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Supplementary Tables

Table S1 Definitions of epitopes, KIR haplotypes and missing self

Epitope	**Corresponding HLA antigens**
Bw4	A23, A24, A25, A32, B13, B27, B37, B38, B44, B47, B49, B51, B52, B53, B57, B58, B59, B63, B77
C1	Cw1, Cw3, Cw7, Cw8, Cw12, Cw14, Cw16
C2	Cw2, Cw4, Cw5, Cw6, Cw15, Cw17, Cw18
KIR haploptype	**Corresponding KIR genes**
BX haplotype	Presence of either KIR2DL2, KIR2DL5, KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS5, KIR3DS1
AA haplotype	Absence of BX haplotype-defining KIR genes
Missing self type	**Definition**
A3/11	Recipient A3 or A11 positive AND recipient KIR3DL2 positive AND donor A3 and A11 negative
Bw4	Recipient BW4 positive AND recipient KIR3DL1 positive AND donor Bw4 negative
C1/2DL2	Recipient C1 positive AND recipient KIR2DL2 positive AND donor C1 negative
C1/2DL3	Recipient C1 positive AND recipient KIR2DL3 positive AND donor C1 negative
C2	Recipient C2 positive AND recipient KIR2DL1 positive AND donor C2 negative

Table S2 Pretransplant determinants of MVI: univariable analysis (N=890 transplantations)

Parameter	Patients	Events	HR	95% CI	P
Recipient age, per 1-year increment	890	222	1.00	0.99-1.01	0.764
Recipient gender					
Male	538	118	1		
Female	352	104	1.44	1.10-1.87	**0.007**
Missing self					
no	501	114	1		
yes	389	108	1.19	0.91-1.54	0.207
Missing self type					
A3/A11	124	35	1.11	0.77-1.59	0.590
Bw4	120	36	1.30	0.91-1.85	0.155
C1/2DL2	37	14	1.75	1.02-3.02	**0.042**
C1/2DL3	78	25	1.39	0.92-2.11	0.122
C2	147	45	1.27	0.92-1.77	0.148
Missing self number, per 1-unit increase	890	222	1.29	1.08-1.53	**0.004**
0	501	114	1		
1	281	67	0.98	0.72-1.32	0.869
2	99	35	1.66	1.13-2.42	**0.009**
3	9	6	3.95	1.74-8.98	**0.001**
Kir haplotype					
AA	281	65	1		
BX	609	157	1.13	0.84-1.50	0.427
Pretransplant HLA-DSA					
no	796	158			
yes	94	64	6.11	4.54-8.23	**<0.001**
Pretransplant HLA-DSA class					
None	796	158	1		
Class I	33	21	4.90	3.10-7.74	**<0.001**
Class II	40	28	7.57	5.02-11.42	**<0.001**
Class I + II	21	15	6.13	3.59-10.45	**<0.001**
HLA-A/B/DR/DQ mismatches, per 1-unit increment	890	222	1.24	1.14-1.35	**<0.001**
HLA-A mismatches, per 1-unit increment	890	222	1.29	1.07-1.56	**0.008**
HLA-B mismatches, per 1-unit increment	890	222	1.47	1.19-1.81	**<0.001**
HLA-C mismatches, per 1-unit increment	890	222	1.33	1.09-1.61	**0.005**
HLA-DRB1 mismatches, per 1-unit increment	890	222	1.45	1.14-1.85	**0.002**
HLA-DQB1 mismatches, per 1-unit increment	890	222	1.51	1.22-1.86	**<0.001**
Retransplantation					
no	764	177	1		
yes	126	45	1.81	1.30-2.52	**<0.001**
CMV status					
D-/R-	246	42	1		
D-/R+	262	71	1.71	1.16-2.51	**0.006**
D+/R-	182	48	1.69	1.12-2.57	**0.014**

