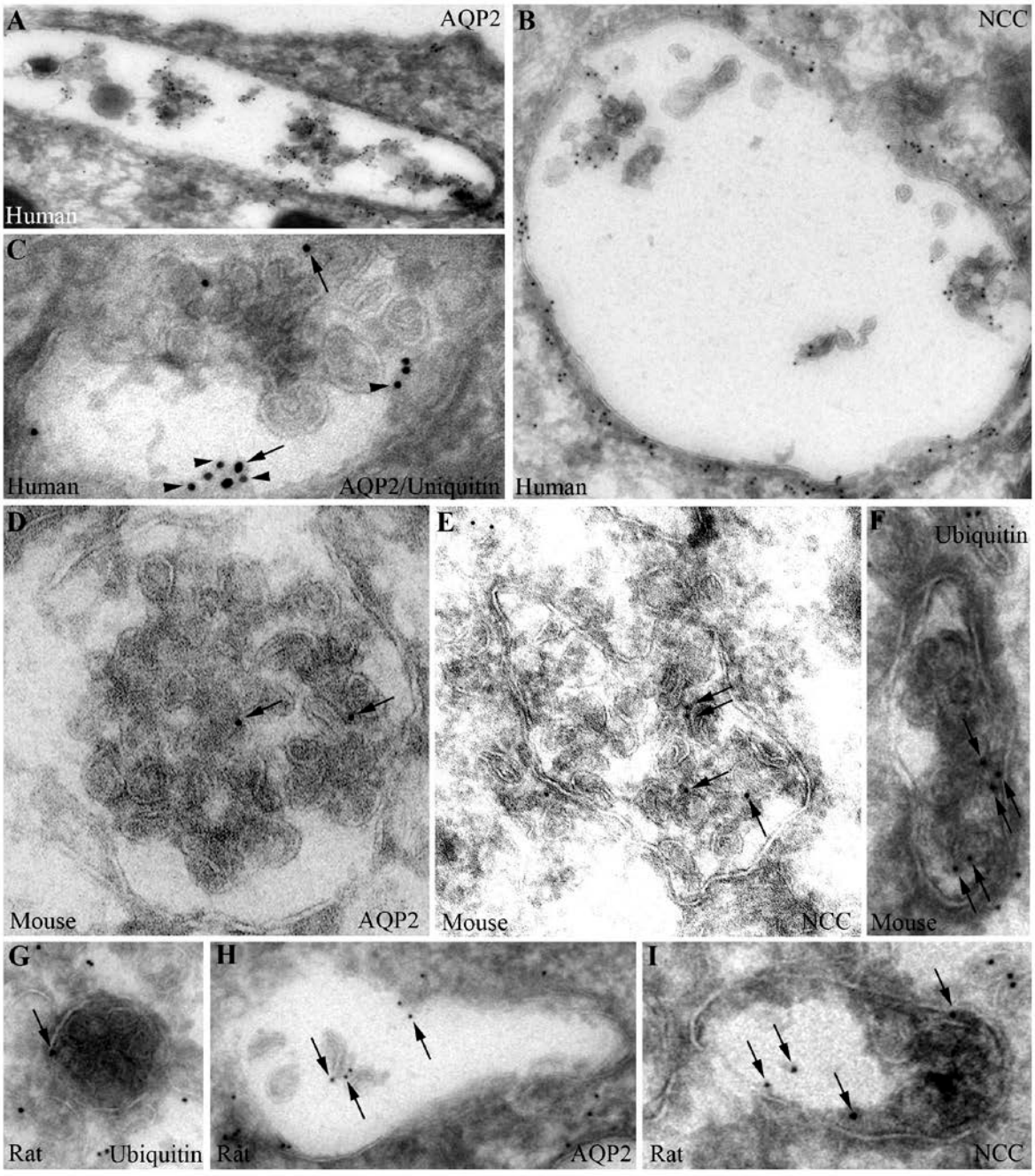
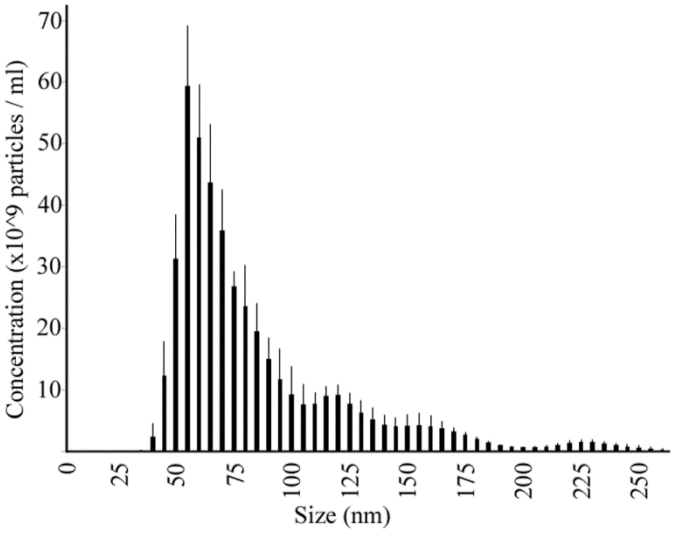
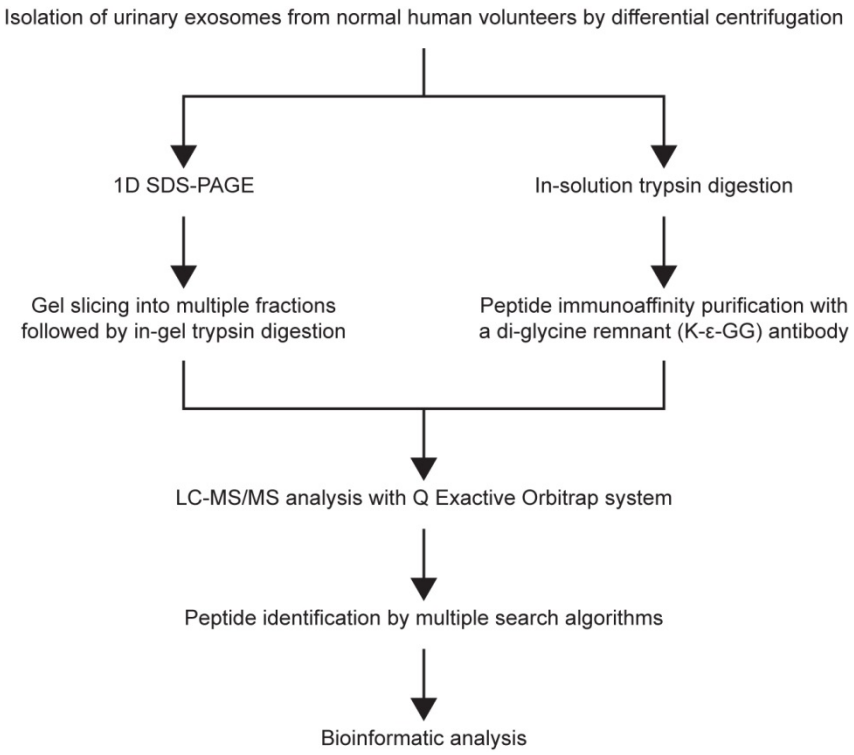
Supplemental Figure 1. Identification of ubiquitin and ubiquitylated membrane proteins in MVBs within human, mouse and rat kidney epithelial cells. A-C) Examples of the detection of the ubiquitylated proteins aquaporin-2 (AQP2) and the NaCl cotransporter (NCC) in human MVBs. Some co-labeling of AQP2 and ubiquitin is detected in association with internal luminal vesicles of an MVB. D-F) Examples of the association of AQP2, NCC and ubiquitin, respectively, in internal luminal vesicles of MVBs from mouse kidney epithelial cells. G-I) Examples of the association of ubiquitin, AQP2 and NCC, respectively, in internal luminal vesicles of MVBs from mouse kidney epithelial cells.



