Supporting information

Supplemental Experimental procedures

Real-time qPCR

Total RNA was extracted from embryonic, postnatal, and adult hearts or NCM and subjected to qRT-PCR, as described previously (1-3)

DNA Isolation and Quantification

Total DNA was prepared from heart tissues or NCM according to standard procedures (2) and digested with 100 µg/ml RNase A for 30 min at 37°C. The relative copy numbers of mitochondrial and nuclear genomes were determined by quantitative PCR with primers specific to the *CoxII* (mitochondrial) and *Dio3* (nuclear) genes (2).

Subcellular fractionation

Cytosolic, mitochondrial, sarcomeric reticulum, and nuclear fractions were isolated with differential centrifugation as described previously (1) by using mouse heart tissues (10-wk-old male). Briefly, ventricular tissues were suspended in homogenizing buffer (10 mM Tris-HCl, pH 8.0, 300 mM sucrose, 1 mM EDTA, 1 mM EGTA) supplemented with protease inhibitors (1 mM PMSF, 2 g/ml aprotinin, 5 g/ml pepstatin A, 10 g/ml leupeptin), homogenized using a Dounce homogenizer, and centrifuged at 600 *g* for 10 min to generate a crude nuclear pellet and a supernatant containing the cytosol, Mito, and SR. The supernatant was then centrifuged at 5,000 *g* for 10 min, giving rise to a pellet (crude mitochondrial fraction) and a supernatant with the crude cytosolic and SR fraction. The crude Mito fraction was washed with homogenizing buffer and centrifuged again at 7,000 *g* for 10 min; the pellet was designated as the mitochondrial fraction. The supernatant from the 5,000 *g* centrifugation was centrifuged again at 14,000 *g* for 20 min; the resulting supernatant was subjected to ultracentrifuge at 14,300 *g* for 100 min using a Beckman Type 45Ti rotor. The resulting supernatant from ultracentrifuge was designated as the cytosolic fraction and the pellet was designated as SR fraction. The first crude nuclear fraction pellet was further washed with Buffer A (20 mM Tris-HCl, pH 8.0, 1.5 mM MgCl2, 10 mM KCl) containing 0.5% (v/v) Nonidet P-40 and centrifuged at 1,000 *g* for 10 min. Nuclear proteins were extracted from the pellet by incubation with Buffer B (20 mM Tris-HCl, pH 8.0, 10% (v/v) glycerol, 0.5 M NaCl, 1.5 mM MgCl2, I mM EDTA, 1 mM EGTA) supplemented with protease inhibitors. The extracted proteins were cleared by centrifugation at 14,000 *g* for 20 min; the final supernatant was designated as the nuclear fraction. All procedures were conducted at 4 °C.

Isolated fractions were subjected to SDS-PAGE and Western blot by using anti-GAPDH, anti-COX, anti-SERCA2a, and anti-Lamin A/C antibodies to determine the purity of the cytosolic, Mito, SR, and nuclear fractionation, respectively.

Western blot

Heart tissues or NCM were homogenized in lysis buffer containing 20 mM Tris-HCl pH 7.8, 150 mM NaCl, 1 mM Na_3VO_4, 5 mM EDTA, 1 % Triton X-100, 5 μl/ml protease inhibitor cocktail (P8340, Sigma, St Louis, MO, USA), and 20 μg/ml phenylmethylsulfonyl fluoride. Protein lysates were resolved by SDS-PAGE and transferred to nitrocellulose membrane (Hybond C Extra; Amersham Biosciences). Western blotting was performed using the antibodies as described previously (1).

Construction of expression vectors

Oligonucleotides used to construct expression vectors are listed in **Supplemental Table 1**. A full-length fragment and different isoforms of mouse Perm1 were amplified by PCR using mouse heart cDNA as a template. PCR products were cloned into the pcDNA3 plasmid vector with EcoRI and XbaI sites. The following constructs were generated:

Plasmid A - Perm1 from exon1 to stop codon;

Plasmid B - Perm1 from exon1 with 2nd AUG mutated to GCG (Alanine);

Plasmid C - Perm1 from 1st AUG without a Kozak sequence;

Plasmid D - Perm1 from 2nd AUG without Kozak sequence;

Plasmid E - Perm1 from 1st AUG with a Kozak sequence (ACC) adjacent to 1st AUG sequence;

Plasmid F - Perm1 from 2nd AUG with a Kozak sequence (ACC). A Methionine (AUG) to Alanine (GCG) point mutation was inserted into Plasmid B, as described in the results section, using previously published methods (4). Primers used for cloning are listed in **Table S3**.

