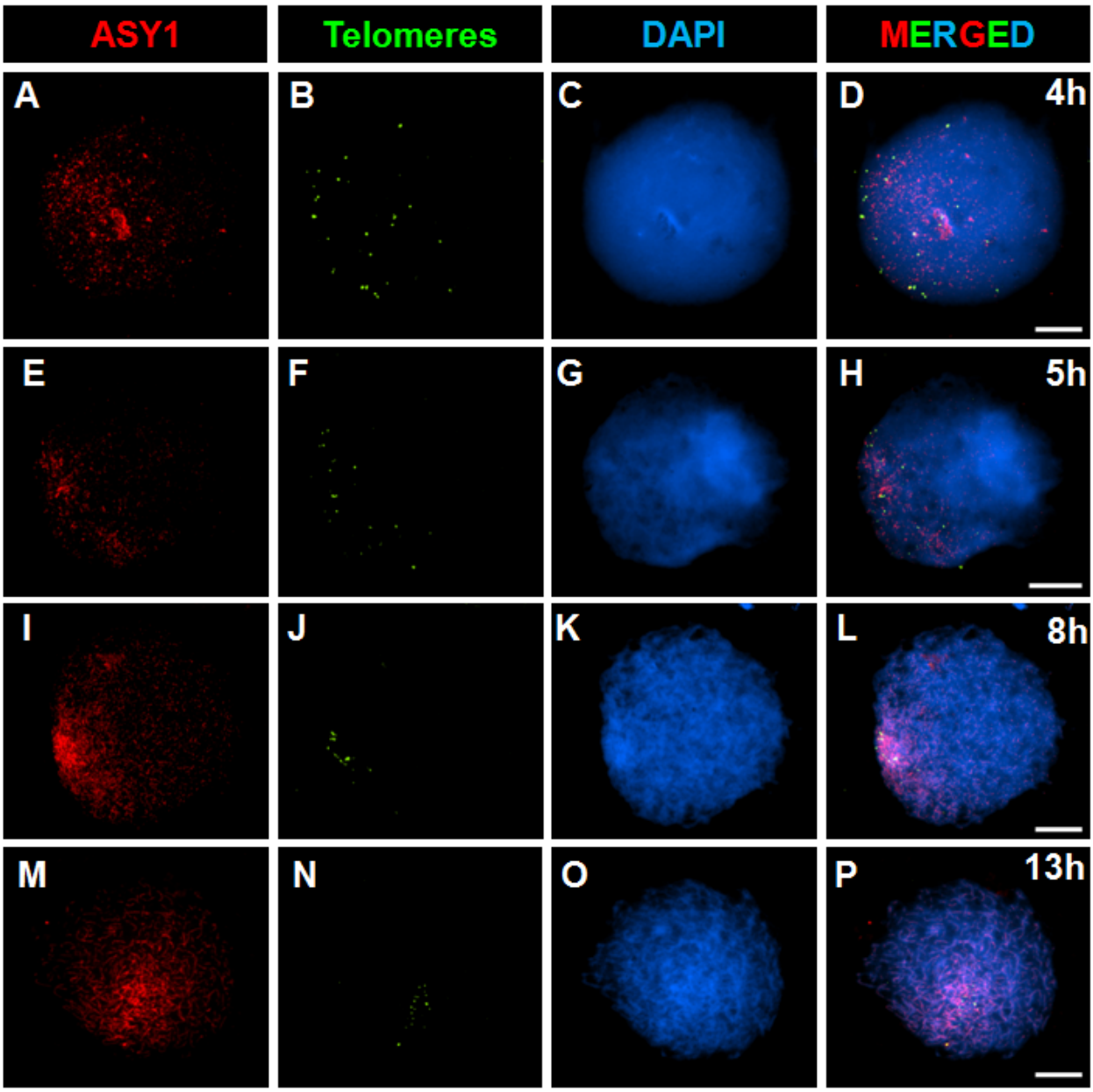
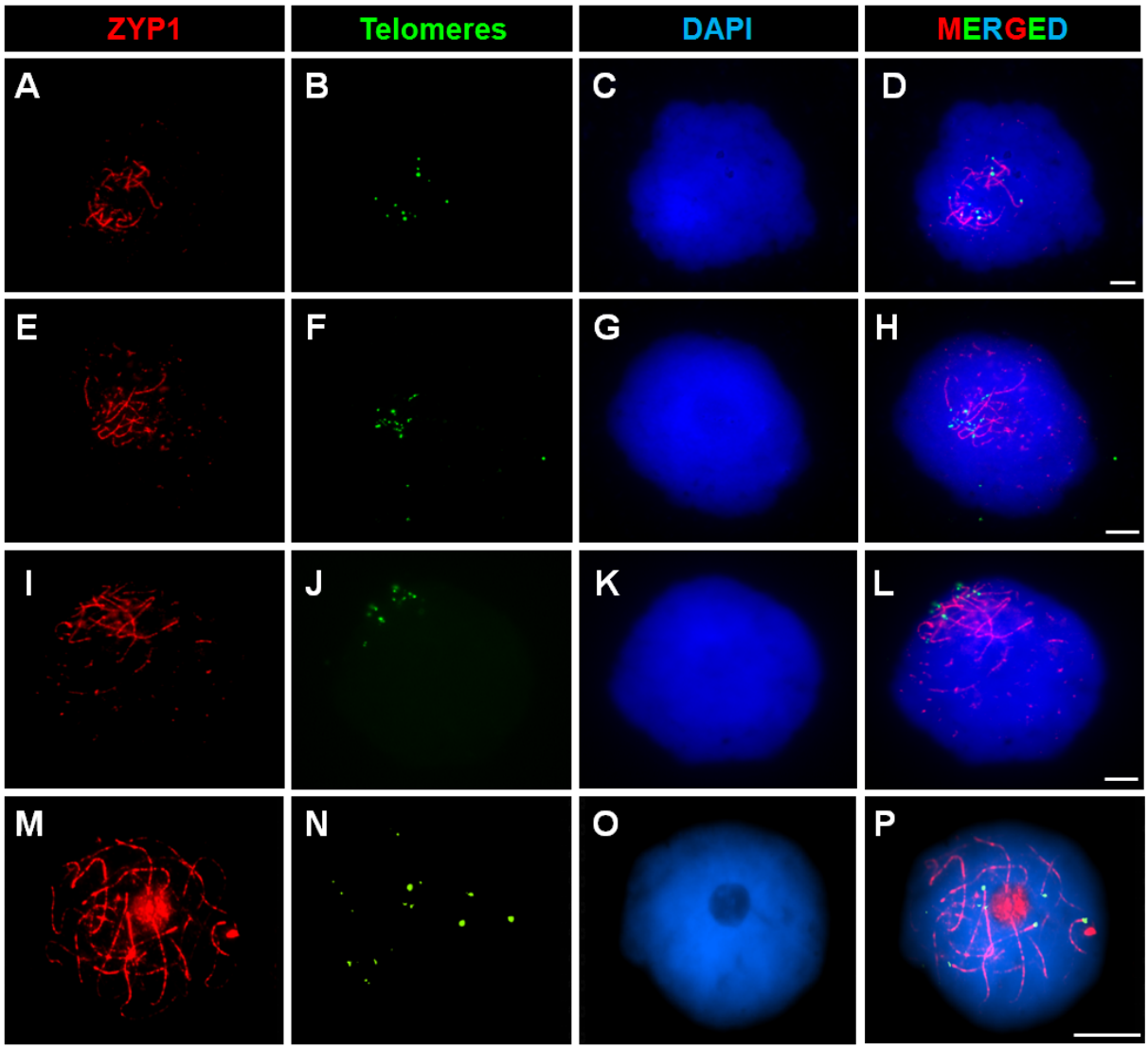
Supplemental Figure 1 *Arabidopsis* and barley meiotic protein ClustalW alignments

