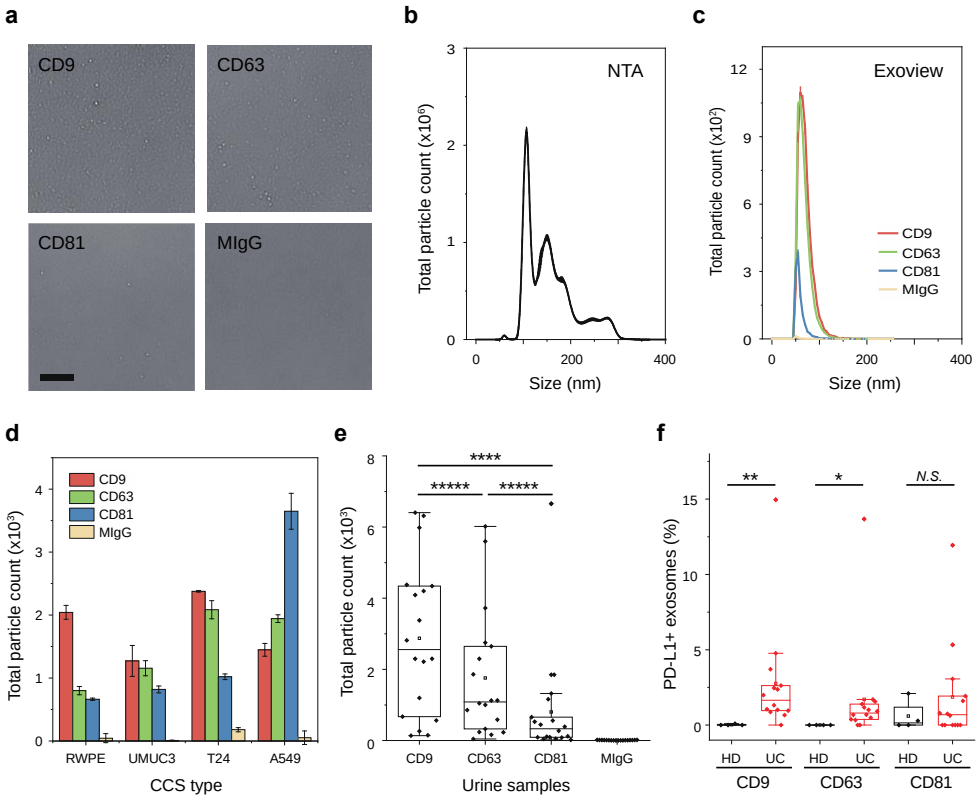
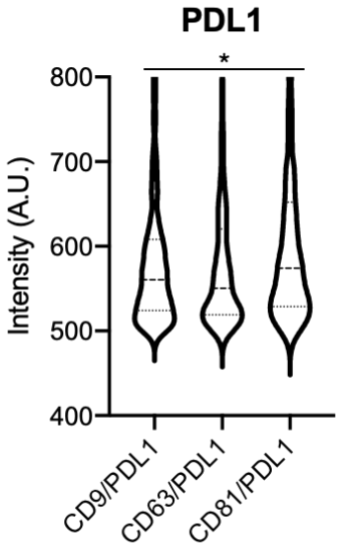
Supplementary Figure 1:

