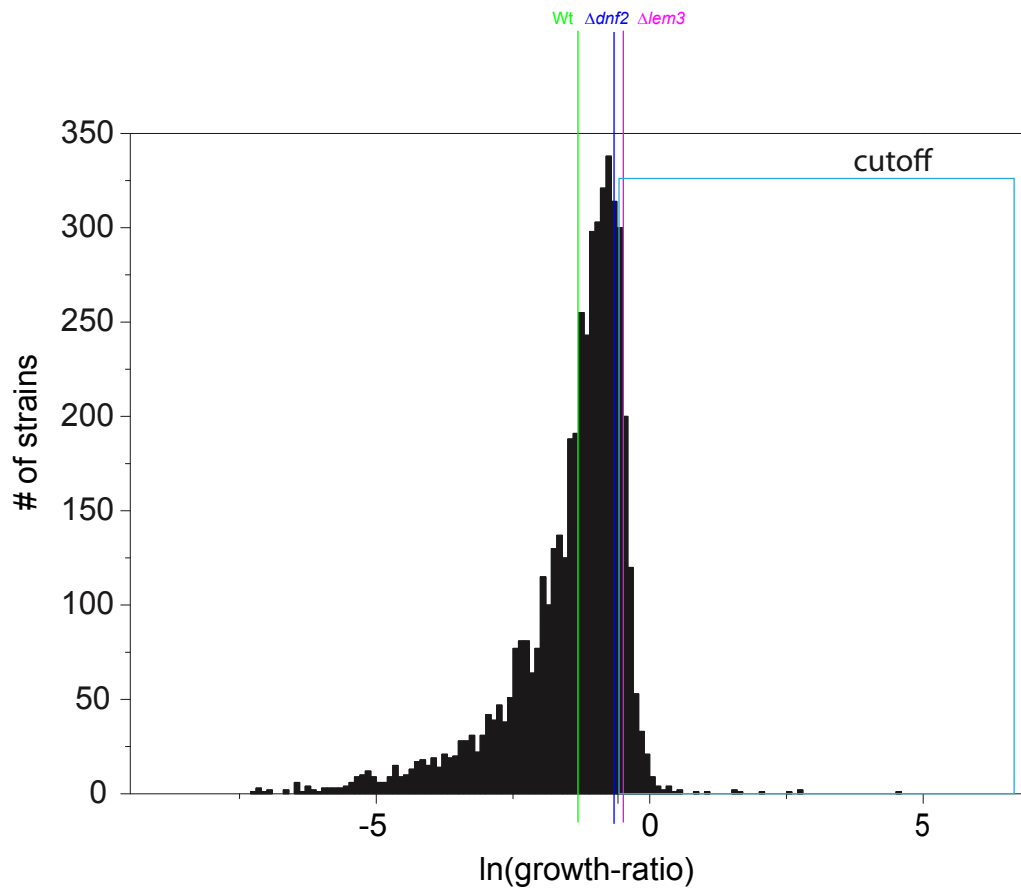


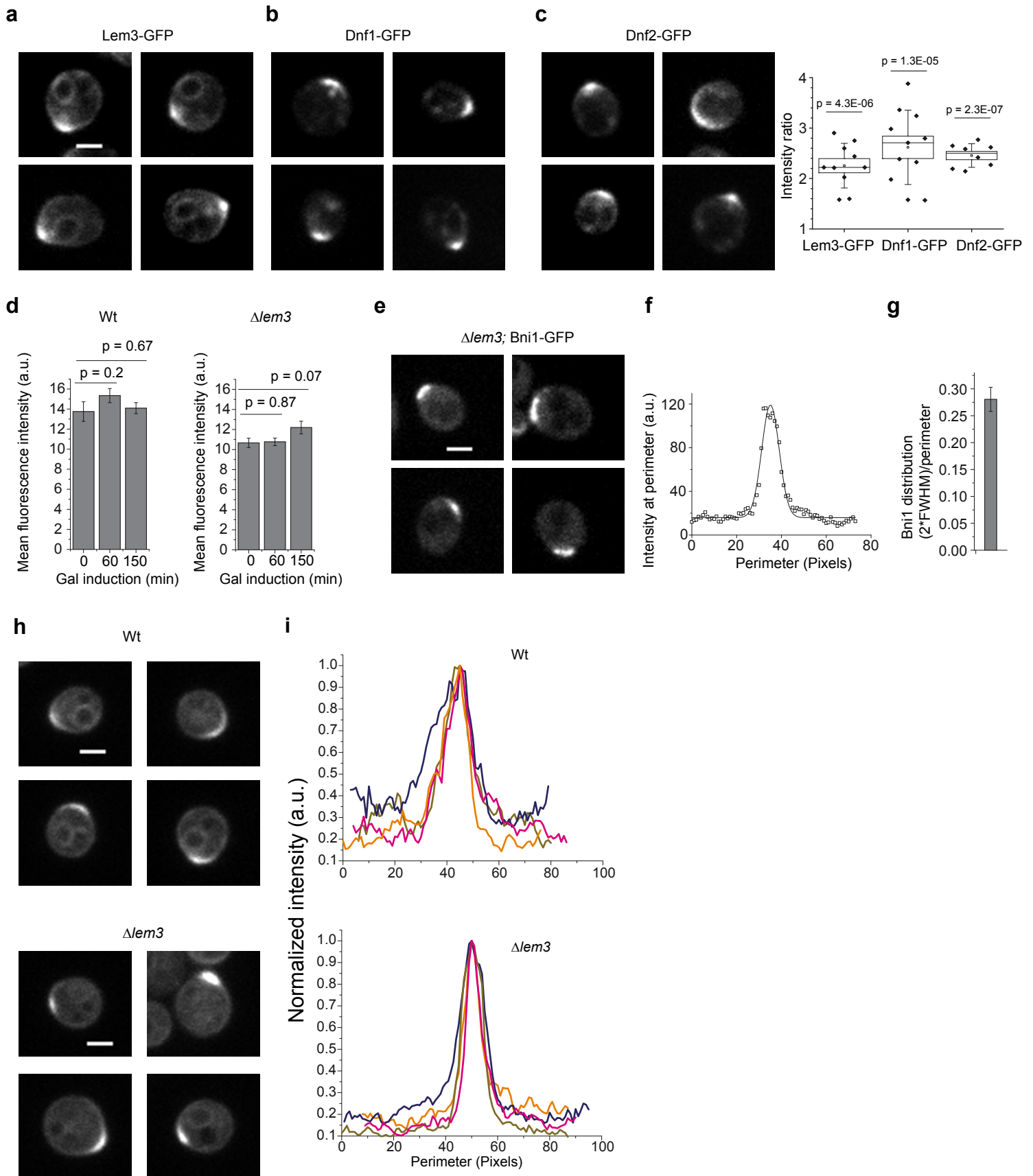
a



b

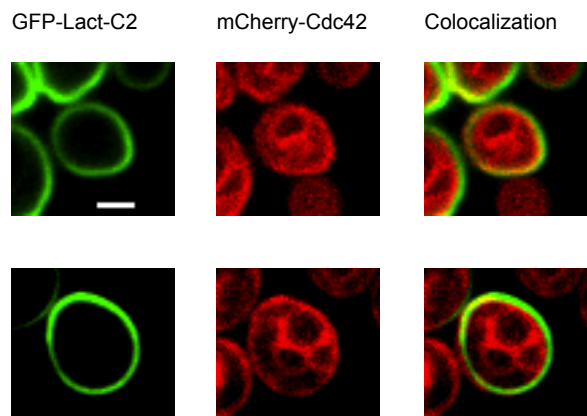
Rescuer candidates that localize to the plasma membrane.

