Supporting information

Materials and Methods

Dye-filling assay

The dye-filling protocol was adapted from previously described methods (1). One day old adult animals were stained with DiI (Molecular Probes) with final concentration of 10 µg/ml for 2-4 hours. The nematodes were washed twice with M9 and mounted on 3% agarose pad for imaging. The nematodes were analyzed with Nomarski optics and fluorescence microscopy, using a ZEISS AxioImager Z1 microscope coupled to CoolSNAP HQ2 camera (Photometric). Multidimensional data were reconstructed as maximum intensity projections using Zeiss Zen software.

Laser ablation

Laser microsurgery was conducted as described previously (2). Briefly, L2 larvae of animal which expressing GFP in specific neurons were mounted on 3% agarose plate with 7 mM sodium azide, and two photon lasers (Chameleon-Ultra 750nm, Coherent Inc.) were used to photobleach the cell body of target neurons (ZEISS LSM 510 META-NLO). After surgery, the animals were recovered on NGM plates and after two days, neuronal loss was confirmed by checking the absence of GFP signal. The animals were subsequently picked to *P. ostreatus* culture and scored for paralysis after 10 minutes of fungal exposure.

Intracellular ROS staining

To stain intracellular ROS accumulation level in nematodes after they exposed to *P. ostreatus*, aldicarb (1.5 mM), levamisole (1 mM), ivermectin (10 µM) and nicotine (20 mM), the paralyzed nematodes were incubated in the M9 with H_2DCFDA (100 µM, D399 Invitrogen) for one hour at

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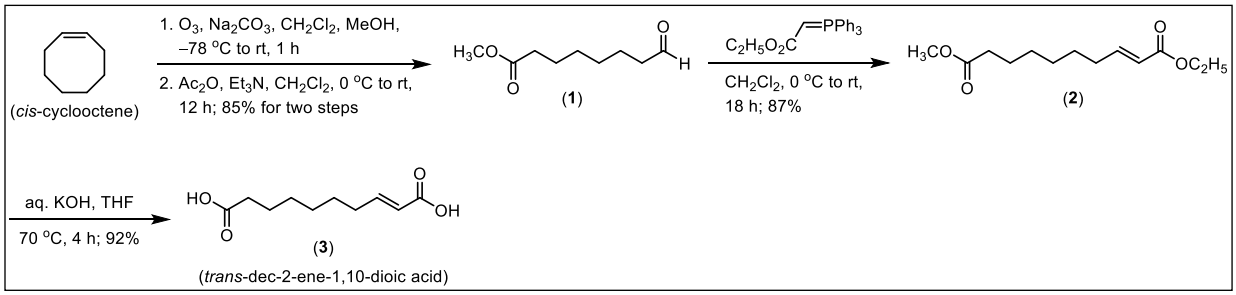
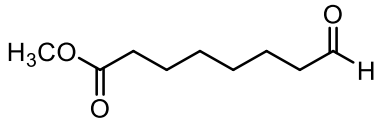
room temperature. The nematodes were imaged by a spinning disk confocal microscopy which was described above in the neuronal necrosis assay.

Synthesis of *trans*-dec-2-ene-1,10-dioic acid



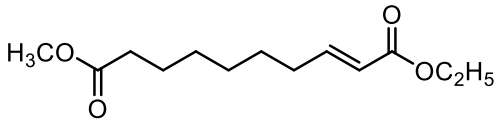
Methyl 8-oxooctanoate (1)



Ozone was bubbled into a mixture of *cis*-cyclooctene (3.90 mL, 30.00 mmol) and Na_2CO_3 (0.82 g, 7.74 mmol) in CH_2Cl_2 (90 mL) and methanol (18 mL) at –78 °C until a faint blue color appeared. Air was then bubbled into the reaction mixture until the blue color was discharged. The cooling bath was removed and the resulting mixture slowly warmed to room temperature. After filtration, toluene (50 mL) was added and the mixture concentrated to ~20 mL with the resulting viscous liquid being diluted with CH_2Cl_2 (80 mL). After cooling to 0 °C, Et_3N (6.2 mL, 44.48 mmol) and acetic anhydride (7.90 mL, 83.57 mmol) were sequentially added dropwise and the mixture was stirred at 0 °C for 30 min and then was warmed to room temperature. After stirring for 12 h at room temperature, the reaction mixture was washed with 0.1 N aqueous HCl (2 × 60 mL), 10% aqueous NaOH (2 × 60 mL), water (60 mL), and dried over $MgSO_4$. The solution was filtered and concentrated in vacuo to give a crude oil, which was purified by

