Supplementary Information

IDT444 Synthesis.

N-Demethylation. (1R,2S,3R,5S)-methyl 3-(4-fluorophenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (2a) and (1R,2S,3S,5S)-methyl 3-(4-fluorophenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (2b). A mixture of the diasterioisomers of (1R,2S,3R,5S)-methyl 3-(4-fluorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate **(1a)** and (1R,2S,3S,5S)-methyl 3-(4-fluorophenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate **(1b)** was prepared using the method described by Meltzer *et al*.[1] This mixture was confirmed to be a 50:50 mixture by GC-MS. Then (0.77g, 2.78mmol) of this mixture was suspended in 1-chloroethyl chloroformate (ACE-Cl; 11ml, 101mmol) under nitrogen and heated to reflux at 135°C for 2 h. The excess ACE-Cl was evaporated, and methanol (25ml) was added to the residue. The reaction mixture was heated to reflux for 1 h and then concentrated to dryness. The residue was diluted with dichloromethane (140ml), washed with saturated $NaHCO_3$ (50ml), dried over $MgSO_4$, filtered and concentrated to dryness to afford a racemic mixture of **(2a)** and **(2b)**, which was purified by flash chromatography (EtOAc 75%, hexanes 20%, Et_3N 5%) to provide the racemic mixture as a colourless waxy solid (0.64g, 87%). *Rf* = 0.15 (EtOAc 75%, hexanes 20%, Et_3N 5%). ^1H NMR ($CDCl_3$) δ 7.21-6.88 (m, 4 ArH), 3.64-4.49 (m, 4H), 3.31 (s, 1H), 3.18-2.99 (m, 1H), 2.36-2.16 (m, 3H), 2.02-1.95 (m, 1H), 1.88-1.51 (m, 3H), 1.22 (t, 1H).

(1R,2S,3R,5S)-methyl 8-(11-(1,3-dioxoisoindolin-2-yl)undecyl)-3-(4-fluorophenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (4a) and (1R,2S,3S,5S)-methyl 8-(11-(1,3-dioxoisoindolin-2-yl)undecyl)-3-(4-fluorophenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (4b). A mixture of the diasterioisomers of **(2a)** and **(2b)** (0.22g, 0.82 mmol) was added to

acetonitrile (100ml) containing potassium iodide (0.18g, 1,1 mmol), potassium carbonate (0.61g, 4.4mmol) and 2-(6-bromoundecyl)isoindoline-1,3-dione **(3)** (0.35g, 1.1 mmol). The reaction mixture was heated to reflux under nitrogen for 2h then concentrated to dryness. The product was purified by flash chromatography (EtOAc 40%, Hexanes 60%) to provide the racemic mixture (0.15g, 54.3%) as a colourless oil (0.218g, 47%). *Rf* = 0.38 (EtOAc 40%, hexanes 60%). ^{1}H NMR ($CDCl_3$) δ 7.77-7.74 (m, 2 ArH), 7.64-7.61 (m, 2 ArH), 7.12-7.09 (m, 2 ArH), 6.88-6.82 (m, 2 ArH), 3.60 (t, 2H) 3.50 (s, 3H), 3.38-3.20 (m, 3H), 2.40- 2.27 (m, 1H), 2.16-2.09 (m, 1H), 2.06-1.88 (m, 4H), 1.61 (t, 2H), 1.49-1.19 (m, 19H).

(1R,2S,3R,5S)-methyl 8-(11-aminoundecyl)-3-(4-fluorophenyl)-8- azabicyclo[3.2.1]octane-2-carboxylate (5a) and (1R,2S,3S,5S)-methyl 8-(11-aminoundecyl)-3-(4-fluorophenyl)-8-azabicyclo[3.2.1]octane-2-carboxylate (5b). A mixture of the diasterioisomers of **(4a)** and **(4b)** (0.074g, 0.13 mmol) was dissolved in ethanol (20ml) and hydrazine monohydrate (2ml) was added. The solution was stirred at ambient temperature for 1 h then evaporated under reduced pressure. The residue was added to methylene chloride (100ml) and stirred at ambient temperature for 24h. It was then filtered and evaporated to yield a mixture of **(5a)** and **(5b)** as a a colourless oil (0.056g, 99%) and was used without further purification. ^{1}H NMR ($CDCl_3$) δ 7.13-7.08 (m, 2 ArH), 6.89-6.83 (m, 2 ArH), 3.59-3.53 (m, 2H), 3.50 (s, 3H), 3.39-3.24 (m, 3H), 2.60 (t, 2H), 2.41-2.27 (m, 2H), 2.15 (t, 2H), 2.09-1.85 (m, 2H), 1.53-1.21 (m, 21H).