<i>LEM3</i>	Membrane protein of the plasma membrane and ER, interacts specifically in vivo with the phospholipid translocase (flippase) Dnf1p; involved in translocation of phospholipids and alkylphosphocholine drugs across the plasma membrane.
<i>THI73</i>	Putative plasma membrane permease proposed to be involved in carboxylic acid uptake and repressed by thiamine; substrate of Dbf2p/Mob1p kinase; transcription is altered if mitochondrial dysfunction occurs
<i>PUN1</i>	Plasma membrane protein with a role in cell wall integrity; co-localizes with Sur7p in punctate membrane patches; null mutant displays decreased thermotolerance; transcription induced upon cell wall damage and metal ion stress
<i>FUS1</i>	Membrane protein localized to the shmoo tip, required for cell fusion; expression regulated by mating pheromone; proposed to coordinate signaling, fusion, and polarization events required for fusion; potential Cdc28p substrate
<i>ROD1</i>	Membrane protein that binds the ubiquitin ligase Rsp5p via its 2 PY motifs; overexpression confers resistance to the GST substrate o-dinitrobenzene, zinc, and calcium; proposed to regulate the endocytosis of plasma membrane proteins
<i>SSO1</i>	Plasma membrane t-SNARE involved in fusion of secretory vesicles at the plasma membrane and in vesicle fusion during sporulation; forms a complex with Sec9p that binds v-SNARE Snc2p; syntaxin homolog; functionally redundant with Sso2p
<i>SSO2</i>	Plasma membrane t-SNARE involved in fusion of secretory vesicles at the plasma membrane; syntaxin homolog that is functionally redundant with Sso1p
<i>YPS1</i>	Aspartic protease, member of the yapsin family of proteases involved in cell wall growth and maintenance; attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
<i>PSR2</i>	Functionally redundant Psr1p homolog, a plasma membrane phosphatase involved in the general stress response; required with Psr1p and Whi2p for full activation of STRE-mediated gene expression, possibly through dephosphorylation of Msn2p
<i>PMP3</i>	Small plasma membrane protein related to a family of plant polypeptides that are overexpressed under high salt concentration or low temperature, not essential for viability, deletion causes hyperpolarization of the plasma membrane potential
<i>YPS3</i>	Aspartic protease, member of the yapsin family of proteases involved in cell wall growth and maintenance; attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor
<i>SNC2</i>	Vesicle membrane receptor protein (v-SNARE) involved in the fusion between Golgi-derived secretory vesicles with the plasma membrane; member of the synaptobrevin/VAMP family of R-type v-SNARE proteins
<i>LDB19</i>	Protein involved in regulating the endocytosis of plasma membrane proteins by recruiting the ubiquitin ligase Rsp5p to its target; localization changes in response to nutrient levels; null mutant has reduced affinity for alcian blue dye
<i>SUR7</i>	Plasma membrane protein that localizes to furrow-like invaginations (MCC patches); component of eisosomes; associated with endocytosis, along with Pil1p and Lsp1p; sporulation and plasma membrane sphingolipid content are altered in mutants
<i>TPN1</i>	Plasma membrane pyridoxine (vitamin B6) transporter; member of the purine-cytosine permease subfamily within the major facilitator superfamily; proton symporter with similarity to Fcy21p, Fcy2p, and Fcy22p