All plasmids were sequenced before use to verify DNA sequence fidelity.

ERRE reporter assay.

U2OS cells were transfected with $ERRE^{ERRa}$-Luc reporter plasmid (40 ng/well) (4), pCMV-beta-galactosidase (10 ng/well), and expression vectors for ERRα, ERRβ, ERRγ, PGC-1α (5), Perm1, or pcDNA control (5-40 ng/well), using PEI MAX™ (Polysciences, Warrington, PA, USA). Total concentrations of plasmids in each experiment were adjusted by the addition of control plasmid vector. Relative luciferase activities were determined as described previously (4).

HEK293 cell culture

For immunoprecipitation *in vitro*, HEK293 cells were transfected with expression vectors for HA-PGC-1a and FLAG-tagged Perm1 (N-terminus Flag-tag with a Kozak sequence inserted ahead of the start codon of FLAG sequence) (1) or control vector (pcDNA3), using PEI MAX™ (Polysciences, Warrington, PA, USA) for immunoprecipitation. Forty-eight hours following transfection, cells were lysed, and lysates were subjected to immunoprecipitation with a FLAG antibody (Clone M2, Sigma, St Louis, MO, USA) (6). Immunoprecipitated proteins were detected by Western blot using appropriate antibodies. HEK293 cells were also transfected with various isoforms of Perm1 using PEI. After 48 hrs, the cells were harvested with the lysis buffer described above. Total protein was subjected to Western blot using anti-PERM1 and GAPDH antibodies.

In vivo immunoprecipitation

Twelve-wk old wild type male mouse whole ventricular tissues were lysed, and lysates were subjected to immunoprecipitation with an anti-PERM1 antibody. Immunoprecipitated proteins were detected by Western blot using appropriate antibodies.

Antibodies.

The antibodies used for Western blotting and ChIP assay are the following : anti-FLAG (Clone M2; Sigma-Aldrich, St Louis, MO, USA); anti-PERM1 (anti-PERM1; Sigma-Aldrich, St Louis, MO, USA); anti-GAPDH (MAB374, Sigma-Aldrich, St Louis, MO, USA); anti-SEARCA2a (2A7-A1, Thermo Fisher, Waltham, MA, USA); anti-Lamin A/C (2032, Cell Signaling Technology, Danvers, MA, USA); anti-COX (11967, Cell Signaling Technology, Danvers, MA, USA); anti-Rt/Ms Total OxPhos Complex Kit (Thermo Fisher, Waltham, MA, USA); anti-ERRα (ab76228; Abcam, Boston, MA, USA); anti-PGC-1α (NBP-04676, NOVUS, Centennial, CO, USA); Horseradish peroxidase-conjugated anti-mouse or anti-rabbit secondary antibodies were purchased from BioRad (Hercules, CA, USA) (1).

Supplemental References

1. Cho, Y., Hazen, B. C., Russell, A. P., and Kralli, A. (2013) Peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1)- and estrogen-related receptor (ERR)-induced regulator in muscle 1 (Perm1) is a tissue-specific regulator of oxidative capacity in skeletal muscle cells. *The Journal of biological chemistry* **288**, 25207-25218

2. Cho, Y., Hazen, B. C., Gandra, P. G., Ward, S. R., Schenk, S., Russell, A. P., and Kralli, A. (2016) Perm1 enhances mitochondrial biogenesis, oxidative capacity, and fatigue resistance in adult skeletal muscle. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **30**, 674-687

