Supplementary Figure 2. (A) D2S receptor activation inhibits forskolin-induced cAMP production. Cells co-transfected with the D2S and DAT, there is a significant decrease in cAMP production upon receptor stimulation with 10 μM quinpirole (30 min). (B) [3H]CFT binding in HEK-293 cells. The estimated whole-cell DAT levels, as indexed by the saturable binding of [3H] CFT, were not significantly different in DAT-expressing cells compared to cells co-transfected with the D2S (DAT/pcD: 52.3±5.5 fmol/10^5 cells; DAT/D2S: 54.2±4 fmol/10^5 cells; n=3; mean ± s.e.m). (C) Coexpression of the D2L variant with DAT in HEK-293 cells does not result in an increase in DA uptake. Control group exhibited a Vmax of 1.3 ± 0.19 pmol/10^5 cells/min, n=4. (D) Representative blots showing the coimmunoprecipitation of the D2 receptor from cells coexpressing DAT with either D2S or D2L reveals no significant difference between the two D2 receptor isoforms (one-way ANOVA, P=0.569, n=4). (E) Western blot of lysates used in the coimmunoprecipitation experiments reveal no change in DAT or D2 protein levels between samples. (F) Co-immunoprecipitation of DAT from HEK-293 cells coexpressing the D2 receptor treated with 1 μM PMA for 30 min at 37°C, revealed no significant difference in the D2-DAT interaction compared to control. Both blots in (A) and (B) depict a ~80 kDa DAT-immunoreactive band. (G) Pretreatment of striatal tissue slices with agonist (10 μM quinpirole: Quin), antagonist (1 μM raclopride: Rac) or both did not affect the co-immunoprecipitation of DAT from solubilized rat striatal tissue with the D2 receptor. Briefly, under deep anesthesia, rat brains were rapidly removed and coronal brain slices (400 μm thickness) containing striatum were cut using a vibrating blade microtome in ice-cold artificial cerebrospinal fluid (ACSF) containing 126 mM NaCl, 2.5 mM KCl, 1 mM MgCl₂, 1 mM CaCl₂, 1.25 mM KH₂PO₄, 26 mM NaHCO₃ and 20 glucose that was bubbled continuously with carbogen (95%O₂/5%CO₂) to adjust the pH to 7.4. Freshly cut slices were placed in an incubating chamber with carbogenated ACSF and recovered at 34°C for 1.5 hr. Slices were then treated with different either quinpirole, raclopride or both in carbogenated ACSF at 34°C for 0.5 hr.