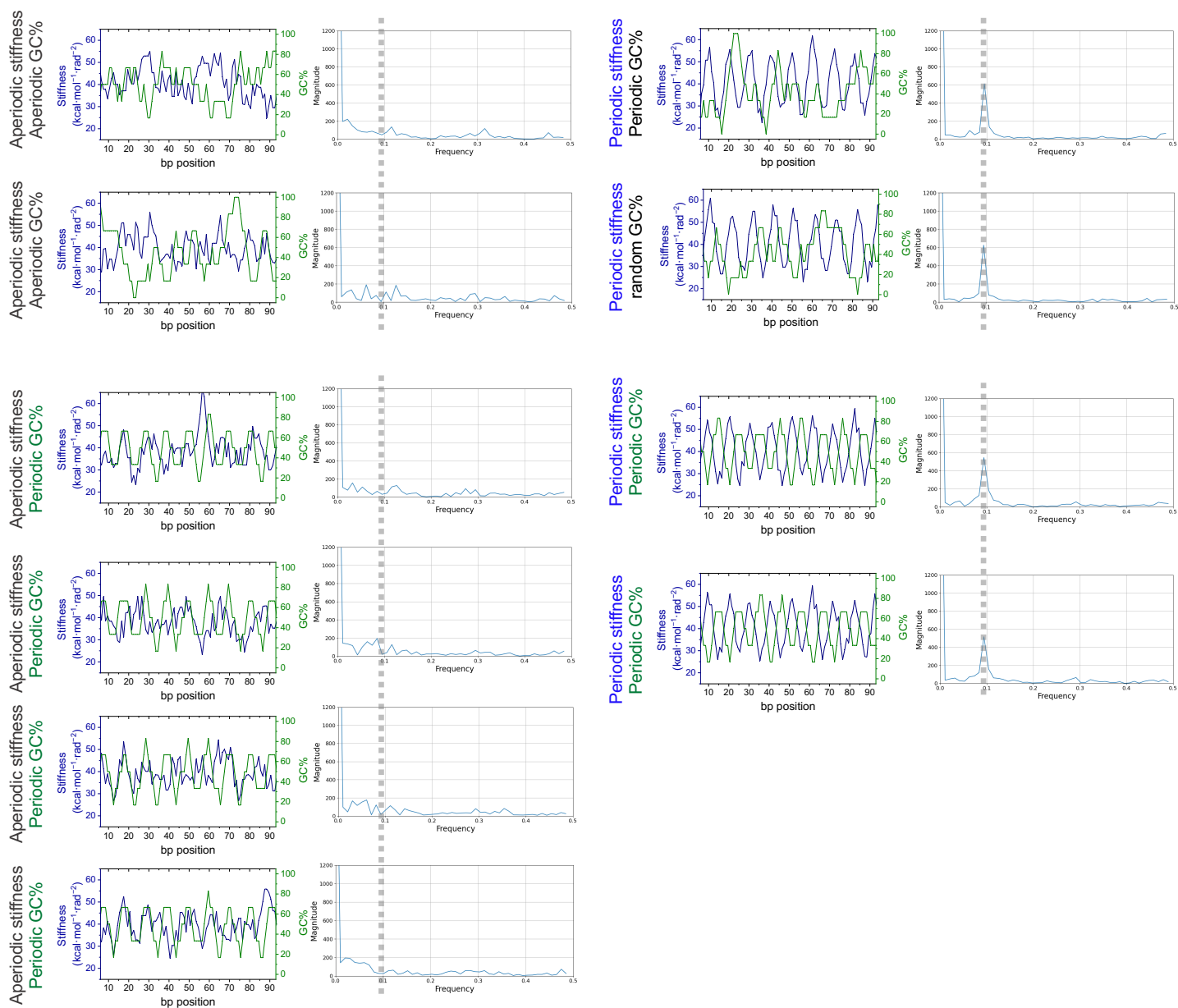
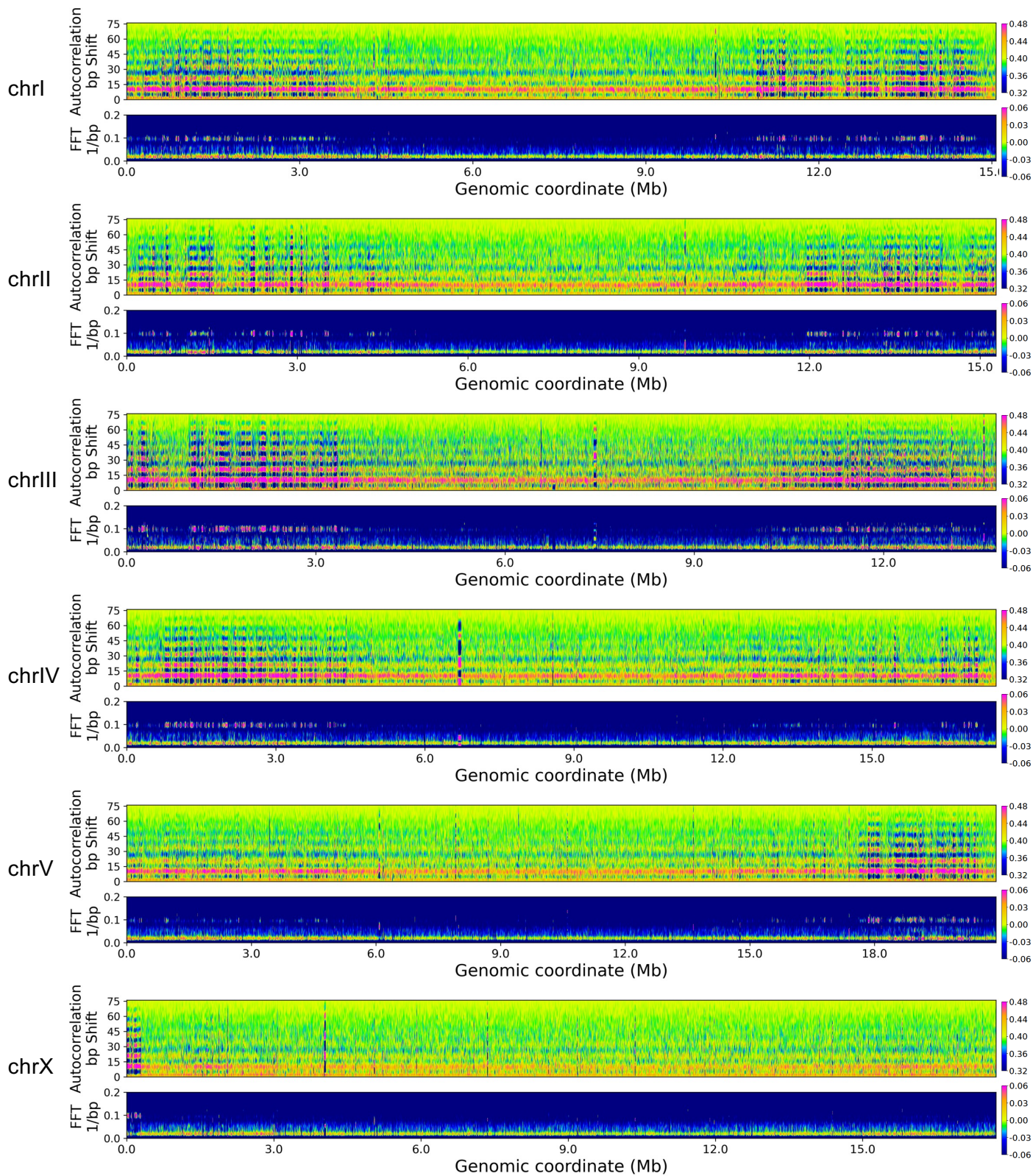


