

Figure S1. Inhibition of human anti-Le^C Abs (isolated from blood serum) binding to Le^C-PAA (as a coating antigen in ELISA) with GlcNAcβ1-OSer and other glycosides, top-down: Galβ1-3GlcNAcβ-OCH₂CH₂CH₂NH₂; Galβ1-3GlcNAcβ-OCH₂CH₂NH₂; GlcNAcβ-OCH₂CH₂CH₂NH₂; GlcNAcβ-OCH₂CH₂NH₂; GlcNAcβ-O-L-Ser(NAc); GlcNAcβ-O-D-Ser(NAc).

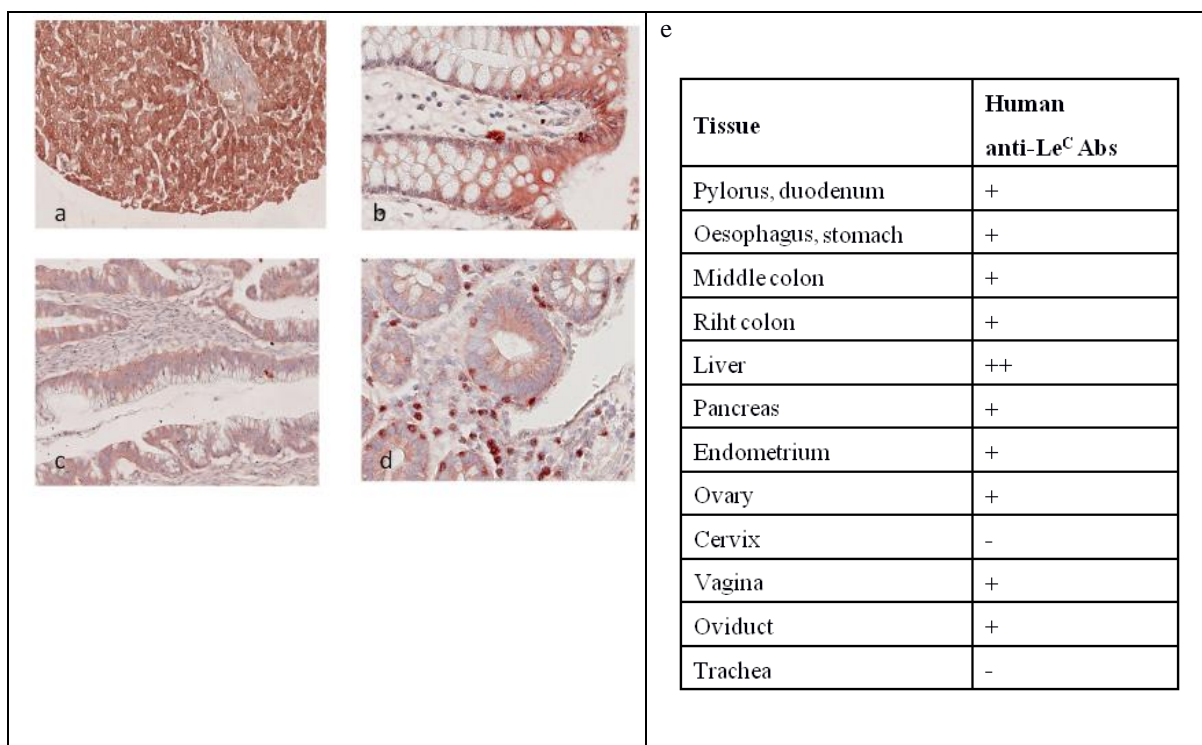


Figure S2. Binding of human anti-Le^C Abs to human tissues. Healthy tissue samples from 10 donors were used to construct a TissueMicroArray that was incubated with biotin-labeled anti-Le^C Abs (a-e). Binding was detected using peroxidase-labeled avidin and counterstaining performed with hematoxylin. Examples of typical staining results are shown. Liver (a), colon mucosa (b), colon carcinoma (c), normal appearing colonic mucosa adjacent to a tumor (d) and staining levels of healthy tissues (e). Blue color on the developed slides is due to hematoxyline staining of DNA/RNA containing cell structures (nuclei) and pink color is due to the bound antibodies.

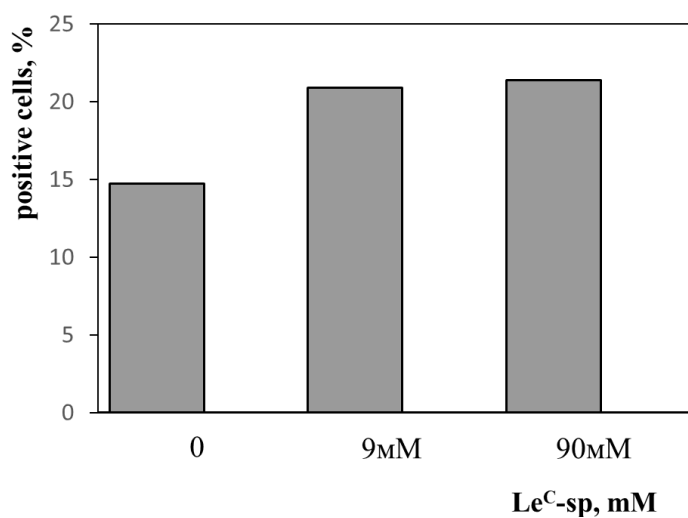


Figure S3. Interaction of human antibodies affinity-isolated using Le^C-Sepharose with ZR 75-1 cells (70% grown density of monolayer) in the presence of free Gal β 1-3GlcNAc β -sp (Le^C-sp) disaccharide, sp = OCH₂CH₂CH₂NH₂. Data of flow cytometry.

