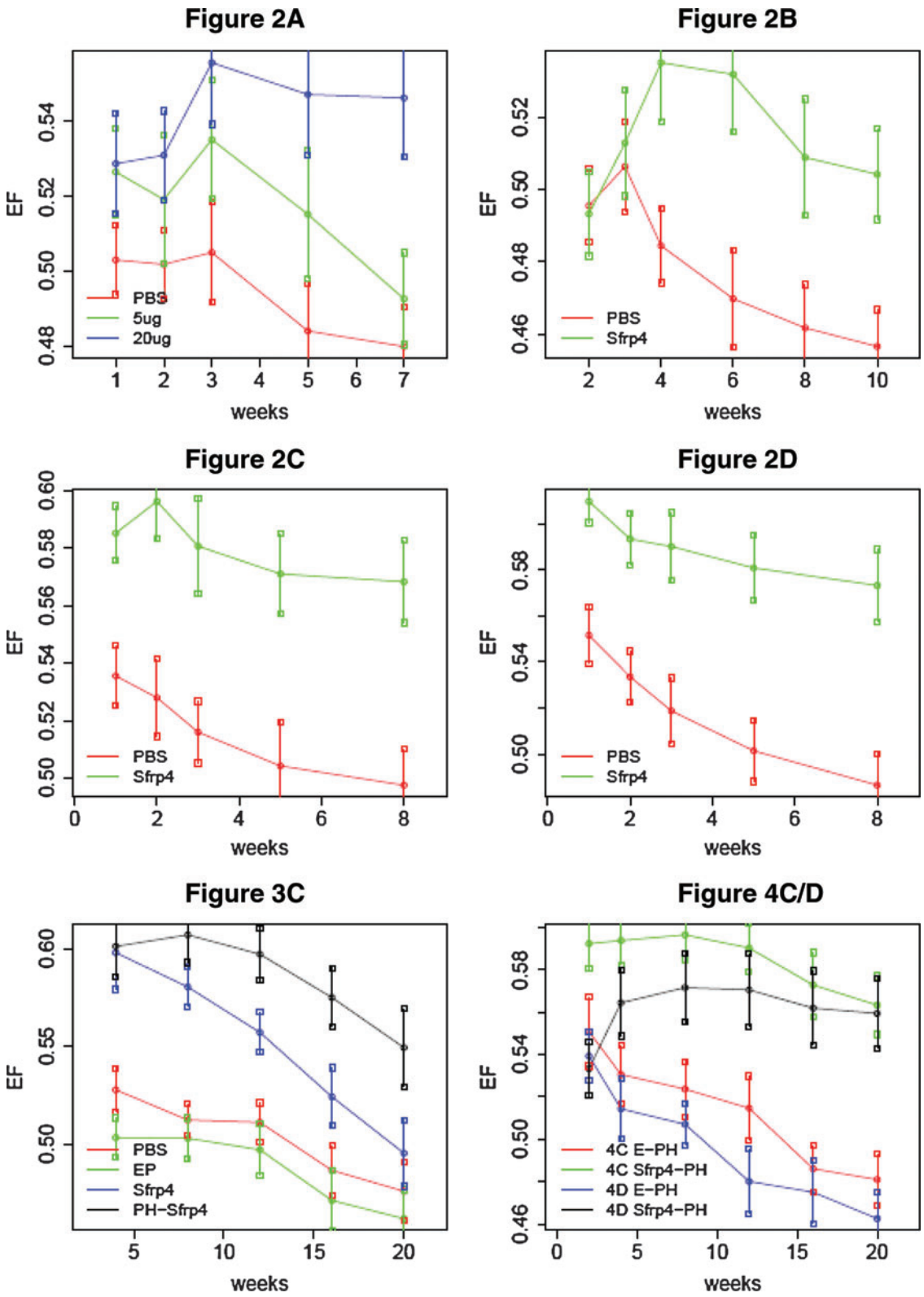
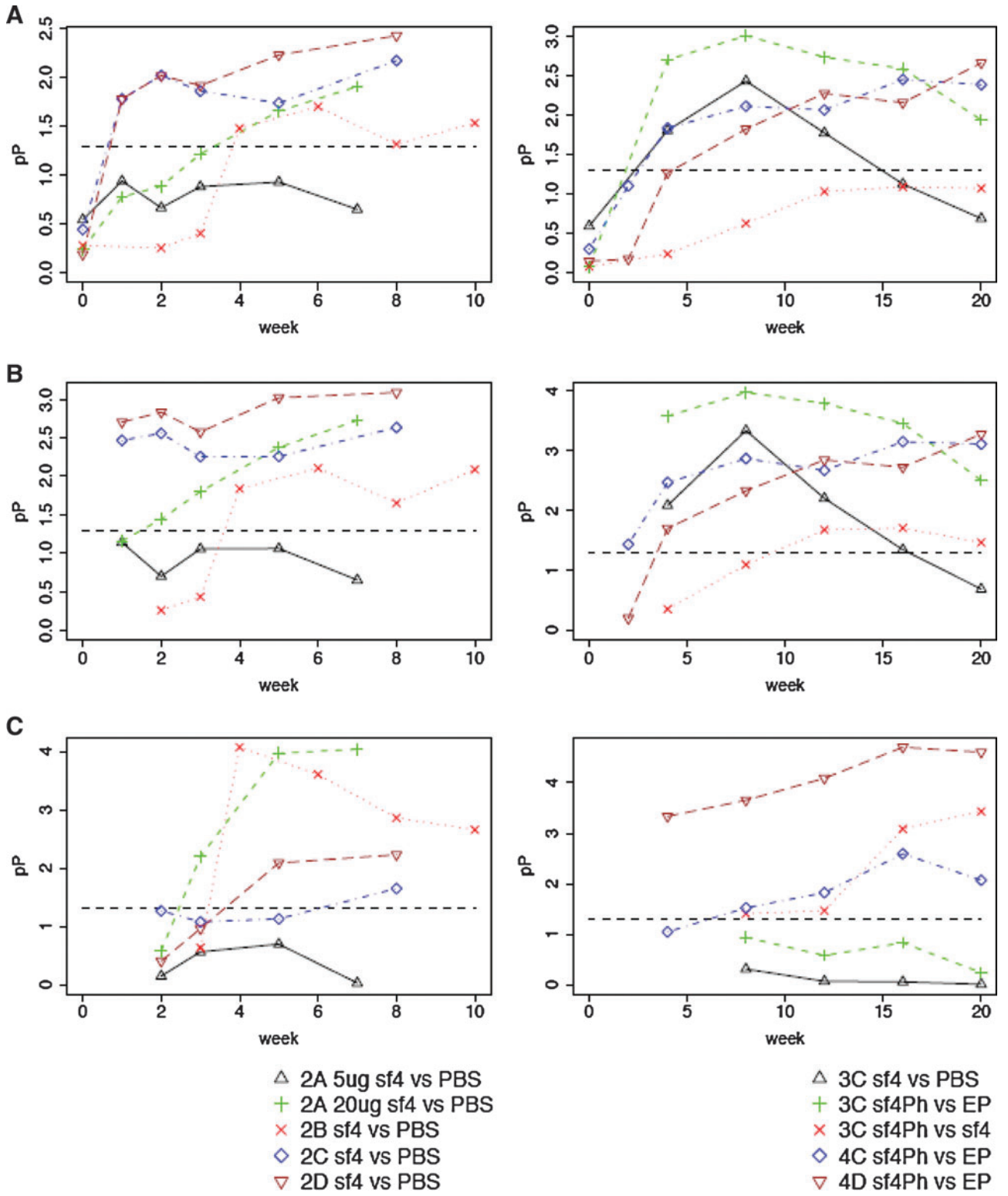
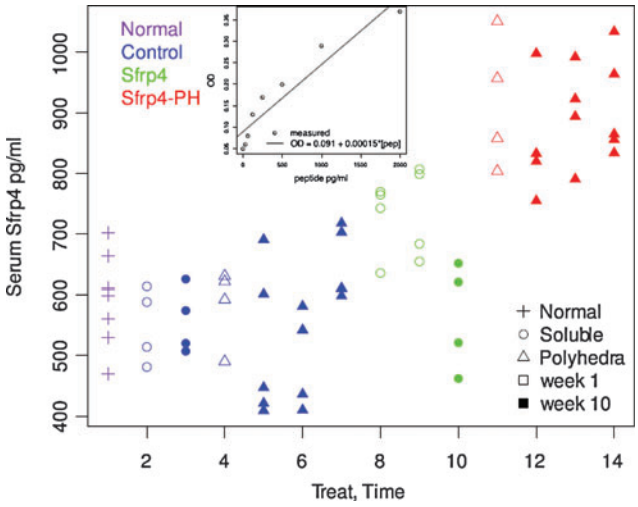


