SUPPLEMENTAL MATERIAL

Data S1.

SUPPLEMENTAL RESULTS

Impact of Breast Cancer

Breast cancer provokes physical characteristic changes

Body weight in MMTV-PyMT+ and MMTV-PyMT- mice is shown in **Figure S1**. Body weight increased in both MMTV-PyMT- and MMTV-PyMT+ mice (p-time<0.001). Despite of significant interaction (p=0.013), it was not possible to identify the difference between groups throughout the study. Body weight after euthanasia was higher in MMTV-PyMT+ mice (p<0.001). This difference was due to tumor burden, since body weight without tumor was similar between groups.

Values of organs and tissues are shown in **Table S1**. Observe that anterior tibial muscle, lung and brown and white fat weight were not different between MMTV-PyMT- and MMTV-PyMT+ mice, liver and spleen were greater in MMTV-PyMT+ mice, while kidney and soleus muscle were lower in MMTV-PyMT+ mice.

Breast cancer alters cardiac morphology and function

All cardiac function and morphology parameters analyzed are shown in **Table S2**. The pooled data (beginning and end of the study) of the LVEDD (PyMT- pre and post vs. PyMT+ pre and post; p-group<0.001, p-interaction=0.417) and LVEDV (PyMT- pre and post vs. PyMT+ pre and post; p-group=0.003, p-interaction=0.437) were lower in the MMTV-PyMT+ mice when compared to MMTV-PyMT-. LVESD and LVESV were similar between MMTV-PyMT- and MMTV-PyMT+ mice. The left atrium responses were statistically different between groups throughout the experimental protocol (p-interaction=0.004). At the beginning of the study, the left atrium was not different between MMTV-PyMT+ and MMTV-PyMT-;

however, at the end of the study, the left atrium was lower in MMTV- PyMT+ mice (0.079±0.001 vs. 0.091±0.008 mm/g, p=0.030) (**Table S2**). The pooled data (beginning and end of the study) of the left ventricular mass was lower in MMTV-PyMT+ mice than in MMTV-PyMT- mice (PyMT- pre: 4.297±0.798 and post: 3.396±0.496vs. PyMT+ pre: 3.454±0.649 and post: 2.968 ± 0.601 mg/g, p-group<0.001, p-interaction=0.365). E/E' wave, E-wave deceleration time and IVRT values were not different between groups. Similarly, heart rate, fractional shortening, ejection fraction and myocardium performance index were not different in MMTV-PyMT- and MMTV-PyMT+ mice.

Longitudinal strain and strain rate at the beginning and at the end of the study are shown in **Table S2**. The pooled data (beginning and end of the study) of the longitudinal strain (PyMT- pre and post vs. PyMT+ pre and post, p-group<0.001, p-interaction=0.126) and strain rate (PyMT_ pre and post vs. PyMT+ pre and post, p-group=0.030, p-interaction=0.168) were impaired in the MMTV-PyMT+ mice when compared to MMTV-PyMT- mice. Both longitudinal strain and strain rate were statistically lower in absolute value in animals with cancer than in control animals. Radial strain and strain rate as well as radial and circumferential short systolic axis were similar between groups.

Exercise training does not reverse morphologic and physiologic changes in MMTV-PyMT+ mice, and does not delay time to tumor growth

Exercise training did not change body weight (**Figure S2**, p-interaction=0.930) and organs and tissues weight (**Table S3**, p>0.05). Also, ET did not influence tumor growth.

Exercise training did not prevent the decline in exercise tolerance (**Figure S3**, p=0.069), despite the increase in citrate synthase activity in the anterior tibial and soleus muscles (**Figure S4**, p<0.001).

Individual data of cardiac morphology and function in sedentary and exercise-trained MMTV-PyMT+ mice are shown in **Figure S5**. Exercise training decreased LVESD (p-

group=0.038, p-interaction=0.226) and LVEDV (p-group=0.026, p-interaction=0.431), but did not change the other parameters associated with cardiac morphology **(Table S4)**. Despite the average difference in longitudinal strain rate responses throughout the experimental protocol (p-interaction=0.032, **Table S4**), it was not possible to identify differences between sedentary and exercise-trained MMTV-PyMT+ mice.

