FANCM promotes class I interfering crossovers and suppresses class II non-interfering crossovers in wheat meiosis

FANCM suppresses crossovers in plants by unwinding recombination intermediates. In wheat, crossovers are skewed toward the chromosome ends, thus limiting generation of novel allelic combinations. Here, we observe that FANCM maintains the obligate crossover in tetraploid and hexaploid wheat, thus ensuring that every chromosome pair exhibits at least one crossover, by localizing class I crossover protein HEI10 at pachytene. FANCM also suppresses class II crossovers that increased 2.6-fold in fancm msh5 quadruple mutants. These data are consistent with a role for FANCM in second-end capture of class I designated crossover sites, whilst FANCM is also required to promote formation of non-crossovers. In hexaploid wheat, genetic mapping reveals that crossovers increase by 31% in fancm compared to wild type, indicating that fancm could be an effective tool to accelerate breeding. Crossover rate differences in fancm correlate with wild type crossover distributions, suggesting that chromatin may influence the recombination landscape in similar ways in both wild type and fancm.
Meiotic recombination is initiated by numerous programmed DNA double-strand breaks (DSBs), catalyzed by SPO11 and MTPV1B2, that are repaired by homologous recombination into crossovers (COs) or non-crossovers (NCOs). COs are characterized by the reciprocal exchange of DNA between homologous chromosomes, whereas NCOs occur at sites of non-reciprocal DNA repair using either the homologue or the sister-chromatid as a template3. In Arabidopsis and wheat, COs form via the class I or class II pathways4–7. The class I pathway accounts for ~85% COs and is required to maintain CO assurance, thus formation of the obligate CO. The class I pathway ensures that each chromosome pair receives at least one CO through the action of meiosis specific proteins MER3/RCK, MSH4, MSH5, SHOC1, ZIP1, PTD and HEI108, thereby promoting correct chromosome segregation. Class I COs are interference-sensitive and therefore more likely to be spaced farther apart than would be expected by random chance4–10. Class II COs are interference-insensitive, accounting for ~15% COs and are partially dependent on the MUS81 endonuclease11,12.

The number of COs per chromosome is tightly constrained in the majority of eukaryotes (~1–3), regardless of physical chromosome size5,13. DSBs usually occur far in excess of COs in plants, suggesting that they do not limit CO formation. In hexaploid wheat the skewed ratio of ~2100 DSBs to ~42 COs per cell reveals that only 2% of potential repair sites mature into COs14, indicating that underlying mechanisms negatively regulate CO formation. In Arabidopsis, class I COs are limited by expression of HEI1015, the presence of PXX116, and the interaction of the synaptonemal complex transverse filament proteins ZYP1a/ZYP1b17,18. Class II COs are limited by three independent, anti-recombination pathways involving FANCM, FIDGETIN and RECQ419–22. The FANCM (Fanconi anemia complementation group M homolog) helicase functions with MHF1 and MHF2 to limit COs by unwinding inter-homolog repair intermediates, such as D-loops, and promotes repair as NCOs, in inbred lines19–21,23,24. Ablation of FANCM in Arabidopsis restores bivalent formation in class I CO deficient mutants to near wild-type levels19,20. Single fancm mutants exhibited a 3-fold increase in COs by pollen fluorescent marker analysis19, but chiasmata (the cytological manifestations of COs) were significantly reduced20, although technical limitations may have precluded detection of more than one closely spaced CO using this analysis. A significant reduction in the number of bivalents and loss of the obligate chiasma was recently observed in lettuce fancm mutants25, although Brassica (diploid and allotetraploid), rice (Oryza sativa) and pea (Pisum sativum) fancm mutants displayed increased COs26,27.

Wheat is an allopolyploid crop species where the predominantly distally distributed COs/chiasma rarely exceed two per chromosome pair7,14,28,29. COs mainly form in gene-rich regions30 that contain a high frequency of TIR-Mariner transposons31 and lower levels of DNA polymorphism32. COs also correlate with enrichment of the Polycomb histone modification H3K27me3 at the distal ends, indicating a potential role for facultative heterochromatin in shaping the recombination landscape33. CO frequency and distribution reduce the probability of creating advantageous novel allelic combinations in crop breeding programmes. Therefore, anti-CO factors, such as FANCM provide a potential way to overcome these limitations34. Here, using wheat fancm null mutants and VIGS, we demonstrate that class I COs are reduced in number, resulting in loss of the obligate CO, but this is offset by an increase in class II COs.

**Results**

**FANCM is conserved in wheat.** To identify FANCM orthologues in wheat, BLAST searches were performed using the Arabidopsis thaliana FANCM amino acid sequence. Two FANCM orthologues were identified in tetraploid wheat T. turgidum: TIFANCM-A1 (TRITD4Av1G171480) and TIFANCM-B1 (TRITD4Av1G035000). Three FANCM orthologues were identified in hexaploid wheat T. aestivum: TaFANCM-A1 (TraesCS4A02G217700), TaFANCM-B1 (TraesCS4B02G096400) and TaFANCM-D1 (TraesCS4D02G092800). All copies are full-length and predicted to produce functional proteins. FANCM is highly conserved between ploidy levels with FANCM-A1 and FANCMB-1 primary amino acid sequences being identical in tetraploid and hexaploid wheat (1447/1447 & 1458/1458, respectively), while the three FANCMB homologues, FANCM-A1, FANCMB-1 and FANCMB-D1, share 94.2% amino acid identity, with polymorphisms at 85 residues (1377/1462). A consensus sequence was created from the three wheat homologues to compare with Arabidopsis. Wheat FANCM shares 38.8% (543/1502) overall amino acid identity with Arabidopsis, with increased homology in the predicted DEXDc (DEAD-like helicases superfamily; 70.7%; 135/191) and HELICc (helicase superfamily c-terminal; 67.9%; 93/137) domains.

