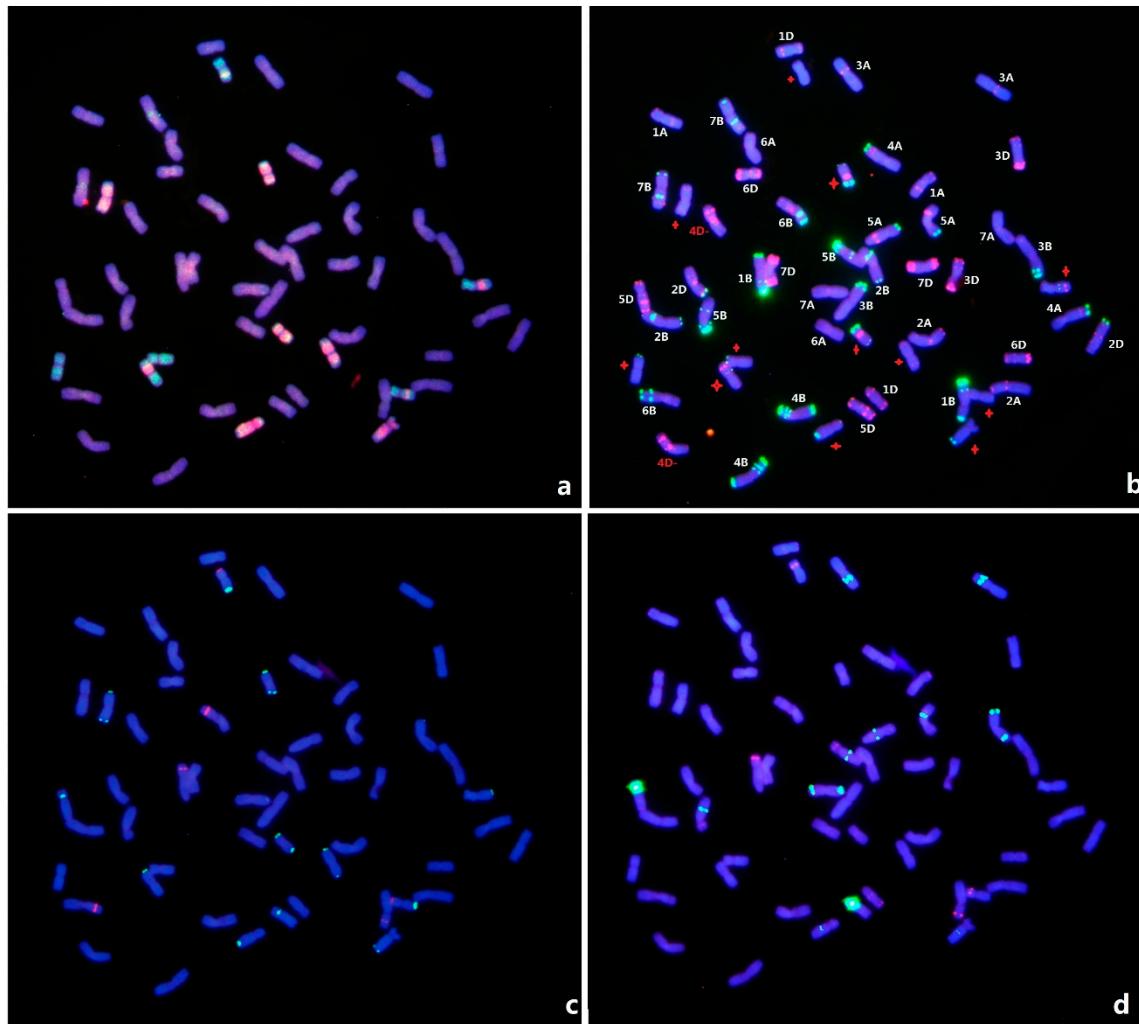


Supplementary Figures:



