

APPENDIX

Appendix Figure S1

Appendix Figure S2

Appendix Figure S3

Appendix Figure Legends

Appendix Table S1

Appendix Table S2

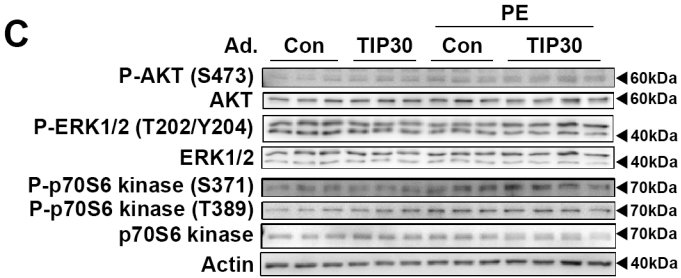
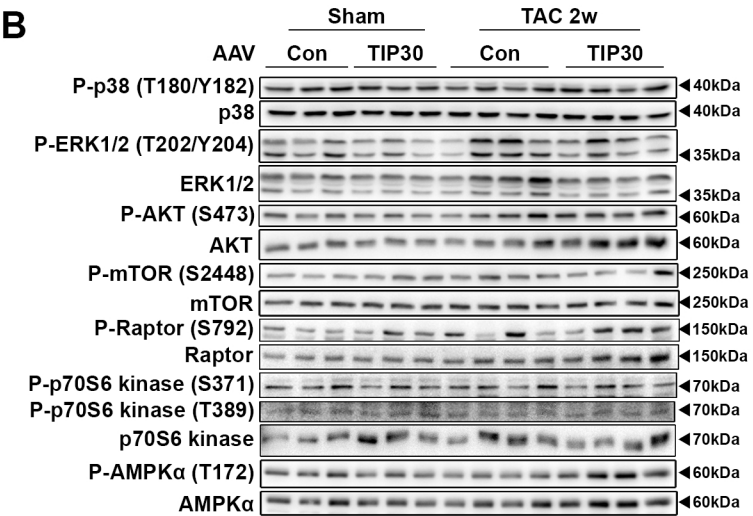
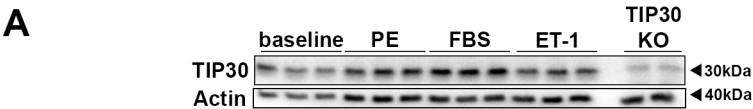
Appendix Table S3

Appendix Table S4

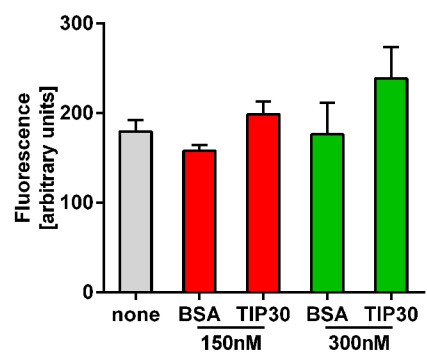
Appendix Table S5

Appendix Table S6

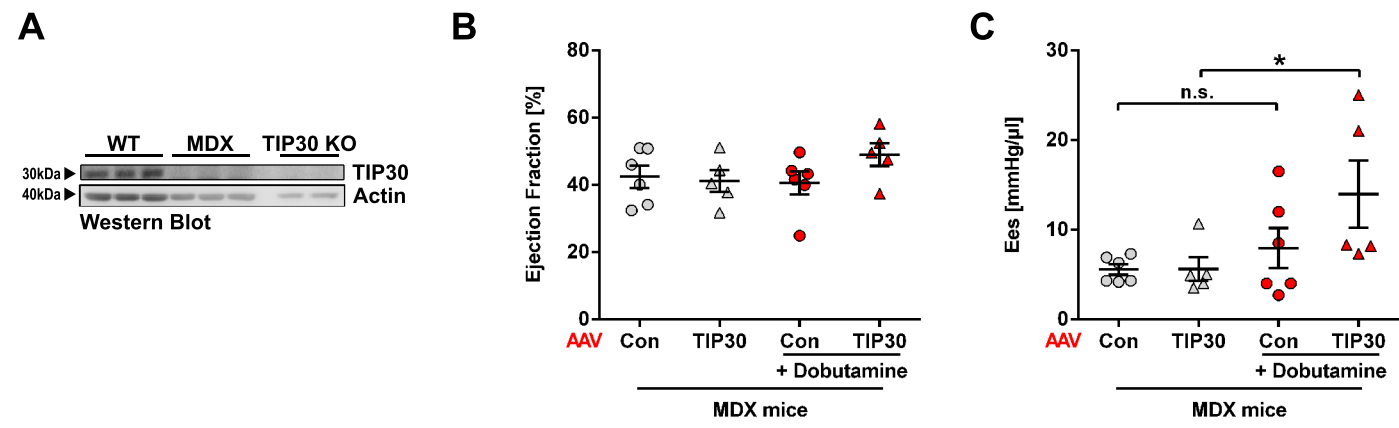
Appendix Figure S1



Appendix Figure S2



Appendix Figure S3



Appendix Figure Legends

Appendix Figure S1: Effects of TIP30 overexpression on hypertrophic signalling.

(A) Western Blot analysis of TIP30 and Actin from NRCM stimulated either with PE, FBS or endothelin-1 (ET-1).

(B) Western Blot analysis of indicated proteins in hearts from C57BL/6 WT mice treated either with AAV9 control virus (AAV-Con) or AAV9-TIP30 followed by 2 weeks TAC or sham surgery.

(C) Western Blot analysis of indicated proteins in neonatal rat cardiomyocytes (NRCM) after adenoviral transduction with control virus (Ad.Con) or Ad.TIP30 and stimulation with phenylephrine (PE) as indicated.

Appendix Figure S2: TIP30 does not interfere with tRNA binding by eEF1A1.

Quantification of fluorescent tRNA bound to eEF1A1 after incubation with purified TIP30-His or bovine serum albumin (BSA) as control (N=2 replicates/group).

