Supplementary Tables

Supplementary Table 1: Hill function parameters. We determined the best fit parameters to describe dose-response curves using TCS and Tango approaches in Fig. 3c and Fig. 4dg. Induction data were fit to the Hill function $y = c + \frac{(S-c) \times X^n}{(K^n + X^n)}$, where *y* is Cerulean expression (in norm. u.), *c* is Cerulean basal expression (in norm. u.), *S* is Cerulean maximum expression (norm. u.), *X* is ligand concentration (nM), *K* is EC50, n is the Hill coefficient. Repression data by the antagonist propranolol were fit to the repressor Hill function $y = c + \frac{b}{(K^n + X^n)}$, where K corresponds to IC50. The values in brackets represent 95% confidence intervals.

Method	Interacting partners	Ligand	Hill parameters			
			K (nM)	S or b (Cerulean, norm. u.)	c (Cerulean, norm. u.)	n
TCS	FKBP/FRB	A/C	1.3 (0.02; 2.6)	0.091	3.8E-09	0.86
	β2AR/β-arrestin	Procaterol	5.0 (3.5; 6.4)	0.030	-8.6E-05	1.09
		Isoproterenol	29.1 (-11.2; 69.4)	0.030	-0.0017	0.52
		Clenbuterol	14.3 (11.1; 17.5)	0.0085	0.00048	1.24
		Propranolol	2.7 (2.2; 3.1)	0.091	8.8E-5	1.36
Tango	β2AR/β-arrestin	Procaterol	11.3 (9.1; 13.5)	0.58	-0.0048	0.70
		Isoproterenol	14.5 (0.5; 28.4)	0.57	-0.053	0.51
		Clenbuterol	18.1(10; 26.3)	0.16	0.013	0.75
		Propranolol	3.2 (2.0; 4.2)	1.32	0.015	1.46

Supplementary Table 2: Quantitative characteristics of the rewired GPCR pathways, related to the experiments in Fig. 5. The On/Off ratio (that is, output fluorescence in the presence of a ligand relative to output fluorescence in its absence) of GPCR-NarX H^{mut} (TCS system) fusion and tTA fusion (Tango assay, Barnea[1] or Presto[2] -like approach) is indicated. On/Off ratio above 2 is indicated with the "+". The constructs are described in Supplementary Fig. 1.

GPCR gene	TCS			Tango		
	Construct	On/Off ratio	> 2	Construct	On/Off ratio	> 2
ADRB2	β2AR$^{\Delta C}$::V2R$^{\Delta N}$::H^{mut}	40.9	+	β2AR$^{\Delta C}$::V2R$^{\Delta N}$::tTA	7.88	+
	β2AR::V2R$^{\Delta N}$::H^{mut}	1.21		β2AR::V2R$^{\Delta N}$::tTA	1.49	
NMBR	NMB-R::V2R$^{\Delta N}$::H^{mut}	28.09	+	NMB-R::V2R$^{\Delta N}$::tTA	2.93	+
AVPR2	V2R::V2R$^{\Delta N}$::H^{mut}	14.03	+	V2R::V2R$^{\Delta N}$::tTA	7.8	+
LPAR1	LPA-1::V2R$^{\Delta N}$::H^{mut}	3.76	+	LPA-1::V2R$^{\Delta N}$::tTA	1.26	
BDKRB2	B2R::V2R$^{\Delta N}$::H^{mut}	0.6		B2R::V2R$^{\Delta N}$::tTA	1.36	
CXCR4	CXC-R4::V2R$^{\Delta N}$::H^{mut}	0.91		CXC-R4::V2R$^{\Delta N}$::tTA	1.49	
NPY1R	NPY1-R::H^{mut}	1.11		NPY1-R::V2R$^{\Delta N}$::tTA	1.07	
NPY5R	NPY5-R$^{\Delta C}$::V2R$^{\Delta N}$::H^{mut}	3.44	+	NPY5-R::V2R$^{\Delta N}$::tTA	1.87	

Supplementary Table 3: Primer Sequences

Primer name	Sequence
PR1021	GCTGCTGCTCTCGAGTCATTATCATTCGTGAGTGTC
PR1023	GCTGCTACCGGTCGCCACCATGGCCCGCTTGCTCCAGCCGTGG
PR1964	CGCGCCTGATTACAAAACTTTAAAAAGTGCTGTAGCGCCGGCTGATTACAAAACTTTAAAAAGTGCTGTCCA
PR1965	TATGGACAGCACTTTTTAAAGTTTTGTAATCAGCCGGCGCTACAGCACTTTTTAAAGTTTTGTAATCAGG
PR2196	TAAGCGGAATTCATCTTGGCTGAGGAATCTT
PR2197	GCGAATTCTAGACTACTTGTACAGCTCGTCC
PR2258	AATGTGAAGCTAGCGCCACCATGGCTGAAGGATCCGTCG
PR2259	AATGTAATCTAGATCACTCTTCCATCACGCCGATC
PR2442	GCTAGCGCTACCGGACTCAGAT
PR2443	TGGGTGGGGTGCGTCCGCGCGCACAGAAGTCCCGGAAACACCG
PR2444	TGGGTGGGGTGCGTCCGCGCGCACACAGAAGCTCCTGGAAGGCAA
PR3687	GGCACAGTCGAGGCTGATTTTC
PR3707	TGGCAGCGGGCGTCAAGCAG
PR3708	ATGGTGGTGGCAGCGGCGAGGTATGGCAACG
PR3709	CTCGCCGCTGCCACCACCATGTTGGCGACG
PR4122	GAAATTAATACGACTCACTATAGGGGAC
PR4122	GAAATTAATACGACTCACTATAGGGGAC
PR4345	GAAATTAATACGACTCACTATAGGGGACCGGTCGCCACCATGGCAGCGGGCGTCAAG
PR4346	CACAGTCGAGGCTGATTTTC
PR4541	GTTTGAGAGCTGCCGAGAGTGCTTCTCTCGCG
PR4542	AGCACTCTCGGCAGCTCTCAAACATAGCCAGG
PR4543	CTTCCAGTGCAGCTTCAATCAGATTTCCCAGTGTG
PR4544	TCTGATTGAAGCTGCACTGGAAGCTCTGGGAC
PR4546	GAAATTAATACGACTCACTATAGGGGACCGGTCGCCACCATGCAAGAGCGGCAGCAGCAG
PR4732	GACGGCCAGTCTTAAGCTCGGGCCCGCTCCGGTGCCCGTCAG
PR4733	GAAGTCGTCGCATGGTGGCGACCGGTTCACGACACCTGAAATGGAAG
PR4734	GACGGCCAGTCTTAAGCTCGGGCCCTGGGCGGGATTCGTCTTG
PR4747	CAAGAGCGGCAGCAGCAG
PR4747	CAAGAGCGGCAGCAGCAG
PR4748	TTCGTGAGTGTCACCCTGC
PR4766	GAAATTAATACGACTCACTATAGGGGACCGGTCGCCACCATGGGCGTGCAGGTGGAG
PR4767	CGCCACCGCCTGAACCGCCTCCACCTTCCAGTTTTAGAAGCTCCACATC
PR4769	GAAATTAATACGACTCACTATAGGGGACCGGTCGCCACCATGGTAGCCATCCTCTGG
PR4770	CGCCACCGCCTGAACCGCCTCCACCTGATATCCGTCTGAACACGTG
PR4771	AGGCGGTTCAGGCGGTGGCGGGTCGCAAGAGCGGCAGCAGCAG
PR4892	CGCGCCTACCCCTATAGGGGTATAGCGCCGGCTACCCCTATAGGGGTATCCA
PR4893	TATGGATACCCCTATAGGGGTAGCCGGCGCTATACCCCTATAGGGGTAGG
PR4971	TTCTTCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGGGCTC
PR4972	CATCGTCATGGAAGAGAGGGCGACTATTGC

PR4973	GCAATAGTCGCCCTCTCTTCCATGACGATG
PR4974	TTCTTCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGGGCGT
PR4975	TTCTTCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGGTAGC
PR4977	TTCTTCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGCAAGAGCGGCAGCAGCAG
PR4978	CCTGATTGGACATGGTGGCGACCGGTTCACGACACCTGA
PR4979	TTCTTCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGCTT
PR4980	TCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGGGGGAGAAACCCGGGAC
PR4981	CTCTTGCGAGCCACCGCCACCGCAGAGTTGATCATCATAGTCGTC
PR4982	GGTGGCGGTGGCTCGCAAGAGCGGCAGCAGCAG
PR4983	TCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGGGGCAACCCGGGAAC
PR4985	CTCTTGCGAGCCACCGCCACCCGATGAAGTGTCCTTGGCC
PR5226	ATTACGCCAAGCTACGGGGGCACCTCGACATACTCGAG
PR5227	CCTTGCTCACCATGGTGGCGAGGTACCGAGCTCGAAATCTC
PR5228	ACCGGGAGAAGCGGGGTCTCG
PR5229	CAGTCGAGGCTGATTTTCTCGCTCAAGCGTAATCTGGAAC
PR5230	GTTCCAGATTACGCTTGAGCGAGAAAATCAGCCTCGACTG
PR5231	CCGGGAGCTTTTTGCAAAAGC
PR5232	GGACGGGGCATGGACTCCGCAG
PR5233	GCACAGTCGAGGCTGATTTTCTCGAGTCATTACTACCCACCGTACTCGTCAATTCC
PR5293	CTCTTGCGAGCCACCGCCACCAATTTTTTCATTGTCGTCGTTGTTG
PR5294	TCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGAATTCAACACTTTTTTCTCAGGTG
PR5295	TGGGTGGGGTGCGTCCGCGCGCAGAGACCAGATCAGCCTTAATG
PR5296	TCCATTTCAGGTGTCGTGAaCCGGTCGCCACCATGGACTTGGAGCTGGATGAG
PR5297	GCGCGCGGACGCACCCCAC
PR5396	GGACGCACCCCACCCAGCCTG
PR5399	TCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGGGGCAACCTGGCAATG
PR5400	CAGGCTGGGTGGGGTGCGTCCACCGGTATCGAT
PR5401	TCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGCTCATGGCCTCTACCAC
PR5402	TCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGTTCTCACCCTGGAAGATTTC
PR5403	TCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGGAGGGGATATCAATCTACACATC
PR5404	TCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGGCCGCTATTAGCACCAG
PR5405	TCCATTTCAGGTGTCGTGAACCGGTCGCCACCATGCCCTCCAAGTCTCTGTC
PR6799	GTGGTATTCTCAGCCTCAAACCATGAGGAAGATGTCG
PR6800	TCATGGTTTGAGGCTGAGAATACCACGATCCTCCC
PR6801	GTGGTATTCTCAAGCTCAAACCATGAGGAAGATGTCG
PR6802	TCATGGTTTGAGCTTGAGAATACCACGATCCTCCC

Supplementary Table 4: List of synthetic DNA sequences used for plasmid constructs

Synthetic DNA name	Sequence
gBlock112	AGATCTCGAGCTCAAGCTTCGAATTCGCCACCATGGGGGAGAAACCCGGGACCAGGGTCTTC AAGAAGTCGAGCCCTAACTGCAAGCTCACCGTGTACTTGGGCAAGCGGGACTTCGTAGATCA CCTGGACAAAGTGGACCCTGTAGATGGCGTGGTGCTTGTGGACCCTGACTACCTGAAGGACC GCAAAGTGTTTGTGACCCTCACCTGCGCCTTCCGCTATGGCCGTGAAGACCTGGATGTGCTG GGCTTGTCCTTCCGCAAAGACCTGTTCATCGCCACCTACCAGGCCTTCCCCCCGGTGCCCAA CCCACCCCGGCCCCCCACCCGCCTGCAGGACCGGCTGCTGAGGAAGCTGGGCCAGCATGCC CACCCCTTCTTCTTCACCATACCCCAGAATCTTCCATGCTCCGTCACACTGCAGCCAGGCCCA GAGGATACAGGAAAGGCCTGCGGCGTAGACTTTGAGATTCGAGCCTTCTGTGCTAAATCACTA GAAGAGAAAAGCCACAAAAGGAACTCTGTGCGGCTGGTGATCCGAAAGGTGCAGTTCGCCCC GGAGAAACCCGGCCCCCAGCCTTCAGCCGAAACCACACGCCACTTCCTCATGTCTGACCGGT CCCTGCACCTCGAGGCTTCCCTGGACAAGGAGCTGTACTACCATGGGGAGCCCCTCAATGTA AATGTCCACGTCACCAACAACTCCACCAAGACCGTCAAGAAGATCAAAGTCTCTGTGAGACAG TACGCCGACATCTGCCTCTTCAGCACCGCCCAGTACAAGTGTCCTGTGGCTCAACTCGAACAA GATGACCAGGTATCTCCCAGCTCCACATTCTGTAAGGTGTACACCATAACCCCACTGCTCAGC GACAACCGGGAGAAGCGGGGTCTCGCCCTGGATGGGAAACTCAAGCACGAGGACACCAACC TGGCTTCCAGCACCATCGTGAAGGAGGGTGCCAACAAGGAGGTGCTGGGAATCCTGGTGTCC T
gBlock113	GCTGGGAATCCTGGTGTCCTACAGGGTCAAGGTGAAGCTGGTGGTGTCTCGAGGCGGGGAT GTCTCTGTGGAGCTGCCTTTTGTTCTTATGCACCCCAAGCCCCACGACCACATCCCCCTCCCC AGACCCCAGTCAGCCGCTCCGGAGACAGATGTCCCTGTGGACACCAACCTCATTGAATTTGAT ACCAACTATGCCACAGATGATGACATTGTGTTTGAGGACTTTGCCCGGCTTCGGCTGAAGGGG ATGAAGGATGACGACTATGATGATCAACTCTGCGGATCCAGCTTGTTTAAGGGACCACGTGAT TACAACCCGATATCGAGCACCATTTGTCATTTGACGAATGAATCTGATGGGCACACAACATCGT TGTATGGTATTGGATTTGGTCCCTTCATCATTACAAACAAGCACTTGTTTAGAAGAAATAATGGA ACACTGTTGGTCCAATCACTACATGGTGTATTCAAGGTCAAGAACACCACGACTTTGCAACAAC ACCTCATTGATGGGAGGGACATGATAATTATTCGCATGCCTAAGGATTTCCCACCATTTCCTCA AAAGCTGAAATTTAGAGAGCCACAAAGGGAAGAGCGCATATGTCTTGTGACAACCAACTTCCA AACTAAGAGCATGTCTAGCATGGTGTCAGACACTAGTTGCACATTCCCTTCATCTGATGGCATA TTCTGGAAGCATTGGATTCAAACCAAGGATGGGCAGTGTGGCAGTCCATTAGTATCAACTAGA GATGGGTTCATTGTTGGTATACACTCAGCATCGAATTTCACCAACACAAACAATTATTTCACAA GCGTGCCGAAAAACTTCATGGAATTGTTGACAAATCAGGAGGCGCAGCAGTGGGTTAGTGGTT GGCGATTAAATGCTGACTCAGTATTGTGGGGGGGCCATAAAGTTTTCATGAGCAAACCTGAAG AGCCTTTTCAGCCAGTTAAGGAAGCGACTCAACTCATGAATGAATTGGTGTACTCGCAATACCC ATACGATGTTCCAGATTACGCTTGAGCGGCCGCGACTCTAGATCATAATCAG
gBlock114	GCGCTACCGGACTCAGATCTCGAGGCCACCATGGACTCCCCGATCCAGATCTTCCGCGGGGA GCCGGGCCCTACCTGCGCCCCGAGCGCCTGCCTGCCCCCCAACAGCAGCGCCTGGTTTCCC GGCTGGGCCGAGCCCGACAGCAACGGCAGCGCCGGCTCGGAGGACGCGCAGCTGGAGCCC GCGCACATCTCCCCGGCCATCCCGGTCATCATCACGGCGGTCTACTCCGTAGTGTTCGTCGT GGGCTTGGTGGGCAACTCGCTGGTCATGTTCGTGATCATCCGATACACAAAGATGAAGACAGC AACCAACATTTACATATTTAACCTGGCTTTGGCAGATGCTTTAGTTACTACAACCATGCCCTTTC AGAGTACGGTCTACTTGATGAATTCCTGGCCTTTTGGGGATGTGCTGTGCAAGATAGTAATTTC CATTGATTACTACAACATGTTCACCAGCATCTTCACCTTGACCATGATGAGCGTGGACCGCTAC ATTGCCGTGTGCCACCCCGTGAAGGCTTTGGACTTCCGCACACCCTTGAAGGCAAAGATCATC AATATCTGCATCTGGCTGCTGTCGTCATCTGTTGGCATCTCTGCAATAGTCCTTGGAGGCACC AAAGTCAGGGAAGACGTCGATGTCATTGAGTGCTCCTTGCAGTTCCCAGATGATGACTACTCC TGGTGGGACCTCTTCATGAAGATCTGCGTCTTCATCTTTGCCTTCGTGATCCCTGTCCTCATCA TCATCGTCTGCTACACCCTGATGATCCTGCGTCTCAAGAGCGTCCGGCTCCTTTCTGGCTCCC GAGAGAAAGATCGCAACCTGCGTAGGATCACCAGACTGGTCCTGGTGGTGGTGGCAGTCTTC GTCGTCTGCTGGACTCCCATTCACATATTCATCCTGGTGGAGGCTCTGGGGAGCACCTCCCAC AGCACAGCTGCTCTCTCCAGCTATTACTTCTGCATCGCCTTAGGCTATACCAACAGTAGCCTGA ATCCCATTCTCTACGCCTTTCTTGATGAAAACTTCAAGCGGTGTTTCCGGGACTTCTGTTTTCC ACTGAAGATGAGGATGGAGCGGCAGAGCACTAGCAGAGTCCGAAATACAGTTCAGGACCCTG CTTACCTGAGGGACATCGATGGGATGAATAAACCAGTATGACAATTGTTGTTGTTAACTTGTTT ATTGC

gBlock115	AGCGGTGTTTCCGGGACTTCTGTGCGCGCGGACGCACCCCACCCAGCCTGGGTCCCCAAGAT GAGTCCTGCACCACCGCCAGCTCCTCCCTGGCCAAGGACACTTCATCGGGATCCGAGAATCT GTACTTTCAGCTGAGATTAGATAAAAGTAAAGTGATTAACAGCGCATTAGAGCTGCTTAATGAG GTCGGAATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAGGTGTAGAGCAGCCTACA TTGTATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGACGCCTTAGCCATTGAGATGTTAGATA GGCACCATACTCACTTTTGCCCTTTAGAAGGGGAAAGCTGGCAAGATTTTTTACGTAATAACGC TAAAAGTTTTAGATGTGCTTTACTAAGTCATCGCGATGGAGCAAAAGTACATTTAGGTACACGG CCTACAGAAAAACAGTATGAAACTCTCGAAAATCAATTAGCCTTTTTATGCCAACAAGGTTTTTC ACTAGAGAATGCATTATATGCACTCAGCGCTGTGGGGCATTTTACTTTAGGTTGCGTATTGGAA GATCAAGAGCATCAAGTCGCTAAAGAAGAAAGGGAAACACCTACTACTGATAGTATGCCGCCA TTATTACGACAAGCTATCGAATTATTTGATCACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCC TTGAATTGATCATATGCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTCCGCGTACAGCCG GGCGCGTACGAAAAACAATTACGGGTCTACCATCGAGGGCCTGCTCGATCTCCCGGACGACG ACGCCCCCGAAGAGGCGGGGCTGGCGGCTCCGCGCCTGTCCTTTCTCCCCGCGGACACAC GCGCAGACTGTCGACGGCCCCCCCGACCGATGTCAGCCTGGGGGACGAGCTCCACTTAGAC GGCGAGGACGTGGCGATGGCGCATGCCGACGCGCTAGACGATTTCGATCTGGACATGTTGG GGGACGGGGATTCCCCGGGTCCGGGATTTACCCCCCACGACTCCGCCCCCTACGGCGCTCT GGATATGGCCGACTTCGAGTTTGAGCAGATGTTTACCGATGCCCTTGGAATTGACGAGTACGG TGGGTAGCAATTGTTGTTGTTAACTTGTTTATTGC
gBlock118	GCTAGCGCTACCGGACTCAGATCTCGAGGCCACCATGGGGCAACCCGGGAACGGCAGCGCC TTCTTGCTGGCACCCAATAGAAGCCATGCGCCGGACCACGACGTCACGCAGCAAAGGGACGA GGTGTGGGTGGTGGGCATGGGCATCGTCATGTCTCTCATCGTCCTGGCCATCGTGTTTGGCA ATGTGCTGGTCATCACAGCCATTGCCAAGTTCGAGCGTCTGCAGACGGTCACCAACTACTTCA TCACTTCACTGGCCTGTGCTGATCTGGTCATGGGCCTGGCAGTGGTGCCCTTTGGGGCCGCC CATATTCTTATGAAAATGTGGACTTTTGGCAACTTCTGGTGCGAGTTTTGGACTTCCATTGATGT GCTGTGCGTCACGGCCAGCATTGAGACCCTGTGCGTGATCGCAGTGGATCGCTACTTTGCCA TTACTTCACCTTTCAAGTACCAGAGCCTGCTGACCAAGAATAAGGCCCGGGTGATCATTCTGAT GGTGTGGATTGTGTCAGGCCTTACCTCCTTCTTGCCCATTCAGATGCACTGGTACCGGGCCAC CCACCAGGAAGCCATCAACTGCTATGCCAATGAGACCTGCTGTGACTTCTTCACGAACCAAGC CTATGCCATTGCCTCTTCCATCGTGTCCTTCTACGTTCCCCTGGTGATCATGGTCTTCGTCTAC TCCAGGGTCTTTCAGGAGGCCAAAAGGCAGCTCCAGAAGATTGACAAATCTGAGGGCCGCTT CCATGTCCAGAACCTTAGCCAGGTGGAGCAGGATGGGCGGACGGGGCATGGACTCCGCAGA TCTTCCAAGTTCTGCTTGAAGGAGCACAAAGCCCTCAAGACGTTAGGCATCATCATGGGCACT TTCACCCTCTGCTGGCTGCCCTTCTTCATCGTTAACATTGTGCATGTGATCCAGGATAACCTCA TCCGTAAGGAAGTTTACATCCTCCTAAATTGGATAGGCTATGTCAATTCTGGTTTCAATCCCCTT ATCTACTGCCGGAGCCCAGATTTCAGGATTGCCTTCCAGGAGCTTCTGTGTCTGCGCAGGTCT TCTTTGAAGGCCTATGGGAATGGCTACTCCAGCAACGGCAACACAGGGGAGCAGAGTGGATA TCACGTGGAACAGGAGAAAGAAAATAAACTGCTGTGTGAAGACCTCCCAGGCACGGAAGACTT TGTGGGCCATCAAGGTACTGTGCCTAGCGATAACATTGATTCACAAGGGAGGAATTGTAGTAC AAATGACTCACTGCTGTAACAATTGTTGTTGTTAACTTGTTTATTGC
gBlock143	ATCTTGGCTGAGGAATCTTCTAACAATTTAGAGCTTAAAAACGCCCACGAGGCGGAGAACGAA ATATCCAGAGAGACGTTAGAAACGTTCAAAAACGTTCGCTAGCGCCACCATGGTGAGCAAGGG CGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGC CACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCTGA AGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCTTCGGC TACGGCCTGATGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCC GCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAA GACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGC ATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCA CAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCA CAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGC GACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCAAGCTGAGCAAAGA CCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCACTC TCGGCATGGACGAGCTGTACAAGTAG
gBlock264	GAAATTAATACGACTCACTATAGGGGACCGGTCGCCACCATGGGCTCGAGCAACCTGGTTGC GCAGCTCGAAAACGAAGTTGCGTCTCTGGAAAATGAGAACGAAACCCTGAAGAAAAAGAACCT GCACAAAAAAGACCTGATCGCGTACCTGGAGAAAGAAATCGCGAATCTGCGTAAGAAAATCGA AGAAGGCGGTGGCGGGTCGCAAGAGCGGCAGCAGCAGCT
gBlock265	TGCAGGGTGACACTCACGAAGGCGGTGGCGGGTCGAACCTGGTTGCGCAGCTCGAAAACGA AGTTGCGTCTCTGGAAAATGAGAACGAAACCCTGAAGAAAAAGAACCTGCACAAAAAAGACCT GATCGCGTACCTGGAGAAAGAAATCGCGAATCTGCGTAAGAAAATCGAAGAATGATAATGACT CGAGAAAATCAGCCTCGACTGTG

gBlock269	GAAATTAATACGACTCACTATAGGGGACCGGTCGCCACCATGGGCTCGAGCGCGCGTAACGCGTATCTGCGTAAGAAAATCGCACGTCTGAAAAAAGACAACCTGCAGCTGGAACGTGATGAACAGAACCTGGAAAAAATCATCGCGAACCTGCGTGACGAAATCGCGCGTCTCGAAAACGAAGTTGCGTCTCACGAACAGGGCGGTGGCGGGTCGCAAGAGCGGCAGCAGCAGCT
gBlock270	TGCAGGGTGACACTCACGAAGGCGGTGGCGGGTCGGCGCGTAACGCGTATCTGCGTAAGAAAATCGCACGTCTGAAAAAAGACAACCTGCAGCTGGAACGTGATGAACAGAACCTGGAAAAAATCATCGCGAACCTGCGTGACGAAATCGCGCGTCTCGAAAACGAAGTTGCGTCTCACGAACAGTGATAATGACTCGAGAAAATCAGCCTCGACTGTG

Supplementary Table 5: Sequences of the coding regions used to build the plasmids.

