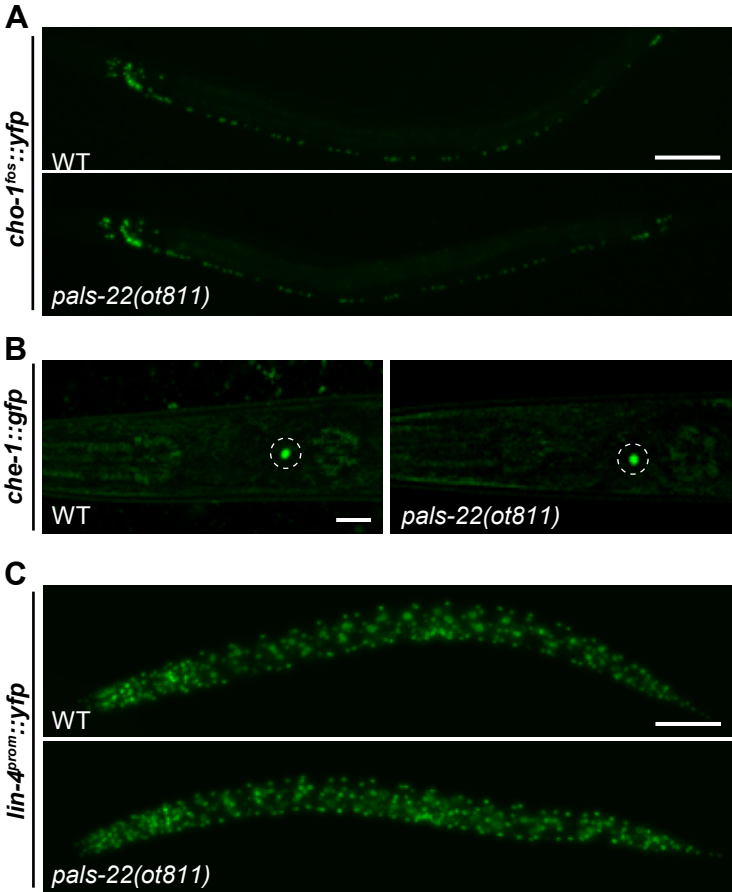


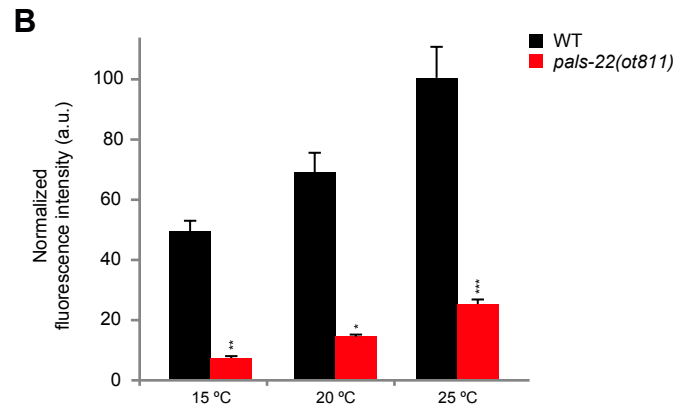
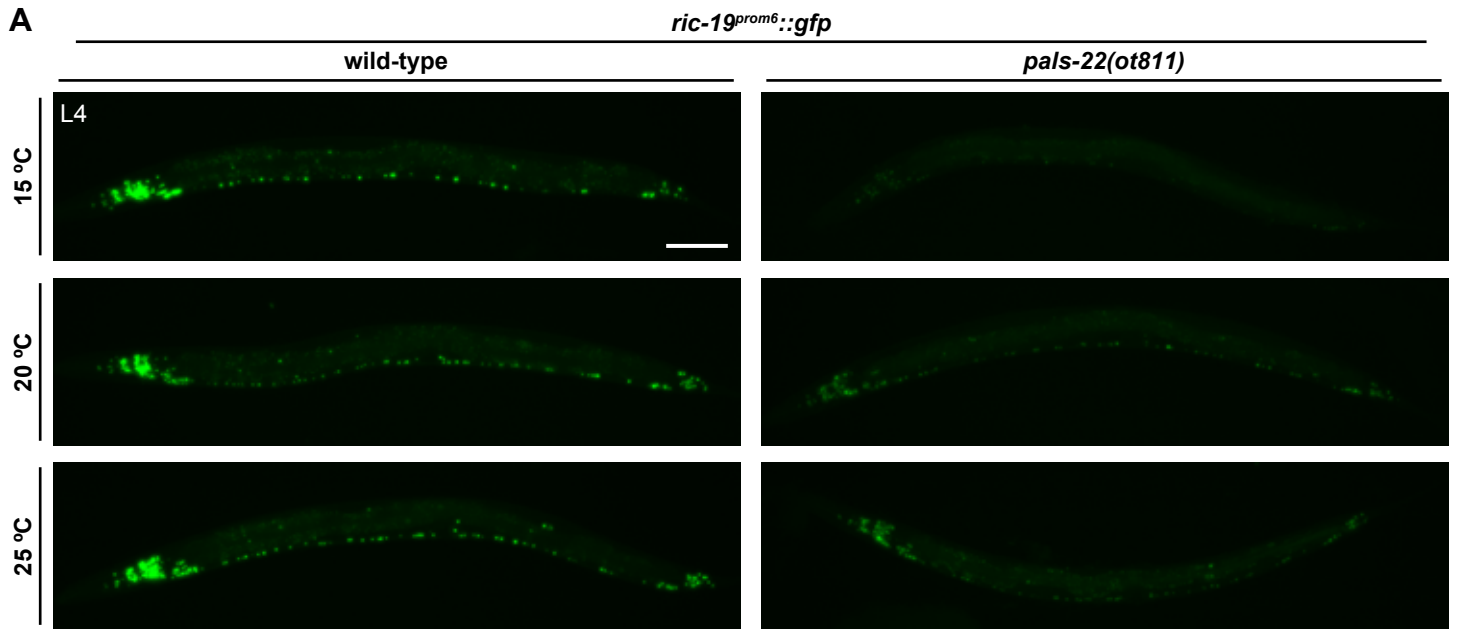
**Table S1: Transgenic strains used in this study.**

