Supplementary Information

5-Hydroxytryptamine 6 receptor (5-HT_6R)-mediated morphological changes via RhoA-dependent pathways

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Supplementary methods

HEK293 cell morphometry

HEK293 cells were seeded on coverslips coated with 1 mg/ml poly-D-lysine. 24 h after transfection, cells were fixed using 4% paraformaldehyde/4% sucrose in PBS for 15 min, washed three times with PBS, and mounted onto microscope slides for confocal imaging and subsequent analysis. For quantification of the cell area in Supplementary Fig. 1 and 2, cell-filled mCherry fluorescence of all images was equally scaled, and individual cell areas were measured using the Metamorph software program.

cAMP activity assay

HEK293 cells coexpressing the cAMP biosensor GloSensor-22F (Promega) and EGFP-, 5-HT_6R-EGFP and 5-HT_6R-ΔCT-EGFP, respectively, were seeded (30,000 ~ 40,000 cells/20 μL/well) into white, clear-bottom, tissue culture plates in DMEM with 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. After a 5- to 6 h recovery, medium was removed from the wells and cells were treated with 3% Glosensor cAMP reagent, luciferin (Promega) 20 μL in filter-sterilized assay buffer including 1X HBSS, 20 mM HEPES and 3rd distilled water, pH 7.4. Then, 10 μL of 3× 5-HT or reference drug, isoproperenol, prepared in assay buffer with 0.1% bovine serum albumin (BSA). After 30 min, cells were treated with 10 μL of drugs prepared above. The luminescence intensity of the accumulated cAMP level was measured by using SpectraMax® i3 (Molecular Devices). The sigmoidal dose-response graph was obtained by using the Prism 6.0 program (GraphPad Software).

Supplementary figures legend

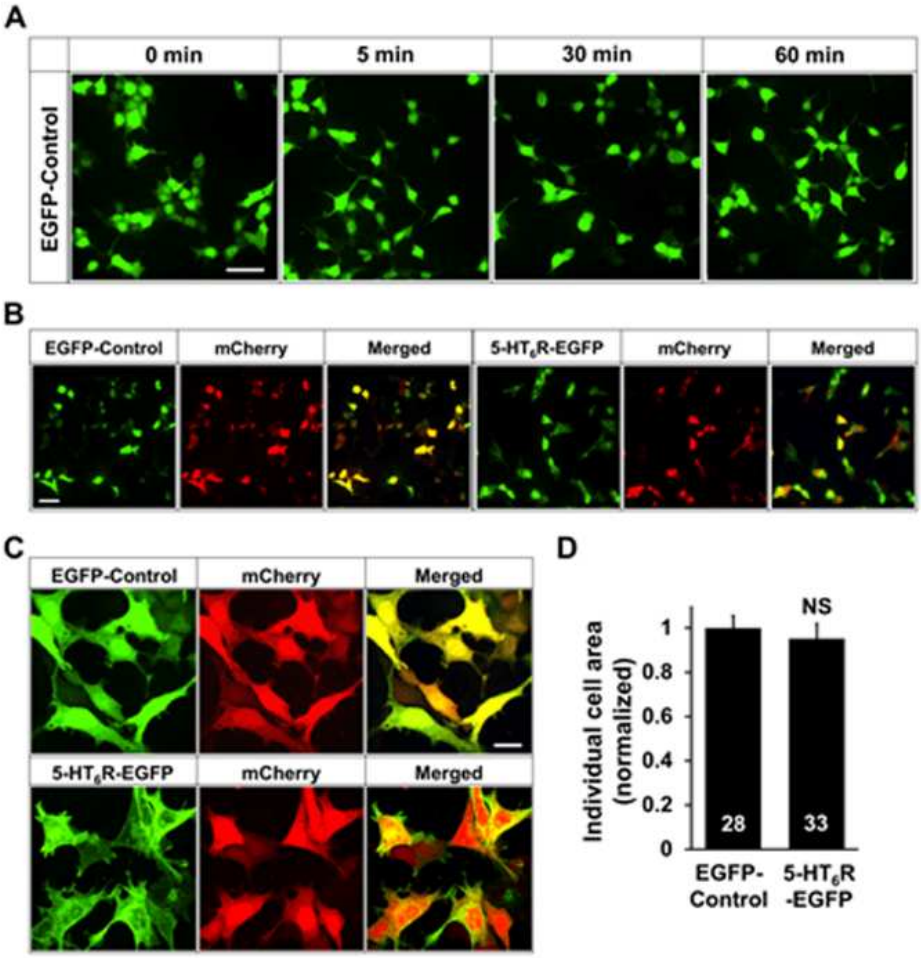
Figure S1. Cell morphology of HEK293 cells expressing EGFP does not react to 5-HT. (A) HEK293 cells expressing EGFP were treated with 5-HT (1 μM) for 0, 5, 30 and 60 min. Scale bar, 50 μm. (B, C) Overexpression of 5-HT_6R-EGFP does not change the cell area in HEK293 cells. EGFP or 5-HT_6R-EGFP together with mCherry was transfected and expressed for 24 h in HEK293 cells prior to imaging. Scale bars, 50 μm in (B) and 20 μm in (C). (D) Individual cell area was analyzed from (C). n = 28 and 33 cells for EGFP control and 5-HT_6R-EGFP samples, respectively. NS, not significant, P = 0.59, student t-test.

