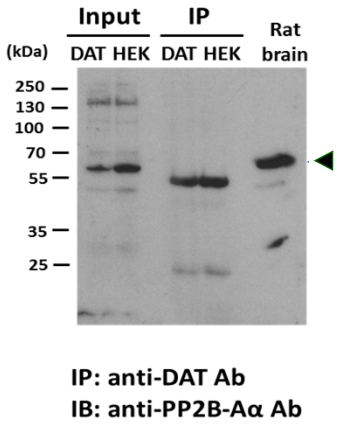

Figure S1. No evidence for interaction between DAT and PP2B in heterologous HEK293 cells. Representative immunoblot for PP2B catalytic Aα subunit (PP2B-Aα) with YFP-tagged hDAT stably expressed in HEK293 cells (DAT; YhDAT-HEK cells). Cell lysates (Input) were obtained from YhDAT-HEK (DAT) and parent HEK293 (HEK) cells and immunoprecipitated (IP) by an anti-DAT antibody. The absence of co-purified PP2B-Aα was observed in IP from both DAT and HEK samples (arrowhead). Rat brain synaptosomal membrane fraction (Rat brain) was subjected to SDS-PAGE to compare the expression of endogenous PP2B-Aα in HEK293 cells. The numbers refer to the positions of prestained molecular weight markers and images represent three experiments with the same results.

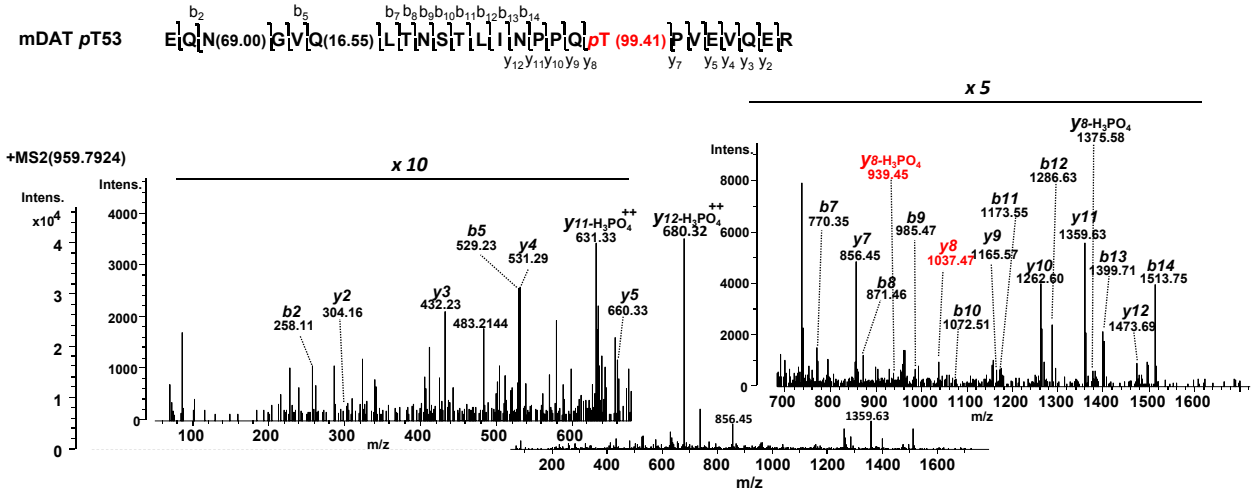

Figure S2. Identification of phosphorylation site at threonine 53 in DAT immunopurified from mouse striatum. The probabilities for the localization of phosphorylation (in red) and deamidation determined by the Mascot delta score are presented in parentheses.

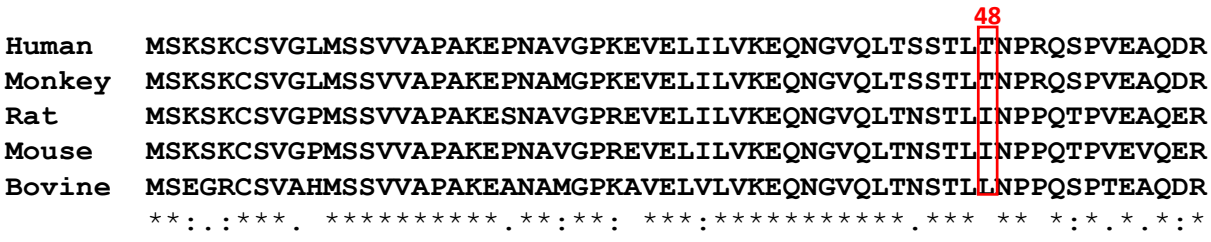

Figure S3. Alignment of DAT amino acid sequences from different species.

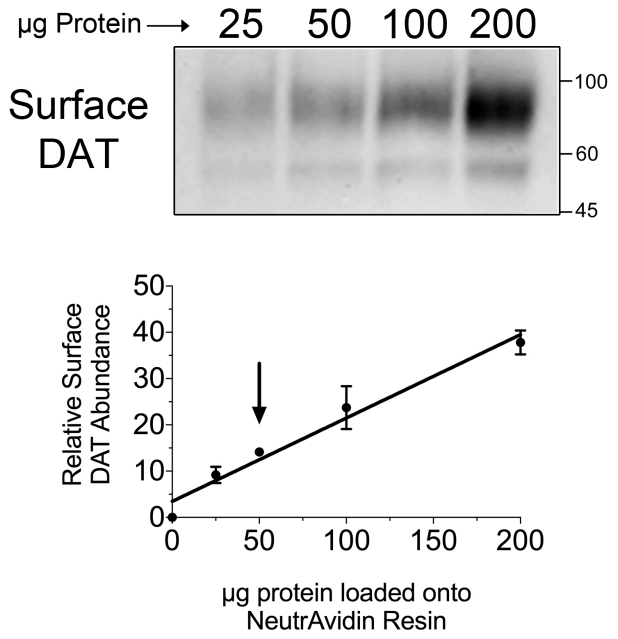

Figure S4. Validation of linear response in the cell surface biotinylation assay. LLC-PK_1 cells expressing WT DAT were subjected to cell surface biotinylation to assess surface levels of DAT. After the cell surface labelling procedure, cells were washed and lysed. Increasing amounts of the cell lysate (25-200 µg) were loaded on equal amounts of NeutrAvidin resin to assess binding capacity of the resin under our experimental conditions. A representative immunoblot of cell surface DAT is shown in the upper panel. Band intensities were quantified and data are shown as mean ± SD with linear regression analysis; n=3. The arrow indicates our standard protein load (50 µg) in our assay for assessing surface DAT levels.