SUPPLEMENTAL MATERIAL

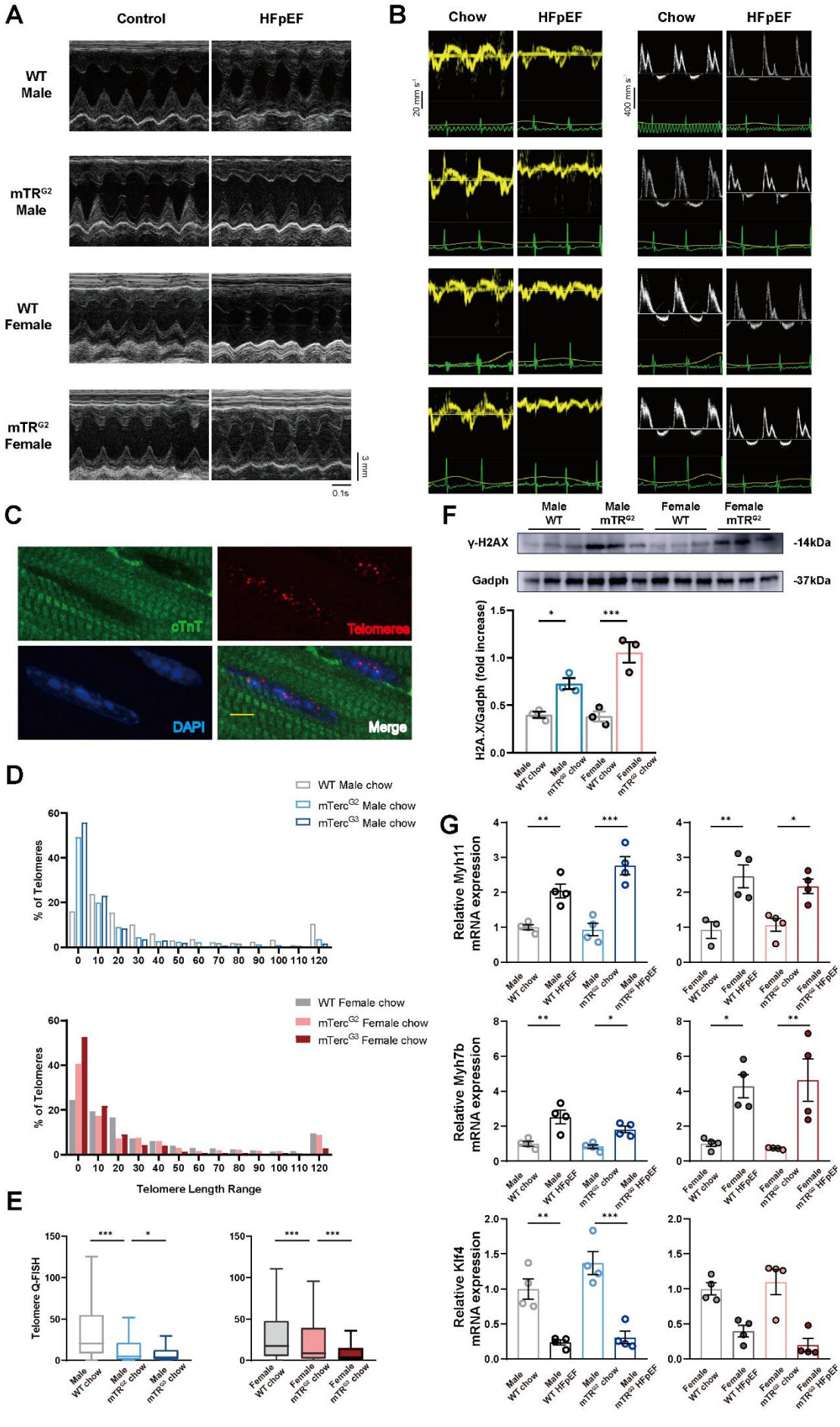
A
Control
HFpEF
WT Male
mTRG2 Male
WT Female
mTRG2 Female
3 mm
0.1s
B
Chow
HFpEF
Chow
HFpEF
20 mm s-1
400 mm s-1
C
cTnT
Telomeres
DAPI
Merge
D
WT Male chow
mTerc G2 Male chow
mTerc G3 Male chow
% of Telomeres
WT Female chow
mTerc G2 Female chow
mTerc G3 Female chow
% of Telomeres
Telomere Length Range
E
Telomere Q-FISH
Male WT chow
Male mTRG2 chow
Male mTRG3 chow
Female WT chow
Female mTRG2 chow
Female mTRG3 chow
F
Male WT
Male mTRG2
Female WT
Female mTRG2
γ-H2AX
-14kDa
Gadph
-37kDa
H2A.X/Gadph (fold increase)
Male WT chow
Male mTRG2 chow
Female WT chow
Female mTRG2 chow
G
Relative Myh11 mRNA expression
Relative Myh7b mRNA expression
Relative Klf4 mRNA expression
Male WT chow
Male WT HFpEF
Male mTRG2 chow
Male mTRG2 HFpEF
Female WT chow
Female WT HFpEF
Female mTRG2 chow
Female mTRG2 HFpEF

Figure S1. Functional and molecular characterization of WT and mTRG2 animals challenged with HFD + L-NAME diet.

