Supplemental Information
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Targeted Expression of Catalase to Mitochondria Prevents Age-Associated Reductions in Mitochondrial Function and Insulin Resistance

Hui-Young Lee, Cheol Soo Choi, Andreas L. Birkenfeld, Tiago C. Alves, Francois R. Jornayvaz, Michael J. Jurczak, Dongyan Zhang, Dong Kyun Woo, Gerald S. Shadel, Warren Ladiges, Peter S. Rabinovitch, Janine H. Santos, Kitt F. Petersen, Varman T. Samuel, and Gerald I. Shulman

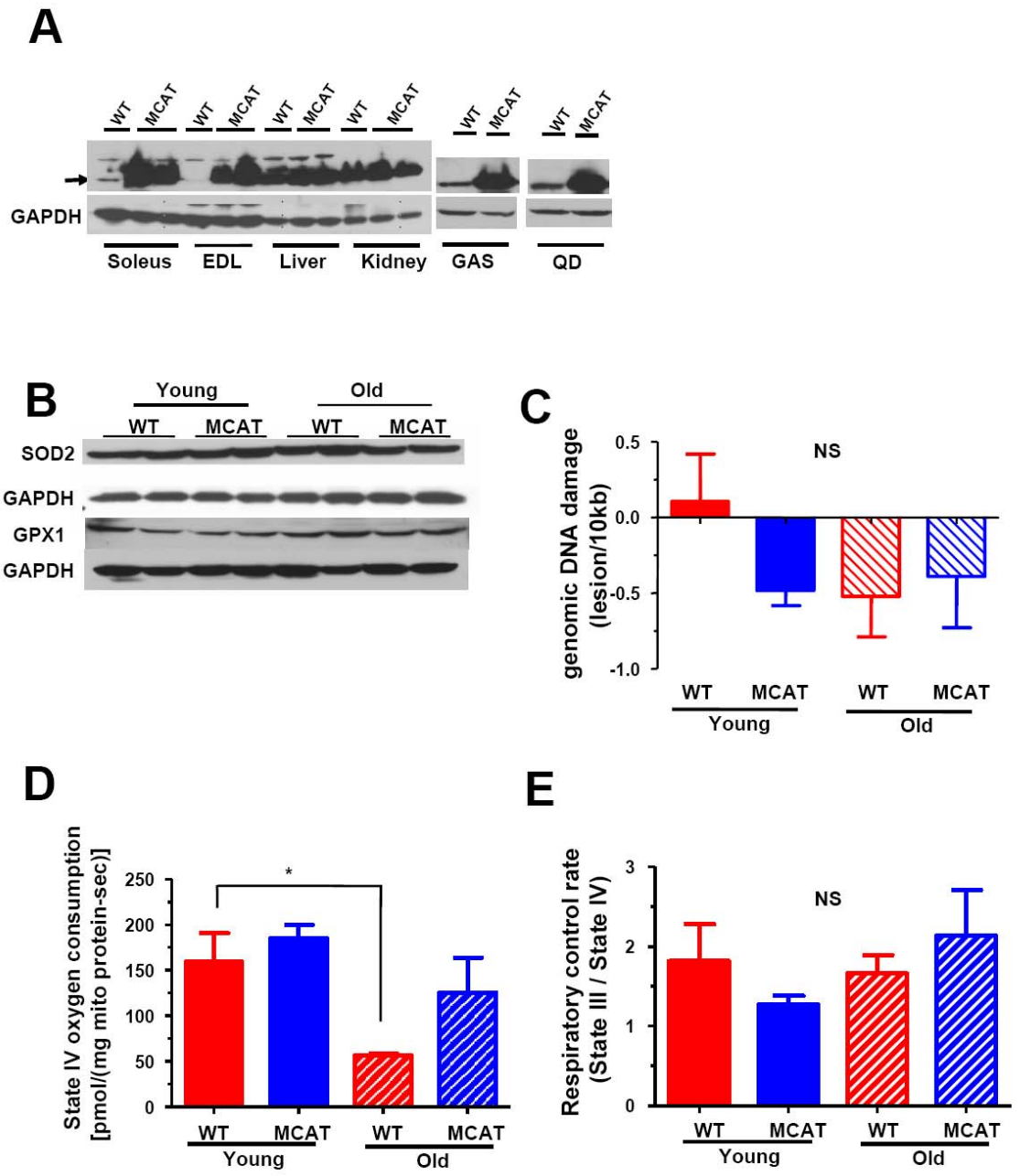

Figure S1, Related to Figure 1. Antioxidant Protein Expression, Genomic DNA Damage, and Mitochondrial Respiration

(A) Catalase protein expression in various tissues.

