Supplemental Figures

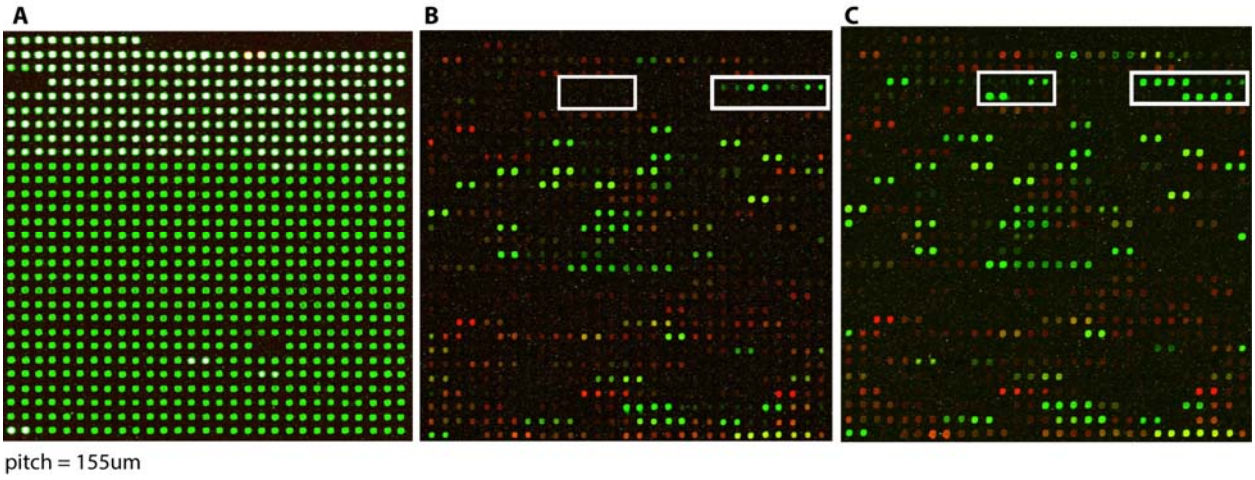

Figure S1, related to Glyco-antigen microarray fabrication, binding assay, and data processing. Representative images of printed glycan microarrays and serum profiling results.

Representative image of printed microarrays (A). In this array, a total of 407 array components were printed in duplicate in a 29 rows × 29 columns format with a pitch of 155 μm. Alexa Fluro 555 azide dye (Thermo Scientific) at 0.7 μg/mL was added to the print buffer to help visualize the printed spots. The printed slides were imaged in GenePix 4000B microarray scanner (Molecular Devices). In this example, six missing spots were identified. They were excluded from further data analysis. After imaging the slides, the soluble dye was washed away and the assay was carried out. Representative images of serum profiling results from the same patient at two time points: baseline at week 8 (B) and post-vaccination at week 48 (C). Serum IgG and IgM bound to the microarray were detected with DyLight 549 anti-Human IgG (green) and DyLight 649 anti-human IgM (red), respectively. Highlighted in the white box are antibodies showing signal changes at two time points.

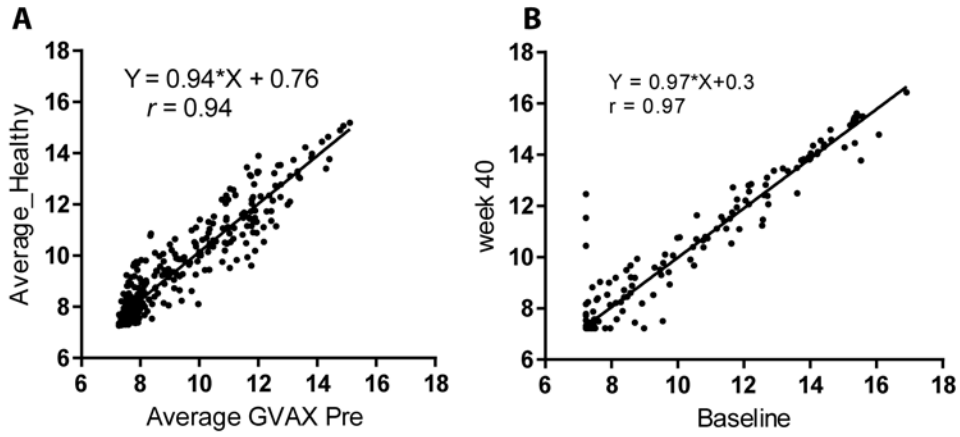

Figure S2, related to Figure 1. Comparison of IgG signals.

