Supplemental Materials

Molecular Biology of the Cell

Matsunaga et al.

Supplemental Information

Supplemental Fig. 1

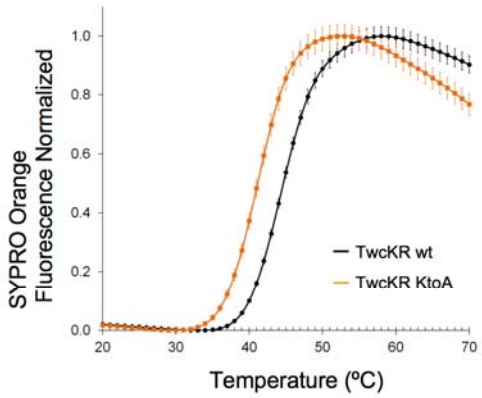


Figure S1. Thermal denaturation of TwcKR wt (black) and TwcKR KtoA (orange) was measured by DSF. Error bars show standard deviation of triplicate measurements collected for each temperature interval.

Supplemental Fig. 2

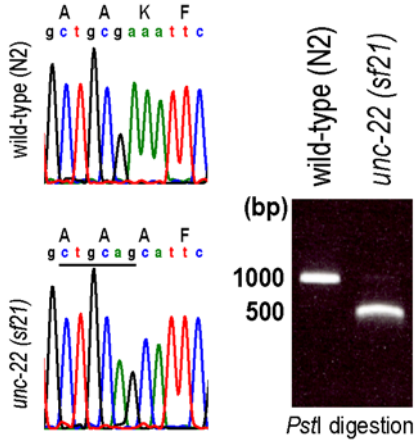


Figure S2. Generation of *unc-22(sf21)* nematodes by CRISPR/Cas9.

On the right is an agarose gel showing PCR fragments digested with *Pst*I that permits discrimination of wild-type from the *unc-22(sf21)* sequence. On the left are portions of the DNA sequence chromatograms of these fragments together with conceptual translation; the underline denotes the *Pst*I recognition site.

Supplemental Fig. 3

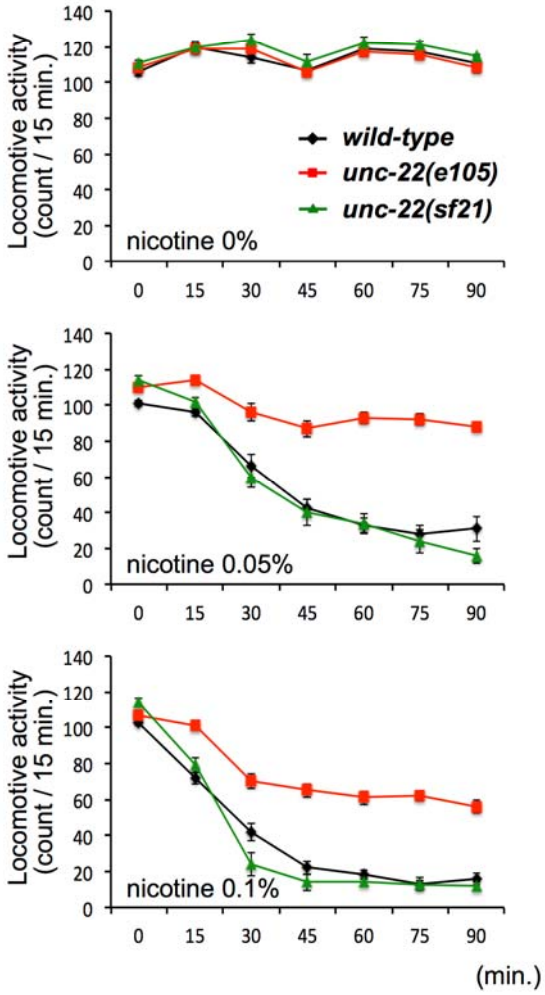


Figure S3. Response of wild-type, *unc-22(e105)* and *unc-22(sf21)* nematodes to nicotine.

A WMicrotracker (DesignPlus) was used to monitor the locomotion of multiple worms per well in a microtiter dish over time during exposure to a solution of 0.00%, 0.05% or 0.1% nicotine.

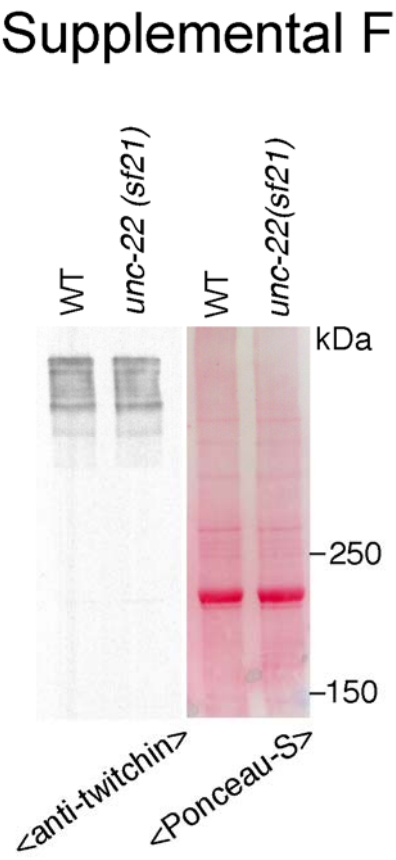


Figure S4. By western blot, the *unc-22(sf21)* mutant expresses normal levels of twitchin isoforms of the appropriate size.

