Supplementary information

Up-regulation of *SPS100* gene expression by an antisense RNA via a switch of mRNA isoforms with different stabilities

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Supplementary Figures and Legends

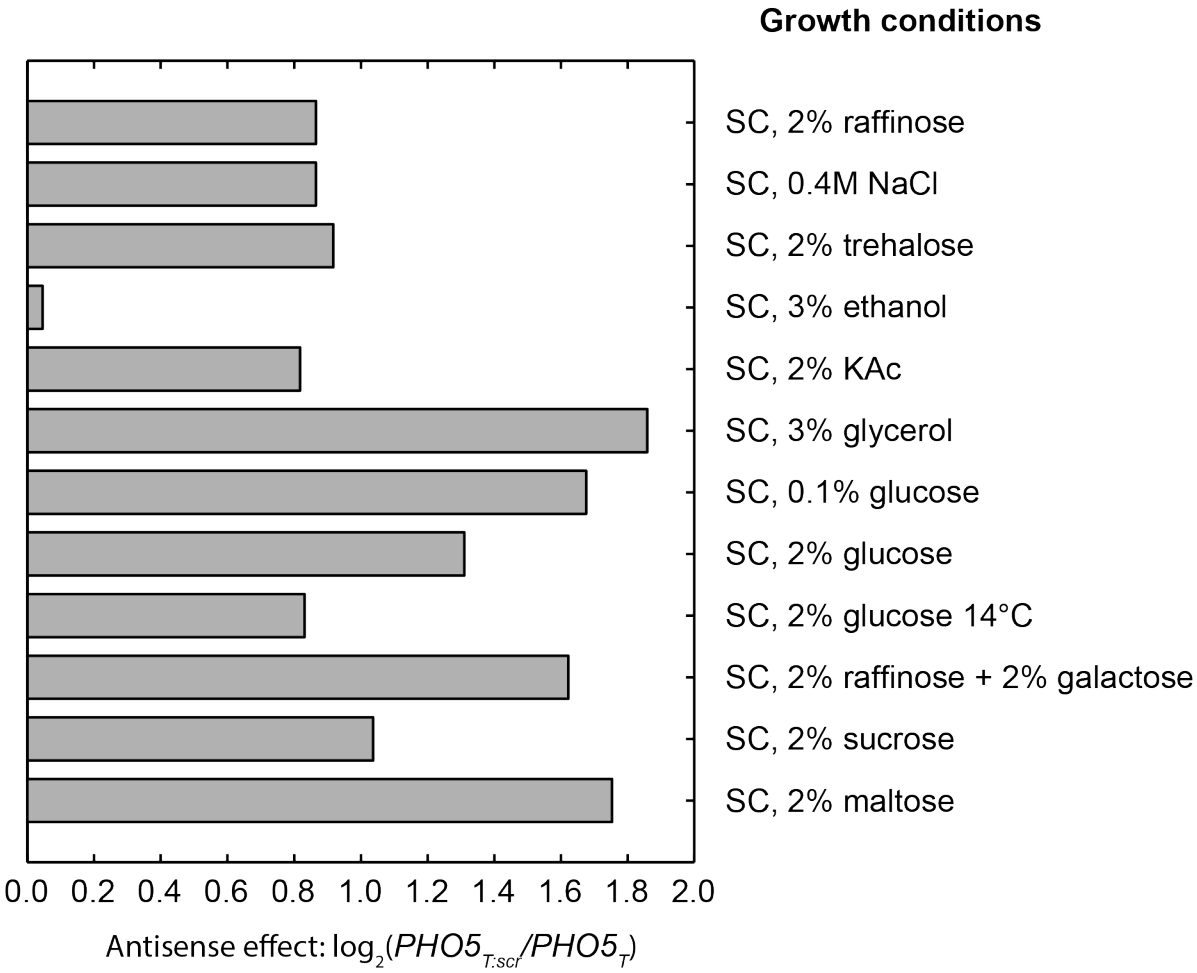

Figure S1. Overview of the antisense effect on *SPS100* across conditions. Raw data for the antisense effect on *SPS100* in all tested conditions. Whole colony fluorescence intensities were measured (Materials and Methods).

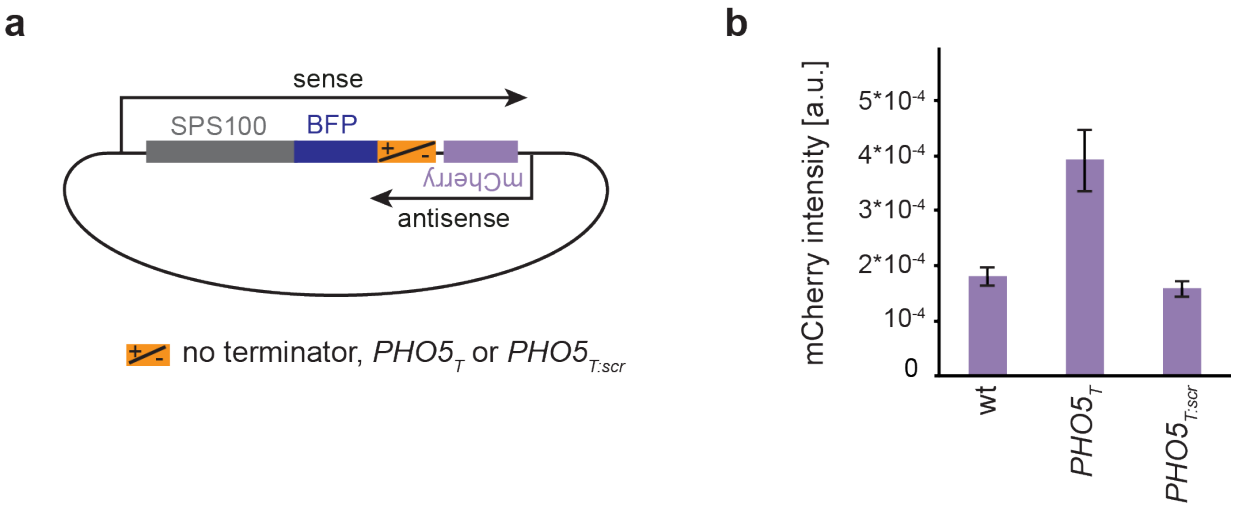

Figure S2. Test run of different versions of the antisense reporter. (**a**) Three different plasmids were tested for the antisense reporter shown in Figure 3a. SPS100-BFP wt, *PHO5*$_T$, and *PHO5*$_{T:scr}$ constructs were cloned along with the SPS100 5'- and 3'-intergenic regions (IGRs) into a centromeric plasmid and mCherry was inserted in antisense direction at the antisense initiation site. (**b**) Fluorescence intensities of the three constructs described in (a) were measured at the colony level after growth for 3 days under starvation conditions (SC, 0.1% glucose). Intensities with the *PHO5*$_T$ construct were higher. Consequently, this plasmid was used as an antisense reporter.

