Supporting Information

Sanei et al. 10.1073/pnas.1103190108

SI Materials and Methods

Plant Growth Conditions and Crossing Approaches. Two genotypes of *Hordeum bulbosum* (Cb2920/4 and Cb3811/3) (1) were vegetatively propagated and vernalized for 7–8 wk at 4 °C, with an 8-h day length. Vegetative propagation is necessary because *H. bulbosum* is self-incompatible (2) and individual genotypes cannot be established from seeds. After vernalization, the two genotypes were maintained separately in cool glasshouses (temperatures <18 °C) with a 16-h day length.

For the *H. vulgare* ("Emir") plants, two environments were used with contrasting temperatures to control chromosome elimination after pollination. One glasshouse was maintained with temperatures greater than 18 °C for chromosome elimination, whereas the other had temperatures less than 18 °C to promote retention of the parental chromosomes after pollination with *H. bulbosum*. Plants were cultivated until ear emergence in a cool glasshouse and were then transferred to their respective environments.

Crossing was done conventionally by emasculating florets of the female parent before anthesis; the spikes covered with bags to prevent out-pollination and pollinated with freshly collected pollen from the male parent. A fine spray of plant growth regulators was applied to florets 1 d (summer) or 1 and 2 d (winter) after pollination to stimulate seed development and improve the quality of the seeds. The mixture comprised 75 mg/L gibberellic acid plus 1 mg/L dicamba, with or without 2 mg/L 2,4-dichlorophenoxyacetic acid. Twelve drops per liter of Tween 20 was added as a surfactant. Immature embryos of various sizes were excised under a stereomicroscope for further analysis.

1. Sanei M, et al. (2010) Interspecific hybrids of *Hordeum marinum* ssp. *marinum* x *H. bulbosum* are mitotically stable and reveal no gross alterations in chromatin properties. *Cytogenet Genome Res* 129:110–116.

2. Bothmer R, Salomon B, Linde-Laursen I (1995) Variation for crossability in a reciprocal, interspecific cross involving *Hordeum vulgare* and *H. lechleri*. *Euphytica* 84:183–187.

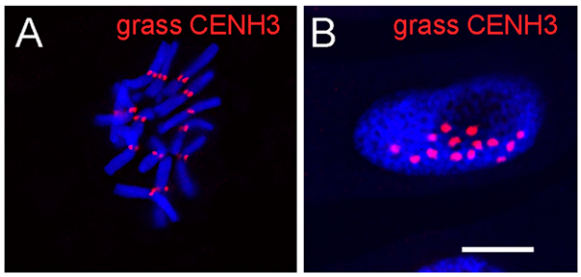

Fig. S1. Confirmation of anti-grass CENH3 cross-reactivity with CENH3s of *H. bulbosum* by indirect immunostaining. Immunostaining of metaphase (*A*) and interphase (*B*) nuclei of *H. bulbosum* with anti-grass CENH3. (Scale bar: 10 μm.)

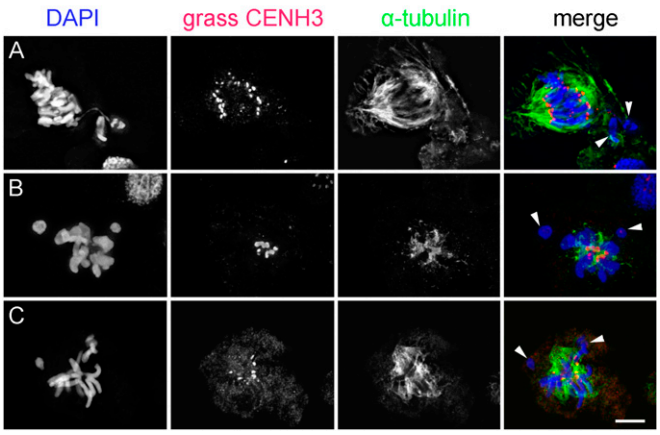

Fig. S2. *H. vulgare* × *H. bulbosum* hybrid anaphase cells show lagging chromosomes (arrowheads). (*A*), (*B*), and (*C*) show different examples. Immunostaining with anti-grass CENH3 and anti–α-tubulin. (Scale bar: 10 μm.)

