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Supplemental Data

**The Biology of Cell-free DNA Fragmentation and the
Roles of DNASE1, DNASE1L3, and DFFB**

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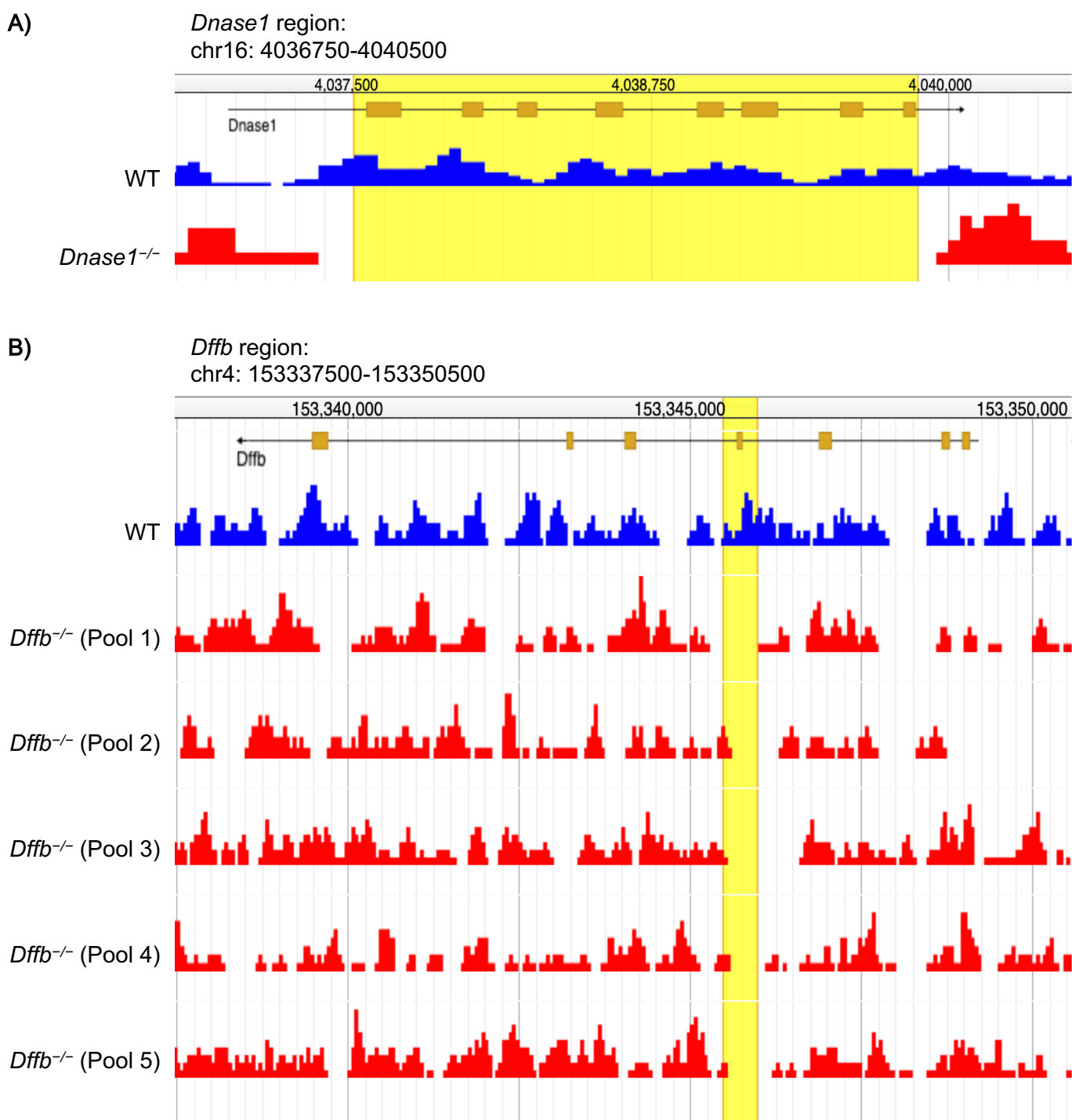


Figure S1. Read coverage of knockout regions.

Sequenced read coverage for plasma of WT (blue), *Dnase1*^{-/-} mice (A, red) and *Dffb*^{-/-} mice (Pool 1-5) (B, red). Knockout regions highlighted in yellow.

