

Supplementary material

IRX2 regulates angiotensin II-induced cardiac fibrosis by transcriptionally activating EGR1 in mice

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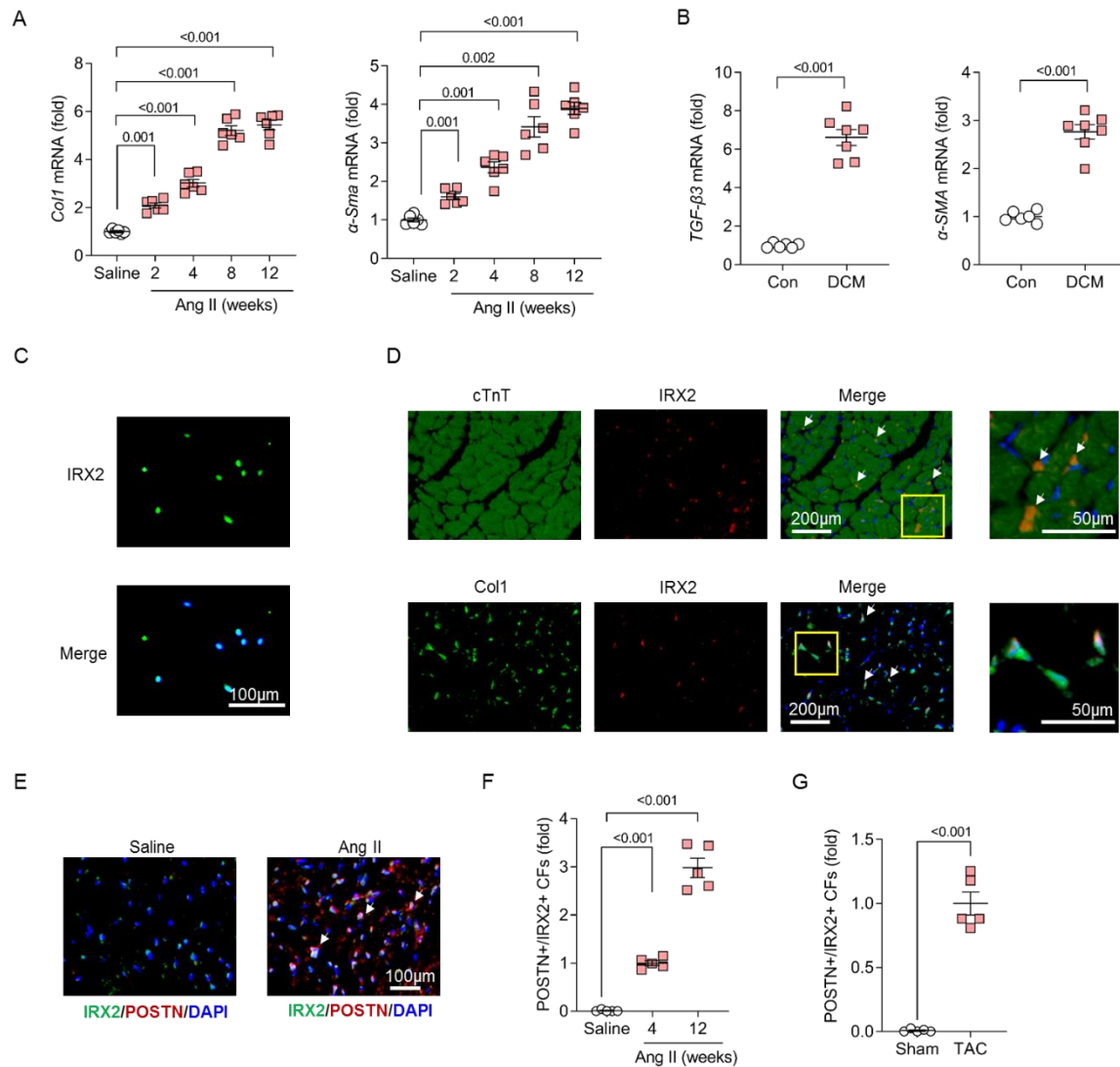
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Figure S1

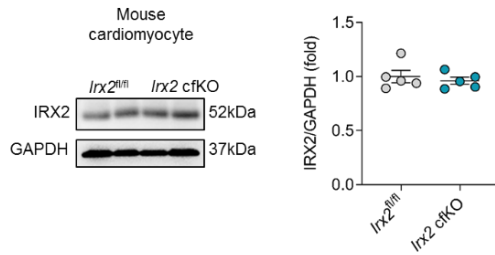


Supplementary Figure 1 IRX2 was mainly expressed in adult cardiac fibroblasts. **A**, Relative mRNA levels of *Col1* and α -*Sma* in the heart after Ang II infusion by an osmotic minipump ($n=6$). **B**, Relative mRNA levels of *TGF- β 3* and α -*SMA* in heart samples obtained from patients with dilated cardiomyopathy (DCM) and control (Con) donors (Con, $n=6$; DCM, $n=7$). **C**, Representative images of IRX2 (green) staining in CFs isolated from wild-type mice. Nuclei were stained with DAPI (blue) ($n=4$). **D**, Representative images of IRX2 (red), cardiac Troponin T (cTnI, green, top), and Col1 (green, bottom) in heart samples. Nuclei were stained with DAPI (blue). Hearts were subjected to immunofluorescence staining to detect the cellular location of IRX2 ($n=4$). **E**, Representative images of IRX2 (green), periostin (POSTN, red) in 12 week-Ang II-infused heart samples. Nuclei were stained with DAPI (blue). Hearts were subjected to immunofluorescence staining to detect POSTN+/IRX2+ CFs ($n=5$). **F-G**, Statistical analysis of POSTN+/IRX2+ CFs in Ang II or transverse aortic constriction (TAC)-treated hearts ($n=5$). Data are shown as the mean $\pm$ SEM, and analysed using one-way

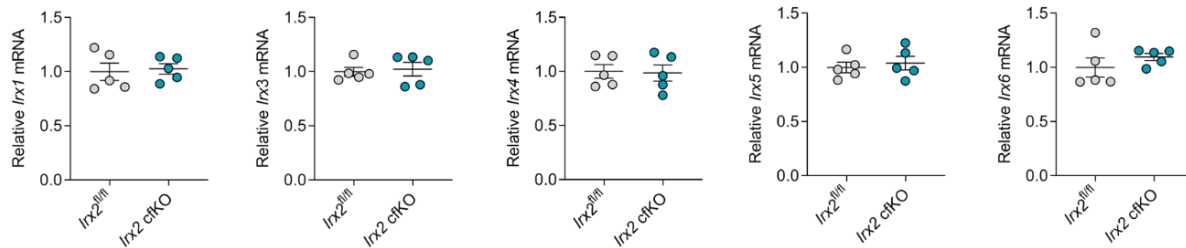
1 ANOVA followed by Tukey post hoc test (**F**) or Tamhane's T2 test (**A**). For the analysis in (**B** and **G**), an
2 unpaired two-tailed Student's *t* test was conducted. Source data are provided as a Source Data file
3

Figure S2

A



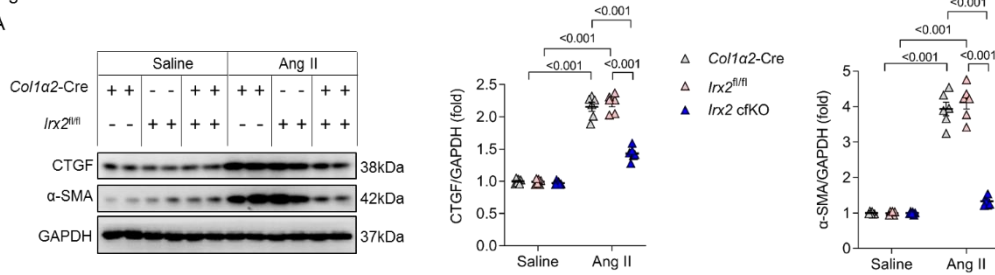
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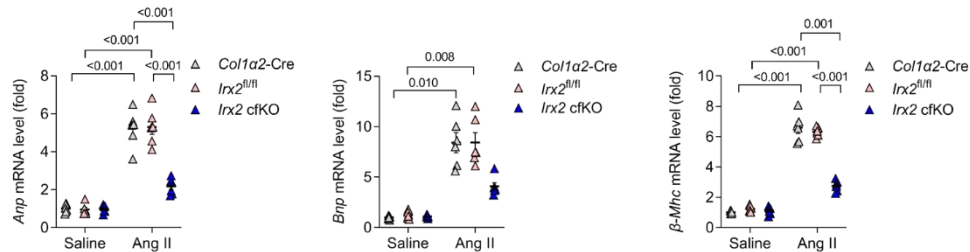
Supplementary Figure 2 Conditional fibroblast-specific *lrx2*-depleted mice were generated to investigate the role of IRX2 in cardiac fibrosis induced by angiotensin II (Ang II) infusion. **A**, Representative western blots and statistical analysis of IRX2 protein expression in cardiomyocytes isolated from *lrx2* cfKO mice and littermate controls ($n=5$). **B**, The mRNA levels of other *lrx* members in *lrx2*-deficient CFs ($n=5$). Data are shown as the mean $\pm$ SEM, and analysed using an unpaired two-tailed Student's *t* test. Source data are provided as a Source Data file.

