## Supplementary Materials

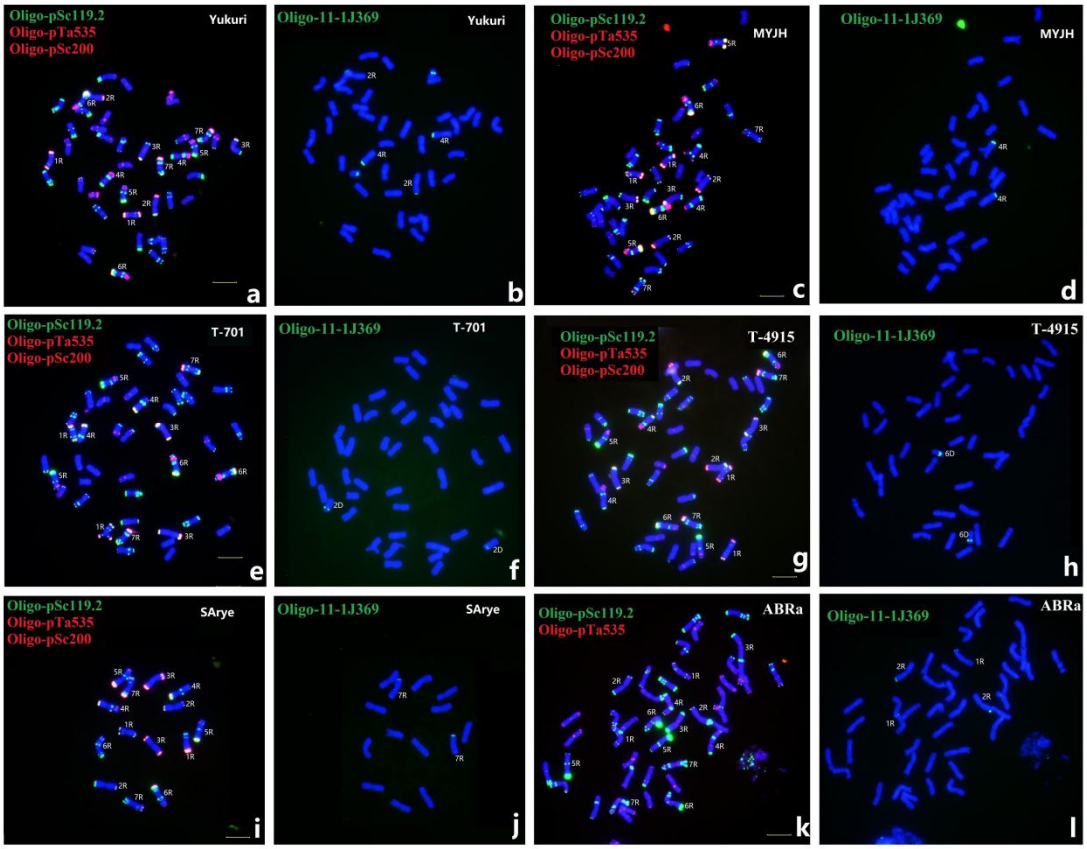



**Figure S1**. Comparative ND-FISH between Yukuri and other triticale and rye lines.The probes of Oligo-pSc119.2 + Oligo-pTa535 + Oligo-pSc200 (a, c, e, g, i, k), and sequential probe of and Oligo-11-1J369 (b, d, f, h, j, l) are shown. The lines Yukuri (a, b), MYJH (c, d), T-701(e,f), T4915 (g, h), SArye (i, j), and durum wheat-S. africanum amphiploid YF (k, l) are indicated. Bar 10μm.

