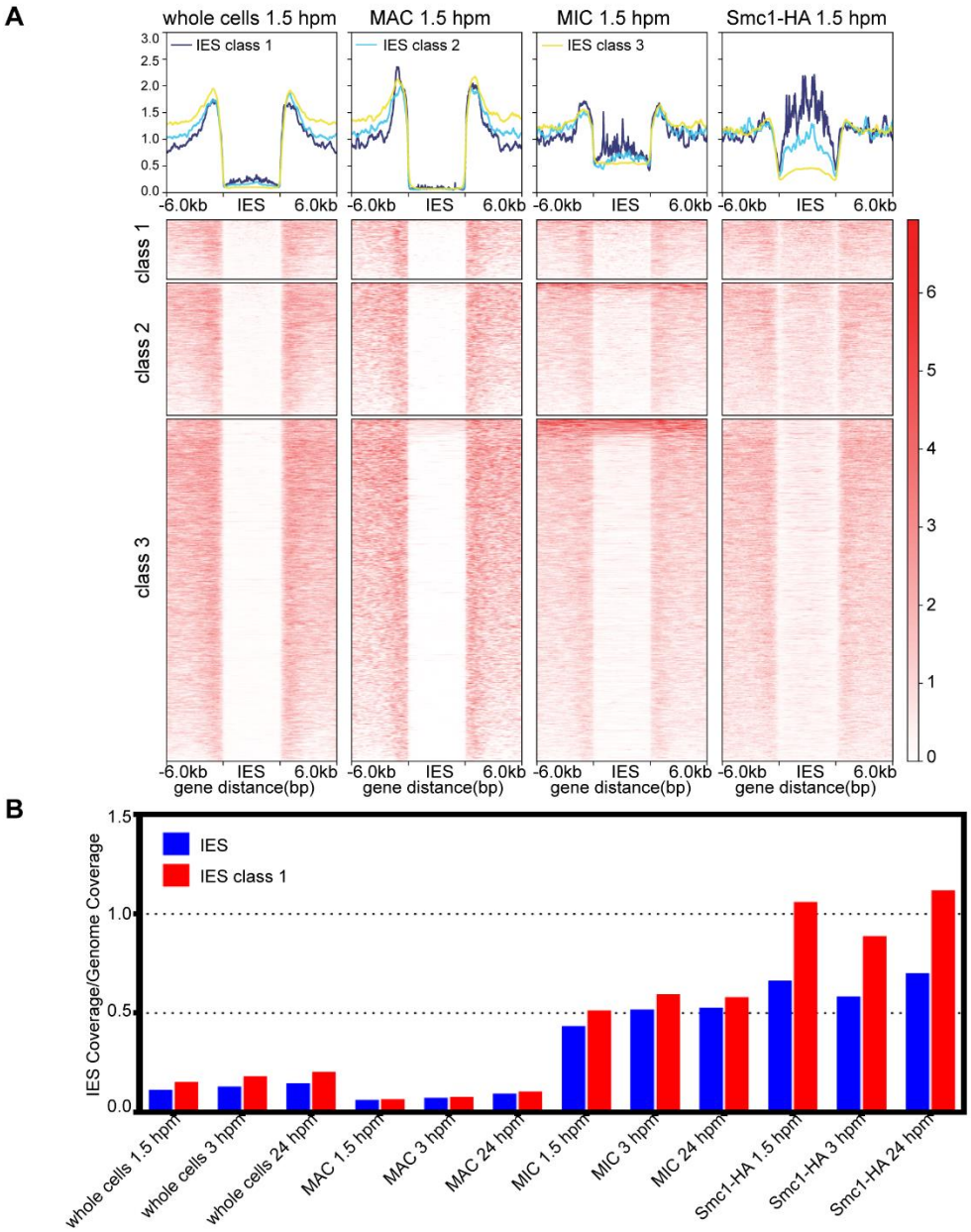
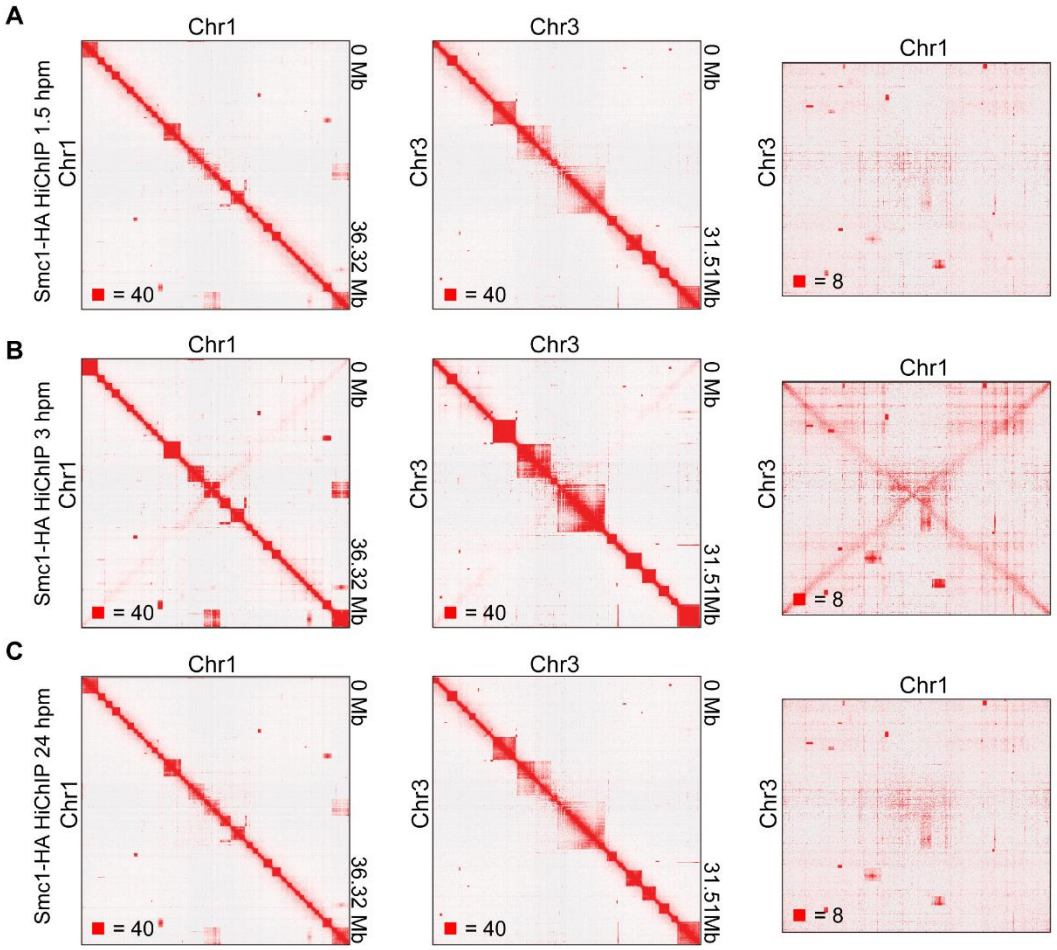
Supplemental Figure S1