Figure S3

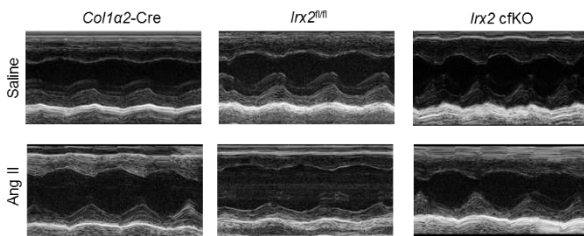
A



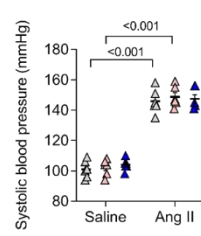
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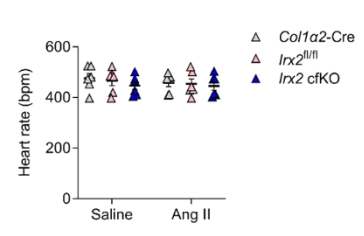
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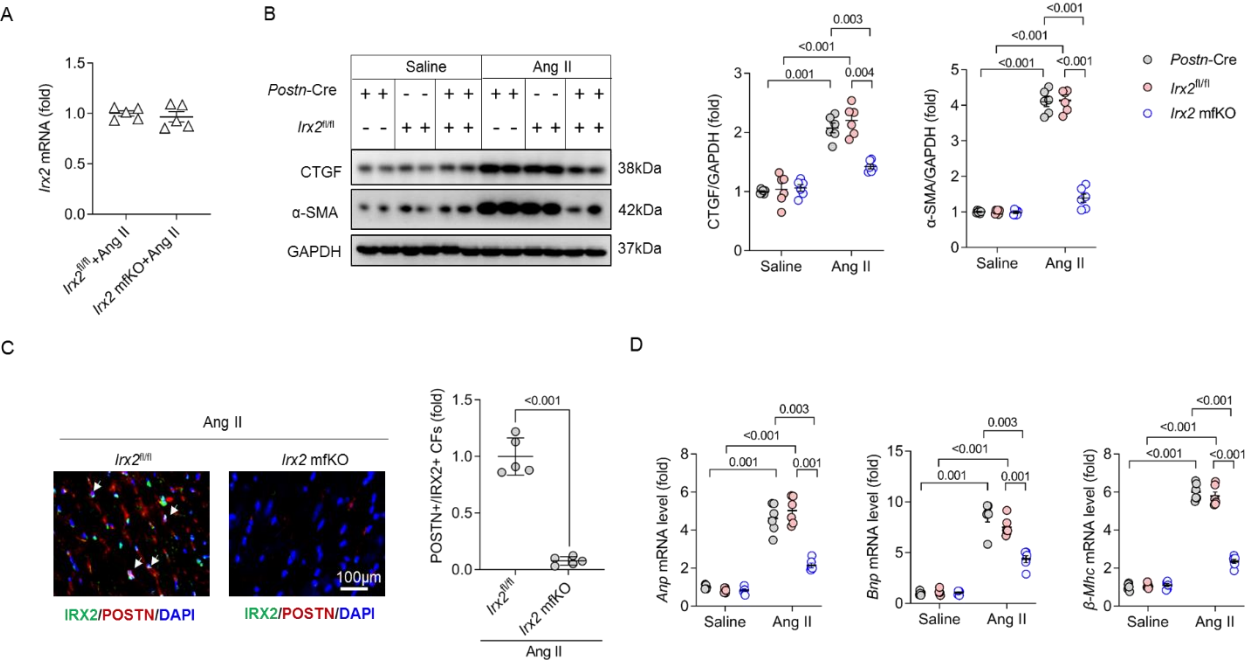


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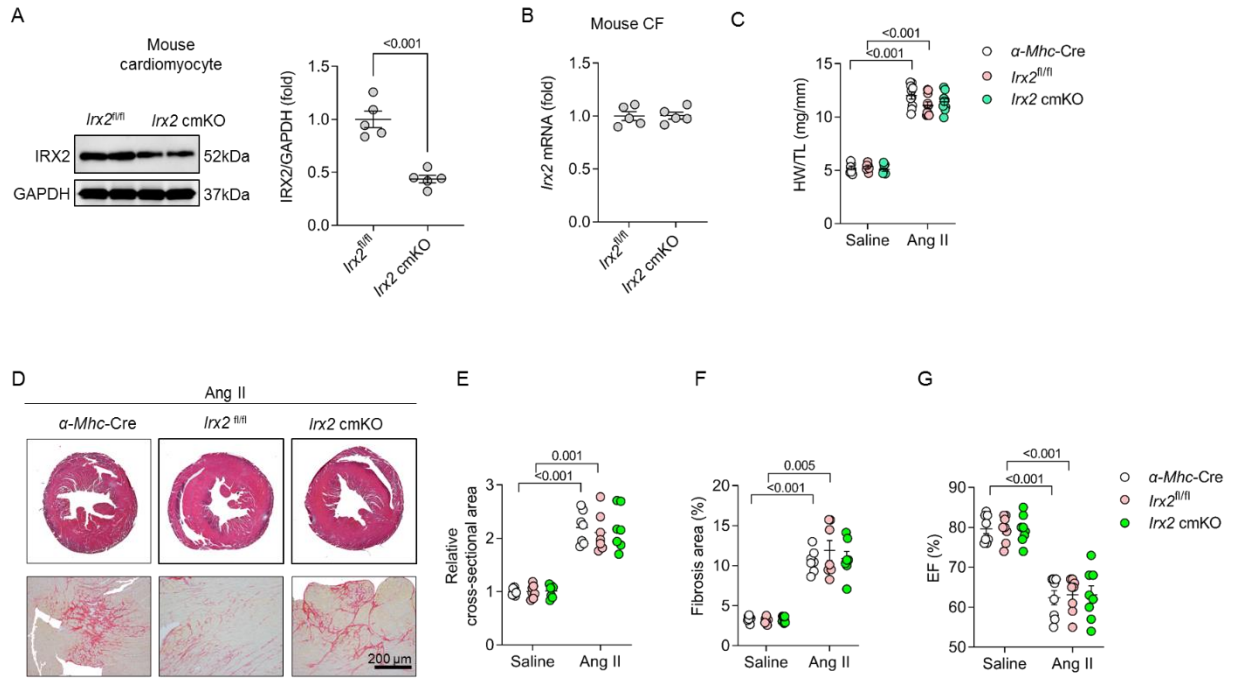
Supplementary Figure 3 Conditional fibroblast-specific *Irx2* depletion attenuated angiotensin II (Ang II) infusion-induced cardiac fibrosis. **A**, Representative western blots and statistical analysis of α -smooth muscle actin (α -SMA) and connective tissue growth factor (CTGF) expression in hearts from *Irx2* cfKO mice and littermate controls after Ang II infusion (n=6). **B**, Relative mRNA levels of *Anp*, *Bnp*, and *β-Mhc* detected in hearts from *Irx2* cfKO mice and control mice (n=6). **C**, Representative echocardiograms in the indicated groups. **D**, Systolic blood pressure of *Irx2* cfKO and littermate controls (n=6). **E**, Heart rate of *Irx2* cfKO and littermate controls (n=6). Data are shown as the mean ± SEM, and analysed using one-way ANOVA followed by Tukey post hoc test (**D-E**) or Tamhane's T2 test (**A-B**). Source data are provided as a Source Data file.

Figure S4



Supplementary Figure 4 Conditional myofibroblast-specific *Irx2* depletion attenuated angiotensin II (Ang II)-induced fibrotic remodelling in mice. **A**, *Irx2* mRNA expression in cardiomyocytes isolated from *Irx2* mflKO mice and littermate controls after Ang II infusion for 12 weeks ($n=5$). **B**, Representative western blots and statistical analysis of α -smooth muscle actin (α -SMA) and connective tissue growth factor (CTGF) in hearts from *Irx2* mflKO mice and control mice ($n=6$). **C**, Representative images and statistical analysis of POSTN+/IRX2+ CFs in hearts ($n=5$). **D**, Relative mRNA levels of *Anp*, *Bnp*, and β -*Mhc* detected in hearts from *Irx2* mflKO mice and control mice after Ang II infusion ($n=6$). Data are shown as the mean $\pm$ SEM, and analysed using one-way ANOVA followed by Tamhane's T2 test (**B** and **D**). For the analysis in (**A** and **C**), an unpaired two-tailed Student's *t* test was conducted. Source data are provided as a Source Data file.

Figure S5



Supplementary Figure 5 Conditional cardiomyocyte-specific *Irx2* depletion did not affect angiotensin II

(Ang II)-induced fibrotic remodelling in mice. Conditional cardiomyocyte-specific *Irx2*-deficient mice (*Irx2*

cmKO) were bred by crossing mice with a conditional knockout allele of *Irx2* (*Irx2*^{fl/fl}) with *α-Mhc-Cre* mice.