3. Garcia-Ruiz, I., Solis-Munoz, P., Fernandez-Moreira, D., Grau, M., Colina, F., Munoz-Yague, T., and Solis-Herruzo, J. A. (2014) High-fat diet decreases activity of the oxidative phosphorylation complexes and causes nonalcoholic steatohepatitis in mice. *Dis Model Mech* **7**, 1287-1296

4. Cho, Y., Noshiro, M., Choi, M., Morita, K., Kawamoto, T., Fujimoto, K., Kato, Y., and Makishima, M. (2009) The basic helix-loop-helix proteins differentiated embryo chondrocyte (DEC) 1 and DEC2 function as corepressors of retinoid X receptors. *Molecular pharmacology* **76**, 1360-1369

5. Gantner, M. L., Hazen, B. C., Conkright, J., and Kralli, A. (2014) GADD45gamma regulates the thermogenic capacity of brown adipose tissue. *Proceedings of the National Academy of Sciences of the United States of America* **111**, 11870-11875

6. Cho, Y., Tachibana, S., Hazen, B. C., Moresco, J. J., Yates, J. R., 3rd, Kok, B., Saez, E., Ross, R. S., Russell, A. P., and Kralli, A. (2019) Perm1 regulates CaMKII activation and shapes skeletal muscle responses to endurance exercise training. *Molecular metabolism* **23**, 88-97

