# Supplemental Tables and Figures

## "Defective responses to oxidative stress in protein L-isoaspartyl repair-deficient *Caenorhabditis elegans*"

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**Supplemental Table S1:**

**PCM-1 activity assay.**

Nematode extracts were prepared either from NGM+OP50 plates or liquid culture and analyzed for PCM-1 methyltransferase activity as described by Kagan et al. (1997). Specific activities determined in triplicate are given with standard deviation values.

| Strain | Grown on NGM plates (pmol/min/mg protein) | Grown on S-Media liquid culture (pmol/min/mg protein) |
|---|---|---|
| N2 | 0.14±0.01 | 1.02±0.02 |
| *pcm-1 (qa201)* | 0.02±0.1 | –0.001±0.02 |
| *pcm-1 (tm363)* | Not determined | 0.016±0.01 |
| *PL51* | 0.24±0.01 | 7.09±0.2 |
| *PL54* | –0.02±0.001 | Not determined |

**Supplemental Table S2:**

**Effect of juglone (236 µM) on young adult nematode survival.**

Young adult nematodes were treated on NGM plates containing 236 µM juglone as described previously (Cysper and Johnson, 2002; De Castro et al., 2004) in three replicate trials (N2 and *pcm-1* (*qa201*), n = 45) or two replicate trials ( *pcm*-1(*tm363*), n = 30). The data shown represents the average percentage of nematodes alive following juglone incubation.

| **Time of Incubation** | | **% Alive** | |
|---|---|---|---|
| **(hour)** | **N2** | ***pcm-1 (qa201)*** | ***pcm-1 (tm363)*** |
| 2 | 0 | 2 | 6 |
| 3 | 0 | 2 | 6 |
| 4 | 0 | 0 | 6 |
| 5 | 0 | 0 | 0 |

**Supplemental Table S3:**

**Paraquat delays the development of wild-type (N2) and *pcm-1* mutant animals.**

Eggs (about 100) were transferred to duplicate NGM+OP50 plates in the presence and absence of 0.2 mM paraquat and the developmental stage of each worm was determined after 48 h at 25 °C as L1, L2, L3, and L4 larvae and as young adult/egg-laying adult (YA/ELA) nematodes. The experiment was replicated a total of four times. The differences between the percentage values of the wild-type and *pcm-1* mutant (both mutants *qa201* and *tm363*) groups was tested in the two-tailed Student's t-test (unequal variance) and p-values were determined. No correction was made for multiple sample correction.

| **L1 control** | | **L1 paraquat** | | **L2 control** | | **L2 paraquat** | | **L3 control** | | **L3 paraquat** | | **L4 control** | | **L4 paraquat** | | **YA/ELA control** | | **YA/ELA paraquat** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant |
| 0 | 0 | 19 | 0 | 0 | 0 | 7 | 11 | 0.6 | 0 | 0 | 6 | 0 | 10.5 | 0 | 0 | 99 | 89.5 | 74 | 83 |
| 0 | 0 | 6 | 0 | 0 | 6 | 5 | 21 | 25 | 8.4 | 10 | 20 | 10 | 7 | 5 | 0 | 65 | 79 | 75 | 60 |
| 0 | 0 | 1 | 22 | 0 | 0 | 6 | 78 | 0 | 0 | 36 | 0 | 0 | 0 | 0 | 0 | 100 | 100 | 57 | 0 |
| 0 | 0 | 0 | 2 | 3.3 | 0 | 0 | 20 | 3.3 | 0 | 46 | 68 | 3.3 | 6 | 53 | 10 | 90 | 94 | 1 | 0 |
| | 0 | | 16 | | 0 | | 58 | | 1 | | 26 | | 5 | | 0 | | 94 | | 0 |
| | 0 | | 7 | | 4 | | 75 | | 4 | | 0 | | 0 | | 0 | | 93 | | 93 |
| | 2 | | 28 | | 4.4 | | 29 | | 3.7 | | 14 | | 3.7 | | 0 | | 87 | | 28 |
| | 0 | | 0 | | 0 | | 25 | | 0 | | 11 | | 2 | | 10 | | 98 | | 6 |
| | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |
| p-value | 0.35 | | 0.64 | | 0.45 | | **0.01** | | 0.46 | | 0.73 | | 0.74 | | 0.42 | | 0.72 | | 0.45 |

