Supplemental Figure 1

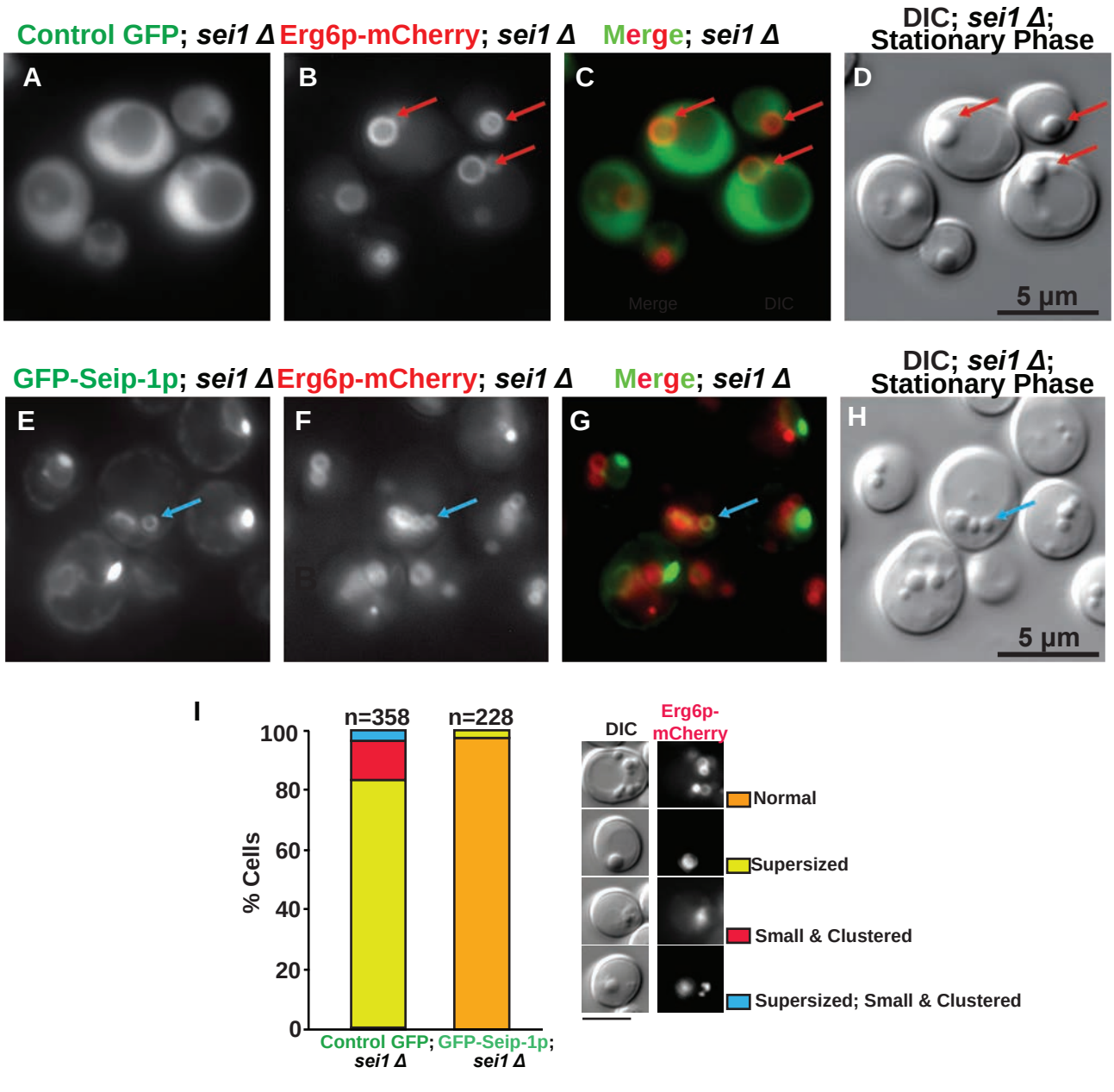
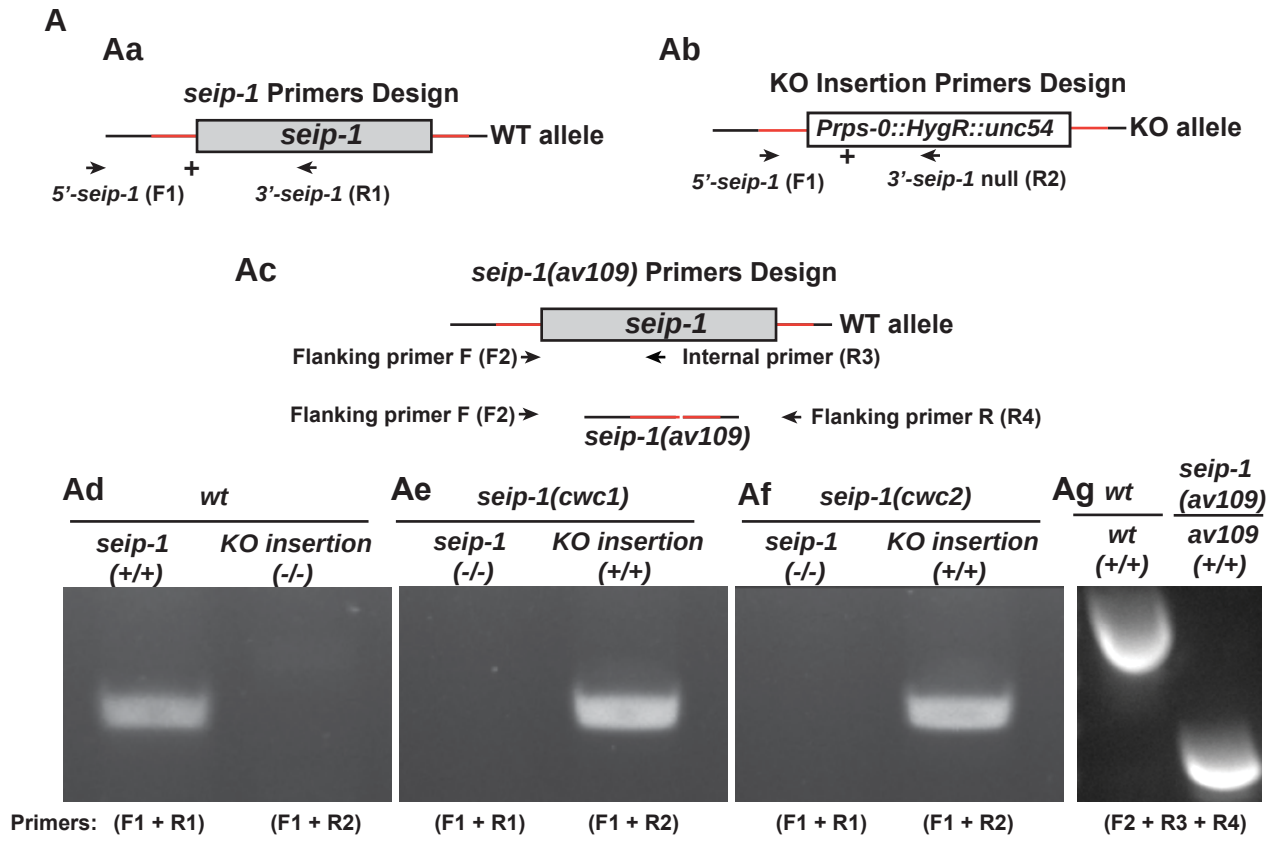
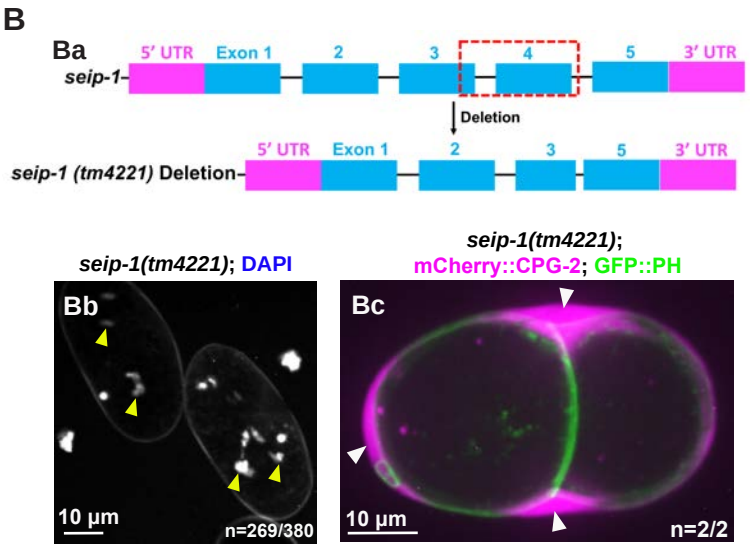