Characterization of exosomes (20-200 nm) isolated using Exodisc. (a-c) Exosomes are isolated from the urine of patients with urothelial cancer and analyzed by NTA and Exoview. (a) The Exoview bright field reflection image revealed the appearance of detected particles on the surface coated with CD9, CD63, CD81, and MIgG. Scale bar: 3 µm. (b) NTA (nanoparticle tracking analysis) results indicated that the size of exosomes was predominantly in the range of 100-200 nm, whereas the results obtained from (c) Exoview (an interferometric reflectance imaging method) showed that the size of exosomes was mainly in the range of 50-100 nm. (d) Single exosome analysis was performed using the CCS of urothelial cell lines (RWPE, UMUC3, and T24) and a lung cancer cell line (A549). The total particle counts for the surface-attached particles in the CCS of urothelial cell lines, including RWPE, UMUC3, and T24, showed a higher concentration for CD9 positive particles, followed by CD63 positive and CD81 positive particles. In contrast, the trend observed for particles isolated from A549 CCS, which is a lung cancer cell line, was opposite. The error bar is denoted as the standard deviation. (e-f) Single exosome analysis was performed on the urine samples obtained from healthy donors (HD; n=4) and patients with urothelial cancer (UC; n=14). (e) The exosomes isolated from urine samples (n=18) showed a similar trend as observed for exosomes from CCS of urothelial cell lines, with the highest particle counts for CD9-positive exosomes and the lowest counts for CD81-positive exosomes. (f) Furthermore, a significant difference in the percentage of PD-L1 positive particles co-localized with CD9 positive particles was observed between HD and UC samples. Significance was tested by Mann-Whitney test with $p < 0.05$, < 0.01, 0.005, 0.001, and 0.0001 noted as *, **, ***, ****, and *****, respectively. All measurements (a-f) were performed in triplicate. The horizontal lines in the box plots denote the minimum, first quartile, median, third quartile, and maximum, while the vertical line denotes the range with a 1.5x interquartile range. The empty square dot in the box plots is denoted as the mean.



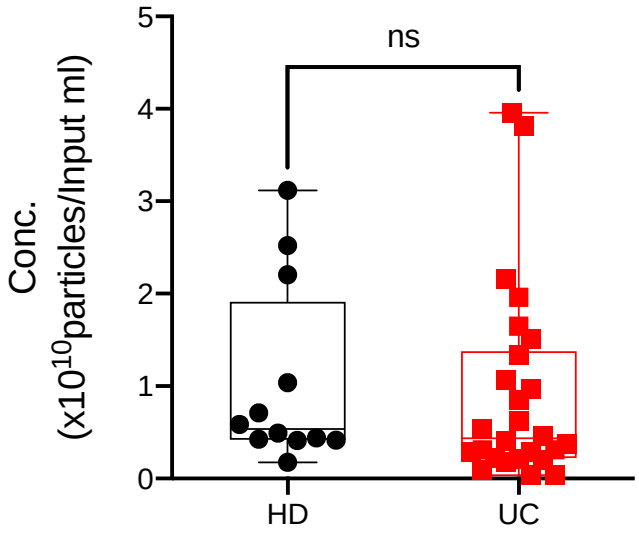
Supplementary Figure 2:

Intensity analysis of single exosomes for PD-L1. The intensity of PD-L1 and tetraspanin markers (**Figure 1D**) of particles, isolated from T24 CCS using Exodisc, attached on the surface coated with CD9, CD63, and CD81 showed varying intensity ranges, whereas Alix showed a uniform intensity range (**Figure 1D**). One-way ANOVA test was used for statistical analysis. * $p < 0.05$; PD-L1, programmed death-ligand 1.



