Supplemental Material for Publication

Supplemental Materials and Methods

Lipid labeling

Strains were grown at 24°C in YPD and diluted into fresh media to an OD_{600} of 0.5. Lipids were labeled by addition of 10 µCi/ml of [9,10-^{3}H]palmitic acid (10 mCi/ml; American Radiolabeled Chemicals, St Louis, MO). 10 OD_{600} units were withdrawn at each time point and cells were pelleted, washed and frozen. Cells were disrupted using glass beads and lipids were extracted with chloroform:methanol (1:1). Aliquots containing equal counts were dried and analyzed by thin layer chromatography (TLC) using silica gel 60 plates (Merck, Darmstadt, Germany), and the solvent system petroleum ether, diethyl ether and glacial acetic acid (70:30:2, v/v/v). Lipids were quantified by radio-TLC scanning (Tracemaster 20, Berthold Technologies, Bad Wildbad, Germany).

Sterol analysis

Lipids were extracted either from whole cells or from isolated microsomes. For total sterol analysis, steryl esters were hydrolyzed under alkaline conditions. Sterols were derivatized using N,O-bis-(trimethylsilyl)-trifluoroacetamide and analyzed on a Voyager Trace 2000 Series GC-MS (Thermo Fisher Scientific, Waltham, MA) equipped with a Zebron ZB-35 capillary column (35% phenyl-methyl polysiloxane; dimensions: 30 m x 0.25 mm x 0.25 mm film thickness) (1). Sterols were identified based on their retention time and their ion fragmentation pattern and quantified relative to cholesterol, which was added as an internal standard to the samples prior to lipid extraction.

References

1. **Quail, MA, Kelly, SL**. 1996. The extraction and analysis of sterols from yeast. Methods Mol Biol **53**:123–131.
2. **Winston, F, Dollard, C, Ricupero-Hovasse, SL**. 1995. Construction of a set of

convenient Saccharomyces cerevisiae strains that are isogenic to S288C. Yeast **11**:53–55.

3. **Hodge, CA, Choudhary, V, Wolyniak, MJ, Scarcelli, JJ, Schneiter, R, Cole, CN**. 2010. Integral membrane proteins Brr6 and Apq12 link assembly of the nuclear pore complex to lipid homeostasis in the endoplasmic reticulum. J Cell Sci **123**:141–151.

4. **Jacquier, N, Choudhary, V, Mari, M, Toulmay, A, Reggiori, F, Schneiter, R**. 2011. Lipid droplets are functionally connected to the endoplasmic reticulum in Saccharomyces cerevisiae. J Cell Sci **124**:2424–2437.

5. **Huh, WK, Falvo, JV, Gerke, LC, Carroll, AS, Howson, RW, Weissman, JS, O'Shea, EK**. 2003. Global analysis of protein localization in budding yeast. Nature **425**:686–691.

Table S1. *S. cerevisiae* strains used in this study:

Name	Relevant genotype	Source
FY86 / RSY3360	*MATα ura3-52 his3Δ200 leu2Δ1*	Winston et al., 1995 (2)
RSY4391	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1 (URA3/2μ)*	This study
SMY 1	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1/195 (URA3/2μ)*	This study
SMY 2 / RSY4392	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1-1(LEU2/CEN)*	This study
SMY 3 / RSY4393	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1-2(LEU2/CEN)*	This study
RSY4530	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1-1(LEU2) pHDEL-RFP(URA3)*	This study
RSY4531	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1-2(LEU2) pHDEL-RFP(URA3)*	This study
RSY4532	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1(URA3) pHDEL-RFP(LEU2)*	This study
RSY4700	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1(URA3) pBRL1-1(LEU2)*	This study
RSY4702	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1(URA3) pBRL1-2(LEU2)*	This study
RSY4697	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1(URA3) pBRL1-1(LEU2) dga1Δ lro1::hPh*	This study
RSY4698	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1(URA3) pBRL1-2(LEU2) dga1Δ lro1::hPh*	This study
RSY4771	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1(URA3) pBRL1-1(LEU2) are1Δ are2::lox-HIS3*	This study
RSY4772	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1(URA3) pBRL1-2(LEU2) are1Δ are2::lox-HIS3*	This study
RSY3363	*MATα ura3Δ0 his3Δ leu2Δ0 lys2 apq12::KanMx*	Hodge et al., 2010 (3)
MWY 2/RSY3361	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 ade2-1 brr6::HIS3 pBRR6-1(LEU2)*	Hodge et al., 2010 (3)
MWY 1	*MATα ura3-52 his3Δ200 leu2Δ1 trp1Δ63 brr6::HIS3 pBRR6 (URA3/CEN)*	Hodge et al., 2010 (3)