The regions of *Arabidopsis* proteins used for raising the antibodies are aligned against the homologous regions in the barley proteins. * denotes identical amino acids; : denotes similar amino acids.



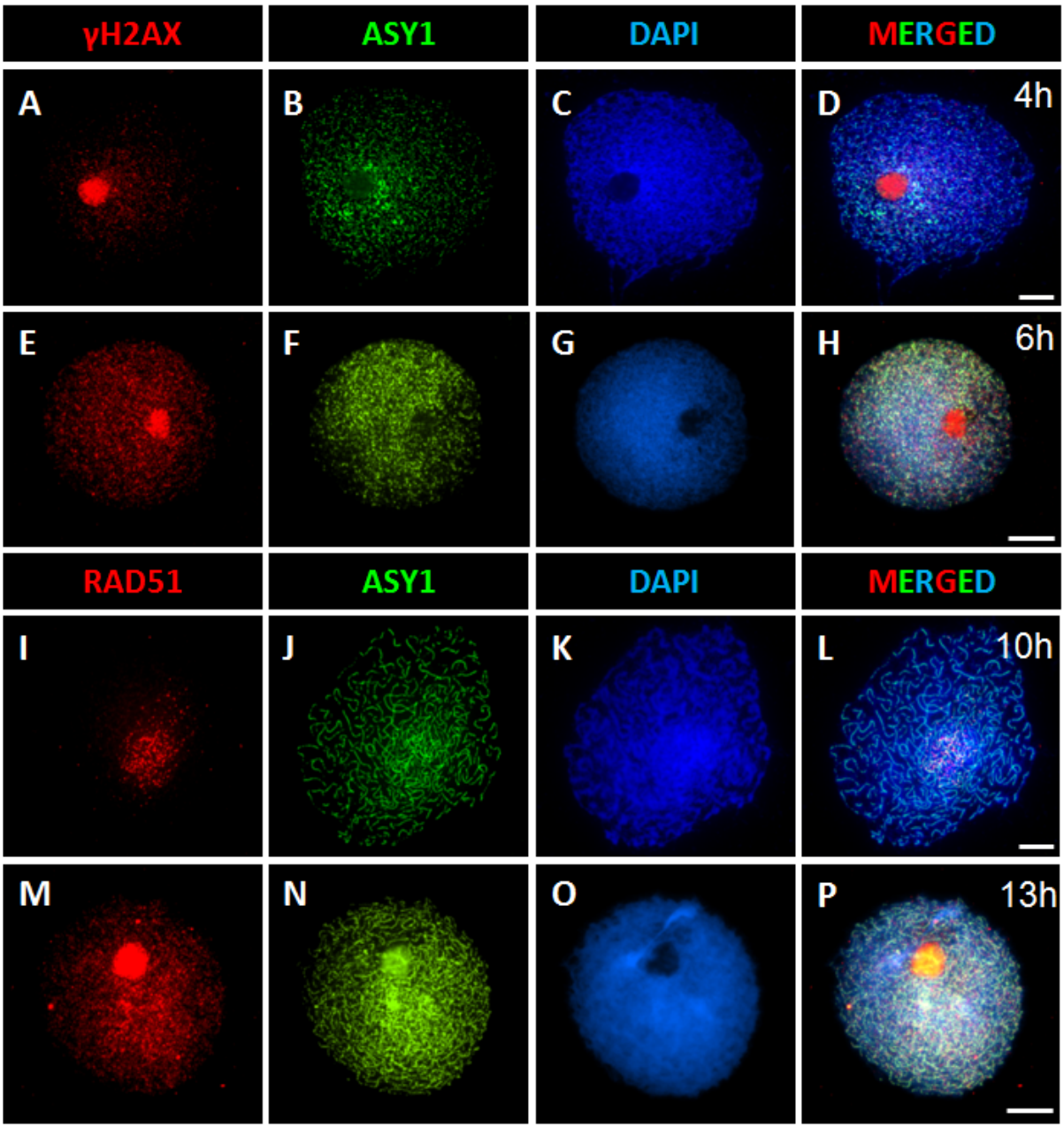
Supplemental Figure 2 Axis morphogenesis in relation to telomere clustering (unmerged images from Figure 2).

