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
Supplementary Movie Legend
Supplementary Tables S1-S6
Supplementary References
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<sub>T</sub>, and PHO5<sub>T;scr</sub> 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<sub>T</sub> 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 (\(\text{O}\) and \(\text{T}\), same orientation of both primers), as well as control primers lying in the same fragment after restriction (\(\text{C1}\) and \(\text{C2}\)). PCR shows expected product sizes from the interaction of restriction fragments \(\text{x}\) and \(\text{z}\) (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 \(\text{x}\) and \(\text{y}\). 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 PHO5T 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 $PHO5_T$

![Comparison of sense and antisense levels up- and downstream of $PHO5_T$.](image)

**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, PHO5T, and PHO5T: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     | MATα                                                                         | (3)       |
| LH175       |            | MATα, 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,scn                                   | (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    | Diploid from yDB14 and yDB302                                               | this study|
|             |            | Δsps100::kanMX6/SPS100-sfGFP                                                |           |
| yMaS200     | Diploid    | Diploid from yDB16 and yDB302                                               | this study|
|             |            | Δsps100::kanMX6/SPS100-sfGFP                                                |           |
| yMaS201     | Diploid    | Diploid from yDB17 and yDB302                                               | this study|
|             |            | Δsps100::kanMX6/SPS100-sfGFP                                                |           |
| yMaS207     | Diploid    | Diploid from yDB302 and YMaM330 (neg. control for yMaS199-201)              | this study|
|             |            | (other control for yMaS199-201)                                             |           |
| 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 tagged)                             | (5)       |
| 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-\(SPS100\_3'IGR\)-\(Cyc1\)term\(\text{rev}\)-\(KanMX\) this study |
| yDB113  | Y8205   | \(CTA1\)-sfGFP-\(PHO5_T\)-\(SPS100\_3'IGR\)-\(Cyc1\)term\(\text{rev}\)-\(KanMX\) this study |
| yDB94   | Y8205   | \(CTA1\)-sfGFP-\(PHO5_T\)-\(SPS100\_3'IGR\)-\(Cyc1\)term\(\text{rev}\)-\(KanMX\) this study |
| yDB108  | Y8205   | \(FBP1\)-sfGFP-\(SPS100\_3'IGR\)-\(Cyc1\)term\(\text{rev}\)-\(KanMX\) this study |
| yDB96   | Y8205   | \(FBP1\)-sfGFP-\(PHO5_T\)-\(SPS100\_3'IGR\)-\(Cyc1\)term\(\text{rev}\)-\(KanMX\) this study |
| yDB97   | Y8205   | \(FBP1\)-sfGFP-\(PHO5_T,scr\)-\(SPS100\_3'IGR\)-\(Cyc1\)term\(\text{rev}\)-\(KanMX\) this study |
| yDB98   | Y8205   | \(UGA2\)-sfGFP-\(SPS100\_3'IGR\)-\(Cyc1\)term\(\text{rev}\)-\(KanMX\) this study |
| yDB100  | Y8205   | \(UGA2\)-sfGFP-\(PHO5_T\)-\(SPS100\_3'IGR\)-\(Cyc1\)term\(\text{rev}\)-\(KanMX\) this study |
| yDB101  | Y8205   | \(UGA2\)-sfGFP-\(PHO5_T,scr\)-\(SPS100\_3'IGR\)-\(Cyc1\)term\(\text{rev}\)-\(KanMX\) this study |
| yMaS107 | ESM356-1| \(ura3::GPD\)prom-\(BFP\)-\(PHO5_T\)-\(SPS100\_3'IGR\)- this study |
KanMx integration of pMaS82 into URA3 locus

yMaS108  ESM356-1  ura3::GPDProm-BFP-SPS100_3'IGR-KanMx

this study

yMaS109  ESM356-1  ura3::GPDProm-BFP-PHO5_Tscr-SPS100_3'IGR-KanMx

this study

yDB188  ESM356-1  ESM356-1 transformed with pDaB27

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔ31..102

(Figure S4)

