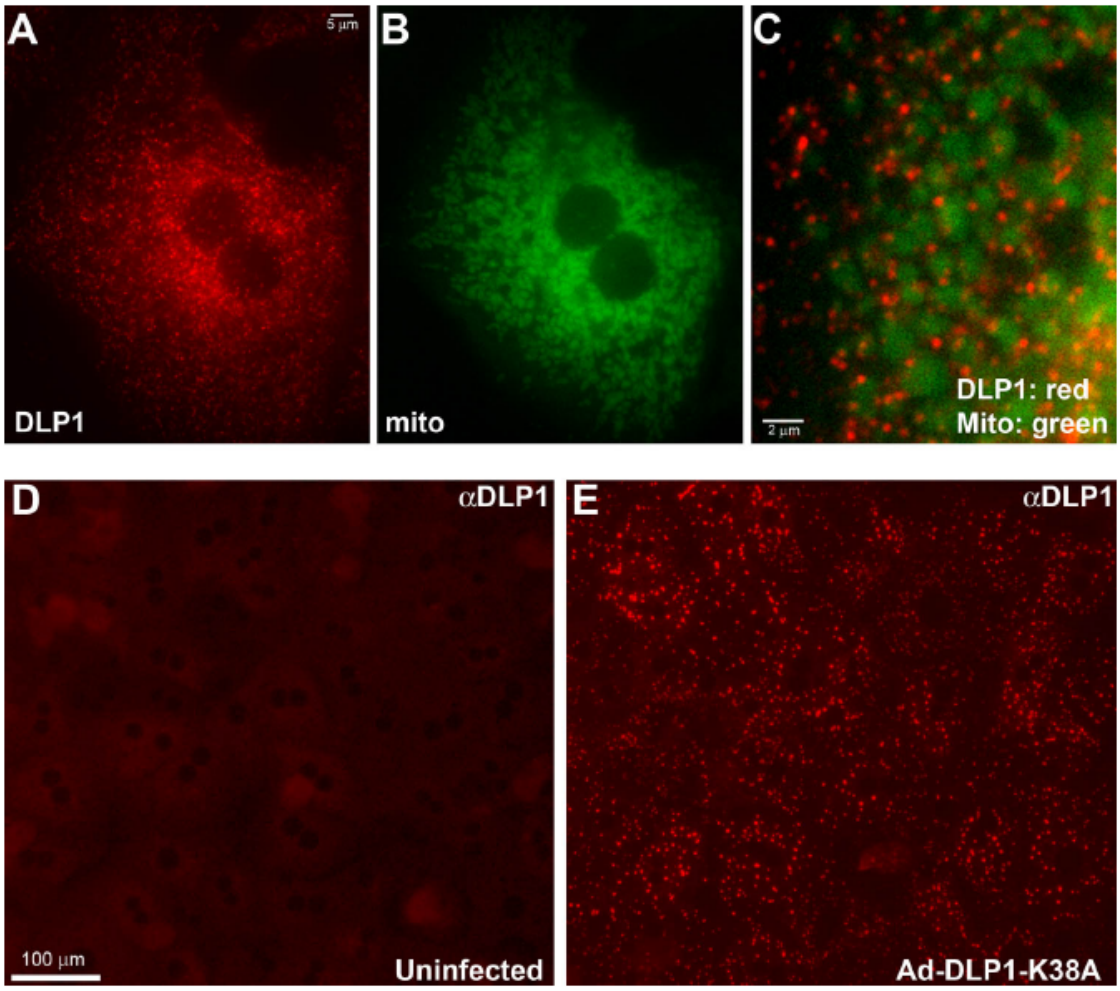