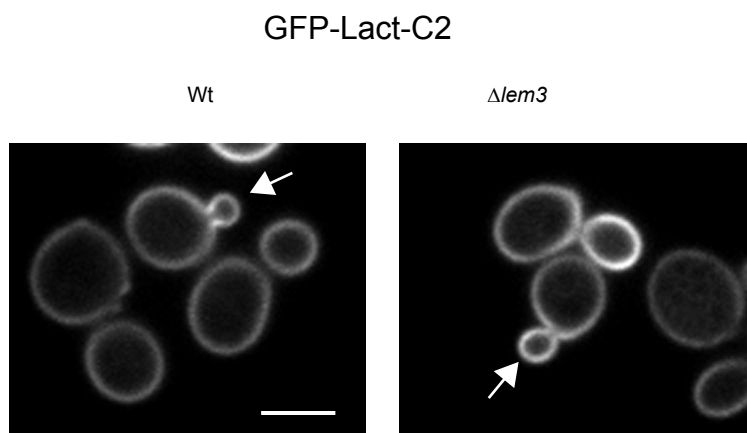


Supplementary Figure 2

a



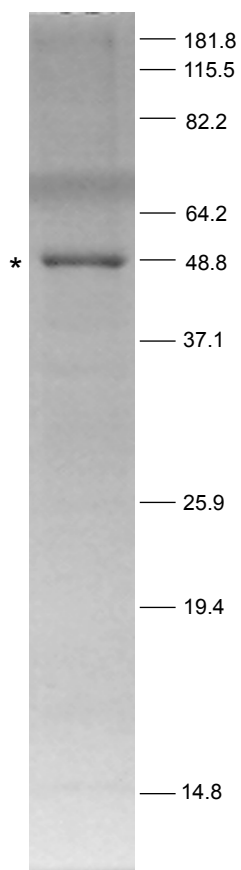
b



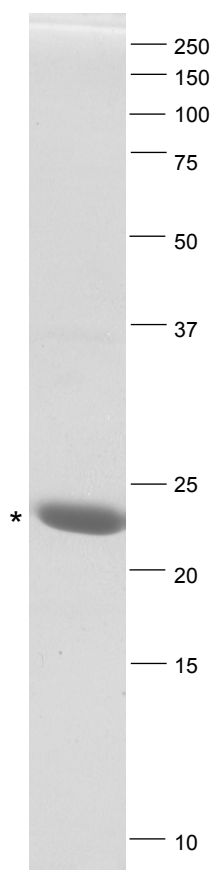
Supplementary Figure 3

a

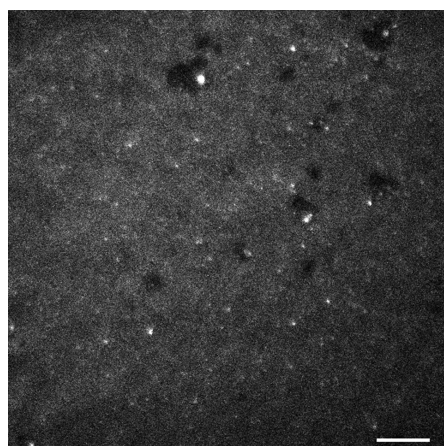
TC-Cdc42

**b**

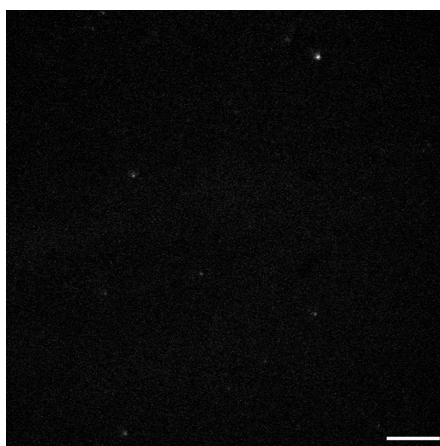
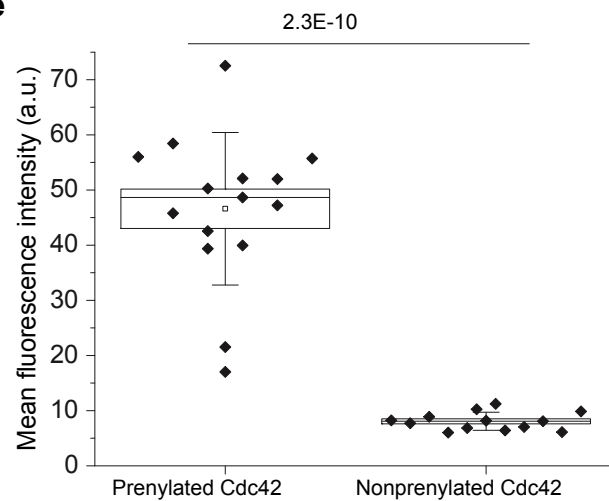
Rdi1-6XHis

