Supplementary Materials: New Oligonucleotide Probes for ND-FISH Analysis to Identify Barley Chromosomes and to Investigate Polymorphisms of Wheat Chromosomes

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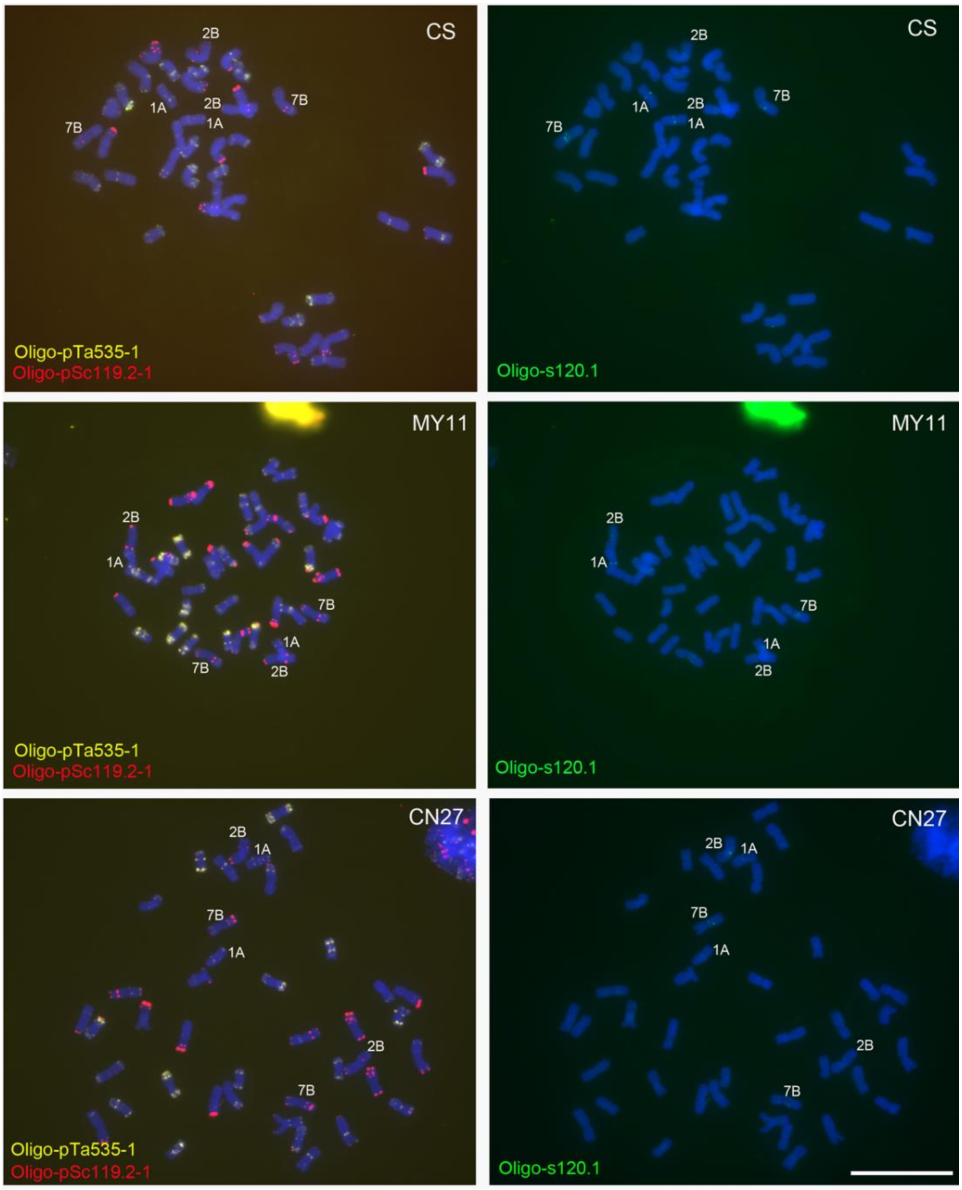

Figure S1. Oligo-s120.1 (**green**) combined with Oligo-pTa535-1 (**yellow**) and Oligo-pSc119.2-1(**red**) was used as probes for non-denaturing fluorescence in situ hybridization (ND-FISH) analysis of root tip metaphase chromosomes of wheat varieties Chinese Spring (CS), Mianyang 11 (MY11), and Chuannong 27 (CN27). Only the chromosomes with signals of Oligo-s120.1 were marked. Chromosomes were counterstained with with 4',6-diamidino-2-phenylindole (DAPI) (**blue**). Scale bar: 10 μm.

