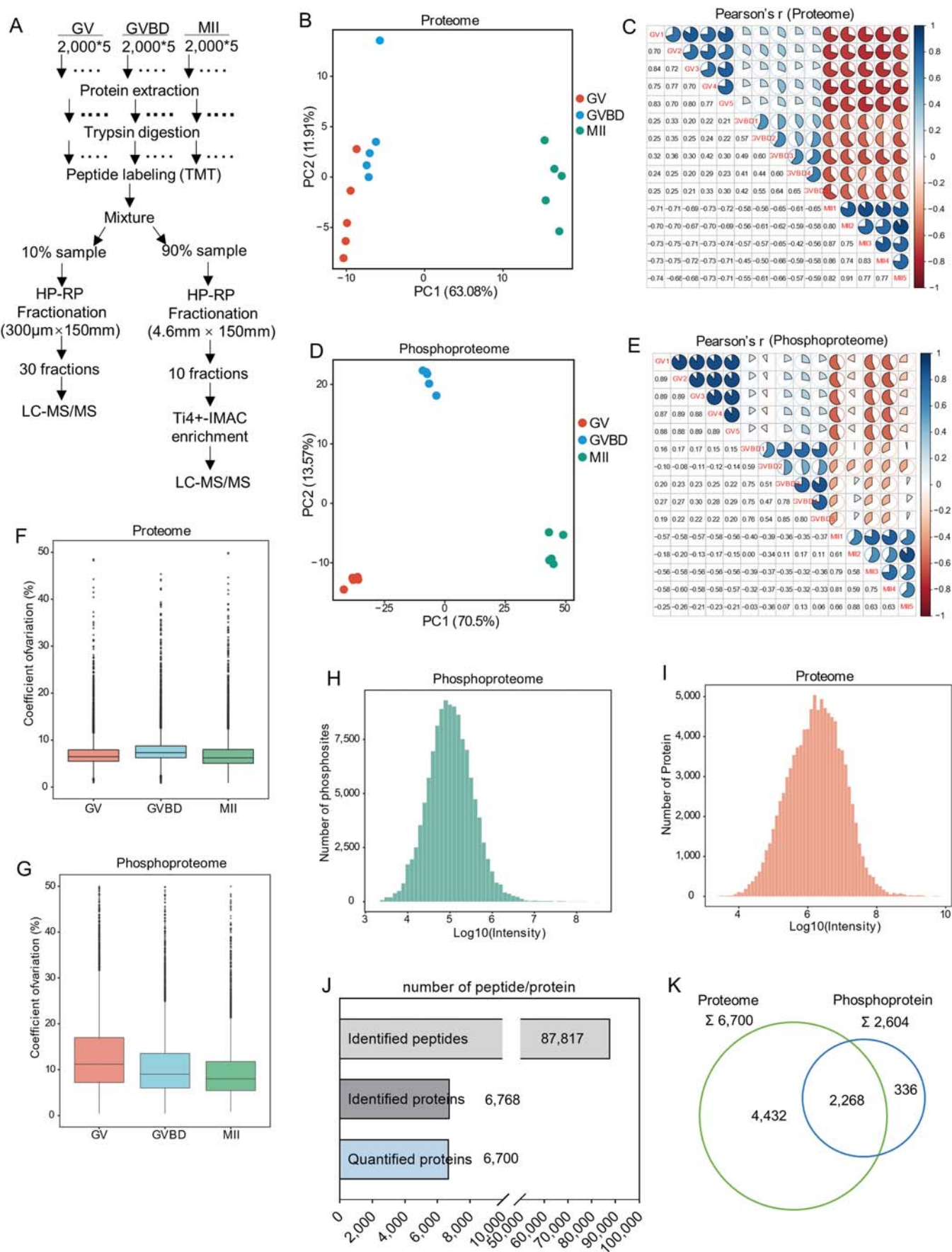
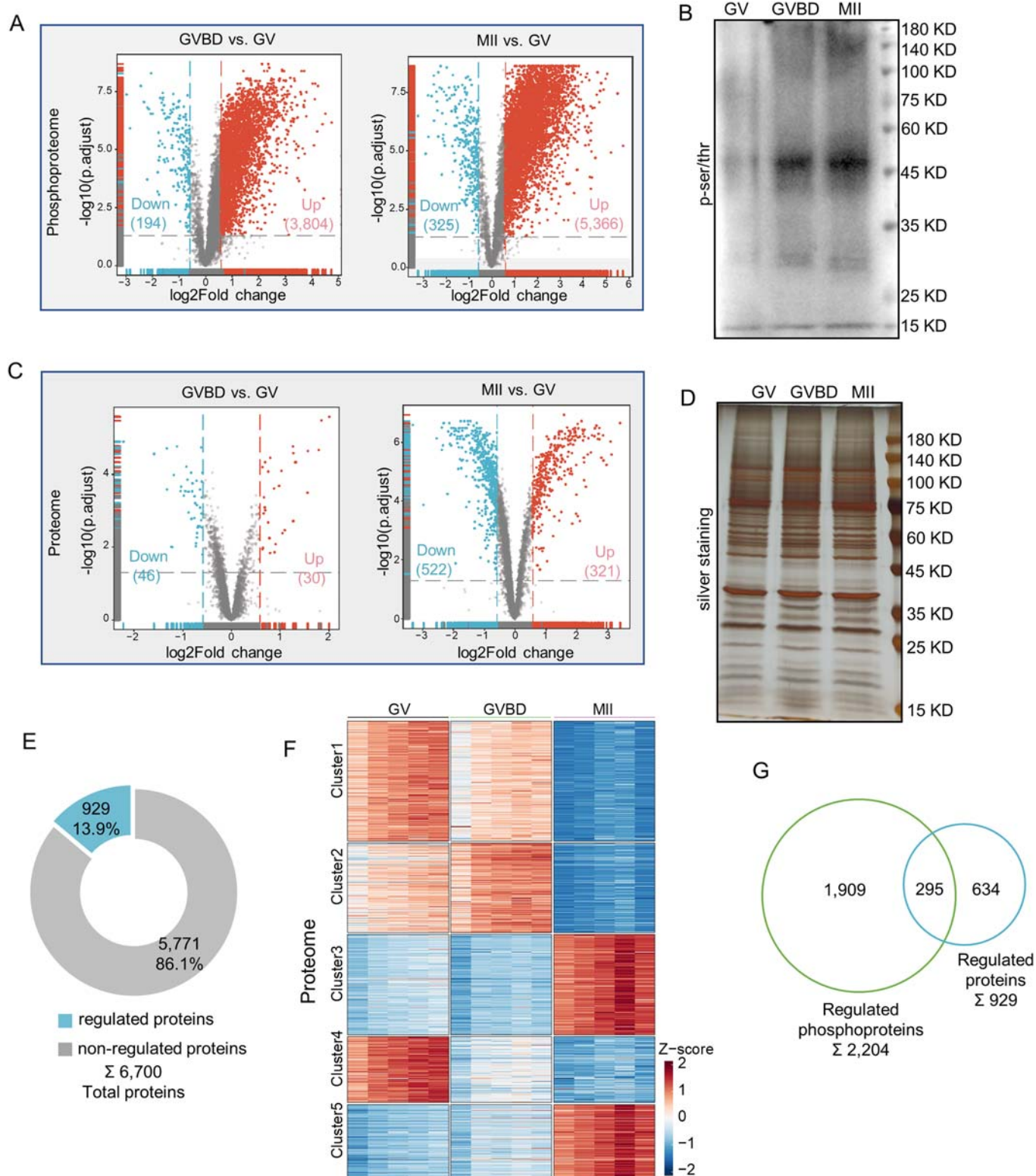