As a marker for chromosome axis formation, ASY1 foci (red) are initially observed adjacent to the telomeres (green) (**A-H**) and as the ASY1 signal progressively linearises (**I**) the telomeres cluster into a 'bouquet' (**J**), which persists through leptotene (**M-P**). Bar, 10μM.



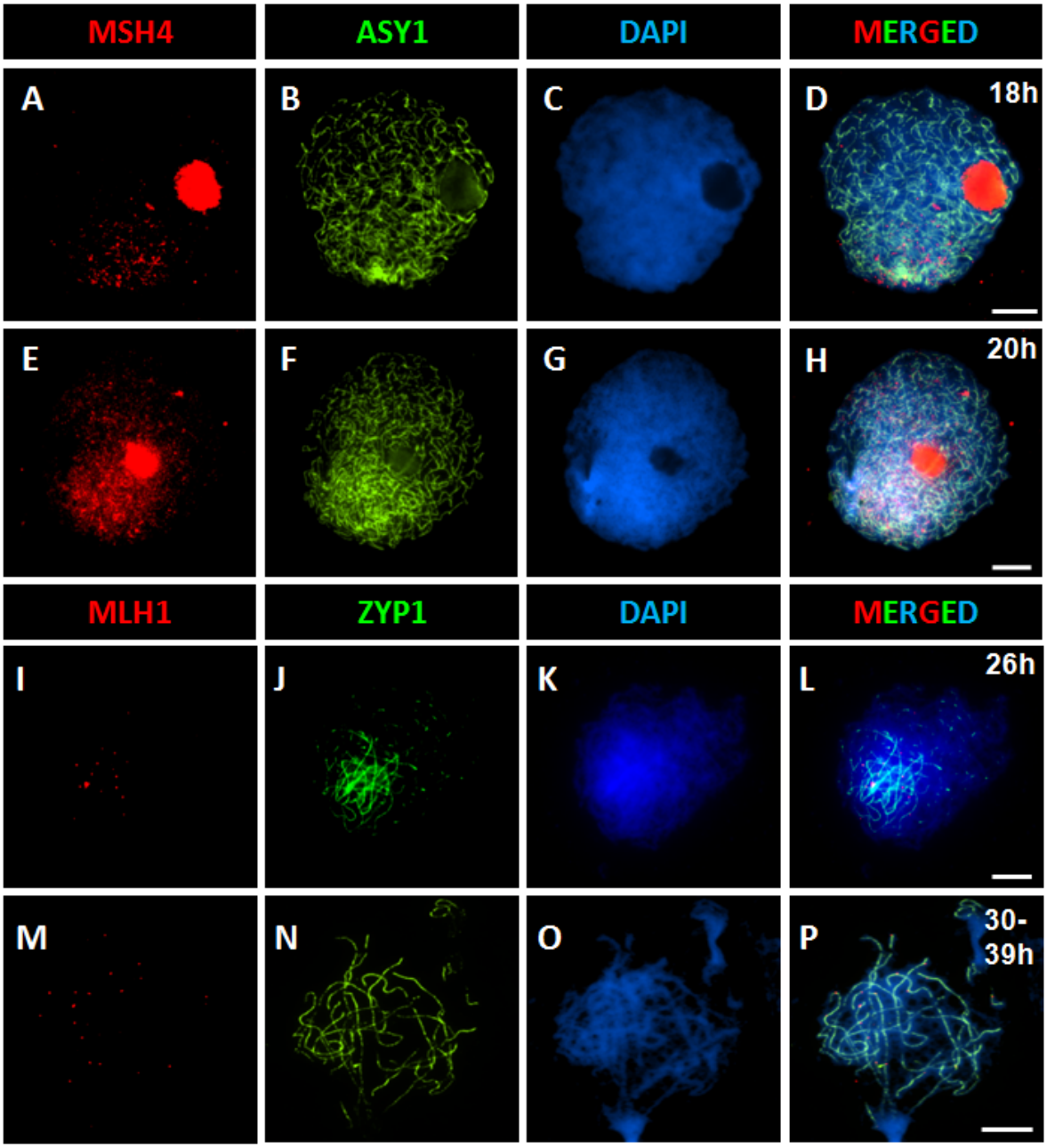
Supplemental Figure 3 Chromosome synapsis initiates from sub-telomeric regions (unmerged images from Figure 2)

As a marker for chromosome synapsis, ZYP1 stretches (red) are initially observed adjacent to the telomeres (green) (**A-H**). As the ZYP1 signal extends from the sub-telomeric regions, further synapsis initiation sites are observed in the interstitial regions (**I-L**) until synapsis is complete (**M-P**). Bar, 10μM.