Figure S1. Expression of *C. elegans seip-1* rescues abnormal LD morphology defects in yeast *sei1*Δ mutants.

(**A-D**) The yeast *sei1Δ* strain co-expressing the LD marker Erg6p-mCherry and GFP alone. Red arrows indicate supersized LDs in the *sei1Δ* cells. (**E-H**) The yeast *sei1Δ* strain co-expressing the LD marker Erg6p-mCherry and GFP-SEIP-1p. GFP-SEIP-1p was observed adjacent to or surrounding the LDs (blue arrows) labeled by Erg6p-mCherry. (**I**) Quantification of LD morphology in the *sei1Δ* cells expressing either GFP alone or GFP-SEIP-1p. n= the number of quantified cells in the study. Scale bars are indicated.

Supplemental Figure 2





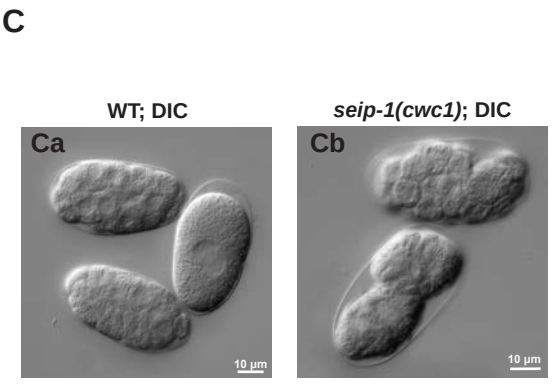


Figure S2. Verification of CRISPR/Cas9 generated deletions in *seip- 1* knockout animals and the disrupted permeability barrier phenotypes

(**A**) Schematic of all *seip-1* knockout alleles and the position of the genotyping primers (F1, R1 and R2) used in this study (Aa-Ac). (Ab) *seip-1(cwc1)* and *seip-1(cwc2)* lines are shown as a replacement of the *seip-1* gene with an expression cassette of a hygromycin B resistance gene (*hygR*). (Ac) *seip-1(av109)* was generated by CRISPR to delete the whole *seip-1* gene. The positions of the genotyping primers (F2, R3 and R4) for verifying the knockout alleles are marked by the black arrows that flank the *seip-1* coding exons. Arrows represent the primer direction from 5' to 3' (Aa-Ac). (Ad-Ag) Representative PCR gel from genotyping single animals for *seip-1(cwc1)* or *seip- 1(cwc2)* candidates. Two pairs of primers [one pair (F1 and R1) for wt (Aa); the other pair (F1 and R2) for KO insertion (Ab)] were used to genotype *seip-1(cwc1)* and *seip-1(cwc2)* candidates. In wild type, PCR product was able to be amplified by wt primers (Ad), but not by KO primers. Homozygous knockout was only able to be amplified by KO primers but not by wt primers (Ae,Af). (Ag) Three primers (two flanking primers F2 and R4 locate outside the deleted region and an internal primer R3) were used to genotype for homozygosity of candidate *seip-1(av109)* deletion animals. Amplicon size of a homozygous deletion with both flanking primers is 422 bp. In wild type, the PCR product amplified by one flanking primer and the internal primer is 877 bp. Heterozygous animals contain both PCR products. **(B)** Diagram of deleted region in the *seip-1(tm4221)* mutant (Ba). The penetration of both DAPI (Bb) and mCherry::CPG-2 (Bc) indicated that *seip-1(tm4221)* embryos also were defective for permeability barrier formation. n=number of embryos with mCherry::CPG-2 penetration / number of embryos imaged. Scale bars are indicated in each panel. **(C)** Deletion of the *seip-1* gene disrupted permeability barrier formation. DIC images show that multicellular *seip-1(cwc1)* mutant embryos (Cb) displayed a shrunken and misshapen morphology compared with wildtype embryos (Ca).

