Supplementary material

Germ cell separation by velocity sedimentation (STA-PUT)

Mouse testicular germ cells were separated using a STA-PUT velocity sedimentation cell separator (ProScience Inc. Scarborough, Ontario, Canada) and a published procedure [41]. Briefly, 10–12 adult mice (45 days) were sacrificed, and their testes were isolated, decapsulated and enzymatically digested, first with collagenase (0.5 mg/ml) for 20 min and then with trypsin (0.5 mg/ml) and DNase I (0.5 µg/ml) together for 13 min. Both of the digestions were performed with constant shaking at 225 rpm in a 32 °C water-bath. The resulting fragile seminiferous tubules were re-suspended by pipetting up and down for 3 min on ice to release the germ cells; this step was performed with a transfer pipette that was cut off at the second mark. This cell suspension was then filtered and washed with 0.5% BSA in KRB media (120 mM NaCl, 4.8 mM KCl, 25.2 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4 \cdot 7H_2O$, 1.3 mM $CaCl_2$, 1 × Pen/Strep/Glu (Life technologies), 1 × essential amino acid, 1 × non-essential amino acid (Lonza, Walkersville, MD, USA), and 11.1 mM dextrose) and complemented with DNase I (0.4 µg/ml). The cells were then resuspended in 25 ml of 0.5% BSA in KRB media and loaded into the 800ml loading chamber of the STA-PUT apparatus. After the cells were loaded into the sedimentation chamber, the clamp between the fleakers containing 2% and 4% BSA in KRB and the clamp between the fleakers and the cell loading chamber were opened in succession to allow the solution to flow into the sedimentation chamber and form a BSA gradient (from 2% to 4%). The cells were allowed to sediment for approximately 1 h 45 min, and 10 ml fractions were then collected. Every fifth fraction, starting from the 21st fraction, was examined using flow cytometry. The purity of the combined fractions was additionally confirmed microscopically. The yield of spermatocytes was about 8x 10^6 cells, and that of spermatids is 1.0 x 10^8cells. For differential plating experiments, the flow cytometry analysis was performed before and after the procedure to confirm the enrichment for tetraplod cells.

Different subtypes of spermatocytes as well as round and elongated spermatids were analyzed together to produce a sufficient amount of lysates for successful proteomic analysis. Fractions with a purity of at least 80 and 90% for primary spermatocytes and spermatids, respectively, were used for analysis.