Element	Sequence
envZ	ATGCGACGACTTCGGTTCTCACCGAGGTCCTCATTTGCGAGGACGCTGCTCCTGATCGTCACACTTTTG TTCGCCAGCCTGGTGACGACTTACTTGGTAGTACTCAACTTCGCGATTCTTCCCTCCTTGCAGCAGTTC AATAAGGTACTGGCGTATGAGGTCAGAATGCTGATGACGGATAAGCTCCAGCTCGAAGATGGGACGCA GCTTGTCGTGCCTCCAGCGTTTCGGCGCGAAATCTACCGCGAGCTGGGAATTTCGCTCTACTCGAATG AGGCTGCGGAAGAAGCGGGGTTGCGCTGGGCACAGCACTATGAATTTCTGTCGCATCAAATGGCGCA ACAGTTGGGAGGTCCGACAGAGGTCAGAGTAGAGGTCAACAAATCGTCGCCCGTGGTGTGGCTTAAG ACCTGGCTCTCCCCTAACATCTGGGTCAGGGTGCCATTGACCGAGATCCATCAGGGCGATTTTTCGCC CCTGTTTCGATACACCCTGGCTATCATGTTGCTTGCGATCGGGGGAGCCTGGCTTTTCATCAGAATCCA AAATCGGCCTCTGGTAGATTTGGAACATGCTGCGCTCCAAGTCGGGAAGGGGATTATTCCTCCACCGC TGAGAGAGTACGGTGCCTCGGAAGTGAGGTCAGTAACACGCGCATTCAATCACATGGCAGCGGGCGT CAAGCAGCTGGCAGACGACCGGACTCTTCTCATGGCCGGAGTCTCACACGATCTCCGCACGCCCCTG ACACGGATTCGCCTTGCGACTGAGATGATGTCCGAGCAGGACGGCTACCTCGCGGAGTCAATTAACAA AGATATCGAGGAGTGCAACGCCATCATTGAGCAGTTCATCGACTATCTGCGCACGGGACAAGAAATGC CCATGGAGATGGCTGACTTGAATGCAGTACTCGGAGAGGTGATCGCGGCAGAGTCAGGATACGAGAG AGAGATTGAAACGGCACTCTATCCGGGGAGCATCGAAGTCAAAATGCATCCGCTCTCGATCAAGAGGG CCGTCGCCAACATGGTGGTGAATGCGGCGAGGTATGGCAACGGGTGGATCAAAGTATCATCGGGAAC GGAACCTAACAGAGCGTGGTTCCAAGTGGAGGACGACGGGCCAGGTATTGCGCCCGAGCAGAGAAAA CACTTGTTTCAGCCCTTTGTCAGAGGGGACTCCGCCCGAACAATCAGCGGAACTGGTTTGGGGTTGGC CATCGTGCAGAGGATCGTGGATAACCACAATGGGATGTTGGAACTTGGTACAAGCGAAAGAGGGGGAC TCTCCATCCGAGCGTGGCTTCCGGTGCCCGTAACAAGAGCCCAGGGAACTACCAAGGAAGGA
ompR	ATGCAAGAAAACTACAAGATTCTCGTGGTGGATGATGACATGCGACTTCGCGCATTGCTCGAAAGATAT CTGACCGAGCAGGGATTTCAAGTGCGCTCCGTGGCCAATGCCGAGCAGATGGATAGGCTCTTGACGA GGGAGTCGTTCCATCTGATGGTGCTGGACTTGATGCTTCCCGGTGAGGACGGATTGTCCATTTGCCGG AGACTTAGGTCGCAGTCAAACCCCATGCCGATCATCATGGTCACAGCGAAGGGAGAGGAGGTCGATAG AATTGTAGGTCTTGAGATTGGGGCAGACGACTACATCCCCAAGCCGTTCAATCCCCGGGAACTTCTTG CGCGAATCCGAGCCGTGCTCAGGCGACAGGCCAACGAGCTGCCCGGAGCTCCATCGCAAGAGGAAG CGGTCATCGCGTTCGGGAAGTTCAAGTTGAACCTCGGCACGAGAGAGATGTTTCGGGAAGATGAACCT ATGCCGCTCACATCGGGGGAGTTTGCGGTCTTGAAAGCACTTGTCTCACACCCGAGAGAACCTCTGTC GCGGGATAAACTCATGAATCTGGCGAGAGGCAGAGAGTATAGCGCGATGGAAAGGTCCATCGATGTCC AGATTAGCCGCCTCCGCCGCATGGTGGAGGAAGATCCAGCCCACCCTCGGTACATCCAGACTGTATG GGGATTGGGGTATGTGTTCGTACCGGATGGGTCAAAAGCA
narX	ATGCTTAAAAGATGTCTCTCACCCCTTACTCTCGTGAACCAGGTGGCGCTTATTGTATTGTTGTCAACCG CGATCGGGCTGGCGGGCATGGCTGTGTCCGGTTGGCTCGTACAGGGGGTGCAGGGGTCGGCACATG CGATCAATAAGGCGGGTTCGCTGAGAATGCAGTCATACCGCCTGTTGGCTGCGGTGCCGCTGTCGGA AAAGGACAAGCCGCTGATCAAGGAGATGGAGCAGACAGCATTTTCAGCCGAGTTGACGAGAGCGGCG GAAAGGGATGGGCAGCTTGCACAACTTCAGGGGCTCCAAGATTATTGGCGGAACGAATTGATCCCAGC GCTTATGAGAGCCCAGAATCGGGAGACAGTCTCAGCAGATGTATCGCAGTTCGTCGCCGGGTTGGATC AGCTTGTCTCCGGGTTCGATCGCACCACAGAAATGAGAATTGAAACTGTCGTACTGGTACATAGGGTGA TGGCAGTCTTTATGGCATTGTTGCTCGTGTTTACTATCATCTGGCTGAGAGCCCGCTTGCTCCAGCCGT GGCGGCAGCTGCTTGCGATGGCTTCGGCGGTGTCCCACCGCGATTTCACTCAGCGGGCTAACATTAG CGGGAGGAATGAGATGGCCATGCTCGGAACTGCCCTTAACAATATGTCCGCCGAACTCGCGGAGTCAT ACGCGGTGCTGGAACAACGGGTGCAAGAGAAAACGGCTGGATTGGAGCACAAGAACCAGATCCTTTC GTTCTTGTGGCAGGCTAACCGACGGTTGCACTCGCGGGCACCCCTGTGCGAGAGGCTGAGCCCTGTA TTGAACGGGCTTCAGAATCTGACTCTCTTGCGCGACATCGAACTCCGAGTCTATGATACGGATGATGAG GAGAATCATCAGGAGTTCACGTGCCAGCCGGACATGACGTGTGACGACAAGGGTTGCCAGTTGTGTCC CAGGGGCGTCCTCCCCGTGGGTGATCGGGGAACCACTTTGAAGTGGCGACTGGCCGATTCACACACG CAGTATGGGATTCTCCTGGCGACCCTCCCGCAAGGACGGCATCTGAGCCACGACCAGCAACAACTTGT CGACACGTTGGTGGAACAGTTGACGGCCACGCTCGCACTCGACCGCCATCAAGAGCGGCAGCAGCAG CTCATCGTCATGGAAGAGAGGGCGACTATTGCCCGGGAGCTTCACGATTCCATTGCACAGTCACTGTC GTGCATGAAGATGCAAGTGAGCTGTCTGCAAATGCAGGGGGACGCGCTCCCCGAGTCGAGCCGCGAA CTGCTGTCCCAGATCCGAAATGAGTTGAATGCGAGCTGGGCACAGCTGAGAGAGCTTCTGACGACATT CCGCCTGCAGCTGACCGAGCCCGGTCTTAGGCCAGCTTTGGAGGCATCGTGCGAAGAGTATTCAGCG AAATTTGGATTTCCCGTCAAACTTGATTACCAACTGCCTCCTCGCCTTGTACCCTCCCACCAGGCGATT CACCTGCTTCAGATCGCGAGAGAAGCACTCTCGAATGCTCTCAAACATAGCCAGGCGAGCGAAGTCGT GGTGACAGTGGCACAGAACGACAACCAGGTGAAATTGACGGTGCAAGACAACGGATGTGGAGTCCCG GAGAATGCGATTAGGTCGAATCATTACGGTATGATCATTATGCGAGATCGCGCGCAATCCTTGAGGGG CGACTGTAGAGTCAGGCGGAGGGAGTCGGGAGGTACGGAGGTCGTAGTAACGTTTATCCCGGAAAAG ACATTCACCGACGTGCAGGGTGACACTCACGAA
narL	ATGTCCAATCAGGAACCCGCGACAATTCTGCTGATCGACGATCACCCCATGCTGCGGACCGGGGTAAA GCAACTTATCTCGATGGCACCCGACATCACAGTCGTCGGAGAGGCGTCGAATGGAGAACAGGGCATC GAACTTGCGGAGAGCCTGGACCCTGACCTTATCCTCCTCGACTTGAATATGCCAGGGATGAACGGATT GGAAACACTCGACAAGCTGCGGGAGAAATCGTTGTCGGGGAGGATCGTGGTATTCTCAGTGTCAAACC ATGAGGAAGATGTCGTGACGGCACTCAAGAGGGGTGCCGACGGATACTTGTTGAAAGACATGGAGCC GGAGGACCTGTTGAAGGCGCTTCACCAAGCCGCAGCTGGAGAAATGGTGTTGTCAGAGGCGCTGACG CCTGTCCTCGCGGCGAGCTTGCGCGCCAACAGAGCTACGACCGAGCGGGACGTAAACCAGCTTACTC

	CGAGAGAGAGGGACATTTTGAAGCTGATTGCGCAGGGGCTTCCCAATAAGATGATTGCCAGACGCCTT GATATCACGGAAAGCACTGTGAAAGTCCACGTGAAACACATGCTCAAAAAGATGAAACTCAAGTCCCGC GTGGAAGCTGCGGTCTGGGTACATCAGGAGCGAATCTTT
VP48	GGACCGGCGGACGCACTGGATGACTTTGACTTGGATATGCTCCCAGCGGATGCGTTGGACGATTTTGA CCTTGACATGTTGCCTGCCGACGCGCTTGACGACTTCGACTTGGACATGCTGCCCGGT
mCherry	ATGGCCATCATCAAGGAGTTCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCCACGAGTT CGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTACGAGGGCACCCAGACCGCCAAGCTGAAGGTGAC CAAGGGTGGCCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATGTACGGCTCCAAGGCCT ACGTGAAGCACCCCGCCGACATCCCCGACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGA GCGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACCCAGGACTCCTCCCTCCAGGACGG CGAGTTCATCTACAAGGTGAAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATGCAGAAGA AGACCATGGGCTGGGAGGCCTCCTCCGAGCGGATGTACCCCGAGGACGGCGCCCTGAAGGGCGAGA TCAAGCAGAGGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTCAAGACCACCTACAAGGC CAAGAAGCCCGTGCAGCTGCCCGGCGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAACG AGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGGGCCGCCACTCCACCGGCGGCATGGACGA GCTGTACAAG
cerulean	ATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGAC GTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCC TGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTG GGGCGTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATG CCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCG AGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGA CGGCAACATCCTGGGGCACAAGCTGGAGTACAACGCCATCAGCGACAACGTCTATATCACCGCCGACA AGCAGAAGAACGGCATCAAGGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCT CGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTAC CTGAGCACCCAGTCCAAGCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTT CGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAG
amCyan (the intron is underlined)	ATGGCCCTGTCCAACAAGTTCATCGGCGACGACATGAAGATGACCTACCACATGGACGGCTGCGTGAA CGGCCACTACTTCACCGTGAAGGGCGAGGTGAGTATGTGCTCGCTTCGGCAGCACATATACTATGTTG AATGAGGCTTCAGTACTTTACAGAATCGTTGCCTGCACATCTTGGAAACACTTGCTGGGATTACTTCTTC AGGTTAACCCAACAGAAGGCTCGAGTGCTGTTGACAGTGAGCGCCGCTTGAAGTTTTTAATTAAATAGT GAAGCCACAGATGTATTTAATTAAAGACTTCAAGCGGTGCCTACTGCCTCGGAGAATTCAAGGGGCTAC TTTAGGAGCAATTATCTTGTTTACTAAAACTGAATACCTTGCTATCTCTTTGATACATTTTTACAAAGCTG AATTAAAATGGTATAAATTAAATCACTTTTTTCAATTGTTTCCTTTTTTTTCCTCAGGGCAGCGGCAAGCC CTACGAGGGCACCCAGACCTCCACCTTCAAGGTGACCATGGCCAACGGCGGCCCCCTGGCCTTCTCC TTCGACATCCTGTCCACCGTGTTCATGTACGGCAACCGCTGCTTCACCGCCTACCCCACCAGCATGCC CGACTACTTCAAGCAGGCCTTCCCCGACGGCATGTCCTACGAGAGAACCTTCACCTACGAGGACGGCG GCGTGGCCACCGCCAGCTGGGAGATCAGCCTGAAGGGCAACTGCTTCGAGCACAAGTCCACCTTCCA CGGCGTGAACTTCCCCGCCGACGGCCCCGTGATGGCCAAGAAGACCACCGGCTGGGACCCCTCCTTC GAGAAGATGACCGTGTGCGACGGCATCTTGAAGGGCGACGTGACCGCCTTCCTGATGCTGCAGGGCG GCGGCAACTACAGATGCCAGTTCCACACCTCCTACAAGACCAAGAAGCCCGTGACCATGCCCCCCAAC CACGTGGTGGAGCACCGCATCGCCAGAACCGACCTGGACAAGGGCGGCAACAGCGTGCAGCTGACC GAGCACGCCGTGGCCCACATCACCTCCGTGGTGCCCTTCT
SYNZIP1	AACCTGGTTGCGCAGCTCGAAAACGAAGTTGCGTCTCTGGAAAATGAGAACGAAACCCTGAAGAAAAA GAACCTGCACAAAAAAGACCTGATCGCGTACCTGGAGAAAGAAATCGCGAATCTGCGTAAGAAAATCG AAGAA
SYNZIP2	GCGCGTAACGCGTATCTGCGTAAGAAAATCGCACGTCTGAAAAAAGACAACCTGCAGCTGGAACGTGA TGAACAGAACCTGGAAAAAATCATCGCGAACCTGCGTGACGAAATCGCGCGTCTCGAAAACGAAGTTG CGTCTCACGAACAG
$MTOR^{2018-113}$ T2098L codes for FRB	ATGGTAGCCATCCTCTGGCATGAGATGTGGCATGAAGGTCTAGAAGAGGCCTCTCGCTTGTACTTTGG GGAGAGGAACGTCAAAGGCATGTTTGAGGTGCTGGAGCCCCTGCATGCTATGATGGAACGCGGTCCC CAGACCCTGAAGGAAACGTCCTTTAATCAGGCATATGGTCGAGATTTAATGGAGGCACAAGAATGGTG CCGAAAGTACATGAAATCAGGGAACGTCAAGGACCTCCTGCAAGCCTGGGACCTCTACTATCACGTGT TCAGACGGATATCA
FKBP1A	ATGGGCGTGCAGGTGGAGACTATCTCCCCAGGAGACGGGCGCACCTTCCCCAAGCGCGGCCAGACCT GCGTGGTGCACTACACCGGGATGCTTGAAGATGGAAAGAAATTTGATTCCTCCCGGGACAGAAACAAG CCCTTTAAGTTTATGCTAGGCAAGCAGGAGGTGATCCGAGGCTGGGAAGAAGGGGTTGCCCAGATGA GTGTGGGTCAGAGAGCCAAACTGACTATATCTCCAGATTATGCCTATGGTGCCACTGGGCACCCAGGC ATCATCCCACCACATGCCACTCTCGTCTTCGATGTGGAGCTTCTAAAACTGGAA
ARRB2	ATGGGGGAGAAACCCGGGACCAGGGTCTTCAAGAAGTCGAGCCCTAACTGCAAGCTCACCGTGTACTT GGGCAAGCGGGACTTCGTAGATCACCTGGACAAAGTGGACCCTGTAGATGGCGTGGTGCTTGTGGAC CCTGACTACCTGAAGGACCGCAAAGTGTTTGTGACCCTCACCTGCGCCTTCCGCTATGGCCGTGAAGA CCTGGATGTGCTGGGCTTGTCCTTCCGCAAAGACCTGTTCATCGCCACCTACCAGGCCTTCCCCCCGG TGCCCAACCCACCCCGGCCCCCCACCCGCCTGCAGGACCGGCTGCTGAGGAAGCTGGGCCAGCATG CCCACCCCTTCTTCTTCACCATACCCCAGAATCTTCCATGCTCCGTCACACTGCAGCCAGGCCCAGAG GATACAGGAAAGGCCTGCGGCGTAGACTTTGAGATTCGAGCCTTCTGTGCTAAATCACTAGAAGAGAA AAGCCACAAAAGGAACTCTGTGCGGCTGGTGATCCGAAAGGTGCAGTTCGCCCCGGAGAAACCCGGC CCCCAGCCTTCAGCCGAAACCACACGCCACTTCCTCATGTCTGACCGGTCCCTGCACCTCGAGGCTTC CCTGGACAAGGAGCTGTACTACCATGGGGAGCCCCTCAATGTAAATGTCCACGTCACCAACAACTCCA