D+/R+	200	61	2.06	1.38-3.06	**<0.001**
Donor age, per 1-year increment	890	222	1.01	1.00-1.02	**0.018**
Donor gender					
Male	473	117	1		
Female	417	105	1.08	0.83-1.40	0.580
Donation type					
Living donation	42	9	1		
Donation after brain death	698	181	1.37	0.70-2.67	0.362
Donation after circulatory death	150	32	1.11	0.53-2.32	0.787
Cold ischemia time, per 1-hour increment	890	222	1.02	0.99-1.04	0.169

Univariable Cox proportional hazards analysis of MVI incidence after transplantation. MVI: microvascular inflammation, HR: hazard ratio, CI: confidence interval, HLA-DSA: donor-specific anti-HLA antibodies, D: donor, R: recipient. Bold P-values indicate statistical significance.

Table S3 The association between NK cell stimuli and kidney transplant rejection phenotypes (N=3476 biopsies)

The estimates and confidence bounds are based on a logistic mixed effect regression model with random intercepts and a linear fixed effect of posttransplant time, corrected for HLA class I and class II mismatch number, donor age, recipient sex and repeat transplantation. All posttransplant biopsies (n=3476) were used in the analysis. Missing self is defined as presence of 2 or more types. MVI: microvascular inflammation, ABMR: antibody-mediated rejection per Banff 2019 definition, TCMR: T-cell mediated rejection, OR: odds ratio, CI: confidence interval, HLA-DSA: donor-specific anti-HLA antibodies, KIR: killer cell immunoglobulin-like receptor. Bold P-values indicate statistical significance

	MVI			**ABMR histology**			**TCMR**			**TCMR or borderline changes**		
Parameter	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**
Missing self	2.16	1.26-3.72	**0.005**	2.27	1.28-4.01	**0.005**	1.39	0.82-2.34	0.222	1.29	0.85-1.97	0.238
HLA-DSA	8.82	5.24-14.87	**<0.001**	28.32	15.7-50.99	**<0.001**	2.23	1.30-3.82	**0.003**	2.13	1.38-3.29	**<0.001**
Prior CMV disease	2.67	1.46-4.87	**0.001**	2.68	1.40-5.21	**0.003**	1.54	0.85-2.81	0.157	1.75	1.10-2.79	**0.018**
Cold ischemia time (hr)	1.00	0.96-1.03	0.828	1.00	0.96-1.04	0.936	0.99	0.95-1.02	0.465	1.00	0.98-1.03	0.926
KIR BX haplotype	1.17	0.77-1.77	0.475	0.91	0.59-1.42	0.686	1.11	0.75-1.63	0.606	1.19	0.87-1.62	0.284

Table S4 The association between missing self and individual histological lesions (N=3476 biopsies)

The estimates and confidence bounds are based on a logistic mixed effect regression model with random intercepts and a linear fixed effect of posttransplant time. Missing self was defined as presence of 2 or more subtypes. Binary definitions were used for presence (Banff score > 0) or absence (Banff score=0) of the histological lesions. The multivariable model is corrected for class I and class II HLA mismatch number, donor age, recipient sex, repeat transplantation, donor-specific antibodies and preceding CMV disease. All posttransplant biopsies (n=3476) were used in the analysis. Bold P-values indicate statistical significance. OR: odds ratio, CI: confidence interval.

	Univariable analysis			**Multivariable analysis**		
Parameter	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**
Intimal arteritis	2.23	1.31-3.79	**0.003**	2.27	1.33-3.87	**0.003**
Glomerulitis	2.27	1.35-3.84	**0.002**	1.89	1.14-3.15	**0.014**
Peritubular capillaritis	1.80	1.17-2.77	**0.008**	1.54	0.95-2.54	0.079
Interstitial inflammation	1.45	1.01-2.07	**0.043**	1.29	0.83-2.00	0.263
C4d deposition peritubular capillaries	1.43	0.95-2.17	0.091	1.27	0.85-1.92	0.246
Transplant glomerulopathy	1.11	0.31-4.03	0.874	1.23	0.48-3.17	0.672
Arterial fibro-intimal thickening	1.06	0.80-1.39	0.704	1.09	0.78-1.31	0.949
Tubulitis	1.04	0.77-1.40	0.784	0.96	0.72-1.29	0.780
Tubular atrophy	0.93	0.70-1.24	0.624	0.89	0.65-1.22	0.474
Arteriolar hyalinosis	0.86	0.65-1.15	0.303	0.81	0.59-1.11	0.188
Interstitial fibrosis	0.85	0.65-1.11	0.223	0.79	0.57-1.08	0.132

