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Supplemental information

A proximity labeling strategy enables proteomic analysis of inter-organelle membrane contacts

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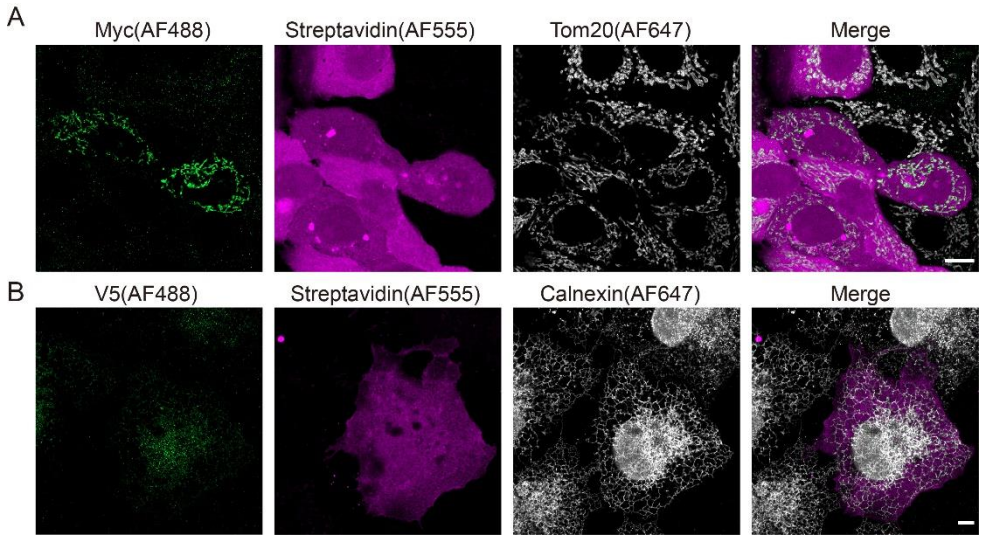

Figure S1. Characterization of the specificity of GNAP2 (Related to Figure 1B)

(A-B) U2OS cells were transiently transfected with mito-myc-2xspGFP11 (A) or spGFP1-10–ER (B) along with GNAP2. After biotin labelling, the cells were fixed and subjected to immunofluorescence labeling. The Alexa 488-conjugated Myc antibody and Alexa 488-conjugated V5 antibody were used to detect mito-myc-2xspGFP11 and spGFP1-10–ER respectively. The Alexa-555 conjugated streptavidin was used to detect biotin labeled proteins. The Alexa 647-conjugated Tom20 antibody and Alexa 647-conjugated Calnexin antibody were used to label mitochondria and ER, respectively. Scale bar, 5 μm. Fragments, green; streptavidin, magenta; mitochondria or ER, grey.