Irx2 cmKO mice and littermate controls were subjected to Ang II infusion for 12 weeks. **A**, Representative

western blots and statistical analysis of IRX2 protein expression in cardiomyocytes isolated from *Irx2* cmKO

mice and littermate controls (*n*=5). **B**, *Irx2* mRNA in CFs isolated from *Irx2* cmKO mice and littermate controls

after Ang II infusion for 12 weeks (*n*=5). **C**, Heart weight-to-tibia length (HW/TL) ratio (*n* = 8 mice, *α-Mhc-*

Cre+Saline; *n* = 8 mice, *Irx2*^{fl/fl}+Saline; *n* = 8 mice, *Irx2* cmKO+Saline; *n* = 10 mice, *α-Mhc-Cre*+TAC; *n* = 10

mice, *Irx2*^{fl/fl}+TAC; *n* = 9 mice, *Irx2* cmKO+TAC). **D**, Histological staining showed heart enlargement and

cardiac fibrosis after Ang II infusion. **E**, The cell area of cardiomyocytes was determined after Ang II infusion

(*n*=7). **F**, Cardiac fibrosis was determined by picrosirius red staining of samples from *Irx2* cmKO mice and

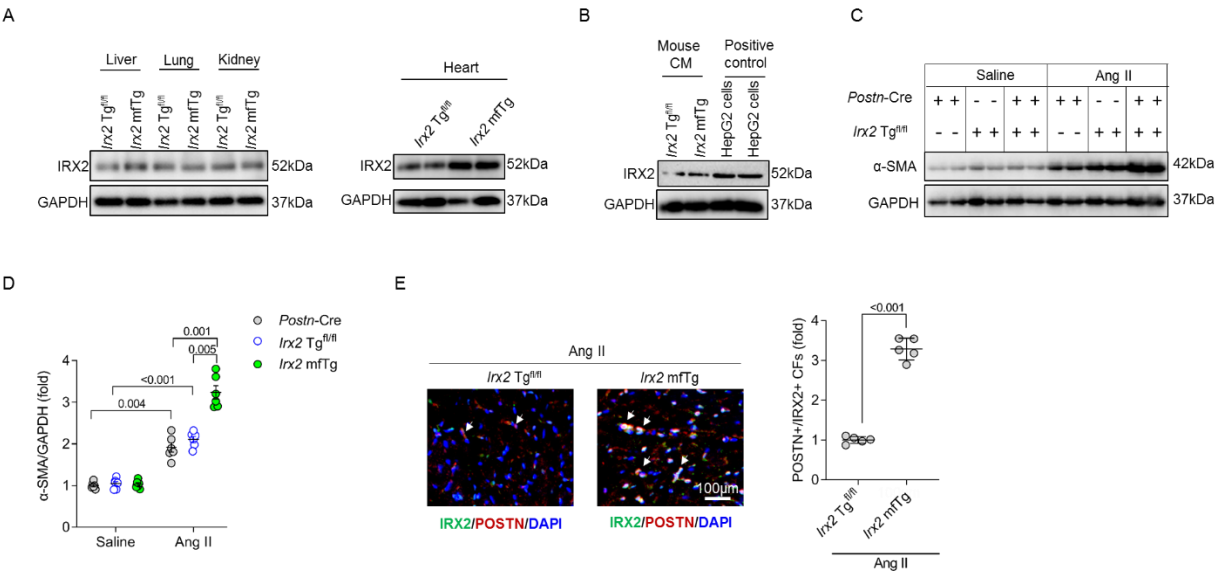
littermate controls after Ang II infusion (*n*=7). **G**, The ejection fraction (EF) was detected in these groups (*n*=8).

Data are shown as the mean ± SEM, and analysed using one-way ANOVA followed by Tukey post hoc test (**G**)

or Tamhane's T2 test (**C**, **E**, **F**). For the analysis in (**A-B**), an unpaired two-tailed Student's *t* test was conducted.

Source data are provided as a Source Data file.

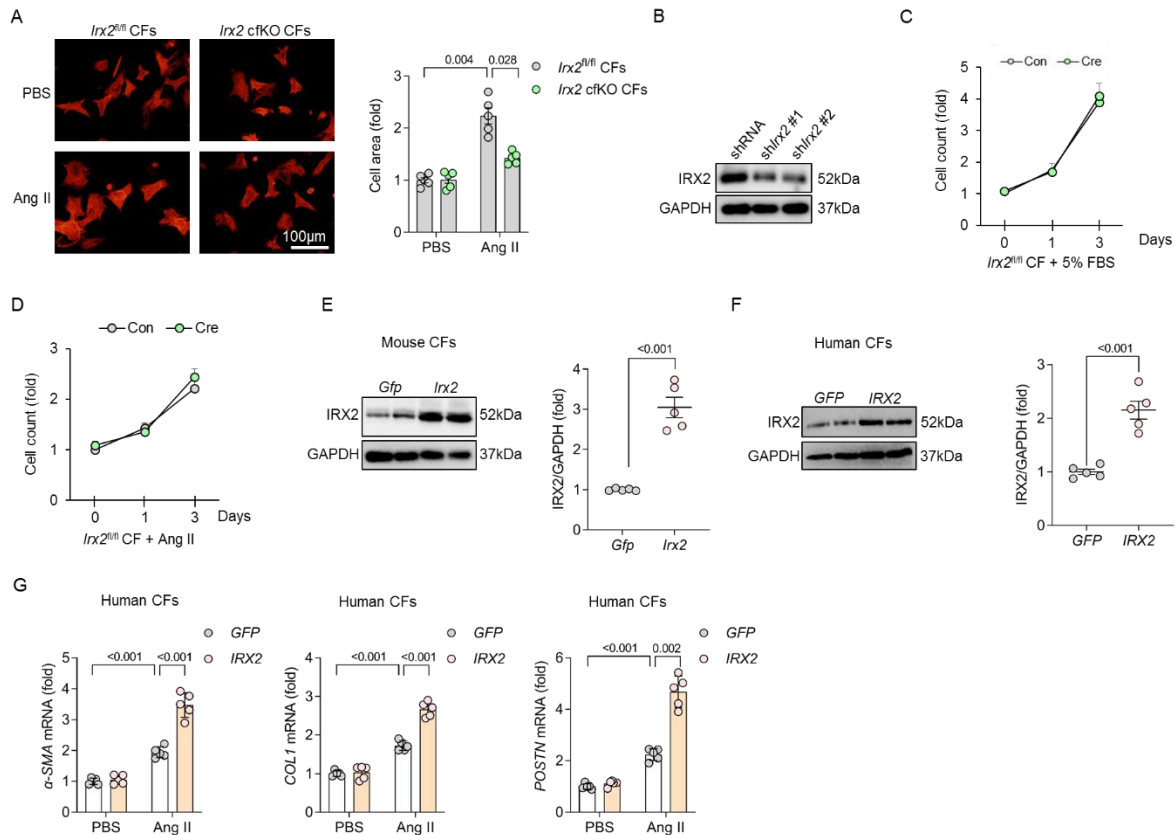
Figure S6



Supplementary Figure 6 Conditional myofibroblast-specific *Irx2*-overexpressing mice were generated.

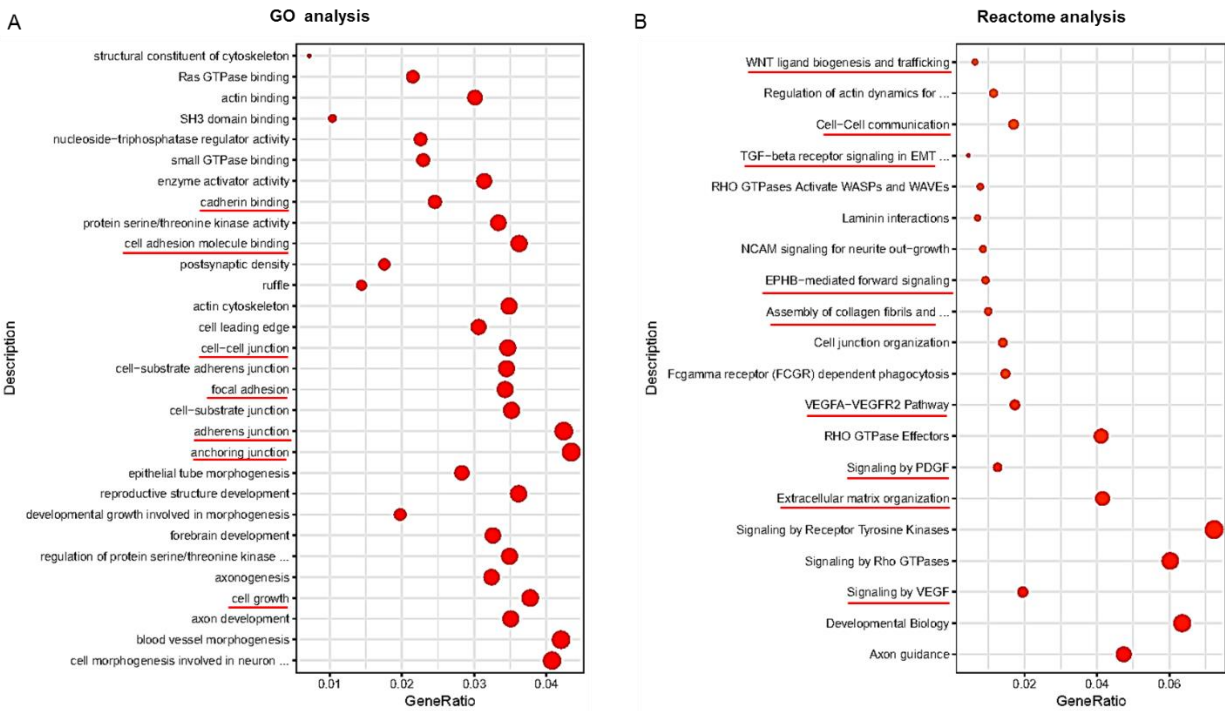
Conditional myofibroblast-specific *Irx2*-overexpressing mice (*Irx2* mfTg) were bred by crossing mice with a conditional transgenic allele of *Irx2* (*Irx2* Tg^{fl/fl}) with *Postn*-Cre mice. *Irx2* mfTg mice and littermate controls were subjected to angiotensin II (Ang II) infusion for 4 weeks. **A**, Representative western blots of IRX2 protein expression in the liver, lungs, kidneys and heart of *Irx2* mfTg mice and littermate controls after Ang II infusion ($n=4$). **B**, Representative western blots of IRX2 protein expression in cardiomyocytes (CMs) isolated from *Irx2* mfTg mice and littermate controls after Ang II infusion for 4 weeks ($n=5$). HepG2 cells were used as the positive control. **C-D**, Representative western blots and statistical analysis of the protein α -smooth muscle actin (α -SMA) in hearts from *Irx2* mfTg mice and littermate controls after Ang II infusion ($n=6$). **E**, Representative images and statistical analysis of POSTN+/IRX2+ CFs in hearts ($n=5$). Data are shown as the mean $\pm$ SEM, and analysed using one-way ANOVA followed by Tamhane's T2 test (**D**). For the analysis in (**E**), an unpaired two-tailed Student's *t* test was conducted. Source data are provided as a Source Data file.