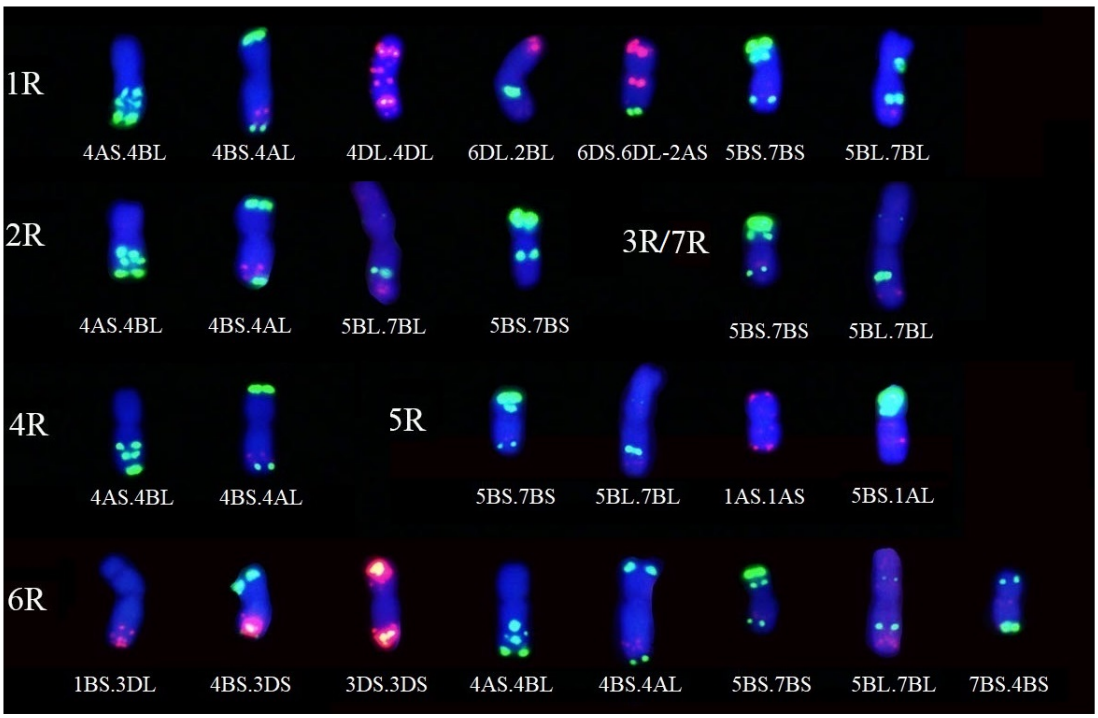


**Figure S2**. Different types of modified wheat chromosomes in wheat-rye additions. The ND-FISH patterns of Oligo-pTa535 (red) and Oligo-pSc119.2 (green) are indicated.

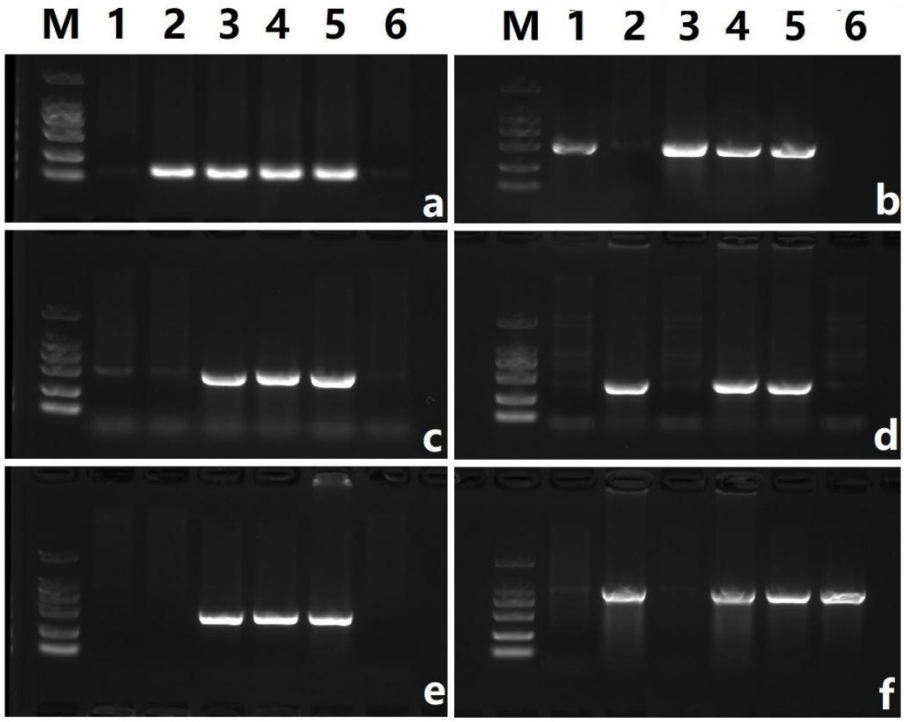



**Figure S3**. The amplification of chromosome 1R specific PCR based molecular markers. (a-c) 1RL specific markers 1RL673 (a), Ku1RL248 (b) and Ku1RL589 (c), (d-f) 1RS specific markers Ku1RS127 (d), 1RS15.3 (e) and 1RS2.29 (f) were used for PCR. The plant materials 1-6 were CS, JH, CSDA1R, Yukuri, MA1R (wheat-monosomic 1R addition from Yukuri), AK58, respectively. M, markers.

