

# **Enhancement of Proteasomal Function Protects Against Cardiac Proteinopathy and Ischemia/Reperfusion Injury in Mice**

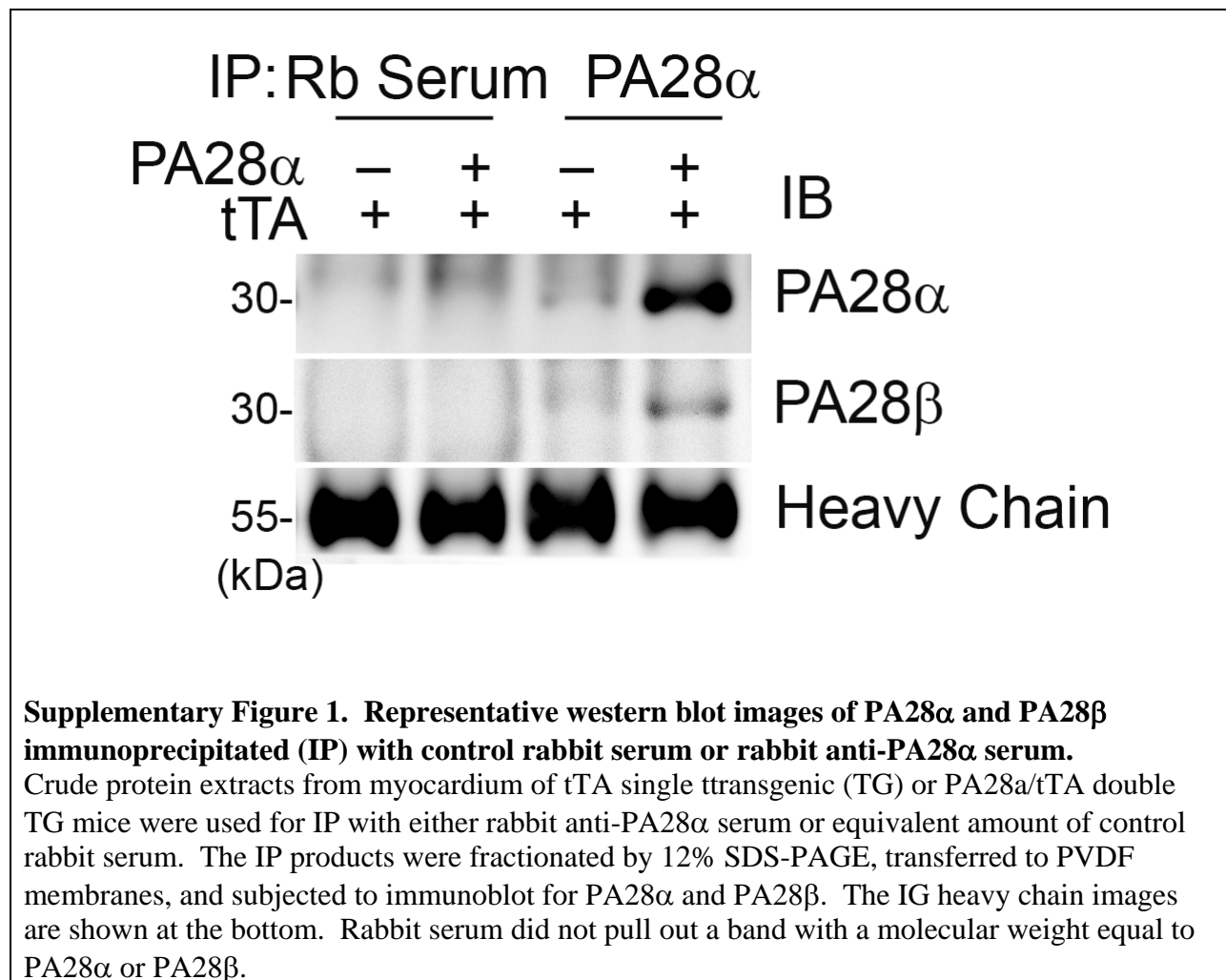