Supplemental Figure 3

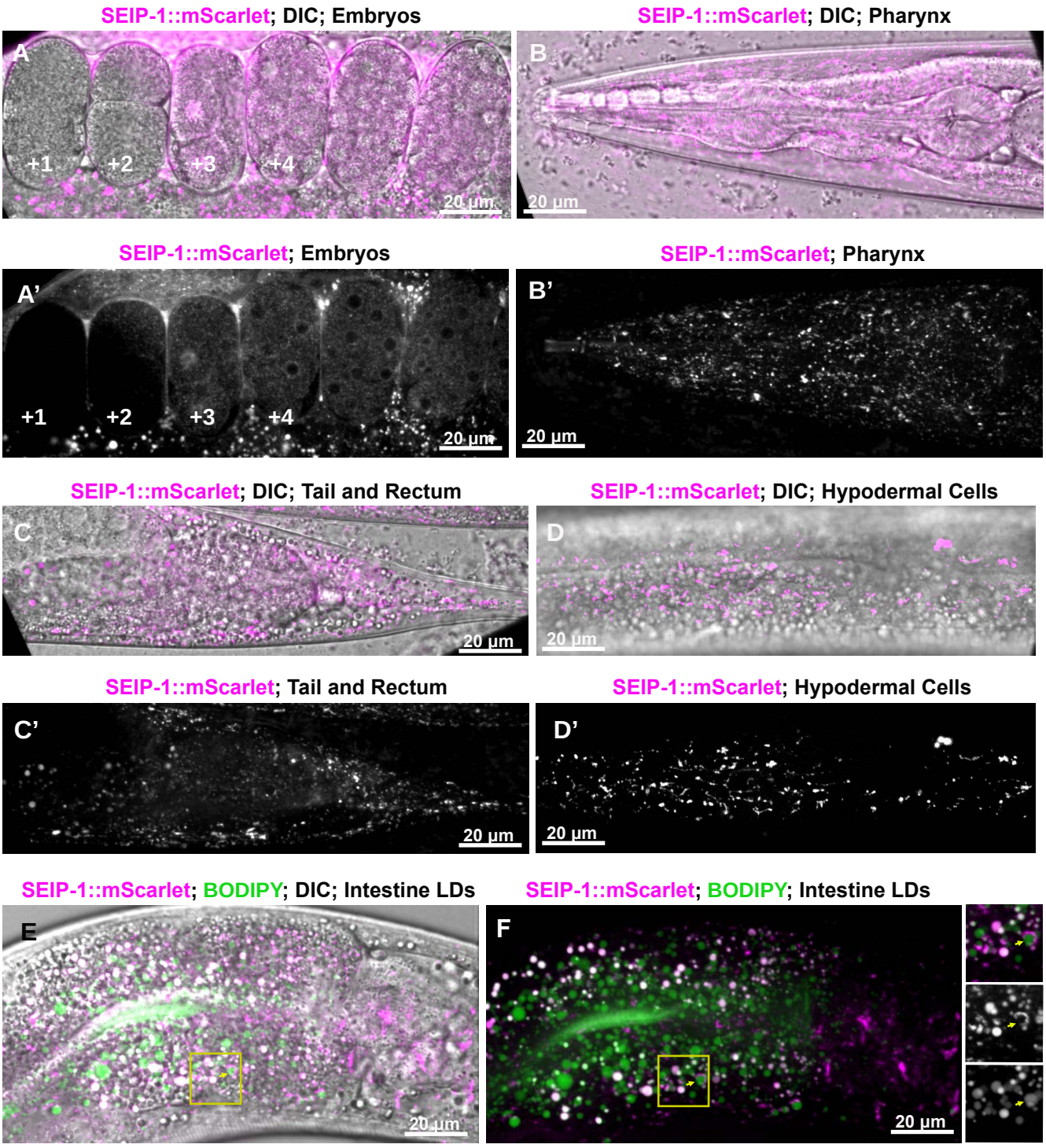


Figure S3. *seip-1* expression pattern in *C. elegans*

(**A-F**) SEIP-1::mScarlet (magenta) presents in a variety of cell types, including embryos (A,A'), pharynx (B,B'**)**, tail and rectum (C,C'), hypodermal cells (D,D'), intestinal cells (E,F). The small panels to the right of F represent enlarged views of the yellow square in panel F. A SEIP-1::mScarlet labeled LD is indicated by yellow arrows. Small top panel represents the merge image, middle panel is SEIP-1::mScarlet only, bottom panel is BODIPY staining image only. Scale bars are indicated in each panel.