**FANCM is required for normal fertility.** Single fancm homo-elog mutants for tetraploid (Triticum turgidum, AABB) (Fig. 1a) and hexaploid wheat (Triticum aestivum, AABBD) (Fig. 1b) were crossed to create null knockout. The tetraploid Tifancm_1 null exhibited a 36% reduction in total number of seeds per plant from 66 ± 2 (n = 4) in wild type to 43 ± 5 (n = 4) in the mutant (p < 0.05, Mann–Whitney U Test; Supplementary Fig. 1). The hexaploid Tafancm null exhibited a 15% reduction in seeds per plant from 164 ± 4 (n = 6) in wild type to 139 ± 6 (n = 6) in the mutant (p < 0.05, Mann–Whitney U Test; Supplementary Fig. 1). Pollen viability decreased from 92% in wild type (n = 2042) to 76% in Tifancm_1 (n = 1994) (Supplementary Fig. 2). This indicates that defects occurring during gamete formation had a direct impact on seed development.

**FANCM is required for crossover assurance.** To determine the cause for the reduction in fertility and pollen viability in fancm null mutants, a cytological analysis was performed on tetraploid wheat meiocytes. At meiotic metaphase I (MI) the number of chiasma, rod and ring bivalents was indistinguishable between wild type and the single homoeolog mutants (Tifancm-A1_m1, Tifancm-A1_m2, and Tifancm-B1; p values > 0.05, Pairwise Wilcoxon Rank Sum Tests). However, in Tifancm_1 nulls, chromosome pairs without chiasma at MI revealed loss of the obligate chiasma and defective CO assurance (Fig. 1c,d, Supplementary Fig. 3). In Tifancm_1, 0.98 ± 0.09 (n = 104) pairs of univalents per cell were observed at MI, compared to only 0.05 ± 0.03 (n = 60) in wild type (p < 0.001, Pairwise Wilcoxon Rank Sum Test), and 64% of cells possessed at least one univalent pair in Tifancm_1, compared with only 5% in wild type. The number of rod bivalents per cell also increased from 1.7 ± 0.16 (n = 60) in wild type to 4.7 ± 0.09 (n = 104) in Tifancm_1 (p < 0.001, Pairwise Wilcoxon Rank Sum Test). The increase in univalent pairs and rod bivalents coincided with an 18% decrease in chiasma per cell, from 26 ± 0.2 (n = 60) in wild type to 22 ± 0.3 (n = 104) in Tifancm_1 (p < 0.001, Pairwise Wilcoxon Rank Sum Test). There was no significant difference between Tifancm_1 and Tifancm_2 null mutants at MI (p values > 0.05, Pairwise Wilcoxon Rank Sum Tests). Chromosome pairs without chiasma mis-segregated in Tifancm_1, resulting in unbalanced gametes (Supplementary Fig. 4). At anaphase I, chromosome mis-segregation was observed in 43% of cells in Tifancm_1 (n = 40), compared with 2.5% in wild type (n = 40). Chromosome mis-segregation through loss of the obligate
chiasma is the most likely cause for reduced pollen viability and seed production in fancm null mutants.

A comparative cytological analysis was then performed on fancm null mutants in hexaploid wheat. In Tafancm, 0.53 ± 0.09 (n = 81) univalents per cell were observed, compared to 0.01 ± 0.01 (n = 76) in wild type (p < 0.001 Pairwise Wilcoxon Rank Sum Test), and 37% of cells possessed at least one univalent pair, compared with 1.3% in wild type (Fig. 1c,d, Supplementary Fig. 3, Supplementary Data 3). The number of rod bivalents per cell also increased from 1.9 ± 0.16 (n = 76) in wild type to 4.6 ± 0.26 (n = 81) in Tafancm (p < 0.001, Mann–Whitney U Test). The increase in frequency of univalents and rod bivalents coincided with a 9% decrease in total chiasmata per cell, from 40 ± 0.2 (n = 76) in wild type to 36 ± 0.4 (n = 81) in Tafancm (p < 0.001, Mann–Whitney U Test), indicating that the requirement for FANCM in maintaining CO assurance is similar in both tetraploid and hexaploid wheat.

