Supplementary Material for

Recipient APOL1 risk alleles associate with death-censored renal allograft survival and rejection episodes

Supplementary Figures

Supplementary Tables

Supplementary Figures

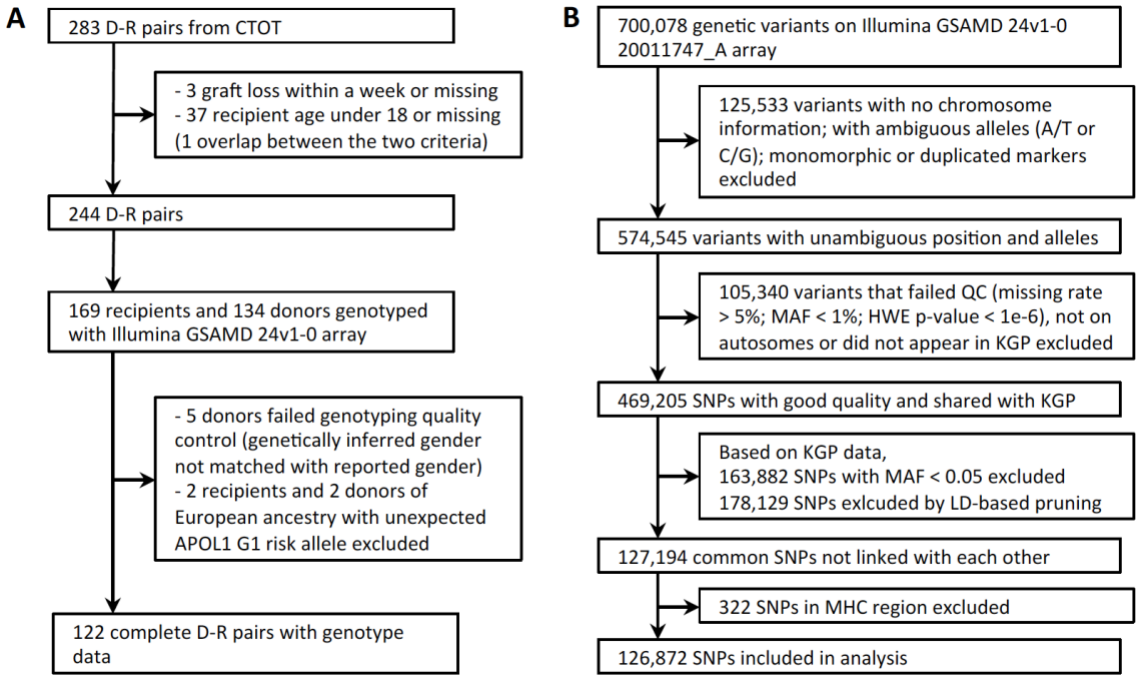

Figure S1. Flowchart of genotyping and quality control for CTOT cohort. (A) Participants; **(B)** Genetic variants. The CTOT data after QC are part of what is shown in Figure 1 in the main text. QC: quality control; MAF: minor allele frequency; HWE: Hardy-Weinberg equilibrium; KGP: 1000 Genomes Project; LD: linkage disequilibrium; MHC: major histocompatibility complex.

