



**Supplementary figure 3.** qRT-PCR of the culture samples used for RNA-seq and interstitial cell culture; Oil Red O staining of these cultures. (A) Hsd3b6 and Hsd17b3, markers of adult Leydig cells, were downregulated in the adult SLC and ST cultures;  $P < 0.05$ . The data are represented as a fold change ( $\log_2$ ) relative to RNA levels in the interstitial cell culture that was established from adult testes and grown for 24 hours before harvest. (B) Leydig cells labeled by Oil Red O staining (arrows) were detected in the interstitial cell culture (1) and ST culture (2) but not in the SLC culture (3). Nuclei were counterstained with hematoxylin. (C) Validation of RNA-seq analysis. RNA expression in the adult SLC culture is represented as a fold change ( $\log_2$ ) relative to RNA expression in the ST culture.  $P < 0.05$  for all genes examined. All PCR data are presented as the mean  $\pm$  SEM from 3 biological replicates. Scale bars: 100  $\mu\text{m}$ .