As the TILLING mutants have a high density of background mutations that may affect the role of FANCM based on previous studies investigating heterozygosity 21,24, TaFANCM–Virus Induced Gene Silencing (VIGS) was performed on hexaploid wheat. Plants inoculated with the Barley Stripe Mosaic Virus: TaFANCM-i construct exhibited 0.58 ± 0.14 (n = 33) univalents per cell, compared to 0.02 ± 0.02 (n = 50) in the empty virus control (p < 0.001, Pairwise Wilcoxon Rank Sum Test), and 40% of cells possessed at least one univalent pair, compared with 2% in the control (Fig. 1c,d, Supplementary Fig. 3, Supplementary Data 3). The number of rod bivalents also increased from 1.56 ± 0.18 (n = 50) in the control to 4.24 ± 0.33 (n = 33) in BSMV:TaFANCM-i (p < 0.001, Pairwise Wilcoxon Rank Sum Test). The increase in frequency of univalents and rod bivalents coincided with a 9% decrease in total chiasmata per cell, from 42 ± 0.2 (n = 50) in the control to 38 ± 0.5 (n = 33) in BSMV:TaFANCM-i (p < 0.001, Pairwise Wilcoxon Rank Sum Test). The same effect was observed in a second, independent construct (BSMV:TaFANCM-ii) where there was no significant difference with construct (i) at MI (p values > 0.05, Pairwise Wilcoxon Rank Sum Tests), indicating that the VIGS and TILLING mutants reproduced the same phenotype for chiasma formation.

The distribution of remaining chiasmata in fancm null mutants, while still deviating significantly from a Poisson distribution ($\chi^2_{(26)} = 38.9, n = 104, p < 0.05$; Fig. 3b), spanned a greater range than wild type (23–28 WT vs 16–27 Ttfancm_1), thus implying a defect in CO control. Therefore, an analysis utilizing pSc119.2-2, 5S rDNA and 45S rDNA synthetic oligonucleotide probes was performed on Ttfancm_2 MI...
chromosomes to determine if there was a pattern in the reduction of chiasmata. Chromosomes 1B and 6B possess 45 S rDNA sites that form the Nucleolar Organization Regions (NOR) and can therefore be reliably identified<sup>2,35</sup> (Supplementary Fig. 5). Chromosomes 1B and 6B exhibited a significant reduction in the number of chiasmata per chromosome in *Tifancm_2* compared to wild type (1.7 ± 0.07, *n* = 45 to 1.3 ± 0.11, *n* = 40 on 1B (*p* < 0.01, Mann–Whitney U Test) and 1.8 ± 0.06, *n* = 45 to 1.3 ± 0.11, *n* = 40 on 6B (*p* < 0.001, Mann–Whitney U Test; Supplementary Fig. 5)). The Fluorescence in situ hybridization (FISH) analysis also revealed a chromosomal bias in chiasmata distribution in the wild type, as chromosomes 1B and 6B were overrepresented among rod bivalents. They accounted for 27%, indicating that chromosomes with NORs were more likely to form a single chiasma than other chromosomes (*χ<sup>2</sup>* = 13.16, *p* < 0.01). Chromosomes 1B and 6B were also overrepresented among the univalent pairs in the *Tifancm_2* null mutant (*χ<sup>2</sup>* = 21.07, *p* < 0.01), accounting for 44% of all observed. However, the overall reduction in chiasmata in *Tifancm_2* occurred throughout all chromosomes and 1B and 6B did not significantly deviate from expected values (*χ<sup>2</sup>* = 5.83, *p* > 0.05).

Therefore, a direct consequence of the *fancm* null mutant appears to be a reduced ability of the NOR chromosomes to buffer against a general loss of chiasma.

**FANCM is required for localization of HEI10 at late prophase I.**

As CO assurance was abrogated in the *fancm* null mutants, localization dynamics of the class I CO protein HEI10 were investigated. HEI10 initially localizes as small, numerous, axis-associated foci during leptotene in Arabidopsis and wheat, that coarsen into fewer, larger foci, that mark class I CO sites at late prophase <sup>29,36,37</sup>. At leptotene, the number of HEI10 foci per meioocyte was not significantly different between *Tifancm_1* (356 ± 32, *n* = 7) and wild type (351 ± 25, *n* = 7) (*p* > 0.05, Mann–Whitney U Test) (Supplementary Fig. 6). At zygote, the number of HEI10 foci was not significantly different between *Tifancm_1* (156 ± 14, *n* = 7) and wild type (156 ± 13, *n* = 9) (*p* > 0.05, Mann–Whitney U Test) (Supplementary Fig. 6). However, at pachytene HEI10 foci decreased by 20% from 39 ± 0.8 (n = 82) in wild type to 32 ± 0.5 (n = 74) in *Tifancm_1* (Mann–Whitney U Test, *p* < 0.001, Fig. 2).

Furthermore, at diakinesis HEI10 foci reduced by 29%, from 34 ± 0.5 (n = 54) in wild type to 24 ± 0.5 (n = 60) in *Tifancm_1* (Mann–Whitney U Test; *p* < 0.001) (Fig. 2) and this also occurred in hexaploid wheat. At pachytene the number of HEI10 foci per meioocyte decreased by 9%, from 58 ± 0.6 (n = 54) in wild type to 53 ± 1.2 (n = 34) in the *Tifancm* null (*p* < 0.001, Mann–Whitney U Test; Fig. 2). This reduction in HEI10 foci at pachytene suggests that formation of a proportion of class I COs is sensitive to the loss of FANCM in wheat.

