

SUPPLEMENTAL MATERIAL

Figure:

Fig. S1

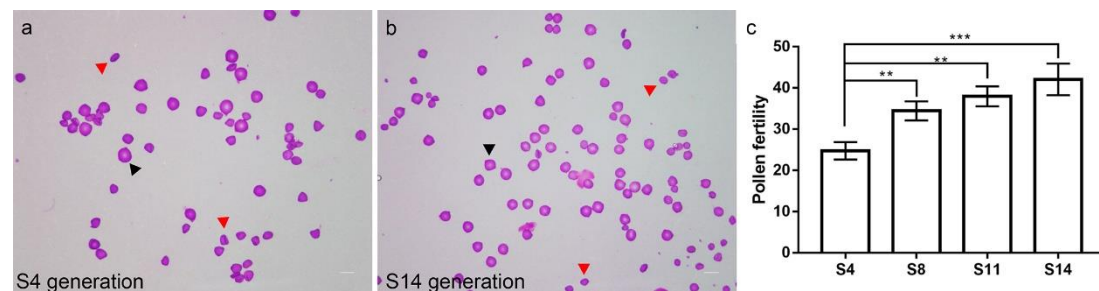


Fig. S1 Comparison of pollen viability in four generations of *C. xhytivus* allotetraploid (S4, S8, S11 and S14). **(a-b)** The plump pollen grains represent pollen fertility (black arrows), while unplump pollen grains represent pollen infertility (red arrows). **(a)** The mean value of S4 generation pollen fertility is 24.7%. **(b)** The mean value of S14 generation pollen fertility is 42.1%. **(c)** Pollen fertility of three generations (S8, S11 and S14) was significantly higher than S4 generation. Error bars indicating the $\pm$ SD over five biological replicates. Asterisks indicating statistically significant differences at ** $p < 0.01$, *** $p < 0.001$.