Expanded View Figures

Figure EV1. Quality control for the phosphoproteomics and proteomics data. Related to Fig. 1.

(A) Workflow of Proteomics and Phosphoproteomics. (B) Principal component analysis depicting the clustering of five proteome replicates from GV, GVBD, and MII oocytes. (C) Heatmap illustrating the Pearson's correlation among the 15 proteome replicates obtained from GV, GVBD, and MII oocytes. (D) Principal component analysis depicting the clustering of five phosphoproteome replicates from GV, GVBD, and MII oocytes. (E) Heatmap illustrating the Pearson's correlation among the 15 phosphoproteome replicates obtained from GV, GVBD, and MII oocytes. (F) Coefficient of variance boxplot of proteome for each stage sample derived from GV, GVBD, and MII oocytes. Box plots: centerlines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the minimum and maximum. $n = 6700$. (G) Coefficient of variance boxplot of phosphoproteome for each stage sample derived from GV, GVBD, and MII oocytes. Box plots: centerlines show the medians; box limits indicate the 25th and 75th percentiles; whiskers extend to the minimum and maximum. $n = 8090$. (H, I) Total phosphopeptide (G) and peptide (H) intensities ranked ascending to illustrate the dynamic range of the dataset. (J) Bar chart showing the number of identified peptides (up), proteins (middle), and quantified proteins (bottom). (K) The overlap of proteins and phosphoproteins.





**Figure EV2. Features of dynamic phosphoproteome and proteome. Related to Fig. 2.**

(A) Volcano plots showing the differentially phosphorylated proteins between GVBD and GV (left), or between MII and GV (right). Student's *t* test followed by Benjamini-Hochberg (BH) *P* value adjustment (*P*.adjust) was used for the statistical analysis. (B) Representative immunoblots of oocytes at GV, GVBD, and MII stages probed with pan-Ser/Thr antibody. (C) Volcano plots showing the differentially expressed proteins between GVBD and GV (left), or between MII and GV (right). Student's *t* test followed by Benjamini-Hochberg (BH) *P*-value adjustment (*p*.adjust) was used for the statistical analysis. (D) Representative silver staining of oocytes at GV, GVBD, and MII stages for protein expression detection. (E) Pie chart showing the number and percentage of regulated proteins. (F) Heatmap illustrating the dynamic changes in regulated proteins during oocyte maturation. (G) The overlap of regulated proteins and regulated phosphoproteins.