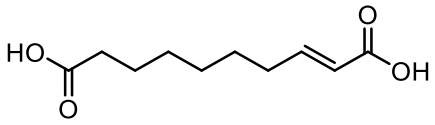
column chromatography on silica gel (20% EtOAc in hexane) to afford the desired product 1 (4.38 g, 85% for two steps). $C_9H_{16}O_3$, colorless oil; TLC (EtOAc/hexane, 1:3) R_f = 0.31; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.72 (1 H, t, J = 1.6 Hz), 3.63 (3 H, s), 2.39 (2 H, ddd, J = 9.2, 5.6, 1.6 Hz), 2.27 (2 H, t, J = 7.6 Hz), 1.61–1.59 (4 H, m), 1.33–1.29 (4 H, m); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.5, 174.0, 51.4, 43.7, 33.9, 28.7, 28.7, 24.6, 21.8; HRMS calcd for $C_9H_{17}O_3$: 173.1178, [ESI] found *m/z*: 173.1179 $[M + H]^+$.

1-Ethyl 10-methyl (*E*)-dec-2-enedioate (2)



To a cooled (0 °C) solution of (carbethoxymethylene)triphenylphosphorane (351 mg, 1 mmol) in anhydrous CH_2Cl_2 (10 mL) was added a solution of compound 1 (173 mg, 1 mmol) in anhydrous CH_2Cl_2 (3 mL). After stirring for 30 min at 0 °C, the reaction mixture was warmed to room temperature and then stirred for 18 h. The solvent was removed and the residual oil was purified by column chromatography on silica gel (10% EtOAc in hexane) to afford the desired product 2 (210 mg, 87%). $C_{13}H_{22}O_4$, colorless liquid; TLC (EtOAc/hexane, 1:4) R_f = 0.30; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (1 H, dt, J = 15.6, 6.9 Hz), 5.78 (1 H, d, J = 15.6 Hz), 4.15 (2 H, q, J = 7.2 Hz), 3.64 (3 H, s), 2.27 (2 H, t, J = 7.5 Hz), 2.19–2.14 (2 H, m), 1.63–1.56 (2 H, m), 1.47–1.40 (2 H, m), 1.32–1.30 (4 H, m), 1.26 (3 H, t, J = 7.2 Hz); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 166.4, 148.9, 121.2, 59.9, 51.2, 33.7, 31.8, 28.6, 28.5, 27.6, 24.6, 14.0; HRMS calcd for $C_{13}H_{23}O_4$: 243.1596, [ESI] found *m/z*: 243.1596 $[M + H]^+$.

trans-Dec-2-ene-1,10-dioic acid (3)



A solution of compound 2 (200 mg, 0.82 mmol) in ethanol (5 mL) was added 2 N aqueous KOH (3 mL). The reaction mixture was heated to 70 ºC and then stirred for 4 h at the same temperature. The reaction solution was concentrated to give the residual liquid, which was diluted with diethyl ether (10 mL) and then acidified by conc. HCl to pH 2. The resulting solution was extracted with diethyl ether (5 × 30 mL), and the combined organic extracts were dried over $MgSO_4$, filtered and concentrated to afford the desired product 3 (151 mg, 92%). $C_{10}H_{16}O_4$, white solid, mp 166–168 ºC; ^{1}H NMR (500 MHz, CD_3OD) δ 6.95 (1 H, dt, *J* = 15.5, 7.0 Hz), 5.79 (1 H, d, *J* = 15.5 Hz), 2.28 (2 H, t, *J* = 7.0 Hz), 2.24–2,20 (2 H, m), 1.62–1.59 (2 H, m), 1.49–1.47 (2 H, m), 1.38–1.36 (4 H, m); ^{13}C NMR (125 MHz, CD_3OD) δ 177.7, 170.2, 151.1, 122.6, 34.9, 33.1, 30.0, 29.9, 29.1, 26.0; HRMS calcd for $C_{10}H_{16}NO_4$: 200.2340, [EI] found *m/z*: 200.2339 [M^+].

