SUPPLEMENTARY DATA

Genome-wide mutational signature of the chemotherapeutic agent mitomycin C in Caenorhabditis elegans

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Table S1: Three-factor mapping data of 69 mitomycin C-treated strains with lethal mutations mapped to chromosomes I or III. Summary of three-factor mapping data used in this study to determine map distance of the lethal mutations to the visible markers.

| Genotype (P₀ hermaphrodite) | Total | Recombinants (Dpy-Unc) | Recombinants (Dpy or Unc) | Lethal Mutation (Distance in map units) |
|-----------------------------|-------|------------------------|---------------------------|----------------------------------------|
| h2715 dpy-5 unc-13 / +++    | 1113  | 12                     | 15 Dpy                    | +3.7 (2.7-4.6)                         |
| h2717 dpy-5 unc-13 / +++    | 1669  | 139                    | 17 Unc                    | -13.2 (11.3-15.2)                      |
| h2718 dpy-5 unc-13 / +++    | 1512  | 61                     | 9 Unc                     | -6.1 (4.1-8.1)                         |
| h2720 dpy-5 unc-13 / +++    | 1742  | 356                    | 18 Dpy 9 Unc              | Chrome III                            |
| h2721 dpy-5 unc-13 / +++    | 1885  | 380                    | 10 Dpy 7 Unc              | Chrome III                            |
| h2722 dpy-5 unc-13 / +++    | 1754  | 352                    | 20 Dpy 14 Unc             | Chrome III                            |
| h2723 dpy-5 unc-13 / +++    | 1517  | 324                    | 18 Dpy 17 Unc             | Chrome III                            |
| h2724 dpy-5 unc-13 / +++    | 2579  | 293                    | 3 Dpy 11 Unc              | Chrome III                            |
| h2726 dpy-5 unc-13 / +++    | 1931  | 4                      | 10 Dpy                    | +2.4 (1.9-2.8)                         |
| h2727 dpy-5 unc-13 / +++    | 2147  | 167                    | 27 Unc                    | -12.6 (11.1-14.2)                      |
| h2728 dpy-5 unc-13 / +++    | 1489  | 21                     | 12 Dpy                    | +4.5 (3.2-5.8)                         |
| h2729 dpy-5 unc-13 / +++    | 1581  | 4                      | 10 Dpy                    | +2.5 (2.1-2.9)                         |
| h2730 dpy-5 unc-13 / +++    | 1418  | 235                    | 11 Dpy 1 Unc              | Chrome III                            |
| h2731 dpy-5 unc-13 / +++    | 924   | 193                    | 7 Dpy 0 Unc               | Chrome III                            |
| h2732 dpy-5 unc-13 / +++    | 2053  | 27                     | 23 Dpy                    | +4.2 (3.2-5.2)                         |
| h2733 dpy-5 unc-13 / +++    | 1277  | 92                     | 3 Unc                     | -11.6 (8.9-14.3)                       |
| h2734 dpy-5 unc-13 / +++    | 2304  | 463                    | 20 Dpy 16 Unc             | Chrome III                            |
| h2736 dpy-5 unc-13 / +++    | 1779  | 392                    | 20 Dpy 18 Unc             | Chrome III                            |
| h2737 dpy-5 unc-13 / +++    | 2201  | 452                    | 16 Dpy 17 Unc             | Chrome III                            |
| h2738 dpy-5 unc-13 / +++    | 2082  | 47                     | 9 Dpy                     | +5.5 (4.5-6.5)                         |
| h2739 dpy-5 unc-13 / +++    | 1704  | 212                    | 19 Dpy 15 Unc             | Chrome III                            |
| h2741 dpy-5 unc-13 / +++    | 2194  | 207                    | 20 Dpy 24 Unc             | Chrome III                            |
| h2744 dpy-5 unc-13 / +++    | 2545  | 262                    | 21 Unc                    | -16.9 (15.0-18.8)                      |
| h2745 dpy-5 unc-13 / +++    | 1515  | 144                    | 3 Dpy 17 Unc              | Chrome III                            |
| h2746 dpy-5 unc-13 / +++    | 2017  | 407                    | 14 Dpy 11 Unc             | Chrome III                            |
| h2747 dpy-5 unc-13 / +++    | 2526  | 567                    | 11 Dpy 22 Unc             | Chrome III                            |
| h2748 dpy-5 unc-13 / +++    | 1529  | 343                    | 8 Dpy 10 Unc              | Chrome III                            |
| h2749 dpy-5 unc-13 / +++    | 2199  | 471                    | 20 Dpy 19 Unc             | Chrome III                            |
| h2750 dpy-5 unc-13 / +++    | 2108  | 448                    | 12 Dpy 12 Unc             | Chrome III                            |
| h2751 dpy-5 unc-13 / +++    | 1828  | 364                    | 13 Dpy 8 Unc              | Chrome III                            |
| h2752 dpy-5 unc-13 / +++    | 2160  | 427                    | 12 Dpy 8 Unc              | Chrome III                            |
| h2753 dpy-5 unc-13 / +++    | 1578  | 339                    | 13 Dpy 9 Unc              | Chrome III                            |
| h2754 dpy-5 unc-13 / +++    | 1649  | 8                      | 12 Dpy                    | +2.8 (2.3-3.3)                         |
| h2755 dpy-5 unc-13 / +++    | 1951  | 0                      | 9 Dpy 11 Unc              | Not applicable^a                        |
| h2756 dpy-5 unc-13 / +++    | 1906  | 227                    | 7 Dpy 13 Unc              | Chrome III                            |
| h2758 dpy-5 unc-13 / +++    | 1978  | 153                    | 11 Unc                    | -12.1 (10.0-14.2)                      |
| h2759 dpy-5 unc-13 / +++    | 1961  | 354                    | 13 Dpy 7 Unc              | Chrome III                            |