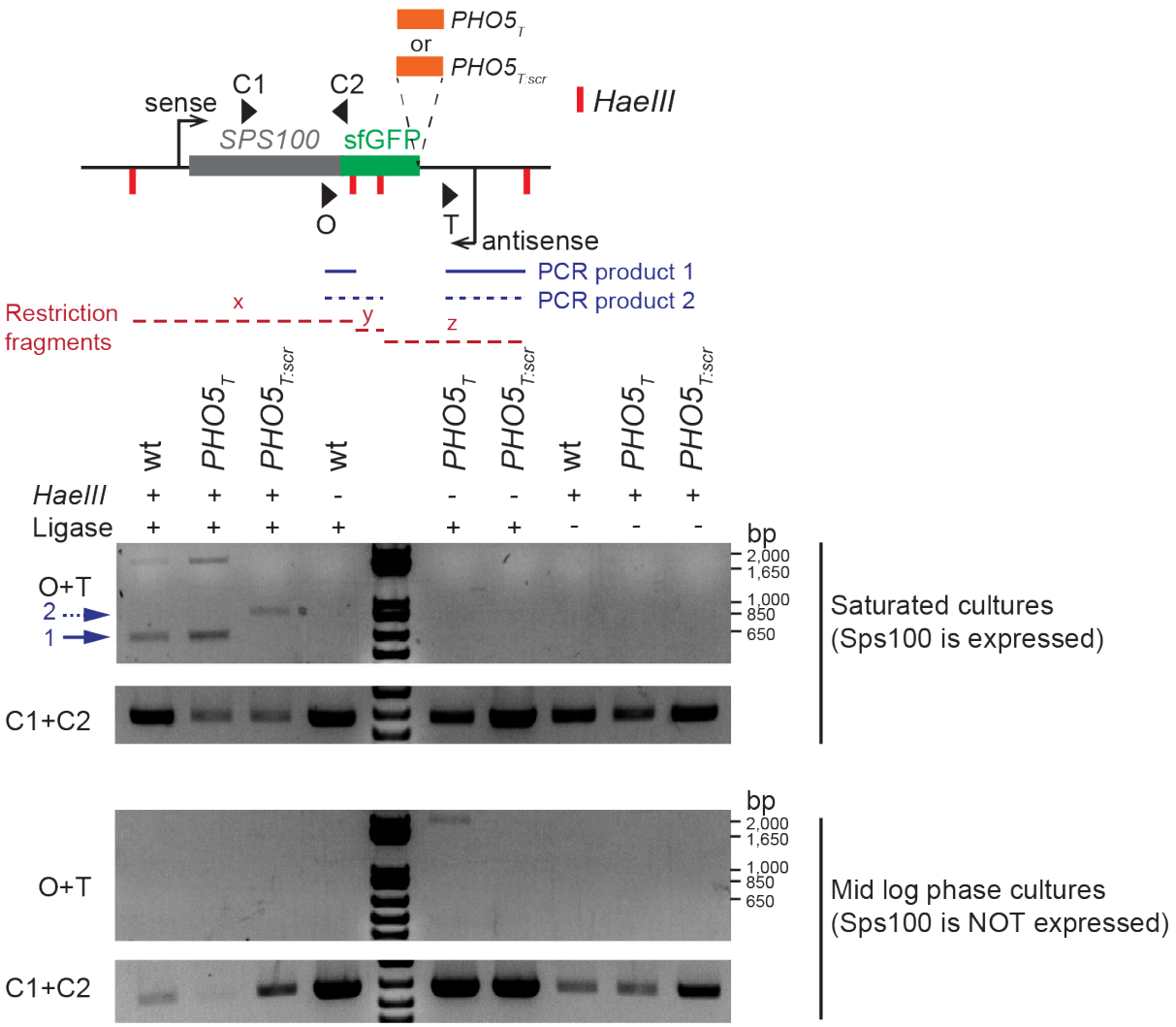

Figure S3. Gene loop formation between the *SPS100* promoter and terminator regions does not depend on antisense but on *SPS100* gene expression. Chromatin extracts from the cells with the indicated *SPS100* constructs were crosslinked with paraformaldehyde and digested with HaeIII enzyme (cut sites shown in red). Fragments after digestion were ligated after strong dilution to enrich for intra-fragment ligations. The cross-links were reversed and DNA was extracted. PCR primers used to detect ligations between different fragments after digestion are shown („O“ and „T“, same orientation of both primers), as well as control primers lying in the same fragment after restriction („C1“ and „C2“). PCR shows expected product sizes from the interaction of restriction fragments „x“ and „z“ (PCR product 1) and a shifted product size (PCR product 2) which is a result of an incomplete digest at the HaeIII site between restriction fragments „x“ and „y“. The PCR products are specific to the interaction and are absent in the controls without HaeIII or ligase (as

opposed to the control product which is present independent on digestion or ligation).