**c**

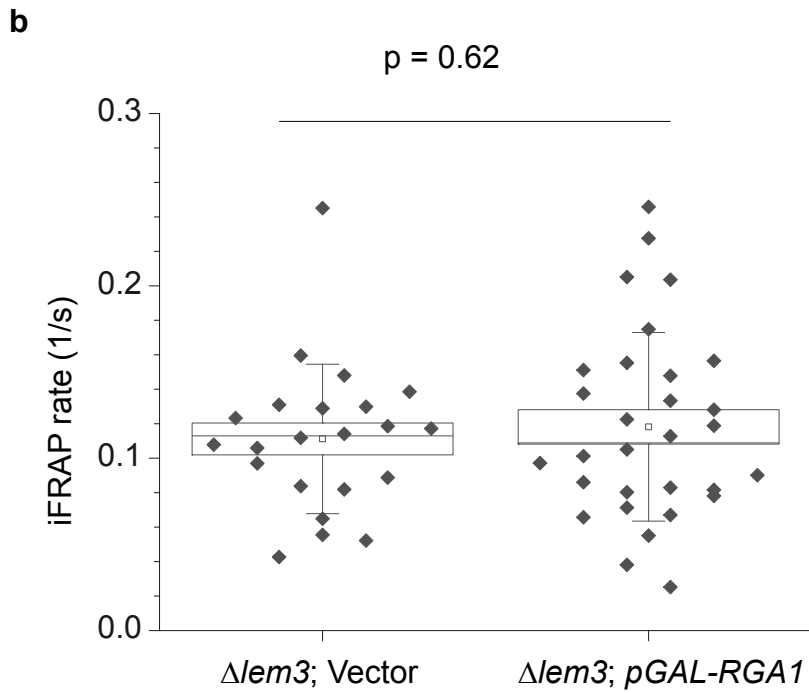
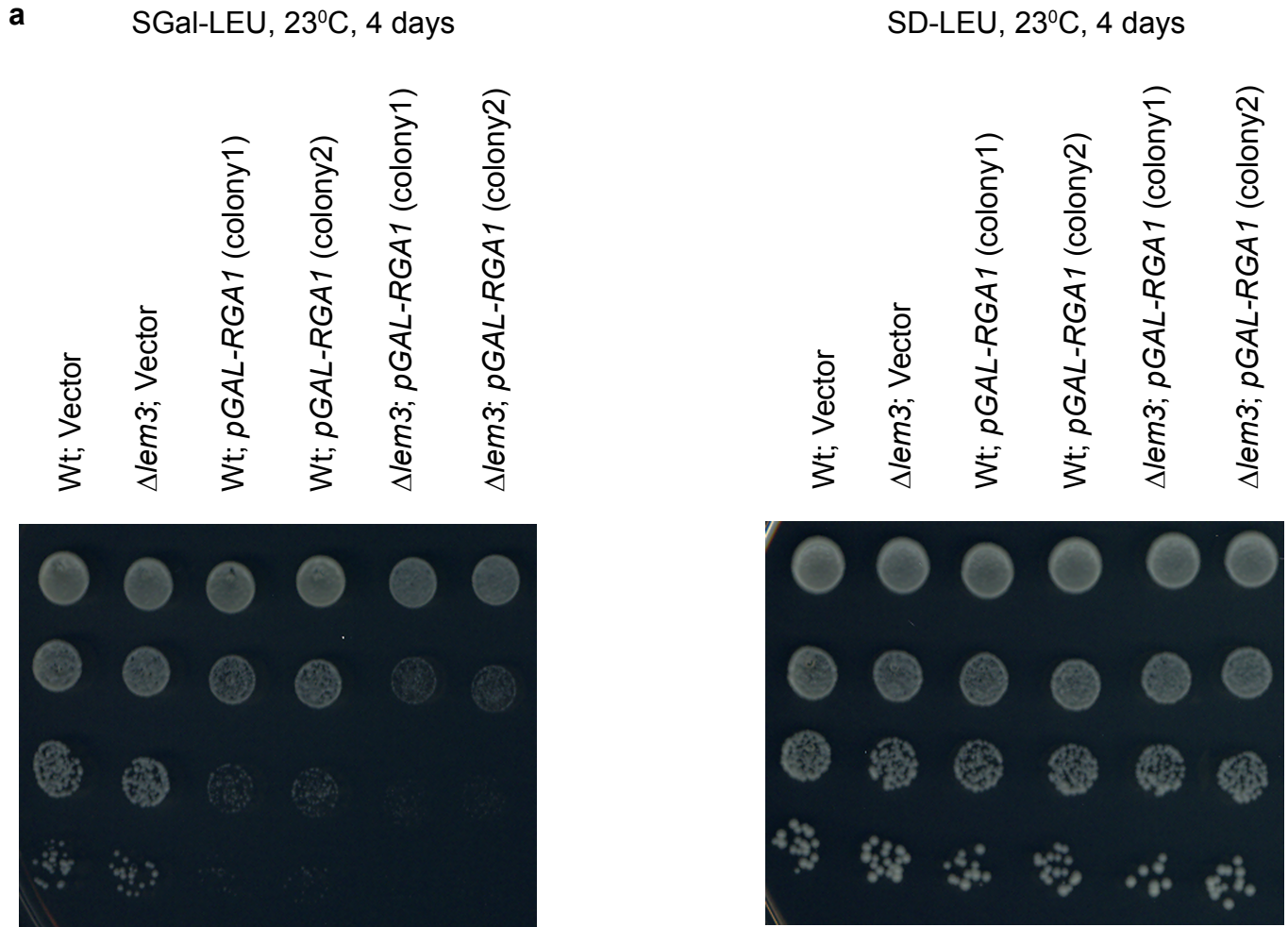
Prenylated Cdc42

