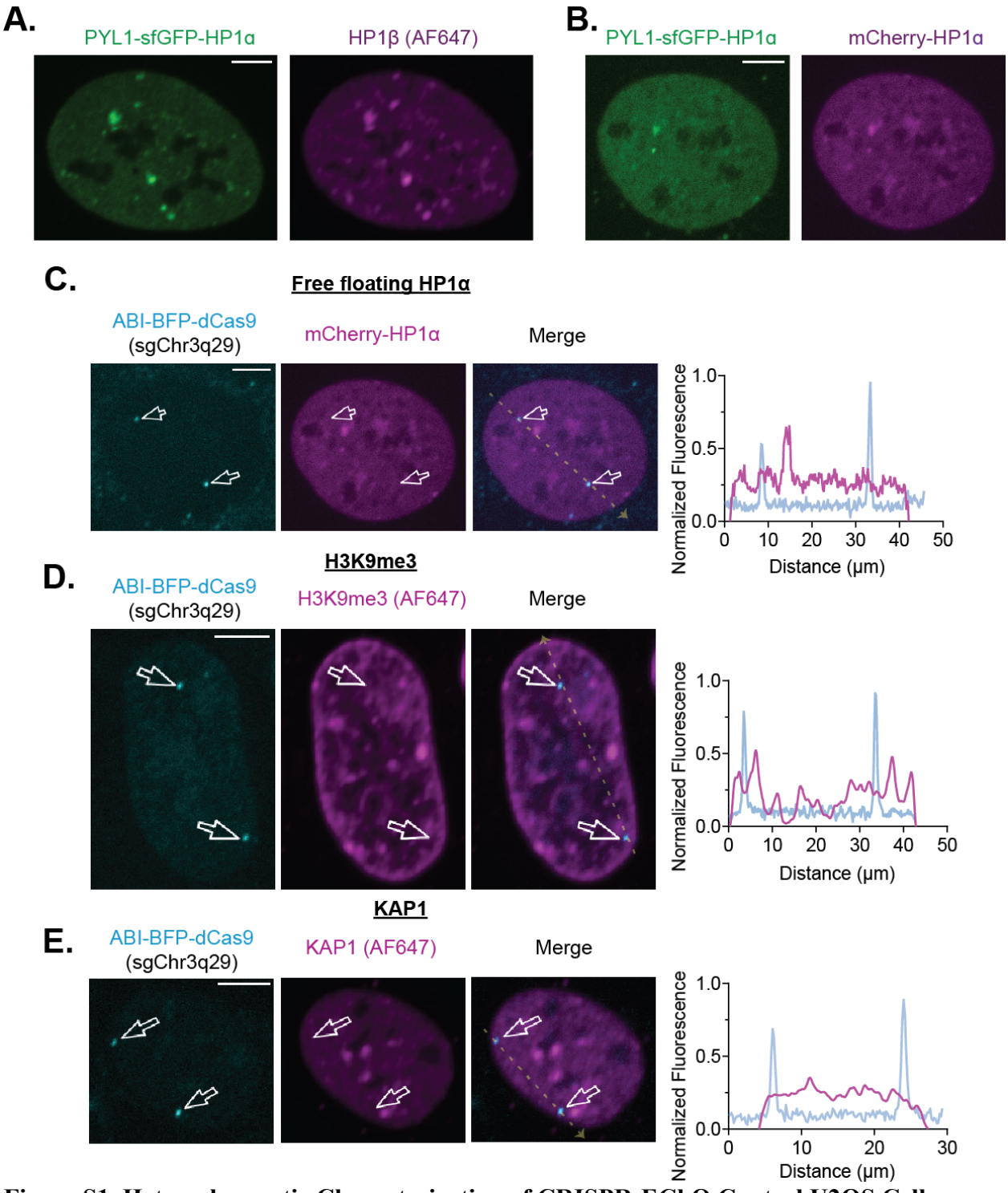

Figure S1. Heterochromatic Characterization of CRISPR-EChO Control U2OS Cells, Related to Figure 1.

