

## Supplementary information

SREP-13-02864A

### Endocrine Protection of Ischemic Myocardium by FGF21

#### from the Liver and Adipose Tissue

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Supplementary Figures

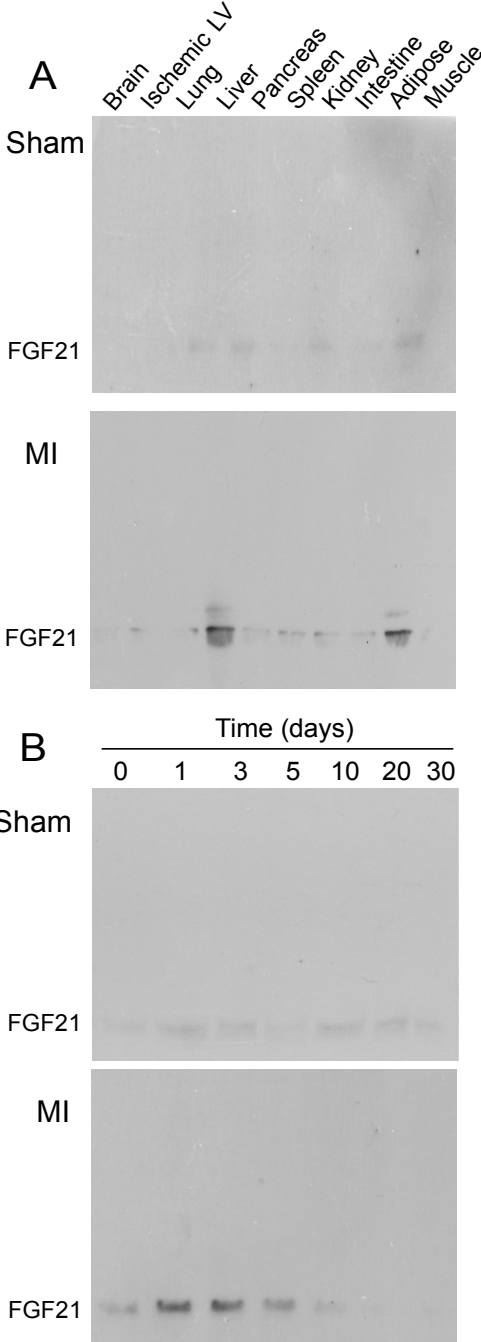


Figure 1. Original immunoblot images for Figure 1 in the main text.

