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Supplemental Information

Genetic and Functional Dissection of the Role of Individual 5-HT_2 Receptors as Entry Receptors for JC Polyomavirus

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A.

5-HT$_{2A}$RΔ1-SVG-A cells

first gRNA target sequence

```
WT  GCCATCTCGCTGGACCGCTA-CGTCGCCATCCAGAATCCCATCC
p1  GCCATCTCGCTGG----------GTCGCCATCCAGAATCCCATCC
p2  GCCATCTCGCTGGACCGCTACCGTCGCCATCCAGAATCCCATCC
```

5-HT$_{2B}$RΔ1-SVG-A cells

first gRNA target sequence

```
WT  AAACAAGCCACCTCAACGCCTA-ACATGGTTGACTGTGTCTACAGT
p1  AAACAAGCCACCTCAACGCCTATACATGGTTGACTGTGTCTACAGT
p2  AAACAAGCCA-GTCA---------ACATGGTTGACTGTGTCTACAGT
p3  AAACAAGCCACCTCA---------ACATGGTTGACTGTGTCTACAGT
p4  AAACAAGCCACCTCA---------AC-------GCTGTGTCTACAGT
```

5-HT$_{2C}$RΔ1-SVG-A cells

first gRNA target sequence

```
WT  CGTCCATCATGCACCTCTGCGCTATATCGCTGGATCGGTATGTA
p1  CGTCCATCATGCACCTCTG--------CGCTGGATCGGTATGTA
```

B.

5-HT$_{2A/2B}$RΔ1-SVG-A cells

first gRNA target sequence

```
2A WT  GCCATCTCGCTGGACCGCTACGTCGCCATCCAGAATCCCATCC
2A_p1  GCCATCTCGCTGGACCGCC--------ATCCAGAATCCCATCC
2A_p2  GCCATCTCGCTGGACCGCT--------ATCCAGAATCCCATCC
```

C.

5-HT$_{2A/2B/2C}$RΔ1-SVG-A cells

first gRNA target sequence

```
2A WT  CGGCCTCCATCATGCACCTCTGCGCCATCTCGCTGGACCGCTACGTCGCCATCCAGAATCCC
2A_p1  CGGCCTCCATCATGCACCTCTGCGCCATCTCGCTGG----------CGCCATCCAGAATCCC
2A_p2  CGGCC----------------------------------------------TCCAGAATCCC
```

first gRNA target sequence

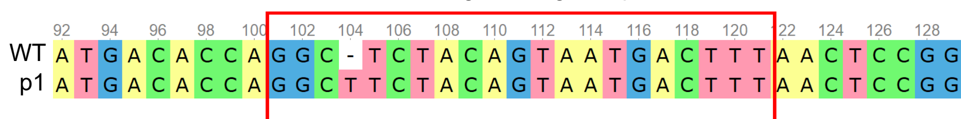
```
2C WT  CGTCCATCATGCACCTCTGCGCTATATCGCTGGATCGGTATGTA
2C_p1  CGTCCATCATGCACCTCTG--------CGCTGGATCGGTATGTA
```

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$_{2A}$RΔ1-SVG-A cells, 5-HT$_{2B}$RΔ1-SVG-A cells and 5-HT$_{2C}$RΔ1-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$_{2B}$RΔ1-SVG-A cell line was used as starting line to generate 5-HT$_{2A/2B}$RΔ1-SVG-A cells and 5-HT$_{2A/2B/2C}$RΔ1-SVG-A cells.

A.

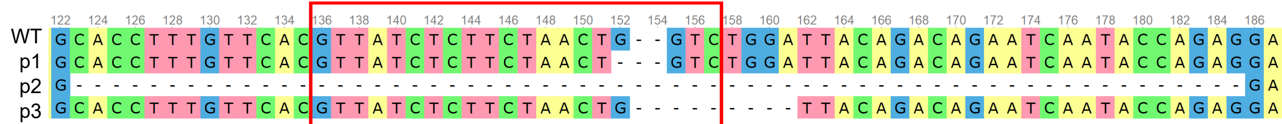
5-HT$_{2A}$RΔ2-SVG-A cells

second gRNA target sequence



5-HT$_{2B}$RΔ2-SVG-A cells

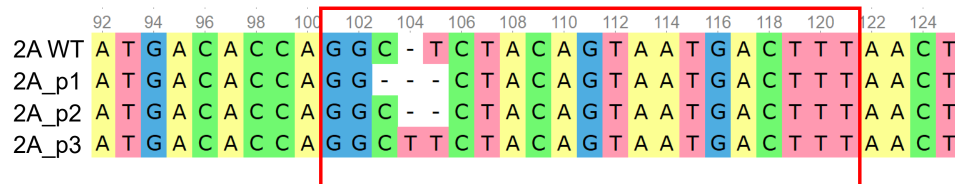
second gRNA target sequence



B.