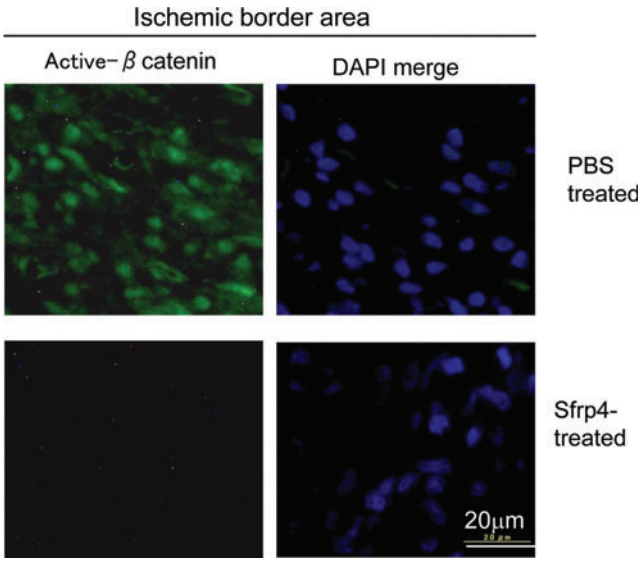
SUPPLEMENTAL FIG. S1. Normalized EF values. Plots show the means and standard errors of EF values normalized by individual preinjury EF values. Y-axis indicates the proportion of preinjury EF values; X-axis indicates week. Error bars indicate standard errors. The data from Fig. 4C and D are presented together since the same time points were used for the analysis; however, it may be wrong to directly compare the levels of EF values, as the injury and treatment model used is slightly different. In Figures 2B and 4D the treatment was applied 2 weeks after the injury. Note the resulting increase in EV values. EF, ejection fraction.