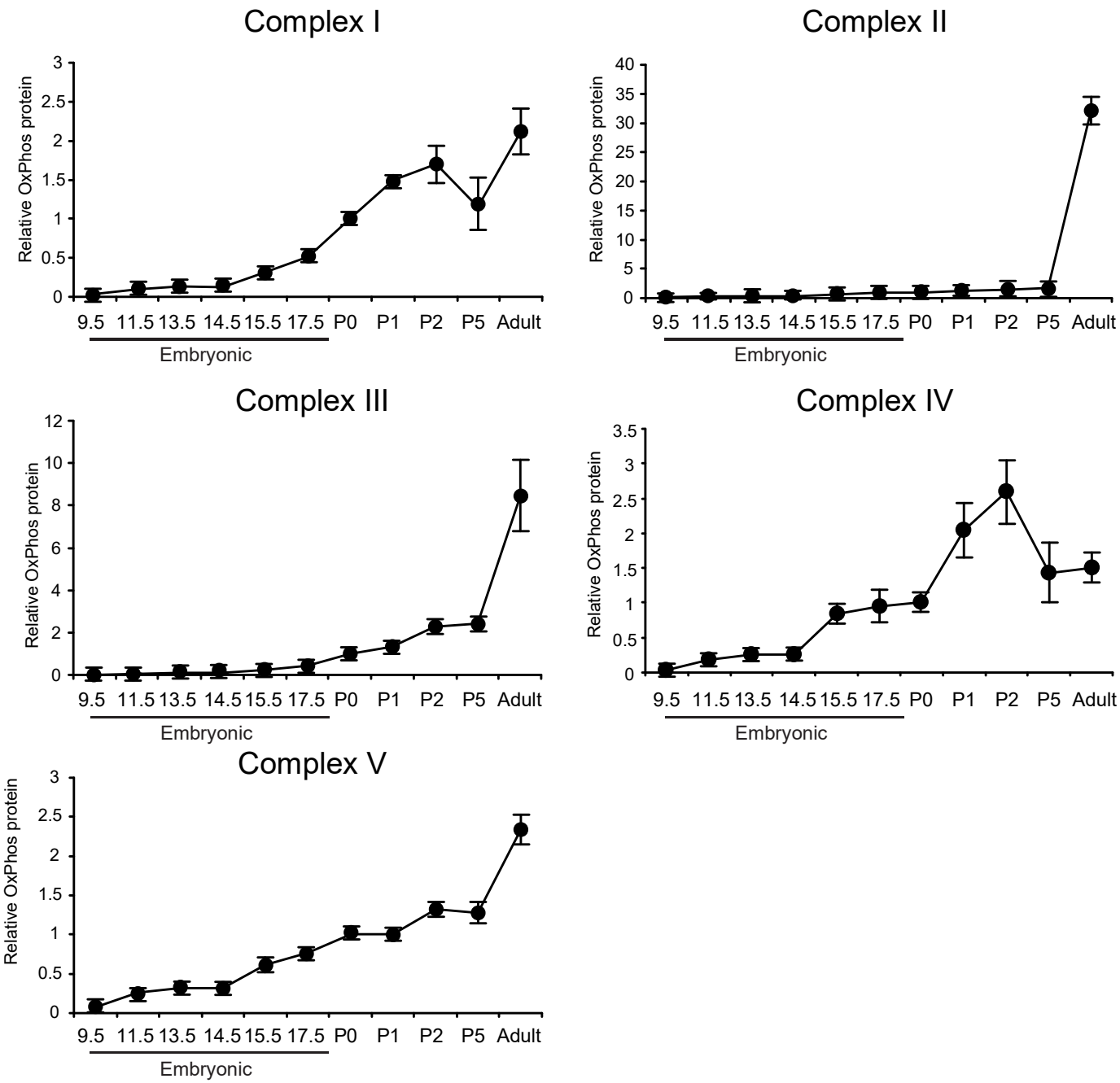

Figure S1. Quantification of OxPhos protein levels from different stages of cardiac development.

Total protein was extracted from cardiac tissues obtained from embryos at varied embryonic stages (determined from timed matings), from neonatal pups, and also from adult mice. Each Oxphos protein was quantified based on Western blot density, normalized to GAPDH, and expressed relative to P0 heart. Data are the mean ± SD (n = 4). (Whole heart tubes were used at E9.5, while ventricular tissue was used for protein extraction at later stages.)

