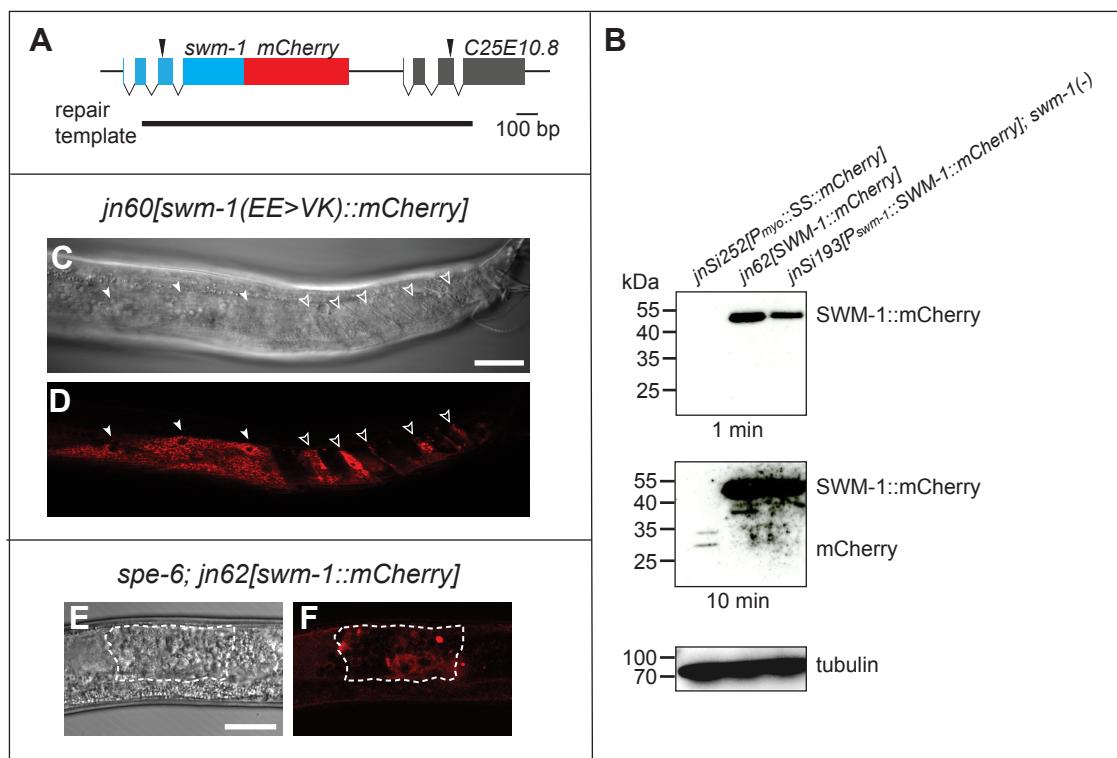
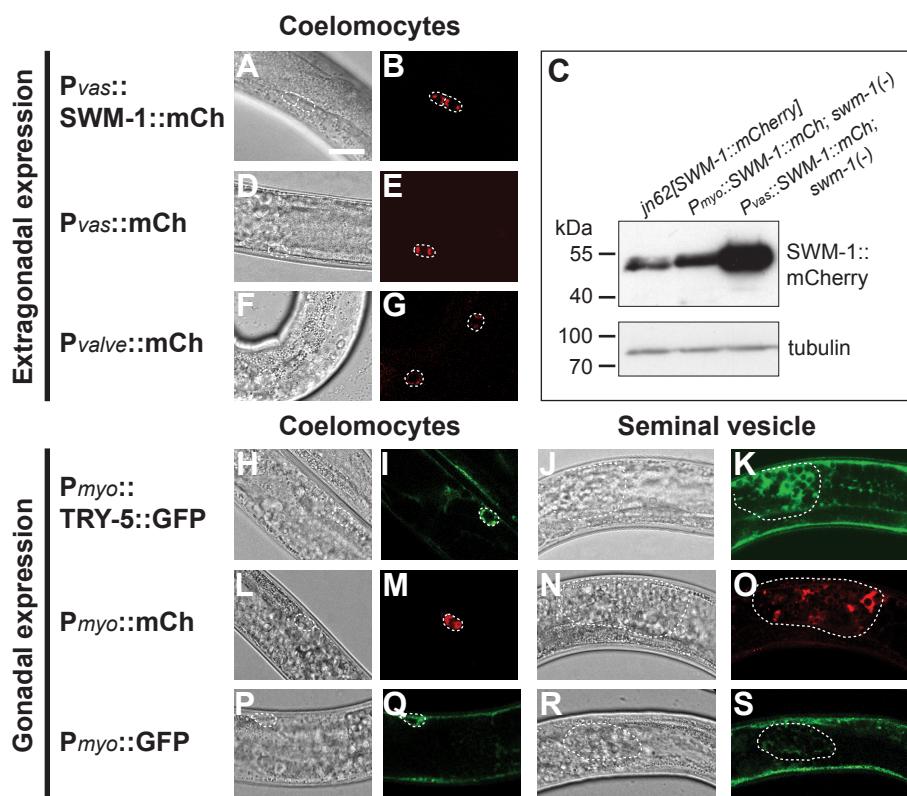


