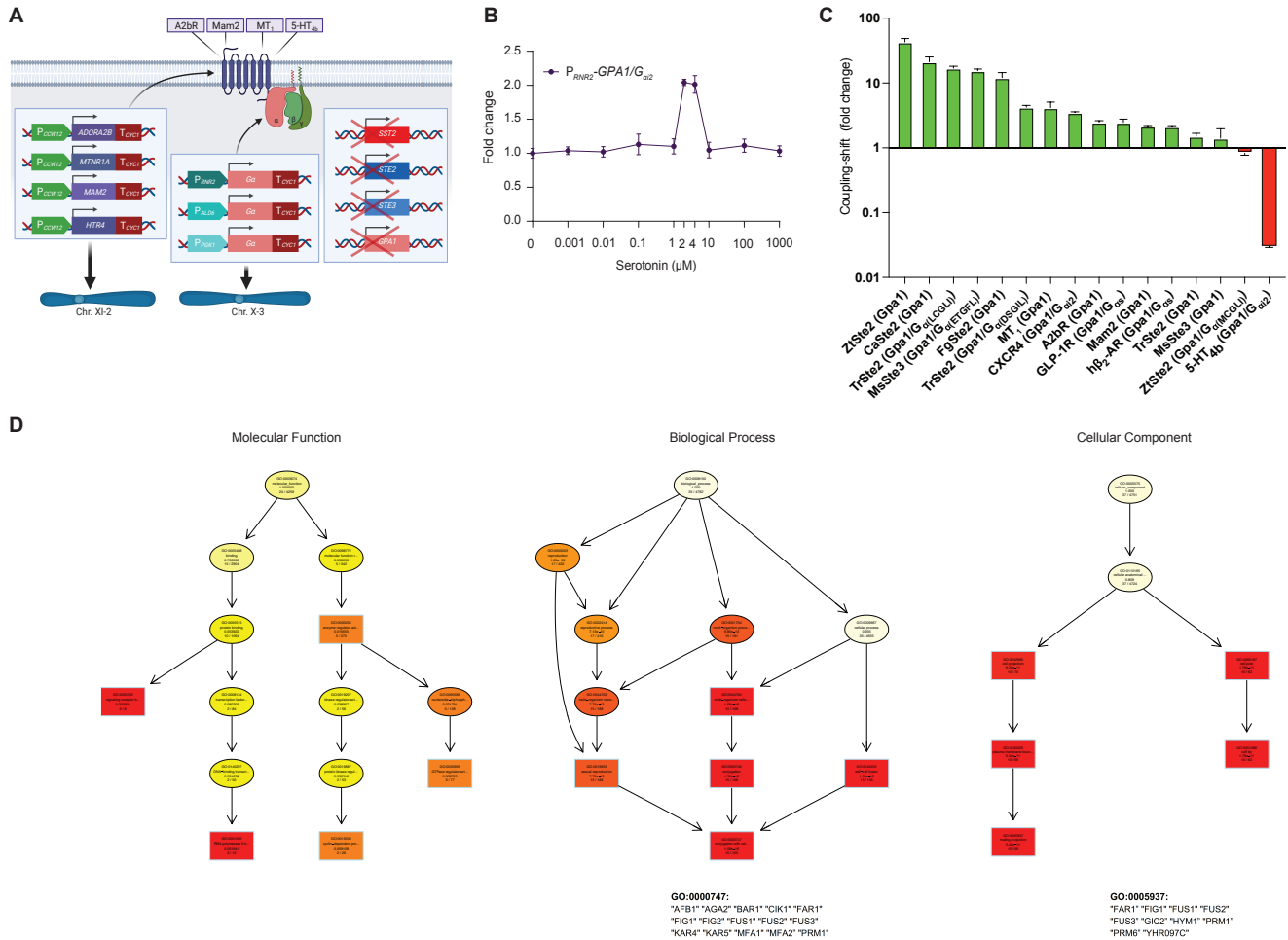
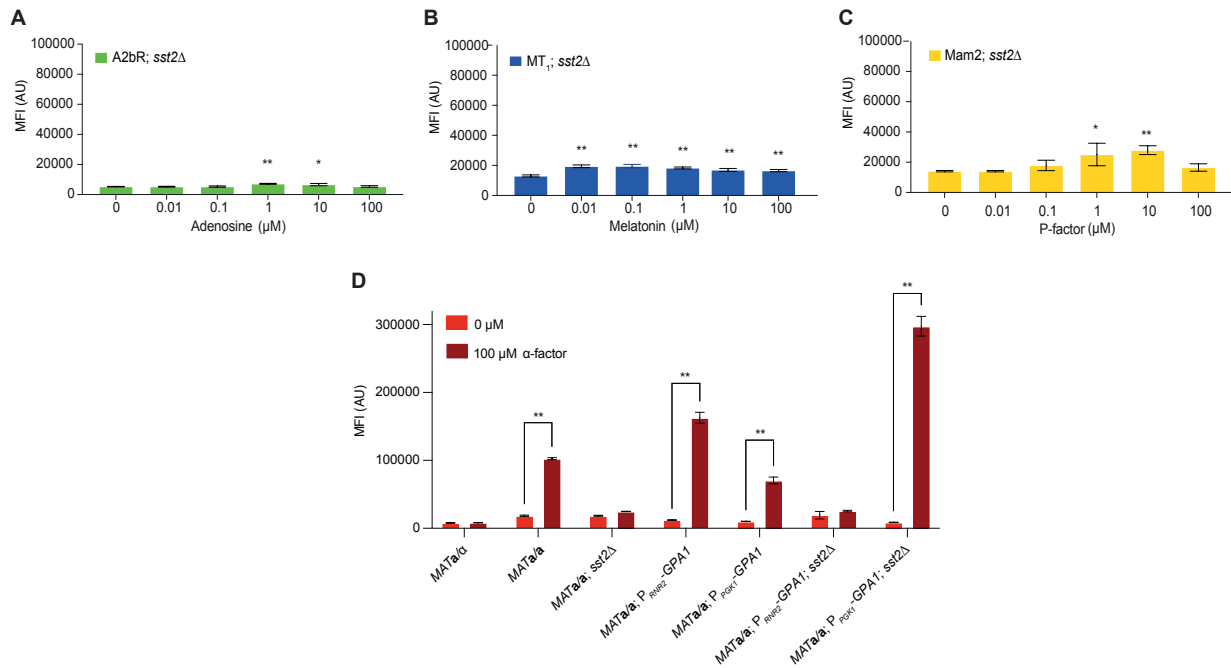
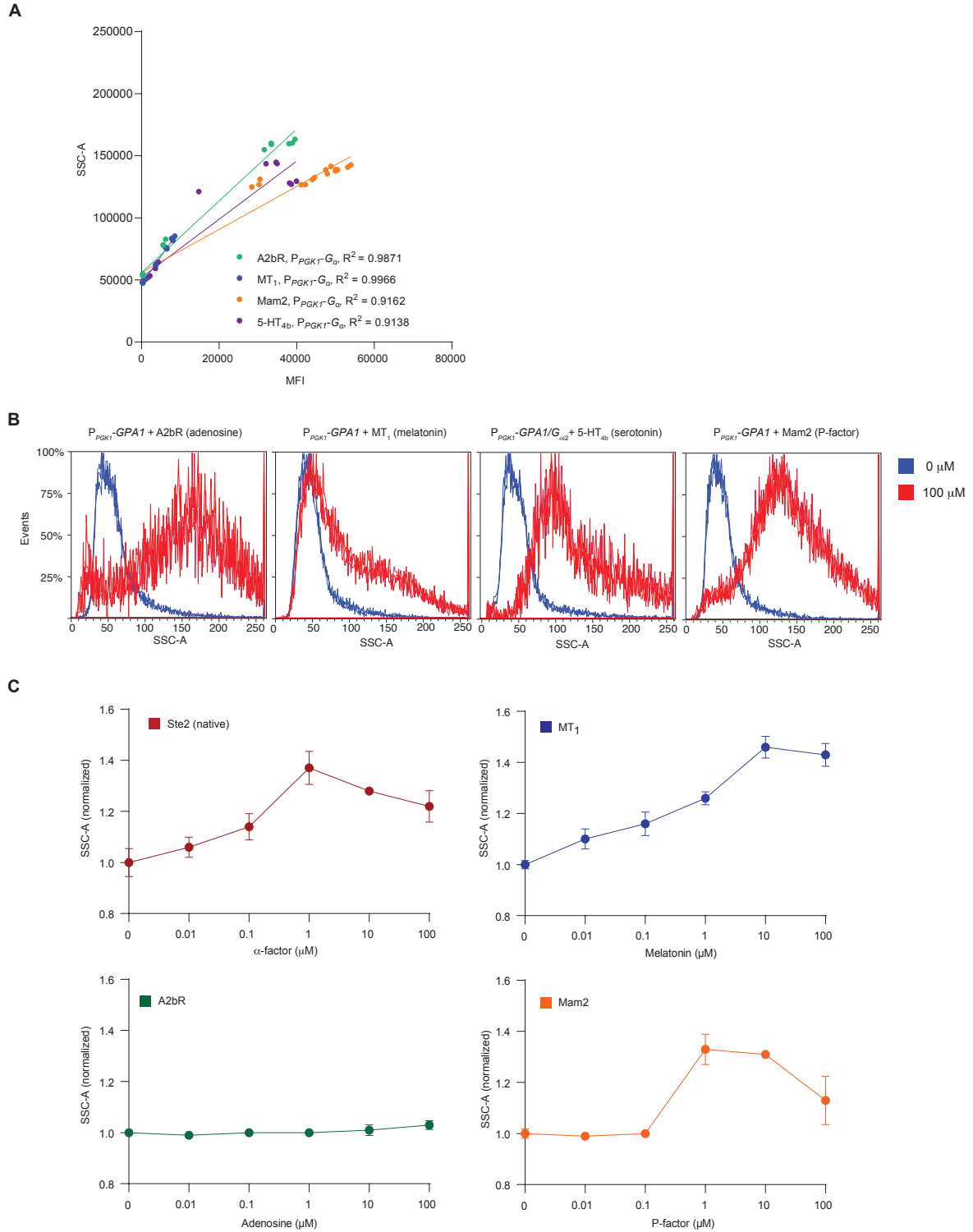




