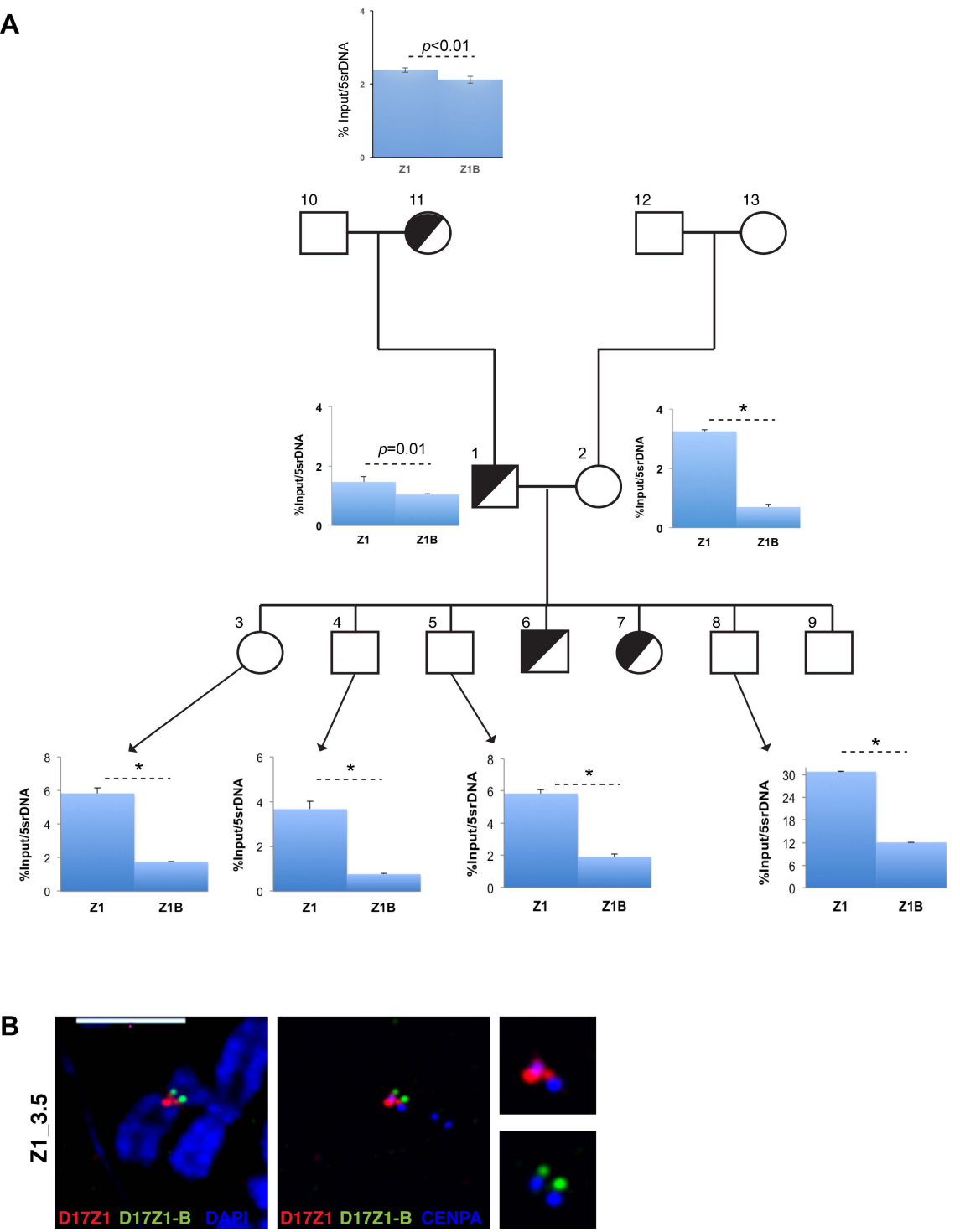
A
% Input/5srDNA
p<0.01
Z1
Z1B
10
11
12
13
1
2
p=0.01
*
3
4
5
6
7
8
9
B
Z1_3.5
D17Z1 D17Z1-B DAPI
D17Z1 D17Z1-B CENPA

Supplemental Figure S1. Determination of centromere location on HSA17 by CENPA ChIP-PCR and CENPA immunostaining-FISH.

A. ChIPs on native chromatin were performed in duplicate using CENPA antibodies and qPCRs for D17Z1, D17Z1-B, and 5s rDNA (control) were performed in triplicate. Relative enrichment of CENPA at D17Z1, D17Z1-B, or both (epiallele individuals) was determined using the percent input method and compared to 5srDNA [1]. In diploid cells, such as the subset of CEPH1345 individuals shown in A, epialleles or functional heterozygotes (Z1/Z1-B) were identified by equal enrichment of CENP-A at D17Z1 and D17Z1-B. ChIP is mainly shown for individuals who had not been previously studied [1]. Asterisks indicate *p* values difference between D17Z1 and D17Z1-B CENPA enrichments that were less than 0.0001. Pedigree of the CEPH 1345 family is shown with the data graphs. Circles represent females, squares denote males, and half shaded circles and squares represent D17Z1/D17Z1-B centromere heterozygotes.

B. Centromere location, especially in diploid lines, was confirmed visually using metaphase CENPA IF-FISH. This allowed us to assign centromere function to specific HSA17 homologs. An example of CENP-A IF-FISH in Z1_3.5, a haploid human cell line, shows that CENPA (blue) co-localizes with D17Z1 (red) and not D17Z1-B (green) (middle panel). Left panel shows FISH image of D17Z1 (red), D17Z1-B (green), and DAPI-stained chromosome (blue). Scale bar equals 5 microns.