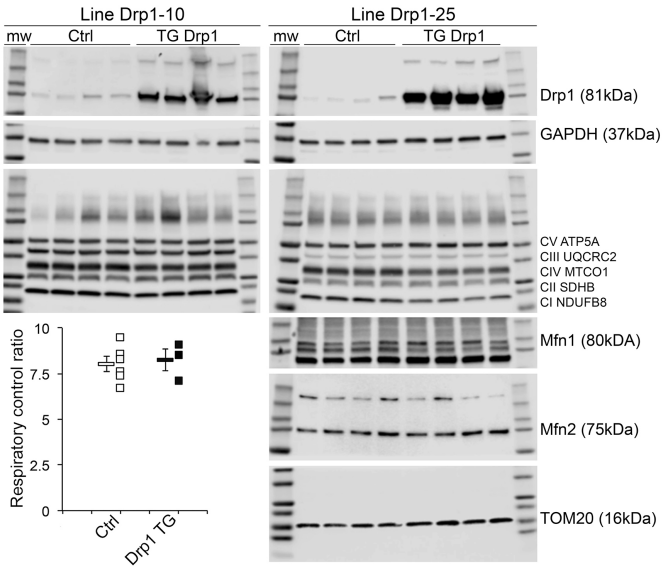

Figure S1. *Characterization of two lines of cardiac Drp1 transgenic mice, Related to Figure 1.* On the left are immunoblots and mitochondrial respiration studies from 10-fold normal expressing TG Drp1-10. On the right are immunoblot data from 25-fold normal expressing TG Drp1-25, which is the same line described in Figure 1. TG Drp1-25 results here are different mice than in Figure 1. TOM20 is translocase of outer mitochondrial membrane 20; multiple bands labeled CI-CV are respiratory complex subunits as annotated. No significant difference (*t*-test).

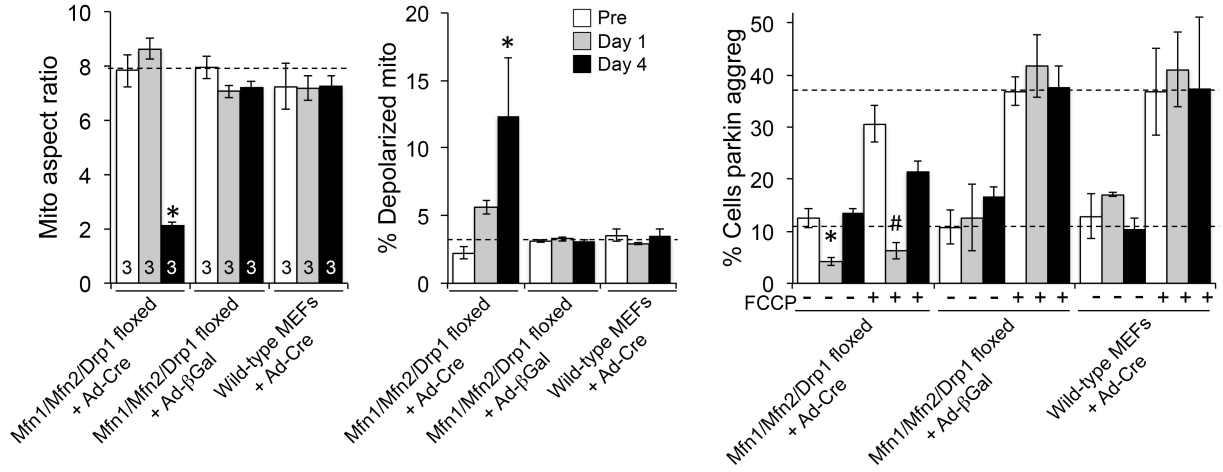

Figure S2. *Studies to control for non-specific adenoviral effects on mitochondria, Related to Figure 2.*
Mfn1/Mfn2/Drp1 triple floxed MEFs or control wild-type MEFs were infected with adenoviri encoding Cre-recombinase (Ad-Cre) or control βGal at 100 MOI. Mitochondrial aspect ratio (left), polarization status (middle) and parkin aggregation (right) were measured before (Pre; white), 1 (grey) and 4 days (black) after Ad-virus application. Mfn1/Mfn2/Drp1 triple floxed MEFs + Ad-Cre data are re-plotted from Figure 2 for comparison. N=3 each treatment condition. Control conditions did not alter any read-out.