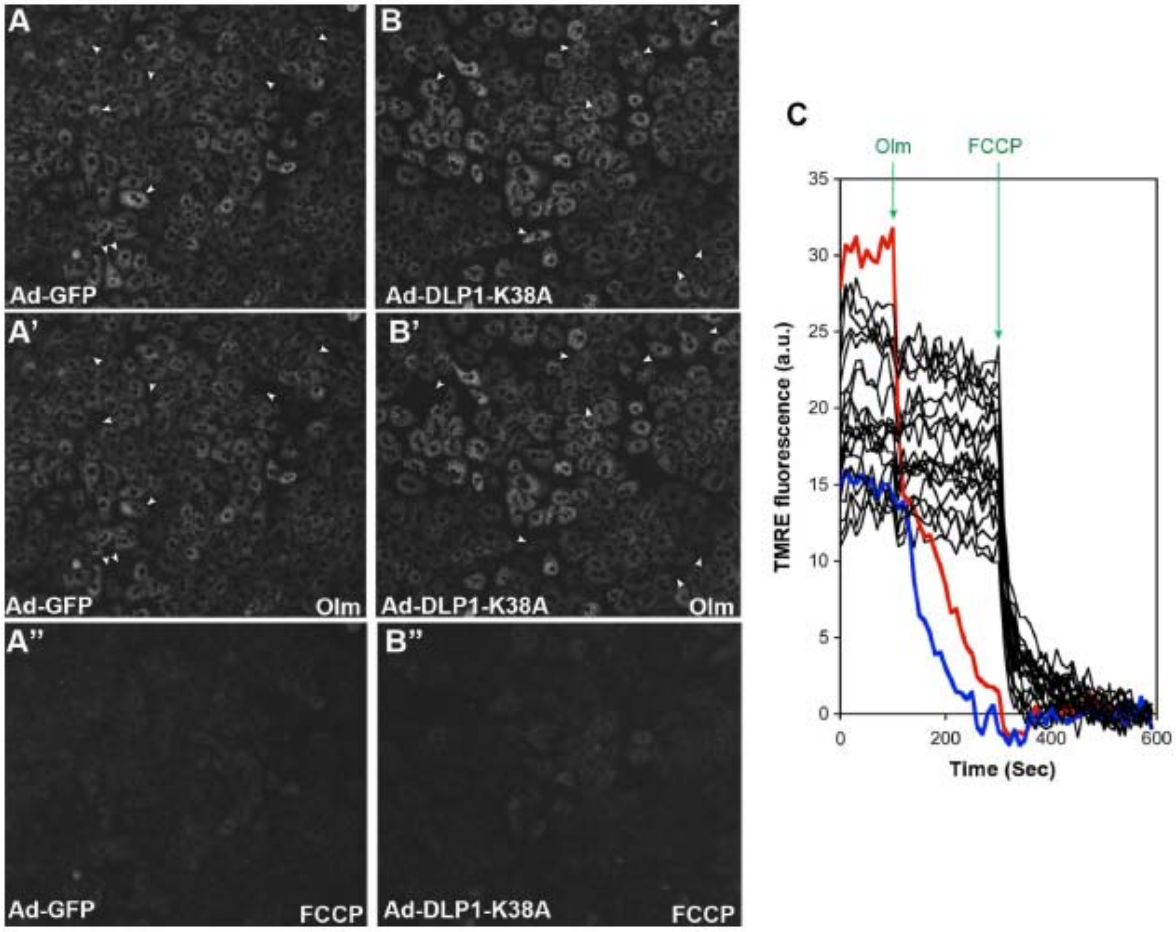
SUPPLEMENTARY DATA