RSY5171	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1-1(LEU2) pAPQ12(URA3)*	This study
RSY5172	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1-2(LEU2) pAPQ12(URA3)*	This study
RSY5173	*MATα ura3-1 his3-11,15 trp1-1 leu2-3,112 ade2-1 brr6::HIS3 pBRR6-1(LEU2) pAPQ12(URA3)*	This study
RSY5174	*MATα ura3Δ0 his3 leu2Δ0 lys2Δ0 apq12::KanMx pBRL1(URA3)*	This study
RSY5249	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1-1(LEU2) pBRR6(URA3)*	This study
RSY5250	*MATα ura3-52 his3Δ200 leu2Δ1 brl1::NatMx pBRL1-2(LEU2) pBRR6(URA3)*	This study
RSY5251	*MATα ura3Δ0 his3Δ leu2Δ0 lys2Δ0 apq12::KanMx pBRR6(URA3)*	This study
RSY1826	*MATα ura3Δ0 his3Δ leu2Δ0 lys2Δ0 are2::KanMx are1::HIS5*	Jacquier et al., 2011 (4)
RSY3290	*MATα ura3Δ0 his3Δ leu2Δ0 lys2Δ0 lro1::KanMx dga1Δ*	Jacquier et al., 2011 (4)
RSY3077	*MATα ura3Δ0 his3Δ leu2Δ0 lys2Δ0 are2::KanMx are1::KanMx lro1Δ dga1Δ*	Jacquier et al., 2011 (4)
Nup60-GFP	*MATa ura3Δ0 his3Δ leu2Δ0 met15Δ0 NUP60-GFP::KanMx*	Invitrogen
Nup82-GFP	*MATa ura3Δ0 his3Δ leu2Δ0 met15Δ0 NUP82-GFP::KanMx*	Invitrogen
CSY 699	*MAT? ura3-53 his3Δ200 leu2Δ0 brl1::KanMx NUP82-GFP::KanMx pBRL1(URA3/CEN)*	This study
CSY 701	*MAT? ura3-53 his3Δ200 leu2Δ0 brl1::KanMx NUP60-GFP::KanMx pBRL1(URA3/CEN)*	This study
CSY 699 + *pBRL1-1*	*MAT? ura3-53 his3Δ200 leu2Δ0 brl1::KanMx NUP82-GFP::KanMx pBRL1-1(LEU2/CEN)*	This study
CSY 699 + *pBRL1-2*	*MAT? ura3-53 his3Δ200 leu2Δ0 brl1::KanMx NUP82-GFP::KanMx pBRL1-2(LEU2/CEN)*	This study
CSY 701 + *pbrl1-1*	*MAT? ura3-53 his3Δ200 leu2Δ0 brl1::KanMx NUP60-GFP::KanMx pBRL1-1(LEU2 CEN)*	This study
CSY 701 + *pbrl1-2*	*MAT? ura3-53 his3Δ200 leu2Δ0 brl1::KanMx NUP60-GFP::KanMx pBRL1-2(LEU2/CEN)*	This study

JSY053	*MATα ura3-52 his3Δ200 leu2Δ1 HIS3MX6-PGAL1-GFP::APQ12*	This study
JSY093	*MATα ura3Δ his3Δ1 leu2Δ lys2Δ BRR6::13MYC-kanMX6*	This study
AAY031	*MATa ura3-52 his3Δ200 leu2Δ1 kanMX6-PGAL1-HA::BRL1 kanMX6-PGAL1-HA::BRR6*	This study
AAY044	*MATa ura3-52 his3Δ200 leu2Δ1 HIS3MX6-PGAL1-GFP::APQ12 kanMX6-PGAL1-HA::BRL1 kanMX6-PGAL1-HA::BRR6*	This study
AAY080	*MATα ura3-52 his3Δ200 leu2Δ1 BRR6::13MYC-kanMX6 kanMX6-PGAL1-HA::BRL1*	This study
AAY082	*MATα ura3-52 his3Δ200 leu2Δ1 BRR6::13MYC-kanMX6 kanMX6-PGAL1HA::BRL1 apq12::CaURA3*	This study
RSY5373	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 BRL1-GFP::HIS3MX6*	Huh et al., 2003 (5)
RSY5374	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 APQ12-GFP::HIS3MX6*	Huh et al., 2003 (5)
RSY5375	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 NUP84-GFP::HIS3MX6*	Huh et al., 2003 (5)
RSY5376	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 BRR6-GFP::HIS3MX6*	Huh et al., 2003 (5)
SWY184 / RSY5412	*MATα ura3-52 his3Δ200 leu2-3,112 SPC42-mCHERRY::kanMX6*	S. Westermann
RSY5444	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 APQ12-GFP::HIS3MX6 SPC42-mCHERRY::kanMX6*	This study
RSY5446	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 NUP84-GFP::HIS3MX6 SPC42-mCHERRY::kanMX6*	This study
RSY5449	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 APQ12-GFP::HIS3MX6 BRL1-mCHERRY::kanMX6*	This study
RSY5450	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 NUP84-GFP::HIS3MX6 BRL1-mCHERRY::kanMX6*	This study
RSY5451	*MATa ura3Δ0 his3Δ1 leu2Δ0 met15Δ0 BRR6-GFP::HIS3MX6 BRL1-mCHERRY::kanMX6*	This study
Nup120Δ	*MATα ura3Δ0 his3Δ1 leu2Δ0 lys2Δ0 nup120Δ::kanMX4*	Euroscarf
RSY5488	*MAT? ura3Δ0 his3Δ1 leu2Δ0 nup120Δ::kanMX4 APQ12-GFP::HIS3MX6*	This study
RSY5490	*MAT? ura3Δ0 his3Δ1 leu2Δ0 nup120Δ::kanMX4 BRL1-*	This study

