Supplemental Data for:

Fatty acid synthase inhibits the O-GlcNAcase during oxidative stress

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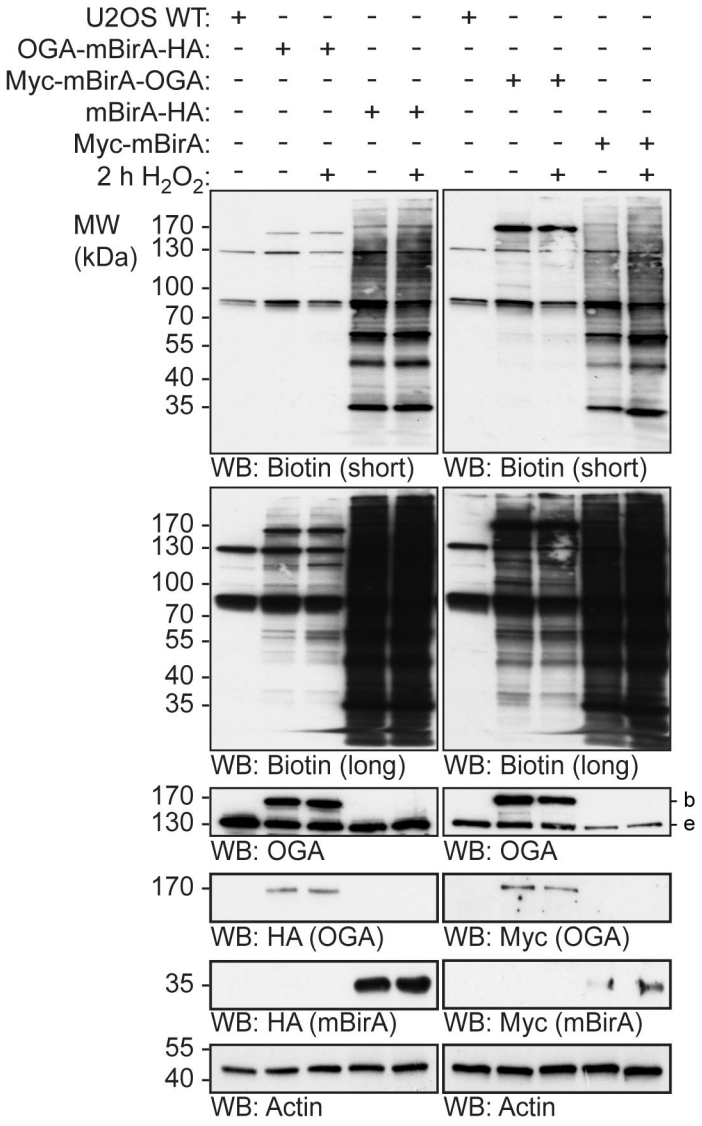
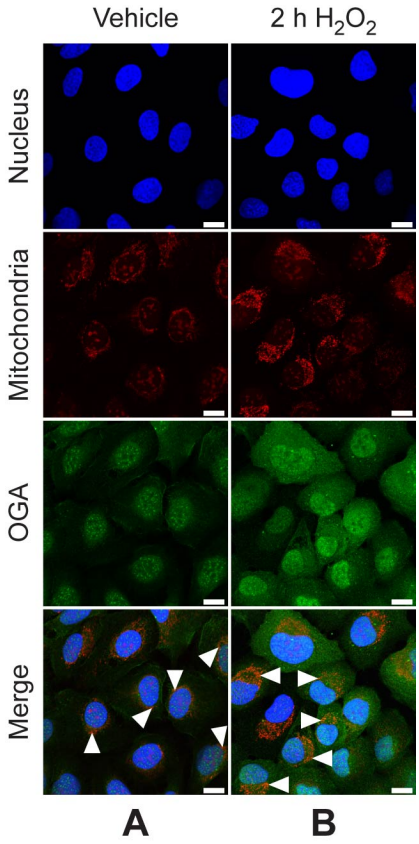
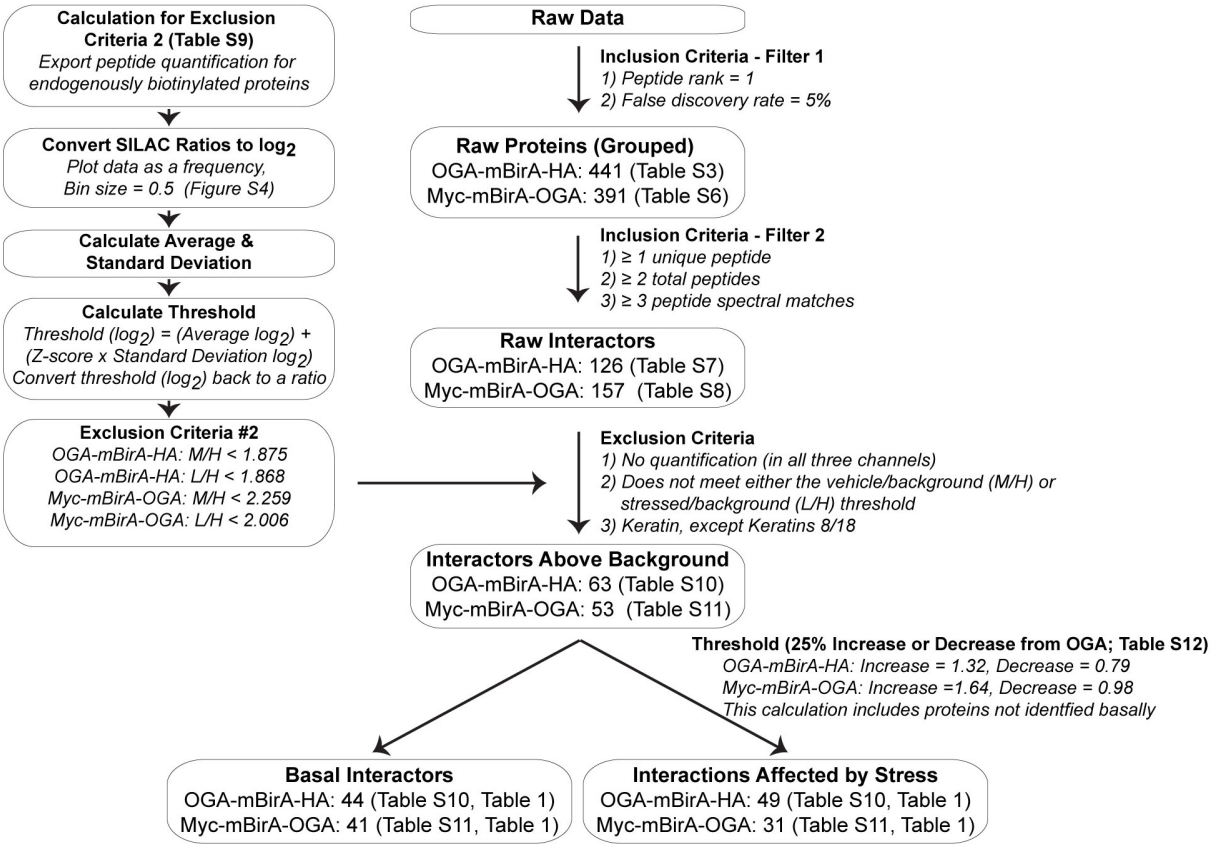