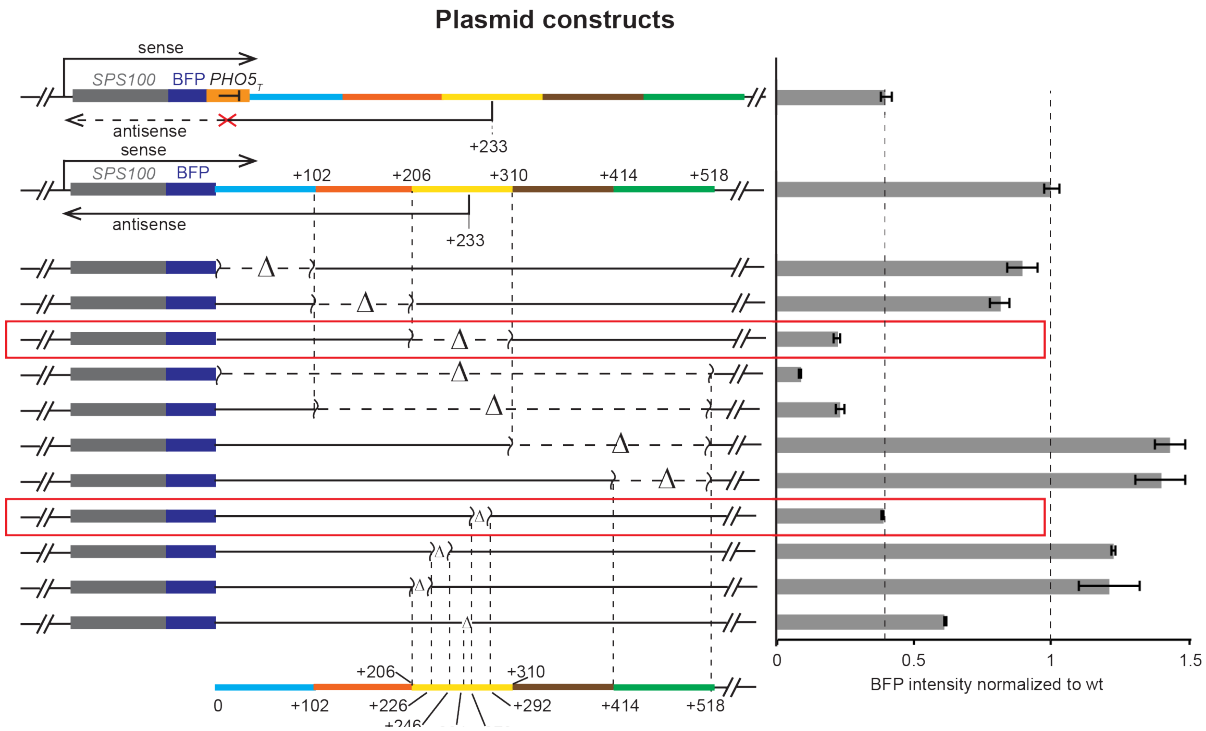

Figure S4. Initial screen for antisense-dependent regulatory regions in the SPS100 3'-IGR. Colony fluorescences of a plasmid with *SPS100-BFP* followed by either $PHO5_T$ and the *SPS100* 3'-IGR (top) or the *SPS100* 3'-IGR directly ("*wt*", second from top) were measured (barplots to the right). Next, selected portions were deleted from the 3'-IGR in the *wt* plasmids and fluorescence intensities were recorded. Coordinates of the deletions are indicated. Error bars denote standard deviations.

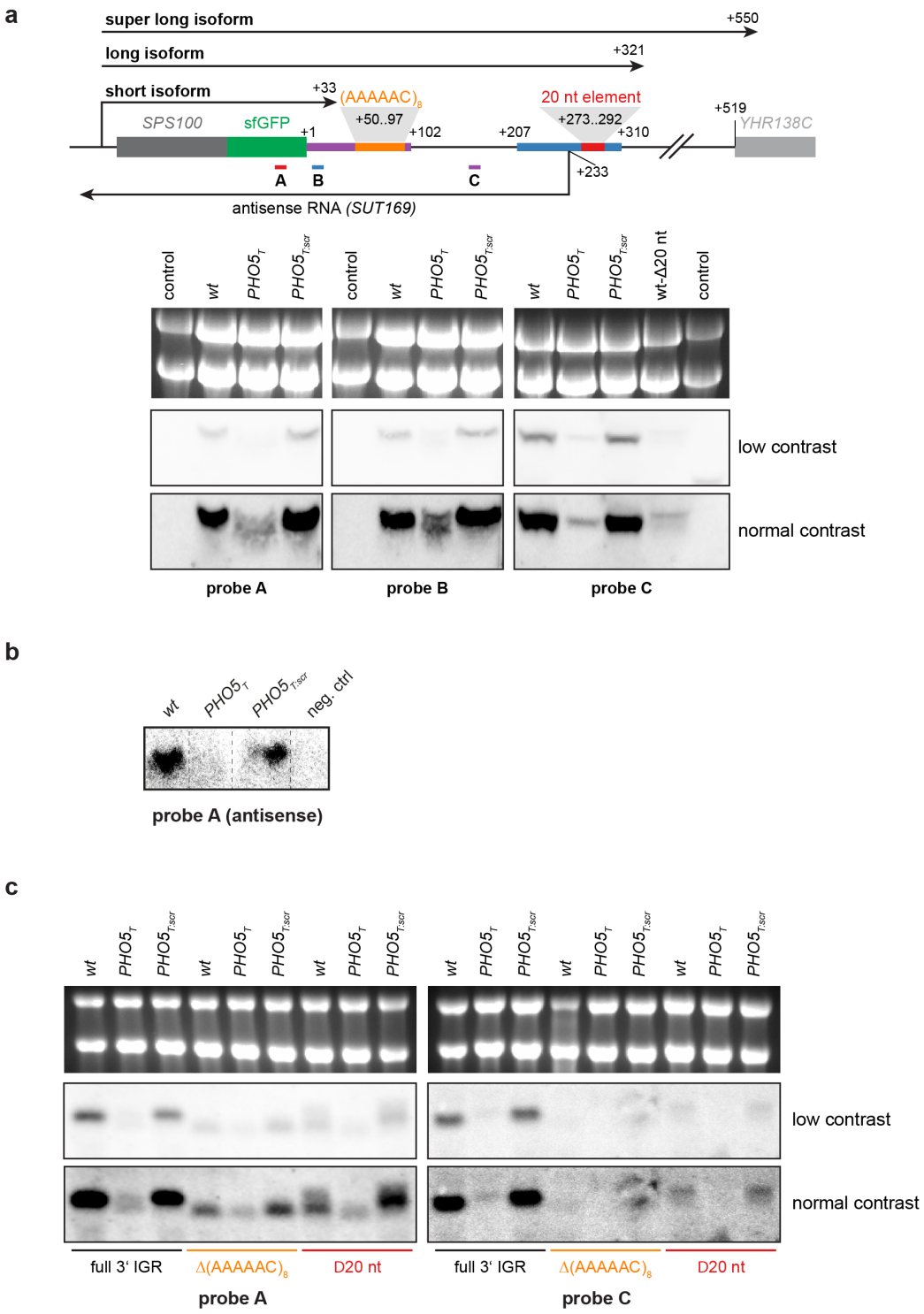

Figure S5. 3'-end mRNA isoforms of *SPS100* determined by Northern blot. Loading controls and low contrast images of Northern blots which correspond to Figures 6b (**a** in supplement) and c (**c** in supplement). (**b**) Northern blots of the *SPS100* strains with the probe to the *SUT169*.

