SUPPLEMENTARY INFORMATION

Rewired DEPTOR-mTORC1/2 signaling stabilizes cardiac fibroblast survival and activation

Sandra García-Carpi[1,2], Carlos Lana[1,2¶], Aida Beà[3¶], Juan García-Valero[4], Lin Y[5], Liu M[5], Hu J[5], Juan Antonio López[6], Jesús Vázquez[6], Patricia Pérez-Galán[4], Junmei Ye[5], Marta Llovera[1,2], Daniel Sanchis[1,2*].

[1] Cell Signaling and Apoptosis Group. Departament de Ciències Mèdiques Bàsiques, Universitat de Lleida (ROR: 050c3cw24), Lleida, 25198, Spain.
[2]Institut de Recerca Biomèdica de Lleida – Fundació Dr. Pifarré, IRBLleida (ROR: 03mfyme49), Av. Alcalde Rovira Roure, 80, 25198, Lleida, Spain.
[3] Department of Experimental and Health Sciences, Pompeu Fabra University (UPF) (ROR: 04n0g0b29), Barcelona.
[4] Department of Hematology-Oncology, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS, ROR: 041gvmd67), Barcelona, Spain and Centro de Investigación Biomédica en Red-Oncología (CIBERONC; ROR: 04hya7017), Barcelona, Spain.
[5] School of Life Science and Technology, China Pharmaceutical University (ROR: 01sfm2718), Nanjing, 211198 Jiangsu Province, China.
[6] Centro Nacional de Investigaciones Cardiovasculares (CNIC). Madrid, Spain (ROR: 02qs1a797) and CIBER de Enfermedades Cardiovasculares (CIBER-CV), Instituto de Salud Carlos III, Madrid, Spain (ROR: 02g87qh62).
[¶] contributed equally
*Lead contact: daniel.sanchis@udl.cat; Tel: +34-973702949. Biomedicina-I Av. Alcalde Rovira Roure, 80; 25198, Lleida, Spain.

