

Title: Gilgamesh is required for the maintenance of germline stem cells in *Drosophila* testis

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Supplementary information

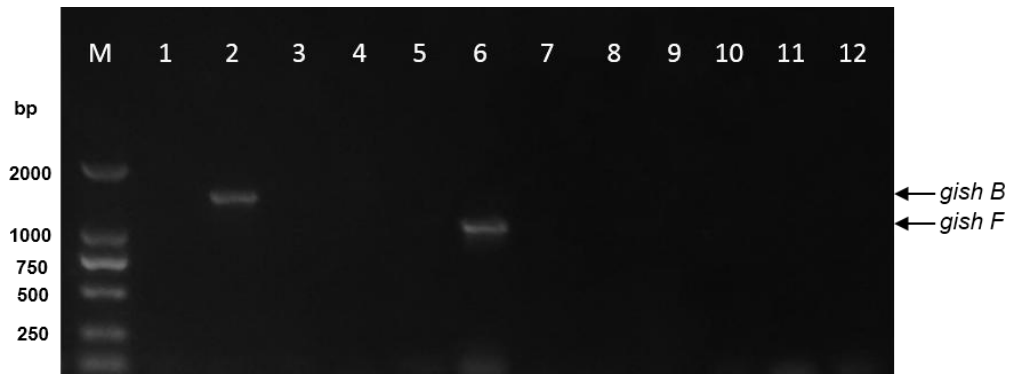


Figure S1. Detection of *gish* splice variants in fly testis by RT-PCR. Agarose gel showing the products of the primer pairs mentioned in S1 Tab. Lanes 1-12 represent the primer pairs that can amplify the *gish* splice variants such as A, B, C, D/L, E, F, G, H, I, J, K and M, respectively. The PCR products (~1400bp size in lane 2, ~1000bp size in lane 6) were detected in agarose gel, which demonstrates that *gishB* (lane 2) and *F* (lane 6) are specifically expressed in fly testis. Lane M denotes DNA ladder.