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Table S1: Three-factor mapping data of 69 mitomycin C-treated strains with lethal mutations mapped to chromosomes I or III. Summary of three-factor mapping data used in this study to determine map distance of the lethal mutations to the visible markers.

| Genotype (P₀, hermaphrodite) | Total  | Recombinants (Dpy-Unc) | Recombinants (Dpy or Unc) | Lethal Mutation (Distance in map units) | Chromosome |
|------------------------------|--------|------------------------|---------------------------|----------------------------------------|------------|
| h2760 dpy-5 unc-13 / +++     | 2319   | 414                    | 15 Dpy 10 Unc             |                                        | III        |
| h2761 dpy-5 unc-13 / +++     | 2039   | 393                    | 13 Dpy 9 Unc              |                                        | III        |
| h2762 dpy-5 unc-13 / +++     | 1415   | 288                    | 9 Dpy 12 Unc              |                                        | III        |
| h2763 dpy-5 unc-13 / +++     | 1926   | 428                    | 9 Dpy 10 Unc              |                                        | III        |
| h2764 dpy-5 unc-13 / +++     | 2174   | 452                    | 8 Dpy 13 Unc              |                                        | III        |
| h2766 dpy-5 unc-13 / +++     | 2153   | 471                    | 14 Dpy 14 Unc             |                                        | III        |
| h2767 dpy-5 unc-13 / +++     | 2061   | 413                    | 13 Dpy 9 Unc              |                                        | III        |
| h2768 dpy-5 unc-13 / +++     | 1908   | 389                    | 21 Dpy 8 Unc              |                                        | III        |
| h2769 dpy-5 unc-13 / +++     | 2190   | 454                    | 23 Dpy 6 Unc              |                                        | III        |
| h2770 dpy-5 unc-13 / +++     | 1906   | 423                    | 7 Dpy 3 Unc               |                                        | III        |
| h2772 dpy-5 unc-13 / +++     | 1876   | 1                      | 8 Unc                     | -0.067 (0.0-0.20)                      | III        |
| h2773 dpy-5 unc-13 / +++     | 2020   | 286                    | 20 Dpy 4 Unc              |                                        | III        |
| h2774 dpy-5 unc-13 / +++     | 2016   | 422                    | 14 Dpy 14 Unc             |                                        | III        |
| h2775 dpy-5 unc-13 / +++     | 1591   | 386                    | 8 Dpy 7 Unc               |                                        | III        |
| h2776 dpy-5 unc-13 / +++     | 1517   | 45                     | 9 Dpy                     | +6.4 (4.4-8.4)                         | III        |
| h2777 dpy-5 unc-13 / +++     | 2080   | 448                    | 10 Dpy 8 Unc              |                                        | III        |
| h2778 dpy-5 unc-13 / +++     | 2175   | 524                    | 9 Dpy 11 Unc              |                                        | III        |
| h2779 dpy-5 unc-13 / +++     | 1470   | 298                    | 8 Dpy 10 Unc              |                                        | III        |
| h2780 dpy-5 unc-13 / +++     | 1588   | 344                    | 8 Dpy 19 Unc              |                                        | III        |
| h2781 dpy-5 unc-13 / +++     | 1467   | 311                    | 9 Dpy 10 Unc              |                                        | III        |
| h2782 dpy-5 unc-13 / +++     | 1927   | 387                    | 17 Dpy 8 Unc              |                                        | III        |
| h2783 dpy-5 unc-13 / +++     | 2140   | 445                    | 19 Dpy 8 Unc              |                                        | III        |
| h2784 dpy-5 unc-13 / +++     | 2383   | 28                     | 11 Unc                    | -1.8 (1.3-2.4)                         | III        |
| h2785 dpy-5 unc-13 / +++     | 2276   | 29                     | 15 Unc                    | -1.8 (0.87-2.7)                        | III        |
| h2786 dpy-5 unc-13 / +++     | 1716   | 360                    | 15 Dpy 11 Unc             |                                        | III        |
| h2787 dpy-5 unc-13 / +++     | 1877   | 28                     | 24 Unc                    | -2.1 (0.44-3.7)                        | III        |
| h2791 dpy-5 unc-13 / +++     | 2201   | 465                    | 16 Dpy 10 Unc             |                                        | III        |
| h2792 dpy-5 unc-13 / +++     | 2272   | 499                    | 15 Dpy 14 Unc             |                                        | III        |
| h2793 dpy-5 unc-13 / +++     | 2678   | 45                     | 35 Dpy                    | +4.7 (3.8-5.5)                         | III        |
| h2795 dpy-5 unc-13 / +++     | 1806   | 279                    | 9 Dpy 16 Unc              |                                        | III        |
| h2796 dpy-5 unc-13 / +++     | 1990   | 94                     | 5 Dpy 4 Unc               |                                        | III        |
| h2798 dpy-5 unc-13 / +++     | 1123   | 6                      | 4 Dpy                     | +3.1 (2.2-3.9)                         | III        |

