

Supplementary Materials for

Obesity Increases Vascular Senescence and Susceptibility to Ischemic Injury Through Chronic Activation of Akt and mTOR

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Published 17 March 2009, *Sci. Signal.* **2**, ra11 (2009)

DOI: 10.1126/scisignal.2000143

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Fig. S1. Increased Akt and mTOR activation following a high-fat diet.

Fig. S2. Endothelial mitochondrial membrane potential.

Figure S1

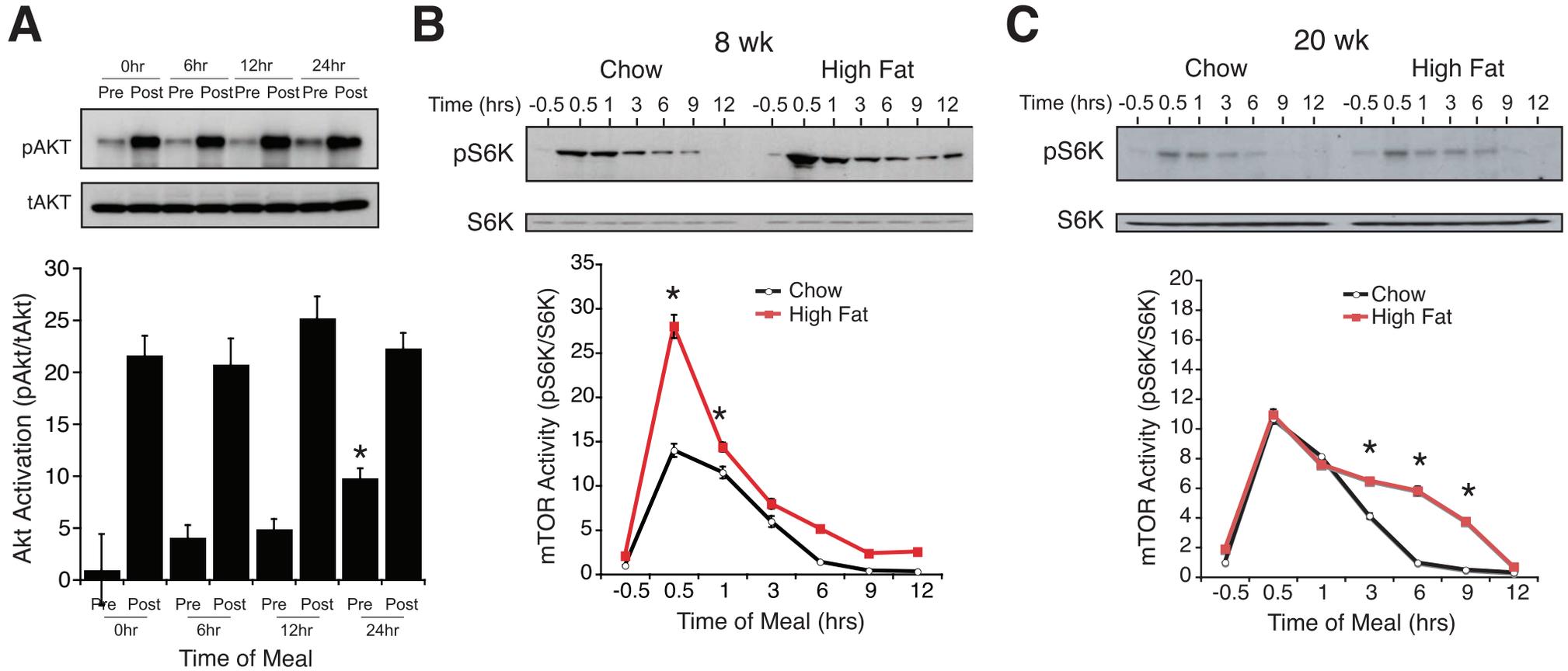
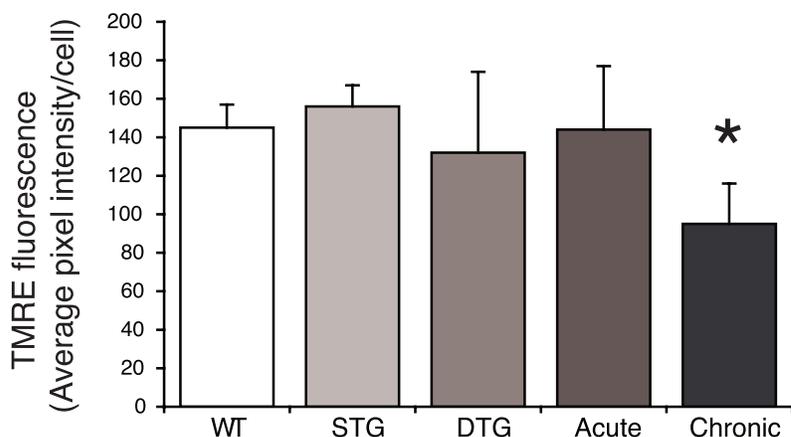


Fig. S1. Increased Akt and mTOR activation following high-fat diet.

