

SUPPLEMENTARY MATERIALS

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Supplementary Table 1: Unadjusted effects of baseline covariates on log percentage of CD28-CD8+ T cells estimated from linear mixed models.

	NA	Mean difference	95% CI	p-value
Biopsy group	0			<0.0001
2 (vs. 1)		0.37	[0.18 ; 0.56]	
3 (vs. 1)		0.43	[0.29 ; 0.57]	
4 (vs. 1)		0.22	[-0.01 ; 0.46]	
5 (vs. 1)		0.17	[-0.01 ; 0.36]	
Transplantation after 2008	0	-0.22	[-0.37 ; -0.07]	0.0032
Recipient age (years)	0	0.01	[0.01 ; 0.01]	<0.0001
Male recipient	0	-0.11	[-0.24 ; 0.02]	0.0920
Retransplantation	0	0.38	[0.21 ; 0.56]	<0.0001
Renal transplantation	0	0.13	[-0.07 ; 0.33]	0.2085
Recurrent initial disease	0	0.10	[-0.05 ; 0.25]	0.2062
DGF	29	0.16	[0.03 ; 0.30]	0.0192
Cold ischemia time (hours)	0	0.02	[0.01 ; 0.02]	<0.0001
History of diabetes	0	0.05	[-0.10 ; 0.19]	0.5476
History of hypertension	0	0.06	[-0.15 ; 0.27]	0.5669
History of cardiovascular disease	0	0.14	[0.01 ; 0.27]	0.0349
Recipient/Donor CMV serology	0			<0.0001
1 (vs. 0)		1.18	[1.04 ; 1.33]	
2 (vs. 0)		0.69	[0.54 ; 0.83]	
3 (vs. 0)		1.25	[1.10 ; 1.40]	
Donor age (years)	0	0.01	[0.00 ; 0.01]	<0.0001
Male donor	1	-0.02	[-0.15 ; 0.11]	0.7707
Deceased donor	0	0.35	[0.14 ; 0.56]	0.0009
HLA-A-B-DR mismatches > 4	0	-0.09	[-0.24 ; 0.07]	0.2566
ABO mismatch	10	-0.43	[-0.90 ; 0.05]	0.0781
Depleting induction	0	0.12	[-0.01 ; 0.25]	0.0644
CSA	0	0.24	[0.03 ; 0.45]	0.0252
Tacrolimus	0	-0.18	[-0.39 ; 0.02]	0.0815
Steroid	0	0.11	[-0.06 ; 0.29]	0.2039
Positive anti-class I immunization	1	0.16	[0.02 ; 0.30]	0.0276
Positive anti-class II immunization	1	0.18	[0.03 ; 0.32]	0.0156
Positive DSA	1	-0.02	[-0.28 ; 0.25]	0.8921
Biopsy rank	0			<0.0001
2 (vs. 1)		0.32	[0.25 ; 0.40]	
3 (vs. 1)		0.47	[0.32 ; 0.62]	
4 (vs. 1)		0.94	[0.50 ; 1.37]	
5 (vs. 1)		0.61	[-0.56 ; 1.79]	
For cause biopsy	0	0.07	[-0.02 ; 0.17]	0.1399
Creatininemia at biopsy (μmol/l)	22	-0.00	[-0.00 ; 0.00]	0.9239
Post-transplantation time of the biopsy (years)	0	0.03	[0.02 ; 0.05]	<0.0001

CI, confidence interval; CMV, cytomegalovirus; CSA, cyclosporin A; DGF, delayed graft function; DSA, donor specific antibodies; HLA, human leucocyte antigens; NA: not available (missing). Recipient/Donor CMV serology definition, 0: negative donor and recipient; 1: negative donor and positive recipient; 2: positive donor and negative recipient; 3: positive donor and recipient.

Supplementary Table 2: Unadjusted effects of baseline covariates on log absolute number of CD28-CD8+ T cells estimated from linear mixed models.