	CCAAGACCGTCAAGAAGATCAAAGTCTCTGTGAGACAGTACGCCGACATCTGCCTCTTCAGCACCGCC CAGTACAAGTGTCCTGTGGCTCAACTCGAACAAGATGACCAGGTATCTCCCAGCTCCACATTCTGTAAG GTGTACACCATAACCCCACTGCTCAGCGACAACCGGGAGAAGCGGGGTCTCGCCCTGGATGGGAAAC TCAAGCACGAGGACACCAACCTGGCTTCCAGCACCATCGTGAAGGAGGGTGCCAACAAGGAGGTGCT GGGAATCCTGGTGTCCTACAGGGTCAAGGTGAAGCTGGTGGTGTCTCGAGGCGGGGATGTCTCTGTG GAGCTGCCTTTTGTTCTTATGCACCCCAAGCCCCACGACCACATCCCCCTCCCCAGACCCCAGTCAGC CGCTCCGGAGACAGATGTCCCTGTGGACACCAACCTCATTGAATTTGATACCAACTATGCCACAGATGA TGACATTGTGTTTGAGGACTTTGCCCGGCTTCGGCTGAAGGGGATGAAGGATGACGACTATGATGATC AACTCTGC
NPY1R	ATGAATTCAACACTTTTTTCTCAGGTGGAGAATCATAGTGTCCATTCCAATTTCAGTGAGAAGAACGCAC AGCTGCTTGCGTTTGAGAATGACGATTGTCACCTGCCACTGGCTATGATTTTCACCCTCGCCCTGGCTT ACGGAGCCGTCATCATACTGGGCGTTTCAGGAAACCTCGCTCTCATCATTATCATTCTTAAGCAAAAAG AAATGAGGAATGTGACGAACATTTTGATAGTCAATCTGTCATTTAGCGATCTCCTCGTAGCAATTATGTG TCTTCCATTTACCTTTGTGTACACTCTGATGGACCACTGGGTGTTTGGCGAGGCCATGTGCAAATTGAA CCCTTTCGTGCAGTGTGTCTCCATTACGGTGTCCATATTCTCTCTCGTCCTGATTGCCGTCGAAAGACA TCAGCTGATTATCAATCCCCGAGGGTGGCGACCTAATAACCGCCATGCTTACGTGGGAATAGCGGTAA TCTGGGTGTTGGCGGTGGCCAGTTCCCTGCCCTTCCTCATTTACCAGGTGATGACAGACGAGCCTTTT CAAAATGTAACTCTTGACGCTTATAAGGATAAGTATGTGTGTTTCGACCAGTTCCCCAGTGACTCCCACA GATTGAGTTATACAACACTGCTGTTGGTTCTTCAGTACTTCGGCCCCCTCTGTTTTATCTTTATATGCTA CTTCAAGATCTATATCAGACTCAAACGCAGGAATAACATGATGGACAAGATGAGAGATAACAAATACCG CAGCTCAGAGACTAAGCGCATCAACATTATGCTGCTGTCCATTGTGGTTGCGTTCGCAGTTTGTTGGCT GCCTCTTACAATCTTTAACACTGTGTTCGATTGGAATCATCAGATCATCGCAACCTGTAACCATAACCTG TTGTTCCTGCTCTGCCATTTGACAGCCATGATCAGTACATGCGTGAACCCGATCTTTTACGGATTTTTGA ACAAGAACTTCCAGCGGGACCTGCAGTTTTTTTTCAACTTTTGCGATTTCCGCTCCCGGGATGACGATT ACGAGACAATAGCAATGAGTACAATGCACACGGACGTTTCTAAAACGAGTCTGAAGCAGGCCAGCCCC GTGGCTTTCAAGAAAATAAACAACAACGACGACAATGAAAAAATT
NPY5R	ATGGACTTGGAGCTGGATGAGTATTACAACAAAACGTTGGCTACTGAGAATAACACCGCAGCCACTAGA AATAGCGATTTCCCCGTTTGGGATGACTATAAATCCTCTGTGGACGATCTGCAGTACTTTCTGATCGGC CTGTACACTTTTGTGAGTCTGCTGGGGTTCATGGGCAATCTGCTCATTCTCATGGCTCTGATGAAAAAG AGGAACCAAAAGACAACAGTGAACTTTCTGATCGGAAACCTCGCCTTCAGTGATATACTGGTGGTCCTC TTCTGCAGCCCCTTCACACTGACCTCAGTTTTGCTTGATCAGTGGATGTTTGGAAAGGTGATGTGCCAC ATTATGCCTTTCCTGCAATGTGTAAGCGTTCTGGTGTCTACCCTCATACTGATCTCAATCGCCATAGTCA GGTACCATATGATCAAGCATCCTATAAGTAACAATCTGACCGCGAACCACGGTTATTTCCTGATCGCTA CCGTGTGGACTCTCGGTTTCGCTATTTGCAGCCCACTGCCTGTGTTTCACTCTTTGGTCGAGCTGCAGG AAACCTTCGGTTCCGCTCTTCTTTCAAGCAGATACCTGTGTGTCGAAAGCTGGCCGAGCGATTCTTACA GGATCGCGTTCACTATCTCACTCCTCCTTGTGCAATACATACTTCCCCTGGTCTGTCTGACGGTGAGCC ATACCAGTGTGTGCAGGAGCATCTCATGCGGCCTCTCAAATAAAGAAAATCGACTGGAGGAGAACGAA ATGATTAATCTTACTCTTCACCCCTCCAAGAAATCAGGGCCTCAGGTGAAGCTGTCTGGCAGCCATAAA TGGAGTTATAGTTTTATCAAGAAACATAGACGACGGTATTCTAAGAAGACCGCTTGTGTGCTGCCTGCC CCCGAACGGCCATCCCAGGAGAATCATTCTAGGATCCTGCCCGAGAACTTCGGCTCCGTCCGCAGCCA ACTTTCATCTTCTAGCAAGTTCATTCCCGGGGTCCCAACTTGTTTTGAGATTAAGCCAGAAGAAAACTCT GATGTGCACGAGCTGAGAGTTAAGCGGTCCGTGACTAGAATTAAGAAGAGATCCAGAAGTGTGTTTTAC AGACTGACCATCCTGATCCTCGTGTTCGCAGTCTCCTGGATGCCACTGCACTTGTTCCATGTGGTTACA GACTTCAACGATAATCTGATCTCAAACCGACACTTTAAGCTCGTGTATTGCATCTGTCATCTCTTGGGAA TGATGTCATGCTGCCTCAACCCCATCCTCTATGGCTTCCTTAATAATGGCATTAAGGCTGATCTGGTCTC TTTGATACACTGCCTCCATATG
ADRB2	ATGGGGCAACCTGGCAATGGATCAGCTTTTCTCCTCGCCCCTAATCGGAGCCACGCTCCCGACCACGA TGTGACTCAGCAGAGAGACGAGGTCTGGGTTGTAGGCATGGGTATTGTGATGTCACTGATCGTCCTGG CAATTGTGTTCGGCAACGTCCTCGTCATTACTGCTATTGCAAAGTTCGAGCGGCTTCAGACGGTTACCA ATTACTTTATTACTTCCCTCGCCTGTGCCGACTTGGTCATGGGCCTTGCGGTTGTACCGTTTGGCGCAG CTCATATCCTGATGAAGATGTGGACTTTTGGTAATTTTTGGTGCGAATTCTGGACTTCAATTGACGTGCT CTGCGTCACAGCGTCAATCGAAACTCTGTGTGTTATTGCCGTTGATAGGTACTTCGCGATTACGAGTCC CTTTAAATACCAGTCTCTGCTGACTAAGAACAAAGCTCGGGTTATCATACTCATGGTGTGGATCGTCTCA GGACTCACAAGCTTTCTGCCTATACAGATGCACTGGTATCGCGCCACACACCAAGAAGCTATCAATTGT TACGCAAACGAAACCTGTTGCGACTTCTTCACTAATCAAGCCTATGCTATCGCTAGTTCAATTGTCTCTT TTTATGTGCCTCTGGTTATCATGGTGTTCGTCTACTCTCGGGTTTTTCAGGAGGCAAAAAGACAGCTCC AGAAAATCGACAAGTCAGAAGGCCGATTTCACGTTCAAAATCTGAGCCAGGTCGAACAAGACGGAAGA ACTGGACATGGTCTTCGGAGATCTTCCAAATTTTGCTTGAAGGAACACAAGGCCCTGAAGACCTTGGG GATAATTATGGGCACATTCACTCTCTGCTGGCTCCCCTTTTTCATTGTCAATATCGTTCACGTTATACAA GACAACTTGATCAGAAAAGAGGTGTATATCCTCCTGAACTGGATTGGCTACGTGAACTCTGGGTTCAAC CCTCTGATTTACTGCCGCAGCCCAGACTTCAGAATCGCATTCCAGGAATTGCTGTGTCTGAGGCGCTCT TCCTTGAAGGCTTATGGAAACGGATACTCCTCTAATGGCAACACGGGCGAACAGTCAGGATACCACGT GGAGCAGGAAAAAGAGAATAAGCTGTTGTGCGAGGACCTCCCTGGCACTGAGGATTTTGTTGGCCACC AAGGAACAGTCCCAAGCGACAATATTGACAGCCAGGGGCGAAACTGCAGCACTAATGATTCACTGCTG
AVPR2	ATGCTCATGGCCTCTACCACAAGCGCTGTGCCCGGACATCCATCTCTTCCAAGTCTTCCCTCCAACAGC TCACAGGAGAGGCCTCTGGATACCCGGGACCCACTTCTTGCAAGAGCGGAGCTCGCCTTGCTCTCCAT CGTTTTCGTTGCCGTCGCGTTGAGCAATGGTCTGGTGCTGGCCGCCCTGGCGCGACGAGGCCGCCGA GGCCACTGGGCGCCTATTCATGTCTTTATCGGCCATCTCTGTCTTGCAGACCTGGCCGTGGCGCTGTT TCAGGTGCTCCCTCAACTGGCTTGGAAAGCCACCGATAGATTCCGGGGGCCAGACGCACTGTGTCGG

	GCCGTGAAATACCTGCAGATGGTGGGAATGTATGCCAGTTCATATATGATACTTGCTATGACCTTGGAC AGACACAGGGCTATCTGTCGCCCCATGCTGGCGTATAGGCACGGTTCCGGAGCCCATTGGAACAGGC CAGTTCTGGTTGCGTGGGCTTTTTCCCTGCTTCTGAGCTTGCCCCAGTTGTTTATTTTCGCACAGAGAA ATGTGGAGGGCGGATCTGGCGTGACCGATTGCTGGGCTTGCTTCGCTGAGCCATGGGGCAGAAGGAC CTACGTGACGTGGATTGCTCTCATGGTGTTCGTTGCTCCCACCCTGGGGATCGCCGCATGCCAGGTCC TGATATTCAGAGAAATACACGCCTCCTTGGTTCCTGGGCCAAGCGAGCGCCCTGGTGGAAGAAGACGG GGTCGAAGGACTGGCTCTCCAGGCGAAGGCGCCCACGTATCAGCTGCGGTAGCTAAGACCGTACGCA TGACACTGGTCATCGTCGTCGTTTACGTTCTTTGCTGGGCCCCGTTTTTTTGGTGCAGCTCTGGGCGG CCTGGGACCCCGAAGCACCCCTGGAAGGTGCTCCATTCGTTCTGCTCATGCTTCTCGCTTCACTGAAT AGCTGTACCAATCCTTGGATTTACGCCTCTTTCTCCTCCAGTGTTAGCTCCGAGCTGCGGAGTCTTCTC TGTTGCGCCCGGGGCAGGACCCCCCCAAGCCTGGGTCCACAGGACGAATCCTGCACTACCGCCTCCA GTTCCCTGGCCAAAGACACCTCTTCT
BDKRB2	ATGTTCTCACCCTGGAAGATTTCTATGTTCCTTTCTGTGCGCGAGGACAGCGTGCCCACAACTGCGAGT TTTAGCGCCGACATGCTTAACGTGACCCTGCAGGGCCCCACTCTCAACGGGACATTTGCTCAGTCTAA ATGTCCTCAAGTGGAATGGCTCGGCTGGTTGAACACTATCCAGCCCCCTTTCCTTTGGGTTCTTTTTGT TCTGGCCACGCTGGAAAACATCTTTGTTCTCTCTGTGTTTTGCCTCCATAAGAGCTCCTGTACGGTGGC CGAAATTTACCTGGGTAATTTGGCCGCAGCGGATCTCATTTTGGCCTGTGGCCTGCCTTTCTGGGCTAT TACTATCAGCAATAATTTTGATTGGTTGTTTGGCGAGACCTTGTGTCGCGTGGTGAATGCAATTATCAGT ATGAATCTGTACAGCTCAATCTGCTTCCTTATGCTCGTCAGCATTGACAGGTACCTCGCCCTTGTAAAG ACTATGAGCATGGGCCGCATGAGGGGCGTACGCTGGGCTAAGCTCTATAGCCTGGTGATTTGGGGTT GCACCCTCCTCCTCAGTTCACCTATGCTGGTCTTCCGAACGATGAAGGAGTACTCTGACGAGGGCCAC AATGTTACCGCATGCGTTATCAGTTACCCATCTTTGATTTGGGAGGTCTTCACAAACATGCTGCTGAAC GTGGTGGGTTTTTTGCTCCCCCTTTCTGTGATTACCTTCTGTACAATGCAGATCATGCAAGTTTTGCGAA ACAACGAAATGCAAAAATTTAAGGAGATACAGACCGAGCGAAGGGCCACAGTGCTGGTCTTGGTCGTT TTGCTGTTGTTCATTATCTGTTGGCTGCCTTTCCAGATCAGCACTTTCCTGGACACGTTGCACAGACTC GGAATCCTGAGTAGTTGTCAGGACGAAAGGATCATCGACGTTATCACTCAAATCGCGTCCTTCATGGCA TACTCCAATAGCTGCTTGAACCCATTGGTGTATGTTATTGTGGGAAAACGGTTTAGGAAGAAGAGCTGG GAAGTGTACCAAGGGGTGTGCCAAAAGGGAGGCTGCCGATCAGAGCCAATCCAAATGGAAAATAGCAT GGGTACCCTGAGAACCTCCATTAGCGTGGAGCGACAGATCCACAAGCTCCAGGACTGGGCTGGCTCT AGACAG
CXCR4	ATGGAGGGGATATCAATCTACACATCAGATAATTACACGGAAGAGATGGGTTCCGGCGATTACGACTCT ATGAAAGAGCCGTGTTTTAGAGAGGAAAACGCGAACTTTAACAAGATCTTTCTCCCCACCATCTACAGC ATCATCTTTCTCACAGGCATCGTAGGGAACGGCCTGGTCATCCTGGTGATGGGATACCAAAAGAAGCT GAGGTCAATGACCGACAAGTATAGGCTCCATCTGTCCGTGGCCGACCTCCTGTTTGTGATTACCCTGC CTTTTTGGGCAGTTGACGCTGTCGCTAATTGGTACTTCGGCAACTTCCTCTGTAAGGCAGTGCACGTTA TCTACACTGTGAATCTTTATAGTTCCGTCTTGATCTTGGCCTTTATCAGTCTCGACAGGTATTTGGCGAT TGTGCACGCTACCAACTCACAACGACCTAGAAAACTCCTGGCTGAGAAGGTGGTTTACGTTGGTGTGT GGATTCCAGCTCTCCTGTTGACAATACCAGACTTCATTTTCGCTAATGTGAGCGAGGCCGATGACAGAT ACATTTGTGACCGATTTTACCCAAACGATCTGTGGGTAGTGGTATTTCAGTTCCAACACATTATGGTCGG GCTGATCTTGCCCGGCATTGTCATACTGTCTTGCTACTGCATCATTATTTCTAAGCTGTCACACTCAAAA GGCCACCAAAAGAGGAAGGCTCTGAAAACAACGGTGATCCTGATACTGGCCTTCTTCGCATGTTGGCT GCCCTACTATATCGGCATCAGCATTGACTCATTTATACTCCTGGAAATTATCAAGCAGGGCTGCGAGTT CGAGAACACCGTTCATAAGTGGATTTCTATAACCGAGGCCCTCGCCTTCTTTCACTGTTGTTTGAATCC GATTCTCTACGCGTTTCTTGGCGCCAAATTTAAAACAAGCGCCCAACATGCACTGACATCAGTGTCTAG GGGGAGCTCTCTGAAAATCCTCTCCAAGGGAAAACGAGGCGGACATAGCAGTGTCAGCACTGAGTCC GAATCCAGCTCATTTCATAGCTCT
LPAR1	ATGGCCGCTATTAGCACCAGTATCCCAGTTATCTCTCAGCCCCAGTTTACAGCGATGAATGAGCCTCAG TGCTTTTACAACGAAAGCATCGCCTTTTTCTATAACAGATCCGGGAAGCATCTGGCCACCGAATGGAAT ACGGTGTCTAAATTGGTCATGGGCCTTGGTATTACCGTTTGCATCTTTATCATGCTTGCAAACTTGCTGG TGATGGTGGCTATCTACGTGAACCGCAGATTCCATTTTCCAATTTACTACTTGATGGCAAATCTTGCCGC AGCTGACTTCTTCGCGGGGTTGGCATATTTCTACTTGATGTTTAATACCGGGCCCAACACCCGCAGACT TACTGTCTCAACTTGGCTGTTGCGCCAGGGACTCATCGACACTTCCCTGACTGCCTCTGTGGCAAACCT GTTGGCCATCGCAATTGAGAGACACATAACGGTGTTCCGAATGCAACTTCATACACGGATGTCAAACCG GAGGGTGGTGGTGGTGATCGTGGTGATCTGGACCATGGCTATCGTTATGGGCGCCATTCCTAGCGTG GGTTGGAATTGCATTTGCGACATCGAGAACTGTTCCAATATGGCACCTCTGTATTCTGACAGTTATCTG GTTTTCTGGGCCATCTTTAATCTCGTGACATTTGTCGTGATGGTGGTACTGTATGCTCACATCTTCGGAT ACGTGAGACAACGCACAATGCGGATGTCTCGACACAGCAGCGGACCCAGACGGAATCGGGACACAAT GATGTCCCTCCTCAAAACCGTCGTAATCGTGTTGGGCGCATTTATCATTTGTTGGACCCCCGGGCTCGT ACTGCTTCTGCTCGACGTGTGTTGTCCCCAGTGTGACGTTCTTGCCTATGAGAAGTTCTTTCTTTTGTTG GCCGAATTCAACTCCGCAATGAATCCTATTATTTACTCTTATCGAGATAAAGAGATGTCCGCAACTTTTC GGCAGATCCTCTGCTGCCAGAGGAGCGAGAATCCTACAGGCCCCACTGAAGGTTCAGATCGGTCAGC TTCATCCCTCAACCACACAATCCTGGCCGGTGTACACTCCAACGATCATTCCGTGGTC
NMBR	ATGCCCTCCAAGTCTCTGTCAAATCTGAGTGTCACCACCGGAGCTAATGAGAGTGGATCTGTTCCTGAG GGATGGGAAAGGGATTTCCTTCCAGCTAGCGATGGGACCACAACAGAGCTTGTGATCCGGTGCGTGAT CCCCTCACTCTACTTGCTGATCATTACAGTCGGGCTTCTCGGCAACATAATGCTCGTTAAGATTTTCATA ACTAATTCAGCCATGCGGTCCGTTCCCAACATCTTTATCAGTAACCTCGCTGCAGGTGATCTGCTCCTG CTTCTGACCTGCGTACCTGTGGACGCATCCCGCTACTTTTTTGATGAATGGATGTTTGGAAAGGTTGGG TGTAAGCTGATCCCTGTCATCCAGCTCACGTCAGTGGGGGTGAGTGTGTTCACGCTTACTGCCCTCTCT GCAGATAGATACCGAGCGATCGTAAACCCCATGGATATGCAGACGTCCGGCGCTCTTCTCCGGACTTG

	CGTGAAGGCAATGGGAATATGGGTGGTGTCAGTGTTGCTGGCTGTTCCCGAGGCCGTGTTTTCAGAGGTTGCTAGGATCTCTTCACTGGATAATAGTTCATTCACGGCCTGTATTCCATATCCCCAGACAGACGAGCTGCACCCTAAGATCCACTCCGTACTTATATTTTTGGTCTATTTTCTGATCCCCCTTGCAATCATCTCAATCTATTACTACCACATCGCAAAAACATTGATCAAATCCGCCCATAACCTCCCCGGGGAGTACAATGAACATACAAAGAAGCAGATGGAGACCAGGAAAAGGCTCGCCAAGATCGTCCTTGTTTTCGTTGGGTGCTTTATCTTCTGCTGGTTTCCCAATCACATACTGTATATGTACCGGAGTTTTAACTATAATGAGATTGATCCCTCACTCGGACATATGATTGTGACCCTCGTGGCCCGGGTGCTCTCCTTCGGGAATAGCTGTGTCAACCCCTTCGCGCTGTACCTGCTCTCCGAGTCTTTTCGCCGACACTTCAATTCACAGCTCTGCTGTGGGAGAAAAAGCTACCAGGAACGAGGAACATCTTATCTGCTTTCATCTAGCGCCGTGCGGATGACATCCCTGAAGAGTAACGCGAAGAACATGGTGACGAACTCAGTCCTCCTGAATGGGCATTCCATGAAGCAGGAGATGGCCCTG
TEV protease	AGCTTGTTTAAGGGACCACGTGATTACAACCCGATATCGAGCACCATTTGTCATTTGACGAATGAATCTGATGGGCACACAACATCGTTGTATGGTATTGGATTTGGTCCCTTCATCATTACAAACAAGCACTTGTTTAGAAGAAATAATGGAACACTGTTGGTCCAATCACTACATGGTGTATTCAAGGTCAAGAACACCACGACTTTGCAACAACACCTCATTGATGGGAGGGACATGATAATTATTCGCATGCCTAAGGATTTCCCACCATTTCCTCAAAAGCTGAAATTTAGAGAGCCACAAAGGGAAGAGCGCATATGTCTTGTGACAACCAACTTCCAAACTAAGAGCATGTCTAGCATGGTGTCAGACACTAGTTGCACATTCCCTTCATCTGATGGCATATTCTGGAAGCATTGGATTCAAACCAAGGATGGGCAGTGTGGCAGTCCATTAGTATCAACTAGAGATGGGTTCATTGTTGGTATACACTCAGCATCGAATTTCACCAACACAAACAATTATTTCACAAGCGTGCCGAAAAACTTCATGGAATTGTTGACAAATCAGGAGGCGCAGCAGTGGGTTAGTGGTTGGCGATTAAATGCTGACTCAGTATTGTGGGGGGGCCATAAAGTTTTCATGAGCAAACCTGAAGAGCCTTTTCAGCCAGTTAAGGAAGCGACTCAACTCATGAATGAATTGGTGTACTCGCAA
tTA	AGATTAGATAAAAGTAAAGTGATTAACAGCGCATTAGAGCTGCTTAATGAGGTCGGAATCGAAGGTTTAACAACCCGTAAACTCGCCCAGAAGCTAGGTGTAGAGCAGCCTACATTGTATTGGCATGTAAAAAATAAGCGGGCTTTGCTCGACGCCTTAGCCATTGAGATGTTAGATAGGCACCATACTCACTTTTGCCCTTTAGAAGGGGAAAGCTGGCAAGATTTTTTACGTAATAACGCTAAAAGTTTTAGATGTGCTTTACTAAGTCATCGCGATGGAGCAAAAGTACATTTAGGTACACGGCCTACAGAAAAACAGTATGAAACTCTCGAAAATCAATTAGCCTTTTTATGCCAACAAGGTTTTTCACTAGAGAATGCATTATATGCACTCAGCGCTGTGGGGCATTTTACTTTAGGTTGCGTATTGGAAGATCAAGAGCATCAAGTCGCTAAAGAAGAAAGGGAAACACCTACTACTGATAGTATGCCGCCATTATTACGACAAGCTATCGAATTATTTGATCACCAAGGTGCAGAGCCAGCCTTCTTATTCGGCCTTGAATTGATCATATGCGGATTAGAAAAACAACTTAAATGTGAAAGTGGGTCCGCGTACAGCCGGGCGCGTACGAAAAACAATTACGGGTCTACCATCGAGGGCCTGCTCGATCTCCCGGACGACGACGCCCCCGAAGAGGCGGGGCTGGCGGCTCCGCGCCTGTCCTTTCTCCCCGCGGGACACACGCGCAGACTGTCGACGGCCCCCCCGACCGATGTCAGCCTGGGGGACGAGCTCCACTTAGACGGCGAGGACGTGGCGATGGCGCATGCCGACGCGCTAGACGATTTCGATCTGGACATGTTGGGGGACGGGGATTCCCCGGGTCCGGGATTTACCCCCCACGACTCCGCCCCCTACGGCGCTCTGGATATGGCCGACTTCGAGTTTGAGCAGATGTTTACCGATGCCCTTGGAATTGACGAGTACGGTGGG
rtTA	ATGTCTAGACTGGACAAGAGCAAAGTCATAAACGGCGCTCTGGAATTACTCAATGGAGTCGGTATCGAAGGCCTGACGACAAGGAAACTCGCTCAAAAGCTGGGAGTTGAGCAGCCTACCCTGTACTGGCACGTGAAGAACAAGCGGGCCCTGCTCGATGCCCTGCCAATCGAGATGCTGGACAGGCATCATACCCACTTCTGCCCCCTGGAAGGCGAGTCATGGCAAGACTTTCTGCGGAACAACGCCAAGTCATTCCGCTGTGCTCTCCTCTCACATCGCGACGGGGCTAAAGTGCATCTCGGCACCCGCCCAACAGAGAAACAGTACGAAACCCTGGAAAATCAGCTCGCGTTCCTGTGTCAGCAAGGCTTCTCCCTGGAGAACGCACTGTACGCTCTGTCCGCCGTGGGCCACTTTACACTGGGCTGCGTATTGGAGGAACAGGAGCATCAAGTAGCAAAAGAGGAAAGAGAGACACCTACCACCGATTCTATGCCCCCACTTCTGAGACAAGCAATTGAGCTGTTCGACCGGCAGGGAGCCGAACCTGCCTTCCTTTTCGGCCTGGAACTAATCATATGTGGCCTGGAGAAACAGCTAAAGTGCGAAAGCGGCGGGCCGGCCGACGCCCTTGACGATTTTGACTTAGACATGCTCCCAGCCGATGCCCTTGACGACTTTGACCTTGATATGCTGCCTGCTGACGCTCTTGACGATTTTGACCTTGACATGCTCCCCGGGTAA
MGSS	ATGGGCTCGAGC
G4S	GGCGGTGGCGGGTCG
(G4S)2	GGTGGAGGCGGTTCAGGCGGTGGCGGGTCG
TCS	GAGAATCTGTACTTTCAGCTG

Supplementary Table 6: Sequences of promoters used to build the plasmids.