SUPPLEMENTAL FIG. S2. Pair-wise *p*-values. Pairwise *p*-values (one-tailed *t*-test) for the indicated comparisons for data from Figures 2 to 4 were calculated using raw EF (**A**), EF values normalized by preinjury EF values (**B**), or EF values normalized by the first postoperative EF measurement (**C**). *p*-Values are plotted as the – log10 *p*-value (pP) on the Y-axis against the week of the measurement (X-axis). Normalizing by preinjury EF values lowers the *p*-values obtained for all treatments, whereas normalizing against the first preoperative EF measurement is only effective for data shown in 2B and 4F where the first postoperative measurement was made before treatment application. These *p*-values have not been corrected for multiple testing, but given that the large majority fall below (inB) the standard 0.05 threshold (indicated by the dashed gray line), this is not a major concern.



SUPPLEMENTAL FIG. S3. *In vivo* release of Sfrp4. Serum concentrations of Sfrp4 were determined by ELISA at 1 or 10 weeks after application of Sfrp4 or controls to ischemic areas of the heart. Measurements from individual rats are plotted (X-axis, different treatments and times; Y-axis, serum level in pg/mL). Inset shows calibration curve and the best-fit line used for optical density (OD) to concentration conversions. Colors: purple, normal nonischemic rats; blue, control treatments (PBS or E-PH); green, soluble Sfrp4; red, S-PH-based treatments. Open and closed symbols indicate serum levels 1 week and 10 weeks after treatment application, respectively. Rats that received Sfrp4-based treatments (soluble or polyhedra based) had higher Sfrp4 serum levels ($p < 1e\text{-}16$) as indicated by a two-way analysis of variance (for time and treatment). Polyhedra-based treatments result in a higher level of Sfrp4 serum than soluble treatments at both weeks 1 and 10 ($p = 0.0003$), but soluble Sfrp4 does show an increase in Sfrp4 levels at week 1 compared to all control treatments ($p = 2.0e\text{-}5$, by *t*-test, uncorrected for multiple testing). Lanes are (1) nonischemic rat, (2) PBS week 1, (3) PBS week 10, (4) E-PH week 1, (5) E-PH week 10, (6) E-PH in fibrin glue week 10, (7) E-PH in collagen sheet week 10, (8) 5 μg Sfrp4 protein week 1, (9) 20 μg Sfrp4 protein week 1, (10) 20 μg Sfrp4 protein week 10, (11) 2.5×10^6 S-PH week 1, (12) 2.5×10^6 S-PH week 10, (13) 5×10^6 S-PH in fibrin glue week 10, and (14) 5×10^6 S-PH in collagen sheet week 10. Sfrp4, secreted frizzled related protein 4; S-PH, Sfrp4 containing polyhedra; PBS, phosphate-buffered saline; E-PH, empty polyhedra.