Comparison of sense and antisense levels up- and downstream of *PHO5*$_T$

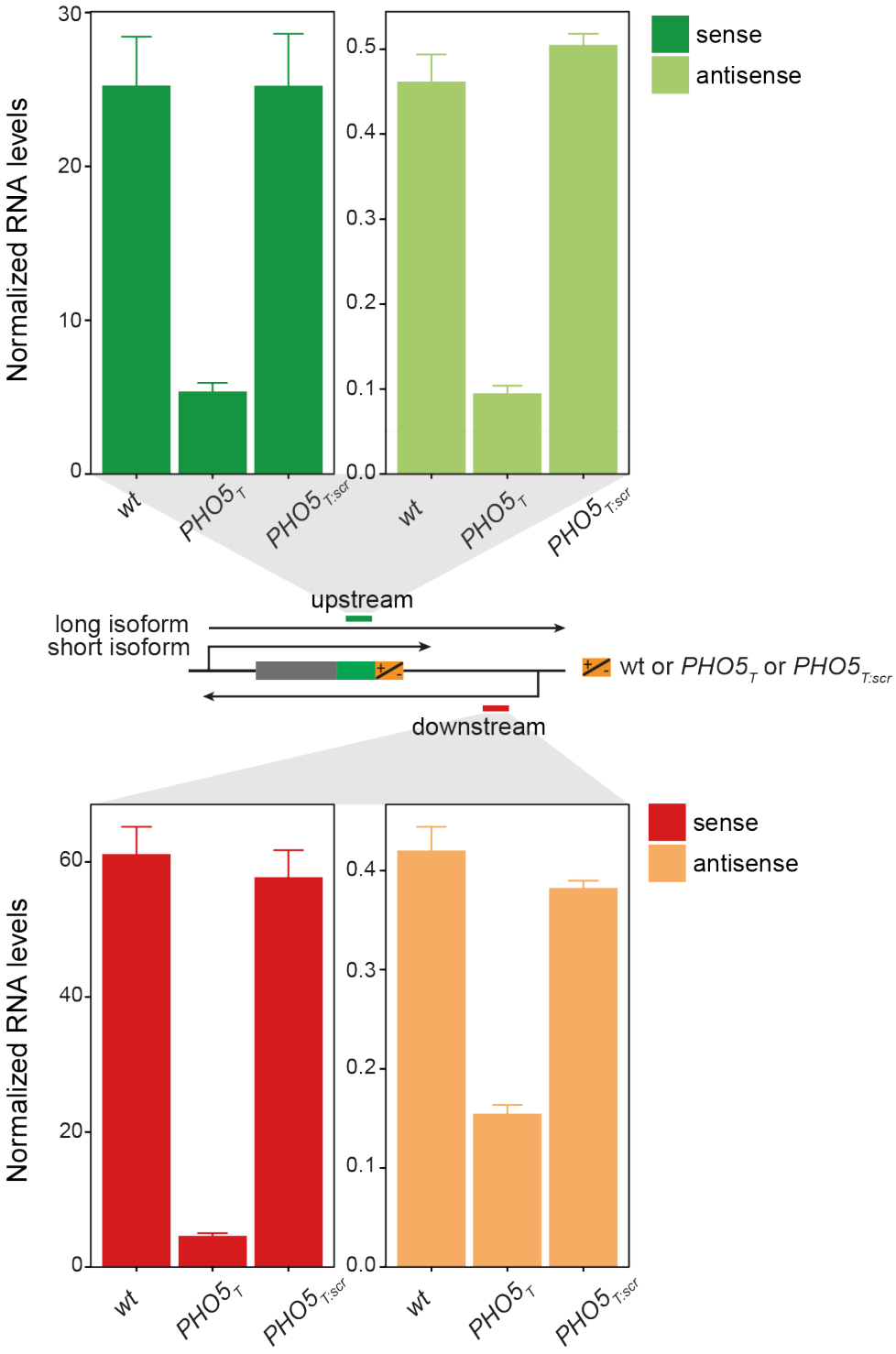

Figure S6. RT-qPCRs with amplicons both up- and downstream of the *PHO5*$_T$/*PHO5*$_{T:scr}$ insertions. Strand-specific RT-qPCRs were conducted in *SPS100-sfGFP wt*, *PHO5*$_T$, and *PHO5*$_{T:scr}$ strains with two different amplicons: the "upstream" amplicon was located within sfGFP (green bar) whereas the

“downstream” amplicon was located in the *SPS100* 3’-IGR downstream of the termination site of the short isoform (red bar). Reverse transcriptions were performed with the same primers that were also used for subsequent qPCR amplification runs. The antisense initiation site is shown schematically, different colours indicate the amplicon and whether sense or antisense was measured (see legends). Error bars show the standard deviation of three technical replicates.

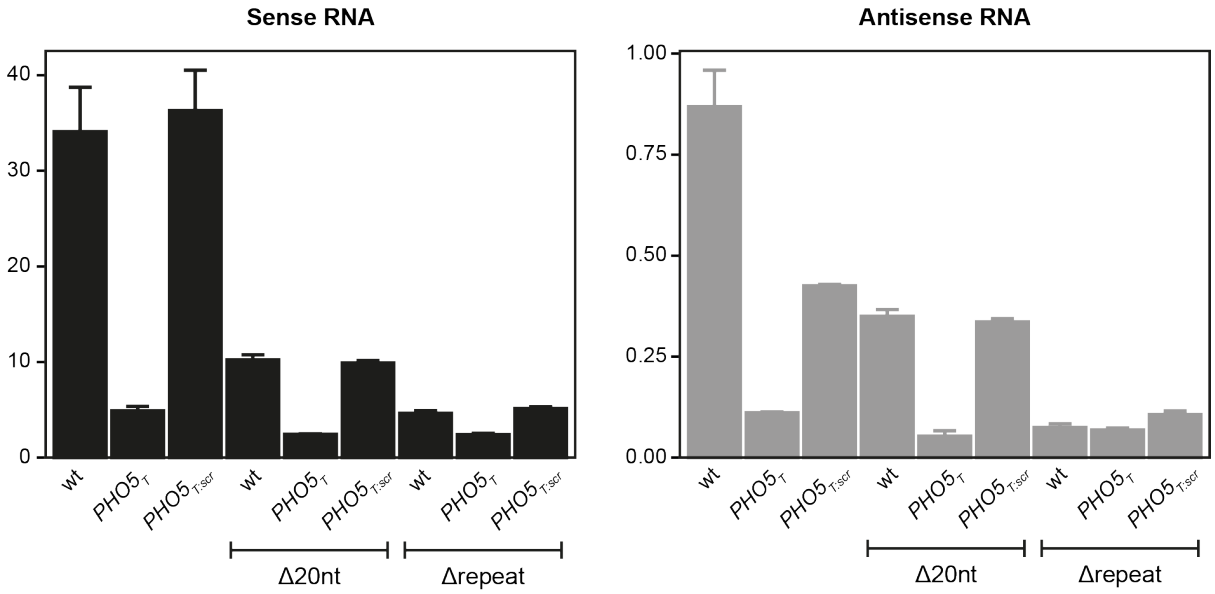

Figure S7. RT-qPCRs of SPS100 antisense library strains with different deletions in the 3'-IGR. Strand-specific RT-qPCRs were conducted in *SPS100-sfGFP* wt, $PHO5_T$, and $PHO5_{T:scr}$ strains with an amplicon binding in sfGFP (see Figure S6) of strains with either no deletion or deletion of the 20 nt element or the repeat as indicated in Figures 5 and 6. Black bars indicate sense, gray bars antisense levels. Error bars show the standard deviation of three technical replicates.