Appendix Figure S3: Expression and functional effects of TIP30 in a mouse model of genetic cardiomyopathy.

(A) Western Blot analysis of TIP30 and Actin expression in WT or mdx (MDX) mice, TIP30 homozygous knock-out mice (KO) served as control.

(B; C) Quantification of ejection fraction (**B**, N=5 to 6 hearts/group) and endsystolic elastance (Ees; **C**, N=5 to 6 hearts/group) by left ventricular catheterization in 6mo old MDX mice with and without AAV9-mediated overexpression of TIP30 and Dobutamine injection as indicated. (n.s.): not statistically significant. * $P < 0.05$. One-way ANOVA with Sidak's multiple comparisons test.

Appendix Table S1: Candidate proteins co-precipitated with GST-TIP30 from cardiomyocyte lysate

Protein	No. of peptides	Coverage (%)	Uniprot Accession Number
Fructose-bisphosphate aldolase A	19	45.9	P05065
Polymerase I and transcript release factor Ptrf	13	43.9	P85125
40S ribosomal protein S3a Rps3a	15	37.5	P49242
Trifunctional enzyme subunit alpha, mitochondrial	44	37.1	Q64428
Glyceraldehyde-3-phosphate dehydrogenase GAPDH	18	35.8	P04797
ADP/ATP translocase 2	14	31.5	Q09073
Nucleolin Ncl	9	30.9	P13383
Basic leucine zipper and W2 domain-containing protein 2 Bzw2	10	24.8	Q9WTT7
Elongation factor 1-alpha 1 eEF1A1	16	24.6	P62630
Heterogeneous nuclear ribonucleoproteins A2/B1 hnRNP A2/B1	5	15	A7VJC2
Succinyl-CoA ligase [GDP-forming] subunit alpha, mitochondrial	6	10.3	P13086
Cytoplasmic dynein 1 heavy chain 1	16	7.2	P38650

Appendix Table S2: Clinical data of the HCM patients

Sex	Diagnosis	Age at operation [years]	IVS [mm]	known mutations
f	HOCM	62	20	negative in MYH7, MYBPC3, TNNT2
f	HOCM	19	33	MYH7, TNNT2
m	HOCM	67	14	negative in MYH7, MYBPC3, TNNT2
m	HOCM	22	50	negative in MYH7, MYBPC3, TNNT2
f	HOCM	15	>>13	MYBPC3
m	FHC	55	n/a	MYH7
m	HOCM	45	n/a	MYBPC3
m	HOCM	32	19 (at age 30)	MYH7

IVS: intraventricular septum; m: male; f: female; HOCM: hypertrophic obstructive cardiomyopathy; FHC: familial hypertrophic cardiomyopathy.

Appendix Table S3: Cardiomyocyte proteins regulated following adenoviral TIP30 overexpression as identified by DIGE

Protein	Regulation	Average Ratio	No. of peptides	Coverage (%)	Uniprot Accession Number
Receptor of activated protein C kinase 1 Rack1	Down	-1.28	5	19.2	P63245
Transitional endoplasmic reticulum ATPase VCP	Down	-1.24	12	17.4	P46462
Vinculin Vcl	Up	1.34	14	16.2	P85972
Chloride intracellular channel protein 5 Clic5	Up	1.36	4	13.9	Q9EPT8
Receptor of activated protein C kinase 1 Rack1	Up	1.41	5	20.2	P63245
ATP synthase subunit alpha, mitochondrial Atp5a1	Up	1.46	4	9.95	P15999
Chloride intracellular channel protein 5 Clic5	Up	2.05	2	9.56	Q9EPT8
NAD-dependent deacetylase sirtuin-5 Sirt5	Up	2.45	4	13.5	Q68FX9
Voltage-dependent anion-selective channel protein 3 Vdac3	Up	2.45	6	23.3	Q9R1Z0
Retinol dehydrogenase 14 Rdh14	Up	2.61	2	7.49	Q9ERI6
ATP synthase subunit alpha, mitochondrial Atp5a1	Up	2.82	6	14.3	P15999
Omega-amidase NIT2 Nit2	Up	2.98	8	33.7	Q497B0
ATP synthase subunit alpha, mitochondrial Atp5a1	Up	3.48	5	12.3	P15999
ATP synthase subunit alpha, mitochondrial Atp5a1	Up	4.89	7	15.4	P15999
ATP synthase subunit alpha, mitochondrial Atp5a1	Up	4.96	9	19.9	P15999
Malate dehydrogenase, mitochondrial Mdh2	Up	4.96	7	25.7	P04636
MICOS complex subunit Mic19 Chchd3	Up	5.66	2	5.3	Q9CRB9
ATP synthase subunit alpha, mitochondrial Atp5a1	Up	5.97	2	4.9	P15999
Oxidoreductase HTATIP2 Tip30	Up	100.80	4	19.4	Q9Z2G9

Appendix Table S4: Reagents, chemicals, plasmids, kits and mice used in this study