A and **B,** Representative left ventricular M-mode echocardiographic and pulsed-wave Doppler (left) and tissue Doppler (right) tracings. **C,** Representative micrographs of quantitative fluorescence *in situ* hybridization (Q-FISH) staining of left murine ventricles. Scale bar is 5μm. **D** and **E,** Cardiomyocyte telomere length distribution and average telomere lengths are shown (n = 3 mice per group; male WT-chow n = 691; male mTR^{G2}-chow n = 849; male mTR^{G3}-chow n = 859; female WT-chow n = 782; female mTR^{G2}-chow n = 915; female mTR^{G3}-chow n = 920). **F,** Representative immunoblots of γ-H2A.X are shown. Histone 3 used as loading control. Densitometric quantifications are shown as mean ± SEM (n = 3 per group). (**G** through **I**)**,** mRNA expression levels of *Myh11*, *Myh7b* and *Klf4* in isolated AMVMs (n = 4 per group). Data are shown as mean ± SEM. Kruskal-Wallis test followed by Dunn's multiple-comparisons test was used for panel **E**; One-way ANOVA followed by lognormal processing and/or Tukey's multiple-comparisons test was used for remaining analyses. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to chow group in the same genotype are shown.

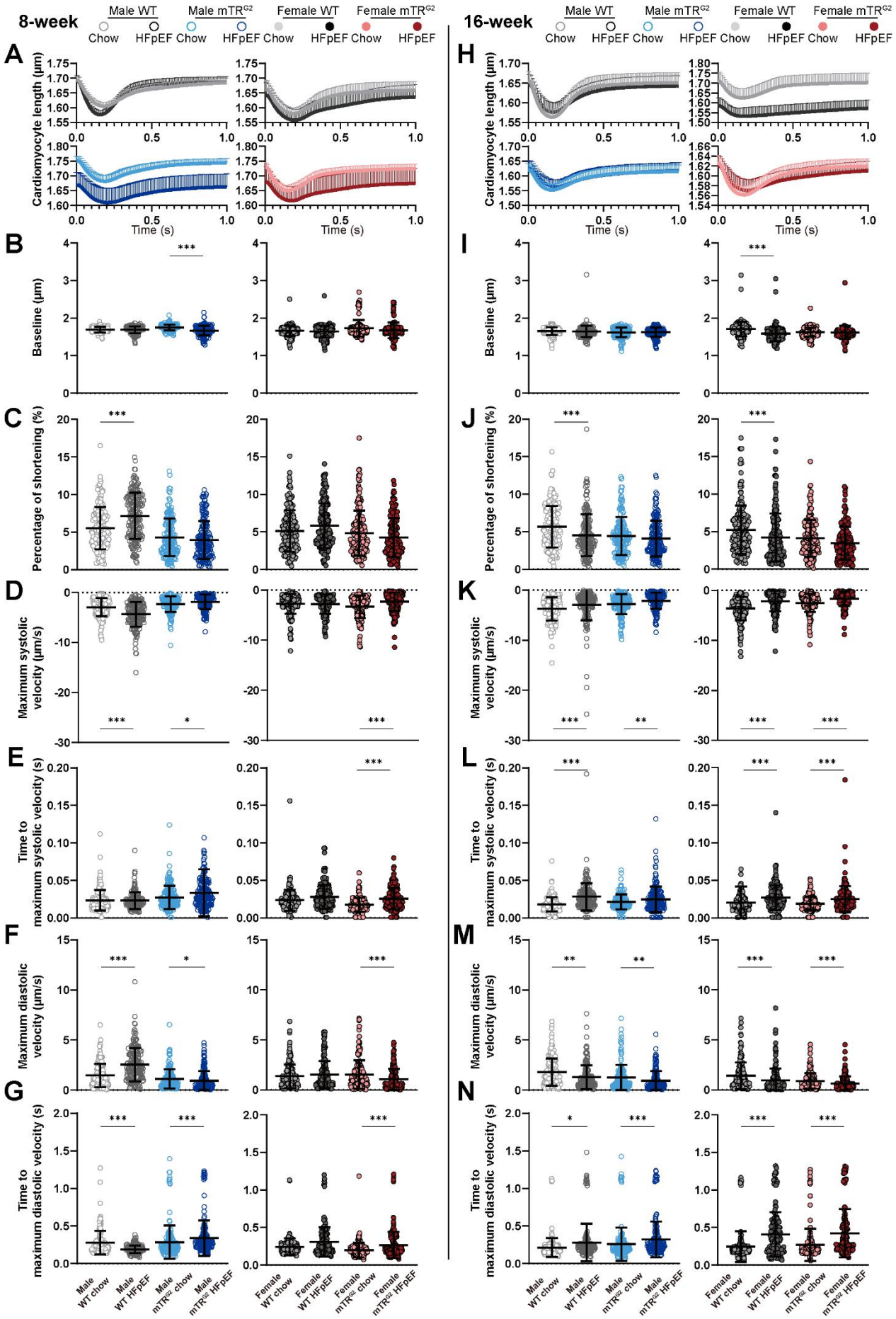
8-week
16-week
Male WT
Male mTRG2
Female WT
Female mTRG2
Chow
HFpEF
A
H
Cardiomyocyte length (µm)
Time (s)
B
I
Baseline (µm)
C
J
Percentage of shortening (%)
D
K
Maximum systolic velocity (µm/s)
E
L
Time to maximum systolic velocity (s)
F
M
Maximum diastolic velocity (µm/s)
G
N
Time to maximum diastolic velocity (s)
Male WT chow
Male WT HFpEF
Male mTRG2 chow
Male mTRG2 HFpEF
Female WT chow
Female WT HFpEF
Female mTRG2 chow
Female mTRG2 HFpEF

