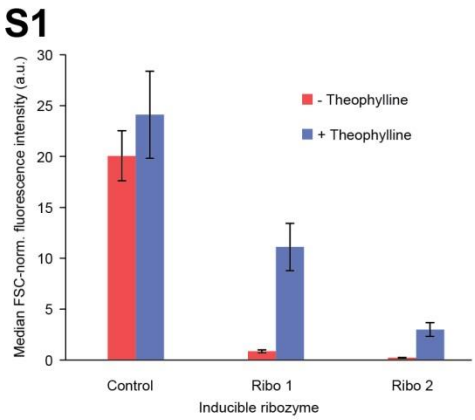

Figure S1. Regulation of expression by different ribozymes. Different genomically integrated reporter constructs are identical in their Tet-promoter and mNeongreen open reading frame (ORF) and differ only in their ribozyme sequences in the 3' regulatory region (3' RR). Both active ribozymes (Ribo 1 and 2) are inhibited by theophylline in their RNase activity and confer a roughly 13-fold range of expression regulation while covering different absolute expression ranges. Ribo 2 has been employed for the noise tuner throughout this work. The control construct harbors an inactive ribozyme. Strains containing the different constructs were grown in presence of 64 ng/µL doxycycline and 0 mM (-) or 25.6 mM (+) theophylline overnight, re-inoculated and grown to mid-exponential phase prior to measuring mNeongreen expression by flow cytometry. mNeongreen fluorescence was normalized for each individual cell to the forward scatter signal. Error bars indicate the MAD of 10,000 cells.

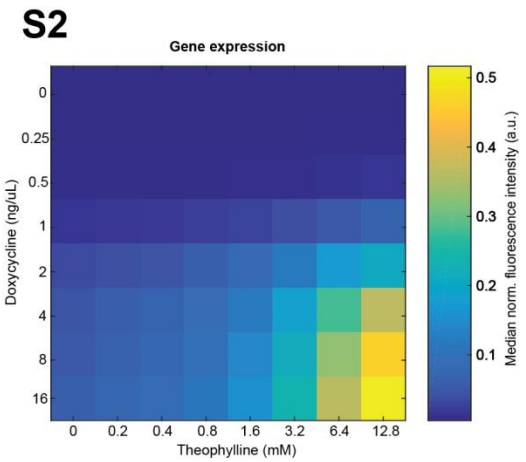

Figure S2. Median mNeongreen expression driven by the noise-tuner. The color code indicates median normalized fluorescence intensity at different combinations of doxycycline and theophylline for the experiment shown in Figure 1c.

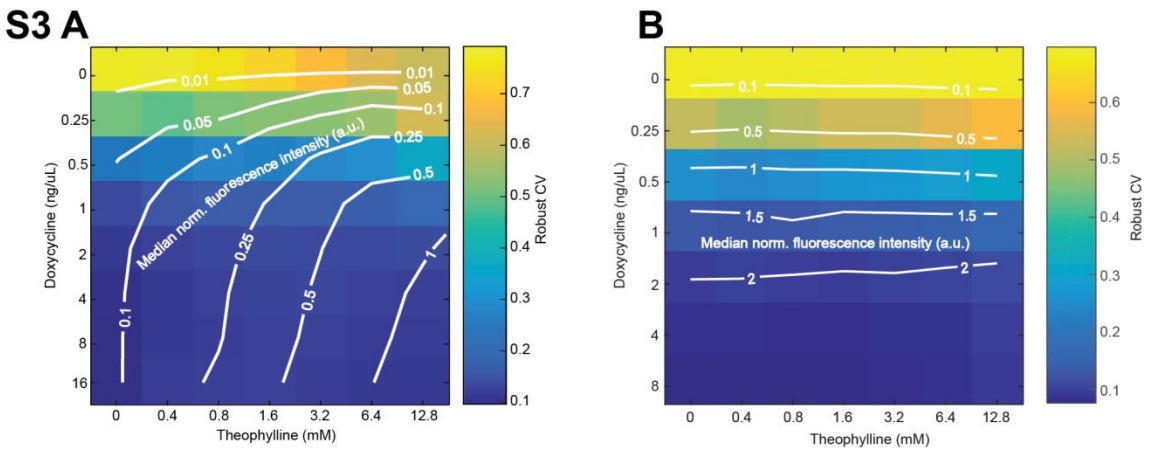

Figure S3. Median-noise relationship for different ribozyme sequences. (a) The absolute expression range of the noise tuner can be changed by employing a different ribozyme sequence. The ribozyme used for the noise tuner in this experiment ("Ribo 1" in Figure S1) confers higher expression than "Ribo 2", which has been employed throughout the remaining experiments in this work (see Figure S1). Accordingly, the range in which noise can be adjusted is shifted to higher median expression (compare this figure to Figure 2a). **(b) A construct with mNeongreen driven by an inducible Tet-promoter and an inactive ribozyme control.** This construct ("Control" in Figure S1) shows no effect of theophylline on either median expression or expression noise. Both, median and noise scale solely with the transcription rate determined by the doxycycline concentration.

