Supporting Information for

PERM1 Gene Therapy Prevents Pressure Overload-induced Heart Failure Through a Sarcomere-Mitochondria Energetic Microdomain

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Table S1. Echocardigraphy of AAV-GFP- and AAV-PERM1-treated hearts in sham and TAC (8 weeks)

Parameter	GFP-SHAM	GFP-TAC	PERM1-SHAM	PERM1-TAC	p-value for effect of Vector	significance for effect of Vector	p-value for effect of Surgery	significance for effect of Surgery	p-value for Interaction	Interaction significance	GFP-SHAM vs GFP-TAC p-value	GFP-SHAM vs GFP-TAC significance	PERM1-SHAM vs PERM1-TAC p-value	PERM1-SHAM vs PERM1-TAC significance	GFP-SHAM vs PERM1-SHAM p-value	GFP-SHAM vs PERM1-SHAM significance	GFP-TAC vs PERM1-TAC p-value	GFP-TAC vs PERM1-TAC significance
LV Diameter systole (mm)	2.27 ± 0.30	3.92 ± 0.89	2.33 ± 0.36	2.56 ± 0.60	0.021	*	0.001	**	0.002	**	<0.001	***	0.788	ns	0.995	ns	0.002	**
LV Diameter diastole (mm)	3.62 ± 0.27	4.62 ± 0.52	3.70 ± 0.26	3.86 ± 0.50	0.1	ns	0.004	**	0.009	**	0.002	**	0.821	ns	0.969	ns	0.016	*
Stroke Volume (ul)	37.46 ± 4.84	29.02 ± 13.07	39.14 ± 4.07	40.00 ± 6.85	0.053	ns	0.347	ns	0.098	ns	0.234	ns	0.993	ns	0.959	ns	0.067	ns
Ejection Fraction (%)	68.05 ± 5.52	32.57 ± 20.03	67.59 ± 7.59	63.31 ± 10.45	0.008	**	<0.001	***	0.001	**	<0.001	***	0.829	ns	1	ns	<0.001	***
Fractional Shortening (%)	37.36 ± 4.46	15.99 ± 10.77	37.24 ± 5.95	34.29 ± 7.60	0.015	*	0.002	**	0.003	**	<0.001	***	0.814	ns	1	ns	0.002	**
Cardiac Output (ml/min)	18.74 ± 2.47	14.35 ± 6.67	19.60 ± 2.29	20.09 ± 2.63	0.041	*	0.33	ns	0.078	ns	0.191	ns	0.989	ns	0.954	ns	0.047	*
LV Mass Corrected (mg)	104.0 ± 28.6	177.4 ± 56.9	106.4 ± 22.	124.9 ± 37.2	0.159	ns	0.007	**	0.055	ns	0.013	*	0.668	ns	0.999	ns	0.088	ns
LVAW systole (mm)	1.51 ± 0.18	1.52 ± 0.13	1.39 ± 0.17	1.51 ± 0.27	0.357	ns	0.316	ns	0.496	ns	1	ns	0.612	ns	0.654	ns	1	ns
LVAW diastole (mm)	1.01 ± 0.18	1.17 ± 0.15	0.94 ± 0.16	1.01 ± 0.21	0.157	ns	0.149	ns	0.556	ns	0.528	ns	0.837	ns	0.872	ns	0.522	ns
LVPW systole (mm)	1.33 ± 0.23	1.06 ± 0.12	1.40 ± 0.25	1.40 ± 0.34	0.099	ns	0.359	ns	0.212	ns	0.404	ns	1	ns	0.952	ns	0.189	ns
LVPW diastole (mm)	0.90 ± 0.26	0.93 ± 0.23	0.96 ± 0.26	1.01 ± 0.29	0.526	ns	0.665	ns	0.93	ns	0.997	ns	0.977	ns	0.97	ns	0.964	ns
Mitral E/A ratio	1.50 ± 0.23	1.37 ± 0.11	1.44 ± 0.20	1.52 ± 0.17	0.787	ns	0.979	ns	0.197	ns	0.735	ns	0.834	ns	0.929	ns	0.625	ns

