Supporting Information

Antibodies against ARHGDIB are associated with long-term kidney graft loss

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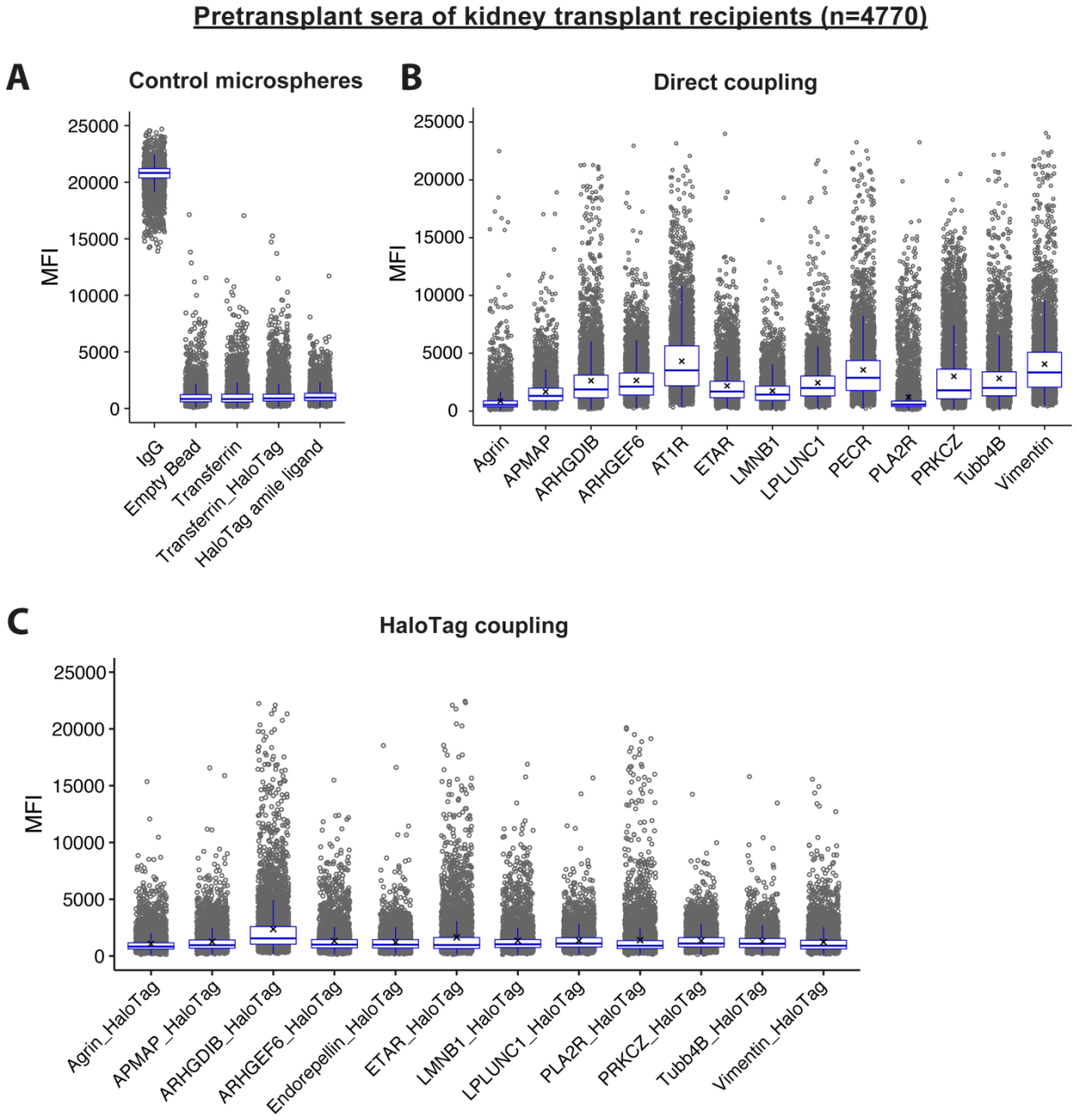
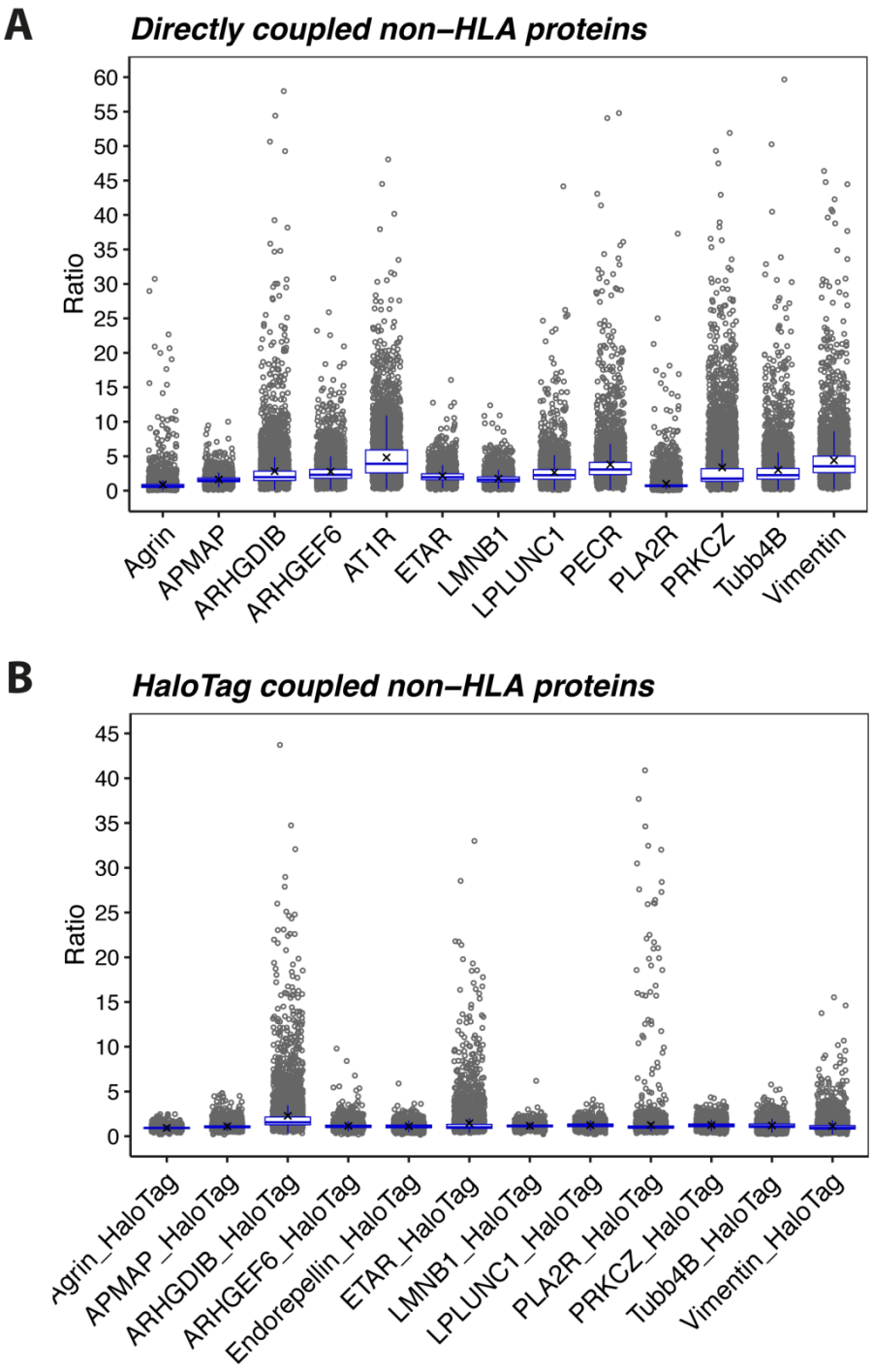
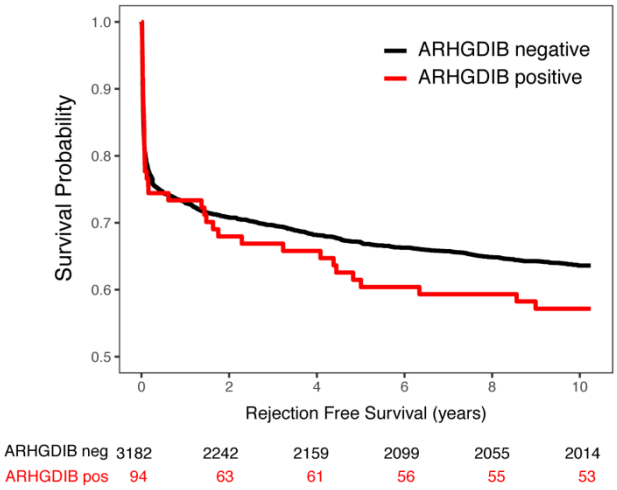