Attachment of (5a) and (5b) to NHS-PEG5000-Biotin. A mixture of **(5a)** and **(5b)** (0.056g, 0.1 mmol) was dissolved in dry methylene chloride (20ml) and NHS-PEG5000-Biotin (0.286g) was added to the solution. This solution was stirred at ambient temperature for 24h and then it was evaporated to dryness. The resulting residue was washed with diethyl ether (4 x 100ml) to yield 0.29g of IDT444 **(6)** as a colourless solid. The product was further purified from unreacted

intermediate (**5a-b**) by size-exclusion chromatography using a PD-10 desalting column (GE Biosciences). The final product was characterized by MALDI-TOF mass spectrometry (Figure S1).[2] MALDI-TOF mass spectrometry was performed on a Voyager-DE STR spectrometer (Applied Biosystems) operating in the reflection mode at 20.00 kV. As matrix, α-cyano-4-hydroxycinnamic acid (10 mg/mL in methanol) was used. Calibration was performed using a standard of linear PEGs in THF (10 mg/mL). Polymeric samples were dissolved in methanol (5 mg/mL) and added to the matrix solution (1 μL each). After 1 μL of potassium chloride solution (1 mg/mL) was added, the mixture was placed on the stainless steel sample probe and evaporated under vacuum. We expected the mass distribution of the polydisperse starting Biotin-PEG5000-NHS material to shift to the right by net ~316 Da, a result of NHS moiety loss (-115 Da) and **(5a-b)** intermediate addition (+431Da). As expected, the mass shift is clearly demonstrated in Figure S1.

Flow Cytometry Gating and Analysis.

Qdot-labeled cells were analyzed on a BD LSRII flow cytometer (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 was collected in log mode. Twenty thousand events were collected per sample. FSC and SSC measurements were used to gate the viable cell region to assay for Qdot fluorescence. SSC-A versus FSC-A plot was used to gate out cellular debris appearing at low fluorescence intensity. SSC-W versus SSC-A and FSC-W versus FSC-A were used to discriminate single cells from doublets/aggregates (doublets and aggregates are typically characterized by wider SSC and FSC pulse width) (Figure S2, Middle). The gated cell population was then used to obtain

a histogram of cell count versus Qdot655 fluorescence intensity (Figure S2, Right). The median fluorescence intensity (MFI) was estimated (FlowJo) for each unimodal, symmetrical histogram as a more robust indicator of the central tendency of the population that is less affected by the outliers. Robust standard deviation (rSD) was calculated using the following equation (FlowJo manual):

$$\%rCV = \frac{rSD}{MFI} \times 100\%,$$

where rCV is robust coefficient of variation and is defined as follows (FlowJo manual):

$$\%rCV = 100\% \times \frac{1}{2} \times \frac{Intensity\ (84.13th\ percentile) - Intensity\ (15.87th\ percentile)}{MFI}.$$

In robust statistics, the data is less affected by the outliers, and analysis is a more reliable measure of the central tendencies of the population examined.

Labeling HeLa Cells with IDT444-SavQdot Conjugates in Solution.

A two-step labeling assay was utilized to specifically target DAT with the IDT444 ligand and SavQdots655. HeLa cells transiently transfected with DAT pcDNA3 were incubated in 24-well culture plates with a solution of the biotinylated ligand (IDT444) in PBS (Gibco) for 10 minutes at 37ºC. Then the contents of each well was pipetted, transferred into 1.5 mL microfuge tubes (Millipore, Billerica, MA), pelleted by centrifugation at 2000 rpm for 5 minutes, and resuspended in a solution of SavQdots655 in PBS for 5 minutes at 4ºC. Qdot-labeled cells were washed two times by centrifugation and resuspension in PBS at 4ºC, transferred to 5 mL polystyrene round-bottom tubes (BD Biosciences, Bedford, MA), and assayed for SavQdot655 labeling with flow cytometry and confocal microscopy. Parallel wells with mock-transfected HeLa cells and DAT pcDNA3-transfected HeLa cells preincubated with 1 μM GBR12909 were also assayed for Qdot fluorescence to determine the degree of nonspecific labeling. The results are

