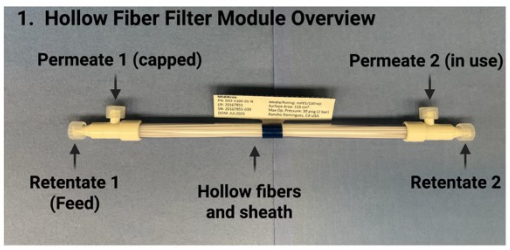
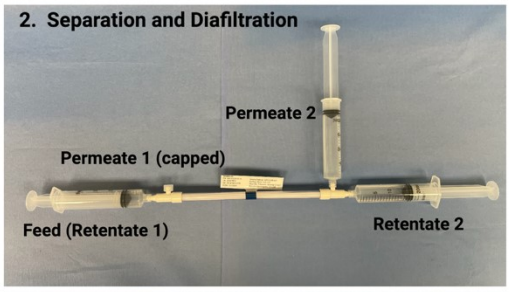
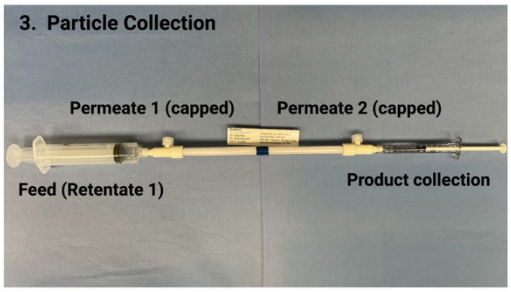


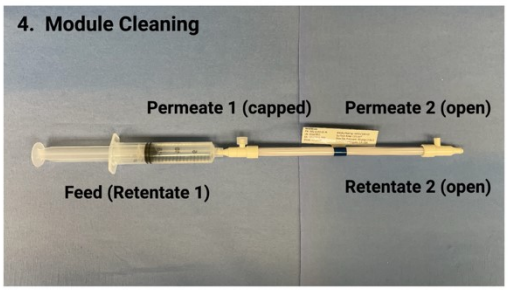
Each connection point is a female Luer-Lok port that will fit any common male Luer-Lok syringe. Permeate 1 is secured with a Luer-Lok cap and remains closed for all process steps. A bundle of hollow fibers is secured within the module sheath and carries out the separation.



Conditioned media or buffer (for diafiltration) is introduced into the Feed port (Retentate 1) via syringe. Two additional syringes are attached at Permeate 2 and Retentate 2. The filtration occurs by actuating Retentate 1 and Retentate 2 syringes back and forth until the Permeate 2 syringe is full. With the exception of the last filter module (100 kD, F4), all permeate fluid is collected and fed to the next filter of the series for further separation. A few milliliters of retentate fluid should always be kept in one retentate syringe to avoid large amounts of product loss during syringe change-over. For diafiltration, introduce 8-10 diavolumes of PBS-H into the feed syringe and collect permeate fluid until the retentate volume is ~2-3 mL in the feed syringe.

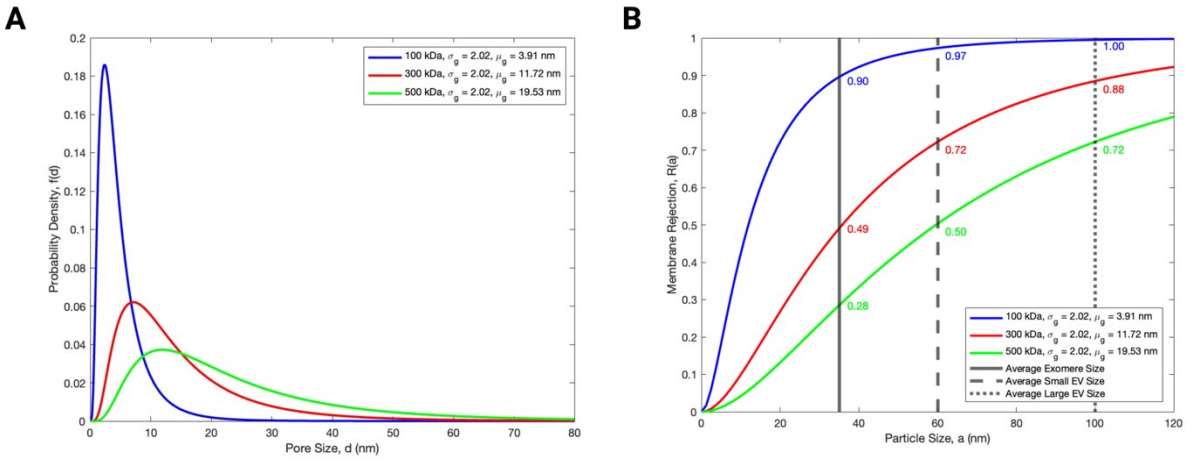


After diafiltration, replace the retentate 2 syringe with a 1 mL Luer-Lok syringe for particle collection. Tilt the filter module toward the product collection syringe and actuate only the 1 mL syringe to collect the particle suspension. Repeat this process until only air is drawn from the filter module.