Supplemental Figure 4

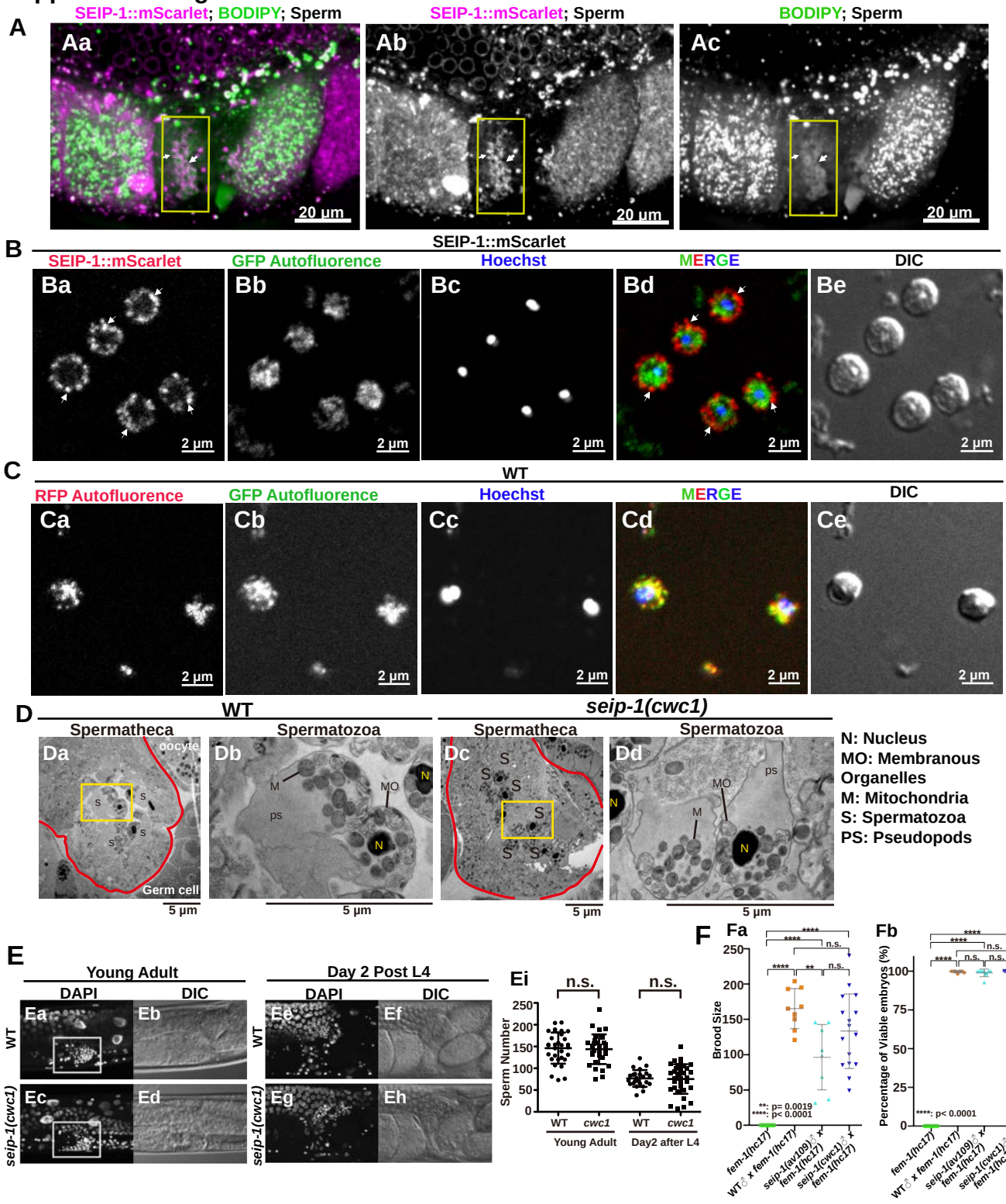
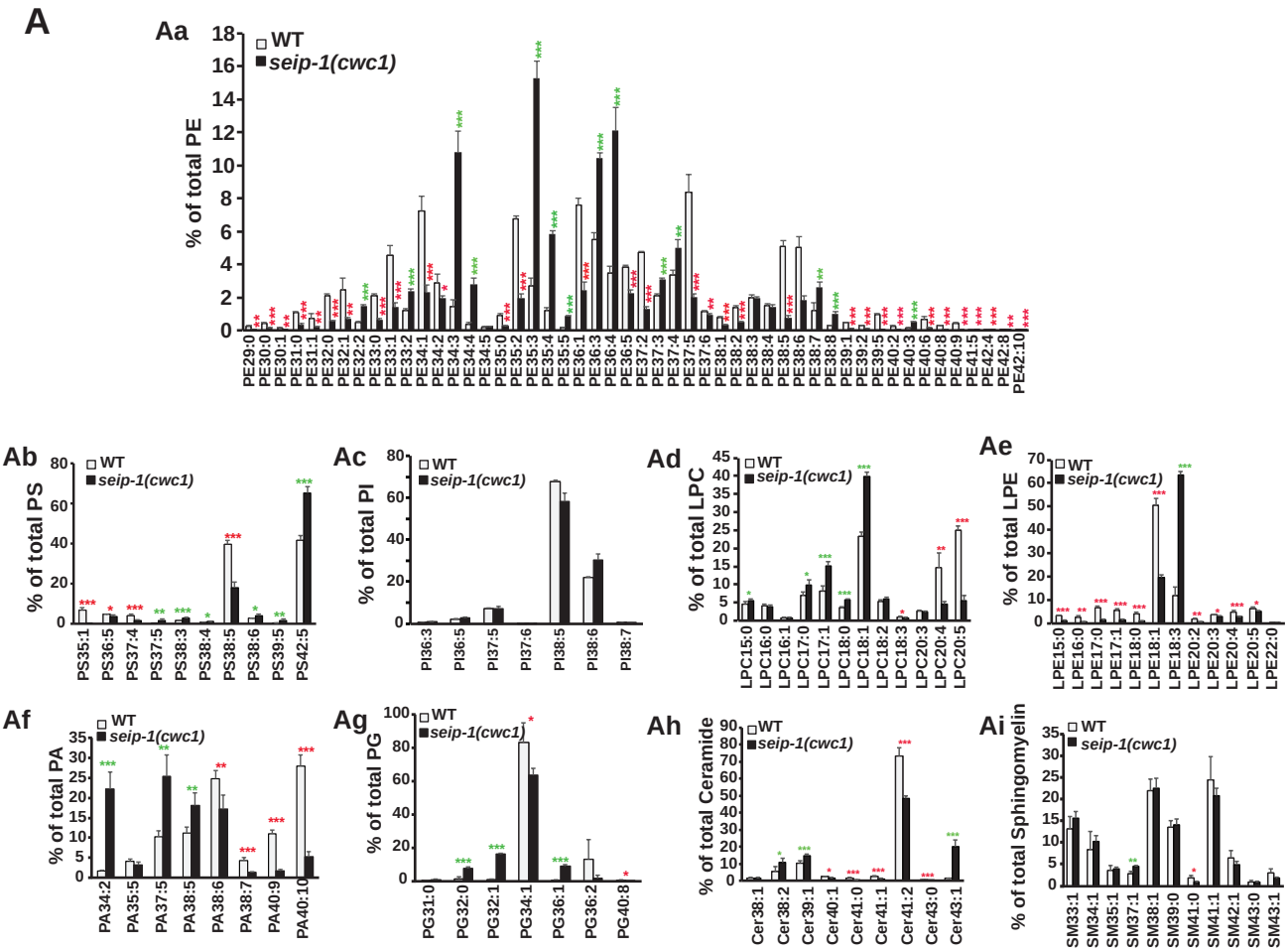


Figure S4. Deletion of the *seip-1* gene does not alter spermatogenesis and sperm function.

