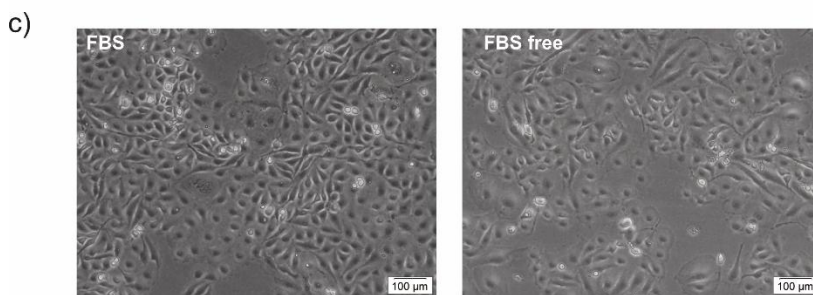
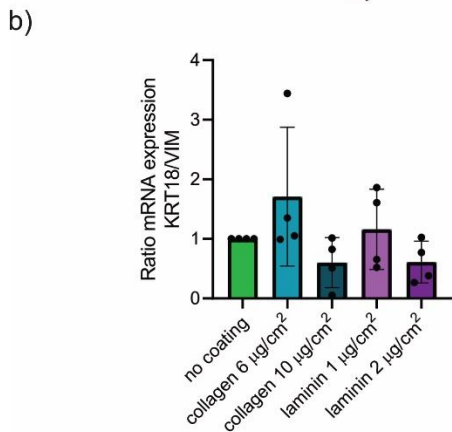
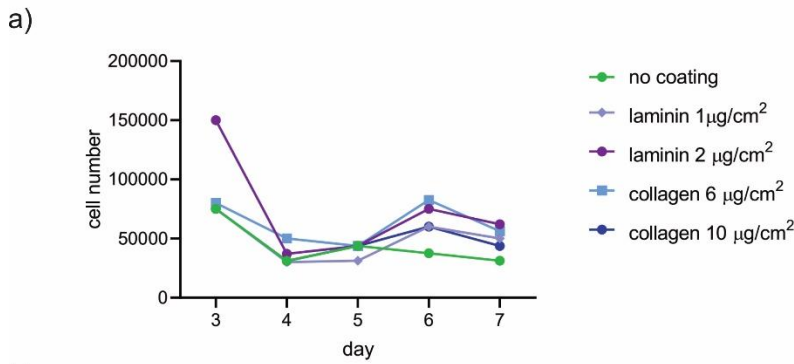


1 **Supplementary Material: Assessing extracellular vesicles from**
 2 **bovine mammary gland epithelial cells cultured in FBS-free**
 3 **medium**

