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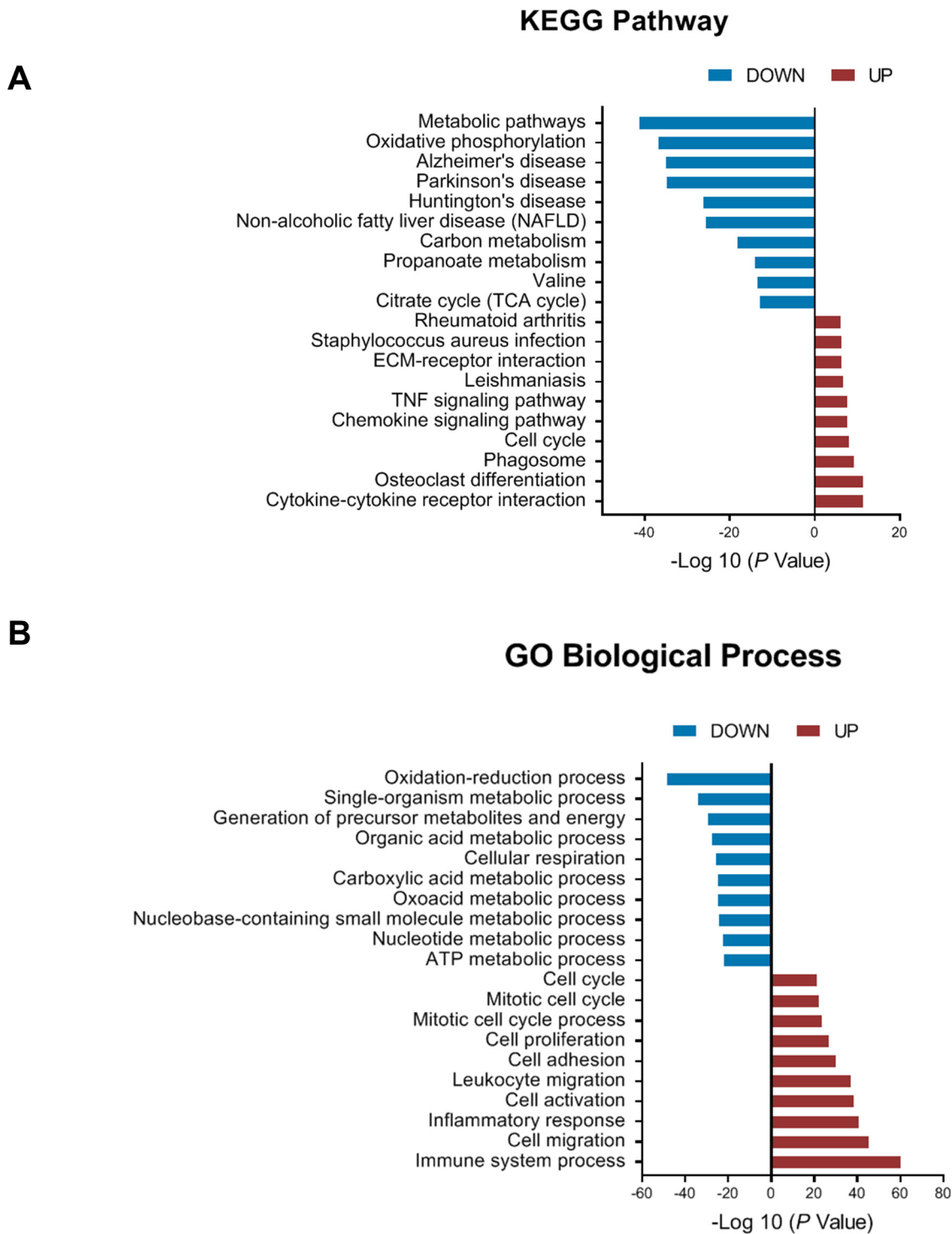
Supplemental Information

Long noncoding RNA *Cfast*

regulates cardiac fibrosis

Feng Zhang, Xuyang Fu, Masaharu Kataoka, Ning Liu, Yingchao Wang, Feng Gao, Tian Liang, Xiaoxuan Dong, Jianqiu Pei, Xiaoyun Hu, Wei Zhu, Hong Yu, Douglas B. Cowan, Xinyang Hu, Zhan-Peng Huang, Jian'an Wang, Da-Zhi Wang, and Jinghai Chen

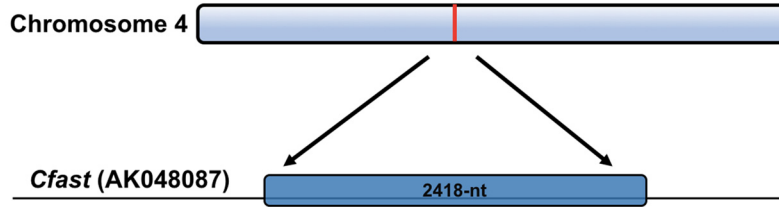
Supplemental Figure 1.



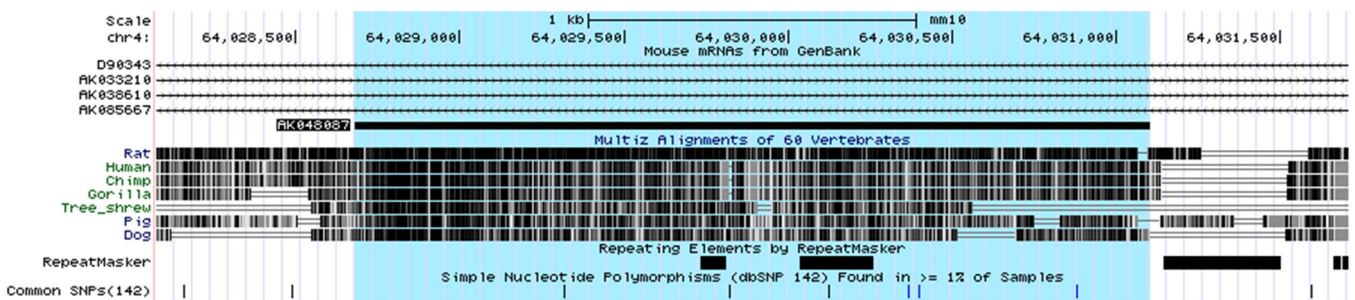
Supplemental Figure 1. Analysis of microarray data after 3days myocardial infarction (MI). (A and B) KEGG pathway (A) and GO terms linked to biological process(B) for the up-regulated and down-regulated genes after myocardial infarction.