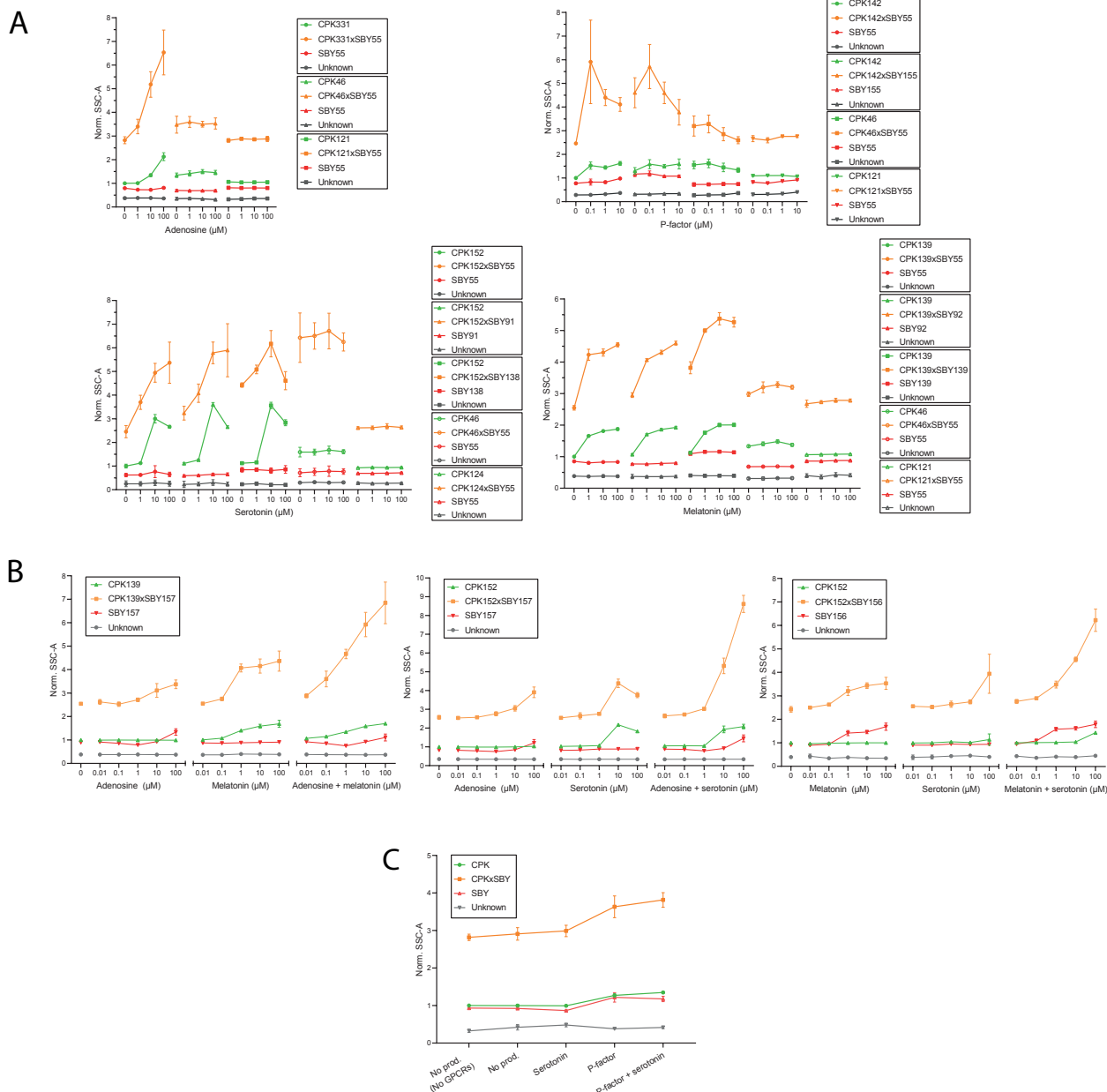
Supplementary Figure S1. A. Schematic overview on engineering of biosensing yeast strains. Heterologous GPCRs (hGPCRs) are overexpressed from the *CCW12* promoter and integrated in the genome, and the *CYC1* terminator attenuated transcription in all designs. Genome-integrated G_{α} subunits were expressed from the *RNR2*, *ALD6*, or *PGK1* promoters. Four native genes, *SST2*, *STE2*, *STE3*, and *GPA1*, were genetically deleted, and G_{β} and G_{γ} subunits are illustrated in a trimeric complex with G_{α} subunit. **B.** Fold change from 0-1000 μ M serotonin supplementation in strain CPK165. **C.** Coupling-shifts are presented as log-scaled fold changes in fluorescence from a P_{FUS1} -GFP reporter following hGPCR integration (+hGPCR: strains SBY143, SBY146, CPK153, CPK156, CPK159, CPK165, and CPK450-459) over background (no hGPCR: SBY123, CPK131, CPK134, CPK343, CPK347, CPK350, and CPK424). Specific G_{α} subunits are indicated for each hGPCR presented in the plot. Means and standard deviations represent three biological replicates. **D.** The GO trees induced by the top 5 GO terms for “molecular function”, “biological process”, and “cellular component” respectively. Rectangles indicate the 5 most significant terms. The color represents the relative significance, ranging from dark red (most significant) to bright yellow (least significant). For each node, the first two lines show the GO information. The third line is the raw p-value, and the fourth line shows the number of significant genes and the total number of genes annotated to the respective GO term.



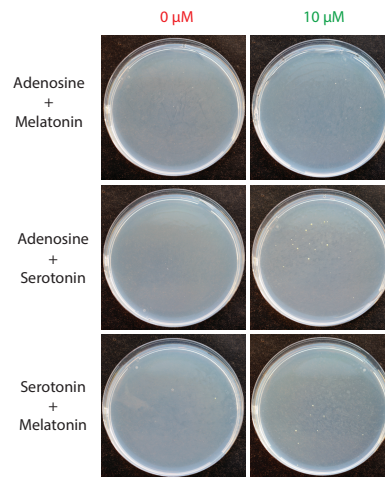
Supplementary Figure S2. A-C: Median fluorescence intensity (MFI) shown in artificial units (AU) from plasmid-based P_{FUS1} -GFP reporter expression in *S. boulardii sst2Δ* biosensing strains for **A.** A2bR sensing adenosine (SB45), **B.** MT₁ sensing melatonin (SB46), and **C.** Mam2 sensing P-factor (SB47). **D.** Mating pathway stimulation with α -factor in engineered *S. boulardii* strains: SB14 (MATa/ α), SB17 (MATa/a), SB36 (MATa/a; *sst2Δ*), SB40 (MATa/a; P_{RNR2}-GPA1), SB39 (MATa/a; P_{PGK1}-GPA1), SB38 (MATa/a; P_{RNR2}-GPA1; *sst2Δ*), and SB37 (MATa/a; P_{PGK1}-GPA1; *sst2Δ*). Means and standard deviations represent three biological replicates. Statistical significance was determined relative to no ligand supplementation (0 μ M) controls using one-way or two-way analysis of variance (ANOVA) for **(A-C)** and for **(D)**, respectively, using GraphPad Prism (* $p > 0.05$, ** $p \leq 0.01$).



Supplementary Figure S3. A. Side-scatter (SSC-A) as a linear function of median fluorescence intensity (MFI) for strains CPK155, CPK158, CPK161, and CPK167. **B.** Histograms showing increased SSC-A during hGPCR-signaling in strains CPK155, CPK158, CPK161, and CPK167 from incubation with cognate ligands (100 μM , red) as compared to no hGPCR-signaling (0 μM , blue). **C.** SSC-A changes with cognate ligand concentration in biosensing *S. boulardii* (SB17 and SB48-50). SSC-A is normalized internally for each biosensing strain to no ligand supplementation controls (0 μM).



Supplementary Figure S4. Alterations in cellular morphology during synthetic mating in the formation of diploids (CPKxSBY) and shmooing haploids (CPK and SBY), interpreted by side-scatter area (SSC-A). SSC-A was measured simultaneously with mating data acquisition. Cells are identified by flow cytometry (see Methods). SSC-A is normalized to the examined CPK-strain without supplementation or production of ligand. CPK46xSBY55 and CPK121xSBY55 or CPK124xSBY55 were used as references for positive and negative mating pair controls with relevant ligand supplementation, respectively. Results are presented as means with standard deviations determined from five biological replicates. **A.** Normalized SSC-A for semi-synthetic mating trials for adenosine in Fig. 4B, P-factor in Fig. 4C, melatonin in Fig. 4D, and serotonin in Fig. 4E. **B.** Normalized SSC-A for full synthetic mating trials for adenosine and melatonin in Fig. 5B, adenosine and serotonin in Fig. 5C, and melatonin and serotonin in Fig. 5D. **C.** Normalized SSC-A for full autonomous mating trials in Fig. 5E.



Supplementary Figure S5. Representative pictures of plated full synthetic mating trials for supplementation (10 μ M) of both ligands in combination, or no supplementation (0 μ M), for crosses with strains corresponding to CPK139xSBY157 (adenosine+melatonin), CPK152xSBY157 (adenosine+serotonin), and CPK152xSBY156 (melatonin+serotonin). CPK139 and CPK152 were transformed with plasmid pEDJ400 (*URA3*) prior to synthetic mating to allow for diploid selection on SC-UW. 95 μ l co-culture was plated on each SC-UW plate, and pictures were taken following incubation at 30 °C for 7 days.

Supplementary Table S4

Strain	Genotype	Plasmid(s)	Parental strain	Reference
yWS677	<i>MATα</i> ; <i>sst2</i> Δ 0; <i>far1</i> Δ 0; <i>bar1</i> Δ 0; <i>ste2</i> Δ 0; <i>ste12</i> Δ 0; <i>gpa1</i> Δ 0; <i>ste3</i> Δ 0; <i>mf</i> (<i>alpha</i>)1 Δ 0; <i>mf(alpha)</i> 2 Δ 0; <i>mfa1</i> Δ 0; <i>mfa2</i> Δ 0; <i>gpr1</i> Δ 0; <i>gpa2</i> Δ 0	N/A	N/A	Shaw et al. 2019
CEN.PK2-1C	<i>MATα</i> ; <i>his3</i> Δ 1; <i>leu2-3_112</i> ; <i>ura3-52</i> ; <i>trp1-289</i> ; <i>MAL2-8c</i> ; <i>SUC2</i>	N/A	N/A	EUROSCARF
BY4741	<i>MATα</i> ; <i>his3</i> Δ 1; <i>leu2</i> Δ 0; <i>met15</i> Δ 0; <i>ura3</i> Δ 0	N/A	N/A	EUROSCARF
SBY1	<i>MATα</i> ; <i>his3</i> Δ 1; <i>leu2</i> Δ 0; <i>met15</i> Δ 0; <i>ura3</i> Δ 0	pEDJ391	BY4741	This study
SBY2	<i>MATα</i> ; <i>his3</i> Δ 1; <i>leu2</i> Δ 0; <i>met15</i> Δ 0; <i>ura3</i> Δ 0; <i>sst2</i> Δ	pEDJ391	SBY1	This study
SBY3	<i>MATα</i> ; <i>his3</i> Δ 1; <i>leu2</i> Δ 0; <i>met15</i> Δ 0; <i>ura3</i> Δ 0; <i>sst2</i> Δ ; <i>ste3</i> Δ	pEDJ391	SBY2	This study
SBY4	<i>MATα</i> ; <i>his3</i> Δ 1; <i>leu2</i> Δ 0; <i>met15</i> Δ 0; <i>ura3</i> Δ 0; <i>sst2</i> Δ ; <i>ste2</i> Δ ; <i>ste3</i> Δ	pEDJ391	SBY3	This study
SBY16	<i>MATα</i> ; <i>his3</i> Δ 1; <i>leu2</i> Δ 0; <i>met15</i> Δ 0; <i>ura3</i> Δ 0; <i>sst2</i> Δ ; <i>ste2</i> Δ ; <i>ste3</i> Δ ; <i>fus1::GFP</i>	pEDJ391	SBY4	This study
SBY53	<i>MATα</i> ; <i>his3</i> Δ 1; <i>leu2</i> Δ 0; <i>met15</i> Δ 0; <i>ura3</i> Δ 0; <i>pTEF1-mRuby2-tADH1-XII-2</i>	pEDJ391	SBY1	This study

