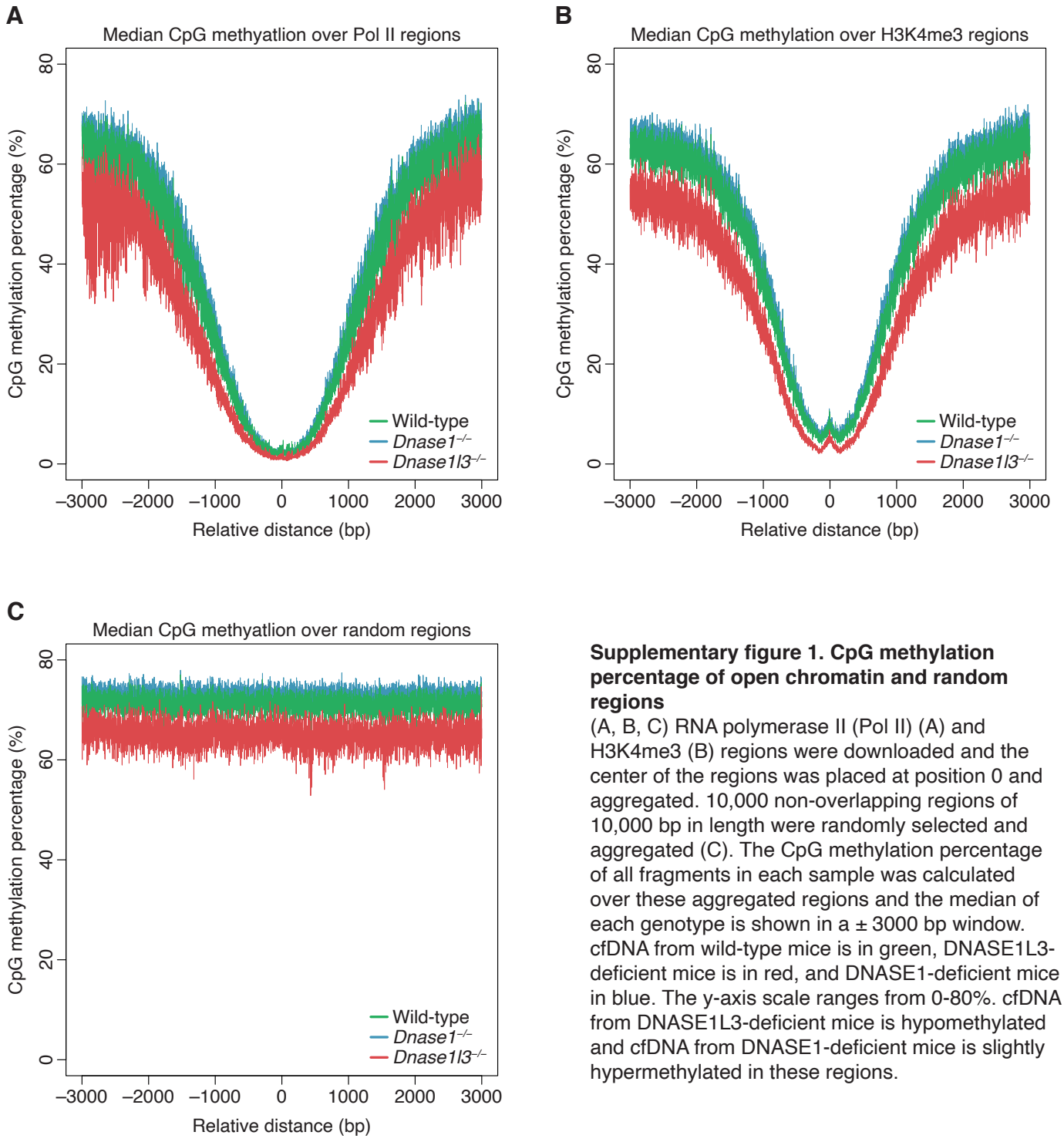
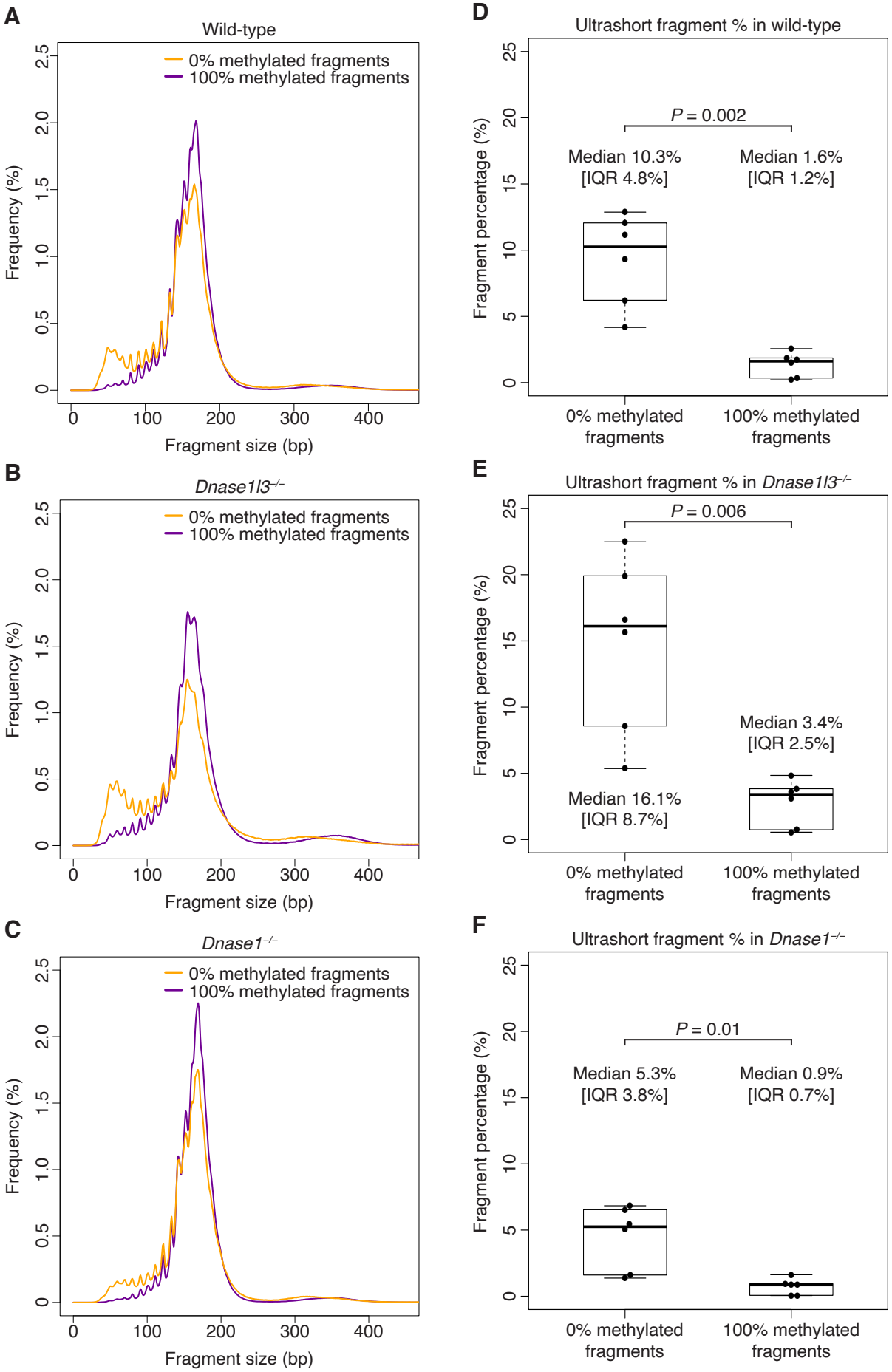