# Supplementary Figure 1



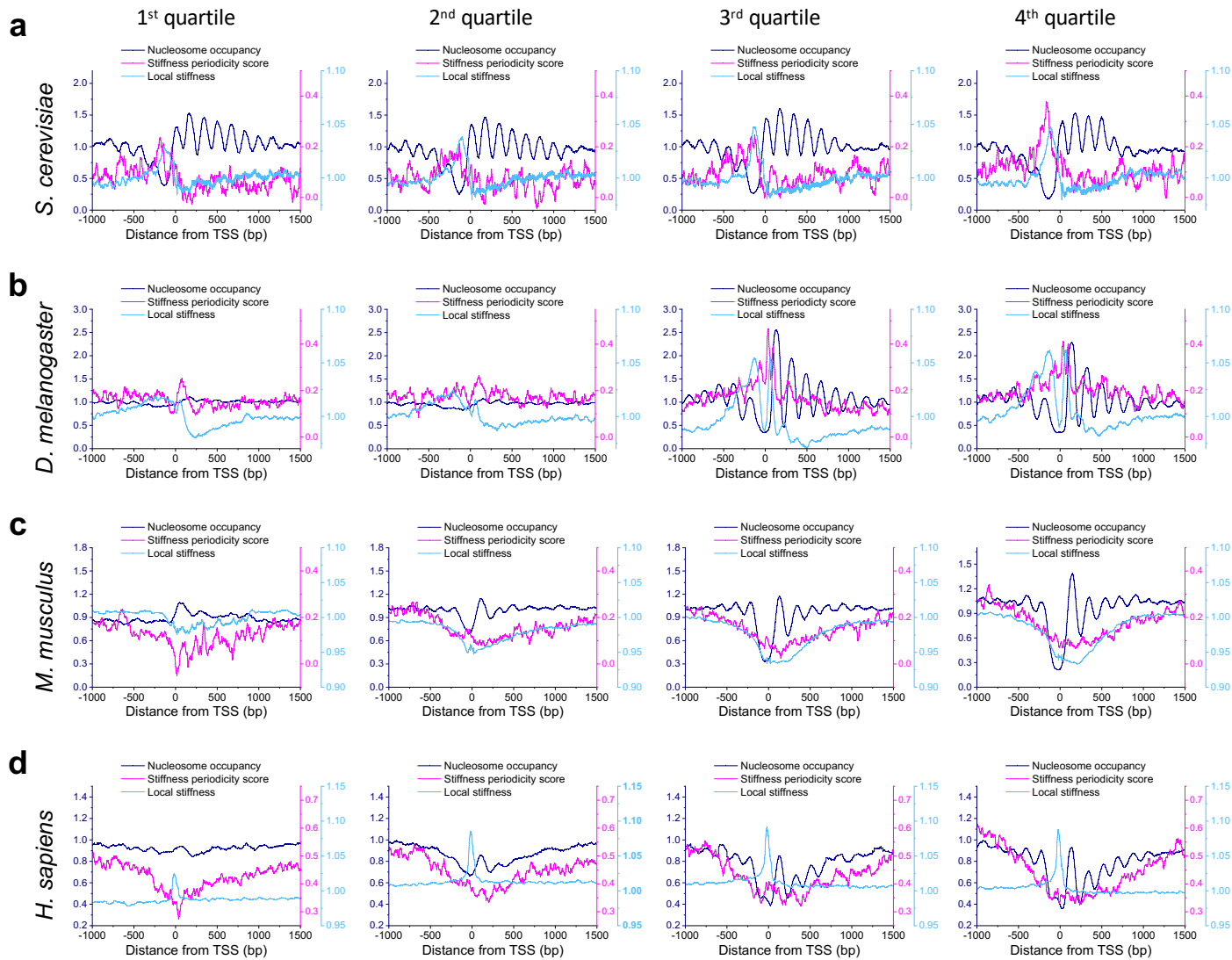
**Stiffness and GC content profiles of all 100 bp DNAs with independently controlled periodicity of stiffness and GC content.** Stiffness and GC content profiles corresponding to all data points in Figure 1H are shown. The right panels display the fast Fourier transform of the stiffness profiles. Gray dotted lines indicate a frequency corresponding to a 10.4 bp periodicity.