**Table S1.** The sequences and hybridization sites of the novel probes by ND-FISH

| Name of probe | Sequences (5'-3') | Hybridization sites on wheat chromosome | Hybridization sites on rye chromosomes |
|---|---|---|---|
| Oligo-pSc119.2 | CCGTTTTGTGGACTATTACTCACCGCTTT GGGGTCCCATAGCTAT | 1A-7D | 1R-7R |
| Oligo-pTa535 | GACGAGAACTCATCTGTTACATGGGCAC TTCAATGTTTTTTAAACTTATTTGAACTC CA | 1A-7D | |
| Oligo-pSc200 | CTCACTTGCTTTGAGAGTCTCGATCAATT CGGA CTCTAGGTTGATTTTTG TATTTTCT | | 1R-7R |
| Oligo-Ku | GATCGAGACTTCTAGCAATAGGCAAAAA TAGTA ATGGTATCCGGGTTCG | | 1R-7R |
| Oligo-K288 | CTTCATAGTCCGGGAGTCCGGCCAAAGG TCATAGTCCG GCCATCC | 1A-7B | |

| | | | |
|---|---|---|---|
| Oligo-5SrDNA | TCAGAACTCCGAAGTTAAGCGTGCTTGGGC GAGAGTAGTAC | 1BS, 1DS, 5AS, 5BS, 5DS, | 5RS |
| Oligo-3A1 | AATAATTTTACACTAGAGTTGAACTAGCTCTATA AGCTAGTTCA | 3AL，5DS，5AL，5BL，7AS，7AL， | 5RL |
| Oligo-V03-71 | CTTCTCCGAGTGAAGAGCGATCCTCTCACTCGGGGGCTTAGCTGCGAGTCTAATC | 4BL，7BS，5A，6DS，7AS，6BL，5BS，1BS，1AS | 1R，4RS，4RL，7RL |
| Oligo-StC12 | ATGTTCTATCACCCATCCCGCTACCACCCTCACTTTTTCACCAG | | 1RS，4RS，5RS，6RS，7RL |
| Oligo-13-J1011 | CATCATGCTTGTTGTGAGAAGCTCTGGTTTGTGAGAAGCATATACCCAAACC | 6D，7A，1A，2A，4A | 1RS，3RS，6RS，7RS |
| Oligo-11-1J369 | GAGTTCGTTCAGAAACTTGTATAGAAACTTTCTTAATGAGCACTCGACA | 6DS | 2RS，4RS |
| Oligo-09-4St142 | GGATCGCTCTACACTCGGAGAAGTTTTTAACTTAGACGAAAGCTGGTTCGTA | 6BL，1B，7B | 2RS，5RS |
| Oligo-6E-571 | ACGTAACGATTTTAGAGGCTAGGAGTGGACACGCCAGCGATTAAATCATC | | 1RS，6RS，7RS |
| Oligo-2S-119 | TTGTTTTCGCCGTGTGGCATGCTGTAACTTATGTGTGTGACAGCCAAGAACCAAAGGGC | | 2RS，4RS |
| Oligo-6E-376 | CGTATTTCAAATCATACGGACCTAAAAATTAGAAGTCATTCCATCTTTA | | 1R，2R，3RL，4RL，5R，6R，7R |
| Oligo-P05 | AATACGCTCTTGTTCTTGGCTGTCACGCACATACTTTATGGGATGTCATAGG | | 2RS，4RS |
| Oligo-2S-248 | AGGAGTTCTTGGGCGACCTTTGTTTCAGAGTGACGTGTGGTAGGGTTTAGGGCAAAT | 6BS，3BL | 4RL，6RS |
| Oligo-7E-369 | AACTTAGGCGAGGACTGGCTCGCGGCTAAGCCCCCGAGTGAGAGGGATGCTCTCA | 6BL，4BL，7BS，5A，6DS，7AS，5BS，1BS，1AS | 4RS，4RL，7RL |
| Oligo-St96-1 | GAGCTGCTATGCACATTTTCGTCCAAGAACGGCGCATGTTCTT | | 1RS，4RS，5RS，6RS，7RL |
| Oligo-St96-2 | CAGGAACTCAATTTTGCAAGCCGGTTTTGGCCTCCGAAACATT | 6B，3B，1BL，4BL，5BS，7B | 1RS，2RS，4R，5R，6R，7R |
| Oligo-2S-163 | GAGCACACGTGCGGTAAGTCTCGGCCCCAGAAAAGGCCGTTTAGACCCTCAAAAAGGCT | | 1R，2R，3RL，4RL，5R，6R，7R |
| Oligo-D01-135 | ACGCGCGCCATGGAAAACAGGGCAAAACCACCGACTCGTCCACGACTCGTAC | 1BS，6BS | 1RS |
| Oligo-StC11 | TTGAGTTTCGGATGATTTTATCTTTGGCGTGTACTCTGCCACCCT | 6BL | 1RS，4RS，5RS，6RS，7RL |
| Oligo-7E-553 | CGGGGGCTTAGCCGCGAGCCAGTCCTCGCCTAAGTTAAAAAATTAC | 4BL，7BS，5A，6DS，7AS，6BL， | 1R，4RS，4RL，7RL |

