Supplementary Information

Life-Cycle-Dependent Toxicities of Mono- and Bifunctional Alkylating Agents in the 3R-Compliant Model Organism *C. elegans*

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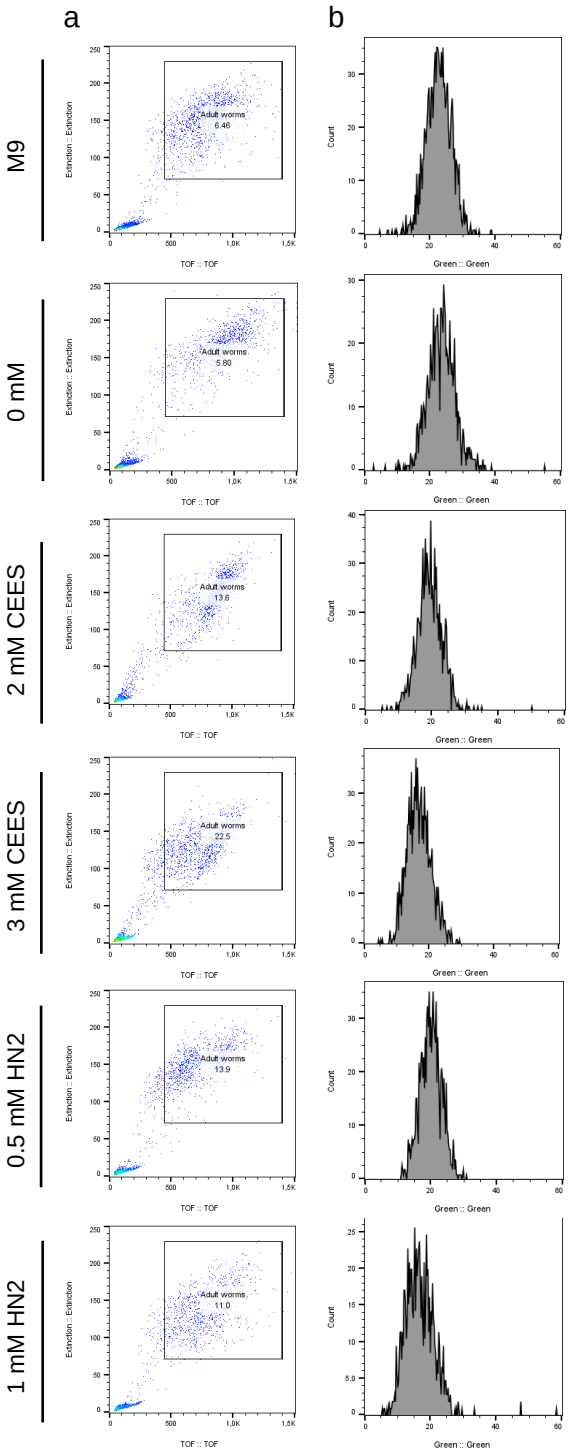

Figure S1: The FlowJo™ analysis of fluorescence signal in BY200 (*dat-1p::*GFP) worms following exposure to alkylating agents. (a) Adult worms were selected based on their size (time of flight; TOF) and optical density (extinction; EXT). (b) For the selected population the mean green fluorescence signal was acquired.

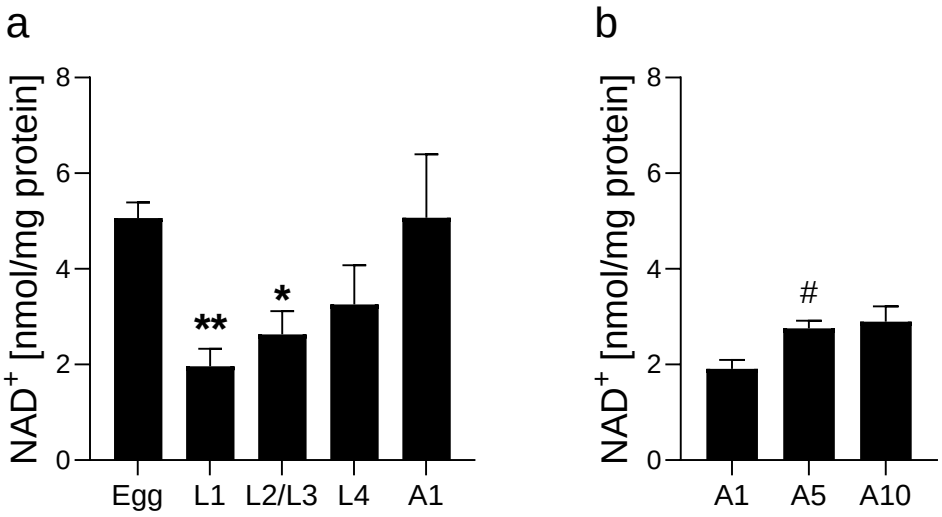

Figure S2: NAD^+ levels in *C. elegans* at different life cycle stages. (a) NAD^+ was extracted from eggs (n=3 biological replicates) or synchronised WT (N2) worms grown on NGM plates until the life cycle stages: L1 (n=6 biological replicates), L2/L3 (n=8 biological replicates), L4 (n=9 biological replicates), A1 (n=7 biological replicates). (b) NAD^+ was extracted from WT (N2) worms grown on NGM + FUdR plates from L4 until respective days of adulthood: day 1 (A1), day 5 (A5), or day 10 (A10) (n=3 biological replicates). NAD^+ was measured with cycling assay and normalised to the total protein levels measured with BCA assay. Results were expressed as mean ±SEM. For statistical analysis unpaired two-tailed t-tests were performed; *p<0.05, ** p<0.01 vs Egg; # p<0.05 vs A1.