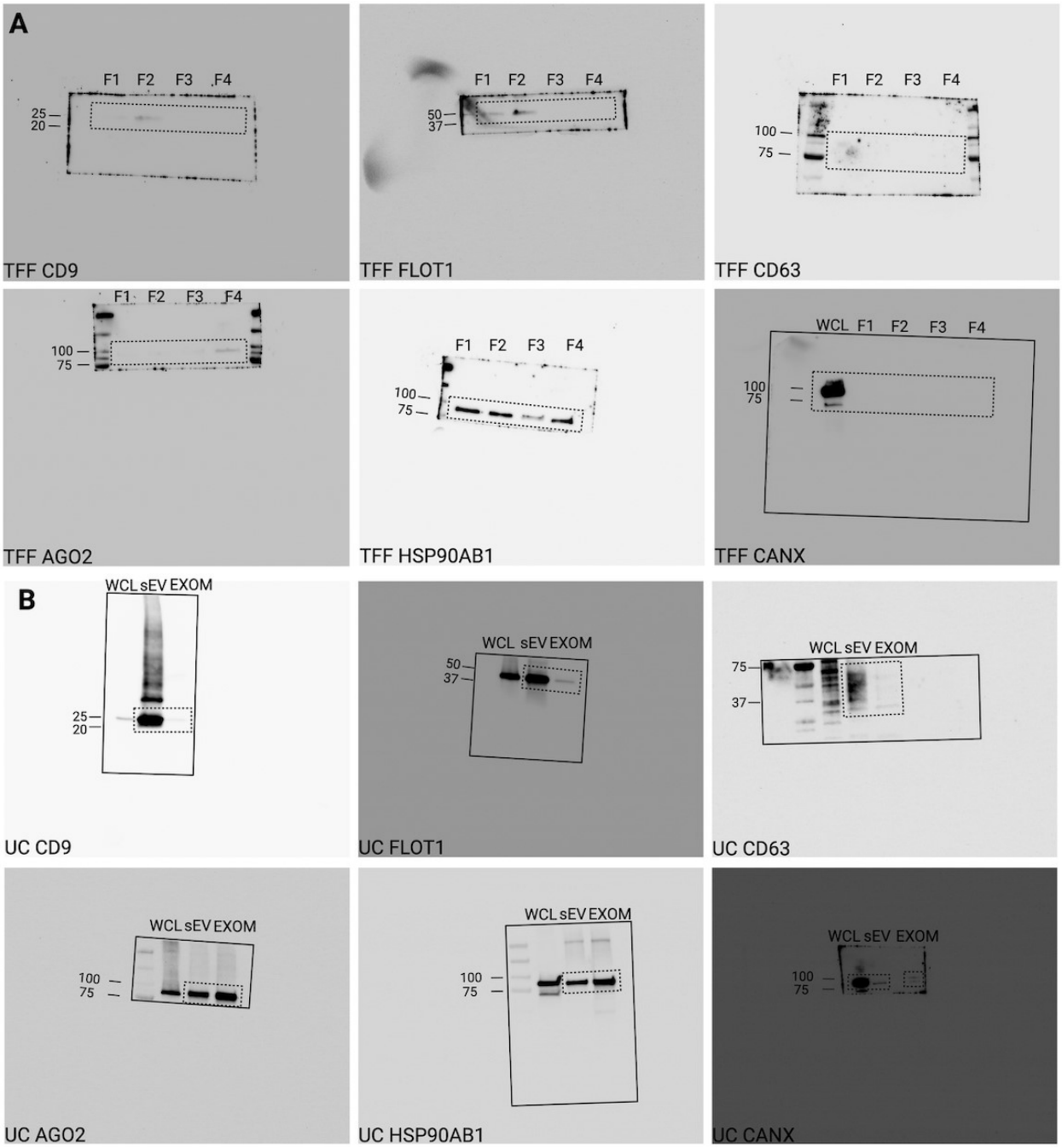


To clean the filter modules after use, remove the Permeate 2 and Retentate 2 syringes and introduce the appropriate cleaning solutions via the Feed port. Dispense over a waste container.

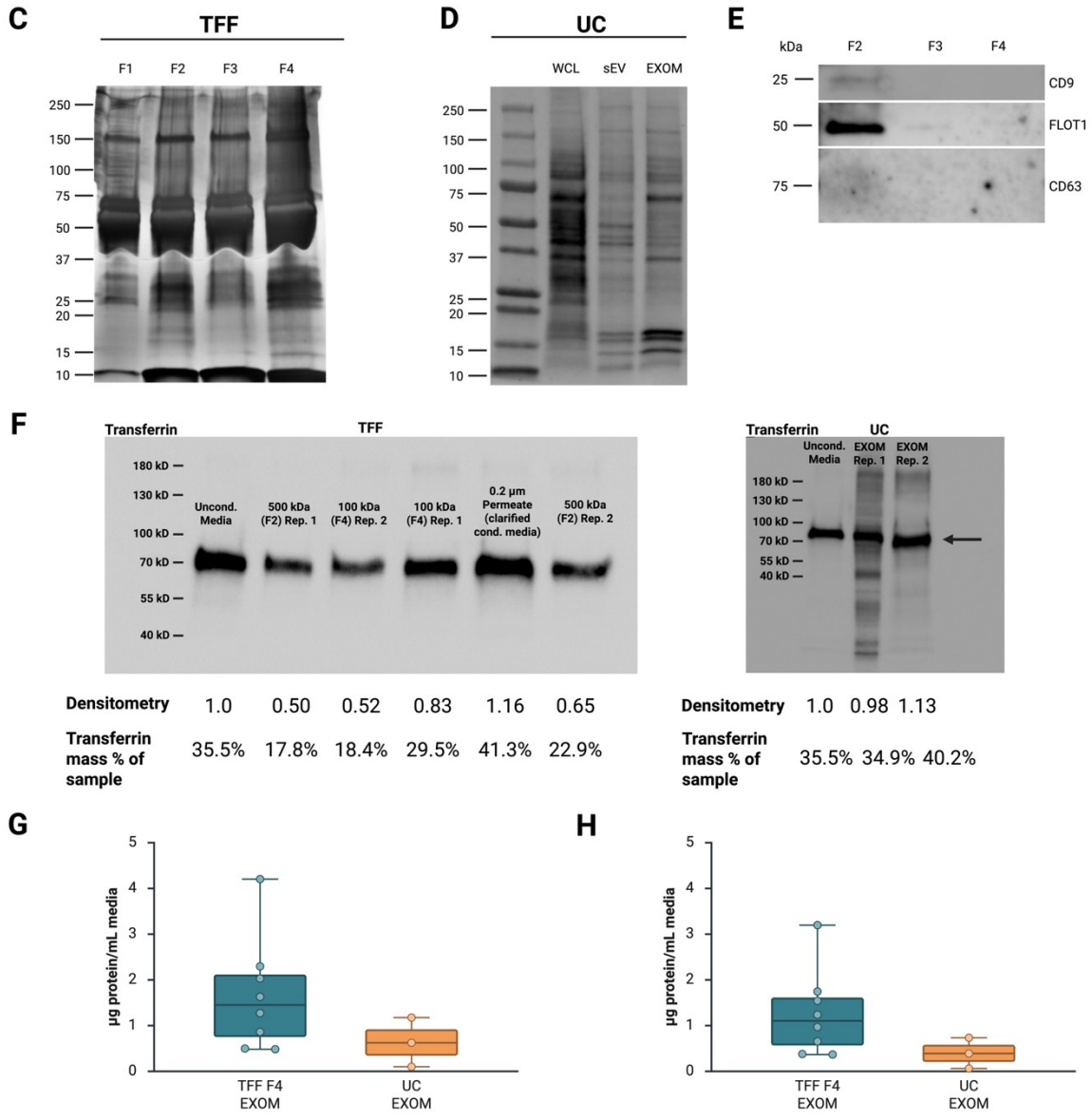
Supplementary Figure 1: TFF process setup for separation, diafiltration, particle collection, and module cleaning.