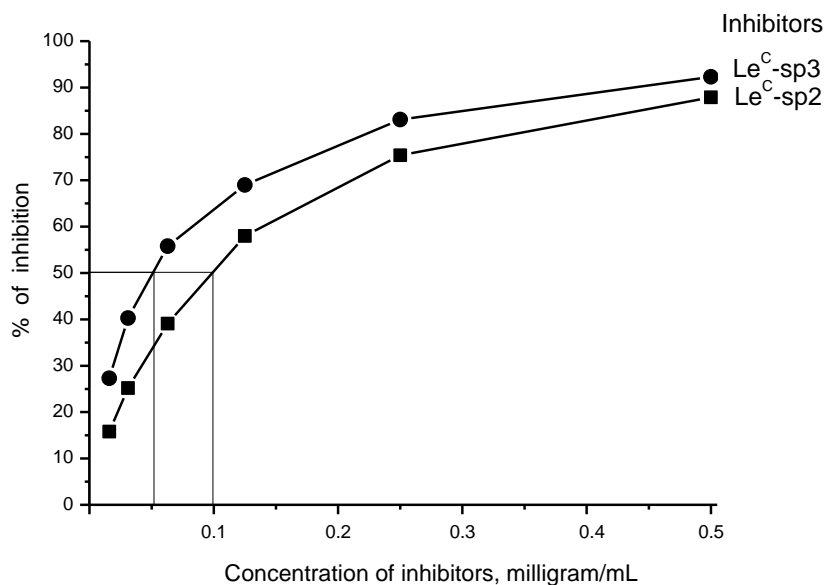


Figure S4. Evaluation of anti-Le^C nAbs affinity towards monomeric disaccharide Gal β 1-3GlcNAc and estimate of K_d for anti-Le^C antibodies using inhibition ELISA. K_d was determined as a value corresponding to the molar concentration of free monomeric antigen-inhibitors Le^C-sp3 (Le^C-OCH₂CH₂CH₂NH₂) and Le^C-sp2 (Le^C-OCH₂CH₂NH₂) necessary for saturation of half of all antigen-binding sites of antibodies, i.e. for 50% inhibition of binding of antibodies to the immobilized antigen (Le^C-PAA): $K_d = [Le^C] = I_{50\%}$ (g/L) = M (g/mol), where $[Le^C]$ is the molar concentration of the monomeric antigen, $I_{50\%}$ is mass concentration of free Le^C disaccharide necessary for 50% inhibition, M is molar mass of the disaccharide. The found values are: $I_{50\%}$ (Le^C-sp3) = 51 μ g/mL, $K_d = 1.4 \times 10^{-4}$ M.

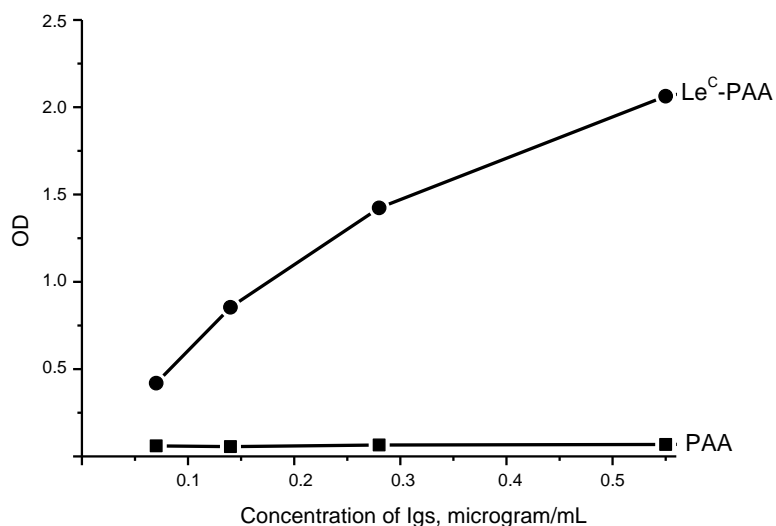


Figure S5. Affinity of the antibodies towards polymeric antigen, Le^C-PAA, estimated using ELISA. Immunological plates (NUNC MaxiSorb) were coated with Le^C-PAA. The affinity constant was evaluated as an amount of Abs equal to the concentration of Abs giving the signal intensity (OD) = 1 in the linear region of the *signal intensity vs. antibody quantity* plot, i.e. $K_d \sim [Abs]$. The concentration of anti-Le^C antibodies (at OD = 1) was 0.2 $\mu\text{g/mL}$, i.e. K_d is equal to 8.6×10^{-10} M.