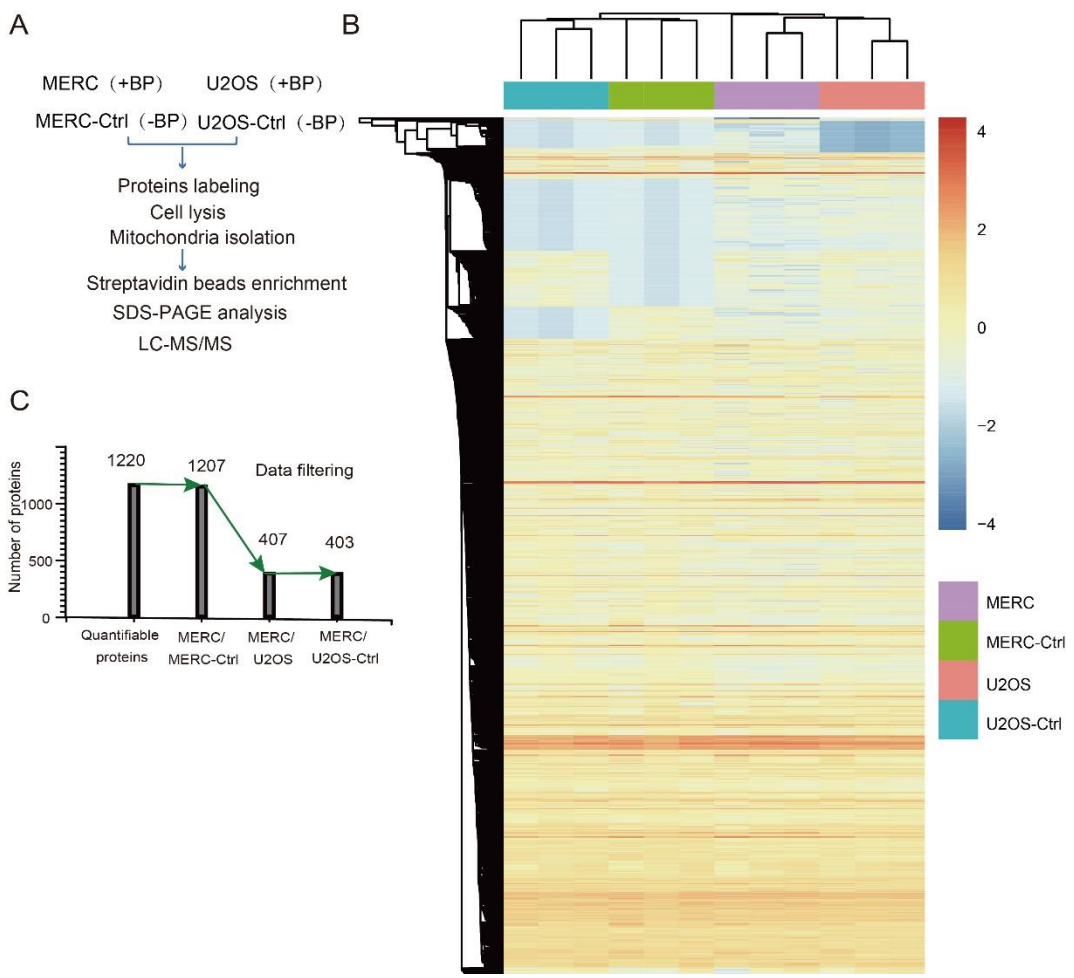

Figure S2. Proteomic characterization of MERC in living cells (Related to Figure 2A)

(A) Flow chart of the experimental procedures. U2OS or MERC cells were transiently transfected with GNAP2 and incubated with or without biotin-phenol. After biotin labelling, the cells were harvested, and biotin labeled proteins were enriched by streptavidin beads, separated by SDS-PAGE. The whole band was cut and analyzed with LC-MS/MS.

(B) The abundance pattern of the quantifiable proteins in all three replicates identified by BiFCPL was shown as heat map.

(C) Data filtering process. Proteins in MERC group were sequentially compared with the other three groups, MERC-Ctrl, U2OS and U2OS-Ctrl, to filter highly enriched MERC proteins. MS data normalization was performed based on the total intensity of all proteins in each biological replicate.

