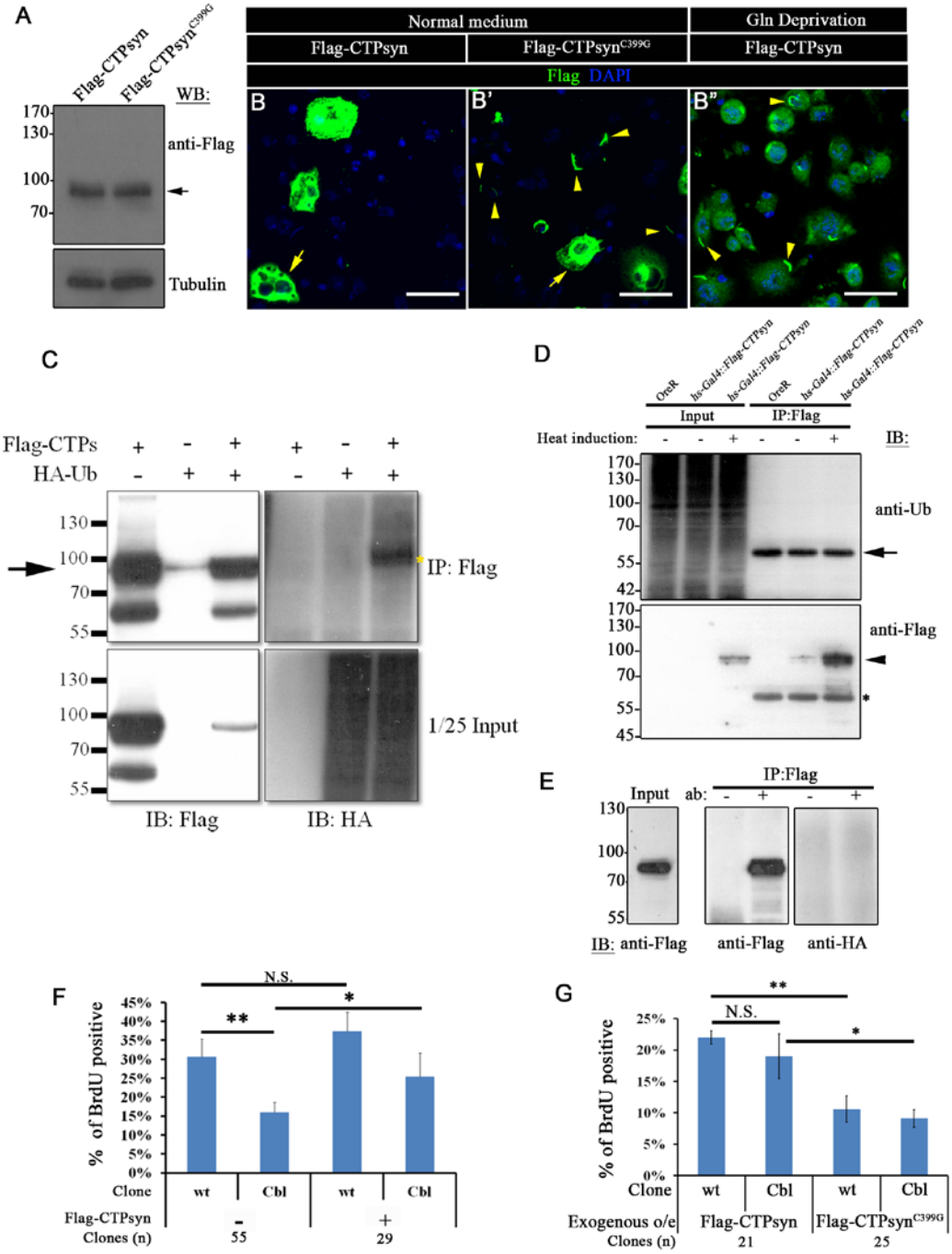

Figure S8. Cbl did not regulate CTPsyn filament structures through the ubiquitination of CTPsyn

(A) Exogenous Flag-CTPsyn expression levels were analyzed in ovarian lysates from *hs-Gal4::Flag-CTPsyn* and *hs-Gal4::UAS-Flag-CTPsyn*^{C399G} females using western

blot. Hs-Gal4 was first expressed at 37°C for 30 minutes and then switched to 25°C to express UAS-Flag- CTPsyn for an additional 3 h before lysing the cells for western blotting using anti-Flag antibody. Tubulin was used as a loading control. (B–B") Multiple merged-confocal sections of S2 cells incubated in complete (B–B') or glutamine-deficient (B") Schneider's medium were stained with anti-Flag (green) and DAPI (blue). The CTPsyn filament structures were observed in Flag-CTPsyn^{C399G}-transfected cells (arrowhead in B') in complete Schneider's medium, and in Flag-CTPsyn-transfected cells in glutamine-deficient medium (arrowhead in B"). Arrows indicate highly expressed cells. (C) Flag-CTPsyn was immunoprecipitated from transfected S2 cells that were co-transfected with HA-ubiquitin using anti-Flag antibody. The immunoprecipitated product was subjected to immunoblotting using anti-HA (right) or anti-Flag antibodies (left). The arrow indicates Flag-CTPsyn. The ubiquitinated protein signal is presented at the right (yellow star). (D and E) Flag-CTPsyn was immunoprecipitated from ovarian (D) or salivary gland (E) cell lysates by anti-Flag antibody. In this assay, ovaries expressed Flag-CTPsyn and salivary glands co-expresses HA-ubiquitin and Flag-CTPsyn. The products were subjected to immunoblotting using anti-Flag antibody (arrowhead in D), anti-ubiquitin (D), or anti-HA (E) antibody. The arrow in D indicates a non-specific band, and the heavy chains of antibodies are labeled by *. (F and G) The percentage of

BrdU-positive versus GFP-negative cells (white line in Figure 7), and GFP-positive cells (yellow line in Figure 7) was quantified per egg chamber. The egg chambers overexpressing Flag-CTPsyn or Flag-CTPsyn^{C399G} in main-body follicle cells. (n = 3 biological repeats). Scale bar: 20 μm. Results are shown as the mean ± s.d.; *$p < 0.05$; **$p < 0.01$, and N.S.: not significant.