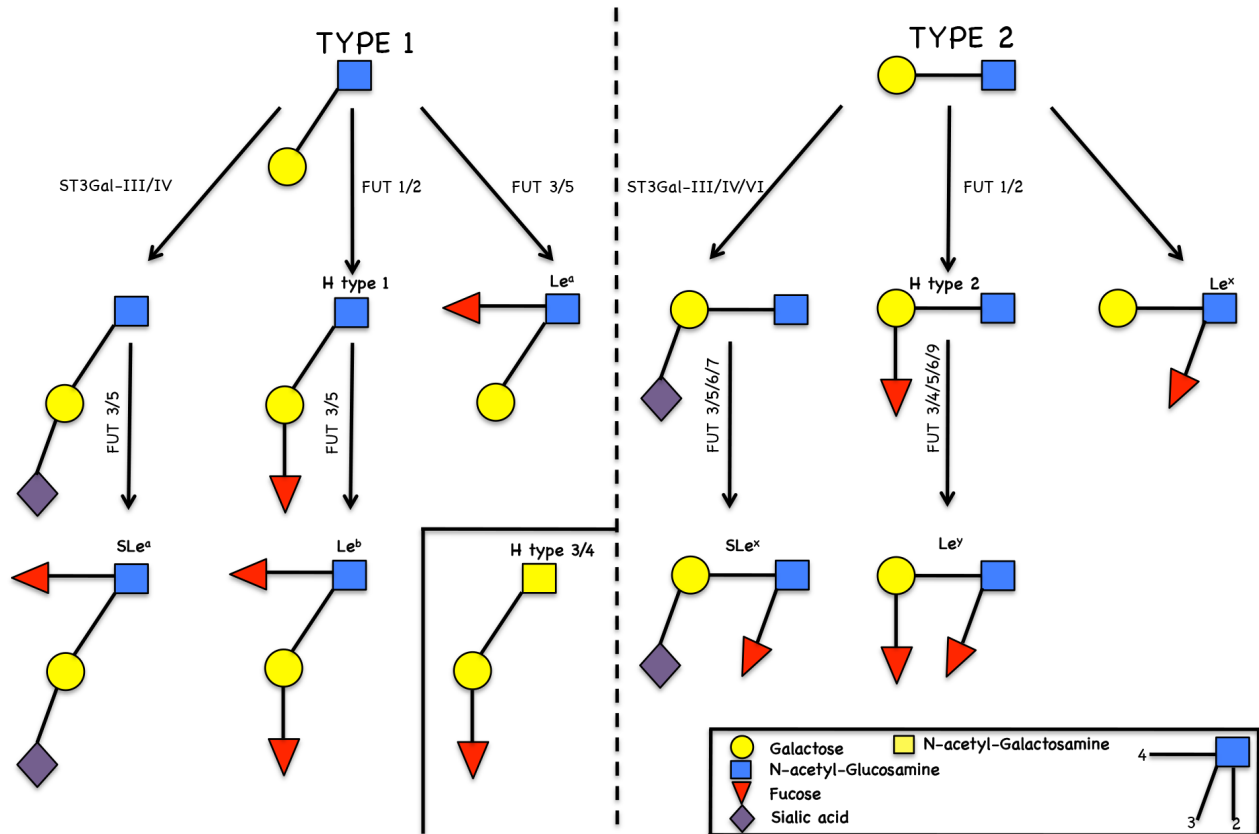


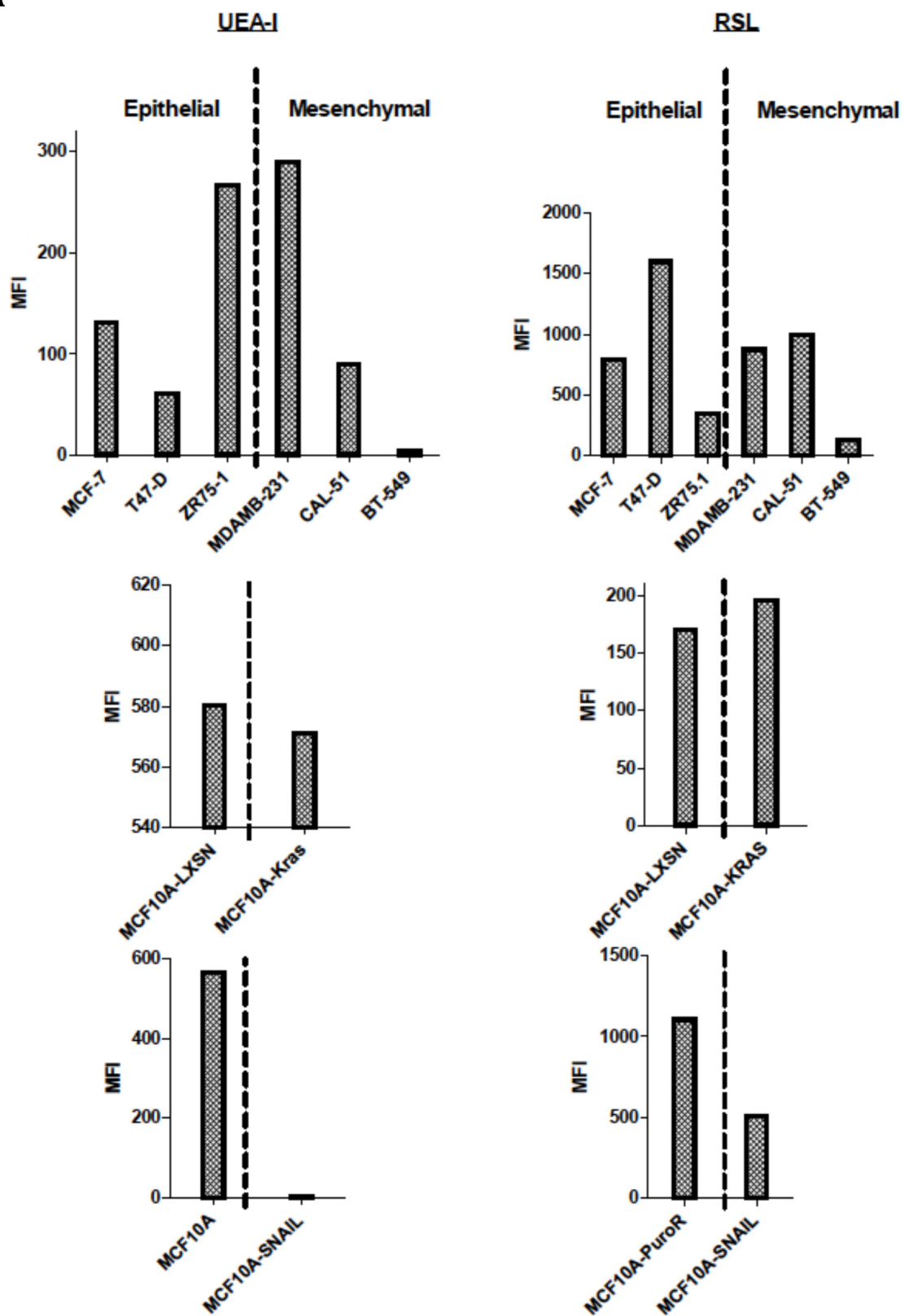
Carcinoma-associated fucosylated antigens are markers of the epithelial state and can contribute to cell adhesion through *CLEC17A* (Prolectin)