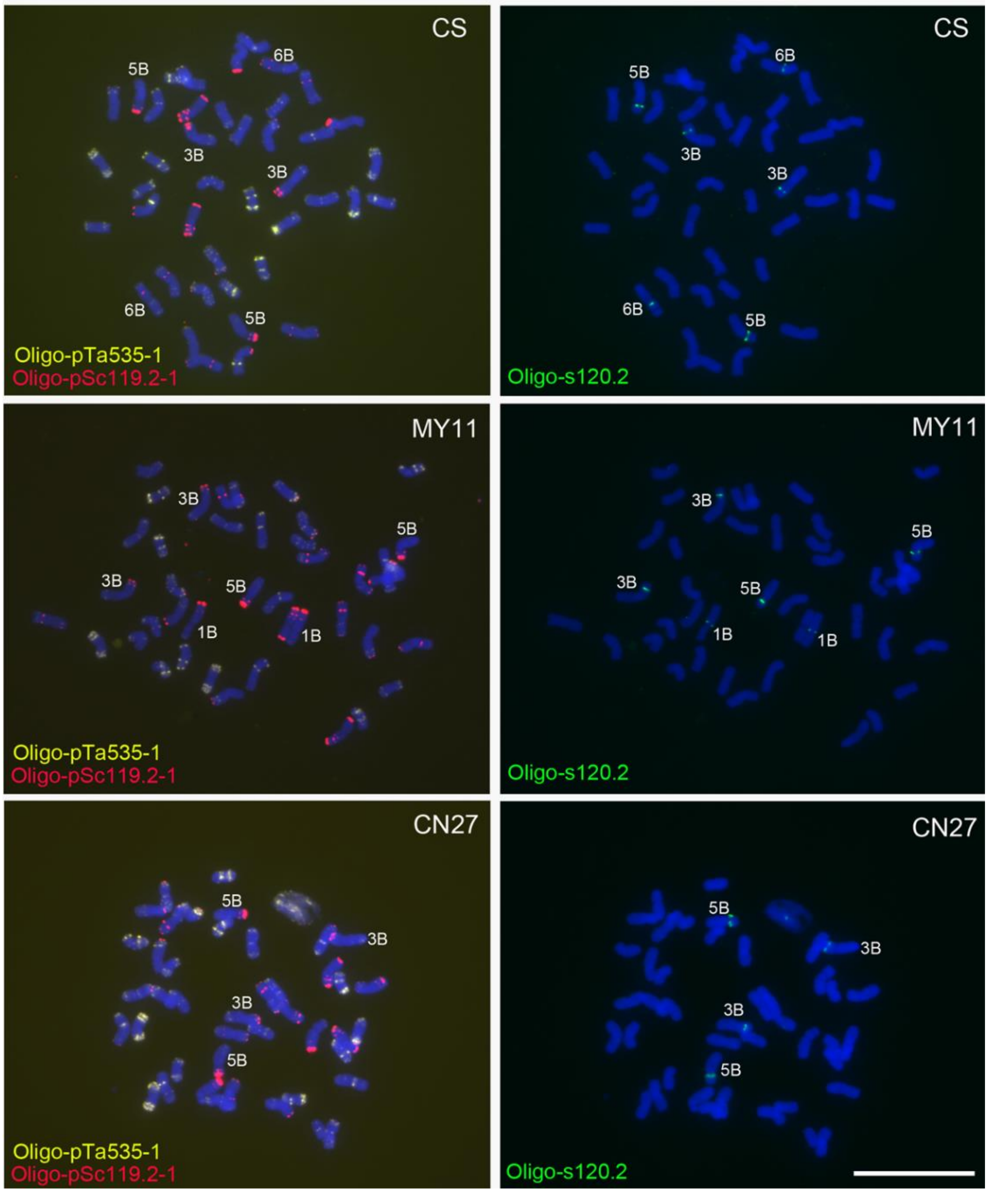

Figure S2. Oligo-s120.2 (**green**) combined with Oligo-pTa535-1 (**yellow**) and Oligo-pSc119.2-1(**red**) was used as probes for ND-FISH analysis of root tip metaphase chromosomes of wheat varieties CS, MY11 and CN27. Only the chromosomes with signals of Oligo-s120.2 were marked. Chromosomes were counterstained with DAPI (**blue**). Scale bar: 10 μm.

