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Supplemental Information

Genetic and Functional Dissection of the Role

of Individual 5-HT₂ Receptors as Entry Receptors

for JC Polyomavirus

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5-HT₂₄RΔ1-SVG-A cells

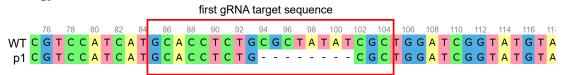
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5-HT_{2B}R∆1-SVG-A cells

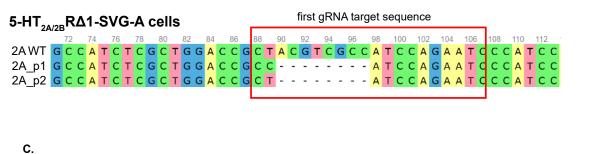
first gRNA target sequence

		112		114	1	116		118		120		122		124		126		128	1	30	1	32	1	134	1	136	1	38	1	40	14	12	144	1	146	1	48	1	50	1	152	1	54	1	Ę
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p1																																													
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р3 (
p4	4	A	A	С	А	А	G	С	С	А	С	С	т	С	А	-	-	-	-	-	-	-	-	A	С	-	-	-	-	-		•	C	т	G	т	G	т	С	Т	A	С	A	G	٦.

5-HT_{2c}RΔ1-SVG-A cells



В.



5-HT _{2A/2B/2C} RΔ1-SVG-A cells	first gRNA target sequence
2A WT 48 50 52 54 56 58 60 62 64 66 68 70 72 74 76 78 80 82 84 86 2A WT C G G C C T C C A T C A T G C A C C T C T G C G C C A T C T C G C T G G A C C G C G G C C T C C G C T G G A C C G C C A T C T C G C T G G A C C G C C A T C T C G C T G G C C A T C T G G C C A T C T G G C C T G G C C A T C T G G C C T G G C C A T C T G G G C C T G G C C A T C T G G G C C A T C T G G G C C T G G C C A T C T G G G C C A T C T G G C C T G G C C A T C T G G G C C A T C T G G C C T G G C C A T C T G G C T G G C C A T C T G G C T G G C C A T C T G G C C A T C T G G C C C A T C T G G C C C A T C T G G C C C A T C T G G C C C A T C T G G C C C C C C C C C C C C C C C	
first gRNA target sequence	-
76 78 80 82 84 86 88 90 92 94 96 98 100 102 10 2C WT C G T C A T G C T C G T C G T C G T C G T C G C T A T C G C C T G C T A T C G C C T A T C G C C T C A T C G C C T C G C C T C G C C T C G C C T C C C T C T C C C T C T C C C C C T C T C T C T C C T C T </td <td></td>	

Figure S1. Genetic modification in single, double and triple mutant clones using gRNAs from published library, related to Figure 2. Single cell isolation and expansion was performed to obtain a clonal population with a single modification. The genome editing was confirmed using the CRISPR deep sequencing service performed at the Center for Computational and Integrative Biology (CCIB) DNA core at the Massachusetts General Hospital (MGH). (A, B, C) Sequence alignments of 5-HT_{2A}R Δ 1-SVG-A cells, 5-HT_{2B}R Δ 1-SVG-A cells and 5-HT_{2C}R Δ 1-SVG-A cells generated using the gRNAs obtained from the GeCKO v2 library show genetic deletions in each receptor gene in the context of single (A), double (B) or triple mutant clones (C). 5-HT_{2B}R Δ 1-SVG-A cell line was used as starting line to generate 5-HT_{2A/2B}R Δ 1-SVG-A cells and 5-HT_{2A/2B/2C}R Δ 1-SVG-A cells.

5-HT₂₄RΔ2-SVG-A cells

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5-HT₂₈R Δ 2-SVG-A cells

second gRNA target sequence

	122	124	1 1	26	128	130	1:	32	134	13	6	138	140	14:	2 '	44	146	148	150	15	52	154	156	13	58	160	16	2 1	64	166	168	17	0 17	72	174	176	6 1 [°]	78	180	18	2 1	84	186
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p2	G		-			-																																					G A
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В.

