

## Expanded View Figures

**A** ALG-1 Wild type sequence 30 amino acids  
-VDREHDSGEGSQPSGTSEDTTLSNMARAVQ-  
# Netphos-3.1b prediction results

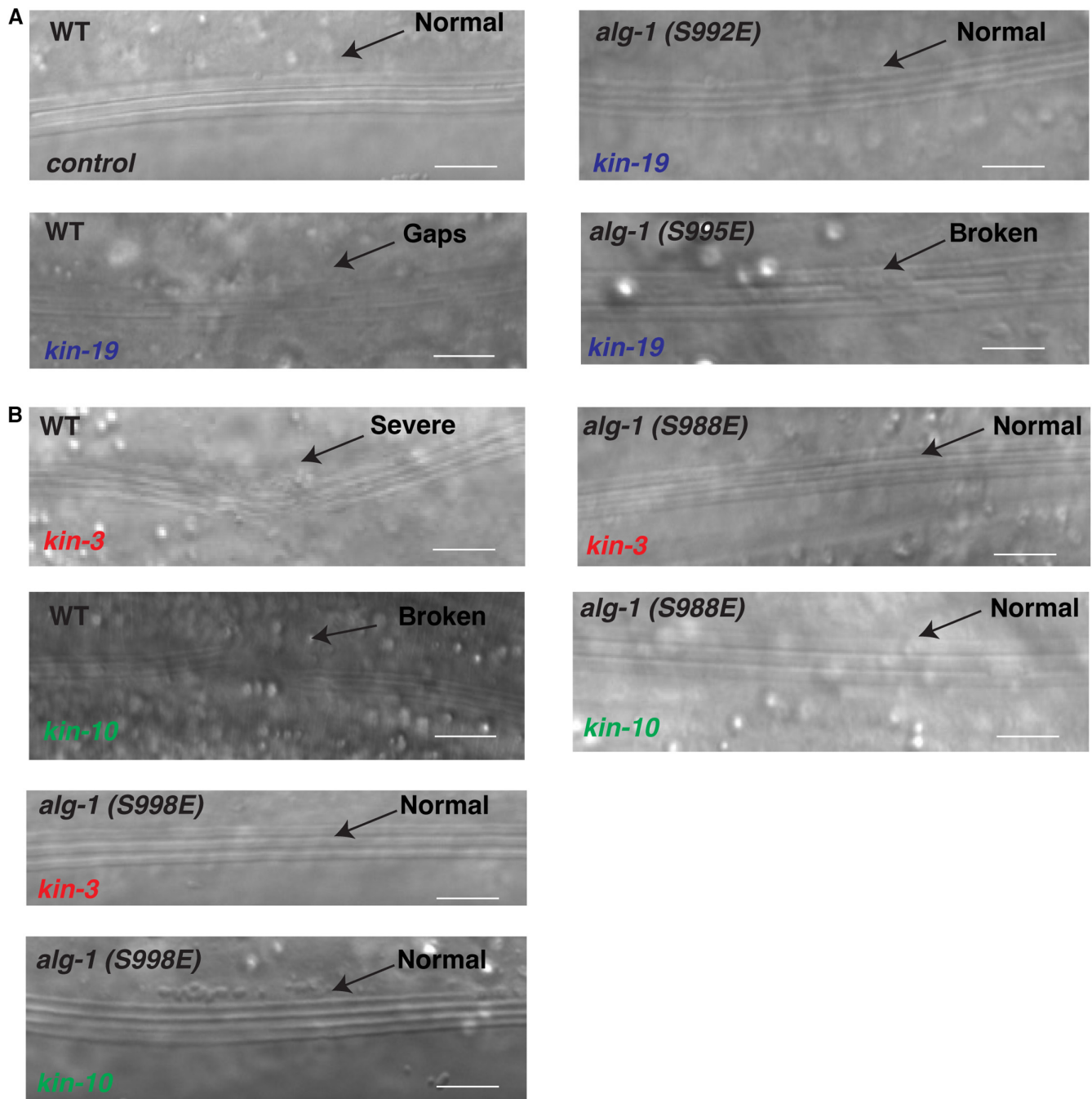
Sequence	# x	Context	Score	Kinase
# Sequence	7 S	REHDSGEGS	0.478	CK II
# Sequence	7 S	REHDSGEGS	0.410	CK I
# Sequence	11 S	SGEGSQPSG	0.398	CK I
# Sequence	11 S	SGEGSQPSG	0.382	CK II
# Sequence	14 S	GSQPSGTSE	0.524	CK I
# Sequence	14 S	GSQPSGTSE	0.359	CK II
# Sequence	16 T	QPSGTSED	0.394	CK II
# Sequence	16 T	QPSGTSED	0.368	CK I
# Sequence	17 S	PSGTSEDTT	0.421	CK I
# Sequence	17 S	PSGTSEDTT	0.404	CK II