SBY55	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; pTEF1-mRuby2-tADH1-XII-2</i>	pEDJ391	SBY53	This study
SBY91	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 ; pTEF1-mRuby2-tADH1-XII-2; tADH1-HsDDC<-pTDH3-pTEF1->SmTPH-tCYC1-XI-3; tADH1-RnPTS<-pTEF1-PGK1p->RnSPR-tCYC1-X-4; tADH1-PaPCBD1<-pTEF1-PGK1p->RnDHPR-tCYC1-XII-4</i>	N/A	SBY55	This study
SBY92	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; pTEF1-mRuby2-tADH1-XII-2; tADH1-HsDDC<-pTDH3-pTEF1->SmTPH-tCYC1-XI-3; tADH1-RnPTS<-pTEF1-PGK1p->RnSPR-tCYC1-X-4; tADH1-PaPCBD1<-pTEF1-PGK1p->RnDHPR-tCYC1-XII-4; tADH1-HsASMT<-pTEF1-PGK1p->BtAANAT-tCYC1-X-2</i>	N/A	SBY91	This study
SBY104	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3</i>	pEDJ391	SBY4	This study
SBY108	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3</i>	pEDJ391	SBY16	This study
SBY116	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	SBY104	This study

SBY120	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; fus1:: GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	SBY108	This study
SBY123	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; fus1:: GFP; pRNR2-GPA1/Gas-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	SBY120	This study
SBY128	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; pPGK1- GPA1-tCYC1-X-3; gpa1Δ0; pTEF1- mKATE2-tCYC1-XII-2</i>	pEDJ391	SBY116	This study
SBY138	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 ; pTEF1-mRuby2-tADH1-XII-2; tADH1-HsDDC<-pTDH3-pTEF1->SmTPH- tCYC1-XI-3; tADH1-RnPTS<-pTEF1- PGK1p->RnSPR-tCYC1-X-4; tADH1- PaPCBD1<-pTEF1-PGK1p->RnDHPR- tCYC1-XII-4; Ty2-LoxP-KIURA3-TAG- pPGK1-SmTPH-tCYC1</i>	N/A	SBY91	This study
SBY139	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; pTEF1-mRuby2-tADH1-XII-2; tADH1-HsDDC<-pTDH3-pTEF1->SmTPH- tCYC1-XI-3; tADH1-RnPTS<-pTEF1- PGK1p->RnSPR-tCYC1-X-4; tADH1- PaPCBD1<-pTEF1-PGK1p->RnDHPR- tCYC1-XII-4; tADH1-HsASMT<-pTEF1- PGK1p->BtAANAT-tCYC1-X-2; Ty2-LoxP- KIURA3-TAG-pPGK1-SmTPH-tCYC1</i>	N/A	SBY92	This study

SBY143	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; fus1:: GFP; pRNR2-GPA1/Gas-tCYC1-X-3; gpa1Δ0; pCCW12:GLP-1R:tCYC1-XI-2</i>	N/A	SBY123	This study
SBY146	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; fus1:: GFP; pRNR2-GPA1/Gas-tCYC1-X-3; gpa1Δ0; pCCW12:ADRB2:tCYC1-XI-2</i>	N/A	SBY123	This study
SBY155	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0 ; pTEF1ro-mRuby2-XII-2; pTEF1- α-leader_P-factor-tCYC1</i>	N/A	SBY55	This study
SBY156	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; X-3- pPGK1:GPA1:tCYC1; gpa1Δ0 ; pTEF1- mKATE2-tCYC1-XII-2; pCCW12- MTNR1A-tCYC1-XI-2</i>	N/A	SBY128	This study
SBY157	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; X-3- pPGK1:GPA1:tCYC1; gpa1Δ0 ; pTEF1- mKATE2-tCYC1-XII-2; pCCW12- ADORA2B-tCYC1-XI-2</i>	N/A	SBY128	This study
SBY172	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; pPGK1- GPA1-tCYC1-X-3; gpa1Δ0 ; pTEF1- mKATE2-tCYC1-XII-2</i>	pEDJ391	SBY128	This study

SBY173	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0 ; pTEF1-mKATE2-tCYC1-XII-2; pCCW12-MAM2-tCYC1-XI-2</i>	N/A	SBY172	This study
SBY174	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0 ; pTEF1-mKATE2-tCYC1-XII-2; pCCW12-MAM2-tCYC1-XI-2; tADH1-HsDDC<-pTDH3-pTEF1->SmTPH-tCYC1-XI-3; tADH1-RnPTS<-pTEF1-PGK1p->RnSPR-tCYC1-X-4; tADH1-PaPCBD1<-pTEF1-PGK1p->RnDHPR-tCYC1-XII-4</i>	N/A	SBY173	This study
SBY175	<i>MATα; his3Δ1; leu2Δ0; met15Δ0; ura3Δ0; sst2Δ; ste2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0 ; pTEF1-mKATE2-tCYC1-XII-2; pCCW12-MAM2-tCYC1-XI-2; tADH1-HsDDC<-pTDH3-pTEF1->SmTPH-tCYC1-XI-3; tADH1-RnPTS<-pTEF1-PGK1p->RnSPR-tCYC1-X-4; tADH1-PaPCBD1<-pTEF1-PGK1p->RnDHPR-tCYC1-XII-4; Ty2-LoxP-KIURA3-TAG-pPGK1-SmTPH-tCYC1</i>	N/A	SBY174	This study
CPK1	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2</i>	pEDJ391	CEN.PK2-1C	This study
CPK2	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ</i>	pEDJ391	CPK1	This study

CPK3	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste3Δ</i>	pEDJ391	CPK2	This study
CPK4	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ</i>	pEDJ391	CPK3	This study
CPK16	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP</i>	pEDJ391	CPK4	This study
CPK46	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; pTEF1-GFP-tCYC1-XII-5</i>	N/A	CPK1	This study
CPK88	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0</i>	pEDJ391	CEN.PK2-1C	This study
CPK109	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1-tCYC1-X-3</i>	pEDJ391	CPK88	This study
CPK112	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1/Gai2-tCYC1-X-3</i>	pEDJ391	CPK88	This study

CPK115	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	CPK109	This study
CPK118	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1/Gai2-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	CPK112	This study
CPK119	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0; pTEF1-GFP-tCYC1-XII-5</i>	pEDJ391	CPK113	This study
CPK121	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; pTEF1-GFP-tCYC1-XII-5</i>	pEDJ391	CPK115	This study
CPK124	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1/Gai2-tCYC1-X-3; gpa1Δ0; pTEF1-GFP-tCYC1-XII-5</i>	pEDJ391	CPK118	This study
CPK125	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3</i>	pEDJ391	CPK16	This study

CPK126	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pALD6-GPA1-tCYC1-X-3</i>	pEDJ391	CPK16	This study
CPK127	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pPGK1-GPA1-tCYC1-X-3</i>	pEDJ391	CPK16	This study
CPK128	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gai2-tCYC1-X-3</i>	pEDJ391	CPK16	This study
CPK129	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pALD6-GPA1/Gai2-tCYC1-X-3</i>	pEDJ391	CPK16	This study
CPK130	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pPGK1-GPA1/Gai2-tCYC1-X-3</i>	pEDJ391	CPK16	This study
CPK131	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	CPK125	This study

CPK132	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pALD6-GPA1-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	CPK126	This study
CPK133	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	CPK127	This study
CPK134	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gai2-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	CPK128	This study
CPK135	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pALD6-GPA1/Gai2-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	CPK129	This study
CPK136	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pPGK1-GPA1/Gai2-tCYC1-X-3; gpa1Δ0</i>	pEDJ391	CPK130	This study
CPK139	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; sst2Δ0; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; pTEF1-GFP-tCYC1-XII-5; pCCW12-MTNR1A-tCYC1-XI-2</i>	N/A	CPK121	This study

CPK142	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; pTEF1-GFP-tCYC1-XII-5; pCCW12-MAM2-tCYC1-XI-2</i>	N/A	CPK121	This study
CPK152	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1/Gai2-tCYC1-X-3; gpa1Δ0; pTEF1-GFP-tCYC1-XII-5; pCCW12-HTR4-tCYC1-XI-2</i>	N/A	CPK124	This study
CPK153	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-MTNR1A-tCYC1-XI-2</i>	N/A	CPK131	This study
CPK154	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pALD6-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-MTNR1A-tCYC1-XI-2</i>	N/A	CPK132	This study
CPK155	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-MTNR1A-tCYC1-XI-2</i>	N/A	CPK133	This study

CPK156	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-MAM2-tCYC1-XI-2</i>	N/A	CPK131	This study
CPK157	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pALD6-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-MAM2-tCYC1-XI-2</i>	N/A	CPK132	This study
CPK158	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-MAM2-tCYC1-XI-2</i>	N/A	CPK133	This study
CPK159	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-ADORA2B-tCYC1-XI-2</i>	N/A	CPK131	This study
CPK160	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pALD6-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-ADORA2B-tCYC1-XI-2</i>	N/A	CPK132	This study

CPK161	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-ADORA2B-tCYC1-XI-2</i>	N/A	CPK133	This study
CPK165	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gai2-tCYC1-X-3; gpa1Δ0; pCCW12-HTR4-tCYC1-XI-2</i>	N/A	CPK134	This study
CPK166	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pALD6-GPA1/Gai2-tCYC1-X-3; gpa1Δ0; pCCW12-HTR4-tCYC1-XI-2</i>	N/A	CPK135	This study
CPK167	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pPGK1-GPA1/Gai2-tCYC1-X-3; gpa1Δ0; pCCW12-HTR4-tCYC1-XI-2</i>	N/A	CPK136	This study
CPK331	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; pTEF1-GFP-tCYC1-XII-5; pCCW12-ADORA2B-tCYC1-XI-2</i>	N/A	CPK121	This study

CPK343	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gα (LCGLI)-tCYC1-X-3; gpa1Δ0</i>	N/A	CPK131	This study
CPK347	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gα (DSGIL)-tCYC1-X-3; gpa1Δ0</i>	N/A	CPK131	This study
CPK350	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gα (ETGFL)-tCYC1-X-3; gpa1Δ0</i>	N/A	CPK131	This study
CPK424	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gα (MCGLI)-tCYC1-X-3; gpa1Δ0</i>	N/A	CPK131	This study
CPK448	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; ste3Δ0; ste2Δ0; sst2Δ0; pPGK1-GPA1/Gai2-tCYC1-X-3; gpa1Δ0; pTEF1-GFP-tCYC1-XII-5; pCCW12-HTR4-tCYC1-XI-2; pTEF1-α-leader_P-factor-tCYC1</i>	N/A	CPK152	This study
CPK450	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-CaSTE2-tCYC1-XI-2</i>	N/A	CPK131	This study

