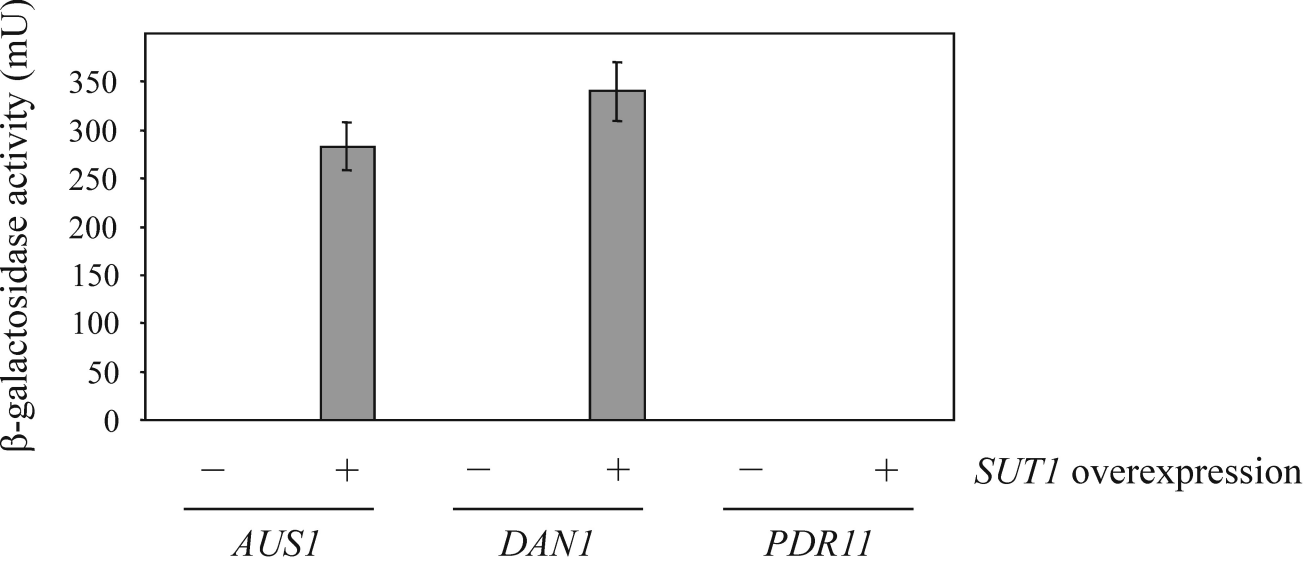
Supplemental figure legends

Figure S1. Overexpression of *SUT1* under aerobic conditions induces expression of *AUS1* and *DAN1*. Wild type cells carried either *SUT1* under control of the strong *PMA1* promoter on a multi copy plasmid (pNF1) or the corresponding empty vector (pNEV-N). In addition, cells harbored a plasmid on which the *lacZ* was fused to the promoter region of *AUS1*, *DAN1* and *PDR11*, respectively. β-galactosidase activity of the indicated strains was determined in at least 6 independent experiments.

Figure S2. Inhibition of sterol uptake by Ste20 is independent of the Ste20 NLS. *STE20* and $STE20^{\Delta NLS}$, respectively, were integrated into *ste20*Δ *hem1*Δ cells. Sterol uptake was measured as described in Figure 11A. Data are normalized to free sterols and steryl ester levels of *STE20* cells after 24 hours of uptake.


β-galactosidase activity (mU)
350
300
250
200
150
100
50
0
−
+
−
+
−
+
SUT1 overexpression
AUS1
DAN1
PDR11

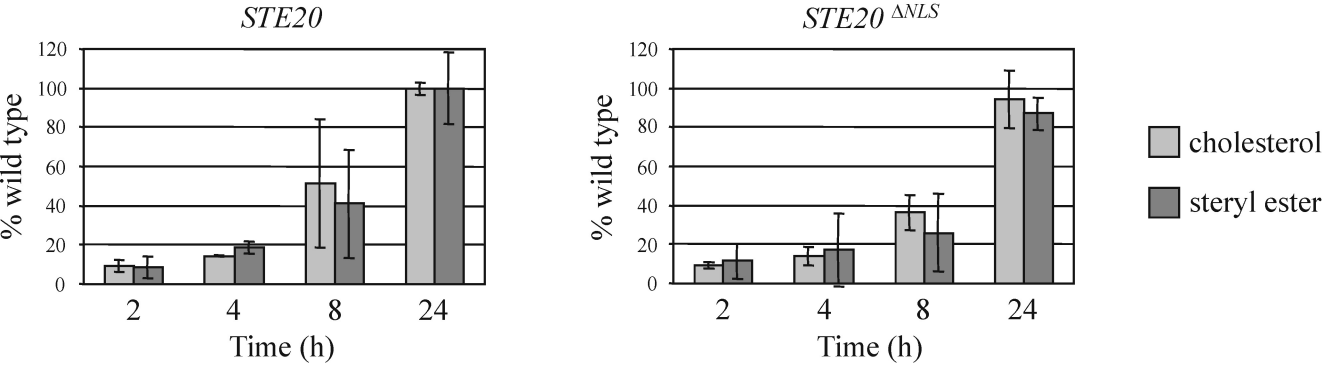

STE20
STE20 ΔNLS
% wild type
120
100
80
60
40
20
0
2
4
8
24
Time (h)
cholesterol
steryl ester

