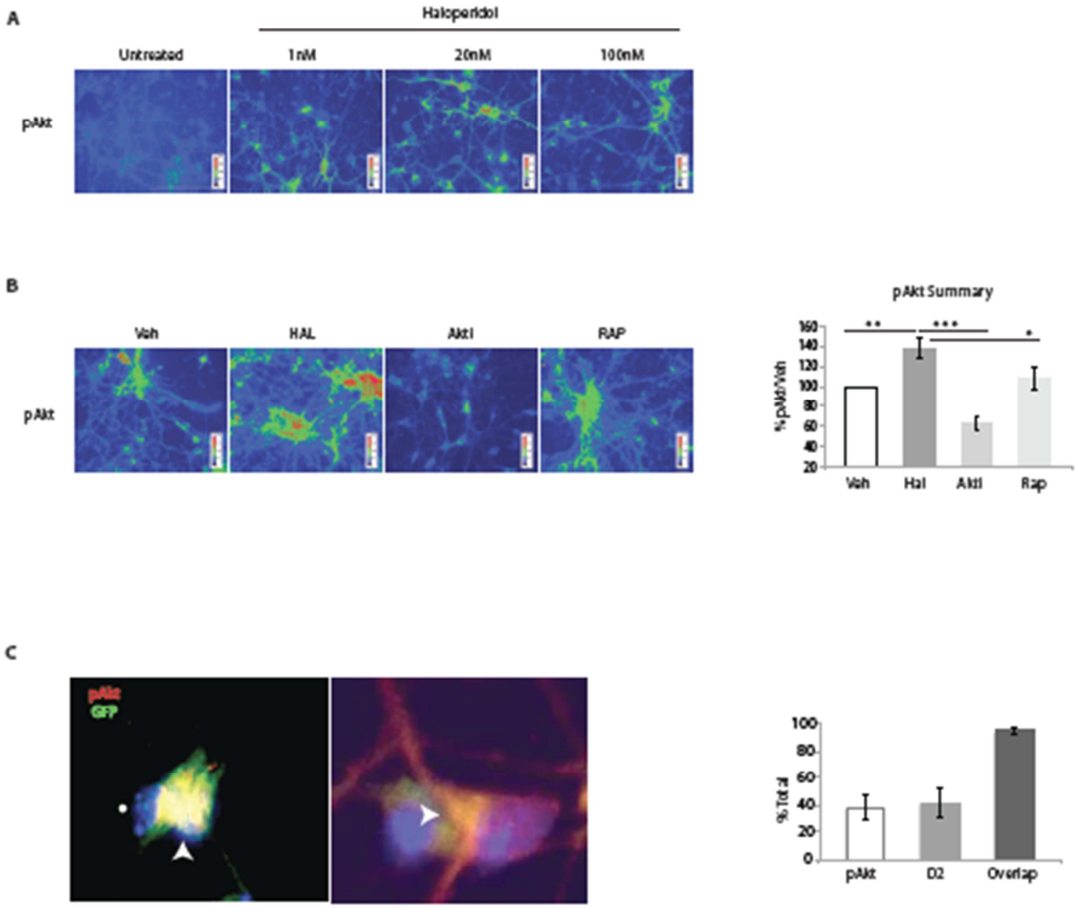

Fig. S1. Characterization of haloperidol-mediated pAkt response. Mouse striatal neurons were cultured for 7 days then serum starved and treated with haloperidol or vehicle for 20 minutes. (**A**) The response of primary striatal neurons to the indicated concentrations of haloperidol. The most intense response and the highest proportion of responding neurons were achieved with 20 nM. Color scale and size scale same as in Figure 1D-E. Representative images are shown. (**B**) Verification of pAkt signal in the presence and absence of an Akt inhibitor (Akti) and rapamycin (RAP) (data are shown as average +/- SEM, n=5, * indicates $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, one-way ANOVA). (**C**) Akt phosphorylation occurs in D_2R-EGFP-positive neurons. Striatal neurons were prepared from D_2R-EGFP transgenic mice and exposed to haloperidol. (Left)The pAkt response is shown in red, D_2R-positive cells are green, and nuclei are blue. (Right) the percent of total cells that respond, the % of total cells that are D_2R positive, and the% overlap between pAkt and D_2R staining (data are shown as average +/- SEM, of 3 independent experiments). The * denotes non-responders (left panel, partially obscured by a D_2R- and pAkt-positive neuron) and the arrow denotes neurons positive for both pAkt and D_2R.

