Supporting Information

Impact of stress on cardiac phenotypes in mice harboring an ankyrin-B disease variant

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Running Title: Impact of stress on the penetrance of ankyrin-B syndrome

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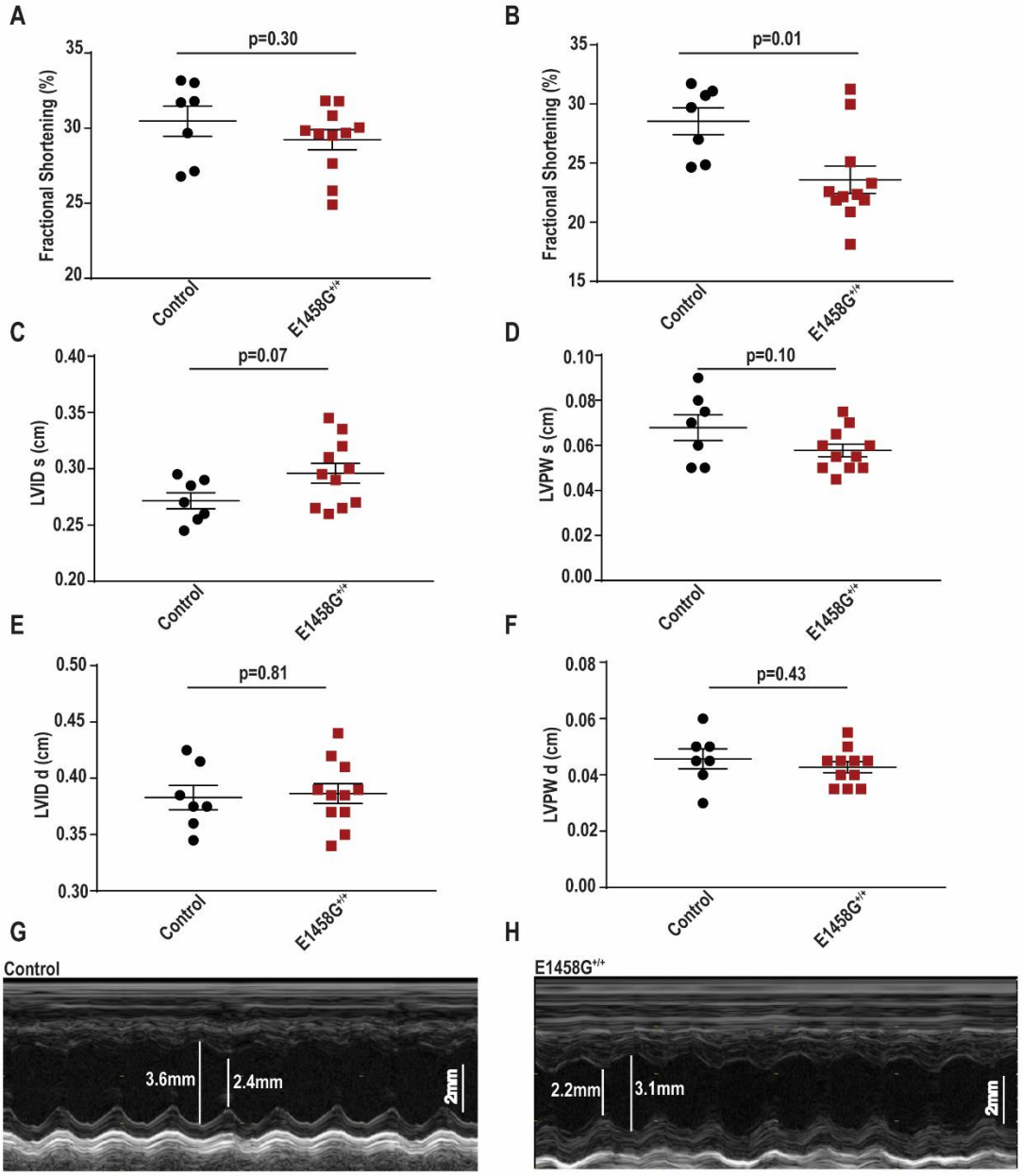
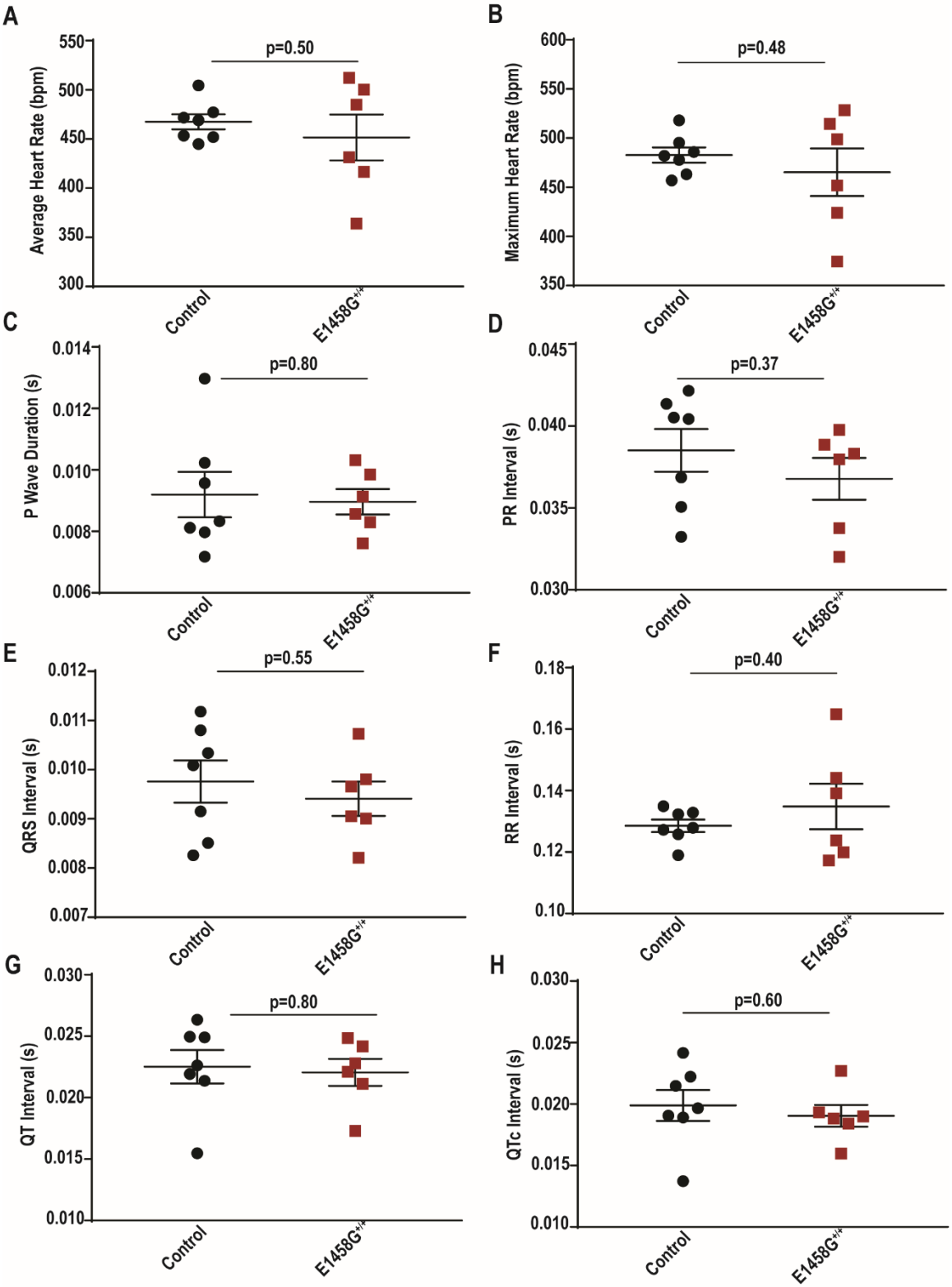


