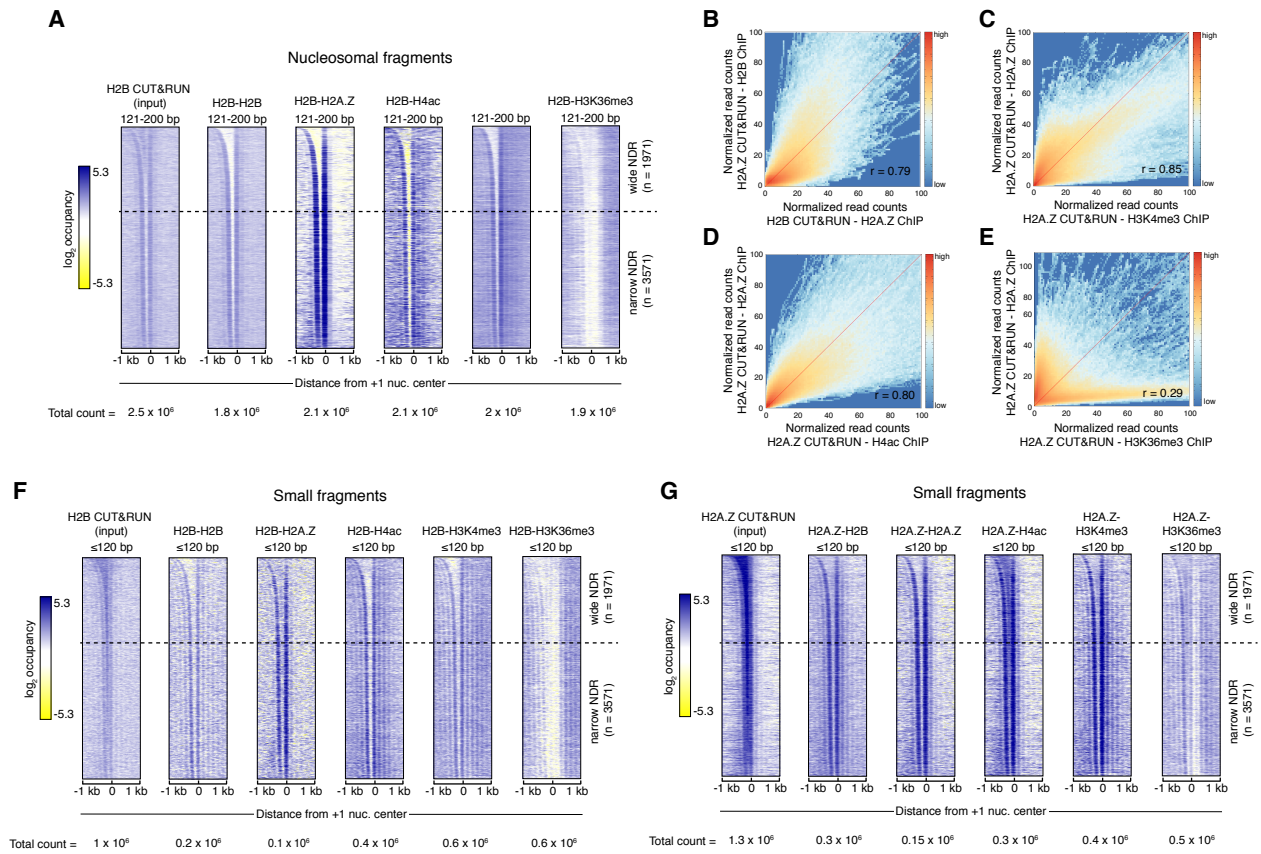
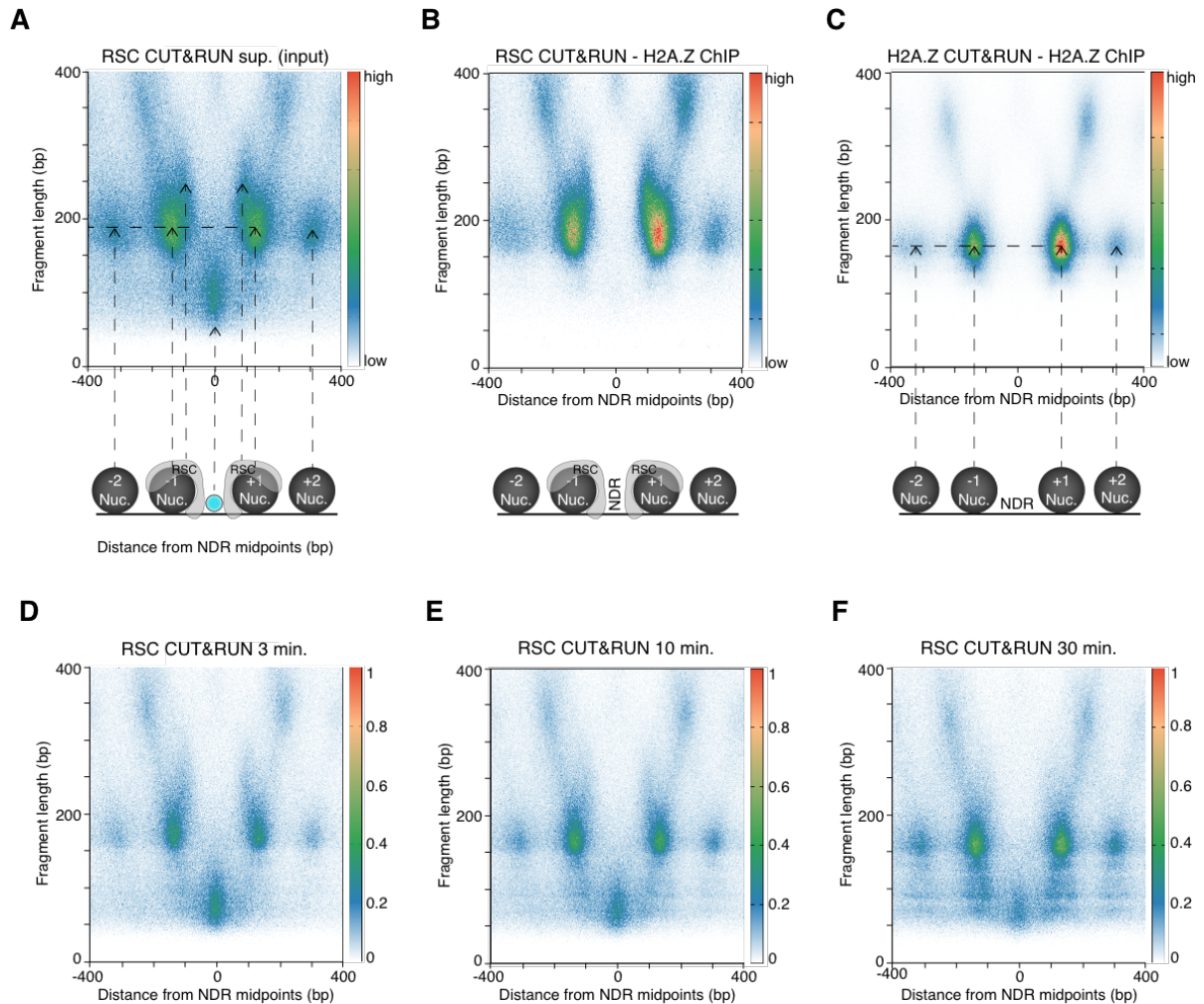


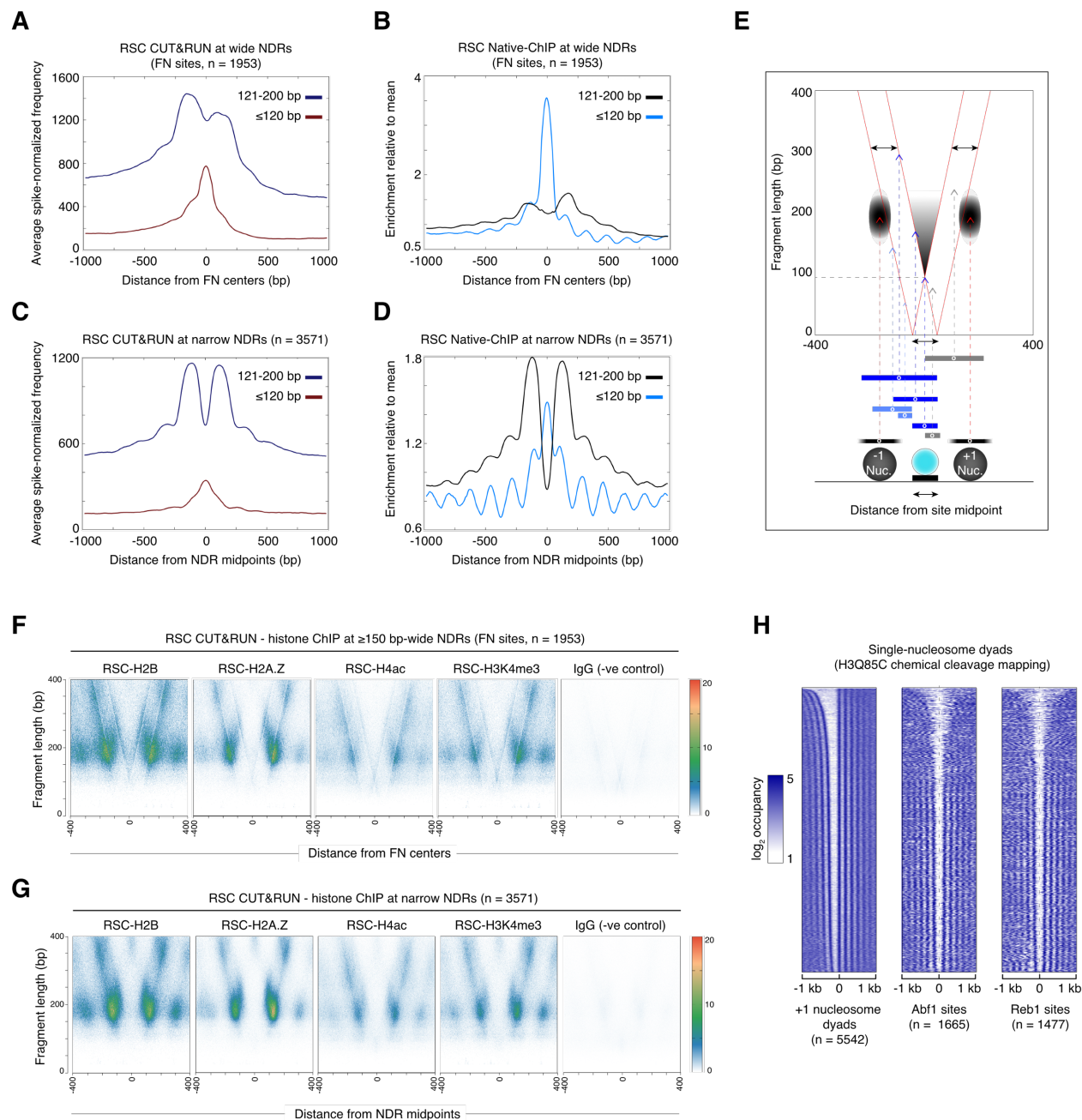
# SUPPLEMENTARY FIGURES



**Figure S1.** Related to Figure 1. (A) Heat maps of 121-200 bp (nucleosomal) fragments from H2B CUT&RUN.ChIP spanning +1 nucleosome centers  $\pm 1$  kb, and sorted by NDR width. (B-E) Scatterplots comparing total-count normalized 121-200 bp reads from CUT&RUN.ChIP with the indicated pairs of first (CUT&RUN) and second (ChIP) antibodies. Pearson correlation coefficient values ( $r$ ) are shown for each scatter plot. (F-G) Heat maps of H2B (F) and H2A.Z (G) CUT&RUN.ChIP similar to (A) and Figure 1D, but for  $\leq 120$  bp (subnucleosomal or short) fragments. All heat maps in the figure are total count-normalized (total number of reads for each sample and size class are mentioned at the bottom of each heat map), and shown in  $\log_2$  scale, with  $\log$  center = 5 and contrast = 3. Dotted lines separate wide NDRs ( $\geq 150$ -bp) from the rest.

