

## **Expanded View Figures**

**Figure EV1. Transcript abundance of ACC1, FAS1, and FAS2 in the cell cycle.** The log<sub>2</sub>-transformed normalized (FPKMs) reads of the indicated transcripts are shown for each cell size pool, as described in Fig 1.



Figure EV2. The ACC1 mRNA does not oscillate in the cell cycle in strains carrying C-terminal TAP-tagged alleles of ACC1 at their endogenous chromosomal locations.

A, B Samples were collected by elutriation and allowed to progress synchronously in the cell cycle. At the indicated cell sizes, the abundance of the indicated mRNAs was queried and quantified as described in Materials and Methods.

Source data are available online for this figure.





## Figure EV3. Lipid droplet and phospholipid content of ACC1 uORF mutant cells in the cell cycle.

- A Representative images to visualize neutral lipids using fluorescence microscopy of wild-type (*uORF*<sup>+</sup>-*ACC1*) cells stained with Nile Red as described in Materials and Methods. The images were taken from cells grown in rich, glucose-containing medium at the following states: proliferating exponentially (Log); arrested in mitosis with nocodazole (M); and 20 or 40 min after they were released from the arrest (20' or 40').
- B Images of ACC1 uORF mutant cells (TG-340AA-ACC1), obtained as in (A).

C Quantification of the lipid droplet (Neutral Lipids) staining from (A and B), displayed with box-plots, normalized for the values of the "Log *uORF*<sup>+</sup>-*ACC1*" sample. The median and the inter-quartile range are indicated, along with the number of cells examined in each case. The whiskers extend to 1.5 times the interquartile range.
 D Strip charts showing the quantification of total phospholipid (PLs) content from the same cell populations shown in (A–C), normalized for the values of the "Log *uORF*<sup>+</sup>-*ACC1*" sample.

Data information: In all microscope images, 5  $\mu m$  scale bars are indicated.



## Figure EV4. Lipid droplet and phospholipid content of ACC1 uORF mutant cells in glycerol medium.

- A, B Representative images to visualize lipid droplets using fluorescence microscopy of wild-type (A) or ACC1 uORF mutant cells (B) proliferating exponentially in poor medium, with glycerol as carbon source. In all microscope images, 5 μm scale bars are indicated.
- C Quantification of the lipid droplet (Neutral Lipids) staining from (A and B), displayed with box-plots, normalized for the values of the "*uORF*<sup>+</sup>-*ACC1*" sample. The median and the inter-quartile range are indicated, along with the number of cells examined in each case. The whiskers extend to 1.5 times the interquartile range.
  D Strip chart showing the quantification of total phospholipid (PLs) content from the same cell populations shown in (A–C), normalized for the values of the "*uORF*<sup>+</sup>-*ACC1*" sample.



## Figure EV5. Model for the nutrient-dependent control of Acc1p levels mediated by the uORF.

- A Top: In rich media with high ribosome content, the uORF does not significantly inhibit the number of scanning ribosomes that initiate from the main ACC1 ORF. Bottom: In poor media with lower ribosome content, the uORF reduces the number of scanning ribosomes that are able to reach the main, downstream AUG, thereby disproportionately reducing Acc1p synthesis.
- B The expected translational efficiency of the wild-type ACC1 mRNA (shown in black) and the mutant lacking the uORF (shown in red) is depicted as a function of the concentration of active initiating ribosomes, R\*, in the cell, adapted from the standard theoretical calculations of Lodish (1974).