Figure S7



Supplementary Figure 7 IRX2 did not affect adult cardiac fibroblast (CF) proliferation in vitro. **A**, CFs were isolated from *Irx2* cfKO mice and *Irx2^{fl/fl}* mice and then stimulated with angiotensin II (Ang II) for another 24 h to induce a fibrotic phenotype. Then, conditioned medium was collected from these myofibroblasts and used to treat neonatal rat cardiomyocytes in the absence or presence of Ang II for 24 h. After that, the cell area of cardiomyocytes was detected by immunofluorescence staining with anti- α -actinin (red) ($n=5$). **B**, Representative western blots of IRX2 protein expression in CFs transfected with shRNA. **C**, The cell counts of CFs with *Irx2* depletion were measured after incubation with 5% FBS ($n=3$ for each time point). **D**, The cell counts of CFs with *Irx2* depletion were measured after incubation with Ang II ($n=4$ for each time point). **E**, Representative western blots and statistical analysis of IRX2 protein expression in mouse CFs infected with an adenovirus carrying *Irx2* ($n=5$). **F**, Representative western blots and statistical analysis of IRX2 protein expression in human CFs infected with an adenovirus carrying *IRX2* ($n=5$). **G**, Human CFs were infected with an adenoviral vector to overexpress *IRX2* and then subjected to Ang II treatment for 24 h. Total RNA was extracted to detect the mRNA levels of α -SMA, *COL1* and *POSTN* ($n=5$). Data are shown as the mean $\pm$ SEM, and analysed using one-way ANOVA followed by Tamhane's T2 test (**A**, **G**). For the analysis in (**E-F**), an unpaired two-tailed Student's *t* test was conducted. For the analysis in (**C-D**), repeated measures ANOVA was conducted. Source data are provided as a Source Data file.

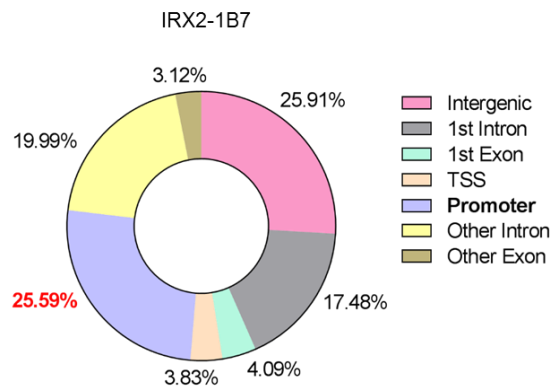
Figure S8



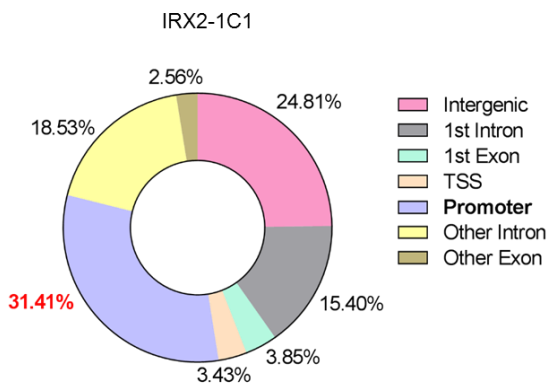
Supplementary Figure 8 GO pathway and Reactome pathway analyses of the RNA-sequencing dataset of *Irx2*-overexpressing cardiac fibroblasts. **A**, GO pathway analysis based on differentially expressed genes identified from the RNA-sequencing dataset of *Irx2*-overexpressing cardiac fibroblasts. **B**, Reactome pathway analysis was performed based on the RNA-sequencing dataset of *Irx2*-overexpressing cardiac fibroblasts. Several signalling pathways, such as the Wnt signalling pathway, the TGF- β receptor signalling, the VEGF signalling, the PDGF signalling, the assembly of collagen fibrils and extracellular matrix organization, were found to be regulated by IRX2. Only significant pathways ($p_{adj} < 0.01$) are shown.

Figure S9

A

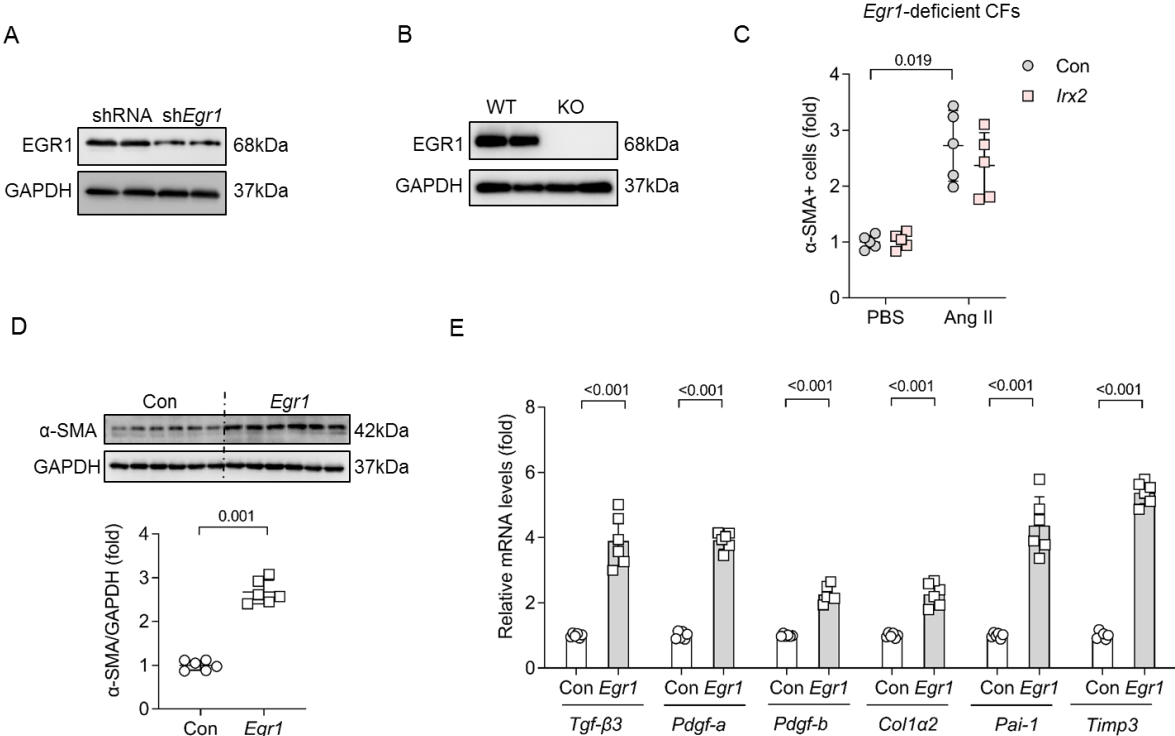


B



Supplementary Figure 9 Pie chart of the distribution of the IRX2 binding sites identified by ChIP-Seq. A, Pie chart of the distribution of the IRX2 binding sites identified by ChIP-Seq with the IRX2-1B7 antibody. **B,** Pie chart of the distribution of the IRX2 binding sites identified by ChIP-Seq with the IRX2-1C1 antibody.

Figure S10

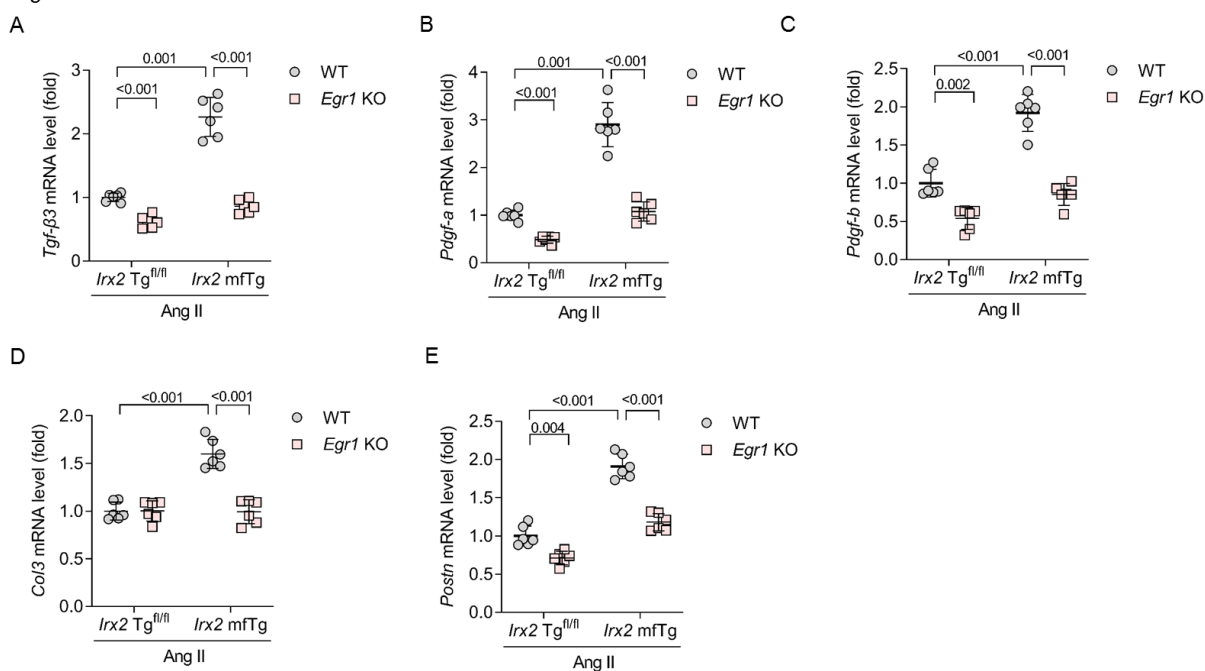


Supplementary Figure 10 EGR1 mediated the fibrotic response in *Irx2*-overexpressing cardiac fibroblasts.

