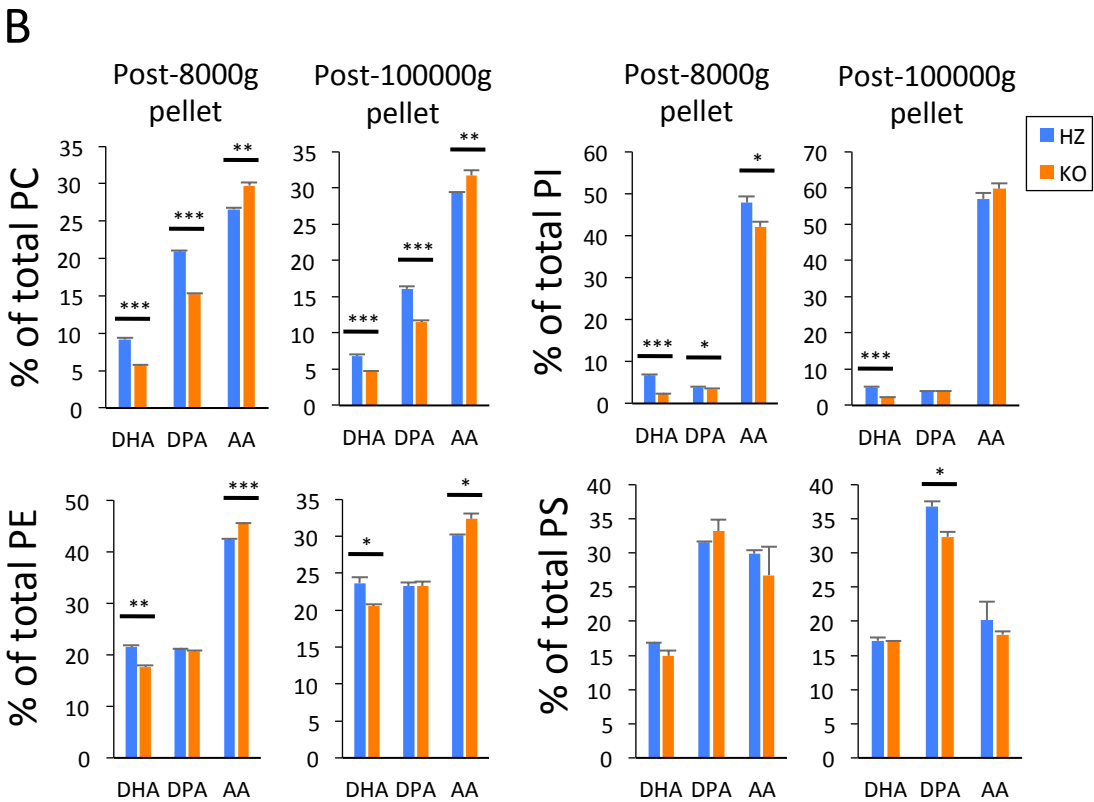
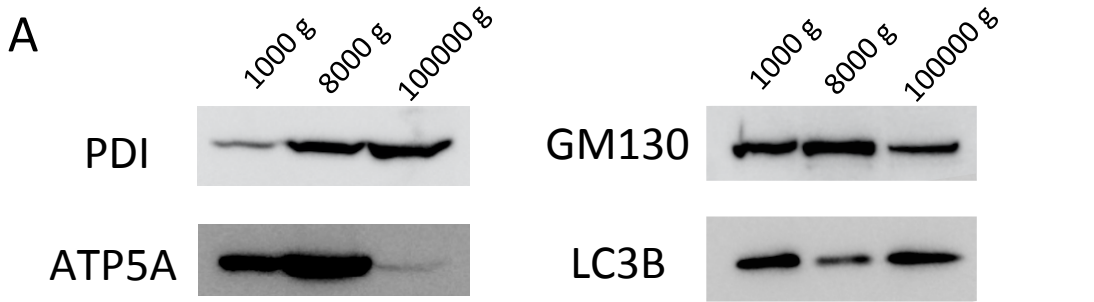


