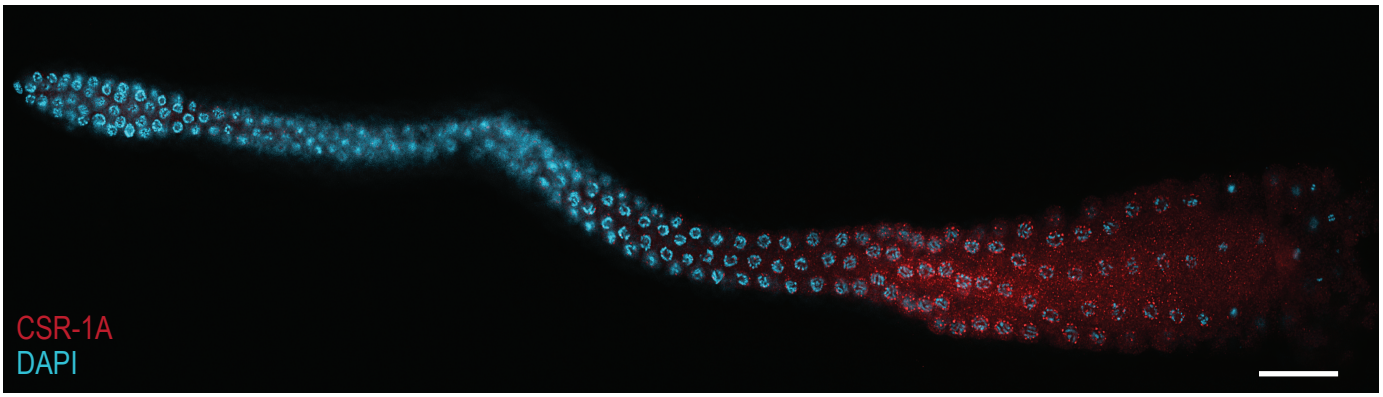
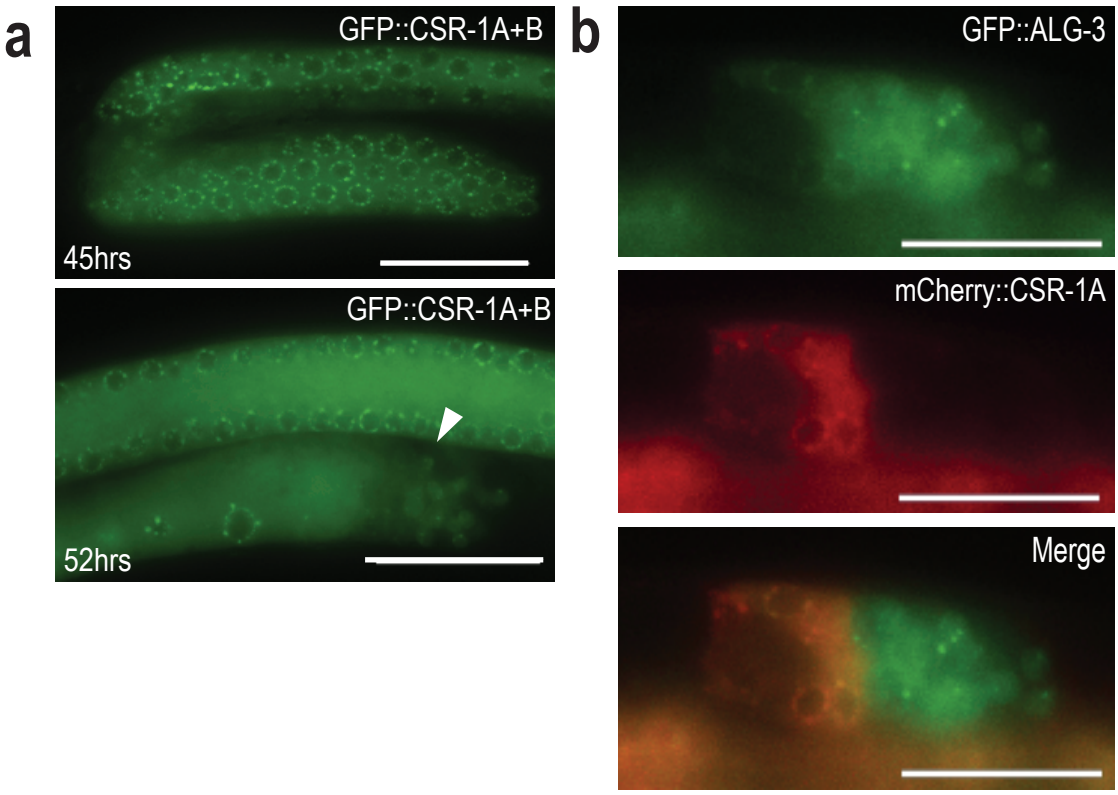
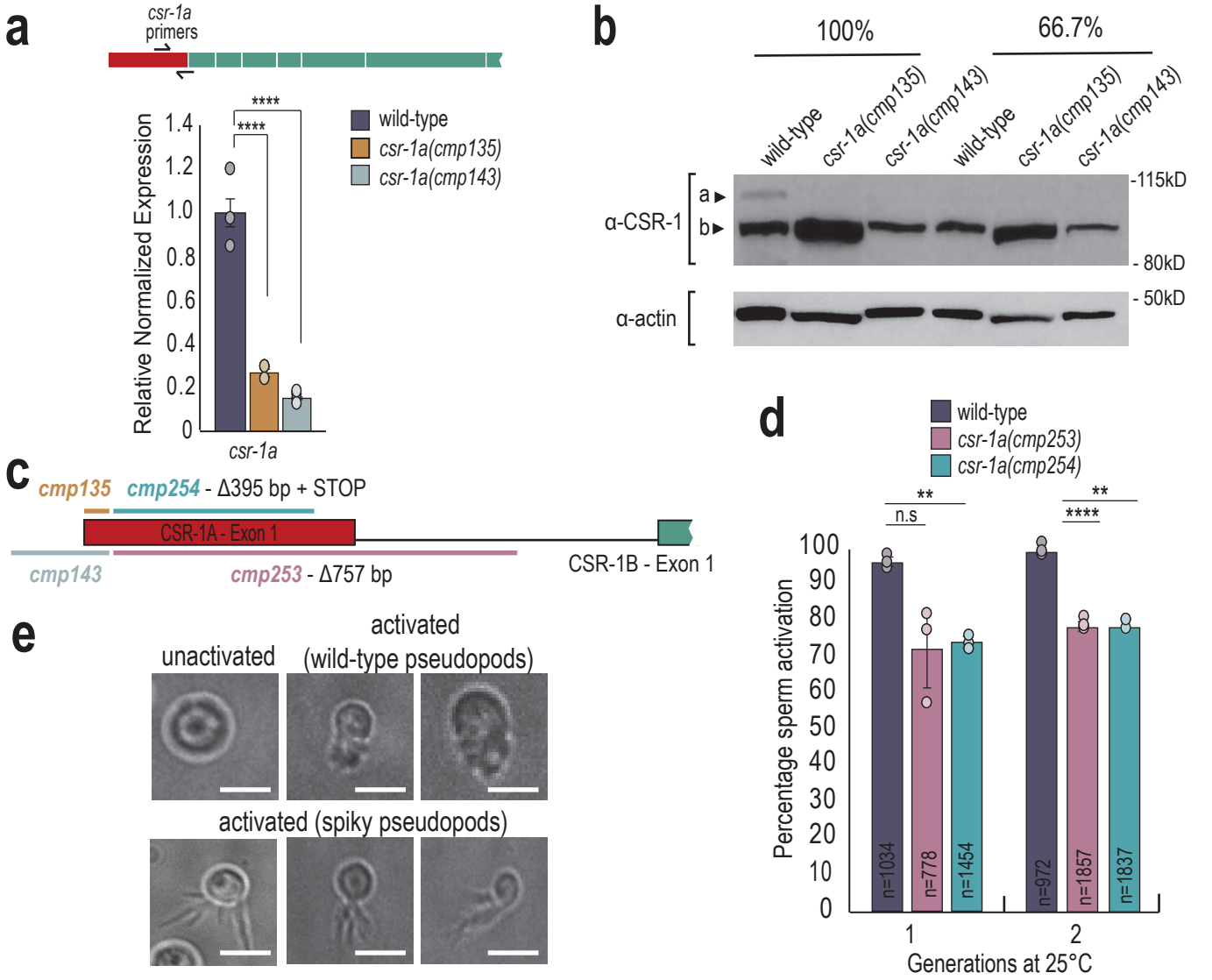


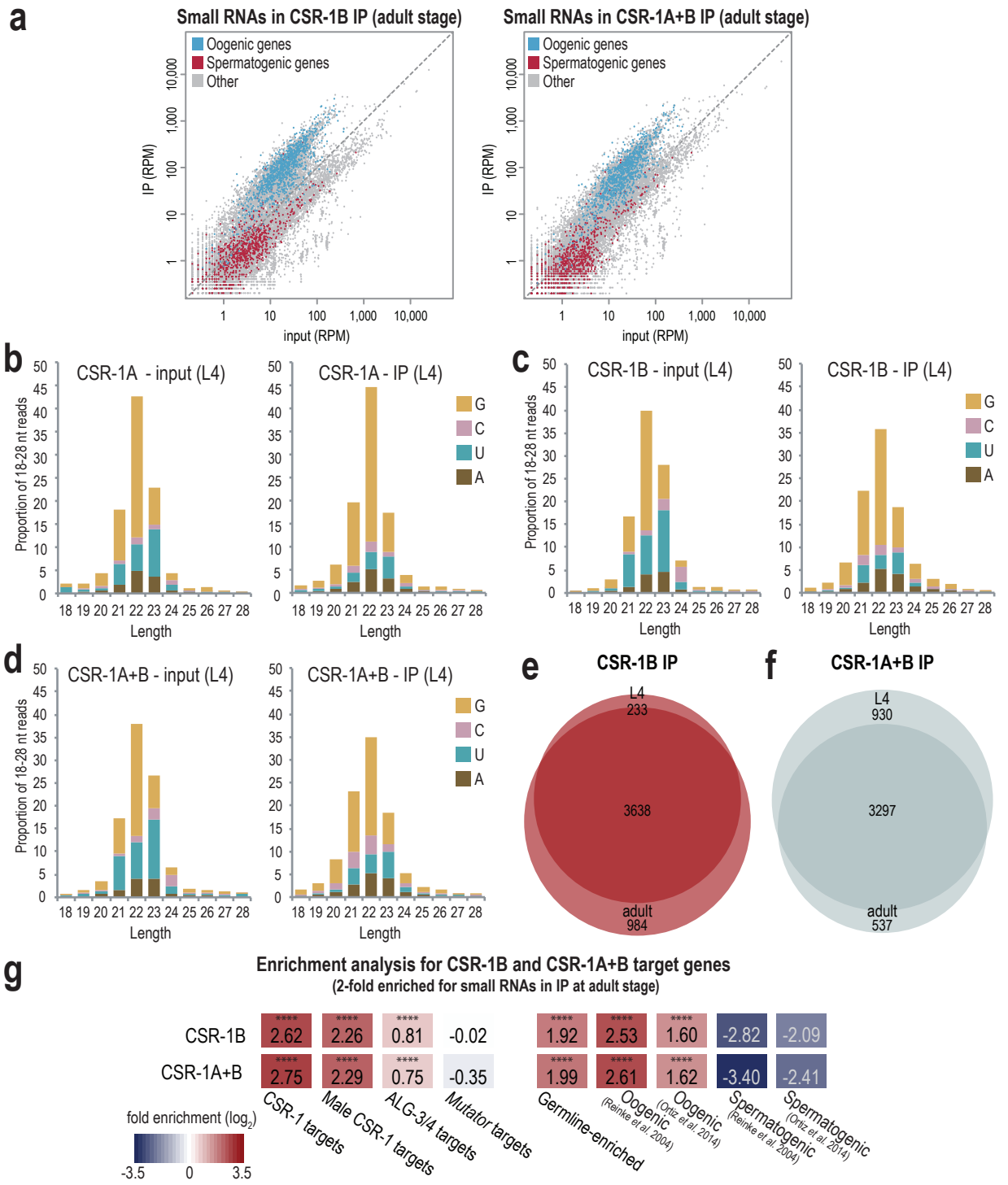
Supplementary Fig. 1 CSR-1A is expressed in the spermatogenesis region of the male germline. Immunofluorescence staining of CSR-1A in a dissected L4 male germline carrying the 2xHA::mCherry::CSR-1A transgene. HA antibodies are used to recognize CSR-1A and DNA is stained with DAPI. The experiment has been reproduced with at least five individual germlines imaged. Scale bar, 20μM.



