

SUPPLEMENTAL MATERIAL

Contents:

Supplemental Tables

Supplemental Figures

Supplemental Table S1: Acceptor list *

Enzyme activity measured	Acceptors used
Galactosyltransferase (5mM):	
β 1,3GalT	GalNAc α -PABA GalNAc α -sp-biotin
β 1,4GalT	Gal β 1,3(GlcNAc β 1,6)GalNAc α -sp-biotin Gal β 1,3(GlcNAc β 1,6)GalNAc α -O-Bn
β 1,3/4GalT	GlcNAc β 1,6GalNAc α -sp-biotin
Sialyltransferase (0.6mM):	
ST3/6[Gal β 1,3GalNAc]	Gal β 1,3GalNAc α -sp-biotin Gal β 1,3GalNAc α -PABA
ST3[Gal β 1,3GalNAc]	Gal β 1,3(GlcNAc β 1,6)GalNAc α -O-Bn
ST3/6[Gal β 1,4GlcNAc]	Gal β 1,4GlcNAc β -sp-biotin Gal β 1,4GlcNAc β 1,3Gal β 1,4GlcNAc β -O-Bn Gal β 1,4GlcNAc β 1,3Gal β - Φ -NO ₂
ST3/6[Gal β 1,3GlcNAc]	Gal β 1,3GlcNAc β 1,3lac β - Φ -NO ₂
Fucosyltransferase (1.5mM) **: 	
α 1,3FT[Gal β 1,4GlcNAc]	Gal β 1,4GlcNAc β 1,3Gal β - Φ -NO ₂ Gal β 1,4GlcNAc β 1,3Gal β 1,4GlcNAc β -O-Bn
α 1,4FT[Gal β 1,3GlcNAc]	Gal β 1,3GlcNAc β 1,3lac β - Φ -NO ₂
α 1,3FT[SialylLacNAc]	Neu5Ac α 2,3Gal β 1,4GlcNAc β -PAA-biotin
* Table lists enzyme activities measured in the current study along with a list of synthetic acceptors used. Concentration of acceptor in individual assays is indicated. These were greater than reaction K _M in all cases. When more than one product may form during a reaction, multiple enzyme activities measured or types of potential glycosidic bonds formed are specified in the left column.	
** α 1,2FT activity is low in breast cancer cell lines and thus enzyme activity for LacNAc based acceptors is attributed to either α 1,3FT or α 1,4FT alone.	

Supplemental Table S2: Enzymes activity in breast cancer cells

	ZR-75-1 (metastatic ER+ ductal carcinoma)	MCF7 (metastatic ER+ ductal adenocarcinoma)	T-47D (metastatic ER+ ductal carcinoma)	DU4475 (non-metastatic triple negative carcinoma)
Cell type	Luminal	Luminal	Luminal	Basal
β 1,3GalT	+	-	++	++
β 1,4GalT	-	-	-	-
ST3[Gal β 1,3GalNAc]	+++	+++	++	+
ST3/6[Gal β 1,4GlcNAc]	+	-	+	-
α 1,3FT[Gal β 1,4GlcNAc]	+	+	+	+
α 1,3FT [SialylLacNAc]	+	+	-	-
α 1,4FT[Gal β 1,3GlcNAc]	++	+	+	+

“-”: Enzyme activity comparable to negative control, “+”: 2-10 times negative control, “++”: 10-30 times negative control, “+++” >30 times negative control.

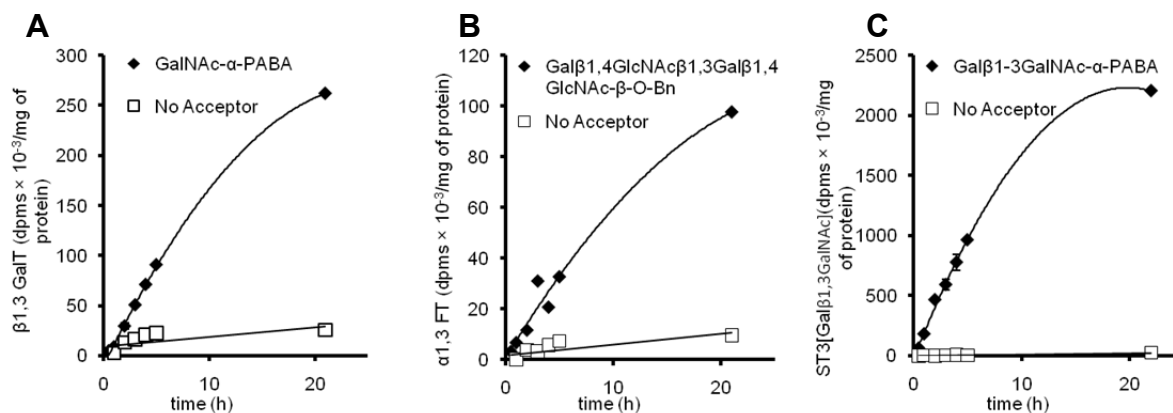


Figure S1: Time course of glycosyltransferase activity in cancer cell line: 1mg/mL ZR-75-1 cell lysate was used as catalyst for $\beta 1,3$ GalT (panel A), $\alpha 1,3$ FucT (panel B) and $\alpha 2,3$ SialylT (panel C) assays. Reaction conditions were identical to Fig. 2-3 in the main manuscript. In this case, samples withdrawn at indicated times were analyzed using RP-TLC. As seen, the extent of enzymatic reactions proceeds approximately linearly during the first 6h for all assays.

