Appendix A: Supplemental Methods

Measurement of EV in plasma

All blood collection was approved by The Royal Veterinary College Ethical Review Board. Venous blood was collected into EDTA tubes and removed to the laboratory for processing as soon as possible. Plasma was centrifuged twice at 2,500 xg for 15 min and the supernatant was decanted and stored at -80°C until use. 5µL plasma was diluted with 45µL Annexin V buffer (eBioscience, Thermo Fisher, Dartford UK) together with 2µL Cy7.7-labelled Annexin V (eBioscience). For feline samples 2µL fluorescently conjugated cross-reactive antibodies against cell surface markers were added (anti human CD235-FITC, anti human CD61-APC, both from eBioscience). Samples were incubated for 15 min on ice before addition of 450 µL Annexin V buffer. 1.1µm latex sizing beads (Sigma, Poole, UK) and 10µL Flowcount Fluorospheres (Beckman Coulter, Hemel Hempstead UK) were added before analysis on a Becton Dickinson FACS CANTO II and Diva analysis software (Becton Dickinson, Oxford UK). Please see Figure 2A for gating strategy. Briefly, events that were 200-1000nm were gated and then analyzed for Annexin V (PS) staining and presence of CD235a (Figure 2d) or CD61 (Figure 2e). Numbers of events in each region were calculated based on the numbers of events counted in an "enumeration gate" based on the number of Flowcount beads calibrated by the manufacturer and according to the equation

Number of gated events of interest per microliter =

$$\frac{\textbf{20 x \#gated MV events x dilution factor of sample}}{\textbf{\# enumeration beads counted (in gate)}}$$

Isolation of exosomes from cow's milk

Milk was obtained from Holstein Friesian cows housed at Bolton's Park Farm (The Royal Veterinary College, Potters Bar, UK). The samples were transported back to the laboratory. 20µL of EDTA was added before centrifugation at 1500×g for 5 minutes. This centrifugation step skimmed the milk, resulting in a layer of cream on the top which was removed. A protocol was optimized for the isolation of exosomes from fresh bovine milk. Fourteen ml of the milk was transferred into a 15ml tube for centrifugation at 2000×g at 4°C for 10 minutes (Thermo Scientific Haraeus^{TM} Multifuge^{TM} X3 FR). Any residual cream forming on the top was removed and a white pellet (possibly formed of proteins and fats) was observed at the bottom of the tube. The supernatant was removed and transferred into Beckman Polyallomer tubes**.** The samples were centrifuged at 17,000×g for 15 minutes at 4 °C in Beckman-Coulter Optima^{TM} L-80 ultracentrifuge (Sw-40-Ti rotor). Acceleration throughout the ultracentrifugation steps was set high, whilst deceleration was set to low. A solid, white pellet was observed on the bottom of the tube. The supernatant was carefully removed and transferred into a clean tube. This ultracentrifugation step was repeated three times until the liquid appeared clear. 10 ml of the supernatant from the final centrifugation step was layered onto a 30% sucrose (Sigma, UK; dissolved in 1xPBS) cushion. These samples were then centrifuged at 100,000×g for 70 minutes at 4°C. A cloudy floating pellet formed over a gelatinous yellow/white pellet on the bottom of the tube. This floating pellet was expected to contain the exosomes and was transferred into a clean ultracentrifugation tube. The tube was filled up with ice-cold 1x PBS and centrifuged again at 100,000×g for 60 minutes at 4°C to purify the exosomes. The resulting floating pellet was

removed and filtered with a 0.22µm filter. The samples were diluted 1:5000 in 1x PBS for analysis by Nanosight LM-10 (Malvern, Malvern UK).

Preparation of EV from Zebrafish embryos

Five zebrafish embryos at 96h post fertilization were placed into an Eppendorf tube with 100µL 1x PBS and homogenized using a single use mini homogenizer (Thermo Fisher). The homogenate was centrifuged at 3,000 xg 5 min 4°C to pellet cellular material and the supernatant was transferred to a clean tube for analysis by Nanosight LM-10 (Malvern, Malvern UK).