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Supporting Information for

HEI10 is Subject to Phase Separation and Mediates RPA1a Degradation during Meiotic Interference-sensitive Crossover Formation

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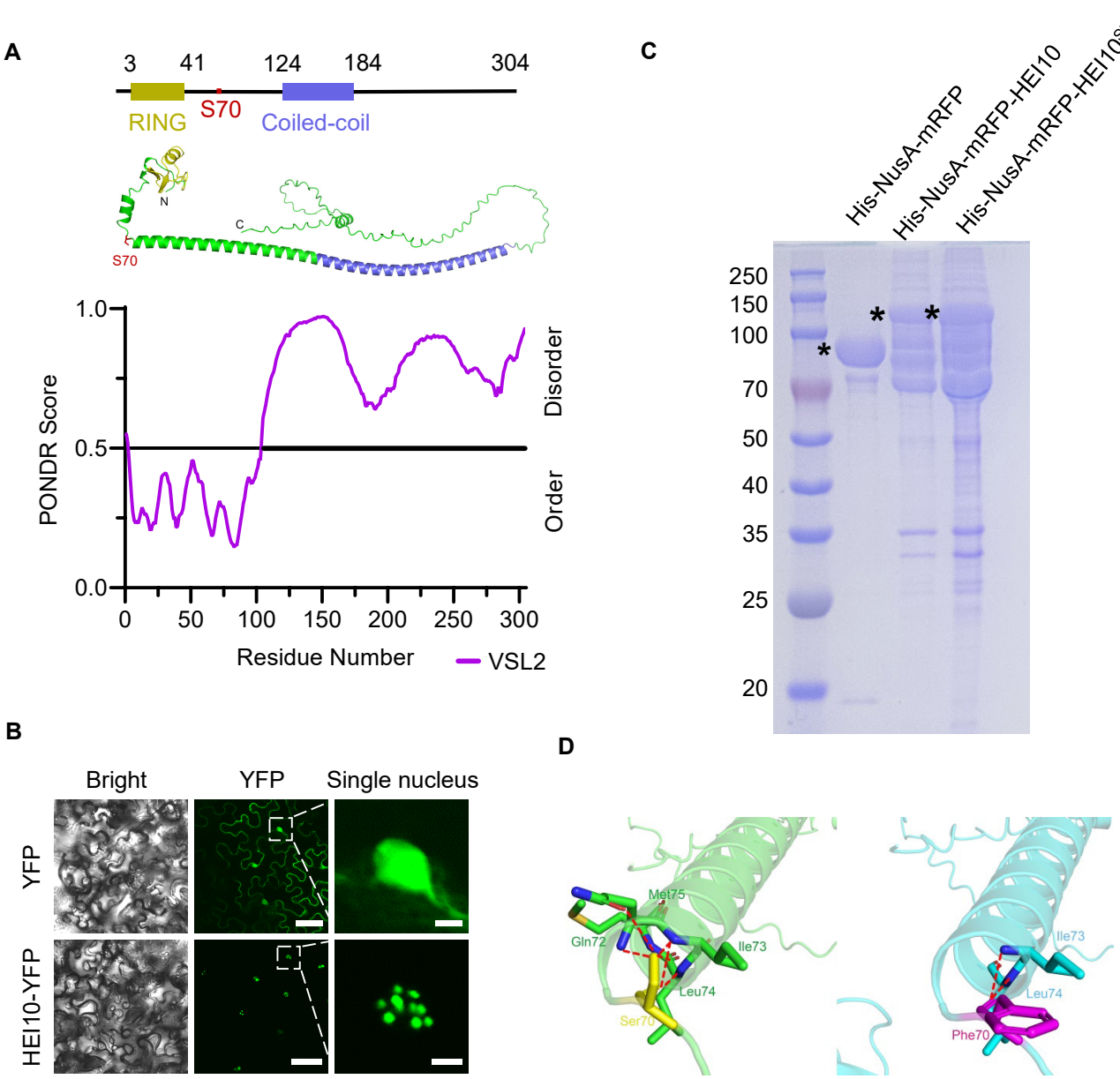

Fig. S1. Characteristics and localization of the HEI10 protein

(A) Top, protein domain structure of HEI10. Middle, 3D structure of HEI10. Bottom, predictions of IDR (intrinsically disordered region), by PONDR (www.pondr.com).