(A) SEIP-1::mScarlet (magenta in a) is expressed in sperm (yellow box represents the constricted spermatheca and white arrows mark individual sperm) (Aa,Ab). (Aa-Ac) There are no visible lipid droplets detected by BODIPY staining (green in Aa) in sperm . **(B)** Localization pattern of SEIP-1::mScarlet in male spermatozoa. (Aa,Ad) SEIP-1::mScarlet is expressed on the membranous organelles (MOs) (white arrows) of male spermatozoa (red in Bd). (Bb,Bd) Strong GFP autofluorescence is observed in the sperm cytosol (green in Bd). (Bc,Bd) DNA was counterstained with Hoechst 33342 (blue in Bd). Differential interference contrast (DIC) images of the spermatozoa are shown in (Be). **(C)** Representative images show that both red (561 nm) (Ca,Cd) (red in Cd) and green (488nm) (Cb,Cd) (green in Cd) autofluorescence are observed in the cytosol of wild type spermatozoa. (Cc,Cd) DNA was counterstained with Hoechst 33342 (blue in Cd). Differential interference contrast (DIC) images of the spermatozoa are also shown in Ce. **(D)** TEM micrographs of high-pressure frozen adult animals demonstrate that sperm morphology was normal in *seip-1(cwc1)* mutant (Dc,Dd) compared with wild type (Da,Db). Panels Db and Dd are the magnified area (5X) of the regions highlighted by a yellow square in panels Da and Dc, showing detailed morphological structure of spermatozoa. **(E)** DAPI staining demonstrated that spermatogenesis was not affected in *seip- 1(cwc1)* mutant (Ec,Ed,Eg,Eh) compared with wild type animals (Ea,Eb,Ee,Ef). (Ei) Quantification of sperm counts in both wildtype and *seip-1(cwc1)* hermaphrodites at different time windows. **(F)** Both *seip-1(av109)* and *seip-1(cwc-1)* males are fertile and sire progeny when mated with *fem-1(hc17ts)* mutants (essentially female animals) (Fa) and most of the F1 cross progeny (>99%) were viable (Fb). Scale bars are indicated in each panel. Data are mean ±s.d. Statistical significance was determined using an unpaired two-tailed Student's *t*-test. P-values: ** = 0.0019 (Fa); **** <0.0001 (Fa-Fb).

Supplemental Figure 5



Supplemental Figure 5

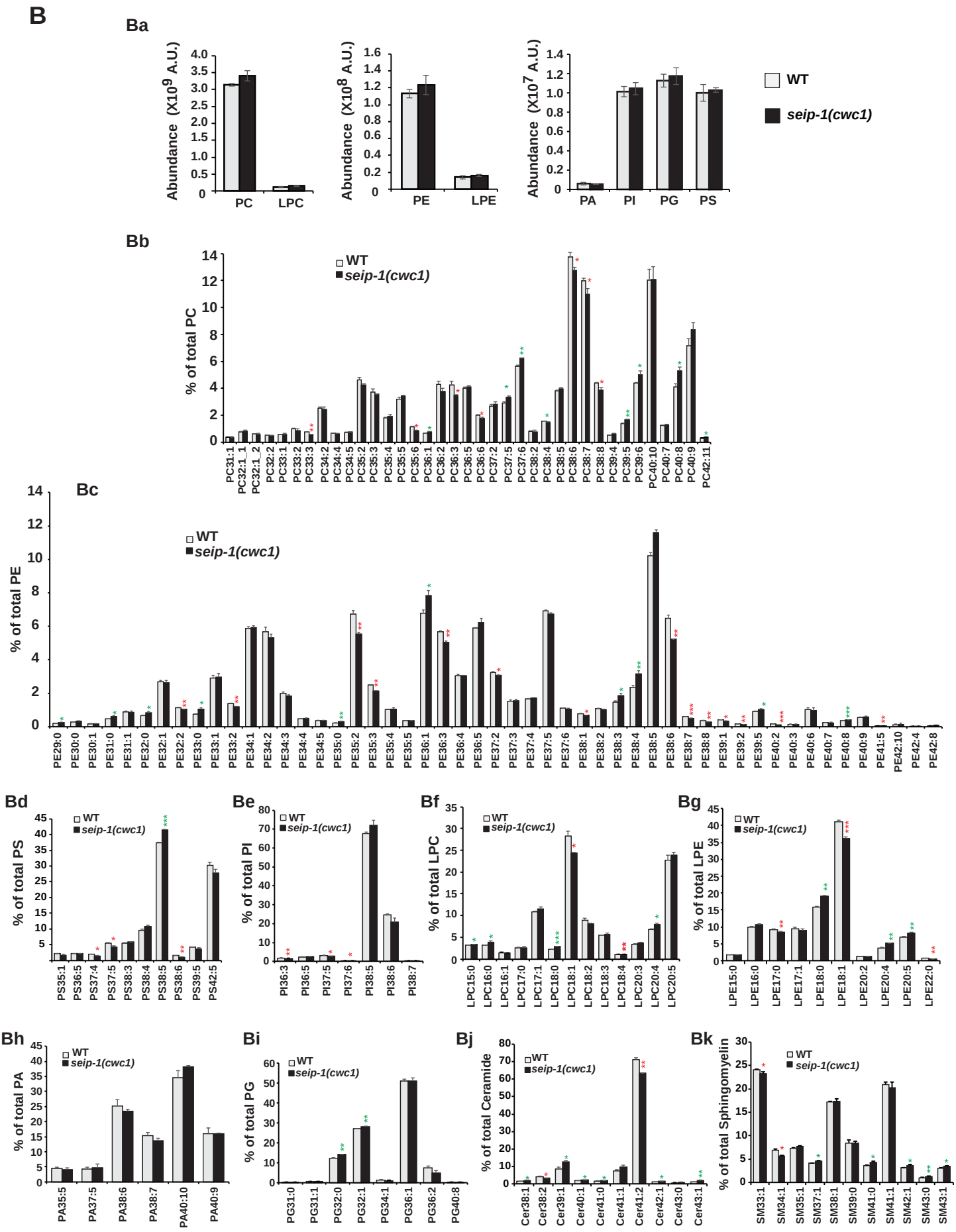


Figure S5. Lipidomic analysis of the *seip-1* mutant embryos

(A) Detailed analysis of the levels of individual lipid species in embryos were compared between *seip-1(cwc1)* mutants and wild type. **(B)** Lipidomic analysis of whole animals indicated that phospholipid levels were affected only subtly in the *seip-1(cwc1)* mutant compared with wild-type animals. Detailed analysis of the levels of individual lipid species in whole animals were compared between *seip-1(cwc1)* mutants and wild type. Green asterisks indicate the data are significantly increased in the *seip-1(cwc1)* mutant embryos compared to wild type; red asterisks indicate the levels are significantly reduced in the *seip-1(cwc1)* mutant embryos compared to wild type. PE, phosphatidylethanoamine; PS, phosphatidylserine; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanoamine; PA, phosphatidic acid; PG, phosphatidylglycerol. Data are mean±s.d. Statistical significance was determined using an unpaired two-tailed Student's *t*-test. P-values: * < 0.05; ** < 0.01; *** < 0.001.