	NA	Mean difference	95% CI	p-value
Biopsy group	0			<0.0001
2 (vs. 1)		0.56	[0.31 ; 0.82]	
3 (vs. 1)		0.60	[0.42 ; 0.79]	
4 (vs. 1)		0.16	[-0.16 ; 0.48]	
5 (vs. 1)		0.33	[0.07 ; 0.59]	
Transplantation after 2008	0	-0.51	[-0.69 ; -0.33]	<0.0001
Recipient age (years)	0	0.00	[-0.00 ; 0.01]	0.1326
Male recipient	0	-0.00	[-0.17 ; 0.16]	0.9553
Retransplantation	0	0.00	[-0.21 ; 0.22]	0.9733
Renal transplantation	0	0.42	[0.17 ; 0.67]	0.0010
Recurrent initial disease	0	0.18	[-0.01 ; 0.37]	0.0576
DGF	29	0.13	[-0.04 ; 0.30]	0.1284
Cold ischemia time (hours)	0	0.02	[0.01 ; 0.03]	<0.0001
History of diabetes	0	-0.08	[-0.26 ; 0.10]	0.3898
History of hypertension	0	0.06	[-0.20 ; 0.31]	0.6582
History of cardiovascular disease	0	0.01	[-0.15 ; 0.18]	0.8791
Recipient/Donor CMV serology	0			<0.0001
1 (vs. 0)		1.18	[0.99 ; 1.37]	
2 (vs. 0)		0.69	[0.49 ; 0.88]	
3 (vs. 0)		1.28	[1.08 ; 1.47]	
Donor age (years)	0	0.00	[0.00 ; 0.01]	0.0468
Male donor	1	-0.03	[-0.18 ; 0.13]	0.7550
Deceased donor	0	0.25	[-0.00 ; 0.51]	0.0529
HLA-A-B-DR mismatches > 4	0	-0.12	[-0.31 ; 0.07]	0.2069
ABO mismatch	10	-0.72	[-1.30 ; -0.15]	0.0141
Depleting induction	0	-0.43	[-0.59 ; -0.28]	<0.0001
CSA	0	0.50	[0.24 ; 0.76]	0.0001
Tacrolimus	0	-0.45	[-0.70 ; -0.19]	0.0006
Steroid	0	-0.19	[-0.40 ; 0.02]	0.0789
Positive anti-class I immunization	1	-0.10	[-0.27 ; 0.08]	0.2739
Positive anti-class II immunization	1	-0.09	[-0.27 ; 0.09]	0.3128
Positive DSA	1	-0.35	[-0.67 ; -0.03]	0.0339
Biopsy rank	0			<0.0001
2 (vs. 1)		0.53	[0.43 ; 0.63]	
3 (vs. 1)		0.83	[0.63 ; 1.04]	
4 (vs. 1)		1.21	[0.60 ; 1.81]	
5 (vs. 1)		1.22	[-0.42 ; 2.86]	
For cause biopsy	0	0.16	[0.04 ; 0.30]	0.0123
Creatininemia at biopsy (µmol/l)	22	-0.00	[-0.00 ; 0.00]	0.2316
Post-transplantation time of the biopsy (years)	0	0.07	[0.05 ; 0.09]	<0.0001

CI, confidence interval; CMV, cytomegalovirus; CSA, cyclosporin A; DGF, delayed graft function; DSA, donor specific antibodies; HLA, human leucocyte antigens; NA, not available (missing). Recipient/Donor CMV serology definition, 0: negative donor and recipient; 1: negative donor and positive recipient; 2: positive donor and negative recipient; 3: positive donor and recipient.

Supplementary Table 3: Characteristics of 510 patients included in the graft survival study (patients who underwent 1-year protocol biopsy with a lymphocyte phenotyping at the same time).

	NA	n	%
Transplantation after 2008	0	421	82.5
Recipient older than 55 years	0	194	38.0
Male recipient	0	327	64.1
Retransplantation	0	65	12.7
Renal transplantation	0	448	87.8
Recurrent initial disease	0	112	22.0
DGF	4	153	30.2
Cold ischemia time (hours)	0		
Less than 12 hours		114	22.4
Between 12 and 24 hours		340	66.7
24 hours and more		56	11.0
History of diabetes	0	139	27.3
History of hypertension	0	455	89.2
History of cardiovascular disease	0	177	34.7
Recipient/Donor CMV serology	0		
0		180	35.3
1		111	21.8
2		113	22.2
3		106	20.8
Donor older than 55 years	0	211	41.4
Male donor	0	296	58.0
Deceased donor	0	461	90.4
HLA-A-B-DR mismatches > 4	0	111	21.8
ABO mismatch	2	11	2.2
Depleting induction	0	191	37.5
CSA	0	20	3.9
Tacrolimus	0	490	96.1
Steroid	0	424	83.1
Positive anti-class I immunization	0	149	29.2
Positive anti-class II immunization	0	124	24.3
Positive DSA	0	26	5.1
	NA	m	SD
Log of CD28-CD8+ T cells (%)	1	2.1	1.1
Log of CD28-CD8+ T cells (absolute value)	2	-2.4	1.3
Creatininemia at biopsy ($\mu\text{mol/l}$)	11	136.3	49.0

