

Cloning

| | | |
|--------------|---------|---|
| <i>msh-2</i> | forward | 5'-GAAGATCTATGAGTGGAGGAAAAGACGAAGCCA-3' |
| | reverse | 5'-ATGCGGCCGCTTATTTGACAAGGCTGAGAATGGCT-3' |

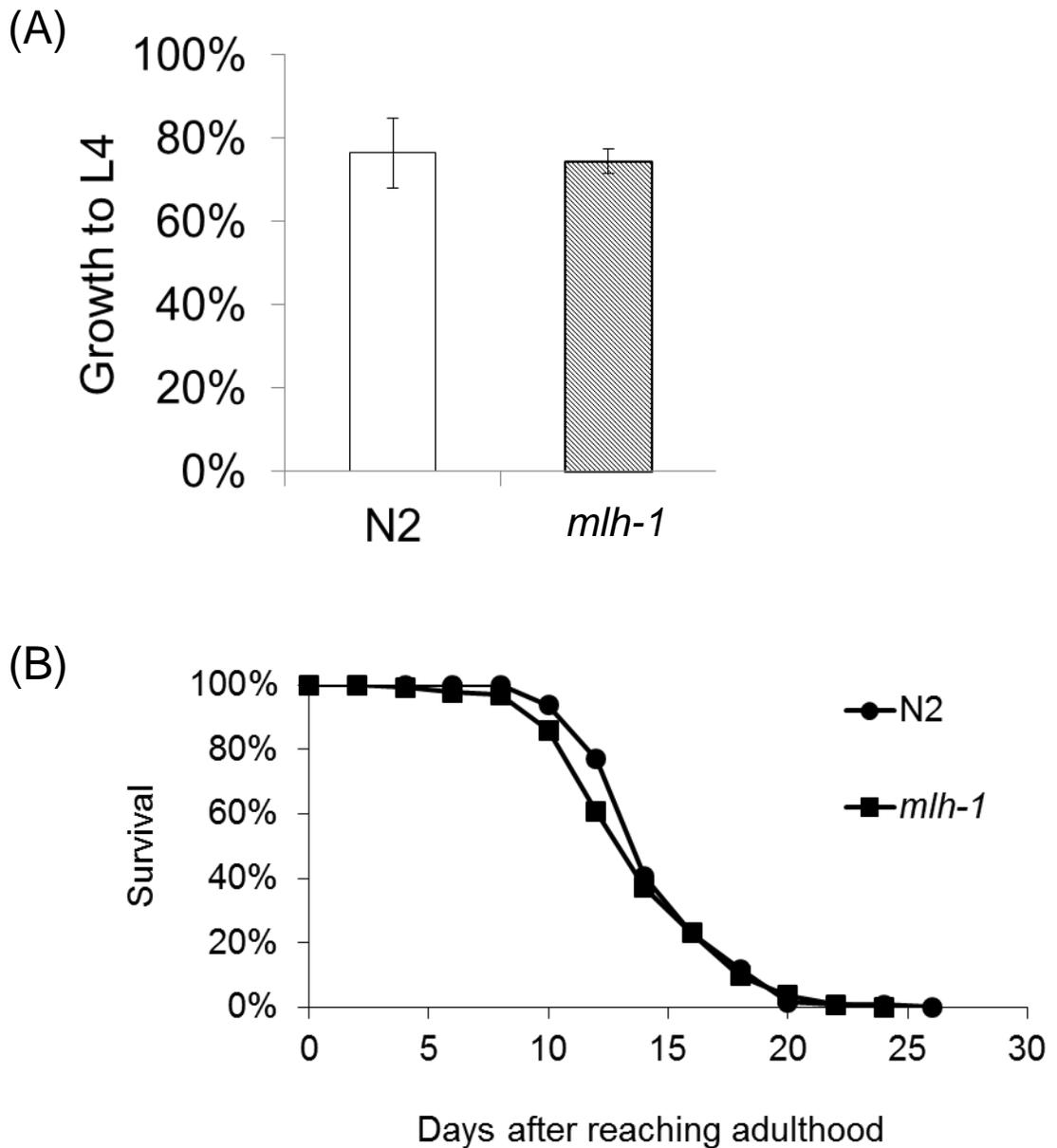
Verification of deletion

| | | |
|-------------------------|---------|-----------------------------------|
| <i>mlh-1</i> (External) | Forward | 5'-CACATCGCTCGAAGACTTTGTACAA-3' |
| | Reverse | 5'-CTGGTGGCCTACCCACCAAACCTCAT-3' |
| <i>mlh-1</i> (Internal) | Forward | 5'-GTCCGCCATTCTCACTAAATCTCGA-3' |
| | Reverse | 5'-CTGGTGGCCTACCCACCAAACCTCAT -3' |
| <i>atm-1</i> (External) | Forward | 5'-CGGAAAAACGATGTACCGATGGCCA-3' |
| | Reverse | 5'-AGGGACCTGCGTCTCTCTTCGCCAC-3' |
| <i>atm-1</i> (Internal) | Forward | 5'-CGGAAAAACGATGTACCGATGGCCA-3' |
| | Reverse | 5'-CCGAAGCATCGCCTTCTCCAACATA-3' |
| <i>msh-6</i> (External) | Forward | 5'-TTATCGAAAGACCGGAAACCAAAA-3' |
| | Reverse | 5'-GAAGCCCTTCGACGAGATTTTCAGC-3' |
| <i>msh-6</i> (Internal) | Forward | 5'-CCGACCCCTTGATCGAAACGG-3' |