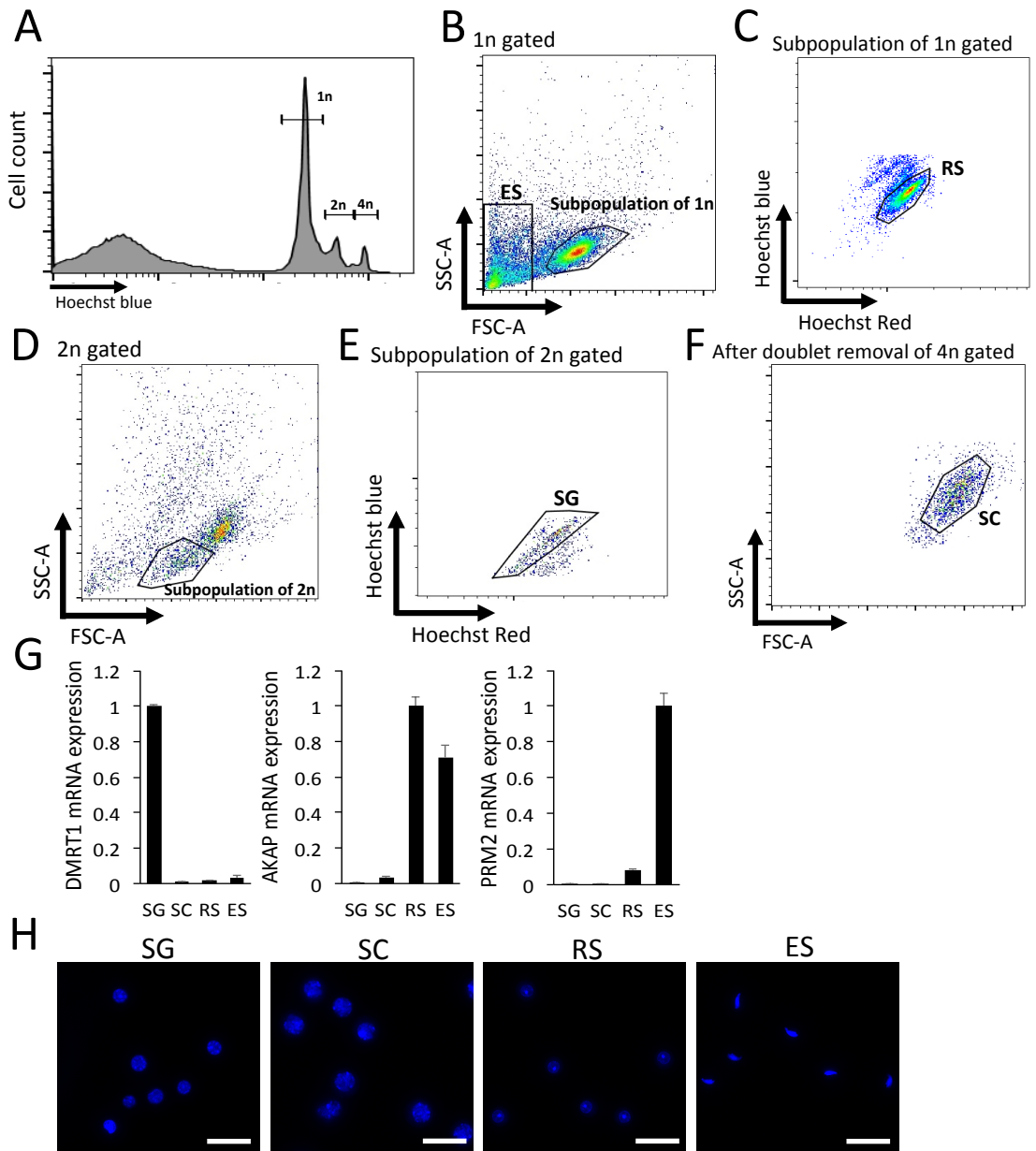
Composition of phospholipid in testis

A-C) percentage of total phosphatidylethanolamine (PE), phosphatidylinositol (PI) and phosphatidylserine (PS). PEs only in excess of 2% are shown. PIs and PSs in excess of 1% are presented. Data are presented as mean \pm SEM (n = 3). * p < 0.05, ** p < 0.01, *** p < 0.001; unpaired student t test.



Subcellular fractionation and lipidomic analysis of the testes

A) Representative subcellular fractionation. Pellets of post-1000g, post-8000g and post-100000g centrifugation were analyzed with western blot for PDI (ER marker), ATP5A (mitochondria marker), GM130 (golgi marker) and LC3B (lysosome marker) respectively. B) Lipidomic analysis of post-8000g and post-100000g pellets. Data are presented as mean \pm SEM (n = 3). * P < 0.01, ** P < 0.01, *** P < 0.001; unpaired student t-test.



FACS sorting of spermatids

A-F) representative sorting of testicular single cells. A, after dead cell removal using propidium iodide, living cells were classified into three populations based on Hoechst 33342 histogram: haploid (1n), diploid (2n), and tetraploid (4n). B-E, haploid cells and diploid cells were gated with forward scattering and side scattering (B, D), followed by further purification using Hoechst blue and red respectively (C, E). F, after doublet cell removal using forward scattering and side scattering, single population of spermatocyte was obtained. spermatogonia (SG), spermatocyte (SC), round spermatid (RS) and elongated spermatid (ES).

G, H) successful purification was confirmed with specific gene expression and nuclear shape in each fraction. Gene expressions of differentiation markers in isolated SG, SC, RS and ES (G). We used expression markers as follows; doublesex and mab-3 related transcription factor 1 (DMRT1), expressed in spermatogonia; A kinase anchoring protein 3 (AKAP3), expressed in haploid spermatids; protamine 2 (PRM2), predominantly expressed in late spermatids. Data are presented as mean \pm SEM (n = 3). H) nuclear shapes of isolated spermatids.