Supporting Information for

The intrinsically disordered protein SPE-56 is required for acrosomal-like exocytosis and fertility in *Caenorhabditis elegans*

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Fig. S1

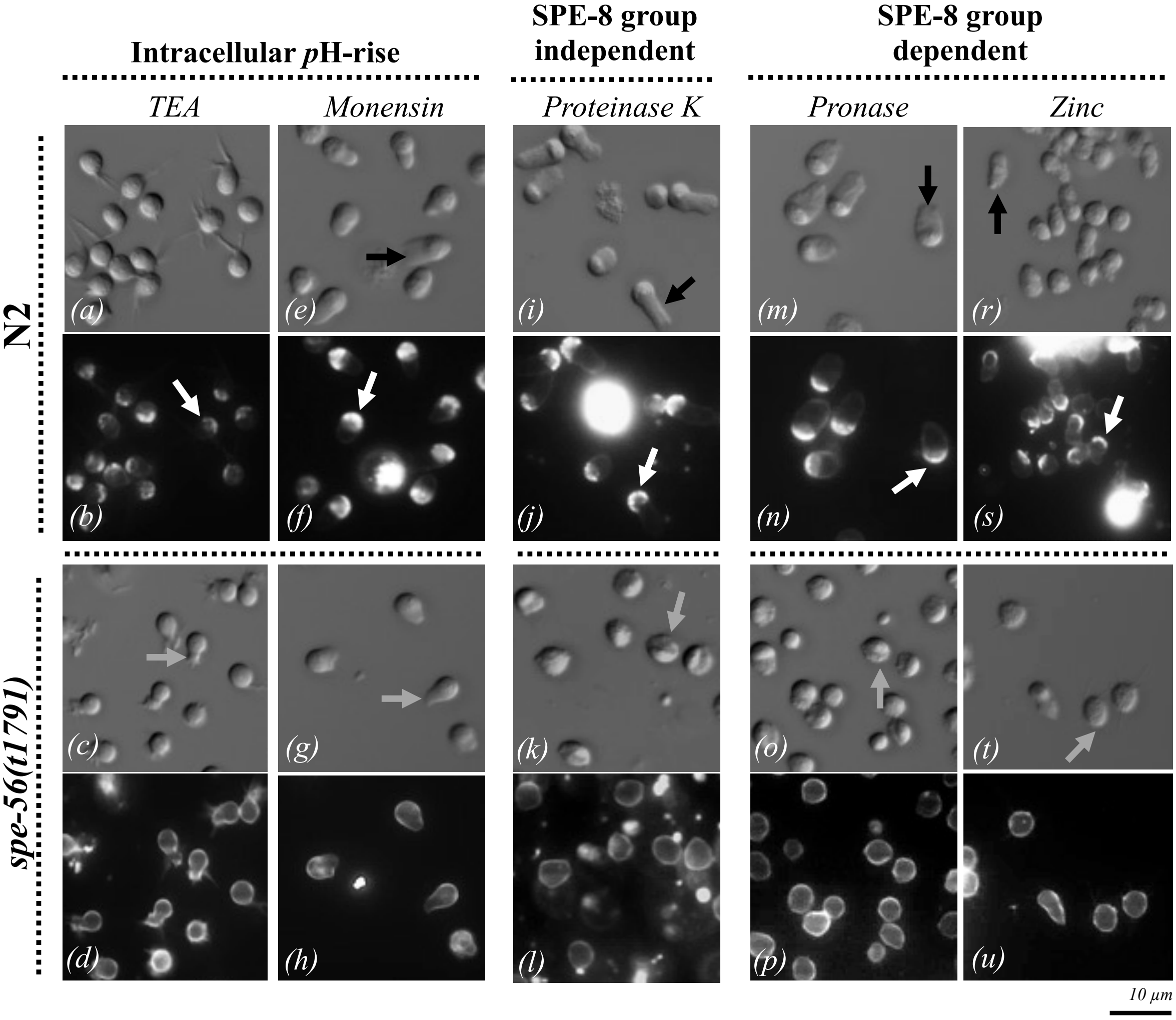

Figure S1. Assessment of *in vitro* activation in *spe-56*-deficient sperm.
DIC and fluorescence images of *in vitro*-activated spermatozoa isolated from N2 wild-type and *spe-56(t1791)* mutant males. Various chemical agents were used to induce activation, including Triethanolamine **(**TEA; ***a–d*)**, Monensin **(*e–h*)**, Proteinase-K **(*i–l*)**, Pronase **(*m–p*)**, and Zinc **(*r–u*)**, which target different activation