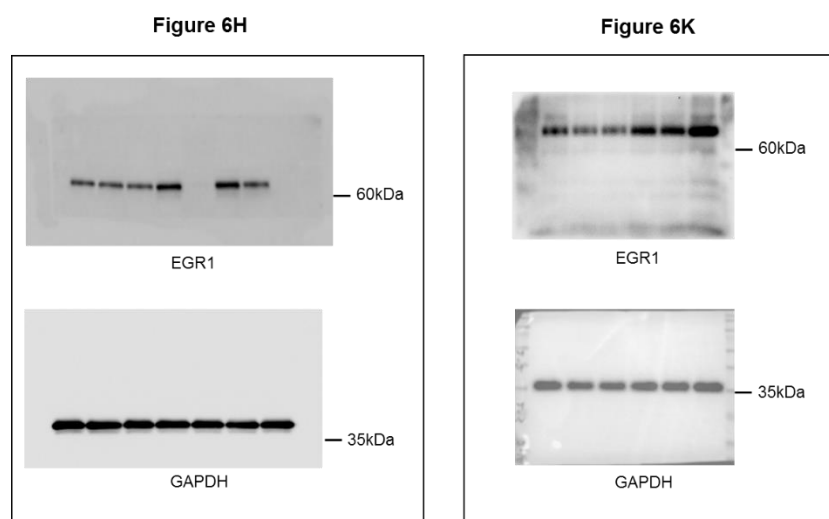
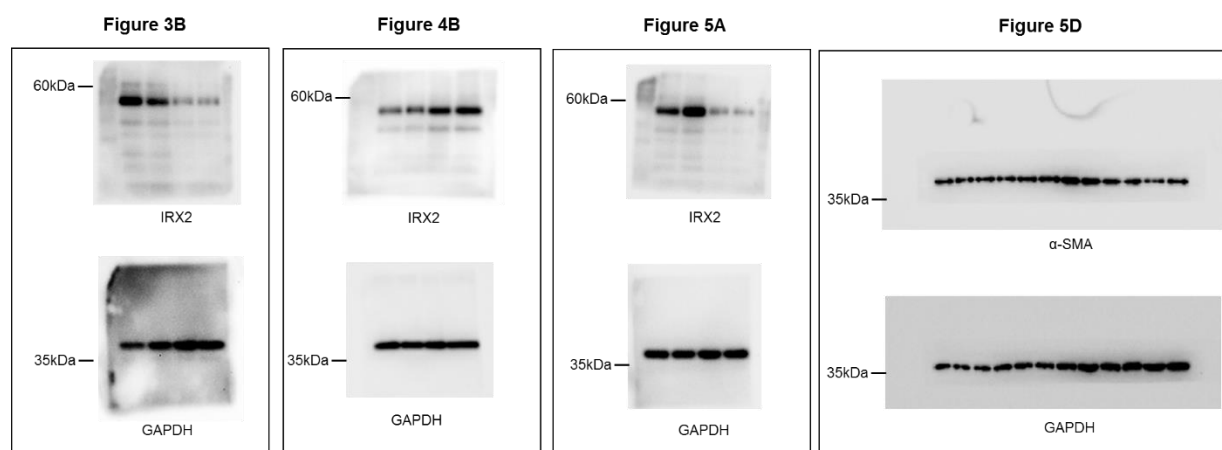
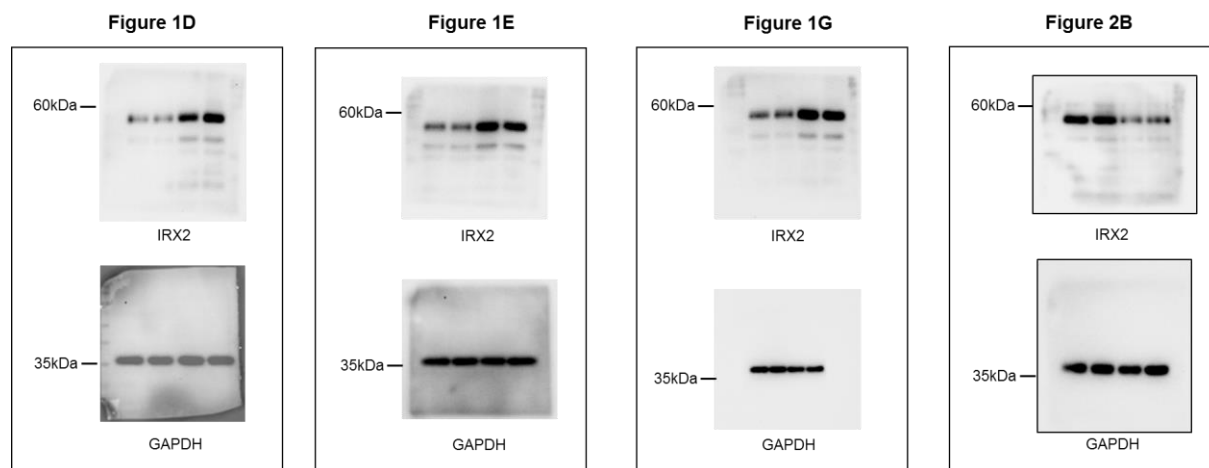
A, Representative western blots of EGR1 protein expression in CFs transfected with shRNA ($n=4$). **B**, EGR1 protein expression was detected in CFs isolated from *Egr1*-deficient mice ($n=4$). **C**, Statistical analysis of α -smooth muscle actin (α -SMA)+ cells in response to Ang II treatment for 24 h ($n=5$, for each experiment, 50-60 fields were counted). **D**, Representative western blots and statistical analysis of α -SMA protein expression in *Egr1*-overexpressing CFs ($n=6$). **E**, The mRNA levels of *Tgf- β 3*, *Pdgf-a*, *Pdgf-b*, *Col1a2*, *Pai-1* and *Timp3* in *Egr1*-overexpressing CFs ($n=5$). Data are shown as the mean $\pm$ SEM, and analysed using one-way ANOVA followed by Tukey test (**C**). For the analysis in (**D-E**), an unpaired two-tailed Student's *t* test was conducted.

Source data are provided as a Source Data file.

Figure S11



Supplementary Figure 11 Genetic depletion of *Egr1* decreased fibrotic markers expression in mice with conditional myofibroblast-specific *Irx2* overexpression after angiotensin II (Ang II) infusion for 4 weeks. Conditional myofibroblast-specific *Irx2*-overexpressing mice (*Irx2* mfTg) were bred with *Egr1* global knockout mice. The resulting mouse line and littermate controls were subjected to Ang II infusion for 4 weeks. **A-E**, Relative mRNA levels of *Tgf-β3* (**A**), *Pdgf-a* (**B**), *Pdgf-b* (**C**), *Col3* (**D**) and *Postn* (**E**) in the heart (n=6). Data are shown as the mean ± SEM, and analysed using one-way ANOVA followed by Tukey post hoc test (**C-E**) or Tamhane's T2 test (**A-B**). Source data are provided as a Source Data file.



Supplementary Figure 12. Uncropped gels relating to indicated figures.

