Supplemental Material

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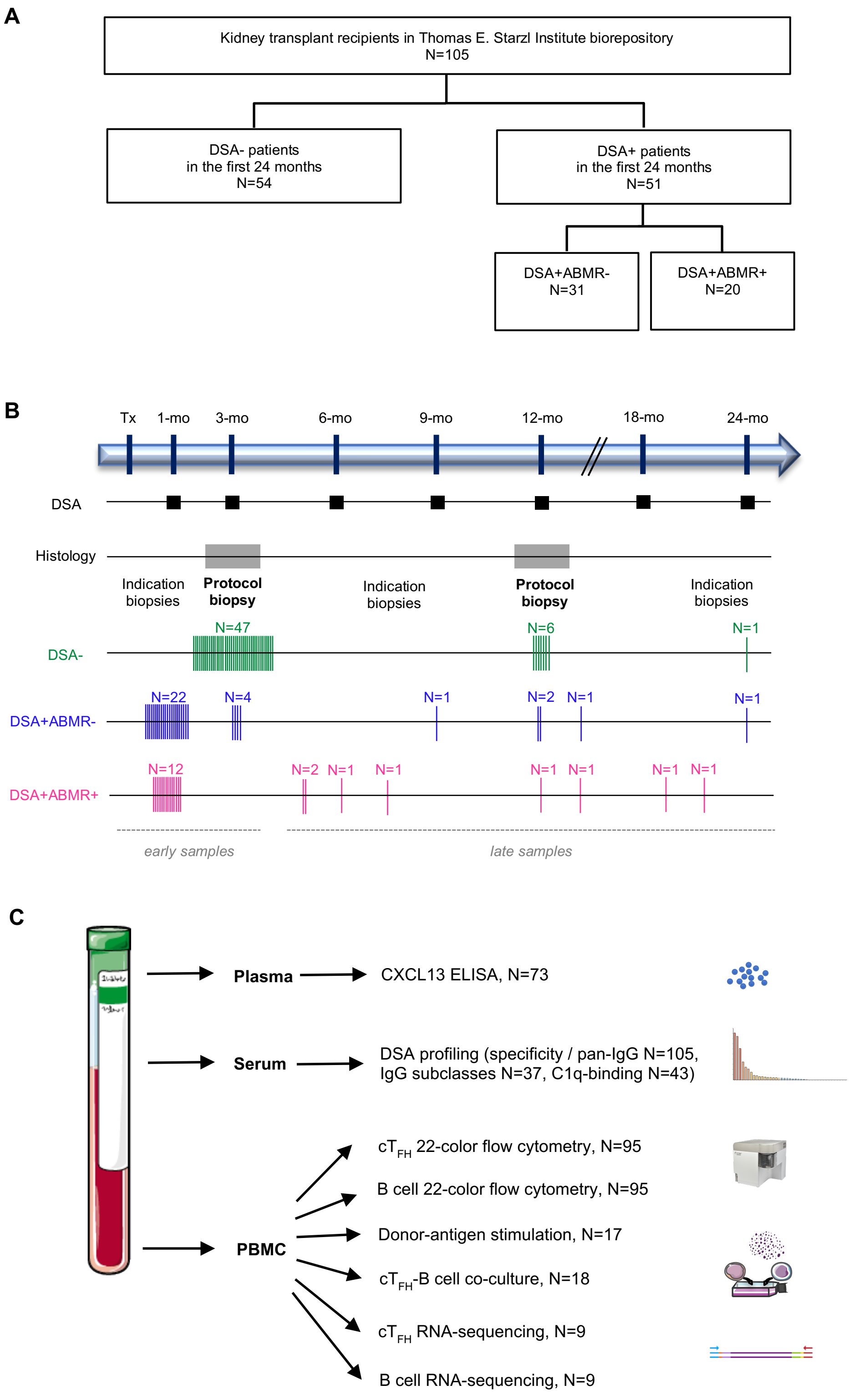

Figure S1. Study outline and cross-sectional blood samples of kidney transplant patients
(A) Study design. Identification of three study groups according to the presence of post-transplant DSA and biopsy-proven ABMR status in the first 24 months post-transplant : patients without

DSA nor ABMR (DSA-), patients with DSA without ABMR (DSA+ABMR-) and patients with DSA and ABMR (DSA+ABMR+). **(B)** Schematic representation of the screening strategy of patients for circulating DSAs; at 1-, 3-, 6-, 9-, 12-, 18- and 24-month post-transplant and at the time of indication biopsies. ABMR was detected by kidney allograft protocol and indication biopsies. Cross-sectional time points of blood samples are represented by vertical colored bars. Early samples are defined by samples collected ≤ 3-month and late samples are those collected > 3-month post-transplant. **(C)** Sample sizes and assays utilized for multidimensional profiling of cT_{FH} and B cell responses in blood of patients.