**DSBs, meiotic progression, and SC formation appear normal in *fancm*.** As a proxy marker for DSB formation during meiosis, localization of the strand-exchange protein RAD51<sup>36</sup> was scored during leptotene. The number of RAD51 foci was not significantly different between *Tifancm_1* (1397 ± 21, *n* = 5) and wild type (1403 ± 24, *n* = 5) (*p* > 0.05, Mann–Whitney U Test) (Supplementary Fig. 7), indicating no apparent effect on the number of early recombination sites. MSH5, an early class I CO recombination protein<sup>7,39</sup> localized to unsynapsed and synapsed chromosomes at zygote, with no significant difference in the number of foci between *Tifancm_1* (336 ± 14 *n* = 5) and wild type (378 ± 22, *n* = 5) (*p* > 0.05, Mann–Whitney U Test) (Supplementary Fig. 8).

Fixed anthers of equivalent sizes from wild type and *Tifancm_1* corresponded to the same meiotic stages, indicating that there was no delay in meiotic progression. In both cases, anthers 0.6–0.7 mm in length contained cells at leptotene, 0.8 mm at zygote, 0.9 mm at pachytene, 0.95–1.1 mm at MI, 1.1 mm at dyad, and 1.3 mm at tetrad. Furthermore, immunolocalization of ASYNAPSIS1 (ASY1)<sup>40</sup> and ZYP1<sup>41</sup> revealed normal axis formation and synopsis in *Tifancm_1*, as previously described for wild type<sup>27,29,42</sup>. In wild type and *Tifancm_1*, ASY1 formed a linear signal on the unsynapsed chromosome axes at leptotene, which depleted during zygote on synapsed chromosomes (Supplementary Fig. 9). ZYP1 was initially detected during late-leptotene as presynaptic foci that extended throughout zygote until a complete linear signal was observed along synapsed chromosomes at pachytene. These data indicate that early stages of meiotic recombination are unperturbed in the *fancm* null mutants.

**FANCM limits class II crossovers.** *TtMsh5B* possesses a natural loss-of-function deletion mutation and therefore the single *TtMsh5a* mutant produces a null allele of MutSy that is defective in class I COs<sup>7</sup>. The *TtMsh5* null was crossed with the *Tifancm_1* null to create the quadruple null *TtMsh5 Tifancm* to investigate whether *fancm* mutants can compensate for loss of class I COs. In *TtMsh5*, 4.29 ± 0.13 (n = 194) bivalents per cell were observed, which increased 2.6-fold to 9.34 ± 0.24 (n = 86) in *TtMsh5 Tifancm* (*p* < 0.001, Pairwise Wilcoxon Rank Sum Test). Furthermore, ring bivalents accounted for only 3.2% of chromosome pairs in *TtMsh5*, which significantly increased to 21% in *TtMsh5 Tifancm* (*p* < 0.001, Pairwise Wilcoxon Rank Sum Test). The increase in ring bivalents coincided with a 2.6-fold increase in the number of chiasmata per cell, from 4.7 ± 0.15 (n = 194) in *TtMsh5* to 12.4 ± 0.41 (n = 86) in *TtMsh5 Tifancm* (*p* < 0.001, Pairwise Wilcoxon Rank Sum Test; Fig. 1c,d, Supplementary Fig. 3, Supplementary Data 3). The additional chiasma in *TtMsh5 Tifancm* did not deviate significantly from a Poisson-predicted distribution (*χ<sup>2</sup>* = 9.74, *n* = 86, *p* > 0.05; Fig. 3d), implying that they formed via the interference-insensitive class II CO pathway.

**FANCM localizes to meiotic chromosomes during prophase I.** To gain further insight into the role of FANCM during meiotic recombination, a wheat FANCM antibody was generated and its specificity verified by immunolocalisation. FANCM was first detected at late-leptotene as numerous (991 ± 42, *n* = 5), small (0.4 µm ± 0.02, *n* = 50), axis-associated foci (Fig. 4a). By early-zygote fewer (20 ± 1.3, *n* = 22), but larger (0.7 µm ± 0.02, *n* = 50) distinct FANCM foci were observed of which 58% (254/441) associated with ASY1 and 42% (187/441) with ZYP1 (Fig. 4b), indicating localization to synapsed and unsynapsed chromosomes. By mid-zygote, the number of large FANCM foci peaked at 29 ± 1.5 (n = 29), which is similar to the number of designated class I CO sites<sup>7</sup> (Fig. 4c). FANCM foci then decreased to 18 ± 1.8 (n = 11) at late-zygote (Fig. 4d), and then 12 ± 1.0 (n = 12) by pachytene (Fig. 4e). When co-immunostained with anti-TaASY1, anti-TaFANCM also gave a linear signal to unsynapsed chromosomes, which was not present when used exclusively or in combination with other antibodies (e.g., anti-HvHEI10, anti-AzYPI), indicating that cross-reactivity with ASY1 may be responsible for the linear signal. Furthermore, the linear signal was present in the *Tifancm_1* null mutant, whereas the FANCM foci were absent (Fig. 4f).

