Supplementary data

Table S1: Association of clinical variables with late sampling-time (T2) anti-Neu5Gc levels in the extended cohort B.

	High level (n=12)	Medium/low level (n=24)	p-value
Delayed graft function	3 (25.0%)	10 (41.7%)	0.468
Cold ischemia time (median, min)	857	1236	0.075
Acute rejection episode	3 (25.0%)	4 (16.7%)	0.664
SSD	5 (41.7%)	6 (25.0%)	0.445
Pregraft PRA	2/9 (22.2%)	13/22 (59.1%)	0.113
class I	2/9 (22.2%)	9/22 (40.9%)	0.429
class II	0/9	7/22	0.077
T2 PRA	3/9 (33.3%)	13/21 (61.9%)	0.236
class I	2/9 (22.2%)	10/21 (47.6%)	0.249
class II	1/9 (11.1%)	7/21 (33.3%)	0.374

Figure S1: Kinetics of blood lymphocyte numbers following ATG induction in recipients of the SSD$^+$ and SSD$^-$ groups.

No significant difference was observed in the rate and magnitude of T cell depletion, as analyzed using blood lymphocyte numbers.

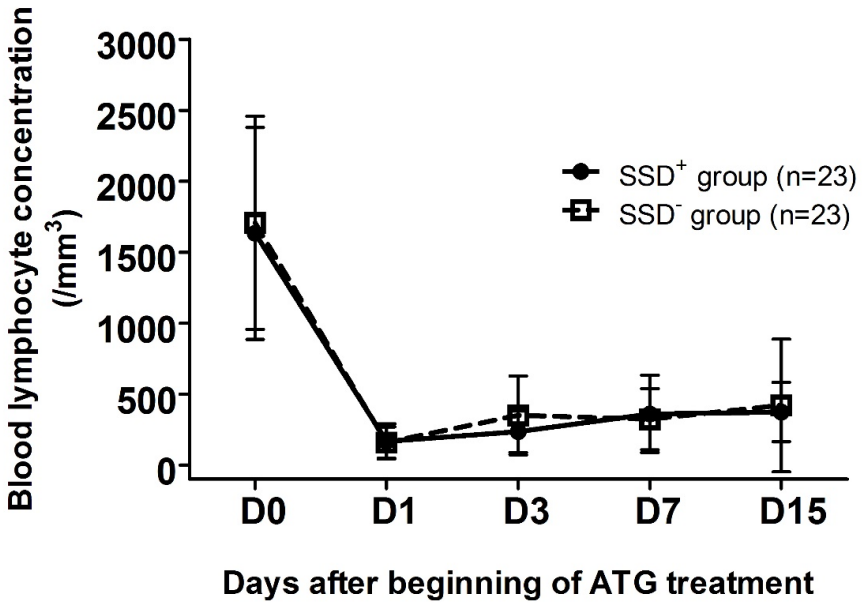

Figure S2 : Total preexisting antibody levels in the SSD$^+$ and SSD$^-$ patients before transplantation compared to healthy volunteers.

(a) Anti-ATG IgGs, (b) anti-Neu5Gc IgGs and (c) anti-Gal IgG and M were quantified using ELISAs. Results are expressed in ng/µl as individual plots, with median and interquartile range for each group. Pregraft sera (T0) of patients from the SSD$^+$ (n=13) and SSD$^-$ (n=13) groups from the cohort B are compared to a group of 70 healthy volunteers matched for age and gender. Pregraft total anti-ATG (mean 9.91± SD 7.14 ng/µl), anti-Neu5Gc (mean 6.7 ± SD 21.02 ng/µl) and anti-Gal levels (mean 5.73 ± SD 4.52 ng/µl) did not differ from age and gender matched healthy individuals (17.55±26.26 ng/µl, 1.07±1.48 ng/µl and 7.63±9.11 ng/µl respectively), whatever the post-graft SSD status. Of note, the values of anti-Neu5Gc and anti-Gal IgGs averaged 1.07 ng/µl and 7.63ng/µl in healthy volunteers.

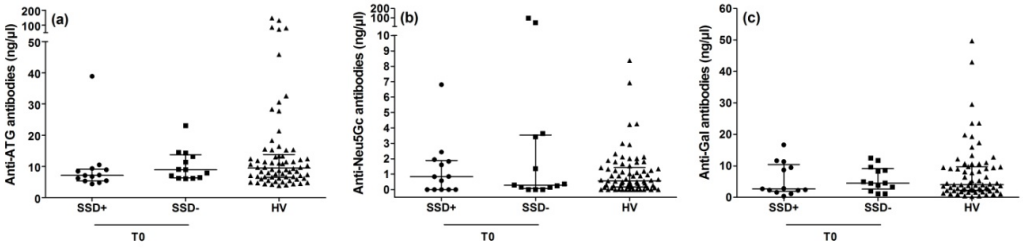

Figure S3: Association between graft survival and antibody serum levels, whatever the SSD status.