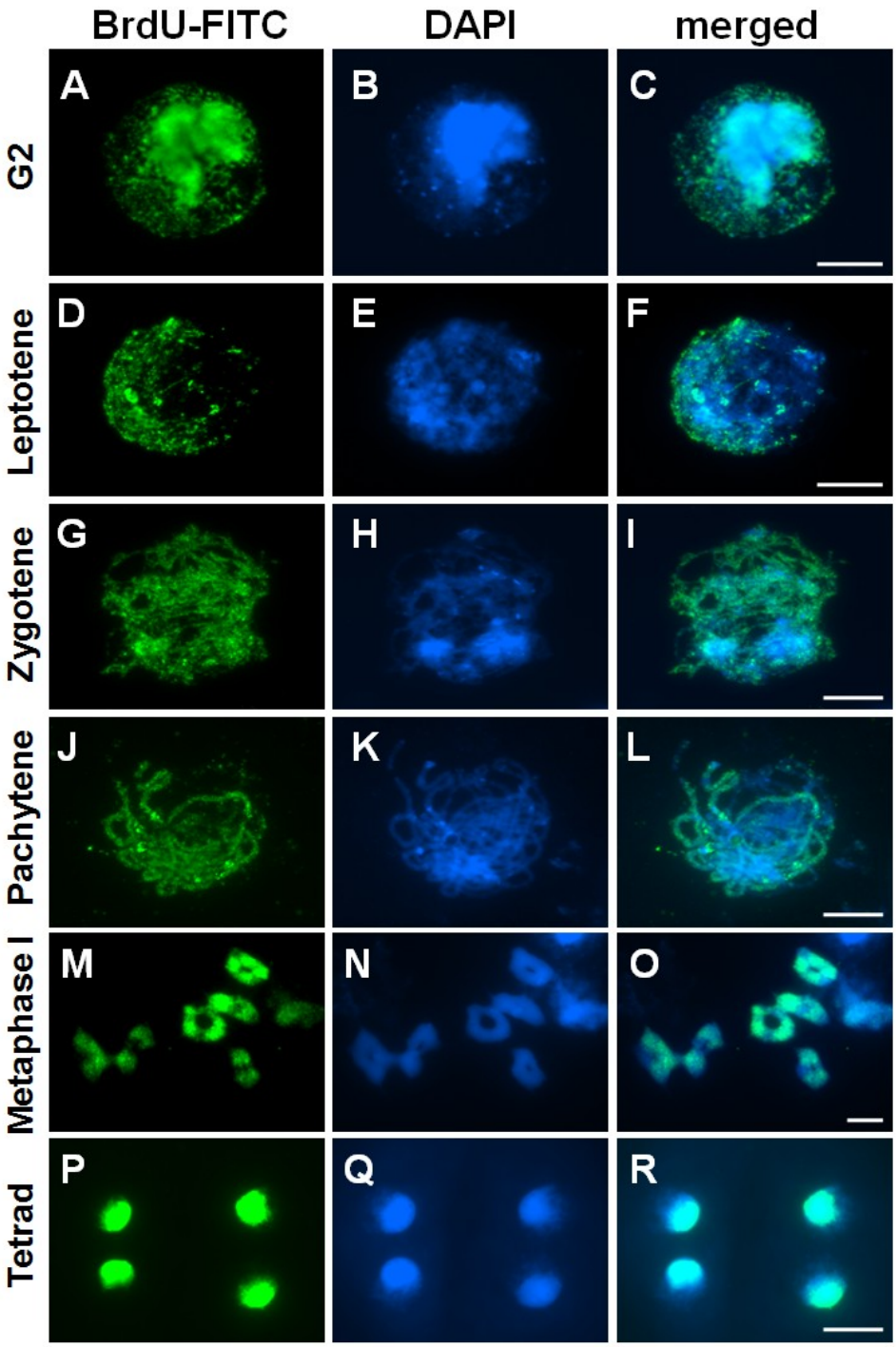
Supplemental Figure 4 Axis morphogenesis in relation to early recombination markers (unmerged images from Figure 2).

Markers for recombination (γH2AX, **A-D**; RAD51, **I-L**) are initially observed as a cluster in the distal regions (corresponding to the most intense region of ASY1 staining) where axis morphogenesis is most advanced and later throughout the nucleus (γH2AX, **E-H**; RAD51, **M-P**). Note: the intense 'ball like' structure is the nucleolus. Bar, 10μM.



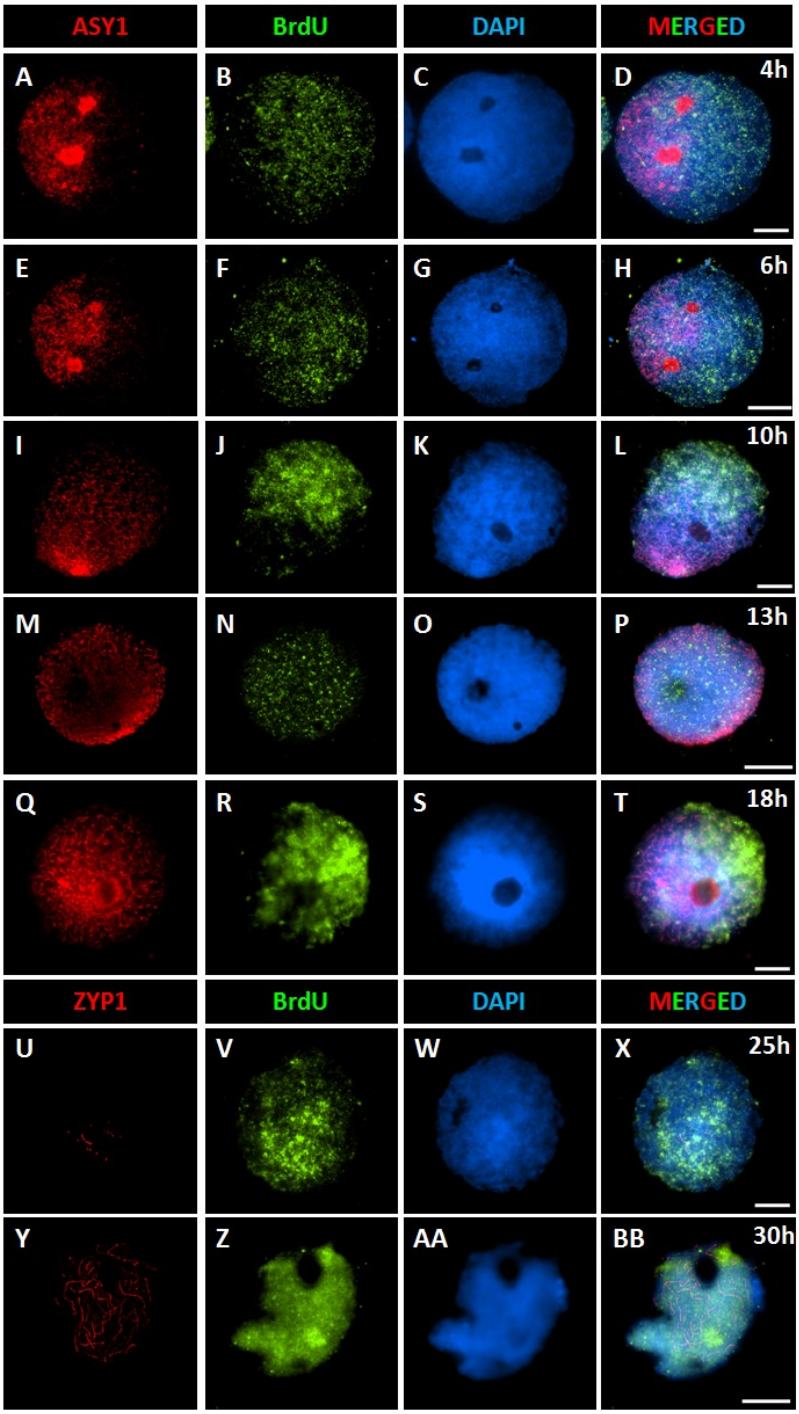
Supplemental Figure 5 Axis morphogenesis in relation to late recombination markers (unmerged from Figure 2)

