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**Organization and replicon interactions within the highly segmented genome of  
*Borrelia burgdorferi***

Zhongqing Ren<sup>1,#</sup>, Constantin N. Takacs<sup>2,3,4,\$,#</sup>, Hugo B. Brandão<sup>5</sup>, Christine Jacobs-  
Wagner<sup>2,3,4\*</sup>, and Xindan Wang<sup>1\*</sup>

<sup>1</sup>Department of Biology, Indiana University, Bloomington, IN 47405, USA;

<sup>2</sup>Department of Biology, Stanford University, Stanford, CA 94305, USA;

<sup>3</sup>Sarafan ChEM-H Institute, Stanford University, Stanford, CA 94305, USA;

<sup>4</sup>Howard Hughes Medical Institute, Stanford, CA 94305, USA;

<sup>5</sup>Illumina Inc., 5200 Illumina Way, San Diego, CA 92122 USA.

<sup>\$</sup>Current address: Department of Biology, College of Science, Northeastern University,  
Boston, MA 02115, USA.

<sup>#</sup>These authors contributed equally.

<sup>\*</sup>Corresponding authors: [jacobs-wagner@stanford.edu](mailto:jacobs-wagner@stanford.edu); [xindan@indiana.edu](mailto:xindan@indiana.edu)

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Running title: Organization of a highly segmented bacterial genome.

## 31 **Abstract**

32 *Borrelia burgdorferi*, a causative agent of Lyme disease, contains the most segmented  
33 bacterial genome known to date, with one linear chromosome and over twenty  
34 plasmids. How this unusually complex genome is organized, and whether and how the  
35 different replicons interact are unclear. We recently demonstrated that *B. burgdorferi* is  
36 polyploid and that the copies of the chromosome and plasmids are regularly spaced in  
37 each cell, which is critical for faithful segregation of the genome to daughter cells.  
38 Regular spacing of the chromosome is controlled by two separate partitioning systems  
39 that involve the protein pairs ParA/ParZ and ParB/SMC. Here, using chromosome  
40 conformation capture (Hi-C), we characterized the organization of the *B. burgdorferi*  
41 genome and the interactions between the replicons. We uncovered that although the  
42 linear chromosome lacks contacts between the two replication arms, the two telomeres  
43 are in frequent contact. Moreover, several plasmids specifically interact with the  
44 chromosome *oriC* region, and a subset of plasmids interact with each other more than  
45 with others. We found that SMC and the SMC-like MksB protein mediate long-range  
46 interactions on the chromosome, but they minimally affect plasmid-chromosome or  
47 plasmid-plasmid interactions. Finally, we found that disruption of the two partition  
48 systems leads to chromosome restructuring, correlating with the mis-positioning of  
49 chromosome *oriC*. Altogether, this study revealed the conformation of a complex  
50 genome and analyzed the contribution of the partition systems and SMC family proteins  
51 to this organization. This work expands the understanding of the organization and  
52 maintenance of multipartite bacterial genomes.

53

## 54 **Author summary**

55 Genomes are highly organized in cells to facilitate biological processes. *Borrelia*  
56 *burgdorferi*, an agent of Lyme disease, carries one linear chromosome and more than  
57 twenty plasmids, in what is known as one of the most segmented bacterial genomes.  
58 How the different replicons interact with each other is unclear. Here we investigate the  
59 organization of this highly segmented genome and the protein factors that contribute to  
60 this organization. Using chromosome conformation capture assays, we determined the  
61 interactions within the chromosome, between chromosome and plasmids, and between

62 the plasmids. We found that the two telomeres of the chromosome interact with each  
63 other; a subset of plasmids interact with the chromosomal replication origin region; and  
64 a subset of plasmids preferentially interact with one another. Finally, we revealed that  
65 two structural maintenance of chromosomes family proteins, SMC and MksB, promote  
66 long-range DNA interactions on the chromosome, and the two partition systems,  
67 ParA/ParZ and ParB/SMC, contribute to chromosome structure. Altogether, we  
68 characterized the conformation of a highly segmented genome and investigated the  
69 functions of different genome organizers. Our study advances the understanding of the  
70 organization of highly segmented bacterial genomes.

71

## 72 **Introduction**

73 *Borrelia burgdorferi* causes Lyme disease, the most prevalent vector-borne infectious  
74 disease in Europe and North America [1, 2]. Although the *B. burgdorferi* genome is only  
75 ~1.5 megabasepairs in size, it includes one linear chromosome and more than 20  
76 plasmids (circular and linear) and is, to our knowledge, the most segmented bacterial  
77 genome [3-6]. Recently, using fluorescence microscopy to visualize loci on the  
78 chromosome and 16 plasmids, we found that *B. burgdorferi* contains multiple copies of  
79 its genome segments *per* cell, with each copy regularly spaced along the cell length [7].

80

81 In bacteria, the broadly conserved *parABS* partitioning system plays an important role in  
82 the segregation of chromosome and plasmids [8-15]. ParA dimerizes upon ATP binding  
83 and non-specifically binds to the DNA [16-19]. Centromeric ParB proteins bind to the  
84 *parS* sequences scattered around the origin of replication and spread several kilobases  
85 to nearby regions, forming a nucleoprotein complex [20-25]. The ParB-DNA  
86 nucleoprotein complex interacts with DNA-bound ParA-ATP dimers and stimulates the  
87 ATPase activity of ParA, leading to the release of ParA from the DNA and the formation  
88 of a ParA concentration gradient along the nucleoid [12, 15, 17, 26]. It is thought that  
89 repeated cycles of ParA and ParB interaction and release, together with the  
90 translocating forces from elastic chromosome dynamics [27-30] or the chemical ParA  
91 gradient [31, 32], promote the segregation of the two newly replicated ParB-origin  
92 complexes from one another [27, 29]. In addition, ParB plays a separate role in

93 recruiting the broadly conserved SMC complex onto the chromosomal origin region [13,  
94 14]. Once loaded, SMC moves away from the loading sites and typically tethers the two  
95 replication arms together, facilitating the resolution and segregation of the two sister  
96 chromosomes [33-35].

97

98 We discovered that in *B. burgdorferi*, the segregation and positioning of the multicopy  
99 chromosomal origins of replication (*oriC*) require the concerted actions of the ParB/SMC  
100 system and a newly discovered ParA/ParZ system [7]. ParZ, a centromere-binding  
101 protein, substitutes ParB to work with ParA and plays a major role in chromosome  
102 segregation [7]. Although *B. burgdorferi* ParB does not appear to partner with ParA, it is  
103 still required to recruit SMC to *oriC*. SMC in turn contributes to *oriC* positioning [7].

104 Overall, these findings advanced our understanding of *oriC* segregation in *B.*

105 *burgdorferi*. However, the information on the organization of the bulk of the

106 chromosome and the interactions among the various genome segments in this

107 bacterium is still lacking.

108

109 Chromosome conformation capture assays (Hi-C) have significantly advanced our  
110 understanding of bacterial genome folding and interactions [34, 36-41]. Along bacterial  
111 genomes, short-range self-interacting domains called chromosome interaction domains  
112 (CIDs) have been observed and are shown to be dictated mostly by transcription, with  
113 domain boundaries correlating with highly transcribed genes. In bacteria that contain the  
114 canonical SMC complex, the two replication arms of the chromosome are juxtaposed  
115 together, whereas bacteria that only encode SMC-like MukBEF and MksBEF analogs  
116 do not show inter-arm interactions [37, 39].

117

118 More recent efforts have begun to reveal the genome conformation of bacteria  
119 containing multiple replicons. In *Agrobacterium tumefaciens*, the origins of the four  
120 replicons are clustered together, which regulates DNA replication and drives the  
121 maintenance of this multipartite genome [41, 42]. Similarly, the two origins of *Brucella*  
122 *melitensis* chromosomes also showed frequent interactions [43]. In *Vibrio cholerae*, the  
123 origin of Chromosome 2 (Ch2) interacts with the *crtS* region on Chromosome 1 (Ch1)

124 for replication control, and the terminus region of Ch1 and Ch2 are interacting for  
125 coordinated replication termination and terminus segregation [40, 44]. These findings  
126 suggest that multipartite genomes harness inter-replicon interactions as a mechanism  
127 for replication regulation and genome maintenance. In this study, we aimed at  
128 understanding how *B. burgdorferi* organizes its ~20 replicons and how the partitioning  
129 proteins and SMC homologues contribute to genome organization.

130

## 131 **Results**

132 **The organization of the linear *B. burgdorferi* chromosome.** To determine the  
133 organization of the highly segmented genome of *B. burgdorferi*, we performed Hi-C on  
134 exponentially growing cultures of the infectious, transformable strain S9 (**Table S1** and  
135 **Fig. 1A, B**). After mapping the reads and plotting the data, we observed many white  
136 lines on the Hi-C map, especially in regions of the map corresponding to the plasmids  
137 (**Fig. 1B**). These white lines indicate the presence of repetitive sequences on the  
138 affected replicons, which were omitted during sequence mapping. The genome-wide Hi-  
139 C interaction map (**Fig. 1B**) has four distinct regions: an intra-chromosome interaction  
140 map in the lower left quadrant, a plasmid-chromosome interaction map with identical,  
141 mirrored copies in the top left and lower right quadrants, and a plasmid-plasmid  
142 interaction map in the top right quadrant. The chromosome displayed strong short-range  
143 interactions as evident by the primary diagonal (**Fig. 1B**, lower left quadrant).  
144 Interestingly, a secondary diagonal representing inter-arm interactions was absent from  
145 the Hi-C map. This was unexpected as *B. burgdorferi* encodes an SMC protein homolog  
146 and all SMC-carrying bacteria tested so far display chromosome with inter-arm  
147 interactions [34, 36, 38, 39, 41, 45, 46]. We note that although *B. burgdorferi* does  
148 contain a homolog of the ScpA subunit of the SMC complex, it does not encode the  
149 other subunit, ScpB [3]. Thus, the absence of the SMC-ScpAB holo-complex might  
150 explain the absence of chromosome arm alignment in *B. burgdorferi* (see Discussion).  
151 Additionally, the two ends of the chromosome, the left and right telomeres (*terCL* and  
152 *terCR*) displayed a striking interaction with each other (**Fig. 1B**, black arrows in lower  
153 left quadrant). Since *B. burgdorferi* is polyploid [7], it is unclear whether the interacting

154 *terCL* and *terCR* are located on the same chromosome or on adjacent chromosome  
155 copies.

156

157 **Interactions between the chromosome and 18 plasmids.** Qualitatively, plasmid-  
158 chromosome interactions were weaker than short-range interactions within the  
159 chromosome (i.e. the primary diagonal of the bottom left quadrant), but were stronger  
160 than long-range interactions within the chromosome (i.e. outside of the primary diagonal  
161 on the bottom left quadrant) (**Fig. 1B**). We plotted the distribution of these types of  
162 interaction frequencies and found that the differences were statistically significant (**Fig.**  
163 **2**). To better show the plasmid-chromosome interactions, we analyzed the interaction of  
164 each plasmid with each 5-kb bin on the chromosome (**Fig. 3A**). Interestingly, a subset  
165 of the linear plasmids, namely lp17, lp21, lp25, and lp28-3, showed stronger interactions  
166 with the chromosomal origin region compared with the rest of the chromosome (**Fig.**  
167 **3A**). These interactions are reminiscent of the origin clustering interactions mediated by  
168 centromeric proteins in *A. tumefaciens*, which are critical for the replication and  
169 maintenance of the secondary replicons in that bacterium [41, 42]. Notably, the plasmid-  
170 chromosome interactions observed here are weaker than those observed in *A.*  
171 *tumefaciens*, and only 4 out of 18 plasmids showed these specific interactions with the  
172 chromosome, thus the biological function of these interactions is unclear (see  
173 Discussion).

174

175 **Plasmid-plasmid interactions.** Plasmid-plasmid interactions are depicted in the top  
176 right quadrant of the Hi-C map (**Fig. 1B**) and appeared stronger than plasmid-  
177 chromosome interactions (**Fig. 1B**, top left quadrant, and **Fig. 2**) and long-range  
178 interactions within the chromosome (**Fig. 1B**, outside of the primary diagonal on the  
179 bottom left quadrant, and **Fig. 2**). To better understand the interactions between every  
180 two plasmids, we recalculated the interaction frequencies after excluding the plasmid-  
181 chromosome interactions from the analysis (**Fig. 3B**). We note that the sizes of the 18  
182 plasmids ranged from 17 kb to 54 kb [3, 4] and that their copy numbers had been  
183 previously determined by microscopy and whole genome sequencing, ranging from 0.5  
184 to 1.3 relative to the copy number of the *oriC* locus [7] (**Fig. 1A**). To understand whether

185 these sizes and copy numbers of the plasmids could impact plasmid-plasmid  
186 interactions, we used these numbers to simulate the plasmid-plasmid interaction  
187 frequencies, assuming that all the plasmids were freely diffusing in the cytoplasm (see  
188 Materials and Methods for simulation details). Our simulation showed that plasmids that  
189 have a bigger size or a higher copy number interacted more with other plasmids in the  
190 raw Hi-C maps before any corrections (**Fig. S1A, B**, top panels). However, these  
191 preferential interactions did not show up after our standard procedure of iterative  
192 corrections for the Hi-C maps [47] (**Fig. S1A, B**, middle panels), unless a very fine color  
193 scale was applied (**Fig. S1A, B**, bottom panels). Interestingly, in our experiment (**Fig.**  
194 **3B**, left), the interactions among the seven cp32 plasmids (cp32-1, cp32-3, cp32-4,  
195 cp32-6, cp32-7, cp32-8, cp32-9) and among the other 11 plasmids were higher than  
196 expected for random encounters based on simulations (**Fig. 3B**, right). Thus, the  
197 preferential interactions between plasmids we observed in our experiment could not be  
198 explained solely by the size and copy number difference in the plasmids. Since  
199 repetitive sequences between different plasmids were removed during mapping, we  
200 believe that these higher-than-expected interactions observed in our experiment are  
201 genuine and not due to erroneous normalization or mapping. The molecular mechanism  
202 of plasmid-plasmid interactions remains to be determined.