Reagent or Resource	SOURCE	IDENTIFIER	
Antibodies			dilution
anti-Actin	Sigma Aldrich	A2066	WB: 1:1.000
anti-Akt	Cell Signaling	9272	WB: 1:1.000
anti-AMPK-alpha	Cell Signaling	2532	WB: 1:1.000
Anti-cleaved caspase-3	Cell Signaling	9661	WB: 1:1.000
anti-eEF1A1	Abcam	118703	WB: 1:1.000; IF 1:100
Anti-eEF1A1+eEF1A2+eEF1A3	Abcam	37969	WB: 1:1.000
Anti-eEF1A1	Sigma Aldrich	sc-21758	WB: 1:1.000
anti-eEF1A2	Abcam	156954	WB: 1:1.000
anti-eEF1B2	Abcam	77043	WB: 1:1.000
anti-GAPDH	Fitzgerald	10R-G109a	WB: 1:3.500
anti-GST	Cell Signaling	2625	WB: 1:1.000
anti-His	Cell Signaling	2365	WB: 1:1.000
anti-His	Cell Signaling	2366	WB: 1:1.000
anti-mTOR	Cell Signaling	2983	WB: 1:1.000
Anti-Myc	Cell Signalling	2276	WB: 1:1.000
anti-p38 MAPK	Cell Signaling	9212	WB: 1:1.000
anti-p44/42 MAPK (Erk1/2)	Cell Signaling	9102	WB: 1:1.000
anti-p70S6 Kinase	Cell Signaling	2708	WB: 1:1.000
Anti-PDGFRa	R&D Systems	AF1062	IF: 1:100
anti-Phospho-4E-BP1	Cell Signaling	2855	WB: 1:1.000
anti-Phospho-Akt	Cell Signaling	2965	WB: 1:1.000
anti-Phospho-AMPK-alpha	Cell Signaling	2535	WB: 1:1.000
anti-Phospho-eEF2	Cell Signaling	2331	WB: 1:1.000
anti-Phospho-mTOR	Cell Signaling	5536	WB: 1:1.000
anti-Phospho-p38 MAPK	Cell Signaling	9211	WB: 1:1.000
anti-Phospho-p44/42 MAPK (Erk1/2)	Cell Signaling	9101	WB: 1:1.000
anti-Phospho-p70S6 Kinase	Cell Signaling	9208	WB: 1:1.000
anti-Phospho-p70S6 Kinase	Cell Signaling	9234	WB: 1:1.000
anti-Phospho-Raptor	Cell Signaling	2083	WB: 1:1.000
anti-Phospho-Rictor	Cell Signaling	3806	WB: 1:1.000
anti-Puromycin	Sigma Aldrich	MABE343	WB: 1:5.000
anti-Raptor	Cell Signaling	2280	WB: 1:1.000
anti-Rictor	Cell Signaling	2114	WB: 1:1.000
anti-SAPK/JNK	Cell Signaling	9252	WB: 1:1.000
Anti-TIP30	Abcam	177961	WB: 1:1.000; IF: 1:100
Anti-TIP30	Abcam	71752	WB: 1:1.000
Anti-Rabbit/Mouse IgG Alexa Fluor® 488 or secondary antibody	New England Biolabs	4412	IF: 1:200
Anti-Rabbit/Mouse IgG Alexa Fluor® 555 secondary antibody	New England Biolabs	4409	IF: 1:200
Alexa Fluor® 488 Phalloidin	Thermo Fisher Scientific	A12379	IF: 1:200
Chemicals			

$^{13}\text{C}_6$, $^{15}\text{N}_2$ -L-Lysine HCl	Silantes	211604102	
$^{13}\text{C}_6$, $^{15}\text{N}_4$ -L-Arginine HCl	Silantes	201604102	
Angiotensin II	Calbiochem	05-23-0101	
Calf Thymus DNA	Thermo Fisher Scientific	15633-019	
cOmplete™ Protease Inhibitor Cocktail	Sigma-Aldrich	11697498001	
Endothelin 1	Sigma-Aldrich	E7764	
Fetal Bovine Serum (FBS) superior	Biochrom	S0615	
Guanosine 5'-Triphosphoric Acid Disodium Salt	AppliChem	A1803	
HardSet Mounting Medium with DAPI	Vector Laboratories	VEC-H-1500	
Hoechst 33258	Sigma-Aldrich	861405	
Isolectin B4	Vector Laboratories	FL-1201	
Lipofectamine 2000 reagent	Invitrogen	11668-027	
Narciclasine	Santa Cruz	Sc-361271	
Normal goat IgG	Santa Cruz	sc-2027	
Normal mouse IgG	Santa Cruz	sc-2025	
Passive Lysis Buffer	Promega	E1941	
peqGOLD TriFast	Peqlab	30-2020	
Phenylephrine	Sigma-Aldrich	P6126	
Puromycin dihydrochloride	Sigma-Aldrich	P7255	
Tissue-Tek® O.C.T™	Sakura	4583	
FITC-conjugated wheat germ agglutinin (WGA)	Sigma-Aldrich	L4895	
Critical Commercial Kits			
AdEasy Adenoviral Vector System	Agilent Technologies	240009	
Duolink In Situ kit	Sigma-Aldrich	DUO92101	
Flexi rabbit reticulocyte lysate	Promega	L4540	
Fluorotect GreenLys in vitro translation Labeling System	Promega	L5001	
GeneTrans II Transfection Reagent	MoBiTec GmbH	0201B	
GlutathioneSepharose 4B	GE Healthcare	17-0756-01	
Maxima H Minus First Strand cDNA Synthesis Kit	Thermo Fisher Scientific	EP0751	
Maxima SYBR Green qPCR Master Mix	Thermo Fisher Scientific	K0253	
Micro BCA Protein Assay Kit	Thermo Fisher Scientific	23235	
Ni-NTA Magnetic Agarose beads	Qiagen	36113	
Ni-NTA Spin columns	Qiagen	31014	
PCR purification kit	Thermo Fisher Scientific	K0701	
PE Annexin V Apoptosis Detection Kit I	BD Biosciences	559763	
Protein A/G PLUS Agarose beads	Santa Cruz	sc-2003	

