

1 **Supporting Information for**

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4 **The intrinsically disordered protein SPE-56 is required for acrosomal-like**

5 **exocytosis and fertility in *Caenorhabditis elegans***

6 **Dieter-Christian Gottschling¹*, Sarah Eiser¹, and Frank Döring¹**

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8 ¹University of Kiel, Department of Molecular Prevention, Kiel, 24118, Germany

9 *gottschling@molprev.uni-kiel.de

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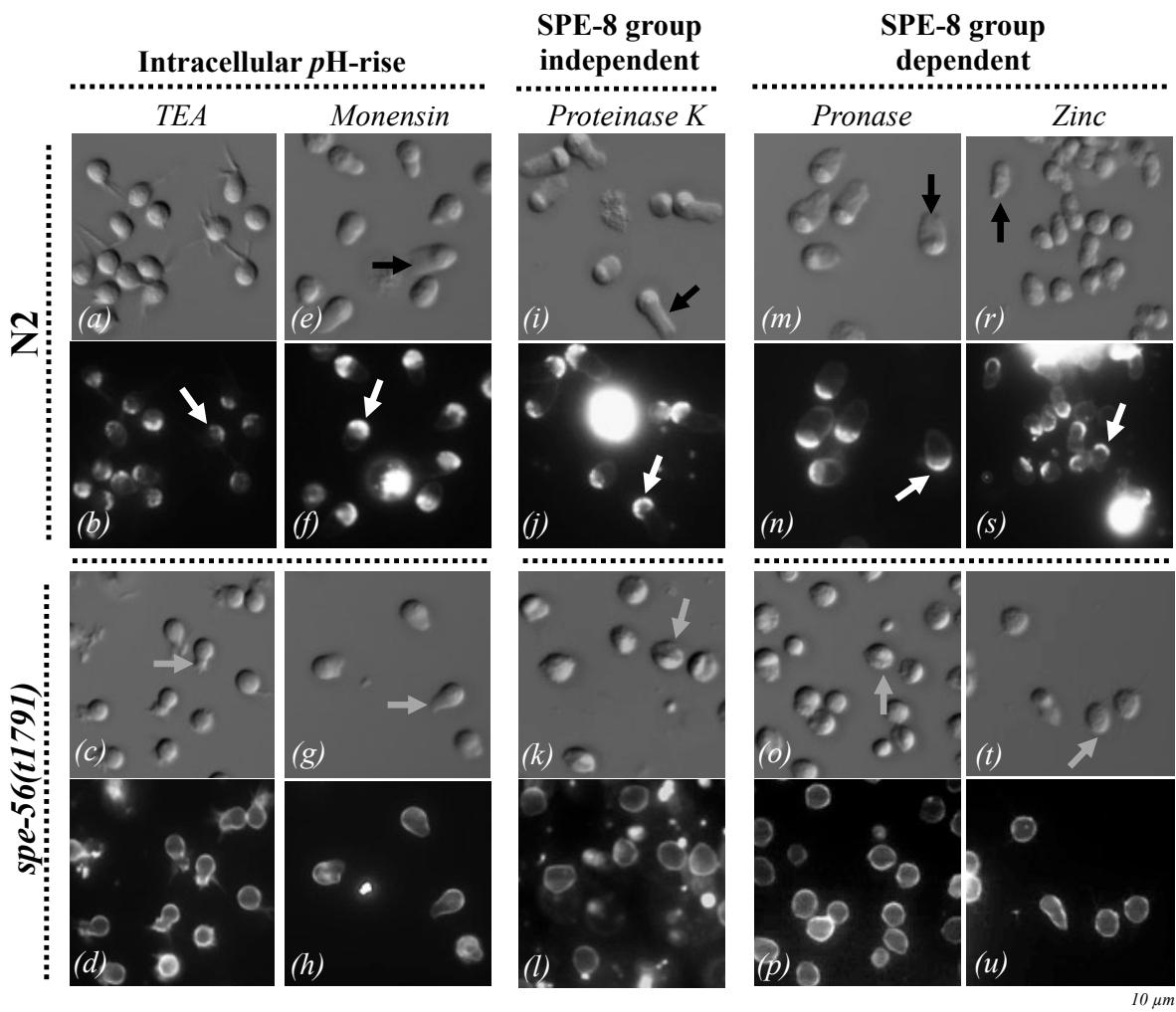
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32 **Fig. S1**

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34 **Figure S1. Assessment of *in vitro* activation in *spe-56*-deficient sperm.**