Figure S2. Cell morphology of HEK293 cells expressing 5-HT_6R-EGFP does not change to SB258585, a selective antagonist of 5-HT_6Rs. (A) HEK293 cells expressing 5-HT_6R-EGFP were treated with SB258585 (20 µM) for 15 min. Scale bar, 50 µm. (B) Overexpression of 5-HT_6R-EGFP does not change the cell area in HEK293 cells. 5-HT_6R-EGFP together with mCherry was transfected and expressed for 24 h in HEK293 cells prior to imaging. Scale bars, 20 µm. (C) Individual cell area was analyzed from n = 28 and 29 cells for 5-HT_6R-EGFP expressing cells in the absence or presence of SB258585, respectively. NS, not significant, P = 0.59, student t-test.

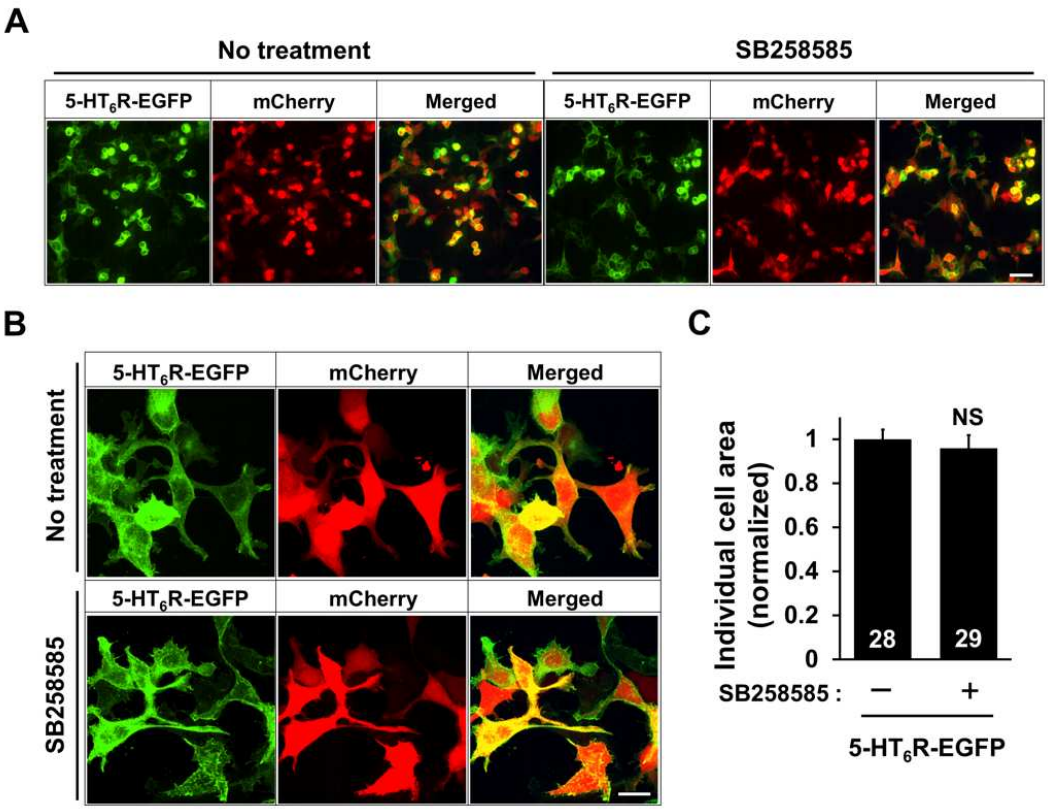
Figure S3. cAMP production was inhibited by ΔCT mutant truncated form. HEK293 cells coexpressing the cAMP biosensor GloSensor-22F and EGFP-control, 5-HT_6R-EGFP and 5-HT_6R-ΔCT-EGFP, respectively, were seeded into tissue culture plates in DMEM. The luminescence intensity of the accumulated cAMP level was measured and the sigmoidal dose-response graph was obtained.

Supplementary figures

Supplementary figure S1



Supplementary figure S2



Supplementary figure S3