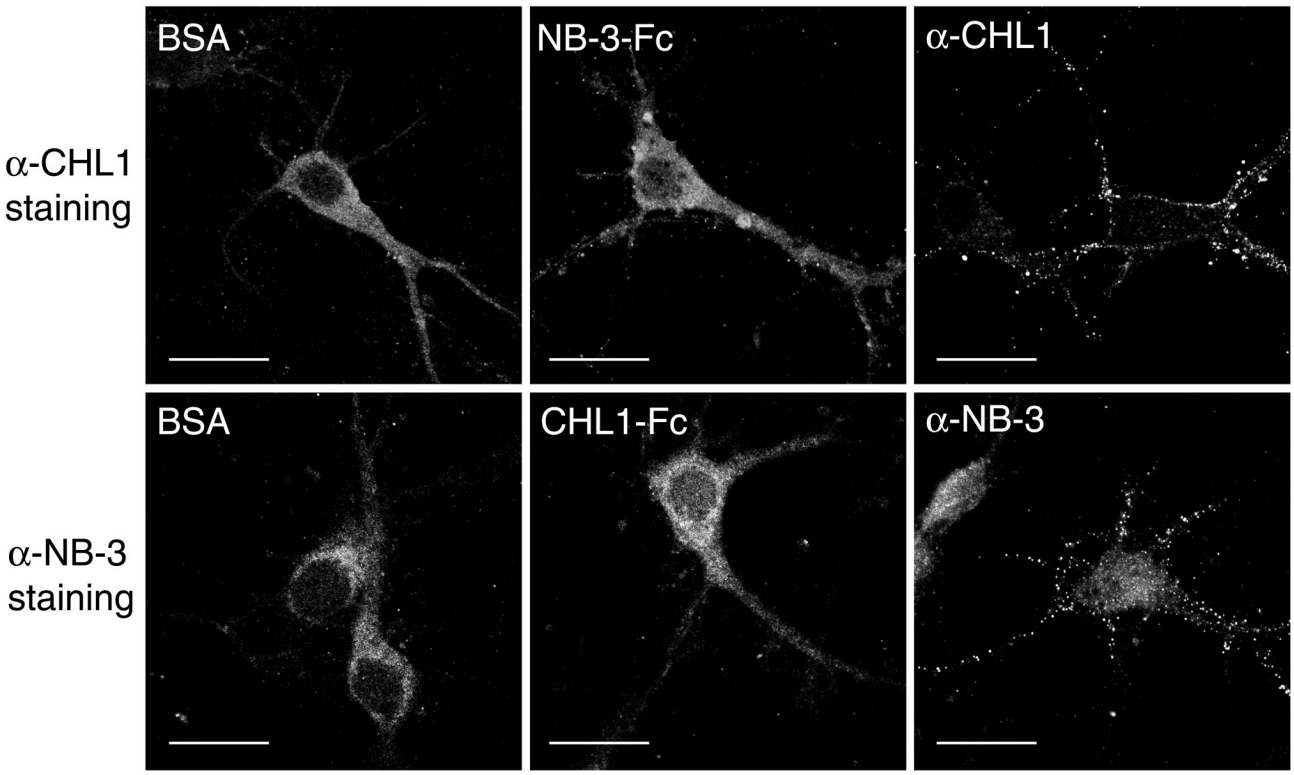
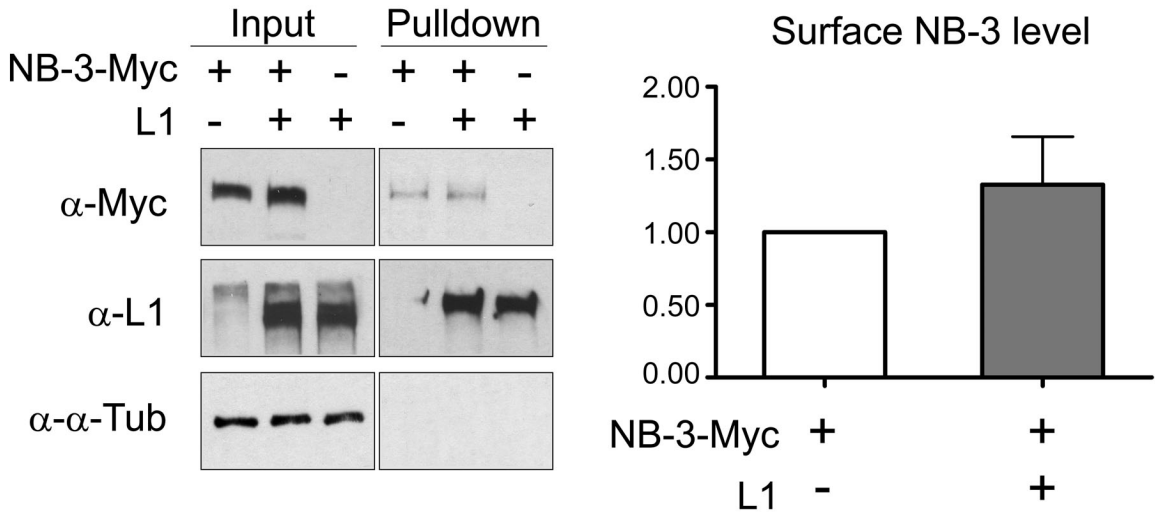
Supplemental figure 1