Figures

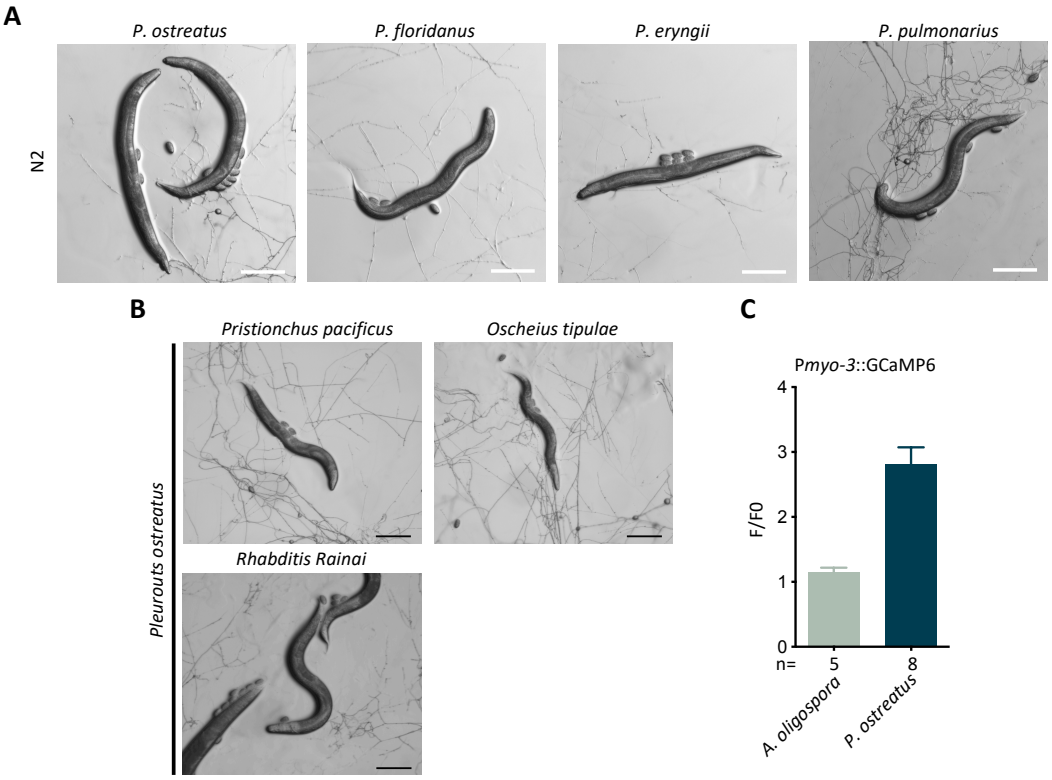


Fig. S1. (A) Wild-type *C. elegans* (N2) on *Pleurotus* hyphae. Scale bar = 200 µm. (B) *Pristionchus pacificus*, *Oscheius tipulae* and *Rhabditis Rainai* on *P. ostreatus* hyphae. Scale bar = 200 µm. (C) GCaMP6 signals in the head muscle cells of *C. elegans* in response to *P. ostreatus* or *A. oligospora* hyphae (mean ± SD, n shown along x-axis).