shown in Figure S3. The presence of a characteristic second peak at higher fluorescence intensity on the flow cytometry histrogram and membrane-associated fluorescent halos on the acquired confocal images indicated specific DAT labeling (Figure 4, Top row). The absence of the characterstic second peak and membrane Qdot labeling were observed for control cell populations indicating a low level of nonspecific binding.

References

1. Meltzer, P. C.; Blundell, P.; Zona, T.; Yang, L.; Huang, H.; Bonab, A. A.; Livni, E.; Fischman, A.; Madras, B. K., A Second-Generation 99mTechnetium Single Photon Emission Computed Tomography Agent That Provides in Vivo Images of the Dopamine Transporter in Primate Brain. *Journal of Medicinal Chemistry* **2003,** *46* (16), 3483-3496.

2. Riebeseel, K., E. Biedermann, et al. Polyethylene Glycol Conjugates of Methotrexate Varying in Their Molecular Weight from MW 750 to MW 40000: Synthesis, Characterization, and Structure-Activity Relationships in Vitro and in Vivo. *Bioconjugate Chemistry* **2002**, *13* (4), 773-785.

LEGEND

Figure S1. MALDI-TOF mass spectra of unconjugated Biotin-PEG5000-NHS (Bottom) and IDT444 (Top).

Figure S2. Flow cytometry gating strategy.

Figure S3. Specific SavQdot-IDT444 labeling of DAT transiently expressed in HeLa cells. Representative flow cytometry histograms and confocal images are shown for DAT-pcDNA3 transfected HeLa cells (Row **A**), DAT-pcDNA3 transfected HeLa cells pretreated with 1 μM GBR12909 (Row **B**), Sham-pcDNA3 transfected HeLa cells (Row **C**), and DAT-pcDNA3 transfected HeLa cells incubated with SavQdot only (Row **D**). All samples were incubated with 100 nM solution of IDT444 in PBS for 5 min at 37°C and subsequently exposed to 1 nM SavQdot in PBS for 5 min at 4°C.