	GFP::HIS3MX6	
RSY5492	*MAT? ura3Δ0 his3Δ1 leu2Δ0 nup120Δ::kanMX4 NUP84-GFP::HIS3MX6*	This study
RSY5539	*MATα BRL1-GFP::HIS3MX6 SPC42-mCHERRY::kanMX6*	This study
RSY5448	*MATa BRR6-GFP::HIS3MX6 SPC42-mCHERRY::kanMX6*	This study
RSY5541	*MAT? ura3Δ0 his3Δ1 leu2Δ0 nup120Δ::kanMX4 BRR6-GFP::HIS3MX6*	This study
RSY5544	*MAT? ura3Δ0 his3Δ1 leu2Δ0 Nup84-GFP::HIS3MX6 BRR6-mCHERRY::kanMX6*	This study
RSY5546	*MAT? ura3Δ0 his3Δ1 leu2Δ0 APQ12-GFP::HIS3MX6 BRR6-mCHERRY::kanMX6*	This study
RSY5554	*MATa ura3Δ0 his3Δ1 leu2Δ0 BRL1-GFP::HIS3MX6 BRR6-mCHERRY::kanMX6*	This study
RSY5735	*MATa BRL1-GFP::HIS3MX6 SPC42-mCHERRY::kanMX6 pGAL-HO-URA3*	This study

Supplementary figure legends

Figure S1. *brl1* mutants have elevated levels of total sterols and accumulate sterol precursors. (A) *brl1* mutant cells accumulate ergosterol and sterol precursors, episterol, zymosterol, and lanosterol (B). Cells were grown at 24°C overnight in YPD medium. Lipids were hydrolyzed under alkaline conditions and total sterols, composed of free sterols and of steryl esters, were quantified by GC-MS using cholesterol as an internal standard. Values are normalized to those of wild-type cells and represent mean ±SD of two independent experiments.

Figure S2. Defects in sterol homeostasis of *brl1* mutant cells are restored by overexpression of *BRR6* or *APQ12*. Strains of indicated genotypes bearing a vector control or a wild-type copy of *BRR6* (pBRR6), *BRL1* (pBRL1), or *APQ12* (pAPQ12) on a high-copy number plasmid were cultivated in SC medium and total sterols were analyzed by GC-MS. Overexpression of *BRR6* and *APQ12* leads to reduction in ergosterol and episterol levels in *brl1-1* (A, B), and *brl1-2* (C, D), as does the overexpression of *BRL1* or *APQ12* in *brr6* cells (E, F). Values are normalized to those of wild-type cells and represent mean ±SD of two independent experiments.