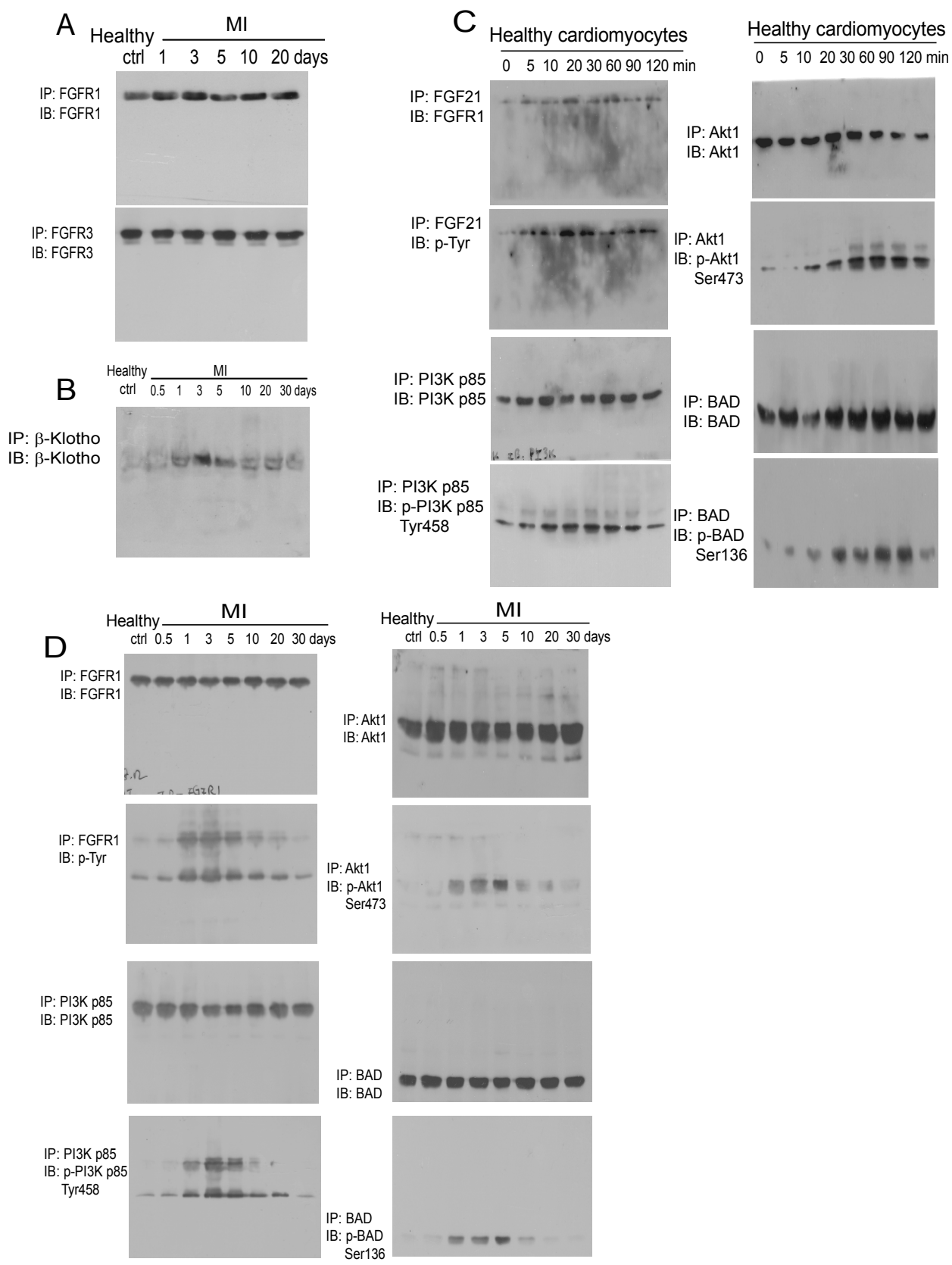


Figure 2. Original immunoblot images for Figure 2 in the main text.