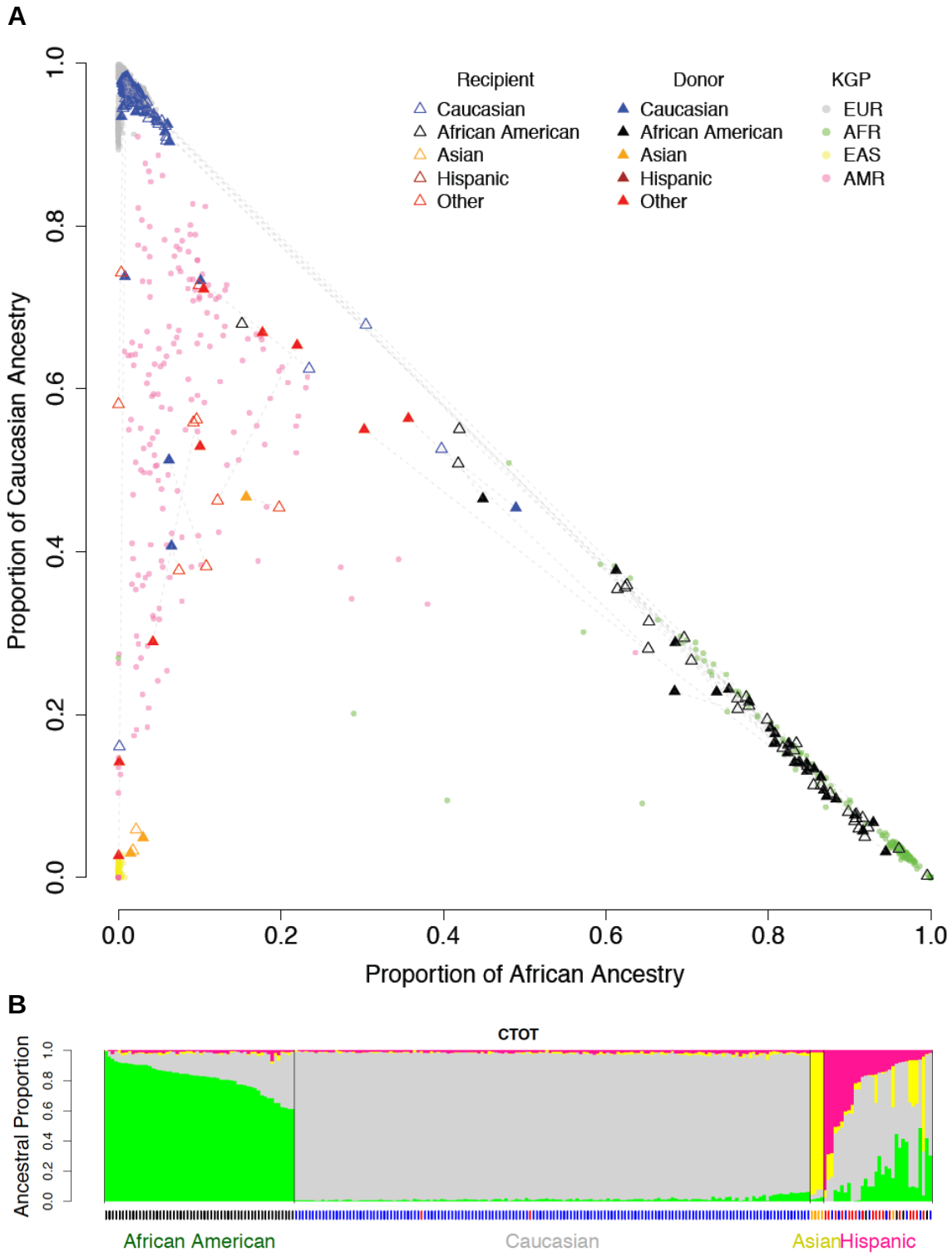

Figure S2. Genetic ancestry of CTOT donors and recipients. (A) Samples from 1000 Genomes Project (KGP) with different ethnicities anchor the location of continental-level ancestries on the space spanned by estimated proportions of African and Caucasian ancestries. CTOT donors (solid triangle) and recipients (empty triangle) are projected onto the same space and colored based on self-reported ancestry. Donor-recipient pairs

are connected by dashed lines. KGP: 1000 Genomes Project; EUR: European; AFR: African; EAS: East Asian; AMR: American. **(B)** The ancestral composition of each individual in CTOT. Each vertical bar represents an individual. The length of colored segments within each bar indicates the estimated proportion of different genetic ancestries. The ticks under the bar plot indicates self-reported race with the same color code in legend of (A).

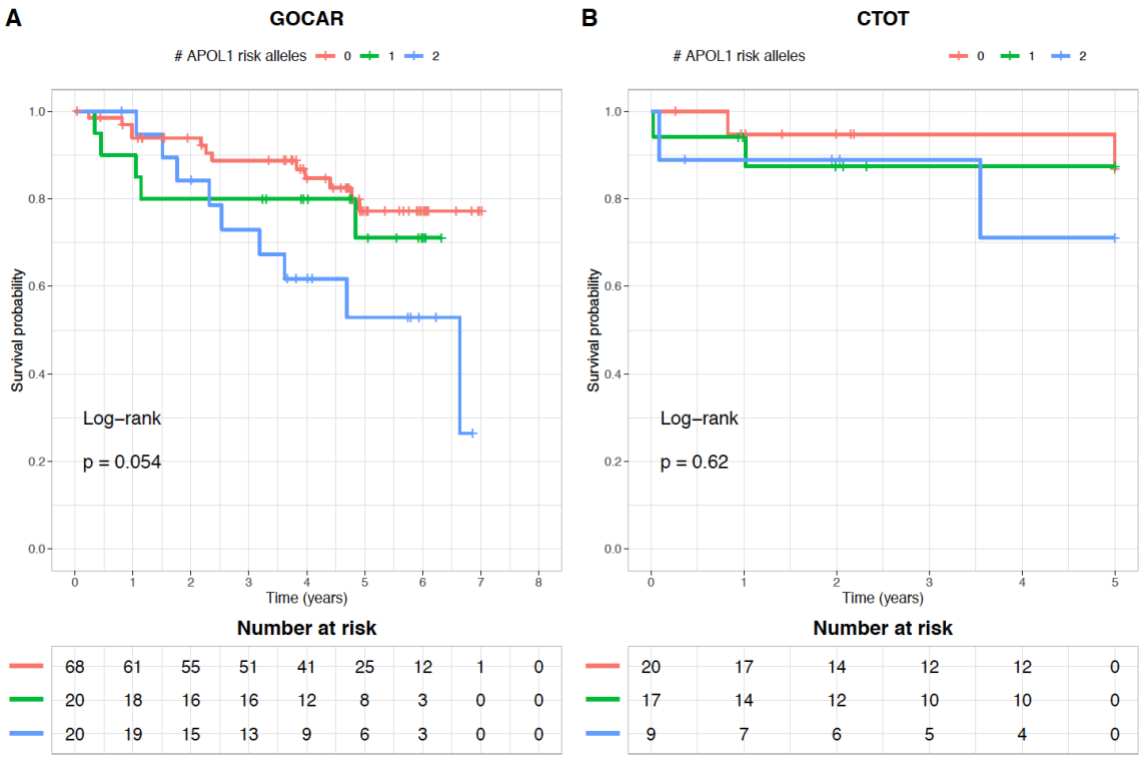

Figure S3. Kaplan-Meier plot of death-censored allograft survival for recipients with different numbers of APOL1 risk alleles. The subset of African American and Hispanic recipients was shown. (**A**) GOCAR; (**B**) CTOT.

