**Supplementary Materials:**

*Supplementary Methods*

Western blot analysis for the validation of ABMR-specific urinary exosomal proteins

Exosomal proteins were lysed using radioimmunoprecipitation assay buffer containing protease inhibitor cocktails. Protein samples (10 μg) were resolved using polyacrylamide gel electrophoresis and transferred into a polyvinylidene difluoride membrane. The membrane was probed with primary antibodies against each protein for 1 h at room temperature, and incubated with horseradish peroxidase-linked secondary antibody for 1 h at room temperature. The blots were developed using an enhanced chemiluminescence detection reagent (Bio-Rad Laboratories, Hercules, CA, USA) and exposed using a Bio-Rad Gel Documentation System (Bio-Rad). Image J Software (NIH, Bethesda, MD, USA) was used for densitometry analysis.

*Immunohistochemistry (IHC)*

Formalin-fixed, paraffin-embedded tissue sections were immunohistochemically stained using the OptiView DAB IHC Detection Kit on a BenchMark XT automatic immunostaining device (Ventana Medical Systems, Tucson, AZ, USA) according to the manufacturer's instructions. Four-micrometer-thick sections obtained with a microtome were transferred onto silanized charged slides and allowed to dry for 10 min at room temperature, followed by incubation for 20 min at 65°C. Sections were processed by heat-induced epitope retrieval method using Cell Conditioning 1 buffer for 32 min and incubated for 16 min with antibodies in an autoimmunostainer. Antigen-antibody reactions were visualized using the Ventana OptiView DAB IHC Detection Kit (Optiview HQ Linker 8 min, Optiview HRP Multimer 8 min, Optiview H2O2/DAB 8 min, Optiview Copper 4 min). Counterstaining was performed using Ventana Hematoxylin II for 12 min and Ventana Bluing reagent for 4 minutes. Finally, all slides were removed from the stainer, dehydrated, and cover-slipped for microscopic examination.