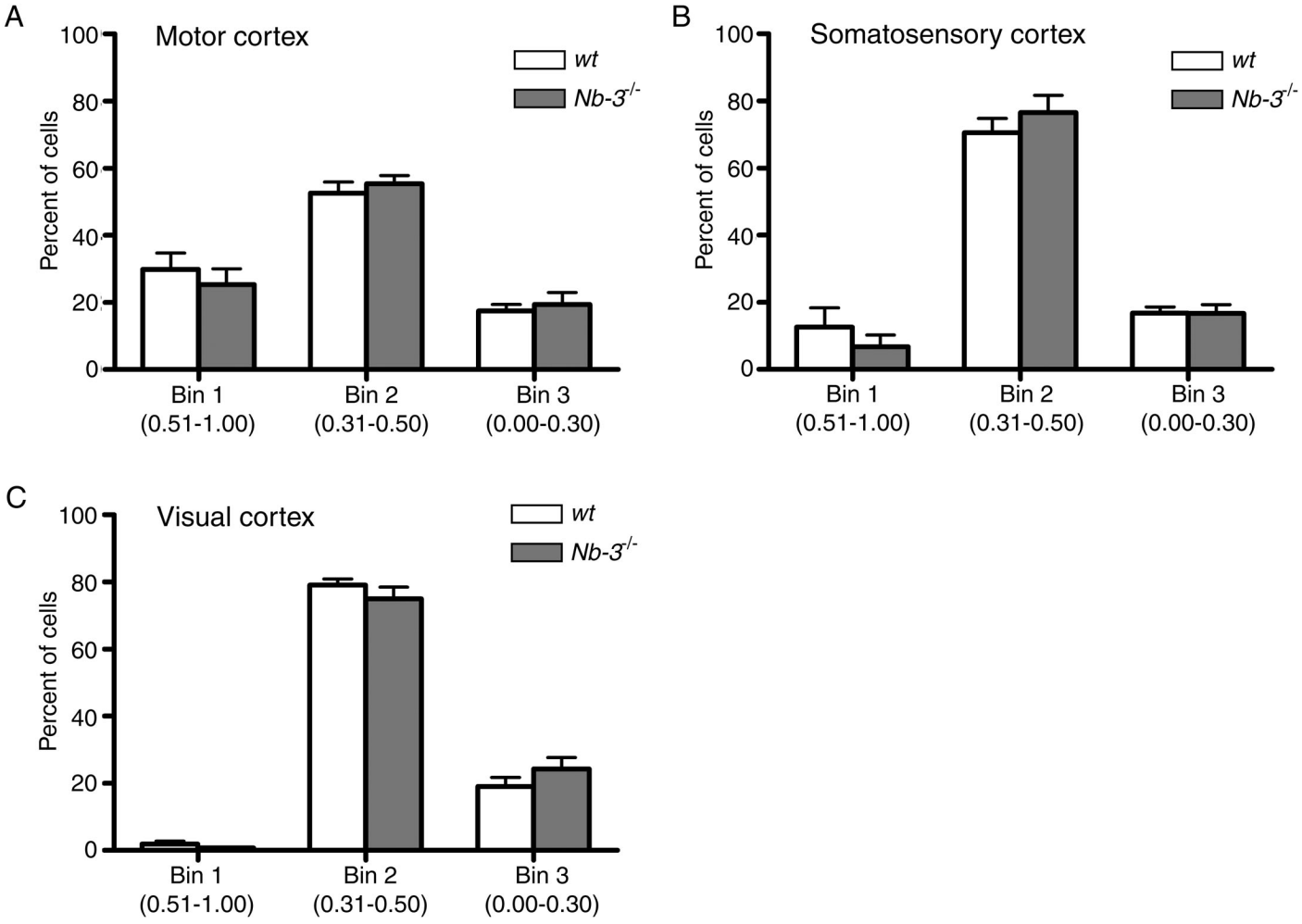
Supplemental figure 1. NB-3/Fc or CHL1/Fc proteins do not induce clustering of CHL1or NB-3 , respectively, on the neuronal surface. Live mouse cortical neurons (7-10 DIV) were treated with bovine serum albumin (BSA), NB-3/Fc, CHL1/Fc (all at 10 μg/ml), or mouse antibodies against CHL1 or NB-3 at 37°C for 2 hours as indicated. Cells were fixed and immunostained for CHL1 or NB-3. Note that antibodies induced clustering of corresponding antigens on the cell surface, while NB-3/Fc did not induce clustering of CHL1, neither did CHL1/Fc have effect on the surface NB-3. Scale bars, 20 μm.

