Palmitoylation by multiple DHHC enzymes enhances dopamine transporter function and stability

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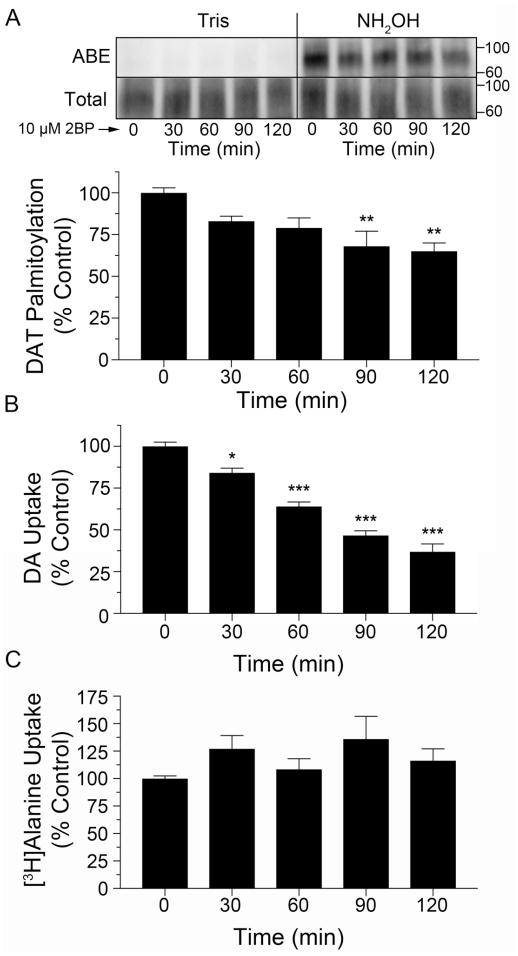

Figure S1. 2BP effects on DAT in N2a cells. rDAT-N2a cells were treated with 7.5 μM 2BP for the indicated times and assessed for A, DAT palmitoylation, B, [^{3}H]DA uptake, or C [^{3}H]alanine uptake. A, DAT levels were determined by immunoblotting, and equal amounts were assessed for palmitoylation. Blots show representative ABE and total DAT samples, and histogram shows quantification of DAT palmitoylation, $^{**}p<0.01$ vs control (ANOVA with Tukey's posttest, n=3). B, [^{3}H]DA transport values normalized to total protein and expressed as % Control, means ± S.E. $^{*}p<0.05$ $^{***}p<0.001$ *vs* control (ANOVA with a Dunnett's posttest, n=4). C, [^{3}H]alanine transport values normalized to total protein and expressed as % Control, means ± S.E., n=3. Lack of 2BP effect on this property indicates no general suppression of Na^+/Cl^- dependent transport by the treatment.

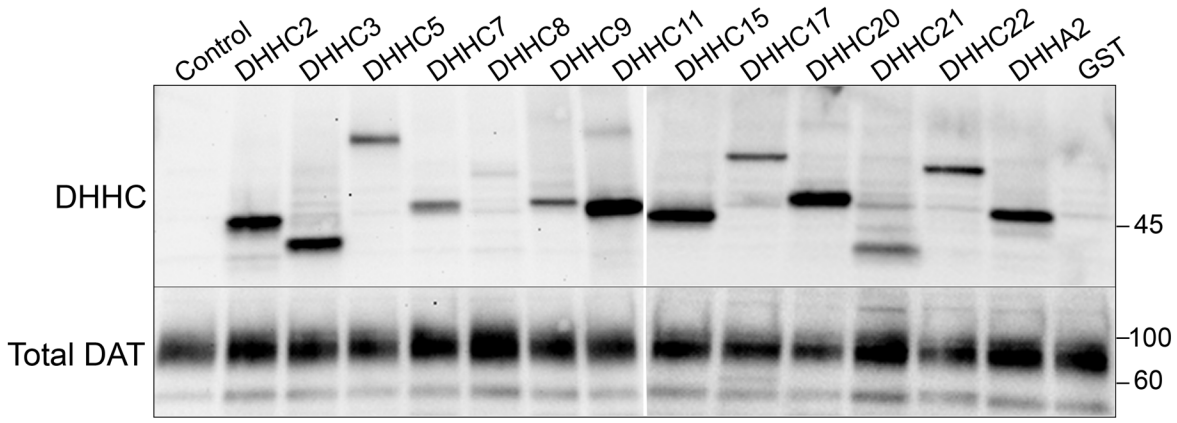

Figure S2. DHHC expression in rDAT-LLCPK$_1$ cells. rDAT LLCPK$_1$ cells were transiently transfected with the indicated HA-tagged DHHC-coding plasmids. Lysates were immunoblotted for DAT (MAb16) and DHHC expression (anti-HA). Blots are representative of three independent experiments. Vertical white dividing lines indicate the merger of two immunoblots performed in parallel.