Supplemental Figure 2.

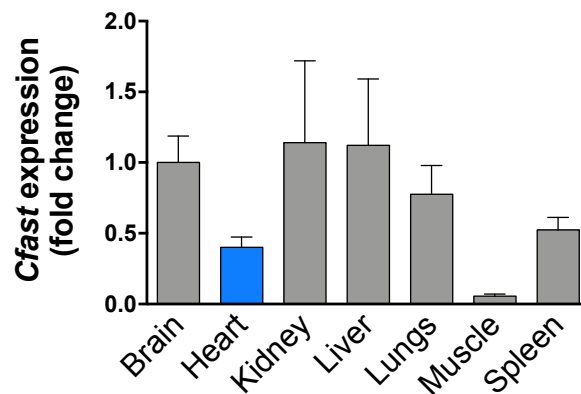
A



B



C



Supplemental Figure 2. Structure of lncRNA *Cfast* and its expression in different tissues.

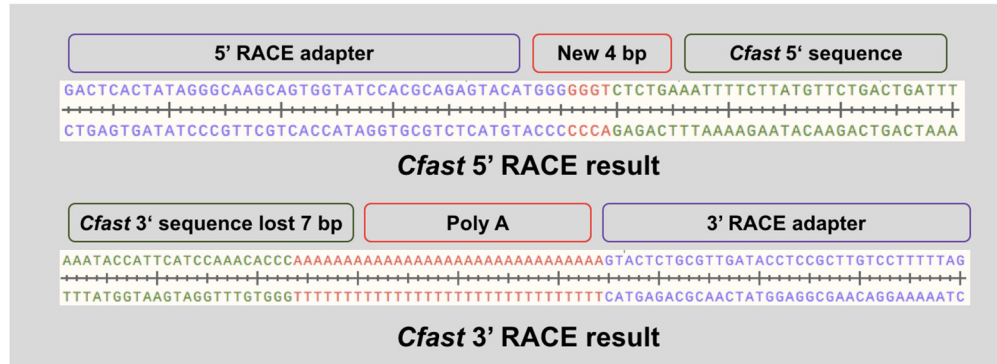
(A) The lncRNA *Cfast* is located on chromosome 4 and appears to a single exon transcript.

(B) Conservation of *Cfast* in the mouse, Rat, Human, Chimp, Gorilla, Pig, Dog. The horizontal lines indicate the mouse *Cfast*. The black boxes show the conserved sequences of *Cfast* in Rat, Human, Chimp, Gorilla, Pig, Dog.

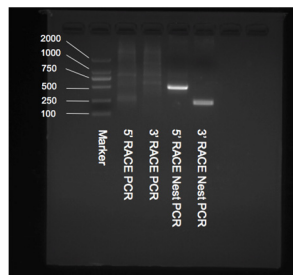
(C) Expression of *Cfast* in different tissues from adult mice. Data represent mean \pm SEM (n = 3).

Supplemental Figure 3.

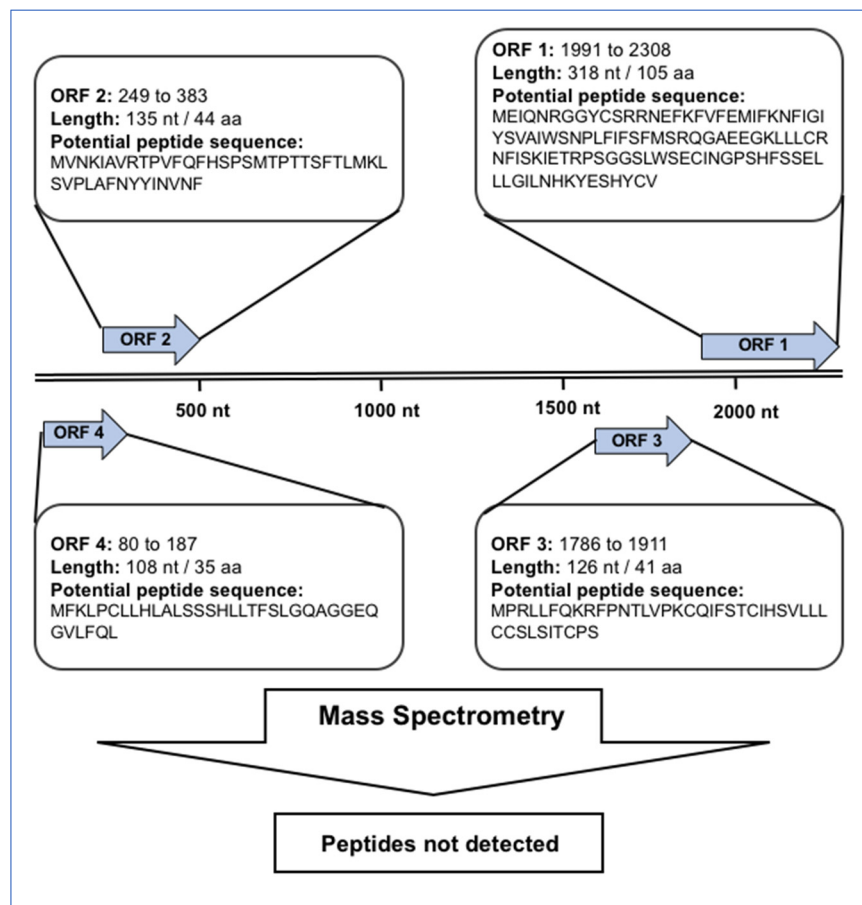
A



B



C



Supplemental Figure 3. Cfast is a long non-coding RNA.