**Supplemental Table S4:**

**Paraquat delays development in *C10F3.4* (*tm2679)* mutant *C. elegans*.**

The number of animals tested and the percentages of larval forms ± the standard deviation from one replicate experiment (two plates) are shown for the summary data presented in Supplemental Figure S1.

| **Paraquat Treatment for Wild-Type (N2) and *tm2679* mutant *C. elegans*** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Strain** | **Condition** | **n** | **% L1** | **% L2** | **% L3** | **% L4** | **%YA/ELA** |
| N2 | control | 198±8.5 | 0 | 0 | 3.6±2.4 | 2.5±0.5 | 94±16.3 |
| N2 | 0.1 mM PQ | 44±8.5 | 0 | 3±4.4 | 52±59 | 45±63 | 0 |
| N2 | 0.2 mM PQ | 37±4.9 | 2 | 0 | 61.4±55 | 27.3±39 | 11.4±16 |
| N2 | 1 mM Vit C | 235±2.12 | 0 | 0 | 0 | 8.5±0.2 | 92±0.2 |
| N2 | 0.1 mM PQ + 1 mM Vit C | 200 | 0 | 0 | 0 | 0 | 100 |
| N2 | 0.2 mM PQ + 1 mM Vit C | 200 | 0 | 0 | 0 | 5±2 | 95±2 |
| *tm2679* | control | 200 | 0 | 0 | 0 | 0 | 100 |
| *tm2679* | 0.1 mM PQ | 32±1.4 | 0 | 0 | 0 | 15±6 | 85±6 |
| *tm2679* | 0.2 mM PQ | 109±3.5 | 0 | 0 | 55±4 | 22±1.4 | 23±5 |
| *tm2679* | 1 mM Vit C | 200 | 0 | 0 | 0 | 0 | 100 |
| *tm2679* | 0.1 mM PQ + 1 mM Vit C | 200 | 0 | 0 | 0 | 10 | 90 |
| *tm2679* | 0.2 mM PQ + 1 mM Vit C | 200 | 0 | 0 | 0 | 5 | 95 |

**Supplemental Table S5:**

***PL51* and *P54* transgenic strains treated with oxidative stress agents show developmental delays similar to wild-type (N2) and *pcm-1* mutant animals, respectively.**

The number of animals tested and the percentages of larval forms ± the standard deviation from three replicate experiments are shown for the summary data presented in Supplemental Figure S2.

| **Paraquat and Homocysteine Compound Treatment for wild-type (N2), *PL51* PCM-1 Overexpressor and *PL54* PCM-1 mutant *C. elegans* strains** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Strain** | **Condition** | **n** | **%L1** | **%L2** | **%L3** | **%L4** | **%YA/ELA** |
| N2 | control | 3200±187 | 0 | 0 | 0 | 0 | 100 |
| N2 | 0.2 mM Pq | 834±31 | 0.4±1.5 | 26.8±7.1 | 66.4±5.4 | 6.34±6.4 | 0 |
| N2 | 20 mM Hcy | 1000±58 | 1.9±3.25 | 98±3.25 | 0 | 0 | 0 |
| N2 | 20 mM HCTL | 974±51 | 12.6±7 | 87±7 | 0 | 0 | 0 |
| *PL51* | control | 152±16 | 0 | 0 | 0 | 97.3±4.7 | 2.7±4.7 |
| *PL51* | 0.2 mM Pq | 162±11 | 0 | 0 | 64.5±40 | 37±25 | 0 |
| *PL51* | 20 mM Hcy | 167±15 | 0 | 61±27 | 39±27 | 0 | 0 |
| *PL51* | 20 mM HCTL | 130±10 | 0 | 87±25 | 12.6±25 | 0 | 0 |
| *PL54* | control | 97±9 | 0 | 0 | 0 | 98.7±3.4 | 1.3±3.4 |
| *PL54* | 0.2 mM Pq | 126±9 | 0 | 62.3±25 | 37.2±25 | 0 | 0 |
| *PL54* | 20 mM Hcy | 82±9 | 78±23 | 21±23 | 0 | 0 | 0 |
| *PL54* | 20 mM HCTL | 135±9 | 46±44 | 27±27 | 0 | 0 | 0 |