Promoter	Sequence
NarL-RE-promoter	CGCGCCTACCCCTATAGGGGTATAGCGCCGGCTACCCCTATAGGGGTATCCATATGCTCTAGAGGGT ATATAATGGGGGCCACTAGTCTACTACCAGAGCTCATCGCTAGCGGGATCCACCGGTCGCCACCATG
OmpR-RE-promoter	CGCGCCATTTACATTTTGAAACATCTATAGCGCCGGCATTTACATTTTGAAACATCTATCCATATGCTCT AGAGGGTATATAATGGGGGCCACTAGTCTACTACCAGAGCTCATCGCTAGCGGGATCCACCGGTCGC CACCATG
TRE promoter	CGAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACGAT GTCGAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAAC GTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGTTTATCCCTATCAGTGATAGAGA ACGTATGTCGAGTTTACTCCCTATCAGTGATAGAGAACGTATGTCGAGGTAGGCGTGTACGGTGGGA GGCCTATATAAGCAGAGCTCGTTTAGTGAACCGTCAGATCGCCTGGAGATTTCGAGCTCGGTACCTC GCCACCATG
EF1α	TTAAGCTCGGGCCCGCTCCGGTGCCCGTCAGTGGGCAGAGCGCACATCGCCCACAGTCCCCGAGAA GTTGGGGGGAGGGGTCGGCAATTGAACCGGTGCCTAGAGAAGGTGGCGCGGGGTAAACTGGGAAA GTGATGTCGTGTACTGGCTCCGCCTTTTTCCCGAGGGTGGGGGAGAACCGTATATAAGTGCAGTAGT CGCCGTGAACGTTCTTTTTCGCAACGGGTTTGCCGCCAGAACACAGGTAAGTGCCGTGTGTGGTTCC CGCGGGCCTGGCCTCTTTACGGGTTATGGCCCTTGCGTGCCTTGAATTACTTCCACGCCCCTGGCTG CAGTACGTGATTCTTGATCCCGAGCTTCGGGTTGGAAGTGGGTGGGAGAGTTCGAGGCCTTGCGCTT AAGGAGCCCCTTCGCCTCGTGCTTGAGTTGAGGCCTGGCCTGGGCGCTGGGGCCGCCGCGTGCGAA TCTGGTGGCACCTTCGCGCCTGTCTCGCTGCTTTCGATAAGTCTCTAGCCATTTAAAATTTTTGATGAC CTGCTGCGACGCTTTTTTTCTGGCAAGATAGTCTTGTAAATGCGGGCCAAGATCTGCACACTGGTATT TCGGTTTTTGGGGCCGCGGGCGGCGACGGGGCCCGTGCGTCCCAGCGCACATGTTCGGCGAGGCG GGGCCTGCGAGCGCGGCCACCGAGAATCGGACGGGGGTAGTCTCAAGCTGGCCGGCCTGCTCTGG TGCCTGGCCTCGCGCCGCCGTGTATCGCCCCGCCCTGGGCGGCAAGGCTGGCCCGGTCGGCACCA GTTGCGTGAGCGGAAAGATGGCCGCTTCCCGGCCCTGCTGCAGGGAGCTCAAAATGGAGGACGCGG CGCTCGGGAGAGCGGGCGGGTGAGTCACCCACACAAAGGAAAAGGGCCTTTCCGTCCTCAGCCGTC GCTTCATGTGACTCCACGGAGTACCGGGCGCCGTCCAGGCACCTCGATTAGTTCTCGAGCTTTTGGA GTACGTCGTCTTTAGGTTGGGGGGAGGGGTTTTATGCGATGGAGTTTCCCCACACTGAGTGGGTGGA GACTGAAGTTAGGCCAGCTTGGCACTTGATGTAATTCTCCTTGGAATTTGCCCTTTTTGAGTTTGGATC TTGGTTCATTCTCAAGCCTCAGACAGTGGTTCAAAGTTTTTTTCTTCCATTTCAGGTGTCGTGAACCGG TCGCCACCATG
EF1α-V1	Altamura et al, in preparation

Supplementary Table 7. Transfection setup for Fig. 1b. Plasmid amounts per transfection are shown.

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng			
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng
CMV-*narL* (pJH004)	100 ng	100 ng	100 ng	100 ng
CMV-*narX* (pJH002)		100 ng		
CMV-$narX^{176\text{-}598}$ (pJH010)			100 ng	
CMV-$narX^{379\text{-}598}$ (pMZ163)				100 ng

Supplementary Table 8. Transfection setup for Fig. 2b. Plasmid amounts per transfection are shown.

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng				
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng
CMV-*narL* (pJH004)	100 ng	100 ng	100 ng	100 ng	100 ng
CMV-*narX* (pJH002)		100 ng			
CMV-*narX* H399Q (pEM014)			100 ng		50 ng
CMV-*narX* N509A (pMZ160)				100 ng	50 ng

Supplementary Table 9. Transfection setup for Fig. 2c. Plasmid amounts per transfection are shown.

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng					
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-narL (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)		100 ng				
EF1α-V1-*narX*$^{379-598}$ (pMZ241)			100 ng			
EF1α-V1-*narX*$^{379-598}$ H399Q (pMZ244)				50 ng	50 ng	50 ng
EF1α-V1-*narX*$^{379-598}$ N509A (pMZ247)				50 ng		
EF1α-V1-*SYNZIP1*::*narX*$^{379-598}$ H399Q (pMZ223)						
EF1α-V1- *SYNZIP1*::*narX*$^{379-598}$ N509A (pMZ225)					50 ng	
EF1α-V1- *SYNZIP2*::*narX*$^{379-598}$ H399Q (pMZ224)						
EF1α-V1- *SYNZIP2*::*narX*$^{379-598}$ N509A (pMZ226)						50 ng

Plasmid	Lane 7	Lane 8	Lane 9	Lane10	Lane11	Lane12
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)						
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α -*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α -V1-*narX* (pMZ239)						
EF1α -V1-*narX*$^{379-598}$ (pMZ241)						
EF1α -V1-*narX*$^{379-598}$ H399Q (pMZ244)						
EF1α -V1-*narX*$^{379-598}$ N509A (pMZ247)	50 ng	50 ng				
EF1α-V1-*SYNZIP1*::*narX*$^{379-598}$ H399Q (pMZ223)	50 ng		50 ng		50 ng	
EF1α-V1- *SYNZIP1*::*narX*$^{379-598}$ N509A (pMZ225)			50 ng			50 ng
EF1α-V1- *SYNZIP2*::*narX*$^{379-598}$ H399Q (pMZ224)		50 ng		50 ng		50 ng
EF1α-V1- *SYNZIP2*::*narX*$^{379-598}$ N509A (pMZ226)				50 ng	50 ng	

Supplementary Table 10. Transfection setup for Fig. 3a. Plasmid amounts per transfection are shown.

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng					
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)		100 ng				
EF1α-V1-*narX*$^{379-598}$ (pMZ241)			100 ng			
EF1α-V1-*narX*$^{379-598}$ H399Q (pMZ244)				50 ng	50 ng	50 ng
EF1α-V1-*narX*$^{379-598}$ N509A (pMZ247)				50 ng		
EF1α-V1-*FKBP1A*::*narX*$^{379-598}$ H399Q (pMZ229)						
EF1α-V1-*FKBP1A*::*narX*$^{379-598}$ N509A (pMZ231)					50 ng	
EF1α-V1-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ H399Q (pMZ230)						
EF1α-V1-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ N509A (pMZ232)						50 ng

Plasmid	Lane 7	Lane 8	Lane 9	Lane 10	Lane 11	Lane 12
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)						
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)						
EF1α-V1-*narX*$^{379-598}$ (pMZ241)						
EF1α-V1-*narX*$^{379-598}$ H399Q (pMZ244)						
EF1α-V1-*narX*$^{379-598}$ N509A (pMZ247)	50 ng	50 ng				
EF1α-V1-*FKBP1A*::*narX*$^{379-598}$ H399Q (pMZ229)	50 ng		50 ng		50 ng	
EF1α-V1-*FKBP1A*::*narX*$^{379-598}$ N509A (pMZ231)			50 ng			50 ng
EF1α-V1-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ H399Q (pMZ230)		50 ng		50 ng		50 ng
EF1α-V1-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ N509A (pMZ232)				50 ng	50 ng	

Supplementary Table 11. Transfection setup for Fig. 3c. Plasmid amounts per transfection are shown.

Plasmid	
EF1α-mCherry (pKH026)	100 ng
NarL_RE-*cerulean* (pMZ219)	100 ng
EF1α-*narL* (pMZ248)	100 ng
EF1α-V1-*FKBP1A*::*narX*$^{379-598}$ N509A (pMZ231)	50 ng
EF1α-V1-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ H399Q (pMZ230)	50 ng

Supplementary Table 12. Transfection setup for Fig. 4b. Plasmid amounts per transfection are shown

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng					
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)		100 ng				
EF1α-V1-*narX*$^{379-598}$ (pMZ241)			100 ng			
EF1α-V1-*narX*$^{379-598}$ H399Q (pMZ244)				50 ng	50 ng	50 ng
EF1α-V1-*narX*$^{379-598}$ N509A (pMZ247)				50 ng		
EF1α-V1-*ARRB2*::*narX*$^{379-598}$ H399Q (pMZ251)						
EF1α-V1-*ARRB2*::*narX*$^{379-598}$ N509A (pMZ252)					50 ng	
EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257)						
EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ N509A (pMZ258)						50 ng

Supplementary Table 14 (cont.)

Plasmid	Lane 7	Lane 8	Lane 9	Lane 10	Lane 11	Lane 12
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)						
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)						
EF1α-V1-*narX*$^{379\text{-}598}$ (pMZ241)						
EF1α-V1-*narX*$^{379\text{-}598}$ H399Q (pMZ244)						
EF1α-V1-*narX*$^{379\text{-}598}$ N509A (pMZ247)	50 ng	50 ng				
EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ H399Q (pMZ251)	50 ng		50 ng		50 ng	
EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ N509A (pMZ252)			50 ng			50 ng
EF1α-V1- *ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*narX*$^{379\text{-}598}$ H399Q (pMZ257)		50 ng		50 ng		50 ng
EF1α-V1- *ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*narX*$^{379\text{-}598}$ N509A (pMZ258)				50 ng	50 ng	

Supplementary Table 13. Transfection setup for Fig. 4d and 4g. Plasmid amounts per transfection are shown.

Plasmid	TCS	Tango
EF1α-mCherry (pKH026)	100 ng	100 ng
Junk-DNA (pBH265)		
NarL_RE-*cerulean* (pMZ219)	100 ng	
EF1α-*narL* (pMZ248)	100 ng	
EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ N509A (pMZ252)	50 ng	
EF1α-V1-*ADRB2*::*AVPR2*::*narX*$^{379\text{-}598}$ H399Q (pMZ257)	50 ng	
tTA_RE-*cerulean* (pMZ290)		100 ng
EF1α-V1-*ARRB2:*:TEV protease (pMZ291)		100 ng
EF1α-V1-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*tTA* (pMZ292)		100 ng

Supplementary Table 14. Transfection setup for Fig. 5a. gray and black bars. Plasmid amounts per transfection are shown

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*ARRB2*::*narX*$^{379-598}$ N509A (pMZ252)	50 ng	50 ng	50 ng	50 ng	50 ng
EF1α-V1- *ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257)	50 ng				
EF1α-V1-*ADRB2*$^{1-413}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ307)		50 ng			
EF1α-V1-*NMBR*$^{1-390}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ312)			50 ng		
EF1α-V1-*AVPR2*$^{1-371}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ308)				50 ng	
EF1α-V1-*LPAR1*$^{1-364}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ311)					50 ng
EF1α-V1-*BDKRB2*$^{1-391}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ309)					
EF1α-V1-*CXCR4*$^{1-358}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ310)					
EF1α-V1-*NPY1R*$^{1-384}$::*narX*$^{379-598}$ H399Q (pMZ295)					
EF1α-V1-*NPY5R*$^{1-438}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ297)					

Plasmid	Lane 6	Lane 7	Lane 8	Lane 9
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*ARRB2*::*narX*$^{379-598}$ N509A (pMZ252)	50 ng	50 ng	50 ng	50 ng
EF1α-V1- *ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257)				
EF1α-V1-*ADRB2*$^{1-413}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ307)				
EF1α-V1-*NMBR*$^{1-390}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ312)				
EF1α-V1-*AVPR2*$^{1-371}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ308)				
EF1α-V1-*LPAR1*$^{1-364}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ311)				
EF1α-V1-*BDKRB2*$^{1-391}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ309)	50 ng			
EF1α-V1-*CXCR4*$^{1-358}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ310)		50 ng		
EF1α-V1-*NPY1R*$^{1-384}$::*narX*$^{379-598}$ H399Q (pMZ295)			50 ng	
EF1α-V1-*NPY5R*$^{1-438}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ297)				50 ng

Supplementary Table 15. Transfection setup for Fig. 5a. ligth brown and brown bars.

Plasmid amounts per transfection are shown

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng
tTA_RE-*cerulean* (pMZ290)	100 ng	100 ng	100 ng	100 ng	100 ng
CMV-*ARRB2*::TEV protease (pBH302)	100 ng	100 ng	100 ng	100 ng	100 ng
CMV-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*tTA* (pBH312)	100 ng				
CMV-*ADRB2*$^{1-413}$::*AVPR2*$^{343-371}$::*tTA* (pMZ300)		100 ng			
CMV-*NMBR*$^{1-390}$::*AVPR2*$^{343-371}$::*tTA* (pMZ305)			100 ng		
CMV-*AVPR2*$^{1-371}$::*AVPR2*$^{343-371}$::*tTA* (pMZ301)				100 ng	
CMV-*LPAR1*$^{1-364}$::*AVPR2*$^{343-371}$::*tTA* (pMZ304)					100 ng
CMV-*BDKRB2*$^{1-391}$::*AVPR2*$^{343-371}$::*tTA* (pMZ302)					
CMV-*CXCR4*$^{1-358}$::*AVPR2*$^{343-371}$::*tTA* (pMZ303)					
CMV-*NPY1R*$^{1-384}$::*AVPR2*$^{343-371}$::*tTA* (pMZ293)					
CMV-*NPY5R*$^{1-445}$::*AVPR2*$^{343-371}$::*tTA* (pMZ295)					

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng
tTA_RE-*cerulean* (pMZ290)	100 ng	100 ng	100 ng	100 ng
CMV-*ARRB2*::TEV protease (pBH302)	100 ng	100 ng	100 ng	100 ng
CMV-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*tTA* (pBH312)				
CMV-*ADRB2*$^{1-413}$::*AVPR2*$^{343-371}$::*tTA* (pMZ300)				
CMV-*NMBR*$^{1-390}$::*AVPR2*$^{343-371}$::*tTA* (pMZ305)				
CMV-*AVPR2*$^{1-371}$::*AVPR2*$^{343-371}$::*tTA* (pMZ301)				
CMV-*LPAR1*$^{1-364}$::*AVPR2*$^{343-371}$::*tTA* (pMZ304)				
CMV-*BDKRB2*$^{1-391}$::*AVPR2*$^{343-371}$::*tTA* (pMZ302)	100 ng			
CMV-*CXCR4*$^{1-358}$::*AVPR2*$^{343-371}$::*tTA* (pMZ303)		100 ng		
CMV-*NPY1R*$^{1-384}$::*AVPR2*$^{343-371}$::*tTA* (pMZ293)			100 ng	
CMV-*NPY5R*$^{1-445}$::*AVPR2*$^{343-371}$::*tTA* (pMZ295)				100 ng

Supplementary Table 16. Transfection setup for Fig. 6. Plasmid amounts per transfection are shown. The experiment was done in 96 well plates.

Plasmid	NarX fusion	Tango	rtTA
EF1α-mCherry (pKH026)	12.5 ng	12.5 ng	12.5 ng
Junk-DNA (pBH265)	25 ng	12.5 ng	37.5 ng
NarL_RE-*cerulean* (pMZ219)	25 ng		
EF1α-*narL* (pMZ248)	12.5 ng		
EF1α-V1-*narX* (pMZ239)			
EF1α-V1-*ARRB2*::*narX*$^{379-598}$ H399Q (pMZ251)	12.5 ng		
EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ N509A (pMZ258)	12.5 ng		
tTA_RE-*cerulean* (pMZ290)		25 ng	25 ng
EF1α-V1-*ARRB2*::TEV protease (pMZ291)		25 ng	
EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*tTA* (pMZ292)		25 ng	
CMV-rtTA (pZ91)			25 ng

Supplementary Table 17. Transfection setup for Supplementary Fig. 2b. Plasmid amounts per transfection are shown.

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng			
OmpR_RE-*cerulean* (pMZ1)	100 ng	100 ng	100 ng	100 ng
CMV-*ompR* (pJH003)	100 ng	100 ng	100 ng	100 ng
CMV-*envZ* (pJH001)		100 ng		
CMV-*envZ*_cyt (pJH009)			100 ng	
CMV-*envZ*$^{223\text{-}450}$ (pMZ123)				100 ng

Supplementary Table 18. Transfection setup for Supplementary Fig. 3. Plasmid amounts per transfection are shown

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng								
NarL_RE-*amCyan* (pJH7)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
CMV-*narL* (pJH004)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
CMV-*narX* (pJH002)		100 ng							
CMV-*narX*$^{379\text{-}598}$ (pMZ163)			100 ng						
CMV-*SYNZIP1*::*narX*$^{379\text{-}598}$ (pMZ200)				100 ng		50 ng			
CMV-*SYNZIP*::*narX*$^{379\text{-}598}$ (pMZ206)					100 ng	50 ng			
CMV-*narX*$^{379\text{-}598}$::*SYNZIP1* (pMZ202)							100 ng		50 ng
CMV-*narX*$^{379\text{-}598}$::*SYNZIP2* (pMZ208)								100 ng	50 ng

Supplementary Table 19. Transfection setup for Supplementary Fig. 4a. Plasmid amounts per transfection are shown.

Plasmid	CMV promoter	EF1α promoter
EF1α-*citrine* (pKH025)	100 ng	100 ng
Junk-DNA (pBH265)	200 ng	
CMV-*iRFP* (pCS12)	100 ng	
EF1α-*iRFP* (pCS184)		100 ng

Supplementary Table 20. Transfection setup for Supplementary Fig. 4b. Plasmid amounts per transfection are shown.

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7	Lane 8	Lane 9
EF1α-*mCherry* (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng								
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
CMV-*narL* (pJH004)	100 ng	100 ng	100 ng						
EF1α-*narL* (pMZ248)				100 ng	100 ng	100 ng			
EF1α-V1-*narL* (pMZ249)							100 ng	100 ng	100 ng
CMV-*narX* (pJH002)		100 ng			100 ng			100 ng	
EF1α-V1-*narX* (pMZ239)			100 ng			100 ng			100 ng

Supplementary Table 21. Transfection setup for Supplementary Fig. 5c. Plasmid amounts per transfection are shown.

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng					
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)		100 ng				
EF1α-V1-*narX*$^{379\text{-}598}$ (pMZ241)			100 ng			
EF1α-V1-*narX*$^{379\text{-}598}$ H399Q (pMZ244)				100 ng		
EF1α-V1-*narX*$^{379\text{-}598}$ N509A (pMZ247)					100 ng	
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ H399Q (pMZ223)						100 ng
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ N509A (pMZ225)						
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ H399Q (pMZ224)						
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ N509A (pMZ226)						

Plasmid	Lane 7	Lane 8	Lane 9
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng
Junk-DNA (pBH265)			
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)			
EF1α-V1-*narX*$^{379\text{-}598}$ (pMZ241)			
EF1α-V1-*narX*$^{379\text{-}598}$ H399Q (pMZ244)			
EF1α-V1-*narX*$^{379\text{-}598}$ N509A (pMZ247)			
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ H399Q (pMZ223)			
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ N509A (pMZ225)	100 ng		
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ H399Q (pMZ224)		100 ng	
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ N509A (pMZ226)			100 ng

Supplementary Table 22. Transfection setup for Supplementary Fig. 5d. Plasmid amounts per transfection are shown.

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng					
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)		100 ng				
EF1α-V1-*narX*$^{379\text{-}598}$ (pMZ241)			100 ng			
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ H399Q (pMZ223)				50 ng		50 ng
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ N509A (pMZ225)					50 ng	50 ng
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ H399Q (pMZ224)					50 ng	
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ N509A (pMZ226)				50 ng		
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ (pMZ221)						
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ (pMZ222)						

Plasmid	Lane 7	Lane 8	Lane 9	Lane 10	Lane 11
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)					
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)					
EF1α-V1-*narX*$^{379\text{-}598}$ (pMZ241)					
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ H399Q (pMZ223)	50 ng				
EF1α-V1-*SYNZIP*::*narX*$^{379\text{-}598}$ N509A (pMZ225)		50 ng			
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ H399Q (pMZ224)					
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ N509A (pMZ226)					
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ (pMZ221)			100 ng		50 ng
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ (pMZ222)	50 ng	50 ng		100 ng	50 ng

Supplementary Table 23. Transfection setup for Supplementary Fig. 5e. Plasmid amounts per transfection are shown.