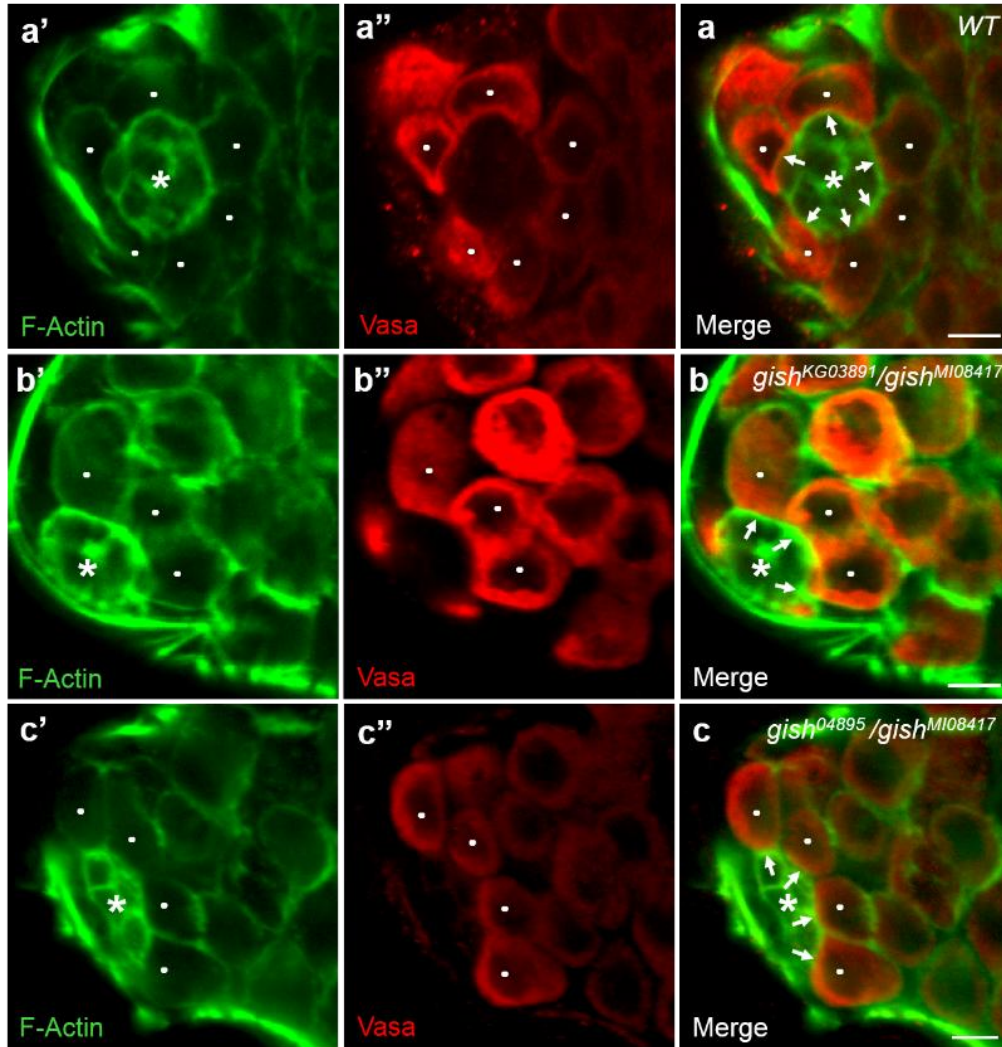


Figure S2. The mutation of *gish* doesn't affect GSCs' adhesion to the hub. Testes from 14-day-old male flies were stained with FITC-conjugated Phalloidin (green, hub cells and germ cells) and anti-Vasa antibody (red, germ cells). The hub was noted by asterisk (green), GSCs were indicated by white dots (red). The boundary of GSCs adhesion to hub were indicated by arrows. (a'-a) Testis from wild-type fly. (b'-b) Testis from *gish*^{KG03891}/*gish*^{MI08417} fly. (c'-c) Testis from *gish*⁰⁴⁸⁹⁵/*gish*^{MI08417} fly. There were no difference in cell-cell adhesions (GSC and hub cell) between *gish* mutants and wild-type testes. Scale bars: 5 μ m.