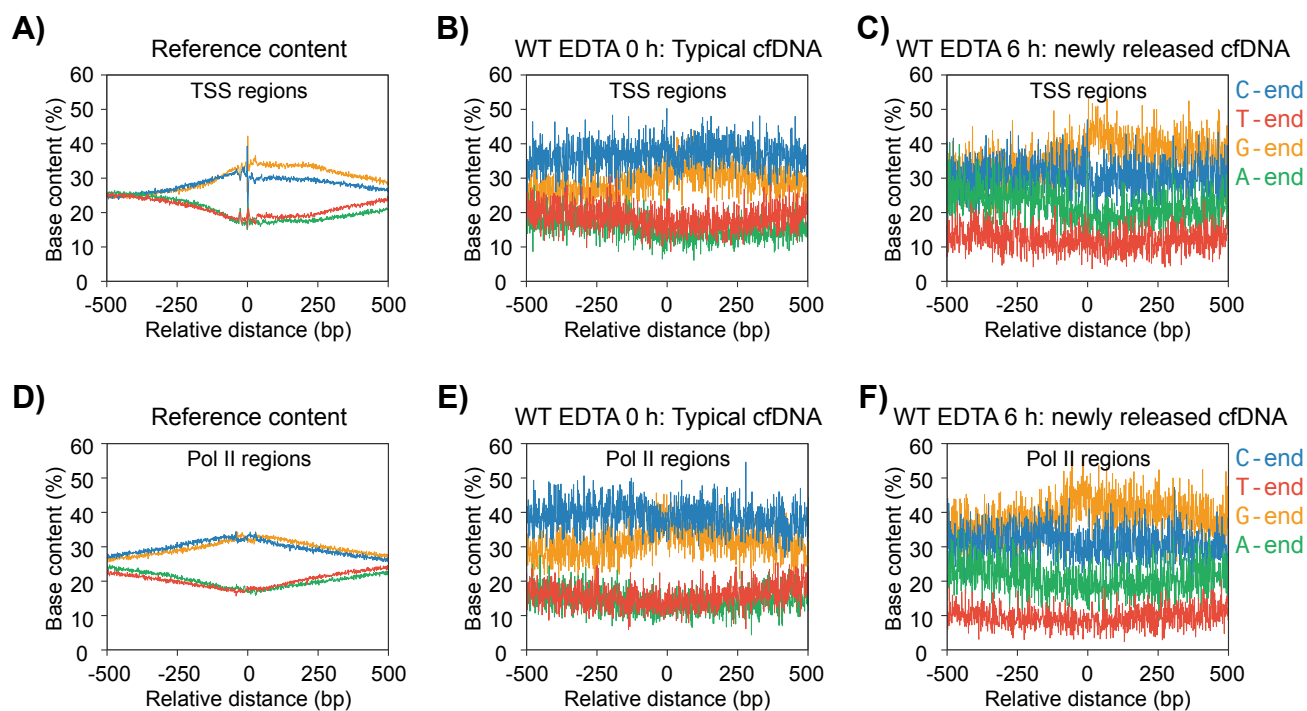


Figure S2. Base content proportions in TSS and Pol II regions.

(A, D) The reference murine genomic content of TSS (A) and Pol II (D) regions.

(B, E) The base content proportions at the 5' end of cfDNA fragments of WT EDTA 0 h samples in TSS (B) and Pol II (E) regions.

(C, F) The base content proportions of WT EDTA 6 h samples enriched with newly released cfDNA in TSS (C) and Pol II regions (C).