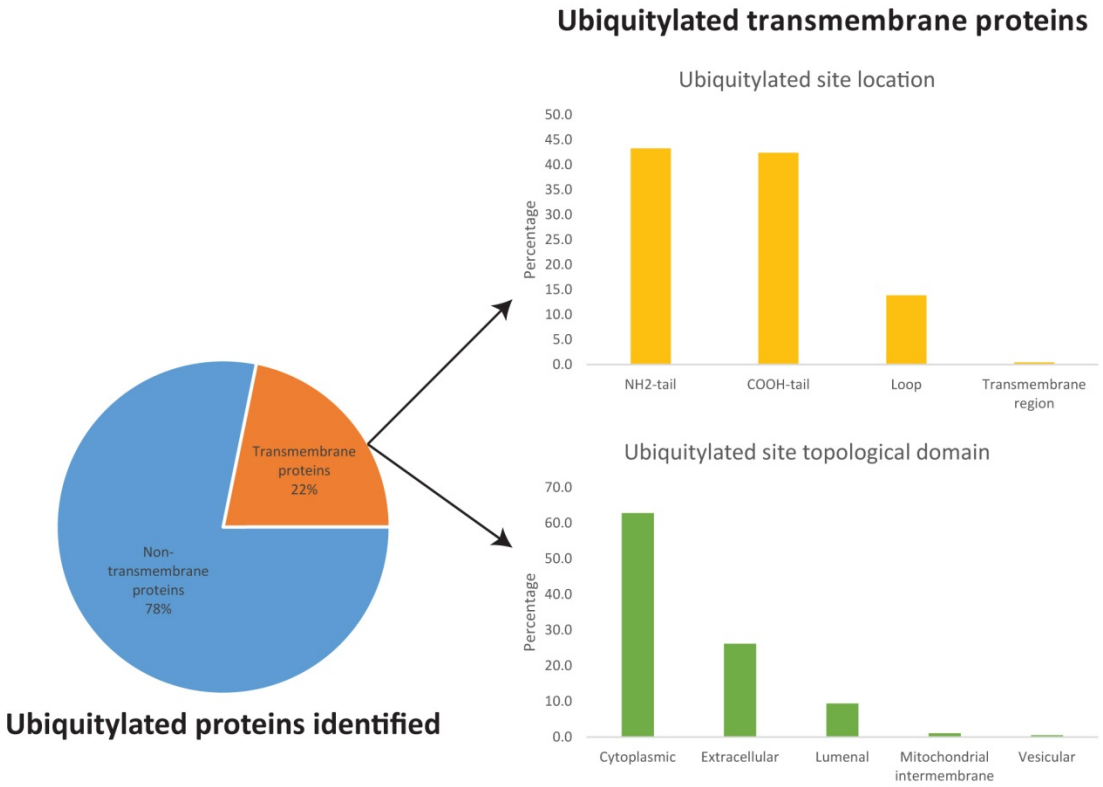
Supplemental Figure 2. Summary of Nanosight analysis of various different purifications of low-density membrane fractions from human urine. The mean diameter of 89.4 ± 2.52 nm (SEM) supports the classification of these fractions as exosomes. No particles were detected above 270 nm in size.