Supplemental Figure 6

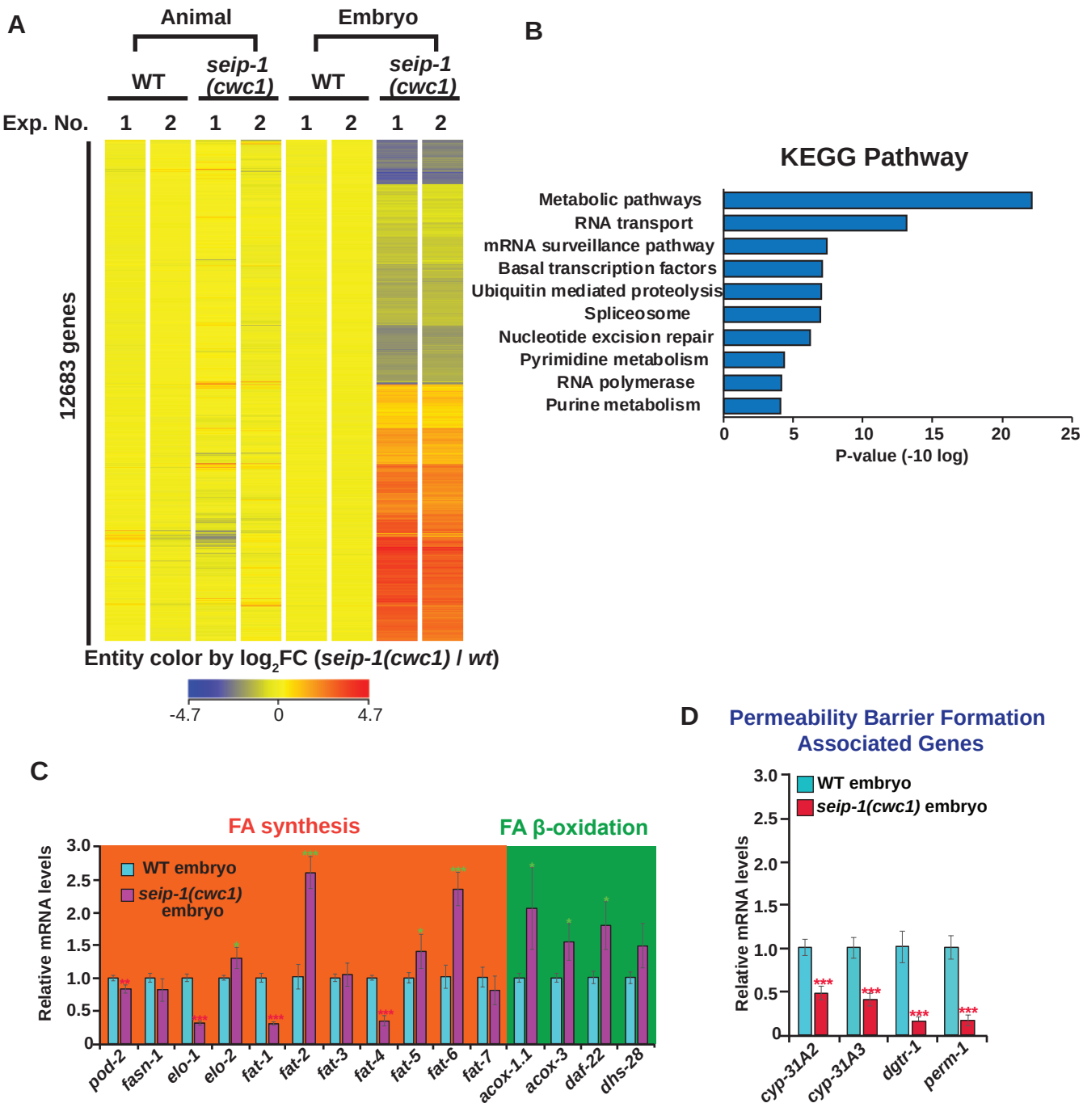


Figure S6. Global gene expression of *seip-1* mutants were analyzed by the microarray and selected genes were further analyzed by qRT-PCR

(**A**) Heatmap shows the variation of 12,683 (fold change >1.5) genes expressed between *seip-1(cwc1)* mutants and wild type. The data were collected from two independent experiments. Mis-regulated gene expression were found in *seip-1(cwc1)* embryos compared to wild type. (**B**) The genes with differential expressions between *seip-1(cwc1)* mutant and wild-type embryos were identified by KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway enrichment analyses. Metabolic pathways genes were significantly affected when the *seip-1* gene was deleted. (**C**) Quantitative transcriptional expression of fatty acid synthesis and fatty acid β-oxidation genes was determined by qRT-PCR. Expression of the *de novo* fatty acid synthetic genes were either up-regulated (*elo-2*, *fat-2*, *fat-5* and *fat-6*) or down-regulated (*pod-2*, *elo-1*, *fat-1*, *fat-4*) in *seip-1(cwc1)* mutant embryos. FA β-oxidation gene expression was up-regulated (*acox-1.1*, *acox-3*, *daf-22*, and *dhs-28*) in *seip-1(cwc1)* mutant embryos. (**D**) Quantitative transcriptional expression of genes associated with the formation of the permeability barrier was determined by qRT-PCR. All of the four selected genes (*cyp-31A2/3*, *perm-1* and *dgtr-1)* were down-regulated in seip-1 mutant embryos compared with wildtype embryos. Data are mean±s.d. Statistical significance was determined using an unpaired two-tailed Student's *t*- test. P-values: * < 0.05; ** < 0.01; *** = 0.001.

Table S1. List of *C. elegans* and yeast strains used in the study.

