

Autophagy controls Nrf2-mediated dichotomy in pressure overloaded hearts

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Supplementary materials

Table S1. Primers for qPCR and genotyping

Primers	Gene access #	Forward (5'—3')	Reverse (5'—3')	Product
qPCR				
ANF	NM_008725.2	CATCACCTGGGCT TCTTCCT	TGGGCTCCAATCC TGTCAATC	405
BNP	NM_008726.4	GCGGCATGGATCTC CTGAAGG	CCCAGGCAGAGTC AGAAACTG	418
α -MHC	NM_010856.3	CCAATGAGTACCGC GTGAA	ACAGTCATGCCGG GATGAT	254
β -MHC	NM_080728.2	ATGTGCCGGACCTT GGAA	CCTCGGGTTAGCT GAGAGATCA	170
SERCA2a	NM_009722.3	CCATCTGCTTGTC ATGTCCT	CAAATGGTTTAGG AAGCGGTTACT	213
Nrf2	NM_010902.3	ATGATGGACTTGGA GTTGCC	TCCTGTTCTTCT GGAGTTG	200
NQO-1	NM_008706.5	CGGTATTACGATCC TCCCTCAACA	AGCCTCTACAGCA GCCTCCTTCAT	120
Agt	NM_007428.3	TTGTCTAGGTTGGC GCTGAA	GGGTGGATGTATA CGCGGTC	143
GAPDH	XM_001479322	ATGTTCCAGTATGA CTCCACTCAGC	GAAGACACCAAGTA GACTCCACGACA	171
Genotyping				
Myh6- MerCreMer +	Cre	AGGTGGACCTGATC ATGGAG	ATACCGGAGATCA TGCAAGC	440
	NW_001030719.1 (Int CTL)	GTAGGTGGAAATTC TAGCATCATCC	CTAGGCCACAGAA TTGAAAGATCT	324
floxedAtg5 (Atg5 ^{fl/fl})	flox	ACAACGTCGAGCAC AGCTGCGCAAGG	GTACTGCATAATG GTTTAACTCTTGC	700
	NT_039492.8 (WT)	GAATATGAAGGCAC ACCCCTGAAATG	GTACTGCATAATG GTTTAACTCTTGC	350
Nrf2 ^{-/-}	LacZ(KO)	TGGACGGGACTATT GAAGGCTG	GCGGATTGACCGT AATGGGATAGG	400
	NM-010902.3 (WT)	TGGACGGGACTATT GAAGGCTG	GCCGCCTTTTCAG TAGATGGAGG	734

Int CTL: internal control (interleukin-2); ANF, atrial natriuretic factor; BNP, B-type natriuretic peptide; α -MHC, alpha-myosin heavy chain; β -MHC, beta-myosin heavy chain; SERCA2a, sarcoplasmic reticulum calcium ATPase2a; NQO-1, NAD(P)H dehydrogenase, quinone-1; Agt, angiotensinogen.

Table S2. Echocardiography of MerCreMer ⁺ (Ctl), MerCreMer ⁺ ::Nrf2KO (Nrf2KO), MerCreMer ⁺ ::Atg5 ^{fl/fl} (Atg5KO), and MerCreMer ⁺ Atg5 ^{fl/fl} ::Nrf2KO (Duo-KO) mice at 2 weeks after TAC.								
	Ctl		Nrf2KO		Atg5KO		Duo-KO	
	Sham	TAC	Sham	TAC	Sham	TAC	Sham	TAC
(n)	(6)	(7)	(6)	(8)	(6)	(6)	(6)	(7)
IVS;d (mm)	0.83±0.11	1.12±0.10 ^A	0.82±0.12	1.19±0.11 ^A	0.84±0.11	1.17±0.14 ^A	0.84±0.05	1.17±0.11 ^A
LVID;d (mm)	3.56±0.23	4.04±0.18 ^A	3.60±0.29	4.24±0.25 ^A	4.02±0.23	4.49±0.17 ^{A,B}	3.70±0.27	4.06±0.19 ^{A,C}
LVPW;d (mm)	0.79±0.08	1.04±0.13 ^A	0.80±0.11	1.17±0.14 ^A	0.80±0.11	1.19±0.16 ^A	0.81±0.11	1.18±0.14 ^A
EF (%)	65.14±8.59	52.22±5.44 ^A	63.89±14.53	43.87±6.6 ^{A,B}	50.93±4.52	31.80±6.2 ^{A,B}	64.19±6.94	45.86±4.2 ^{A,C}
FS (%)	33.15±4.62	25.67±2.38 ^A	32.72±7.76	21.64±3.4 ^{A,B}	25.81±2.71	15.76±3.1 ^{A,B}	32.44±3.69	22.80±2.2 ^{A,C}
LVID;d, left ventricular internal dimension diastolic; LVPW;d, left ventricular posterior wall diastolic; FS, fractional shortening; EF, ejection fraction. BW, body weight; HW, heart weight; TIBIA, tibia length; LW, lung weight; TIBIA, tibia length; HW/TIBIA, heart weight/tibia length ratio; HW/BW, heart weight/body weight ratio; LW/TIBIA, lung weight/tibia length ratio.; LV, left ventricle; IVS, interventricular septum. ^A , p<0.05, TAC vs sham in the same group; ^B , p<0.05, vs. MerCreMer ⁺ TAC; ^C , p<0.05, vs. Atg5KO TAC;								