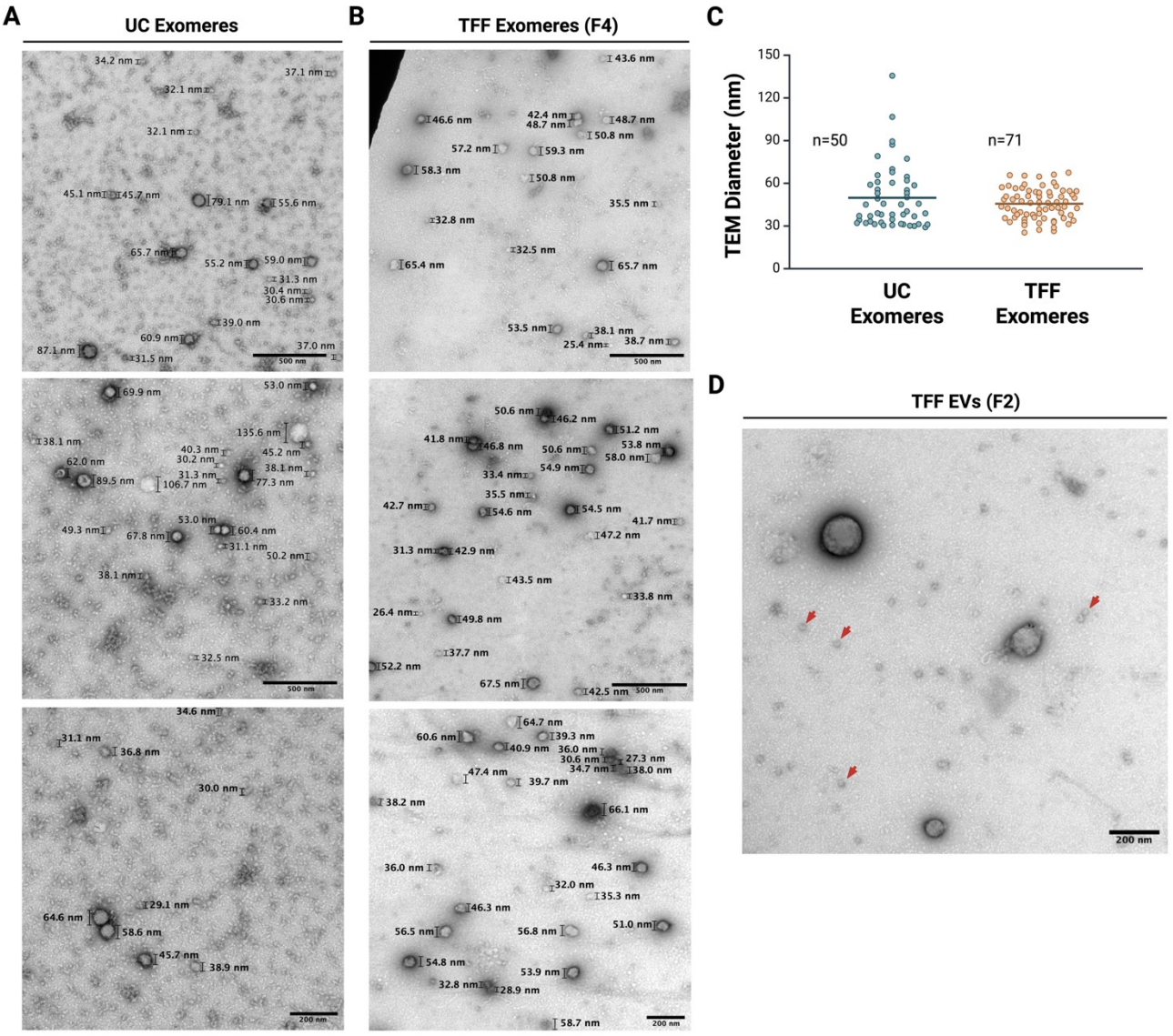
Supplementary Figure 2: Filter rejection predictions using log-normal models previously described.[1] **A.** Probability density functions showing the theoretical pore size distribution for 100, 300, and 500 kDa MWCO membranes. **B.** Membrane rejection performance for particles with a size of 35 nm (exomeres), 60 nm (sEVs), and 100 nm (LEVs). The value chosen for geometric standard deviation (σ_g) was adopted from prior literature based on measurements of asymmetric, modified polyethersulfone (PES) membranes which well-represent the commercial membranes used in this study.[2] Geometric means (μ_g) were calculated from σ_g and the mean pore diameter reported by the manufacturer.