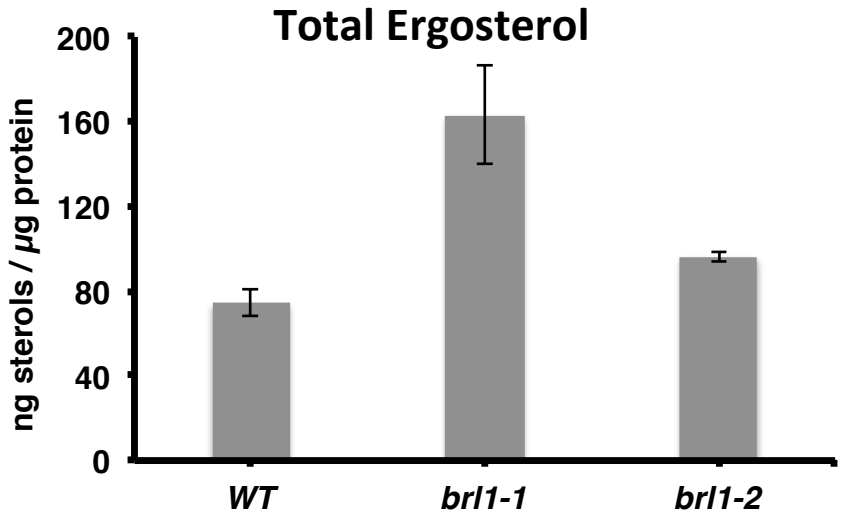
Figure S3. Elevated synthesis of steryl esters in *brl1*. Incorporation of [^{3}H]-palmitic acid into steryl esters (STE) in *brl1-1* and *brl1-2* cells. Strains were pre cultured in YPD media and lipids were radiolabeled with [^{3}H]-palmitic acid (10 μCi/ml) for the indicated period of time. Lipids were extracted, separated by TLC and quantified by radioscanning. The fraction of [^{3}H]-palmitic acid incorporated into STE is plotted [%]. Values are mean ±SD of two independent experiments.

Figure S4. Recovery of wild-type and *brl1-1* mutant cells from treatment with BA. Cells were cultivated at 24°C and treated with BA (0.2%) for 5 h. Cells were washed with fresh medium and re-cultivated for another 5 h or 10 h. Lipids were extracted and fatty acid profiles were recorded by GC and plotted relative to the levels present at the zero time point. Values are mean ±SD of three independent experiments.

Figure S5. Transient colocalization of Brr6 and Brl1 with the SPB. Cells were treated with alpha factor (10 µg/ml for 2 h), the pheromone was washed out and colocalization of Brr6-GFP or Brl1-GFP with the SPB marker, Spc42, was analyzed in synchronized cells by microscopy. Bar, 5 µm.

Figure S6. Localization of Brl1, Brr6, and Apq12 in cells treated with BA. Strains bearing the indicated fluorescently tagged proteins were cultivated in SC media and treated with BA (0.2%) for 2 h or 4 h prior to microscopic analysis of their subcellular distribution. Bar, 5 µm.

A


Total Ergosterol
ng sterols / µg protein
200
160
120
80
40
0
WT
brl1-1
brl1-2

B

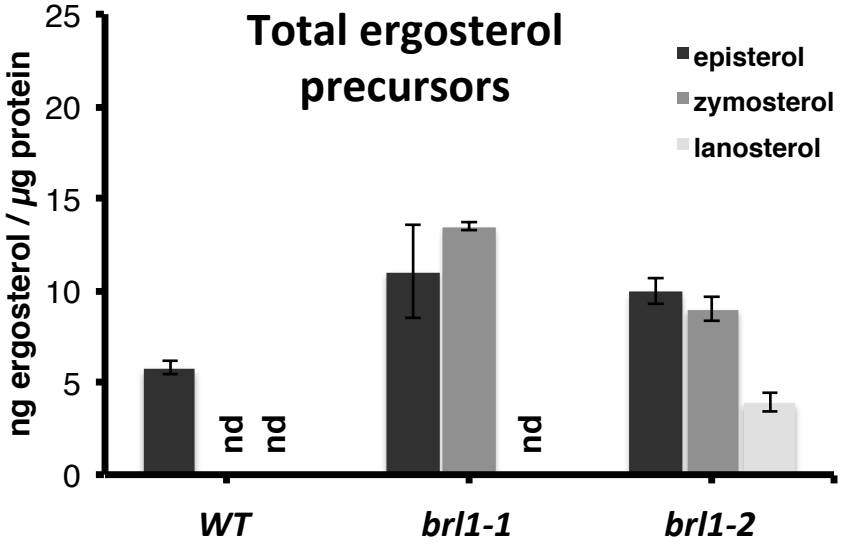

Total ergosterol precursors
ng ergosterol / µg protein
25
20
15
10
5
0
episterol
zymosterol
lanosterol
nd
nd
nd
WT
brl1-1
brl1-2