MSH4 is initially observed as a cluster of foci in the distal regions **(A-D)** and later throughout the nucleus **(E-H)**. MLH1 is initially observed contiguous with synapsis in the distal regions **(I-L)** and later still appears to be in these regions **(M-P)**. Note: the intense 'ball like' structure is the nucleolus. Bar, 10µM.



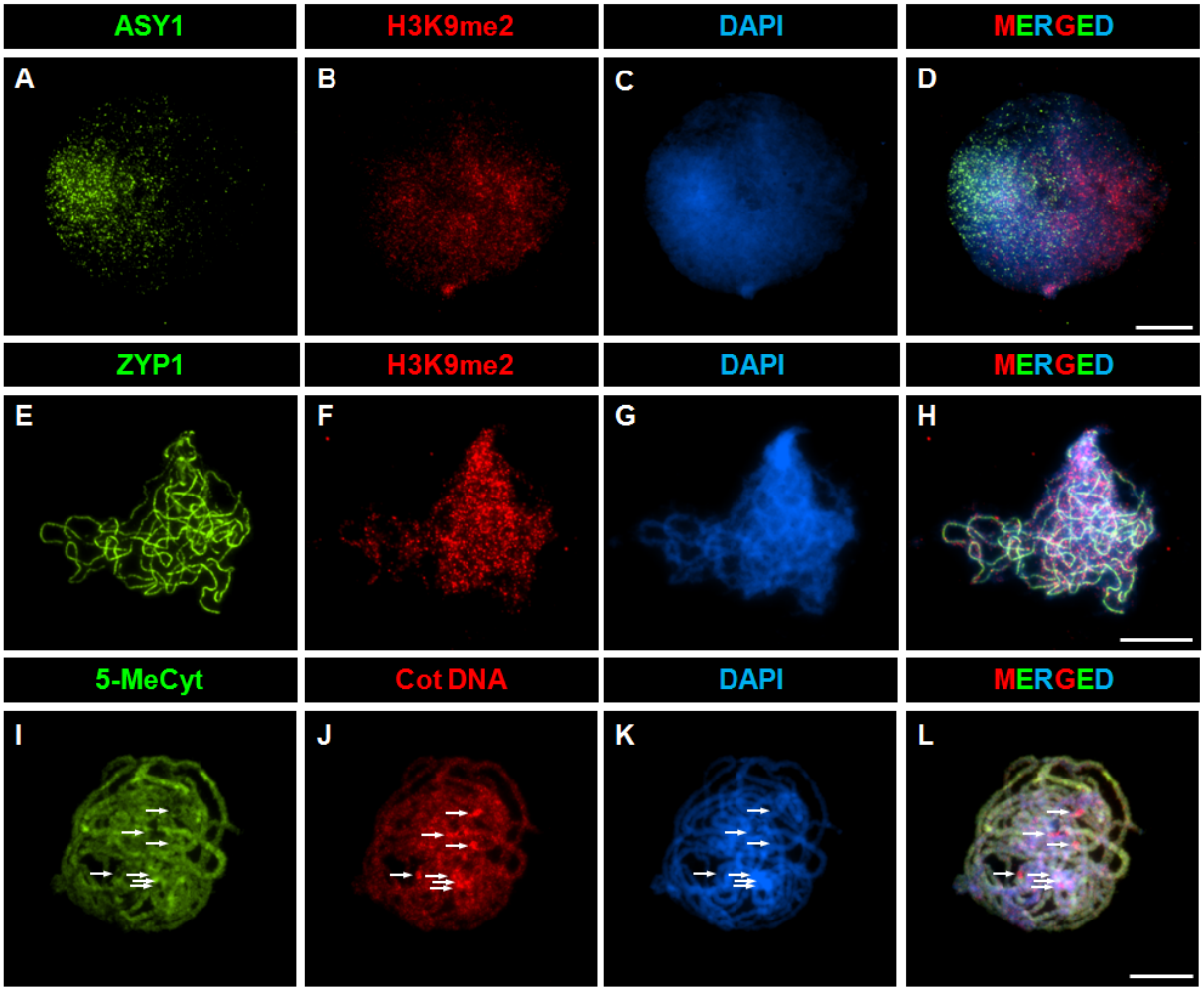
Supplemental Figure 6 A cytological meiotic time-course

5-bromo-2'-deoxyuridine(BrdU) is incorporated into newly synthesised DNA during pre-meiotic S-phase and detected with an anti-BrdU antibody labelled with FITC (green). Leptotene nuclei with labelled BrdU were observed after 13h (**D-F**), followed by zygotene at 25h (**G-I**), pachytene 30h (**J-L**), metaphase I 40h (**M-O**) and tetrad 43h (**P-R**). Nuclei were counterstained with DAPI (blue). Bar, 10µM.



