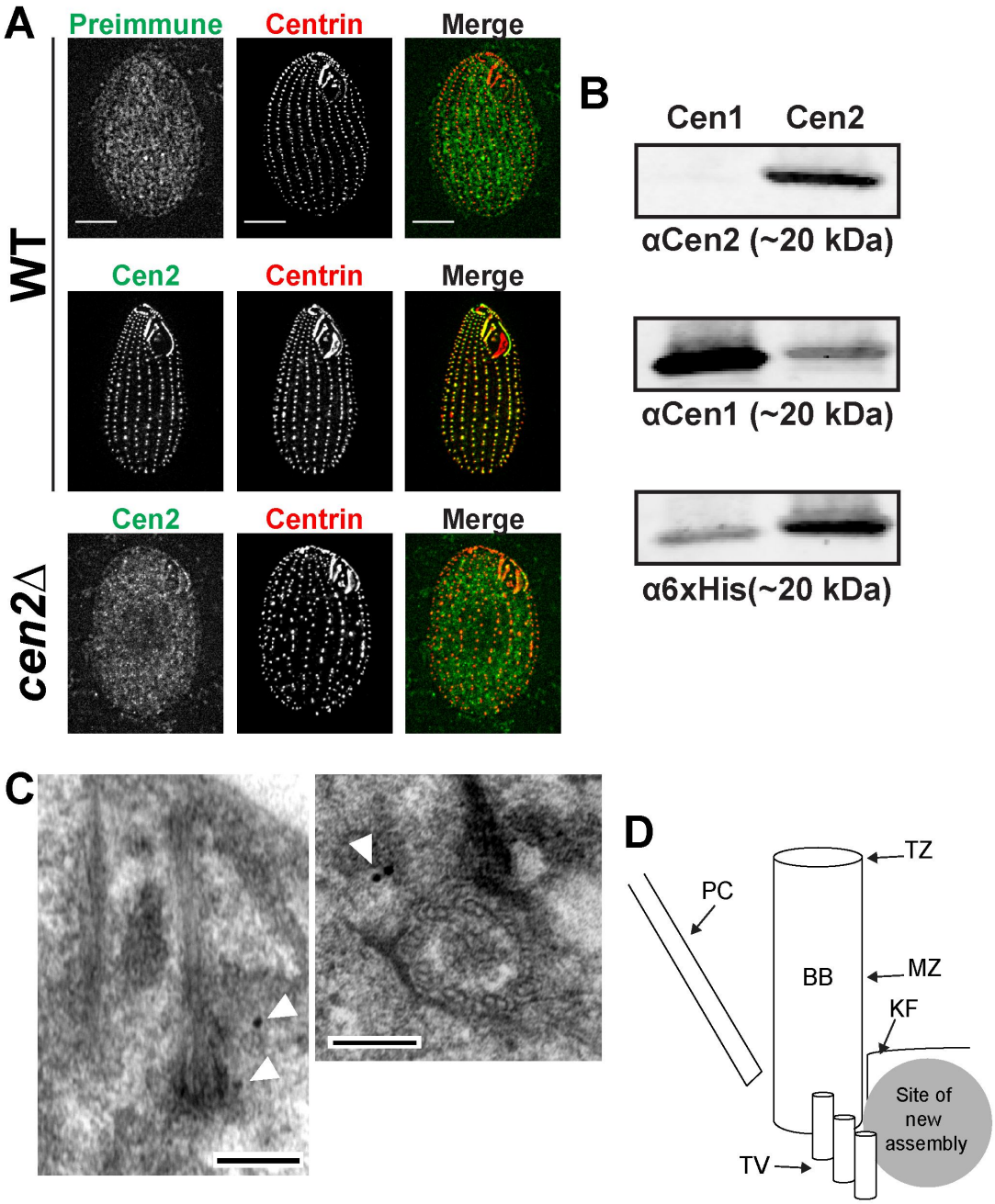
Supplemental Figure 1. Alignment of *Tetrahymena* and human centrins. Black boxes indicate conserved residues and gray boxes indicate similar residues. The red box indicates the N-terminal tail region, and the green box indicates the epitope used to raise the anti-Cen2 antibody. The red line indicates where the two domains separate. The asterisk indicates the sequence for the previously annotated *CEN2* gene. tt, *Tetrahymena thermophila*; hs, *Homo sapiens.*