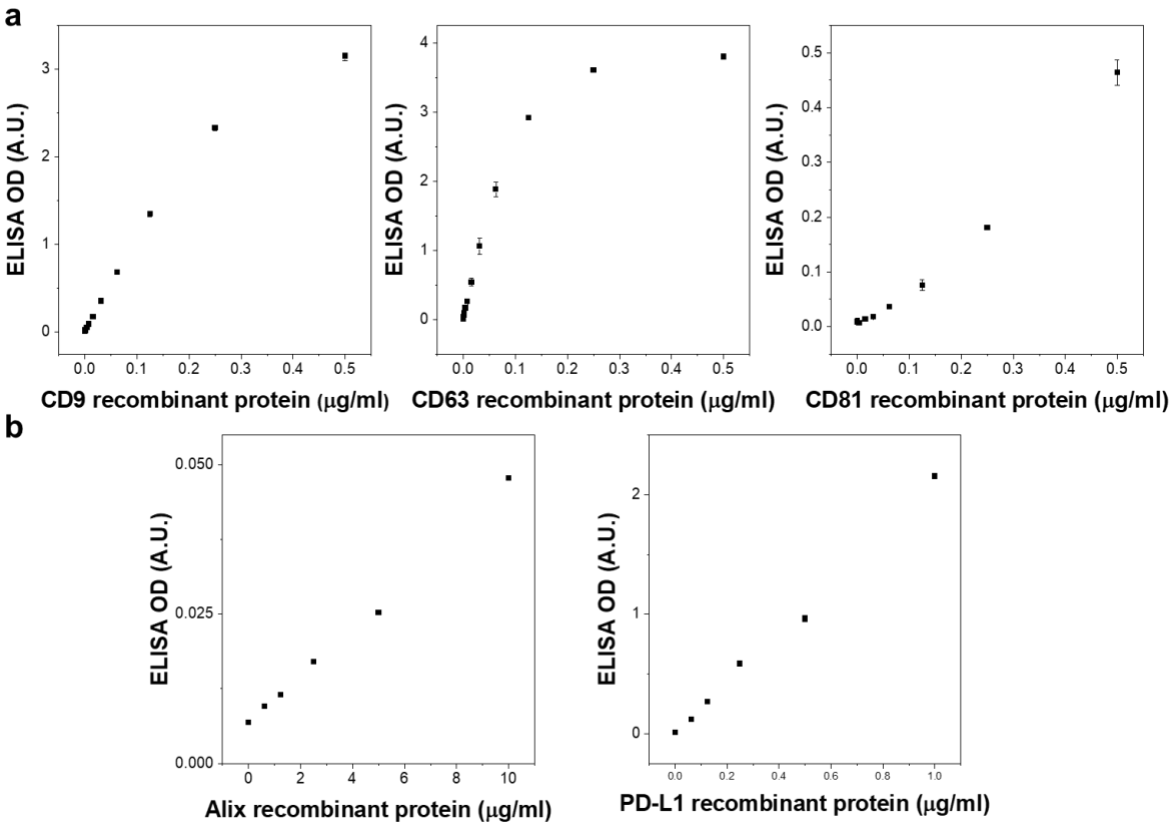
Supplementary Figure 3:

Nanoparticle tracking analysis (NTA) of the number of exosomes isolated from urine. The number of exosomes isolated from the urine of healthy donors (HD; n=12) and patients with urothelial cancer (UC; n=26) did not show any significant difference. The horizontal lines in the box plot denote the minimum, first quartile, median, third quartile, and maximum, while the vertical line denotes the range with 1.5x interquartile range. The empty square dot denotes the mean. ns represents not significant.



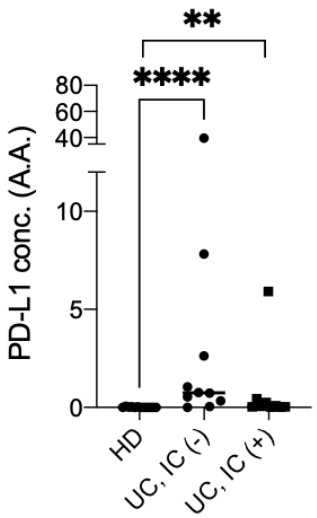
Supplementary Figure 4:

Calibration curves for recombinant proteins including CD9, CD63, CD81, Alix and PD-L1. The error is denoted as standard deviation.