(B) Localization of HEI10-YFP in tobacco nuclei by fluorescence microscopy after treated with MMS. The images of single nucleus in the right pane are enlarged regions in the yellow boxes. Bar= 50μm (left), 5μm (right).

(C) Coomassie staining of His-NusA-mRFP, His-NusA-mRFP-HEI10 and His-NusA-mRFP-HEI10^{S70F}. Asterisks indicate the fusion protein bands.

(D) The hydrogen bonding interactions of the residue 70 and adjacent α-helix predicted by ESMFold. The hydrogen-bonding interactions are shown with red dotted lines. The Ser70 residue (yellow) can interact with Gln72, Ile73, Leu74 and Met75, and the Phe70 (purple) residue can only interact with Ile73 and Leu74.

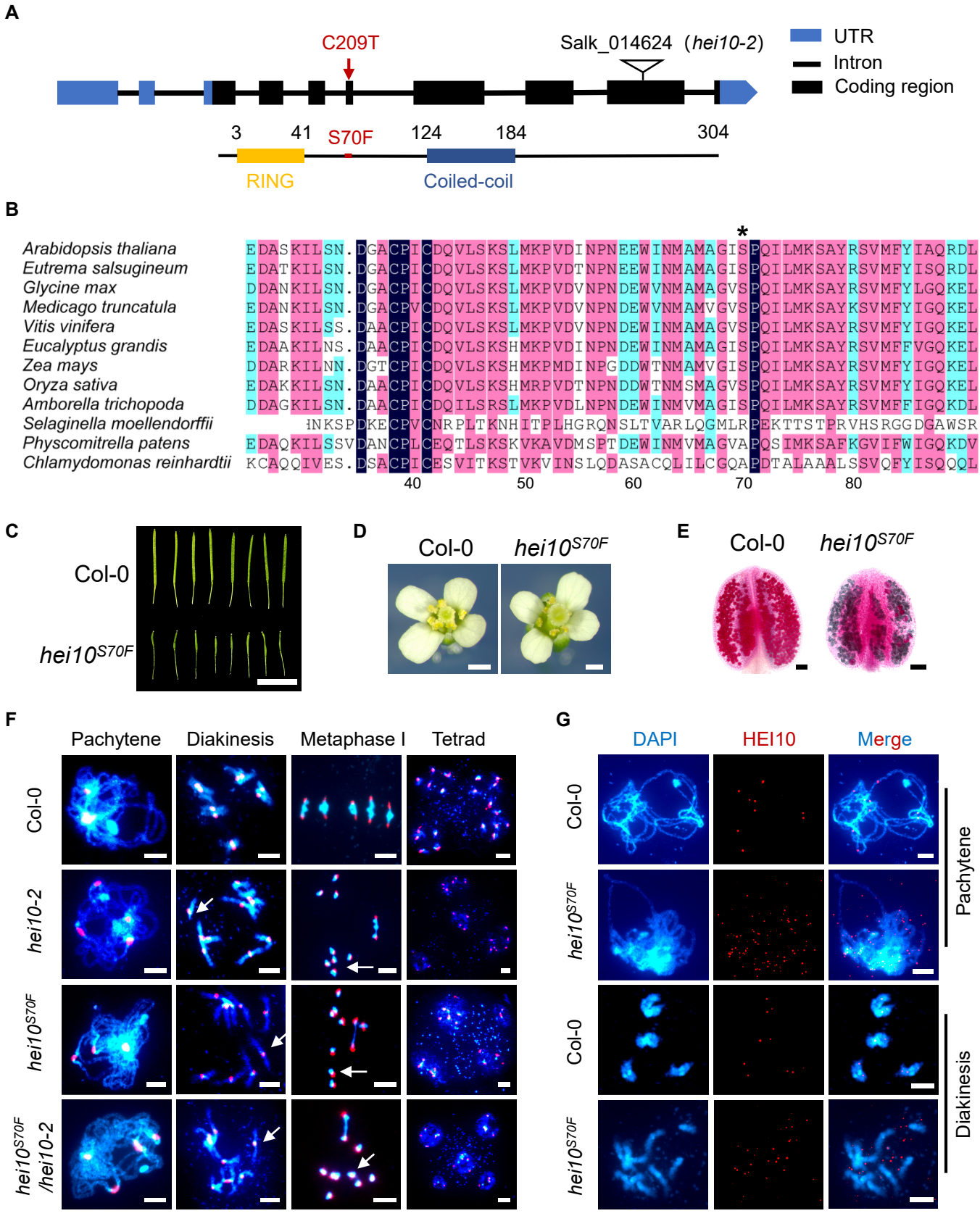

Fig. S2. *hei10*^{S70F} exhibits meiotic defects similar to *hei10-2* allele

(A) Diagrams showing genomic and protein structures of HEI10. Mutations of *hei10-2* and *hei10*^{S70F} are marked above the gene structure.

(B) Multiple sequence alignment of HEI10 orthologs in plants using DNAMAN 7.0. Asterisk indicates the Ser70 site.

(C) Siliques of Col-0 and *hei10*^{S70F}. Bar=1cm.

(D) Open flowers of Col-0 and *hei10*^{S70F}. Bar=1mm.

(E) Alexander staining of Col-0 and *hei10*^{S70F} anthers. Bar=50μm.

(F) The centromere FISH of meiotic chromosomes at pachytene, diakinesis, metaphase I and tetrad stage in Col-0, *hei10-2*, *hei10*^{S70F} and *hei10*^{S70F}*/hei10-2*. Bar=5μm. Arrows indicate univalents at diakinesis and metaphase I.

(G) Immunostaining of HEI10 at pachytene and diakinesis in Col-0 and *hei10*^{S70F} with HEI10 antibody. Bar=5μm.