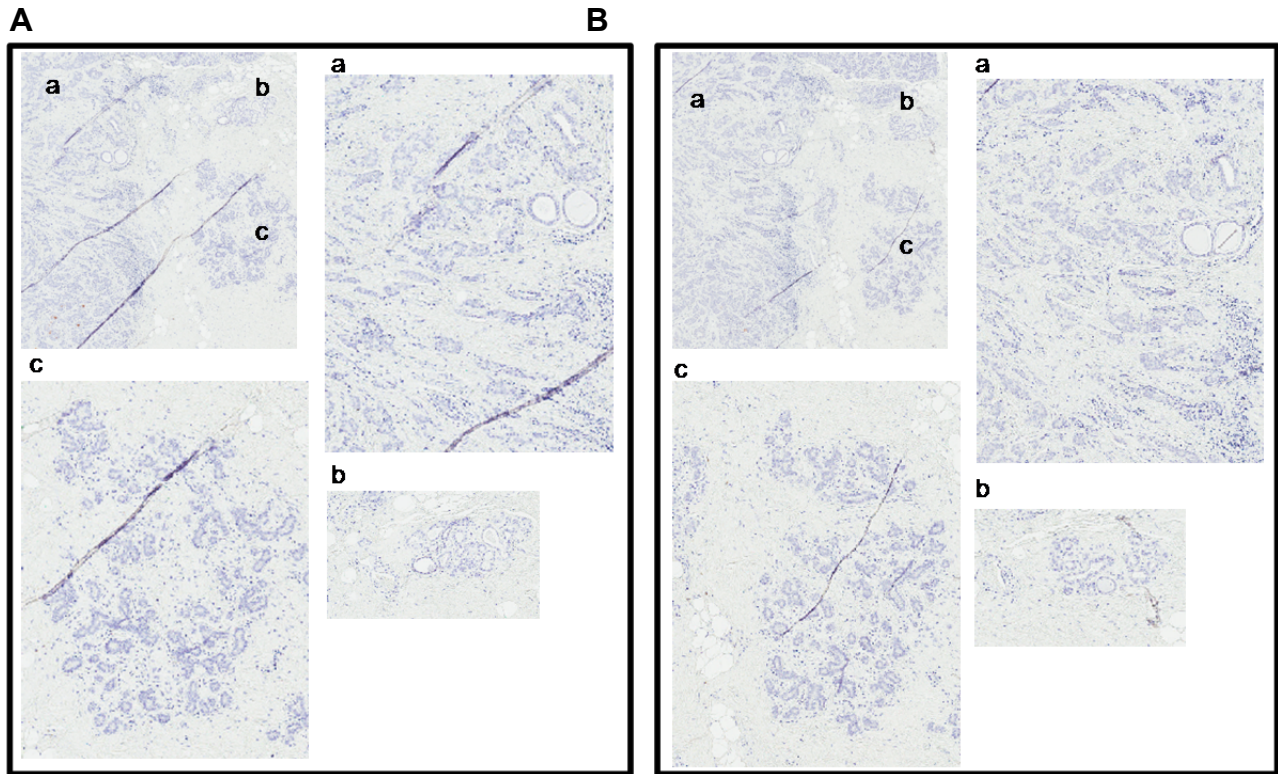


Figure S2: Histochemistry images without sialidase. *A.* Staining with anti T-antigen antibody for breast tumor section with adjacent normal. The section is labeled as a. Tumor section b. Ductal region in normal section c. Lobular section in normal section. *B.* Staining with PNA lectin for breast tumor section with adjacent normal. The section is labeled identical to panel *A.*

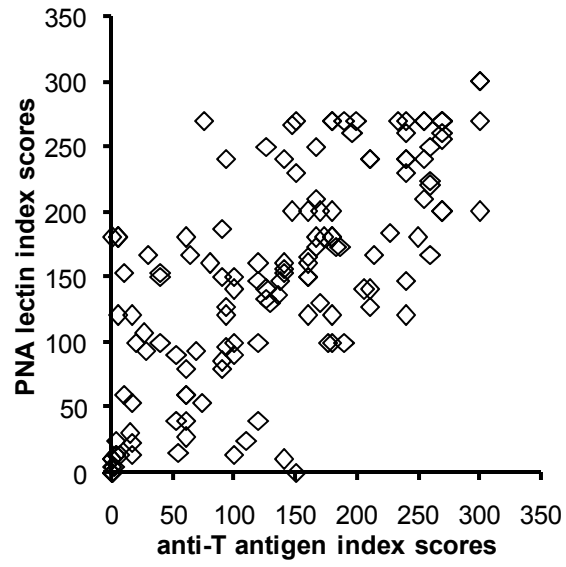


Figure S3: Correlation between PNA lectin and anti T-antigen mAb staining in breast TMA after neuraminidase treatment: Pearson correlation score = 0.725.