Table S5 Association of missing self and peritransplantation factors with early occurrence of MVI (n=748 biopsies)

The first biopsy within three posttransplant months was included for transplantations with available information on tacrolimus trough levels. The estimates and confidence bounds are based on a logistic regression model. The multivariable model is corrected for class I and class II HLA mismatch number, donor and recipient age, recipient sex, previous transplantation and days after transplantation. Missing self is defined as presence of 2 or more types. HLA-DSA: donor-specific anti-HLA antibodies, OR: odds ratio, CI: confidence interval.

	Univariable analysis			**Multivariable analysis**		
Parameter	**OR**	**95% CI**	**P**	**OR**	**95% CI**	**P**
Missing self	2.53	1.50-4.26	**<0.001**	3.25	1.71-6.18	**<0.001**
HLA-DSA	9.42	5.73-15.47	**<0.001**	9.73	5.35-17.7	**<0.001**
Cold ischemia time, per 1 hour increase	1.04	1.01-1.08	**0.024**	1.02	0.97-1.08	0.415
Delayed graft function	2.38	1.48-3.84	**<0.001**	1.57	0.88-2.81	0.129
Induction therapy	1.23	0.81-1.86	0.330	0.78	0.45-1.34	0.365
Average tacrolimus trough level, per 1 ng/ml increase	1.06	0.96-1.16	0.254	0.89	0.80-0.99	**0.040**
Previous CMV disease	1.99	0.40-10.01	0.402	4.13	0.72-23.77	0.112

Table S6. Risk factors for development of MVI after CMV disease (n=72 transplantations)

Cox proportional hazards analysis of MVI incidence after the first episode of CMV disease, in transplantation with further histological follow-up and without previous occurrence of MVI. HLA-DSA: anti-HLA donor-specific antibodies, HR: hazard ratio, CI: confidence interval.

			Univariable			Multivariable		
Parameter	**Patients**	**Events**	**HR**	**95% CI**	**P**	**HR**	**95% CI**	**P**
Reduction of maintenance immunosuppression								
No	51	15	1			1		
Yes	21	7	1.27	0.52-3.12	0.600	1.49	0.59-3.74	0.396
HLA-DSA								
No	69	20	1			1		
Yes	3	2	2.79	0.64-12.22	0.174	3.22	0.66-15.70	0.148
Missing self types								
0-1	59	18	1			1		
2-3	13	4	1.02	0.35-3.03	0.965	0.87	0.28-2.68	0.810
Primo infection								
No	9	2	1			1		
Yes	63	20	1.54	0.36-6.59	0.562	0.78	0.16-3.77	0.755
Induction therapy at transplantation								
No	51	17	1			1		
Yes	21	5	0.67	0.25-1.81	0.670	0.52	0.18-1.49	0.223

Table S7 Determinants of transplant glomerulopathy after diagnosis of MVI (N=190 cases)

Univariable and multivariable Cox proportional hazards analysis of transplant glomerulopathy incidence after the first biopsy showing MVI, corrected for time after transplantation. Cases with transplant glomerulopathy at the time of MVI diagnosis, without subsequent histological follow-up or without information on previous CMV disease were excluded from the analysis (n=32). Bold P-values indicate statistical significance. MVI: microvascular inflammation, HR: hazard ratio, CI: confidence interval, HLA-DSA: donor-specific anti-HLA antibodies, TCMR: T-cell mediated rejection, eGFR: estimated glomerular filtration rate.

