SUPPLEMENTARY MATERIAL

Identification of a serum and urine extracellular vesicles signature predicting renal outcome after kidney transplant

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Extended methods

Patient recruitment and sampling strategy

We recruited 58 patients who underwent kidney transplant for end-stage renal disease at the Nephrology, Dialysis and Renal Transplant Unit, IRCCS S. Orsola Hospital (University of Bologna, Italy) between October 2015 and January 2017. All patients who gave written informed consent, were consecutively recruited and included in the study. Patients were excluded from the analysis in case of concomitants infections, acute inflammatory disease, or active cancer. The study complied with the Declaration of Helsinki; we obtained the approval from the local ethics committee (protocol n° 133/2015/U/Sper). Patient outcome was defined according to glomerular filtration rate estimated by CKD-EPI equation (eGFR) at 12 months, using a cut-off of 45 mL/min. Post-transplant treatment included thymoglobulin, basiliximab, steroid, FK-506, and/or mycophenolic acid. For each patient, peripheral blood and urine samples were collected before kidney transplant (baseline, or T0), 10-14 days (T1), 3 months (T2), and 12 months (T3) after transplant (urine was not available for 38 anuric patients at T0; Figure 1A).

Blood and urine samples handling

Venous blood (5 ml) was collected, for each donor at different time points (see above), in serum separator tubes and stored 30 minutes at room temperature (RT). After clot formation a first centrifugation at 1600 g for 15 min at 4°C was performed to separate serum from cellular components. Serum was transferred in a new clean tube and centrifuged at 3,000 g for 20 min. Supernatant was transferred in new clean tube and underwent centrifugation step at 10,000 g for 15 min, followed by a second centrifugation at 20,000 g for 30' to remove intact cells, cellular debris and larger EVs. Supernatant was transferred in a new tube and stored. Second morning urine samples (10-20 ml) were collected in parallel. A first centrifugation at 3000 g for 15 min at 4°C was performed to separate urine from cellular components. Urine was transferred in a new clean tube and centrifuged at 3,000 g for further 15 min. Supernatant was transferred in a clean tube and stored. High-speed centrifugation steps were not performed for urine to avoid precipitation of Tamm-Horsfall protein. Samples were processed immediately after collection and pre-cleared aliquots were then stored at -80°C and never thawed prior to analysis. The above-described standard protocol was compared to an alternative protocol including a pre-isolation step by ultracentrifugation (100,000 g for 3 hours at 4-10°C; Beckman Coulter Optima L-90K; Beckman Coulter, Fullerton, CA, USA) with pellet re-suspended in 100 uL PBS prior to further analysis. For urine a pre-treatment with 100 mM DTT (dithiothreitol) was performed to eliminate Tamm-Horsfall protein, prior to ultracentrifugation.

Nanoparticle tracking analysis

Particle concentration and diameter were measured by nanoparticle tracking analysis (NTA) using NanoSight LM10 (Malvern Instruments) equipped with a 405 nm laser and NTA 2.3 analytic software; 1 uL of serum or 100 uL of urine were diluted in a total volume of 1 mL of phosphate buffered saline (PBS) sterile solution (1:1000 for serum; 1:10 for urine). Brownian movements of Particle were recorded by a camera and size and number of EVs per mL were calculated by Stokes-Einstein equation; 3 videos of 60 s were analysed for each sample.

Analysis of EV surface antigens by flow cytometry

EVs were isolated by capture beads coated with antibodies against specific EV surface antigens and analyzed by flow cytometry (FC) using MACSPlex human Exosome Kit (Miltenyi Biotec; Bergisch Gladbach, Germany), as previously described (see Figure 1B). Polystyrene capture beads are labeled with a different amount of two dyes (PE, phycoerythrin, and FITC, fluorescein isothiocyanate), to obtain 37 different beads subsets discriminable at FC. Each subset is conjugated with a different antibody against a specific EV surface epitope. EV epitopes included in the analysis are: CD3 (T-cell transmembrane co-receptor), CD4 (T-/B-cell transmembrane glycoprotein), CD19 (Surface molecule co-stimulating B-cell activation), CD8 (T-cell transmembrane glycoprotein), HLA-II (Type II-Major Histocompatibility Complex DR/-DP/-DQ), CD56 (Neural Cell Adhesion Molecule), CD105 (Endoglin), CD2 (T-/NK-cell transmembrane glycoprotein), CD1c (T-cell surface glycoprotein), CD25 (Interleukin-2 Receptor alpha-chain), CD49e (Integrin alpha-5), ROR1 (Neurotrophic Tyrosine Kinase receptor-related 1), CD209 (Dendritic Cell-Specific Intercellular adhesion molecule-3), CD9 (Tetraspanin super-family), SSEA-4 (Stage-Specific Embryonic Antigen-4), HLA-I, (Type I-Major Histocompatibility Complex -A/-B/-C), CD63 (Tetraspanin super-family), CD40 (Antigen Presenting Cells co-stimulatory receptor), CD62P (P-selectin), CD11c (Integrin, alpha-X), CD81 (Tetraspanin super-family), MCSP (Melanoma-associated Chondroitin Sulphate Proteoglycan), CD146 (Melanoma Cell Adhesion Molecule), CD41b (Platelet membrane glycoprotein II-b), CD42a (Platelet membrane glycoprotein IX), CD24 (Heat stable antigen 24), CD86 (Antigen Presenting Cells co-stimulatory protein), CD44 (Homing Cell Adhesion Molecule or Phagocytic glycoprotein-1), CD326 (Epithelial Cell Adhesion Molecule), CD133/1 (Prominin-1), CD29 (Integrin beta-1), CD69 (Transmembrane C-Type Lectin protein), CD142 (Platelet Tissue Factor-III), CD45 (Protein Tyrosine Phosphatase, Receptor type-C), CD31 (Platelet-Endothelial Cell Adhesion Molecule-1), CD20 (B-lymphocyte antigen-20), and CD14 (Lipopolysaccharide co-receptor binding protein).

Serum (50 uL) and urine (250 uL) samples were incubated overnight (14-16 h) with 15 uL MACSPlex Exosome Capture Beads, protected from light on an orbital shaker (800 rpm at 10°C). After incubation, 1 mL of MACSPlex buffer was added to each tube and then tubes were centrifuged 3.000 g for 10 minutes at 10°C to wash beads. After careful aspiration of 1 mL of supernatant, beads-EV complexes were than labeled with 15 uL APC (allophycocyanin)- conjugated detection antibodies against CD9, CD63 and CD81 (1 hour protected from light on an orbital shaker 450 rpm at 10°C). After two washing steps (1 mL PBS sterile solution, 15 minutes protected from light on an orbital shaker 450 rpm at 10°C), samples were loaded to and acquired by MACSQuant Analyzer 10 flow cytometer (Miltenyi Biotec; Bergisch Gladbach, Germany). Samples were analyzed by the instrument, resulting in approximately 10.000-15.000 single bead events being recorded for each sample. MACSPlex Exosome Setup Beads were used to setup MACSQuant analyzer: measurements were confine on capture beads by setting a trigger for side scatter (SSC) and forward scatter (FSC); single beads were gated to exclude doublets and non-bead events. FITC and PE voltage were adapted to optimize the discrimination of the 37 bead subsets; single bead subsets were each gated to allow the measurement of the APC median fluorescence signal intensity. APC- median fluorescence intensity (MFI) was corrected by subtracting the respective value of blank control, normalized by the average MFI of CD9-CD63-CD81, and expressed as normalized MFI (nMFI; %).

Super-resolution microscopy

Single vesicles analysis was performed by super-resolution microscopy using Nanoimager S Mark II microscope from ONI (Oxford Nanoimaging, Oxford, UK) equipped with a 100x, 1.4NA oil immersion objective, an XYZ closed-loop piezo 736 stage, and triple emission channels split at 640, 488 and 555 nm. All the components (buffer, reagents, and chip) of EV profiler Kit (ONI) were used following manufacturer's protocol, apart from antibodies. We utilized CD105 FITC conjugated, SSEA-4 (APC conjugated, clone REA101) and CD133/1 (PE conjugated, clone REAA753), all from Miltenyi Biotec. A pool of urinary EVs isolated by ultracentrifugation from control subjects was tested. Images were acquired in dSTORM mode, sequentially in total reflection fluorescence (TIRF) mode. Single-molecule data was filtered using NimOS software (v.1.18.3, ONI). Data has been processed with the Collaborative Discovery (CODI) online analysis platform www.alto.codi.bio from ONI with drift correction pipeline version 0.2.3.

Statistics and diagnostic modelling

Python 3.5 (library, scikit-learn), IBM SPSS Statistics 26 (IBM Corp, Armonk, NY) and GraphPad Prism 8.0a (GraphPad, La Jolla, CA) were used for analyses and figure preparation. Variable

distribution was assessed by with Kolmogorov−Smirnov test. Normally distributed variables are expressed as mean ± standard deviation (SD) and analyzed by T student test for independent or matched data (when appropriated). Non-normally distributed variables are expressed as median [interquartile range] and analyzed by Mann-Whitney test (independent data) or Wilcoxon test (matched data). A mixed-effects analysis was performed to take into account the presence of missing values among repeated measures. Categorical variables are expressed as absolute number (percentage) and compared with chi-square tests. Correlations were evaluated by Pearson's test (R coefficient) and analysis of regression curves. Odds ratio (OR) were calculated by univariate logistic regression analysis. Receiver operating characteristics (ROC) curves were used to assess the area under the curve (AUC) and compare diagnostic performances of selected variables.

Machine learning (ML) supervised algorithms were used to train and validate diagnostic models to predict renal outcome at T3 (n=58), using nMFI of serum- or urine- EV surface antigens (n=194 [urine]; n=232 [serum]). Four different machine learning classifiers (linear discriminant analysis, random forest, support vector machine with linear or gaussian kernel) and 3 algorithms for data imbalance correction (synthetic minority over-sampling technique [SMOTE], SMOTE and nearest neighbors [SMOTE&NN], and random oversampling [RO] methods) were applied to the overall cohort, generating 616 different models.

After tuning of hyperparameters, the model with the highest accuracy was then selected to be tested by a leave-one out validation method to exclude overfitting bias and assess how the model could generalize in an independent cohort; briefly, the algorithm trains the model on all patients except one (n – 1) and predicts the outcome of the excluded patient; the process is repeated n-times, where "n" is the total number of patients included in the analysis. At each n-round, the patient used for the test is rotated, then the accuracy of the validation model is computed from the ratio between the number of patients correctly predicted and the total number of patients included by the validation algorithm. Overfitting effect was calculated as accuracy at training minus accuracy at validation.

Linear discriminant analysis (LDA) employs linear combinations of variables to maximize the separation between groups by increasing the precision estimates by variance reduction. The algorithm computes a set of coefficients for linear combination of each variable to predict the outcome. The estimation is derived from the following equation: "$LDAcoeff_1$*$Variable_1$ + $LDAcoeff_1$*$Variable_1$ + … + $LDAcoeff_n$*$Variable_n$ > cut-off".

Random forest (RF) algorithm uses "n" classification trees with a fixed number of splits for each tree. The predicted outcome resulted from the outcome of each classification tree of the forest; if at least "(n/2) + 1" out of "n" trees of the RF predicted persistent renal dysfunction, then the patient is classified accordingly.

Linear support vector machine (l-SVM) builds a classification model to assign patients to their outcome given a linear boundary. The model finds out the plane which best separates groups of patients (persistent renal dysfunction *vs.* renal recovery), maximizing the distances between them. Patients are classified according to the following equation: "$SVMcoeff_0 + SVMcoeff_1*Variable_1 + SVMcoeff_2*variable_2 + \ldots. + SVMcoeff_n*Variable_n$".

Gaussian SVM (g-SVM) allows to divide patients using a non-linear boundary. The corresponding equation in this case is: "$SVMcoeff_0 + SVMcoeff_1*f(Variable_1) + SVMcoeff_2*f(variable_2) + \ldots. + SVMcoeff_n*f(Variable_n)$", where "f" is an exponential function coefficient.

Table S1. Characteristics of patients at follow-up

	Variable	Entire cohort [n=58]	Renal Recovery [n=35]	Persistent renal dysfunction [n=23]	*P*-value
Characteristics at T1	Creatinine (mg/dL)	2.2 ± 1.49	2.2 ± 1.59	2.3 ± 1.35	0.768
	eGFR* (mL/min)	42 ± 19.5	45 ± 20.9	36 ± 15.4	0.055
	PU (mg/dL)	20 [10; 50]	20 [10; 50]	20 [10; 50]	0.717
	DGF, n (%)	12 (20.7)	7 (20.0)	5 (21.7)	0.873
	Vesical-ureteral reflux, n (%)	6 (10.3)	5 (14.3)	1 (4.3)	0.386
	Bacterial Infection				
	No, n (%)	49 (84.5)	31 (88.5)	18 (78.3)	
	UTI, n (%)	2 (3.4)	1 (2.9)	1 (4.3)	0.560
	Sepsis, n (%)	7 (12.1)	3 (8.6)	4 (17.4)	
	KPC colonization, n (%)	10 (17.2)	4 (11.4)	6 (26.1)	0.172
	Viral infection				
	No, n (%)	49 (84.5)	31 (88.5)	18 (78.3)	
	BKV, n (%)	2 (3.4)	1 (2.9)	1 (4.3)	0.563
	CMV, n (%)	7 (12.1)	3 (8.6)	4 (17.4)	
	BKV + CMV, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	
	NODAT, n (%)	7 (12.1)	4 (11.4)	3 (13.0)	1.000
	Graft rejection, n (%)				
	No evidence, n (%)	56 (96.6)	33 (94.2)	23 (100.0)	
	Acute cellular rejection, n (%)	1 (1.7)	1 (2.9)	0 (0.0)	0.506
	Humoral rejection, n (%)	1 (1.7)	1 (2.9)	0 (0.0)	
	Donor Specific Antibodies, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	1.000
Characteristics at T2	Creatinine (mg/dL)	1.6 ± 0.46	1.5 ± 0.46	1.7 ± 0.45	0.074
	eGFR* (mL/min)	48 ± 13.7	53 ± 13.7	41 ± 10.1	**0.001**
	PU (mg/dL)	10 [0; 20]	10 [0; 20]	10 [0; 30]	0.287
	Vesical-ureteral reflux, n (%)	2 (3.4)	1 (2.9)	1 (4.3)	1.000
	Bacterial Infection				
	No, n (%)	57 (98.3)	35 (100.0)	22 (95.7)	
	UTI, n (%)	1 (1.7)	0 (0.0)	1 (4.3)	0.397
	Sepsis, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	
	Viral infection				
	No, n (%)	46 (79.4)	29 (82.9)	17 (74.0)	
	BKV, n (%)	1 (1.7)	0 (0.0)	1 (4.3)	0.269
	CMV, n (%)	2 (3.4)	2 (5.7)	0 (0.0)	
	BKV + CMV, n (%)	9 (15.5)	4 (11.4)	5 (21.7)	
	NODAT, n (%)	8 (13.8)	5 (14.3)	3 (13.0)	1.000
	Graft rejection, n (%)				
	No evidence, n (%)	55 (94.8)	33 (94.3)	22 (95.7)	
	Acute cellular rejection, n (%)	3 (5.2)	2 (5.7)	1 (4.3)	1.000
	Humoral rejection, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	
	Donor Specific Antibodies, n (%)	2 (3.4)	1 (2.9)	1 (4.3)	0.761
Characteristics at T3	Creatinine (mg/dL)	1.6 ± 0.61	1.3 ± 0.29	2.0 ± 0.64	**<0.001**
	eGFR* (mL/min)	51 ± 18.8	63 ± 13.7	34 ± 7.9	**<0.001**
	PU (mg/dL)	0 [0; 20]	0 [0; 20]	10 [0; 30]	**0.047**
	Vesical-ureteral reflux, n (%)	4 (6.9)	3 (8.6)	1 (4.3)	1.000
	Bacterial Infection				
	No, n (%)	54 (93.1)	33 (94.2)	21 (91.3)	
	UTI, n (%)	3 (5.2)	1 (2.9)	2 (8.7)	0.453
	Sepsis, n (%)	1 (1.7)	1 (2.9)	0 (0.0)	
	Viral infection				
	No, n (%)	51 (87.9)	32 (91.3)	19 (82.6)	
	BKV, n (%)	3 (5.2)	1 (2.9)	2 (8.7)	0.456
	CMV, n (%)	1 (1.7)	1 (2.9)	0 (0.0)	
	BKV + CMV, n (%)	3 (5.2)	1 (2.9)	2 (8.7)	
	NODAT, n (%)	8 (13.8)	6 (17.1)	2 (8.7)	0.458

Graft rejection, n (%)				
No evidence, n (%)	56 (96.6)	34 (97.1)	22 (95.7)	
Acute cellular rejection, n (%)	2 (3.4)	1 (2.9)	1 (4.3)	1.000
Humoral rejection, n (%)	0 (0.0)	0 (0.0)	0 (0.0)	
Donor Specific Antibodies, n (%)	2 (3.4)	1 (2.9)	1 (4.3)	0.761

Clinical and biochemical characteristics of patients included in the analysis after stratification for post-transplant renal outcome: renal recovery (n=35) *vs.* persistent renal dysfunction (n=23; eGFR ≤ 45 mL/min at T3) at follow up: T1 (10-14 days after transplant); T2 (3 months after transplant); T3 (12 months after transplant). eGFR, glomerular filtration rate; PU, proteinuria; DGF, delayed graft function; UTI, urinary tract infection; BKV, BK polyomavirus; CMV, Cytomegalovirus; NODAT, new-onset diabetes mellitus after transplantation; KPC, Klebsiella Pneumoniae carbapenemase-producing bacteria. A $p<0.05$ was considered significant and shown in bold. *eGFR: glomerular filtration rate was estimated by CKD-EPI equation.

Table S2. Quantitative analysis of serum- vs. urine- EVs

Variable	**Serum** [n=232]	**Urine** [n=194]	***P*-value**
EV Diameter (nm)	181 [163; 201]	183 [168; 201]	0.342
EV concentration (n/mL) [all vesicles]	2.4e12 [1.3e12; 3.8e12]	5.7e9 [3.5e9; 11.4e9]	**<0.001**
EV concentration (n/mL) [30-150nm]	0.9e12 [0.5e12; 1.5e12]	2.1e9 [1.2e9; 4.1e9]	**<0.001**
EV concentration (n/mL) [151-500nm]	1.3e12 [0.7e12; 2.0e12]	3.4e9 [1.9e9; 6.5e9]	**<0.001**
EV concentration (n/mL) [501-1000nm]	2.8e10 [1.1e10; 6.4e10]	1.4e7 [0.5e7; 6.3e7]	**<0.001**
CD9 (MFI; a.u.)	10.7 [4.0; 23.3]	10.5 [5.6; 22.1]	0.857
CD63 (MFI; a.u.)	19.2 [9.8; 43.7]	14.8 [7.7; 29.9]	**0.016**
CD81 (MFI; a.u.)	65.2 [33.2; 156.1]	24.3 [8.4; 57.1]	**<0.001**
Mean MFI for CD9, CD63, CD81	31.4 [17.6; 78.8]	22.1 [12.5; 36.3]	**<0.001**
Correlation of serum EV concentration (n/mL)	**R coefficient**	***P*-value**	
CD9 (MFI; a.u.)	0.336	**<0.001**	
CD63 (MFI; a.u.)	0.334	**<0.001**	
CD81 (MFI; a.u.)	0.493	**<0.001**	
Mean MFI for CD9, CD63, CD81	0.534	**<0.001**	
Creatinine (mg/dL)	0.327	**<0.001**	
Correlation of urine EV concentration (n/mL)	**R coefficient**	***P*-value**	
CD9 (MFI; a.u.)	0.117	0.105	
CD63 (MFI; a.u.)	0.321	**<0.001**	
CD81 (MFI; a.u.)	0.406	**<0.001**	
Mean MFI for CD9, CD63, CD81	0.461	**<0.001**	
Creatinine (mg/dL)	-0.020	0.785	

Serum- and urine- extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81). Median and interquartile range are reported for each variable. Correlation between EV concentration and MFI for CD9-CD63-CD81 or creatinine were assessed by Pearson's R test. A $p<0.05$ was considered significant and shown in bold.