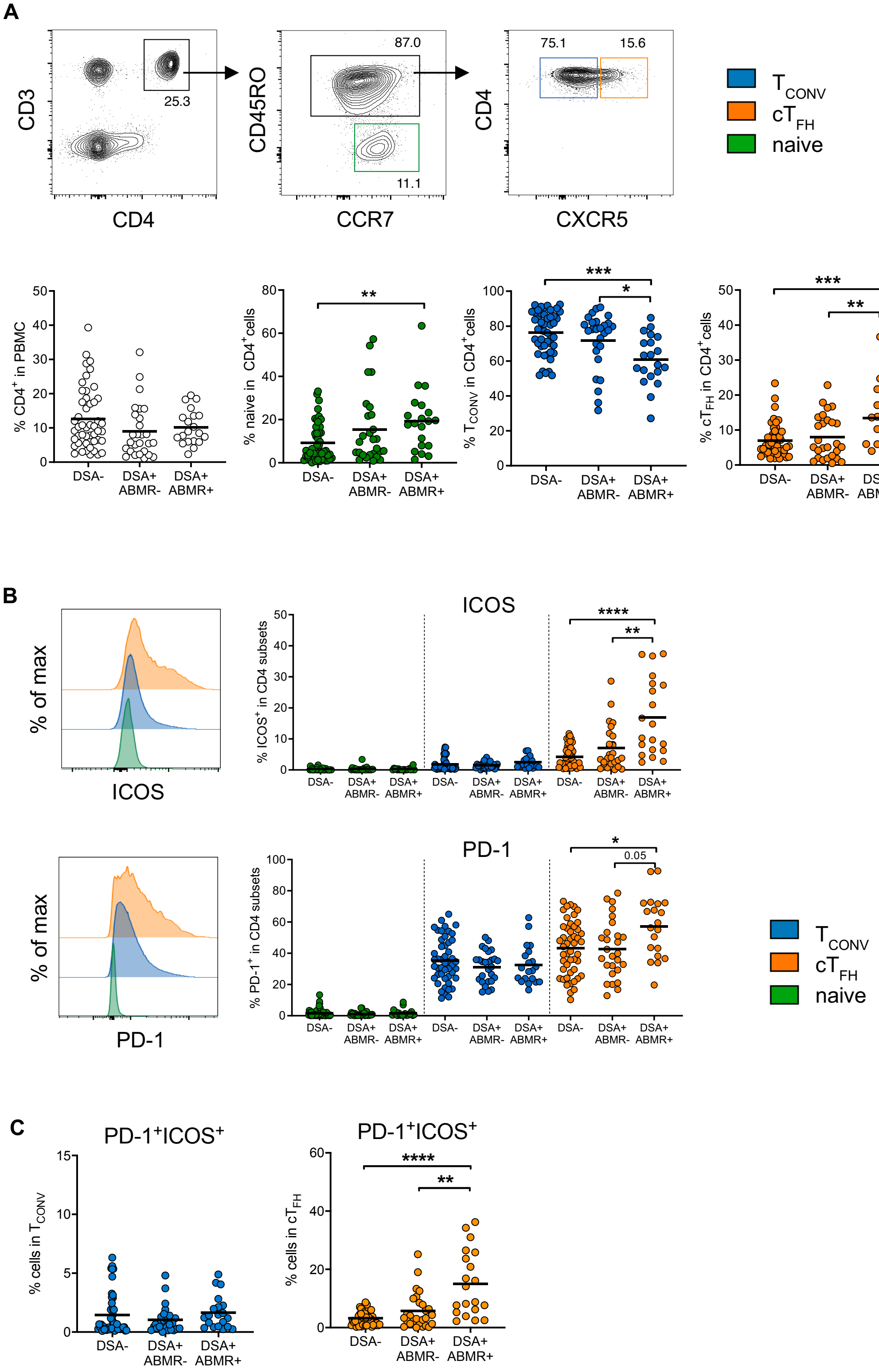

Figure S2. Flow cytometry analyses of blood CD4^{+}T cells

(A) Representative example of the gating strategy by flow cytometry (upper panel) and dot plots of percentages of CD4, naive, T$_{CONV}$ and cT$_{FH}$ cells are displayed; DSA- (N=48), DSA+ABMR- (N=27) and DSA+ABMR+ (N=20) (lower panel). **(B)** Representative histograms and dot plots of percentages of ICOS and PD-1 in naive, T_{CONV} and cT_{FH} cells are displayed; DSA- (N=48), DSA+ABMR- (N=27) and DSA+ABMR+ (N=20). **(C)** Dot plots of percentages of PD-1^+ICOS$^+$ cells in T_{CONV} and cT_{FH} are displayed; DSA- (N=48), DSA+ABMR- (N=27) and DSA+ABMR+ (N=20). Kruskal-Wallis with Dunn's post-test for panels **A, B** and **C**. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. Each dot represents one subject and horizontal lines are mean values.

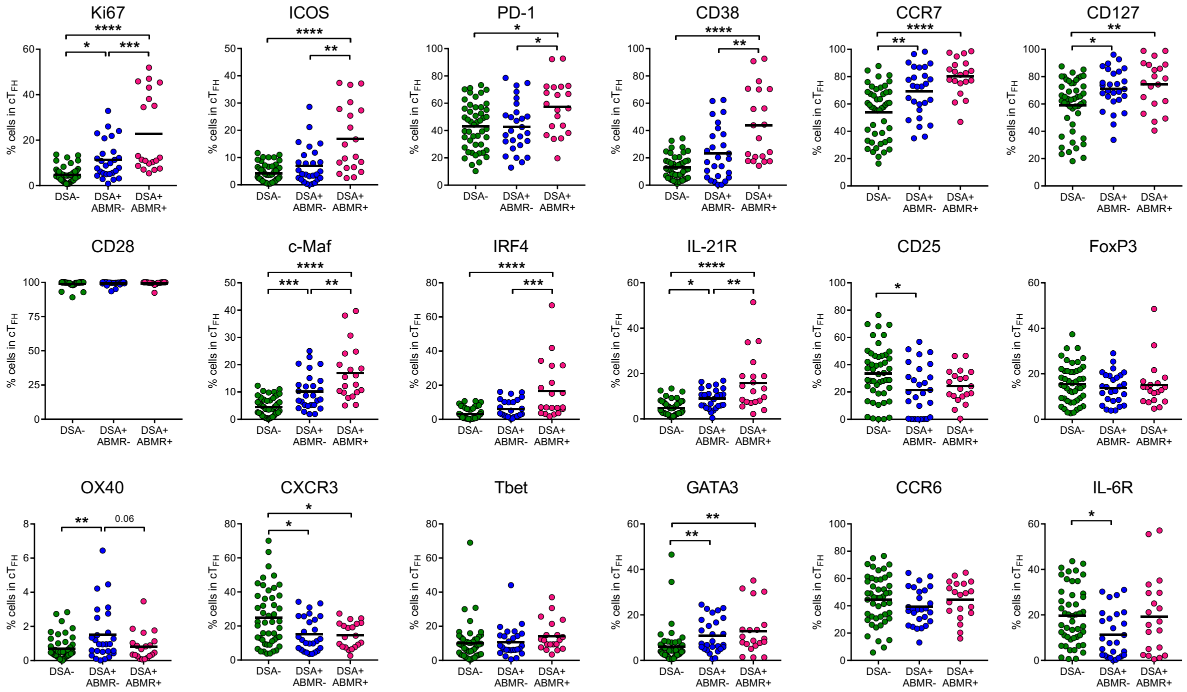

Figure S3. Biaxial flow cytometry analyses of cT$_{FH}$

Dot plots of percentages of indicated marker expression in cT$_{FH}$ by flow cytometry are displayed; DSA- (N=48), DSA+ABMR- (N=27) and DSA+ABMR+ (N=20) patients. One-way ANOVA with Tukey post-test or Kruskal-Wallis with Dunn’s post-test. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Each dot represents one subject and horizontal lines are mean values.

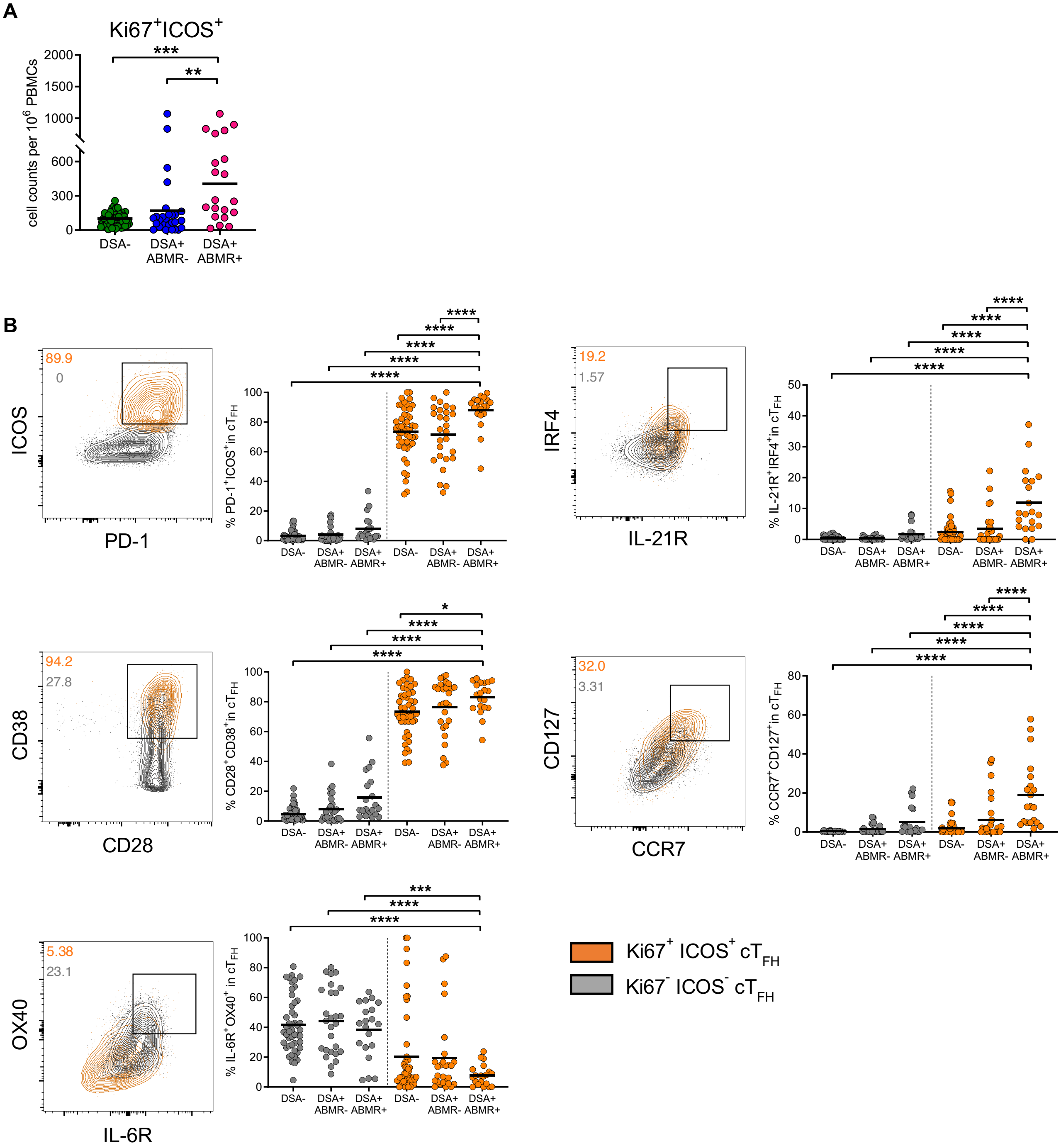

Figure S4. Biaxial flow cytometry analyses of Ki67$^+$ICOS$^+$ compared to Ki67$^-$ICOS$^-$ cT$_{FH}$
(A) Dot plot of Ki67$^+$ICOS$^+$ cT$_{FH}$ as cell counts per million PBMCs by flow cytometry, are displayed; DSA- (N=48), DSA+ABMR- (N=27) and DSA+ABMR+ (N=20) patients. Kruskal-Wallis with Dunn's post-test. **(B)** Representative examples of flow cytometry analysis and dot plots of percentages of indicated marker expression on Ki67$^-$ICOS$^-$ or Ki67$^+$ICOS$^+$ cT$_{FH}$ are displayed; DSA- (N=48), DSA+ABMR- (N=27) and DSA+ABMR+ (N=20). One-way ANOVA with Dunnett post-test. *P < 0.05; ***P < 0.001; ****P < 0.0001. Each dot represents one subject and the horizontal lines are the mean values.