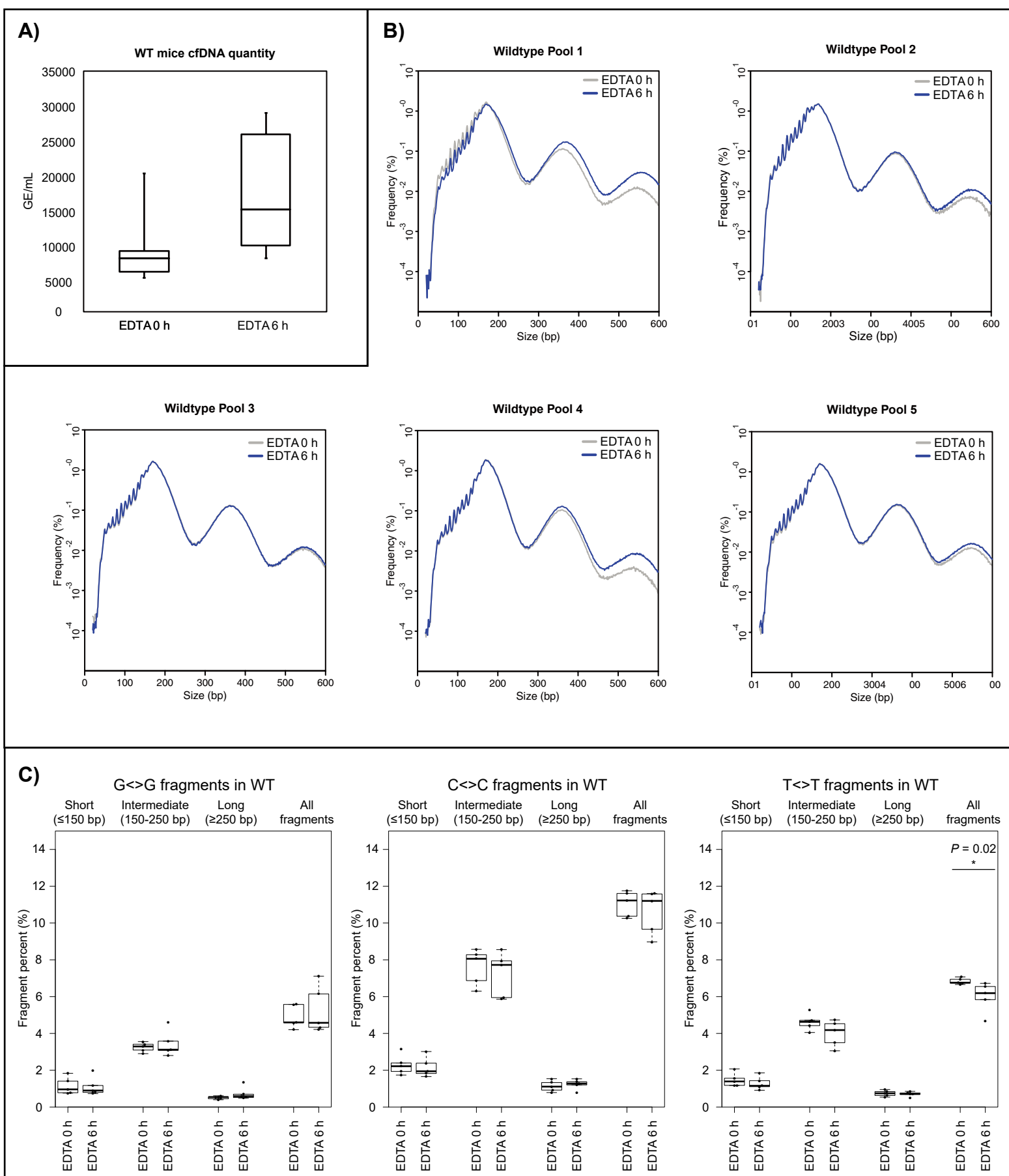
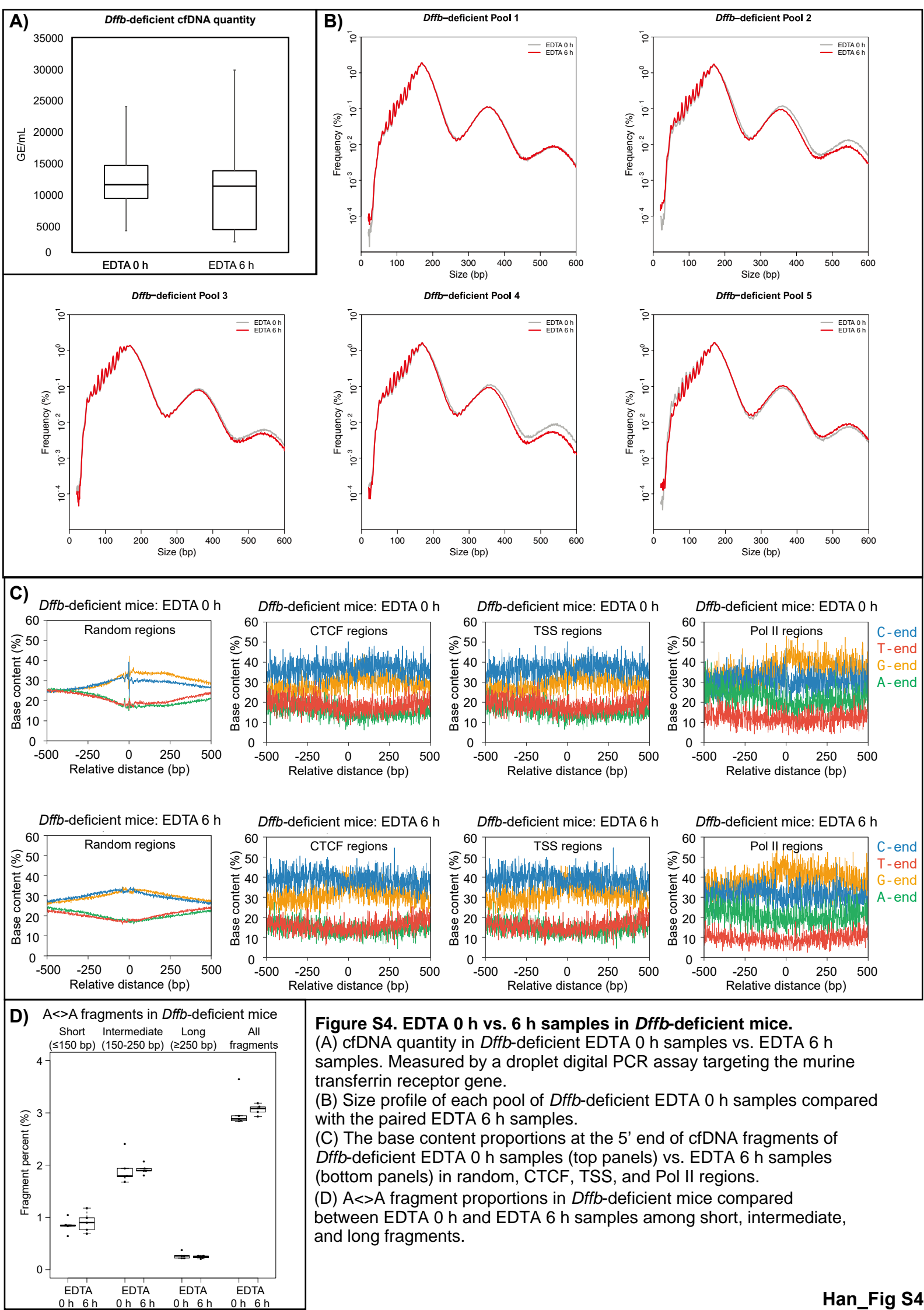