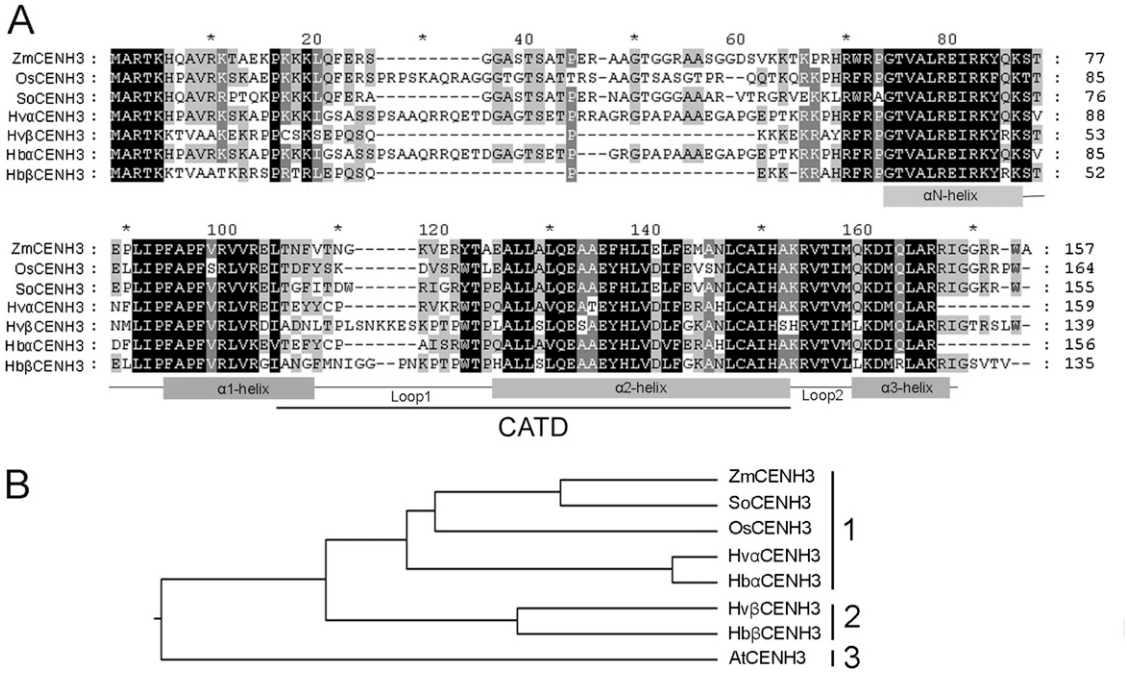

Fig. S3. Comparison of αCENH3 and βCENH3 of *H. vulgare* and of *H. bulbosum* with CENH3s of maize, rice, and sugar cane. Hb, *H. bulbosum*; Hv, *H. vulgare*; Os, rice; So, sugar cane; Zm, maize. (*A*) Alignment of deduced amino acid sequences. The CENH3-typical α*N*-helix, α1-helix, α2-helix, α3-helix, loop 1 region, and CAT domain are indicated. (*B*) Phylogenetic analysis shows that the αCENH3s form a distinct subcluster with the CENH3s of maize, rice, and sugar cane. βCENH3s of *Hordeum* species form a separate cluster. CENH3 of *A. thaliana* was used as an outgroup. At, *A. thaliana*.