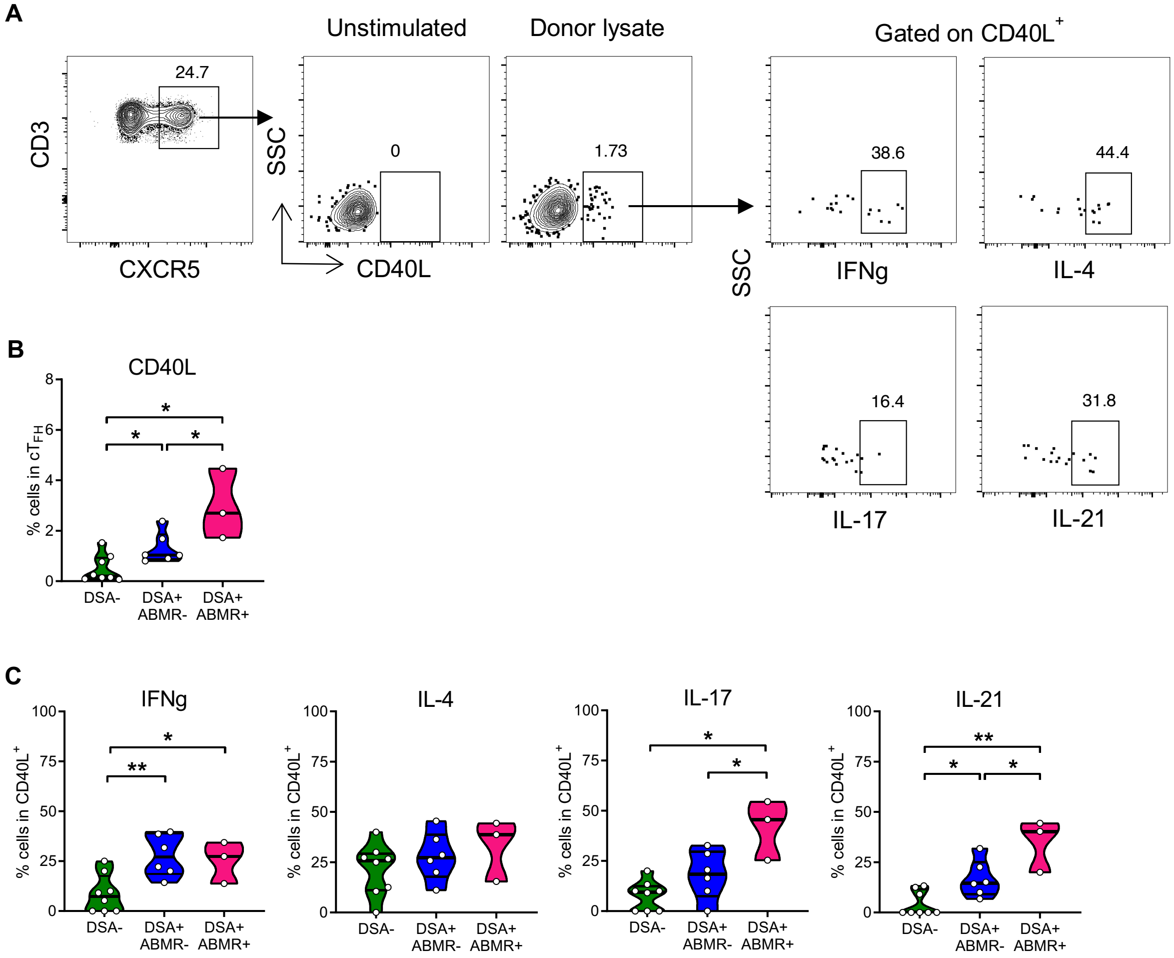

Figure S5. Flow cytometry analyses of cT_{FH} cytokine production in response to donor-antigen stimulation

Short (6-hour) stimulation of PBMCs from patients pulsed with their donor PBMC lysate. **(A)** Representative example of the gating strategy by flow cytometry to identify CD40L$^+$ cells in cT_{FH} (pre-gated on CD3$^+$CD8$^-$CXCR5$^+$) and cytokines expression among CD40L$^+$ cells in indicated experimental conditions. **(B-C)** Violin plots of percentages of CD40L$^+$ cells in cT_{FH} and percentages of indicated cytokines expression within CD40L$^+$ cells are displayed; DSA- (N=8), DSA+ABMR- (N=6) and DSA+ABMR+ (N=3). Unpaired t-test for panels **B** and **C**. *P < 0.05; **P < 0.01. Each dot represents one subject and horizontal lines of violin plots are the median and quartiles values.

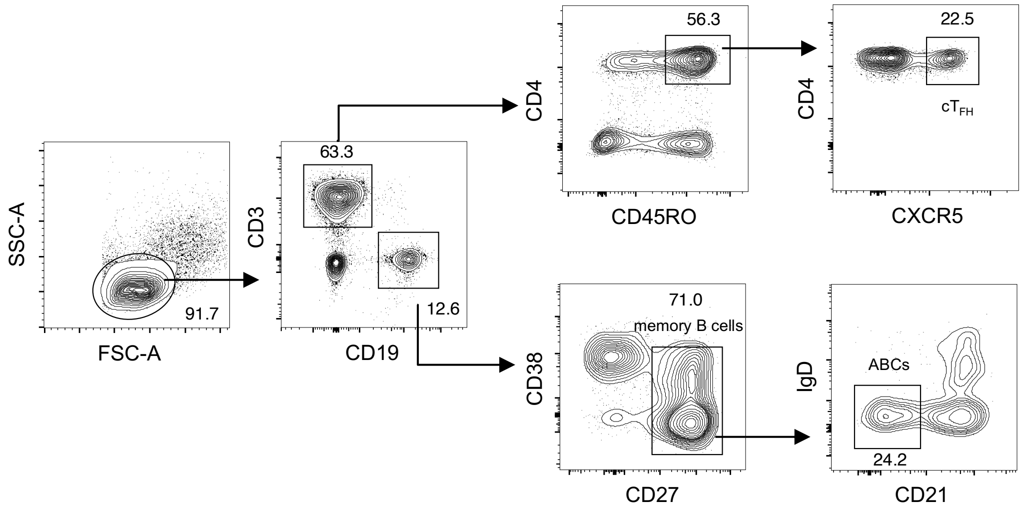

Figure S6. Gating strategy for sorting cT$_{FH}$, memory B cells and ABCs
Representative example of the gating strategy for FACS sorting of cT$_{FH}$ (CD19$^-$CD3$^+$CD4$^+$CD45RO$^+$CXCR5$^+$), memory B cells (CD19$^+$CD3$^-$CD38^{lo}CD27$^+$) and ABCs (CD19$^+$CD3$^-$CD38^{lo}CD27$^+$IgD$^-$CD21^{lo}) from PBMC samples.

