Landscape of innate immune system transcriptome and acute T-cell mediated rejection of human kidney allografts

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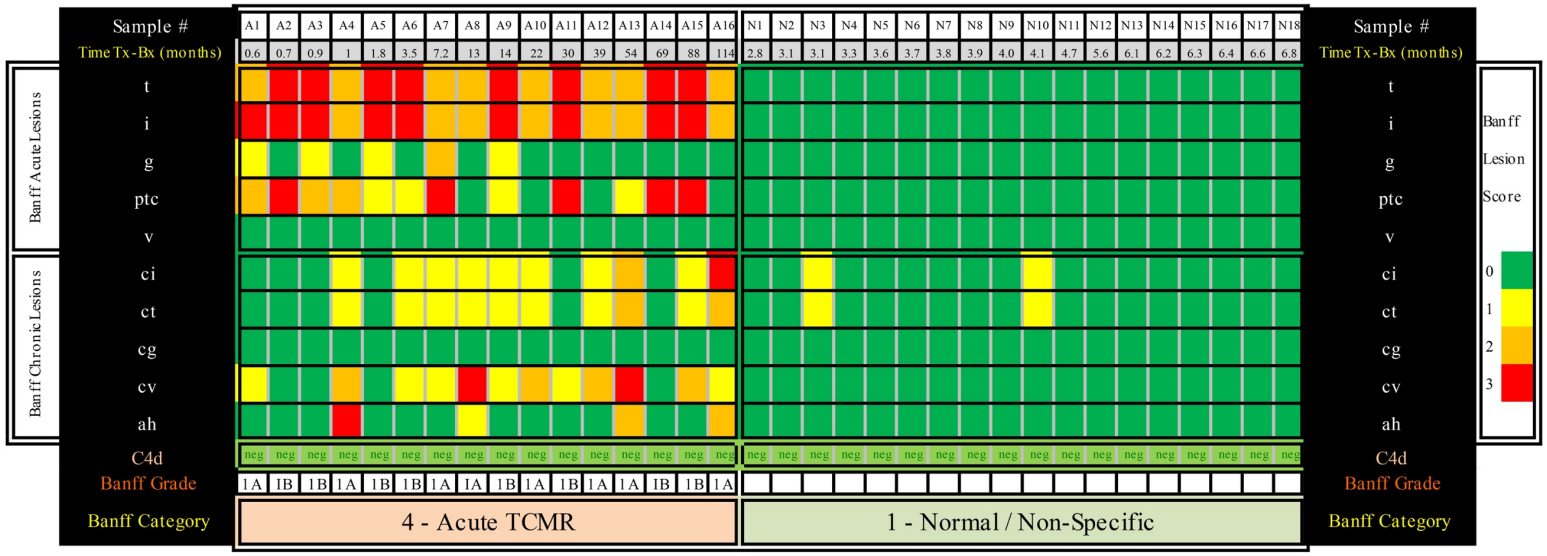
Supplemental Materials

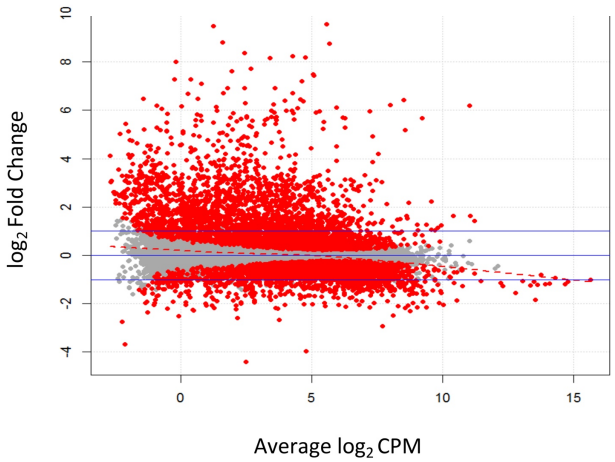
Supplemental Figure 1. Banff histopathology scores and global intragraft expression of mRNAs in human kidney allografts.

A.

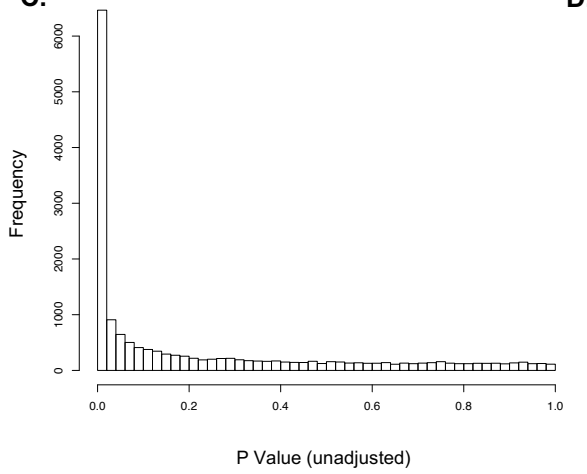


TCMR Biopsies (N=16) vs. Normal Biopsies (N=18)

B.



C.



D.

Gene Expression	P-FDR P<0.05	P<0.01	P<0.001
Decreased	2,819	1,768	866
Increased	3,622	2,704	1,995
Differentially Expressed	**6,441**	**4,472**	**2,861**
Unchanged	9,940	11,909	13,520
% DE	39.3%	27.3%	17.5%
Dispersion	19.94%		
BCV	44.66%		

(A) Banff histopathological scores of the 34 kidney allograft biopsies obtained from 34 kidney allograft recipients. Biopsy tissue sections were stained with hematoxylin and eosin, periodic acid Schiff, and Masson trichrome for light microscopic evaluation. Staining for polyoma virus was done using affinity-purified and agarose-conjugated IgG_{2a} mouse monoclonal antibody (Calbiochem, San Diego, CA) that recognizes a 94-kDa SV40 large T antigen. Indirect immunofluorescence for complement factor 4 degradation (C4d) product was done on cryosections using a monoclonal anti-C4d antibody (Quidel, Santa Clara, CA). The biopsies were categorized using the Banff 2017 update of the Banff '97 classification (1). The Banff diagnostic categories include acute T-cell mediated rejection (Acute TCMR, Sample # A1 through A16) and normal/non-specific (Normal, Sample # N1 through N18). Immunostaining of the biopsies for CD3 confirmed the higher abundance of CD3+ T lymphocytes in TCMR (315 cells per high power field (40x), median) compared to Normal (40 cells). All biopsies classified as acute TCMR were performed because of acute graft dysfunction. All biopsies classified as Normal were performed for surveillance purpose in recipients without acute graft dysfunction. The median number of glomeruli per biopsy sample was 14 (range 9-18). Colors represent Banff scores from 0 to 3. Banff grade and Banff acute scores for tubulitis (t), interstitial inflammation (i), glomerulitis (g), peritubular capillary inflammation (ptc) score and vascular inflammation (v). Banff chronic scores for interstitial fibrosis (ci), tubular atrophy (ct)) score, chronic glomerulopathy (cg), chronic vascular lesions (cv) score and arteriolar hyaline thickening (ah) are shown for each biopsy. Also shown in the figure is the staining for complement factor 4d (C4d) in the peritubular capillaries.

(B) Smear plot of the mRNA expression between TCMR and Normal biopsies. Library preparation for RNA sequencing was done by poly-A selection of mRNA and conversion to single-stranded cDNA using random hexamer primer followed by second strand generation to create double stranded cDNA. The first batch contained 15 samples (10 TCMR and 5 Normal) and were sequenced single-end, 6 pooled libraries per lane of a flow cell, on a HiSeq 2500 (Illumina, Inc., San Diego, CA) sequencer. The second batch contained 19 samples (6 TCMR and 13 Normal) and were sequenced paired-end, 10 pooled libraries per lane of a flow cell, on a HiSeq 4000 sequencer. The raw sequencing data were stored in FASTQ format. The 101 bp single and paired-end reads were aligned to human genome (GRCh37/hg19 assembly) using Tophat2 (2). The human UCSC reference annotation was used to align and quantitate the transcripts. A gene was considered expressed in the TCMR or Normal biopsy when its normalized mRNA count was $\geq$1 count per million mapped reads (cpm) in at least 1 sample in that group. The X-axis is the average RNA counts per million mapped reads (CPM), depicted as average $\log_2$

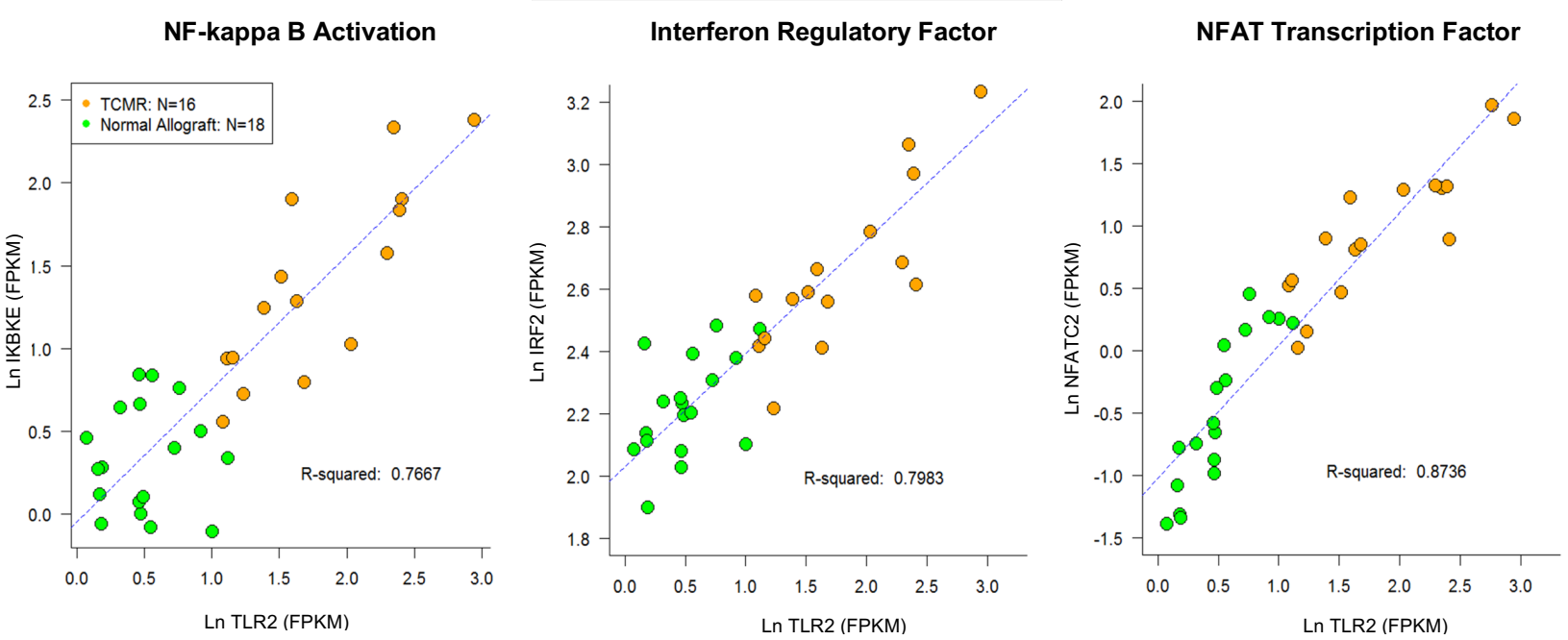
CPM. The differential intragraft expression of mRNAs between TCMR and Normal is depicted as log_2Fold Change on the Y-axis. Fold change was determined using edgeR, a software package released by Bioconductor, an R programming language-based open source, open development software project. edgeR fits a negative binomial model of the sequence data and derives probability values for the differential expression using an exact test, which uses a quantile-adjusted conditional maximum likelihood method (3). Knowing the conditional distribution for the sum of counts in a group, the exact P values are computed by summing over all sums of counts that have a probability less than the probability under the null hypothesis of the observed sum of counts. The exact test for the negative binomial distribution has strong parallels with Fisher's exact test. Probability values were adjusted for FDR using the Benjamini-Hochberg method and is the expected proportion of false positives among all the statistically significant P values (<0.05). We considered P-FDR<0.05 as statistically significant. A value of 0 on the Y-axis corresponds to no difference between the expression in TCMR and Normal (TCMR/Normal =1). A value above 0 denotes mRNA abundance in TCMR is greater than in Normal biopsy. A value below 0 denotes mRNA abundance in TCMR is less than in Normal biopsy. Each dot represents a mRNA. Red dots are mRNAs whose difference in expression between TCMR and Normal is statistically significant at P<0.05.

