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Supplementary Materials for

BAP31 regulates mitochondrial function via interaction with Tom40 within ER-mitochondria contact sites

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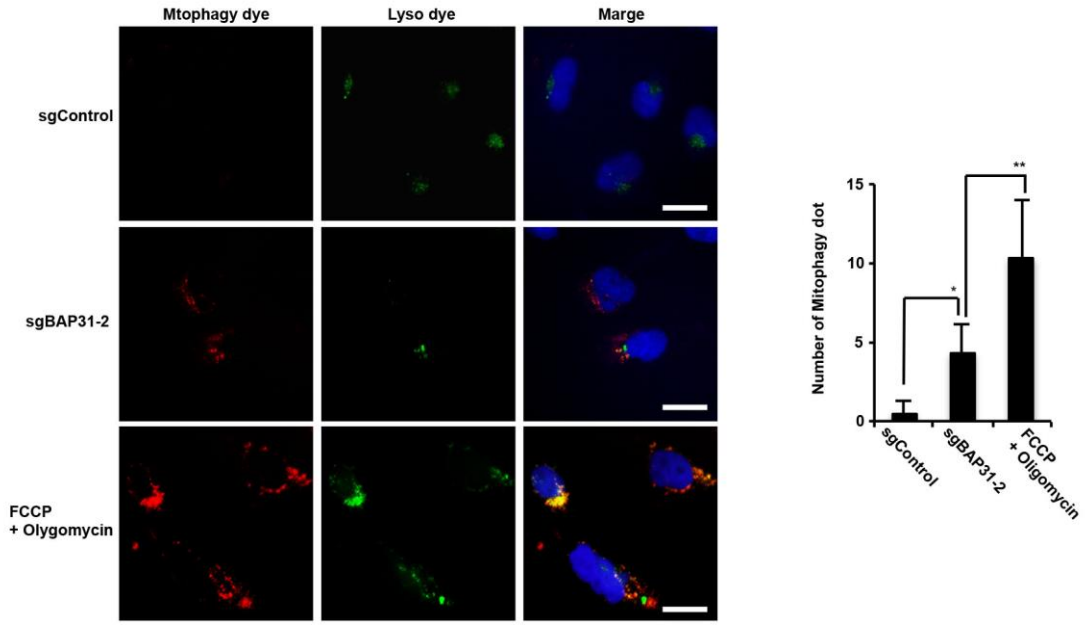

Fig. S1. BAP31 depletion induces mitophagy. Assessment of mitophagy in U2OS sgBAP31-2 or sgControl cells with or without FCCP (1 μM) and oligomycin (1 μM) treatment for 12 h (left panel). Blue represents nuclear DAPI staining. Scale bar: 10 μm. Colocalization between the mitophagy (red) and lysosome dyes (green) were quantified, and data are presented as mean ± SD (n = 6) (right panel). P-value was calculated using a two-way ANOVA: *P < 0.05, **P < 0.01.