(A) Representative confocal microscopy image showing PYL1-sfGFP-HP1α with similar nuclear localization as endogenous HP1β. U2OS cells expressing PYL1-sfGFP-HP1α are immunostained for HP1β and visualized using an AlexaFluor 647-conjugated secondary. **(B)** Representative confocal microscopy image showing PYL1-sfGFP-HP1α with similar nuclear localization as

ectopic mCherry-labeled HP1α. **(C)** Representative confocal microscopy image showing that ABI-tagBFP-dCas9 bound Chr3q29 loci do not exhibit high mCherry-HP1α signal. The same cell is visualized in **(B)** and **(C)** for different purposes. **(D)** Representative confocal microscopy image showing that ABI-tagBFP-dCas9 bound Chr3q29 loci do not exhibit high H3K9me3 signal. Cells were immunostained for H3K9me3 and visualized with an AlexaFluor 647-conjugated secondary. **(E)** Representative confocal microscopy image showing that ABI-tagBFP-dCas9 bound Chr3q29 loci do not exhibit high KAP1 signal. Cells were immunostained for KAP1 and visualized with an AlexFluor 647-conjugated secondary. In **(C-E)**, white arrows indicate nuclear positions of Chr3q29. Right plots show line scans of fluorescence intensities for each color channel along the dotted yellow line in each left image. Fluorescence intensities are normalized to the maximum (1) and minimum (0) intensities within the nucleus in the image. Scale bars represent 10 μm.