(C) Histogram of P values derived from comparing mRNAs between TCMR and Normal. We identified a total of 16,381 protein-coding genes including mitochondrial genes that were expressed in the kidney allograft. A gene was considered expressed in the TCMR or Normal group when its normalized mRNA count was ≥1 count per million mapped reads in at least 1 sample in that group. The difference in the expression of a gene between TCMR and Normal was compared using the exact test implemented in edgeR. Graph depicts the histogram of all the 16,381 P values. Each P value tests the null hypothesis of no difference in gene expression between TCMR and Normal. If all 16,381 null hypotheses are true, then the P values, by definition, follow a uniform distribution between 0 to 1 and the histogram is flat (4). Among the 16,381 P values, 8658 (53%) were >0.05. As depicted, the distribution of P values (except those closer to 0) was flat and represents the null P values. On the contrary, if the null hypothesis is not true, then there is greater occurrence of low P values close to 0. Among the 16,381 P values, 7723 (47%) were <0.05. As depicted, the peak of the histogram is close to 0 which represents the alternate hypothesis. Thus, the shape of the histogram reassured us that the vast majority of 7723 statistically significant (<0.05) P values were not false positives.

(D) Differentially expressed mRNAs between TCMR and Normal. Table depicts the number of mRNAs with higher or lower abundance in TCMR compared to Normal, and the number of mRNAs differentially expressed (DE) at different levels of significance. Among the 7723 genes that were statistically significant at P<0.05 between TCMR and Normal, 6441 (83%) were statistically significant at P-FDR<0.05. Among the 6441 (39.3% of the 16,381 total genes) differentially expressed mRNAs, the abundance of 3622 (56%) were higher and that of 2819 (44%) were lower in TCMR compared to Normal. The P-FDR is the expected proportion of false positives among all the statistically significant P values (<0.05). Thus, among the 6441 mRNAs that were significantly different between TCMR and Normal, 322 (5%) are expected to be false positive. Biological coefficient of variation (BCV), estimated as the square root of dispersion, is the coefficient of variation with which the (unknown) true abundance of the gene varies between biological replicates. It represents the CV that would remain between biological replicates if the sequencing depth could be increased indefinitely.

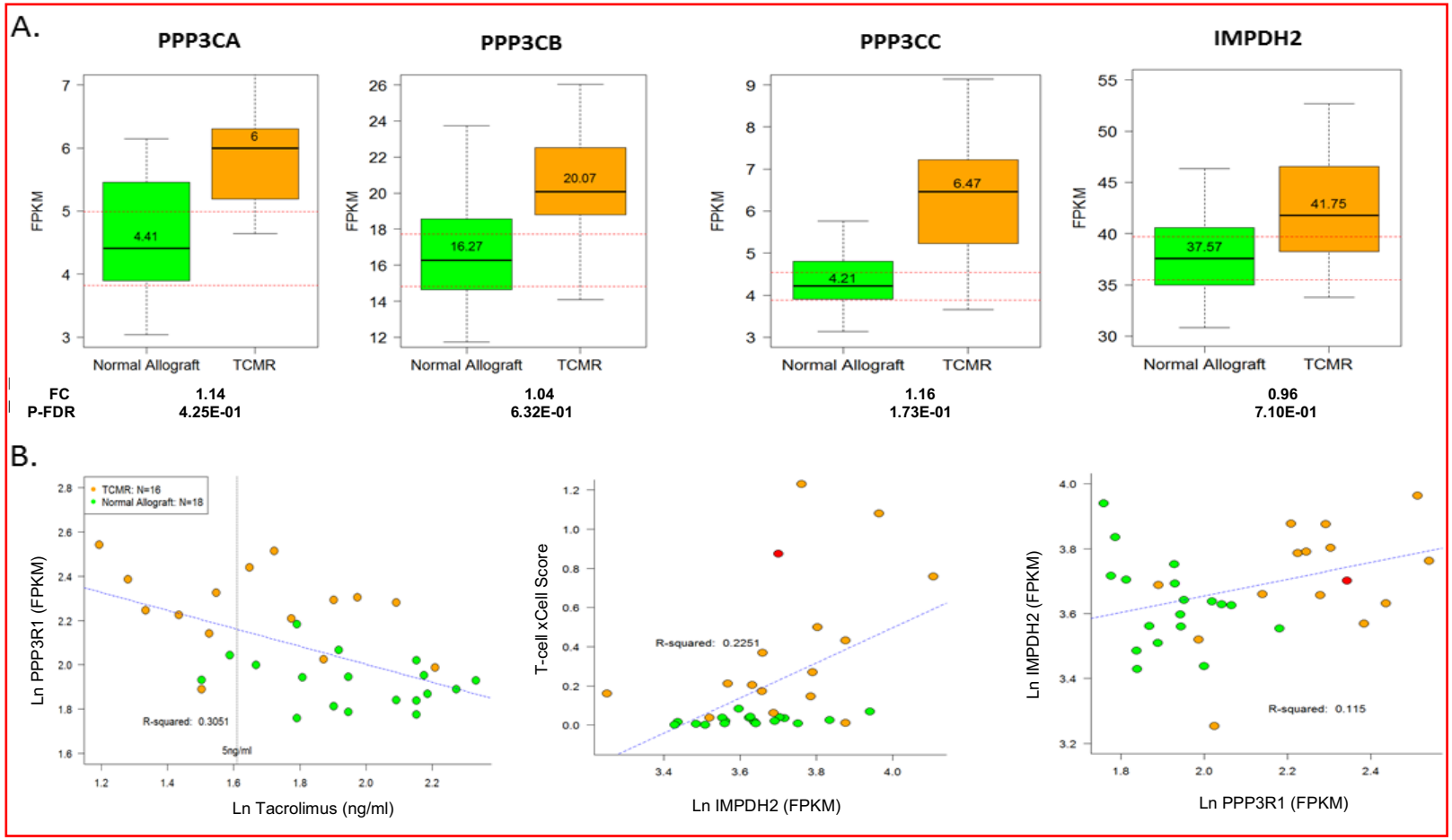
1. *Haas M, Loupy A, Lefaucheur C, Roufosse C, Glotz D, Seron D, et al. The Banff 2017 Kidney Meeting Report: Revised diagnostic criteria for chronic active T cell-mediated rejection, antibody-mediated rejection, and prospects for integrative endpoints for next-generation clinical trials. Am J Transplant. 2018;18(2):293-307.*
2. *Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, and Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. Genome Biol. 2013;14(4):R36.*
3. *Robinson MD, McCarthy DJ, and Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010;26(1):139-140. https://bioconductor.org/packages/release/bioc/html/edgeR.html*
4. *Breheny P, Stromberg A, Lambert J. p-Value Histograms: Inference and Diagnostics. High Throughput. 2018;7(3).pii:E23.*

Supplemental Figure 2. Relationship between the intragraft abundance of mRNAs for pattern recognition receptor TLR2 and its downstream signaling molecules in kidney allografts.



TLR signaling results in the activation of NF-kappa-B, Interferon regulatory factors, and NAFT pathways. Panels depict the significant positive association between TLR2 and IKBKE, a key gene of NF-kappa-B pathway **(A)**; between TLR2IRF2, an interferon regulatory factor **(B)**; and between TLR2 and NFATC2, a member of the NFAT family **(C)**. FPKM is the number of fragments per kilobase of exon per million reads mapped value, which normalizes the RNA sequencing data for sequencing depth (per million scaling factor) and gene length (per kilobase scaling factor). mRNA abundance is represented as $\log_e$FPKM values. The strength of the association is expressed as the coefficient of determination (r^2).