**B** AGO-2 Wild type sequence 30 amino acids  
-VDKEHDSAEGSHTSGQSNRGRDHQALAKAVQ-  
# Netphos-3.1b prediction results

Sequence	# x	Context	Score	Kinase
# Sequence	7 S	KEHDSAEGS	0.508	CK I
# Sequence	7 S	KEHDSAEGS	0.453	CK II
# Sequence	11 S	SAEGSHTSG	0.415	CK I
# Sequence	11 S	SAEGSHTSG	0.335	CK II
# Sequence	13 T	EGSHTSGQS	0.389	CK II
# Sequence	13 T	EGSHTSGQS	0.371	CK I
# Sequence	14 S	GSHTSGQSN	0.450	CK I
# Sequence	14 S	GSHTSGQSN	0.305	CK II
# Sequence	17 S	TSGQSNRGRD	0.378	CK I
# Sequence	17 S	TSGQSNRGRD	0.323	CK II

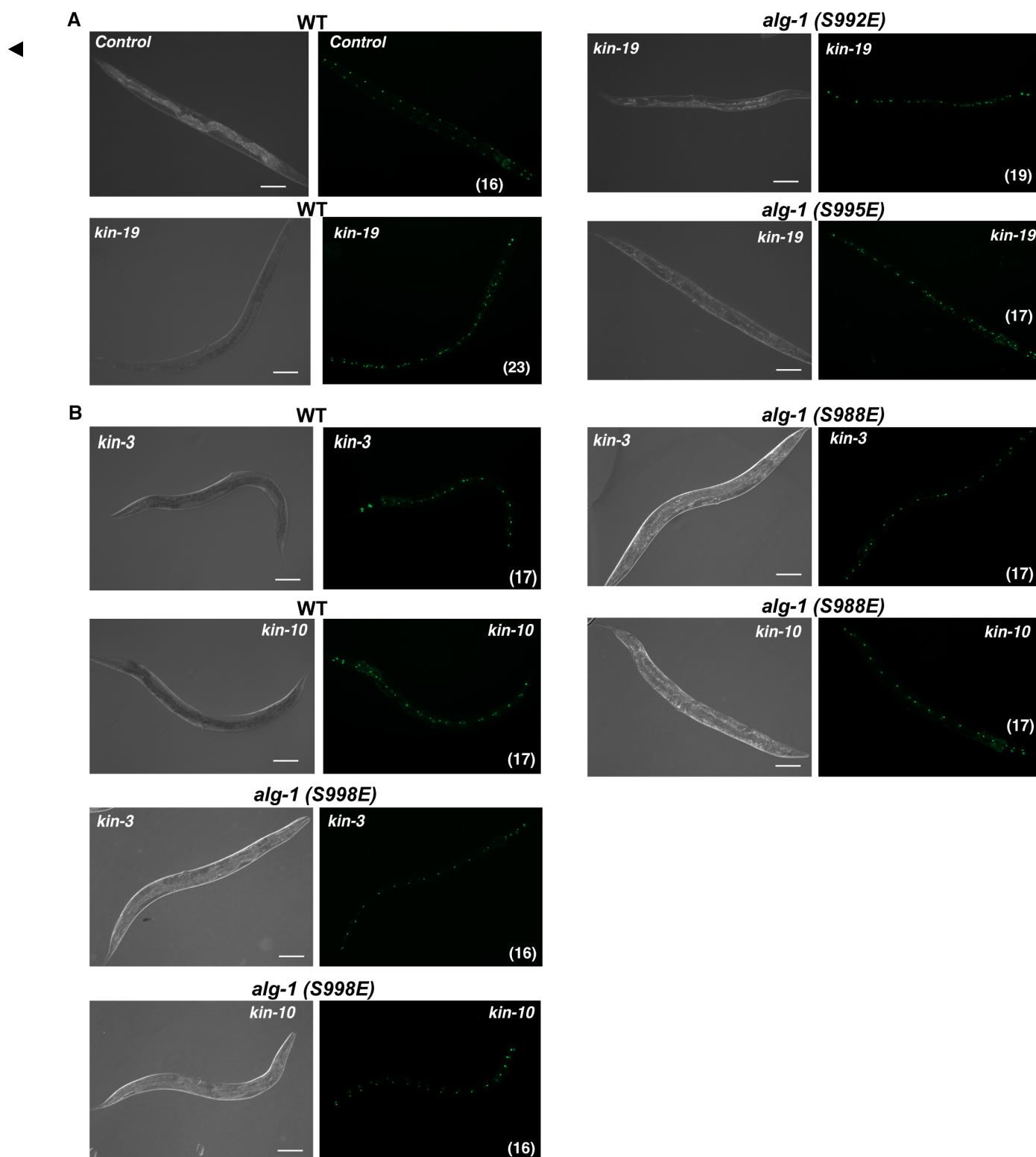
**Figure EV1.** CK1A1 and CK2 were both predicted to be phosphorylating the S988:S998 cluster on ALG-1 and S824:S834 cluster on AGO2 by Netphos3.1 online database.