Table S3. Flow cytometric analysis of EV antigens in serum- vs. urine- EVs

Variable	**Serum** [n=232]			**Urine** [n=194]			***P*-Value**
	25th	50th	75th	25th	50th	75th	
CD3 (nMFI; %)	0.2	6.2	27.8	3.5	11.5	21.5	0.085
CD4 (nMFI; %)	1.1	8.1	35.7	0.0	1.9	5.5	**<0.001**
CD19 (nMFI; %)	3.2	15.5	40.4	1.5	5.1	12.2	**<0.001**
CD8 (nMFI; %)	12.8	31.8	58.2	0.8	4.4	11.3	**<0.001**
HLA-II (nMFI; %)	24.5	64.0	109.2	1.8	6.1	13.8	**<0.001**
CD56 (nMFI; %)	0.0	2.1	13.1	10.2	34.1	111.0	**<0.001**
CD105 (nMFI; %)	0.0	0.0	0.0	1440.9	2492.2	5175.0	**<0.001**
CD2 (nMFI; %)	0.0	4.2	30.5	5.8	16.0	36.7	**<0.001**
CD1c (nMFI; %)	2.2	10.7	40.8	0.8	3.6	9.6	**<0.001**
CD25 (nMFI; %)	0.0	5.0	20.6	1.1	5.8	15.3	0.618
CD49e (nMFI; %)	1.4	7.5	34.1	0.0	4.2	13.5	**<0.001**
ROR1 (nMFI; %)	1.6	8.3	35.9	1.2	5.8	16.1	**0.008**
CD209 (nMFI; %)	2.4	9.7	30.0	0.0	2.7	9.7	**<0.001**
CD9 (nMFI; %)	17.0	32.0	55.4	28.8	49.6	123.1	**<0.001**
SSEA-4 (nMFI; %)	0.0	21.5	83.1	100.3	193.0	361.6	**<0.001**
HLA-I (nMFI; %)	0.0	14.1	50.9	68.2	252.2	688.3	**<0.001**
CD63 (nMFI; %)	31.6	66.2	95.4	44.3	77.0	121.8	**0.001**
CD40 (nMFI; %)	3.8	12.8	39.7	0.0	3.7	9.8	**<0.001**
CD62P (nMFI; %)	38.5	76.7	144.0	1.4	3.7	7.5	**<0.001**
CD11c (nMFI; %)	0.8	6.8	37.1	0.0	2.3	8.1	**<0.001**
CD81 (nMFI; %)	153.4	208.9	253.9	58.1	150.8	217.3	**<0.001**
MCSP (nMFI; %)	1.5	6.4	23.4	0.0	1.6	8.2	**<0.001**
CD146 (nMFI; %)	0.0	2.1	10.9	0.0	2.6	8.1	0.629
CD41b (nMFI; %)	19.1	48.0	91.4	6.1	16.5	37.6	**<0.001**
CD42a (nMFI; %)	39.6	80.8	147.9	1.3	5.1	12.2	**<0.001**
CD24 (nMFI; %)	2.5	9.8	28.0	20.1	35.3	61.0	**<0.001**
CD86 (nMFI; %)	0.0	2.2	16.7	14.5	33.1	53.4	**<0.001**
CD44 (nMFI; %)	1.2	7.6	23.5	3.3	9.4	21.3	0.271
CD326 (nMFI; %)	0.0	0.0	10.7	0.0	0.5	10.3	0.337
CD133/1 (nMFI; %)	5.9	15.7	50.2	8.7	20.0	39.0	0.569
CD29 (nMFI; %)	6.3	17.4	43.0	0.0	2.7	6.7	**<0.001**
CD69 (nMFI; %)	3.7	12.1	36.1	2.4	5.5	13.4	**<0.001**
CD142 (nMFI; %)	2.6	9.4	36.4	3.9	9.8	20.4	0.227
CD45 (nMFI; %)	1.7	9.2	29.6	0.5	5.8	16.6	**0.003**
CD31 (nMFI; %)	7.1	16.7	45.8	8.4	23.1	38.5	0.417
CD20 (nMFI; %)	0.7	7.7	22.8	7.6	17.7	31.6	**<0.001**
CD14 (nMFI; %)	1.2	10.2	36.2	1.6	7.1	19.3	**<0.001**

Serum- and urine- extracellular vesicle (EV) surface antigens were characterized by flow cytometry. Median fluorescence intensity (MFI) was normalized by mean MFI of CD9, CD63 and CD81 and reported as median and interquartile range (nMFI; %). A $p<0.05$ was considered significant and shown in bold.

Table S4. EV Quantification in serum and urine at patient follow-up

	Variable	T0 [n=58*]	T1 [n=58]	T2 [n=58]	T3 [n=58]	Pairwise Comparison					
						T0 *vs* T1	**T0 *vs* T2**	**T0 *vs* T3**	**T1 *vs* T2**	**T1 *vs* T3**	**T2 *vs* T3**
Serum EVs	EV Diameter (nm)	180 [165; 202]	183 [168; 198]	188 [170; 203]	175 [151; 204]	0.428	0.086	0.425	0.260	0.194	**0.017**
	EV concentration (n/mL) [all vesicles]	2.8e12 [2.1e12; 5.7e12]	2.6e12 [1.3e12; 3.8e12]	2.0e12 [1.3e12; 3.0e12]	1.9e12 [0.9e12; 3.4e12]	**0.002**	**<0.001**	**<0.001**	0.191	0.152	0.876
	EV concentration (n/mL) [30-150nm]	1.3e12 [0.7e11; 2.8e12]	0.9e12 [0.5e12; 1.6e12]	0.7e12 [0.5e12; 1.2e12]	0.9e12 [0.5e12; 1.5e12]	**0.003**	**<0.001**	**0.007**	0.071	0.930	0.098
	EV concentration (n/mL) [151-500nm]	1.5e12 [1.2e12; 2.9e12]	1.3e12 [0.7e12; 2.0e12]	1.2e12 [0.7e12; 2.0e12]	1.0e12 [0.5e12; 1.8e12]	**0.002**	**0.002**	**<0.001**	0.474	**0.035**	0.087
	EV concentration (n/mL) [501-1000nm]	4.1e10 [2.0e10; 8.2e10]	3.0e10 [1.4e10; 6.5e10]	2.7e10 [1.2e10; 6.1e10]	1.2e10 [0.3e10; 3.9e10]	0.629	0.070	0.055	0.160	0.179	0.707
	CD9 (MFI; a.u.)	16.1 [6.2; 32.8]	10.2 [3.5; 18.0]	9.7 [3.8; 22.4]	10.4 [2.8; 31.9]	0.081	0.062	0.883	0.876	0.103	0.081
	CD63 (MFI; a.u.)	24.0 [12.0; 57.2]	17.2 [7.4; 38.0]	20.4 [10.1; 31.1]	17.4 [8.8; 43.1]	0.090	**0.017**	0.696	0.579	0.346	0.165
	CD81 (MFI; a.u.)	71.1 [36.5; 199.1]	61.5 [33.6; 125.9]	74.2 [33.2; 123.5]	65.2 [23.1; 213.7]	**0.014**	**0.045**	0.482	0.612	**0.042**	0.079
	Mean MFI for CD9-63-81	42.7 [19.6; 120.2]	29.8 [17.5; 70.8]	30.5 [16.1; 57.5]	33.0 [14.1; 89.8]	**0.009**	**0.011**	0.374	0.975	0.066	**0.046**
Urine EVs	EV Diameter (nm)	185 [173; 206]	187 [167; 205]	184 [168; 203]	180 [168; 196]	0.870	0.786	0.210	0.494	0.206	0.085
	EV concentration (n/mL) [all vesicles]	7.0e9 [3.7e9; 9.7e9]	5.9e9 [3.9e9; 13.1e9]	5.7e9 [2.9e9; 13.7e9]	5.2e9 [3.2e9; 10.4e9]	0.160	0.333	0.478	0.226	0.186	0.739
	EV concentration (n/mL) [30-150nm]	2.5e9 [1.1e9; 3.9e9]	2.1e9 [1.4e9; 4.8e9]	1.9e9 [1.0e9; 3.7e9]	2.1e9 [1.2e9; 3.6e9]	0.078	0.587	0.520	0.151	0.173	0.965
	EV concentration (n/mL) [151-500nm]	4.0e9 [2.1e9; 5.9e9]	3.5e9 [2.2e9; 7.9e9]	3.1e9 [1.7e9; 7.3e9]	3.3e9 [1.8e9; 5.0e9]	0.296	0.244	0.499	0.348	0.245	0.642
	EV concentration (n/mL) [501-1000nm]	1.9e7 [1.1e7; 7.8e7]	1.9e7 [0.5e7; 6.7e7]	1.1e7 [0.4e7; 7.8e7]	1.3e7 [0.4e7; 4.8e7]	**0.001**	**0.049**	0.912	0.598	0.165	0.125
	CD9 (MFI; a.u.)	6.7 [2.0; 11.9]	13.8 [7.1; 22.1]	8.1 [5.1; 15.1]	15.6 [6.0; 33.2]	0.218	0.116	**0.003**	0.562	0.836	0.664
	CD63 (MFI; a.u.)	10.8 [5.3; 23.0]	23.8 [12.5; 38.5]	11.4 [5.6; 23.5]	14.4 [5.7; 33.6]	0.480	0.403	0.679	**0.016**	0.490	0.139
	CD81 (MFI; a.u.)	47.5 [17.1; 70.7]	26.9 [11.4; 60.6]	23.5 [7.7; 52.0]	18.3 [5.9; 54.8]	0.992	**0.034**	0.501	**0.009**	0.471	0.108
	Mean MFI for CD9-63-81	25.0 [9.9; 38.3]	25.2 [15.8; 39.4]	17.2 [10.5; 28.0]	20.1 [10.4; 45.6]	0.465	0.338	0.540	**0.024**	0.627	0.118

Serum- and urine- extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81). Median and interquartile range are reported for each variable at the different time points: T0, (before transplant); T1 (10-14 days after transplant); T2 (3 months after transplant); T3 (12 months after transplant); *urine were not available for 38 anuric patients at T0. A $p<0.05$ was considered significant and shown in bold.

Table S5. Serum EV surface antigens at follow-up

Variable	**T0** [n=58]			**T1** [n=58]			**T2** [n=58]			**T3** [n=58]			**Pairwise Comparison**					
	25th	50th	75th	25th	50th	75th	25th	50th	75th	25th	50th	75th	**T0*vs*T1**	**T0*vs*T2**	**T0*vs*T3**	**T1*vs*T2**	**T1*vs*T3**	**T2*vs*T3**
CD3 (nMFI; %)	0.0	7.1	32.0	0.1	5.8	24.0	0.0	5.6	18.5	1.8	9.0	43.4	0.359	0.645	0.167	0.791	0.055	0.076
CD4 (nMFI; %)	0.9	11.5	48.1	1.7	8.6	39.2	0.4	6.6	21.1	1.6	7.9	45.7	0.324	0.318	0.851	0.853	0.240	0.166
CD19 (nMFI; %)	3.9	17.4	58.3	2.0	17.6	34.2	2.4	14.1	36.2	3.6	14.2	47.7	0.085	0.126	0.540	0.933	0.194	0.206
CD8 (nMFI; %)	17.3	41.7	63.3	8.7	29.6	45.1	12.5	28.3	46.7	18.8	40.8	76.2	0.056	0.824	0.921	0.202	**0.015**	0.726
HLA-II (nMFI; %)	22.5	68.4	118.7	17.2	44.1	89.5	25.8	61.1	91.7	33.5	76.7	125.5	0.076	0.378	0.988	0.340	**0.043**	0.278
CD56 (nMFI; %)	0.0	3.6	22.8	0.0	0.2	9.5	0.0	2.1	11.3	0.0	4.6	17.1	0.118	0.073	0.333	0.576	0.467	0.209
CD105 (nMFI; %)	0.0	0.0	8.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.159	0.112	0.076	0.839	0.336	0.346
CD2 (nMFI; %)	1.1	8.3	42.0	0.0	2.8	19.9	0.0	2.3	17.4	0.8	5.6	42.2	0.139	0.136	0.415	0.898	0.063	**0.042**
CD1c (nMFI; %)	2.2	14.0	56.5	3.3	9.2	38.2	0.8	8.1	34.7	3.1	12.4	53.7	0.332	0.444	0.890	0.929	0.337	0.348
CD25 (nMFI; %)	0.0	6.7	22.3	0.0	4.1	15.7	0.0	2.3	13.4	0.0	5.5	26.8	0.318	0.154	0.763	0.475	0.545	0.218
CD49e (nMFI; %)	2.0	6.9	47.4	1.4	8.6	38.4	0.7	3.9	22.7	3.0	11.0	39.9	0.193	**0.034**	0.408	0.140	0.487	**0.043**
ROR1 (nMFI; %)	2.1	8.0	50.6	1.2	6.8	24.1	1.1	8.1	26.4	3.2	10.4	38.1	0.382	0.555	0.585	0.980	0.709	0.786
CD209 (nMFI; %)	3.2	10.8	38.0	2.2	9.9	26.1	0.5	5.4	21.6	1.9	10.1	34.3	0.415	0.098	0.355	0.405	0.951	0.294
CD9 (nMFI; %)	17.0	39.3	77.0	17.1	32.5	50.6	17.7	27.3	45.7	16.1	32.9	56.6	**0.023**	0.053	0.109	0.793	0.554	0.421
SSEA-4 (nMFI; %)	0.0	16.3	79.9	0.0	11.4	77.0	0.0	20.2	56.6	0.0	30.5	115.7	0.391	0.517	0.244	0.905	0.053	**0.049**
HLA-I (nMFI; %)	0.0	13.2	54.2	0.0	13.9	40.7	0.0	7.5	41.5	0.0	25.6	70.1	0.402	0.644	0.389	0.851	0.106	0.115
CD63 (nMFI; %)	34.3	73.8	93.3	30.9	63.1	90.9	30.8	64.7	105.8	30.8	64.6	96.7	0.567	0.615	0.240	0.898	0.653	0.413
CD40 (nMFI; %)	2.5	13.7	42.1	3.8	11.3	44.2	3.5	13.7	37.8	6.5	15.1	41.7	0.379	0.547	0.522	0.283	0.873	0.294
CD62P (nMFI; %)	33.6	84.9	159.7	33.5	68.1	117.6	37.6	70.1	113.8	44.6	107.4	164.4	0.505	0.576	0.602	0.863	0.652	0.823
CD11c (nMFI; %)	1.0	6.6	43.1	2.2	7.9	41.2	0.6	4.5	28.6	0.0	7.2	39.2	0.570	0.158	0.941	0.078	0.627	0.146
CD81 (nMFI; %)	137.8	193.7	250.6	175.5	211.2	255.5	159.7	210.3	259.9	159.4	198.2	244.8	**0.021**	**0.000**	**0.021**	0.408	0.299	**0.018**
MCSP (nMFI; %)	2.2	5.4	30.7	1.2	6.8	24.1	0.4	6.3	19.3	1.5	7.5	22.8	0.471	0.484	0.396	0.892	0.917	0.951
CD146 (nMFI; %)	0.0	2.0	11.7	0.0	2.4	11.0	0.0	2.4	6.6	0.1	2.1	12.4	0.616	0.326	0.364	0.358	0.323	0.939
CD41b (nMFI; %)	22.3	58.1	96.6	14.9	52.1	99.0	17.1	36.9	82.4	20.0	51.2	96.1	0.376	0.121	0.621	0.588	0.686	0.302
CD42a (nMFI; %)	31.7	79.4	192.9	36.3	71.6	144.4	37.5	65.7	116.2	64.5	101.3	162.7	0.366	0.340	0.324	0.827	0.732	0.959
CD24 (nMFI; %)	3.3	11.0	39.7	1.1	9.7	23.8	4.1	9.4	24.8	2.4	9.1	32.6	0.530	0.822	0.470	0.826	0.917	0.729
CD86 (nMFI; %)	0.0	4.1	19.4	0.0	0.0	13.8	0.0	1.1	15.8	0.0	3.7	25.8	0.452	0.210	0.747	0.651	0.664	0.277
CD44 (nMFI; %)	1.3	10.1	36.1	0.9	6.1	19.7	0.5	6.3	15.9	1.8	7.4	34.3	0.152	**0.036**	0.267	0.431	0.766	0.171
CD326 (nMFI; %)	0.0	0.0	20.4	0.0	0.0	3.8	0.0	0.0	8.2	0.0	0.0	15.4	0.213	0.094	0.695	0.602	0.403	0.133
CD133/1 (nMFI; %)	6.2	22.8	69.2	5.8	13.8	50.7	6.1	16.6	35.9	3.8	16.5	48.0	0.116	0.229	0.178	0.805	0.695	0.963
CD29 (nMFI; %)	7.6	20.2	58.4	4.7	15.4	51.0	4.7	13.1	31.6	6.8	18.6	40.2	0.412	**0.029**	0.136	0.058	0.435	0.062
CD69 (nMFI; %)	4.7	19.1	55.4	3.1	8.6	31.6	4.2	12.1	28.3	2.9	13.0	34.5	0.068	0.140	**0.047**	0.808	0.993	0.788
CD142 (nMFI; %)	2.5	12.1	69.3	3.5	8.8	26.7	2.4	7.9	25.5	2.2	9.2	38.0	**0.021**	**0.007**	0.052	0.728	0.641	0.345
CD45 (nMFI; %)	2.8	10.4	43.2	1.0	9.0	29.2	1.5	8.9	21.6	0.7	7.1	31.6	0.191	0.335	0.659	0.965	0.200	0.194
CD31 (nMFI; %)	9.2	20.6	73.1	5.9	17.1	52.6	6.3	13.3	33.3	7.2	15.8	43.3	**0.015**	**0.016**	**0.017**	0.974	0.781	0.804
CD20 (nMFI; %)	0.5	9.8	28.7	0.5	5.2	16.9	0.4	6.5	24.5	2.1	9.3	28.8	0.109	0.862	0.407	0.370	0.384	0.704
CD14 (nMFI; %)	0.0	8.9	37.4	2.1	10.9	37.9	0.6	10.0	39.0	2.6	10.0	45.3	0.866	0.460	0.852	0.537	0.982	0.465

Serum extracellular vesicle (EV) surface antigens were characterized by flow cytometry at the different time points: T0, (before transplant); T1 (10-14 days after transplant); T2 (3 months after transplant); T3 (12 months after transplant). Median fluorescence intensity (MFI) was normalized by mean MFI of CD9, CD63 and CD81 and reported as median/interquartile range (nMFI; %). A $p<0.05$ was considered significant and shown in bold.

Table S6. Urine EV surface antigens at follow-up

Variable	**T0** [n=20]			**T1** [n=58]			**T2** [n=58]			**T3** [n=58]			**Pairwise Comparison**					
	25th	50th	75th	25th	50th	75th	25th	50th	75th	25th	50th	75th	**T0*vs*T1**	**T0*vs*T2**	**T0*vs*T3**	**T1*vs*T2**	**T1*vs*T3**	**T2*vs*T3**
CD3 (nMFI; %)	1.5	6.8	13.1	0.8	5.5	14.2	7.0	12.7	23.0	9.0	15.5	30.4	**0.028**	**<0.001**	**0.002**	0.216	0.134	0.689
CD4 (nMFI; %)	0.0	0.9	3.4	0.0	0.8	3.6	0.0	1.9	5.7	0.0	2.7	12.5	0.367	0.842	0.308	0.588	0.757	0.144
CD19 (nMFI; %)	1.4	2.2	4.9	0.3	2.5	5.8	2.7	6.5	12.2	4.2	11.6	18.9	0.340	**0.002**	**<0.001**	**0.010**	**<0.001**	0.069
CD8 (nMFI; %)	0.0	1.4	5.0	0.4	2.3	5.3	0.1	4.3	9.6	4.1	9.6	21.2	0.480	0.235	**0.007**	0.587	**0.005**	**0.006**
HLA-II (nMFI; %)	2.8	7.9	18.5	0.3	4.0	9.7	1.6	5.0	12.6	4.0	8.4	17.9	0.111	0.077	0.204	0.663	0.521	**0.040**
CD56 (nMFI; %)	0.0	0.0	11.8	3.5	21.9	46.4	13.1	45.7	136.7	23.9	61.5	214.1	**0.005**	**<0.001**	**<0.001**	**0.037**	**0.002**	0.189
CD105 (nMFI; %)	861.8	1318.6	1749.8	1210.7	2189.2	3636.4	1888.6	2887.7	5721.4	2022.3	4445.3	6295.6	**0.001**	**<0.001**	**<0.001**	**0.034**	**<0.001**	0.374
CD2 (nMFI; %)	1.8	6.8	11.1	2.4	8.7	25.4	9.1	26.5	42.4	13.3	25.7	47.9	**0.015**	**<0.001**	**<0.001**	**0.030**	**0.031**	0.962
CD1c (nMFI; %)	0.0	1.3	3.5	0.0	1.9	6.0	0.6	4.5	10.2	2.3	8.5	13.4	**0.013**	**0.002**	**<0.001**	0.175	**0.003**	0.087
CD25 (nMFI; %)	0.0	10.8	26.1	0.8	3.6	7.8	1.6	5.9	14.8	1.4	11.5	20.4	**0.039**	**0.049**	0.157	0.922	0.226	**0.036**
CD49e (nMFI; %)	0.0	3.1	6.8	0.0	2.3	9.8	0.3	4.0	11.0	1.3	9.8	21.9	0.741	0.782	0.168	0.847	0.136	**0.033**
ROR1 (nMFI; %)	0.6	7.4	13.9	0.7	3.6	10.8	1.3	5.5	12.7	3.5	11.2	24.8	0.861	0.760	0.241	0.663	0.153	**0.008**
CD209 (nMFI; %)	0.0	0.3	4.3	0.0	2.0	7.4	0.0	2.4	9.1	0.0	6.6	14.1	0.089	**0.023**	**0.001**	0.491	**0.026**	0.135
CD9 (nMFI; %)	14.5	30.3	46.6	30.2	48.4	122.4	24.4	44.9	138.5	33.7	77.7	140.6	**0.015**	**0.010**	**<0.001**	0.612	0.071	0.252
SSEA-4 (nMFI; %)	35.7	111.3	165.1	65.9	140.2	257.7	107.1	222.8	377.3	188.8	345.8	450.9	0.068	**0.002**	**<0.001**	**0.002**	**<0.001**	0.172
HLA-I (nMFI; %)	4.2	27.4	141.4	29.8	115.1	321.3	179.9	456.2	833.7	170.5	379.2	1118.0	0.079	**0.002**	**<0.001**	**<0.001**	**<0.001**	0.256
CD63 (nMFI; %)	24.5	49.4	126.1	56.7	97.6	131.3	41.4	65.8	96.0	49.1	77.8	135.9	0.340	0.967	0.491	**0.023**	0.306	0.293
CD40 (nMFI; %)	0.0	3.1	9.1	0.1	2.7	4.9	0.0	3.9	7.2	0.4	6.3	14.9	0.827	0.722	0.141	0.640	0.382	**0.044**
CD62P (nMFI; %)	1.6	3.4	8.7	0.6	2.9	5.9	1.5	3.6	6.8	1.3	4.7	12.6	0.826	0.355	**0.045**	0.338	0.055	0.389
CD11c (nMFI; %)	0.4	2.2	7.9	0.0	1.7	4.4	0.0	0.8	5.1	0.7	7.1	16.4	0.088	0.603	**0.003**	0.444	0.351	**0.010**
CD81 (nMFI; %)	119.6	213.9	246.4	85.4	140.8	206.6	39.6	159.7	221.3	34.7	106.5	206.3	**0.007**	**0.021**	**0.001**	0.756	0.079	0.078
MCSP (nMFI; %)	0.0	0.2	7.5	0.0	1.0	3.6	0.0	1.5	7.4	0.3	5.1	13.6	0.284	0.880	**0.032**	0.513	0.373	**0.037**
CD146 (nMFI; %)	0.0	0.3	1.8	0.1	2.6	7.6	0.0	2.9	9.0	0.0	4.3	12.8	**<0.001**	**0.002**	**<0.001**	0.698	0.756	0.323
CD41b (nMFI; %)	1.3	7.3	17.2	5.2	9.5	25.1	5.7	17.1	36.9	15.2	30.0	53.1	0.493	0.183	**0.028**	0.366	**0.002**	**0.004**
CD42a (nMFI; %)	0.2	2.5	6.2	1.0	3.6	7.6	1.0	5.1	10.6	2.9	9.9	14.9	0.723	0.388	0.103	0.524	**0.031**	0.192
CD24 (nMFI; %)	15.2	32.1	75.5	12.1	23.0	38.9	22.2	33.9	55.3	27.8	55.8	78.2	0.069	0.430	0.210	0.104	**<0.001**	**0.004**
CD86 (nMFI; %)	1.5	12.2	31.4	9.3	22.0	42.3	16.8	34.4	60.1	28.7	45.5	66.1	0.332	0.085	**0.006**	0.312	0.071	0.391
CD44 (nMFI; %)	0.7	5.2	16.6	2.8	7.5	14.4	2.7	9.4	19.0	7.0	16.7	31.2	**0.012**	**0.048**	**0.002**	0.989	0.100	**0.028**
CD326 (nMFI; %)	0.0	3.3	16.2	0.0	0.0	4.9	0.0	0.0	10.6	0.0	2.5	11.1	0.718	0.344	0.270	0.467	0.473	0.956