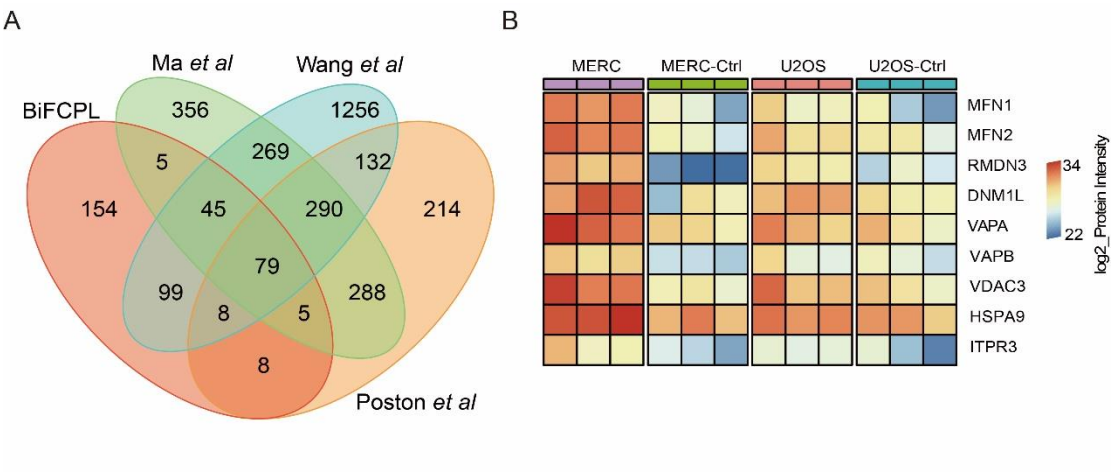

Figure S3. Specificity of the MERC proteins identified by BiFCPL(Related to Figure 2)

(A) A Venn diagram between MERC proteins and other studies via subcellular fraction. The organism and cell types were as follows: BiFCPL, human, U2OS cells; Ma *et al*, mouse, brain; Wang *et al*, human, testes; Poston *et al*, mouse, brain.

(B) The abundance pattern of the known MERC proteins in all three replicates identified by BiFCPL was shown as heat map.