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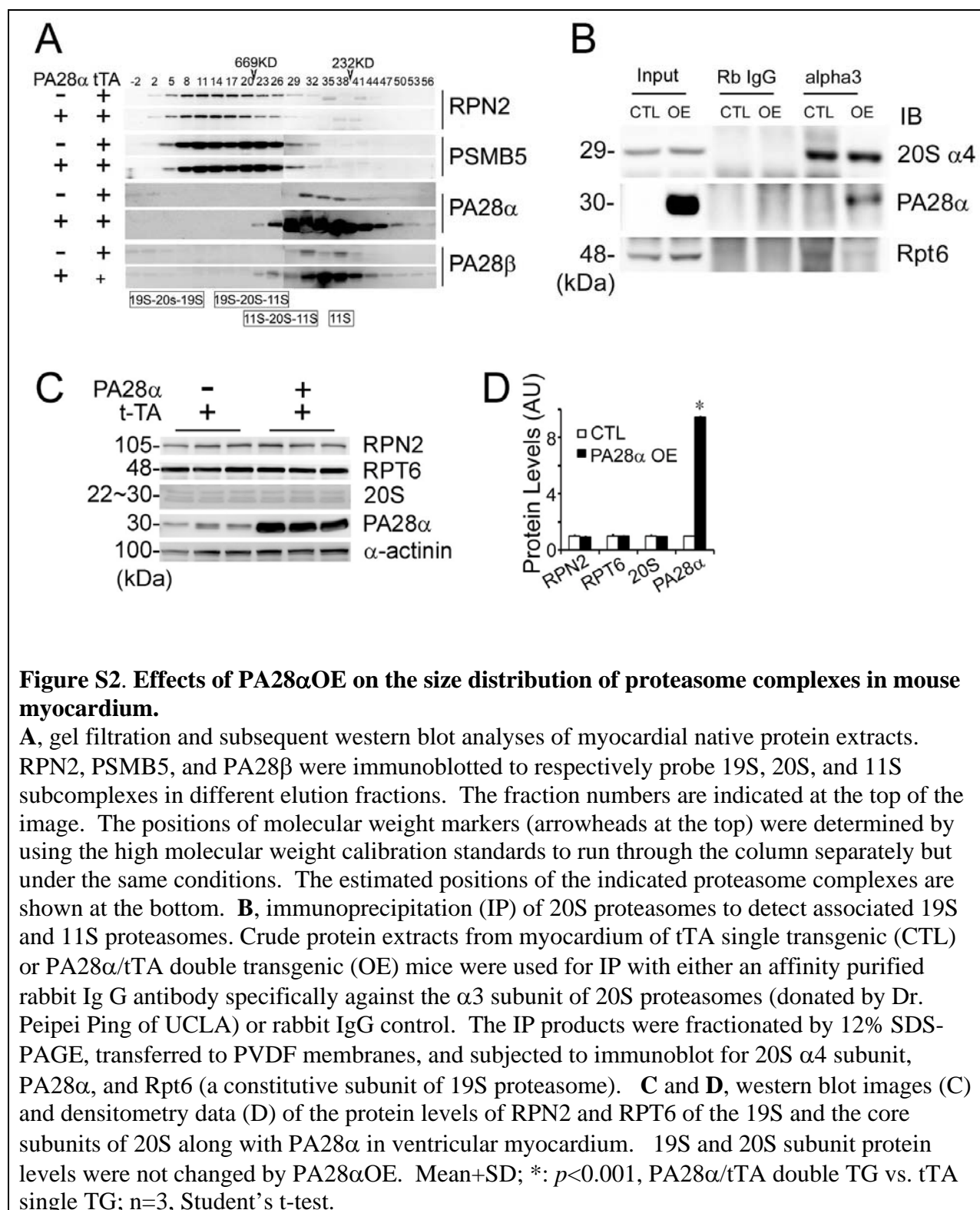
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