Results of 2-way ANOVA performed on factors of Vector (GFP vs. PERM1) and Surgery (SHAM vs. TAC) using Python script presented in Dataset S1.
Post-hoc pairwise comparisons are performed using Tukey HSD test.
LV: left ventricle; LVAW: left ventricular anterior wall; LVPW: left ventricular posterior wall;
E/A ratio: the ratio of peak velocity of the blood flow caused by LV relaxation in early diastole (the E wave) to peak velocity flow caused by atrial contraction in late diastole (the A wave).
Significance thresholds: *p < 0.05, **p < 0.01, ***p < 0.001.
ns: not significant.

Supplemental Results

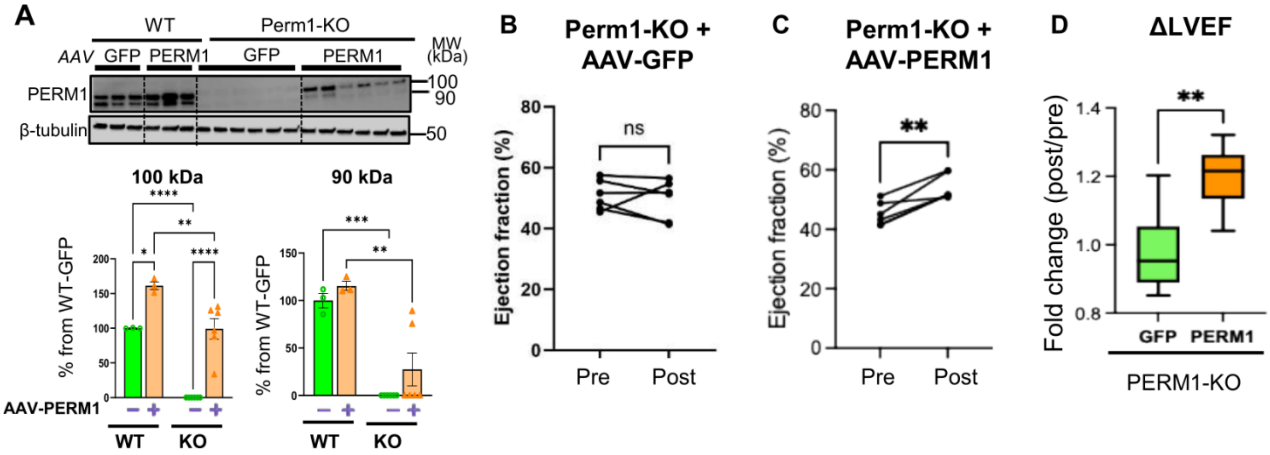

Figure S1. AAV-PERM1 enhances systolic function in PERM1-KO mice. A, Western blot analysis shows the partial restoration of PERM1 expression in the heart, more predominantly with the 100-kDa PERM1 expression levels than 90-kDa isoform. **B-C,** Left ventricular ejection fraction (LVEF), showing that AAV-PERM1 enhances EF significantly in PERM1-KO mice, while there was no change by AAV-GFP (control). **D,** Delta LVEF shows the significant increase of systolic function in Perm1-KO mice by AAV-PERM1. *Student's t*-test; **: p<0.01, *: $p < 0.05$; ns, not significant.