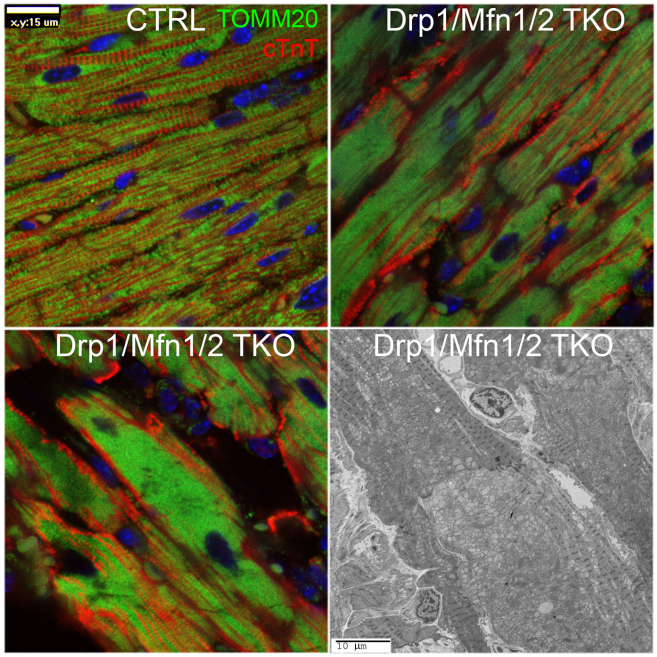

Figure S3. *Sarcomere and mitochondrial distribution in Mfn1/Mfn2/Drp1 TKO mouse hearts, Related to Figure 5.*
Fluorescence microscopy of cardiac Troponin T (red) and TOMM20 (green) stained Ctrl (left upper) or Mfn1/Mfn2/Drp1 TKO (right upper and left bottom) mouse hearts. Cardiac troponin T is an essential component of sarcomere; TOMM20 is a mitochondrial outer membrane protein. Right bottom, representative transmission electron micrograph of Mfn1/Mfn2/Drp1 TKO mouse hearts.