this study

yDB189  ESM356-1  ESM356-1 transformed with pDaB28

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔ102..518

this study

yDB187  ESM356-1  ESM356-1 transformed with pDaB26

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔ103..206

this study

yDB186  ESM356-1  ESM356-1 transformed with pDaB25

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔ207..310

this study

yDB183  ESM356-1  ESM356-1 transformed with pDaB22

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔ414..518

this study

yDB184  ESM356-1  ESM356-1 transformed with pDaB23

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔ311..518

this study

yDB185  ESM356-1  ESM356-1 transformed with pDaB33

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔα41..518

this study

yDB204  ESM356-1  ESM356-1 transformed with pDaB33

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔ265..272

this study

yDB205  ESM356-1  ESM356-1 transformed with pDaB34

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔ207..226

this study

yDB206  ESM356-1  ESM356-1 transformed with pDaB35

pRS415-SPS100_5'IGR-SPS100-BFP-SPS100_3'IGRΔ227..246

this study

yDB207  ESM356-1  ESM356-1 transformed with pDaB36

pRS415-SPS100_5'IGR-SPS100-BFP-
| Code   | Strain | Constructs                                                                 | Notes                                      |
|--------|--------|----------------------------------------------------------------------------|--------------------------------------------|
| yDB43  | Y8205  | SPS100-sfGFP-SPS100_3′IGR\textsubscript{Δ273..292}                         | analogous to yDB92                         |
| yDB45  | Y8205  | SPS100-sfGFP-PHO5\textsubscript{T}-SPS100_3′IGR                           | analogous to yDB92                         |
| yDB46  | Y8205  | SPS100-sfGFP-PHO5\textsubscript{T,scr}-SPS100_3′IGR                      | analogous to yDB92                         |
| yMaS248| Y8205  | SPS100-sfGFP-SPS100_3′IGR\textsubscript{Δ1..102}                        |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
|        |        | made as in Figure 4b, used for Figure 5                                     |                                                                                          |
| yMaS212| Y8205  | SPS100-sfGFP-SPS100-PHO5\textsubscript{T}-3′IGR\textsubscript{Δ1..102}    |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yMaS214| Y8205  | SPS100-sfGFP-SPS100-PHO5\textsubscript{T,scr}-3′IGR\textsubscript{Δ1..102} |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yMaS249| Y8205  | SPS100-sfGFP-SPS100_3′IGR\textsubscript{Δ(AAAAAC)8}                      |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yMaS250| Y8205  | SPS100-sfGFP-SPS100-PHO5\textsubscript{T}-3′IGR\textsubscript{Δ(AAAAAC)8} |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yMaS251| Y8205  | SPS100-sfGFP-SPS100-PHO5\textsubscript{T,scr}-3′IGR\textsubscript{Δ(AAAAAC)8} |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yMaS220| Y8205  | SPS100-sfGFP-SPS100_3′IGR\textsubscript{Δ207..310}                       |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yMaS213| Y8205  | SPS100-sfGFP-SPS100-PHO5\textsubscript{T}-3′IGR\textsubscript{Δ207..310}  |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yMaS215| Y8205  | SPS100-sfGFP-SPS100-PHO5\textsubscript{T,scr}-3′IGR\textsubscript{Δ207..310} |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yDB224 | Y8205  | SPS100-sfGFP-SPS100_3′IGR\textsubscript{Δ273..292}                       |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yDB225 | Y8205  | SPS100-sfGFP-SPS100-PHO5\textsubscript{T}-3′IGR\textsubscript{Δ272..292}  |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yDB226 | Y8205  | SPS100-sfGFP-SPS100-PHO5\textsubscript{T,scr}-3′IGR\textsubscript{Δ272..292} |                                                                                          |
|        |        | Cycl1\text{term}(rev)-KanMX                                                |                                                                                          |
| yMaS139| ESM356-1| ura3::GPDprom-22PP7-BFP-SPS100_3′IGR-NatNT2 + NOP1prom-PCP-3mCherry-KanMX |                                                                                          |
| yMaS140| ESM356-1| ura3::GPDprom-22PP7-BFP-PHO5\textsubscript{T}                           |                                                                                          |
|        |        | SPS100_3′IGR-NatNT2 + NOP1prom-PCP-3mCherry-KanMX                          |                                                                                          |
| yMaS141| ESM356-1| ura3::GPDprom-22PP7-BFP-PHO5\textsubscript{T,scr}                        |                                                                                          |
|        |        |                                                                            |                                                                                          |
yDK475-1  Y8205  SPS100-PHO5-T-S2 in BY4741 background  this study
yDK476-6  Y8205  SPS100-PHO5_Tscr-S2 in BY4741 background  this study