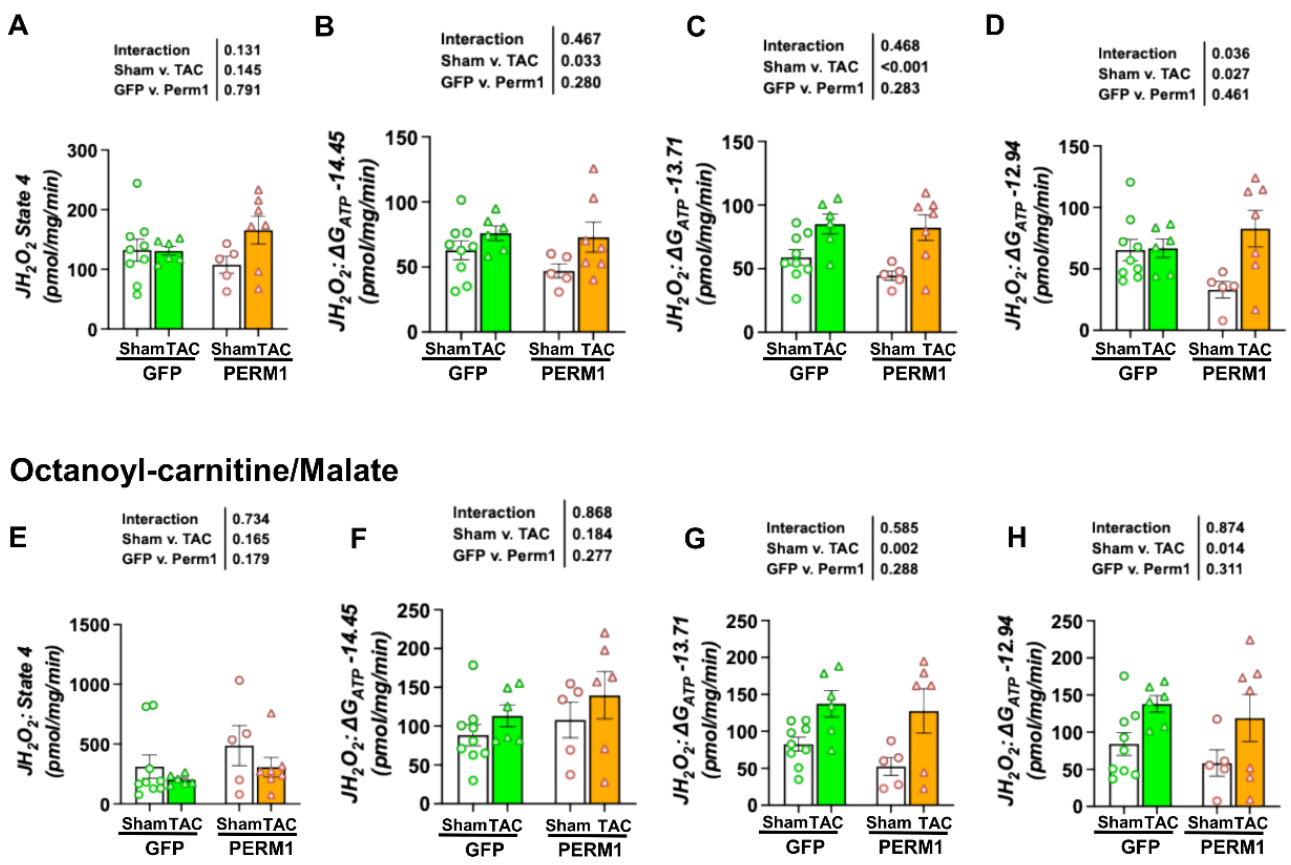

Figure S2. Mitochondrial H_2O_2 emission (JH_2O_2, **A** and **E**) and corresponding electron leak measured at three energetic states (ΔG_{ATP} = −14.45, −13.71, and −12.94 kcal mol−1) during respiration supported by pyruvate/malate (**B-D**) and octanoylcarnitine/malate (**F-H**).