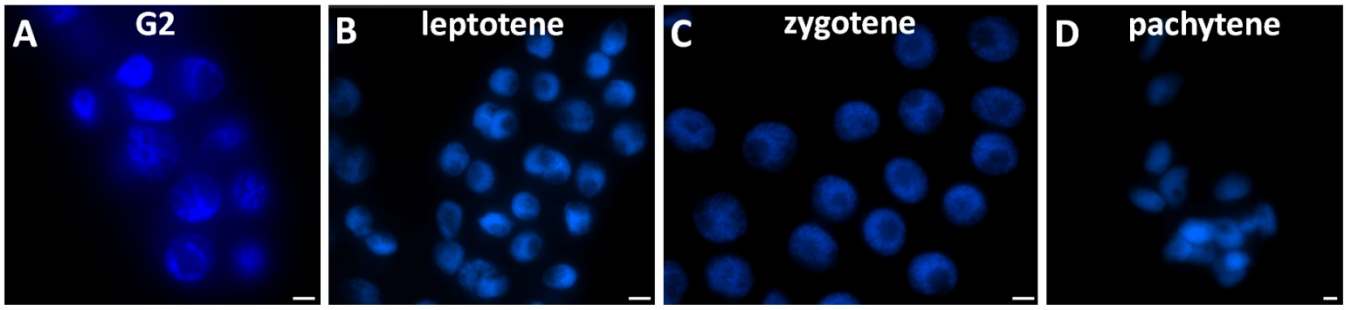
Supplemental Figure 7 An immunological time-course of barley meiotic prophase I

5-bromo-2'-deoxyuridine (BrdU) is incorporated into newly synthesised DNA during pre-meiotic S-phase and detected with an anti-BrdU antibody labelled with FITC (green). By dual labelling with markers for chromosome axis formation (ASY1, red) (**A-T**) and synapsis (ZYP1, red) (**U,BB)** the duration of meiotic stages can be determined. Bar, 10µM.



Supplemental Figure 8 Heterochromatin, methylated DNA and repetitive Cot DNA, are abundant throughout the barley chromosomes

The heterochromatin mark histone 3 lysine 9 dimethylation (H3K9me3) (**B,F**) occurs throughout the nucleus during prophase I but appears less abundant in the chromosome distal regions where ASY1 is loading (**A, D**). The methylated DNA nucleoside 5-methyl cytidine (5-MeCyt) is also abundant throughout the chromosomes during pachytene (**I**) along with fluorescence *in situ* hybridization of barley repeat sequence Cot DNA (**J**), DAPI (**K**) and merged (**L**), showing centromeres with white arrows. Bar, 10μM.

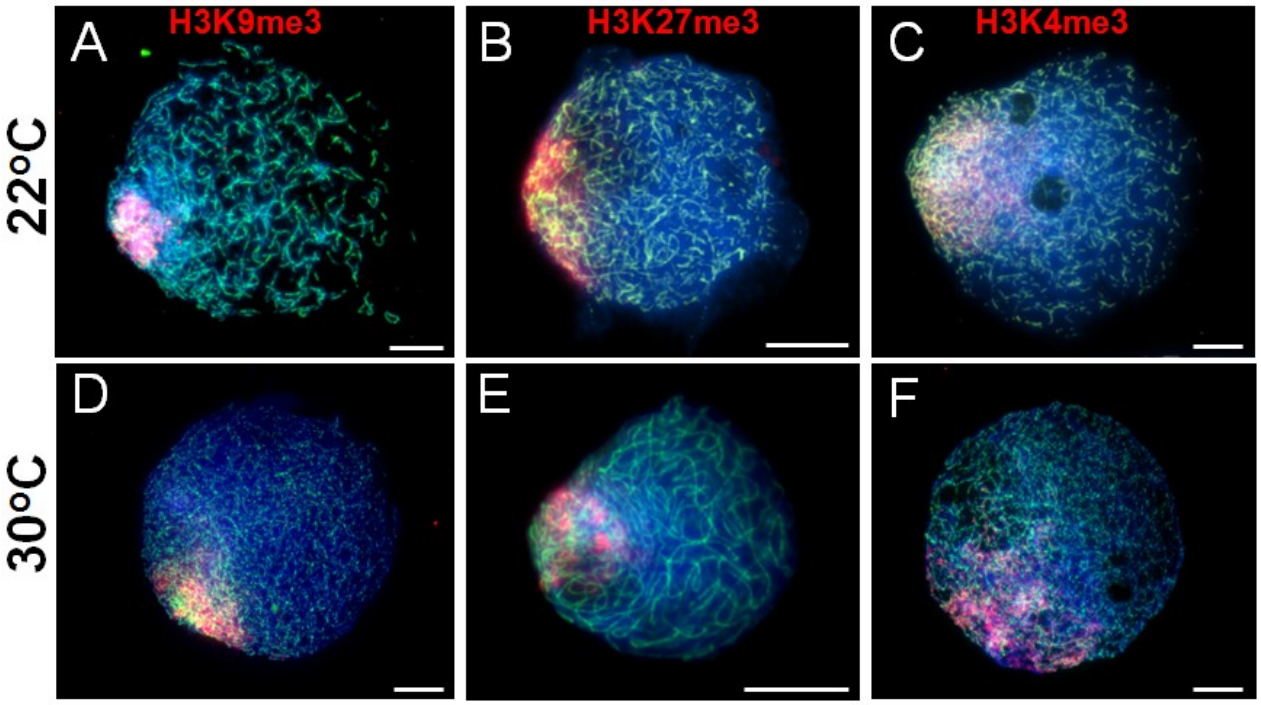


Supplemental Figure 9 Undigested meiocytes squeezed out of the anther locules shows that nuclear volume changes *in vivo,* but not as much as cell wall free nuclei.

Meiotic stages were determined by measuring anther lengths and sizes of nuclei by tracing round the DAPI (blue) stained areas. **A**, G2 = 485μm^2 (n=45); **B**, leptotene = 479μm^2 (n=45); **C**, zygotene = 449μm^2 (n=45); **D**, pachytene =283μm^2 (n=45). Bar, 10μM.

