**Supplementary Information**

**Supplementary results**

GDNF induces global phosphorylation changes in proliferating SPCs

To obtain sufficient material for large-scale quantitative proteomic and phosphoproteomic analyses, we established cell cultures using validated protocols for the long-term *in vitro* propagation of mouse SPCs. We enriched spermatogonia from the testes of B6;129S-Gt(Rosa)26Sor/J (ROSA) pups by MACS with CD90.2-conjugated beads. Following culture in a serum-free defined medium with continuous replenishment of growth factors including GDNF, we obtained 3 independent lines that proliferated as clumps with the typical grape-like shape of SPCs (Supplementary Figure 1A, upper and middle panels). After transplantation into germ cell-depleted testes, cells of all 3 lines engrafted and formed extensive spermatogenic colonies, validating that the SPC cultures had retained *in vivo* regeneration potency (Supplementary Figure 1A, lower panel).

In normal growing cells, phosphoserine encompasses approximately 90% of all phosphor-amino acids. We therefore used an anti-total phosphoserine antibody to profile phosphorylation changes in cultured cells in response to changes in the availability of GDNF and bFGF, which are both growth factors considered essential for the maintenance of SPCs *in vitro*. Major shifts in global phosphoserine levels in cells were apparent after removal and subsequent replenishment of GDNF from the culture medium (Supplementary Figure 1B, whereas bFGF depletion and re-exposure were associated with a small amount of change (Supplementary Figure 1C).
**Supplementary figures**

**Supplementary figure 1**

GDNF induced phosphorylation changes in proliferating SPCs. (A) Clump forming grape-shaped SPCs (upper panel; scale bar, 20 μm) transgenic for beta-galactosidase were X-gal positive (middle panel; scale bar, 20 μm) and generate colonies in recipient testis (bottom panel; scale bar, 100 mm). (B) Global serine phosphorylation changed in cells treated with GDNF, and (C) bFGF. N, cells grown in normal culture conditions; -12 h, after depletion of growth factor for 12 h; +(time period), after replenishment of growth factor for time period indicated. Western blot analysis was performed using an anti-phosphoserine antibody.

**Supplementary figure 2**

Western Blotting experiments showed that the regulation of GDNF on two known GDNF-regulated proteins, CCND2 and GFRA1, which were identified by Mass Spec with differential expression.

**Supplementary figure 3**

Numbers of sites were identified by IMAC, and TiO2. A total of 4429 sites were identified by IMAC, and a total of 10458 sites were identified by TiO2, and 2746 sites were shared in both methods.

**Supplementary figure 4**

Volcano plots for both proteins and sites. (A) Among these, 34 proteins were upregulated and 22 proteins were downregulated in response to GDNF. (B) A total of 145 differentially phosphorylated proteins containing 248 sites were upregulated, and 194 phosphorylated
proteins containing 322 sites were downregulated.

**Supplementary figure 5**

The relative expression of genes associated to SPC fate regulation following the inhibition of ERK1/2 kinase. (A) Self-renewal associated genes (Etv5, Bcl6b, Zbtb16, Pou5f1), (B) differentiation associated genes (Kit, Stra8), (C) cell cycle associated genes (Ccna2, Ccnb1, Ccnd2,Ccne1), and (D) apoptosis associated genes (Bcl2, Bax, Casp3).

**Supplementary figure 6**

Identified phospho-sites of Raptor. Representative Phospho-sites of Raptor, Thr^{853} that identified by Mass was showed.

**Supplementary figure 7**

ZBTB16, p-ERK1/2 and p-RPS6 from control testis (WT) staining without GDNF-virus injection.

**Supplementary tables**

**Supplementary table 1. Quantitative proteome of mouse SSCs.**

Sheet A listed the detailed information for protein identification and quantification including protein identity, protein name, gene name, expression values and statistical results. Sheet B provided the identified peptides.

**Supplementary table 2. Quantitative phosphoproteome of mouse SSCs.**

Sheet A provided detailed information for site identification and quantification including site composition, corresponding protein, position, corresponding peptides, scores,
expression values and statistical results. Sheet B provided representative annotated spectra for each site.

**Supplementary table 3. Detailed results of functional enrichment analysis for proteins with DE sites.**

Sheet A provided the enriched GO terms for proteins with DE sites, while Sheet B provided the enrichment results for KEGG pathway analysis. Sheet A and B provided detailed information for each enriched term including statistical results and corresponding proteins.

**Supplementary table 4. Detailed results of predicted kinases motif that overrepresented for DE sites.**

Sheet A provided the predicted kinases with motifs overrepresented among significantly up-regulated sites, while Sheet B provided the predicted kinases with motifs overrepresented among significantly down-regulated sites. Sheet A and B provided detailed information for each predicted kinase motif including binding motif, kinase description, statistical results, and corresponding substrate sites.

**Supplementary table 5. Global kinases predicted for DE sites based on PhosphoMotif Finder.**

Sheet A provided the global predicted kinases with motifs among up-regulated sites, while Sheet B provided the global predicted kinases with motifs among down-regulated sites.

**Supplementary table 6. Detailed results for the network of proteins with up regulated sites.**

Detailed relations and scores of evidences were provided for each paired genes.
Supplementary figure 7