Supplemental Figure S1. Detection of non-HLA antibodies in 4770 kidney transplant recipients. Shown are the individual MFI values with box and whisker plots (median and interquartile range in blue, and the black X represents the mean) for the positive control microsphere (IgG) and the 4 negative control microspheres (**A**), the 13 directly-coupled non-HLA proteins (**B**), and the 12 HaloTag-coupled non-HLA proteins (**C**). Directly-coupled Endorepellin was excluded from further analysis due to cross reactivity as mentioned in the manuscript describing the development and validation of this assay. The box plots (Tukey) were drawn in R 3.4.1. using ggplot package (geom_boxplot). The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The upper whisker extends from the hinge to the largest value no further than 1.5 * IQR from the hinge (where IQR is the inter-quartile range, or distance between the first and third quartiles). The lower whisker extends from the hinge to the smallest value at most 1.5 * IQR of the hinge. Data beyond the end of the whiskers are called "outlying" points and are plotted individually.



Supplemental Figure S2. Detection of non-HLA antibodies in 4770 kidney transplant recipients. Shown are the individual signal-to-background ratios with box and whisker plots (median and interquartile range in blue, and the black X represents the mean) for the 13 directly-coupled non-HLA proteins (**A**), and the 12 HaloTag coupled non-HLA proteins (**B**). Transferrin or Transferrin_HaloTag microspheres were used as background accordingly. The box plots (Tukey) were drawn in R 3.4.1. using ggplot package (geom_boxplot). The lower and upper hinges correspond to the first and third quartiles (the 25th and 75th percentiles). The upper whisker extends from the hinge to the largest value no further than 1.5 * IQR from the hinge (where IQR is the inter-quartile range, or distance between the first and third quartiles). The lower whisker extends from the hinge to the smallest value at most 1.5 * IQR of the hinge. Data beyond the end of the whiskers are called "outlying" points and are plotted individually.



Supplemental Figure S3. 10-year rejection free survival for patients with ARHGDIB antibodies transplanted with a deceased donor kidney (N=3276). Rejection free survival was defined as whether a patient had a registered rejection (treatment) or not. This uncorrected analysis showed that the rejection free survival was comparable (not significantly different) between the positive and negative groups.

Supplemental Table S1. Overview of the selected cut-offs based on the maximal graft survival difference in 4770 kidney transplant patients.

						Graft survival difference between the non-HLA positive and negative group at:			Numbers at risk for the non-HLA positive group at:		
Coupling	**Protein**	**Ratio cut-off**	**Absolute MFI cut-off**	**Number of positive patients**	**% Positive patients**	**1 year**	**5 years**	**10 years**	**1 year**	**5 years**	**10 years**
Direct	Agrin	3	1000	97	2·0 %	5·0 %	5·9 %	6·4 %	82	69	45
	ETAR	5	1500	83	1·7 %	3·5 %	8·3 %	7·3 %	72	58	42
	PLA2R	7	500	29	0·6 %	-1·7 %	-1·5 %	9·5 %	27	24	18
	LPLUNC1	9	500	62	1·2 %	-0·5 %	10·8 %	6·7 %	54	40	28
	ARHGDIB	10	500	134	2·8 %	5·9 %	10·9 %	13·1 %	113	92	66
	ARHGEF6	10	500	53	1·1 %	0·9 %	2·0 %	4·5 %	48	41	29
	PECR	12	500	114	2·4 %	3·8 %	10·7 %	7·6 %	100	78	56
	AT1R	15	500	106	2·2 %	2·0 %	4·5 %	7·3 %	91	75	48
	APMAP	NA	NA								
	LMNB1	NA	NA								
	PRKCZ	NA	NA								
	TUBB4B	NA	NA								
	Vimentin	NA	NA								
HaloTag	ARHGEF6_HaloTag	2	2000	55	1·2 %	4·2%	7·1 %	0·7 %	49	38	28
	LPLUNC1_HaloTag	2	2000	41	0·9 %	-0·3 %	3·6 %	6·6 %	38	31	20
	APMAP_HaloTag	2·5	500	54	1·1 %	0·7%	0·0 %	0·0 %	47	36	23
	TUBB4B_HaloTag	2·5	500	65	1·4 %	3·8%	-1·5 %	-1·7 %	56	49	32
	PRKCZ_HaloTag	2·5	1500	45	0·9 %	4·8 %	8·0 %	9·4 %	38	31	17
	PLA2R_HaloTag	7	500	42	0·9 %	-1·4 %	1·4 %	12·5 %	39	34	23
	ETAR_HaloTag	8	500	62	1·3 %	1·1 %	-0·8 %	4·7 %	56	49	33
	Agrin_HaloTag	NA	NA								
	ARHGDIB_HaloTag	NA	NA								
	Endorepellin_HaloTag	NA	NA								
	LMNB1_HaloTag	NA	NA								
	Vimentin_HaloTag	NA	NA								