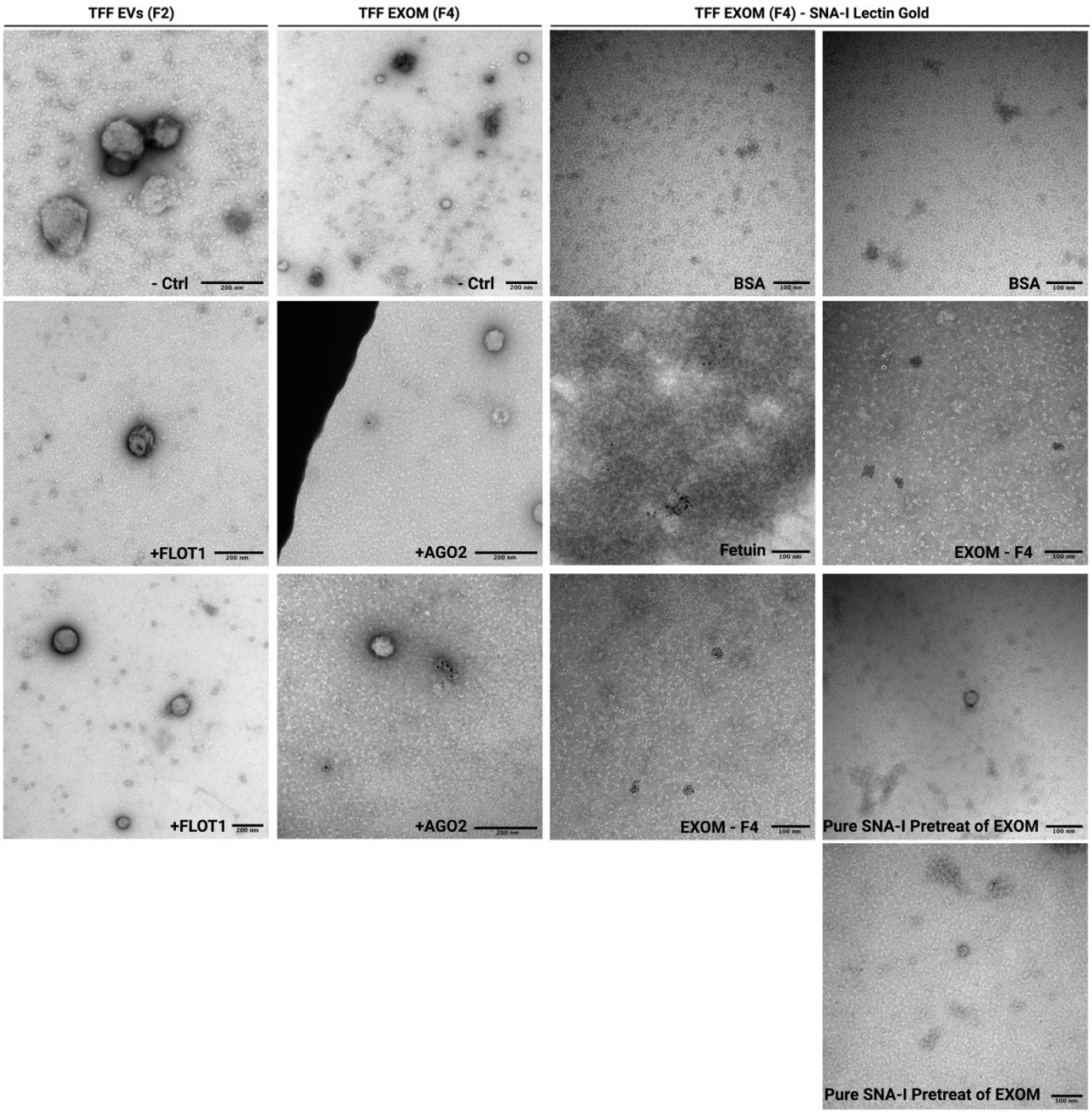
Supplementary Figure 3: Unprocessed Western blots for **A.** Tandem TFF and **B.** Differential UC. Dotted boxes represent the regions of the blots presented in **Fig. 2**. Solid boxes represent the outline of the complete blot.



