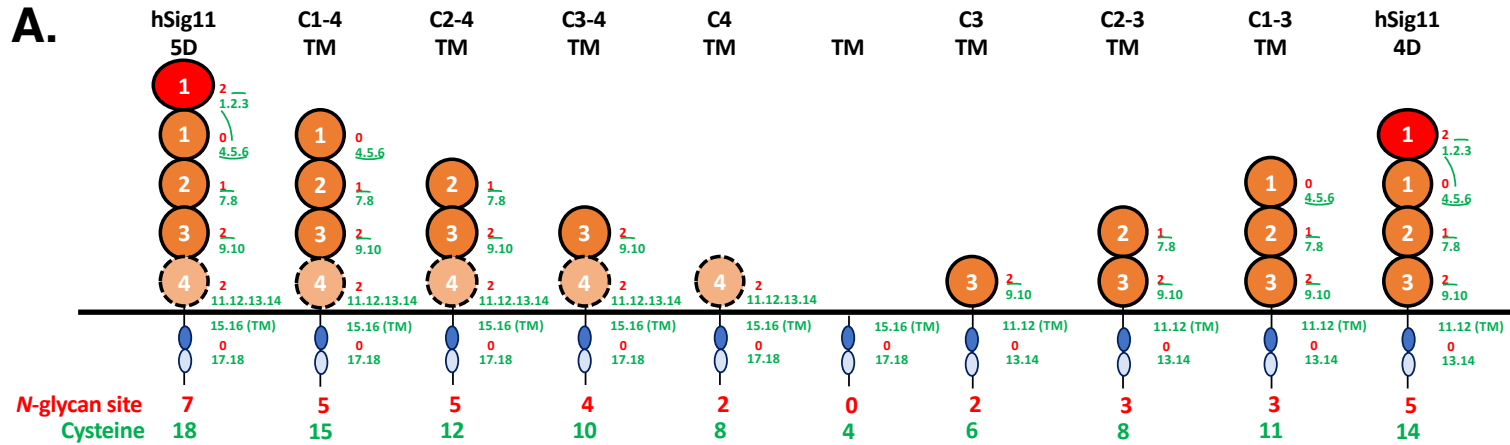
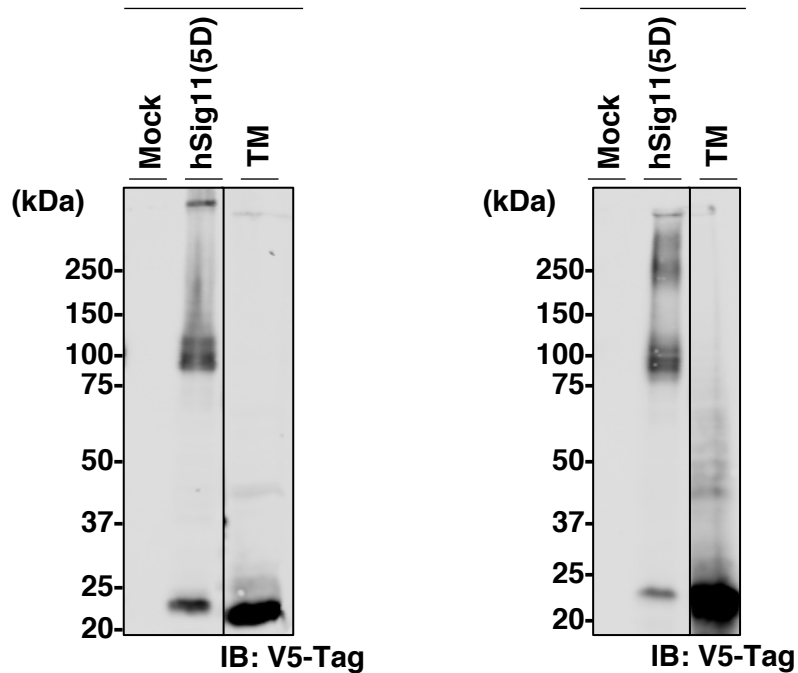