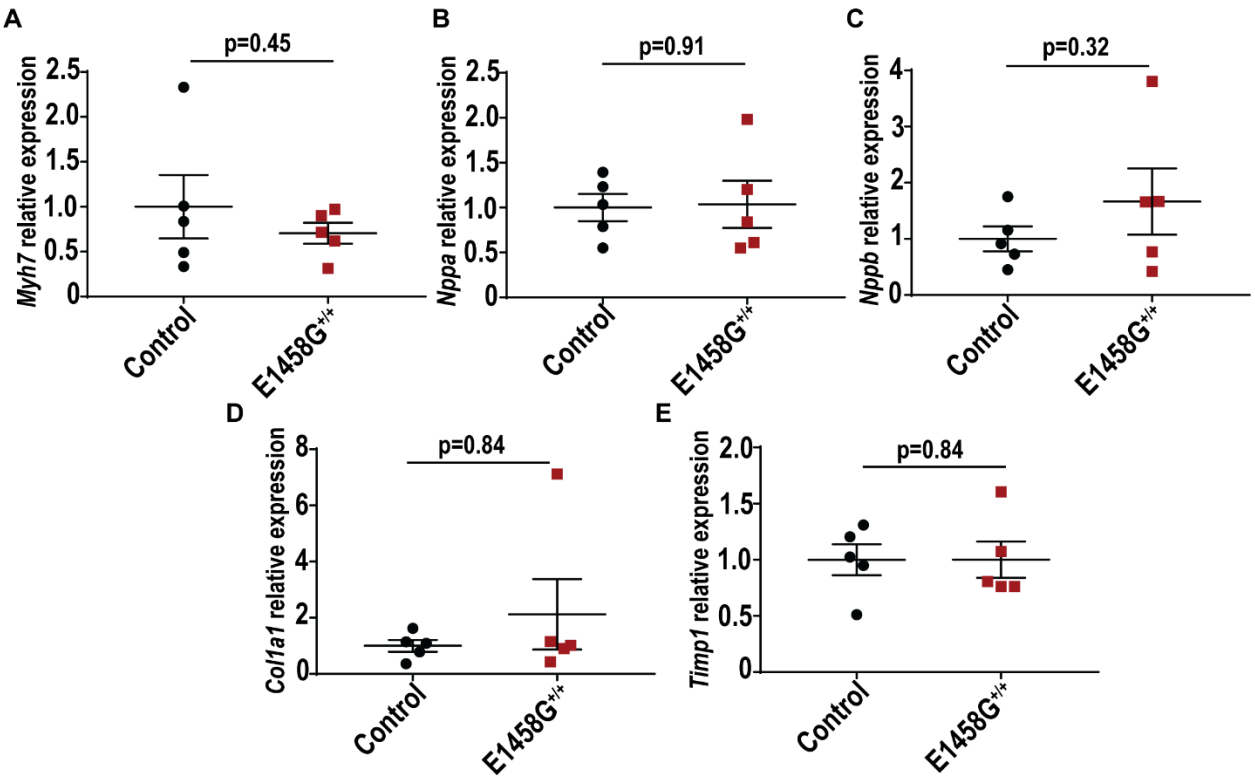
Suppl. Figure 1. AnkBp.E1458G$^{+/+}$ mice do not display an early structural phenotype. A. Control and AnkBp.E1458G$^{+/+}$ mice do not display changes in fractional shortening around three months of age (Con, N=7 and AnkBp.E1458G$^{+/+}$, N=11). Data passed Shapiro-Wilk normality test and unpaired t-test was performed. **B.** AnkBp.E1458G$^{+/+}$ mice display a significant reduction in fractional shortening around six months of age (Con, N=7 and AnkBp.E1458G$^{+/+}$, N=11). Data passed Shapiro-Wilk normality test and unpaired t-test was performed. **C-D** AnkBp.E1458G$^{+/+}$ mice display a trending increase in left ventricle internal diameter (LVID) and a trending decrease in left ventricle posterior wall (LVPW) during systole around six months of age (Con, N=7 and AnkBp.E1458G$^{+/+}$, N=11). Data passed Shapiro-Wilk normality tests and unpaired t-tests were performed. **E-F.** No significant changes in the left ventricle internal diameter (LVID)