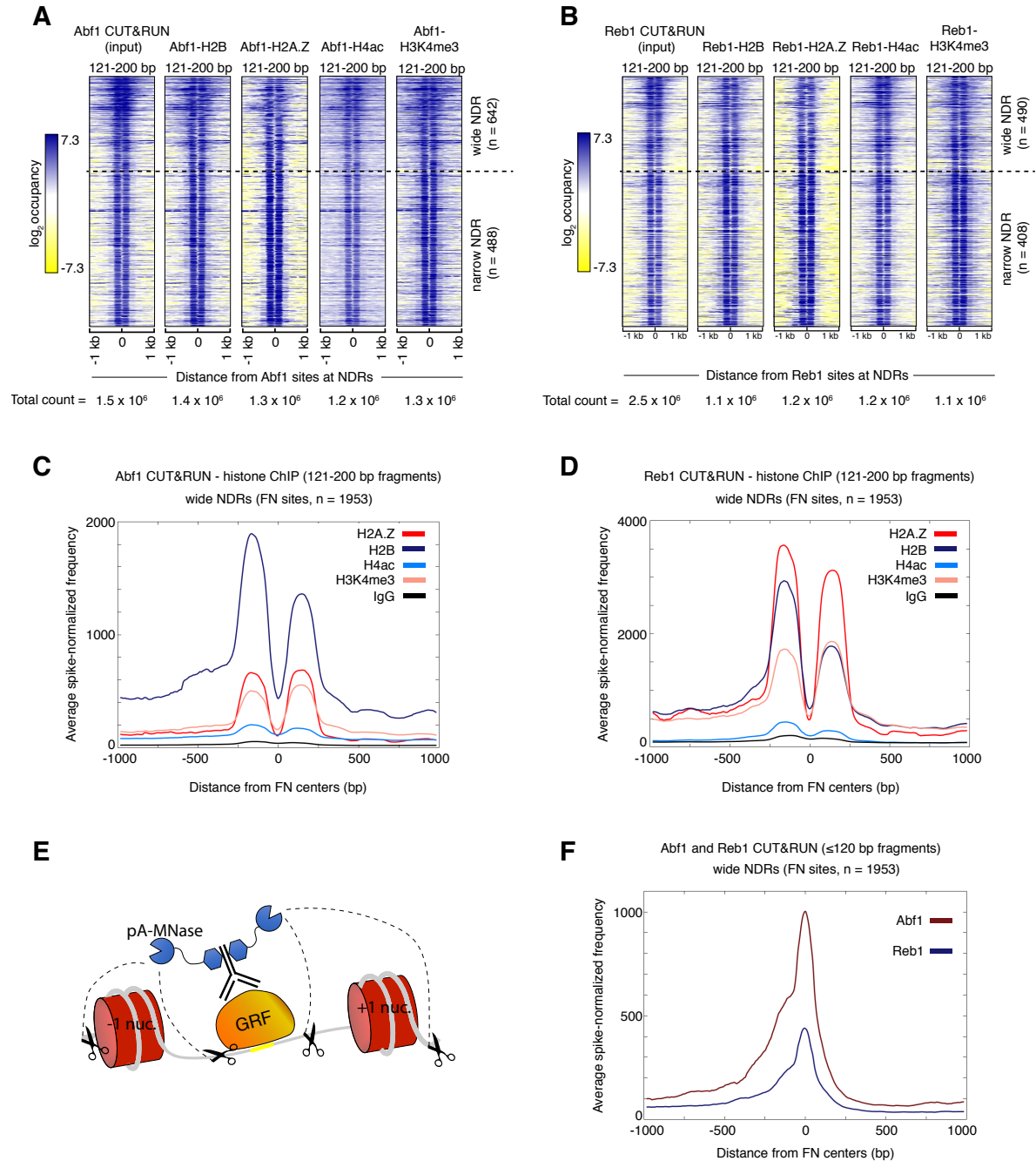


**Figure S2.** Related to Figure 2. (A-C) V-plots of spike-in normalized reads mapped within the 800-bp regions spanning the centers of NDRs from the experiments indicated at the top of each plot. A fragment that spans an MNase-protected region results in a pixel on the plot, where pixel color scales with fragment-count frequency. For detailed interpretation of V-plots see Figure S3E. In panel B (illustration at the bottom), arrows show the positions on the plot where fragments/pixels corresponding to free nucleosomes, RSC-engulfed nucleosomes, and a small footprint over the NDR map. Horizontal dotted lines in A and C show the average size of nucleosomal fragments obtained from RSC versus H2A.Z CUT&RUN. (D-F) V-plots of reads mapped within the 800-bp regions spanning the centers of NDRs from RSC CUT&RUN time-course (D= 3 min, E= 10 min, F= 30 min) ([Skene and Henikoff, 2017](#)).



