Figure S1

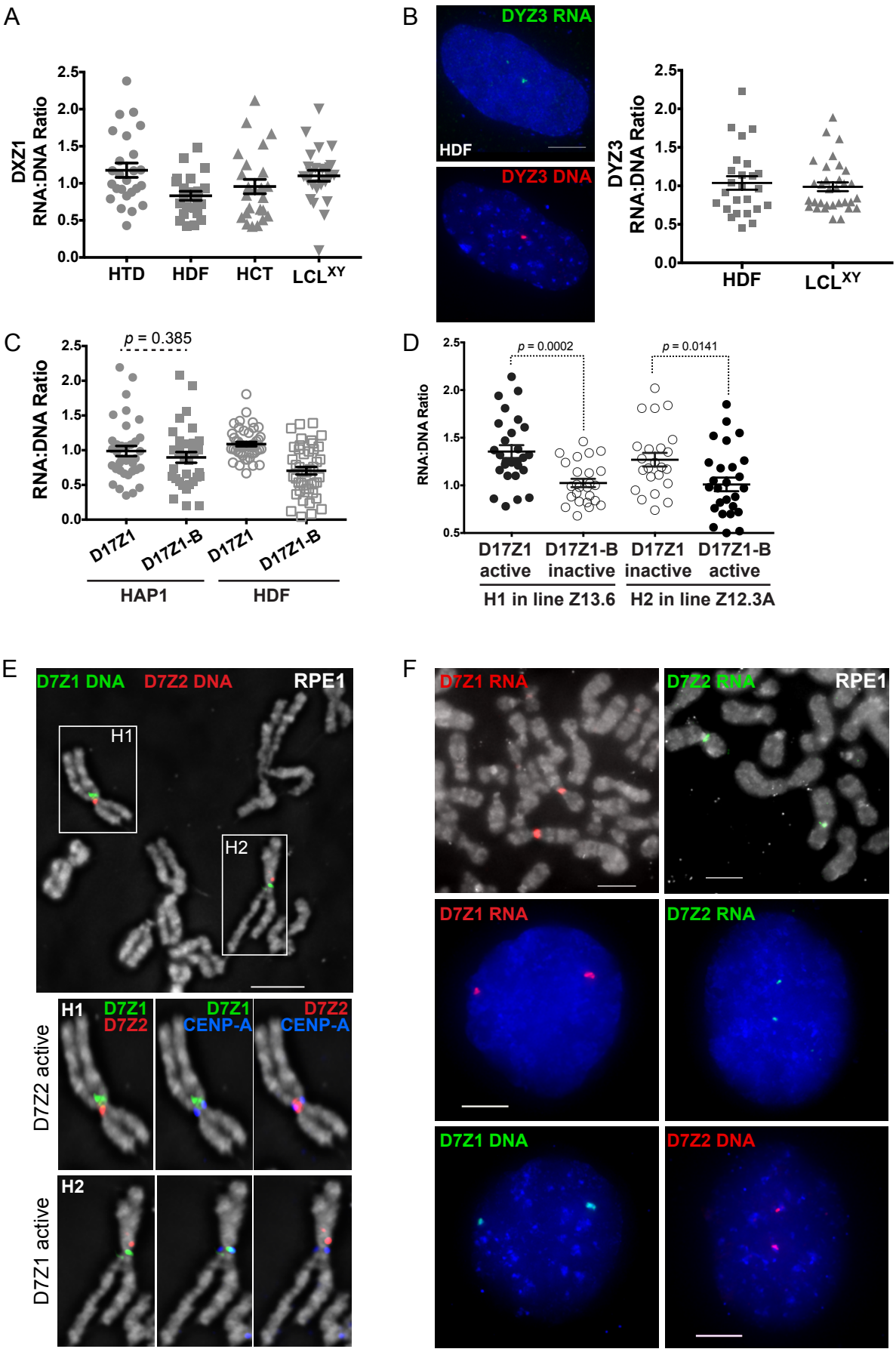

A
DXZ1 RNA:DNA Ratio
2.5
2.0
1.5
1.0
0.5
0.0
HTD
HDF
HCT
LCL XY
B
DYZ3 RNA
HDF
DYZ3 DNA
DYZ3 RNA:DNA Ratio
HDF
LCL XY
C
p = 0.385
RNA:DNA Ratio
D17Z1
D17Z1-B
D17Z1
D17Z1-B
HAP1
HDF
D
p = 0.0002
p = 0.0141
RNA:DNA Ratio
D17Z1 active
D17Z1-B inactive
D17Z1 inactive
D17Z1-B active
H1 in line Z13.6
H2 in line Z12.3A
E
D7Z1 DNA
D7Z2 DNA
RPE1
H1
H2
H1
D7Z1 D7Z2
D7Z1 CENP-A
D7Z2 CENP-A
D7Z2 active
H2
D7Z1 active
F
D7Z1 RNA
D7Z2 RNA
RPE1
D7Z1 RNA
D7Z2 RNA
D7Z1 DNA
D7Z2 DNA