**Supplementary Figures**

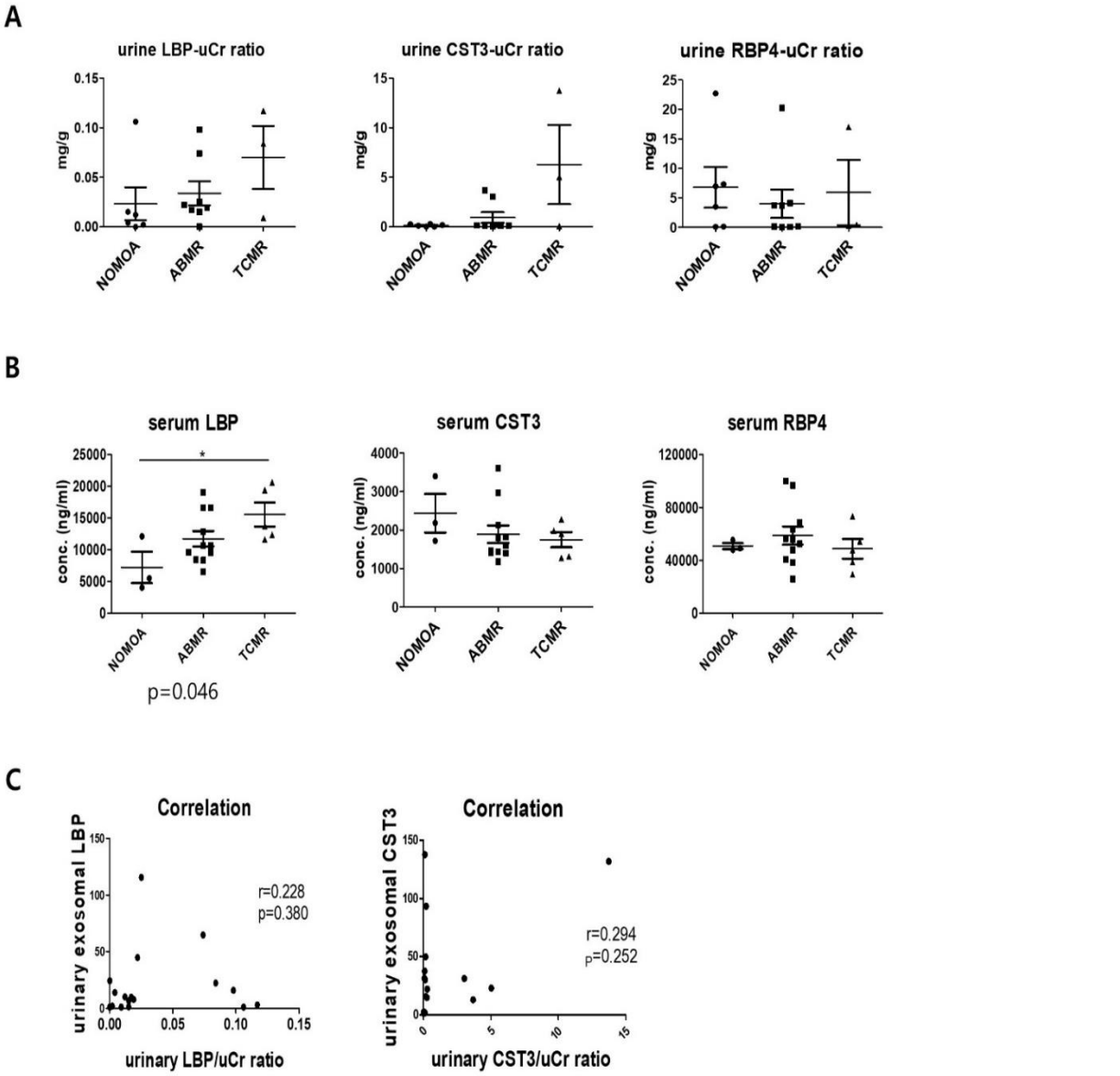


**Figure S1.** Validation of proteomic biomarkers in urine and serum. A. The creatinine (Cr) levels of urine and serum were evaluated from matched patients in WB-based validation (urine; 6 NOMOA, 8 ABMR, and 3 TCMR, serum; 6 NOMOA, 8 ABMR, and 4 TCMR). B. The protein concentration in urine was measured by ELISA and adjusted to urine creatinine level (9 NOMOA, 10 ABMR, and 4 TCMR). C. The protein concentration in serum was measured by ELISA (3 NOMOA, 11 ABMR, and 5 TCMR). Error bars indicate mean +/− SEM of individuals included in each group. Kruskal-Wallis One-way ANOVA p values are indicated for each protein. Dunn's Multiple Comparison test post hoc test significance values are indicated as *$P$< 0.05, **$P$< 0.01, and ***$P$< 0.001. D. Analysis of correlation between urinary exosomal proteins and urinary free proteins was assessed using Spearman's rank correlation (n=17). The r of 0.2-0.29 in Spearman's is considered to have a weak correlation. ABMR, antibody-mediated rejection; NOMOA, no major abnormality; TCMR, T cell-mediated rejection; CST3, Cystatin-C; LBP, lipopolysaccharide-binding protein; uCr. urine creatinine.

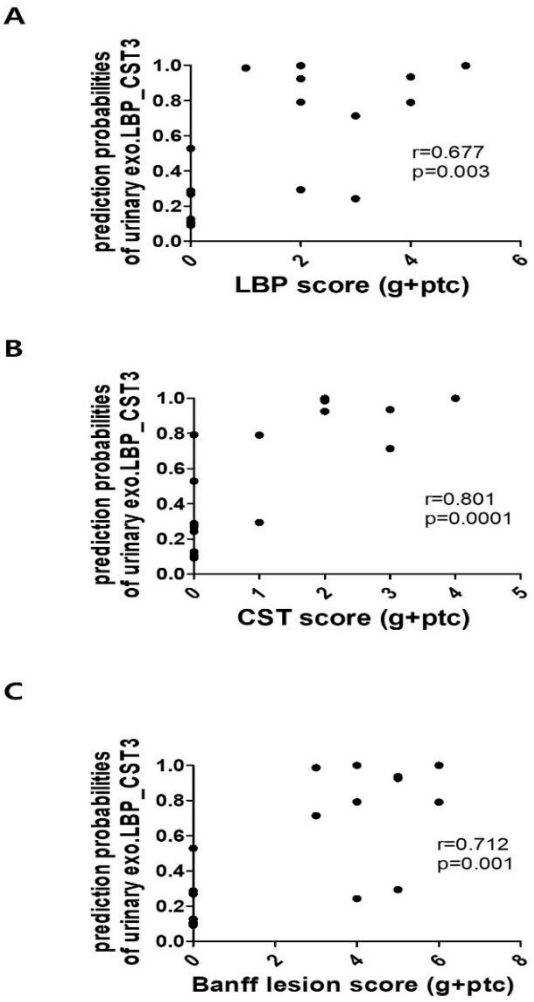


**Figure S2.** Association of prediction probabilities of combined urinary biomarkers with expression in allografts. Correlation between prediction probabilities of combined urinary biomarkers with (A) LBP score, (B) CST3 score, and (C) Banff lesion score (g+ptc) from matched samples (n= 17) are shown. The results were categorized as strong (r=0.7–1) and moderate (r=0.5–0.7) according to the degree of association. ABMR, antibody-mediated rejection; TCMR, T cell-mediated rejection; CST3, Cystatin-C; LBP, lipopolysaccharide-binding protein; g, glomeruli; ptc, peritubular capillaries.