**Supplemental Table S6:**

**Effects of homocysteine and homocysteine derivatives on wild-type (N2) and *pcm-1* mutant *C. elegans* development.**

Eggs (about 100) were transferred to duplicate NGM+OP50 plates containing 0, 10 mM and 20 mM homocysteine (Hcy), homocysteine thiolactone (HCTL), and homocystine (Hcy-Hcy) and the developmental stage of each worm was determined after 48 h at 25 °C as L1, L2, L3, and L4 larvae and as young adult/egg-laying adult (YA/ELA) nematodes. The experiment was replicated a total of five times. The percentage of nematodes in each developmental stage was determined for each plate from combined data of the untreated and the homocystine treated groups (control), and for the combined data of the 20 mM homocysteine, 10 mM and 20 mM homocysteine thiolactone treated groups (variable). The differences between the percentage values of the control and variable groups was tested in the two-tailed Student's t-test (unequal variance) and p-values were determined. The data for the *pcm-1* mutants are combined. No correction was made for multiple sample correction.

| **L1 control** | | **L1 variable** | | **L2 control** | | **L2 variable** | | **L3 control** | | **L3 variable** | | **L4 control** | | **L4 variable** | | **YA control** | | **YA variable** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant | N2 | mutant |
| 0 | 0 | 0 | 0 | 0 | 0 | 39 | 13 | 3 | 14 | 39 | 50 | 20 | 0 | 0 | 0 | 78 | 86 | 21 | 38 |
| 0 | 0 | 0 | 48 | 0 | 0 | 8 | 27 | 0 | 0 | 14 | 0 | 5 | 20 | 12 | 17 | 95 | 80 | 66 | 7 |
| 0 | 0 | 19 | 71 | 0 | 0 | 42 | 0 | 1 | 2 | 21 | 10 | 3 | 35 | 0 | 0 | 96 | 64 | 18 | 20 |
| 0 | 0 | 0 | 36 | 0 | 0 | 93 | 48 | 1 | 0 | 0 | 0 | 0 | 10 | 7 | 12 | 99 | 90 | 0 | 4 |
| 0 | 0 | 12 | 29 | 3 | 0 | 69 | 9 | 6 | 0 | 5 | 30 | 10 | 0 | 10 | 0 | 81 | 100 | 4 | 32 |
| 0 | 0 | 0 | 0 | 0 | 0 | 28 | 0 | 0 | 0 | 47 | 25 | 0 | 0 | 2 | 0 | 100 | 100 | 23 | 75 |
| 0 | 0 | 15 | 14 | 0 | 0 | 10 | 43 | 0 | 9 | 34 | 0 | 36 | 30 | 7 | 0 | 64 | 62 | 35 | 42 |
| 0 | 0 | 18 | 100 | 0 | 0 | 59 | 0 | 2 | 4 | 24 | 0 | 1 | 22 | 0 | 0 | 97 | 74 | 0 | 0 |
| 0 | 0 | 0 | 67 | 0 | 0 | 0 | 20 | 17 | 0 | 1 | 0 | 21 | 61 | 0 | 13 | 62 | 39 | 99 | 0 |
| 0 | 0 | 0 | 14 | 3.2 | 0 | 0 | 43 | 6.4 | 9 | 24 | 0 | 17 | 30 | 24 | 0 | 73 | 62 | 53 | 42 |
| 0 | 0 | 4 | 0 | 0 | 0 | 73 | 36 | 0 | 0 | 16 | 15.3 | 0 | 0 | 0 | 19 | 100 | 100 | 7 | 31 |
| 0 | 3 | 17 | 42 | 0 | 3 | 83 | 39 | 3 | 8 | 0 | 3 | 6 | 15 | 0 | 0 | 91 | 70 | 0 | 16 |
| 0 | 0 | 64 | 0 | 0 | 0 | 31 | 54 | 1 | 0 | 5 | 21 | 2 | 42 | 0 | 0 | 97 | 58 | 0 | 26 |
| 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 11 | 0 | 18 | 5 | 12 | 0 | 21 | 17 | 71 | 100 | 61 | 78 |
| 0 | 0 | 0 | 13 | 0 | 0 | 64 | 13 | 0 | 0 | 10 | 0 | 13 | 30 | 0 | 26 | 88 | 70 | 27 | 47 |
| | 9 | | 20 | | 9 | | 56 | | 22 | | 0 | | 0 | | 0 | | 59 | | 24 |
| | 0 | | 65 | | 0 | | 15 | | 15 | | 19 | | 9 | | 0 | | 76 | | 0 |
| | 0 | | 96 | | 0 | | 4 | | 11 | | 0 | | 46 | | 0 | | 42 | | 0 |
| | 0 | | 2 | | 0 | | 20 | | 0 | | 44 | | 13 | | 13 | | 87 | | 22 |
| | 0 | | 65 | | 0 | | 15 | | 15 | | 19 | | 9 | | 0 | | 76 | | 0 |
| | 0 | | 0 | | 0 | | 8 | | 15 | | 42 | | 21 | | 0 | | 63 | | 50 |
| | 0 | | 94 | | 0 | | 2 | | 0 | | 2 | | 71 | | 0 | | 29 | | 3 |
| | 0 | | 67 | | 0 | | 23 | | 0 | | 2 | | 0 | | 0 | | 100 | | 8 |
| | 0 | | 28 | | 0 | | 45 | | 0 | | 27 | | 18 | | 0 | | 82 | | 0 |
| | 0 | | 0 | | 0 | | 30 | | 0 | | 15 | | 45 | | 20 | | 55 | | 35 |
| | 0 | | 0 | | 0 | | 76 | | 33 | | 0 | | 0 | | 0 | | 67 | | 24 |
| | 0 | | 100 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 100 | | 0 |
| | 0 | | 100 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 100 | | 0 |
| | 0 | | 28 | | 0 | | 56 | | 0 | | 17 | | 46 | | 0 | | 53.7 | | 0 |
| | 0 | | 100 | | 0 | | 0 | | 0 | | 0 | | 0 | | 0 | | 100 | | 0 |
| | | | | | | | | | | | | | | | | | | | |
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| | | | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | | | |
| p-value | 0.21 | | **0.001** | | 0.44 | | 0.08 | | 0.36 | | 0.23 | | 0.05 | | 0.71 | | 0.03 | | 0.44 |