mechanisms, including intracellular *p*H-raise, an SPE-8–independent pathway, and the canonical SPE-8 pathway. Black arrows indicate the pseudopods of the activated spermatozoa; white arrows indicate the MO fusion pores. Gray arrows indicate the short pseudopods that are typical of *spe-56*-deficient spermatozoa.

Fig. S2

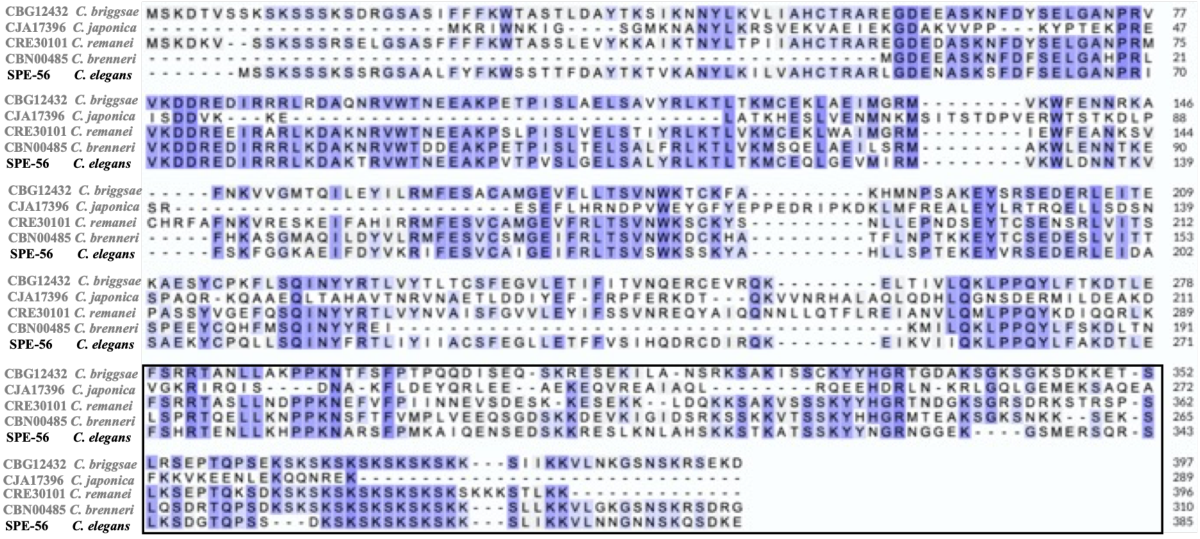

Figure S2. Multiple sequence alignment of SPE-56 homologs of rhabditid nematodes. Amino acid alignments with *Clustal Omega* and *Clustal* color scheme *(94)* shows sequence similarities in *C. elegans* compared with *C. brigsae*, *C. japonica*, *C. remanei*, and *C. brenneri*. The black border indicates disorder prediction by MobiDB *(92).*

