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Supplementary Information for

Genome-wide CRISPR screens reveal multitiered mechanisms through which mTORC1 senses mitochondrial dysfunction

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This PDF file includes:

Other supplementary materials for this manuscript include the following:

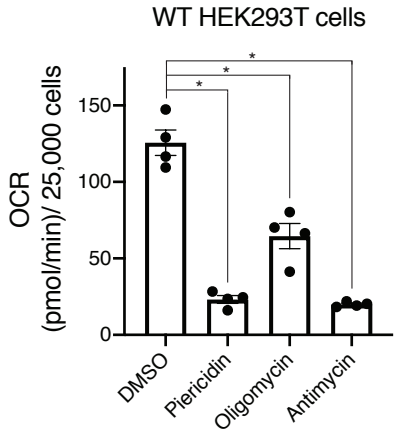

Fig. S1. Mitochondrial inhibition with piericidin, antimycin, or oligomycin, lowers oxygen consumption rate (OCR). Wild-type HEK293T cells were treated with vehicle (DMSO), piericidin (500 nM), oligomycin (1 μM), or antimycin (500 nM) and OCRs were measured with a Seahorse XFe96 Analyzer. OCRs are shown as mean ± s.e.m. for n=4 biologically independent experiments. P values were determined using a two-sided Student's t-test. *P < .05.

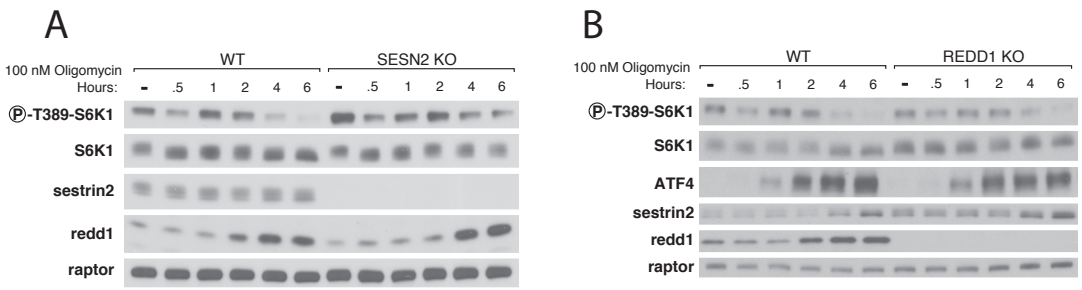

Fig. S2. Loss of Sestrin2 or Redd1 alone is not sufficient to render mTORC1 signaling resistant to oligomycin treatment. (A) Immunoblot analyses of mTORC1 signaling over the course of a 6-hour treatment with 100 nM oligomycin in wild-type and Sestrin2 KO HEK293T cells. (B) Immunoblot analyses of mTORC1 signaling over the course of a 6-hour treatment with 100 nM oligomycin in wild-type and Redd1 KO HEK293T cells.

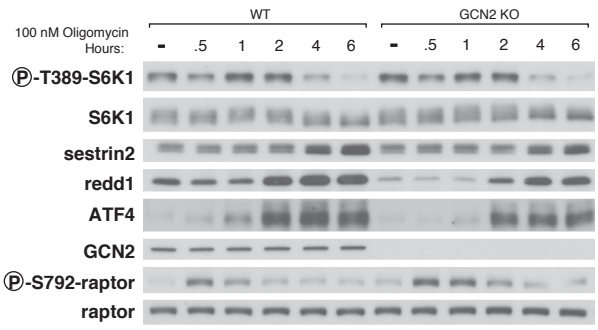

Fig. S3. mTORC1 signaling remains sensitive to oligomycin in cells lacking GCN2. Immunoblot analyses of mTORC1 signaling over the course of a 6-hour treatment with 100 nM oligomycin in wild-type and GCN2 KO HEK293T cells.