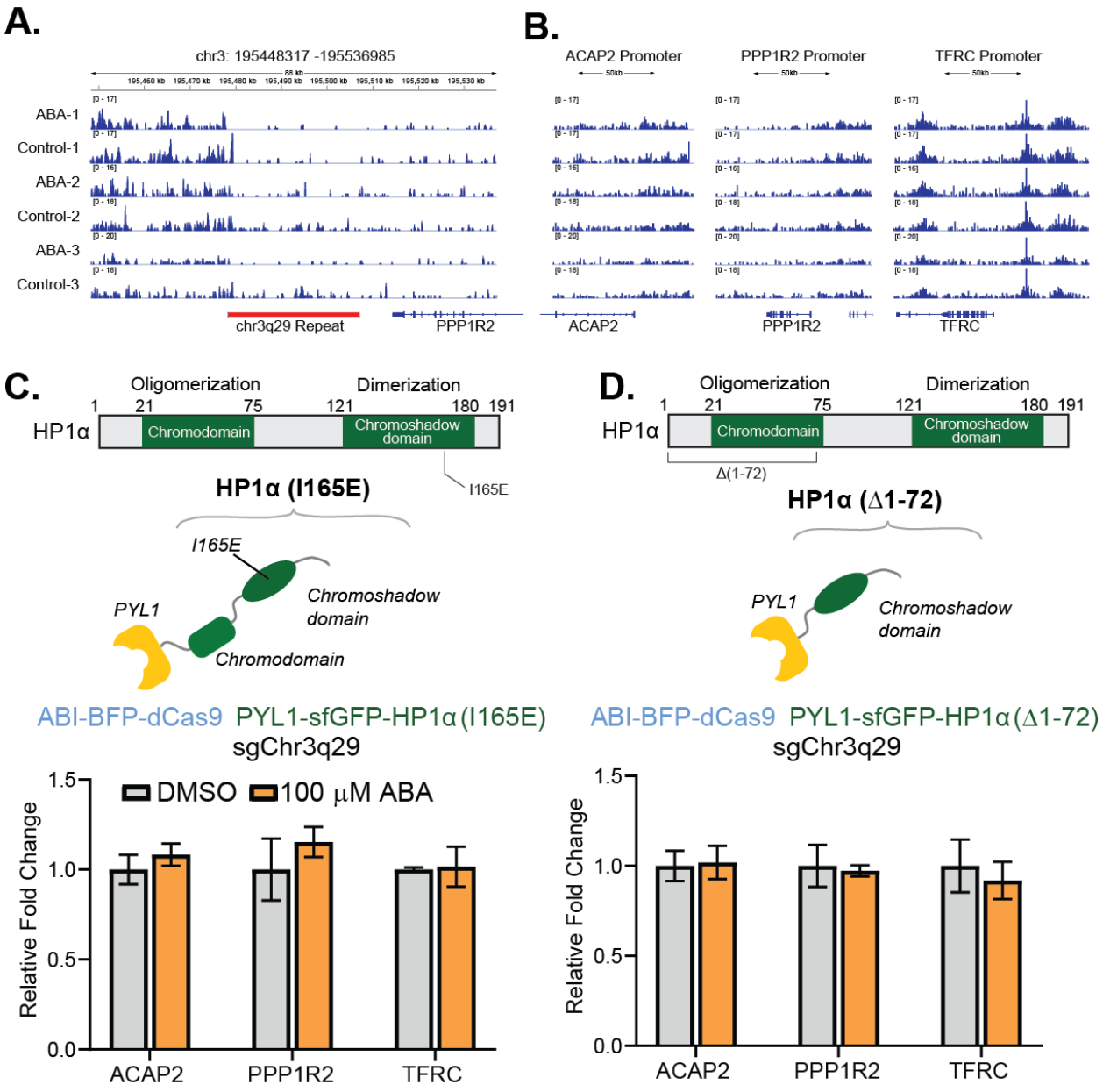

Figure S2. Heterochromatic Properties of CRISPR-EChO at Chr3q29, Related to Figure 2. **(A-B)** H3K9me3 ChIP-seq IGV read coverage histogram tracks of **(A)** a region of Chr3q29 spanning 30 kb on each side of the sgRNA targeted repeat site or **(B)** the promoters of three adjacent genes, mapped in **Fig. 2B**, for three replicate samples of CRISPR-EChO U2OS cells targeting Chr3q29 and treated with 100 μM ABA or DMSO control for two days. Track heights are normalized to total mapped reads for each sample. **(C-D)** RT-qPCR quantifying mRNA levels of the three indicated genes in U2OS cells expressing sgChr3q29 and a mutant HP1α CRISPR-EChO system with either **(C)** a point mutation to abolish dimerization, I165E, or **(D)** deletion of the N-terminus and chromodomains, Δ1-72 a.a. Schematic for mutant effectors shown on top, with fused fluorescent marker not depicted. Cells were treated with DMSO or 100 μM ABA for 5 days before RNA extraction. Data represents mean ± SD for four biological replicates from two independent experiments.

