Supplemental Data to

Single-cell quantitative proteomic analysis of human oocyte maturation revealed high heterogeneity in *in vitro* matured oocytes

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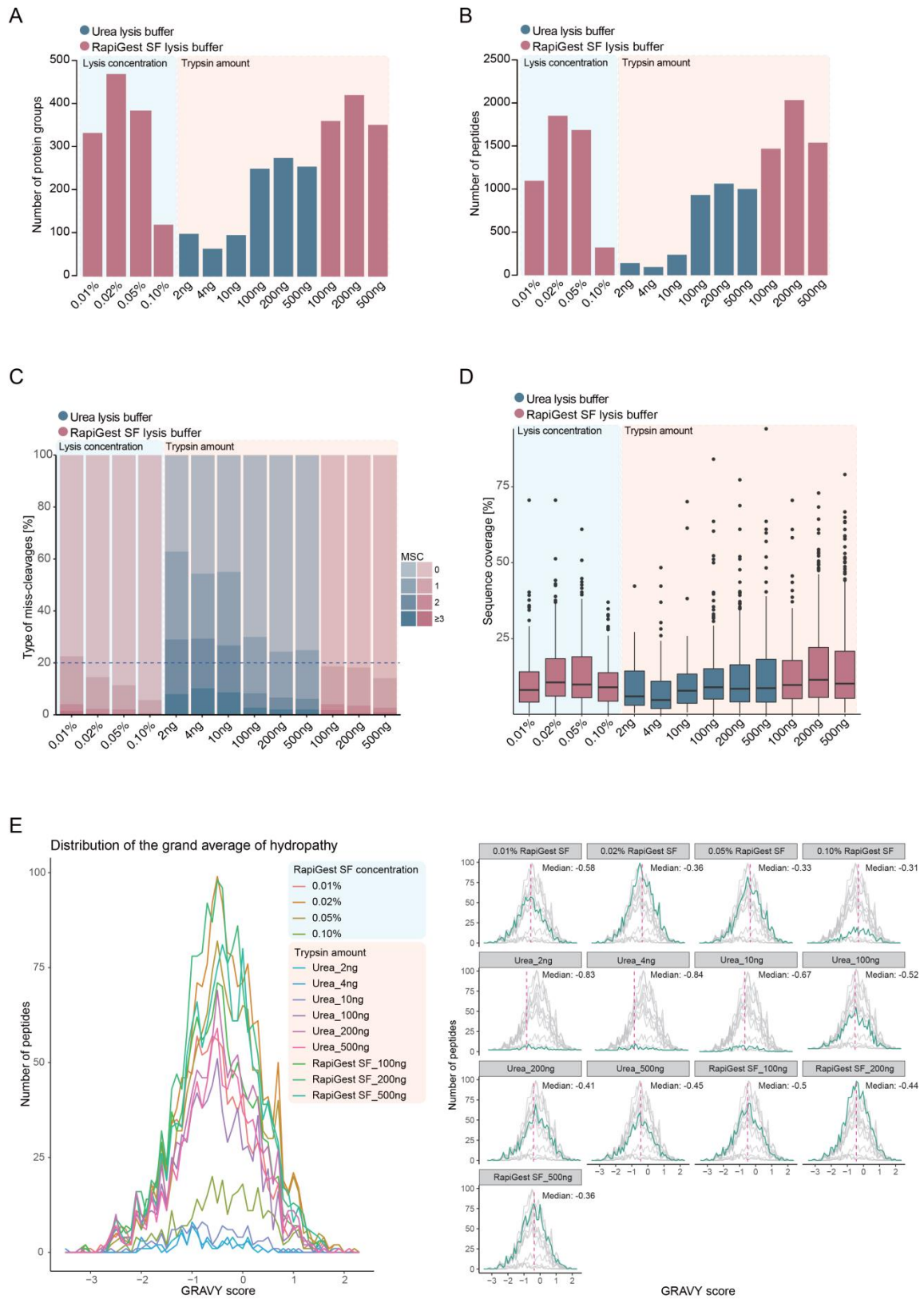Running title: Single-cell Proteomics of Human Oocytes