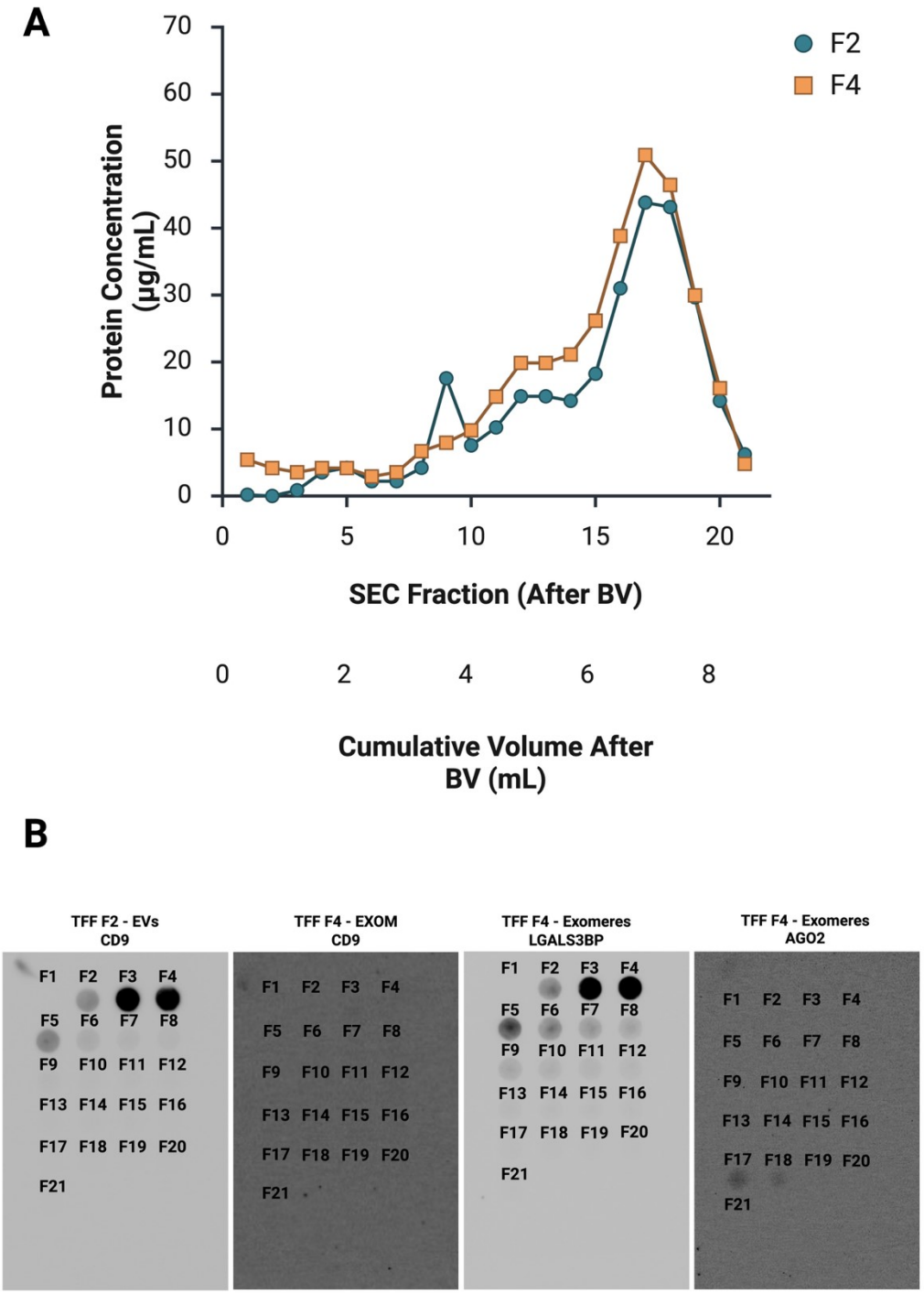
Supplementary Figure 3 (continued): Total protein analysis of TFF and UC isolates. **C**. Silver staining for TFF fractions and **D.** Revert700 fluorescent staining for UC fractions. **E.** Femto ECL Western blot showing absence of EV markers in NVEP fractions F3 and F4 in comparison to EVs in F2. **F.** Transferrin Western blots, densitometry, and abundance in TFF and UC samples by mass percent. 7.5 μg of total protein were loaded per lane. **G.** Total protein content per mL of conditioned media processed for UC and TFF exomeres. **H.** Total protein content in TFF and UC exomere fractions after adjusting for copurifying transferrin. Figure created with BioRender.com.



