

Supplemental Materials and Methods

Immunoblotting. Cells transfected with siRNA were washed with PBS and lysed in RIPA buffer (Upstate, Charlottesville, VA) containing protease inhibitor cocktail (Roche, Sydney, Australia). Proteins were separated by SDS-PAGE, transferred to nitrocellulose membranes (Invitrogen) and probed with primary antibodies. HRP-conjugated antibodies (Chemicon) were used to detect the primary antibodies. Chemiluminescence detection was performed using ECL-Plus (Amersham Biosciences, Little Chalfont, UK). Immunoreactive bands quantitated and analyzed using ImageJ software (NIH, Bethesda, MD). Both bands in the doublet labeled Dysbindin in Figure S1A were reduced by siRNA to the same degree, and the intensities were added prior to statistical analyses.

Flow cytometric analysis of phosphorylated CREB protein (pCREB) - effect of haloperidol. The transfected cells were incubated with serum-free RPMI-1640 for 1 hr and treated with 20 μ M haloperidol (Tocris Bioscience) for 30 min at 37°C. After PBS washes, cells were fixed with Phosflow Fix Buffer I (BD Bioscience) for 30 min at 37°C. Cells were permeabilized by Phosflow Perm/Wash Buffer I for 30 min at room temperature. After washing, cells were labeled with PE-conjugated anti-pCREB antibody for 2 hrs at 4°C. After washing, cells were analyzed using FACScan.