Figure S1, related to Figure 1 and Figure 2. Quantitation of RNA:DNA Ratios at Chromosome-Specific Alpha Satellite Arrays and on Epiallele Chromosomes HSA17 and HSA7.

(A) DXZ1 RNA:DNA ratios (mean ± SEM) in four different human cell lines.

(B) Representative RNA (green) – DNA (red) FISH in HDF cells using an HOR probe for DYZ3, the alpha satellite array on HSAY. Quantitation of RNA:DNA ratios in two additional human cell lines (mean ± SEM).

(C) Quantitation of RNA:DNA ratios for D17Z1 and D17Z1-B on HSA17 measured by RNA-DNA FISH in two additional cell lines (mean ± SEM). In both, D17Z1 is the active array and D17Z1-B is inactive. The RNA:DNA ratio in HAP1, a haploid line, is not significant between D17Z1 and D17Z1-B. We could not make a similar statistical comparison for diploid line HDF since values for two HSA17s are represented by the data.

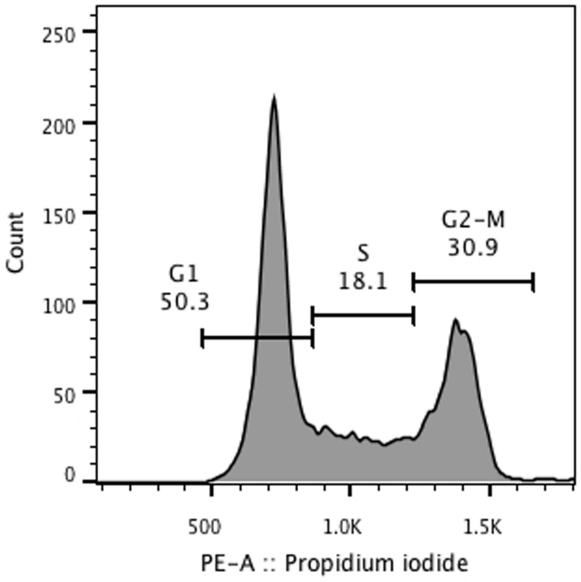
(D) RNA:DNA ratios for RNA-DNA FISH for HSA17 epiallele chromosomes (mean ± SEM) derived from parental line HTD. Each HSA17 was isolated into a somatic cell hybrid background, yielding cell line Z13.6 that contains homolog 1 (H1) and cell line Z12.3A containing H2. D17Z1 and D17Z1-B transcripts were detected in each functional configuration (centromere active or inactive).

(E) Discovery of centromeric epialleles on HSA7 in RPE1 cells. Immunostaining for CENP-A (blue) and DNA FISH for D7Z1 (green) and D7Z2 (red) on metaphase chromosomes identified one homologue with active D7Z1 and the other homologue with active D7Z2. HSA7 epialleles were also identified in cell lines HDF and HTD (not shown).

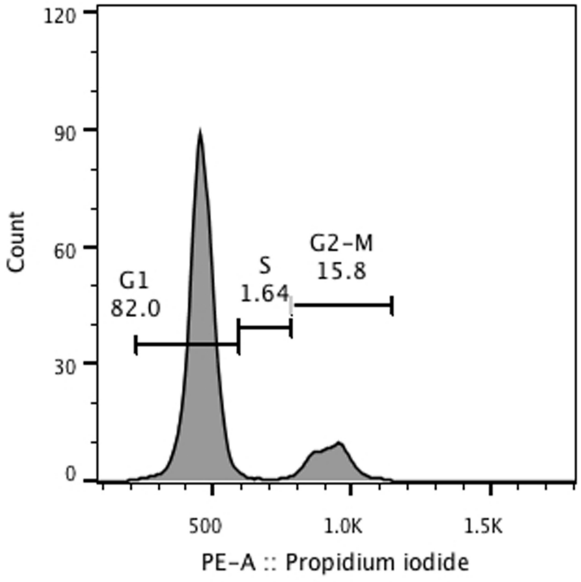
(F) RNA FISH with HOR probes specific for D7Z1 and D7Z2 detects transcripts on RPE1 metaphase chromosomes. RNA-DNA FISH shows that array-specific transcripts are present in interphase and localized *in cis*.