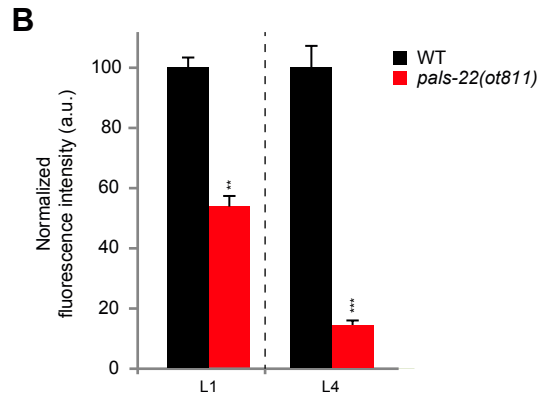
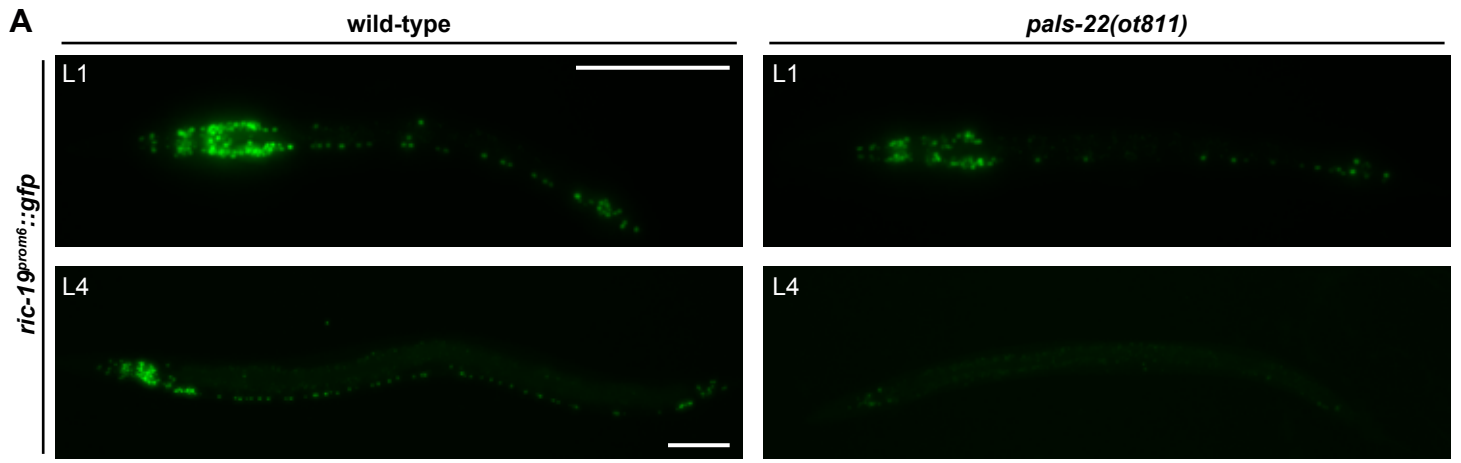
Strain name	Transgene name	Transgene	Microinjection conditions for transgenesis				Notes	Reference
			Injection conc. (ng/μL)	Co-injection marker *	Marker conc. (ng/μL)	Array format		
OH11062	<i>otIs381</i>	<i>ric-19<sup>prom6</sup>::NLS::gfp</i>	50	<i>elt-2<sup>prom</sup>::DsRed2</i>	50	Simple	Promoter coordinates (-1, -147). Integrated in chromosome V.	1
OH11061	<i>otIs380</i>	<i>ric-19<sup>prom6</sup>::NLS::gfp</i>	50	<i>elt-2<sup>prom</sup>::DsRed2</i>	50	Simple	Promoter coordinates (-1, -147). Integrated in chromosome V.	1
PD4251	<i>ccls4251</i>	<i>myo-3<sup>prom</sup>::gfp</i>		<i>dpy-20</i>		Simple		2
OH8908	<i>otIs251</i>	<i>cat-2<sup>prom</sup>::gfp</i>	100	<i>rgef-1<sup>prom</sup>::DsRed2</i>	50	Simple		3
OH10689	<i>otIs355</i>	<i>rab-3<sup>prom1</sup>::NLS::tagrfp</i>	50	-	-	Simple	Promoter coordinates (+2921, -1462). Integrated in chromosome IV.	1
OH11746	<i>otIs447</i>	<i>unc-3<sup>prom</sup>::mChOpti</i>	50	<i>pha-1</i>	50	Simple	Promoter coordinates (-336, -558).	4
OH14861	<i>otIs644</i>	<i>tdc-1<sup>prom</sup>::ChR2::yfp</i>	50	-	50	Simple		5
OH14942	<i>otEx6944</i>	<i>ric-4<sup>prom26</sup>::NLS::yfp</i>	15	<i>ttx-3<sup>prom</sup>::mChOpti</i>	30	Simple	Promoter coordinates (+5362, +6592).	This study