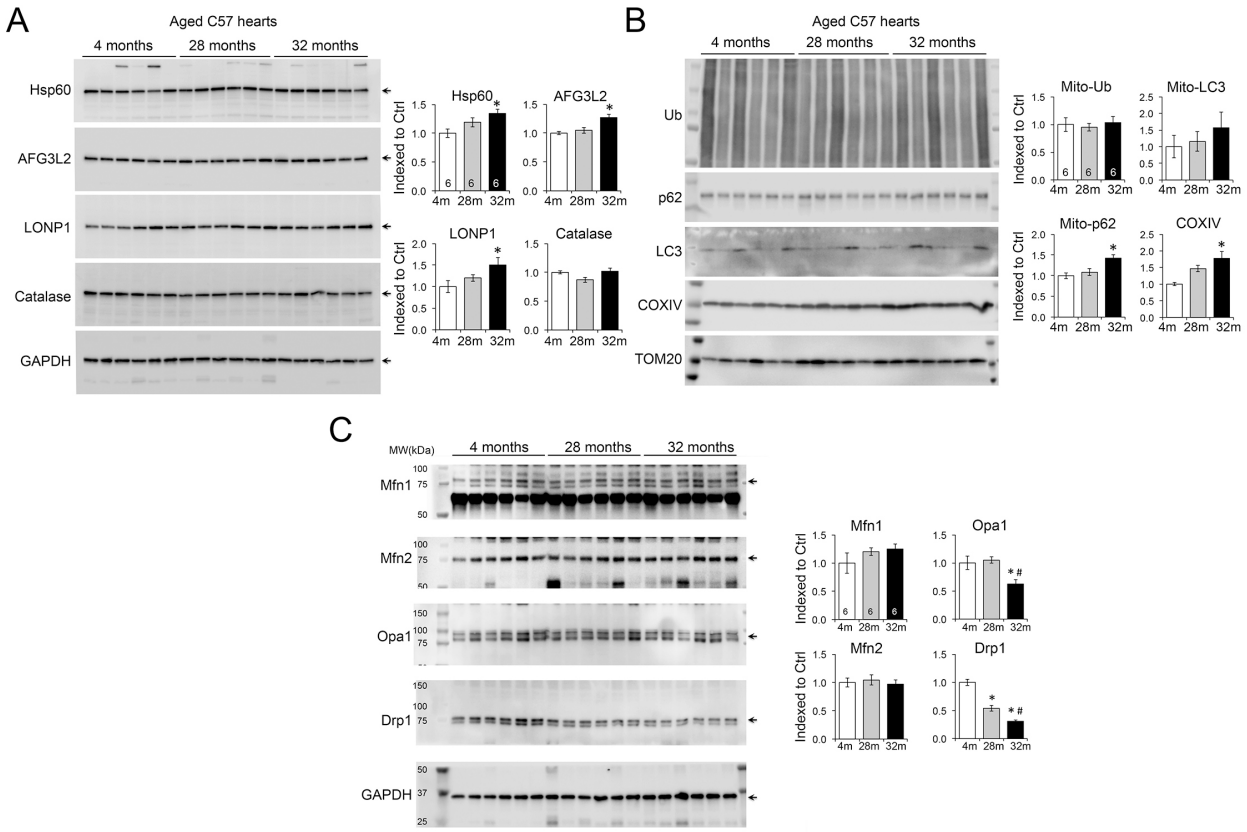

Figure S4. *Proteostasis, mitophagy and abundance of mitochondrial dynamics proteins in genetically normal aged mouse hearts, Related to Figure 7.*

(A) Induction of the mitochondrial unfolded protein response. Samples are myocardial homogenates; GAPDH is loading control. * is $p<0.05$ vs 4 month hearts (ANOVA).

(B) Mitophagy proteins in cardiac mitochondrial fractions. TOM20 is loading control. * is $p<0.05$ vs 4 month hearts (ANOVA).

(C) Mitochondrial dynamics proteins in myocardial homogenates. GAPDH is loading control. * is $p<0.05$ vs 4 month hearts; # is $p<0.05$ vs 28 month hearts (ANOVA).

