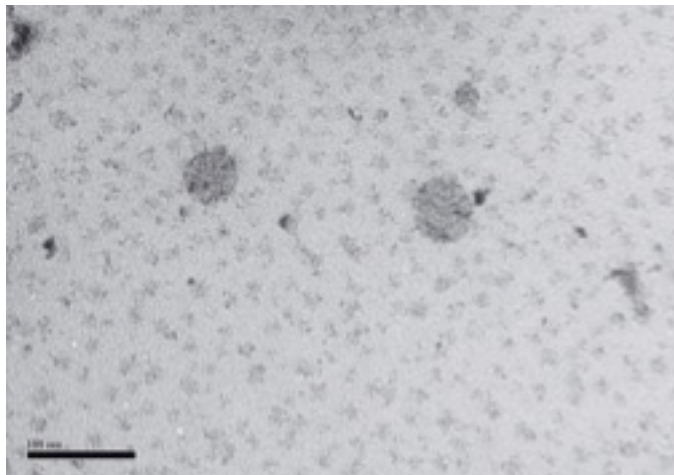


Vasopressin regulates extracellular vesicle uptake by kidney collecting duct cells

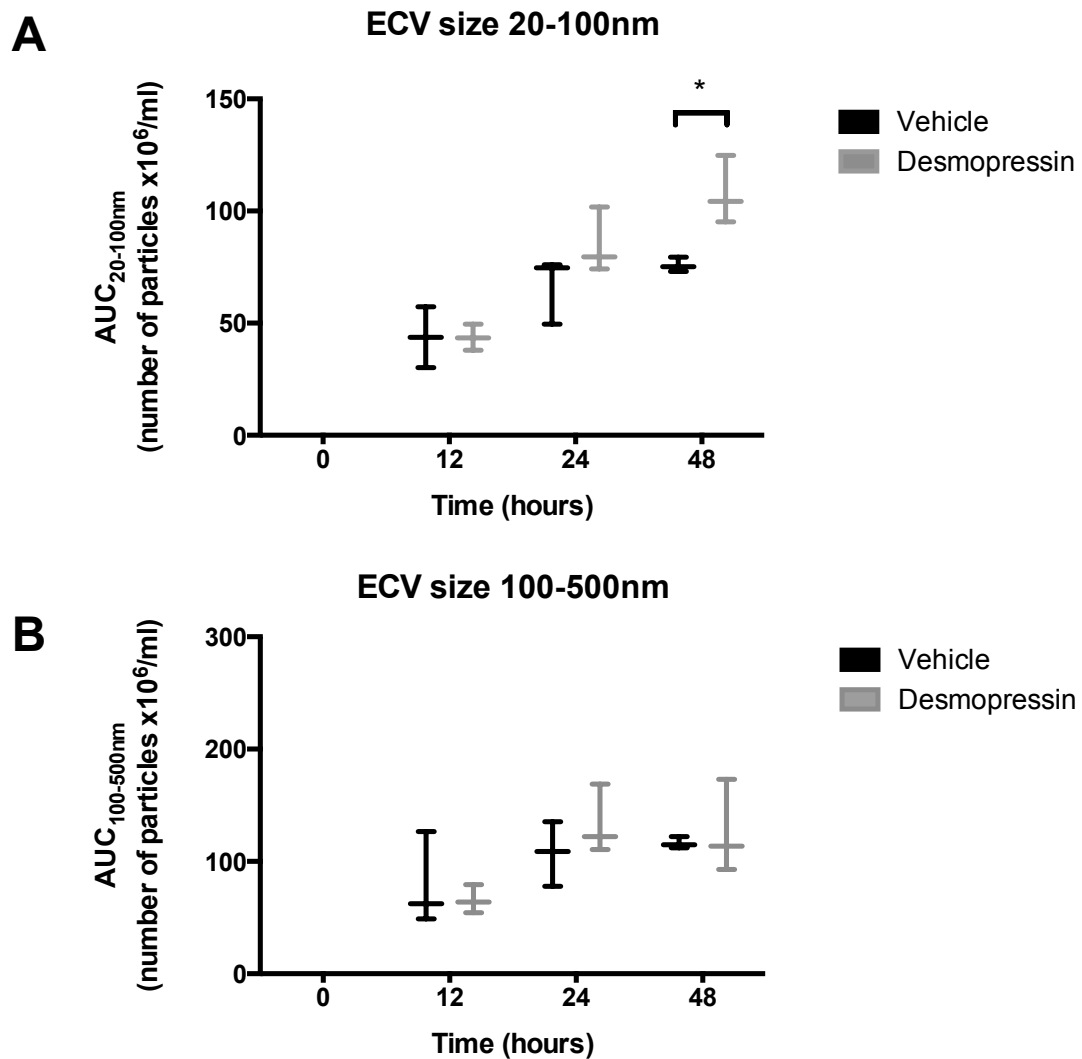
Authors: Wilna Oosthuyzen, Kathleen M Scullion, Jessica R. Ivy, Emma E Morrison, Robert W Hunter, Philip J Starkey Lewis, Eoghan O'Duibhir, Jonathan M. Street, Andrea Caporali, Christopher D. Gregory, Stuart J Forbes, David J. Webb, Matthew A. Bailey and James W. Dear

