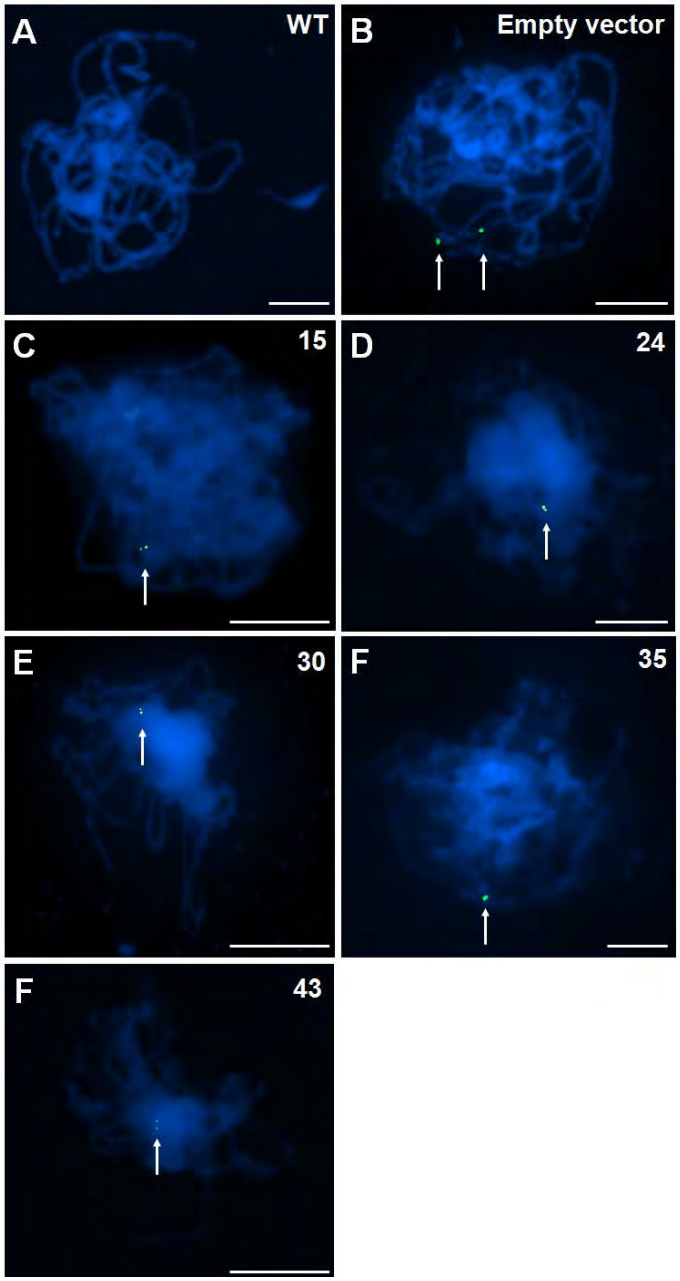
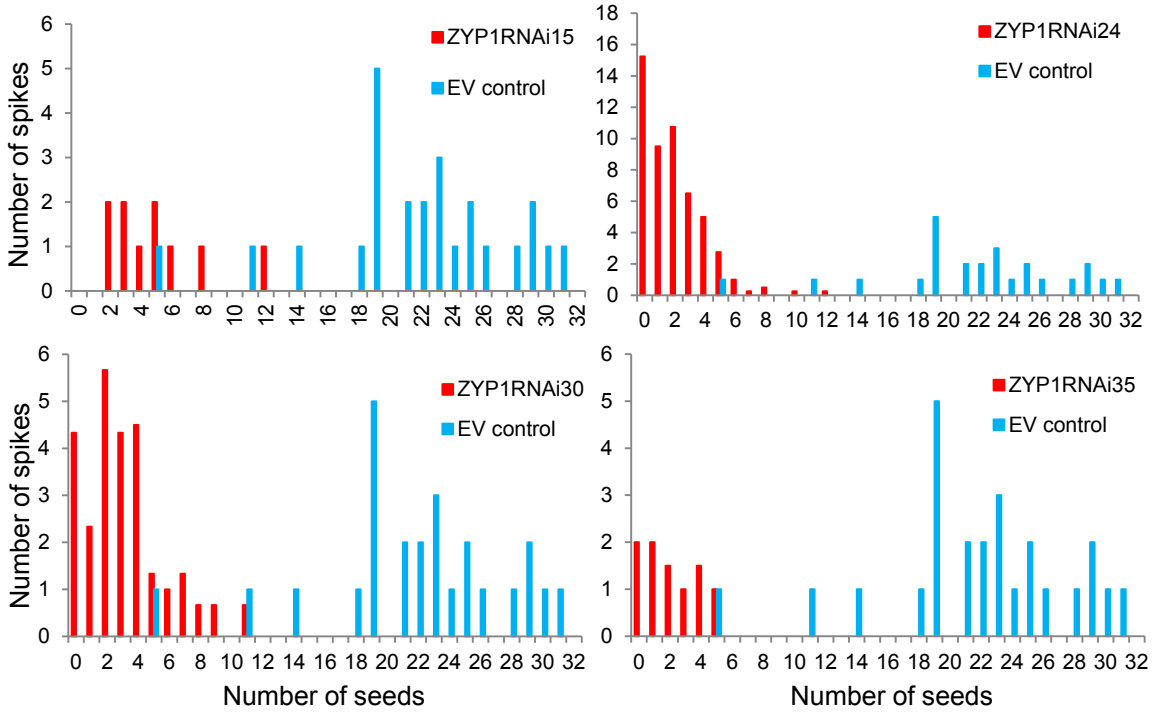