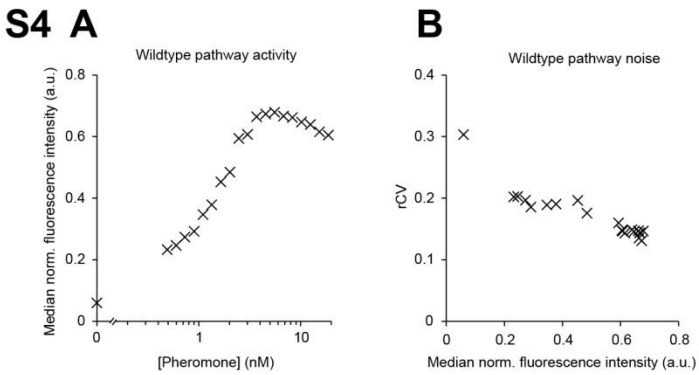

Figure S4. Mating pathway reporter dose-response (a) and noise (b) of wild type strain. Pathway reporter and experimental conditions are the same as for results shown in Figure 4 for the *SST2* noise-tuner strain. No doxycycline and theophylline were present in the media. The employed strain was YAA328 (see description with Table S3).

S5

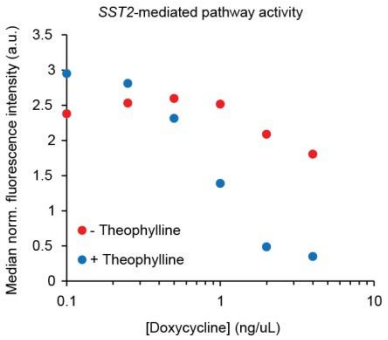

Figure S5. Mating pathway activity for different *SST2* expression regimes. *SST2* expression was titrated overnight and subsequently in a day culture with doxycycline concentrations ranging from 0 to 4 ng/µL either without (red) or with 12.8 mM theophylline (blue). Cells were measured after stimulation with 2.47 nM pheromone for 180 minutes. Addition of theophylline strongly represses the pathway activity, indicating effective mRNA stabilization of the negative pathway regulator *SST2*.

Table S1. Plasmids used in this study.

Plasmid	Description
pAA207	*In vivo* tagging plasmid with 3' regulatory region (3' RR) T_{FZF1} with *HPH* marker gene.
pAA208	*In vivo* tagging plasmid with 3' RR T_{GIC1} with *HPH* marker gene.
pAA209	*In vivo* tagging plasmid with 3' RR T_{TPS1} with *HPH* marker gene.
pAA263	*In vivo* tagging plasmid with an inducible $P_{TetO7\text{-}ACT1}$ promoter comprising seven Tet-operator sites and the *ACT1* core promoter and 5'-UTR with *kanMX* marker gene.
pMFM046	*HIS3*-integrative plasmid with mNeongreen driven by TetO7 *ACT1* promoter and sTRSV control ribozyme and T(Synth27) terminator with *CgHIS3* marker gene.
pMFM047	*HIS3*-integrative plasmid with mNeongreen driven by TetO7 *ACT1* promoter and L2b8-t47 ribozyme and T(Synth27) terminator with *CgHIS3* marker gene.
pMFM048	*HIS3*-integrative plasmid with mNeongreen driven by TetO7 *ACT1* promoter and L2b8-a1-t41 ribozyme and T(Synth27) terminator with *CgHIS3* marker gene.
pMFM058	*In vivo* tagging plasmid with L2b8-a1-t41 ribozyme and T(Synth27) terminator with *natNT* marker gene.
pMFM065	*In vivo* tagging plasmid with 3' RR T_{ADH1} with *natNT* marker gene.

Table S2. Primers used for in vivo tagging.

