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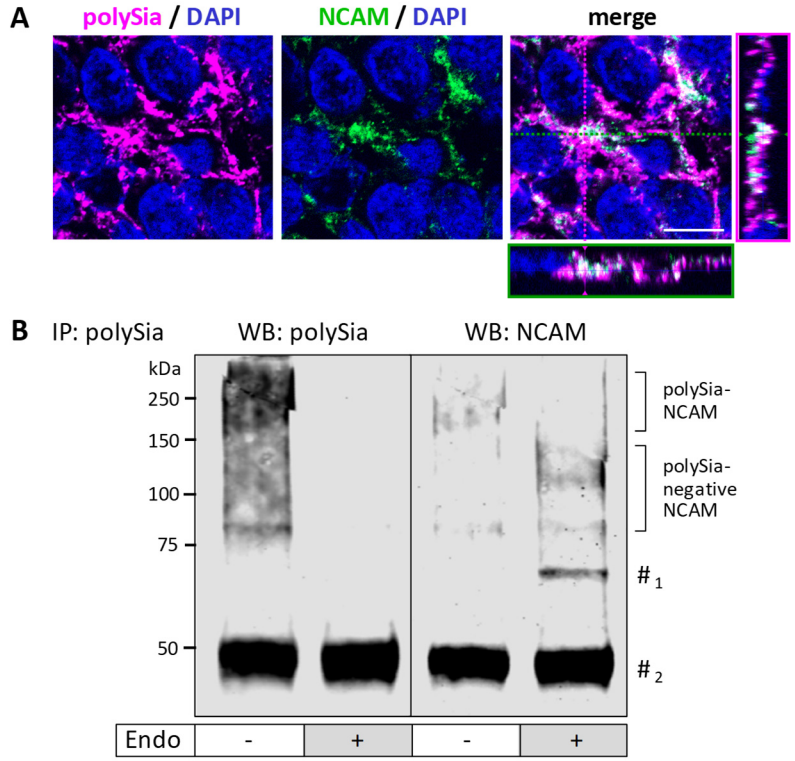

Supplementary Figure S4. PolySia on GB tumor cells is associated with NCAM. **A,** Double staining of polySia (magenta) and NCAM (green) with 3D reconstruction for colocalization analysis. Nuclei were counterstained with DAPI (blue). Scale bar, 10 µm. **B,** Immunoprecipitation (IP) of polysialylated proteins from polySia-positive GB tissue lysate using polySia-specific mAb 735-conjugated magnetic beads followed by Western blot (WB) detection with polySia-specific antibody (left), and, after stripping, with mAb 123C3, specific for the extracellular part of NCAM (right). Where indicated, IP fractions were treated with endosialidase (Endo +), to remove polysialic acid. Despite the reduced access of mAb 123C3 to polysialylated NCAM (1), the high molecular weight smear obtained with polySia-specific antibody was partially detected by mAb 123C3. The shift of the signal towards a smaller apparent molecular weight denotes the loss of polySia-NCAM and the appearance of polySia-negative NCAM, as indicated. $\#_1$ designates a band appearing after endosialidase treatment that migrates at the apparent molecular weight of the endosialidase monomers (2) and cross-reacts with the IgG1- but not the IgG2a-specific secondary antibodies applied for the detection of NCAM and polySia, respectively. $\#_2$ designates a signal caused by the heavy chain of the antibody used for IP.

References

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