Supplementary Information for

The Dsc ubiquitin ligase complex identifies transmembrane degrons to degrade orphaned proteins at the Golgi

The Supplementary information consists of:

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Supplementary Methods



Supplementary Figure 1. Quantitative proteomic SILAC profiling of the Gld1-Dsc complex **a**, **b** Volcano plots showing the H/L peptide ratios of proteins from heavy ¹³C₆, ¹⁵N₂-L-lysine labeled *vld1* Δ cells against light ¹²C₆, ¹⁴N₂-L-lysine L-lysine labeled *gld1* Δ cells **a** total proteome changes and **b** only transmembrane proteins from n=3 independent experiments. See also **Supplementary Data 1, 2. c** Scatter plot of the membrane proteins quantified in WT/*tul1* Δ (x-axis) and in *vld1* Δ /*gld1* Δ (y-axis), only displaying proteins with -log₂> -1. Membrane proteins that are significantly upregulated (-log₂ \geq 0.3, p \leq 0.05) in both datasets are highlighted. See also **Supplementary Data 3. a-c** The p-values were calculated by using the background based t-test, the adjusted p-values by using the Benjamin-Hochberg method.



Supplementary Figure 2. Impact on lipid and sphingolipid synthesis in $tull \Delta$, $gld l \Delta$, and $ORM2^{KR}$ mutant cells

a Principal component analysis of lipid extracts from WT (dark gray), *tull* (light blue), *gldl* (soft purple), and $ORM2^{KR}$ (pale yellow) mutant cells. **b** Abundance (in % of total lipids) of phosphatidic acid (PA), phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and phosphatidylserine (PS) of lipid extracts from WT (dark gray), *tull* Δ (light blue), *gld* 1Δ (soft purple), and *ORM* 2^{KR} (pale yellow) mutant cells normalized to the total lipid content. c Abundance of ceramides (Cer), inositolphosphoryl-ceramides (IPC), mannosyl-inositolphosphoryl-ceramides (MIPC), mannosyl-diinositolphosphorylceramides (M(IP)2C) classes of lipid extracts from WT (dark gray), $tull\Delta$ (light blue), $gldl\Delta$ (soft purple), and ORM2^{KR} (pale yellow) mutant cells normalized to the total lipid content. **d-f** Abundance of different **d** ceramide, **e** IPC, and **f** MIPC species of lipid extracts from WT (dark gray), $tull\Delta$ (light blue), gld1 Δ (soft purple), and ORM2^{KR} (pale yellow) mutant cells shown as mol% normalized to the respective lipid class. Lipid extracts were measured using LC-MS. Data is presented as mean \pm SD from four independent experiments (n=4 independent experiments) and legend for all graphs is shown on the right-hand side of the figure. Downregulated species are labeled in red. Data were analyzed by multiple unpaired t-test with the two-stage linear stepup procedure of Benjamini, Krieger and Yekutieli. P-values > 0.05 (ns), ≤ 0.05 (*), ≤ 0.01 (**) and < 0.001 (***). The exact p-values are provided in the source data.



Brightfield

NG-Hmx1

rps5^{G747E}

 $tul1\Delta rps5^{G747E}$

Supplementary Figure 3. Hmx1 is a substrate of the Gld1-Dsc complex

a Densitometric quantification of FLAG-Hmx1 protein levels from Western blots of cell lysates of the indicated cells after the addition of CHX (WT: n=6 independent experiments, all others: n=3 independent experiments), presented as mean \pm standard error of the mean (SEM). **b**, **c** SDS-PAGE and Western blot analysis with the indicated antibodies of WT cells and the indicated mutants that were either untreated (0 min) or treated with 50 µg/mL cycloheximide (CHX) to block protein synthesis for the indicated time points. Coomassie and Pgk1 served as a loading control. d Live cell epifluorescence microscopy of the indicated cells, induced (500 nM β-estradiol for the indicated times) mNeongreen-ALFA-Hmx1 (NG-Hmx1) (green) and Elo3-mCherry (red). Scale bars 5μm. e NG-Hmx1 expression was induced with 500 nM β-ES and samples were collected at the indicated time points. Densitometric quantification of NG-Hmx1 and free NG protein levels. Each experiment was repeated three times (n=3 independent experiments), the sum of NG-Hmx1 and free NG levels were set to 1, and their respective fraction was related to the sum. Data are presented as mean \pm SEM. **f** Epifluorescence microscopy of living $rsp5^{G747E}$ and $tull \Delta rsp5^{G747E}$ mutant cells expressing inducible NG-Hmx1 (green). NG-Hmx1 expression was induced with 500 nM β-ES and imaged after 310 min. Scale bars 5 µm. Representative micrographs from 3 independent experiments with similar results are shown in **d**, **f**.



Supplementary Figure 4. Dsc complex substrate ubiquitination and extraction

a, d, e Densitometric quantification of a, d FLAG-Hmx1 or e NG-Hmx1 clipping (relative ratio of NG-Hmx1 / clipped NG) protein levels from Western blots of cell lysates. Data are presented as mean \pm SEM. **a**, **d** Each experiment was repeated three times (n=3 independent experiments), protein levels were normalized to Pgk1 loading controls, and each time point was related to t=0 min (set to 1). e Each experiment was repeated three times, the sum of NG-Hmx1 and free NG levels were set to 1, and their respective fraction was related to the sum. b SDS-PAGE and Western blot analysis with the indicated antibodies of denaturing FLAG-Hmx1 immunoprecipitations (IP) from the indicated cells. Two top panels: eluates from a 2nd IP step with either K48 (top panel) or K63-linkage specific nanobodies. Third panel: 1st IP step with α-Flag antibody. c SDS-PAGE and Western blot analysis with the indicated antibodies of denaturing FLAG-Hmx1 immunoprecipitations (IP) from the indicated cells that cannot form K63, K48 or K11 polyubiquitin chains. b, c Representative blots from 3 independent experiments with similar results are shown. f SDS-PAGE and Western blot analysis with the indicated antibodies of WT cells and the indicated mutants that were either untreated (0 min) or treated with 50 µg/mL cycloheximide (CHX) to block protein synthesis for the indicated time points. FLAG-Orm2 was co-expressed from a plasmid. Densitometric quantification of FLAG-Orm2 protein levels from Western blots of cell lysates. Each experiment was repeated three times (n=3 independent experiments), protein levels were normalized to Pgk1 loading controls, and each time point was related to t=0 min (set to 1). Data are presented as mean \pm (SEM). g Epifluorescence microscopy of living WT and $ubx3^{\Delta UBX}$ cells co-expressing GFP-Orm2 (green) from a plasmid. Scale bars 5 µm.



Supplementary Figure 5. Endogenous Yif1 is a Dsc complex substrate and Dsc2 is required for substrate recognition

a-b SDS-PAGE and Western blot analysis with the indicated antibodies. Cells were left untreated (0 min) or treated with 50 µg/mL cycloheximide (CHX) for the indicated time points. Densitometric quantification of Yif1-HA protein levels from Western blots of the indicated cell lysates. Each experiment was repeated at least three times (WT, *tul1* Δ : n=4 independent experiments; all others: n=3 independent experiments) protein levels were normalized to Pgk1 loading controls, and each time point was related to t=0 min (set to 1). Data are presented as mean ± SEM. **a** Middle panel: *pdr5* Δ cells were incubated with 50 µM MG-132 or vehicle (DMSO) 10 min prior to the addition of CHX. **b** Cells were shifted to semi-permissive temperature (30°C). **c** SDS-PAGE and Western blot analysis with the indicated antibodies. Elution (upper panel) and input (lower panel) from non-denaturing FLAG-Hmx1, FLAG-Orm2-KR, and Yif1-HA immunoprecipitations from the indicated cells. Control (Ctrl) cells were untagged WT strains. Representative blots from 3 independent experiments with similar results are shown.

Supplementary Figure 6. MD simulation of Dsc2 and lipid interactions

a Overlay of the lipid density map with a top view (from the luminal site) of the rhomboid domain of Dsc2 or Dsc2^{Y52AR,53A} displaying the distance of the average center of mass in the lipid bilayer over the final 40 ns and the lipids. **b** Snapshots of the molecular dynamics simulation of the rhomboid domain of Dsc2 showing the entire system including a single asymmetric PC (10:0; 16:0), all other lipids and water or just the Dsc2 rhomboid domain with water in orthographic and perspective views. The C10 acyl chain is shown in yellow. For clarity, also another snapshot with the different angle is provided. **c** Lipid dissociation plots show the distance in (Å) of the first 5 C atoms in the C10 chain with respect to L21 of Dsc2 over a period of 40 ns. PC (10:0; 16:0) (red), PC (18:1; 16:0) (black) or PC (10:1; 16:0) (blue). **d** Alignment of the L1 loop region of Dsc2 with other indicated cell lysates. Each experiment was repeated three times (n=3 independent experiments), protein levels were normalized to Pgk1 loading controls, and each time point was related to t=0 min (set to 1). Data are presented as mean \pm SEM.





Supplementary Figure 7. The TMD of Hmx1 is a Dsc complex degron

a SDS-PAGE and Western blot analysis with the indicated antibodies of whole cell lysates from WT cells expressing the indicated versions of the Hmx1 TMD. Representative blots from 3 independent experiments with similar results are shown. b, c Densitometric quantification of protein levels from Western blots of Figure 7b cell lysates Figure 7c immunoprecipitated proteins. Each experiment was repeated three times (n=3 independent experiments), protein levels were normalized to Pgk1 loading controls. Data are presented as mean \pm SEM. Data were analyzed by a two-tailed unpaired t-test. d, g, i SDS-PAGE and Western blot analysis with the indicated antibodies of total cell lysates from the indicated cells expressing different model substrates: **d**, **g** expressing truncated eGFP-ALFA-Hmx1²⁶⁴ or eGFP-ALFA-Hmx1²⁸⁴ **i** or eGFP-Pep12 or eGFP-Pep12-Hmx1, including densitometric quantification (n=3 independent experiments). **d**, **g** Cells were left untreated (0 min) or treated with 50 µg/mL cycloheximide (CHX) to block protein synthesis for 45 min to 90 min. Coomassie served as a loading control. e Epifluorescence and phase contrast microscopy of living WT and *tull* mutants expressing truncated pHluorine-Hmx1 (pH-Hmx1²⁶⁴) from residue 264-317 (green) with mCherry-Cps1 (red, MVB cargo). Scale bars 5 µm. f SDS-PAGE and Western blot analysis with the indicated antibodies of input and elution of denaturing eGFP-ALFA-Hmx1²⁶⁴ immunoprecipitations (IP) from WT cells and the indicated mutants. Coomassie served as a loading control. The two top panels show the eluates from a 2nd IP step with K48- or K63-linkage specific nanobodies decorated with an anti-ubiquitin antibody. Representative blots from 3 independent experiments with similar results are shown in **d**, **f**, **g**. **h** Epifluorescence and phase contrast microscopy of living WT and $ubx3^{\Delta UBX}$ (ΔUBX) mutants expressing truncated GFP-Hmx1 from residue 264-317. Cells additionally expressed either VPS4 or vps4-E233Q (to block vacuolar sorting) from an episomal plasmid. Panel shows GFP-Hmx1²⁶⁴ (green) with FM4-64 (red, vacuolar membrane). E indicates class E compartments; scale bars 5 µm.

Supplementary Tables

Supplementary Table 1.

Gene ontology - cellular component for upregulated membrane proteins

	WT/tull	$l\Delta$ Hits:	21			WT/vps4 Δ	Hits:	72		
Cellular										
Component	ER	Golgi	PM	vacuole	mitos	ER	Golgi	PM	vacuole	mitos
Count	6	3	2	4	5	12	5	34	15	1
Frequency	29%	14%	10%	19%	24%	17%	7%	47%	21%	1%
Genes	ALG3	GNT1	FTR1	CPR8	COXI	CSG2	AKR1	AGP1	CCC2	FIS1
	CSG2	NEO1	VHT1	CTS1	COX10	SUR1	KHA1	AQR1	COS8	
	DGK1	TPO5		VPS33	ECM19	EMC1	SMF2	BAP2	COTI	
	ELO2			YML018C	RCF1	ERG3	TLG1	CANI	CPR8	
	HMX1				TIM17	FLC2	VTI1	CCH1	FET5	
	ORM2					HMX1		DIP5	PMC1	
						INP54		ENA1	PRB1	
						NSG2		ENA2	PRM4	
						PMT5		ENA5	SMF3	
						PRM8		FCY2	SNA3	
						YDR476C		FET3	VPS62	
						YET3		FRE1	YML018C	
								FTR1	YOL019W	
								FUI1	COS6	
								GAP1	PEP12	İ I
								HIP1		İ I
								HXT1		Í I
								HXT2		Í I
								MMP1		
								PHO90		Í I
								PTR2		İ I
								PUNI		
								STE2		
								STE6		
								SUL2		
								TPN1		
								SUL1		
								YRO2		
								TNA1		
								GNP1		
								LYP1		
								BAP3		
								SIT1		
								RSN1		

Supplementary Table 2.