CPK451	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-FgSTE2-tCYC1-XI-2</i>	N/A	CPK131	This study
CPK452	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-ZtSTE2-tCYC1-XI-2</i>	N/A	CPK131	This study
CPK453	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-TrSTE2-tCYC1-XI-2</i>	N/A	CPK131	This study
CPK454	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0; pCCW12-MsSTE3-tCYC1-XI-2</i>	N/A	CPK131	This study
CPK455	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gai2-tCYC1-X-3; gpa1Δ0; pCCW12:CXCR4:tCYC1-XI-2</i>	N/A	CPK134	This study

CPK456	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gα (MCGLI)-tCYC1-X-3; gpa1Δ0; pCCW12-ZtSTE2-tCYC1-XI-2</i>	N/A	CPK424	This study
CPK457	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gα (LCGLI)-tCYC1-X-3; gpa1Δ0; pCCW12-TrSTE2-tCYC1-XI-2</i>	N/A	CPK343	This study
CPK458	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gα (DSGIL)-tCYC1-X-3; gpa1Δ0; pCCW12-TrSTE2-tCYC1-XI-2</i>	N/A	CPK347	This study
CPK459	<i>MATα; his3D1; leu2-3_112; ura3-52; trp1-289; MAL2-8c; SUC2; sst2Δ; ste2Δ; ste3Δ; fus1::GFP; pRNR2-GPA1/Gα (ETGFL)-tCYC1-X-3; gpa1Δ0; pCCW12-MsSTE3-tCYC1-XI-2</i>	N/A	CPK350	This study
Sb.MYA-796	<i>MATα/α</i>	N/A	N/A	ATCC MYA-796
DD277	<i>MATα/α; ura3Δ</i>	N/A	N/A	This study
DD313	<i>MATα/α; ura3Δ; his3Δ</i>	N/A	N/A	This study
SB4	<i>MATα/α; ura3Δ; his3Δ</i>	pDAM215	DD313	This study

SB5	<i>MATa/α; ura3Δ; his3Δ; sst2Δ</i>	pDAM215	SB4	This study
SB6	<i>MATa/α; ura3Δ; his3Δ; sst2Δ; ste3Δ</i>	pDAM215	SB5	This study
SB8	<i>MATa/α; ura3Δ; his3Δ</i>	N/A	SB4	This study
SB9	<i>MATa/α; ura3Δ; his3Δ; sst2Δ</i>	N/A	SB5	This study
SB14	<i>MATa/α; ura3Δ; his3Δ</i>	pDAM194	Sb.MYA-796	This study
SB17	<i>MATa/α; ura3Δ; his3Δ</i>	pDAM194	SB8	This study
SB19	<i>MATa/α; ura3Δ; his3Δ; sst2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0</i>	pDAM215	SB6	This study
SB20	<i>MATa/α; ura3Δ; his3Δ; sst2Δ; ste3Δ; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0</i>	pDAM215	SB6	This study
SB21	<i>MATa/α; ura3Δ; his3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0</i>	pDAM215	SB4	This study
SB22	<i>MATa/α; ura3Δ; his3Δ; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0</i>	pDAM215	SB4	This study
SB23	<i>MATa/α; ura3Δ; his3Δ; sst2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM215	SB19	This study
SB24	<i>MATa/α; ura3Δ; his3Δ; sst2Δ; ste3Δ; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0</i>	N/A	SB20	This study

SB25	<i>MATa/a; ura3Δ; his3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM215	SB21	This study
SB26	<i>MATa/a; ura3Δ; his3Δ; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0</i>	N/A	SB22	This study
SB30	<i>MATa/a; ura3Δ; his3Δ; sst2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0</i>	N/A	SB19	This study
SB31	<i>MATa/a; ura3Δ; his3Δ; sst2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	N/A	SB23	This study
SB33	<i>MATa/a; ura3Δ; his3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	N/A	SB25	This study
SB35	<i>MATa/a; ura3Δ; his3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0</i>	N/A	SB21	This study
SB36	<i>MATa/a; ura3Δ; his3Δ; sst2Δ</i>	pDAM194	SB9	This study
SB37	<i>MATa/a; ura3Δ; his3Δ; sst2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0</i>	pDAM194	SB30	This study
SB38	<i>MATa/a; ura3Δ; his3Δ; sst2Δ; ste3Δ; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0</i>	pDAM194	SB24	This study
SB39	<i>MATa/a; ura3Δ; his3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0</i>	pDAM194	SB35	This study

SB40	<i>MATa/a; ura3Δ; his3Δ; pRNR2-GPA1-tCYC1-X-3; gpa1Δ0</i>	pDAM194	SB26	This study
SB41	<i>MATa/a; ura3Δ; his3Δ; sst2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM194	SB31	This study
SB42	<i>MATa/a; ura3Δ; his3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM194	SB33	This study
SB45	<i>MATa/a; ura3Δ; his3Δ; sst2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM194+pDAM71	SB41	This study
SB46	<i>MATa/a; ura3Δ; his3Δ; sst2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM194+pDAM72	SB41	This study
SB47	<i>MATa/a; ura3Δ; his3Δ; sst2Δ; ste3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM194+pDAM74	SB41	This study
SB48	<i>MATa/a; ura3Δ; his3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM194+pDAM71	SB42	This study
SB49	<i>MATa/a; ura3Δ; his3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM194+pDAM72	SB42	This study
SB50	<i>MATa/a; ura3Δ; his3Δ; pPGK1-GPA1-tCYC1-X-3; gpa1Δ0; ste2Δ</i>	pDAM194+pDAM74	SB42	This study

Supplementary Table S5

Plasmid	Design	Plasmid marker	Backbone	Origin of replication	Reference
pEDJ391	pTEF1- <i>Cas9</i> -tCYC1	<i>LEU2</i>	N/A	CEN/ARS	Jensen et al. 2021
pEDJ400	USER cloning site	<i>URA3</i>	N/A	2μ	Jensen et al. 2021
pEDJ437	USER cloning site	<i>HIS3</i>	N/A	2μ	Jensen et al. 2021
pDAM1	gRNA_ <i>STE2</i> (TACAGTTTGAAACCA AACCA)	<i>NatR</i>	pCfB3050	2μ	This study
pDAM2	gRNA_ <i>STE3</i> (GATATTGGTGTACCT ACACA)	<i>NatR</i>	pCfB3050	2μ	This study
pDAM3	gRNA_ <i>FUS1</i> (AGAAGTATAACGACA CCCAG)	<i>NatR</i>	pCfB3050	2μ	This study
pDAM4	gRNA_ <i>SST2</i> (ATCCCAACCCACGCTTG GACA)	<i>NatR</i>	pCfB3050	2μ	This study
pDAM6	gRNA_ <i>GPA1-C-term</i> (GTTTTGCTGGATGATT AGAT)	<i>NatR</i>	pCfB3050	2μ	This study
pDAM7	gRNA_ <i>STE2</i> (TACAGTTTGAAACCA AACCA)	<i>URA3</i>	pEDJ400	2μ	This study

pDAM8	gRNA_ <i>STE3</i> (GATATTGGTGTACCT ACACA)	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM22	gRNA_X-4_XI-3_XII-4	<i>NatR</i>	pTAJAK-71	2μ	This study
pDAM23	<i>tADH1-RnPTS</i> <- <i>pTEF1</i> - <i>pPGK1</i> -> <i>RnSPR-tCYC1</i> (X-4)	Markerfree	pCfB3035	N/A	This study
pDAM24	<i>tADH1-PaPCBD1</i> <- <i>pTEF1-pPGK1</i> - > <i>RnDHPR-tCYC1</i> (XII- 4)	Markerfree	pCfB3040	N/A	This study
pDAM25	<i>tADH1-HsASMT</i> <- <i>pTEF1-pPGK1</i> - > <i>BtAANAT-tCYC1</i> (X-2)	Markerfree	pCfB2899	N/A	This study
pDAM30	pPGK1- <i>GPA1</i> -tCYC1	<i>LEU2</i>	pRS415U	CEN/ARS	This study
pDAM32	pPGK1- <i>GPA1/Gai2</i> - tCYC1	<i>LEU2</i>	pRS415U	CEN/ARS	This study
pDAM47	pRNR2- <i>GPA1</i> -tCYC1	<i>LEU2</i>	pRS415U	CEN/ARS	This study
pDAM49	pRNR2- <i>GPA1/Gai2</i> - tCYC1	<i>LEU2</i>	pRS415U	CEN/ARS	This study
pDAM50	pALD6- <i>GPA1</i> -tCYC1	<i>LEU2</i>	pRS415U	CEN/ARS	This study
pDAM52	pALD6- <i>GPA1/Gai2</i> - tCYC1	<i>LEU2</i>	pRS415U	CEN/ARS	This study

pDAM71	pCCW12: <i>ADORA2B</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM72	pCCW12: <i>MTNR1A</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM74	pCCW12: <i>MAM2</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM76	pCCW12: <i>HT4R</i> :tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM77	gRNA_ <i>GPA1</i> (GCATCACATCAATAAT CCAG) - promoter target	<i>NatR</i>	pDAM1	2μ	This study
pDAM78	gRNA_ <i>GPA1</i> (CTGTTTCCGAAGATG CAAGA) - terminator target	<i>NatR</i>	pDAM1	2μ	This study
pDAM82	gRNA_ <i>GPA1</i> _promote r+terminator target	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM122	pTEF1- <i>mKate2</i> -tCYC1	Markerfree	pCfB3039	N/A	This study
pDAM123	pTEF1- <i>α-leader_P-</i> <i>factor</i> -tCYC1	Markerfree	pCfB2899	N/A	This study
pDAM182	gRNA_ <i>MATα</i> (CAAATCATACAGAAA CACAG)	<i>HIS3</i>	pEDJ437	2μ	This study

pDAM194	pFUS1- <i>yEGFP</i> -tFUS1	<i>URA3</i>	pJV452	CEN/ARS	This study
pDAM215	pTEF1- <i>Cas9</i> -tCYC1	<i>URA3</i>	pRS416U	CEN/ARS	This study
pDAM216	pCCW12: <i>ADRB2</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM217	pCCW12: <i>CXCR4</i> : <i>tCYC1</i>	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM218	pCCW12: <i>GLP-1R</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM219	pCCW12: <i>CaSTE2</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM220	pCCW12: <i>FgSTE2</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM221	pCCW12: <i>ZtSTE2</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM222	pCCW12: <i>TrSTE2</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
pDAM223	pCCW12: <i>MsSTE3</i> : tCYC1	<i>HIS3</i>	pEDJ437	2μ	This study
ID6911	gRNA_X-3	<i>URA3</i>	N/A	2μ	This study
PL_12_I3	gRNA_ <i>MATa</i> (ATCAATATCACCCCAA GCAC)	<i>URA3</i>	N/A	2μ	This study

