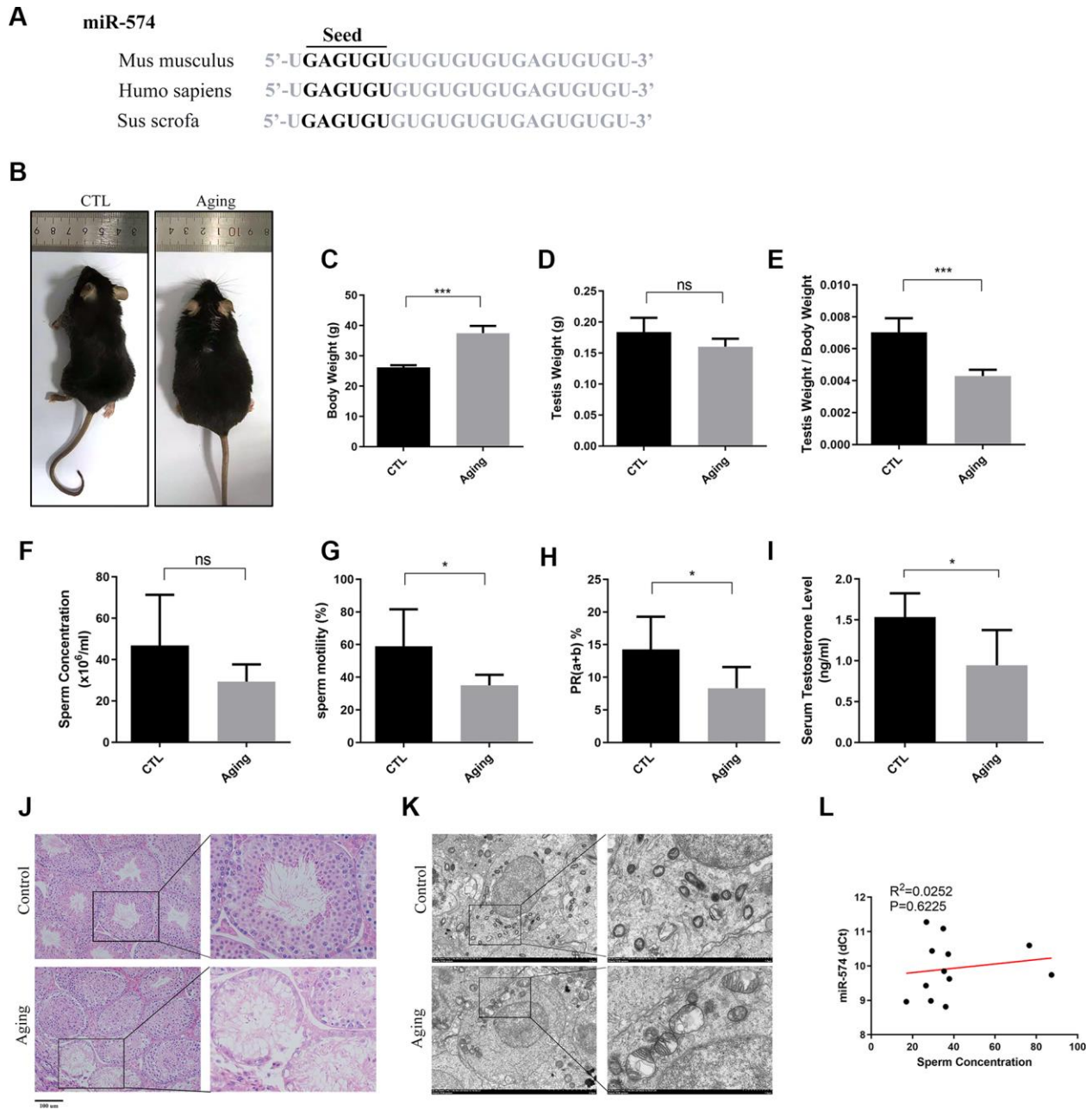