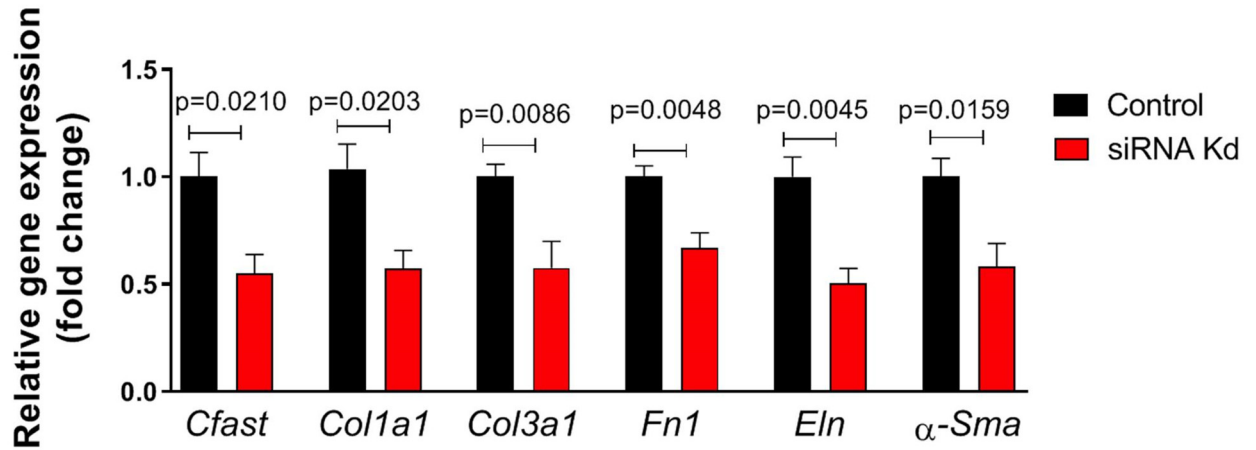
(A) Graphical representation of 3' and 5' rapid amplification of cDNA (complementary DNA) ends (RACE) results of Cfast.

(B) PCR product Gel image of 3' and 5' RACE of Cfast

(C) Presentation of the open reading frames (ORFs) potentially encoded by the Cfast sequence.

Supplemental Figure 4.

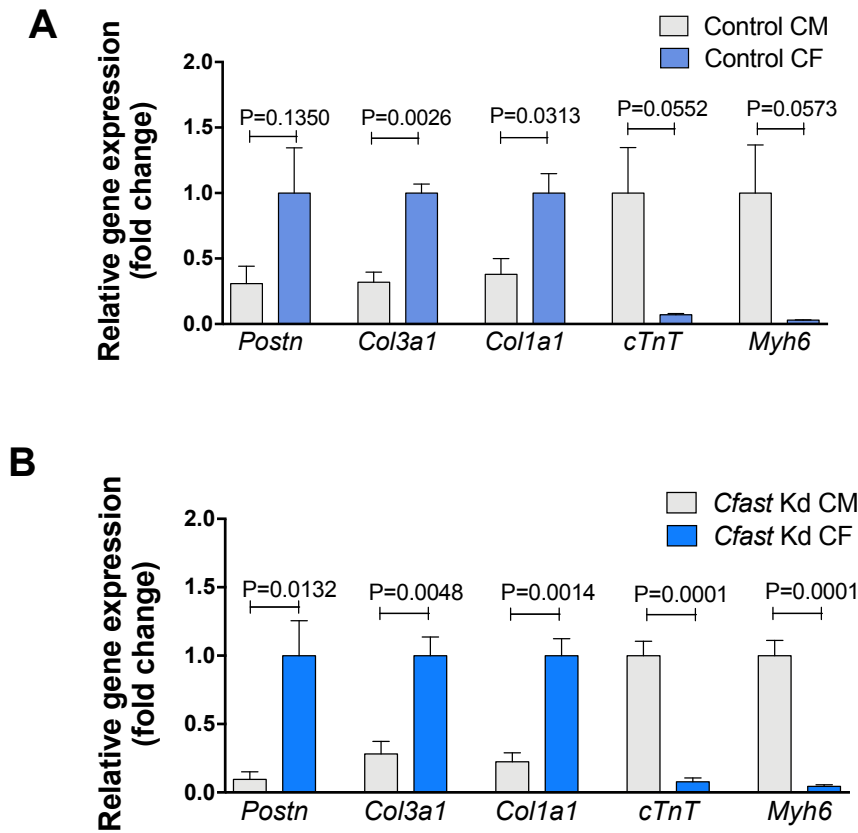
A



Supplemental Figure 4 Knock-down of *Cfast* by siRNA inhibits expression of cardiac fibrotic genes.

(A) qRT-PCR of *Cfast* and fibrosis genes in control and *Cfast* siRNA treated cardiac fibroblasts. $n \geq 4$, P values were determined by Student's t test..

