FIGURE S3

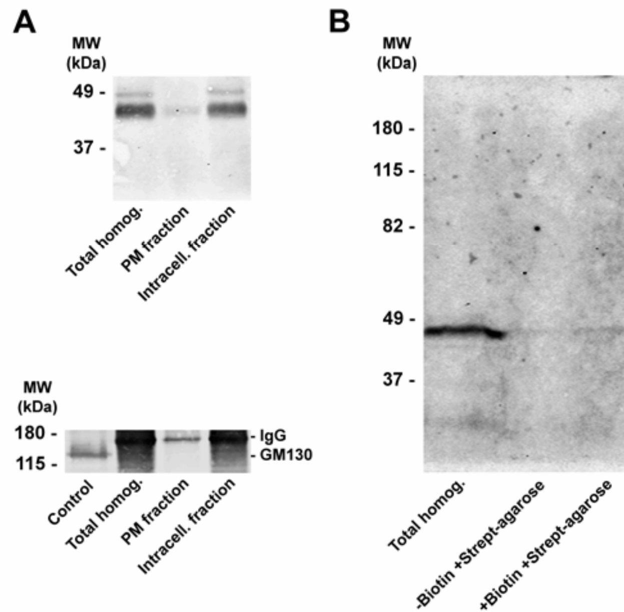

Figure S3: Quantification of ecto-Sial-T2 expression level in CHO-K1 cells. (A) Intact CHO-K1$^{Sial\text{-}T2+}$ cells in suspension were incubated at 4ºC with antibody to HA for 45 min. After washing, cells were lysed and the Sial-T2-HA/antibody complex recovered using protein A-sepharose beads (PM fraction). The supernatant, containing the intracellular fraction of Sial-T2-HA, was subjected to a second cycle of immunoprecipitation with antibody to HA and protein A-sepharose beads (Intracell. fraction). Additionally, total Sial-T2-HA was also recovered from homogenates of CHO-K1$^{Sial\text{-}T2+}$ cells (Total homog.). Fractions from immunoprecipitates were Western blotted with antibody to HA. Molecular masses of the markers in kDa are indicated on the left (upper part). The purity of the fractions was evaluated by Western blot analysis of the cis-Golgi protein GM130. IgG indicates the position of the immunoglobulin G used in the immnoprecipitation assay (antibody to HA). Control was CHO-K1 homogenate (lower part). (**B**) CHO-K1$^{Sial\text{-}T2+}$ cells were incubated in PBS buffer without (-Biotin) or with EZ-link Sulfo-NHS-SS-Biotin (+Biotin) at 4°C for 1 h. After washing, biotinylated cells were scraped off the plates in lysis buffer and centrifuged at 14,000 *g*. The resulting supernatant was incubated with streptavidin-agarose beads at 4°C for 3 h. The beads were recovered by centrifugation and then washed in lysis buffer without Triton X-100. The resulting biotinylated cell surface proteins were resolved by SDS-PAGE gel and Western blotted with antibody to HA to detect the presence of Sial-T2 as mentioned in Experimental Procedures. Molecular masses of the markers in kDa are indicated on the left. The relative contribution of individual bands was calculated using the computer Odyssey Application Software v2.1 (LI-COR Odyssey).