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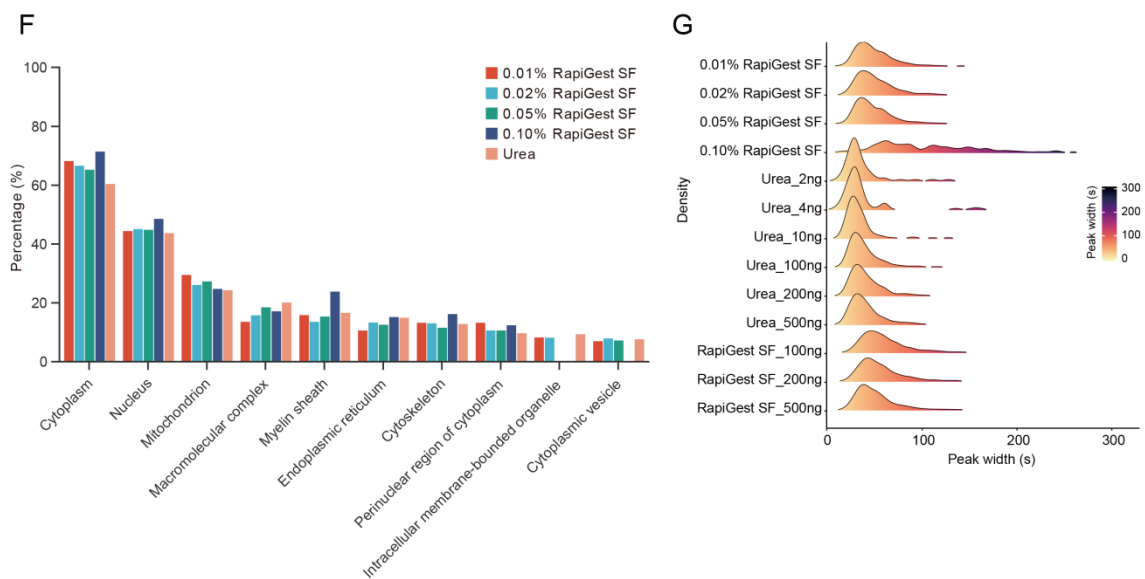Feiyang Diao, E-mail: diaofeiyang@njmu.edu.cn.

Supplemental Figure 1

Figure S1. **Analysis of single-cell sample preparation in different conditions.** The number of proteins (A) and peptides (B), types of peptide miss-cleavages (MSC) (C), sequence coverages (D), distribution of grand average of hydropathy (GRAVY) scores (E), enriched cellular component terms (F) and distribution of peak widths (G) for the data of mouse single-cell oocyte samples using different concentrations of RapiGest SF lysis buffer or urea lysis buffer for protein lysis and different amounts of trypsin for protein digestion.

Supplemental Figure 2

A

B

C

D

E

Figure S2. Single cell proteomic analysis of human oocyte maturation. A, The number of proteins and peptides identified in each of the human oocyte. B, Venn diagram showing the overlap between our data and Virant-Klun et.al.'s single human oocyte as well as 100 human oocytes data. C-E, Correlation analysis of log2 transformed LFQ values among GV (C), IVM (D) and IVO (E) oocytes respectively. Scatter plots of each pairing sample are presented in the lower left panel, and the Pearson's correlation coefficient is presented in the upper right panel.
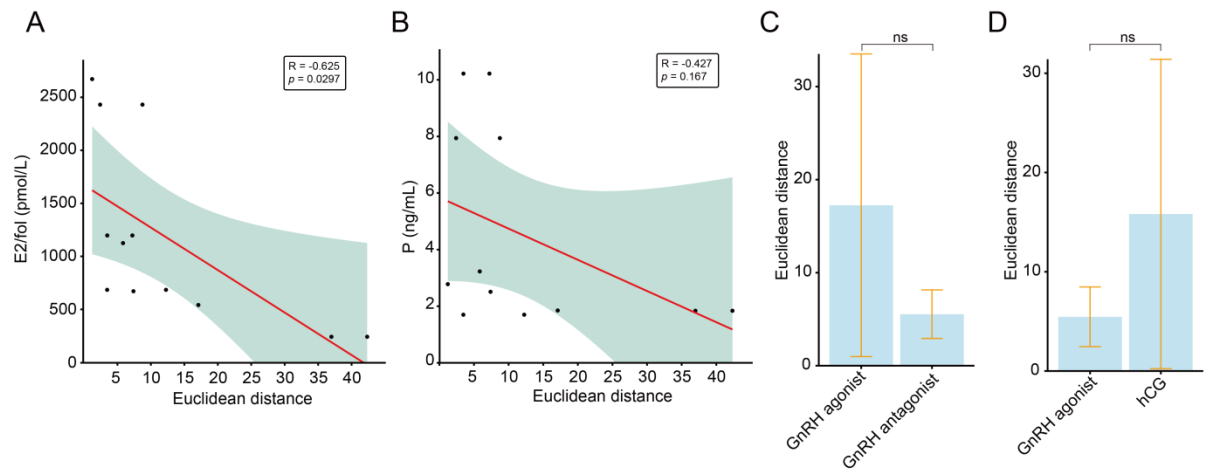
Supplemental Figure 3