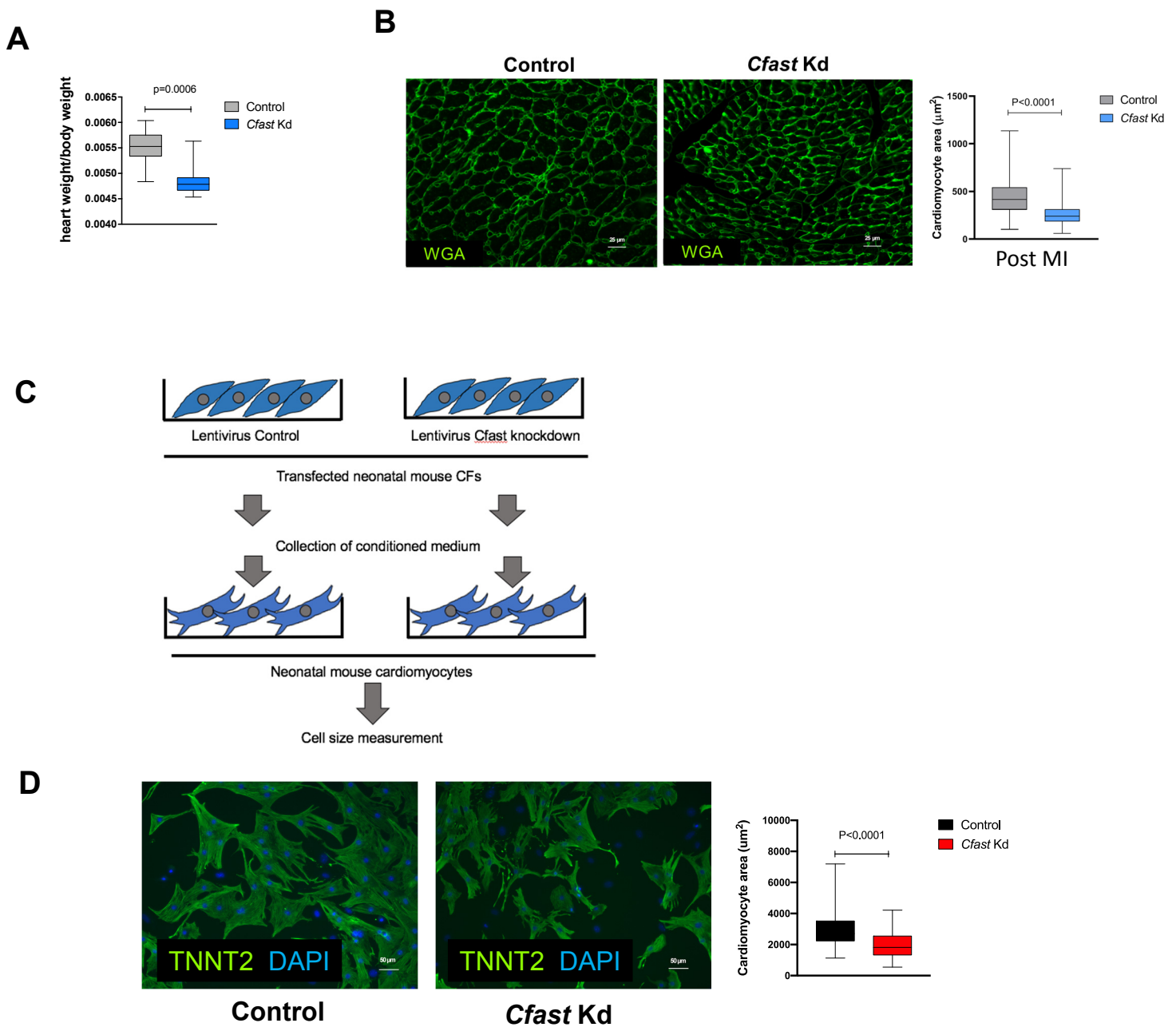
Supplemental Figure 5.



Supplemental Figure 5 Expression of cardiomyocyte and cardiac fibroblast gene expression in mouse hearts after *Cfast* knockdown.

8 weeks old adult mice were subjected to myocardial infarction surgery. Immediately after MI surgery, control (Control) or *Cfast* knocking down lentivirus (*Cfast* Kd) was injected into myocardium adjacent to the infarcted area. Seven days later, cardiomyocytes (CM) and cardiac fibroblasts (CF) were isolated from the hearts of Control (A) or *Cfast* kd (B) mice. Expression of cardiomyocyte (*cTnT*, *Myh6*) or cardiac fibroblast (*Postn*, *Col3a1*, *Col1a1*) marker genes was determined by qRT-PCR. Data was represented as (CM/CF) ratio over mean fold change \pm SEM ($n \geq 3$). P value was determined by Student's t test.

Supplemental Figure 6.



Supplemental Figure 6 Cfast knocking down reduces cardiomyocyte hypertrophy post MI.