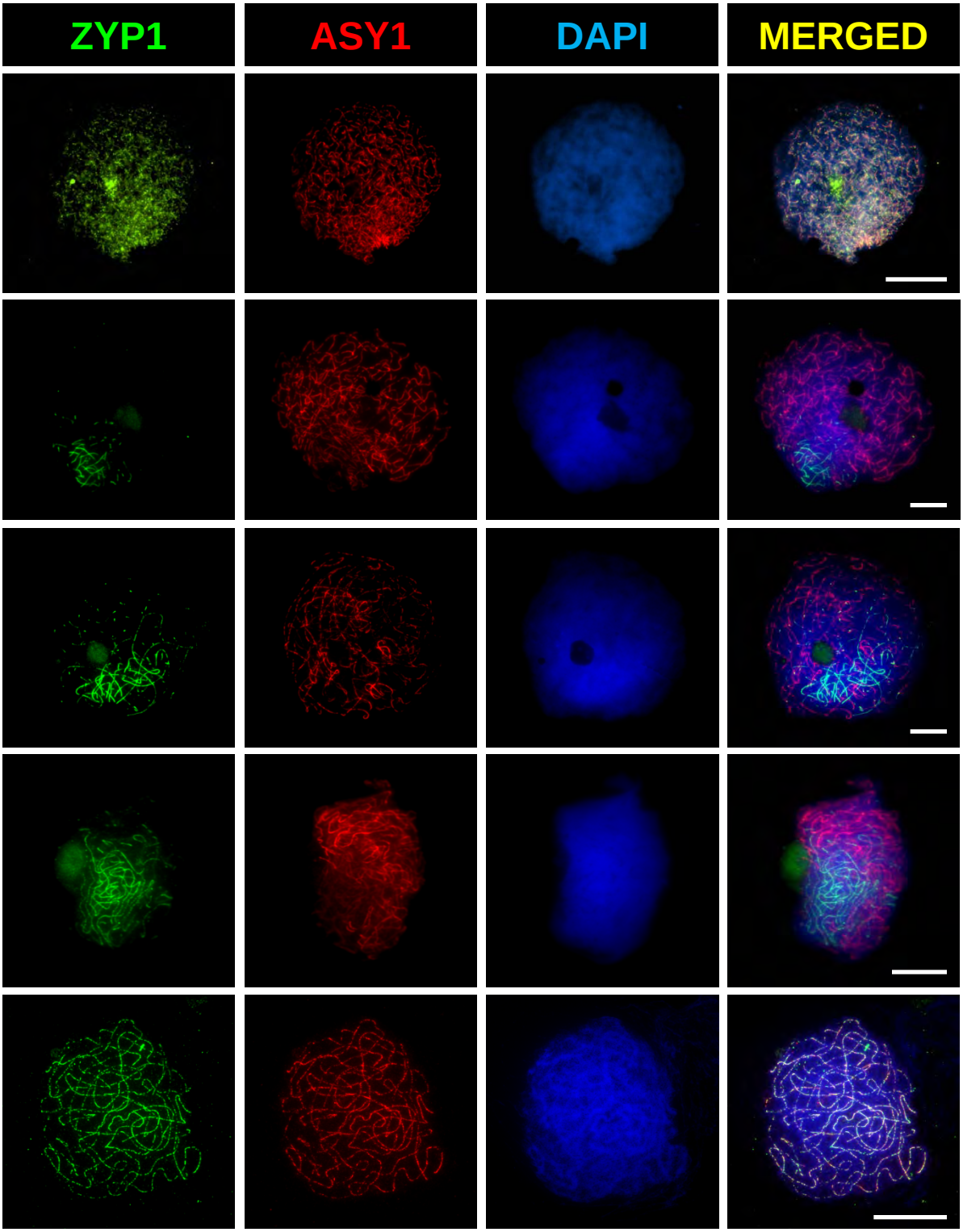
Supplemental Figure 1. Determining the number of T-DNA inserts in *ZYP1*^{RNAi} lines using fluorescence *in situ* hybridization with pBRACT probe on meiotic prophase I nuclei.

(A) Wild-type 'Golden Promise' was used to test the specificity of the probe. (B) In the empty vector control, two T-DNA insertion sites (green spots) are indicated by the white arrows. (C-F) For each of the *ZYP1*^{RNAi} lines 15, 24, 30, 35 and 43, there is one homozygous insertion site per cell (green), highlighted by white arrows. Note, when two small signals are observed such as in (F) this is due to the homologues being spaced apart but only represents one homozygous insertion site. Nuclei are stained with DAPI. Bar = 10 µm.