and left ventricle posterior wall thickness (LVPW) during diastole around six months of age (Con, N=7 and AnkBp.E1458G$^{+/+}$, N=11). Data passed Shapiro-Wilk normality tests and unpaired t-tests were performed**. G-H.** Representative echocardiographs denoting no changes in cardiac function in the mice carrying the human AnkBp.E1458G variant and the control littermates around three months of age (Con, N=7 and AnkBp.E1458G$^{+/+}$, N=11), Scale bars equal 2mm.

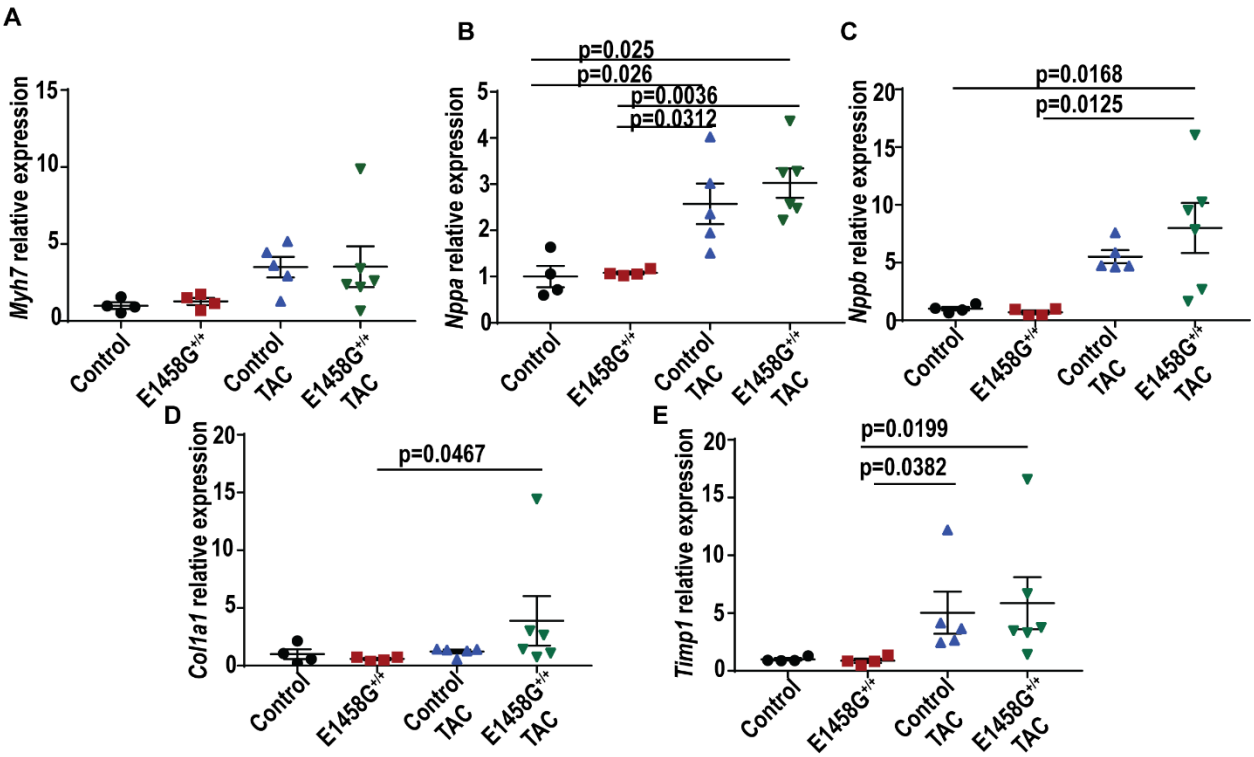


Suppl. Figure 2. AnkBp.E1458G$^{+/+}$ mice do not display electrical changes in subsurface ECG around three months of age. A. Average Heart rate**, B.** Maximal heart

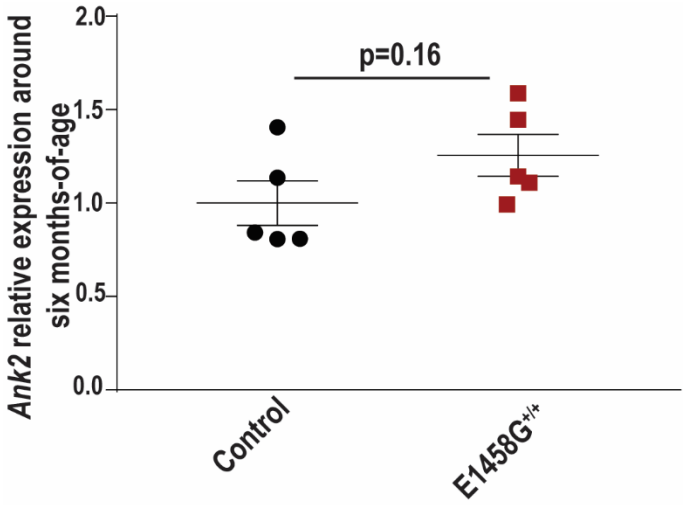
rate, **C.** P Wave Duration, **D.** PR Interval, **E.** QRS Interval, **F.** RR Interval, **G.** QT Interval and **H.** QTc (Mitchell) (Con, N=7 and AnkBp.E1458G$^{+/+}$, N=6). All data passed Shapiro-Wilk normality tests and unpaired t-tests were performed**.**