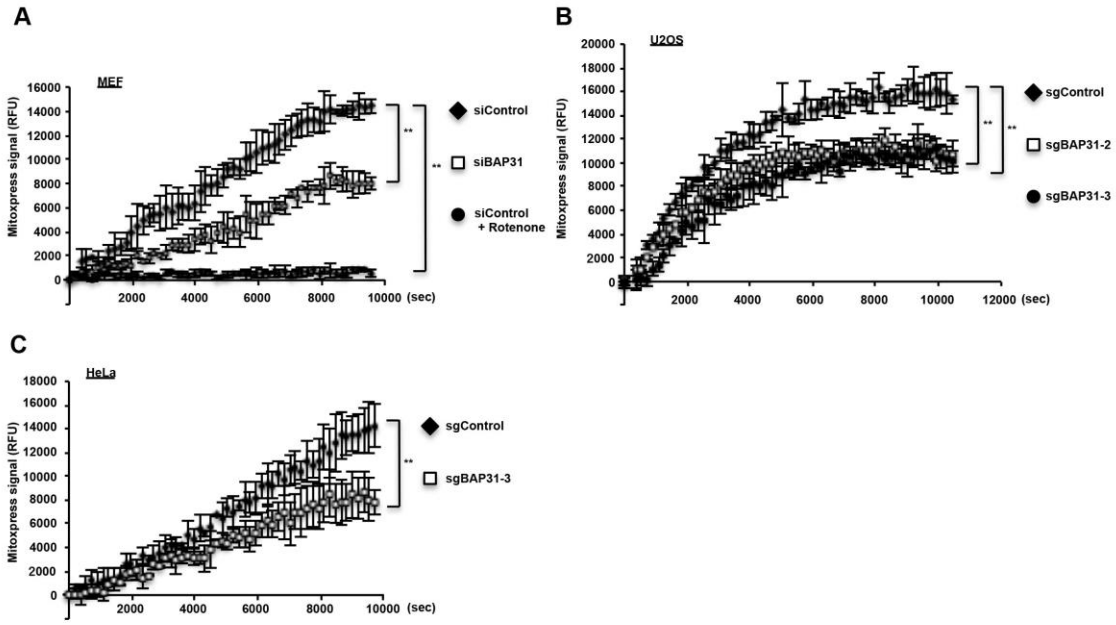

Fig. S2. Mitochondrial oxygen consumption is suppressed by BAP31 knockdown. (A) MEF cells transfected with siBAP31 or siControl for 24 h received additional treatment with or without rotenone (5 μM) for 6 h, and (B) U2OS sgControl, sgBAP31-2, sgBAP31-3 and (C) HeLa sgControl, sgBAP31-3 cells were cultured for 24 h. Time-resolved fluorescence of the MitoXpress probe was converted to phosphorescence values and normalized to the cell number. Data are expressed as mean TR-F. Data are presented as mean ± SD of three simultaneously performed experiments. *P*-value was calculated using a two-way ANOVA: $^{**}P < 0.01$.

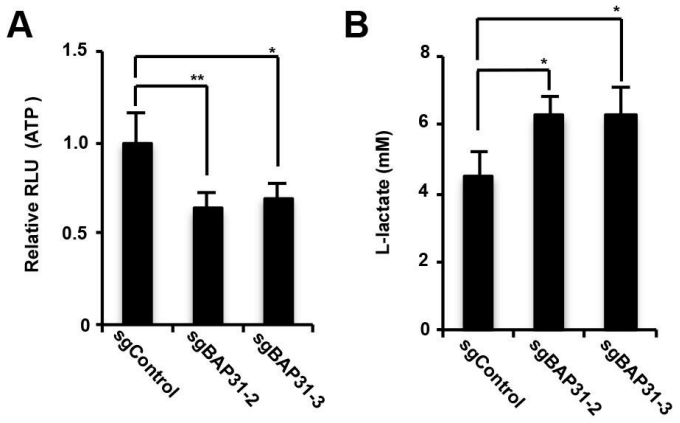

Fig. S3. Depletion of BAP31 expression decreases ATP levels and stimulates glycolysis. (A) ATP level was determined using a CellTiter-Glo assay (n = 3). (B) L-lactate levels were determined using a Glycolysis Cell-Based Assay Kit (n = 3). P-value was calculated using a two-way ANOVA: *P < 0.05, **P < 0.01.

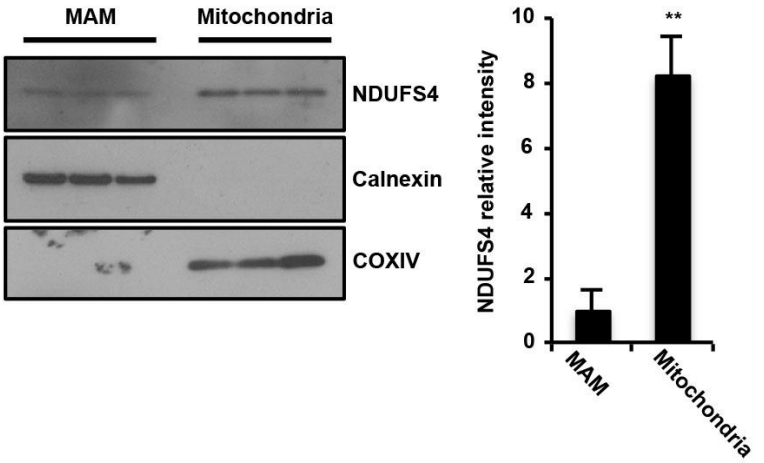

Fig. S4. The abundance of NDUFS4 localization at the MAM is lower than at the mitochondria. U2OS cells were fractionated into two parts (MAM and mitochondria) and blotted using the indicated antibodies. The isolated mitochondria and MAM were resuspended in equal volumes of isolation buffer, and the same volumes were added per lane (left panel). The intensity of the NDUSF4 band was determined and expressed relative to the results obtained within the MAM (n = 3) (right panel). Data are presented as mean ± SD of three simultaneously performed experiments. P-value was calculated using a two-way ANOVA: *P < 0.05, **P < 0.01.

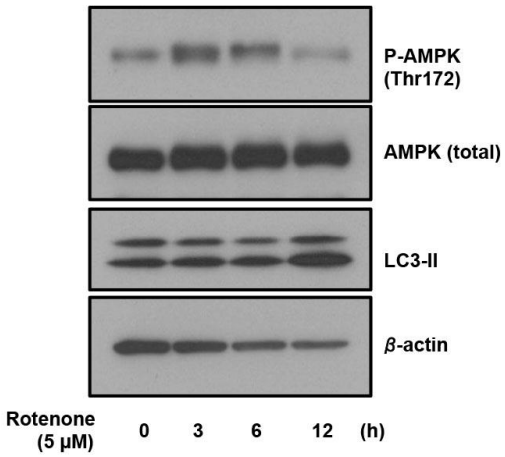

Fig. S5. Inhibition of mitochondrial complex I induces the activation of AMPK and LC3-II expression. U2OS cells were treated with rotenone (mitochondrial complex I inhibitor) at the indicated concentrations and time periods. Cell lysates were subjected to immunoblotting using the indicated antibodies.