The averaged IgG signals of the pre-vaccination samples from GVAX Pancreas patients (n=28) were compared with healthy subjects (n=220) (A). The IgG signals from the same patient at baseline (week 8) were compared with week 40 (B). Fluorescence intensity of IgG signals were Log-transformed (base 2). r: Pearson coefficiency.

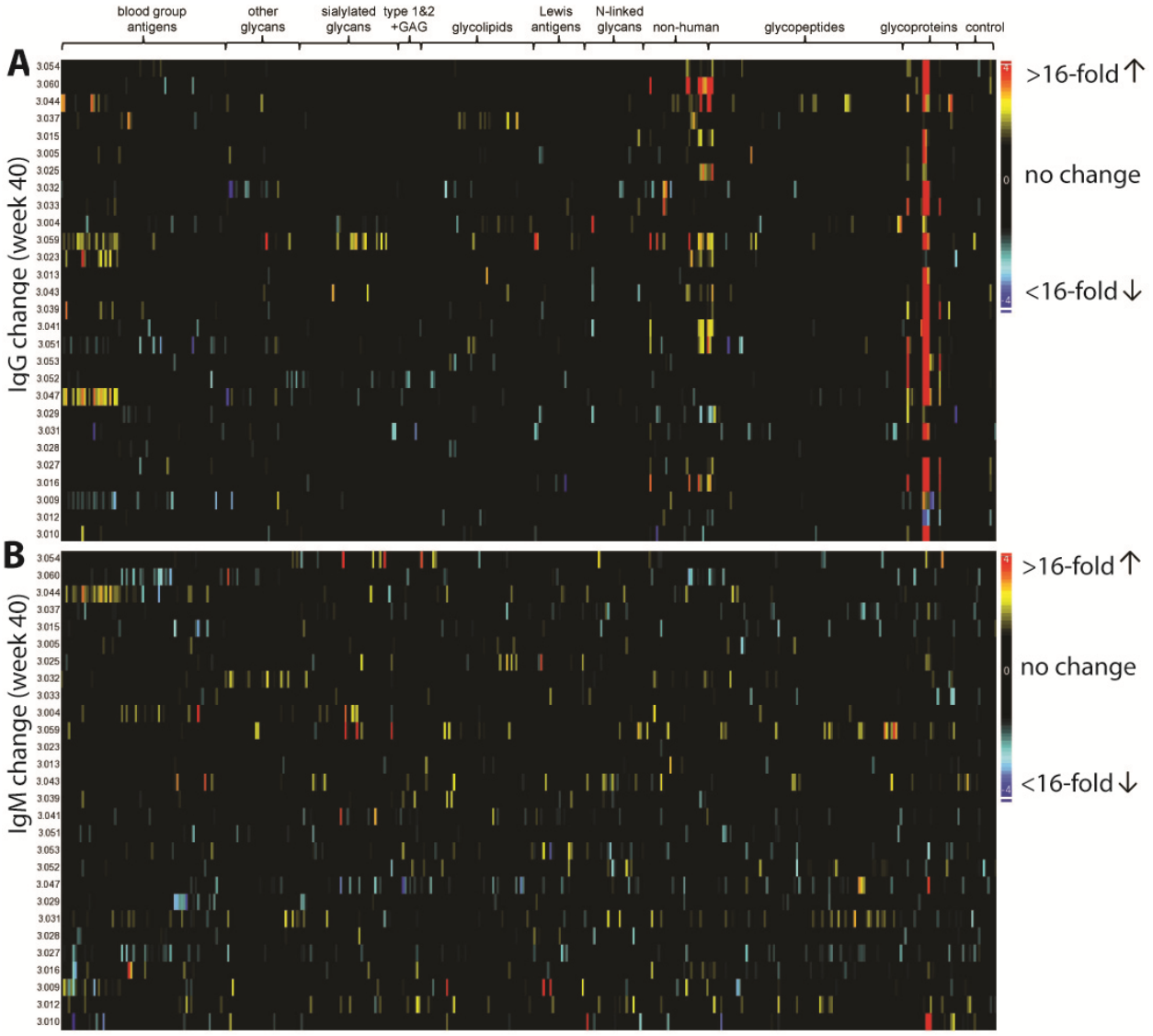

Figure S3, related to Figure 1: Heatmap of antibody changes at week 40 in GVAX Pancreas patients.