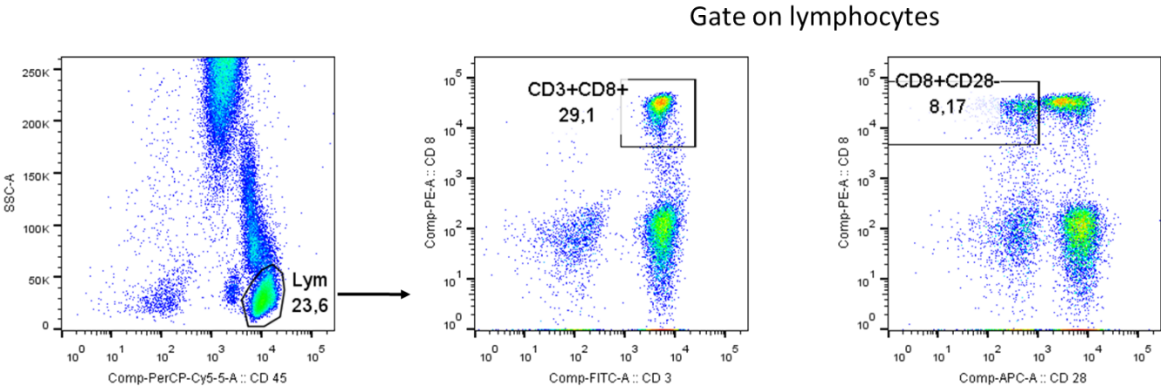
CMV, cytomegalovirus; CSA, cyclosporin A; DGF, delayed graft function; DSA, donor specific antibodies; HLA, human leucocyte antigens; m, mean; NA, not available (missing); SD, standard deviation. Recipient/Donor CMV serology definition, 0: negative donor and recipient; 1: negative donor and positive recipient; 2: positive donor and negative recipient; 3: positive donor and recipient; Log: natural logarithm.

Supplementary Table 4: Unadjusted Cox models studying the associations between different variables and graft survival.

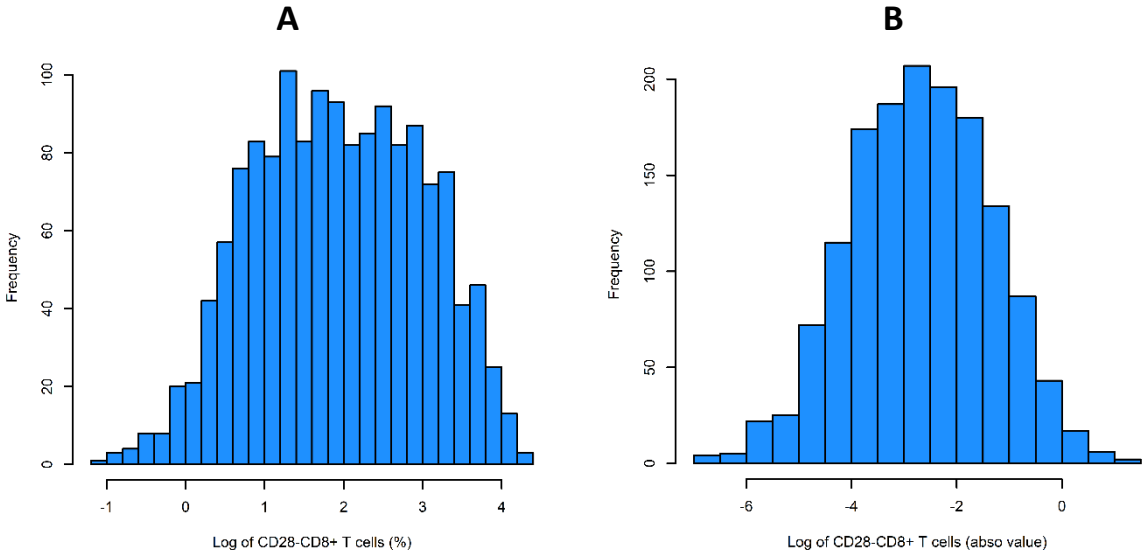
	NA	HR	95% CI	p-value
Log of CD28-CD8+ T cells (%)	1	1.14	[0.89 ; 1.45]	0.2969
Log of CD28-CD8+ T cells (absolute value)	2	1.10	[0.89 ; 1.35]	0.3715
Transplantation after 2008	0	0.81	[0.46 ; 1.42]	0.4640
Recipient older than 55 years	0	1.18	[0.70 ; 1.97]	0.5341
Male recipient	0	1.49	[0.85 ; 2.61]	0.1607
Retransplantation	0	1.63	[0.80 ; 3.32]	0.1818
Renal transplantation	0	0.97	[0.42 ; 2.26]	0.9452
Recurrent initial disease	0	0.80	[0.41 ; 1.53]	0.4917
DGF	4	1.70	[1.02 ; 2.86]	0.0434
Cold ischemia time (hours)	0			0.0816
Between 12 and 24 (vs. Less than 12)		3.20	[1.15 ; 8.89]	
24 and more (vs. Less than 12)		3.16	[0.97 ; 10.32]	
History of diabetes	0	1.35	[0.77 ; 2.36]	0.3006
History of HTA	0	0.58	[0.29 ; 1.19]	0.1375
History of cardiovascular disease	0	1.11	[0.65 ; 1.88]	0.7064
Recipient/Donor CMV serology	0			0.9977
1 (vs. 0)		0.98	[0.49 ; 1.96]	
2 (vs. 0)		1.05	[0.54 ; 2.07]	
3 (vs. 0)		0.99	[0.49 ; 1.97]	
Donor older than 55 years	0	1.49	[0.90 ; 2.47]	0.1186
Male donor	0	1.07	[0.64 ; 1.77]	0.8051
Deceased donor	0	2.29	[0.56 ; 9.40]	0.2506
HLA-A-B-DR mismatches > 4	0	1.60	[0.91 ; 2.80]	0.1013
ABO mismatch	2	1.27	[0.17 ; 9.23]	0.8141
Depleting induction	0	1.11	[0.65 ; 1.90]	0.6894
CSA	0	2.77	[1.19 ; 6.43]	0.0179
Tacrolimus	0	0.63	[0.23 ; 1.73]	0.3696
Steroid	0	0.78	[0.44 ; 1.38]	0.3905
Positive anti-class I immunization	0	1.00	[0.57 ; 1.76]	0.9929
Positive anti-class II immunization	0	1.54	[0.89 ; 2.67]	0.1268
Positive DSA	0	1.46	[0.46 ; 4.69]	0.5222
Creatininemia at biopsy (μmol/l)	11	1.02	[1.01 ; 1.02]	<0.0001

