Supporting Information

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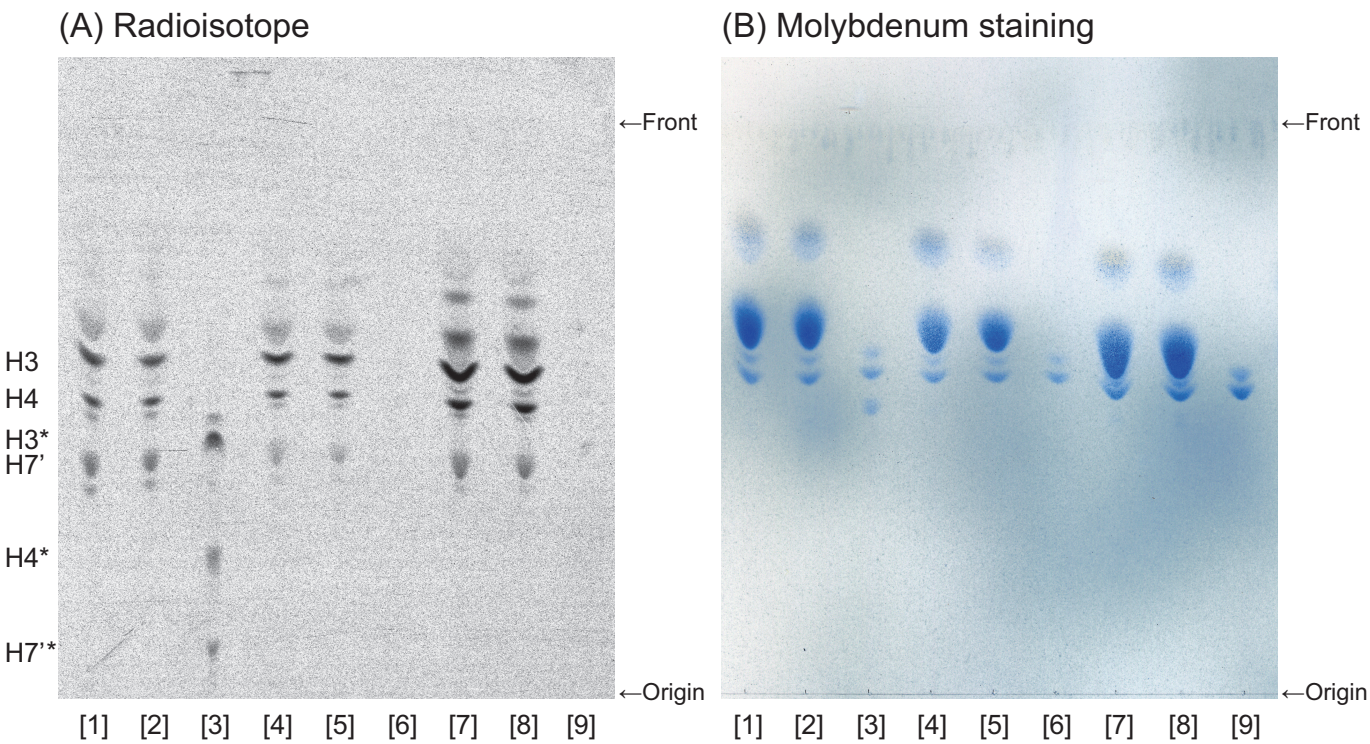

Fig. S1. Quantitative recovery of lipids after alkali-treatment of GPI-containing samples. Lipids extracted from parental CHO-K1 cells (lanes 1–3), DHAP-ATase defective NRel-4 cells (lanes 4–6), and alkyl-DHAP synthase defective NZel-1 (lanes 7–9) cells were metabolically labeled with D-[2-^{3}H]mannose in the presence of BE49385A, PIG-N-inhibitor, to accumulate late GPI precursors. The extracted lipids were untreated (lanes 1, 4, and 7), treated with methanol as a control (lanes 2, 5, and 8) or 0.1 N KOH in methanol (lanes 3, 6, and 9) for 1 h, and analyzed by TLC with a solvent system of chloroform/methanol/H_2O (10:10:3). After recording radioactive mannolipid spots with phosphoimager (panel *A*), the TLC plate was stained with molibdate for phospholipids (panel *B*). Phospholipid species were seen in lanes 6 and 9 as well as lane 3, indicating quantitative recovery of lipids after alkali-treatment.