Supplementary Figure 1. (A-C) DLP1 distribution in hepatocytes. Endogenous DLP1 distributes throughout the hepatocyte cytoplasm as punctate spots (A). The enlarged image overlaid with mitochondria (C) shows that DLP1 puncta are frequently associated with mitochondria. (D-E) Expression of DLP1-K38A. The dominant-negative DLP1 mutant was expressed in hepatocytes by adenoviral infection (Ad-DLP1-K38A; E). Bright DLP1 aggregates were formed in the cytoplasm of all cells in culture upon Ad-DLP1-K38A infection whereas no such aggregates were found with endogenous DLP1 in uninfected control cells (D).



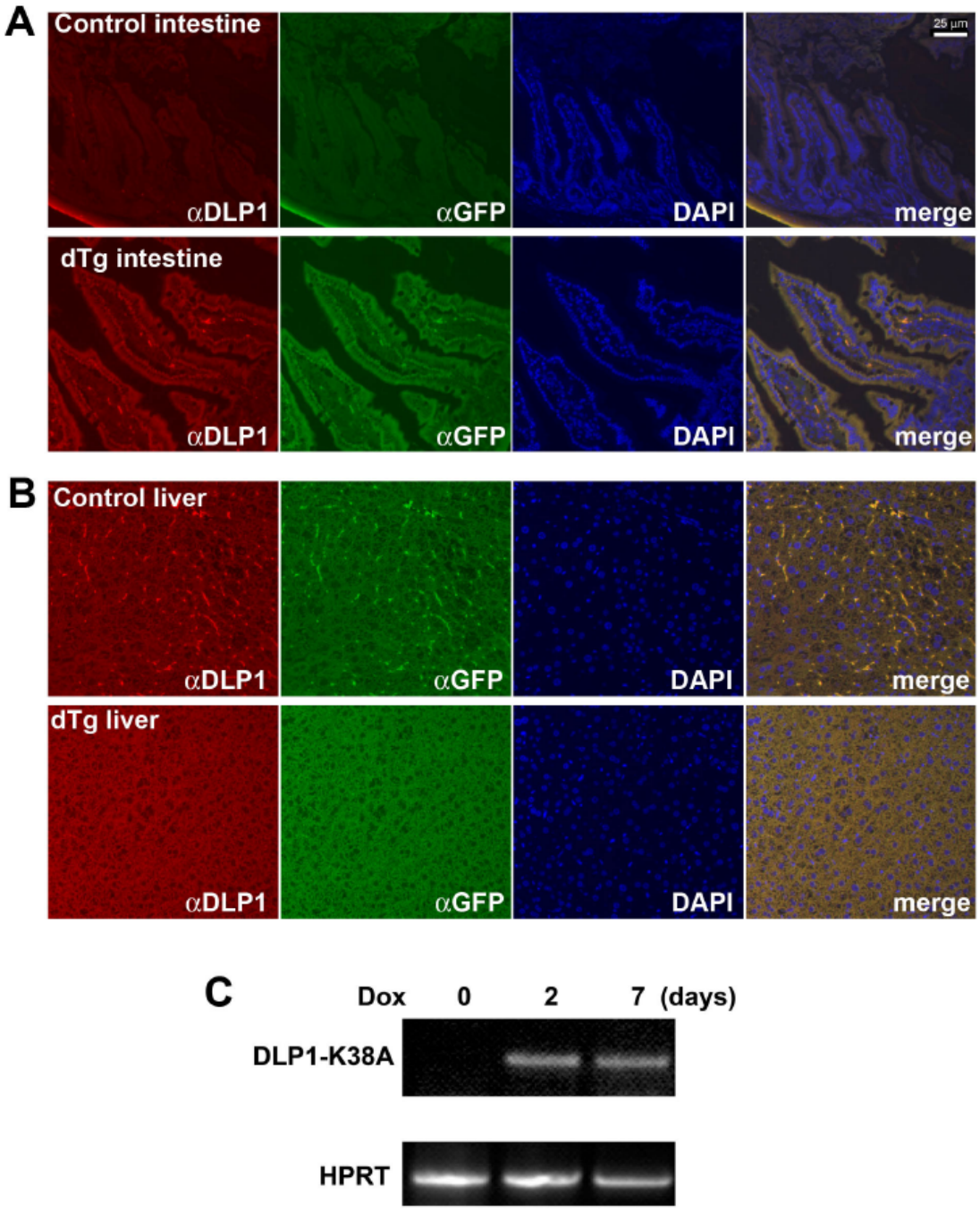
SUPPLEMENTARY DATA

Supplementary Figure 2. Oligomycin treatment had no significant effect on the mitochondrial inner membrane potential in cells expressing DLP1-K38A. Both control and DLP1-K38A-expressing cell cultures had similar numbers of cells showing a rapid loss of TMRE fluorescence upon oligomycin treatment (pointed by arrowheads in A, A' and B, B'). FCCP completely dissipated the inner membrane potential (A", B"). (C) TMRE fluorescence quantification in individual cells during sequential treatments of oligomycin and FCCP. A couple of cells display rapid loss of TMRE fluorescence upon oligomycin treatment (red and blue lines) whereas the rest of the cells maintain the normal membrane potential that dissipates with the FCCP treatment.



SUPPLEMENTARY DATA

Supplementary Figure 3. Transgene expression in dTg[rtTA/K38A]. (A and B) Tissue sections from control and dTg[rtTA/DLP1-K38A] Dox-fed for 2 days were immunostained with anti-DLP1 and anti-GFP antibodies. Increased fluorescence signals for DLP1 and GFP were observed in both intestine (A) and liver (B) from the dTg[rtTA/DLP1-K38A]. Note that the liver from control animals shows nonspecific fluorescent patches from residual blood in sinusoids, which are masked by an increased fluorescence signal in the dTg liver. (C) Kidney RT-PCR shows DLP1-K38A expression in dTg[rtTA/K38A] after 2 and 7 days of Dox induction.