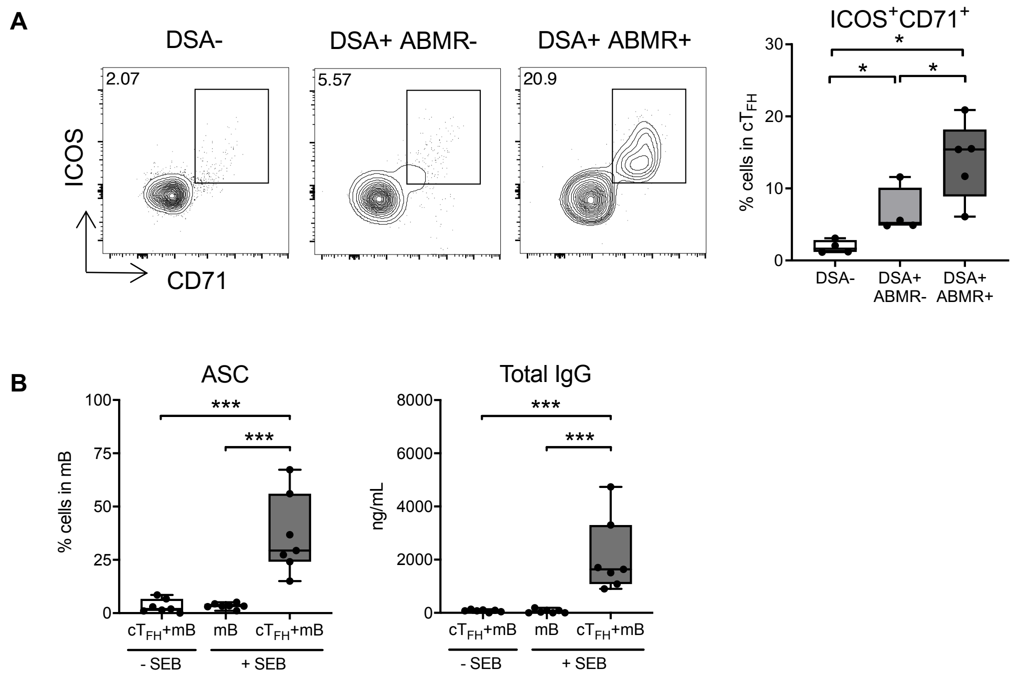

Figure S7. Co-culture analyses of cT$_{FH}$ with autologous memory B cells

Co-culture of sorted cT$_{FH}$ with autologous memory B cells in presence of SEB (6 days). **(A)** Representative examples of individual experiments by flow cytometry analysis and dot plot of percentages of ICOS^{+}CD71^{+} cells among cT$_{FH}$ in co-cultures are displayed; DSA- (N=4), DSA+ABMR- (N=4) and DSA+ABMR+ (N=5). (**B**) Dot plots of percentages of CD27^{+}CD38^{hi} antibody-secreting cells (ASC) in memory B cells and total IgG measured by ELISA in supernatants after 6 days of co-culture in DSA+ABMR+ patients, are displayed; N=7 per condition. Mann-Whitney U test for panel **A** and **B**. *$P < 0.05$; ***$P < 0.001$.

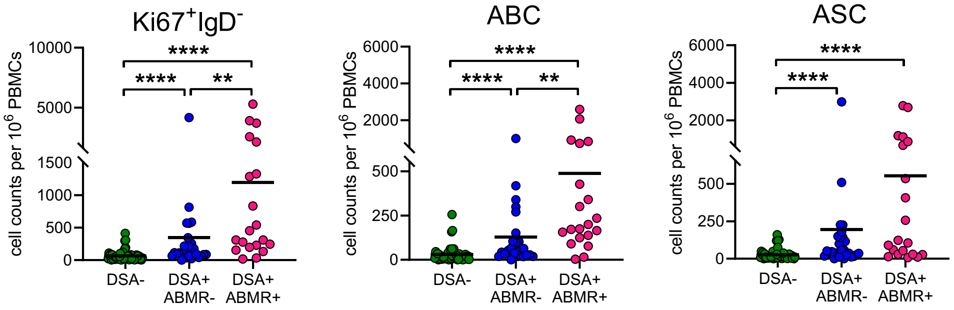

Figure S8. Flow cytometry analyses of ABCs and ASCs

Dot plot of Ki67$^+$IgD$^-$, ABCs and ASCs as cell counts per million PBMCs by flow cytometry, are displayed; DSA- (N=48), DSA+ABMR- (N=27) and DSA+ABMR+ (N=20) patients. Mann-Whitney U test. *P < 0.05; **P < 0.01; ****P < 0.0001. Each dot represents one subject and the horizontal lines are the mean values.

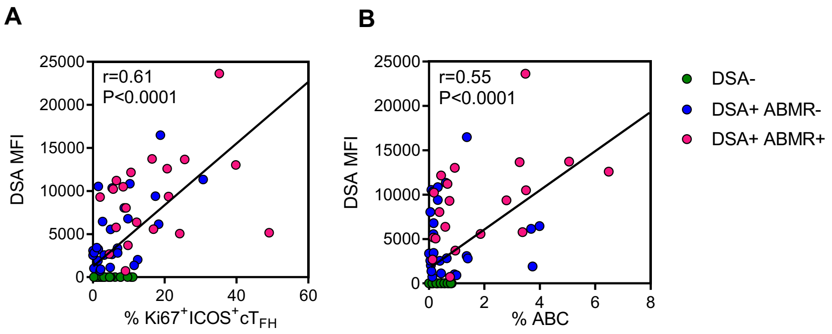

Figure S9. Correlation of cT_{FH}, ABCs with DSAs
Spearman correlation analysis of $Ki67^{+}ICOS^{+}$ cT_{FH} **(A)** or ABCs **(B)** with DSA levels in sera measured by Luminex at the time of flow cytometry analysis, are displayed ; DSA- (N=48), DSA+ABMR- (N=27) and DSA+ABMR+ (N=20) patients.

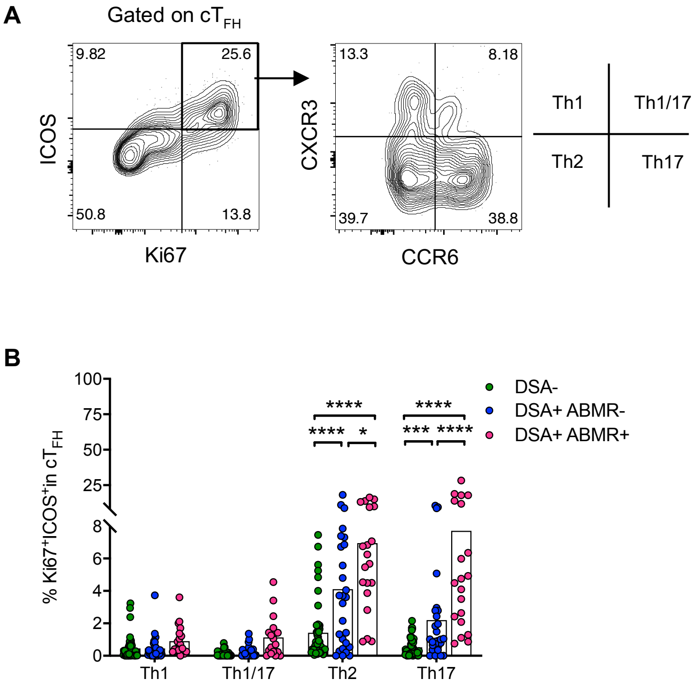

Figure S10. Flow cytometry analyses of cT$_{FH}$ polarization
(A) Representative example of the gating strategy by flow cytometry to identify Th1 (CXCR3$^+$CCR6$^-$), Th1/17 (CXCR3$^+$CCR6$^+$), Th2 (CXCR3$^-$CCR6$^-$) and Th17 (CXCR3$^-$CCR6$^+$) subsets among Ki67$^+$ICOS$^+$ cT$_{FH}$ is displayed. **(B)** Percentages of Ki67$^+$ICOS$^+$ Th1, Th1/17, Th2 and Th17 cells in cT$_{FH}$ are displayed; DSA- (N=48), DSA+ABMR- (N=27) and DSA+ABMR+ (N=20). Multiple t-test with Holm-Sidak correction. *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001. Each dot represents one subject and horizontal lines are mean values.