pJV452	USER cloning site	<i>URA3</i>	pEDJ400	CEN/ARS	This study
pCfB2899	USER integration site (X-2)	Markerfree	N/A	N/A	Jessop-Fabre et al. 2016
pCfB3020	gRNA_X-2	<i>NatR</i>	N/A	2μ	Jessop-Fabre et al. 2016
pCfB3035	USER integration site (X-4)	Markerfree	N/A	N/A	Jessop-Fabre et al. 2016
pCfB3039	USER integration site (XII-2)	Markerfree	N/A	N/A	Jessop-Fabre et al. 2016
pCfB3040	USER integration site (XII-4)	Markerfree	N/A	N/A	Jessop-Fabre et al. 2016
pCfB3042	gRNA_X-4	<i>NatR</i>	N/A	2μ	Jessop-Fabre et al. 2016
pCfB3044	gRNA_XI-2	<i>NatR</i>	N/A	2μ	Jessop-Fabre et al. 2016
pCfB3045	gRNA_XI-3	<i>NatR</i>	N/A	2μ	Jessop-Fabre et al. 2016
pCfB3048	gRNA_XII-2	<i>NatR</i>	N/A	2μ	Jessop-Fabre et al. 2016
pCfB3049	gRNA_XII-4	<i>NatR</i>	N/A	2μ	Jessop-Fabre et al. 2016

pCfB3050	gRNA_XII-5	<i>NatR</i>	N/A	2μ	Jessop-Fabre et al. 2016
pCfB9221	<i>tADH1-HsDDC<- pTDH3-pTEF1- >SmTPH-tCYC1 (XI-3)</i>	Markerfree	N/A	N/A	Gift from Dr. Nicholas Milne
pCfB1248	<i>pX-4-LoxP-SpHIS5- PaPCBD1<-pTEF1- pPGK1->RnDHPR</i>	<i>SpHIS5</i>	N/A	N/A	Germann et al. 2016
pCfB1251	<i>pX-3-LoxP-KILEU2- RnPTS<-pTEF1-pPGK1- >RnSPR</i>	<i>KILEU2</i>	N/A	N/A	Germann et al. 2016
pCfB1252	<i>pXII-1-LoxP-KILEU2- HsASMT<-pTEF1</i>	<i>KILEU2</i>	N/A	N/A	Germann et al. 2016
pCfB2772	<i>Ty2::KIURA3-pPGK1- SmTPH-tCYC1</i>	N/A	N/A	N/A	Germann et al. 2016
pCfB2628	<i>pXI-5-LoxP-SpHIS5- HsDDC<-pTEF1- pPGK1->BtAANAT</i>	<i>SpHIS5</i>	N/A	N/A	Germann et al. 2016
pTAJAK-71	USER cloning site	<i>NatR</i>	N/A	2μ	Jakočiūnas et al. 2015
pRS416U	USER cloning site	<i>URA3</i>	N/A	CEN/ARS	Jensen et al. 2017
pRS415U	USER cloning site	<i>LEU2</i>	N/A	CEN/ARS	Jensen et al. 2017

pYR11	pTDH3:his6- <i>mKate2</i> - <i>MBP</i> -tIDP1 (XII-5)	N/A	N/A	N/A	Romero-Suarez et al. 2021
pDD110	pCCW12: <i>Cas9</i> :tENO2: pSNR52: <i>GFP</i> _dropout:tSUP1	<i>NatR</i>	N/A	CEN/ARS	This study
pDD111	<i>gRNA_URA3</i> (TAGCGGTTTGAAGCA GGCGG)	<i>NatR</i>	DD110	CEN/ARS	This study
pDD115	<i>gRNA_HIS3</i> (CATTTGTAATACGCTT TACT)	<i>NatR</i>	DD110	CEN/ARS	This study
pDD118	pTEF1: <i>Cas9</i> :tENO2	<i>NatR</i>	N/A	CEN/ARS	Durmusoglu et al. 2021

Supplementary Table S6

Oligo Name	Oligo Sequence
DAM1	TACAGTTTGAAACCAAACAGTTTTAGAGCTAGAAATAGCAAG
DAM2	GATATTGGTGACCTACACAGTTTTAGAGCTAGAAATAGCAAG
DAM3	AGAAGTATAACGACACCCAGGTTTTAGAGCTAGAAATAGCAAG
DAM4	ATCCCAACCACGCTTGGACAGTTTTAGAGCTAGAAATAGCAAG
DAM7	GTGGCTACTCTGATTAGTATG
DAM8	CGAAGGTCACGAAATTACTTTTTCAAAGCCGTAAATTTTGATTTTGATTCTTGGATATGG
DAM9	ACTTAAAAATGCACCGTTAAGAACCATATCCAAGAATCAAAATCAAAATTTACGGCTTTG
DAM10	GTTATCTCATTAGTAATCC
DAM13	GATTCGCCAAAGTATTCTTACC
DAM14	TACTCCTAGTCCAGTAAATATAATGCGACACTCTTGTTGGAAAATTTTGATAGTATTTTGC
DAM15	TGTAGGAAAGGCCAAAATACTATCAAAATTTTCCACAAGAGTGTCGCATTATATTTACTGG
DAM16	GAATATACTGACGTATCCATTG
DAM19	GCAAGTTATTACAGTCTTAC
DAM20	AATTGTACCTGAAGATGAGTAAGACTCTCAATGAACTTACAACCTCTATCTTTAATTACC
DAM21	GTTATAGGTTCAATTTGGTAATTAAGATAGAGTTGTAAGTTTCATTGAGAGTCTTACTC
DAM22	GCAAGAAAACACACTACCTG
DAM31	CGTGTCGGCGATGCAGAAGCG
DAM34	CCCGAGTATTTACCAGGGAG
DAM37	GAGTAGTTCGTACTGTTTTAAGGTTTTGCTGGATGATCAAATCCGTGACTGCACTCAATAC
DAM38	TGATCATCCAGCAAAACCTTAAACAGTACGAACTACTCTGAATCGCGTGCATTCATCCGC
DAM39	CATCTACACAATTAGCAAGG
DAM40	TAAAAAAGGAGTAGAAACATTTTGAAGCTATGGTGTGTGCcataacgcgttacacggaag
DAM41	ccctccttactgctctcctccgtgtaacgcgttatgGCACACACCATAGCTTCAAATG
DAM42	GATTAACCTCTCTCTTTGGACACCATTGTTTTTTGTAATTAAACTTAGATTAGATTGC
DAM43	AGCATAGCAATCTAATCTAAGTTTTAATTACAAAAACAATGGTGTCCAAAGGAGAGGAG
DAM44	aattcgctatttagaagtgtcaacaacgtatctacTTACTTATACAATTCATCCATACC
DAM45	CTGGCTTAGGCGGTGGTATGGATGAATTGTATAAGTAAGtagatacgttggtgacacttc
DAM46	gcagtacaaggacgcgttaagaaaaatttcgagagagtcggagcgacctcatgctatacc
DAM47	ggtatagcatgaggtcgctccgactctctcgaaattttcttaacgcgtccttgactgc
DAM48	AGCGAACGTAAGAGAGG

DAM53	CATCAACAACAGGGTCAGCAG
DAM54	ACAACACCAGTGAATAATTCTTCACCTTTAGACATTGTTTTTTGATTTTCAGAACTTG
DAM55	TGCTGGTATTACCCATGGTATGGATGAATTGTACAAATAATGAAAATAATATTGACGTTC
DAM56	CTGAAGTATCCTATATCAAC
DAM58	CGTGCGAUCGAAGAAGTACCTTCAAAGAATGG
DAM59	ATGACAGAUATCCGCTCTAACCGAAAAGG
DAM63	ACCTGCACUAAAACAATGTTGTTGGAAACACAAGATGCG
DAM64	ATCTGTCAUTCAGGATCCGAGTCCAACGCC
DAM65	ACCTGCACUAAAACAATGCAAGGTAATGGTTC
DAM66	ATCTGTCAUTCAGGATCCAACGGAGTC
DAM67	ACCTGCACUAAAACAATGAGGCAACCTTGGTGG
DAM68	ATCTGTCAUTCAGGATCCCGTCCAC
DAM76	ATCTGTCAUAAAACAATGAGATTCCTTC
DAM77	ACCTGCACUAAAACAATGAGGCAGGTTTGG
DAM78	ACCTGCACUTTACAAATTGGGCCTATCTGG
DAM80	CACGCGAUAGTGCAGGUATCCGCTCTAACCGAAAAGG
DAM81	TTCTCAAGCAAGGTTTTTCAG
DAM85	GTCTCTATTGGAAACTGAATGC
DAM115	CACGCGAUTTACAAATTGGGCCTATCTGG
DAM116	AGTGCAGGUGGTAATTGGACAAATAAATACGTGTATTAAG
DAM117	CGTGCGAUGAAACTAACTAAAAACCGTAC
DAM118	AGTGCAGGUTGTATTCTGATAGTATG
DAM160	ACCTGCACUAAAACAATGGATAAATTGGATGCTAATGTTTC
DAM162	ATCTGTCAUCCTCTCAATAGGGAAAACCTGGCC
DAM191	CGTGCGAUGCGGCCGCAACCAGGGCAAAGCAAATAAAAAG
DAM192	AGTGCAGGUTATTGATATAGTGTTTAAGCG
DAM209	caataggcaagaagtaggcgagagccgacatacgagactAGCCACAGCTGTGCATTACGC
DAM210	aataggcaagaagtaggcgagagccgacatacgagactGAAACTAACTAAAAACCGTAC
DAM211	acaataggcaagaagtaggcgagagccgacatacgagactCGAAGAAGTACCTTCAAAG
DAM212	cttttactagcatatcaatatccgtttcattgaaaagtggTTCTCAAGCAAGGTTTTTCAG
DAM218	GCATCACATCAATAATCCAGGTTTTAGAGCTAGAAATAGCAAG
DAM219	CTGTTTCCGAAGATGCAAGAGTTTTAGAGCTAGAAATAGCAAG