**Supplemental Table S7:**

**Effects of oxidative stress agents on wild-type (N2) and *pcm-1* mutant *C. elegans* development.**

The number of animals tested and the percentages of larval forms ± the standard deviation from four to five replicate experiments are shown for the summary data presented in Figures 3, 4, and 5.

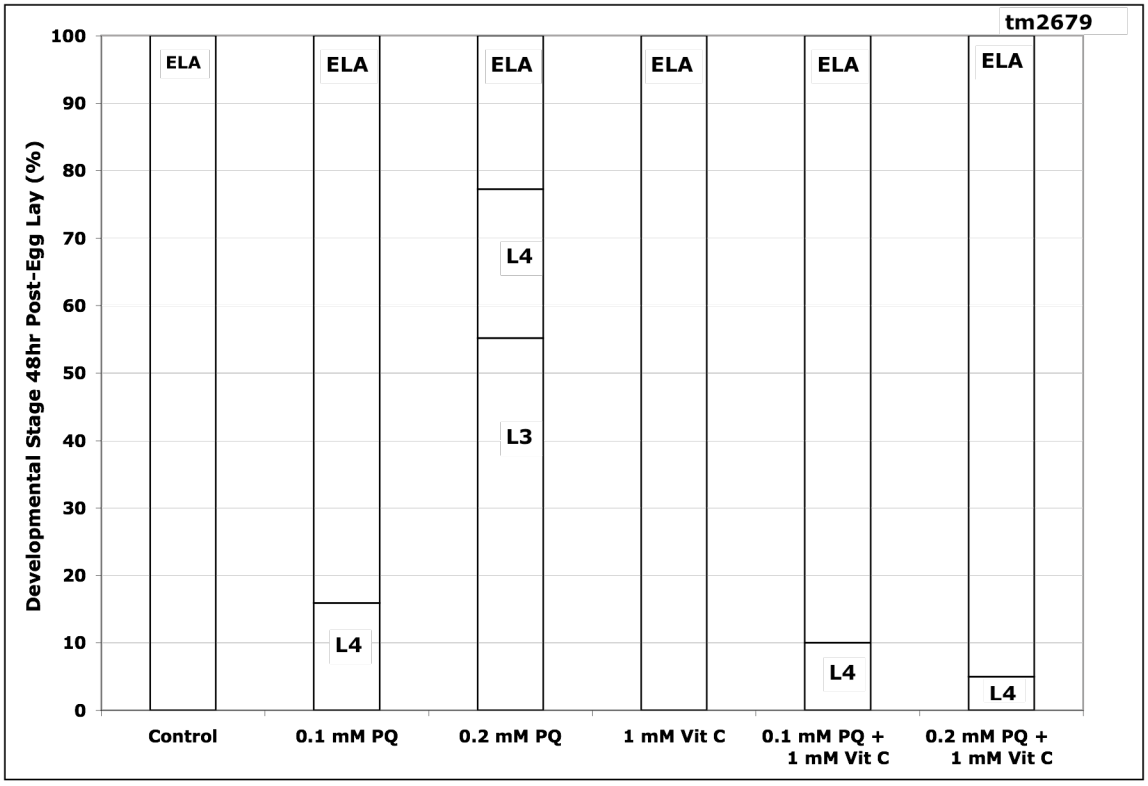
| **Paraquat Treatment for Wild-Type (N2) and *pcm-1* mutant *C. elegans*** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Strain** | **Condition** | **n** | **%L1** | **%L2** | **%L3** | **%L4** | **%YA/ELA** |
| N2 | control | 523±60 | 0 | 0.8±1.7 | 7.2±11.9 | 3.3±4.7 | 88.5±16.3 |
| N2 | 0.1 mM PQ | 206±35 | 0 | 2±3 | 17.5±15 | 15.9±19 | 64±37 |
| N2 | 0.2 mM PQ | 412±82 | 6.5±8.7 | 4.5±3.1 | 23±21 | 15±26 | 51.8±34 |
| N2 | 1 mM Vit C | 491±95 | 0 | 0 | 0 | 0±4.5 | 100±4.5 |
| N2 | 0.1 mM PQ + 1 mM Vit C | 290±92 | 0 | 0 | 0 | 9±5.2 | 91±5.2 |
| N2 | 0.2 mM PQ + 1 mM Vit C | 243±103 | 0 | 0 | 0 | 5±15 | 95±14.6 |
| *pcm-1* mutants | control | 1233±169 | 0.25±0.7 | 1.8±2.5 | 2.1±3 | 4.3±3.6 | 92±6.6 |
| *pcm-1* mutants | 0.1 mM PQ | 226±28 | 4.7±9.1 | 8.3±8.8 | 12.2±17 | 19±15 | 56±34 |
| *pcm-1* mutants | 0.2 mM PQ | 618±118 | 9.4±11.2 | 30±25 | 18±22 | 11±28 | 33±39 |
| *pcm-1* mutants | 1 mM Vit C | 857±198 | 0 | 0 | 0 | 0.4±1.1 | 99.6±1.1 |
| *pcm-1* mutants | 0.1 mM PQ + 1 mM Vit C | 491±204 | 0 | 0 | 0 | 7.2±8 | 92.8±7.9 |
| *pcm-1* mutants | 0.2 mM PQ + 1 mM Vit C | 863±208 | 0 | 0 | 0.9±2.3 | 14±27 | 84.9±26 |
| **Homocysteine Treatment for Wild-Type (N2) and *pcm-1* mutant *C. elegans*** | | | | | | | |
| **Strain** | **Condition** | **n** | **%L1** | **%L2** | **%L3** | **%L4** | **%YA/ELA** |
| N2 | control | 503±59 | 0 | 0.6±1.3 | 2.2±2.4 | 7.6±7.8 | 90±9.6 |
| N2 | 10 mM Hcy | 210±27 | 1.6±3.6 | 10.4±6.1 | 14.6±10.4 | 12.2±15 | 61±18 |
| N2 | 20 mM Hcy | 117±10 | 6.2±8.8 | 50.2±32 | 15.8±15 | 6±5.5 | 22±26 |
| N2 | 10 mM HCTL | 395±45 | 6.6±9 | 19.4±25 | 26±17 | 6.6±10 | 42±37 |