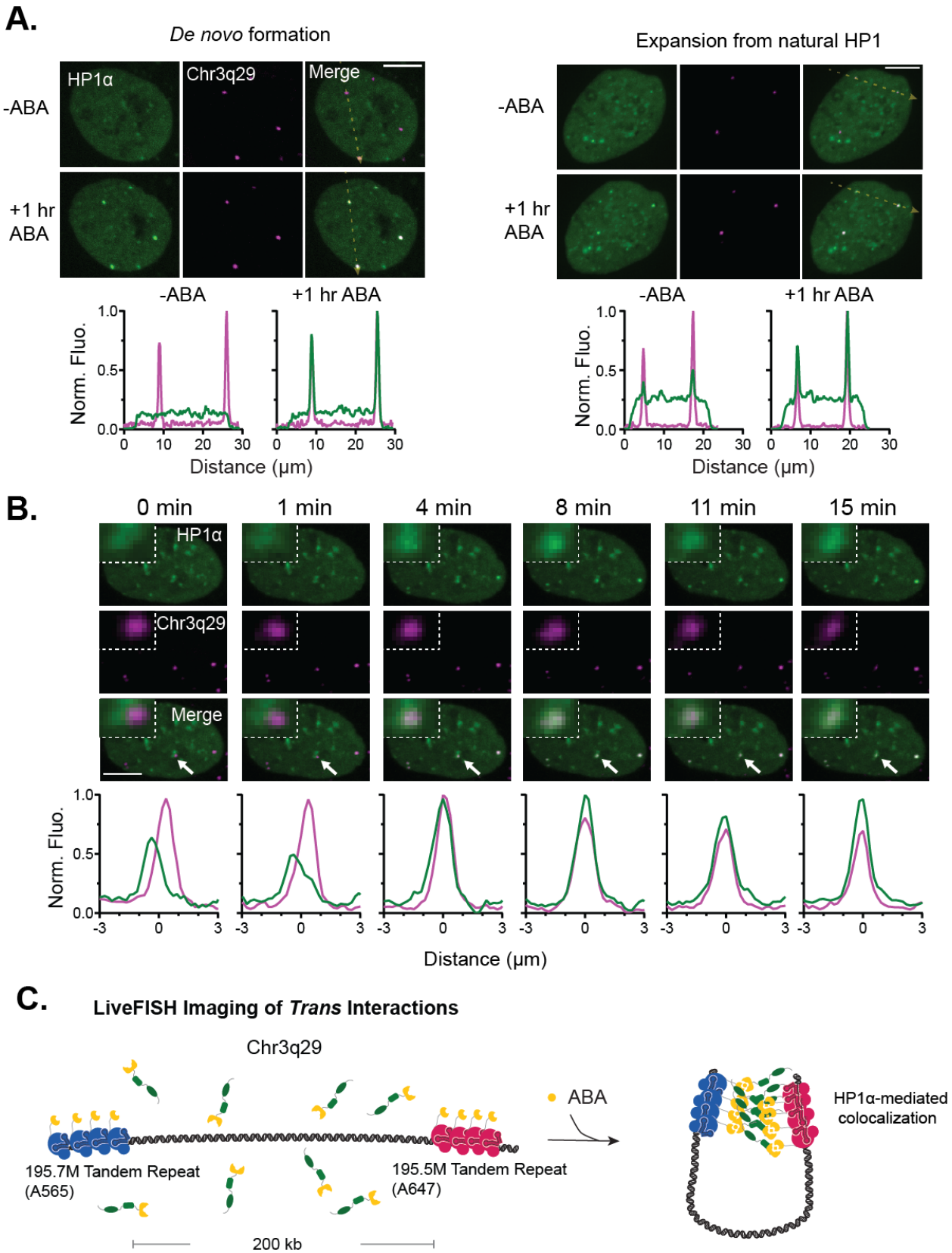

Figure S3. HP1α Assembly and Long-Range Interactions at Chr3q29, Related to Figure 3.

(A) Representative time lapse confocal microscopy images of a U2OS cell expressing the HP1α CRISPR-EChO system, MCP-mCherry, and the sgChr3q29-2xMS sgRNA before and after 1 hour 100 μM ABA treatment. Left two panels show a cell that forms HP1α puncta *de novo* at Chr3q29 after ABA addition. Right two panels show a cell with pre-existing HP1α puncta at Chr3q29 that expand after ABA addition. Bottom plots show line scans of fluorescence intensities for each color channel along the yellow dotted line in each Merge image. **(B)** A second representative time-lapse confocal microscopy image (along with **Fig. 3D**) showing incorporation of Chr3q29 into natural HP1α aggregates in the nucleus mediated by CRISPR-EChO. Time 0 min represents image taken immediately before 100 μM ABA addition, and 1 min represents the first image taken of the same cell after addition. Inset image represents 6x magnification of the region indicated by the white arrow. Bottom plots show line scans of fluorescence intensities for each color channel for region indicated by the white arrow. In **(A)** and **(B)**, fluorescence intensities are normalized to the maximum (1) and minimum (0) intensities observed across line scans for all displayed time points. Scale bars represent 10 μm. **(C)** Illustration of the LiveFISH experimental set-up. Atto565-labeled sgRNA targeting Chr3-195.7M and Atto647-labeled sgRNA targeting Chr3-195.5M tandem repeats on Chr3q29 are simultaneously introduced to U2OS cells expressing the CRISPR-EChO system. ABA addition leads to recruitment of HP1α to each tandem repeat and facilitates their interaction and co-localization with each other.