Extracts from wild-type (WT) or *unc-22(sf21)* mutant animals were separated on a 5% SDS-PAGE and transferred to a membrane. On the right is shown the blot after Ponceau-

S staining; the positions of molecular weight markers are indicated. On the left is shown the result of reaction to anti-twitchin antibodies.

Supplemental Fig.5

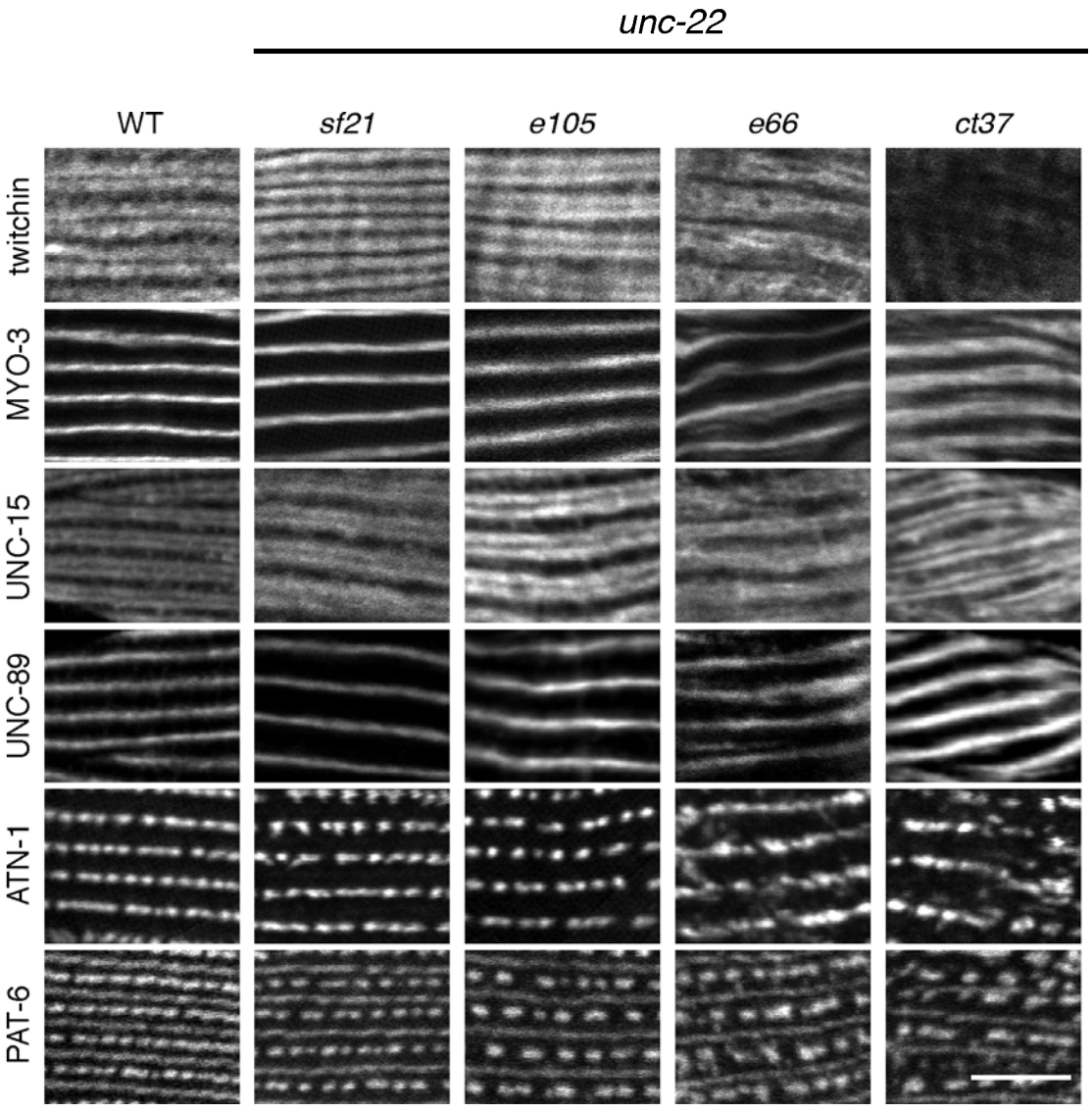
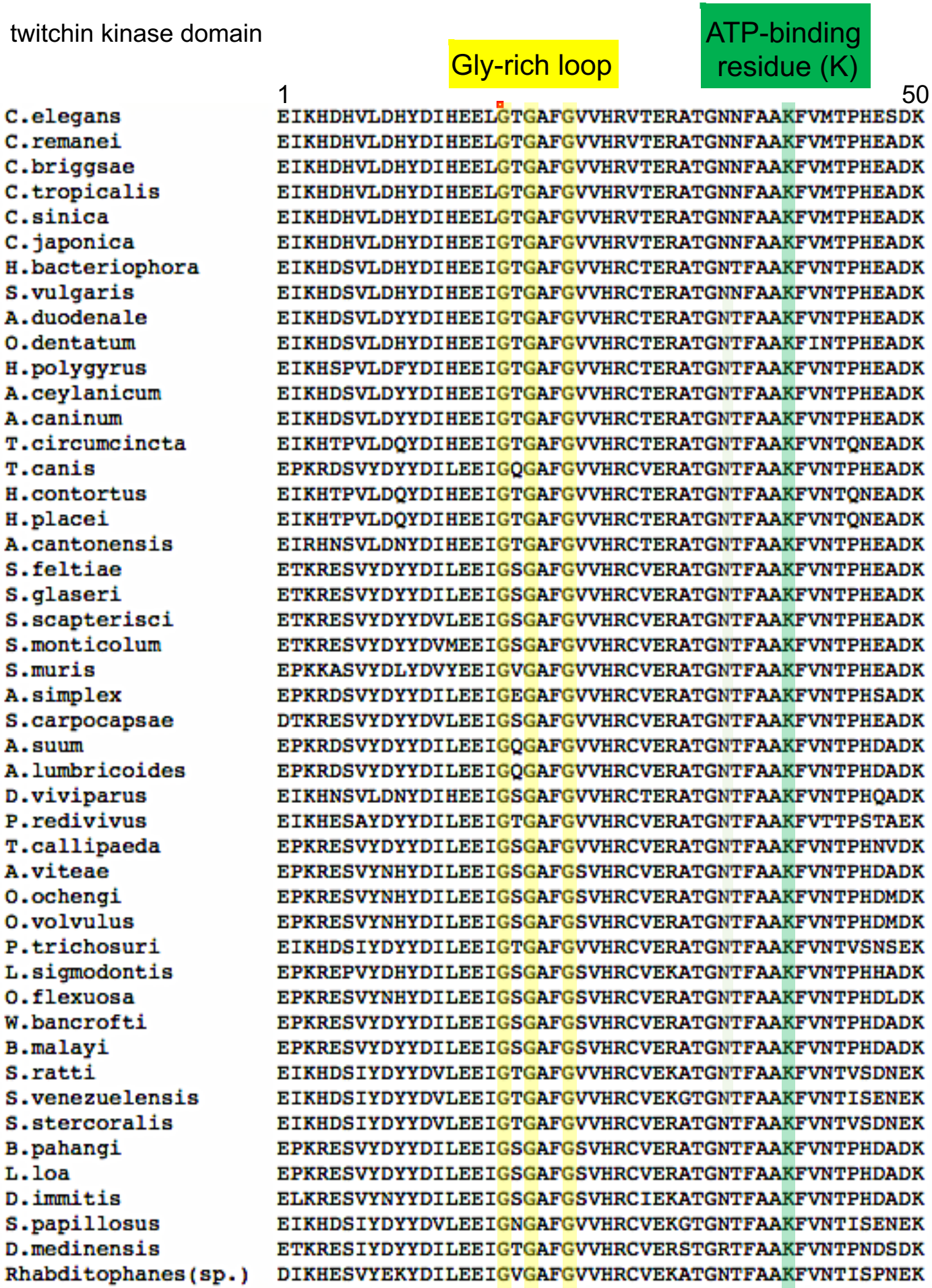
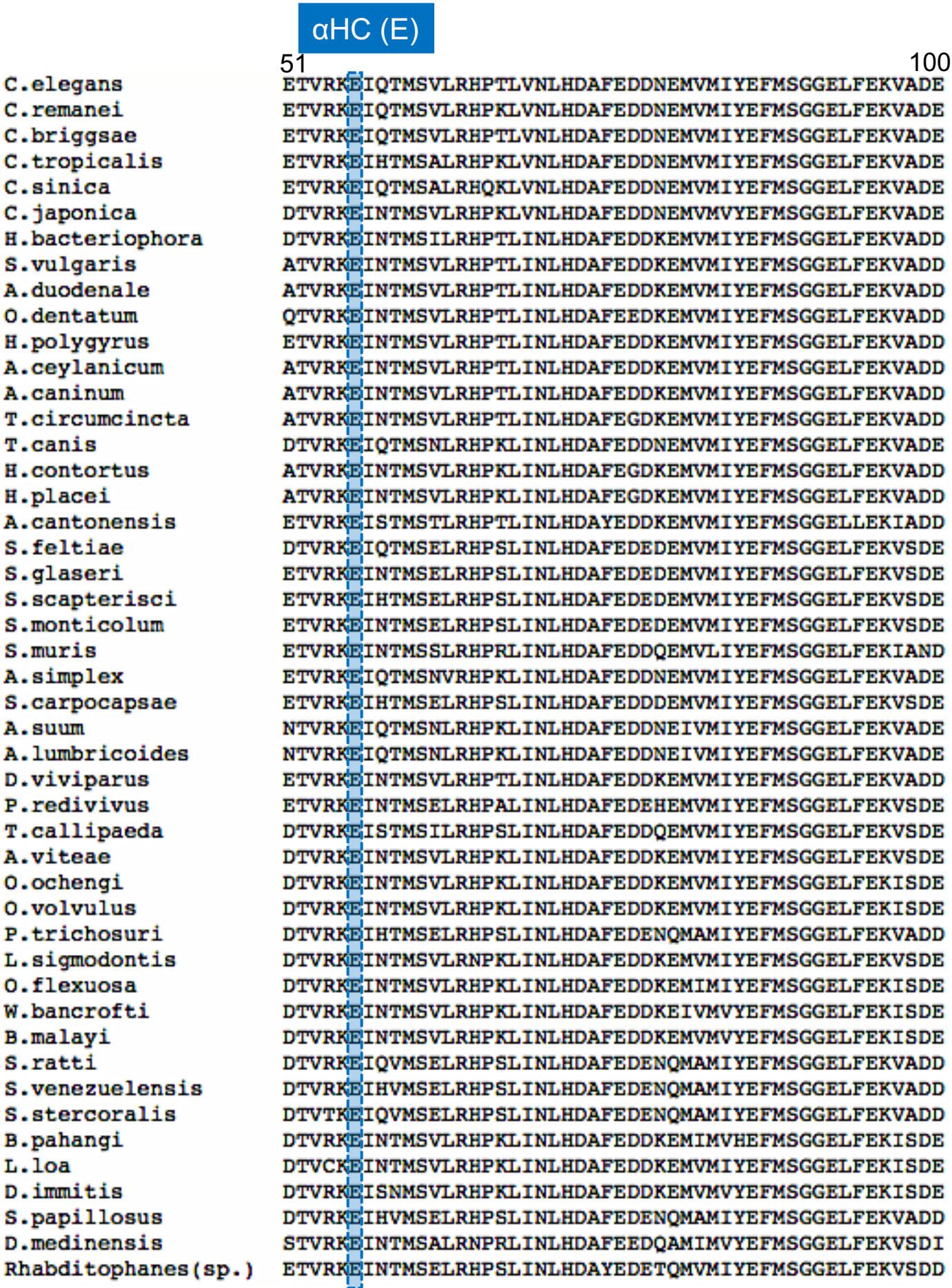