Figure S2. Evaluation of contractile function in WT- and mTR^{G2}-HFpEF cardiomyocytes.

A and **H,** Representative tracings of cardiomyocyte contraction–relaxation in different experimental groups at 8-weeks and 16-weeks. **B** and **I,** Baseline sarcomere length at 8-weeks and 16-weeks. **C** and **J,** Percentage of cardiomyocyte shortening at 8-weeks and 16-weeks. **D** and **K,** Maximum diastolic velocity at 8-weeks and 16-weeks. **E** and **L,** Maximum systolic velocity at 8-weeks and 16-weeks. **F** and **M,** Time to maximum diastolic velocity at 8-weeks and 16-weeks. **G** and **N,** Time to maximum systolic velocity at 8-weeks and 16-weeks. n = 6 mice per group; n = 30 cardiomyocytes per mouse were used. Data are shown as mean ± SD. Kruskal-Wallis test followed by Dunn's multiple-comparisons test was used. *P < 0.05, **P < 0.01, ***P < 0.001 compared to chow group in the same genotype are shown.

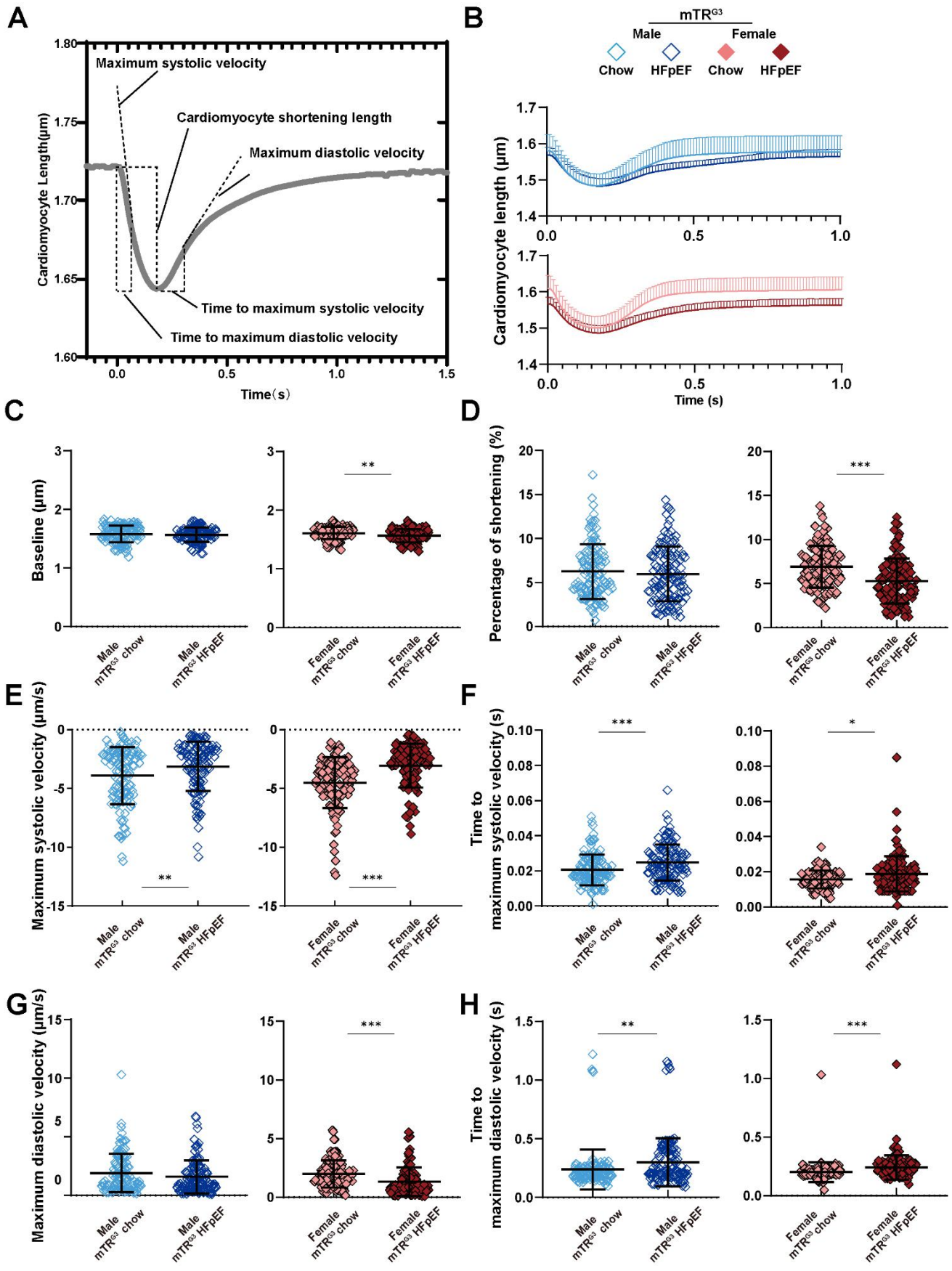
A
Maximum systolic velocity
Cardiomyocyte shortening length
Maximum diastolic velocity
Time to maximum systolic velocity
Time to maximum diastolic velocity
Cardiomyocyte Length(μm)
Time(s)
B
mTR^G3
Male
Female
Chow
HFpEF
Chow
HFpEF
Cardiomyocyte length (μm)
Time (s)
C
Baseline (μm)
D
Percentage of shortening (%)
E
Maximum systolic velocity (μm/s)
F
Time to maximum systolic velocity (s)
G
Maximum diastolic velocity (μm/s)
H
Time to maximum diastolic velocity (s)
Male mTR^G3 chow
Male mTR^G3 HFpEF
Female mTR^G3 chow
Female mTR^G3 HFpEF

