Supporting information

Mitochondrial respiratory chain dysfunction alters ER sterol sensing and mevalonate pathway activity

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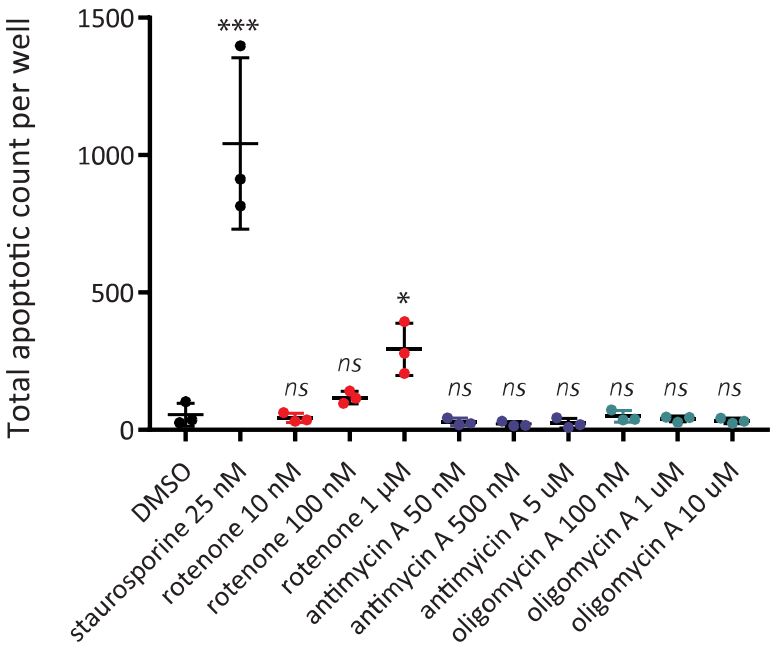

Figure S1. Dose response of cellular apoptosis to complex I inhibition.

Apoptosis of human fibroblasts treated with a range of inhibitor concentrations. Apoptosis was followed using Annexin V Green marker in a ZOOM Live-Cell analysis system (see Materials and Methods). Apoptotic cell counts were measured after growth for 5 days in the absence or presence of respiratory chain inhibitors. Rotenone (red circles), antimycin A (blue circles) or oligomycin (green circles) were used at the indicated concentrations. Staurosporine (black circles) was included as a positive control. Results from three independent experiments ± standard deviation are shown. P-values were calculated with t-tests followed by Benjamini-Hochberg multiple testing correction. *ns: not significant; *** p<0.001 compared to DMSO control.*

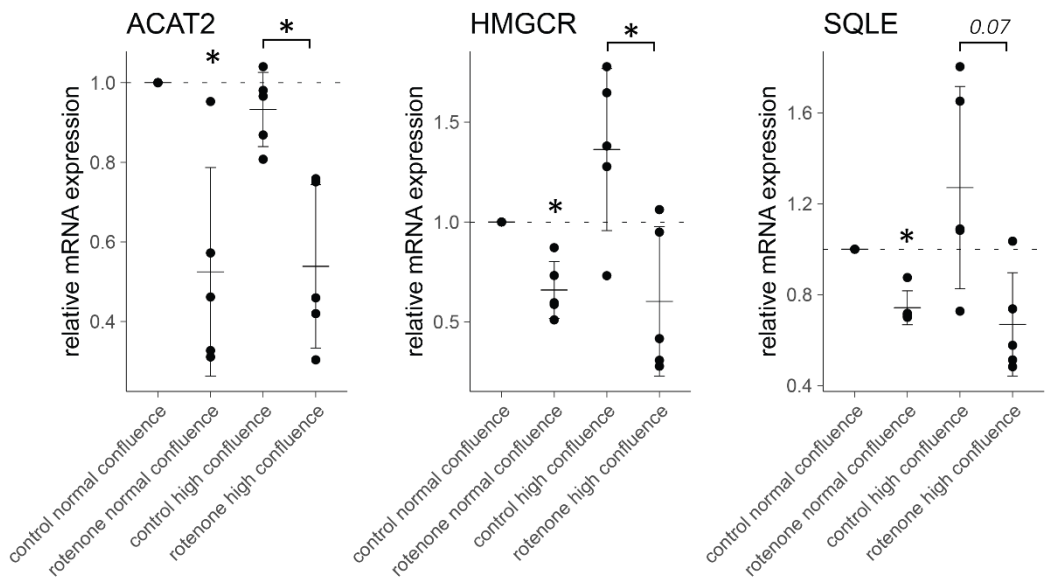

Figure S2. Expression of mevalonate pathway genes in growth inhibited fibroblasts

Mevalonate pathway gene expression as measured by qPCR in cells grown in high or low confluency treated with either DMSO (0.1%) or rotenone (100 nM) for 24 hours. Horizontal lines indicate the mean relative expression of five independent experiments. Error bars indicate mean ± standard deviation. Significance of difference versus normal control or indicated comparison was calculated by t-tests with Benjamini-Hochberg multiple testing correction. * *p<0.05.*