DAM235	GGAGAAGAAAAATAGTAATTTTTCTGGTGCTGCTGCTCCTTCTGTGATGCTAAAATACC
DAM236	AGTAGAAGCTATTCTACTGTAAATTGGTATTTTAGCATCACAGAAGGAGCAGCAGCACC
DAM288	ATCTGTCAUAAAACAATGGTTTCTGAACTCATCAAGG
DAM289	CACGCGAUTTATCTGTGTCCCACTTAGATGG
DAM335	ACCTGCACUAAAACAGAATTCAACGTTGGATC
DAM336	ATCTGTCAUTTATAGAAGAGAGTCGTTGG
DAM337	ACCTGCACUAAAACAATGTCCATACCGTTGCCGCTTC
DAM338	ATCTGTCAUTCAGGAGCTGTGAAAAGAGG
DAM339	ACCTGCACUAAAACAATGGCCGGAGCACCCG
DAM340	ATCTGTCAUTCATGAGCAAGAGGCCTGAC
DAM341	ACCTGCACUAAAACAATGAACATTAATTCTACGTTCATACC
DAM342	ATCTGTCAUCTATACACGTTTGATTGTAATCTGC
DAM343	ACCTGCACUAAAACAATGTCCAAAGAAGTATTCGACC
DAM344	ATCTGTCAUCTACAAGGGGGCACGTATCCTCTCCTCTCTCTG
DAM345	ACCTGCACUAAAACAATGGTAGTTACCGCACCTCCC
DAM346	ATCTGTCAUCTAATCGGACCTTACTGAGTAAGAACGCCC
DAM347	ACCTGCACUAAAACAATGGCCCCACACTTTG
DAM348	ATCTGTCAUCAATCTGTTACTAGTGACGCTGAAATTACG
DAM349	ACCTGCACUAAAACAATGTCAGGCTTTGCTAC
DAM350	ATCTGTCAUAGCGAAACGATTAGTTTTTCGTGTCATC
DAM418	TGATCATCCAGCAAAACCTTAAACTATGTGGCCTTATCTGAATCGCGTGCATTTCATCCGC
DAM419	GATAAGGCCACATAGTTTAAGGTTTTGCTGGATGATCAAATCCGTGACTGCACTCAATAC
DAM422	TGATCATCCAGCAAAACCTTAAAGATTCAGGGATTTTGTGAATCGCGTGCATTTCATCCGC
DAM423	CAAAATCCCTGAATCTTTAAGGTTTTGCTGGATGATCAAATCCGTGACTGCACTCAATAC
DAM426	TGATCATCCAGCAAAACCTTAAAGAAACGGGGTTCTTGTGAATCGCGTGCATTTCATCCGC
DAM427	CAAGAACCCCGTTTCTTTAAGGTTTTGCTGGATGATCAAATCCGTGACTGCACTCAATAC
DAM485	CAAATCATACAGAAACACAGGTTTTAGAGCTAGAAATAGCAAG
DAM550	TGATCATCCAGCAAAACCTTAAATGTGTGGGTTGATTTGAATCGCGTGCATTTCATCCGC
DAM551	AATCAACCCACACATTTTAAGGTTTTGCTGGATGATCAAATCCGTGACTGCACTCAATAC
DAM594	5/PHOS/GATCATTTATCTTCACTGCGGAGAAG
EDJ134	CGTGCGAUAGCCACAGCTGTGCATTACG
EDJ318	AGTGCAGGUTGTTTTATTTGTTGTAAAAAGTAG

EDJ325	CACGCGAUTCACACCTTCCTCTTCTTCTTGG
1564	CGTGCGAUGCACACACCATAGCTTC
1565	ATGACAGAUUTTGTAAATTAACCTTAG
TJOS-62	CGTGCGAUagggaacaaaagctggagct
TJOS-63	AGTGCAGGUagggaacaaaagctggagct
TJOS-64	ATCTGTCAUagggaacaaaagctggagct
TJOS-65	CACGCGAUtaactaattacatgactcga
TJOS-66	ACCTGCACUtaactaattacatgactcga
TJOS-67	ATGACAGAUtaactaattacatgactcga
MAB1	CGTGCGAUGGCAACATAGCAGTACGTCGCCAAA
ID904	CCGTGCAATACCAAAATCG
JV498	ttacaggcaagcgatccgtcACCTGCACTCAACGGAATGC
JV499	tttatagcacgtgatactccTTCAGGTGGCACTTTTCGGG
JV500	CCCGAAAAGTGCCACCTGAAGgagtatcacgtgctataaaaataatt
JV501	GCATTCCGTTGAGTGCAGGTgacggatcgcttgctgtaa
JV502	CGTGCGAUATAATCAGAACTCCAACAATAGTCA
JV503	CACGCGAUATTCACCAGACCCGCTCCT
JZ1	ctaattgtgtccgcgtttctaGTTTTAGAGCTAGAAATAGCAAG
MAD1	ATCAATATCACCCCAAGCACGTTTTAGAGCTAGAAATAGCAAG
PR_26_D7	TGTTACACTCTCTGGTAACTTAG
PR_26_D8	AAGATAAGAACAAGAATGATGCTAAG
PR_26_D8	AAGATAAGAACAAGAATGATGCTAAG
DDpr097	GCCGCCGTTGTTGTTTTT
DDpr098	TCATAACACAGTCCTTTCCCGC
DDpr254	GGTTCTGGCGAGGTATTGGATAGTTC
DDpr255	TGGCTGTGGTTTCAGGGTCCATAAAGC
DDpr262	gatcTAGCGGTTTGAAGCAGGCGG
DDpr263	aaacCCGCCTGCTTCAAACCGCTA
DDpr264	gatcCATTTGTAATACGCTTTACT
DDpr265	aaacAGTAAAGCGTATTACAAATG
DDpr273	CGACGTTGAAATTGAGGCTAC

DDpr274	CTACACGTTTCGCTATGCTTC
DDpr276	GCCGCCGTTGTTGTTATTGT
DDpr277	CTGTTCCCTAGCATGTACGT
5	acctgcacuttgtaattaaacttag
8	acctgcacuttgtaattaaacttag
350	CGTGCGAUTTATTCTCCTTTGTAGACCACAAT
389	CACGCGAUTTAAATGTCATAGAAGTCCACGTG
1564	CGTGCGAUGCACACACCATAGCTTC
1565	CGTGCGAUGCACACACCATAGCTTC
1761	atctgtcaUaaaacaatgagcaccgagcattcattg
1762	CACGCGAUTTAACGATCGCTATTACGACGCAGTG
2254	AGTGCAGGUAAAACAATGGGTAGCAGCGAAGATC
2255	CGTGCGAUTTATTTACGTGCCAGGATTGCATC
2149	CGTGCGAUTTACTTTCTACCTTCAGCAG
2153	CACGCGAUTTAGAAGTAAGCTGGAGTC

Supplementary Table S7

ID	Insert	Sequence
gDAM1	<i>GPA1/Gai2</i>	AAAACAATGGGGTGTACAGTGAGCACGCAAACAATAGGAGACGAAAG TGATCCTTTTCTACAGAACAAAAGAGCCAATGATGTCATCGAGCAATC GTTGCAGCTGGAGAAACAACGTGACAAGAATGAAATAAAACTGTTACT ATTAGGTGCCGGTGAGTCAGGTAAATCAACGGTTTTTAAACAATTAAA ATTATTACATCAAGGCGGTTTTCTCCCATCAAGAAAGGTTACAGTATGC TCAAGTGATACGGGCAGATGCCATACAATCAATGAAAATTTTGATTATT CAGGCCAGAAAACTAGGTATTCAACTTGACTGTGATGATCCGATCAAC AATAAAGATTTGTTTGCATGTAAGAGAATACTGCTAAAGGCTAAAGCTT TAGATTATATCAACGCCAGTGTTGCCGGTGTTCTGATTTTCTAAATG ATTATGTAAGTACTCAGAAAGGTATGAACTAGGAGGCGTGTTT AGAGTACCGGACGAGCAAAAGCTGCTTTTCGATGAAGACGGAAATATT TCTAATGTCAAAAGTGACACTGACAGAGATGCTGAAACGGTGACGCA AAATGAGGATGTTGATAGAAACAACAGTAGTAGAATTAACCTACAGGA TATTTGCAAGGACTTGAACCAAGAAGGCGATGACCAGATGTTTGTTAG AAAACATCAAGGGAAATTCAAGGACAAAATAGACGAAATCTTATTCA CGAAGACATTGCTAAGGCAATAAAGCAACTTTGGAATAACGACAAAGG TATAAAGCAGTGCTTTGCACGTTCTAATGAGTTTCAATTGGAGGGCTC AGCTGCATACTACTTTGATAACATTGAGAAATTTGCTAGTCCGAATTAT GTCTGTACGGATGAAGACATTTTGAAGGGCCGTATAAAGACTACAGG CATTACAGAAACCGAATTTAACATCGGCTCGTCCAAATTCAAGGTTCT CGACGCTGGTGGGCAGCGTTCTGAACGTAAGAAGTGGATTCAATTGTT TCGAAGGAATTACAGCAGTTTTATTTGTTTTAGCAATGAGTGAATACGA CCAGATGTTGTTTGAGGATGAAAGAGTGAACAGAATGCATGAATCAAT AATGCTATTTGACACGTTATTGAACTCTAAGTGGTTCAAAGATACACC GTTTATTTTGTGTTTTAAATAAAATTGATTTGTTTCGAGGAAAAGGTAAAA GCATGCCCATAAGAAAGTACTTTCCTGATTACCAGGGACGTGTCGGC GATGCAGAAGCGGGTCTAAAATATTTTGAGAAGATATTTTGAGCTTG AATAAGACAAACAAACCAATCTACGTGAAACGAACCTGCGCTACCGAT ACCCAAATATGAAGTTCGTATTGAGTGCAGTCACGGATTTGATCATC CAGCAAAACCTTAAAGATTGCGGCCTGTTCTGA

gDAM2

ADORA2B

TTGTTGGAAACACAAGATGCGTTGTATGTTGCGTTGGAATTGGTAATT
GCTGCGCTATCGGTTGCGGGAAATGTTTTGGTTTGTGCTGCGGTTGG
AACGGCGAATACCTTGCAAACGCCTACTAATTATTTTTGGTTTCATTG
GCAGCGGCTGATGTTGCTGTTGGACTATTTGCTATTCCTTTTCGCTATT
ACTATTTCTTTGGGATTTTGTACCGATTTTATGGATGTCTATTTCTAG
CTTGTTTTGTTTTGGTTCTAACGCAATCTTCAATTTTTCTCTATTGGCT
GTTGCCGTAGATAGGTATTTGGCTATTTGCGTACCGCTAAGGTACAAG
TCCCTTGTAACGGGAACTCGTGCCAGGGGAGTAATAGCAGTACTATG
GGTACTAGCTTTTCGGAATTGGACTTACCCCTTTTTTGGGATGGAATTC
CAAGGATTCCGCTACTAATAATTGTACAGAGCCTTGGGACGGAACTAC
GAACGAGTCTTGTTGTCTAGTTAAATGCCTATTCGAAAACGTTGTACC
TATGTCTTATATGGTGTACTTTAACTTTTTTCGGATGCGTGTTGCCTCCT
TTGCTAATCATGTTGGTTATTTATATAAAAATTTTTTTGGTTGCTTGTAG
GCAACTACAACGTACCGAATTGATGGATCATTGAGGACTACTCTACA
AAGGGAAATTCACGCCGCTAAATCCTTGGCTATGATAGTTGGAATATT
CGCTTTGTGTTGGCTCCCTGTTACGCAGTGAATTGCGTAACCCTATT
TCAACCTGCACAAGGCAAGAACAAACCTAAATGGGCCATGAACATGG
CTATACTATTGTCCACGCTAACTCCGTGGTAAACCCTATAGTATACG
CATATAGGAATCGTGATTTTCGTTATACCTTCCATAAGATAATTTCAAG
GTACCTACTATGTCAGGCCGACGTAAAATCCGGAAACGGCCAAGCAG
GAGTGCAACCTGCACTAGGCGTTGGACTCGGATCC