**d**

Nonprenylated Cdc42

**e**

Supplementary Figure 4



Supplementary Figure 5

Type of file: table

Label: Table 1

Filename: 2011-1023-LIBPB-325 SupplementaryTable1.xlsx

Strains

RLY 2530

RLY3458

RLY3556

RLY3557

RLY3811

RLY3856

RLY3857

RLY3858

RLY4153

RLY4154

RLY4155

RLY4273

RLY4277

RLY4300

RLY4796

RLY4797

RLY4826

RLY4839

RLY4875

RLY4921

RLY4922

RLY4923

RLY6593

RLY6617

RLY6766

RLY6768

RLY6875

RLY6907

RLY6908

RLY6909

RLY7104

RLY7498

RLY7544

RLY7550

RLY7551

RLY7552

RLY7553

All the strains were o

* All strains used in t

1

2

Genotype*

his3Δ1;leu2Δ0;met15Δ0;ura3Δ0

RDII-mCHERRY::HIS

pGAI1-GFP-myc6-CDC42^{R66E} CEN URA3 RDII-mCHERRY::HIS5

pGAI1-GFP-myc6-CDC42^{C188S} CEN URA3 RDII-mCHERRY::HIS5

pGAL1-RDII CEN HIS5

LEM3-GFP::HIS5

DNF1-GFP::HIS5

DNF2-GFP::HIS5

lem3Δ :: KAN pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3)

dnf1Δ :: KAN pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3)

dnf2Δ :: KAN pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3)

lem3Δ :: KAN pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3) pGAL1-RDII CEN HIS5

pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3)

pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3 pGAL1-RDII CEN HIS5

pGAI1-GFP-myc6-CDC42^{S185A} CEN URA3

pGAI1-GFP-myc6-CDC42^{S185K} CEN URA3

rdi1Δ :: LEU2 pGAL1-GST-HA-FLASH-CDC42/ pRS316 URA3)

pGAI1-GFP-myc6-CDC42^{S185D} CEN URA3

CEN LEU2

lem3Δ :: KAN pGAI1-GFP-myc6-CDC42^{S185K} CEN URA3

lem3Δ :: KAN pGAI1-GFP-myc6-CDC42^{S185D} CEN URA3

lem3Δ :: KAN pGAI1-GFP-myc6-CDC42^{S185A} CEN URA3

dnf1Δ :: KAN dnf2Δ :: KAN pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3)

lem3Δ :: KAN ; rdi1Δ :: LEU2 pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3)

GFP-LACT-C2 CEN URA3

lem3Δ :: KAN GFP-LACT-C2 CEN URA3

pCDC42-mCHERRY-CDC42/ pRS305 LEU2 GFP-LACT-C2 CEN URA3

GFP-PHx2-PLCd CEN URA

lem3Δ :: KAN GFP-PHx2-PLCd CEN URA

lem3Δ :: KAN BNII-GFP::HIS5

rdi1Δ :: LEU2 pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3)

pil1Δ :: KAN pRL369(pCDC42-GFP-myc6-CDC42/ pRS306 URA3)

lem3Δ :: KAN CEN LEU2

pGAL1-RGA1 CEN LEU2 (colony1)

pGAL1-RGA1 CEN LEU2 (colony2)

lem3Δ :: KAN pGAL1-RGA1 CEN LEU2 (colony1)

lem3Δ :: KAN pGAL1-RGA1 CEN LEU2 (colony2)

originally created for this study except RLY3557¹ and RLY3856-RLY3858².

this study are the derivatives of S288C background with the following genotype: MATa his3 Δ 1;leu2 Δ 0;me

Slaughter, B. D., Das, A., Schwartz, J. W., Rubinstein, B. & Li, R. Dual modes of Cdc42 recycling fine-tune polarized morphogenesis. *Dev. Cell* **17**, 823-835 (2009).

Huh, W. K. *et al.* Global analysis of protein localization in budding yeast. *Nature* **425**, 686-691 (2003).

t15Δ0;ura3Δ0