Supplementary Figures 1-3

Supplementary Tables 1-3

SUPPLEMENTARY FIGURES

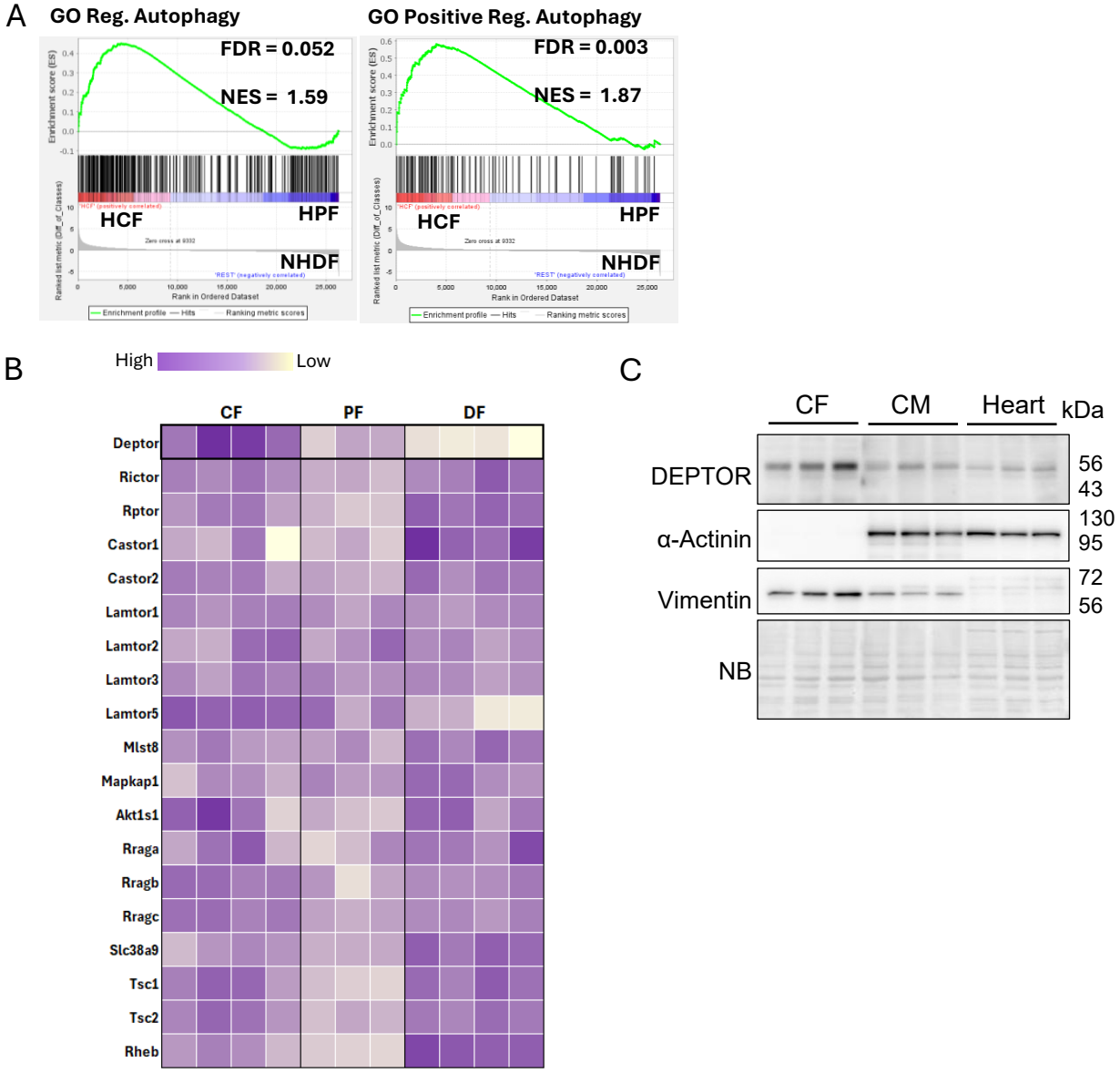


Figure S1. Autophagy-related transcriptional enrichment, mTOR-associated protein expression, and DEPTOR abundance in cardiac fibroblasts. A. GSEA plots for GO Regulation of Autophagy and Positive Regulation of Autophagy from human cardiac fibroblast (HCF, n=4) compared with pulmonary fibroblasts (HPF, n=5) and normal dermal fibroblasts (NHDF, n=7). The leading edge of each gene set, FDR (false discovery rate) and NES (normalized enrichment score) values are displayed. B. Heatmap of a curated set of mTOR-associated regulatory proteins detected in the proteomic dataset from rat cardiac (CF, n=4), pulmonary (PF, n=3) and dermal (DF, n=4) fibroblasts. C. DEPTOR expression in cultured rat cardiac fibroblasts, cardiomyocytes and total ventricle tissue samples. Alpha actinin and Vimentin were used as cardiomyocyte and fibroblast markers, respectively. Naphthol blue staining of membrane was used to assess protein loading (n=3).