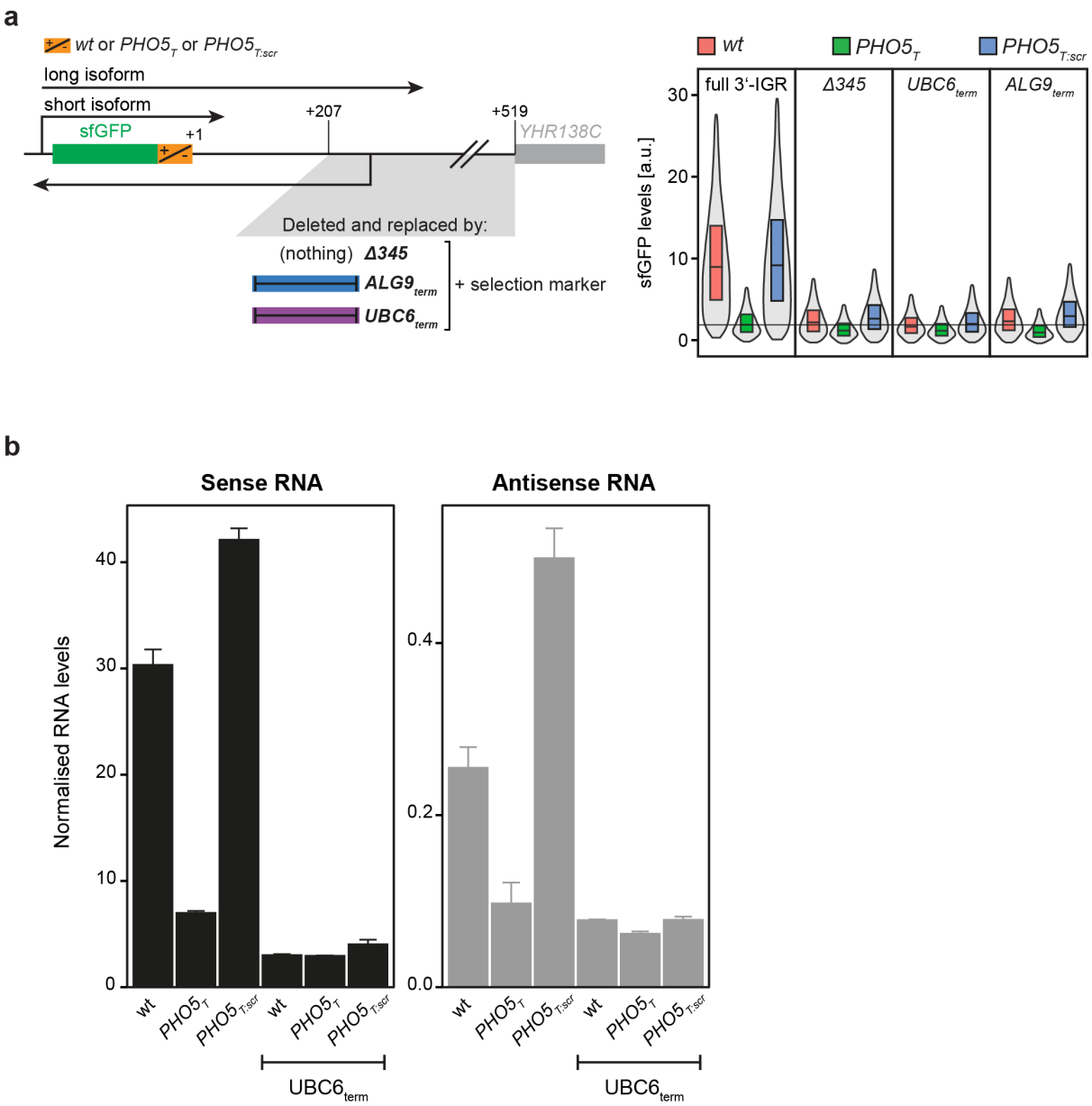

Figure S8. Sps100 expression levels and RT-qPCRs of SPS100 antisense library strains with and without heterologous terminators. (**a**) The region spanning nucleotides 207-519 of the *SPS100* 3'-IGR was deleted or replaced by two different bidirectional terminators ($ALG9_{term}$ and $UBC6_{term}$, see main text) in each of *wt,* $PHO5_T$ or $PHO5_{T:scr}$ strains. The resulting strains were grown into starvation and sfGFP intensities were measured by flow cytometry. The boxes show the first and third quartiles and the median. The grey violin plots show the distribution densities ranging from the first quartile minus 1.5 * interquartile range (IQR) to the third quartile + 1.5 * IQR. (**b**) Strand-specific RT-qPCRs were conducted in *SPS100-sfGFP wt*, $PHO5_T$, and $PHO5_{T:scr}$ strains with an amplicon binding in sfGFP (see

Figure S6) of the strains shown in (a). Black bars indicate sense, gray bars antisense levels. Error bars show the standard deviation of three technical replicates.

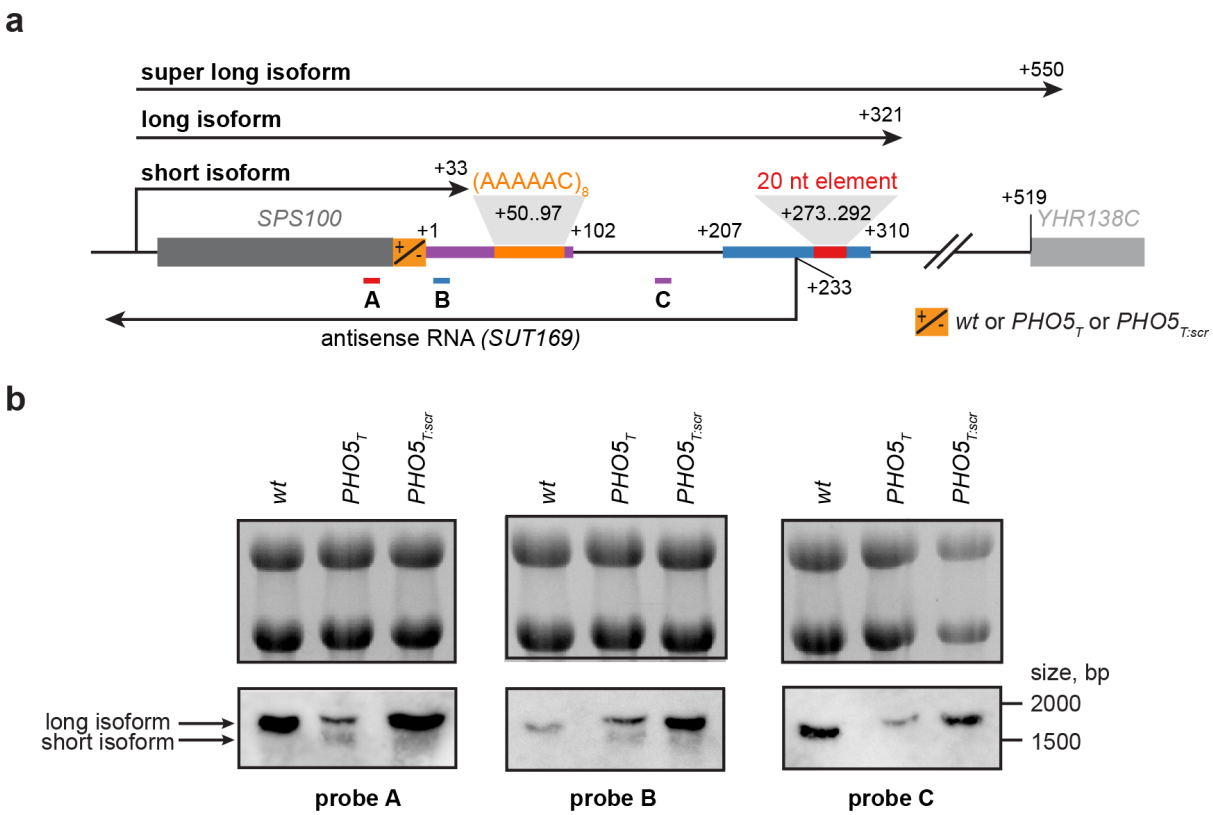

Figure S9. 3'-end mRNA isoforms of *SPS100* determined by Northern blot in the strains without the sfGFP tag. (a) Scheme of the locus of *SPS100* (no sfGFP) with the sequence motifs of Figure 5 and the three mRNA isoforms indicated. (**b**) Northern blots on SPS100 mRNAs with three different probes as indicated in (a). Arrows indicate long and short isoform bands. Upper panel is loading control (rRNA bands on the RNA gel stained with ethidium bromide).