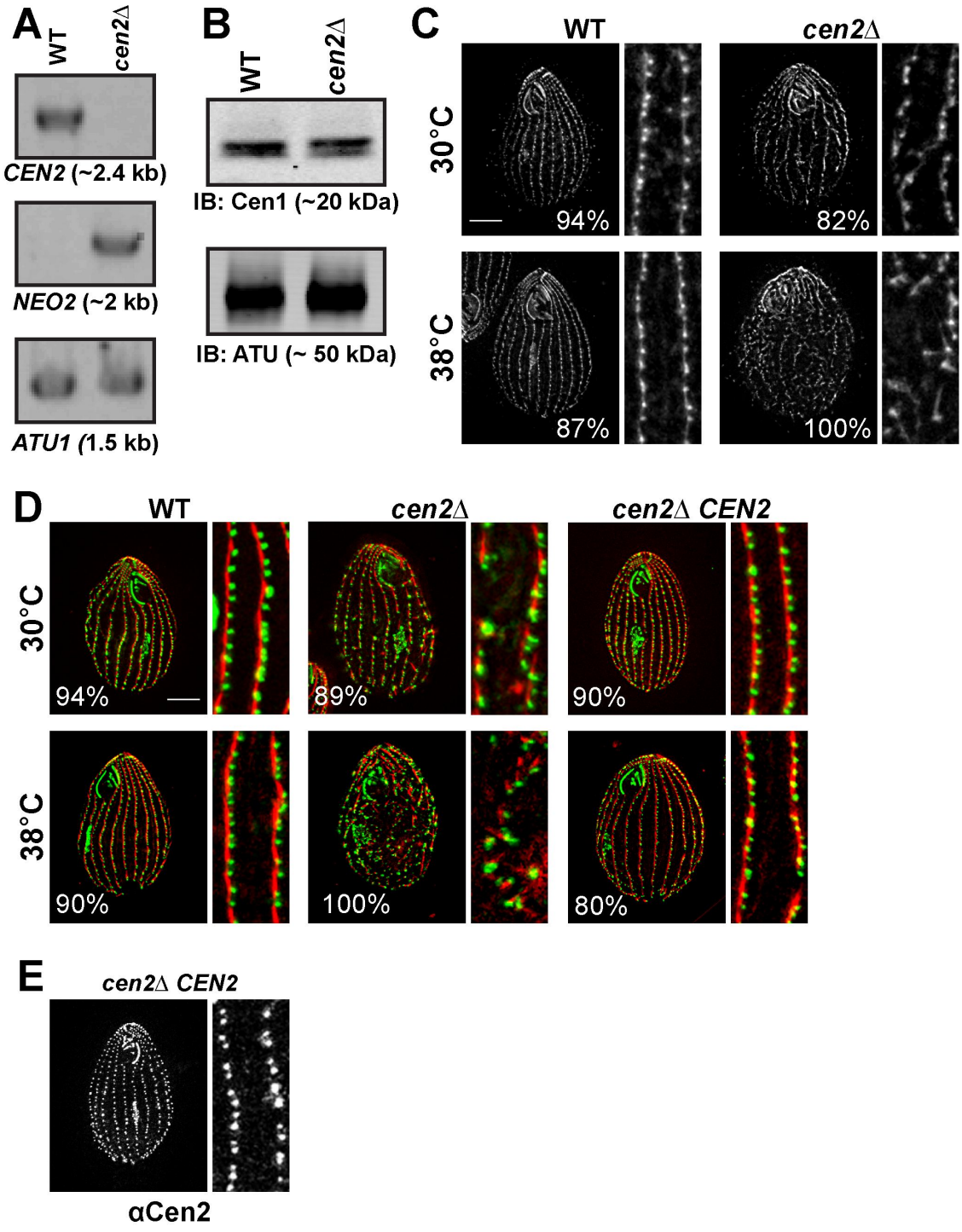


E

Particles per BB	TZ	MZ	PC	TV	Site of new assembly	Proximal
0.9 (n = 64 BB)	1 (1.8%)	1 (1.8%)	7 (13%)	9 (16%)	32 (57%)	7 (13%)