For SynZip2::H^{mut}+ SynZip2::N^{mut} titration:

E1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	93.75 ng	87.5 ng	75 ng	50 ng	
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*SYNZIP2*::*narX*$^{379-598}$ H399Q (pMZ224)	3.125 ng	6.25 ng	12.5 ng	25 ng	50 ng
EF1α-V1-*SYNZIP2*::*narX*$^{379-598}$ N509A (pMZ226)	3.125 ng	6.25 ng	12.5 ng	25 ng	50 ng

For SynZip1::H^{mut}+ SynZip1::N^{mut} titration:

EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	93.75 ng	87.5 ng	75 ng	50 ng	
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*SYNZIP1*::*narX*$^{379-598}$ H399Q (pMZ223)	3.125 ng	6.25 ng	12.5 ng	25 ng	50 ng
EF1α-V1-*SYNZIP1*::*narX*$^{379-598}$ N509A (pMZ225)	3.125 ng	6.25 ng	12.5 ng	25 ng	50 ng

For SynZip1::H^{mut}+ SynZip2::N^{mut} titration:

EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	93.75 ng	87.5 ng	75 ng	50 ng	
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*SYNZIP1*::*narX*$^{379-598}$ H399Q (pMZ223)	3.125 ng	6.25 ng	12.5 ng	25 ng	50 ng
EF1α-V1-*SYNZIP2*::*narX*$^{379-598}$ N509A (pMZ226)	3.125 ng	6.25 ng	12.5 ng	25 ng	50 ng

For SynZip1::N^{mut}+ SynZip2::H^{mut} titration:

EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	93.75 ng	87.5 ng	75 ng	50 ng	
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*SYNZIP1*::*narX*$^{379\text{-}598}$ N509A (pMZ225)	3.125 ng	6.25 ng	12.5 ng	25 ng	50 ng
EF1α-V1-*SYNZIP2*::*narX*$^{379\text{-}598}$ H399Q (pMZ224)	3.125 ng	6.25 ng	12.5 ng	25 ng	50 ng

For NarX titration:

EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	93.75 ng	87.5 ng	75 ng	50 ng	
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)	6.25 ng	12.5 ng	25 ng	50 ng	100 ng

Supplementary Table 24. Transfection setup for Supplementary Fig. 6c. Plasmid amounts per transfection are shown

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng					
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)		100 ng				
EF1α-V1-*narX*$^{379\text{-}598}$ (pMZ241)			100 ng			
EF1α-V1-*narX*$^{379\text{-}598}$ H399Q (pMZ244)				100 ng		
EF1α-V1-*narX*$^{379\text{-}598}$ N509A (pMZ247)					100 ng	
EF1α-V1-*FKBP1A*::*narX*$^{379\text{-}598}$ H399Q (pMZ229)						100 ng
EF1α-V1-*FKBP1A*::*narX*$^{379\text{-}598}$ N509A (pMZ231)						
EF1α-V1-*MTOR*$^{2018\text{-}113}$ T2098L::*narX*$^{379\text{-}598}$ H399Q (pMZ230)						
EF1α-V1-*MTOR*$^{2018\text{-}113}$ T2098L::*narX*$^{379\text{-}598}$ N509A (pMZ232)						

Plasmid	Lane 7	Lane 8	Lane 9
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng
Junk-DNA (pBH265)			
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)			
EF1α-V1-*narX*$^{379\text{-}598}$ (pMZ241)			
EF1α-V1-*narX*$^{379\text{-}598}$ H399Q (pMZ244)			
EF1α-V1-*narX*$^{379\text{-}598}$ N509A (pMZ247)			
EF1α-V1-*FKBP1A*::*narX*$^{379\text{-}598}$ H399Q (pMZ229)			
EF1α-V1-*FKBP1A*::*narX*$^{379\text{-}598}$ N509A (pMZ231)	100 ng		
EF1α-V1-*MTOR*$^{2018\text{-}113}$ T2098L::*narX*$^{379\text{-}598}$ H399Q (pMZ230)		100 ng	
EF1α-V1-*MTOR*$^{2018\text{-}113}$ T2098L::*narX*$^{379\text{-}598}$ N509A (pMZ232)			100 ng

Supplementary Table 25. Transfection setup for Supplementary Fig. 7. Plasmid amounts per transfection are shown

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng					
NarL_RE-*amCyan* (pJH007)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
CMV-*narL* (pJH004)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
CMV-*narX* (pJH002)		100 ng				
CMV-*narX*$^{379-598}$ (pMZ163)			100 ng			
CMV-*FKBP1A*::*narX*$^{379-598}$ (pMZ214)				100 ng		50 ng
CMV-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ (pMZ215)					100 ng	50 ng

Supplementary Table 26. Transfection setup for Supplementary Fig. 8c. Plasmid amounts per transfection are shown.

Plasmid	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
Junk-DNA (pBH265)	100 ng					
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)		100 ng				
EF1α-V1-*narX*$^{379\text{-}598}$ (pMZ241)			100 ng			
EF1α-V1-*narX*$^{379\text{-}598}$ H399Q (pMZ244)				100 ng		
EF1α-V1-*narX*$^{379\text{-}598}$ N509A (pMZ247)					100 ng	
EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ H399Q (pMZ251						100 ng
EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ N509A (pMZ252)						
EF1α-V1-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*narX*$^{379\text{-}598}$ H399Q (pMZ257)						
EF1α-V1-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*narX*$^{379\text{-}598}$ N509A (pMZ258)						

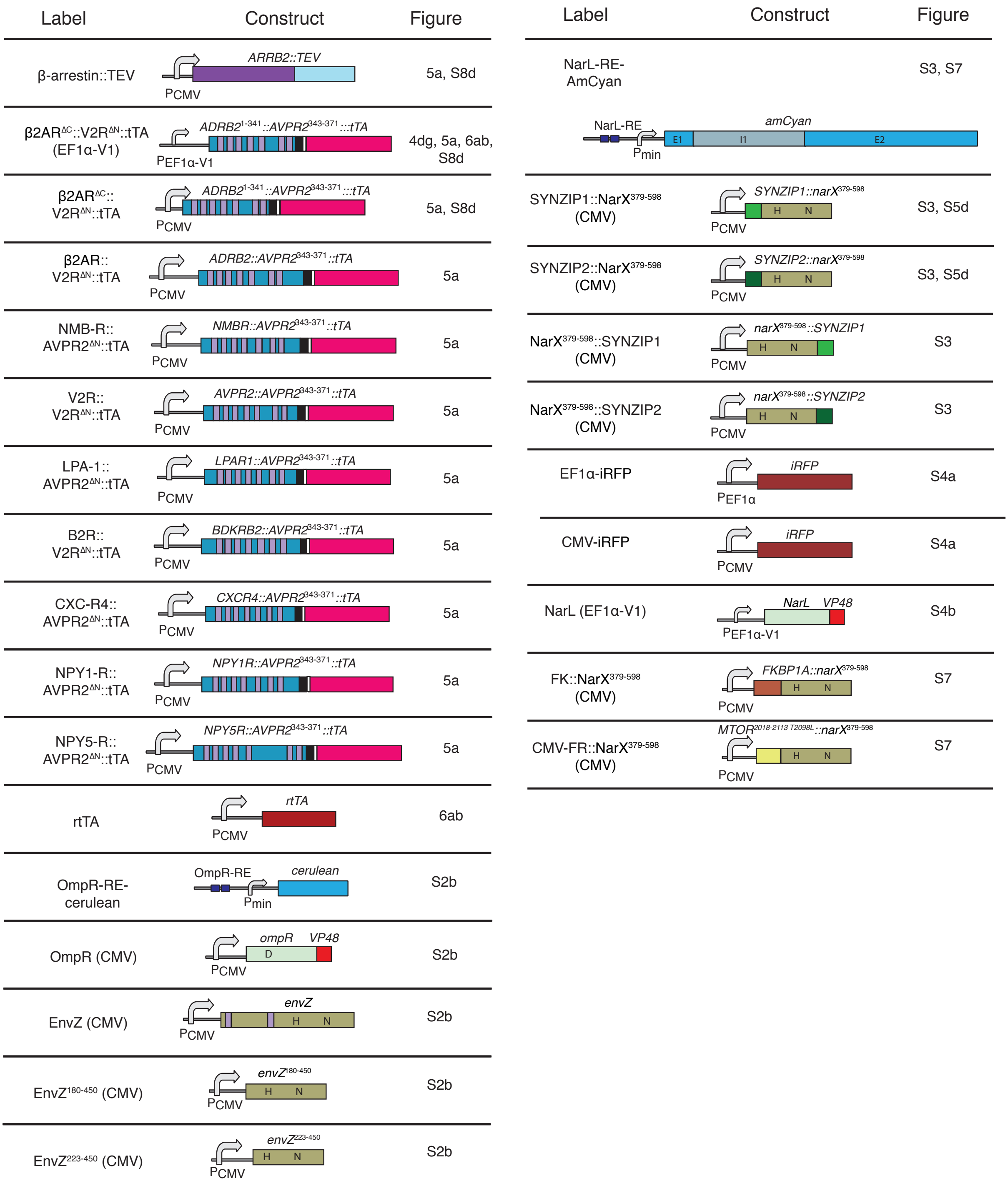
Plasmid	Lane 7	Lane 8	Lane 9
EF1α-mCherry (pKH026)	100 ng	100 ng	100 ng
Junk-DNA (pBH265)			
NarL_RE-*cerulean* (pMZ219)	100 ng	100 ng	100 ng
EF1α-*narL* (pMZ248)	100 ng	100 ng	100 ng
EF1α-V1-*narX* (pMZ239)			
EF1α-V1-*narX*$^{379\text{-}598}$ (pMZ241)			
EF1α-V1-*narX*$^{379\text{-}598}$ H399Q (pMZ244)			
EF1α-V1-*narX*$^{379\text{-}598}$ N509A (pMZ247)			
EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ H399Q (pMZ251			
EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ N509A (pMZ252)	100 ng		
EF1α-V1-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*narX*$^{379\text{-}598}$ H399Q (pMZ257)		100 ng	
EF1α-V1-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*narX*$^{379\text{-}598}$ N509A (pMZ258)			100 ng

Supplementary Table 27. Transfection setup for Supplementary Fig. 8d. Plasmid amounts per transfection are shown

Plasmid	CMV-Tango	EF1α-V1-Tango
EF1α-mCherry (pKH026)	100 ng	100 ng
tTA_RE-*cerulean* (pMZ290)	100 ng	100 ng
CMV-*ARRB2::*TEV protease (pBH302)	100 ng	
CMV-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*tTA* (pBH312)	100 ng	
EF1α-V1-*ARRB2::*TEV protease (pMZ291)		100 ng
EF1α-V1-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*tTA* (pMZ292)		100 ng

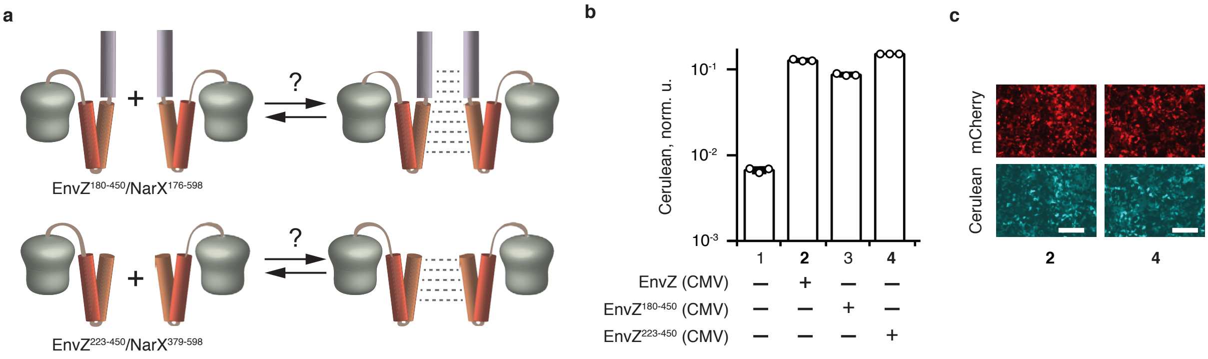
Supplementary Figures

Label	Construct	Figure
NarL-RE-cerulean	NarL-RE; P_{min}; *cerulean*	1b, 2bc, 3ac, 4bdg, 5a, 6 ab, S4b, S5cde, S6c, S8c
NarL (CMV)	P_{CMV}; *narL* (D) *VP48*	1b, 2b, S3, S4b, S7
NarX (CMV)	P_{CMV}; *narX* (H, N)	1b, 2b, S3, S4b, S7
NarX$^{176\text{-}598}$ (CMV)	P_{CMV}; *narX*$^{176\text{-}598}$ (H, N)	1b
NarX$^{379\text{-}598}$ (CMV)	P_{CMV}; *narX*$^{379\text{-}598}$ (H, N)	1b, S3, S7
NarL	$P_{EF1α}$; *narL* (D) *VP48*	2c, 3ac, 4bdg, 5a, 6 ab, S4b, S5cde S6c, S8c
NarX H399Q (CMV)	P_{CMV}; *narX*^{H399Q} (Q, N)	2b
NarX N509A (CMV)	P_{CMV}; *narX*^{N509A} (H, A)	2b
NarX	$P_{EF1α\text{-}V1}$; *narX* (H, N)	2c, 3a, 4b, S4b, S5cd, S6c, S8c
NarX$^{379\text{-}598}$	$P_{EF1α\text{-}V1}$; *narX*$^{379\text{-}598}$ (H, N)	2c, 3a, 4b, S4ab, S5cd, S6c, S8c
NarX$^{379\text{-}598}$ H399Q (H^{mut})	$P_{EF1α\text{-}V1}$; *narX*$^{379\text{-}598\ H399Q}$ (Q, N)	2c, 3a, 4b, S5c, S6c, S8c
NarX$^{379\text{-}598}$ N509A (N^{mut})	$P_{EF1α\text{-}V1}$; *narX*$^{379\text{-}598\ N509A}$ (H, A)	2c, 3a, 4b, S5c, S6c, S8c
SYNZIP1::H^{mut}	$P_{EF1α\text{-}V1}$; *SYNZIP1::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	2c, S5cde
SYNZIP1::N^{mut}	$P_{EF1α\text{-}V1}$; *SYNZIP1::narX*$^{379\text{-}598\ N509A}$ (H, A)	2c, S5cde
SYNZIP2::H^{mut}	$P_{EF1α\text{-}V1}$; *SYNZIP2::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	2c, S5cde
SYNZIP2::N^{mut}	$P_{EF1α\text{-}V1}$; *SYNZIP2::narX*$^{379\text{-}598\ N509A}$ (H, A)	2c, S5cde
FK::H^{mut}	$P_{EF1α\text{-}V1}$; *FKBP1A::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	3a, S6c
FK::N^{mut}	$P_{EF1α\text{-}V1}$; *FKBP1A::narX*$^{379\text{-}598\ N509A}$ (H, A)	3ac, S6c
FR::H^{mut}	$P_{EF1α\text{-}V1}$; *MTOR*$^{2018\text{-}2113\ T2098L}$*::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	3ac, S6c
FR::N^{mut}	$P_{EF1α\text{-}V1}$; *MTOR*$^{2018\text{-}2113\ T2098L}$*::narX*$^{379\text{-}598\ N509A}$ (H, A)	3a, S6c
β-arrestin::H^{mut}	$P_{EF1α\text{-}V1}$; *ARRB2::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	4b, 6ab, S8c
β-arrestin::N^{mut}	$P_{EF1α\text{-}V1}$; *ARRB2::narX*$^{379\text{-}598\ N509A}$ (H, A)	4bdg, 5a, S8c
β2AR^{ΔC}:: V2R^{ΔN}::H^{mut}	$P_{EF1α\text{-}V1}$; *ADRB2*$^{1\text{-}341}$*::AVPR2*$^{343\text{-}371}$*::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	4bdg, 5a, S8c
β2AR^{ΔC}:: V2R^{ΔN}::N^{mut}	$P_{EF1α\text{-}V1}$; *ADRB2*$^{1\text{-}341}$*::AVPR2*$^{343\text{-}371}$*:::narX*$^{379\text{-}598\ N509A}$ (H, A)	4b, 6ab, S8c
β2AR:: V2R^{ΔN}::N^{mut}	$P_{EF1α\text{-}V1}$; *ADRB2-AVPR2*$^{343\text{-}371}$*::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	5a
NMB-R:: V2R^{ΔN}::H^{mut}	$P_{EF1α\text{-}V1}$; *NMBR::AVPR2*$^{343\text{-}371}$*::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	5a
V2R:: V2R^{ΔN}::H^{mut}	$P_{EF1α\text{-}V1}$; *AVPR2::AVPR2*$^{343\text{-}371}$*::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	5a
LPA-1:: V2R^{ΔN}::H^{mut}	$P_{EF1α\text{-}V1}$; *LPAR1::AVPR2*$^{343\text{-}371}$*::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	5a
B2R:: V2R^{ΔN}::H^{mut}	$P_{EF1α\text{-}V1}$; *BDKRB2::AVPR2*$^{343\text{-}371}$*::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	5a
CXC-R4:: V2R^{ΔN}::H^{mut}	$P_{EF1α\text{-}V1}$; *CXCR4::AVPR2*$^{343\text{-}371}$*::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	5a
NPY1-R::H^{mut}	$P_{EF1α\text{-}V1}$; *NPY1R::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	5a
NPY5-R^{ΔC}:: V2R^{ΔN}::H^{mut}	$P_{EF1α\text{-}V1}$; *NPY5R*$^{1\text{-}438}$*::AVPR2*$^{343\text{-}371}$*::narX*$^{379\text{-}598\ H399Q}$ (Q, N)	5a
Tet-RE-Cerulean	Tet-RE; P_{min}; *cerulean*	4dg, 5a, 6ab, S8d
β-arrestin::TEV (EF1α-V1)	$P_{EF1α\text{-}V1}$; *ARRB2::TEV*	4dg, 6ab, S8d

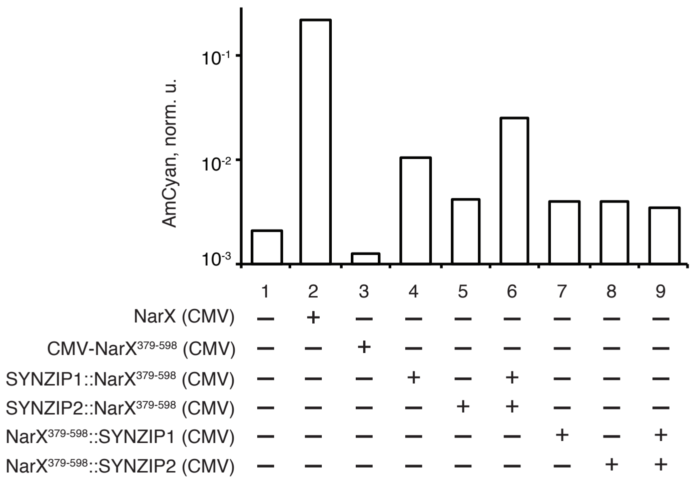


Supplementary Fig. 1 | Schematic representation of the transfected DNA constructs. The promoters, the genes (for gene fragments the numbers indicate the first and the last amino acid encoded by the sequence, mutated codons are also indicated), and the DNA binding elements are indicated. RE, response element; P_{CMV}, early-late cytomegalovirus promoter; $P_{EF1\alpha}$, human elongation factor-1 alpha promoter; $P_{EF1\alpha\text{-}V1}$, modified human elongation factor-1 alpha promoter. P_{min} is a minimal TATA box known as "YB-TATA", described previously (Angelici, B., Mailand, E., Haefliger, B. & Benenson, Y. Synthetic

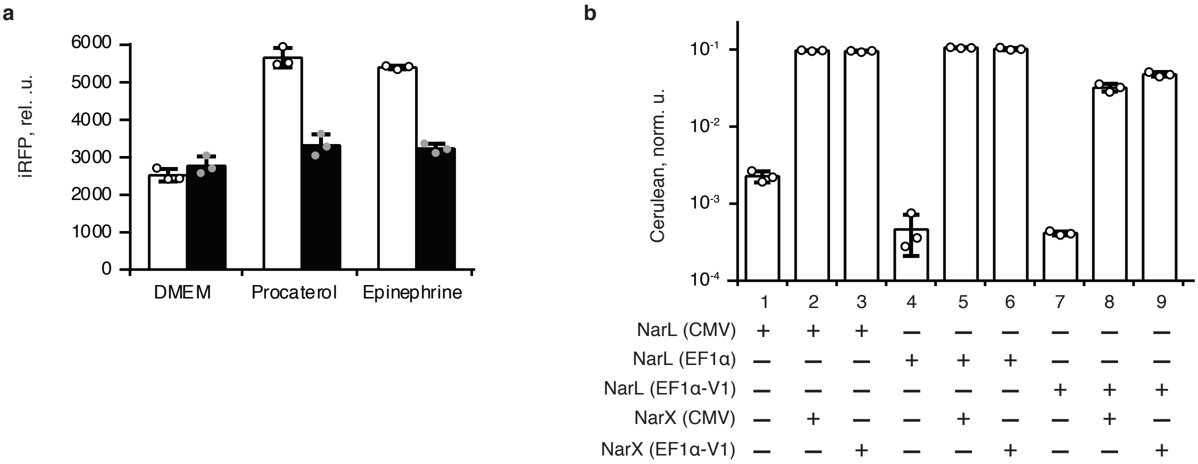
Biology Platform for Sensing and Integrating Endogenous Transcriptional Inputs in Mammalian Cells. *Cell Reports* **16**, 2525-2537 (2016)). The label D in the coding sequence of the RRs OmpR and NarL, corresponds to the phosphorylated aspartate. The labels H and N in the coding sequence of the HKs EnvZ and NarX correspond, respectively, to the phosphorylatable histidine and the asparagine important for the ATP binding of the HK. The labels Q and A present in the coding sequence of NarX correspond, respectively, to the mutants of the phosphorylatable histidine and the asparagine important for the ATP binding. The labels E1, I1 and E1 indicate the first exon, the first intron, and the second exon of *amCyan*. The violet stripes indicate the sequence coding for the transmembrane domains. The black strip corresponds to the AVPR2$^{343\text{-}371}$. The white bar between AVPR2$^{343\text{-}371}$ and *tTA* represents the sequence coding for TEV protease cleavage site. The size and the localization of the transmembrane domain have been collected from UniProt website (UniProt, C. UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res* **47**, D506-D515 (2019)).