Exercise training did not improve phospholamban (p=0.006) and did not change SERCA2a (p=0.345), p-phospholamban (p=0.554), NCX (p=0.247) and SERCA2a/phospholamban (p=0.111) (**Figure S6**).

Table S1. Organ, tissue and tumor weight in MMTV-PyMT- and MMTV-PyMT+.

Variable	MMTV-PyMT-		MMTV-PyMT+		P
	median (IQR)	N	median (IQR)	N	
Anterior tibial	0.039 (0.036; 0.041)	12	0.036 (0.033; 0.039)	14	0.060
Soleus	0.007 (0.007; 0.007)	14	0.006 (0.006; 0.007)	15	**0.029**
Lung	0.15 (0.14; 0.17)	14	0.15 (0.14; 0.16)	15	0.983
Liver	0.98 (0.94; 1.04)	14	1.24 (1.16; 1.37)	15	**<0.001**
Spleen	0.069 (0.06; 0.081)	14	0.121 (0.094; 0.147)	15	**<0.001**
Kidney	0.26 (0.25; 0.28)	14	0.23 (0.22; 0.25)	15	**0.001**
Brown fat	0.063 (0.046; 0.082)	13	0.057 (0.042; 0.08)	14	0.583
White fat	0.15 (0.13; 0.2)	13	0.12 (0.09; 0.16)	14	0.068
Breast tumor			1.41 (1.22; 1.56)	15	
All tumors			2.95 (2.55; 4.64)	15	

Mann-Whitney's test. Values are expressed as medians and interquartile ranges (IQR). Anterior tibial was not collected in two mice in the MMTV-PyMT- group and brown and white fats were not collected in one mouse in the MMTV-PyMT- group and one mouse in the MMTV-PyMT+ group.

Table S2. Morphological and functional cardiac parameters in MMTV-PyMT- and MMTV-PyMT+ mice in the pre and post experimental protocol.

Variable	MMTV-PyMT-		MMTV-PyMT+		p Group	p Time	p Interaction
	pre	post	pre	post			
Morphology							
LVEDD/weight (mm/g)	0.185±0.019	0.168±0.01	0.177±0.014	0.151±0.014	**<0.001**	**<0.001**	0.417
LVESD/weight (mm/g)	0.125±0.022	0.112±0.019	0.12±0.018	0.096±0.014	0.060	**0.001**	0.309
LV Mass/weight (mg/g)	4.297±0.798	3.396±0.496	3.454±0.649	2.968±0.601	**<0.001**	**0.002**	0.365
LA/weight (mm/g)	0.099±0.012	0.091±0.008	0.104±0.008	0.079±0.01	0.270	**<0.001**	**0.004**
Volumes							
LVEDV/weight (µl/g)	2.99±0.41	2.64±0.38	2.61±0.35	2.45±0.34	**0.003**	**0.039**	0.437
LVESV/weight (µl/g)	1.18±0.41	1.01±0.4	1.04±0.31	0.84±0.25	0.132	0.063	0.897
Functional							
FS (%)	33.5±6.7	34.8±7.8	32.2±6.6	36.1±6.7	0.995	0.220	0.532
EF (%)	62.4±8.8	64.1±10.4	60.8±9.1	66±8.4	0.946	0.232	0.539
Ewave DECc. (ms)	1.36±0.55	1.37±0.52	1.21±0.41	1.38±0.5	0.568	0.547	0.618
IVRTc (ms)	1.46±0.13	1.59±0.23	1.52±0.29	1.49±0.25	0.707	0.538	0.334
E/E' nor	28.4±5	26.1±5.9	28.3±6.3	26.7±6.5	0.876	0.376	0.871
HR (bpm)	417.6±29.9	435.3±40.5	400.9±44.9	427.5±37.7	0.260	**0.046**	0.687
MPI	15.6±2.8	14.6±2.3	15.8±2.1	14.8±3.5	0.761	0.245	0.971
RLA Strain	18.4±4.6	16.1±6.4	20.2±7.1	15.9±3.9	0.667	**0.034**	0.520
RLA Strain Rate	4.82±1.16	4.72±1.21	4.37±1.14	4.3±1.29	0.249	0.806	0.967
LLA Strain	-14.4±4.8	-14.5±2.7	-12.4±2.4	-9.8±1.7	**<0.001**	0.137	0.126
LLA Strain Rate	-5.07±2.27	-6.03±3.56	-4.39±1.47	-3.66±0.92	**0.030**	0.852	0.168
RSA Strain	21.9±8.6	20.1±8	23.6±9.8	22.2±6.4	0.517	0.423	0.926
RSA Strain Rate	5.45±1.5	5.15±1.22	4.88±1.37	5.28±1.3	0.619	0.899	0.331
CSA Strain	-17.9±2.7	-16.5±3.8	-17.5±4.9	-18.9±3	0.446	0.984	0.129
CSA Strain Rate	-5.57±1.16	-4.96±1.3	-5.28±1.44	-5.41±0.51	0.834	0.417	0.218