## Supplementary Figure 2



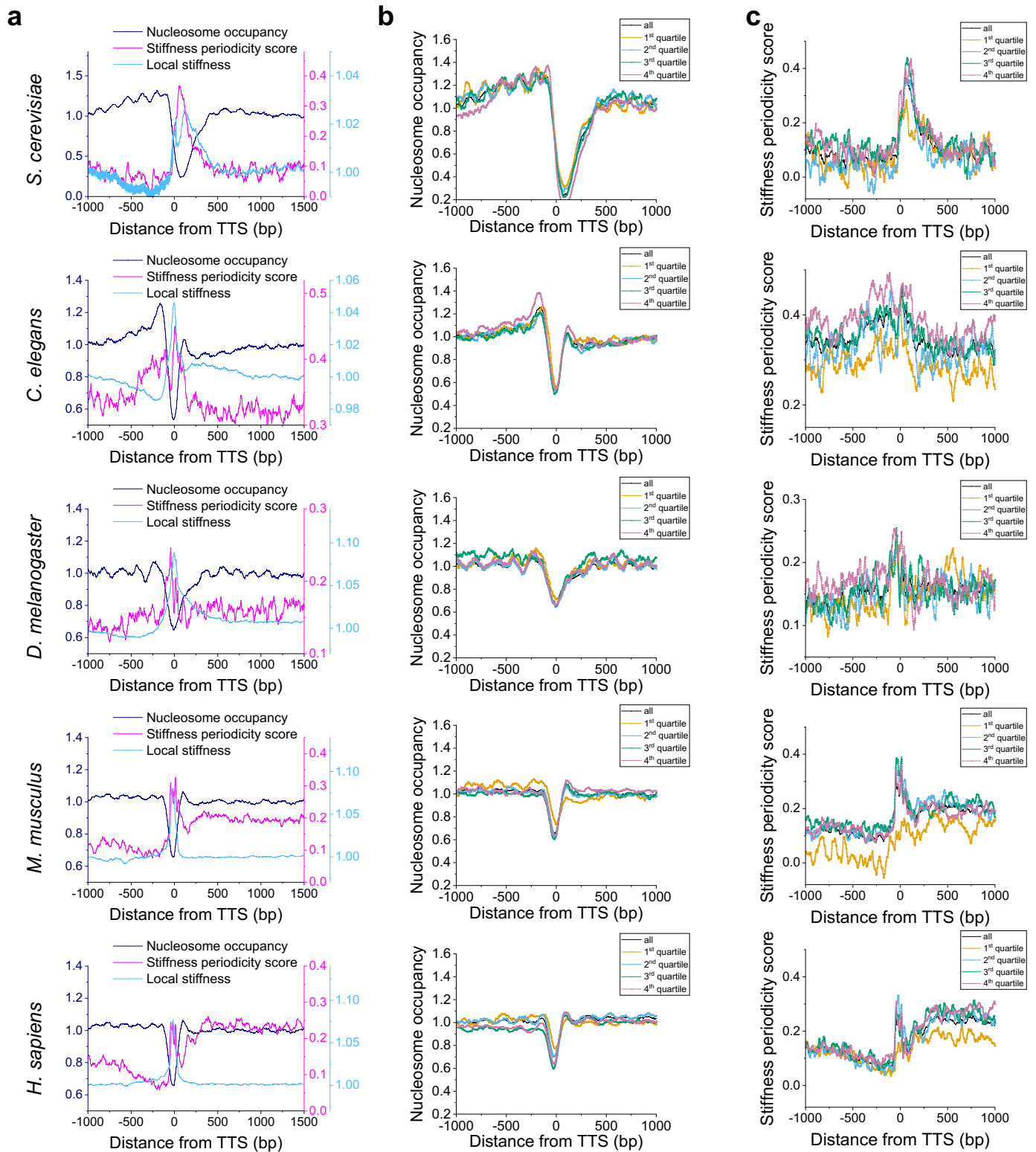
**Genome-wide stiffness autocorrelation spectrograms of the *C. elegans* genome.** Stiffness autocorrelation spectrograms for the six chromosomes (chrI–V and chrX; top panels), along with the corresponding fast Fourier transform spectra (bottom panels), reveal the distribution of stiffness periodicity across the genome. Stiffness periodicity is concentrated in specific regions of each chromosome.

## Supplementary Figure 3



**Stiffness periodicity, local stiffness, and nucleosome occupancy profiles near TSS across gene expression quartiles.** **a-d**, Stiffness periodicity score (magenta), local stiffness (cyan), and nucleosome occupancy (navy) near TSS were averaged across genes in each gene expression quartile and are shown for *S. cerevisiae* (**a**), *D. melanogaster* (**b**), *M. musculus* (**c**), and *H. sapiens* (**d**). Y-axes are color-matched to their corresponding profiles. Higher expressed genes exhibit more pronounced variations in stiffness periodicity and/or local stiffness, which are correlated with patterns in nucleosome occupancy in a species-specific manner.