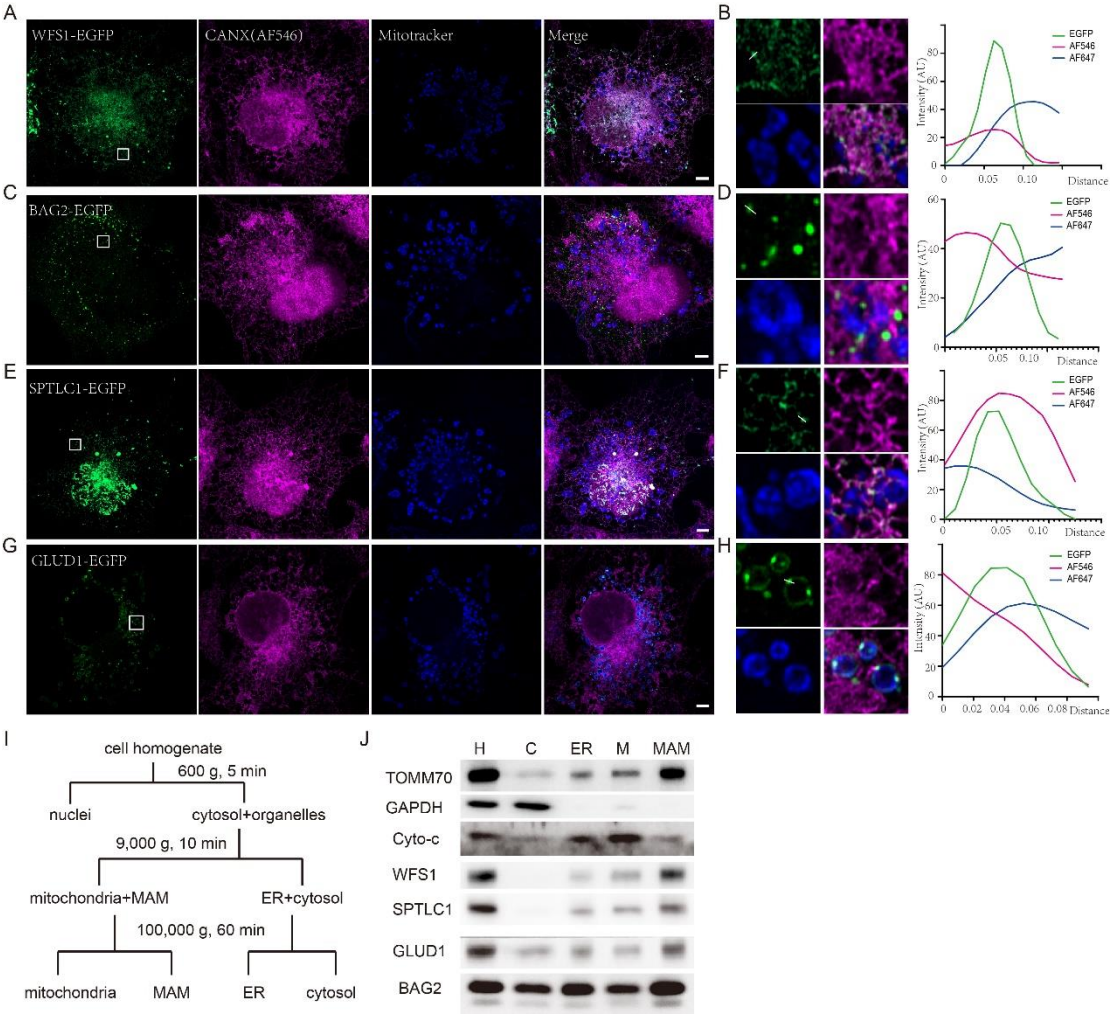

Figure S4. Validation of novel MERC proteins (Related to Figure 3)

(A, C, E, G) Fluorescent images of fixed cells. Wild-type U2OS cells were transiently transfected with the indicated plasmid to express WFS1-EGFP (A), BAG2-EGFP (C), SPTLC1-EGFP (E) or GLUD1-EGFP (G). The cells were then grown on coverslips to allow adhering and spreading. MitoTracker (deep red) was used to label mitochondria and an Alexa 546-conjugated antibody against calnexin was used to label ER. The images were taken with LSM 980 confocal microscopy equipped with a 63×/1.40 NA oil immersion objective (Zeiss). (B, D, F, H) The smaller images were amplification of the boxed region in the larger images. Plot analysis was used to assess fluorescence distribution of the indicated channels. Scale bar, 5 μm. Candidates, green; ER, magenta; mitochondria, blue.

(I) Flow chart of the subcellular fraction analysis.

(J) Western blotting analysis of the different fractions against the indicated proteins.

