Supplemental Figure 1. GPRC6A Homology Modelling. (A) MSA between 16 sequences shown using clustralx color code. Alignment is shown for helix-4, extracellular loop-2 and helix-5 regions. (B) Sequence similarity scores between 16 sequences after MSA. Sequence 1: GPRC6A, Sequences 2-9: class-C GPCR's, Sequences 10-16: class-A GPCR's. mGluR-1 (sequence-2) and mGluR-5 (sequence-3) taken as main templates for transmembrane domain modelling (C, D) GPRC6A transmembrane homology models based on the mGlu-1 and mGlu-5 receptor structures.

Supplemental Figure 2. Generation of GPRC6A knockdown PC-3 cells using CRISPR-Cas9 system.

(A) The lentiCRISPR vector from Addgene. Location and sequences of GPRC6A sgRNA-3 (B), and sequence mutagenesis of GPRC6A in PC-3 cells with CRISPR-Cas9 system (C). GPRC6A messenger RNA (D) and protein expression (E) in GPRC6A knockdown PC-3 cells using CRISPR-Cas9 system.

Supplemental Figure 3. Docking of DJ-I-267R to GPRC6A. Residues surrounding DJ-I-267R in the binding pocket.

Supplemental Figure 4. Docking of DJ-I-267S to GPRC6A. Residues surrounding DJ-I-267S in the binding pocket.

Α											
	1000	1010	1020	1030	1040	1050	1060	1070	1080	1090 1100	1110 1120
GPRC6a/1-926	FLKCLYRPILI	IIFTCTGIOVVICT	L-WLIF		· AAPTVEVN · · ·		VSLPRV····		CEE0	SILAFOTMLOY	AILAFICFIFAFK
MGIuR1_HUMAN/1-1194										SNLOVVAPLOY	
GRM5_HUMAN/1-1212										· · · · · · · · · TNLGVVTPLGY	
MGluR3_MOUSE/1-879										KD <mark>S</mark> SML <mark>S</mark> LTY	
										· · · · · · · · · SLMALOFLIGY	
GABAR1_HUMAN/1-961										· · · · · · · · · MNTWLG IFYGY	
										· · · · · · · · DYMTAVAEFLF	
										· LSA · · PRRNEDF VLLLTY	
										N <mark>SLOF</mark> ILAFL <mark>Y</mark>	
										TNNE · · · · SFVIYMFVVHF	
										• TNR • • • • AYA I ASSI I SF	
Beta 2_HUMAN/1-467										· TNR · · · · AYATASSIISF	
A2AR_HUMAN/1-412										VV <mark>P</mark> M····NYMVYFNFFAC	
HT2B_HUMAN/1-481										· KERF · · GDFMLFGSLAAF	
										• DHI • • • • LYTVYSTVGAF	
NTS1_RAT/1-424	LMSR - SRTKK	AIWLASALLAI	P · MLF T · MG	LONRSOD	🧕 · · · · · · · · · · · · ·		THPO	V	PTPI	- VDTATVKV <mark>VI</mark> Q <mark>V</mark> NT <mark>FM</mark> SF	- LFPMLVISILNTVI