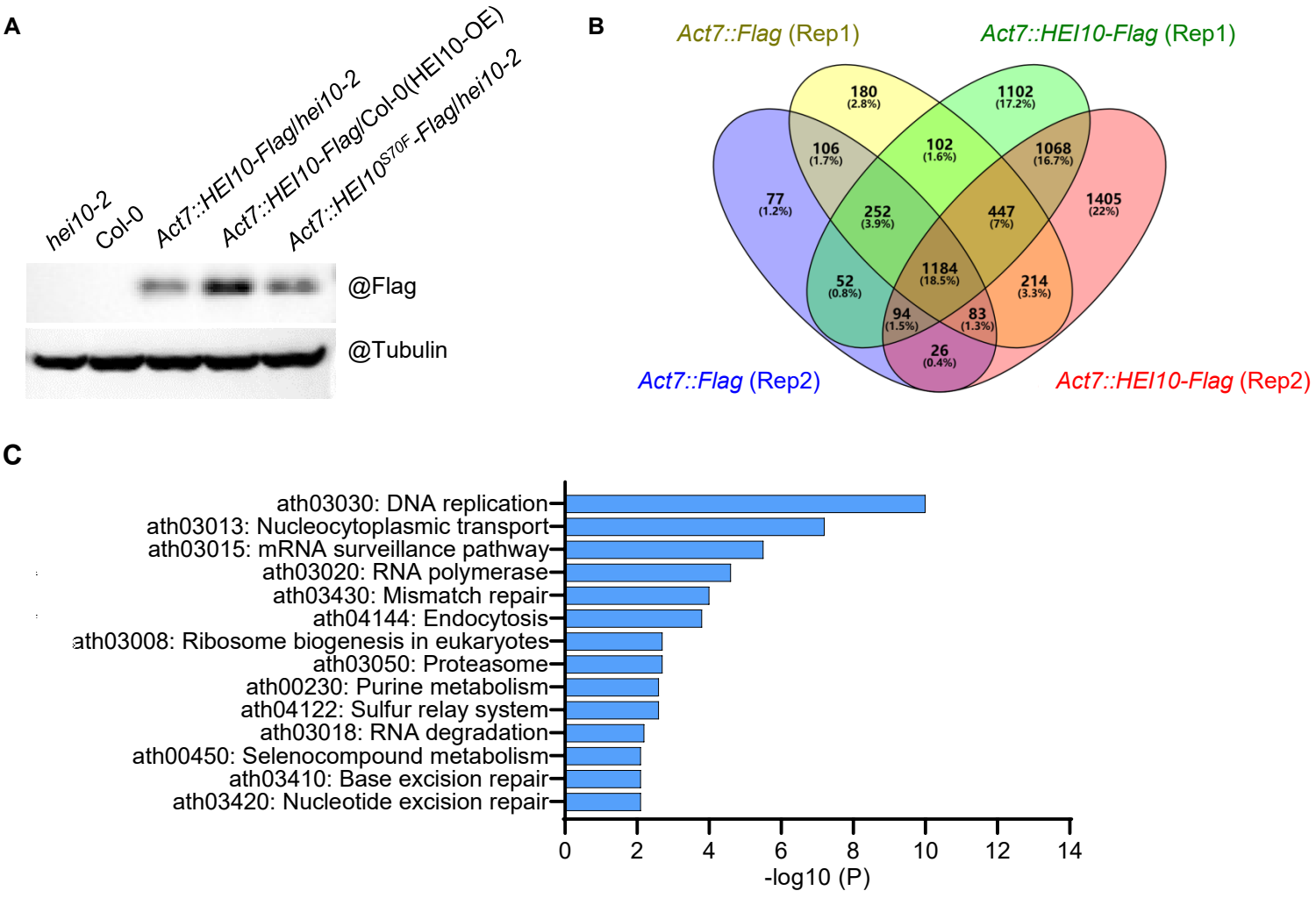

Fig. S3. Identification of HEI10 interacting proteins by IP-MS

(A) Detection of the HEI10 protein levels in HEI10 transgenic lines by anti-Flag antibody.

(B) Venn diagram for the identified proteins by IP-MS using the inflorescences of *Act7::HEI10-Flag* and *Act7::Flag*. The venn diagram is drawn by Venny 2.1 (https://bioinfogp.cnb.csic.es/tools/venny/).

(C) KEGG pathway enrichment of the 1,068 candidates identified in (B) is analyzed by Metascape (https://metascape.org).