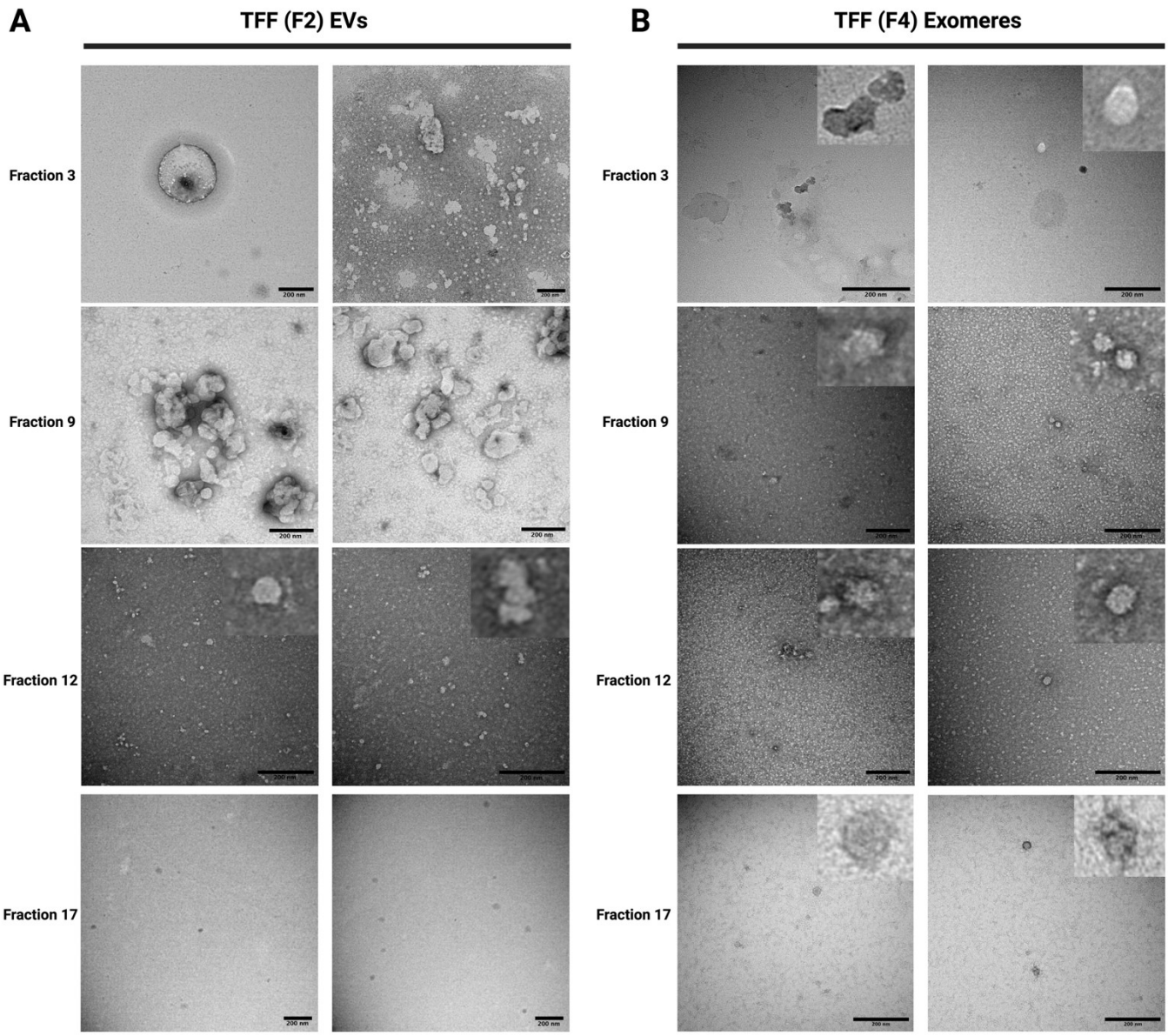
Supplementary Figure 4: TEM micrographs of particles measured for **A.** UC exomeres and **B.** TFF exomeres (F4). **C.** Size distribution of particles within each fraction based on manual measurement using the analyze > measure feature in ImageJ 1.54g (Java 1.8.0_345). **D.** Exomere-shaped particles co-purify in TFF EV fraction F2. Figure created with BioRender.com.



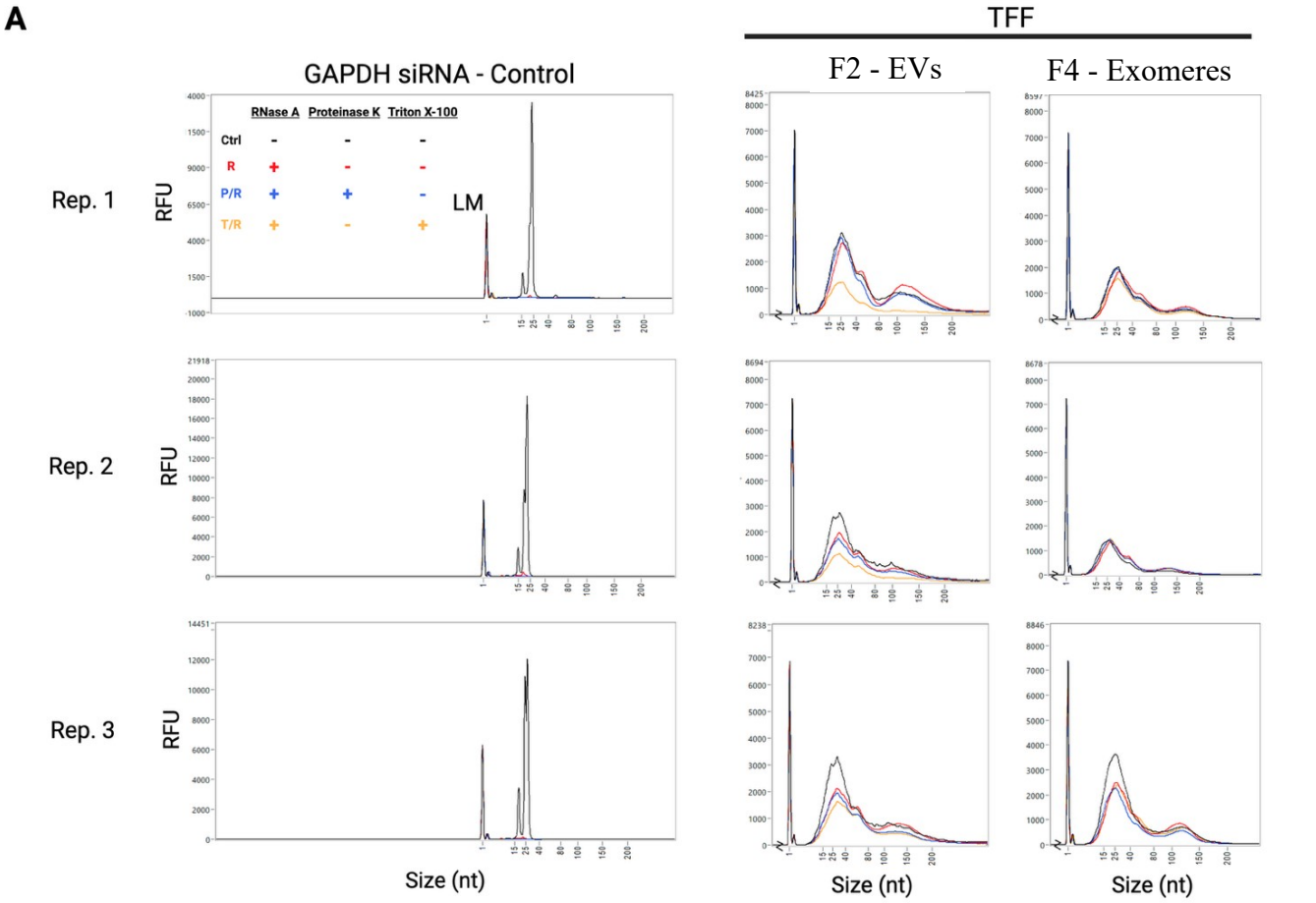
Supplementary Figure 5: Unprocessed TEM micrographs for immunogold and SNA-I lectin gold experiments.