203  
204 **Clustering analysis of *smc* and *par* mutants.** The highly conserved SMC family  
205 proteins and the DNA partitioning proteins are central players in bacterial chromosome  
206 organization and segregation [48, 49]. *B. burgdorferi* has a canonical SMC, encoded by  
207 gene *bb0045*, as well as an MksB protein, encoded by gene *bb0830*, but lacks the  
208 genes encoding the accessory proteins ScpB, MksE, and MksF [3]. Additionally, *B.*  
209 *burgdorferi* employs two partition systems for the positioning of its multicopy *oriC* loci,  
210 ParB/SMC and ParA/ParZ [7]. To understand the contribution of these factors to *B.*  
211 *burgdorferi* genome interactions, we performed Hi-C on a collection of mutants (**Table**  
212 **S1**). Essentially, the genes of interest were replaced with a gentamycin or kanamycin  
213 resistance gene. The control strain CJW\_Bb284 had the gentamycin marker inserted in  
214 a non-coding region located in between the convergently-oriented *parZ* and *parB* genes,  
215 in the otherwise wild-type (WT) *parAZBS* locus. The Hi-C maps of strain CJW\_Bb284

216 were almost identical to the maps generated using the parental WT strain S9 (**Fig. S2**).  
217 Additionally, our Hi-C experiments on WT, control, and every mutant were done in two  
218 biological replicates that showed nearly identical results (**Fig. S3**).

219  
220 To compare the different mutants, we performed a clustering analysis using the contact  
221 probability curves of our 22 Hi-C samples so that mutants that had similar profiles of  
222 contact probabilities would be grouped together (**Fig. 4, S4**). Using the Silhouette  
223 method [50], we found that the mutants could be divided into six groups (**Fig. 4A, B**)  
224 (see Materials and Methods): group 1 includes WT and the control strain CJW\_Bb284  
225 (**Fig. 4B, C, Fig. S2**); group 2 includes  $\Delta smc$  (**Fig. 4B, D**); group 3 includes  $\Delta mksB$  (**Fig.**  
226 **4B, E**); group 4 includes  $\Delta parB$ ,  $\Delta parS$  and  $\Delta parBS$  (**Fig. 4B, F**); group 5 includes  
227  $\Delta parA$ ,  $\Delta parZ$  and  $\Delta parAZ$  (**Fig. 4B, G**); and group 6 includes  $\Delta parAZBS$  (**Fig. 4B, H**)  
228

229 This grouping analysis based on Hi-C results indicates that the control strain  
230 CJW\_Bb284 behaves the same as its parental WT strain; SMC and MksB have different  
231 effects on chromosome folding; ParB and *parS* work as a unit; ParA and ParZ work  
232 together; and ParB/*parS* and ParA/ParZ have additive effects because  $\Delta parAZBS$   
233 formed its own group. Notably, our recent ChIP-seq and microscopy analyses [7] have  
234 indicated that ParB binds to *parS* and recruits SMC to the origin region, and ParZ works  
235 with ParA; disrupting *parBS* barely changed *oriC* spacing; deleting *parA*, *parZ* or *parAZ*  
236 had similar effects and dramatically changed the even spacing of *oriC* in the polyploid  
237 cells; finally, deleting *parBS* and *parA* caused a stronger defect in *oriC* spacing than  
238  $\Delta parAZ$  alone [7]. Therefore, the grouping of mutants based on Hi-C analysis here (**Fig.**  
239 **4B**) is largely consistent with our previous cytological characterization of these mutants  
240 [7]. This agreement reveals the robustness of our assays.

241  
242 **SMC and MksB mediate long-range interactions within the chromosome.** In our  
243 clustering analysis, the two biological replicates of  $\Delta smc$  fell in one group (group 2) and  
244 replicates of  $\Delta mksB$  fell into a separate group (group 3) (**Fig. 4B, D, E**). To understand  
245 how  $\Delta smc$  and  $\Delta mksB$  affect genome contacts, we analyzed the  $\log_2$  ratios of the Hi-C  
246 maps between each mutant strain and the relevant control. (**Fig. 5A-F**). We observed



247 that both  $\Delta smc$  and  $\Delta mksB$  strains had decreased long-range DNA contact compared  
248 with the control (**Fig. 5D-F**, blue pixels in black trapezoid). Specifically, as seen on the  
249 Hi-C contact probability decay curves (**Fig. 5G-I**), in  $\Delta smc$ , loci separated by ~60 kb or  
250 greater had decreased frequency of contacts compared with the control, and in  $\Delta mksB$ ,  
251 loci separated by ~100 kb or greater had decreased frequency of contact compared with  
252 the control (**Fig. 5H, I**, black dotted lines). These data indicate that both SMC and MksB  
253 promote long-range DNA contacts and that their effects are different enough to fall into  
254 different groups in our clustering analysis. We noted that *B. burgdorferi* is missing the  
255 ScpB subunit of the SMC complex, as well as the MksE and MksF subunits of the  
256 MksBEF complex. However, previous work showed that purified *B. subtilis* SMC protein  
257 (in the absence of ScpA and ScpB) is able to form DNA loops *in vitro* [51]. Our results  
258 suggest that the incomplete SMC/Mks complexes may form DNA loops in *B.*  
259 *burgdorferi*. Curiously, the absence of SMC or MksB enhanced the *terCL-terCR*  
260 interactions (**Fig. 5E, F**, black arrows), suggesting that these proteins reduce the  
261 contacts between the telomeres. Finally, we note that both SMC and MksB mainly affect  
262 interactions within the chromosome and not between chromosome and plasmid or  
263 among the plasmids (**Fig. 5A-F, S5-7**).

264  
265 **Contribution of ParB/*parS* and ParA/ParZ to chromosome organization.** In the  
266 grouping analysis,  $\Delta parS$ ,  $\Delta parB$  and  $\Delta parBS$  fell in the same group (group 4) (**Fig. 4B,**  
267 **F**), consistent with previous finding that ParB and *parS* act as a unit [7]. The absence of  
268 *parB* and/or *parS* caused similar changes to genome interactions compared with the  
269 control (**Fig. 6A-F**): *terCL-terCR* interactions decreased (**Fig. 6D-F**, blue pixels indicated  
270 by black arrows); longer range (>150 kb) interactions within the chromosome increased  
271 (**Fig. 6D-F**, red pixels within black trapezoid); and short-range interactions (50-150 kb)  
272 decreased (**Fig. 6D-F**, blue pixels between black trapezoid and the red line). These  
273 trends are opposite to those observed in  $\Delta smc$  or  $\Delta mksB$  (**Fig. 5E, F**). Since ParB  
274 recruits SMC to the *oriC* region in *B. burgdorferi* [7], the loss of *parBS* could lead to  
275 increased non-specific loading of SMC on the chromosome. Thus, these results are  
276 consistent with a scenario in which non-specific loading of SMC to the chromosome

277 outside of the *oriC* region (i.e. independent of ParB/*parS*) is the major contributor to  
278 long-range chromosome interactions.

279

280 Group 5 contains  $\Delta parA$ ,  $\Delta parZ$ ,  $\Delta parAZ$  (**Fig. 4B, G, 6G-I**), consistent with the idea that  
281 ParA and ParZ works in the same pathway [7]. The absence of *parA* and/or *parZ*  
282 caused two major changes in chromosome folding: loci separated by 100 to 300 kb had  
283 increased interactions (**Fig. 6K-M**, red pixels below the black line) and loci separated by  
284 300 kb or more had decreased interactions (**Fig. 6K-M**, blue pixels above the black  
285 line). Thus, ParA/ParZ acts to reduce mid-range (100-300 kb) and enhance long-range  
286 (>300 kb) DNA interactions on the chromosome. Since ParA/ParZ promotes  
287 chromosome segregation and spacing, we speculate that loss of ParA acting on DNA  
288 caused these changes in DNA interactions.

289

290 Finally,  $\Delta parAZBS$ , which lacked both *parBS* and *parAZ*, formed its own group (group 6)  
291 (**Fig. 4B, H, 6J, N**). This mutant essentially exhibited an additive effect of  $\Delta parBS$  (**Fig.**  
292 **6C, F**) and  $\Delta parAZ$  (**Fig. 6I, M**): decreased interactions below 150 kb (like in  $\Delta parBS$ ),  
293 increased mid-range (100-300 kb) interactions (as seen in  $\Delta parAZ$ ), and a complete  
294 loss of *terCL-terCR* interactions (**Fig. 6J, N**, black arrows). These effects can be  
295 explained by the independent actions of ParB/*parS* and ParA/ParZ that we discussed  
296 above.

297

298 Overall, our Hi-C analyses of these mutants indicate that the perturbation of genome  
299 interactions is correlated to the previously observed cytological defects in chromosome  
300 positioning and segregation [7]. Interestingly, although DNA interactions within the  
301 chromosome were changed in cells missing *parBS* or *parAZ*, the interactions between  
302 replicons (plasmid-chromosome and plasmid-plasmid interactions) remained similar to  
303 the control (**Fig. S5-S7**). Only in  $\Delta parAZBS$ , plasmid-chromosome interactions were  
304 reduced, and plasmid-plasmid interactions were more evened out, which could be due  
305 to the entanglement of different copies of chromosomes in the polyploid cells [7].

306

307 **Discussion**

308 In this study, we characterized the organization of the highly segmented genome of *B.*  
309 *burgdorferi* and the contribution of the chromosome partitioning proteins and SMC  
310 homologs to this organization. *B. burgdorferi* contains a linear chromosome and  
311 expresses an SMC protein, which is recruited by ParB/*parS* to the chromosomal origin  
312 like in many other bacteria. Notably, the *B. burgdorferi* chromosome does not have  
313 inter-arm interactions observed in other SMC-carrying bacteria [34, 36, 38, 39, 41, 45 ,  
314 46]. Nonetheless, SMC and its analog MksB contribute to long-range DNA contacts  
315 possibly through DNA looping. Interestingly, the absence of ParB/*parS* enhances SMC's  
316 loop forming ability, suggesting that SMCs that load non-specifically outside of the  
317 chromosomal origin regions are more productive at forming DNA loops, while SMCs  
318 recruited by ParB to the origin is less so. Since *B. burgdorferi* is lacking ScpB and  
319 MksEF to form complete SMC and Mks complexes, it is possible that the loop formation  
320 mechanism by the incomplete complexes is different from the loop-extrusion activity of  
321 the holocomplexes [51-55]. For instance, it is possible that SMC or MksB alone can only  
322 facilitate long-range loop formation by binding to and bridging two DNA segments that  
323 are already in proximity.

324  
325 The *B. burgdorferi* strain used in this study contains 18 plasmids. These plasmids  
326 showed differential interactions with the chromosome. Namely, plasmids lp17, lp21,  
327 lp25, and lp28-3 formed specific interactions with the chromosome at the *oriC* region,  
328 but the other 14 plasmids did not (**Fig. 3A, S6**). This pattern was highly reproducible in  
329 different mutants (**Fig. S5, S6**), suggesting that these plasmid-chromosome interactions  
330 are real, specific interactions. What are the molecular mechanism and biological  
331 function of these interactions? In *A. tumefaciens*, the secondary replicons cluster with  
332 the primary replicon at their origin regions through interactions between ParB homologs  
333 [41, 42], which prevents the loss of the secondary replicons [42]. In *B. burgdorferi*, we  
334 note that these interactions did not require ParB/*parS* or ParA/ParZ (**Fig. S5, S6**),  
335 suggesting that the molecular mechanism for these interactions is different from the  
336 centromeric clustering observed in *A. tumefaciens*. Although it is still possible that the  
337 four plasmids that interact with the chromosome may “piggyback” the chromosome to  
338 facilitate their own segregation and maintenance, it is also possible that these plasmid-

339 chromosome interactions have functions unrelated to plasmid segregation. Indeed, 14  
340 out of 18 plasmids did not interact with the chromosome origin, indicating that *B.*  
341 *burgdorferi* plasmids segregate largely independently from the chromosome. Notably, *B.*  
342 *burgdorferi* is polyploid with unequal copy number for each replicon [7] while *A.*  
343 *tumefaciens* newborn cells are haploid [41]. We postulate that the difference in ploidy  
344 might be one underlying factor accounting for the difference in organizing strategies  
345 between these two species. Our findings suggest that different species might take  
346 diverse strategies to organize and maintain segmented genomes.