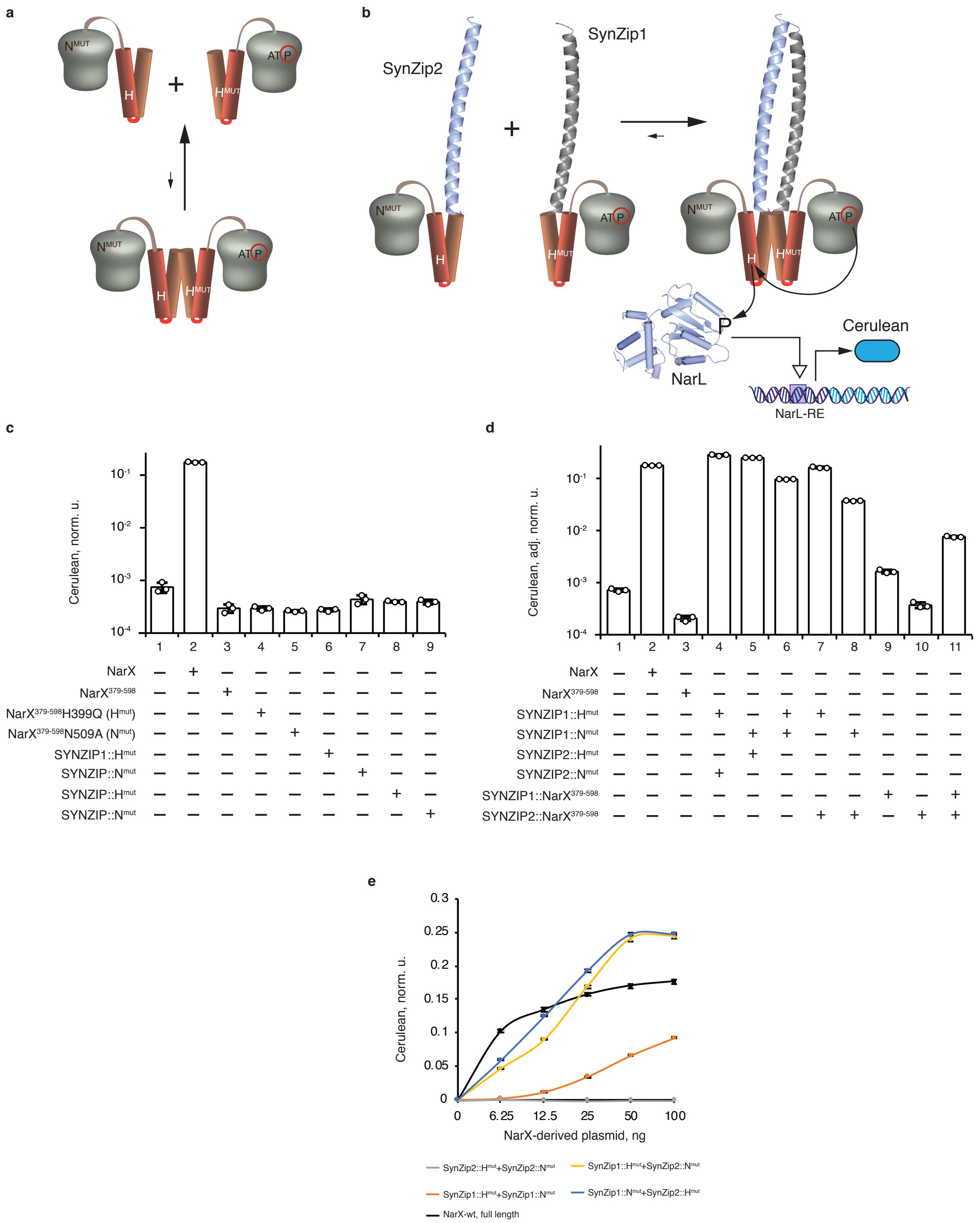
Supplementary Fig. 2 I Identification of truncated HK cytoplasmic domains with reduced intrinsic signaling (see also Fig.1). **a**, Schematics of the dimerization testing of the various truncated cytoplasmic domains of the HK receptors. The corresponding EnvZ and NarX domains are indicated in the panel. **b**, Dimerization tests for EnvZ. The bars show Cerulean expression in cells transfected with response regulator OmpR, OmpR-inducible cerulean reporter and the indicated truncated mutant when applicable. The bar height indicates Cerulean expression normalized to the transfection control and averaged across independent biological triplicate, shown as mean ± SD. The circles indicate individual measurements. **c**, Representative microscopy images of HEK293 cells are shown for transfections indicated in bold in panel **b**. The top and the bottom row of images show, respectively, the expression of mCherry transfection reporter (red), and pathway-induced Cerulean protein output (cyan) in the same transfection. The white scale bars correspond to 200 µM. Constructs are described in Supplementary Fig. 1. The results were reproduced at least once in an independent experiment.



Supplementary Fig. 3 | Restoration of two-component signalling via forced dimerization of protein moieties fused at the C or the N-termini of NarX. The bars represent signalling levels in mammalian cells expressing the response regulator NarL from the CMV promoter and NarL-inducible AmCyan fluorescent reporter, alone or with different combinations of SYNZIP1 and SYNZIP2 fused at the C-terminus or at the N-terminus of NarX, as indicated in the chart. The bar height indicates AmCyan expression normalized to the transfection control. Similar experiments were done with small modifications, leading to similar conclusions. Constructs are described in Supplementary Fig. 1.

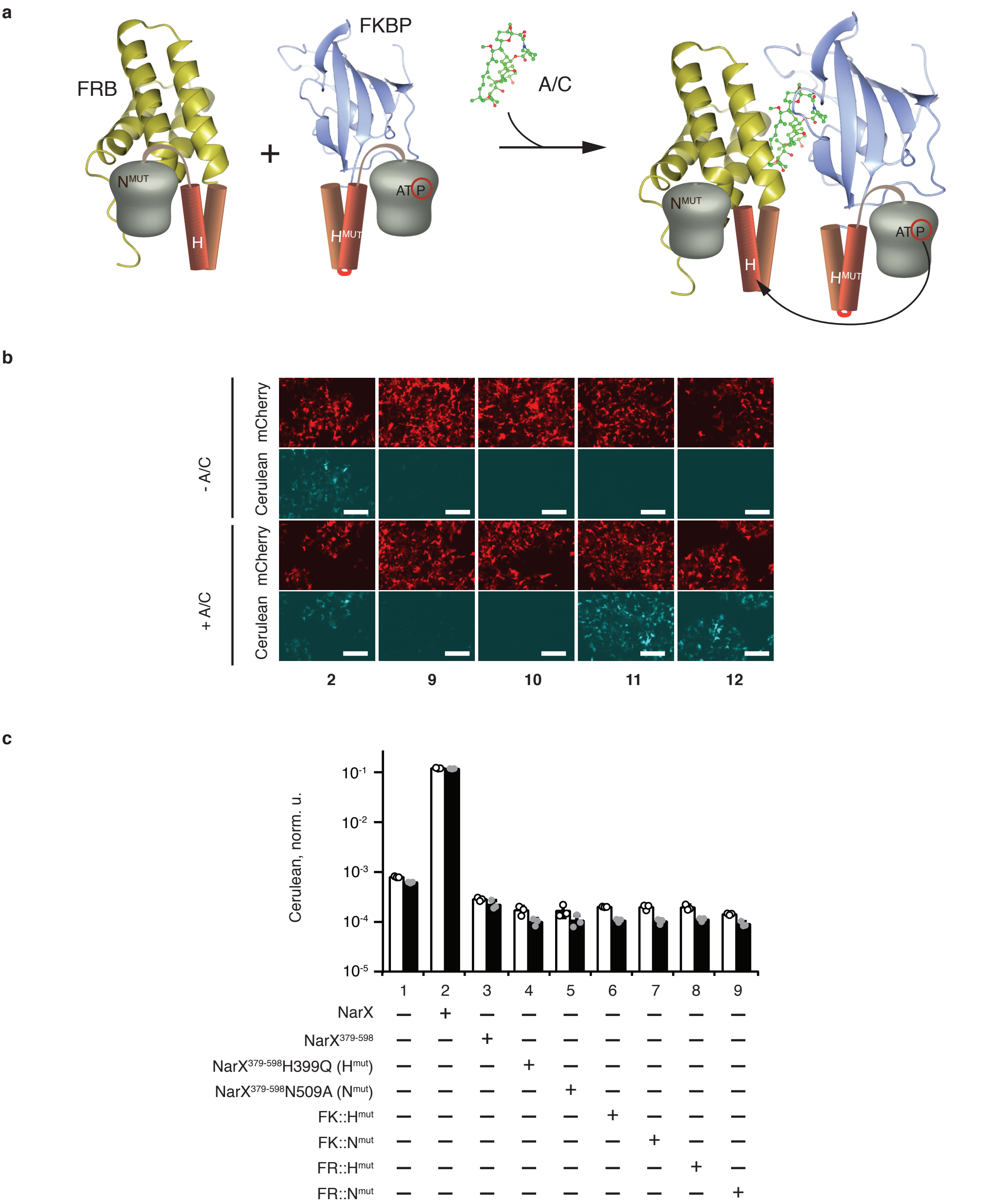


Supplementary Fig. 4 | Comparison of CMV and EF1α promoters. a, iRFP fluorescence of HEK cells transfected with the plasmid expressing iRFP from the CMV promoter (white bars) or from the EF1α promoter (black bars). The reporter expression in DMEM without any ligand or in the presence of 1µM of procaterol or 2 µM of epinephrine is shown as indicated. The bar chart displays iRFP level normalized to the frequency of the transfection marker Citrine-positive cells (rel. u.) as mean ± SD of independent biological triplicates. **b**, Activity of the NarX/NarL pathway expressed from CMV or EF1α promoters. Every transfection contains NarL-inducible Cerulean reporter and plasmids expressing NarL and NarX from CMV, EF1α or EF1α-V1 promoters, as indicated in the chart. The bar height indicates Cerulean expression normalized to the transfection control and averaged across independent biological triplicate, shown as mean ± SD. The circles indicate the individual measurements. Constructs are described in Supplementary Fig. 1. The results were reproduced at least once in an independent experiment.

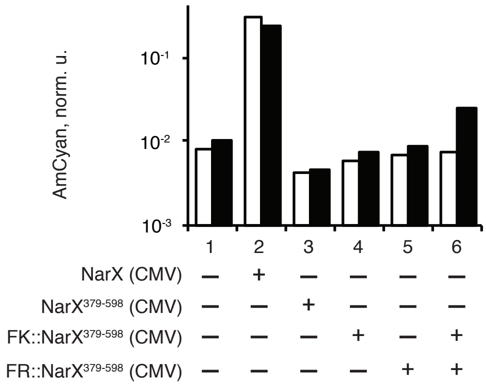


Supplementary Fig. 5 | Control experiment demonstrating the restoration of two-component signaling via forced dimerization (see also Fig. 2). **a**, The baseline dissociated state of the truncated NarX mutants. **b**, Schematics of forced dimerization with the help of fused protein domains of strong mutual affinity. The fusions of SYNZIP2 to ATP binding mutant and SYNZIP1 to the histidine mutant are shown schematically, structures based on a published study (PDB ID: 3HE5, Reinke, A.W., Grant, R.A. & Keating, A.E. A Synthetic coiled-coil interactome provides heterospecific modules for molecular engineering. *J. Am. Chem. Soc.*

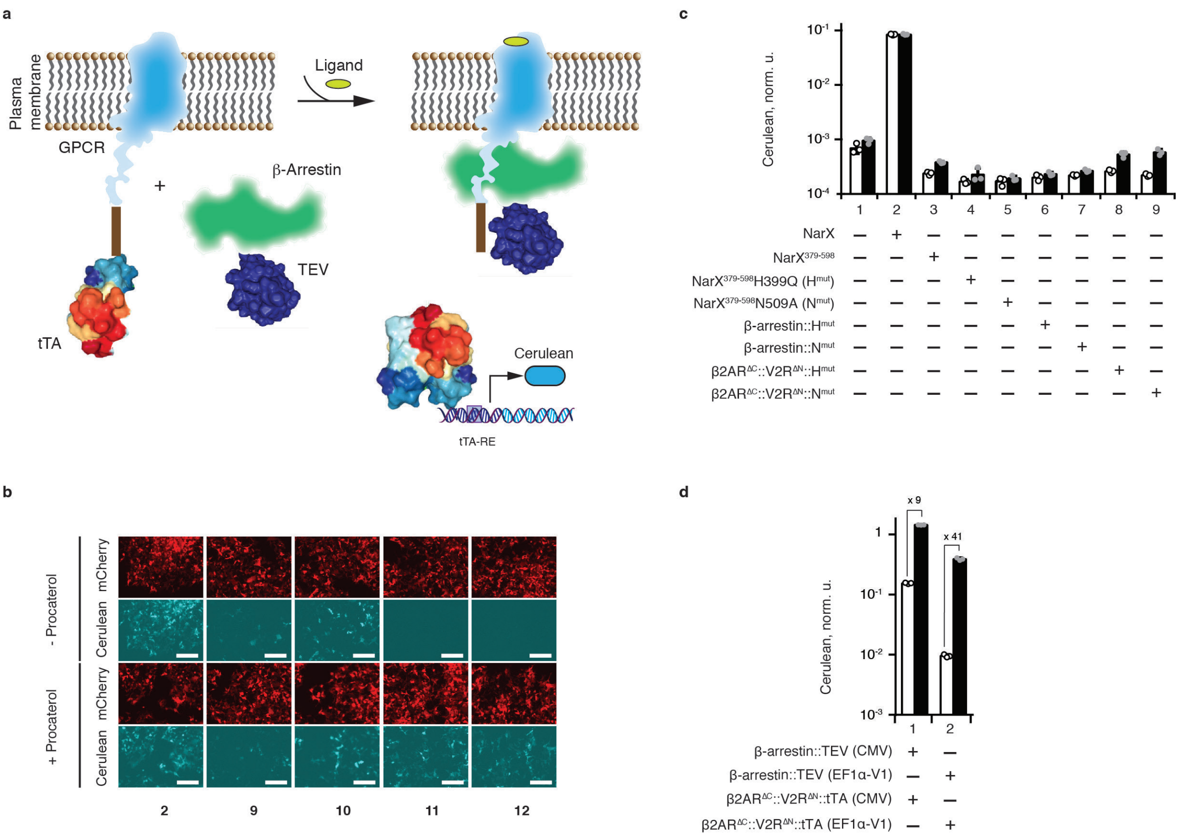
132, 6025-6031 (2010)). Upon dimerization, phosphate is transferred to the histidine and then to the aspartate in NarL (PDB: 1RNL, Baikalov, I. et al. Structure of the *Escherichia coli* response regulator NarL. *Biochemistry* **35**, 11053-11061 (1996)), resulting in reporter gene expression. **c**, Cerulean expression in the presence of NarL, driven by the promoter EF1α, and NarL-inducible Cerulean reporter, alone or with individual variants of NarX mutant fused to SYNZIP1 or SYNZIP2, as indicated. **d**, Cerulean expression in the presence of NarL and NarL-inducible Cerulean reporter, alone or with different combinations of SYNZIP1 and SYNZIP2 fused to wild-type NarX or various NarX mutants, as indicated in the chart. **e**, Dose-dependency of signaling intensity for different interacting components and varying plasmid amounts as indicated in the plot. The ng amount indicates the total amount of NarX-containing plasmids, for example for NarX-wt 100 ng corresponds to 100 ng of this construct, but for combinations of two constructs this means 50 ng of each. The bar height and the dots' Y coordinate indicates Cerulean expression normalized to the transfection control and averaged across independent biological triplicate, shown as mean ± SD. The circles in the panels **c**, **d** indicate individual measurements. Constructs are described in Supplementary Fig. 1. The results were reproduced at least once in independent experiments.



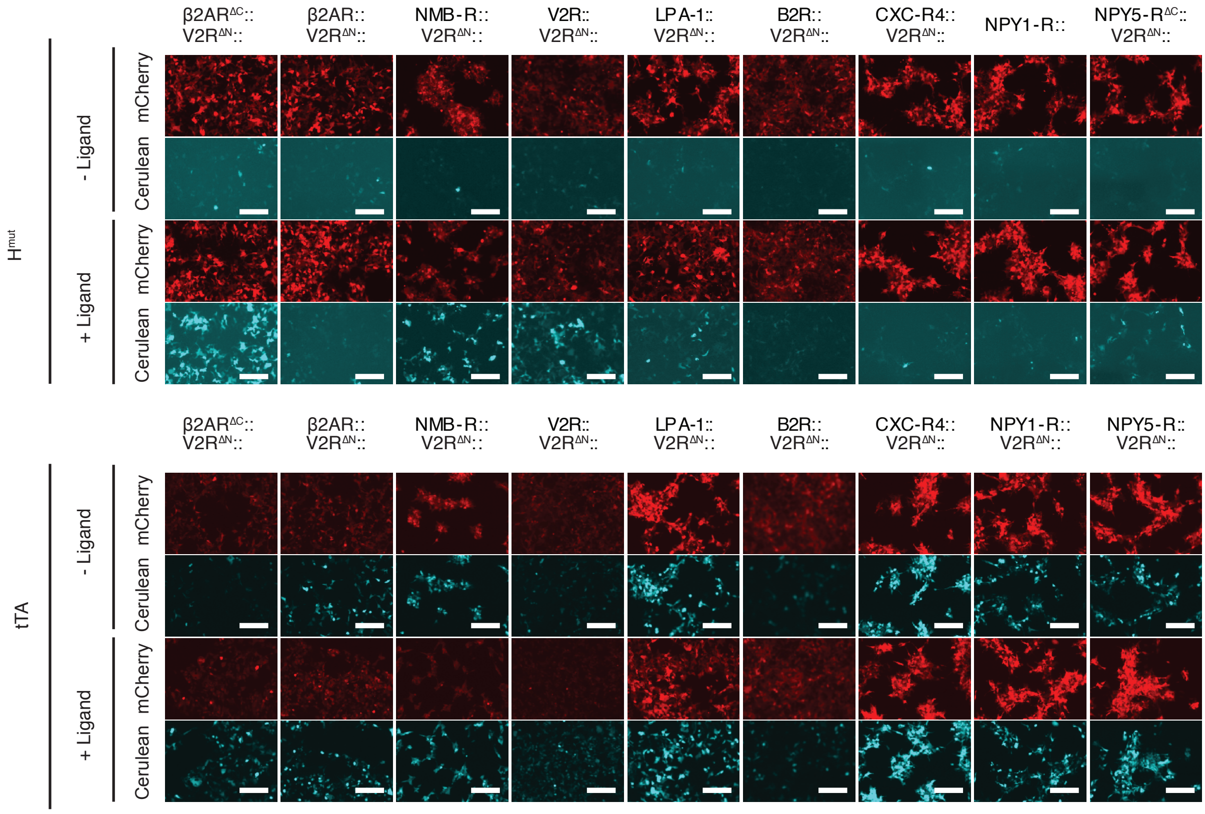
Supplementary Fig. 6 | Control experiments and additional data related to the transduction of cytoplasmic ligand to gene expression (see also Fig. 3). **a**, Schematics of ligand-induced signaling. The structures of FKBP and FRB domains and the dimerizer ligand are based on the report describing the complex between FKBP, FRB and rapamycin (PDB: 3AFP, Marz, A.M., Fabian, A.K., Kozany, C., Bracher, A. & Hausch, F. Large FK506-binding proteins shape the pharmacology of rapamycin. *Mol. Cell. Biol.* **33**, 1357-1367 (2013)), an analog of A/C ligand. **b**, Representative microscopy images of HEK293 cells are shown for selected transfections indicated in Fig. 3a. In all panels the top and the bottom row of images show, respectively, the expression of mCherry transfection reporter (red), and pathway-induced Cerulean protein output (cyan) in the same transfection with or without the ligand (A/C). The white scale bar in **b** corresponds to 200 μM. **c**, Cerulean expression in the presence of the RR NarL and NarL-inducible Cerulean reporter, alone or with individual fusion variants of NarX and FKBP or FRB, as indicated. For each pair of bars, the bar on the left (white) represents reporter expression without the A/C ligand, and the bar on the right (black) represents reporter expression with the ligand (100 nM). The bar height indicates Cerulean expression normalized to the transfection control and averaged across independent biological triplicate, shown as mean ± SD. The circles indicate the individual measurements. Constructs are described in Supplementary Fig. 1. The results were reproduced at least once in independent experiments.



Supplementary Fig. 7 | Restoration of two-component signalling via ligand induced dimerization of protein moieties fused at the N-termini of NarX. AmCyan expression in the presence of NarL and NarL-inducible AmCyan fluorescent protein reporter, alone or with individual fusion variants of NarX and FKBP or FRB, as indicated. For each pair of bars, the bar on the left (white) shows reporter expression without the A/C ligand, and the bar on the right (black) shows reporter expression with the ligand (1 μM). The bar height indicates AmCyan expression normalized to the transfection control. Similar experiments were done with small modifications, leading to similar conclusions. Constructs are described in Supplementary Fig. 1.