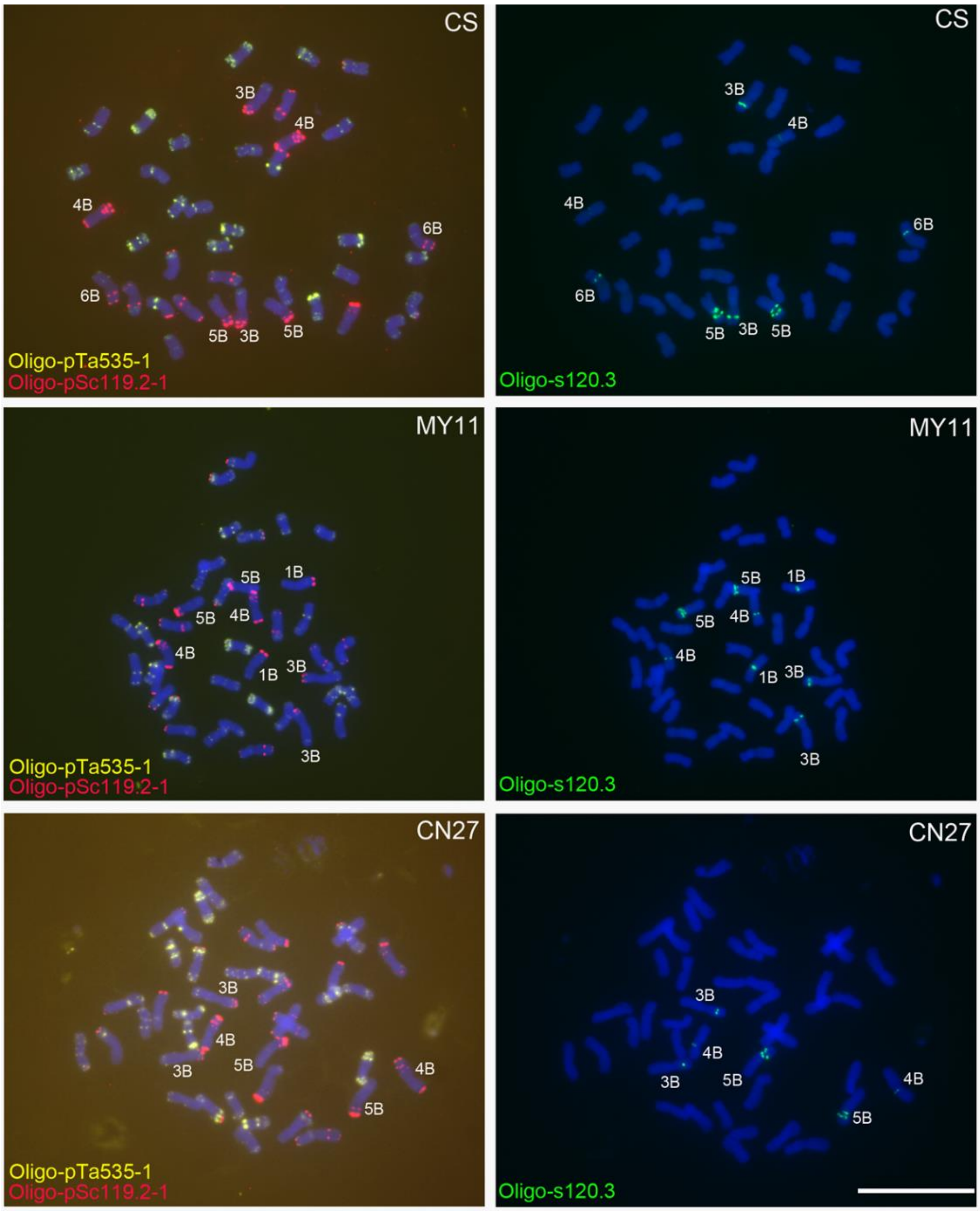

Figure S3. Oligo-s120.3 (**green**) combined with Oligo-pTa535-1 (**yellow**) and Oligo-pSc119.2-1(**red**) was used as probes for ND-FISH analysis of root tip metaphase chromosomes of wheat varieties CS, MY11 and CN27. Only the chromosomes with signals of Oligo-s120.3 were marked. Chromosomes were counterstained with DAPI (**blue**). Scale bar: 10 μm.