Figure S2

A



A'



B

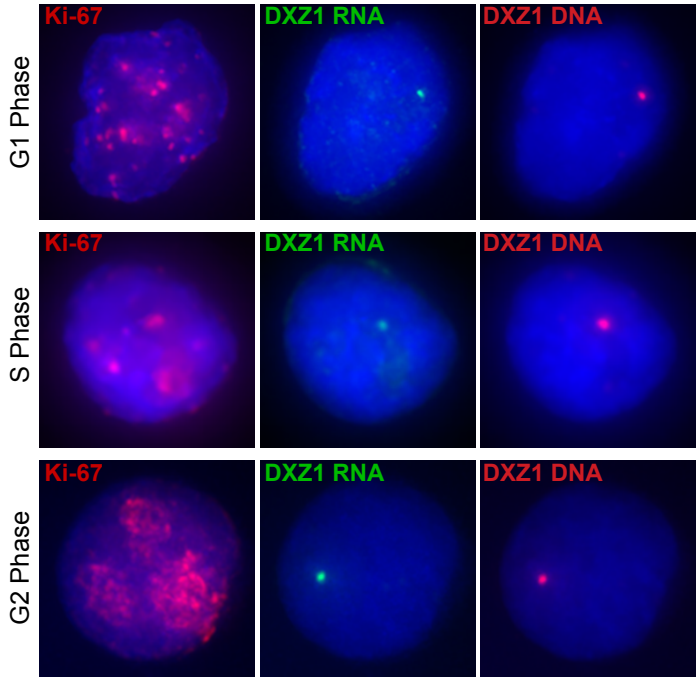


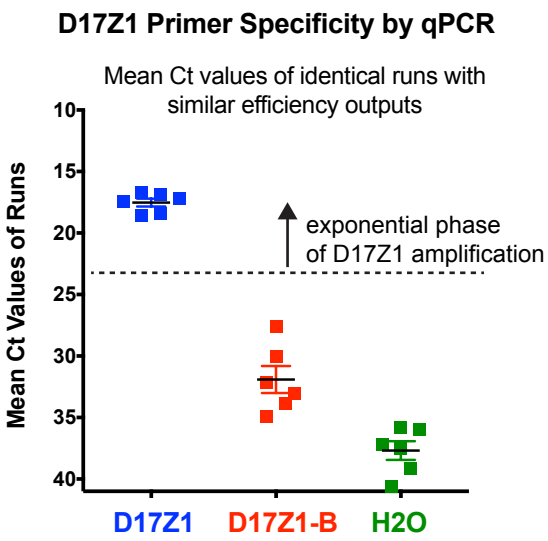
Figure S2, related to Figure 2. Assessment of Cell Synchronization Efficiency by FACS and Cell Cycle Staging by Ki-67 Immunostaining.

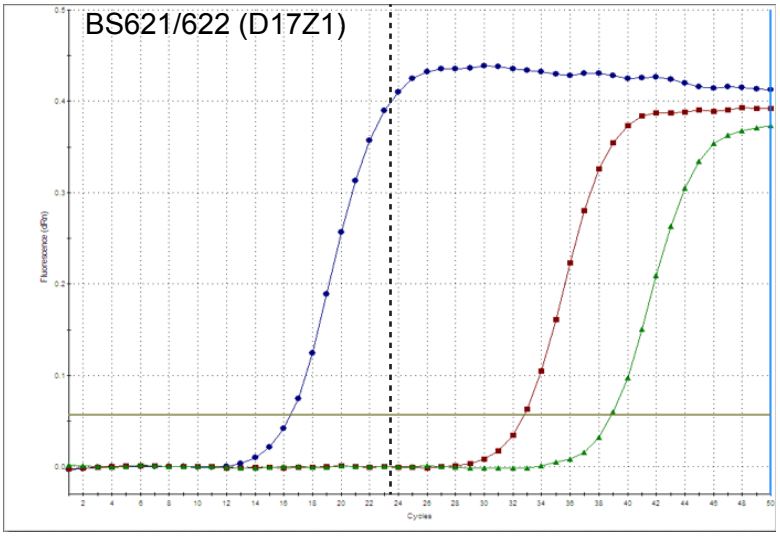
(A, A') FACS analysis of unsynchronized HT1080 cells stained with propidium iodide (A) and HT1080 cells synchronized in G1 by release from nocodazole arrest and stained with propidium iodide (A').