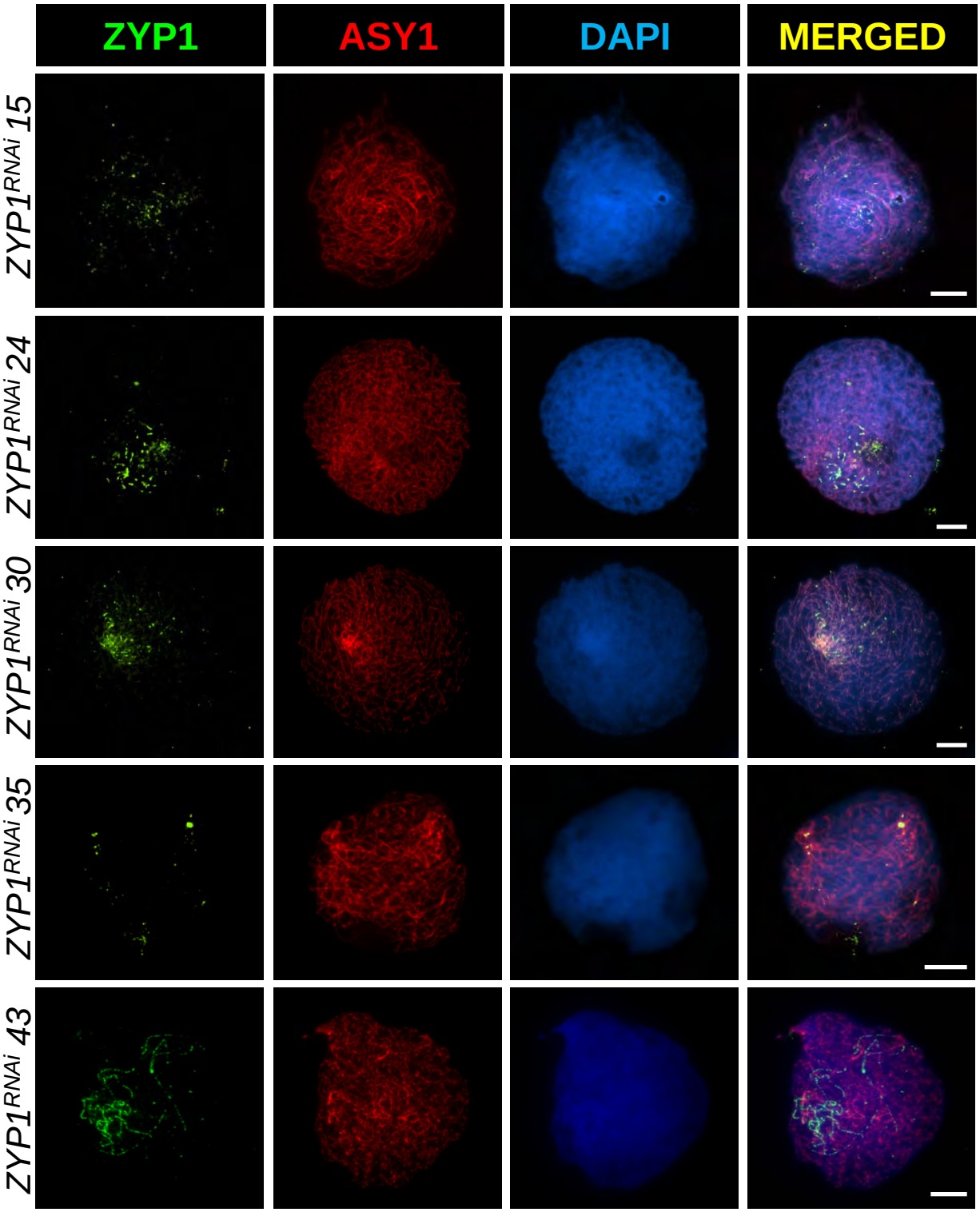


Supplemental Figure 2: *HvZYP1* suppression induces sterility in barley.

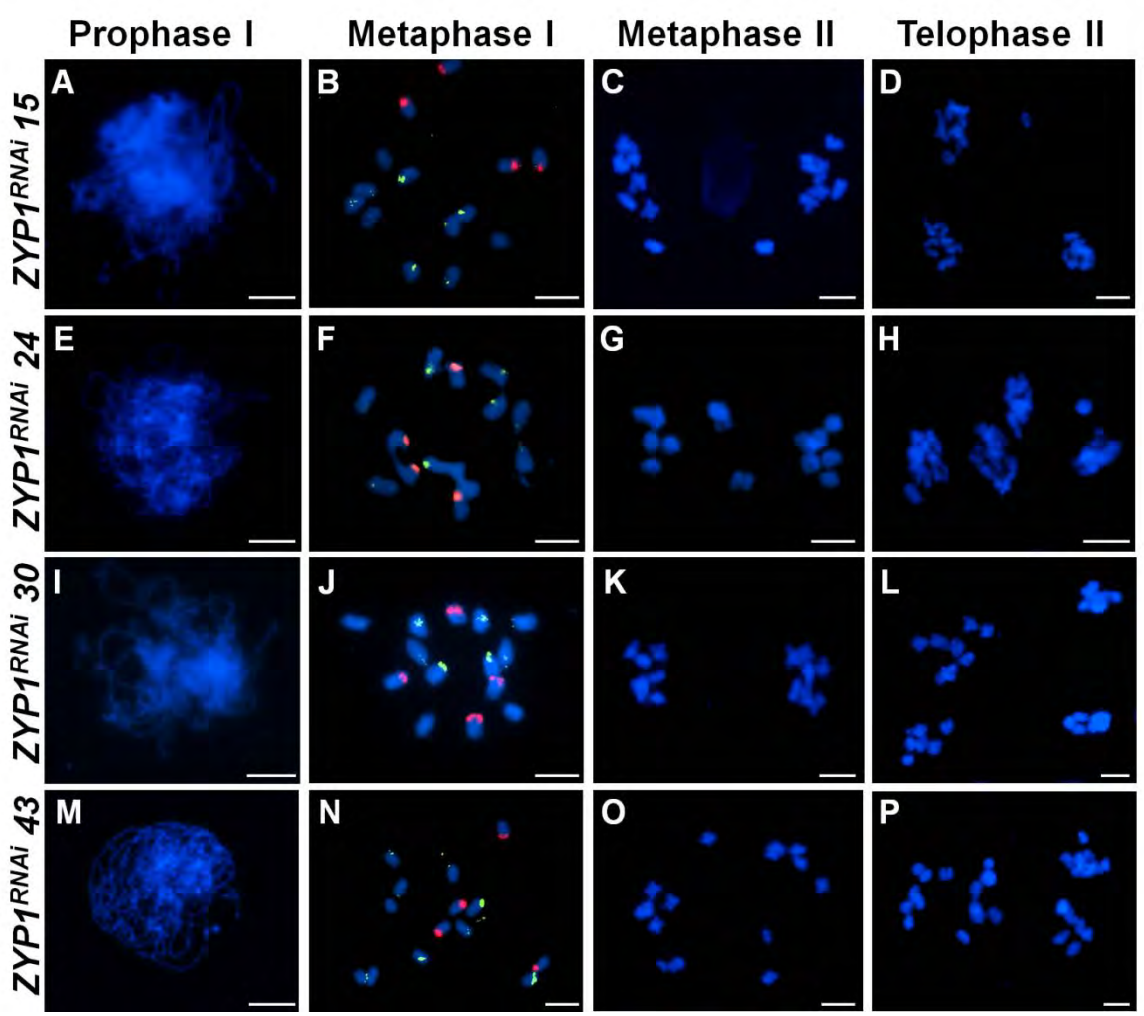
Distribution of seed number per spike in the RNAi lines *ZYP1*^{RNAi}*15, ZYP1*^{RNAi}*24, ZYP1*^{RNAi}*30* and *ZYP1*^{RNAi}*35*. Spikes of the empty vector control (EV) have higher number of seeds than all *ZYP1*^{RNAi} lines. Note, the same EV plants were used for all comparisons but scales on each graph differ due to differences between the RNAi lines.