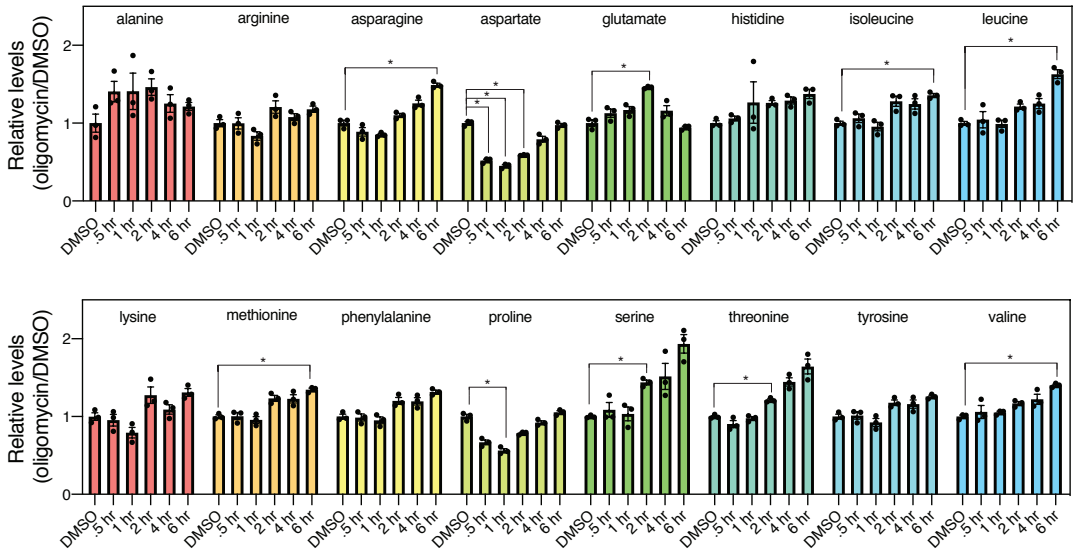

Fig. S4. The levels of most amino acid do not decrease after a 6-hour treatment with oligomycin. Relative amino acid levels in wild-type HEK293T cells after a 6-hour treatment with 100 nM oligomycin. Relative amino acid levels are shown as mean ± s.e.m. for *n*=3 biologically independent experiments. **P* <0.05 (**P* <0.000625 after Bonferroni correction).

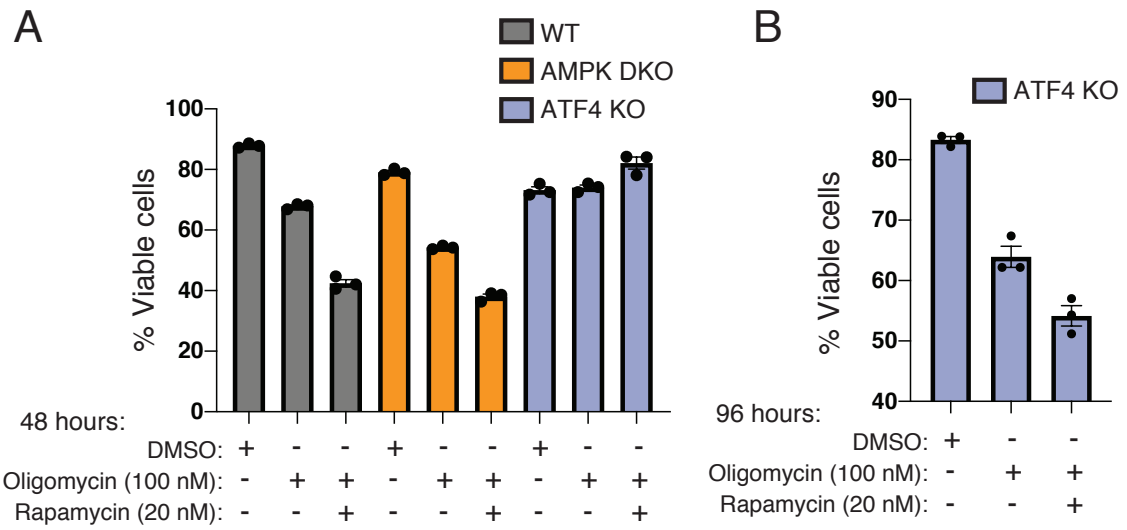

Fig. S5. Impact of loss of AMPK or ATF4 and/or rapamycin treatment on oligomycin-induced cell death. (A) Cell viability measured by Propidium Iodide (PI) staining in wild-type, AMPK DKO, and ATF4 KO HEK293T cells after a 48-hour treatment with the DMSO vehicle, 100 nM oligomycin, or 100 nM oligomycin plus 20 nM rapamycin. Viability measurements are shown as mean ± s.e.m. for *n*=3 independent experiments. (B) Cell viability of ATF4 KO HEK293T cells after 96-hour treatments as in (A). Measurements are displayed as in (A).

Dataset S1 (separate file).
Primary screen CS values (Fig. 1D)
List of sgRNAs in the mTOR focused sublibrary
Focused sublibrary CS values (Fig. 2C, 3A-D)
Focused sublibrary day 8 and day 16 CS values (Fig. 3E)
MAGeCK analysis

Dataset S2 (separate file).
Western blot quantification values (Fig. 4, 5)
Relative AMP levels (Fig. 4,5)

Dataset S3 (separate file).
List of sgRNAs used in this study
Oxygen consumption rates (OCRs) (Fig. S1)
Relative amino acid levels (Fig. S4)
Viability data (Fig. S5)