**Figure S1. Transgenes with 1.3 kb of sequence upstream of *swm-1* rescue premature sperm activation in a *swm-1* null mutant.** Males harboring *swm-1* transgenes showed strong rescue of the activated sperm defect. Rescue was slightly reduced in strains expressing SWM-1::mCherry as compared to those expressing untagged SWM-1. Individual males were scored as containing only activated sperm (black), a mixture of activated and non-activated sperm (grey), or only non-activated sperm (white). Columns show the percent of males with the indicated phenotypes. n=29-57 animals/genotype at each time point.



**Figure S2. Localization of SWM-1::mCherry.** (A) Schematic of CRISPR-CRISPR-mediated insertion of mCherry at the *swm-1* genomic locus. Arrowheads indicate Cas9 cut sites. (B) Western blot of worm extracts probed with antibodies against mCherry (upper panels show different exposures) and tubulin (lower panel). mCherry and SWM-1::mCherry protein from *jnSi252[Pmyo::SS::mCherry]*, *jn62[swm-1::mCherry]* and *jnSi193[Pswm-1::swm-1::mCherry]* males are detected near their predicted sizes of 41 kDa and 27 kDa, respectively. (C,D) *jn60[swm-1(EE>VK)::mCherry]* adult male. As compared to *jn62*, SWM-1 is visible at elevated levels in body wall muscle cells (arrowheads) and male-specific diagonal tail muscle cells (open arrowheads). (E,F) Seminal vesicle of a *dpy-18 spe-6; jn62[swm-1::mCherry]* adult male. SWM-1::mCherry is present throughout the entire seminal vesicle. The *dpy-18* mutation is present in the strain as a marker linked to *spe-6*; it does not affect sperm phenotypes. All images were taken at 24 hr post L4. Scale bar, 25 $\mu$ m.



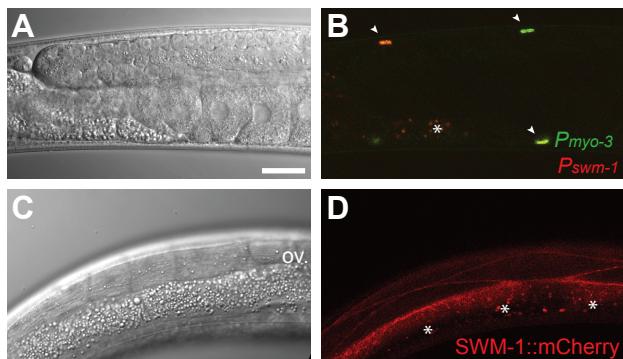
**Figure S3. Secreted proteins move between extragonadal and gonadal tissues.**

(A,B,D-S) Transmitted-light and confocal images of 24 hr adult males. (A,B,D-G)

Proteins secreted by gonadal tissues can localize to coelomocytes in the body cavity.