Table S1. Yeast strains used in this study

Name	Genotype	Source or reference
		This study
CTY34	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 His3MX6-GAL1-3HA-YCK1*	This study
HUY28	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6*	This study
HUY31	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6 ste20Δ::klTRP1*	This study
HUY35	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 KanMX6-GAL1-3HA-STE20 hem1Δ::His3MX6*	This study
HUY37	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 KanMX6-GAL1-3HA-SKM1*	This study
HUY42	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6 ste20Δ::klTRP1 sut1Δ::KanMX6*	This study
HUY43	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 ste11Δ::hphNT1*	This study
HUY44	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 ste11Δ::klTRP1 KanMX6-GAL1-3HA-STE20*	This study
HUY45	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 ste11Δ::His3MX6*	This study
HUY47	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 ste11Δ::hphNT1 KanMX6-GAL1-3HA-STE20*	This study
HUY50	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 His3MX6-GAL1-3HA-CLA4*	This study
HUY52	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 KanMX6-GAL1-3HA-CLA4*	This study
HUY55	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6 ste20Δ::klTRP1 STE20^{K649A}-URA3*	This study
MLY48	*MAT***a** *his3::hisG leu2::hisG trp1::hisG ura3-52 ste20Δ::KanMX6 HIS3-STE20$^{\Delta 272-288}$*	This study
MLY49	*MAT***a** *his3::hisG leu2::hisG trp1::hisG ura3-52 ste20Δ::KanMX6 HIS3-STE20*	This study
MLY107	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 His3MX6-GAL1-3HA-SKM1*	This study
MLY109	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 skm1Δ::His3MX6*	This study
MLY113	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 skm1Δ::klTRP1 His3MX6-GAL1-GFP-SKM1-LEU2*	This study
MLY114	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 His3MX6-GAL1-GFP-SKM1$^{\Delta 213-230}$-LEU2 skm1Δ::klTRP1*	This study
MLY180	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hem1Δ::LEU2 ste20Δ::KanMX6 STE20-His3*	This study
MLY181	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hem1Δ::LEU2 ste20Δ::KanMX6 STE20$^{\Delta 272-288}$-His3*	This study
MLY185	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hem1Δ::LEU2 His3MX6-GAL1-3HA-SKM1 KanMX6-GAL1-3HA-CLA4*	This study
MLY186	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::hphNT1 cla4Δ::klTRP1*	This study
MLY187	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 skm1Δ::hphNT1 hem1Δ::klTRP1*	This study
MLY197	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 His3MX6-GAL1-GFP-SKM1$^{K213A/R214A}$-LEU2 skm1Δ::klTRP1*	This study

MLY198	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 skm1Δ::klTRP1 His3MX6-GAL1-GFP-SKM1*$^{R227A/K228A/K230A}$*-LEU2*	This study
MLY199	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 skm1Δ::klTRP1 SKM1-URA3*	This study
MLY200	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 skm1Δ::klTRP1 SKM1*$^{\Delta NLS}$*-URA3*	This study
MLY201	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 skm1Δ::hphNT1 SKM1-URA3*	This study
MLY202	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 skm1Δ::hphNT1 SKM1*$^{\Delta NLS}$*-URA3*	This study
PPY966	*MAT***a** *his3::hisG leu2::hisG trp1::hisG ura3-52*	Tiedje et al. (2007)
SHY41	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 sut1Δ::His3MX6 KanMX6-GAL1-3HA-STE20*	This study
SHY43	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 sut1Δ::His3MX6 ste20Δ::klTRP1*	This study
SHY44	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6 sut1Δ::klTRP1 KanMX6-GAL1-3HA-STE20*	This study
SHY50	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 sut1Δ::His3MX6*	This study
SHY70	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 sut1Δ::hphNT1 KanMX6-GAL1-3HA-SKM1*	This study
SHY71	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 sut1Δ::hphNT1 His3MX6-GAL1-3HA-SKM1*	This study
SHY72	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 sut1Δ::hphNT1 hem1Δ::klTRP1 KanMX6-GAL1-3HA-CLA4*	This study
SHY73	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 ste20Δ::klTRP1 STE20-URA3*	This study
SHY74	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6 ste20Δ::klTRP1 STE20-URA3*	This study
SHY75	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 sut1Δ::klTRP1 His3MX6-GAL1-3HA-CLA4*	This study
SHY79	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 KanMX6-GAL1-3HA-STE20-URA3 ste20Δ::klTRP1*	This study
SHY80	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6 ste20Δ::klTRP1 KanMX6-GAL1-3HA-STE20-URA3*	This study
THY310	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 ste20Δ::klTRP1*	Tiedje et al. (2007)
THY547	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 sut1Δ::His3MX6*	This study
THY566	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 KanMX6-GAL1-3HA-STE20*	This study
THY608	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 cla4Δ::klTRP1*	This study
THY696	*MAT***a** *his3::hisG leu2::hisG trp1::hisG ura3-52 ste20Δ::KanMX6*	This study
THY725	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hem1Δ::LEU2 ste20Δ::KanMX6*	This study
THY732	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 His3MX6-GAL1-3HA-YCK1*	This study
THY734	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hem1Δ::LEU2 skm1Δ::KanMX6*	This study
THY737	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hem1Δ::LEU2 cla4Δ::KanMX6*	This study
THY738	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::klTRP1 KanMX6-GAL1-3HA-GIC2*	This study
THY741	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 KanMX6-GAL1-3HA-GIC2*	This study
THY742	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6 ste20Δ::klTRP1 STE20*$^{\Delta 272-284}$*-URA3*	This study