Supplemental Figure 3A. Unmerged images from Figure 4 of ZYP1 (green) and ASY1 (red) of prophase I in empty vector control nuclei. Bar = 10 μm.

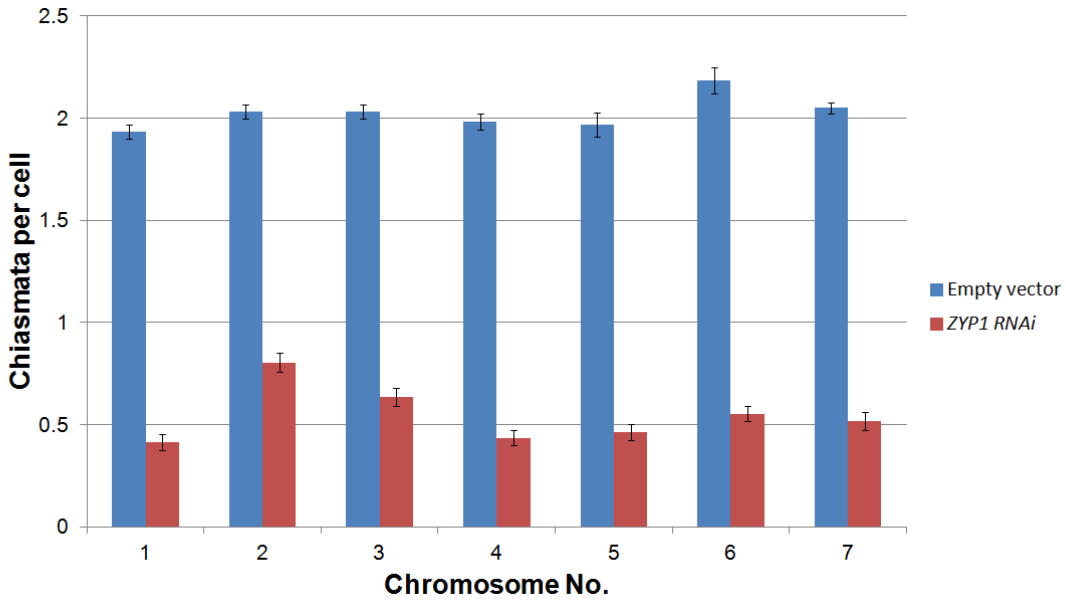


Supplemental Figure 3B. Unmerged images from Figure 4 of ZYP1 (green) and ASY1 (red) of prophase I in *ZYP1*^{RNAi} nuclei. Bar = 10 µm.



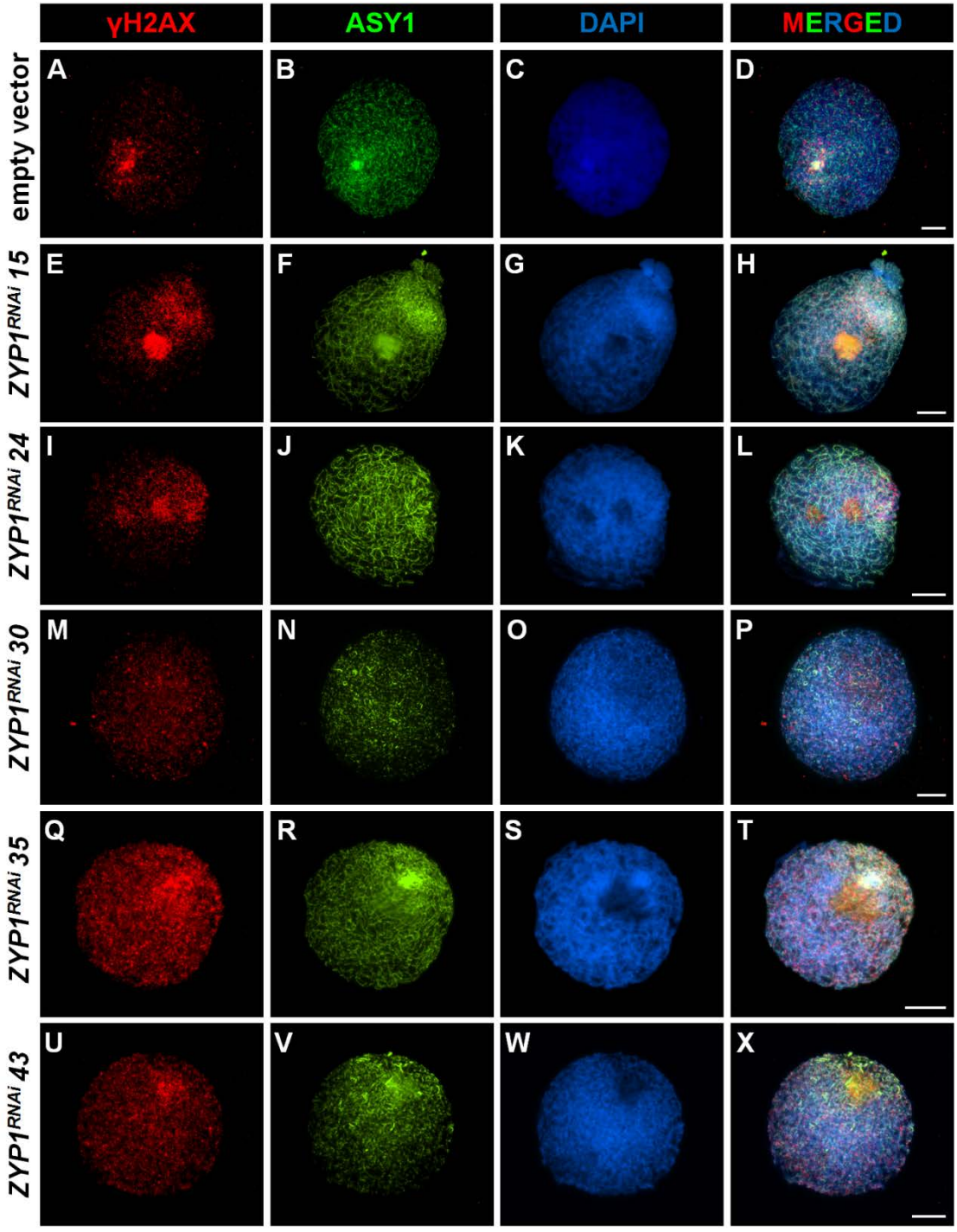
Supplemental Figure 4. A cytological analysis of meiotic chromosomes in *ZYP1*^{RNAi} lines which were not shown in Figure 5.