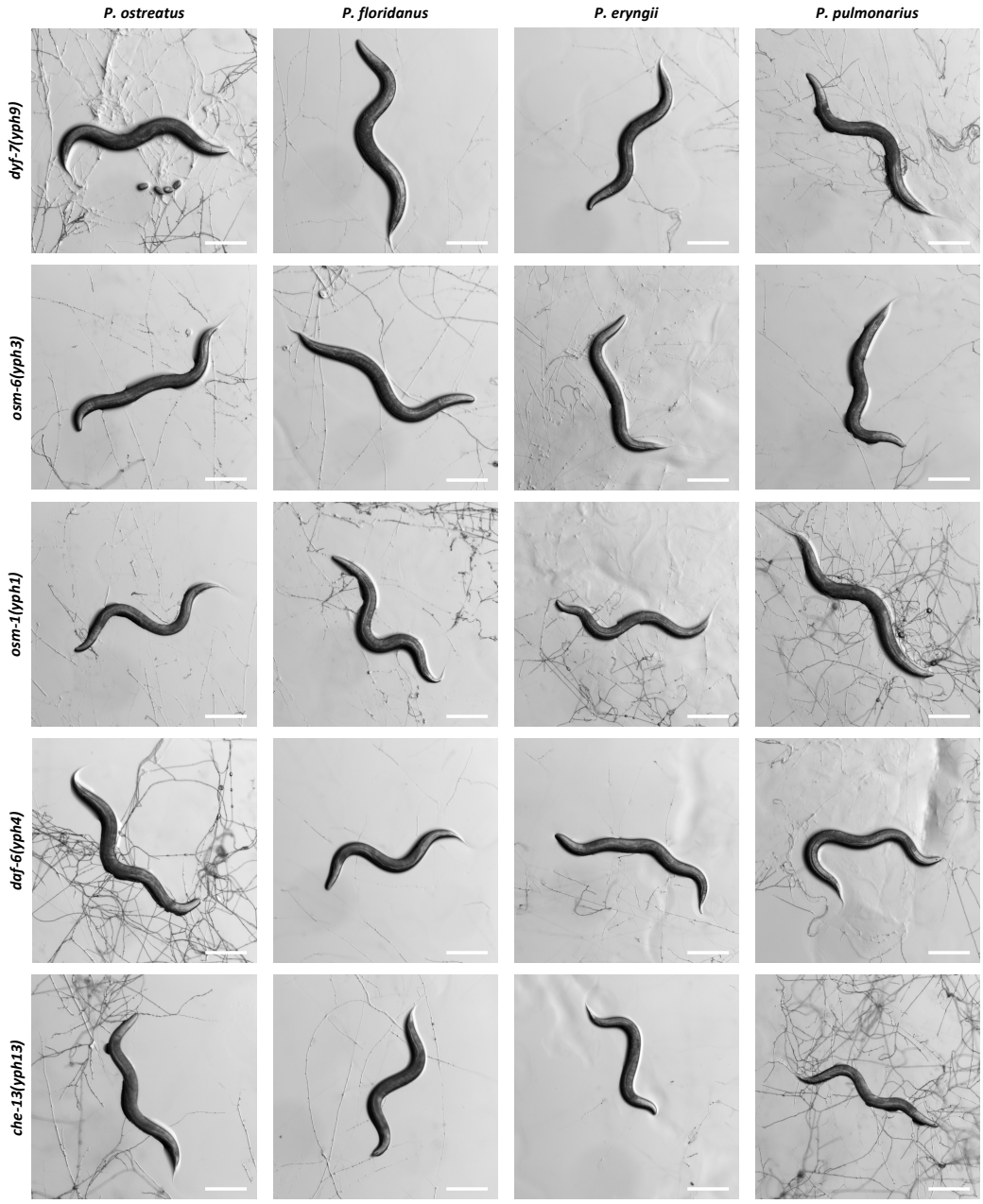


Fig. S2. *P. ostreatus* -resistant mutants on hyphae of four other *Pleurotus* species. Scale bar = 200 μm.