(B) Expression of superoxide dismutase 2 (SOD2) and glutathione peroxidase 1 (GPX1) in quadriceps muscle.

(C) Genomic DNA damage assessed by QPCR (n=4-6).

(D and E) State IV oxygen consumption (D) and respiratory control rate of isolated mitochondria from skeletal muscle (E) (n=6). All data are mean ± SEM. *P<0.05; NS, not significant by ANOVA.

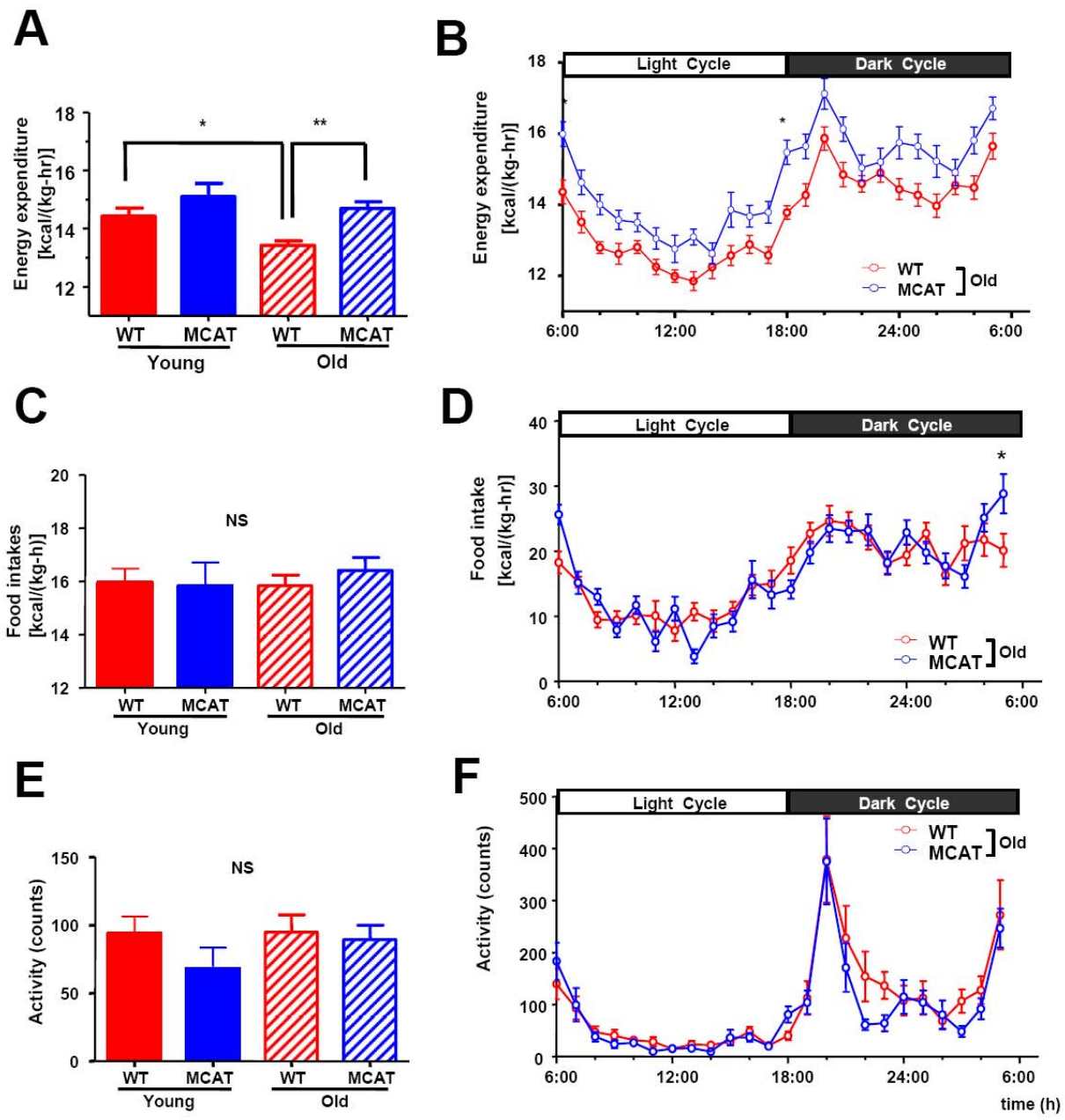

Figure S2, Related to Figure 2. Whole-Body Energy Expenditure, Food Intakes and Locomoter Activity