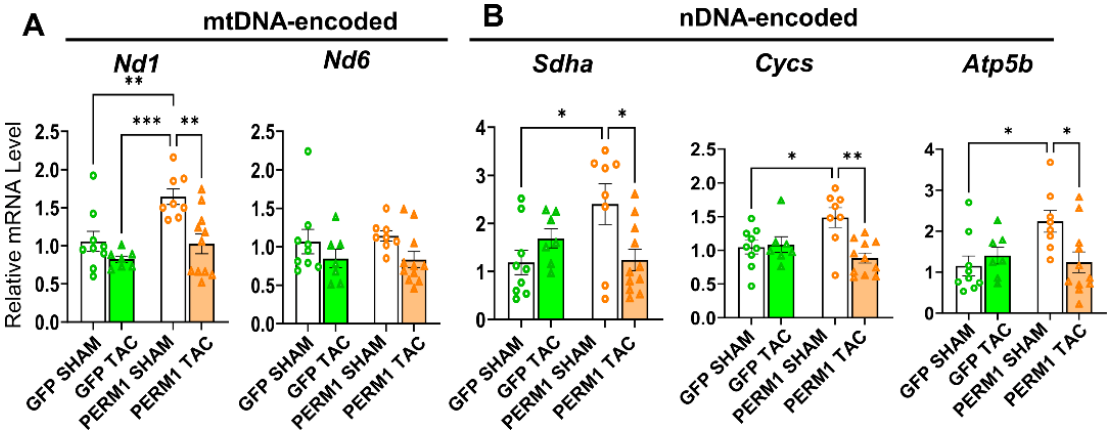

Figure S3. qPCR analysis of mitochondrial DNA (mtDNA)-encoded (**A**) and nuclear DNA (nDNA)-encoded (**B**) genes involved in the electron transport chain (ETC). Pressure overload did not significantly alter the mRNA expression of these ETC genes. AAV-PERM1 significantly increased the transcript levels of ND1, SDHA, CYCS, and ATP5B under basal conditions; however, the expression of these genes remained downregulated in AAV-PERM1-treated TAC hearts. *: p<0.05, **: p<0.01, ***:p<0.001, by two-way ANOVA.