OH13606	<i>otIs620</i>	<i>unc-11<sup>prom8</sup>::NLS::gfp</i>	10	-	-	Complex	Promoter coordinates (-775, -1067).	This study
OH10687	<i>otIs353</i>	<i>ric-4<sup>fosmid</sup>::SL2::NLS-YFP-H2B</i>	15	<i>pha-1</i>	2.5	Complex		1
OH12543	<i>otIs534</i>	<i>cho-1<sup>fosmid</sup>::SL2::NLS-YFP-H2B</i>	15	<i>pha-1</i>	2.5	Complex		1
OH15146	<i>otTi32</i>	<i>lin-4<sup>prom</sup>::yfp</i>					MiniMos strain.	6
CA1208	<i>ieSi60</i>	<i>myo-2<sup>prom</sup>::TIR1::mRuby</i>					MiniMos strain.	7
OH14130	<i>ot856</i>	<i>gfp</i> insertion in <i>che-1</i> locus					CRISPR/Cas9 strain.	8
OH14420	<i>otEx6761</i>	<i>pals-22<sup>PCR</sup></i>	5	<i>ttx-3<sup>prom</sup>::mChOpti</i>	3	Complex	Injected into <i>pals-22(ot811);otIs381</i> .	This study
OH14429	<i>otEx6769</i>	<i>pals-22<sup>fosmid</sup></i>	10	<i>myo-2<sup>prom</sup>::mChOpti</i>	3	Complex	Injected into <i>pals-22(ot811);otIs381</i> . <i>NotI</i> digested WRM0616DC09 fosmid.	This study
OH15144	<i>otEx7036</i>	<i>pals-22<sup>prom</sup>::gfp</i>	5	<i>pha-1</i>	3	Complex	<i>pals-22</i> transcriptional reporter. Promoter coordinates (-1, -791).	This study
OH15145	<i>otEx7037</i>	<i>pals-22<sup>prom</sup>::pals-22::gfp</i>	5	<i>pha-1</i>	3	Complex	<i>pals-22</i> translational reporter. Promoter coordinates (-1, -791).	This study
ERT520	<i>gySi37</i>	<i>pals-22<sup>prom</sup>::pals-22::gfp</i>					MosSCI insertion. Promoter coordinates (-1, -2001).	9

