

Related to Fig. 3.



**a**, Immunofluorescent labeling of 0- and 30- day cultured gonadal tissues for LIN28A (red). DNA was counterstained with Hoechst 33342 (blue). LIN28A+ cells (white triangles). Scale bars,  $10 \mu m$ .

**b**, Immunostaining of cultured tissues for SYCP3 (green) and  $\gamma$ H2AX (red). DNA was counterstained with Hoechst 33342 (blue). XY body by  $\gamma$ H2AX staining (white triangles). Scale bars, 10  $\mu$ m.

**c**, Immunostaining for PNA in cultured tissues (white triangles). DNA was counterstained with Hoechst 33342 (blue). Scale bars,  $10 \mu m$ .

**d**, Immunostaining for PNA in haploid cells isolated after FACS. DNA was counterstained with Hoechst 33342 (blue). Scale bars, 10 μm.

e, FACS analysis of cultured tissue at day 134.

**f**, FISH using both fluorescent probes specific for chromosomes X and Y for haploid cells from (a) sorted by FACS. Scale bars, 10 μm.

g, Transmission electron microscopy shows ultramicrostructural findings of different cell types; primary spermatocytes with nuclear synaptonemal complexes: black triangles; and early tail structure: white triangles. Scale bars, Spermatocyte:2  $\mu$ m. Tail structure: 2  $\mu$ m.

**h**, H&E staining of 0-, 10- and 50-day cultured tissues and human adult testis. Spermatocytes in vitro (triangle), and spermatids in vitro or in vivo (arrow) are indicated; Scale bars, 10 μm.