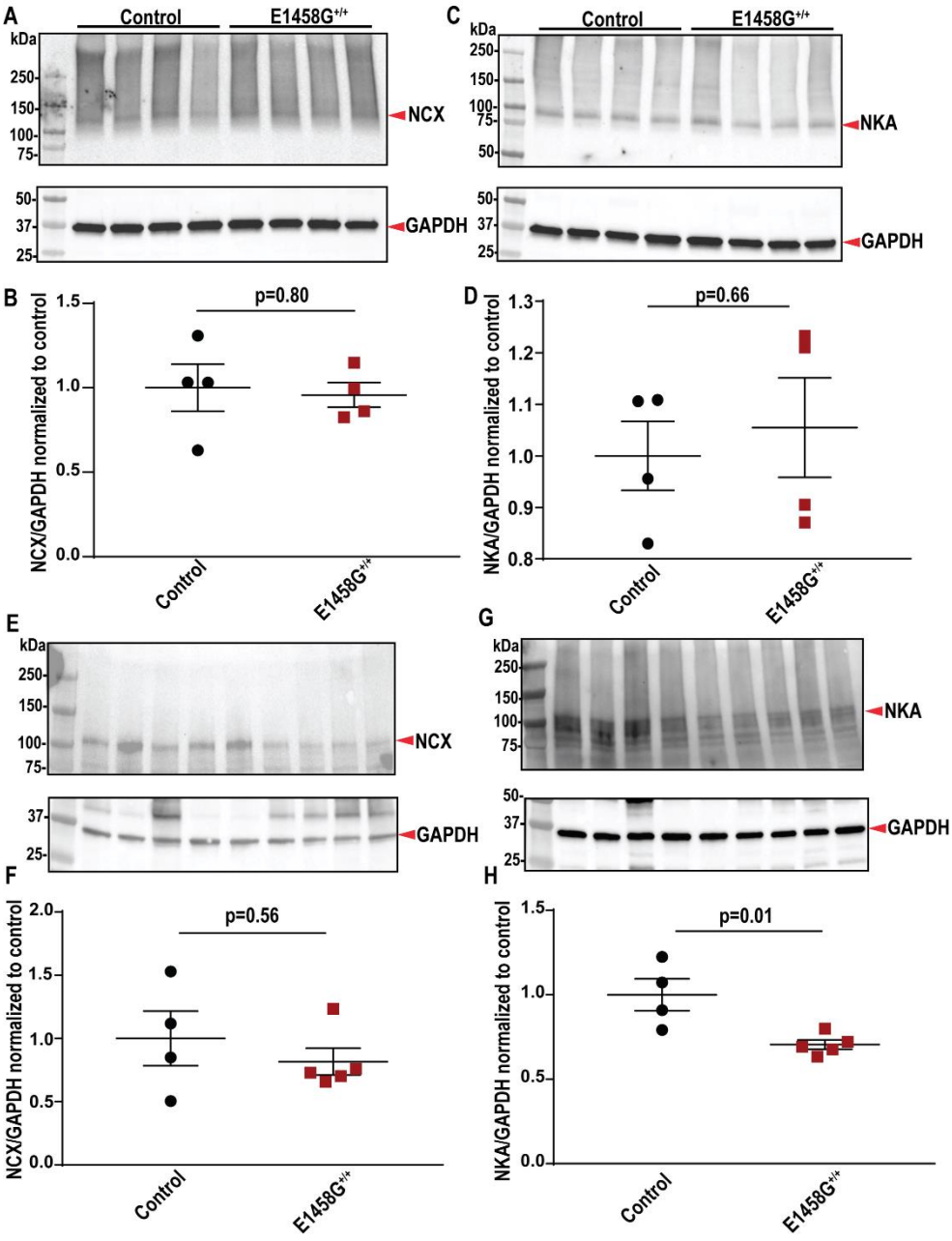
Suppl. Figure 3. No changes in hypertrophic gene expression markers; (A) myosin heavy chain 7 (*Myh7*), (B) atrial natriuretic peptide (*Nppa*), (C) brain natriuretic peptide (*Nppb*), (D) Collagen type I alpha 1 chain (*Col1a1)* and (E) Tissue inhibitor matrix metalloproteinase 1 (*Timp1)* in WT and AnkBp.E1458G$^{+/+}$ mice at ~six months of age (N=5/ genotype). Data passed Shapiro-Wilk normality tests and unpaired t-tests were performed (A-C). Data did not pass Shapiro-Wilk normality tests and Mann-Whitney tests were performed (D-E).