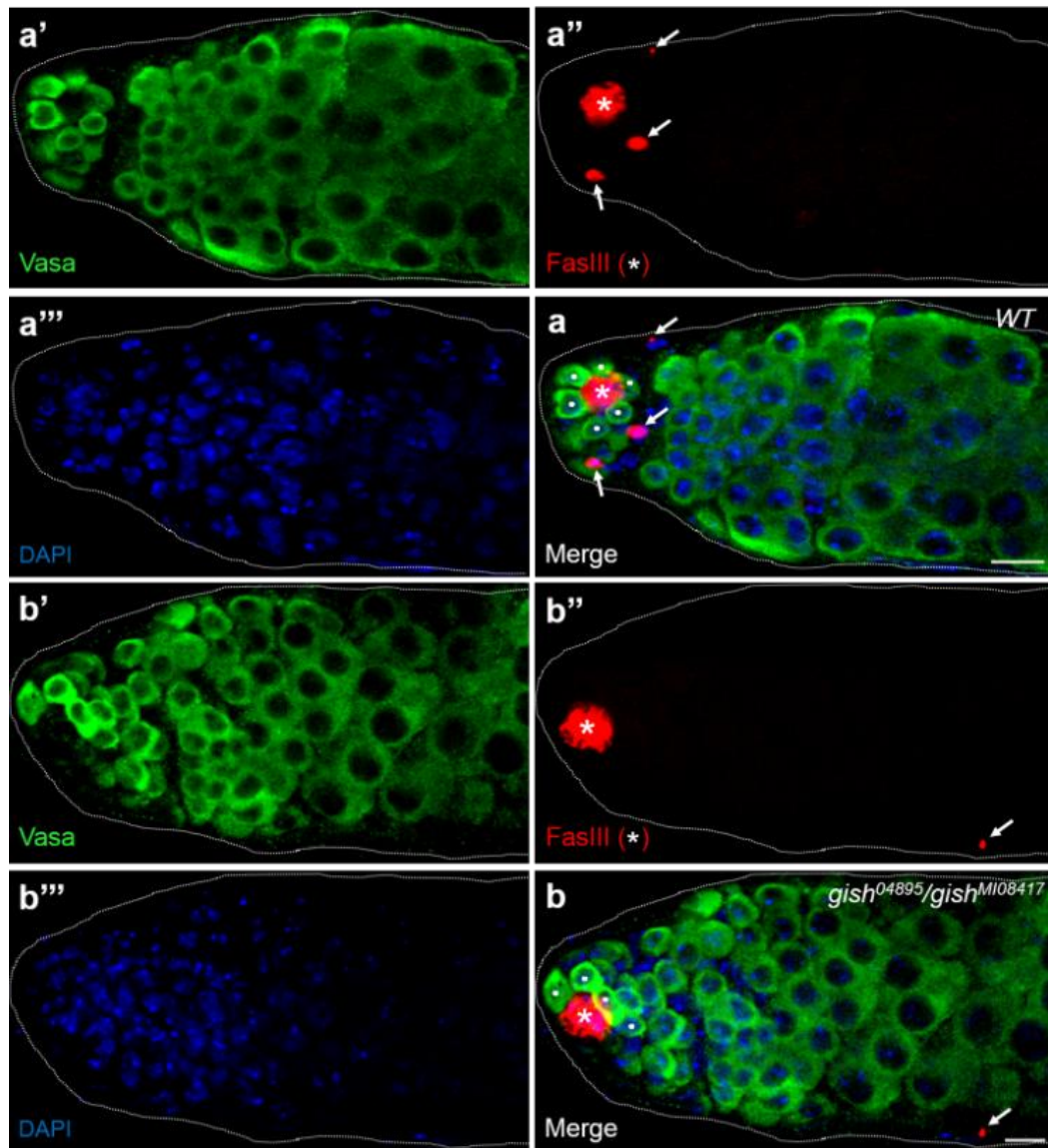


Figure S3. Loss of GSC in *gish* mutant is not caused by cell death. Testes from male flies were labeled by TUNEL (red, indicated by arrows), stained with anti-Vasa antibody (green, germ cells) and anti-Fas III antibody to label the hub (red, asterisk). Germline stem cells (GSCs) were indicated by white dots. The outlines of testes were drawn by broken lines. (a'-a) Testis from wild-type fly. (b'-b) Testis from *gish*⁰⁴⁸⁹⁵/*gish*^{MI08417} fly. Cell death was not detected in GSCs (indicated by white dots), but in somatic cells (indicated by arrows, negative for anti-Vasa staining). Scale bars: 10 μm.

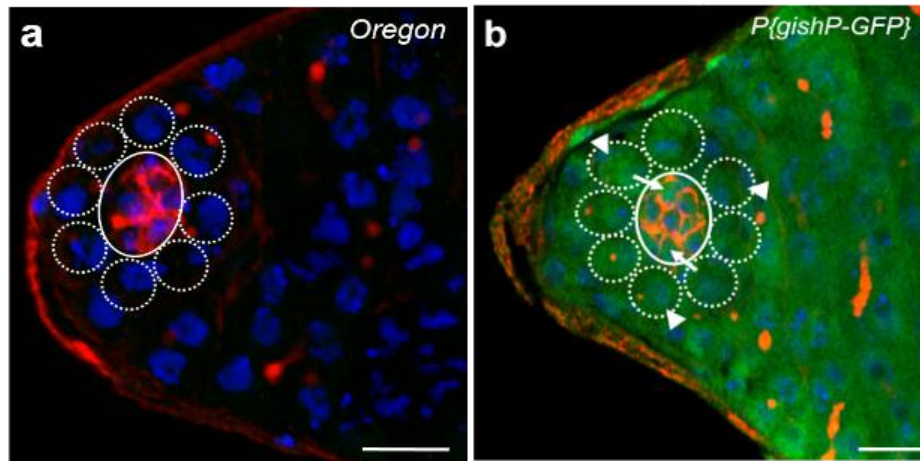


Figure S4. the gene *gish* expresses broadly in adult fly testis. (a) Testis from *Oregon* fly (*wild-type*). (b) Testis bearing a transgene P{*gishP-GFP*}. (a-b) Testes were labeled by with anti-GFP (green), anti-Fas III antibody (red, marking the hub), anti-Hts antibody (red, labeling the germ cells) and DAPI (blue). The gene *gish* expresses (green) both in GSCs (broken lines, arrowhead) and in hub cells (a circle, arrow). Scale bars: 10 μ m.