Figure S3. Evaluation of contractile function in mTR^{G3}-HFpEF cardiomyocytes.

A, Ionoptix schematic diagram. **B,** Representative tracings of cardiomyocyte contraction–relaxation of each experimental group at 8-weeks. (**C** through **H**), Baseline sarcomere length, percentage of cardiomyocyte shortening, maximum systolic velocity, time to maximum systolic velocity, maximum diastolic velocity, and time to maximum diastolic velocity at 8-weeks are shown (n = 4 mice per group; n = 30 cardiomyocytes per mouse were used). Data are shown as mean ± SD. Mann-Whitney test was used. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 compared to chow group in the same genotype are shown.

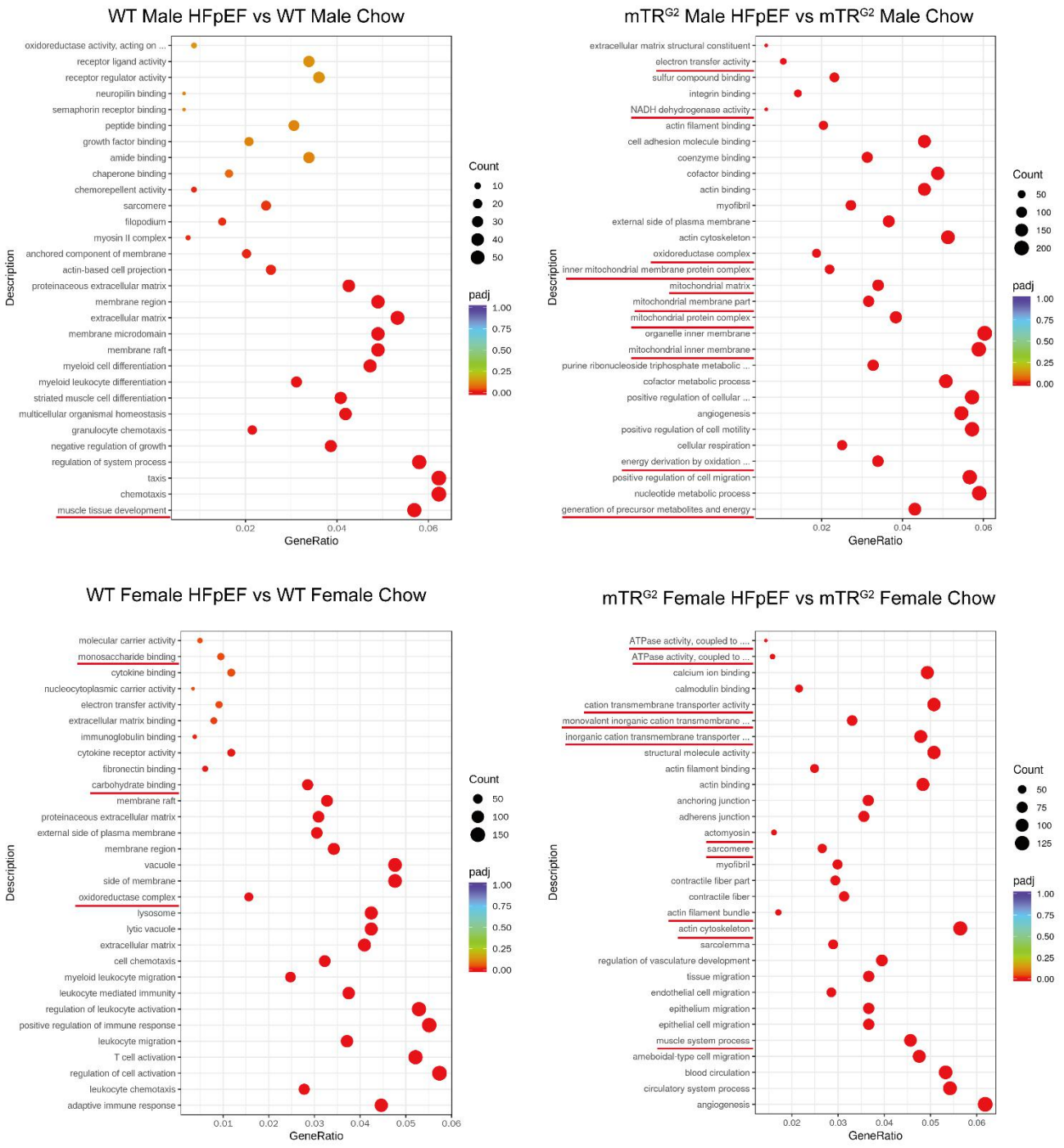

Figure S4. Gene ontology analysis of differentially expressed genes.

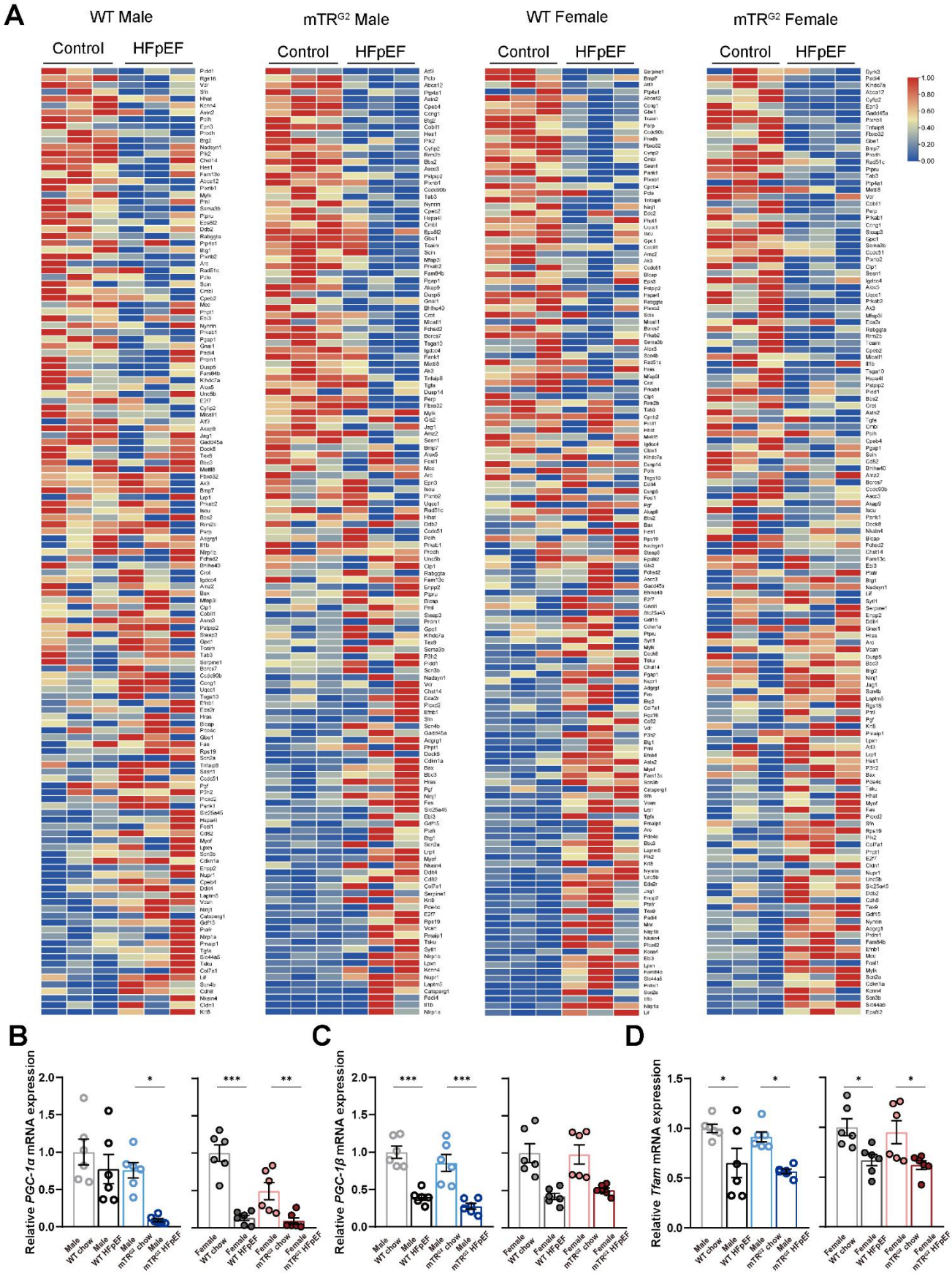
A
WT Male
mTRG2 Male
WT Female
mTRG2 Female
Control
HFpEF
1.00
0.80
0.60
0.40
0.20
0.00
B
C
D
Relative PGC-1α mRNA expression
Relative PGC-1β mRNA expression
Relative Tfam mRNA expression
Male WT chow
Male WT HFpEF
Male mTRG2 chow
Male mTRG2 HFpEF
Female WT chow
Female WT HFpEF
Female mTRG2 chow
Female mTRG2 HFpEF

Figure S5. Transcriptomic profiling of p53-mitochondrial related genes in HFpEF cardiomyocytes.