Fig. S1

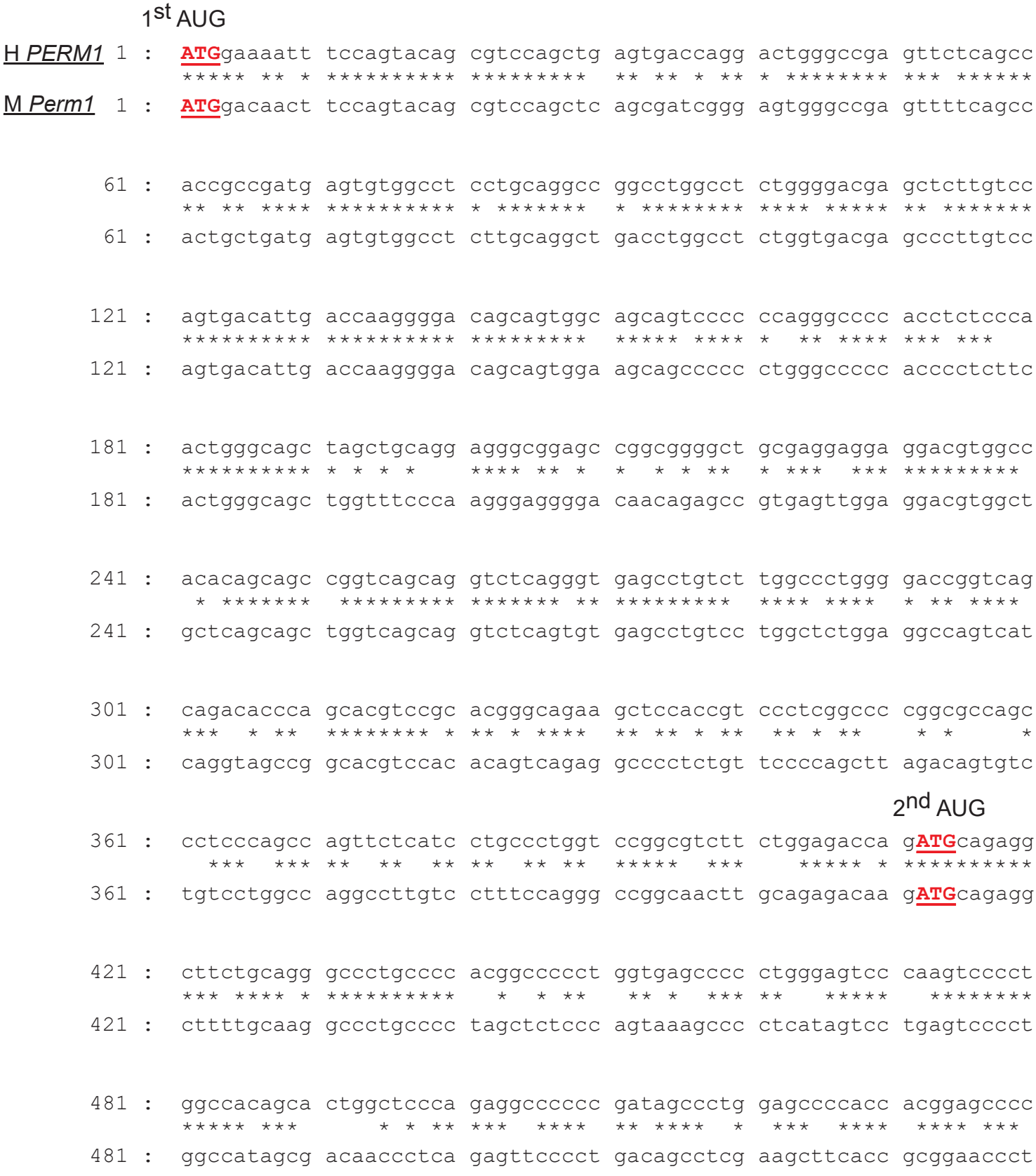

Figure S2. cDNA alignment of Human (H) and Mouse (M) *PERM1*. cDNA sequences from the start codon (1st AUG) to 540 bp of human and mouse *PERM1* were aligned. The conserved 1st and 2nd AUGs are highlighted.