Supplementary Materials

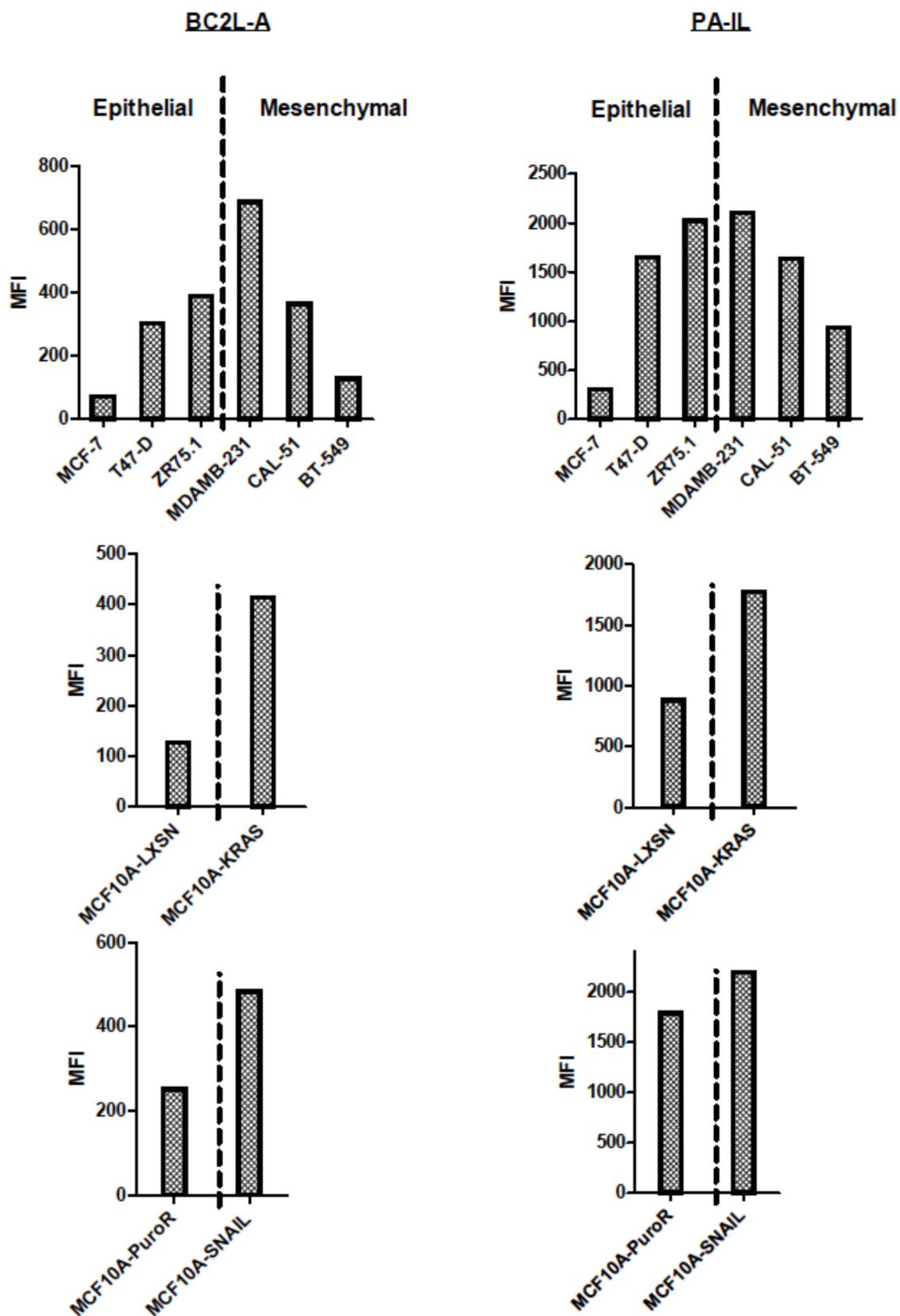


Supplementary Figure S1: Biosynthetic pathway of the histo-blood group antigens. α 1, 2 fucosyltransferases (FUT), α 1-3/4 FUT and α 2-3 sialyltransferases (ST3Gal) mainly responsible for synthesis of the various intermediates are indicated. N-acetyl galactosamine or galactose moieties can be added to the terminal galactose to make the A or B antigens respectively.