Supplementary Fig. 2 CSR-1A is excluded from secondary spermatocytes.
a 3xFLAG::GFP::CSR-1A+B at 45 hrs (early L4 stage) or 52 hrs (young adult stage) post-L1 arrest. White arrowhead indicates region of secondary spermatocytes. Scale bars, 25μM. **b** Live imaging of double-transgenic animal labelled for both GFP::ALG-3 and mCherry::CSR-1A at the young adult stage. Scale bars, 25μM. At least 5 individual germlines were imaged for each strain and condition.

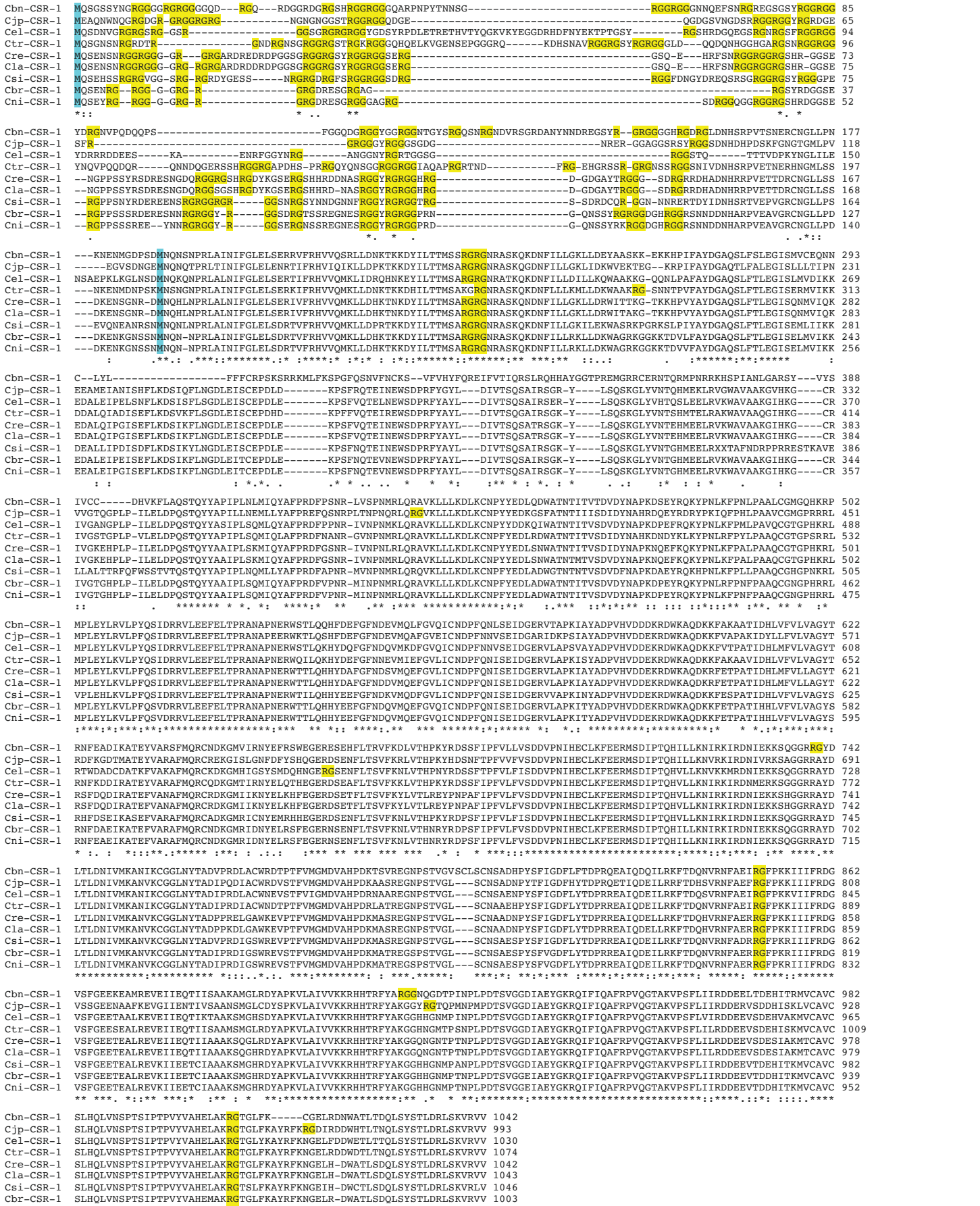


Supplementary Fig. 3 CSR-1A is required for optimal male fertility.
