Supporting Information

Combined sialic acid and HDAC inhibitor treatment upregulates the neuroblastoma antigen GD2

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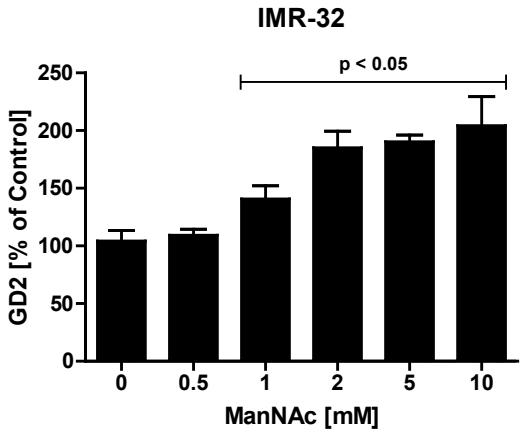

Figure S1. ManNAc enhances GD2 expression on IMR-32 cells.

Effect of ManNAc supplementation on GD2 expression of IMR-32 cells. Cells were cultured for three days with 0-10 mM ManNAc and GD2 expression was assessed by flow cytometry. Bar diagram shows mean percentage GD2 expression ± SEM normalized to control (n = 3).

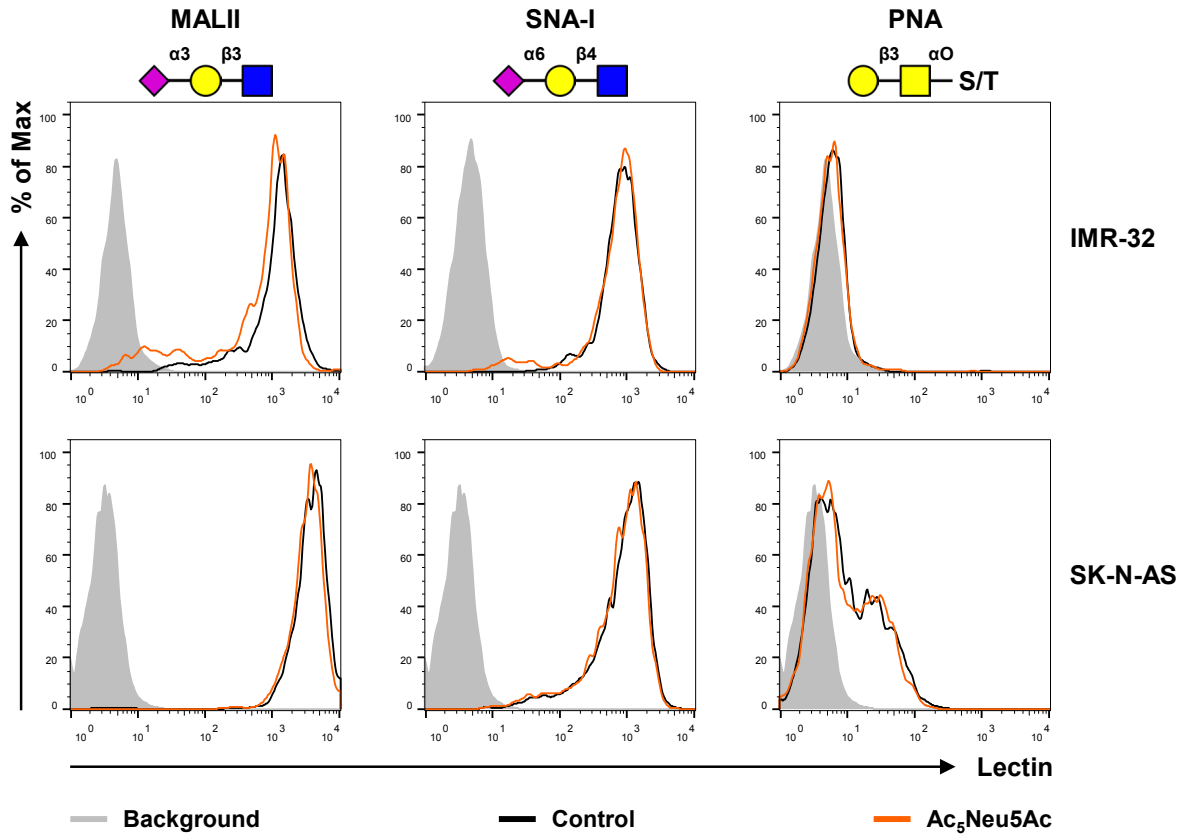

Figure S2. Cell surface sialylation of IMR-32 and SK-N-AS cells.

IMR-32 (upper row) and SK-N-AS (lower row) cells were cultured for 3 days with control or $Ac_5Neu5Ac$, stained with lectins and analyzed by flow cytometry. Histograms show representative binding of MALII (left), SNA-I (middle) and PNA (right).