CD133/1 (nMFI; %)	0.0	5.3	9.9	7.3	14.7	24.7	12.3	23.6	38.0	13.1	33.5	57.7	**0.009**	**<0.001**	**<0.001**	0.704	0.051	**0.005**
CD29 (nMFI; %)	0.0	1.4	5.3	0.0	1.8	5.0	0.0	2.6	7.2	0.0	4.3	10.8	0.119	**0.042**	**0.016**	0.965	0.468	0.341
CD69 (nMFI; %)	1.5	5.1	10.3	1.5	3.8	7.0	2.2	5.4	13.9	4.1	10.8	15.8	0.629	**0.030**	**0.002**	0.163	**0.004**	0.503
CD142 (nMFI; %)	1.8	5.1	11.8	2.6	5.5	12.3	4.5	12.6	20.1	9.0	17.4	26.3	0.702	0.245	**0.029**	**0.030**	**<0.001**	**0.030**
CD45 (nMFI; %)	0.0	1.1	3.8	0.5	4.1	8.7	0.4	7.1	17.4	3.0	14.5	23.4	**0.001**	**<0.001**	**<0.001**	0.779	0.382	0.342
CD31 (nMFI; %)	45.2	72.3	143.5	4.7	12.0	26.7	7.6	22.3	33.5	11.9	30.0	43.5	**<0.001**	**<0.001**	**<0.001**	0.766	0.060	**0.028**
CD20 (nMFI; %)	3.5	28.7	48.9	5.9	10.7	24.3	7.6	16.0	23.8	15.6	25.7	35.3	0.099	0.053	0.440	0.869	0.124	**0.006**
CD14 (nMFI; %)	1.6	8.0	13.8	0.0	3.4	13.6	2.1	8.8	20.4	3.4	9.6	24.0	0.516	0.603	0.780	0.754	0.462	0.505

Urine extracellular vesicle (EV) surface antigens were characterized by flow cytometry at the different time points: T0, (before transplant); T1 (10-14 days after transplant); T2 (3 months after transplant); T3 (12 months after transplant). Median fluorescence intensity (MFI) was normalized by mean MFI of CD9, CD63 and CD81 and reported as median/interquartile range (nMFI; %). A $p<0.05$ was considered significant and shown in bold.

Table S7. Prediction of renal recovery: analysis of serum EVs at T0

Variable	Renal Recovery [n=35]			Persistent renal dysfunction [n=23]			*P*-Value
	25th	50th	75th	25th	50th	75th	
EV Diameter (nm)	158	177	194	170	180	208	0.328
EV conc (n/mL) [all vesicles]	1.9E+12	2.8E+12	6.5E+12	2.2E+12	3.2E+12	4.8E+12	0.937
EV conc (n/mL) [30-150nm]	6.7E+11	1.4E+12	2.9E+12	7.9E+11	9.4E+11	2.7E+12	0.573
EV conc (n/mL) [151-500nm]	1.2E+12	1.5E+12	2.6E+12	1.3E+12	1.6E+12	3.0E+12	0.639
EV conc (n/mL) [501-1000nm]	2.1E+10	3.9E+10	8.3E+10	1.8E+10	4.5E+10	8.1E+10	0.685
CD9 (MFI; a.u.)	7.7	16.3	31.7	5.7	14.1	34.8	0.805
CD63 (MFI; a.u.)	12.3	17.2	47.6	11.2	45.6	60.8	0.287
CD81 (MFI; a.u.)	25.9	56.1	292	48.8	75	174.6	0.551
Mean MFI for CD9-CD63-CD81	17.5	29.9	128	28.7	47.2	82.6	0.617
CD3 (nMFI; %)	0.0	5.6	27.8	0.0	8.7	40.5	0.454
CD4 (nMFI; %)	0.5	9.6	32.1	1.2	16.5	56.1	0.326
CD19 (nMFI; %)	3.5	12.9	47.1	6.5	18.5	69.6	0.224
CD8 (nMFI; %)	17.3	40.7	62.6	17.1	44.9	87.4	0.668
HLA-II (nMFI; %)	15.8	38.1	103.2	43.6	96.7	125.2	**0.048**
CD56 (nMFI; %)	0.0	2.7	14.4	0.0	4.5	36.7	0.451
CD105 (nMFI; %)	0.0	0.0	0.0	0.0	0.0	35.1	0.916
CD2 (nMFI; %)	0.8	4.3	33.9	3.4	16.3	56.9	0.072
CD1c (nMFI; %)	1.3	10.4	38.2	4.6	15.3	77.0	0.206
CD25 (nMFI; %)	0.0	6.7	20.8	0.0	6.3	49.8	0.718
CD49e (nMFI; %)	0.7	6.1	57.2	2.4	13.7	39.2	0.528
ROR1 (nMFI; %)	1.0	5.8	40.5	3.2	10.5	64.0	0.173
CD209 (nMFI; %)	3.2	9.4	29.7	2.7	18.7	51.9	0.855
CD9 (nMFI; %)	17.2	35.7	75.7	16.4	40.8	84.1	0.843
SSEA-4 (nMFI; %)	0.0	32.9	80.2	0.0	0.0	79.5	0.527
HLA-I (nMFI; %)	0.0	13.0	50.8	0.0	22.7	66.9	0.889
CD63 (nMFI; %)	29.6	68.4	88.6	36.1	75.7	109.9	0.283
CD40 (nMFI; %)	1.6	10.9	35.1	3.1	16.7	89.7	0.394
CD62P (nMFI; %)	19.5	44.6	74.1	127.4	260.6	463.1	**<0.001**
CD11c (nMFI; %)	1.0	5.9	23.4	0.9	8.5	69.5	0.368
CD81 (nMFI; %)	143.8	209.1	262.2	123.0	190.8	226.2	0.369
MCSP (nMFI; %)	0.6	4.4	26.8	4.1	9.8	67.4	0.163
CD146 (nMFI; %)	0.0	1.4	9.6	0.0	3.8	31.1	0.185
CD41b (nMFI; %)	13.6	37.0	82.5	49.1	82.0	142.8	**0.003**
CD42a (nMFI; %)	16.2	41.4	65.5	149.0	236.0	466.6	**<0.001**
CD24 (nMFI; %)	1.5	9.0	38.9	5.0	14.2	45.0	0.133
CD86 (nMFI; %)	0.0	4.4	18.9	0.0	0.0	25.2	0.409
CD44 (nMFI; %)	1.2	7.9	16.2	3.1	17.9	82.0	0.141
CD326 (nMFI; %)	0.0	0.0	20.0	0.0	0.0	36.8	0.979
CD133/1 (nMFI; %)	8.7	20.5	63.9	4.8	25.0	74.5	0.867
CD29 (nMFI; %)	4.6	12.6	50.7	11.9	34.1	75.8	**0.036**
CD69 (nMFI; %)	2.2	8.7	55.3	7.3	26.3	68.1	0.133
CD142 (nMFI; %)	2.2	9.4	44.8	5.0	22.7	78.8	0.129
CD45 (nMFI; %)	2.2	10.6	34.0	3.0	10.2	52.5	0.818
CD31 (nMFI; %)	3.6	14.9	37.1	22.4	72.6	169.3	**<0.001**
CD20 (nMFI; %)	2.0	10.3	26.0	0.0	9.8	46.1	0.755
CD14 (nMFI; %)	0.0	6.1	29.5	2.6	17.4	56.7	0.125

Serum extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable at T0 (before transplant) in patients with renal recovery *vs.* persistent renal dysfunction (Glomerular Filtration Rate, eGFR ≤ 45 mL/min at T3). A $p<0.05$ was considered significant and shown in bold.

Table S8. Prediction of renal recovery: analysis of serum EVs at T1

Variable	Renal Recovery [n=35]			Persistent renal dysfunction [n=23]			*P*-Value
	25th	50th	75th	25th	50th	75th	
EV Diameter (nm)	166	182	197	169	183	199	0.818
EV conc (n/mL) [all vesicles]	1.5E+12	2.6E+12	3.4E+12	1.2E+12	2.7E+12	4.1E+12	0.861
EV conc (n/mL) [30-150nm]	5.7E+11	8.2E+11	1.5E+12	5.0E+11	1.0E+12	1.7E+12	0.787
EV conc (n/mL) [151-500nm]	6.8E+11	1.3E+12	1.8E+12	7.3E+11	1.5E+12	2.6E+12	0.594
EV conc (n/mL) [501-1000nm]	1.2E+10	2.5E+10	8.4E+10	1.6E+10	3.0E+10	4.8E+10	0.987
CD9 (MFI; a.u.)	2.7	5.7	14.2	9.5	12.1	19.8	**0.044**
CD63 (MFI; a.u.)	6.3	14.5	22.2	12.6	19.6	44.7	0.177
CD81 (MFI; a.u.)	36.1	56.2	89.6	21.6	84.3	143.8	0.662
Mean MFI for CD9-CD63-CD81	16.6	25.5	39.2	17.8	42.5	74.5	0.324
CD3 (nMFI; %)	0.0	2.5	19.3	1.5	11.8	32.1	**0.034**
CD4 (nMFI; %)	1.3	3.3	27.6	3.2	23.8	43.5	0.080
CD19 (nMFI; %)	1.4	7.9	28.9	3.3	24.5	54.7	0.164
CD8 (nMFI; %)	6.1	23.6	43.7	12.6	29.9	56.6	0.287
HLA-II (nMFI; %)	13.9	32.3	72.4	21.7	68.8	133.7	**0.048**
CD56 (nMFI; %)	0.0	0.0	5.6	0.0	1.3	28.6	0.188
CD105 (nMFI; %)	0.0	0.0	0.0	0.0	0.0	84.8	0.051
CD2 (nMFI; %)	0.0	1.3	13.5	0.9	5.5	32.1	0.114
CD1c (nMFI; %)	2.0	7.5	35.8	4.3	18.2	50.5	0.230
CD25 (nMFI; %)	0.0	3.9	15.1	0.1	5.5	52.0	0.374
CD49e (nMFI; %)	0.8	5.2	24.5	4.3	17.8	46.7	0.084
ROR1 (nMFI; %)	0.3	6.2	16.4	1.5	14.3	58.8	0.122
CD209 (nMFI; %)	1.9	8.2	21.2	2.4	12.4	41.1	0.583
CD9 (nMFI; %)	12.3	28.6	49.5	25.4	34.3	64.4	0.114
SSEA-4 (nMFI; %)	0.0	3.3	64.8	0.0	29.9	95.0	0.451
HLA-I (nMFI; %)	0.0	6.2	32.4	2.6	19.0	50.3	0.149
CD63 (nMFI; %)	27.8	65.4	81.4	48.6	60.6	94.4	0.413
CD40 (nMFI; %)	3.5	10.7	18.2	5.5	43.2	64.7	**0.026**
CD62P (nMFI; %)	25.6	45.8	77.4	68.6	108.7	207.8	**<0.001**
CD11c (nMFI; %)	0.9	7.6	24.7	4.4	16.8	62.5	0.202
CD81 (nMFI; %)	180.5	221.5	264.1	148.5	209.5	221.1	0.071
MCSP (nMFI; %)	0.0	4.5	12.4	4.4	11.8	53.8	**0.012**
CD146 (nMFI; %)	0.0	1.2	9.8	0.0	5.0	16.9	0.173
CD41b (nMFI; %)	12.5	24.1	68.3	54.4	82.4	114.5	**0.005**
CD42a (nMFI; %)	20.3	44.3	82.5	82.1	137.3	259.1	**<0.001**
CD24 (nMFI; %)	0.4	5.3	15.1	4.9	19.9	63.2	**0.005**
CD86 (nMFI; %)	0.0	0.0	8.6	0.0	1.7	39.0	0.110
CD44 (nMFI; %)	0.0	2.7	11.2	4.2	12.3	50.2	**0.003**
CD326 (nMFI; %)	0.0	0.0	3.0	0.0	0.0	19.0	0.489
CD133/1 (nMFI; %)	4.0	11.4	26.8	9.4	18.4	69.2	0.061
CD29 (nMFI; %)	4.0	9.3	30.9	6.9	38.3	77.9	**0.037**
CD69 (nMFI; %)	1.5	6.3	20.8	4.2	24.9	46.7	0.079
CD142 (nMFI; %)	3.2	6.0	16.8	6.9	18.1	57.6	**0.010**
CD45 (nMFI; %)	1.0	8.0	24.9	2.8	19.2	38.9	0.425
CD31 (nMFI; %)	3.6	8.2	17.4	34.9	52.1	66.9	**<0.001**
CD20 (nMFI; %)	0.5	5.6	13.5	0.7	5.0	35.2	0.477
CD14 (nMFI; %)	2.7	10.5	36.2	2.0	11.9	58.8	0.968

Serum extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable at T1 (10-14 days after transplant) in patients with renal recovery *vs.* persistent renal dysfunction (Glomerular Filtration Rate, eGFR ≤ 45 mL/min at T3). A $p<0.05$ was considered significant and shown in bold.

Table S9. Prediction of renal recovery: analysis of serum EVs at T2

Variable	Renal Recovery [n=35]			Persistent renal dysfunction [n=23]			*P*-Value
	25th	50th	75th	25th	50th	75th	
EV Diameter (nm)	175	184	200	167	193	204	0.968
EV conc (n/mL) [all vesicles]	1.4E+12	2.1E+12	3.4E+12	1.2E+12	2.0E+12	2.7E+12	0.455
EV conc (n/mL) [30-150nm]	4.5E+11	7.3E+11	1.4E+12	4.7E+11	6.9E+11	1.1E+12	0.470
EV conc (n/mL) [151-500nm]	7.1E+11	1.4E+12	2.0E+12	6.4E+11	1.1E+12	2.0E+12	0.422
EV conc (n/mL) [501-1000nm]	1.7E+10	3.4E+10	7.4E+10	1.1E+10	2.2E+10	4.3E+10	0.179
CD9 (MFI; a.u.)	3.4	10.7	24.5	3.9	9.4	19.2	0.479
CD63 (MFI; a.u.)	12.7	22.9	38.3	7.6	13.2	23.6	0.068
CD81 (MFI; a.u.)	33.5	77	123.4	32	54.2	123.6	0.645
Mean MFI for CD9-CD63-CD81	21.5	30.6	71	15.1	30.3	49.7	0.404
CD3 (nMFI; %)	0.0	3.6	17.6	0.0	8.8	24.8	0.355
CD4 (nMFI; %)	0.4	7.0	19.4	0.9	6.0	35.0	0.720
CD19 (nMFI; %)	2.6	8.3	30.1	1.1	20.7	37.6	0.633
CD8 (nMFI; %)	8.7	27.3	44.2	20.6	36.7	81.8	0.139
HLA-II (nMFI; %)	21.1	49.5	82.9	38.4	74.9	121.5	0.155
CD56 (nMFI; %)	0.0	0.1	11.3	0.0	2.8	11.3	0.659
CD105 (nMFI; %)	0.0	0.0	4.6	0.0	0.0	0.0	0.479
CD2 (nMFI; %)	0.0	1.6	15.8	0.0	2.5	22.3	0.876
CD1c (nMFI; %)	0.9	8.9	29.2	0.5	7.3	40.6	0.650
CD25 (nMFI; %)	0.0	2.4	13.0	0.0	2.2	17.8	0.808
CD49e (nMFI; %)	1.4	6.2	24.2	0.0	2.0	22.2	0.275
ROR1 (nMFI; %)	0.9	9.4	34.2	1.3	6.9	20.1	0.811
CD209 (nMFI; %)	0.7	4.4	25.7	0.0	12.3	20.3	0.701
CD9 (nMFI; %)	15.2	25.5	44.8	18.5	28.2	47.4	0.408
SSEA-4 (nMFI; %)	0.0	26.1	50.5	0.0	16.4	85.3	0.865
HLA-I (nMFI; %)	0.0	6.1	44.4	0.0	8.9	40.5	0.897
CD63 (nMFI; %)	26.5	62.4	97.9	31.3	75.0	117.3	0.382
CD40 (nMFI; %)	3.3	11.8	37.5	7.9	20.5	44.7	0.290
CD62P (nMFI; %)	28.4	62.6	83.7	70.5	112.9	231.8	**<0.001**
CD11c (nMFI; %)	0.8	4.5	35.1	0.0	4.6	21.1	0.434
CD81 (nMFI; %)	150.2	209.3	260.6	161.0	211.3	244.2	0.924
MCSP (nMFI; %)	0.1	4.9	14.5	2.5	7.7	35.8	0.406
CD146 (nMFI; %)	1.5	3.9	9.5	0.0	0.0	4.0	**0.027**
CD41b (nMFI; %)	14.0	32.0	60.1	31.9	42.3	85.6	0.094
CD42a (nMFI; %)	22.1	51.7	88.6	51.7	115.9	175.2	**0.001**
CD24 (nMFI; %)	1.9	6.5	24.1	8.4	11.2	26.8	0.083
CD86 (nMFI; %)	0.0	0.4	19.1	0.0	1.7	15.1	0.816
CD44 (nMFI; %)	1.0	7.4	17.2	0.0	4.4	15.4	0.322
CD326 (nMFI; %)	0.0	0.0	8.8	0.0	0.0	5.0	0.709
CD133/1 (nMFI; %)	3.5	12.8	32.5	14.4	28.3	55.3	**0.011**
CD29 (nMFI; %)	4.7	11.8	31.0	4.6	14.3	33.1	0.975
CD69 (nMFI; %)	3.0	8.5	26.0	9.8	17.4	46.2	0.144
CD142 (nMFI; %)	1.6	6.8	25.0	4.1	15.1	32.0	0.408
CD45 (nMFI; %)	0.5	8.3	16.3	1.6	14.8	31.2	0.348
CD31 (nMFI; %)	5.0	8.9	18.1	6.9	24.3	43.0	**0.040**
CD20 (nMFI; %)	0.3	5.4	11.6	0.4	10.1	27.7	0.397
CD14 (nMFI; %)	0.2	6.8	30.3	5.5	18.2	57.3	0.099

Serum extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable at T2 (3 months after transplant) in patients with renal recovery *vs.* persistent renal dysfunction (Glomerular Filtration Rate, eGFR ≤ 45 mL/min at T3). A $p<0.05$ was considered significant and shown in bold.