Figure S3. **Statistical analysis of factors related to IVM oocyte heterogeneity.** Correlation analysis between the E2/fol (A) or progesterone level (B) and the Euclidean distance from each oocyte to the group median. The red line indicates the linear predictor, while the green shape indicates the 95% confidence interval. Bar plot of the Euclidean distance from each oocyte to the group median in different regimens of ovarian stimulation (C) or ovulation trigger strategies (D). ns, not significant (Student's t-test).
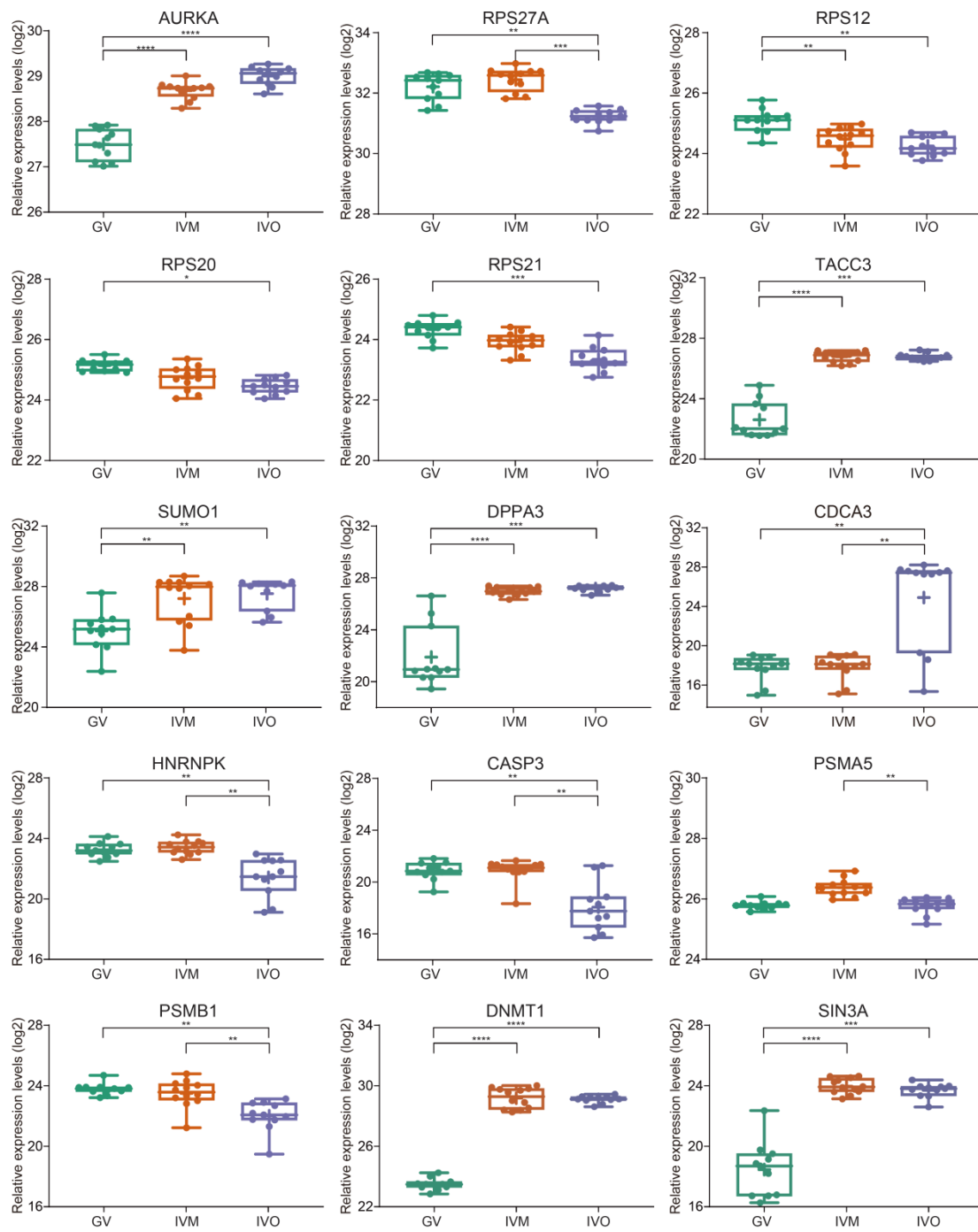
Supplemental Figure 4

Figure S4. **Expression levels of representative differential proteins.** Box and whiskers plots depicting expression levels of representative proteins with differential expression patterns between oocyte groups. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (Linear mixed effects model with Tukey's posthoc test, fold change > 1.5).