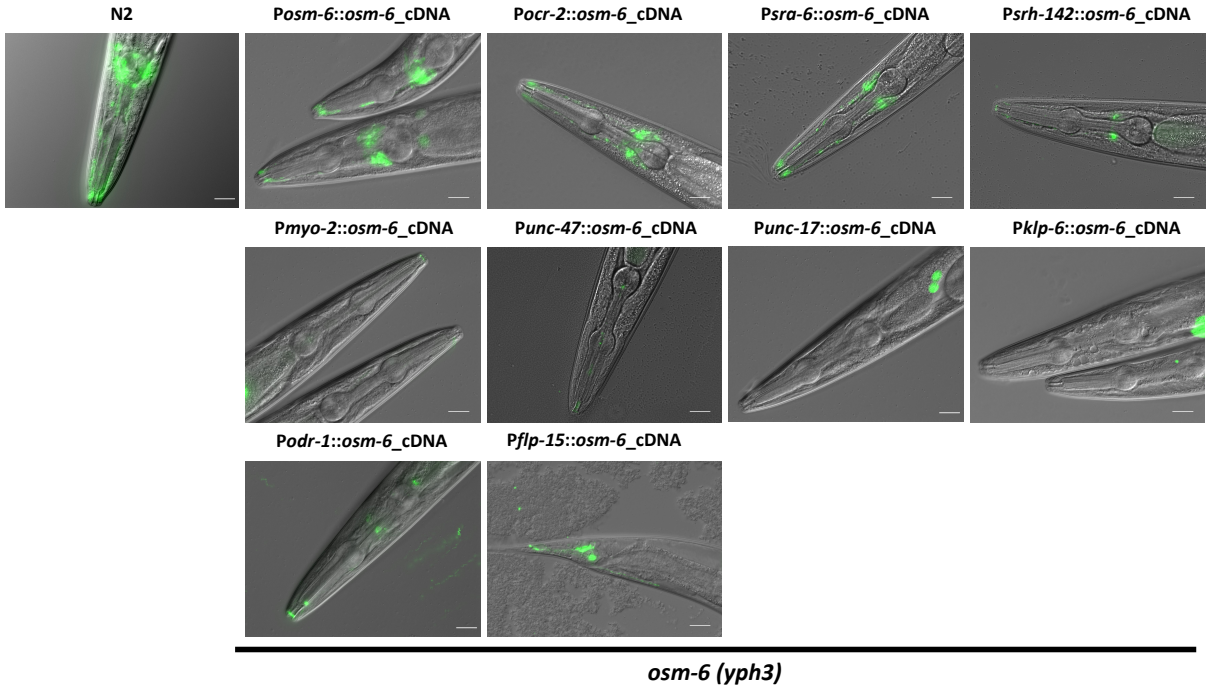


Fig. S3. Images of DiI staining of N2 and the *osm-6* (*yph3*) mutant expressing wild-type *osm-6* cDNA under various promoters. Scale bar = 20 μm.