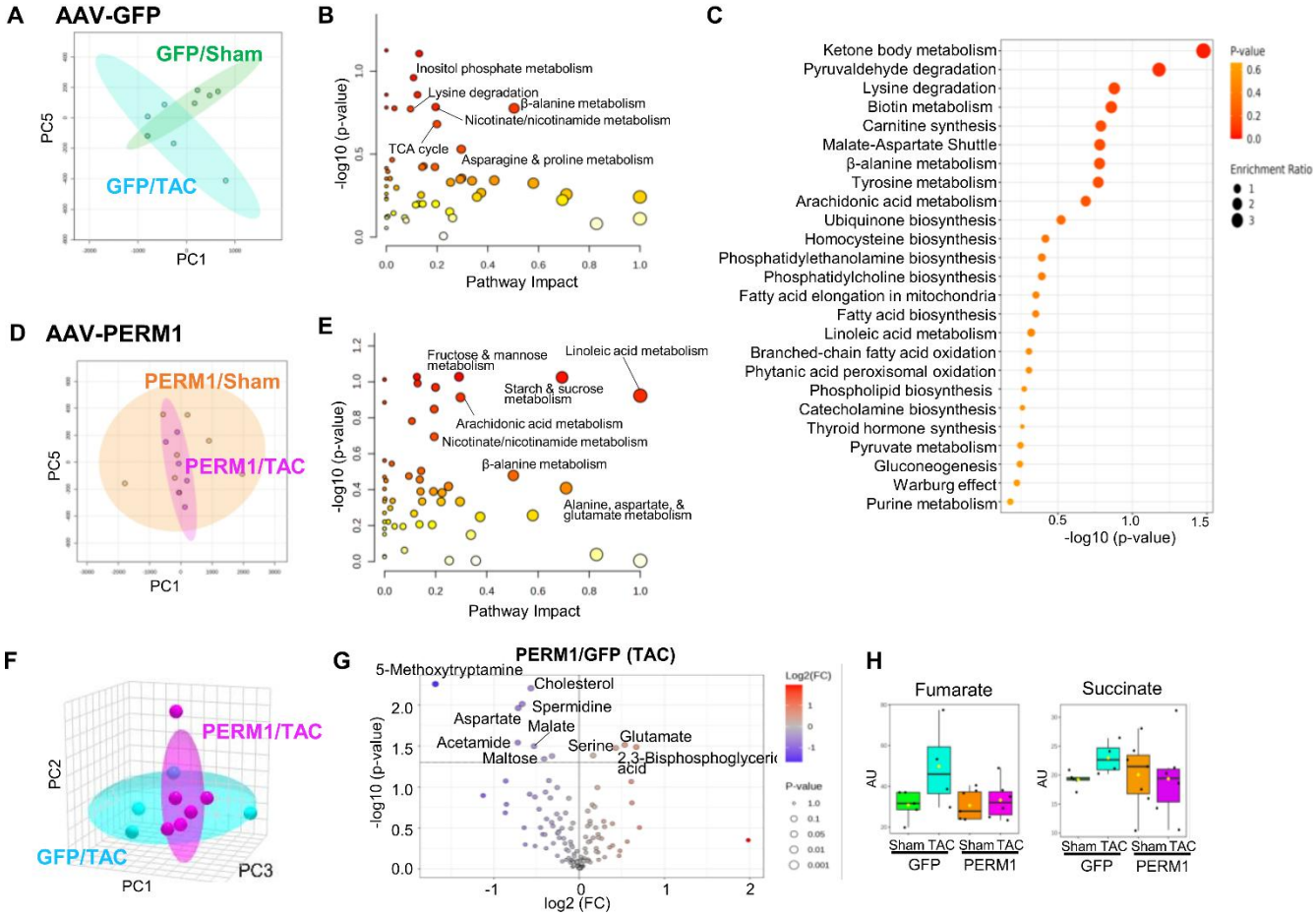

Figure S4. Metabolomic profiling of AAV-GFP and AAV-PERM1 hearts subjected to Sham or TAC. A-C, Metabolomic profiles of AAV-GFP Sham and AAV-GFP TAC hearts. The principal component analysis (PCA) plot shows a distinct separation between TAC and Sham groups, indicating altered metabolomic profiles in response to TAC (**A**, $p<0.05$). Pathway analysis (**B**) and enrichment analysis (**C**) highlight specific metabolic pathways affected by TAC. **D-E**, Comparison of AAV-PERM1 Sham and AAV-PERM1 TAC hearts. The PCA plot shows no significant separation between the two groups (**D**, $p>0.05$), suggesting that AAV-PERM1 preserves a metabolomic profile in TAC hearts comparable to Sham. **F-H**, Comparison of AAV-GFP TAC and AAV-PERM1 TAC hearts. A distinct separation in metabolomic profiles is observed (**F**). TAC-induced accumulation of TCA cycle intermediates (malate, fumarate, succinate) and spermidine is attenuated in AAV-PERM1 hearts (**G-H**).