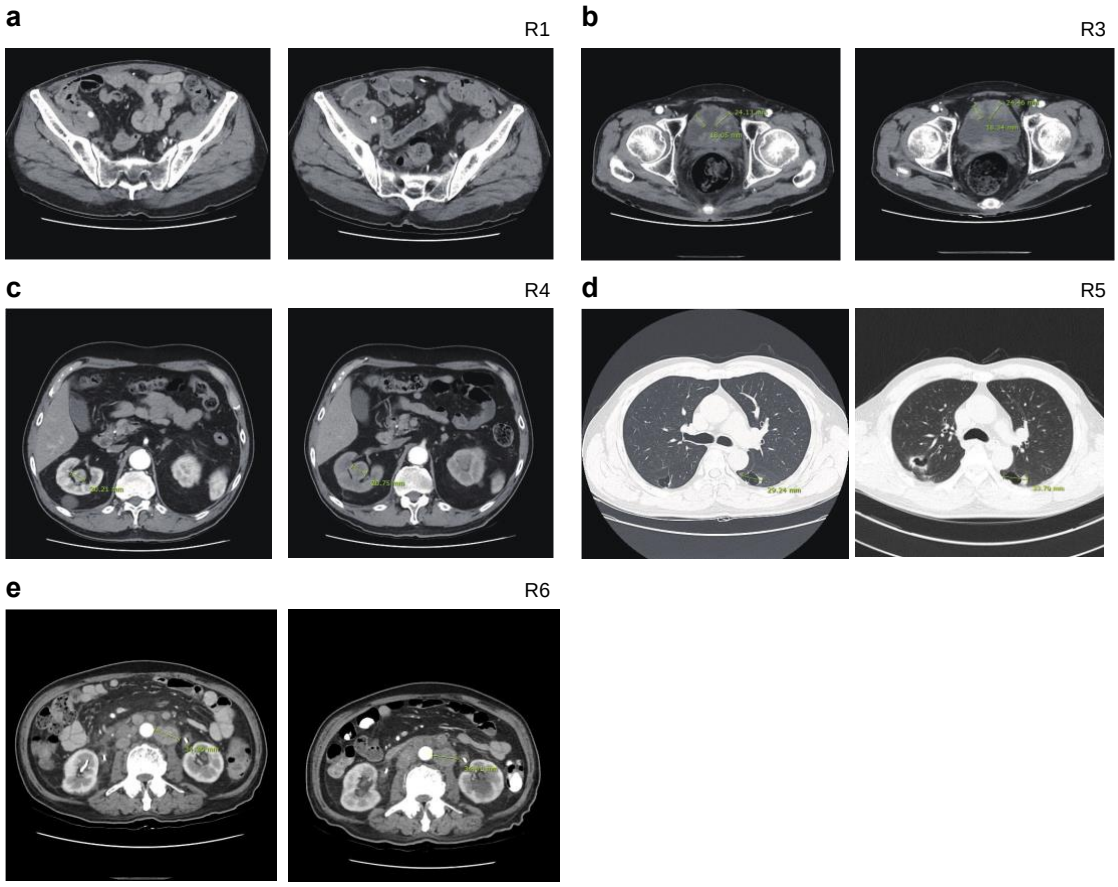
Supplementary Figure 5:

Exosomal PD-L1 concentration with immune cell (IC) score. Exosomal PD-L1 was quantified from EVs isolated from clinical samples (HD; n=12, UC; n=19, excluding tissue data non-available patients (n=7) from total patients (n=26)). Among patients with urothelial cancer (n=19), ten patients were IC negative, and nine patients were IC positive (IC ≥ 1). The PD-L1 concentration showed elevated expression in both IC-positive patients and IC-negative patients compared to HD (Mann-Whitney test, p-value = 0.0012 and < 0.0001, respectively). Exosomal PD-L1 was detected in all cohort patients, while only 47% of tumor tissues were positive for PD-L1.



