

Fig. S1. Effects of rapamycin on protein synthesis. (A) Western blot analysis of mTOR target S6K1. Flies were fed with rapamycin and extracts were made from whole flies. Antibodies used were against p-S6k1 and dS6K1. (B) Level of ^{35}S -methionine incorporation in proteins located in the cytosol and in the mitochondria of flies treated with rapamycin and vehicle control (ethanol). * $p < 0.05$ versus control as determined by t-test. t-test [$t = 3.4572$, $df = 6$, $p\text{-value} = 0.01351$]. (C) Amount of nuclear DNA and mitochondrial DNA in flies treated with rapamycin and vehicle control (ethanol).

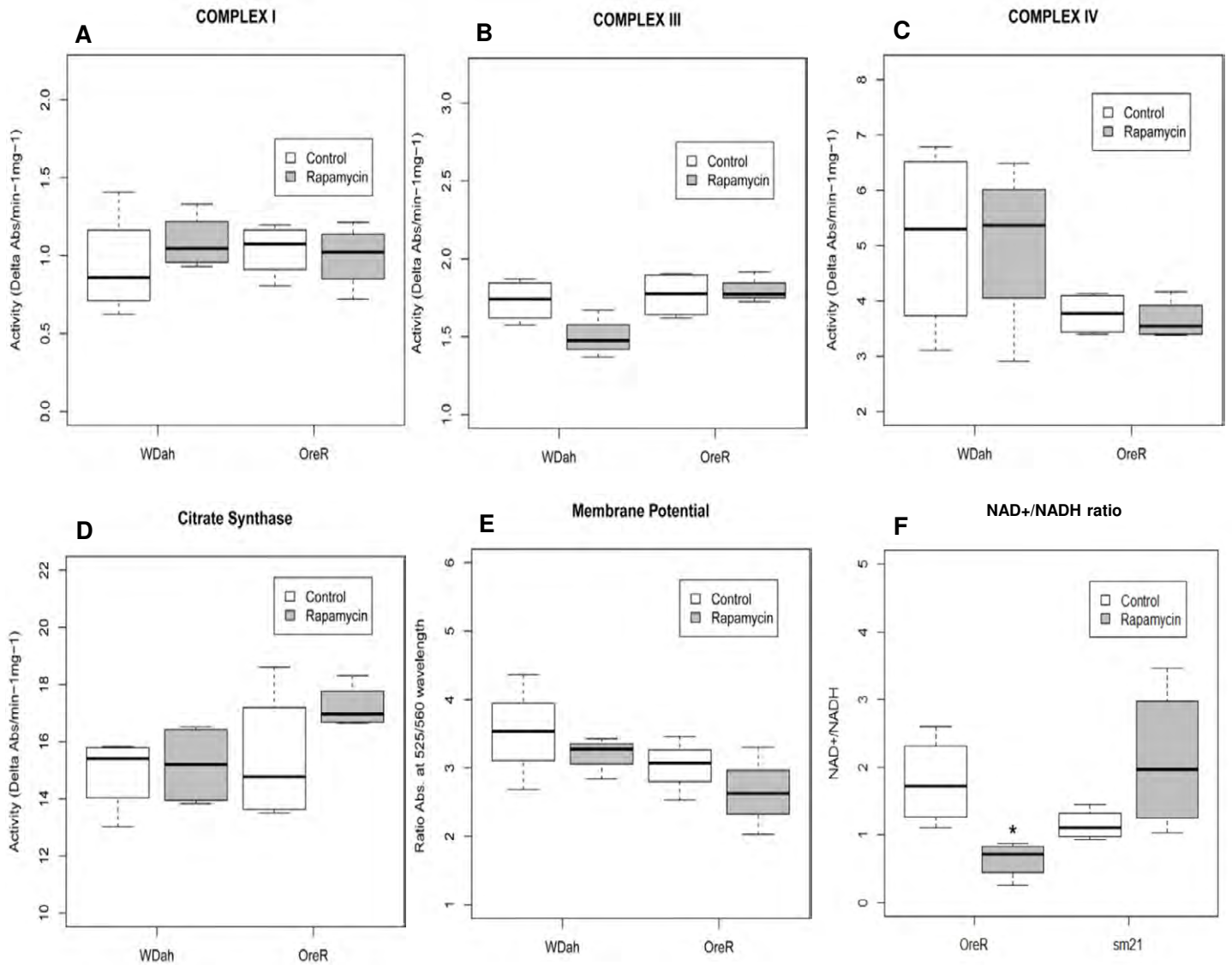


Fig. S2. Effects of rapamycin on ETC complexes, citrate synthase, mitochondrial membrane potential and NAD⁺/NADH ratio. Related to Figure 1. Enzymatic activity of complex I (A), complex III (B), and complex IV(C), citrate synthase (D) in isolated mitochondria from *w^{Dah}* and *OreR* flies treated with rapamycin or vehicle control for 10 days. Enzymatic activity was normalized to sample protein content. (E) mitochondrial membrane potential (measured as a ratio of fluorescence at 525/590 emission wavelength (monomer/dimer of JC-1 protocol) higher membrane potential have higher 525/590 ratios. (F) (Ratio between NAD⁺ and NADH in *OreR* and *sm21* mitochondria isolates from flies treated with rapamycin or vehicle control for 10 days. **p* < 0.025 versus control as determined by t-test.