(C) Higher levels of SWM-1::mCherry are produced in the vas deferens as compared to muscle. Western blot of worm extracts probed with anti-mCherry (upper panel) or anti-tubulin (lower panel). Strains shown: UX790, UX786, UX886. (H-S) Different proteins secreted from muscle can localize to coelomocytes in the body cavity and to the seminal vesicle lumen. Strains shown: UX960, UX872, UX928, UX786, UX941, UX957.

Full genotypes are listed in Table 1. Scale bar, 25  $\mu$ m.



**Figure S4. SWM-1 is produced in hermaphrodite muscle.**

(A,B) *jnSi130[Pswm-1::mCherry::H2B]/jnSi226[Pmyo::gfp::H2B]* hermaphrodite. *swm-1*-positive cells coincide with a marker of body wall muscle (arrowheads). (C,D) *jn62[swm-1::mCherry]* hermaphrodites showing localization of SWM-1::mCherry to body wall muscle. All images were taken at 24 hr post L4. \*, intestinal autofluorescence. Scale bar, 25 $\mu$ m.

**Table S1. *C. elegans* strain genotypes.**

Strain #	Genotype
AV322	<i>swm-1(me87)</i> <i>him-5(e1490)</i> V
CB1490	<i>him-5(e1490)</i> V
UX590	<i>jnSi130[Pswm-1_1.3::mCherry::H2B::3'swm-1_0.7, Cb-unc-119(+)] II; unc-119(ed3) III; him-5(e1490)</i> V
UX580	<i>jnSi131[Pswm-1_1.3::swm-1::3'swm-1_0.7, Cb-unc-119(+)] II; unc-119(ed3) III; swm-1(me87) him-5(e1490)</i> V
UX730	<i>jnSi193[Pswm-1_1.3::swm-1::mCherry::3'swm-1_0.7, Cb-unc-119(+)] II; unc-119(ed3) III; swm-1(me87) him-5(e1490)</i> V
UX950	<i>jnSi193[Pswm-1_1.3::swm-1::mCherry::3'swm-1_0.7, Cb-unc-119(+)] II; swm-1(me87) try-5(tm3813) him-5(e1490)</i> V
UX790	<i>swm-1(jn62{swm-1::mCherry}) him-5(jn64)</i> V
UX888	<i>swm-1(jn62{swm-1::mCherry}) him-5(jn64) V; ncls13[ajm-1::GFP]</i>
UX895	<i>dpy-18(e364) spe-6(hc163) III; swm-1(jn62{swm-1::mCherry}) him-5(jn64) V</i>
UX946	<i>dpy-11(e224) swm-1(jn62{swm-1::mCherry}) fog-2(q71)</i> V
UX791	<i>swm-1(jn60{swm-1::mCherry EE&gt;VK}) him-5(e1490)</i> V
UX838	<i>jnSi226[Pmyo-3::GFP::H2B::3'unc-54, Cb-unc-119(+)] II; unc-119(ed3) III</i>
UX876	<i>jnSi253[Pmyo-3::swm-1::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490)</i> V
UX886	<i>jnSi255[Pmyo-3::swm-1::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490)</i> V
UX969	<i>jnSi253[Pmyo-3::swm-1::3'unc-54, Cb-unc-119(+)] IV; him-5(e1490)</i> V
UX873	<i>jnSi252[Pmyo-3::swm-1::mCherry::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490)</i> V
UX915	<i>jnSi234[Pmyo-3::swm-1(ΔSS)::mCherry::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490)</i> V
UX872	<i>jnSi251[Pmyo-3::swm-1SS::mCherry::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490)</i> V
UX928	<i>arl37[Pmyo-3::secreted GFP::3'unc-54, Cb-unc-119(+)] II, dpy-18(e364) spe-6(hc163) III; him-5(e1490)</i> V
UX960	<i>jnSi276[Pmyo-3::try-5::GFP::3'unc-54, Cb-unc-119(+)] II; unc-119(ed9) III; swm-1(me87) try-5(tm3813) him-5(e1490)</i> V