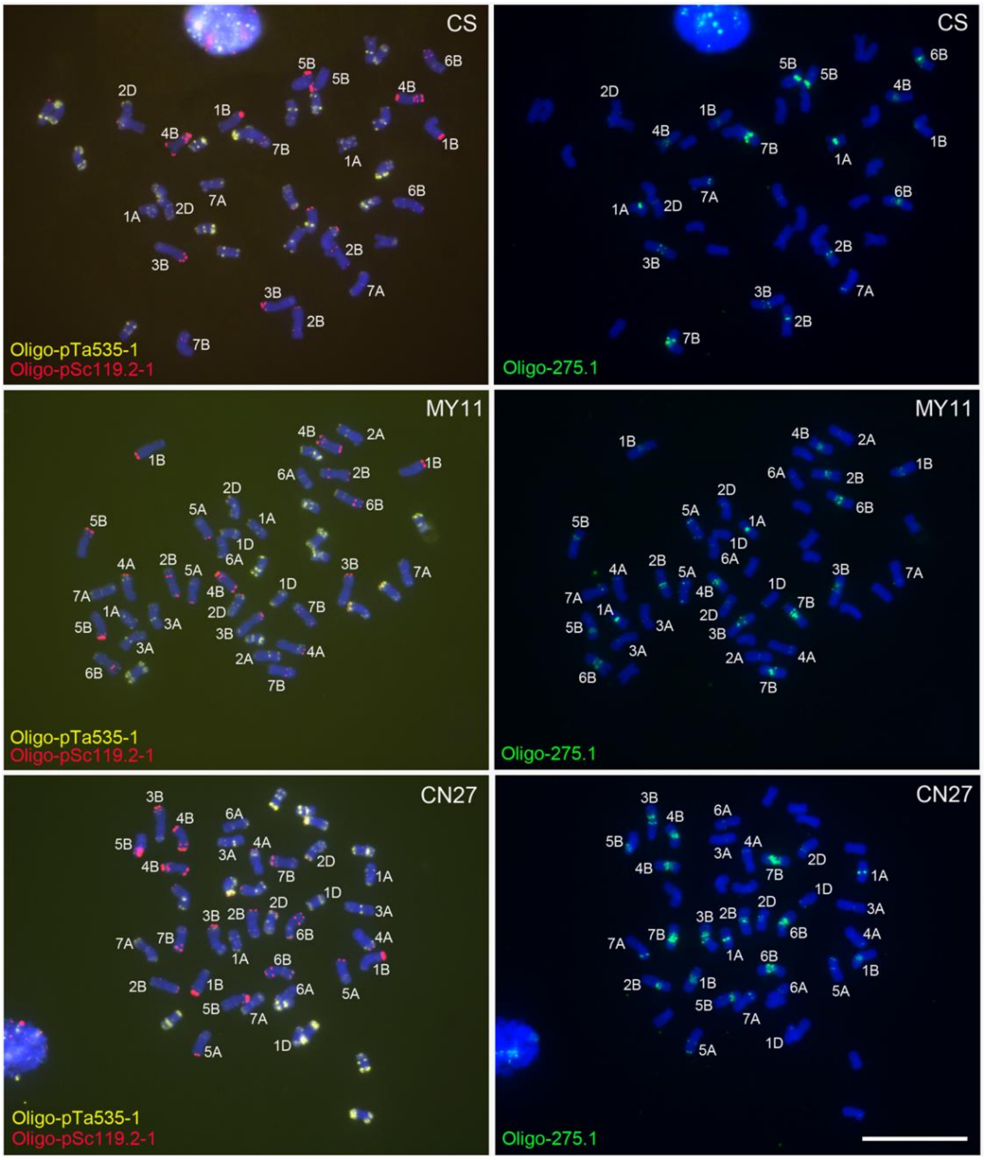

Figure S4. Oligo-275.1 (**green**) combined with Oligo-pTa535-1 (**yellow**) and Oligo-pSc119.2-1 (**red**) was used as probes for ND-FISH analysis of root tip metaphase chromosomes of wheat varieties CS, MY11 and CN27. Only the chromosomes with signals of Oligo-275.1 were marked. Chromosomes were counterstained with DAPI (**blue**). Scale bar: 10 µm.