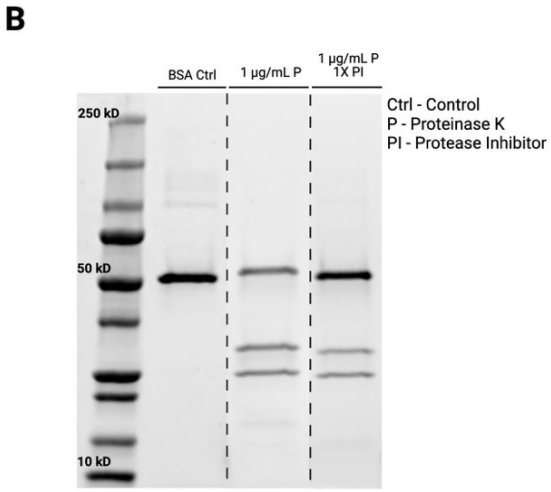


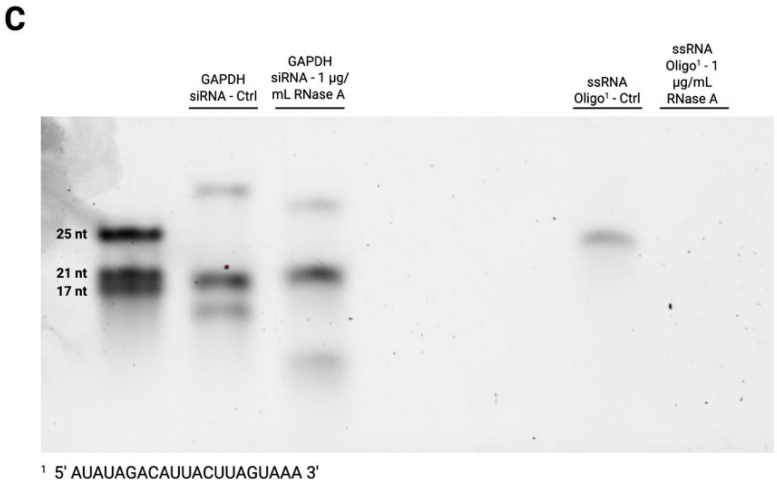
Supplementary Figure 6: Size exclusion chromatography (SEC) of TFF fractions F2 and F4. **A.** Protein concentration of SEC fractions as measured by BCA. 2 mL of buffer (void) volume (BV) was collected before elution of particle-containing fractions. **B.** Dot blots of SEC fractions for TFF F2 EVs and F4 exomeres. Blot brightness was uniformly adjusted in ImageJ 1.54g (Java 1.8.0_345) to visualize weaker signals. Figure created with BioRender.com.



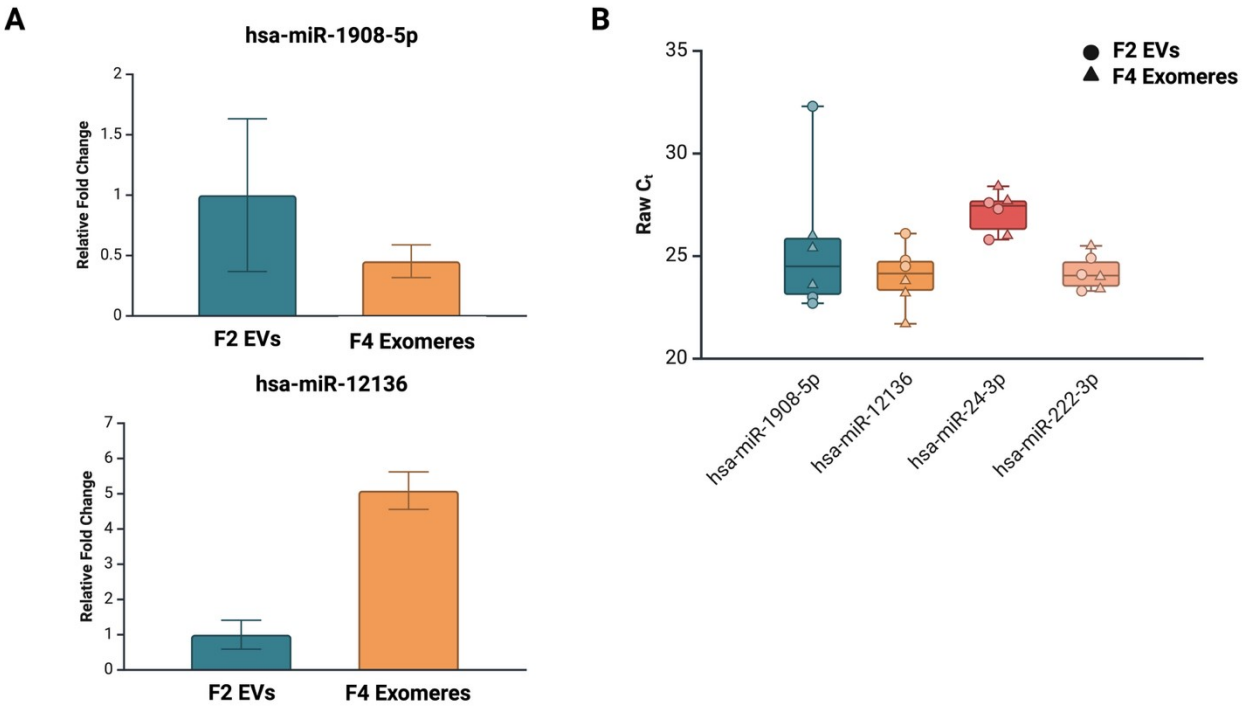
Supplementary Figure 7: TEM of SEC fractions for TFF F2 EVs (**A**) and F4 exomeres (**B**).