\* All strains were injected either in N2 or *pha-1(e2123)* background. For complex arrays, transgenes were injected with 100 ng/μL of sheared OP50 bacterial genomic DNA. References: 1: (STEFANAKIS *et al.* 2015), 2: (FIRE *et al.* 1998), 3: (DOITSIDOU *et al.* 2013), 5: (SERRANO-SAIZ *et al.* 2013) 4: (KERK *et al.* 2017), 6:

Kindly provided by Haosheng Sun. Generated as detailed in (FROKJAER-JENSEN *et al.* 2014), 7: (ZHANG *et al.* 2015), 8: Kindly provided by Dylan Rahe. Generated as described in (DICKINSON *et al.* 2015). 9: Kindly provide by E. Troemel.

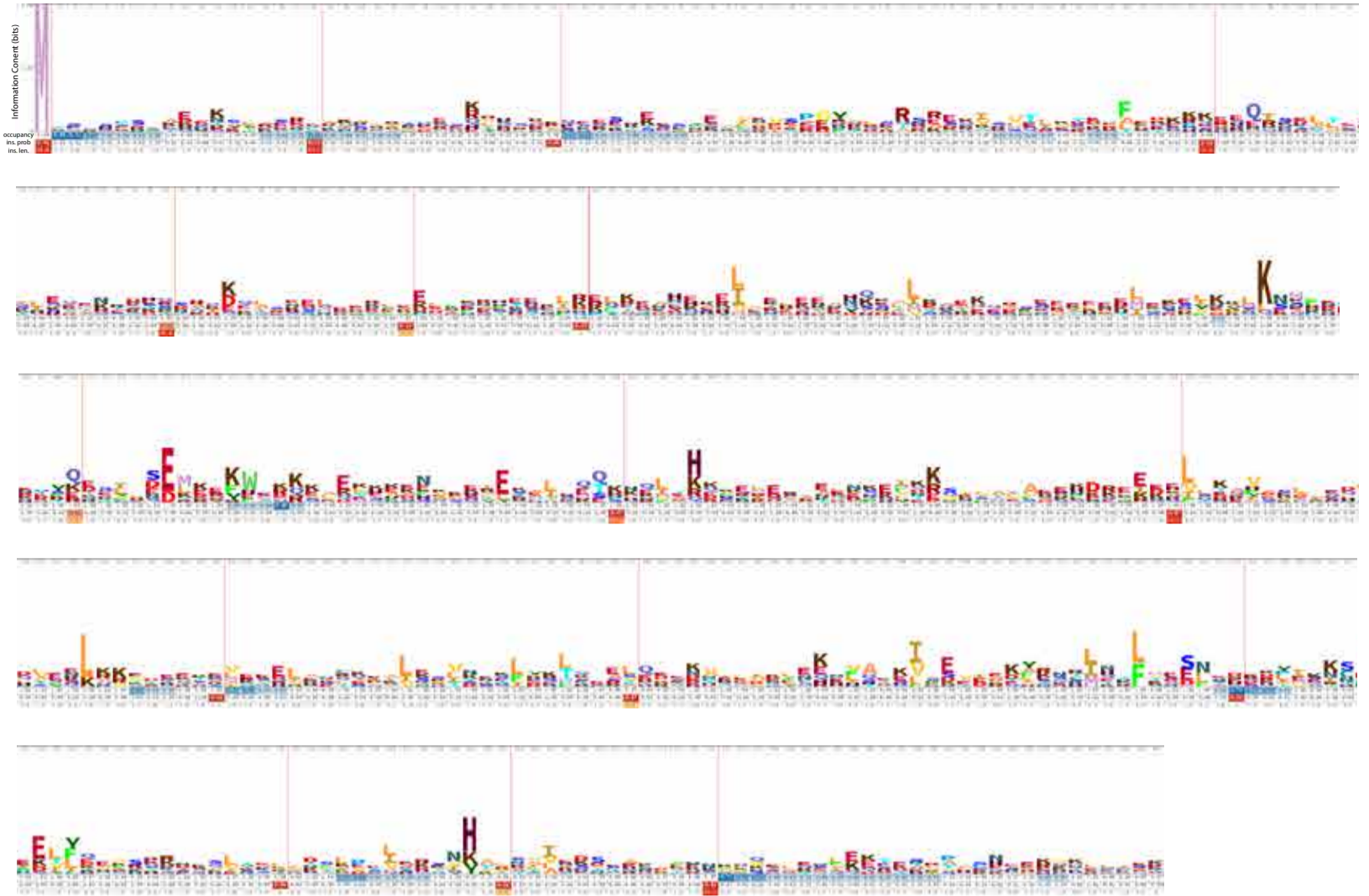






# A ALS2CR12 signature logo

Skylign.org Logo  
 PANTHER DATABASE HMM: PTHR21707.SF42  
 Alphabet: AA  
 Letter Height: Information Content - Above Background  
 Number of Sequences:27  
 Length:404



# B PALS-22 paralogue alignment

T-COFFEE, Version\_11.00.d625267 (2016-01-11 15:25:41 - Revision d625267 - Build 507)  
 Cedric Notredame  
 CPU TIME:0 sec.  
 SCORE=569

\*  
 BAD AVG GOOD

\*  
 PALS-22 C. eleg : 46  
 PALS-23 C. eleg : 50  
 PALS-24 C. eleg : 48  
 PALS-25\_C.\_eleg : 52  
 cons : 56

```
PALS-22_C._eleg MSSNENSDDVPEQRQI--VRRSRVYNPRDPSSPEPPYDPYPPGIHAQAGCATRPIISA-----