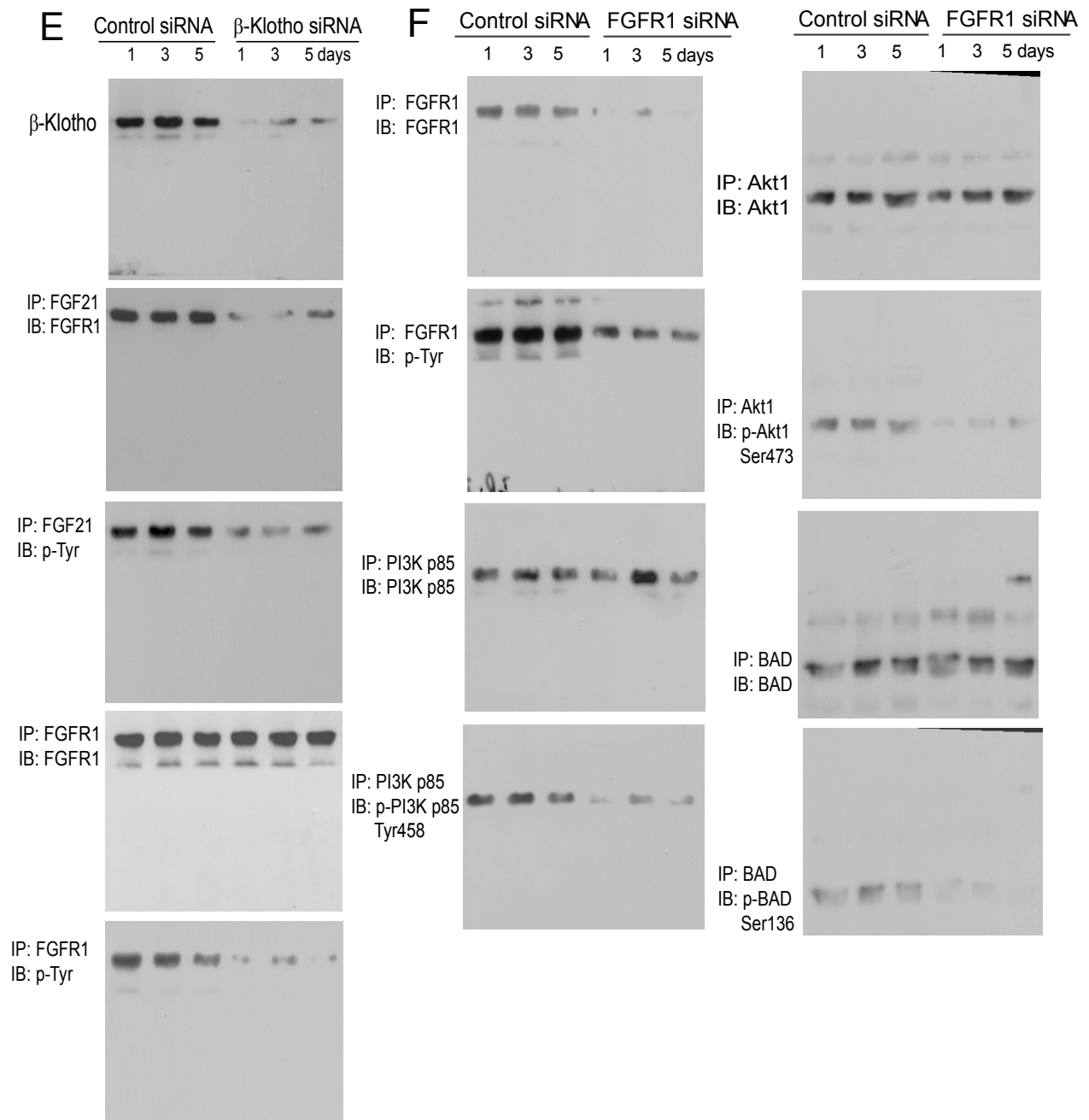


Figure 3. Original immunoblot images for Figure 3 in the main text.

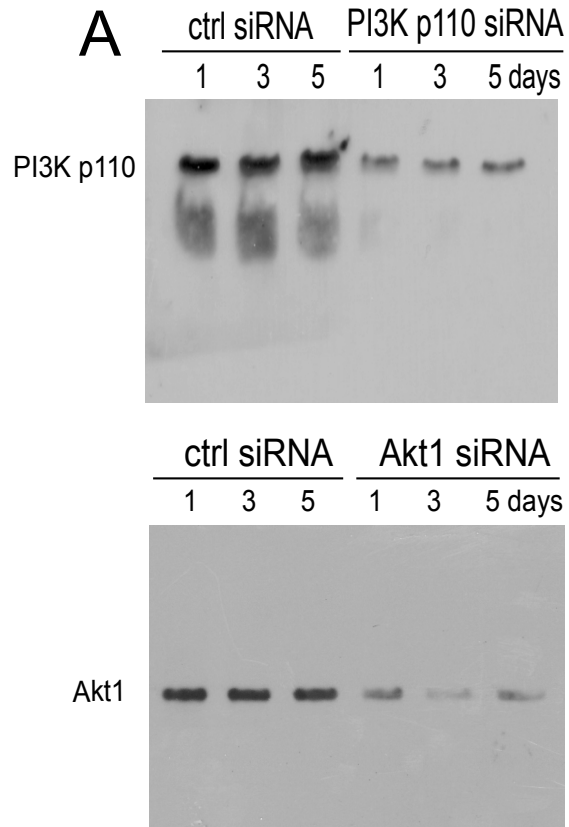


Figure 4. Original immunoblot images for Figure 7 in the main text.

## Supplementary Tables

The following tables present P values from post-hoc pairwise multiple comparisons tests by using the Tukey, Bonferroni, Fisher's least-significant-difference, and Scheffé methods for the listed parameters. The most conservative method was selected and used for difference evaluation.