As the number of zygote FANCM foci correlated with the number of designated class I CO sites, FANCM was co-immunostained with HEI10. Due to difficulties staging the meiocytes without ASY1, an alternative anti-HvHEI10 was used which produces a linear signal on unsynapsed chromosomes and forms prominent foci on synapsed chromosomes<sup>7</sup>. As the HEI10
**Fig. 2** Class I crossover recombination protein HEI10 is reduced in fancm null mutants at late prophase I. a–f Co-immunofluorescence of HEI10 (white) and ZYP1 (red) on meiotic prophase I chromosome spreads. Representative micrographs are shown from replicates, (a) (n = 82), (b) (n = 74), (c) (n = 54), (d) (n = 60), (e) (n = 54), and (f) (n = 34). Pachytene (a, c, e and f) and early diakinesis (b and d). Scale bars = 10 µm. g Counts of HEI10 foci per cell with mean values ± SD. The number of meiocytes sampled for each line is shown in brackets. *** = p < 0.001 (Mann-Whitney U Test). Source data are provided as a Source Data file.

**Fig. 3** FANCM limits class II crossovers. a–d Observed and Poisson-predicted distributions of chiasma frequency per cell. a The observed wild-type distribution of chiasmata deviates significantly from a Poisson-predicted distribution ($\chi^2_{(28)} = 113.16$, n = 60, $p < 0.01$, Chi-squared test). b The distribution of chiasmata in Tfancm_1 deviates significantly from a Poisson-predicted distribution ($\chi^2_{(26)} = 38.9$, n = 104, $p < 0.05$, Chi-squared test). c The distribution of chiasmata in Tmsh5 does not deviate significantly from a Poisson-predicted distribution ($\chi^2_{(14)} = 5.63$, n = 194, $p \geq 0.975$, Chi-squared test). d The distribution of chiasmata in Tmsh5 Tfancm_1 does not deviate significantly from a Poisson-predicted distribution ($\chi^2_{(20)} = 9.74$, n = 86, $p > 0.95$, Chi-squared test). Source data are provided as a Source Data file.
Fig. 4 FANCM localizes to meiotic chromosomes at prophase I. a–g Co-immunofluorescence of FANCM (red), ASY1 (green) and ZYP1 (blue) on meiotic prophase I chromosome spreads. h Co-immunofluorescence of FANCM (red), HEI10 (green) and ZYP1 (blue) on meiotic chromosome spreads at mid-zygotene. Representative micrographs are shown from replicates, (a) \(n = 5\), (b) \(n = 22\), (c) \(n = 29\), (d) \(n = 11\), (e) \(n = 12\), (f) \(n = 16\), (g) \(n = 20\), (h) \(n = 5\). Insets, 2× magnified view of outlined region, showing co-localization of FANCM and HEI10 foci on synapsed chromosomes. Scale bars = 10 µm.
foci on unsynapsed chromosomes were obscured by the non-specific linear signal, only FANCM foci localizing on synapsed chromosomes were scored. A mean of 14 ± 2.7 (n = 5) FANCM foci per cell were observed of which 8 ± 2.2 (59%) overlapped with emergent HEI10 foci (Fig. 4h). Furthermore, FANCM immunolocalisation to class I-deficient mutant Ttmsh5 displayed a 51% reduction in the number of FANCM foci at mid-zygotene, from 29 ± 1.5 (n = 29) in wild type to 15 ± 0.8 (n = 20) in Ttmsh5 (Fig. 4g). Taken together, these data are consistent with 51–59% FANCM foci being present at designated class I CO sites and promoting class I interfering COs, while 41–49% FANCM foci are located at potential NCO sites.

Genetic mapping reveals an overall increase in crossovers in fancm mutants. Genetic mapping using molecular marker analysis was performed on segregating tetraploid and hexaploid fancm null mutants as an additional tool to measure recombination frequency and distribution. The hexaploid wheat Cadenza fancm null mutant was crossed with Avalon to produce F2 plants and the F3 segregants were used for genetic map construction (Fig. 5a). Segregating Cadenza/Avalon genetic markers on the 35 K Axiom chip provided 21 intervals on ten chromosomes that were tested for recombination using a LOD 8 threshold. This revealed an increase in COs (>10%) in 11 intervals, a reduction in five (>–10%) and no difference in 4 (between 10% and –10%) (Table 1, Fig. 5b, Supplementary Data 4, 5 & 6). Chromosome 1A exhibited the largest increase in genetic map length in the fancm null mutant and is shown as an example (Fig. 5c). The map length was increased by 19.1% in fancm over WT when adding the cM distances across all regions, or by 31.3% when averaging the percentage cM increase from the 21 intervals.

Recombination in tetraploid wheat fancm mutants was measured with KASP markers designed against mapped segregating SNPs from the mutation donor lines. Four F3 populations, two homozygous WT at FANCM and two fixed homozygous for the mutations, consisting of 95 plants, were tested across seven genetic intervals on 5 chromosomes. Recombination was increased in the chromosome 1B interval but decreased within the 2A, 2B and 3A intervals, but were the same in another 3A interval and 3B (Table 2, Supplementary Data 7). The trend was an overall decrease, but statistically the mutant and wild type were indistinguishable in genetic map length for the intervals tested.