CI, confidence interval; CSA, cyclosporin A; CMV, cytomegalovirus; DGF, delayed graft function; DSA, donor specific antibodies; HLA, human leucocyte antigens; HR, hazard ratio; NA: not available (missing). Recipient/Donor CMV serology definition, 0: negative donor and recipient; 1: negative donor and positive recipient; 2: positive donor and negative recipient; 3: positive donor and recipient; Log: natural logarithm.

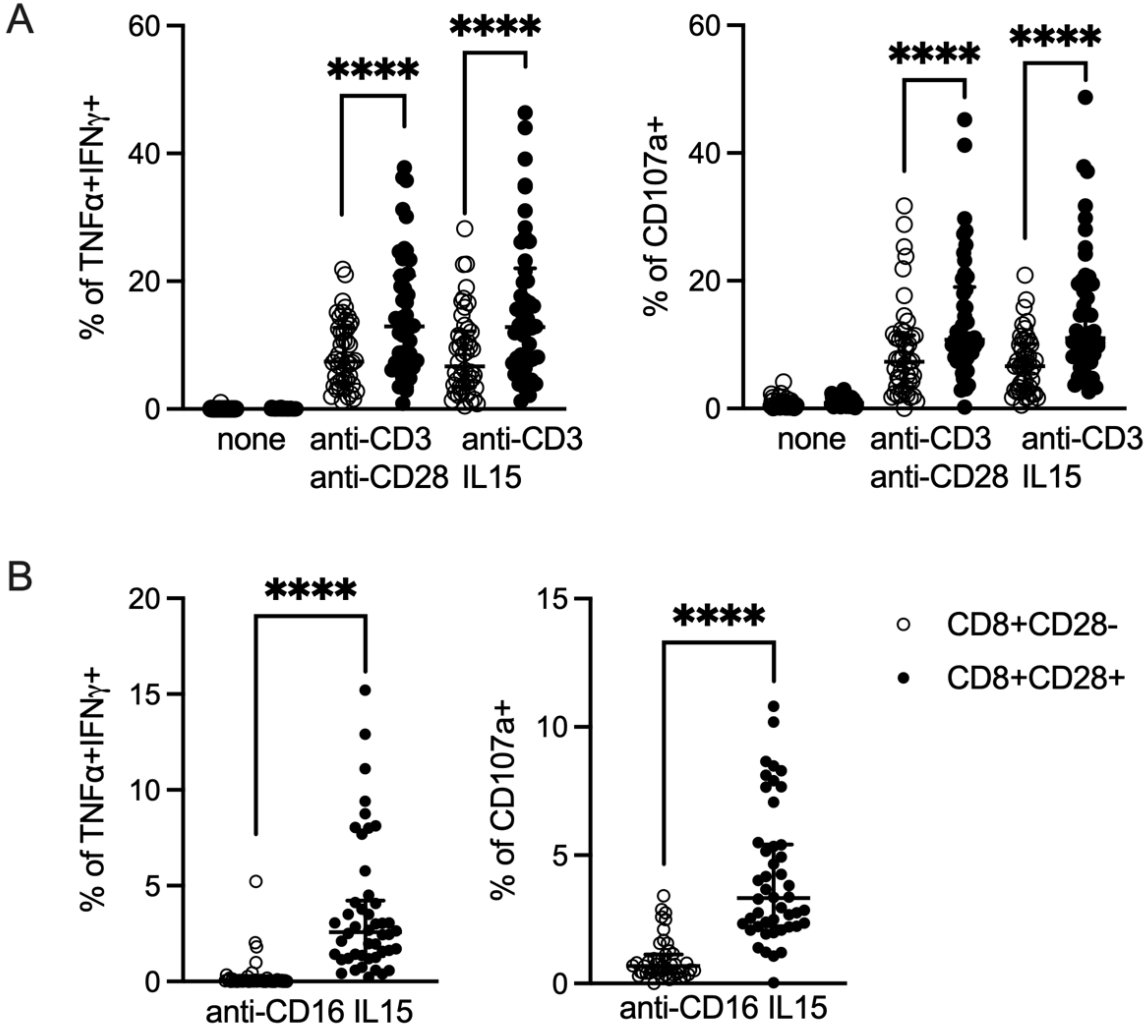
Supplementary Figure 1: Gating strategy to identify the CD8+ and CD8+ CD28- T cell subsets from whole blood using flow cytometry. Numbers indicate the percentage of the gated population. Lym: lymphocytes.



