Supplemental Materials

Molecular Biology of the Cell

Luo et al.

SUPPLEMENTAL MATERIALS:

Supplemental Figure 1. Ectopic targeting of Tom20-mCherry-tagged exocyst subunits to mitochondria. Colocalization of Tom20-mCherry-tagged exocyst subunits with Cit1-GFP, a marker for mitochondria.

Supplemental Figure 2. Sec3-GFP was recruited to mitochondria in cells expressing Tom20-mCherry tagged exocyst subunits Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo84p, but not Exo70p.

Supplemental Figure 3. Sec4p, Bgl2p and Chs3p associated with mitochondria in cells expressing Tom20-mCherry-Sec3p, but not those expressing Tom20-mCherry. (A) Total lysates from cells expressing Tom20-mCherry or Tom20-mCherry-Sec3p were separated by SDS-PAGE. The amounts of Tom20-mCherry, Tom20-mCherry-Sec3p, Sec4p, Bgl2p, Chs3p, and alcohol dehydrogenase ("Adh1p", as a loading control) were analyzed by immunoblotting. Molecular weights (in "kD") are indicated to the left. (B) Mitochondria were purified as previously described (Meisinger et al., 2006). The association of Sec4p, Bgl2p and Chs3p to mitochondria in cells expressing Tom20-mCherry or Tom20-mCherry-Sec3p was analyzed by immunoblotting.

Supplemental Figure 4. Sec4p was not recruited to mitochondria by Tom20-mCherry tagged exocyst subunits Sec5p, Sec6p, Sec8p, Sec10p, Sec15p, Exo70p, and Exo84p. With either DMSO or latrunculin B treatment, Sec4p was not recruited to mitochondria in these cells.

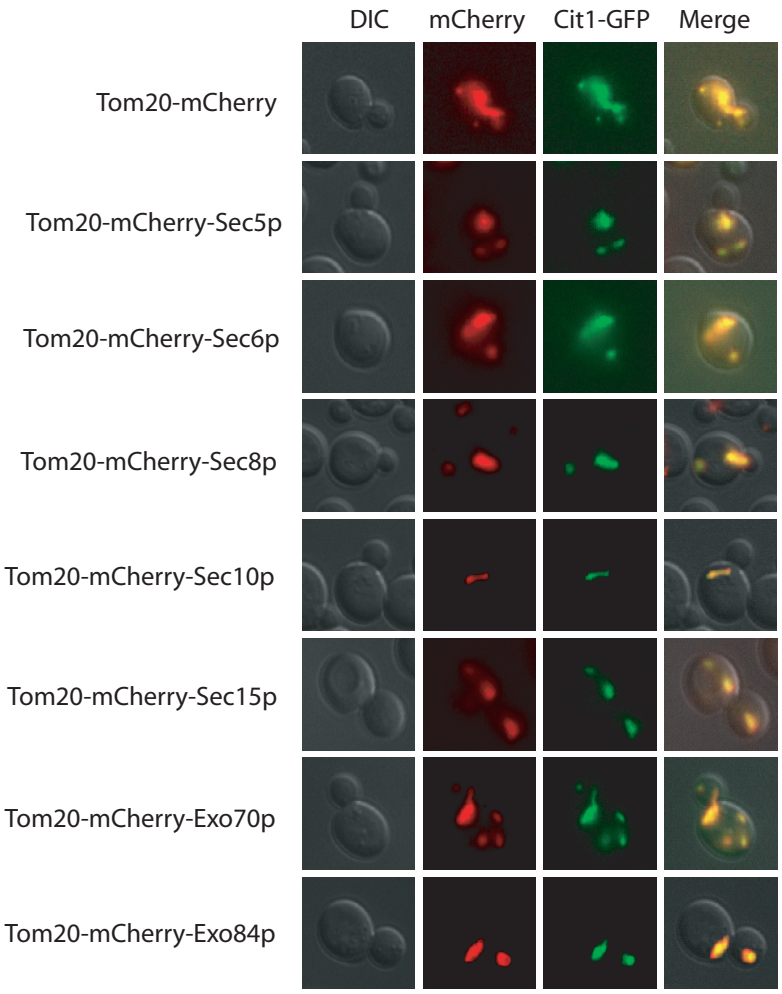
Supplemental Figure 5. Latrunculin-treatment does not affect the recruitment of Sec8p to mitochondria in cells expressing Tom20-mCherry-Sec3p. Localization of Sec8-GFP was examined in cells expressing Tom20-mCherry or Tom20-mCherry-Sec3p treated with DMSO (left) or latrunculin B (right).