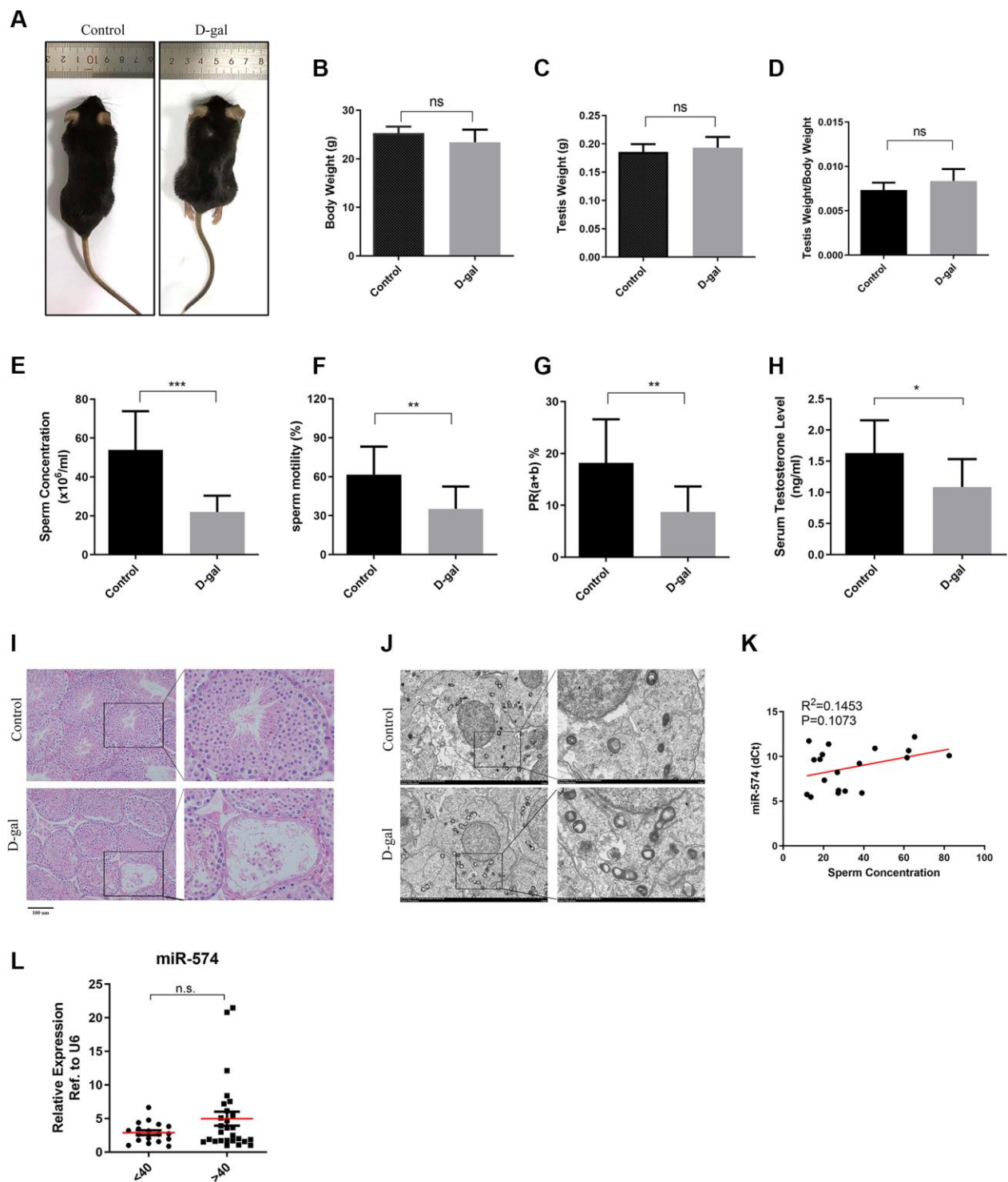