(B) Ki-67 immunostaining showing cell cycle stage-specific morphology used to determine proportion of cells in G1, S, or G2.

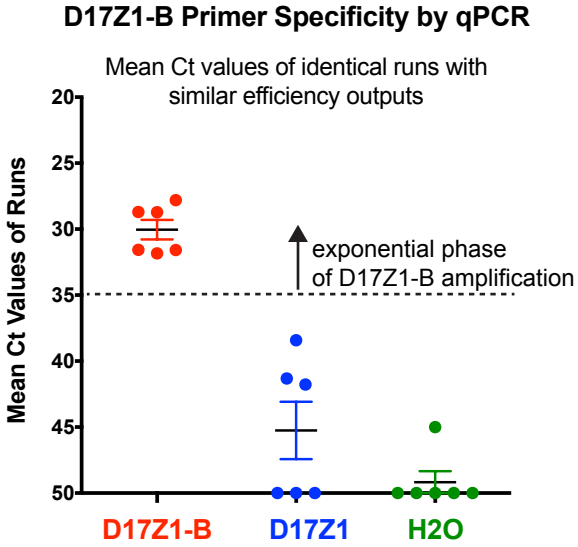
Figure S3

A


D17Z1 Primer Specificity by qPCR
Mean Ct values of identical runs with similar efficiency outputs
Mean Ct Values of Runs
10
15
20
25
30
35
40
exponential phase of D17Z1 amplification
D17Z1
D17Z1-B
H2O


BS621/622 (D17Z1)

B


D17Z1-B Primer Specificity by qPCR
Mean Ct values of identical runs with similar efficiency outputs
Mean Ct Values of Runs
20
25
30
35
40
45
50
exponential phase of D17Z1-B amplification
D17Z1-B
D17Z1
H2O

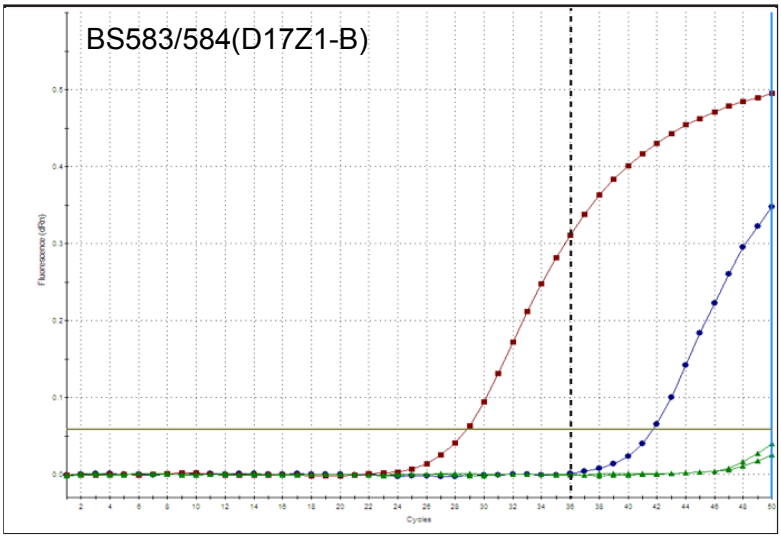

BS583/584(D17Z1-B)

Figure S3, related to Figure 2, Figure 5. Establishment of Array-Specific qPCR/RT-qPCR Primer Specificity.

(A) Mean Ct values (left) and representative amplification plot (right) using D17Z1 primers (BS621/622) in reactions containing D17Z1 DNA, D17Z1-B DNA, or H_2O.

(B) Mean Ct values (left) and representative amplification plot (right) using D17Z1-B primers (BS583/584) in reactions containing D17Z1-B DNA, D17Z1 DNA, or H_2O.