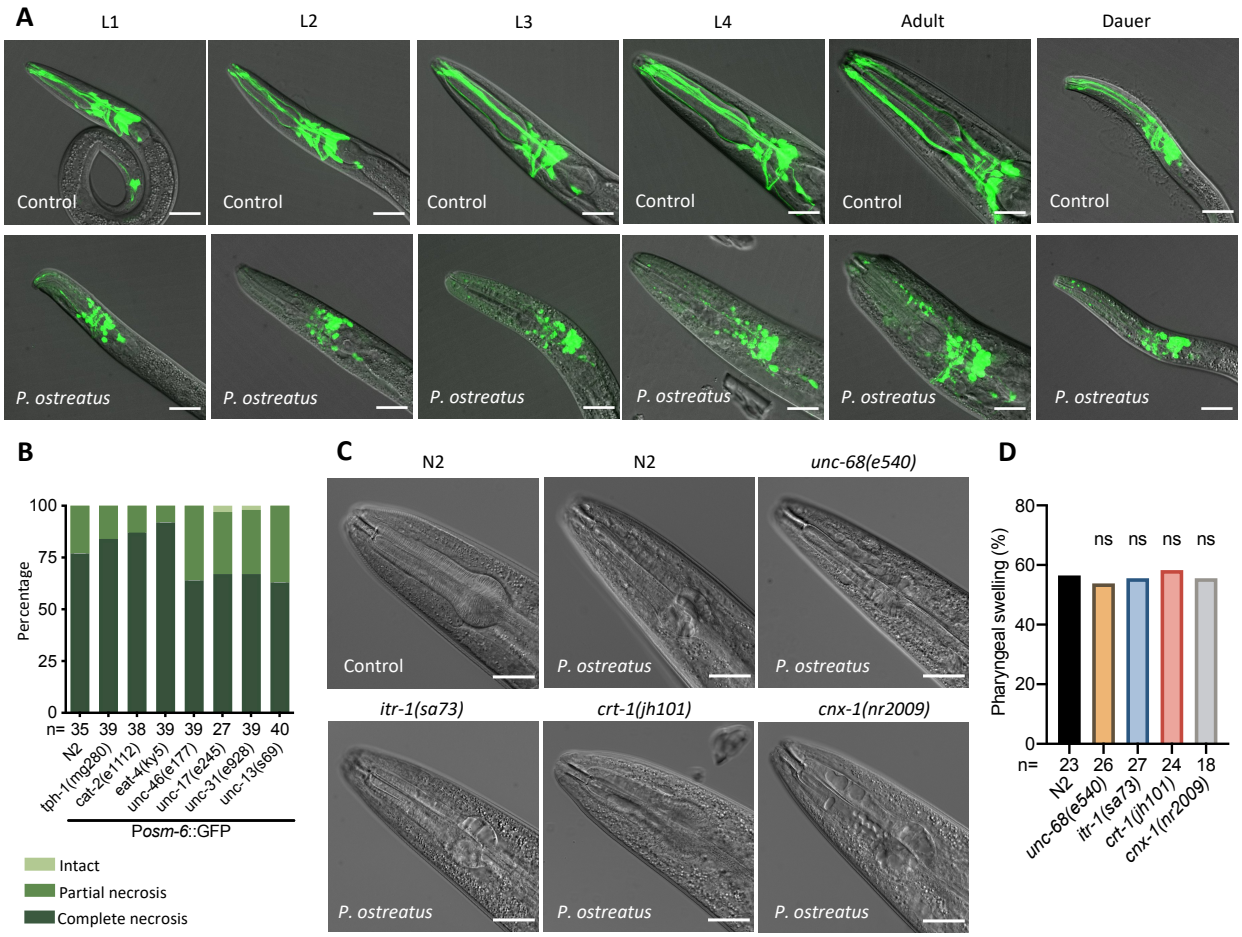


Fig. S4. (A) Images of cell necrosis in ciliated sensory neurons (P*osm-6*::GFP) in different developmental stages of *C. elegans* wild-type (N2). Scale bar = 20 µm. (B) Quantification of necrosis of ciliated sensory neuron (P*osm-6*::GFP) in neurotransmitter mutants. (C) Differential interference contrast images of the pharyngeal corpus in N2 and mutants that exposed to *P. ostreatus*. Scale bar = 20 µm. (D) Quantification of pharyngeal corpus swelling in N2 and mutants upon contact with *P. ostreatus* hyphae.

Movie S1. *C. elegans* contacts the hyphae of *P. ostreatus*.

Movie S2. Calcium imaging of the pharyngeal muscles

Movie S3. Calcium imaging of the body wall muscles

Movie S4. The *osm-6 (yph3)* mutant contacts the hyphae of *P. ostreatus.*

Movie S5. Calcium imaging of the IL2 neurons.

Movie S6. Cell necrosis in the ciliated sensory neurons (P*osm-6*::GFP).

Movie S7. *P. pacificus* and the *osm-1 (tu1129)* mutant contact the hyphae of *P. ostreatus.*

Strain list:

Identifier	Species	Genotype	Source
N2	*Caenorhabditis elegans*	wild type	CGC
CB4856	*Caenorhabditis elegans*	wild type (Hawaiian)	CGC
TWN135	*Caenorhabditis elegans*	*dyf-7(yph9)*	This study
TWN112	*Caenorhabditis elegans*	*daf-6(yph4)*	This study
TWN116	*Caenorhabditis elegans*	*osm-6(yph5)*	This study
TWN111	*Caenorhabditis elegans*	*osm-6(yph3)*	This study
TWN172	*Caenorhabditis elegans*	*osm-1(yph10)*	This study
TWN109	*Caenorhabditis elegans*	*osm-1(yph1)*	This study
TWN183	*Caenorhabditis elegans*	*osm-1(yph12)*	This study
TWN195	*Caenorhabditis elegans*	*osm-1(yph16)*	This study
TWN184	*Caenorhabditis elegans*	*che-13(yph13)*	This study
CB3330	*Caenorhabditis elegans*	*che-11(e1810)*	CGC
CB1387	*Caenorhabditis elegans*	*daf-10(e1387)*	CGC
PR813	*Caenorhabditis elegans*	*osm-5(p813)*	CGC

CB1033	*Caenorhabditis elegans*	*che-2(e1033)*	CGC
SP1234	*Caenorhabditis elegans*	*dyf-2(m160)*	CGC
DR86	*Caenorhabditis elegans*	*daf-19(m86)*	CGC
TWN1475	*Caenorhabditis elegans*	*tax-2(ok3403)*	CGC
TWN1474	*Caenorhabditis elegans*	*tax-4(ok3371)*	CGC
CX10	*Caenorhabditis elegans*	*osm-9(ky10)*	CGC
PS4322	*Caenorhabditis elegans*	*trp-1(ok323)*	CGC
CX2205	*Caenorhabditis elegans*	*odr-3(n2150)*	CGC
NL335	*Caenorhabditis elegans*	*gpa-3(pk35)*	CGC
PT1194	*Caenorhabditis elegans*	*klp-6(my8); him-5(e1490)*	CGC
MT15434	*Caenorhabditis elegans*	*tph-1(mg280)*	CGC
CB1112	*Caenorhabditis elegans*	*cat-2(e1112)*	CGC
MT6308	*Caenorhabditis elegans*	*eat-4(ky5)*	CGC
CB177	*Caenorhabditis elegans*	*unc-46(e177)*	CGC
PS34	*Caenorhabditis elegans*	*unc-17(e245)*	CGC
CB928	*Caenorhabditis elegans*	*unc-31(e928)*	CGC
EG9631	*Caenorhabditis elegans*	*unc-13(s69)*	CGC
MT1522	*Caenorhabditis elegans*	*ced-3(n717)*	CGC
CB540	*Caenorhabditis elegans*	*unc-68(e540)*	CGC
JT73	*Caenorhabditis elegans*	*itr-1(sa73)*	CGC
NS2938	*Caenorhabditis elegans*	*cnx-1(nr2009)*	CGC
KJ216	*Caenorhabditis elegans*	*crt-1(jh101)*	CGC
TWN1916	*Caenorhabditis elegans*	*yphIs4[Pmyo-3::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN1915	*Caenorhabditis elegans*	*yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study

