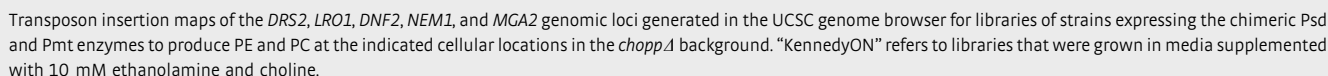


LR01 DNF2 CH01



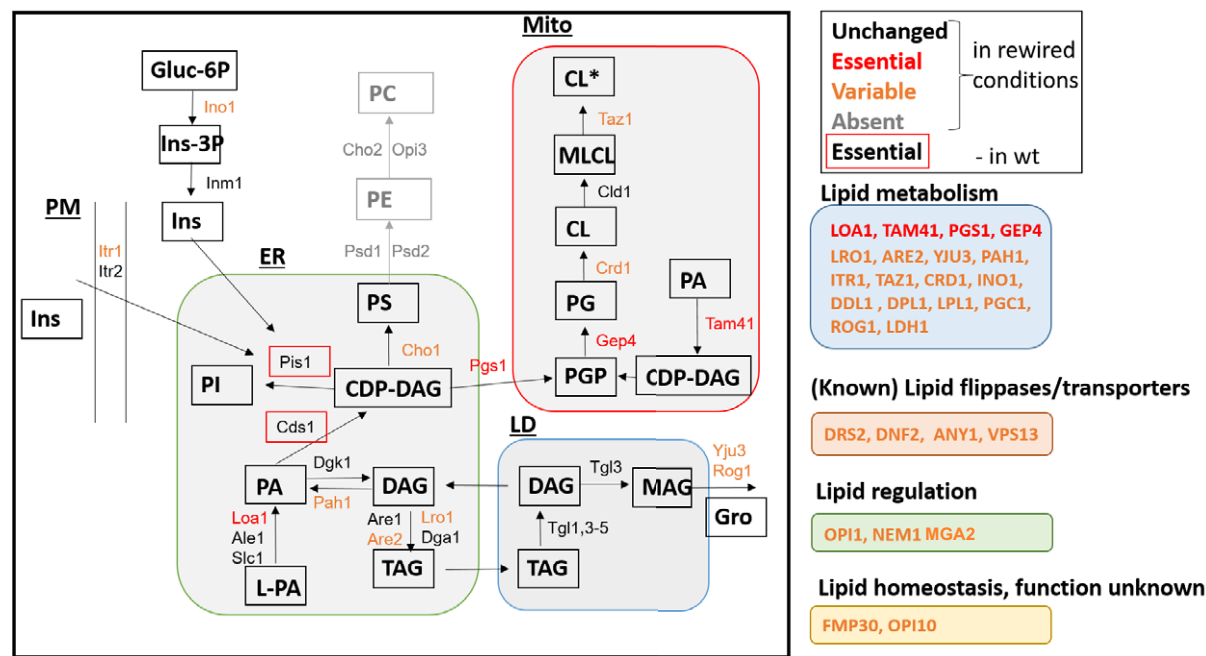


Figure EV2. Requirement for lipid metabolic genes in rewired yeast.
Schematic illustration of lipid synthesis pathways in yeast. Genes that are essential or absent in all libraries are depicted in red or gray, respectively. Genes variably required in one or more rewired libraries, as assessed by manual inspection or volcano plots, are depicted in orange. Genes that are essential in wild-type conditions are boxed in red.

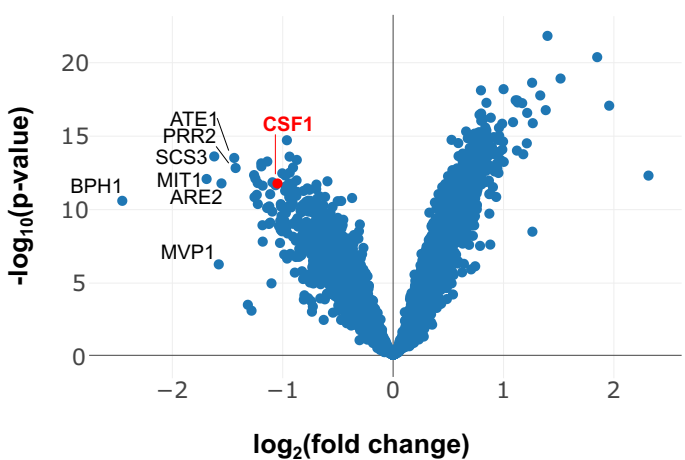


Figure EV3. The requirement for Csf1 is specific to the Psd-MIM/Pmt-pex library grown in Kennedy_{OFF} conditions.
Volcano plot comparing the number of transposon insertions per gene in the Psd-MIM / Pmt-pex Kennedy_{OFF} condition versus all other libraries. CSF1 is highlighted in red.