Fig. S2

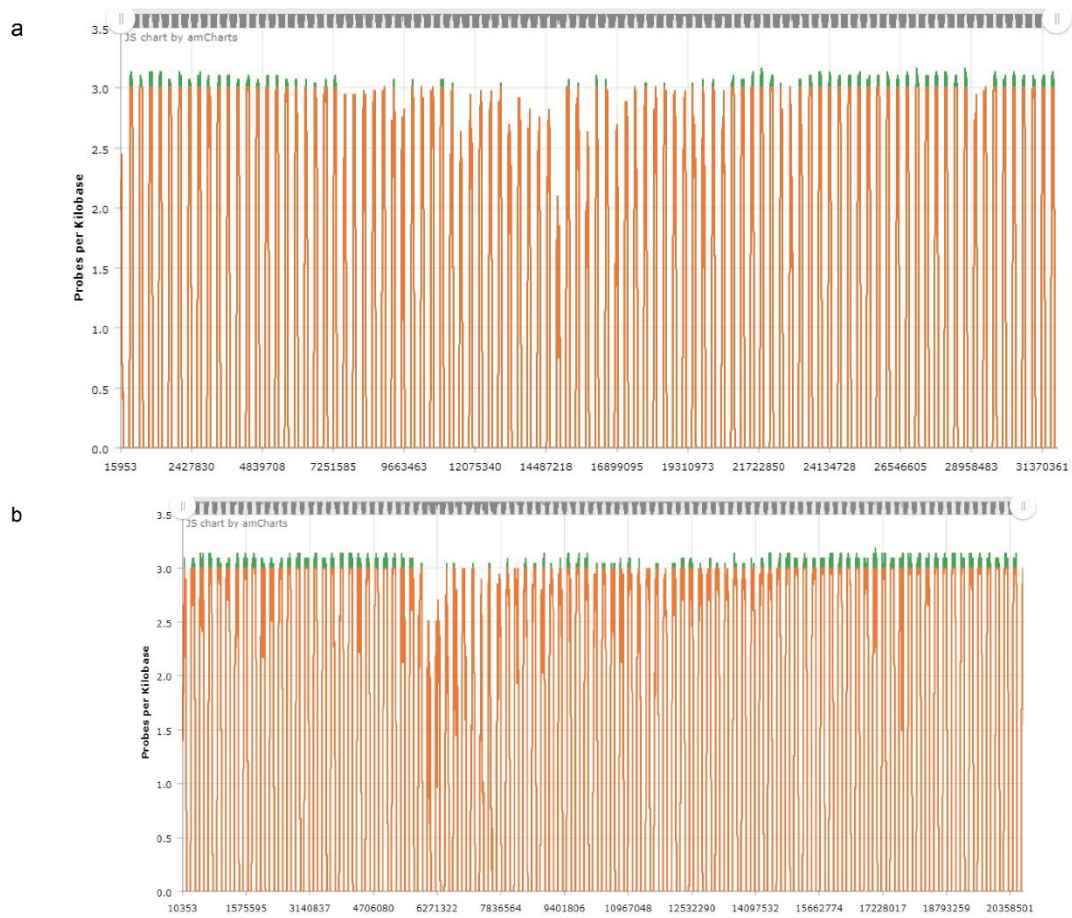


Fig. S2 Locations and density of 27,392 oligos along the sequence map of cucumber chromosomes. (a) The oligos information of cucumber chromosome 5. (b) The oligos information of cucumber chromosome 7. Oligos targeting 100kb per 300kb were chosen for chromosomes C5 and C7.

Fig. S3

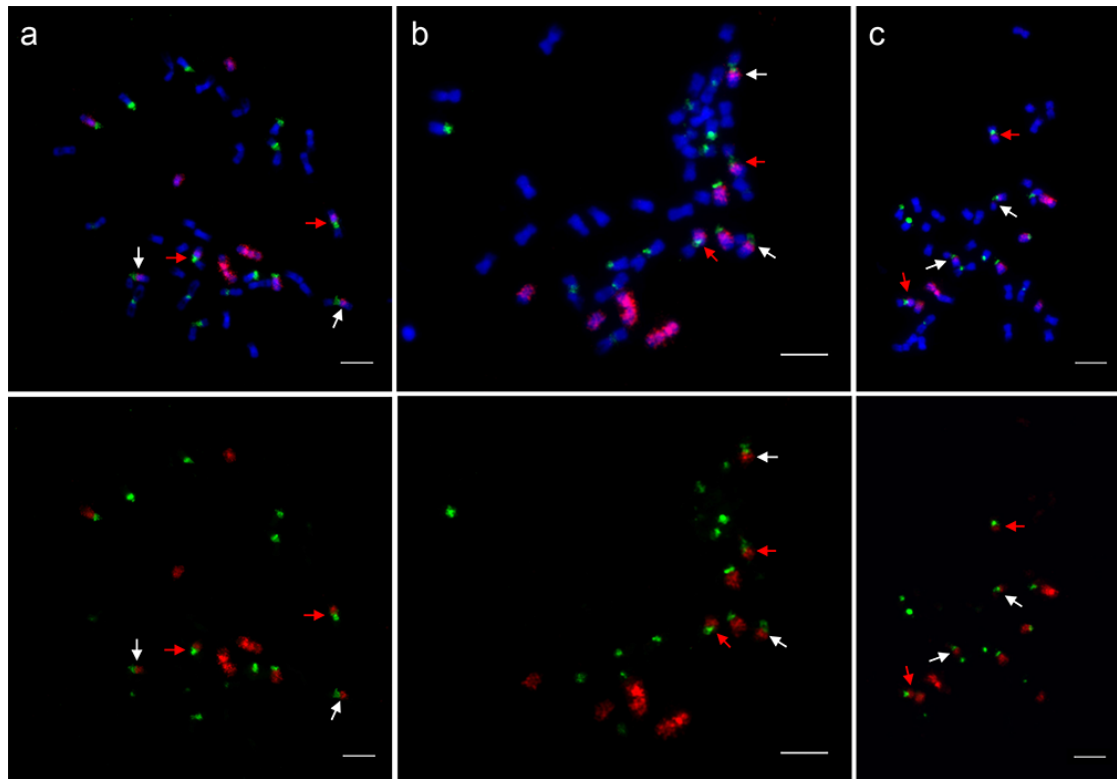


Fig. S3 Two translocations carrying C5-oligo signals were detected in other cells from same S₁₄ individual as Fig. 5. **(a-c)** Red FISH signals from C-oligo probes; green FISH signals from 45S rDNA probes. Red arrows indicating one translocation anchored on C2; white arrows indicating another translocation anchored on C4.