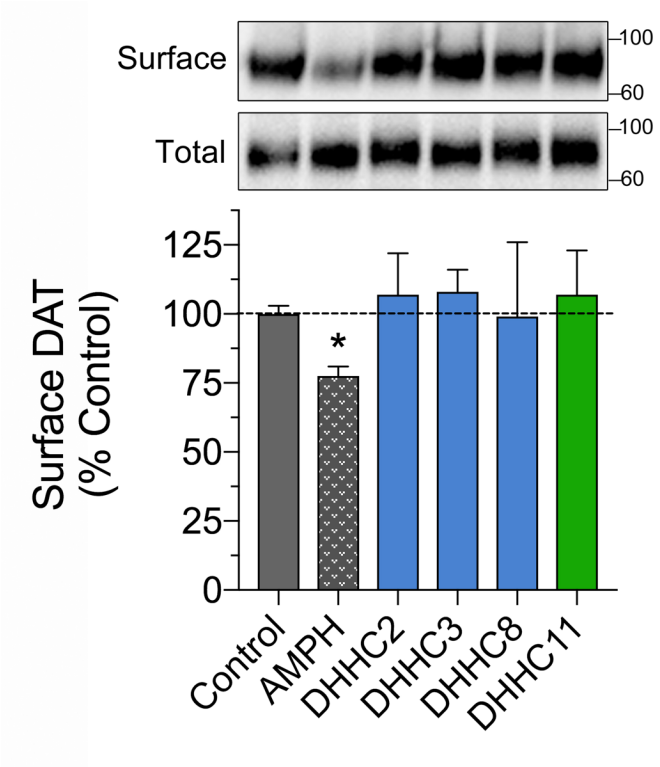

Figure S3. Surface biotinylation analysis of rDAT-LLCPK$_1$ cells transfected with control, DHHC2, DHHC3, DHHC8, or DHHC11 plasmids, and control cells treated with amphetamine (1 μM, 30 min). Upper and lower panels show representative blots of biotinylated (surface) or total DATs from equal amounts of protein. Histogram shows quantification of surface band densities (% Control, means ± S.E., n=2). * $p<0.05$, AMPH vs control, (t-test). Shading indicates control (gray), control plus amphetamine (stippled gray), cells transfected with DHHC enzymes that stimulate palmitoylation (blue), and cells transfected with DHHC enzyme that does not stimulate palmitoylation (green).

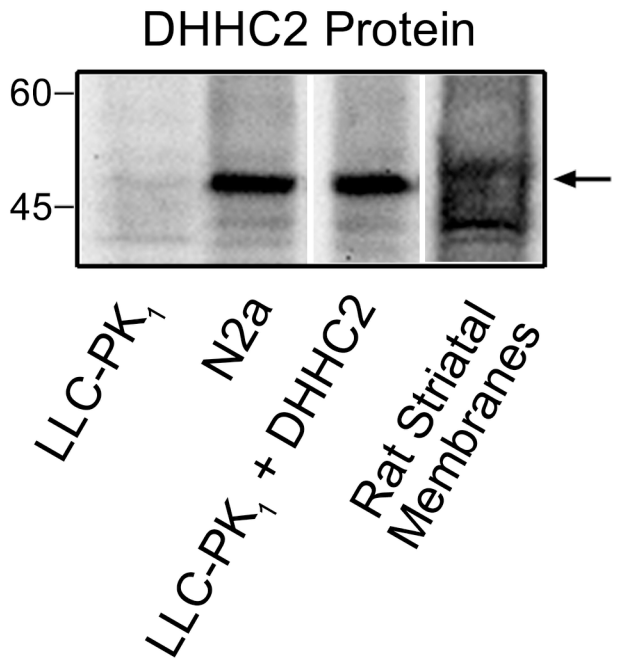

Figure S4. DHHC2 expression in rat striatum and N2a cells. Lysates were prepared from rat striatum, N2a cells, and rDAT-LLC-PK_1 cells with or without DHHC2 transfection, as indicated, and immunoblotted using anti-DHHC2 Ab. Arrow indicates DHHC2 monomer.