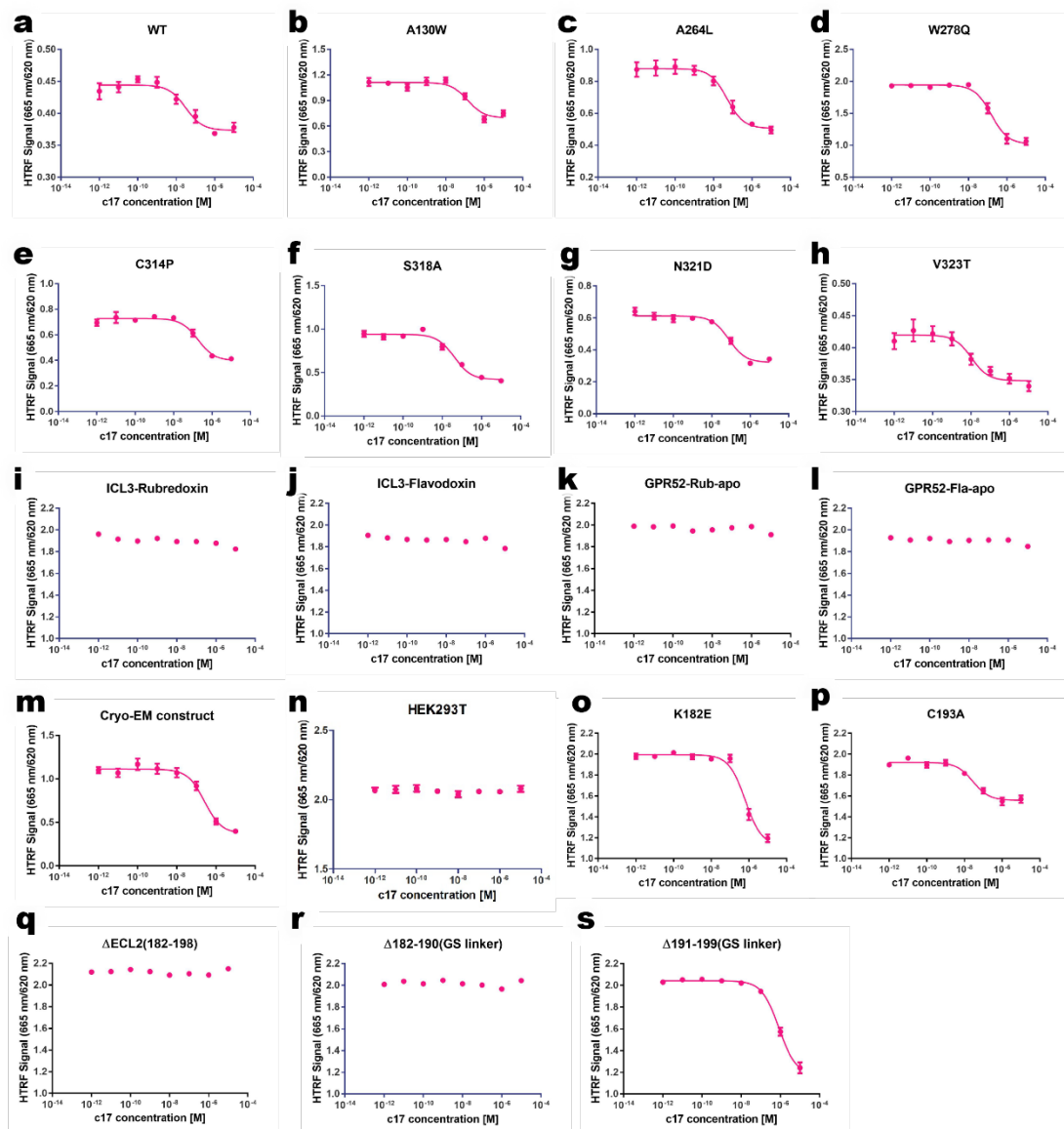

Supplementary information

Structural basis of ligand recognition and self-activation of orphan GPR52

In the format provided by the
authors and unedited

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Supplemental Figure 1. Effects of GPR52 mutations on potency of c17. a-s, Dose-dependent raw curves of c17 on wild-type (WT) and mutant GPR52 as well as HEK293T cells. Error bars representing the standard error of the mean (s.e.m.). Data represent mean $\pm$ s.e.m. (n = 3 independent experiments).