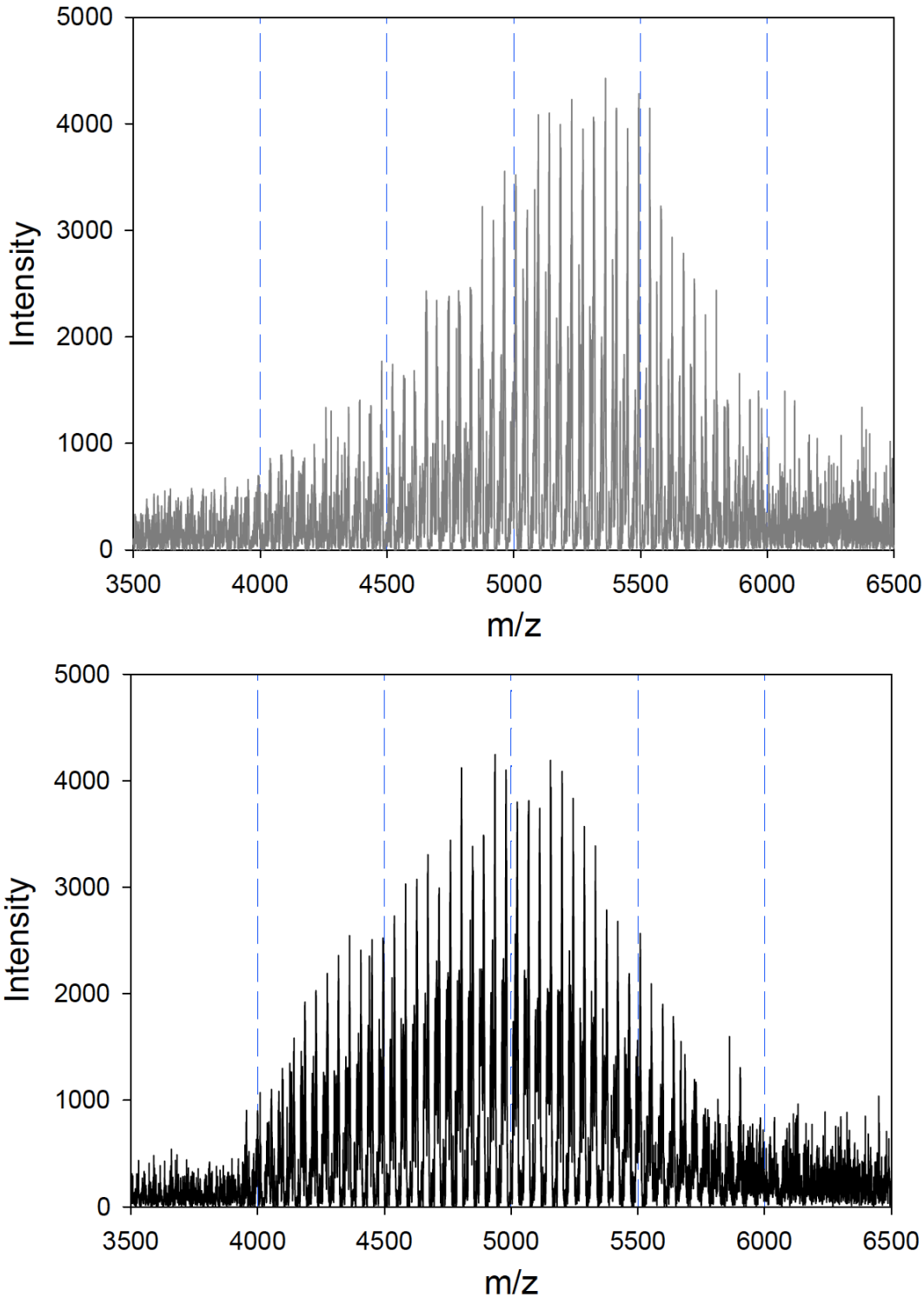
5000
4000
3000
2000
1000
0
Intensity
3500
4000
4500
5000
5500
6000
6500
m/z
5000
4000
3000
2000
1000
0
Intensity
3500
4000
4500
5000
5500
6000
6500
m/z

Figure S1.

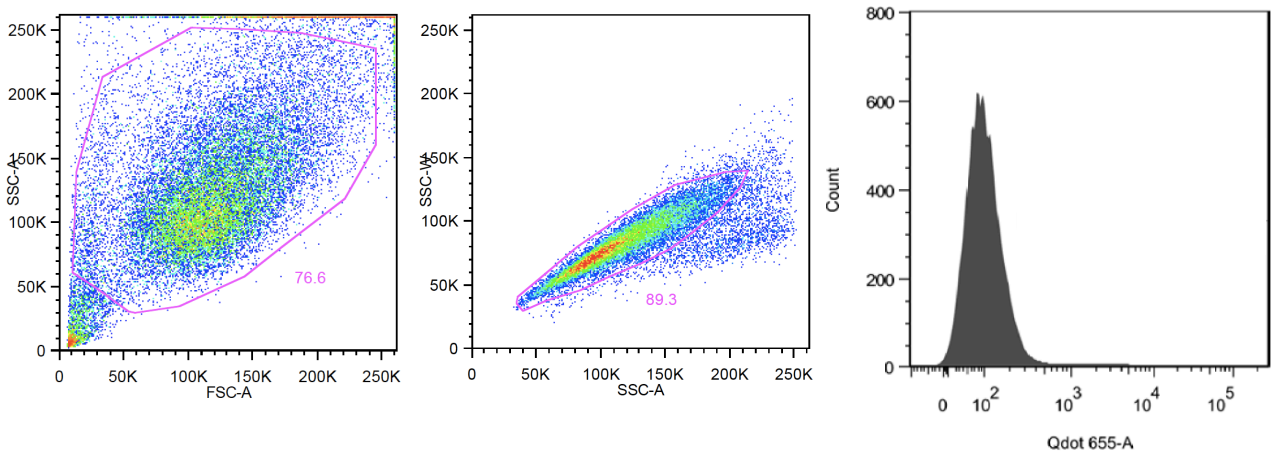
250K
200K
150K
100K
50K
0
SSC-A
0
50K
100K
150K
200K
250K
FSC-A
76.6
SSC-W
SSC-A
89.3
800
600
400
200
Count
10^2
10^3
10^4
10^5
Qdot 655-A

Figure S2.