UX948	<i>jnSi203[Pclec-197::swm-1::3'unc-54, Cb-unc-119(+)] IV; him-5(e1490) V</i>
UX966	<i>jnSi203[Pclec-197::swm-1::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490) V</i>
UX786	<i>jnSi199[Pclec-197::swm-1::mCherry::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490) V</i>
UX954	<i>dpy-18(e364) spe-6(hc163) III; jnSi199[Pclec-197::swm-1::mCherry::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490) V</i>
UX941	<i>unc-119(ed3) III; jnSi268[Pclec-197::swm-1SS::mCherry::3'unc-54, Cb-unc-119(+)] IV</i>
UX955	<i>dpy-18(e364) spe-6(hc163) III; jnSi269[Pclec-197::swm-1SS::mCherry::3'unc-54, Cb-unc-119(+)] IV; him-5(e1490) V</i>
UX924	<i>jnSi72[Pclec197::try-5::GFP::3'unc-54, Cb-unc-119(+)] II; dpy-18(e364) spe-6(hc163) III; swm-1(me87) try-5(tm3813) him-5(e1490) V</i>
UX945	<i>jnSi262[Pdpy-7::swm-1::mCherry::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490) V</i>
UX961	<i>dpy-18(e364) III; jnSi262[Pdpy-7::swm-1::mCherry::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490) V</i>
UX962	<i>dpy-18(e364) spe-6(hc163) III, jnSi262[Pdpy-7::swm-1::mCherry::3'unc-54, Cb-unc-119(+)] IV; swm-1(me87) him-5(e1490) V</i>
UX957	<i>dpy-18(e364) spe-6(hc163) III; jnSi272[Pins-31::swm-1SS::mCherry::3'unc-54, Cb-unc-119(+)] IV; him-5(e1490) V</i>
UX968	<i>dpy-18(e364) spe-6(hc163) III; jnSi214[Pins-31::secreted GFP::3'unc-54, Cb-unc-119(+)] IV; him-5(e1490) V</i>
UX467	<i>jnSi92[Ptry-5::try-5::GFP::3'try-5, Cb-unc-119(+)] II; swm-1(me87) try-5(tm3813) him-5(e1490) V</i>
UX958	<i>jnSi92[Ptry-5::try-5::GFP::3'try-5, Cb-unc-119(+)] II; dpy-18(e364) spe-6(hc163) III; try-5(tm3813) him-5(e1490) V</i>
UX967	<i>jnSi92[Ptry-5::GFP::3'try-5, Cb-unc-119(+)] II; dpy-18(e364) spe-6(hc163) III; swm-1(jn60{swm-1::mCherry EE&gt;VK}) him-5(e1490) V</i>
UX381	<i>jnSi68[Prab-3::try-5::GFP::3'try-5, Cb-unc-119(+)] II; swm-1(me87) try-5(tm3813) him-5(e1490) V</i>