Figure 7H

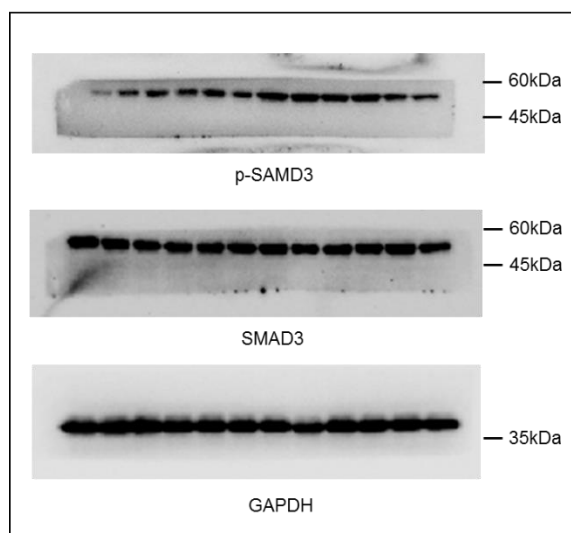


Figure 7I

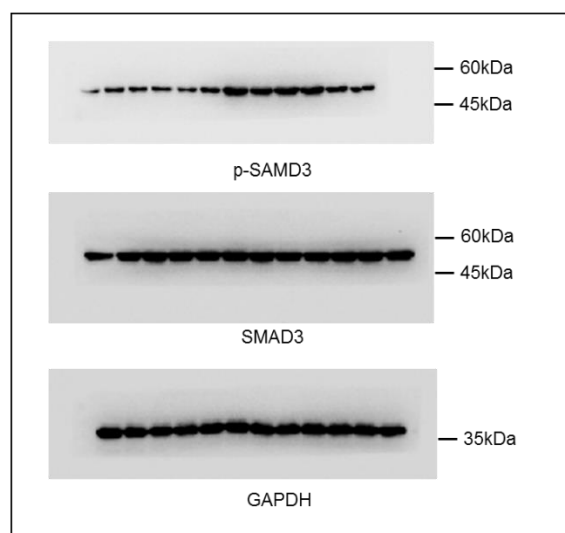


Figure S2A

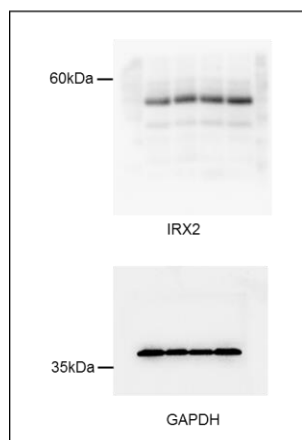


Figure S3A

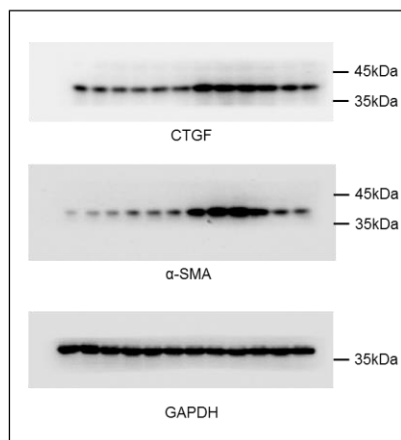


Figure S4B

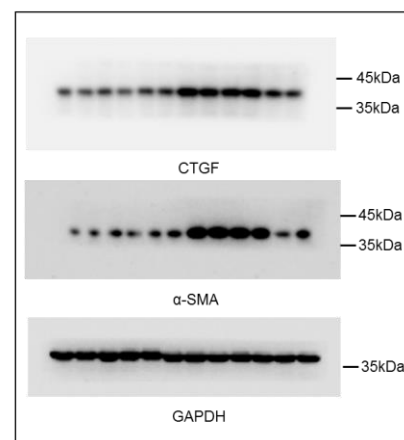


Figure S5A

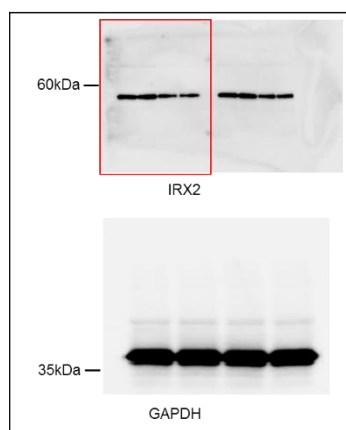


Figure S6A

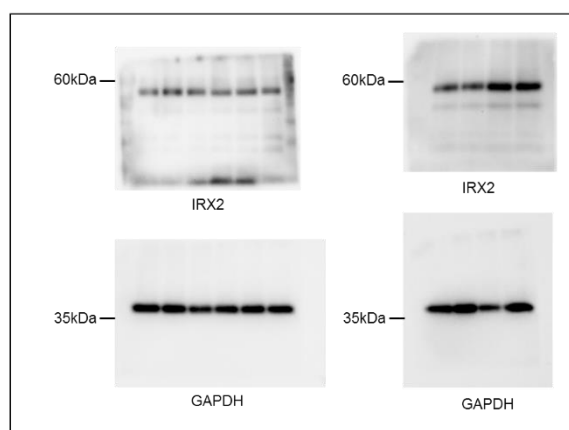
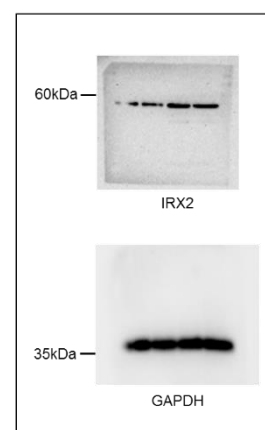
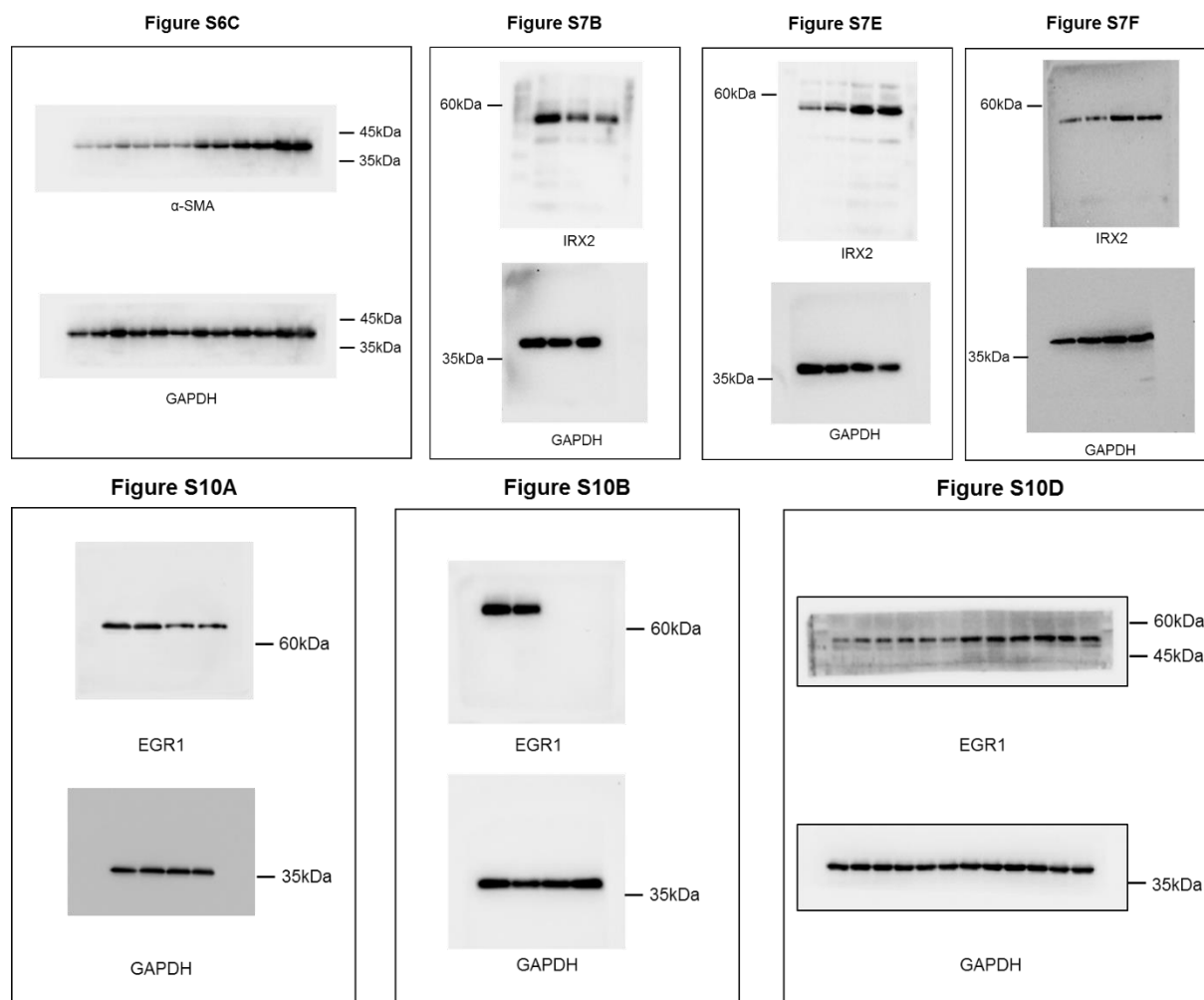


Figure S6B



Supplementary Figure 12. Uncropped gels relating to indicated figures (continued).



Supplementary Figure 12. Uncropped gels relating to indicated figures (continued).

1 **Supplementary Table 1 Primer sequences used in our study**

Gene	Species	Sequence	
<i>GAPDH</i>	Human	Forward	AGCCACATCGCTCAGACAC
		Reverse	GCCCAATACGACCAAATCC
<i>IRX1</i>	Human	Forward	CCAGTTCCAATACGGGGACC
		Reverse	CCATCGTGCTCGTCGATCTT
<i>IRX2</i>	Human	Forward	CCGAGAAACAAAAGCGAAGA
		Reverse	AGCACGAGTGATCCGTGAG
<i>IRX3</i>	Human	Forward	AAAAGTTACTCAAGACAGCTTTCCA
		Reverse	GGATGAGGAGAGAGCCGATA
<i>IRX4</i>	Human	Forward	ACTACCCTTACGAGCCAGC
		Reverse	CCTTGAGCGTGCTGGTGG
<i>IRX5</i>	Human	Forward	CCTATCCGCAGGGCTACTTG
		Reverse	CTCAGCTCCTCCTGCTTCG
<i>IRX6</i>	Human	Forward	CTCAACGAGCACCGCAAAAA
		Reverse	TCTCTGGGGACTGAGAGTCG
<i>EGR1</i>	Human	Forward	ACCTGACCGCAGAGTCTTTTC
		Reverse	GATGAGCTGGGACTGGTAGC
<i>Irx1</i>	Mouse	Forward	TTATCCCTATGGTCAGTTTCAATACG
		Reverse	CGTTGAGCCAGGCTTTTCAG
<i>Irx2</i>	Mouse	Forward	ACGCACACCACCGGAATG
		Reverse	ATGGATAGGCCGCACTGC
<i>Irx3</i>	Mouse	Forward	CGCCTCAAGAAGGAGAACAAGA
		Reverse	CGCTCGCTCCCATAAGCAT
<i>Irx4</i>	Mouse	Forward	GCGGGCCGGCTCTTTCTTG
		Reverse	AGTTCTAGCTCCTTGTCGTCTTTG
<i>Irx5</i>	Mouse	Forward	GGCTACAACCTCGCACCTCCA
		Reverse	CCAAGGAACCTGCCATACCG
<i>Irx6</i>	Mouse	Forward	GGCGGCCTGCTCCTGCAGCCC
		Reverse	GGATGTGCTGCCATACGGGTGT
<i>Gapdh</i>	Mouse	Forward	ACTCCACTCACGGCAAATTC
		Reverse	TCTCCATGGTGGTGAAGACA
<i>Anp</i>	Mouse	Forward	ACCTGCTAGACCACCTGGAG
		Reverse	CCTTGGCTGTTATCTTCGGTACCGG
<i>Bnp</i>	Mouse	Forward	GCTGCTTTGGGCACAAGATAG
		Reverse	GGTCTTCCTACAACAACCTTCAG