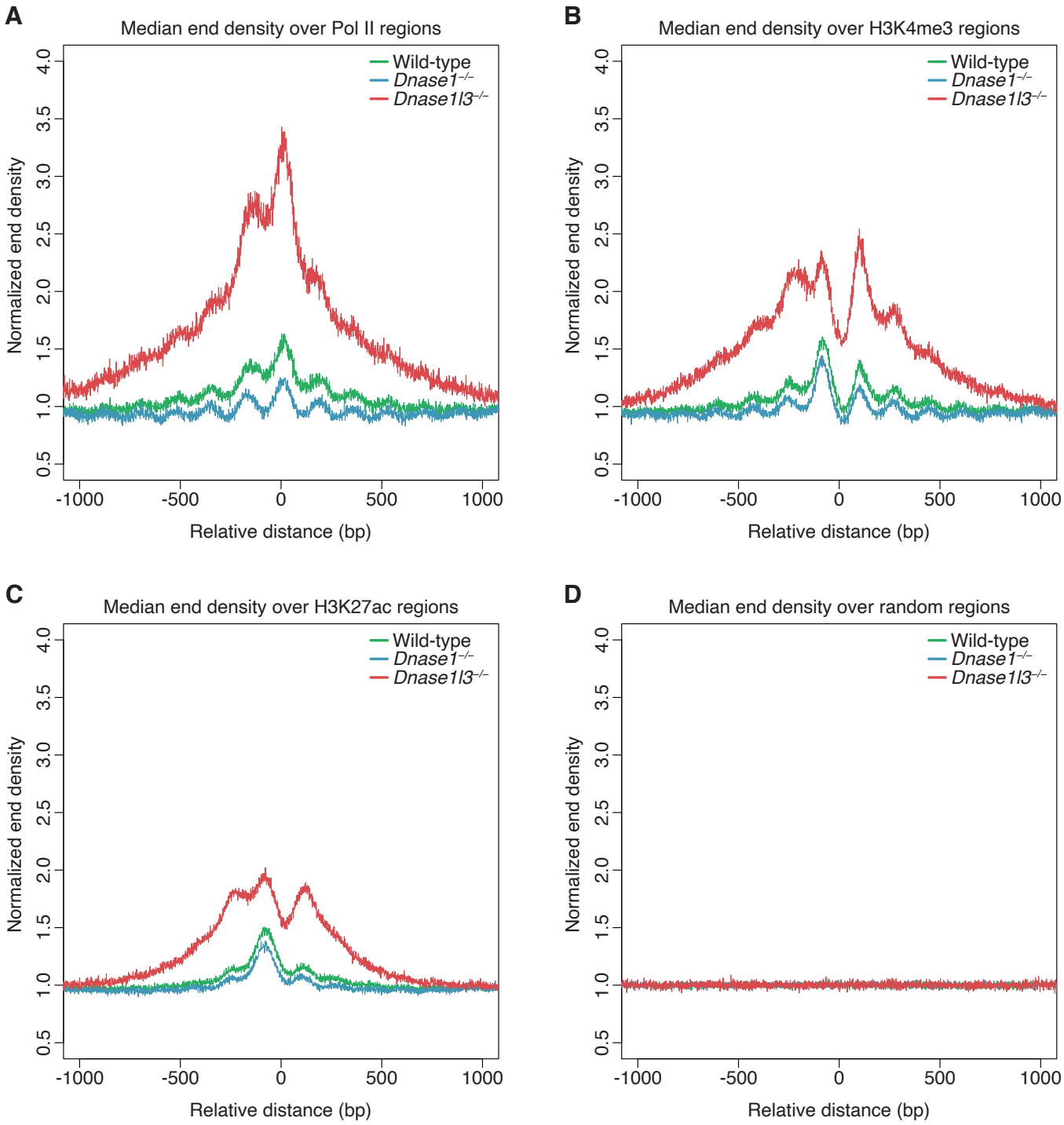
Supplementary figure 1. CpG methylation percentage of open chromatin and random regions

(A, B, C) RNA polymerase II (Pol II) (A) and H3K4me3 (B) regions were downloaded and the center of the regions was placed at position 0 and aggregated. 10,000 non-overlapping regions of 10,000 bp in length were randomly selected and aggregated (C). The CpG methylation percentage of all fragments in each sample was calculated over these aggregated regions and the median of each genotype is shown in a ± 3000 bp window. cfDNA from wild-type mice is in green, DNASE1L3-deficient mice is in red, and DNASE1-deficient mice in blue. The y-axis scale ranges from 0-80%. cfDNA from DNASE1L3-deficient mice is hypomethylated and cfDNA from DNASE1-deficient mice is slightly hypermethylated in these regions.