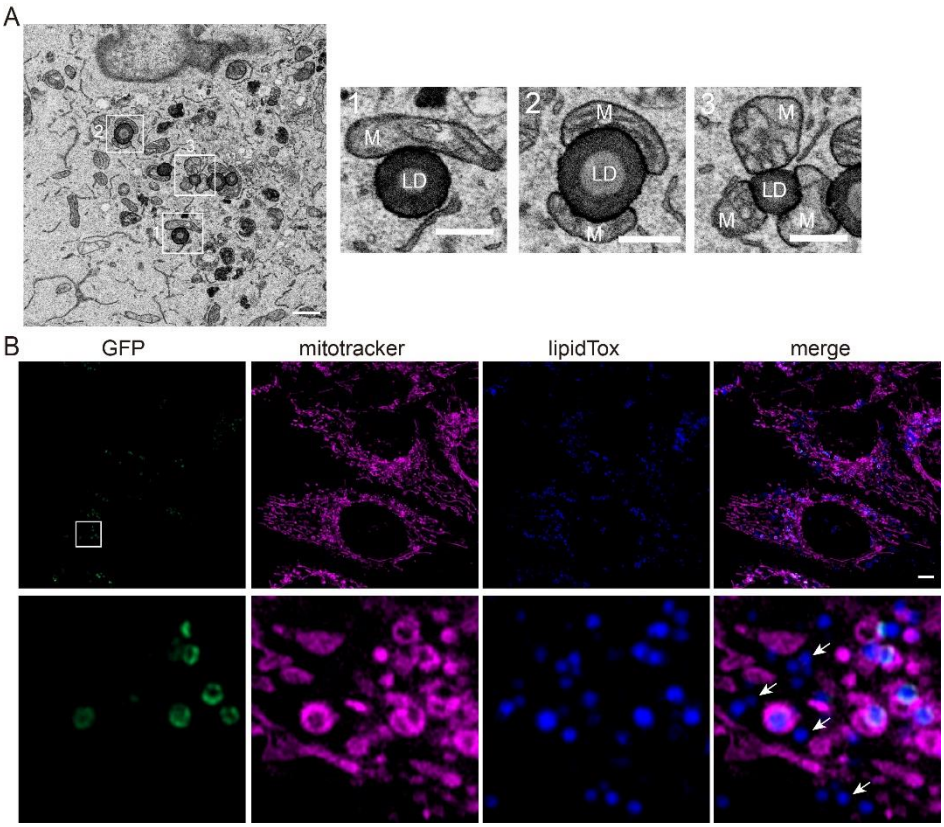

Figure S5. Electron microscopy and fluorescence images of the MLDCS cells (Related to Figure 4B)

(A) 1, 2 and 3 represents different types of the contact between mitochondria and LDs. Scale bar, 1 μm in the bigger image and 500 nm in the smaller images.

(B) Fluorescence images of the MLDCS cells 4 h post exposure to 100 μM oleic acids. MitoTracker and LipidTox were used to label mitochondria and LDs, respectively. Scale bar, 5 μm. The lower images were a zoom-in version of the boxed region in the upper images. The white arrows marked free LDs. Contact, green; mitochondria, magenta; LDs, blue.