An assessment of the association between kidney graft survival and anti-Neu5Gc (a), anti-Gal (b) and global anti-ATG (c) antibodies serum levels was tentatively performed in patients treated with ATG with available sera at T0 and at a late time point of 7 ± 3 years (median of the T2 sampling time), regardless of their SSD status (n=36, including those from the cohort B). The third of patients with the highest values (n=12,"high level" group) and the other patients (n=24, "low level" group) were compared. The same procedure was performed for anti-Neu5Gc, anti-ATG, and anti-Gal antibody levels. (a) Graft survival of patients according to anti-Neu5Gc levels, at a late sampling point (7 ± 3 years). Graft survival was lower in the "high anti-Neu5Gc level" group compared to the "low level" group (**p=0.004). (b,c) Graft survival of patients according to to anti-Gal levels (b) or anti-ATG levels (c), at a late sampling time (7 ± 3 years). No significant survival difference was observed.

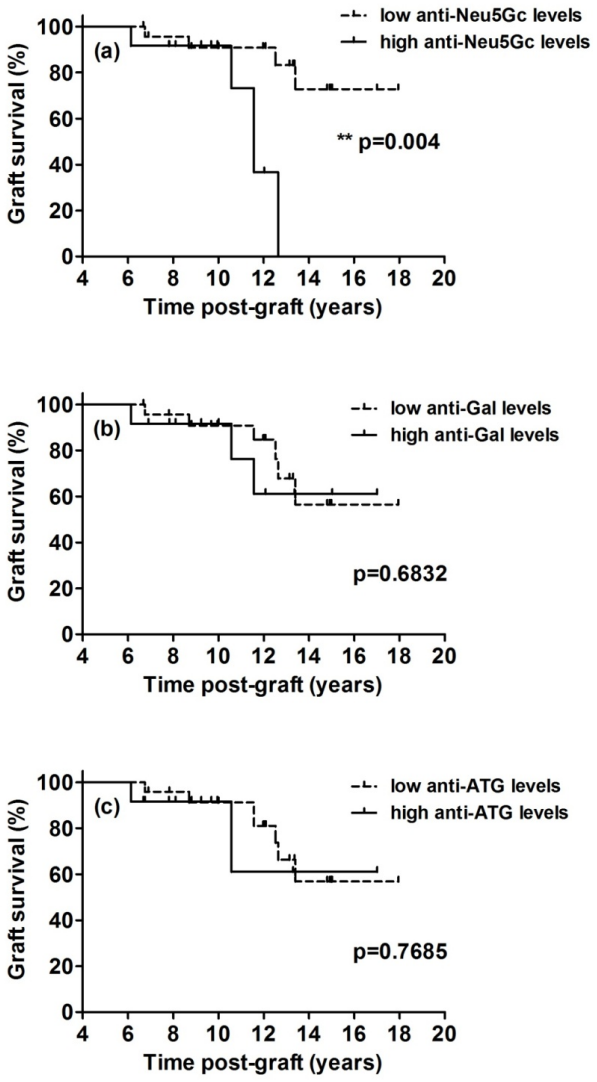

Figure S4: Short-term analysis of total IgG and IgM levels.

In a supplemental cohort of 17 patients, serum was sampled pre-graft (day 0) and at 7, 14, 21, 60 and 90 days post-graft, for the purpose of a short term analysis of the humoral response to ATG. Results are expressed in ng/µl as individual plots, with median and interquartile range for each group, for anti-ATG IgGs (a), anti-Neu5Gc IgGs (b), anti-Gal IgGs (c), as well as total anti-ATG IgMs (d). In addition, serum levels of Thymoglobulin® were quantified using a Rabbit IgG ELISA Quantitation Set (Bethyl Laboratories Inc., USA). Results are represented as individual plots for each patient at each sampling time point, in µg/ml, and show a scattering of serum levels after treatment, especially at day 7 post-graft (e). * p<0.05, ** p<0.01, *** p<0.001 using a Wilcoxon non parametric paired test.