Fig. S4

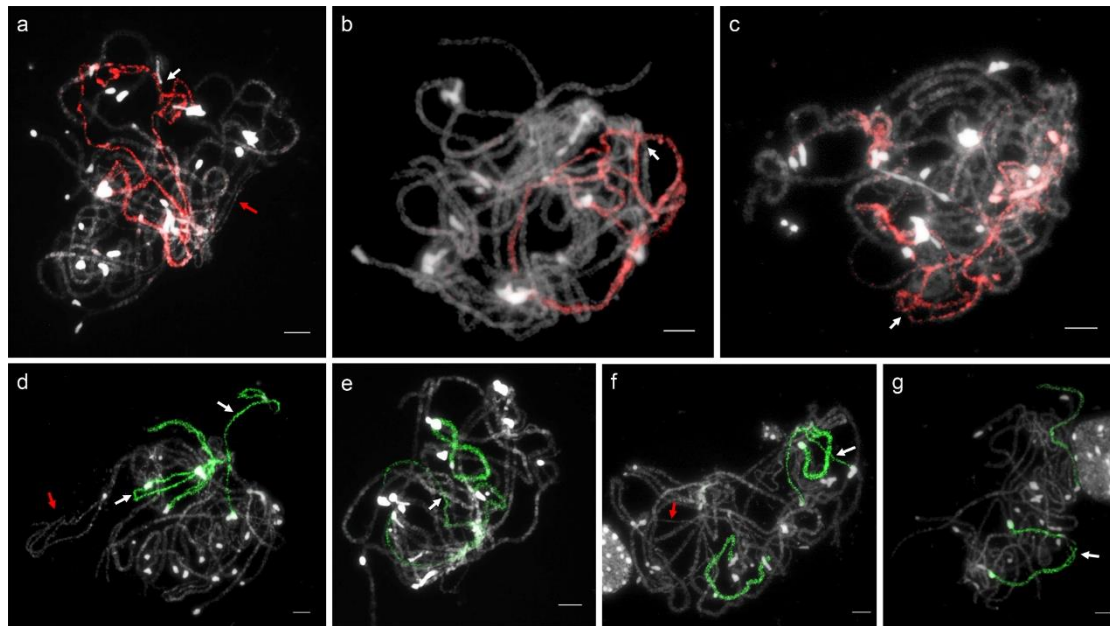


Fig. S4 Tracing chromosome pairing at meiotic pachytene of the S₄ generation. **(a-c)** Chromosome painting by C5-oligo probes (red). The chromosomes C5 and H9 or H10 were partially paired (white arrows). **(d-g)** Chromosome painting by C7-oligo probes (green). **(d)** An unpaired single chromosome string of C7 (white arrow). **(e)** An unpaired chromosome region of H1 (white arrow). **(f, g)** Unpaired chromosome region of C7 (white arrow). **(a, d, f)** Red arrows indicating unpaired single chromosome strings and/or chromosome regions. Bars = 5μm.

