Supplementary Figure 4. Expression patterns of NGL mRNAs and proteins. (a) Tissue distribution patterns of NGL-2 and NGL-3 mRNAs in rat Northern blot analysis. Sk. M, skeletal muscle. (b) Widespread distribution of mRNAs of NGL isoforms revealed by in situ hybridization analysis on horizontal and sagittal sections of adult rat brain (6 weeks). Ob, olfactory bulb; Ctx, cortex; Hc, hippocampus; Cb, cerebellum. (c) Expression patterns of mRNAs of netrin-G and NGL isoforms in the early (2 weeks) postnatal rat brain revealed by in situ hybridization. (d) NGL proteins are mainly expressed in brain. Whole tissue homogenates were immunoblotted with an antibody that recognizes all three NGL isoforms (#1583). (e) Widespread distribution of NGL proteins in various brain regions. OR, other regions of the brain. (f) NGL proteins were detected in synaptic rat brain fractions. H, homogenates; P2, crude synaptosomes; S2, supernatant after P2 precipitation; S3, cytosol; P3, light membranes; LP1, synaptosomal membranes; LS2, synaptosomal cytosol; LP2, synaptic vesicle-enriched fraction. (g) Enrichment of NGL in PSD fractions; extracted with Triton X-100 once (PSD I), twice (PSD II), or with Triton X-100 and Sarkosyl (PSD III). (h) A sharp increase in the expression levels of NGL is seen during postnatal rat brain development. Whole brain homogenates were used. E, embryonic day; P, postnatal day; Adult, 6 weeks. (i) Modification of NGL by N-glycosylation. The crude synaptosomal fraction of adult rat brain was treated with N-glycosidase F (PNGase F) at 37 C. Synaptophysin (SynPhy) and α-tubulin were used as positive and negative controls, respectively.