| N2 | 20 mM HCTL | 201±18 | 17±27 | 50±34 | 10±7.5 | 4.2±9 | 19±26 |
| N2 | 10 mM Hcy-Hcy | 338±69 | 0 | 0.6±1.4 | 5.1±7.2 | 15±15 | 79±18 |
| N2 | 20 mM Hcy-Hcy | 245±27 | 0 | 1.4±3.3 | 3±4.6 | 6.6±5.8 | 89.4±11 |
| *pcm-1* mutants | control | 656±118 | 0 | 0 | 3.2±5 | 20±20 | 77±19 |
| *pcm-1* mutants | 10 mM Hcy | 323±13 | 5.1±8 | 7.8±6.7 | 11.8±12 | 20±14 | 55±25 |
| *pcm-1* mutants | 20 mM Hcy | 153±15 | 41±34 | 18±18 | 13±18 | 5±7 | 24±25 |
| *pcm-1* mutants | 10 mM HCTL | 376±33 | 26±34 | 26±21 | 12±15 | 8±10 | 27±24 |
| *pcm-1* mutants | 20 mM HCTL | 351±34 | 46±44 | 27±27 | 12±14 | 2±7 | 13±19 |
| *pcm-1* mutants | 10 mM Hcy-Hcy | 278±16 | 1.5±3 | 1.5±3.2 | 7±8.5 | 16±18 | 74±21 |
| *pcm-1* mutants | 20 mM Hcy-Hcy | 194±17 | 0 | 0 | 6±13 | 19±29 | 75±28 |
| **Vitamin C Prevents The Developmental Delay Induced by Homocysteine Thiolactone in Wild-Type (N2) and *pcm-1* mutant *C. elegans*** | | | | | | | |
| **Strain** | **Condition** | **n** | **%L1** | **%L2** | **%L3** | **%L4** | **%YA/ELA** |
| N2 | control | 523±60 | 0 | 0.66±1.5 | 1.4±1.9 | 1.1±1.6 | 97±4.6 |
| N2 | 1 mM Vit C | 491±95 | 0 | 0 | 0 | 2.25±4.5 | 98±4.5 |
| N2 | 10 mM HCTL | 330±42 | 0 | 8.8±10.2 | 56±18 | 30±22 | 6.25±7 |
| N2 | 10 mM HCTL + 1 mM Vit C | 376±101 | 0 | 0 | 0.3±0.6 | 0 | 99.7±0.6 |
| N2 | 20 mM HCTL | 550±57 | 0 | 39±30 | 39±20 | 15±15 | 7±11 |
| N2 | 20 mM HCTL + 1 mM Vit C | 537±68 | 0 | 0 | 1.5±2.4 | 9.6±8 | 89±10 |
| *pcm-1* mutants | control | 1209±174 | 0.2±0.6 | 0.5±1.5 | 0.6±1.3 | 4.4±3.8 | 94±4.5 |
| *pcm-1* mutants | 1 mM Vit C | 857±198 | 0 | 0 | 0 | 0.4±1.1 | 99.6±1.1 |
| *pcm-1* mutants | 10 mM HCTL | 441±131 | 0 | 30±32 | 39±16 | 26±23 | 5±9 |
| *pcm-1* mutants | 10 mM HCTL + 1 mM Vit C | 713±194 | 0 | 0 | 0 | 4.6±7 | 95.4±7 |
| *pcm-1* mutants | 20 mM HCTL | 845±205 | 34±38 | 39±26 | 15±13 | 4±5 | 13±13 |
| *pcm-1* mutants | 20 mM HCTL + 1 mM Vit C | 1098±180 | 0 | 0.14±0.4 | 7.6±14 | 13±22 | 79±36 |