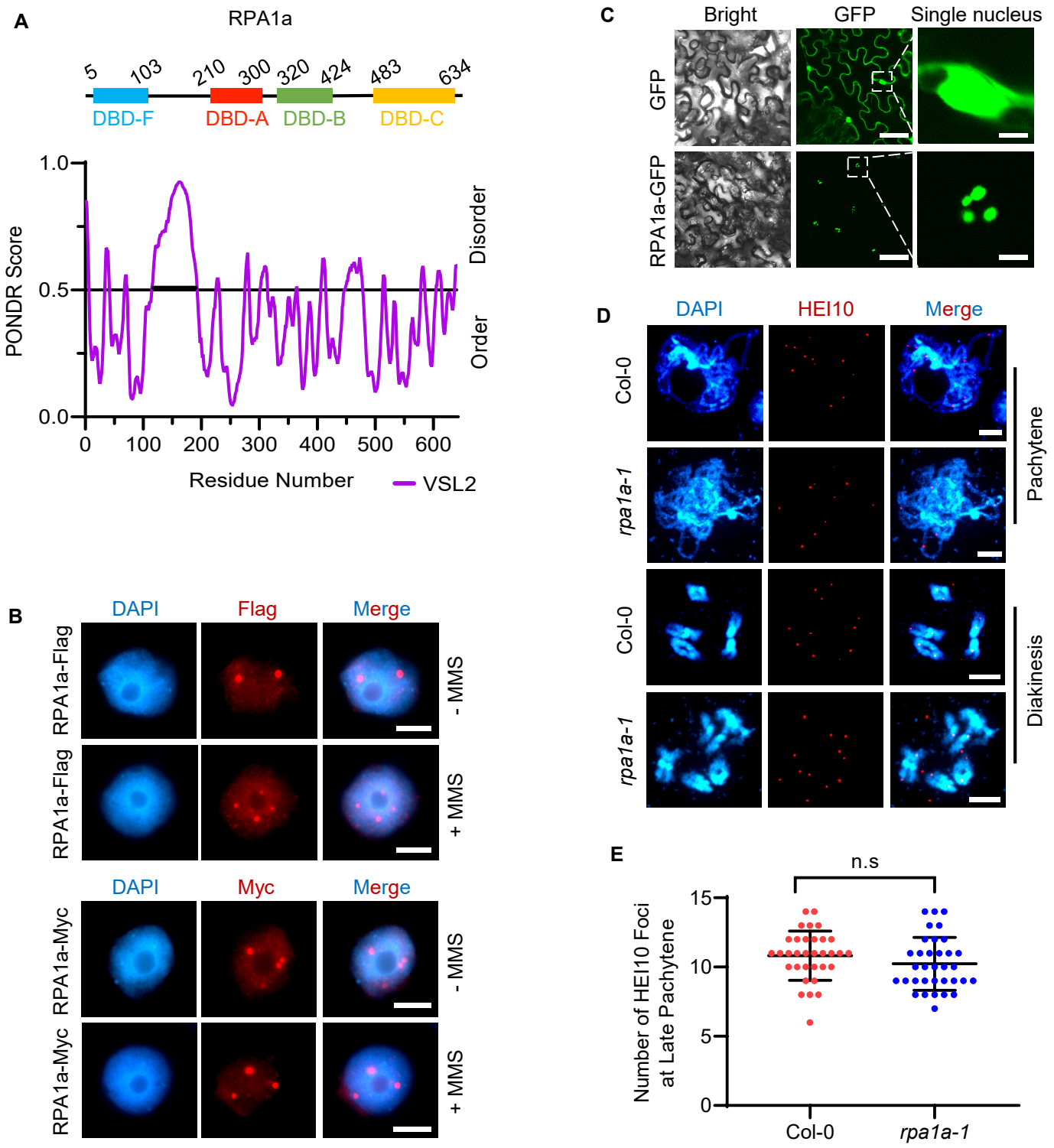

Fig. S4. RPA1a can undergo phase separation independent of HEI10 *in vivo*

(A) Top, protein domain structure of RPA1a. Bottom, predictions of IDRs by PONDR.

(B) Immunostaining of tobacco nuclei that express RPA1a-Flag/Myc. Bar=5μm.

(C) Localization of RPA1a-GFP in tobacco nuclei by fluorescence microscopy. The images of single nucleus in the right pane are enlarged regions in the white boxes. Bar= 50 μm (left), 5 μm (right).

(D) Immunostaining of HEI10 in Col-0 and *rpa1a-1* at pachytene and diakinesis. Bar=5μm.

(E) Statistical analysis of the HEI10 foci at late pachytene in Col-0 and *rpa1a-1* (two-tailed student's t-test).

