Supplemental Tables and Figures

Table S1. ***eRpL22-like* knockout phenotypes after heat shock (HS)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Genotype** | **Time of HS after egg laying** | **# of heat-shocked embryos** | **Phenotypes** |
| CKO; HS-FLP/+ | 1 hour | 16 | Embryonic lethality |
|  |  | 2 | L1 lethality |
|  |  | 1 | Developmental arrest in L1-like stage, defects in tracheal branching and growth, comparatively smaller Malpighian tubules |
|  |  | 1 | Midpupal lethality, male genitalia deformation, gut development defects |
|  |  | 1 | Early adult lethality (within 24h post-eclosure), tissue (bristles, midgut, eye) death, larval mobility defects |
|  |  | 1 | Eye bristle defects in adult female |
|  | 24-36 hours | 15 | Lethality |
|  |  | 1 | Severe testis/seminal vesicle defects; sterile |
|  |  | 1 | Ovary defects including egg chamber polarity defects; not sterile |
|  |  | \*3 | Normal gonad development |
|  | 48-60 hours | 12 | Normal gonad development; sporadic rough eye |
| Wildtype | 1 hour | 35 | Embryonic lethality |
|  |  | 16 | Wildtype |
|  | 24-36 hours | 35 | Lethality |
|  |  | 62 | Wildtype |

* \*Timing of HS was likely later in the 24-36 hour window.

|  |  |  |
| --- | --- | --- |
| **Genotype** | **Length measurements (μm)** | **Average length (μm)** |
| Wildtype (3 larvae) | 2398.2, 2564.6, 3021.2 | 2661.3 |
| Wildtype with heat shock (1 larva) | 3525.9 |  |
| CKO (2 larvae) | 2493.5, 2341.6 | 2417.5 |
| CKO with heat shock (1 larva) | 707.1 |  |

Table S2. Larval length measurements for Figure 2

**Table S3. Primer Table**

|  |  |  |
| --- | --- | --- |
| **Primer Name** | **Sequence (5’ -> 3’)** | **Use** |
| F\_Act5C | 5’-GAGCGCGGTTACTCTTTCAC-3’ | qRT-PCR |
| R\_Act5C | 5’-GCCATCTCCTGCTCAAAGTC-3’ | qRT-PCR |
| F\_eRpL22 | 5’-TGCTGAGGATAGCATCATGG-3’ | qRT-PCR |
| R\_eRpL22 | 5’-AAAAGTGAACGTCGGAGCTG-3’ | qRT-PCR |
| F\_eRpL22-like | 5’-CAAAAAGAAGGCTTGGCAAC-3’ | qRT-PCR |
| R\_eRpL22-like | 5’-TCCTTCAGCTGGTTGAGCTT-3’ | qRT-PCR |
| F\_eRpL22-like\_pENTR | 5’-CACCGCGAGATCTATGAGTTC  CCAGACGCAG-3' | Creation of *eRpL22-like-FLAG* transgenic |
| R\_eRpL22-like\_FLAG | 5’-GTCACGGATCCTTACTTGTCATCGTCATCCTT GTAGTCGCCGCGGCCGATGGCAAAGGTTTTTC  CGCCATTGTCGTCGGCAA-3' | Creation of *eRpL22-like-FLAG* transgenic |
| F\_pENTR\_FLAG | 5’-CACCATGATCGGCCGCGGCGACTAC  AAGGATGAC GATGACAAG-3' | Creation of *FLAG-eRpL22* transgenic |
| R\_eRpL22 | 5’-GCCCTGAGGCTCTTGGGAGCAGCGGCAGC-3' | Creation of *FLAG-eRpL22* transgenic |
| F\_DsRed | 5' - gctcttcaagggagtgaaatacatc - 3' | Genotype for DsRed |
| R\_DsRed | 5' - gatccacaaggccctgaagc - 3' | Genotype for DsRed |
| F\_Dmel\_Hsp70 | 5'- gcctcgaatgttcgcgaaaagagc - 3' | Genotype for HSp70-Fippase |
| RFLP1 | 5’- CTGACGAACAAGCACCTTAGGTGGTG – 3’ | Genotype for HSp70-Flippase |
| F\_Flank | 5’- GCCTTGTTTGCTCTTGGCAG - 3’ | Genotype for eRpL22-like locus / flanking sequence |
| R\_Flank | 5’- AATTAAGCCGCCTGGCATCG- 3’ | Genotype for eRpL22-like locus / flanking sequence |
| F\_Actin\_Gal4 | 5’-catgatgaaataacataaggtggtccc-3’ | Genotype for *Actin*-Gal4 |
| R\_Actin\_Gal4 | 5’-CTCCAGTCACAGCTTTGCAGC-3’ | Genotype for *Actin*-Gal4 |
| F\_DmL22likeBamHI | 5′-GTCACGGATCCATGAGTTCCCAGACGCAGAAAAAG  AATGCTTCCAA-3′ | Genotyping for *eRpL22-L22-like-FLAG* construct |
| R\_DmL22likeBamHI | 5′-GTCACGGATCCTTAGGCAAAGGTTTTT  CCGCCATTGTCGTCGGCAA-3′ | Genotyping for *eRpL22-L22-like-FLAG* construct |