PALS-23_C._eleg MSHELLFSKVKLKINQFIDDLKSTDSLWNE-----VRFDAHVKISDCRTS IKE-
PALS-24_C._eleg MTARKNDEDALQNLK--INLENTNSESNVKYLFLQNLKHEKMMETEQAERKMLRLKQEEQ
PALS-25_C._eleg -MVDLRLDVIRKKVHHIVESLQRISTLCNPKIGEPS-----VNPGGHEADVEVLVAGRED-
```

cons . . . . :

```
PALS-22_C._eleg -----HIVPQALIEPIRLHREE-----DELGTSSCRPVPOCDF-----
PALS-23_C._eleg -----LITNFLEKTNKNGQFLTAQGNFATSILGILNDRFMIAI--VTKGLSK--ADG
PALS-24_C._eleg LEEVMKLIETTKNSLKMDFQKKKQILKQH----AEKWRMLKEQHKEIEEFKQNDQKETDG
PALS-25_C._eleg -----LRDKIYELTNNSGRIHAEQVRILSDIQYITQETYFSTICFHLSQCVD--GDC
```

cons : : : : . : :

```
PALS-22_C._eleg NLESDRWEEDQLENPQIKSQWKGSETR-----PVANFARRQQRVLRDWDKHDVQITVWE
PALS-23_C._eleg EEFKSYGELADSLINNFRVQISSLNSV-----KQE-LTRKREVRG-----NAKPTID
PALS-24_C._eleg TSLGKLQDPLKPLLPFFKSQRDEFERIRYDPELAEHF-IEAYKRKKS-----VITEVFD
PALS-25_C._eleg EDLKKYGEFAELLVQQILEQLRNFDSV-----EVK-QATKAESLE-----TARQTFR
```

cons . . . : \* :

```
PALS-22_C._eleg ELKKVLEAAEIVDVTLNMMKTL----NPDTKA--MWLLKFHTTSLTEYLEDVESRAEYFNEML
PALS-23_C._eleg KFQITILYWTNAIRTNMSIIGAIYPMQRTVVKVNAKTLNSQFSAMQKNREILEQCHDKFKEFL
PALS-24_C._eleg EFNILWSVCSFSDKLTISICYKEMDPDC-----LIENVNTLSNELENFEKVTAEFTKYL
PALS-25_C._eleg KIKSLTSPVFSIEKVLRIETLCLPPDGDAPSIGVRELDVFNISIKSFTEEFETSASDFDTYL
```