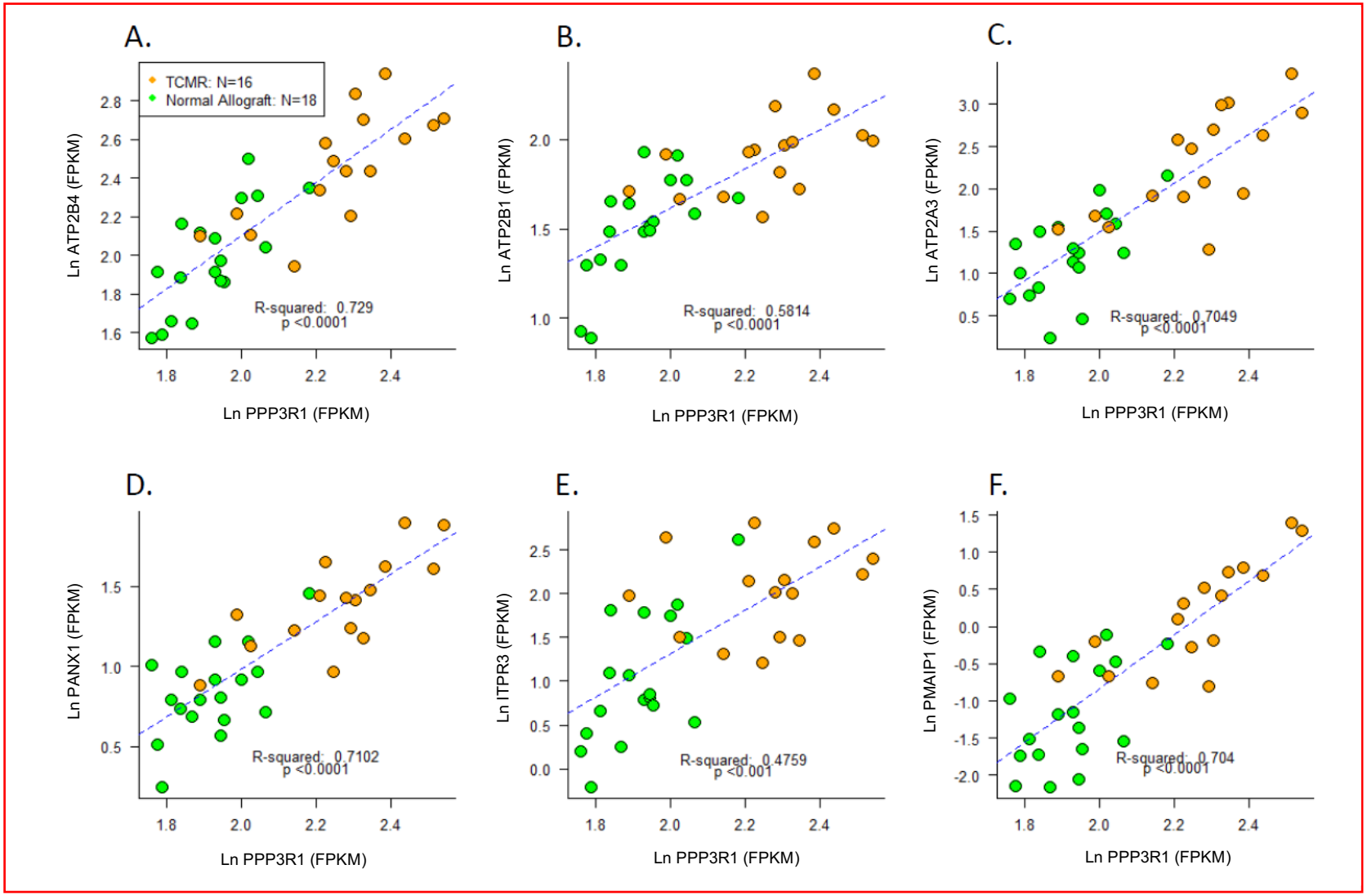
Supplemental Figure 3. Intragraft abundance of mRNA calcineurin subunits and IMPDH isoform 2 in TCMR and Normal Biopsies.



Box plots depict the intragraft abundance of mRNA for calcineurin isoforms PPP3CA (catalytic subunit alpha), PPP3CB (catalytic subunit beta), and PPP3CC (catalytic subunit gamma), and inosine-5'-monophosphate dehydrogenase isoform 2 (IMPDH2) in TCMR and Normal biopsies **(A).** The horizontal dotted red lines represent the 95% confidence interval of the Normal group. Scatter plots **(B)** depict the relation between mRNA abundance of calcineurin isoform PPP3R1 (regulatory subunit alpha) and tacrolimus whole blood trough level at the time of kidney allograft biopsy (left panel), IMPDH2 and a T-cell enrichment score, derived by xCell (1), an in-silico cell-type enrichment analysis (middle panel), and PPP3R1 and IMPDH2 (right panel).

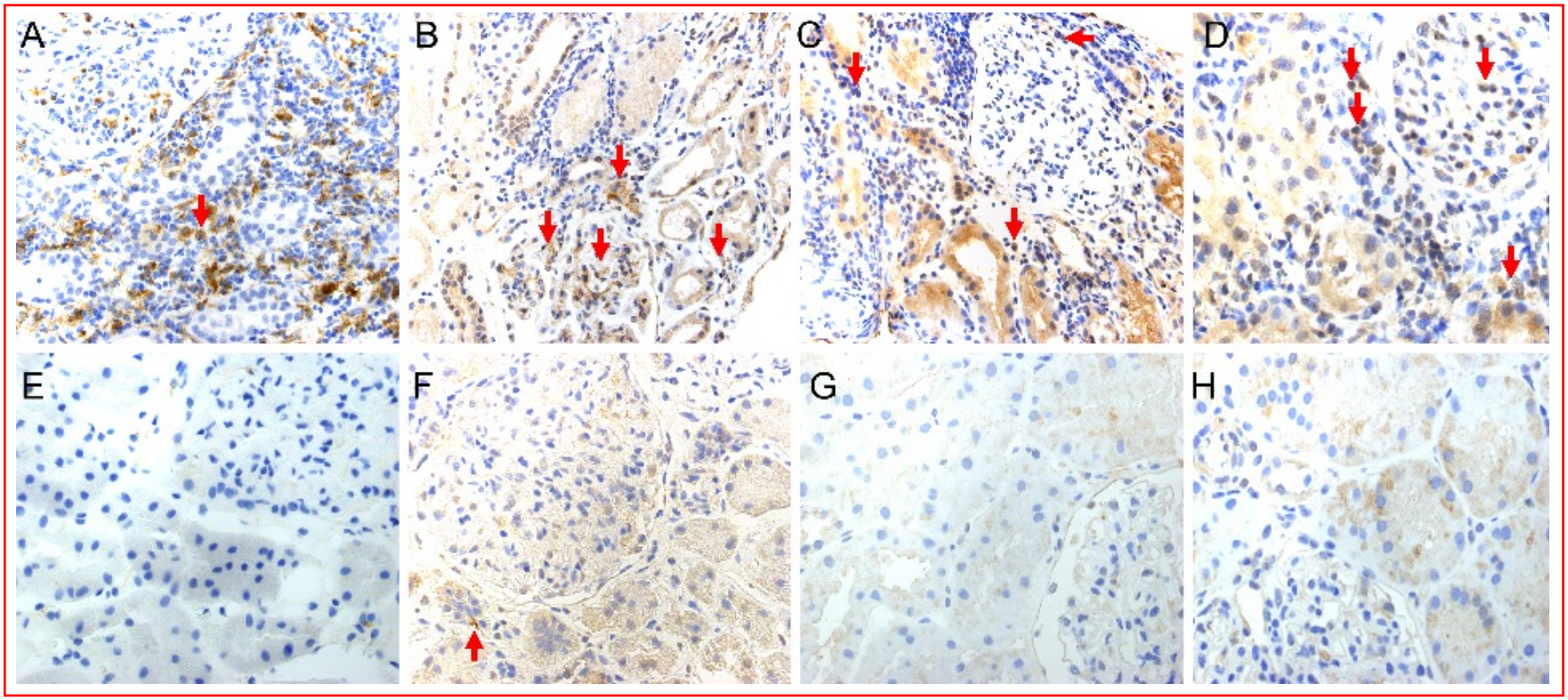
1. Aran D, Hu Z, and Butte AJ. xCell: digitally portraying the tissue cellular heterogeneity landscape. Genome Biol. 2017;18(1):220.

Supplemental Figure 4. Relationship between intragraft abundance of mRNAs for calcineurin subunit PPP3R1 and Ca^{2+}ATPases in kidney allografts.



All four calcineurin subunits were expressed in the kidney allograft. The intragraft abundance of PPP3CA (catalytic subunit alpha), PPP3CB (catalytic subunit beta), PPP3CC (catalytic subunit gamma), and PPP3R1 (regulatory subunit alpha) were higher in TCMR compared to Normal biopsy. However, the difference in abundance was statistically significant only for PPP3R1. The top panel depicts the positive association between the abundance of PPP3R1 mRNA and the abundance of mRNA for ATP2B4 **(A)**, mRNA for ATP2B1 **(B)**, and mRNA for ATP2A3 **(C)**. The bottom panel depicts the positive relationship between the abundance of PPP3R1 mRNA and the abundance of PANX1 mRNA **(D)**, ITPR3 mRNA **(E)**, and PMAIP1 mRNA **(F)**. The plasma membrane calcium ATPase (PMCA) ATP2B4 pumps Ca^{2+} from the cytosol to the exterior and ATP2B1is another PMCA. The sarcoplasmic reticular Ca^{2+}ATPase (SERCA) ATP2A3 pumps Ca^{2+} from cytosol into the endoplasmic reticulum. PANX1 is a Ca^{2+} leak channel that regulates endoplasmic reticulum Ca^{2+} homeostasis; ITPR3, is an intracellular channel that mediates Ca^{2+} release from the endoplasmic reticulum following stimulation by inositol 1,4,5-trisphosphate; and PMAIP1 (Noxa) promotes mitochondrial membrane changes and efflux of apoptogenic proteins from the mitochondria.

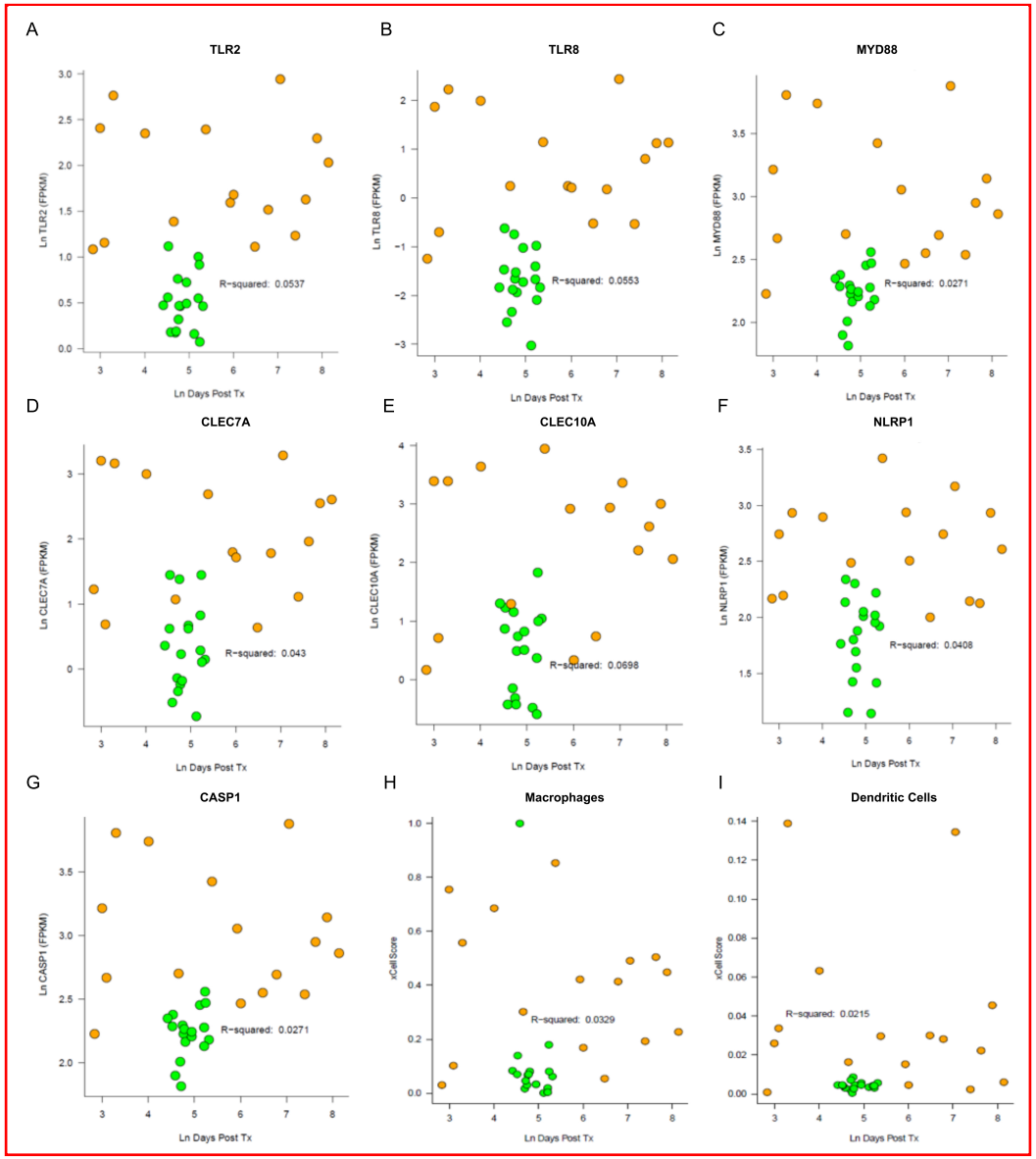
Supplemental Figure 5. Immunostaining of kidney allografts for markers of innate immune system.