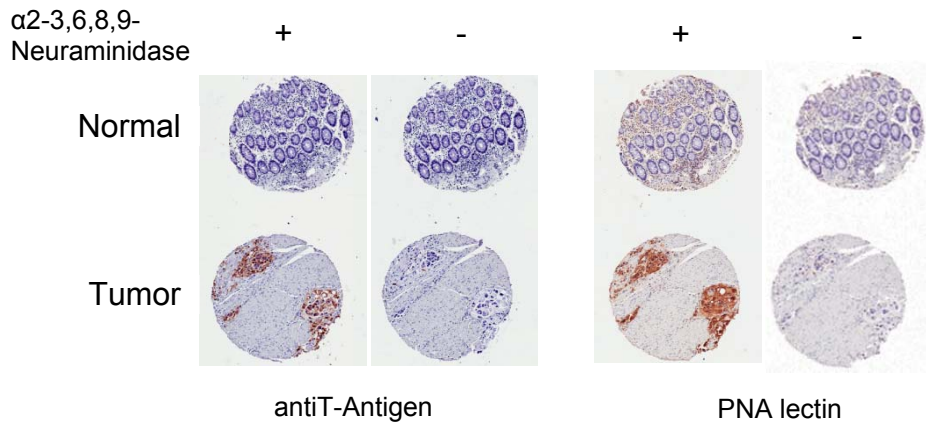


Figure S4: Specific case of matched colon tumor and normal sample. Images of matched normal and tumor section of colon cancer patient stained with anti T-antigen and PNA lectin, after treatment with (‘+’) or without (‘-’) neuraminidase.

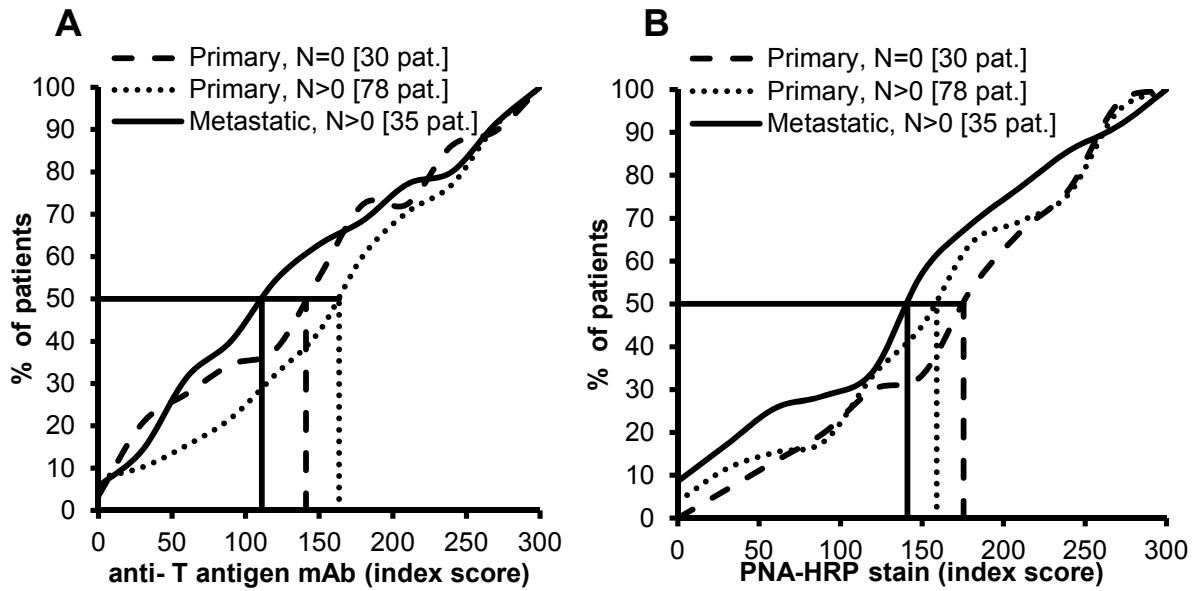


Figure S5: Correlation of amount of staining with metastatic stage. *A.* Plot showing variation of distribution of number of patients in the 3 different groups viz. non-metastatic (Primary, N=0), primary metastatic tumor (Primary, N>0), metastasized tumor (Metastatic, N>0) with anti-T antigen staining (panel **A**) and PNA-HRP staining (panel **B**). Number of patients in each category is provided in square brackets. As seen, metastasized tumors have less staining of both anti-T-antigen mAb and PNA-HRP.