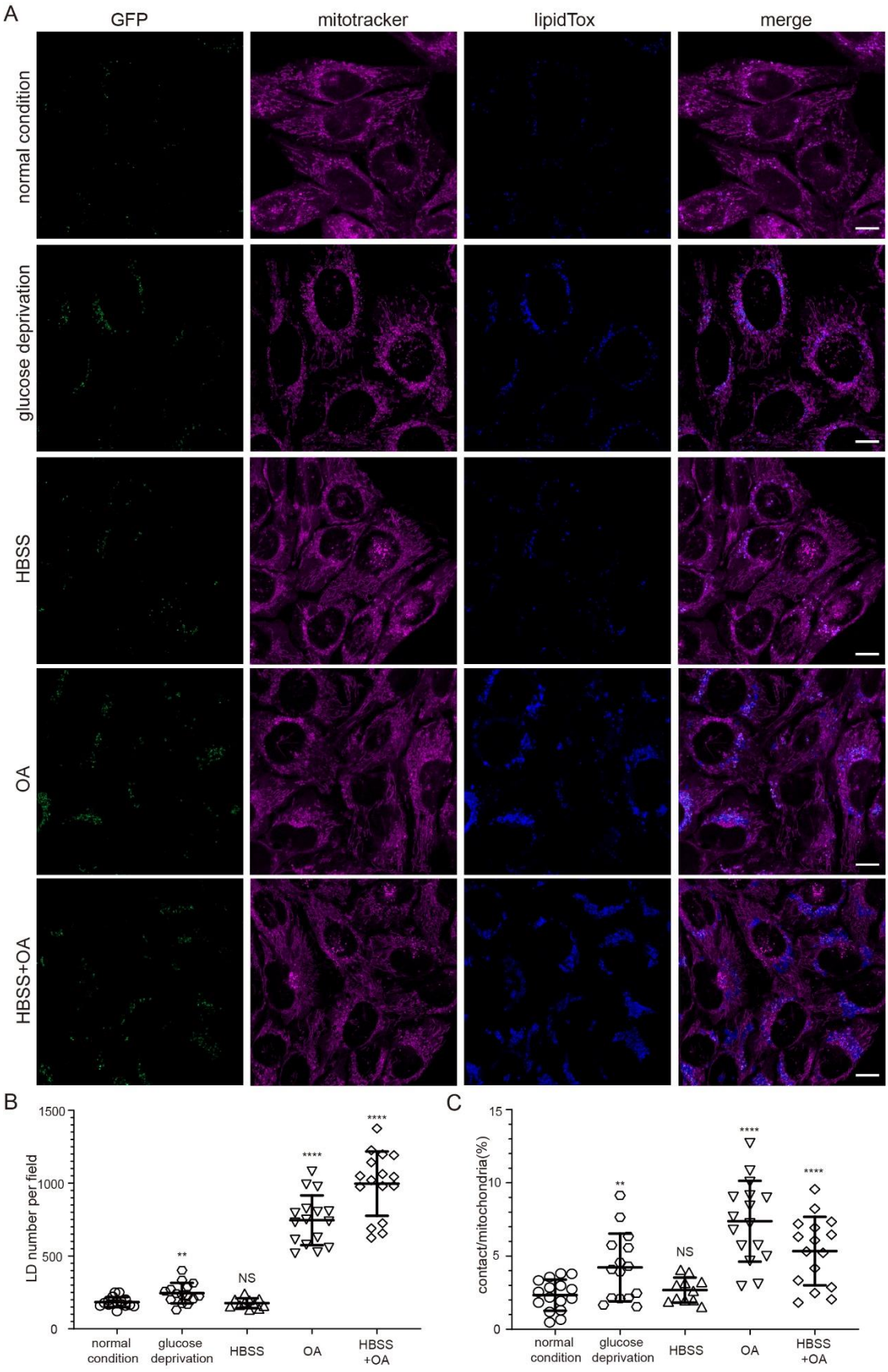

Figure S6. Mitochondria-LD contact is sensitive to nutrient status (Related to Figure 5A)

(A) Representative single plane images of the MLDCS cells under normal condition, glucose deprivation, starvation with HBSS, oleic acids (OA) treatment and starvation with HBSS supplemented with OA treatment (HBSS+OA) condition. The duration of the nutritional stress was 16 h. Scale bar, 5 μm. Contact, green; mitochondria, magenta; LDs, blue.

(B-C) Quantification of the numbers of LDs (B) and contacts (C) per field under the indicated conditions. The contacts were defined as the ratio of the area of GFP normalized to the area of mitochondria. N=11-16 fields were analyzed. The experiments shown were a representative of three biological replicates. Data were presented as mean ± SEM. NS, no significance. ** $P<0.01$, **** $P<0.0001$ (two-tailed t-test).