Fig. S3

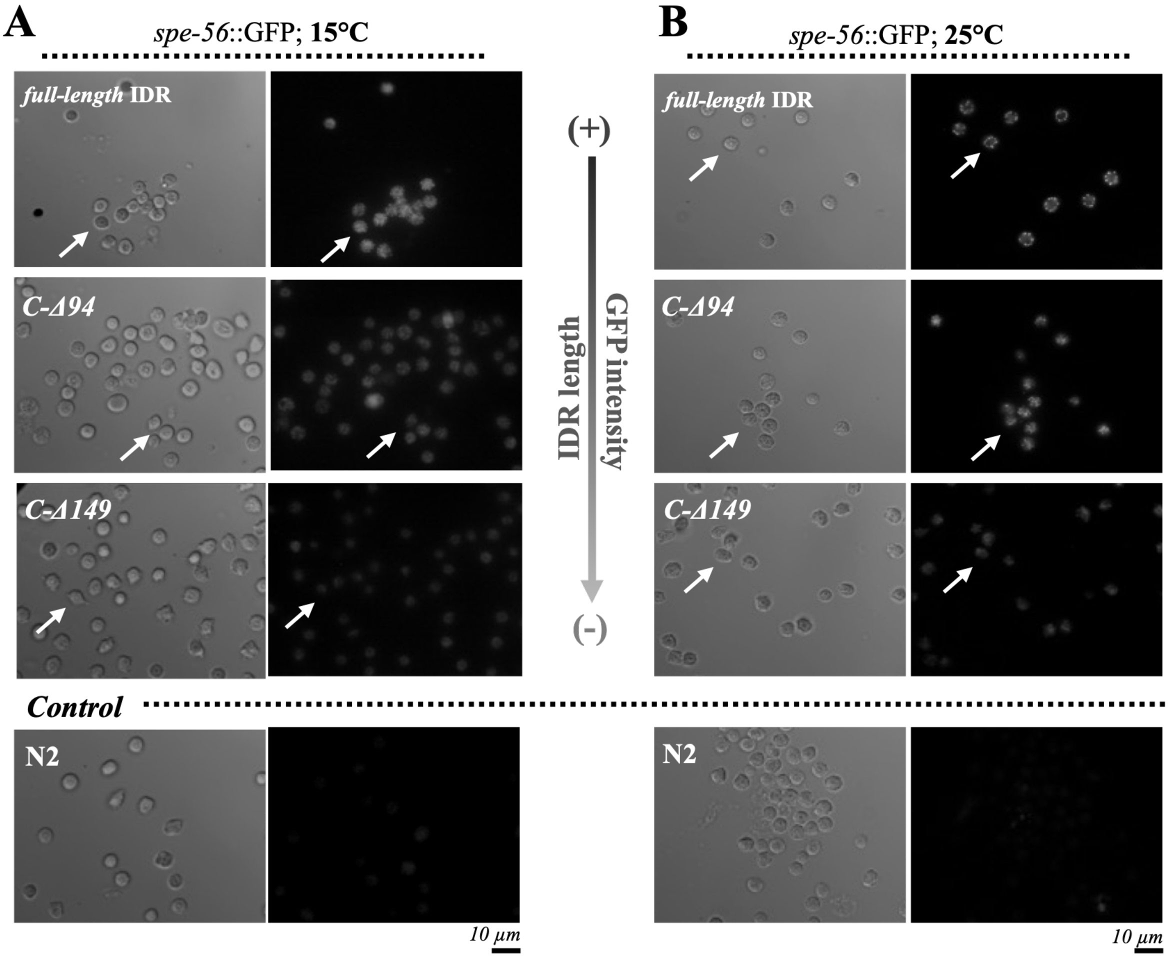

Figure S3. Temperature- and IDR-dependent segregation of SPE-56 in C-terminal deletion mutants.

DIC and fluorescence images of *spe-56(fed113)* spermatid groups carrying SPE-56::GFP with either the full-length C-terminal IDR or CRISPR/Cas9-engineered IDR-deletion variants (*C-Δ94* or *C-Δ149*), grown at 15 °C **(A)** or 25 °C **(B)** . Autofluorescence-control images depicting N2 wild-type spermatids are shown below. White arrows indicate spermatids. The gray long arrow between the images indicates the relative loss of GFP fluorescence with increasing IDR deletion.