Supplementary Information

Supplementary Methods

Plasmids

p*UAS*p-DACK (*UAS-DACK*) was generated by PCR amplification of the full-length *DACK* transcript from pOT2-LD25841 (*Drosophila* Genomics Resource Center) as a Not*I*/Bam*HI* fragment and cloning into the corresponding sites of vector p*UAS*p. Full-length human CTP synthase 1 (*CTPS1*) was PCR-amplified as a Bam*HI*-Sal*I* fragment from pCDNA4.1/hCTPS1 and cloned into the Bgl*II* and Sal*I* sites of vector pEGFP-C1 (Clontech). The QuikChange Kit (Agilent) was used to introduce the E161K mutation into pEGFP-CTPS1 and the K156A mutation into p*UAS*p-DACK. Mutagenic primer sequences are available upon request. All constructs were fully sequenced.

Transgenic Fly Lines

DACK and *CTPS1-E161K* were PCR-amplified using *attB*-modified primers and were cloned into pDONR-Zeo (Invitrogen). The cloned inserts were transferred into destination vectors pPFW and pPGW, respectively, using Gateway cloning technology to create N-terminally-tagged constructs for *Drosophila* embryo microinjection. Transgenic flies were generated by BestGene, Inc. *UAS-DACK*, *UAS-DACK-K156A*, and *UAS-GFP-CTPS1-E161K* map to the 2nd chromosome. *UAS-FLAG-DACK* maps to the X chromosome.

Mammalian Cell Culture

HEK293 and MCF7 cells were grown in Dulbecco's Modified Eagles Medium (DMEM) supplemented with 10% fetal bovine serum, L-glutamine (2mM), and penicillin/streptomycin (100μg/ml). Cells were transfected for 24 hours with Lipofectamine 2000 (Invitrogen) according to the

manufacturer's protocol. For treatment with 6-diazo-5-oxo-L-norleucine (DON, Sigma), MCF7 cells were seeded on glass coverslips and treated overnight with 200 μM DON followed by fixation and processing for immunofluorescence microscopy.

Immunofluorescence Microscopy

Fly ovaries were dissected and processed for immunostaining as described [1] using indicated primary antibodies followed by incubation with species-specific secondary antibodies conjugated to Alexa-488 or Cy3 (Jackson Immunoresearch Laboratories, Inc.). Fluorescently-conjugated phalloidin (Invitrogen) was used to label F-actin and Draq5 (Cell Signaling Technology) was used to label nuclei. After staining, samples were mounted in Vectashield mounting medium (Vector Laboratories). Images were collected at room temperature (~ 22°C) using 40x (1.25 NA) or 63x (1.4 NA) oil immersion lenses (Leica) on an upright microscope (DM 5000; Leica) coupled to a confocal laser scanner (TCS SP5; Leica). LAS AF SP5 software (Leica) was used for data acquisition.

Filament measurements were quantified with Imaris 7.5.0 using the Filament Tracer module. Filaments within germline cells were first rendered by manually selecting beginning and end points. The filaments were then traced and volume-rendered using the AutoPath algorithm without any additional manual input. The minimum and maximum diameters used for the calculations were 0.05 and 20 μm, respectively. The raw statistics were exported directly from Imaris into Microsoft Excel.

RNA Isolation and Quantitative RT-PCR

Whole flies or ovaries, as indicated, were dounce-homogenized and total RNA was isolated using an RNeasy kit (Qiagen). RNA concentrations were determined with a spectrophotometer (NanoDrop; Thermo Fisher Scientific). RNA was reverse transcribed using Moloney murine leukemia virus reverse transcriptase (Ambion) and a mixture of anchored oligo-dT and random decamers. Two

reverse-transcription reactions were performed for each sample using either 100 or 25 ng of input RNA. Aliquots of the cDNA were used to measure CTP synthase and RpII140 levels. The following oligonucleotides were used: CTP synthase variant C (NM_168606.1): forward, 5'-CTGTGGTCTGGATGTAACCTCG-3'; and reverse, 5'-CAAAACGTAAACCTCGCCATG-3', RpII140 forward: CGCACGTGGAAGTTGGTAAT, reverse: ACAATCAGAGTCCGCGTACACA, probe: 6fam-ACGCCCATAGCTTGCTTACCCATAGC-bhq1. Assays were used in combination with Universal Master mix and run on a 7900 HT sequence detection system (Applied Biosystems). Cycling conditions were 95°C, 15 min, followed by 40 (two-step) cycles (95°C, 15 s; 60°C, 60 s). Ct (cycle threshold) values were converted to quantities (in arbitrary units) using a standard curve (four points, four-fold dilutions) established with a calibrator sample.