Supplementary Movie Legend

Movie S1. Live monitoring of the transcription process. A GPD-22PP7-BFP-*SPS100* 3'-IGR strain was used for the analysis of transcriptional activity. Left: Maximum projection images which were recorded over ~ 30 min. Individual transcription events were detected and the Hidden Markov model was used for the analysis of the transcription site intensity traces (right part, intensity axis, blue lines). Resulted ON/OFF states are depicted in the right axis in green/red lines. Green and red circles and lines correspond to the transcription events in two different cells.

Supplementary Tables S1-S6

Table S1 – Yeast strains used in this study

Strain	Background	Description	Reference
Y8205		*MATα* *his3Δ1 ura3Δ0 met15Δ0* *can1Δ::STE2pr-his5* *lyp1Δ::STE3pr-LEU2*	(1)
yMaM330	Y8205	insertion of Gal-inducible I-SceI cassette *leu2Δ0::GAL1pr-I-SCEI-natNT2*	(2)
ESM356-1	FY1676	*MATa* *ura3-52 leu2Δ1 his3Δ200 trp1Δ63*	(3)
LH175		*MAT**a**, ho:hisG, lys2 ura3 leu2 his3 trp1ΔFA (SK1 background)*	(4)
yDB14	YMaM330	SPS100-sfGFP-S2 site (otherwise seamlessly tagged) in YMaM330 background	(5)
yDB16	YMaM330	As yDB14 but with sfGFP followed by $PHO5_T$	(5)
yDB17	YMaM330	As yDB14 but with sfGFP followed by $PHO5_{T:scr}$	(5)
yDB218	ESM356-1	ESM356-1 transformed with pDaB38	this study
yMaS221	Y8205	yDB14 transformed with pMaS135	this study
yMaS222	Y8205	yDB16 transformed with pMaS135	this study
yMaS223	Y8205	yDB17 transformed with pMaS135	this study
yMaS224	Y8205	yDB14 transformed with pMaS136	this study
yMaS225	Y8205	yDB16 transformed with pMaS136	this study
yMaS226	Y8205	yDB17 transformed with pMaS136	this study
yDB302	LH175	*Δsps100::kanMX6* (SK1 background)	this study
yMaS199		Diploid from yDB14 and yDB302 *Δsps100::kanMX6/SPS100-sfGFP*	this study
yMaS200		Diploid from yDB16 and yDB302 *Δsps100::kanMX6/SPS100-sfGFP*	this study
yMaS201		Diploid from yDB17 and yDB302 *Δsps100::kanMX6/SPS100-sfGFP*	this study
yMaS207		Diploid from yDB302 and YMaM330 (neg. control for yMaS199-201)	this study
yDB18	Y8205	yDB14 transformed with pDB6 (pRS413-*SPS100*)	this study
yDB20	Y8205	yDB16 transformed with pDB6 (pRS413-*SPS100*)	this study
yDB21	Y8205	yDB17 transformed with pDB6 (pRS413-*SPS100*)	this study
yDB51	YMaM330	*CTA1-sfGFP-S2 site* (otherwise seamlessly	(5)

		tagged) in YMaM330 background	
yDB53	YMaM330	As yDB51 but with sfGFP followed by *PHO5$_T$*	(5)
yDB54	YMaM330	As yDB51 but with sfGFP followed by *PHO5$_{T:scr}$*	(5)
yDB59	YMaM330	*UGA2-sfGFP-S2 site* (otherwise seamlessly tagged) in YMaM330 background	(5)
yDB61	YMaM330	As yDB51 but with sfGFP followed by *PHO5$_T$*	(5)
yDB62	YMaM330	As yDB51 but with sfGFP followed by *PHO5$_{T:scr}$*	(5)
yDB55	YMaM330	*FBP1-sfGFP-S2 site* (otherwise seamlessly tagged) in YMaM330 background	(5)
yDB57	YMaM330	As yDB51 but with sfGFP followed by *PHO5$_T$*	(5)
yDB58	YMaM330	As yDB51 but with sfGFP followed by *PHO5$_{T:scr}$*	(5)
yDB92	Y8205	yDB51 (*CTA1-sfGFP*) tagged with PCR product of S2/S3 primers on pDB10 (Figures 4b-c): *CTA1-sfGFP-SPS100_3'IGR-Cyc1term(rev)-KanMX*	this study
yDB113	Y8205	*CTA1-sfGFP-PHO5$_T$-SPS100_3'IGR-Cyc1term(rev)-KanMX* analogous to yDB92	this study
yDB94	Y8205	*CTA1-sfGFP-PHO5$_T$-SPS100_3'IGR-Cyc1term(rev)-KanMX* analogous to yDB92	this study
yDB108	Y8205	*FBP1-sfGFP-SPS100_3'IGR-Cyc1term(rev)-KanMX* analogous to yDB92	this study
yDB96	Y8205	*FBP1-sfGFP-PHO5$_T$-SPS100_3'IGR-Cyc1term(rev)-KanMX* analogous to yDB92	this study
yDB97	Y8205	*FBP1-sfGFP-PHO5$_{T:scr}$-SPS100_3'IGR-Cyc1term(rev)-KanMX* analogous to yDB92	this study
yDB98	Y8205	*UGA2-sfGFP-SPS100_3'IGR-Cyc1term(rev)-KanMX* analogous to yDB92	this study
yDB100	Y8205	*UGA2-sfGFP-PHO5$_T$-SPS100_3'IGR-Cyc1term(rev)-KanMX* analogous to yDB92	this study
yDB101	Y8205	*UGA2-sfGFP-PHO5$_{T:scr}$-SPS100_3'IGR-Cyc1term(rev)-KanMX* analogous to yDB92	this study
yMaS107	ESM356-1	*ura3::GPDprom-BFP-PHO5$_T$-SPS100_3'IGR-*	this study