SUPPLEMENTAL FIG. S4. One hundred microliter PBS or 20 μg Sfrp4 protein was injected into ischemic border areas just after lateral anterior descendent ligation as in Figure 6B–D. Heart sections 3 days after lateral anterior descendent ligation were stained with anti-active β-catenin antibody and Alexa 488 (green). 4′6-diamidino-2-phenylindole (DAPI) (blue) was used to mark the positions of nuclei. Active β-catenin is also observed in nuclei in PBS-treated heart, but not in Sfrp4-treated heart.

Supplemental Table S1. Expression of *Secreted Frizzled Related Protein* 4, *Midkine,* and *Pleiotrophin* in Rat Hearts in a Myocardial Infarction and Treatment Model[8,9] Determined Using the Affymetrix Rat_230A Oligonucleotide Microarray

gene description	Affymetrix gene ID	normal	MI	col sheet	myo inject	myo sheet
sfrp 4 (1)	1368394_at	11.1	242.3	141.1	1884.3	2326.2
sfrp 4 (2)		30.3	734.7	168.7	562.8	4265.6
midkine (1)	1367682_at	37.3	193.9	130.6	296	432.1
midkine (2)		90.6	474.4	341	223.8	678.4
pleiotrophin (1)	1369968_at	47.8	735.4	386.3	1846.6	3061.9
pleiotrophin (2)		189.9	1571.1	925.1	979.2	4311.6

The heart lateral anterior descendent (LAD) branch was ligated followed by a range of experimental treatments applied 2 weeks after the LAD ligation: (i) a collagen sheet (col sheet, control), or (ii) a myoblast sheet (myo sheet) was patched to or (iii) 1×10^6 myoblasts were injected (myo injection) to the ischemic area. The hearts were obtained 4 weeks after respective treatments for gene chip analysis. Normal: normal rat heart without manipulation. Mas-5 expression levels from two independent experiments are shown.

Supplemental Table S2. List of Primers Used for Quantitative Reverse Transcriptase-Polymerase Chain Reaction and Antibodies Used for Immunostaining

List for primers used for qRT-PCR

gene	forward	reverse
Sfrp4	tga aaa gtg gag aga tca act cag ta	ggc tgg cta tct gct tct tgt c
Collagen type IIIa	aac tac ctt ggt cag tcc tat gag tct a	tcc cga gtc gca gac aca
Cyclin D2	tgg gta agc tga agt gga acc t	tgc gaa gga tgt gct caa tg
TCF4	gcg gag gat ggc caa taa	cct tga aag cct cgt tga tat ca
β-catenin	ccc agt cct tca cgc aag a	ccc tct gag ccc gag tca t
GAPDH	agc cca gaa cac cat tcc tac	atg cct gct tca cca cat tc

List for antibodies used for immuno staining

molecule	primary antibody Cat#, supplier, dilution	sencondary antibody, dilution
β -catenin	C7207, Sigma 1:200	Alexa 488 Molecular Probes 1:1000
active β -catenin	05-665, Upstate 1:200	Alexa 488 Molecular Probes 1:1000
phospho serine9 GSK-3β	#9336S, Cell Signaling Technology 1:200	Alexa 594 Molecular Probes 1:1000
Sfrp4	AF-1827, R&D System Inc. 1:100	Alexa 594 Molecular Probes 1:1000
collagen type IIIa	LB-1393, LSL Inc. Japan 1:250	Alexa 488 Molecular Probes 1:1000
cardiac troponin T (cTn-T)	MS-295-P, Neomarkers 1:100	Alexa 594 Molecular Probes 1:1000