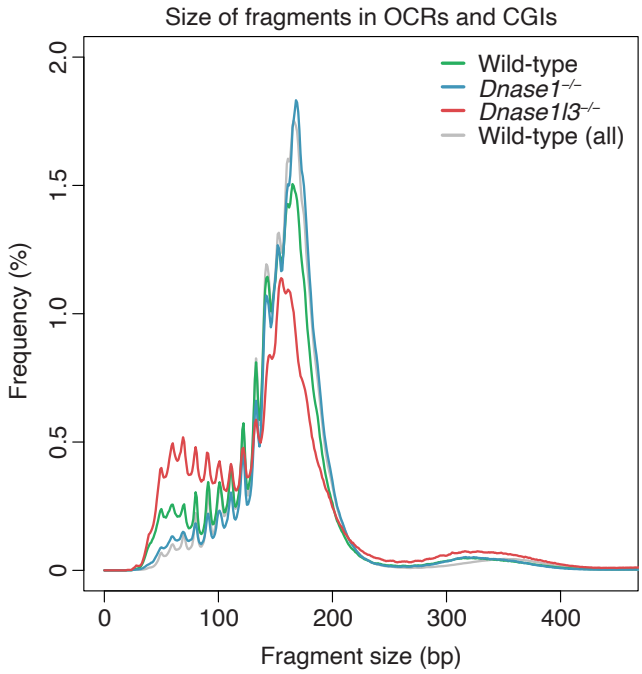


Supplementary figure 2. cfDNA size profile is shorter in 0% methylated fragments than in 100% methylated fragments in all three genotypes

(A, B, C) The size profile of 0% methylated fragments (orange) and 100% methylated fragments (purple) are compared within cfDNA of wild-type (A), DNASE1L3-deficient (B), and DNASE1-deficient mice (C). The y-axis scale ranges from 0-2.5%.
(D, E, F) The proportion of fragments that are ultrashort (≤ 80 bp) was calculated wild-type (D), DNASE1L3-deficient (E), and DNASE1-deficient mice (F) and median and IQR is shown. Welch *t*-test was performed for significance testing. The y-axis scale ranges from 0-25%.