| | Reverse | 5'-CTGGTGGCCTACCCACCAAACCTCAT -3' |

Semi-quantitative PCR

| | | |
|---------------|---------|-----------------------------------|
| <i>ama-1</i> | forward | 5'-TTCCAAGCGCCGCTGCGCATTGTCTC-3' |
| | reverse | 5'-CAGAATTTCCAGCACTCGAGGAGCGGA-3' |
| <i>tbg-1</i> | forward | 5'-TGATGACTGTCCACGTTGGA-3' |
| | reverse | 5'-CGTCATCAGCCTGGTAGAACA-3' |
| <i>msh-2</i> | forward | 5'-GAAGAAAAGTCGATCCGCAA-3' |
| | reverse | 5'-CAAGAGGAAGAAGATTCGGTC-3' |
| <i>egl-1</i> | forward | 5'-ATGCTGATGCTCACCTTTGCC-3' |
| | reverse | 5'-GATGGAAGAGGCTTCTGTCCG-3' |
| <i>vps-34</i> | forward | 5'-CGGGATCCCAAACCAATGCC-3' |
| | reverse | 5'-GTGAATAATATCAGAAATCATAGCC-3' |

Supplementary Table. The primers used in this study. These primers were used for the cloning of *msh-2* for knockdown, Verification of deletion of mutants and semi-quantitative PCR.

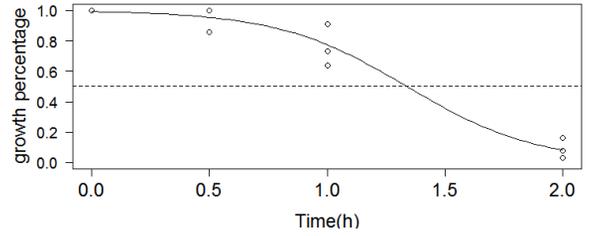
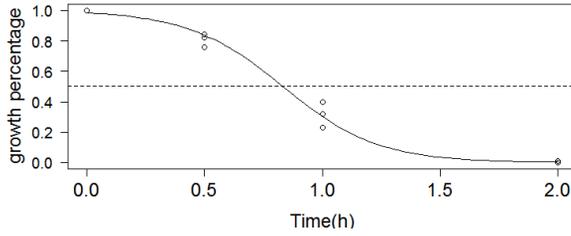
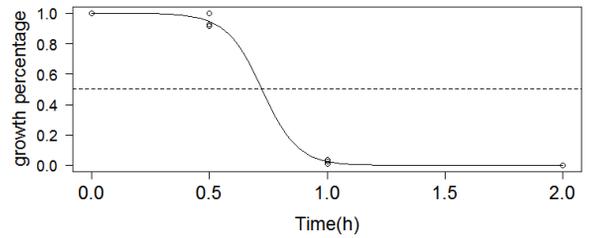
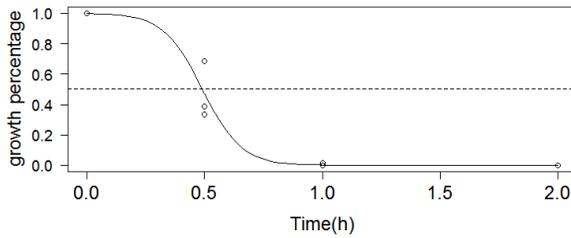