SUPPLEMENTARY DATA

Supplementary Figure 4. Long-term low-level expression of DLP1 K38A does not result in detrimental systemic effects. Control and dTg[rtTA/K38A] mice were Dox-fed for 11 months. (A, B) Blood was collected by sub-mandibular draw from mice fasted overnight and serum samples were analyzed for lipid profile and liver panel. Values represent the mean and standard error (n=3; * n=2 for ALT and AST from dTg[rtTA/K38A]). (C, D) The relative abundance of HNE and nitrotyrosine modifications on serum proteins. Anti-HNE (C) and anti-nitrotyrosine antibodies (D) were used for immunoblot of serum proteins. Non-saturated immunoblot signals were normalized to total protein. Error bars are SEM. n = 3.

A

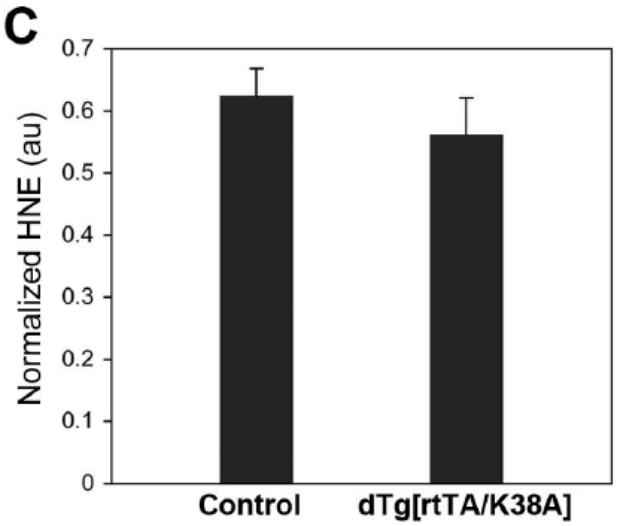
Lipid Profile	Cholesterol	Triglycerides	HDL
Control	74.0 ± 6.11	97.7 ± 5.17	74.7 ± 7.88
dTg[rtTA/K38A]	87.0 ± 4.58	102.3 ± 6.74	91.0 ± 4.00

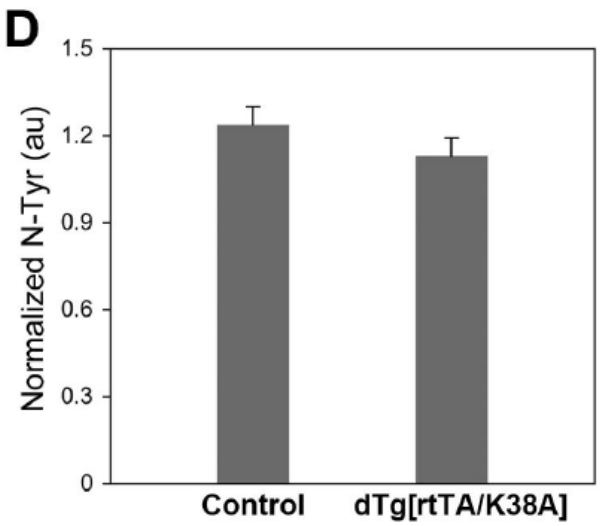
Values are in mg/dL

B

Liver Panel	ALP	AST	ALT	Albumin	Bilirubin (D)
Control	62.3 ± 1.86	84.0 ± 14.05	24.7 ± 0.33	3.4 ± 0.09	0.1
dTg[rtTA/K38A]	56.7 ± 6.17	92.0*	29.5*	3.6 ± 0.06	0.1