347  
348 The interactions between the plasmids on average are more frequent than plasmid-  
349 chromosome interactions and long-range intra-chromosomal interactions (**Fig. 1B, 2**).  
350 Interestingly, we observed all seven cp32 plasmids interact more frequently with one  
351 another, and cp26 and the ten linear plasmids preferentially interact with one another  
352 (**Fig. 3B**). This grouping does not seem to be correlated with plasmid size or copy  
353 number (**Fig. 1A, 3B**), and the mechanism for these preferential interactions remains to  
354 be explored.

355  
356 Unlike in other bacteria studied to date, in *B. burgdorferi*, there are two partitioning  
357 system pairs, ParA/ParZ and ParB/*parS*, which co-regulate the spacing of the *oriC*  
358 copies in the cell. ParA/ParZ plays a more important role than ParB/*parS*. While  
359 removing ParB/*parS* only caused very mild defects in maintaining *oriC* spacing in the  
360 presence of ParA/ParZ, deleting both *parA* and *parBS* further disrupted the spacing  
361 pattern [7]. By Hi-C, we observed a similar trend in genome reorganization in these  
362 mutants: removing *parAZ* caused a significant increase of the medium-range (100-300  
363 kb) interactions but double deletion of *parAZ* and *parBS* led to an additive increase in  
364 these interactions. Thus, the segregation defect is correlated with increased mid-range  
365 genome interactions. The causal relationship between chromosome segregation and  
366 genome folding is unclear and remains to be examined. We speculate that the tension  
367 exerted through the partitioning system leads to the change in DNA folding over the  
368 length of the chromosome, which in our case is the decrease of DNA interactions in the  
369 100-300 kb range.

370  
371 Despite the absence of inter-arm interactions on the chromosome, the two ends of the  
372 linear chromosome *terCL* and *terCR* interact, which requires ParA/ParZ and ParB/*parS*.  
373 The contribution of ParA/ParZ and ParB/*parS* to *terCL-terCR* interactions might be  
374 through different mechanisms. ParA/ParZ is required for the spacing of *oriC* copies [7].  
375 Thus, it is possible that mis-positioning of chromosome copies reduces the frequency of  
376 *terCL-terCR* contacts. For ParB/*parS*, although it does not contribute much to the  
377 spacing of chromosome copies [7], it recruits SMC to the origin. Since SMC reduced  
378 *terCL-terCR* contacts (**Fig. 5F**), it is possible that ParB-mediated recruitment of SMC to  
379 the *oriC*-proximal *parS* site and away from chromosome arms lifts SMC's inhibitory role  
380 in *terCL-terCR* interactions.

381  
382 Altogether, our study identifies intrachromosomal, chromosome-plasmid, and plasmid-  
383 plasmid interactions of the most segmented bacterial genome known to date. We  
384 explored the contribution of SMC-family proteins and two partitioning systems to the  
385 folding and interactions of the genome. Although the exact mechanism for replicon  
386 interactions remains to be investigated, our study presents one step forward in the  
387 understanding of multipartite genome architecture and maintenance.

388

## 389 **Materials and methods**

### 390 **General Methods**

391 The *B. burgdorferi* strains used in this study are listed in **Table S1**. Cells were grown in  
392 exponential growth in complete Barbour-Stoenner-Kelly (BSK)-II liquid medium at 34°C  
393 in a humidified incubator and under 5% CO<sub>2</sub> atmosphere [56, 57]. Complete BSK-II  
394 medium contained 50 g/L bovine serum albumin (Millipore, Cat. 810036), 9.7 g/L  
395 CMRL-1066 (US Biological, Cat. C5900-01), 5 g/L Neopeptone (Difco, Cat. 211681), 2  
396 g/L Yeastolate (Difco, Cat. 255772), 6 g/L HEPES (Millipore, Cat. 391338), 5 g/L  
397 glucose (Sigma-Aldrich, Cat. G7021), 2.2 g/L sodium bicarbonate (Sigma-Aldrich, Cat.  
398 S5761), 0.8 g/L sodium pyruvate (Sigma-Aldrich, Cat. P5280), 0.7 g/L sodium citrate  
399 (Fisher Scientific, Cat. BP327), 0.4 g/L N-acetylglucosamine (Sigma-Aldrich, Cat.  
400 A3286), 60 mL/L heat-inactivated rabbit serum (Gibco, Cat.16120), and had a pH of

401 7.60. When noted, the following antibiotics were used: gentamicin at 40 µg/mL,  
402 streptomycin at 100 µg/mL, and kanamycin at 200 µg/mL [58-60]. Lists of strains,  
403 plasmids, oligonucleotides and Next-Generation-Sequencing samples can be found in  
404 Tables S1-S4.

405

#### 406 **Growing cells for Hi-C**

407 For Hi-C biological replicates, pairs of 100 mL cultures of each strain were inoculated  
408 and grown for two or three days. The cultures were fixed by addition of 37 mL 37%  
409 formaldehyde (Sigma-Aldrich, Cat. F8775) followed by rocking at room temperature for  
410 30 min. Formaldehyde was inactivated using 7 mL 2.5 M glycine and rocking for 5 min.  
411 The samples were chilled on ice for 10 min, then pelleted at 4°C and 4,300 x g for 30  
412 min in an Allegra X-14R centrifuge (Beckman Coulter) equipped with a swinging bucket  
413 SX4750 rotor. The pellet was resuspended in 1 mL ice-cold HN buffer (50 mM NaCl, 10  
414 mM HEPES, pH 8.0) [61], then pelleted at 4°C and 10,000 x g for 10 min. The pellet  
415 was resuspended in 400 µL cold HN buffer, and 100 µL aliquots were frozen in a dry ice  
416 ethanol bath then stored at below -80°C.

417

#### 418 **Hi-C**

419 The detailed Hi-C procedure for *B. burgdorferi* was adapted from previously described  
420 protocols in *B. subtilis* [34] and *A. tumefaciens* [41]. Briefly,  $5 \times 10^8$  *B. burgdorferi* cells  
421 were used for each Hi-C reaction. Cells were lysed using Ready-Lyse Lysozyme  
422 (Epicentre, R1802M) in TE for 60 min, followed by 0.5% SDS treatment for 30 min.  
423 Solubilized chromatin was digested with DpnII and incubated for 2 hours at 37°C. The  
424 digested chromatin ends were repaired with Klenow and Biotin-14-dATP, dGTP, dCTP,  
425 dTTP. The repaired products were ligated in dilute reactions by T4 DNA ligase at 16°C  
426 overnight (about 20 hrs). Ligation products were reverse-crosslinked at 65°C overnight  
427 (about 20 hrs) supplemented with EDTA, 0.5% SDS and proteinase K. The DNA was  
428 then extracted twice with phenol/chloroform/isoamylalcohol (25:24:1) (PCI), precipitated  
429 with ethanol, and resuspended in 40 µl 0.1XTE buffer. Biotin at non-ligated ends was  
430 removed using T4 polymerase (4 hrs at 20°C) followed by extraction with PCI. The DNA  
431 was then resuspended in 105 µl ddH<sub>2</sub>O and sheared by sonication for 12 min with 20%

432 amplitude using a Qsonica Q800R2 water bath sonicator. The sheared DNA was used  
433 for library preparation with the NEBNext Ultrall kit (E7645) following the manufacturer's  
434 instructions for end repair, adapter ligation, and size selection. Biotinylated DNA  
435 fragments were purified using 5  $\mu$ l streptavidin beads following the manufacturer's  
436 instructions. All DNA-bound beads were used for PCR in a 50  $\mu$ l reaction for 14 cycles.  
437 PCR products were purified using Ampure beads (Beckman, A63881) and sequenced  
438 at the Indiana University Center for Genomics and Bioinformatics using NextSeq 500.  
439 Paired-end sequencing reads were mapped to the genome file of *B. burgdorferi* B31  
440 (NCBI Reference Sequence GCA\_000008685.2 ASM868v2) using the default setting  
441 with MAPQ30 filter of Distiller (<https://github.com/open2c/distiller-nf>). Plasmids are  
442 arranged in this order: cp26, cp32-1, cp32-3, cp32-4, cp32-6, cp32-7, cp32-8, cp32-9,  
443 lp17, lp21, lp25, lp28-1, lp28-2, lp28-3, lp28-4, lp36, lp38 and lp54. Plasmids cp9, lp5  
444 and lp56 are absent from our strain. The *B. burgdorferi* B31 genome was divided into 5-  
445 kb bins. Subsequent analysis and visualization were done using R and Python scripts.

446

#### 447 **Hi-C analysis**

448 The mapped Hi-C contact frequencies were stored in multi-resolution cooler files [62]  
449 and the Hi-C matrices were balanced using the iterative correction and eigenvector  
450 decomposition method [47]. The iterative correction method is a standard way to  
451 balance the Hi-C map such that the rows and columns sum to a constant value  
452 (typically 1), which helps to correct for biases in genomic coverage (e.g. how easy it is  
453 to capture or amplify specific genome regions). The iterative correction process and  
454 intuition for the procedure can be approximately summarized as follows: each individual  
455 value within a row is divided by the sum of values for that row to achieve a sum of 1 for  
456 every row. However, this normalization of the rows breaks the required symmetry of the  
457 Hi-C matrix. Therefore, row normalization is followed by column normalization where  
458 each individual value in a column is divided by the resulting sum of values for that  
459 column, which subsequently "unbalances" the rows and the row sum is no longer 1. As  
460 such, the process can be iteratively repeated until the row and column sums converge  
461 to 1 within a pre-defined error tolerance. This results in a balanced Hi-C matrix in which  
462 genomic coverage biases are minimized. We described the process starting with

463 normalization of rows followed by columns. However, the procedure could equally have  
464 been applied by starting with columns instead of rows since the Hi-C matrix is  
465 symmetric about the primary diagonal. Unless otherwise specified, all Hi-C plots and  
466 downstream analyses were performed with this iterative correction.

467

468 Plots were generated with R or Python 3.8.15 using Matplotlib 3.6.2 [63]. Data were  
469 retrieved for plotting at 5-kb resolution.  $P_c(s)$  curves show the averaged contact  
470 frequency between all pairs of loci on the chromosome separated by set distance ( $s$ ).  
471 The x-axis indicates the genomic distance of separation in kb. The y-axis represents  
472 averaged contact frequency in a logarithmic scale. The curves were computed for data  
473 binned at 5 kb. For the  $\log_2$  ratio plots, the Hi-C matrix of each mutant was divided by  
474 the matrix of the control. Then,  $\log_2(\text{mutant/control})$  was calculated and plotted in a  
475 heatmap using R.

476

#### 477 **Clustering of strains based on Hi-C data**

478 Clustering of strains based on the contact probability curves was done using the scikit-  
479 learn 1.1.3 k-means algorithm [50]. To determine the optimal number of clusters, we  
480 maximized the average Silhouette score. The silhouette score,  $s(i)$  is a metric that  
481 determines, for some collection of objects  $\{i\}$ , how well each individual object,  $i$ , matches  
482 the clustering at hand [64]. In our case, the collection of objects were the log-  
483 transformed contact frequency  $P_c(s)$  curves, which were computed as the average  
484 value of the contact frequency of pairs of loci separated by a fixed genomic distance.  
485 Average silhouette scores were computed for data clustered using k-means with varying  
486 the number of clusters ranging from 2 to 21. We found that the number of clusters that  
487 maximized the average silhouette score was 6, suggesting that 6 is the optimal number  
488 of clusters in the data.

489

#### 490 **Generating expected plasmid-plasmid interaction frequencies map**

491 Expected plasmid-plasmid interaction frequencies were computed using either copy  
492 number of the plasmids alone, as obtained by marker frequency analysis, or in  
493 combination with information on the plasmid lengths (**Fig. 1A**).