Supplemental Table S3. Ejection Fraction Analysis of Variance *p*-Values

	Multivariate					Univariate			
Figure / subset	Treatment	Treatment:Time				Treatment	Treatment:time		
	P/W/H/R	Pillai	Wilks	Hotelling-Lawley	Roy	raw	raw	G-G	H-F
2A	6.16E-2	8.55E-4	2.35E-4	7.85E-5	5.53E-5	6.16E-2	3.08E-7	5.42E-6	6.82E-7
2A(5ug-20ug)	2.76E-1	2.38E-4	2.38E-4	2.38E-4	2.38E-4	2.76E-1	1.38E-5	2.65E-4	5.34E-5
2A(5ug-PBS)	2.14E-1	1.76E-1	1.76E-1	1.76E-1	1.76E-1	2.14E-1	2.13E-1	2.28E-1	2.13E-1
2B	8.13E-2	5.81E-2	5.81E-2	5.81E-2	5.81E-2	8.13E-2	1.33E-7	1.34E-5	1.33E-7
2B-post-treatment	4.85E-2	3.59E-2	3.59E-2	3.59E-2	3.59E-2	4.85E-2	5.29E-5	7.61E-4	9.05E-5
2C	5.78E-3	6.15E-1	6.15E-1	6.15E-1	6.15E-1	5.78E-3	2.65E-1	2.73E-1	2.65E-1
2D	1.87E-3	3.16E-1	3.16E-1	3.16E-1	3.16E-1	1.87E-3	4.37E-2	7.12E-2	5.08E-2
3C	1.13E-4	3.46E-2	1.51E-2	6.57E-3	4.92E-4	1.13E-4	8.75E-5	4.20E-4	1.11E-4
3C(Sfrp4_Sfrp4-PH)	1.23E-1	1.59E-2	1.59E-2	1.59E-2	1.59E-2	1.23E-1	5.70E-4	2.16E-3	5.70E-4
4E	2.96E-3	3.40E-2	3.40E-2	3.40E-2	3.40E-2	2.96E-3	6.75E-4	6.38E-3	1.40E-3
4F	8.51E-3	7.67E-3	7.67E-3	7.67E-3	7.67E-3	8.51E-3	1.26E-12	2.02E-9	1.26E-12
4F-post-treatment	3.50E-3	4.47E-2	4.47E-2	4.47E-2	4.47E-2	3.50E-3	8.13E-5	8.93E-4	1.40E-4

The EF data from individual experiments were analyzed by a two-way (time and treatment) repeated measures ANOVA using the "ANOVA" function of the "car" package implemented in the R implementation of the S language. The model was specified to identify significant treatment and time-treatment interaction effects. The "ANOVA" function performs both multivariate and univariate repeated measures ANOVA, and provides *p*-value estimates based on four different *F*-value approximations (Pillai, Wilks, Hotelling-Lawley, and Roy) for the multivariate ANOVA. Univariate *p*-values are reported using both Greenhouse-Geisser (G-G) and Huynh-Feldt (H-F) corrections for departures from sphericity for time–treatment interaction effects. *p*-Value estimates for the primary treatment effect were identical for the multivariate and univariate effect; however, large differences were observed for the treatment–time interaction effects. Significant ($p < 0.05$) effects were seen in all experiments using the most conservative Pillai estimate. 2A(5–20 μg), 2A(5 μg-PBS), and 3C(Sfrp4-S-PH) indicate analyses performed on the indicated subsets of the data to test for differences in dosage, effect of 5 μg Sfrp4, and an effect for polyhedra versus nonpolyhedra. Similarly, analyses of data points restricted to after the application of the treatment were performed for data from Figures 2B and 4D.

ANOVA, analysis of variance; Sfrp4, secreted frizzled related protein 4; PBS, phosphate-buffered saline.