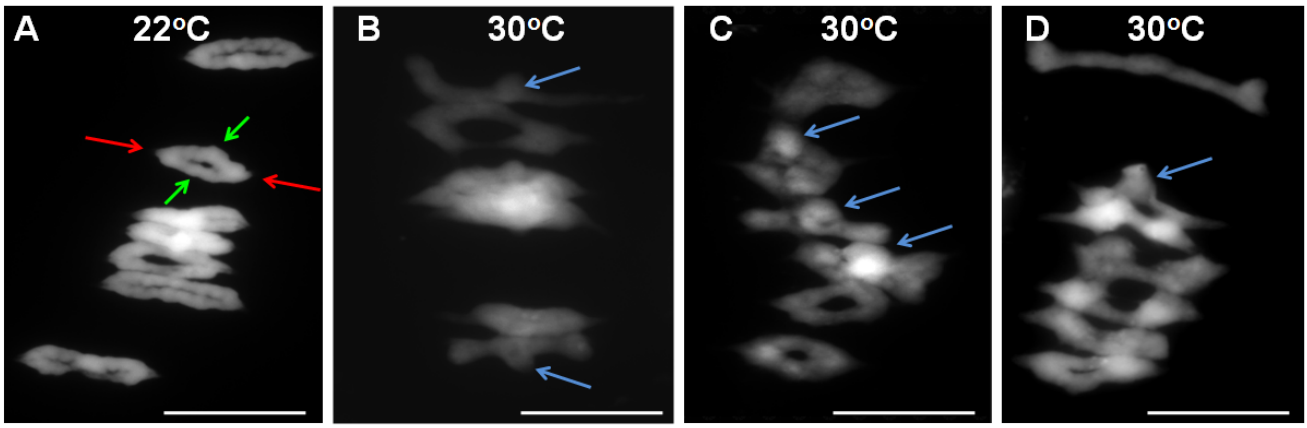
Supplemental Figure 10 Meiotic defects at 35°C

(A) In a mid-prophase I nucleus ZYP1 foci (green) are observed but do not elongate when ASY1 is linear (red). **(B)** Metaphase I chromosomes have unusual shapes that are agglomerated, leading to aberrant segregation **(C)**. Bar, 10μM.



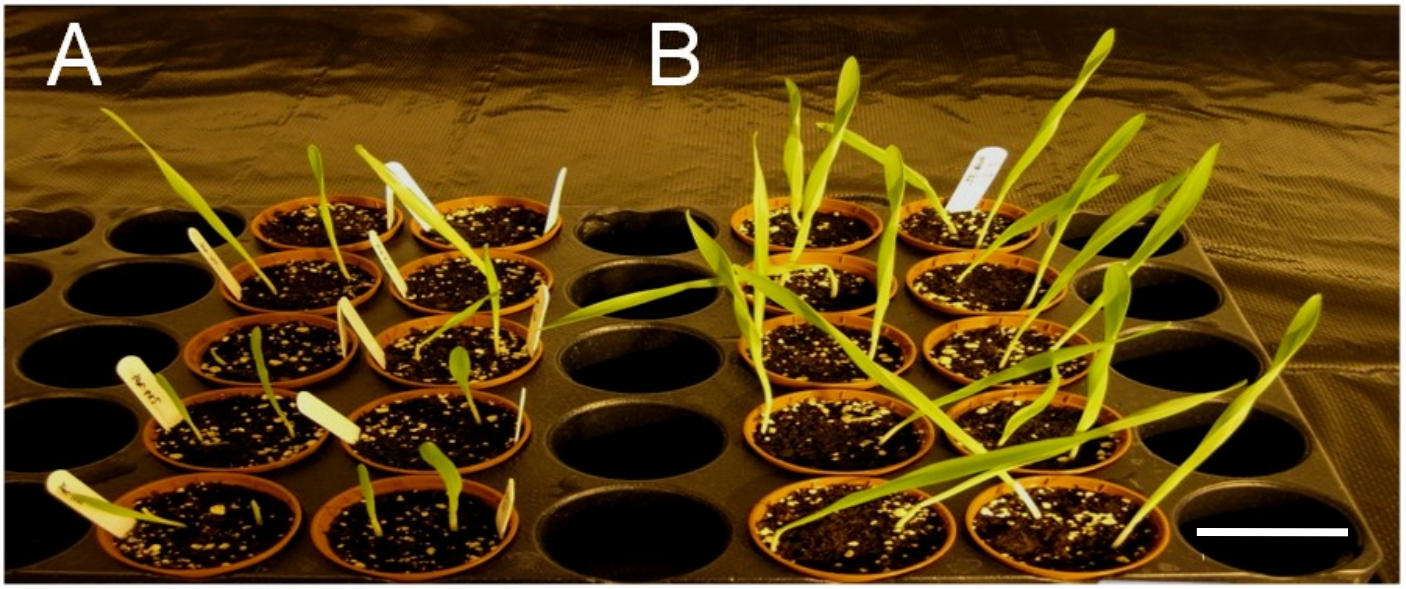
Supplemental Figure 11 Sub-telomeric euchromatic histone marks are indistinguishable at 22°C and 30°C

Histone 3 tri-methylated lysine 9 (H3K9me3) (**A,D**), histone 3 tri-methylated lysine 27 (H3K27me3) (**D,E**) and histone 3 tri-methylated lysine 4 (H3K4me3) (**C,F**) (red) immunolocalisation was indistinguishable between 22°C (**A,B,C**) and 30°C (**D,E,F**). Stages were identified with anti-ASY1 (green) counterstained with DAPI (blue). Bar, 10μM.



Supplemental Figure 12 Metaphase I chromosome spreads reveal increased interstitial chiasmata in plants grown at 30°C compared to 22°C

(A) At 22°C seven ring bivalents typically form with distal chiasmata (green arrows) and centromeres (red arrows) highlighted. (**B,C,D**) At 30°C, rod bivalents are more prevalent with increased interstitial chiasmata (blue arrows). Bar, 10μM.