494

495 For the simulated plasmid-plasmid contact map using both the copy numbers and  
496 plasmid lengths (**Fig. S1A**), we first multiplied the average plasmid copy number relative  
497 to the *oriC* (i.e. which have values ranging between 0.5 and 1.4, see **Fig. 1A**) by the  
498 plasmid lengths in numbers of 5-kb bins (i.e. which have values between 3 and 10 bins  
499 per plasmid, see **Fig. 1A**) and rounded the resulting number to the nearest integer,  $n_p$   
500 for each plasmid  $p$ . The values of  $n_p$  ranged between 2 and 14, and the total sum over  
501 all the plasmids,  $p$ , was  $N = \sum_p n_p = 80$ . The simulated plasmid-plasmid “contact  
502 frequency” matrix was computed using the probability of randomly drawing a given pair  
503 of plasmids. The probability for drawing a plasmid,  $p$ , is  $n_p/N$ . The resulting probability  
504 matrix from this calculation can be seen in **Fig. S1A** (top panel). To best compare the  
505 simulated plasmid-plasmid contact probability map with the experimental Hi-C data, we  
506 applied the iterative correction procedure [47] to this map. The resulting matrix is shown  
507 both with the same scale bar as the experimental Hi-C map (**Fig. S1A**, middle panel)  
508 and with a very fine color scale (**Fig. S1A**, bottom panel). We note that the iterative  
509 correction scheme tends to minimize the effects of copy number variation from one  
510 genome segment to another and this is why the expected (i.e. simulated) plasmid-  
511 plasmid contact map looks largely uniform when plotted with the same dynamic range  
512 as experimental data (**Fig. 3B, S1**).

513

514 The simulated plasmid-plasmid contact map computed using only copy numbers was  
515 made in a similar fashion (**Fig. S1B**). For this method, instead of multiplying copy  
516 number by the length of the plasmid, a fixed integer number was used (in our case, 10)  
517 to convert the relative ratios into integer numbers. The method of computation was the  
518 same as that described above.

519

520 We make two important assumptions for this calculation: 1) plasmids constitute  
521 independent units of interaction, and 2) these independent units are “well mixed”. The  
522 independence of contacts assumption implies there are no restrictions on how many  
523 DNA segments may be simultaneously in contact with one another within a “Hi-C  
524 contact volume” and the identity of the DNA segments in contact does not matter. The

525 “well mixed” assumption stipulates that independent DNA segments interact with equal  
526 probability with other DNA segments. Together, these assumptions allow us to compute  
527 the plasmid-plasmid interaction frequencies while safely ignoring other types of contacts  
528 such as plasmid-chromosome and chromosome-chromosome contacts.

529

### 530 **Plasmid construction**

531 Plasmid pΔmksB(gent) was generated in the following manner: (i) nucleotides 874996  
532 through 876527 of the B31 chromosome were PCR-amplified with primers NT968 and  
533 NT969; (ii) the gentamicin cassette of pKIGent\_parSP1\_phoU [7] was PCR-amplified  
534 with primers NT970 and NT971; (iii) nucleotides 879168 through 880691 of the B31  
535 chromosome were PCR-amplified with primers NT972 and NT973; (iv) the suicide  
536 vector backbone of pΔparA(kan) [7] was PCR-amplified with primers NT974 and NT975;  
537 and (v) the four PCR fragments listed above were digested with DpnI (New England  
538 Biolabs), gel-purified, and subjected to Gibson assembly [65] using New England  
539 Biolabs’ platform. The assembled plasmid was introduced into *Escherichia coli* strain  
540 NEB 5-alpha (New England Biolabs) by heat shocking. The resulting strain (CJW7512)  
541 was grown at 30°C on LB plates or in Super Broth liquid medium with shaking, while 15  
542 µg/mL gentamicin was used for selection.

543

### 544 **Strain construction**

545 To generate strain CJW\_Bb605, 75 µg of plasmid pΔmksB(gent) were digested with  
546 ApaI (New England Biolabs) in a 500 µL reaction volume for 4 hours. The DNA was  
547 then ethanol precipitated [66], dried, and resuspended into 25 µL sterile water. The  
548 resulting DNA suspension was then electroporated at 2.5 kV, 25 µF, 200 Ω, 2 mm-gap  
549 cuvette [67, 68] into 100 µL of electrocompetent cells made [69] using *B. burgdorferi*  
550 strain S9. The electroporated bacteria were transferred immediately to 6 mL BSK-II  
551 medium and allowed to recover overnight at 34°C. The next day, a fraction of the culture  
552 was embedded in 25 mL of semisolid BSK-agarose medium containing gentamicin per  
553 10-cm round Petri dish, as previously described [70]. The semisolid BSK-agarose mix  
554 was made by mixing 2 volumes of 1.7% agarose in water, sterilized by autoclaving, then  
555 melted and pre-equilibrated at 55°C, with 3 volumes of BSK-1.5 medium, which was

556 also equilibrated at 55°C for at most 5 minutes. BSK-1.5 contained 69.4 g/L bovine  
557 serum albumin, 12.7 g/L CMRL-1066, 6.9 g/L Neopeptone, 3.5 g/L Yeastolate, 8.3 g/L  
558 HEPES, 6.9 g/L glucose, 6.4 g/L sodium bicarbonate, 1.1 g/L sodium pyruvate, 1.0 g/L  
559 sodium citrate, 0.6 g/L N-acetylglucosamine, and 40 mL/L heat-inactivated rabbit serum,  
560 and had a pH of 7.50. After 10 days of growth in the BSK-agarose semisolid matrix, an  
561 individual colony was expanded in liquid culture and confirmed by PCR to have  
562 undergone correct double crossover homologous recombination of the suicide vector,  
563 thus yielding strain CJW\_Bb605. This strain was also confirmed by multiplex PCR [71]  
564 to contain all endogenous plasmids contained by its parent.

565

566 Further information and requests for strains, plasmids, resources, reagents, and  
567 analytical scripts should be directed to and will be fulfilled by the corresponding authors  
568 with appropriate Material Transfer Agreements.

569

## 570 **Acknowledgements**

571 We thank the Wang and Jacobs-Wagner labs for discussions and support, the Indiana  
572 University Center for Genomics and Bioinformatics for assistance with high-throughput  
573 sequencing. Support for this work comes in part from the Pew Innovation Fund (C.J.-  
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578 Medical Institute.

579

## 580 **Supplemental Information**

581 Supplemental information includes seven figures and four tables.

582

## 583 **Author Contributions**

584 Z.R., C.N.T., C.J.-W. and X.W. designed the study. Z.R. and X.W. performed Hi-C  
585 experiments and analyses. C.N.T. generated plasmids and strains and collected cells  
586 for Hi-C experiments. H.B.B. developed methods for analysis and generated figure

587 plots. C.J.-W. and X.W. supervised the project and acquired funding. Z.R. and X.W.  
588 wrote the manuscript with input from all authors.

589

590 **Declaration of Interests**

591 The authors declare no competing interests. H.B.B is an employee of Illumina, Inc.

592

593

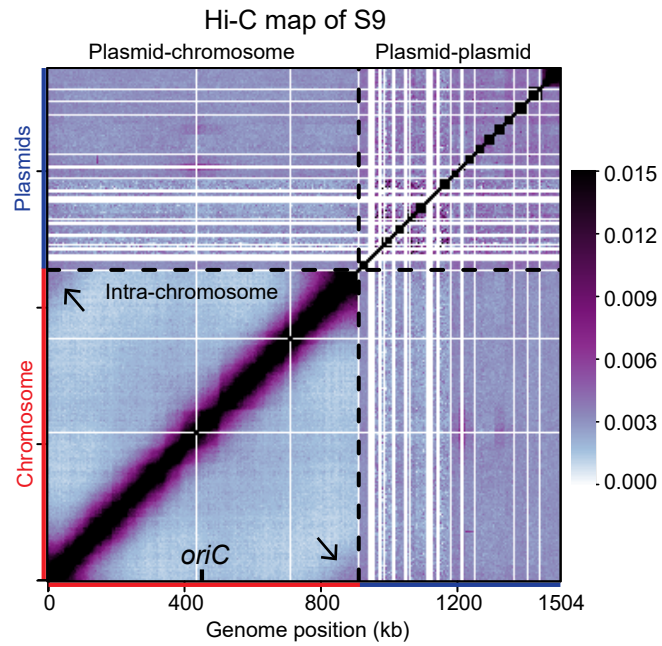
**A** *Borrelia burgdorferi* replicons

Chromosome (Chr)  
 $terCL$   $oriC$   $terCR$   
 (Size=911 kb,  $oriC$  copy=1.0)

18 plasmids

	Name	Size (kb)	Copy
Circular	cp26	26	1.3
	cp32-1	31	0.6
	cp32-3	30	0.5
	cp32-4	30	0.6
	cp32-6	30	0.6
	cp32-7	31	0.5
	cp32-8	31	0.6
	cp32-9	31	0.5
	Linear	lp17	17
lp21		19	1.0
lp25		24	0.6
lp28-1		28	0.6
lp28-2		30	0.7
lp28-3		29	0.7
lp28-4		27	0.7
lp36		37	1.1
lp38		39	0.9
lp54	54	1.4	

**B**



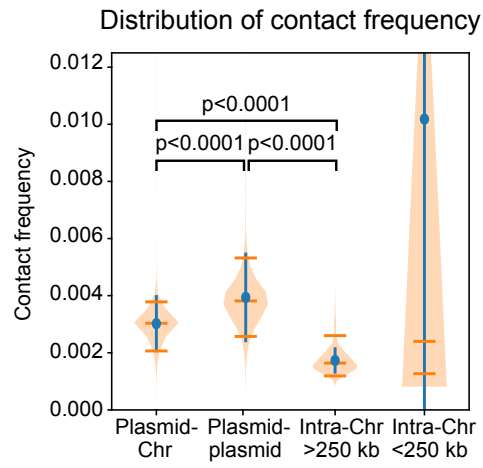
603 **Figure 1. Genome-wide organization of *B. burgdorferi* replicons.**

604 **(A)** The *B. burgdorferi* S9 wild-type strain has a linear chromosome (Chr), 8 circular  
605 plasmids and 10 linear plasmids. The replication origin of the chromosome is labeled as  
606 *oriC*. The sizes (in kb) and relative copy numbers of the plasmids are listed. The relative  
607 copy number of each plasmid were previously measured using whole genome  
608 sequencing analysis [7], and is shown relative to the copy number of *oriC*.

609 **(B)** Normalized Hi-C matrix showing interaction frequencies for pairs of 5-kb bins across  
610 the genome of *B. burgdorferi* S9. x and y-axes show genome positions. The  
611 chromosome and the plasmids are indicated by red and blue bars, respectively. *oriC* is  
612 labeled on the x-axis. The boundary between the chromosome and the plasmids are  
613 indicated by black dotted lines. The plasmids are ordered alphabetically from cp26 to  
614 lp54, from left to right and bottom to top, respectively. The whole map was divided into  
615 four regions: the bottom left region shows intra-chromosomal interactions, the top left  
616 and bottom right regions show plasmid-chromosome interactions, and the top right  
617 region represents plasmid-plasmid interactions. We used the same convention for all  
618 whole-genome Hi-C and Hi-C derivative plots in this study. The color scale depicting Hi-  
619 C interaction scores in arbitrary unit is shown at the right.

620

621



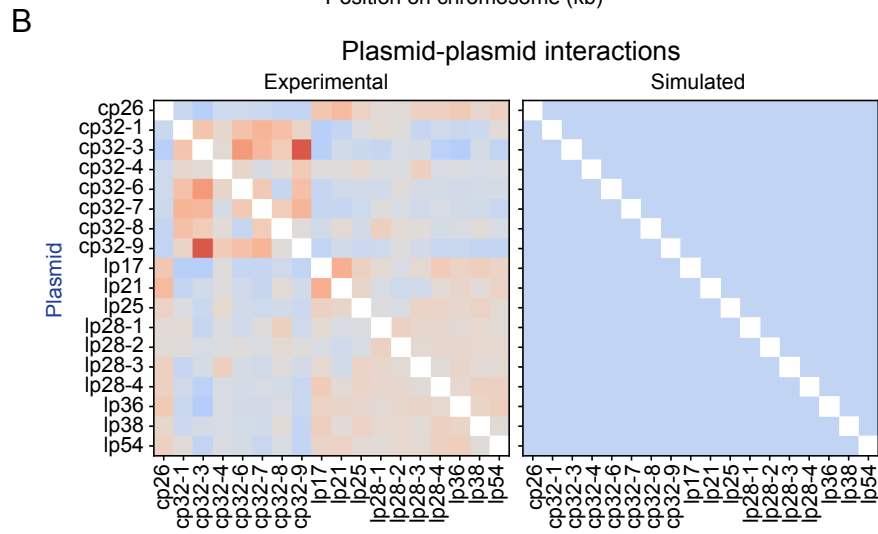
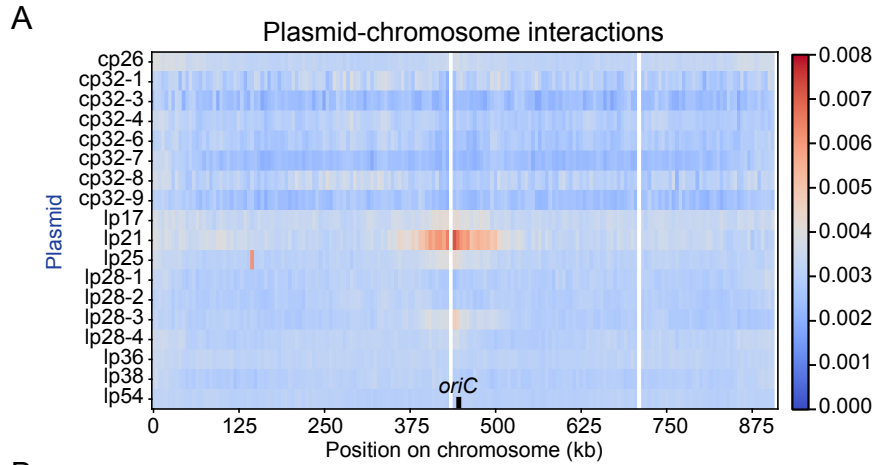
622 **Figure 2. Hi-C contact frequencies for different types of interactions.**

623 Distributions of Hi-C contact frequencies measured for different types of interactions are  
624 shown as violin plots. Blue lines indicate standard deviations of the values. Orange lines  
625 indicate the median, 5<sup>th</sup> and 95<sup>th</sup> percentile of the data. The *p*-values were computed  
626 using a Mann-Whitney U test. All comparisons were done for data binned at 5 kb  
627 resolution.

