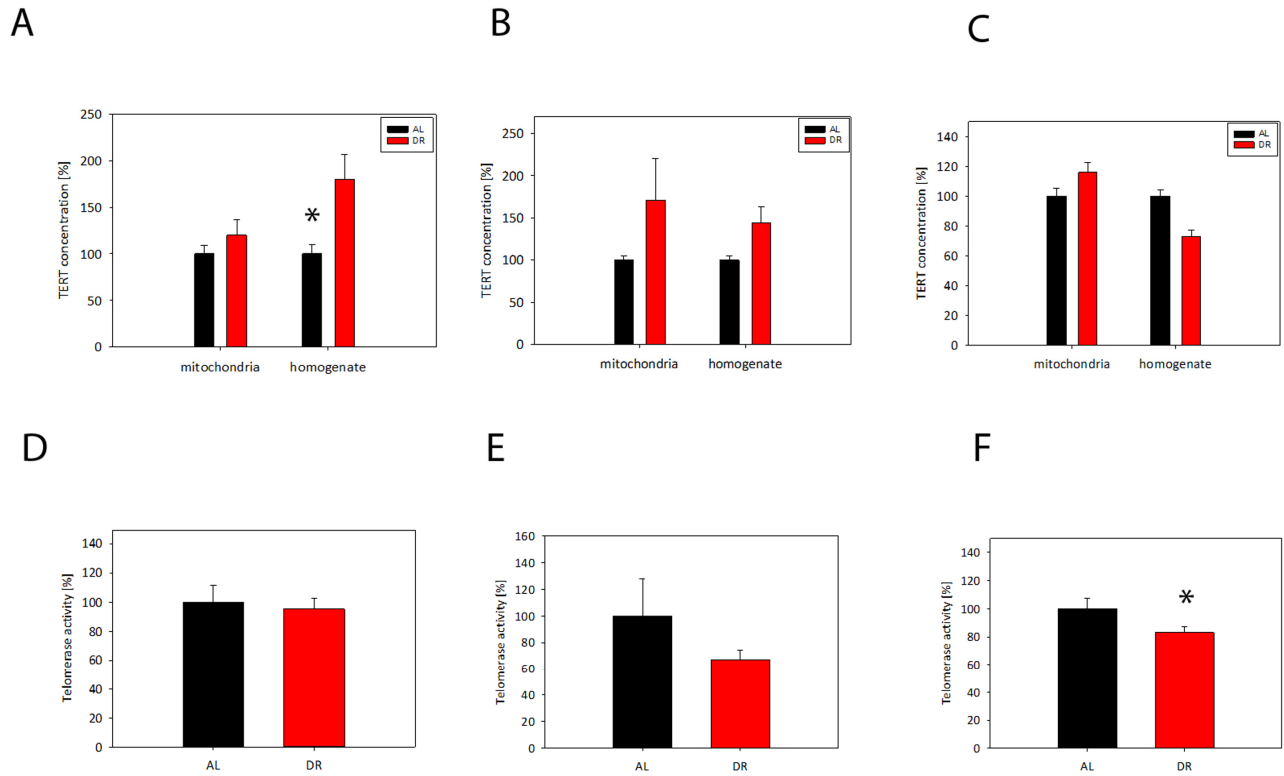
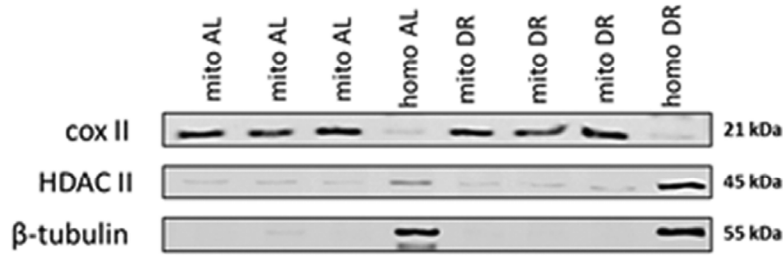