No. Fig	Strain	Genotype
Fig.1	N2	Bristol (wild-type)
	AG363	*seip-1(av109) V. CRISPR/Cas9 Edit. Deletion of coding region*
	AST1	*seip-1(cwc1) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
	AST2	*seip-1(cwc2) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
Fig.2	N2	Bristol (wild-type)
	AG363	*seip-1(av109) V. CRISPR/Cas9 Edit. Deletion of coding region*
	AST1	*seip-1(cwc1) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
	OD344	*unc-119(ed3) III; ltIs151 [pSO33; Pcpg-2::cpg-1SigSeq:: mCherry::cpg-2; unc-119(+)]; ltIs38 [pAA1; pie-1p::GFP::PH(PLC1δ1); unc-119(+)] III*
	POM6	*unc-119(ed3)III; pmnSi5 [pSO58; Pperm-4::perm-4::mCherry; unc-119 (+) II]*
	OD367	*unc-119(ed3) III; ltIs150[pSO31; Pcpg-1::cpg-1SigSeq::mCherry-TEV-STag::cpg-1 genomic; unc-119(+)]; ltIs38 [pAA1; Ppie-1::GFP::PH(PLC1delta1); unc-119 (+)] III*
Fig.3	N2	Bristol (wild-type)
	AST1	*seip-1(cwc1) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
	AG363	*seip-1(av109) V. CRISPR/Cas9 Edit. Deletion of coding region*
Fig.4	AG444	*seip-1(av169[seip-1::mScarlet]) V. CRISPR/Cas9 Edit*
	AG547	*seip-1(av169[seip-1::mScarlet]) V; unc-119(ed3) III; ojIs23 [pie-1p::GFP::SP12 + unc-119(+)]*
	AG548	*seip-1(av169[seip-1::mScarlet]) V; pwIs23 [vit-2::GFP]*
Fig. 5	N2	Bristol (wild-type)
	AST1	*seip-1(cwc1) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
Fig.6	N2	Bristol (wild-type)
	AST1	*seip-1(cwc1) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
	AG363	*seip-1(av109) V. CRISPR/Cas9 Edit. Deletion of coding region*
	AG549	*seip-1(av109) V; unc-119(ed3) III; ltIs151 [pSO33; Pcpg-2::cpg-1SigSeq:: mCherry::cpg-2; unc-119(+)]; ltIs38 [pAA1; pie-1p::GFP::PH(PLC1δ1); unc-119(+)] III*
Fig.7	N2	Bristol (wild-type)
	AG429	*seip-1(av160[A185P]) V. CRISPR/Cas9 Edit.*
Fig.S1	CWY3115	*sei1Δ::HIS ERG6-mCherry::KAN*
Fig.S2	AG444	*seip-1(av169[seip-1::mScarlet]) V. CRISPR/Cas9 Edit*
	AG363	*seip-1(av109) V. CRISPR/Cas9 Edit. Deletion of coding region*
	AST1	*seip-1(cwc1) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
	AST2	*seip-1(cwc2) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
	FX14734	*seip-1(tm4221)/hT1 V*

	AG550	*seip-1(tm4221) V; unc-119(ed3) III; ltIs151 [pSO33; Pcpg-2::cpg-1SigSeq::mCherry::cpg-2; unc-119(+)]; ltIs38 [pAA1; pie-1p::GFP::PH(PLC1δ1); unc-119(+)] III*
Fig.S3	AG444	*seip-1(av169[seip-1::mScarlet]) V. CRISPR/Cas9 Edit*
Fig.S4	N2	Bristol (wild-type)
	AG444	*seip-1(av169[seip-1::mScarlet]) V. CRISPR/Cas9 Edit*
	AST1	*seip-1(cwc1) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
	AG363	*seip-1(av109) V. CRISPR/Cas9 Edit. Deletion of coding region*
Fig.S5	N2	Bristol (wild-type)
	AST1	*seip-1(cwc1) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
Fig.S6	N2	Bristol (wild-type)
	AST1	*seip-1(cwc1) V. CRISPR/Cas9 Edit. seip-1 coding region replaced by HygR expression cassette.*
Video.S1	AG444	*seip-1(av169[seip-1::mScarlet]) V. CRISPR/Cas9 Edit*

Table S2. List of sequences for the CRISPR design

Strain	Genotype	Description	Sequence name	Sequence 5'-3'	PAM
AG444	*seip-1(av169[seip-1::mScarlet]) V*	Knock in mScarlet at C-terminus of *seip-1*, *mScarlet* was amplified from plasmid pMS050	NEST1 crRNA	TTTTCTAAGATCCAAAAGTC	CGG
			Repair Template	AAAGAAGTGAAAAAAGAGATCAAAAAAGAAGAACC**A**GG**T**CT**C**TTA**G**A**C**CT**C**AG**G**AA**G**AG**G**AA**G** GTCTCCAAGGGAGAGGCCGTCATCAAGGAGTTCATGCGTTTCAAGGTCCAAGCGCTCCGAGGGAC GTCACTCCACCGGAGGAATGGACGAGCTCTACAAGTAGtgcttttcgattcaacatttaatactcaattt	
			Genotyping F1	gaaaattccatccggcatcc	
			Genotyping R1	ggcgctgacattaccacata	
			NEST2 crRNA	TTCAAGGTCCAAGCG**C**TCCG	
			Repair Template F1	GCCGTCATCAAGGAGTTCATGCGTTTCAAGGTCCACATGGAGGGATCCATGAACG	
			Repair Template R1	TAGAGCTCGTCCATTCCTCCGGTGGAGTGACGTCC**T**TC**T**GA**A**CGCTCGTATTGCTCGACGACGGTG	
AG363	*seip-1(av109) V*	Deletion of coding region of *seip-1*	crRNA N-terminus	ccaaaagctacaatgccATG	AGG
			crRNA C-terminus	ACGAGCAGTCACGATTAACT	CGG
			Genotyping F1	caaacttctctcgtggcacc	
			Genotyping R1	ggcgctgacattaccacata	
			Genotyping Internal	gaaaattccatccggcatcc	
AST1 and AST2	*seip-1(cwc1) V and seip-1(cwc2) V*	Knock out *seip-1* by replacing the *seip-1* coding sequence with *HygR* expression cassette.	crRNA N-terminus	ccaaaagctacaatgccATG	AGG
			crRNA C-terminus	aaagtagaaaatgaccagcg	AGG
			Repair Template	tggagcattttatttgacgcgcgatatctcttagcgaaaactacagtaagaacttttataacttaaataaaaaaatcgtttcaaatcggtaaaaattaccaatatcagtcag aaaattaacttattttggttttctgattagttttcttttcgatttttgaaaatgttatgaatttcaattgaaatttcatcgcccacgtttttaaataaatcgtttcataataaatcatgc attcatcaatgcgagaccatggtctcgcattgatgaatgcatctacgctccaccgcactctccaaaatatttcatcgccttccggtgagtgtttgtgtgcacatctccgc gtctctattcatctcataatctctgctctactaatttctctatttctcttctgcttttccatctcaatcatcgttctcttcgtcttcttcttcgtcttcttctcgtctttaatcactgttacc gctcccatgacagttaattatattcaaacttctctcgtggcacccgcggctgcttcaaaaatgatgaaatattattttatattatcacatttattaacagactggaacggga aaagttaccaaaagctacaatgcctggtcattttctactttgccttttataatttttataaacgatttttcgaatagattttcttaaactattgtgaatgtgctatattactggttgtt cagccatgttttctatctgataatgtttatttttcaatttatttccccattattataaaatttattctttcagtttagtatgtggtaatgtcagcgccgccatgtcatcattgtctggc cacgcgtccacaattcagccatcatgtctcggattctgcggtcgaaccgtcctcgtcggcaactattctgaagacgtggaggcgacgacgacggcggccggatcc acgtcgttgtcaagatgtggaccatgttcatttggatatcgaaacaatgccatgtcaatatgcgaatcatgtgatactccactacaaccatacgattggatgtacctttttgt ttatcgccttactcccattgcttctgcatatgcaatttatcagaatagccagaaagtagtaagtgattttcttcaataaaaacactgaataaattgtgtgactttaagtattag gtattcaaaaaagacgtttctttagtattctatcgataaataaataaattaattcatttagaaaattgagccc	
			Genotyping F1 (*seip-1*)	acggcgttagtgctcgtgtcaaagg	
			Genotyping R1(*seip-1*)	atgatgaactgacgacggaagaagg	
			Genotyping R2 (*HygR*)	agaagtactcgccgatagtggaaaccgacg	
AG429	*seip-1(av160[A185P]) V*	Generate a point mutation A185P in *seip-1*	crRNA	ACGAGCAGTCACGATTAACT	CGG
			Repair Template	TATCAAGCCAAAACGAGCAGTCACGATTAACTCGG**GC**TC**C**TCGATctggaaatagagaaataattagagttggac	
			Genotyping F1	gaaaattccatccggcatcc	
			Genotyping R1	ggcgctgacattaccacata	