628

629





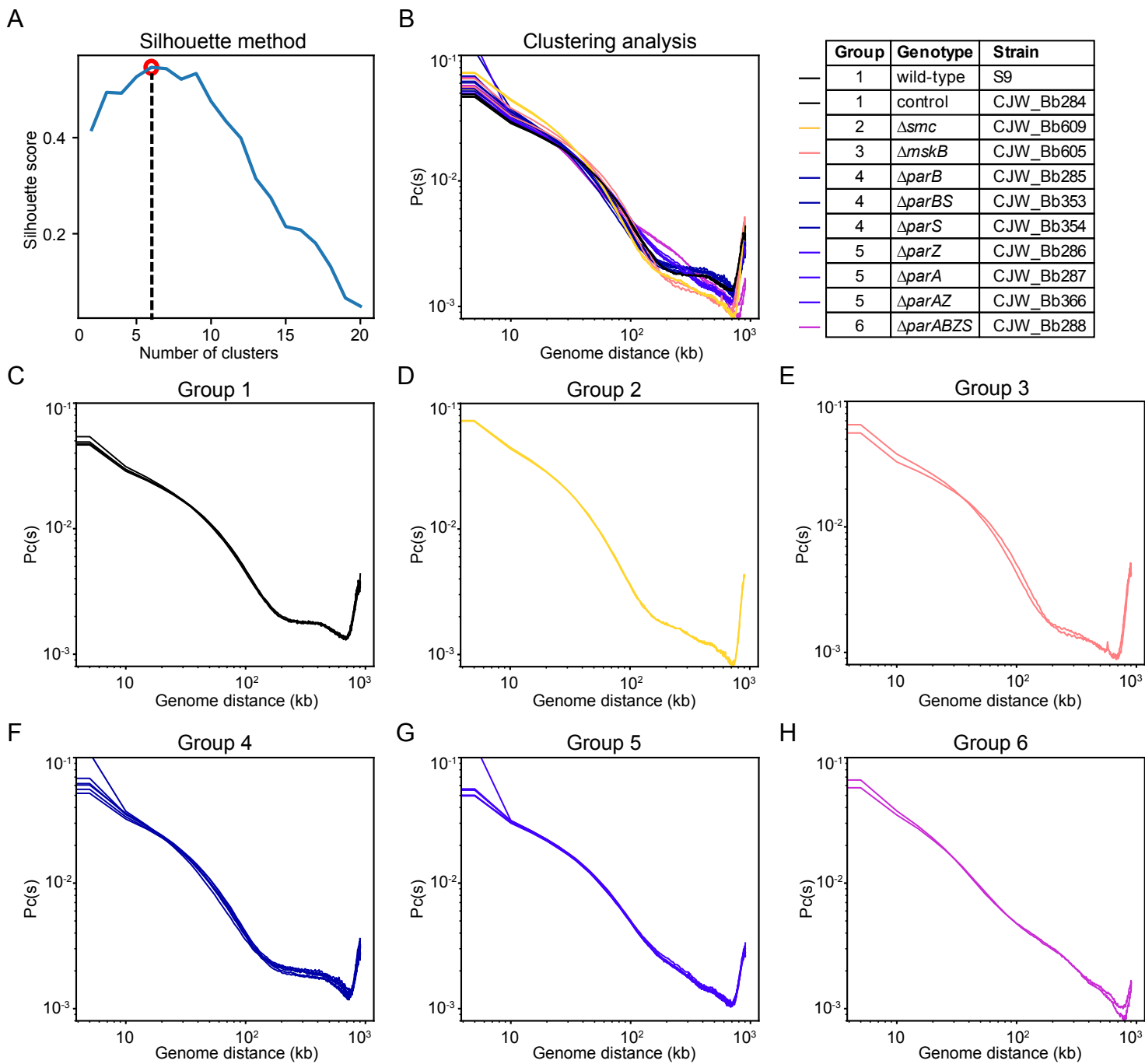
630 **Figure 3. Plasmid-chromosome and plasmid-plasmid interactions.**

631 **(A)** The heatmap of plasmid interactions with chromosome loci in WT *B. burgdorferi*  
632 train S9. To generate the interaction score between each plasmid and each  
633 chromosome locus, the Hi-C interaction scores in consecutive bins are summed  
634 according to each plasmid. The plot shows averaged data of two replicates. The x-axis  
635 indicates the genome position on the chromosome. The y-axis specifies different  
636 plasmids. The color scale depicting interaction scores in arbitrary unit is shown at the  
637 right. The color scale depicting relative interaction frequency in arbitrary unit is shown at  
638 the right.

639 **(B)** Left, the experimentally measured interaction frequencies between plasmids. To  
640 generate the interaction score within every pair of plasmids, the Hi-C interaction scores  
641 in consecutive bins are summed according to each plasmid. The data are normalized  
642 such that each row has the same total score. This normalization ignores the plasmid-  
643 chromosome interactions. The plot shows averaged data of two replicates. The x-axis  
644 and y-axis indicate the different plasmids of *B. burgdorferi* strain S9. the simulated  
645 interaction frequencies between plasmids based on plasmid copy number and plasmid  
646 sizes (see Materials and Methods). The normalization method is the same as the  
647 experimental data shown on the left. The color scale is the same as in **(A)**. The  
648 simulated maps with iterative correction or in a finer color scale can be found in **Fig. S1**.

649

650



651 **Figure 4. Clustering analysis of different mutants.**

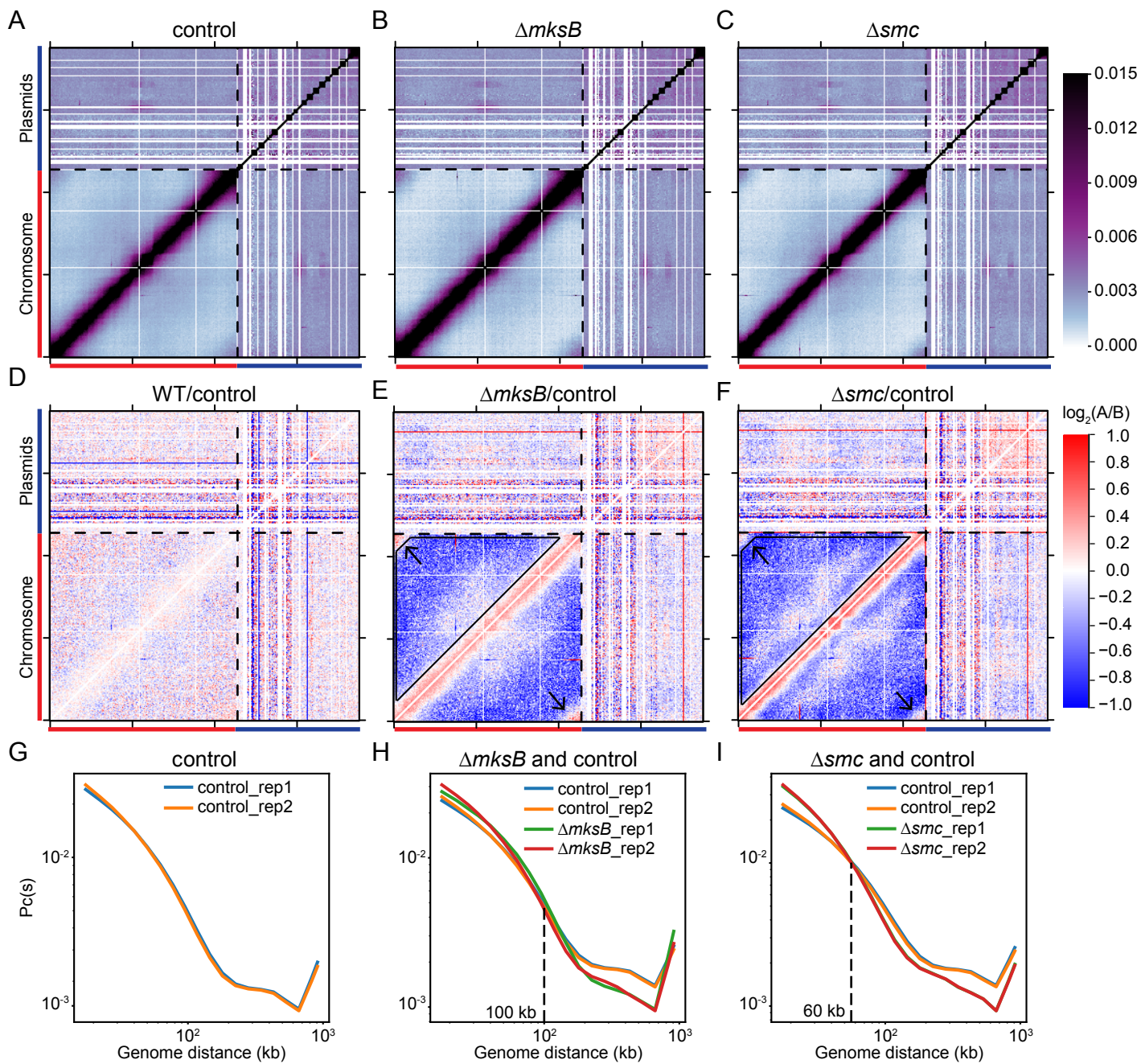
652 **(A)** Determination of the optimal number of clusters of contact probability curves,  $P_c(s)$ ,  
653 for k-means clustering (see Materials and Methods). The number of clusters was  
654 determined by identifying the peak in Silhouette score. This analysis suggests six  
655 optimal groupings, which is indicated by the red circle and black dotted line.

656 **(B)**  $P_c(s)$  curves of all the samples. Grouping results of the 11 strains are listed on the  
657 right. Two biological replicates of each strain are plotted. Individual  $P_c(s)$  curves can be  
658 found in **Fig. S4**.

659 **(C-I)** Curves of the same group in **(B)** are plotted in different panels.