Figure S1

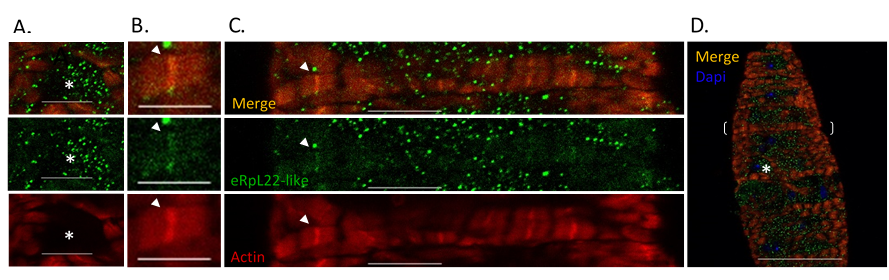
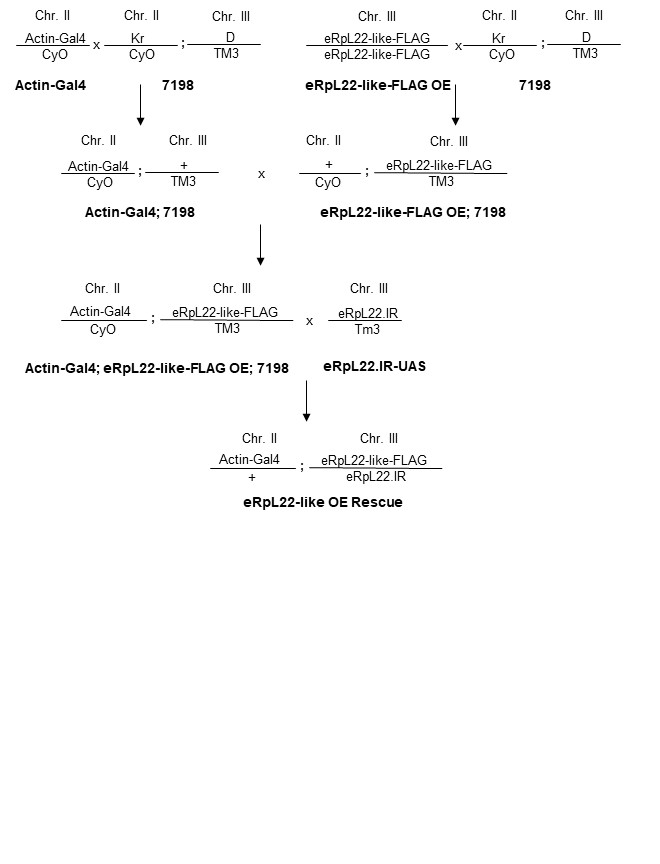


Figure S2

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Figure S3



**Supplemental Information**

**Figure S1. IHC of wildtype first instar larva gut**. IHC of wildtype first instar larva gut. eRpL22-like (green) co-localizes with actin (red) in muscle (arrows) (B-C), does not co-localize in non-muscle regions of gut (asterisks) (A). Magnification: A) scale bar 10µm, B) scale bar 5µm, C) scale bar 10µm, D) scale bar 50µm.

**Figure S2.** **Complete germline development is retained in eRpL22-like-depleted testes. A)** Phase contrast microscopy of light (top) and heavy (bottom) testis squashes shows various stages of germline development in control (bam>GFP) and eRpL22-like-depleted tissue, including mitotic cells and primary spermatocytes (bracket), elongating spermatids (short open arrow), sperm bundles (long open arrow), and individual mature sperm (closed arrow). Asterisk denotes the apical tip, where germline stem cells are located and germline development begins. (10X magnification) **B)** IHC of eRpL22-like-depleted testis. eRpL22 (red) shows increased cytoplasmic co-localization with eRpL22-like (green) compared to eRpL22 in the control (wildtype). Degenerating cysts (arrowhead) are also evident in eRpL22-like-depleted testes. Asterisks denote apical tips. (20x magnification). Images in A and B are representative from numerous examples.

**Figure S3.** **Schematic of *eRpL22-like* rescue cross strategy.** Genotypes are listed on each chromosome and name of parent fly is shown in bold below genotypes.