4 **Supplementary Figure 1**



5

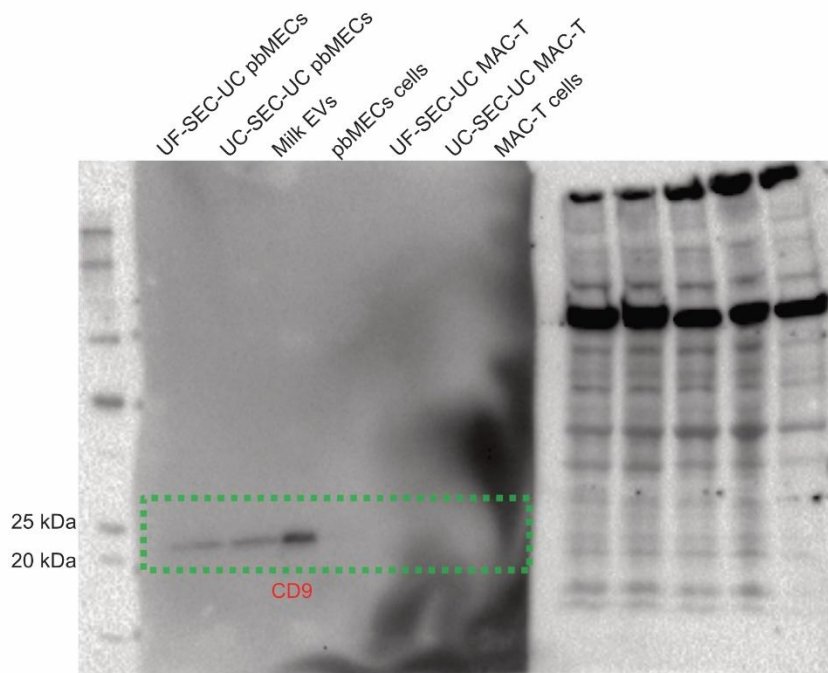
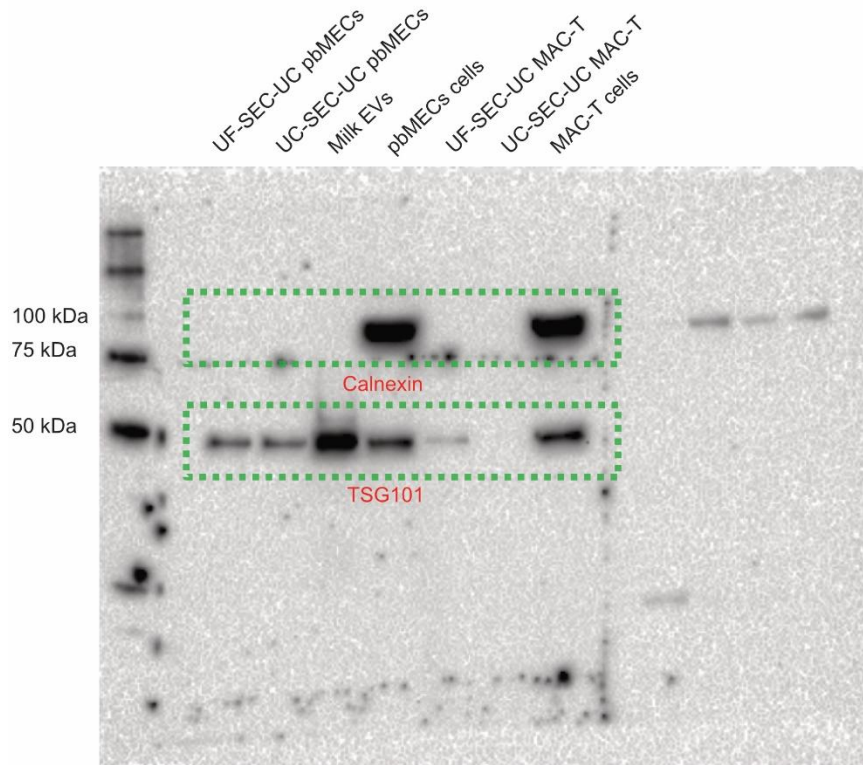
6 **Supplementary Figure 1.** a) Growth curve of pbMECs in FBS-free medium. Cells were plated on
 7 6-well multiwell dishes and counted every day from day 3 to day 7; the culture dishes were uncoated or
 8 coated with laminin 1 or 2 µg/cm² or collagen I 6 or 10 µg/cm²; b) Ratio of the mRNA expression of
 9 KRT18/VIM of pbMECs at 80% confluence plated in coated or coated wells as in a); c) MAC-T cells
 10 grown in FBS 10% or FBS-free medium.



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11 **Supplementary Figure 2**



12

13 **Supplementary Figure 2.** Full-length western blots shown in figure 4c. The selected areas are in green.

