

Supplementary Figure 1. Inactivation of *C. elegans psr-1* causes a mild increase in germ cell corpses in response to Ultraviolet (UV) radiation. Quantification of germ cell corpses in wild-type (white bars) and *psr-1(tm469)* (solid bars) hermaphrodites was carried out in the absence or presence of 100 Jm⁻² UV radiation. Animals were allowed to develop to the young adult stage (24 hours post L4 to the adult molt) at 20°C, treated with UV, and scored for the number of germ cell corpses in gonads 2, 4, or 6 hours after UV radiation, respectively. Error bars indicate s.e.m. (*n*=15 at each time point). The asterisk (*) indicates statistically significant difference at *P* < 0.05, according to Student's *t*-test.



Supplementary Figure 2. Analysis of eggs laid in N2 and *psr-1(tm469)* animals. The numbers of eggs laid in N2 and *psr-1(tm469)* animals were counted at 24 hours, 36 hours, and 48 hours post L4 to the adult molt. Single L4 larva was placed in a plate and transferred to a new plate every 12 hours at 20°C. Error bars indicate s.e.m. (*n*=12).



Supplementary Figure 3. A *mec-4(u231)* gain-of-function mutation causes necrotic death of touch receptor neurons. (a, b) The Differential Interference Contrast (DIC) microscopy and GFP images of the PLM touch receptor neurons. The PLM neuron displayed a normal, oval-shape morphology with both anterior and posterior axonal processes visible in a *bzIs8* animal (a). In a *mec-4(u231) bzIs8* animal, a dying PLM neuron adopted a vacuolar morphology characteristic of necrotic cells and lost its axonal processes (b). Arrowheads indicate the PLM neurons. Scale bars represent 10 µm.



Supplementary Figure 4. Analyses of PSR-1 protein and mRNA levels in C. elegans. a, The enzyme-linked immunosorbent assay (ELISA) analysis of monoclonal antibodies (mAbs) to PSR-1 was performed as described in Methods. The upper limit value of OD450nm-OD630nm, which reflects the mAb binding affinity to the antigen, is 4.0. The data indicate that all mAbs bound GST-PSR-1(293-400), but not GST, with high affinity and specificity. b, c, e, f, Analysis of PSR-1 protein expression in C. elegans using different PSR-1 antibodies. 100 L4 larvae from the indicated strains were solubilized with SDS sample buffer, resolved on 12% SDS PAGE, and subjected to immunoblotting using mouse mAb 8F7 (b), purified rat polyclonal antibodies to PSR-1 (c), and mouse M2 and AA4.3 monoclonal antibodies to the Flag epitope and alpha-tubulin, respectively (e, f). 5 ng of GST-PSR-1(293-400) were used as a positive control (b, c). Asterisks indicate non-specific, cross-reactive bands to the antibodies. d, The relative mRNA levels of rpl-26, ced-1 and psr-1 genes in C. elegans. Real-time PCR analysis of rpl-26, ced-1 and psr-1 mRNA levels in wild type N2 animals was performed as described in Methods. Error bars represent the mean ± SD of three independent experiments.



ced-1(e1735); psr-1::3xflag SCl; psr-1(tm469); Ex(P_{ced-1}CED-1 (C::GFP)

Supplementary Figure 5. Immunostaining of *C. elegans* gonads. (a-c)

Exposed gonads of adult hermaphrodites (24 hours post L4 to the adult molt) or embryos of the indicated genotypes were stained with Hoechst 33342 (or DAPI) and 8F7 mouse monoclonal antibody to PSR-1 (**a**) or the M2 monoclonal antibody to the Flag epitope (**b**,**c**) using fixed, permeabilized conditions (see Methods). Images of Hoechst 33342 or DAPI staining, 8F7 or anti-Flag staining (Rhodamine), and the merged image of Rhodamine and Hoechst (or DAPI) are shown. (d) Exposed gonads from the same stage adult hermaphrodites of the indicated genotype were stained with the M2 antibody using the non-permeabilized conditions (see Methods). Images of anti-Flag staining (Rhodamine), CED-1 Δ C::GFP, DIC, and the merged image of Rhodamine and CED-1 Δ C::GFP are shown. CED-1 Δ C::GFP without CED-1 cytoplasmic domain clusters on the surface of apoptotic cells but does not rescue the *ced-1(e1735)* engulfment defect¹. "Ex" indicates an extrachromosomal transgene array carrying P_{ced-1}CED-1 Δ C::GFP. Arrowheads indicate apoptotic germ cells. Scale bars represent 10µm



Supplementary Figure 6. CED-2 associates with PSR-1 in *C. elegans*.

Lysates from C. elegans animals carrying integrated transgene expressing TRA-2::3xFlag (TRA-2) or 3xFlag::PSR-1 (PSR-1) were prepared as described in Methods. One portion of the worm lysate was used in the immunoblotting (IB) analysis to examine the expression levels of CED-2 (lower panel) and two Flagtagged proteins (upper panel) in worms. The remaining portion of the lysate was incubated and precipitated (IP) with Anti-Flag M2 Affinity Gel. The amount of the CED-2 protein pulled down by the two Flag-tagged proteins (indicated by pink arrows) was analyzed by immunoblotting using rabbit anti-CED-2 antibodies (lower panel), which detected a 31 kD band (the estimated size of CED-2; indicated by green arrows) that was present in wild-type (N2) lysate, but absent in ced-2(e1752) mutant lysate. An additional band with a slightly larger size (green arrowheads) was also present in N2 lysate and absent in ced-2(e1752) lysate and may be an isoform or a modified form of CED-2. TRA-2::3xFlag and 3xFlag::PSR-1 showed similar sizes (slightly over 50 kD) on the blot². Asterisks indicate antibody light chain. Uncropped scans of the blots are shown in Supplementary Figure 7.





Supplementary Figure 7. Uncropped scans of anti-Flag and anti-CED-2 blots. Areas indicated by dash boxes are shown in Supplementary Figure 6.

Supplementary References

- 1. Zhou, Z., Hartwieg, E., & Horvitz, H.R., CED-1 is a transmembrane receptor that mediates cell corpse engulfment in *C. elegans. Cell* **104**, 43-56. (2001).
- Mapes, J., Chen, J.T., Yu, J.S., & Xue, D., Somatic sex determination in Caenorhabditis elegans is modulated by SUP-26 repression of *tra-2* translation. Proc Natl Acad Sci U S A **107**, 18022-18027 (2010).