The splicing variants for <i>gish</i>	The sequences of primer pairs	The length of RT-PCR products (bp)
<i>gish</i> A	Forward: CCAACCAGATGTGCAATAAAAGTG	800
	Reverse: TAATTGTATAGCTATCAGAAGTACGC	
<i>gish</i> B	Forward: ATGCAGCGACGAGAACGGCAAGC	1407
	Reverse: TCATTTTTGGCGCGTCGATTTC	
<i>gish</i> C	Forward: CCAACCAGATGTGCAATAAAAGTG	810
	Reverse: AGTTGTTTCGCTATCATCAATACAG	
<i>gish</i> L&D	Forward: GCAGATTCGTTTTTTAGTCCCAAATC	2640
	Reverse: TTACAACGGAAGTGTCCGGTGTG	
<i>gish</i> E	Forward: ATGCAGCGACGAGAACGGCAAGC	500
	Reverse: TAATTGTATAGCTATCAGAAGTACGC	
<i>gish</i> F	Forward: ATGCAGCGACGAGAACGGCAAGC	1077
	Reverse: TAATTGCTTGCAATCACTATGTCTC	
<i>gish</i> G	Forward: ATGTTGCGCAAATATTTATAC	1410
	Reverse: TCATTTTTGGCGCGTCGATTTC	
<i>gish</i> H	Forward: CGGACGCTAAATCCTGCAGA	1607
	Reverse: TCATTTTTGGCGCGTCGATTTC	
<i>gish</i> I	Forward: ATGCAGCGACGAGAACGGCAAGC	1370
	Reverse: CTGGAATATAAGGAACCATTT	
<i>gish</i> J	Forward: ATGCAGCGACGAGAACGGCAAGC	1300
	Reverse: CTGGAGTATTATATATTCTTCATTCTC	
<i>gish</i> K	Forward: GTCGGCGAATGTGTTGCTTTGGTG	1205
	Reverse: CTGGAATATAAGGAACCATTT	
<i>gish</i> M	Forward: ATGCAGCGACGAGAACGGCAAGC	2400
	Reverse: AAGTGATTGATCTTTGCGGAACTTG	

Table S1. Primers for detecting of *gish* mRNA splicing variants in *Drosophila* testis.

Genotype	The average number of GSCs in fly testis in different day after eclosion (Mean \pm SD)	
	Day 1	Day 20
<i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{KG03891}	6.7 \pm 1.4 (n=67)	3.7 \pm 1.0 (n=64)
P{ <i>gishP-gishF</i> }; <i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{KG03891}	7.9 \pm 1.2 (n=62)	7.5 \pm 1.0 (n=65) *
<i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{MI08417}	6.8 \pm 1.3 (n=64)	3.5 \pm 1.0 (n=65)
P{ <i>gishP-gishF</i> }; <i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{MI08417}	7.7 \pm 1.4 (n=62)	7.5 \pm 1.2 (n=65) *
<i>gish</i> ^{KG03891} / <i>gish</i> ^{MI08417}	6.9 \pm 1.0 (n=66)	3.3 \pm 1.3 (n=67)
P{ <i>gishP-gishF</i> }; <i>gish</i> ^{KG03891} / <i>gish</i> ^{MI08417}	7.8 \pm 1.0 (n=64)	7.5 \pm 1.2 (n=64) *

Table S2. The rescue assay of *gish* mutants by the transgenic line of P{*gishP-gishF*}. SD, standard deviation. n, Number of testes examined. **P* < 0.01, unpaired *t*-test, compared with the corresponding *gish* mutant at day 20.