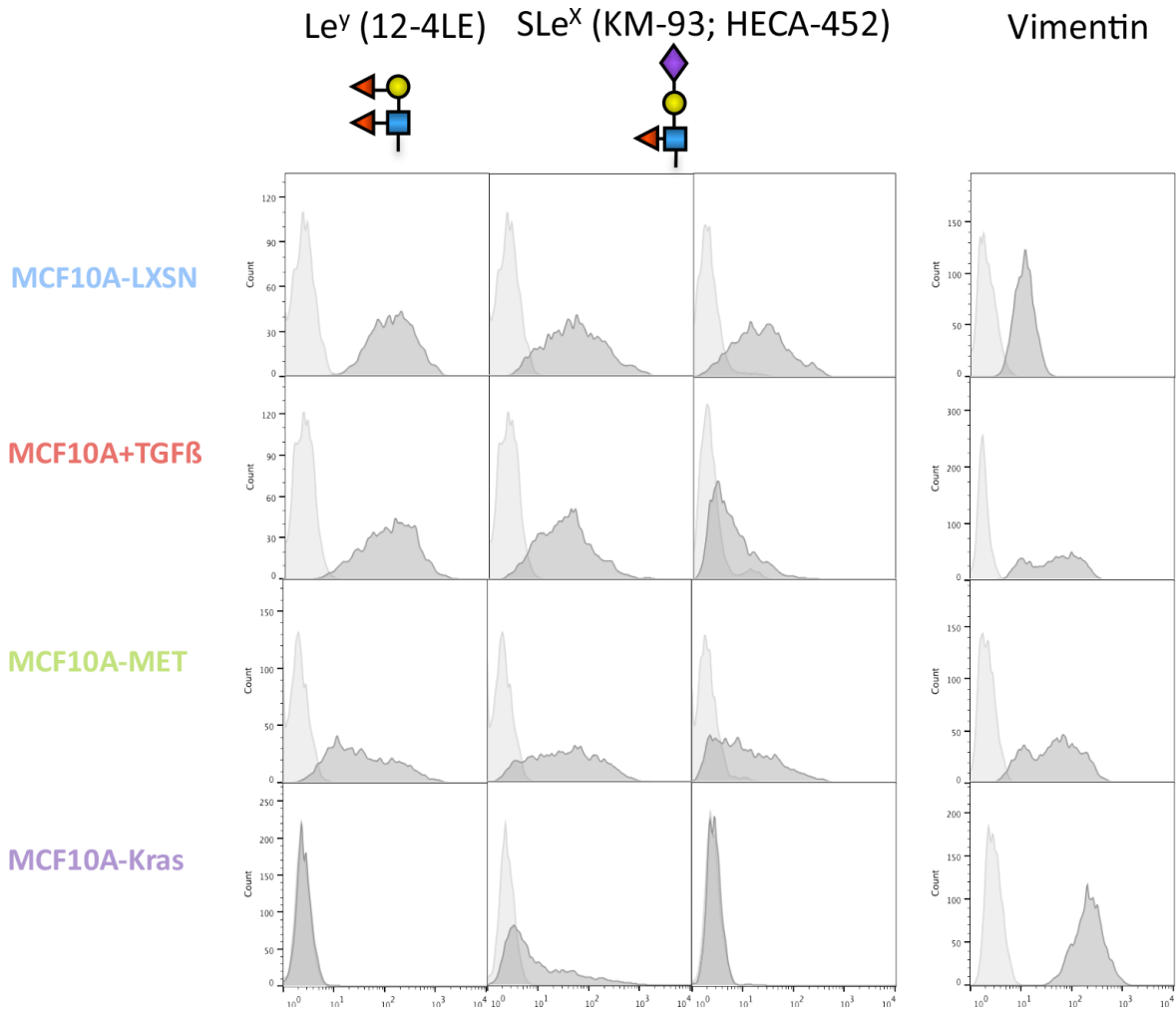
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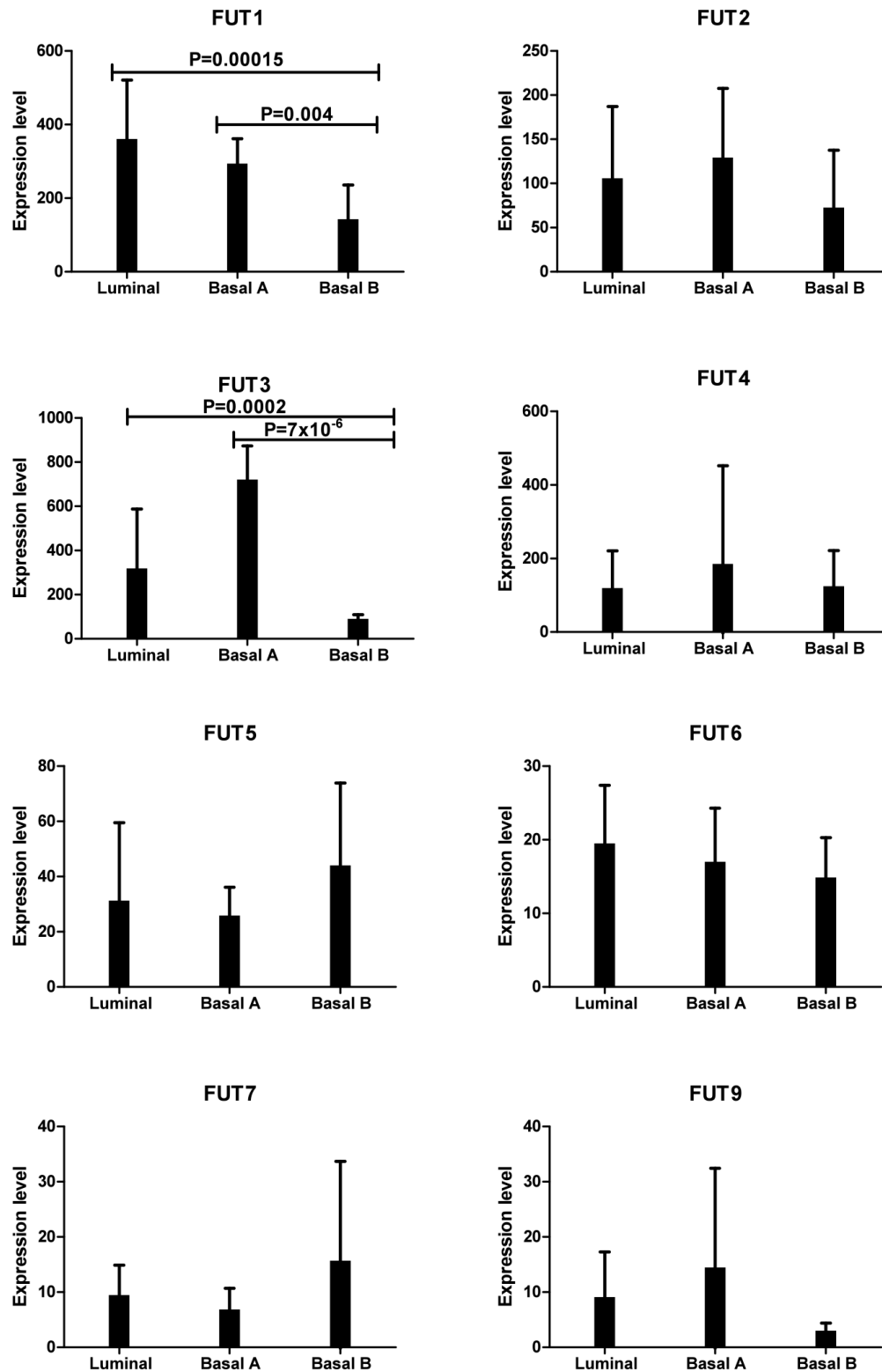
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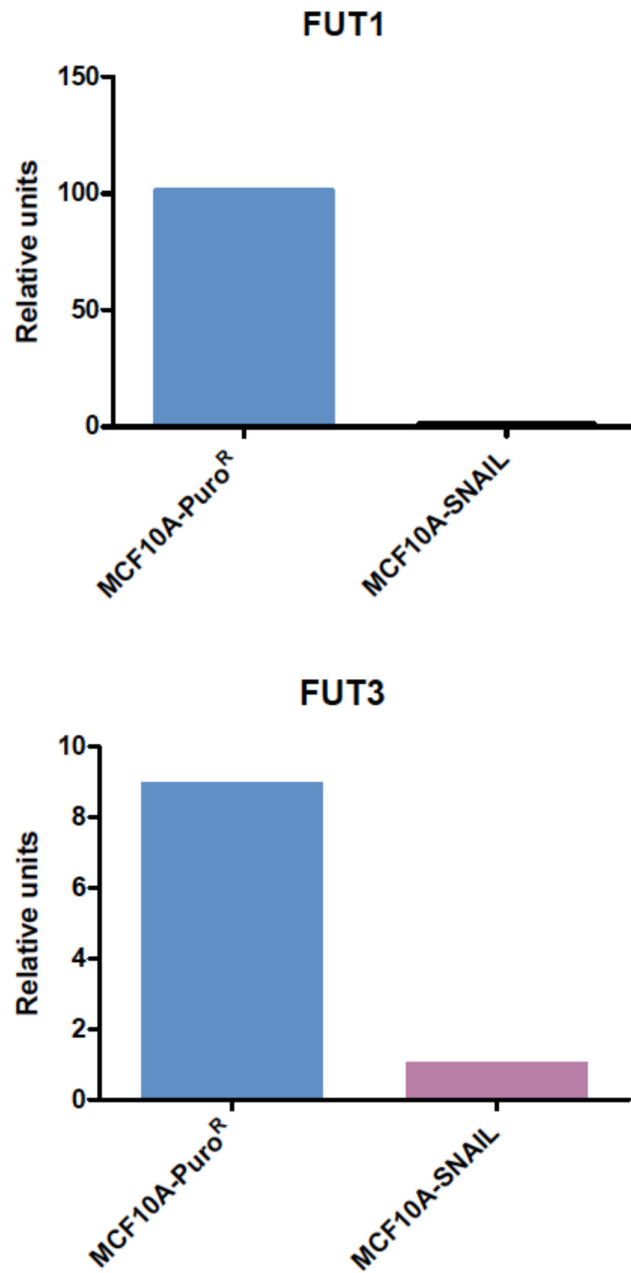
Supplementary Figure S2: Lectin binding on breast cell lines. The indicated breast cell lines have been stained with recombinant biotinylated lectins followed by streptavidin-PE and analysed by flow cytometry. Mean fluorescence intensities are presented. The results are representative of at least two experiments.