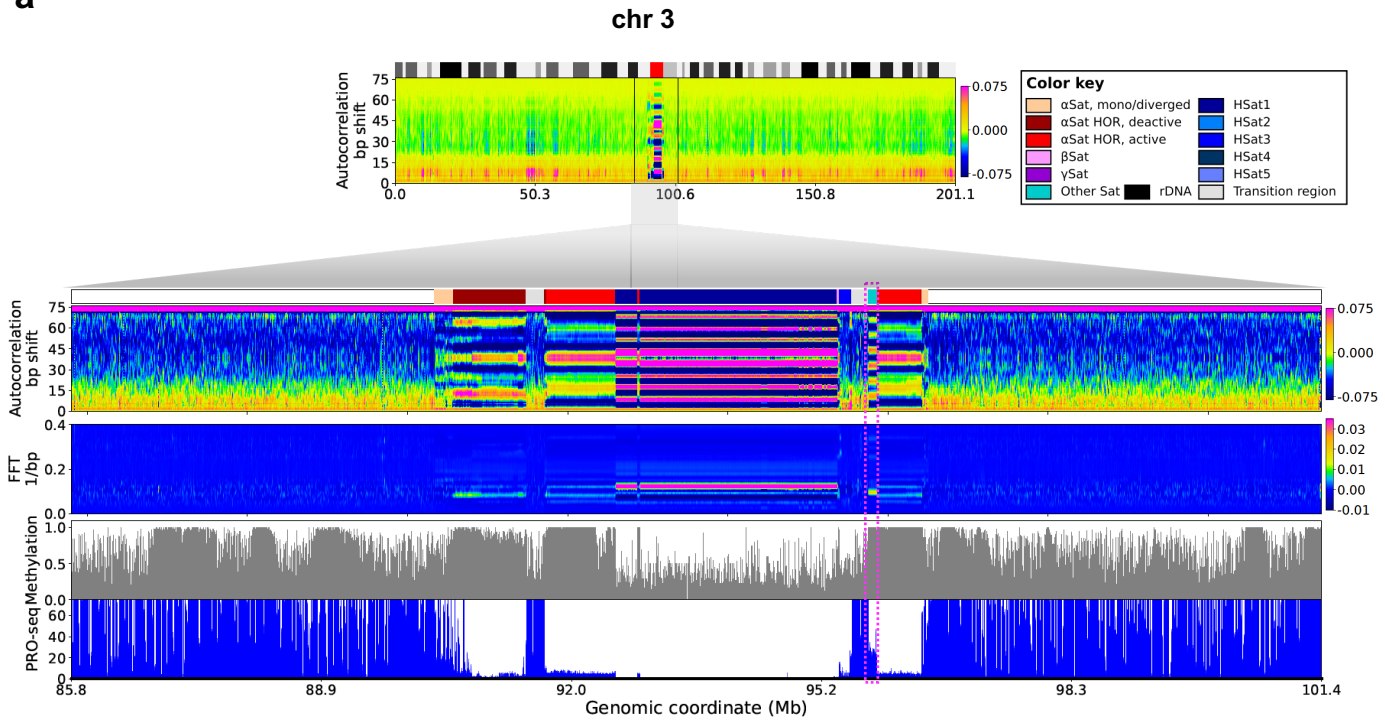
# Supplementary Figure 4



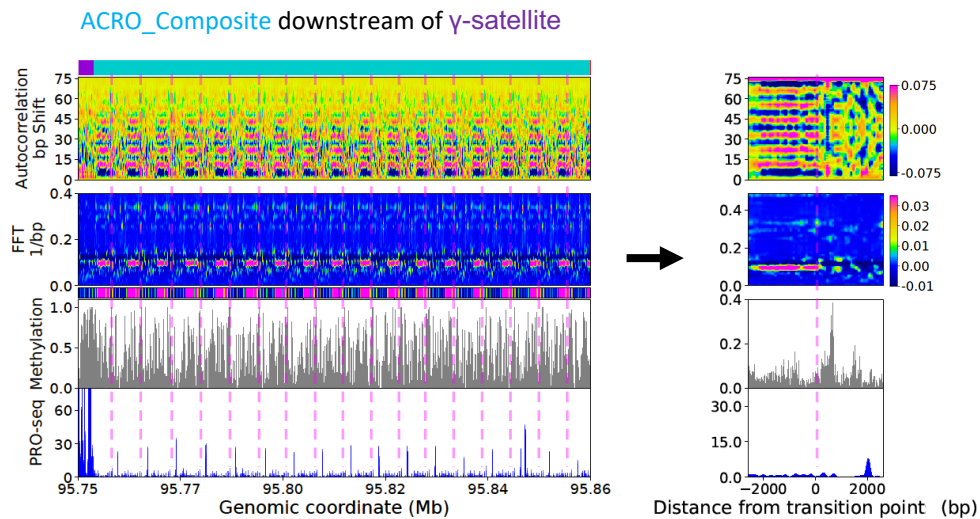
**Stiffness periodicity, local stiffness, and nucleosome occupancy profiles near TTS across species. a**, Stiffness periodicity score (magenta), local stiffness (cyan), and nucleosome occupancy (navy) near TTS were averaged across genes and are shown for each species. Y-axes are color-matched to their corresponding profiles. **b**, Nucleosome occupancy profiles near TTS for each gene expression quartile. **c**, Stiffness periodicity profiles near TTS for each gene expression quartile.