A, Heatmap of p53 downstream target genes are shown. (n = 3 per group). (**B** through **D**) mRNA levels of *Pgc1-α, Pgc1-β* and *Tfam* measured by RT-qPCR are shown as mean ± SEM (n = 6 per group). One-way ANOVA followed by lognormal processing and/or Tukey's multiple-comparisons test was used. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 compared to chow group in the same genotype are shown.

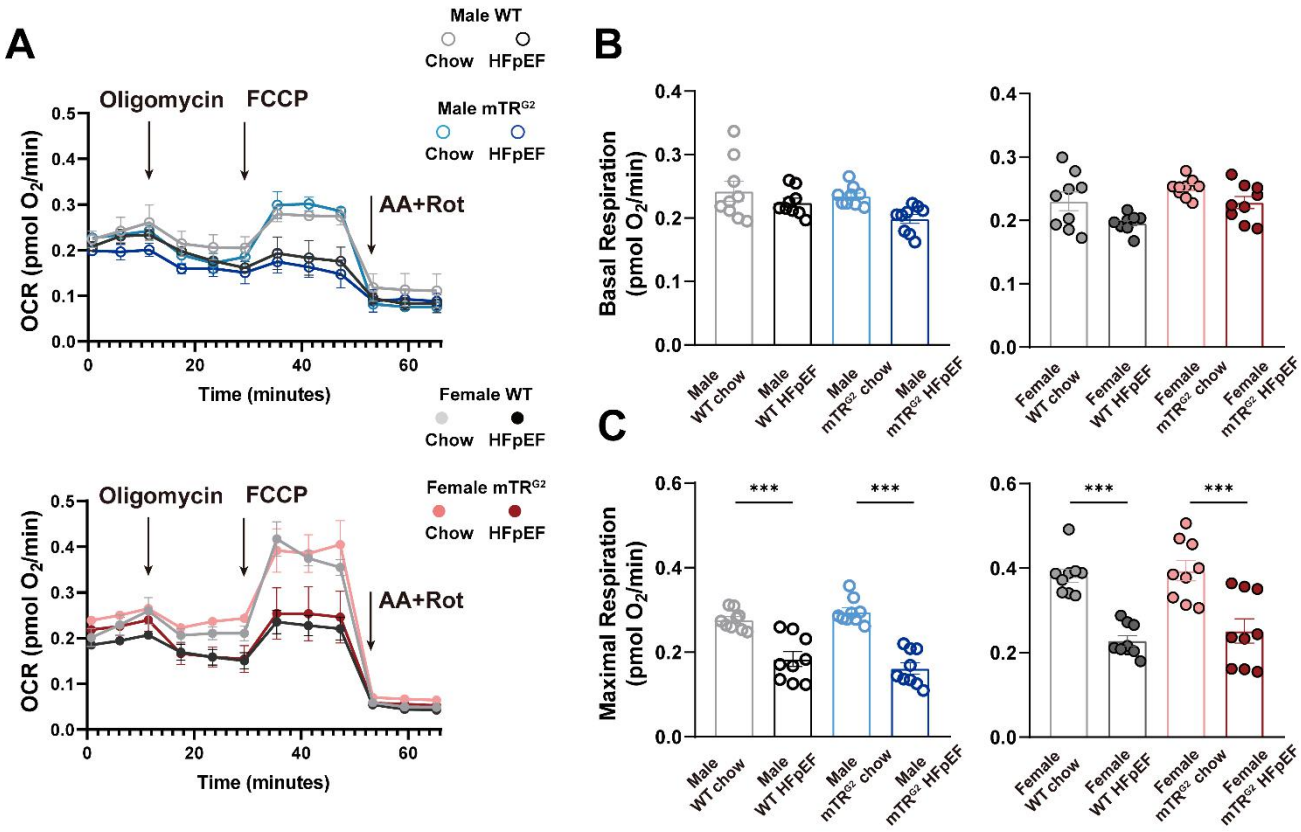

Figure S6. Evaluation of mitochondrial respiration of 16-weeks mTR^{G2}-HFpEF cardiomyocytes.

A, Real-time mitochondrial respiration of isolated AMVMs (n = 3 per group). **B** and **C,** Quantification of basal and maximal OCR of AMVMs. Results are shown as mean ± SEM (n = 3 per group). One-way ANOVA followed by lognormal processing and/or Tukey's multiple-comparisons test was used. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to chow group in the same genotype.