TWN1350	*Caenorhabditis elegans*	*dyf-7(yph9); yphEx26[WRM0632aE04; Pofm-1::RFP]*	This study
TWN1352	*Caenorhabditis elegans*	*osm-1(yph1); yphEx28[WRM0622aH04; Pofm-1::RFP]*	This study
TWN1353	*Caenorhabditis elegans*	*daf-6(yph4); yphEx29[WRM0629aE09; Pofm-1::RFP]*	This study
TWN1331	*Caenorhabditis elegans*	*osm-6(yph3); yphEx23[WRM0617dH10; Pofm-1::RFP]*	This study
TWN2538	*Caenorhabditis elegans*	*che-13(yph13); yphEx203[WRM0625bc12; Pofm-1::RFP]*	This study
TWN2379	*Caenorhabditis elegans*	*osm-6(yph3); yphEx95[Posm-6::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2386	*Caenorhabditis elegans*	*osm-6(yph3); yphEx102[Pmyo-2::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2395	*Caenorhabditis elegans*	*osm-6(yph3); yphEx111[Punc-17::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2405	*Caenorhabditis elegans*	*osm-6(yph3); yphEx121[Pklp-6::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2418	*Caenorhabditis elegans*	*osm-6(yph3); yphEx134[Pocr-2::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study

TWN2431	*Caenorhabditis elegans*	*osm-6(yph3); yphEx147[Punc-47::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2442	*Caenorhabditis elegans*	*osm-6(yph3); yphEx151[Psra-6::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2448	*Caenorhabditis elegans*	*osm-6(yph3); yphEx157[Psrh-142::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2461	*Caenorhabditis elegans*	*osm-6(yph3); yphEx170[Pflp-15::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2765	*Caenorhabditis elegans*	*osm-6(yph-3); yphEx248[Podr-1::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2371	*Caenorhabditis elegans*	*yphEx88[Pklp-6::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2474	*Caenorhabditis elegans*	*osm-6(yph-3); yphEx175[Pklp-6::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2480	*Caenorhabditis elegans*	*osm-6(yph-3); yphEx181[Pklp-6::GCaMP6s::unc-54 3'UTR; Pklp-6::osm-6 cDNA::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2473	*Caenorhabditis elegans*	*yphEx174[Pklp-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
PY1058	*Caenorhabditis elegans*	*lin-15(n765); oyIs14[Psra-6::GFP + lin-15(+)]*	CGC
TWN2638	*Caenorhabditis elegans*	*osm-6(yph-3); yphEx233[Posm-6::osm-6 cDNA::unc-54 3'UTR;*	This study

		Posm-6::TeTx::mCherry; Pofm-1::RFP]	
TWN2617	*Caenorhabditis elegans*	*yphEx216[Posm-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2598	*Caenorhabditis elegans*	*yphEx209[Podr-1::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2817	*Caenorhabditis elegans*	*yphEx265[Punc-17::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
NC1687	*Caenorhabditis elegans*	*wdIs52[PF49H12.4::GFP]*	CGC
CLP1166	*Caenorhabditis elegans*	*twnEx501[Pgcy-8::GFP; Pmec-3::GFP]*	Dr. Chun-liang Pan's lab
TWN2847	*Caenorhabditis elegans*	*yphEx279[Pptr-10::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2644	*Caenorhabditis elegans*	*osm-6(yph-3); yphEx239[Pklp-6::osm-6 cDNA::unc-54 3'UTR; Posm-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2849	*Caenorhabditis elegans*	*osm-6(yph-3); yphEx281[Psrh-142::osm-6 cDNA::unc-54 3'UTR; Posm-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2851	*Caenorhabditis elegans*	*ced-3(n717); yphEx216[Posm-6::GFP::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2815	*Caenorhabditis elegans*	*unc-68(e540); yphEx216[Posm-6::GFP::unc-54 3'UTR; Pofm-1::RFP]*	This study