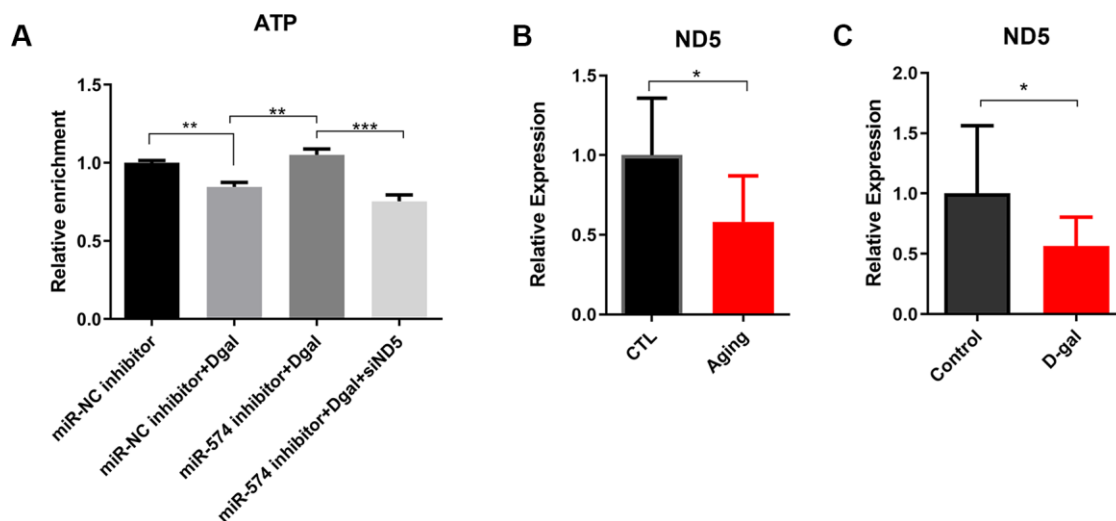
SUPPLEMENTARY FIGURES



Supplementary Figure 1. Establishment of natural aging mouse model. (A) MiR-574 is highly conserved in multiple species. (B) The older-age mice presented thinning of hair, hypoactivity and other characteristics of aging. (C–E) The body weight, testicular weight and testicular organ index of the natural aging mouse model. (F–I) The sperm concentration, sperm motility, progressive motility and serum testosterone of the natural aging mouse model. (J–K) H&E staining and electron microscopy observations in the testes of the natural aging mouse model. (L) Scatter plot of sperm concentration and the miR-574 expression in the sperm of the natural aging mouse model.



Supplementary Figure 2. Establishment of the D-gal-induced aging mouse model. (A) The D-gal-treated mice presented few characteristics of aging in appearance. (B–D) The body weight, testicular weight and testicular organ index of the D-gal-treated mouse model. (E–H) The sperm concentration, sperm motility, progressive motility and serum testosterone of the D-gal-treated mouse model. (I–J) H&E staining and electron microscopy observations in the testes of the D-gal-treated mouse model. (K) Scatter plot of sperm concentration and the miR-574 expression in the D-gal-treated mouse model. (L) miR-574 was detected in the sperm of males less than 40 years old (n=18) and more than 40 years old (n=28).

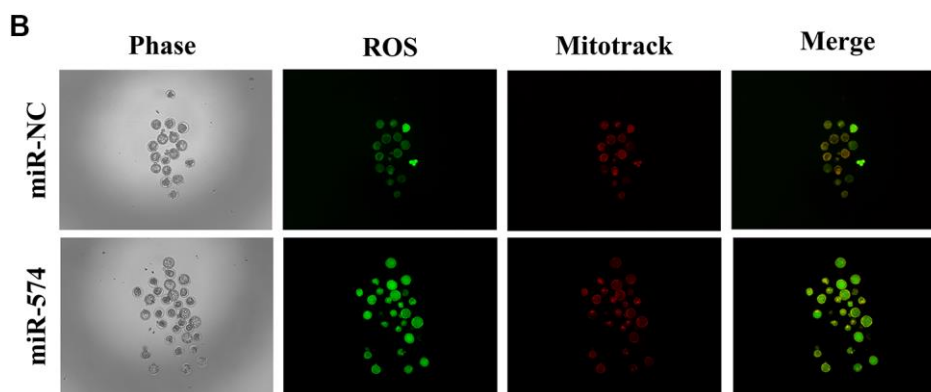


Supplementary Figure 3. mt-ND5 depletion alleviated the increase effects of miR-574 inhibitor on ATP levels. (A) The ATP levels was detected in different groups as shown. (B–C) The expression of mt-ND5 was detected in the sperm of the natural aging mouse model and D-gal-induced aging mouse model. Data are shown as mean±S.D.

A Preimplantation development of embryos with or without miR-574 supplementation

Injected content	No. 2PN	No. 2-cell (% of 2-cell / 2PN)	No. 4-cell (% of 4-cell / 2-cell)	No. Blastocyst (% of Blastocyst / 2-cell)
miR-NC	81	74 (91.36)	71 (95.95)	57 (77.03)
miR-574	120	107 (89.17)	97 (90.65)	70 (65.42)

Note: 2PN, 2 pronuclei;



Supplementary Figure 4. Effects of miR-574 on early embryonic development. (A) The preimplantation development of embryos with or without miR-574 supplementation. (B) The ROS (green) and MitoTracker (red) were detected in the embryos with or without miR-574 supplementation.