**Supplementary data:**

**Fig. 1.** Lethality in *loe*. A) Lethality during development. There is no significant lethality during embryonic development because the percentage of 1.instar *loe* larvae hatched from eggs (set as a 100%) is similar to the wild type. Comparing the number of pupae reveals that only 64% of the 1.instar *loe* larvae reach the pupal stage instead of 96% in wild type. The number of adult *loe* flies hatching from pupae (95%) is not different from wild type (96%). B) Life span of adults. The *loe* mutation reduces the life span to approximately four weeks (wild type 8-10 weeks). Expressing the *loe* cDNA in neurons restores the wild type life span. Double mutants of *loe* and *Appl* have an even shorter life span of approximately 10d. *Appl* mutants alone have also a shortened life span similar to *loe*.

**Fig. 2.** *loe* mRNA expression. A) A full-length loei probe, containing also the conserved 3' end, detects several transcripts in bodies and heads. The transcript (arrow), which is recognized by a loei specific probe and which is affected in the *loe* mutant (B), is strongly enriched in the head fraction. C) The loei transcript (arrow) is weakly expressed in larval stages and probably not expressed in embryo and pupae.

**Fig. 3.** In-situ hybridization of *loe* RNA on tissue sections. A) A probe comprised of the loei specific exons reveals staining of the entire brain cortex, in the thoracic ganglia, and a few cells in the male sex organs (arrow). B) An antisense probe to the 3' part, which is conserved in the different splice forms, labels in addition to the nervous system the ovaries and weaker the flight muscles. C) Control with a sense probe. D) Magnification from (B). In ovaries staining is detectable in nurse cells.
(arrow) as well as in the oocyte (arrowhead). (E) In contrary, the loeI specific probe does not stain the ovaries. F) Staining on head sections shows that the loeI transcript is not equally abundant in all nerve cells. Strongest expression is found in the cortex of the central brain whereas the staining is weaker (in some regions very weak, arrow) in the cortex of the optic lobes, some regions. G) A magnification from (A) shows expression in the neuronal cell bodies surrounding the thoraxic ganglia (arrows). The thoracic ganglia has, like the brain, a vacuolar appearance in loe mutant flies (data not shown).

**Methods:**

**Measurement of lethality:** For the developmental measurements 25 females and 25 males were kept in food vials for one day. The number of laid eggs was counted and two days later the number of unhatched embryos (eggs that were still intact). Approximately two weeks later, when most adults had eclosed, we counted the pupal cases and the number of adult flies. Vials were kept for another week and checked daily for flies eclosing later. There was no difference in the duration of any developmental stage between loe and wild type. For the determination of the life span between 30 and 40 newly eclosed male flies were kept on food vials and counted after 1-4 days. Vials were exchange after 6-10 days. All measurements were done at 25°C.

**In situ Hybridization:** Frozen head sections of adult wild type flies were fixed and hybridized as described in Poeck et al. (1993). Digoxigenin-labeled RNA probes were transcribed following the protocol of the Boehringer RNA labeling kit, using
loel fragments in bluescript pSK. Hybridizations were carried out overnight at
50°C using 25 µl per slide of hybridization solution consisting of Boehringer
standard hybridization buffer containing 50% formamide and digoxigenin-labeled
antisense RNA at 2.5-5 µg RNA per ml.
A

Lethality during development

B

Adult life span