Supplementary Figure Legends

Figure S1 - Supplement to Figure 2.

A OPA1 oligomerization was analysed in isolated mouse liver mitochondria incubated with or without the indicated ETC substrates as Figure 2A for 1 hour at 37°C and subsequently cross-linked with BMH [10 mM] for 30 minutes at room temperature and quenched with BME. OPA1 oligomers were then analysed by gradient gel western blot.

B The effect of varying digitonin concentrations on the mobilization of cytochrome C from intracristae stores. Mitochondria were incubated in a no substrate buffer for 1 hour, and then varying concentrations of digitonin (0.5 µg/µg mitochondria, 0.05 %; 1 µg/µg mitochondria, 0.1%; 1.5 µg/µg mitochondria, 0.15 %) were incubated with the mitochondria for 30 minutes at 4°C on a rotator. Mobilized cytochrome c was then separated from retained CytC by centrifugation and analysed by western blot where released CytC was mobilized from the pellet (P) to the supernatant (SN) fraction. The highest concentration of digitonin that showed no change in Tom20 release (1ug/ug protein) was used in subsequent experiments.

C The reversibility of cytochrome c mobilization was analysed as previously described where mitochondria were incubated with or without complex I substrates, spun down and resuspended in the indicated buffer for 10 minutes and analysed for cytochrome c retention.

D Isolated liver mitochondria were incubated for 1 hour in the indicated buffers and processed for electron microscopy. Left panel, representative EM images from samples incubated with the indicated buffer. Right panel, quantification of orthodox, condensed and other mitochondria from 50 images per condition (over 500 mitochondria per condition) (averages ± SEM of 3 independent experiments).

E Cristae width was quantified from orthodox and condensed mitochondria from 10 fields of no substrate incubated mitochondria (minimum 25 mitochondria per class per experiment) (averages ± SEM of 3 independent experiments).

Student t-tests were performed as indicated *p<0.05, ***p<0.005.

Figure S2 - OPA1 dependent starvation-induced cell death.

A Representative images of cells quantified in Figure 3A and S1B, live-cell stained with Hoescht and PI.

B Cells were treated as in Figure 3A, but quantified as the percent condensed nuclei (averages ± SEM of 3 independent experiments).

C Cells were treated as in Figure 3E, but quantified as the percent condensed nuclei (averages ± SEM of 3 independent experiments).

Student t-tests were performed as indicated, *p<0.05, **p<0.01 and ***p<0.005.
Figure S3 - OPA1(Q297V) after long-term reintroduction is fusion incompetent, but rescues cristae structure.

A Longterm reintroduced OPA1 KO cells were seeded on glass coverslips 24 hours prior to fixation and analysed for mitochondrial length by immunofluorescence for mtHSP70.

B Parallel cultures were seeded onto square coverslips, fixed, and mitochondrial cristae structure was assessed by EM.

Figure S4 - OPA1 is required for ATP-linked and reserve OCR.

A WT and OPA1 KO MEFs transiently or stably expressing GFP, starved or not for 2 hours, were analysed with the Seahorse XF-24 analyzer. At the indicated times, oligomycin (O), FCCP (F), and antimycin A (AA) with rotenone (R) were injected (averages ± SEM of 4 independent experiments).

B Quantification of ATP-linked (resting OCR minus oligomycin insensitive OCR) and reserve OCR (Maximal minus resting) in A (averages ± SEM of 4 independent experiments).

Student t-tests were performed as indicated, *p<0.05 and ***p<0.005.

Figure S5 - WT and OPA1(Q297V) rescue cell growth in galactose media.

Cell doubling times were calculated within the second week of growth in either glucose or galactose medium from Figure 4F using http://www.doubling-time.com/compute.php.

Figure S6 - OPA1 interacting partner identification by Stable Isotope Labeling by Amino Acids in Cell Culture (SILAC).

A Schema of SILAC experiment to identify OPA1 binding partners.

B Representation of the mass spectroscopy data as the relative abundance of the peptide fragments to the log SILAC ratio of the heavy to light fraction. A log SILAC ratio of 1 was set as the threshold for putative interactors to consider validating and previously reported or functionally suggested OPA1 interacting partners are identified on the graph.

C Pie graph representing the localization of the 35 potential OPA1 interactors identified from this screen.

Figure S7 - Fusion proteins for DIC, OGC, AGC1, and AGC2 are all mitochondrially targeted.

A MEFs were transiently transfected with mito-YFP-3xFLAG, DIC-3xFLAG, OGC-3xFLAG, AGC1-3xFLAG, and AGC2-3xFLAG for 24 hours, seeded onto coverslips for 24 hours, fixed and analysed by immunofluorescence with anti-flag antibodies.
Endogenous OPA1 was immunoprecipitated from mouse liver mitochondrial lysates with or without 15 mM phenylsuccinate (PhS) and the eluted samples were analysed by western blot.

Figure S8 - Overexpression or knockdown of OGC does not affect mitochondrial length.

A MEFs were transiently transfected with no plasmid, mito-YFP-3xFlag, or OGC-3xFlag for 48 hours. Mitochondrial morphology was then analysed by Tom20 immunofluorescence and binned as fragmented, intermediate or elongated (right panels, averages ± SEM of 3 independent experiments).

B MEFs were transfected twice with siOGC for 120 hrs total and mitochondrial morphology was then analysed by Tom20 immunofluorescence and binned as fragmented, intermediate or elongated (right panels, averages ± SEM of 3 independent experiments).

Figure S9 - Supplement for Figure 6.

A Representative EM images of mitochondria from siCtrl or siOGC MEFs. Scale bars: 500 nm.

B Cell death of siCtrl or siOGC MEFs starved or not for 6 hours was analysed following PI and Hoescht staining where dead cells are expressed as the percentage of PI+ to all cells (Hoechst+) (averages ± SEM of 3 independent experiments).

C Cells were treated as in B, but quantified as the percent condensed nuclei (averages ± SEM of 3 independent experiments).

D Quantification of Figure 6D of the OPA1 oligomer to monomer ratio, percent of control (averages ± SEM of 4 independent experiments). Right panel, quantification of same experiments, but expressed as the change in OPA1 oligomers from incubation with complex I substrates to no substrates (AU: Arbitraty Units).

E Quantification of Figure 6E of the ratio of cytochrome c in the supernatants to that remaining in the pellets after incubation with digitonin and expressed as percent of siCtrl with NS (averages ± SEM of 4 independent experiments).

Student t-tests were performed as indicated *p<0.05, **p<0.005.