## Supplemental Figures

### Supplemental Figure S1:

### Paraquat delays development of *C10F3.4* (*tm2679)* mutant *C. elegans*.

Eggs were transferred to each of two NGM+OP50 plates containing 0, 0.1 mM and 0.2 mM paraquat (PQ) in the presence and absence of 1 mM vitamin C at 25 °C and were then scored for larval development after 48 h as previously mentioned in Figure 3. Data from one replicate experiment are shown. A total of 198 wild-type (data not shown) and 200 *tm2679* mutant control animals were analyzed. For the paraquat experiments, 44 and 37 wild-type animals and 32 and 109 *tm2679* mutant animals were analyzed following treatment with 0.1 mM paraquat and 0.2 mM paraquat, respectively. For the paraquat plus vitamin C experiments, 200 wild-type animals and 200 *pcm-1* mutant animals were analyzed following treatment with 0.1 mM paraquat + 1 mM vitamin C and 0.2 mM paraquat + 1 mM vitamin C each. The total number of animals analyzed following treatment with 1 mM vitamin C was 235 (wild-type) and 200 (*pcm-1* mutant). No significant statistical difference was observed between the wild-type and *tm2679* mutant nematodes following treatment with paraquat or paraquat and vitamin C as determined by the two-tailed Student's T-test of unequal variance. Standard deviations along with numerical values for each developmental stage are provided in Supplemental Table 4.