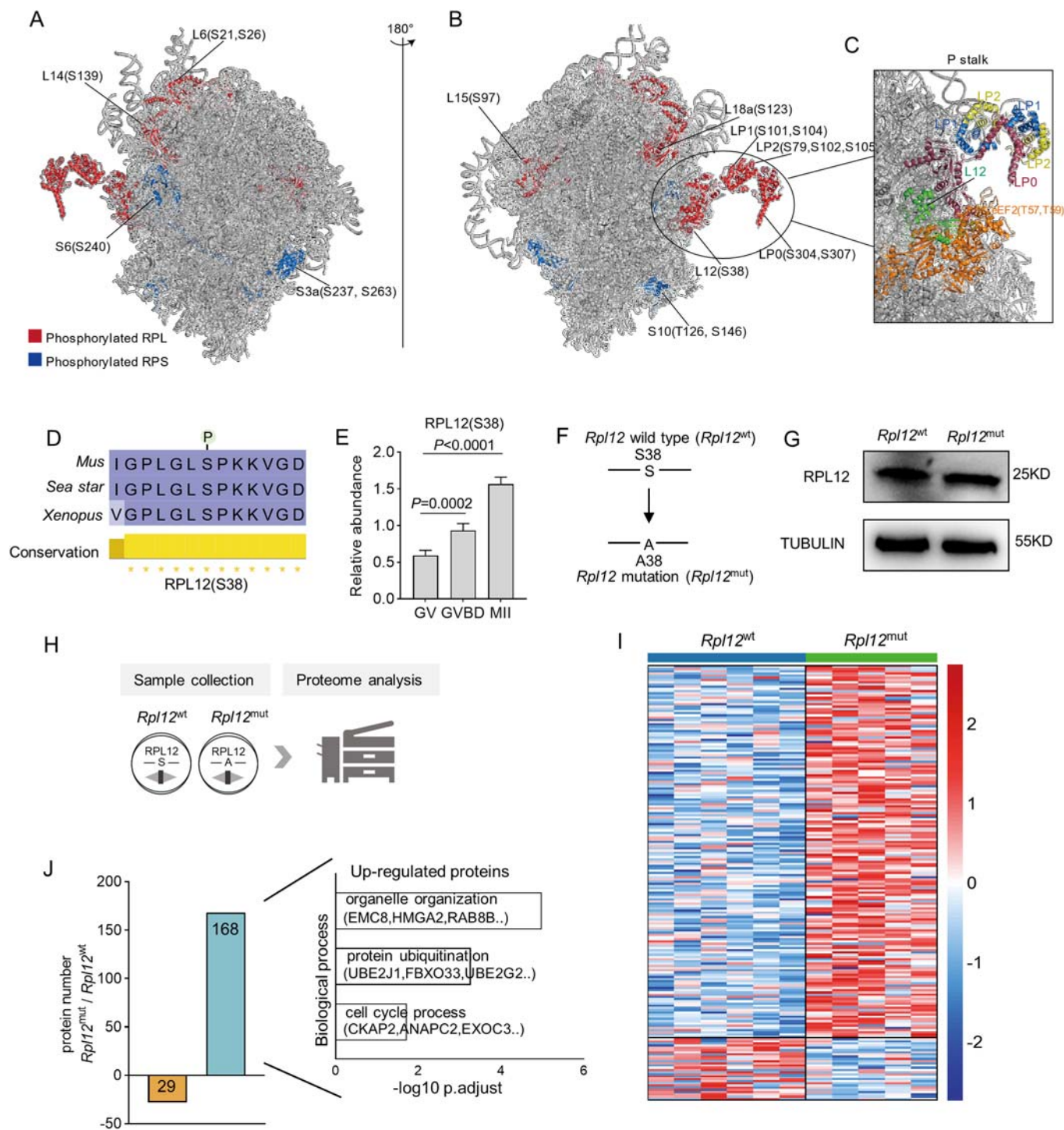