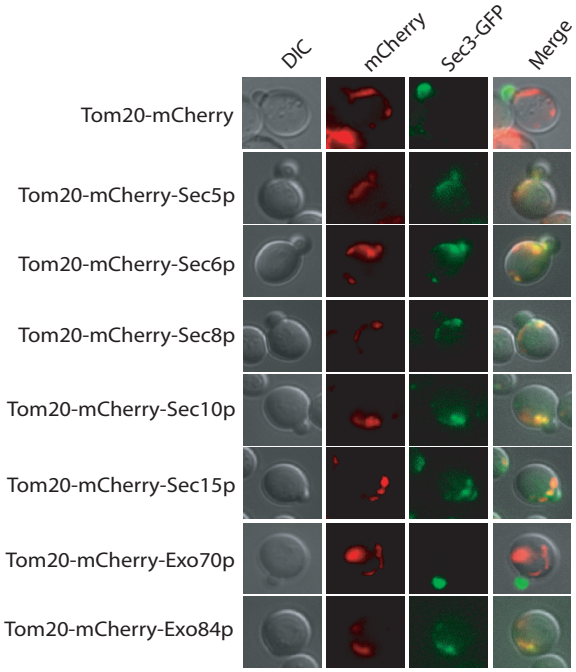
Table S1. Yeast strains used in this study

Name	Genotype	Source
NY1490	*Mata ura3-52 leu2-3, 112, his3Δ200 trp1 Gal-LA-*	Peter Novick lab
NY180	*Matα ura3-52 leu2-3, 112*	Peter Novick lab
NY768	*Matα ura3-52 leu2-3, 112 sec1-1*	Peter Novick lab
NY770	*Matα ura3-52 leu2-3, 112 sec2-41*	Peter Novick lab
NY774	*Matα ura3-52 leu2-3, 112 sec4-8*	Peter Novick lab
NY877	*Matα ura3-52 leu2-3, 112 sec7-1*	Peter Novick lab
NY782	*Matα ura3-52 leu2-3, 112 sec9-4*	Peter Novick lab

Table S2. Plasmids used in this study

Name	Description	Source /Comments
pRCLG	*pRS416-CIT1-GFP*	Liza Pon Lab
p415TEF	*CEN LEU2*	Mumberg*et al.*, 1995
pGV369	*p415TEF-mCherry*	This study
pGV373	*p415TEF-TOM20-mCherry*	This study
pG1872	*p415TEF-TOM20-mCherry-SEC3*	This study
pG1891	*p415TEF-TOM20-mCherry-SEC3N (a.a.1-320)*	This study
pG1892	*p415TEF-TOM20-mCherry-SEC3C (a.a. 321-1336)*	This study
pG1889	*p415TEF-TOM20-mCherry-SEC5*	This study
pG1884	*p415TEF-TOM20-mCherry-SEC6*	This study
pG1886	*p415TEF-TOM20-mCherry-SEC8*	This study
pG1887	*p415TEF-TOM20-mCherry-SEC10,*	This study
pG1890	*p415TEF-TOM20-mCherry-SEC15*	This study
pG1876	*p415TEF-TOM20-mCherry-EXO70*	This study
pG1880	*p415TEF-TOM20-mCherry-EXO84*	This study
pNB882	*pRS306-SEC6-GFP,* digest with BsaBI for chromosomal integration	Peter Novick Lab
pNB885	*pRS306-SEC8-GFP,* digest with BglII for chromosomal integration	Peter Novick Lab
pGV379	*p415TEF-TOM20-2FLAG*	This study
pG1898	*p415TEF-TOM20-2FLAG-SEC3*	This study
pGV377	*p415TEF-Pex3N-mCherry*	This study
pG1895	*p415TEF-Pex3N-mCherry-SEC3*	This study
pG1896	*p415TEF-Pex3N-mCherry-EXO70*	This study
pG379	*pRS313-CHS3-GFP*	Randy Schekman lab