gDAM3

MTNR1A

AAAACAATGCAAGGTAATGGTTCTGCTTTGCCAAATGCTTCTCAACCA
GTTTTGAGAGGTGATGGTGCTAGACCTTCTTGGTTGGCTTCTGCTTTA
GCTTGTGTTTTGATTTTCACCATCGTTGTCGATATCTTGGGTAACTTGT
TGGTTATCTTGTCCGTCTACCGTAACAAGAAATTGAGAAACGCTGGTA
ACATCTTCGTTGTTTCTTTGGCTGTTGCTGATTTGGTTGTTGCTATCTA
TCCATATCCACTGGTCTTGATGTCCATTTTTAACAACGGTTGGAAC TTG
GGTACTTGCAATTGTCAAGTTTCTGGTTTCTTGATGGGTTTGTCCGTTA
TTGGTTCCATTTTCAACATTACCGGTATCGCCATTAAACAGGTACTGTTA
CATTTGCCACTCACTGAAGTACGACAAGTTGTACTCTTCTAAGAACTC
CTTGTGCTACGTTTTGTTGATCTGGTTGTTAACTTTGGCTGCTGTTTTG
CCTAATTTGAGAGCTGGTACATTGCAATACGATCCAAGAATCTACTCT
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TTTTCCATTTTTTGGTCCCAATGATTATCGTCATCTTCTGCTACTTGAG
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GGTTTGGCAGTTGCTTCTGATCCAGCTTCTATGGTTCCAAGAATTCCA
GAATGGTTGTTGTTGCTTCTTACTACATGGCTTACTTCAACTCTTGTT
TGAACGCCATTATCTACGGCTTGTTGAATCAGAACTTTAGGAAAGAGT
ACAGGCGTATCATCGTTTCTTTGTGTACTGCTAGAGTTTTCTTCGTCG
ATTCCTCTAATGATGTTGCCGATAGAGTTAAGTGGAAACCATCTCCAT
TGATGACCAACAACAATGTTGTCAAGGTTGACTCCGTTGGATCCTGA

gDAM4

MAM2

AAAACAATGAGGCAACCTTGGTGGAAAGACTTTACTATTCCCGATGCA
AGCGCAATTATTCACCAAAATATTACCATTGTGTCTATTGTCTGGAGAG
ATTGAAGTGCCTGTTTCAACAATTGATGCATATGAAAGGGATAGGTTA
TTAACTGGAATGACTTTATCTGCCCAATTAGCTTTAGGAGTGTTAACCA
TTTTAATGGTTTGTCTGTTATCATCAAGCGAAAAACGTAAACACCCTGT
TTTTGTTTTTAATTCGGCAAGTATTGTTGCAATGTGTTTACGCGCCATT
TTAAATATCGTGACCATCTGCTCGAATTCGTACAGTATCCTCGTTAATT
ACGGGTTTATCTTAAACATGGTTCATATGTATGTGCATGTGTTTAATAT
TTAATTTTATTATTAGCACCGGTGATCATTTTTACTGCTGAGATGTCG
ATGATGATTCAAGTTAGGATCATTTGTGCACATGATAGGAAAACACAA
CGTATCATGACTGTTATTAGTGCCTGCTTAACTGTTTTAGTTCTCGCAT
TTTGGATTACTAACATGTGTCAACAGATTCAGTATCTCTTATGGTTAAC
TCCTTTATCGTCGAAAACCATTGTTGGATACTCTTGGCCCTACTTTATT
GCTAAAATCTTATTTGCTTTTTTCGATTATTTTTCACAGTGGTGTTTTTTC
ATACAAACTCTTTAGGGCCATCTTAATCCGCAAAAAAATTGGGCAATT
CCTTTTGGTCCGATGCAGTGTATTTTAGTTATTTTCGTGCCAATGTTTAA
TTGTTCTGCTACCTTTACTATCATCGATAGTTTTATCCATACGTATGA
TGGCTTTTCGTCTATGACTCAATGTCTCCTGATCATTTCTTTACCATT
TCGAGTTTATGGGCGTCTAGTACAGCTCTCAAATTACAATCGATGAAA
ACTTCATCTGCGCAAGGAGAAACACCGAGGTTTCGATTAGGGTTGA
TAGGACGTTTGATATCAAACATACTCCCAGTGACGATTATTCGATTTCT
GATGAATCTGAAACTAAAAAGTGGACGGGATCCTGA

gDAM5

HT4R

AAAACAATGGATAAATTGGATGCTAATGTTTCTTCTGAAGAAGGTTTTG
GTTCTGTTGAAAAAGTTGTTTTGTTGACTTTTTTGTCTACTGTTATTTTG
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GACAATTGAGAAAAATTAATACTAATTATTTTATTGTTTCTTTGGCTTTT
GCTGATTTGTTGGTTTCTGTTTTGGTTATGCCATTTGGTGCTATTGAAT
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ATTCTACTTATTGTGTTTTTATGGTTAATAAACCATATGCTATTACTTGT
TCTGTTGTTGCTTTTTATATTCCATTTTTGTTGATGGTTTTGGCTTATTA
TAGAATTTATGTTACTGCTAAAGAACATGCTCATCAAATTCAAATGTTG
CAAAGAGCTGGTGCTTCTTCTGAATCTAGACCACAATCTGCTGATCAA
CATTCTACTCATAGAATGAGAACTGAAACTAAAGCTGCTAAAACTTTGT
GTATTATTATGGGTGTTTTTGTGTTGGGCTCCATTTTTTGTACT
AATATTGTTGATCCATTTATTGATTATACTGTTCCAGGTCAAGTTTGGA
CTGCTTTTTTGTGGTTGGGTATATTAATTCTGGTTTGAATCCATTTTT
GTATGCTTTTTTGAATAAATCTTTTAGAAGAGCTTTTTTGATTATTTGT
GTTGTGATGATGAAAGATATAGAAGACCATCTATTTTGGGTCAAACCTG
TTCCATGTTCTACTACTACTATTAATGGTTCTACTCATGTTTTGAGAGA
TGCTGTTGAATGTGGTGGTCAATGGGAATCTCAATGTCATCCACCAGC
TACTTCTCCATTGGTTGCTGCTCAACCATCTGATACTTGA
AAAACAATGAGCAAAGGAGAAGAACTTTTCACTGGAGTTGTCCCAATT
CTTGTTGAATTAGATGGTGATGTTAATGGGCACAAATTTTCTGTCAGA
GGAGAGGGTGAAGGTGATGCTACAATCGGAAAACCTACCCTTAAATTT
ATTTGCACTACTGGAAAACCTACCTGTTCCATGGCCAACACTTGTCACT
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AAAAGGCATGACTTTTTCAAGAGTGCCATGCCCGAAGGTTATGTACAG
GAACGCACTATATCTTTCAAAGATGACGGGAAATACAAGACGCGTGCT
GTAGTCAAGTTTGAAGGTGATACCCTTGTTAATCGTATCGAGTTAAAG
GGTACTGATTTTAAAGAAGATGGAAACATTCTCGGACACAAACTCGAG
TACAACTTTAACTCACACAATGTATACATCACGGCAGACAAACAAAAG
AATGGAATCAAAGCTAACTTCACAGTTCGCCACAACGTTGAAGATGGT
TCCGTTCAACTAGCAGACCATTATCAACAAAATACTCCAATTGGCGAT
GGCCCTGTCCTTTTACCAGACAACCATTACCTGTGACACAAACTGTC
CTTTCGAAAGATCCCAACGAAAAGCGTGACCACATGGTCCTTCATGAG
TACGTAAATGCTGCTGGGATTACACATGGCATGGATGAGCTCTACAAA
TGA

gDAM6

yEGFP

gDAM7

ADRB2

AAAACAGAATTCAACGTTGGATCCAAGAATCAAAAATGTCTGATGCGG
CTCCTTCATTGAGCAATCTATTTTATGATGTGACGCAACAGAGAGACG
AGGTGTGGGTAGTCGGGATGGGTATCGTCATGAGCCTGATAGTTCTG
GCGATAGTCTTCGGAAACGTATTGGTGATTACAGCTATTGCGAAATTT
GAAAGACTACAAACCGTGACCAACTACTTCATTACTAGCCTAGCCTGT
GCTGATTTGGTAATGGGACTAGCGGTTGTACCGTTTGGCGCAGCGCA
TATTTTGATGAAGATGTGGACATTCGGTAATTTTGGTGTGAGTTTTGG
ACATCTATTGATGTACTTTGCGTGACCGCCTCAATCGAAACATTGTGT
GTTATTGCGGTGGACCGTTATTTTGCCATTACCAGCCCATTTAAGTAT
CAGTCCCTGCTAACAAAGAATAAGGCACGTGTTATAATACTTATGGTA
TGGATTGTCTCTGGCCTAACCTCATTCTGCCCATACAAATGCACTGG
TATCGTGCTACACATCAGGAGGCAATCAATTGTTACGCGAACGAGAC
GTGTTGCGACTTCTTCACAAACCAAGCGTATGCAATAGCTAGTTCCAT
AGTTTCATTTTACGTCCCTTTGGTAATAATGGTTTTTGTCTACTCCAGG
GTTTTCCAAGAAGCAAAGCGTCAGCTGCAAAAAATTGATAAATCAGAA
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gDAM9

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gDAM11

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gDAM13

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gDAM14

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gDAM15

α -leader_P-factor

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DDgb009

gRNA_URA3_KO

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DDgb008

pSNR52, GFP dropout, sgRNA, tSUP4

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agaccag

Supplementary Table S8

Heterologous GPCR	Uniprot ID	Species of origin	Gα used in study	Amino acid sequence
<i>MTNR1A</i>	P48039	Human	<i>GPA1</i>	MQGNGSALPNASQPVLRGDGARP SWLASALACVLIFTIVVDILGNLLVIL SVYRNKKLRNAGNIFVVS LAVADLV VAIYPYPLV LMSIFNNGWNLGYLHC QVSGFLMGLSVIGSIFNITGIAINRY CYICHSLKYDKLYSSKNSLCYVLLI WLLTLAAVLPNL RAGTLQYDPRIYS CTFAQSVSSAYTIAVVVFHFLVPMII VIFCYLRIWILVLQVRQRVKPDRKP KLKPQDFRN FVTMFVV FVLFAICWA PLNFIGLAVASDPASMVPRIPEWLF VASYYMAYFNSCLN AIYGLLNQNF RKEYRRIIVSLCTARVFFVDSSNDV ADRVKWKPSPLMTNNNVVKVDSV MLEETQDALYVALELVIAALSVAGN VLVCAAVGTANTLQTPTNYFLVSLA AADVAVGLFAIPFAITISLGFCTDFY GCLFLACFVLVLTQSSIFSLLAVAVD RYLAICVPLRYKSLVTGTRARGVIA VLWVLAFGIGLTPFLGWNSKDSAT NNCTEPWDGTTNESCCLVKCLFEN VVPMSYMVYFNFFGCVLPPLLIMLV IYIKIFLVACRQLQRTELM DHSRTTL QREIHA AKSLAMIVGIFALCWLPVH AVNCVTLFQPAQGKNKPKWAMNM AILLSHANSV VNP IVYAYRNRDFRY TFHKIISRYLLCQADV KSGNGQAGV QPALGVGL
<i>ADORA2B</i>	P29275	Human	<i>GPA1</i>	

