

Figure S1. Images of experimental observations in vivo.

Each column displays observational snapshots from separate channels. From left to right: TetO/TetR-GFP, SPC42-RFP, Trans. Each row displays images obtained from distinct experimental group. From top to bottom: WT, SPT10Δ, ycg1-2 permissive 24°C, ycg1-2 restrictive 37°C.



Figure S2. Illustration of simulation models.

(A) Initialization of the condensin model. The blue beads represent the substructure formed by a single condensin complex. (B) The condensin starts to extrude a loop. (C) The condensin forms a large, dynamic loop. (D) In the simulation, a histone creates small constant loops at its attachment site. (E) The snapshot of the simulated dicentric plasmid after reaching equilibrium. Small red beads are the discretized unit and are connected through springs, which are black segments in figures. Two red boxes represent centromeres and are fixed in space. Large spheres with the same color are two attachment sites of one condensin complex.



Figure S3. Illustration of the behavior of condensins in the model

(A) A complete process of a condensin taking one move. Red spheres are DNA beads in the simulation and black line segments are springs connecting DNA beads. The dark blue bead (left) is the strong sites of the condensin. The light blue bead (right) is the weak sites of the condensin. Spring (blue line) connects to the monomer (red bead) that is closest to the point in space (gray dot) that is a 10 nm extension from the vector (gray, dotted line) formed by the current condensin spring (blue line)(B-C) Illustrative configurations of a loop extruded for a same period of time by a condensin possessing (B) tension-independent extrusion rate; (C) tension-dependent extrusion rate. Two end beads (black) are fixed in space. (D) A special case where the condensin takes a move and reduces the size of the extruded loop. (E) The condensin loop is disrupted and then the condensin begins to extrude a new loop.



Figure S4. Experimental WT data and simulated chromosome data.

(A) Plasmid signal length box plots from experimental observation. Each box represents data from a single timelapse on an independent plasmid. 35 groups of data are sorted by the ascending order of the median of signal lengths. Each dot represents one datapoint. (B) The boxplots of the signal length changes for aggregated simulation data as well as the experimental data. For both box plots, the red line

represents the median, and the bottom and the top edges of the box indicate the 25<sup>th</sup> and 75<sup>th</sup> percentiles, respectively. The whiskers extend to the most extreme data points not considered as outliers, and the outliers are plotted individually using the "+" symbol. Each dot represents one datapoint.

	Experiment WT	Raw Chain	Histone	Condensin	Histone + Condensin
# Observations (total timepoints)	925	183	183	183	168
Mean	804.2872419	871.3079	838.0402	805.5568	719.9247691
Median	774.5504821	875.1215	837.9084	818.9072	692.2718123
SD	301.8242902	69.15537	31.55819	167.068	177.3216677
Skewness	0.3541	-0.0456	0.0374	0.0815	0.5352
Kurtosis	2.2286	2.4196	2.6822	2.5848	2.4438
Normalized ranksum p-val against experimental WT		0.6532	0.6646	0.7372	0.9351
Normalized kstest2 p-val against experimental WT		0.429	0.4298	0.1631	0.8986

Table S1. Aggregated statistics of experimental WT data and simulated data.

The second column includes statistics of signal lengths from the experimental WT data. The third to last columns are statistics of signal lengths from Simulated data in different conditions. The statistics are indicated by their names in the first column. The last two rows are p-values of ranksum test and Two Sample Kolmogorov-Smirnov test between the experimental WT data and simulated data in each condition, using MATLAB's built-in packages (*ranksum* and *kstest2*). The higher p-value infers the higher probability in failure to reject the null hypotheses, for ranksum test that two data sets obtain the same median, and for two sample K-S test that two datasets obtain the same distribution.



Figure S5. Boxplots of aggregated data for *spt10*  $\Delta$  condition and *ycg1-2* condition, combining experimental data and simulated data for direct comparison.

(A)(B) Boxplots of aggregated plasmid signal lengths and plasmid signal length changes, respectively. First two boxes in each panel correspond to experimental data, from WT and from *spt10*  $\Delta$  mutant, respectively. The third and fourth boxes in each panel correspond to simulated WT plasmid and simulated *spt10*  $\Delta$  mutant, respectively. (C)(D) Boxplots of aggregated plasmid signal length and plasmid signal length changes, respectively. First two boxes in each panel correspond to experimental data, from *ycg1-2* permissive condition and from restrictive condition, respectively. The third and fourth boxes in each panel correspond to experimental data, from *ycg1-2* permissive condition and from restrictive condition, respectively. The third and fourth boxes in each panel correspond to simulated *ycg1-2* mutant in permissive condition and in restrictive condition, respectively. The boxplot syntax is the same as Figure S1.

	WT	SPT10 Δ	Simulation full histone	Simulation 50% histone
# Observations (total timepoints)	1172	1141	168	183
Mean	804.2872	798.5033151	719.9247691	683.6846198
Median	774.5505	729.5506347	692.2718123	652.0028894

SD	301.8243	369.292146	177.3216677	172.9937196	
Skewness	0.354064	0.771137973	0.535165175	0.817635339	
Kurtosis	2.228635	2.980276332	2.443767638	3.200481729	
Normalized ranksum against SPT10 $\Delta$			0.938535233	0.879596625	
Normalized kstest2 against SPT10 $\Delta$			0.277218538	0.529191381	

Table S2. Aggregated statistics of experimental SPT10 data and its simulated data.

The statistics are indicated by their names in the first column. The second column includes statistics of signal lengths from the experimental WT data. The third column includes the statistics of signal lengths from the experimental *spt10*  $\Delta$  mutants. The fourth column is from simulated WT data, which contains 6 condensins and a full load of histones. The last column is from simulated *spt10*  $\Delta$  mutant, where the histone capacity is reduced to 50% and all condensins remains.

	ycg1-2 permissive	ycg1-2 restrictive	6 condensins	3 condensins
# Observations (total timepoints)	753	357	168	122
Mean	598.8414521	770.1964309	719.9247691	757.330969
Median	567.0976222	808.9456428	692.2718123	780.8858083
SD	201.9735071	266.2989357	177.3216677	187.0110082
Skewness	0.656860608	-0.034800354	0.535165175	0.097111416
Kurtosis	2.754140885	1.899233189	2.443767638	1.910838279
Normalized ranksum against ycg1-2 restrictive			0.806333988	0.936293108
Normalized kstest2 against ycg1-2 restrictive			0.059461923	0.70040946

Table S3. Aggregated statistics of experimental ycg1-2 data and its simulated data.

The statistics are indicated by their names in the first column. The second column includes statistics of signal lengths from the experimental *ycg1-2* mutants in permissive condition. The third column includes the statistics of signal lengths from the experimental *ycg1-2* mutants in restrictive condition. The fourth column is from simulated WT data, which contains 6 condensins and a full load of histones. The last column is from simulated *ycg1-2* mutants in restrictive condition, where the number of condensins is reduced from 6 to 3.