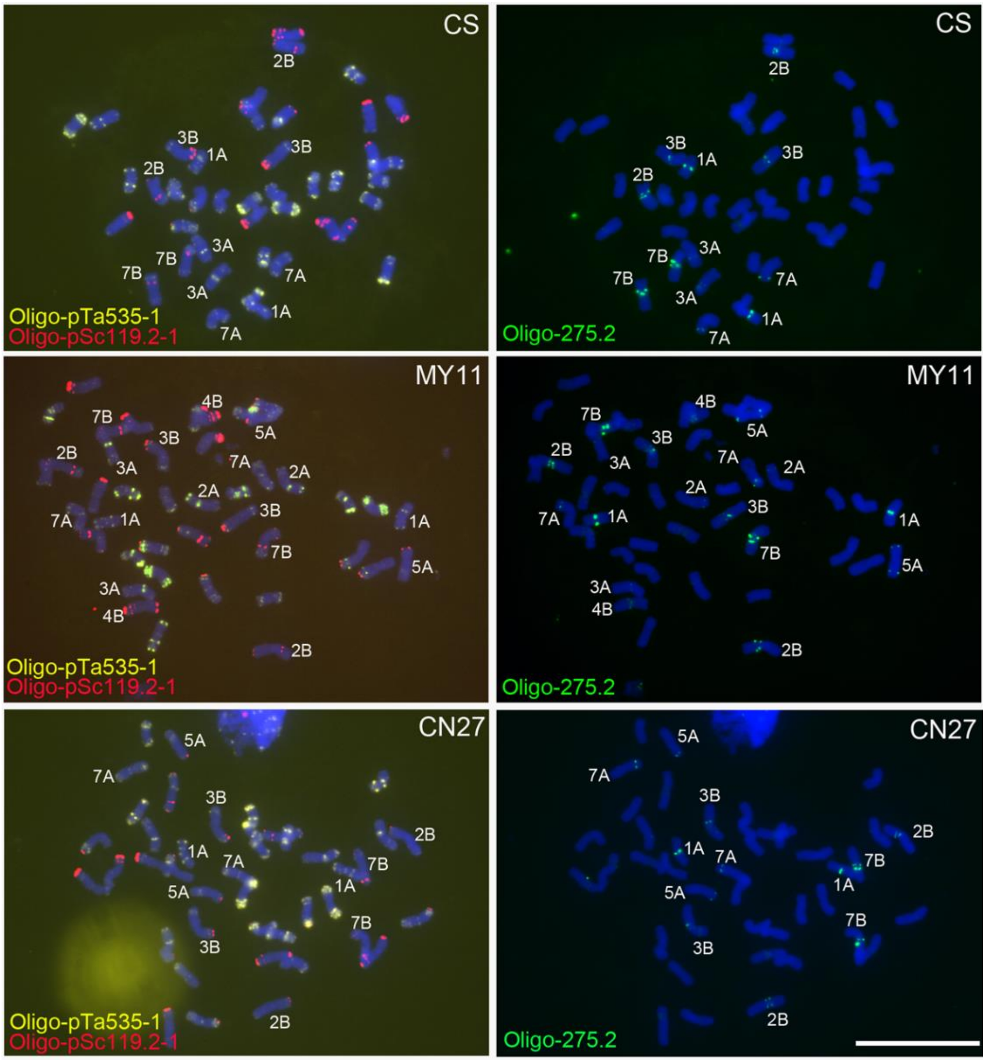

Figure S5. Oligo-275.2 (**green**) combined with Oligo-pTa535-1 (**yellow**) and Oligo-pSc119.2-1 (**red**) was used as probes for ND-FISH analysis of root tip metaphase chromosomes of wheat varieties CS, MY11 and CN27. Only the chromosomes with signals of Oligo-275.2 were marked. Chromosomes were counterstained with DAPI (**blue**). Scale bar: 10 μm.