Supplementary Figure 8: A. RNA protection assay results representing three biological replicates. Treatment conditions: Ctrl (Control), R (RNase A only), P/R (Proteinase K/RNase A), T/R (Triton X-100/RNase A). **B**. Coomassie blue-stained PAGE gel of BSA treated with Proteinase K or Proteinase K and inhibitor. Gel was cropped to remove conditions not used in the RNase protection experiment. **C.** SYBR™ gold-stained PAGE gel of dsRNA and ssRNA treated with RNase A.



C

Normalized Read Counts (Median of Ratios)							
miR	**F2 EVs - 1**	**F2 EVs - 2**	**F2 EVs - 3**	**F4 EXOM - 1**	**F4 EXOM - 2**	**F4 EXOM - 3**	**baseMean**
hsa-miR-1908-5p	7188.83	3132.79	11269.26	298.90	541.99	375.06	3801.14
hsa-miR-12136	74.93	122.28	125.30	214.87	538.92	494.58	261.81
hsa-miR-24-3p	194.82	127.17	29.48	121.24	127.38	83.20	113.88
hsa-miR-222-3p	1147.94	1261.92	978.41	1162.58	1391.40	1170.08	1185.39

Supplementary Figure 9: RT-qPCR validation of differentially and stably expressed miRNAs. **A.** Relative fold change calculated using the $\Delta\Delta C_t$ method. F2 EVs were taken as the basis. The geometric mean of hsa-miR-24-3p and hsa-miR-222-3p was used for normalization of each sample. **B.** Raw C_t values for DE miRs and putative reference miRs. **C.** Normalized mean read counts from RNA sequencing for the selected miRs. Figure created with BioRender.com.

Sample	Reference miRs C_t			Sample	DE miRs from RNA Seq C_t	
	hsa-miR-24-3p	hsa-miR-222-3p	Geo Mean C_t		hsa-miR-1908-5p	hsa-miR-12136
F2 EVs - 1	27.3	24.1	25.7	F2 EVs - 1	32.3	26.1
F2 EVs - 2	27.6	24.9	26.2	F2 EVs - 2	23.0	24.8
F2 EVs - 3	25.8	23.3	24.5	F2 EVs - 3	22.7	24.5
F4 Exomeres - 1	28.4	25.5	26.9	F4 Exomeres - 1	25.4	23.8
F4 Exomeres - 2	27.7	24.0	25.8	F4 Exomeres - 2	26.0	23.2
F4 Exomeres - 3	26.0	23.4	24.7	F4 Exomeres - 3	23.6	21.7

Sample	ΔC_t		Sample	$\Delta\Delta C_t$ (F2 EVs as basis)	
	hsa-miR-1908-5p	hsa-miR-12136		hsa-miR-1908-5p	hsa-miR-12136
F2 EVs - 1	6.6	0.4	F2 EVs - 1	6.1	0.8
F2 EVs - 2	-3.2	-1.4	F2 EVs - 2	-3.8	-1.1
F2 EVs - 3	-1.8	0.0	F2 EVs - 3	-2.4	0.3
F4 Exomeres - 1	-1.5	-3.1	F4 Exomeres - 1	-2.0	-2.8
F4 Exomeres - 2	0.2	-2.6	F4 Exomeres - 2	-0.3	-2.3
F4 Exomeres - 3	-1.1	-3.0	F4 Exomeres - 3	-1.6	-2.6

Avg. ΔC_t (F2 as basis)	0.5	-0.3

Sample	$2^{-\Delta\Delta Ct}$ (F2 EVs as basis)		Sample	Relative Fold Change	
	hsa-miR-1908-5p	hsa-miR-12136		hsa-miR-1908-5p	hsa-miR-12136
F2 EVs - 1	0.01447	0.58329	F2 EVs - 1	0.00233	0.49783
F2 EVs - 2	13.49239	2.12492	F2 EVs - 2	2.17271	1.81358
F2 EVs - 3	5.12293	0.80681	F2 EVs - 3	0.82496	0.68860
F4 Exomeres - 1	4.14042	6.88334	F4 Exomeres - 1	0.66674	5.87479
F4 Exomeres - 2	1.25052	4.77619	F4 Exomeres - 2	0.20137	4.07638
F4 Exomeres - 3	3.04107	6.22430	F4 Exomeres - 3	0.48971	5.31231

Average $2^{-\Delta\Delta Ct}$ (F2 EVs as basis)	6.20993	1.17167

Supplementary Figure 9 (continued): Relative fold change calculations for F2 EV and F4 exomere miRs validated with RT-qPCR.

References:

1. J. Ren, Z. Li and F.-S. Wong, *Journal of Membrane Science*, 2006, **279**, 558–569.
2. S. Singh, K. C. Khulbe, T. Matsuura and P. Ramamurthy, *Journal of Membrane Science*, 1998, **142**, 111–127.