During prophase I in the *ZYP1*^{RNAi} lines chromosomes form thin strands which align only in small regions (A, E, I, M). Metaphase I chromosomes contain a mixture of univalents and bivalents which can be distinguished with the 5*S* (green) and 45*S* (red) ribosomal DNA fluorescence *in situ* hybridization probes (B, F, J, N). During metaphase II mis-segregation becomes apparent (C, G, K, O). At telophase II, mis-segregation leads to unbalanced gametes (D, H, L, P). Nuclei were stained with DAPI and bar = 10 µm.



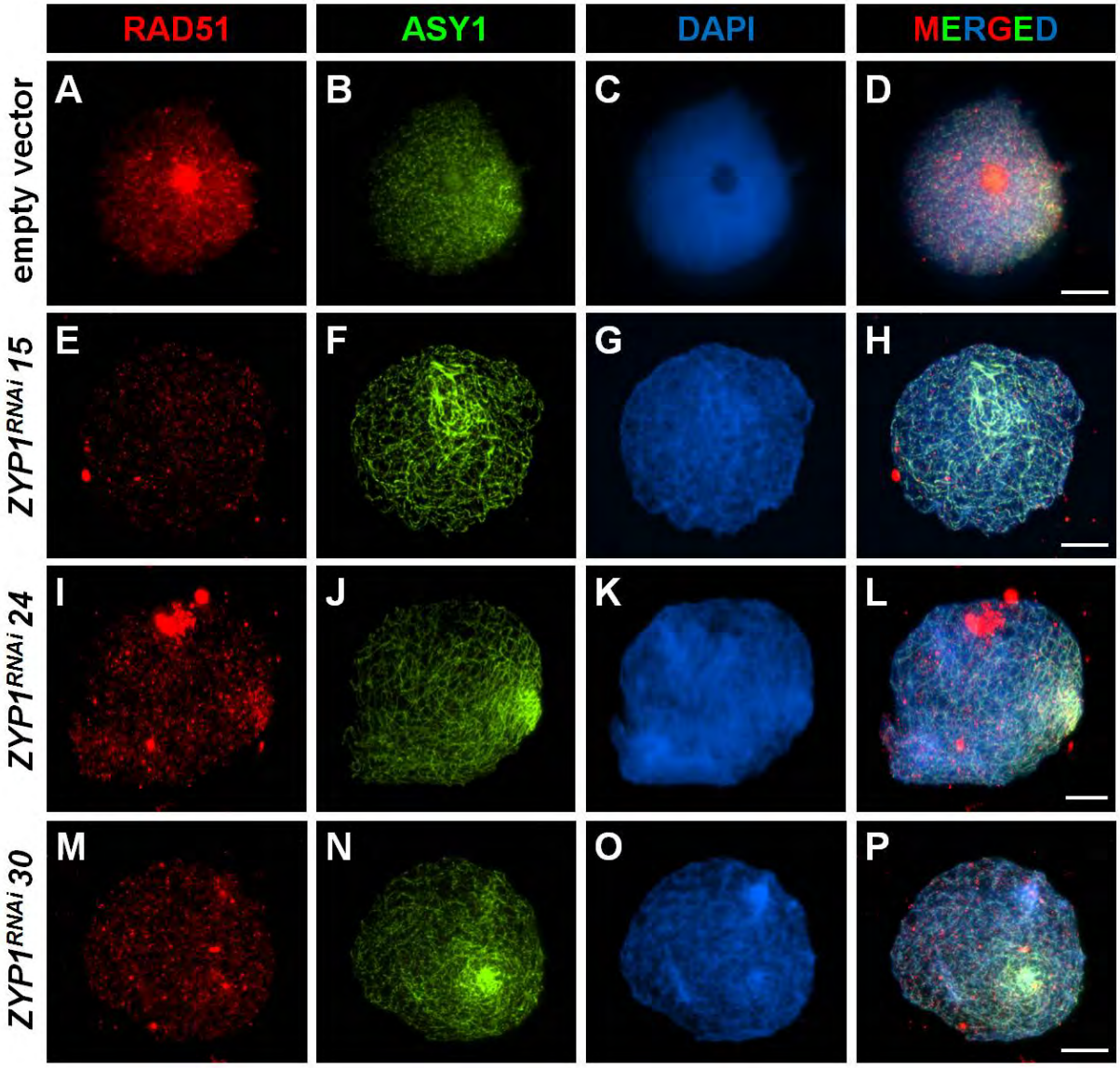
Supplemental Figure 5. The numbers of chiasmata in individual chromosomes are differentially affected in the *ZYP1*^{RNAi} lines.