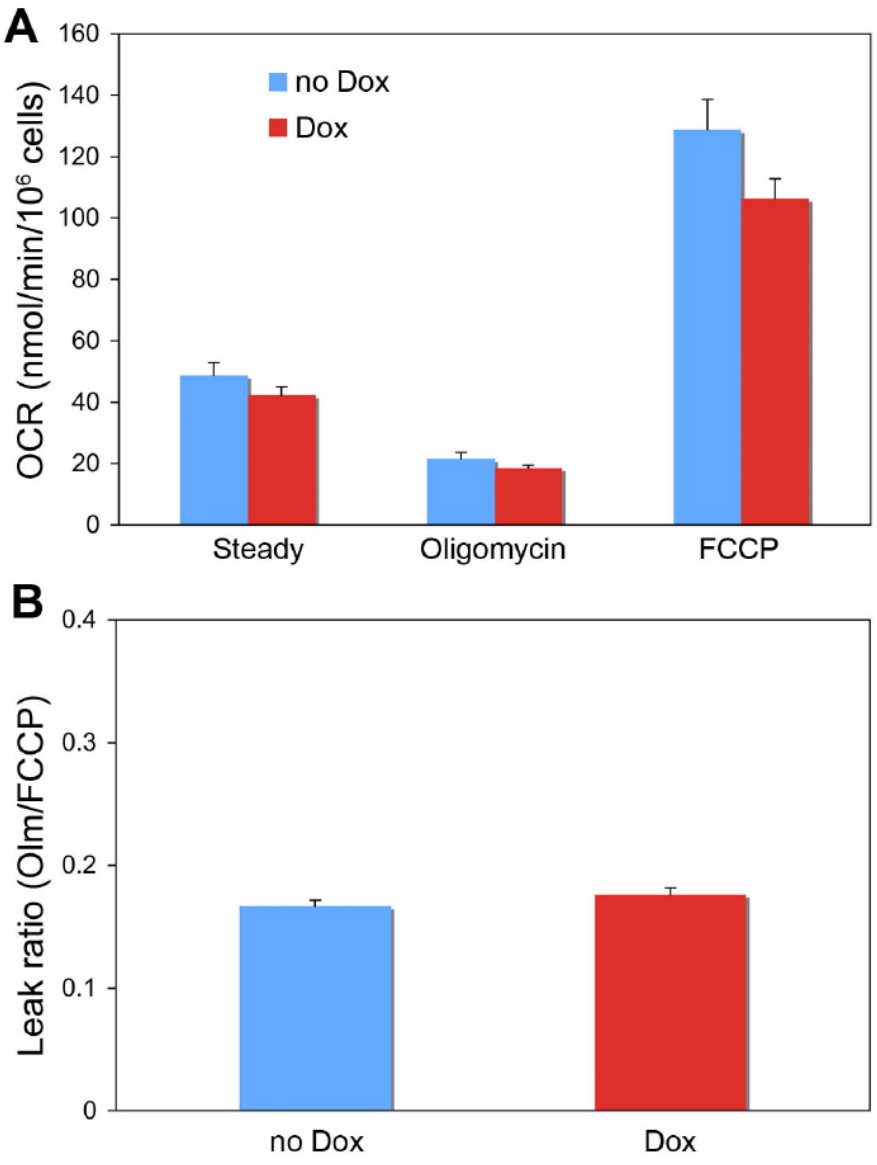
*ALP, alkaline phosphatase in U/L; AST, aspartate transaminase in U/L; ALT, alanine transaminase in U/L; albumin in g/dL; direct billirubin in mg/dL. * n = 2*





