Supplemental Materials

Molecular Biology of the Cell

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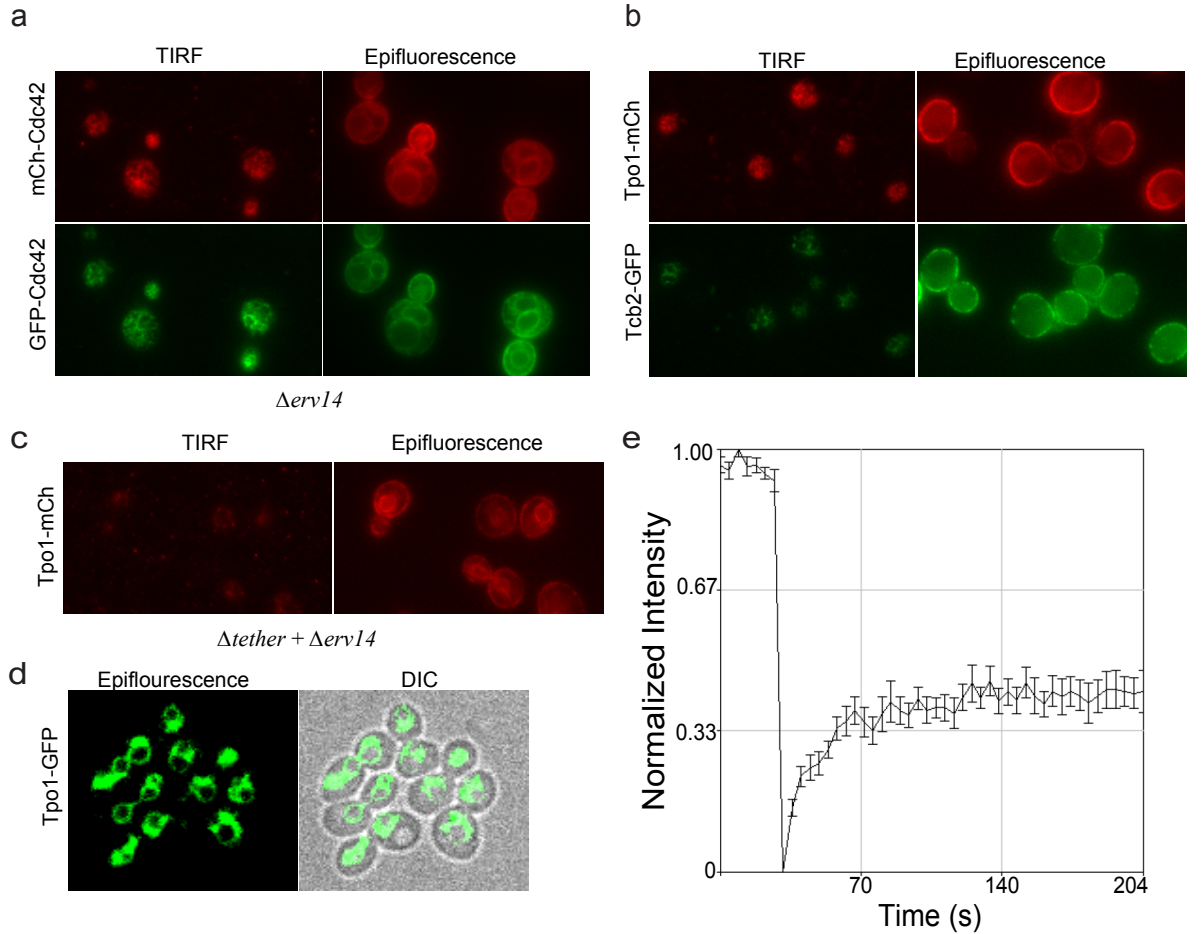
Supplemental Figure 1. Tpo1 is localized in cortical ER in Δ*erv14*. (a) Comparative visualization of membrane associated mCh-Cdc42 and GFP-Cdc42 on plasma membrane using TIRF and epifluorescence microscopy (positive control). (b) Comparative visualization of plasma membrane protein Tpo1-mCh and cortical ER protein Tcb2 in TIRF and epifluorescence microscopy. Tcb2-GFP is not observed in TIRF plane while Tpo1 does, confirming that TIRF plane does not collect signal from cortical ER. (c) Comparative visualization of Tpo1-mCh in Δ*erv14* through TIRF and epifluorescence microscopy suggested no signal on the PM confirming that Tpo1 gets trapped in ER in *erv14* mutant. (d) Tpo1-GFP localized with collapsed cER in Δ*erv14* mutant in the background of the deleted ER-PM tethering proteins *Δtether* (*ist2*Δ, *scs2/22*Δ, and *tcb1/2/3*Δ). (e) Average fluorescence recovery plot for Tpo1-GFP in Δ*erv14* mutant.

