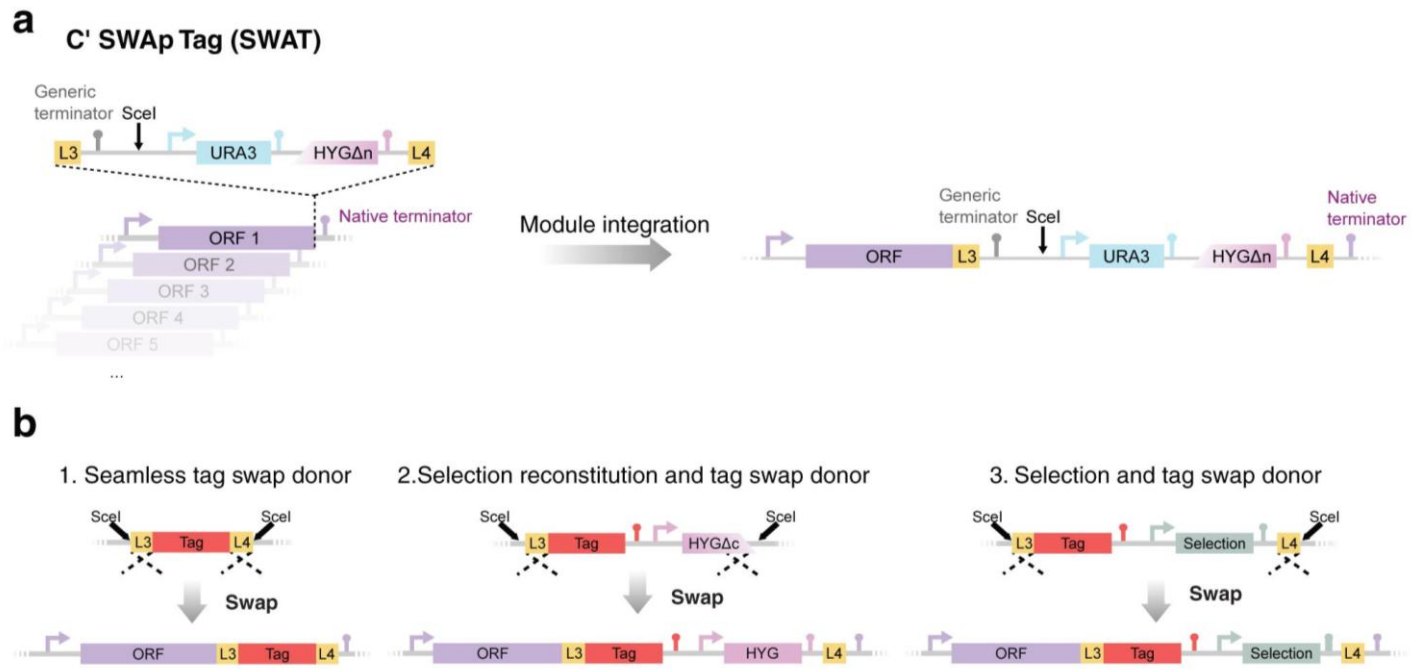
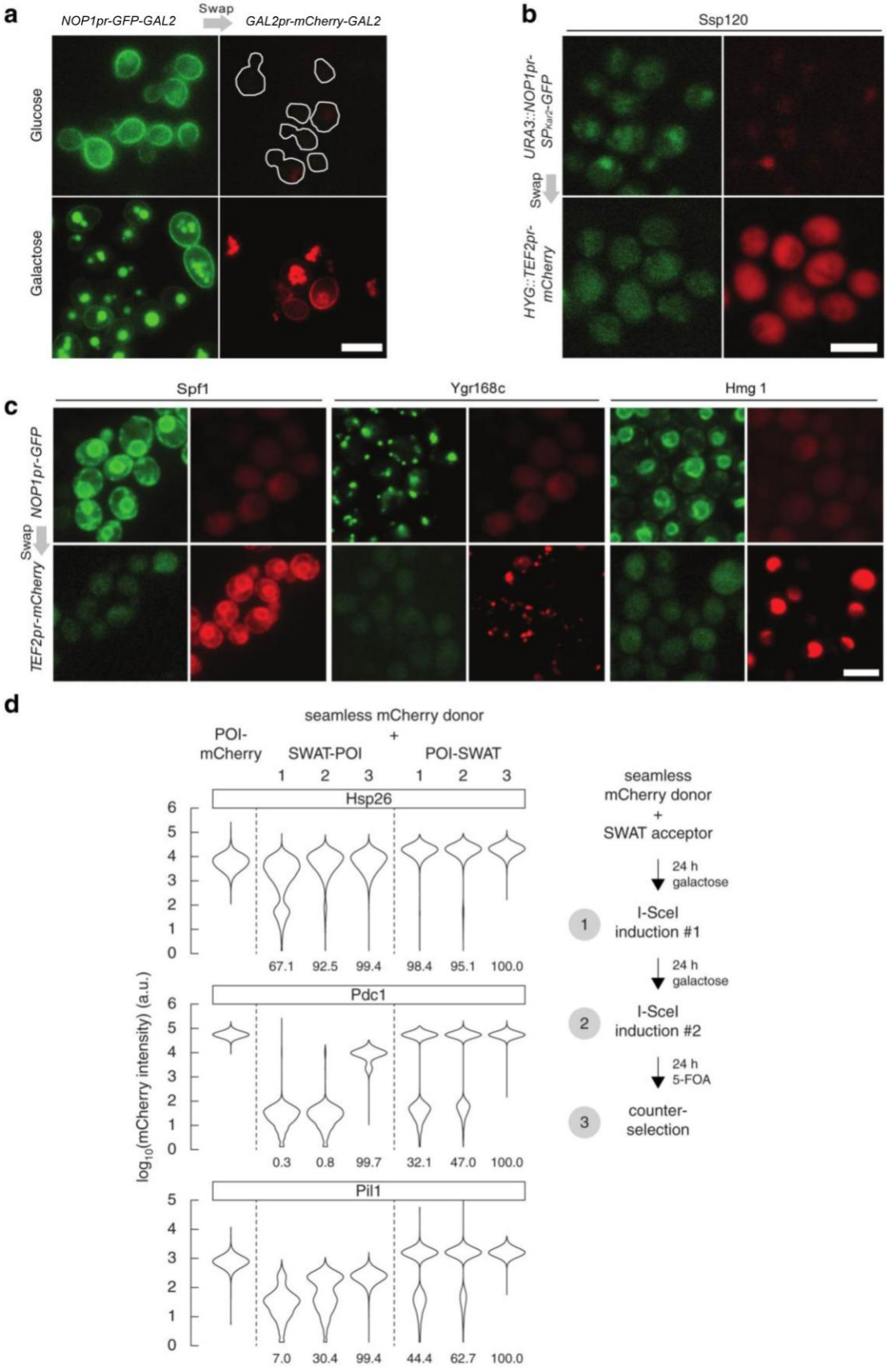