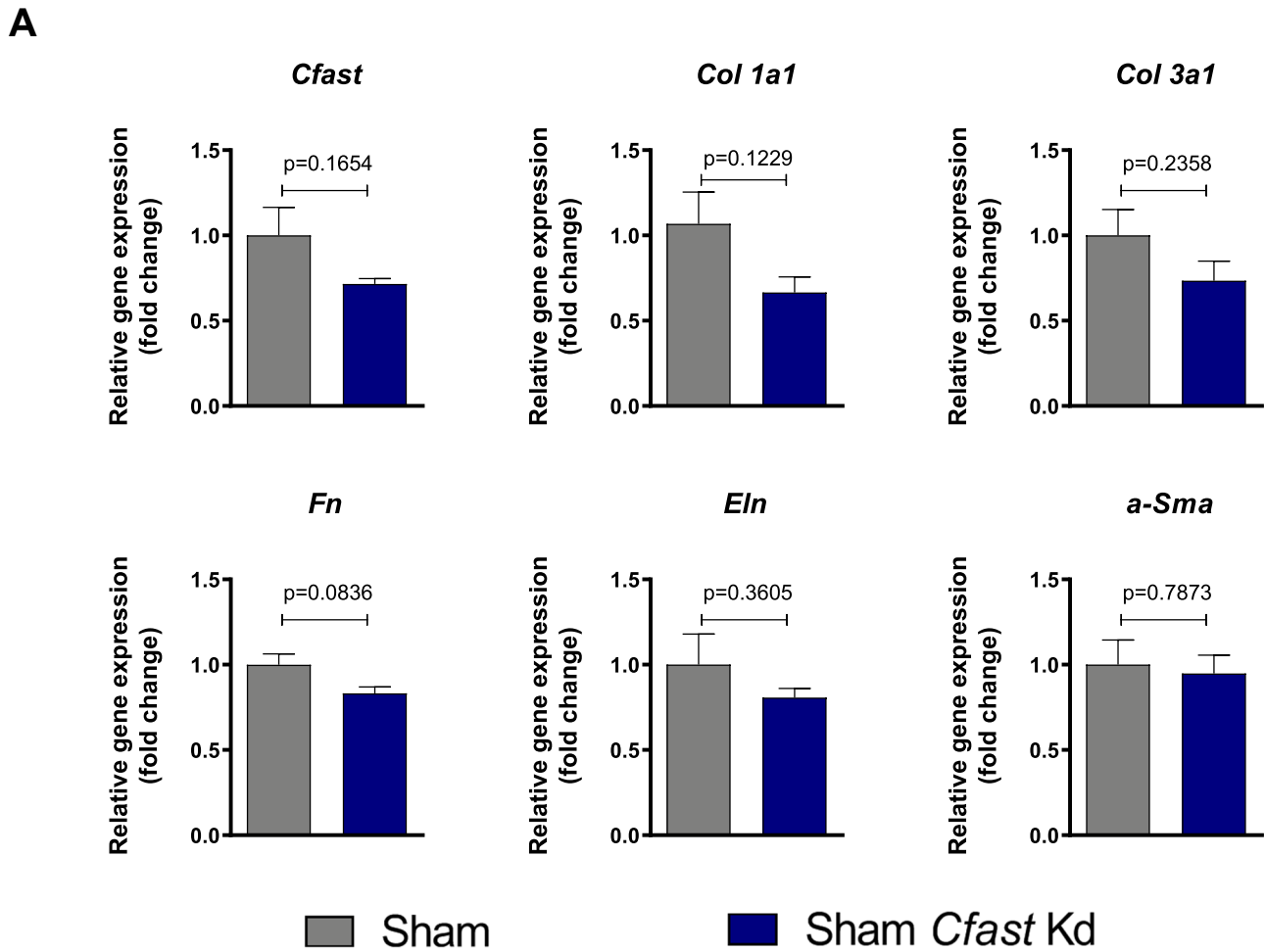
(A) Heart weight to body weight ratio of mouse hearts 28 days after cardiac-injection of Cfast depletion lentivirus and MI (Control $n=8$, Cfast Kd $n=10$). P value was determined by Student's t test.

(B) Determination of cardiomyocyte cross-sectional area in histological sections stained with wheat germ agglutinin (WGA, green) from control and Cfast Kd groups post-MI (scale bar= $25\ \mu\text{m}$, $n=6/6$). P value was determined by Student's t test.

(C) Neonatal cardiac fibroblasts (CFs) have been transfected with lentivirus control or lentivirus Cfast knockdown. The conditioned medium was collected 48 hours after transfection and added to cultured neonatal mouse cardiomyocytes (NMCMs). Cell sizes were measured 48 hours after addition of CF-conditioned medium.

(D) NMCMs cultured in CFs conditioned medium for 48 hours were then stained with TNNT2 (Green) for cardiomyocytes and DAPI (blue) for nuclear. Cell size measurement in NMCMs ($n=4$ independent replicates, 127-127 cells each, scale bar= $50\ \mu\text{m}$). P value was determined by Student's t test.

Supplemental Figure 7.

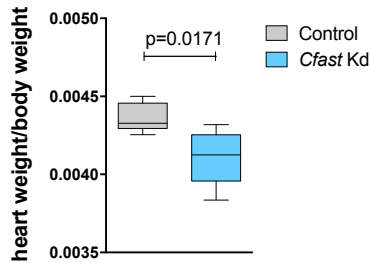


Supplemental Figure 7 Knock-down of *Cfast* does not affect fibrotic gene in Sham.

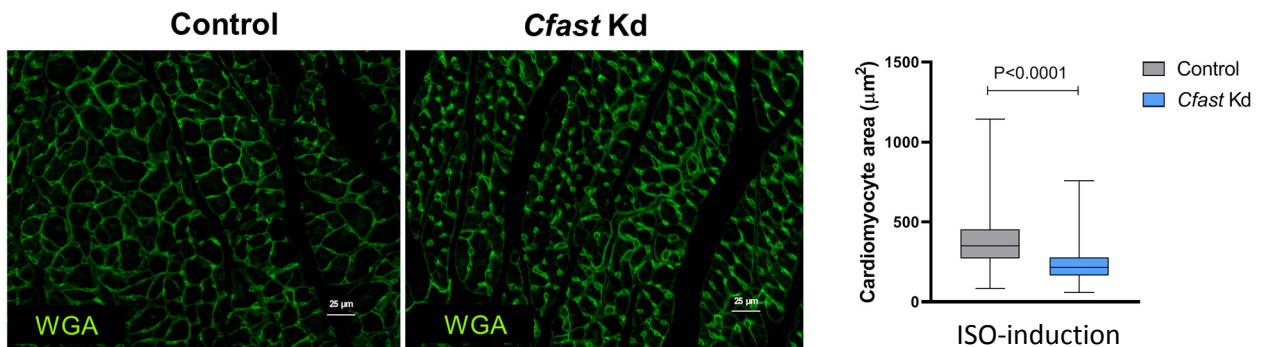
(A) qRT-PCR of *Cfast* and fibrosis genes in *Cfast* depletion and control treated with sham. n=3, P values were determined by Student's t test..

Supplemental Figure 8.