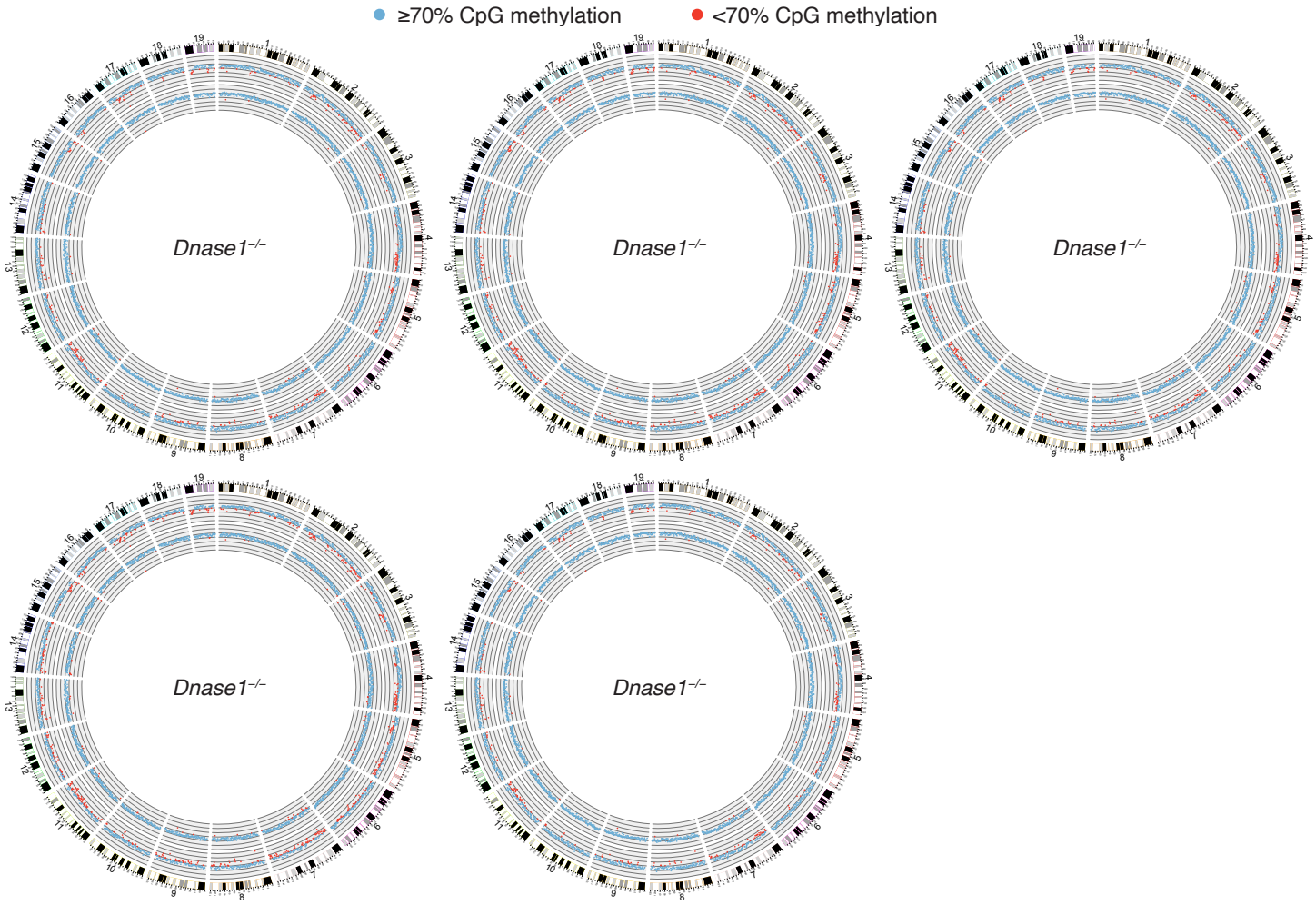


Supplementary figure 3. The normalized end density of other open chromatin regions and CGIs. The normalized end density is calculated from fragment end counts divided by the median end counts in the ± 3000 bp region. The median normalized end density for each genotype is shown in a ± 1000 bp window over the aggregated RNA polymerase II (Pol II) (A), H3K4me3 (B), H3K27ac (C), and random (D) regions. cfDNA from wild-type mice is in green, DNASE1L3-deficient mice is in red, and DNASE1-deficient mice in blue. The y-axis scale ranges from 0.5-4%.

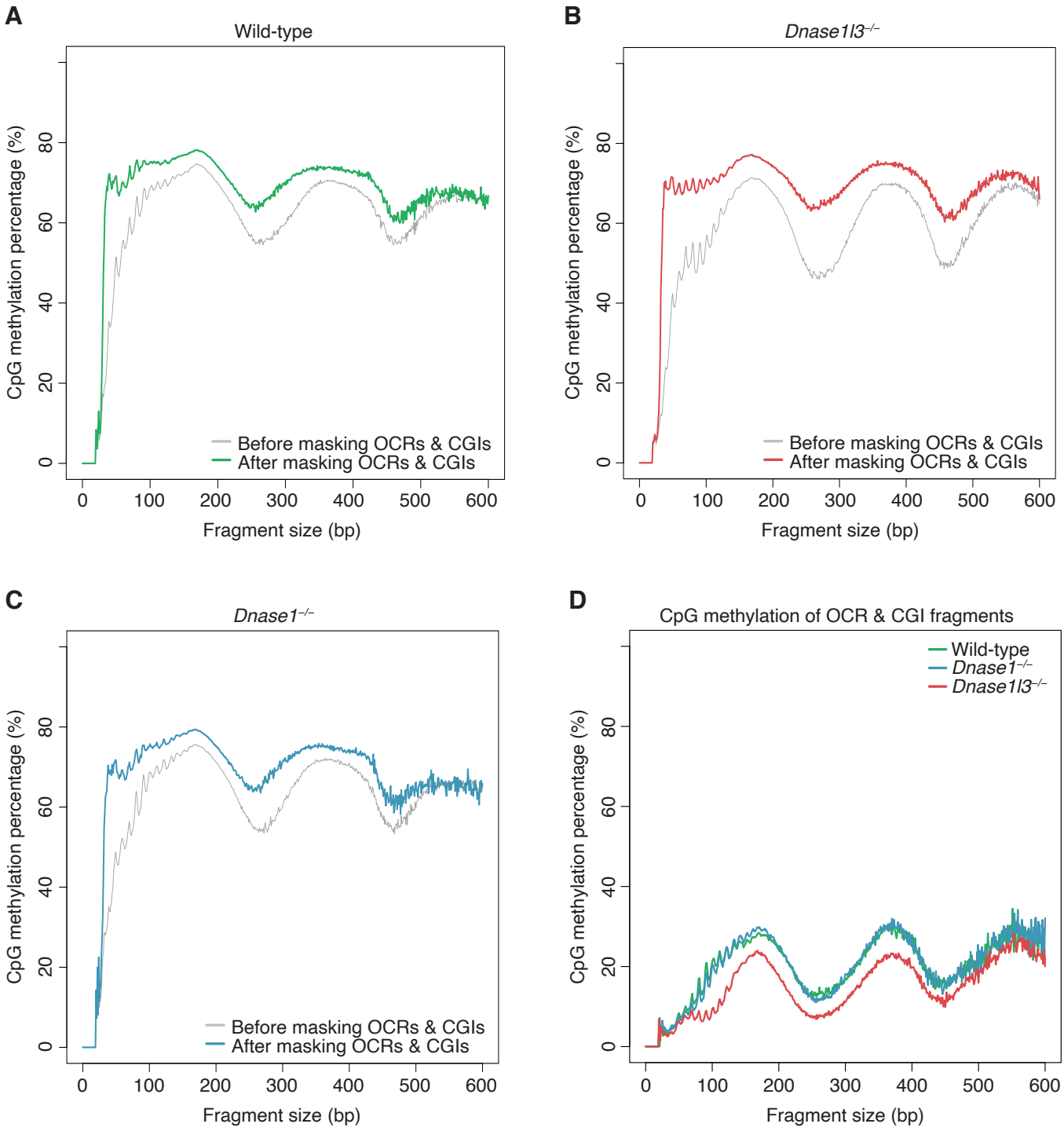


Supplementary figure 4. Size profile of fragments in OCRs and CGIs
The median cfDNA size profile of fragments inside OCRs and CGIs is shown. cfDNA from wild-type mice is in green, DNASE1L3-deficient mice is in red, and DNASE1-deficient mice in blue. The cfDNA size profile of all wild-type fragments is shown as a comparison in gray. The y-axis scale ranges from 0-2%.

