

Supplementary Material

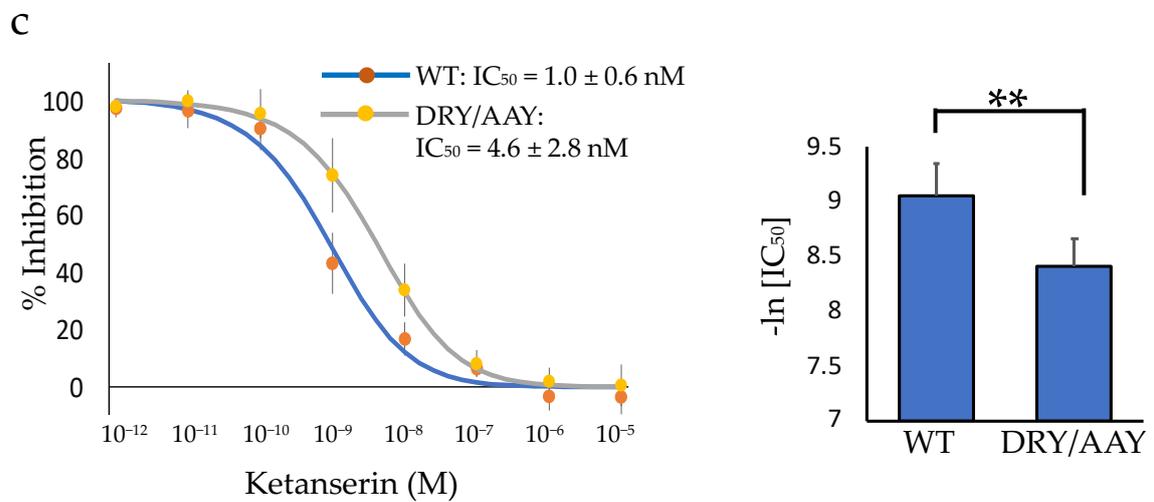
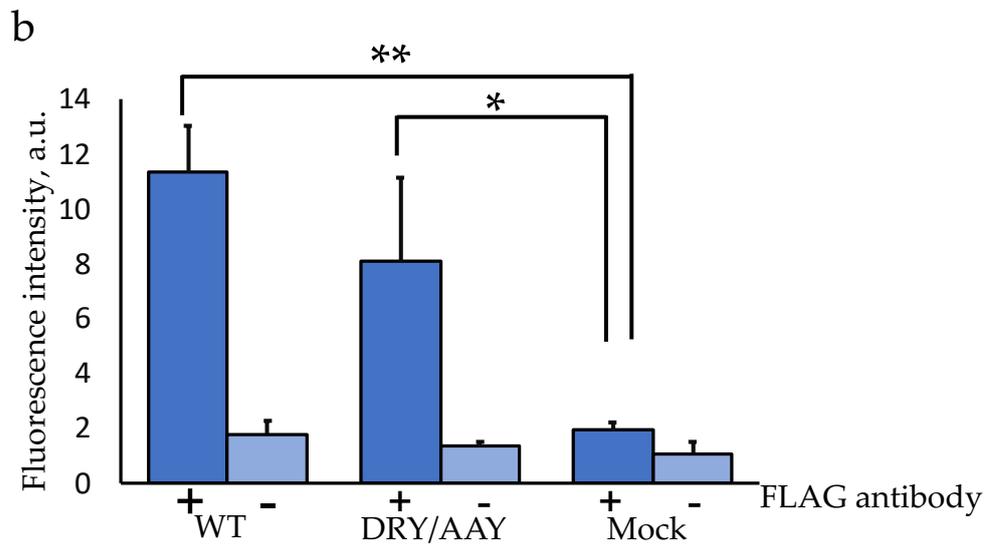
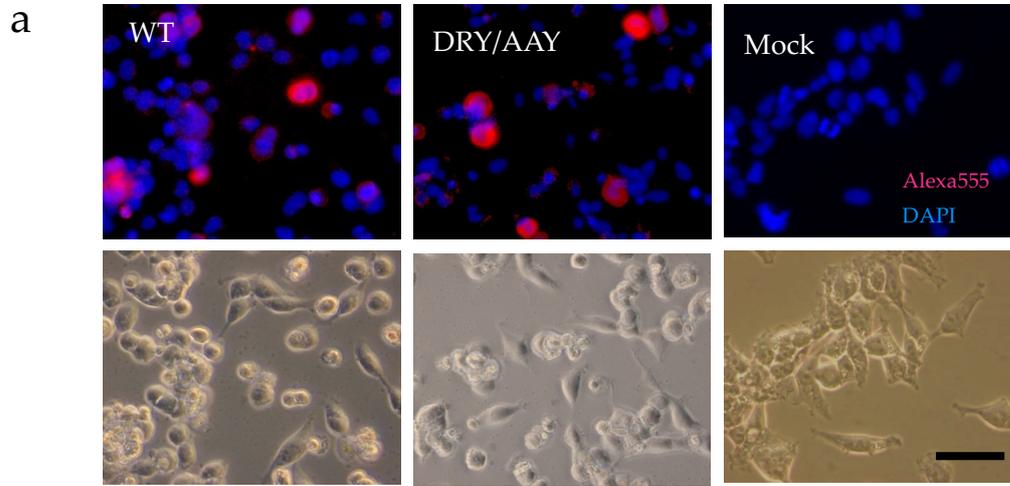