$5-HT_{2A/2B/2C}R\Delta 2-SVG-A$ cells

													se	eco	nd	gF	RN	At	arg	jet	se	que	ene	ce									
	92		94		96		98		100	10	2	104	1	106	5	108		110		112		14						120		122		124	
2A WT																																	
2A_p1																																	
2A_p2	A	т	G	А	С	А	С	С	А	GÖ	G C	: -	-	С	т	А	С	А	G	т	Α	A	т	G	A	С	т	т	т	А	А	С	т
2A p3																																	

first gRNA target sequence 76 78 80 82 84 86 88 90 92 94 96 98 100 102 104 106 108 110 112 114 116 118 2C WT C G T C C A T C A T G C A C C T C T G C G C T A T A - T C G C T G G A T C G G T A T G T A 2C_p1 C G T C C A T C A T G C A C C T C T G C G C T A T A T T C G C T G G A T C G G T A T G T A 2C_p2 C G T C C A T C A T C A T G C A C C T C T G C G C T A T A T T C G C T G G A T C G G T A T G T A

Figure S2. Genetic modification in single and triple mutant cell clones using custom designed gRNAs, related to Figure 3. Single cell isolation and expansion was performed. The genome editing was confirmed using the CRISPR deep sequencing service performed at the Center for Computational and Integrative Biology (CCIB) DNA core at the Massachusetts General Hospital (MGH). (A) Sequence alignments of $5-HT_{2A}R\Delta 2$ -SVG-A cells and $5-HT_{2B}R\Delta 2$ -SVG-A cells generated using custom designed gRNAs show genetic deletions causing out of frame translation in each receptor gene in the context of single (A) or triple mutant clones (B). In the $5-HT_{2AZBI2C}R\Delta 2$ -SVG-A cells custom designed gRNAs were used to target $5-HT_{2A}R$ and $5-HT_{2B}RA$ obtained from the GeCKO v2 library was used to target $5-HT_{2C}R$. $5-HT_{2R}R\Delta 2$ -SVG-A cells.



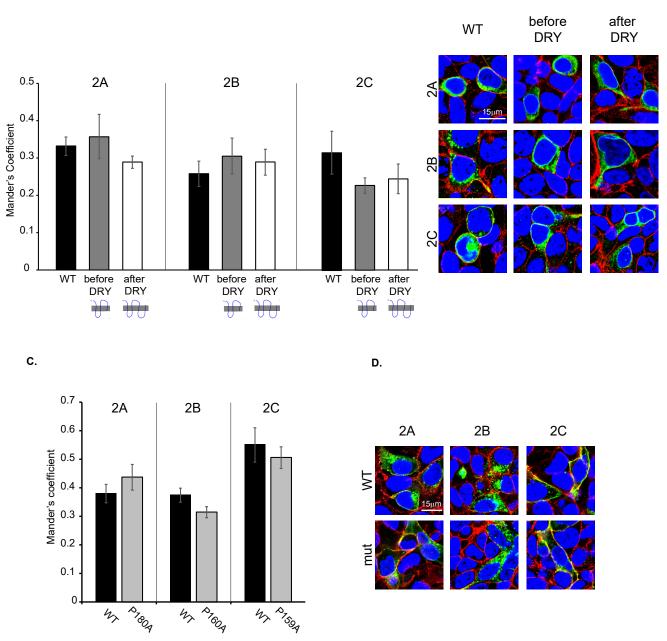


Figure S3. WT and mutant receptors are equally expressed at the cell surface, related to Figure 4. (A) Constructs expressing $5-HT_{2A}R$, $5-HT_{2B}R$ and $5-HT_{2C}R$ were truncated before the DRY (before DRY) or after the DRY motif (after DRY) and a His tag was added at this site. A His tag was also added at the C-terminal end of the WT receptors. HEK293A cells were transfected with WT or truncated constructs for 48 hours. Cells were stained with a pan-Cadherin antibody, a cell surface marker, and the His tag. DAPI was used to counterstain the nuclei. (B) Representative images of each condition are shown. (C) The proline located 6 amino acids downstream of the DRY motif was mutated to an alanine in three constructs expressing $5-HT_{2A}R$ (2A), $5-HT_{2B}R$ (2B), or $5-HT_{2C}R-YFP$ (2C). HEK293A cells were transfected with WT or mutated constructs. Cells were stained for cadherins and DAPI was used to counterstain the nuclei. (D) Representative images are shown. Cells were imaged using a scanning confocal microscope and colocalization between each form of the serotonin receptors, either directly tagged with YFP or stained for the His tag, and cadherins were measured using Mander's coefficient. The experiments were performed in duplicates and the error bars represent SEM.