Photomicrographs of representative kidney allograft biopsies with TCMR (top panel) and Normal (bottom panel) depict staining for CD68+ macrophages (**A and E**), Toll-like receptor TLR4 (**B and F**), NOD-like receptor NLRP1 (**C and G**), and inflammatory caspase CASP1 (**D and H**), using CD68, TLR4, NLRP1, and CASP1 antibodies, respectively, on paraffin-embedded tissue sections. In the top panel, TCMR biopsies show positive staining for TLR4, NLRP1, and CASP1 in the macrophages. Some of the stained macrophages are shown by a red arrow. In the bottom panel, the Normal biopsies are negative for CD68, TLR4, NLRP1, and CASP1, except for an occasional cell that stained positive for TLR4 (F). Photomicrographs A to C are shown magnified 400 times and photomicrographs D to H are shown magnified 600 times.

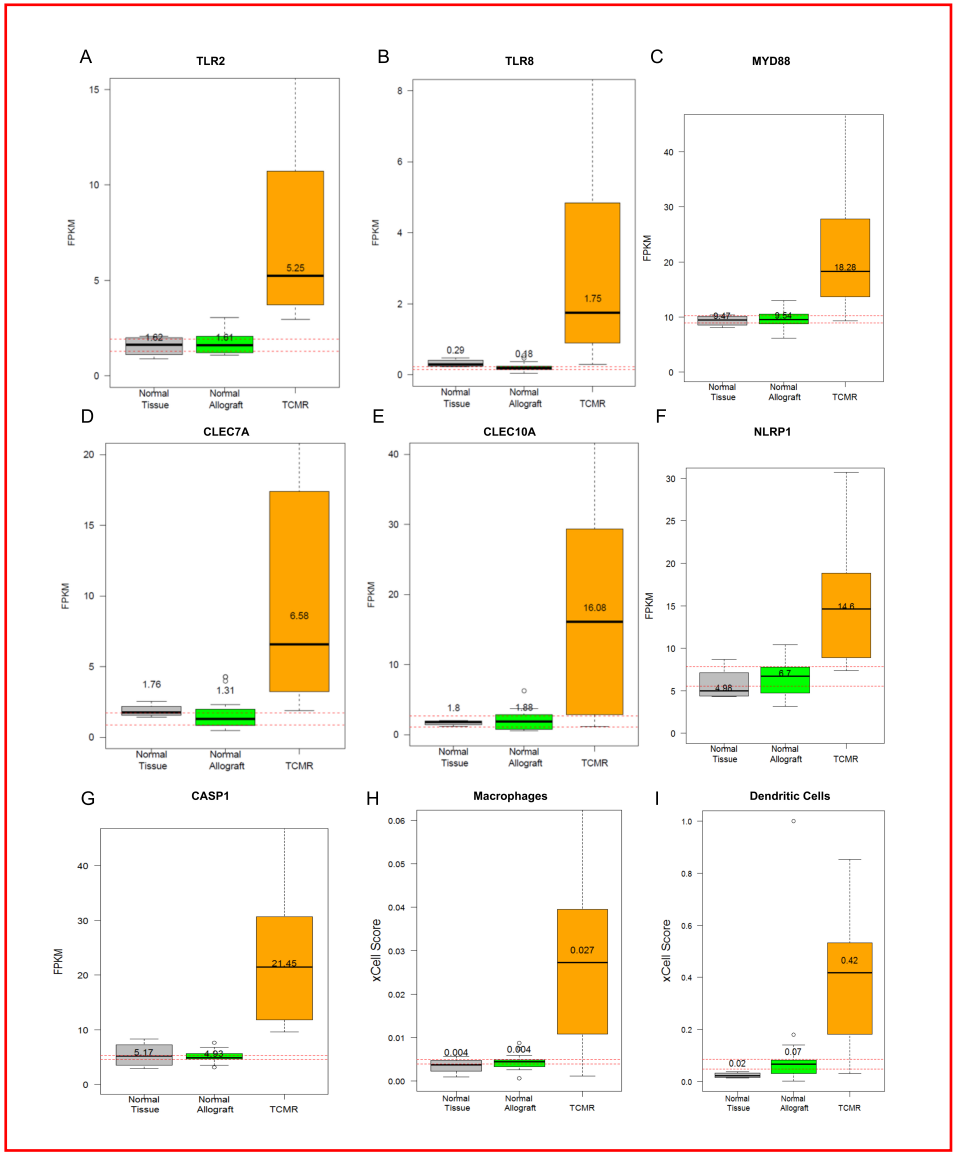
Immunohistochemical staining was done using the respective antibodies on paraffin-embedded kidney allograft tissue sections on a Leica Bond system (Buffalo Grove, IL) using the modified protocol F provided by the manufacturer. The section was pretreated using heat-mediated antigen retrieval with Tris-EDTA buffer (pH = 9, epitope retrieval solution 2) for 20 min and incubated with the antibodies for 15 min at room temperature. CD3, CD68, TLR4, NLRP1, and CASP1 were detected using a horseradish peroxidase–conjugated compact polymer system and 3,3’-Diaminobenzidine as the chromogen. Each section was counterstained with hematoxylin and mounted with Leica Micromount.

Supplemental Figure 6. Relationship between intragraft abundance of mRNAs and time from transplantation to allograft biopsy.



Scatter plots depict the relation between mRNA abundance of TLR2 **(A)**, TLR8 **(B)**, MYD88 **(C)**, CLEC7A **(D)**, CLEC10A **(E)**, NLRP1 **(F)**, or CASP1 **(G)** and time from transplantation to the allograft biopsy. Also shown are the relation between xCell scores of macrophages **(H)** or dendritic cells **(I)** and time from transplantation to the allograft biopsy.

Supplemental Figure 7. Intragraft abundance of mRNAs in TCMR and Normal compared to abundance of mRNAs in native non-transplant kidneys.



Box plots depict the intragraft and native kidney abundance of mRNAs of TLR2 **(A)**, TLR8 **(B)**, MYD88 **(C)**, CLEC7A **(D)**, CLEC10A **(E)**, NLRP1 **(F)**, and CASP1 **(G)**. We analyzed the RNA-sequencing data of four native non-transplant kidneys (Normal Tissue) available from a public database (proteinatlas.org) and compared the expression of a select panel of mRNAs with that of TCMR and Normal. The horizontal dotted red lines represent the 95% confidence interval of the Normal group. Also shown are the xCell scores of macrophages **(H)** and dendritic cells **(I)** in the three groups.

Supplemental Table 1. Characteristics of kidney allograft recipients and RNA samples.

Characteristics	Kidney Allograft Biopsy	
	Acute T Cell Mediated Rejection	Normal
	N=16	N=18
At the time of transplant		
Recipient information		
Age, years, mean (SD)	47 (12)	49 (12)
Women, n (%)	8 (50)	9 (50)
Racial categories, Black, n (%)	6 (38)	4 (22)
Donor information		
Age (years), mean (SD)	42 (17)	46 (13)
Women, n (%)	10 (63)	14 (78)
Racial categories, Black, n (%)	1 (16)	1 (6)
Deceased donor, n (%)	8 (50)	7 (39)
HLA-ABDR mismatch, median (IQR)	5 (3-6)	5 (3-6)
Induction therapy, n (%)	16 (100)	18 (100)
Antithymocyte globulin, n (%)	12 (75)	18 (100)
After transplant and before the index biopsy		
Delayed graft function, n (%)	4 (25)	0 (0)
Calcineurin inhibitors, n (%)	15 (94)	18(100)
Corticosteroids maintenance, n (%)	3 (19)	2 (11)
BK virus nephropathy	0 (0)	0 (0)
Biopsy proven acute rejection, n (%)	0 (0)	0 (0)
At the time of index allograft biopsy		
Reason for biopsy, clinically indicated: surveillance, n	16:0	0:18
Time from transplantation to biopsy, months, median (IQR)	12.5 (1-49)	3.5 (3-6)
Serum creatinine, mg/dl, median (IQR)	2.55 (1.77-3.83)	1.34 (1.05-1.48)
Serum tacrolimus trough level, ng/dl, median (IQR)	5.2 (4.2-6.7)	7.0 (6.0-8.7)
Allograft biopsy histopathology		
Number of glomeruli, median (IQR)	13 (9-18)	15 (10-18)
Positive staining for SV40 large T antigen, n (%)	0 (0)	0 (0)
Positive staining for complement component 4d, n (%)	0 (0)	0 (0)
Banff category 4 – acute T cell mediated rejection, n (%)	16 (100)	0 (0)
Banff category 1 – normal / nonspecific, n (%)	0 (0)	18 (100)
Banff acute T cell mediated rejection grade 1A:1B, n	8:8	0:0
Allograft biopsy tissue RNA		
Total RNA isolated from kidney allograft biopsy tissue stored in RNAlater™, ng, median (IQR)	2143 (1270-3030)	2085 (1545-2758)
Total RNA purity, A260/A280, median (IQR)	2.06 (1.96-2.13)	2.00 (1.97-2.02)
Total RNA integrity, RNA integrity number, median (IQR)	7.75 (7.0-8.5)	8.2 (7.4-8.7)
Quantity of total RNA used for mRNA sequencing, ng	400	400

Kidney allograft biopsies were categorized using the Banff 2017 update of the Banff '97 classification. All biopsies reported as acute T cell mediated rejection (TCMR) were performed because of acute graft dysfunction. All biopsies reported as Normal were done for surveillance purpose in recipients without acute graft dysfunction. All but one patient received calcineurin inhibitor (Tacrolimus)-based maintenance immunosuppression. One recipient in the TCMR group received ASKP1240, a fully human antibody targeting CD40 in antigen presenting cells. This patient was converted to Tacrolimus after the rejection. All 34 recipients received Mycophenolate Mofetil. We assessed RNA purity by the ratio of absorbance at 260 nm and 280 nm. A ratio of ~2.0 is accepted as 'pure' for RNA. We assessed RNA integrity using the RNA integrity number (RIN), an algorithm developed by Agilent Technologies, that assigns a numerical score from 1 to 10 for total RNA. A score of 1 is the most degraded profile while a score of 10 is the most intact RNA.

