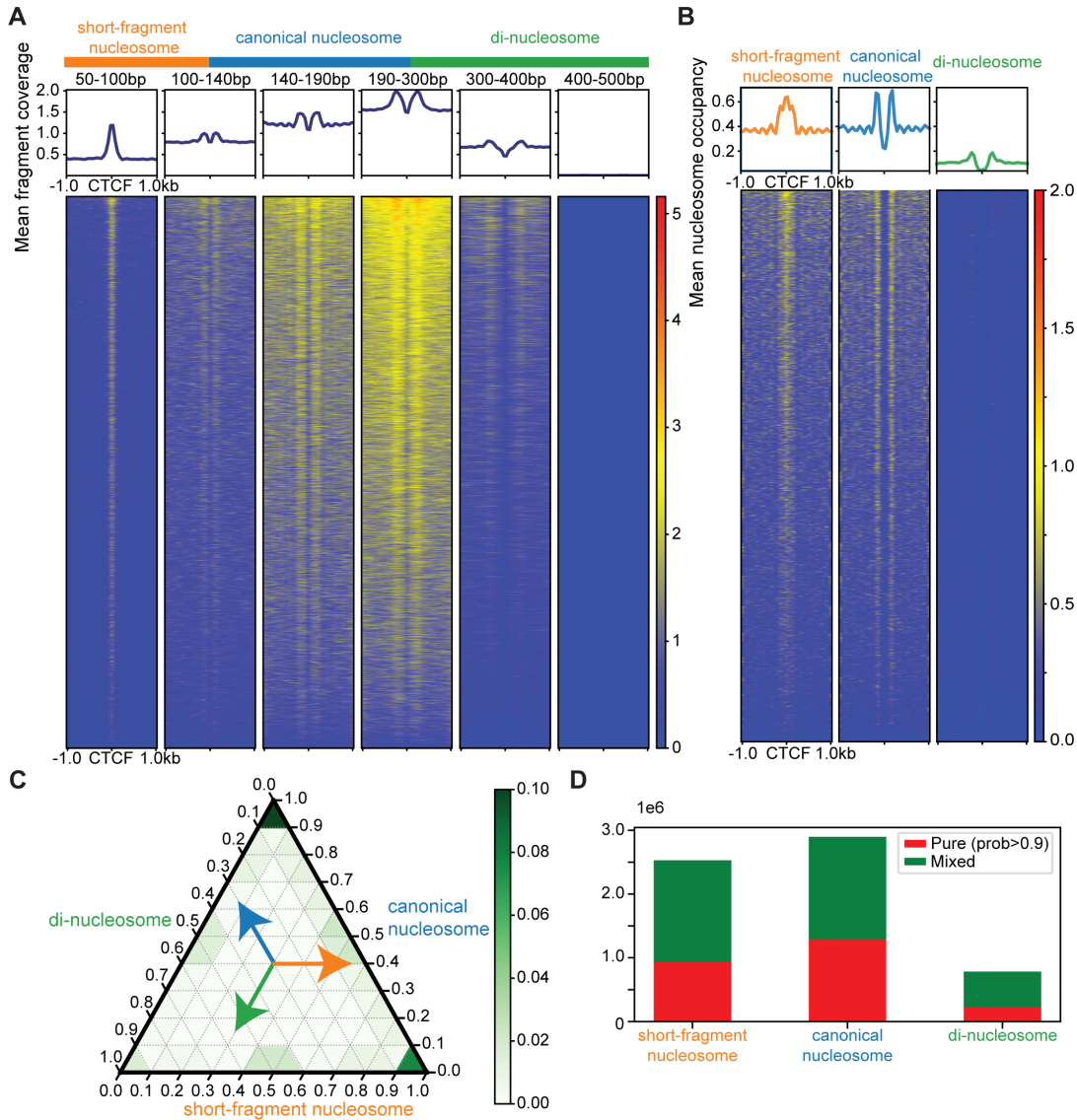
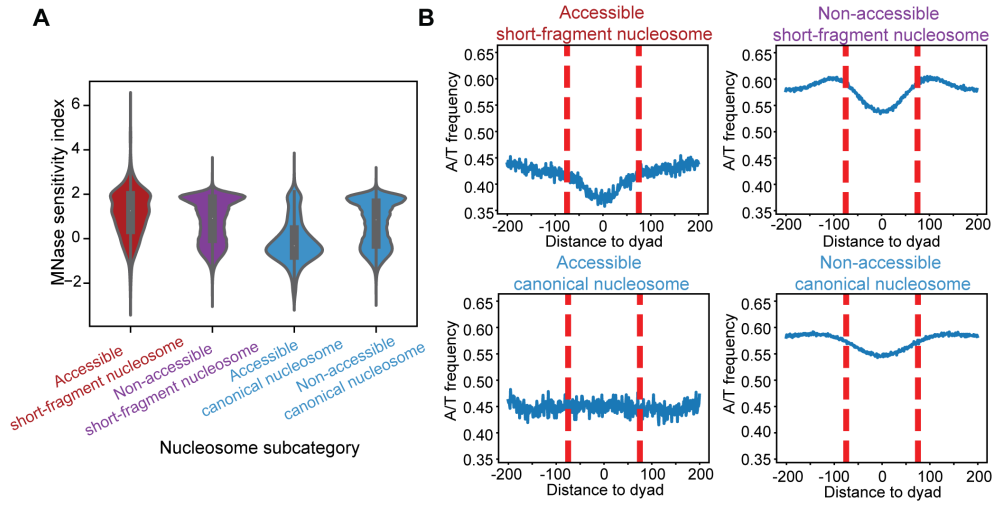