Supplementary Figure 5



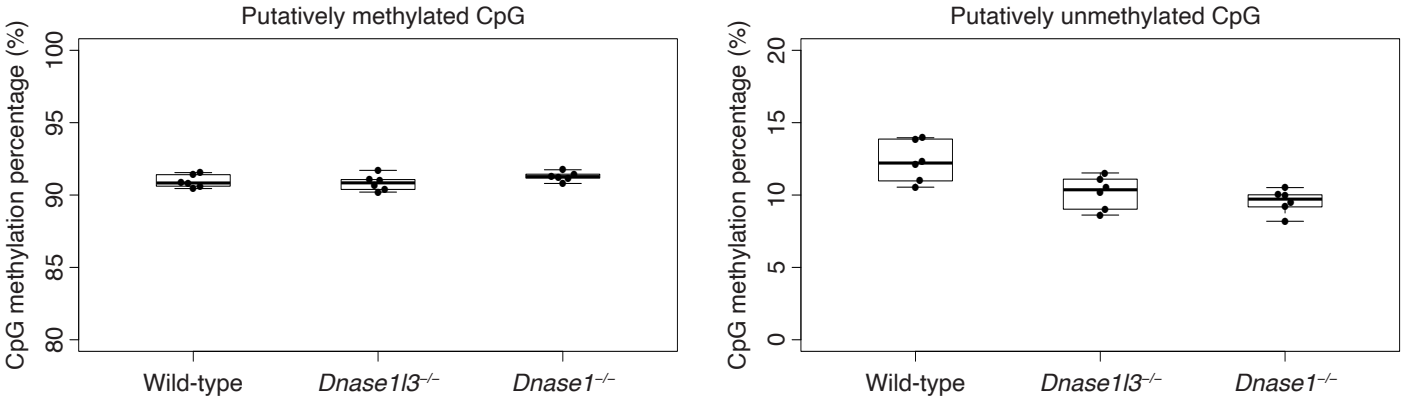
Supplementary figure 5. Circos plots for each pooled sample
Circos plots showing genome-wide CpG methylation percentages before (outer ring) and after (inner ring) masking OCR and CGI fragments. Each dot represents the CpG methylation percentage in a 1 Mb bin of the mouse autosome and colored in blue if ≥ 70% and in red if < 70%. A Circos plot is shown for each wild-type, DNASE1L3-deficient, and DNASE1-deficient mouse.



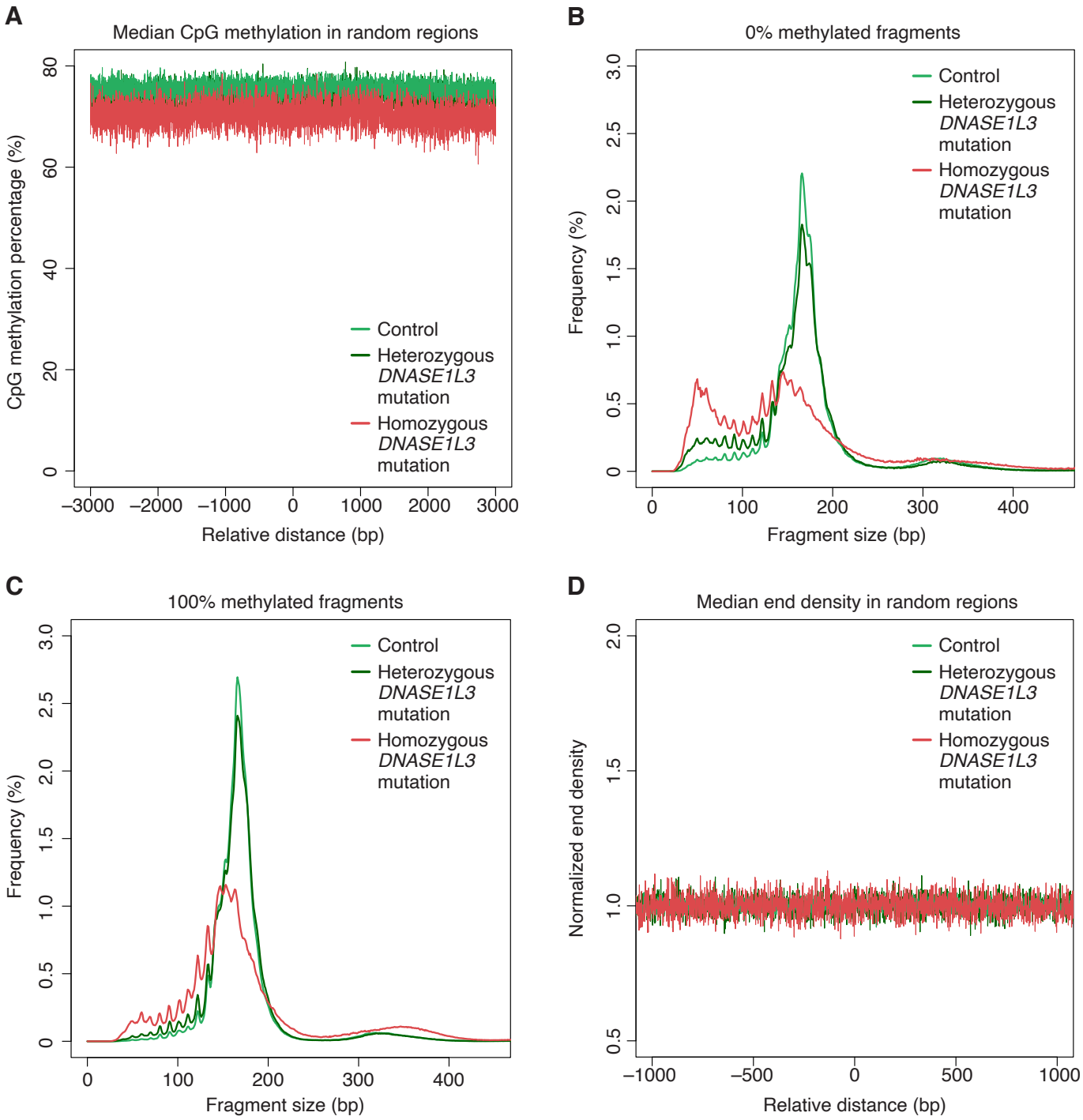
Supplementary figure 6. Effect of masking OCR and CGI fragments on CpG methylation by fragment size

(A, B, C) CpG methylation percentage with each fragment size before (gray) and after (green, red, blue) masking OCR and CGI fragments in wild-type (A), DNASE1L3-deficient (B), and DNASE1-deficient mice (C).