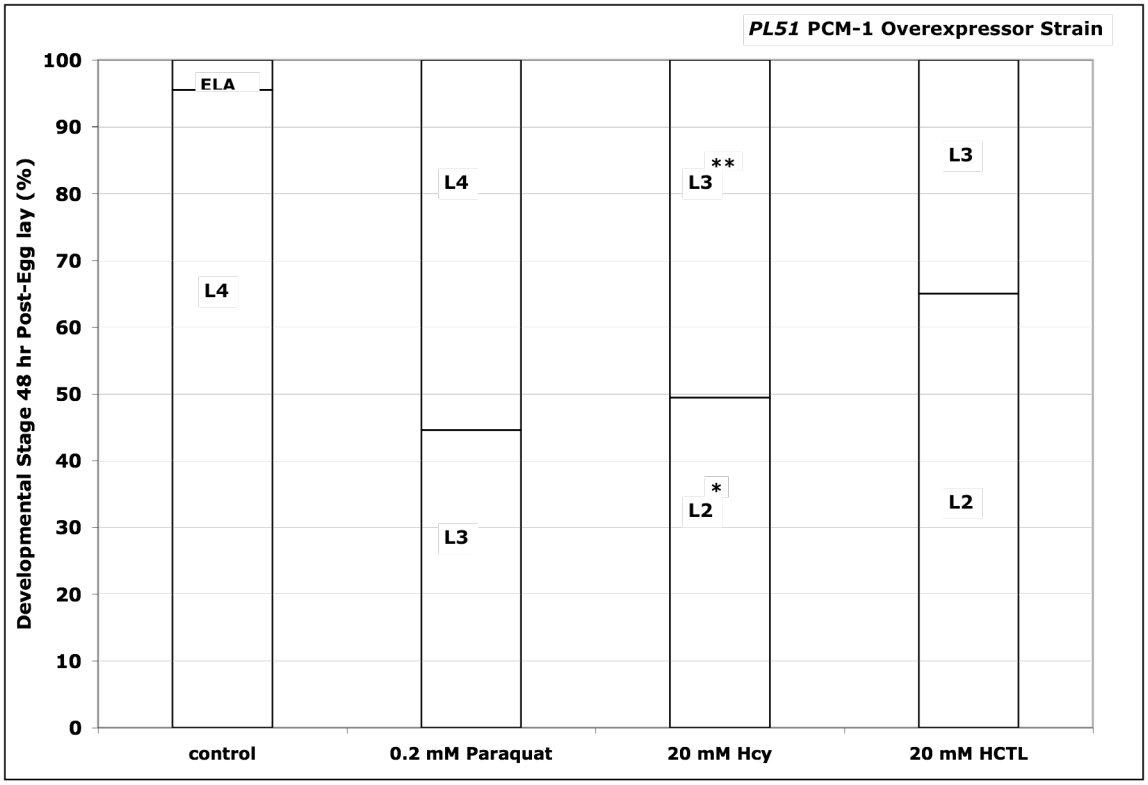


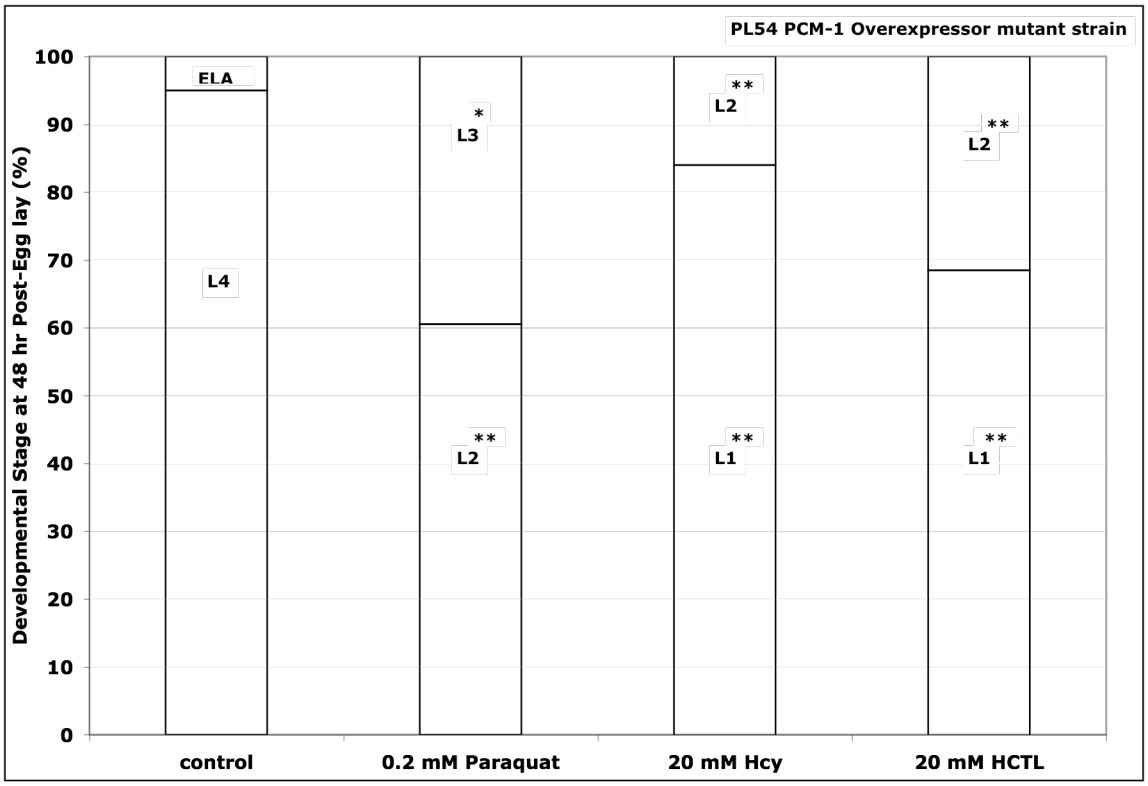
**Supplemental Figure S2:**

***PL51* (PCM-1 overexpressing) and *PL54* (mutant PCM-1 overexpressing) transgenic strains treated with oxidative stress agents show developmental delays similar to wild-type (N2) and *pcm-1* mutant animals, respectively.**

Upper panel. *PL51* PCM-1 overexpressor strain shows a developmental delay similar to wild-type (N2) nematodes. Eggs were transferred to each of two NGM+OP50 plates containing 0, 0.2 mM paraquat, 20 mM homocysteine (Hcy), and 20 mM HCTL at 25 °C and were then scored for larval development after 48 h as previously mentioned in Figure 3 and Figure 4. Data averaged from three replicate experiments are shown. A total of 3200 wild-type and 152 GFP-positive *PL51* transgenic control animals were analyzed. For the 0.2 mM paraquat experiment, 834 wild-type animals and 162 GFP-positive *PL51* transgenic animals were analyzed. 1000 and 974 wild-type animals and 167 and 130 GFP-positive *PL51* transgenic animals were examined following treatment with 20 mM Hcy and 20 mM HCTL, respectively. Statistically significant differences in developmental delay compared to the wild-type animals treated under the same conditions are noted (two-tailed Student's T-test of unequal variance: *$p<0.05$, **$p<0.0005$). Standard deviations along with numerical values for each developmental stage are provided in Supplemental Table 5.