Fig. S5

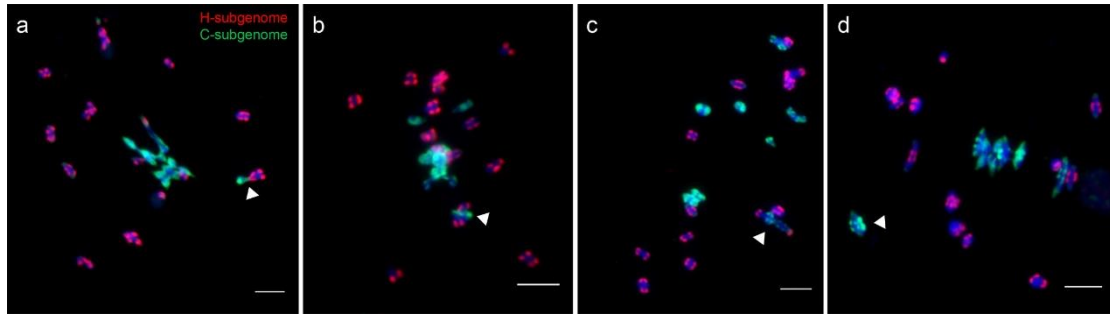


Fig. S5 Three representative PMCs with multivalents and one lagged C-bivalent at metaphase I in S_4 generation. **(a)** A HH-C trivalent (white arrow). **(b)** A H-CC-H tetravalent (white arrow). **(c)** A H-C-H trivalent (white arrow). **(d)** A C-bivalent did not reach the equatorial plate (white arrow). Bars = 5 μ m.

Fig. S6

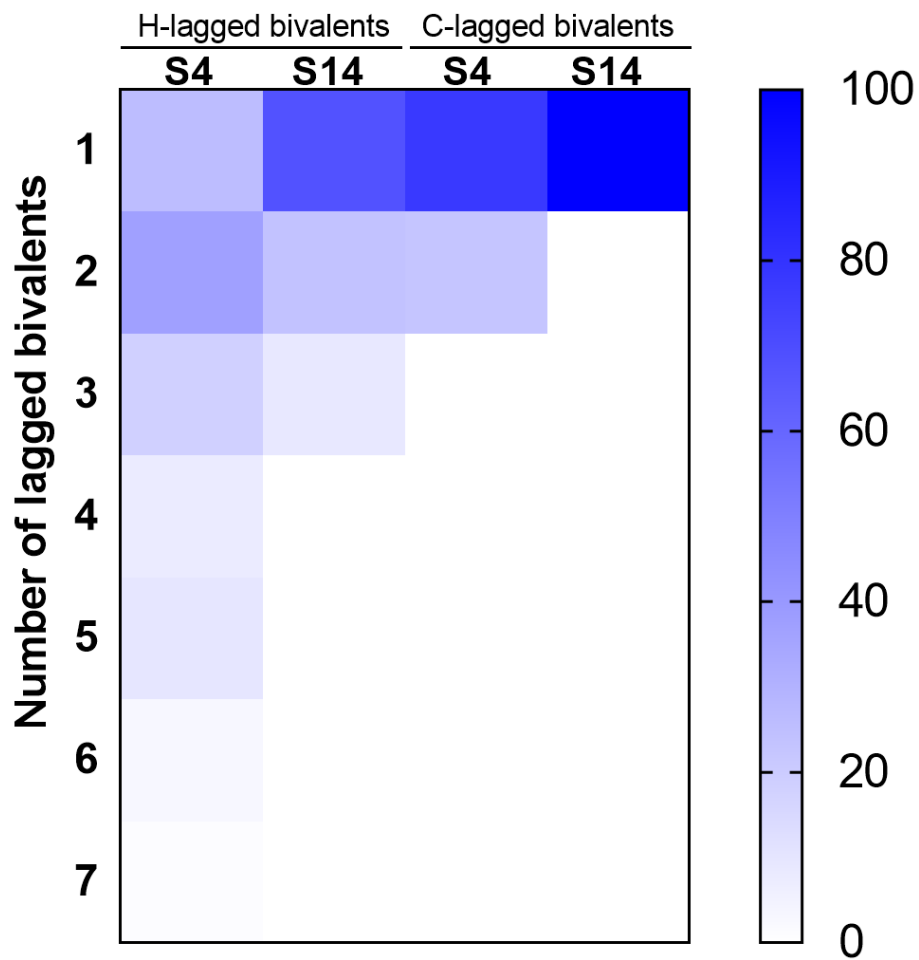


Fig. S6 A heatmap depicting the frequency of different lagged bivalent numbers in the asynchronous meiotic PMCs of S₄ and S₁₄ generations. The y-axes denote the number of bivalents that did not reach the equatorial plate in each MI PMCs with asynchronous meiosis. The colored vertical bars refer to the frequencies of PMCs falling to each of different lagged bivalents in all asynchronous meiotic PMCs.