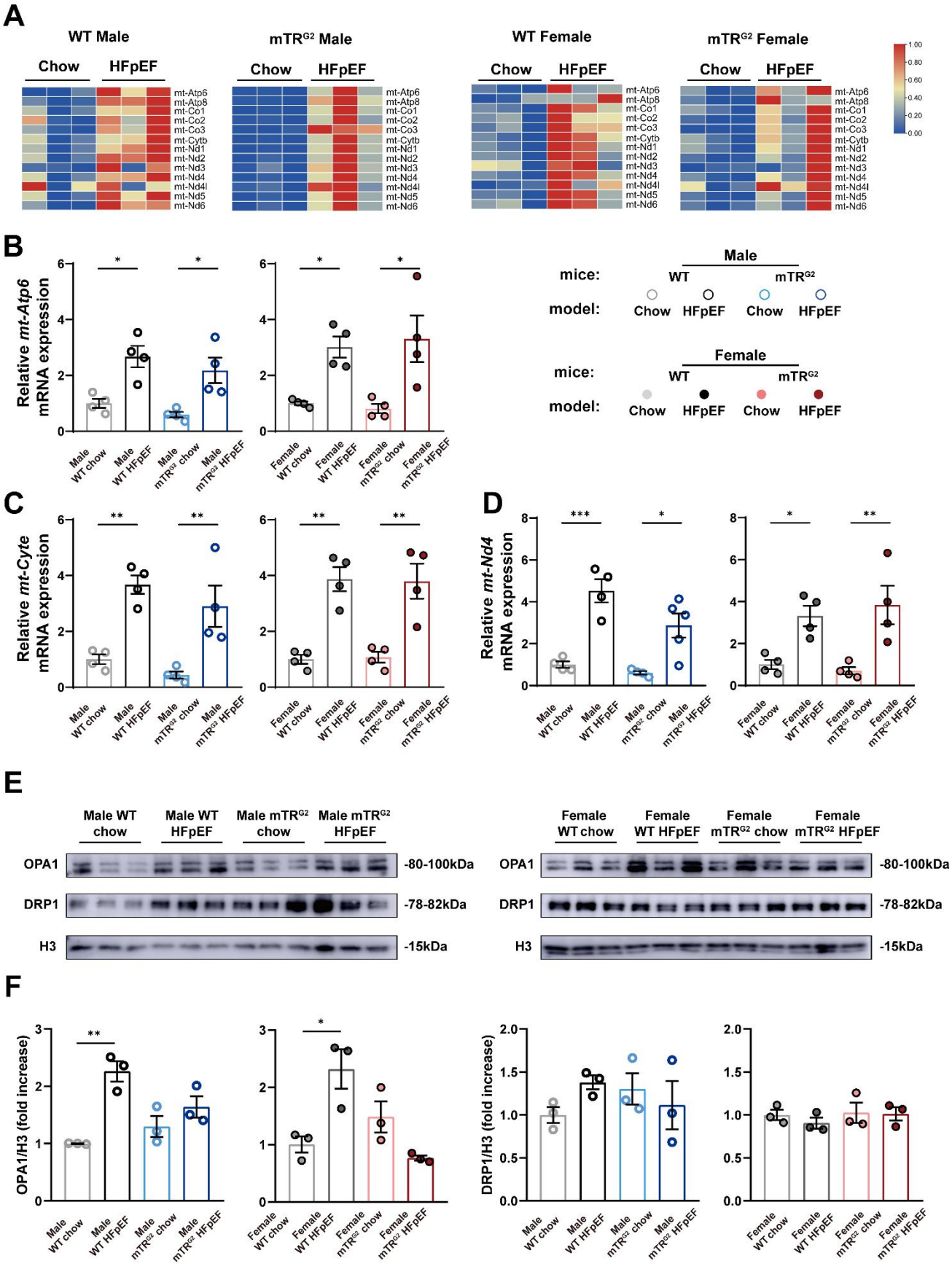
A
WT Male
mTR^G2 Male
WT Female
mTR^G2 Female
Chow
HFpEF
mt-Atp6
mt-Atp8
mt-Co1
mt-Co2
mt-Co3
mt-Cytb
mt-Nd1
mt-Nd2
mt-Nd3
mt-Nd4
mt-Nd4l
mt-Nd5
mt-Nd6
B
Relative mt-Atp6 mRNA expression
mice:
model:
Male
Female
WT
mTR^G2
Chow
HFpEF
C
Relative mt-Cyte mRNA expression
D
Relative mt-Nd4 mRNA expression
E
Male WT chow
Male WT HFpEF
Male mTR^G2 chow
Male mTR^G2 HFpEF
Female WT chow
Female WT HFpEF
Female mTR^G2 chow
Female mTR^G2 HFpEF
OPA1
DRP1
H3
-80-100kDa
-78-82kDa
-15kDa
F
OPA1/H3 (fold increase)
DRP1/H3 (fold increase)

Figure S7. Upregulation of mitochondrial DNA-encoding genes in 8-weeks mTR^{G3}-HFpEF cardiomyocytes.

A, Heatmap of mitochondrial DNA-encoding genes (n = 3 per group). (**B** through **D**), mRNA levels of *mt-Atp6*, *mt-Cyte* and *mt-Nd4* measured by RT-qPCR are shown as mean ± SEM (n = 6 per group). **E,** Representative images of OPA1 and DRP1 expression in AMVMs assayed by immunoblotting. Histone H3 used as loading control. **F,** Densitometric quantifications of OPA1 and DRP1 protein expression. Results are shown as mean ± SEM (n = 3 per group). One-way ANOVA followed by Tukey's multiple-comparisons test was used. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 compared to chow group in the same genotype are shown.