Supplementary Figure 1

The SWAp-Tag (SWAT) strategy is also suitable for C′ protein tagging and swapping.

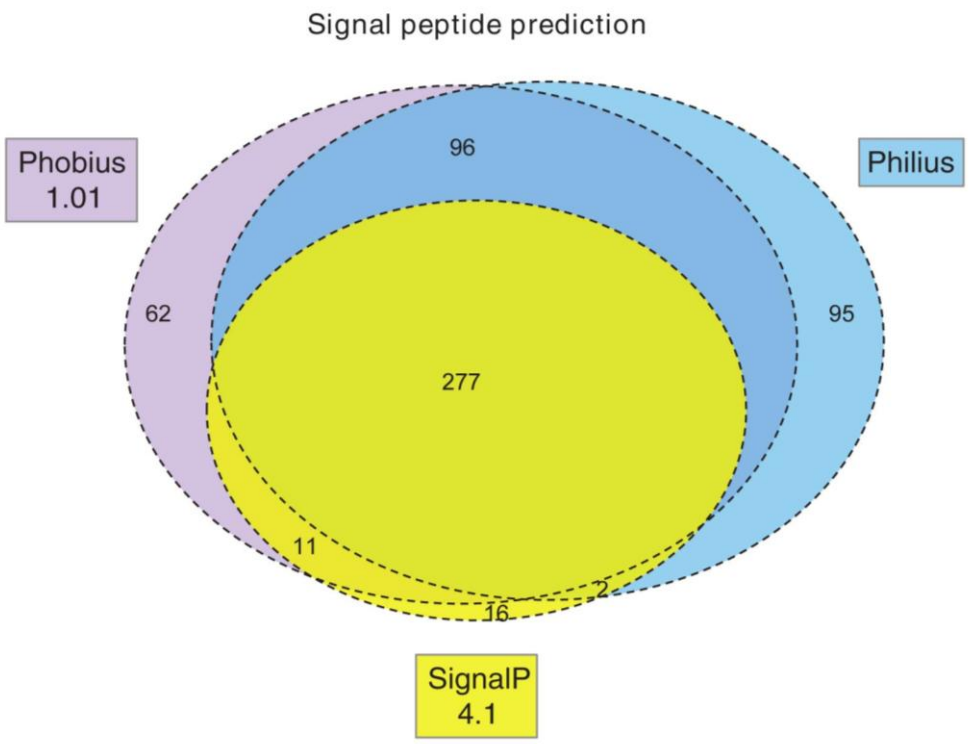
(**a**) The C' Swap-tag (SWAT) acceptor tagging module contains several components: the restriction site for the I-SceI endonuclease (SceI), a *URA3* selection marker (URA3), a truncated Hygromycin B selection marker (HYG⊿n), and a generic terminator, and are flanked by two generic sequences for homologous recombination that also serve as linkers for protein fusion (L3 and L4). (**b**) Donor plasmid features (top), which, after swapping, result in several types of tagged-gene libraries (bottom).


a
NOP1pr-GFP-GAL2
Swap
GAL2pr-mCherry-GAL2
Glucose
Galactose
b
Ssp120
URA3::NOP1pr-SPKar2-GFP
Swap
HYG::TEF2pr-mCherry
c
Spf1
Ygr168c
Hmg 1
NOP1pr-GFP
Swap
TEF2pr-mCherry
d
seamless mCherry donor
+
POI-mCherry
SWAT-POI
POI-SWAT
1 2 3 1 2 3
Hsp26
Pdc1
Pil1
log10(mCherry intensity) (a.u.)
67.1 92.5 99.4 98.4 95.1 100.0
0.3 0.8 99.7 32.1 47.0 100.0
7.0 30.4 99.4 44.4 62.7 100.0
seamless mCherry donor + SWAT acceptor
24 h galactose
1 I-SceI induction #1
24 h galactose
2 I-SceI induction #2
24 h 5-FOA
3 counter-selection

Supplementary Figure 2

Several types of tag-swap strategies can be implemented to achieve desired protein-tagging states.