660

661



662 **Figure 5. SMC and MksB mediate long-range DNA interactions.**

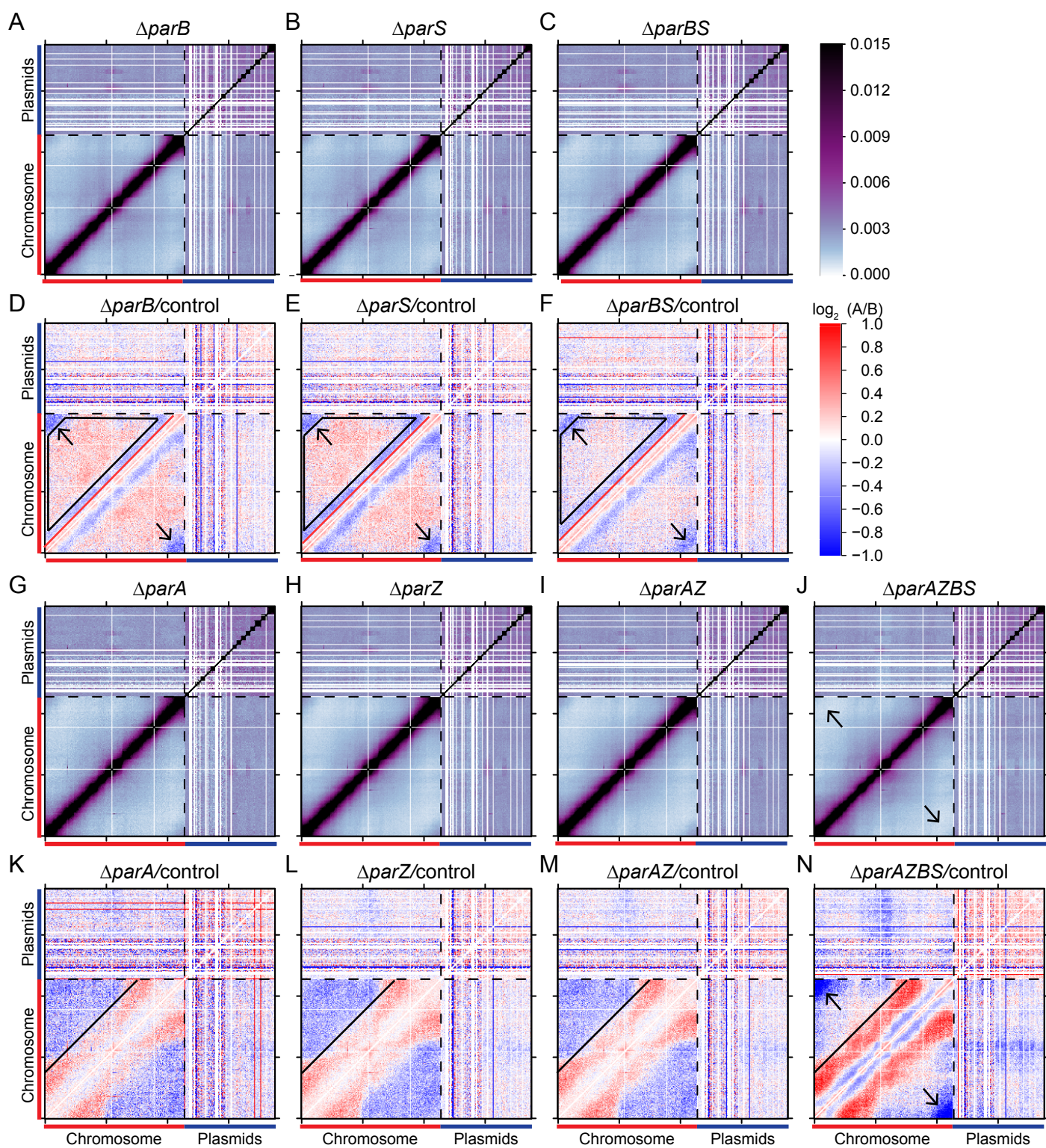
663 **(A-C)** Normalized Hi-C interaction maps of the control (CJW\_Bb284),  $\Delta mksB$   
664 (CJW\_Bb605,) and  $\Delta smc$  (CJW\_Bb609) strains. Black dotted lines mark the boundary  
665 between the depiction of the chromosome and that of the plasmids. The color scale  
666 depicting Hi-C interaction scores in arbitrary unit is shown at the right.

667 **(D-F)**  $\log_2$  ratio plots comparing different Hi-C matrices.  $\log_2(\text{matrix 1}/\text{matrix 2})$  was  
668 calculated and plotted in the heatmaps. Matrix 1/ matrix 2 are shown at the top of each  
669 plot. The color scale is shown at the right of panel **(F)**. Black arrows point to *terCL*-  
670 *terCR* interactions. Black trapezoids indicate reduced interactions in the mutants.

671 **(G-I)** Contact probability decay  $P_c(s)$  curves of indicated Hi-C matrices.  $P_c(s)$  curves  
672 show the average contact frequency between all pairs of loci on the chromosome  
673 separated by set distance ( $s$ ). The x-axis indicates the genomic distance of separation  
674 in kb. The y-axis represents averaged contact frequency. The curves were computed for  
675 data binned at 5 kb. The intersection points of mutant and control curves are indicated  
676 by black dotted lines.

677

678

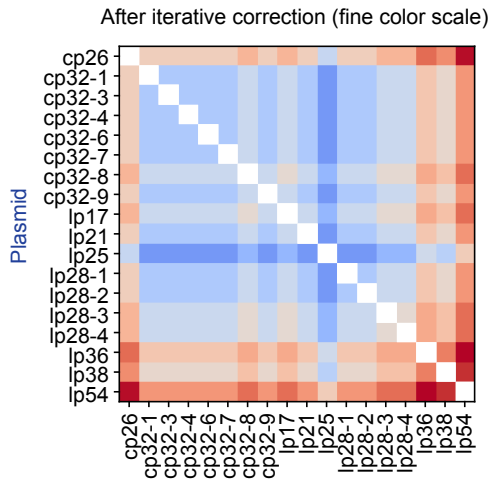
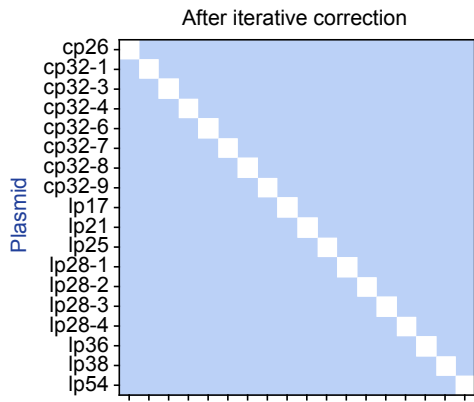
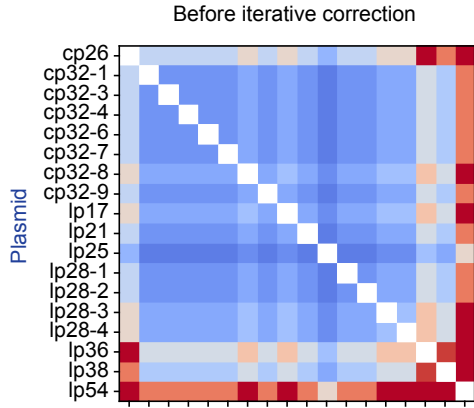


679 **Figure 6. Disruption of the partition systems re-structures the genome.**  
680 **(A-C)** Normalized Hi-C interaction maps of the  $\Delta parB$  (CJW\_Bb353),  $\Delta parS$   
681 (CJW\_Bb354), and  $\Delta parBS$  (CJW\_Bb285) strains. Black dotted lines indicate the  
682 boundary between the chromosome and the plasmids. The color scale depicting Hi-C  
683 interaction scores in arbitrary unit is shown at the right.  
684 **(D-F)** Log<sub>2</sub> ratio plots comparing  $\Delta parB$  (CJW\_Bb353),  $\Delta parS$  (CJW\_Bb354), and  
685  $\Delta parBS$  (CJW\_Bb285), respectively, with the control (CJW\_Bb284) strain. Black arrows  
686 point to blue pixels *terCL-terCR* interactions. Black trapezoids indicate area of read  
687 pixels. Red lines indicate the boundary between red and blue pixels. The color scale is  
688 shown at the right.  
689 **(G-J)** Normalized Hi-C interaction maps of the  $\Delta parA$  (CJW\_Bb366),  $\Delta parZ$   
690 (CJW\_Bb286),  $\Delta parAZ$  (CJW\_Bb287) and  $\Delta parAZBS$  (CJW\_Bb288) strains. Black  
691 arrows indicate *terCL-terCR* interactions.  
692 **(I-N)** Log<sub>2</sub> ratio plots comparing  $\Delta parA$  (CJW\_Bb366),  $\Delta parZ$  (CJW\_Bb286),  $\Delta parAZ$   
693 (CJW\_Bb287), or  $\Delta parAZBS$  (CJW\_Bb288) with the control (CJW\_Bb284) strain. Solid  
694 black lines indicate the boundary between red and blue pixels. Black arrows indicate  
695 *terCL-terCR* interactions.  
696  
697



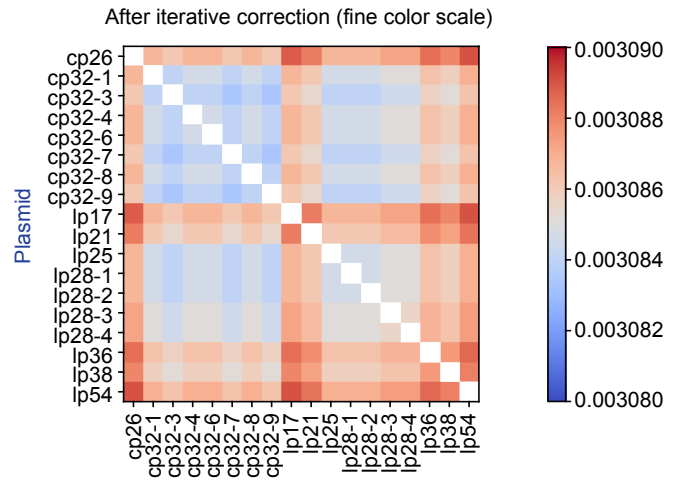
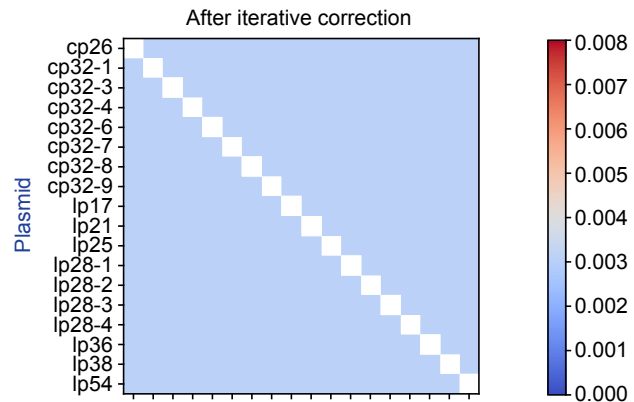
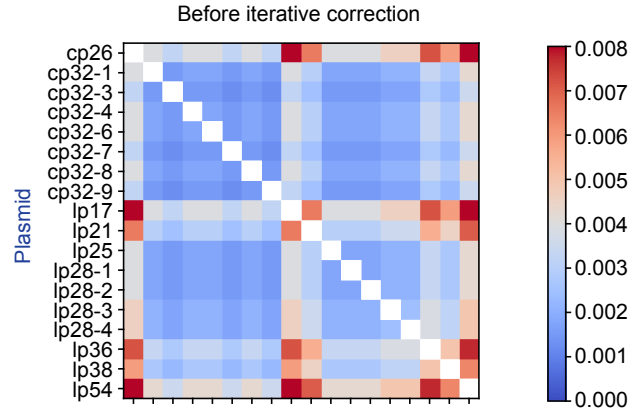
A

Simulated plasmid-plasmid interactions  
using plasmid copy number and size



B

Simulated plasmid-plasmid interactions  
using plasmid copy number only

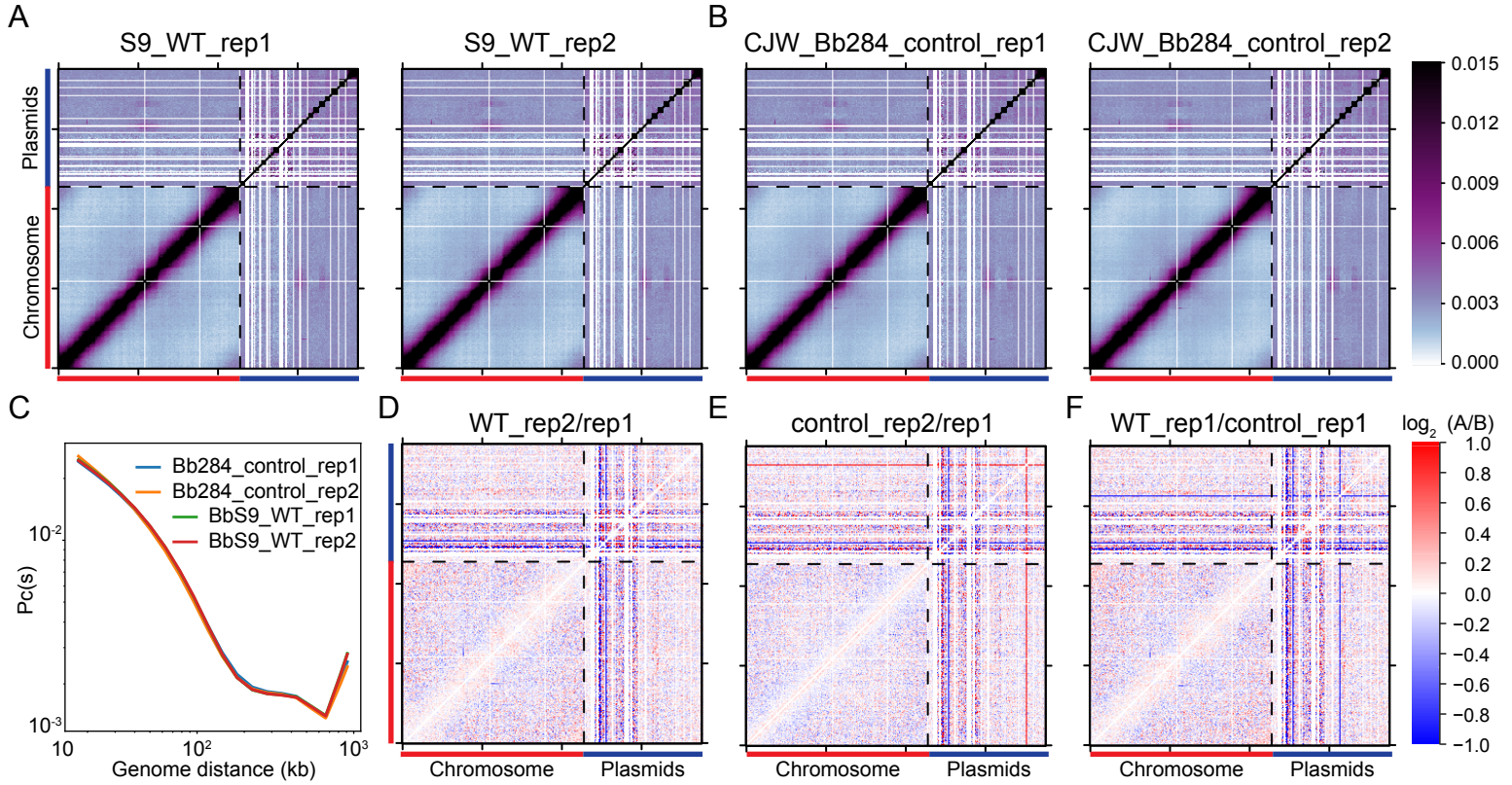


698 **Figure S1. Simulated plasmid-plasmid interaction frequency.**

699 The expected contact probability between plasmids was calculated under the  
700 assumptions that plasmids are independent of one another and are “well mixed” within  
701 the cytoplasm. The calculation was performed using copy number and plasmid length  
702 together (**A**) or using only plasmid copy numbers (**B**). Top panels, the exact contact  
703 frequency expected between plasmid segments. Middle panels, the contact frequency  
704 expected between plasmids after application of the iterative correction normalization  
705 procedure. Bottom panels, the same as middle panels, but shown with a much finer  
706 color scale. The color scale depicting contact frequency in arbitrary unit is shown at the  
707 right. We note that the residual resemblance between bottom and top panels results  
708 from the fact that the iterative correction procedure only asymptotically approaches 1  
709 (see Materials and Methods).