Lower panel. *PL54* mutant PCM-1 overexpressor strain shows a developmental delay similar to *pcm-1* mutant nematodes. Eggs were transferred to each of two NGM+OP50 plates containing 0, 0.2 mM paraquat, 20 mM homocysteine (Hcy), and 20 mM HCTL at 25 °C and were then scored for larval development after 48 h as previously mentioned in Figure 2 and Figure 3. Data averaged from three replicate experiments are shown. The total amounts of wild-type nematodes treated are mentioned above. A total of 97 GFP-positive *PL54* transgenic control animals were analyzed. For the 0.2 mM paraquat experiment, 126 GFP-positive *PL54* transgenic animals were analyzed. 82 and 135 GFP-positive *PL54* transgenic animals were examined following treatment with 20 mM Hcy and 20 mM HCTL, respectively. Statistically significant differences in developmental delay compared to the wild-type animals (data not shown) treated under the same conditions are noted (two-tailed Student's T-test of unequal variance: *$p<0.05$, **$p<0.0005$). Standard deviations along with numerical values for each developmental stage are provided in Supplemental Table 5.


*PL51* PCM-1 Overexpressor Strain
Developmental Stage 48 hr Post-Egg lay (%)
100
90
80
70
60
50
40
30
20
10
0
ELA
L4
L4
L3
**
L3
*
L2
L3
L2
control
0.2 mM Paraquat
20 mM Hcy
20 mM HCTL


PL54 PCM-1 Overexpressor mutant strain
Developmental Stage at 48 hr Post-Egg lay (%)
100
90
80
70
60
50
40
30
20
10
0
ELA
L4
*
L3
**
L2
**
L2
**
L2
**
L1
**
L1
control
0.2 mM Paraquat
20 mM Hcy
20 mM HCTL