Reagents, strains and plasmid

Antibodies (dilution)	SOURCE	IDENTIFIER
		Cat. # A4416;
Goat anti mouse IgG-HRP (1:5,000)	Sigma	RRID:AB_258167
		Cat. # A0545;
Goat anti rabbit IgG-HRP (1:5,000)	Sigma	RRID:AB_257896
		Cat. # ab131368
		RRID:AB_289511
Rat anti-mouse IgG-HRP for IP (1:10,000)	Abcam	4
		Cat. # 459250;
Mouse monoclonal anti-PGK1 (22C5D8)		RRID:AB_253223
(1:10,000)	Invitrogen	5
		Cat. #
Mouse monoclonal anti-GFP (IgG1K, clones 7.1		11814460001;
and 13.1) (1:1,000)	Roche Diagnostics	RRID:AB_390913
		Cat # PABG1-100;
		RRID:AB_256500
Rabbit polyclonal anti-GFP (1:1,000)	ChromoTek	6
		Cat. # F3165;
Mouse monoclonal anti-FLAG M2 (1:5,000)	Sigma	RRID:AB_259529
		Cat. # 901502;
Mouse monoclonal anti-HA (IgG1K, clone		RRID:AB_256500
16B12) (1:2,000)	Bio Legend	6
		Cat. # 3724,
Rabbit monoclonal anti-HA (IgG, clone C29F4)		RRID:AB_154958
(1:2,000)	Cell Signaling	5
Mouse monoclonal anti-ubiquitin (P4D1)		Cat. # sc-8017;
(1:1,000)	Santa Cruz	RRID:AB_628423
		Cat. #8081;
Rabbit monoclonal anti-K48-linkage specific		RRID:AB_108598
poly-ubiquitin (clone, D9D5) (1:1,000)	Cell signaling	93
	Gift from Peter	1
Rabbit polyclonal anti-Tul1 (1:2,000)	Espenshade	1
	Gift from Peter	1
Rabbit polyclonal anti-Ubx3 (1:2,000)	Espenshade	1
	Gift from Peter	1
Rabbit polyclonal anti-Dsc2 (1:100)	Espenshade	1
	Gift from Peter	1
Rabbit polyclonal anti-Dsc3 (1:2,000)	Espenshade	1
Rabbit monoclonal anti-mNeonGreen (IgG, clone		
E6M3D) (1:1,000)	Cell Signaling	Cat. #41236
	NanoTag	
Rabbit polyclonal anti-ALFA (1:2,000)	Biotechnologies	Cat. #N1581

Chemicals, Peptides, and Recombinant Proteins	SOURCE	IDENTIFIER
[13C6,15N2]-L-lysine	Sigma	Cat. # 608041

		Cat. # M8823;
		RRID:AB_263708
anti-FLAG magnetic beads M2	Sigma	9
3xFLAG elution peptide	Sigma	Cat. # F4799
MG-132	Sigma	Cat. # 474787
Cycloheximide	Sigma	Cat. # C7698
	NanoTag	
anti-ALFA selector ST magnetic beads	Biotechnologies	Cat. # N1516
	NanoTag	
anti-ALFA selector CE agarose beads	Biotechnologies	Cat. # N1512-L
	NanoTag	
ALFA elution peptide	Biotechnologies	Cat. # N1520
	NanoTag	
anti-Ubiquitin K48 Selector agarose beads	Biotechnologies	Cat. # N1810
	NanoTag	
anti-Ubiquitin K63 Selector agarose beads	Biotechnologies	Cat. # N1910
	Thermo Fisher	
Pierce [™] anti-HA magnetic beads	Scientific	Cat. # 88836
MagStrep"type3" XT Beads	IBA	Cat. #2-4090-002
cOmplete [™] , EDTA-free Protease Inhibitor		
Cocktail	Sigma	Cat. # C7698
N-ethylmaleimide (NEM)	Sigma	Cat. # E3876
phenylmethane sulfonyl fluoride (PMSF)	Sigma	Cat. # P7626
protease inhibitor cocktail	Sigma	Cat. # P8215
glyco-diosgenin (GDN)	Sigma	Cat. # 850525
β-Estradiol	Sigma	Cat. # E2758
	Thermo Fisher	
FM4-64 Dye	Scientific	Cat. # T3166

Yeast strains:	SOURCE	IDENTIFIER
SEY6210 wildtype (MATalpha leu2-3, 112 ura3-		
52 his3-200 trp1-901 lys2-801 suc2-9)	2	SEY6210
SEY6210.1 wildtype (MATa leu2-3, 112 ura3-52		
his3-200 trp1-901 lys2-801 suc2-9)	2	SEY6210.1
SEY6210.1 vps4::TRP1	3	MBY4
SEY6210.1 tul1::HIS3	4	SSY17
SEY6210.1 vps4::TRP1, tul1::HIS3	4	SSY31
SEY6210.1 orm2::TRP1	4	YWY005
SEY6210.1 vps4::TRP1, pdr5::HIS3	this study	YWY007
SEY6210.1 orm2::TRP1, tul1::HIS3	4	YWY027
SEY6210 orm2::TRP1, ubx3::HIS3	4	YWY047
SEY6210.1 ubx3\alphaUBX::HIS3	this study	YWY050
SEY6210.1 gld1::HIS3	this study, ⁴	YWY051
SEY6210.1 vld1::HIS3	this study, ⁴	YWY052
SEY6210 orm2::TRP1, dsc3::HIS3	4	YWY055
SEY6210.1 orm2::TRP1, dsc2::HIS3	4	YWY056
SEY6210 YIF1-3xHA::TRP1	this study	YWY089
SEY6210 YIF1-3xHA::TRP1, tul1::HIS3	this study	YWY090
SEY6210 UBX3-3xFLAG::HIS3	this study	YWY118
SEY6210? YIF1-3xHA::TRP1, pdr5::HIS3	this study	YWY122

SEY6210 ubx3/AUBX::TRP1	this study	YWY146
SEY6210.1 YIF1-3xHA::TRP1, ubx3::HIS3	this study	YWY167
SEY6210 YIF1-3xHA::TRP1. dsc2::HIS3	this study	YWY168
SEY6210.1 YIF1-3xHA::TRP1, dsc3::HIS3	this study	YWY169
SEY6210.1 UBX3-3xFLAG::HIS3. dsc3::HIS3	this study	YWY204
SEY6210.1 <i>YIF1-3xHA::TRP1 cdc48-3</i>	this study. ⁵	YWY268
SEY6210.1 UBX3-3xFLAG::HIS3. dsc2::HIS3.		
<i>YIF1-3xHA::TRP1</i>	this study	YWY320
SEY6210.1 hmx1::HIS3	this study	YWY322
SEY6210.1 hmx1::3xFLAG-HMX1-TRP1	this study	YWY329
$SEY62101 hmx1\cdots3xFLAG-HMX1-TRP1$		
tul1HIS3	this study	YWY337
SEY6210 vif1··HIS3 P ^{ADH1} -eGFP-YIF1-nRS406	this study	YWY345
SEY 6210 yif1::HIS3, $tul1::KanMX4 P^{ADH1}$ -	uns study	1 11 10 10
oGFP_VIF1_nRS406	this study	YWY352
SFY6210.1 hmr1···3rFIAG-HMX1·TRP1	uns study	1 1 1 352
$vns4 \cdots TRP1$ ndr5 $\cdots HIS3$ cdc48-3	this study 5	VWV421
SEV6210.1 tull::KanMX4 hmx1::HIS3	this study.	YWY461
SEV6210.1 ubr3. HIS3 hmr1. HIS3	this study	VWV/60
$SEV6210.1 \ dsc^3 \cdots HIS3 \ hmr1 \cdots HIS3$	this study	1 W 1409 VWV/71
SEV6210, $usc3$, $TDD1$, $hmx1$, $HIS3$	this study	1 W 14/1 VWV/72
SEV6210 ups4TRF1, untalIIISS		1 W 14/2
SE10210 Vps41KF1, 1001KunwiX4, hms1HIS2	this study	VWVA7A
1000000000000000000000000000000000000	this study	1 W 14/4 VWV475
SE10210 <i>usc2::HIS3</i> , <i>Mmx1::HIS3</i>	this study	1 W 14/5
SEY0210.1 <i>asc2::HIS5, nmx1::HIS5</i>		1 W 14/0
SEY6210.1 <i>hmx1::3xFL</i> AG-HMX1-1KP1,	the at a day	VWV470
$\frac{UDX}{UDX} = \frac{UDX}{UDX} = $		YWY4/8
$SEY6210.1 hmx1::HIS3, ubx3\Delta UBX::TRP1$	this study	Y W Y 487
SEY6210.1 hmx1::HIS3, rsp3::HIS +	this study, gift from	NUN 405
<i>rsp5(G/4/E)</i> /pRS415	Ming Li	Y W Y 495
SEY6210.1 hmx1::HIS3, tul1::NatR, rsp5::HIS +	this study, gift from	MANAOC
rsp5(G/4/E)/pRS415	Ming Li	Y W Y 496
$SEY 6210.1 hmx1::HIS3, ubx3\Delta UBX::HIS3$	this study	ISY 198
$SEY6210 hmx1::HIS3, ubx3\Delta UBX::HIS3,$		
vps4::TRP1		1011100
	this study	ISY199
SEY6210 UBX3-3xFLAG::HIS3, YIF1-	this study	ISY199
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1	this study this study	ISY199 ISY213
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3,	this study this study	ISY199 ISY213
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1	this study this study this study	ISY199 ISY213 ISY215
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3,	this study this study this study	ISY199 ISY213 ISY215
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3, YIF1-3xHA::TRP1	this study this study this study this study	ISY199 ISY213 ISY215 ISY216
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3, YIF1-3xHA::TRP1 SEY6210 YIP(URA3)-SEC7pr-SEC7-6xmCherry	this study this study this study this study this study; ⁴	ISY199 ISY213 ISY215 ISY216 ISY223
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3, YIF1-3xHA::TRP1 SEY6210 YIP(URA3)-SEC7pr-SEC7-6xmCherry SEY6210.1 gld1::HIS3, YIP(URA3)-SEC7pr-	this study this study this study this study this study; ⁴	ISY199 ISY213 ISY215 ISY216 ISY223
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3, YIF1-3xHA::TRP1 SEY6210 YIP(URA3)-SEC7pr-SEC7-6xmCherry SEY6210.1 gld1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry	this study this study this study this study this study; ⁴ this study	ISY199 ISY213 ISY215 ISY216 ISY223 ISY222
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3, YIF1-3xHA::TRP1 SEY6210 YIP(URA3)-SEC7pr-SEC7-6xmCherry SEY6210.1 gld1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry SEY6210.1 tul1::HIS3, YIP(URA3)-SEC7pr-	this study this study this study this study this study; ⁴ this study	ISY199 ISY213 ISY215 ISY216 ISY223 ISY222
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3, YIF1-3xHA::TRP1 SEY6210 YIP(URA3)-SEC7pr-SEC7-6xmCherry SEY6210.1 gld1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry SEY6210.1 tul1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry	this study this study this study this study this study; ⁴ this study this study	ISY199 ISY213 ISY215 ISY216 ISY223 ISY222 ISY225
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3, YIF1-3xHA::TRP1 SEY6210 YIP(URA3)-SEC7pr-SEC7-6xmCherry SEY6210.1 gld1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry SEY6210.1 tul1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry SEY6210 tul1::HIS3, ELO3-3xHA-	this study this study this study this study this study; ⁴ this study this study	ISY199 ISY213 ISY215 ISY216 ISY223 ISY222 ISY225
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3, YIF1-3xHA::TRP1 SEY6210 YIP(URA3)-SEC7pr-SEC7-6xmCherry SEY6210.1 gld1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry SEY6210.1 tul1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry SEY6210 tul1::HIS3, ELO3-3xHA- mCherry::TRP1	this study this study this study this study this study; ⁴ this study this study this study	ISY199 ISY213 ISY215 ISY216 ISY223 ISY222 ISY225 ISY227
SEY6210 UBX3-3xFLAG::HIS3, YIF1- 3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, tul1::HIS3, YIF1-3xHA::TRP1 SEY6210.1 UBX3-3xFLAG::HIS3, dsc3::HIS3, YIF1-3xHA::TRP1 SEY6210 YIP(URA3)-SEC7pr-SEC7-6xmCherry SEY6210.1 gld1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry SEY6210.1 tul1::HIS3, YIP(URA3)-SEC7pr- SEC7-6xmCherry SEY6210 tul1::HIS3, ELO3-3xHA- mCherry::TRP1 SEY6210 UBX3-mNeongreen::TRP1, tul1::HIS3,	this study this study this study this study this study; ⁴ this study this study this study	ISY199 ISY213 ISY215 ISY216 ISY223 ISY222 ISY225 ISY227