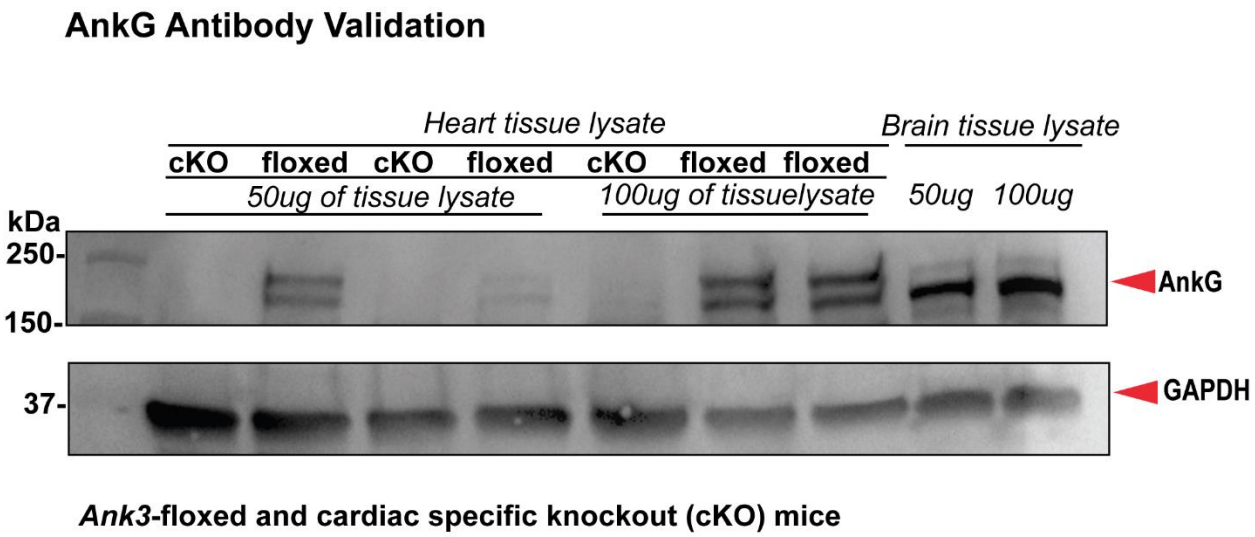
Suppl. Figure 4. Hypertrophic gene expression markers: (A) myosin heavy chain 7 (*Myh7*), data did not pass Shapiro-Wilk normality test and Kruskal-Wallis test was performed followed by Dunn's multiple comparisons test. (B) atrial natriuretic peptide (*Nppa*), data passed Shapiro-Wilk normality test and ANOVA test was performed followed by Tukey's multiple comparisons test. (C) brain natriuretic peptide (*Nppb*), data passed Shapiro-Wilk normality test and ANOVA test was performed followed by Tukey's multiple comparisons test. (D) Collagen type I alpha 1 chain (*Col1a1),* data did not pass Shapiro-Wilk normality test and Kruskal-Wallis test was performed followed by Dunn's multiple comparisons and (E) Tissue inhibitor matrix metalloproteinase 1 (*Timp1)*, data did not pass Shapiro-Wilk normality test and Kruskal-Wallis test was performed followed by Dunn's multiple comparisons test. WT and AnkBp.E1458G$^{+/+}$ hearts were used at baseline (~2-3 months of age) and 4 weeks post TAC (N=4-5/group).