Supplementary Figures and Legends

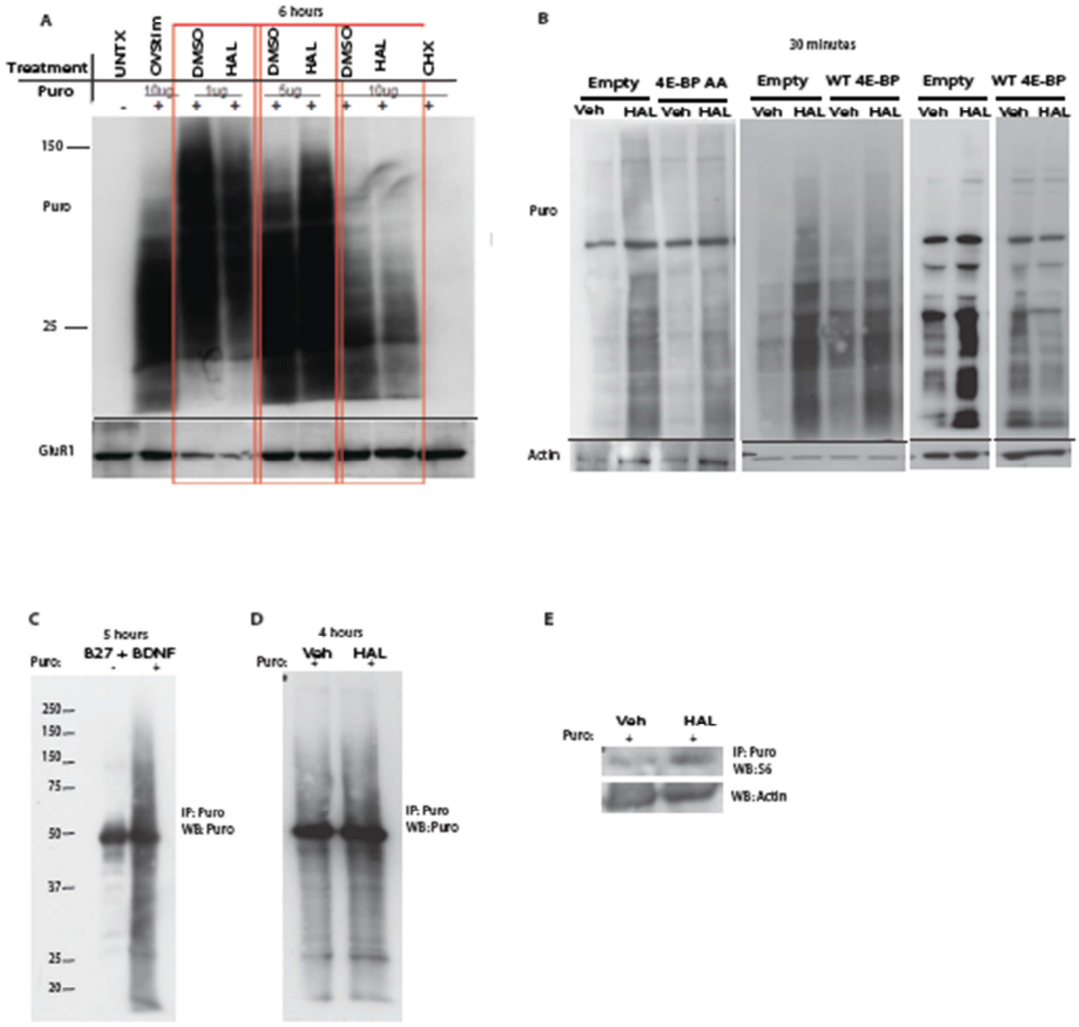

Fig. S2. Puromycin dosing and control experiments for genetic manipulations. (**A**) Determining the optimal concentration of puromycin. Primary striatal neurons were incubated with 1, 5, or 10 μg/ml puromycin for 6 hours and DMSO, haloperidol (HAL), serum (overstim), or cycloheximide for 6 hours and then samples were analyzed by Western blot using an antibody recognizing puromycin. Groups treated with the same dose of puromycin are outlined in red. GluR1 served as a loading control. 1 μg/ml was selected because it had the fewest truncated proteins (shorter proteins would indicate incomplete translation due to puromycin inhibiting the ribosome). (**B**) Striatal neurons expressing the empty vector, the doxycycline-inducible 4E-BP AA, or wild-type (WT) 4E-BP were incubated with puromycin and haloperidol or DMSO (Veh) for 30 minutes. Actin is a loading control. These blots demonstrate the effectiveness of the 4E-BP constructs used. (**C**) Striatal neurons were stimulated with an endogenous ligand, brain-derived neurotrophic factor (BDNF), and serum (B27) in the presence or absence of puromycin for 5 hours. The cells were lysed and immunoprecipitated with an antibody recognizing puromycin. Newly synthesized proteins were detected by Western blot with the antibody to puromycin only in the puromycin-treated group, suggesting that the puromycin antibody works well for immunoprecipitation. (**D**) Striatal neurons were exposed to DMSO (Veh) or haloperidol in the presence of puromycin for 4 hours, lysed, and immunoprecipitated with an antibody recognizing puromycin. Samples were analyzed by Western blot with an antibody to puromycin. This demonstrates that immunoprecipitation with the puromycin antibody can enable detection of changes in nascent proteins in 4 hours. (**E**) Changes in the synthesis of specific proteins can be detected by Western blot following immunoprecipitation with the antibody recognizing puromycin. Striatal neurons were exposed to DMSO or haloperidol in the presence of puromycin for 4 hours and lysed. Nascent proteins were precipitated using an antibody recognizing puromycin, and probed for S6, a protein identified in the 5-hour screen as upregulated with haloperidol treatment. Actin is a loading control. Data in all panels are representative of multiple puromycin-treatment experiments. Black lines indicate that blots have been stripped after treatment with the antibody recognizing puromycin and re-probed for the loading control.