Supplementary Figure S2. Basic phenotypes of *mlh-1(ok1917)* worms. (A) Percent growth (L1 to adult). Data represent the mean \pm S.D. from three independent experiments. No significant difference was observed (p -value >0.05 by Student's t -test). (B) The lifespan survival curve. ● and ■ represent N2 ($n=126$) and backcrossed *mlh-1(ok1917)* ($n=135$), respectively. Data represent the results from three independent experiments. No significant difference was observed in mean or 50% lifespan (p -value >0.05 by Student's t -test).

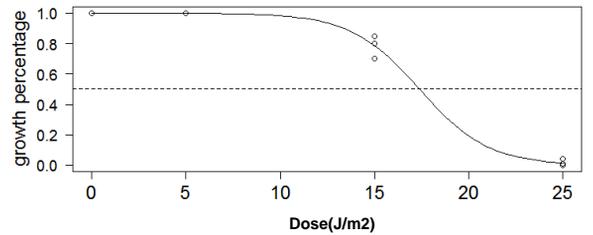
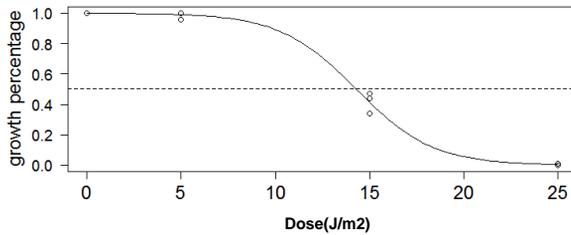
N2

mlh-1(ok1917)

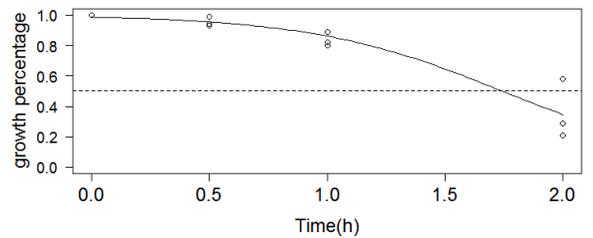
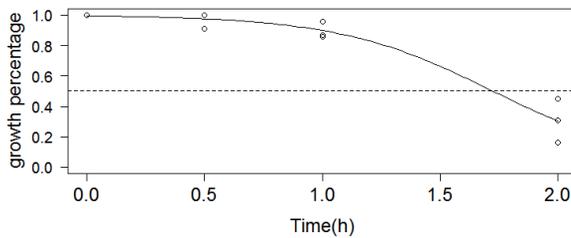
MNNG

NaHSO₃

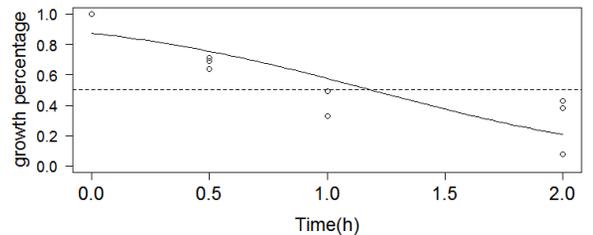
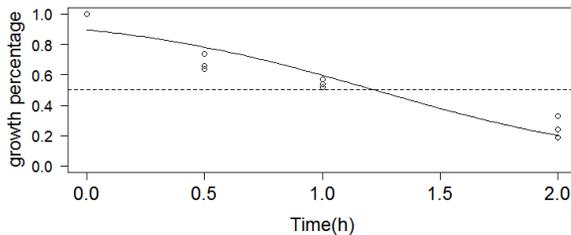
UVC