(D) The CpG methylation percentage was calculated within OCR and CGI fragments of a particular size and the median of each genotype was plotted. cfDNA from wild-type mice is in green, DNASE1L3-deficient mice is in red, and DNASE1-deficient mice in blue. The y-axis scale ranges from 0-100%.

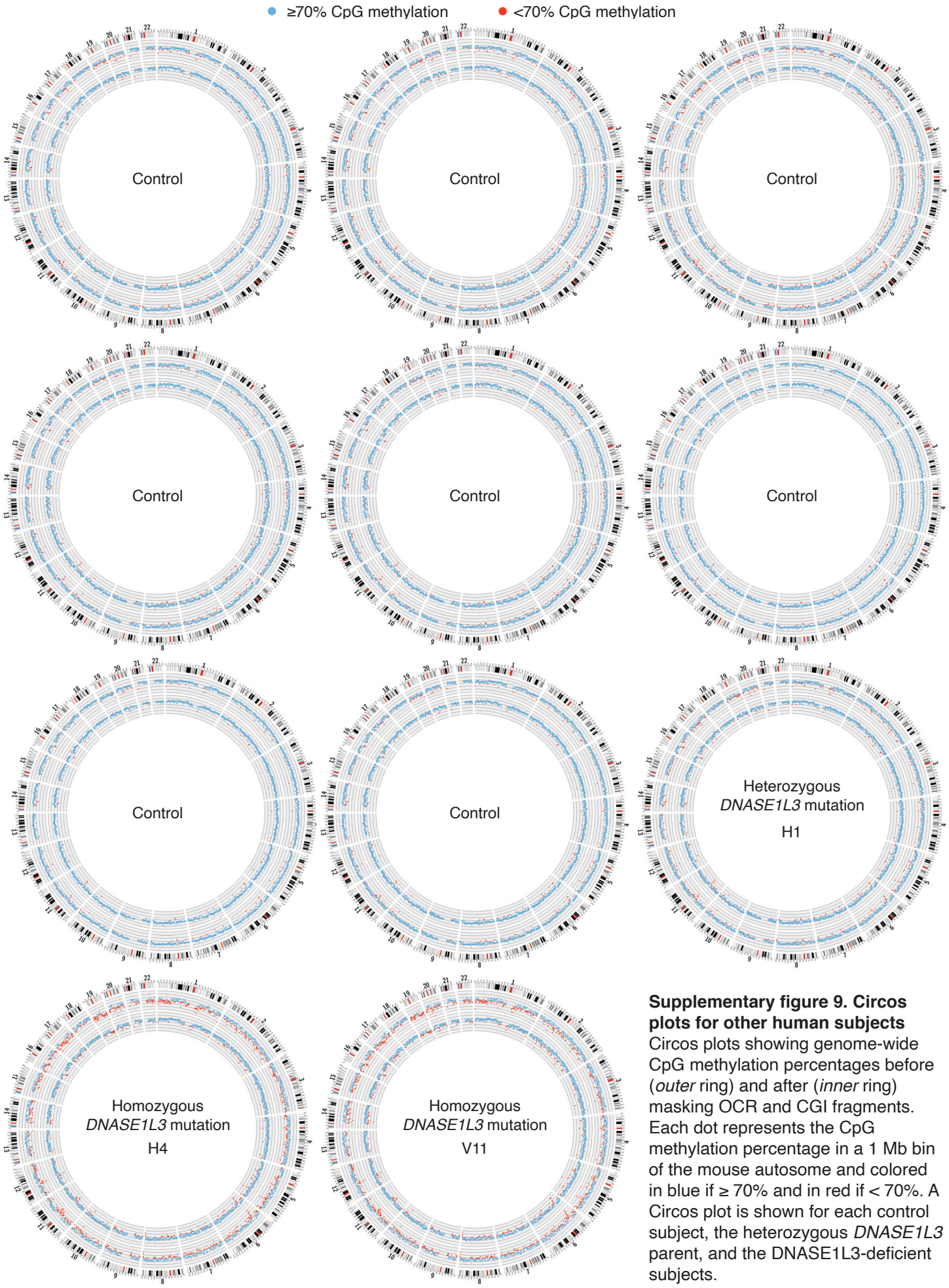


Supplementary figure 7. Plasma cfDNA methylation percentage of the putatively unmethylated CpG (A) and putatively methylated CpG (B) in wild-type, DNASE1L3-deficient, and DNASE1-deficient mice. The y-axis scale ranges from 80-100% in putatively methylated CpGs and from 0-20% in putatively unmethylated CpGs.