<i>HT4R</i>	Q13639-1	Human	<i>GPA1/Gai2 (DCGLF)</i>	MDKLDANVSSEEGFGSVEKVLLT FLSTVILMAILGNLLVMVAVCWDRQ LRKIKTNYFIVSLAFADLLVSVLVMP FGAIELVQDIWIYGEVFCLVRTSLDV LLTTASIFHLCCISLDRYYAICCQPL VYRNKMTPLRIALMLGGCWVIPTFI SFLPIMQGWNNIGIIDLIEKRKFNQ SNSTYCVFMVNKPYAITCSVAFYI PFLLMVLAYRYIYVTAKEHAHQIQM LQRAGASSESRPQSADQHSTHRM RTETKAAKTLCIIMGCFCLCWAPFF VTNIVDPFIDYTPGQVWTAFLWLG YINSGLNPFYAFLNKSFRRAFLIILC CDDERYRRPSILGQTVPCSTTTING STHVLRDAVECGGQWESQCHPPA TSPLVAAQPSDT
<i>GLP-1R</i>	P43220	Human	<i>GPA1/Gas (QYELL)</i>	MAGAPGPLRLALLLLGMVGRAGPR PQGATVSLWETVQKWREYRRQCQ RSLTEDPPPATDLFCNRTFDEYAC WPDGEPGSFVNVSCPWYLPWASS VPQGHVYRFCTAEGWLQKDNSSL PWRDLSECEESKRGERSSPEEQLL FLYIIYTVGYALSFSALVIASAILLGF RHLHCTRNYIHLNLFASFILRALSVEI KDAALKWMYSTAAQQHQWDGLLS YQDSLSCRLVFLLMQYCVAANYYW LLVEGVYLYTLAFSVLSEQWIFRLY VSIGWGVPLLFPVWPWGIVKYLYEDE GCWTRNSNMNYWLIIRLPILFAIGV NFLIFVRVICIVVSKLKANLMCKTDIK CRLAKSTLTLIPLLGTHEVIFAFVMD EHARGTLRFIKLFTELSFTSFQGLM VAILYCFVNNEVQLEFRKSWERWR LEHLHIQRDSSMKPLKCPTSSLSSG ATAGSSMYTATCQASCS

<i>ADRB2</i>	P07550	Human	<i>GPA1/Gαs (QYELL)</i>	MGQPGNGSAFLLAPNGSHAPDHDVTQ ERDEVWVVGMGIVMSLIVLAIVFGNVL VITAIKFERLQTVTNYFITSACADLVM GLAVVPFGAAHILMKMWTFGNFWCEF WTSIDVLCVTASIELCVIAVDRIYFAITSP FKYQSLTKNKARVILMVWIVSGLTSFL PIQMHWYRATHQEAINCYANETCCDFF TNQAYAIASSIVSFYVPLVIMVFVYSRVF QEAQRQLQKIDKSEGRFHVQNLSQVEQ DGRGTGHGLRRSSKFCLKEHKALKTLGIIM GTFTLCWLPFFIVNIVHVIQDNLRKEVYI LLNWIGYVNSGFNPLIYCRSPDFRIAFQE LLCLRRSSLKAYGNGYSSNGNTGEQSGY HVEQEKENKLLCEDLPGTEDFVGHQGT VPSDNIDSQGRNCSTNDSLL
<i>CXCR4</i>	P61073-1	Human	<i>GPA1/Gαi2 (DCGLF)</i>	MEGISIYTSNDYTEEMGSGDYDSM KEPCFREANANFNKIFLPTIYSIIFLT GIVGNGLVILVMGYQKKLRSMTDKY RLHLSVADLLFVITLPFWAVDAVAN WYFGNFLCKAVHVIYTVNLYSSVLIL AFISLDRIYLAIVHATNSQRPRKLLAE KVVYVGVWIPALLLTIPDFIFANVSE ADDRYICDRFYPNDLWVVVFQFQH IMVGLILPGIVILSCYCIISKLSHSKG HQKRKALKTTVILILAFFACWLPYYI GISIDSFILLEIKQGCEFENTVHKWI SITEALAFFHCCLNPILYAFLGAKFK TSAQHALTSVSRGSSLKILSKGKRG GHSSVSTESESSSFHSS

MAM2	Q00619	<i>Schizosaccharomyces pombe</i>	GPA1	MRQPWWKDFTIPDASAIHQNITIVS IVGEIEVPVSTIDAYERDRLLTGMTL SAQLALGVLTIMVCLLSSEKRRKH PVFVFNSASIVAMCLRAILNIVTICSN SYSILVNYGFILNMVHMYVHVFNILI LLLAPVIFTAEMSMMIQVRIICAHDR KTQRIMTVISACLTVLVLAFWITNMC QQIQYLLWLTPLSKTIVGYSWPYFI AKILFAFSIIFHSGVFSYKLFRAILIRK KIGQFPFGPMQCILVISCQCLIVPAT FTIIDSFIHTYDGFSSMTQCLLIISLPL SSLWASSTALKLQSMKTSSAQGET TEVSIRVDRTFDIKHTPSDDYSISDE SETKKWT
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CaSTE2	A0A1D8PTB4	<i>Candida albicans</i>	GPA1	MNINSTFIPDKPGDIIISYSIPGLDQPI QIPFHSLDSFQTDQAKIALVMGITIG SCSMTLIFLISIMYKTNKLTNLKLKLK LKYILQWINQKIFTKKRNDNKQQQQ QQQQQIESSSYNNTTTTTSGSYKL FLFYLNSLILLIGIIRSGCYLNYNLGP LNSLSFVFTGWYDGSSFISSDVTN GFKCILYALVEISLGFQVYVMFKTS NLKIWGIMASLLSIGLGLIVVAFQINL TILSHIRFSRAISTNRSEEESSSSLS SDSVGYVINSIWMDLPTILFSISINIM TILLIGKLIIRTRRYLGLKQFDSFHI LLIGFSQTLIIPSIILVVHYFYLSQNKD SLLQQISLLLILMLPLSSLWAQTAN NTHNINSSPSLSFISRHHSFDSRS GGSNTIVSNGGSNGGGGGGNFPV SGIDAQLPPDIEKILHEDNNYKLLNS NNEVNDGDIINDEGMITKQITIKRV
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<i>FgSTE2</i>	I1RG07	<i>Fusarium graminearum</i>	<i>GPA1</i>	MSKEVFDPFQTQNVTFAPDGKTEIS IPVAAIDQVRRMMVNTTINYATQLG ACLIMLVLLVMVPKEKFRPFMIL QITSLVISCCRMLLLSIFHSSQFLDF YVFWGDDHSRIPRSAYAPSVAGNT MSLCLVISVETMLMSQAWTMVRLW PNVWKYIIAGVSLIVSIMAISVRLAYT IIQNNAVLKLEPAFHMFWLIKWTVIM NVAISWWCAIFNIKLVWHLISNRGI LPSYKTFTPMEVLMITNGILMIIPVIF ASLEWAHFVNFESASLTLTSAVIL PLGTLAAQRIASSAPSSANSTGASS GIRYGVSGPSSFTGFKAPSFSTGTT DRPHVSIYARCEAGTSSREHINPQ GVELAKLDPETDHHVRVDRAFLQR EERIRAPL
<i>ZtSTE2</i>	F9X131	<i>Zymoseptoria tritici</i>	<i>GPA1, GPA1/Gα (MCGLI)</i>	MVVVTAPPSVDRTYFIPNSTFDPYQQDLT LVYPDGVHALVANVDDIVYFMGLAVKS TLIFAIQIGISFVLMVLIALTKPERRVTLV FFLNMTALFTIFIRAILMCTTFVGTYYNFY NWIMGNYPNSGLADRVSIAAEVFAFLIIL SLELSMMFQVRIVCINLSSFRRRIITFSSIV VAMIVCTVRFALMVLSCDWIRVNIGDA TQEKNRINRVASGYNICTIASIIFNTIFV SKLAVAIAKHRRSMGMKQFGPMQIIFVM GCQTLIPAIFGIISYFALASTQVYSLMPM VVAIFLPLSSMWASFNTNKTNSVTNMR QPNVYRPNMIIIGQDTTQNSGKNTNISG TSNSTATTSSFASDKRRLNLSFNTQGTLV NSISEEEVNNPQKLGPSATVAVMDRDSL ELEMQRHGIAQGRSYSVRSD

<i>TrSTE2</i>	A0A022VRI2	<i>Trichophyton rubrum</i>	<i>GPA1, GPA1/Gα (LCGLI), GPA1/Gα (DSGIL)</i>	MAPHFDPFQQSVTFLRSDGTSFPISMA DFNKFMLYAVRTSIAAASQLGASVVMA VLLALLTAPDKRRSIVFYLNITTLLVNVCR TLSTTIFFTSSWVEIYTYFSGDYSRITTGAY ANSVMGTIATGIMVILIELSLLIQTHVLCS TLRDLYRNILLAWSCLVAAVPIAFRIA VANVKAIMTQSSLGKNVWIQSSSNISIT VSICYFSLLFLAKLGYAIYTRRLGMMKGFG VMQIIFIMACQTMILPAIMSILQYFIPEFE VNTNILTLLALSLPLTTLWSAAAVRHGH NKNQGSGRHFHWGGSSEKSLFDHNKTS GGFSHPTSTLIGSMPGPEKVK SADHFD RLYPELHDTGNITIERNFSVTSNRL MSGFATPIFAILAVIVSLLPVPSHWRARN FVILGLVFWLVVGNLNIFVNRIIWMSNA KNSAPIWCDLSVKLMSMASFSLPCSALLI SSKLYDIASLKYSKRTPEEKQRLWILELCI TVLPVFYSLTLVSQGHFRFNIVYGRGCEP AVYFSSVSIVIDYGIPVSLCITSLVYSVLSLR HFFIHKKDFDAILSKSGSGISTKKFLRMIS FALIDILINFPILLAAFALEVSYMKIIPYTS WDFVHKRFSDVWIYPRTAISSSRFKHFL TMTSFATWSQCMMGFIFFFMFGLNTDI KSDYVKAFMKVKGMFHLKTQKATREYK DDNICSTDDISDVSNHKSSEDNIHPDGV SVEFSHVDLEASGHEASNYSISIPSNDT KTNRFA
<i>MsSTE3</i>	A0A1M8A1X3	<i>Malassezia sympodialis</i>	<i>GPA1, GPA1/Gα (ETGFL)</i>	