## Supplementary Tables

**Table S1.** Comparison of histologic findings of for-cause biopsies of each group.

| | Discovery cohort | | | | | Validation cohort | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **ABMR (N=12)** | **BKVN (N=5)** | **TCMR (N=8)** | **NOMOA (N=10)** | **P** | **ABMR (N=25)** | TCMR (N=10) | NOMOA (N=19) | P |
| Banff score | | | | | | | | | |
| g | 2.00±1.04 | 0.20±0.45 | 0.38±0.45 | 0.33±0.50 | <0.001 | 1.96±0.84 | 0.20±0.42 | 0.33±0.49 | <0.001 |
| ptc | 2.33±0.78 | 0.80±0.84 | 0.50±1.07 | 0.11±0.33 | <0.001 | 2.40±0.65 | 0.40±0.70 | 0.16±0.50 | <0.001 |
| t | 0.50±0.91 | 1.20±1.10 | 2.38±0.52 | 0.11±0.33 | <0.001 | 1.04±0.20 | 2.50±0.71 | 0.21±0.42 | <0.001 |
| i | 1.00±0.85 | 1.40±1.14 | 2.25±0.46 | 0.44±0.53 | 0.001 | 1.40±0.65 | 2.10±0.57 | 0.42±0.51 | <0.001 |
| cg | 1.67±1.16 | 0 | 0 | 0.67±0.71 | <0.001 | 1.32±1.11 | 0.20±0.42 | 0.28±0.75 | <0.001 |
| ci | 2.08±1.00 | 1.40±1.34 | 1.13±0.84 | 1.56±1.13 | 0.223 | 1.88±0.93 | 1.20±0.79 | 1.16±0.96 | 0.027 |
| ct | 2.17±0.84 | 1.20±1.10 | 1.25±0.71 | 1.56±1.13 | 0.12 | 1.92±0.86 | 1.40±0.70 | 1.21±0.92 | 0.032 |
| cv | 1.33±0.89 | 1.00±0.71 | 1.13±0.99 | 1.44±1.13 | 0.846 | 0.92±0.98 | 1.50±0.97 | 1.11±0.94 | 0.28 |
| v | 0.17±0.39 | 0 | 0.63±0.92 | 0 | 0.114 | 0 | 0.20±0.63 | 0.05±0.23 | 0.344 |
| ti | 2.00±0.85 | 2.20±1.30 | 2.50±0.54 | 1.33±0.87 | 0.063 | 1.84±0.85 | 2.20±0.79 | 1.0±0.67 | 0.001 |
| C4d > 0 on IHC | 5 (41.7) | 1 (20) | 1 (12.5) | 1 (11.1) | 0.083 | 12(48.0) | 3(30.0) | 3(15.8) | 0.229 |

Values are mean ± standard deviation or n (%).

ABMR, antibody-mediated rejection; BKVN, BK virus nephropathy; TCMR, T cell-mediated rejection; NOMOA, no major abnormality; BMI, body mass index; ESRD, end-stage renal disease; FSGS, focal segmental glomerulosclerosis; PCKD, polycystic kidney disease; KT, kidney transplantation; ATG, anti-thymocyte globulin; HLA, human leukocyte antigen; DDKT, deceased donor kidney transplantation.