| | | | |
|---|---|---|---|
| | | 5BS，1BS，1AS | |
| Oligo-TB553-2 | TTTGTAATGGAAGGATGGCGCATTGTTC TATATGTTATTGTCCATATA | | 1RS，6RS，7RS |
| Oligo-10-7J89 | CAGCCTGAAGTGAAGAGATCTCCCGCTG AGCTTCCGCAGCGGCC | | 1R，2R，3RL，4RL，5R，6R，7R |
| Oligo-7E-716 | GTACAGGACTGCAGCTAAGCCCCCGAG TGAGAGGGTTGCTCATCACTCGGTAGGA TT | 6BL，1B，7B | 2RS，5RS |
| Oligo-Y372 | AATTTCGTCACCGTATCTTCTCTAATGTG ACACCAACACCCTATCGTAGACATCCGT ATCT | | 1R，2R，3RL，4RL，5R，6R，7R |
| Oligo-$(GAA)_7$ | GAAGAAGAAGAAGAAGAAGAA | 1B-7B，2A-7A，2D，7D | 2RL，3RS，6R |
| Oligo-$(CAA)_7$ | GAAGAAGAAGAAGAAGAAGAA | 7A， 3D， 2A，1B-7B，4A，4D | 1R-7R |
| Oligo-1B-3 | CAATGGGTTCAGAAACTTGTCTGAAAAA CAGCGACGAATGATG | | 2RS，4RS |
| Oligo-2H-18-50 | TAATAAGCTTAGTTAGCTCCAAAATGAC ATATTTTC | 6BL，7BL，3BS， | 5RS，7RL |
| Oligo-pTa1-360 | CCTTCAAAAGGAAGTGCCAATGGGTTCA GAAACTTGTCCGAAAAACAGCGGCGAA TG | | 2RS，4RS |
| Oligo-pAWRC | CGTAGGCGCCGATCTTGAAAGAGACTTG CACGGTGTGCTCGACTCGAAGAATTCCG GCGT | | 1R-7R |
| Oligo-CCS1 | CCGTTTGATAGAGGCAAAGGTGTCCCGT CT TTTGATGAGA | 1A-7D | 1R-7R |
| Oligo-pTa1-335 | TCTATAATCAACATAATCTCTCGAATTT CCTTCAGAAGGAAGTGCCAATGGGCTC AG | 6BL，1B，5B | 2RS，4RS |

**Table S2.** Sequences and location of the 1R-specific markers

| Primers | Forward | Reverse | Location on 1R of Lo7 (Mb) | Reference |
|---|---|---|---|---|
| 1RS2.29 | GTTGCTCCCATGGCAT GACAG | GCTGGTAGTTAATG CACCGAG | 2.29 | This study |
| 1RS15.3 | GTTTGGAGCGATAAC TAGTGA | GGAACTGGTGGATA CGGTTC | 15.30 | This study |
| KU1RS127 | TGAGATGAGAGAGGG GTCCT | AATGTGGAGGGTAG CTGCAA | 139.89 | Qiu et al. (2016) |
| KU1RL589 | GCACAAGATGTAGCC | GCACGTAATTTGGC | 535.09 | Qiu et al. |

| | | | | |
|---|---|---|---|---|
| | AGCTG | GATGG | | (2016) |
| 1RL673 | GGTGCGTCTTAAACT | GATCGTAGTGGGTA | 673.99 | This study |
| | GATTC | GTATCT | | |
| KU1RL248 | TTTCAGAGAGGCATG | CCCTCAAAGGCGAT | 685.49 | Qiu et al. |
| | AATGCTA | TTTACA | | (2016) |