DIC
mCherry
Cit1-GFP
Merge
Tom20-mCherry
Tom20-mCherry-Sec5p
Tom20-mCherry-Sec6p
Tom20-mCherry-Sec8p
Tom20-mCherry-Sec10p
Tom20-mCherry-Sec15p
Tom20-mCherry-Exo70p
Tom20-mCherry-Exo84p


DIC
mCherry
Sec3-GFP
Merge
Tom20-mCherry
Tom20-mCherry-Sec5p
Tom20-mCherry-Sec6p
Tom20-mCherry-Sec8p
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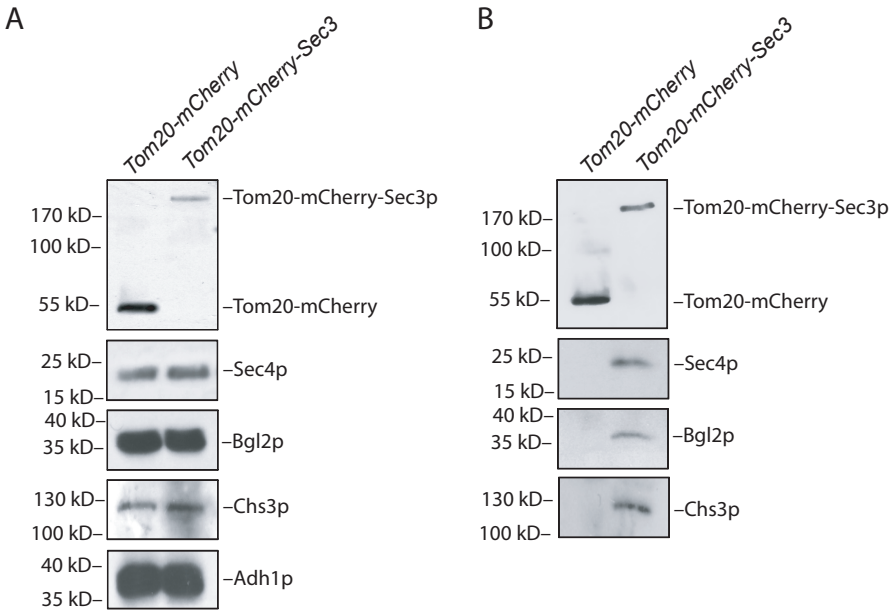
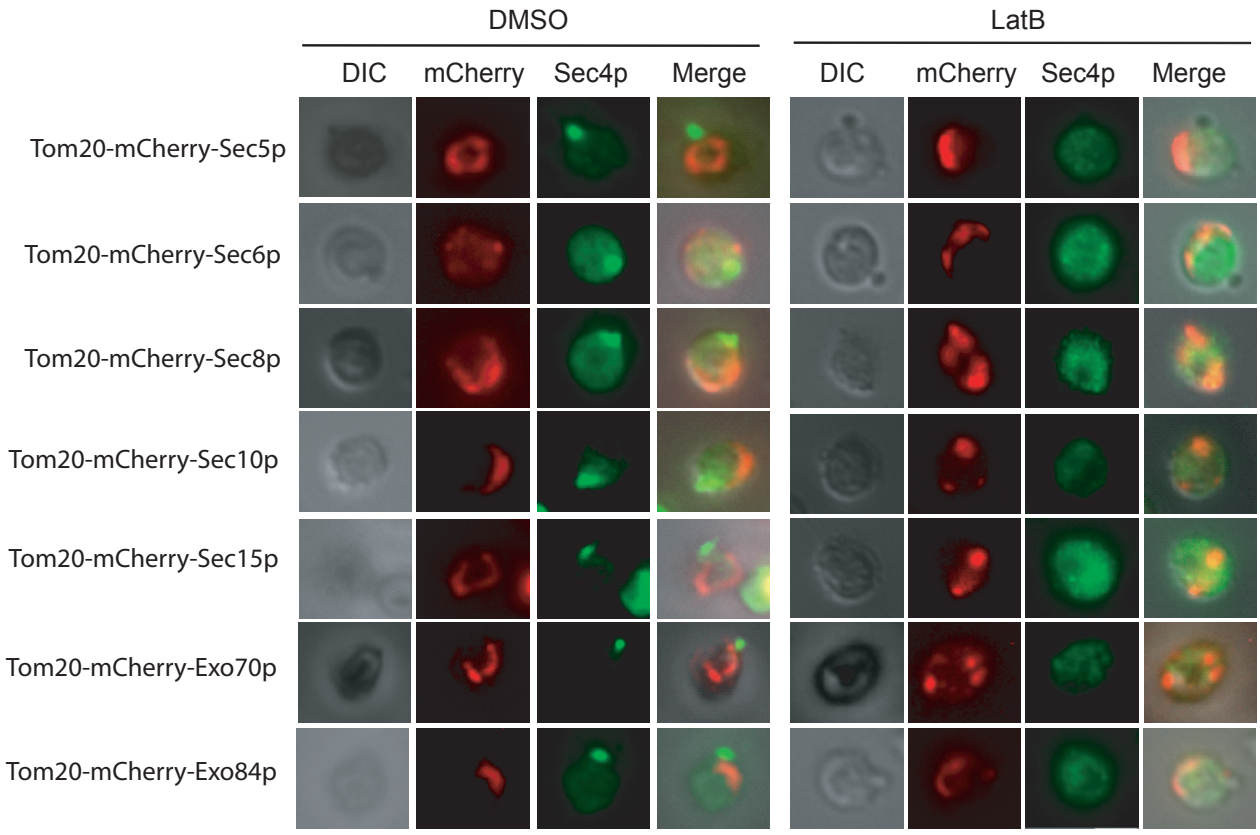
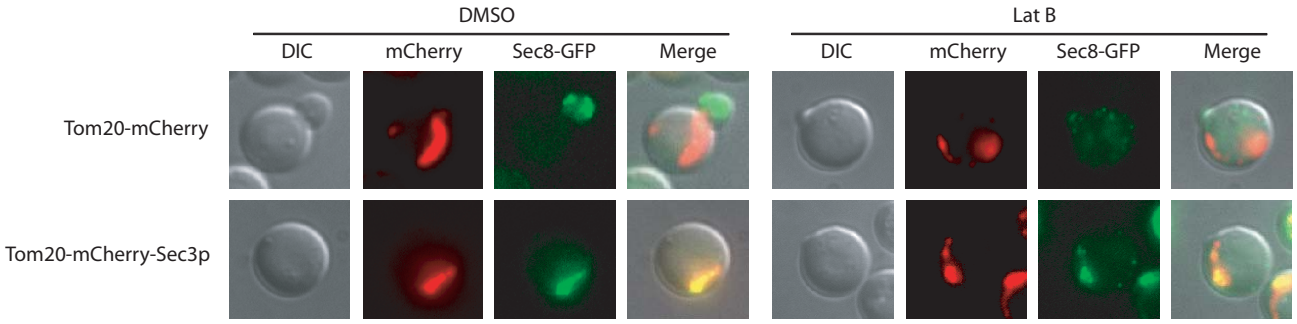
Tom20-mCherry-Sec15p
Tom20-mCherry-Exo70p
Tom20-mCherry-Exo84p