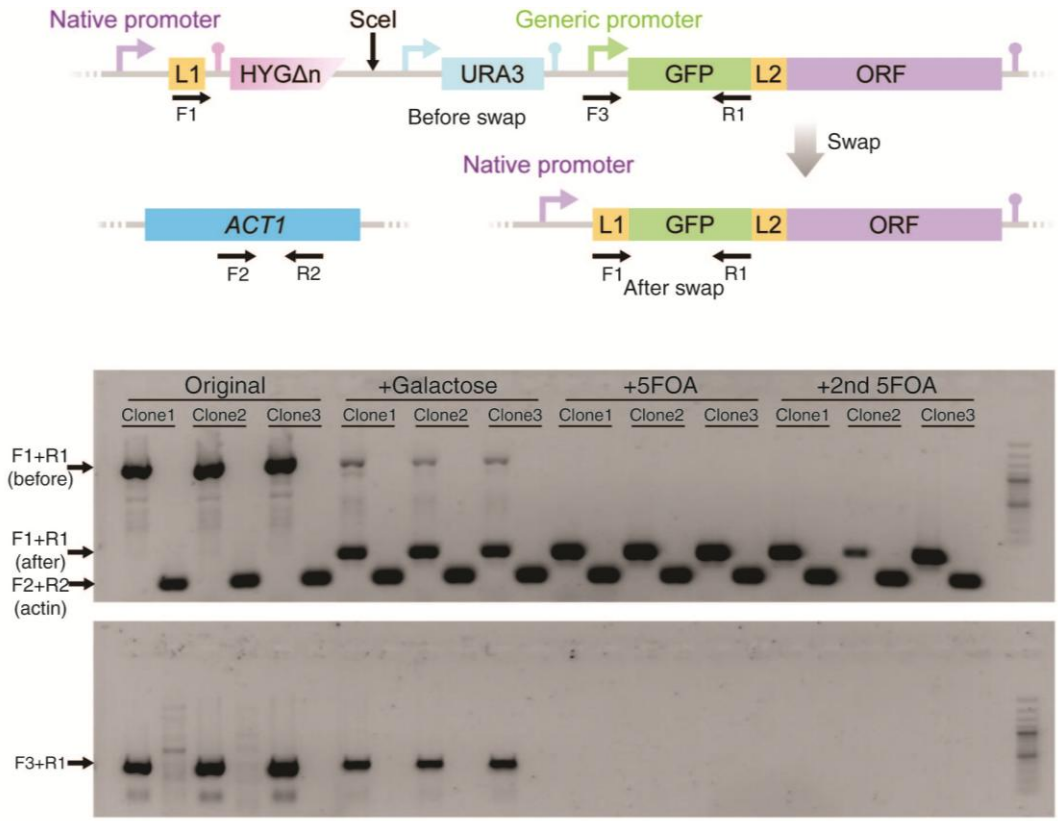
(**a**) An example for a seamless tag swap. The galactose induced gene *GAL2* was tagged with an N' swat module containing a GFP (SWAT-GFP), and was replaced by a seamless mCherry donor plasmid. GFP-Gal2 that is under control of the constitutive *S.p NOP1* promoter in the SWAT-GFP cassette is expressed under both glucose and galactose containing media. Following swapping the native *GAL2* promoter is restored leaving a seamless mCherry tag (N'). All four images are overlay images of both GFP and mCherry channels, to portray that only the GFP tag is seen before the swap, and only the mCherry tag in seen post swap. (**b**) An example for selection reconstitution and tag swap. *SSP120* was tagged with an N' swat module containing a GFP and the Kar2 signal peptide (SWAT-SP-GFP), and was replaced by a HYGΔc::*TEF2*pr-mCherry donor plasmid. After swapping using Hygromycin the SP-GFP module was replaced by an mCherry tag with no SP, resulting in the loss of the GFP signal (left) and the expression of a mis-localized, cytoplasmic, mCherry-Ssp120 fusion protein. (**c**) An example for selection and tag swap. The three indicated genes were N' tagged with the SWAT-GFP module, replaced by *NAT::Tef2pr-mCherry* donor plasmid. After swapping the GFP tag was replaced with the mCherry, resulting in the loss of the GFP signal (left) and expression of the mCherry fused proteins (right). (**d**) Comparison of seamless tagging efficiency with N'-SWAT and C'-SWAT acceptor sites. Strains carrying a GAL1pr-I-SceI construct and expressing the indicated proteins of interest (POI) tagged with N'-SWAT (SWAT-POI) or C'-SWAT (POI-SWAT) acceptor modules were transformed with seamless mCherry donor plasmids. Distributions of single cell fluorescence intensities were measured with flow cytometry the indicated step of the swapping procedure (1-3). The percentage of cells with fluorescence above background is indicated under the plots. Fluorescence intensities of strains expressing POI-mCherry fusions are shown for comparison. Scale bars, 5 μm.



Supplementary Figure 3

Computational prediction of *Saccharomyces cerevisiae* proteins bearing a signal peptide.