Figure S3. EDTA 0 h vs. 6 h samples in WT mice.

(A) cfDNA quantity in WT EDTA 0 h samples vs. EDTA 6 h samples. Measured by a droplet digital PCR assay targeting the murine transferrin receptor gene.

(B) Size profile of each pool of WT EDTA 0 h samples compared with the paired EDTA 6 h samples.

(C) G<>G, C<>C, and T<>T fragment proportions in WT mice compared between EDTA 0 h and EDTA 6 h among short, intermediate, and long fragments. P-value calculated by Mann-Whitney *U* test.



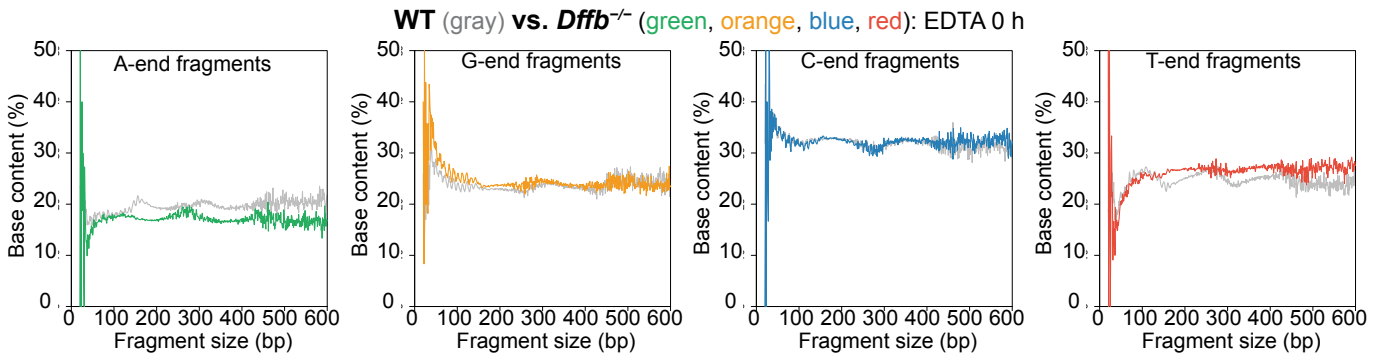


Figure S5. WT vs. *Dffb*-deficient baseline cfDNA.