Renilla Luciferase Assay System	Promega	E2810	
Zeba Spin desalting columns 7K MWCO	Thermo Fisher Scientific	89882	
Experimental Models: Cell Lines			
COS-1	Laboratory of Kai C. Wollert	N/A	
HEK293	Leibniz Institute DSMZ	Cell Line 293 ACC: 305	
Experimental Models: Organisms/Strains			
E.coli: BJ5183-AD-1 Electroporation Competent Cells	Agilent Technologies	200157	
E.coli: DH5 α TM Competent Cells	Thermo Fisher Scientific	18265-017	
E.coli: One Shot [®] BL21 Star TM (DE3) Chemically Competent	Thermo Fisher Scientific	18265-017	
E.coli: XL10-Gold Ultracompetent Cells	Agilent Technologies	200315	
Mouse: B6.129P2-Htatip2 ^{tm1Hx}	Laboratory of H.Xiao	[(Ito et al., 2003)]	
Mouse: C57BL/6N	The Jackson Laboratory	JAX: 005304	
Mouse: C57BL/mdx	Laboratory of O. J. Müller		
Recombinant DNA			
Plasmid: pcDNA3.1/V5-eEF1A1-HIS	This paper	N/A	
Plasmid: pcDNA3.1/V5-eEF1A1- Δ AS1-240-HIS (Δ Dom1)	This paper	N/A	
Plasmid: pcDNA3.1/V5-eEF1A1- Δ AS241-336-HIS (Δ Dom2)	This paper	N/A	
Plasmid: pcDNA3.1/V5-eEF1A1- Δ AS336-462-HIS (Δ Dom3)	This paper	N/A	
Plasmid: pcDNA3.1/V5-eEF1A2-HIS	This paper	N/A	
Plasmid: pcDNA3.1/V5-TIP30-HIS	This paper	N/A	
Plasmid: pcDNA3.1/V5-TIP30- Δ 102-107-HIS	This paper	N/A	
Plasmid: pcDNA3.1/V5-TIP30- Δ C15-HIS	This paper	N/A	
Plasmid: pcDNA3.1/V5-TIP30- Δ N25-HIS	This paper	N/A	
Plasmid: pcDNA3.1/V5-TIP30- Δ N52-HIS	This paper	N/A	
Plasmid: pds-CMV _{enh} -MLC260-TIP30	This paper	N/A	
Plasmid: pds-TntT-TIP30	This paper	N/A	
Plasmid: pGEX-4T1-eEF1A1	This paper	N/A	
Plasmid: pGEX-4T1-eEF1A2	This paper	N/A	
Plasmid: pGEX-4T1-TIP30	This paper	N/A	

Plasmid: pGEX-4T1-TIP30-AS1-130	This paper	N/A	
Plasmid: pGEX-4T1-TIP30-AS1-180	This paper	N/A	
Plasmid: pGEX-4T1-TIP30-AS1-230	This paper	N/A	
Plasmid: pGEX-4T1-TIP30-AS1-50	This paper	N/A	
Plasmid: pshuttleCMV-eEF1A1-Myc	This paper	N/A	
Plasmid: pshuttleCMV-hnRNPA2/B1-Myc	This paper	N/A	
Plasmid: pshuttleCMV-Nucleolin-Myc	This paper	N/A	
Plasmid: pshuttleCMV-Rps3a-Myc	This paper	N/A	
Plasmid: pshuttleCMV-TIP30	This paper	N/A	
Plasmid: pshuttleCMV-TIP30-HIS	This paper	N/A	
Vector: pcDNA3.1/V5-His A	Thermo Fisher Scientific	V81020	
Vector: pDP9rs	Laboratory of O. J. Müller	N/A	
Vector: pds-CMV _{enh} -MLC260 (AAV9)	Laboratory of O. J. Müller	N/A	
Vector: pds-TntT-Cre	Laboratory of O. J. Müller	N/A	
Vector: pGEX-4T1	GE Healthcare	28-9545-49	
Vector: pShuttleCMV	Agilent Technologies	240007	
Adenovirus expressing eEF1A1-Myc	This paper	N/A	
Adenovirus expressing TIP30	This paper	N/A	
Adenovirus expressing TIP30-HIS	This paper	N/A	
Adenovirus expressing β -Gal (Ad.Control)	Previous work	[(Froese et al., 2011)]	
cDNA clone eEF1A1	GE Healthcare	MRN1768-202843669	
cDNA clone eEF1A2	GE Healthcare	MRN1768-202780416	
cDNA clone eEF1B2	GE Healthcare	ERN1146-202843321	
cDNA clone hnRNPA2/B1	GE Healthcare	MHS6278-202826002	
cDNA clone Nucleolin	GE Healthcare	MRN1768-202780611	
cDNA clone Rps3a	GE Healthcare	MRN1768-202843526	
Sequence-Based Reagents			
Silencer® Negative Control No. 1 siRNA	Thermo Fisher Scientific	AM4611	
siRNA eEF1A1	Sigma Aldrich	SASI_Rn02_00269532	

Primer	Forward (5'-to-3' sequence)	Reverse (5'-to-3' sequence)	
Cloning primer for human hnRNPA2/B1-Myc	TATAGGTACC ATGGAGAGAG AAAAGGAACA	TTAAGCGGC CGCCTACAG ATCTTCTTCA GAAATAAGTT TTTGTTCCTG GACCGTAGT TAGAAGGT	
Cloning primer for rat eEF1A1-His	GCGATCTAGA ATGGGAAAGG AAAAGACTCA	GCGAACCGG TTTTAGCCTT CTGAGCTTT CT	
Cloning primer for rat eEF1A1-Myc	TGACGGTACC ATGGGAAAGG AAAAGACTCA	ATATCTCGA GCTACAGAT CTTCTTCAGA AATAAGTTTT TGTTCTTTAG CCTTCTGAG CTTTCT	
Cloning primer for rat eEF1A1-Δ 241-336-His (ΔDom2) I	TGACGGTACC ATGGGAAAGG AAAAGACTCA	ATATCTCGA GACGAGTTG GCGGCAGAA T	
Cloning primer for rat eEF1A1-Δ 241-336-His (ΔDom2) II	ATATCTCGAG GCAGCTGGCT TCACTGCT	ATATACCGG TTTTAGCCTT CTGAGCTTT CTG	
Cloning primer for rat eEF1A1-Δ 336-462-His (ΔDom3)	ATATCTCGAG ACGAGTTGGC GGCAGAAT	ATATCTCGA GTTCCATTG GTGGGTCAT TT	
Cloning primer for rat eEF1A1-Δ1-240-His (ΔDom1)	TTATGGTACC ATGCCAACTG ACAAGCCTCT G	ATATACCGG TTTTAGCCTT CTGAGCTTT CTG	
Cloning primer for rat eEF1B2-His	ATATGAATTCA TGGGTTTCGG AGACCTGA	ATATCTCGA GGATTTTGTT AAAAGCAGC CACAT	
Cloning primer for rat GST-eEF1A1	TCTCGAATTC GGAAAGGAAA AGACTCA	ATATGTCTGA CAGGTGTTA GGGATAATA T	
Cloning primer for rat GST-eEF1B2	ATATCTGAATT CGGTTTCGGA GACCTGAAAA	ATATCTCTCG AGTTAGATTT TGTTAAAAG CAGCCA	