Left ventricle end diastolic diameter (LVEDD), left ventricle end systolic diameter (LVESD), left ventricle mass (LV), Left atrium (LA), left ventricle end diastolic volume (LVEDV) and left ventricle end systolic volume (LVESV). E wave (E), E wave deceleration time corrected (E wave DECc.) and isovolumetric relaxation time corrected (IVRTc), heart rate (HR), E' wave (E'), ejection fraction (EF), fractional shortening (FS), myocardium performance index (MPI), radial long axis (RLA), longitudinal long axis (LLA), radial short axis (RSA), circumferential short axis (CSA). The morphological parameters and volumes were corrected for weight. E wave DECc. and IVRTc were corrected for heart rate. GEE with normal distribution and identity link function using AR(1) correlation matrix between evaluations. Values are expressed as means ± SD. Four mice in the MMTV-PyMT- and 2 mice in the MMTV-PyMT+ were excluded from this analysis because of poor imaging quality in the echocardiography. Thus, 10 mice in the MMTV-PyMT- and 13 mice in the MMTV-PyMT+ were involved in this analysis. Of note, two left atrium measures were not obtained in the MMTV-PyMT+ mice.

Table S3. Organs, tissues and tumor weight in sedentary and exercise-trained MMTV-PyMT+ mice.

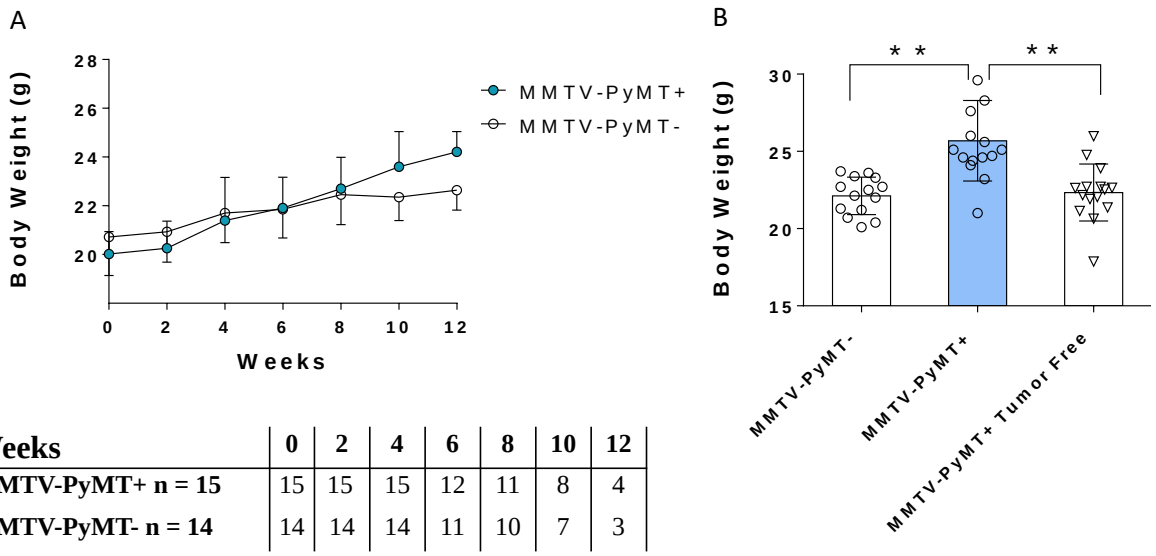
Variable	Sedentary		Exercise-trained		p
	median (IQR)	N	median (IQR)	N	
Heart	0.116 (0.11; 0.124)	6	0.113 (0.105; 0.148)	6	0.937
Anterior tibial	0.036 (0.033; 0.039)	6	0.029 (0.026; 0.037)	6	0.180
Soleus	0.006 (0.006; 0.007)	6	0.006 (0.005; 0.006)	6	0.394
Liver	1.14 (1.08; 1.27)	6	1.21 (1.12; 1.28)	6	0.699
Spleen	0.15 (0.077; 0.183)	6	0.095 (0.088; 0.13)	6	0.485
Kidney	0.222 (0.204; 0.241)	6	0.219 (0.198; 0.227)	6	0.589
Brown fat	0.027 (0.015; 0.032)	6	0.025 (0.018; 0.034)	6	0.937
White fat	0.131 (0.075; 0.149)	6	0.128 (0.055; 0.222)	6	0.937
Breast tumor	1.53 (1.5; 2.35)	7	1.59 (1.09; 2.08)	10	0.475
All tumors	3.45 (2.37; 4.33)	8	3.2 (1.53; 5.29)	10	0.696