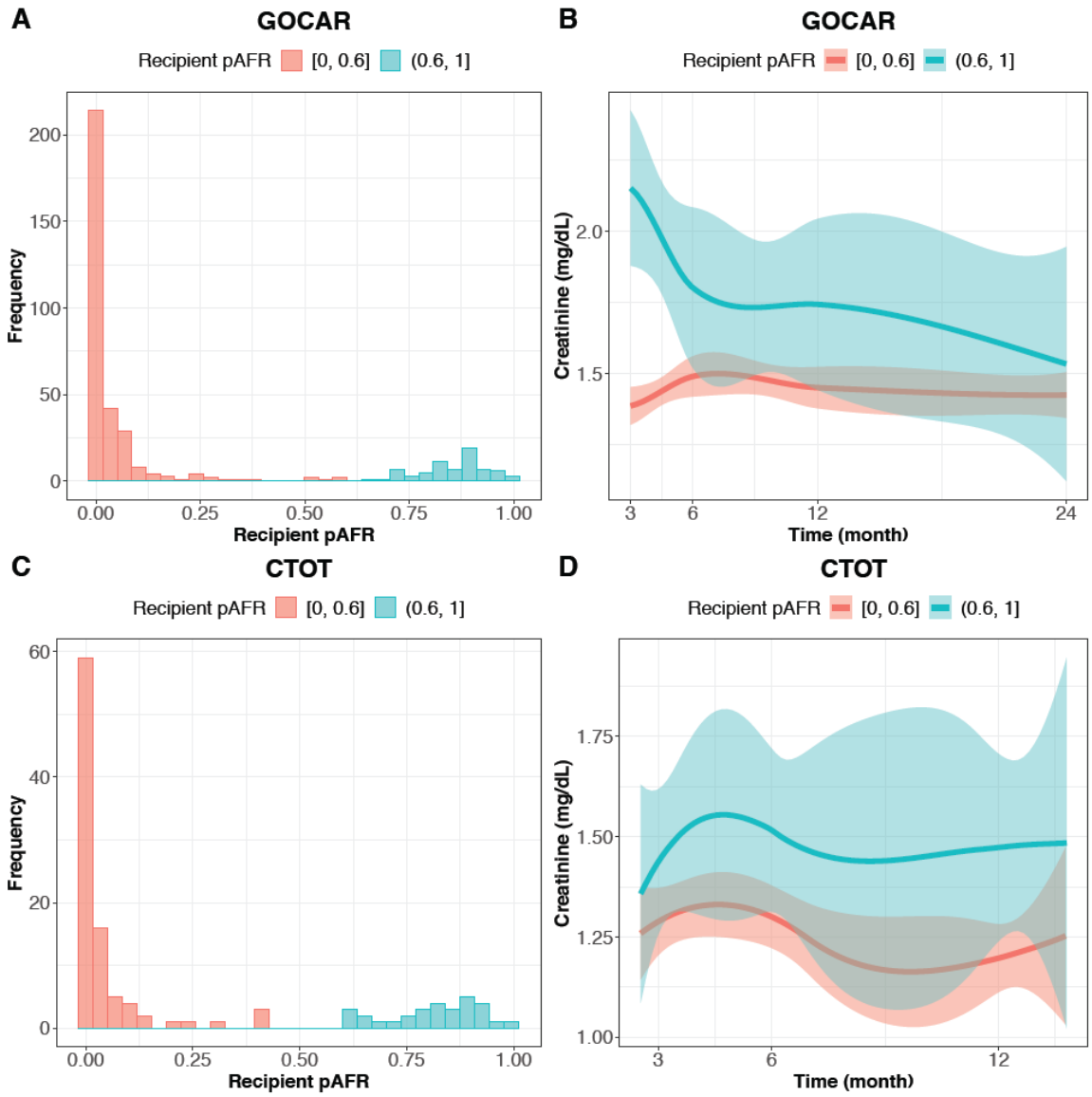

Figure S4. Recipient pAFR and creatinine in GOCAR and CTOT cohorts. (**A**) and (**C**) Histogram of the distribution of recipient pAFR values in GOCAR and CTOT cohorts. Recipients were categorized in 2 groups by their pAFR corresponding to non-African American and African American. (**B**) and (**D**) Smoothed curves with 95% confidence band for longitudinal creatinine levels grouped by recipient pAFR as shown in (A) and (C) for GOCAR and CTOT cohorts.