Percentages of A-ends (green), G-ends (orange), C-ends (blue), and T-ends (red) in *Dffb*-deficient EDTA 0 h cfDNA compared with the percentages in WT EDTA 0 h cfDNA (gray).

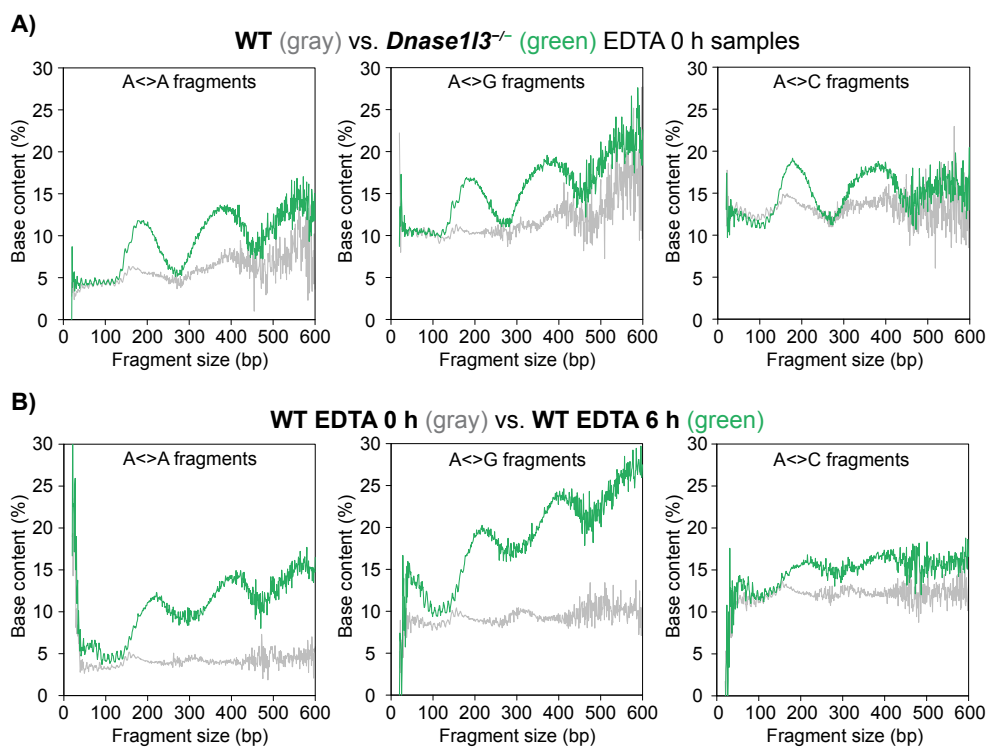


Figure S6. Similarities in *Dnase1/3*-deficient cfDNA compared with WT fresh cfDNA (EDTA 6 h samples).

(A) Percentages of A<->A, A<->G, and A<->C fragments in *Dnase1/3*-deficient EDTA 0 h cfDNA compared with the baseline representation of WT EDTA 0 h cfDNA (gray).

(B) Percentages of A<->A, A<->G, and A<->C fragments in WT EDTA 6 h samples enriched with newly released cfDNA compared to the baseline representation of WT EDTA 0 h cfDNA (gray).