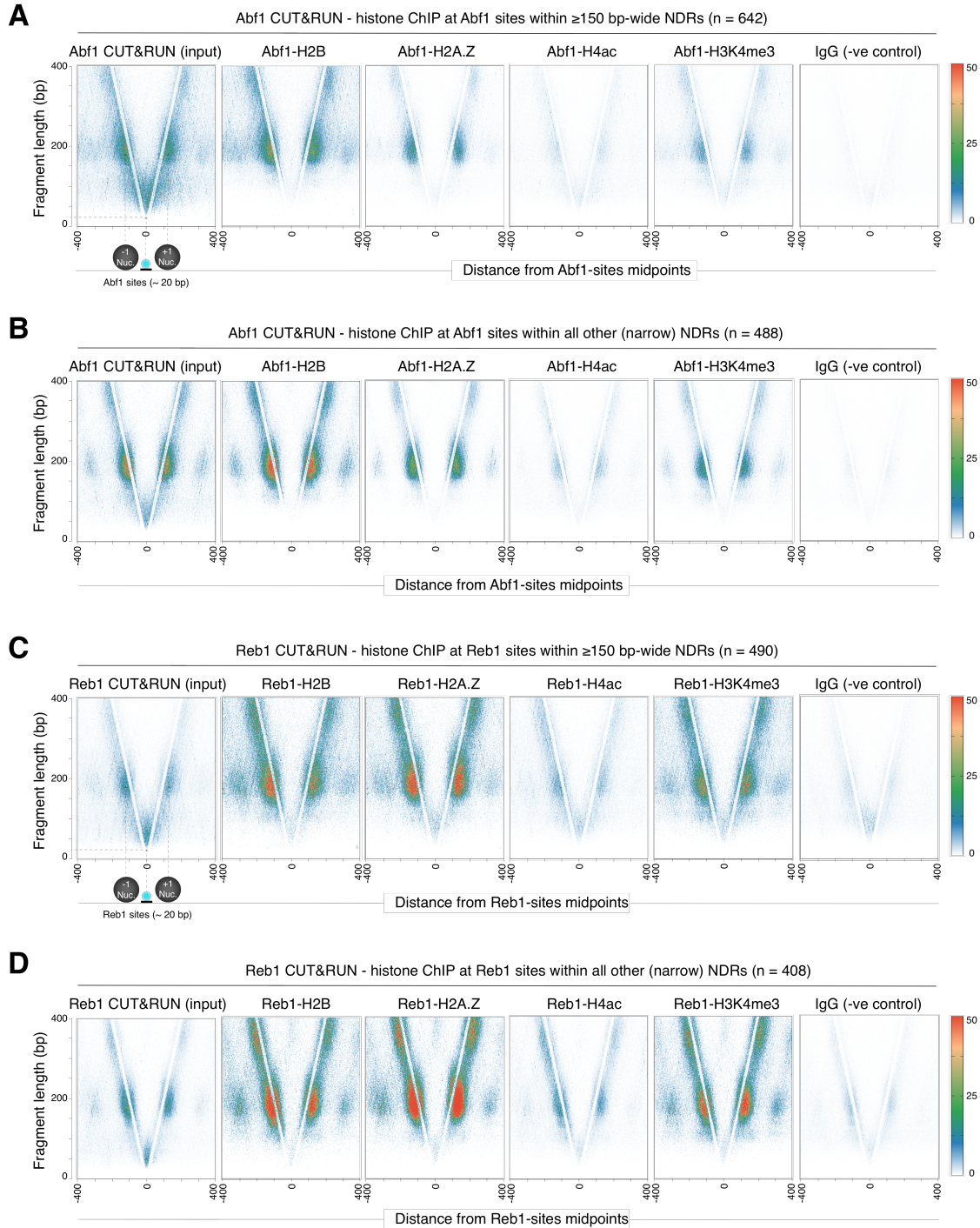
**Figure S3.** Related to Figures 2 and 3. (A-D) Relative enrichments of spike-in normalized densities of 121-200 bp (nucleosomal) and  $\leq 120$  bp (subnucleosomal) fragments from RSC CUT&RUN (A, C) and RSC Native-ChIP (B, D) over the  $\pm 1$ -kb region spanning the NDRs, plotted separately for  $\geq 150$  bp-wide NDRs containing previously annotated ([Kubik et al., 2015](#)) fragile nucleosome (FN) sites (A, B) and narrow (all other) NDRs (C, D). (E) Schematic diagram illustrates how the V-plots

shown in Figure 3A, B and panels F and G in this figure are interpreted. Dot clusters over the midpoint in the plot form a V-shape, and the y-axis position of the vertex shows limit digestion, indicated by a horizontal dotted line. Representative fragments are shown below the graph, and arrows show where each fragment maps, resulting in a dot. A fragment that spans the central protected region (lower blue bar) results in a dot at the vertex of the V. The inner left diagonal results from fragments cleaved precisely on the right side of the protected region (remaining blue bars) and likewise for the right diagonal. The outer left diagonal results from fragments cleaved precisely on the left side of the protected region (cyan bars) and likewise for the right diagonal. Representative fragments spanning the phased nucleosomes are shown as black bars, and grey bars indicate fragments resulting from a cleavage within the central protected region. (F, G) V-plots of spike-in normalized reads from RSC CUT&RUN-histone ChIP-seq (RSC CUT&RUN.ChIP) experiments. V-plots spanning  $\pm 400$  bp from the centers of fragile nucleosome (FN) sites at  $\geq 150$  bp-wide NDRs (F) or the midpoints of narrow (all other) NDRs (G) are shown separately. (H) Heat maps of H3Q85C chemical cleavage mapping ([Chereji et al., 2018](#)) spanning  $\pm 1$  kb of +1 nucleosomes (left), Abf1 binding sites (middle), and Reb1 binding sites (right). Shown here are densities of 44-58 bp fragments, which correspond to single-nucleosome dyads. Heat maps are shown in  $\log_2$  scale, with  $\log$  center = 1 and contrast = 5.