Figure S1. Expression and function of 5-HT_{2A}R used in this experiment. (a) Expression of N-FLAG 5-HT_{2A} receptors (WT) and DRY/AAY in HEK293 cells was confirmed by immunocytochemical studies using anti-FLAG antibodies. The cells were stained with monoclonal anti-FLAG M2 antibody (Sigma-Aldrich: F1804) followed by Alexa Fluor 555 anti-mouse IgG (Thermofisher: A31622). Both WT and DRY/AAY expressing cells were positive for FLAG antibodies. About 40% of the cells were positive for Alexa555 in this transient expression, while no positive cells were observed in the mock transfection. Lower panels: corresponding bright-field images. Scale bar, 50µm. (b) Live-cell based immunofluorescence assay. Living cells in suspension were labelled with anti-FLAG M2 antibody, followed by Alexa Fluor 488-conjugated secondary antibody (Thermofisher: A11001, Ex/Em = 493/519 nm). The fluorescence intensities were measured with a 96 well plate using the FlexStation 3 system. Both the intensities of WT and DRY/AAY were significantly higher than those of mock transfected cells, suggesting the expression of 5-HT_{2A}R on the cell surface. Experiments without the primary FLAG antibody were presented as a negative control. Data are presented as mean values ± SD (standard deviation) from four different experiments. Two-tailed paired Student *t*-test *p*-values indicate statistical significance (**p* < 0.05 and ***p* < 0.01). (c) Inhibition of α-MS induced calcium influx by ketanserin. HEK 293 cells transfected with WT or DRY/AAY were pretreated with ketanserin at 10⁻⁵ – 10⁻¹² M, then calcium influx by 10µM α-MS was measured using the FlexStation 3 system with the FLIPR Calcium 6 reagent (Molecular Devices Inc.). The IC₅₀ were calculated to be 1.0 ± 0.6 nM for WT, and 4.6 ± 2.8 nM for DRY/AAY, respectively. Data are presented as mean ± SD from five different experiments. Right panel; concentration of ketanserin at IC₅₀ was analyzed by the Student *t*-test (***p* < 0.01).