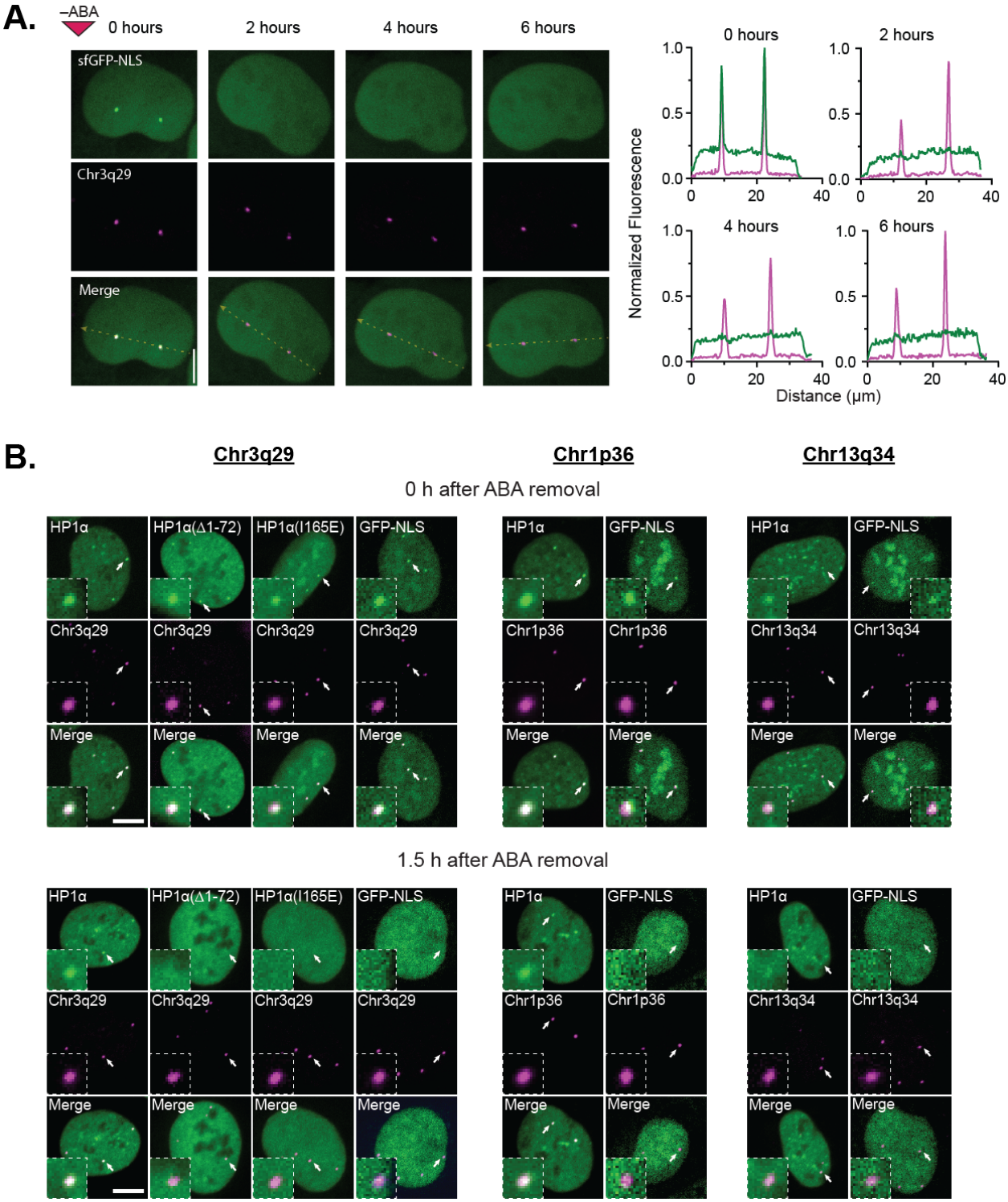

Figure S4. CRISPR-EChO Disassembly Kinetics Upon ABA Removal, Related to Figure 4. **(A)** Representative time-lapse confocal microscopy images of a U2OS cell expressing the CRISPR-EChO system with a PYL1-sfGFP-NLS control effector. Cells were treated for 24 hrs with 100 μM ABA prior to imaging. The 0 hour image represents the first image taken immediately after ABA removal. Bottom plots show line scans of fluorescence intensities for each color channel along the yellow dotted lines in each Merge image. Fluorescence intensities

are normalized to the maximum (1) and minimum (0) intensities observed across line scans for all displayed time points. **(B)** Representative confocal microscopy images of U2OS cells expressing the CRISPR-EChO system with various effectors and targeted to Chr3q29, Chr1p36, or Chr13q34. Images were taken immediately after ABA removal or at 1.5 hours after ABA removal. Scale bars represent 10 μm.