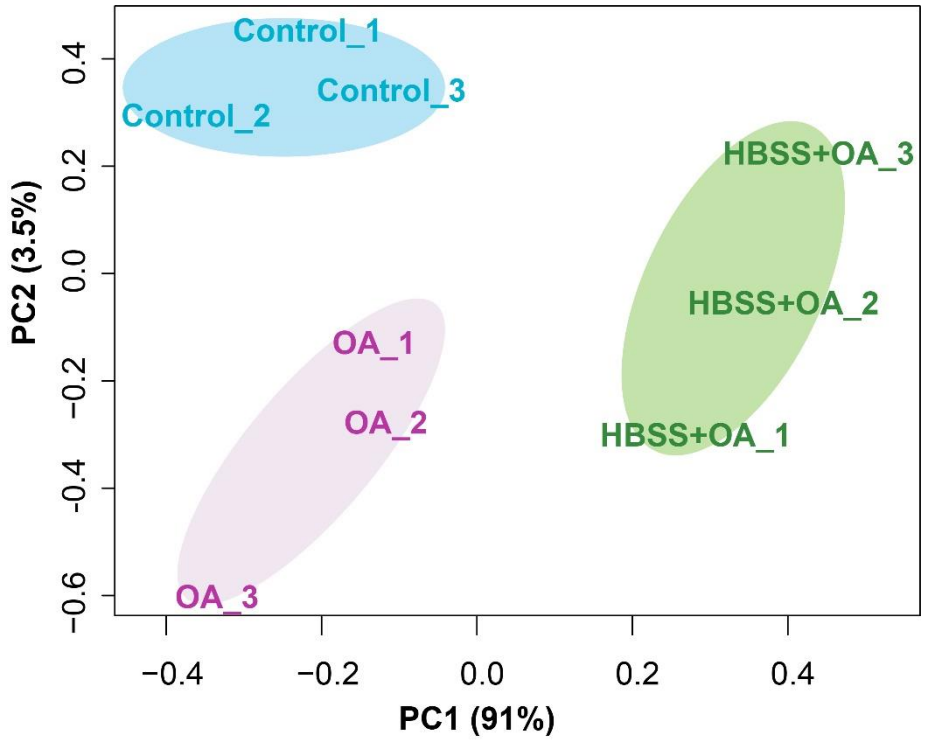

Figure S7. Principal components analysis (PCA) of the three biological replicates (Related to Figure 5A)

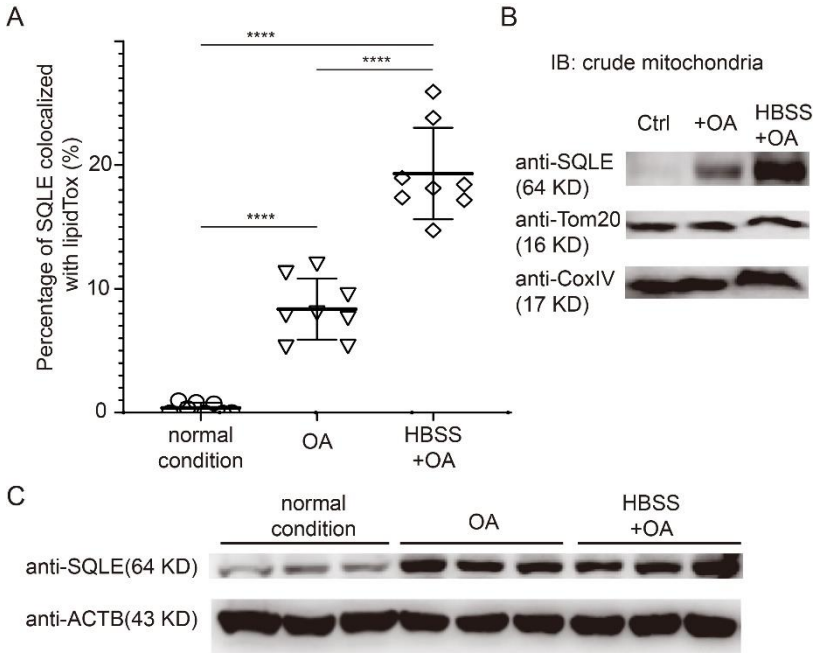

Figure S8. SQLE accumulates at mitochondria-LD contact sites under metabolic challenge (Related to Figure 6)

(A) Quantification of the percentage of SQLE protein colocalized with LDs. N=8 images were analyzed. The experiments shown were a representative of three biological replicates. Data were presented as mean ± SEM. **** P<0.0001 (two-tailed t-test).

(B) Western blotting analysis of the isolated mitochondria. Wild-type HepG2 cells were incubated under normal, OA or HBSS+OA conditions for 16 h. Then, the cells were harvested and crude mitochondria were isolated. The samples were then subjected to SDS-PAGE and immunoblotted with antibodies against SQLE, Tom20 (mitochondria outer membrane marker) and CoxIV (mitochondria inner membrane marker).

(C) Protein expression analysis. HepG2 cells were incubated under normal, OA or HBSS+OA conditions. The cells were then washed, lysed and subjected for western blotting analysis using antibodies against SQLE and ACTB.

