Document S1. Supplementary Materials and Methods (Plasmid constructions for supporting information)

All plasmids used in this study are summarized in Table S2. All oligonucleotides are listed in Table S1. The bait proteins (X) were fused with a C-terminal ubiquitin moiety linked to an artificial transcription factor (X-Cub) in pBT3-C (Dualsystems Biotech AG, Schlieren, Switzerland). The prey proteins (Y) were fused with an N-terminal moiety of split-ubiquitin with I13G mutation (Y-NubG) in pPR3-C (Dualsystems Biotech AG).

**Bait vectors:** For bait vectors, several promoters exhibiting distinctive expression strength were substituted for the original weak CYC1 promoter of pBT3-C as follows. 

*PHO5* promoter (stronger than *CYC1* promoter) was PCR-amplified with oligonucleotides o1 and o2. The *SacII*-XbaI *PHO5* promoter was inserted at the *SacII*-XbaI site on pBT3-C, resulting in the plasmid pBPH3-C.

*TP11* promoter (stronger than *PHO5* promoter) was PCR-amplified with oligonucleotides o3 and o4. The *SacII*-XbaI *TP11* promoter was inserted at the *SacII*-XbaI site on pBT3-C, resulting in the plasmid pBTP3-C.

*TDH3* promoter (stronger than *TP11* promoter) was PCR-amplified with oligonucleotides o5 and o6. The *SacII*-XbaI *TDH3* promoter was inserted at the *SacII*-XbaI site on pBT3-C, resulting in the plasmid pBTD3-C.

**GPCR expression plasmids:** The bait and prey plasmids used for the expression of GPCRs were constructed as follows. Full length *STE2* genes encoding yeast pheromone receptor were PCR-amplified with oligonucleotide pairs: o7 and o8; o9 and o10. The *XbaI*-HindIII *STE2* gene fragments were inserted at the *XbaI*-HindIII site on pBT3-C, pBPH3-C, pBTP3-C and pBTD3-C, resulting in the plasmids pBT3-STE2, pBPH3-STE2, pBTP3-STE2 and pBTD3-STE2, respectively. The *SpeI*-EcoRI *STE2* gene fragment was inserted at the *SpeI*-EcoRI site on pPR3-C, resulting in the plasmid pPR3-STE2.

Truncated *STE2* genes that lack the C-terminal domain (Ste2ΔC) were PCR-amplified
with oligonucleotide pairs: o7 and o11; o9 and o12. The XbaI-HindIII STE2ΔC gene fragments were inserted at the XbaI-HindIII site on pBT3-C and pBTP3-C, resulting in the plasmid pBT3-STE2ΔC and pBTP3-STE2ΔC, respectively. The SpeI-EcoRI STE2ΔC gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-STE2ΔC.

Deletional STE2 genes that lack the domains from TM6 until C-terminal tail (TM1-5) were PCR-amplified with oligonucleotide pairs: o7 and o13; o9 and o14. The XbaI-HindIII TM1-5 gene fragment was inserted at the XbaI-HindIII site on pBT3-C, resulting in the plasmid pBT3-STE2TM1-5. The SpeI-EcoRI TM1-5 gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-STE2TM1-5.

Deletional STE2 genes that lack the domains from the N-terminal tail to TM5 and C-terminal domain (TM6-7) were PCR-amplified with oligonucleotide pairs: o15 and o11; o16 and o12. The XbaI-HindIII TM6-7 gene fragment was inserted at the XbaI-HindIII site on pBT3-C, resulting in the plasmid pBT3-STE2TM6-7. The SpeI-EcoRI TM6-7 gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-STE2TM6-7.

The HXT1 gene encoding glucose transporter was PCR-amplified with oligonucleotides o17 and o18. The SpeI-EcoRI HXT1 gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-HXT1.

GABBR1a genes encoding GABA\textsubscript{B1a} receptor were PCR-amplified with oligonucleotide pairs: o19 and o20; o21 and o22. The XbaI-HindIII GABBR1a gene fragment was inserted at the XbaI-HindIII site on pBTP3-C, resulting in the plasmid pBTP3-GABBR1a. The SpeI-EcoRI GABBR1a gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-GABBR1a.

