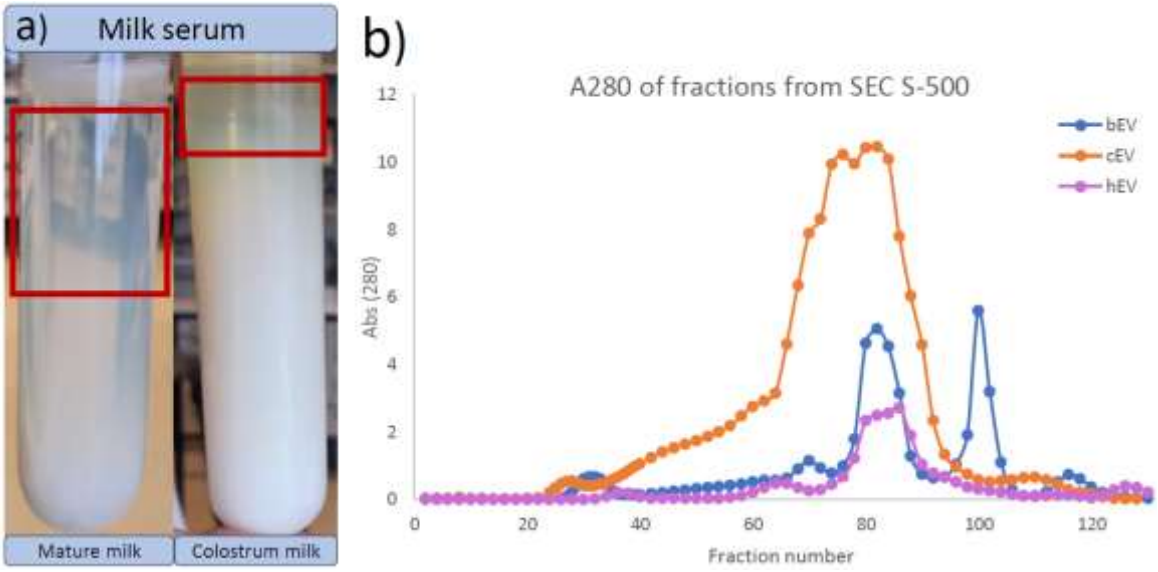
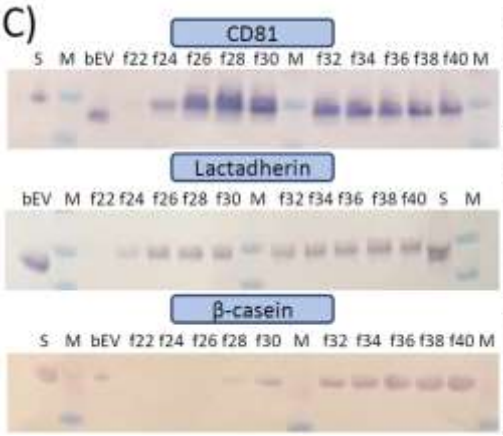
Supplementary Material

Isolation of extracellular vesicles





D) NTA measurements

Sample	Mean EV size (nm)	Particle/mg protein in EV isolates
bEV	186.8 ± 7.0	5.68E+12
cEV	168.3 ± 1.4	1.42E+12
hEV	255.7 ± 4.1	2.63E+12
Wet wEV	n.d.	n.d.
Dry wEV	n.d.	n.d.
Wet mcEV	153.3 ± 3.0	1.14E+12
Dry mcEV	n.d.	n.d.
ProLip	123.0 ± 0.7	4.76E+11

Figure S1. Analysis of EV isolations. (a) Pictures of milk after the 2. centrifugation step. The red box indicates where the milk serum was collected. (b) SEC S-500 elution profiles of mature milk serum (blue graph), human milk serum (purple graph) and colostrum serum (orange graph). The S-500 SEC column ran with a flow of 32 ml/hour and a fraction size of 4.5 ml. (c) Western blot of colostrum fractions 22-40 after SEC (the first peak). Equal volumes of the given fractions were run in each lane. Abbreviations: S, colostrum serum; M, marker (kDa). (d) Particle to protein ratios are calculated from total number of particles and protein concentration in EV isolates. All nano particle tracking analysis data are averages from five measurements on each sample. n.d.: not determined.