Supplementary Figure S3: Expression of fucosylated antigens by MCF10A cells treated with TGF- β . MCF10A-LXSN epithelial cells were treated with either 0,5 μ M A83-01 (MCF10A-LXSN) or 5 ng/ml TGF- β R&D (MCF10A-TGF) during four days. TGF- β was then withdrawn and cells were grown four more days in presence of 0,5 μ M A83-01 (MCF10A-MET). Cells were analyzed by flow cytometry as described in Figure 1.



Supplementary Figure S4: Fucosyltransferases genes expression in breast cell lines. Expression levels of the FUT α 1-2 (FUT1 and 2) and FUT α 1-3/4 (FUT3, 4, 5, 6, 7 and 9) genes were extracted from a transcriptomic dataset of 51 breast cancer cell lines available in the NCBI database (GEO n° GSE12777) thanks to the online analysis tool BioGPS (<http://www.biogps.org>) and cell lines were sorted according to their overall gene profile subtypes (according to the classification of Neve RM et al, *Cancer Cell* 2006 and Kao J et al *PlosOne* 2009). Mean expression level for each *FUT* gene and standard deviations are shown.



Supplementary Figure S5: Fucosyltransferases genes expression in MCF10A-Puro^R/MCF10A-SNAIL. Total RNAs were extracted from the different cell lines, cDNAs were then synthesized using oligo-dT and subjected to qPCR analysis with *FUT1*, *FUT3* and *GAPDH* specific primers and probes. *FUT1* and *FUT3* expression levels are presented relatively to *GAPDH* expression. Data shown are representative of three independent experiments.