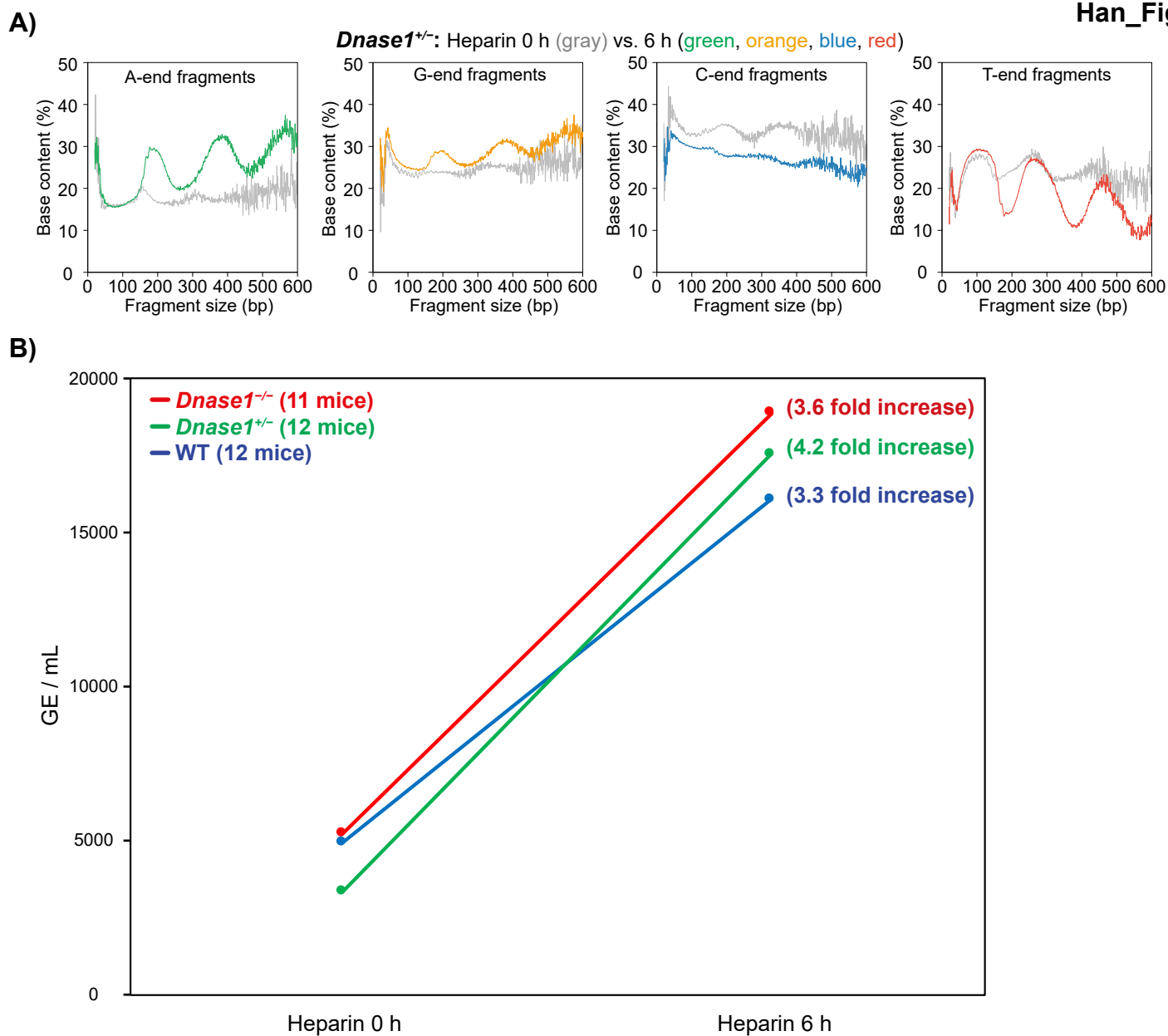


Figure S7. Heparin effect in WT, *Dnase1^{+/-}*, and *Dnase1^{-/-}* mice.

(A) Percentages of cfDNA with A-ends (green), G-ends (orange), C-ends (blue), and T-ends (red) in *Dnase1^{+/-}* cfDNA after 6 h heparin incubation compared with its baseline at 0 h incubation (gray).

(B) cfDNA quantity increases in WT, *Dnase1^{+/-}*, *Dnase1^{-/-}* mice in heparin 6 h samples.