**Figure S4.** Related to Figure 4. (A, B) Heat maps of 121-200 bp (nucleosomal) fragments from Abf1 (A) and Reb1 (B) CUT&RUN.ChIP spanning  $\pm 1$  kb of the respective GRF binding sites. Heat maps are total count-normalized and shown in log<sub>2</sub> scale, with log center = 5 and contrast = 5. Dotted lines separate wide NDRs ( $\geq 150$ -bp) from the rest. (C, D) Enrichment of spike-in normalized densities of 121-200 bp

(nucleosomal) fragments from Abf1 (C) and Reb1 (D) CUT&RUN.ChIP over the  $\pm 1$ -kb region spanning the fragile nucleosome (FN) sites within  $\geq 150$  bp-wide NDRs. (E) An illustration showing that CUT&RUN with pA-MNase targeted to a GRF or a transcription factor releases the factor binding site as well as the flanking nucleosomes due to flexibility of the MNase tether ([Skene and Henikoff, 2017](#)). (F) Enrichment of spike-in normalized densities of  $\leq 120$  bp (subnucleosomal) fragments from Abf1 and Reb1 CUT&RUN used as input for subsequent histone ChIP-seq experiments shown in Figure 4C, D; over the  $\pm 1$ -kb region spanning FN sites within  $\geq 150$  bp-wide NDRs.



**Figure S5.** Related to Figure 4. V-plots of spike-in normalized reads from Abf1 and Reb1 CUT&RUN-histone ChIP-seq experiments. V-plots spanning  $\pm 400$  bp from the centers of Abf1 (A, B) and Reb1 (C, D) sites within  $\geq 150$  bp-wide NDRs (A, C) or  $< 150$  bp (narrow) NDRs (B, D) are shown separately. In panels A and C, arrows show the

positions on the plots where fragments/pixels correspond to nucleosomes or the bound GRF. Horizontal dotted lines show the length in base pairs of limit MNase digestion around the central protected region.