Figure S5. Comparison of sarcomeric structure of wild-type and *unc-22* mutant nematodes assessed by immunofluorescent staining.

Wild-type (WT) and the indicated *unc-22* mutant nematodes were fixed and immunostained with antibodies to the indicated sarcomeric proteins: twitchin, MYO-3 (myosin heavy chain A) and UNC-15 (paramyosin) of the A-bands; UNC-89 (obscurin) of the M-lines; ATN-1 (α-actinin) of dense bodies (Z-disk analogs); and PAT-6 (α-parvin) of M-lines and dense bodies. As indicated, *unc-22(sf21)* shows normal localization of every sarcomeric protein tested, including twitchin. For comparison, *unc-22(e105)* shows the same normal localization pattern as *unc-22(sf21)*, but the loss of function allele *unc-22(e66)* and the null allele *unc-22(ct37)* show disorganization of each of these marker proteins. Scale bar, 10 μm.

Supplemental Fig. 6

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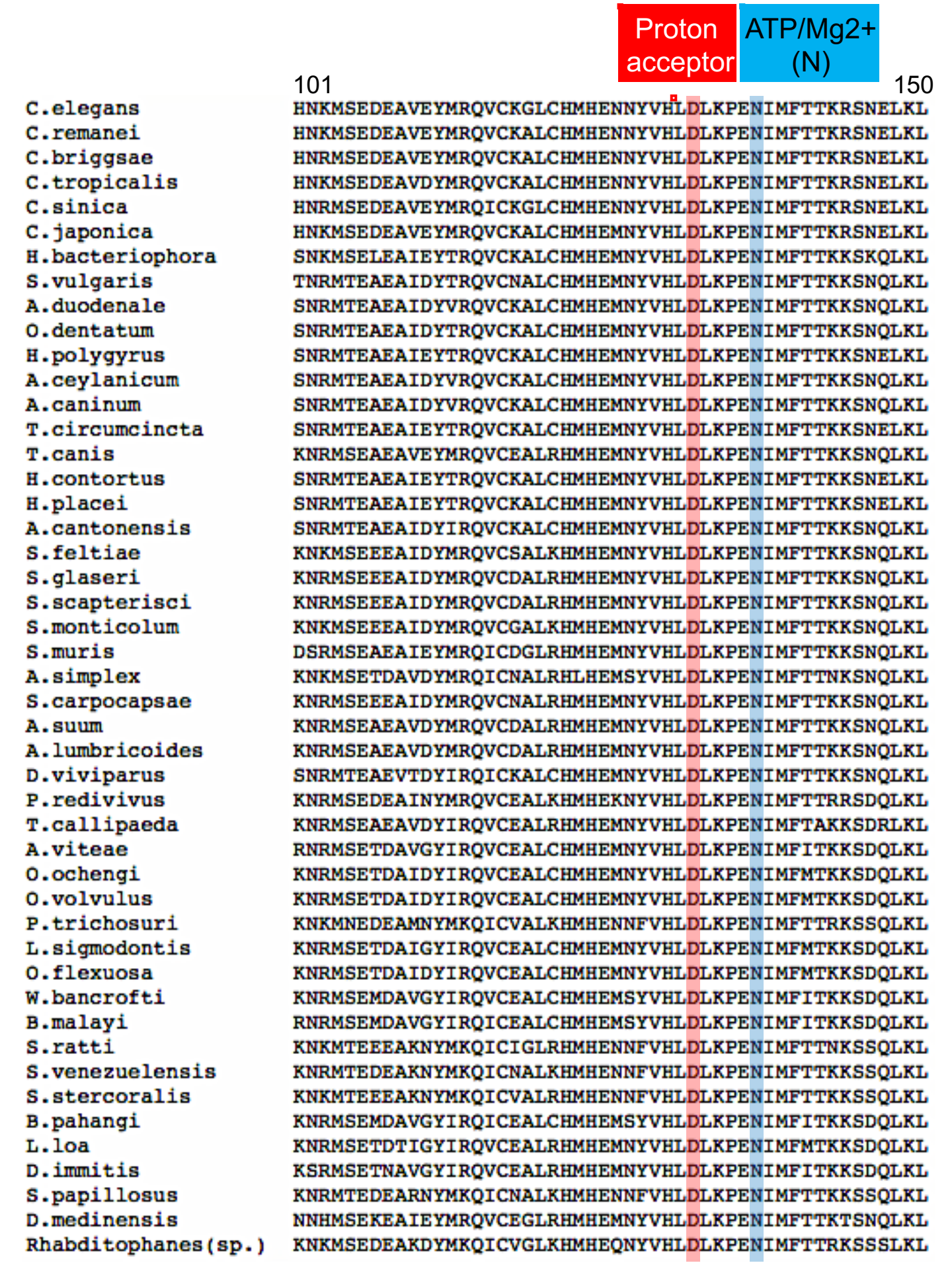
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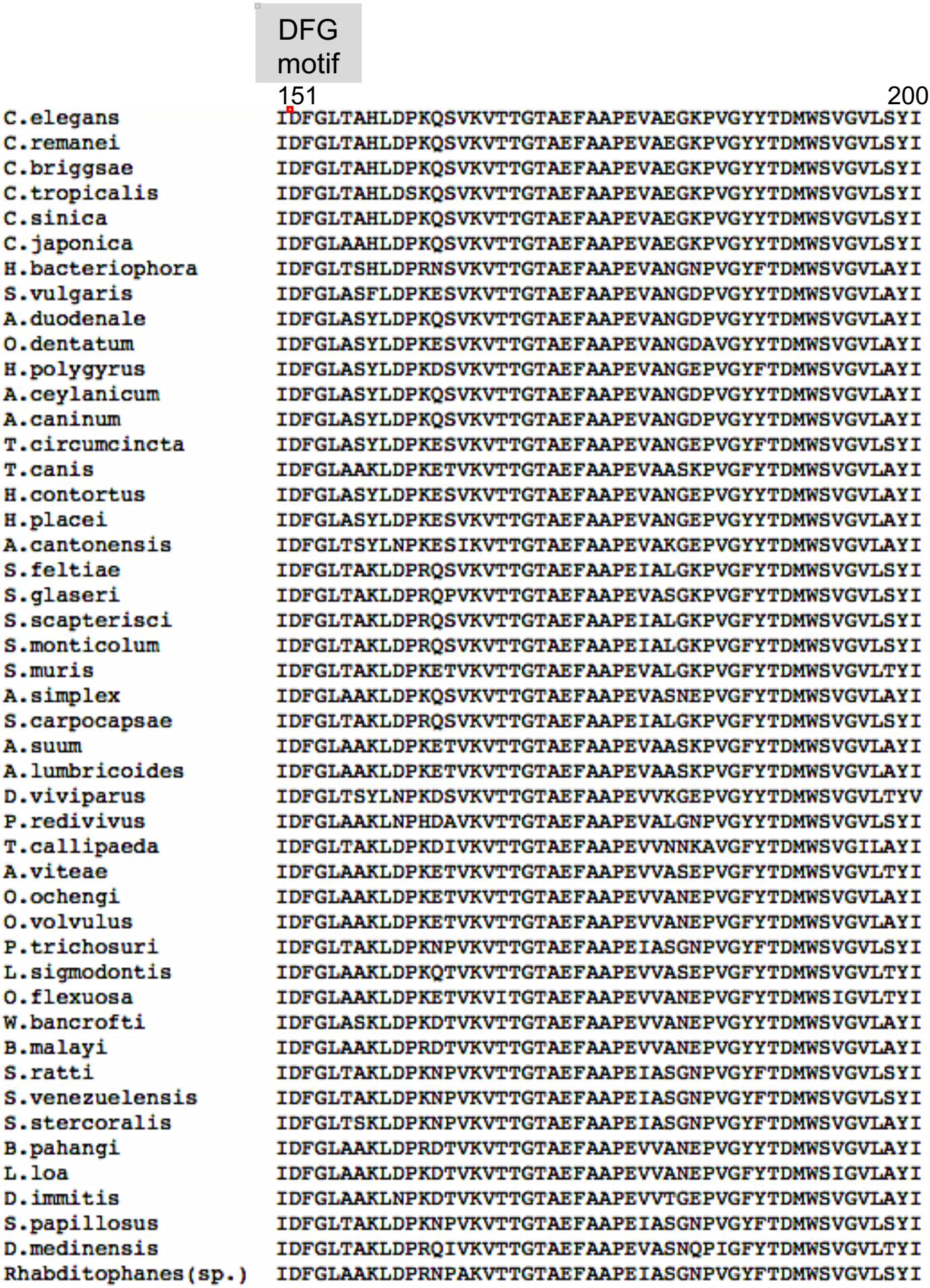
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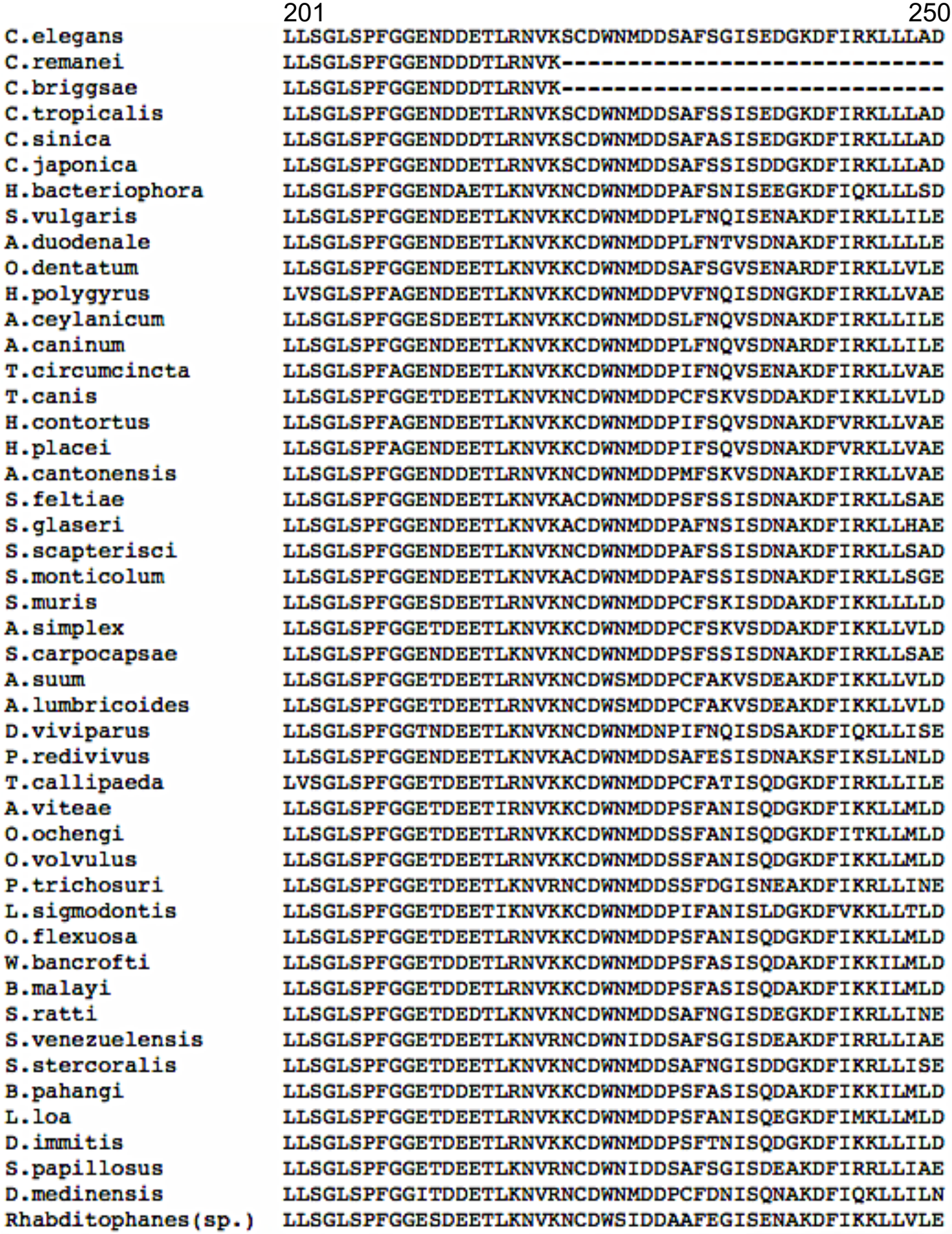
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O.ochengi LLSGLSPFGGETDEETLRNVKKCDWNMDDSSFANISQDGKDFITKLLMLD
O.volvulus LLSGLSPFGGETDEETLRNVKKCDWNMDDSSFANISQDGKDFIKKLLMLD
P.trichosuri LLSGLSPFGGETDEETLKNVRNCDWNMDDSSFDGISNEAKDFIKRLLINE
L.sigmodontis LLSGLSPFGGETDEETIKNVKKCDWNMDDPIFANISLDGKDFVKKLLTLD