Table S10. Prediction of renal recovery: analysis of serum EVs at T3

Variable	Renal Recovery [n=35]			Persistent renal dysfunction [n=23]			*P*-Value
	25th	50th	75th	25th	50th	75th	
EV Diameter (nm)	152	175	206	140	171	188	0.309
EV conc (n/mL) [all vesicles]	9.8E+11	1.8E+12	3.4E+12	7.9E+11	1.9E+12	3.5E+12	0.818
EV conc (n/mL) [30-150nm]	4.9E+11	7.4E+11	1.5E+12	4.1E+11	1.1E+12	1.6E+12	0.431
EV conc (n/mL) [151-500nm]	5.2E+11	1.0E+12	1.8E+12	4.5E+11	1.0E+12	1.7E+12	0.745
EV conc (n/mL) [501-1000nm]	3.2E+09	1.3E+10	4.6E+10	3.1E+09	1.1E+10	3.0E+10	0.465
CD9 (MFI; a.u.)	5.5	13.3	31.6	2.5	5.3	39	0.159
CD63 (MFI; a.u.)	9.6	21.2	40.9	6.2	16.9	49.8	0.691
CD81 (MFI; a.u.)	25.9	66.2	198.9	19.6	50.8	251.9	0.805
Mean MFI for CD9-CD63-CD81	18.3	39.8	78.8	10.4	26.3	103.8	0.763
CD3 (nMFI; %)	1.6	7.0	55.7	2.5	11.2	24.8	0.817
CD4 (nMFI; %)	0.6	6.9	66.8	2.4	9.2	39.3	0.987
CD19 (nMFI; %)	3.1	18.8	63.7	4.1	13.8	41.0	0.520
CD8 (nMFI; %)	11.1	32.6	76.1	25.3	44.6	76.5	0.520
HLA-II (nMFI; %)	31.9	75.7	120.3	35.9	85.5	134.8	0.328
CD56 (nMFI; %)	0.0	4.9	11.5	0.0	0.6	24.5	0.744
CD105 (nMFI; %)	0.0	0.0	0.0	0.0	0.0	0.0	0.479
CD2 (nMFI; %)	0.3	5.1	39.0	2.5	7.7	43.0	0.661
CD1c (nMFI; %)	1.5	11.8	71.0	3.3	14.3	50.1	0.874
CD25 (nMFI; %)	0.0	7.9	20.1	0.0	2.4	34.0	0.717
CD49e (nMFI; %)	4.2	10.6	27.5	1.9	17.1	56.0	0.968
ROR1 (nMFI; %)	3.3	10.6	38.1	1.7	5.5	41.1	0.757
CD209 (nMFI; %)	1.8	9.9	34.2	3.9	12.6	40.5	0.556
CD9 (nMFI; %)	16.7	33.6	57.0	14.5	29.9	51.1	0.441
SSEA-4 (nMFI; %)	0.0	42.0	108.5	0.0	28.7	149.7	0.866
HLA-I (nMFI; %)	0.0	27.9	68.7	0.0	14.3	91.1	0.796
CD63 (nMFI; %)	31.1	63.8	99.8	20.7	64.9	95.7	0.968
CD40 (nMFI; %)	8.0	14.6	33.3	4.2	15.6	49.7	0.981
CD62P (nMFI; %)	35.3	82.8	157.6	87.1	110.8	175.3	0.137
CD11c (nMFI; %)	0.0	5.4	59.1	1.9	10.2	35.1	0.625
CD81 (nMFI; %)	158.9	204.9	251.5	162.5	197.7	228.2	0.994
MCSP (nMFI; %)	1.0	8.5	32.8	2.6	6.0	22.2	0.994
CD146 (nMFI; %)	0.0	2.2	11.9	0.4	1.9	14.7	0.538
CD41b (nMFI; %)	14.3	47.2	83.4	27.2	62.6	128.7	0.159
CD42a (nMFI; %)	58.0	92.7	154.3	77.7	104.5	172.0	0.249
CD24 (nMFI; %)	2.4	7.7	32.6	1.5	10.9	32.8	0.744
CD86 (nMFI; %)	0.5	6.9	26.9	0.0	2.1	13.0	0.153
CD44 (nMFI; %)	3.2	7.6	35.1	0.0	6.0	32.9	0.339
CD326 (nMFI; %)	0.0	2.1	15.8	0.0	0.0	8.5	0.292
CD133/1 (nMFI; %)	3.8	15.4	54.2	3.8	21.4	46.3	0.994
CD29 (nMFI; %)	6.6	16.1	45.6	8.2	20.5	38.6	0.628
CD69 (nMFI; %)	2.2	9.7	38.7	3.2	16.5	33.2	0.769
CD142 (nMFI; %)	2.4	10.1	38.7	1.7	9.0	37.8	0.605
CD45 (nMFI; %)	0.0	4.6	32.3	4.2	9.9	31.4	0.262
CD31 (nMFI; %)	7.2	12.2	40.6	6.0	22.3	53.8	0.445
CD20 (nMFI; %)	2.4	7.3	21.1	1.8	11.8	39.1	0.622
CD14 (nMFI; %)	2.2	13.1	69.3	2.7	6.5	32.1	0.422

Serum extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable at T3 (12 months after transplant) in patients with renal recovery *vs.* persistent renal dysfunction (Glomerular Filtration Rate, eGFR ≤ 45 mL/min at T3). A $p<0.05$ was considered significant and shown in bold.

Table S11. Prediction of renal recovery: univariate logistic regression analysis

Logistic Regression (ref. Renal Recovery) [n=58]		**OR (95% CI)**	***P*-Value**
Serum EVs at T0	HLA-II (nMFI; %)	0.993 (0.986-1.001)	0.079
	CD62P (nMFI; %)	0.944 (0.910-0.980)	**0.003**
	CD41b (nMFI; %)	0.983 (0.971-0.995)	**0.006**
	CD42a (nMFI; %)	0.835 (0.683-0.991)	**0.019**
	CD29 (nMFI; %)	0.985 (0.970-1.001)	0.069
	CD31 (nMFI; %)	0.973 (0.956-0.991)	**0.003**
Urine EVs at T1	CD19 (nMFI; %)	1.154 (0.996-1.339)	0.057
	CD56 (nMFI; %)	1.013 (0.999-1.028)	0.074
	CD105 (nMFI; %)	1.011 (1.002-1.023)	**0.001**
	CD2 (nMFI; %)	1.027 (0.996-1.058)	0.084
	CD1c (nMFI; %)	1.148 (1.039-1.198)	**0.018**
	SSEA-4 (nMFI; %)	1.018 (1.008-1.028)	**<0.001**
	HLA-I (nMFI; %)	1.002 (0.999-1.003)	0.112
	CD42a (nMFI; %)	1.113 (0.999-1.241)	0.053
	CD133/1 (nMFI; %)	1.074 (1.015-1.136)	**0.013**
	CD45 (nMFI; %)	1.082 (0.978-1.197)	0.128
	CD20 (nMFI; %)	1.012 (0.986-1.038)	0.371

Univariate logistic regression analysis was performed to assess the association between each serum- or urine- extracellular vesicle (EV) surface antigen (expressed as normalized median fluorescence intensity, nMFI, %) and renal outcome after kidney transplant. nMFI levels were considered at T0 (before transplant) for serum EV surface antigens, and at T1 (10-14 days after transplant) for urine EV surface antigens. Odds ratios (ORs) are reported for each EV antigen together with its 95% confidence interval; an OR greater than 1 is associated with an increased likelihood of renal recovery; an OR less than 1 is associated with a decreased likelihood. A $p<0.05$ was considered significant and shown in bold.

Table S12. Prediction of renal recovery: analysis of urine EVs at T0

Variable	Renal Recovery [n=12]			Persistent renal dysfunction [n=8]			*P*-Value
	25th	50th	75th	25th	50th	75th	
EV Diameter (nm)	169	194	206	173	175	199	0.536
EV conc (n/mL) [all vesicles]	3.2E+09	8.5E+09	1.2E+10	3.7E+09	5.1E+09	8.5E+09	0.589
EV conc (n/mL) [30-150nm]	1.0E+09	3.2E+09	5.4E+09	1.1E+09	2.1E+09	3.3E+09	0.589
EV conc (n/mL) [151-500nm]	1.9E+09	5.0E+09	5.9E+09	2.1E+09	3.2E+09	5.8E+09	0.817
EV conc (n/mL) [501-1000nm]	1.1E+07	2.8E+07	2.5E+08	7.9E+06	1.5E+07	4.7E+07	0.487
CD9 (MFI; a.u.)	0.7	7.8	11.9	3.1	6.1	22.3	0.643
CD63 (MFI; a.u.)	2.2	10.8	41.3	7.2	10.5	20.1	0.939
CD81 (MFI; a.u.)	15.8	45.5	70.7	22.8	48.0	70.4	0.643
Mean MFI for CD9-CD63-CD81	9.7	26.0	38.2	11.7	24.7	38.4	0.939
CD3 (nMFI; %)	1.0	6.6	10.6	1.5	10.0	19.4	0.418
CD4 (nMFI; %)	0.0	0.9	3.3	0.0	1.1	13.5	0.813
CD19 (nMFI; %)	1.9	2.5	4.2	0.7	1.6	5.6	0.512
CD8 (nMFI; %)	0.0	0.3	4.9	0.3	1.9	10.7	0.386
HLA-II (nMFI; %)	3.0	9.5	18.5	0.6	5.0	33.9	0.316
CD56 (nMFI; %)	0.0	0.0	5.7	0.0	13.5	30.6	0.164
CD105 (nMFI; %)	912.9	1361.2	1825.5	834.2	1267.4	1613.5	0.643
CD2 (nMFI; %)	1.5	3.4	8.2	3.6	10.6	18.4	0.064
CD1c (nMFI; %)	0.3	1.7	3.5	0.0	0.4	4.5	0.348
CD25 (nMFI; %)	0.6	8.8	66.4	0.0	10.8	20.9	0.697
CD49e (nMFI; %)	0.0	4.2	6.8	0.2	1.1	23.5	0.938
ROR1 (nMFI; %)	0.0	5.7	13.6	2.7	8.1	14.9	0.586
CD209 (nMFI; %)	0.0	0.0	1.4	0.0	4.1	6.4	0.201
CD9 (nMFI; %)	6.9	27.1	36.7	17.6	36.0	70.6	0.190
SSEA-4 (nMFI; %)	31.5	103.4	165.1	85.8	124.4	184.5	0.537
HLA-I (nMFI; %)	3.1	12.5	40.9	11.3	124.6	280.0	0.082
CD63 (nMFI; %)	19.9	53.7	176.9	26.5	47.0	117.5	0.939
CD40 (nMFI; %)	0.1	3.4	12.3	0.0	2.8	3.7	0.458
CD62P (nMFI; %)	2.5	3.5	10.5	0.4	2.9	7.7	0.280
CD11c (nMFI; %)	0.1	1.7	8.0	1.3	4.8	7.4	0.440
CD81 (nMFI; %)	103.8	211.3	258.7	129.2	214.9	235.8	0.939
MCSP (nMFI; %)	0.0	1.7	7.5	0.0	0.1	20.7	0.968
CD146 (nMFI; %)	0.0	0.3	1.8	0.0	0.3	1.7	0.875
CD41b (nMFI; %)	1.3	5.7	15.3	1.3	7.8	22.9	0.699
CD42a (nMFI; %)	0.2	2.5	7.2	0.5	3.7	6.0	0.876
CD24 (nMFI; %)	14.0	36.9	75.7	18.2	32.1	75.5	0.877
CD86 (nMFI; %)	1.5	5.4	17.6	3.1	28.7	54.1	0.121
CD44 (nMFI; %)	0.7	5.0	16.6	0.7	5.2	18.2	0.908
CD326 (nMFI; %)	0.0	0.3	13.1	2.9	7.9	88.8	0.059
CD133/1 (nMFI; %)	0.0	6.1	11.4	0.0	4.1	8.4	0.344
CD29 (nMFI; %)	0.0	1.0	3.7	0.4	3.9	7.3	0.214
CD69 (nMFI; %)	0.3	5.4	10.0	2.8	5.1	12.2	0.373
CD142 (nMFI; %)	1.6	3.7	10.2	3.4	5.3	12.5	0.316
CD45 (nMFI; %)	0.0	1.8	3.8	0.0	0.3	10.0	0.524
CD31 (nMFI; %)	45.9	94.2	143.5	43.2	64.7	138.0	0.487
CD20 (nMFI; %)	1.2	37.3	48.9	8.5	25.1	55.3	1.000
CD14 (nMFI; %)	2.3	6.0	11.9	0.2	10.0	28.9	0.699

Urine extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable at T0 (before transplant) in patients with renal recovery *vs.* persistent renal dysfunction (Glomerular Filtration Rate, eGFR ≤ 45 mL/min at T3). A $p<0.05$ was considered significant and shown in bold.

Table S13. Prediction of renal recovery: analysis of urine EVs at T1

Variable	Renal Recovery [n=35]			Persistent renal dysfunction [n=23]			*P*-Value
	25th	50th	75th	25th	50th	75th	
EV Diameter (nm)	162	186	204	169	188	208	0.583
EV conc (n/mL) [all vesicles]	4.2E+09	7.3E+09	1.7E+10	3.7E+09	5.4E+09	1.1E+10	0.162
EV conc (n/mL) [30-150nm]	1.5E+09	2.1E+09	6.6E+09	1.4E+09	2.1E+09	4.4E+09	0.361
EV conc (n/mL) [151-500nm]	2.4E+09	4.1E+09	9.1E+09	1.8E+09	2.6E+09	6.2E+09	0.108
EV conc (n/mL) [501-1000nm]	5.7E+06	2.0E+07	6.4E+07	3.6E+06	1.8E+07	7.7E+07	0.633
CD9 (MFI; a.u.)	6.5	14.7	22.7	7.5	12.6	17.7	0.662
CD63 (MFI; a.u.)	14.3	23.5	40.8	10.8	23.9	37.7	0.763
CD81 (MFI; a.u.)	11.5	27.7	60.6	10.0	26.0	60.6	0.968
Mean MFI for CD9-CD63-CD81	18.0	26.6	40.1	14.0	24.1	36.9	0.656
CD3 (nMFI; %)	1.8	7.3	18.7	0.6	8.8	17.8	0.132
CD4 (nMFI; %)	0.0	1.7	6.9	0.0	0.6	2.1	0.363
CD19 (nMFI; %)	0.9	4.1	7.9	0.0	1.1	3.1	**0.006**
CD8 (nMFI; %)	0.2	2.3	9.4	0.4	2.0	4.7	0.582
HLA-II (nMFI; %)	0.0	3.6	10.2	2.6	4.8	9.5	0.428
CD56 (nMFI; %)	13.8	34.0	81.2	0.0	6.5	34.3	**0.003**
CD105 (nMFI; %)	1780.9	3060.6	5061.8	773.8	1397.8	1685.2	**<0.001**
CD2 (nMFI; %)	5.8	14.6	34.8	0.7	5.6	15.9	**0.008**
CD1c (nMFI; %)	0.4	4.5	9.1	0.0	1.6	2.9	**0.016**
CD25 (nMFI; %)	0.5	3.9	7.9	0.9	3.4	5.1	0.472
CD49e (nMFI; %)	0.0	3.5	10.5	0.0	1.7	3.8	0.277
ROR1 (nMFI; %)	0.7	4.6	16.1	0.5	1.7	6.7	0.323
CD209 (nMFI; %)	0.0	2.0	9.3	0.4	1.7	3.6	0.987
CD9 (nMFI; %)	28.4	59.9	157.9	22.9	33.7	153.4	0.084
SSEA-4 (nMFI; %)	137.8	216.9	305.2	34.7	75.2	120.7	**<0.001**
HLA-I (nMFI; %)	61.7	170.2	371.4	2.4	76.1	260.1	**0.030**
CD63 (nMFI; %)	58.7	94.4	131.3	51.6	112.3	119.8	0.943
CD40 (nMFI; %)	0.0	2.0	5.8	0.7	3.4	4.7	0.428
CD62P (nMFI; %)	0.9	3.7	8.0	0.6	2.1	5.0	0.192
CD11c (nMFI; %)	0.4	2.7	6.9	0.0	0.9	3.3	0.064
CD81 (nMFI; %)	40.4	132.8	210.5	101.1	160.4	205.3	0.195
MCSP (nMFI; %)	0.0	1.4	7.9	0.0	0.9	2.7	0.468
CD146 (nMFI; %)	0.2	4.5	11.1	0.1	1.4	4.0	0.125
CD41b (nMFI; %)	6.3	12.6	29.6	4.9	8.1	18.6	0.200
CD42a (nMFI; %)	2.2	4.1	13.2	0.2	2.4	4.8	**0.036**
CD24 (nMFI; %)	13.0	26.6	44.3	11.3	19.8	35.8	0.125
CD86 (nMFI; %)	12.9	27.9	51.8	3.8	14.5	39.4	0.066
CD44 (nMFI; %)	4.7	10.2	18.3	1.7	6.5	8.6	0.064
CD326 (nMFI; %)	0.0	0.0	2.9	0.0	1.5	11.6	0.360
CD133/1 (nMFI; %)	10.6	22.0	48.2	7.2	10.2	16.1	**0.003**
CD29 (nMFI; %)	0.0	3.1	5.3	0.0	1.5	2.8	0.254
CD69 (nMFI; %)	2.5	4.5	11.0	1.1	3.3	5.5	0.335
CD142 (nMFI; %)	3.1	5.9	14.2	2.4	4.5	8.8	0.201
CD45 (nMFI; %)	2.4	6.0	11.2	0.2	2.5	4.2	**0.033**
CD31 (nMFI; %)	4.9	12.0	29.2	4.4	12.0	22.1	0.892
CD20 (nMFI; %)	7.2	13.5	26.9	3.8	6.6	12.5	**0.043**
CD14 (nMFI; %)	0.0	6.6	20.9	0.0	3.2	4.7	0.088

Urine extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable at T1 (10-14 days after transplant) in patients with renal recovery *vs.* persistent renal dysfunction (Glomerular Filtration Rate, eGFR ≤ 45 mL/min at T3). A $p<0.05$ was considered significant and shown in bold.

Table S14. Prediction of renal recovery: analysis of urine EVs at T2

Variable	Renal Recovery [n=35]			Persistent renal dysfunction [n=23]			*P*-Value
	25th	50th	75th	25th	50th	75th	
EV Diameter (nm)	168	184	203	168	182	217	0.994
EV conc (n/mL) [all vesicles]	3.7E+09	6.1E+09	1.7E+10	2.7E+09	3.6E+09	7.0E+09	0.087
EV conc (n/mL) [30-150nm]	1.1E+09	2.5E+09	6.7E+09	6.9E+08	1.4E+09	2.5E+09	0.174
EV conc (n/mL) [151-500nm]	2.1E+09	3.9E+09	9.3E+09	1.4E+09	2.4E+09	4.6E+09	0.064
EV conc (n/mL) [501-1000nm]	2.3E+06	9.0E+06	7.6E+07	4.3E+06	1.1E+07	1.0E+08	0.956
CD9 (MFI; a.u.)	5.2	8.4	25.0	2.8	7.6	10.2	0.283
CD63 (MFI; a.u.)	5.3	10.4	17.9	7.3	11.5	28.9	0.324
CD81 (MFI; a.u.)	7.7	23.9	56.9	7.7	21.0	51.0	0.775
Mean MFI for CD9-CD63-CD81	13.2	18.8	28.6	8.5	16.8	25.4	0.224
CD3 (nMFI; %)	7.8	13.7	28.0	4.8	10.9	17.0	0.094
CD4 (nMFI; %)	0.0	2.1	7.4	0.0	1.3	3.5	0.552
CD19 (nMFI; %)	3.8	7.4	13.9	0.8	3.9	9.0	0.079
CD8 (nMFI; %)	0.0	5.2	10.3	1.1	2.9	7.5	0.936
HLA-II (nMFI; %)	1.3	5.8	15.2	1.8	4.5	11.5	0.656
CD56 (nMFI; %)	17.8	93.1	208.1	10.5	22.0	50.0	**0.007**
CD105 (nMFI; %)	2671.9	4856.3	6713.6	782.0	1631.6	2763.5	**<0.001**
CD2 (nMFI; %)	12.4	30.1	43.0	2.9	9.6	37.7	**0.027**
CD1c (nMFI; %)	0.7	6.3	12.7	0.2	2.4	5.7	0.108
CD25 (nMFI; %)	2.4	7.2	15.3	0.7	3.9	11.0	0.111
CD49e (nMFI; %)	0.0	4.8	16.3	1.9	3.9	9.3	0.905
ROR1 (nMFI; %)	2.4	5.6	13.3	0.3	4.9	11.5	0.385
CD209 (nMFI; %)	0.0	3.5	9.0	0.0	1.8	9.7	0.827
CD9 (nMFI; %)	23.5	63.9	179.2	12.3	41.8	166.2	0.081
SSEA-4 (nMFI; %)	222.0	365.7	576.9	52.0	100.7	158.0	**<0.001**
HLA-I (nMFI; %)	267.2	582.4	992.7	71.6	235.4	505.5	**0.001**
CD63 (nMFI; %)	40.8	64.4	89.1	41.6	73.6	142.2	0.509
CD40 (nMFI; %)	0.0	4.3	9.6	2.6	3.6	6.4	0.772
CD62P (nMFI; %)	2.3	4.4	7.7	1.3	2.5	5.4	0.111
CD11c (nMFI; %)	0.0	1.0	5.2	0.0	0.4	5.1	0.825
CD81 (nMFI; %)	28.1	120.6	217.4	72.2	163.9	227.3	0.230
MCSP (nMFI; %)	0.0	0.7	7.8	0.0	2.1	7.3	0.400
CD146 (nMFI; %)	0.0	3.4	8.0	0.0	1.5	9.6	0.767
CD41b (nMFI; %)	8.9	26.0	39.4	3.4	9.0	28.0	0.083
CD42a (nMFI; %)	2.0	6.2	12.5	0.7	2.4	8.6	0.163
CD24 (nMFI; %)	26.4	38.7	49.1	11.2	22.9	66.4	0.146
CD86 (nMFI; %)	25.7	47.6	76.1	14.3	22.1	35.1	**0.011**
CD44 (nMFI; %)	1.7	9.6	20.0	2.8	8.3	18.2	0.905
CD326 (nMFI; %)	0.0	0.0	7.8	0.0	0.0	13.0	0.812
CD133/1 (nMFI; %)	23.0	33.2	65.6	5.8	11.6	19.8	**<0.001**
CD29 (nMFI; %)	0.0	3.7	7.5	0.0	1.6	4.6	0.556
CD69 (nMFI; %)	2.5	7.1	15.4	0.0	3.2	9.1	0.115
CD142 (nMFI; %)	6.6	12.9	20.3	2.9	10.6	18.2	0.294
CD45 (nMFI; %)	1.9	11.7	20.3	0.0	3.9	12.2	0.105
CD31 (nMFI; %)	11.4	21.3	26.6	6.6	26.0	39.5	0.256
CD20 (nMFI; %)	9.0	16.7	24.0	5.1	10.7	22.4	0.123
CD14 (nMFI; %)	4.8	14.8	22.4	0.0	4.0	12.8	**0.014**

Urine extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable at T2 (3 months after transplant) in patients with renal recovery *vs.* persistent renal dysfunction (Glomerular Filtration Rate, eGFR ≤ 45 mL/min at T3). A $p<0.05$ was considered significant and shown in bold.