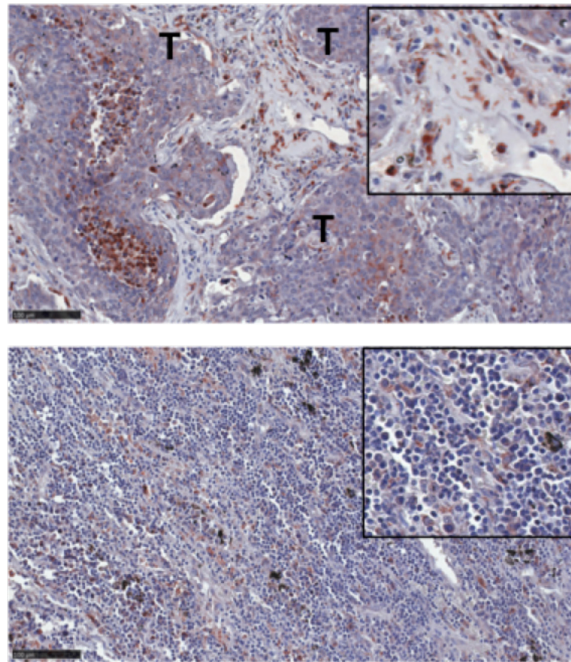
A

-pFUT1

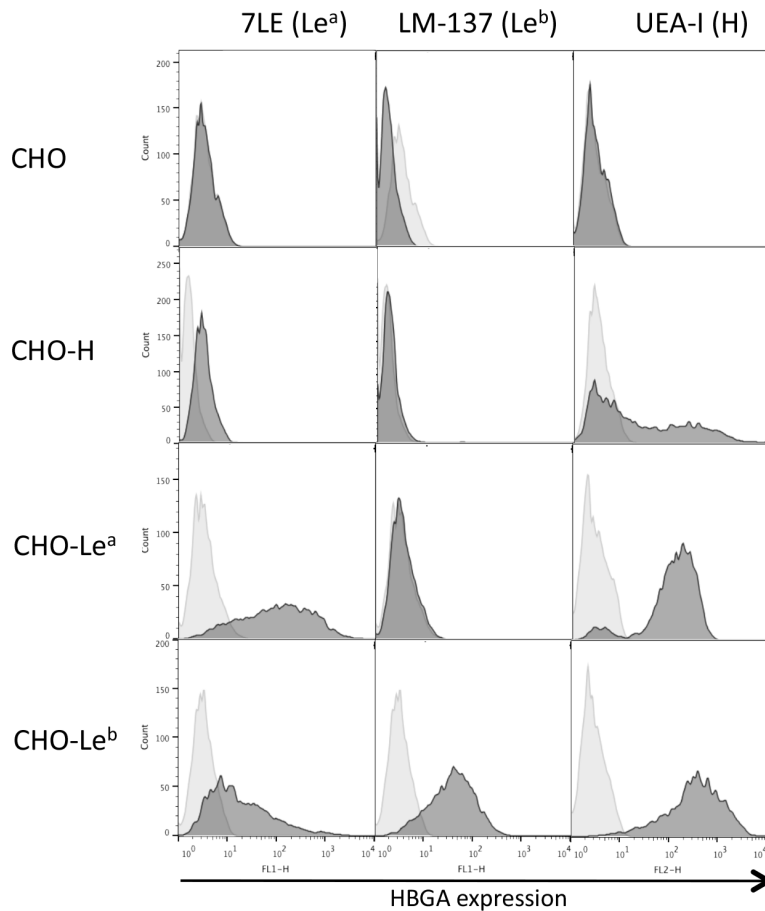
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-pFUT3

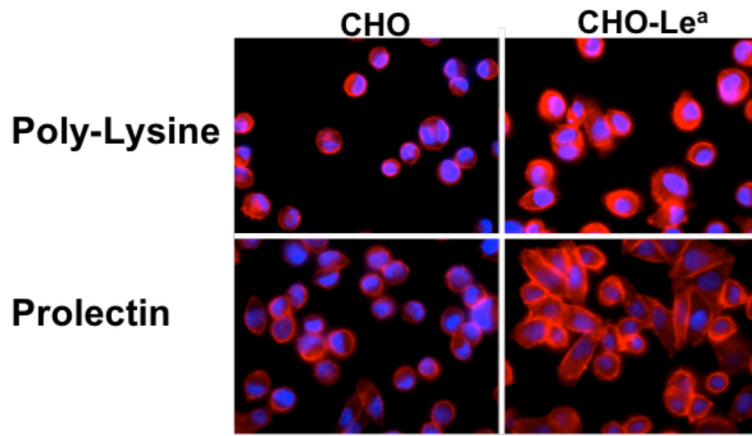
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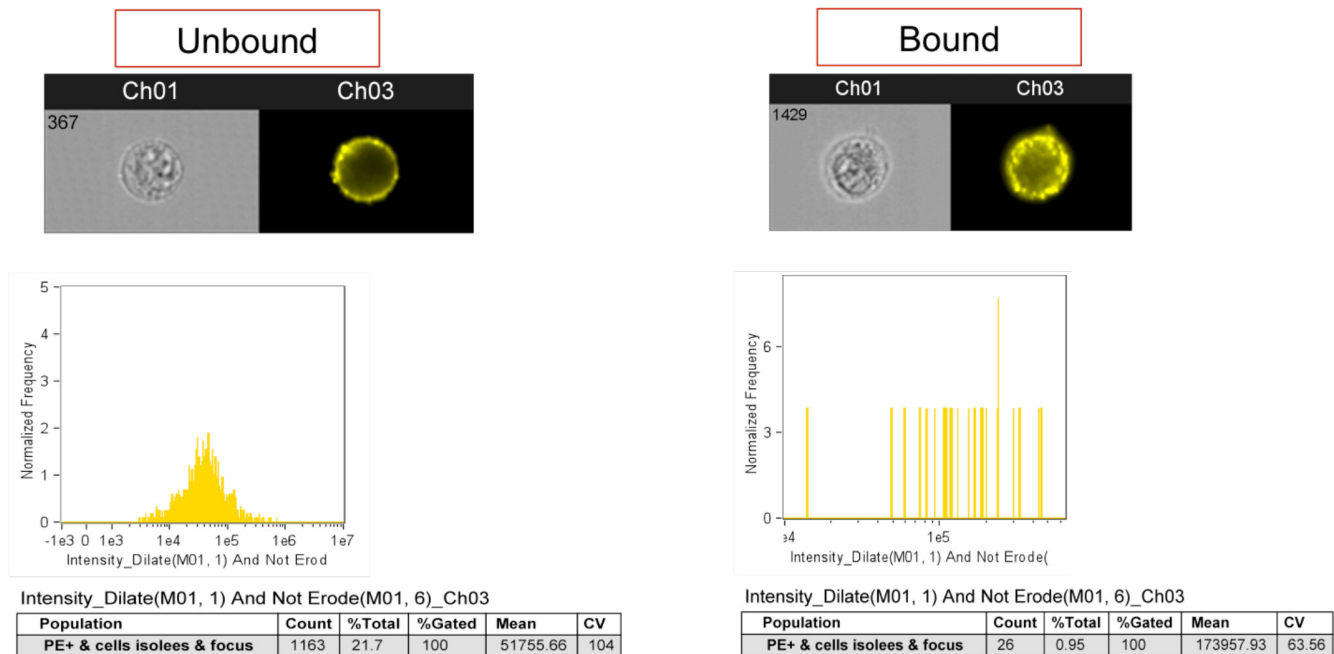
Supplementary Figure S7: Staining by prolectin of leucocyte populations infiltrated in tumors. Tetramers of biot-prolectin CRD/streptavidin-HRP were bound to a paraffin embedded TMA from lung cancer. An example of tumor is shown with infiltrating leucocytes strongly stained by prolectin as well lymphoid tissue from the same patient. 20x magnifications are shown with inlets at 40x magnification. (T = Tumor).