Figure S1

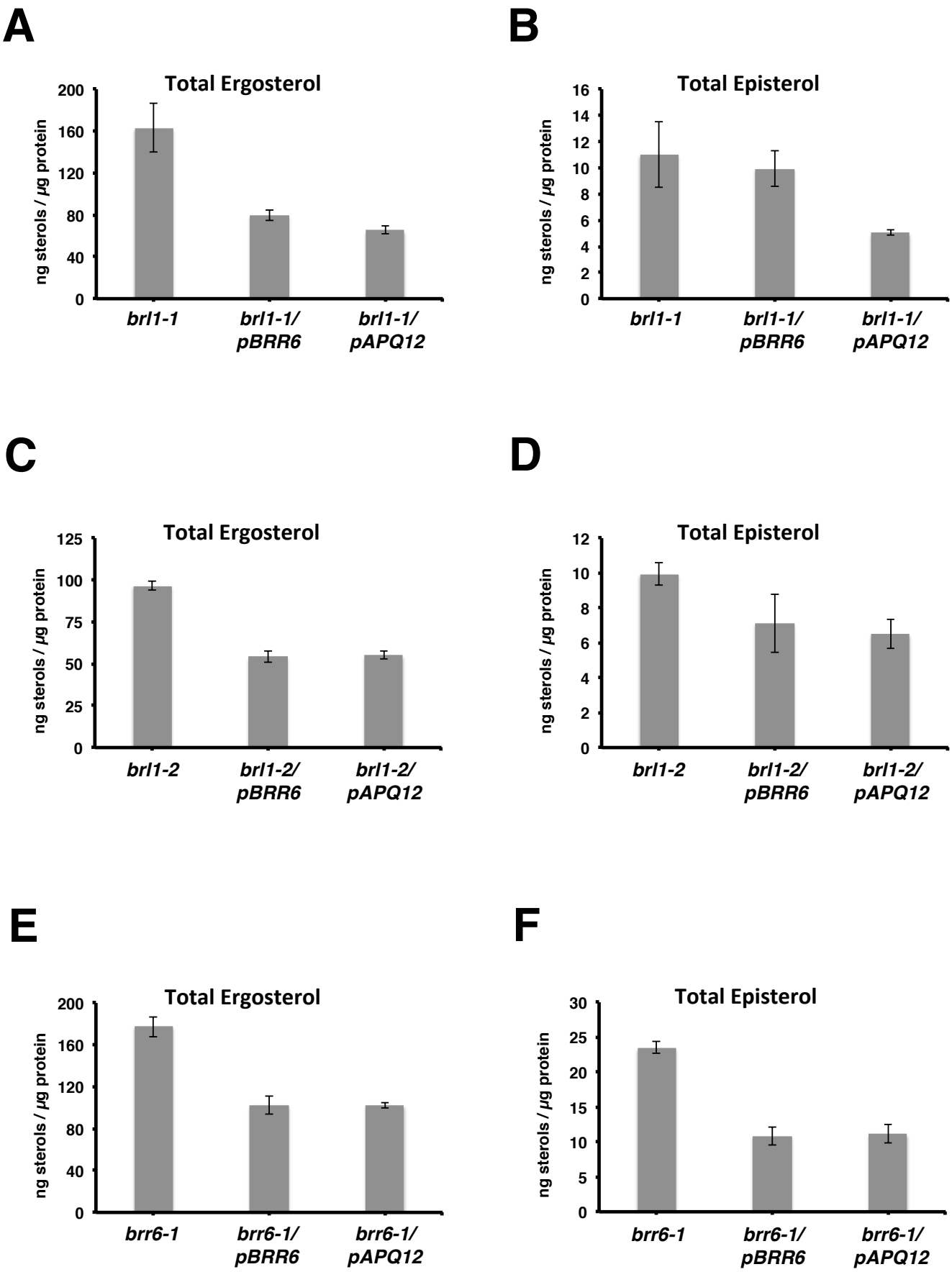

A
Total Ergosterol
ng sterols / µg protein
200
160
120
80
40
0
brl1-1
brl1-1/ pBRR6
brl1-1/ pAPQ12
B
Total Episterol
ng sterols / µg protein
16
14
12
10
8
6
4
2
0
brl1-1
brl1-1/ pBRR6
brl1-1/ pAPQ12
C
Total Ergosterol
ng sterols / µg protein
125
100
75
50
25
0
brl1-2
brl1-2/ pBRR6
brl1-2/ pAPQ12
D
Total Episterol
ng sterols / µg protein
12
10
8
6
4
2
0
brl1-2
brl1-2/ pBRR6
brl1-2/ pAPQ12
E
Total Ergosterol
ng sterols / µg protein
200
160
120
80
40
0
brr6-1
brr6-1/ pBRR6
brr6-1/ pAPQ12
F
Total Episterol
ng sterols / µg protein
30
25
20
15
10
5
0
brr6-1
brr6-1/ pBRR6
brr6-1/ pAPQ12