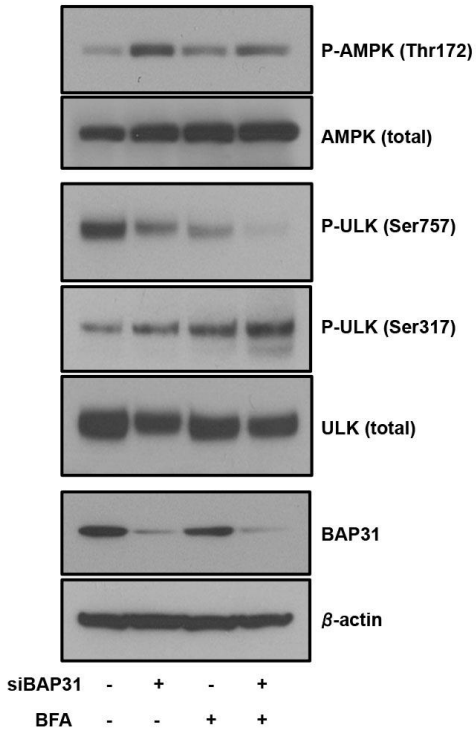

Fig. S6. AMPK signaling is activated by ER stress. AMPK signaling is activated by ER stress. U2OS cells were transfected with siBAP31 and siControl for 18 h and then treated with or without BFA (1 μg/ml) for 8 h. Cells were subjected to immunoblotting using the indicated antibodies.

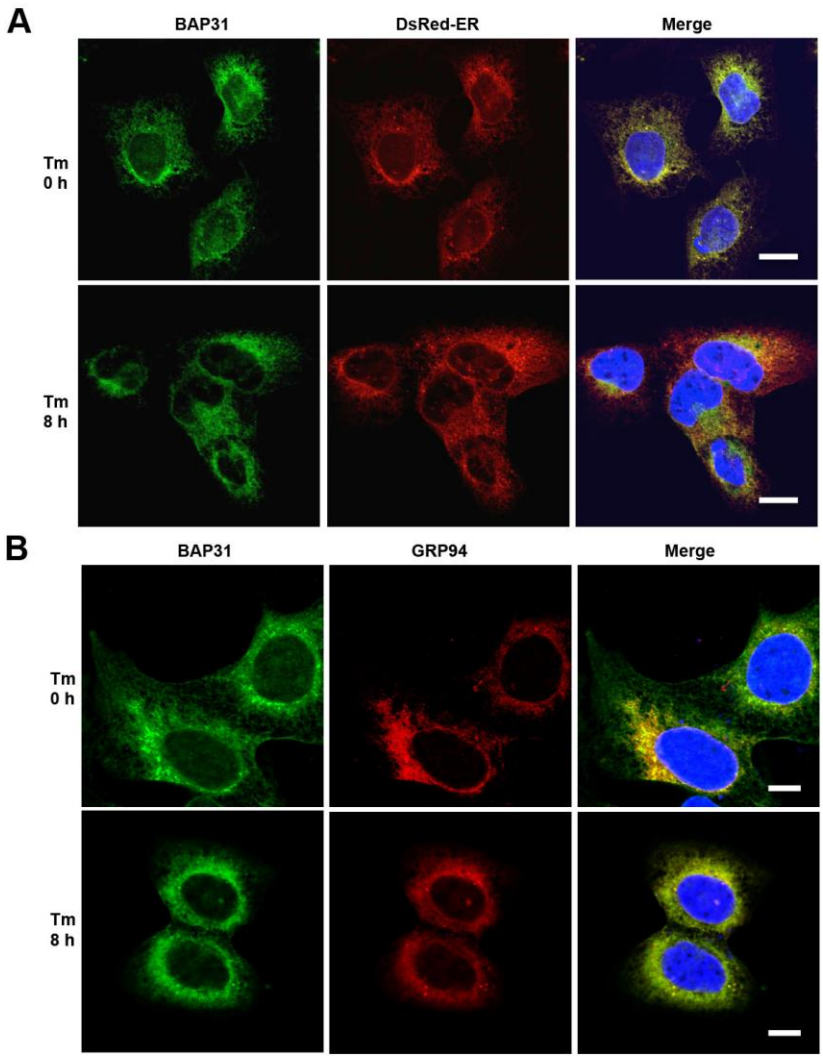

Fig. S7. The effect of ER stress on BAP31 localization. (A, B) U2OS cells were treated with Tm for 8 h and subjected to immunostaining using BAP31 (green) antibody and DsRed-ER (ER marker, red) (A) or BAP31 (green) and GRP94 (red) antibodies (B). Merged images are also shown, and colocalization is indicated in yellow. Blue represents nuclear DAPI staining. Scale bar: 10 μm.