

Supplemental data summary

Supplemental Figure S1 – The overlaps among phosphorylation site identifications from five replicates including two replicates for IMAC and three replicates for TiO₂.

Supplemental Figure S2 – The KSPN constructed with the iGPS predicted KSRs from the phosphoproteome in this study. This figure is a detailed version of Figure 3A. Different colored proteins were from different datasets. “IMAC”, “TiO₂” and “Kinase” in the colored box represent that the protein is a phosphoprotein identified by IMAC, TiO₂ and a kinase, respectively, while “/” means both.

Supplemental Figure S3 – The KSPN subnetwork for the spermatogenesis-related proteins. This figure is a detailed version of Figure 3B. Different colored proteins were from different datasets. “IMAC”, “TiO₂” and “Kinase” in the colored box represent that the protein is a phosphoprotein identified by IMAC, TiO₂ and a kinase, respectively, while “/” means both.

Supplemental Figure S4 – The spermatogenesis-related KSPN subnetwork of Mapk1. MAPK1 was predicted to phosphorylate 47 spermatogenesis-related proteins, which were identified as phosphorylated in this study. Different colored proteins were from different datasets. “IMAC”, “TiO₂” and “Kinase” in the colored box represent that the protein is a phosphoprotein identified by IMAC, TiO₂ and a kinase, respectively, while “/” means both.

Supplemental Figure S5 – The snapshots of the pTestis database for phosphorylation in the testis. (A) The search option. (B) Mapk1 as a representative result of using the search option. (C) The blast option. (D) The identified phosphorylation sites of Mapk1 and the kinase annotations.

Supplemental Figure 6 –The kinase activity analyses for the five replicates in this study. The top15 significantly high--activity kinases for the mouse testis phosphoproteome from five replicates are presented (p-value <0.05). E-ratio: the proportion in the specific dataset divided by that in the mouse phosphoproteome atlas.

Supplemental Figure S7 – The KSPN subnetwork of the PLKs. 122 phosphorylated proteins from the phosphoproteome identified in this study were predicted as phosphorylated by the PLKs. Different colored proteins were from different datasets. “IMAC”, “TiO₂” and “Kinase” in the colored box represent that the protein is a phosphoprotein identified by IMAC, TiO₂ and a kinase, respectively, while “/” means both.

Supplemental Figure S8 –The detection of the expression of PLK1-3 mRNAs by RT-PCR in GC2 cells.

Supplemental Table S1 – The mouse testis phosphoproteome identified in this study, including the detailed information for the identified proteins, peptides and sites.

Supplemental Table S2 – The top 15 enriched biological processes, molecular functions and cellular components in the four datasets, including Total, IMAC, TiO₂ and Gygi’s in this study. a. The number of proteins annotated; b. The proportion of proteins annotated; c. Enrichment ratio: the proportion in the mouse testis phosphoproteome from this study divided by that in the mouse proteome.

Supplemental Table S3 – The significantly high-activity kinases for the four datasets, including Total, IMAC, TiO₂ and Gygi’s in this study. a. The number of p-sites annotated with the kinase; b. The proportion of p-sites annotated; c. Enrichment ratio: the proportion in the mouse testis phosphoproteome from this study divided by that in mouse phosphoproteome atlas.