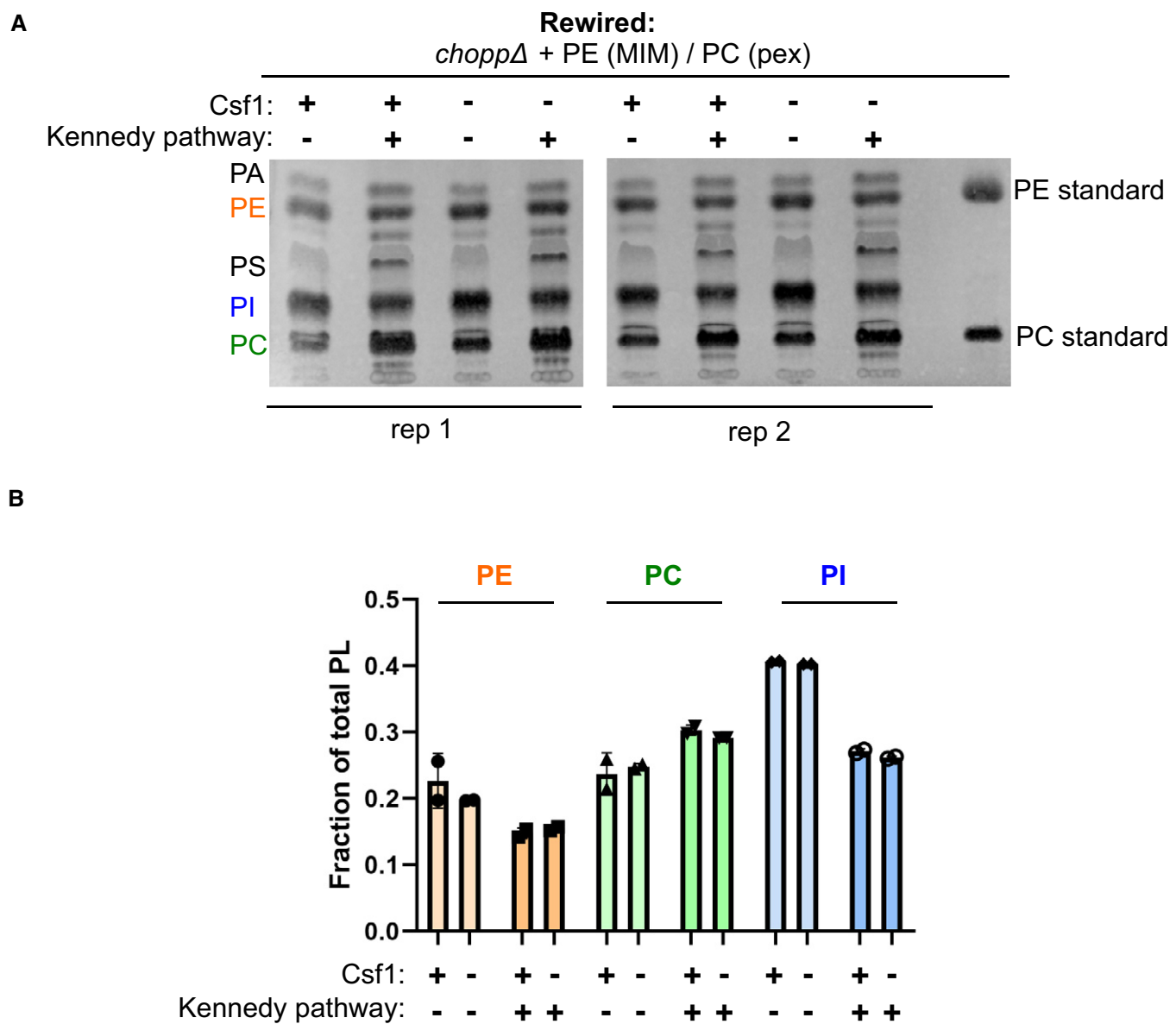


Figure EV4. The absence of Csf1 in rewired conditions does not affect steady state PE or PC production.

A Thin-layer chromatography (TLC) analysis of the steady-state lipid profiles of the indicated strains grown in Kennedy_{OFF} or Kennedy_{ON} conditions (+10 mM ethanolamine and choline).