Mann-Whitney's test. Values are expressed as medians and interquartile ranges (IQR).

Table S4. Morphological and functional cardiac parameters in sedentary and exercise-trained MMTV-PyMT+ mice in the pre and post experimental protocol.

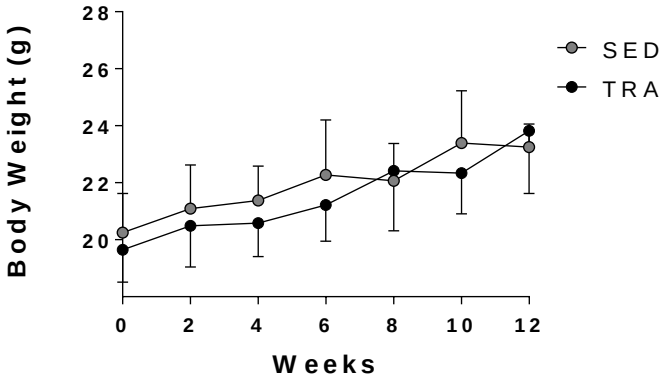
Variable	Sedentary		Exercise-trained		p Group	p Time	p Interaction
	Pre	Post	Pre	Post			
Morphology							
LVEDD/weight (mm/g)	0.182±0.018	0.161±0.016	0.175±0.013	0.148±0.013	0.084	<0.001	0.367
LVESD/weight (mm/g)	0.12±0.017	0.111±0.011	0.113±0.016	0.094±0.017	0.038	0.001	0.226
LV Mass/weight (mg/g)	3.875±0.939	3.726±0.861	3.392±0.533	3.284±0.613	0.062	0.549	0.925
LA/weight (mm/g)	0.103±0.007	0.088±0.009	0.1±0.014	0.089±0.006	0.780	<0.001	0.456
Volumes							
LVEDV/weight (µl/g)	2.84±0.58	2.72±0.5	2.48±0.35	2.2±0.54	0.026	0.058	0.431
LVESV/weight (µl/g)	1.05±0.33	1.11±0.2	0.89±0.31	1.03±0.56	0.350	0.452	0.765
Functional							
FS (%)	34.1±5.1	30.9±2.8	35.4±6.2	39±11.6	0.109	0.922	0.080
EF (%)	63.6±7.1	59.1±3.9	65.5±8.2	63±16.4	0.417	0.291	0.763
Ewave DECc. (ms)	1.75±0.61	1.29±0.33	1.7±0.67	1.43±0.5	0.821	0.018	0.558
IVRTc (ms)	1.69±0.21	1.55±0.2	1.55±0.15	1.55±0.23	0.207	0.352	0.313
E/E' nor	31.1±6.4	26.8±4.1	27.4±6.7	31.3±11.2	0.893	0.914	0.069
HR (bpm)	399.3±45.4	424.4±25.3	413.4±56.7	433.4±19.6	0.360	0.091	0.848
MPI	13.7±2.3	14.6±4.4	13.2±1.8	13.1±1.4	0.264	0.620	0.594
RLA Strain	18.4±8.2	15.5±6.4	18.4±5.4	16.4±6.3	0.812	0.302	0.830
RLA Strain Rate	5.58±2.86	4.54±1.18	4.53±1.27	5.2±1.87	0.783	0.720	0.095
LLA Strain	-14.2±2.7	-11.4±3.1	-13±3.8	-16.4±6	0.117	0.838	0.055
LLA Strain Rate	-5.03±2.12	-3.76±1.51	-4.41±1.48	-5.75±2.2	0.265	0.957	0.032
RSA Strain	26±7.1	21.9±10.5	18.2±7	21.1±9.8	0.092	0.848	0.280
RSA Strain Rate	5.66±1.49	4.94±1.44	4.33±1.03	5.51±1.48	0.263	0.669	0.080
CSA Strain	-19.4±2.4	-17±4.1	-16.3±2.8	-17.4±4.1	0.235	0.581	0.144
CSA Strain Rate	-5.38±0.61	-4.75±0.75	-4.54±0.67	-5.02±1.13	0.223	0.823	0.093