Heatmap of IgG changes (A) and IgM changes (B) in 28 patients at week 40 (relative to baseline profile). Each row represents a patient and each column represents an antigen (grouped by families). Scale bar represents the magnitude of antibody changes (in Log2 scale). Signal decrease is indicated with cyan to blue; signal increase is indicated with yellow to red.

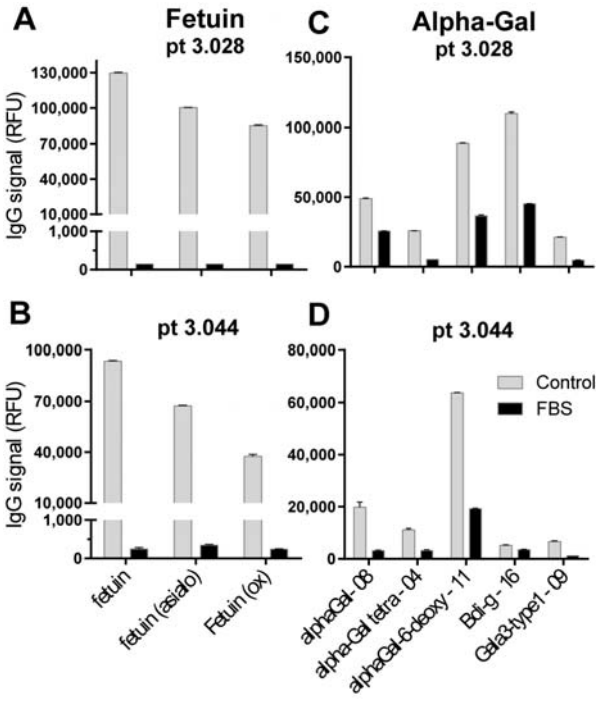

Figure S4, related to Figure 2. Anti-fetuin and anti-alpha-Gal IgG signals were inhibited by addition of FBS.

Post vaccination sera from patients 3.028 and 3.044 (week 48) were incubated with 20% (v/v) FBS or PBS buffer (control) on the microarray. IgG signals specific to 3 bovine fetuin antigens (A and B) and 5 representative alpha-Gal antigens (C and D) were inhibited. Error bars represent SEM (n=2).