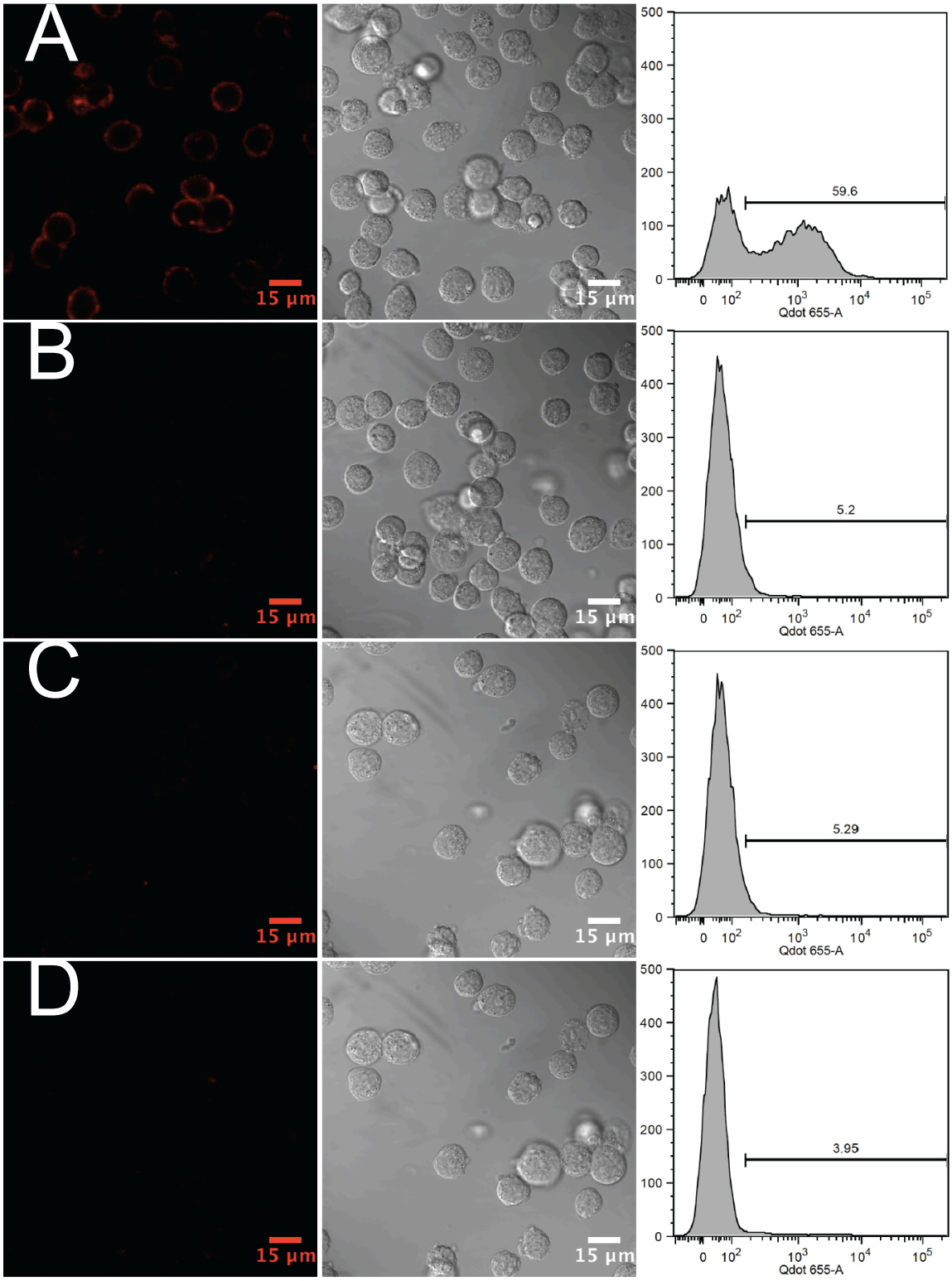

Figure S3.