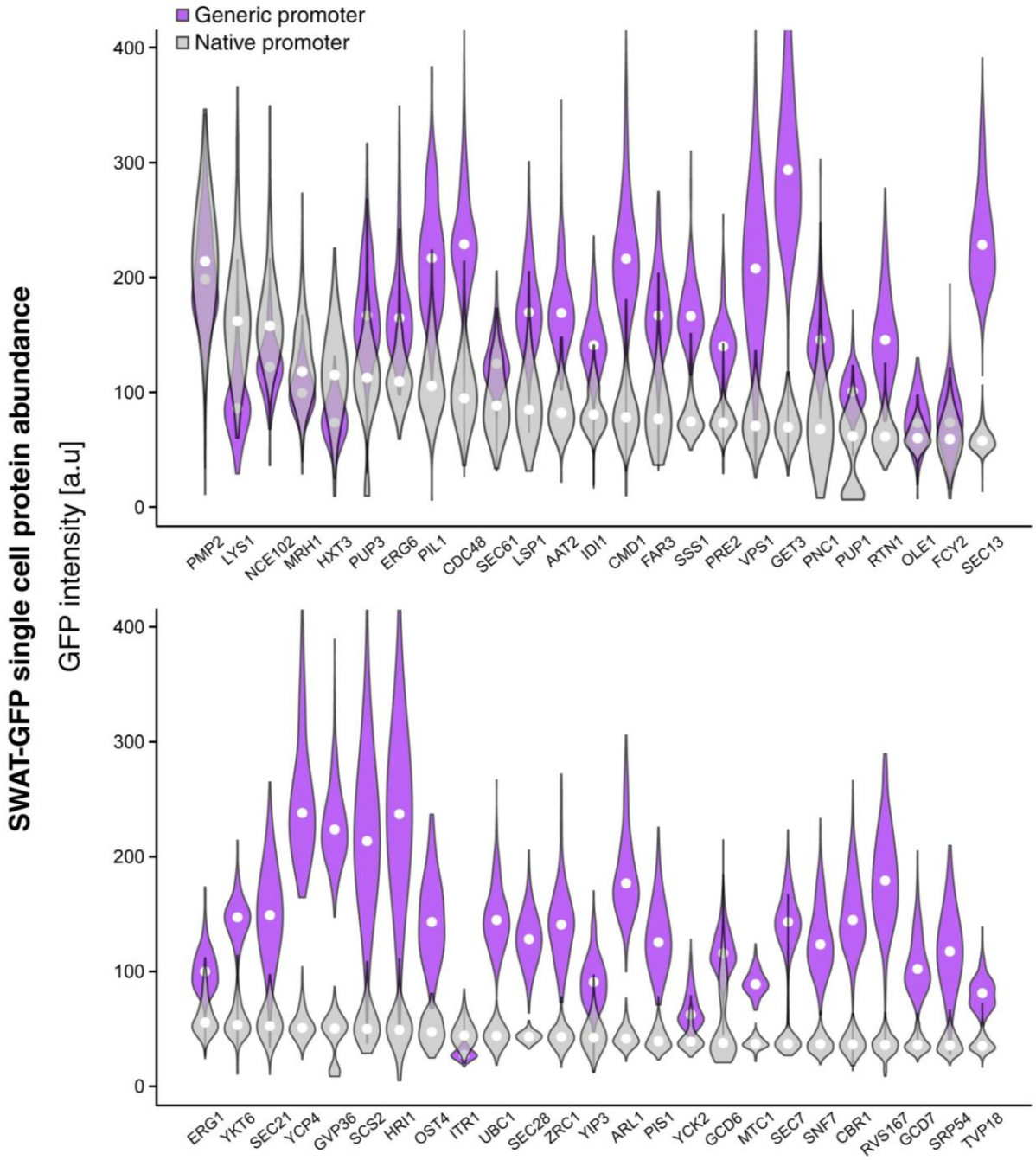
In order to perform correct N' tagging and swapping of proteins containing an N' signal peptide, prediction of the presence and cleavage site of this motif was performed for all yeast proteins using three prediction algorithms- SignalP, Phobius and Philius. The Venn diagram illustrates that a signal peptide was predicted by all programs for 277 proteins, while some predictions were only predicted by some of the programs. We tagged all proteins predicted to contain a SP by at least two programs with a SWAT module that contained the Kar2 SP.



Supplementary Figure 4

Seamless swapping of SWAT-GFP cassettes is validated by PCR.

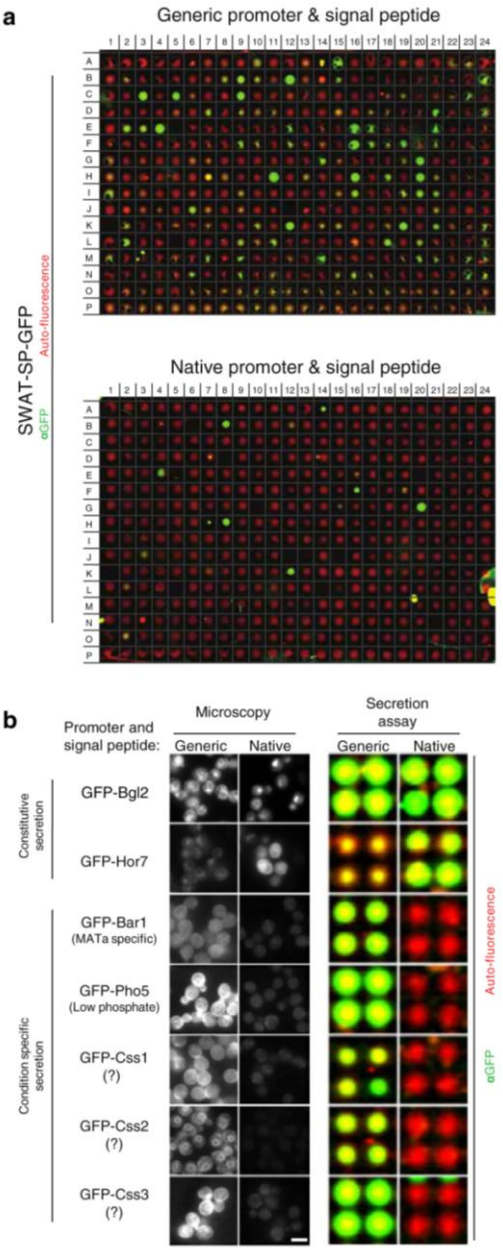
Validation (check) PCRs were performed for three picked clones containing a SWAT-GFP tagged *HMG2* gene before and after swapping with a seamless GFP donor plasmid. Induction of the I-SceI endonuclease by plating on galactose, results in a mixture of un-swapped and swapped conditions. Further application of two rounds of negative selection by 5FOA, completely abolishes the un-swapped condition, leaving only the swapped one demonstrating that this is a robust process that gives rise to homogenous populations of cells.