(A-F) Energy expenditure (A), food intake (C), and locomotor activity (E) during 72 hr analysis; hour to hour average energy expenditure (B), food intake (D), and locomotor activity (F) for old groups during light/dark period. N=8 in young groups; n=12-15 in old groups. All data are mean ± SEM. *P<0.05; NS, not significant by ANOVA.

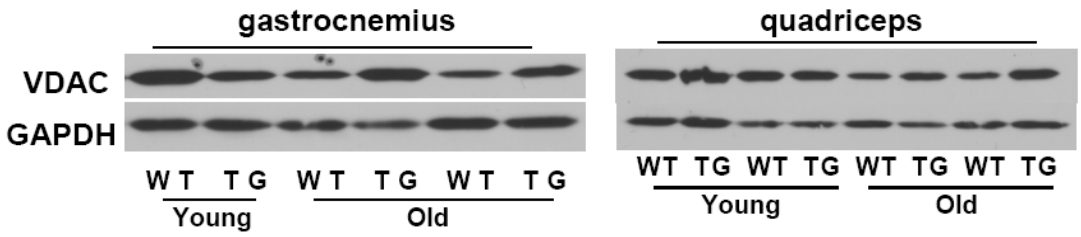

Figure S3, Related to Figure 4. VDAC Mitochondrial Membrane Protein Expression

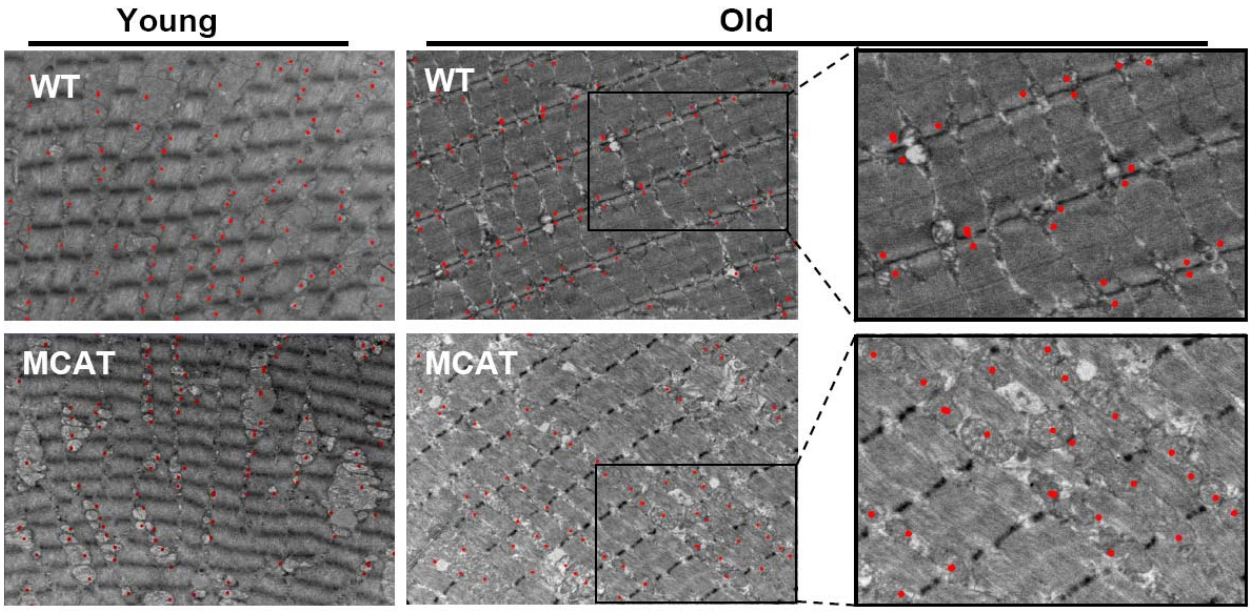

Figure S4, Related to Figure 4. EM of Muscle in Young and Old WT and MCAT Mice
In contrast to the young WT and old MCAT mice intramyofiblilar mitochondria are rarely found in A-band of the old WT mice and most of the mitochondria are singly located within the space between I band and Z-line, while young WT and MCAT mitochondria are located in A-band and connected together across the Z-line. Furthermore, the shape of old WT mitochondria are round or slightly elongated, while young WT and MCAT mitochondria are large and amorphous. Red spots denote mitochondria; far right panel is a close up view of the old WT and MCAT groups.

Table S1. Basal Characterization for Animals

	Young		Old		*P-value*	*P-value*
	WT (n=8)	MCAT (n=8)	WT (n=10)	MCAT (n=10)	Young WT vs. old WT	Old WT vs. old MCAT
Age (month)	6.6 ± 0.3	6.9 ± 0.3	15.8 ± 0.1	15.7 ± 0.2	<0.001	NS
Body weight (g)	32.3 ± 0.7	31.6 ± 0.5	34.9 ± 0.5	34.3 ± 1.2	<0.05	NS
Body fat (%)	12.5 ± 1.5	10.4 ± 1.1	13.6 ± 1.0	11.7 ± 1.8	NS	NS
Lean body mass (%)	73.9 ± 1.0	74.9 ± 1.1	72.4 ± 0.7	74.0 ± 1.4	NS	NS