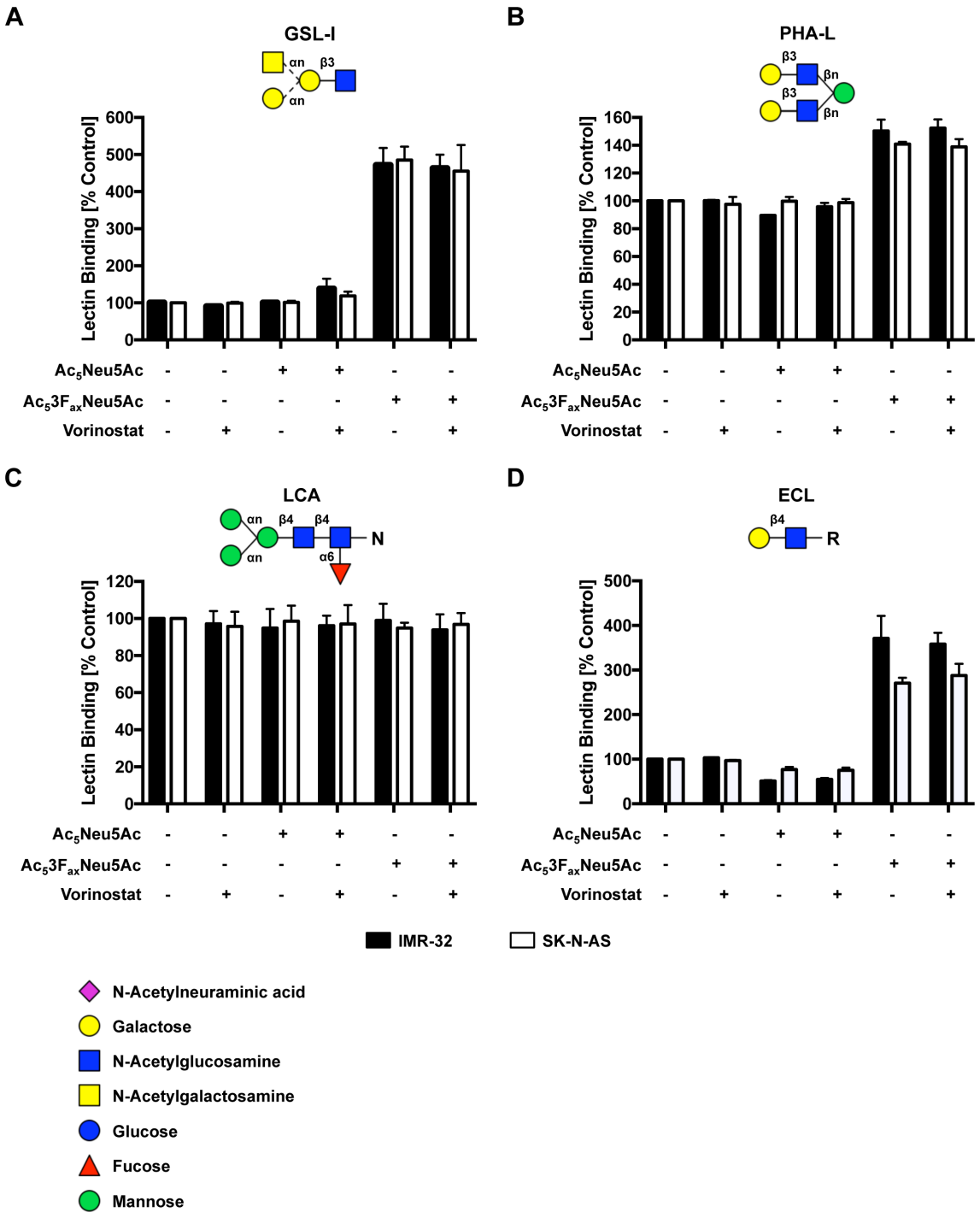

Figure S3. Effect of sialic acid analogues combined with Vorinostat on sialylation and glycosylation.

IMR-32 and SK-N-AS cells were cultured for 3 days with control, $Ac_5Neu5Ac$ or $Ac_53F_{ax}Neu5Ac$ and the last 24 hours of culture Vorinostat was added. Cells were stained with lectins and analyzed by flow cytometry. The presence of GalNAc and galactose was determined with GSL-I (**A**), complex branched glycans by PHA-L (**B**), branched (N-)glycans and mannose by LCA (**C**), and uncapped terminal Galβ1-4GlcNAc residues by ECL (**D**). Fluorescence intensities of the lectins were normalized to the respective untreated cell controls and calculated as mean percentage ± SEM lectin binding (n = 2-4).