O.flexuosa LLSGLSPFGGETDEETLRNVKKCDWNMDDPSFANISQDGKDFIKKLLMLD
W.bancrofti LLSGLSPFGGETDDETLRNVKKCDWNMDDPSFASISQDAKDFIKKILMLD
B.malayi LLSGLSPFGGETDDETLRNVKKCDWNMDDPSFASISQDAKDFIKKILMLD
S.ratti LLSGLSPFGGETDEDTLKNVKNCDWNMDDSAFNGISDEGKDFIKRLLINE
S.venezuelensis LLSGLSPFGGETDEETLKNVRNCDWNIDDSAFSGISDEAKDFIRRLLIAE
S.stercoralis LLSGLSPFGGETDEETLKNVKNCDWNMDDSAFNGISDDGKDFIKRLLISE
B.pahangi LLSGLSPFGGETDDETLRNVKKCDWNMDDPSFASISQDAKDFIKKILMLD
L.loa LLSGLSPFGGETDEETLRNVKKCDWNMDDPSFANISQEGKDFIMKLLMLD
D.immitis LLSGLSPFGGETDEETLRNVKKCDWNMDDPSFTNISQDGKDFIKKLLILD
S.papillosus LLSGLSPFGGETDEETLKNVRNCDWNIDDSAFSGISDEAKDFIRRLLIAE
D.medinensis LLSGLSPFGGITDDETLKNVRNCDWNMDDPCFDNISQNAKDFIQKLLILN
Rhabditophanes(sp.) LLSGLSPFGGESDEETLKNVKNCDWSIDDAAFEGISENAKDFIKKLLVLE