Supplementary Figure 5

Seamless swapping of SWAT-GFP cassettes is an efficient and accurate process.

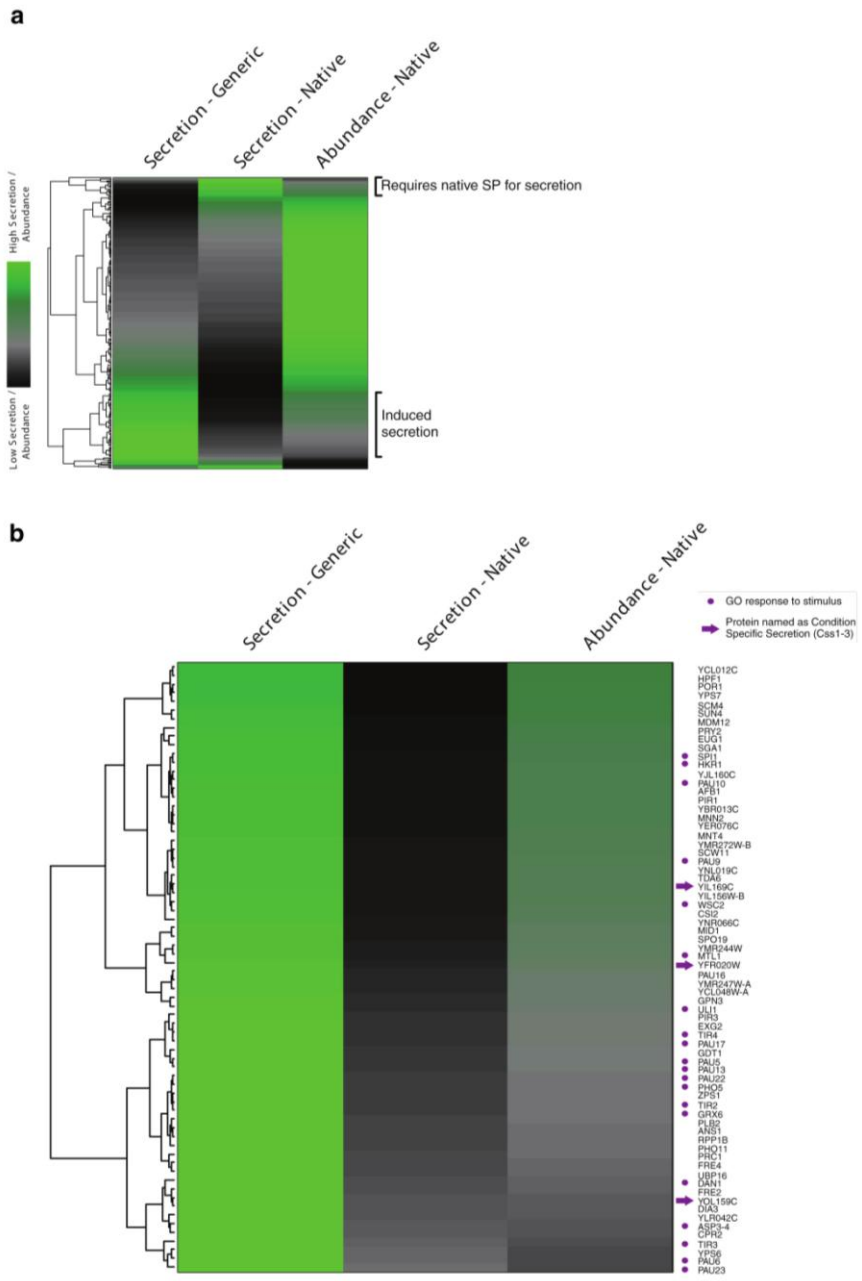
Seamless GFP swap results in a homogenous population. Distribution of single cell protein abundance in strains of the SWAT-GFP library before (generic promoter, purple) and after (native promoter, grey) seamless swapping. Protein abundance was measured by microscopy, and strains were selected randomly, spanning a wide range of protein abundance under native regulation. White circles show the medians; polygons represent density estimates of data and extend to extreme values.



Supplementary Figure 6

Systematic characterization of signal peptide–guided proteins by the SWAp-Tag system uncovers three new condition-specific secreted proteins.