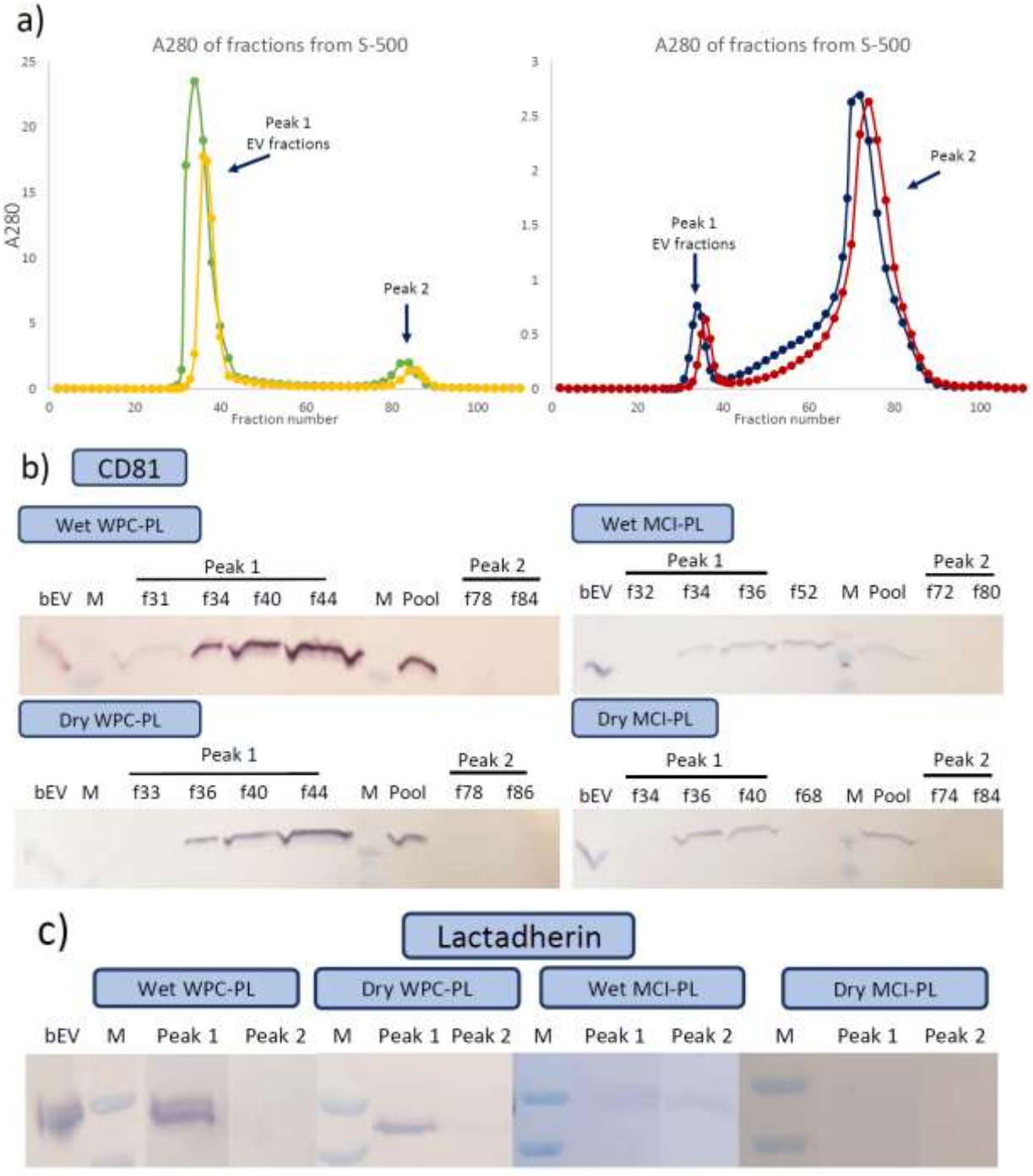


Figure S2. Analysis of EV isolation from wet and dry WPC-PL and MCI-PL. (a) SEC elution profiles of the following samples: wet WPC-PL (green) and dry WPC-PL (yellow) and wet MCI-PL (blue) and dry MCI-PL (red). The S-500 SEC column ran with a flow of 32 ml/h and a fraction size of 4.5 ml. (b) Western blot stained for CD81, made over the peaks on the chromatogram. 6 μg protein was put on the gel. Abbreviations: M, marker (kDa); Pool, all fractions of peak 1 pooled together. (c) Western blot over the two peaks from all EV isolations from AFI samples, stained against lactadherin.