**Table S2. Oligonucleotides used to generate Gateway vector donor plasmids.**

Fragment	Length (bp)	Forward primer <sup>1</sup>	Reverse primer <sup>1</sup>	Gateway vector	Plasmid name
<i>swm-1</i> promoter	1365	GGGGACAACCTTGTAT <b>AGAAAAGTTGTGCCCA</b> <b>TTTCTCACACAGAC</b>	GGGGACTGCTTTTG TACAAACTG <b>TTTCACA</b> <b>ATAACGAAGGGCAAC</b>	pDONR P4-P1r	pAKS4
<i>swm-1</i> coding region	630	GGGGACAAGTTGTAC AAAAAACGAGGCTTGA <b>AAATGGTAGTTTTG</b> <b>AG</b>	GGGGACCACTTGTAC AAGAAAGCTGGGTATT <b>ATTTGGACAATCTTC</b> <b>TTATCAATG</b>	pDONR 221	pAKS2
<i>swm-1</i> coding region, no stop codon	626	GGGGACAAGTTGTAC AAAAAACGAGGCTTGA <b>AAATGGTAGTTTTG</b> <b>AG</b>	GGGGACCACTTGTAC AAGAAAGCTGGGTCTT <b>TTGGACAATCTTC</b> <b>TCAATG</b>	pDONR 221	pDRC1
3' <i>swm-1</i> region	729	GGGGACAGCTTCTTG TACAAAGTGGTTGTTT <b>TTGAGTAAACATTCC</b> <b>AAG</b>	GGGGACAACTTGTAT AATAAAGTT <b>ACGCAC</b> <b>AAATCCTTCTTGC</b>	pDONR P2r-P3	pAKS3
<i>mCherry::3'swm-1</i> region	1599	GGGGACAGCTTCTTG TACAAAGTGGTAATGG <b>TCTCAAAGGGTGAAGA</b> <b>AG</b>	GGGGACAACTTGTAT AATAAAGTT <b>ACGCAC</b> <b>AAATCCTTCTTGC</b>	pDONR P2r-P3	pDRC2
<i>swm-1</i> no secretion signal, no stop codon	498	GGGGACAAGTTGTAC AAAAAACGAGGCT <b>GCA</b> <b>TGAGTAAGTAAATATA</b> <b>TGTG</b>	GGGGACCACTTGTAC AAGAAAGCTGGGTCTT <b>TTGGACAATCTTC</b> <b>TCAATG</b>	pDONR 221	pDRC41
<i>swm-1</i> secretion signal	109	GGGGACAAGTTGTAC AAAAAACGAGGCT <b>GGA</b> <b>GGATACTTGT</b> <b>CATAATCAC</b>	GGGGACCACTTGTAC AAGAAAGCTGGGTCTG <b>TGGCGGTAGCAACAG</b> <b>CAA</b>	pDONR 221	pDRC39
<i>clec-197</i> (vas) promoter	4048	GGGGACAACCTTGTAT AGAAAAGTT <b>AGCCTC</b> <b>ATTCATCGCTCGTCA</b> <b>GTG</b>	GGGGACTGCTTTTG TACAAACTG <b>TGCCTG</b> <b>AAGTGCCACAAAG</b>	pDONR P4-P1r	pAKS7
<i>ins-31</i> (valve) promoter	600	GGGGACAACCTTGTAT AGAAAAGTT <b>TGCAGA</b> <b>ATTGGAAAGAAAAGAG</b>	GGGGACTGCTTTTG TACAAACTG <b>GGTGAT</b> <b>GGTTGAAGGCAGGTG</b>	pDONR P4-P1r	pAKS9
GFP with secretion signal	593	GGGGACAAGTTGTAC AAAAAACGAGGCT <b>TATG</b> <b>CATAAGGTTTGCTG</b>	GGGGACCACTTGTAC AAGAAAGCTGGGT <b>CTA</b> <b>TTGTATAGTCATCCA</b> <b>TGC</b>	pDONR 221	pAKS10

<sup>1</sup> Primer sequences include *att* sites for recombination with Gateway vectors; *C. elegans* genomic sequence is in bold.

**Table S3. Gene-specific oligonucleotides used for CRISPR gene editing.**

Fragment description	Length	Forward primer <sup>1</sup>	Reverse primer <sup>2</sup>
<i>swm-1</i> gRNA5	483	CCTCCTATTGCGA GATGTCTT <b>CACGATACCAAGCT</b> <b>CCTCATGTTAAG</b> AGCTATGCTGGA	NA
<i>C25E10.8</i> gRNA3	483	CCTCCTATTGCGA GATGTCTT <b>ATCACTGTGAGAC</b> <b>ACAATGGTTAAG</b> AGCTATGCTGGA	NA
<i>him-5</i> gRNA1	484	CCTCCTATTGCGA GATGTCTT <b>ATGAACAAACGAAA</b> <b>AACAGTGTAAAG</b> AGCTATGCTGGA	NA
<i>him-5</i> gRNA3	484	CCTCCTATTGCGA GATGTCTT <b>AAGTGGATCAGG</b> <b>AATCAGCGGTTA</b> AGAGCTATGCTGG A	NA
<i>swm-1::mCherry</i> repair template	2044	TAATCACATGCAT CGTTG	ACGCACAAATC CTTTCTTGC

<sup>1</sup> Primers for gRNA fragments were designed for use with the sgRNA(F+E)

pJW1311 template as in Ward (2015). Gene-specific target sequence is in bold.

<sup>2</sup> NA, not applicable.