Supplemental figure 2



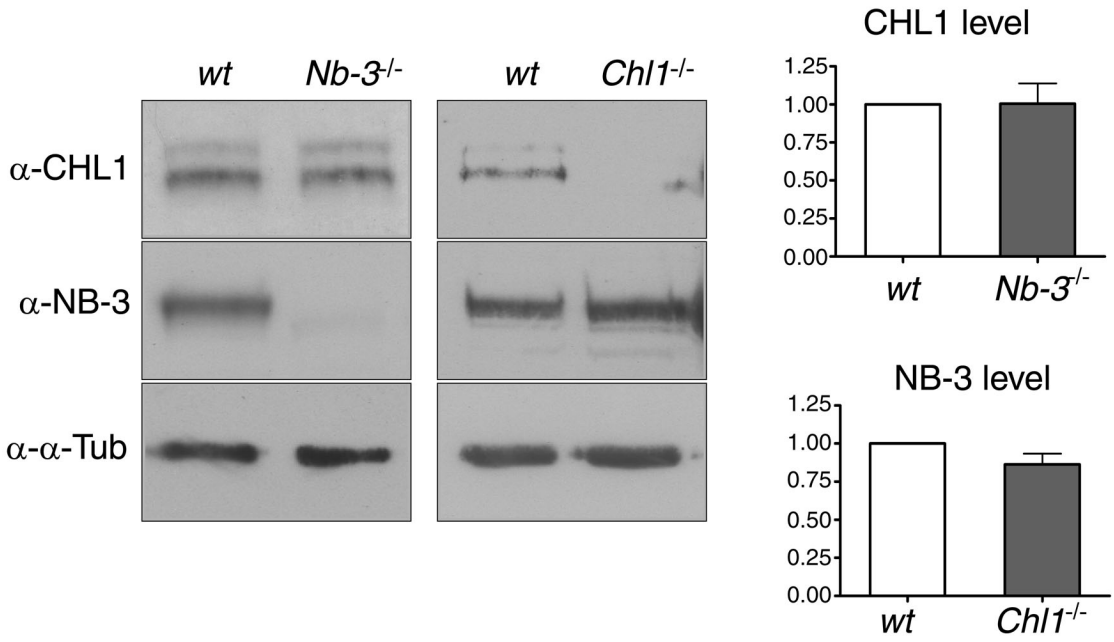
Supplemental figure 2. L1 has no effect on the surface expression of NB-3 in transfected COS1a cells. 48 hours after transfection, live cells were biotinylated. After cell lysis, biotinylated cell surface proteins were pulled down using a NeutrAvidin Gel. Pull-down samples were blotted with anti-Myc or anti-L1 antibodies to assess the level of cell surface NB-3-Myc and L1 proteins. Levels of cell surface proteins were normalized to corresponding input proteins. Results from four independent experiments (n=4) are presented as Mean ± SEM. No statistical significance was detected ($p>0.05$); one-sample t-test.