Genotype	The percentage of apoptotic GSCs between wild type and <i>gish</i> mutant fly testes
<i>Oregon-R</i>	0.45% (N=220)
<i>gish</i> ^{KG03891} / <i>gish</i> ^{MI08417}	0.40% (N=246)
<i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{MI08417}	0.38% (N=237)

Table S3. The analysis of the cell death in *gish* mutant testes at day 5 after eclosion. N, the total number of GSCs examined.

Genotype	The average number of GSCs in fly testis in different day after eclosion (Mean \pm SD)	
	Day 1	Day 20
<i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ⁰⁴⁸⁹⁵	6.7 \pm 1.4 (n=67)	3.6 \pm 1.1 (n=66)
P{ <i>nosP-gishF</i> }; <i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ⁰⁴⁸⁹⁵	7.7 \pm 1.3 (n=65)	7.5 \pm 1.1 (n=66) *
<i>gish</i> ^{KG03891} / <i>gish</i> ^{MI08417}	6.8 \pm 1.0 (n=70)	3.3 \pm 1.3 (n=69)
P{ <i>nosP-gishF</i> }; <i>gish</i> ^{KG03891} / <i>gish</i> ^{MI08417}	7.6 \pm 1.1 (n=64)	7.4 \pm 1.1 (n=67) *

Table S4. The rescue asssay of *gish* mutants by the transgenic line of P{*nosP-gishF*}. SD, standard deviation. n, Number of testes examined. **P* < 0.01, unpaired *t*-test, compared with the corresponding *gish* mutant at day 20.

Genotype	The average number of GSCs in fly testis in different day after eclosion (Mean \pm SD)	
	Day 1	Day 20
<i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{M108417}	6.8 \pm 1.1 (n=69)	3.6 \pm 1.2 (n=68)
P{ <i>c587-gal4</i> };P{ <i>UASp-gishF</i> }; <i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{M108417}	6.9 \pm 1.1 (n=65)	3.5 \pm 1.2 (n=66) *
<i>gish</i> ^{KG03891} / <i>gish</i> ^{M108417}	6.8 \pm 1.2 (n=70)	3.4 \pm 1.2 (n=69)
P{ <i>c587-gal4</i> };P{ <i>UASp-gishF</i> }; <i>gish</i> ^{KG03891} / <i>gish</i> ^{M108417}	6.8 \pm 1.1 (n=66)	3.3 \pm 1.0 (n=68) *

Table S5. The rescue assay of *gish* mutants by combining the transgenic lines of P{*c587-gal4*} and P{*UASp-gishF*}. SD, standard deviation; n, Number of testes examined. **P* > 0.05, unpaired *t*-test, compared with the corresponding *gish* mutant at day 20.

Genotype	The average number of GSCs in fly testis in different day after eclosion (Mean \pm SD)	
	Day 1	Day 20
<i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{KG03891}	6.7 \pm 1.4 (n=65)	3.6 \pm 1.1 (n=66)
P{ <i>UASp-gishF</i> }/P{ <i>nos-gal4</i> }; <i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{KG03891}	7.7 \pm 1.3 (n=66)	7.5 \pm 1.1 (n=67) *
<i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{M108417}	6.8 \pm 1.0 (n=70)	3.4 \pm 1.2 (n=69)
P{ <i>UASp-gishF</i> }/P{ <i>nos-gal4</i> }; <i>gish</i> ⁰⁴⁸⁹⁵ / <i>gish</i> ^{M108417}	7.6 \pm 1.1 (n=64)	7.4 \pm 1.1 (n=67) *
<i>gish</i> ^{KG03891} / <i>gish</i> ^{M108417}	6.8 \pm 1.0 (n=69)	3.3 \pm 1.3 (n=69)
P{ <i>UASp-gishF</i> }/P{ <i>nos-gal4</i> }; <i>gish</i> ^{KG03891} / <i>gish</i> ^{M108417}	7.5 \pm 1.1 (n=67)	7.4 \pm 1.1 (n=69) *

Table S6. The rescue assay of *gish* mutants by combining the transgenic lines of P{*nos-gal4*} and P{*UASp-gishF*}. SD, standard deviation. n, Number of testes examined. **P* < 0.01, unpaired *t*-test, compared with the corresponding *gish* mutant at day 20.