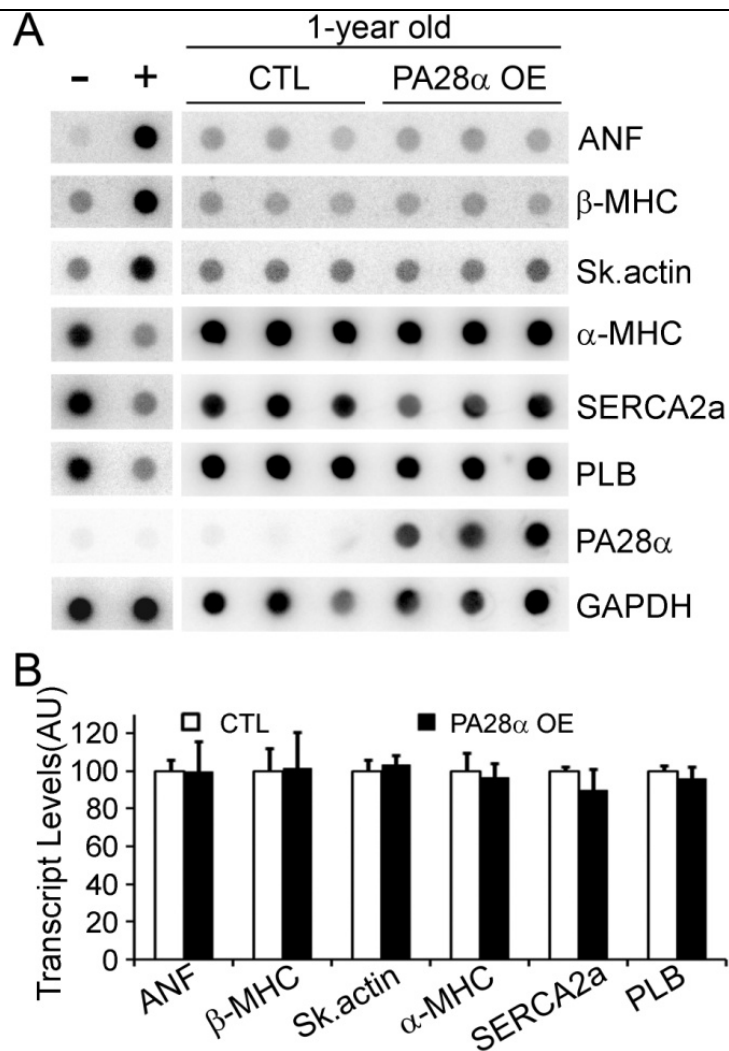
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**Online Supplementary Data**