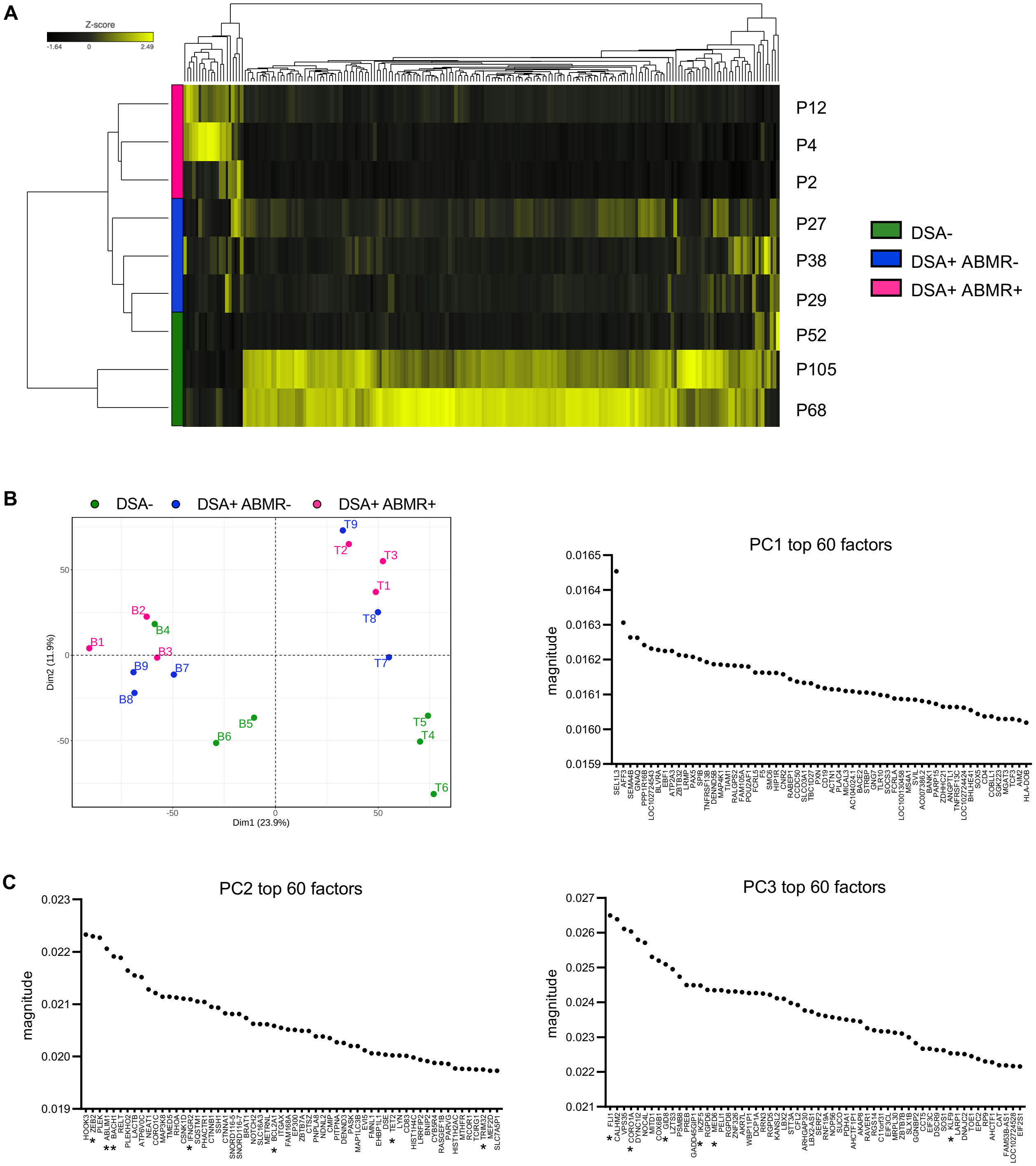

Figure S11. Transcriptional profiling of cT$_{FH}$ and ABCs by hierarchical clustering and PCA RNA-seq analysis of sorted cT$_{FH}$ and ABCs in three patient groups; DSA- (N=3), DSA+ABMR- (N=3) and DSA+ABMR+ (N=3). **(A)** Heatmap generated by hierarchical clustering of genes expressed in ABCs and the three types of patient samples. Genes used for clustering were differentially expressed (fold change >2, false discovery rate P-Value <0.05). **(B)** PCA of the cT$_{FH}$ and ABC RNA-seq patterns for each patient group (N=3 patients per group). PC1 and PC2 delineated the cT$_{FH}$ (T1-9) and ABCs (B1-9) from DSA- (green), DSA+ABMR- (blue) and

DSA+ABMR+ (pink) patients as separate clusters (left panel). Top 60 genes as factors contributing to PC1, aligned by magnitude. (right panel). **(C)** Top 60 genes as factors contributing to PC2 and PC3, aligned by magnitude. * refers to selected genes displayed in **Figure 6B**.

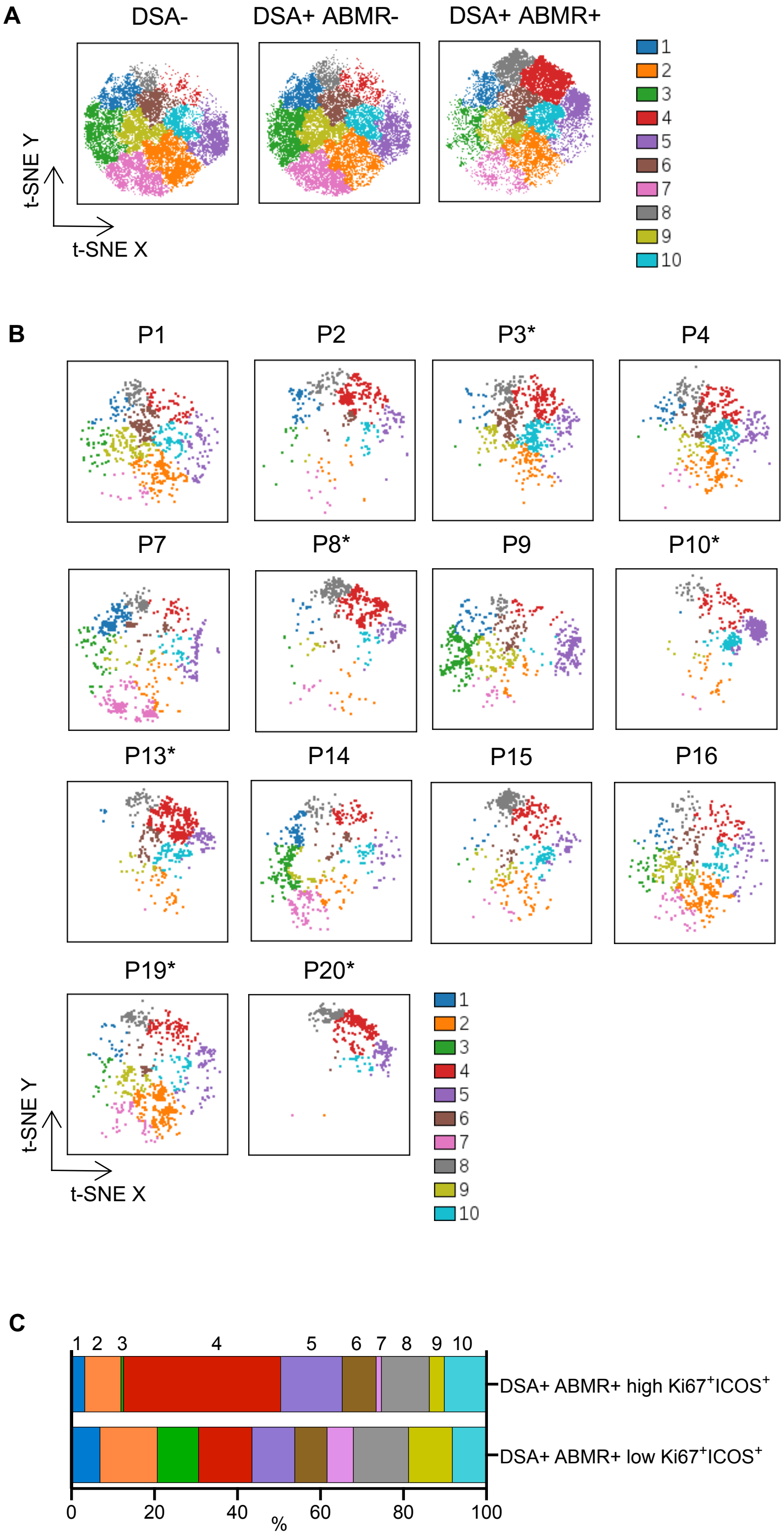

Figure S12. High-dimensional flow cytometry and t-SNE analyses of cT_{FH} in individual patients

(A) t-SNE projections of cT_{FH} cells were generated using N=18,700 cells from each patient group and were overlaid with 10 cT_{FH} cell clusters delineated by SPADE clustering (as in **Figure 1C**);

DSA- (N=20), DSA+ABMR- (N=20) and DSA+ABMR+ (N=20) patients. **(B)** t-SNE projections of cT_{FH} cells generated for individual patients (N=20) from DSA+ABMR+ group. Each t-SNE map is based on N=935 cells. * indicates the 10 patients from the DSA+ABMR+ high $Ki67^+ICOS^+$ subgroup as in **Figure 8**. **(C)** Stacked bar plot showing cT_{FH} cell cluster distribution based on SPADE clustering as in panel **B**. Cluster 4 is significantly different in proportion across the indicated groups, by Mann-Whitney U test.