<i>β-Mhc</i>	Mouse	Forward	CCGAGTCCCAGGTCAACAA
		Reverse	CTTCACGGGCACCCTTGGA
<i>Col1</i>	Mouse	Forward	CCCAACCCAGAGATCCCATT
		Reverse	GAAGCACAGGAGCAGGTGTAGA
<i>Col3</i>	Mouse	Forward	CCCAACCCAGAGATCCCATT
		Reverse	GAAGCACAGGAGCAGGTGTAGA
<i>α-Sma</i>	Mouse	Forward	TGGCTCTGTGCAAGAAGCGGAT
		Reverse	AATCTGGGCGTTGTCACTGCGT
<i>Egr1</i>	Mouse	Forward	CCTTTTCTGACATCGCTCTGAA
		Reverse	CGAGTCGTTTGGCTGGGATA
<i>Tgf-β3</i>	Mouse	Forward	ATGACCCACGTCCCCTATCA
		Reverse	ACTCAGACTCCGAGGTCTCC
<i>Pdgf-a</i>	Mouse	Forward	GTGCGACCTCCAACCTGA
		Reverse	GGCTCATCTCACCTCACATCT
<i>Pdgf-b</i>	Mouse	Forward	CGGCCTGTGACTAGAAGTCC
		Reverse	GAGCTTGAGGCGTCTTGG
<i>Pai-1</i>	Mouse	Forward	AGGATCGAGGTAAACGAGAGC
		Reverse	TTGGTTGAGGGAATCATTCAT
<i>Timp3</i>	Mouse	Forward	CACGGAAGCCTCTGAAAGTC
		Reverse	TCCCACCTCTCCACAAAGTT
<i>TGF-β3</i>	Human	Forward	ACTTGCACCACCTTGGACTTC
		Reverse	GGTCATCACCGTTGGCTCA
<i>α-SMA</i>	Human	Forward	CCCTAAAGCTTCCCAGACTTCCG
		Reverse	AGTCCCGGGGATAGGCAAA
<i>PERIOSTIN</i>	Human	Forward	TTGAGACGCTGGAAGGAAAT
		Reverse	AGATCCGTGAAGGTGGTTTG

1

2

1 **Supplementary Table 2 Differentially expressed genes in IRX2-overexpressed mouse cardiac fibroblasts**

Gene	<i>Irx2_1</i>	<i>Irx2_2</i>	<i>Irx2_3</i>	<i>Gfp_1</i>	<i>Gfp_2</i>	<i>Gfp_3</i>	FC	<i>P</i> value	padj
							(<i>Irx2</i> vs		
							<i>Gfp</i>)		
Fibrotic genes									
<i>Tgf-β3</i>	1425.88	1358.89	1570.59	1141.46	1249.39	807.58	1.36	0.001	0.006
<i>Tgf-βr2</i>	1897.76	1814.48	1690.65	1395.87	1453.55	1336.82	1.29	0.000	0.000
<i>Pdgf-a</i>	1084.04	1135.03	1211.12	942.21	981.67	852.82	1.24	0.001	0.007
<i>Pdgf-B</i>	317.69	305.04	252.05	121.58	117.36	156.31	2.21	0.000	0.000
<i>Pdgfra</i>	4482.92	4252.44	3992.83	3302.81	3276.30	3641.64	1.25	0.000	0.001
<i>Pdgfrβ</i>	4301.78	4209.87	3874.17	3204.87	3359.43	2540.65	1.36	0.000	0.000
<i>Bmp8α</i>	73.38	84.34	80.74	40.53	42.79	39.76	1.94	0.000	0.002
<i>Thbs3</i>	117.04	111.93	131.29	52.91	39.12	78.15	2.12	0.000	0.000
<i>Igf2</i>	126.33	137.15	163.59	50.66	51.34	24.68	3.37	0.000	0.000
<i>Igfbp6</i>	852.74	838.66	872.71	200.37	193.15	167.27	4.57	0.000	0.000
<i>Smad3</i>	1466.75	1468.45	1374.71	944.46	1007.34	921.38	1.50	0.000	0.000
<i>Col9a3</i>	112732.54	106615.93	88615.93	75023.45	77222.82	62836.17	1.43	0.000	0.000
<i>Col1a1</i>	121.69	121.39	77.23	64.16	58.68	53.47	1.82	0.001	0.006
<i>Col18a1</i>	17035.28	16837.12	14996.10	10714.42	11128.41	10392.94	1.52	0.000	0.000
<i>Col7a1</i>	11111.62	10708.73	8320.56	5971.84	6163.84	5500.86	1.71	0.000	0.000
<i>Col6a2</i>	961.42	955.32	929.58	480.67	444.99	300.27	2.32	0.000	0.000
<i>Col6a1</i>	414.29	420.91	334.90	233.02	240.83	226.23	1.67	0.000	0.000
<i>Col6a3</i>	1694.33	1678.90	1352.24	947.84	900.98	1205.20	1.55	0.000	0.000
<i>Col27a1</i>	31.58	12.61	33.70	10.13	7.33	1.37	4.14	0.002	0.009
<i>Col8a2</i>	132.83	118.23	95.49	45.03	61.12	41.13	2.35	0.000	0.000
<i>Col4a4</i>	17.65	16.55	13.34	2.25	1.22	4.11	6.27	0.001	0.006
<i>Col26a1</i>	447.73	466.62	398.79	84.43	64.79	86.38	5.57	0.000	0.000
<i>Mmp24</i>	3204.74	3217.51	3079.40	2395.49	2482.90	2191.02	1.34	0.000	0.000
<i>Mmp28</i>	682.75	621.12	680.33	462.66	366.75	361.97	1.67	0.000	0.000
<i>Mmp2</i>	531.34	507.61	425.47	177.86	155.26	172.76	2.89	0.000	0.000
<i>Mmp11</i>	16.72	18.92	16.15	1.13	1.22	1.37	13.92	0.000	0.001
<i>Mmp15</i>	148.63	143.46	108.83	51.78	61.12	54.84	2.39	0.000	0.000
<i>Timp3</i>	17119.82	16223.89	12263.54	7815.74	8066.05	4846.84	2.20	0.000	0.000
<i>Fgf1</i>	107.75	100.10	126.38	57.41	35.45	58.96	2.20	0.000	0.000
<i>Fgf 2</i>	819.30	823.69	856.56	566.23	550.12	525.13	1.52	0.000	0.000
<i>Fgf 22</i>	68.74	71.73	61.08	18.01	15.89	10.97	4.49	0.000	0.000
<i>Fgfr1</i>	5289.21	5076.12	4534.15	4115.56	3759.18	4409.46	1.21	0.002	0.008
<i>Fgfr3</i>	155.13	146.61	153.06	49.53	52.57	54.84	2.90	0.000	0.000
<i>Lama5</i>	854.60	908.82	1025.06	271.29	305.62	274.22	3.28	0.000	0.000
<i>Smoc2</i>	983.72	1033.35	744.22	453.66	435.21	377.05	2.18	0.000	0.000

<i>Icam2</i>	46.45	25.22	37.91	16.89	11.00	13.71	2.63	0.002	0.010
<i>Icam5</i>	42.73	56.75	40.72	27.02	8.56	13.71	2.84	0.001	0.003
<i>Pcdh12</i>	65.02	63.85	60.38	28.14	33.01	30.16	2.07	0.001	0.003
<i>Pcdhgc5</i>	508.11	446.92	339.82	296.06	276.28	301.64	1.48	0.000	0.002
<i>Cdh24</i>	177.42	189.17	184.65	33.77	26.89	28.79	6.16	0.000	0.000
<i>Cdh3</i>	294.46	244.35	223.27	73.17	47.68	67.18	4.05	0.000	0.000
<i>Itga3</i>	151.41	160.80	133.40	51.78	48.90	47.99	3.00	0.000	0.000
<i>Itga7</i>	1167.64	1142.13	1117.74	363.60	426.65	303.01	3.14	0.000	0.000
<i>Itga8</i>	305.61	303.46	229.59	164.35	154.03	179.61	1.68	0.000	0.000
<i>Itga2b</i>	185.78	204.94	211.33	75.42	106.36	69.93	2.39	0.000	0.000
<i>Itgb4</i>	94.75	110.35	96.89	36.02	25.67	32.91	3.19	0.000	0.000
<i>Wnt5b</i>	70.60	107.20	76.53	9.01	2.44	4.11	16.34	0.000	0.000
<i>Wnt9a</i>	89.18	75.67	82.15	10.13	13.45	6.86	8.12	0.000	0.000
<i>Wnt6</i>	61.31	78.82	56.17	32.65	20.78	28.79	2.39	0.000	0.001
<i>Wnt10b</i>	27.87	40.99	42.83	4.50	1.22	1.37	15.74	0.000	0.000
<i>Wnt7a</i>	61.31	59.90	55.47	2.25	4.89	8.23	11.50	0.000	0.000
<i>Wnt4</i>	13.93	10.25	16.15	2.25	1.22	1.37	8.32	0.002	0.007
<i>Wnt3</i>	624.23	588.80	464.79	352.34	365.53	270.11	1.70	0.000	0.000
<i>Wnt11</i>	52.95	45.72	42.13	5.63	2.44	4.11	11.55	0.000	0.000
<i>Wnt8b</i>	361.35	329.48	350.35	47.28	46.45	79.52	6.01	0.000	0.000
<i>Ctnnbip1</i>	488.61	502.88	436.70	338.84	367.97	320.84	1.39	0.000	0.001
<i>Tnfrsf9</i>	78.96	81.97	113.04	12.38	22.00	34.28	3.99	0.000	0.000
<i>Tnfrsf12a</i>	8874.81	8888.73	8179.44	6484.04	6270.20	6632.01	1.34	0.000	0.000
<i>Tnfrsf18</i>	156.99	174.20	146.04	42.78	50.12	41.13	3.56	0.000	0.000
<i>Tnfrsf25</i>	66.88	51.23	56.17	23.64	20.78	16.45	2.86	0.000	0.000
<i>Il34</i>	296.32	282.18	251.35	178.99	202.93	156.31	1.54	0.000	0.001
<i>Il12rb1</i>	48.30	35.47	33.70	5.63	8.56	4.11	6.42	0.000	0.000
<i>Irf3</i>	1093.33	1097.20	1092.46	898.31	931.54	872.02	1.22	0.001	0.005
<i>Irf8</i>	183.92	227.79	183.25	127.20	102.69	135.74	1.63	0.000	0.001
<i>Ifnlr1</i>	58.52	51.23	51.96	23.64	23.23	28.79	2.14	0.001	0.005