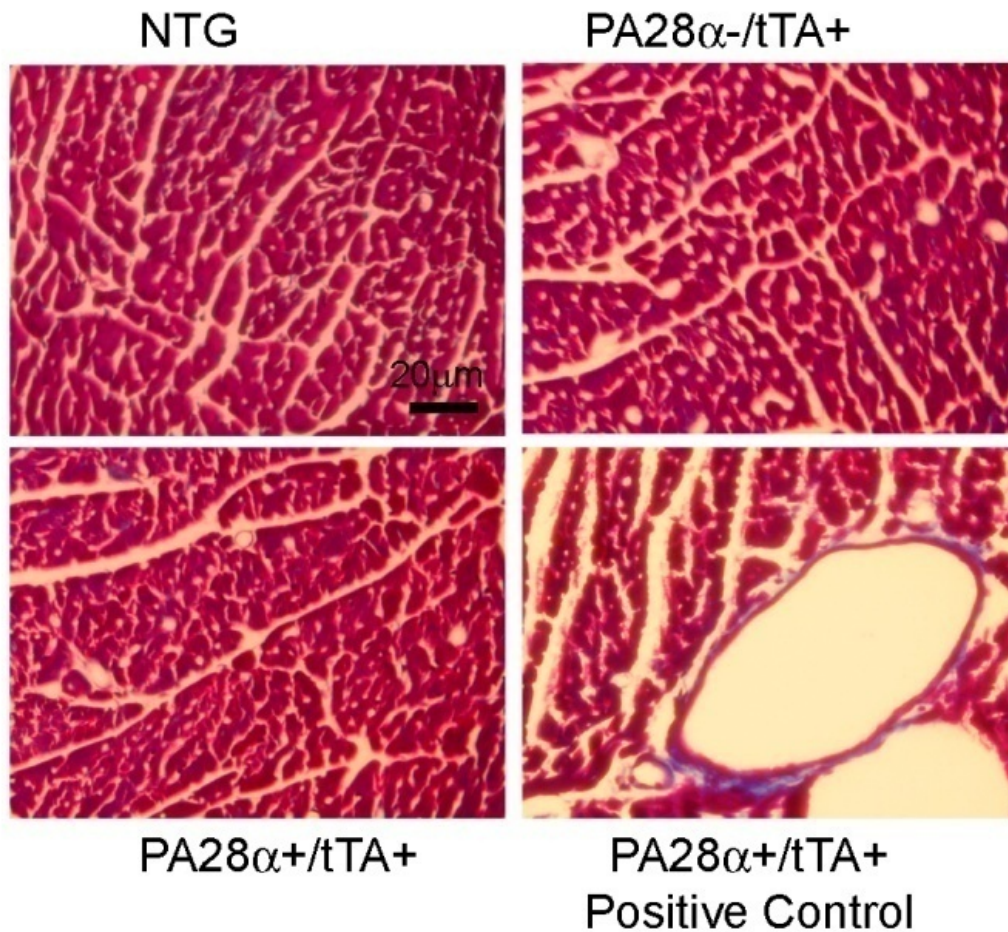
## Supplementary Figures and Legends



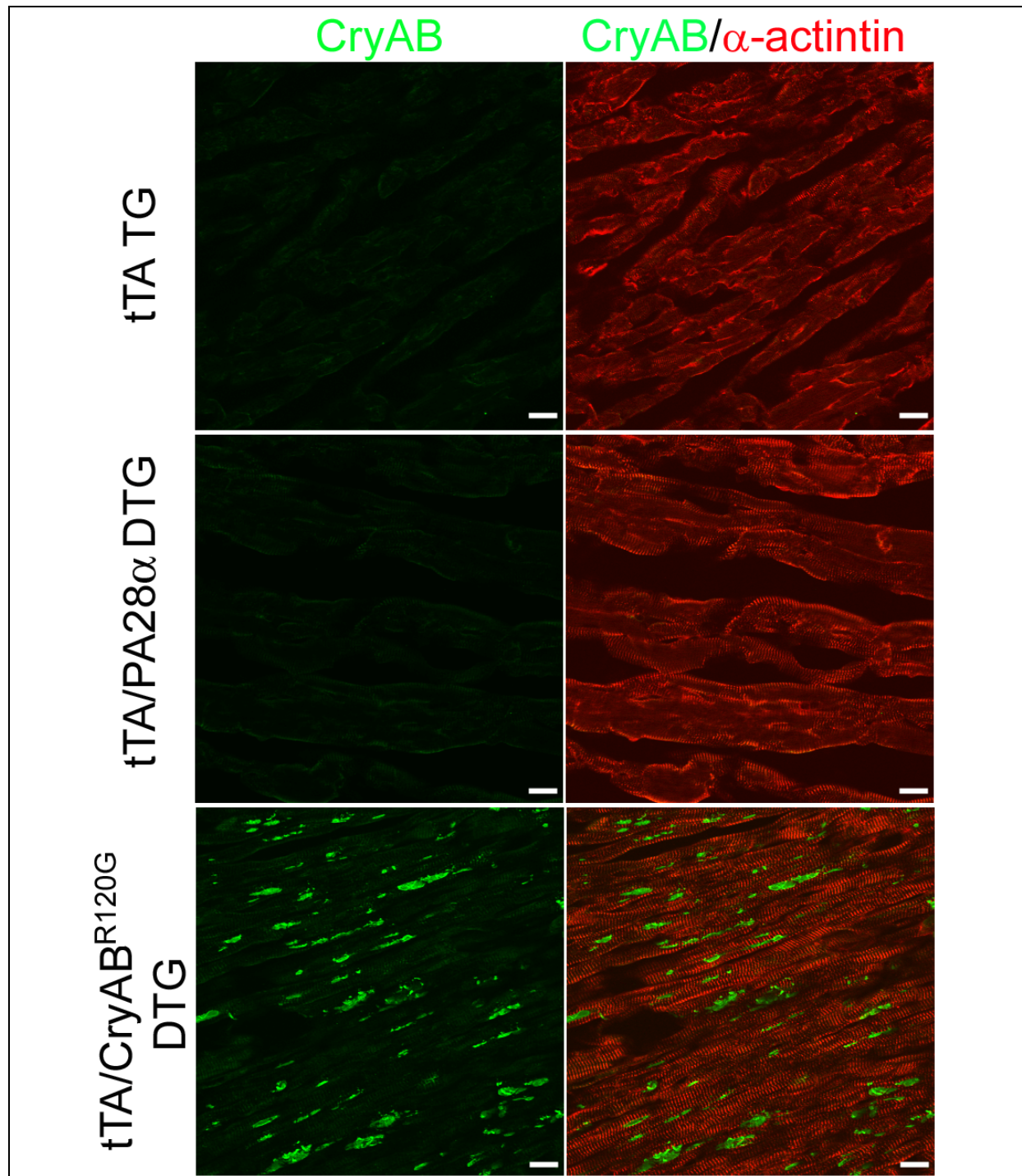




**Figure S3. PA28 $\alpha$ OE did not change the fetal gene expression profile.** RNA dot blot images (A) and densitometric analysis (B) of the fetal gene panel in 1-year old tTA TG (CTL) and PA28 $\alpha$ /tTA double TG (PA28 $\alpha$ OE) mouse hearts were presented. A pair of RNA samples from normal (-) and known cardiomyopathic (+) mouse ventricles were included in the profiling for validating the method used. N=3, Student's t-test,  $p > 0.05$ .



**Figure S4. Representative micrographs of trichrome-stained myocardium.** Trichrome staining of ventricular tissue samples from 1-year old mice. NTG, tTA TG, or PA28 $\alpha$ /tTA double TG genotypes were analyzed. No discernable fibrosis was evident in the myocardial interstitium. The bottom right panel is a positive control from a PA28 $\alpha$ /tTA double TG mouse which shows the blue vessel wall staining.



**Figure S5.** Confocal micrographs of double immunofluorescence staining for CryAB (green) and  $\alpha$ -actinin (red) in ventricular myocardium obtained from mice of the indicated genotypes. Note that CryAB positive protein aggregates are only detectable in CryAB<sup>R120G</sup> transgenic hearts. TG, transgenic; DTG, double TG; Scale bar = 20 $\mu$ m