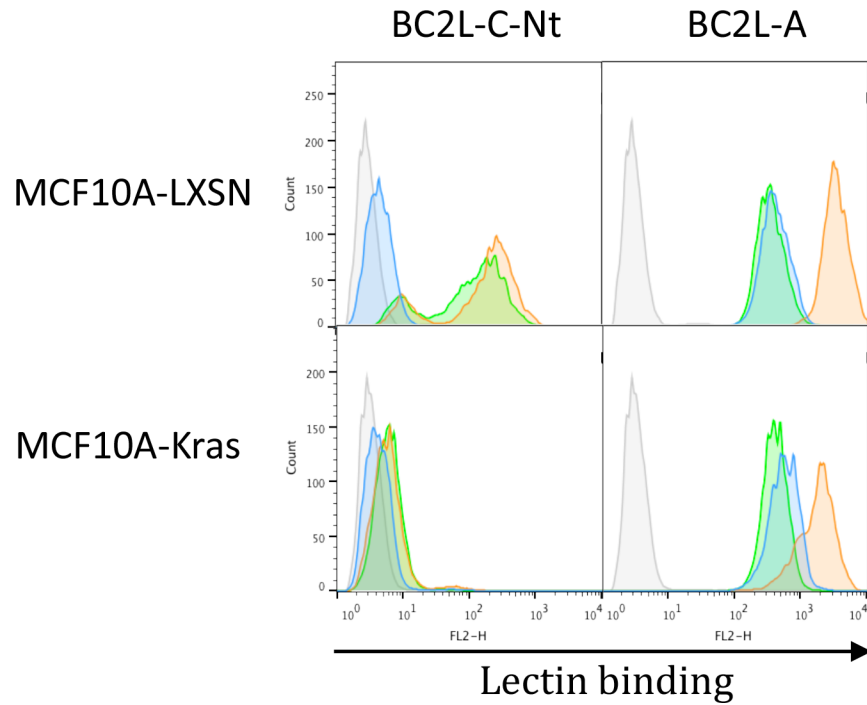
Supplementary Figure S8: Phenotyping of the CHO, CHO-H, CHO-Le^a and CHO-Le^b. The CHO cell lines were analysed by flow cytometry with the antibodies 7LE (anti-Le^a) and LM137 (anti-H type 1/Le^b) followed by anti-mouse-FITC and the biotinylated lectin UEA-I (α 2-Fucose specific) followed by streptavidin-PE.



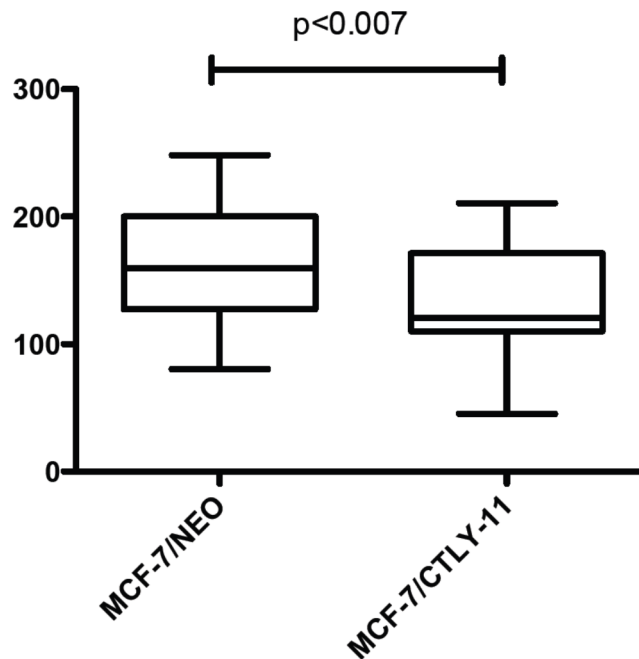
Supplementary Figure S9: Comparison of CHO-Le^a binding to poly-lysine and a prolectin coating. Lab-tek chamber slides have been coated with prolectin/streptavidin tetramers or poly-L lysine. CHO and CHO-Le^a cells were bound for 2 hours. After careful and thorough washes, the cells were stained with Hoechst (nuclei) and phalloidin (actin). Pictures were taken at ×63 magnification with a Zeiss Axiovert 200 microscope.



Supplementary Figure S10: The cells that bind to prolectin under shear flow conditions are those having the highest expression of prolectin ligands. DU-145 were injected into Prolectin-coated BioFlux channels. Unbound cells were washed away with PBS (Ca,Mg) and collected in the output well. Bound cells were detached by trypsin treatment. Both bound and unbound cells were stained with Prolectin/Strep-PE tetramers and analyzed with the Amnis Image Stream X II flow cytometer.