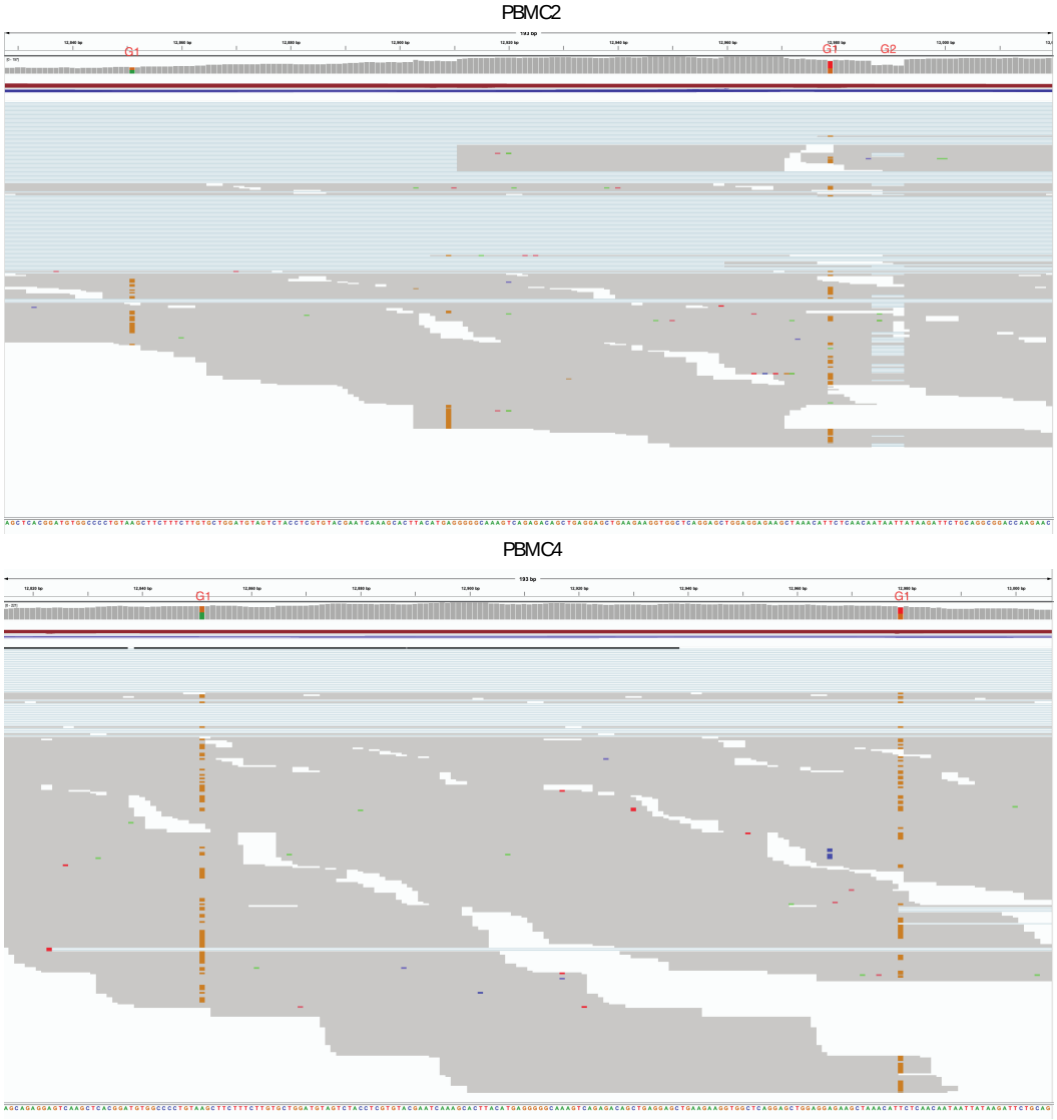

Figure S5. Allele specific expression of APOL1 in single cell RNA sequencing data from pre-transplant PBMCs of GOCAR recipients. Visualization of the expression of APOL1 risk alleles in two GOCAR recipients using Integrative Genomics Viewer (IGV).(1) PBMC2 carries G1/G2 genotype and PBMC4 carries G1/G0 genotype. The short reads are represented by gray thick segments. The G1 allele (the two SNPs in almost perfect linkage disequilibrium) is indicated by red dots, while the G2 allele (the 6 bp micro-deletion) is indicated by short blue segments.

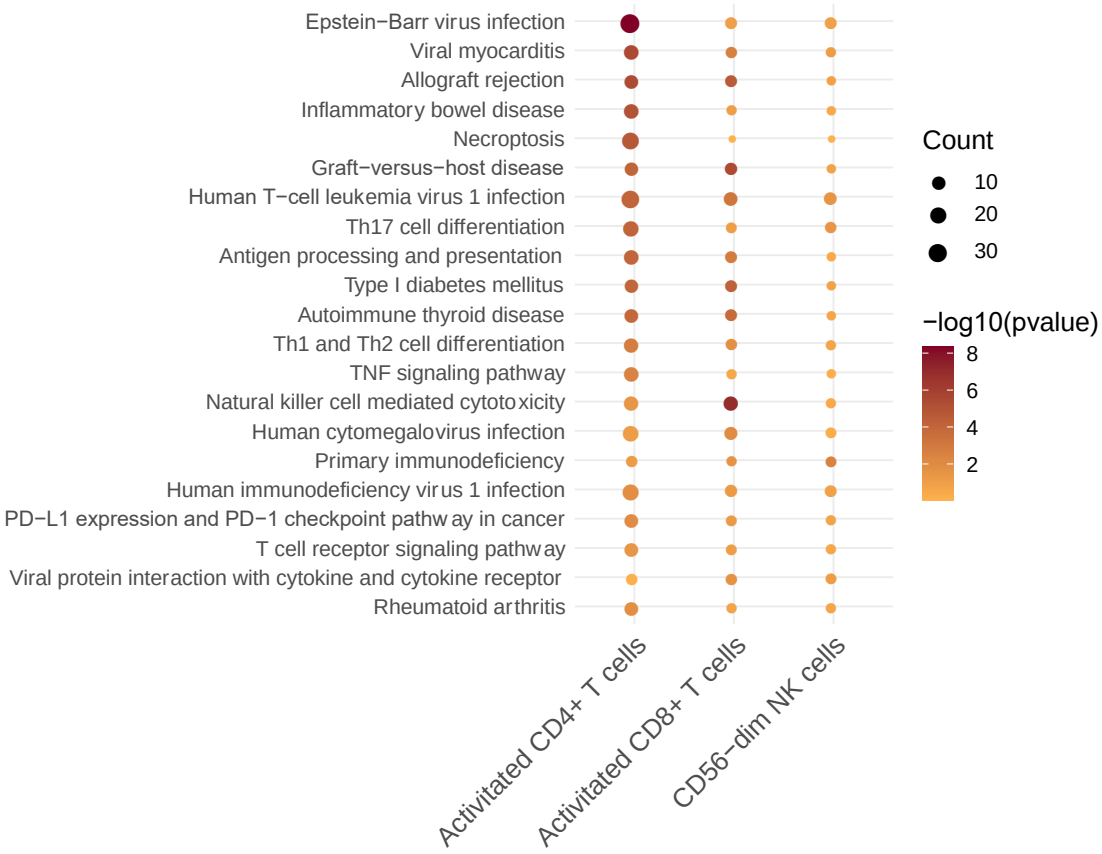

Figure S6. Enrichment in immune related pathways of DEGs in scRNAseq data from PBMCs of two AA/H ESRD patients. DEGs were identified by comparing the patient with APOL1 G2/G0 genotype vs. the patient with G0/G0 genotype in activated $CD4^{+}$ and $CD8^{+}$ T cells and $CD56^{dim}$ NK cells.