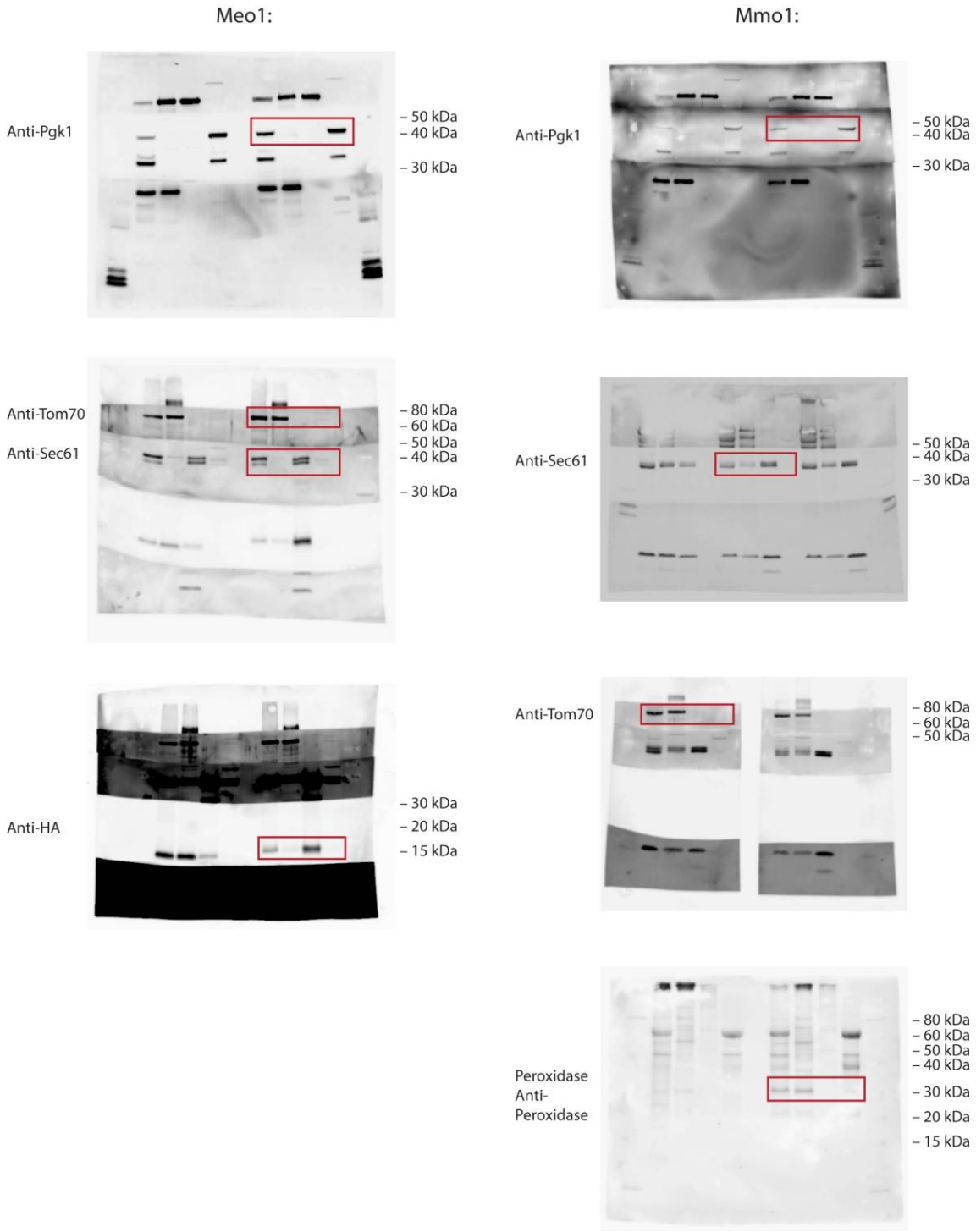
(**a**) Protein secretion was assayed for all SWAT-SP-GFP strains either under regulation of a generic (top) or native (bottom) promoter and signal peptide. Images are an overlay of the αGFP antibody (green) and auto-fluorescence to verify presence of the colony (red). For the complete secretion measurements see Supplementary Table 5. (**b**) Some proteins, such as Bgl2, show high levels of expression and secretion under both generic and native regulation, while others, such as Hor7 show higher expression and secretion levels under native regulation. A subset of proteins are only secreted in unique conditions and therefore were only expressed and secreted under regulation of the generic promoter such as BAR1 (mating type specific) and Pho5 (phosphate depletion). Three uncharacterized proteins behaved in the same manner and were therefore classified as condition specific secreted, and were named Css1, Css2, and Css3. Images of the secretion assay (right) are as described for panel a, with four repeats for each gene. Scale bars, 5 µm.



Supplementary Figure 7

Clustering of protein abundance and secretion levels identifies conditionally expressed and secreted proteins.