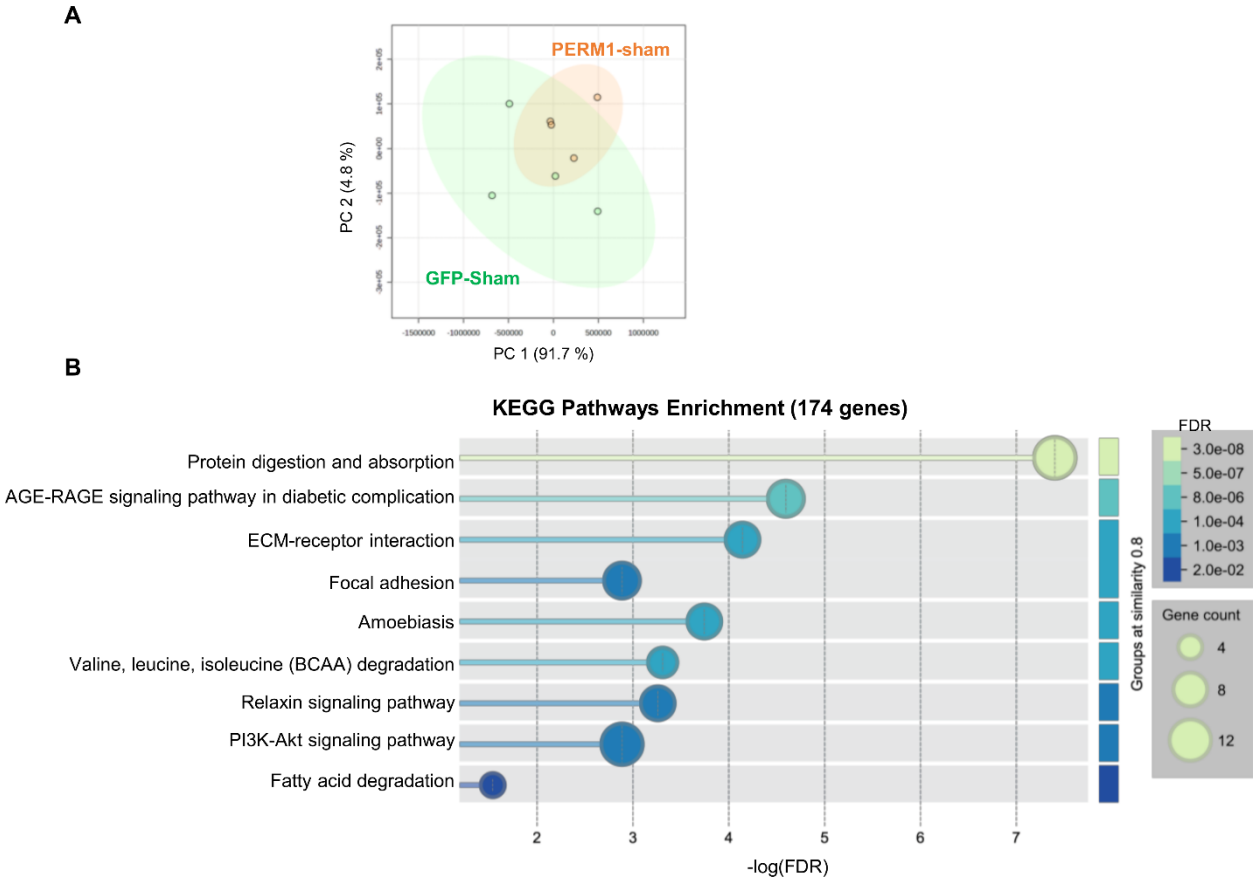

Figure S5. (**A**) PCA plot showing the similar profile of transcripts expressed in AAV-GFP-sham and AAV-PERM1-sham hearts, indicating that AAV-PERM1 alone did not alter transcriptome. (**B**) KEGG pathway enrichment analysis of 174 genes commonly altered by pressure overload in both AAV-GFP and AAV-PERM1-treated hearts, as identified by Venn analysis shown in Figure 5L of the main text. (see Dataset 6 for the complete gene list and pathway annotations).