Supplementary Tables

Table S1. Demographic and clinicopathologic characteristics of GOCAR and CTOT donors and recipients with genome-wide genotype data.

Variable	GOCAR D-R pairs with genotype (n = 385) [a]	CTOT D-R pairs with genotype (n = 122) [b]	p-value[c]
Recipient			
Death censored graft loss (years)			
mean ± SD; median (range)	4.6 ± 1.7; 4.9 (0.04, 7.3)	3.7 ± 1.8; 5.0 (0.02, 5.0)	**<0.001**
# events (%)	50 (13.0%)	6 (4.9%)	**0.01**
TCMR >= borderline, # events (%)	126 (32.7%)	15 (12.3%)	**<0.001**
TCMR > borderline, # events (%)	36 (9.4%)	1 (0.8%)	**<0.001**
Recurrent TCMR >= borderline, # events (%)	59 (15.3%)	-	-
Recurrent TCMR > borderline, # events (%)	25 (6.5%)	-	-
Age (years), mean ± SD; median (range)	49.9 ± 13.5; 50 (18, 83)	48.8 ± 13.6; 50 (18, 89)	0.44
Gender, male, n (%)	257 (66.8%)	74 (60.7%)	0.23
Genetic ancestry[d], n (%)			0.30
African American	70 (18.2%)	30 (24.6%)	
Asian	13 (3.4%)	2 (1.6%)	
Caucasian	235 (61.0%)	74 (60.7%)	
Hispanic	67 (17.4%)	16 (13.1%)	
HLA mismatch score[e], n (%)	2.0 ± 1.0	3.3 ± 1.8	**0.03**[f]
Induction, n (%)			0.11
No induction	78 (20.3%)	36 (29.5%)	
Non-depletional (IL2 antagonist)	130 (33.8%)	36 (29.5%)	
Depletional (Thymoglobulin or Campath)	177 (46.0%)	50 (41.0%)	
# APOL1 risk alleles[g], n (%)			**0.001**
0	316 (82.1%)	94 (77.0%)	
1	20 (5.2%)	17 (13.9%)	
2	20 (5.2%)	9 (7.4%)	
N/A	29 (7.5%)	2 (1.6%)	

Donor				
Age (years), mean ± SD; median (range)		42.6 ± 14.7; 45 (3, 73)	40.3 ± 12.3; 39 (6, 65)	0.09
Gender, male, n (%)		196 (50.9%)	49 (40.2%)	**0.05**
Genetic ancestry, n (%)				**0.003**
	African American	33 (8.6%)	26 (21.3%)	
	Asian	7 (1.8%)	2 (1.6%)	
	Caucasian	293 (76.1%)	78 (63.9%)	
	Hispanic	52 (13.5%)	16 (13.1%)	
Donor type, live donor, n (%)		194 (50.4%)	105 (86.8%)	**<0.001**
# APOL1 risk alleles[g], n (%)				**<0.001**
	0	355 (92.2%)	99 (81.1%)	
	1	10 (2.6%)	16 (13.1%)	
	2	6 (1.6%)	5 (4.1%)	
	N/A	14 (3.6%)	2 (1.6%)	

[a]: Genome-wide genotype data is available for 385 donor-recipient (D-R) pairs from the parent GOCAR study after data processing and quality control detailed elsewhere.(2)

[b]: Genome-wide genotype data is available for 122 donor-recipient (D-R) pairs from the parent CTOT study after data processing and quality control (see Methods).

[c]: P-value was calculated from unpaired t-test for continuous variables and from Fisher's exact test for categorical variables unless otherwise specified. Bold p-value < 0.05.

[d]: Genetic ancestry was inferred from genome-wide genotype data and considered more accurate than self-reported race.(2)

[e]: HLA mismatch score was derived from 2-digit HLA allele typing. Following previous reports for GOCAR,(2-4) the raw mismatch score (scaling from 0 to 6) was categorized into: 0 (no mismatches), 1 (1-2 mismatches), 2 (3-4 mismatches), and 3 (5-6 mismatches); while for the CTOT cohort, the raw mismatch score (scaling from 0 to 6) was used. In subsequent statistical analyses, this variable was used as numeric covariate in regression models.

[f]: In order to calculate the p-value, the raw HLA mismatch score used in CTOT was hereby categorized in the same way as GOCAR so that the HLA mismatch scores originally defined on different scales in the two cohorts are comparable. The p-value was calculated by Fisher's exact test.

[g]: There are missing data in the APOL1 genotype of 29 recipients and 14 donors for GOCAR cohort and of 2 recipients and 2 donors for CTOT cohort (see supplementary Table S3 for details).

Table S2. Genetic ancestry and self-reported ancestry of donors and recipients in CTOT. Genetic ancestry is inferred from genome-wide genetic data for n = 122 D-R pairs.