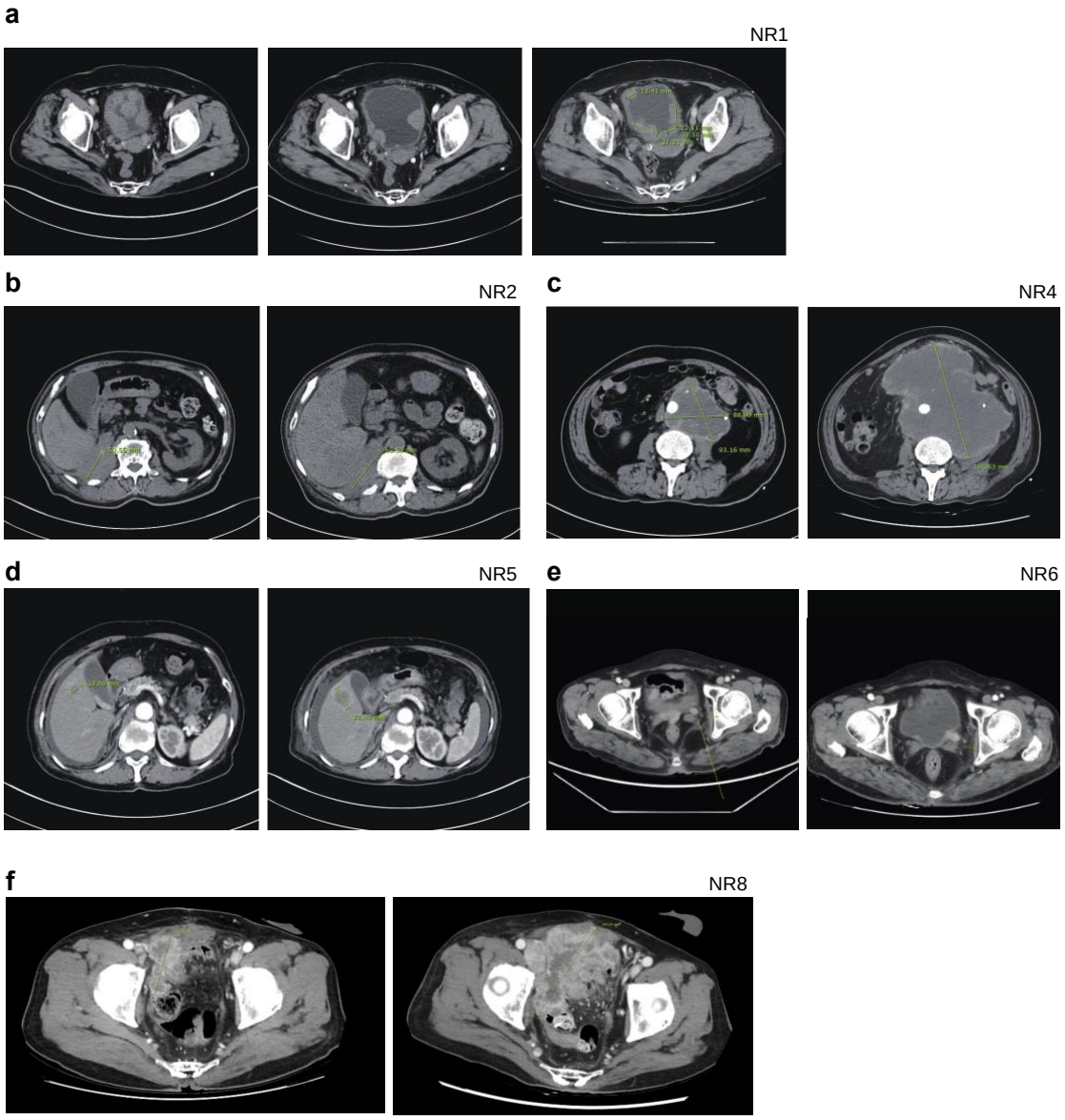
Supplementary Figure 6:

CT images of the patients for responder including R1-R6 (CT images for R2 is included in **Figure 4**).



Supplementary Figure 7:

CT images of the patients for non-responder including NR1-NR8 (CT images for NR3 are included in **Figure 4**, and CT images for NR7 are not available due to the cancer-related death before taking follow-up CT image).



Supplementary Figure 8:

A longitudinal follow-up study on a patient (R4), tracking serial time points both before and during treatment. The PD-L1/Alix ratio reached its peak at the third treatment cycle (T3, 9 weeks), while the PD-L1 concentration demonstrated fluctuating patterns of decreases and increases. These variations might be attributed to the dependency on exosome amount.