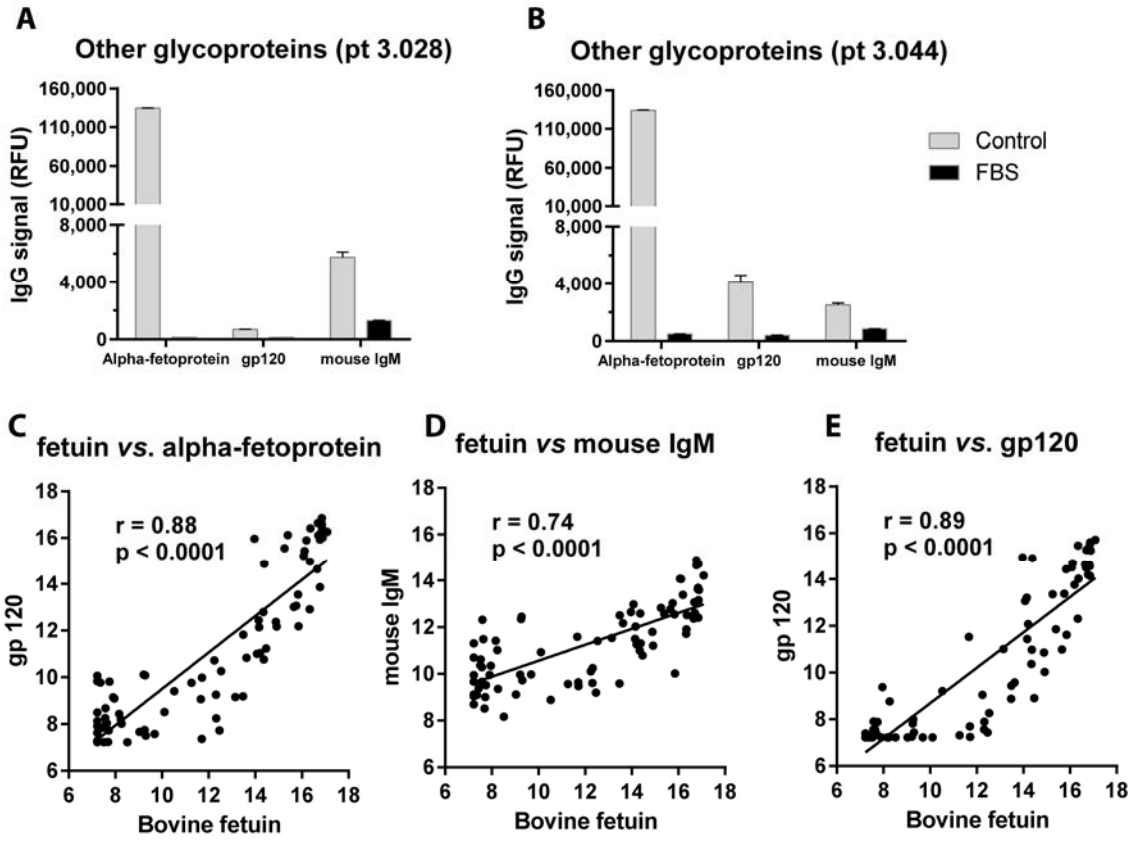

Figure S5, related to Figure 2. Other signals that are inhibited by FBS.

In addition to fetuin and alpha-Gal antigens, IgG signals to three glycoproteins (alpha-fetoprotein, gp120 and mouse IgM) were also inhibited by FBS in both patients 3.028 (A) and 3.044 (B). IgG signals to fetuin in all 84 samples (28 patients, 3 samples per patient) are strongly associated with the IgG signals to alpha-fetoprotein (C, $p < 0.0001$), gp120 (D, $p < 0.0001$) and mouse IgM (E, $p < 0.0001$). These high correlations indicated that alpha-fetoprotein, gp120 and mouse IgM likely contain FBS residues from cell culture. Error bars represent SEM (n=2), r: Pearson coefficiency. *Note*: these three proteins were found all derived from cell culture: alpha-fetoprotein was from cell culture of human hepatocellular carcinoma (Biodesign #A32260H); Mouse IgM was purified from mouse supernatant induced by hybridoma cells (Invitrogen #026800); gp120 was expressed in HEK293 cells.

Supplemental Tables

Table S1, related to Glyco-antigen microarray fabrication, binding assay, and data processing: List of array components used for glyco-antigen microarray production.

See excel sheet named "A411 list of array components".

Table S2 (related to Table 2). Summary of Kaplan-Meier estimates for all possible stratifications.

In the Kaplan-Meier analysis, patients were stratified into upper and lower strata based on their responses to the antigen. Only 15 cutoffs that include at least 7 patients in both strata were considered. The number of cutoffs resulting in Log-rank p-value less than 0.05 and the cutoff with best p-value are listed.

Array components	Time point of antibody change	# of cutoffs with p<0.05	Total cutoffs considered	Best Log rank p-value
alphaGal- 08	week 40	10	15	0.005
	week 48	2	15	0.022
alpha-Gal tetra - 04	week 40	6	15	0.002
	week 48	6	15	0.014

Supplemental Experimental Procedure, related to Glyco-antigen microarray fabrication, binding assay, and data processing

Glyco-antigen microarray binding assay

In the assay, arrays were blocked at 4 °C overnight with 3% BSA in PBS buffer (200 μL/well) and then washed six times with PBS buffer containing 0.05% Tween 20 (PBST). A reference serum and 15 patient sera both diluted to 1:50 with 3% BSA and 1% HSA in PBST buffer were added onto each slide. All samples were measured in duplicate wells and samples from the same patient at different time points were arranged on the same slide. After gentle agitation at 37 °C for 4h, the slides were washed with PBST six times (200 μL/well), and the bound serum antibodies were detected by incubation with DyLight 549 anti-Human IgG and DyLight 649 anti-human IgM (Jackson ImmunoResearch) at 2 μg/L in PBS buffer with 1% BSA and 3% HSA (100 μL/well) at 37 °C for 2h. After washing with PBST seven times (200 μL/well), slides were dried by centrifugation at 1000× rpm and then scanned with a Genepix 4000B microarray scanner (Molecular Devices) at 10 μm resolution. The fluorescence intensity of each array component was quantified with GenePix 7.0 software (Molecular Devices). The signals of each slide were normalized to the median signal of a reference serum on the same slide. The normalized signals from two duplicate arrays were then averaged and Log-transformed (base 2) to give a final signal value for each array component (4 replicates per component).