Left ventricle end diastolic diameter (LVEDD), left ventricle end systolic diameter (LVESD), left ventricle mass (LV), Left atrium (LA), left ventricle end diastolic volume (LVEDV) and left ventricle end systolic volume (LVESV). E wave (E), E wave deceleration time corrected (E wave DECc), isovolumetric relaxation time corrected (IVRTc), heart rate (HR), E' wave (E'), ejection fraction (EF), fractional shortening (FS), myocardium performance index (MPI), radial long axis (RLA), longitudinal long axis (LLA), radial short axis (RSA), circumferential short axis (CSA). The morphological parameters and volumes were corrected by weight. E wave DECc. and IVRTc were corrected for heart rate. GEE with normal distribution and identity link function using AR(1) correlation matrix between evaluations. Values are expressed as means ± SD. Two measurements of LVESV in MMTV-PyMT+ mice, and one measure of LA in each group were not obtained.



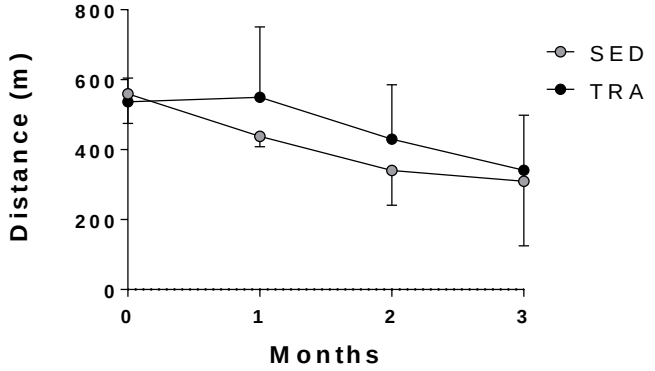
Weeks	0	2	4	6	8	10	12
MMTV-PyMT+ n = 15	15	15	15	12	11	8	4
MMTV-PyMT- n = 14	14	14	14	11	10	7	3

Figure S1. Body weight of MMTV-PyMT- and MMTV-PyMT+. The greater body weight in the MMTV-PyMT+ mice can be attributed to the increase in the volume of tumors. (A) Evolution of body weight during experimental protocol. Numbers in the table means number of mice throughout the weeks. (B) Body weight after euthanasia between MMTV-PyMT-, MMTV-PyMT+ and MMTV-PyMT+ without tumors ** $P<0.001$. GEE with normal distribution and identity link function using AR(1) correlation matrix between weeks followed by Bonferroni's multiple comparison. Values are expressed as means and SD.