Supplementary figure 1



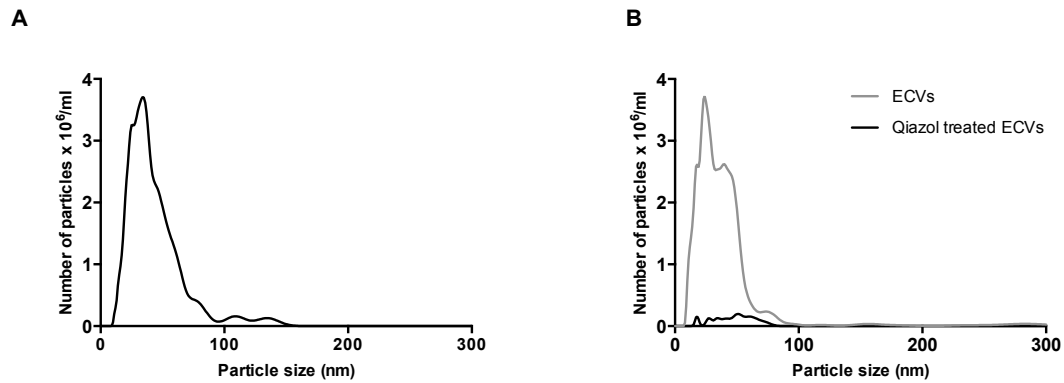
Supplementary figure 1. Structures of exosome size and shape are visible in the mCCD_{C11} cell culture medium using transmission electron microscopy. Bar, 100 nm.

Supplementary figure 2



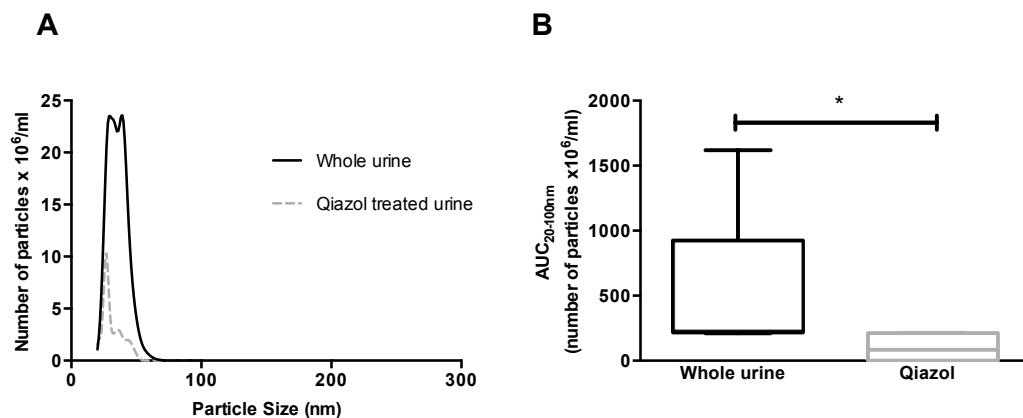
Supplementary figure 2. ECVs of exosome size are released from mCCDC₁₁ cells following stimulation by desmopressin. A) Number of particles in the supernatant from mCCDC₁₁ cells stimulated with desmopressin (3.16ng/ml) for the time indicated. Particles measured by NTA. Data are presented as the area under the concentration curve (AUC) for particles sized between 20-100nm. n=5. *p=0.02. B) Number of particles in the supernatant from mCCDC₁₁ cells stimulated with desmopressin (3.16ng/ml) for the time indicated. Particles measured by NTA. Data are presented as the area under the concentration curve (AUC) for particles sized between 100-500nm. n=5.

Supplementary figure 3



Supplementary figure 3. Kidney collecting duct cell ECVs were loaded with fluorescent Cell Tracker label and had an exosomal size distribution, as measured by NTA (A). Membrane disruption with QIAzol cell lysis reagent substantially reduced this NTA signal, consistent with fluorescent loading of membrane-bound ECVs (B).

Supplementary figure 4



Supplementary figure 4. Particles of exosome size are present in mouse urine after intravenous injection of fluorescently-loaded ECVs derived from mCCD_{C11} cells. Treatment of the urine with QIAzol cell lysis reagent substantially reduced this NTA signal, consistent with the presence of fluorescently-loaded membrane-bound ECVs. A). NTA trace demonstrating particle size and concentration. B) Tukey plot of urine before and after QIAzol treatment. Data are presented as the area under the concentration curve (AUC) for particles sized between 20-100nm. n=5. *p<0.05.