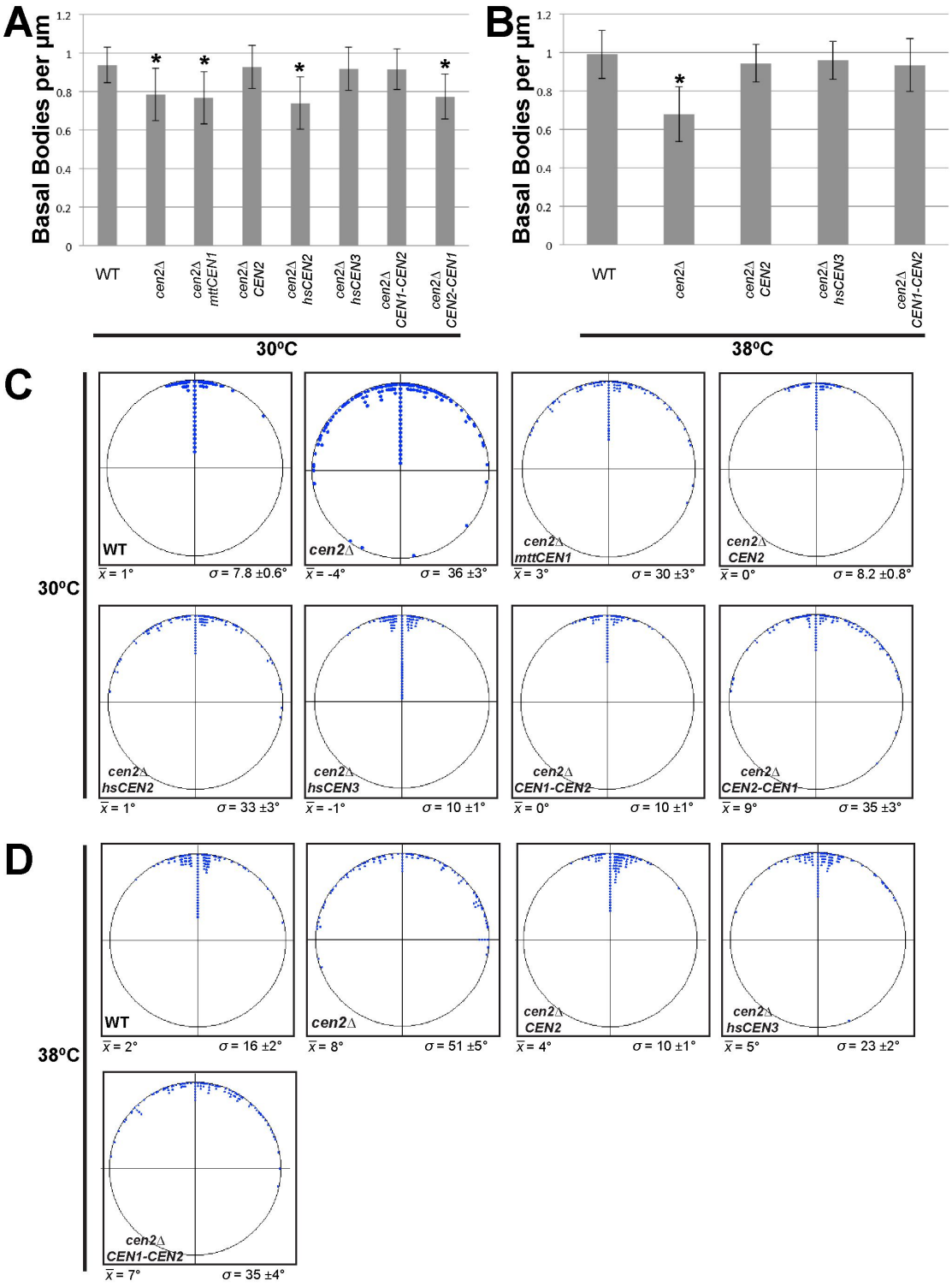
Supplemental Figure 2. The anti-Cen2 antibody shows that Cen2 localizes to basal bodies. (A) Immunofluorescence images showing labeling by the anti-Cen2 antibody or the preimmune serum in WT or *cen2* null (*cen2*Δ) cells. The anti-centrin antibody is the

20H5 antibody, which is a general centrin antibody and recognizes Cen1. Bar, 10 μm. (B) A Western blot showing that the anti-Cen2 antibody recognizes only recombinant Cen2. (C) Immuno-electron microscopy shows that Cen2 localizes to the site of new assembly. Left micrograph: Longitudinal view, Bar, 100 nm; Right micrograph: cross section, Bar, 100 nm. (D) Schematic showing the regions of the basal bodies. (E) Particle distribution to various regions of the basal body. BB, basal body; KF, kinetodesmal fiber; MZ, mid-zone; PC, postciliary microtubules; TV, transverse microtubules; TZ, transition zone.



Supplemental Figure 3. Cen1 expression levels do not change in the *cen2*Δ. (A) PCR analysis confirming the integration of *NEO2* into the *CEN2* locus and deletion of *CEN2* in *cen2*Δ cells. *ATU1* served as a positive control. (B) A western blot showing the levels of

Cen1 in WT and *cen2*Δ cells. Detection of alpha tubulin (ATU) served as a loading control. (C) Labeling by the anti-Sas6a antibody shows that the *cen2*Δ has the same basal body phenotypes seen in Figure 3. (D) Immunofluorescence images showing that *CEN2* rescues the basal body phenotypes in the *cen2*Δ. (E) An immunofluorescence image showing that the anti-Cen2 antibody recognizes Cen2 in the *cen2*Δ rescued with *CEN2*. Green, Cen1; Red, kinetodesmal fibers; Bar, 10 μm; Width of insets, 6 μm; Percentages indicate the frequency of observed phenotype for 100 cells.



Supplemental Figure 4. Plots showing the number of basal bodies per µm and the basal body angle distribution. (A) A plot showing the number of basal bodies per µm at 30°C. (B) A plot showing the number of basal bodies per µm at 38°C. (C) Circular plots showing basal body angle distribution at 30°C. (D) Circular plots showing basal body

angle distribution at 38°C. Asterisk, P<0.001%, N = 50 measurements; $\bar{x}$, average angle; σ, standard deviation. Each point in the circular plots for WT at 30°C, the Cen1-Cen2 rescue at 30°C, and the *CEN2* rescue 30°C represents two measurements. All the rest of the points represent one measurement. N = 100 measurements.