Crossover rate differences in fancm correlate wild type crossover distributions. Genomic regional variation in CO rate differences between fancm and wild type shows a significant positive correlation with a previously published wild type CO rate map.
Table 1 Recombination analysis in the hexaploid Avalon x Cadenza F2 fancm mutant mapping population.

| Chr. | Markers | Marker region         | Position       | Control | fancm     | Delta | % Difference |
|------|---------|-----------------------|----------------|---------|-----------|-------|--------------|
| 1A_1 | 162     | AX-94733072 - AX-94752690 | 2378195-332208434 | 93.76   | 126.60    | 32.84 | 35.0%        |
| 1A_2 | 12      | AX-94874424 - AX-95152377 | 5484944355-586914258 | 3.33    | 11.44     | 8.11  | 243.3%       |
| 1B_1 | 116     | AX-95025932 - AX-95731046 | 571060391-645252540 | 67.91   | 83.51     | 15.61 | 23.0%        |
| 1B_2 | 13      | AX-94813152 - AX-94804524 | 658531550-662842760 | 12.24   | 10.66     | -1.58 | -12.9%       |
| 2B   | 63      | AX-95630341 - AX-94643790 | 2177758-52907517 | 46.89   | 40.27     | -6.62 | -14.1%       |
| 3B_1 | 19      | AX-94653790 - AX-94633883 | 655306481-672099880 | 11.79   | 18.27     | 6.48  | 55.0%        |
| 3B_2 | 17      | AX-95427865 - AX-94990660 | 82107029-829286625 | 7.34    | 11.99     | 4.65  | 63.3%        |
| 3D_1 | 15      | AX-94534357 - AX-94667914 | 132604563-240524132 | 37.08   | 27.06     | -10.02 | -27.0%       |
| 3D_2 | 9       | AX-95010529 - AX-95272113 | 564307274-574193365 | 19.87   | 20.92     | 1.05  | 5.3%         |
| 4A_1 | 10      | AX-9519296 - AX-95990960 | 626191038-649686665 | 14.14   | 13.24     | -0.80 | -12.7%       |
| 4A_2 | 4       | AX-94775503 - AX-94877844 | 713251841-723516355 | 10.52   | 20.58     | 10.06 | 95.6%        |
| 4A_3 | 32      | AX-9513315 - AX-94555122 | 282124614-742002004 | 28.21   | 29.18     | 0.97  | 3.4%         |
| 5A_1 | 25      | AX-94654340 - AX-95257149 | 607681540-619778700 | 5.34    | 9.57      | 4.24  | 79.4%        |
| 5A_2 | 24      | AX-94995752 - AX-95230303 | 676592388-685543180 | 10.22   | 8.99      | -0.33 | -3.2%        |
| 5B_1 | 13      | AX-95106649 - AX-95258242 | 535359866-544608954 | 20.53   | 4.51      | -16.02 | -78.0%       |
| 5B_2 | 74      | AX-95151783 - AX-94657489 | 584127473-633943938 | 20.12   | 23.19     | 3.08  | 15.3%        |
| 5B_3 | 14      | AX-94885467 - AX-94743639 | 693674319-708229373 | 18.74   | 20.17     | 1.43  | 7.6%         |
| 7A_1 | 48      | AX-94485699 - AX-94664110 | 1704442-20294508 | 36.84   | 38.44     | 1.60  | 4.4%         |
| 7A_2 | 30      | AX-94802515 - AX-9505573 | 61887949-83631459 | 22.92   | 26.30     | 3.38  | 14.8%        |
| 7B_1 | 22      | AX-94733039 - AX-95191125 | 3338679-21668399 | 26.66   | 32.04     | 5.38  | 20.2%        |
| 7B_2 | 48      | AX-94582074 - AX-95104628 | 724954096-750605855 | 29.68   | 70.99     | 41.31 | 139.2%       |
In tetraploid wheat, ~29 FANCM foci coarsened during zygotene of which ~51–59% localized with HEI10. In the absence of FANCM, HEI10 foci reduced from 34 to 24 per nucleus at diakinesis, suggesting that FANCM is required to promote 29% of class I COs. Our data are consistent with a model in which FANCM facilitates second-end capture at class I CO sites. FANCM may release the DSB second-end from association with the sister-chromatid, or other homologous/homeologous templates, similarly to budding yeast, but at a later stage. This model is consistent with current data from rice where FANCM interacts with RPA1a during meiosis to promote CO formation36, and in Arabidopsis RPA1a plays a crucial role in second-end capture37. Our data supports the molecular function of FANCM as a DNA helicase that unwinds strand invasion D-loop intermediates during zygotene. The requirement for FANCM to function at a subset of designated class I CO sites may be due to a stochastic propensity for DSB second-ends to invade the sister chromatid, rather than the homolog. This would also imply that CO site designation may occur at the D-loop stage in wheat.

A role for FANCM in limiting the class II CO pathway was revealed by crossing the mutant with the class I CO mutant msh57. Chiasmata increased over ~2.6-fold in the msh5/fanCM quadruple mutant compared to the msh5 mutant, although this was insufficient to restore CO levels, as reported in Arabidopsis19,20. Therefore, FANCM may unwind a small proportion of D-loop intermediates between homologous chromosomes that were destined to form NCOs, but in its absence may be processed by the class II CO pathway.