Fig. S2

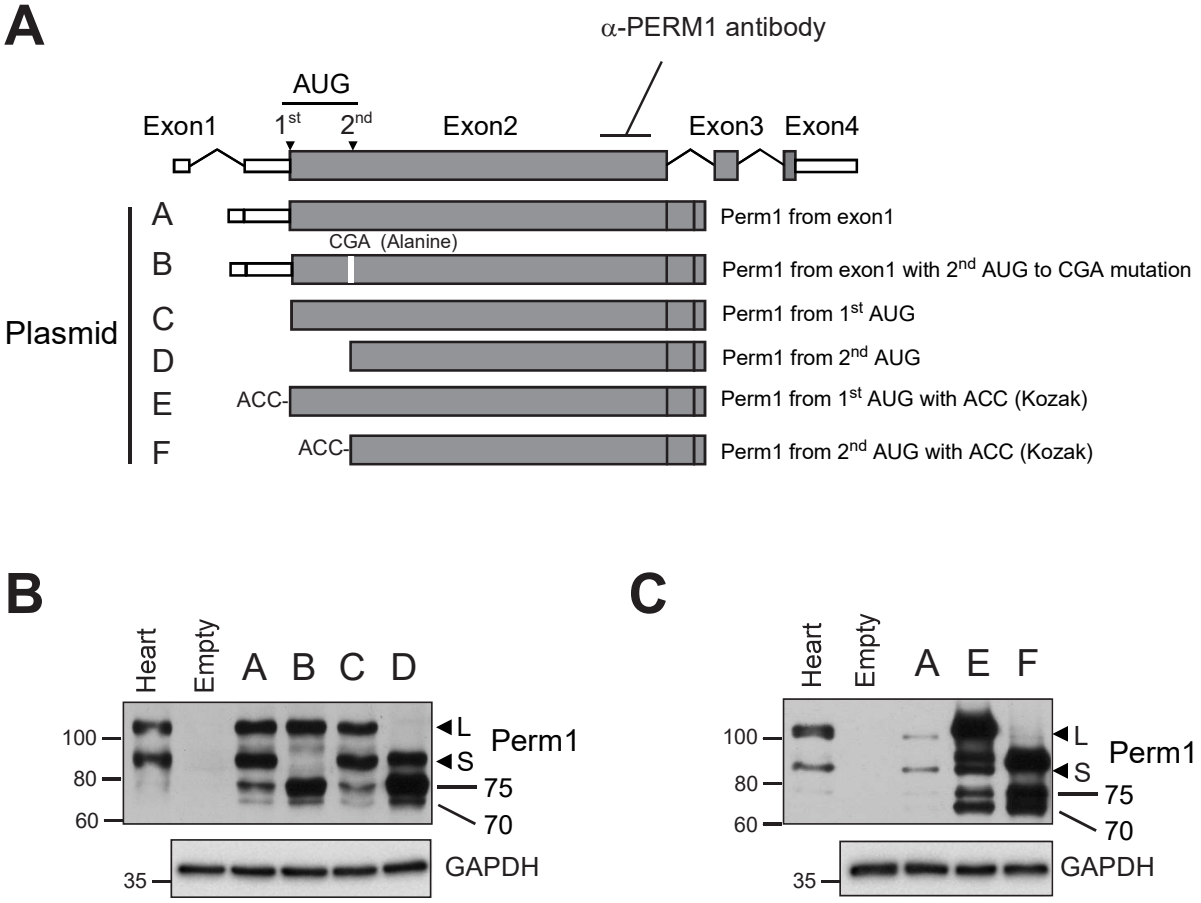

Figure S3. An internal translational initiation site leads to production of the short isoform of Perm1.

(A) Schematic of Perm1 fragments derived from mouse Perm1 cDNAs. Each cDNA fragment was cloned into the pcDNA3 vector.

(B and C) Each plasmid (above) was transfected into HEK293 cells. Forty-eight hours following transfection, cells were harvested, and total cell protein was subjected to Western blot with anti-PERM1 and GAPDH antibodies. L, long; S, short; 70, 70 kDa Perm1; 75, 75 kDa Perm1. Heart = whole heart tissue lysates; Empty = no plasmid; A-F above each lane refers to plasmid construct.

Fig. S3

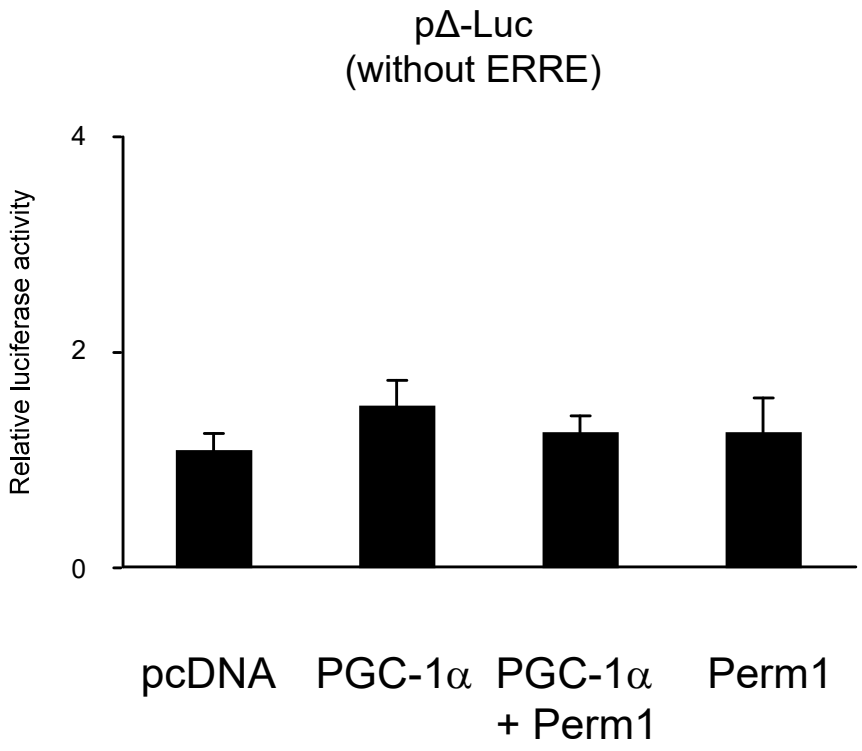

Figure S4. Perm1 and PGC-1a do not affect luciferase activity produced from a reporter plasmid lacking ERRE.