Figure S4

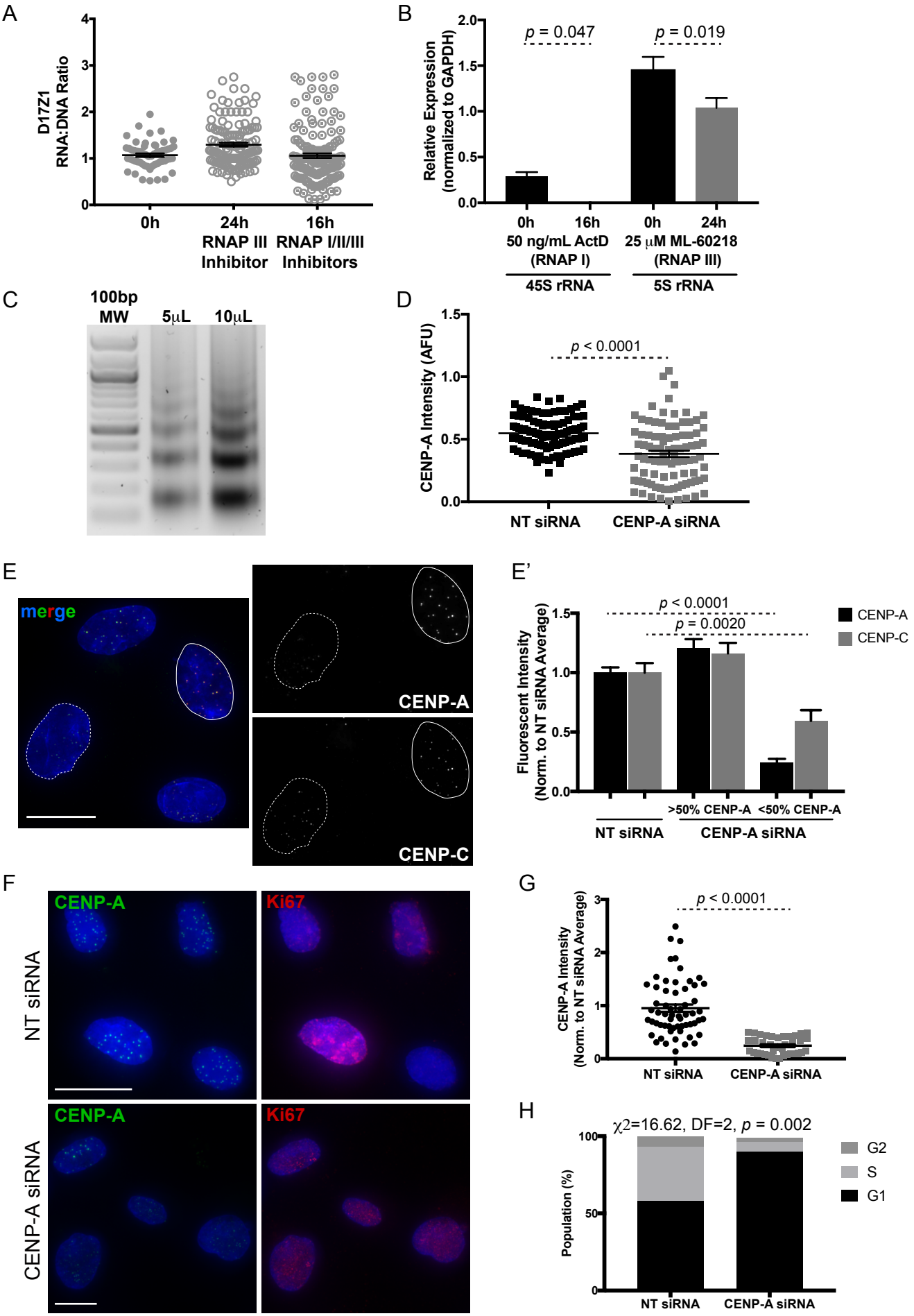

A
D17Z1 RNA:DNA Ratio
0h
24h RNAP III Inhibitor
16h RNAP I/II/III Inhibitors
B
Relative Expression (normalized to GAPDH)
p = 0.047
p = 0.019
0h
16h
50 ng/mL ActD (RNAP I)
45S rRNA
0h
24h
25 μM ML-60218 (RNAP III)
5S rRNA
C
100bp MW
5μL
10μL
D
CENP-A Intensity (AFU)
p < 0.0001
NT siRNA
CENP-A siRNA
E
merge
CENP-A
CENP-C
E'
Fluorescent Intensity (Norm. to NT siRNA Average)
p < 0.0001
p = 0.0020
CENP-A
CENP-C
>50% CENP-A
<50% CENP-A
NT siRNA
CENP-A siRNA
F
NT siRNA
CENP-A siRNA
CENP-A
Ki67
G
CENP-A Intensity (Norm. to NT siRNA Average)
p < 0.0001
NT siRNA
CENP-A siRNA
H
χ2=16.62, DF=2, p = 0.002
Population (%)
G2
S
G1
NT siRNA
CENP-A siRNA

Figure S4, related to Figure 4 and Figure 5. Effect of RNA Ppolymerase Inhibition, Verification of Native Chromatin Nucleosomal Content, and Effect of CENP-A Depletion on RNA:DNA Ratios.