A



B

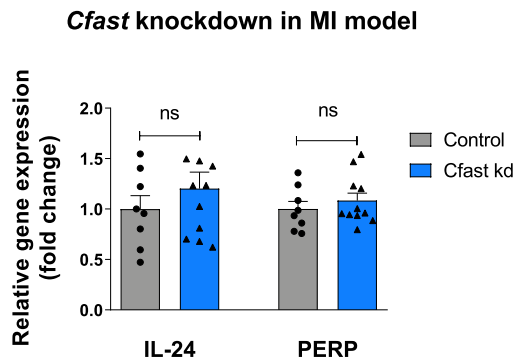


Supplemental Figure 8. Cfast knocking down reduces cardiac hypertrophy post ISO treatment.

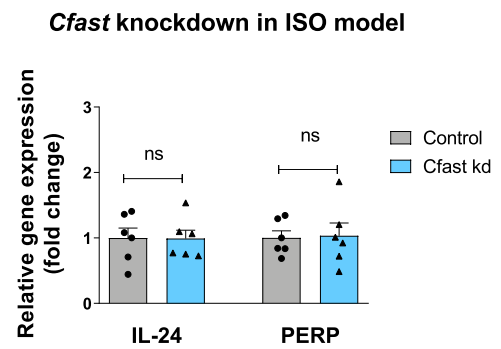
- (A) Heart weight to body weight ratio of mouse hearts 21 days after cardiac injection of Cfast depletion lentivirus and ISO-infusion. Bars represent means normalized to Control \pm SEM (n=6). P value was determined by Student's t test.
- (B) Determination of cardiomyocyte cross-sectional area in histological sections stained with wheat germ agglutinin (WGA, green) from control and Cfast Kd groups post-ISO treatment (scale bar=25 μm , n=6/6). P value was determined by Student's t test.

Supplemental Figure 9.

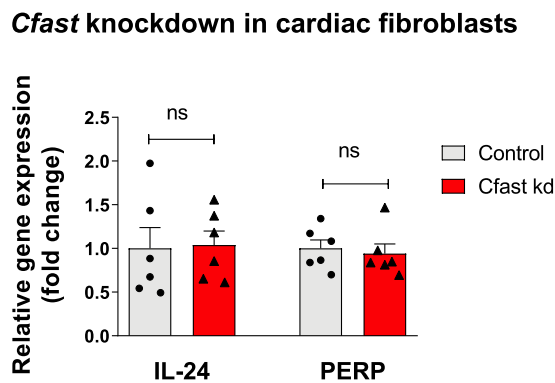
A



B



C



Supplemental Figure 9. Impact of *Cfast* knocking down in non-canonical TGF-beta signaling.
(A-C) Level of IL-24 and PERP were determined by qRT-PCR on the samples of *Cfast* knocking down and control groups in both in vivo model of (A) myocardial infarction (MI) and (B) isoproterenol (ISO) treated hearts, as well as (C) in vitro model of isolated cardiac fibroblasts. n=6, P values were determined by Student's t test..

List of Supplemental Tables

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Supplemental Table 1. Echocardiography examination of cardiac function after Cfast-depletion and 4-week of myocardial infraction or Sham

Echocardiography examination of cardiac function after Cfast-depletion and 4-week of myocardial infraction (MI)													
Group	Ear tag	IVS;d	IVS;s	LVID;d	LVID;s	LVPW;d	LVPW;s	EF	FS	LV Mass	LV Mass (Corrected)	LV Vol;d	LV Vol;s
Control + MI	5161	1.04386	1.528509	3.72807	2.736404	0.902193	1.357018	52.820535	26.599983	137.803582	110.242866	59.187023	27.924121
	5163	0.939474	1.289912	4.592982	3.862281	0.514474	0.589035	33.598601	15.909076	130.800512	104.64041	96.988415	64.401664
	5164	1.08114	1.543421	4.108333	3.131579	0.477193	0.917105	47.890866	23.774947	118.589714	94.871771	74.580482	38.863243
	5166	0.939474	1.014035	4.637719	3.892105	0.260965	0.969298	33.888607	16.077171	104.497831	83.598265	99.215474	65.592732
	5180	1.0625	1.706439	4.475379	3.953788	0.347727	0.341288	25.386983	11.654678	120.296885	96.237508	91.262184	68.093469
	3534	0.820175	0.857456	3.646053	2.870614	0.730702	0.872368	44.015705	21.267903	96.759712	77.40777	56.117074	31.416748
	3535	0.924561	1.237719	4.190351	3.288158	0.924561	1.282456	44.018605	21.530249	154.488415	123.590732	78.152109	43.750641
3540	0.820175	1.230263	4.369298	3.467105	0.551754	0.842544	42.3518	20.648466	111.436135	89.148908	86.256018	49.725042	
Cfast kd + MI	5169	1.006579	1.550877	3.578947	2.244298	0.782895	1.461404	68.25463	37.291667	114.64575	91.7166	53.670932	17.038036
	5170	0.984211	1.476316	3.854825	2.736404	0.663596	0.932018	56.440525	29.013535	115.127142	92.101714	64.105734	27.924121
	5171	1.014035	1.640351	3.549123	2.341228	0.685965	1.357018	63.98113	34.033619	105.220914	84.176731	52.602725	18.946907
	5175	1.02	1.4875	4.030417	2.734167	0.6375	1.1475	60.898419	32.161685	124.829589	99.863671	71.270343	27.867831
	5177	0.94693	1.431579	4.160526	2.669298	0.775439	1.498684	65.822735	35.842295	138.552993	110.842394	76.842963	26.262823
	5178	0.984211	1.342105	3.832456	2.684211	0.827632	1.222807	57.883326	29.961075	130.073582	104.058866	63.222309	26.627134
	3537	0.529386	1.036404	3.899561	2.400877	0.715789	1.088596	69.376679	38.432121	80.947784	64.758227	65.892337	20.178422
	3538	0.669697	0.95303	3.889394	2.826894	0.721212	1.101136	53.799374	27.31788	93.071439	74.457151	65.483979	30.254008
	3539	0.812719	1.334649	4.15307	2.840789	0.61886	0.976754	59.981823	31.597854	107.978434	86.382747	76.517538	30.620924
	3533	0.909649	1.461404	3.899561	2.848246	1.021491	1.215351	53.228485	26.95983	146.290977	117.032782	65.892337	30.818844