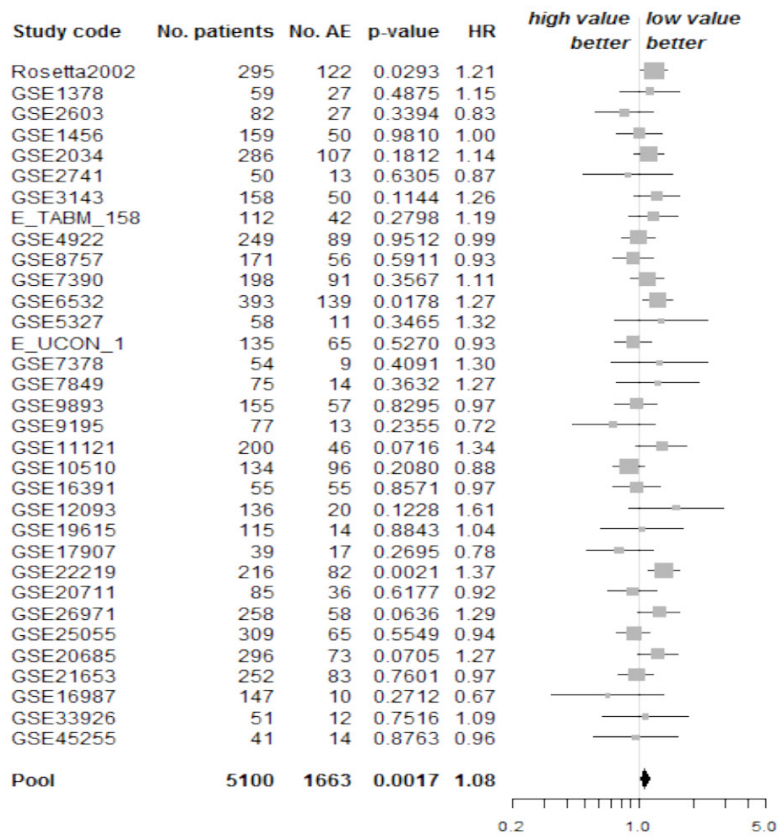


Supplementary Figure S11: Inhibition of fucosylation by 2F-Fuc treatment and increase of mannosylation by KFN treatment on MCF10A cells. MCF10A-LXSN and MCF10A-Kras(v12) were treated with DMSO (green), 5 μM kifunensin (orange) or 400 μM 2 fluoro-fucose (blue) during four days. Cells were then harvested, probed with BC2L-C-Nt-biot (to stain fucosylated structures) or BC2L-A-biot (to stain high mannose structures) followed by streptavidin-PE and analyzed on FACSCalibur flow cytometer.

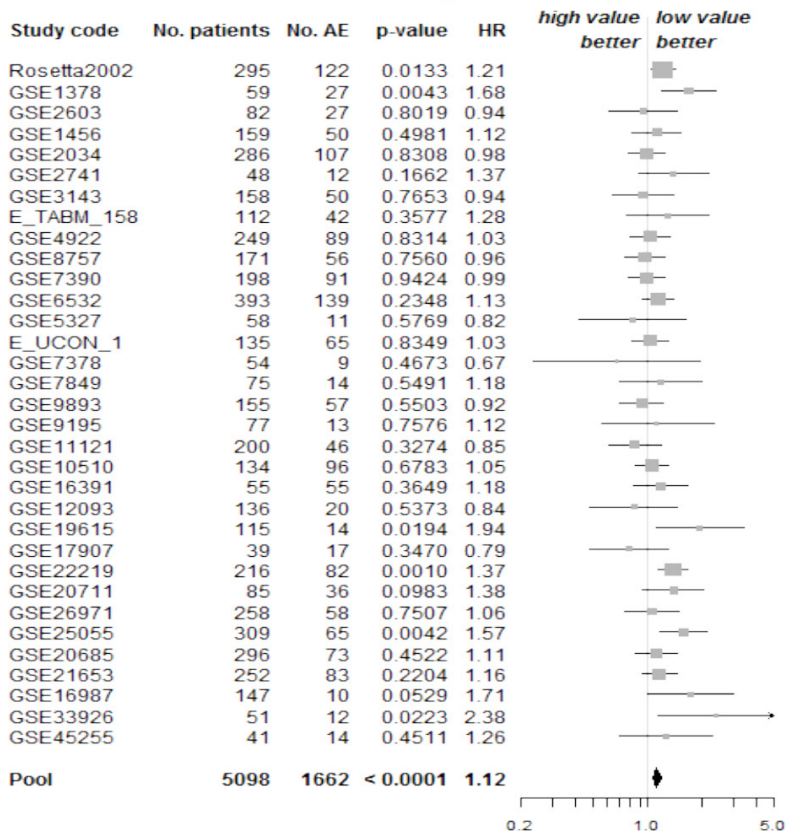


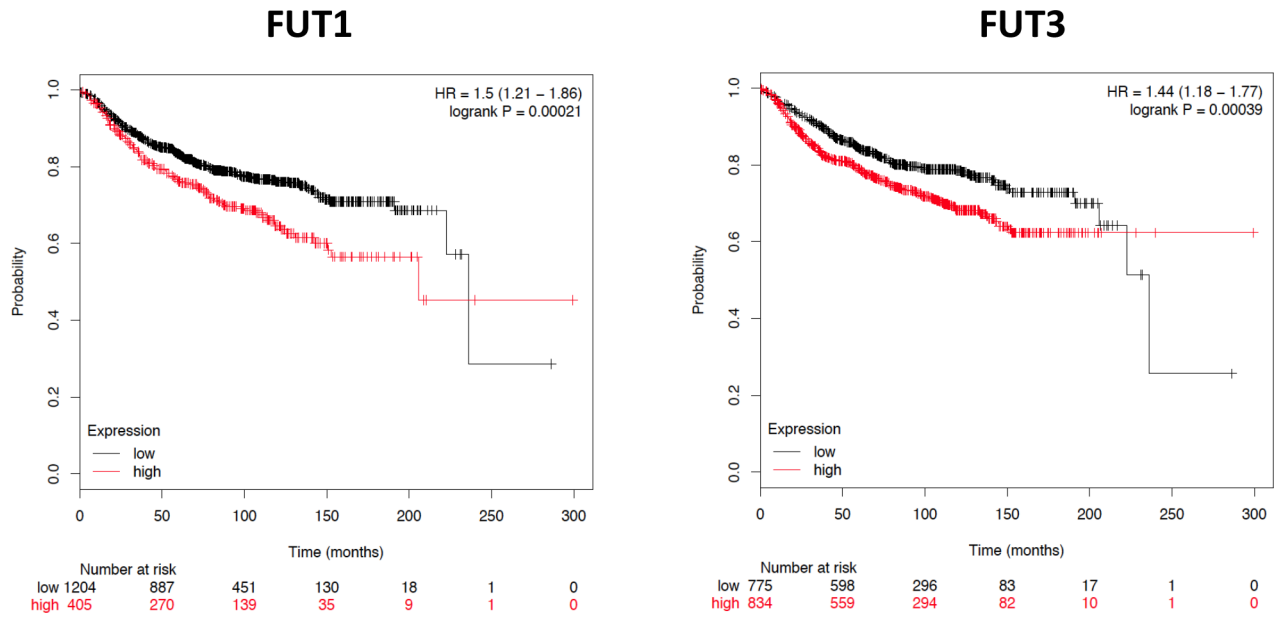
Supplementary Figure S12: Interaction of MCF-7 cells with prolectin-expressing fibroblasts. Rat6-Neo and Rat6-CTLY11 or Rat6-CTLY11 in presence of 25 mM galactose or fucose were injected in BioFlux channels covered with a layer of MCF-7 cells under a pressure of 0,05 Dyn/cm². Films of 1 minute (600 pictures) were acquired on a Leica DMI 6000B microscope using the Metamorph software. Chronophotographic images, corresponding to 10 pictures segments of the films, were constructed using the Fiji software and distances covered by individual cells were measured. Student *t*-tests were performed to assess statistical significance.