a RT-qPCR on *csr-1a* mutants and wild-type animals at L4. Relative expression was normalized to *rpl-32* and calculated relative to wild-type. Bar graphs representing mean for three biological replicates and error bars indicate SEM. Two-tail t-tests were performed to determine statistical significance. **b** Western blot detecting for both CSR-1 isoforms expression in wild-type and *csr-1a* mutants L4 hermaphrodites at 100% and 66.7% loading volumes, using CSR-1 antibody. Actin is shown as a loading control. The blot has been reproduced. **c** Schematic representation of two new *csr-1a* mutants (*cmp253* and *cmp254*). **d** *in vitro* sperm activation assay on additional *csr-1a* alleles and wild-type. Animals were raised at 25°C for either one or two generations and at least 150 spermatids were counted for each replicate at each generation. Three biological replicates are shown, with bar graphs representing the mean and error bars indicating standard deviation. Two-tail t-tests were performed to determine statistical significance. **e** Representative images from the *in vitro* sperm activation assay of *csr-1b(cmp258)* mutants. Unactivated spermatids and activated spermatids with wild-type pseudopods were observed in wild-type and all mutant strains, while activated spermatids with spiky pseudopods were observed only in *csr-1b(cmp258)* mutants. The phenotype was observed in in all replicates. Scale bars, 5μM. n.s. denotes not significant and indicates a p-value > 0.05, ** indicates a p-value ≤ 0.01, and **** indicates a p-value ≤ 0.0001. See Supplementary Data 7 for more details regarding statistical analysis. Source data are provided as a Source Data file.

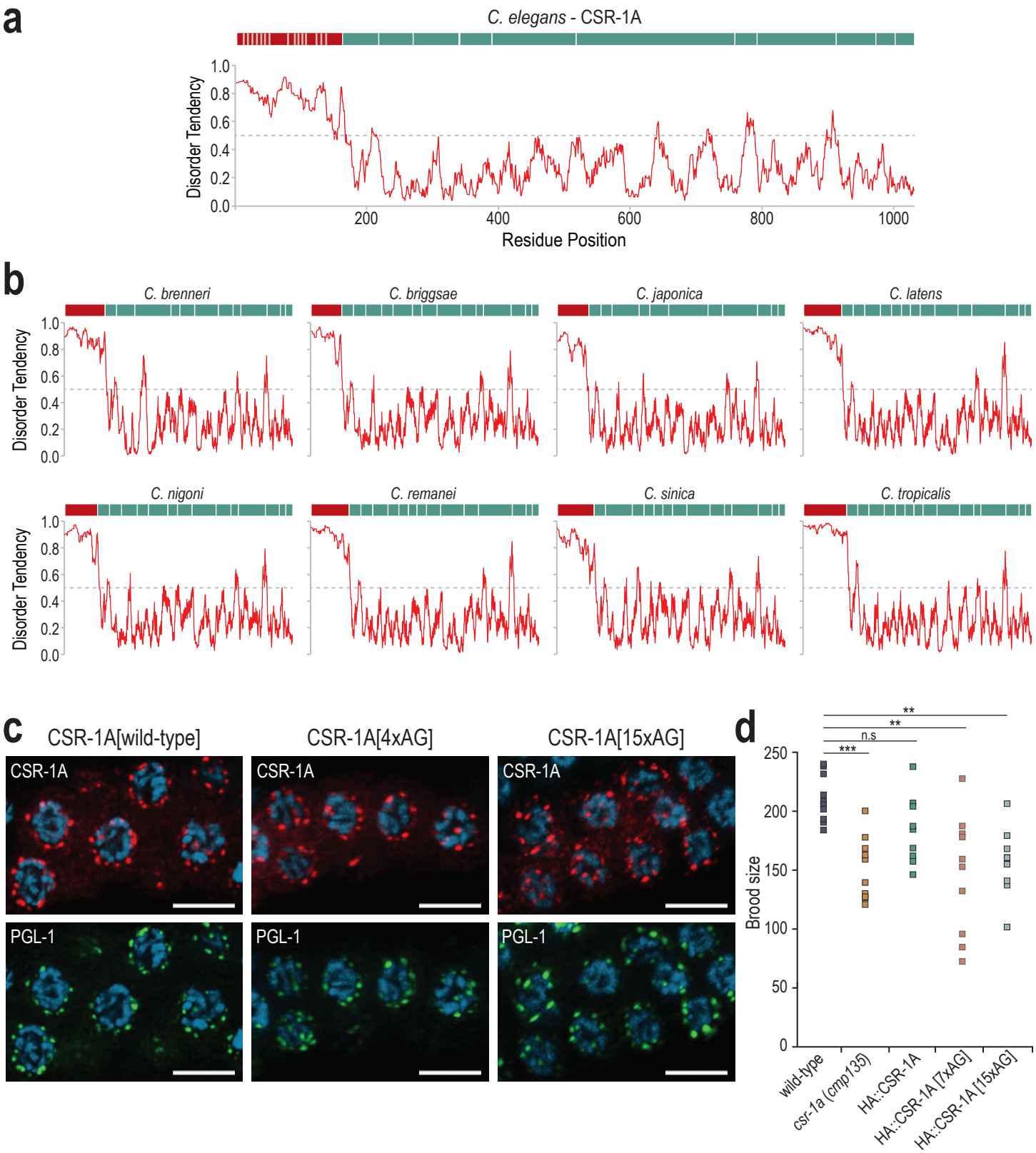


Supplementary Fig. 4 Both CSR-1 isoforms bind germline-enriched 22G-RNAs.

a Normalized reads for oogenic and spermatogenic genes (defined by Reinke *et al*, 2004) at adult stage from FLAG::CSR-1B IP and FLAG::CSR-1A+B IP compared to input. Oogenesis and spermatogenesis genes are indicated in blue and red respectively. **b-d** 5' length and nucleotide distribution in representative input and IP libraries