SEY6210.1 UBX3-mNeongreen::TRP1,		
tul1::HIS3, YIP(TRP1)-MNN9pr-MNN9-		
mTagBFP2	this study	ISY237
SEY6210.1 orm2::KL-URA3	this study	OSY583
SEY6210 ELO3-3xHA-mCherry::TRP1	this study, ⁴	OSY847
SEY6210 ubx3/AUBX::TRP1, vps4::TRP1,		
pdr5::HIS3	this study	OSY1243
SEY6210 UBX3-mNeonGreen::TRP1	6	MWY218
SEY6210.1 UBX3-mNeonGreen::TRP1.		
tul1::HIS3	this study	MWY221
SEY6210 UBX3-mNeonGreen TRP1 dsRed-		
HDEL::TRP1	this study	MWY248
SEY6210 UBX3-mNeonGreen TRP1 dsRed-		
HDEL: TRP1 tull: HIS3	this study	MWY250
SEY6210.1 UBX3-mNeonGreen: TRP1_dsRed-	ling study	111111250
HDFL.:TRP1 dsc2:HIS3	this study	MWY296
SFY6210 UBX3-mNeonGreen··TRP1 dsRed-	this study	10100 1290
HDEL ··TRP1 dsc2··HIS3	this study	MWY297
SEV6210.1 orm2::TRP1_3rELAG_ORM2	this study	TMY002
SEV $6210.1 \text{ orm} 2 \cdots TRP 1 - 3xELAG - ORM 2^{K25R,K33R}$	this study	TMV008
SEV6210.1 orm2TRP1 3xELAG-ORM2		11111000
5E10210.1 of m21 KI 1-5 AU EAO-O KM2,	this study	TMV010
SEV6210.1 hmr13rELAC HMV1 TDD1		11111019
SET0210.1 $IIIIIIISXI LAO-IIIIIXI-IIXI 1,ndr5HIS3$	this study	VNV045
SEV6210.1 herests 2. ELAC HMV1 TDD1		VIN1043
$SEI 0210.1 \text{ mmx} 1.75\text{XF} LAG-mmxI-IKF1,$ $wbw^2 \cdots HIS2$	this study	VNIV047
uox3IIIS3 SEX6210.1 hereal2xELAC_HMV1_TDD1		VIN1047
SE 10210.1 Imm 1SXF LAG-HIMAT-TKF1,	this study	VNIV052
USC2IIISS		VIN1035
$SEI 0210.1 \ \text{MM} \times 1.5 \times \Gamma LAG - \Pi \text{M} \times 1 - 1 \text{KP1},$	this study	VNIV055
Vps4::TRF1		VINTUSS
$SEY0210.1 \ nmx1::SXFLAG-HMX1-TKP1,$	this stude.	VNIVOCO
pars::HIS3, vps4::TKP1	this study	VIN Y 060
$SEY0210.1 \ nmx1::SXFLAG-HMX1-1RF1,$	41-1	VNIVOCC
<i>tull::HIS5, vps4::TKP1</i>	this study	VIN Y 000
$SEY0210.1 \ nmx1::3xFLAG-HMX1-1KP1,$	41-1	VNIVOC7
	this study	VIN Y 067
SEY6210.1 hmx1::3xFLAG-HMX1-1KP1,	41-1	VNIVOCO
gla1::HISS		VIN 1009
SEY6210.1 hmx1::3xFLAG-HMX1-	.1 1	
IRP1, vla1::HIS3	this study	VNY0/1
SEY 6210.1 ELO3-mCherry::1RP1, dsc2::HIS3,	.1 • 1	DUNOO1
GFP-ORM2:TRP1	this study	BKY001
SEY6210 rsp5::HIS + rsp5($G/4/E$)/pRS415	1	YML981
SEY6210 tul1::NatR, rsp5::HIS +		
rsp5(G/4/E)/pKS415	Gift from M. Li	Y X Y 462
SUB280 lys2-801 leu2-3,112 ura3-52 his3- $\Delta 200$		
<i>trp1-1 ubi1::TRP1, ubi2-</i> \(\Delta:ubi3-\Delta\)		
$ubi4-\Delta 2$::LEU2, pUB100-RSP31 (2 μ , HIS3),	8	GLID 200
$pUB39-P^{COT}-Ub (2\mu, LYS2)$	°, gift from S. Leon	SUB280
SUB280, $pUB39-P^{CUP1}-Ub^{K11K}$ (2 μ , LYS2)	°, gift from S. Leon	SUB516
SUB280, $pUB39-P^{CUP1}-Ub^{KOSK}$ (2 μ , LYS2)	°, gift from S. Leon	SUB413

100 ura3-1 ade2-1 his3-11,15) W303 P ^{GAL1} DSC2··cloNAT_dsc3··HIS3	Fröhlich this study	ARY001 ARY005
W303 wildtype (MATa leu2-3,112 trp1-1 can1-	Gift from F.	
$pUB115-P^{CUP1}-Ub^{K48R} (2\mu, LYS2)$	⁸ , gift from S. Leon	SUB333
SUB280, <i>pUB146-P^{GAL1}-Ub</i> (2µ, URA3),		

Primer for gene knock out / knock in	Used for strain:	Primer name:
GGCTATTGTGGAGAAAAAGCCAACTAAAA		
CCCGAACCCTCTTCTTGACGGATCCCCGGG		UBX3-354-STOP-
TTAATTAA	YWY050 / YW146	F
CTTTAGAATAAACGTTTTGAGATGACTATT		
TTTGAAATTCCTTGAATTCGAGCTCGTTTA	YWY050 / YW118 /	
AAC	YW146	UBX3-KO-R
CCAAAATATCTGACGTTAGCGGCATATAT		
CCATCAGGAAATATTGTAATGGAACGCCT		
GGACGAACGGATCCCCGGGTTAATTAA	YWY118	UBX3-TAG-F2
CCTATTCGTCTATGGCTTCATTTGGCAAAA		
TGTTCTAATGTGGTTAATGGGTCGGATCCC		
CGGGTTAATTAA	YWY089	YIF1-TAG-F
GATAATAATAATGCGCATGAAATTAAATC		
CTCTCTTTGATCTCTTCAATCAAGAGAATT	YWY089 /	
CGAGCTCGTTTAAAC	YWY179	YIF1-TAG-R
CGCTACTGAGTATCAATAGCAGAAAACGA		
AAACCAAAGGCCCATACCAAGCGGATCCC		
CGGGTTAATTAA	YWY179	YIF1-KO-F
GCACAATATAACACAGCATATATACACAC		
ACACACATAAAATAACCGCAAAACGGATC		
CCCGGGTTAATTAA	YWY322	HMX1-KO-F
GATATTATTTCATGTATATATTATGTTTGT		
ATTTAGACTTTTTTTTTTTTATACGGAATTCG	YWY322 /	
AGCTCGTTTAAAC	YWY329	HMX1-KO-R
AGTTTACTAGAGCATCGTCG	YWY329	HMX1-GT-F
CAACGACATAAAAAGCAAACACAATAGTC		Dsc2 N-tag pYM
TACAAATAATGcgtacgctgcaggtcgac	ARY003	F
TGACGGGCATAGCCGTAAGCCCCACAGGA		Dsc2 N-tag pYM
GGCTCCATAGAcatcgatgaattctctgtcg	ARY003	R
CCCTAGAGGCAAGATTGTAGCTGAAGCTG		
G	TMY002 / TMY008	ORM2-integr-R
TTCTGCGAATGCGTGACCGTATCCGCAAA		
GAAC		
GAAGATTAAATTTAGGGCGGATCCCCGGG		
TTAA		
TTAA	TMY002 / TMY008	ORM2-INT-LG-F

Primer for plasmid cloning	Used for	Primer name:
	plasmid(s):	
gcggagctcGTGCTAATTCATCTAGCAACTGG	pYW133 and	SacI-Dsc2-200-F
	pYW150 and all	
	derivatives thereof	