Fasting glucose (mg/dl)	98.1 ± 2.3	103.1 ± 4.0	102.2 ± 4.0	99.1 ± 3.9	NS	NS
Fasting insulin (μU/ml)	3.9 ± 1.3	3.7 ± 1.8	5.7 ± 0.6	6.1 ± 1.8	NS	NS
Fasting FA (mg/dl)	1.5 ± 0.1	1.4 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	<0.05	NS
Fasting EGP [mg/(kg-min)]	9.5 ± 0.4	11.0 ± 0.4	11.5 ± 0.5	10.4 ± 0.4	<0.05	NS
Triglyceride in GAS muscle (mg/g tissue)	7.1 ± 0.9	5.1 ± 1.0	7.6 ± 0.9	5.7 ± 0.8	NS	NS

Data are expressed as mean values ± SEM. *P-value* evaluated by one-way ANOVA followed by post hoc analysis using the Bonferroni's Multiple Comparison Test. NS, not significant.

Table S2. Characterization of Animals during Hyperinsulinemic-Euglycemic Clamp Study

	Young		Old		*P-value*	*P-value*
	WT (n=8)	MCAT (n=8)	WT (n=10)	MCAT (n=10)	Young WT vs. old WT	Old WT vs. old MCAT
Clamped glucose (mg/dl)	126 ± 3.1	127 ± 4.3	122 ± 1.9	124 ± 1.6	NS	NS
Insulin (µU/ml)	50.7 ± 3.4	54.9 ± 0.1	67.4 ± 3.9	59.9 ± 0.2	<0.05	NS
FA (mg/dl)	0.35 ± 0.07	0.29 ± 0.03	0.37 ± 0.04	0.27 ± 0.04	NS	NS
FA suppression (%)	76 ± 4.7	77 ± 3.4	63 ± 3.3	73 ± 4.7	<0.05	0.09
EGP [mg/(kg-min)]	8.4 ± 1.0	8.7 ± 1.4	10.9 ± 1.1	9.0 ± 0.9	NS	NS
EGP suppression (%)	10.1 ± 12.8	22.4 ± 11.2	0.4 ± 14.4	12.9 ± 7.9	NS	NS
Glycolysis [mg/(kg-min)]	36.0 ± 4.0	29.1 ± 2.7	28.9 ± 1.4	31.8 ± 2.2	NS	NS
Glycogen synthesis [mg/(kg-min)]	23.3 ± 4.1	20.5 ± 2.7	9.3 ± 1.5	20.2 ± 3.13	<0.01	<0.05

Data are expressed as mean values ± SEM. *P-value* evaluated by ANOVA followed by post hoc analysis using the Bonferroni's Multiple Comparison Test. NS, not significant