Supplementary Figures and Legends

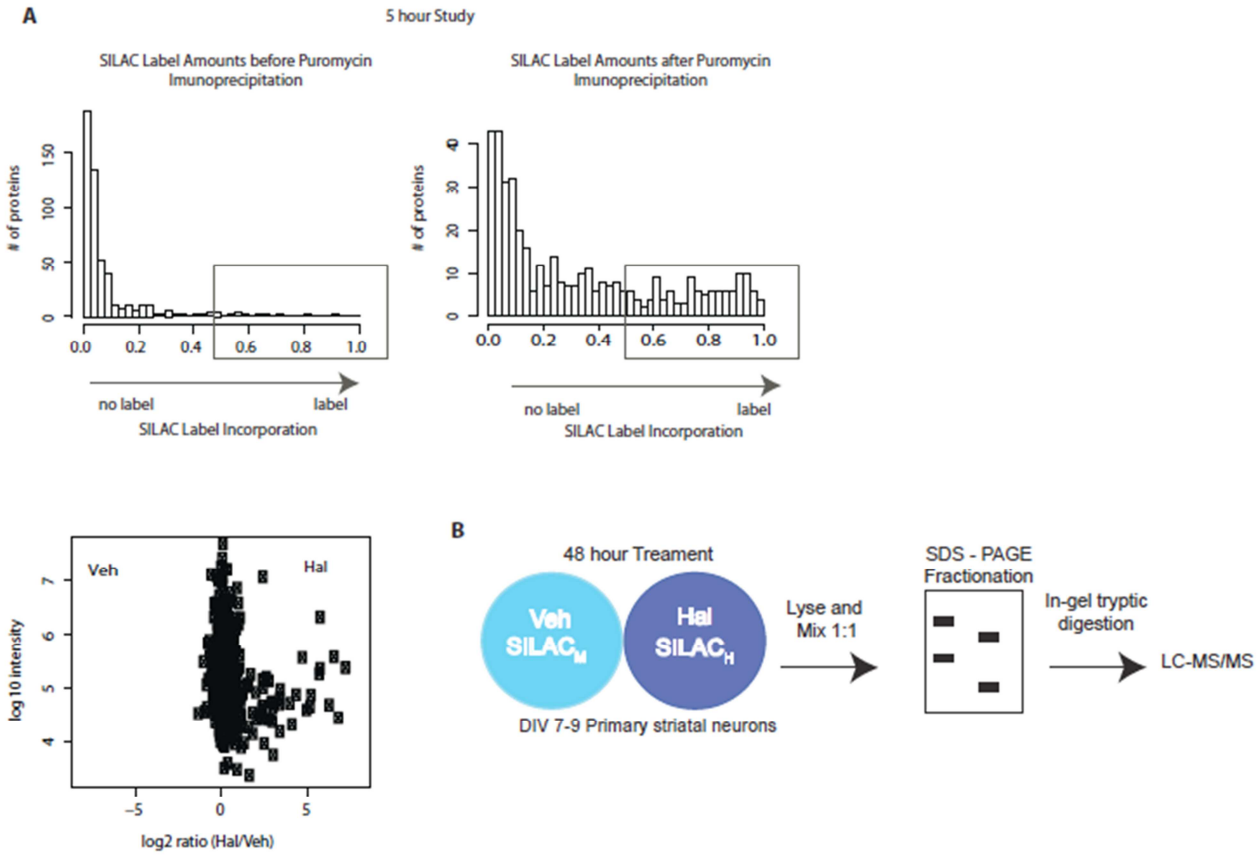

Fig. S3. Proteomic analysis of measured proteins. (**A**) Immunoprecipitation of puromycin-labeled proteins increases the amount of SILAC-labeled proteins. **Top**: Following 5 hours of incubation with heavy arginine and lysine (SILAC) and puromycin, lysates of striatal neurons were collected and immunoprecipitated with a puromycin-specific antibody to enrich for SILAC-labeled proteins. The regions in the boxes represent the portion with significant SILAC labeling. **Bottom**: Measured proteins were normalized and the ratio of haloperidol to vehicle was plotted. Clustering around 0 represents basal translation in the neurons; the points (proteins) that are farther from 0 indicate more protein synthesis in the haloperidol-treated cells. (**B**) Overview of the 48-hour study method. DIV7 striatal primary cultures were exposed for 48 hours to haloperidol, SILAC labeled, and analyzed by mass spectrometry**.**