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C.elegans              PNTRMTIHQALEHPWL
C.remanei              ----------------
C.briggsae             ----------------
C.tropicalis           PNTRMTIHQALEHPWL
C.sinica               PNTRMTIHQALEHPWL
C.japonica             PNSRMTVHQALEHPWL
H.bacteriophora        TSSRMTIHQALEHPWL
S.vulgaris             P---------------
A.duodenale            PDKRMTIHEALAHPWL
O.dentatum             PDKRMTVHEALAHPWL
H.polygyrus            PGGRMTVHEALNHPWL
A.ceylanicum           PDKRMTVHEALAHPWL
A.caninum              PDKRMTIHEALAHPWL
T.circumcincta         PSKRMTIHEALNHPWL
T.canis                PASRMTVHEALEHPWL
H.contortus            PGGRMTIHEALNHPWL
H.placei               PGSRMTIHEALNHPWL
A.cantonensis          PEKRITIHEALAHPWL
S.feltiae              PTERMNIHDALDHPWL
S.glaseri              PSSRMNIHEALDHPWL
S.scapterisci          PTERMNIHEAMDHPWL
S.monticolum           PTERMNIHEALDHPWL
S.muris                PSSRMTVHQALEHPWL
A.simplex              PSSRMTIHEALEHPWL
S.carpocapsae          PTERINIHEALDHPWL
A.suum                 PTSRMTVHEALEHPWL
A.lumbricoides         PTSRMTVHEALEHPWL
D.viviparus            PSKRMTVHETLSHPWL
P.redivivus            PKSRLTVHDALDHPWL
T.callipaeda           PKNRMSIHEALEHPWL
A.viteae               PKSRMTVHEALEHPWL
O.ochengi              PKSRMTVHEALEHPWL
O.volvulus             PKSRMTVHEALEHPWL
P.trichosuri           SDKRMTIHEALDHPW-
L.sigmodontis          PKSRMTVHEALEHPWI
O.flexuosa             PKSRMTVHEALEHPWL
W.bancrofti            PKSRMTVHEALEHPWL
B.malayi               PKSRMTVHEALEHPWL
S.ratti                PEKRMNIHEALDHPWL
S.venezuelensis        PEKRMNIYEALDHPW-
S.stercoralis          PEKRMNIHEAIDHPW-
B.pahangi              PKSRMTVHEALEHPWL
L.loa                  PKSRMTVHEALEHPWL
D.immitis              PENRMTVHEALEHPWL
S.papillosus           PEKRMNIYEALDHPW-
D.medinensis           PGNRMNIHEALQHPWL
Rhabditophanes(sp.)    SGSRMGIHDALDHPW-
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Figure S6. Sequence analysis suggests that twitchin kinase is likely to be an active protein kinase in most nematodes.