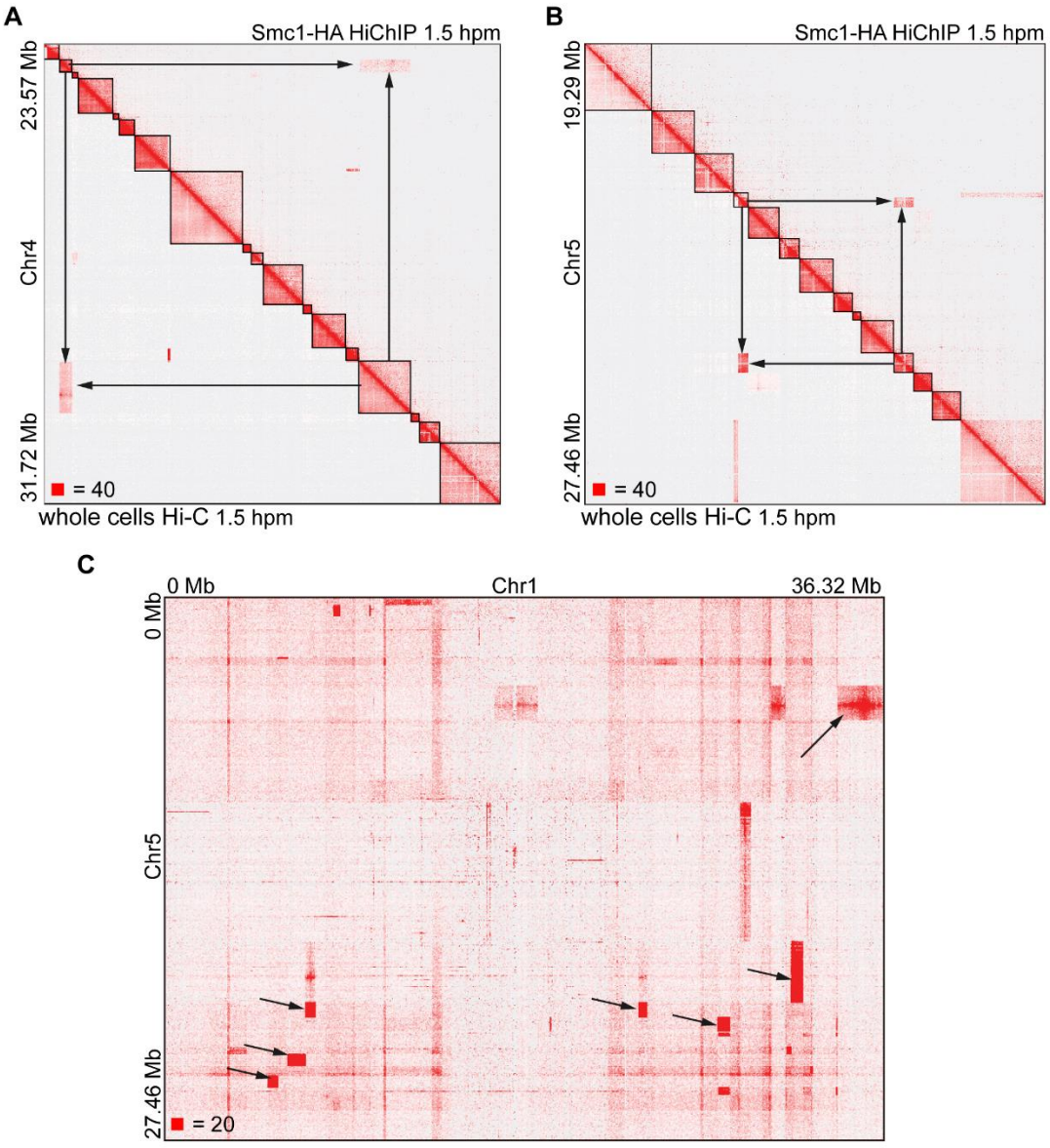
Supplemental Figure S1. Evaluation of MAC contamination in the MIC Hi-C data according to the reads coverage on IES regions. (A) Reads coverage of Hi-C data of whole *T. thermophila* cells, the isolated MACs, the isolated MICs and HiChIP data of Smc1-HA on IES regions were calculated by deepTools and normalized by Reads Per Kilobase per Million mapped reads (RPKM). The heatmaps showed reads coverage on three different classes of IES regions at 1.5 hpm. (B) The bar plot showed the ratio of Hi-C and HiChIP reads coverage on IES regions compared to other genomic regions. All IESs and class 1 of IESs were on display.