			Univariable			Multivariable		
Parameter	**Patients**	**Events**	**HR**	**95% CI**	**P**	**HR**	**95% CI**	**P**
Missing self types								
0-1	154	22	1			1		
2-3	36	9	2.04	0.92-4.41	0.080	2.51	1.12-5.62	**0.025**
HLA-DSA								
no	127	14	1			1		
yes	63	17	2.91	1.41-6.00	**0.004**	3.55	1.59-7.92	**0.002**
TCMR								
no	107	19	1					
yes	83	12	0.85	0.40-1.79	0.665	0.90	0.37-2.19	0.816
Prior CMV disease								
No	173	29	1			1		
yes	17	2	0.82	0.19-3.45	0.455	1.99	0.41-9.80	0.396
eGFR								
per 1 ml/min/1.73m² increase	190	31	1.00	0.98-1.01	0.556	0.99	0.97-1.02	0.993
Indication biopsy								
no	83	12	1			1		
yes	107	19	1.24	0.59-2.60	0.570	1.06	0.38-2.91	0.914

Table S8 Predictors of allograft failure after diagnosis of MVI (N=222 cases)

Univariable and multivariable Cox proportional hazards analysis of allograft survival after the first biopsy showing MVI, corrected for time after transplantation. Bold P-values indicate statistical significance. MVI: microvascular inflammation, HR: hazard ratio, CI: confidence interval, HLA-DSA: donor-specific anti-HLA antibodies, TCMR: T-cell mediated rejection, eGFR: estimated glomerular filtration rate.

			Univariable			Multivariable		
Parameter	**Patients**	**Events**	**HR**	**95% CI**	**P**	**HR**	**95% CI**	**P**
Missing self types								
0-1	181	47	1			1		
2-3	41	12	1.14	0.60-2.14	0.695	1.23	0.65-2.33	0.526
HLA-DSA								
no	146	30	1			1		
yes	76	29	2.34	1.40-3.92	**0.001**	2.31	1.36-3.94	**0.002**
TCMR								
no	129	30	1					
yes	93	29	1.28	0.77-2.14	0.338	0.96	0.52-1.79	0.964
Prior CMV disease								
No	201	56	1			1		
yes	21	3	0.50	0.16-1.62	0.507	0.90	0.27-3.04	0.871
eGFR								
per 1 ml/min/1.73m² increase	222	59	0.97	0.96-0.99	**<0.001**	0.96	0.94-0.99	**0.001**
Indication biopsy								
no	100	19	1			1		
yes	122	40	1.90	1.10-3.28	**0.022**	0.78	0.35-1.74	0.547

Supplementary Figures

Figure S1 Study cohort overview MVI: microvascular inflammation.

