018 (vs. untreated)		
		IGF1 (20 ng/ml)	0.024 (vs. untreated)		
Fig. 5E	TNF-α	M0+IGF1	0.028 (vs. M0)		
	MRC1	M0+IGF	0.035 (vs. M0)		
	IGF1	M0+IGF1	0.002 (vs. M0)		
Fig. 6B	CD206⁺	IGF1	0.041 (vs. control)		

All P-values are rounded to three decimals. EF = ejection fraction, FAC = fraction area change, SV = stroke volume.

Table S6: Primer sequences

Gene	Primer sequence
Tumor Necrosis Factor Alpha	forward: GCCTCTTCTCATTCCCTGCTTG reverse: CTGATGAGAGGGAGGCCATT
Interleukin 12A	forward: TACTAGAGAGACTTCTCCACAACAAGAG reverse: TCTGGTACATCTTCAAGTCCTCATAGA
iNOS	forward: CATCAACCAGTATTATGGCTC reverse: TTCCTTTGTTACAGCTTCC
Macrophage mannose receptor 1	forward: CTCTGTTTCAGCTATTGGACGC reverse: CGGAATTTCTGGGATTTCAGCTTC
Arginase 1	forward: CTCCAAGCCAAAGTCCTTAGAG reverse: AGGAGCTGTCATTAGGGACATC
Resistin-like alpha	forward: TCACAGGTCTGGCAATTCTTCTG reverse: TTTGTCCTTAGGAGGGCTTCCTCG
Insulin like growth factor 1	forward: CTGGACCAGAGACCTTTGC reverse: GGACGGGGACTTCTGAGTCTT
Vascular Endothelial Growth Factor	forward: GCACATAGAGAGAATGAGCTTCC reverse: CTCCGCTCTGAACAAGGCT
Endoplasmic reticulum membrane protein complex 10	forward: GTCCCCTAAACAGCCACTCT reverse: CTCCTCCCAGAGTTCGGAAG
beta-Actin	forward: GATGTATGAAGCTTTGGTC reverse: TGTGCACTTTTATTGGTCTC
Nuclear distribution C	forward: AGAACTCCAAGCTATCAGAC reverse: CTCAGGATTTCCIGTTTCTTC
My-IGF1RKO	forward: TTCACCAGTACCATGGGCTCC reverse: CTCAGCTTTGCAGGTGCACG