Cloning primer for rat Nucleolin-Myc	GCGCAGATCT ATGGTGAAAC TCGCAAAGGC	GCGCCTCGA GCTACAGAT CTTCTTCAGA AATAAGTTTT TGTTCTTCAA ACTTCGTCTT CTTTC	
Cloning primer for rat Rps3a-Myc	TATAGGTACC ATGGCGGTCTG GGAAGAACAA	ATATCTCGA GCTACAGAT CTTCTTCAGA AATAAGTTTT TGTTCAACG GATTCTTGG ACTGGTG	
Cloning primer for rat TIP30 (AAV9-TIP30)	ATATGGATCC ACCGGTCGCC ACCATGGCGG ACAAGGAAAC ACTGCT	GCGCTGTAC ATCAGTTATC TAGATCCGG TGA	
Cloning primer for rat TIP30 (Ad.TIP30)	AGAGGGTACC ATGGCGGACA AGGAAACAC	TTGGCTCGA GAGTTTGGG TCCGTCGCT GT	
Cloning primer for rat TIP30 (GST-TIP30)	ATATGGATCC GCGGACAAGG AAACACTGCT	TTGGGAATT CCGGCATGG TCACAGTTT G	
Cloning primer for rat TIP30 (GST-TIP30-AS1-130)	ATATGGATCC GCGGACAAGG AAACACTGCT	GTGTGAATT CCAGCAAGT TGAAATGTTT GC	
Cloning primer for rat TIP30 (GST-TIP30-AS1-180)	ATATGGATCC GCGGACAAGG AAACACTGCT	GTGTGAATT CGCCTGGAC GAGACTGTT GCC	
Cloning primer for rat TIP30 (GST-TIP30-AS1-230)	ATATGGATCC GCGGACAAGG AAACACTGCT	GTGTGAATT CGATGGCCT TATTTTCCAG AA	
Cloning primer for rat TIP30 (GST-TIP30-AS1-50)	ATATGGATCC GCGGACAAGG AAACACTGCT	GTGTGAATT CAATGAGCG TTACTTTGGA AA	
Cloning primer for rat TIP30-His	AGAGGGTACC CAATGGCGGA CAAGGAAACA	ATCGACCGG TCAGTTTGG GTCCGTCGC TGT	
Cloning primer for rat TIP30-Δ102-107-His I	AGAGGGTACC CAATGGCGGA CAAGGAAACA	ATATCTCGA GCGCTCCAG CTTTTCTCCT	

Cloning primer for rat TIP30-Δ102-107-His II	ATATCTCGAG CGAGATTATG TGCTGAAGT	ATATACCGG TCAGTTTGG GTCCGTGC	
Cloning primer for rat TIP30-ΔC15-His	AGAGGGTACC CAATGGCGGA CAAGGAAACA	ATATACCGG TCTTATTTTC CAGAAGTTC CATTT	
Cloning primer for rat TIP30-ΔN25-His	TTATGGTACC ATGGCCAGCG GTGAAACCGG	ATATACCGG TCAGTTTGG GTCCGTGC	
Cloning primer for rat TIP30-ΔN52-His	TTATGGTACC ATGAGGAAGC TCACATTTGA	ATATACCGG TCAGTTTGG GTCCGTGC	
qRT-PCR primer for human GAPDH	TCGACAGTCA GCCGCATCTT CTTT	ACCAAATCC GTTGACTCC GACCTT	
qRT-PCR primer for human TIP30	ACCGGCAGAG TGCTCTTAAA	TCTCGGTCA ACACGAACA AA	
qRT-PCR primer for mouse Acta1	TGTCGTCCCA GTTGGTGATA	ACCGCTCTT GTGTGTGAC AA	
qRT-PCR primer for mouse eEF1A1	CGGCCACCTG ATCTACAAAT	CACGCTCAG CTTTCAGTTT G	
qRT-PCR primer for mouse eEF1A2	TGAGATGGTC CCTGGAAAAC	GCTTTCTGA GCCTTCTGT GC	
qRT-PCR primer for mouse GAPDH	ACCCAGAAGA CTGTGGATGG	CACATTGGG GGTAGGAAC AC	
qRT-PCR primer for mouse L7	TGGAACCATG GAGGCTGT	TCTCAGTGC GGTACATCT GC	
qRT-PCR primer for mouse Myh6	ACTGTGGTGC CTCGTTCC	GCCTCTAGG CGTTCCTTCT C	
qRT-PCR primer for mouse Myh7	AGGCAAGGCA AAGAAAGGCT CATC	GCGTGGAGC GCAAGTTTG TCATAA	
qRT-PCR primer for mouse TIP30	GAAGTGGAAG CCAAGGTTGA	CTGGGACTC ACCAGGTTG TT	
qRT-PCR primer for rat GAPDH	ACCACCATGG AGAAGGCTGG	CTCAGTGTA GCCCAGGAT GC	
qRT-PCR primer for rat L7	GAAGCTCATC TATGAGAAGG C	AAGACGAAG GAGCTGCAG AAC	

qRT-PCR primer for rat TIP30	CGACCGAGAT TATGTGCTGA	GGACGAGAC TCTTGCCTG TC	
qRT-PCR primer for renilla	CCAGGGATTT CAGTCGATGT	AATCTCACG CAGGCAGTT CT	
qRT-PCR primer for mouse Rheb1	CCATGGCAAG TTGTTGGATAT G	TCTTCATAGC TGATCACCC TTTC	
qRT-PCR primer for mouse Hrd1	CCAACATCTC CTGGCTCTTC CA	CAGGATGCT GTGATAAGC GTGG	
qRT-PCR primer for mouse Xbp1	CTCACGGCCT TGTGGTTGA	TCCATTCCC AAGCGTGTT C	
qRT-PCR primer for mouse Manf	TCAATGAGGT GTCGAAGCCC	GTCCACTGT GCTCAGGTC AA	
Software and Algorithms			
Graph Pad Prism 6	Graph Pad software	N/A	
ImageJ (Schneider et al., 2012)	National Institutes of Health, USA	N/A	
Quantity One	Bio-Rad	N/A	
Ion Wizard software	IonOptix		

