

SUPPLEMENTARY FIGURE LEGNEDS

Figure S1. The defect of *mili* mutant in germline stem cell self-renewal. All panels are stained with hematoxylin and eosin and shown under the same magnification for easy comparison. sg: spermatogonia; sc: spermatocytes; st: spermatids; stc: Sertoli cells.

Figure S2. Further characterization of germline stem cell maintenance defects in *mili*(-/-) testes. **A-B**, testicular sections of eight-day old *mili*^{+/-} (A) and *mili*^{-/-} (B) mice stained for TUNNEL (red), laminin (green), and DNA (blue). Equally few *mili*^{+/-} and *mili*^{-/-} spermatogonia are undergoing cell death. **C-D**, testicular sections of nine-week old *mili*^{+/-} (C) and *mili*^{-/-} (D) mice stained for MILI (red), Sertoli cells (by anti-TSX antibody, green), and DNA (blue). Sertoli cells are present in the mutant testis but MILI protein is absent since this is a protein-null allele. **E-F**, testicular sections of nine-week old *mili*^{+/-} (E) and *mili*^{-/-} (F) mice stained for BC7 antibody and DAPI, which label primary spermatocytes (green) and DNA (blue). A small number of germ cells in the mutant testis have differentiated into spermatocytes, as also indicated by histological staining (Figure S1). **G-H**, testicular sections of six-month old *mili*^{+/-} (G) and *mili*^{-/-} (H) mice stained for EE2 to label spermatogonia (red), phosphohistone 3 to label mitotic chromosomes (green), and DNA (blue). In the *mili*^{+/-} testis, many spermatogonia and spermatocytes are undergoing active mitosis, yet in the *mili*^{-/-} testis spermatogonia are absent and mitosis is not detectable. **I-J**, testicular sections of six-month old *mili*^{+/-} (I) and *mili*^{-/-} (J) mice stained for laminin (green) to outline seminiferous tubules, TUNNEL labeling to identify apoptotic cells (red), and DAPI to visualize DNA (blue). In the *mili*^{+/-} testis, apoptosis is barely detectable, yet the *mili*^{-/-} testis is undergoing catastrophic apoptosis.

Figure S3. MILI antibody specifically recognizes MILI. Western blot showing that the antibody recognizes a single band of expected MILI size that is present in *mili*(+/+) and *mili*(+/-) testes, but not *mili*(-/-) testes. Tubulin serves as a loading control.

Figure S4. MILI-mediated translational regulation likely occurs in germline stem cells. A, Western blot showing the co-presence of MILI, eIF3a, eIF4GII, and ribosomal protein S6 in 4,6,8,10 dpp testicular extract. **B,** MILI antibody co-precipitated MILI and eIF3a from the 8dpp testicular extract. The co-immunoprecipitation is abolished by MILI-blocking peptide.

