**Supporting Information**

# The sulfur mustard analog mechlorethamine (bis(2-chloroethyl)methylamine) modulates cell cycle progression via the DNA damage response in human lung epithelial A549 cells

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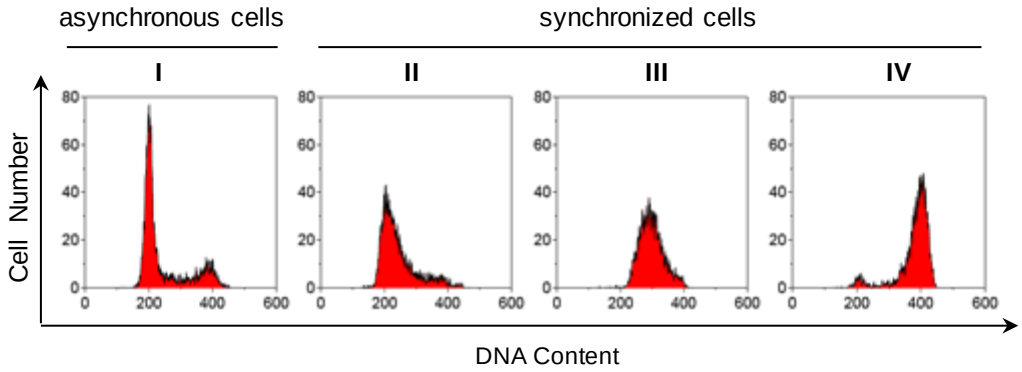
**Figure S1**

**A**




**B** Percentage of cells in the G0/G1 phase after HN2 treatment

| HN2 (μM) | asynchronous cells (I) | synchronized cells (II) | synchronized cells (III) | synchronized cells (IV) |
|---|---|---|---|---|
| 0 | 73.8 ± 2.4 | 73.3 ± 3.8 | 71.8 ± 2.2 | 74.8 ± 6.4 |
| 1 | 49.7 ± 4.6 | 50.0 ± 2.3 | 49.3 ± 3.6 | 49.2 ± 3.2 |
| 2 | 28.5 ± 3.5 | 25.1 ± 0.8 | 24.2 ± 1.2 | 24.5 ± 4.4 |
| 5 | 19.5 ± 5.5 | 14.8 ± 2.5 | 18.5 ± 2.2 | 25.4 ± 4.2 |
| 10 | 16.3 ± 1.9 | 14.2 ± 1.5 | 18.4 ± 1.2 | 19.3 ± 2.9 |
| 20 | 26.8 ± 1.5 | 17.5 ± 4.5 | 23.2 ± 2.4 | 28.8 ± 1.6 |

Percentage of cells in the S phase after HN2 treatment

| HN2 (μM) | asynchronous cells (I) | synchronized cells (II) | synchronized cells (III) | synchronized cells (IV) |
|---|---|---|---|---|
| 0 | 15.4 ± 2.1 | 17.6 ± 0.8 | 14.5 ± 1.2 | 13.8 ± 2.7 |
| 1 | 19.1 ± 2.7 | 13.7 ± 0.2 | 18.4 ± 2.1 | 16.8 ± 1.5 |
| 2 | 35.4 ± 5.5 | 36.7 ± 1.1 | 40.2 ± 4.1 | 38.5 ± 4.3 |
| 5 | 58.9 ± 4.1 | 54.6 ± 3.1 | 59.5 ± 3.6 | 52.6 ± 2.2 |
| 10 | 69.0 ± 3.2 | 64.5 ± 4.5 | 58.4 ± 3.5 | 62.1 ± 5.5 |
| 20 | 58.9 ± 1.7 | 67.4 ± 5.9 | 57.6 ± 2.0 | 52.9 ± 2.7 |

Percentage of cells in the G2/M phase after HN2 treatment

| HN2 (μM) | asynchronous cells (I) | synchronized cells (II) | synchronized cells (III) | synchronized cells (IV) |
|---|---|---|---|---|
| 0 | 9.2 ± 0.7 | 7.5 ± 0.8 | 12.4 ± 1.4 | 9.5 ± 1.8 |
| 1 | 29.9 ± 4.6 | 33.3 ± 4.3 | 30.3 ± 1.0 | 32.2 ± 2.3 |
| 2 | 32.8 ± 1.9 | 34.1 ± 0.6 | 34.2 ± 1.9 | 35.0 ± 1.1 |
| 5 | 18.9 ± 2.1 | 26.1 ± 2.6 | 20.5 ± 1.2 | 18.5 ± 2.0 |
| 10 | 12.4 ± 1.0 | 17.7 ± 0.5 | 20.4 ± 1.9 | 17.1 ± 3.1 |
| 20 | 12.2 ± 1.0 | 11.7 ± 1.9 | 17.3 ± 1.4 | 15.8 ± 4.0 |

**Figure S1.** Effects of HN2 on cell cycle distribution in asynchronous and synchronized A549 cells. Cells were cultured in DMEM growth medium (asynchronous cells) or synchronized at the G1/S boundary using a serum starvation (24 h)-thymidine (2 mM, 24 h) block. After the cell cycle block, cells were washed with HBSS and then cultured in complete growth medium for 0-12 h. Cells were then treated with increased concentrations of HN2 (1-20 μM) or vehicle controls. After 24 h, cell cycle profiles were analyzed by flow cytometry. (A) Representative cytograms of asynchronous (I) and synchronized cells after serum starvation/thymidine block release at 0 h (II), 3 h (III), and 8 h (IV). (B) Percentage of cells in each phase of cell cycle in asynchronous and synchronized A549 cells after HN2 treatment. Data are presented as means ± SE, n = 3. For each data set, a non-linear regression analysis comparing synchronized and asynchronous cells was performed and tested for statistical significance by chi-square test using SigmaPlot software. Cell cycle distribution in asynchronous and synchronous cells were not significantly different at $p < 0.05$.