B Quantification of the fraction of PE, PC, and PI of total phospholipids measured by TLC analysis shown in A. Quantification was done using Fiji/ImageJ software as described in the Materials and Methods.

Figure EV5. Rate of growth and ¹⁵N incorporation in PS, PE, and PC during the experiment shown in Fig 6C.

A OD₆₀₀ measurements of the indicated genotypes at different time points taken during the ¹⁵N-serine labeling experiment.

B Line plots depict quantification of the amount of indicated ¹⁵N-labeled species normalized by the amount of its corresponding unlabeled species.

Source data are available online for this figure.

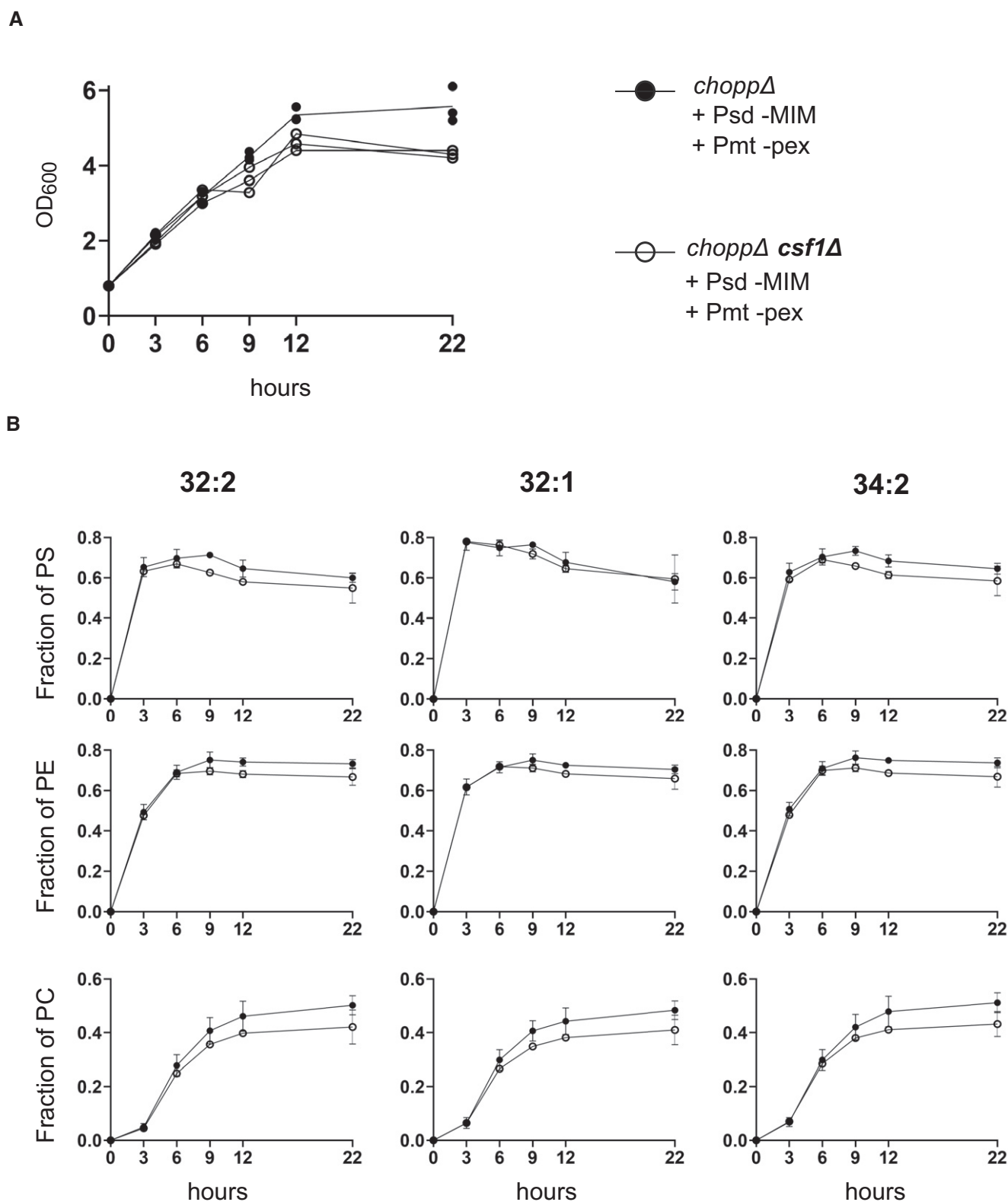


Figure EV5.