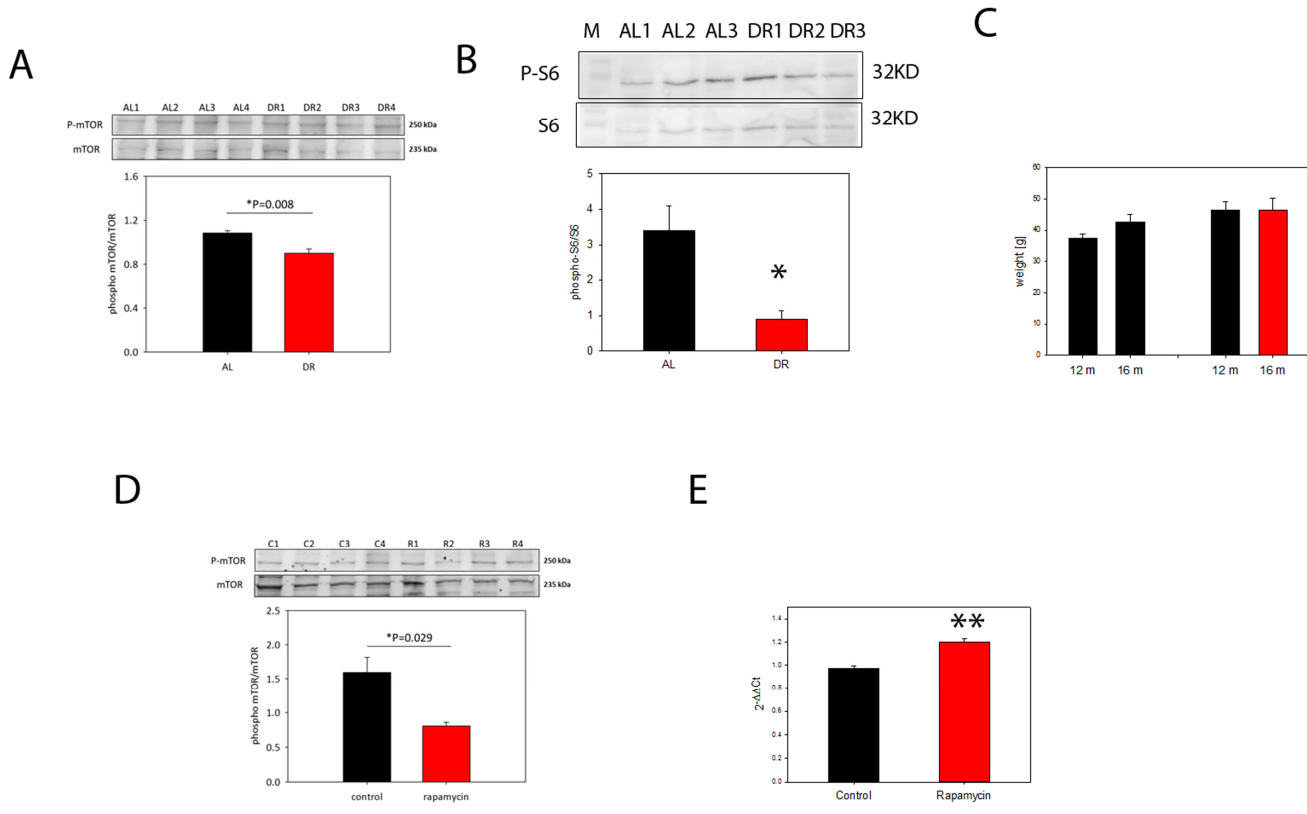
SUPPLEMENTARY MATERIAL



Supplementary Figure S1. TERT protein abundance and telomerase activity in mouse liver fractions of 3 short term DR experiments. (A-C) TERT protein abundance in liver mitochondria and homogenates from the same mice as used for figure 3 D-F (percentage of *ad libitum*-fed controls). (D-F) telomerase activity (TA, measured by TRAP assay as percentage of *ad-libitum*-fed controls) in liver homogenates in the same DR experiments as above. (A, D) Experiment 1; 4 animals per group. (B, E) Experiment 2, n=4 per group. (C, F) Experiment 3, n=7 per group for mitochondria and n=3 per group for homogenates. *Statistical significance was tested by t- test. * p<0.05.



Supplementary Figure S2. Purity of the brain mitochondrial fraction. Isolated mitochondria and tissue homogenates were subjected to Western blotting with antibodies localized to mitochondria (Cox II, cytochrome c oxidase II), the nucleus (HDAC II, histone deacetylase II) and the cytoplasm (β -tubulin). Mito: mitochondrial fraction; homo: whole brain homogenate.



Supplementary Figure S3. Downregulation of the mTOR pathway in mouse brain after rapamycin treatment and short term DR. (A) mTOR phosphorylation after short term dietary restriction in mouse brain tissue after short term DR (exp. 1). Upper panels: immuno-blot with indicated antibodies, AL1-AL4 – *ad libitum* fed, DR1-DR4 – dietary restricted; lower panels: densitometric quantification, as the average of the ratios of phosphorylated to non-phosphorylated protein. * p=0.008, t-test. (B) S6 phosphorylation in the same experiment as in A. *P<0.05, t-test. (C) Body weight (in g) of rapamycin-treated and control wild type mice at ages of 12 months (before rapamycin) and 16 months (after rapamycin). (D) mTOR phosphorylation in brain tissue after 4 months of rapamycin treatment, C1-C4 –controls R1-R4 –rapamycin. * P<0.05, t-test (E) TERT mRNA abundance in mouse brain tissue after 4 months of rapamycin treatment measured by qPCR, n=3-4 per group. **P<0.001, t-test.

SUPPLEMENTARY METHODS

Immunoblotting

Tissue homogenates and mitochondria were lysed using CHAPS buffer (Roche). 60µg protein per sample was run on 10% polyacrylamide gel and blotted to ECL membrane (GE Healthcare) at 100V for 90 min. at 4°C, blocked and incubated with the primary antibody diluted over night at 4°C, After washing with TBST buffer and incubation with a peroxidase labelled secondary antibody membranes were developed with t a chemiluminescence detection kit (GE Healthcare) images were taken with a LAS 3000[®] camera (Fujifilm) and quantified by densitometry and normalised to the loading control (β-tubulin) using AIDA[®] software (Raytest).

TRAP assay

Telomerase was determined using the TeloTTAGGG telomere repeat assay (Roche) as described previously [7, 10] using 100ng liver tissue per reaction and a serial dilution of Hela cells (100, 10, 1, 0.1ng) as controls.

Supplementary Table 1. Primary antibodies used for immunoblotting

antibody	manufacturer	dilution
HDACII	Abcam	1:800
CoxII	Santa Cruz	1:200
phospho-mTOR (Ser2448)	Abcam	1:500
mTOR	Abcam	1:500
phospho-S6	Cell Signalling	1:1000
S6	Cell Signalling	1:1000
β-tubulin	Abcam	1:1000