		KanMx integration of pMaS82 into *URA3* locus	
yMaS108	ESM356-1	*ura3::GPDprom-BFP-SPS100_3'IGR-KanMx* integration of pMaS83 into *URA3* locus	this study
yMaS109	ESM356-1	*ura3::GPDprom-BFP-PHO5$_{T:scr}$-SPS100_3'IGR-KanMx* integration of pMaS84 into *URA3* locus	this study
yDB188	ESM356-1	ESM356-1 transformed with pDaB27 *pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 1..102}$* (Figure S4)	this study
yDB189	ESM356-1	ESM356-1 transformed with pDaB28 pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 102..518}$	this study
yDB187	ESM356-1	ESM356-1 transformed with pDaB26 pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 103..206}$	this study
yDB186	ESM356-1	ESM356-1 transformed with pDaB25 pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 207..310}$	this study
yDB183	ESM356-1	ESM356-1 transformed with pDaB22 pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 414..518}$	this study
yDB184	ESM356-1	ESM356-1 transformed with pDaB23 pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 311..518}$	this study
yDB185	ESM356-1	ESM356-1 transformed with pDaB33 pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 1..518}$	this study
yDB204	ESM356-1	ESM356-1 transformed with pDaB33 pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 265..272}$	this study
yDB205	ESM356-1	ESM356-1 transformed with pDaB34 pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 207..226}$	this study
yDB206	ESM356-1	ESM356-1 transformed with pDaB35 pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGR$_{\Delta 227..246}$	this study
yDB207	ESM356-1	ESM356-1 transformed with pDaB36 pRS415-SPS100_5'IGR-SPS100-BFP-	this study

		SPS100_3'IGR$_{\Delta 273..292}$	
yDB43	Y8205	*SPS100-sfGFP-SPS100_3'IGR* analogous to yDB92	this study
yDB45	Y8205	*SPS100-sfGFP-PHO5$_{T}$-SPS100_3'IGR* analogous to yDB92	this study
yDB46	Y8205	*SPS100-sfGFP-PHO5$_{T:scr}$-SPS100_3'IGR* analogous to yDB92	this study
yMaS248	Y8205	*SPS100-sfGFP-SPS100_3'IGR$_{\Delta 1..102}$-Cyc1term(rev)-KanMX* made as in Figure 4b, used for Figure 5	this study
yMaS212	Y8205	*SPS100-sfGFP-SPS100-PHO5$_{T}$-3'IGR$_{\Delta 1..102}$-Cyc1term(rev)-KanMX*	this study
yMaS214	Y8205	*SPS100-sfGFP-SPS100-PHO5$_{T:scr}$-3'IGR$_{\Delta 1..102}$-Cyc1term(rev)-KanMX*	this study
yMaS249	Y8205	*SPS100-sfGFP-SPS100_3'IGR$_{\Delta (AAAAAC)8}$-Cyc1term(rev)-KanMX*	this study
yMaS250	Y8205	*SPS100-sfGFP-SPS100-PHO5$_{T}$-3'IGR $_{\Delta (AAAAAC)8}$-Cyc1term(rev)-KanMX*	this study
yMaS251	Y8205	*SPS100-sfGFP-SPS100-PHO5$_{T:scr}$-3'IGR $_{\Delta (AAAAAC)8}$-Cyc1term(rev)-KanMX*	this study
yMaS220	Y8205	*SPS100-sfGFP-SPS100_3'IGR$_{\Delta 207..310}$-Cyc1term(rev)-KanMX*	this study
yMaS213	Y8205	*SPS100-sfGFP-SPS100-PHO5$_{T}$-3'IGR $_{\Delta 207..310}$-Cyc1term(rev)-KanMX*	this study
yMaS215	Y8205	*SPS100-sfGFP-SPS100-PHO5$_{T:scr}$-3'IGR $_{\Delta 207..310}$-Cyc1term(rev)-KanMX*	this study
yDB224	Y8205	*SPS100-sfGFP-SPS100_3'IGR$_{\Delta 273..292}$-Cyc1term(rev)-KanMX*	this study
yDB225	Y8205	*SPS100-sfGFP-SPS100-PHO5$_{T}$-3'IGR $_{\Delta 272..292}$-Cyc1term(rev)-KanMX*	this study
yDB226	Y8205	*SPS100-sfGFP-SPS100-PHO5$_{T:scr}$-3'IGR $_{\Delta 272..292}$-Cyc1term(rev)-KanMX*	this study
yMaS139	ESM356-1	*ura3::GPDprom-22PP7-BFP-SPS100_3'IGR-NatNT2 + NOP1prom-PCP-3mCherry-KanMX*	this study
yMaS140	ESM356-1	*ura3::GPDprom-22PP7-BFP-PHO5$_{T}$-SPS100_3'IGR-NatNT2 + NOP1prom-PCP-3mCherry-KanMX*	this study
yMaS141	ESM356-1	*ura3::GPDprom-22PP7-BFP-PHO5$_{T:scr}$-*	this study

		SPS100_3'IGR-NatNT2 + NOP1prom-PCP-3mCherry-KanMX	
yDK475-1	Y8205	*SPS100-PHO5$_T$-S2* in BY4741 background	this study
yDK476-6	Y8205	*SPS100-PHO5$_{T:scr}$-S2* in BY4741 background	this study

Table S2 – Plasmids used in this study

Plasmid	Backbone	Description	Reference
pFA6a		*E. coli* plasmid with AmpR cassette	(6)
pRS413		Centromeric plasmid, histidin selectable	(7)
pRS415		Centromeric plasmid, leucin selectable	(7)
pRS306K		Integrative plasmid for *URA3* site, *kanMX4* resistance cassette	(8)
pMaM175	pFA6a	contains *S3-sfGFP-ISceIsite-S.Parad.Tcyc1-ScURA3-SceIsite-sfGFPΔN–S2* Used for seamless tagging with sfGFP	(5)
pMaM201	pFA6a	Like pMaM175 but with *PHO5$_T$* following sfGFP	(5)
pMaM203	pFA6a	Like pMaM175 but with *PHO5$_{T:scr}$* following sfGFP	(5)
pDaB38	pRS415	NotI site of pRS415 contains *SPS100_5'IGR-ORF-TagBFP-PHO5$_T$-SPS100_3'IGR* with mCherry inserted in antisense direction at position 227 of 3'IGR Used as antisense reporter (Figure 3)	this study
pMaS135	pRS413	Like pDaB38 but without mCherry and with histidine selectable marker	this study
pMaS136	pRS413	Like pDaB38 but with histidine selectable marker	this study
pDB6	pRS413	*SPS100* ORF including 1572 bp upstream (= 5'IGR) and 519 bp downstream (=3' IGR) was amplified from genome ESM356-1 and inserted into pRS413 cut with XhoI + SpeI Used for Figure 4a	this study
pMaS9	pRS415	NotI site of pRS415 contains *SPS100_5'IGR-ORF-TagBFP-SPS100_3'IGR*	this study
pMaS10	pRS415	Like pMaS9 but with *TagBFP-PHO5$_T$*	this study
pDB10	pFA6a	SalI site of pFA6a contains SPS100_3'IGR-CYC1Term(rev)-KanMX	this study

		Used for tagging strategy in Figure 4b-c	
pMaS133	pDB10	pDB10 with deletion of nucleotides 1-102 of *SPS100* 3' IGR	this study
pMaS134	pDB10	pDB10 with deletion of nucleotides 207-310 of *SPS100* 3' IGR	this study
pFH17	pDB10	pDB10 with deletion of $(AAAAAC)_8$ repeat of *SPS100* 3' IGR (nucleotides 50-97)	this study
pDaB49	pDB10	pDB10 but with deletion of 20 nt motif of *SPS100* 3' IGR (nucleotides 273-292)	this study
pMaS82	pRS306K	*pGPD-TagBFP-SPS100_3'IGR* integrative plasmid targeting the *URA3* locus	this study
pMaS83	pRS306K	Like pMaS82 but with *TagBFP* followed by $PHO5_T$	this study
pMaS84	pRS306K	*Like pMaS82 but with TagBFP followed by* $PHO5_{T:scr}$	this study