710

711



712 **Figure S2. Comparison of WT and control.**

713 **(A-B)** Normalized Hi-C interaction maps of *B. burgdorferi* strains S9 (WT) and the  
714 control strain CJW\_Bb284. Two biological replicates of each strain (rep1 and rep2) are  
715 shown. The color scale depicting Hi-C interaction scores in arbitrary unit is shown at the  
716 right. We note that *PflaB-aadA* sequence from the chromosome is inserted in *bbe02*  
717 region lp25. Short-range intra-chromosomal interactions involving the *flaB* promoter  
718 region could be assigned to lp25 and account for the interactions between lp25 and the  
719 promoter region of *flab* on the chromosome at ~150 kb.

720 **(C)** Pc(s) curves of the four samples. Pc(s) curves show the averaged contact  
721 frequency between all pairs of loci on the chromosome separated by set distance (s).  
722 The x-axis indicates the genomic distance of separation in kb. The y-axis represents  
723 averaged contact frequency. The curves were computed for data binned at 5 kb.

724 **(D-F)** Log<sub>2</sub> ratio plots comparing different Hi-C matrices. Log<sub>2</sub>(matrix 1/matrix 2) was  
725 calculated and plotted in the heatmaps. Matrix 1 / matrix 2 are shown at the top of each  
726 plot. The color scale is shown at the right of panel **(F)**.

727

728

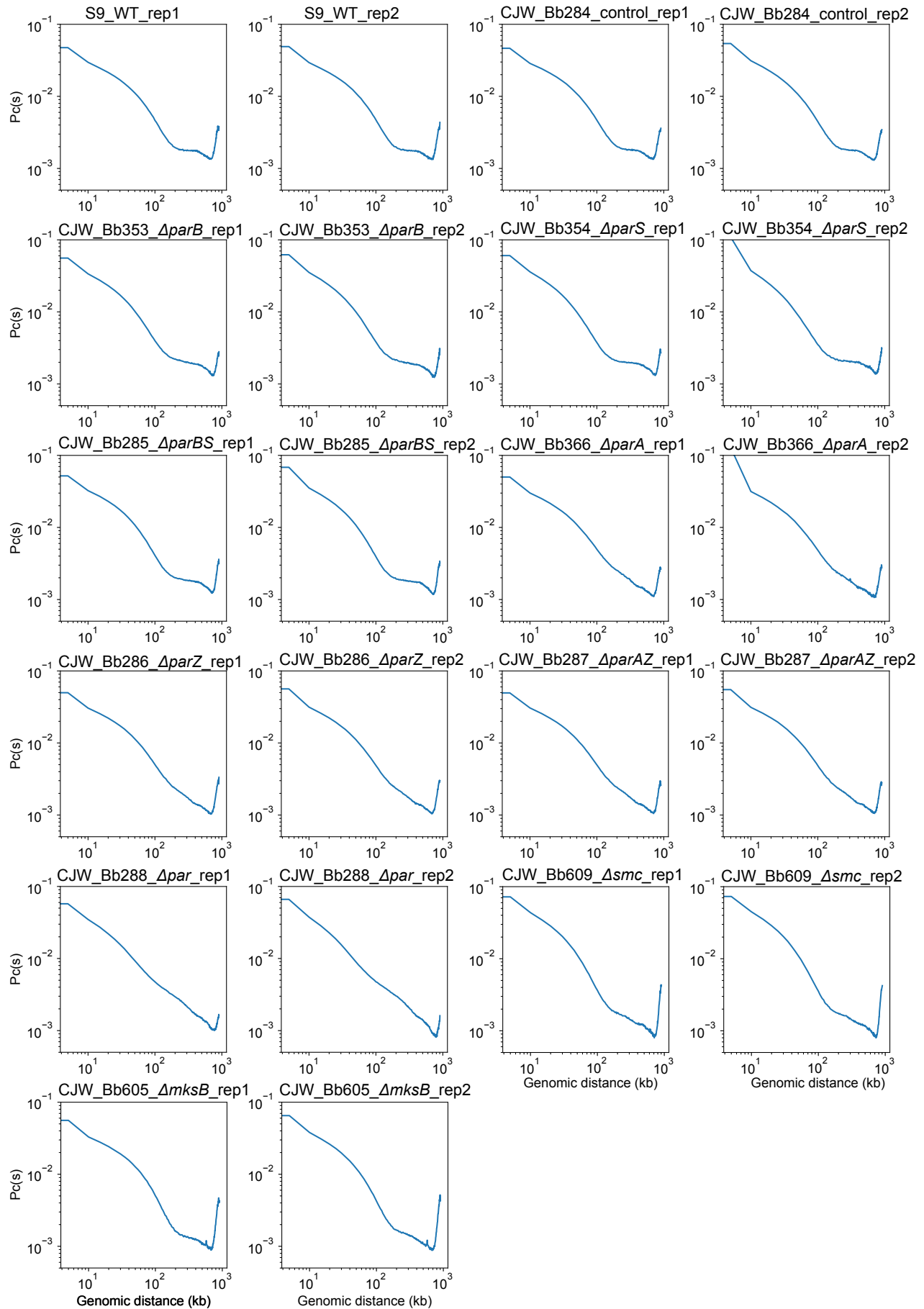


729 **Figure S3. Hi-C samples used in this study.**

730 The normalized Hi-C plots of all the 22 experiments. The color scale depicting Hi-C  
731 interaction scores is shown in  $\log_{10}$ .

732

733



734 **Figure S4. Individual  $P_c(s)$  curves of all the samples analyzed in this study.**

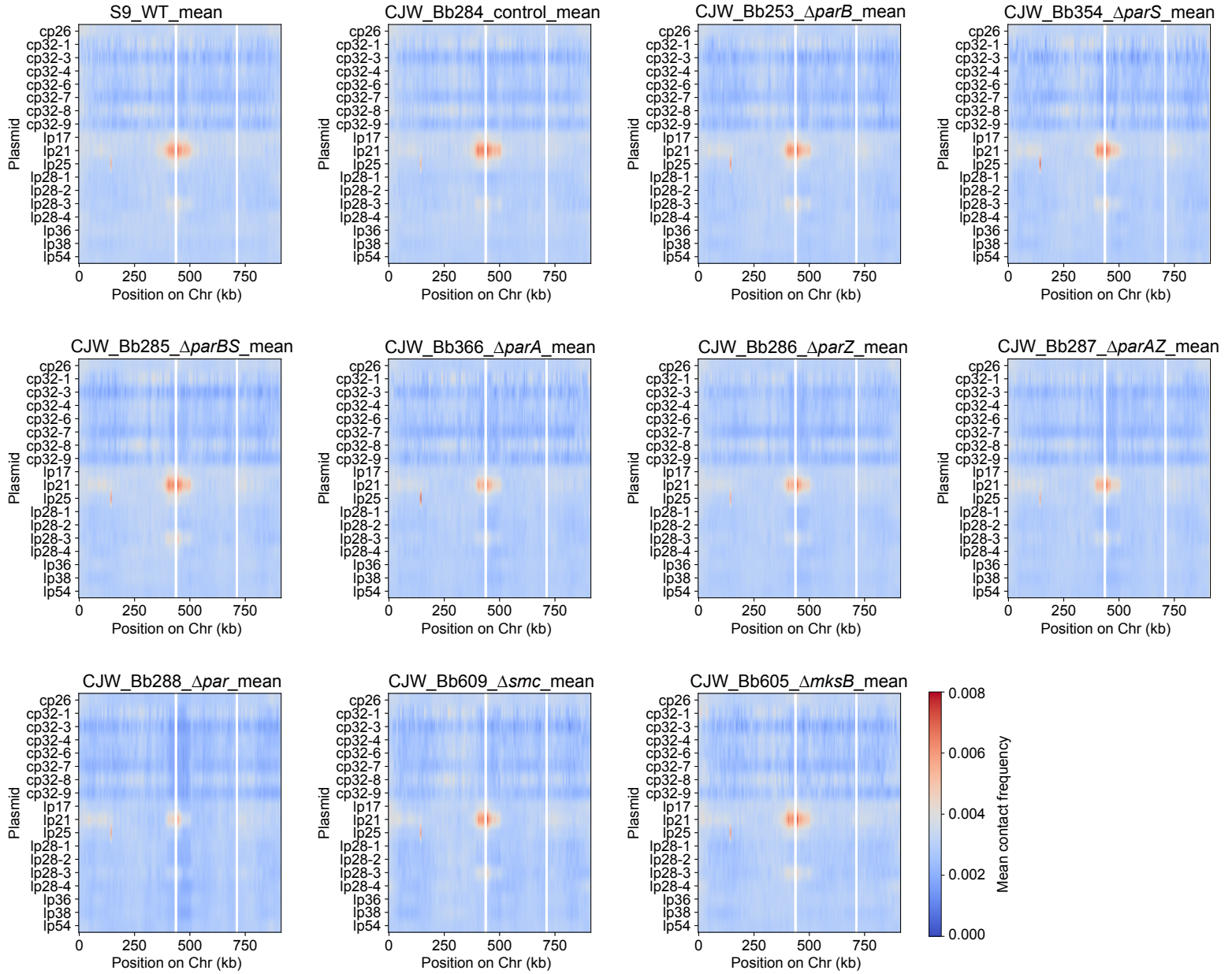
735  $P_c(s)$  curves of all the 22 Hi-C experiments. x-axis indicates genomic distance and y-

736 axis shows averaged contact frequency.