# Supplementary Figure 5

**a**

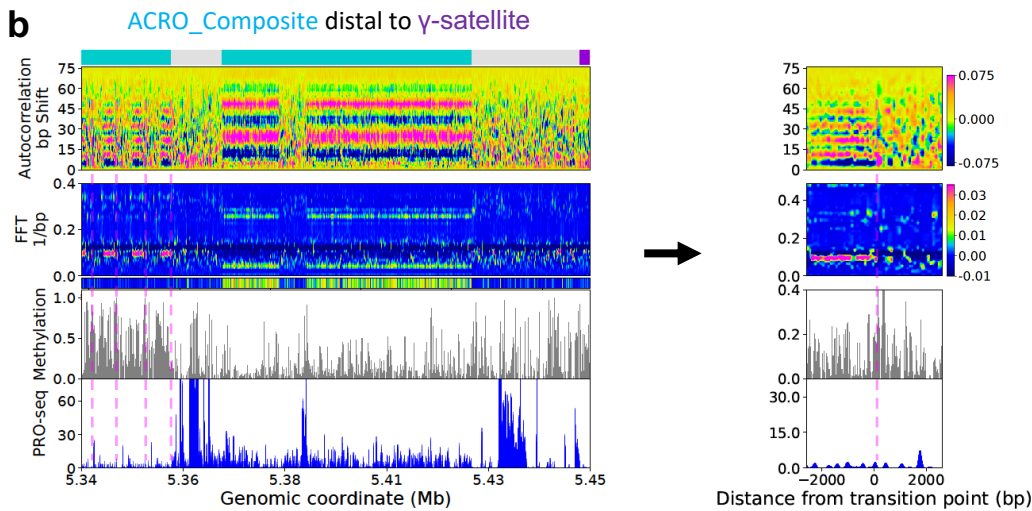
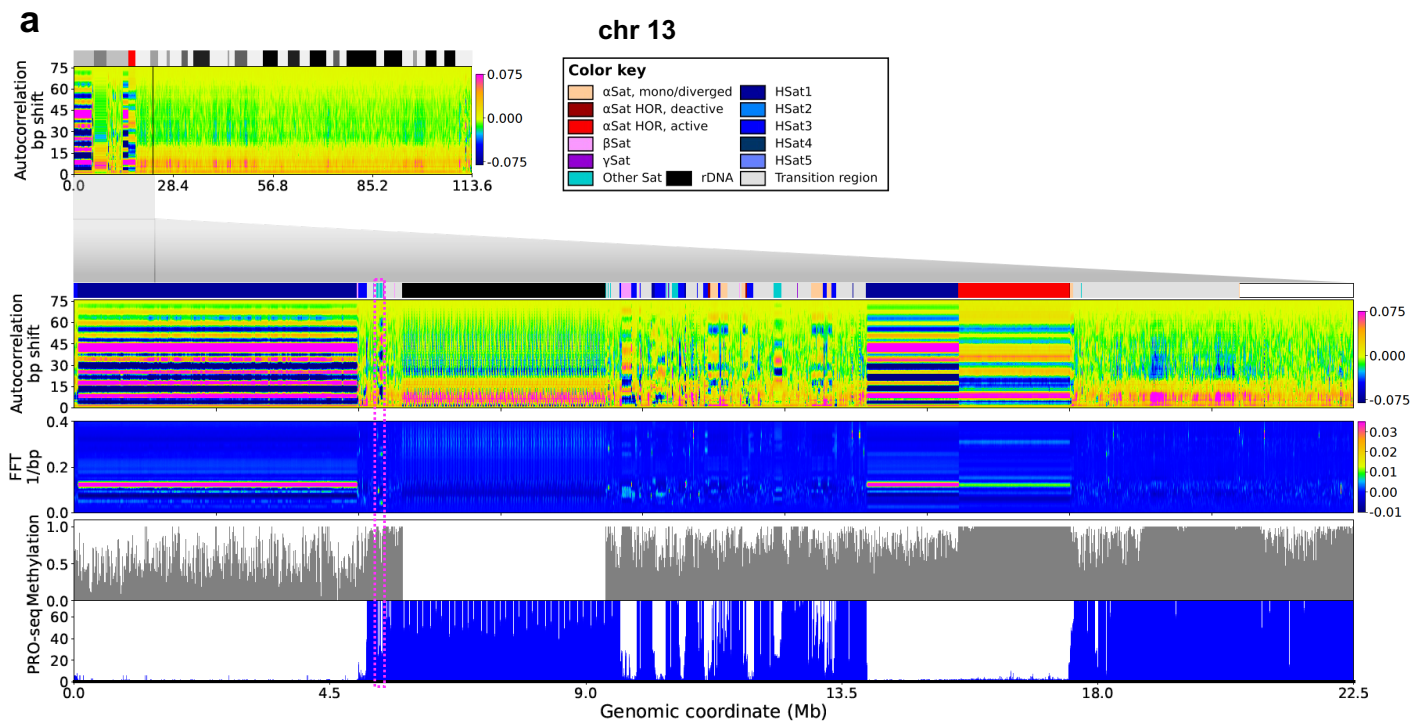