a Map units were determined by three-factor mapping. Listed map units are lethal mutations identified on chromosome I.  
b Putative lethal mutation mapped between the two visible markers.
Table S2: Heterozygosity determined by the ratio of reference:variant reads of *dpy-5* and *unc-13* markers

| Allele | Reference : Variant read ratio |
|--------|-------------------------------|
|        | *dpy-5* markers | *unc-13* marker |
|        | C>T :5,432,433 bp | C>A :5,432,448 bp | C>T :7,434,404 bp |
| h2717  | 50% : 50%         | 56% : 44%         | 35% : 65%         |
| h2718  | 58% : 42%         | 55% : 45%         | 61% : 39%         |
| h2727  | 45% : 55%         | 46% : 54%         | 44% : 56%         |
| h2733  | 59% : 41%         | 56% : 44%         | 58% : 42%         |
| h2744  | 50% : 50%         | 38% : 62%         | 38% : 62%         |
| h2755  | 53% : 47%         | 50% : 50%         | 25% : 75%         |
| h2758  | 48% : 52%         | 33% : 67%         | 44% : 56%         |
| h2784  | 43% : 57%         | 50% : 50%         | 41% : 59%         |
| h2785  | 49% : 51%         | 47% : 53%         | 63% : 37%         |
| h2787  | 38% : 62%         | 33% : 67%         | 50% : 50%         |
Table S3: 22 mitomycin C-induced deletions identified genome-wide in the unbalanced chromosomes using Pindel.

| Allele   | I  | II | III | IV | V  | X  | Total |
|----------|----|----|-----|----|----|----|-------|
| h2717    | 1  | 1  |     |    |    |    | 2     |
| h2718    | 3  | 2  | 1   |    |    |    | 6     |
| h2727    |    | 1  | 1   | 2  |    |    | 4     |
| h2733    | 1  |    |     | 3  |    |    | 4     |
| h2744    |    |    |     | 1  |    |    | 1     |
| h2755    |    |    |     | 2  |    |    | 2     |
| h2758    |    | 1  |     |    |    |    | 1     |
| h2784    |    |    |     |    |    |    | 0     |
| h2785    |    |    |     |    |    |    | 0     |
| h2787    |    | 1  | 1   |    |    |    | 2     |
| Size     | 15.1 Mb | 15.3 Mb | 13.8 Mb | 17.5 Mb | 20.9 Mb | 17.7 Mb | **100.3 Mb** |
Table S4: Total single nucleotide variants identified in untreated spontaneously mutating N2, EMS-treated, and MMC-treated strains.

| Base-difference | 10 spontaneously mutating N2 strains | 10 EMS-treated strains | 10 MMC-treated strains |
|-----------------|-------------------------------------|------------------------|------------------------|
| G:C to A:T      | 80                                  | 1700                   | 61                     |
| A:T to T:A      | 86                                  | 118                    | 57                     |
| G:C to T:A      | 110                                 | 39                     | 44                     |
| A:T to G:C      | 41                                  | 59                     | 40                     |
| A:T to C:G      | 44                                  | 32                     | 21                     |
| G:C to C:G      | 30                                  | 17                     | 22                     |
| Total           | 391                                 | 1965                   | 245                    |
Map position of the hT2 breakpoint was determined by McKim et al. (1993), which was used to identify the hT2 physical breakpoint at 11 Mbp. The physical location was inferred from the position of pes-2.2, the gene closest to the hT2 physical breakpoint.

Figure S1: Genetic map of mitomycin C-induced lethal mutations in the hT2-balanced region of chromosome I. The h alleles depicted were mapped to chromosome I using three-factor mapping. The numbers displayed beside each h allele represent the mapped genetic location, and the dotted lines represent 95% confidence intervals of three-factor mapping. The genes bli-3, dpy-5, unc-13, and unc-101 are shown as references. Map positions of lethal mutations are displayed as map units.
Figure S2: Determination of the hT2 breakpoint using Integrative Genomics Viewer. The hT2 breakpoint was determined by arranging segments of chromosome I and III into 10 Kb and 5 Kb bins, followed by a script that identified major drops in average coverage at the target region. (A) The state of the genome observed from the sequence. Breakpoints were identified on IGV. A, B, C, D represents chromosomal segments of DNA flanked by the breakpoints. (B) The order of chromosomal segments rearranged to fit the sequence. (C) Proposed structure of the original chromosome segments prior to gamma-irradiation, identified by lining up breakpoints. (D) The hT2 breakpoint on chromosome III visualized using IGV. Multicolored alignments indicate mismatched reads.