Table S15. Prediction of renal recovery: analysis of urine EVs at T3

Variable	Renal Recovery [n=35]			Persistent renal dysfunction [n=23]			*P*-Value
	25th	50th	75th	25th	50th	75th	
EV Diameter (nm)	166	180	189	168	180	202	0.298
EV conc (n/mL) [all vesicles]	3.1E+09	5.2E+09	1.1E+10	3.6E+09	5.6E+09	1.0E+10	0.918
EV conc (n/mL) [30-150nm]	1.2E+09	2.0E+09	3.9E+09	1.2E+09	2.1E+09	3.3E+09	0.685
EV conc (n/mL) [151-500nm]	1.8E+09	3.3E+09	4.9E+09	2.3E+09	3.0E+09	6.5E+09	0.981
EV conc (n/mL) [501-1000nm]	3.0E+06	1.3E+07	2.5E+07	6.3E+06	1.4E+07	6.6E+07	0.441
CD9 (MFI; a.u.)	11.8	18.4	39.1	3.5	6.2	19.5	**0.003**
CD63 (MFI; a.u.)	8.5	17.4	51.5	4.4	9.7	22.1	**0.017**
CD81 (MFI; a.u.)	6.7	17.6	59.2	3.4	22.3	49.4	0.556
Mean MFI for CD9-CD63-CD81	11.7	25.7	53.4	6.7	16.0	35.8	**0.042**
CD3 (nMFI; %)	12.6	18.2	31.4	3.0	10.0	27.7	**0.023**
CD4 (nMFI; %)	0.0	2.8	12.6	0.0	2.2	10.0	0.530
CD19 (nMFI; %)	6.0	12.2	22.6	1.7	6.1	17.1	0.084
CD8 (nMFI; %)	7.3	12.6	24.9	0.0	4.7	15.3	**0.001**
HLA-II (nMFI; %)	3.3	12.9	18.9	4.4	7.7	17.3	0.691
CD56 (nMFI; %)	42.6	110.9	309.2	16.2	29.7	73.8	**<0.001**
CD105 (nMFI; %)	4255.2	5337.9	7708.5	1338.8	1846.9	3337.9	**<0.001**
CD2 (nMFI; %)	21.3	30.5	60.6	4.6	13.6	35.8	**0.003**
CD1c (nMFI; %)	3.9	9.8	17.6	1.4	4.2	10.5	**0.038**
CD25 (nMFI; %)	1.5	13.6	21.3	1.1	4.4	18.5	0.247
CD49e (nMFI; %)	4.1	14.9	25.9	0.5	4.5	14.3	0.066
ROR1 (nMFI; %)	3.8	9.0	19.6	1.7	16.6	36.0	0.206
CD209 (nMFI; %)	1.2	8.9	15.4	0.0	2.3	10.8	0.191
CD9 (nMFI; %)	58.1	113.6	152.1	29.2	87.7	162.9	0.094
SSEA-4 (nMFI; %)	283.0	370.5	645.9	107.0	175.3	383.6	**<0.001**
HLA-I (nMFI; %)	279.1	674.3	1264.0	59.3	195.3	589.1	**0.002**
CD63 (nMFI; %)	54.8	85.0	136.4	36.5	65.4	104.8	0.249
CD40 (nMFI; %)	0.0	7.4	15.3	0.4	4.7	12.7	0.570
CD62P (nMFI; %)	1.5	5.3	13.6	1.1	4.5	10.7	0.673
CD11c (nMFI; %)	2.3	11.4	17.7	0.0	2.8	8.9	**0.030**
CD81 (nMFI; %)	27.8	129.4	199.0	74.8	168.2	215.8	0.112
MCSP (nMFI; %)	0.5	6.9	13.9	0.0	1.1	11.5	0.132
CD146 (nMFI; %)	0.0	7.0	19.2	0.0	1.5	6.2	0.121
CD41b (nMFI; %)	17.2	41.9	65.2	11.0	20.3	38.0	**0.045**
CD42a (nMFI; %)	3.0	11.9	15.7	1.1	8.5	14.6	0.567
CD24 (nMFI; %)	51.2	60.9	85.9	20.5	34.9	68.7	**0.004**
CD86 (nMFI; %)	35.9	49.9	75.3	18.6	35.7	52.3	**0.009**
CD44 (nMFI; %)	8.9	22.3	32.1	4.8	11.5	28.3	0.087
CD326 (nMFI; %)	0.0	2.6	17.3	0.0	2.5	7.6	0.747
CD133/1 (nMFI; %)	22.7	44.2	68.5	9.9	17.5	37.5	**0.003**
CD29 (nMFI; %)	0.0	4.3	12.4	0.0	4.3	8.8	0.583
CD69 (nMFI; %)	6.7	13.9	16.5	1.8	5.8	9.5	**0.003**
CD142 (nMFI; %)	11.9	18.3	29.3	4.7	9.6	23.9	0.060
CD45 (nMFI; %)	3.2	16.5	26.1	2.3	10.3	21.1	0.524
CD31 (nMFI; %)	12.4	29.8	36.6	10.2	35.0	46.8	0.305
CD20 (nMFI; %)	20.5	31.3	38.2	11.4	20.3	28.5	**0.005**
CD14 (nMFI; %)	5.5	17.3	26.7	2.5	8.2	16.0	0.129

Urine extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable at T3 (12 months after transplant) in patients with renal recovery *vs.* persistent renal dysfunction (Glomerular Filtration Rate, eGFR ≤ 45 mL/min at T3). A $p<0.05$ was considered significant and shown in bold.

Table S16. Correlation between EV surface antigens and clinical parameters

	EV antigens	Creatinine (mg/dL)	eGFR (mL/min)	PU (mg/dL)
Serum EVs	CD62P (nMFI; %)	0.110 *0.093*	**-0.144** ***0.028***	0.014 *0.852*
	CD41b (nMFI; %)	0.076 *0.247*	-0.107 *0.105*	0.029 *0.702*
	CD42a (nMFI; %)	0.090 *0.173*	**-0.130** ***0.048***	0.014 *0.856*
	CD31 (nMFI; %)	**0.213** ***0.001***	**-0.247** ***<0.001***	**0.264** ***<0.001***
Urine EVs	CD105 (nMFI; %)	**-0.274** ***<0.001***	**0.378** ***<0.001***	-0.118 *0.122*
	CD1c (nMFI; %)	**-0.177** ***0.013***	**0.187** ***0.009***	-0.070 *0.358*
	SSEA-4 (nMFI; %)	**-0.275** ***<0.001***	**0.384** ***<0.001***	**-0.206** ***0.006***
	CD133/1 (nMFI; %)	**-0.261** ***<0.001***	**0.304** ***<0.001***	-0.119 *0.118*

The correlation between clinical parameters (creatinine, mg/dL; glomerular filtration rate, eGFR, mL/min; proteinuria, PU, mg/dL) and EV antigens discriminating patients according to renal outcome at T3, was evaluated by Pearson's R test. Pearson's R coefficient (above) and *p*-values (below) are reported for each comparison. A $p<0.05$ was considered significant and shown in bold.

Table S17. Prediction of renal recovery: ROC curve analysis

	ROC curve analysis (ref. Renal Recovery) [n=58]	**AUC (95% CI)**	***P*-Value***
Serum EVs at T0	HLA-II (nMFI; %)	0.653 (0.505-0.802)	0.052
	CD62P (nMFI; %)	0.970 (0.935-1.000)	**<0.001**
	CD41b (nMFI; %)	0.730 (0.601-0.860)	**0.003**
	CD42a (nMFI; %)	0.999 (0.995-1.000)	**<0.001**
	CD29 (nMFI; %)	0.664 (0.523-0.805)	**0.036**
	CD31 (nMFI; %)	0.818 (0.707-0.929)	**<0.001**
	Compound EV biomarker	0.836 (0.736-0.936)	**<0.001**
Urine EVs at T1	CD19 (nMFI; %)	0.712 (0.578-0.846)	**0.007**
	CD56 (nMFI; %)	0.730 (0.594-0.866)	**0.003**
	CD105 (nMFI; %)	0.852 (0.753-0.952)	**<0.001**
	CD2 (nMFI; %)	0.707 (0.566-0.848)	**0.008**
	CD1c (nMFI; %)	0.686 (0.551-0.822)	**0.017**
	SSEA-4 (nMFI; %)	0.856 (0.760-0.952)	**<0.001**
	HLA-I (nMFI; %)	0.670 (0.523-0.816)	**0.030**
	CD42a (nMFI; %)	0.663 (0.523-0.804)	**0.037**
	CD133/1 (nMFI; %)	0.730 (0.601-0.859)	**0.003**
	CD45 (nMFI; %)	0.666 (0.523-0.808)	**0.034**
	CD20 (nMFI; %)	0.658 (0.511-0.805)	**0.043**
	Compound EV biomarker	0.901 (0.823-0.978)	**<0.001**

Receiver operating characteristics (ROC) curve analysis was performed to assess the diagnostic performance of each serum- or urine- extracellular vesicle (EV) surface antigen (expressed as normalized median fluorescence intensity, nMFI, %) to predict renal outcome after kidney transplant. nMFI levels were considered at T0 (before transplant) for serum EV surface antigens, and at T1 (10-14 days after transplant) for urine EV surface antigens. Areas under the curve (AUCs) are reported for each EV antigen together with its 95% confidence interval for each EV antigen and for a compound EV marker derived from the weighted linear combination of EV antigens significantly associated to renal outcome at univariate logistic regression analysis (CD62P, CD41b, CD42a, and CD31 for serum EV antigens; CD105, CD1c, SSEA-4, and CD133/1 for urine EV antigens). A $p<0.05$ was considered significant and shown in bold (*asymptotical difference compared to the referral line).

Table S18. Supervised learning to predict renal recovery through EV profiling

	Model	Data Imbalance	Acc (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Serum EVs at T0	**LDA**	None	74.1 [70.7]	34.8 [30.4]	100.0 [97.1]	100.0 [87.3]	70.0 [68.0]
		SMOTE	81.1 [74.1]	60.9 [47.8]	94.3 [91.4]	87.5 [78.5]	78.6 [72.7]
		SMOTE&NN	72.4 [69.0]	56.5 [39.1]	82.9 [88.6]	68.5 [69.3]	74.4 [68.9]
		RO	81.0 [70.7]	65.2 [34.8]	91.4 [94.3]	83.3 [80.0]	80.0 [68.8]
	RF	None	98.3 [96.5]	100.0 [95.7]	97.1 [97.1]	95.8 [95.6]	100.0 [97.2]
		SMOTE	**98.3 [98.3]**	**100.0 [95.7]**	**97.1 [100.0]**	**95.8 [100.0]**	**100.0 [97.3]**
		SMOTE&NN	98.3 [94.8]	95.7 [91.3]	100.0 [97.1]	100.0 [95.4]	97.3 [94.4]
		RO	98.3 [96.5]	100.0 [95.7]	97.1 [97.1]	95.8 [95.6]	100.0 [97.2]
	l-SVM	None	98.3 [93.1]	95.7 [87.0]	100.0 [97.1]	100.0 [95.2]	97.3 [91.9]
		SMOTE	100.0 [91.4]	100.0 [82.6]	100.0 [97.1]	100.0 [94.9]	100.0 [89.5]
		SMOTE&NN	87.9 [87.9]	69.6 [73.9]	100.0 [97.1]	100.0 [94.4]	83.3 [85.0]
		RO	100.0 [96.5]	100.0 [95.7]	100.0 [97.1]	100.0 [95.6]	100.0 [97.2]
	g-SVM	None	98.3 [93.1]	95.7 [87.0]	100.0 [97.1]	100.0 [95.2]	97.3 [91.9]
		SMOTE	98.3 [96.5]	95.7 [95.7]	100.0 [97.1]	100.0 [95.6]	97.3 [97.2]
		SMOTE&NN	94.8 [93.1]	87.0 [91.3]	100.0 [94.3]	100.0 [91.3]	92.1 [94.3]
		RO	96.5 [93.1]	95.7 [91.3]	97.1 [94.3]	95.6 [91.3]	97.2 [94.3]
Urine EVs at T1	**LDA**	None	82.8 [72.4]	82.6 [69.6]	82.9 [74.3]	76.0 [64.0]	87.9 [78.8]
		SMOTE	84.5 [74.1]	95.7 [87.0]	77.1 [65.7]	73.3 [62.5]	96.5 [88.5]
		SMOTE&NN	74.1 [62.1]	100.0 [65.2]	57.1 [60.0]	60.5 [51.7]	100.0 [72.4]
		RO	84.5 [77.6]	95.7 [91.3]	77.1 [68.6]	73.3 [65.6]	96.5 [92.3]
	RF	None	82.8 [77.6]	78.3 [69.6]	85.7 [82.9]	78.3 [72.8]	85.7 [80.6]
		SMOTE	82.8 [75.9]	82.6 [78.3]	82.9 [74.3]	76.0 [66.7]	87.9 [83.9]
		SMOTE&NN	75.9 [75.8]	78.3 [73.9]	74.3 [77.1]	66.7 [68.0]	83.9 [81.8]
		RO	86.2 [74.1]	95.7 [78.3]	80.0 [71.4]	75.9 [64.3]	96.6 [83.4]
	l-SVM	**None**	**84.5 [80.1]**	**73.9 [71.6]**	**91.4 [85.7]**	**85.0 [76.7]**	**84.2 [82.2]**
		SMOTE	81.0 [75.8]	91.3 [82.6]	74.3 [71.4]	70.0 [65.5]	92.9 [86.2]
		SMOTE&NN	74.1 [67.3]	78.3 [65.2]	71.4 [68.6]	64.3 [57.7]	83.4 [75.0]
		RO	84.5 [77.6]	91.3 [82.6]	80.0 [74.3]	75.0 [67.9]	93.3 [86.7]
	g-SVM	None	82.8 [75.9]	82.6 [69.6]	82.9 [80.0]	76.0 [69.6]	87.9 [80.0]
		SMOTE	77.6 [75.9]	60.9 [60.9]	88.6 [85.7]	77.8 [73.7]	77.5 [76.9]
		SMOTE&NN	77.6 [75.9]	69.6 [60.9]	82.9 [85.7]	72.8 [73.7]	80.6 [76.9]
		RO	79.3 [74.2]	69.6 [60.9]	85.7 [82.9]	76.2 [70.1]	81.1 [76.3]

Supervised learning was used to train and validate a prediction model able to discriminate patients with renal recovery (n=35) from those with persistent renal dysfunction (Glomerular Filtration Rate, eGFR $\leq$ 45 mL/min at T3; n=23). Median fluorescence intensity (MFI) of serum- and urine-extracellular vesicle (EV) surface antigen differentially expressed was used to derive the prediction models (MFI levels at T0 for serum EVs, or at T1 for urine EVs). Four different machine learning classifiers (linear discriminant analysis, LDA, random forest, RF, support vector machine with linear or gaussian kernel, l-SVM / g-SVM) and 3 algorithms for data imbalance correction (synthetic minority over-sampling technique [SMOTE], SMOTE and nearest neighbors [SMOTE&NN], and random oversampling methods [RO]) were applied to the overall cohort (n=58), generating 616 different models. For each classifier and correction technique, we reported the best model after optimization of hyperparameters, accuracy (Acc), sensitivity (Sens), specificity (Spec), and positive / negative prediction value (PPV / NPV). Performance at validation by leave-one-out algorithm is reported in squared brackets. Prediction models with the highest performance are shown in bold and red characters (a RF with 10 tree and 20 leaves with SMOTE correction for serum EV markers, and a l-SVM without correction for dataset imbalance for urine EV markers).

Table S19. Multivariate analysis on donor age/type, renal outcome, and EV markers

Logistic Regression (ref. Renal Recovery) [n=58]		Multivariate model including Donor Age				Multivariate model including Donor Type			
		EV antigen (nMFI; %)		**Donor age** (years)		**EV antigen** (nMFI; %)		**Donor type** (ref. deceased)	
		OR (95% CI)	***P*-Value**	**OR (95% CI)**	***P*-Value**	**OR (95% CI)**	***P*-Value**	**OR (95% CI)**	***P*-Value**
Serum EVs at T0	CD62P	0.942 (0.903-0.982)	**0.004**	0.998 (0.938-1.062)	0.950	0.937 (0.900-0.976	**0.002**	0.460 (0.003-19.159)	0.767
	CD41b	0.985 (0.973-0.997)	**0.014**	0.979 (0.946-1.014)	0.242	0.984 (0.972-0.996)	**0.009**	0.496 (0.048-5.116)	0.556
	CD42a	0.264 (0.003-20.461)	0.966	0.529 (0.023-11.667)	0.975	0.846 (0.696-1.027)	0.092	0.002 (0.001-41.213)	0.959
	CD31	0.969 (0.948-0.990)	**0.004**	0.966 (0.928-1.005)	0.086	0.971 (0.953-0.990)	**0.003**	0.409 (0.035-4.733)	0.474
Urine EVs at T1	CD105	1.001 (1.001-1.002)	**0.002**	0.985 (0.949-1.021)	0.409	1.002 (1.001-1.003)	**0.002**	8.724 (0.373-15.010)	0.178
	CD1c	1.258 (1.037-1.526)	**0.020**	0.972 (0.941-1.004)	0.088	1.262 (1.043-1.527)	**0.017**	0.321 (0.029-3.580)	0.355
	SSEA-4	1.017 (1.007-1.027)	**0.001**	0.977 (0.941-1.015)	0.237	1.018 (1.008-1.028)	**<0.001**	1.099 (0.053-12.754)	0.951
	CD133/1	1.085 (1.021-1.153)	**0.008**	0.962 (0.928-0.997)	**0.035**	1.081 (1.018-1.148)	**0.011**	0.706 (0.055-9.049)	0.789

Multivariate logistic regression analysis was performed to assess the association between each serum- or urine- extracellular vesicle (EV) surface antigen (expressed as normalized median fluorescence intensity, nMFI, %), donor age, donor type (deceased *vs.* living donor), renal outcome after kidney transplant. Two distinct regression models were examined, including each serum- and urine- EV marker associated to renal outcome and donor age (on the left), or donor type (on the right). nMFI levels were considered at T0 (before transplant) for serum EV surface antigens, and at T1 (10-14 days after transplant) for urine EV surface antigens. Odds ratios (ORs) are reported for each EV antigen together with its 95% confidence interval; an OR greater than 1 is associated with an increased likelihood of renal recovery; an OR less than 1 is associated with a decreased likelihood. A $p<0.05$ was considered significant and shown in bold.