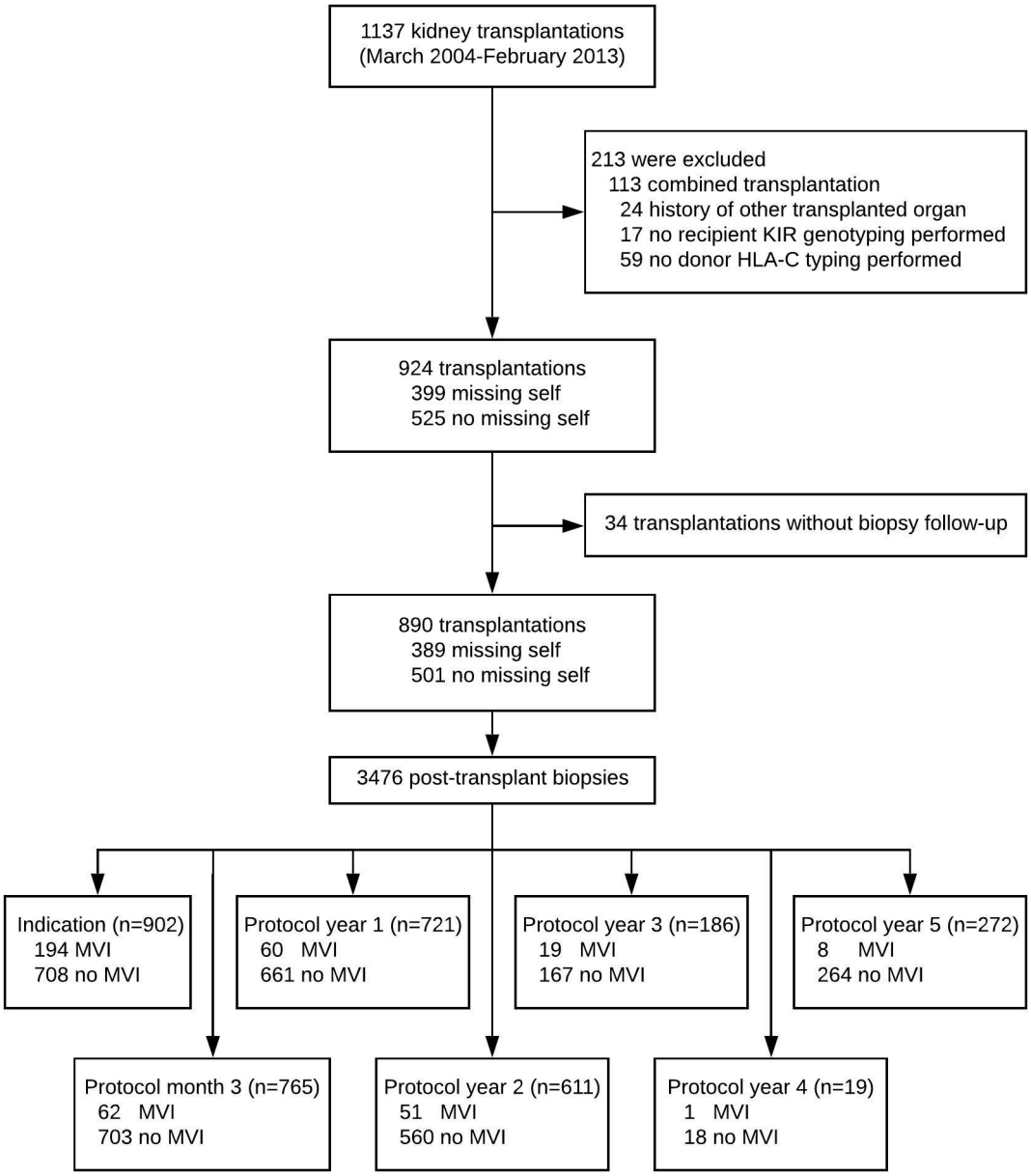


Figure S2 Tacrolimus exposure associates with decreased risk of early MVI in transplantations with missing self (n=748 biopsies)

The first biopsy within three posttransplant months was included for transplantations with tacrolimus as maintenance immunosuppression and ≥5 available trough levels. The estimates and confidence bounds are based on a logistic regression model. **a.** Odds ratio for MVI in function of the average tacrolimus exposure in presence (n=89) or absence (n=659) of high missing self. **b.** Odds ratio plot of the probability of MVI in the presence (n=86) or absence (n=662) of HLA-DSA. MVI: microvascular inflammation, HLA-DSA: donor-specific anti-HLA antibodies, Low MS: < 2 missing self types, High MS: ≥ 2 missing self types