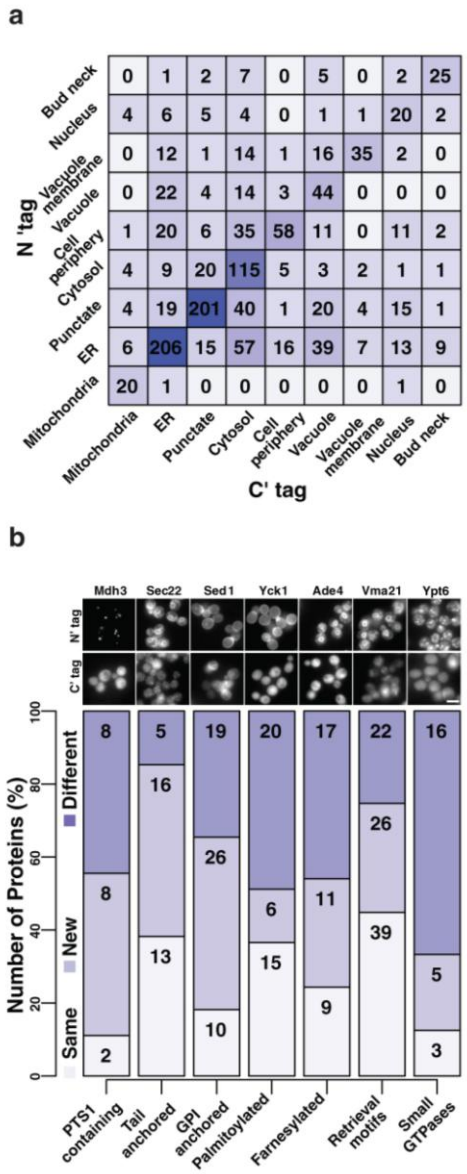
(**a**) Clustering was performed for three parameters measured for the SWAT-SP-GFP library: Protein abundance under native regulatory factors (promoter and signal peptide) and protein secretion levels under generic or native regulation (array before or after seamless GFP swap). Protein abundance was measured by microscopy and given as median GFP intensity. Secretion level was measured by western blot and given as ratio of GFP and auto-fluorescence. (**b**) A cluster of proteins presenting high secretion levels under generic regulation and low secretion and intra-cellular protein abundance under their native regulation. This cluster ("Induced secretion" in panel a) shows a high enrichment of "response to stimulus" GO annotation (purple dots). This enrichment indicates that many proteins may be expressed and secreted under specific conditions. Three unstudied proteins (purple arrows) were found in this cluster and were named as condition specified secretion (Css1-3) proteins.



Supplementary Figure 8

Original images of western blots shown in **Figure 4e**.

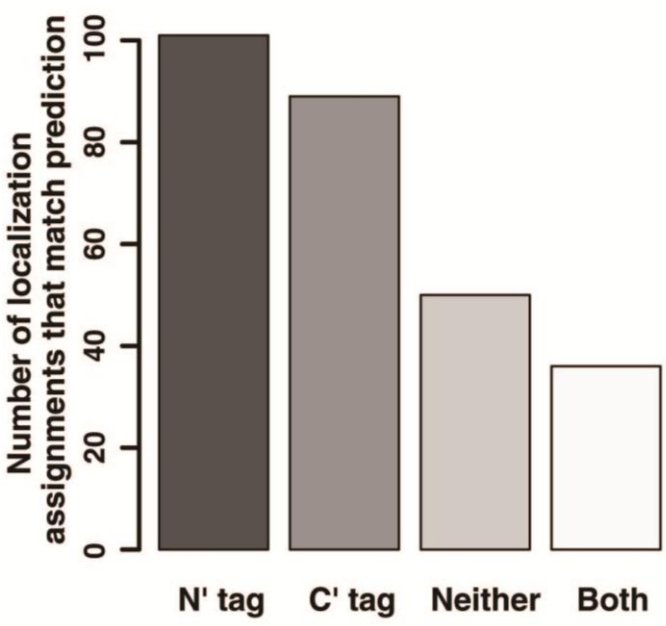
Red boxes indicate approximate image sections used for the figure.



Supplementary Figure 9

The N′ SWAT-GFP library complements the previous C′-tagged GFP library.

(**a**) Table demonstrating the distribution of localization differences between N′ and C′ tagging reveals that the most prevalent cases are ones where tagging on either side conferred a cytosolic localization, possibly due to interference of the tag with targeting signals, while the other showed targeting to a specific organelle. For all localization assignments please see (Supplementary Table 4). N′ tag – SWAT-GFP library, C′ tag – original GFP collection[4]. (**b**) Different localizations of proteins when tagged at their N′ or C′ are explained by interference of targeting/processing motifs by the tag. Seven groups of proteins with C′ processing signals indeed tend to have different localization assignments between the N′ and C′ tagged libraries. Images illustrate that for such proteins, while the C′ GFP tag causes an abnormal, cytosolic localization, the N′ GFP tag confers correct targeting of the proteins to organelles. Retrieval motifs include: HDEL, KDEL, KKXX, KXKXX, and XXRR. Scale bars, 5 μm.



Supplementary Figure 10

The N′ and C′ GFP libraries are complementary.

Proteins that were assigned different intracellular localizations when tagged at their N vs. C termini, were compared to GO ontology obtained from previous knowledge. It was found that ~30% comply with the N' localization, while about another 30% with the C' localization, and the rest either contain a GO term for both N' and C' assignments or for none. This demonstrates the importance of having complementary libraries for studying yeast cell biology. N' tag – SWAT-GFP library, C' tag – original GFP collection[4].

Yofe, Weill, et al., Supplementary Table 1.