PYW150 and all derivatives thereofCTGCAGACCTACCATCAATACgeCgcTCTGCPYW150, pNM003Dsc2-Y53R54A-FGCAGAACTGGAACTGGAGAGGCGGAGPYW150, pNM003Dsc2-Y53R54A-RTGATGGTAGGTCTGCAGPYW1212, pNM003IYW-Dsc2-ALFA-FGAAGAGGAATTACCTCGTCTGCAACCTGGAGPYW212, pNM003IYW-Dsc2-ALFA-RGCACGACGTAATTCCCFFCGACGACGTAATTCCCCTCTCCAACCTGGAGPYW185, pYW242, pYW243 and all derivatives thereofSacI-HMX1-FgcgggatecGGCAACAAGGATCACTGGeerivatives thereofBamHI-HMX1-RcacacacataAAATAACCGCAAAAATGGACTAC ACAGCATGACGGpYW185, pYW242, pYW243 and all derivatives thereofBamHI-HMX1-RcacacacataAAATAACCGCAAAAATGGACTAC GGTCATGGTCTTGTAGTCCATTTTGCG GTTATTTatgtgtggpYW185pHMX1-FLAG-FCAGGATGACGATGACAAGGAGGACAGT AGCAATCAATCATACpYW185sxFLAG-HMX1-FGTATGATGTGTCTGTGTCTCCTCTGT CGCCCTTGCTACCATGCTTGpYW185sxFLAG-HMX1-FGCATGAACGACGACAAAGGAGGACAGT AGCAATACATCCpYW185gCGCCTGCACACATGACGACGACGACGAGGGCGpYW242, pYW243p(HMX1)-Fluo-FCGCCCTTGCTCACCATTTTTGCGGTTATTT tgtgtgtgpYW242, pYW243p(HMX1)-Fluo-RGCATGGACGAGCTGTACAAGGGCAAGTTC AGGAGGCAGCTGTACAAGGCGCAGCTGTACAAGGCGCAGCTpYW242HMX1-264-317-FGAAGAGGACTTCCAGGCpYW243HMX1-284-317-FGAAGAGGACTTCACAGCGCGCAGCTTGACAGCpYW243HMX1-284-317-FGAAGAGGAATTACCTCGAGCGAGCTGCACCpYW243HMX1-284-317-FGAAGAGGAATTACCTCGAGCGAGCTTGACCAGpYW243HMX1-284-317-FGAAGAGGAATTACCTCGAGCGTTGAAGCTGCCApYW243HMX1-284-3	cgcactagtGGCTGCAAGTGGATGTTTG	pYW133 and	Dsc2-SpeI-R
derivatives thereofCTGCAGACCTACCATCAATACgcCgeTCTGCPYW150, pNM003Dsc2-Y53R54A-FGCAGAACTGGAATATGAGCAGAgeGgeGTAPYW150, pNM003Dsc2-Y53R54A-RTTGATGGTAGGTCGTCGTCGTTGACCGAPYW121, pNM003tYW-Dsc2-ALFA-FGCAGAGGAATTACGTCGTCGTCGTCGTCGTGACGPYW121, pNM003tYW-Dsc2-ALFA-FGCACGACGTAATCCCCCAACCTGGAGPYW185, pYW242, pYW243 and allderivatives thereofgegggatecGGCAACAAGGATCACTGGPYW185, pYW242, pYW243 and allderivatives thereofgegggatecGGCAACAAGGATCACTGGPYW185PHMX1-FLAG-FCGTCATCGTGACGTGACCATGGGCGPYW185PHMX1-FLAG-FCGTCATGGTCTTTGTAGTCCATTTTTGCGPYW185SxFLAG-HMX1-FGTATATACACGACATGACAAGGAGGACAGTPYW185SxFLAG-HMX1-FCAAGGATGACCAAGGAGGAGGACAGTPYW185SxFLAG-HMX1-FGCATGACAAGTCAACAGGAGGACAGTPYW185SxFLAG-HMX1-FGCATGACAAGCAATGACAAGGAGGACAGTPYW185RecacacataAAATAACCGCAAAAATGGTGAGCPYW185RAAGGACGTGTACAAGGGAGGACAGTPYW185RGCATGACAGACTGCCCATTTTTGCGGGTTATTTTPYW185RGCATGACGAGCTGTACAAGGCAAGTTCPYW242, PYW243p(HMX1)-Fluo-FGCGCCTGCCACCATTTTTGCGGTTACTAGCTGCPYW242HMX1-264-317-FGCATGGACGAGCTGTACAAGGCAGCTTGACAGPYW243HMX1-284-317-FGCATGGACGAGCTGTACAAGGCAGCTGCACCPYW243HMX1-284-317-FGAAGAGCACTCCPYW243HMX1-284-317-FGAAGAGCACTCCPYW257 and allALFA-264-2KR-FGAAGAGGAATTACCTCGTACTGCGGCGPYW257 and allACCCCGCAAGCTGCACACCGGCAGGTTCAACCTGCAGC<		pYW150 and all	-
CTGCAGACCTACCATCAATACgeCgeTCTGC PYW150, pNM003 Dsc2-Y53R54A-F TCATATTCCAGTTCTGC GCAGAACTGGAATATGAGCCAGAgeGgeGTA TTGATGGTGGTCTGCCAG GAAGAGGAATTACGTCGTCGTTGACCGA PYW150, pNM003 IW-Dsc2-ALFA- GAAGAGGAATTACGTCGTCGTTGACCGA PYW212, pNM003 IW-Dsc2-ALFA- GGCCGCGGTAATTCCTCTCCCACCTGGAG GTCCCTAAAGGTATCCAAAAATTGG geggagetcGTATTGTGATCTTGGGCGC derivatives thereof geggagetcGTATTGTGATCTTGGGCGC derivatives thereof GacacatatAATTACCGCCAAAAATTGGACTAC AAAGACCATGACGACGCACATTGTGGCGCA GTTATTTTatatgtgtg CGACGACGACGACAAGGATCACTGG GTTATTTTGTGATGCTGTGATCTTGGGGGACAGT AAGACATGACGAGGAGCACAT GTATTTTGTGATGCAATATCC CGACGACGACGACGAGGACAGT AGCAATACAATCATAC CGACGATGACGAGGAGCAGCT AGCAATACAATCATAC CGCCCTTGCTCACCAGGAGGACAGT AGCAATACAATCATAC ACCAGGGCG CGCCCTTGCTCACCATGTTTGCGGTTATTTT atgtgtgg GCATGGACGACGGCCCACAAAATGGTGAGC AAGGACGACGACGGCCACTGC GTCTGCTCCTCGCCCCTGTCCCTGTC CGCCCTTGCTCACCATTTTTGCGGTTATTTT atgtgtgg GCATGGACGAGCGGCCAGGCACGT AGCACTGGCCGCACCATTTTGCGGTTATTTT atgtgtgg GCATGGACGAGCGGACCGTC GCCTGGCACGACCGACCCCTGTCCCTGTC CTCGAAGCGAGCGGACGTGCACAGGCAAGTC GCATGGACGAGCGGACGTGCCAGGCC AGGGCG GTCGGCAGCGCGCCCTGTACAAGGCAAGTC GCATGGACGAGCTGTACAAGGCAGGTC GCATGGACGAGCTGTACAAGGCGAGCTGC GCCTGGACCGAGCTGTACAAGGCGCAGTC GCATGGACGAGCTGTACAAGGCGCAGCTC GCATGGACGAGCTGTACAAGGCGCAGCTC GCATGGACGAGCTGTACAAGGCGCAGCT GCATGGACGAGCTGTACAAGGCCGCACTCC GCACGGACGAGCTGTACAAGGCGCAGCT GCATGGACGAGCTGTACAAGGCGCAGCT GCATGGACGAGCTGTACAAGGCGCAGCT GCATGGACGAGCTGTACAAGGCGCACTCC GCACGGACGTAATTACGTCGTCGGCGCAGCT GCATGGACGAGCTGTACAAGGCCGCG GCTCGTACAAGCTGAAGCTGCACCC GCTCGTACAAGCTGAAGCTGCACCC GCTCGTACAAGCTGAAGCTCCACCTCC GTTGACCGGAACCTGCCGCG GTTGGACCAAGCCGGCAGGCTTCAACCCGCAG GCTTGTACACCTGAAGCTGCACCGCG GTTGGACCAACCCGGAAGCTGCACCG GTTGGACCGAACCCGGCAGGTTCAGCCTCC GTTGGACCGAACCCGGCAGGTTCAGCCTCC GTTGGACCGAACCCGGCAGGTTCGCGCG GTTGGTCAAACCCGGAAGCTGCACCGCG GTTCGGTCAAAC GCTTGTACACCTGAAGCTGACCTGCCGG GTTGGTACAACCCGGAAGCTGCCGCG GTTGGTACAACCCGGAAGCTGCCGCG GTTCGGTCAAAC GTTGGTCAACCCGGAACCTGCCGG GTTCGGTCAAAC GTTGGTCAAACC GTTGGTCAACCCGGAAGCTGCCGCG		derivatives thereof	
TCATATTCCAGTTCTGCPYW150, pNM003Dsc2-Y53R54A-RGCAGAACTGGAATTACGTCGCAGpYW150, pNM003Dsc2-Y53R54A-RGAAGAGAATTACGTCGTCGCAGpYW212, pNM003IYW-Dsc2-ALFA-FGACGACGTAATTCCTCTCCAACCTGGAGpYW212, pNM003IYW-Dsc2-ALFA-FGGTCTCCTAAAGGTATTCCTCTCCAACCTGGAGpYW212, pNM003IYW-Dsc2-ALFA-FGGTCTCCTAAAGGTATTCCTCTCCAACCTGGAGpYW185, pYW242, pYW243IYW-Dsc2-ALFA-FgeggagetcGGCAACAAGGATCACTGGpYW185, pYW242, pYW243 and allderivatives thereofgegggatcCGCCAACAAGGATCACTGGgergagatccGCCAACAAGGATCACTGGpYW185AAGACCATGACGGpYW185pHMX1-FLAG-FCCGTCATGGTCTTTGTAGTCCATTTTTGCGgrYW185pHMX1-FLAG-RCCATGGACGAGCAAGCAAGGAGGACAGTpYW185pHMX1-FLAG-RCAAGGATGACAAGGAGGACAGTpYW185RCacacacatataAATTAACCGCAAAAATGGTCACCpYW185RAAGGACGGpYW242, pYW243p(HMX1)-Fluo-FGCATGGACGAGCGACGTTCAAGGAGGAAGTTCpYW242, pYW243p(HMX1)-Fluo-FGCATGGACGAGCTGACAAGGCGAAGTTCpYW242pYW242HMX1-264-317-FGCATGGACGAGCTGACAAGGCGAAGTTCpYW242HMX1-264-317-FGGAGGCGCTGTACAAGGCGAAGTTCpYW243HMX1-284-317-FGGAGGCTGTACAAGTCGACAGCTGCTACCApYW243HMX1-284-317-FGAAGAGAATTACGTCGTCGTCGTGTGACCGApYW257 and allALFA-264-2KR-FGAAGAGAATTACGTCGTCGCGTTGAACCApYW257 and allALFA-264-2KR-FGAAGAGGAATTACGTCGTCGCGCGCGAGCTTCAACCpYW257 and allALFA-264-2KR-FGAAGAGGAATTACGTCGTCGAGCTTGCACGpYW257 and allALFA-264-2KR-F	CTGCAGACCTACCATCAATACgcCgcTCTGC	pYW150, pNM003	Dsc2-Y53R54A-F
GCAGAACTGGAATATGAGCAGAgcGgCGTA TTGATGGTAGGTCTGCAGpYW150, pNM003Dsc2-Y53R54A-R TW-Dsc2-ALFA- FGAGAGGGAATTACGTCGTCGTTTGACCGA ATGATTATATCAGCATATACCpYW212, pNM003tYW-Dsc2-ALFA- FGGACGACGTAAATCCCTCTCCAACCTGGAG GGTCTCCTAAAGGTATCCAAAAATTGGpYW12, pNM003tYW-Dsc2-ALFA- RgcggagctcGTATTGTGATCTAGACCAACATGGCpYW185, pYW242, pYW243 and all derivatives thereofSacI-HMX1-FgcggagctcGTATTGTGATCTTGGGCGCpYW185, pYW242, pYW243 and allBamHI-HMX1-RcacacacataAAATAACCGCAAAAATGGACTAC AAAGACCATGACGGpYW185pHMX1-FLAG-FCGCCATGGCTTTGTAGTCCATTTTGCG GTTATTTtatgtgtgpYW185pHMX1-FLAG-RCAAGGATGACGATGACAAGGAGGACAGT GCAATGACAATCATACpYW1853xFLAG-HMX1-FGAAGAGGCGpYW1853xFLAG-HMX1-FGTAGGATGACGAGGACAAGT GCATGGACGAGGCCAACAATGGTGGACCpYW1853xFLAG-HMX1-FGCATGGACGAGGCAAAATGGTGAGC AAGGCGpYW1853xFLAG-HMX1-FGCATGGACGAGGCAGTTCT GCATGGACGAGGCTGTACAAGGCAAGTCC GCATGGACGAGCTGTACAAGGCCAAGTCT GCATGGACGAGCTGTACAAGGCCAAGTCT GCATGGACGAGCTGTACAAGGCCAGCTC GCATGGACGAGCTGTACAAGCCTGCTCCT GCATGGACGAGCTGTACAAGCCTGCCCTGTCACAGCTCCCCpW242HMX1-284-317-FGCATGGACGAGCTGTACAAGCTGTGCCACT GCATGGACGAGCTGTACAAGCCTGCCCAGCpW243HMX1-284-317-FGAAGAGGAATTACGTCGTCGTTGACAG GCATGGCAGAGTATACGTCGTCGTTGACAGC GCATGGACAAGCCGCAAGGTTGACAAGCCTGCCGA GCACGGCAATTACGTCCTGCCAGCGCAGGCTGACAACCTGCCGA GCTTGTACAGCTCGCAGCGCAGGTTCAACGCGCAGGTTCACCGCGA GCTTGTACACGCGAAGCTGCACGCAGCGCAGGACTCCCCGCAGGTCAACCCGCAGGCTCAACC GCTTGTACACCTGGAACCTGCCAGC GCTTGTACACCTGGAACCACGCGCAGGTCACCGCGCG GTTGGTCAAACC GCTTGTACAACCTGCAACCTGCCGG GTTGGT	TCATATTCCAGTTCTGC		
TTGATGGTAGGTCTGCAGPW212, pNM003t/W-Dsc2-ALFA-FGACGACGTAATTACCTCTTCCAACCTGGAGpYW212, pNM003t/W-Dsc2-ALFA-FGGACGACGTAATTCCTCTTCCAACCTGGAGpYW212, pNM003t/W-Dsc2-ALFA-RGGTCTCCTAAAGGTATCCAAAAATTGGpYW185, pYW242, pYW243 and all derivatives thereofsacI-HMX1-FgcggggatcCGGCAACAAGGATCACTGGpYW185, pYW242, pYW243 and all derivatives thereofsacI-HMX1-FgcggggatcCGGCAACAAGGATCACTGGpYW185, pYW242, pYW243 and all derivatives thereofsacI-HMX1-FccacacatatAAATAACCGCAAAATGGACTAC AAAGACCATGACGGpYW185pHMX1-FLAG-FCCGTCATGGTCTTTGTAGTCCATTTTTGCGpYW185sxFLAG-HMX1-FGTATGTATTGCTACTGTCCTCCTGTGT CAAGGATGACAAGGAGGACAGT AGCAATACAATCAACCGAAAAATGGTGACC AAGGCCGpYW185sxFLAG-HMX1-FCACGGCGCGpYW185RsxFLAG-HMX1-FCACGGACGACGCGACACGTC AGGACGAGCGCGpYW242, pYW243p(HMX1)-Fluo-FCGCCTGGCCACGACGTGACAAGGGCAAGTC AGGGCGGpYW242, pYW243p(HMX1)-Fluo-RGCATGGACGACGTGTACAAGGGCAAGTC GCATGGACGACGTGACAAGTCGCCACGTC GCATGGACGACGTGACAAGTCGCCACGTC GCATGGACGACGTGACAAGGCAGACTGTCACAG GCATGGACGACGTCACAGGTCAAAGGCACAGTC GAAGAGGAATTACGTCGTCGCTTGTACACGCA GCATCGCAAGTTCACGCTGCTGCAGCACGTCGCAGAGTTCACGCTGCAGC GCATGGACGAACTCCCGCAGGTTCAACGCGCAAGTTCCACGCAG GCATGGACGAACTCCCGCAGGTTCAACGCTGCAGC GCATGGACGAACTCCCGAACGTCGCAAGCTCGCAGAGCTGAACCTGCCAG GCTTGACACGCTGAACCTGCAGCG GCTCGTACAACCTGAACCTGCAGCG GCTCGTACAACCTGAACCTGCAGCG GCTAGTATACCCGAACCTGCAGCGTCACCTGC GCTAGTACACCGGAAGCTGACACGCCGCA GCTTGACACCTGAACCTGCAGCG GCTAGTACACCGGAAGCTGACCTGCCGG GTTCGGCAAACALFA-264-2KR-FGAAGGGAATTACCTCTCAACCTGGAACCTGCCGG GTTCGGCA	GCAGAACTGGAATATGAGCAGAgcGgcGTA	pYW150, pNM003	Dsc2-Y53R54A-R
GAAGAGGAATTACGTCGTCGTTTGACCGA ATGATTTATATCAGCATATACCPYW212, pNM003 FW212, pNM003IYW-Dsc2-ALFA-F FCGACGACGTAATTCCTCTCTCAACCTGGAG GGTCTCCTAAAGGTATCCAAAAATTGGCpYW212, pNM003 FW212, pNW003IYW-Dsc2-ALFA-R FgcggagctcGTATTGTGATCTTGGGCGCpYW212, pNW003 PWW185, pYW242, pYW243 and all derivatives thereofSacI-HMX1-FgcgggatcGGCAACAAGGATCACTGG CacacacatatAAATAACCGCAAAAATGGACTAC AAAGACATGACGGpYW185 PHMX1-FLAG-FCGTCGTCATGGTCTTTGTAGTCCATTTTTGCG GTTATTTrtatgtgtgpYW185pHMX1-FLAG-RCAAGGATGACGATGACAAGGAGGACAGT AGCAATACAATCAATCpYW1853xFLAG-HMX1-FACACATCATCTTG CATCGTCATCTGTCCTCCTGT CATCGTCATCGTGCACATTTTTGCGGTTATTT TatgtgtgpYW1853xFLAG-HMX1-FCAGGAGGACGAGGCG CAGGGCGpYW1853xFLAG-HMX1-FCGCCCTTGCTCACCATTTTGCGGTTATTT TatgtgtgpYW185RRCCCCCTTGCTCACCATTTTTGCGGTTATTT TatgtgtgpYW185RRCCATGGACGAGGCGGAAAAATGGTGAGC AGGGCGpYW242, pYW243p(HMX1)-Fluo-FCGCCCTTGCTCACCATTTTTGCGGTTATTT TatgtgtgpYW242, pYW243p(HMX1)-Fluo-RGCATGGACGAGCTGTACAAGGCCGCTGTACAAGGCCGCTGTACAAGGCCGCC GCTCGTCAAGCTGACACTGCCCCTGTTGACCAGCTC GCAGGGAATTACGTCGTCGTTGACCAGpYW243HMX1-284-317-FGCATGGACGAGCTGTACAAGGCTGTGACAG GCTCGTCATGCCGAGGCTGTACAAGCTGCACGAG GCACGACGACTCCCpYW243HMX1-284-317-FGCACGGCAAGTTCAGCTGCAGCGCGA GAAGAGGAATTACGTCGTCGTGTGTGACAGCTGCAGG GGCTGTACAAGCTGCAAGCTGCACGGAGGTTCACCGGAGGTCACCTGCAGG GCTGGACAAACpYW257, pW258, pYW251 and all 	TTGATGGTAGGTCTGCAG		
ATGATTTATATCAGCATATACCFCGACGACGTAATTCCCTCTTCAACCTGGAGpW212, pNM003tYW-Dsc2-ALFA-GGTCTCCTAAAGGTATCCAACATGGACpYW185, pYW242, pYW243 and allgcgggatccGTATTGTGATCTTGGGCGCpW185, pYW242, pYW185, pYW242, pYW185, pYW243 and allgcgggatccGGCAACAAGGATCACTGGpYW185, pYW242, pYW185pW185, pYW242, pYW185pW185, pYW242, pYW185cacacacatatAATAACCGCAAAAATGGACTACpYW185pHMX1-FLAG-FCCGTCATGGTCTTTGTAGTCCATTTTTGCGpYW185pHMX1-FLAG-RCAAGGATGACGATGACAAGGAGGACAGT AGCAATACAATCATACpYW1853xFLAG-HMX1-FGAAGGGCGpYW185sxFLAG-HMX1-FCAACGACGAAGAAATGGTGAGCpYW185RCacacacatatAAATAACCGCAAAAATGGTGAGC AAGGGCGpYW185RCacacacatatAAATAACCGCAAAAATGGTGAGC AGGGCGpYW185RCacacacatatAAATAACCGCAAAAATGGTGAGC AGGGCGpYW242, pYW243p(HMX1)-Fluo-FCGCCTTGCACCATTTTTGCGGTTATTTt atgtgtgtgpYW242, pYW243p(HMX1)-Fluo-FCGCTGGACGAGCTGTACAAGGCCAGTC GCATGGACGAGCTGTACAAGTCGCCTTGTACAAGCTC GCATGGACGAGCTGTACAAGTCGCCTGTACAAGTCGCCT GCATGGACGAGCTGTACAAGCCGCGCAGCTTGACAAGpYW242HMX1-284-317-FGGAGGGAGTTCAGGCGCGGCGTCAAGGCTCTCAAG GCATGGACGAAGTTCAGCTCGTCGTTGTACAGCpYW243HMX1-284-317-FGAAAGGGAATTACGTCGTCGTTTGACCGA GCATGGACGAAGTTCAGCTCGCGCGCGGCAGCTTCAAGCpYW257, nPW258, pYW251HMX1-284-317-FGAAAGGGAATTACGTCGTCGTGTTTGACCGA GCCTGTACAAGCCGCAAGTTCACGCGCG GCTTGTACAAGCTCGCAGCGTCAAGCpYW251ALFA-284-FGAAAGGGAATTACGTCGTCGTGTTGACCGG GCTTGAACAGCTCCAGCGCAGGTCACCTGCCGG GCTTGTACAAGCCGCAAGCTGCAGCGpYW25	GAAGAGGAATTACGTCGTCGTTTGACCGA	pYW212, pNM003	tYW-Dsc2-ALFA-
CGACGACGTAATTCCTCTTCCAACCTGGAG GGTCTCCTAAAGGTATCCAAAAATTGGpYW121, pNM003 PW185, pYW242, pYW185, pYW242, pYW185, pYW243 and all derivatives thereof(YW-Dsc2-ALFA-R RgcggagctcGTATTGTGATCTTGGGCGCerivatives thereofSacl-HMX1-FgregggatccGGCAACAAGGATCACTGGpYW185, pYW242, pYW185, pYW242, pYW185, pYW243 and all derivatives thereofBamHI-HMX1-RcacacacataAAATAACCGCAAAAATGGACTACpYW185pHMX1-FLAG-FCGTCATGGTCTTTGTAGTCCATTTTGCG GTTATTTtatgtgtgtpYW185pHMX1-FLAG-RCAAGGATGACGATGACAAGGAGGACAGT AGCAATACAATCATACpYW185sxFLAG-HMX1-FGTATGATTGTATGCTACTGTCCTCTTGT GCACTCATCATGGpYW185sxFLAG-HMX1-FGCACGCAGCGCpYW185p(HMX1)-FLuo-FCGCCCTTGCTCACCATTTTGCGGTTATTTt tatgtgtgtpYW242, pYW243p(HMX1)-Fluo-FGCATGGACGAGCTGTACAAGGGCGAAGTTC GCATGGACGAGCTGTACAAGGCGAACTTGCCTGT GCATGGACGAGCTGTACAAGGCGACTGTACAAGGCGACTGT GCATGGACGAGCTGTACAAGGCGACTGTACAAGGCGACTGT GCATGGACGAGCTGTACAAGGCGACTGTACAAGGCAAGTTC GCATGGACGAGCTGTACAAGGCGACTGTACAAG GCCTGGCCAAGTCCAGCT GCAGGGAATTACGTCGTCGTGTATGACGAG ACCCCGGCAAGTTCAGCTGCATGAGCAGACTGC GAAGAGGAATTACGTCGTCGTGTTTGACGAG ACCCCTGCCATAGTCCCCpYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTTTGACGAG GGCTGTACAAGGCACTCCpYW243HMX1-284-317-RGAAGAGGAATTACCTCGGCGTTTGAACGA GGCTGTACAAGCTGCAGCTGTCAAGCTGCAGC GGCTGTACAAGCTGCAAGCTGCACCTGC GGTGATACACCTGCAGCGCAGGTCAAGCTGCAGCTCA GGTGATTACGCpYW261ALFA-264-2KR-FGAAGAGGAATTACCTCGAAGCTGAACCTGCCGG GTTCGACAACCCGGCAGGTTCAGCTGCACTGC GTTGACACACCGGAAGCTCCACGCGG GTTCGGTCAAACpYW261ALFA-264-2KR-FGAAGGGGCAACCCGGAAGGTTCAACCT	ATGATTTATATCAGCATATACC		F
GGTCTCCTAAAGGTATCCAAAAATTGGRgcggagctcGTATTCTGATCCTAAAGTTGGPW185, pYW242, pW243 and all derivatives thereofSacI-HMX1-FgcgggatccGGCAACAAGGATCACTGGPW185, pYW242, pYW243 and all derivatives thereofSacI-HMX1-FgcgggatccGGCAACAAGGATCACTGGPW185, pYW242, pYW243 and all derivatives thereofBamHI-HMX1-RcacacacataAATAACCGCAAAAATGGACTAC AAAGACCATGACGGPW185pHMX1-FLAG-FCGTCATGGTCTTTGTGAGTCCATTTTGCG GTTATTTtatgtgtgtgpW185pHMX1-FLAG-RCAAGGATGACGACGACAGAGAGGAGCACGT AGCAATACAATCATACpYW1853xFLAG-HMX1-FCACGGCGpYW185RsacI-Ad-HMX1-FCacacacataAAATAACCGCAAAAATGGTGCGC CAGGCGGpW185pHMX1-FLAG-RCACGGACGAGCTGTACAAGGAGGACAGTT CAGGCGGpYW185sxFLAG-HMX1-FCGCATGGACGAGCTGTACAAGGCAAGTTC AGGCTCACACATTGCCCTTGTACAGCTC GCCATGGACGAGCTGTACAAGGCAAGTTC GCATGGACGAGCTGTACAAGTCGCACAGTCC GCATGGACGAGCTGTACAAGTCGCACAGTCC GCATGGACGAGCTGTACAAGTCGCGCCAGCT GCATGGACGAGCTGTACAAGTCGCGCTTGACAGCTC GCATGGACGAAGTTACGTCGTCGTTGACAGC GCATGGACGAAGTTACGTCGTCGTGTGACAG CCCTCTGCATGCpYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTTGACAGC GCATGGACGAAGTTCCCGCGCGCGCGCGCGCGCGCGCGCG	CGACGACGTAATTCCTCTTCCAACCTGGAG	pYW212, pNM003	tYW-Dsc2-ALFA-
pYW185, pYW242, pYW243 and all derivatives thereof acacacataAAATAACCGCAAAAATGGACTAC AAAGACCATGACGGSac1-HMX1-FgcgggatccGGCAACAAGGATCACTGG cacacacataAAATAACCGCAAAAATGGACTAC AAAGACCATGACGGderivatives thereof derivatives thereofBamHI-HMX1-RCAAGGATGACGATGACAAGGAGGACAGT GGCATGACGATGACAATCATACpYW185pHMX1-FLAG-FCCGCCATGGTCTTTGTAGTCCATTTTGCG GTTATTTatgtgtgt CAAGGATGACAATCATACpYW185pHMX1-FLAG-RCAAGGATGACAATCATAC GGCATGACAATCATCATGpYW185sxFLAG-HMX1-FGCATGACAATCATCGTCCTCCTTGT CATCGTCATCCTTGpYW185RcacacacataAAATAACCGCAAAAATGGTGGAGC AAGGGCGpYW242, pYW243p(HMX1)-Fluo-FCGCCCTTGCTCACCATTTTGCGGTTATTTt tatgtgtgt ggggpYW242, pYW243p(HMX1)-Fluo-RGCATGGACGAGCTGTACAAGGGCAAGTTC AGCATGGACGAGCTGTACAAGGCCAAGTTC GCATGGACGAGCTGTACAAGGCCAAGTTC GCATGGACGAGCTGTACAAGGCCAAGTTC GCATGGACGAGCTGTACAAGTCGCCTCT GAAGAGGAATTACGTCGTCGTCGTACAAGTCGCAC GCTCGTCCATGCpYW242HMX1-264-317-FGGAGTGGCCTTTAGTAGCAAGTCGCACTCC GCATGGACGAGCTGTACAAGTCGCGCTACTA GAAGAGGAATTACGTCGTCGTTTGACCGA ACCCGGCAAGTTCAGCGCGCTGTTGACAGCA ACCCGGCAAGTTACGTCGTCGTTGACCGA ACCCGGCAAGTTCACCAGCGCCGCAGGTCACTCCpYW243HMX1-284-317-FGAAGAGGAATTACGTCGTCGTCGTTGACCGA GCTTGTACAGCTCCAAGCACGCCCpYW257 and all derivatives thereofALFA-264-FPYW257, PYW258 PYW292 and all derivatives thereof, GCACGGCAAGTTCACCACCGCGAGGTCAGCTCA PYW258 GYW292 and all derivatives thereof, PYW251ALFA-264-2KR-FCGACGGCAAGTCGCGCGCTCCATGC GTTGAACACCTGAAGCTGAACCTGCCGG GTTCGACAACpYW261ALFA-264-2KR-FGAATGGATGTACAAC GCTCGTCAAAC <td< td=""><td>GGTCTCCTAAAGGTATCCAAAAATTGG</td><td></td><td>R</td></td<>	GGTCTCCTAAAGGTATCCAAAAATTGG		R