Supplemental Table 1. Effect of binding pocket mutations on agonist c17 potency.

Data were acquired with WT GPR52 and mutants expressed in HEK293T cells by using HTRF assay (cisbio) to measure G α s-mediated cAMP stimulation. Error bars representing the standard error of the mean (s.e.m.). Data represent mean $\pm$ s.e.m. (n = 3 independent experiments).

	EC50 (nM)	pEC50$\pm$s.e.m
WT	4.472	8.35 $\pm$ 0.12
Y34A (N-term)	13.533	7.87 $\pm$ 0.07
V39F (1.31)	3.83	8.42 $\pm$ 0.02
I47A (1.39)	No activity	No activity
L101A (2.64)	0.709	9.15 $\pm$ 0.11
F117A (3.28)	1.815	8.74 $\pm$ 0.40
I189A (ECL2)	0.567	9.25 $\pm$ 0.08
F300A (7.36)	27.266	7.76 $\pm$ 0.10
T303F (7.39)	403.233	6.39 $\pm$ 0.01
W304A (7.40)	0.918	9.04 $\pm$ 0.26

Supplemental Table 2. Pocket volume comparison of GPR52 with Class-A peptide receptors. * Organism(s): *Rattus norvegicus*

Receptor name	Abbreviation	PDB	Volume (Å ³)
Orphan GPCR-52	GPR52-c17	--	1054.375
Orphan GPCR-52	GPR52-apo	--	958.875
Apelin receptor	APJ	5VBL	2573.875
Angiotensin II type-1 receptor	AT1	4YAY	3272.125
C5a anaphylatoxin chemotactic receptor 1	C5A1	5O9H	3055
C-C chemokine receptor type 2	CCR2	5T1A	2700.875
C-C chemokine receptor type 5	CCR5	5UIW	3251.875
C-C chemokine receptor type 9	CCR9	5LWE	1458
C-X-C chemokine receptor type 4	CXCR4	3ODU	3186.75
Endothelin receptor type B	ETB	5GLI	3822.625
Neurokinin 1 receptor = Substance-P receptor	NK1	6HLO	2054.875
Neuropeptide Y receptor type 1	NPY1	5ZBH	2261.875
Neurotensin receptor type 1	NTS1	4BUO*	1610.625
Delta-type opioid receptor	OPRD	4N6H	2447.625
Kappa-type opioid receptor	OPRK	4DJH	2699.125
Mu-type opioid receptor	OPRM	4DKL	3060
Nociceptin receptor	OPRX	4EA3	2993
Orexin receptor type 1	OX1	5ZJ8	3526.5
Orexin receptor type 2	OX2	5WQC	2562.625

Supplemental Table 3. List of structures used to compare ligand locations.

Small molecule receptor	Abbreviation	PDB
5-hydroxytryptamine receptor 1B	5HT1B	4IAQ
5-hydroxytryptamine receptor 2B	5HT2B	5TVN
5-hydroxytryptamine receptor 2C	5HT2C	6BQG
Muscarinic acetylcholine receptor M1	M1	5CXV
Muscarinic acetylcholine receptor M2	M2	6OIK
Muscarinic acetylcholine receptor M3	M3	4U15
Muscarinic acetylcholine receptor M4	M4	5DSG
Beta-1 adrenergic receptor	B1AR	6H7M
Beta-2 adrenergic receptor	B2AR	3P0G
Adenosine receptor A1	A1	6D9H
Adenosine receptor A2A	A2A	5G53
P2Y purinoceptor 12	P2Y12	4PXZ
Dopamine D2 receptor	D2	6CM4
Dopamine D3 receptor	D3	3PBL
Dopamine D4 receptor	D4	5WIV
Histamine H1 receptor	H1	3RZE
Melatonin receptor type 1A	MT1	6ME2
Melatonin receptor type 1B	MT2	6ME6
Lipid receptor	Abbreviation	PDB
Free fatty acid receptor 1	GPR40	5TZR
Sphingosine 1-phosphate receptor 1	S1P1	3V2Y
Platelet-activating factor receptor	PAF	5ZKP
Cannabinoid receptor 1	CB1	5XRA
Cannabinoid receptor 2	CB2	5ZTY
Prostaglandin E2 receptor EP3 subtype	EP3	6M9T
Leukotriene B4 receptor 1	BLT1	5X33
Lysophosphatidic acid receptor 1	LPA1	4Z35
Lysophosphatidic acid receptor 6	LPA6	5XSZ