These data suggest that FANCM reduces the probability of non-interfering class II CO’s from forming, whilst promoting interfering class I CO’s, thus producing a landscape where COs are spaced further apart. FANCM appears to ensure the fate of a subset of recombination intermediates, rather than being involved in CO interference per se, but a recent analysis of the role of FANCM and its interacting partner FANCD2 in Arabidopsis suggests that it directly promotes interference of class I COs, based on HEI10 foci frequency and distribution25. However, due to the significant reduction of HEI10 foci in wheat, this effect may be obscured, but still active.

The data presented here suggest that FANCM is required at CO designated sites/NCOs during zygotene. In wild type tetraploid wheat, the ~10-fold excess of HEI10 foci at mid-zygotene (351) compared to the eventual (34) foci at diakinesis superficially seems to be sufficient to enable CO assurance. However, in the absence of the 29 FANCM foci (of which ~51–59% localized with HEI10), the eventual HEI10 foci reduced to 24 at diakinesis in the mutant. Therefore, these data are consistent with class I CO designation occurring before FANCM coarsens at mid-zygotene, as the majority of the remaining HEI10 were unable to compensate. Recent studies have shown that CO interference is mediated by ZYP1 in Arabidopsis17,18, although modelling of CO designation with the coarsening of HEI10 foci occurring during mid/late prophase I may also play a role37. The FANCM data here indicates that CO designation occurs earlier than mid-zygotene, consistent with budding yeast3. This also raises the question of how FANCM is regulated to function at both designated class I CO sites and non-designated sites to ensure the optimum fate of recombination intermediates.

The wheat fanCM mutants exhibited a reduction in fertility (36% in tetraploid and 15% in hexaploid wheat), due to the loss of the obligate chiasma. This is consistent with a previous study utilizing FANCM-VIGS on tetraploid wheat F1 hybrids that showed a significant reduction in fertility, but no differences in recombination on chromosome 1A38. The immunohistochemistry, cytological and molecular marker analysis presented here reveal that loss of class I COs are offset by an increase of class II COs in the tetraploid, thus resulting in no net change. However, fewer HEI10 foci were reduced in hexaploid wheat fanCM mutants, compared to the tetraploid, leading to an overall increase of COs by 31%. Based on an increase of class II chiasmata detected in the tetraploid, it is likely that the increase in COs in the hexaploid arose via the class II CO pathway. The molecular marker data revealed that COs increased in the majority of intervals tested, thus providing an opportunity to modulate recombination in wheat breeding programs. The distal H3K27me3-enriched regions experienced the greatest increase in COs, whereas the interstitialial proximal regions were more likely to be lower, similar to recombination increases in the barley rec8 mutant39.

**Methods**

**Identification of wheat FANCM**. Wheat FANCM orthologues were identified using the Arabidopsis thaliana amino acid sequences (AT5g53360) against publicly available databases: Triticum turgidum50, Svevo.v1.1 https://plants.ensembl.org/Triticum_turgidum and T. aestivum50. IWGSC https://plants.ensembl.org/Triticum_aestivum. Wheat cds were aligned using the Clustal W algorithm (gap open cost = 12, gap extend cost = 3), translated, and the primary sequence ran through the Conserved Domain Database (NCBI).

**Plant material.** Triticum turgidum cv. ‘Kronos’ was used as a wild-type control for experiments involving the tetraploid Kronos TILLING mutant lines obtained from www.SeedStor.ac.uk51. We selected knockout mutants (premature termination codon; Supplementary Data 1) in TifFANCM-A1 (TRITD4A41G17480) exon 2 (Kronos3873; abbreviated K3873 hereafter) and exon 8 (K0234). Similarly, we selected a knockout mutant (K3842) in the third exon of TifFANCM-B1 (TRITD4B1G135000). We confirmed the mutations via genotyping (Supplementary Data 2) and named the single mutants as TifFANCM-A1_m (K3873), TifFANCM-A1_m2 (K0234), and TifFANCM-B1 (K3842). We also generated double null mutants: TifFANCM-1 (K3873 x K3842) and TifFANCM-2 (K0234 x K3842) and characterized a Tms5-A1 mutant (K663), which is functionally a null (Tms5-A1) as there is a natural loss-of-function deletion in TMS5-B1 (Desjardins et al. 2020), and a Tms5-A1 double mutant (K3873 x K3842 x K663). Triticum aestivum cv. ‘Cadenza’ was used as a wild-type control for experiments involving hexaploid Cadenza TILLING mutant lines. We selected knockout mutants in all three TaFANCM homologues including TaFANCM-A1 (Traces4SA02G217700; CadenzaT061, abbreviated I(C061 hereafter), TaFANCM-B1 (Traces4SA02G597600; C1195) and TaFANCM-D1 (Traces4SA02G598200; C0827). The single mutants were crossed to generate a TafanCM null mutant (C0641 x C1195 x C0827). Plants were grown under controlled environmental growth conditions: photo-period 16 h, temperature 21 °C (day)/16 °C (night) and relative humidity ~60%.

**VIGS experiments.** VIGS experiments were conducted in a Level 3 biological containment facility at Rothamsted Research.