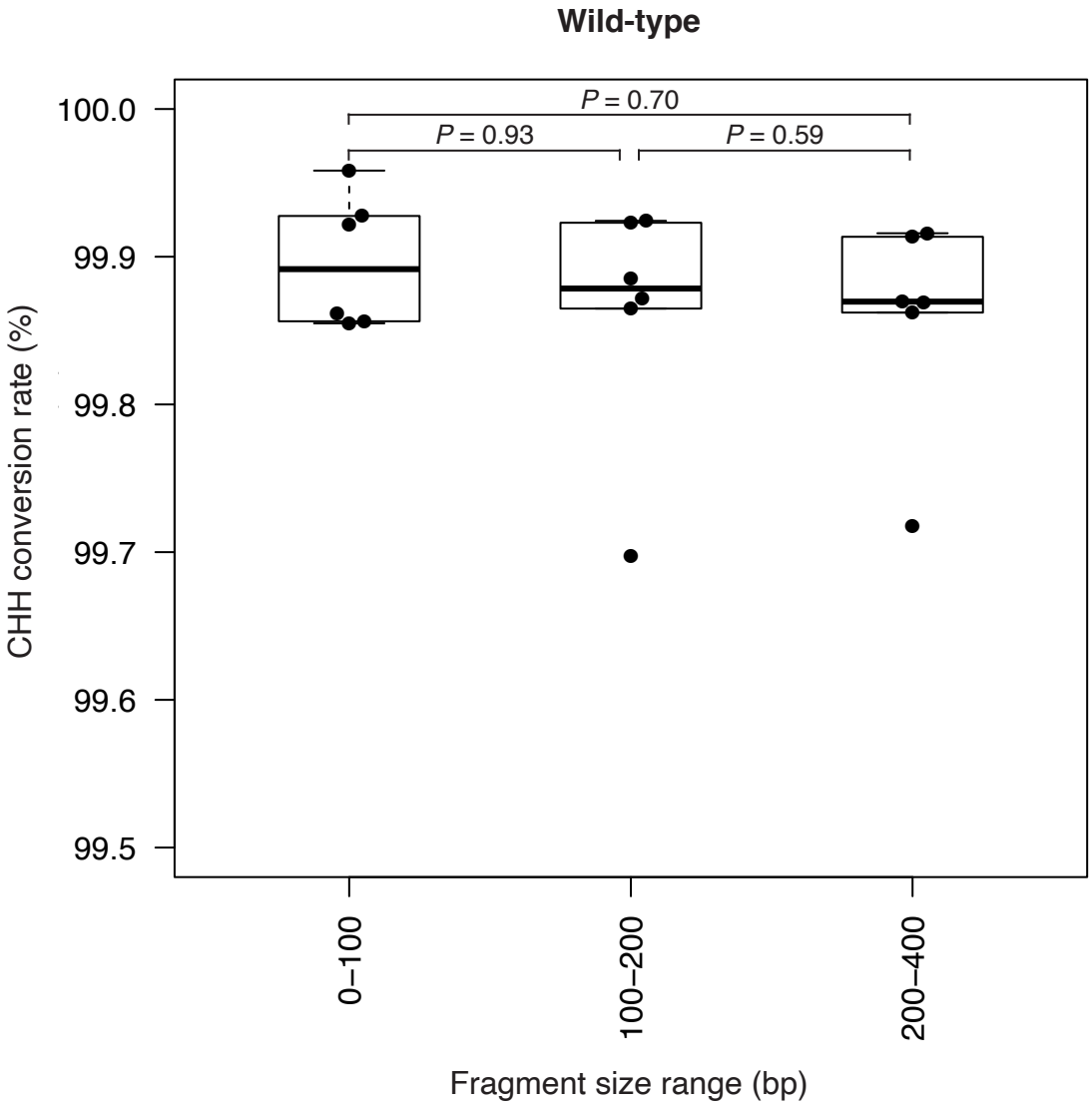


Supplementary figure 8. CpG methylation and end density of random regions and size profile of human subjects

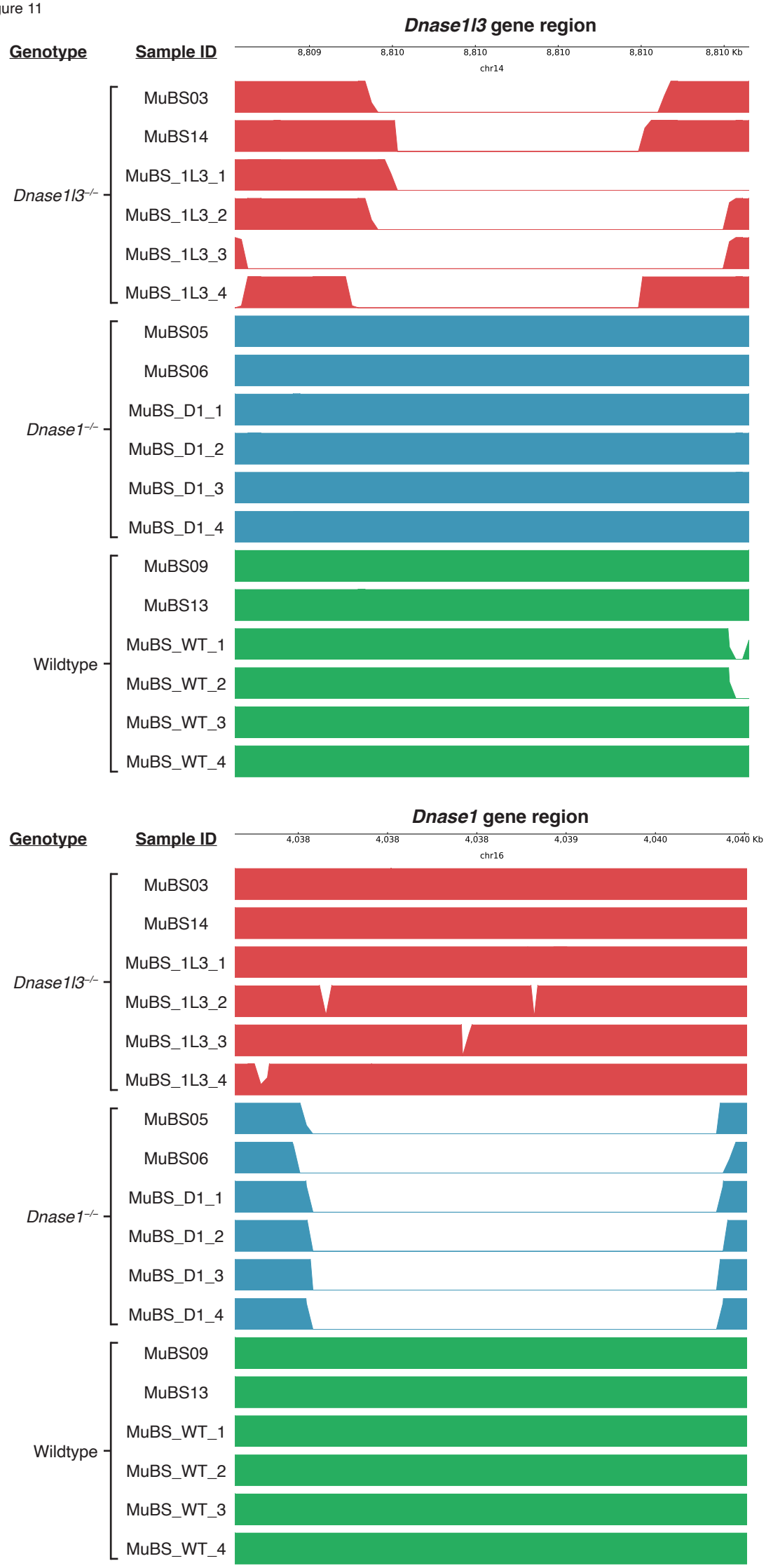
(A) The CpG methylation percentage of fragments from each sample was calculated over aggregated random regions, and the median of each sample type is shown in a ± 3000 bp window. The y-axis ranges from 0-80%.
(B, C) The median cfDNA size profile of each subject type was plotted using only 0% methylated fragments (B), or only 100% methylated fragments (C). The y-axis ranges from 0-3%.
(D) The median normalized end density for each sample type is shown in a ± 1000 bp window over the aggregated random regions. The y-axis ranges from 0.5-2%.
cfDNA from control samples is in light green, the heterozygous *DNASE1L3* parent is in dark green, and DNASE1L3-deficient subjects is in red.