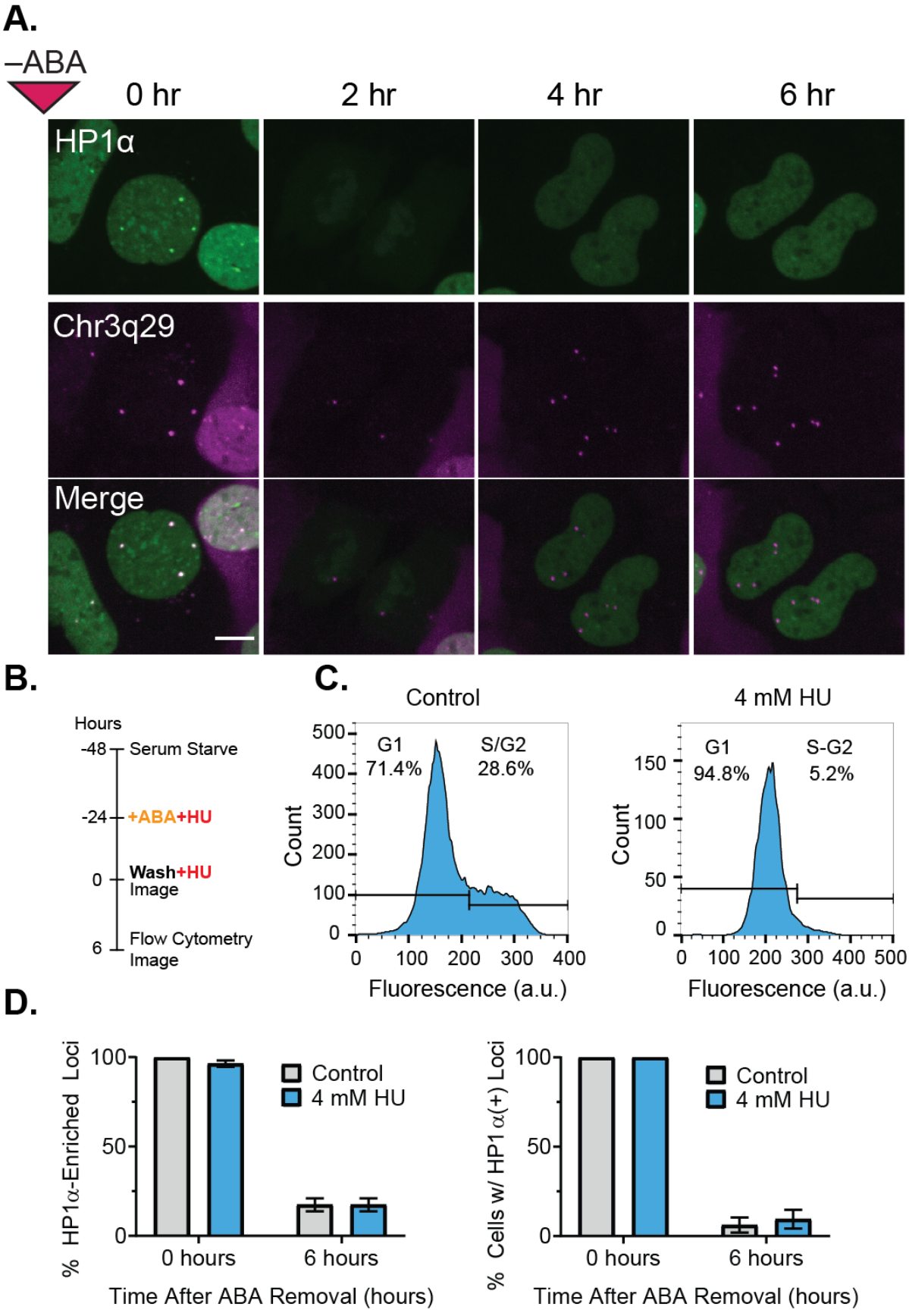
A.
–ABA
0 hr
2 hr
4 hr
6 hr
HP1α
Chr3q29
Merge
B.
Hours
-48
Serum Starve
-24
+ABA+HU
0
Wash+HU
Image
6
Flow Cytometry
Image
C.
Control
4 mM HU
G1
71.4%
S/G2
28.6%
G1
94.8%
S-G2
5.2%
Count
Fluorescence (a.u.)
D.
% HP1α-Enriched Loci
% Cells w/ HP1 α(+) Loci
Control
4 mM HU
0 hours
6 hours
Time After ABA Removal (hours)

Figure S5. Cell Cycle Arrest Does Not Delay Disassembly of HP1α at Chr3q29, Related to Figure 4.

