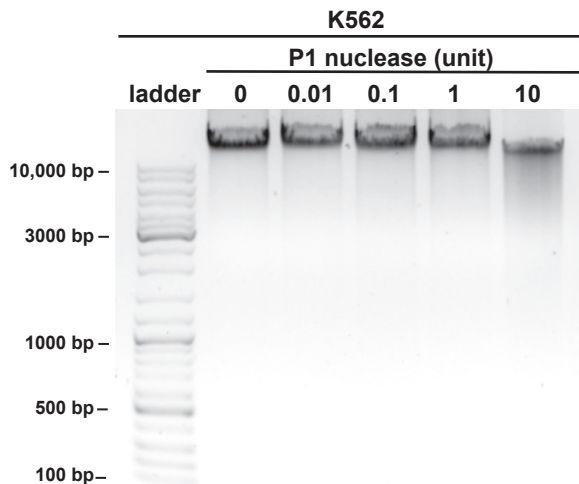
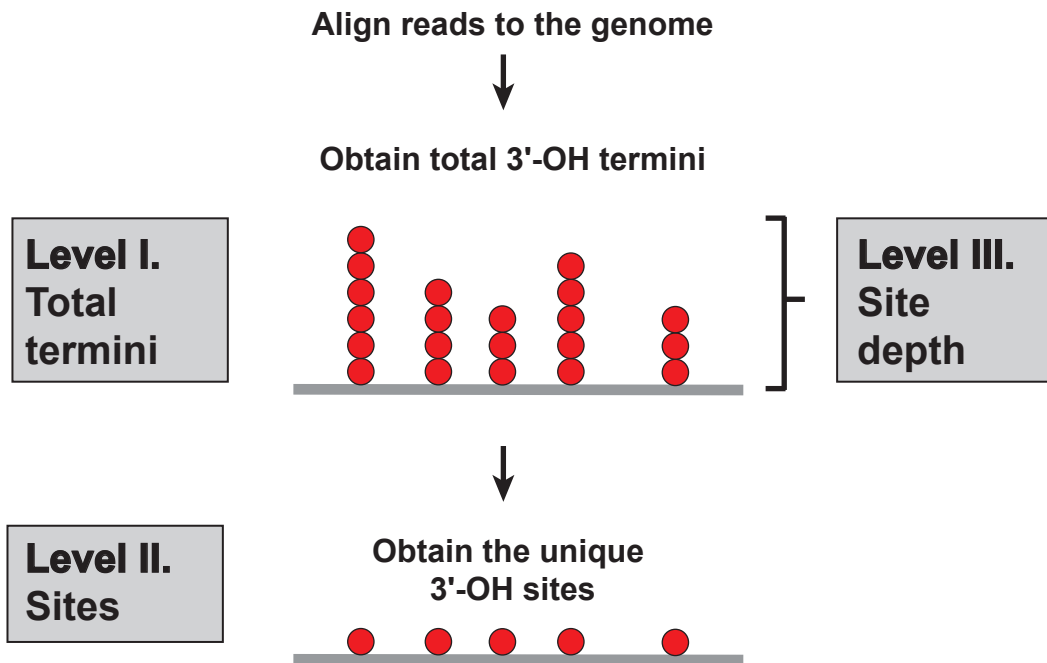