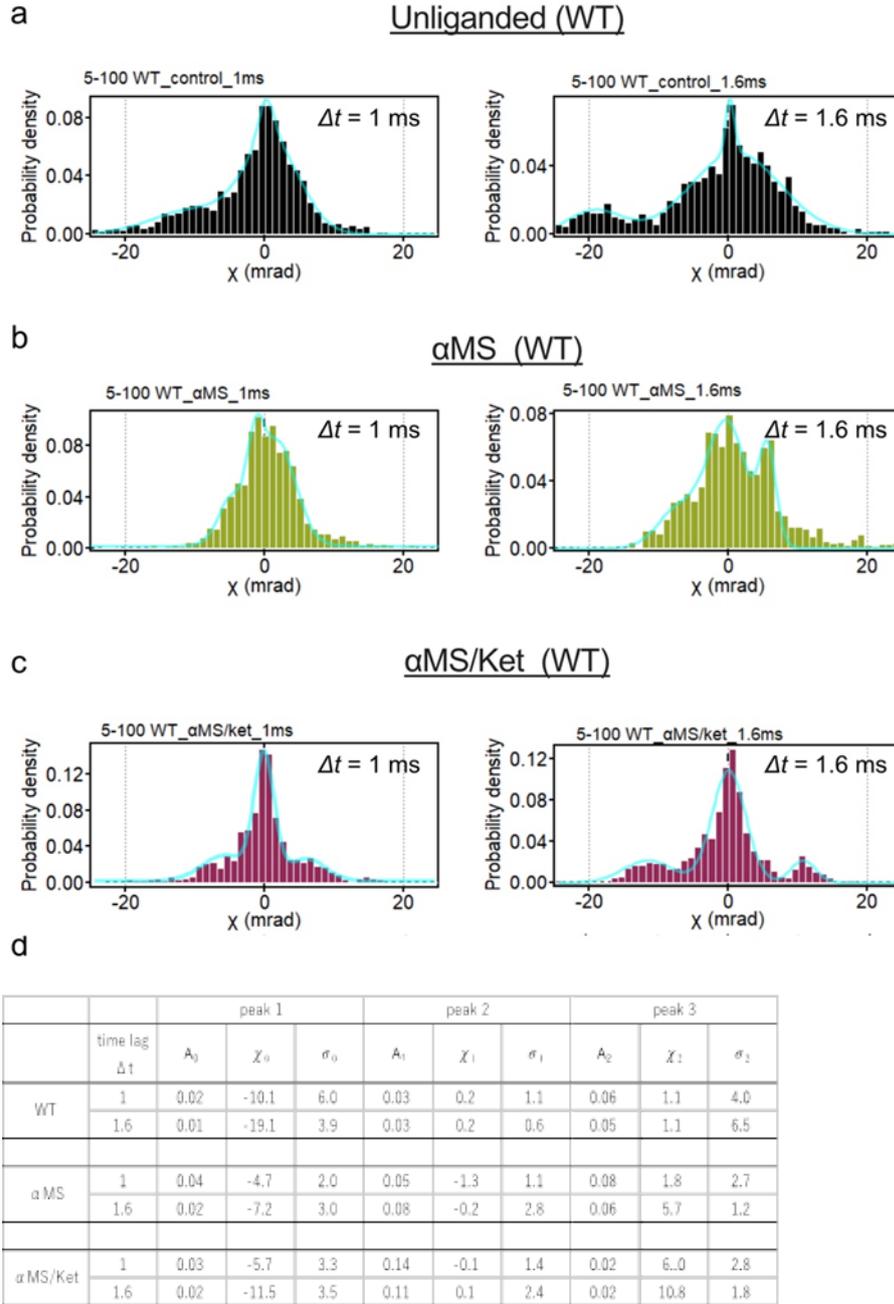


Figure S2. Three Gaussian curve fitting of distribution of angular displacement for wild type 5-HT_{2A}R. **(a–c)** Distribution of the absolute angular displacement of the wild-type 5-HT_{2A}R over an interval time t (left panels, $\Delta t = 1$ ms, and right panels, $\Delta t = 1.6$ ms) fitted by three Gaussian curves. **(d)** Statistical parameters for fitted curves. Curves were fitted with the following equation, where A represents amplitude, χ represents angular displacement, and σ represents standard deviation.

$$f(\chi) = A_0 \exp\left(-\frac{(\chi - \chi_0)^2}{2\sigma_0^2}\right) + A_1 \exp\left(-\frac{(\chi - \chi_1)^2}{2\sigma_1^2}\right) + A_2 \exp\left(-\frac{(\chi - \chi_2)^2}{2\sigma_2^2}\right)$$