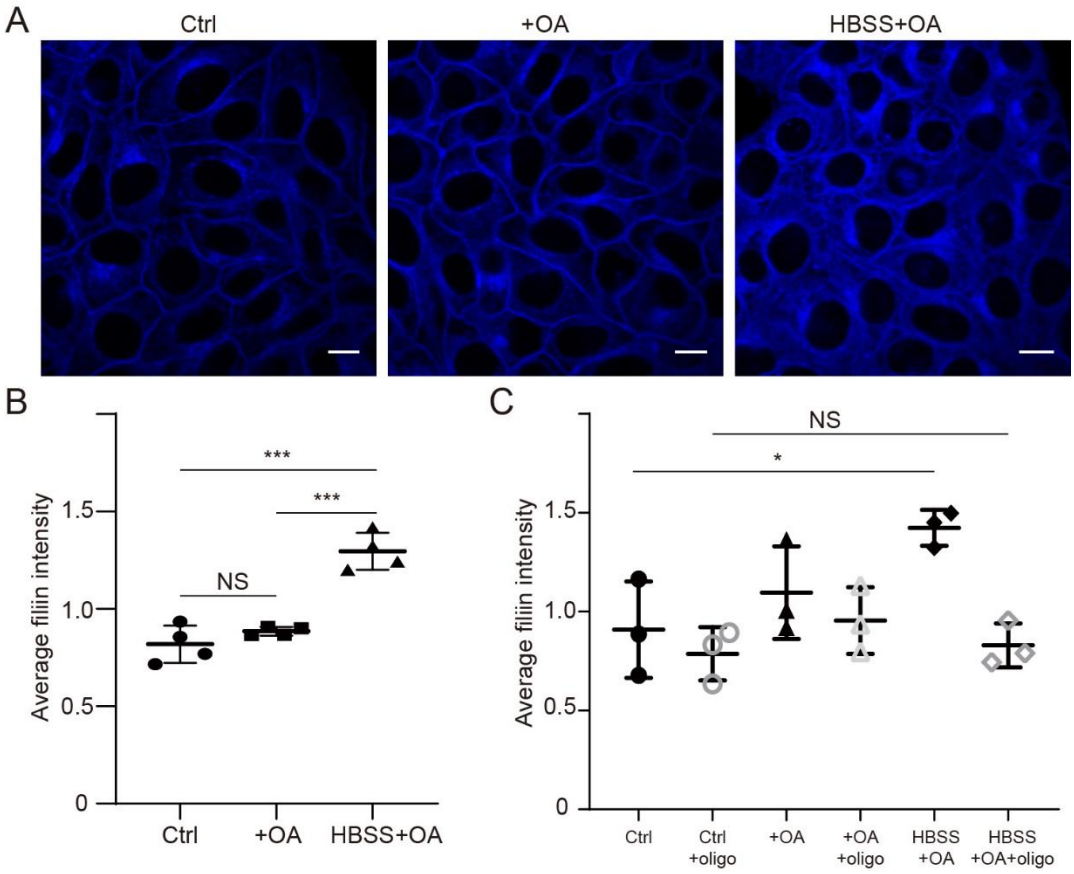

Figure S9. SQLE accumulates at mitochondria-LD contact sites probably to utilize local mitochondrial ATP for cholesterol synthesis (Related to Figure 6)

(A) Representative images of the filipin stained HepG2 cells. The cells were incubated under normal condition or with either OA or HBSS+OA condition for 16 h. The cells were then fixed and subjected for filipin staining. The fluorescent images were acquired by confocal microscopy. Scale bar, 10 μm.

(B) Statistical analysis of the average filipin staining intensity of the three groups in four biological replicates. HepG2 cells were incubated under normal condition or with either OA or HBSS+OA condition for 16 h. The cells were then fixed and subjected for filipin staining. Fluorescent images were acquired by confocal microscopy and fluorescent intensity was measured and compared. The fluorescent intensity of each group was normalized to the average value of all the three groups for each replicate. Data were presented as mean ± SEM. NS, no significance. *** $p<0.001$ (two tailed t-test).

(C) Statistical analysis of the average filipin staining intensity in the indicated groups in three biological replicates. Same as in (B) except the cells were incubated with or without 1 μM oligomycin A. NS, no significance. * $p<0.05$ (two tailed t-test).