(A) Quantitation of D17Z1 RNA:DNA ratios (mean ± SEM) following RNA polymerase III (RNAP III) or all polymerase (RNAP I/II/III) inhibition. Each data point represents a single interphase centromere.

(B) Semi-quantitative RT-PCR (mean ± SEM) of 45S rRNA following RNAP I inhibition with 50ng/mL Actinomycin D and 5S rRNA following RNAP III inhibition with 25μM ML-60218 to test efficacy of inhibition.

(C) Agarose gel showing DNA from HAP1 chromatin preparation that was prepared by digestion with micrococcal nuclease. The 100bp molecular weight ladder was used to size chromatin fragments.

(D) CENP-A intensity in RPE1 cells treated with NT siRNA or CENP-A siRNA. Each point represents an individual interphase cell; mean ± SEM is shown.

(E, E') Representative CENP-A and CENP-C immunostaining in CENP-A siRNA-treated RPE1 cells (bar, 15μm) (E) and comparison of CENP-A and CENP-C intensity in non-targeting (NT) siRNA-treated cells and CENP-A siRNA-treated cells (divided into two groups based on level of CENP-A depletion).

(F) Representative CENP-A and Ki67 immunostaining in NT siRNA-treated and CENP-A siRNA-treated RPE1 cells (bars, 15μm).

(G) Comparison of CENP-A intensity in NT siRNA-treated cells and CENP-A siRNA-treated cells used in cell cycle analysis (restricted to cells containing less than 50% of the average

amount of CENP-A, based on NT siRNA-treated cells). Each point represents an individual interphase cell; mean ± SEM is shown.

(H) Quantitation of Ki-67-staged (G1/S/G2) interphase cells from NT siRNA and CENP-A siRNA-treated cells. Data in this figure were statistically analyzed by a t-test, except for (H) in which a Chi-square test was performed.