Supplemental figure 3



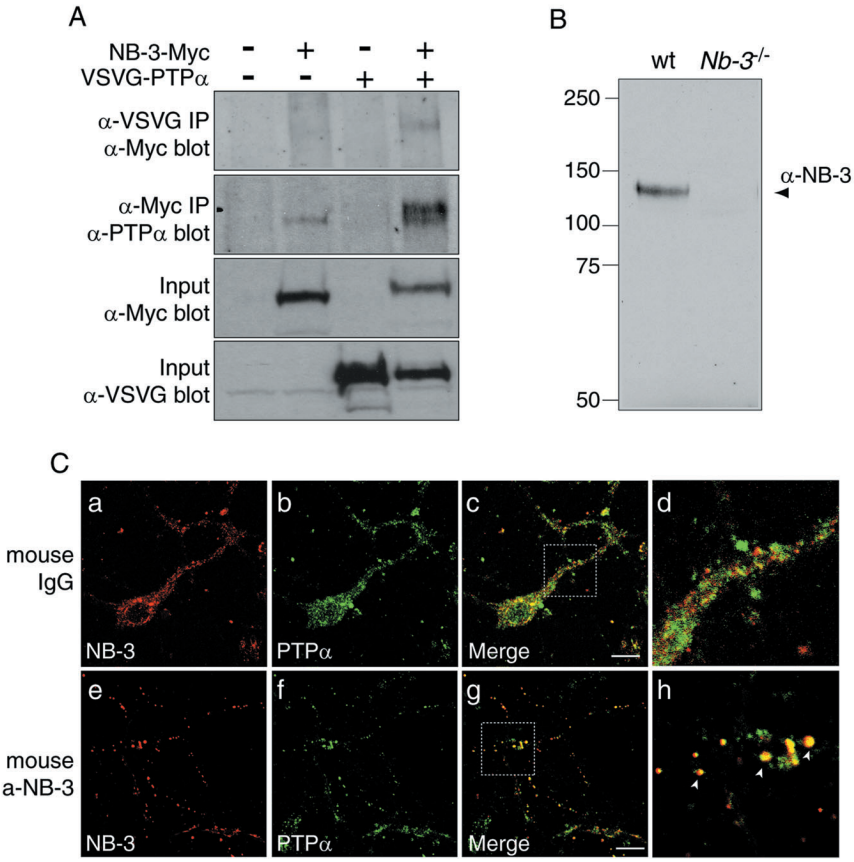
Supplemental figure 3. Distribution of YFP-labeled pyramidal neurons in each cortical region of *Nb-3*$^{+/+}$;Thy1-YFPH and *Nb-3*$^{-/-}$;Thy1-YFPH littermates (1-month old, 3 pairs). The longitudinal position was determined for 112 to 644 neurons per genotype and cortical region. The percent of cells in each bin was calculated. Results from three independent experiments (n=3) are presented as Mean ± SEM. No statistical significance was detected for all three cortical areas checked ($p>0.05$); one-way ANOVA with repeated measures.

Supplemental Figure 4



Supplemental figure 4. Expression level of NB-3 and CHL1 proteins in the *Nb-3*$^{-/-}$ and *Chl1*$^{-/-}$ mice brains. Whole brain homogenates from P7 wildtype and knockout mice were resolved in SDS-PAGE and blotted with rabbit polyclonal antibodies against NB-3 and CHL1. Levels of NB-3 or CHL1 were normalized to input a-tubulin levels. Results from four independent experiments (n=4) are presented as Mean ± SEM. CHL1 level is not changed in the *Nb-3*$^{-/-}$ brains ($p>0.05$, one-sample t-test). NB-3 level is slightly reduced in the *Chl1*$^{-/-}$ brains, but the change is not statistically significant ($p>0.05$, one-sample t-test).

Supplemental figure 5



Supplemental figure 5. NB-3 associates with PTPα. (A) Association of ectopically expressed NB-3-Myc and VSVG-PTPα in HEK293T cells. HEK293T cells were transfected with mock, NB-3-Myc, VSVG-PTPα cDNA or both. Whole cell lysates were immunoprecipitated with anti-VSVG or anti-Myc antibodies, and were probed with anti-Myc or anti-PTPα antibodies, respectively. Note that in lysates from NB-3-Myc-expressing cells, anti-Myc antibody also brought down endogenous PTPα, which had a lower molecular weight due to the lack of VSVG epitop. Input, whole cell lysates of mock or transfected HEK293T cells before immunoprecipitation. (B) Specificity of mouse monoclonal antibody against NB-3 used to induce clustering of NB-3 protein in cultured cortical neurons. Whole brain homogenates from wild-type and *Nb-3*$^{-/-}$ mice were resolved in SDS-PAGE, followed by immunoblotting with the anti-NB-3 antibody. A single band around 135 kD was recognized in the wild-type lane. (C) Live mouse cortical neurons (7-10 DIV) were treated with nonspecific mouse IgG (a-d) or with mouse monoclonal antibody against NB-3 (e-h) at 37°C for 2 hours, followed by fixation and immunostaining for NB-3 and PTPα. Note that NB-3 antibody induced clustering of NB-3 at the cell surface and redistribution of PTPα to the NB-3 clusters (arrowheads in h). (d and h) Higher magnification of selected areas in c and g, respectively. Scale bars, 10 mm.