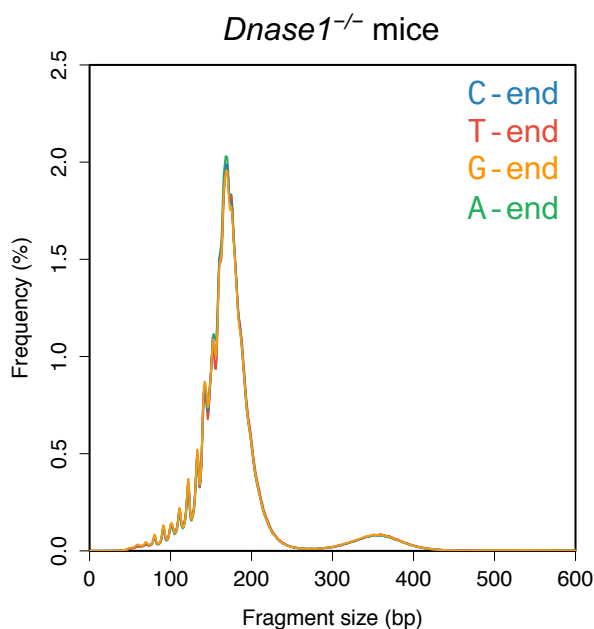
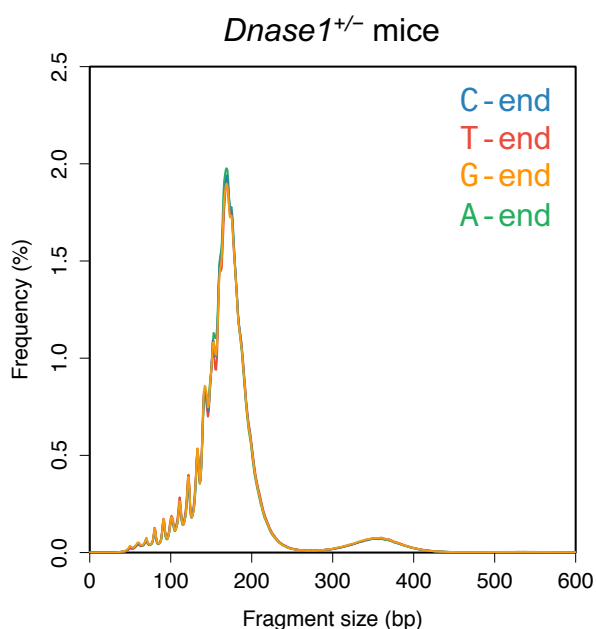
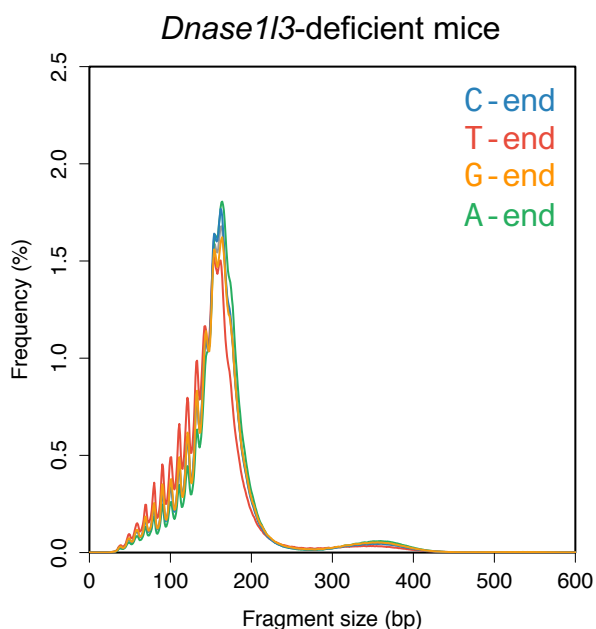
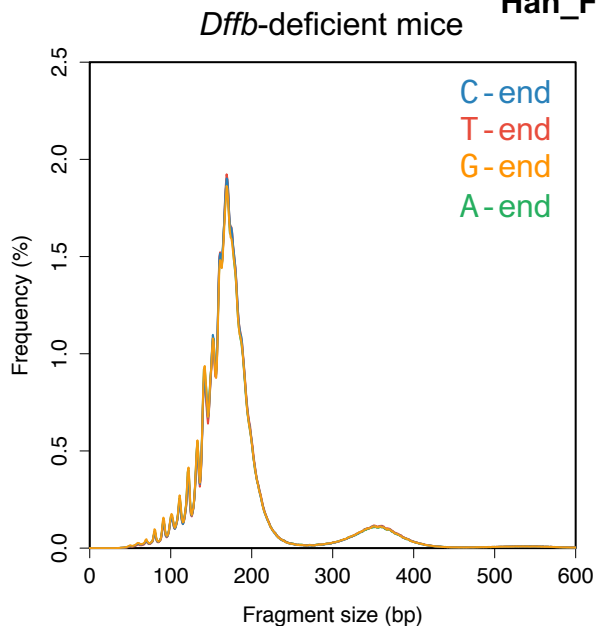
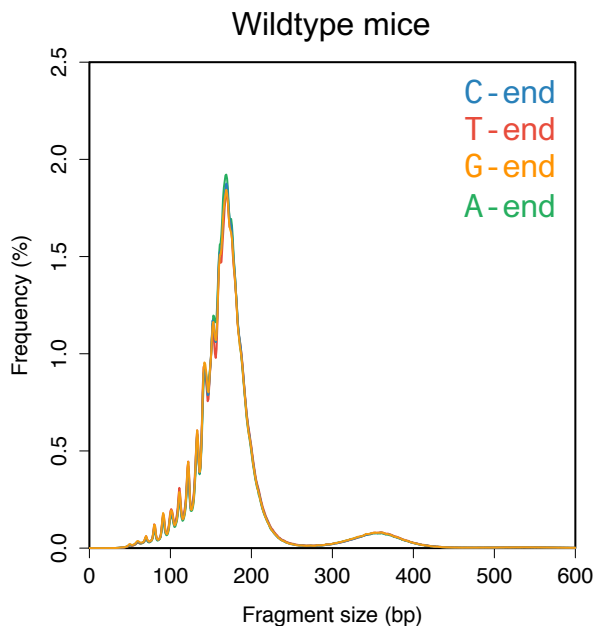