Supplemental Figure S2



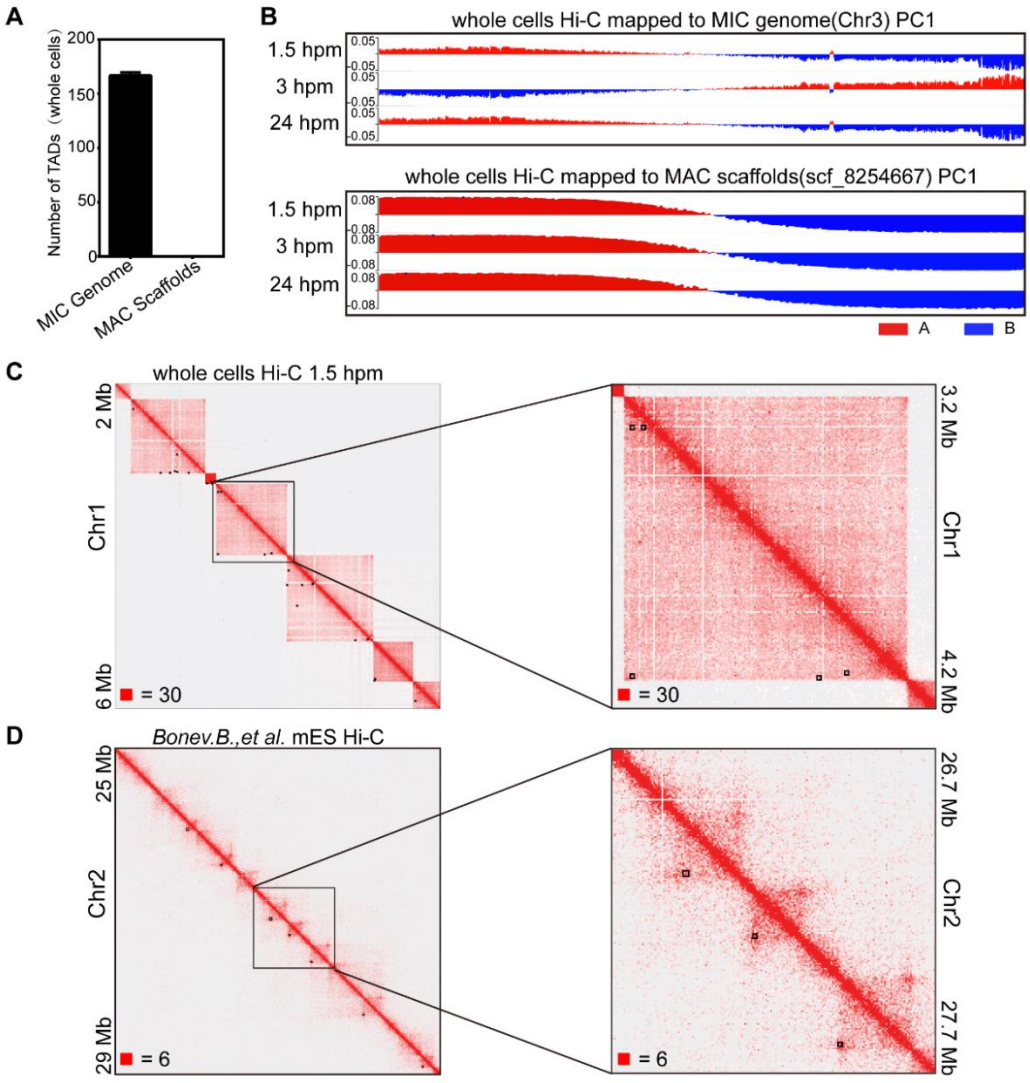
Supplemental Figure S2. **The X-shape chromosome interactions in the MIC at 3 hpm.** (A-C) Heatmaps of Smc1-HA HiChIP showing the intra-chromosome interactions on Chromosome 1 (left), Chromosome 3 (middle) and the inter-chromosome interactions between Chromosome 1 and 3 (right) in the MIC at 1.5 hpm (A), 3 hpm (B) and 24 hpm (C). Numbers at the lower left of heatmaps corresponded to the maximum signal in the matrix. hpm: hours post-mixing.

Supplemental Figure S3



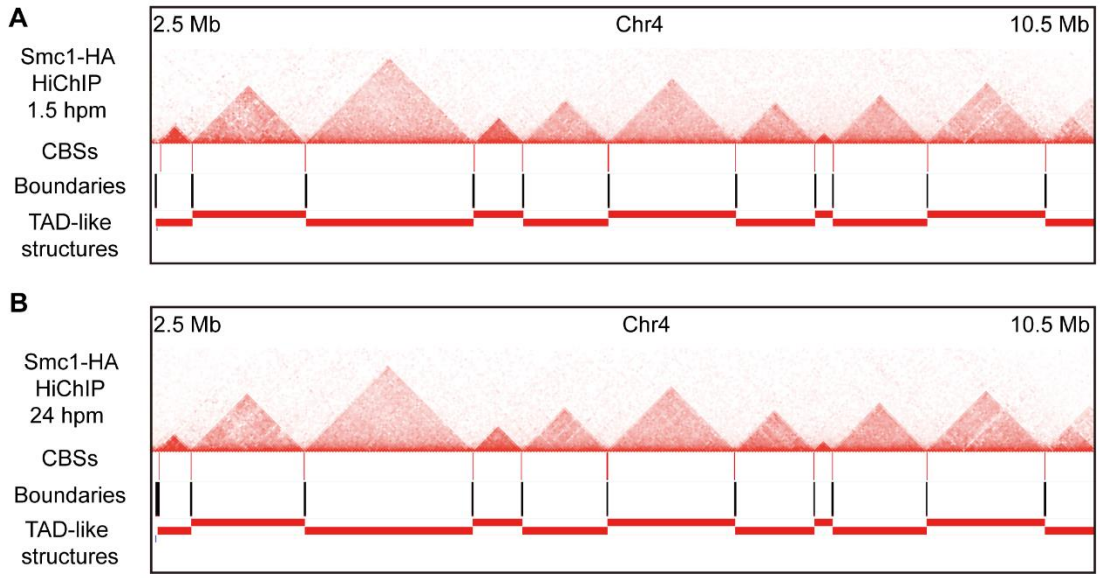
Supplemental Figure S3. 3D genome data might be helpful to optimize *T. thermophila* genome assembly. (A, B) Heatmaps showing the Hi-C interactions from 23.57 Mb to 31.72 Mb on the MIC Chromosome 4 (A) and from 19.29 Mb to 27.46 Mb on the MIC Chromosome 5 (B) at 1.5 hpm (Smc1-HA HiChIP data, upper right; *T. thermophila* whole cells Hi-C data, lower left). Chromatin interactions indicated by the black arrows were some strong larger-scale intra-chromosome interactions separated by several TAD-like structures. (C) Heatmaps showing the inter-chromosome Hi-C interactions between Chromosomes 1 and 5, the larger-scale inter-chromosome interactions indicated by the black arrows were some strong inter-chromosome interactions with clear boundaries. Numbers at the lower left of heatmaps corresponded to the maximum signal in the matrix. hpm: hours post-mixing.