Supplemental Table 4. List of structures used to construct the structure similarity network. * not human protein. ** agonist-bound inactive state.

Receptor Name	Abbreviation	PDB
5-hydroxytryptamine receptor 1B	5HT1B	5V54
5-hydroxytryptamine receptor 2A	5HT2A	6A94
5-hydroxytryptamine receptor 2B	5HT2B	6DS0
5-hydroxytryptamine receptor 2C	5HT2C	6BQH
Adenosine receptor A1	A1	5UEN
Adenosine receptor A2A	A2A	4EIY
Angiotensin II type-1 receptor	AT1	4YAY
Apelin receptor	APJ	5VBL
Beta-1 adrenergic receptor	B1AR	5A8E*
Beta-2 adrenergic receptor	B2AR	2RH1
C5a anaphylatoxin chemotactic receptor 1	C5A1	5O9H
Cannabinoid receptor 1	CB1	5TGZ
Cannabinoid receptor 2	CB2	5ZTY
C-C chemokine receptor type 2	CCR2	5T1A
C-C chemokine receptor type 5	CCR5	5UIW
C-C chemokine receptor type 9	CCR9	5LWE
C-X-C chemokine receptor type 4	CXCR4	3ODU
Dopamine D2 receptor	DRD2	6CM4
Dopamine D3 receptor	DRD3	3PBL
Dopamine D4 receptor	DRD4	5WIU
Endothelin receptor type B	ETB	5GLI
Free fatty acid receptor 1	FFA1	5TZR
Histamine H1 receptor	H1	3RZE
Leukotriene B4 receptor 1	BLT1	5X33*
Lysophosphatidic acid receptor 1	LPA1	4Z35
Lysophosphatidic acid receptor 6	LPA6	5XSZ*
Melatonin receptor type 1A	MT1	6ME2
Melatonin receptor type 1B	MT2	6ME6
Muscarinic acetylcholine receptor M1	M1	5CXV
Muscarinic acetylcholine receptor M2	M2	3UON
Muscarinic acetylcholine receptor M3	M3	4U15*
Muscarinic acetylcholine receptor M4	M4	5DSG
Neurokinin 1 receptor = Substance-P receptor	NK1	6HLO
Neuropeptide Y receptor type 1	NPY1	5ZBH
Neurotensin receptor type 1	NTS1	4BUO*
Delta-type opioid receptor	OPRD	4N6H
Kappa-type opioid receptor	OPRK	4DJH
Mu-type opioid receptor	OPRM	4DKL*
Nociceptin receptor	OPRX	4EA3

Orexin receptor type 1	OX1	4ZJ8
Orexin receptor type 2	OX2	5WQC
P2Y purinoceptor 1	P2Y1	4XNW
P2Y purinoceptor 12	P2Y12	4PXZ**
Prostaglandin D2 receptor 2	DP2	6D27
Prostaglandin E2 receptor EP3 subtype	EP3	6M9T
Prostaglandin E2 receptor EP4 subtype	EP4	5YWY
Proteinase-activated receptor 1	PAR1	3VW7
Proteinase-activated receptor 2	PAR2	5NDD
Rhodopsin	RHO	1U19*
Sphingosine 1-phosphate receptor 1	S1P1	3V2Y
Thromboxane A2 receptor	TP	6IU
Platelet-activating factor receptor	PAF	5ZKQ