TWN2852	*Caenorhabditis elegans*	*itr-1(sa73); yphEx216[Posm-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2853	*Caenorhabditis elegans*	*cnx-1(nr2009); yphEx216[Posm-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2866	*Caenorhabditis elegans*	*crt-1(jh101); yphEx216[Posm-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2861	*Caenorhabditis elegans*	*dyf-7(yph9); yphEx216[Posm-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2862	*Caenorhabditis elegans*	*che-13(yph13); yphEx216[Posm-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2648	*Caenorhabditis elegans*	*osm-6(yph3); yphEx243[Posm-6::GFP:: unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2863	*Caenorhabditis elegans*	*unc-68(e540); yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN3006	*Caenorhabditis elegans*	*tph-1(mg280); yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN3010	*Caenorhabditis elegans*	*cat-2(e1112); yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN3014	*Caenorhabditis elegans*	*eat-4(ky5); yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study

TWN3017	*Caenorhabditis elegans*	*unc-46(e177); yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2898	*Caenorhabditis elegans*	*unc-17(e245); yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2719	*Caenorhabditis elegans*	*unc-31(e928); yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2771	*Caenorhabditis elegans*	*unc-13(s69); yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
TWN2971	*Caenorhabditis elegans*	*yphEx285[Posm-6::TeTx::mCherry; Pofm-1::RFP]; yphIs3[Pmyo-2::GCaMP6s::unc-54 3'UTR; Pofm-1::RFP]*	This study
SJ4103	*Caenorhabditis elegans*	*zcIs14[Pmyo-3::mitoGFP]*	CGC
TWN3020	*Caenorhabditis elegans*	*yphEx286[Pmyo-2::GCaMPer::unc-54 3'UTR; Pofm-1::RFP]*	This study
RS2333	*Pristionchus pacificus*	wild type	**(3)**
Fu1098	*Pristionchus pacificus*	*dyf-2(tu1098)*	**(3)**
Fu1129	*Pristionchus pacificus*	*osm-1(tu1129)*	**(3)**
	Pristionchus pacificus	*osm-3(tu1190); klp-20(tu1131)*	**(3)**
TWN1898	*Caenorhabdits briggsae*	wild type	This study
PHw2	*Diploscapter coronatus*	wild type	This study
TWN1998	*Oscheius myriophilus*	wild type	This study
TWN1422	*Rhabditis colombiana*	wild type	This study
TWN1327	*Oscheius tipulae*	wild type	This study

TWN301	*Rhabditis rainai*	wild type	This study
TWN1985	*Pristionchus pacificus*	wild type	This study
TWN1840	*Panagrellus redivivus*	wild type	This study
TWN1305	*Acrobeloides apiculatus*	wild type	This study
Northw1	*Cephalobus cubaensis*	wild type	This study
TWN1306	*Mesorhabditis sp.*	wild type	This study
TWN1451	*Pelodera teres*	wild type	This study
BCRC36436	*Pleurotus sapidus*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36432	*Pleurotus djamor*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36222	*Pleurotus flabellatus*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36217	*Pleurotus dryinus*	wild type	Bioresource Collection and Research Center (Taiwan)

BCRC36211	*Pleurotus calyptratus*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36210	*Pleurotus floridanus*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36230	*Pleurotus pulmonarius*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36237	*Pleurotus sajor-caju*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36069	*Pleurotus ostreatus*	wild type	Bioresource Collection and Research Center (Taiwan)

BCRC36940	*Pleurotus nebrodensis*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36163	*Pleurotus eryngii*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36214	*Pleurotus fuscus var. ferulae*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36226	*Pleurotus subareolatus*	wild type	Bioresource Collection and Research Center (Taiwan)
BCRC36924	*Pleurotus citrinopileatus*	wild type	Bioresource Collection and Research Center (Taiwan)

BCRC36171	*Pleurotus cystidiosus*	wild type	Bioresource Collection and Research Center (Taiwan)
AT8	*Coprinopsis cinerea*	wild type	Dr. Ursula Kues's lab

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