Figure S2

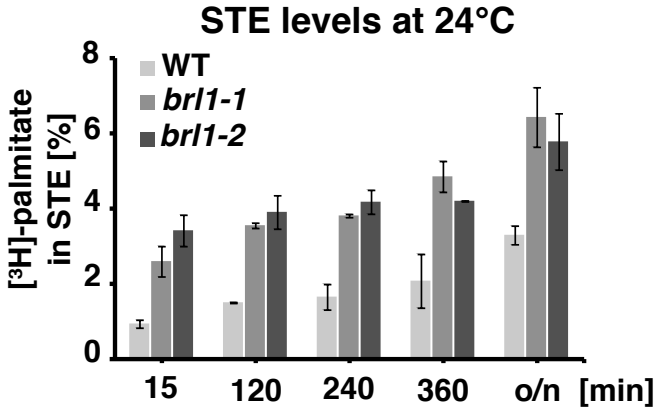
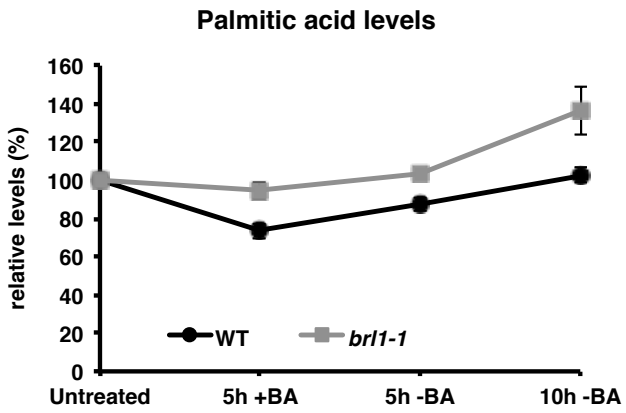
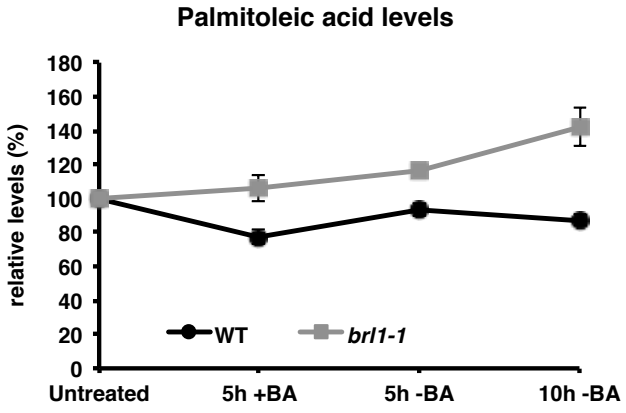
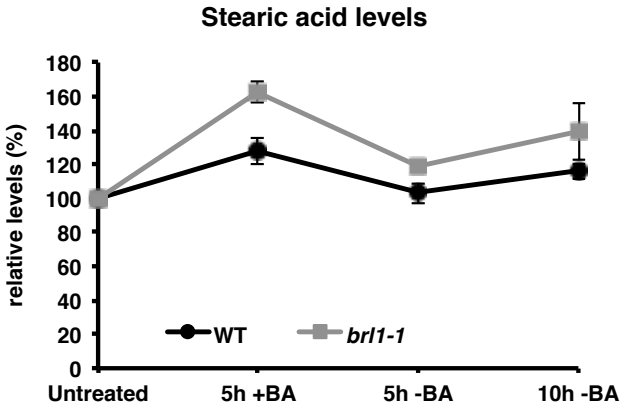

STE levels at 24°C
WT
brl1-1
brl1-2
[3H]-palmitate in STE [%]
8
6
4
2
0
15
120
240
360
o/n
[min]

Figure S3


Palmitic acid levels
relative levels (%)
160
140
120
100
80
60
40
20
0
WT
brl1-1
Untreated
5h +BA
5h -BA
10h -BA


Palmitoleic acid levels
relative levels (%)
180
160
140
120
100
80
60
40
20
0
WT
brl1-1
Untreated
5h +BA
5h -BA
10h -BA


Stearic acid levels
relative levels (%)
180
160
140
120
100
80
60
40
20
0
WT
brl1-1
Untreated
5h +BA
5h -BA
10h -BA