Table S1. Patients demographics

	DSA-	DSA+ ABMR-	DSA+ ABMR+	P value****
	N=54	N=31	N=20	
Characteristics at the time of transplantation				
Recipient age (years), mean ± SD	49.9 ±13.2	46.7 ±14.5	45.3 ±15.1	0.358
Recipient male sex, n(%)	39 (72.2)	17 (54.8)	11 (55.0)	0.182
Caucasian, n(%)	49 (90.7)	27 (87.1)	15 (75.0)	0.208
Retransplantation, n(%)	4 (7.4)	7 (22.6)	8 (40)	0.004
Time in dialysis (months), mean ± SD	23.6 ± 28.1	41.4 ± 48.0	37.0 ± 28.8	0.064
Native kidney disease, n(%)				
Glomerular*	10 (18.5)	11 (35.5)	5 (25.0)	0.218
Hypertensive	6 (11.1)	3 (9.7)	4 (20.0)	0.507
Tubulointerstitial nephropathy	1 (1.8)	3 (9.7)	1 (5.0)	0.264
Polycystic kidney disease	10 (18.5)	5 (16.1)	2 (10.0)	0.677
Diabetes	13 (24.1)	3 (9.7)	5 (25.0)	0.230
Other nephropathy**	13 (24.1)	7 (22.6)	5 (25.0)	0.979
Donor age (year), mean ± SD	43.9 ±11.7	39.3 ±15.3	39.4 ±13.7	0.223
Donor male sex, n(%)	23 (42.6)	17 (54.8)	10 (50.0)	0.538
Living donor, n(%)	28 (51.8)	13 (41.9)	5 (25.0)	0.114
Cold ischemia time (min), mean ± SD	297.3 ± 371.7	440.7 ± 395.4	417.4 ± 299.3	0.174
Thymoglobulin induction therapy, n(%)	54 (100)	31 (100)	20 (100)	–
Negative flow cytometry crossmatch, n(%)	54 (100)	31 (100)	20 (100)	–
HLA mismatches, mean ± SD***	4.9 ± 1.9	5.0 ± 2.1	5.0 ± 2.0	0.981
Characteristics at the time of cross-sectional sample collection				
Time to transplantation (months), mean ± SEM	4.3 ± 0.4	3.6 ± 1.0	5.3 ± 1.4	0.439
TCMR lesions at the time of sample collection, n(%)	0 (0)	0 (0)	17 (85)	<0.0001
Tacrolimus, n(%)	53 (98.1)	31 (100)	18 (90.0)	0.091
Tacrolimus trough level (µg/L), mean ± SD	9.6 ± 2.8	9.1 ± 3.0	8.8 ± 5.3	0.614
Mycophenolate mofetil, n(%)	54 (100)	31 (100)	20 (100)	–

ABMR, antibody-mediated rejection ; DSA, donor-specific antibody ; HLA, human leukocyte antigen ; TCMR, T-cell mediated rejection

*Glomerulonephritis includes chronic glomerulonephritis, crescentic glomerulonephritis, focal segmental glomerulosclerosis, Wegener's granulomatosis, IgA nephropathy, membranous nephropathy

**other nephropathy includes familial nephropathy, renal hypoplasia and other noncategorized conditions

***HLA mismatches at A, B, DR and DQ locus

****One-way ANOVA and chi-squared test were used for statistical comparison of continuous and categorical variables, respectively

Table S2. Data and assay table

Patients	Patient groups	Cross-sectional cT$_{\mathrm{FH}}$ flow cytometry	cT$_{\mathrm{FH}}$ RNA-seq	Cross-sectional B cells flow cytometry			ABC RNA-seq	cT$_{\mathrm{FH}}$-mB cells co-culture		Serum DSA profiling			Plasma CXCL13 ELISA (pg/mL)	Donor-antigen stimulation	Longitudinal cT$_{\mathrm{FH}}$-B cells flow cytometry
		% Ki67+ICOS+ in cT$_{\mathrm{FH}}$		% Ki67+IgD- in CD27+	% ABC in CD27+	% ASC in CD27+		ASC at 6 days (cell counts)	Total IgG at 6 days (ng/mL)	Specificity Pan-IgG	IgG subclasses	C1q-binding			
		Fig. 1F	Fig. 2, 6	Fig. 4A	Fig. 4B	Fig. 4B	Fig. 6, S11	Fig. 3A	Fig. 3B	Fig. S9A, S9B	Fig. 5A	Fig. 5A	Fig. 4C	Fig. S5	Fig. 7
P1	DSA+ABMR+	4.56 %		0.28 %	0.13%	0.06%				X	X	X	32		
P2	DSA+ABMR+	6.44 %	X	5.17 %	3.38%	1.63%	X	5513	4739	X	X	X	63		
P3	DSA+ABMR+	16.5 %		9.00 %	5.06%	3.55%				X	X	X	75		
P4	DSA+ABMR+	8.45 %	X	9.01 %	3.50%	4.73%	X			X	X	X	58		
P5	DSA+ABMR+	21.1 %		7.60 %	2.80%	3.60%				X	X	X	36		X
P6	DSA+ABMR+	9.78 %		1.74 %	0.95%	0.46%		2803	1635	X		X	46		X
P7	DSA+ABMR+	9.08 %		1.53 %	0.76%	0.50%				X			52		
P8	DSA+ABMR+	20.7 %		15.1 %	6.49%	8.38%				X	X	X	28		X
P9	DSA+ABMR+	9.23 %		0.51 %	0.38%	0.03%				X	X	X	83	X	
P10	DSA+ABMR+	49.1 %		0.28 %	0.16%	0.05%		6130	3301.9	X	X	X	259		
P11	DSA+ABMR+	24.2 %		2.07 %	0.24%	0.54%				X	X	X	143	X	
P12	DSA+ABMR+	16.9 %	X	9.35 %	1.86%	6.80%	X	1732	1705	X	X	X	259		
P13	DSA+ABMR+	39.8 %		3.31 %	0.93%	2.12%				X	X	X	127		
P14	DSA+ABMR+	5.67 %		1.01 %	0.18%	0.64%				X	X	X	28		
P15	DSA+ABMR+	10.6 %		0.77 %	0.44%	0.31%				X	X	X	35		
P16	DSA+ABMR+	6.57 %		1.26 %	0.67%	0.42%		2069	1512	X	X	X	15		
P17	DSA+ABMR+	35.2 %		5.26 %	3.48%	1.51%		7061	1082	X	X	X	32	X	X
P18	DSA+ABMR+	2.01 %		1.06 %	0.74%	0.06%		2548	902	X	X	X	45		
P19	DSA+ABMR+	12.2 %		0.75 %	0.59%	0.11%				X	X	X	27		
P20	DSA+ABMR+	25.6 %		10.2 %	3.27%	6.68%				X	X	X	470		
P21	DSA+ABMR-	0.28 %		2.44 %	0.92%	1.28%				X		X	37		
P22	DSA+ABMR-									X				X	
P23	DSA+ABMR-	17.4 %		0.89 %	0.32%	0.46%		983	861	X	X	X			
P24	DSA+ABMR-							382	159.5	X				X	
P25	DSA+ABMR-	30.7 %		1.21 %	0.61%	0.52%				X	X	X	70		
P26	DSA+ABMR-									X				X	
P27	DSA+ABMR-	0.043 %	X	1.85 %	1.36%	0.38%	X			X	X	X		X	X
P28	DSA+ABMR-	1.49 %		0.13 %	0.09%	0.02%				X	X	X	18		
P29	DSA+ABMR-	1.99 %	X	0.19 %	0.12%	0.05%	X			X			33		
P30	DSA+ABMR-	5.29 %		0.16 %	0.14%	0.00%				X	X	X	24		
P31	DSA+ABMR-	4.94 %		0.25 %	0.16%	0.08%		211	400.1	X	X	X	7		
P32	DSA+ABMR-	5.35 %		0.24 %	0.15%	0.05%				X	X	X	21		
P33	DSA+ABMR-	18.8 %		2.35 %	1.37%	0.90%				X	X	X	259		X
P34	DSA+ABMR-	1.41 %		15.5 %	3.74%	11.08 %		1680	193	X	X	X	135		
P35	DSA+ABMR-									X				X	X
P36	DSA+ABMR-	1.49 %		0.22 %	0.13%	0.07%				X		X	50		
P37	DSA+ABMR-	12.5 %		0.14 %	0.07%	0.05%				X		X	44		
P38	DSA+ABMR-	2.68 %	X	7.63 %	3.99%	3.02%	X	655	1341.8	X	X	X	60		X
P39	DSA+ABMR-	0 %		1.04 %	0.39%	0.41%				X	X	X	29	X	X
P40	DSA+ABMR-	9.76 %		1.39 %	0.17%	1.10%				X	X	X	25		