(A) Time lapse microscopy images showing a U2OS cell expressing HP1α CRISPR-EChO undergoing mitosis. Bright HP1α puncta observed at Chr3q29 loci at 0 hr are lost during and following mitosis. Cells were treated for 24 hrs with 100 μM ABA prior to imaging. The 0 hour image represents the first image taken immediately after ABA removal. Scale bar represents 10 μm. **(B)** Experimental set-up for serum starvation and hydroxyurea induced cell cycle arrest. Control cells are serum starved but grown in complete media upon ABA addition. **(C)** Flow cytometry quantification of DNA content in Hoechst 33342-stained U2OS HP1α CRISPR-EChO cells with or without hydroxyurea-induced cell cycle arrest. Control cells show 28.6% of cells in S/G2 phase, while 4mM HU-treated cells show 5.2% of cells in S/G2. **(D)** Comparison of HP1α disassembly rates at Chr3q29 between control and cell cycle arrested U2OS HP1α CRISPR-EChO cells. Left panel displays the percentage of all Chr3q29 loci with HP1α-enrichment at the indicated time points (n=109-116 loci). Right panel shows percentage of cells with >50% Chr3q29 loci with GFP enrichment (n=31-34 cells). Error bars represent SEM calculated from Bernoulli distributions.

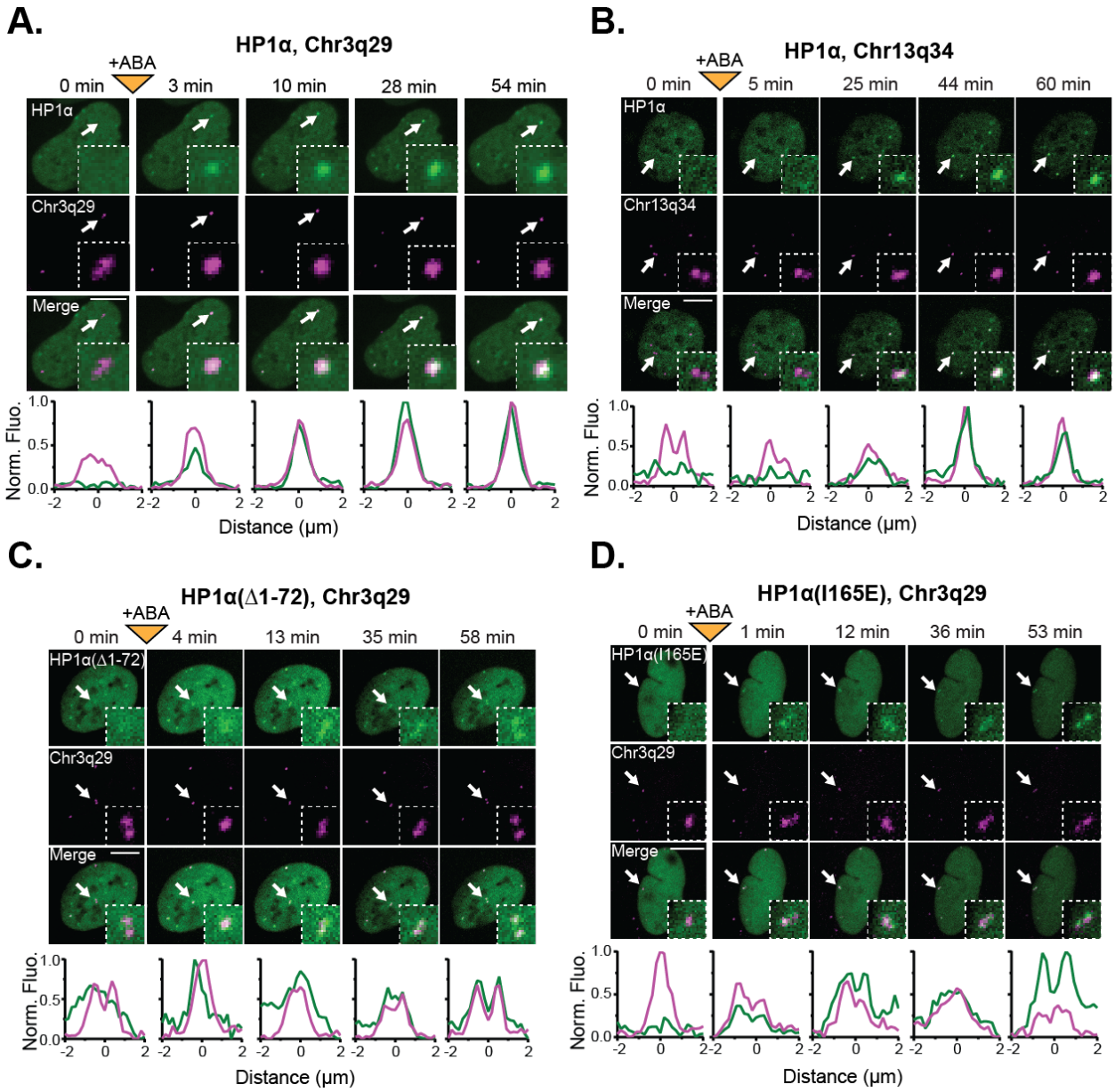

Figure S6. HP1α Recruitment to Tandem Repeat Sites Induces Chromatin Compaction, Related to Figure 5.