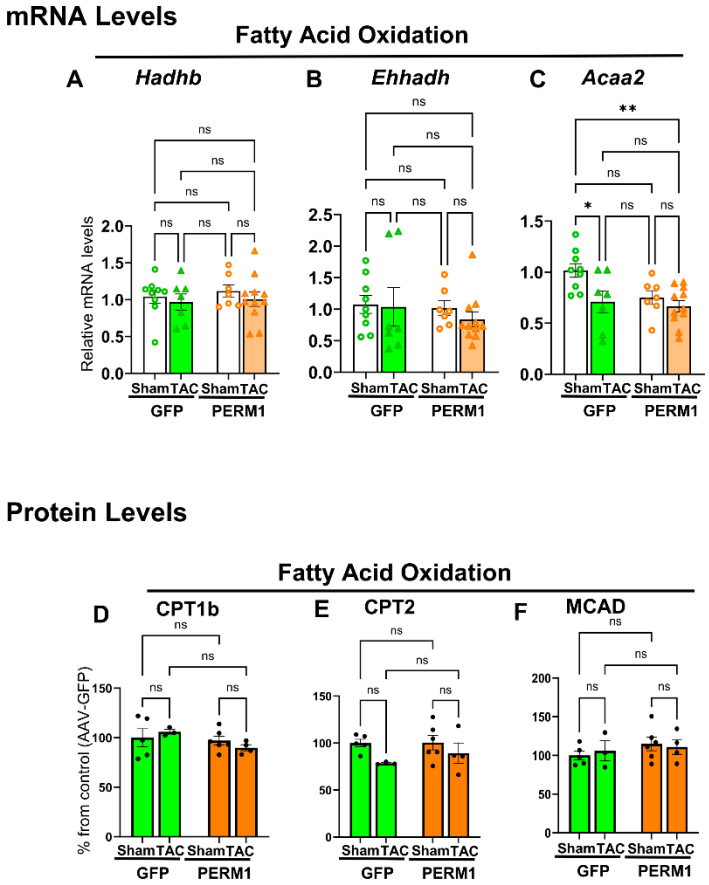

Figure S6. Expression levels of genes (**A-C**) and proteins (**D-H**) involved in fatty acid oxidation in AAV-GFP- and AAV-PERM1-treated hearts, analyzed by qPCR and western blotting assays, respectively. *: p<0.05, **: p<0.01, ***: p<0.001, ns: not significant by two-way ANOVA.