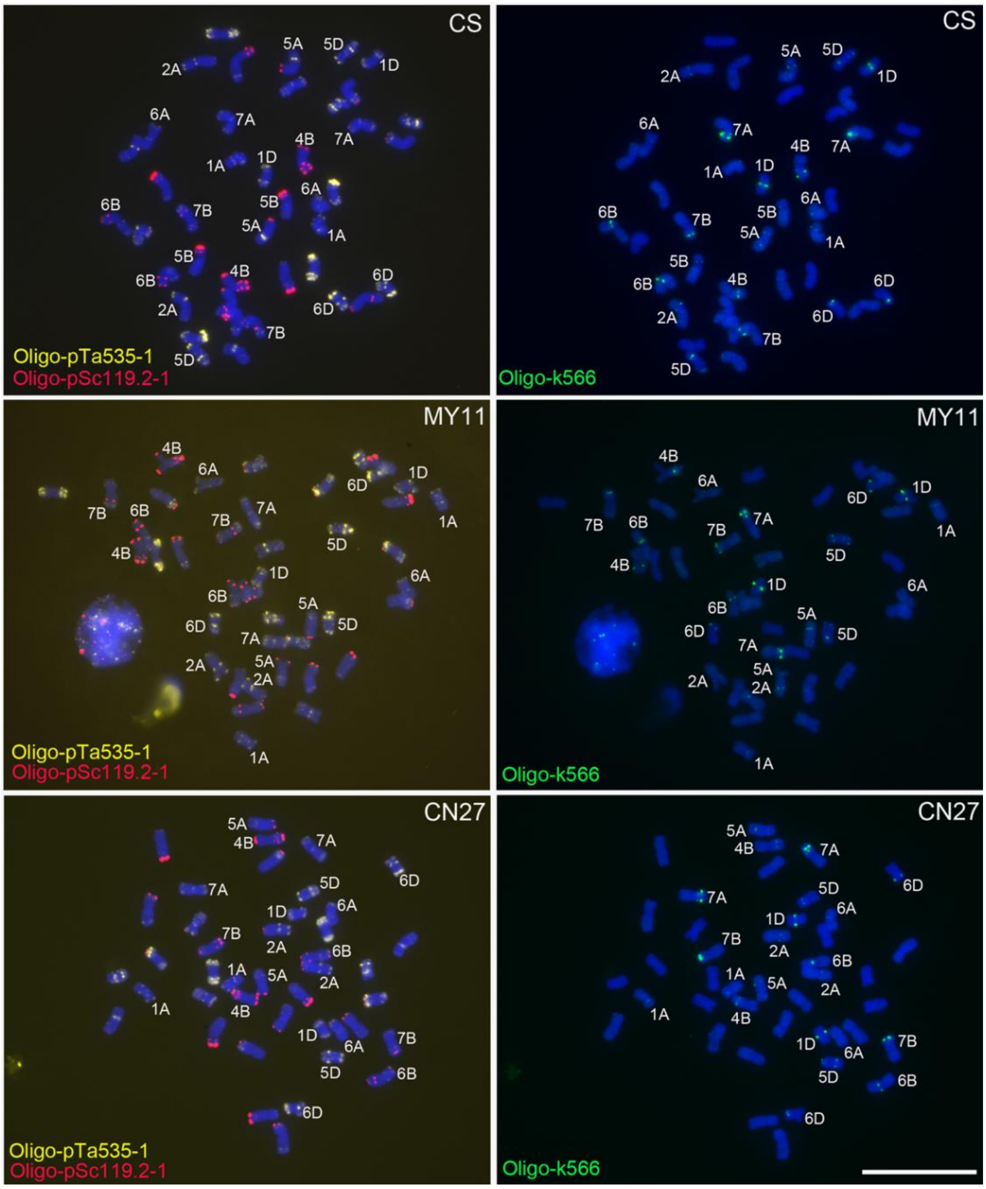

Figure S6. Oligo-k566 (**green**) combined with Oligo-pTa535-1 (**yellow**) and Oligo-pSc119.2-1 (**red**) was used as probes for ND-FISH analysis of root tip metaphase chromosomes of wheat varieties CS, MY11 and CN27. Only the chromosomes with signals of Oligo-k566 were marked. Chromosomes were counterstained with DAPI (**blue**). Scale bar: 10 μm.

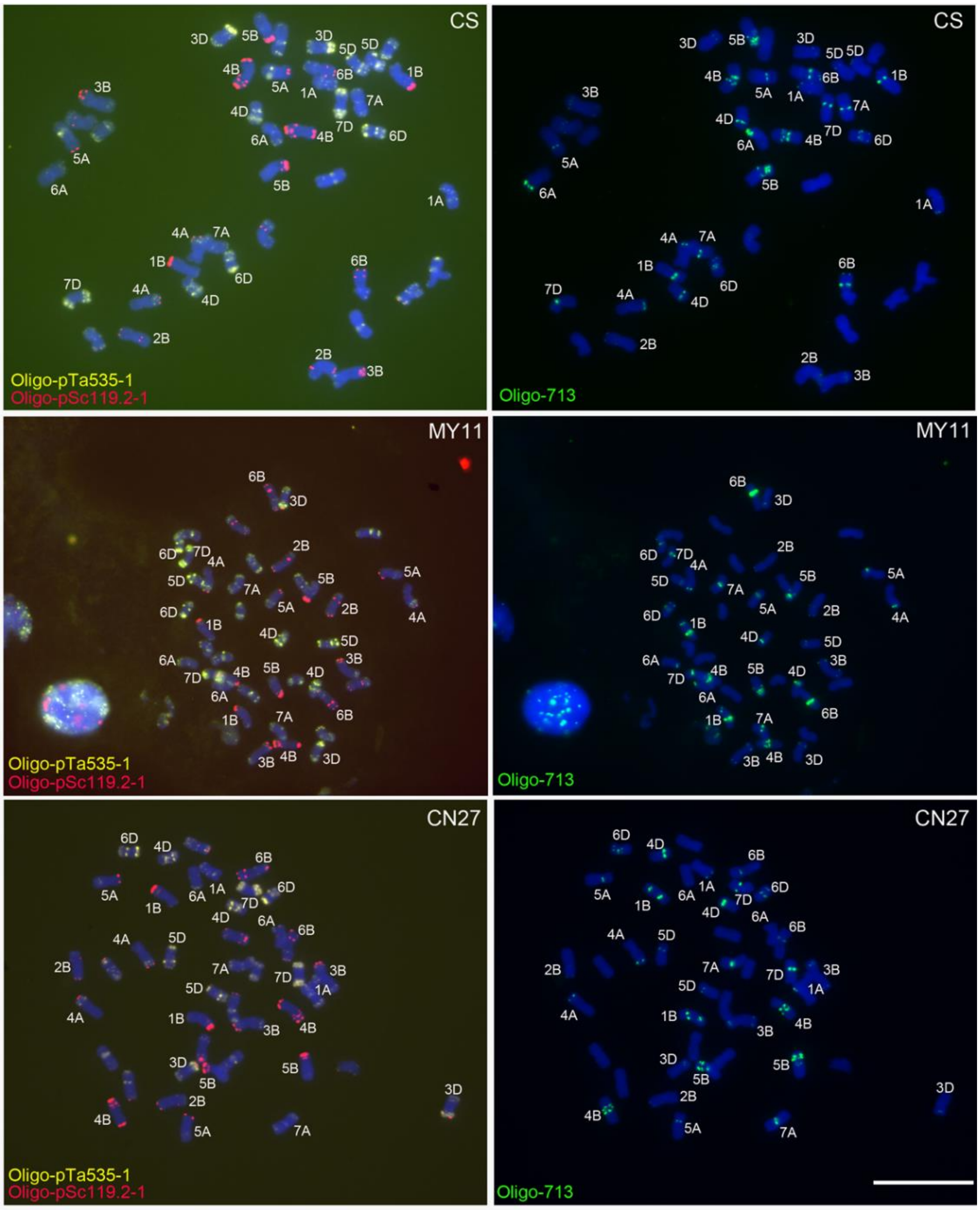

Figure S7. Oligo-713 (**green**) combined with Oligo-pTa535-1 (**yellow**) and Oligo-pSc119.2-1 (**red**) was used as probes for ND-FISH analysis of root tip metaphase chromosomes of wheat varieties CS, MY11 and CN27. Only the chromosomes with signals of Oligo-713 were marked. Chromosomes were counterstained with DAPI (**blue**). Scale bar: 10 μm.