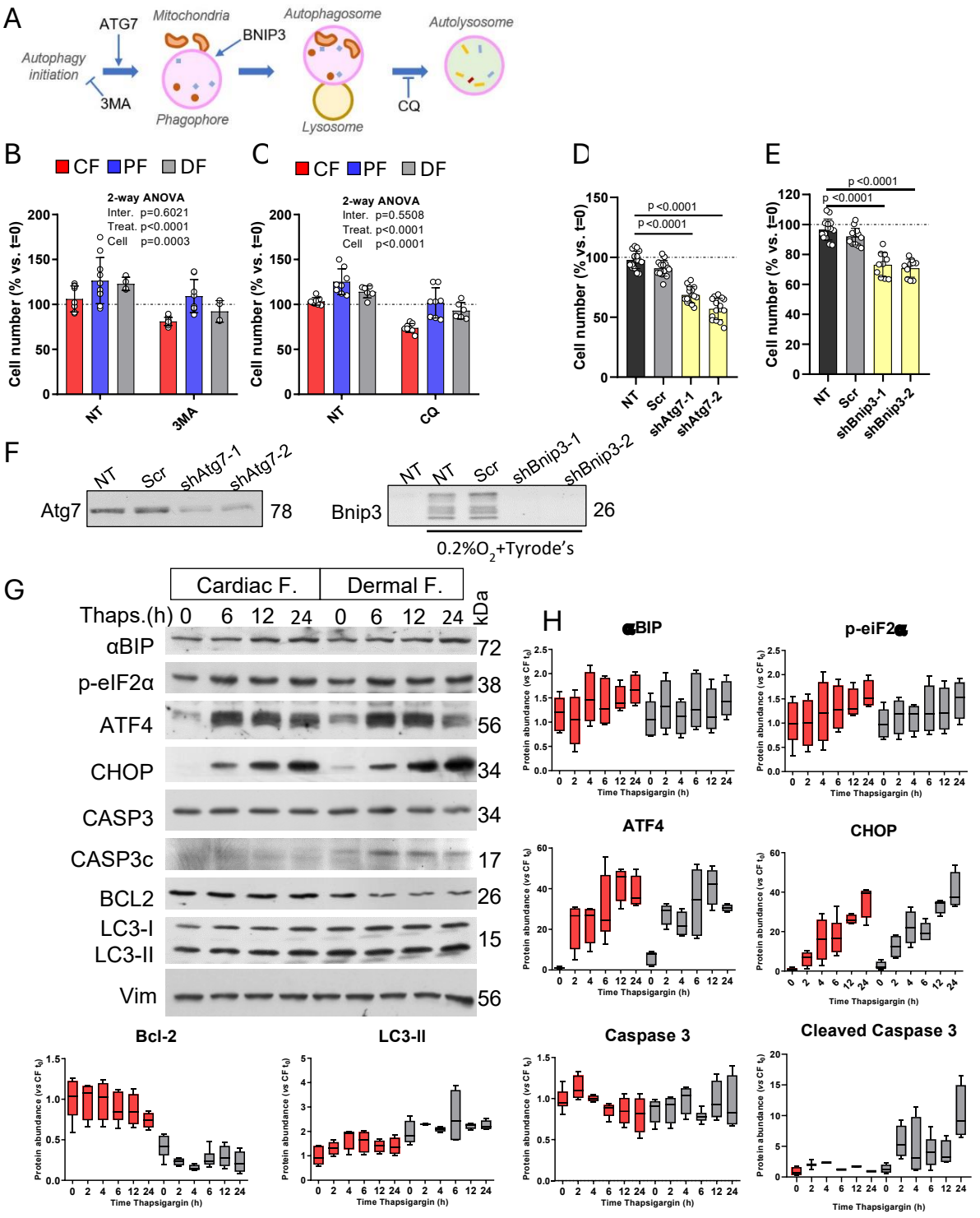
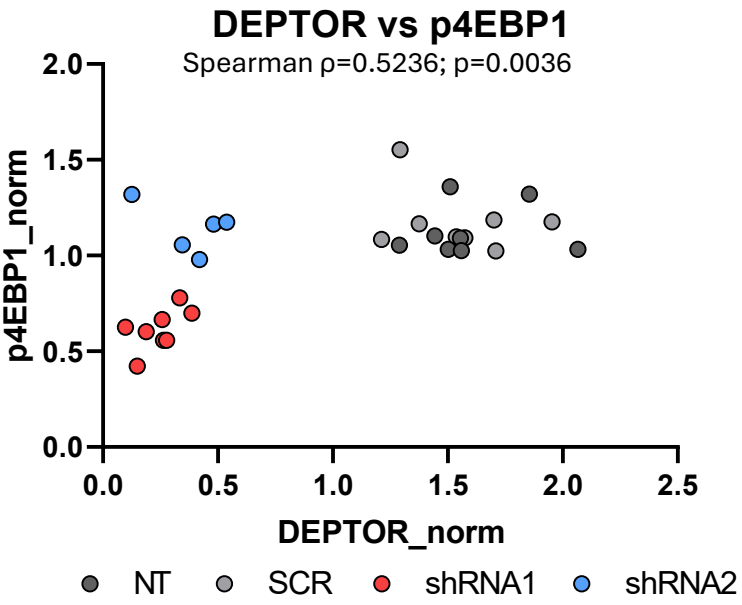