Supplemental Figure 13 Post-meiotic fertility

Seeds collected from plants grown at 30°C were sown and 15/20 germinated (**A**) compared to the control where seeds were collected from plants grown at 22°C and 19/20 germinated (**B**). Bar, 5cm.

Gene name	cDNA fragment	Oligonucleotide	5'-Sequence-3'
RAD51	3'RACE	Rad51_F1	CCTTCAGAAATTAGCGGATGA
		Rad51_F2	**ATTGGAGGGAACATCATGGCTCAC**
	5'RACE	Rad51_R1	AGCCTTTCCTTCACCACCACCTTG
		Rad51_R2	**CTAGTGAACCCCAGTGGAACCAAC**
	Full length	Rad51_FL_F1	AACCCCAAAACCCGCCTCCCCTGC
		Rad51_FL_F2	**CGCCTCCCCTGCTAGAGAAGAGAG**
		Rad51_FL_R1	GGACCCAAGGAATATCGCTTCTTAC
		Rad51_FL_R2	**TCTTACGGAGGGGCAAATTCCATC**
DMC1	3'RACE	DMC1_F1	CGCATACACCTATGAGCACCAGTAC
		DMC1_F2	**TTGCTGAGGAGTTCAATGTTGCAG**
	5'RACE	DMC1_R1	CAACACGGAATAGCGCAATCACAG
		DMC1_R2	**AGCAGCCTCGCAGATCTTATCAAC**
	Full length	DMC1_FL_F1	GAACGTCAGCGCCCCGTTCA
		DMC1_FL_F2	**ATGGGCCTAGCTGGCAGCAGCAAAG**
		DMC1_FL_R1	AAGCAAGAATCAAGAGACGGCGTA
		DMC1_FL_R2	**AAGAGACGGCGTATTTCATTCTTC**
MSH4	3'RACE	Msh4_F1	TTGAAGTACTCGAACCAAGGGGATG
		Msh4_F2	**CAACCGTGAAGCATTGCAGGACCTC**
	5'RACE	Msh4_R1	GTCCACTTTCAGTATCGGACTCTG
		Msh4_R2	**TTGCCTGCAGCGTAACTCTCCTT**
	Full length	Msh4_FL_F1	CGCGCACACACCAGCTAGCAGC
		Msh4_FL_F2	**CGTAGTTGCATCCTCCCAGGGTTC**
		Msh4_FL_R1	GCTTGTACTTTTAGGACATATCAC
		Msh4_FL_R2	**CGCCACAACTTTATGTGTCCCCAG**

MSH5	**3'RACE**	Msh5_F1	GGTTCTACTACCATACTCAAAAGACA
		Msh5_F2	**TTACATCAAGTTGGAACAATGCTC**
	5'RACE	Msh5_R1	TCACGTACAACGCTCTGCTGGTTC
		Msh5_R2	**AATCATAATTTGCAAAATGACTGATGG**
	Full length	Msh5_FL_F1	GGACACTGACATGGACTGAAGGAG
		Msh5_FL_F2	**ACATGGACTGAAGGAGTAGAAAAG**
		Msh5_FL_R1	TGGATGGTCAAAACCGATTCCTC
		Msh5_FL_R2	**CCGATTCCTCGTTTGAATATGCTG**
MLH1	**3'RACE**	Mlh1_F1	GTCCAGTCGCCATGAGCTTGTTAC
		Mlh1_F2	**GATCAGTACACTCCTGATATGGATC**
	5'RACE	Mlh1_R1	GCAGCTGCTGCCGTTCTGAAGCAC
		Mlh1_R2	**TGCAAACCGACTGATGAAGTCCAC**
	Full length	Mlh1_FL_F1	TCCGCCCGGTCCCCAGACGC
		Mlh1_FL_F2	**AGACGCGGCGCGGCGGGCATG**
		Mlh1_FL_R1	AGCAGAGCCAAATCATTCGTGCAT
		Mlh1_FL_R2	**CAGGGCTGAGCTGGAGCCCAGC**
ZYP1	**3'RACE**	Zyp1_F1	AATGGATCAGAGTTACAAGGAACAG
		Zyp1_F2	**AGCTTCTAGGAAGAATGCAGTCAG**
	5'RACE	Zyp1_R1	TGTTGCTCTTCTCAGCTTGGTATG
		Zyp1_R2	**CTTCACCGAGGCCTGCTCCTTGAC**
	Full length	Zyp1_FL_F1	CCCTCTCCCCCCTCCGCGTTCGTG
		Zyp1_FL_F2	**GGCCTGGTGCTGCCGCCGTCGACG**
		Zyp1_FL_R1	AACGACAGGAAACCTGAGATTCC
		Zyp1_FL_R2	**CAAGACGGTCATTGGCTGGGTGTG**

ASY1	**3'RACE**	Asy1_F1	CTAATGATGCTAACAGTGACGATG
		Asy1_F2	**TACTGGAAGTTGATATCACTGAAG**
	5'RACE	Asy1_R1	AACCTTGTTAACAGCATAACCATC
		Asy1_R2	**TTGCTCCCTGTGCGACTCATGTTC**
	Full length	Asy1_FL_F1	CCCTCCCACGCGCACACACAGCAC
		Asy1_FL_F2	**CACCACCAGGGCGGCAAAATGGTG**
		Asy1_FL_R1	GCAGAAAATGACTTGCTCCGATTC
		Asy1_FL_R2	**AAATGACTTGCTCCGATTCAGTAC**

Supplemental Table 1: Primers used for cloning barley meiotic cDNAs

Two successive PCR reactions were carried out for each fragment. 1 µl of the barley inflorescence cDNA (see Material and Methods) was used as a template with the forward (F1) and reverse (R1) primers. 1 µl of this primary reaction was added in the secondary reaction with the corresponding nested primers indicated in **bold** (forward (F2) and reverse (R2)). The 3'RACE and 5'RACE oligonucleotides were used in combination with the GeneRacer 3' and 5' primers (Invitrogen), respectively. Oligonucleotides are shown 5'-3'.