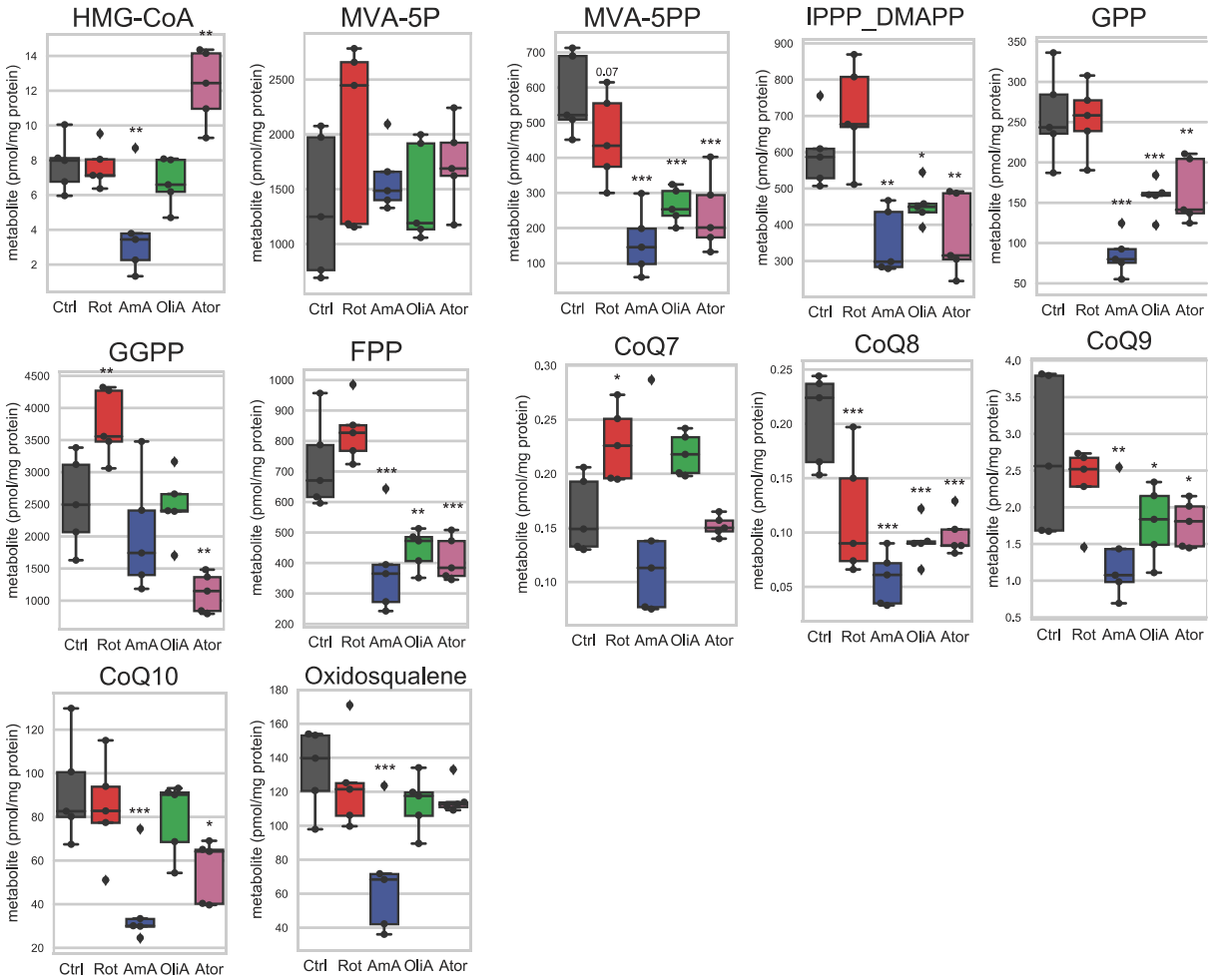

Figure S3. Additional mevalonate pathway metabolites altered by mitochondrial respiratory chain inhibition

Boxplots depicting additional mevalonate pathway metabolites in pmol/mg protein. Cells cultured and analyzed as in figure 3. The box plot shows the median and the interquartile range (IQR). Individual data points as shown. Significance of difference versus control was calculated with ANOVA with Tukey's post-hoc test. *ns: not significant; * p<0.05; ** p<0.01; *** p<0.001.*