Helix-4

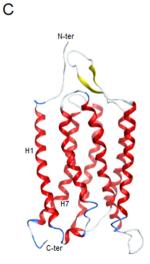
В

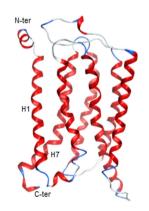
EL-2

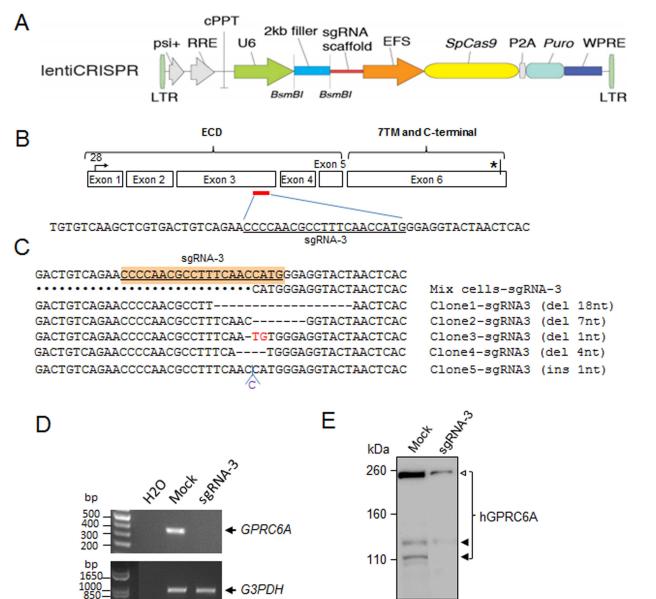
Helix-5

D

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1:GPRC6a		33.5	34.2	44.7	45.0	27.5	17.9	29.1	49.2	27.6	22.5	22.5	21.4	22.9	22.1	25.9
2:MGluR1_HUM	43.2		73.8	61.3	44.0	29.9	25.2	35.3	43.5	24.7	24.2	24.2	23.5	23.7	19.7	23.1
3:GRMS_HUMAN	44.7	75.0		60.9	42.7	30.1	23.9	32.8	43.6	25.9	22.3	22.3	22.3	22.5	20.0	22.9
4:MGluR3_MOU	42.4	45.1	44.1		38.9	30.2	16.9	31.9	40.1	25.6	20.1	20.1	19.2	18.3	21.0	22.4
5:CASR_HUMAN	52.4	39.7	38.0	47.7		27.4	20.7	31.9	49.3	26.4	20.3	20.3	19.2	22.5	20.0	23.3
6:GABAR1_HUM	28.5	24.0	23.8	33.0	24.4		19.8	26.3	30.6	26.1	22.3	22.3	19.7	20.4	23.6	26.9
7:GPCR_orpha	23.4	25.6	23.9	23.3	23.4	25.0		26.9	24.3	24.7	21.0	21.0	23.5	18.7	19.2	22.9
8:GPRC5a_RAI	11.2	10.6	9.7	13.0	10.6	9.8	7.9		11.1	19.8	16.1	16.1	16.3	16.4	16.7	18.2
9:TASR1_HUMA	44.7	30.7	30.3	38.3	38.5	26.7	16.8	26.1		26.1	19.5	19.5	18.0	19.8	19.2	23.6
10:Rhodopsin	10.4	7.2	7.4	10.1	8.5	9.5	7.1	19.3	10.8		26.6	26.6	29.6	27.2	33.3	29.7
11:Betal_Turk	11.3	9.5	8.6	10.7	8.8	10.8	8.1	21.0	10.8	35.6		99.8	40.8	37.2	46.9	34.2
12:Beta2_HUMA	11.3	9.5	8.6	10.7	8.8	10.8	8.1	21.0	10.8	35.6	99.8		40.8	37.2	46.9	34.2
13:A2AR_HUMAN	9.5	8.1	7.6	9.0		8.4	8.0	18.8	8.8	35.1	36.0	36.0		33.1	35.1	29.0
14:HT2B_HUMAN	11.9	9.5	8.9	10.0	10.0	10.2	7.4	22.1	11.3	37.6	38.3	38.3	38.6		44.4	34.2
15:HT1B_HUMAN	9.3	6.4	6.4	9.3	7.2	9.6	6.2	18.2	8.9	37.4	39.2	39.2	33.3	36.0		31.8
16:NTS1_RAT/1	11.9	8.2	8.0	10.8	9.2	11.9	8.0	21.6	11.9	36.2	31.0	31.0	29.9	30.1	34.6	



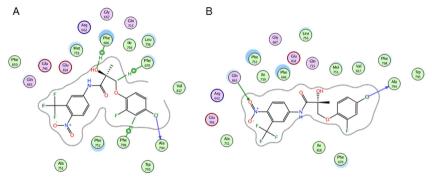


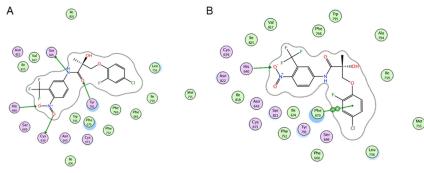


50 -

t-ERK

650





NAME	ACTIVITY	STRUCTURE
DJ-I-17	++	O_2N \rightarrow H \rightarrow OH $O-CI$ F_3C
DJ-I-47	-	$O_2N \xrightarrow{O}_H \xrightarrow{O}_{OH} O \xrightarrow{O}_{OH} O$
DJ-I-157	+	
DJ-I-225S	++	
<u>DJ-I-267S</u>	++	O2N - N - N - O - O - O - CI
DJ-III-127	++	$NC \rightarrow H$ $H \rightarrow H$ H \rightarrow H $H \rightarrow H$ H \rightarrow H $H \rightarrow H$
DJ-III-129	+	$O_2N \rightarrow H$ H H H H H H H H H
DJ-III-159	++	$O_2N \xrightarrow{O}_{H} \xrightarrow{O}_{OH} \xrightarrow{F} \xrightarrow{F}$
DJ-IV-5S	-	NC- F ₃ C Br

Supplemental Table 1. The list of SARMs.