Supplemental Table S4. FS and Functional Area Change Determined by Echocardiography

acute ischemic treatment model (Fig.2A)

FS

pre-ligation	58.3 ± 4.48		
ligation	PBS n=7	sfrp4 (5)n=6	sfrp4 (20)n=9
1 week	31.3 ± 6.64	35.5 ± 5.26	37.7 ± 4.4
2 weeks	32.6 ± 4.29	36.3 ± 3.11	36.9 ± 3.76
3 weeks	30.4 ± 2.46	34.8 ± 3.75	35.3 ± 4.73
5 weeks	27.7 ± 3.56	33.4 ± 4.31	35.0 ± 3.67
7 weeks	25.5 ± 4.6	32.1 ± 3.54	34.1 ± 2.98

FAC

pre-ligation	79.4 ± 5.72		
ligation	PBS n=7	sfrp4 (5)n=6	sfrp4 (20)n=9
1 week	32.6 ± 6.98	38.3 ± 4.9	44.1 ± 3.87
2 weeks	30.8 ± 7.38	37.7 ± 5.76	42.6 ± 3.3
3 weeks	29.3 ± 6.2	36.1 ± 3.83	40.9 ± 4.04
5 weeks	28.2 ± 2.78	34.8 ± 3.35	39.8 ± 4.76
7 weeks	28.0 ± 3.79	34.2 ± 4.9	38.2 ± 3.21

sub acute ischemic treatment model (Fig.2B)

FS

pre-ligation	55.7 ± 6.22	
ligation	PBS n=5	sfrp4 n=6
2 weeks	30.1 ± 5.32	36.5 ± 4.39
3 weeks	29.4 ± 3.37	35.0 ± 4.07
4 weeks	28.3 ± 3.64	35.4 ± 3.08
6 weeks	27.5 ± 2.2	33.6 ± 4.33
8 weeks	26.0 ± 2.78	31.7 ± 3.51
10 weeks	24.3 ± 2.29	30.9 ± 3.04

FAC

pre-ligation	76.4 ± 7.75	
ligation	PBS n=5	sfrp4 n=6
2 weeks	31.0 ± 4.56	43.1 ± 5.36
3 weeks	29.4 ± 3.01	41.3 ± 4.04
4 weeks	28.1 ± 2.88	40.0 ± 3.43
6 weeks	27.7 ± 4.43	39.7 ± 5.87
8 weeks	25.3 ± 3.71	38.2 ± 4.7
10 weeks	23.8 ± 2.27	37.1 ± 4.99

recanalization injury treatment model (Fig.2C)

FS

pre-ligation	57.8 ± 5.12	
ligation	PBS n=5	sfrp4 n=6
1 week	35.8 ± 3.37	38.9 ± 4.4
2 weeks	35.2 ± 2.89	36.5 ± 3.64
3 weeks	33.3 ± 4.03	37.3 ± 3.77
5 weeks	32.7 ± 3.88	36.0 ± 4.44
8 weeks	31.1 ± 3.3	34.7 ± 5.48

FAC

pre-ligation	81.9 ± 3.41	
ligation	PBS n=5	sfrp4 n=6
2 weeks	38.9 ± 6.28	42.1 ± 4.43
3 weeks	34.3 ± 3.78	41.8 ± 4.2
4 weeks	32.2 ± 3.76	41.2 ± 3.9
6 weeks	31.0 ± 4.79	40.3 ± 5.68
8 weeks	26.5 ± 3.08	39.9 ± 4.03

recanalization injury prevention model (Fig.2D)

FS

pre-ligation	60.3 ± 5.37	
ligation	PBS n=6	sfrp4 n=7
1 week	32.6 ± 6.88	37.8 ± 5.42
2 weeks	33.3 ± 5.32	35.3 ± 4.4
3 weeks	30.7 ± 3.96	35.7 ± 3.26
5 weeks	29.2 ± 3.32	33.3 ± 3.8
8 weeks	28.1 ± 3.4	34.1 ± 4.08

FAC

pre-ligation	79.4 ± 4.03	
ligation	PBS n=6	sfrp4 n=7
2 weeks	33.9 ± 5.03	43.4 ± 4.62
3 weeks	32.3 ± 3.65	41.8 ± 3.12
4 weeks	32.0 ± 3.4	39.7 ± 5.35
6 weeks	31.1 ± 4.04	37.0 ± 3.38
8 weeks	27.6 ± 3.88	36.3 ± 4.03

acute ischemic treatment model with polyhedra injection (Fig.3C)