Chiasmata were scored on meiotic chromosomes at metaphase I using the 5*S* and 45*S* FISH probes in the empty vector control and *ZYP1*^{RNAi} lines. A reduction of chiasmata was observed for all chromosomes in the *ZYP1*^{RNAi} lines compared to the control, although chromosome 2 was least affected.



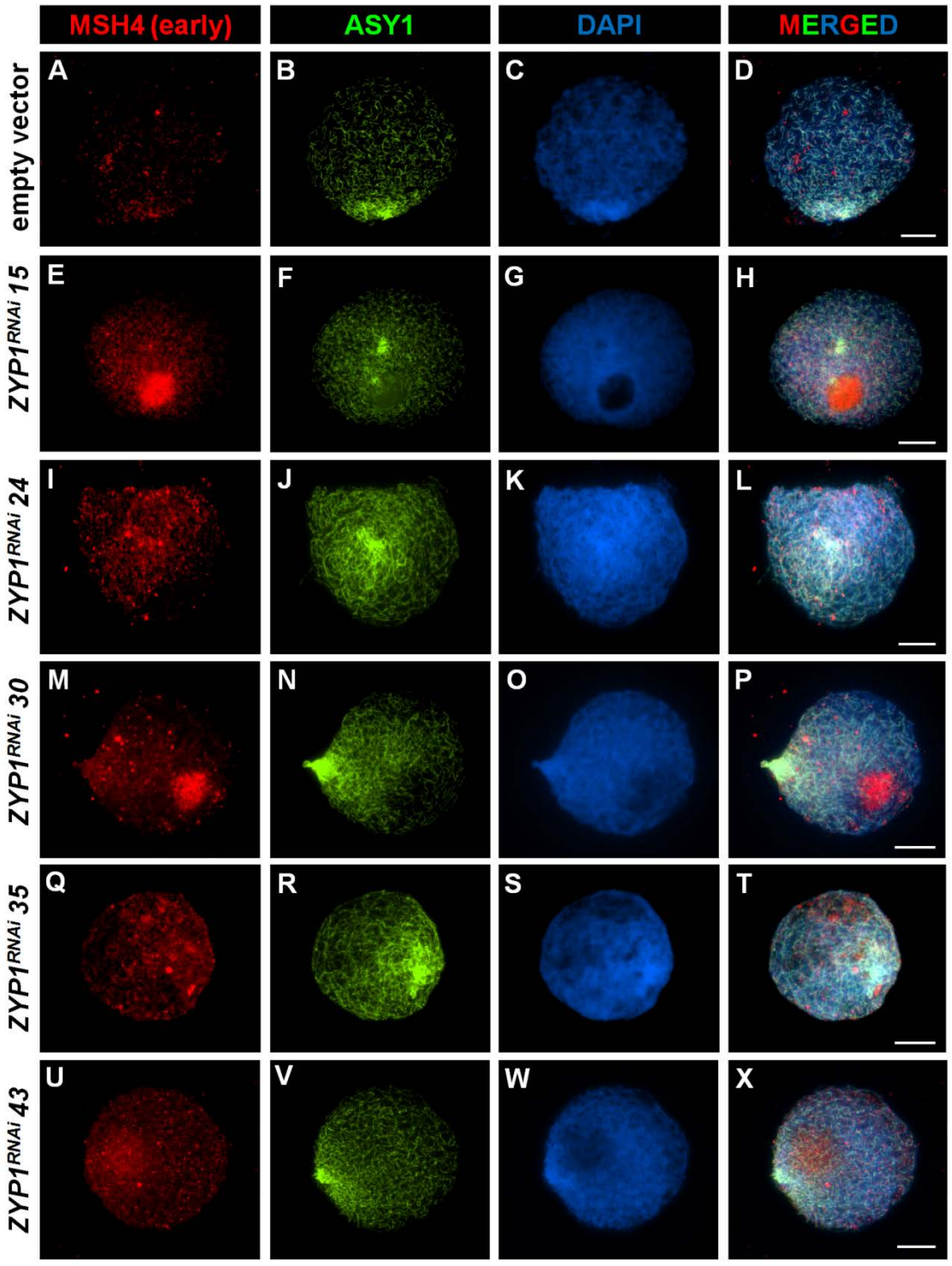
Supplemental Figure 6. Immunolocalisation of γH2AX in the empty vector control and *ZYP1*^{RNAi} lines.

(A, E, I, M, Q, U) γH2AX foci (red); (B, F, J, N, R, V) ASY1 (green); (C, G, K, O, S, W) DAPI (blue); and (D, H, L, P, T, X) merged images. Bar =10 μm.