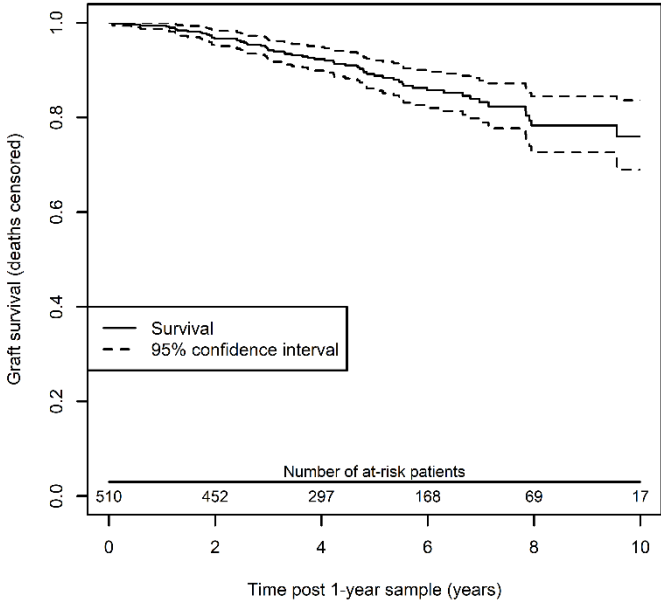
Supplementary Figure 2: Distribution of the natural logarithm of the percentage (A) and absolute number (B) of CD28-CD8+ T cells.



Supplementary Figure 3: Functional studies of CD8+ T cells in kidney recipients with normal/subnormal biopsies (group 1). Frozen PBMCs (n=46) from kidney recipients belonging to group 1 (normal/subnormal biopsies) were retrieved from our biocollection, thawed, kept in the resting state overnight in complete RPMI medium. The next day, PBMCs were stimulated for 4 h with plate-bound anti-CD3 mAb combined with soluble anti-CD28 mAb or IL-15 (A), and plate-bound anti-CD16 mAb combined with soluble IL-15 (B) as indicated. Cells were stained for surface CD8, CD28, and CD107a, followed by intracellular staining for TNF α and IFN γ and analyzed by flow cytometry. The percentage of cells double positive for TNF α and IFN γ (left) and positive for CD107a (right) among CD28+CD8+ (open circles) and CD28-CD8+ (filled circles) T cells are shown. ****: p<0.0001.



Supplementary Figure 4: Unadjusted graft survival curve (deaths censored) estimated by the Kaplan-Meier method in the 510 recipients with a 1-year protocol biopsy.



Supplementary Figure 5: Cumulative incidence curves of death-censored graft survival estimated from the Kaplan-Meier estimator and the Aalen-Johansen estimator taking into account deaths with functioning graft as competing events in the 510 recipients with a 1-year protocol biopsy.