Name	Sequence	Description
MFM216	AATTGAACTTCAAGGAATGGCAA AAGGCTTTCACTGACGTTATGGG TTAACGTACGCTGCAGGTCGAC	Forward primer used to create PCR fragments containing terminator regions from pAA207, pAA208, pAA209, and pMFM065 with homology to mNeongreen ORF 3' end.
MFM193	ATATACACATGTATATATATCGTA TGCTGCAGCTTTAAATAATCGGT GTCATCGATGAATTCGAGCTCG	Reverse primer used to create PCR fragments containing terminator regions from pAA207, pAA208, pAA209, and pMFM065 with homology to *HIS3* 3' UTR.
AA491	CTGAGGCGTTATAGGTTCAATTT GGTAATTAAAGATAGAGTTGTAA GATGCGTACGCTGCAGGTCGAC	Forward primer used to create PCR fragments containing TetO7 promoter from pAA263 with homology to *SST2* promoter region.
AA492	CTGCTGAAATTTTTGGAAGATAA TTCATGCAACGTCCTATTTTTATC CACCATCGATGAATTCTCTGTCG	Reverse primer used to create PCR fragments containing TetO7 promoter from pAA263 with homology to *SST2* ORF 5' end.
MFM184	CAGACGTATACAAAGATGCTAGC GCTTTAATAGAAATCCAAGAAAA GTGCCGTACGCTGCAGGTCGAC	Forward primer used to create PCR fragments containing TetO7 promoter from pMFM058 with homology to *SST2* ORF 3' end.
MFM185	ACTGTTTGTGCAATTGTACCTGAA GATGAGTAAGACTCTCAATGAAA TTAATCGATGAATTCGAGCTCG	Reverse primer used to create PCR fragments containing TetO7 promoter from pMFM058 with homology to *SST2* 3' UTR.

Table S3. Strains used in this study. All *Saccharomyces cerevisiae* strains described here are derivatives of SEY6210a (*MATa leu2-3,112 ura3-52 his3Δ200 trp1Δ901 lys2-801 suc2Δ9*).

Strain	Relevant genotype	Description
YMFM034	*leu2Δ::*[P_{GPD}-*mTurquoise2*-T_{GPD}:*LEU2*] *lys2Δ::*[P_{ADH1}-*rtTA*-T_{ADH1}:*LYS2*]	Strain with constitutively expressed *mTurquoise2* and the reverse tetracycline trans-activator *rtTA*.
YMFM048	*his3Δ::*[$P_{TetO7\text{-}ACT1}$-*mNeongreen*-T_{sTRSV}:*CgHIS3*]	Derivative of YMFM034 with mNeongreen driven by inducible promoter and inactive ribozyme 3' RR.
YMFM049	*his3Δ::*[$P_{TetO7\text{-}ACT1}$-*mNeongreen*-$T_{L2b8\text{-}t47\text{-}T(Synth27)}$:*CgHIS3*]	Derivative of YMFM034 with mNeongreen driven by inducible promoter and "Ribo 2" 3' RR.
YMFM050	*his3Δ::*[$P_{TetO7\text{-}ACT1}$-*mNeongreen*-$T_{L2b8\text{-}a1\text{-}t41\text{-}T(Synth27)}$:*CgHIS3*]	Derivative of YMFM034 with mNeongreen driven by inducible promoter and "Ribo 1" 3' RR.
YMFM101	*his3Δ::*[$P_{TetO7\text{-}ACT1}$-*mNeongreen*-T_{FZF1}:*HPH*]	Derivative of YMFM034 with mNeongreen driven by inducible promoter and *FZF1* 3' RR.
YMFM102	*his3Δ::*[$P_{TetO7\text{-}ACT1}$-*mNeongreen*-T_{GIC1}:*HPH*]	Derivative of YMFM034 with mNeongreen driven by inducible promoter and *GIC1* 3' RR.
YMFM103	*his3Δ::*[$P_{TetO7\text{-}ACT1}$-*mNeongreen*-T_{TPS1}:*HPH*]	Derivative of YMFM034 with mNeongreen driven by inducible promoter and *TPS1* 3' RR.
YMFM104	*his3Δ::*[$P_{TetO7\text{-}ACT1}$-*mNeongreen*-T_{ADH1}:*natNT*]	Derivative of YMFM034 with mNeongreen driven by inducible promoter and *ADH1* 3' RR.
YAA328	*LYS2::rtTA mfα2Δ::*[$_{PTetO7\text{-}ACT1}$-*mCherry*-T_{ADH1}:*CgHIS3*] *mfα1Δ::klTRP1 leu2Δ::*[P_{GPD}-*mTurquoise2*-T_{GPD}:*CgLEU2*] *bar1Δ::*[P_{FUS1}-*mNeongreen*-T_{FUS1}:*URA3*]	Strain with constitutively expressed *mTurquoise2* and *mNeongreen* pathway activity reporter.
YMFM074	[*kanMX:*$P_{TetO7\text{-}ACT1}$]*::SST2::*[$T_{L2b8\text{-}a1\text{-}t41\text{-}T(Synth27)}$:*natNT*]	Derivative of YAA328 with *SST2* driven by inducible promoter and "Ribo 2" 3' RR.