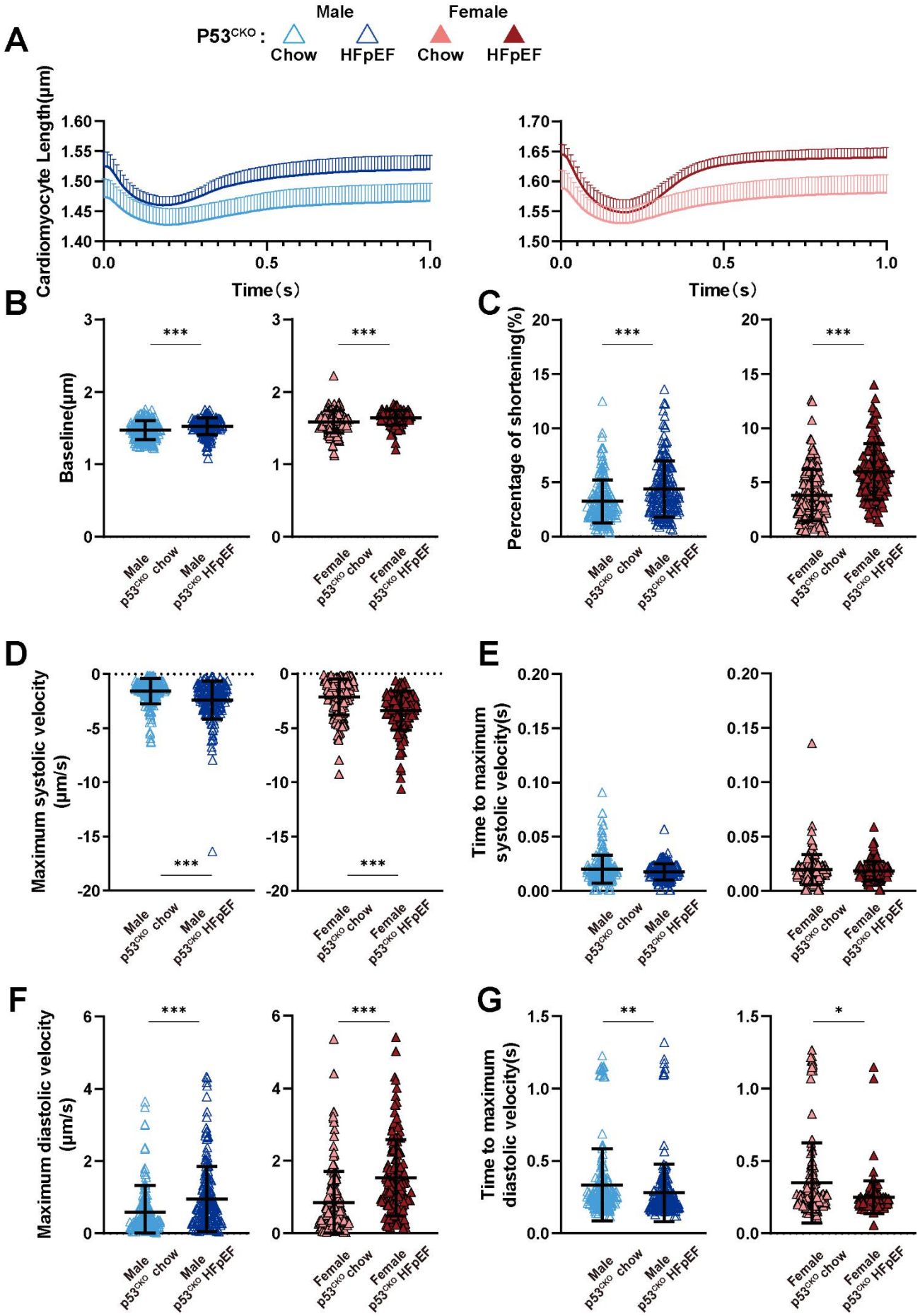
A
Male
Female
P53CKO :
Chow
HFpEF
Chow
HFpEF
Cardiomyocyte Length(μm)
Time(s)
B
Baseline(μm)
C
Percentage of shortening(%)
D
Maximum systolic velocity (μm/s)
E
Time to maximum systolic velocity(s)
F
Maximum diastolic velocity (μm/s)
G
Time to maximum diastolic velocity(s)
Male p53CKO chow
Male p53CKO HFpEF
Female p53CKO chow
Female p53CKO HFpEF

Figure S8. Evaluation of contractile function in p53^{CKO}-HFpEF cardiomyocytes.

A, Representative tracings of cardiomyocyte contraction–relaxation in different experimental groups. Results are shown as mean ± SEM. (**B** through **G**), Baseline sarcomere length, percentage of cardiomyocyte shortening, maximum systolic velocity, time to maximum systolic velocity, maximum diastolic velocity, and time to maximum diastolic velocity at 8-weeks are shown (n = 6 mice per male p53^{CKO} group, n = 5 per female p53^{CKO} group; n = 30 cardiomyocytes per mouse were used). Mann-Whitney test was used. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared to chow group are shown.