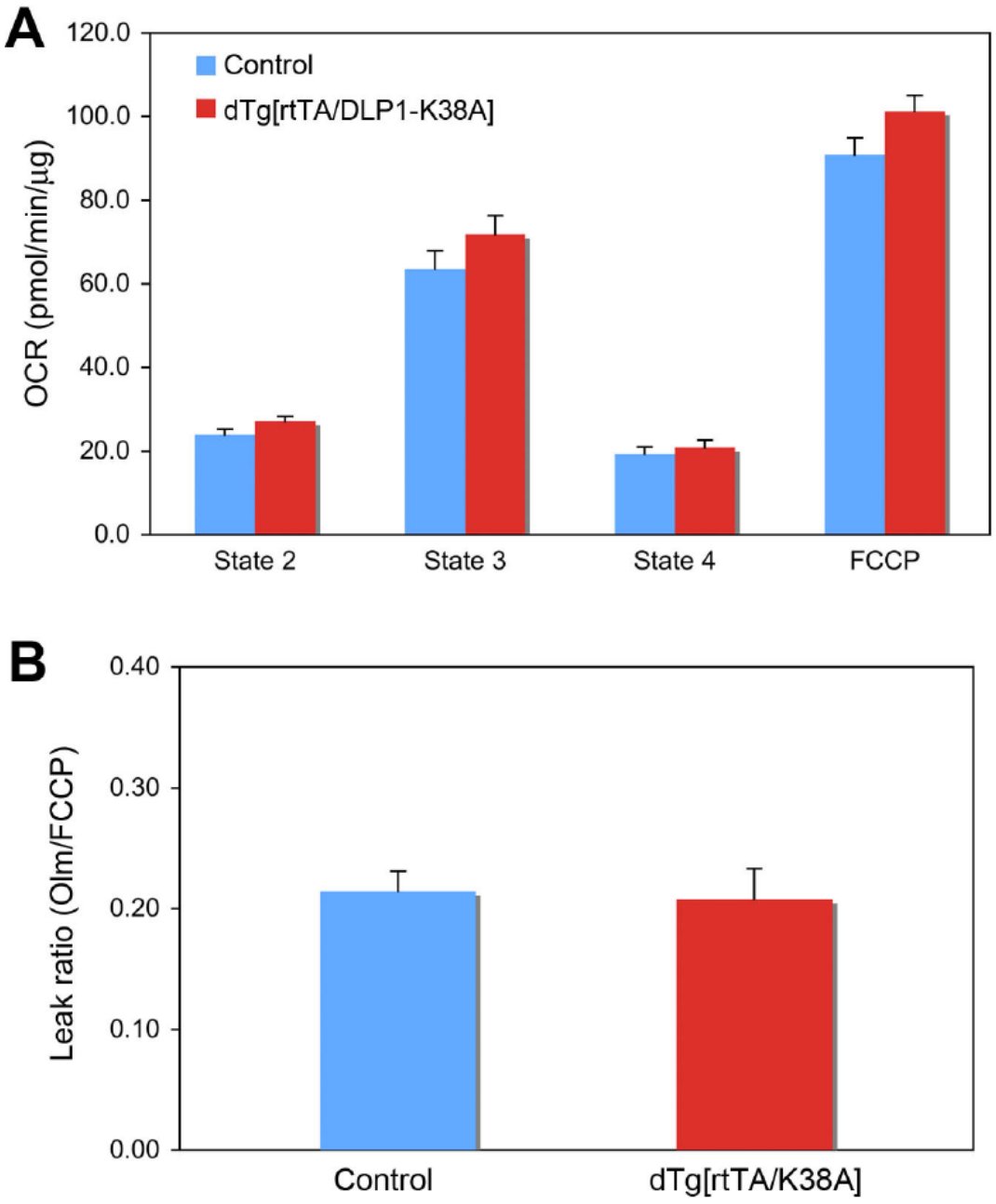
SUPPLEMENTARY DATA

Supplementary Figure 5. Dox administration alone has no effect on respiration in mice. Control single transgenic rtTA mice were chow- and Dox-fed for 4-5 days and OCR was measured 2 hours after hepatocyte isolation (A). The leak ratio indicates no difference between chow and Dox feedings (B). n = 11 for Dox-fed and 8 for normal chow-fed mice. Error bars represent SEM.



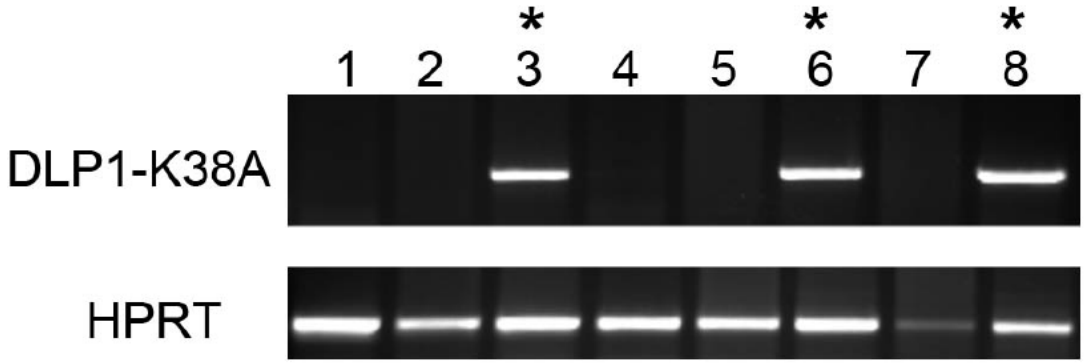
SUPPLEMENTARY DATA

Supplementary Figure 6. Isolated mitochondria from control and dTg[rtTA/DLP1-K38A] mice show no difference in the inner membrane proton leak. The OCR was measured in mitochondria isolated from kidney with pyruvate/malate as substrates. State 3 respiration was initiated by adding 200 μM ADP and state 4 respiration was measured after ADP depletion. (A) Mitochondria from control and dTg[rtTA/K38A] displayed no differences in OCRs throughout respiration states. (B) The calculated leak ratios showed no difference between control and dTg[rtTA/K38A]. n = 4. Error bars are SEM.