NA: not available, since there was no effect on the graft survival independent for all possible cut-offs.

Supplemental Table S2. Multivariable analyses of 10-year death-censored graft failure for the presence of different non-HLA antibodies in the total cohort (N=4770).

	No. (%) of Transplants with non-HLA antibody	HR	95% CI	p-value
Agrin	97 (2·0 %)	1·273	0·867-1·868	0·218
ETAR	83 (1·7 %)	1·513	1·009-2·270	0·045
PECR	114 (2·4%)	1·401	0·995-1·973	0·053
PLA2R	29 (0·6 %)	1·580	0·817-3·057	0·174
LPLUNC1	62 (1·2 %)	1·397	0·864-2·259	0·172
ARHGEF6	53 (1·1 %)	1·210	0·713-2·054	0·480
AT1R	106 (2·2 %)	1·297	0·896-1·878	0·169
ARHGEF6_HaloTag	55 (1·2 %)	1·113	0·644-1·925	0·701
LPLUNC1_HaloTag	41 (0·9 %)	1·430	0·827-2·475	0·201
APMAP_HaloTag	54 (1·1 %)	1·259	0·743-2·134	0·393
TUBB4B_HaloTag	65 (1·4 %)	1·013	0·573-1·792	0·964
PRKCZ_HaloTag	45 (0·9 %)	1·488	0·860-2·573	0·155
PLA2R_HaloTag	42 (0·9 %)	0·977	0·313-3·053	0·968
ETAR_HaloTag	62 (1·3 %)	1·242	0·758-2·037	0·389

In this multivariable analysis we evaluated the effect of the presence of pretransplant single non-HLA antibodies on the 10-year death-censored graft failure and adjusted for differences in the following covariates: recipient age (quadratic), donor age (quadratic), donor type (living or deceased), cold ischemia time in hours for donation after brain death (DBD) and donation after cardiac death (DCD), time on dialysis in years (quadratic), induction therapy with interleukin- 2 receptor–blocking antibody and the presence of pretransplant donor-specific HLA antibodies against HLA-A/B/DR/DQ. CI, confidence interval; HR, hazard ratio. P<0·002 was considered statistically significant.

Supplemental Table S3. Multivariable analyses of 1-year death-censored graft failure for the presence of antibodies against ARHGDIB.

	No. (%) of Transplants with non-HLA antibody	HR	95% CI	p-value
ARHGDIB				
Total cohort (N=4770)	134 (2·8%)	1·597	1·006-2·536	0·047
Deceased donors (N=3276)	94 (2·9%)	1·620	0·993-2·643	0·053
Living donors (N=1494)	40 (2·7%)	1·387	0·336-5·726	0·651

In this multivariable analysis we evaluated the effect of the presence of pretransplant ARHGDIB on the 1-year death-censored graft failure and adjusted for differences in the following covariates: recipient age (quadratic), donor age (quadratic), donor type (living or deceased, only for the total cohort), cold ischemia time in hours for donation after brain death (DBD) and donation after cardiac death (DCD), time on dialysis in years (quadratic), induction therapy with interleukin- 2 receptor–blocking antibody and the presence of pretransplant donor-specific HLA antibodies against HLA-A/B/DR/DQ. CI, confidence interval; HR, hazard ratio.