- A Netphos3.1 scores of CKI and CK2 phosphorylating the different phosphorylatable Serine residues (S988, S992, S995, and S998) and a Threonine residue (T997) within the S988:S998 cluster on ALG-1 in *Caenorhabditis elegans*.
- B Netphos3.1 scores of CKI and CK2 phosphorylating the different phosphorylatable Serine residues (S824, S828, S831, and S834) and a Threonine residue (T830) within the S824:S834 cluster on AGO2 in humans.



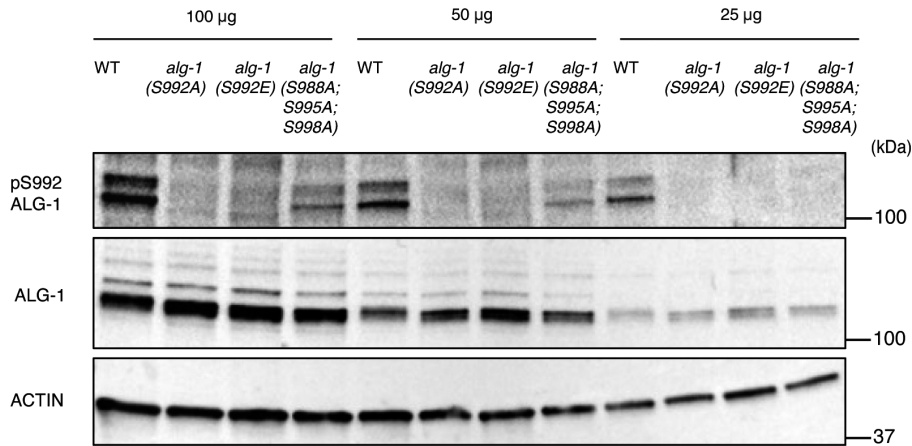
**Figure EV2. CK1A1 depletion in *alg-1(S992E)* and *alg-1(S995E)* animals and CK2 depletion in *alg-1(S988E)* and *alg-1(S998E)* animals rescue alae defects (a phenotype associated with reduced miRNA pathway function).**

A, B Representative images with alae structure (normal and defective) are shown for all conditions. The scale bar represents 10  $\mu\text{m}$ .



**Figure EV3.** CK1A1 depletion in *alg-1(S992E)* and *alg-1(S995E)* animals and CK2 depletion in *alg-1(S988E)* and *alg-1(S998E)* animals rescue seam cell defects (a phenotype associated with reduced miRNA pathway function).

A, B Representative pictures of seam cell *gfp* reporter expression (normal and abnormal) are shown for all conditions. The number in brackets indicates the number of seam cells. The scale bar represents 100  $\mu$ m.



**Figure EV4. Specificity of the phospho-specific antibody to the S992 site within the S988:S998 cluster on ALG-1.**

Representative Western blot of pALG-1 (S992) and ALG-1 in wild-type, *alg-1*(S992A), *alg-1*(S992E), and *alg-1*(S988A;S995A;S998A) young adult worm extracts at different protein concentrations of 100, 50, and 25 µg to demonstrate the specificity of the phospho-specific antibody. ACTIN is used as a loading control.