**Table 1.** Caspase 3 activity in ischemic cardiomyocytes at 24 hrs. The P value from the global ANOVA test (one-way) is < 0.0001.

Treatments	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
a vs. b	< 0.0001	< 0.0001	< 0.0001	< 0.0001
a vs. c	< 0.0001	< 0.0001	< 0.0001	< 0.0001
a vs. d	< 0.0001	< 0.0001	< 0.0001	< 0.0001
b vs. c	< 0.005	< 0.005	< 0.002	< 0.01
b vs. d	> 0.5	> 0.5	> 0.5	> 0.5
c vs. d	< 0.002	< 0.002	< 0.0001	< 0.005

The letters a, b, c, and d represent wild-type sham controls, wild-type myocardial injury (MI), FGF21<sup>-/-</sup> MI with PBS administration, and FGF21<sup>-/-</sup> MI with FGF21 administration, respectively.

**Table 2.** Fraction of TUNEL-positive cell nuclei in the ischemic myocardium at 24 hrs. The P value from the global ANOVA test (one-way) is < 0.0001.

Treatments	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
a vs. b	< 0.0001	< 0.0001	< 0.0001	< 0.0001
a vs. c	< 0.0001	< 0.0001	< 0.0001	< 0.0001
a vs. d	< 0.0001	< 0.0001	< 0.0001	< 0.0001
b vs. c	< 0.0001	< 0.0001	< 0.0001	< 0.0001
b vs. d	< 0.002	< 0.005	< 0.0001	< 0.005
c vs. d	< 0.0001	< 0.0001	< 0.0001	< 0.0001

The letters a, b, c, and d represent wild-type sham controls, wild-type myocardial injury (MI), FGF21<sup>-/-</sup> MI with PBS administration, and FGF21<sup>-/-</sup> MI with FGF21 administration, respectively.

**Table 3.** Fraction of acute myocardial infarcts in reference to the area at risk at 24 hrs. The P value from the global ANOVA test (one-way) is < 0.001.

Treatments	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
a vs. b	< 0.02	< 0.02	< 0.005	< 0.02
a vs. c	< 0.5	< 0.5	< 0.2	< 0.5
b vs. c	< 0.0001	< 0.0001	< 0.0001	< 0.0001

The letters a, b, and c represent wild-type myocardial injury (MI), FGF21<sup>-/-</sup> MI with PBS administration, and FGF21<sup>-/-</sup> MI with FGF21 administration, respectively.

**Table 4.** Fraction of myocardial infarcts at 5, 10, and 30 days.

Table 4.1. Multiple comparisons in reference to times. The P value from the global ANOVA test (two-way) is < 0.0001.

Times (days)	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
5 vs. 10	< 0.0001	< 0.0001	< 0.0001	< 0.0001
5 vs. 30	< 0.0001	< 0.0001	< 0.0001	< 0.0001
10 vs. 30	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 4.2. Multiple comparisons in reference to treatments. The P value from the global ANOVA test (two-way) is < 0.0001.

Treatments	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
a vs. b	< 0.02	< 0.02	< 0.01	< 0.05
a vs. c	< 0.0001	< 0.0001	< 0.0001	< 0.0001
c vs. d	< 0.02	< 0.02	< 0.01	< 0.02

The letters a, b, and c represent wild-type myocardial injury (MI), FGF21<sup>-/-</sup> MI with PBS administration, and FGF21<sup>-/-</sup> MI with FGF21 administration, respectively.

**Table 5.** Left ventricular (LV) dp/dt and -dp/dt (absolute value) at 1, 5, 10, and 30 days.

Table 5.1. Multiple comparisons in LV dp/dt in reference to times. The P value from the global ANOVA test (two-way) is  $> 0.5$ .

Times (days)	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
1 vs. 5	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
1 vs. 10	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
1 vs. 30	$> 0.5$	$> 0.5$	$< 0.5$	$> 0.5$
5 vs. 10	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
5 vs. 30	$> 0.5$	$> 0.5$	$< 0.5$	$> 0.5$
10 vs. 30	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$

Table 5.2. Multiple comparisons in LV dp/dt in reference to treatments. The P value from the global ANOVA test (two-way) is  $< 0.0001$ .