Complete Western Blots used for figure 5

Iκ-Bα **β-actin**

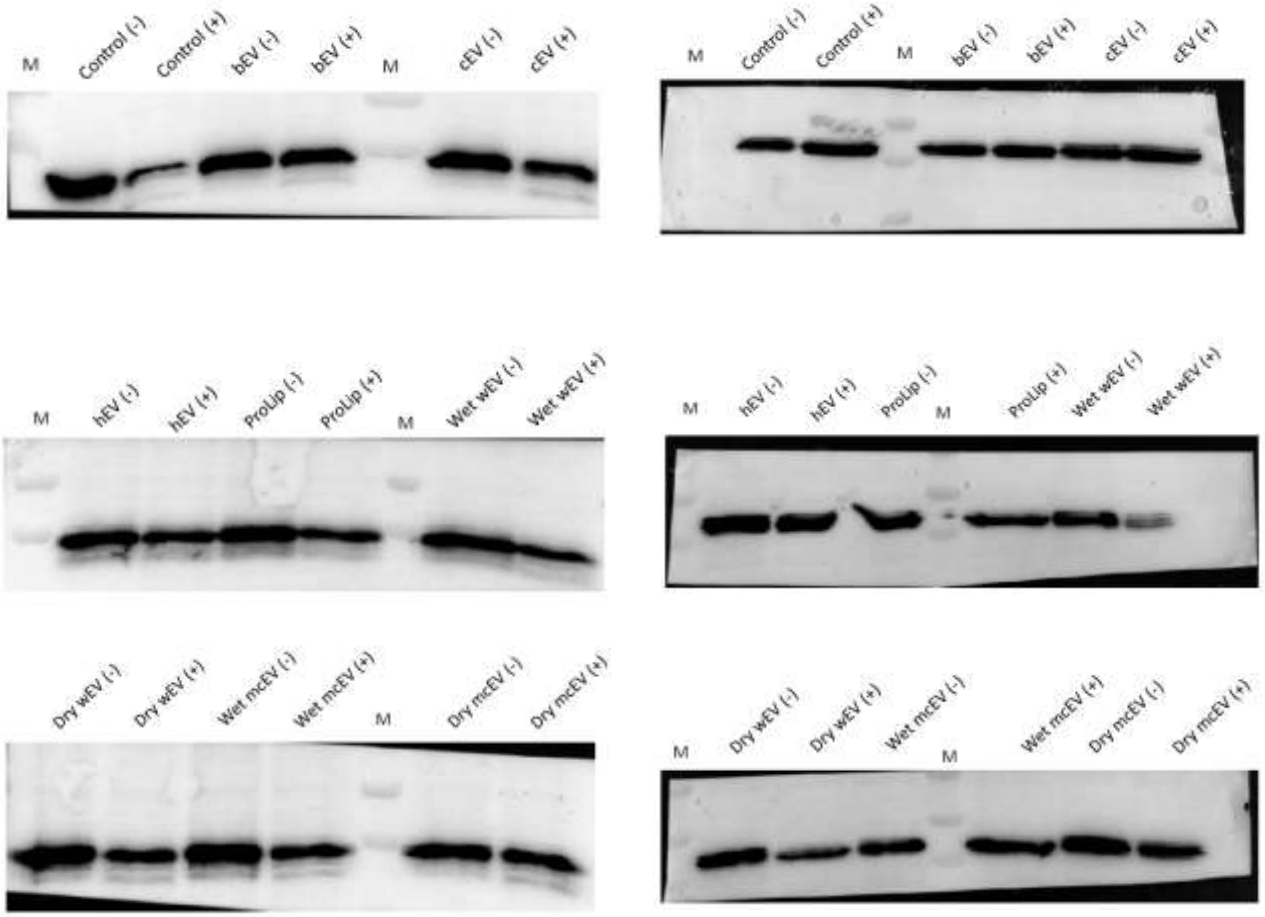


Figure S3. Total western blots used in figure 5. The left column shows the western blots stained against Iκ-Bα. The right column shows western blots used as controls; these are stained against the classical loading control β-actin. Before incubation with the primary antibody the membranes were cut into smaller sizes. This representative data is the result of minimum two independent experiments.