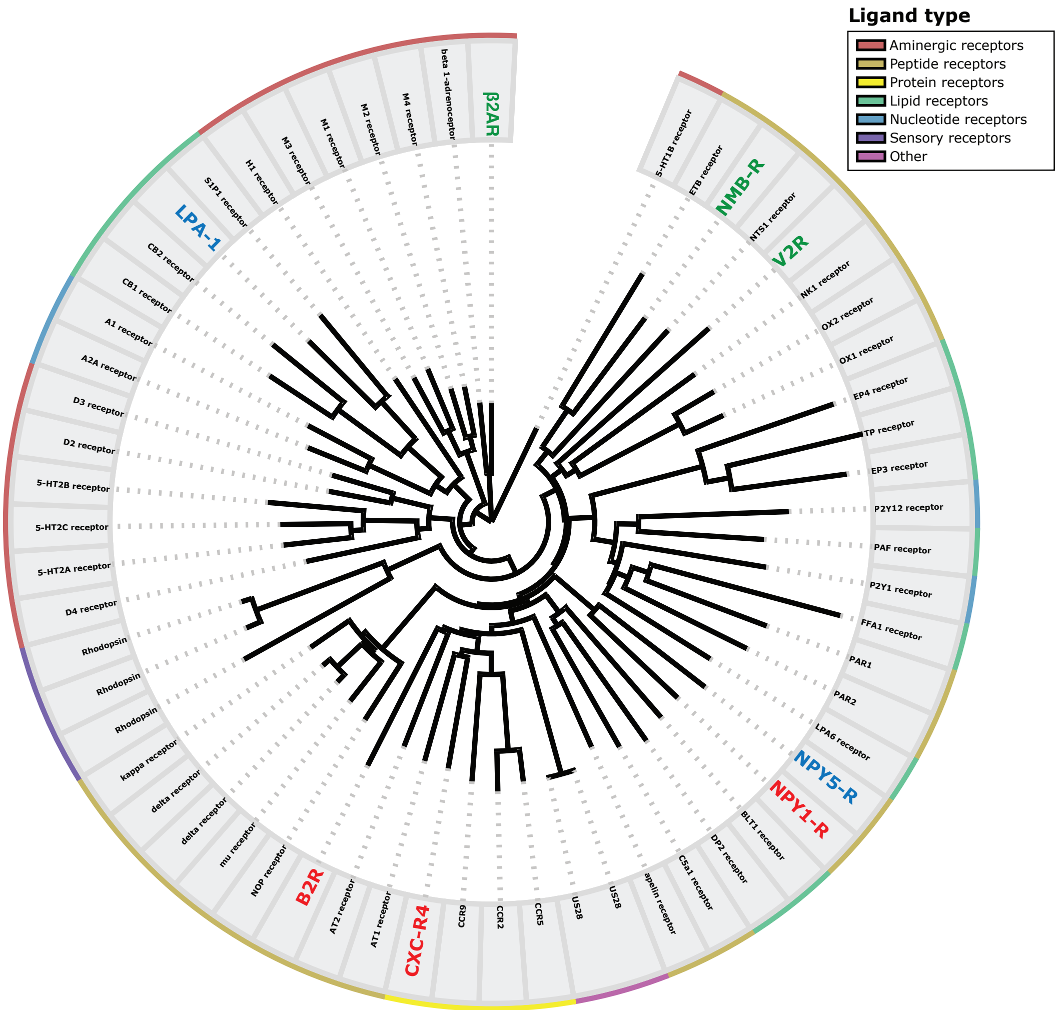


Supplementary Fig. 8 | Data related to Figure 4. **a**, Schematics of transducing ligand-induced GPCR-β/arrestin interaction (Shukla, A.K. et al. Visualization of arrestin recruitment by a G-protein-coupled receptor. *Nature* **512**, 218-222 (2014)) into gene expression using the Tango assay. The proteins and the DNA binding domain are indicated (tTA: PDB: 2TRT, Hinrichs, W., et al., Structure of the Tet repressor-tetracycline complex and regulation of antibiotic resistance. (1994) Science 264: 418-420; TEV: PDB: 1Q31, Nunn, C. M. et al., Crystal structure of tobacco etch virus protease shows the protein C terminus bound within the active site. (2005) J. Mol. Biol. 350: 145-155). The brown segment between the GPCR and tTA represents the TEV protein cleavage site. **b**, Representative microscopy images of the transfections whose images are shown in Fig. 4, here showing the expression of the mCherry transfection control. In all panels the top and the bottom row of images show, respectively, the expression of mCherry transfection reporter (red) and pathway-induced Cerulean protein output (cyan) in the same transfection with or without ligand. **c**, Signalling levels in mammalian cells expressing the response regulator NarL and NarL-regulated Cerulean fluorescent protein reporter, alone or with the indicated individual protein domains or fusions. **d**, Signalling levels in mammalian cells expressing the Tango assay components from CMV or EF1α-V1 promoters. For each pair of bars in panels **a** and **d**, the bar on the left (white) represents reporter expression without procaterol, and the bar on the right (black) represents reporter expression with procaterol (2 μM). The bar height indicates Cerulean expression normalized to the transfection control and averaged across independent biological triplicate, shown as mean ± SD. The value above the bars in **d** indicates the fold change of the Cerulean expression between cells grown with and without procaterol. The circles indicate individual measurements. Constructs are described in Supplementary Fig. 1. The scale bar in **b** corresponds to 200 µM. The results were reproduced at least once in independent experiments.



Supplementary Fig. 9 I Supplementary imaging data for Fig. 5. Representative microscopy images of the conditions described in Fig. 5, here also showing the expression of the mCherry transfection control. In all panels the top and the bottom row of images show, respectively, the expression of mCherry transfection reporter (red), and pathway-induced Cerulean protein output (cyan) in the same transfection with or without ligand. The modified GPCRs used for the transfection are indicated on top and the fused domains (H^{mut} or tTA) are shown on the left. The scale bars correspond to 200 µM.

a



b

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V2R    (329-354)    SSSVSSELRS LLCCARGRTP PSLGP
β2AR   (330-355)    -PDFRIAFQE LLCLRRSSLK AYGNGY
NMB-R  (328-353)    SESFRRHFNS QLCCGRKSYQ ERGTS
LPA-1  (316-341)    -KEMSATFRQ ILCCQRSENP TGPTEG
NPY5-R (429-445)    NNGIKADLVS LIHCLHM
                             s llcc r

NPY1-R (321-346)    GFLNKNFQRD LQFFFNFCDF RSRDD
B2R    (336-361)    GKRFRKKSWE VYQGVCQKGG CRSEP
CXC-R4 (303-328)    AFLGAKFKTS AQHALTSVSR GSSLK
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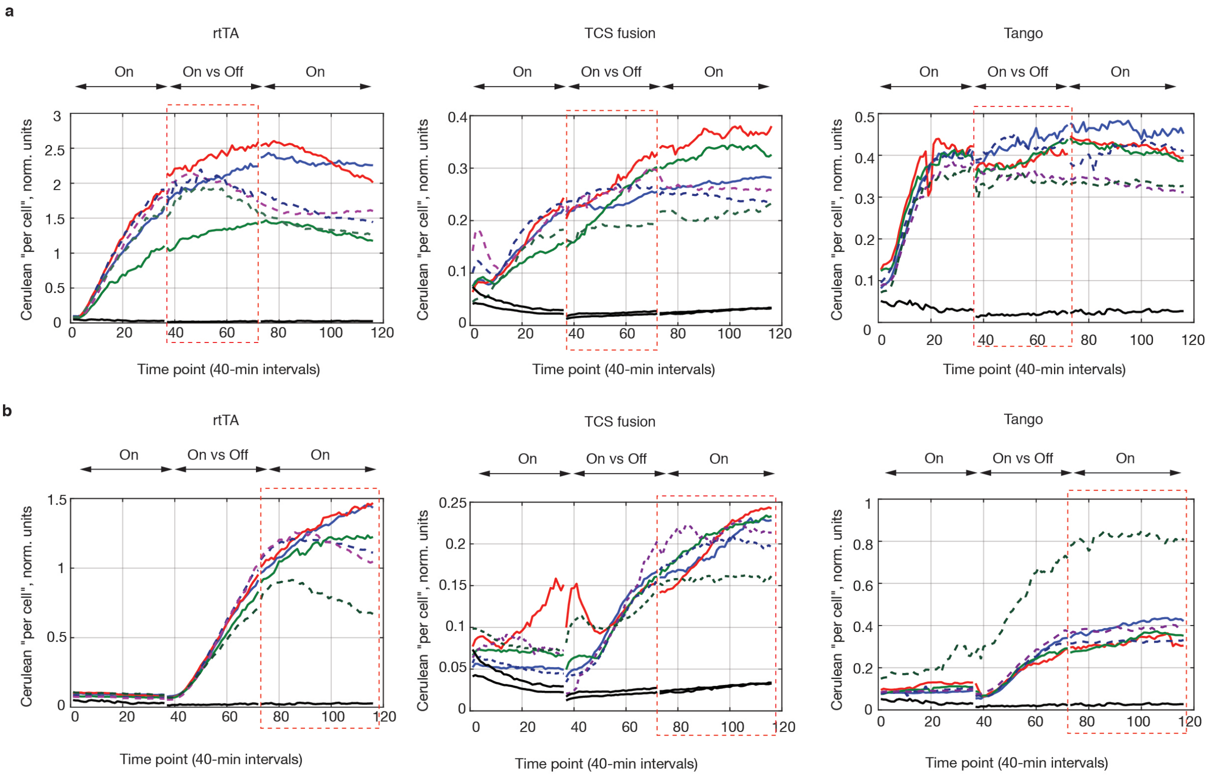
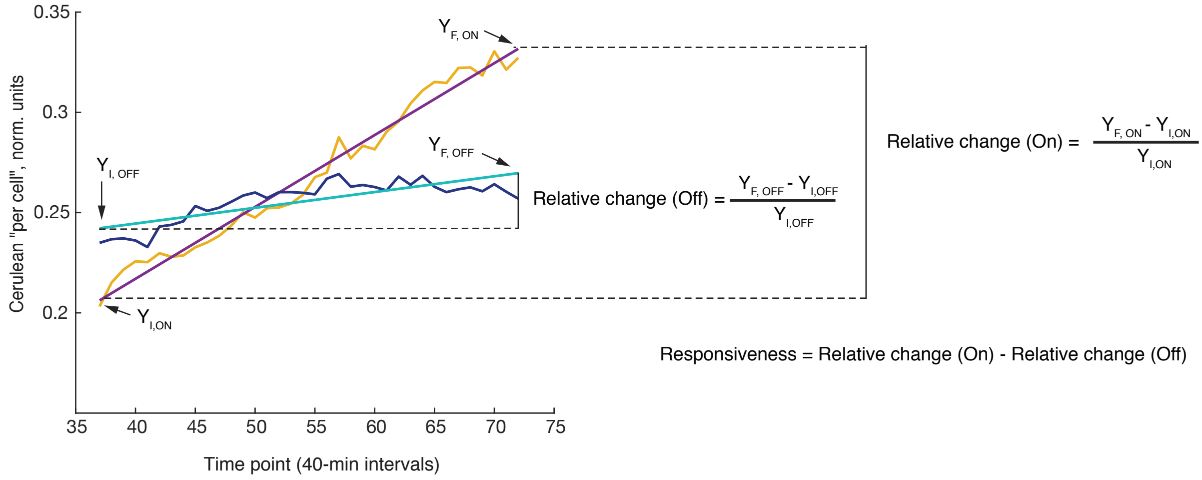
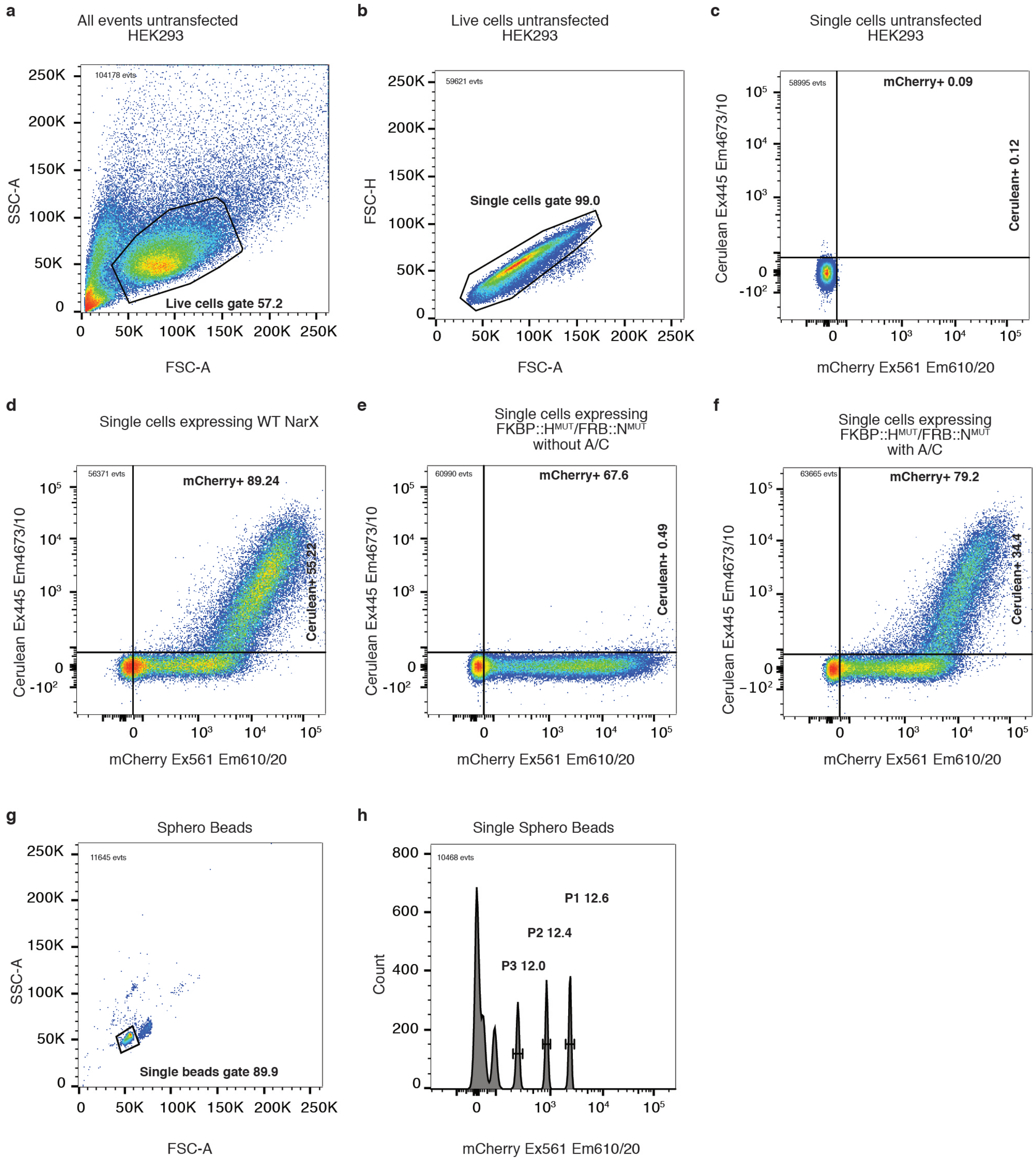
Supplementary Fig. 10 | Sequence analysis of the GPCRs used in this study. a, Phylogenetic trees of selected class A GPCRs based on full sequence. The eight GPCRs used in this study are highlighted in green for the GPCR that we were able to rewire either using the TCS fusions or the Tango assay, in blue for GPCRs that we were able to rewire using the TCS fusions only, and in red for GPCR that we were unable to rewire using either method. The type of ligand is indicated by color code in the outside circle and indicated in the box. **b**, Multiple sequence alignment based on MULTALIN of the 5 GPCR rewireable with TCS. The alignment of the 25 amino acids following the end of the 7th transmembrane helix of the V2R, β2AR, NMB-R, LPA-1 and NPY5-R has been realized with the software Multalin version 5.4.1. The amino acids conserved in 60% or 80% of the sequences are indicated in blue and red, respectively. The sequence of the GPCR NPY1-R, B2R and CXC-R4 are shown below the alignment. The numbers in brackets indicate the position number in the protein of the first and the last amino acid shown. Note that NPY5-R has only 17 amino acid after the end of the 7th transmembrane helix. GPCR name is highlighted with the same color as the one used in panel **a**.



Supplementary Fig. 11 | Time lapse traces obtained in the dynamic characterization (See also Fig. 6). Charts represent, from left to right, rtTA-Dox (rtTA), GPCR signaling via TCS pathway (TCS fusions) and GPCR signaling via a Tango assay (Tango). The top row shows the comparison of On-On-On to On-Off-On sequence for the indicated signaling approaches. The solid lines show On-On-On sequences while the dotted traces are On-Off-On sequences. The bottom row compares the Off-On-On (solid lines) to Off-On-Off (dotted lines) sequences. The dotted red frames highlight the time intervals in which the treatments diverged between the samples. Lines of the same color represent consecutive time lapse traces obtained from the same well. Black lines represent background readout.



Supplementary Fig. 12 | Illustration of responsiveness evaluation method (see also Fig. 6). Time-lapse traces corresponding to On and Off induction interval are fitted with a linear fit and the relative change in signal is calculated according to the formulas in the panel. The responsiveness is defined as a difference between the two, as shown. For rtTA time course, the fit does not use the first 18 time points for both On and Off traces due to biphasic behavior of the Off traces.



Supplementary Fig. 13 I Illustration of gating in flow cytometry analyses. **a-f**, Cell gating examples. **a-c,** Gating using untransfected HEK293 cells. **a**, Live cells gating is determined by plotting all events recorded from the biological sample on SSC-A and FSC-A density plot. **b**, Single cell live gating is done by plotting live cells on FSC-A and FSC-H density plot. **c**, Cerulean+ gating and mCherry+ gating are determined from untransfected HEK293 cells with 99.9% Cerulean+ and mCherry+ outside of the gate, respectively. **d–f**, Examples of gating applied to HEK293 expressing different TCS pathways. **d**, HEK293 cells expressing the WT NarX/NarL TCS. **e,** HEK293 cells expressing FKBP::H^{MUT}/FRB::N^{MUT} in absence of the heterodimezer. **f**, HEK293 cells expressing FKBP::H^{MUT}/FRB::N^{MUT} in the presence of the heterodimerizer. **g-h**, Beads gating examples. **g**, The single bead gating is determined by plotting beads on FSC-A and SSC-H density plot. **h**, The three brighter populations of colored flow cytometry calibration particles are determined by plotting all single beads on mCherry histogram plot. The number of events shown on each plot is indicated in the top left corner of the plot. The value on the right of the name of each gate indicates the percentage of the events present inside the gate.

Supplementary Notes

Supplementary Note 1: Recombinant DNA cloning protocols.

OmpR_RE-*cerulean* (pMZ1): The Cerulean coding sequence from EF1α-*cerulean* (pKH024[3]) was digested with *Not*I and *Sma*I and cloned into the plasmid OmpR_RE-*amCyan* (pJH008[4]) digested with *Afe*I and *Psp*OMI.

CMV-*envZ* N347A (pMZ37): The 5' and the 3' fragments of *envZ* were PCR amplified with PR3687/PR3708 and PR3707/PR3709 from the plasmid CMV-*envZ* (pJH001[4]). The primers were designed to introduce a mutation exchanging the codon encoding for the asparagine (N) at the 347th position to codon encoding for an alanine (A). Both PCR products and the plasmid CMV-*envZ* (pJH001[4]), digested with *Xho*I and *Pvu*II, were assembled using Gibson mix.

CMV-*envZ*$^{223\text{-}450}$ (pMZ123): The 3' fragment of *envZ* was PCR amplified with PR4345/PR4346 from the plasmid CMV-*envZ* (pJH001[4]). The primers were designed to amplify the sequence from the 20th codon upstream of the codon encoding for the phosphorylatable histidine at the position 243 till the end of the gene, and to insert ATG sequence in front of this amplified sequence. The PCR product and the plasmid CMV-*envZ* (pJH001[4]), digested with *Xho*I and *Age*I, were assembled using Gibson mix.

CMV-*narX* N509A (pMZ160): The 5' and the 3' fragments of *narX* were PCR amplified with PR4122/PR4541 and PR4346/PR4542 from the plasmid CMV-*narX* (pJH002[4]). The primers were designed to introduce a mutation exchanging the codon encoding for the asparagine (N) at the 509th position to codon encoding for an alanine (A). Both PCR products and the plasmid CMV-*envZ* (pJH001[4]), digested with *Xho*I and *Age*I, were assembled using Gibson mix.

CMV-*narX*$^{379\text{-}598}$ (pMZ163): The 3' fragment of *narX* was PCR amplified with PR4345/PR4546 from the plasmid CMV-*narX* (pJH002[4]). The primers were designed to amplify the sequence from the 20th codon upstream the codon encoding for the phosphorylatable histidine at the position 399 till the end of the gene, and to insert ATG sequence in front of this amplified sequence. The PCR products and the plasmid CMV-*envZ* (pJH001), digested with *Xho*I and *Age*I, were assembled using Gibson mix.

EF1α-V1-*envZ-mCherry* (pMZ194): The EF1α-V1, as shortened version of EF1α, was PCR amplified with PR4733/PR4734 from the plasmid pRA114 (Altamura et al, manuscript in preparation). The promoter and the plasmid EnvZ-GGGGS-mCherry (pEM017[4]), digested with *Psp*OMI and *Age*I, were assembled using Gibson mix.

CMV-*SYNZIP1*::*narX*$^{379-598}$ (pMZ200): We performed de novo synthesis of gBlock sequence encoding for MGSS starting sequence, SYNZIP1 and G4S linker (gBlock264) via IDT. The coding sequence of NarX$^{379-598}$ was PCR amplified with PR4346/PR4747 from the plasmid CMV-*narX*$^{176-598}$ (pJH010). The gBlock, the PCR product and the plasmid CMV-*envZ* (pJH001[4]), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

CMV-*narX*$^{379-598}$::*SYNZIP1* (pMZ202): We performed de novo synthesis of gBlock sequence encoding for G4S linker and SYNZIP1 (gBlock265) via IDT. The coding sequence of NarX$^{379-598}$ was PCR amplified with PR4122/PR4747 from the plasmid CMV-*narX*$^{379-598}$ (pMZ163). The gBlock, the PCR product and the plasmid CMV-*envZ* (pJH001[4]), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

CMV-*SYNZIP2*::*narX*$^{379-598}$ (pMZ206): We performed de novo synthesis of gBlock sequence encoding for MGSS starting sequence, SYNZIP2 and G4S linker (gBlock269) via IDT. The coding sequence of NarX$^{379-598}$ was PCR amplified with PR4346/PR4747 from the plasmid CMV-*narX*$^{176-598}$ (pJH010). The gBlock, the PCR product and the plasmid CMV-*envZ* (pJH001[4]), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

CMV-*narX*$^{379-598}$::*SYNZIP2* (pMZ208) : We performed de novo synthesis of gBlock sequence encoding for G4S linker and SYNZIP2 (gBlock270) via IDT. The coding sequence of NarX$^{379-598}$ was PCR amplified with PR4122/PR4748 from the plasmid CMV-*narX*$^{379-598}$ (pMZ163).

The gBlock, the PCR product and the plasmid CMV-*envZ* (pJH001[4]), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

CMV-*MTOR*$^{2018\text{-}113}$ T2098L::*CBRC* (pMZ211): The sequences coding for N-terminus of FRB and the C-terminus of FRB with CBRC were PCR amplified with PR4122/PR4541 and PR4346/PR4542 from the plasmid *MTOR*$^{2018\text{-}113}$::*CBRC*[5]. The primers were designed to introduce a mutation exchanging the codon coding for the threonine (T) at 2098th position (relative to the full protein Serine/Threonine-protein kinase TOR1) to a codon coding for a leucine (L). Both PCR products and the plasmid *MTOR*$^{2018\text{-}113}$::*CBRC*, digested with *Bam*HI and *Age*I, were assembled using Gibson mix.