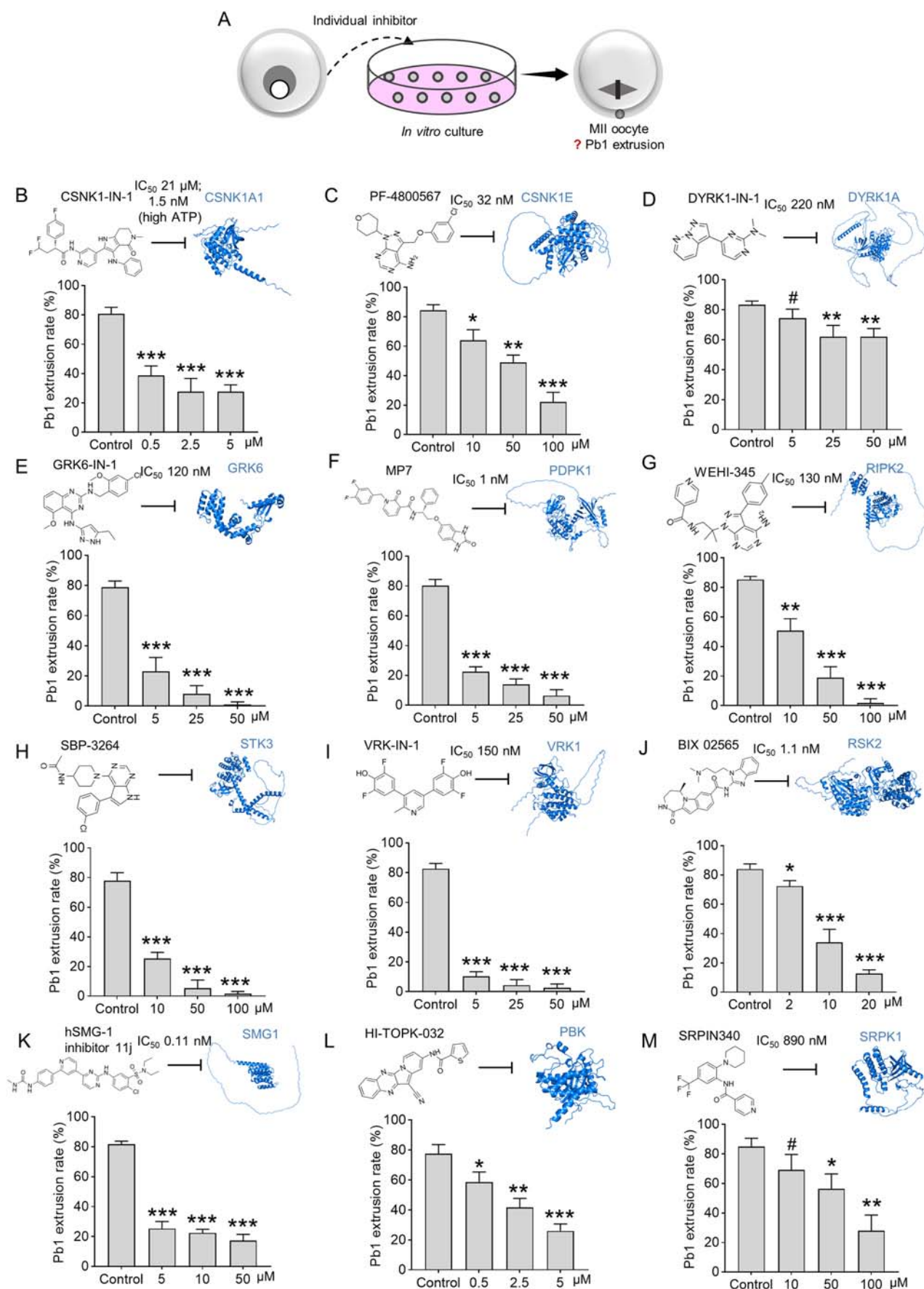