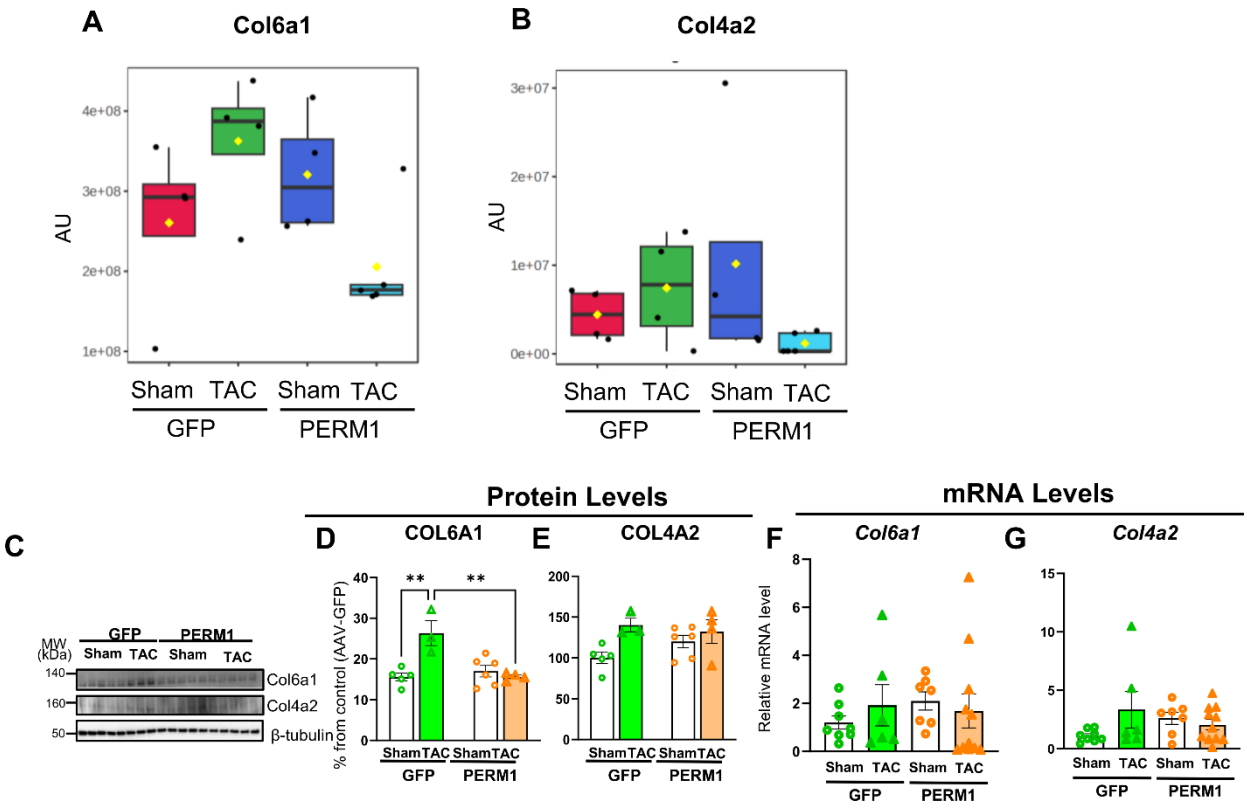

Figure S7. AAV-PERM1 increases Col6a1 and Col4a2 expression in the pressure-overloaded heart. A-B, Box-Whisker plots showing the prominent changes in Col6a1 and Col4a2 protein expression by TAC and AAV-PERM1, derived from proteomics analysis. **C-E**, Western blot analysis of Col6a1 and Col4a2. **F-G**, Relative mRNA levels of Col6a1 and Col4a2 measured by qPCR. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

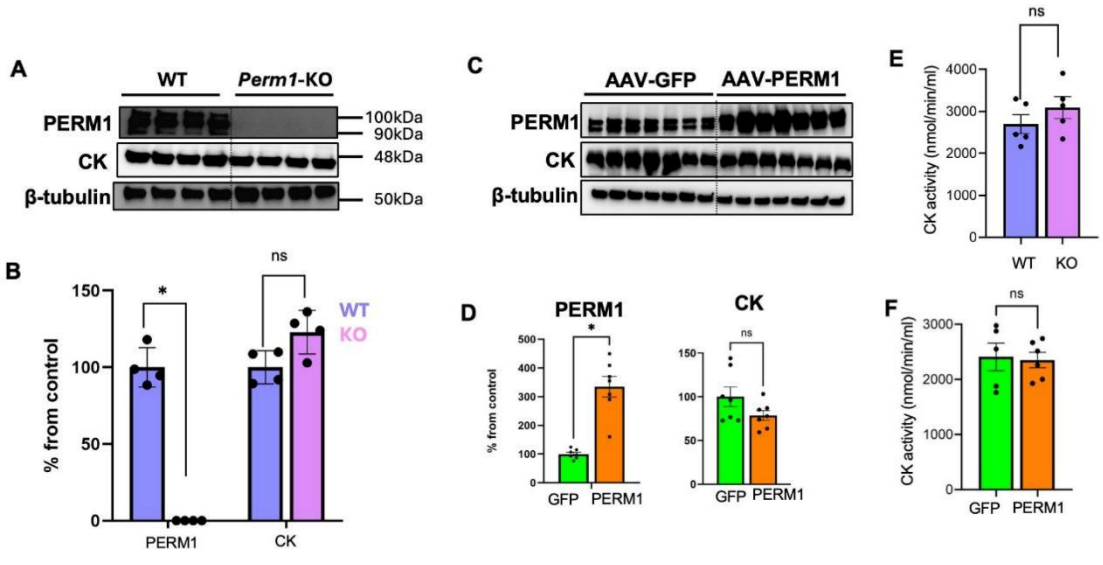

Figure S8. (**A-B**) Western blot analysis shows no change in creatine kinase expression in *Perm1*-KO hearts. (**C-D**) AAV-mediated PERM1 overexpression does not alter creatine kinase expression levels. (**E-F**) Creatine kinase activity assays show no significant change in enzymatic activity in *Perm1*-KO or AAV-PERM1 hearts. *Student's t-*test; $p<0.05$; ns, not significant.