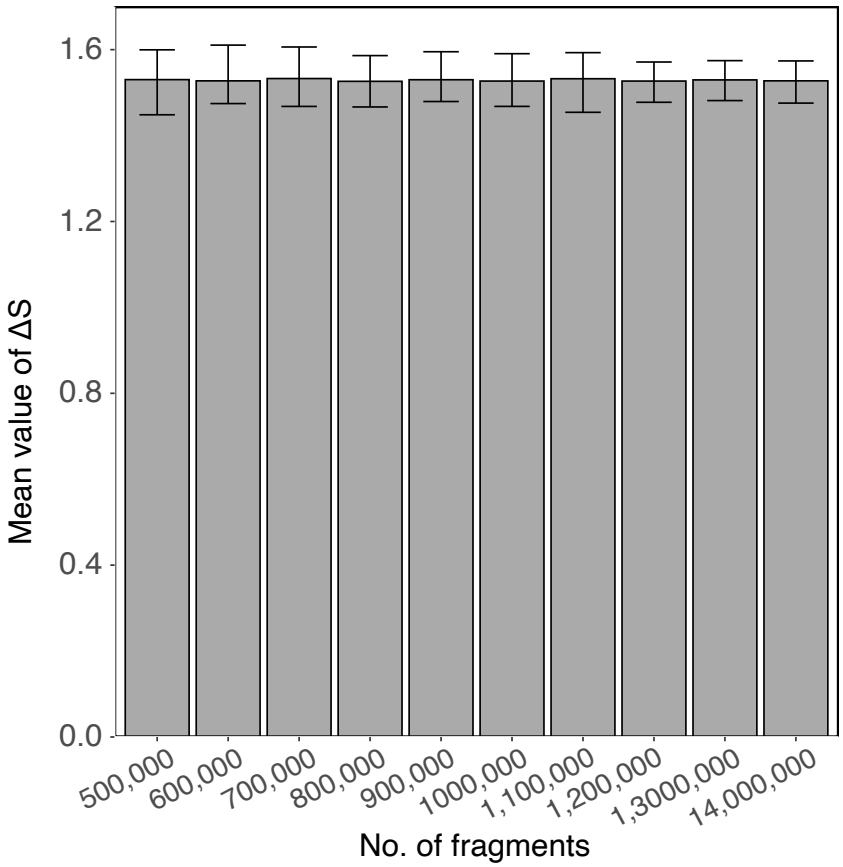
Supplementary figure 9. Circos plots for other human subjects
Circos plots showing genome-wide CpG methylation percentages before (*outer* ring) and after (*inner* ring) masking OCR and CGI fragments. Each dot represents the CpG methylation percentage in a 1 Mb bin of the mouse autosome and colored in blue if ≥ 70% and in red if < 70%. A Circos plot is shown for each control subject, the heterozygous *DNASE1L3* parent, and the DNASE1L3-deficient subjects.



Supplementary figure 10. CHH conversion rate of different fragment sizes in cfDNA of wild-type mice. The CHH site conversion efficiency was calculated among all the fragments ranging from 0-100, 100-200, and 200-400 bp in the cfDNA of WT mice. There is no significant difference in CHH conversion efficiency between the different fragment sizes in the cfDNA of WT mice (Significance testing by Mann-Whitney *U* test). The y-axis ranges from 99.5-100%.



Supplementary figure 11. Plasma cfDNA coverage of each sample over the deleted regions.



Supplementary figure 12. Mean delta S simulation.
The difference in cumulative frequency (delta S) between 0% and 100% methylated fragments at 80 bp was calculated in a simulation using different fragment counts. The simulation was repeated 50x at each fragment size and the mean (bar), minimum (lower whisker), and maximum (upper whisker) is shown. Even at 0.5M counts, the mean difference between 0% and 100% methylated fragments at 80 bp is not significantly different than if the analysis was done with 1.4M counts. Beginning from 1.2M counts, the max-min range is slightly narrower.