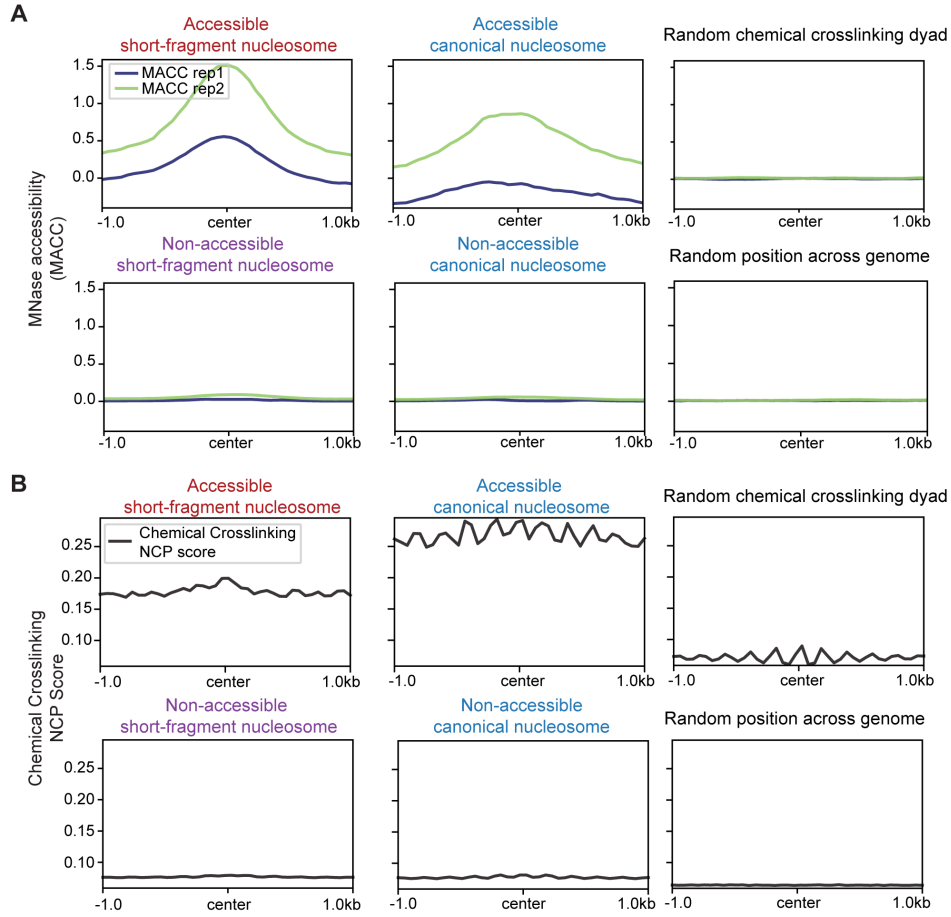
## SUPPLEMENTARY FIGURES



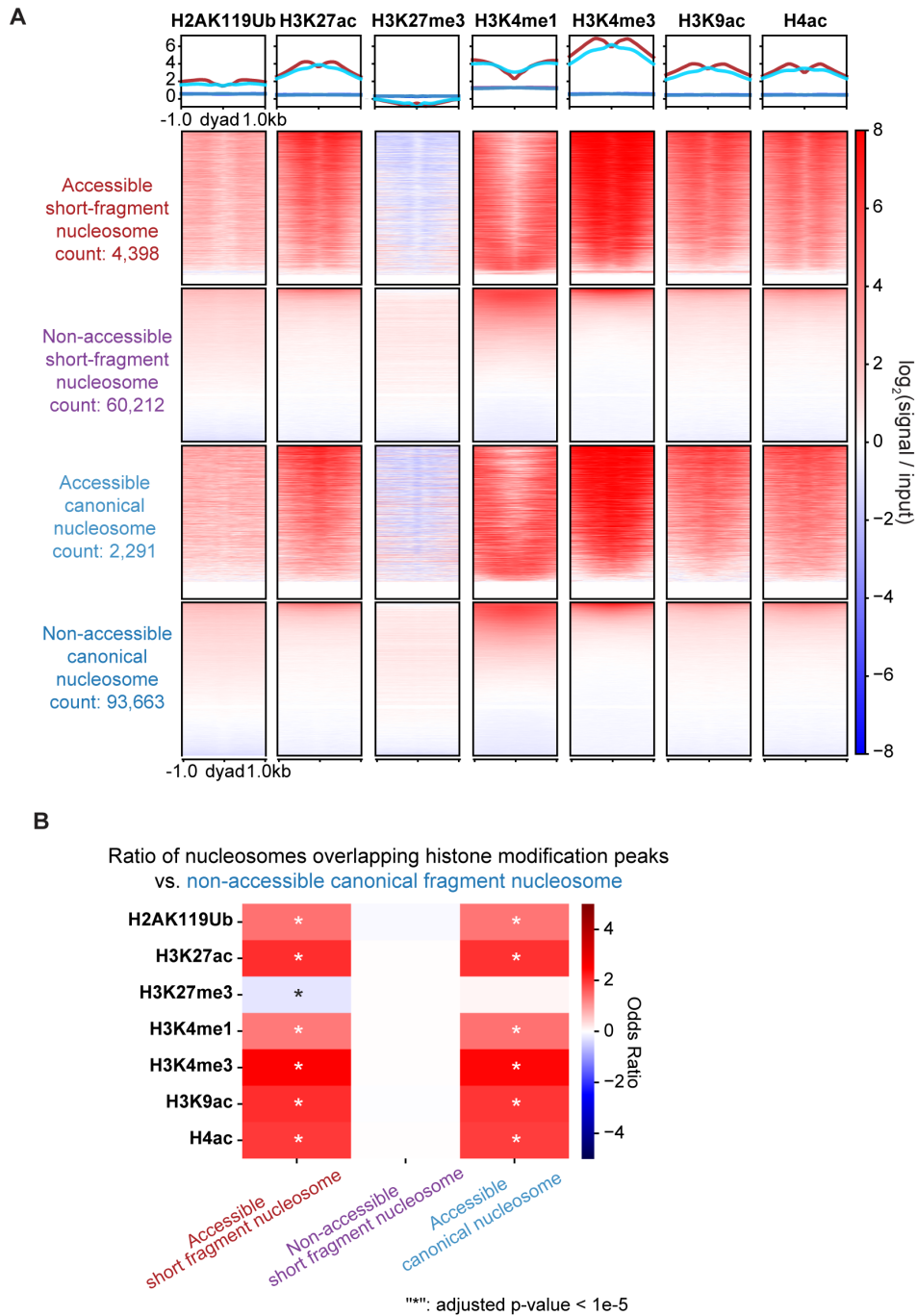
**Figure S1:** Extended analysis of SEM-characterized nucleosome subtypes in mESCs (related to Figure 3). **A)** Heatmap and profile plot of MNase-seq fragments split by fragment size (50-100bp, 100-140bp, 140-190bp, 190-300bp, 300-400bp, 400-500bp) around CTCF binding sites. **B)** Heatmap and profile plot of each nucleosome subcategory around CTCF binding sites. **C)** Ternary plot showing the distribution of nucleosome mixture type probabilities. **D)** Bar plot shows the proportion of mixed and "pure" (subtype probability > 0.9) nucleosomes for each of the nucleosome subtypes.



**Figure S2:** Accessible short-fragment nucleosomes display high MNase sensitivity. **A)** Violin plot shows the distribution of MNase sensitivity index in each nucleosome subcategory. **B)** A/T nucleotide frequency around each nucleosome subcategory, red dash lines mark the nucleosome entry/exit sites ( $\pm 75$ bp).