(a) Donor: Genetic ancestry versus self-reported race

		Self-reported Race				
		African American	Asian	Caucasian	Hispanic	Other/ Unreported
Genetic Ancestry	African American	26	0	0	0	0
	Asian	0	2	0	0	0
	Caucasian	0	0	76	0	2
	Hispanic	1	1	5	0	9

(b) Recipient: Genetic ancestry versus self-reported race

		Self-reported Race				
		African American	Asian	Caucasian	Hispanic	Other/ Unreported
Genetic Ancestry	African American	30	0	0	0	0
	Asian	0	2	0	0	0
	Caucasian	0	0	74	0	0
	Hispanic	3	0	4	0	9

(c) Donor and recipient genetic ancestry

		Recipient Genetic Ancestry				
		African American	Asian	Caucasian	Hispanic	Total
Donor Genetic Ancestry	African American	24	0	1	1	26
	Asian	0	2	0	0	2
	Caucasian	4	0	72	2	78
	Hispanic	2	0	1	13	16
	Total	30	2	74	16	122

Table S3. APOL1 risk genotype in donor-recipient pairs of GOCAR and CTOT.

(A) GOCAR (n = 385 D-R pairs)

		Recipient APOL1 risk genotype				
		G0/G0	G0/G1 or G0/G2	G1/G1, G1/G2, or G2/G2	N/A	Total
Donor APOL1 risk genotype	G0/G0	305	17	14	19	355
	G0/G1 or G0/G2	6	0	1	3	10
	G1/G1, G1/G2, or G2/G2	0	2	2	2	6
	N/A	5	1	3	5	14
	Total	316	20	20	29	385

(B) CTOT (n = 122 D-R pairs)

		Recipient APOL1 risk genotype				
		G0/G0	G0/G1 or G0/G2	G1/G1, G1/G2, or G2/G2	N/A	Total
Donor APOL1 risk genotype	G0/G0	87	7	5	0	99
	G0/G1 or G0/G2	5	8	3	0	16
	G1/G1, G1/G2, or G2/G2	2	2	1	0	5
	N/A	0	0	0	2	2
	Total	94	17	9	2	122

Table S4. Summary of APOL1 risk alleles in GOCAR and CTOT cohorts stratified by recipients and donors with different genetic ancestries.

Genetic Ancestry	Recipient #APOL1 risk alleles					Donor #APOL1 risk alleles				
	0	1	2	N/A	Total	0	1	2	N/A	Total
GOCAR (n = 385 D-R pairs)										
African American	12	16	15	27	70	9	7	6	11	33
Asian	13	0	0	0	13	7	0	0	0	7
Caucasian	235	0	0	0	235	293	0	0	0	293
Hispanic	56	4	5	2	67	46	3	0	3	52
All	316	20	20	29	385	355	10	6	14	385
CTOT (n = 122 D-R pairs)										
African American	8	14	8	0	30	6	15	5	0	26
Asian	2	0	0	0	2	2	0	0	0	2
Caucasian	72	0	0	2	74	76	0	0	2	78
Hispanic	12	3	1	0	15	15	1	0	0	16
All	94	17	9	2	122	99	16	5	2	122

Table S5. Association of recipient APOL1 risk alleles with death-censored allograft loss under different genetic models in multivariable Cox regression analysis of the GOCAR cohort.

Genetic Model[a,b]	beta[c]	HR (95% CI)	p-value	R^2 [d]	AIC[e]	BIC[f]
GOCAR: recipients of all ancestries[g], (n = 343 [h]; 44 [12.8%] graft loss events)						
Additive model	0.76	2.14 (1.25, 3.67)	0.006	0.1151	451.45	463.94
Dominant model	1.27	3.57 (1.24, 10.3)	0.018	0.1114	452.88	465.37
Recessive model	1.16	3.19 (1.25, 8.15)	0.016	0.1108	453.09	465.58
GOCAR: recipients of African American and Hispanic, (n = 108 [h]; 26 [24.1%] graft loss events)						
Additive model	0.84	2.32 (1.33, 4.06)	0.003	0.1820	212.48	220.03
Dominant model	1.45	4.27 (1.41, 12.9)	0.010	0.1708	213.94	221.49
Recessive model	1.27	3.56 (1.36, 9.33)	0.010	0.1662	214.54	222.09
GOCAR: recipients of all ancestries[g], within the stratum of donors carrying APOL1 low-risk genotype[i] (n = 330 [h]; 41 [12.4%] graft loss events)						
Additive model	0.66	1.93 (1.06, 3.49)	0.030	0.1033	419.34	431.34
Dominant model	0.98	2.66 (0.86, 8.22)	0.089	0.0994	420.79	432.79
Recessive model	1.07	2.91 (1.04, 8.10)	0.041	0.1019	419.88	431.88
GOCAR: recipients of African American and Hispanic, within the stratum of donors carrying APOL1 low-risk genotype[i] (n = 97 [h]; 23 [23.7%] graft loss events)						
Additive model	0.74	2.11 (1.14, 3.88)	0.017	0.1562	185.15	191.96
Dominant model	1.17	3.23 (0.99, 10.6)	0.053	0.1434	186.61	193.42
Recessive model	1.18	3.26 (1.14, 9.33)	0.028	0.1482	186.06	192.87

[a]: In the additive model, the number of recipient APOL1 risk alleles was treated as numeric variable; in the dominant model, the recipients carrying 1 or 2 risk alleles were compared with those with no risk alleles; in the recessive model, the recipients carrying 2 risk alleles were compared with those with 0 or 1 risk allele.
[b]: In the multivariable Cox regression model, adjusted covariates include recipient genetic ancestry, induction, donor type and HLA mismatch score. For concise presentation, only the results for the recipient APOL1 risk genotype variable were shown in the table.
[c]: Beta is the coefficient of the recipient APOL1 risk genotype variable in the linear predictor of the Cox regression model.
[d]: An approximate R^2 based on Nagelkerke NJD, *Biometrika*, 1991, 78(3): 691-692. A larger value indicates better goodness-of-fit.
[e]: AIC: Akaike's Information Criterion, defined as -2*log-likelihood + 2*npar, where npar is the number of parameters in the model. A smaller value indicates better goodness-of-fit.
[f]: BIC: Bayesian Information Criterion, defined as -2*log-likelihood + log(n)*npar, where npar is the number of parameters in the model, and n is the sample size. A smaller value indicates better goodness-of-fit.
[g]: The "Asian" category was excluded due to limited sample size which led to instable model fitting.
[h]: Sample size was reduced due to missing data in APOL1 risk alleles.
[i]: Donor APOL1 high-risk genotype is defined as 2 copies of G1/G2 alleles and low-risk genotype as 0 or 1 G1/G2 allele. In this analysis, we focused on the stratum of donors with APOL1 low-risk genotype, because the model fitting would not have converged if the donor APOL1 risk genotype had been included as a covariate due to the limited number of donors with APOL1 high-risk genotype.