Weeks	0	2	4	6	8	10	12
SED n = 9	9	9	9	8	5	5	3
TRA n = 12	12	12	12	12	12	8	8

Figure S2. Body weight in exercise-trained (TRA) and sedentary MMTV-PyMT+ mice (SED). Numbers in the table means number of mice throughout the weeks. GEE with normal distribution and identity link function using AR(1) correlation matrix between weeks. Values are expressed as means and SD.



Months	0	1	2	3
SED n = 9	9	9	8	5
TRA n = 12	12	12	12	11

Figure S3. Running distance in the exercise tolerance test in sedentary and exercise-trained groups. Numbers in the table mean number of mice throughout the months. GEE with normal distribution and identity link function using AR(1) correlation matrix between weeks. Values are expressed as means and SD. Reduction in the distance covered in the exercise tolerance tests performed during the experimental protocol (p-group=0.263, p-time<0.001 and p-interaction=0.069).

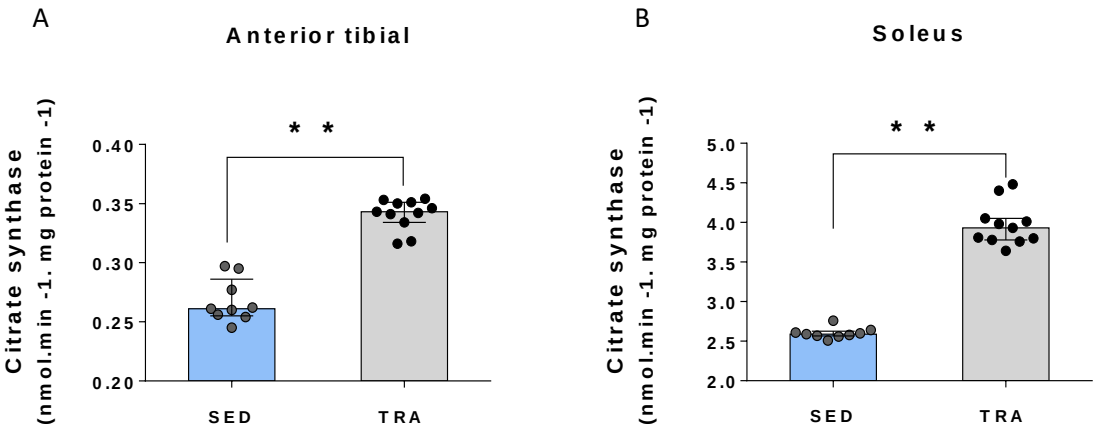


Figure S4. Measurement of the citrate synthase enzyme activity in the anterior tibial (A) and soleus muscle (B) in sedentary and trained MMTV-PyMT+. SED=Sedentary; TRA=Exercise-trained. ** $P<0.001$, between group difference. Mann-Whitney's test. Values are expressed as medians and interquartile ranges (IQR). Animals/group: 9 SED and 11 TRA.