Supplemental Figure S4



Supplemental Figure S4. Higher-order chromatin organization in the MAC of *T. thermophila*. (A) The bar plot showed the number of TADs identified by HiCExplorer when mapped the whole cells Hi-C data to the MIC genome (left) and the MAC scaffolds (right) at 1.5 hpm, 3 hpm and 24 hpm. (B) Compartment eigenvector of the whole cells Hi-C data was plotted along the MIC Chromosome 3 (mapped to the MIC genome, up) and the MAC scaffold 8254667 (mapped to the MAC scaffolds, below) at three different conjugation stages and used as A (red) and B (blue) compartments segmentation. (C) Heatmaps showing the Hi-C interactions of a 4 Mb region on Chromosome 1 (2 to 6 Mb, left) at 1.5 hpm. Zoomed-in views of a 1 Mb region (right) were also shown.(D) Heatmaps showing the Hi-C interactions of a 4 Mb region on Chromosome 2 (25 to 29 Mb, left) of mouse published mESC Hi-C data (Bonev et al. 2017). Zoomed-in views of a 1 Mb region (right) were also shown. The black points/boxes on (C) and (D) showed the chromatin loops identified by HiCCUPS in juicer. Numbers at the lower left of heatmaps corresponded to the maximum signal in the matrix.

Supplemental Figure S5



Supplemental Figure S5. Boundaries of TAD-like structures in the MIC consistent with CBSs. (A) The location of CBSs, boundaries and TAD-like structures compared to the heatmaps on the Chromosome 4 (2.5 Mb - 10.5 Mb) at 1.5 hpm. (B) The location of CBSs, boundaries and TAD-like structures compared to the heatmaps on the Chromosome 4 (2.5 Mb - 10.5 Mb) at 24 hpm. hpm: hours post-mixing.

Supplemental Table S1. MIC centromeres midpoints based on Hi-C results.

MIC Chromosome	Previous Cen midpoint (Mb)	Chromosome midpoint (Mb)	Cen midpoint from Hi-C (Mb)
1	18.61	18.16	17.71
2	12.33	12.76	12.65
3	15.15	15.76	15.33
4	15.29	15.86	14.79
5	13.88	13.74	12.79

Previous work defined the centromere regions (Hamilton et al. 2016) from L-Cbs and R-Cbs, thus the centromere midpoint is the middle of L-Cbs and R-Cbs. Here, we defined the centromere midpoints from our Hi-C and HiChIP data, and the intersection of crescent specific chromosome interactions stood for the midpoint of the centromere. Locations in Mb were the distances from the end of the left arm (telomere).

Supplemental Reference

Bonev B, Mendelson Cohen N, Szabo Q, Fritsch L, Papadopoulos GL, Lubling Y, Xu X, Lv X, Hugnot J-P, Tanay A et al. 2017. Multiscale 3D Genome Rewiring during Mouse Neural Development. *Cell* **171**: 557-572.e524.

Hamilton EP, Kapusta A, Huvos PE, Bidwell SL, Zafar N, Tang H, Hadjithomas M, Krishnakumar V, Badger JH, Caler EV et al. 2016. Structure of the germline genome of Tetrahymena thermophila and relationship to the massively rearranged somatic genome. *Elife* **5**: e19090.