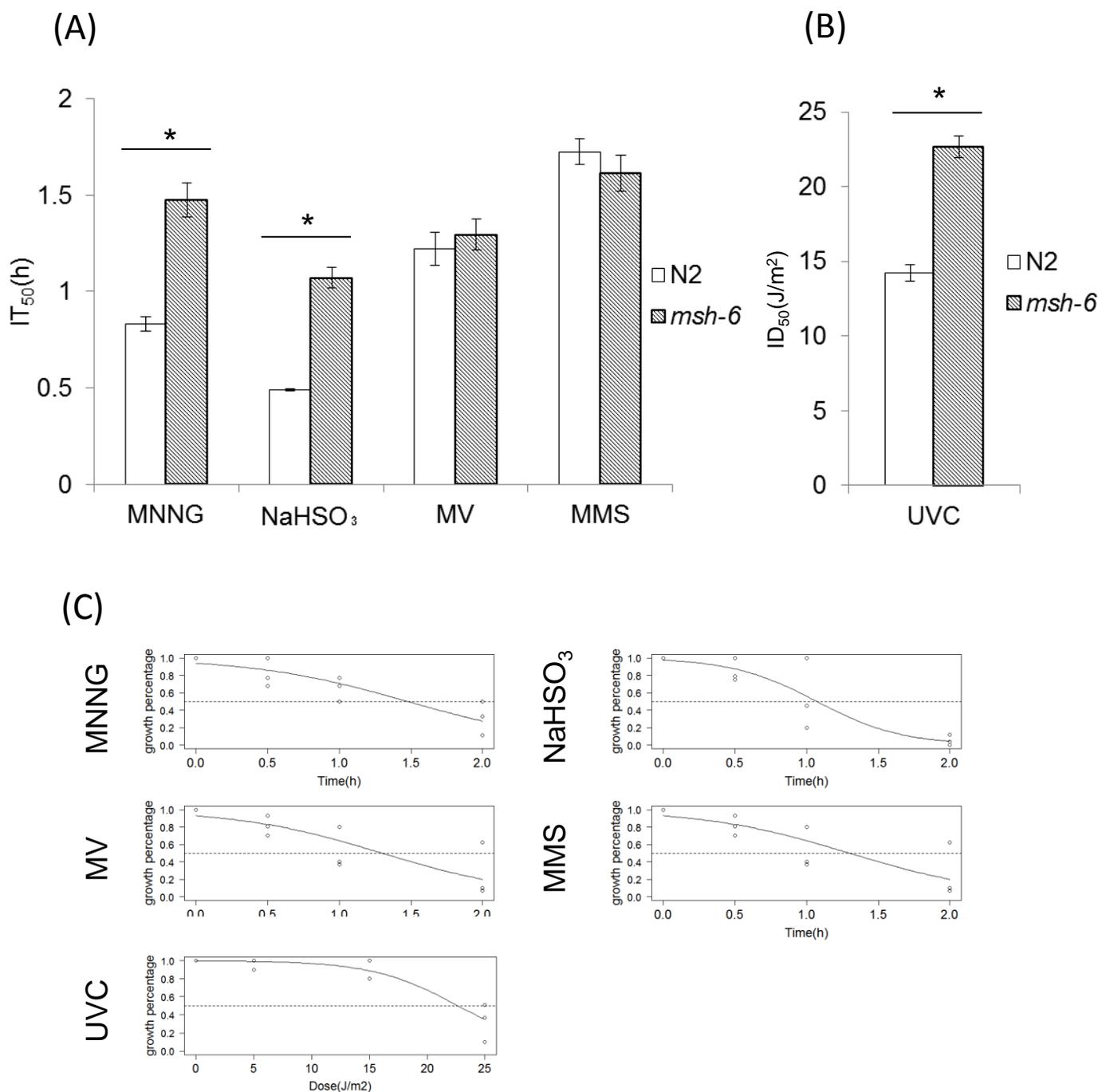
MMS



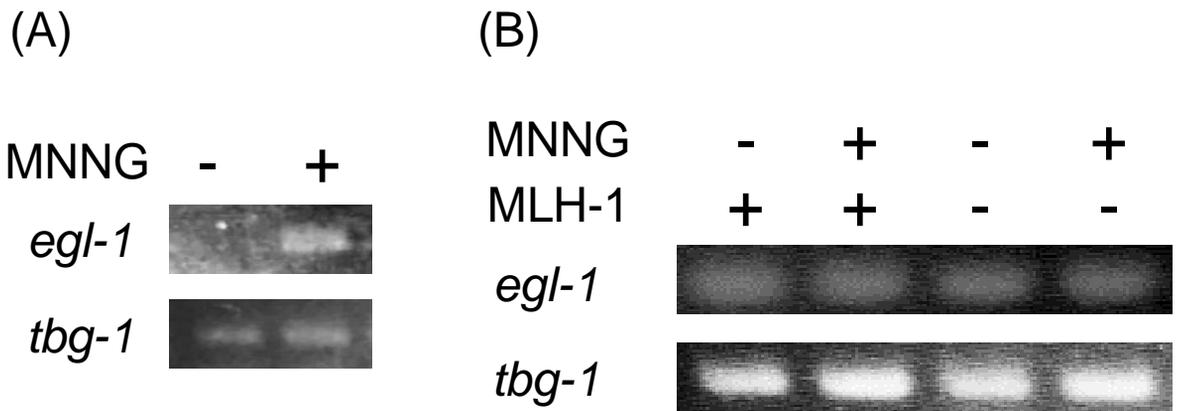
MV



Supplementary Figure S3. The logistic curve of L1 growth assays. The synchronized starved L1 larvae of N2 and *mlh-1(ok1917)* were subjected to time-course drug or irradiation treatments with 0.6 mM MNNG, 60 mM NaHSO₃, 40 mM MV, 0.05% MMS or UVC irradiation. After the treatments or irradiation, worms were cultured for 4 days on NGM plates at 20 °C. Then, the normalized percent growth (L1 to L4) was measured. The time- and dose-response curves were obtained with three-parameter logistic curves using R. The dotted lines represent 50% line.

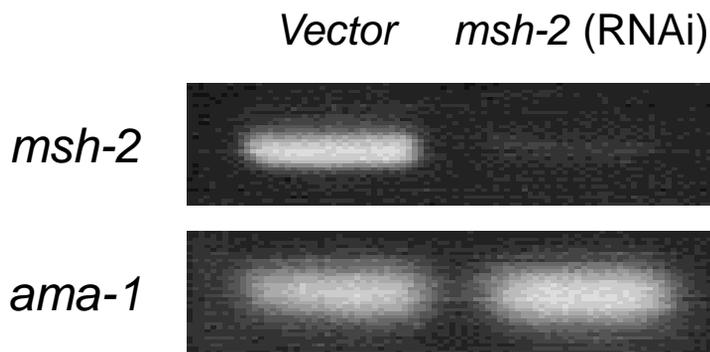


Supplementary Figure S4. L1 growth assay of *msh-6* (*pk2504*) worms. (A) The synchronized starved L1 larvae of N2 and *msh-6* (*pk2504*) were treated with (A) 0.6 mM MNNG, 60 mM NaHSO₃, 40 mM MV, or 0.05% MMS and (B) irradiated with UVC. After the treatments or irradiation, worms were cultured for 4 days on NGM plates at 20 °C. We then calculated IT_{50} or ID_{50} from the normalized percent growth (L1 to L4). Error bars show 95% confidence interval. * means p-value < 0.05 by Student's t-test. (C) The logistic curve of L1 growth assays. The time- and dose-response curves were obtained with three-parameter logistic curves using R. The dotted lines represent 50% line.



Supplementary Figure S5. The expression levels of *egl-1*. (A) MNNG treatment induces *egl-1* expression in N2 adult worms. The N2 adult worms were treated with 3.4 mM MNNG for 1 hour at 20°C. Then, total RNA was purified and reverse transcribed. The expression levels of *egl-1* were determined by semi-quantitative PCR. *tbg-1* was used as the loading control. (B) The adult worms of wild-type or *ok1917* germline deficient worms (*glp-4(bn2)*) were treated with 3.4 mM MNNG for 1 hour at 20°C. Then, total RNA was purified and reverse transcribed. The expression levels of *egl-1* were determined by semi-quantitative PCR. *tbg-1* was used as the loading control.