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

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47 Fig. S2

CBG12432	<i>C. briggsae</i>	MSKDTVSSKSKSSSKSDRGSAISIFFKWTASTLDAYTKS1KNNYLKV1IAHCTRAREGDEEASKNFDYSELGANPRV	77
CJA17396	<i>C. japonica</i>	MKRIWNKG----SGMKNANYLKRSVEKVAEIKGDAKVVPP---KYPTEKPRE	47
CRE30101	<i>C. remanei</i>	MSKDKV--SSKSSSSRSELGSASFVFFKWTASSLEVYKKA1TKNTLTP1IAHCTRAREGDEEASKNFDYSELGANPRM	75
CBN00485	<i>C. brenneri</i>	-----MSKSSSSKSSRSGSALEYFKWSSTFDAYTKVAKNLYLKLIVAHCTRARL-----MGDEEASKNFDYSELGANPR	21
SPE-56	<i>C. elegans</i>	-----MSKSSSSKSSRSGSALEYFKWSSTFDAYTKVAKNLYLKLIVAHCTRARL-----MGDEEASKNFDYSELGANPR	79
CBG12432	<i>C. briggsae</i>	VKDDRED IRRRLRDAQNRYWVNTNEAKPETPIISLAELSAVYRLKTLTKMCEKLAEIMGRM-----VKWFENNRA	146
CJA17396	<i>C. japonica</i>	ISDVKV---KE-----LATKHES1VENNNMMSITSTDPVERWSTKSDLP	88
CRE30101	<i>C. remanei</i>	VKDDRED IRARLKDAKNRWVWTNEAKPSLPISLVELSTIYRKLTKVLMCEKLAW1MGRM-----IEWFANKS	144
CBN00485	<i>C. brenneri</i>	-----VKDDREDIRRRLKDAKNRWVWTDEAKPETPIISLTEL SALFRKLTKVMSQELAE1LSRM-----AKWLLENNTKE	90
SPE-56	<i>C. elegans</i>	-----VKDDREDIRRRLKDAKTRVWVNTNEAKPVTPVSLGELSALYRKLTKMCEQ-----GVEMVRM-----VKWLDDNNTK	139
CBG12432	<i>C. briggsae</i>	-----FNKVVGMMTQILEYIILRNFESACAMGEVFLLTSVNWKTCFK-----KHMNPSSAKEYSRSEDERLEITE	209
CJA17396	<i>C. japonica</i>	SR-----ESEFLHRNDPWFVWYEGFYEPPEDRIPKDKLMFREALEY1LRTQELLSDSN	139
CRE30101	<i>C. remanei</i>	CHRFAFNKVRESKEIFAH1RNMFESVCA-----NLLEPNDSSEYTCSENSRUVIT	212
CBN00485	<i>C. brenneri</i>	-----FHAKASGMAQILD0YVLRMFESVCSMGEI-----TFLNPTKKEY1TCSEDEESLVIIT	153
SPE-56	<i>C. elegans</i>	-----FSKFGGKAEI-----F0YVVKR1FESVCAIGE1-----HLLSPTKEKEY1VRSEDEEDIDA	202
CBG12432	<i>C. briggsae</i>	KAESYCPKFLSQINNYYRTLVTLTCSEFGVLET-----F1T1VNRQERCEVRQK-----ELT1V1LQK1PPQYLF1TKDTLE	278
CJA17396	<i>C. japonica</i>	SPADR-KQAAEQLTAHAVTNVRNAETLDD1YEF-----RPRFERK-----QKVVNRHALAQDHLQNSDERMILDEAK	211
CRE30101	<i>C. remanei</i>	PASSYYVGEFQPSQINNYRTV1LTVYVNAISFGVVVLEYI-----ESSVNREQYAIQONNLLQTFLRE1IANV1LQML1PPQYKDIQORLK	289
CBN00485	<i>C. brenneri</i>	SPEEYQCHFMSSQINNYREI-----KML1QKL1PPQYLF1SKDLT	191
SPE-56	<i>C. elegans</i>	-----SAEKYCOPQLLSQINYFRTL1YI1ACSFEGLLETFFVSI1HQDRCD1RQK-----EIKVII1QK1PPQYLF1KDCTLE	271
CBG12432	<i>C. briggsae</i>	FSRRT1ANLLAKPPKNTFSEPTPOQDIDSEQ-----SKRESEKILA-NSRNSKA1SSCKYH1GRTGDAKSSGKSGKSDKKT-----S	352
CJA17396	<i>C. japonica</i>	FVQYR1QKS1----DNA-KFLDEYQRLEE---AEKEQVREAI1AQL-----RQEEDHRLN-KRLQGQLGEMEQA	321
CRE30101	<i>C. remanei</i>	FSRRT1ASL1NDPPKNEFVYPI1NNEVSDESK-KESEK1-----LDDQGKKS1AKVSS1SKY1HGR1TNDGKGSRSRDKSTR-----S	362
CBN00485	<i>C. brenneri</i>	LSPPRTQELLKPPKPKNSFTFVMPVLEEEQSGDSKKDEVK1G1D1DSRKSSKKV1TSSK1YHGR1TNDGKGSRSRDKSTR-----S	265
SPE-56	<i>C. elegans</i>	FSHRTEN1LLKHPK1NARSEPMKA1QENSEDSKKRESLKNL1AHSKK1STKATSSSKY1NGRNGGEK-----GSMERSQR-----S	343
CBG12432	<i>C. briggsae</i>	LRSEPTQPSSKSKSKSKSKSK-----S11KKV1LNKGNSNSKRSEKD	397
CJA17396	<i>C. japonica</i>	FKKVKEENLEKQONREK-----S11KKV1LNKGNSNSKRSEKD	289
CRE30101	<i>C. remanei</i>	LKSEPTQKSDSKSKSKSKSKSK-----S11KKV1LNKGNSNSKRSEKD	396
CBN00485	<i>C. brenneri</i>	LGSDRTQPSSKSKSKSKSK-----S11KKV1LNKGNSNSKRSEKD	310
SPE-56	<i>C. elegans</i>	LKSDGTQPSS-----S11KKV1LNKGNSNSKRSEKD	385

49 Figure S2. Multiple sequence alignment of SPE-56 homologs of rhabditid nematodes

50 Amino acid alignments with *Clustal Omega* and *Clustal* color scheme (94) shows sequence
51 similarities in *C. elegans* compared with *C. briggsae*, *C. japonica*, *C. remanei*, and *C. brenneri*.
52 The black border indicates disorder prediction by MobiDB (92).

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