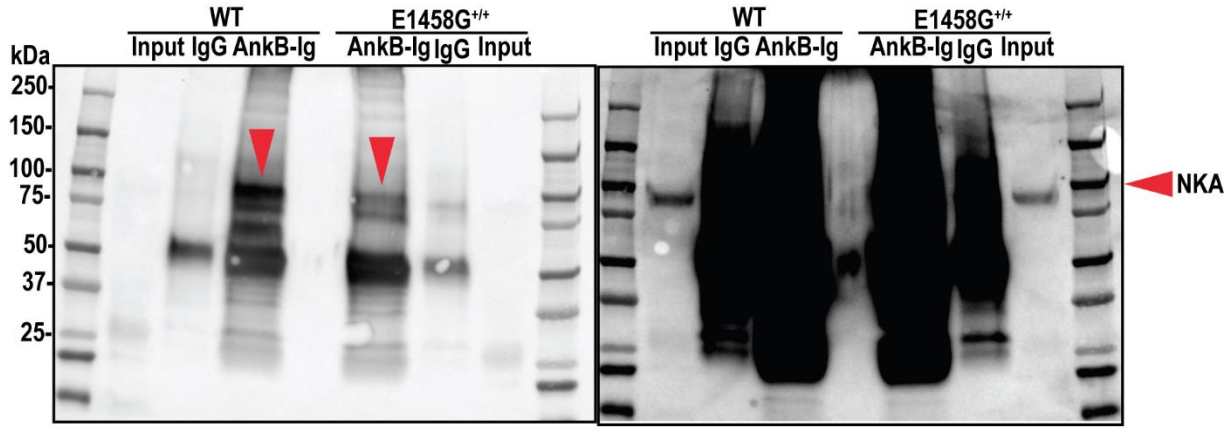
Suppl. Figure 5. *Ank2* relative expression in control and AnkBp.E1458G$^{+/+}$ mice around six months of age (N=5 mice/genotype). Data passed Shapiro-Wilk normality test and unpaired t-test was performed**.**



Suppl. Figure 6. Expression of ankyrin-B and ankyrin membrane partners in the AnkBp.E1458G$^{+/+}$ heart lysates in young mice at baseline (A-D) and 4 weeks post TAC (E-H). A-B. Immunoblotting and quantitative analysis of Na^+/Ca^{2+} exchanger (NCX) normalized to GAPDH. **C-D.** Immunoblotting and quantitative analysis of Na^+, K^+-ATPase (NKA) normalized to GAPDH. Data passed Shapiro-Wilk normality tests and unpaired t-test were performed (B-D). **E-F.** Immunoblotting and quantitative analysis of Na^+/Ca^{2+} exchanger (NCX) normalized to GAPDH (data did not pass Shapiro-Wilk normality test and Mann-Whitney test was performed). **G-H.** Immunoblotting and quantitative analysis of Na^+, K^+-ATPase (NKA) normalized to GAPDH (data passed Shapiro-Wilk normality test and unpaired t-test was performed). Con and AnkBp.E1458G$^{+/+}$, N=4.



Suppl. Figure 7. Validation of AnkG antibody using floxed and *Ank3*-cardiac specific Knockout (KO).



Suppl. Figure 8. Immunoprecipitation experiments illustrating the interaction between AnkB-Ig or IgG control and NKA using control and AnkBp.E1458G$^{+/+}$ lysates. Data are representative of experiments repeated three times.

Suppl. Table 1. Summary of echocardiographic parameters in control and the AnkBp.E1458G$^{+/+}$ mice at ~ six months of age.