(A)



(B)

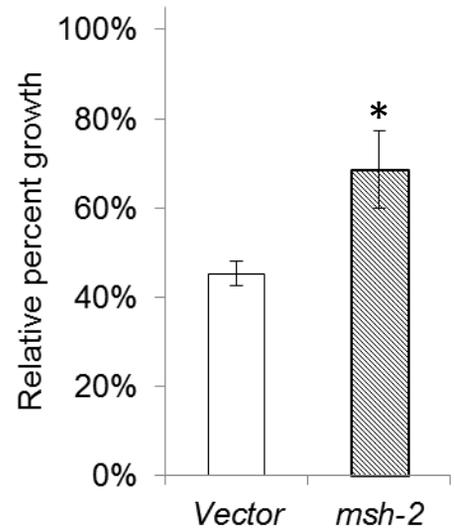


Figure S6. Confirmation of knockdown of the *msh-2*-gene by semi-quantitative PCR. (A) The knockdown analysis was performed using standard feeding methods. Worms were cultured on RNAi plates for 4 days from the egg stage, and total RNA was then prepared. The decrease of mRNA level was analyzed by semi-quantitative PCR. Considering the difference of rate of germ cells between vector and *msh-2* (RNAi), *ama-1* was used as the loading control. (B) *msh-2* knockdown induces the resistance to MNNG. The synchronized L1 larvae of vector or *msh-2* RNAi were treated with 0.6 mM MNNG for 1 hour at 20 °C. After the treatment, worms were cultured at 20 °C for 4 days. We then calculated the normalized percent growth (L1 to L4). The data represents the mean \pm S.D. from three independent experiments. * means p -value $<$ 0.05 by Student's t -test.

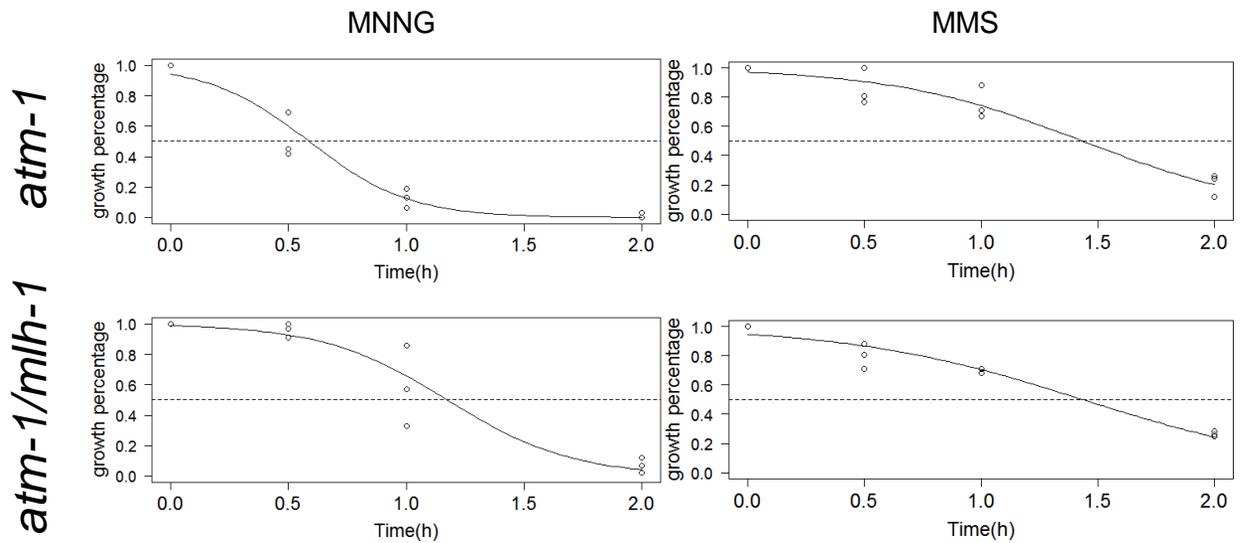


Figure S7. MNNG induces DSB in *C. elegans* dividing cells. The logistic curve of L1 growth assays of *atm-1(tm5027)* and *atm-1(tm5027)/mlh-1(ok1917)* worms. The synchronized starved L1 larvae were subjected to time-course drug treatment with 0.6 mM MNNG or 0.05% MMS. After the treatment, worms were cultured for 4 days on NGM plates at 20°C. Then, the normalized percent growth (L1 to L4) was measured. The time-response curve was obtained with a three-parameter logistic curve using R. The dotted lines represent 50% line.