Supplemental Table 2. Intragraft expression of mRNAs encoding Toll-Like Receptors in TCMR and Normal kidney allograft biopsies.

Supplemental Table 2A. Plasma membrane and endosomal/lysosomal TLRs.

Plasma membrane TLRs

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
TLR1	1.40	0.65	3.59	3.20	2.73	2.32E-11	7.37E-10
TLR2	1.61	0.74	5.25	6.75	3.89	6.81E-18	1.34E-15
TLR4	2.02	1.35	6.27	4.08	2.48	5.55E-10	1.35E-08
TLR5	1.35	2.44	3.18	3.23	1.61	9.86E-03	2.85E-02
TLR6	0.18	0.14	0.79	0.58	4.68	1.02E-14	7.05E-13

Endosomal/lysosomal TLRs

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
TLR3	2.78	3.08	5.34	3.78	1.34	3.11E-03	1.15E-02
TLR7	0.32	0.30	2.43	2.32	5.96	5.86E-17	7.54E-15
TLR8	0.18	0.10	1.75	2.96	14.32	6.43E-19	1.57E-16
TLR10	0.15	0.10	1.00	1.36	6.37	2.22E-11	6.75E-10

The median and inter quartile range (IQR) values for intragraft mRNA abundance in TCMR and Normal biopsies are shown as fragments per kilobase of exon per million reads mapped (FPKM) value which normalizes the RNA sequencing data for sequencing depth (per million scaling factor) and gene length (per kilobase scaling factor). Median and IQR of the FPKM were computed using Cufflinks bioinformatics tool (1). The difference in intragraft expression of mRNAs between TCMR and Normal biopsies is expressed as Fold Change (FC). A FC value of 1 corresponds to no difference between the groups (TCMR/Normal=1); FC > 1 denotes expression of TCMR > Normal, and FC with a negative sign denotes expression of Normal > TCMR. Fold change was calculated using edgeR. Probability (P) values are adjusted for false discovery rate (FDR) by applying the Benjamini-Hochberg method on the P value. Thus, for a given gene, the median (IQR) FPKM value for each group is derived from Cufflinks, while the FC and P-FDR value is derived from edgeR.

1.Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, et al. Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc. 2012;7(3):562-78. http://cole-trapnell-lab.github.io/cufflinks/cufflinks/

Supplemental Table 2B. Association between intragraft abundance of mRNAs for TLRs and mRNAs for their key signal adapters/transducers in human kidney allografts.

			TLR1	TLR2	TLR3	TLR4	TLR5	TLR6	TLR7	TLR8	TLR10
		FC	2.73	3.89	2.48	1.61	4.68	1.34	5.96	14.32	6.37
Symbol	FC	P-FDR	3.00E-10	3.42E-16	1.15E-02	8.33E-09	2.50E-02	4.39E-13	7.55E-15	1.57E-16	6.76E-10
MYD88	2.03	1.50E-07	0.934	0.949	0.297	0.843	0.096	0.928	0.902	0.953	0.764
MAP3K1	1.62	4.11E-03	0.35	0.301	0.866	0.561	0.924	0.34	0.409	0.19	0.149
MAPK1	1.09	2.79E-01	0.324	0.27	0.839	0.547	0.842	0.33	0.368	0.198	0.137
IKBKE	3.07	2.01E-09	0.894	0.904	0.281	0.837	0.076	0.906	0.844	0.921	0.67
IRAK1	1.14	2.87E-01	0.686	0.708	-0.138	0.496	-0.293	0.657	0.583	0.675	0.567
IRF1	4.92	6.21E-15	0.905	0.913	0.325	0.863	0.11	0.894	0.838	0.92	0.649
IRF2	1.43	1.49E-04	0.929	0.923	0.434	0.907	0.273	0.915	0.878	0.896	0.66
IRF4	18.70	2.30E-17	0.84	0.855	0.176	0.663	0.107	0.831	0.795	0.789	0.706
NFATC1	1.63	5.50E-04	0.887	0.865	0.441	0.881	0.262	0.862	0.803	0.841	0.514
NFATC2	3.68	8.10E-12	0.954	0.941	0.547	0.943	0.359	0.951	0.928	0.885	0.683
TP53	1.40	1.82E-04	0.915	0.889	0.461	0.832	0.217	0.892	0.852	0.796	0.702

The relationship intragraft abundance of mRNAs for TLRs (columns) and mRNAs for their key signal adapters/transducers (rows) are expressed as correlation coefficients (r). Yellow/red represents r values that are significant at false discovery rate adjusted (by Benjamini-Hochberg method) P value <0.001.

Supplemental Table 3. Intragraft expression of mRNAs encoding C-Type Lectins, C-Type Lectin Receptors, and Mannose Receptors in TCMR and Normal biopsies.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
CLEC12A*	0.000	0.000	3.398	11.689	15.12	4.99E-17	6.51E-15
CLEC7A	1.305	1.095	6.584	12.708	5.47	1.37E-13	6.56E-12
CLEC10A	1.882	2.043	16.082	25.863	7.15	2.21E-11	6.76E-10
CD209	0.844	0.551	2.896	2.971	3.92	2.55E-11	7.67E-10
CD69	0.458	0.367	2.774	5.480	6.46	5.43E-11	1.53E-09
CLEC4E	0.446	0.482	2.946	3.436	5.74	8.30E-11	2.23E-09
CLEC2B	2.866	1.213	9.172	7.680	2.95	2.80E-10	6.90E-09
CLEC4A	1.949	1.793	6.609	6.442	3.37	1.89E-09	3.93E-08
CLEC4D	0.044	0.062	0.262	0.306	8.10	7.54E-08	1.12E-06
CLEC5A**	0.000	0.000	0.000	0.000	0.00	1.07E-07	1.54E-06
CLEC4C	0.108	0.060	0.398	0.514	6.15	2.46E-06	2.60E-05
CLEC17A	0.093	0.033	0.367	0.414	4.78	3.31E-06	3.36E-05
CLEC9A	0.403	0.265	1.268	2.050	3.50	5.19E-06	4.98E-05
MRC1	1.313	0.635	5.453	5.540	2.65	1.76E-05	1.43E-04
CD302	8.791	3.624	12.986	5.112	1.48	1.02E-04	6.54E-04
CLEC4G	0.186	0.146	0.338	0.314	3.14	1.72E-04	1.02E-03
CLEC4F	0.310	0.141	0.684	0.839	2.52	4.28E-04	2.20E-03
CLEC6A	0.021	0.033	0.095	0.261	3.97	2.22E-03	8.71E-03
CD207	0.100	0.127	0.210	0.471	2.72	6.09E-03	1.99E-02
CLEC18A	57.896	41.250	15.823	18.684	-3.23	1.20E-04	7.55E-04
CLEC18B	72.131	41.502	23.702	31.562	-2.56	5.86E-04	2.87E-03
CLEC3B	71.991	22.846	48.734	31.795	-1.52	2.26E-03	8.83E-03
PLA2R1	5.576	7.308	2.541	2.784	-1.77	6.56E-03	2.11E-02
CLEC11A	7.542	2.804	9.162	4.217	-1.01	9.51E-01	9.69E-01
CLEC14A	6.015	2.753	7.894	3.416	1.07	4.20E-01	5.51E-01
CLEC16A	5.714	1.804	7.296	1.947	1.16	5.59E-02	1.17E-01
CLEC1A	1.243	0.367	1.959	1.041	1.26	5.86E-02	1.22E-01
MRC2	11.075	4.331	15.889	10.014	1.19	2.30E-01	3.52E-01

* expressed in 3 Normals only

** expressed in 1 Normal and 3 TCMR

Supplemental Table 4. Intragraft expression of mRNAs encoding NOD-Like Receptors in TCMR and Normal Biopsies.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
NLRP1	6.70	2.82	14.60	9.88	2.26	4.5E-10	1.1E-08
NLRP2	1.81	1.28	2.39	2.25	1.18	4.3E-01	5.6E-01
NLRP3	0.37	0.23	1.29	1.32	3.82	1.3E-10	3.6E-09
NLRP6	0.79	0.32	0.80	0.59	-1.10	6.9E-01	7.9E-01
NLRP7	0.04	0.09	0.19	0.12	4.90	1.3E-04	7.7E-04
NLRP9	0.21	0.19	0.18	0.15	-1.42	4.8E-02	1.0E-01
NLRP11	0.16	0.09	0.22	0.14	1.07	7.5E-01	8.3E-01
NLRP12	0.15	0.07	0.27	0.24	1.76	8.2E-03	2.5E-02
NLRP14	0.12	0.07	0.10	0.08	-1.40	1.5E-01	2.5E-01
NOD1	1.10	0.50	2.47	3.62	1.48	1.9E-05	1.5E-04
NOD2	0.20	0.18	1.56	2.32	6.00	3.7E-15	3.0E-13
NAIP	3.02	1.19	7.71	4.20	1.92	1.7E-06	1.8E-05
NLRC4	0.34	0.18	0.88	1.07	3.12	1.3E-08	2.5E-07
NLRC5	4.18	2.25	20.70	20.34	4.31	2.2E-17	3.8E-15
NLRX1	6.30	1.41	6.62	1.32	-1.11	1.2E-01	2.2E-01
AIM2	0.32	0.27	3.14	3.95	12.74	1.2E-15	1.1E-13
IFI16	10.39	3.66	30.84	29.43	3.10	8.6E-16	8.1E-14
MEFV	0.26	1.99	0.79	2.14	4.87	4.0E-09	8.1E-08
CIITA	1.62	0.94	13.17	14.68	2.55	2.0E-12	7.9E-11

Supplemental Table 5. Gene set enrichment analysis of mRNAs encoding Pattern Recognition Receptors in TCMR and Normal.

Gene set analysis of PRRs

PRRs	Gene Count	ROAST PropDown*	ROAST PropUp*	Direction	ROAST P-FDR	Camera P-FDR
TLR	9	0%	100.0%	Up	1.00E-03	9.20E-07
CLR	27	11%	81.5%	Up	1.00E-03	1.06E-05
NLR	18	11%	66.7%	Up	1.00E-03	3.26E-04
RLR	5	0%	80.0%	Up	1.00E-03	4.91E-03
Secreted	12	25%	41.7%	Up	1.00E-03	6.22E-01
PRRs	**71**	**11%**	**73%**	**Up**	**1.00E-03**	**6.18E-10**

Gene set testing is a differential expression analysis in which a P value is assigned to a set of genes as a unit. We used ROAST (1) and CAMERA (2) for gene set testing. ROAST uses rotation, a Monte Carlo method, for multivariate regression. CAMERA estimates and incorporates the variance inflation factor associated with inter-gene correlation.