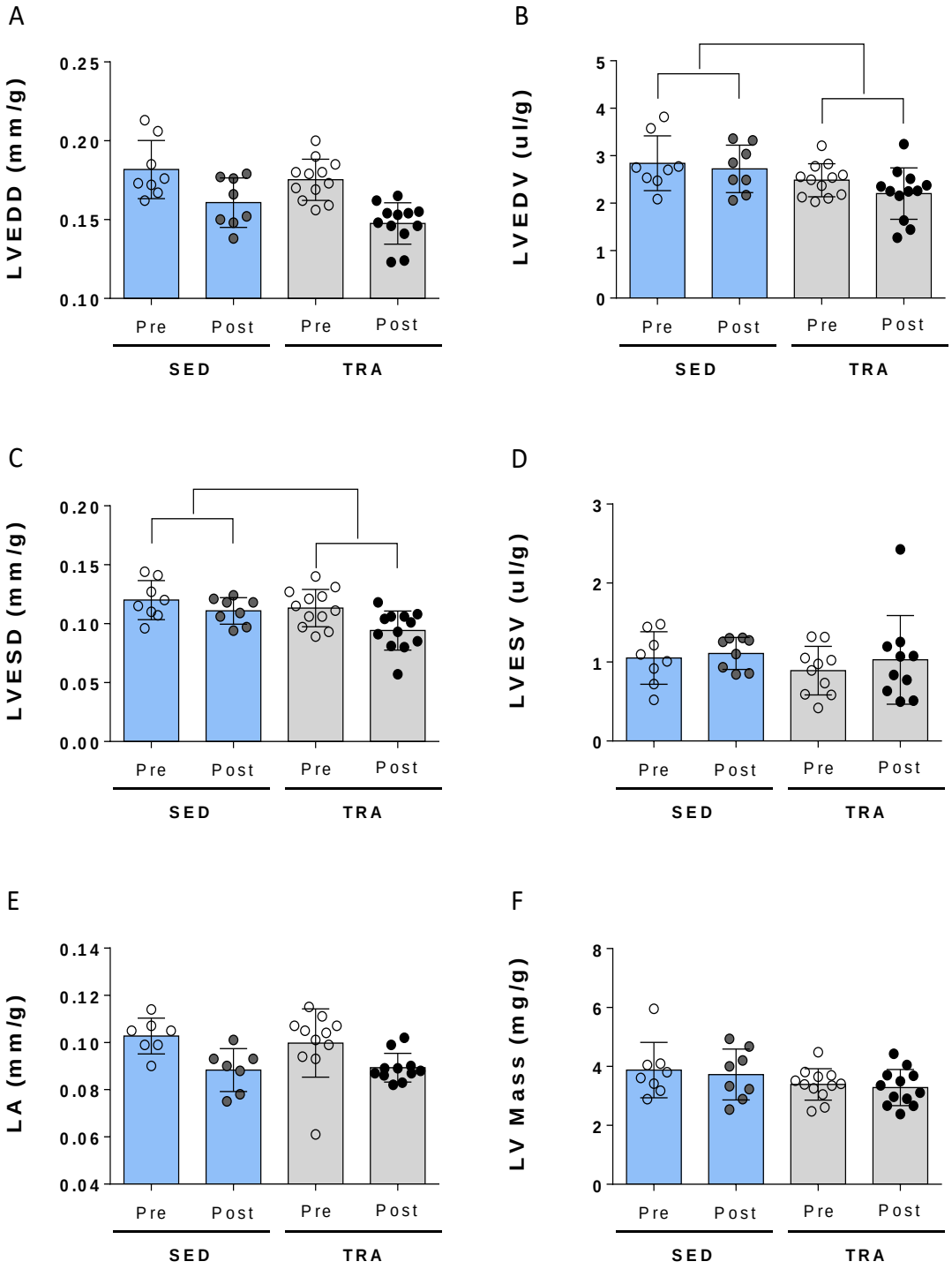


Figure S5. Morphological cardiac parameter in sedentary and exercise-trained MMTV-PyMT+ mice in the pre and post experimental protocol. A, Left ventricle end diastolic diameter (LVEDD); B, Left ventricle end diastolic volume (LVEDV); C, Left ventricle end systolic diameter (LVESD); D, Left ventricle end systolic volume (LVESV); E, Left atrium

(LA); F, Left ventricle mass (LV Mass). LVESD (p-group=0.038, p-interaction=0.226) and LVEDV (p-group=0.026, p-interaction=0.431) SED=Sedentary; TRA=Exercise-trained. GEE with normal distribution and identity link function using AR(1) correlation matrix between weeks. Values are expressed as means and SD. Animals/group: 8 SED and 12 TRA MMTV-PyMT+ mice. Two measures of LVESV in the exercise-trained MMTV-PyMT mice, and one measure of LA in each group were not obtained.