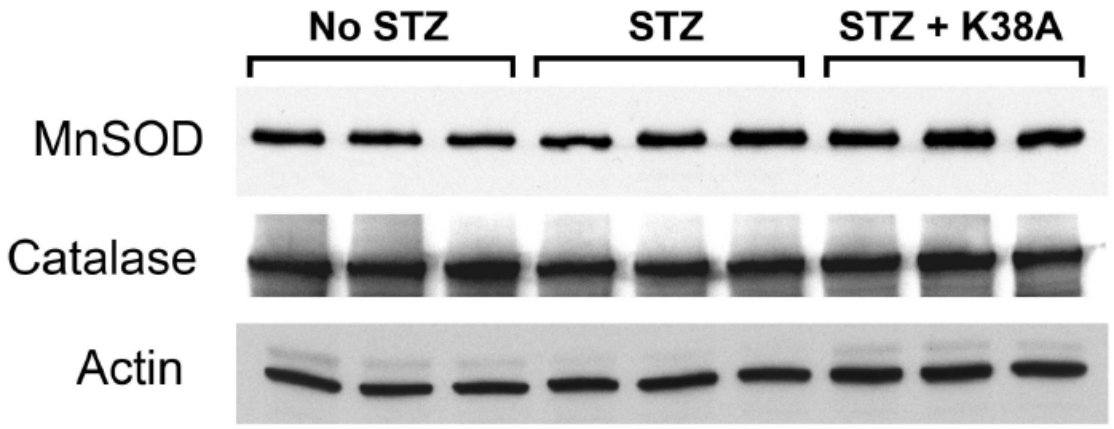


SUPPLEMENTARY DATA

Supplementary Figure 7. Transgene expression in diabetic dTg[rtTA/K38A] mice. Kidneys from mice at 3 weeks post STZ injection were subjected to RT-PCR: non-diabetic control (lane 7), diabetic control (lanes 1, 2, 4, 5), and diabetic dTg[rtTA/K38A] (lanes 3, 6, 8; asterisks) mice. DLP1-K38A expression was observed in diabetic dTg[rtTA/K38A] mice, indicating no effect of hyperglycemia on transgen expression.



Supplementary Figure 8. STZ treatment did not affect the level of MnSOD and catalase. Kidney extracts from control mice injected with buffer (No STZ) or STZ (STZ) and dTg[rtTA/K38A] injected with STZ (STZ+K38A) were analyzed for the level of antioxidant enzymes. Analyses of three mice from each group show no significant changes in the level of MnSOD and catalase.



SUPPLEMENTARY DATA

Supplementary Movie 1. Mouse hepatocytes expressing DLP1-K38A and control cells infected with Ad-GFP were labeled with TMRE and subjected to time-lapse imaging. Images were acquired at every 10 seconds for 100 frames. Movie plays at 5 frames/sec.

Supplementary Movie 2. TMRE flickering is independent of oligomycin. Hepatocytes expressing DLP1-K38A were treated with 10 mM oligomycin. Images were acquired at every 10 seconds for 100 frames. Movie plays at 5 frames/sec.

Supplementary Movie 3. Cyclosporine A, DIDS, 4-chlorodiazepam, and MnTMPyP do not block the TMRE flickering. Hepatocytes expressing DLP1-K38A were treated with 5 mM cyclosporine A, 100 mM DIDS, 30 mM 4-chlorodiazepam, or 50 mM MnTMPyP, and imaged for TMRE. Images were acquired at every 10 seconds for 60 frames. Movie plays at 5 frames/sec.

Supplementary Movie 4. Normal phenotype and behavior of diabetic dTg[rtTA/K38A]. Control and dTg[rtTA/K38A] mice were injected with vehicle or STZ. Mice were filmed at 3 weeks after the injection. Vehicle-injected control mice show normal behavior whereas STZ-injected Dox-fed control mice are sluggish and look sick. STZ-injected Dox-fed dTg[rtTA/K38A] show normal phenotype and behavior.