## SUPPLEMENTAL TABLE OF CONTENTS

**Table S1.** Left: list of antibody panel used for CyTOF with conjugated unique metal isotopes. Right: level of clustering analysis that antibodies were used in.

**Table S2.** Baseline characteristics of DSA<sup>POS</sup> recipients and their donors, stratified by development of ABMR.

**Table S3.** Allograft outcomes of DSA<sup>POS</sup> recipients, stratified by development of ABMR.

**Figure S1.** Analysis of calcineurin inhibitor (CNI) adherence in study participants. Panel A demonstrates the measured CNI levels in DSA<sup>NEG</sup> (black) and DSA<sup>POS</sup> recipients within the first month and at 3, 6, 9, 12, and 15 months post-transplant compared to the desired CNI concentration (in grey). Panel B compares CNI intra-patient variability (IPV), a marker of CNI adherence, between DSA<sup>NEG</sup> (black) and DSA<sup>POS</sup> (red) patients after 6 months post-transplant when CNI levels stabilize.

**Figure S2.** Depiction of changes in major cell cluster percentages between DSA<sup>POS</sup> and DSA<sup>NEG</sup> in the PBMC samples collected pre-DSA. The increased percentage of monocytes in the DSA<sup>POS</sup> recipients is accounted for by increases in several lymphocyte populations in DSA<sup>NEG</sup> recipients, although none are statistically significant in and of themselves.

Metal	Marker	Clustering
Rh103Di	Viability	
Nd142Di	CD19	
ND143Di	CD45RA*	
Nd145Di	CD4	
Nd146Di	CD8	
Nd148Di	CD16	
Nd150Di	CD1c	
Eu141Di	CD123	Major Immune
Sm152Di	CD66b	Compartments
Gd155Di	CD27*#	
Gd160Di	CD14	
Dy161Di	CD56	
Er168Di	CD3	
Er170Di	CD38*#	
Yb174Di	HLADR*	
In113Di	CD57*	
In115Di	CD11c <sup>#</sup>	
Nd144Di	KLRG1*#	
Pr141Di	IgD#	
Sm147Di	2B4*	
Sm149Di	CD127*#	
Eu153Di	PD-1*	
Sm154Di	ICOS*	Lymphocyte
Gd156Di	TIM3*	Compartments
Gd158Di	CCR6*#	*CD4 <sup>+</sup> and CD8 <sup>+</sup> T cell
Dy163Di	CXCR5* <sup>#</sup>	subclustering
Dy164Di	CD21 <sup>#</sup>	#B cell subclustering
Er166Di	CD25*#	
Er167Di	CCR7*#	
Er169Di	OX40*	
Yb171Di	CD95*#	
Yb172Di	IgM <sup>#</sup>	
Yb173Di	ČXCR3*#	
Lu175Di	TIGIT*	
Yb176Di	CD45RO*#	

Table S1. CyTOF Antibodies.

	Total DSA <sup>POS</sup> (n=10)	ABMR <sup>POS</sup> (n=5)	ABMR <sup>NEG</sup> (n=5)	p-value
Donors		, <i>t</i>	\$ <i>t</i>	
Age (years)	11.0 ± 5.8	12.0 ± 5.4	10.0 <u>+</u> 6.6	0.61
Male no. (%)				1.00
Male	8 (80)	4 (80)	4 (80)	
Recipients				
Age (years)	10.8 ± 6.4	11.6 ± 7.2	10.0 <u>+</u> 6.2	0.72
Male no. (%)				1.00
Male	8 (80)	4 (80)	4 (80)	
Race (%)				1.00
White	9 (90)	4 (80)	5 (100)	
Hispanic	1 (10)	1 (20)	0 (0)	
Primary Renal Disease				1.00
no. (%)				
Alport Syndrome	1 (10)	0 (0)	1 (20)	
ARPKD	2 (20)	1 (20)	1 (20)	
CAKUT	6 (60)	4 (80)	2 (40)	
Nephronophthisis	1 (10)	0 (0)	1 (20)	
Months on Dialysis	17.2 ± 10.2	17.4 ± 11.0	17.0 ± 10.7	0.95
Cold ischemia time (min)	816.8 <u>+</u> 184.9	723.2 ± 91.8	910.4 <u>+</u> 215.9	0.13
Year of Transplant	$2009 \pm 3.2$	2010 ± 3.4	2008 ± 4.5	0.45
HLA Mismatch (A+B+DQ+DR)	4.6 ± 1.3	4.6 ± 1.1	4.6 ± 1.5	1.00

Table S2. Baseline Characteristics of Study Donor and Recipients for Patients that Developed DSA.

All donors were white and deceased. ARPKD: Autosomal recessive polycystic kidney disease; CAKUT: congenital anomalies of the kidney and urinary tract; HLA: human leukocyte antigen. Continuous variables represented as mean  $\pm$  SD. Categorical variables are expressed as percentages. \* p<0.05, \*\*p<0.01, \*\*\*p<0.001 by unpaired t-test for continuous variables or Fisher exact test for categorical variables.