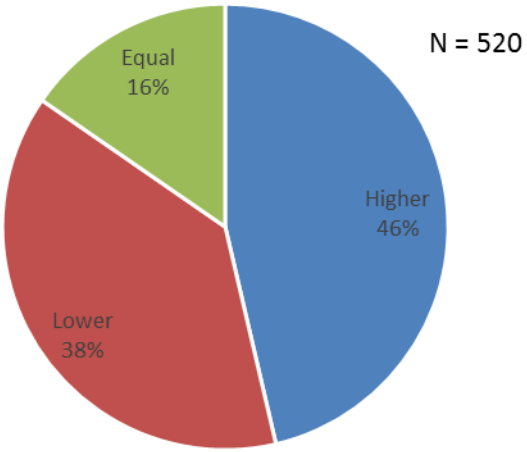
Supplemental Figure 3. Flow diagram of proteomic techniques for large-scale identification of ubiquitylated proteins in human urinary exosomes.



Supplemental Figure 4. Additional features of the ubiquitylated peptides identified.



Supplemental Figure 5. Comparison of the molecular masses of ubiquitylated forms identified relative to the unmodified form for each ubiquitylated protein



Supplemental Figure 6. Schematic for multivesicular body (MVB) formation in renal epithelial cells followed by ILV formation, MVB exocytosis and exosome secretion into urine. Apical and basolateral plasma membrane proteins, both ubiquitylated and non-ubiquitylated, are endocytosed and sorted in the endosomal pathway. Fusion with the MVB outer membrane and internalization of the ubiquitylated cargo proteins into MVB lumen is assisted by endosomal sorting complexes required for transport (ESCRT)-protein machinery (ESCRT-0, ESCRT-I, ESCRT-II, and ESCRT-III), VPS4, and ALIX. In this novel model, deubiquitylation of the ubiquitylated cargo proteins during ILV formation is not an essential step in exosome formation. Thus, a variety of ubiquitylated forms of cargo proteins (i.e. mono-, multi-mono- and polyubiquitylated forms) are present in ILVs and exosomes. During the invagination of the MVB outer membrane to form an ILV, ubiquitylated and non-ubiquitylated cytosolic proteins can enter and become trapped inside the ILV lumen. Non-ubiquitylated cargo proteins might also get recruited to MVB via an ESCRT-independent mechanism.