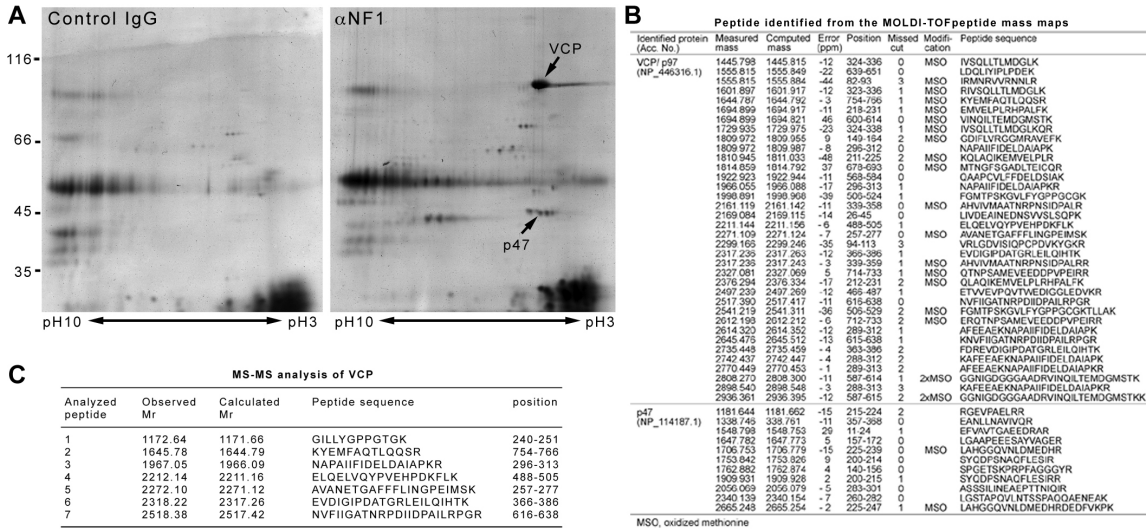
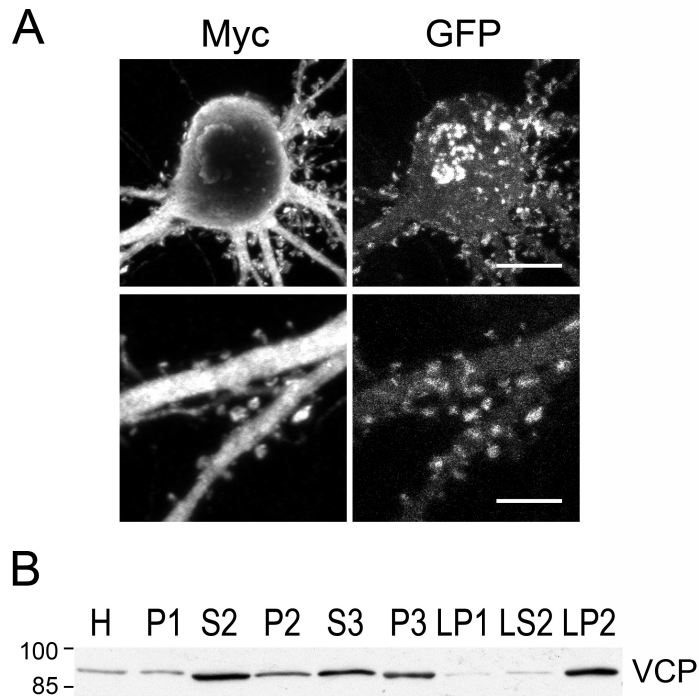


Supplementary Figure S1



Supplementary Figure S1

Identification of VCP as a neurofibromin-associated protein by 2-D gel electrophoresis and mass spectrometry analysis. (A) Two-dimensional gel electrophoresis of neurofibromin protein complex. To identify neurofibromin-associated proteins, brain extract containing 5 mg of protein was subjected to immunoprecipitation using 25 µg of neurofibromin antibody NF1 or control non-immune IgG. After immunoprecipitation, the beads were treated with 150 µl denaturing buffer (40 mM Tris, 9 M Urea, 4% CHAPS, and 100 mM DTT) overnight at room temperature. Protein samples were then loaded onto IPG gel strips (pH 3–10, non-linear gradient, length of 13 cm, Amersham Biosciences) for separation in the first dimension. The protein samples were then further separated in the second dimension using 8% SDS-PAGE and visualized by Sypro Ruby (Molecular Probes) staining. The associated proteins were analyzed by MALDI-Q-TOF (Proteomic Core Facility at the Institute of Biological Chemistry, Academia Sinica). Proteins were identified from the peptide mass fingerprints by searching databases for matches using the ProFound software (<http://www.unb.br/cbsp/paginiciais/profound.htm>) and MASCOT MS/MS Ions Search (http://www.matrixscience.com/cgi/search_form.pl?FORMVER=2&SEARCH=MIS). A specifically associated protein (arrow) was analyzed by MALDI-Q-TOF. The results of the MOLDI-TOF and MS-MS analyses are summarized in (B, C). In addition to VCP and p47, we did not identify other potential neurofibromin-associated protein from the 2D gel.



Supplementary Figure S2

Subcellular distribution of VCP in neurons. **(A)** Expression of Myc-tagged VCP in cultured hippocampal neurons examined by immunostaining using Myc tag antibody. Cultured neurons were cotransfected with Myc-tagged VCP and GFP-actin. GFP-actin is highly enriched at dendritic spines. The upper panel focuses on soma; the lower panel highlights dendrites and dendritic spines. Scale bars, upper panel, 10 μm ; lower panel, 5 μm . **(B)** VCP distribution in a series of biochemical subcellular fractions prepared from P21 rat brain. H, total homogenate; P1, nuclei and cell debris; S2, supernatant of P2; P2, crude synaptosomal fraction; S3, soluble cytosolic fraction; P3, light membrane fraction; LP1, lysed synaptosomal membrane; LS2, synaptic cytosol; LP2, crude synaptic vesicles.