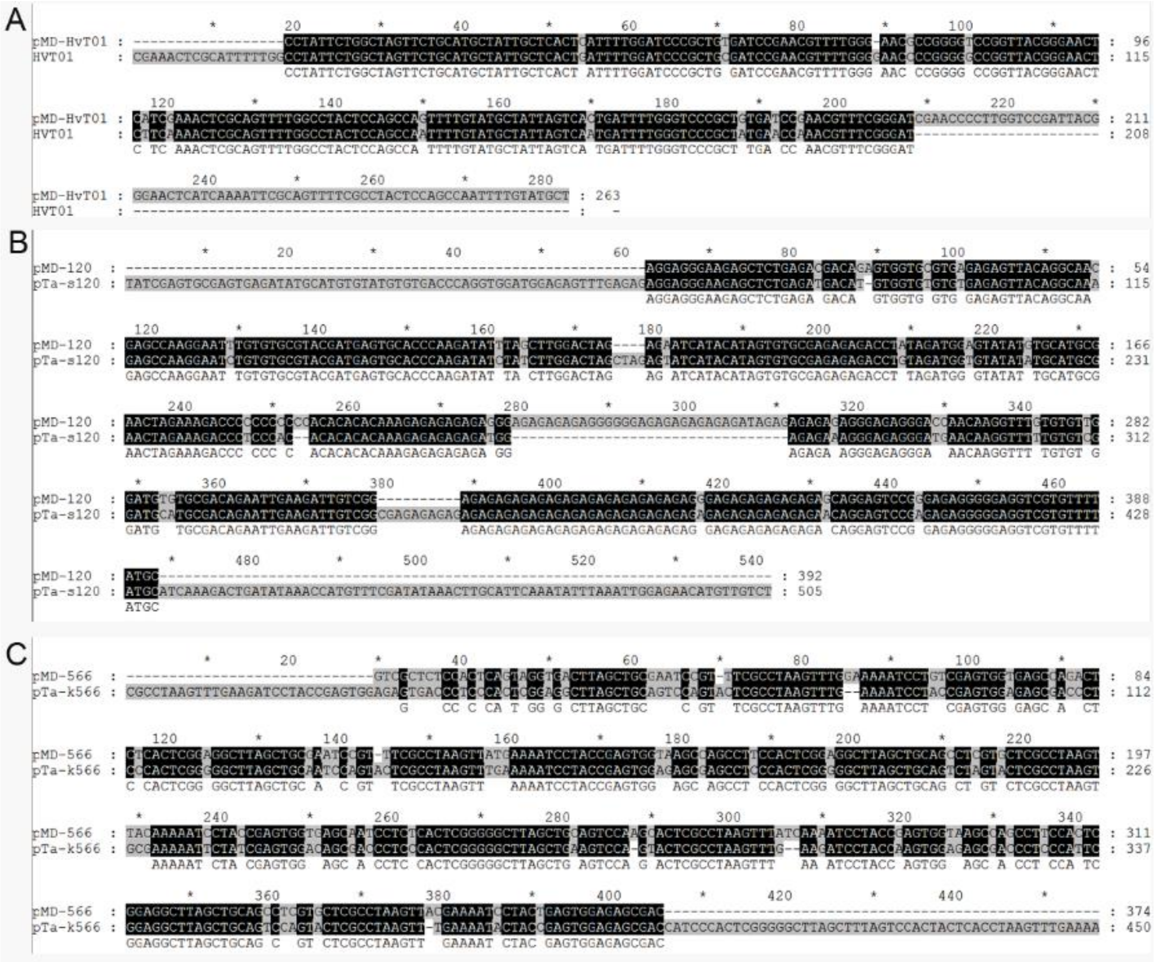

Figure S8. Sequence alignment between cloned repetitive sequences and their corresponding original repetitive sequences: (**A**) alignment between pMD-HvT01 and HVT01; (**B**) alignment between pMD-120 and pTa-s120; and (**C**) alignment between pMD-566 and pTa-k566.