Supplementary Figures and Legends

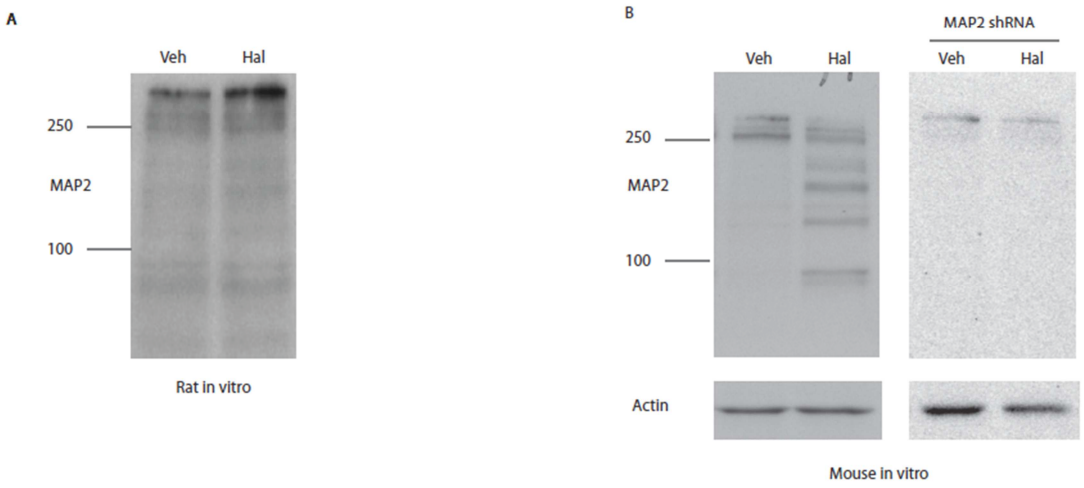

Fig. S4. Haloperidol-induced proteins that react with a MAP2 antibody in mouse striatal neurons or rat striatal neurons in vitro. (**A**) Rat striatal neurons were cultured for 7 days and then exposed to 20 nM haloperidol for 24 hours time. Lysates of vehicle and haloperidol-treated neurons were analyzed by Western blot with an antibody recognizing MAP2. (**B**) Mouse striatal neurons were cultured for 7 days and then exposed to 20 nM haloperidol for 24 hours. Samples on the right were from neurons expressing a MAP2-targeted shRNA. Lysates of vehicle and haloperidol-treated neurons were analyzed by Western blot with an antibody recognizing MAP2. Note the increase in MAP2-dependent immunoreactive bands in the haloperidol-treated neurons that are not present in the MAP2 shRNA lanes.

Fig. S5. DARPP32 can label spines in DIV14 cultured striatal neurons. To ensure that the DARPP32 antibody labeled spines, we transfected striatal neurons with a GFP antibody and cocultured them with cortical neurons. Neurons were grown to DIV14, fixed, and stained. Arrows indicate examples of DARPP32 (red) and GFP (green) in spines.

Supplementary Figures and Legends

Table S1. Complete results of the 5-hour proteomic screen. Primary striatal neurons were exposed for 5 hours to haloperidol or vehicle and puromycin and SILAC, lysed, and puromycin-tagged nascent proteins were immunoprecipitated and prepared for mass spectrometry. Protein ratios are presented as vehicle to haloperidol and thus proteins with lower ratios were increased with haloperidol treatment. (Excel file)

Table S2. Complete results of the 48-hour proteomic screens. Primary striatal neurons were exposed for 48 hours to haloperidol or vehicle and SILAC, lysed, and prepared for mass spectrometry. (Excel file)

Table S3. Haloperidol-induced increases in proteins linked to mTORC1 signaling. In Thoreen *et al*. (*26*) the ribosomal association of mRNAs with known TOP sequence or established mTORC1-dependency, and suspected TOP-like sequences were measured in the presence and absence of a mTORC1 inhibitor. Those mRNAs with decreased ribosomal association with inhibitor treatment (Column B), were considered