Legends for Supporting Information Figures

Figure S1. rHIgM12 binding to neuronal membranes is sensitive to trypsin treatment. (a) The control hippocampal neurons treated with ethanol (vehicle of EDO-P4) were rHIgM12 (a1**,** green), GM1 (a2**,** labeled by CTB, red) and A2B5 (which binds to ganglioside, GT3, Dubois *et al*. 1990) (a3, blue) positive. (b) Following treatment with EDO-P4 (blocking glucosylceramide formation), rHIgM12 strongly labeled neuronal surfaces (b1), whereas both CTB (b2) and A2B5 (b3) staining were substantially decreased. (c) Of note, rHIgM12 staining in neurons treated with trypsin was substantially decreased (c1). In comparison, the GM1 levels were not significantly affected (c2), suggesting that rHIgM12 binds to epitopes that are sensitive to trypsin treatment. Scale bar = 50 μm.

Figure S2. rHIgM12 does not associate with tubulin directly. (a) N2A neuroblastoma cells were stained with rHIgM12 (a1, green) and β3-tubulin (a2**,** red). The nuclei were labeled with DAPI (a3, blue). N2A cells were negative for rHIgM12 staining, but expressed β3-tubulin (β3-Tub). (b) Supernatants of N2A cell lysates (Super) were incubated with rHIgM12, and molecules associated with rHIgM12 were pulled down by protein-l agarose and subject to western blotting. Neither was rHIgM12 detected in the pellet nor pulled down β3-tubulin (β3-Tub). Scale bar = 50 μm.

Figure S3. rHIgM12 pulls down actin, but does not co-localize with bundled F-actin. (a) A small amount of actin was pulled down by rHIgM12, and none of the anti-actin antibodies tested worked in immunoprecipitation (three antibodies were tested). The band at the similar position as β3-tubulin (β3-Tub) was the IgG heavy chain (empty arrowhead). (b) DIV1 live hippocampal neurons were stained at 4°C with rHIgM12 (b1**,** green), and F-actin (b2**,** red) was labeled with Texas-red phalloidin after fixation. In the growth cone region, F-actin receded and was enriched in the central domain, whereas rHIgM12 evenly stained the *puncta* structures distributing across the growth cone surface.