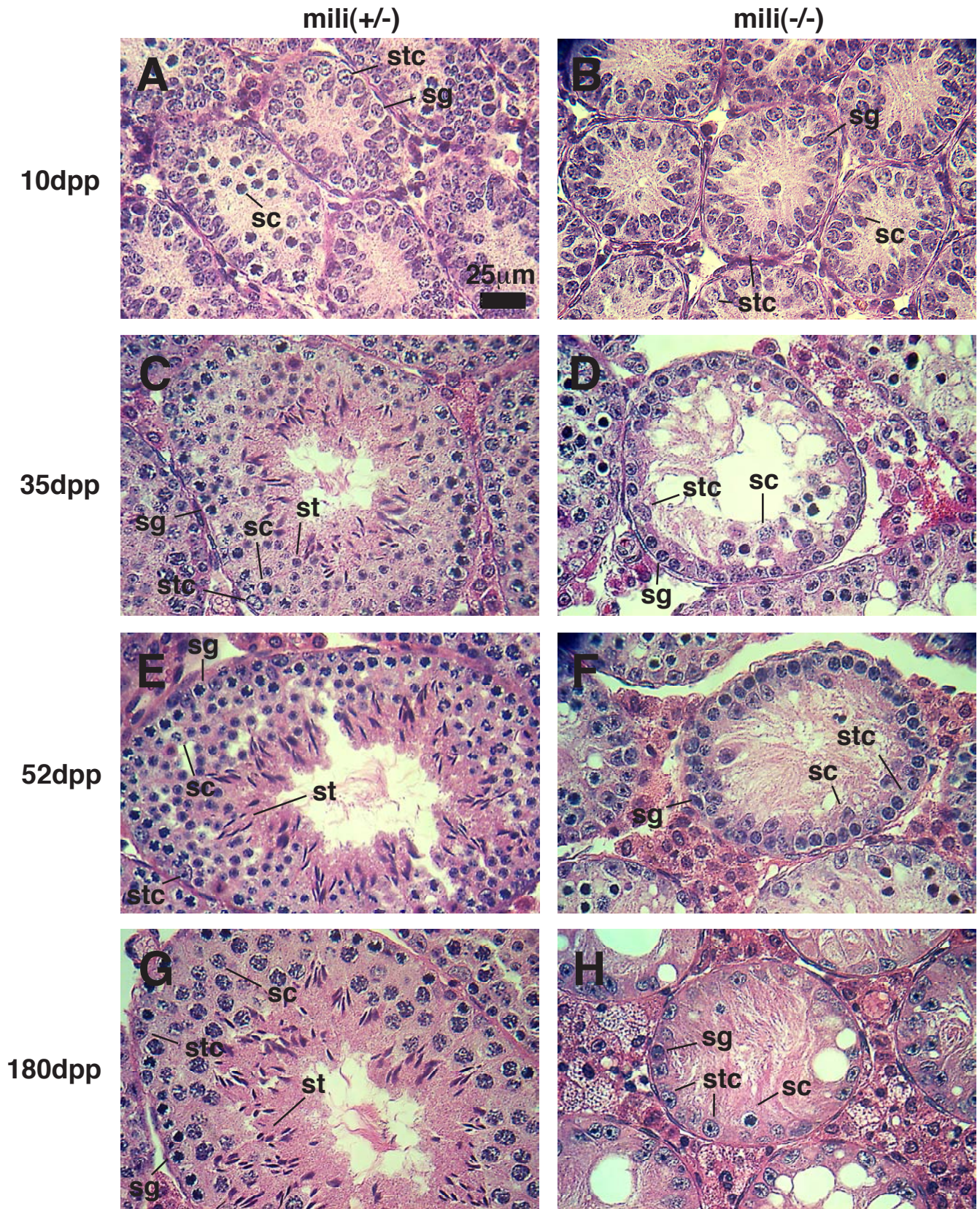


Fig. S1 by Unhavaithaya et al.