mTORC1-sensitive. Here we compared these mTORC1-sensitive mRNAs with our proteomic screen (5 hrs) and study (48 hrs), to see if any of those mRNAs were altered at the protein level following mTORC1 induction by haloperidol. Several of these mTORC1-sensitive mRNAs were increased at the protein level with haloperidol treatment at both time points. Increased or decreased denotes the direction of change in at least 2 detections by mass spectrometry unless otherwise noted. The term candidate refers to those proteins that met low stringency criteria. Their protein classification is provided if the class is not one that is highlighted in Table 2 or table S4. (Excel File)

Table S4. **Candidate proteins from cytoskeleton, translation, mRNA, membrane, and release protein groups.** Candidate proteins that met the low stringency, medium stringency, or high stringency criteria. Proteins that were detected and identified in common with previous antipsychotic treatment proteomic studies Chan *et al*. (*28*) and Ma *et al*. (*27*). (Excel File)

Supplementary Figures and Legends

Table S5. Statistical significance for three biological replicates for selected proteins of interest. Significance was calculated using non-normalized peptide ratios subjected to t-test analysis for each mass spectrometry experiment. Direction indicates the proteins increased (up) with haloperidol treatment. ARMS was significantly increased in the first experiment and trend-level increased in the second. MAP2 was significantly increased in all experiments, and S6 was increased in 2/3 of the mass spectrometry experiments. Proteins are listed by their full name and UniProtKB ID number.

Protein	Direction	Forward p value	Reverse 1 p value	Reverse 2 p value
Ankyrin repeat-rich membrane spanning protein/ Kinase D-interacting substrate of 220 kDa **UniProtKB**:Q9EQG6 **(ARMS)** Required for dendritic branching/ outgrowth	up	$p = 0.01$	$p = 0.09$	Not detected
Microtubule-associated protein 2 **UniProtKB**: P15146 **(MAP2)** Dendritic outgrowth/branching	up	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
40S ribosomal protein S6 **UniProtKB**: P62755 **(RpS6)** Ribosomal protein, phosphorylation correlates with increased translation	up	$p < 0.0005$	Variable	$p = 0.02$