cons :::: . . : : : \* . : . \* : \* \* \*

```
PALS-22_C._eleg LELDGIHPDLFTECSNYISQLKDVTHSRELOAVQVHLPKAVKNSDAQKRVFYQLAGEAKKDI
PALS-23_C._eleg NNDARVPPPELFEFSRFQNLAEELMNNADFIACVTHLSKAVEENNEEMILYGGKVRALNQYF
PALS-24_C._eleg VNTDGIHPDLYKACSDYKMRLETVMNDENVFAILMQLPEAIAERNQEDIDFYVDLAESL----
PALS-25_C._eleg SSTSHLALIEIFYSSSTLLQELGDMVNNRELTSVCTHLPIDQKNKDKITFYGQQVAELCNKM
```

cons . : : : \* . \* : : . : : : \* . \* : : : \* . .

```
PALS-22_C._eleg -----AVVLNNKVKTLIDNGFT-----L
PALS-23_C._eleg AKVTGDEEELHQIVLENVQEPSFLQVPSNPT
PALS-24_C._eleg -----ETELLPFLPESI-----
PALS-25_C._eleg TDNRKKIERSLRQLQKDHVSQASYVG-----L
```

cons \* . :

## SUPPLEMENTARY FIGURE LEGENDS

### Figure S1: *pals-22* effect on complex and single copy reporters.

(A) *cho-1<sup>fosmid</sup>::yfp* (cholinergic neurons) is equally expressed in wild-type (top) and *pals-22(ot811)* mutant (bottom) worms. Complex array transgene.

(B) *che-1(ot856[che-1::gfp])* is equally expressed in wild-type (left) and *pals-22(ot811)* mutant (right) worms. Dotted outline: ASE neuron. Endogenously tagged locus using CRISPR/Cas9 technology.

(C) *lin-4<sup>prom</sup>::yfp* (ubiquitous) is equally expressed in wild-type (top) and *pals-22(ot811)* mutant (bottom) worms. Single copy miniMos strain.

At least 50 animals examined for each genotype. Scale bars represent 50  $\mu\text{m}$  (A and C) and 10  $\mu\text{m}$  (B).

### Figure S2: *pals-22* effects are more apparent at 15 °C.

(A) *ric-19<sup>prom6</sup>::NLS::gfp* expression (pan-neuronal) in *pals-22(ot811)* mutant (right) compared to wild-type (left) is more reduced at 15 °C (top) than at 20 °C (middle) or 25 °C (bottom).

(B) Quantification of the data represented in (A). The data are presented as mean + SEM, normalized to the wild-type fluorescence at 25 °C (fluorescence measured in the head region). Unpaired *t*-test, \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , \* $p < 0.05$ ;  $n \geq 7$  for all conditions.

### Figure S3: *pals-22* effects are more apparent in the adult.

(A) *ric-19<sup>prom6</sup>::NLS::gfp* expression (pan-neuronal) in *pals-22(ot811)* mutant (right) compared to wild-type (left) is more severely affected in L4-stage worms (bottom) than in L1-stage worms (top). Scale bars represent 50  $\mu\text{m}$ .

(B) Quantification of the data represented in (A). The data are presented as mean + SEM, normalized to the wild-type fluorescence (fluorescence measured in the head region). Unpaired *t*-test, \*\*\* $p < 0.001$ , \*\* $p < 0.01$ ;  $n \geq 8$  for all conditions.

### Figure S4: Protein alignment of PALS orthologs.

(A) ALS2CR12 signature logo. See Methods. Occupancy: the probability of observing a letter at position *k*; ins. prob: insert probability; ins. len: insert length (see [skylign.org/help](http://skylign.org/help) for details).

**(B)** PALS-22 and three PALS protein sequences from the LG III cluster (-26.98 cM), which are among the genes most closely related to PALS-22 (Table 2) were aligned with M-Coffee (Wallace *et al.* 2006) using default settings, demonstrating the low level of conservation for even the most closely related PALS proteins.