<u>Table 33. Allogian Outcomes of Fatients by Development of Abwik.</u>						
-	Total DSA <sup>POS</sup>	ABMRPOS		p-value		
	(n=10)	(n=5)	(n=5)			
eGFR post-transplant ¤						
3 months	98.8 ± 24.9	99.1 ± 28.8	98.4 ± 23.8	0.97		
6 months	97.7 <u>+</u> 24.9	97.6 ± 28.2	97.7 <u>+</u> 24.5	0.99		
9 months	94.7 <u>+</u> 22.7	93.6 ± 22.4	95.8 ± 25.6	0.89		
12 months	95.2 ± 23.2	94.2 ± 23.0	96.2 ± 26.1	0.90		
DSA HLA Class reactivity	10 (100)	5 (100)	5 (100)	1.00		
Class I	<b>3</b> <sup>f</sup> (30)	2 <sup>f</sup> (40)	1 <sup>f</sup> (20)			
Class II						
DGF no. (%)	1 (10)	0 (0)	1 (20)	1.00		
ACR no. (%)	3 (30)	2 (40)	1 (20)	1.00		
ACR time post-	17.0 ± 1.0	$16.5 \pm 0.7$	18.0 ± 0.0	1.00		
transplant (months)						
Graft Loss no. (%)	3 (30)	3 (30)	0 (0)	0.17		
Graft loss time post-		48.0 ± 25.1	N/A			
transplant (months)						
DSA Titers (MFI)	12531 ± 6920.9	13686.6 ± 9300.8	11375.4 ± 4234.5	0.63		

## Table S3. Allograft Outcomes of Patients by Development of ABMR.

**a**eGFR: estimated glomerular filtration rate calculated by the Schwartz formula (Schwartz, Haycock et al. 1976); DSA: DGF: delayed graft function; ACR: acute cellular rejection. <sup>*f*</sup>2 patients did not have Class I DSA at onset but developed them after detection of Class II DSA. Continuous variables represented as mean  $\pm$  SD. Categorical variables as a percentage. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 by unpaired t-test for continuous variables or Fisher exact test for categorical variables.

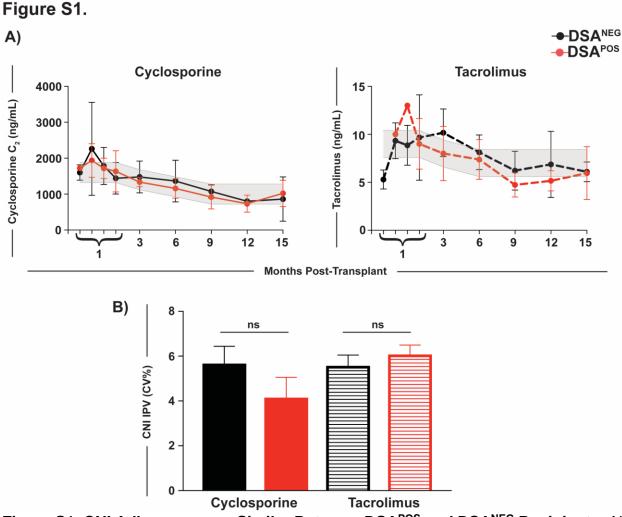
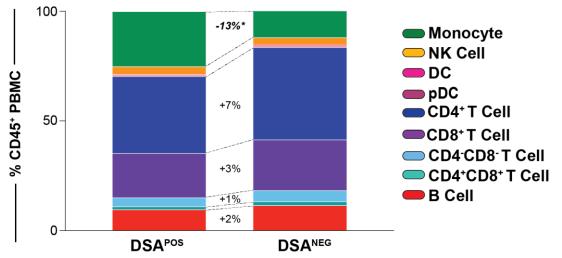


Figure S1. CNI Adherence was Similar Between DSA<sup>POS</sup> and DSA<sup>NEG</sup> Recipients. All patients received mycophenolate mofetil (MMF) and a calcineurin inhibitor (CNI), with or without steroids, as maintenance immunosuppression. A) Twelve patients received cyclosporine as the CNI (left; 7 DSA<sup>POS</sup>, 4 DSA<sup>NEG</sup>) and nine patients received tacrolimus (right;3 DSA<sup>POS</sup>, 6 DSA<sup>NEG</sup>). Error bars represent standard deviation (SD) and target values of the CNI are shown in grey. B) CNI intra-patient variability (IPV), as measured by the coefficient of variation (CV%), was not different in patients on tacrolimus during the "stable" phase of immunosuppression ( $\geq$ 6-months post-transplant). Error bars represent standard error of the mean (SD). \*p<0.05, \*\*p<0.01 by unpaired t-test.

Three patients switched from cyclosporine to tacrolimus; 2 were DSA<sup>NEG</sup> and 1 was DSA<sup>POS</sup>ABMR<sup>POS</sup>. Coefficient of variation was calculated by formula:  $(CV\%) = \sqrt{1 + (CV\%)^2}$ 

$$\sqrt{\{\Sigma(X_j-\bar{X})^2/(n-1)/\bar{X}\}*100}$$
.

Figure S2.



**Figure S2. Increases in several lymphocyte populations account for the decreased monocytes seen in DSA<sup>NEG</sup> recipients prior to DSA detection.** Analysis of the breakdown of major immune cell classes prior to DSA detection in DSA<sup>POS</sup> (left) and DSA<sup>NEG</sup> (right) recipients. Percentage change in larger cell subsets annotated between bars. \*p<0.05, \*\*p<0.01 by unpaired t-test.

## REFERENCES

Schwartz, G. J., et al. (1976). "A simple estimate of glomerular filtration rate in children derived from body length and plasma creatinine." Pediatrics **58**(2): 259-263.