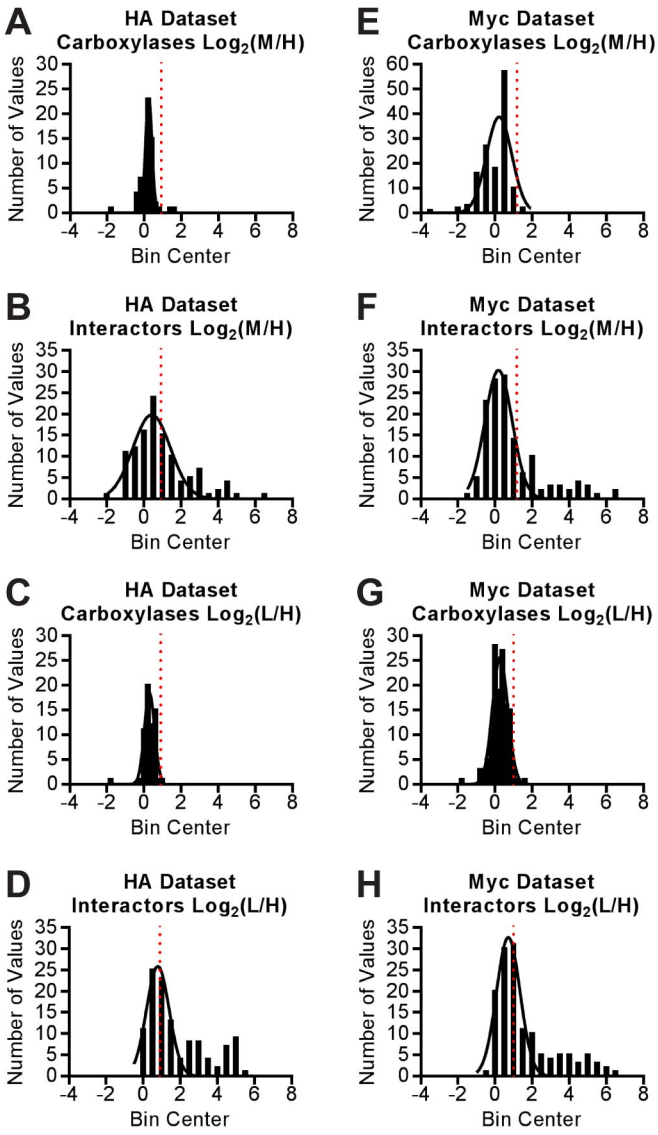
Supplemental Figure 1. The biotinylation pattern produced by OGA-mBirA-HA and Myc-mBirA-OGA is different to that of mBirA alone. Cells were transfected with mBirA-HA, OGA-mBirA-HA, Myc-mBirA, or Myc-mBirA-OGA, and treated with or without H_2O_2 (2.5 mM, 2 h). Non-transfected wild type (WT) U2OS cells were treated with vehicle. All cells were treated with biotin (25 μM, 16 h). Equal amounts of protein (5 μg; TCL lysates) were separated by SDS-PAGE and the following were detected by western blot: biotin, OGA, HA, Myc, and actin. The migration of endogenous OGA (e), mBirA-tagged OGA (b), and the molecular weight markers (MW) are indicated.