Pathway analysis of PRRs

PRR Pathways	Pathway Genes	Expressed in Kidney	EdgeR DE Analysis DE Genes	% DE	DE Up	% Up	DE Down	% Down	GSEA (GAGE) stat. mean	q-val Up
Toll-like receptor signaling pathway	104	86	58	67.4%	55	94.8%	3	5.2%	3.615	3.76E-45
NOD-like receptor signaling pathway	168	147	101	68.7%	90	89.1%	11	10.9%	4.247	1.94E-62
RIG-I-like receptor signaling pathway	70	54	33	61.1%	31	93.9%	2	6.1%	1.962	6.49E-15
Cytosolic DNA-sensing pathway	64	48	29	60.4%	25	86.2%	4	13.8%	2.021	1.94E-15
C-type lectin receptor signaling pathway	104	97	58	59.8%	55	94.8%	3	5.2%	*	1.28E-08

*enrichment assessed with Camera

Enriched PRR pathways (Kyoto Encyclopedia of Genes and Genomes [KEGG] database (3)) in TCMR biopsies. We used GAGE (Generally Applicable Gene-set Enrichment (4)) for this analysis. For a given pathway, the GAGE algorithm tests whether specific gene sets are significantly differentially expressed (each TCMR vs. all Normal) relative to the background whole gene set of 16,381 genes (each TCMR vs. all Normal) that we identified, using a rank-based two-sample T test (equivalent to the non-parametric Mann-Whitney test). For each gene set, a global P value is derived on a meta-test on the negative log sum of all P values from the individual TCMR vs. Normal comparisons. The stat mean is the mean of the individual statistics from multiple gene set tests. Its absolute value measures the magnitude of gene-set level changes, and its sign indicates direction of the changes (positive: up regulated in TCMR, negative: down regulated in TCMR). The q value is the false discovery rate adjustment, by Benjamini-Hochberg method, on the global P value.

1. Wu D, Lim E, Vaillant F, Asselin-Labat ML, Visvader JE, and Smyth GK. ROAST: rotation gene set tests for complex microarray experiments. Bioinformatics. 2010;26(17):2176-82.
2. Wu D, and Smyth GK. Camera: a competitive gene set test accounting for inter-gene correlation. Nucleic Acids Res. 2012;40(17):e133.
3. Kanehisa M, Furumichi M, Tanabe M, Sato Y, and Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 2017;45(D1):D353-D61. https://www.kegg.jp
4. Luo W, Friedman MS, Shedden K, Hankenson KD, and Woolf PJ. GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinformatics. 2009;10:161.

Supplemental Table 6. Association between intragraft abundance of mRNAs for NLRs and mRNAs for Caspases in TCMR and Normal biopsies.

			NLRP1	NLRP3	NLRP7	NLRP12	NAIP	NOD1	NOD2	AIM2	IFI16	NLRP2	NLRP6	NLRP9	NLRP11	NLRP14
		FC	2.26	3.82	4.90	1.76	1.92	1.48	6.00	12.74	3.10	1.18	-1.10	-1.42	1.07	-1.40
Symbol	FC	P-FDR	5.60E-09	2.83E-09	6.90E-04	2.15E-02	9.02E-06	7.15E-05	2.26E-13	7.44E-14	3.46E-14	5.36E-01	8.05E-01	1.41E-01	7.81E-01	2.87E-01
Inflammatory Caspases																
CASP1	3.94	1.71E-15	0.867	0.928	0.757	0.766	0.728	0.845	0.95	0.974	0.978	0.395	0.082	-0.152	-0.002	-0.345
CASP4	2.30	1.04E-10	0.747	0.828	0.735	0.647	0.721	0.789	0.858	0.973	0.904	0.462	-0.038	-0.187	-0.135	-0.347
CASP5	7.39	3.66E-06	0.562	0.688	0.574	0.629	0.623	0.672	0.742	0.912	0.814	0.426	-0.034	-0.187	-0.108	-0.256
CASP12	-1.09	8.79E-01	-0.039	-0.084	-0.076	0.142	0.252	0.138	-0.021	-0.137	-0.039	0.456	-0.526	0.79	0.234	0.309
Apoptosis Caspases																
CASP3	1.94	3.12E-10	0.838	0.885	0.706	0.707	0.771	0.857	0.898	0.937	0.948	0.432	-0.023	-0.074	-0.048	-0.299
CASP6	1.05	5.88E-01	0.191	0.192	0.213	0.151	0.181	0.369	0.182	0.217	0.186	0.045	0.119	0.041	0.259	-0.06
CASP7	1.28	1.05E-02	0.577	0.574	0.396	0.46	0.736	0.72	0.637	0.596	0.725	0.59	-0.379	0.42	0.069	-0.205
CASP8	1.64	1.54E-07	0.887	0.91	0.716	0.767	0.727	0.874	0.903	0.918	0.933	0.356	0.173	-0.087	0.117	-0.281
CASP9	-1.45	1.43E-04	-0.391	-0.449	-0.331	-0.542	-0.52	-0.425	-0.409	-0.406	-0.363	0.127	-0.302	-0.062	-0.309	-0.334
CASP10	1.58	4.85E-06	0.774	0.765	0.629	0.675	0.841	0.869	0.8	0.782	0.852	0.695	-0.319	0.27	0.071	-0.13

The relation between NLRs (columns) and caspases (rows) are expressed as correlation coefficients (r). Yellow/red represents r values that are significant at false discovery rate adjusted (by Benjamini-Hochberg method) P value <0.001.

Supplemental Table 7. Intragraft expression of mRNAs encoding secreted PRRs and Complements in TCMR and Normal.

Supplemental Table 7A. Secreted PRRs.

Symbol	Normal N=18 Median	Normal N=18 IQR	TCMR N=16 Median	TCMR N=16 IQR	FC	PValue	P-FDR
COLEC12	1.536	2.110	3.110	3.026	1.66	1.11E-02	3.15E-02
COLEC11	25.589	17.896	14.979	12.765	-2.66	2.88E-05	2.20E-04
APCS	0.019	0.040	0.027	0.108	10.13	1.84E-03	7.46E-03
FCN1	1.617	1.784	17.047	44.733	11.74	1.87E-13	8.65E-12
FCN2	0.151	0.303	0.463	0.549	-1.19	6.35E-01	7.41E-01
FCN3	6.196	6.619	2.520	4.528	-1.94	5.00E-02	1.07E-01
NPTX1	0.177	0.142	0.148	0.120	-1.73	6.23E-02	1.27E-01
NPTX2	0.095	0.057	0.162	0.181	4.01	1.42E-04	8.64E-04
NPTXR	0.550	0.546	1.320	0.641	1.57	1.94E-03	7.80E-03
PTX3*	0.000	0.000	0.000	2.129	4.12	1.69E-06	1.85E-05
SFTPD	0.801	0.374	0.678	0.837	-1.10	7.65E-01	8.43E-01
SVEP1	4.207	1.765	8.179	5.810	1.55	7.84E-03	2.43E-02

* expressed in 7 TCMR only

Supplemental Table 7B. Classical complement pathway.

Symbol	Normal N=18 Median	IQR	TCMR N=16 Median	IQR	FC	PValue	P-FDR
C1QA	46.796	20.830	168.449	184.756	6.34	4.7E-14	2.7E-12
C1QB	34.206	20.662	183.209	181.760	8.67	6.9E-17	9.9E-15
C1QC	28.110	13.161	118.803	150.781	7.78	8.0E-16	7.7E-14
C1R	106.538	47.354	271.671	171.570	2.05	2.2E-06	2.4E-05
C1S	32.087	40.264	126.252	83.637	2.53	7.3E-09	1.4E-07
C2*	0.000	5.700	0.000	35.371	4.55	1.1E-08	2.1E-07
C4A	78.914	51.799	99.512	84.184	-1.01	9.8E-01	9.9E-01
C4B	73.238	55.346	99.047	85.090	1.12	6.2E-01	7.3E-01
C4BPA	0.064	0.101	0.141	0.273	2.02	3.9E-02	8.8E-02
C4BPB	0.275	0.150	0.454	0.223	1.78	4.2E-02	9.2E-02
C5	2.900	0.832	2.418	1.190	-1.31	1.7E-02	4.4E-02
C6	0.753	0.560	1.399	0.769	1.33	1.5E-01	2.6E-01
C7	167.570	90.072	202.066	109.502	-1.03	8.7E-01	9.2E-01
C8G	1.416	1.204	1.460	1.604	-1.17	4.6E-01	5.9E-01
CD46	69.849	25.235	83.227	25.354	1.05	6.9E-01	7.8E-01
CD55	8.528	3.633	14.217	5.734	1.24	2.5E-02	6.1E-02
CFHR1	2.597	1.276	6.716	6.851	2.54	4.8E-03	1.6E-02
CSMD1	0.347	0.649	0.415	0.768	-1.95	6.4E-02	1.3E-01
CSMD2	0.109	0.036	0.162	0.190	1.64	1.0E-02	3.0E-02
SERPING1	131.155	46.509	264.290	210.620	1.90	5.0E-06	4.8E-05

*C2 expressed in 6 TCMR and 7 Normals; C9 not expressed

Supplemental Table 7C. Alternative and lectin complement pathway.

Symbol	Normal N=18 Median	IQR	TCMR N=16 Median	IQR	FC	PValue	P-FDR
C3	16.091	23.699	32.132	93.807	8.21	5.3E-11	1.6E-09
CFB	28.843	19.835	87.223	68.369	3.56	2.6E-10	6.7E-09
CFD	2.934	2.734	11.729	8.275	2.90	5.8E-06	5.5E-05
CFH	9.194	2.167	18.331	10.912	1.72	2.5E-04	1.4E-03
CFI	121.265	44.086	131.254	52.137	-1.02	8.9E-01	9.3E-01
CFP	1.046	0.983	4.797	8.559	4.25	2.5E-08	4.3E-07
MASP1	3.236	1.629	2.591	2.406	-1.35	1.3E-01	2.3E-01
MASP2	0.853	1.137	1.627	1.006	1.12	3.8E-01	5.1E-01

Supplemental Table 7D. Complement receptors & inhibitors.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
C3AR1	2.114	1.694	10.114	8.221	5.11	9.0E-21	6.7E-18
C5AR1	2.984	1.042	5.467	2.680	1.69	9.5E-05	6.0E-04
CR1	0.547	0.277	1.095	0.932	1.99	6.7E-04	3.1E-03
CR2	2.912	6.748	0.409	1.154	-2.41	4.6E-02	1.0E-01
ITGAM	1.196	0.695	4.188	4.155	3.57	2.7E-14	1.6E-12
ITGAX	1.391	1.013	10.609	22.879	7.84	9.9E-16	9.1E-14
ITGB2	7.629	4.952	32.321	61.485	6.65	6.1E-18	1.2E-15
VTN	3.393	14.758	0.000	0.000	-2.59	4.1E-03	1.4E-02
CD59	186.974	93.021	254.029	105.379	1.01	9.0E-01	9.4E-01
CLU	480.871	241.785	941.003	615.386	1.33	8.4E-02	1.6E-01
VSIG4	5.746	3.335	18.001	18.046	4.85	4.8E-14	2.8E-12