Immunoprecipitation and Western Blotting

Whole ovaries were dissected in Grace's insect medium and lysed by dounce homogenization in RIPA buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 1% deoxycholic acid) containing protease inhibitors (Roche). HEK293 cells were lysed in lysis buffer (20 mM Tris, pH 7.5, 150 mM NaCl, 1% Triton X-100) containing protease inhibitors (Roche) and phosphatase inhibitors (1 mM Na_3VO_4, 10 mM NaF, and 1 mM β-glycerophosphate). Lysates were incubated with anti-myc antibodies followed by antibody capture using protein A/G agarose beads (Santa Cruz Biotechnology). Beads were washed four times in lysis buffer, and bound proteins were eluted with 2X sample buffer. Proteins were resolved by SDS-PAGE and transferred to nitrocellulose followed by immunoblotting with the indicated antibodies.

Supplementary References

1. O'Reilly AM, Lee HH, Simon MA (2008) Integrins control the positioning and proliferation of follicle stem cells in the Drosophila ovary. *J Cell Biol* **182**: 801-815

Supplementary Figure Legends

Supplementary Figure S1: Specificity of the anti-DAck antibody.

(A) Egg chambers from flies of the indicated genotype were dissected and immunostained with the DAck antibody. Scale bar = 50 μm.

(B) Localization of FLAG-DAck in the female germline using an anti-FLAG antibody. Shown is a three-dimensional confocal projection of an egg chamber of the indicated genotype. Scale bar = 10 μm.

Supplementary Figure S2: Decreased PIP_2 levels in egg chambers of *CTPS*$^{e/f}$ flies.

Expression of a *UAS-GFP-PH-PLCδ* transgene (green) was driven in the female germline using *nanos*-Gal4:VP16 in *w*^{1118} and *CTPS*$^{e/f}$ flies. Shown are three-dimensional confocal projections of stage 10 egg chambers stained with Draq5 (blue). Scale bar = 20 μm.

Supplementary Figure S3: CTP synthase protein expression in early and late oogenesis.

Western blot analysis of early (germarium [G] through stage 9) and late (stage 10-14) egg chambers from *w*^{1118} and *DAck*^{86} flies using anti-CTP synthase, anti-DAck, and anti-β-tubulin antibodies. Arrow indicates the band corresponding to CTP synthase.

Supplementary Figure S4: Transgenic expression of human CTPS1-E161K in *Drosophila* ovarian germline cells.

(A) Evolutionary conservation of glutamate residue 161 (red) of CTP synthase. An alignment of the corresponding regions of CTP synthase from *H. sapiens, D. melanogaster, and S. cerevisiae* is shown. **(B)** Comparable expression of endogenous GFP-CTP synthase and Gal4-driven GFP-CTPS1-E161K in the female germline as demonstrated by immunoblotting of ovary lysates with an anti-GFP antibody.

Supplementary Figure S5: Ack does not phosphorylate CTP synthase.

HEK293 cells were either untransfected, transfected with a plasmid encoding myc-CTPS1, or co-transfected with plasmids encoding myc-CTPS1 and FLAG-Ack1-1-789. Anti-myc antibody was used to immunoprecipitate myc-CTPS1 from cell lysates, and the immunoprecipitates were probed with anti-myc and anti-phosphotyrosine antibodies. The bottom panel demonstrates expression of FLAG-Ack1-1-789 in the relevant cell lysate.

Supplementary Movie S1: Three-dimensional confocal projection of *Drosophila* egg chambers stained with an anti-DAck antibody.