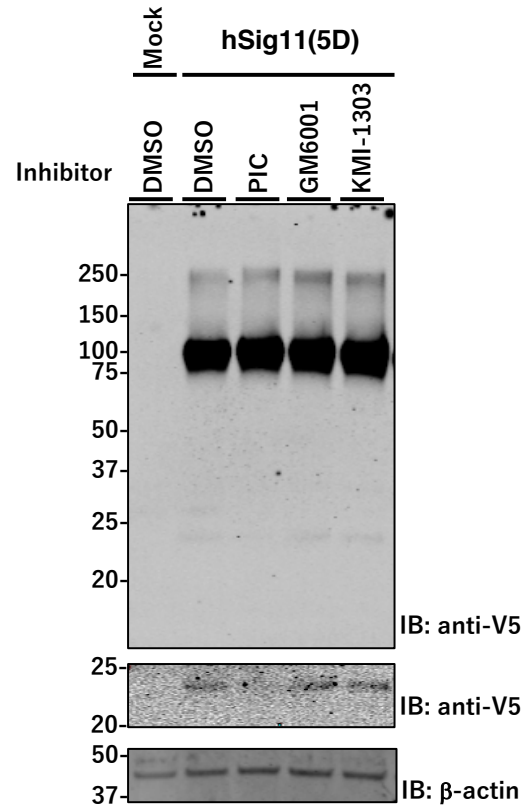
S.Fig.1



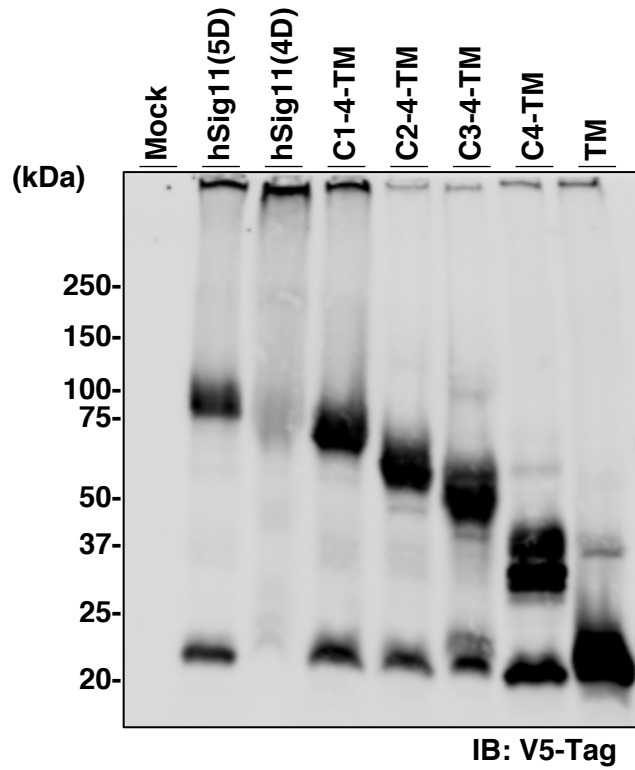
B. Reducing (with 2-ME) 100°C 5 min Non-reducing (without 2-ME) 100°C 5 min



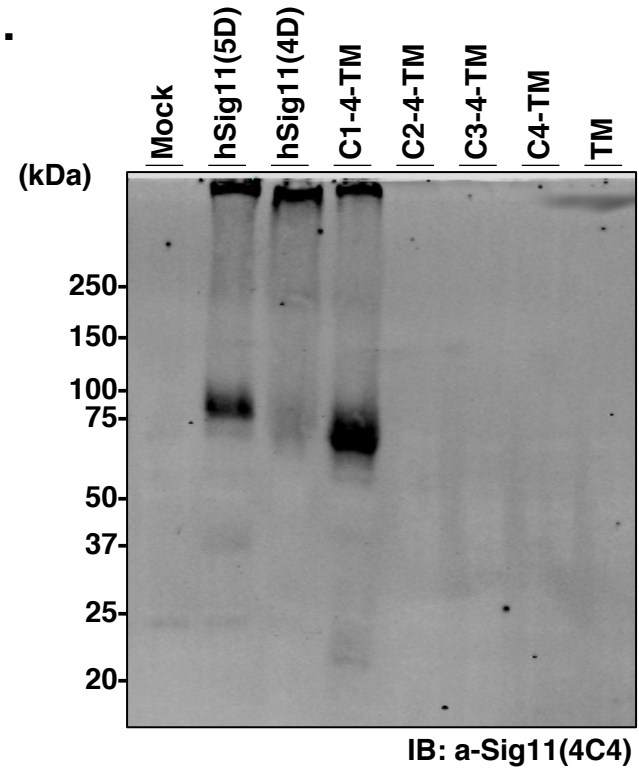
S.Fig.2



A.