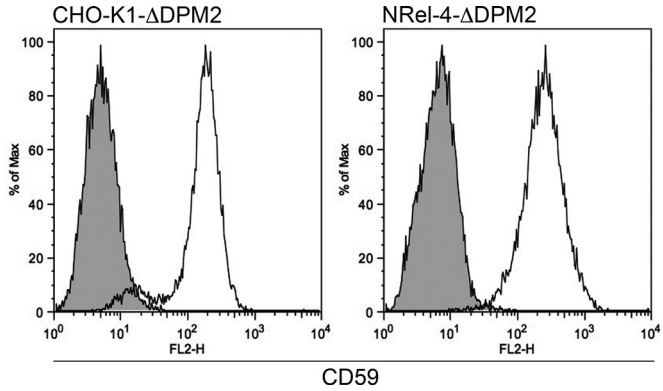

Fig. S2. Characterization of the surface CD59 expression on DPM2-defective-CHO-K1 (CHO-K1-ΔDPM2) and -NRel-4 (NRel-4-ΔDPM2) cells. CHO-K1-ΔDPM2 (left panel) and NRel-4-ΔDPM2 (right panel) cells were transfected with DPM2 cDNA, stained 72 h later for CD59 and analyzed by flow cytometry. Closed area, ΔDPM2 cells; open area, DPM2-transfected ΔDPM2 cells.

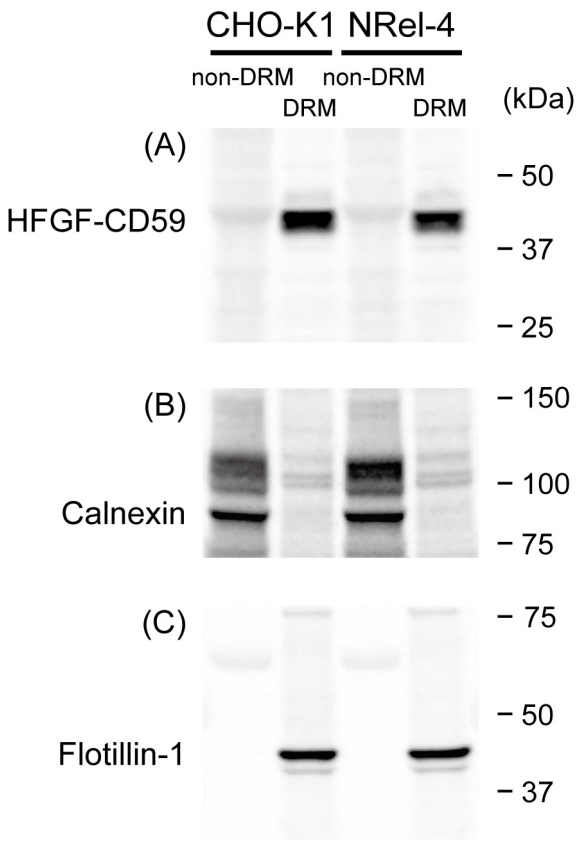

Fig. S3. Detergent-resistant membrane (DRM) association of GPI-APs bearing diacyl form GPI. CD59 tagged with His-FLAG-GST-FLAG (HFGF-CD59) was expressed in wild-type CHO-K1 and NRel-4 mutant cells. The cells were first incubated in ice-cold 1% Triton X-100 for 20 min and supernatant separated after centrifugation at 21,900 × *g* for 15 min as a non-DRM fraction. The pellets were then incubated in 60 mM octyl-β-glucoside on ice for 30 min to solubilyze DRM and supernatant separated as a DRM fraction. Calnexin (non-DRM marker); Flotillin-1 (DRM marker). (*A*) SDS/PAGE under nonreducing conditions and Western blotting against anti-CD59; (*B*) and (*C*) SDS/PAGE under reducing conditions and Western blotting against anti-Calnexin (*B*) and anti-Flottilin-1 (*C*).