**RT-PCR.** Total RNA was extracted from T. turgidum cv. ‘Cronos’ and T. aestivum cv. ‘Cadenza’ spikes using ISOLATE II RNA Mini Kit (Bioline), and cDNA was synthesized with the Tetro cDNA synthesis kit (Bioline). The coding sequences for the two varieties were amplified and sequenced using homoeolog-specific primers: TA_F_5′-ATGTGGTTGCTTTCAGAGGTA-3′ and TaFANCM-B1_R_5′-ATGTTGGTTGCTTTCAGAGGTA-3′, FANCM_B_F_5′-AGAGGCTATGGTTCTACATCCC-3′ and FANCM_B_R_5′-GATCTGATGTATGTTCTACATCCC-3′, and TaFANCM-vigs-f5-5′-AAAGGAGGAAGGTGGGAAAG-3′ and TaFANCM-D_F_5′-CCAGCCAGAGCATGATACCT-3′. Wheat FANCM orthologues were identified using the Clustal W algorithm (gap open cost = 12, gap extend cost = 3). The coding sequences for both varieties were amplified and sequenced using homoeolog-specific primers: FANCM_A_F_5′-AAGGAGGTTTAAGGTAGCTATACAGG-3′ and TaFANCM-vigs-i_F_5′-AACCACACACGCCTACATCCAGG-3′, and TaFANCM-vigs-i_R_5′-AACCACACACGCCTACATCCAGG-3′. Plants were grown in 3:1 (v/v) ethanol: acetic acid at 14-days post-infected sap at the 4–4.5 leaf stage (~28 days post-sowing)35. For cytological analysis, anthers were dissected and fixed in 3:1 (v/v) ethanol: acetate at 14-days post-infection.
FANCM antibody production. Primers Abf 5′-AGCA-TATGTGGGTGGCAGCTGGTGTG-3′ and Abf 5′-TCTCGGA-GATTGTTGGTGGCAGCTGGTGTG-3′ were used to amplify 1107–1395 bp of the TraesCS4B02G096500 (FANCM-B1) coding region with Q5 DNA polymerase (NEB). The PCR product was ligated into pDrive (Qiagen) and sequenced (Eurofins). pDrive was then digested with NdeI and XhoI, and the insert was ligated into pET21b (Merck-Millipore). Transformed BL21 (DE3) cells expressed a 32 kDa recombinant protein that was used to produce a rat anti-FANCM antibody (DC Biosciences).

Fertility assessment. Fertility was assessed in Tifacm_1 and Tifacm null mutants by counting the total number of seeds per plant and comparing with wild type using Mann–Whitney U tests (Minitab v 18.1.0.0). The ratio of viable to non-viable pollen grains was also determined for Tifacm_1 using Alexander staining25.

Cytological procedures. Meiotic chromosome spreads were prepared from fixed anthers. Following digestion (30–60 min at 37°C) in citrate buffer containing 0.33% cellulose (Duchefa Biochemie) and 0.33% pectolytic (PM Biomedicals), the anthers were macerated in a drop of water, spread in 60–80% acetic acid at 45°C, and refixed in 3:1 (v/v) ethanol:acetic acid24,26. Slides were mounted in VECTASHEILD Mounting Medium with DAPI (Vector Laboratories). Fluorescence in situ hybridization (FISH) was conducted using three labelled synthetic oligonucleotides: pSc119.2-2 [A647]35, pTAT749-1 (5′-TTdRd7 and pTA71-4 (5′-A647)35. Nikon Ni-E and Eclipse Ci microscopy microscopes equipped with NIS elements software were used to capture and quantify images. Chiasmata number was scored in situ hybridization (FISH) was conducted using three labelled synthetic oligo-}

sibrings fixed as either homozygous wild type for the three FANCM homoelogues, or homozygous for all three mutations at fancm were self-pollinated to obtain F1 seed. F1 populations of 50 and 100 plants respectively, were sown from populations arising from fixed WT FANCM and triple mutant fancm F1 siblings. F3 seed from an Avalon x Cadenza cross were germinated and grown in pots filled with peat-based soil and kept in a glasshouse at 15–18°C with 16 h light, 8 h dark. Leaf-tissue was harvested from individual plants 14 days post-sowing, when the plants were at an early flowering stage. DNA was extracted following the protocol with minor modifications. DNA concentration was assessed using a Qubit 2.0 Fluorometer and then normalized to 23 ng/μl ready for analysis with the Axiom® Wheat Breeder’s array37. Sample preparation for array genotyping was performed with the Beckman Coulter Biomek FX. Samples were then genotyped using the Axiom® 35 K Wheat Breeder array in conjunction with the GeneTitan® using standard Affy-}

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**Author contributions**
S.D.D., J.S., I.G., A.J.B., and K.K. performed the research; J.D.H., S.D.D., J.S., C.U., and K.J.E. designed the study; J.D.H., S.D.D., A.J.T., I.R.H., J.S., C.U., and K.J.E. analyzed the data; S.D.D., A.J.T., E.S.M., F.C.H.F., I.R.H., C.U., K.J.E., and J.D.H. wrote the paper. J.D.H. agrees to serve as the author responsible for contact and ensures communication.

**Competing interests**
The authors declare no competing interests.

**Additional information**
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