Sequence alignment of the catalytic domain of twitchin kinase orthologs from 47 nematode species indicating conservation of 6 residues or motifs known to be crucial for protein kinase activity (highlighted or boxed in color). The roles or names of these motifs are indicated in the top row.

Supplemental Fig. 7

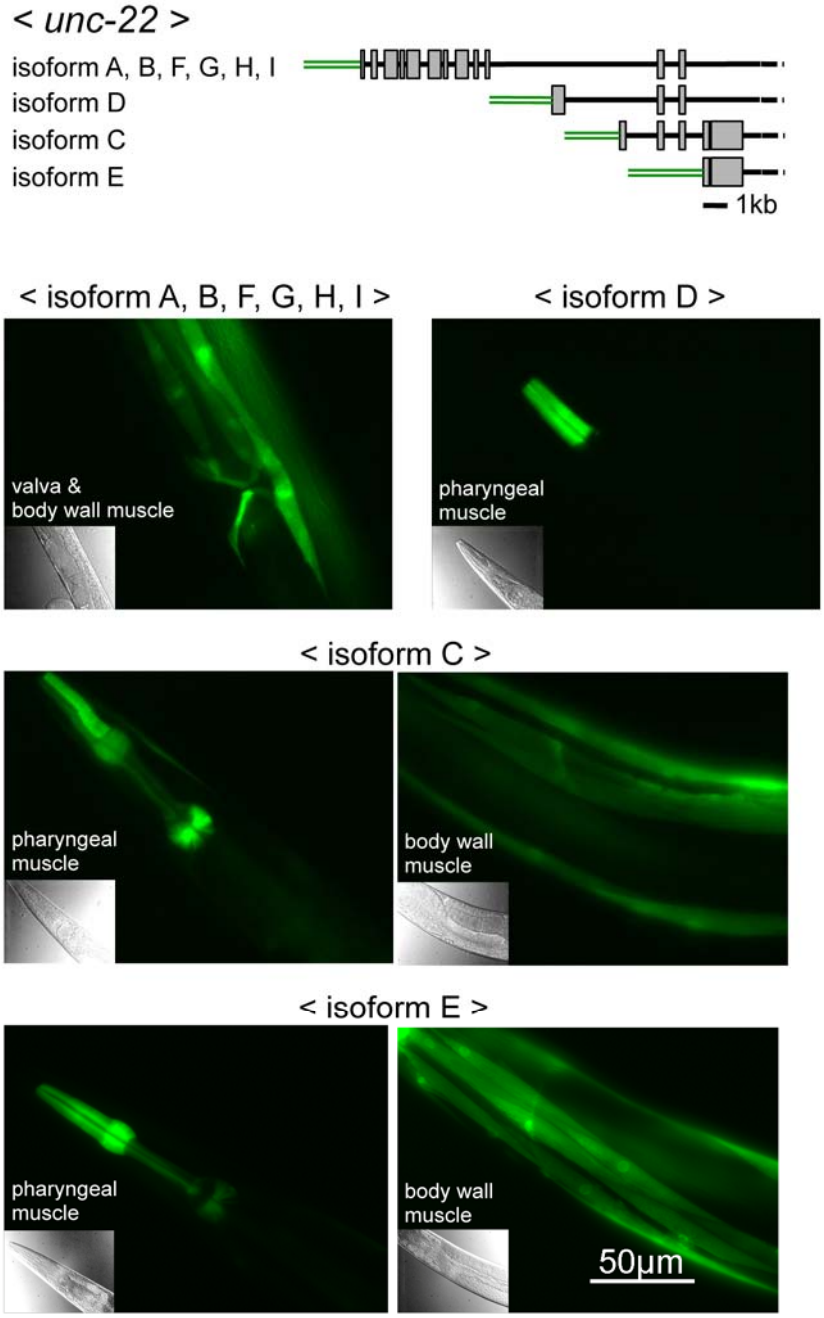

Figure S7. *unc-22* are promoters expressed in body wall muscle, pharyngeal muscle and/or vulva muscle.

As depicted on WormBase, *unc-22* has 9 isoforms that include the protein kinase domain. To investigate their expression patterns in adults, we generated transgenic worms expressing VENUS driven by each upstream sequence of *unc-22*. As shown, isoforms (A, B, F, G, H, and I) share the same initiator codon, and this promoter reporter is expressed in body wall and vulva muscles. In contrast, the isoform D promoter reporter is only expressed in pharyngeal muscles. For isoforms C and D, promoter reporters are expressed in body wall and pharyngeal muscles.