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gcggagctcGTATTGTGATCTTGGGCGCderivatives thereofSacI-HMX1-FpYW185, pYW243 and allpYW243 and allgcgggatccGGCAACAAGGATCACTGGderivatives thereofAAAGACCATGACGGpYW185CGTCATGGTCTTTGTAGTCCATTTTGCGpYW185CGTCATGGTCATGACAGGAGGACAGTpYW185AGGAATACAATCATACpYW185ATCGTCATCTTGaxFLAG-HMX1-FGTATGTATGTATGCTACTGTCCTCTGTaxFLAG-HMX1-FCACGGCACACACCACACACAGGAGGACAGTpYW185AGGGCGpYW185CACGGCACTCTGCCACCTTGTaxFLAG-HMX1-FCGCCCTTGCTCACCATTTTGCGGTTATTTtaxFLAG-HMX1-FatgtgtggpYW242, pYW243CGCCCTTGCTCACCATTTTGCGGTTATTTtpYW242, pYW243atgtgtggpYW242, pYW243CTTGAAGCTGAACTGCCCTGTACAAGGGCAAGTTCpYW242AGGTCAAGCTGAACTTGCCCTTGTACAGGTCpYW242GCATGGACGAGCTGTACAAGGCCAGTCpYW243GAAGAGACACTCCpYW243GAAGAGACACTCCpYW243GAAGAGGAATTACGTCGTGTGTGACAGpYW243GAAGAGGAATTACGTCGTCGTTTGACCGApYW257 and allACCCGGCAAGTTCAGCGTCGTCTGTGACGAGderivatives thereofALFA-264-FpYW258 and allACCCTGTACTAGAGAGCACTCCpYW258 and allGAGAGAGAATTACGTCGTCTTCCACACGGAGderivatives thereofGACGACGACGAAGTCAGCAGCAGCTGACACTGCpYW258 and allGCACGACGTAATTCCTCTCTCCAACCGGAGderivatives thereofGCACGACGAAGTCAGCAGCGAGGTCAGCTCApYW261ALFA-264-FKpYW261GAATGACGAACCTGGCAGGTCAGCTGCGpYW261GTTTGACCGAACCTGGCAGGTCAGCTGCAGpYW261 <td< td=""><td></td><td>pYW243 and all</td><td></td></td<>		pYW243 and all	
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pYW243 and all derivatives thereofBamHI-HMX1-RcacacacataAATAACCGCAAAAATGGACTAC AAAGACCATGACGGpYW185pHMX1-FLAG-FCCGTCATGGTCTTTGTAGTCCATTTTGCG GTTATTTatagtgigtgpYW185pHMX1-FLAG-RCAAGGATGACAAGCAGAGAAGGACAGT AGCAATACAATCATACpYW185sxFLAG-HMX1-FGTATGATGTATTGCTACTGTCCTCCTTGT GTATGATTGTATTGCTACCGCAAAAATGGTGAGC AGGGCGpYW185sxFLAG-HMX1-FCacacacataAATAACCGCAAAAATGGTGAGC AGGGCGpYW242, pYW243p(HMX1)-Fluo-FCGCCCTTGCTCACCATTTTTGCGGTTATTTt atgtgigtgpYW242, pYW243p(HMX1)-Fluo-RGCATGGACGAGCTGTACAAGGGCAAGTCC GCCTTGCAACCTGCCCTTGTCACAGGCAAGTCC GCATGGACGAGCTGTACAAGGCCAAGTCC GCATGGACGAGCTGTACAAGGCCAGACTTGCCCT TCAAGpYW242HMX1-264-317-FCTCGACG GCATGGACGAGCTGTACAAGGCCAGACT GCATGGACGAGCTGTACAAGTCTGCCAC GCATGGACGAAGTTCCCpYW243HMX1-284-317-FGCATGGACGAAGTTCC GCATGGCAAGTTCAGCGCA GCTCGTCCATGCpYW243HMX1-284-317-FGAAGAGGAATTACGTCGTGTTGTGACGAA GCTCGTCCATGCpYW243HMX1-284-317-FGAAGAGGAATTACGTCGTGTTGTGACCGA ACCCGGCAAGTTCAGCTCCCpYW243HMX1-284-317-FGAAGAGGAATTACGTCGTGTTGTGACCGA ACCCTGCATAGTACGACGCGCGCGGCAGGTTCAGCTGCpYW257, pYW258, pYW292 and all derivatives thereofALFA-264-FGAAGAGGAATTACGTCGTCTTCCCAGCGCGGCAGGTTCAGCTCC GGTTGTACAGCTGAACCTGCCGCG GGTTGTACAGCTGAAAGCTGCACCGGCAGGTTCAGCTCA GTTGACCGAACCCGGCAGGTTCAGCTGCG GCTTGAACACCTGAAGCTGAACCTGCCGG GTTCGACAACpYW261ALFA-264-2KR-FGAATGGATGTACAAC GAATGATGCAAACCTGCACGG GTTCAAACCCGAAACTCGCGCGG GTTCAACACCTGAAACCTGCCGCGpYW261ALFA-264-2KR-F		pYW185, pYW242,	
gcgggatcCGGCAACAAGGATCACTGGderivatives thereofBamHI-HMX1-RcacacacataAAATAACCGCAAAAATGGACTACpYW185pHMX1-FLAG-FCCGTCATGGCTCTTGTAGTCCATTTTGCGpYW185pHMX1-FLAG-RCCGTCATGGTCTTGTGAGCAAGGAGGACAGTpYW185pHMX1-FLAG-RCAAGGATGACGATGACAAGGAGGACAGTpYW1853xFLAG-HMX1-FGTATGATTGTATTGCTACTGTCCTCTGTpYW1853xFLAG-HMX1-FGTATGATTGTATTGCTACTGTCCTCTGTpYW185RcacacacataAAATAACCGCAAAAATGGTGAGCpYW242, pYW243p(HMX1)-Fluo-FCGCCCTGCTCACCATTTTGCGGTATTTtpYW242, pYW243p(HMX1)-Fluo-RGCATGGACGAGCTGTACAAGGGCAAGTTCpYW242HMX1-264-317-FCTTGAAGCTGAACTTGCCCTTGTACAGCTCpYW242HMX1-264-317-RGCATGGACGAGCTGTACAAGGCCAGCTCpYW243HMX1-264-317-RGCATGGACGAGCTGTACAAGTCTGCTACTpYW243HMX1-264-317-RGGATGGACGAGCTGTACAAGTCTGCTACTpYW243HMX1-284-317-RGGAGGGCAAGTTCAGCAGCpYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTTTGACCGApYW257 and allACCCGGCAAGTTCAGCAGCACTCCpYW257, pYW258, pYW292 and allACCCGGCAAGTTCAGCAGCTTCAAGderivatives thereofALFA-284-FpYW257, pYW258, pYW292 and allCGACGACGTAATTCCTCTTCCAACCTGGAGpYW251GACGAGCACCTGCAGCCGCAgGTTCAGCTCCApYW261ALFA-264-2KR-FCTAATACACCTGAAGCTGCAAGCTCCAGGTGTATTACGpYW261ALFA-264-2KR-FCTAATACACCTGAAGCTGCAGCGCGGGTTCGGTCAAACpYW261ALFA-264-2KR-FCTAATACACCTGAAGCTGCAGCGCGGGTTGGTCAAACpYW261ALF		pYW243 and all	
cacacacataAAATAACCGCAAAAATGGACTAC AAAGACCATGACGG pYW185 pHMX1-FLAG-F CCGTCATGGTCTTTGTAGTCCATTTTGCG GTTATTTitatgtgtg GTATTTitatgtgtg GAAGGATGACGATGACAAGGAGGACAGT AGCAATACAATCATAC pYW185 3xFLAG-HMX1-F GTATGATTGTATTGCTACTGTCCTCCTTGT CATCGTCATCCTTG pYW185 R cacacacataAAATAACCGCAAAAATGGTGAGC AAGGGCG pYW242, pYW243 p(HMX1)-Fluo-F CGCCTGGCACCATTTTTGCGGTTATTTt atgtgtgtg GCATGGACGAGCTGTACAAGGGCAAGTTC AGCTTCAAG pYW242, pYW243 p(HMX1)-Fluo-R GCATGGACGAGCTGTACAAGGGCAAGTTC AGCTTCAAG pYW242, pYW243 p(HMX1)-Fluo-R GCATGGACGAGCTGTACAAGGGCAAGTTC AGCTTCAAG pYW242 HMX1-264-317-F CTTGAAGCTGAACTTGCCCTTGTACAGCTC GCCATGC GC GCCATGGCAGCTGTACAAGGTCGTACT AGAAGAGCACTCC pYW243 HMX1-264-317-R GCATGGACGAGCTGTACAAGTCTGCTACT AGAAGAGGAATTACGTCGTCGTTTGACCGA ACCCGGCAAGTTCAGCAGACTTGTACA GCTCGTCCATGC pYW243 HMX1-284-317-R GAAGAGGAATTACGTCGTCGTTTGACCGA ACCCGGCAAGTTCAGCAGCACTCT GAAGAGGAATTACGTCGTCGTTTGACCGA ACCCGGCAAGTTCAGCAGACCTCG GCATGCAGCTCCC pYW257 and all derivatives thereof ALFA-264-F GAAGAGGAATTACGTCGTCGTTTGACCGA ACCCCGGCAAGTTCAGAGAGCACTCC PYW258 and all derivatives thereof, GGCTTGTACAAGCTGCAGCTCA GTTTGACCGAACCCGGCAgGTTCAGCTCA GTTTGACCGAACCCGGCAgGTTCAGCTCA GTTGACAGCCGGCAGGTCAGCTCA GTTTGACCGAACCCGGCAgGTTCAGCTGCA GTTGACAGCCGGCAGGTCAGCTCA GTTGACCGAACCCGGCAgGTTCAGCTGCA GTTGACAAGCCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCTGCA GTTCGACCGAACCCGGCAGGTCAGCGCG GTTCGGTCAAAC GAATGGATGTCAACAGGGATTCTGCTACT AG GAATGGATGTCAACAGGGATTCTGCTACT AG	gcgggatccGGCAACAAGGATCACTGG	derivatives thereof	BamHI-HMX1-R
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AGCTTCAAGpYW242HMX1-264-317-FCTTGAAGCTGAACTTGCCCTTGTACAGCTCpYW242HMX1-264-317-RGCATGGACGAGCTGTACAAGTCTGCTACTpYW243HMX1-284-317-FAGAAGAGCACTCCpYW243HMX1-284-317-FGGAGTGCTCTTCTAGTAGCAGACTTGTACApYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTCGTTTGACCGApYW257 and allACCCGGCAAGTTCAGCTCGTCGTTTGACCGAderivatives thereofALFA-264-FGAAGAGGAATTACGTCGTCGTCGTTTGACCGApYW258 and allACCCTCTGCTACTAGAAGAGCACTCCderivatives thereofALFA-284-FGACGACGTAATTCCTCTTCCAACCTGGAGpYW257, pYW258, pYW292 and allCGACGACGTAATTCCTCTTCCAACCTGGAGderivatives thereof, pYW283, pYW302ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCA GGTGTATTACGpYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGG GTTCGGTCAAACpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACT AGpYW261HMX1-K281R-F	GCATGGACGAGCTGTACAAGGGCAAGTTC	p ==, p ==.e	P(11) 1100 11
CTTGAAGCTGAACTTGCCCTTGTACAGCTC GTCCATGCPW242HMX1-264-317-RGCATGGACGAGCTGTACAAGTCTGCTACT AGAAGAGCACTCCpYW243HMX1-284-317-FGGAGTGCTCTTCTAGTAGCAGACTTGTACA GCTCGTCCATGCpYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTCGTTTGACCGA ACCCGGCAAGTTCAGCTTCAAGpYW257 and all derivatives thereofALFA-264-FGAAGAGGAATTACGTCGTCGTTTGACCGA ACCCTGCTACTAGAAGAGCACTCCpYW258 and all derivatives thereofALFA-284-FGAAGAGGAATTACGTCGTCGTTTGACCGA ACCCTCTGCTACTAGAAGAGCACTCCpYW257, pYW258, pYW257, pYW258, pYW292 and allALFA-284-FCGACGACGTAATTCCTCTTCCAACCTGGAGG GGCTTGTACAGCTCGCATGCpYW257, pYW258, pYW292 and allALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCA GGTGTATTACGpYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGG GTTCGGTCAAACpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACT AGpYW261HMX1-K281R-F	AGCTTCAAG	pYW242	HMX1-264-317-F
GTCCATGCpYW242HMX1-264-317-RGCATGGACGAGCTGTACAAGTCTGCTACTpYW243HMX1-284-317-FAGAAGAGCACTCCpYW243HMX1-284-317-FGGAGTGCTCTTCTAGTAGCAGACTTGTACApYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTTGACCGApYW257 and allALFA-264-FGAAGAGGAATTACGTCGTCGTTGACCGApYW258 and allALFA-264-FACCCTGCTACTAGAAGAGCACTCCderivatives thereofALFA-284-FGAAGAGGAATTACGTCGTCGTCGATGGAGpYW257, pYW258, pYW292 and allALFA-284-FCGACGACGTAATTCCTCTTCCAACCTGGAGderivatives thereof, pYW292 and allALFA-284-FCGACGACGTAATTCCTCTTCCAACCTGGAGpYW261ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCApYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGGpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261HMX1-K281R-F	CTTGAAGCTGAACTTGCCCTTGTACAGCTC	•	
GCATGGACGAGCTGTACAAGTCTGCTACT AGAAGAGCACTCCPW243HMX1-284-317-FGGAGTGCTCTTCTAGTAGCAGAACTTGTACA GCTCGTCCATGCpYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTCGTTTGACCGA ACCCGGCAAGTTCAGCTTCAAGpYW257 and all derivatives thereofALFA-264-FGAAGAGGAATTACGTCGTCGTTTGACCGA ACCCTCTGCTACTAGAAGAGCACTCCpYW258 and all derivatives thereofALFA-284-FGAAGAGGAATTACGTCGTCGTTTCAACCGA ACCCTCTGCTACTAGAAGAGCACTCCpYW257, pYW258, pYW257, pYW258, pYW292 and all derivatives thereof, GGCTTGTACAGCTCGTCCATGCALFA-284-FGAACGACGTAATTCCTCTTCCAACCTGGAG GGCTTGTACAGCTCGTCCATGCpYW261ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCA GGTGTATTACGpYW261ALFA-264-2KR-FCGAAAGCGTCAAACCpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACT AGpYW261HMX1-K281R-F	GTCCATGC	pYW242	HMX1-264-317-R
AGAAGAGCACTCCpYW243HMX1-284-317-FGGAGTGCTCTTCTAGTAGCAGACTTGTACApYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTTGACCGApYW257 and allLAFA-264-FACCCGGCAAGTTCAGCTCGTCGTTGACCGApYW258 and allALFA-264-FACCCTCTGCTACTAGAAGAGCACTCCpYW257, pYW258, pYW292 and allLAFA-284-FCGACGACGTAATTCCTCTTCCAACCTGGAGderivatives thereofALFA-284-FGTTGACCGAAGCTCGTCGTCGTCGTGGAGpYW257, pYW258, pYW292 and allALFA-284-FCGACGACGTAATTCCTCTTCCAACCTGGAGderivatives thereof,ALFA-284-FGGTTTGTACAGCTCGTCCATGCpYW283, pYW302ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCApYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGGpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261HMX1-K281R-F	GCATGGACGAGCTGTACAAGTCTGCTACT	•	
GGAGTGCTCTTCTAGTAGCAGACTTGTACA GCTCGTCCATGCpYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTTTGACCGA ACCCGGCAAGTTCAGCTTCAAGpYW257 and all derivatives thereofALFA-264-FGAAGAGGAATTACGTCGTCGTTTGACCGA ACCCTCTGCTACTAGAAGAGCACTCCpYW258 and all derivatives thereofALFA-284-FGCACGACGTAATTCCTCTTCCAACCTGGAG GGCTTGTACAGCTCGTCCATGCpYW257, pYW258, pYW292 and all derivatives thereof, gGCTTGTACAGCTCGTCCATGCpYW283, pYW302ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCA GGTGTATTACGpYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGG GTTCGGTCAAACpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACT AGpYW261HMX1-K281R-F	AGAAGAGCACTCC	pYW243	HMX1-284-317-F
GCTCGTCCATGCpYW243HMX1-284-317-RGAAGAGGAATTACGTCGTCGTTGACCGApYW257 and allALFA-264-FACCCGGCAAGTTCAGCTCGTCGTTGACCGApYW258 and allALFA-284-FGAAGAGGAATTACGTCGTCGTTGACCGAderivatives thereofALFA-284-FACCCTCTGCTACTAGAAGAGCACTCCderivatives thereofALFA-284-FCGACGACGTAATTCCTCTTCCAACCTGGAGpYW257, pYW258, pYW292 and all	GGAGTGCTCTTCTAGTAGCAGACTTGTACA		
GAAGAGGAATTACGTCGTCGTTTGACCGApYW257 and allACCCGGCAAGTTCAGCTTCAAGderivatives thereofALFA-264-FGAAGAGGAATTACGTCGTCGTTTGACCGApYW258 and allACCCTCTGCTACTAGAAGAGCACTCCderivatives thereofALFA-284-FPYW257, pYW258, pYW292 and allpYW292 and allCGACGACGTAATTCCTCTTCCAACCTGGAGderivatives thereof,GGCTTGTACAGCTCGTCCATGCpYW283, pYW302ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCApYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGGpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261HMX1-K281R-F	GCTCGTCCATGC	pYW243	HMX1-284-317-R
ACCCGGCAAGTTCAGCTTCAAGderivatives thereofALFA-264-FGAAGAGGAATTACGTCGTCGTTTGACCGApYW258 and allACCCTCTGCTACTAGAAGAGCACTCCderivatives thereofALFA-284-FpYW257, pYW258, pYW292 and allpYW292 and allCGACGACGTAATTCCTCTTCCAACCTGGAGderivatives thereof, pYW283, pYW302ALFA-FLUO-RGTTTGACCGAACCTGGCAgGTTCAGCTTCApYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGGpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261HMX1-K281R-F	GAAGAGGAATTACGTCGTCGTTTGACCGA	pYW257 and all	
GAAGAGGAATTACGTCGTCGTTTGACCGA ACCCTCTGCTACTAGAAGAGCACTCCpYW258 and all derivatives thereofALFA-284-FPYW257, pYW258, pYW292 and allpYW292 and allCGACGACGTAATTCCTCTTCCAACCTGGAG GGCTTGTACAGCTCGTCCATGCderivatives thereof, pYW283, pYW302ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCA GGTGTATTACGpYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGG GTTCGGTCAAACpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACT AGpYW261HMX1-K281R-F	ACCCGGCAAGTTCAGCTTCAAG	derivatives thereof	ALFA-264-F
ACCCTCTGCTACTAGAAGAGCACTCCderivatives thereofALFA-284-FPYW257, pYW258, pYW292 and allpYW292 and all	GAAGAGGAATTACGTCGTCGTTTGACCGA	pYW258 and all	
pYW257, pYW258, pYW292 and allpYW292 and allCGACGACGTAATTCCTCTTCCAACCTGGAGderivatives thereof, pYW283, pYW302ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCApYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGGpYW261ALFA-264-2KR-FGATGGATGTTCAACApYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261HMX1-K281R-F	ACCCTCTGCTACTAGAAGAGCACTCC	derivatives thereof	ALFA-284-F
pYW292 and allCGACGACGTAATTCCTCTTCCAACCTGGAGGCTTGTACAGCTCGTCCATGCGTTTGACCGAACCCGGCAgGTTCAGCTTCAGTTTGACCGAACCCGGCAgGTTCAGCTTCAGGTGTATTACGCGTAATACACCTGAAGCTGAACcTGCCGGGTTCGGTCAAACGAATGGATGTTCAACAgGGATTCTGCTACTAGPYW261HMX1-K281R-F		pYW257, pYW258,	
CGACGACGTAATTCCTCTTCCAACCTGGAGderivatives thereof, pYW283, pYW302ALFA-FLUO-RGGCTTGTACAGCTCGTCCATGCpYW283, pYW302ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTTCApYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACCTGCCGGpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261HMX1-K281R-F		pYW292 and all	
GGCTTGTACAGCTCGTCCATGCpYW283, pYW302ALFA-FLUO-RGTTTGACCGAACCCGGCAgGTTCAGCTCApYW261ALFA-264-2KR-FGGTGTATTACGpYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACcTGCCGGpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261HMX1-K281R-F	CGACGACGTAATTCCTCTTCCAACCTGGAG	derivatives thereof,	
GTTTGACCGAACCCGGCAgGTTCAGCTTCAPYW261ALFA-264-2KR-FGGTGTATTACGpYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACcTGCCGGFHIX1-K281R-FGAATGGATGTTCAACAgGGATTCTGCTACTFHMX1-K281R-F	GGCTTGTACAGCTCGTCCATGC	pYW283, pYW302	ALFA-FLUO-R
GGTGTATTACGpYW261ALFA-264-2KR-FCGTAATACACCTGAAGCTGAACcTGCCGGpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTpYW261HMX1-K281R-F	GTTTGACCGAACCCGGCAgGTTCAGCTTCA	· · ·	
CGTAATACACCTGAAGCTGAACcTGCCGG GTTCGGTCAAACpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACT AGpYW261HMX1-K281R-F	GGTGTATTACG	pYW261	ALFA-264-2KR-F
GTTCGGTCAAACpYW261ALFA-264-2KR-RGAATGGATGTTCAACAgGGATTCTGCTACTAGpYW261HMX1-K281R-F	CGTAATACACCTGAAGCTGAACcTGCCGG	•	
GAATGGATGTTCAACAgGGATTCTGCTACT AG pYW261 HMX1-K281R-F	GTTCGGTCAAAC	pYW261	ALFA-264-2KR-R
AG pYW261 HMX1-K281R-F	GAATGGATGTTCAACAgGGATTCTGCTACT	1	
	AG	pYW261	HMX1-K281R-F