Figure S2. Autophagy supports fibroblast survival and modulates ER-stress responses in cardiac fibroblasts. A. Diagram showing the main autophagy components indicating the involvement of ATG7 and BNIP3 and steps interfered by pharmacological autophagy inhibitors 3-methyladenine (3MA) and Chloroquine (CQ). B-E. Survival of cardiac, pulmonary and dermal fibroblasts (CF, PF, DF) treated with 3MA, CQ or transduced with two independent knockdown constructs for Atg7 and Bnip3 (n=8-15). F, Western blot assessment of knockdown efficiency of the constructs used in E and F (hypoxia was used to stabilize and detect Bnip3). G, Representative Western blots and corresponding densitometric quantification of the main components of the Unfolded protein response (UPR), including also caspase-3 activation, BCL2 and LC3, during Thapsigargin treatment in cardiac (red) and dermal (grey) fibroblasts (n=4-6).

A



B

	XY pairs	ρ	p
p4E-BP1 Thr37/46	29	0.5236	0.0036
p70S6K Thr389	22	0.6533	0.0010
pAKT Ser473	37	0.2428	0.1477
BCL2	37	0.5946	0.0001

Figure S3. Quantitative association between DEPTOR and mTOR signaling outputs in adult cardiac fibroblasts. A. Scatter plot showing the monotonic association between DEPTOR abundance and p4E-BP1 Thr37/46 phosphorylation across pooled, experiment-normalized measurements from control and Deptor shRNA conditions. Each point represents an individual measurement. Spearman correlation coefficient (ρ) and two-tailed p value are indicated. B. Summary table of Spearman correlation analyses between DEPTOR abundance and downstream signaling nodes, including S6K Thr389, p4E-BP1 Thr37/46, and pAKT Ser473 as well as BCL2. The number of observations (XY pairs), correlation coefficients (ρ), and p values are shown.

Supplementary Table S1.

Supplementary Table S1. Echocardiography measurements at baseline

		AAV9-Ctrl		**AAV9-Deptor-shRNA**		**Student's t.**
Parameter	Unit	mean	SD	mean	SD	2 tails
Heart Rate	BPM	422.3841	44.110737	385.05539	38.727357	0.0528357
LV Diameter; s	mm	2.3637893	0.4108254	2.6257561	0.3124282	0.1222300
LV Diameter; d	mm	3.300459	0.4086997	3.4970189	0.2588621	0.2197333
LV Volume; s	µL	20.340341	8.5896441	25.832113	7.2076022	0.1290688
LV Volume; d	µL	45.133784	13.037526	51.204953	8.9648408	0.2400744
Stroke Volume	µL	24.793443	6.7602456	25.37284	5.3783992	0.8298773
Ejection Fraction	%	55.616823	10.803733	49.938102	9.3044613	0.2093098
Fractional Shortening	%	28.534083	7.0336974	25.016087	5.901951	0.2279882
Cardiac Output	mL/min	10.565034	3.3030986	9.7825249	2.408722	0.5466101
LV Mass	mg	96.186374	19.00332	105.01423	22.442112	0.3165430
LV Mass Corrected	mg	76.949099	15.202656	84.011384	17.953689	0.3165430
LVAW;s	mm	1.1101452	0.0855491	1.125376	0.2796722	0.8466759
LVAW;d	mm	0.8255669	0.0840072	0.8561754	0.1997545	0.6076091
LVPW;s	mm	1.1861394	0.3740545	1.0319445	0.2423273	0.2905202
LVPW;d	mm	0.9143833	0.4036954	0.8654757	0.3089808	0.7592833
		n=9		n=15		

Echocardiographic parameters at baseline. Cardiac parameters were quantified at baseline, three weeks after AAV injection using a VisualSonics Vevo 3100LT ultrasound system equipped with a 30-MHz transducer. Left ventricular dimensions were obtained in M-mode, and ejection fraction (EF) and fractional shortening (FS) were calculated using standard echocardiographic formulas (see materials and Methods section). LV: left ventricle; s: systole; d: diastole. Statistical significance between the two groups was assessed using a two-tailed Student's T-test.