from L4 stage HA::CSR-1A (**b**), FLAG::CSR-1B (**c**), and FLAG::CSR-1A+B (**d**). **e-f** Venn diagram indicates overlap of L4 and adult target genes from CSR-1B (**e**) and CSR-1A+B (**f**) immunoprecipitations. Target genes are defined are at least 2-fold enriched in the IP, with at least 10 RPM in IP samples and a DESeq2 adjusted p-value ≤ 0.05. **g** Enrichment analysis (log2(fold enrichment)) examining the overlap of adult stage CSR-1B and CSR-1A+B target genes with known targets of the CSR-1, male CSR-1, ALG-3/4, and *mutator* small RNA pathways and oogenesis and spermatogenesis-enriched genes. See Materials and Methods for gene list information. Two-tailed p-values for enrichment was calculated using the Fisher's Exact Test function in R. n.s. denotes not significant and indicates a p-value > 0.05 and **** indicates a p-value ≤ 0.0001. See Supplementary Data 7 for more details regarding statistical analysis.



Supplementary Fig. 5 Clustal Omega alignment of CSR-1 in Caenorhabditis species.
Arginine/glycine motifs are highlighted in yellow and start codons for both CSR-1A and CSR-1B are highlighted in blue. See Materials and Methods for protein sequence information.



Supplementary Fig. 6 The N-terminal exon of CSR-1 is disordered across Caenorhabditis species.
a-b Graphs examining the disorder tendencies of CSR-1A in C. elegans (**a**) and CSR-1 in related nematode species (**b**). Residue position is plotted on the x-axes and disorder tendency scores (y-axes) above 0.5 indicate disorder. Red bar above each plot marks region of first exon and green bars mark all other exons. **c** Immunofluorescence staining of dissected L4 hermaphrodite germlines in HA::CSR-1A, HA::CSR-1A[4xAG], and HA::CSR-1A[15xAG], using antibodies against HA and PGL-1. Scale bars, 5μM. At least five individual germlines were imaged for each strain. d Brood size assay on wild-type and mutant animals raised at 25°C (n=10). Two-tail t-tests were performed to determined statistical significance. ** indicates p-value ≤ 0.01, *** indicates a p-value ≤ 0.001. See Supplementary Data 7 for more details regarding statistical analysis.