P41	DSA+ABMR-	2.16 %		3.37 %	0.99%	2.16%				X			200		
P42	DSA+ABMR-	4.81 %		0.75 %	0.44%	0.23%				X			37		X
P43	DSA+ABMR-	3.16 %		0.23 %	0.05%	0.12%				X	X	X	17		
P44	DSA+ABMR-	0.39 %		1.30 %	0.64%	0.35%				X	X	X	18		X
P45	DSA+ABMR-	6.85 %		0 %	0.00%	0.00%		894	186	X	X	X	34		
P46	DSA+ABMR-	1.06 %		0.38 %	0.17%	0.15%				X		X	19		
P47	DSA+ABMR-	10.3 %		1.08 %	0.32%	0.75%				X	X	X	44		
P48	DSA+ABMR-	6.88 %		3.20 %	1.40%	1.65%				X	X	X	39		
P49	DSA+ABMR-	8.71 %		0.077 %	0.05%	0.02%				X	X	X	132		
P50	DSA+ABMR-	18.3 %		11.1 %	3.69%	6.95%				X	X	X	25		
P51	DSA+ABMR-	11.5 %		0.24 %	0.09%	0.13%				X		X	11		
P52	DSA-	1.88 %	X	1.06 %	0.82%	0.22%	X	25	325	X			28		X
P53	DSA-	1.99 %		0.072 %	0.06%	0.01%				X			12		
P54	DSA-	2.28 %		1.12 %	0.58%	0.55%				X			29		
P55	DSA-	2.83 %		0.16 %	0.09%	0.07%				X					
P56	DSA-	0.52 %		0.35 %	0.16%	0.12%				X			20		
P57	DSA-	6.16 %		0	0.00%	0.00%				X			51		
P58	DSA-	1.40 %		0.74 %	0.33%	0.31%				X			16		
P59	DSA-	0.73 %		0.32 %	0.12%	0.17%				X					
P60	DSA-	1.18 %		0.46 %	0.28%	0.18%				X					
P61	DSA-	2.91 %		0.084 %	0.03%	0.05%				X			21		
P62	DSA-	7.75 %		0.17 %	0.17%	0.00%				X					
P63	DSA-	0.27 %		0.76 %	0.47%	0.22%				X			36		X
P64	DSA-	0.52 %		0.082 %	0.04%	0.03%				X					
P65	DSA-	4.98 %		0.42 %	0.15%	0.19%				X			20		
P66	DSA-	1.06 %		0.073 %	0.03%	0.04%				X			22		
P67	DSA-	2.33 %		0.30 %	0.15%	0.08%				X			28		
P68	DSA-	0.41 %	X	0.00874	0.47%	0.38%	X	1302	806.8	X					X
P69	DSA-	10.6 %		0.60 %	0.27%	0.28%				X			29		
P70	DSA-	0.26 %		0.098 %	0.03%	0.04%				X					
P71	DSA-	0.76 %		1.27 %	0.72%	0.47%				X					
P72	DSA-	1.22 %		0.063 %	0.04%	0.01%				X			35		
P73	DSA-	2.22 %		0.47 %	0.06%	0.31%				X			21		
P74	DSA-	1.01 %		0.094 %	0.04%	0.02%				X			32		
P75	DSA-									X				X	
P76	DSA-	4.34 %		0.0005	0.00%	0.04%				X			88		
P77	DSA-	1.89 %		0.35 %	0.10%	0.10%				X			26		
P78	DSA-	2.28 %		0.10 %	0.07%	0.02%				X			24		
P79	DSA-	0.11 %		0.45 %	0.10%	0.31%				X					
P80	DSA-	1.87 %		0.43 %	0.17%	0.24%				X			15		
P81	DSA-	0.26 %		0.20 %	0.10%	0.10%				X			18		
P82	DSA-	0.93 %		0.053 %	0.00%	0.00%				X			7		
P83	DSA-	2.94 %		0.11 %	0.05%	0.05%				X			27		
P84	DSA-	0.52 %		0.25 %	0.16%	0.04%				X			15		
P85	DSA-	7.70 %		0.066 %	0.04%	0.02%				X			29		
P86	DSA-	2.53 %		0.12 %	0.06%	0.05%				X			13		
P87	DSA-	11.1 %		1.13 %	0.42%	0.59%				X					
P88	DSA-	0.48 %		0.62 %	0.55%	0.04%				X			11		X
P89	DSA-	0.79 %		1.25 %	0.67%	0.31%				X					
P90	DSA-	3.27 %		0.74 %	0.25%	0.41%				X			25		
P91	DSA-	1.83 %		0.30 %	0.09%	0.13%				X					
P92	DSA-	0.49 %		0.35 %	0.13%	0.02%				X					
P93	DSA-	3.10 %		0.018 %	0.00%	0.01%				X					
P94	DSA-	0.37 %		0.032 %	0.01%	0.01%				X					
P95	DSA-	3.28 %		0.21 %	0.07%	0.13%				X				X	

P96	DSA-							607	349	X				X	
P97	DSA-	0.44 %		0.040 %	0.00%	0.02%				X			34	X	
P98	DSA-							384	257.7	X				X	
P99	DSA-	9.65 %		0.15 %	0.06%	0.05%				X					
P100	DSA-									X				X	
P101	DSA-	4.68 %		0.15 %	0.06%	0.06%				X					
P102	DSA-									X				X	
P103	DSA-	0.44 %		0.11 %	0.07%	0.02%				X					
P104	DSA-							1277	787.12	X				X	
P105	DSA-	0.20 %	X	1.32 %	0.79%	0.39%	X			X					X

Table S3. Phenotypic patterns of cT$_{FH}$ cell clusters

cluster	markers	polarization	differentiation	activation	proliferation
1	PD-1^{low} CD38^{+} CD127$^{+/-}$ OX40^{+} IL-6R^{+}	Th0	effector memory	semi-activated	no
2	CD127$^{+/-}$ CD28^{+} c-Maf^{+} CD25^{+} OX40^{+} CXCR3^{+} CCR6^{+} IL-6R^{+}	Th1/17	effector memory	resting	no
3	CD127$^{+/-}$ CD25^{+} FoxP3^{+} OX40^{+} CXCR3^{+} Tbet^{+} IL-6R^{+}	Th1	effector memory	resting	no
4	Ki67^{hi} ICOS^{hi} PD1^{hi} CD38^{+} CCR7^{+} CD127^{+} CD28^{+} c-Maf^{+} IRF4^{+} IL-21R^{+} GATA3^{+} CCR6^{+}	Th2/Th17	central memory	activated	yes
5	CD38^{+} CD25^{+} FoxP3^{+} IL-6R^{+}	Treg	effector memory	semi-activated	no
6	CD38^{+} CD28^{+} c-Maf^{+} CD25^{+} OX40^{+} IL-6R^{+}	Th0	effector memory	semi-activated	no
7	CD127^{+} CD28^{+} IRF4^{+} CD25^{+} OX40^{+} IL-6R^{+}	Th0	effector memory	resting	no
8	Ki67^{low} ICOS^{low} PD1^{hi} CD38^{+} CCR7^{+} CD127^{+} CD28^{+} c-Maf^{+} IRF4^{+} IL-21R^{+} Tbet^{+}	Th1	central memory	activated	yes
9	CD38^{+} CD127^{+} CD28^{+} c-Maf^{+} CD25^{+} OX40^{+} CXCR3^{+} IL-6R^{+}	Th1	effector memory	semi-activated	no
10	PD-1^{low} CD38^{+} CD28^{+} c-Maf^{+} CD25^{+} OX40^{+} CCR6^{+} IL-6R^{+}	Th17	effector memory	semi-activated	no