A
Tom20-mCherry
Tom20-mCherry-Sec3
170 kD–
100 kD–
55 kD–
–Tom20-mCherry-Sec3p
–Tom20-mCherry
25 kD–
15 kD–
–Sec4p
40 kD–
35 kD–
–Bgl2p
130 kD–
100 kD–
–Chs3p
40 kD–
35 kD–
–Adh1p
B
Tom20-mCherry
Tom20-mCherry-Sec3
170 kD–
100 kD–
55 kD–
–Tom20-mCherry-Sec3p
–Tom20-mCherry
25 kD–
15 kD–
–Sec4p
40 kD–
35 kD–
–Bgl2p
130 kD–
100 kD–
–Chs3p


DMSO
LatB
DIC
mCherry
Sec4p
Merge
DIC
mCherry
Sec4p
Merge
Tom20-mCherry-Sec5p
Tom20-mCherry-Sec6p
Tom20-mCherry-Sec8p
Tom20-mCherry-Sec10p
Tom20-mCherry-Sec15p
Tom20-mCherry-Exo70p
Tom20-mCherry-Exo84p


DMSO
Lat B
DIC
mCherry
Sec8-GFP
Merge
DIC
mCherry
Sec8-GFP
Merge
Tom20-mCherry
Tom20-mCherry-Sec3p