**Figure S1.** Sequential ND-FISH of Zhong 2  
Oligo-B113 (green) +Oligo-pDb12H (red) (a), Oligo-pSc119.2 (green) + Oligo-pTa535 (red) (b), Oligo-pTa71 (red)+ Oligo-pSt122 (green)(c), and Oligo-3A1(green) + Oligo-5SrDNA (red) (d) as probes, respectively.

|                                 |                       |                     |                     |                     |                     |     |
|---------------------------------|-----------------------|---------------------|---------------------|---------------------|---------------------|-----|
| Wheat Pina-D1 DQ363911          | M K A L F L I G L L   | A L V A S T A F A Q | Y S E V V G S Y D V | A G G G G A Q Q C P | V E T K L N S C R N | 50  |
| Thinopyrum intermedium MH745805 | . . T F . . L - - .   | . . . V . . . . .   | . . G . . . . .     | . . A . . . . .     | . . L . . . . .     | 47  |
| Thinopyrum intermedium MH745804 | . . . F . . V V . .   | . . . . . . . . .   | . . G . . . . .     | . . . . . . . .     | . . I . . . . .     | 50  |
| Hy36-1 MH745807                 | . . . F . . . . .     | . . . . . . . .     | . . R . . . . .     | . . . . . . . .     | . . L D . . . .     | 50  |
| Dasypyrum villosum MH745812     | . . T F . . L - - .   | . . V . . . . .     | . . G . . . . .     | . . . . . . . .     | . . L . . . . .     | 47  |
| Wheat Pina-D1 DQ363911          | Y L L D R C S T M K   | D F P V T W R W W K | W W K G G C Q E L L | G E C C S R L G Q M | P P Q C R C N I I Q | 100 |
| Thinopyrum intermedium MH745805 | . . . . .   T . . . . | . . . . . . . . .   | . . R . . . . .     | . . L . . . . .     | . . . . . . . .     | 97  |
| Thinopyrum intermedium MH745804 | . . . . .   T . . . . | . . . . . . . .     | . . H . . . . .     | . . . . . . . .     | . . . . . . . .     | 100 |
| Hy36-1 MH745807                 | . . . . .   T . . . . | . . L . . . . .     | . . R . . . . .     | . . . . . . . .     | . . . . . . . .     | 100 |
| Dasypyrum villosum MH745812     | . . . . .   T . . . . | . . R . . . . .     | . . . . . . . .     | . . S . . . . .     | . . . . . . . .     | 97  |
| Wheat Pina-D1 DQ363911          | G S I Q G D L G G I   | F G F Q R D R A S K | V I Q E A K N L P P | R C N Q G P P C N I | P G T I G Y Y W     | 148 |
| Thinopyrum intermedium MH745805 | . . . . . . . . . .   | . . . . . . . . . . | . . . . . . . . . . | . . . . . . . . . . | . . S . S . . . .   | 145 |
| Thinopyrum intermedium MH745804 | . . . . . . . . . .   | . . . . . . . . . . | . . . . . . . . . . | . . . . . . . . . . | . . S . . . . . .   | 148 |
| Hy36-1 MH745807                 | . . . . . . . . . .   | . . . . . . . . . . | . . . . . . . . . . | . . K . . . . . .   | . . D . . . . . .   | 148 |
| Dasypyrum villosum MH745812     | . . . . . . . . . .   | . . . . . . . . . . | . . . . . . . . . . | . . . . . . . . . . | . . S . S . . . .   | 145 |

**Figure S2.** Alignment of PINA genes sequences among wheat, *Thinopyrum intermedium* and *Dasyperymum villosum*. The dots reflect the identical amino acids among the sequences. Triangles show the tryptophan-rich region (WRWWKWWK) of *Pina* genes. Boxes indicated the Cysteine residue of the *Pina* genes.



**Figure S3.** Spikes (a) and grains (b) of CS (A), Zhong 5(B), Hy36(C), and Hy37(D).