Echocardiography examination of cardiac function after Cfast-depletion and 4-week of Sham													
Group	Ear tag	IVS;d (mm)	IVS;s (mm)	LVID;d (mm)	LVID;s (mm)	LVPW;d (mm)	LVPW;s (mm)	EF (%)	FS (%)	LV Mass (mg)	LV Mass (Corrected) (mg)	LV Vol;d (ul)	LV Vol;s (ul)
Control + Sham	9208	1.040517	1.626724	3.326724	1.67069	0.791379	0.981897	82.181321	49.779723	105.785152	84.628122	45.003066	8.018952
	9204	0.893966	1.450862	2.740517	1.231034	1.011207	1.289655	87.168154	55.080228	83.906025	67.12482	28.027783	3.596482
	9205	0.981897	1.201724	2.637931	1.157759	1.128448	1.568103	88.028739	56.111096	93.399738	74.71979	25.505634	3.053346
Cfast kd + Sham	9203	0.718103	1.143103	2.637931	1.24569	0.87931	1.406897	85.448352	52.777764	60.671408	48.537126	25.505634	3.71149
	9206	0.85	1.289655	2.989655	1.480172	0.967241	1.289655	83.142882	50.490207	88.818117	71.054494	34.705659	5.850374
	9207	0.674138	0.835345	2.799138	1.406897	0.703448	1.157759	82.658998	49.738205	53.630676	42.904541	29.52838	5.120517

Supplemental Table 2 Echocardiography examination of cardiac function after Cfast-depletion and 3-week of isoproterenol injection

Echocardiography examination of cardiac function after Cfast-depletion and 3-week of isoproterenol (ISO) injection													
Group	Ear tag	IVS;d	IVS;s	LVID;d	LVID;s	LVPW;d	LVPW;s	EF	FS	LV Mass	LV Mass (Corrected)	LV Vol;d	LV Vol;s
Control + ISO	8602	0.676136	0.6375	3.750247	2.938043	0.813043	0.794565	44.600568	21.657347	95.913296	76.730637	60.032316	33.257562
	8603	0.760128	0.913834	3.303409	2.272826	0.804644	0.869318	60.247318	31.19756	83.527583	66.822066	44.243611	17.588022
	8610	0.63666	0.720652	2.842292	1.977174	0.760968	0.794565	59.685993	30.437337	56.081623	44.865298	30.66076	12.360581
	8611	0.734091	0.865119	3.492391	2.328261	0.714773	0.74417	63.075338	33.333324	82.186345	65.749076	50.60286	18.684935
	8612	0.714773	0.714773	3.148864	2.376136	0.579545	0.656818	50.079425	24.539898	59.488849	47.591079	39.387251	19.662342
Cfast kd + ISO	8527	0.772727	0.734091	3.651136	2.743182	0.734091	0.540909	50.101803	24.867712	93.245457	74.596366	56.304766	28.095063
	8601	0.693775	0.826482	3.048913	1.962055	0.560227	0.626581	66.709742	35.647393	54.046809	43.237447	36.410217	12.121055
	8606	0.699654	0.824382	3.687253	2.450469	0.519071	0.882337	63.163663	33.542152	71.550127	57.240102	57.648167	21.235473
	8608	0.52957	0.810524	3.267712	1.794911	0.594664	0.713093	77.608537	45.071322	52.465347	41.972278	43.094474	9.649483
	8614	0.628261	0.729891	3.464674	2.217391	0.628261	0.74837	66.704392	36.00001	67.017316	53.613853	49.641088	16.528302
	8530	0.560227	1.120455	3.825	2.298864	0.560227	0.85	71.239793	39.89898	68.435919	54.748735	62.929315	18.098601
	8531	0.683696	0.572826	3.326087	2.051087	0.628261	0.73913	69.832225	38.333333	66.312722	53.050178	44.982223	13.570136