Treatments	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
a vs. b	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
a vs. c	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
a vs. d	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
b vs. c	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
b vs. d	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
c vs. d	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$

The letters a, b, c, and d represent wild-type sham controls, wild-type myocardial injury (MI), FGF21<sup>-/-</sup> MI with PBS administration, and FGF21<sup>-/-</sup> MI with FGF21 administration, respectively.



Table 5.3 Multiple comparisons in LV  $-dp/dt$  in reference to times. The P value from the global ANOVA test (two-way) is  $> 0.5$ .

Times (days)	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
1 vs. 5	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
1 vs. 10	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
1 vs. 30	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
5 vs. 10	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
5 vs. 30	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
10 vs. 30	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$

Table 5.4. Multiple comparisons in LV  $-dp/dt$  in reference to treatments. The P value from the global ANOVA test (two-way) is  $< 0.0001$ .

Treatments	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
a vs. b	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
a vs. c	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
a vs. d	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
b vs. c	$< 0.01$	$< 0.01$	$< 0.002$	$< 0.02$
b vs. d	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
c vs. d	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$

The letters a, b, c, and d represent wild-type sham controls, wild-type myocardial injury (MI), FGF21<sup>-/-</sup> MI with PBS administration, and FGF21<sup>-/-</sup> MI with FGF21 administration, respectively.

**Table 6.** Fractional shortening of the left ventricle at 1, 5, 10, and 30 days.

Table 6.1. Multiple comparisons in reference to times. The P value from the global ANOVA test (two-way) is  $< 0.1$ .

Times (days)	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
1 vs. 5	$> 0.5$	$> 0.5$	$< 0.5$	$> 0.5$
1 vs. 10	$> 0.5$	$> 0.5$	$< 0.5$	$> 0.5$
1 vs. 30	$< 0.5$	$> 0.5$	$< 0.2$	$> 0.5$
5 vs. 10	$< 0.5$	$< 0.5$	$< 0.1$	$< 0.5$
5 vs. 30	$< 0.2$	$< 0.2$	$< 0.05$	$< 0.2$
10 vs. 30	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$

Table 6.2. Multiple comparisons in reference to treatments. The P value from the global ANOVA test (two-way) is  $< 0.0001$ .

Treatments	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
a vs. b	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
a vs. c	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
a vs. d	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
b vs. c	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
b vs. d	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$
c vs. d	$< 0.0001$	$< 0.0001$	$< 0.0001$	$< 0.0001$

The letters a, b, c, and d represent wild-type sham controls, wild-type myocardial injury (MI), FGF21<sup>-/-</sup> MI with PBS administration, and FGF21<sup>-/-</sup> MI with FGF21 administration, respectively.

**Table 7.** Influence of siRNA-mediated FGFR1,  $\beta$ -Klotho, PI3K p110, or Akt1 gene silencing on the fraction of myocardial infarcts at day 5. The P value from the global ANOVA test (one-way) is  $< 0.005$ .

Treatments	P values			
	Tukey	Bonferroni	Fisher's	Scheffé
a vs. b	$< 0.01$	$< 0.01$	$< 0.002$	$< 0.05$
a vs. c	$< 0.1$	$< 0.2$	$< 0.02$	$< 0.2$
a vs. d	$< 0.002$	$< 0.002$	$< 0.0001$	$< 0.005$
a vs. e	$< 0.01$	$< 0.01$	$< 0.002$	$< 0.05$
b vs. c	$> 0.5$	$> 0.5$	$< 0.5$	$> 0.5$
b vs. d	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
b vs. e	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$
c vs. d	$< 0.5$	$> 0.5$	$< 0.2$	$> 0.5$
c vs. e	$> 0.5$	$> 0.5$	$< 0.5$	$> 0.5$
d vs. e	$> 0.5$	$> 0.5$	$> 0.5$	$> 0.5$

The letters a, b, c, d, and e represent control, FGFR1,  $\beta$ -Klotho, PI3K p110, and Akt1 siRNA treatments, respectively.