CTAGTAGCAGAATCCcTGTTGAACATCCAT		
TC	pYW261	HMX1-K281R-R
CTTAGTTTCGACGGATTCTAGAATGAGTAA		
AGGAGAAGAACTTTTCACTGG	pYW263	pTDH3-pH-F
CCAGTGAAAAGTTCTTCTCCTTTACTCATT		
CTAGAATCCGTCGAAACTAAG	pYW263	pTDH3-pH-R
CATGGCATGGATGAACTATACAAACCCTC		
CAGGTTGGAAGAG	pYW263	pH-ALFA-F
CTCTTCCAACCTGGAGGGTTTGTATAGTTC		
ATCCATGCCATG	pYW263	pH-ALFA-R
GAGCACTCCACACGGTCATTCTTGTCATGC		
TGCTGGTGCTTTC	pYW290	HMX1-N+3-F
GAAAGCACCAGCAGCATGACAAGAATGAC		
CGTGTGGAGTGCTC	pYW290	HMX1-N+3-R
GAAGAGGAATTACGTCGTCGTTTGACCGA		ALFA-HMX1-FL-
ACCCGAGGACAGTAGCAATACAATCATAC	pYW292, pYW311	F
CGACGACGTAATTCCTCTTCCAACCTGGAG		
GGCATTCTAGAATCCGTCGAAACTAAGTT		
CTGG	pYW311	pTDH3-ALFA-R
	pYW292, pIS099,	
GCGctcgagATGGTGAGCAAGGGCG	pIS100, pIS101	XhoI-eGFP-F
	pYW292, pIS099,	
GCGggtaccGGCAACAAGGATCACTGG	pIS100, pIS101	HMX1-KpnI-R
GAAGAGCACTCCACACGgtaatcctgGTCATTC		
TTGTCATGCTGC	pYW300	HMX1-N+6-F
GCAGCATGACAAGAATGACcaggattacCGTG		
TGGAGTGCTCTTC	pYW300	HMX1-N+6-R
CATGCGGTACCAAAAACGTTCTGCTACTA		Pep12-Hmx1-
GAAGAGCACTCC	pYW302	TMD-F
GGAGTGCTCTTCTAGTAGCAGAACGTTTTT		Pep12-Hmx1-
GGTACCGCATG	pYW302	TMD-R
CGTTTGACCGAACCCTCGGAAGACGAATT		
TTTTGG	pYW283, pYW302	ALFA-Pep12-F