1 **Supplementary Table 3 Genes identified by a bioinformatics intersection analysis between RNA-seq and**
2 **ChIP-seq**

Gene	RNA-seq			ChIP-seq_1B7		ChIP-seq_1C1	
	Log FC (<i>Irx2</i> vs <i>Gfp</i>)	<i>P</i> value	padj	LogFC	<i>P</i> value	LogFC	<i>P</i> value
<i>Gna11</i>	0.43	1.86E-06	1.93E-05	0.90	0.017	0.92	0.017
<i>Ano8</i>	0.65	2.13E-07	2.70E-06	0.87	0.012	0.82	0.023
<i>Mlf2</i>	0.35	4.85E-05	3.66E-04	0.97	0.010	1.21	0.001
<i>Gadd45b</i>	0.94	1.40E-25	1.64E-23	1.39	0.000	1.35	0.000
<i>Cby1</i>	0.94	4.37E-21	3.45E-19	1.11	0.010	1.15	0.006
<i>Pck2</i>	0.64	2.48E-15	1.03E-13	1.08	0.023	1.14	0.016
<i>Pxn</i>	0.95	6.37E-14	2.22E-12	1.33	0.001	1.03	0.021
<i>Asic1</i>	0.72	6.59E-04	3.56E-03	1.17	0.004	0.99	0.021
<i>Vegfa</i>	0.34	3.37E-05	2.64E-04	0.76	0.018	1.02	0.001
<i>Egr1</i>	0.32	7.02E-04	3.75E-03	0.81	0.006	1.02	0.001
<i>Tcf15</i>	4.75	3.33E-05	2.61E-04	1.49	0.001	1.66	0.000
<i>Nectin2</i>	0.79	2.76E-14	1.02E-12	1.07	0.000	0.83	0.009
<i>Zswim4</i>	0.85	3.30E-11	7.99E-10	1.16	0.001	0.96	0.006
<i>Kmt5c</i>	0.61	6.61E-07	7.59E-06	0.82	0.011	0.78	0.018
<i>Csnk1g2</i>	0.31	1.00E-03	5.09E-03	1.00	0.002	0.88	0.009
<i>Serpine2</i>	0.35	7.32E-05	5.27E-04	1.60	0.000	1.06	0.034
<i>Napa</i>	0.30	1.13E-05	9.87E-05	0.95	0.004	0.99	0.002
<i>Lsm14b</i>	0.40	1.01E-05	8.97E-05	0.65	0.048	0.76	0.018
<i>Klf10</i>	0.35	2.40E-04	1.48E-03	0.81	0.013	0.75	0.031
<i>Rnf126</i>	0.44	7.30E-06	6.71E-05	1.08	0.001	0.87	0.011
<i>Lrwd1</i>	0.61	1.46E-07	1.91E-06	0.77	0.043	0.77	0.044
<i>Bcl6b</i>	1.93	5.99E-07	6.95E-06	0.98	0.009	1.34	0.000
<i>Ccdc96</i>	1.49	4.03E-06	3.92E-05	1.37	0.000	1.69	0.000
<i>Six5</i>	0.62	3.76E-04	2.19E-03	0.99	0.001	1.16	0.000
<i>Lrrc8a</i>	0.46	2.03E-03	9.34E-03	0.87	0.014	1.19	0.001
<i>Bet1l</i>	0.39	3.29E-04	1.95E-03	1.18	0.000	1.35	0.000
<i>Fam212a</i>	0.52	1.83E-03	8.53E-03	1.16	0.002	1.27	0.001
<i>Ppp1cc</i>	0.35	1.96E-04	1.24E-03	0.98	0.007	1.16	0.001
<i>Mrpl55</i>	0.37	1.71E-03	8.05E-03	1.07	0.004	0.92	0.021
<i>Efs</i>	1.57	1.60E-35	3.39E-33	2.56	0.000	1.91	0.000
<i>Sft2d1</i>	0.32	1.73E-03	8.12E-03	1.40	0.000	1.50	0.000
<i>Rab13</i>	0.62	2.48E-09	4.49E-08	1.10	0.003	0.82	0.041
<i>Dusp5</i>	0.57	3.30E-06	3.27E-05	1.53	0.000	0.78	0.046
<i>Kcnb1</i>	2.00	5.47E-12	1.47E-10	1.03	0.034	1.34	0.004
<i>Ada</i>	0.65	6.90E-07	7.89E-06	0.98	0.019	1.18	0.003

<i>Tbcc</i>	0.42	8.92E-04	4.60E-03	1.34	0.000	1.20	0.000
<i>Ninj1</i>	0.43	2.88E-05	2.30E-04	1.19	0.001	0.99	0.009
<i>Rilpl1</i>	0.56	1.30E-08	2.07E-07	0.94	0.019	1.35	0.000
<i>Arrdc2</i>	0.76	1.84E-04	1.18E-03	0.92	0.015	1.23	0.001
<i>Fam131a</i>	1.35	4.22E-12	1.15E-10	1.06	0.003	1.01	0.004
<i>Cdkn2d</i>	0.81	7.80E-14	2.69E-12	0.86	0.013	0.98	0.005
<i>Cnp</i>	0.58	6.89E-08	9.66E-07	41	0.81	0.77	0.041
<i>Arsa</i>	0.35	8.31E-04	4.34E-03	1.34	0.000	0.98	0.015
<i>Tspan4</i>	0.36	1.29E-06	1.39E-05	0.78	0.017	1.09	0.001
<i>Prmt5</i>	0.36	1.38E-05	1.19E-04	0.75	0.022	0.69	0.037
<i>Acadvl</i>	0.50	9.11E-06	8.19E-05	0.83	0.046	1.23	0.001
<i>Arntl</i>	0.53	9.08E-04	4.68E-03	1.58	0.000	1.01	0.011
<i>Pacsin2</i>	0.34	1.53E-04	1.00E-03	0.87	0.023	1.40	0.000
<i>Vav2</i>	0.37	3.40E-04	2.00E-03	1.17	0.002	0.84	0.042
<i>Dolpp1</i>	0.57	4.72E-05	3.57E-04	0.89	0.006	0.97	0.004
<i>Ppm1m</i>	0.68	1.09E-11	2.84E-10	1.25	0.002	1.31	0.002
<i>Btg2</i>	0.42	3.28E-08	4.88E-07	1.24	0.000	0.71	0.036
<i>Kifc2</i>	1.60	3.72E-08	5.50E-07	1.06	0.001	0.74	0.038
<i>Mapkapk3</i>	0.49	4.85E-08	7.00E-07	0.96	0.005	0.84	0.018
<i>Nfic</i>	0.47	1.99E-06	2.06E-05	0.76	0.047	0.85	0.024
<i>Smardc2</i>	0.40	1.01E-04	6.96E-04	1.00	0.001	1.24	0.000
<i>Col6a2</i>	0.77	2.39E-13	7.78E-12	1.49	0.001	1.32	0.006
<i>Snhg12</i>	0.46	6.72E-07	7.71E-06	1.24	0.006	1.13	0.018
<i>Tcf7</i>	2.90	8.31E-13	2.51E-11	1.01	0.034	1.24	0.008
<i>Ttyh2</i>	0.64	6.82E-11	1.57E-09	1.03	0.005	1.11	0.002
<i>Cd320</i>	0.80	2.08E-06	2.14E-05	0.90	0.014	0.89	0.017
<i>Nubp2</i>	0.30	5.90E-04	3.24E-03	1.13	0.001	1.26	0.000
<i>Tmem263</i>	0.31	1.54E-03	7.35E-03	1.08	0.002	1.03	0.003
<i>Slc2a4</i>	5.03	4.67E-14	1.67E-12	0.87	0.028	1.24	0.001
<i>Mif4gd</i>	0.58	2.10E-04	1.32E-03	1.18	0.001	1.02	0.006
<i>Prkcsh</i>	0.21	8.35E-04	4.35E-03	1.21	0.000	1.22	0.000
<i>Ruvbl2</i>	0.24	1.56E-03	7.46E-03	1.41	0.000	1.23	0.000
<i>Nudt18</i>	0.52	1.79E-05	1.50E-04	1.09	0.001	1.02	0.002
<i>Cisd3</i>	0.63	4.14E-06	4.02E-05	1.34	0.000	1.02	0.006
<i>Rab11fip4</i>	1.98	3.72E-04	2.17E-03	1.61	0.001	1.24	0.016