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### Table S2 – Plasmids used in this study

| Plasmid    | Backbone | Description                                                                 | Reference |
|------------|----------|-----------------------------------------------------------------------------|-----------|
| pFA6a      | E. coli  | plasmid with AmpR cassette                                                  | (6)       |
| pRS413     | Centromeric | plasmid, histidine selectable                                               | (7)       |
| pRS415     | Centromeric | plasmid, leucine selectable                                                 | (7)       |
| pRS306K    | Integrative | plasmid for URA3 site, kanMX4 resistance cassette                          | (8)       |
| pMaM175    | pFA6a   | contains S3-sfGFP-Scelsite-S. Parad. Tyc1-ScURA3-Scelsite-sfGFPΔN-S2       | (5)       |
| pMaM175    | pFA6a   | Like pMaM175 but with PHO5_T following sfGFP                                | (5)       |
| pMaM201    | pFA6a   | Like pMaM175 but with PHO5_Tscr following sfGFP                             | (5)       |
| pMaM203    | pFA6a   | Like pMaM175 but with PHO5_Tscr following sfGFP                             | (5)       |
| pDaB38     | pRS415  | NotI site of pRS415 contains SPS100_5'IGR-ORF-TagBFP-PHO5_T-SPS100_3'IGR  | this study|
|            |         | with mCherry inserted in antisense direction at position 227 of 3'IGR     |           |
| 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 | this study|
|            |         | Used for Figure 4a                                                       |           |
| 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|
Table S3 – Oligonucleotides used in this study

| Primer | Sequence 5’ – 3’ |
|--------|------------------|
| **RT-qPCR primers:** | |
| *SPS100* sense (reverse) | CCATGTGATCACGCTTTTCATTCGGA |
| *SPS100* antisense (reverse) | AAGAGCTATTTACTGGGGTTGTACC |
| *SPS100* sense/antisense forward (qPCR) | GGCCAACCCCTAGTAACAACTTTG |
| *SPS100* sense/antisense reverse (qPCR) | CACGTAGCCTTCTGGCATAG |
| *BFP* sense A (reverse) | TTCAGGGCCATGTCGTTT |
| *BFP* sense B (reverse) | CGTAGTACACAACACAATAATCATC |
| *BFP* antisense (reverse) | TTCACCGAGACGCTGTACC |
| *BFP* sense/antisense forward (qPCR) | TTCACCGAGACGCTGTACC |
| *BFP* sense/antisense reverse A (qPCR) | TTCAGGGCCATGTCGTTT |
| *BFP* sense/antisense reverse B (qPCR) | CGTAGTACACAACACAATAATCATC |
| **3’ RACE primers:** | |
| reverse transcription (Q_T) | CCAGTGAGCAGAGTGACGACTCGAGCTCAAGCTTTTTTTTTTTTTTTTTTTTTTTTTTVN |
| first amplification reverse (Q_O) | CCAGTGAGCAGAGTGACG |
|                     | Sequences                                      |
|---------------------|-----------------------------------------------|
| **second amplification reverse (Q)** | 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:**

| Name     | Targeting Sense                  | Sequence                                                                 |
|----------|----------------------------------|--------------------------------------------------------------------------|
| **ProbeA** | sfGFP                            | GTAGTGATTATCGGGTAACAAGACTGGACCATCAACAAATAG GGGT                         |
| **ProbeB** | SPS100 after STOP                | CGTAGTACACAACACATAATCATCTTAATCGATGAATTTCGA GCTCG                        |
| **ProbeC** | SPS100 long isoform              | GAACACTGATAATAACTGTACTGAAGACAAACATTTAGGA AGTAAC                          |
| **ProbeA** | ORF                              | GGATTGGTGACAAGTACCACCGACAAATTGCACCTTTGTGG AATACTTTGTG                   |
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 gene | tagged | 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                  |                   |
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.

| Gene    | Condition 1 | Condition 2 | Condition 3 |
|---------|-------------|-------------|-------------|
| 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 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 PHO5T and PHO5T:scr; not reported = we could not identify other studies reporting antisense-dependent regulation of this gene.
Supplementary References

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