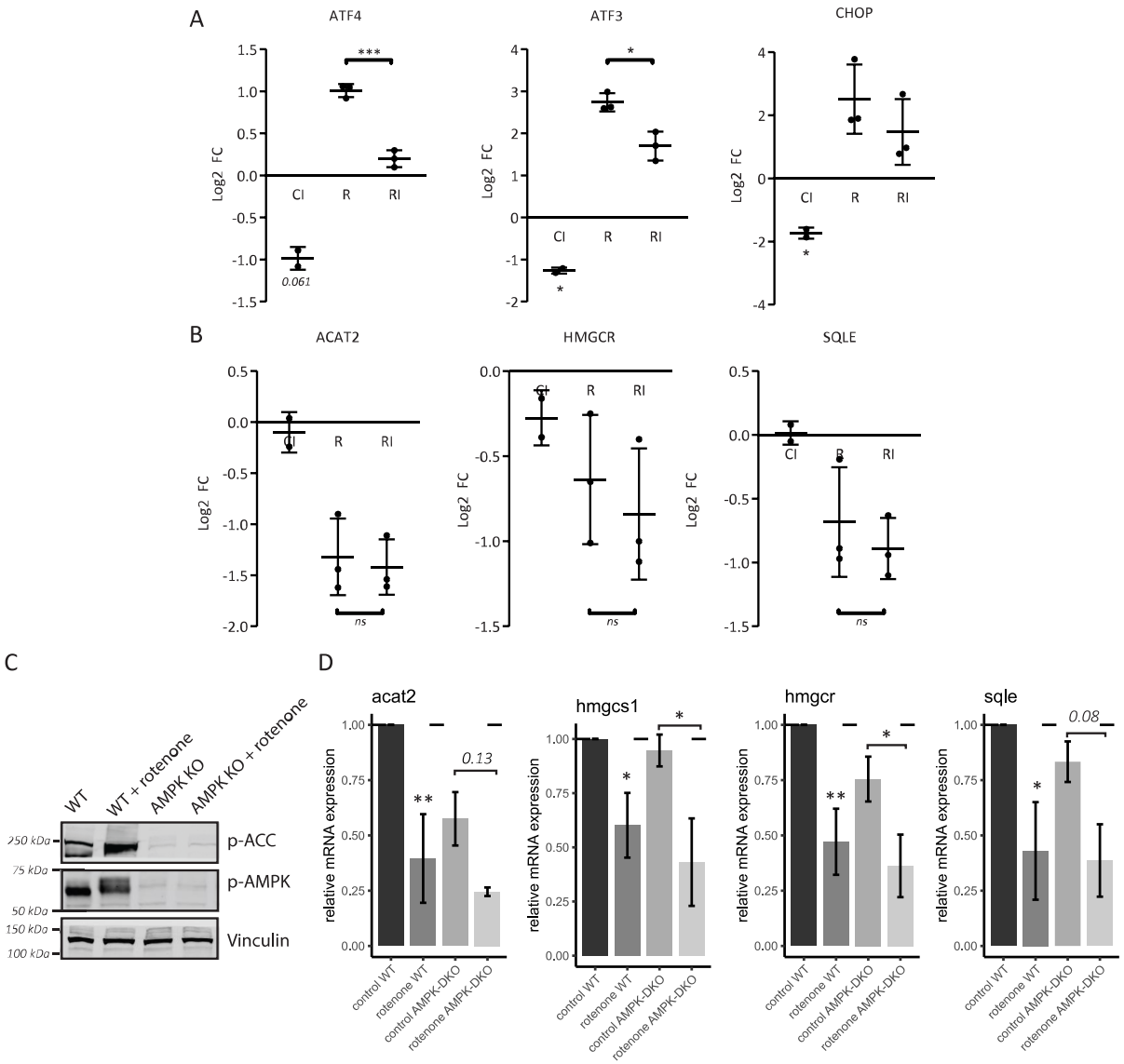

Figure S4. Downregulation of mevalonate pathway genes is not affected by stress or energy sensing pathways

(A) Gene expression of transcription factors induced by the Integrated Stress Response (ISR), as measured by qPCR. Primary human fibroblasts were treated for 24 hours as indicated (CI: 0.1% DMSO + 500 nM ISRIB, R: 100 nM rotenone, RI: 100 nM rotenone + 500 nM ISRIB). Bar indicates mean of three independent experiments ± standard deviation. Significance calculated with t-tests followed by Benjamini-Hochberg multiple testing correction. (B) Gene expression of mevalonate pathway genes as measured by qPCR. Cells treated as in A. Significance calculated as in A. (C) Western blot of phosphorylated-AMPK (Thr172), phosphorylated-ACC (Ser79) in wild-type mouse embryonic fibroblasts (MEFs) and MEFs lacking AMPKα1 and α2, treated with either DMSO (0.1%) or rotenone (100 nM). Vinculin was used as loading control. (D) Gene expression of mevalonate pathway genes in MEFs under conditions as described in A as measured by qPCR. Relative expression as mean of three independent experiments. Error bars indicate mean ± sd. Significance of difference versus control was calculated with ANOVA followed by Tukey's post-hoc test. *ns: not significant; * p<0.05; ** p<0.01; *** p<0.001.*