5-HT$_{2A/2B/2C}$RΔ2-SVG-A cells

second gRNA target sequence



first gRNA target sequence

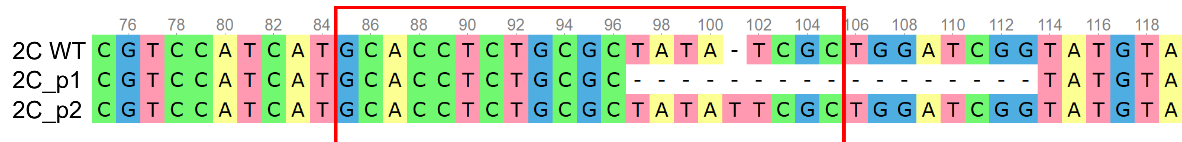


Figure S2. Genetic modification in single and triple mutant cell clones using custom designed gRNAs, related to Figure 3. Single cell isolation and expansion was performed. The genome editing was confirmed using the CRISPR deep sequencing service performed at the Center for Computational and Integrative Biology (CCIB) DNA core at the Massachusetts General Hospital (MGH). (A) Sequence alignments of 5-HT$_{2A}$RΔ2-SVG-A cells and 5-HT$_{2B}$RΔ2-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$_{2A/2B/2C}$RΔ2-SVG-A cells custom designed gRNAs were used to target 5-HT$_{2A}$R and 5-HT$_{2B}$R and the gRNA obtained from the GeCKO v2 library was used to target 5-HT$_{2C}$R. 5-HT$_{2B}$RΔ2-SVG-A cell line was used as starting line to generate 5-HT$_{2A/2B/2C}$RΔ2-SVG-A cells.

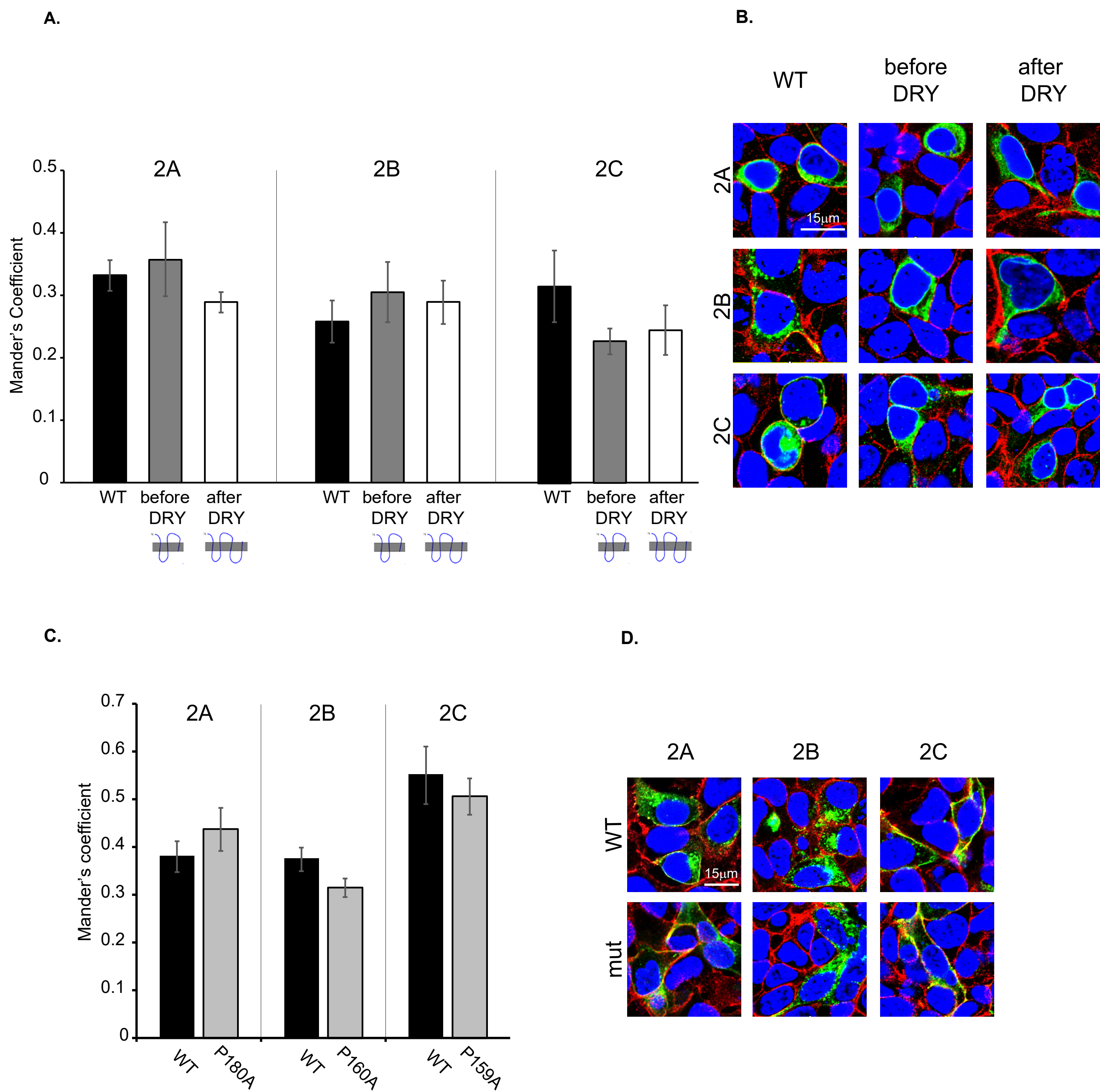


Figure S3. WT and mutant receptors are equally expressed at the cell surface, related to Figure 4. (A) Constructs expressing 5-HT_{2A}R, 5-HT_{2B}R and 5-HT_{2C}R were truncated before the DRY (before DRY) or after the DRY motif (after DRY) and a His tag was added at this site. A His tag was also added 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.