	Ctl		Nrf2KO		Atg5KO		Duo-KO	
	Sham	TAC	Sham	TAC	Sham	TAC	Sham	TAC
Echo (n)	(8)	(7)	(10)	(8)	(12)	(6)	(11)	(7)
IVS;d (mm)	1.18±0.12	1.35±0.12 ^A	28.65±2.10	1.16±0.12 ^{A,C}	0.92±0.16 ^B	1.03±0.11 ^C	0.75±0.09 ^{B,D}	1.07±0.10 ^A
LVID;d (mm)	3.67±0.44	4.06±0.43 ^A	3.72±0.36	3.80±0.32	4.16±0.24 ^B	4.93±0.24 ^{A,C}	3.72±0.21 ^D	4.00±0.40 ^E
LVPW;d (mm)	0.92±0.13	1.40±0.23 ^A	0.84±0.07	1.12±0.19 ^{A,C}	0.82±0.14	1.00±0.14 ^{A,C}	0.89±0.09	1.04±0.16 ^A
EF (%)	65.78±8.50	39.69±4.79 ^A	61.85±8.73	45.63±5.0 ^{A,C}	47.35±8.22 ^B	24.7±6.40 ^{A,C}	57.67±5.53 ^B	38.1±8.37 ^{A,E}
FS (%)	35.77±5.97	19.03±2.54 ^A	33.34±6.62	22.52±2.7 ^{A,C}	24.11±4.45 ^B	11.5±3.17 ^{A,C}	29.8±3.68 ^{B,D}	18.4±4.67 ^{A,E}
Pathology (n)	(8)	(7)	(10)	(8)	(12)	(6)	(11)	(7)
BW (g)	28.95±1.91	28.65±2.10	28.26±1.90	29.06±2.30	29.3±2.35	28.85±1.66	25.4±2.11 ^{B,D}	26.50±2.95
HW (mg)	140.0±15.1	238.57±24 ^A	136.0±10.7	197.5±24 ^{A,C}	172.5±20.9 ^B	270±20.9 ^{A,C}	120.9±15 ^{B,D}	201.4±16 ^{A,E}
LW (mg)	187.5±22.5	398.6±112 ^A	177.0±15.7	346.3±56.6 ^A	272.5±64.9 ^B	453.3±66.5 ^A	165.5±23.4 ^D	377.1±53 ^{A,E}
Tibia (mm)	18.05±0.42	17.66±0.52	17.95±0.30	17.76±0.34	18.17±0.34	18.00±0.21	17.89±0.31	17.97±0.29
HW/Tibia (mg/mm)	7.76±0.84	13.51±1.25 ^A	7.58±0.67	11.13±1.4 ^{A,C}	9.51±1.26 ^B	15.0±1.13 ^{A,C}	6.76±0.89 ^{B,D}	11.21±0.9 ^{A,E}
LW/Tibia (mg/mm)	10.39±1.18	22.61±6.46 ^A	9.86±0.90	19.52±3.26 ^A	14.98±3.53 ^B	25.19±3.73 ^A	9.26±1.40 ^D	21.01±3.1 ^{A,E}
Remodeling (n)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Mvocytes (µm ²)	207.1±37.6	403.2±59.8 ^A	206.4±43.1	325.2±56 ^{A,B}	286.1±52.7 ^B	438.8±65 ^{A,C}	202±31.6	332.6±61 ^{A,E}
Fibrosis (%)	3.40±1.83	23.85±6.72 ^A	3.3±1.9	18.9±4.9 ^{A,B}	12.75±4.24 ^B	28.45±6.2 ^{A,C}	7.4±3.5 ^D	23.1±5.9 ^{A,E}
αPCR (n)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
α-MHC	1.02±0.18	0.60±0.17 ^A	0.98±0.12	0.94±0.22 ^C	0.81±0.18 ^B	0.37±0.10 ^{A,C}	1.08±0.14 ^D	0.63±0.15 ^{A,E}
β-HMC	1.02±0.19	10.16±4.19 ^A	1.25±0.18 ^B	4.43±1.63 ^{A,C}	11.42±2.35 ^B	87.9±12.2 ^{A,C}	2.71±0.52 ^{B,D}	37.2±12.6 ^{A,E}
ANF	1.05±0.30	27.80±2.96 ^A	1.03±0.21	14.4±5.6 ^{A,C}	23.46±5.92 ^B	47.51±12 ^{A,C}	1.35±0.13 ^{B,D}	19.6±3.56 ^{A,E}
BNP	1.03±0.25	5.42±1.56 ^A	1.03±0.24	3.25±0.86 ^{A,C}	4.22±1.30 ^B	10.8±1.9 ^{A,C}	2.35±0.54 ^{B,D}	5.85±0.89 ^{A,E}
SERCA2α	1.02±0.20	0.54±0.09 ^A	0.98±0.21	0.71±0.15 ^{A,C}	0.65±0.15 ^B	0.38±0.09 ^{A,C}	1.06±0.19 ^D	0.72±0.23 ^{A,E}

^A, p<0.05, TAC vs sham in the same group; ^B, p<0.05, vs. Ctl sham; ^C, p<0.05, Ctl TAC; ^D, p<0.05, vs. Atg5KO sham; ^E, p<0.05 vs. Atg5KO TAC