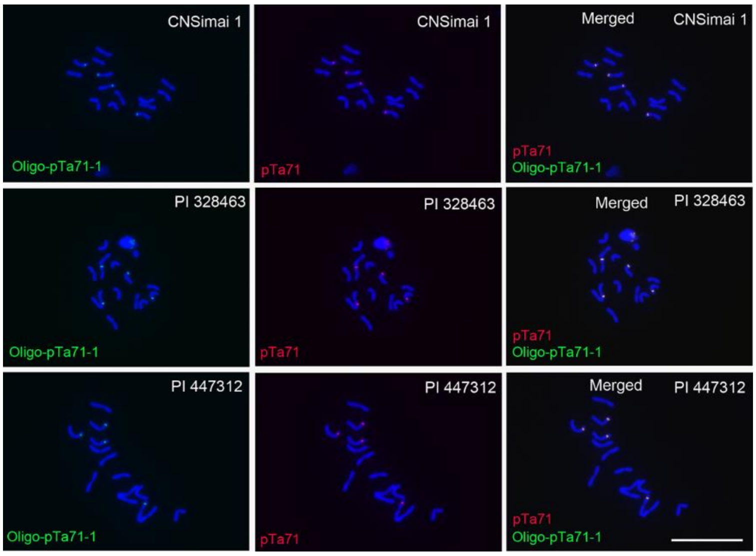

Figure S9. Oligo-pTa71-1 (**green**) and pTa71 (**red**) were used as probes for denaturing FISH analysis of root tip metaphase chromosomes of three barley varieties. Chromosomes were counterstained with DAPI (**blue**). Scale bar: 10 μm.

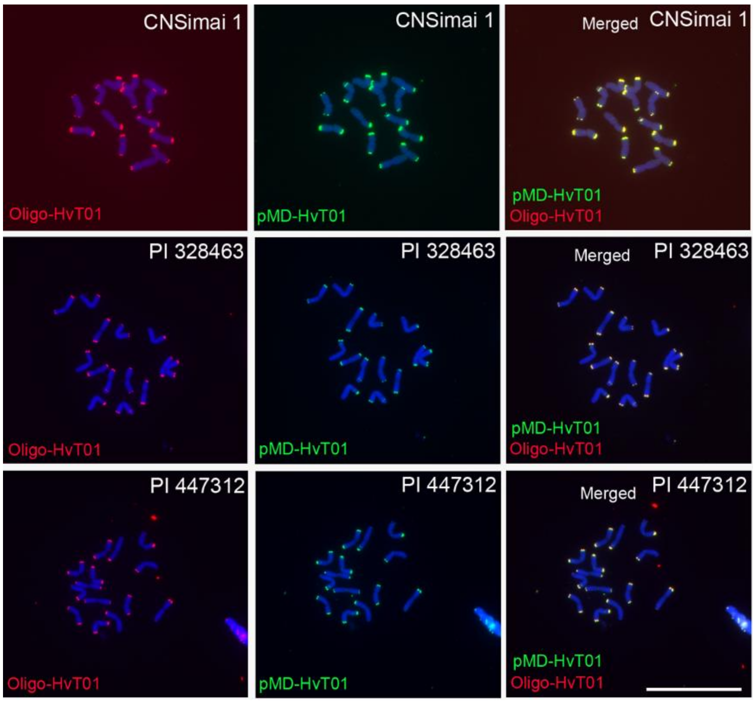

Figure S10. Oligo-HvT01 (**red**) and pMD-HvT01 (**green**) were used as probes for denaturing FISH analysis of root tip metaphase chromosomes of three barley varieties. Chromosomes were counterstained with DAPI (**blue**). Scale bar: 10 μm.

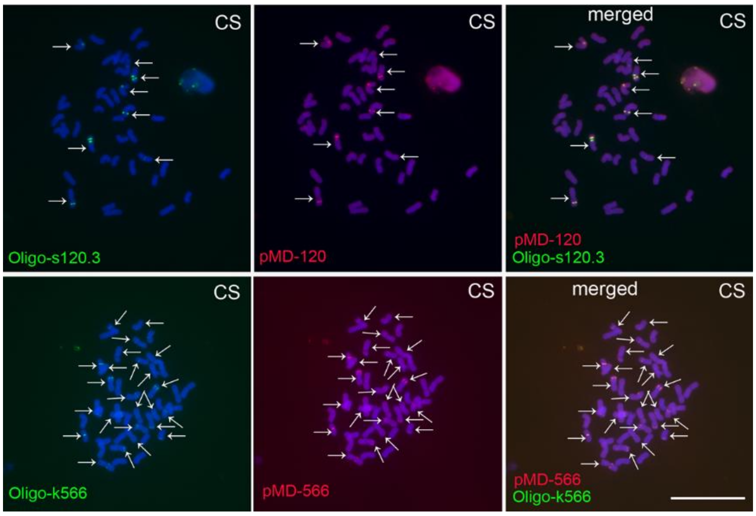

Figure S11. Oligo-s120.3 (**green**), Oligo-k566 (**green**), pMD-120 (**red**) and pMD-566 (**red**) were used as probes for denaturing FISH analysis of root tip metaphase chromosomes of wheat CS. Arrows indicate the chromosomes with hybridization signals. Chromosomes were counterstained with DAPI (**blue**). Scale bar: 10 μm.