**b**



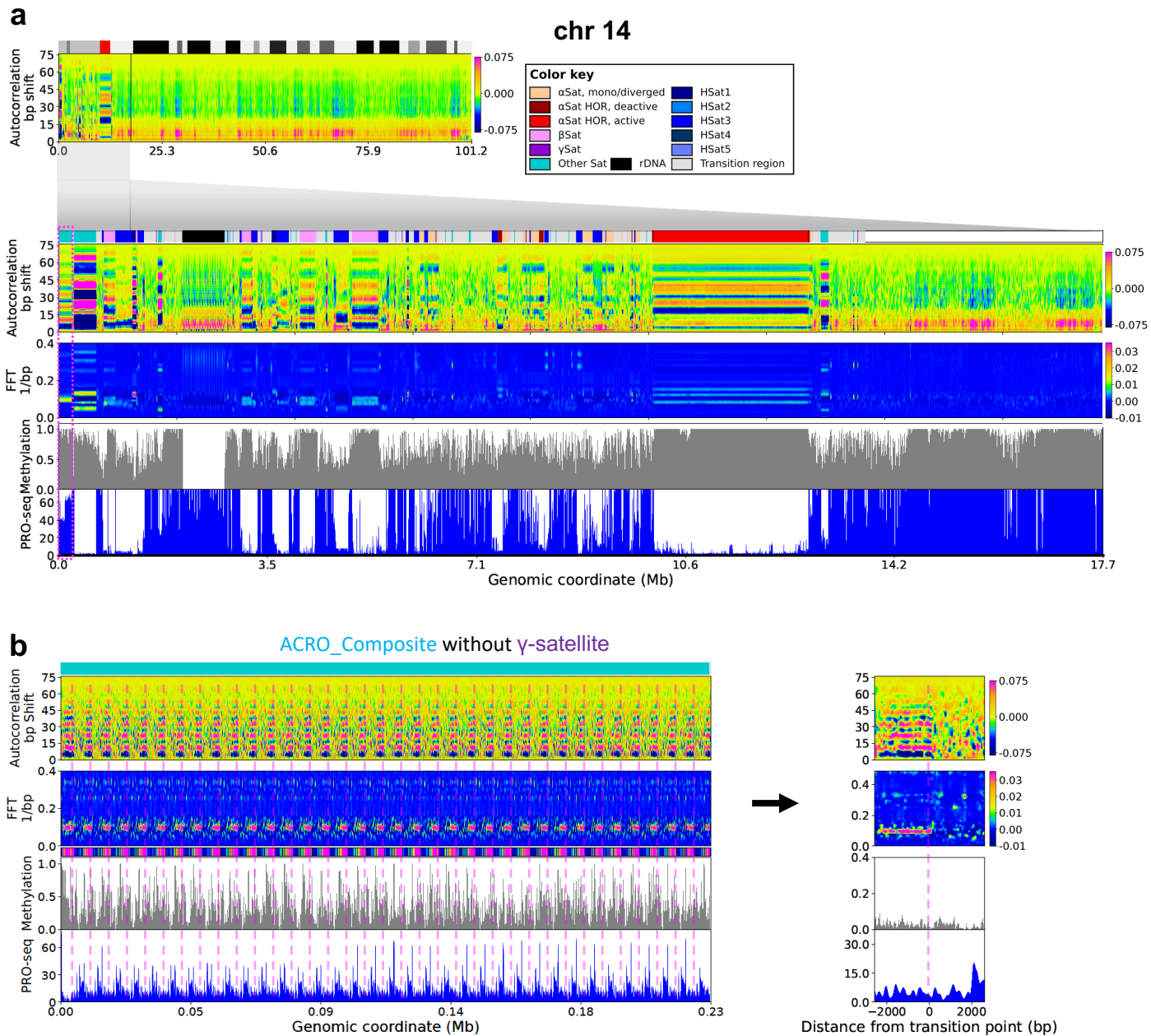
**Periodic stiffness patterns in chromosome 3 centromeric region.** **a**, Top: Stiffness autocorrelation spectrogram of chromosome 3. Bottom: Spectrogram of the centromeric region, shown together with its fast Fourier transform (FFT), CpG methylation, and PRO-seq profiles. **b**, Expanded view of the ACRO\_Composite region downstream of  $\gamma$ -satellite (magenta box in (a)). Spectrogram, its FFT, CpG methylation, and PRO-seq profiles of ACRO\_Composite monomers are shown on the right, overlaid by alignment at the periodic-aperiodic boundary.

# Supplementary Figure 6



**Periodic stiffness patterns in chromosome 13 centromeric region.** **a**, Top: Stiffness autocorrelation spectrogram of chromosome 13. Bottom: Spectrogram of the centromeric region, shown together with its FFT, CpG methylation, and PRO-seq profiles. **b**, Expanded view of the ACRO\_Composite region distal to  $\gamma$ -satellite (magenta box in (a)). Spectrogram, its FFT, CpG methylation, and PRO-seq profiles of ACRO\_Composite monomers are shown on the right, overlaid by alignment at the periodic-aperiodic boundary.