Capital letters represent the ORF or exon sequence, small letters indicate the sequence from intron. Bolded letters indicate the optimized base needed for the CRISPR design.

Table S3. List of qPCR primer sequences

Tested gene	Sequence name	Sequence 5'-3'	Tested gene	Sequence name	Sequence 5'-3'
acox-1.1	acox-1.1_F	CAAGTGGGCAAAGGAAAGTCC	*fasn-1*	fasn-1_F	TAAGCTGAAAAGTGTTCGCGGTA
	acox-1.1_R	ACTGACGGAAGAACATCTGTCTTGT		fasn-1_R	CCAGCCCAGAGCCTATCCA
acox-3	acox-3_F	AAGATGGGTTTGCGATTTGG	*fat-1*	fat-1_F	AAGACCGCCGGAATCATG
	acox-3_R	GGATCCTTGTGATCTCTTTTGCA		fat-1_R	CCTTTGCCTTCTCCTCGAGAGT
act-1	act-1_F	CTTCCCTCTCCACCTTCCAAC	*fat-2*	fat-2_F	CTTCACTACAACGTTACCCTCGACTA
	act-1_R	CGTCGTATTCTTGCTTGGAGATC		fat-2_R	GACACCCTTTGCTTTATGAGTCAA
cyp-31A2	cyp-31A2_F	TCGTTCGCCCGGTCACT	*fat-3*	fat-3_F	CCACGTTGCAATCTGAATGC
	cyp-31A2_R	TGGGACGACGTCTGGTGAG		fat-3_R	TTTGCACCATTCTTTCACATATTTC
cyp-31A3	cyp-31A3_F	GATTATCGTTCGCCCAGTCAC	*fat-4*	fat-4_F	GCACCATCTTTTCCCAACGA
	cyp-31A3_R	CGGCGTCTGGTAAGCTTCAT		fat-4_R	TGGCATAACAGTGTTCAAGTTGTG
daf-22	daf-22_F	AATTGGTGGAGCTGGAGTAGTTG	*fat-5*	fat-5_F	GCAAGAAGTTCGGCTGTGAAA
	daf-22_R	GCTCCAGGGAATCCCAATCTA		fat-5_R	TCCCAATTTGTGGAGCATTTT
dgtr-1	dgtr-1_F	CAATCTGTTCGAGGAGTACAAGCA	*fat-6*	fat-6_F	ATTATCGGCCGGCAGGTATC
	dgtr-1_R	TGAGTGTCGGGAGGAATGG		fat-6_R	TTTTCCTCGTTGAATATCACATCC
dhs-28	dhs-28_F	TTCTTGAAAAGGCGAAGAAGTCA	*fat-7*	fat-7_F	GAGTTTATCAGCCGGCAGGTT
	dhs-28_R	ATTGAACGCTTCTGTCTGTTTACAA		fat-7_R	TTTTCTTGATTCTTCACTTCCGTG
elo-1	elo-1_F	TCACCAATGCCAACTGTGATTT	*perm-1*	perm-1_F	TGGACCTTTTTCAACGCTACG
	elo-1_R	AAACTGCGAGCTTGAATACTGATG		perm-1_R	TGGCTTGTATCCCAACATCAGA
elo-2	elo-2_F	CAAAAACGCTCACCAACCAA	*pod-2*	pod-2_F	TTGGAATCGGAGCCTACACG
	elo-2_R	CACAATGTTTATCTACTCCTGCTTGC		pod-2_R	TGTGCTGAACGATTCGATGAG

Movie 1. SEIP-1 expression pattern during ovulation

Ovulation imaged in the genome-edited animals expressing SEIP-1::mScarlet (magenta). arrows in left and right panels indicate SEIP-1::mScarlet expression in the fifth gonadal sheath cell until the oocyte completed the ovulation. Left panel indicates the mScarlet (magenta) channel only, middle channel indicates DIC only, right channel indicates the merged image of mScarlet and DIC. Images are z-max with 10 z planes taken every 30 seconds with 1 µm step size. Timing is indicated in lower right of the left panel. Playback rate is 2 frames/second. Scale bar is indicated in the left panel.