737

738



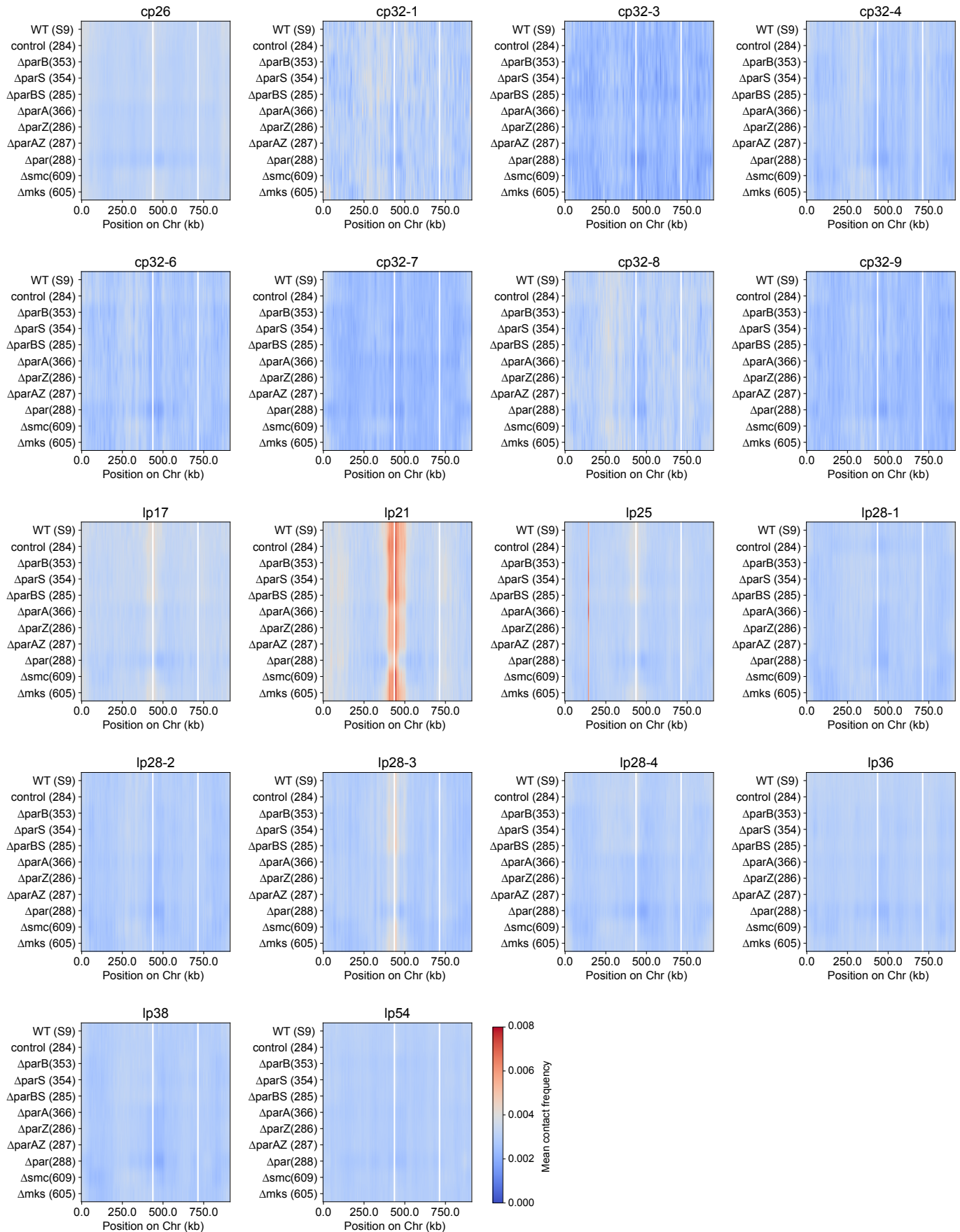


739 **Figure S5. Plasmid-chromosome interactions in different mutants.**

740 Heatmap of plasmid-chromosome interaction frequencies are shown. The x-axis shows  
741 chromosome location in kb. The y-axis specifies the different plasmids analyzed. The  
742 color indicates the contact frequency between plasmid and chromosome loci. Each  
743 graph plots the mean value of two biological replicates found in **Fig. S3**. Data are  
744 binned at 5-kb resolution.

745

746

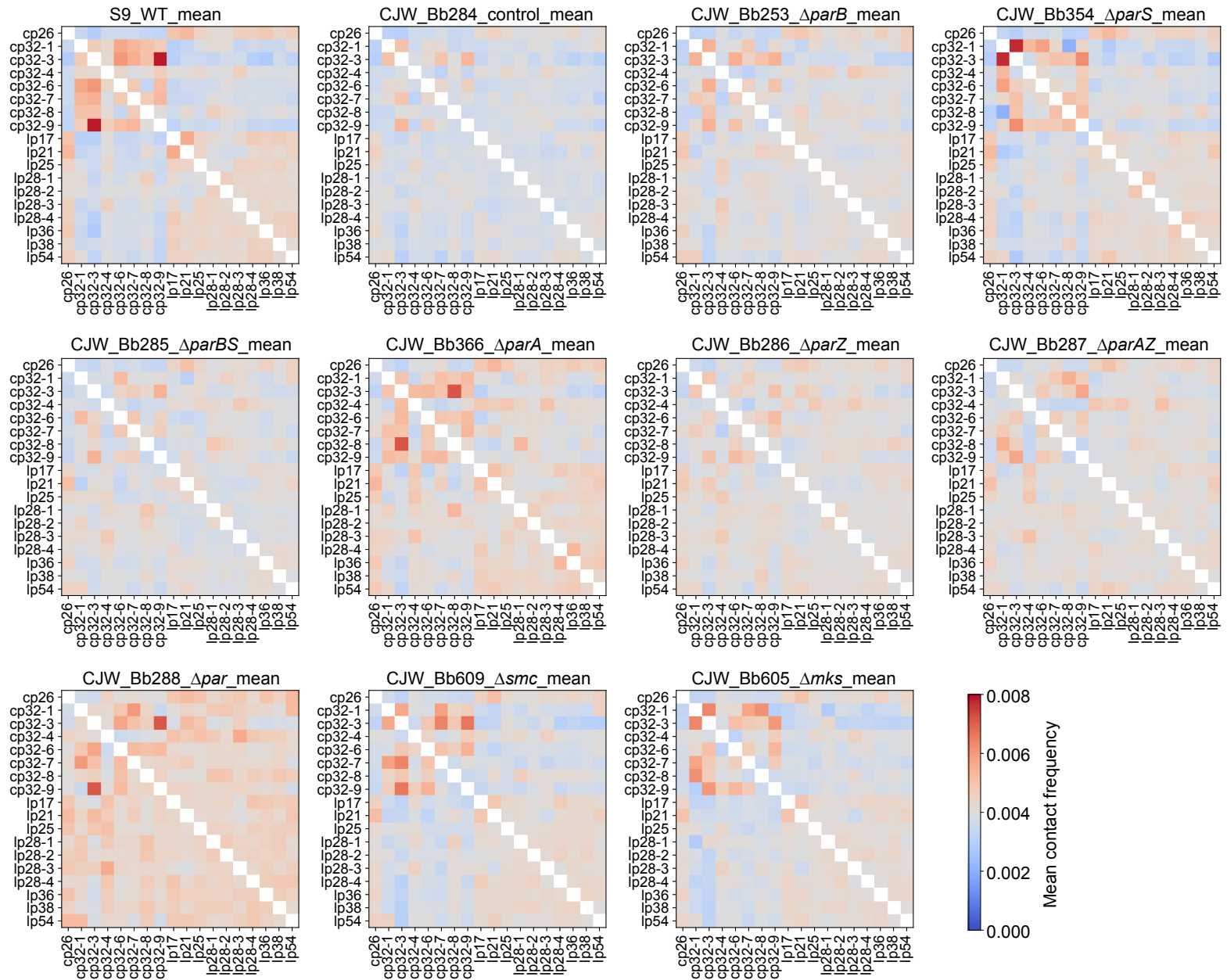


747 **Figure S6. Plasmid-chromosome interactions in different mutants organized by**  
748 **plasmids.**

749 Heatmaps of plasmid-chromosome interaction frequencies are shown. The x-axis  
750 shows the chromosome location in kb. The y-axis specifies the different mutants. The  
751 color indicates the contact frequency between plasmid and chromosome loci. Each  
752 graph plots the mean value of two biological replicates found in **Fig. S3**. Data are  
753 binned at 5-kb resolution.

754

755



756 **Figure S7. Plasmid-plasmid interactions in different mutants.**

757 Plasmid-plasmid contact frequencies in different strains. The x and y axes indicate the  
758 plasmids analyzed. The color shows the computed contact frequency. Each graph plots  
759 the mean of two biological replicates found in **Fig. S3**. Data are normalized such that  
760 the sum of each row has the same total score.

761

762

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## Supplementary Information for

### **Organization and replicon interactions within the highly segmented *Borrelia burgdorferi* genome**

Zhongqing Ren, Constantin N. Takacs, Hugo B. Brandão, Christine Jacobs-Wagner, and Xindan Wang

Corresponding authors: [jacobs-wagner@stanford.edu](mailto:jacobs-wagner@stanford.edu); [xindan@indiana.edu](mailto:xindan@indiana.edu)

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Tables S1 to S4  
SI References

**Table S1. Bacterial strains used in this study.**

Strain	Genotype	Antibiotic resistance	Reference	Figure
S9	Transformable derivative of the <i>B. burgdorferi</i> type strain B31; lacks endogenous plasmids cp9, lp5, and lp56; also known as B31-A3-68- $\Delta$ <i>bbe02::PflaB-aadA</i>	Sr	[1]	1-4, S2-S7
CJW_Bb284	S9-derived control strain; has gentamicin resistance cassette inserted between <i>parZ</i> and <i>parB</i>	Sr, Gm	[2]	4A-C, 5A, 5D-I, S2-S7
CJW_Bb285	S9-derived $\Delta$ <i>parBS</i> strain	Sr, Gm	[2]	4ABF, 6CF, S3-7
CJW_Bb286	S9-derived $\Delta$ <i>parZ</i> strain	Sr, Gm	[2]	4ABG, 6HL, S3-7
CJW_Bb287	S9-derived $\Delta$ <i>parAZ</i> strain	Sr, Gm	[2]	4ABG, 6IM, S3-7
CJW_Bb288	S9-derived $\Delta$ <i>parAZBS</i> strain	Sr, Gm	[2]	4ABH, 6JN, S3-7
CJW_Bb353	S9-derived $\Delta$ <i>parB</i> strain	Sr, Gm	[2]	4ABF, 6AD, S3-7
CJW_Bb354	S9-derived $\Delta$ <i>parS</i> strain	Sr, Gm	[2]	4ABF, 6BE, S3-7
CJW_Bb366	S9-derived $\Delta$ <i>parA</i> strain	Sr, Km	[2]	4ABG, 6GK, S3-7
CJW_Bb605	S9-derived $\Delta$ <i>mksB</i> strain	Sr, Gm	This study	4ABE, 5BEH, S3-7
CJW_Bb609	S9-derived $\Delta$ <i>smc</i> strain	Sr, Gm	[2]	4ABD, 5CFI, S3-7

Sr, streptomycin resistance; Gm, gentamicin resistance; Km, kanamycin resistance.

**Table S2. Plasmids used in this study.**

<b>Plasmid</b>	<b>Description</b>	<b>Reference</b>
<i>pΔmksB(gent)</i>	Plasmid to make replace $\Delta mksB$ with gentamycin resistance gene	This study
<i>pKIGent_parS<sup>P1</sup>_phoU</i>	Plasmid to insert <i>parS<sup>P1</sup></i> near <i>phoU</i>	[2]
<i>pΔparA(kan)</i>	Plasmid to delete <i>parA</i> from <i>B. burgdorferi</i> chromosome	[2]

**Table S3. Oligonucleotides used in this study.**

<b>Oligo</b>	<b>Sequence</b>
NT968	5'-tggtagcagctcggatccgggattctttgctgtttgtagatctactacatgcc-3'
NT969	5'-tttgtttttaccgggcccggattgtctaaaagaagtgtatcgaaattcaactcatg-3'
NT970	5'-cttctttaagacaatcgggcccgggtaaaaaacaaaagatccttaaggatctttg-3'
NT971	5'-tatccaattgtcgcccggtcaaggaagattcctattaaggtgaactaagagc-3'
NT972	5'-aatctcctgaaccgcccggcgacaaattggcataattcccatgcttctattgaagg-3'
NT973	5'-ctctagatgcatgcatgcaataacccaaaaagatataaccgcaaaagacaataatgc-3'
NT974	5'-tcttttggttattgcaatgcatgcatctagaggccaattcgccctatagtgagtcg-3'
NT975	5'-aaacaacgcaaaagaaatcccggatccgagctcggtagccaagcttgatgcatagcttgag-3'

**Table S4. Next generation sequencing samples used in this study.**

<b>Sample name</b>	<b>Figure</b>	<b>Reference</b>
HiC_CJW_Bb284_rep1	4A-C, 5A, 5D-I, S2-7	This study
HiC_CJW_Bb284_rep2	4A-C, S2-7	This study
HiC_CJW_Bb285_rep1	4ABF, 6CF, S3-7	This study
HiC_CJW_Bb285_rep2	4ABF, S3-7	This study
HiC_CJW_Bb286_rep1	4ABG, 6HL, S3-7	This study
HiC_CJW_Bb286_rep2	4ABG, S3-7	This study
HiC_CJW_Bb287_rep1	4ABG, 6IM, S3-7	This study
HiC_CJW_Bb287_rep2	4ABG, S3-7	This study
HiC_CJW_Bb288_rep1	4ABH, 6JN, S3-7	This study
HiC_CJW_Bb288_rep2	4ABH, S3-7	This study
HiC_CJW_Bb353_rep1	4ABF, 6AD, S3-7	This study
HiC_CJW_Bb353_rep2	4ABF, S3-7	This study
HiC_CJW_Bb354_rep1	4ABF, 6AD, S3-7	This study
HiC_CJW_Bb354_rep2	4ABF, S3-7	This study
HiC_CJW_Bb366_rep1	4ABG, 6GK, S3-7	This study
HiC_CJW_Bb366_rep2	4ABG, S3-7	This study
HiC_CJW_Bb605_rep1	4ABE, S3-7	This study
HiC_CJW_Bb605_rep2	4ABE, 5BEH, S3-7	This study
HiC_CJW_Bb609_rep1	4ABD, 5CFI, S3-7	This study
HiC_CJW_Bb609_rep2	4ABD, S3-7	This study
HiC_CJW_S9WT_rep1	1-4, S2-7	This study
HiC_CJW_S9WT_rep2	4A-C, S2-7	This study



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