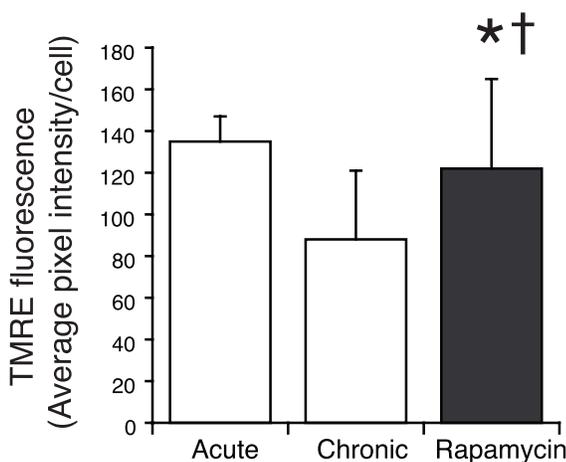
(A) Akt is activated to a greater extent after each high-fat diet meal. Aortic tissues were isolated from mice trained to receive 3 high-fat diet meals a day at 0, 6 or 12 hours. Akt phosphorylation was determined before (Pre) or after (Post) each meal. Each group contained 3 mice. * $P < 0.05$ when compared to value at 0 hr. **(B&C)** Immunoblots showing time-dependent effects of high fat or chow diet on S6K phosphorylation and pS6K/S6K ratio following feeding at 8 and 20 weeks. * $P < 0.05$ when compared to chow diet. Densitometric analyses of the ratio for each blot are shown in the bottom panels. Results are presented as mean \pm SD. Each lane represents one or two mice. Each experiment was performed three times.

Figure S2

A



B



C

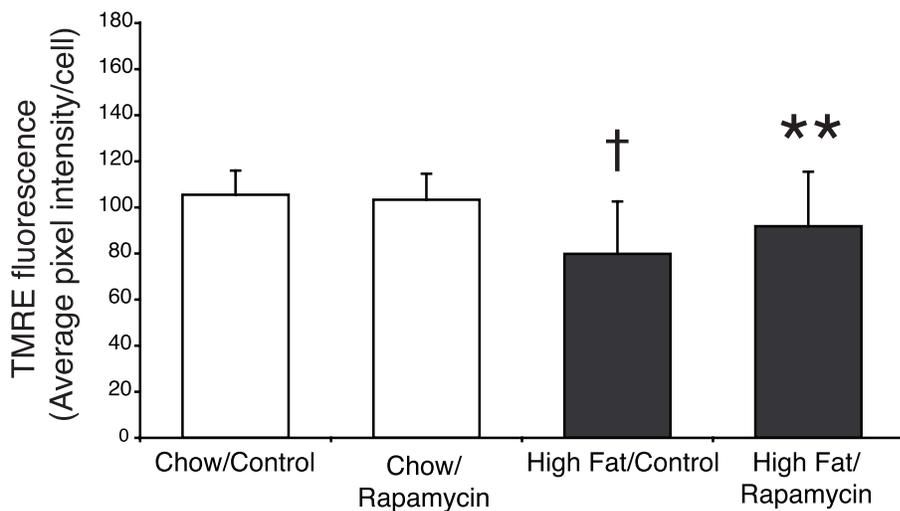


Fig. S2. Endothelial mitochondrial membrane potential.

Mitochondrial membrane potentials were analyzed in aortic endothelial cells from **(A)** WT, wild-type; STG, single transgenic mice (tet-myrAkt); DTG, double transgenic mice (VE-cadherin-tTA/tet-myrAkt) without induction and with short-term (ST) or long-term (LT) Akt induction. (* $P < 0.05$ when compared to DTG and DTG-ST), **(B)** Double mutant Akt mice (DTG) with short-term (DTG-ST) or long-term Akt activation with vehicle (DTG-LT) or rapamycin (DTG-LT/Rapa) treatment (* $P < 0.05$ when compared to DTG-LT, † $P = 0.23$ when compared to DTG-ST) and **(C)** Chow diet or High fat diet fed mice receiving rapamycin or vehicle (control) treatment (** $P < 0.05$ when compared to high fat fed group without rapamycin; † $P = 0.017$ when compared to chow diet fed group). Experiments were performed three times using the fluorescence probe, tetramethylrhodamine ethyl ester (TMRE, Molecular probes).