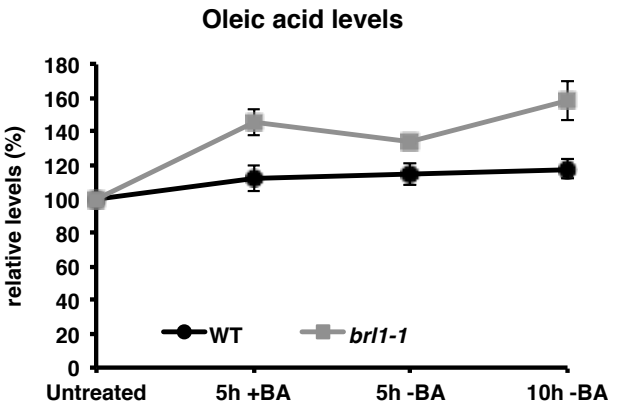

Oleic acid levels
relative levels (%)
180
160
140
120
100
80
60
40
20
0
WT
brl1-1
Untreated
5h +BA
5h -BA
10h -BA

Figure S4

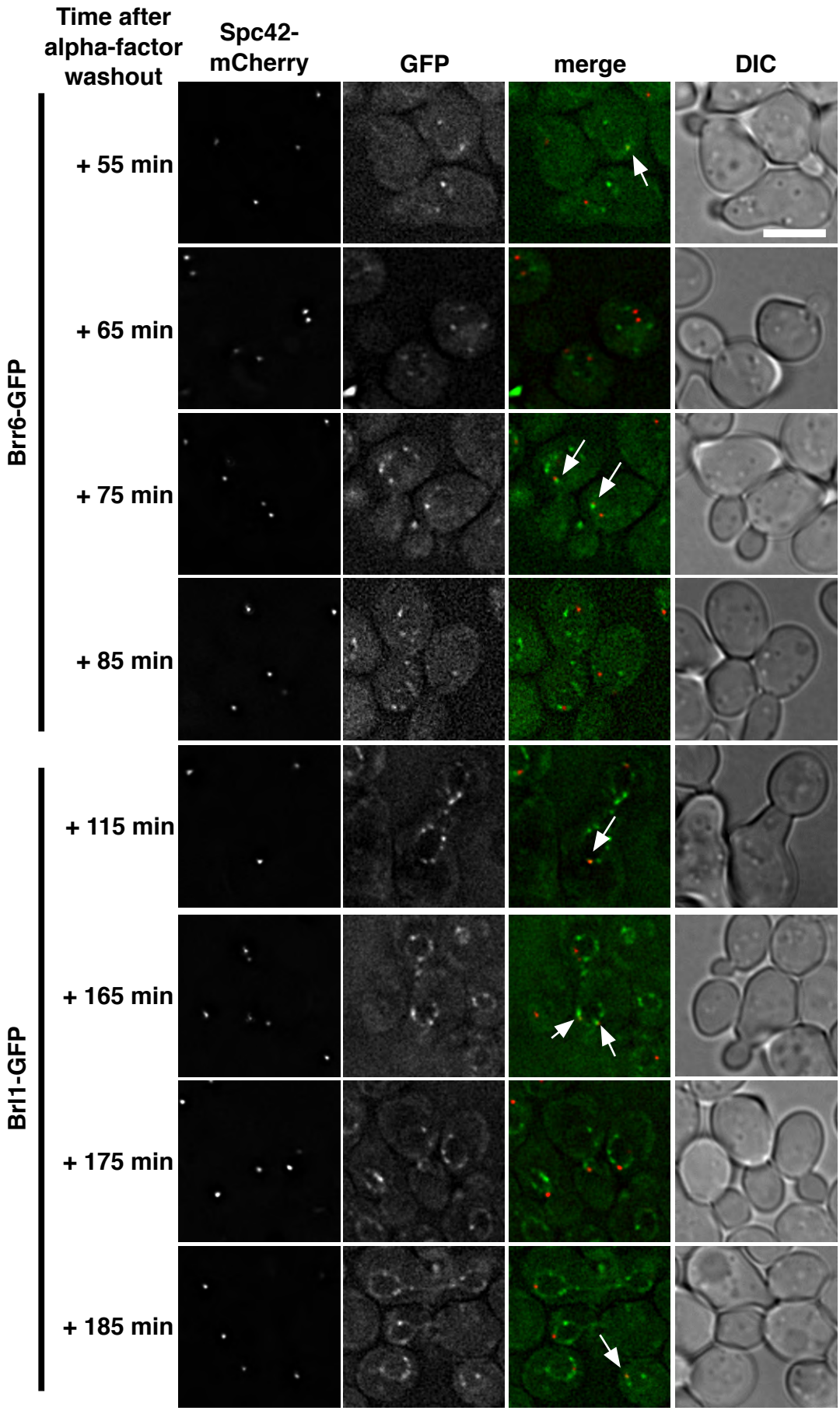

Time after alpha-factor washout
Spc42-mCherry
GFP
merge
DIC
Brr6-GFP
+ 55 min
+ 65 min
+ 75 min
+ 85 min
Brl1-GFP
+ 115 min
+ 165 min
+ 175 min
+ 185 min