FS

pre-ligation	61.4 ± 5.08			
ligation	PBS n=5	empty-p n=6	sfrp4 n=6	sfrp4-p n=6
1 week	30.3 ± 3.88	31.0 ± 4.72	35.3 ± 3.49	39.7 ± 4.66
2 weeks	29.6 ± 3.56	30.1 ± 3.42	347 ± 3.78	38.0 ± 3.32
3 weeks	28.5 ± 2.95	29.4 ± 3.02	33.9 ± 3.40	37.4 ± 3.28
5 weeks	26.2 ± 4.61	27.6 ± 2.8	33.1 ± 4.13	36.6 ± 4.02
7 weeks	25.0 ± 2.2	24.2 ± 3.43	32.7 ± 4.4	36.0 ± 3.31

FAC

pre-ligation	82.5 ± 4.22			
ligation	PBS n=5	empty-p n=6	sfrp4 n=6	sfrp4-p n=6
1 week	33.1 ± 3.45	34.5 ± 4.12	40.1 ± 5.11	42.7 ± 4.2
2 weeks	32.6 ± 4.44	31.6 ± 3.78	38.6 ± 4.37	41.0 ± 2.84
3 weeks	30.9 ± 5.78	31.3 ± 4.08	36.4 ± 3.9	40.5 ± 4.47
5 weeks	29.2 ± 3.68	30.5 ± 3.94	36.0 ± 4.74	39.4 ± 3.8
7 weeks	28.7 ± 4.4	29.0 ± 4.05	35.5 ± 3.66	38.9 ± 4.39

acute ischemic treatment model with polyhedra in sheet (Fig.4E)

FS

pre-ligation	55.9 ± 5.98	
ligation	PBS n=5	sfrp4-p n=6
2 weeks	32.4 ± 4.79	35.0 ± 3.32
4 weeks	30.2 ± 3.6	34.4 ± 4.9
8 weeks	31.7 ± 3.91	33.3 ± 3.27
12 weeks	27.2 ± 3.5	33.7 ± 4.88
16 weeks	25.9 ± 3.88	32.3 ± 5.3
20 weeks	24.4 ± 4.6	32.1 ± 3.22

FAC

pre-ligation	76.2 ± 4.33	
ligation	PBS n=5	sfrp4-p n=6
2 weeks	30.5 ± 3.38	41.7 ± 5.98
4 weeks	29.3 ± 3.4	40.2 ± 4.76
8 weeks	27.7 ± 4.4	38.3 ± 4.22
12 weeks	24.3 ± 3.87	39.3 ± 4.03
16 weeks	23.8 ± 2.7	38.0 ± 3.42
20 weeks	22.1 ± 4.25	37.9 ± 3.93

sub acute ischemic treatment model with polyhedra in fibrin glue (Fig.4F)

FS

pre-ligation	61.1 ± 6.46	
ligation	PBS n=6	sfrp4-p n=6
2 weeks	30.5 ± 3.9	35.2 ± 3.88
4 weeks	28.7 ± 3.42	34.1 ± 4.06
8 weeks	28.2 ± 4.33	33.0 ± 3.76
12 weeks	27.0 ± 3.9	33.7 ± 3.8
16 weeks	25.5 ± 2.63	32.4 ± 3.45

FAC

pre-ligation	80.6 ± 4.55	
ligation	PBS n=6	sfrp4-p n=6
2 weeks	28.7 ± 4.62	42.9 ± 3.66
4 weeks	27.4 ± 4.02	41.8 ± 3.72
8 weeks	25.5 ± 3.4	39.1 ± 4.55
12 weeks	25.9 ± 2.48	38.0 ± 3.8
16 weeks	23.1 ± 3.66	38.6 ± 4.42

FS (fractional shortening) and FAC (fractional area change) of control or Sfrp4-treated rat hearts corresponding to EF (ejection fraction) shown in figures (Figs. 2A–D, 3C, and 4E, F) are shown in the table. The values represent means + standard deviation.