Supplementary Table S2. ECG post-MI

AAV9-ctrl+sham

Index	Unit	T88	T84	T91	T86	T130	T132	T129	Mean	SD
Diameter;s	mm	2.884243	2.174291	2.420905	2.855139	2.52089	2.442529	2.716092	2.57341271	0.25781438
Diameter;d	mm	4.193654	3.23743	3.697382	4.134774	3.663793	3.732184	3.947126	3.80090614	0.3263036
Volume;s	µL	31.784165	15.729847	20.601683	31.00242	22.78402	21.064201	27.415308	24.3402491	5.923411274
Volume;d	µL	78.297821	42.132578	58.028	75.722126	56.773603	59.343342	67.820953	62.5883461	12.4515089
Stroke Volume	µL	46.513656	26.402631	37.426317	44.719706	33.989583	38.279141	40.405645	38.248097	6.75749577
Ejection Fraction	%	59.406067	62.685596	64.496996	59.057647	59.868638	64.504525	59.576935	61.3680577	2.44796081
Fractional Shortening	%	31.22363	32.83898	34.523806	30.848124	31.199989	34.554968	31.18811	32.3538439	1.61929966
LV Mass Cor	mg	83.052796	67.886455	77.467001	82.553077	79.549878	79.228973	81.055742	78.684846	5.14451318
LVAW;s	mm	1.070532	1.131729	1.078403	1.079923	1.094253	1.152874	1.006322	1.08771943	0.04706941
LVAW;d	mm	0.645858	0.768204	0.733608	0.687896	0.742529	0.742529	0.742529	0.72330757	0.04179831
LVPW;s	mm	0.725484	0.850511	0.782296	0.70269	0.83046	0.791379	0.713218	0.77229114	0.05886619
LVPW;d	mm	1.159006	1.227754	1.276477	1.153881	1.240805	1.240805	1.143103	1.20597729	0.05282492

AAV9-ctrl+MI: 21 days

Index	Unit	A355	A378	A383	B167	B172	B166	B170	Mean	SD
Diameter;s	mm	2.688805	2.541412	2.273706	2.781364	3.076583	3.123111	3.274386	2.82433957	0.35540451
Diameter;d	mm	3.735303	3.772917	3.366674	4.083213	4.586541	4.640618	4.188451	4.05388814	0.46541038
Volume;s	µL	26.765752	23.252656	17.621826	29.358662	37.25405	38.662695	43.316401	30.8902917	9.21103725
Volume;d	µL	59.463547	60.913727	46.350882	73.546054	96.695083	99.362102	78.086205	73.4882286	19.66556
Stroke Volume	µL	32.697795	37.661071	28.729056	44.187391	59.441033	60.699407	34.769804	42.5979367	12.8445218
Ejection Fraction	%	54.987966	55.331906	43.755286	43.712154	30.190578	45.317535	44.52746	45.4032693	8.44807992
Fractional Shortening	%	27.989632	28.617889	21.535893	21.804514	14.397502	22.629024	21.823215	22.6853813	4.74099891
LV Mass Cor	mg	55.140796	72.531084	62.686825	84.575927	75.260221	101.121139	77.29776	75.5162503	14.8861899
LVAW;s	mm	0.846685	1.117058	1.085848	1.299378	0.939375	1.192616	0.90153	1.05464143	0.16521601
LVAW;d	mm	0.667447	0.720388	0.791167	0.800536	0.520252	0.764733	0.638788	0.700473	0.10011604
LVPW;s	mm	0.613969	0.82255	0.953475	1.045734	0.762313	0.984092	0.78967	0.85311471	0.14984686
LVPW;d	mm	0.496203	0.69881	0.670665	0.639313	0.599677	0.631246	0.660198	0.62773029	0.06570449