Figure S8. 10 bp periodicity present in short 150 bp cfDNA fragments of all mice genotypes.
cfDNA size profile of A-end, G-end, C-end, and T-end fragments in EDTA 0 h samples of WT, *Dffb*^{-/-}, *Dnase113*^{-/-}, *Dnase1*^{+/-}, and *Dnase1*^{-/-} mice.

Table S1. Number of unique fragments in each sample sequenced by an Illumina platform

| Pool | Genotypes | Condition | Number of non-duplicate fragments |
|-------------------------------------|------------------------------|-------------|-----------------------------------|
| <i>Dnase1</i>-deficient pool | WT | Heparin 0hr | 16430394 |
| | WT | Heparin 6hr | 20736728 |
| | <i>Dnase1</i> ^{+/-} | Heparin 0hr | 17338183 |
| | <i>Dnase1</i> ^{+/-} | Heparin 6hr | 23974725 |
| | <i>Dnase1</i> ^{-/-} | Heparin 0hr | 20737940 |
| | <i>Dnase1</i> ^{-/-} | Heparin 6hr | 14322424 |
| <i>Dffb</i>-deficient Pool 1 | WT | EDTA 0hr | 11247117 |
| | WT | EDTA 6hr | 13550536 |
| | <i>Dffb</i> ^{-/-} | EDTA 0hr | 14072590 |
| | <i>Dffb</i> ^{-/-} | EDTA 6hr | 13719670 |
| <i>Dffb</i>-deficient Pool 2 | WT | EDTA 0hr | 11386335 |
| | WT | EDTA 6hr | 14182499 |
| | <i>Dffb</i> ^{-/-} | EDTA 0hr | 12071679 |
| | <i>Dffb</i> ^{-/-} | EDTA 6hr | 11096029 |
| <i>Dffb</i>-deficient Pool 3 | WT | EDTA 0hr | 18443065 |
| | WT | EDTA 6hr | 17309666 |
| | <i>Dffb</i> ^{-/-} | EDTA 0hr | 18487335 |
| | <i>Dffb</i> ^{-/-} | EDTA 6hr | 15276638 |
| <i>Dffb</i>-deficient Pool 4 | WT | EDTA 0hr | 21106472 |
| | WT | EDTA 6hr | 20338278 |
| | <i>Dffb</i> ^{-/-} | EDTA 0hr | 14178723 |
| | <i>Dffb</i> ^{-/-} | EDTA 6hr | 16550591 |
| <i>Dffb</i>-deficient Pool 5 | WT | EDTA 0hr | 18899353 |
| | WT | EDTA 6hr | 20161869 |
| | <i>Dffb</i> ^{-/-} | EDTA 0hr | 25148138 |
| | <i>Dffb</i> ^{-/-} | EDTA 6hr | 17422602 |