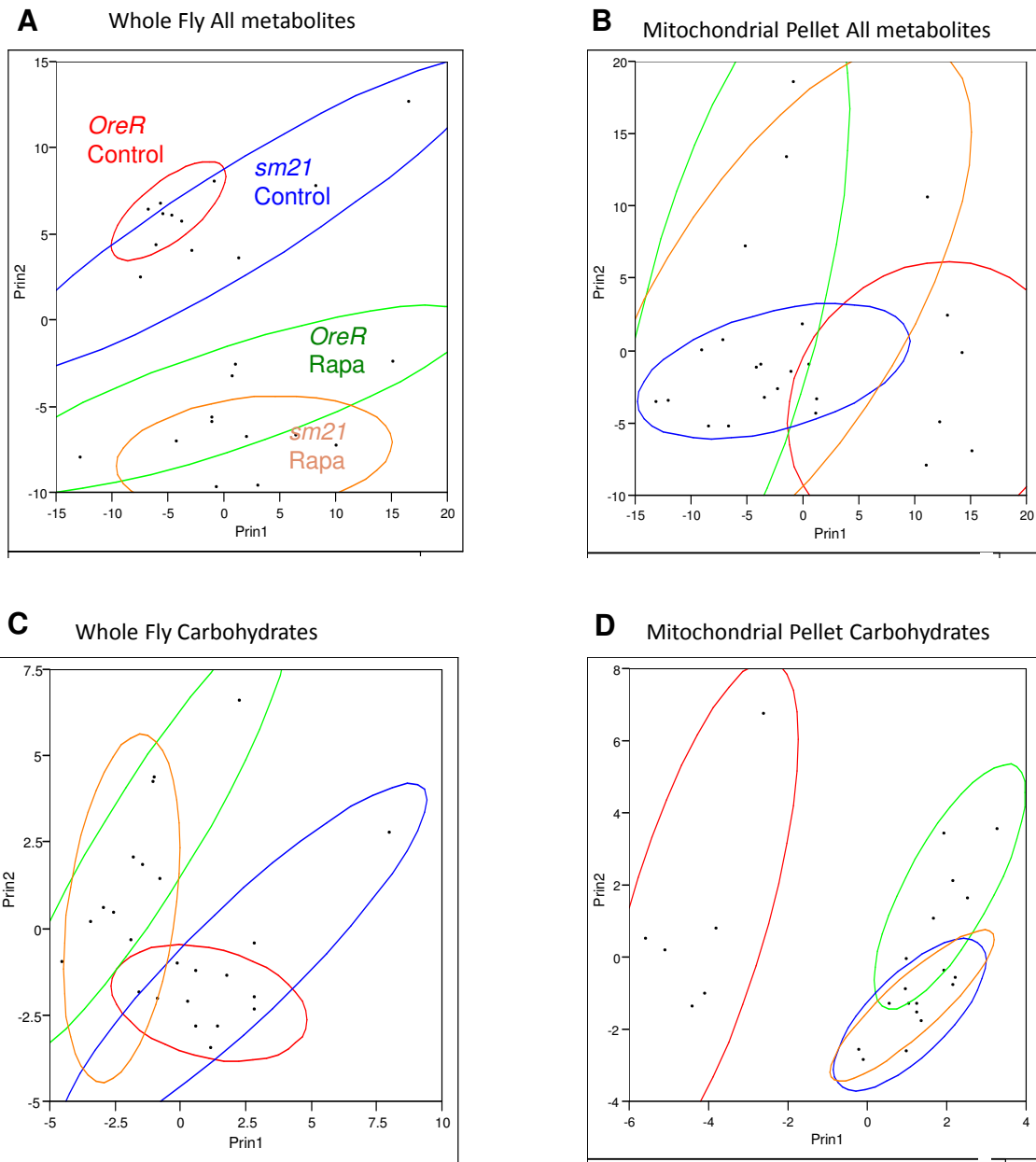


Fig. S3. PCA analysis using default options of Multivariate Analyses of the statistical software JMP. Principle components were extracted from the correlation matrices for each plot, using the same input data set as used Figure 3, 4 and 5. Some factor axes are reverse relative to Figure 3A and 5A, but all points are in same relative positions. Ellipses are fit as bivariate normal distributions defining 95% confidence limits of the six replicate samples for each genotype-rapamycin treatment. Non-overlapping ellipses can be taken as significantly different samples in metabolite space.

Table S1. List of metabolites described in Figs 3, 4 and 5

[Download Table S1](#)

Table S2. List of PCA summaries from Figs 3, 4 and 5

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