Figure S5

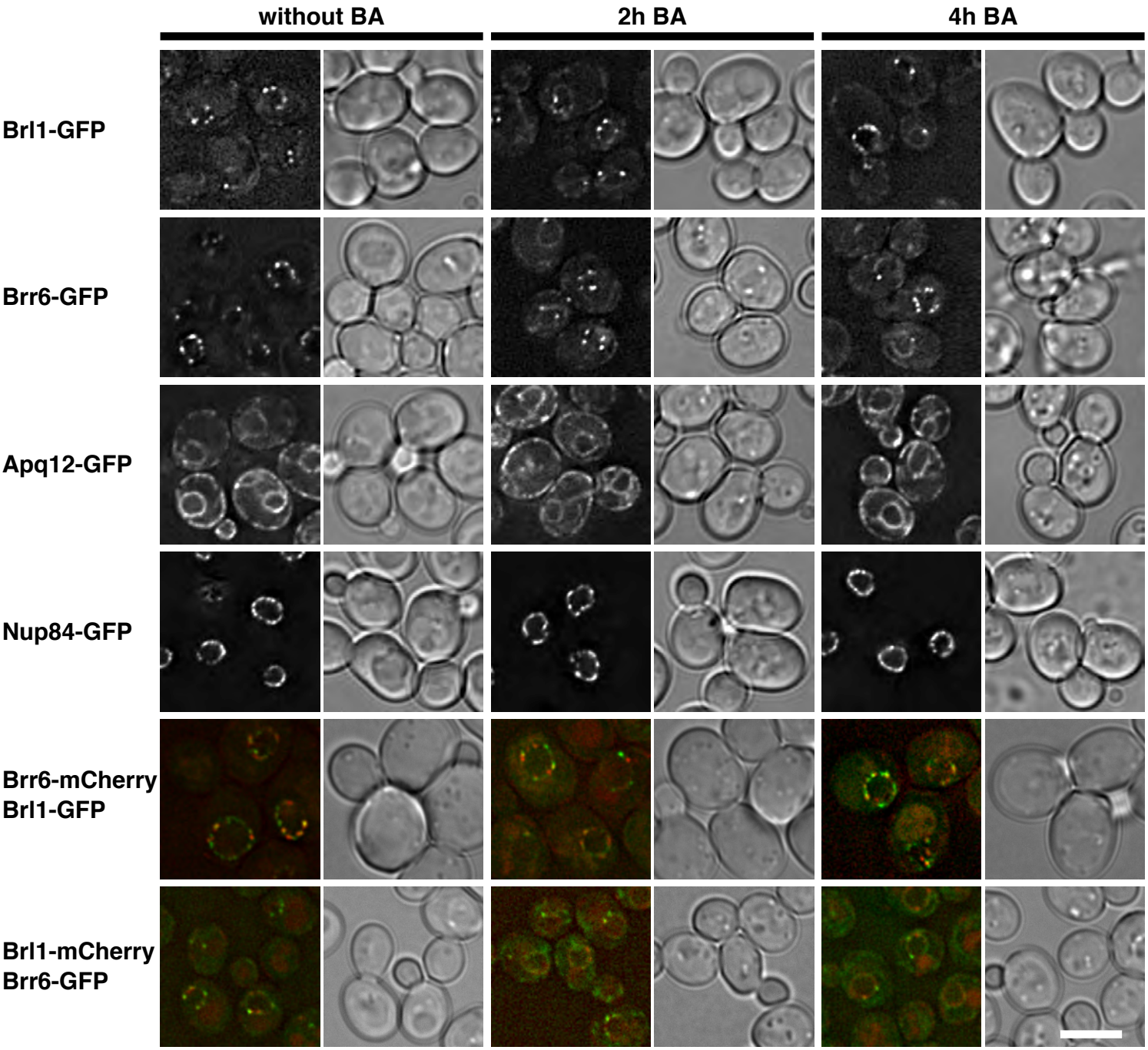

without BA
2h BA
4h BA
Brl1-GFP
Brr6-GFP
Apq12-GFP
Nup84-GFP
Brr6-mCherry
Brl1-GFP
Brl1-mCherry
Brr6-GFP

Figure S6