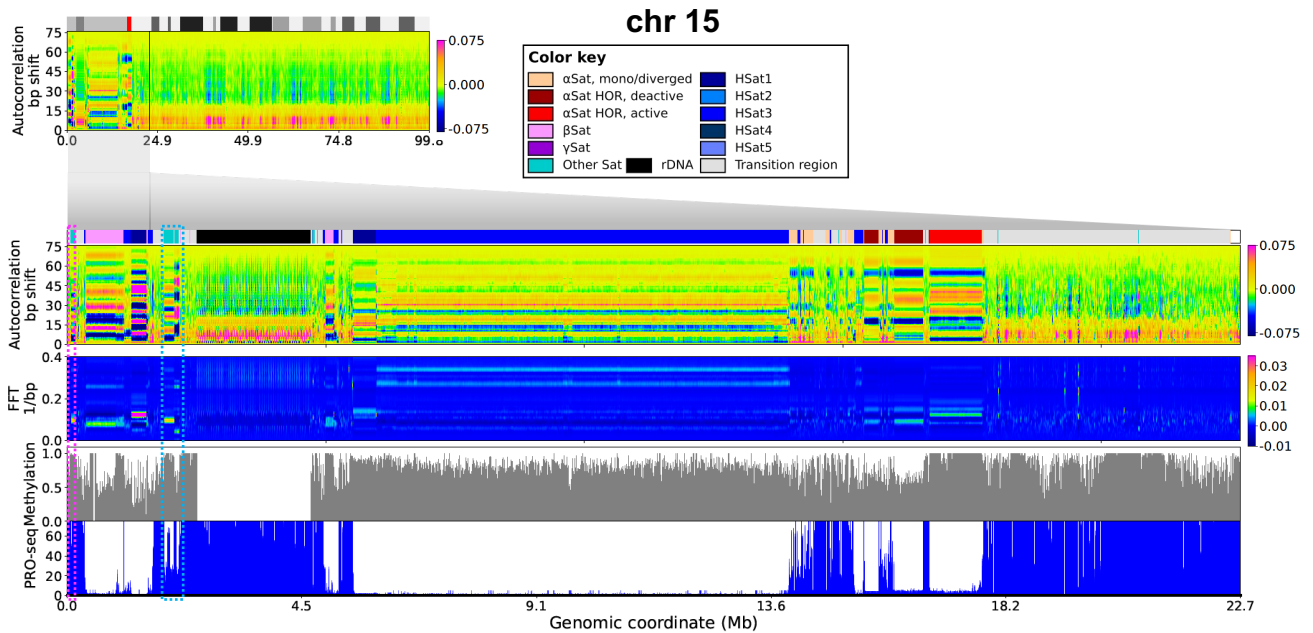
# Supplementary Figure 7



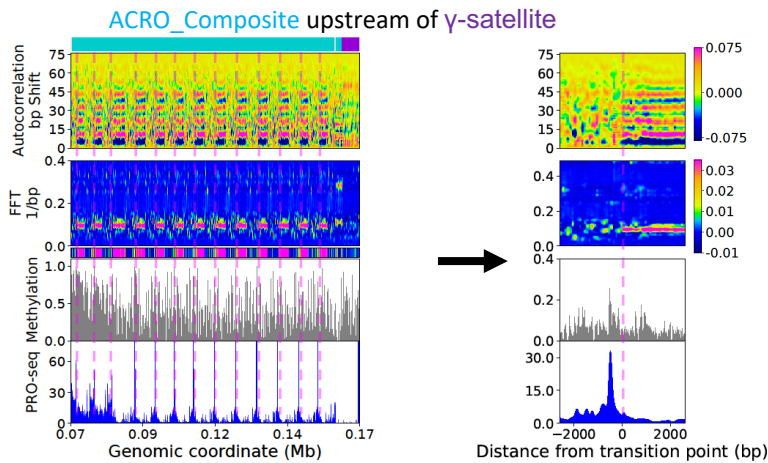
**Periodic stiffness patterns in chromosome 14 centromeric region.** **a**, Top: Stiffness autocorrelation spectrogram of chromosome 14. Bottom: Spectrogram of the centromeric region, shown together with its FFT, CpG methylation, and PRO-seq profiles. **b**, Expanded view of the ACRO\_Composite (magenta box in (a)). Spectrogram, its FFT, CpG methylation, and PRO-seq profiles of ACRO\_Composite monomers are shown on the right, overlaid by alignment at the periodic-aperiodic boundary.