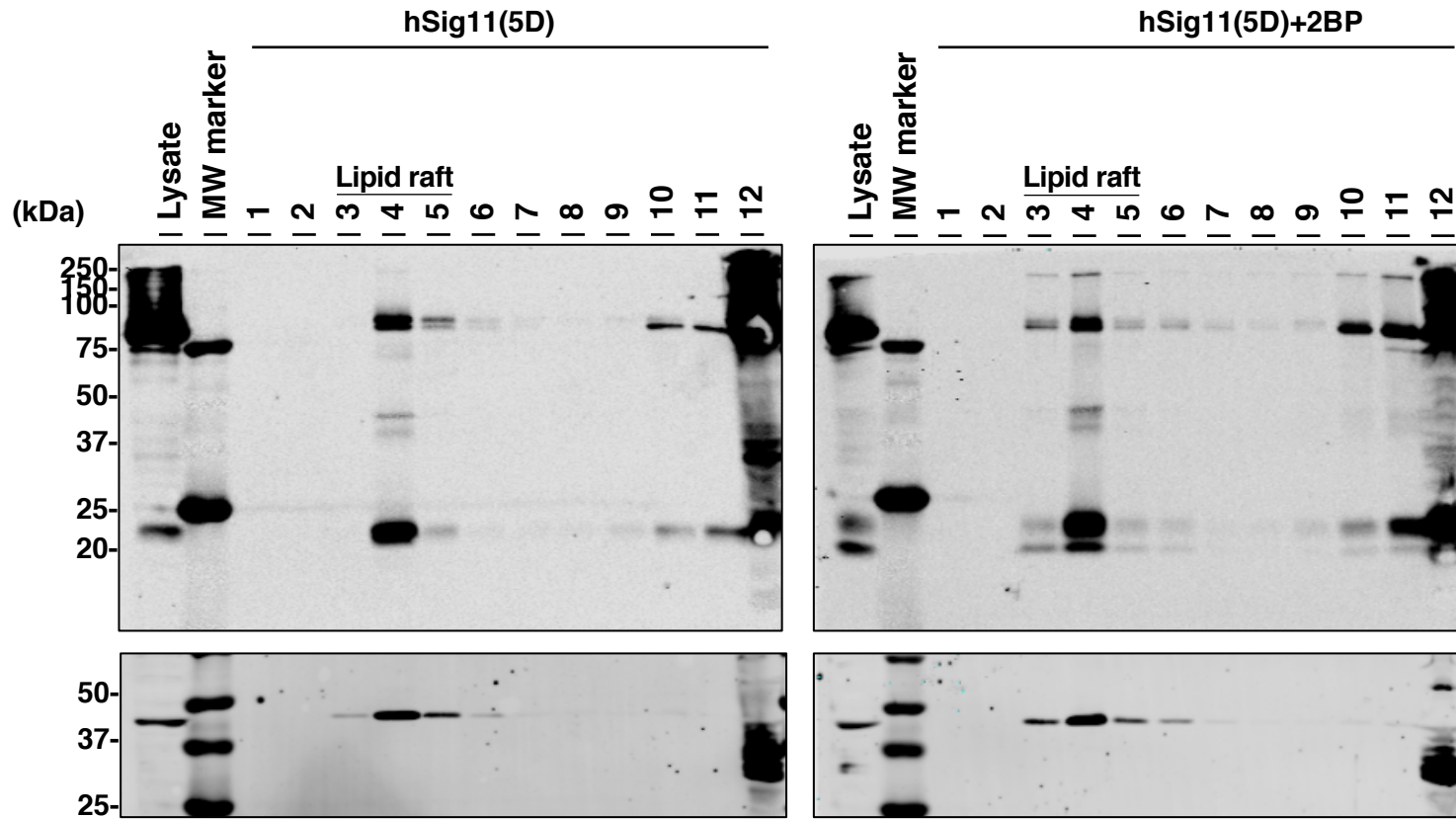


B.



S.Fig.4

Lipid raft purification after inhibition of palmitoylation



Supporting Information

S. Figure 1. Only TM and ICD (without ECD) is enough for aggregation. (A) Schematic image of full length and the different truncated mutants of hSiglec-11. (B) HEK293 cells were transiently transfected with V5-tagged full length hSiglec-11 or truncated mutant (TM) and cell lysates were analyzed by Western blotting under reducing (left panel) and non-reducing condition (right panel) with anti-V5 tag antibody. Lysates were heated to 100 °C for 5 minutes. fMock indicates transfection without the plasmid.

S. Figure 2. Inhibition of hSiglec-11(5D) ECD proteolytic cleavage. HEK293 cells were transiently transfected with V5-tagged full length hSiglec-11(5D) for 2 days, then DMSO or one of the inhibitors (PIC, GM6001 or KMI-1303) was added. Cells were harvested on Day 3. Cell lysates were analyzed by Western blotting under reducing condition with anti-V5 tag antibody. Expression levels of full length Siglec-11 (higher molecular weight membrane) and proteolytical fragment (lower molecular weight) were different, requiring different exposures. β -actin was used as loading control. Mock indicates transfection without the plasmid.

S. Figure 3. Recognition epitope of 4C4 antibody on Siglec-11. HEK293 cells were transiently transfected with V5-tagged full length hSiglec-11 or truncated mutants and cell lysates were analyzed by Western blotting under reducing condition with either anti-V5 tag antibody (A) and 4C4 antibody (B).

S. Figure 4. Effect of palmitoylation on hSiglec-11 for lipid raft localization. Cell lysates of V5-tagged Siglec-11 transfected HEK293 cells cultured with or without 2BP were fractionated by density gradient separation and analyzed by Western blotting with anti-V5 tag antibody. The left panel is hSiglec-11(5D) without 2BP and the right panel is hSiglec-11(5D) with 2BP. Flotillin-1 was used as a lipid raft marker.