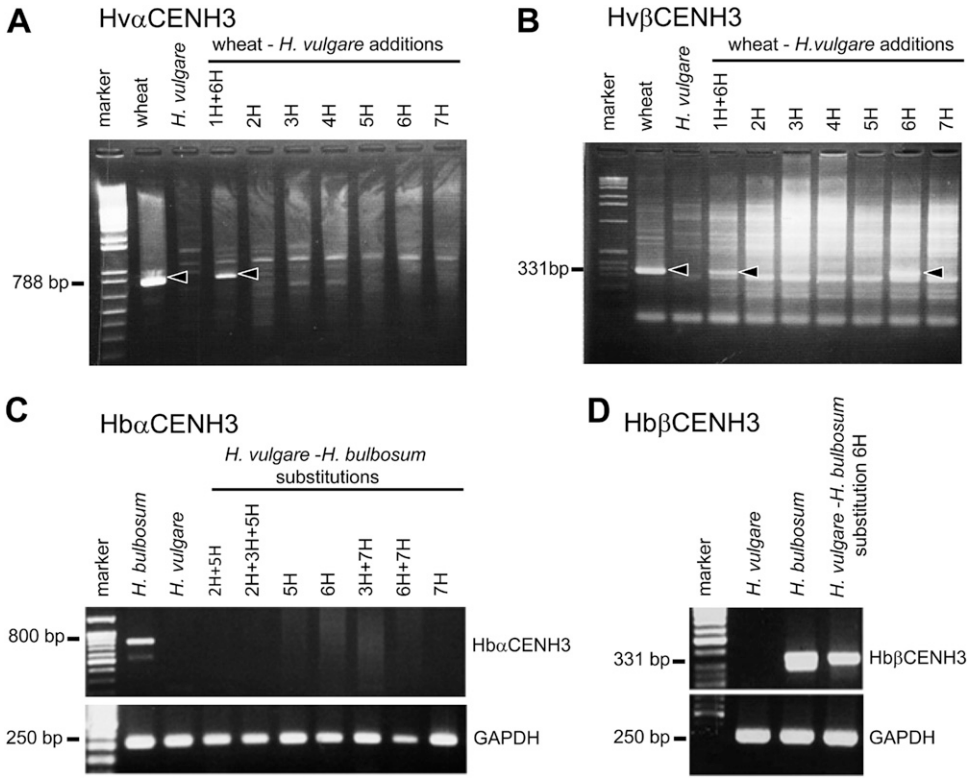

Fig. S4. Chromosome mapping of α*CENH3s* and β*CENH3s* in *H. vulgare* (*A* and *B*) and in *H. bulbosum* (*C* and *D*). Mapping of α*CENH3s* (*A*) and β*CENH3s* (*B*) of *H. vulgare* using wheat-barley addition lines. The bands corresponding to the barley CENH3s are indicated by arrowheads. PCR was performed on the genomic DNA samples of (from left to right) wheat (Chinese Spring), barley (Betzes), and seven barley (Betzes) chromosome addition lines in wheat (Chinese Spring) (1, 2). Because the 1H addition line causes severe sterility, double-disomic addition 1H and 6H was used. For amplification of *HvαCENH3* and *HvβCENH3*, the primer pairs 8/9 and 3/10 were used, respectively. Chromosome mapping of α*CENH3s* (*C*) and β*CENH3s* (*D*) of *H. bulbosum* using *H. vulgare* lines with substituted *H. bulbosum* chromosomes. (*C*) Amplification of α*CENH3* in *H. bulbosum* but not in the substitution lines (2H–7H) (3, 4), except for 1H, indirectly indicates that α*CENH3* is located on 1H of *H. bulbosum* A substitution with 1H of *H. bulbosum* is not available. (*D*) Amplification of the chromosomal location of *HbβCENH3* using the 6H *H. bulbosum* substitution line in the barley genome; the result shows that this gene is located on chromosome 6 of *H. bulbosum*. For amplification of HbαCENH3 and HbβCENH3, the primer pairs 4/5 and 3/11 were used, respectively. Amplification of *GAPDH* with primers 12 and 13 was used as a positive control.

1. Islam AKMR, Shepherd KW (2000) Isolation of a fertile wheat-barley addition line carrying the entire barley chromosome 1H. *Euphytica* 111:145–149.
2. Islam AKMR, Shepherd KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. *Heredity* 46:161–174.
3. Pickering RA (1992) Monosomic and double monosomic substitutions of *Hordeum bulbosum* L. chromosomes into *H. vulgare* L. *Theor Appl Genet* 84:466–472.
4. Thiele V, Pickering RA, Melz G, Pohler W (1992) Identification of *Hordeum bulbosum* chromosomes in *H. vulgare–H. bulbosum* substitutions using isozyme markers. *Genome* 35: 454–460.