(A) A second representative image (along with **Fig. 5B**) showing compaction of a Chr3q29 locus following 100 μM ABA addition to recruit HP1α. Compaction is concomitant with the assembly of the HP1α puncta at Chr3q29. **(B)** A representative image showing compaction of a Chr13q34 locus following 100 μM ABA addition to recruit HP1α. **(C-D)** Representative images show Chr3q29 loci remaining diffuse following 100 μM ABA addition to recruit mutant **(C)** HP1α(Δ1-72) or **(D)** HP1α(I165E). Inset image represents 6x magnification in **(A)** and 4x magnification in **(B-D)** of the region indicated by the white arrow. For all panels, bottom plots show fluorescence intensities of each color channel for region indicated by the white arrow. Fluorescence intensities are normalized to the maximum (1) and minimum (0) intensities observed across line scans for all displayed time points. Scale bars represent 10 μm.

Table S1: Plasmid constructs used in this study, Related to Figures 1 to 5.

Construct	Plasmid number (pSLQ)
pHR: pmU6 sgRNA pCMV Puro	pSLQ1844
pHR: pTRE3G dCas9-2xNLS-HaloTag	pSLQ4218
pHR: pPGK ABI-tagBFP-dCas9-2xNLS	pSLQ4356
pHR: pPGK PYL1-sfGFP-HP1A	pSLQ6901
pHR: pPGK PYL1-sfGFP-HP1Adel72	pSLQ6903
pHR: pPGK PYL1-sfGFP-HP1AI165E	pSLQ6904
pHR: pPGK mCherry-HP1A	pSLQ6909
pHR: pPGK PYL1-sfGFP-NLS	pSLQ6918
pHR: pmU6 sgRNA 2xMS2 pUbC MCP-mCherry-p2a-Puro	pSLQ6941

Table S2: sgRNAs used in this study, Related to Figures 1 to 5.

sgRNA	Spacer	Genomic Target (hg38)	# sgRNA Sites	Reference
NT	GTACGTTCTCTATCACTGATA	Non-targeting		This study
Chr3q29	TGATATCACAG	chr3: 195478317 - 195506985	529	(Ma et al., 2016)
Chr1p36	AGATGCTCACC	chr1: 2648588 - 2777187	>482*	(Ma et al., 2016)
Chr13q34	ACCATTCCTTC	chr13: 112277011 - 112319170	358	(Ma et al., 2016)

*Approximately 40 kbp of the 120 kbp tandem repeat region has no mapped sequence on hg38.

Table S3: Fluorescently labeled crRNAs used in this study, Related to Figure 3.

crRNA	Spacer	Genomic Target (hg38)	# sgRNA Sites	Conjugated Fluorophore	Reference
Chr3-195.7M	AGGTATGGGTG	chr3:195775389-195777939	116	Atto565	This study
Chr3-195.5M	TGATATCACAG	chr3: 195478317 - 195506985	529	Atto647	(Ma et al., 2016)

Table S4: RT-qPCR Primers used in this study, Related to Figure 2.

Gene	Forward	Reverse	Amplicon Size (bp)	IDT Catalog #
GAPDH	CAATGACCCCTTCATTGACC	TTGATTTTGGAGGGATCTCG	159	N/A
ACAP2	CCTGAGGTCTTCAACTACCAC	GTTCAAACGAGCCAGCAATG	124	Hs.PT.58.4848886
PPP1R2	CTCCACTGCTTTCTTGTTCCT	GATGAAGATGCCTGTAGTGACA	127	Hs.PT.58.45684411
TFRC	CCCAGTTGCTGTCCTGATATAGß	TCTGGATAAAGCGGTTCTTGG	137	Hs.PT.39a.22214826