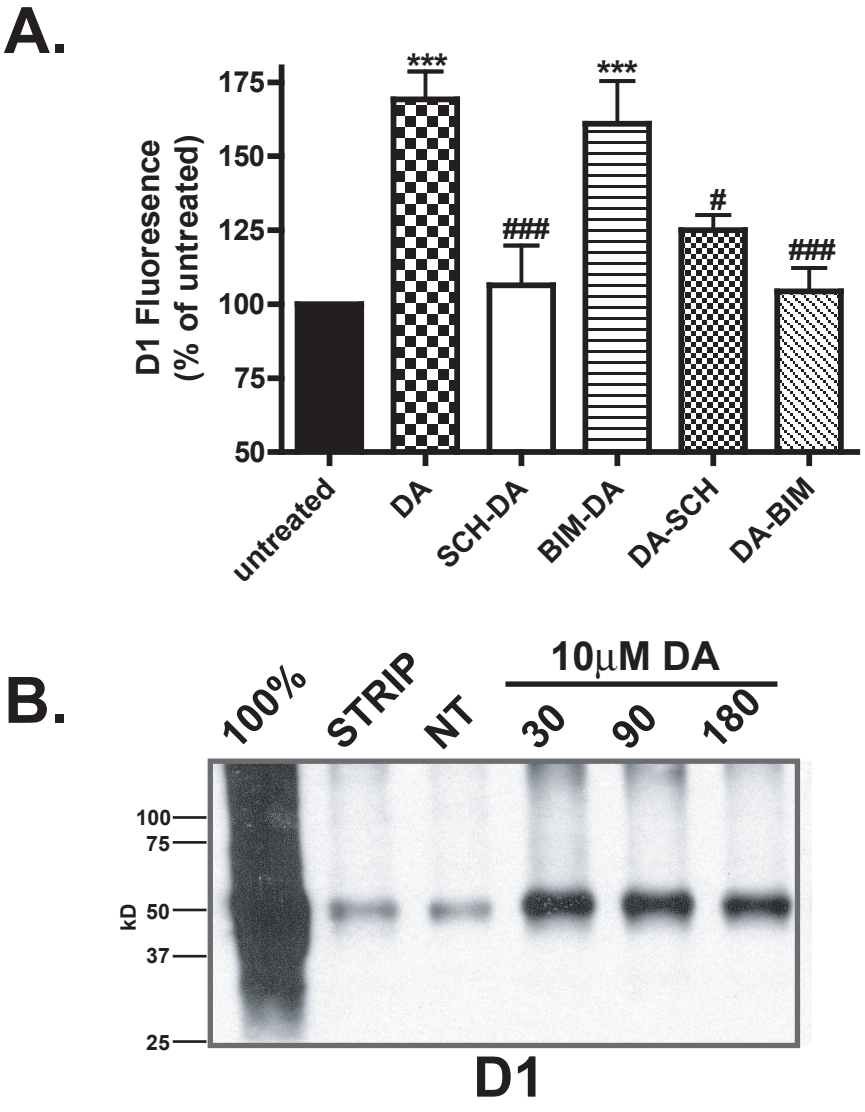
Supplemental Figure 1. The D1 dopamine receptor recycles.
(A) The recycling of the D1 dopamine receptor was analyzed using flow cytometry. Cells were labeled with anti-FLAG M1-conjugated Alexa 647 antibody for 20 minutes and were either left untreated or pretreated with antagonist (ant) (10 μM SCH23390) or PKC inhibitor (BIM 1 μM) for 30 minutes. Cells were then stimulated with dopamine (DA, 10 μM) for 45 minutes, washed in PBS/EDTA to remove antibody from any remaining cell surface receptors and either fixed to monitor degree of endocytosis (DA, ant-DA, BIM-DA) or returned to the incubator in the presence of PBS/EDTA containing either antagonist (DA-ant) or PKC inhibitor (DA-BIM) to monitor recycling. Data is represented as the mean of at least 4 independent experiments performed in duplicate analyzed using One-way ANOVA with Bonferroni t-test. *** $p \leq 0.001$ compared to untreated controls or ### $p \leq 0.001$, # $p \leq 0.05$ compared to DA treated samples. (B). HEK293 cells expressing FLAG-tagged D1 receptor were surface biotinylated and incubated in the absence (NT) or presence of DA (10 μM D4) for 30, 90 or 180 minutes. The fate of the surface labeled receptors that had been protected after endocytosis was assessed after receptor immunoprecipitation followed by SDS PAGE and streptavidin overlay. 100% lane shows total surface receptor labeled. Strip lane shows efficiency of thio cleavage. A representative immunoblot is shown.



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