AAV9-shDEPTOR+MI: 21 days

Index	Unit	C178	C182	C190	C191	C193	C194	C196	C197	C201	C215	Mean	SD
Diameter;s	mm	2.677685	2.691978	2.485562	3.038806	2.709195	3.090343	3.344059	2.777353	2.788051	2.556187	2.8161219	0.26407156
Diameter;d	mm	3.771714	3.77395	3.453461	4.018562	3.788887	4.176637	4.354467	3.902352	3.804937	3.585307	3.8630374	0.26574924
Volume;s	µL	26.509529	26.821131	22.051828	36.126804	27.2601	37.638229	45.605883	29.047597	29.246451	23.66067	30.3968222	7.24553712
Volume;d	µL	60.860002	60.965488	49.27615	70.788527	61.526142	77.571672	85.56897	66.225268	62.233657	54.008468	64.9024344	10.7353675
Stroke Volume	µL	34.350472	34.144357	27.224321	34.661723	34.266042	39.933443	39.963088	37.177671	32.987206	30.347798	34.5056121	3.93321756
Ejection Fraction	%	56.441787	56.006042	55.248475	48.965171	55.693468	51.479415	46.70278	56.138197	53.005411	56.190815	53.5871561	3.4644494
Fractional Shortening	%	29.006141	28.689493	28.026319	24.380766	28.498181	26.008831	23.203948	28.828724	26.725418	28.648036	27.1996457	2.05752809
LV Mass Cor	mg	69.544064	56.179629	71.522399	71.878258	63.021306	77.899303	91.423716	60.887989	80.137841	79.944815	72.243932	10.5487464
LVAW;s	mm	0.908899	0.948888	1.268073	1.198765	1.0521	1.116739	1.081095	0.895841	1.073726	1.015187	1.0557313	0.12058433
LVAW;d	mm	0.718621	0.687887	0.938077	0.818656	0.779738	0.77555	0.769202	0.714744	0.982791	0.827883	0.8013149	0.09545409
LVPW;s	mm	0.820385	0.752892	0.853788	0.587688	0.732973	0.714903	0.820766	0.84294	0.884151	1.058366	0.8068852	0.12381606
LVPW;d	mm	0.654879	0.476625	0.62296	0.478679	0.486762	0.53629	0.636245	0.47076	0.528121	0.791494	0.5682815	0.10549009

One way ANOVA followed by Tukey's test

	Ctrl.Sham vs Control MI	Ctrl.Sham vs Deptor MI	Ctrl.MI vs Deptor MI
Diameter;s	ns	ns	ns
Diameter;d	ns	ns	ns
Volume;s	ns	ns	ns
Volume;d	ns	ns	ns
Stroke Vol	ns	ns	ns
Ejection Fraction	<0.0001	0.017	0.012
Fractional Shortening	<0.0001	0.0059	0.0159
LV Mass Cor	ns	ns	ns
LVAW;s	ns	ns	ns
LVAW;d	ns	ns	ns
LVPW;s	ns	ns	ns
LVPW;d	<0.0001	<0.0001	0.3284

Supplementary Table S2.

Echocardiographic parameters post-Myocardial infarction. Individual values, mean and SD for cardiac parameters quantified three weeks after sham operation or LAD occlusion, using a VisualSonics Vevo 3100LT ultrasound system equipped with a 30-MHz transducer. Left ventricular dimensions were obtained in M-mode, and ejection fraction (EF) and fractional shortening (FS) were calculated using standard echocardiographic formulas as described in the materials and methods section. LV: left ventricle; s: systole; d: diastole. Statistical significance between groups was assessed using one way ANOVA followed by Tukey's test. Data are available in the source data files.

Supplementary Table 3. Key reagents and resources.