GABBR2 genes encoding GABA\textsubscript{B2} receptor were PCR-amplified with oligonucleotide pairs: o23 and o24; o25 and o26. The XbaI-HindIII GABBR2 gene fragments were inserted at the XbaI-HindIII site on pBTP3-C and pBTD3-C, resulting in the plasmid pBTP3-GABBR2 and pBTD3-GABBR2, respectively. The SpeI-EcoRI GABBR2 gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-GABBR2.
AGTR1 genes encoding AT₁ (angiotensin type 1) receptor were PCR-amplified with oligonucleotide pairs: o27 and o28; o29 and o30. The XbaI-HindIII AGTR1 gene fragments were inserted at the XbaI-HindIII site on pBT3-C and pBTP3-C, resulting in the plasmid pBT3-AGTR1 and pBTP3-AGTR1, respectively. The SpeI-EcoRI AGTR1 gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-AGTR1.

AGTR2 gene encoding AT₂ (angiotensin type 2) receptor was PCR-amplified with oligonucleotides o31 and o32. The SpeI-EcoRI AGTR2 gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-AGTR2.

MTNR1A genes encoding MT₁ (melatonin 1A) receptor were PCR-amplified with oligonucleotide pairs: o33 and o34; o35 and o36. The XbaI-HindIII MTNR1A gene fragments were inserted at the XbaI-HindIII site on pBT3-C, resulting in the plasmid pBT3-MTNR1A and pBPH3-MTNR1A, respectively. The SpeI-EcoRI MTNR1A gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-MTNR1A.

MTNR1B gene encoding MT₂ (melatonin 1B) receptor was PCR-amplified with oligonucleotides o37 and o38. The SpeI-EcoRI MTNR1B gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-MTNR1B.

SSTR2 genes encoding somatostatin receptor 2 were PCR-amplified with oligonucleotide pairs: o39 and o40; o41 and o42. The XbaI-HindIII SSTR2 gene fragments were inserted at the XbaI-HindIII site on pBT3-C, pBPH3-C, pBTP3-C and pBTD3-C, resulting in the plasmid pBT3-SSTR2, pBPH3-SSTR2, pBTP3-SSTR2 and pBTD3-SSTR2, respectively. The SpeI-EcoRI SSTR2 gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-SSTR2.

SSTR5 genes encoding somatostatin receptor 5 were PCR-amplified with oligonucleotide pairs: o43 and o44; o45 and o46. The XbaI-HindIII SSTR5 gene fragment was inserted at the XbaI-HindIII site on pBTD3-C, resulting in the plasmid pBTD3-SSTR5. The SpeI-EcoRI SSTR5 gene fragment was inserted at the SpeI-EcoRI site on pPR3-C, resulting in the plasmid pPR3-SSTR5.

ADRB2 genes encoding β₂-adrenergic receptor were PCR-amplified with
oligonucleotide pairs: o47 and o48; o49 and o50. The *XbaI-HindIII* *ADRB2* gene fragments were inserted at the *XbaI-HindIII* site on pBT3-C, pBTP3-C and pBTD3-C, resulting in the plasmid pBT3-ADRB2, pBTP3-ADRB2 and pBTD3-ADRB2, respectively. The *SpeI-EcoRI* *ADRB2* gene fragment was inserted at the *SpeI-EcoRI* site on pPR3-C, resulting in the plasmid pPR3-ADRB2.

*HTR1A* genes encoding 5-hydroxytryptamine (serotonin) receptor 1A were PCR-amplified with oligonucleotide pairs: o51 and o52; o53 and o54. The *XbaI-HindIII* *HTR1A* gene fragments were inserted at the *XbaI-HindIII* site on pBPH3-C and pBTP3-C, resulting in the plasmid pBPH3-HTR1A and pBTP3-HTR1A, respectively. The *SpeI-EcoRI* *HTR1A* gene fragment was inserted at the *SpeI-EcoRI* site on pPR3-C, resulting in the plasmid pPR3-HTR1A.

*EDNRB* gene encoding endothelin receptor type B was PCR-amplified with oligonucleotides o55 and o56. The *SpeI-ClaI* *EDNRB* gene fragment was inserted at the *SpeI-ClaI* site on pPR3-C, resulting in the plasmid pPR3-EDNRB.

*NTSR1* gene encoding neurotensin receptor 1 was PCR-amplified with oligonucleotides o57 and o58. The *SpeI-EcoRI* *NTSR1* gene fragment was inserted at the *SpeI-EcoRI* site on pPR3-C, resulting in the plasmid pPR3-NTSR1.

*NTSR2* gene encoding neurotensin receptor 2 was PCR-amplified with oligonucleotides o59 and o60. The *SpeI-EcoRI* *NTSR2* gene fragment was inserted at the *SpeI-EcoRI* site on pPR3-C, resulting in the plasmid pPR3-NTSR2.