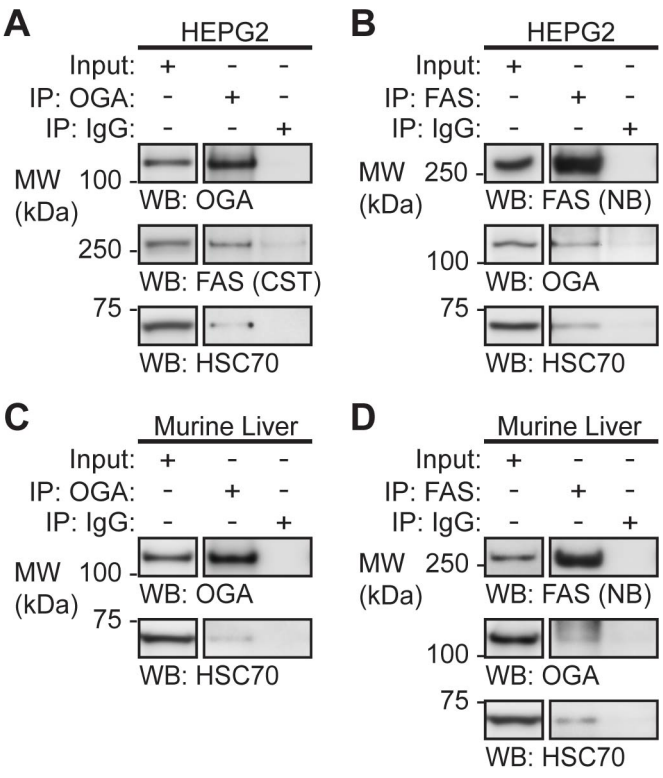
Supplemental Figure 2. Endogenous OGA localizes to the nucleus, cytoplasm, and mitochondria of U2OS cells. U2OS cells were fixed, permeabilized, and stained for OGA. Nuclei and mitochondria were stained with Hoechst 33342 and MitoTracker Orange CMTMRos, respectively. White triangles indicate co-localization (orange) of MitoTracker and OGA. Images were acquired at 63x magnification on a Zeiss Axio Examiner 710NLO-Meta multiphoton microscope. N=3. The scale bar represents 15 μm. (A) Cells were treated with vehicle. (B) Cells were treated with H_2O_2 (2.5 mM, 2 h).