Supplementary Table 1. Serial Echocardiographic analysis of mice

	LVIDd	LVPWd	LVIDs	LVPWs	LVAWd	LVAWs	LVVd	LVVs	%EF	% FS	HR
<b>2 month</b>											
NTG (n=10)	3.65 ± 0.34	0.81 ± 0.11	2.34 ± 0.35	1.24 ± 0.25	0.62 ± 0.07	1.09 ± 0.14	62.9 ± 11.8	22.2 ± 6.4	54.0 ± 11.8	38.2 ± 5.3	456 ± 34
α-/tTA+ (n=9)	3.64 ± 0.26	0.72 ± 0.11	2.35 ± 0.28	1.19 ± 0.15	0.66 ± 0.09	1.06 ± 0.09	57.9 ± 8.6	20.3 ± 6.5	56.3 ± 12.6	39.7 ± 2.9	482 ± 53
α+/tTA- (n=12)	3.64 ± 0.26	0.74 ± 0.115	2.32 ± 0.33	1.11 ± 0.17	0.65 ± 0.09	1.10 ± 0.10	57.0 ± 8.6	21.1 ± 3.9	52.1 ± 14.2	39.5 ± 6.1	465 ± 43
α+/tTA+ (n=11)	3.71 ± 0.3	0.80 ± 0.12	2.36 ± 0.35	1.25 ± 0.20	0.67 ± 0.10	1.16 ± 0.20	63.2 ± 10.1	21.4 ± 3.0	56.9 ± 12.0	41.5 ± 6.5	477 ± 22
<b>8 month</b>											
NTG (n=9)	3.88 ± 0.11	0.86 ± 0.15	2.43 ± 0.22	1.31 ± 0.12	0.85 ± 0.18	1.24 ± 0.16	65.2 ± 4.2	23.5 ± 4.6	64.2 ± 4.9	34.6 ± 5	512 ± 30
α-/tTA+ (n=11)	3.78 ± 0.34	0.86 ± 0.10	2.58 ± 0.42	1.24 ± 0.25	0.81 ± 0.12	1.21 ± 0.18	61.8 ± 12.5	25.1 ± 8.5	60.6 ± 8.3	32.1 ± 6.1	502 ± 31
α+/tTA- (n=9)	3.86 ± 0.19	0.89 ± 0.06	2.49 ± 0.16	1.34 ± 0.11	0.87 ± 0.12	1.30 ± 0.17	64.6 ± 7.4	22.2 ± 3.4	65.5 ± 3.8	35.5 ± 3.8	491 ± 45
α+/tTA+ (n=11)	3.80 ± 0.26	0.86 ± 0.17	2.50 ± 0.28	1.24 ± 0.24	0.85 ± 0.14	1.20 ± 0.15	62.5 ± 10.1	25.5 ± 4.0	62.5 ± 6.8	33.4 ± 4.9	474 ± 37
<b>12 month</b>											
NTG (n=9)	3.97 ± 0.19	0.89 ± 0.23	2.77 ± 0.3	1.32 ± 0.25	0.84 ± 0.20	1.19 ± 0.21	68.9 ± 7.9	29.3 ± 6.1	57.7 ± 7.7	30.3 ± 5	482 ± 19
α-/tTA+ (n=10)	3.83 ± 0.17	0.93 ± 0.18	2.57 ± 0.19	1.27 ± 0.21	0.82 ± 0.11	1.19 ± 0.18	63.2 ± 6.7	24.0 ± 4.5	60.7 ± 5.1	33.0 ± 3.4	493 ± 19
α+/tTA- (n=11)	3.96 ± 0.34	0.92 ± 0.14	2.79 ± 0.35	1.28 ± 0.16	0.88 ± 0.14	1.26 ± 0.17	69.3 ± 13.4	29.9 ± 9.1	57.4 ± 7.2	29.9 ± 4.7	496 ± 34
α+/tTA+ (n=13)	3.92 ± 0.20	0.86 ± 0.09	2.76 ± 0.18	1.17 ± 0.14	0.82 ± 0.11	1.14 ± 0.12	67.8 ± 9.2	28.3 ± 4.2	57.2 ± 4.2	30.2 ± 2.3	495 ± 17

α-/tTA+: PA28α NTG/tTA TG; α+/tTA-: PA28α TG/tTA NTG; α+/tTA+: PA28α TG/tTA TG; n: number of animals; LVIDd : Diastolic Left Ventricle Interdiameter; LVIDs: Systolic Left Ventricle Interdiameter; LVPWd: Diastolic Left Ventricle Posterior Wall; LVPWs: Systolic Left Ventricle Posterior Wall; LVAWd: Diastolic Left Ventricle Anterior Wall; LVAWs: Systolic Left Ventricle Anterior Wall; LVVd: Diastolic Left Ventricle Volume; LVVs: Systolic Left Ventricle Volume; EF: Ejection Fraction; FS: Fractional Shortening; HR: Heart Rate; Mean ±SD, One way ANOVA. No significant difference observed between groups at any time point.