Table S20. Diagnosis of graft rejection: analysis of serum EVs

Variable	Rejecting patients [n=7]	Non-rejecting patients [n=225]	*P*-Value
EV Diameter (nm)	175 [167; 189]	181 [163; 202]	0.404
EV conc (n/mL) [all vesicles]	5.6e12 [5.0e12; 6.7e12]	2.3e12 [1.3e12; 3.6e12]	**0.001**
EV conc (n/mL) [30-150nm]	2.4e12 [2.0e12; 3.4e12]	0.9e12 [0.5e12; 1.6e12]	**0.001**
EV conc (n/mL) [151-500nm]	3.1e12 [1.6e12; 3.4e12]	1.3e12 [0.7e12; 2.0e12]	**0.006**
EV conc (n/mL) [501-1000nm]	10.1e10 [1.9e10; 15.9e10]	2.8e10 [1.1e10; 5.8e10]	0.054
CD9 (MFI; a.u.)	99.6 [48.5; 153.4]	10.3 [3.9; 21.6]	**<0.001**
CD63 (MFI; a.u.)	95.8 [63.9; 167.1]	18.3 [9.4; 38.8]	**<0.001**
CD81 (MFI; a.u.)	244.9 [96.9; 302.1]	64.5 [32.1; 146.8]	**0.004**
Mean MFI for CD9, CD63, CD81	127.5 [80.4; 211.0]	30.6 [17.5; 74.4]	**0.002**
CD3 (nMFI; %)	54.1 [27.9; 89.8]	5.9 [0.1; 25.1]	**0.010**
CD4 (nMFI; %)	57.9 [0.4; 105.5]	7.7 [1.1; 34.6]	0.106
CD19 (nMFI; %)	72.7 [22.3; 111.4]	14.3 [3.2; 38.8]	**0.039**
CD8 (nMFI; %)	76.2 [74.7; 109.4]	31.3 [12.6; 55.5]	**0.005**
HLA-II (nMFI; %)	84.2 [40.2; 110.5]	63.4 [23.5; 108.2]	0.736
CD56 (nMFI; %)	20.0 [0.0; 41.6]	1.9 [0.0; 13.0]	0.136
CD105 (nMFI; %)	2.5 [0.0; 18.2]	0.0 [0.0; 0.0]	0.067
CD2 (nMFI; %)	64.3 [0.0; 110.8]	4.1 [0.0; 24.7]	0.061
CD1c (nMFI; %)	61.9 [0.0; 103.8]	10.4 [2.3; 39.1]	0.302
CD25 (nMFI; %)	47.3 [22.0; 74.8]	4.7 [0.0; 17.9]	**0.003**
CD49e (nMFI; %)	68.0 [35.8; 80.2]	6.7 [1.4; 27.8]	**0.003**
ROR1 (nMFI; %)	52.0 [6.9; 70.4]	8.1 [1.5; 34.4]	**0.046**
CD209 (nMFI; %)	85.2 [4.4; 115.5]	9.4 [2.3; 28.9]	**0.047**
CD9 (nMFI; %)	60.4 [46.1; 11.8]	30.0 [16.5; 52.4]	**0.003**
SSEA-4 (nMFI; %)	107.7 [0.1; 108.5]	21.4 [0.0; 80.0]	0.107
HLA-I (nMFI; %)	47.8 [16.9; 91.0]	13.7 [0.0; 50.5]	0.091
CD63 (nMFI; %)	92.5 [79.2; 115.9]	65.0 [31.3; 94.2]	0.078
CD40 (nMFI; %)	50.3 [18.3; 91.3]	12.6 [3.7; 38.2]	0.066
CD62P (nMFI; %)	102.9 [77.4; 115.0]	77.9 [41.5; 144.6]	0.338
CD11c (nMFI; %)	93.1 [8.5; 107.8]	6.2 [0.7; 34.8]	**0.005**
CD81 (nMFI; %)	145.1 [120.5; 224.5]	209.1 [155.9; 254.8]	0.122
MCSP (nMFI; %)	48.2 [0.0; 88.3]	6.2 [1.6; 22.2]	0.143
CD146 (nMFI; %)	15.0 [1.0; 41.1]	2.1 [0.0; 10.2]	0.154
CD41b (nMFI; %)	105.1 [26.0; 123.1]	46.4 [18.9; 86.7]	0.206
CD42a (nMFI; %)	104.5 [74.7; 134.6]	84.3 [45.0; 150.6]	0.473
CD24 (nMFI; %)	55.7 [5.1; 78.3]	9.2 [2.5; 25.3]	0.093
CD86 (nMFI; %)	85.5 [0.0; 111.7]	2.1 [0.0; 15.8]	**0.030**
CD44 (nMFI; %)	54.6 [10.6; 124.2]	7.5 [1.1; 23.2]	**0.030**
CD326 (nMFI; %)	58.0 [0.0; 78.7]	0.0 [0.0; 9.6]	**0.008**
CD133/1 (nMFI; %)	61.4 [13.3; 108.3]	15.6 [5.8; 47.5]	0.079
CD29 (nMFI; %)	63.0 [0.0; 68.4]	17.0 [6.3; 41.3]	0.412
CD69 (nMFI; %)	47.4 [16.2; 69.9]	11.7 [3.5; 33.8]	**0.030**
CD142 (nMFI; %)	53.9 [3.1; 107.5]	9.4 [2.5; 34.4]	0.067
CD45 (nMFI; %)	47.0 [11.7; 83.5]	8.3 [1.6; 28.5]	**0.038**
CD31 (nMFI; %)	57.5 [8.4; 93.3]	15.4 [6.6; 42.3]	0.166
CD20 (nMFI; %)	71.1 [9.2; 84.9]	7.3 [0.6; 21.8]	**0.010**
CD14 (nMFI; %)	55.2 [0.0; 109.0]	9.8 [1.2; 35.1]	0.167

Serum extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable in patients diagnosed with graft rejection (n=7) compared to non-rejecting subjects (n=225), independently from time point of sampling. A $p<0.05$ was considered significant and shown in bold.

Table S21. Diagnosis of graft rejection: analysis of urine EVs

Variable	Rejecting patients [n=7]	Non-rejecting patients [n=187]	*P*-Value
EV Diameter (nm)	193 [149; 209]	183 [168; 201]	0.737
EV conc (n/mL) [all vesicles]	14.1e9 [10.0e9; 58.3e9]	5.4e9 [0.3e9; 11.1e9]	**0.003**
EV conc (n/mL) [30-150nm]	8.6e9 [2.9e9; 22.5e9]	2.1e9 [1.1e9; 3.7e9]	**0.017**
EV conc (n/mL) [151-500nm]	8.6e9 [5.5e9; 34.8e9]	3.2e9 [1.9e9; 6.3e9]	**0.002**
EV conc (n/mL) [501-1000nm]	30.4e7 [0.7e7; 103.0e7]	1.4e7 [0.5e7; 6.0e7]	**0.043**
CD9 (MFI; a.u.)	58.1 [5.8; 145.6]	10.1 [5.3; 22.7]	**0.039**
CD63 (MFI; a.u.)	54.5 [11.1; 156.8]	14.3 [7.4; 28.9]	0.104
CD81 (MFI; a.u.)	24.5 [20.7; 69.5]	24.9 [8.0; 56.3]	0.438
Mean MFI for CD9, CD63, CD81	77.0 [26.6; 97.3]	21.5 [11.7; 35.7]	**0.002**
CD3 (nMFI; %)	15.5 [7.9; 40.5]	11.2 [3.4; 21.4]	0.265
CD4 (nMFI; %)	8.2 [0.0; 13.9]	1.7 [0.0; 5.0]	0.183
CD19 (nMFI; %)	19.1 [3.8; 26.6]	5.0 [1.4; 12.1]	**0.038**
CD8 (nMFI; %)	6.1 [3.5; 19.0]	4.1 [0.7; 11.1]	0.297
HLA-II (nMFI; %)	12.4 [0.0; 41.0]	6.1 [1.8; 13.7]	0.507
CD56 (nMFI; %)	170.5 [41.7; 281.5]	31.7 [9.4; 101.2]	**0.011**
CD105 (nMFI; %)	7436.4 [2329.5; 10403.4]	2472.9 [1442.0; 5131.2]	**0.016**
CD2 (nMFI; %)	26.3 [17.5; 51.6]	14.7 [5.5; 36.7]	0.118
CD1c (nMFI; %)	13.0 [7.4; 22.5]	3.5 [0.8; 9.1]	**0.006**
CD25 (nMFI; %)	11.6 [0.0; 17.7]	5.7 [1.1; 15.3]	0.807
CD49e (nMFI; %)	9.6 [2.9; 15.2]	4.1 [0.0; 13.5]	0.218
ROR1 (nMFI; %)	16.1 [5.0; 36.6]	5.7 [1.1; 15.7]	**0.045**
CD209 (nMFI; %)	4.7 [3.5; 22.0]	2.3 [0.0; 9.5]	**0.036**
CD9 (nMFI; %)	179.2 [50.5; 214.5]	48.4 [28.4; 120.8]	**0.040**
SSEA-4 (nMFI; %)	545.9 [165.0; 649.9]	193.9 [105.7; 360.5]	0.069
HLA-I (nMFI; %)	521.7 [174.7; 1040.8]	240.6 [67.7; 674.3]	0.155
CD63 (nMFI; %)	91.3 [41.7; 141.3]	76.2 [45.0; 121.4]	0.609
CD40 (nMFI; %)	4.3 [0.0; 15.3]	3.6 [0.0; 9.6]	0.666
CD62P (nMFI; %)	3.9 [2.3; 14.6]	3.7 [1.3; 7.1]	0.412
CD11c (nMFI; %)	9.1 [0.0; 21.8]	2.3 [0.0; 7.9]	0.267
CD81 (nMFI; %)	79.8 [26.9; 213.0]	151.0 [60.8; 217.4]	0.360
MCSP (nMFI; %)	6.8 [0.0; 25.1]	1.4 [0.0; 8.0]	0.281
CD146 (nMFI; %)	4.6 [0.0; 24.3]	2.6 [0.0; 8.0]	0.461
CD41b (nMFI; %)	46.6 [0.0; 82.8]	16.3 [6.3; 36.3]	0.465
CD42a (nMFI; %)	14.6 [9.7; 15.7]	4.6 [1.1; 11.9]	**0.020**
CD24 (nMFI; %)	31.6 [20.5; 70.5]	35.8 [19.8; 60.8]	0.760
CD86 (nMFI; %)	52.3 [25.7; 121.5]	31.9 [12.9; 52.9]	**0.041**
CD44 (nMFI; %)	25.1 [4.4; 55.6]	9.4 [3.2; 20.0]	0.086
CD326 (nMFI; %)	21.0 [0.0; 97.3]	0.1 [0.0; 8.8]	0.066
CD133/1 (nMFI; %)	41.8 [11.5; 68.5]	19.8 [8.7; 37.5]	0.079
CD29 (nMFI; %)	3.1 [0.0; 28.6]	2.5 [0.0; 6.6]	0.559
CD69 (nMFI; %)	7.1 [5.8; 12.2]	5.5 [2.2; 13.4]	0.230
CD142 (nMFI; %)	22.0 [6.6; 32.2]	9.5 [3.6; 20.1]	0.063
CD45 (nMFI; %)	13.1 [2.8; 41.6]	5.3 [0.5; 15.1]	0.142
CD31 (nMFI; %)	36.7 [15.1; 47.0]	23.0 [7.8; 37.9]	0.484
CD20 (nMFI; %)	33.4 [15.9; 57.0]	17.5 [7.3; 30.6]	0.058
CD14 (nMFI; %)	20.7 [14.8; 47.2]	6.8 [1.6; 17.3]	**0.024**

Urine extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81; normalized MFI, nMFI, % for 37 EV surface antigens). Median and interquartile range are reported for each variable in patients diagnosed with graft rejection (n=7) compared to non-rejecting subjects (n=187), independently from time point of sampling. A $p<0.05$ was considered significant and shown in bold.

Table S22. Diagnosis of graft rejection: univariate logistic regression analysis

	Logistic Regression (ref. Rejection)	OR (95% CI)	*P*-Value
Serum EVs [n=232]	CD3 (nMFI; %)	1.02 (1.01-1.03)	**0.016**
	CD19 (nMFI; %)	1.01 (1.00-1.02)	0.055
	CD8 (nMFI; %)	1.00 (0.99-1.01)	0.220
	CD25 (nMFI; %)	1.03 (1.01-1.06)	**0.001**
	CD49e (nMFI; %)	1.01 (1.00-1.03)	**0.027**
	ROR1 (nMFI; %)	1.01 (0.99-1.02)	0.070
	CD209 (nMFI; %)	1.02 (1.01-1.04)	**0.003**
	CD9 (nMFI; %)	1.03 (1.01-1.05)	**0.002**
	CD11c (nMFI; %)	1.01 (1.00-1.03)	**0.016**
	CD86 (nMFI; %)	1.03 (1.01-1.04)	**0.001**
	CD44 (nMFI; %)	1.02 (1.01-1.03)	**0.003**
	CD326 (nMFI; %)	1.04 (1.02-1.06)	**<0.001**
	CD69 (nMFI; %)	1.01 (0.99-1.02)	0.145
	CD45 (nMFI; %)	1.01 (0.99-1.03)	0.057
	CD20 (nMFI; %)	1.01 (1.00-1.02)	0.052
Urine EVs [n=194]	CD19 (nMFI; %)	1.06 (1.01-1.11)	**0.024**
	CD56 (nMFI; %)	1.02 (1.01-1.04)	**0.026**
	CD105 (nMFI; %)	1.02 (1.01-1.03)	**0.002**
	CD1c (nMFI; %)	1.05 (0.99-1.10)	0.065
	ROR1 (nMFI; %)	1.03 (1.01-1.05)	**0.011**
	CD209 (nMFI; %)	1.04 (0.98-1.11)	0.143
	CD9 (nMFI; %)	1.02 (1.01-1.03)	**0.027**
	CD42a (nMFI; %)	1.04 (0.99-1.09)	0.110
	CD86 (nMFI; %)	1.02 (1.01-1.03)	**0.007**
	CD14 (nMFI; %)	1.02 (1.01-1.04)	**0.010**

Univariate logistic regression analysis was performed to assess the association between each serum- or urine- extracellular vesicle (EV) surface antigen (expressed as normalized median fluorescence intensity, nMFI, %) and a diagnosis of graft. Odds ratios (ORs) are reported for each EV antigen together with its 95% confidence interval; an OR greater than 1 is associated with an increased likelihood of graft rejection. A $p<0.05$ was considered significant and shown in bold.

Table S23. Diagnosis of graft rejection: ROC curve analysis

	ROC curve analysis (ref. Rejection)	AUC (95% CI)	*P*-Value*
Serum EVs [n=232]	CD3 (nMFI; %)	0.784 (0.576-0.993)	**0.010**
	CD19 (nMFI; %)	0.730 (0.524-0.935)	**0.039**
	CD8 (nMFI; %)	0.815 (0.694-0.936)	**0.005**
	CD25 (nMFI; %)	0.828 (0.695-0.961)	**0.003**
	CD49e (nMFI; %)	0.827 (0.684-0.970)	**0.003**
	ROR1 (nMFI; %)	0.721 (0.535-0.908)	**0.046**
	CD209 (nMFI; %)	0.720 (0.469-0.971)	**0.047**
	CD9 (nMFI; %)	0.834 (0.725-0.942)	**0.003**
	CD11c (nMFI; %)	0.808 (0.625-0.992)	**0.005**
	CD86 (nMFI; %)	0.731 (0.475-0.988)	**0.037**
	CD44 (nMFI; %)	0.740 (0.520-0.960)	**0.031**
	CD326 (nMFI; %)	0.764 (0.530-0.999)	**0.017**
	CD69 (nMFI; %)	0.742 (0.569-0.914)	**0.030**
	CD45 (nMFI; %)	0.730 (0.508-0.953)	**0.038**
	CD20 (nMFI; %)	0.784 (0.631-0.937)	**0.010**
	Compound EV biomarker	0.857 (0.702-1.000)	**0.001**
Urine EVs [n=194]	CD19 (nMFI; %)	0.730 (0.520-0.940)	**0.039**
	CD56 (nMFI; %)	0.785 (0.656-0.913)	**0.011**
	CD105 (nMFI; %)	0.768 (0.592-0.944)	**0.016**
	CD1c (nMFI; %)	0.804 (0.699-0.910)	**0.006**
	ROR1 (nMFI; %)	0.723 (0.552-0.894)	**0.045**
	CD209 (nMFI; %)	0.731 (0.584-0.878)	**0.038**
	CD9 (nMFI; %)	0.729 (0.531-0.926)	**0.040**
	CD42a (nMFI; %)	0.759 (0.609-0.909)	**0.020**
	CD86 (nMFI; %)	0.728 (0.558-0.898)	**0.041**
	CD14 (nMFI; %)	0.750 (0.582-0.918)	**0.025**
	Compound EV biomarker	0.770 (0.578-0.962)	**0.015**

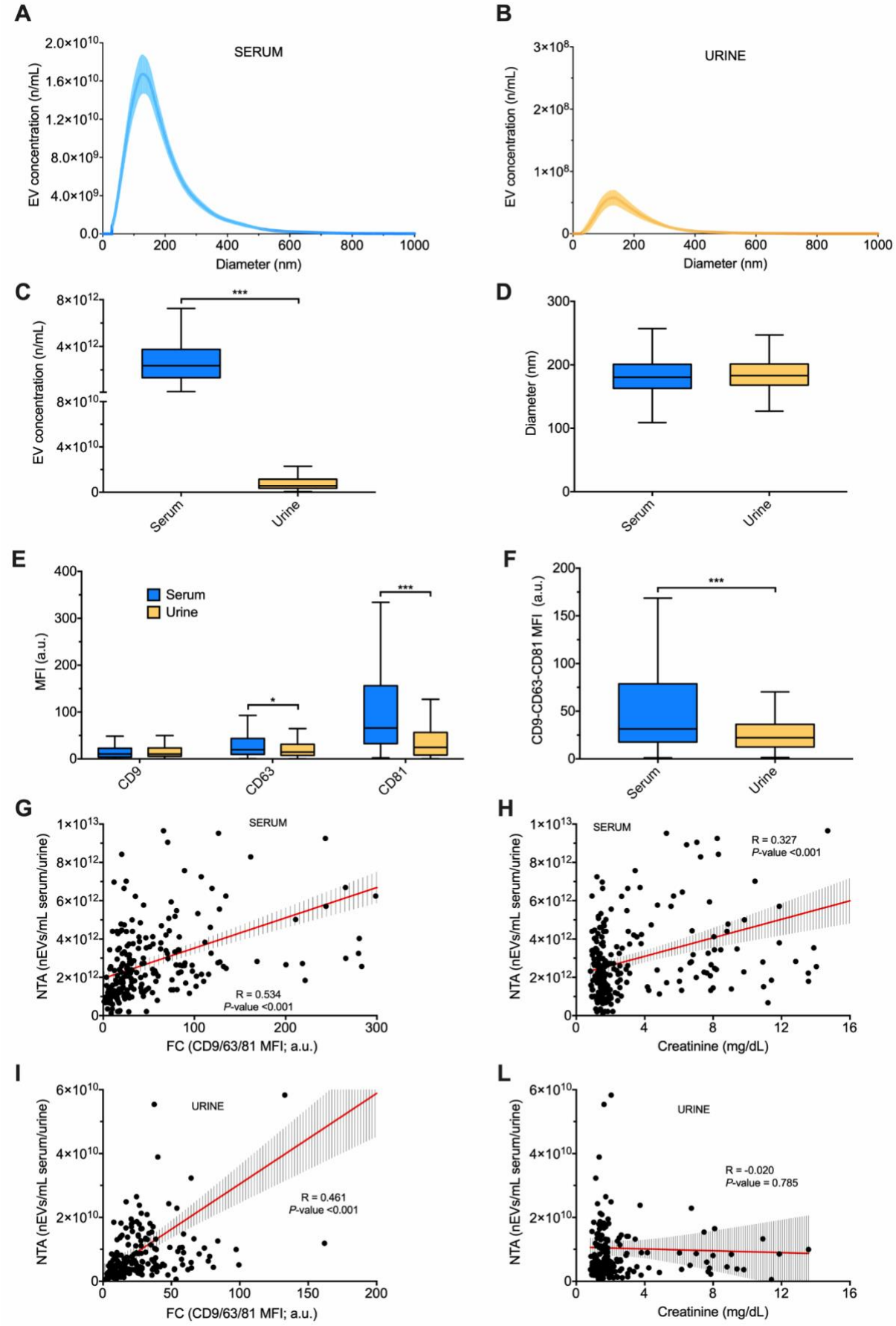
Receiver operating characteristics (ROC) curve analysis was performed to assess the performance of each serum- or urine- extracellular vesicle (EV) surface antigen (expressed as normalized median fluorescence intensity, nMFI, %) to diagnose graft rejection. Areas under the curve (AUCs) are reported for each EV antigen together with its 95% confidence interval for each EV antigen and for a compound EV marker derived from the weighted linear combination of EV antigens significantly associated to graft rejection at univariate logistic regression analysis (CD25, CD49e, CD209, CD9, CD11c, CD86, CD44, and CD326 for serum EV antigens; CD19, CD56, CD105, ROR1, CD9, CD86, and CD14 for urine EV antigens). A $p<0.05$ was considered significant and shown in bold (*asymptotical difference as compared to the referral line).