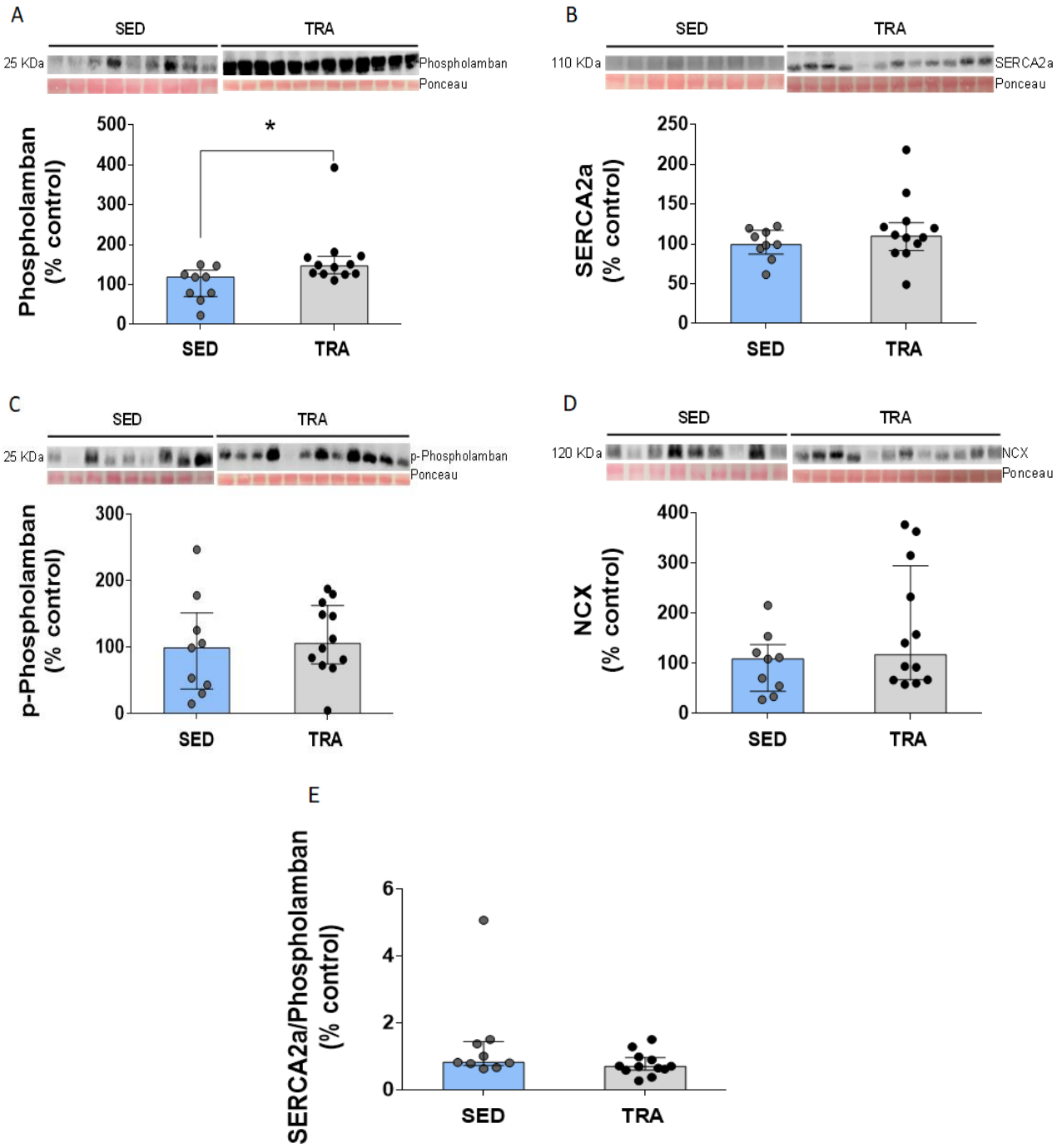


Figure S6. Cardiac myocyte calcium handling proteins in sedentary and exercise-trained MMTV-PyMT+ mice – A, Phospholamban; B, SERCA2a; C, phosphorilated phospholamban (p-phospholamban); D, sodium/calcium exchanger (NCX) and E, SERCA2a/phospholamban ratio. Western blot bands are shown for each figure. Exercise training did not improve phospholamban (p=0.006) and did not change SERCA2a (p=0.345), p-phospholamban

(p=0.554), NCX (p=0.247) and SERCA2a/phospholamban (p=0.111). $^{*}P<0.05$, between group difference. Animals/group: 9 sedentary (SED) and 12 exercise-trained (TRA). Mann-Whitney's test. Values are expressed as medians and interquartile ranges (IQR).