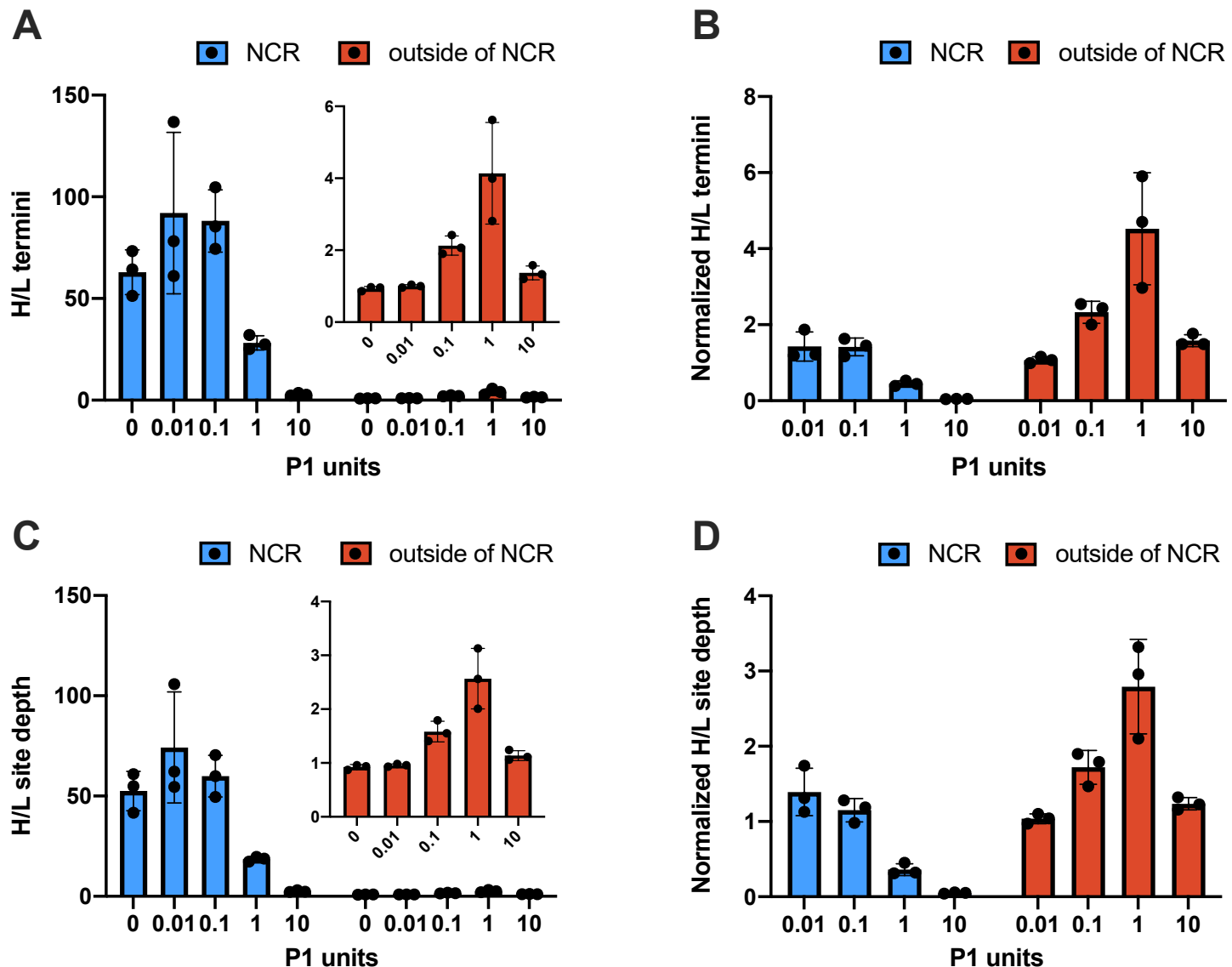
**Figure S1. Analytical pipeline used in this work for detection and analysis of the essDNA regions.**



**Figure S2. Agarose gel analysis of P1 digested DNA.** Genomic DNA isolated from K562 crosslinked nuclei digested with the indicated amounts of P1 is shown. For each treatment, three biological replicates were combined into a single lane.



**Figure S3. Analytical pipeline for calculation of total 3'-OH termini, 3'-OH sites with unique genomic coordinates and the depth of sites in the mitochondrial genome.**



**Figure S4. Analysis of the H vs. L strand distribution of essDNA signal using additional metrics.** (A, B) The H/L ratios (A) and H/L normalized ratios (B) calculated using total 3'-OH termini. (C, D) The H/L ratio (C) and H/L normalized ratio (D) using the depth of sites. The normalized ratios were obtained by dividing the H/L ratios of the P1 treated samples to the H/L ratio of the untreated control. The error bars indicate SD of three independent biological replicates.