CMV-*FKBP1A*::*narX*$^{379\text{-}598}$ (pMZ214): The sequence coding for FKBP was PCR amplified with PR4766/PR4767 from the plasmid *CBRN*::*FKBP1A*[5]. The coding sequence of NarX$^{379\text{-}598}$ was PCR amplified with PR4346/PR4771 from the plasmid CMV-*narX*$^{176\text{-}598}$ (pJH010). The primers were designed to insert sequence encoding for (G4S)2 linker between the amplified fragment. Both PCR products and the plasmid CMV-*envZ* (pJH001[4]), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

CMV-*FRB* T2098L::*narX*$^{379\text{-}598}$ (pMZ215): The sequence coding for FRB T2098L was PCR amplified with PR4769/PR4770 from the plasmid CMV-*MTOR*$^{2018\text{-}113}$ T2098L::*CBRC* (pMZ211). The coding sequence of NarX$^{379\text{-}598}$ was PCR amplified with PR4346/PR4771 from the plasmid CMV-*narX*$^{176\text{-}598}$ (pJH010). The primers were designed to insert sequence coding for (G4S)2 linker between the amplified fragment. Both PCR products and the plasmid CMV-*envZ* (pJH001[4]), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

NarL_RE-*cerulean* (pMZ219): The minimal response element NarL_RE was formed by annealing the primers PR4892 and PR4893. The annealed product and the plasmid OmpR_RE-*cerulean* (pMZ1), digested with *Asc*I and *Nde*I, were assembled using Gibson mix.

EF1α-V1-*SYNZIP1*::*narX*$^{379-598}$ (pMZ221): The sequence coding for MGSS starting sequence, SYNZIP1, G4S linker, and NarX^{379to383} was PCR amplified with PR3687/PR4971 from the plasmid CMV-*SYNZIP1*:: *NarX*$^{379-598}$ (pMZ200). The PCR product and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*SYNZIP2*::*narX*$^{379-598}$ (pMZ222): The sequence coding for MGSS starting sequence, SYNZIP2, G4S linker, and NarX^{379to383} was PCR amplified with PR3687/PR4971 from the plasmid CMV-*SynZip2*::*narX*$^{379-598}$ (pMZ206). The PCR product and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*SYNZIP1*::*narX*$^{379-598}$ H399Q (pMZ223): The sequence coding for MGSS starting sequence, SYNZIP,1 and G4S linker was PCR amplified with PR4971/PR4973 from the plasmid CMV-*SYNZIP1*::*narX*$^{379-598}$ (pMZ200). The sequence coding for NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR4972 from the plasmid CMV-*narX* H399Q (pEM014[4]). Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*SYNZIP2*::*narX*$^{379-598}$ H399Q (pMZ224): The sequence coding for MGSS starting sequence, SYNZIP2, and G4S linker was PCR amplified with PR4971/PR4973 from the plasmid CMV-*SYNZIP2*::*narX*$^{379-598}$ (pMZ206). The sequence coding for NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR4972 from the plasmid CMV-*narX* H399Q (pEM014[4]). Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*SYNZIP1*::*narX*$^{379-598}$ N509A (pMZ225): The sequence coding for MGSS starting sequence, SYNZIP1, and G4S linker was PCR amplified with PR4971/PR4973 from the plasmid CMV-*SYNZIP1*::*narX*$^{379-598}$ (pMZ200). The sequence coding for NarX$^{379-598}$ N509A was PCR amplified with PR3687/PR4972 from the plasmid CMV-*narX* N509A (pMZ160).

Both PCR products and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*SYNZIP2*::*narX*$^{379-598}$ N509A (pMZ226): The sequence coding for MGSS starting sequence, SYNZIP2, and G4S linker was PCR amplified with PR4971/PR4973 from the plasmid CMV-*SYNZIP2*::*narX*$^{379-598}$ (pMZ206). The sequence coding for NarX$^{379-598}$ N509A was PCR amplified with PR3687/PR4972 from CMV-*narX* N509A (pMZ160). Both PCR products and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*FKBP1A*::*narX*$^{379-598}$ H399Q (pMZ229): The sequence coding for FKBP and (G4S)2 linker was PCR amplified with PR4974/PR4973 from the plasmid CMV-*FKBP1A*:: *NarX*$^{379-598}$ (pMZ214). The sequence coding for NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR4972 from the plasmid CMV-*narX* H399Q (pEM014[4]). Both PCR products and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ H399Q (pMZ230): The sequence coding for FRB T2098L and (G4S)2 linker was PCR amplified with PR4975/PR4973 from the plasmid CMV-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ (pMZ215). The sequence coding for NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR4972 from the plasmid CMV-*narX* H399Q (pEM014[4]). Both PCR products and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*FKBP1A*::*narX*$^{379-598}$ N509A (pMZ231): The sequence coding for FKBP and (G4S)2 linker was PCR amplified with PR4974/PR4973 from the plasmid CMV-*FKBP1A*::*narX*$^{379-598}$ (pMZ214). The sequence coding for NarX$^{379-598}$ N509A was PCR amplified with PR3687/PR4972 from the plasmid CMV-*narX* N509A (pMZ160). Both PCR products and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ N509A (pMZ232): The sequence coding for FRB T2098L and (G4S)2 linker was PCR amplified with PR4975/PR4973 from the plasmid CMV-*MTOR*$^{2018-113}$ T2098L::*narX*$^{379-598}$ (pMZ215). The sequence coding for NarX$^{379-598}$ N509A was PCR amplified with PR3687/PR4972 from the plasmid CMV-*narX* N509A (pMZ160). Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*narX* (pMZ239): The sequence coding for NarX was PCR amplified with PR3687/PR4979 from the plasmid CMV-*narX* (pJH002[4]). The PCR product and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*narX*$^{379-598}$ (pMZ241): The 3’ fragment of *narX* was PCR amplified with PR4977/PR3687 from the plasmid CMV-*narX* (pJH002[4]). The primers were designed to amplify the sequence from the 20th codon upstream the codon encoding for the phosphorylatable histidine at the position 399 till the end of the gene and to insert the ATG sequence in front of this amplified sequence. The PCR product and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*narX* H399Q (pMZ242): The sequence coding for NarX was PCR amplified with PR3687/PR4979 from the plasmid CMV-*narX* H399Q (pEM014[4]). The PCR product and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*narX*$^{379-598}$ H399Q (pMZ244): The 3’ fragments of *narX* was PCR amplified with PR4977/PR3687 from the plasmid CMV-*narX* H399Q (pEM014[4]). The primers were designed to amplify the sequence from the 20th codon upstream of the codon encoding for the phosphorylatable histidine at the position 399 till the end of the gene and to insert the ATG sequence in front of this amplified sequence. The PCR product and the plasmid EF1α-

V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*narX* N509A (pMZ245): The sequence coding for NarX was PCR amplified with PR3687/PR4979 from the plasmid CMV-*narX* N509A (pMZ160). The PCR product and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*narX*$^{379\text{-}598}$ N509A (pMZ247): The 3' fragment of *narX* was PCR amplified with PR4977/PR3687 from the plasmid CMV-*narX* N509A (pMZ160). The primers were designed to amplify the sequence from 20 codon upstream the codon encoding for the phosphorylatable histidine at the position 399 to the end of the gene and to insert the ATG sequence in front of this amplified sequence. The PCR product and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-*NarL* (pMZ248): The EF1α promoter was PCR amplified with PR4732/PR4978 from the plasmid pRA58 (Altamura et al, manuscript in preparation). The PCR product and the plasmid CMV-*narL* (pJH004[4]), digested with *Psp*OMI and *Age*I, were assembled using Gibson mix.

EF1α-V1-*NarL* (pMZ249): The EF1α-V1 promoter was PCR amplified with PR4734/PR4978 from the plasmid pRA114 (Altamura et al, manuscript in preparation). The PCR product and the plasmid CMV-*narL* (pJH004), digested with *Psp*OMI and *Age*I, were assembled using Gibson mix.

EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ (pMZ250): The sequence coding for β-arrestin-2 was PCR amplified with PR4980/PR4981 from the plasmid CMV-*ARRB2*::TEV protease (pBH302). The sequence coding for NarX$^{379\text{-}598}$ was PCR amplified with PR3687/PR4982 from the plasmid CMV-*narX* (pJH002[4]). The primers were designed to insert sequence encoding for G4S linker between the amplified fragment. Both PCR products and EF1α-V1-*envZ-mCherry* the plasmid (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ H399Q (pMZ251): The sequence coding for β-arrestin-2 was PCR amplified with PR4980/PR4981 from the plasmid CMV-*ARRB2:*:TEV protease (pBH302). The sequence coding for NarX$^{379\text{-}598}$ H399Q was PCR amplified with PR3687/PR4982 from the plasmid CMV-*narX* H399Q (pEM014[4]). The primers were designed to insert sequence encoding for G4S linker between the amplified fragment. Both PCR products and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*ARRB2*::*narX*$^{379\text{-}598}$ N509A (pMZ252): The sequence coding for β-arrestin-2 was PCR amplified with PR4980/PR4981 from the plasmid CMV-*ARRB2:*:TEV protease (pBH302). The sequence coding for NarX$^{379\text{-}598}$ N509A was PCR amplified with PR3687/PR4982 from the plasmid CMV-*narX* N509A (pMZ160). The primers were designed to insert sequence encoding for G4S linker between the amplified fragment. Both PCR products and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*narX*$^{379\text{-}598}$ H399Q (pMZ257): The sequence coding for β2AR$^{1\text{-}341}$::V2R$^{343\text{-}371}$ was PCR amplified with PR4983/PR4985 from the plasmid CMV-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*tTA* (pBH312). The sequence coding for NarX$^{379\text{-}598}$ H399Q was PCR amplified with PR3687/PR4982 from the plasmid CMV-*narX* H399Q (pEM014). The primers were designed to insert sequence encoding for G4S linker between the amplified fragment. Both PCR products and the plasmid EF1α-V1-*envZ-mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*narX*$^{379\text{-}598}$ N509A (pMZ258): The sequence coding for β2AR$^{1\text{-}341}$::V2R$^{343\text{-}371}$ was PCR amplified with PR4983/PR4985 from the plasmid CMV-*ADRB2*$^{1\text{-}341}$::*AVPR2*$^{343\text{-}371}$::*tTA* (pBH312). The sequence coding for NarX$^{379\text{-}598}$ N509A was PCR amplified with PR3687/PR4982 from the plasmid CMV-*narX* N509A (pMZ160). The primers were designed to insert sequence encoding for G4S linker between the amplified fragment. Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

DcuR_RE-*cerulean* (pMZ259): The minimal response element DcuR_RE was formed by annealing the primers PR1964 and PR1965. The annealed product and the plasmid OmpR_RE-*cerulean* (pMZ1), digested with *Asc*I and *Nde*I, were assembled using Gibson mix.

tTA_RE-*cerulean* (pMZ290): The promoter regulated by tTA was PCR amplified with PR5226/PR5227 from the plasmid tTA_RE-*mCherry* (pIM003[6]). The PCR product and the plasmid DcuR_RE-*cerulean* (pMZ259), digested with *Asc*I and *Age*I, were assembled using Gibson mix.

EF1α-V1-*ARRB2:*:TEV protease (pMZ291): The sequence coding for β-arrestin-2$^{283-409}$ and for the TEV protease was PCR amplified with PR5228/PR5229 from the plasmid CMV-*ARRB2:*:TEV protease (pBH302). The sequence of the bGH poly(A) signal was PCR amplified with PR5230/PR5231 from the plasmid EF1α-V1-*ARRB2*::*narX*$^{379-598}$ (pMZ250). Both PCR products and the plasmid EF1α-V1-*ARRB2*::*narX*$^{379-598}$ (pMZ250), digested with *Bsa*I and *Avr*II, were assembled using Gibson mix.

EF1α-V1-*NPY1R*::*narX*$^{379-598}$ H399Q (pMZ295): The sequence coding for NPY1-R was PCR amplified with PR5293/PR5294 from the plasmid CMV-*NPY1R*::*AVPR2*$^{343-371}$::*tTA* (pMZ293). The sequence coding for NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR4982 from the plasmid EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257). The primers were designed to insert sequence encoding for G4S linker between the amplified fragment. Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*NPY5R*$^{1-438}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ297): The sequence coding for NPY5-R$^{1-338}$ was PCR amplified with PR5295/PR5296 from the plasmid CMV- *NPY5R*$^{1-445}$::*AVPR2*$^{343-371}$::*tTA* (pMZ294). The sequence coding for V2R$^{343-371}$::NarX$^{379-598}$ H399Q was

PCR amplified with PR3687/PR5297 from the plasmid EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257). The primers were designed to insert sequence encoding for G4S linker between the amplified fragment. Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*ADRB2*$^{1-413}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ307): The sequence coding for β2AR$^{1-413}$::V2R$^{343-349}$ was PCR amplified with PR5399/PR5400 from the plasmid CMV-*ADRB2*$^{1-413}$::*AVPR2*$^{343-371}$::*tTA* (pMZ300). The sequence coding for V2R$^{343-371}$::NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR5396 from the plasmid EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257). Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*AVPR2*$^{1-371}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ308): The sequence coding for V2R^{-371}::V2R$^{343-349}$ was PCR amplified with PR5401/PR5400 from the plasmid CMV-*AVPR2*$^{1-371}$::*AVPR2*$^{343-371}$::*tTA* (pMZ301). The sequence coding for V2R$^{343-371}$::NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR5396 from the plasmid EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257). Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*BDKRB2*$^{1-391}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ309): The sequence coding for B2R$^{1-391}$::V2R$^{343-349}$ was PCR amplified with PR5402/PR5400 from the plasmid CMV-*BDKRB2*$^{1-391}$::*AVPR2*$^{343-371}$::*tTA* (pMZ302). The sequence coding for V2R$^{343-371}$::NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR5396 from the plasmid EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257). Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*CXCR4*$^{1-358}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ310): The sequence coding for CXC-R4$^{1-358}$::V2R$^{343-349}$ was PCR amplified with PR5403/PR5400 from the plasmid CMV-*CXCR4*$^{1-358}$::*AVPR2*$^{343-371}$::*tTA* (pMZ303). The sequence coding for V2R$^{343-371}$::NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR5396 from the plasmid EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257). Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*LPAR1*$^{1-364}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ311): The sequence coding for LPA-R1$^{1-364}$::V2R$^{343-349}$ was PCR amplified with PR5404/PR5400 from the plasmid , CMV-*LPAR1*$^{1-364}$::*AVPR2*$^{343-371}$::*tTA* (pMZ304). The sequence coding for V2R$^{343-371}$::NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR5396 from the plasmid EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257). Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*NMBR*$^{1-390}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ312): The sequence coding for NMB-R$^{1-390}$::V2R$^{343-349}$ was PCR amplified with PR5405/PR5400 from the plasmid , CMV-*NMBR*$^{1-390}$::*AVPR2*$^{343-371}$::*tTA* (pMZ305). The sequence coding for V2R$^{343-371}$::NarX$^{379-598}$ H399Q was PCR amplified with PR3687/PR5396 from the plasmid EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257). Both PCR products and the plasmid EF1α-V1-*envZ*-*mCherry* (pMZ194), digested with *Age*I and *Xho*I, were assembled using Gibson mix.

EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*tTA* (pBH292): The sequence coding for β2AR$^{254-341}$::V2R$^{343-371}$::tTA was PCR amplified with PR5232/PR5233 from the plasmid CMV-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*tTA* (pBH312). The PCR product and the plasmid EF1α-V1-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*narX*$^{379-598}$ H399Q (pMZ257), digested with *Bgl*II and *Xho*I, were assembled using Gibson mix.

CMV-*ARRB2::*TEV protease (pBH302): We performed de novo synthesis of gBlock sequence encoding for β-arrestin-2 fused with the TEV protease with 2 gBlock (gBlock112 and gBlock 113) via IDT. The gBlock and the plasmid pZsYellow1-N1 (Clontech 632445), digested with *Not*I and *Eco*RI, were assembled using Gibson mix.

CMV-*OPRK1*$^{1-345}$::*AVPR2*$^{343-371}$::*tTA* (pBH309): Via IDT, We performed de novo synthesis of gBlock (gBlock114) sequence encoding for KOR-1 and of gBlock (gBlock115) sequence encoding for V2R fused to tTA. The sequence coding for KOR-1$^{1-345}$ was PCR amplified with PR2442/PR2443 from gBlock114. The PCR product, gBlock115 and the plasmid pZsYellow1-N1 (Clontech 632445), digested with *Xho*I and *Mfe*I, were assembled using Gibson mix.

CMV-*ADRB2*$^{1-341}$::*AVPR2*$^{343-371}$::*tTA* (pBH312): Via IDT, We performed de novo synthesis of gBlock (gBlock118) sequence encoding for β2AR. The sequence coding for β2AR$^{1-341}$ was PCR amplified with PR2442/PR2444 from gBlock118. The PCR product and the plasmid CMV-*OPRK1*$^{1-345}$::*AVPR2*$^{343-371}$::*tTA* (pBH309), digested with *Xho*I and *Bss*HII, were assembled using Gibson mix.

EF1α::*iRFP* (pCS184): The iRFP coding sequence from CMV-*iRFP* (pCS12[7]) was PCR amplified with PR2258/PR2259. The PCR product and the plasmid EF1α::*citrine* (pRA001, Altamura et al, manuscript in preparation), digested with *Bmt*I and *Xba*I, were assembled using ligation mix.

CMV-*narX*$^{176-598}$ (pJH010): The 3’ fragment of *narX* was PCR amplified with PR1021/PR1023 from CMV-*narX* (pJH002). The primers were designed to amplify the sequence of NarX from the codon encoding the alanine at the position 176 to the end of the gene and to insert the ATG sequence in front of this amplified sequence. The PCR products and CMV-*narX* (pJH002[4]) were digested with *Xho*I and *Age*I. The two digested products are then ligated together.

The following plasmids were reported previously: CMV-*envZ* (pJH001), CMV-*narX* (pJH002), CMV-*ompR* (pJH003), CMV-*narL* (pJH004), OmpR_RE-*amCyan* (pJH008), CMV-*envZ*_cyt (pJH009), CMV-*envZ* H243V (pEM013), CMV-*narX* H399Q (pEM014), EnvZ-GGGGS-mCherry (pEM017)[4], *CBRN*::*FKBP1A* and *MTOR*$^{2018-113}$::*CBRC*[5], Ef1α-*Cerulean* (pKH024), Ef1α-*citrine* (pKH025), Ef1α-*mCherry* (pKH026) and Junk-DNA (pBH265)[3], pTRE Bidirectional mCherry-pA (pIM003)[6], CMV-rtTA (pZ91) [8]. The plasmid CMV-*iRFP* (pCS12) was obtained from Addgene (plasmid 31857[7]). The plasmid CMV-*NPY1R*::*AVPR2*$^{343-371}$::*tTA* (pMZ293, addgene number 66453), CMV-*NPY5R*$^{1-445}$::*AVPR2*$^{343-371}$::*tTA* (pMZ294, addgene number 66456), CMV-*ADRB2*$^{1-413}$::*AVPR2*$^{343-371}$::*tTA* (pMZ300, addgene number 66220), CMV-*AVPR2*$^{1-371}$::*AVPR2*$^{343-371}$::*tTA* (pMZ301, addgene number 66227)), CMV-*BDKRB2*$^{1-391}$::*AVPR2*$^{343-371}$::*tTA* (pMZ302, addgene number 66230), CMV-*CXCR4*$^{1-358}$::*AVPR2*$^{343-371}$::*tTA* (pMZ303, addgene number 66262) , CMV-*LPAR1*$^{1-364}$::*AVPR2*$^{343-371}$::*tTA* (pMZ304, addgene number 66418), CMV-*NMBR*$^{1-390}$::*AVPR2*$^{343-371}$::*tTA* (pMZ305, addgene number 66445) were obtained from Addgene[2].

Supplementary Note 2: Preparation of chemicals used in this study

A/C Heterodimerizer (Clontech; Cat# 635057) solution at 50 μM was prepared in ethanol (Honeywell; Cat# 02860) and diluted in DMEM to have final concentration of 500 nM of A/C Heterodimerizer.

Procaterol (Sigma; Cat# P9180-10MG) solution at 200 μM was prepared in DMSO (Sigma; Cat# D4540, BCBT0803) and diluted in DMEM to have final concentration of 2 μM of procaterol.

Bradykinin acetate salt (Sigma; Cat#B3259) solution at 10 mM was prepared in water (Invitrogen; Cat#10977-035) and diluted in DMEM to have final concentration of 10 μM of Bradykinin.

Lysophosphatidic Acid (Santa cruz Biotech; Cat#SC201053) solution at 2 mM was prepared in PBS (Gibco; Cat#10010-015) and diluted in DMEM without serum to have final concentration of 10 μM of Lysophosphatidic Acid.

[Arg8]-Vasopressin (Tocris; Cat#2935) solution at 1mM was prepared in water (Invitrogen; Cat#10977-035) and diluted in DMEM to have final concentration of 10 μM of [Arg8]-Vasopressin.

NMB (Tocris; Cat#1908) solution at 1 mM was prepared in DMSO (Sigma; Cat#D4540) and diluted in DMEM without serum to have final concentration of 10 μM of NMB.

Recombinant Human CXCL12 (SDF-1α, Lys22 to lys89) (Biolegend; Cat#581202) at 25 μM (200 μg/ml) was diluted in DMEM without serum to have final concentration of 0.2 μM of CXCL12.

NPY (Sigma; Cat#N5017) was prepared in water (Invitrogen; Cat#10977-035) and diluted in DMEM without serum to have final concentration of 0.5 μM of NPY.

For the titration experiment 5 μl of the chemical tested at 100x of the desired final concentration were added to the 500 μl of DMEM present in the wells. The different stock solution used were prepared as indicated below:

A/C Heterodimerizer (Clontech; Cat# 635057) stock solution was prepared in ethanol (Honeywell; Cat# 02860): 250μM, 50 μM, 20 μM, 8 μM, 3.2 μM, 1.28 μM, 512 nM, 205 nM, 81.9 nM, 32.8 nM, 13.1 nM, 5.24 nM, 1.04 nM.

Procaterol (Sigma; Cat# P9180-10MG) stock solution was prepared in DMSO (Sigma; Cat# D4540, BCBT0803): 1mM, 286 μM, 81.6 μM, 23.3 μM, 10 μM, 6.6 μM, 1.9 μM, 544 nM, 155 nM, 44.4 nM and 12.7 nM.

Isoproterenol (Sigma; Cat# I6504) stock solution was prepared in DMSO (Sigma; Cat# D4540): 1mM, 286 μM, 81.6 μM, 23.3 μM, 6.6 μM, 1.9 μM, 544 nM, 155 nM, 44.4 nM and 12.7 nM.

Clenbuterol (Sigma; Cat# C5423) stock solution was prepared in DMSO (Sigma; Cat# D4540): 1mM, 286 μM, 81.6 μM, 23.3 μM, 6.6 μM, 1.9 μM, 544 nM, 155 nM, 44.4 nM and 12.7 nM.

Propranolol (Sigma; Cat# P0884) stock solution was prepared in water (Invitrogen; Cat#10977-035): 1mM, 286 μM, 81.6 μM, 23.3 μM, 6.6 μM, 1.9 μM, 544 nM, 155 nM, 44.4 nM and 12.7 nM.

Supplementary References

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