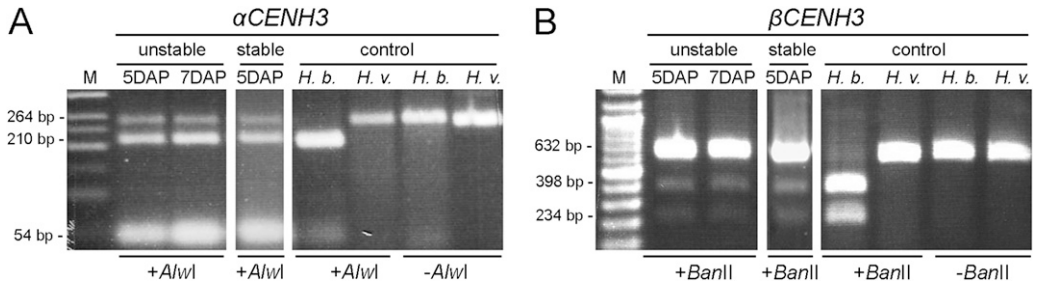

Fig. S5. Transcriptional analysis of αCENH3 (*A*) and βCENH3 (*B*) of *H. vulgare* and *H. bulbosum* in stable and unstable plants 5 and 7 d after pollination (DAP) of old *H. vulgare* × *H. bulbosum* hybrid embryos by RT-PCR. Only *H. bulbosum*-derived α*CENH3* and β*CENH3* transcripts were digestible with AlwI and BanII, respectively. As a control, RT-PCR products of *H. vulgare* and *H. bulbosum* plants were used. DAP, days after pollination; H.b., *H. bulbosum*; H.v., *H. vulgare*.

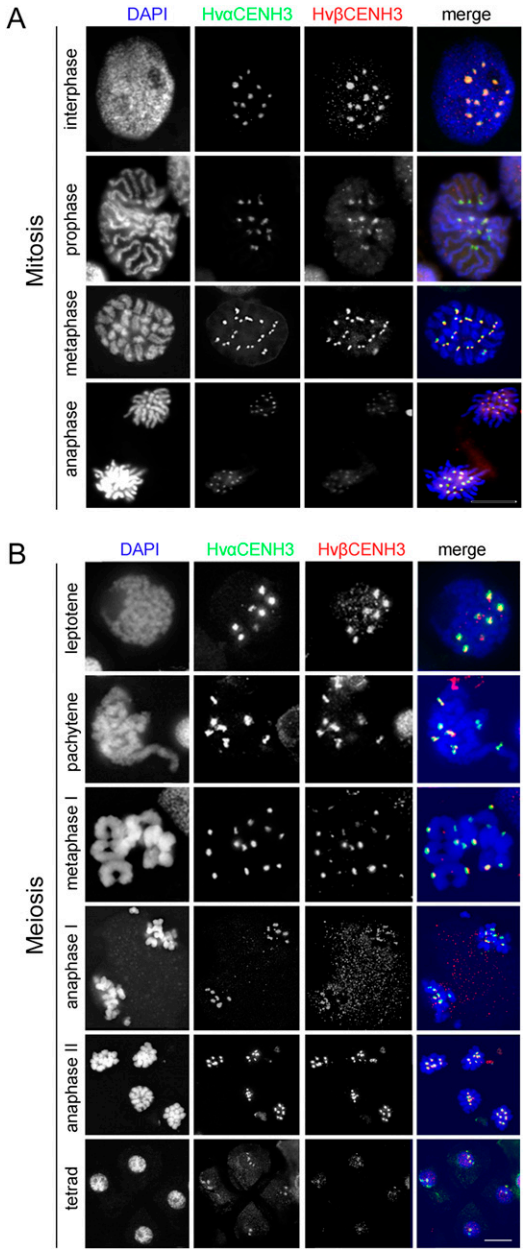

Fig. S6. Distribution of αCENH3 and βCENH3 at different stages of mitosis (*A*) and meiosis (*B*) in *H. vulgare* is demonstrated by immunostaining. (Scale bars: 10 μm.)

