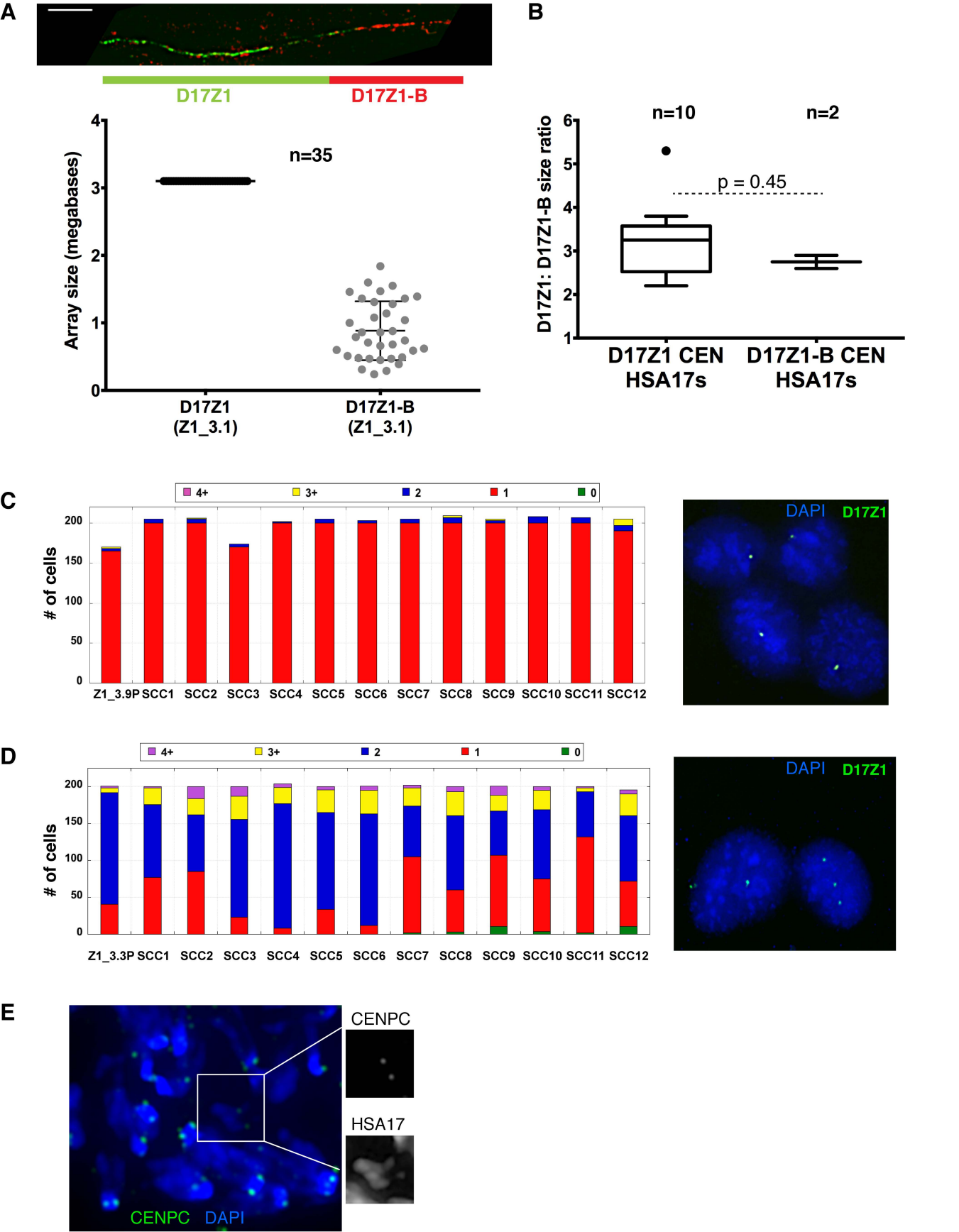
A
D17Z1
D17Z1-B
n=35
Array size (megabases)
D17Z1
(Z1_3.1)
D17Z1-B
(Z1_3.1)
B
n=10
n=2
p = 0.45
D17Z1: D17Z1-B size ratio
D17Z1 CEN
HSA17s
D17Z1-B CEN
HSA17s
C
4+
3+
2
1
0
# of cells
Z1_3.9P SCC1 SCC2 SCC3 SCC4 SCC5 SCC6 SCC7 SCC8 SCC9 SCC10 SCC11 SCC12
DAPI
D17Z1
D
4+
3+
2
1
0
# of cells
Z1_3.3P SCC1 SCC2 SCC3 SCC4 SCC5 SCC6 SCC7 SCC8 SCC9 SCC10 SCC11 SCC12
DAPI
D17Z1
E
CENPC
HSA17
CENPC
DAPI

Supplemental Figure S4. HSA17 alpha satellite arrays sizes and chromosome stability.

A. Prior to this study, D17Z1-B sizes were not known. We estimated D17Z1-B total array size on individual HSA17s using DNA fiber-FISH. Probes specific to D17Z1 (green) and D17Z1-B (red) were co-hybridized onto DNA fibers (n=35 in this example) and the length of each fluorescence signal was measured (in microns). Based on the sizes of D17Z1 that had been determined by PFGE (Table 1), the average size of D17Z1-B was calculated. The range in D17Z1-B sizes is largely due to variation in mechanical stretching of the DNA fiber during preparation. Scale bar equals 15 microns.

B. The ratio of D17Z1:D17Z1-B total array sizes was calculated to test the hypothesis that smaller ratios are linked to centromere assembly at D17Z1-B. Although D17Z1 arrays in our dataset were generally smaller when D17Z1-B was the functional centromere, there was not a statistically significant difference between D17Z1:D17Z1-B array size ratios.

C. Mitotic stability of individual HSA17s of single cell clones (SSC) derived from a parental line containing a single HSA17 was measured in somatic cell hybrids using FISH with probes to D17Z1 (green). The parental line of Z1_3.9P and multiple versions of this chromosome (SCC # lines) were analyzed to account for random mutations in a single line that might affect overall chromosome stability. Line Z1_3.9 contains an HSA17 with a large D17Z1 array composed almost exclusively of wild-type HORs. All versions of this HSA17 were highly stable. In these lines containing a single HSA17, any deviation from 1 equals instability. Nuclear DNA is counterstained with DAPI (blue). Colors in bar graph represent the number of HSA17s per cell (green = 0; red =1, blue = 2, yellow = 3, magenta = 4+).

D. HSA17s with moderate to extreme D17Z1 variation exhibited mitotic chromosome instability. The D17Z1 array in line Z1_3.3 contains ~60% variant HORs. This HSA17 exhibited instability within the parental line (Z1_3.3P) as well as in all the other independent versions of the HSA17 that were analyzed. The instability suggests that the HSA17 undergoes multiple rounds of non-disjunction.

E. Immunostaining for CENPC (green), a CCAN protein that links CENPA chromatin to the outer kinetochore, was performed to measure the amount of centromere proteins on the unstable HSA17 Z1_4.0 compared to the other normal chromosomes.