Supplemental Table 3. Primers used for qRT-PCR

Supplemental Table 3. Primers used for qRT-PCR			
Species	lncRNA	Forward primer sequences	Reverse primer sequences
Mouse	NR-102296	GCGGGAGTAACAAGGACACA	AAAACGGAGCCTGACACCAT
Mouse	NC-000072	CACTTTGCTATGGCAGGCTC	AGGCAGATGAAGCAAGGTCC
Mouse	NR-038009	GCTGCGGCAGTTTGTACTC	TAGCAGATGCTTGGGTGAGC
Mouse	NR-015519	TCTGCAAACTACCTCTGCCG	CCGAAAACCAGGACCAGTCA
Mouse	NR-033498	AGCCCCACATAAGGAACTGC	ACTGCCACTGCACTCTCATC
Mouse	NR-024720	TCACAGCCGAAATCAAAGCAC	TTCACCAAGAAGCATTGCGTTC
Mouse	NC-000071	GGCCTTTTGCTTAGCACTGG	GCTTTGTAAGGCCACGGTTC
Mouse	lncRNA-Cfast	TCCTGGGTTTGGTAACTGCC	GAATTCTGCTGGCCTGAGGT
Human	n338538	AAAATAAACTCTTTGGCTCAGTTTGT	GATCTCCTCGGGGCTCAG
Human	n384785	CAGCTTCTTCTGCATCTGAGG	TGCGGTTAGAAACTCAGCTAGAA
Human	lncRNA-Cfast-1	AGACAGGGGCATTGCCTTTA	CAAGGCTCCCCAGCAGATAA
Human	lncRNA-Cfast-2	TCTTTCCCGATCCAATGTGCT	GTCAATGGCTCCATCTGGCT
Species	mRNA	Forward primer sequences	Reverse primer sequences
Mouse	Nppa	CACAGATCTGATGGATTCAAGA	CCTCATCTTCTACCGGCATC
Mouse	Nppb	GTCAGTCGTTTGGGCTGTAAC	AGACCCAGGCAGAGTCAGAA
Mouse	Myh7	CGCATCAAGGAGCTCACC	CTGCAGCCGCAGTAGGTT
Mouse	Col1a1	CATGTTCACTTTGTGGACCT	GCAGCTGACTTCAGGGATGT
Mouse	Col3a1	TCCCCTGGAATCTGTGAATC	TGAGTCGAATTGGGGAGAAT
Mouse	Fn1	CCTTCTGTGGCTCCAGA T	GCTGCCCCCATTCATAACA
Mouse	Ein	TGGAGCAGGACTTGGAGGT	CCTCCAGCACCATACTTAGCA
Mouse	α -Sma	CCAGCACCATGAAGATCAAG	TCCACATCTGCTGGAAGGTA
Mouse	Cotl1	ACCGACAAGACGCTGGTG	GCTGATCACAAATTCCTTTGC
Mouse	Myh6	GGGCTGGAGCACTGAGAG	GAGAGAGGAACAGGCAGGAA
Mouse	Gapdh	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG
Mouse	18S RNA	TCCGACCATAAACGATGCCG	CAATCTGTCAATCCTGTCCGTGTC
Mouse	Neat1	TGGCCCCTTTTGTTCATTAGC	TGGAAGGCCATTGTTTCAGG
Mouse	Xist	GGAAAGCCCCAAGTAAAAGG	CCAGGAACCAATTCTTGCCCTA
Mouse	Postn	AAGCTGCGGCAAGACAAG	TCAAATCTGCAGCTTCAAGG
Human	Nppa	CAGGATGGACAGGATTGGA	TGTCCTCCCTGGCTGTTATC
Human	Nppb	GATGGTGCAAGGGTCTGG	TAATGCCGCCTCAGCACT
Human	β -actin	CCAACCGCGAGAAGATGA	CCAGAGGCCGTACAGGGATAG

Supplemental Table 4. Primers used for RACE

Supplemental Table 4. Primers used for RACE	
Primer name	Primer sequence
5' RACE-GSP-Cfast	CTGGAAGAGAACACCCTGCTCCC
5' RACE-GSP2-Cfast	GGGAGCAGGGTGTTCTCTTC
3' RACE-GSP-Cfast	GAAACCAGACCATCAGGAGGTAGC
3' RACE-GSP2-Cfast	GCTACCTCCTGATGGTCTGG
Cfast full sequence primer F	CCCATGTA CTCTGCGTGGATACC
Cfast full sequence primer R	ATGTCGTATACATACATTTATTGCATGGCTCC

Supplemental Table 5. Sequences for shRNAs and siRNAs

Sequences for shRNAs		
Name	Sense (5'-3')	Antisense (5'-3')
Control shRNA	GTACGTCGTGAACCCCTTGAAGATTAGT	ACTAATCTTCAAGGGTTCACGACGTAC
Cfast shRNA	GTCTGAATGTATCAATGGGCCTAGTCA	TGACTAGGCCCATGATACATTCAGAC
Sequences for siRNAs		
Name	Sense (5'-3')	Antisense (5'-3')
Cfast siRNA-1	CCAACAACUUCAUUCACAUTT	AUGUGAAUGAAGUUGUUGGTT
Cfast siRNA-2	CCUGCAUUUGUAUGGCUAATT	UUAGCCAUACAAAUGCAGGTT

Supplemental Table 6. List of the antibodies used in immunofluorescence (IF), western blot (WB), RNA immunoprecipitation (RIP) and Immunoprecipitation (IP)

List of the antibodies used in immunofluorescence (IF), western blot (WB), RNA immunoprecipitation (RIP) and Immunoprecipitation (IP)				
Primary antibodies	Source	Catalog #	Origin	Application
COL3A1	Abclonal	A3795	Rabbit	1:1000for WB
Collagen I	Bioss	bs10423R	Rabbit	1:1000for WB
β -ACTIN	CST	4970s	Rabbit	1:2000for WB
COTL1	Abcam	ab235833	Rabbit	1:1000for WB
TGF β RAP1	Abclonal	A14386	Rabbit	1:1000for WB
phospho-Histone H3	Millipore	06-570	Rabbit	1:400 for IF
α -Smooth Muscle Actin-FITC	SIGMA	F3777	Mouse	1:400 for IF
myosin, sarcomere (MHC)	Developmental Studies Hybridoma Bank	MF20	Mouse	5 μ g/ml for IF
Monoclonal ANTI-FLAG	SIGMA	F1804	Mouse	RIP and IP
IgG1 Isotype Control	Invitrogen	MA5-14453	Mouse	RIP and IP
Secondary antibodies				
Alexa Fluor® 594 Goat Anti-Rabbit	Invitrogen	A-11012	Goat	1:400 for IF
Alexa Fluor® 488 Goat Anti-Mouse	Invitrogen	A-11001	Goat	1:400 for IF
Alexa Fluor™ 594 Conjugate, Wheat Germ Agglutinin (WGA)	Invitrogen	W11262		1:400 for IF
Goat anti-mouse IgG HRP	Hangzhou Huaan Biotechnology	HA1006	Goat	1:2000for WB
Goat anti-rabbit IgG HRP	Hangzhou Huaan Biotechnology	HA1001	Goat	1:2000for WB