Supplemental Table 8. Intragraft expression of mRNAs encoding Damage Associated Molecular Patterns in TCMR and Normal.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
AGER*	0.00	0.00	0.00	1.58	1.35	6.68E-03	2.14E-02
CD36	2.95	2.61	7.76	5.06	2.38	2.06E-04	1.19E-03
CD44	7.08	4.43	20.96	17.87	2.56	1.51E-10	3.86E-09
DDB1	46.83	13.24	68.34	21.23	-1.16	9.29E-02	1.74E-01
DNAJA1	62.10	9.62	90.66	20.25	1.16	3.65E-02	8.33E-02
FPR1	0.42	0.25	3.46	7.67	12.41	1.65E-17	2.62E-15
FPR2	0.16	0.10	0.41	0.85	9.25	8.32E-09	1.59E-07
FPR3	1.28	0.69	7.31	5.28	6.23	2.80E-21	3.28E-18
HIF1A	23.43	11.41	49.65	28.17	1.73	1.35E-04	8.29E-04
HMGB1	84.56	15.90	124.52	47.19	1.01	6.75E-01	7.73E-01
HMGN1	67.35	12.48	84.63	36.80	1.14	4.51E-02	9.85E-02
HMOX1	46.18	17.99	50.90	35.60	1.09	6.85E-01	7.81E-01
HSP90AA1	201.39	41.34	211.47	31.10	-1.08	6.09E-01	7.19E-01
HSP90AB1	200.89	27.10	224.47	30.98	-1.11	4.53E-01	5.82E-01
HSP90B1	177.76	32.58	237.03	86.60	1.18	1.20E-01	2.12E-01
HSPA6	0.79	0.48	3.09	3.11	3.58	1.78E-10	4.66E-09
HSPB3	0.06	0.10	0.03	0.09	4.47	1.83E-02	4.71E-02
HSPBAP1	4.53	1.12	7.58	3.24	1.46	7.23E-05	4.78E-04
IL1RL1	5.05	5.24	1.47	2.87	-1.80	9.59E-02	1.78E-01
LRP1	18.32	8.40	30.05	11.08	1.63	3.16E-04	1.71E-03
P2RX7	0.61	0.90	2.75	2.91	5.14	1.06E-11	3.42E-10
S100A8	2.47	2.02	5.75	14.89	3.68	1.21E-05	1.04E-04
S100A9	3.67	2.54	16.59	23.24	5.28	4.55E-12	1.58E-10
SAP130	4.55	1.38	5.46	1.05	1.01	6.78E-01	7.76E-01
SP1	7.82	4.53	12.14	4.05	1.31	5.48E-04	2.71E-03
SYK	4.97	2.41	12.05	8.09	1.53	2.69E-03	1.02E-02
TXNIP	132.47	36.27	245.88	149.22	1.76	8.83E-06	7.87E-05

* not expressed in Normals

Supplemental Table 9. Intragraft expression of mRNAs encoding DNA repair genes in TCMR and Normal.

Supplemental Table 9A. Genes recruited to location of DNA double-strand breaks.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
ATM	2.319	1.873	5.570	1.173	1.81	2.08E-08	3.64E-07
BRCA1	0.587	0.189	1.068	0.633	1.80	2.30E-05	1.78E-04
CMPK2	1.328	0.422	3.055	3.392	2.09	4.40E-05	3.11E-04
DNASE1L3	16.886	8.683	19.588	18.707	-1.11	4.48E-01	5.75E-01
H2AFX	4.454	1.605	6.765	2.830	1.59	7.11E-04	3.29E-03
MDC1	7.535	1.867	10.987	2.549	1.22	4.95E-02	1.05E-01
MRE11A	1.854	1.443	3.909	1.629	1.59	1.37E-04	8.08E-04
NBN	6.587	2.071	9.942	2.466	1.29	4.38E-04	2.19E-03
PRKDC	17.607	6.906	23.909	8.145	1.22	1.06E-02	3.01E-02
RAD50	5.676	2.487	7.905	2.062	1.05	4.95E-01	6.20E-01
SMC1A	6.170	2.654	8.006	2.497	1.13	1.66E-01	2.71E-01
TBKBP1	5.092	1.315	5.749	1.794	-1.06	5.77E-01	6.92E-01
TMEM173	17.229	6.068	28.036	14.793	1.52	5.42E-05	3.73E-04
TP53BP1	6.976	2.248	9.985	3.114	1.34	4.04E-04	2.05E-03

Supplemental Table 9B. Genes recruited to location of DNA replication stress.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
ATR	9.808	8.001	14.093	13.147	1.30	3.68E-04	1.89E-03
ATRIP	2.543	1.067	2.749	1.085	1.00	9.70E-01	9.80E-01
BLM	0.280	0.145	0.911	1.084	3.72	1.77E-09	3.85E-08
HUS1	4.173	0.963	5.232	1.691	1.01	9.34E-01	9.57E-01
RAD1	6.655	2.279	9.048	2.441	1.03	6.49E-01	7.52E-01
RAD17	9.513	2.135	11.272	3.898	-1.03	6.70E-01	7.69E-01
RPA1	8.482	3.104	21.777	15.237	1.18	1.01E-02	2.90E-02
TREX1	2.543	1.067	2.749	1.085	-1.08	9.70E-01	9.80E-01

Supplemental Table 9C. Cytosolic nucleic acid sensor genes.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
ADAR	25.992	8.420	39.861	6.555	1.32	1.19E-03	5.01E-03
EIF2AK2	3.794	1.257	5.977	2.053	1.35	4.38E-04	2.19E-03
IFIT1	21.448	6.033	20.128	8.059	-1.22	7.10E-02	1.40E-01
MB21D1	0.398	0.252	1.579	1.240	4.14	2.30E-13	1.12E-11
OAS1	13.017	4.628	25.978	21.509	1.50	1.04E-02	2.96E-02
PNPT1	6.949	3.023	9.662	2.814	-1.04	5.68E-01	6.84E-01
PQBP1	40.512	8.704	38.090	12.414	-1.30	1.61E-02	4.24E-02
RNASEL	3.187	0.752	4.935	0.852	1.32	5.59E-04	2.69E-03
SUPV3L1	17.725	4.124	15.985	5.912	-1.20	1.08E-02	3.06E-02

Supplemental Table 10. Gene set enrichment analysis of mRNAs encoding DAMPs, DNA damage and repair sensors in TCMR and Normal.

Damage & Repair	Gene Count	ROAST PropDown*	ROAST PropUp*	Direction	ROAST P-FDR	Camera P-FDR
DAMPs	**22**	**9.1%**	**68.2%**	**Up**	**1.00E-03**	**2.58E-03**
DNA Damage & Nucleic Acid Sensing						
DSB	14	0.0%	78.6%	Up	1.00E-03	3.25E-03
RDS	7	0.0%	42.9%	Up	1.00E-03	1.81E-01
NAS	9	33.3%	55.6%	Up	3.00E-03	2.41E-01
DNA Damage & NAS	**30**	**10.0%**	**63.3%**	**Up**	**1.00E-03**	**7.81E-04**
Damage & Repair Sensors	**52**	**9.6%**	**65.4%**	**Up**	1.00E-03	**3.80E-05**

* proportion of genes in set with z < -sqrt(2) for down or z > sqrt(2) for up

DAMPs: Danger Associated Molecular Patterns; DSB: DNA double strand break sensing genes;

RDS: Replication DNA Stress sensing; NAS: Nucleic Acid sensing genes

Supplemental Table 11. Intragraft expression of mRNAs encoding key intracellular Ca^{2+} regulators in TCMR and Normal.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
SLC8A3	0.081	0.093	0.144	0.047	2.21	3.73E-03	1.29E-02
SLC24A4	0.092	0.082	0.317	0.414	3.84	1.18E-06	1.33E-05
ATP2A3	3.471	2.240	9.908	9.209	2.79	2.45E-10	6.32E-09
ATP2B1	4.608	1.352	6.929	1.794	1.30	8.73E-04	3.90E-03
ATP2B4	6.992	2.138	11.748	5.512	1.48	1.45E-05	1.21E-04
KCNN4	0.712	0.488	2.944	4.433	5.67	6.74E-13	2.92E-11
KCNMA1	1.163	1.505	2.537	2.104	1.91	2.77E-03	1.01E-02
ORAI1	5.160	1.609	7.445	2.886	1.29	1.46E-02	3.94E-02
ORAI2	4.609	2.451	9.028	2.656	1.35	2.60E-03	9.59E-03
ORAI3	15.549	3.800	12.903	3.112	-1.42	6.03E-03	1.92E-02
ITPR3	2.295	3.693	8.041	7.107	1.96	5.21E-06	4.96E-05
CAMK2D	6.086	2.562	9.585	2.720	1.29	1.59E-04	9.23E-04
STIM1	8.473	1.901	11.383	3.720	1.20	6.03E-02	1.23E-01
STIM2	7.955	1.353	8.820	2.508	1.11	1.65E-01	2.70E-01
CALB1	92.093	73.110	15.427	70.490	-2.66	1.04E-02	2.98E-02
CALM1	132.711	32.467	124.094	18.231	-1.37	5.60E-03	1.80E-02
CALML3	12.176	23.025	0.550	3.142	-4.05	7.79E-03	2.36E-02

Supplemental Table 12. Intragraft expression of mRNAs encoding mitochondrial Ca^{2+} uniporter genes in TCMR and Normal.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
CAMK2D	6.09	2.56	9.58	2.72	1.29	1.59E-04	9.21E-04
MCUB	1.06	0.55	4.19	3.00	3.94	3.60E-13	1.65E-11
MCU	6.65	1.19	8.52	1.86	1.16	3.73E-02	8.38E-02
MICU1	53.82	22.38	29.06	10.60	-1.83	4.02E-07	5.17E-06
MICU2	36.67	3.79	31.01	5.39	-1.32	8.09E-05	5.21E-04
SMDT1	91.15	19.08	69.42	26.54	-1.67	9.37E-07	1.09E-05

Supplemental Table 13. Intragraft expression of mRNAs encoding Endoplasmic Reticulum stress and Unfolded Protein Response in TCMR and Normal.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
EIF2AK3	2.457	1.401	5.223	1.646	1.61	7.21E-07	8.64E-06
EIF2AK4	6.026	2.905	7.722	1.247	1.27	5.09E-03	1.67E-02
EDEM1	6.420	1.203	9.615	2.883	1.29	1.27E-03	5.29E-03
EDEM3	6.024	1.754	7.358	1.201	1.23	1.73E-02	4.50E-02
ERO1L	4.153	0.748	6.896	2.968	1.48	4.27E-06	4.15E-05
TXNDC5	42.025	13.302	67.308	40.416	2.01	4.99E-03	1.64E-02
DDIT3 (CHOP)	28.740	5.964	0.000	24.868	-1.30	1.87E-02	4.79E-02
ERN1	1.919	0.988	3.326	0.555	1.50	1.71E-05	1.40E-04
PPP1R15A	9.138	2.212	12.771	3.219	1.24	1.54E-02	4.09E-02
MAN1C1	21.693	10.299	16.015	7.572	-1.65	1.24E-03	5.20E-03