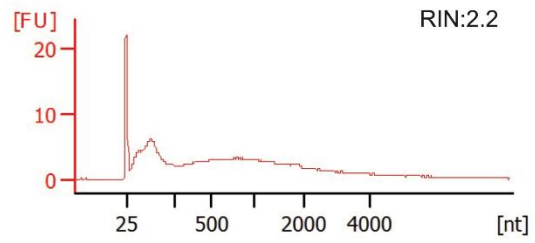
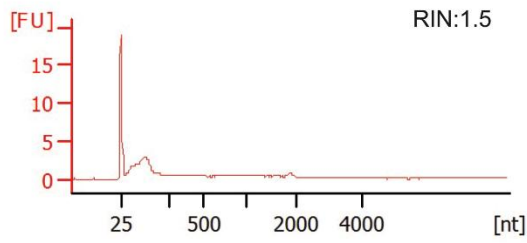
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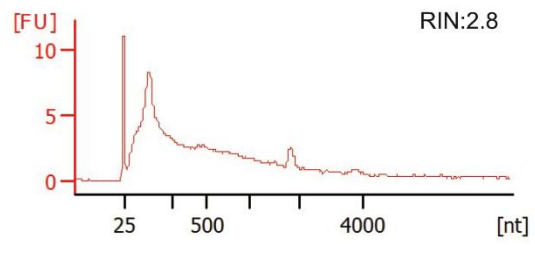
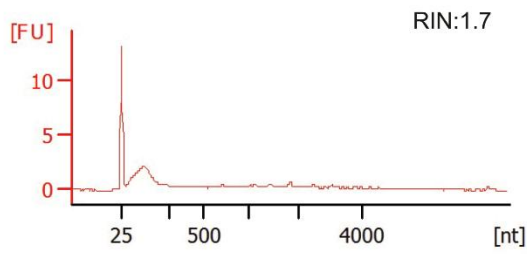
16 **Supplementary Figure 3**

a) pbMEC

UF-SEC-UC

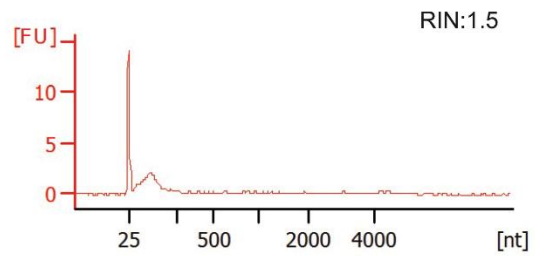
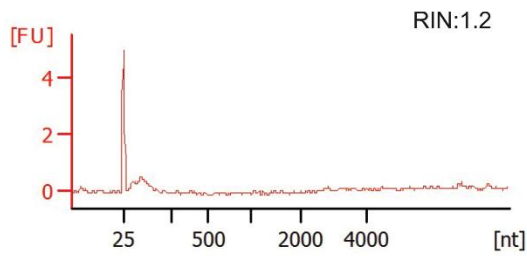


UC-SEC-UC

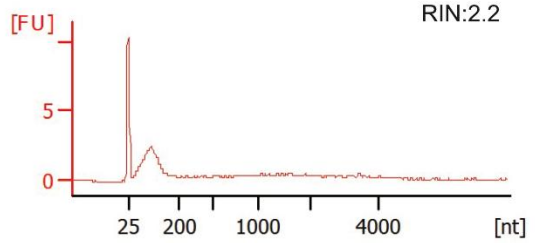
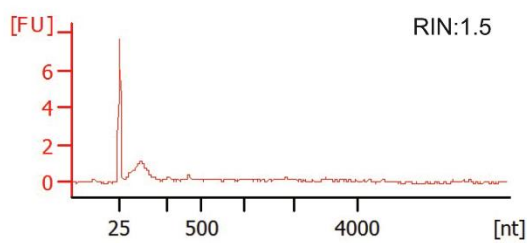


b) MAC-T

UF-SEC-UC



UC-SEC-UC



17

18 **Supplementary figure 3.** Agilent 2100 Bioanalyzer data from EVs RNA isolated from pbMEC (a) or
19 MAC-T (b) with either UF-SEC-UC or UC-SEC-UC

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24 **Supplementary Table 1**

25 **Table 1.** Summary of TRPS measurements. The data come from one replicate and were measured with a nanopore
 26 NP150.

Route	Pellet resuspension volume [μL]	Raw concentration [particles/mL]	Size range	Mean particle size [nm] \pm SD	Particle rate [particles/min]
UCx2	30	4.39E+11	67-512	119 \pm 39.7	192.8
SEC-UC	30	-	-	-	-
CP	100	2.95E+09	108-781	195 \pm 70.1	119.5
CP-SEC-UC	100	4.99E+08	123-708	210 \pm 89.4	20.02
UF-SEC-UC	30	5.30E+10	70-473	139 \pm 36.5	277.4
UC-SEC-CP	30	1.47E+10	81-564	151 \pm 70.0	701.2
UC-SEC-UF	150	-	-	-	-
UC-SEC-UC	30	6.87E+09	77-559	158 \pm 60.8	327.0

27