Figure EV3. RPL12-Ser38 phosphorylation participates in maintaining translational homeostasis in oocytes. Related to Fig. 5.

(A, B) Quantified phosphorylation sites of ribosome proteins (RPs) mapped to the ribosome structure (PDB: 4V6X). Phosphorylated RPs and RPLs are shown in blue and red, respectively. RPL: Large ribosomal subunit proteins; RPS: Small ribosomal subunit proteins. (C) An enlarged view of the P stalk showing that RPL12-S38 is proximal to the ribosomal GTPase EEF2, RPLP0, RPLP1, and RPLP2. (D) Alignment and conservation analyses of RPL12 sequences flanking conserved phosphosites. (E) Bar chart showing the phosphorylation level of RPL12-S38 during oocyte maturation. The p value is labeled in the figure. Data are expressed as mean percentage $\pm$ SD from five independent replicates. Two-tailed Student's t test was used for statistical analysis, comparing to GV oocytes. (F) Schematic representation of the design for RPL12 phosphomutant. (G) Immunoblotting showing the overexpression of exogenous RPL12 (RPL12^{wt} and RPL12^{mut}) protein in oocytes. (100 oocytes per lane). (H) Diagram showing the sample collection for proteomic analysis. (I). Heatmap showing the differentially expressed proteins between Rpl12^{wt} and Rpl12^{mut} oocytes. (J) Bar chart showing the differentially expressed proteins between Rpl12^{wt} and Rpl12^{mut} oocytes. The number of upregulated (up-) and downregulated (down-) proteins in Rpl12^{mut} oocytes (left). Representative biological processes enriched for upregulated proteins in Rpl12^{mut} oocytes (right). Benjamini-Hochberg (BH) corrected p-value adjustment (p.adjust) was used for the enrichment analyses.



◀ Figure EV4. Effects of different kinase inhibitors on oocyte maturation. Related to Fig. 6.

(A) Schematic presentation of the inhibitor treatment experiment. (B–M) Quantitative analysis of the Pb1 extrusion rate in oocytes treated with different inhibitors. Data are expressed as mean percentage $\pm$ SD from three independent replicates in which at least 100 oocytes were analyzed for each group. Two-tailed Student's *t* test was used for statistical analysis, comparing to control group (DMSO treatment). **P* > 0.05, **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