Supplemental Figure 3. Flowchart for mass spectrometry dataset processing to generate the protein lists in the supplemental tables and table 1.



Supplemental Figure 4. Determination of the threshold for non-specific interactors using Log_2 SILAC ratios and frequency histograms of the endogenously biotinylated carboxylases. Frequency histograms were generated from: (A) Log_2(M/H) SILAC ratios for the carboxylases (HA dataset; Supplemental Table 9); (B) Log_2(M/H) SILAC ratios for raw interactors (HA dataset; Supplemental Table 7); (C) Log_2(L/H) SILAC ratios for the carboxylases (HA dataset; Supplemental Table 9); (D) Log_2(L/H) SILAC ratios for raw interactors (HA dataset; Supplemental Table 7); (E) Log_2(M/H) SILAC ratios for the carboxylases (Myc dataset; Supplemental Table 9); (F) Log_2(M/H) SILAC ratios for raw interactors (Myc dataset; Supplemental Table 8); (G) Log_2(L/H) SILAC ratios for the carboxylases (Myc dataset; Supplemental Table 9); and (H) Log_2(L/H) SILAC ratios for raw interactors (Myc dataset; Supplemental Table 8). (A-H) Red dotted line represents the Log_2 threshold with a one-sided 95% confidence interval.



Supplemental Figure 5. FAS, OGA, and HSC70 interact basally in human hepatocytes and murine liver tissue. Cells and tissue were lysed in NETN buffer and pre-cleared. Immunoprecipitations were performed with an anti-OGA antibody (IP: OGA; A, C), an anti-FAS antibody (IP: FAS; B, D), or a rabbit isotype control immunoglobulin (IP: IgG; A-D). (A) Endogenous OGA was enriched from HEPG2 cell lysate (500 µg), of which 2% (input) and 50% (immunoprecipitate) were analyzed by SDS-PAGE. OGA, FAS, and HSC70 were detected by western blot. N=4, except for WB: FAS (N=2). (B) Endogenous FAS was enriched from HEPG2 cell lysate (250-600 µg), of which 1.7-4% (input) and 33-80% (immunoprecipitate) were analyzed by SDS-PAGE. FAS, OGA, and HSC70 were detected by western blot. N=4. (C) Endogenous OGA was enriched from normal murine liver tissue lysate (400 µg), of which 3.8% (input) and 63% (immunoprecipitate) were analyzed by SDS-PAGE. OGA and HSC70 were detected by western blot. N=3. (D) Endogenous FAS was enriched from normal murine liver tissue lysate (400 µg), of which 3.8% (input) and 63% (immunoprecipitate) were analyzed by SDS-PAGE. FAS, OGA, and HSC70 were detected by western blot. N=3. (A-D) FAS (CST) and FAS (NB) represent anti-FAS antibody from Cell Signaling Technology and Novus Biologicals, respectively. To ensure that images were in the linear range, western blot exposures from the input and immunoprecipitated fractions are often different. The exposure lengths for the test and control isotype antibody immunoprecipitates are always identical. The migration of molecular weight (MW) markers is indicated.