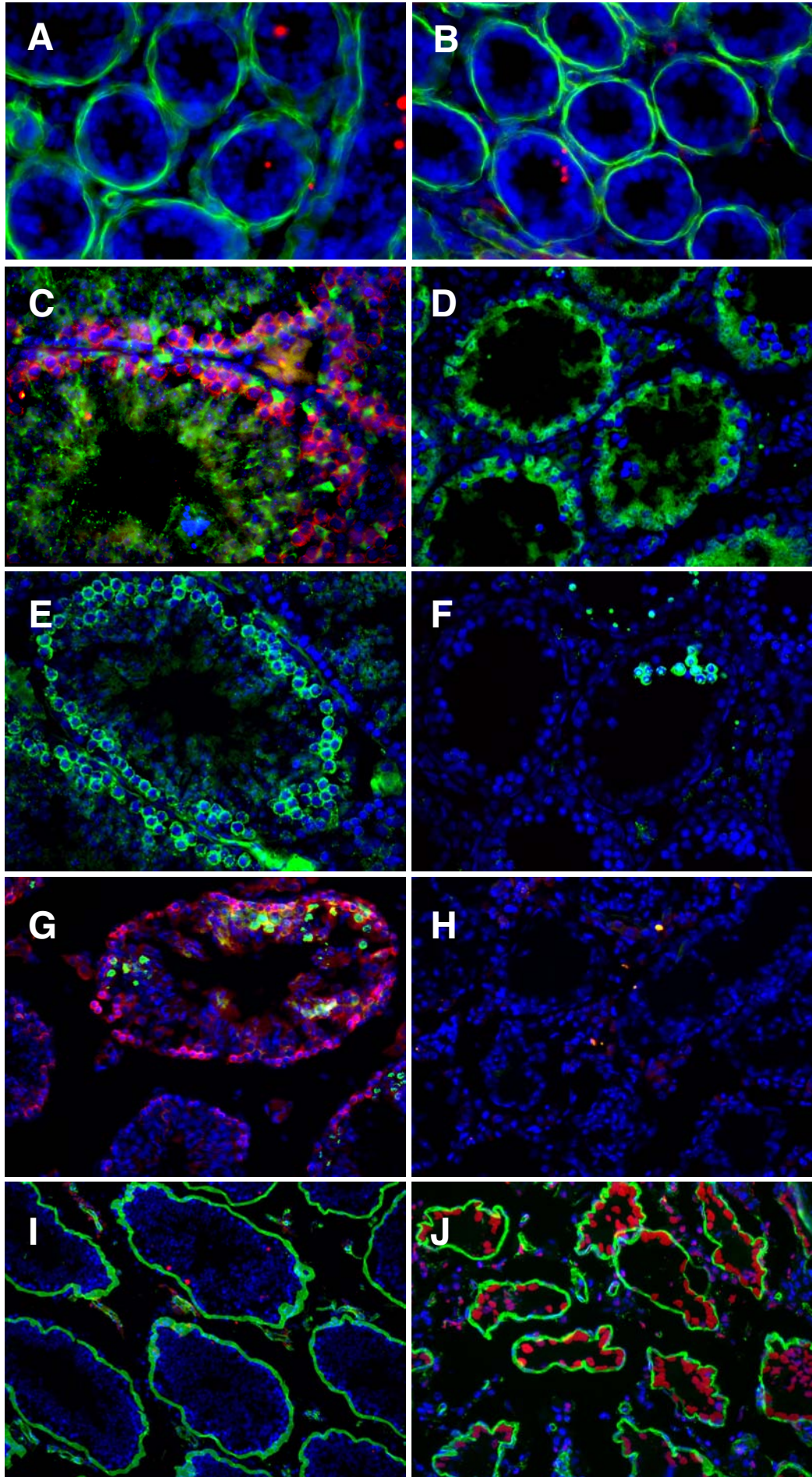


Figure S2 by Unhavaithaya et al.

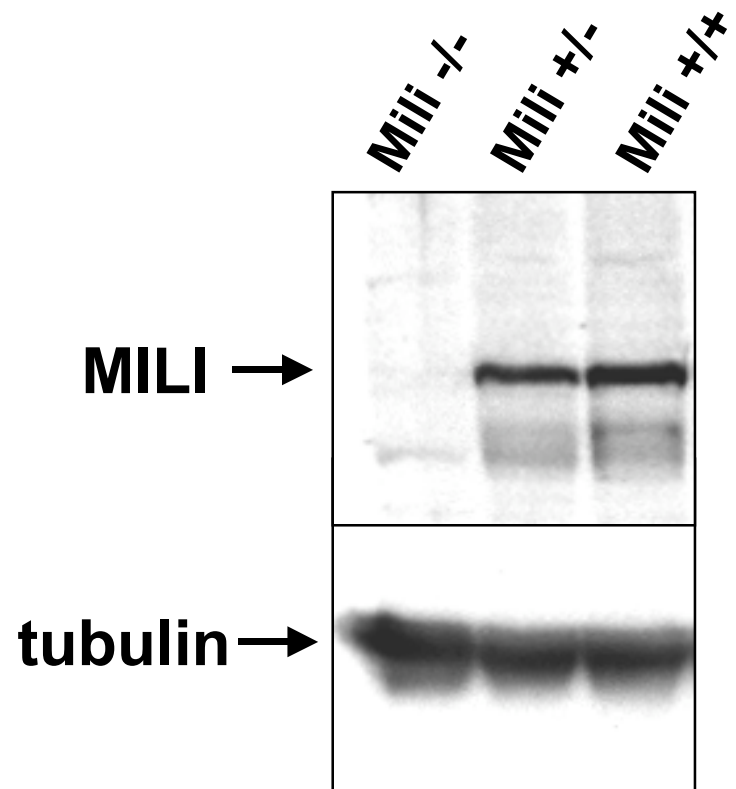


Fig. S3 by Unhavaithaya et al.

A

Adult 4 dpp 6 dpp 8 dpp 10 dpp

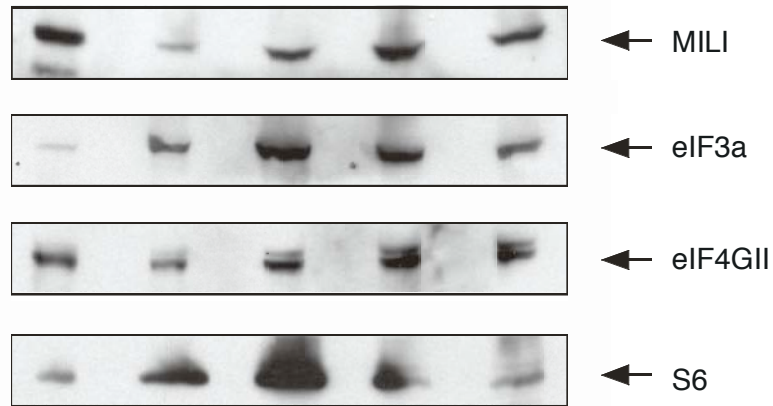
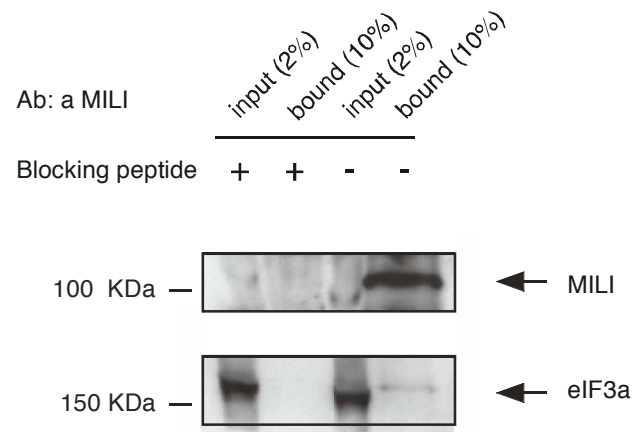
**B**

Fig 4 by Unhavaithaya et al.