Table S24. Supervised learning to predict graft rejection through EV profiling

	Model	Acc (%)	Sens (%)	Spec (%)	PPV (%)	NPV (%)
Serum	**LDA**	89.7 [86.2]	85.7 [57.1]	89.8 [87.1]	20.7 [12.1]	99.5 [98.5]
	RF	**99.1 [96.1]**	**100.0 [71.4]**	**99.1 [96.9]**	**77.6 [41.7]**	**100.0 [99.1]**
	l-SVM	87.1 [85.3]	71.4 [57.1]	87.6 [86.2]	15.2 [11.4]	99.0 [98.5]
	g-SVM	81.5 [81.0]	71.4 [71.4]	81.8 [81.3]	10.9 [10.6]	98.9 [98.9]
Urine	**LDA**	78.3 [76.8]	71.4 [28.6]	78.6 [78.6]	11.1 [4.8]	98.7 [96.7]
	RF	73.7 [79.3]	85.7 [42.9]	73.3 [80.7]	10.7 [7.7]	99.3 [97.4]
	l-SVM	80.9 [77.3]	71.4 [42.9]	81.3 [78.6]	12.5 [7.0]	98.7 [97.4]
	g-SVM	71.6 [72.3]	71.4 [42.9]	71.7 [73.4]	8.6 [5.7]	98.5 [97.2]

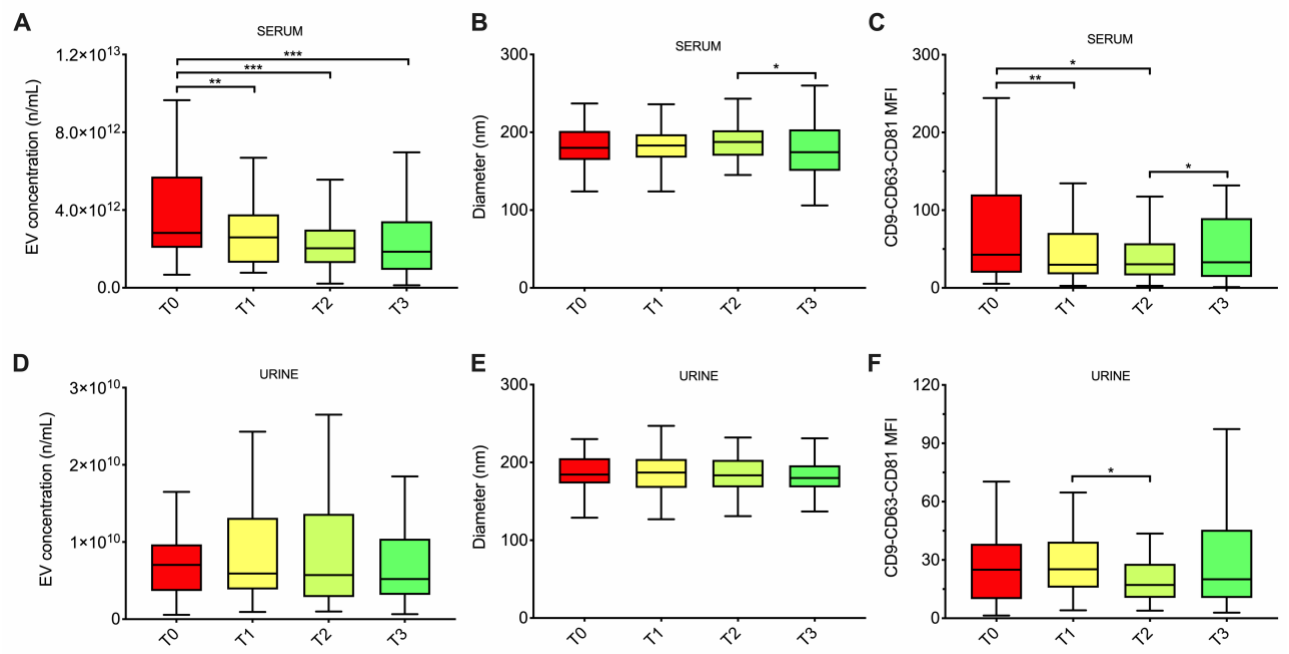
Supervised learning was used to train and validate a prediction model able to discriminate patients with a diagnosis of graft rejection (n=7) from those with a normal follow-up. Median fluorescence intensity (MFI) of serum- and urine- extracellular vesicle (EV) surface antigen was used to derive the prediction models (the total number of samples included in the analysis was 232 for serum EVs and 194 for urine EVs, independently from time point of sampling). Four different machine learning classifiers (linear discriminant analysis, LDA, random forest, RF, support vector machine with linear or gaussian kernel, l-SVM / g-SVM) and 3 algorithms for data imbalance correction (synthetic minority over-sampling technique [SMOTE], SMOTE and nearest neighbors, and random oversampling methods) were applied to the overall cohort, generating 616 different models. For each classifier, we reported the best model after optimization of hyperparameters, accuracy (Acc), sensitivity (Sens), specificity (Spec), and positive/negative prediction value (PPV / NPV). Performance at validation by leave-one-out algorithm is reported in squared brackets. The prediction model with the highest performance is shown in bold.

Figure S1. Quantitative comparison between serum- and urine- EVs



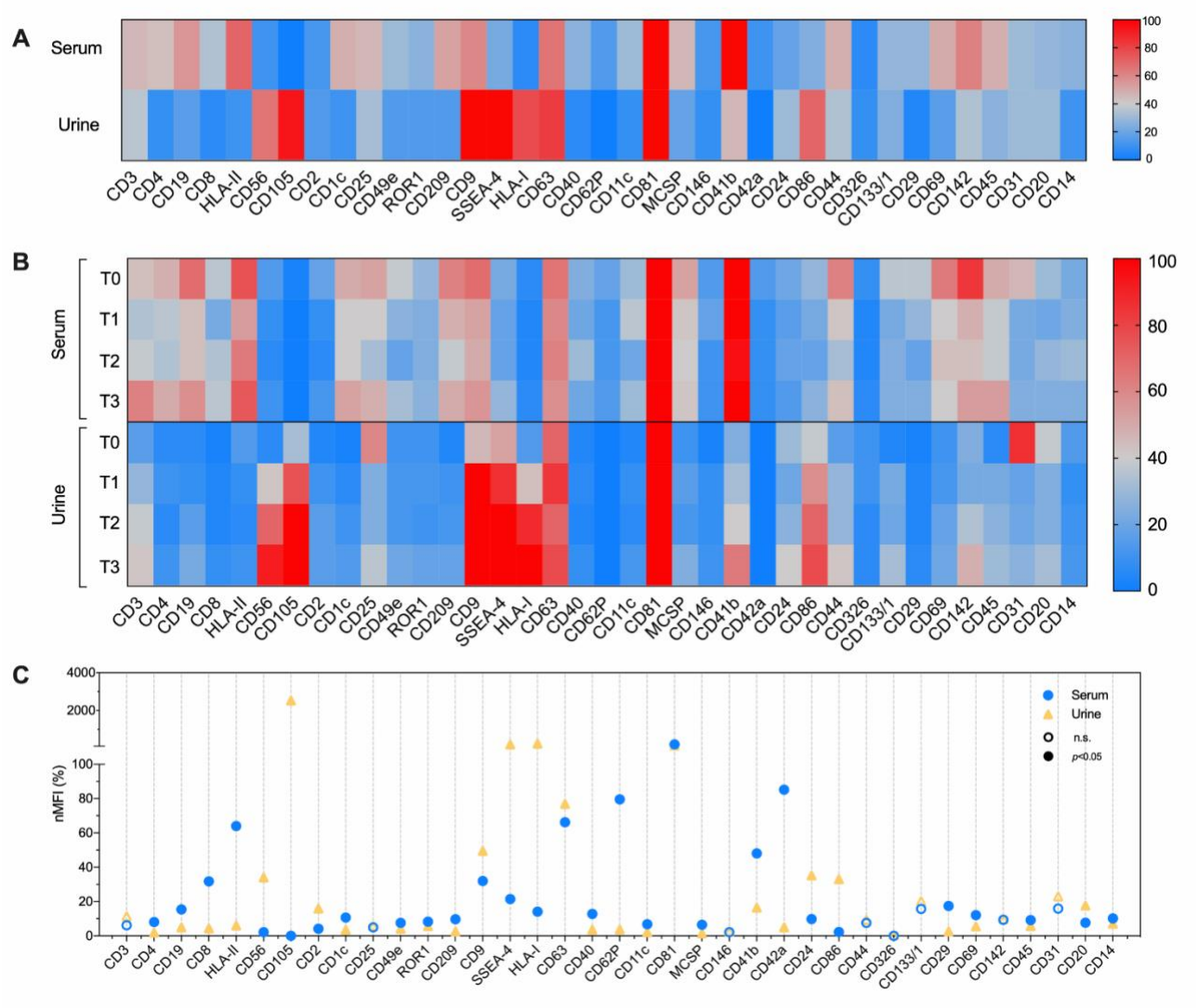
Characterization of serum- and urine- extracellular vesicles (EVs) by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81). The total number of samples included in the analysis was 232 for serum EVs, and 194 for urine EVs. (**A**) Cumulative distribution plot showing serum EV concentration and diameter at NTA. (**B**) Cumulative distribution plot showing urine EV concentration and diameter at NTA. (**C**) EV concentration (number of particles per mL) at NTA. (**D**) EV diameter (nm) at NTA. (**E-F**) Median fluorescence intensity (MFI) for CD9, CD63 and CD81 at FC. (**G**) Correlation between serum EV concentration at NTA and CD9-CD63-CD81 MFI at FC. (**H**) Correlation between serum EV concentration at NTA and creatinine. (**I**) Correlation between urine EV concentration at NTA and CD9-CD63-CD81 MFI at FC. (**L**) Correlation between urine EV concentration at NTA and creatinine. **p*<0.05; ****p*<0.001; statistics is reported in Table S2.

Figure S2. Quantification of EV in serum and urine at patient follow-up



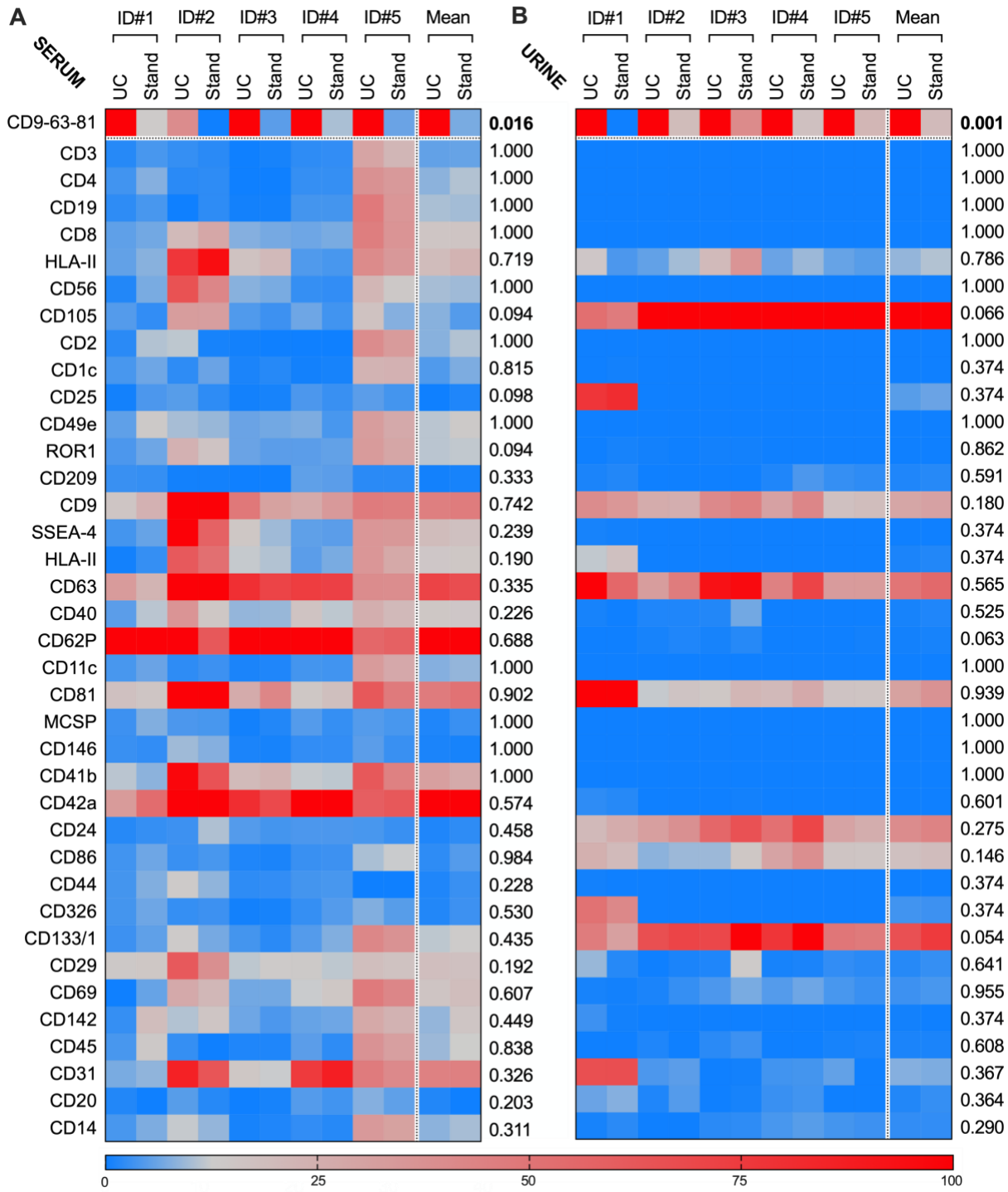
Characterization of serum- and urine- extracellular vesicles (EVs) by nanoparticle tracking analysis (NTA; EV concentration and diameter) and flow cytometry (median fluorescence intensity, MFI, expressed as arbitrary unit, a.u., for CD9, CD63 and CD81) after stratification for time point: T0, (before transplant); T1 (10-14 days after transplant); T2 (3 months after transplant); T3 (12 months after transplant). The total number of samples included in the analysis was 232 for serum EVs, and 194 for urine EVs. (**A**) Serum EV concentration (number of particles per mL) at NTA. (**B**) Serum EV diameter (nm) at NTA. (**C**) Median fluorescence intensity (MFI) for CD9, CD63 and CD81 at FC for serum EVs. (**D**) Urine EV concentration (number of particles per mL) at NTA. (**E**) Urine EV diameter (nm) at NTA. (**F**) Median fluorescence intensity (MFI) for CD9, CD63 and CD81 at FC for urine EVs. **p*<0.05; ***p*<0.01; ****p*<0.001; statistics is reported in Table S4.

Figure S3. EV surface antigens in serum- vs. urine- EVs



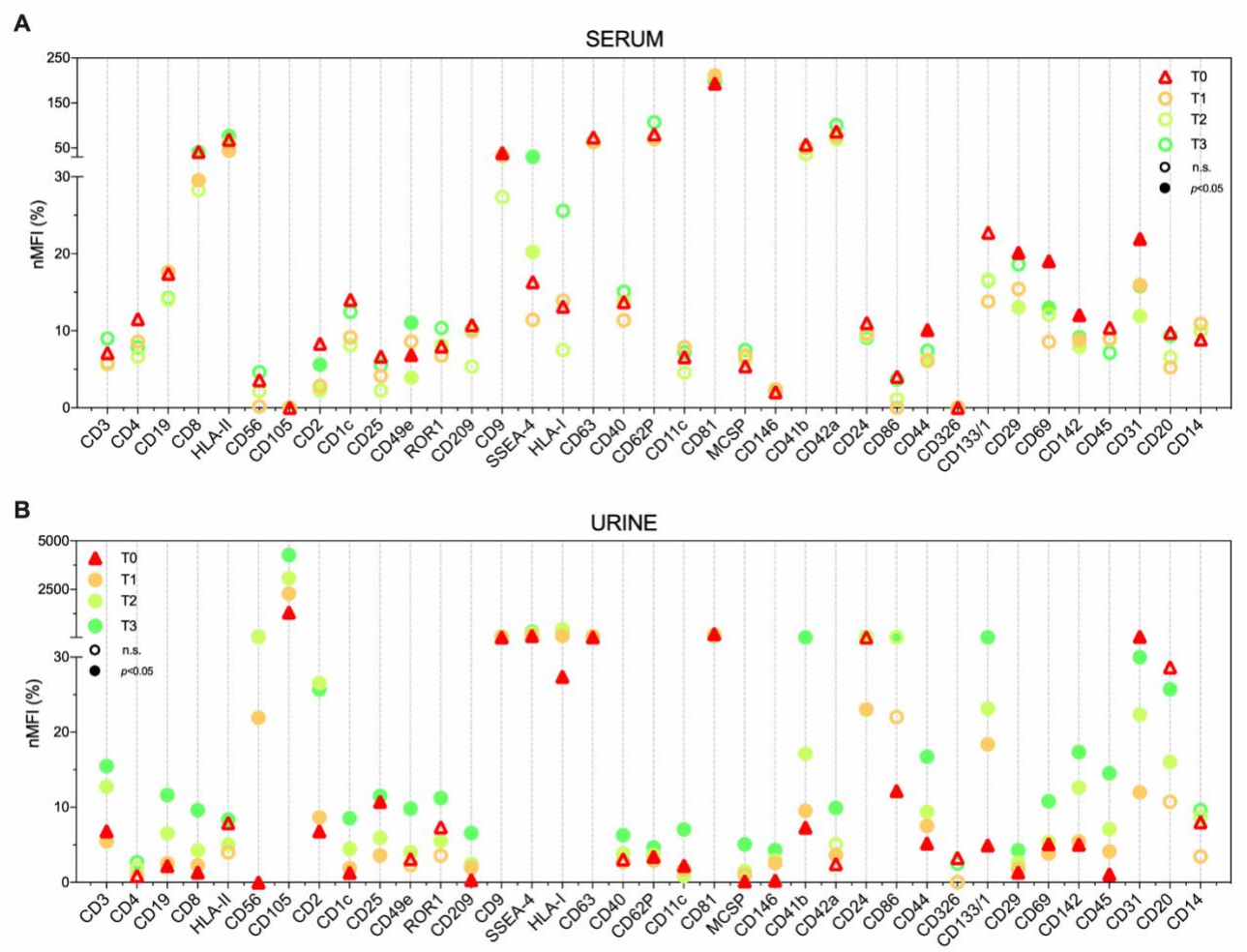
Serum- and urine- extracellular vesicle (EV) surface antigens were evaluated by flow-cytometry (the total number of samples included in the analysis was 232 for serum EVs, and 194 for urine EVs). Median fluorescence intensity (MFI; %) was reported after normalization for mean MFI of CD9, CD63 and CD81 (nMFI; %) for 37 different EV surface antigens. (**A**) Heatmap showing EV surface antigen expression in serum *vs.* urine EVs (blue, low MFI; red, high MFI). (**B**) Heatmap showing EV surface antigen expression in serum *vs.* urine EVs (blue, low MFI; red, high MFI) after stratification for time point: T0, (before transplant); T1 (10-14 days after transplant); T2 (3 months after transplant); T3 (12 months after transplant). (**C**) Molecular signature by EV surface antigens: serum *vs*. urine EVs. Statistics is reported in Tables S3-S5-S6.

Figure S4. Serum- and urine- EV profiling including pre-isolation by ultracentrifugation



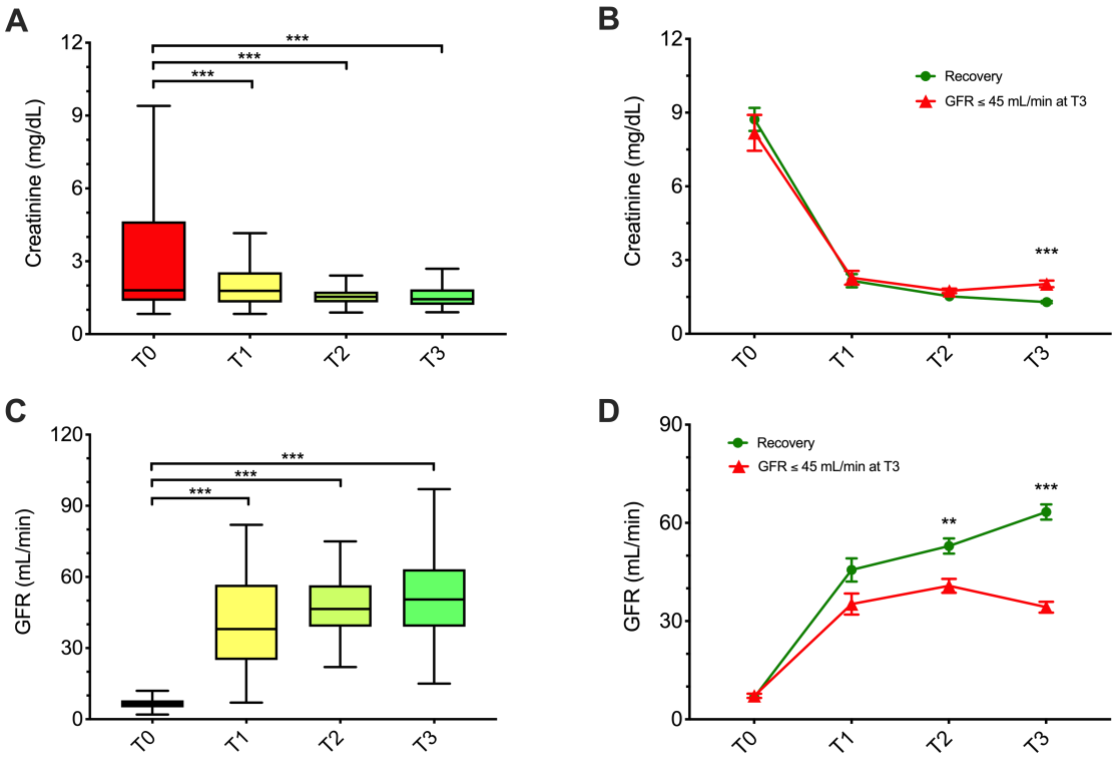
Comparison of different protocols for the profiling of serum- (**A**) and urine- (**B**) EV surface antigens by flow cytometry. The standard protocol (see methods) was compared to an alternative protocol which included a pre-isolation step by ultracentrifugation. The heat map reports median fluorescence intensity (MFI) for CD9-CD63-CD81 (first row) and the normalized MFI (nMFI) for the 37 evaluated surface antigens after normalization for mean MFI of CD9, CD63, and CD81 (n=5). A $p<0.05$ was considered significant and shown in bold.