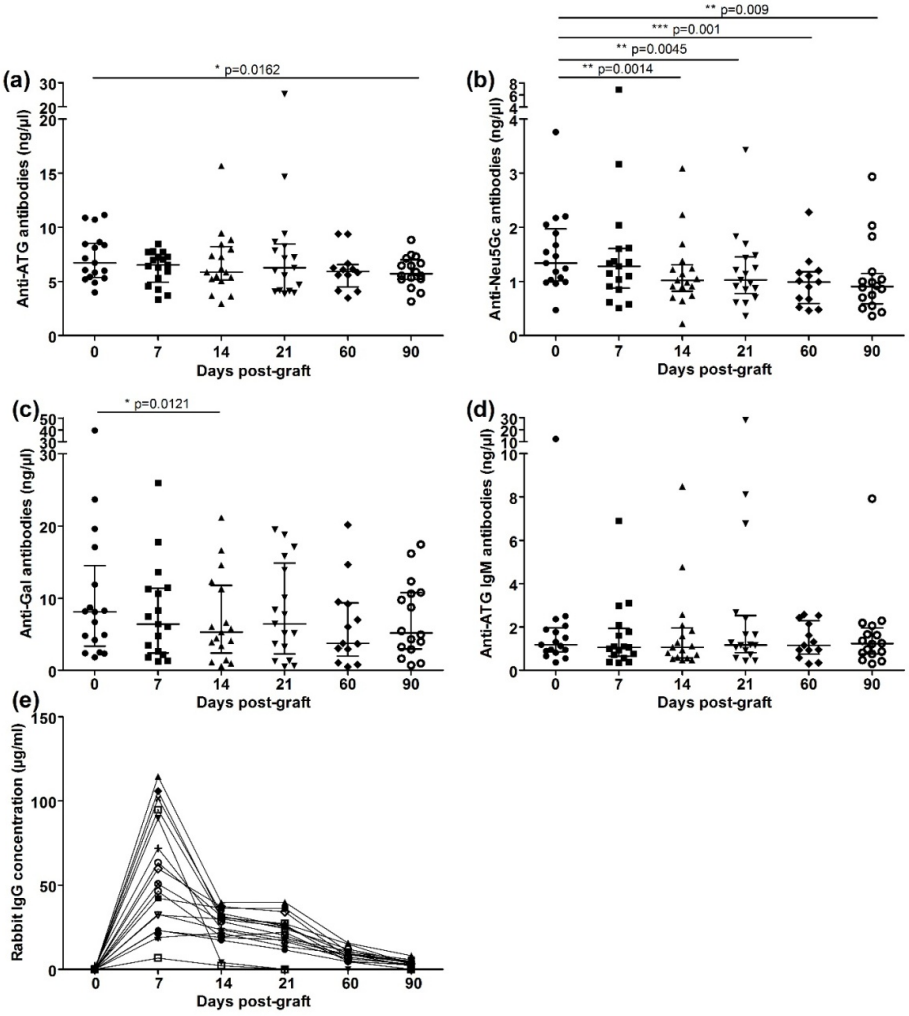

Figure S5: MALDI-TOF mass spectrum of rabbit Thymoglobulin tryptic glycopeptides.

(a) Glycoforms of two main peptides were observed, Peptide 1 (EQQFNSTIR from Fc, in red) and Peptide 2 (undetermined sequence from Fab, in blue). These glycopeptides are the only glycosylated species identified out the tryptic digest of the protein and detected by the mass spectrometer.. Three glycopeptides had Neu5Gc, at m/z 2890, 3052 and 3093 (circled in grey, confirmed by MS/MS). No Neu5Ac residues were detected. Species at m/z 2745 and 2948 are labelled with two isomeric possibilities, one with two Gal residues attached β-1,4 to GlcNAc and the other with a Galα-1,3Galβ-1,4GlcNAc branch (circled in green). (b) After treatment with β-galactosidase, all terminal Gal linked in β were removed, leaving the residual species shown in the spectrum. It is suggested that products circled in green contain an α-Gal linkage. Asterisks (*) denote Na+ adducts present in the enzyme buffer.