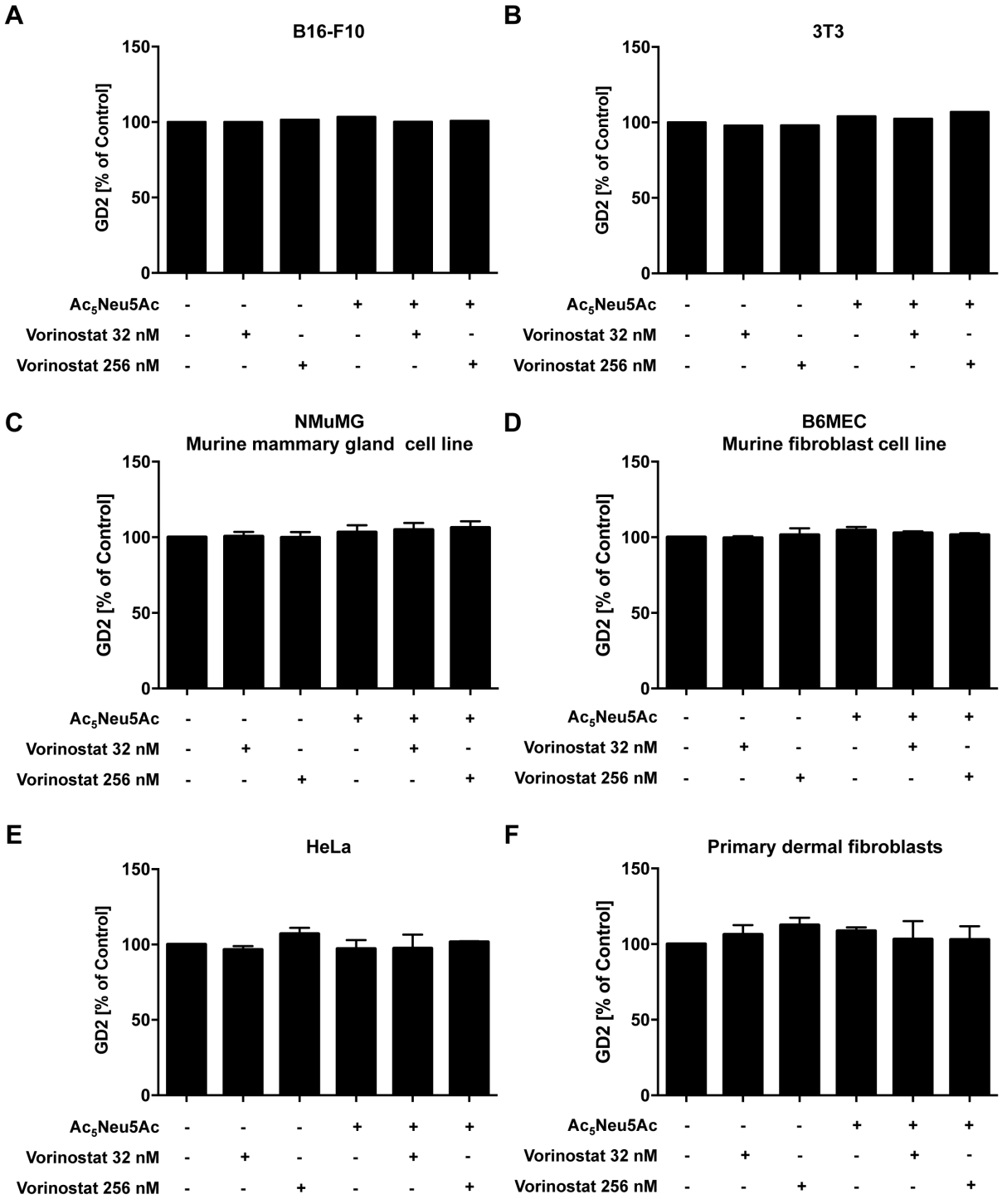

Figure S4. **Ac_5Neu5Ac and Vorinostat do not induce GD2 expression in non-neuroblastoma cells.** Effect of Ac_5Neu5Ac, Vorinostat or the combination on GD2 expression in B16-F10 (**A**), 3T3 (**B**), NMuMG (**C**), B6MEC (**D**) and HeLa (**E**) cells and primary human dermal fibroblasts (**F**). These cells were treated for three days with 2 mM Ac_5Neu5Ac. The last day of the culture, 32 nM or 256 nM Vorinostat was added and GD2 expression was assessed by flow cytometry. Bar diagram shows mean percentage GD2 expression ± SEM normalized to control (n = 1 for B16-F10 and 3T3 cells, n = 2 for HeLa cells, n=3 for NMuMG, B6MEC cells and primary dermal fibroblasts).