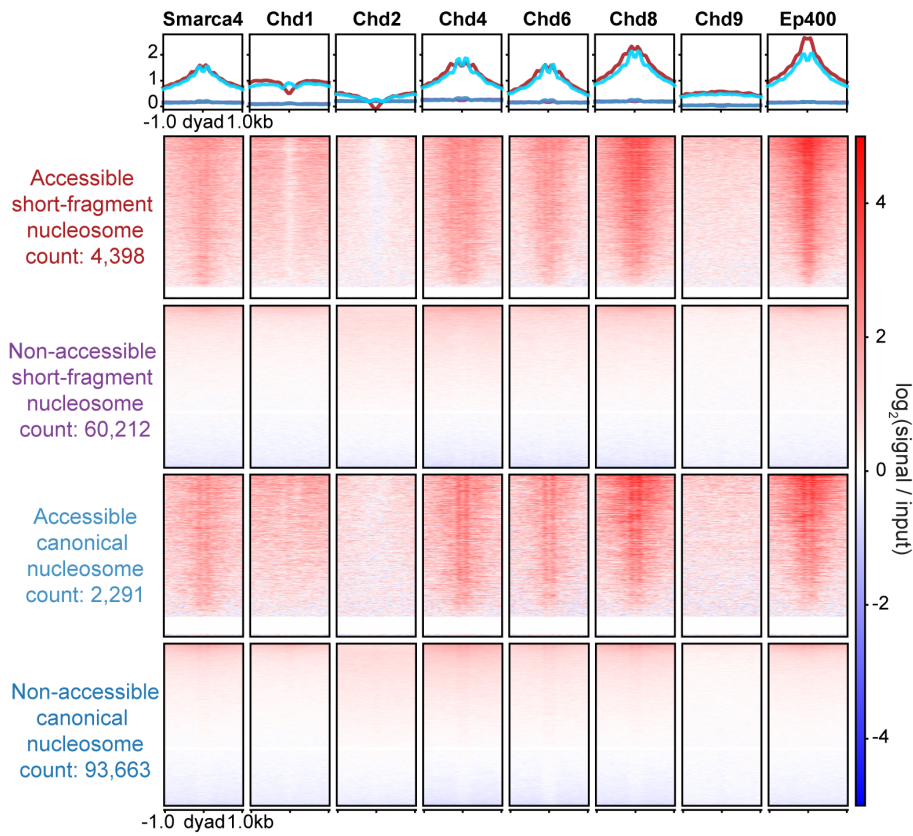


**Figure S3:** Accessible short-fragment nucleosomes display high MNase accessibility and nucleosome centering positioning scores. **A)** Profile plot of MACC value around nucleosome dyad locations for each nucleosome subcategory. **B)** Profile plot of Chemical Crosslinking NCP score around nucleosome dyad locations for each nucleosome subcategory.



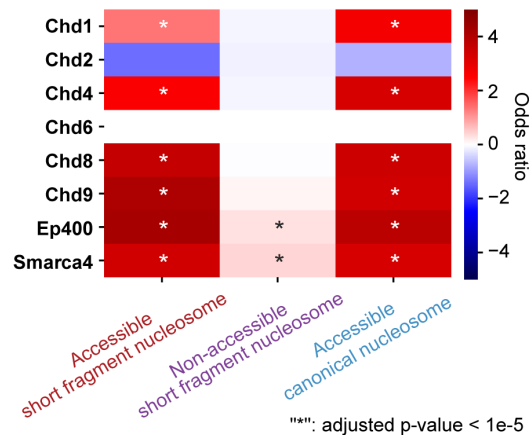
**Figure S4:** Active histone modifications are enriched at sites adjacent to accessible short-fragment nucleosomes. **A)** Heatmap and profile plot of histone modifications around nucleosome dyad locations. **B)** Chi-square test on the ratio of nucleosomes overlapping histone modification peaks compared to that of non-accessible canonical nucleosomes.

**A**



**B**

Ratio of nucleosomes overlapping chromatin remodeler peaks  
vs. *non-accessible canonical fragment nucleosome*



**Figure S5:** Several chromatin remodelers are enriched at accessible short-fragment nucleosome dyad locations. **A)** Heatmap and profile plot of chromatin remodelers around nucleosome dyad locations. **B)** Chi-square test on the ratio of nucleosomes overlapping chromatin remodeler peaks compared to that of non-accessible canonical nucleosomes.