Supplementary Table 2. Gravimetric measurements of 1-year old mice

	BW(g)	HW(mg)	HW/BW (mg/g)	VW/BW (mg/g)	LvW/BW (mg/g)	RvW/BW (mg/g)	LW/BW (mg/g)	LiW/BW (mg/g)	KW/BW (mg/g)	HW/TL (mg/g)
Ntg(n=6)	36.1 ± 6.0	135.7 ± 24.5	3.9 ± 0.2	3.4 ± 0.2	2.6 ± 0.1	0.8 ± 0.1	4.8 ± 0.8	39.7 ± 5.4	11.3 ± 0.8	7.3 ± 0.8
α-/tTA+(n=8)	33.5 ± 4.7	141.1 ± 17.0	4.3 ± 0.5	3.9 ± 0.5	3.0 ± 0.4	0.8 ± 0.1	4.4 ± 0.5	43.3 ± 4.3	12.1 ± 1.5	7.7 ± 0.6
α+/tTA-(n=7)	36.4 ± 4.2	143.1 ± 15.1	3.9 ± 0.2	3.5 ± 0.1	2.8 ± 0.2	0.8 ± 0.1	4.4 ± 0.5	43.3 ± 4.3	12.1 ± 1.5	7.7 ± 1.5
α+/tTA+(n=11)	36.3 ± 5.3	148.8 ± 20.5	4.1 ± 0.5	3.7 ± 0.4	2.8 ± 0.3	0.8 ± 0.1	4.7 ± 0.6	44.1 ± 3.9	11.9 ± 2.1	7.9 ± 1.1

α-/tTA+: PA28α NTG/tTA TG; α+/tTA-: PA28α TG/tTA NTG; α+/tTA+: PA28α TG/tTA TG; BW: Body Weight; HW: Heart Weight; VW: Ventricle Weight; TL: Tibia Length; LvW: Left ventricle Weight; RvW: Right ventricle Weight; LW: Lung Weight; LiW: Liver Weight; KW: kidney Weight; mean ±SD, One way ANOVA; no significant difference observed between groups.



**Supplementary Table 3. Echocardiographic Findings at 12 Weeks of Age**

	tTA n=7	tTA/mCryAB n=13	PA28 $\alpha$ /tTA/mCryAB n=13
BW (g)	27.98 $\pm$ 2.92	30.56 $\pm$ 4.26	27.34 $\pm$ 4.40
HR (bpm)	474 $\pm$ 44	449 $\pm$ 34	479 $\pm$ 50
LVID;d (mm)	3.73 $\pm$ 0.2	3.89 $\pm$ 0.39	3.72 $\pm$ 0.25
LVPW;d (mm)	0.85 $\pm$ 0.14	1.05 $\pm$ 0.17*	0.90 $\pm$ 0.09†
LVID;s (mm)	2.56 $\pm$ 0.25	2.46 $\pm$ 0.41	2.24 $\pm$ 0.37
LVPW;s (mm)	1.17 $\pm$ 0.17	1.53 $\pm$ 0.21**	1.42 $\pm$ 0.13*
LVAW;d (mm)	0.78 $\pm$ 0.07	0.97 $\pm$ 0.10**	0.85 $\pm$ 0.09†
LVAW;s (mm)	1.12 $\pm$ 0.12	1.51 $\pm$ 0.14**	1.39 $\pm$ 0.12**†
LV %EF	59.83 $\pm$ 7.66	66.69 $\pm$ 9.03	70.74 $\pm$ 8.84*
LV % FS	31.49 $\pm$ 5.53	36.85 $\pm$ 7.32	40.01 $\pm$ 7.53*
LV Mass (mg)	86 $\pm$ 16	124 $\pm$ 19**	95 $\pm$ 16††

\* P< 0.05 vs tTA; \*\* P< 0.001 vs tTA; † P<0.05 vs tTA/mCryAB; †† P<0.001 vs tTA/mCryAB; one way ANOVA and Holm-Sidak test.