Table S6. Association of APOL1 risk alleles with death-censored allograft loss in an additive manner in CTOT cohort.

Variable[a]	HR	95% CI	p-value[d]
CTOT: recipients all ancestries[b] ***(n = 117***[c]***; 6 [5.1%] graft loss events)***			
# APOL1 risk alleles	2.73	(1.04, 7.20)	**0.04**
Donor type (ref: LD) DD	3.85	(0.71, 20.8)	0.12
HLA mismatch score	1.16	(0.73, 1.83)	0.52
CTOT: recipients of African American and Hispanic (n = 46[c]***; 6 [13.0%] graft loss events)***			
# APOL1 risk alleles	1.32	(0.46, 3.81)	0.60
Donor type (ref: LD) DD	3.96	(0.70, 22.4)	0.12
HLA mismatch score	1.08	(0.64, 1.84)	0.77

[a]: In the multivariable Cox regression model, donor type and HLA mismatch score were forced into model, while other covariates adjusted in GOCAR data, including recipient ancestry and induction, that were not significant in multivariable analysis were not included in order to increase statistical power for the CTOT cohort with limited sample size.

[b]: The "Asian" category was excluded due to limited sample size which led to instable model fitting.

[c]: Sample size was reduced due to missing data in donor APOL1 risk alleles.

[d]: Bold p-value < 0.05.

Table S7. Association of recipient APOL1 risk alleles with death-censored allograft loss using multivariable Cox regression, within the stratum of donors carrying APOL1 low-risk genotype in the GOCAR cohort.

Variable	HR	95% CI	p-value[d]
GOCAR: recipients of all ancestries[a], within the stratum of donors carrying APOL1 low-risk genotype[b] (n = 330 [c]; 41 [12.4%] graft loss events)			
# APOL1 risk alleles	1.93	(1.06, 3.49)	**0.03**
Recipient genetic ancestry (ref: Caucasian)			
African American	1.02	(0.29, 3.57)	0.98
Hispanic	2.70	(1.23, 5.95)	**0.01**
Induction (ref: No)			
Non-depletional	2.84	(0.92, 8.79)	0.07
Depletional	3.56	(1.14, 11.1)	**0.03**
Donor type (ref: LD) DD	2.62	(1.24, 5.51)	**0.01**
HLA mismatch score	1.20	(0.81, 1.77)	0.36
GOCAR: recipients of African American and Hispanic, within the stratum of donors carrying APOL1 low-risk genotype[b] (n = 97 [c]; 23 [23.7%] graft loss events)			
# APOL1 risk alleles	2.11	(1.14, 3.88)	**0.02**
Recipient genetic ancestry (ref: AA) Hispanic	2.98	(0.90, 9.82)	0.07
Induction (ref: No)			
Non-depletional	5.46	(0.62, 47.7)	0.13
Depletional	4.93	(0.60, 40.7)	0.14
Donor type (ref: LD) DD	2.90	(0.88, 9.55)	0.08
HLA mismatch score	1.67	(0.85, 3.30)	0.14

[a]: The "Asian" category was excluded due to limited sample size which led to instable model fitting.

[b]: Donor APOL1 high-risk genotype is defined as 2 copies of G1/G2 alleles and low-risk genotype as 0 or 1 G1/G2 allele.

[c]: Sample size was reduced due to missing data in APOL1 risk alleles.

[d]: Bold p-value < 0.05.

Table S8. Association of recipient APOL1 risk alleles with death-censored allograft loss using multivariable Cox regression, within the stratum of donors carrying APOL1 low-risk genotype in the CTOT cohort.

Variable[a]	HR	95% CI	p-value[e]
CTOT: recipients all ancestries[b], within the stratum of donors carrying APOL1 low risk genotype[c] (n = 112 [d]; 6 [5.4%] graft loss events)			
# APOL1 risk alleles	2.84	(1.05, 7.70)	**0.04**
Donor type (ref: LD) DD	3.61	(0.67, 19.4)	0.13
HLA mismatch score	1.13	(0.71, 1.79)	0.62
CTOT: recipients of African American and Hispanic, within the stratum of donors carrying APOL1 low risk genotype[c] (n = 41 [d]; 6 [14.6%] graft loss events)			
# APOL1 risk alleles	1.32	(0.45, 3.91)	0.62
Donor type (ref: LD) DD	3.76	(0.67, 21.1)	0.13
HLA mismatch score	1.04	(0.60, 1.80)	0.88

[a]: In the multivariable Cox regression model, donor type and HLA mismatch score were forced into model, while other covariates adjusted in GOCAR data, including recipient ancestry and induction, that were not significant in multivariable analysis were not included in order to increase statistical power for the CTOT cohort with limited sample size.

[b]: The "Asian" category was excluded due to limited sample size which led to instable model fitting.

[c]: Donor APOL1 high-risk genotype is defined as 2 copies of G1/G2 alleles and low-risk genotype as 0 or 1 G1/G2 allele.