Appendix Table S5: Statistics

Figure		Exact <i>P</i> -value	Statistical test
1	B	WT TAC/KO TAC: *=0.0475; WT TAC/Het TAC: ****< 0.0001	one-way ANOVA followed by the Sidak's multiple comparisons test
	C	WT TAC/Het TAC: ****< 0.0001	one-way ANOVA followed by the Sidak's multiple comparisons test
	D	WT TAC/Het TAC: *=0.0273	one-way ANOVA followed by the Sidak's multiple comparisons test
	E	WT Sham/WT TAC: **=0.0069; WT TAC/KO TAC: ***=0.0003; WT TAC/Het TAC: ****< 0.0001	one-way ANOVA followed by the Sidak's multiple comparisons test
	F	WT TAC/ Het TAC: *= 0.0161	one-way ANOVA followed by the Sidak's multiple comparisons test
	G	WT Sham/WT TAC: *=0.0279; WT TAC/Het TAC: **=0.0051	one-way ANOVA followed by the Sidak's multiple comparisons test
	I	WT TAC/Het TAC: **=0.0081	one-way ANOVA followed by the Sidak's multiple comparisons test
	L	WT Sham/WT TAC: *=0.0479; WT TAC/Het TAC: *=0.0292	one-way ANOVA followed by the Sidak's multiple comparisons test
	N	WT TAC/Het TAC: **=0.0055	one-way ANOVA followed by the Sidak's multiple comparisons test
2	Q	WT AAV-Con TAC/Het AAV-Con TAC: *=0.0133; Het AAV-Con TAC/Het AAV-TIP30 TAC: *=0.0257; WT AAV-Con Sham/WT AAV-Con TAC: ***=0.0008	one-way ANOVA followed by the Sidak's multiple comparisons test
	R	WT AAV-Con TAC/Het AAV-Con TAC: *=0.0163; Het AAV-Con TAC/Het AAV-TIP30 TAC: *=0.0354	one-way ANOVA followed by the Sidak's multiple comparisons test
	B	Ad.Con ET-1/Ad.TIP30 ET-1: *=0.0348; Ad.Con/Ad.TIP30: ***=0.0001; Ad.Con PE/Ad.TIP30 PE: ***=0.0002; Ad.Con/Ad.Con FBS: ***=0.0008; Ad.Con/Ad.Con PE: ****<0.0001	one-way ANOVA followed by the Sidak's multiple comparisons test
	C	Ad.Con/Ad.Con PE: *=0.0162; Ad.Con PE/Ad.TIP30 PE: ****<0.0001	one-way ANOVA followed by the Sidak's multiple comparisons test
	D	Ad.Con/Ad.Con ET-1: *=0.0152; Ad.Con ET-1/Ad.TIP30 ET-1: *=0.0435; Ad.Con PE/Ad.TIP30 PE: **=0.0014; Ad.Con/Ad.Con PE: ****<0.0001	one-way ANOVA followed by the Sidak's multiple comparisons test
	H	AAC-Con Sham/AAV-Con TAC: *=0.0196	one-way ANOVA followed by the Sidak's multiple comparisons test
	I	AAC-Con Sham/AAV-Con TAC: *=0.0118; AAV-Con TAC/AAV-TIP30 TAC: *=0.0438	one-way ANOVA followed by the Sidak's multiple comparisons test

	J	AAV-Con 2wk TAC/AAV-TIP30 2wk TAC: **=0.0028; AAV-Con 4wk TAC/AAV-TIP30 4wk TAC: **=0.0048	one-way ANOVA followed by the Sidak's multiple comparisons test
3	J	eEF1A1 mRNA expression neonatal/adult sham: *=0.0468; eEF1A1 mRNA expression adult sham/adult TAC: *=0.0180; eEF1A2 mRNA expression neonatal/adult sham: ***=0.0003	one-way ANOVA followed by the Sidak's multiple comparisons test
4	C	Ad.Con PE/Ad.TIP30 PE: *=0.0131	two-sided Student's t -test
	E	Ad.Con/Ad.Con PE: ****<0.0001; Ad.Con PE/Ad.TIP30 PE: ****<0.0001	one-way ANOVA followed by the Sidak's multiple comparisons test
	G	WT Sham/WT TAC: *=0.0355; WT TAC/Het TAC: *=0.0277	one-way ANOVA followed by the Sidak's multiple comparisons test
5	D	ratio TIP30/GAPDH expression: ****<0.0001; ratio eEF1A1/GAPDH expression: **= P = 0.0083	two-sided Student's t -test
	H	ratio TIP30/Actin expression: *=0.0228; ratio TIP30/eEF1A1 expression: **=0.0039	two-sided Student's t -test
	J	ratio TIP30/Actin expression: ***=0.0005; ratio TIP30/eEF1A1 expression: **=0.0058	two-sided Student's t -test
6	A	Tip30 mRNA expression: ***=0.0003; ratio TIP30/eEF1A1 mRNA expression: *=0.0137	two-sided Student's t -test
	B	Tip30 mRNA expression: **=0.0077; ratio TIP30/eEF1A1 mRNA expression: *= P = 0.0492	two-sided Student's t -test
	C	Tip30 mRNA expression: ****<0.0001; eEF1A1 mRNA expression: **= P = 0.0020; ratio TIP30/eEF1A1 mRNA expression: ****<0.0001	two-sided Student's t -test
	E	AAV-Con 3mo/AAV-TIP30 3mo: *=0.0132; AAV-Con 9mo/AAV-TIP30 9mo: **=0.0017	one-way ANOVA followed by the Sidak's multiple comparisons test
	F	AAV-Con/AAV-TIP30: *=0.0116	two-sided Student's t -test
7	B	Ad.Con/Ad.Con PE: **=0.0028; Ad.Con PE/Ad.TIP30 PE: *=0.0491	one-way ANOVA followed by the Sidak's multiple comparisons test
	C	WT ET-1/Het ET-1: *=0.0143; Het ET-1/Het ET-1 + Narci: ***=0.0001	one-way ANOVA followed by the Sidak's multiple comparisons test
	E	WT TAC/Het TAC: ***=0.0002	one-way ANOVA followed by the Sidak's multiple comparisons test
	F	WT TAC/Het TAC: ***=0.0007; Het TAC/Het TAC Narci: P=0.09	one-way ANOVA followed by the Sidak's multiple comparisons test
	G	WT TAC/Het TAC: **=0.0052; Het TAC/Het TAC Narci: *=0.0235	one-way ANOVA followed by the Sidak's multiple comparisons test
	H	Het TAC/Het TAC Narci: **=0.0063	one-way ANOVA followed by the Sidak's multiple comparisons test
	I	Het TAC/Het TAC Narci: *=0.0102; WT TAC/Het TAC: **=0.0086	one-way ANOVA followed by the Sidak's multiple comparisons test
EV1	A	WT TAC/ Het TAC: **=0.0100	one-way ANOVA followed by the Sidak's multiple comparisons test