Figure S5

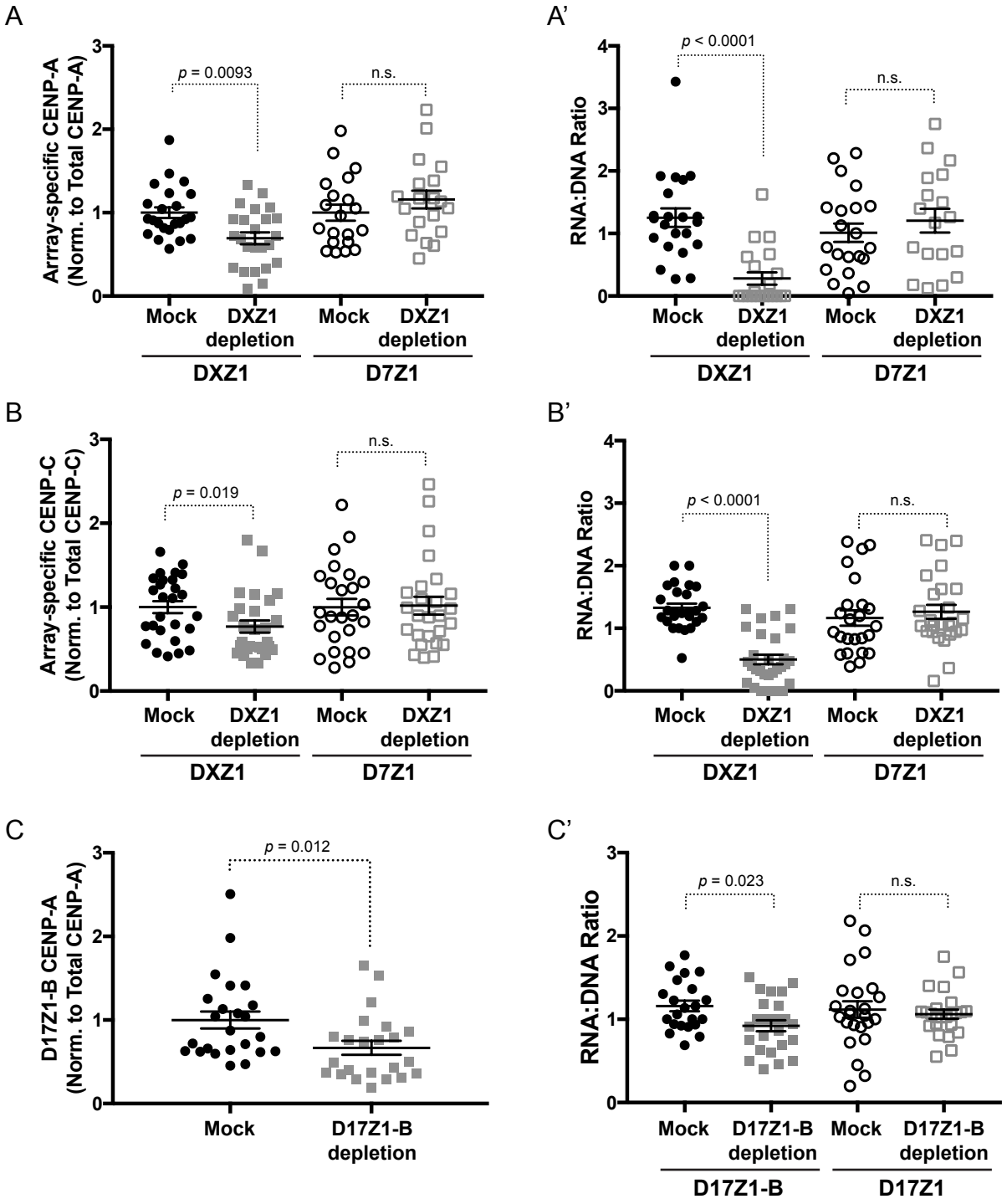

A
Arrray-specific CENP-A (Norm. to Total CENP-A)
p = 0.0093
n.s.
Mock
DXZ1 depletion
Mock
DXZ1 depletion
DXZ1
D7Z1
A'
RNA:DNA Ratio
p < 0.0001
n.s.
Mock
DXZ1 depletion
Mock
DXZ1 depletion
DXZ1
D7Z1
B
Array-specific CENP-C (Norm. to Total CENP-C)
p = 0.019
n.s.
Mock
DXZ1 depletion
Mock
DXZ1 depletion
DXZ1
D7Z1
B'
RNA:DNA Ratio
p < 0.0001
n.s.
Mock
DXZ1 depletion
Mock
DXZ1 depletion
DXZ1
D7Z1
C
D17Z1-B CENP-A (Norm. to Total CENP-A)
p = 0.012
Mock
D17Z1-B depletion
C'
RNA:DNA Ratio
p = 0.023
n.s.
Mock
D17Z1-B depletion
Mock
D17Z1-B depletion
D17Z1-B
D17Z1

Figure S5, related to Figure 6. Effect of Array-Specific dsRNA Depletion on Centromere Protein Abundance and RNA:DNA Ratios.

(A, A') Replicate CENP-A and RNA:DNA ratio quantitation at targeted DXZ1 and control D7Z1 after DXZ1 dsRNA depletion.

(B, B') Replicate CENP-C and RNA:DNA ratio quantitation at targeted DXZ1 and control D7Z1 after DXZ1 dsRNA depletion.

(C, C') Replicate CENP-A and RNA:DNA ratio quantitation at targeted D17Z1-B and control D17Z1 after D17Z1-B dsRNA depletion. All CENP and RNA:DNA data in this figure are presented as mean ± SEM. Each data point represents a single interphase centromere. Data were statistically tested using a t-test.