[d]: Sample size was reduced due to missing data in donor APOL1 risk alleles.

[e]: Bold p-value < 0.05.

Table S9. Association of recipient APOL1 risk alleles with different TCMR outcomes in the CTOT cohort.

TCMR outcome	$n_{control}$[a,b]	n_{case}[a]	HR	95% CI	p-value[d]
CTOT: recipient of all ancestries					
TCMR >= borderline (Univariate)	106	14	2.32	(1.06, 4.87)	**0.03**
TCMR >= borderline (Multivariable[c])	100	14	2.32	(1.02, 5.16)	**0.04**
CTOT: recipients of African American and Hispanic					
TCMR >= borderline (Univariate)	39	7	2.95	(1.01, 10.3)	0.06
TCMR >= borderline (Multivariable[c])	34	7	3.39	(1.07, 13.6)	**0.05**

[a]: Sample size was reduced due to missing data in APOL1 risk alleles for univariate analysis and due to missing data in APOL1 risk alleles and HLA mismatch score for multivariable analysis.

[b]: Controls (no TCMR) were defined as patients with either (a) no TCMR or borderline TCMR on obtained biopsies at anytime, or (b) no reported biopsies during follow up.

[c]: In the multivariable Cox regression model, we focused on the stratum of donors with APOL1 low-risk genotype carrying 0 or 1 G1/G2 allele, because the model fitting would not have converged if the donor APOL1 risk genotype had been included as a covariate due to the limited number (n = 5) of donors with APOL1 high-risk genotype carrying 2 G1/G2 alleles. HLA mismatch score were forced into model, while other covariates adjusted in GOCAR data, including recipient ancestry, induction, and donor type that were not significant in multivariable analysis were not included in order to increase statistical power for the CTOT cohort with limited sample size.

[d]: Bold p-value < 0.05.

Table S10. Association of APOL1 risk alleles with death-censored allograft loss independent of APOL1 SNP-wise mismatch in GOCAR and CTOT.

Variable[a]	HR	95% CI	p-value[d]
GOCAR: recipients of all ancestries[b] (n = 343[c]; 44 [12.8%] graft loss events)			
# APOL1 risk alleles	2.24	(1.30, 3.86)	**0.004**
APOL1 SNP-wise mismatch	0.75	(0.40, 1.44)	0.39
GOCAR: recipients of African American and Hispanic (n = 108[c]; 26 [24.1%] graft loss events)			
# APOL1 risk alleles	2.46	(1.38, 4.40)	**0.002**
APOL1 SNP-wise mismatch	0.75	(0.32, 1.76)	0.51
CTOT: recipients of all ancestries (n = 117[c]; 6 [5.1%] graft loss events)			
# APOL1 risk alleles	2.56	(0.98, 6.73)	0.06
APOL1 SNP-wise mismatch	1.39	(0.73, 2.66)	0.31
CTOT: recipients of African American and Hispanic (n = 46[c]; 6 [13.0%] graft loss events)			
# APOL1 risk alleles	1.10	(0.35, 3.41)	0.87
APOL1 SNP-wise mismatch	1.62	(0.78, 3.35)	0.19

[a]: In the Cox regression model for GOCAR, covariates include recipient ancestry, number of APOL1 risk alleles, and APOL1 SNP-wise mismatch, induction, donor type, and HLA mismatch score. In the multivariable Cox regression model for CTOT, donor type and HLA mismatch score were forced into model, while other covariates adjusted in GOCAR data, including recipient ancestry and induction, that were not significant in multivariable analysis were not included in order to increase statistical power for the CTOT cohort with limited sample size. For concise presentation, only recipient number of APOL1 risk alleles and APOL1 SNP-wise mismatch were shown in the table.

[b]: The "Asian" category was excluded due to limited sample size which led to instable model fitting.

[c]: Sample size was reduced due to missing data in APOL1 risk alleles.

[d]: Bold p-value < 0.05.

Table S11. Enrichment in KEGG pathways of DEGs identified in immune cells in DICE data.

(Supplementary_Tables_S11_S13_S14.xlsx)

Table S12. GOCAR recipients with generated single cell RNA-seq data.

Sample ID	Age	Gender	Genetic Ancestry	APOL1 Genotype	Recurrent TCMR	Graft Loss during follow-up
PBMC1	68	Female	African American	G0/G0	No	No
PBMC2	41	Male	African American	G1/G2	Yes	Yes
PBMC3	71	Male	African American	G0/G0	No	No
PBMC4	58	Male	African American	G1/G0	Yes	Yes

Table S13. Enrichment in KEGG pathways of DEGs identified from four GOCAR recipients with single cell RNA sequencing data of pre-transplant PBMCs.

(Supplementary_Tables_S11_S13_S14.xlsx)

Table S14. Enrichment in immune related pathways of DEGs identified from a subset of GOCAR recipients with PBMC bulk RNA sequencing data.

(Supplementary_Tables_S11_S13_S14.xlsx)

Table S15. Genes used to define cell types in the scRNAseq data analysis.

Cell type	Genes used to define cell type
$CD4^+$ T cell	CD3D, CD3E, LTB, IL7R
$CD8^+$ T cell	CD3D, CD3E, CD8A, CD8B
Activated $CD4^+$ T cell	CD3D, CD3E, LTB, TNF, STAT1, MAF
Activated $CD8^+$ T cell	CD3D, CD3E, CD8A, CD8B, GZMB, GNLY, PRF1
$CD56^{dim}$ NK cell	GNLY, NKG7, PRF1, GZMB, GZMH, FGFBP2
Monocyte	CD14, LYZ, S100A8, S100A9
B Cell	CD19, CD79A, CD74, MS4A1

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