THY743	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 ste20Δ::klTRP1 STE20$^{\Delta 272-284}$-URA3*	This study
THY747	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6 ste20Δ::klTRP1 KanMX6-GAL1-3HA-STE20^{K649A}-URA3*	This study
THY748	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 hem1Δ::His3MX6 ste20Δ::klTRP1 KanMX6-GAL1-3HA-STE20$^{\Delta 272-284}$-URA3*	This study
THY751	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 KanMX6-GAL1-3HA-STE20^{K649A}-URA3 ste20Δ::klTRP1*	This study
THY752	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 KanMX6-GAL1-3HA-STE20$^{\Delta 272-284}$-URA3 ste20Δ::klTRP1*	This study
THY754	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1 ste20Δ::klTRP1 STE20^{K649A}-URA3*	This study
YPH499	*MAT***a** *ura3-52 lys2-801 ade2-101 trp1Δ63 his3Δ200 leu2Δ1*	Sikorski and Hieter (1989)
YRS1707	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hem1Δ::LEU2*	Reiner et al. (2006)
YRS1962	*MATα his3Δ1 leu2Δ0 ura3Δ0 lys2Δ0 hem1Δ::LEU2 aus1Δ::KanMX4 pdr11Δ::KanMX4*	Reiner et al. (2006)

Table S2. Plasmids used in this study

Name	Genotype	Source or reference
pCT2	pRS313 carrying *pMET25-RDI1-CUbiquitin-RURA3*	Tiedje et al. (2007)
pCT18	pET28c carrying *SUT1*	This study
pCT54	pET28c carrying *SEC6*	This study
pHU31	YEp367 carrying *pAUS1*	This study
pHU32	YEp367 carrying *pPDR11*	This study
pKA86	pRS316 carrying *pGAL1-STE20-3HA*	Tiedje et al. (2007)
pML32	pRS313 carrying $STE20^{\Delta 272-288}$	This study
pML33	pRS313 carrying *STE20*	This study
pML34	pPP1843 carrying $STE20^{272-400}$	This study
pML40	pPP1843 carrying $STE20^{272-288**}$	This study
pML41	pPP1843 carrying $STE20^{285-400}$	This study
pML42	pPP2022 carrying *STE20*	This study
pML47	pPP1843 carrying $STE20^{272-600}$	This study
pML49	pPP1843 carrying $STE20^{272-288*}$	This study
pML55	pPP2022 carrying $STE20^{\Delta 272-284}$	This study
pML56	pPP2022 carrying $STE20^{\Delta 272-288}$	This study
pML78	pPP1843 carrying *CLA4*	This study
pML88	pPP1843 carrying $CLA4^{181-400}$	This study
pML89	pPP1843 carrying $CLA4^{181-550}$	This study
pML90	pPP1843 carrying $CLA4^{400-550}$	This study
pML93	pPP1843 carrying $CLA4^{450-550}$	This study
pML94	pPP1843 carrying $SKM1^{201-230}$	This study
pMR216	YEp367 carrying *pDAN1*	Regnacq et al. (2001)
pNEV-N	2µm, *URA3*, *pPMA1*	Sauer and Stolz (1994)
pNF1	pNEV-N carrying *SUT1*	Ness et al. (2001)
pPP1843	pRS426 carrying *pGAL1-GST-GFP*	Winters et al. (2005)
pPP2022	pRS316 carrying *pGAL1-GST-GFP*	Peter Pryciak
pTH197	pRS313 carrying *pMET25-STE20-CUbiquitin-RURA3*	Tiedje et al. (2007)
pTH283	pGEX-5X-1 carrying *SUT1*	This study
pTH345	pADNX carrying *pADH1-NUbiquitin-UBC6*	Tiedje et al. (2007)
pTH348	pADNX carrying *pADH1-NUbiquitin-SUT1*	This study
YEp367	2µm, *LEU2*, *lacZ*	Myers et al. (1986)

*, fusion with the peptide SGAGAGAGAGAIL
**, fusion with the peptide LGYFLFFEGGPGTQFAL