# Supplementary Figure 8

**a**

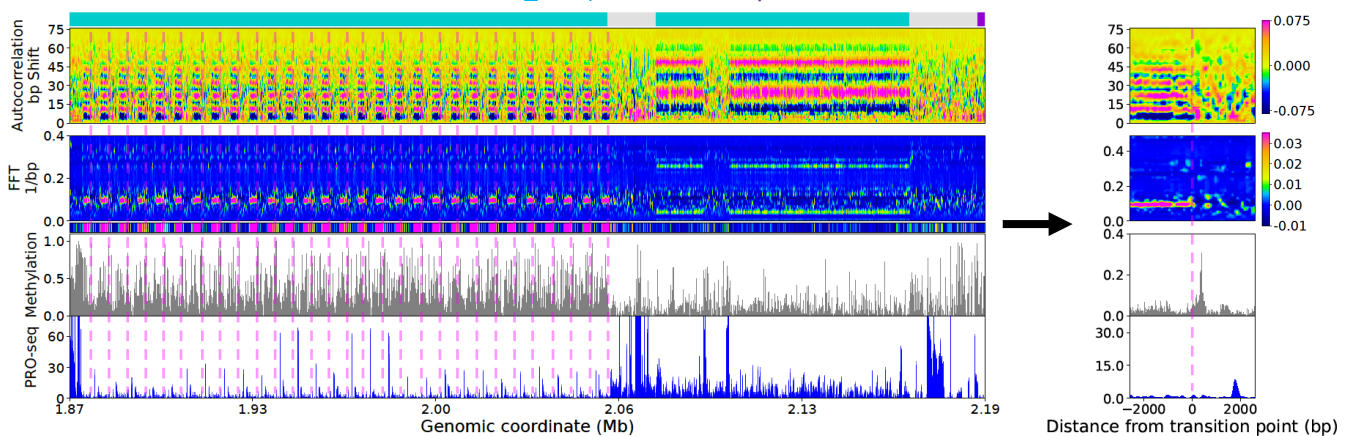


**b**



**c**

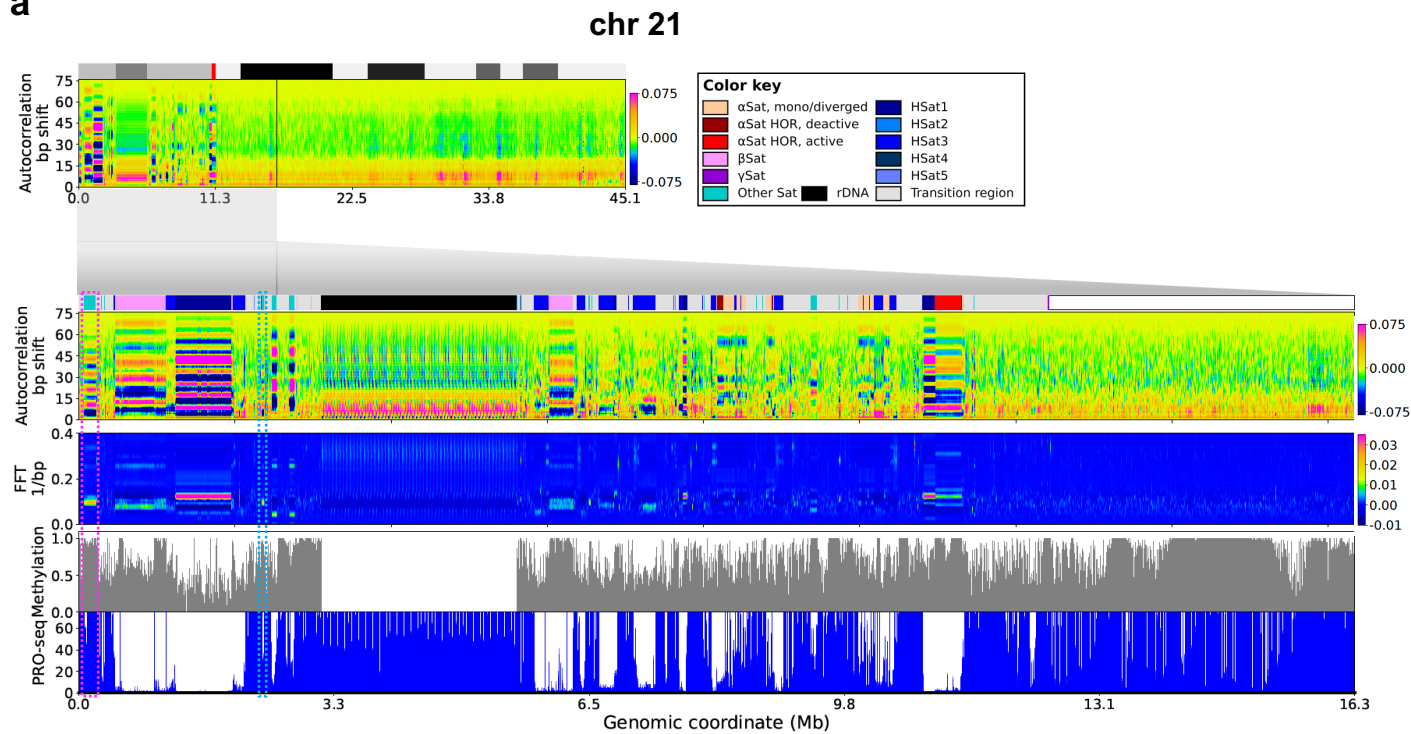
**ACRO\_Composite distal to  $\gamma$ -satellite**



**Periodic stiffness patterns in chromosome 15 centromeric region.** **a**, Top: Stiffness autocorrelation spectrogram of chromosome 15. Bottom: Spectrogram of the centromeric region, shown together with its FFT, CpG methylation, and PRO-seq profiles. **b-c**, Expanded view of the ACRO\_Composite regions upstream of (**b**; magenta box in **(a)**) and distal to (**c**; cyan box in **(a)**)  $\gamma$ -satellite. Spectrogram, its FFT, CpG methylation, and PRO-seq profiles of ACRO\_Composite monomers are shown on the right, overlaid by alignment at the periodic-aperiodic boundary.

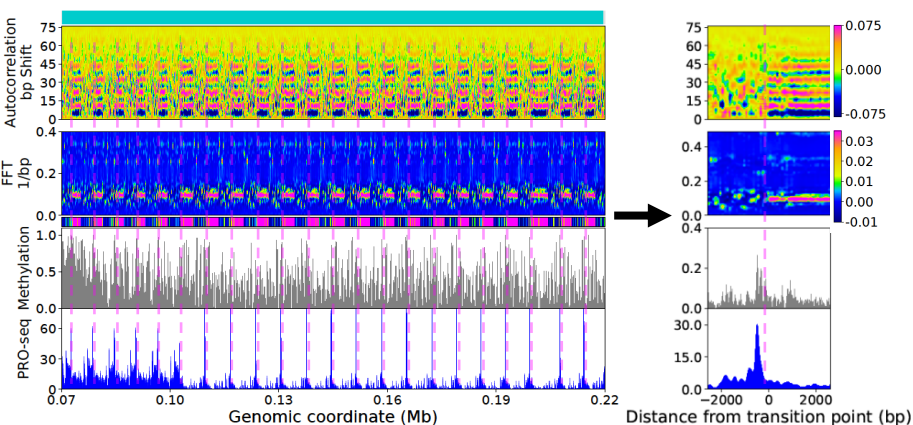
# Supplementary Figure 9

**a**



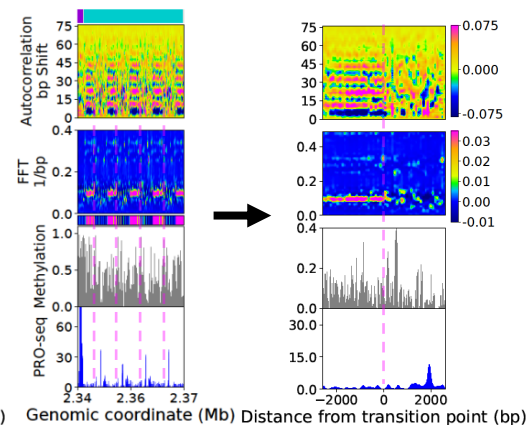
**b**

ACRO\_Composite upstream of  $\gamma$ sat



**c**

ACRO\_Composite downstream of  $\gamma$ sat



**Periodic stiffness patterns in chromosome 21 centromeric region.** (a) Top: Stiffness autocorrelation spectrogram of chromosome 21. Bottom: Spectrogram of the centromeric region, shown together with its FFT, CpG methylation, and PRO-seq profiles. (b, c) Expanded view of the ACRO\_Composite regions upstream (b; magenta box in (a)) and downstream (c; cyan box in (a)) of  $\gamma$ -satellite. Spectrogram, its FFT, CpG methylation, and PRO-seq profiles of ACRO\_Composite monomers are shown on the right, overlaid by alignment at the periodic-aperiodic boundary.