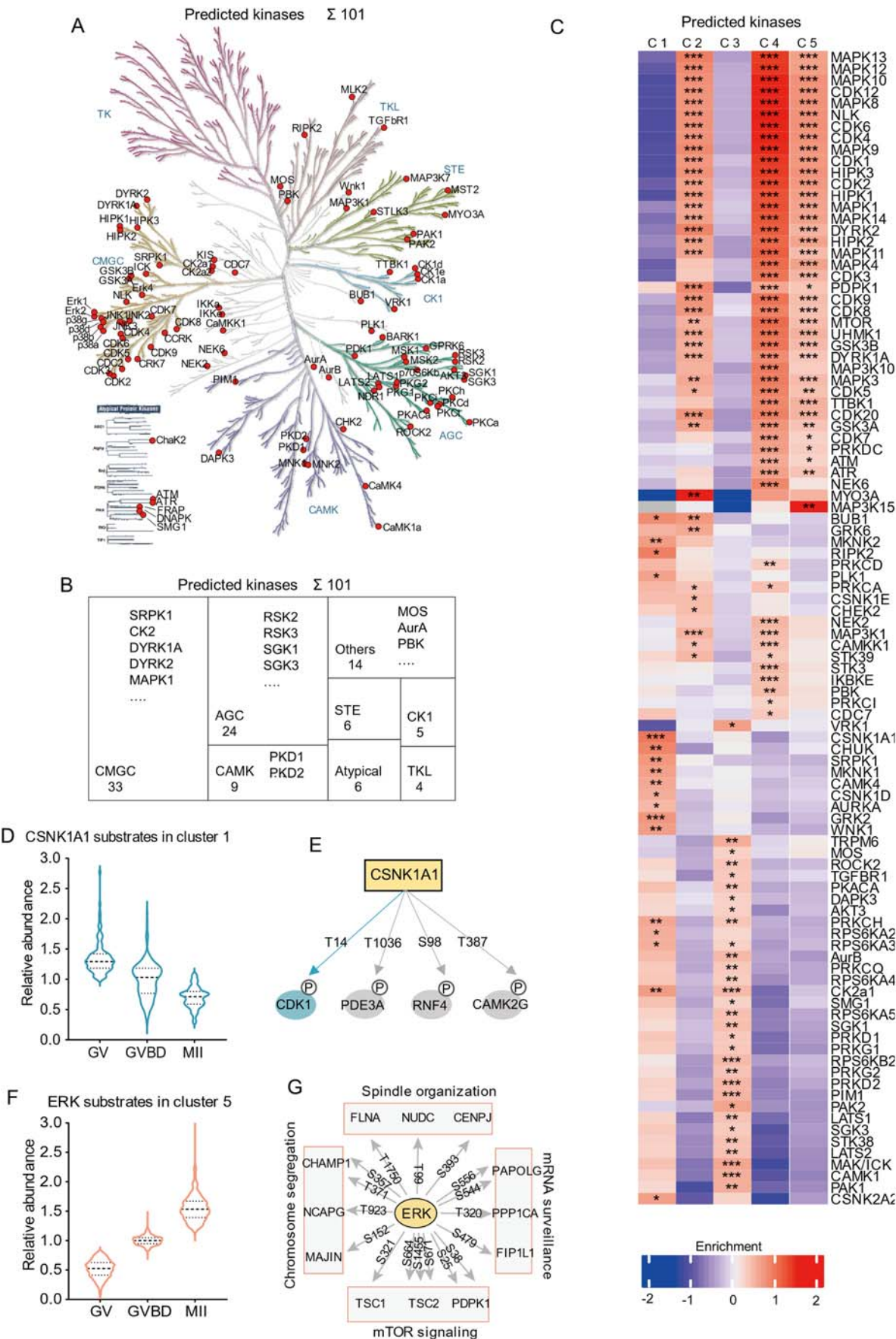


Figure EV5. Enrichment analysis of the predicted kinases in oocytes. Related to Fig. 6.

(A) Kinase tree showing the predicted kinases annotated to the major kinase families. (B) The number of predicted kinases in each kinase family. (C) Heatmap showing the predicted kinases enriched in each cluster. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Benjamini-Hochberg (BH) corrected P value adjustment (P_{adjust}) was used for the enrichment analyses. (D) Phosphorylation levels of the predicted CSNK1A substrates in cluster 1 during oocyte maturation. $n = 191$. (E). Networks showing the representative phosphosites in annotated substrates (KSPN) of CSNK1A1 kinase. Proteins are shown as nodes and phospho-residue is indicated by the number. (F) Phosphorylation levels of the predicted ERK substrates in cluster 5 during oocyte maturation. $n = 672$. (G) Networks showing the representative phosphosites in annotated substrates (KSPN) of ERK kinases. Proteins are shown as nodes and phospho-residue is indicated by the number.