Table S3 – Oligonucleotides used in this study

Primer	Sequence 5' – 3'
RT-qPCR primers:	
SPS100 sense (reverse transcription)	CCATGTGATCACGCTTTTCATTCGGA
SPS100 antisense (reverse transcription)	AAGAGCTATTTACTGGGGTTGTACC
SPS100 sense/antisense forward (qPCR)	GGCCAACCCTAGTAACAACTTTG
SPS100 sense/antisense reverse (qPCR)	CACGTAGCCTTCTGGCATAG
BFP sense A (reverse transcription)	TTCAGGGCCATGTCGTTT
BFP sense B (reverse transcription)	CGTAGTACACAACACATAATCATC
BFP antisense (reverse transcription)	TTCACCGAGACGCTGTACC
BFP sense/antisense forward (qPCR)	TTCACCGAGACGCTGTACC
BFP sense/antisense reverse A (qPCR)	TTCAGGGCCATGTCGTTT
BFP sense/antisense reverse B (qPCR)	CGTAGTACACAACACATAATCATC
3' RACE primers:	
reverse transcription (Q_T)	CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTT TTTTTTTTTTTTVN
first amplification reverse (Q_O)	CCAGTGAGCAGAGTGACG

second amplification reverse (Q_I)	GAGGACTCGAGCTCAAGC
SPS100 first amplification forward (GSP1)	TGGGTACTTGTCACCAATCC
BFP first amplification forward (GSP1)	CCTGAGGGCTTCACATGG
SPS100 second amplification forward (GSP1)	AGCGAGTTACAACAAATCTTCC
BFP second amplification forward (GSP2)	GGCTGCCTCATCTACAACG
Northern blot probes:	
ProbeA (targeting sense sfGFP)	GTAGTGATTATCGGGTAACAAGACTGGACCATCACCAATAGGGGT
ProbeB (targeting sense *SPS100* after STOP)	CGTAGTACACAACACATAATCATCTTAATCGATGAATTCGAGCTCG
ProbeC (targeting *SPS100* long isoform)	GAACACTGATAATAACTGTACTGAAGACAAACAATTAGGAAAGTAAC
ProbeA* (targeting sense *SPS100* ORF)	GGATTGGTGACAAGTACCCAGCAGAAATTGCACCTTGTGGAATACTTGTG

Table S4 – Growth conditions

Media	Condition	Concentration	Temperature
SC	raffinose	2 % w/v	30 °C
SC	sodium chloride	0.4 M	30 °C
SC	trehalose	2 % w/v	30 °C
SC	ethanol	3 % w/v	30 °C
SC	potassium acetate	2 % w/v	30 °C
SC	glycerol	3 % w/v	30 °C
SC	glucose	0.1 % w/v	30 °C
SC	glucose	2 % w/v	30 °C
SC	glucose	2 % w/v	14 °C
SC	raffinose + galactose	2 + 2 % w/v	30 °C
SC	sucrose	2 % w/v	30 °C
SC	maltose	2 % w/v	30 °C

SC – synthetic complete.

Table S5 – Detection of sfGFP fusions by the plate colony assay

sfGFP tagged gene	T=14°C 2% glucose	T=30°C 0.1% glucose	T=30°C 2% glucose
KAP123	5.63	5.62	4.66
HNM1	4.28	NA	NA
AMS1	4.31	4.08	3.27
HXT5	13.43	20.47	23.54
BCY1	8.12	5.05	4.95
ELO1	7.03	2.93	NA
PTM1	5.63	NA	3.36
RCK2	4.55	NA	NA
TMA7	9.61	3.98	4.47
CHS5	3.89	NA	NA
RPL6B	10.76	5.53	4.98
CCS1	3.43	NA	NA
PUB1	21.38	7.65	9.04
SUR1	2.07	NA	NA
CTR1	6.39	2.93	3.36
HXT3	13.24	NA	NA
GLC3	15.58	6.93	9.02
HMF1	10.17	4.73	4.61
ICL1	64.70	50.79	39.52
KRS1	14.64	4.50	6.10
YHR087W	47.09	19.74	41.20
COX5B	3.08	NA	NA
PRY1	5.29	NA	NA
SPC1	3.70	NA	NA
CYC1	5.30	NA	NA
FBP1	10.74	13.74	7.20
SUR7	5.20	3.31	3.76
ADH6	6.26	3.49	NA
YGP1	3.70	NA	NA
HTZ1	5.44	3.39	3.26
UGA2	3.21	3.04	4.94
JEN1	11.62	19.80	4.58
YMR178W	4.49	NA	NA
FBA1	245.67	125.15	119.46
LEM3	3.38	NA	NA
GTT1	4.32	4.09	4.40
ARA1	14.69	12.58	8.19
NPC2	3.37	3.47	NA
VCX1	4.71	NA	NA
INH1	8.59	8.12	7.78
YNL194C	3.25	9.01	5.29
PDC1	211.42	85.75	156.83
MRPL23	3.03	NA	NA

SVP26	3.90	NA	NA
CTA1	NA	8.75	NA
SPS100	NA	27.97	5.07
YKL187C	NA	3.63	NA
CYB2	NA	3.40	NA
YBL029C-A	NA	NA	4.22
YJR096W	NA	NA	4.42

Table of the 50 genes which were detected above background at 3 different growth conditions. Values correspond to the fold increase of colony fluorescence above background. NA means that the gene was not detected at this particular growth condition.

Table S6 – Detection of sfGFP fusions by the plate colony assay

Gene	Regulation (this study)	Regulated (Huber et al., 2016)	Regulated (other studies)
SPS100	↑	n/a (not expressed)	not reported
PDC1	↑	n/a (overexposed)	not reported
FBA1	↑	n/a (overexposed)	not reported
CTA1	↑	no	not reported
AMS1	↑	↑	not reported
HXT5	↑ for 0.1% and SC, 30 °C, ↓ for 14 °C	no	not reported
HXT3	↓	no	not reported
YNL194C	↓	no	not reported
COX5B	↓	↓	not reported
SPC1	↓	↓	not reported
YHR087W	↓	↓	not reported
SUR7	↓	no	yes, but only at low levels (9)
SUR1	↓	↓	not reported
ELO1	↓	↓	not reported
UGA2	↓	no	not reported

Comparison with previous studies. The genes identified in this study to be regulated by antisense transcription were compared to our previous study where we used exponential growth conditions, and to reports in the literature. Legend: ↑ = antisense increases expression; ↓ = antisense decreases expression; **n/a** = was not tested for regulation; **no** = gene was tested for regulation but no difference was observed between $PHO5_{T}$ and $PHO5_{T:scr}$; **not reported** = we could not identify other studies reporting antisense-dependent regulation of this gene.

Supplementary References

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