**Table S2.** List of eighteen urinary exosomal biomarker candidates.

| Accession | Gene name | Description | Peptides | Unique Peptides | Abundance Ratio: (ABMR/NOMOA) | Fold change [ABMR /NOMOA ($log^2$)] | P-Value |
|---|---|---|---|---|---|---|---|
| **P20062** | **TCN2** | **Transcobalamin-2** | **3** | **3** | 4.436 | 2.149259365 | 0.00095445 |
| P02652 | APOA2 | Apolipoprotein A-II | 3 | 3 | 4.085 | 2.030336078 | 9.45E-07 |
| Q9UGM5 | FETUB | Fetuin-B | 7 | 7 | 4.342 | 2.118359726 | 1.28E-08 |
| O14896 | IRF6 | Interferon regulatory factor 6 | 4 | 4 | 4.395 | 2.135863165 | 0.00045703 |
| P05546 | SERPIND1 | Heparin cofactor 2 | 19 | 19 | 3.667 | 1.874600266 | 7.34E-06 |
| Q99972 | MYOC | Myocilin | 11 | 11 | 5.61 | 2.488000771 | 6.48E-09 |
| P06681 | C2 | Complement C2 | 28 | 28 | 3.37 | 1.752748591 | 2.43E-05 |
| P26572 | MGAT1 | Alpha-1,3-mannosyl-glycoprotein 2-beta -N-acetylglucosaminyltransferase | 8 | 8 | 3.104 | 1.634128558 | 6.07E-06 |
| P18428 | LBP | Lipopolysaccharide-binding protein | 15 | 15 | 3.655 | 1.869871406 | 7.69E-06 |
| P27169 | PON1 | Serum paraoxonase/arylesterase 1 | 13 | 13 | 4.935 | 2.303050085 | 6.45E-08 |
| P08697 | SERPINF2 | Alpha-2-antiplasmin | 14 | 14 | 3.009 | 1.589284107 | 0.00010906 |
| P00734 | F2 | Prothrombin | 36 | 36 | 3.597 | 1.846794159 | 9.72E-06 |
| P02647 | APOA1 | Apolipoprotein A-I | 23 | 23 | 3.143 | 1.652142272 | 6.21E-05 |
| P29622 | SERPINA4 | Kallistatin | 22 | 22 | 3.368 | 1.751892138 | 2.45E-05 |
| P15291 | B4GALT1 | Beta-1,4-galactosyltransferase 1 | 12 | 12 | 3.045 | 1.606442228 | 9.35E-05 |
| P01034 | CST3 | Cystatin-C | 6 | 6 | 4.863 | 2.281846592 | 1.24E-08 |
| P01860 | IGHG3 | Immunoglobulin heavy constant gamma 3 | 13 | 5 | 4.256 | 2.089498151 | 7.56E-07 |
| P02753 | RBP4 | Retinol-binding protein 4 | 11 | 11 | 3.902 | 1.964213778 | 2.92E-06 |

**Table S3.** Area under the curve of the receiver operating characteristics curves of the four candidate biomarkers in differentiating AMBR from NOMOA in the validation cohort.

| | AUC | 95% CI | p-value | Cut-off | Specificity | Sensitivity | PPV | NPV |
|---|---|---|---|---|---|---|---|---|
| **LBP** | 0.813 | 0.562–0.954 | 0.0094 | >6.04 | 62.5 | 100 | 76.9 | 100 |
| **CST3** | 0.802 | 0.555–0.951 | 0.0068 | >22.06 | 100 | 70 | 100 | 72.7 |
| **PON1** | 0.619 | 0.349–0.844 | 0.4008 | >5.130 | 85.71 | 55.56 | 83.3 | 60 |
| **RBP4** | 0.532 | 0.273–0.779 | 0.8418 | <8.403 | 57.14 | 66.67 | 66.7 | 57.1 |
| **LBP-CST3** | 0.888 | 0.651–0.986 | <0.0001 | >0.529 | 80 | 87.5 | 81.8 | 85.7 |

ABMR, antibody-mediated rejection; NOMOA, no major abnormality; CI, confidential interval; PPV, positive predictive value; NPV, negative predictive value; LBP, lipopolysaccharide-binding protein; CST3, Cystatin-C; PON1, serum paraoxonase/arylesterase 1; RBP4, retinol-binding protein 4.

**Table S4.** List of antibodies used for Western blot analysis and Immunohistochemistry.

| Antibody | Host | Clonality | Company | Dilution factor | Application |
|---|---|---|---|---|---|
| CFD | mouse | monoclonal | Invitrogen | 1:200 | Western blot analysis |
| PON1 | mouse | monoclonal | Abcam | 1:200 | Western blot analysis |
| CST3 | goat | polyclonal | R&D systems | 1:500 | Western blot analysis |
| RBP4 | goat | polyclonal | R&D systems | 1:2000 | Western blot analysis |
| LBP | goat | polyclonal | R&D systems | 1:2000 | Western blot analysis |
| CD63 | mouse | monoclonal | BD Biosciences | 1:1000 | Western blot analysis |
| TSG101 | mouse | monoclonal | GeneTex | 1:1000 | Western blot analysis |
| Alix | mouse | monoclonal | Cell signaling Technology | 1:1000 | Western blot analysis |
| CST3 | goat | polyclonal | R&D systems | 1:100 | Immunohistochemistry |
| RBP4 | goat | polyclonal | Affinity Biosciences | 1:500 | Immunohistochemistry |
| LBP | goat | polyclonal | Invitrogen | 1:300 | Immunohistochemistry |