Plasmids	SOURCE	IDENTIFIER
gene deletion(/insertion) cassette HIS3	9	pFA6a-HIS3
gene deletion(/insertion) cassette TRP1	9	pFA6a-TRP1
	Gift from Scott D.	pFA6a-3xFLAG-
gene insertion cassette 3xFLAG::HIS3	Emr	HIS3
empty centromer vector HIS3	10	pRS413
empty centromer vector TRP1	10	pRS414
empty centromer vector LEU2	10	pRS415
empty centromer vector URA3	10	pRS416
yeast integrative plasmid (YIp) URA3	10	pRS406
pRS416 - <i>VPS4-3xHA</i>	6	pDT82
pRS416 - VPS4-3xHA ^{E233Q} (endogenous 5' and 3')	6	pDT84
pRS415 - VPS4 (endogenous 5' and 3')	6	pOS014
pRS415 - VPS4 ^{E233Q} (endogenous 5' and 3')	6	pOS015
pRS416 - 3xFLAG-ORM2 (endogenous 5' and 3')	4	pOS129
pRS415 - GFP-ORM2 (endogenous 5' and 3')	4	pOS173

pRS415 - P ^{TDH3} -GFP-ORM2	this study	pOS200
pRS415 - P ^{TPI1} -mCherry-CPS1	6	pOS246
pRS416 - ORM2 (endogenous 5' and 3')	4	pVB17
pRS416 - GFP-ORM2 (endogenous 5' and 3')	4	pYW006
pRS416 - 3xFLAG-ORM2 ^{K25R,K33R} (endogenous 5'		
and 3')	4	pYW015
pRS416 - <i>GFP-ORM2^{K25R,K33R}</i> (endogenous 5' and		
3')	4	pYW028
pRS416 - TUL1 (endogenous 5' and 3')	4	pYW060
pRS415 - DSC2 (endogenous 5' and 3')	this study	pYW133
pRS415 - <i>DSC2</i> ^{Y53A,R54A} (endogenous 5' and 3')	this study	pYW150
pRS416 - 3xFLAG-HMX1 (endogenous 5' and 3')	this study	pYW185
pRS415 - DSC2-ALFA (endogenous 5' and 3')	this study	pYW212
pRS416 - <i>eGFP-HMX1</i> (264-317) (endogenous 5'		
and 3')	this study	pYW242
pRS416 - <i>eGFP-HMX1</i> (284-317) (endogenous 5'		
and 3')	this study	pYW243
pRS416 - <i>P</i> ^{TDH3-} <i>eGFP-HMX1</i> (284-317)	•	
(endogenous 3')	this study	pYW255
pRS416 - P^{TDH3} -eGFP-ALFA-HMX1(264-317)	•	
(endogenous 3')	this study	pYW257
pRS416 - P ^{TDH3-} eGFP-ALFA-HMX1(284-317)	•	
(endogenous 3')	this study	pYW258
pRS416 - P ^{TDH3-} eGFP-ALFA-HMX1 ^{K265,269,282R}	•	
(264-317) (endogenous 3')	this study	pYW261
pRS416 - P ^{TDH3-} pHluorin-ALFA-HMX1(264-317)	•	
(endogenous 3')	this study	pYW263
pRS416 - P ^{TDH3-} eGFP-ALFA-PEP12 (endogenous	•	
3')	this study	pYW283
pRS416 - <i>P</i> ^{TDH3-} <i>eGFP-ALFA-HMX1</i> (264-317)	•	
T292_V293insVIL (endogenous 3')	this study	pYW290
pRS416 - yZ3EV- P ^{Z3EV} -mNeonGreen-ALFA-	this study; gift from	
HMX1	D. Botstein	pYW292
pRS416 - <i>P</i> ^{TDH3-} <i>eGFP-ALFA-HMX1</i> (264-317)		
T292_V293insVILVIL (endogenous 3')	this study	pYW300
pRS416 - P ^{TDH3} -eGFP-ALFA-PEP12(2-263)-	•	
<i>HMX1</i> (284-317) (<i>HMX1</i> 3')	this study	pYW302
pRS416 - P ^{TDH3} -ALFA-HMX1 (endogenous 3')	this study	pYW311
pRS410 - 3xFLAG-HMX1 (endogenous 5' and 3')	this study	pAP117
pRS418 - VPS4 ^{E233Q} (endogenous 5' and 3')	⁶ , this study	pAP118
pRS416 - yZ3EV- P ^{Z3EV} -mNeonGreen-ALFA-	this study; gift from	
<i>HMX1</i> (264-317)	D. Botstein	pIS099
pRS416 - vZ3EV- P ^{Z3EV} -mNeonGreen-ALFA-	this study; gift from	1
<i>HMX1</i> (264-317) T292 V293insVIL	D. Botstein	pIS100
pRS416 - yZ3EV- P ^{Z3EV} -mNeonGreen-ALFA-	this study; gift from	1
HMX1(264-317) T292 V293insVILVIL	D. Botstein	pIS101
	this study: gift from	*
pRS414 - yZ3EV- P ^{Z3EV} -eGFP-ALFA-HMX1	D. Botstein	pKS022
pRS415 - DSC2 ^{Y53A,R54A} -ALFA (endogenous 5' and		*
3')	this study	pNM003
pRS415 - Vps4-3xHA-mCherry	11	pSS52