Supplemental Table 14. Intragraft expression of mRNAs encoding cellular oxidative stress in TCMR and Normal biopsies.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
CYBB (NOX2)	3.84	2.95	20.43	29.11	6.41	1.50E-18	3.75E-16
NOX4	138.14	151.36	52.50	50.77	-2.57	2.01E-03	7.76E-03
NCF1	1.61	0.71	7.21	8.56	5.37	2.01E-12	7.93E-11
NCF2	1.57	0.98	7.73	15.33	5.85	3.80E-16	4.08E-14
NCF4	2.67	0.82	10.46	9.97	3.95	1.09E-13	5.69E-12
SOD1	295.43	150.35	217.87	101.07	-1.74	1.30E-04	7.78E-04
SOD2	85.62	38.00	186.45	57.67	2.04	1.40E-06	1.55E-05
TXNIP	132.47	36.27	245.88	149.22	1.76	1.14E-05	9.79E-05
PRDX1	361.68	102.23	448.64	230.45	-1.26	8.06E-02	1.55E-01
PRDX4	29.04	8.10	31.21	9.95	1.04	7.62E-01	8.39E-01
PRDX2	204.07	43.40	148.61	47.93	-1.88	1.38E-08	2.52E-07
PRDX3	163.31	27.00	125.89	27.49	-1.51	8.53E-05	5.46E-04
PRDX5	237.50	110.25	183.41	45.94	-1.62	1.34E-04	7.98E-04
PRDX6	147.50	43.32	125.85	23.03	-1.48	6.56E-04	3.09E-03
CAT	155.39	63.28	112.57	45.97	-1.68	1.67E-04	9.63E-04
COX4I1	451.76	159.19	388.34	61.69	-1.62	5.53E-05	3.80E-04
GPX3*	14380.00	16036.53	3810.78	6671.59	-3.58	5.13E-04	2.51E-03
GPX4	305.63	120.54	243.44	100.81	-1.76	5.03E-04	2.47E-03

Supplemental Table 15. Intragraft expression of mRNAs encoding proteins involved in glycolysis and gluconeogenesis in TCMR and Normal biopsies.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
ACSS1	31.75	13.68	27.97	4.99	-1.29	2.32E-03	8.78E-03
ACSS2	53.38	16.11	32.55	19.36	-1.85	1.78E-05	1.45E-04
ADH1A	0.00	0.00	0.00	1.79	1.66	3.94E-02	8.77E-02
ADH1B	27.88	25.21	60.24	43.93	1.13	5.57E-01	6.75E-01
ADH4	0.32	0.42	0.35	0.33	-1.89	4.03E-02	8.94E-02
ADH5	98.66	16.93	71.31	28.67	-1.61	1.71E-05	1.40E-04
ADH6	35.00	10.83	16.42	22.31	-2.19	1.20E-02	3.36E-02
ADPGK	14.41	1.42	20.51	6.07	1.43	5.51E-06	5.21E-05
AKR1A1	339.72	134.74	227.08	108.35	-1.86	2.05E-03	7.90E-03
ALDH1A3	1.02	0.45	2.94	2.55	2.91	3.04E-07	4.07E-06
ALDH1B1	45.53	35.33	26.14	29.18	-1.87	6.30E-03	1.99E-02
ALDH2	374.94	146.85	178.93	122.88	-2.44	4.25E-06	4.14E-05
ALDH3A1	0.77	0.32	0.74	0.30	-1.07	7.56E-01	8.35E-01
ALDH3A2	71.94	10.23	41.81	35.67	-1.82	2.18E-05	1.72E-04
ALDH3B1	21.34	7.98	13.88	5.62	-1.75	2.36E-03	8.90E-03
ALDH3B2	0.44	0.20	0.46	0.26	-1.13	5.49E-01	6.67E-01
ALDH7A1	155.04	36.67	83.27	72.42	-2.05	6.19E-06	5.75E-05
ALDH9A1	65.92	21.38	46.00	24.52	-1.54	2.14E-04	1.19E-03
ALDOA	605.01	158.48	579.03	333.04	-1.36	1.46E-02	3.93E-02
ALDOB	3546.19	3255.70	1306.18	1746.17	-2.93	1.05E-02	2.99E-02
ALDOC	4.40	1.36	5.13	3.17	1.03	8.35E-01	8.92E-01
BPGM	16.01	2.50	17.24	4.44	-1.07	3.95E-01	5.24E-01
DLAT	0.00	16.07	15.58	20.05	-1.24	5.19E-03	1.69E-02
DLD	63.78	62.16	42.59	52.90	-1.35	7.66E-04	3.51E-03
ENO1	524.34	67.75	554.43	105.02	-1.13	3.19E-01	4.47E-01
ENO2	3.49	2.64	7.58	4.64	1.68	7.55E-04	3.47E-03
ENO4	0.59	0.17	0.78	0.21	1.09	5.27E-01	6.49E-01
FBP1	380.12	341.27	103.91	149.49	-3.92	1.65E-06	1.79E-05
FBP2	0.07	0.13	0.11	0.11	1.02	9.52E-01	9.69E-01
G6PC	42.72	45.21	13.48	19.27	-3.70	8.36E-04	3.78E-03
G6PC3	56.60	14.28	50.81	6.31	-1.44	1.59E-04	9.23E-04
G6PD	14.88	3.90	17.74	8.23	1.01	9.53E-01	9.69E-01
GALM	80.60	19.63	41.99	27.60	-2.03	2.26E-05	1.76E-04
GAPDH	968.66	349.66	831.61	367.60	-1.53	1.26E-02	3.48E-02
GCK	0.23	0.17	0.33	0.23	-1.29	3.99E-01	5.28E-01
GPI	135.18	12.83	127.32	25.29	-1.30	5.13E-03	1.68E-02
HK1	11.22	4.69	20.46	17.80	1.42	1.29E-02	3.57E-02
HK2	0.29	0.13	1.54	1.42	4.65	3.81E-15	3.02E-13
HK3	0.56	0.43	2.25	7.23	8.06	2.46E-12	9.55E-11
HKDC1	5.50	2.12	8.66	4.84	1.29	6.51E-02	1.31E-01
LDHA	123.34	41.09	215.93	83.57	1.38	4.12E-03	1.41E-02
LDHB	907.85	225.94	642.24	219.97	-1.64	6.22E-04	2.95E-03
LDHC	0.59	0.73	0.36	0.53	-1.18	6.33E-01	7.39E-01
MINPP1	9.41	1.63	9.17	2.64	-1.14	1.56E-01	2.59E-01
PCK1	510.05	237.38	217.61	359.20	-1.96	4.34E-02	9.48E-02
PCK2	235.89	200.95	90.07	91.76	-3.12	1.44E-04	8.48E-04
PDHA1	196.91	75.52	225.91	91.43	-1.21	1.60E-02	4.23E-02
PDHB	56.61	5.67	52.73	11.26	-1.36	1.45E-04	8.54E-04
PDHX	11.76	1.77	10.95	2.39	-1.23	4.03E-03	1.38E-02
PFKL	109.93	32.09	100.04	43.19	-1.38	5.43E-03	1.76E-02
PFKM	36.01	7.60	32.43	12.84	-1.30	6.29E-04	2.98E-03
PFKP	18.30	7.07	36.69	21.13	1.61	1.96E-05	1.57E-04
PGAM1	103.47	22.65	91.76	17.78	-1.38	5.40E-04	2.62E-03
PGAM4	5.18	2.25	7.29	2.37	1.18	7.90E-01	8.59E-01
PGK1	302.63	74.09	345.71	115.71	-1.19	9.53E-02	1.77E-01
PGM1	43.02	5.98	31.50	7.93	-1.69	2.80E-11	8.63E-10
PGM2	5.76	1.17	9.24	3.51	1.37	4.57E-04	2.28E-03
PKLR	42.73	18.98	16.68	34.73	-2.48	8.66E-03	2.57E-02
PKM	205.38	106.08	305.48	165.67	1.16	2.93E-01	4.19E-01
TPI1	397.25	114.77	322.97	40.17	-1.42	2.94E-03	1.06E-02

Supplemental Table 16. Intragraft expression of mRNAs encoding proteins involved in citric acid cycle in TCMR and Normal biopsies.

	Normal N=18		TCMR N=16				
Symbol	Median	IQR	Median	IQR	FC	PValue	P-FDR
ACLY	34.56	9.81	35.84	14.57	-1.15	2.15E-01	3.31E-01
ACO1	60.53	37.77	40.59	36.17	-1.86	6.75E-04	3.16E-03
ACO2	146.74	50.26	72.16	38.56	-2.06	7.60E-06	6.89E-05
CS	90.15	24.02	89.10	31.83	-1.19	1.83E-02	4.72E-02
DLAT	0.00	16.07	15.58	20.05	-1.24	5.19E-03	1.69E-02
DLD	63.78	62.16	42.59	52.90	-1.35	7.66E-04	3.51E-03
DLST	70.81	14.95	52.26	23.91	-1.46	3.29E-04	1.72E-03
FH	58.44	9.40	39.76	11.67	-1.66	2.69E-06	2.78E-05
IDH1	79.93	42.83	36.80	27.48	-1.96	1.00E-04	6.27E-04
IDH2	171.38	40.72	121.16	42.73	-1.66	4.24E-07	5.43E-06
IDH3A	17.18	6.41	23.18	8.21	-1.03	6.45E-01	7.49E-01
IDH3B	62.85	13.82	53.01	10.87	-1.44	7.83E-05	5.09E-04
IDH3G	51.08	19.82	35.86	12.25	-1.51	4.39E-05	3.11E-04
MDH1	204.45	64.74	164.78	72.42	-1.56	4.25E-04	2.14E-03
MDH2	101.04	46.81	121.48	56.64	-1.62	2.42E-05	1.86E-04
OGDH	82.44	21.32	47.47	21.70	-1.77	4.39E-06	4.26E-05
OGDHL	160.61	101.92	80.13	51.17	-2.11	2.03E-04	1.14E-03
PC	66.81	42.51	28.33	44.90	-2.39	8.43E-05	5.41E-04
PCK1	510.05	237.38	217.61	359.20	-1.96	4.34E-02	9.48E-02
PCK2	235.89	200.95	90.07	91.76	-3.12	1.44E-04	8.48E-04
PDHA1	196.91	75.52	225.91	91.43	-1.21	1.60E-02	4.23E-02
PDHB	56.61	5.67	52.73	11.26	-1.36	1.45E-04	8.54E-04
PDHX	11.76	1.77	10.95	2.39	-1.23	4.03E-03	1.38E-02
SUCLA2	31.72	3.01	26.62	11.17	-1.47	5.38E-06	5.10E-05
SUCLG1	292.31	168.80	131.85	110.73	-2.47	4.13E-06	4.05E-05
SUCLG2	79.33	19.97	51.66	21.59	-1.63	1.29E-04	7.71E-04