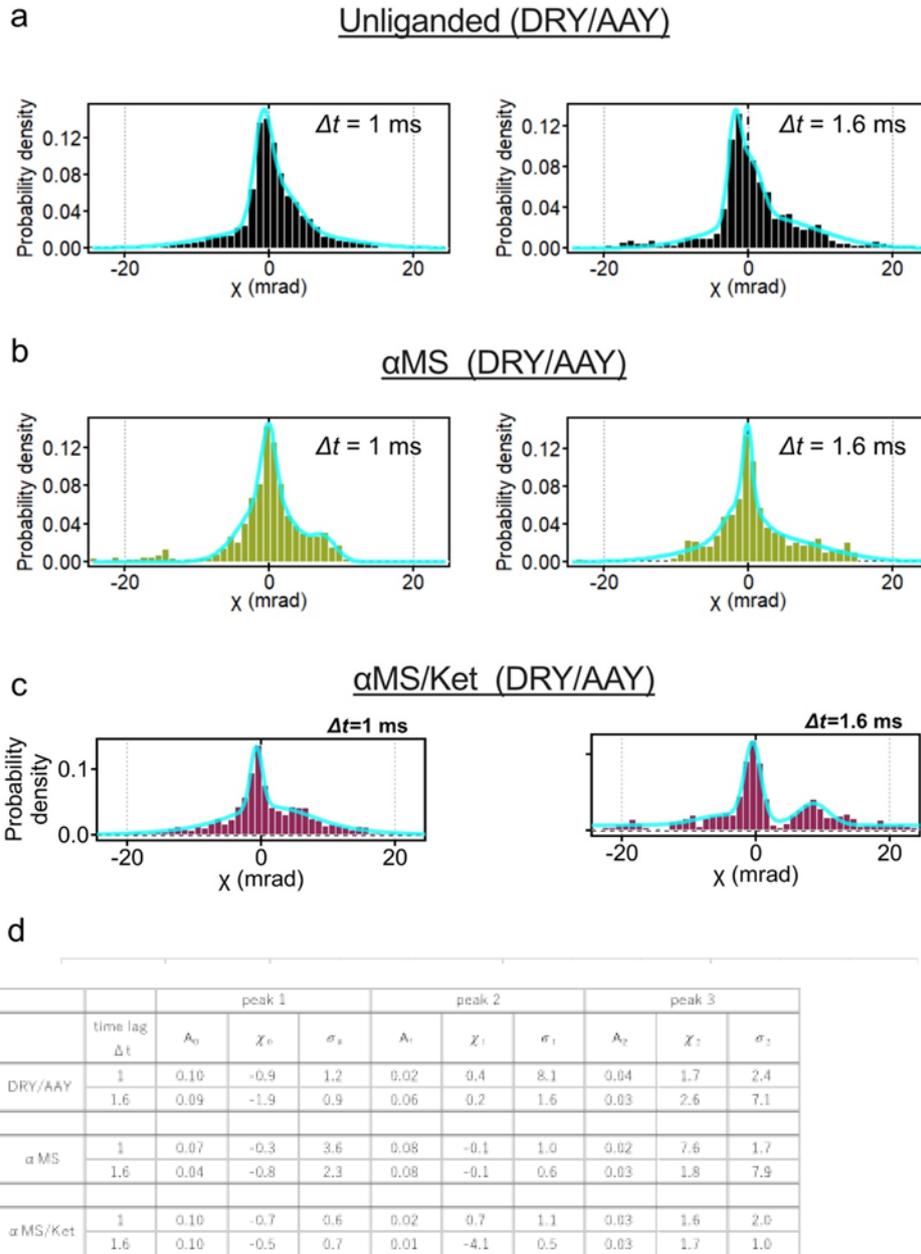


Figure S3. Three Gaussian curve fitting of distribution of angular displacement for DRY/AAY mutant 5-HT_{2A}R. (a–c) Distribution of the absolute angular displacement of the DRY/AAY mutant 5-HT_{2A}R over an interval time t (left panels, $\Delta t = 1$ ms, and right panels, $\Delta t = 1.6$ ms) fitted by three Gaussian curves. (d) Statistical parameters for fitted curves.

Table S1. Parameters of MSD curves for wild type 5-HT_{2A}R. The MSD curves were fitted by the following function: $\delta^2(t) = D_\alpha t^\alpha + 2\beta^2$ on the range of 0–4 ms. D_α is the anomalous diffusion constant, a non-linear relationship to time, α called subdiffusion ($1 > \alpha > 0$) or superdiffusion ($\alpha > 1$) and β called measurement error.

<u>WT theta</u>			
	Ligand-free	α MS	α MS/ket
D_α (mrad ² /ms)	5.57	3.31	2.64
α	0.99	0.84	0.46
β^2 (mrad ²)	0.003	-0.001	0.003

<u>WT kai</u>			
	Ligand-free	α MS	α MS/ket
D_α (mrad ² /ms)	66.6	32.2	37.8
α	1.67	1.47	0.97
β^2 (mrad ²)	0.889	1.016	0.002

Table S2. Parameters for single Gaussian fitting. The probability density was fitted by the Gaussian function: $f(x) = a \exp(-(x-\mu)^2/2\sigma^2)$.

<u>WT</u>				
	Time interval (ms)	a	μ	σ^2
Ligand free	1	0.08	0.54	2.6
	1.6	0.05	0.98	4.2
α -MS	1	0.10	0.08	2.7
	1.6	0.07	0.36	4.0
α -MS/ket	1	0.14	-0.18	1.3
	1.6	0.11	0.05	1.7

<u>DRY/AAV</u>				
	Time interval (ms)	a	μ	σ^2
Ligand free	1	0.13	-0.14	1.8
	1.6	0.11	-0.64	1.8
α -MS	1	0.12	-0.20	1.6
	1.6	0.10	-0.30	1.9