pRS414- P^{TDH3} -mCherry-CPS1 $^{\Delta 250}$	12	pMM29
GAL1pr::natNT2	gift from F. Föhlich	pYM-N23
		RRID:
YIP(TRP1)-MNN9pr-MNN9-mTagBFP2	gift from Z. Xie	Addgene_133662
		RRID:
YIP(URA3)-SEC7pr-SEC7-6xmCherry	⁴ ; gift from B. Glick	Addgene 105267

Software and Algorithms	SOURCE	IDENTIFIER
GIMP	GIMP Development Team	Version 2.10
Inkscape	Inkscape Project	Version 1.3.1
Proteome Discoverer 2.5	Thermo Scientific	Version 2.5
Excel	Microsoft	Version 16.0.5422.1000
Word	Microsoft	Version 16.0.5413.1000
ImageJ	Schneider et al. 2012 ¹³	Version 1.54g
Visi View	Visitron	Version 2.1.4
Leica Application Suite X (LAS X)	Leica	Version 3.9.1.28433
R Studio	Posit PBC	Version 2023.09.1 +494
PyMOL	Schrodinger LLC	Version 2.5.4
Prism	GraphPad	Version 10.1.2

Supplementary Table 3.

MD Simulation checklist

Reliability and reproducibility checklist for molecular	Yes	No	Response
dynamics simulations			(Please state where this
*All boxes must be marked YES by acceptance unless			information can be found
"Response not needed if No".			in the text)
1. Convergence of simulations and analysis			
1a. Is an evaluation presented in the text to show that the property being measured has equilibrated in the simulations (<i>e.g.</i> time-course analysis)?			The precise temporal evolution of these properties after the equilibration phase was appended. From these data, it should be possible to observe the convergence, as they are visually stable. (membrane thinning videos, and the supplementary figure 6)
1b. Then, is it described in the text how simulations are split into equilibration and production runs and how much data were analyzed from production runs?			(SI 'Supplementary Methods for MD Simulations' session, also shortly repeated in discussion)
1c. Are there at least 3 simulations per simulation condition with statistical analysis?	X		(SI 'Supplementary Methods for MD Simulations' session)
1d. Is evidence provided in the text that the simulation results presented are independent of initial configuration?			For the three or more repeats we tried, provided that the membrane is stabilized and the protein is not affected by inhomogeneity near the edges, the results are reproducible. (SI 'Supplementary Methods for MD Simulations' session)
2. Connection to experiments	1	1	
2a. Are calculations provided that can connect to experiments (<i>e.g.</i> loss or gain in function from mutagenesis, binding assays, NMR chemical shifts, J-couplings, SAXS curves, interaction distances or FRET distances, structure			From a variety of arbitrary starting points, we can demonstrate the thinning of the membrane and the binding of truncated lipids,

factors, diffusion coefficients, bulk modulus and other mechanical properties, <i>etc.</i>)?		which align with the experimental findings and are consistent with the substrate recognition hypothesis. (discussion on membrane thinning in the main text and on truncated lipid association in SI)
3. Method choice		
3a. Do simulations contain membranes, membrane proteins, intrinsically disordered proteins, glycans, nucleic acids, polymers, or cryptic ligand binding?		(SI 'Supplementary Methods for MD Simulations' session)
3b. Is it described in the text whether the accuracy of the chosen model(s) is sufficient to address the question(s) under investigation (e.g. all-atom vs. coarse-grained models, fixed charge vs. polarizable force fields, implicit vs. explicit solvent or membrane, force field and water model, etc.)?		The most accurate setting is employed, utilising all- atom, explicit solvent and membrane models. The force fields have fixed charge, as previous work in our group demonstrated that the polarizable force fields would be too slow to converge even for a simpler system and the secondary structure also risks to break. (details in SI for the sake of reference limits)
3c. Is the timescale of the event(s) under investigation beyond the brute-force MD simulation timescale in this study that enhanced sampling methods are needed?		The converged statistics were obtained within a reasonable timeframe using the classical molecular dynamics (MD) method. Enhanced sampling was not employed, as it would be necessary to conduct connected classical MD in order to recover the energy landscape in such a case. (SI 'Supplementary Methods for MD Simulations' session)
If YES , are the parameters and convergence criteria for the enhanced sampling method clearly stated?		
If NO , is the evidence provided in the text?	\boxtimes	(SI 'Supplementary Methods for MD Simulations' session)
4. Code and reproducibility		 T • • 1 • . •
4a. Is a table provided describing the system setup that includes simulation box dimensions, total number of atoms,		Lipid compositions are described. The remaining

total number of water molecules, salt concentration, lipid composition (number of molecules and type)?			numbers are a consequence of the box described in SI. (SI 'Supplementary Methods for MD Simulations' session)
4b. Is it described in the text what simulation and analysis software and which versions are used?			(SI 'Supplementary Methods for MD Simulations' session)
4c. Are other parameters for the system setup described in the text, such as protonation state, type of structural restraints if applied, nonbonded cutoff, thermostat and barostat, etc.?			The protocol to obtain the protonation state and structural restraints are described. (SI 'Supplementary Methods for MD Simulations' session)
4d. Are initial coordinate and simulation input files and a coordinate file of the final output provided as supplementary files or in a public repository?	\boxtimes		10.5281/zenodo.13889274 10.5281/zenodo.13889576
4e. Is there custom code or custom force field parameters?		\mathbf{X}	
If YES , are they provided as supplementary files or in a public repository?			

Supplementary Methods

Supplementary Methods for MD Simulations

We retrieved the Dsc2 starting structure from the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/, accession: AF Q08232 F1). We focused the analysis on the rhomboid like domain of Dsc2, and therefore omitted an β -sheet (aa 148 – 182) that connects TM3 with TM4 as well as the undefined C-terminal portion (aa 241 - 322). Omitting these regions also accelerate the convergence of the simulation. Preparation and equilibration for the systems packed in membranes and aqueous phases are detailed in SI. Preparation of the initial structure, comprising the protonation and minimization were conducted in the MOE platform (Molecular Operating Environment, version 2022.02, Molecular Computing Group Inc, Montreal, Canada). Dsc2 was oriented in the membrane using PPM 2.0¹⁴ and embedded in a lipid bilayer comprising 45% POPC, 45% POPE and 10% cholesterol with the CHARMM-GUI Membrane Builder¹⁵. A 75 Å*75 Å membrane with a default 22.5 Å aqueous layer was added to ensure the efficient embedding of the Dsc2 and minimize artificial interference among neighboring images during the simulation. The resulting system was parameterized with LEaP from the AmberTools23¹⁶ using TIP3P¹⁷, FF14SB¹⁸ and Lipid21¹⁹ as force fields for water, protein and lipids. We equilibrated the system by minimizing gradually all hydrogen atoms, heavy atoms of water, lipids and the protein. The system was then heated up from 100K to 300K, minimized to cool down before being heated up again to 300K. Finally, we equilibrated the system with 1ns isotropic position scaling followed by 10ns pressure scaling, as was suggested by previous studies ^{20,21}. After equilibration the system was simulated for 100ns with the Langevin thermostat and the Berendsen barostat. Asymmetric lipids are prepared by truncating the fatty acid chains following the previously described protocol ²². To sample the preferred association sites of truncated lipids, we simulated an excessive amount of 6% truncated lipids close to but outside the first solvation shell of the dsc2. To further retrieve the statistics on associated lipids, we simulate the systems with single PC (C10:0;C16:0) or PC (C10:1;C16:0) at the sampled binding site, corresponding to 1%-2% of the total lipids sampled similar to the physiological condition. These systems with asymmetric lipids are simulated for 200ns, leading to an aggregated trajectory of 140ns*3 per system before inhomogeneity at the edges of membrane disrupts the equilibrium. The last 40ns with stabilized binding statistics are used to plot the distance between dsc2 and truncated lipids. The equilibrated system was simulated with periodic boundary conditions using the SHAKE algorithm for hydrogens under constant pressure for 100ns with amber 22¹⁶. For non-bonding interactions a 8 Å cut off was applied and long-range electrostatics were estimated with the particle mesh Ewald method ²³.

We took the last 40ns where the simulation converges to obtain statistics on membrane thickness changes with the membplugin ²⁴ and visualized the dynamics with VMD ²⁵.

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