Treg, T regulatory cells
Polarization was defined according to expression of CXCR3 and CCR6
Differentiation was defined according to expression of CCR7
Activation was defined according to expression of ICOS, PD-1 and CD38
Proliferation was defined according to expression of Ki67

Table S4. Impacted canonical pathways in cT$_{FH}$ from DSA+ABMR+ versus DSA- patients predicted by Ingenuity Pathway Analysis

Ingenuity Canonical Pathways	**-log(p-value)***	**Genes**
LXR/RXR Activation	7.18	IL1A,TNFRSF1A,CD36,IL6,ABCA1,TLR4,LYZ,CCL2,IL1RN,ITIH4,CD14,IL1B,S100A8,PTGS2,RXRA,IL1RAP,CLU,CYP51A1
iCOS-iCOSL Signaling in T Helper Cells	4.75	**CD40LG,CAMK4,PRKCQ,CD3E,**NFKBIE,**FGFR2,CD3D,CD28,**NFKBID,**CD3G,LCK,**HLA-DMA**,ICOS**,HLA-DRA**,LAT**,FCER1G,**ITK**
CD28 Signaling in T Helper Cells	4.24	**CAMK4,PRKCQ,CD3E**,NFKBIE,**FGFR2,CD3D,CTLA4,CD28**,NFKBID,**CD3G,LCK**,HLA-DMA,SYK,HLA-DRA**,LAT**,FCER1G,**ITK**
Role of NFAT in Regulation of the Immune Response	3.99	**CAMK4,PRKCQ,CD3E,FCGR2A,**NFKBIE**,GNB5,FGFR2,CD3D,CD28,**NFKBID,**CD3G,LCK**,HLA-DMA,SYK,HLA-DRA,**LAT**,FCER1G,LYN,PLCB1,**ITK**
Th1 Pathway	3.93	**CD40LG**,ICAM1,**PRKCQ,CD3E,HAVCR2**,IFNGR2,**FGFR2**,IL6,**CD3D,CD3G,CD28**,NFIL3,HLA-DMA,HLA-DRA,**ICOS,DLL4**
Calcium-induced T Lymphocyte Apoptosis	3.54	**CD3G,LCK,PRKCQ,CAMK4,CD3E**,HLA-DMA,HLA-DRA,NR4A1,FCER1G,**CD3D**
Th2 Pathway	3.45	CCR1,ICAM1**,PRKCQ,CD3E,MAF,FGFR2,CD3D**,SPI1,**CD3G,CD28**,HLA-DMA,HLA-DRA,**ICOS,S1PR1**,JAG1,ACVR2A
PPAR Signaling	3.01	NFKBID,IL1A,SRA1,IL1RN,TNFRSF1A,NFKBIE,IL1B,PTGS2,INSR,RXRA,IL1RAP,PDGFC

Genes in bold are the genes upregulated in DSA+ABMR+ versus DSA- group
*Statistical analysis was performed using Fisher's exact test.

Table S5. Upstream regulators of differentially expressed genes in cT_{FH} from DSA+ABMR+ versus DSA- patients predicted by Ingenuity Pathway Analysis

Upstream regulator	Molecule type	Predicted activation state	Activation z-score	P-value
STAT3	transcription regulator	activated	2.121	9.90E-04
IL-2	cytokine	inhibited	-2.478	3.03E-11

Table S6. DSA characteristics

	Overall DSA+	**DSA+ ABMR-**	**DSA+ ABMR+**	**P value***
	N=51	**N=31**	**N=20**	
DSA present before transplantation, n(%)	19 (37.3)	11 (35.5)	8 (40.0)	0.745
Post-tranplant DSA detection (months), mean ± SEM	3.3 ± 0.7	2.9 ± 0.8	3.9 ± 1.1	0.920
Early post-transplant DSA detection ≤ 3-month, n(%)	39 (76.5)	26 (83.9)	13 (65.0)	0.121
Transient DSA, n(%)**	6 (11.8)	6 (19.4)	0 (0)	0.070
HLA class specificity, n(%)				
HLA class I	14 (27.5)	11 (35.5)	3 (15.0)	0.198
HLA class II	23 (45.1)	16 (51.6)	7 (35.0)	0.244
HLA class I + II	14 (27.4)	4 (12.9)	10 (50.0)	0.009
MFI, mean ± SEM***	6456 ± 688	4736 ± 712	9122 ± 1149	0.001
IgG subclasses reactivity, n(%)****	25 (67.6) /37	11 (57.9) /19	14 (77.8) /18	0.197
Single IgG subclass reactivity	13 (35.1)	8 (42.1)	5 (27.8)	0.362
Multiple IgG subclasses reactivity	12 (32.4)	3 (15.8)	9 (50)	0.038

ABMR, antibody-mediated rejection; DSA, donor-specific antibody; HLA, human leukocyte antigen; MFI, mean fluorescence intensity

*Mann-Whitney and chi-squared test were used for statistical comparison of continuous and categorical variables, respectively

**one single DSA detection post-transplant during the first 24-months post-transplant

***MFI of the DSA with the highest MFI value

****14 out of 51 samples were not tested for IgG subclasses

Table S7. Antibodies

Marker	Dye	Clone	Vendor
Blimp1	AF647	6D3	BD
c-Maf	eFluor 660	sym0F1	Invitrogen
CCR6	PE-Cy7	11A9	BD
CCR7	BV711	3D12	BD
CD11c	BV510	B-ly6	BD
CD127	BV510	HIL-7R-M21	BD
CD19	APC-eFluor 780	SJ25C1	Invitrogen
CD20	BV570	2H7	Biolegend
CD21	APC	Bu32	Biolegend
CD24	BV605	ML5	BD
CD25	APC-Cy7	M-A251	BD
CD27	PE-Cy7	O323	Invitrogen
CD28	PE-Cy5	CD28.2	BD
CD3	BV750	SK7	Biolegend
CD3	BV510	SK7	Biolegend
CD38	PE-CF594	HIT2	BD
CD4	BV605	RPA-T4	BD
CD40L	eFluor 450	24-31	eBiosciences
CD45RO	BV570	UCHL1	Biolegend
CD71	PE	CY1G4	Biolegend
CD8	PerCp-Cy5.5	SK1	BD
CD86	PE-Cy5	IT2.2	Biolegend
CD95	PerCP-Cy5.5	DX2	BD
CXCR3	AF700	1C6/CXCR3	BD
CXCR3	BV605	G025H7	Biolegend
CXCR5	AF488	RF8B2	BD
FoxP3	APC	PCH101	Invitrogen
GATA3	BV421	L50-823	BD
ICOS	PerCP-eFluor 710	ISA-3	Invitrogen
IFNg	Alexa 700	B27	BD
IgD	BV421	IA6-2	BD
IL-17a	PE	N49-653	BD
IL-21	APC	3A3-N2.1	BD
IL-21R	BV786	17A12	BD
IL-4	PE-CF594	MP4-25D2	BD
IL-6R	BB515	M5	BD
IRF4	eFluor 450	3E 4	Invitrogen
IRF8	PerCP-eFluor 710	V3GYWCH	Invitrogen
Ki67	BV480	B56	BD
OX40	PerCP-Cy5.5	ACT35	BD
PD-1	BV650	EH12.1	BD
RORgt	BV650	Q21-559	BD
Tbet	PE	4B10	BD