	B	WT TAC/ Het TAC: *=0.0344	one-way ANOVA followed by the Sidak's multiple comparisons test
	C	WT TAC/ Het TAC: ***=0.0003	one-way ANOVA followed by the Sidak's multiple comparisons test
	E	WT Sham/WT TAC: ***=0.0002; Het Sham/Het TAC: ****<0.0001	one-way ANOVA followed by the Sidak's multiple comparisons test
	F	WT TAC/Het TAC: *=0.0214; WT Sham/WT TAC: ***=0.0003	one-way ANOVA followed by the Sidak's multiple comparisons test
EV2	A	WT /Het: ***=0.0004	two-sided Student's t -test
	C	WT /Het: ***=0.0002	two-sided Student's t -test
	G	WT /Het: *=0.0478	two-sided Student's t -test
EV3	B	P-p70S6 (S371) expression: WT sham/Het sham: *=0.0485; WT TAC/Het TAC: *=0.0169 P-p70S6 (T389) expression: WT TAC/Het TAC: *=0.0298; WT Sham/WT TAC: ****<0.0001 P-mTOR expression: WT TAC/Het TAC: *=0.0120 P-AMPKα expression: WT TAC/Het TAC: **=0.0029 P-SAPK/JNK expression: WT Sham/WT TAC: **=0.0087 P-ERK1/2 expression: WT TAC/Het TAC: *=0.0351; WT sham/Het sham: **=0.0035	one-way ANOVA followed by the Sidak's multiple comparisons test
EV4	D	Ad.Con PE/Ad.TIP30 PE: *=0.0280; Ad.Con/Ad.Con PE: **=0.0038	one-way ANOVA followed by the Sidak's multiple comparisons test
	E	Ad.Con PE/Ad.TIP30 PE: *=0.0190; Ad.Con/Ad.Con PE: *=0.0017	one-way ANOVA followed by the Sidak's multiple comparisons test
	G	AAV-Con Sham/AAV-Con TAC: *=0.0138; AAV-Con TAC/AAV-TIP30 TAC: **0.0011	one-way ANOVA followed by the Sidak's multiple comparisons test
EV5	A	WT/Het: *=0.0393	one-way ANOVA followed by the Sidak's multiple comparisons test
	B	Hrd1 expression: WT TAC/KO TAC: **=0.0016 Xbp1 expression: WT TAC/KO TAC: *=0.0475 Manf expression: WT TAC/KO TAC: **=0.0019 Rheb1 expression: WT TAC/Het TAC: **=0.0017; WT TAC/KO TAC: ***=0.0009	one-way ANOVA followed by the Sidak's multiple comparisons test
	D	Ad.Con Scrl/Ad.TIP30 Scrl: **= 0.0067; Ad.Con Scrl/Ad.Con eEF1A1: **= 0.0080	one-way ANOVA followed by the Sidak's multiple comparisons test
	E	Ad.Con PE/Ad.Con PE +Narci: *=0.0348; Ad.Con/Ad.Con PE: **=0.0061; Ad.Con PE/Ad.TIP30 PE: ****<0.0001; Ad.Con PE +Narci/Ad.TIP30 PE +Narci: P=0.08	one-way ANOVA followed by the Sidak's multiple comparisons test
	F	TIP30-HIS ΔN52/TIP30-HIS full: *=0.0394; BSA/TIP30-HIS full: ***=0.0002	one-way ANOVA followed by the Sidak's multiple comparisons test

Appendix S3	C	AAV-TIP30/AAV-TIP30 +Dobutamine: $\ast=0.0382$; AAV-Con/AAV-Con +Dobutamine: $P=0.8132$	one-way ANOVA followed by the Sidak's multiple comparisons test
--------------------	---	--	---