Figure S6

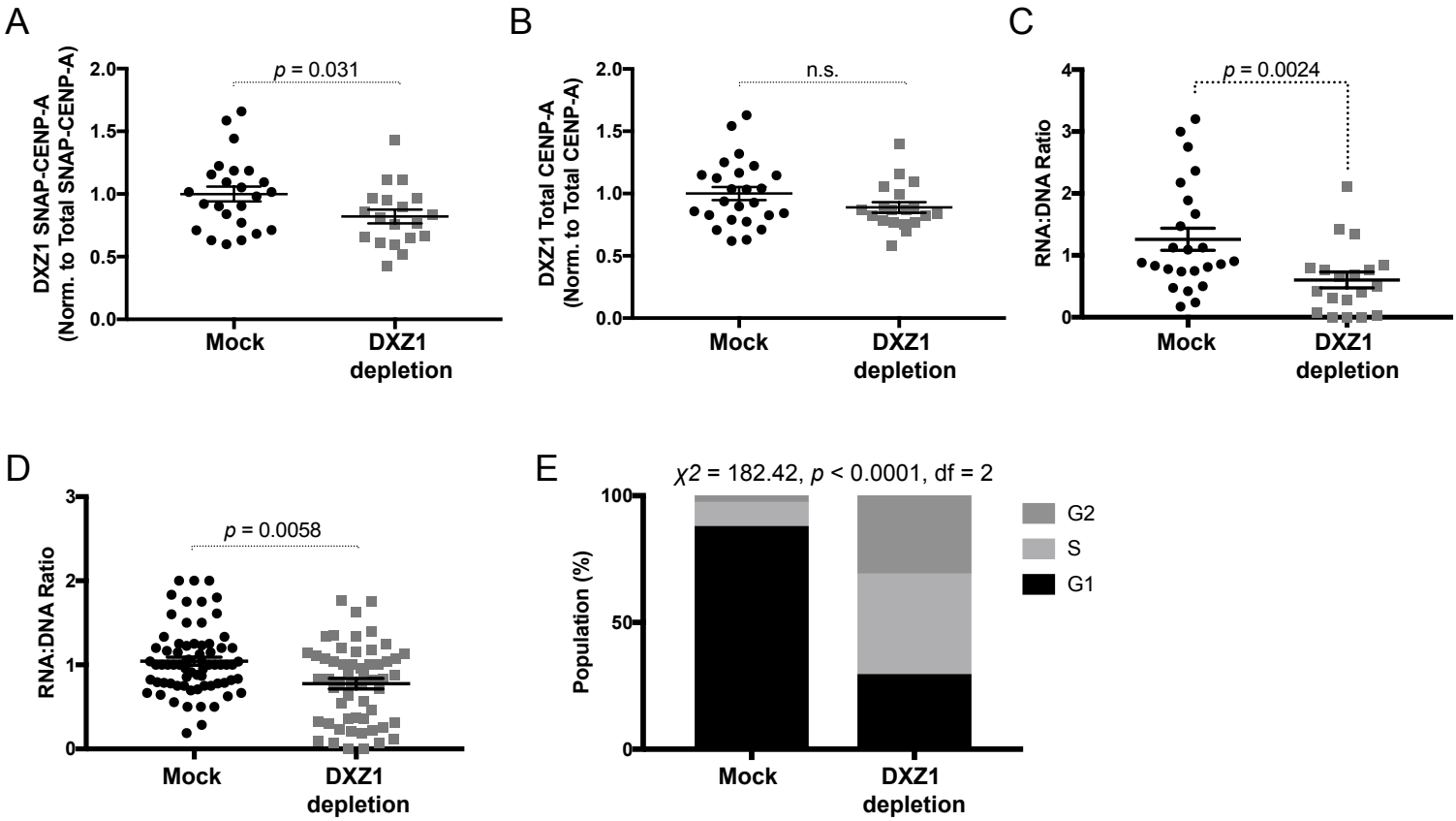

A
DXZ1 SNAP-CENP-A (Norm. to Total SNAP-CENP-A)
p = 0.031
Mock
DXZ1 depletion
B
DXZ1 Total CENP-A (Norm. to Total CENP-A)
n.s.
Mock
DXZ1 depletion
C
RNA:DNA Ratio
p = 0.0024
Mock
DXZ1 depletion
D
RNA:DNA Ratio
p = 0.0058
Mock
DXZ1 depletion
E
χ2 = 182.42, p < 0.0001, df = 2
Population (%)
G2
S
G1
Mock
DXZ1 depletion

Figure S6, related to Figure 7. Effect of Array-Specific dsRNA Depletion on CENP-A Loading and Cell Cycle Progression.

(A) Replicate SNAP-CENP-A quantitation at targeted DXZ1 after DXZ1 dsRNA depletion.

(B) Replicate total CENP-A quantitation at targeted DXZ1 after DXZ1 dsRNA depletion.

(C) Replicate RNA:DNA ratios at targeted DXZ1 after DXZ1 dsRNA depletion.

(D) Replicate RNA:DNA ratios at targeted DXZ1 in cells used for cell cycle analysis after DXZ1 dsRNA depletion.

(E) Replicate quantitation of Ki-67-staged (G1/S/G2) interphase cells from mock-treated and DXZ1 RNA depleted cells. All CENP-A and RNA:DNA data in this figure are presented as mean ± SEM. Each data point represents a single interphase centromere. Data were statistically tested using a t-test except for (E) in which a Chi-square test was performed.