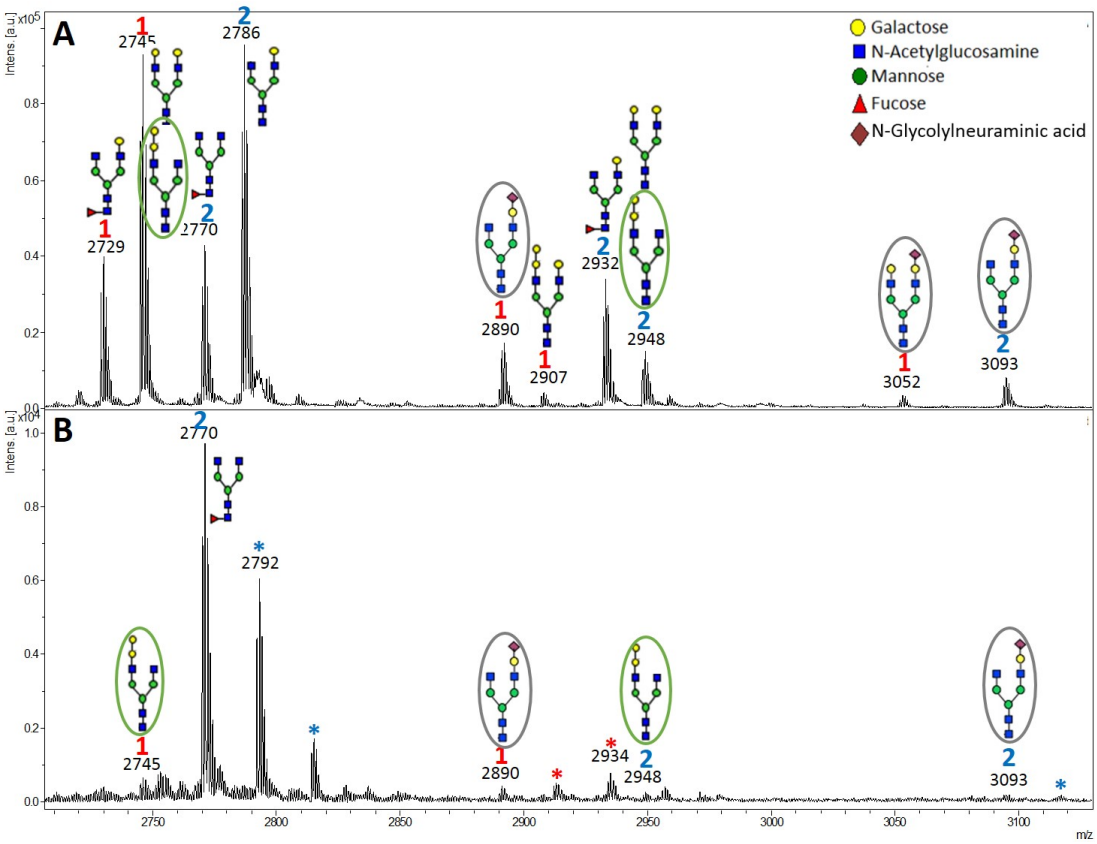

Figure S6 : Flow cytometry of fresh human aortic endothelial cells before any culture step or following two weeks of culture in the presence of 10 % FCS.
Supplementary Figure 6a shows the Neu5Gc pattern of human aortic ECs stained by polyclonal chicken anti-Neu5Gc. Left panel: fresh cells tested immediately following harvesting. Rigth panel: ECs cultured two weeks in medium containing FCS as an exogenous source of Neu5Gc when the cells are used in experiments of stimulation by patients sera with anti-Neu5Gc (Figure S6b). Fresh aortic ECs display substantial amounts of membranous Neu5Gc (MFI:125). FCS cultured ECs display roughly equivalent pattern of membranous Neu5Gc (MFI:141).

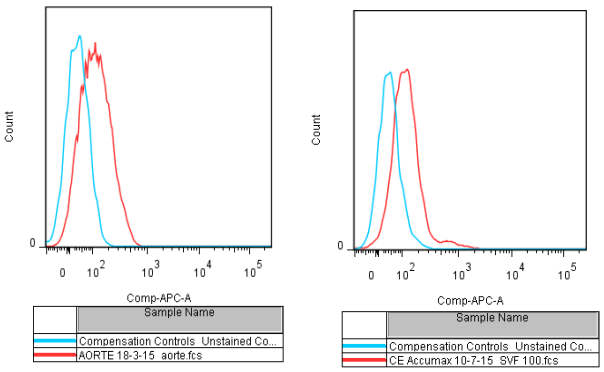

Figure S7: Inflammatory cytokine transcript accumulation following in vitro activation of Neu5Gc positive aortic human ECs with patients sera.

Q-PCR analysis of VCAM-1, ICAM-1, E-Selectin, ADAM-10, TNFa, IL-1b, IL-6 and IL-8 transcripts accumulation in Neu5Gc positive ECs stimulated 4 hours by patients sera containing (High anti-N) or not anti-Neu5Gc Abs (No anti-N) (see Results section for details). Controls correspond to medium (negative) or TNF added medium (positive).