SWAT tagging modules

Name	Description	N' / C' tagging	5' -3' Generic recombination sequences	Tag	Generic Promoter (N') / Terminator (C')
pST-N1	N' SWAT	N	L1 – L2	-	*S.p NOP1*
pST-N2	SWAT-GFP	N	L1 – L2	sfGFP	*S.p NOP1*
pST-N3	SWAT-SP_{Kar2}-GFP	N	L1 – L2	SP_{Kar2}-sfGFP	*S.p NOP1*
pST-N4	SWAT-SP_{Pep4}-GFP	N	L1 – L2	SP_{Pep4}-sfGFP	*S.p NOP1*
pST-N5	SWAT-SP_{Prc1}-GFP	N	L1 – L2	SP_{Prc1}-sfGFP	*S.p NOP1*
pST-N6	SWAT-$SP_{Mf\alpha1}$-GFP	N	L1 – L2	$SP_{Mf\alpha1}$-sfGFP	*S.p NOP1*
pST-N7	SWAT-GFP (*S.p EF1*pr)	N	L1 – L2	sfGFP	*S.p EF1*
pST-C1	SWAT C'	C	L3 – L4	-	*S.p CYC1*
pST-C2	GFP-SWAT	C	L3 – L4	sfGFP	*S.p CYC1*

Yofe, Weill, et al., Supplementary Table 2.

SWAT donor plasmids

Name	Description	N' / C' swapping	5' – 3' recombination sequences	Swap tag	Swap selection	Swap promoter (N') / terminator (C')	HYGΔc	Plasmid selection
pSD-N1	Donor template	N	L1 – L2	-	-	-	-	kanMX4
pSD-N2	Donor template	N	L1 – L2	-	-	-	-	*S.c MET15*
pSD-N3	Donor template	N	L1 – L2	-	-	-	-	natPP[3]
pSD-N4	HYGΔc Donor template	N	HYGΔc – L2	-	hphNT1	-	+	kanMX4
pSD-N5	HYGΔc Donor template	N	HYGΔc – L2	-	hphNT1	-	+	*S.c MET15*
pSD-N6	HYGΔc Donor template	N	HYGΔc – L2	-	hphNT1	-	+	natPP[3]
pSD-N7	HYGΔc::*TEF2*pr template	N	HYGΔc – L2	-	hphNT1	*S.c TEF2*	+	*S.c MET15*
pSD-N8	HYGΔc::*TEF2*pr- SP_{Kar2} template	N	L1 – L2	SP_{Kar2}[1]	hphNT1	*S.c TEF2*	-	napPP[3]
pSD-N9	Seamless GFP	N	L1 – sfGFPΔc	-	-	-	-	kanMX4
pSD-N10	Seamless GFP	N	L1 – sfGFPΔc	-	-	-	-	*S.c MET15*
pSD-N11	Seamless GFP	N	L1 – sfGFPΔc	-	-	-	-	natPP[3]
pSD-N12	Seamless mCherry	N	L1 – L2	mCherry	-	-	-	kanMX4
pSD-N13	Seamless mCherry	N	L1 – L2	mCherry	-	-	-	*S.c MET15*
pSD-N14	Seamless mCherry	N	L1 – L2	mCherry	-	-	-	natPP[3]
pSD-N15	NAT::*TEF2*pr-mCherry	N	L1 – L2	mCherry	natMX6	*S.c TEF2*	-	*S.c MET15*
pSD-N16	NAT::*TEF2*pr- SP_{Kar2}-mCherry	N	L1 – L2	SP_{Kar2}-mCherry[1]	natMX6	*S.c TEF2*	-	*S.c MET15*
pSD-N17	HYGΔc::*TEF2*pr-mCherry	N	HYGΔc – L2	mCherry	hphNT1	*S.c TEF2*	+	natPP[3]
pSD-N18	HYGΔc::*CYC1*pr-BFP	N	HYGΔc – L2	BFP	hphNT1	*S.c CYC1*	+	natPP[3]
pSD-N19	HYGΔc::Z4pr	N	HYGΔc – L2	-	hphNT1	Z_4EV inducible[2]	+	*S.c MET15*
pSD-N20	HYGΔc::Z4pr	N	HYGΔc – L2	-	hphNT1	Z_4EV inducible[2]	+	natPP[3]
pSD-C1	Donor template	C	L3-L4	-	-	-	-	kanMX4
pSD-C2	KAN Donor template	C	L3-L4	-	kanMX4	*S.c ADH1*	-	kanMX4
pSD-C3	HYGΔc Donor template	C	L3- HYGΔc	-	hphNT1	*S.c ADH1*	+	kanMX4
pSD-C4	Seamless sfGFP	C	L3-L4	sfGFP	-	-	-	kanMX4
pSD-C5	sfGFP::KAN	C	L3-L4	sfGFP	kanMX4	*S.c ADH1*	-	kanMX4
pSD-C6	sfGFP:: HYG	C	L3- HYGΔc	sfGFP	hphNT1	*S.c ADH1*	+	kanMX4
pSD-C7	Seamless mCherry	C	L3-L4	mCherry	-	-	-	kanMX4
pSD-C8	mCherry::KAN	C	L3-L4	mCherry	kanMX4	*S.c ADH1*	-	kanMX4
pSD-C9	mCherry:: HYG	C	L3- HYGΔc	mCherry	hphNT1	*S.c ADH1*	+	kanMX4

[1] SP_{Kar2} – a codon changed version of *S.c* Kar2 signal peptide. See full sequence in experimental procedures.

[2] Z_4EV inducible – A modified GAL4 promoter engeneered to be activated by an estradiol binding transcription factor (Z4EV), see McIsaac & Noyes et al. 2013.
[3] natPP – A Nourseothricin (NAT) selection marker was created by combining the coding sequence from natMX6 with S.c PGK1 promoter and PGK1 terminator (PP)