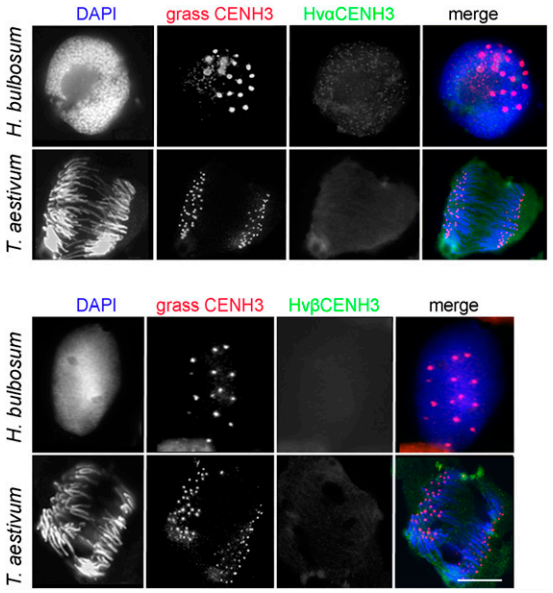

Fig. S7. Confirmation of anti-HvαCENH3 and anti-HvβCENH3 species specificity by indirect immunostaining of *H. bulbosum* and *T. aestivum* chromosomes. Anti-grass CENH3 was used as a positive control. (Scale bar: 10 μm.)

