

Supplemental Information

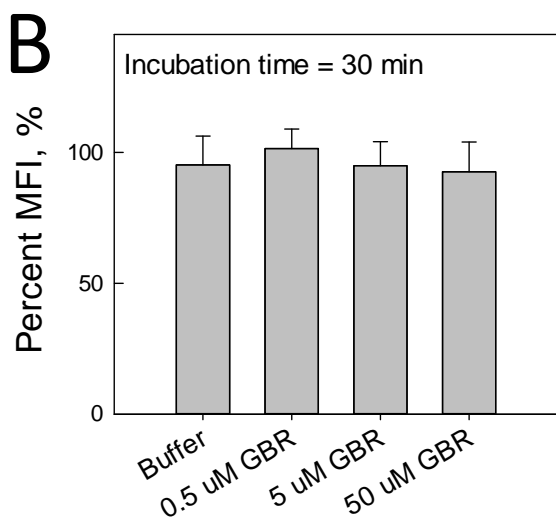
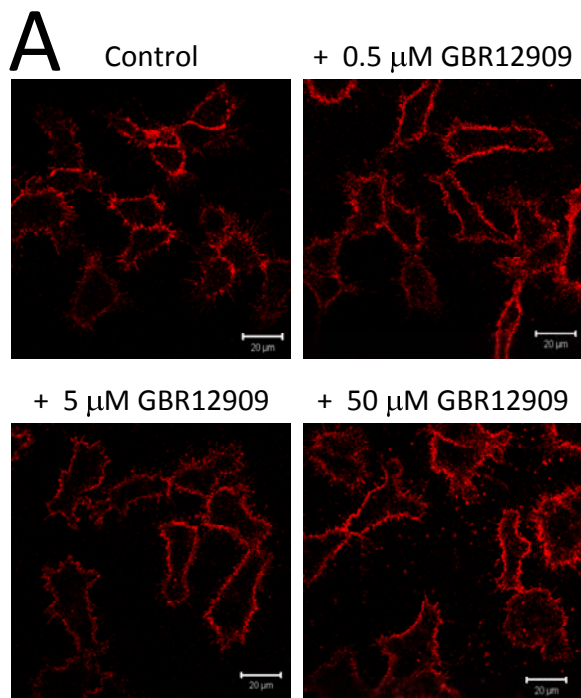


Figure S1. Temporal stability of QD-IDT444 conjugate binding to DAT in FIp-In-HEK-293 cells. (A) Confocal images were obtained at least 30 min after incubation with either imaging buffer or 30-min incubation with GBR12909. Images are representative of two independent experiments. (B) Bar graph of median fluorescence intensity (MFI) of QD-labeled DAT-expressing HEK cells was obtained by normalizing MFI to control cell population that was assayed for fluorescence immediately after QD labeling (n = 3 independent experiments).

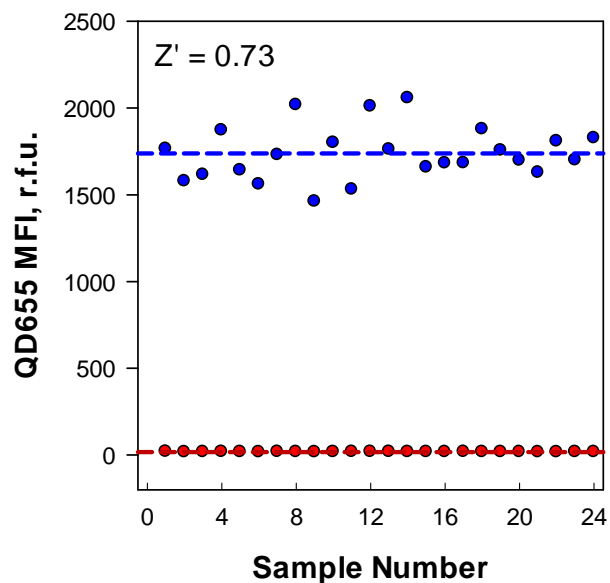


Figure S2. Statistical evaluation of assay quality and performance using negative (red) and positive (blue) control data. Mean MFI is indicated by the dashed line.

Experimental

Materials. Streptavidin-conjugated quantum dots with a maximum fluorescence emission at 655 nm (SavQdots655) were obtained as a 1 μ M solution dissolved in borate buffer at pH 8.5 from Invitrogen (Carlsbad, CA). GBR12909 was obtained from Tocris Bioscience (Ellisville, MI). The IDT444 ligand was synthesized as previously described.

Cell Line Maintenance. The Flp-In 293 host cell line (Invitrogen) was obtained from Dr. Randy Blakely's lab (Vanderbilt University) and was grown in complete medium (D-MEM with 2 mM L-glutamine, 10% FBS, 1% pen/strep) supplemented with 100 μ g/mL Hygromycin B in a 37°C incubator with 5% CO₂. The wild-type human DAT (hDAT) cDNA cloned in the pcDNA5/FRT expression vector was transfected into Flp-In-293 cells using the Fugene 6 transfection reagent (Roche, NJ). After 48 h recovery, the cells were grown in medium with 100 μ g/ml hygromycin B (100 μ g/ml) added for several weeks to select for resistant cells where the cDNA construct had been recombined into the Flp-In site in the Flp-In-293 cells. Prior to fluorescent imaging and flow cytometry experiments, cells were seeded in 24-well polylysine-coated culture plates (BD Biosciences, Bedford, MA).

Confocal Microscopy. HEK cells were seeded in 8-well chamber slides (Nalge Nunc, NY). Prior to confocal microscopy, the old medium was aspirated from the chamber slide wells, and cells were washed 3x with warm imaging buffer (Phenol Red-free DMEM/1%FBS). HeLa cells were then incubated with 100 nM IDT444, washed 3x with warm imaging buffer, incubated with 1 nM SavQdot655, washed 3x with warm imaging buffer, and visualized in 200 μ L of warm imaging buffer at 37°C. DIC and fluorescent images were acquired on the Zeiss LSM 510 inverted confocal microscope with the 488-nm excitation laser, a 650-nm long pass filter, and Zeiss Plan-Apo oil immersion objective (63X, NA 1.40).

Flow Cytometry. HEK cells grown in 24-well culture plates were washed several times with warm KRH buffer and then exposed to GBR12909 or PMA for 20 minutes at 37°C and 5% CO₂. Cells were then washed several times with cold KRH buffer and then incubated at RT for 10 minutes in presence of 100 nM IDT444/GBR12909 mixture or 100 nM IDT444 only respectively. After another round of triplicate washes with KRH buffer, HEK cells were incubated with 1 nM SavQD/1%BSA mixture at RT for 5 minutes. Following QD labeling step, HEK cells were nonenzymatically dissociated using CellStripper (Mediatech Inc., Manassas, VA) and subsequently assayed for fluorescence via flow cytometry. Samples were analyzed on a BD LSRII flow cytometer using either polystyrene disposable tubes or a BD HTS Cube attachment (BD Biosciences). Qdot655 fluorescence was detected with the 488-nm excitation laser on the FL3 channel (640-nm long pass filter). Forward (FSC) and side scatter (SSC) data were collected in linear mode, while the FL3 channel data were collected in log mode. Ten thousand events were collected per sample. FSC and SSC measurements were used to gate the viable cell region to assay for Qdot fluorescence. Median fluorescence intensity (MFI) values were then determined for the gated cell population using Flow Jo (Tree Star, Ashland, OR).

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