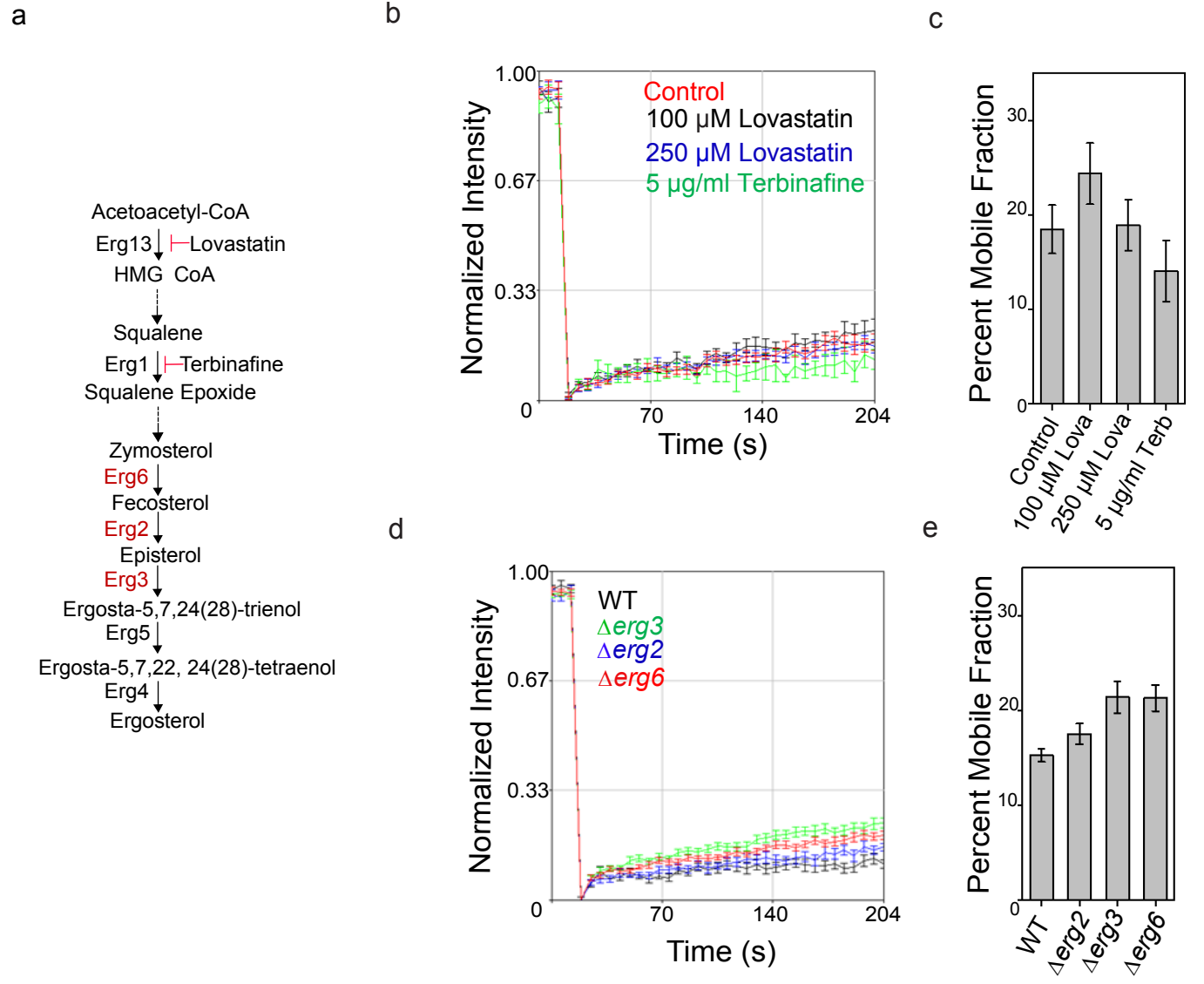
Supplemental Figure 2. Ergosterol-rich domains do not restrict Tpo1 mobility in the plasma membrane. (a) Schematic of ergosterol biosynthetic pathway in yeast showing inhibitors and enzymes in the pathway. Lovastatin (lova) inhibits the conversion of acetyl-CoA into HMG-CoA whereas terbinafine (terb) inhibits the conversion of squalene to squalene epoxide in the ergosterol biosynthetic pathway. Different enzymes involved in the ergosterol biosynthetic pathway are shown on the left side of each step. (b) Average fluorescence recovery traces for Tpo1-GFP in control (red, n=15), 100 μM lova (black, n=13), 250 μM lova (blue, n=14), and 5 μg/ml terb (green, n=11)-treatment conditions. (c) Percent mobile fractions for Tpo1-GFP in control, 100 μM lova, 250 μM lova, and 5 μg/ml terb-treatment conditions. (d) Average fluorescence recovery plot for Tpo1-GFP in WT (black, n=13), *Δerg3* (green, n=15), *Δerg2,* (black, n=11) and *Δerg6* (red, n=14) different ergosterol mutants. (e) Percent mobile fractions for Tpo1-GFP in WT, *Δerg2*, *Δerg3* and *Δerg6.* Mean ± SEM are indicated. Statistical analysis showed no significant difference between drug-treated or mutant samples and respective control.

Supplementary Figure 3. Decline in Tpo1 level is significantly higher in C18SL with RLS. (a) Decay rate measurement for Tpo1-GFP in single cell time lapse microscopy with replicative life span post gal promoter induction on microfluidics device. Modeling of Tpo1 protein level for (b) WT and (c) C18SL with RLS. (d) Tpo1 protein level with yeast RLS.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3