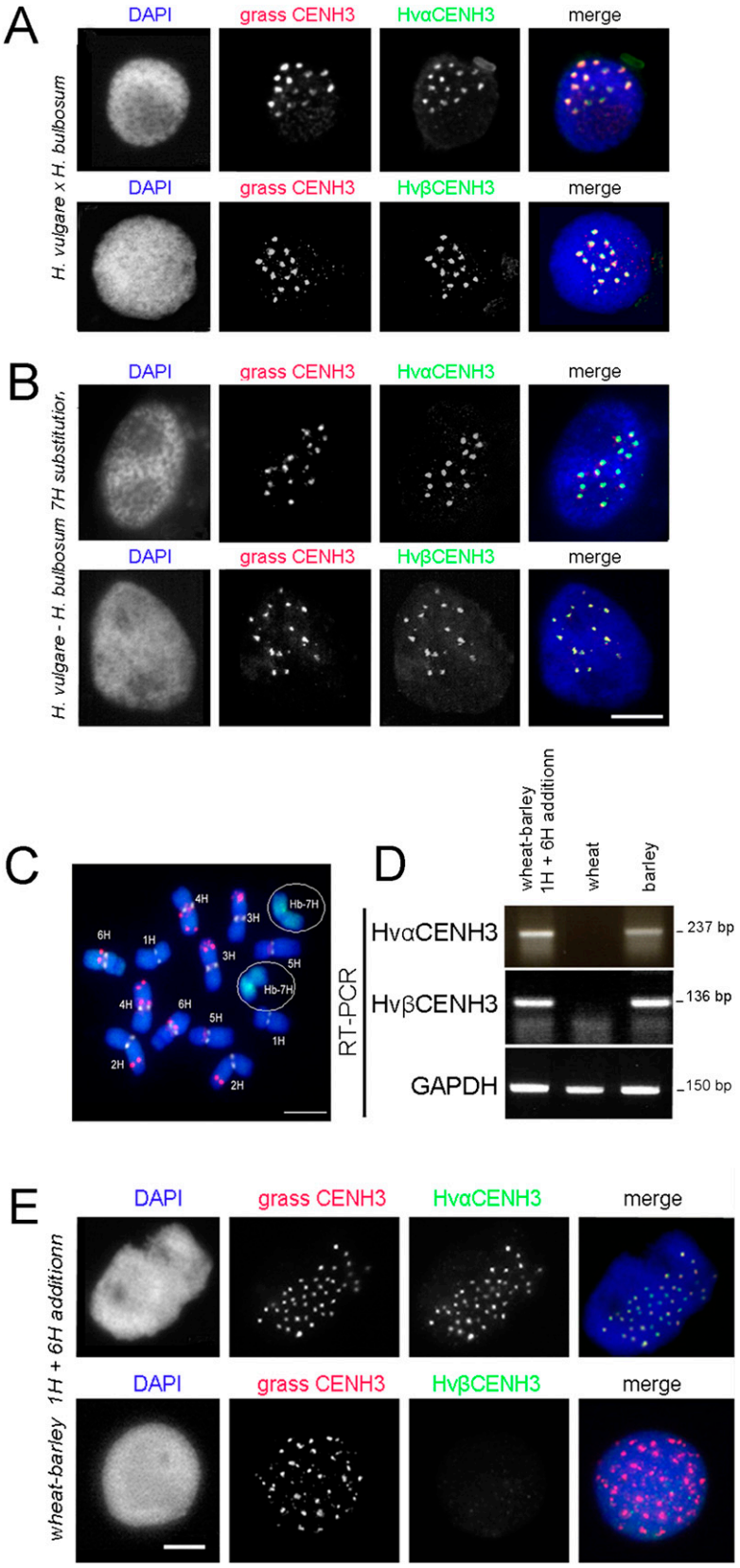

Fig. S8. Cross-species incorporation analysis of αCENH3 and βCENH3 of *H. vulgare* in a closely related species. All centromeres of stable *H. vulgare* × *H. bulbosum* hybrid nuclei (*A*) and of an *H. vulgare-H. bulbosum* 7H substitution incorporating αCENH3 and βCENH3 derived from *H. vulgare* (*B*) are shown. (*C*) Genotype confirmation of an *H. vulgare-H. bulbosum* 7H substitution line by in situ hybridization. Mitotic *H. vulgare-H. bulbosum* 7H substitution chromosomes after FISH using genomic *H. bulbosum* DNA (green); (AGGGAG)n, a barley-specific centromere repeat (yellow); and 5S rDNA (red) as probes. Circles

Legend continued on following page

indicate chromosome 7H of *H. bulbosum* (*D*) Transcription and cross-species incorporation analysis of αCENH3 and βCENH3 of *H. vulgare* in a wheat-barley double-disomic 1H + 6H addition line. RT-PCR demonstrates transcription of both *CENH3* variants of barley in the addition line. (*E*) All barley and wheat centromeres incorporate αCENH3 (*Upper*) but not βCENH3 (*Lower*) of *H. vulgare* despite transcription. Anti-grass CENH3 was used as an internal control. (Scale bar: 10 μm.)

Table S1. List of primers

No.	Name of primer	Sequence 5′—3′′	Annealing temperature, °C
1	Degenerate-F	GTRGCRCTGCGGGAGATCAGGA	68
2	Degenerate-R	CTBGCRAGYTGYATGTCCTTTT	61
3	Race-CENH3-F	GTGGCCACTGCGGGAGATCAGGAAGTACC	72
4	αHv + Hb-F	CGGGCACGTCCGAGACTCC	69
5	αHv + Hb-R	GTAGAATTCGGTGACCTCCTTGACC	66
6	βCENH3-F	ATGGCTCGCACGAAGAAAACGG	64.5
7	βCENH3-3′-R	GCAAAGGCCGAGAAGTCAGATG	64.5
8	αCENH3-F	AGAAGAAGATCGGGTCCGCTA	64.5
9	αCENH3-R	GTGCAAACGGGATGAGAAAATT	59
10	βCENH3-R	GTCGGCTTGCTCTCCTTCTTGTTCG	68
11	βHbCENH3-R	ATGGCGTCGGCTTGTTGGACCC	68
12	GAPDH-F	CAATGATAGCTGCACCACCAACTG	59
13	GAPDH-R	CTAGCTGCCCTTCCACCTCTCCA	59

F, forward; R, reverse.