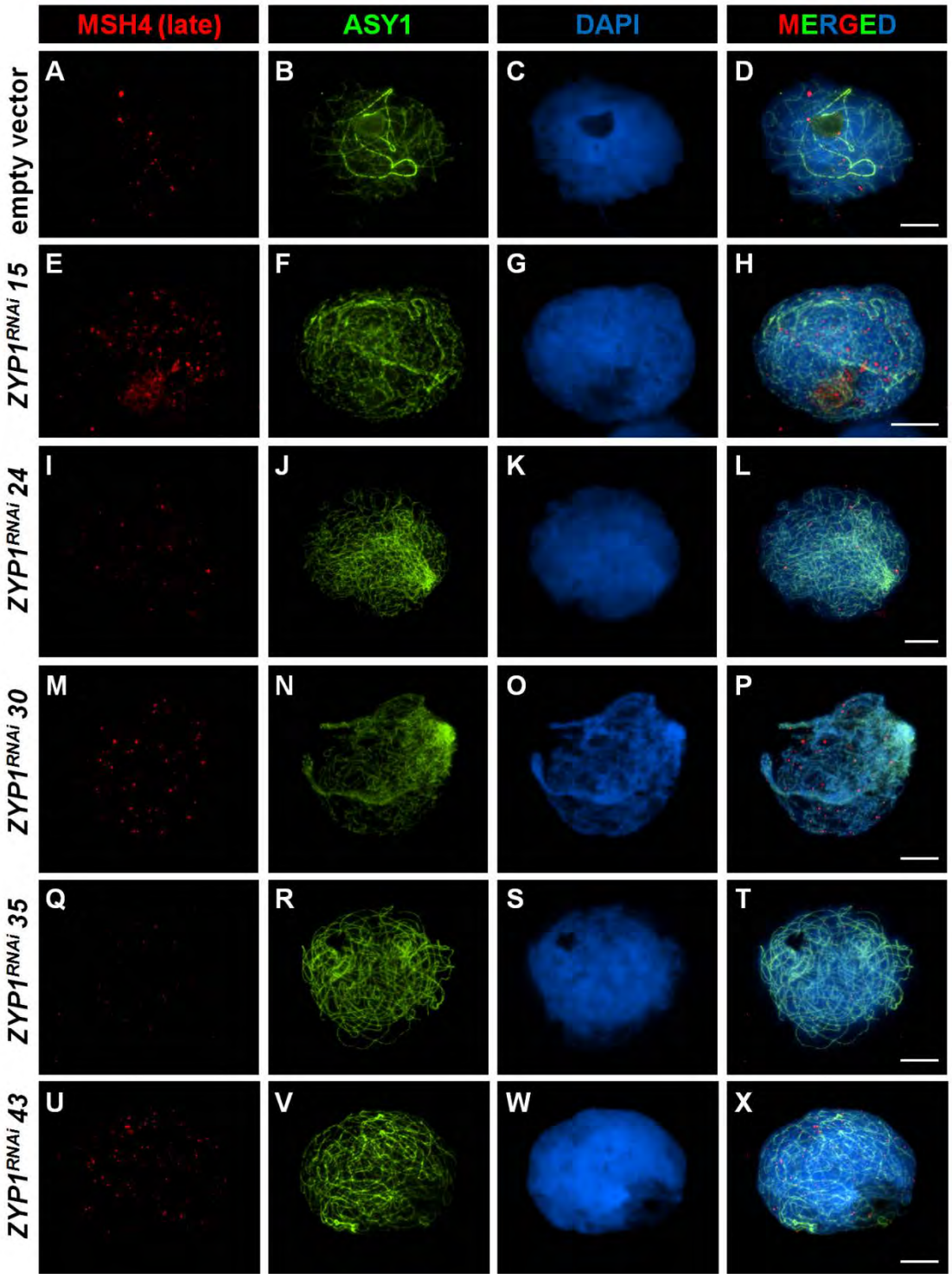
Supplemental Figure 7. Immunolocalisation of RAD51 in the empty vector control and selected *ZYP1*^{RNAi} lines.

(A, E, I, M) RAD51 foci (red); (B, F, J, N) ASY1 (green); (C, G, K, O) DAPI (blue); and (D, H, L, P) merged images. Bar =10 μm.



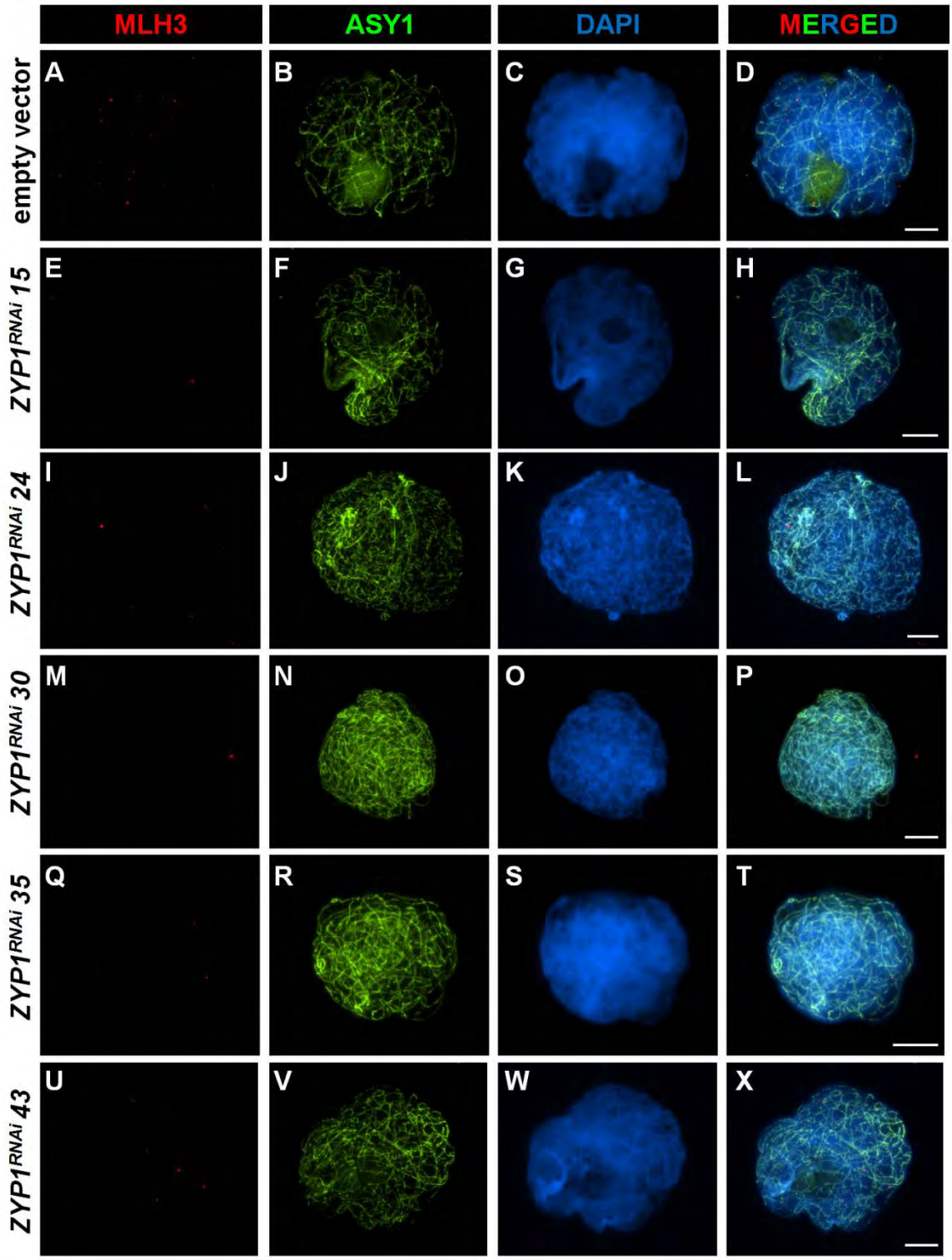
Supplemental Figure 8. Immunolocalisation of MSH4 (early leptotene) in the empty vector control and *ZYP1*^{RNAi} lines.