Source data are available online for this figure.

**Figure EV5. Functional analysis using a *let-7* miRNA reporter.**

A, B CK1A1 depletion in *alg-1*(S992E) and *alg-1*(S995E) animals and CK2 depletion in *alg-1*(S988E) and *alg-1*(S998E) animals rescue *let-7* miRNA reporter de-repression. Representative pictures of *let-7* miRNA reporter expression are shown for all conditions. The scale bar represents 20 µm.

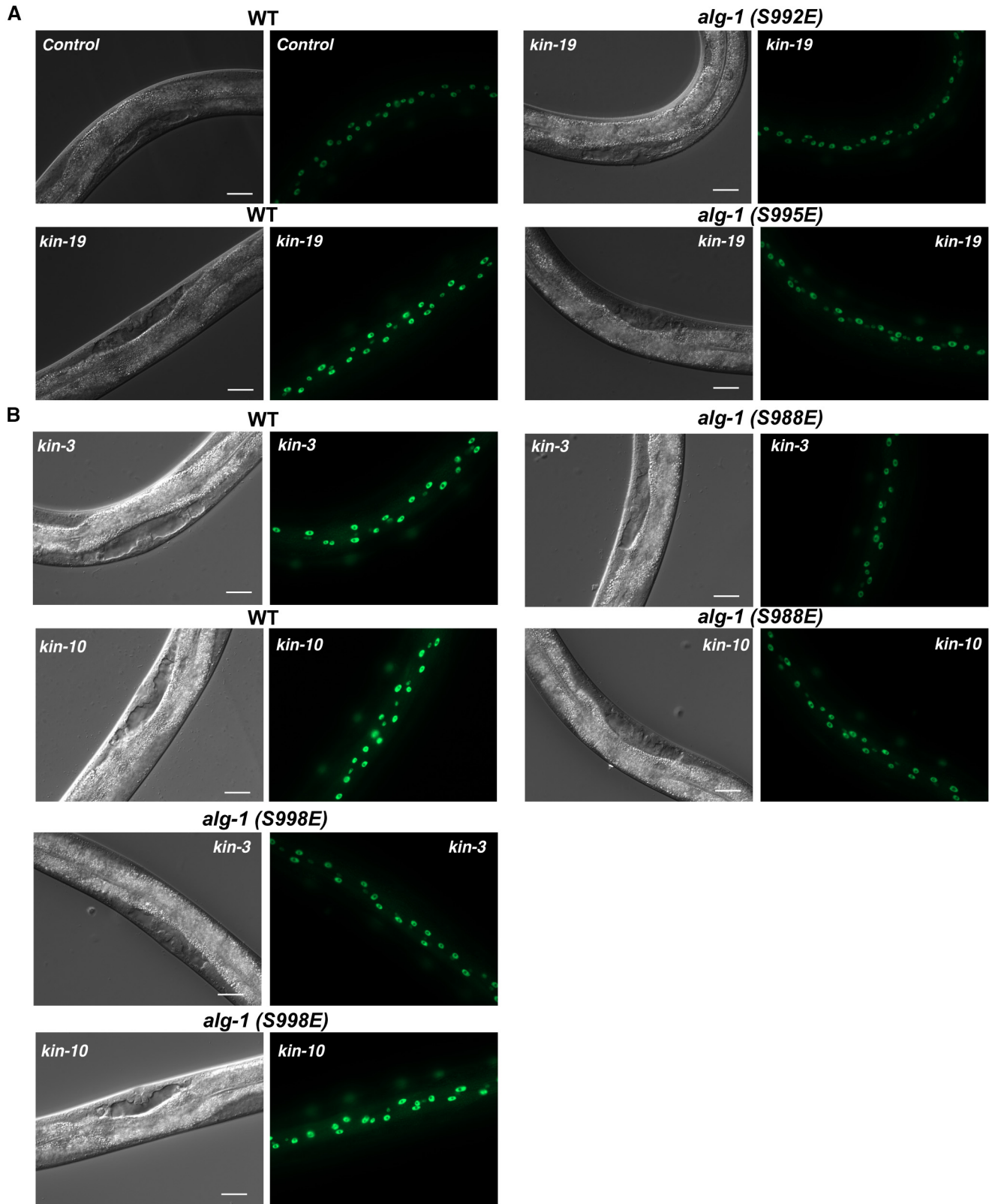


Figure EV5.