U2OS cells were transfected with the control LUC reporter (40 ng), pΔ-LUC (the identical vector, but without an ERRE), together with pcDNA3 control, PGC-1α (5 ng), and Perm1 (40 ng) plasmids, as indicated on the figure graph. Data are the mean ± SD, and are displayed as the mean of eight experimental replicates, from two representative experiments. No significant differences were detected between groups.

Fig. S4

	Sham	TAC
BW (g)	28.186 ± 2.68	27.18 ± 1.77
HR (bpm)	527.80 ± 13.57	538.00 ± 21.69
IVSd (mm)	0.73 ± 0.02	0.70 ± 0.02
LVPWd (mm)	0.66 ± 0.04	0.90 ± 0.13*
LVIDd (mm)	3.3 ± 0.16	4.46 ± 0.45**
LVIDs (mm)	2.03 ± 0.34	3.68 ± 0.51*
EF (%)	69.84 ± 11.58	36.20 ± 8.41**
FS (%)	39.01 ± 8.50	17.16 ± 4.76**

Table S1. Echocardiographic parameters of mice 8-wk post TAC or Sham surgeries

Ventricular dilation (LVIDd and LVIDs), increased posterior wall thickness (LVPWd) and reduction of function (as measured by ejection fraction (EF) and fractional shortening (FS) was evident in the mice subject to 8 weeks of transverse aortic constriction (TAC) surgery.

BW= body weight; HR= heart rate; IVSd= interventricular septal thickness in diastole; LVPWd= left ventricular posterior wall thickness in diastole; LVIDd= left ventricular internal dimension in diastole; LVIDs= left ventricular internal dimension in systole; EF=Ejection fraction; FS=Fractional shortening. *, $P<0.05$; **, $P<0.01$ vs. Sham. n=5. Data are presented as mean ± SD.

Table S1

Primers used for ChIP assays

Gene	Forward	Reverse
Sirt3 ERRE	GGAACGCAGGGAGTCTAGAT	ACGAAGAAGTGAGCTCCGAG
Ckmt2 ERRE	TGGCCCACCGTTTCTTATTA	CGACAGACTCCTCCAGCTTC
Esrrα Distal ERRE (negative control region)	GGTGGTCCCTGTGCTCACAT	GCTGCAGGGGCACACTGTAT

Table S2. List of PCR primers used for ChIP assays in **Fig. 7**.

Table S2

Primers used for cloning different Perm1 isoforms

Plasmid	Forward	Reverse
A (Perm1 from exon1)	tataGAATTCAGGCCTCTAGATGATAGCTCCA	tataTCTAGA**CTA**GCAGCTGGGGTTTGAGCTG
C (Perm1 from 1st AUG)	tataGAATTC**ATG**GACAACTTCCAGTACAGCGT	tataTCTAGA**CTA**GCAGCTGGGGTTTGAGCTG
D (Perm1 from 2nd AUG)	tataGAATTC**ATG**CAGAGGCTTTTGCAAGG	tataTCTAGA**CTA**GCAGCTGGGGTTTGAGCTG
E (Perm1 from 1st AUG with ACC)	tataGAATTC*ACC* **ATG**GACAACTTCCAGTACAGCGT	tataTCTAGA**CTA**GCAGCTGGGGTTTGAGCTG
F (Perm1 from 2nd AUG with ACC)	tataGAATTC*ACC* **ATG**CAGAGGCTTTTGCAAGG	tataTCTAGA**CTA**GCAGCTGGGGTTTGAGCTG

Plasmid A - Perm1 from exon1 to stop codon;
Plasmid B - Perm1 from exon1 with 2nd AUG mutated to GCG (Alanine);
Plasmid C - Perm1 from 1st AUG without a Kozak sequence;
Plasmid D - Perm1 from 2nd AUG without Kozak sequence;
Plasmid E - Perm1 from 1st AUG with a Kozak sequence (ACC) adjacent to 1st AUG sequence;
Plasmid F - Perm1 from 2nd AUG with a Kozak sequence (ACC).

Table S3. List of PCR primers used for cloning different Perm1 isoforms in **Fig. S3**.

Table S3