(A, E, I, M, Q, U) MSH4 foci (early)(red); (B, F, J, N, R, V) ASY1 (green); (C, G, K, O, S, W) DAPI (blue); and (D, H, L, P, T, X) merged images. Bar =10 μm.



Supplemental Figure 9. Immunolocalisation of MSH4 (late leptotene) in the empty vector control and *ZYP1*^{RNAi} lines.

(A, E, I, M, Q, U) MSH4 foci (late)(red); (B, F, J, N, R, V) ASY1 (green); (C, G, K, O, S, W) DAPI (blue); and (D, H, L, P, T, X) merged images. Bar =10 µm.



Supplemental Figure 10. Immunolocalisation of MLH3 in the empty vector control and *ZYP1*^{RNAi} lines.

(A, E, I, M, Q, U) MLH3 foci (red); (B, F, J, N, R, V) ASY1 (green); (C, G, K, O, S, W) DAPI (blue); and (D, H, L, P, T, X) merged images. Bar =10 µm.

Transgenic line	Resistant	Sensitive	Not germinating	% Sensistive (of germinated seeds)
ZYP1^{RNAi} 15	15	3	2	16.7
ZYP1^{RNAi} 24	11	3	6	21.4
ZYP1^{RNAi} 30	16	3	1	15.8
ZYP1^{RNAi} 35	14	4	2	22.2
ZYP1^{RNAi} 43	15	4	1	21.1
EV	18	2	0	10

Supplemental Table 1: Segregation of hygromycin resistance in the T1 seed of *ZYP1^{RNAi}* lines and empty vector control line (EV) when seeds were germinated on hygromycin-containing medium.

For lines with a single transgenic locus, 25% of segregating T1 seedlings will have lost the transgenic locus and be hygromycin sensitive. For lines with two transgenic loci, only 6.25% of segregating T1 seed will have lost both transgenic loci and be hygromycin sensitive. The percentage of sensitive seedlings for all of the *ZYP1^{RNAi}* lines is closest to 25%, indicating that these lines have a single transgenic locus and this was confirmed by FISH (see Supplemental Figure 1). (Note: These analyses can not determine how many transgenes have inserted at each transgenic locus).

Plant line	γH2AX[1]	RAD51[2]	MSH4 early[3]	MSH4 late[4]	MLH3[5]
EV	465 ± 15	395 ± 11	394 ± 6	23 ± 1.5	15 ± 2.4
ZYP1^{RNAi}*15*	521 ±24	392 ± 13	342 ± 30	41 ± 9	3.0 ± 0.5
ZYP1^{RNAi}*24*	518 ± 35	388 ± 32	376 ± 46	39 ± 2	2.0 ± 0.5
ZYP1^{RNAi}*30*	484 ± 50	416 ± 22	406 ± 18	45 ± 3	2.0 ± 0.2
ZYP1^{RNAi}*35*	473 ± 24	-	450 ± 53	53 ± 6	1.5 ± 0.7
ZYP1^{RNAi}*43*	437 ± 57	-	466 ± 25	53 ± 6	1.0 ± 0.3

Supplemental Table 2: Quantification of recombination protein loading in the empty vector control and *ZYP1*^{RNAi} lines.

Numbers represent average number of foci labelled per cell with antibodies raised against γH2AX, RAD51, MSH4 or MLH3 at leptotene (γH2AX; RAD51), mid-leptotene (MSH4 early), late leptotene (MSH4 late) and zygotene (MLH3) (n = 5–10). Standard error is given in brackets.

[1]For exemplar images see Supplemental Figure 6
[2]For exemplar images see Supplemental Figure 7
[3]For exemplar images see Supplemental Figure 8
[4]For exemplar images see Supplemental Figure 9
[5]For exemplar images see Supplemental Figure 10

Target gene	Oligonucleotide	5′-Sequence-3′ *
ZYP1	attB1-HvZYP1	GGGGACAAGTTTGTACAAAAAAGCAGGCTAGTCAACAAGTTGACCAGGAAGTG
ZYP1	attB2-HvZYP1	GGGGACCACTTTGTACAAGAAAGCTGGGTATATGACAGCAGCTGGACGGTATC
Hygromycin	HygF	TTGCATCGGCCGCGCTCCCGATTC
Hygromycin	HygR	TCGACCCTGCGCCCAAGCTGCATC
ZYP1	HvZYP1L	CTTCGGAACTTCAAGGGAGA
ZYP1	HvZYP1R	TTCTGGTTTTCAGCAAGGCTA
SKP1	HvSKP1L	TTGACCAGGCAACCCTCTT
SKP1	HvSKP1R	GCCCCTTGATGTTGAGGTAG

Supplemental Table 3. Oligonucleotides used in this study

* attB1 and attB2 sequences are underlined.