FUT1: Forest plot



FUT3: Forest plot





Supplementary Figure S13: Prognostic value of *FUT1* and *FUT3* in breast cancer. (A) Computational analysis of breast cancer patients data were performed using the online platform bc genexminer (<http://bcgenex.centregauducheau.fr>). *FUT1* and *FUT3* expression are grouped into high and low level expression by comparison with the median expression of each gene. Analysis shown was done on all the patients irrespective of their nodal or estrogen receptor status. (B) Computational analysis was also performed using the online platform KM plotter (<http://kmplot.com/analysis/index.php?p=service&cancer=breast>) using the Jetset best probe set and automatic selection of the best cutoff to split patients. Analyses are shown on all patients and the time represents distant metastasis free survival.

Supplementary Table S1: Lectins

Lectin	Species	Specificity	Main Ligands	Working concentration
PA-IL	<i>Pseudomonas aeruginosa</i>	Gal	Gb3	20 µg/ml
RSL	<i>Ralstonia solanacearum</i>	Fuc	Blood group antigens	0,1 µg/m
BC2L-A	<i>Burkholderia cenocepacia</i>	Man	Dimannoside	2 µg/ml
BC2L-C-Nt	<i>Burkholderia cenocepacia</i>	Fuc	H type 1/3, Lewis Y	5–10 µg/ml
UEA-I	<i>Ulex europeaus</i>	Fuc (α 1, 2)	H type 2	1 µg/ml

Supplementary Table S2: Primers and probes used for qRT-PCR analysis

Gene	Acces. nb	Forward primer	Reverse primer	Probe
<i>FUT1</i>	NM_000148	AGGTATAAACACAC CCTCTGTGCTT	GAGTTCAGGGACAG ACAGT GGTT	AAACTGGCAGGTACCGT GCTCATGTC
<i>FUT3</i>	NM_000149	GGGATCCCTTTTCG TCACACT	CGAACTGGTCTAAG CCTTGCA	AGGTGACCTACAGGCTC CGCTCGA
<i>CDH1</i>	NM_004360.3	GGGCGAGTGCCAA CTGGACC	CCAGCGGCCCTTC ACAGTC	/
<i>SNAIL</i>	AF155233.1	GACCCAGTGCCTC GACCACTA	CAGCAGGTGGGCCT GGTCGTA	/
<i>SNAIL2</i>	NM_003068.4	GACCCGCTGGCAA GATGCCG	GCTGGGCGTGGAAT GGAGCA	/
<i>b-Actin</i>	X00351.1	AGAAAATCTGGCAC CACACC	CAGAGGCGTACAGG GATAGC	/

Supplementary Table S3: Synthetic oligosaccharides used for the ELISA

Poly-Acrylamide (PAA)	A type 2	GalNAc α 3[Fuc α 2]Gal β 4GlcNAc β -R1
	A tri	GalNAc α 3[Fuc α 2]Gal β -R1
	A di	GalNAc α 3Gal β -R1
	B type 2	Gal α 3[Fuc α 2]Gal β 4GlcNAc β -R1
	B tri	Gal α 3[Fuc α 2]Gal β -R1
	B Di	Gal α 3Gal β -R1
	H Di	[Fuc α 2]Gal β -R1
	H type 2	[Fuc α 2]Gal β 4GlcNAc β -R1
	H type 3	[Fuc α 2]Gal β 3GalNAc α -R1
	Le^a	Gal β 3[Fuc α 4]GlcNAc β -R1
	Le^b	[Fuc α 2]Gal β 3[Fuc α 4]GlcNAc β -R1
	Le^x	Gal β 4[Fuc α 3]GlcNAc β -R1
	Le^y	[Fuc α 2]Gal β 4[Fuc α 3]GlcNAc β -R1
	Human serum albumine (HSA)	A tri
A hepta		GalNAc α 3[Fuc α 2]Gal β 3[Fuc α 4]GlcNAc β 3Gal β 4Glc β -R2
B tri		Gal α 3[Fuc α 2]Gal β -R2
H type 1		[Fuc α 2]Gal β 3GlcNAc β -R2
H type 2		[Fuc α 2]Gal β 4GlcNAc β -R2
Le^a		Gal β 3[Fuc α 4]GlcNAc β -R2
Le^b		[Fuc α 2]Gal β 3[Fuc α 4]GlcNAc β -R2
Le^x		Gal β 4[Fuc α 3]GlcNAc β -R2
Le^y		[Fuc α 2]Gal β 4[Fuc α 3]GlcNAc β -R2

Supplementary Table S4: Surface expression of Le^x and Le^y on the tumor cell lines used for the prolectin binding experiments

Cell lines	Le^x (3E1)	Le^y (12-4Le)
MCF-7	+++	+++
HT-29	+++	+
OVCAR-3	+++	+++
DU-145	++	+/-