Appendix Table S6: N values

Figure		N
1	B	WT sham=6; Het Sham=8; KO Sham=6; WT TAC=18; Het TAC=17; KO TAC=11
	C	WT sham=6; Het Sham=8; KO Sham=6; WT TAC=18; Het TAC=17; KO TAC=11
	D	WT sham=6; Het Sham=8; KO Sham=6; WT TAC=16; Het TAC=15; KO TAC=14
	E	WT sham=7; Het Sham=8; KO Sham=6; WT TAC=16; Het TAC=15; KO TAC=14
	F	WT sham=4; Het Sham=5; WT TAC=11; Het TAC=7
	G	WT sham=4; Het Sham=5; WT TAC=11; Het TAC=8
	H	WT sham=5; Het Sham=6; WT TAC=10; Het TAC=10
	I	WT sham=5; Het Sham=6; WT TAC=10; Het TAC=10
	K	WT sham=3; Het Sham=3; WT TAC=5; Het TAC=5
	L	WT sham=4; Het Sham=4; WT TAC=7; Het TAC=11
	N	WT sham=5; Het Sham=6; WT TAC=7; Het TAC=6
	Q	WT AAV-Con Sham=5; Het AAV-Con Sham=3; WT AAV-TIP30 Sham=5; Het AAV-TIP30 Sham=6; WT AAV-CON TAC=5, Het AAV-Con TAC=6; WT AAV-TIP30 TAC=9; Het AAV-TIP30 TAC=10
	R	WT AAV-Con Sham=5; Het AAV-Con Sham=3; WT AAV-TIP30 Sham=5; Het AAV-TIP30 Sham=6; WT AAV-CON TAC=5, Het AAV-Con TAC=6; WT AAV-TIP30 TAC=9; Het AAV-TIP30 TAC=10
2	B	Ad. Con=8; Ad.TIP30=8; Ad.Con ET-1=8; Ad.TIP30 ET-1=8; Ad.Con FBS=6; Ad.TIP30 FBS=6, Ad.Con PE=8; Ad.TIP30 PE=8
	C	Each group N=9
	D	Each group N=3
	E	Each group N=7
	H	AAV-Con Sham=8; AAV-TIP30 Sham=8; AAV-Con TAC=13; AAV-TIP30 TAC=11
	I	AAV-Con Sham=4; AAV-TIP30 Sham=3; AAV-Con TAC=5; AAV-TIP30 TAC=5
	J	AAV-Con Sham=10 each time point; AAV-TIP30 Sham=10 each time point; AAV-Con TAC 2wk=14; AAV-Con TAC 4wk=13; AAV-Con TAC 6wk=13; AAV-TIP30 TAC 2wk=14; AAV-TIP30 TAC 4wk=14; AAV-TIP30 TAC 6wk=13
3	J	Neonatal=3; Adult Sham=3; Adult TAC=4
4	C	Each group N=3
	E	Ad.Con=3; Ad.TIP30=3; Ad.Con PE=4; Ad.TIP30=4
	G	WT Sham=7; Het Sham=8; WT TAC=11; Het TAC=12
5	A	Each group N=4
	D	Sham=4; TAC 2wk= 8
	F	Sham=4; TAC 6wk= 8
	H	WT TAC=3; Het TAC 2wk= 4
	J	Each group N=4
6	A	Control=6; Failing=8

	B	Control=4; HCM=8
	C	WT=3; MDX=4
	E	AAV-Con baseline=7; AAV-TIP30 baseline=7; AAV-Con 1mo=7; AAV-TIP30 1mo=6; AAV-Con 3mo=7; AAV-TIP30 3mo=7; AAV-Con 6mo=7; AAV-TIP30 6mo=7; AAV-Con 9mo=6; AAV-TIP30 9mo=6
	F	AAV-Con=6; AAV-TIP30=5
7	B	Each group N=3
	C	Each group N=6
	E	WT Sham=6; WT TAC=8; Het TAC=9; WT TAC +Narci=10; Het TAC +Narci=6
	F	WT Sham=6; WT TAC=8; Het TAC=9; WT TAC +Narci=10; Het TAC +Narci=6
	G	Each group N=5
	H	WT Sham=6; WT TAC=8; Het TAC=9; WT TAC +Narci=10; Het TAC +Narci=6
	I	WT Sham=2; Het Sham=2; WT TAC=3; Het TAC=3; WT TAC +Narci=4; Het TAC +Narci=4
EV1	A	WT sham=7; Het Sham=8; KO Sham=6; WT TAC=16; Het TAC=17; KO TAC=14
	B	WT sham=7; Het Sham=8; KO Sham=6; WT TAC=16; Het TAC=17; KO TAC=14
	C	WT sham=7; Het Sham=8; KO Sham=6; WT TAC=16; Het TAC=17; KO TAC=14
	D	WT sham=7; Het Sham=8; KO Sham=6; WT TAC=16; Het TAC=17; KO TAC=14
	E	WT Sham=5; Het Sham=5; WT TAC=5; Het TAC=7
	F	WT Sham=4; Het Sham=5; WT TAC=11; Het TAC=8
	G	Each group N=5 mice
	I	WT sham=4; Het Sham=4; KO Sham=2; WT TAC=5; Het TAC=5; KO TAC=4
	J	WT sham=3; Het Sham=3; WT TAC=5; Het TAC=5
EV2	A	Each group N=4
	B	Each group N=4
	C	Each group N=4
	D	Each group N=4
	E	Each group N=4
	G	Each group N=4
EV3	B	p38, SAPK/JNK, ERK1/2, AKT, p70S6 kinase, 4E-BP1, eEF2: WT sham=3; Het Sham=3; WT TAC=4; Het TAC=4 mTOR, AMPK α : WT Sham=2, Het Sham=2, WT TAC=3, Het TAC=3
EV4	D	Ad.Con=7; Ad.TIP30=7; Ad.Con PE=8; Ad.TIP30=8
	E	Ad.Con=7; Ad.TIP30=7; Ad.Con PE=8; Ad.TIP30=8
	G	AAV-Con Sham=3; AAV-TIP30 Sham=3; AAV-Con TAC=7; AAV-TIP30 TAC=6
EV5	A	WT=3; Het=4; KO=3
	B	WT sham=6; Het Sham=6; KO Sham=4; WT TAC=10; Het TAC=10; KO TAC=5
	D	Ad.Con si-Scrl=12; Ad.TIP30 si-Scrl=12; Ad.Con si-eEF1a1=9; Ad.TIP30 si-eEF1A1=9

	E	Each group N=3
	F	Each group N=4
Appendix S2		Each group N=2
Appendix S2	B	AAV-Con=6; AAV-TIP30=5; AAV-Con +Dobutamine=6; AAV-TIP30 +Dobutamine=5
	C	AAV-Con=6; AAV-TIP30=5; AAV-Con +Dobutamine=6; AAV-TIP30 +Dobutamine=5