Supplementary Table 3. Reagents and Resources						
Category	**Reagent / Resource**	**Target / Purpose**	**Supplier / Manufacturer**	**Catalogue number**	**Concentration / Dilution**	**Application**
TaqMan probe	Deptor	Deptor transcript	ThermoFisher	Rn01504430_m1	-	RT-qPCR
TaqMan probe	Gapdh	Gapdh transcript	ThermoFisher	Rn01775763_g1	-	RT-qPCR
Antibody	DEPTOR/DEPDC6 (D9F5)	DEPTOR	CST	11816	1:1000	WB
Antibody	Vimentin	Vimentin	abcam	ab45939	1:1000	WB
Antibody	αBIP	alpha-BIP	SCBT	sc-1051	1:3000	WB
Antibody	p-eIF2α	phospho-eIF2α	CST	9721	1:1000	WB
Antibody	ATF4	ATF4	CST	11815	1:1000	WB
Antibody	GADD153 (F-168) CHOP	CHOP	Santa Cruz Biotech.	SC575	1:1000	WB
Antibody	Caspase-3	Full length caspase 3	CST	9662	1:3000	WB
Antibody	Caspase-3 (8G10)	Caspase 3 cleaved	CST	9665	1:1000	WB
Antibody	BCL2	BCL2	BD biosciences	610538	1:500	WB
Antibody	LC3	LC3A/B	Abcam	ab128025	1:2000	WB
Antibody	ATG7	ATG7	CST	2631	1:1000	WB
Antibody	BNIP3	BNIP3	CST	3769	1:1000	WB
Antibody	P-4E-BP1 (T37/46)(23684)	P-4E-BP1 (T37/46)	CST	2855	1:1000	WB
Antibody	4E-BP1 (53H11)	4EBP1	CST	9644	1:1000	WB
Antibody	Akt1 (C-20)	AKT	Santa Cruz Biotech.	sc-1618	1:1000	WB
Antibody	Alfa-actinin (EA53)	alpha-actinin	SIGMA-ALDRICH	A7811	1:1000	WB
Antibody	P-p70 S6Kinase (T389)(108D2)	P-p70 S6Kinase (T389)	CST	9234T	1:1000	WB
Antibody	p70 S6 Kinase (E8K6T) XP®	p70 S6 Kinase	CST	34475	1:1000	WB
Antibody	Phospho-S473 Akt1	Phospho-S473 Akt1	CST	4060	1:1000	WB
Antibody	anti Rabbit IgG HRP	rabbit antibodies	SIGMA-ALDRICH	A0545	1:10000	WB
Antibody	anti Mouse IgG HRP	mouse antibodies	SIGMA-ALDRICH	A9044	1:10000	WB
Antibody	anti Goat IgG HRP	goat antibodies	SIGMA-ALDRICH	A5420	1:10000	WB
shRNA / Vector	Scrambled construct	Silencing control	SIGMA-ALDRICH	TRCN0000000001	-	Lentiviral transduction
shRNA / Vector	Deptor shRNA #1	Deptor silencing	SIGMA-ALDRICH	TRCN0000110158	-	Lentiviral transduction
shRNA / Vector	Deptor shRNA #2	Deptor silencing	SIGMA-ALDRICH	TRCN0000288335	-	Lentiviral transduction
shRNA / Vector	Atg7 shRNA #1	Atg7 silencing	SIGMA-ALDRICH	TRCN0000305991	-	Lentiviral transduction
shRNA / Vector	Atg7 shRNA #2	Atg7 silencing	SIGMA-ALDRICH	TRCN0000375444	-	Lentiviral transduction
shRNA / Vector	Bnip3 shRNA #1	Bnip3 silencing	SIGMA-ALDRICH	TRCN0000009690	-	Lentiviral transduction
shRNA / Vector	Bnip3 shRNA #2	Bnip3 silencing	SIGMA-ALDRICH	TRCN0000009691	-	Lentiviral transduction
Drug / Inhibitor	Rapamycin	mTORC1/inhibitor	MP Biomedicals	215934680	DMSO 1 mM stock	Pharmacological inhibition
Drug / Inhibitor	JR-AB2-011	RICTOR/mTORC2 inhibitor	Dismed	HY-122022-5MG	DMSO 10 mM stock	Pharmacological inhibition
Drug / Inhibitor	LY294002	PI3K/inhibitor	SIGMA-ALDRICH	440202-5MG	DMSO 20 mM stock	Pharmacological inhibition
Drug / Inhibitor	Thapsigargin 95%	SERCA pump/autophagy inducer	Fisher Scientific	J62866.M	DMSO 2.5 mM stock	Pharmacological inhibition
Drug / Inhibitor	3-methyladenine	PI3K (III)/autophagy inducer	ApexBio	A8353	HBSS 40mM stock	Pharmacological inhibition
Drug / Inhibitor	Chloroquine	lysosome/autophagy inducer	SIGMA-ALDRICH	C6628	PBS 50mM stock	Pharmacological inhibition
Deptor shRNA	AAV9-Tcf21-Deptor shRNA	*Deptor* knockdown	Vigene Biosciences	Gene ID : 97998	5.39×10^{13}vg/mL	AAV transduction
Control shRNA	AAV9-Tcf21-Control shRNA	Negative control	Vigene Biosciences	N/A	8.85×10^{13}vg/mL	AAV transduction