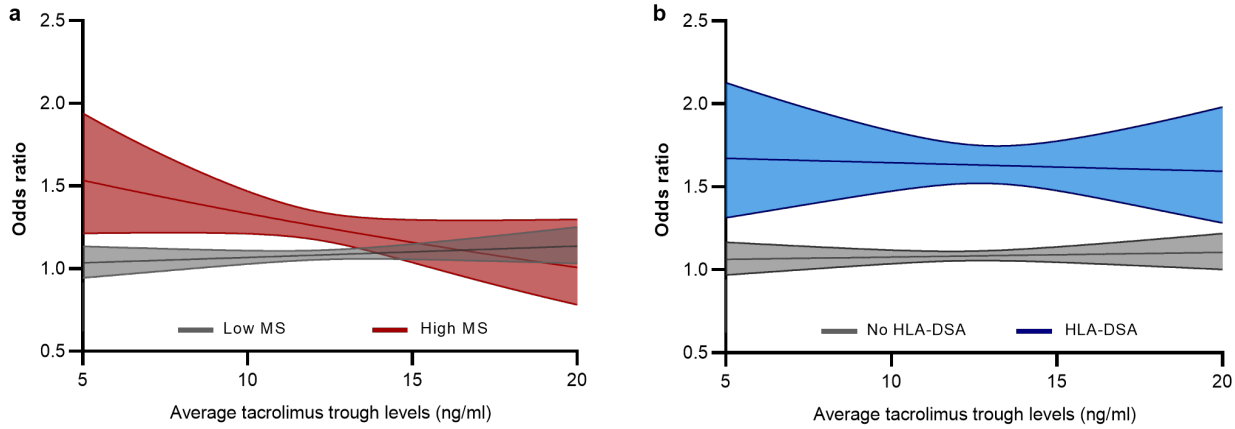
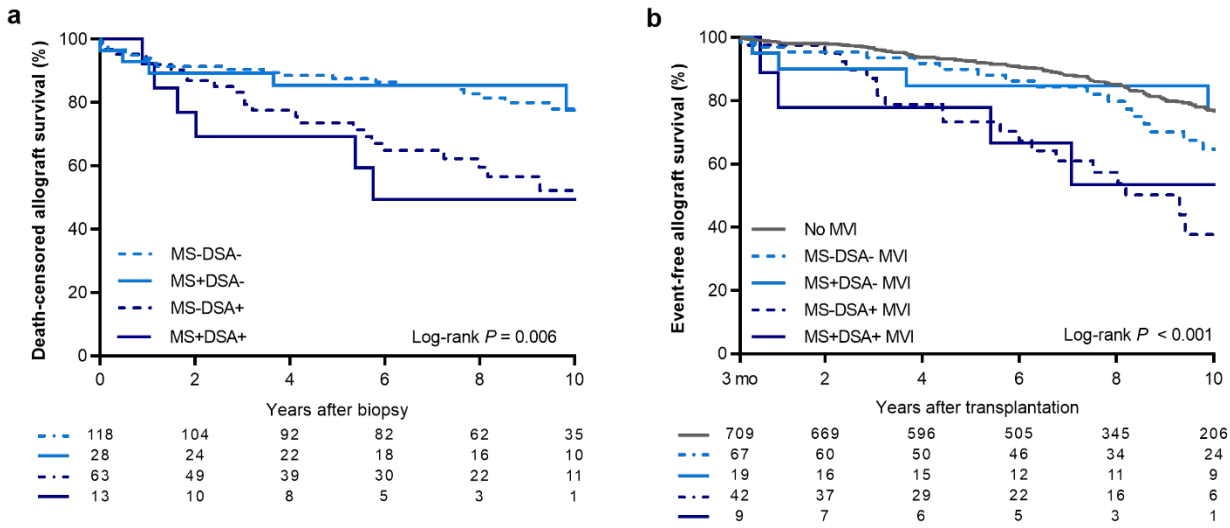


Figure S3 Missing self does not influence allograft function after MVI diagnosis



a. Kaplan-Meier survival curve of death-censored allograft failure after MVI diagnosis (N=222), stratified according to high or low missing self (i.e. 2-3 vs. 0-1 types) and HLA-DSA status (i.e. previous or current positivity). **b.** Landmark analysis of death-censored allograft failure or persistent 50% eGFR decline after the third posttransplant month, with stratification based on the presence of MVI within the first three months, high missing self and HLA-DSA status at three months. MVI: microvascular inflammation, HLA-DSA: anti-HLA donor-specific antibodies, MS: high missing self.