Supplementary Material

#### Specification of isolated nanoparticles by qEVsingle size exclusion chromatography column

# qEVsingle® size exclusion chromatography column (SEC) was used to isolate EVs, and a total of 20 fractions were collected (each 200 µl) as mentioned in materials and methods section “Isolation of extracellular vesicles from follicular fluid”. The EV concentration was determined using ZetaView® nanoparticle tracking analyser (NTA). NTA analysis revealed that fraction 6-9 contains the highest number of nanoparticles/EVs (SU Figure 1) and no significant protein contamination. The fractions 1-5 contained no particles (void volume), whereas fractions from 10-20 contained fewer nanoparticles and relatively higher protein concentration. Based on these results, fraction 6-9 were pooled together, concentrated with Amicon® Ultra 2 centrifugal filter units (10 kDa), and were used for downstream experiments.

# C:\Users\mohamm92\Desktop\2nd project Human FF\pic and graph\EV-PROTEIN-2.tif

**Supplementary Figure S1**. Evaluating the profile of nanoparticles/EVs and protein concentration of fractions isolated on qEVsingle® column. Fraction 6-9 (EV fraction) contained the highest number of nanoparticles and relatively low protein contamination. Particle concentration was analyzed using ZetaView® nanoparticle tracking analyzer (NTA), and the protein concentration was measured using Quick Start™ Bradford Protein Assay

A screenshot of a cell phone

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**Supplementary Figure S2** Histograms of p-values with 1 000 samples drawn from the EV size distributions with sizes of (**A**) 100, (**B**) 1 000, (**C**) 2 000, and (**D**) 5 000. P-values indicating statistical significance are clearly overrepresented even with the sample size of 100.

**Small RNA sequencing read size distribution in granulosa cells, cell-free follicular fluid and extracellular vesicles**

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**Supplementary Figure S3**. Average length distribution of sequenced RNA molecules from all sample types (mean ±SD). Read length corresponding to miRNAs is marked by black box. EV-extracellular vesicles, FF-follicular fluid, MGC-granulosa cells.

**Differentially Expressed miRNAs between sample types in the healthy follicle**

**A close up of a map

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**Supplementary Figure S4.** Differentially expressed miRNAs between sample types. (**A**) Cell-free follicular fluid (FF) versus granulosa cells (MGC). (**B**) Extracellular vesicles of the follicular fluid (EV) versus FF.

**Validation of sequencing results by RT-qPCR**

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**Supplementary Figure S5.** Comparison of RNA sequencing and RT-qPCR results of miRNA expression levels between PCOS and control samples. (**A**) Granulosa cell samples (MGC). (**B**) Cell-free follicular fluid (FF). (**C**) Extracellular vesicles of the follicular fluid (EV). Results are displayed as a mean of fold change ±SEM on log­2 scale (#=0.051, \*p < 0.05, \*\*<0.01 \*\*\*p < 0.001, Student’s t-test

**Functions of the predicted targets for the novel miRNA sequence**



**Supplementary Figure S6.** Treemap of functional enrichment analysis results of the novel miRNA targets. Gene ontology terms reflecting enriched biological processes are categorized into superclusters depicted in different colours.