Figure S5. Serum-/Urine EV specific signature at follow-up



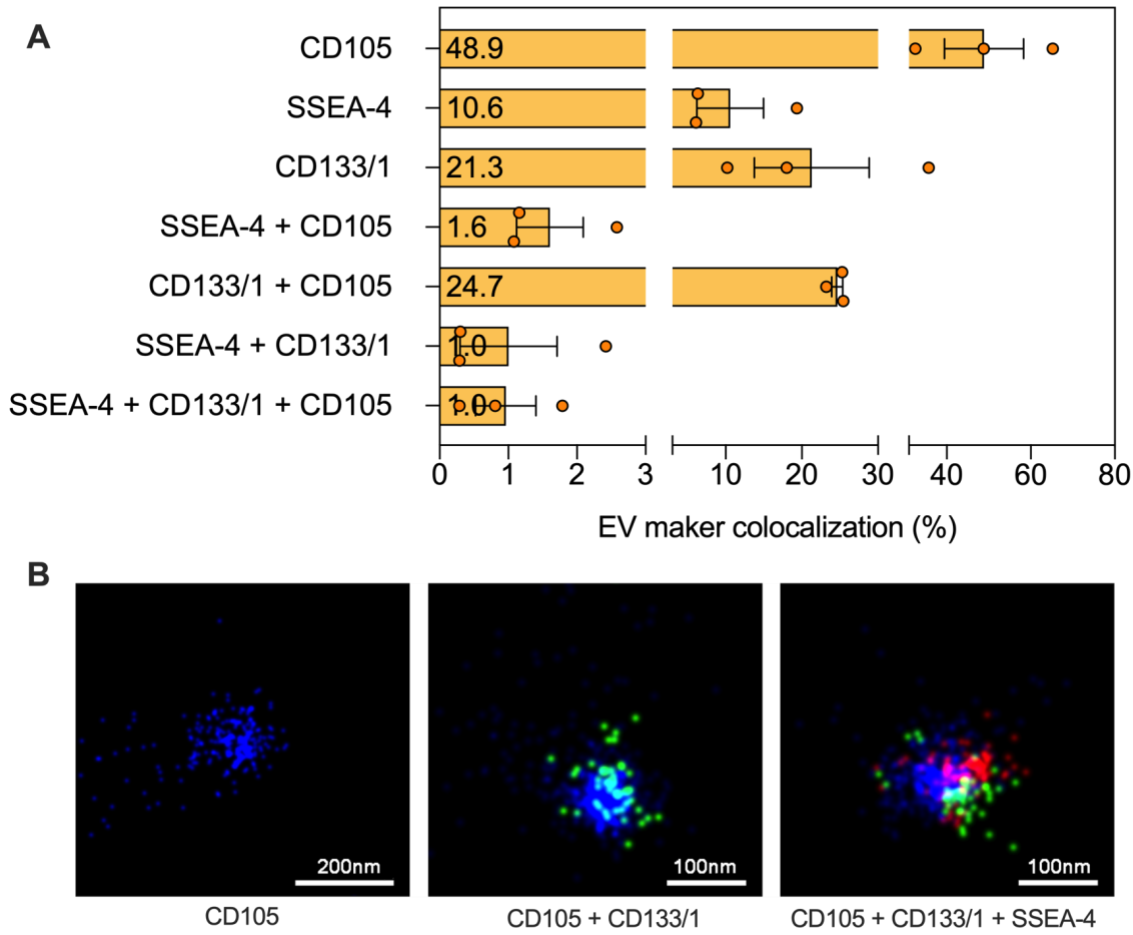
Serum- and urine- extracellular vesicle (EV) surface antigens were evaluated by flow-cytometry (the total number of samples included in the analysis was 232 for serum EVs, and 194 for urine EVs). Median fluorescence intensity (MFI; %) was reported after normalization for mean MFI of CD9, CD63 and CD81 (nMFI; %) for 37 different EV surface antigens. Molecular signature by EV surface antigens at the different time points (T0, [before transplant]; T1 [10-14 days after transplant]; T2 [3 months after transplant]; T3 [12 months after transplant]) was shown for serum- (**A**) and urine- EVs (**B**). Statistics is reported in Tables S5-S6.

Figure S6. Evaluation of creatinine and eGFR at follow-up



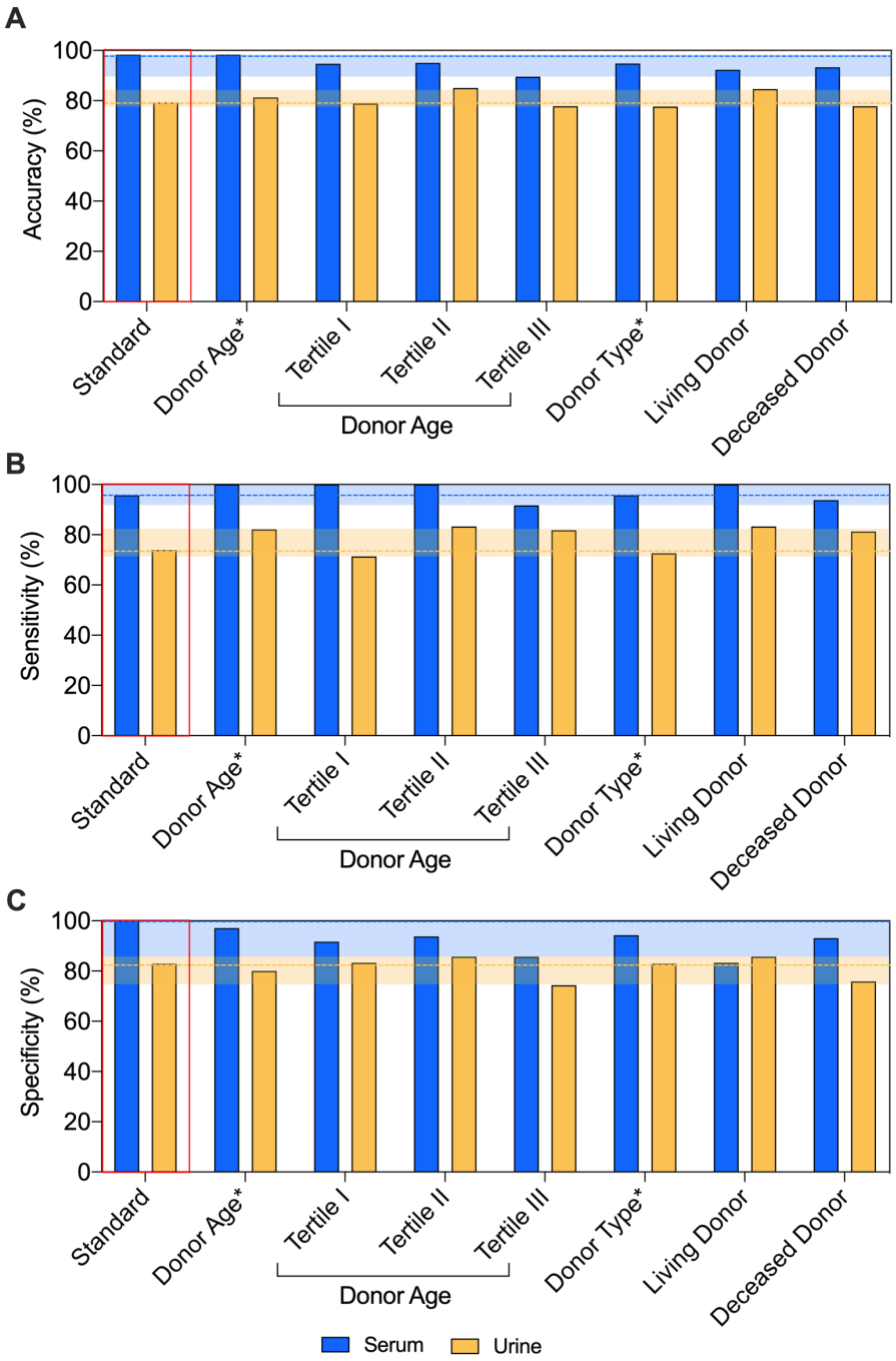
Patient creatinine and glomerular filtration rate (eGFR) at different time points: T0, (before transplant); T1 (10-14 days after transplant); T2 (3 months after transplant); T3 (12 months after transplant. (**A**) Creatinine (mg/dL); (**B**) Mean creatinine is shown at the different time points in patients displaying renal recovery (green line) or persistent renal dysfunction (red line; eGFR ≤ 45 mL/min at T3). (**C**) eGFR (mL/min); (**D**) Mean eGFR is shown at the different time points in patients displaying renal recovery (green line) or persistent renal dysfunction at T3 (red line). ***p*<0.01; ****p*<0.001; statistics is reported in Table S1.

Figure S7. Single vesicle analysis on urine-derived surface antigens



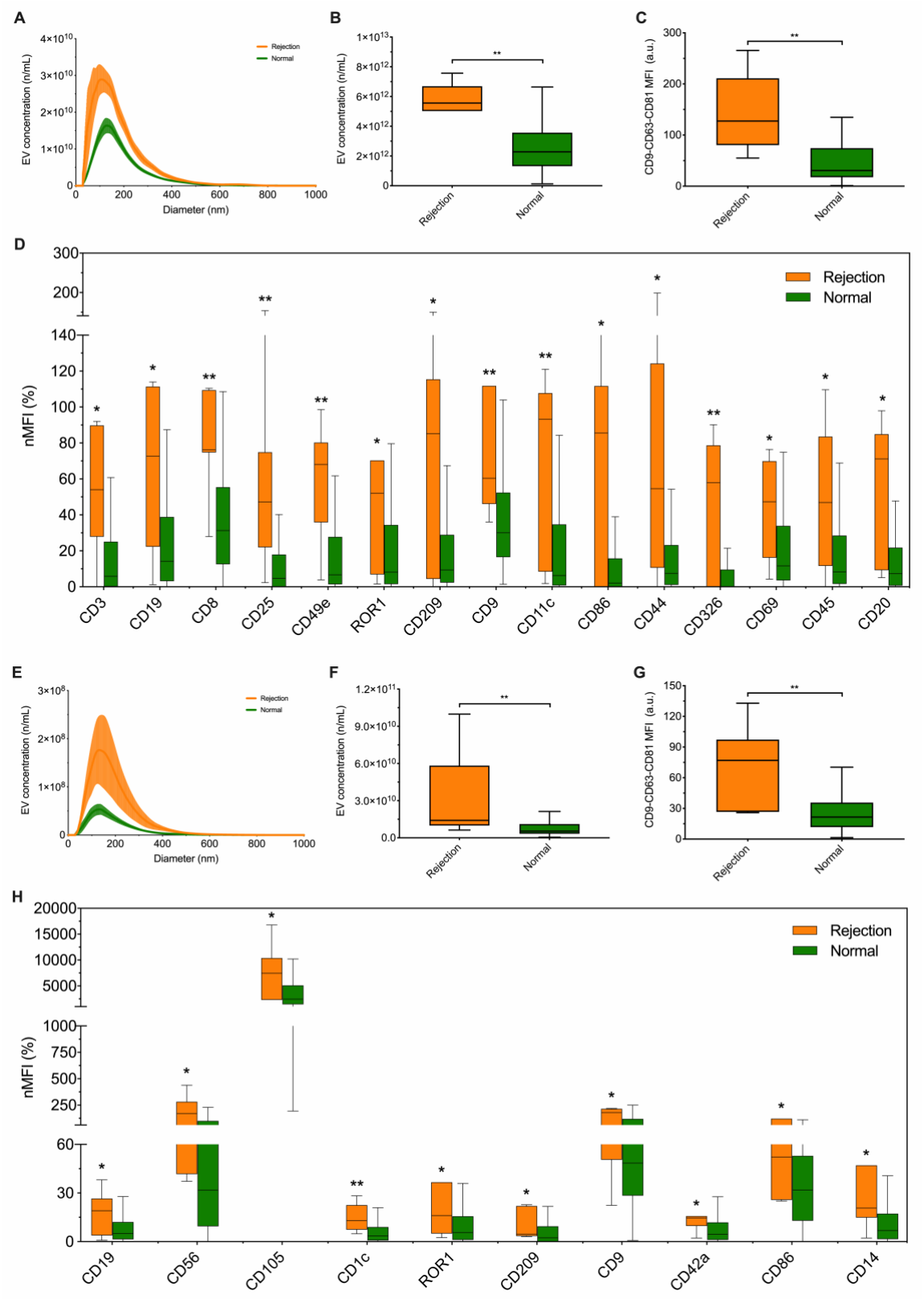
Distribution of urine EV antigens by super-resolution microscopy analysis. (**A**) Clustering analysis showing single, double, and triple positive EV fractions expressing CD105, SSEA4 and CD133/1. Analyses were performed in triplicates of a pool of urine EVs from control subjects; the graph shows mean ± SEM of a cumulative analysis of 3 fields for each preparation and expressed as percentage. (**B**) Representative super-resolution microscopy images of urine EVs showing single (CD105), double (CD105 and CD133/1), and triple expression of CD105 (blue), CD133 (green), SSEA4 (red). The scale bares are below each EV image (100 or 200nm).

Figure S8. Sensitivity analysis for age and donor type



Sensitivity analysis evaluating the impact of donor age and type on supervised learning models to predict renal recovery using serum- / urine- EV markers. The prediction model was trained and validated including donor age (Donor Age*) or donor type (Donor Type*) between discriminating features and compared with the standard model (red square). We also applied the standard model on the cohort after stratification for donor age (increasing age from tertile I to III) or type (deceased *vs.* living donor). (**A**) Accuracy ranging between 89.5 and 98.3% for serum EV marker-based model, and between 77.6 and 85.0% for urine EV marker-based model. (**B**) Sensitivity ranging between 91.7 and 100% for serum EV marker-based model, and between 71.4 and 83.3% for urine EV marker-based model. (**C**) Specificity ranging between 83.3 and 100.0% for serum EV marker-based model, and between 74.4 and 85.7% for urine EV marker-based model.

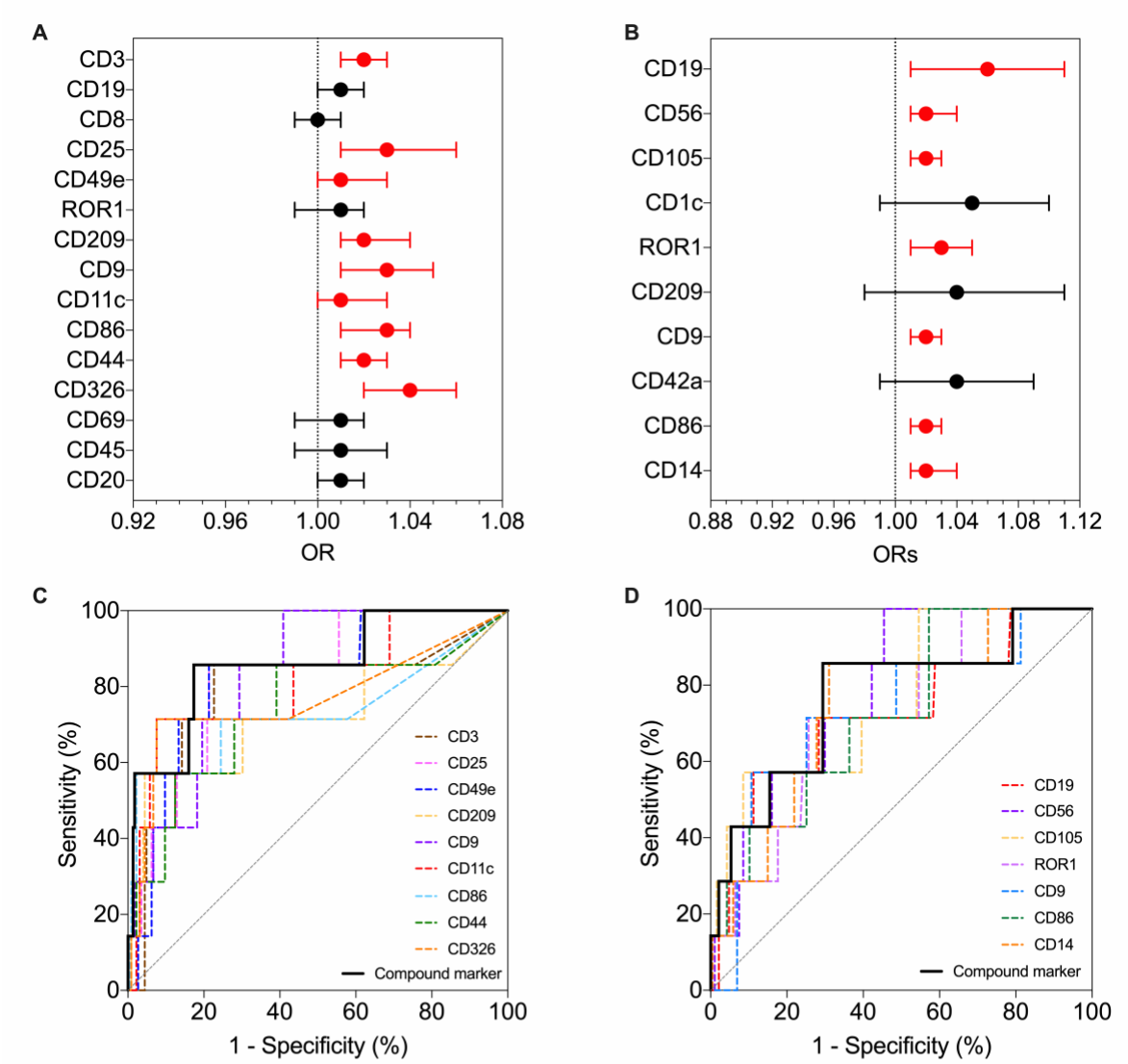
Figure S9. Diagnosis of graft rejection by a specific EV signature



Serum- and urine- extracellular vesicles (EVs) were characterized by nanoparticle tracking analysis (NTA) and flow cytometry (FC) in transplanted patients with or without a diagnosis of graft rejection at follow-up (the total number of samples included in the analysis was 232 for serum EVs, and 194 for urine EVs, independently from time point of sampling; 7 serum/urine samples were collected from patients with graft rejection). (**A**) Cumulative distribution plot showing serum EV concentration

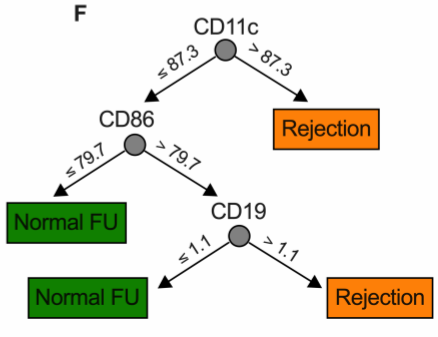
and diameter at NTA. (**B**) Serum EV concentration (number of particles per mL). (**C**) Median fluorescence intensity (MFI) for CD9, CD63 and CD81 at FC for serum EVs. (**D**) Normalized MFI (nMFI) for differentially expressed serum EV surface antigens in patients with or without graft rejection. (**E**) Cumulative distribution plot showing urine EV concentration and diameter at NTA. (**F**) Urine EV concentration (number of particles per mL). (**G**) MFI for CD9, CD63 and CD81 at FC for urine EVs. (**H**) nMFI for differentially expressed urine EV surface antigens in patients with or without graft rejection. **p*<0.05; ***p*<0.01; statistics is reported in Tables S20-S21.

Figure S10. Supervised learning to diagnose graft rejection



E

Prediction Model		PREDICTED at TRAINING		PREDICTED at VALIDATION	
		Normal Follow-up	Graft Rejection	Normal Follow-up	Graft Rejection
REAL	Normal Follow-up	223	2	218	7
	Graft Rejection	0	7	2	5
Diagnostic metrics		Accuracy (%) 99.1 Sensitivity (%) 100.0 Specificity (%) 99.1 Positive PV (%) 77.6 Negative PV (%) 100.0		Accuracy (%) 96.1 Sensitivity (%) 71.4 Specificity (%) 96.9 Positive PV (%) 41.7 Negative PV (%) 99.1	



Supervised learning was used to train and validate a prediction model able to discriminate patients with graft rejection (n=7) from those with a normal follow up. Normalized median fluorescence intensity (nMFI) of serum- and urine- extracellular vesicle (EV) surface antigen was used to derive the prediction models (the total number of samples included in the analysis was 232 for serum EVs and 194 for urine EVs, independently from time point of sampling). (**A**) The association of differentially expressed serum EV antigens with graft rejection was assessed by univariate regression analysis. Odds ratios (ORs) are reported for each EV antigen together with its 95% confidence interval; an OR greater than 1 is associated with an increased likelihood of graft rejection (significant associations were highlighted in red). (**B**) Association of differentially expressed urine EV antigens with graft rejection by univariate analysis. (**C**) Analysis of receiver operating characteristic (ROC) curves for serum EV surface antigens associated with graft rejection at univariate analysis. Diagnostic performance was assessed also for a compound EV marker derived by linear combination of all the others (black line) (**D**) Analysis of receiver operating characteristic (ROC) curves for urine EV surface antigens associated with graft rejection at univariate analysis. (**E-F**) Machine learning algorithms were used to train and validate 616 different diagnostic models based on serum- or urine-EVs (see methods). Confusion matrix and a representative tree are shown for the best model at training and validation: a random forest regressor with random oversampling correction for dataset imbalance, 10 classification trees and a maximum split number of 10, employing nMFI levels for serum EV surface antigens. Validation is provided by leave-one-out algorithm (see extended methods). Statistics is reported in Tables S22 to S24.