Mouse	Ejection Fraction (%)	Fractional Shortening (%)	LVIDs (cm)	LVIDd (cm)	LVPWs (cm)	LVPWd (cm)	IVSs (cm)	IVSd (cm)
E1458G$^{+/+}$1	51.355	22.155	0.29	0.37	0.075	0.055	0.055	0.05
E1458G$^{+/+}$2	51.675	22.355	0.3	0.385	0.05	0.05	0.075	0.06
E1458G$^{+/+}$3	43.64	18.15	0.345	0.42	0.045	0.035	0.055	0.035
E1458G$^{+/+}$4	65.985	31.25	0.265	0.385	0.065	0.045	0.065	0.045
E1458G$^{+/+}$5	48.97	20.87	0.31	0.39	0.06	0.035	0.055	0.035
E1458G$^{+/+}$6	52.19	22.585	0.27	0.35	0.05	0.045	0.055	0.04
E1458G$^{+/+}$7	56.455	25.115	0.295	0.39	0.055	0.035	0.06	0.045
E1458G$^{+/+}$8	64.195	29.975	0.26	0.37	0.07	0.045	0.06	0.045
E1458G$^{+/+}$9	53.12	23.285	0.335	0.44	0.06	0.045	0.055	0.035
E1458G$^{+/+}$10	50.79	21.9	0.32	0.41	0.055	0.04	0.05	0.045
E1458G$^{+/+}$11	50.92	21.88	0.265	0.34	0.05	0.04	0.04	0.04
Control 1	66.715	31.73	0.245	0.36	0.09	0.06	0.05	0.05
Control 2	59.595	27.005	0.27	0.375	0.05	0.03	0.06	0.04
Control 3	63.62	29.705	0.295	0.425	0.06	0.04	0.07	0.055
Control 4	65.12	30.725	0.285	0.415	0.08	0.045	0.08	0.05
Control 5	56.205	24.86	0.26	0.345	0.05	0.045	0.055	0.045
Control 6	55.73	24.665	0.29	0.385	0.07	0.05	0.065	0.035
Control 7	65.83	31.09	0.255	0.375	0.075	0.05	0.06	0.04

Suppl. Table 2. Summary of electrocardiographic parameters using implanted radio-telemeters in control and the AnkBp.E1458G$^{+/+}$ mice around six months of age.

Mouse	Min HR (BPM)	Max HR (BPM)	RR Interval (S)	HR (BPM)	PR Interval (S)	P Duration (S)	QRS Interval (S)	QT Interval (S)	QTc (S)
E1458G$^{+/+}$1	596.1	678.6	0.09562	627.9	0.03699	0.008297	0.00962	0.02357	0.02412
E1458G$^{+/+}$2	503.1	663.9	0.1062	568	0.03635	0.009818	0.009136	0.02326	0.02262
E1458G$^{+/+}$3	463.9	577.9	0.1191	505.6	0.03878	0.01005	0.009507	0.02065	0.01897
E1458G$^{+/+}$4	467.2	631.5	0.1105	545	0.03449	0.008551	0.01122	0.02524	0.02391
E1458G$^{+/+}$5	420.7	623.4	0.1275	473.4	0.03608	0.009651	0.01206	0.02509	0.02224
E1458G$^{+/+}$6	527	716.7	0.09559	630	0.03547	0.009153	0.008932	0.02064	0.02116
E1458G$^{+/+}$7	498.4	700.7	0.1014	596.9	0.032	0.01118	0.009545	0.01978	0.01975
E1458G$^{+/+}$8	527.1	759.8	0.0862	700.3	0.0327	0.008767	0.009745	0.02432	0.02635
E1458G$^{+/+}$9	361.1	736.8	0.09929	612.9	0.02713	0.00507	0.01076	0.02462	0.02478
E1458G$^{+/+}$10	577.6	706.5	0.09337	644.3	0.03233	0.008422	0.009906	0.02347	0.02433
Control 1	599.2	724.3	0.08939	672	0.03034	0.009515	0.009764	0.0212	0.02243
Control 2	420.6	669.2	0.1032	590.1	0.03342	0.00959	0.009573	0.02237	0.02221
Control 3	530.8	775.1	0.08389	717.9	0.03063	0.007569	0.008603	0.02102	0.023
Control 4	573.7	722.7	0.08965	670.6	0.03191	0.008708	0.009684	0.01716	0.01818
Control 5	602.6	743.6	0.09042	665.2	0.03293	0.008028	0.009291	0.02106	0.02218
Control 6	479.1	712.5	0.09673	626.6	0.03455	0.007702	0.007723	0.02193	0.02241
Control 7	551.6	757.7	0.09011	668.8	0.03038	0.0085	0.01287	0.01948	0.02065