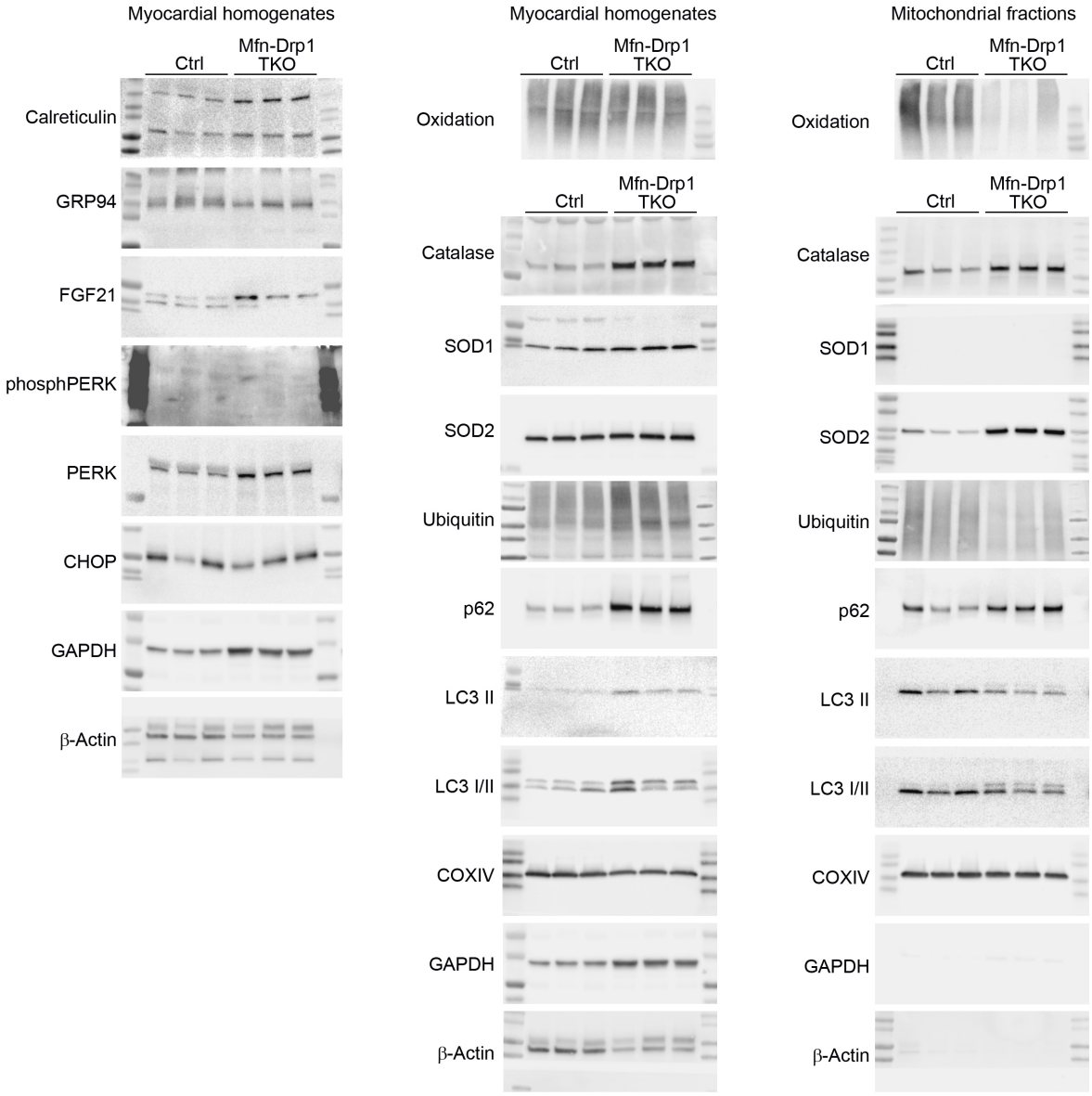

Figure S5. *ER stress and autophagy/mitophagy markers in Mfn1/Mfn2/Drp1 TKO mouse hearts, Related to Figure 7.*
(Left) Protein levels of ER stress markers in Mfn1/Mfn2/Drp1 TKO mouse hearts 16 weeks after gene ablation. *(Middle and right)* Autophagy and ROS markers in myocardial homogenates (middle) and mitochondria-enriched fractions (right) of Mfn1/Mfn2/Drp1 TKO mouse hearts 16 weeks after gene ablation. These independent studies reproduce and extend findings in the time course results presented in Figure 7.

Table S1. *qPCR primer sequences, Related to Figures 3 and 6.*

Oligonucleotides	SOURCE	IDENTIFIER
mtDNA forward primer: CCTATCACCCTTGCCATCAT	Chen et al., 2010	N/A
mtDNA Reverse primer: GAGGCTGTTGCTTGTGTGAC	Chen et al., 2010	N/A
Nuclear DNA forward primer: ATGGAAAGCCTGCCATCATG	Chen et al., 2010	N/A
Nuclear DNA Reverse primer: TCCTTGTTGTTCAGCATCAC	Chen et al., 2010	N/A
Tfam (Mm00447485_m1)	Thermo Fisher Scientific	Cat#4331182
PGC-1α (Mm01208835_m1)	Thermo Fisher Scientific	Cat#4331182
PGC-1β (Mm00504720_m1)	Thermo Fisher Scientific	Cat#4331182
PPARγ (Mm01184322_m1)	Thermo Fisher Scientific	Cat#4331182
ANP (Mm01255747_g1)	Thermo Fisher Scientific	Cat#4331182
BNP (Mm01255770_g1)	Thermo Fisher Scientific	Cat#4331182
SERCA (Mm01201431_m1)	Thermo Fisher Scientific	Cat#4331182
GAPDH (Mm03302249_g1)	Thermo Fisher Scientific	Cat#4331182