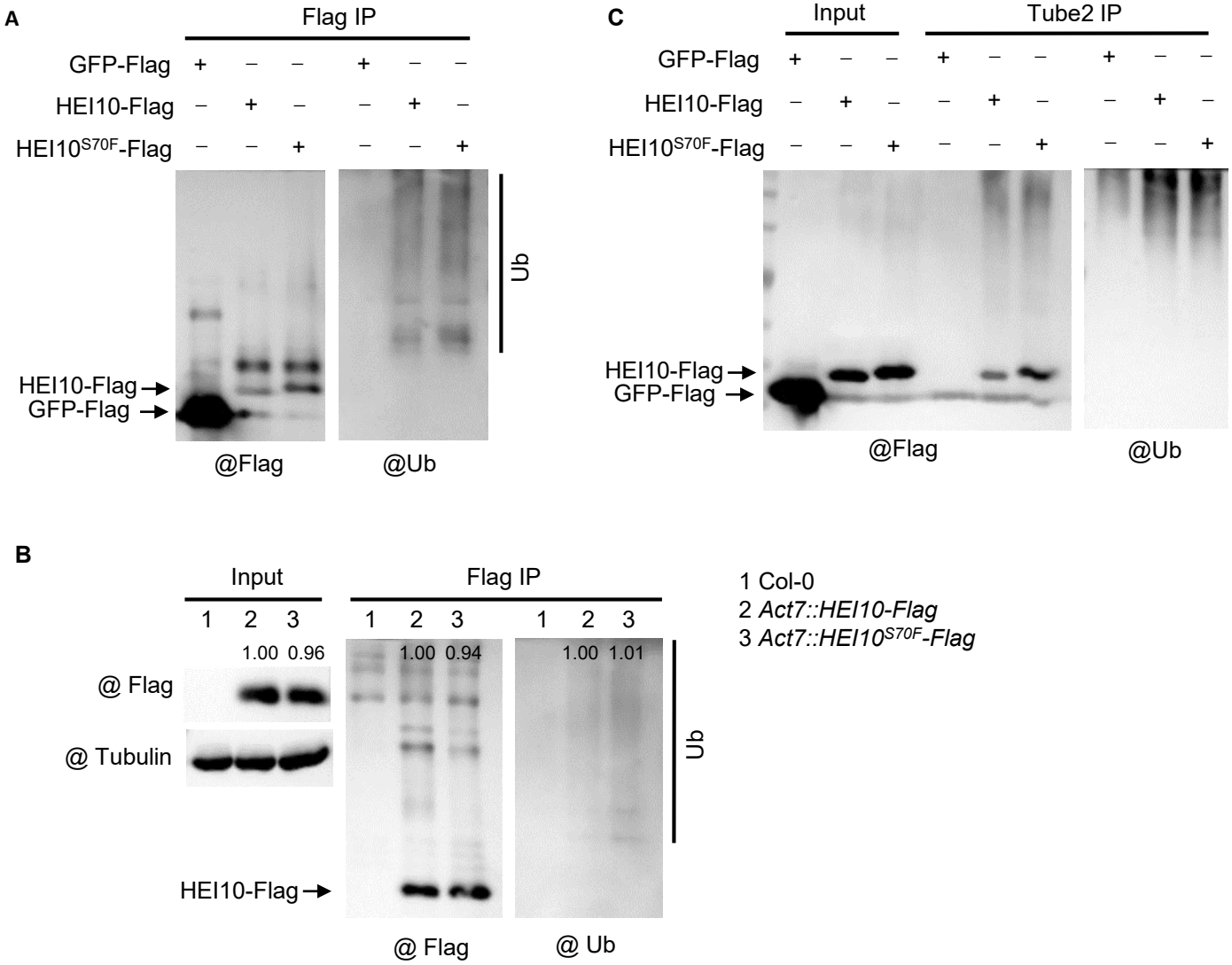

Fig. S5. HEI10 has the ubiquitination E3 ligase activity

(A) Detection of ubiquitination levels of HEI10 and HEI10^{S70F} immunoprecipitated in tobacco cells by anti-Flag and anti-UBQ11 antibodies. GFP-flag is used as the negative control.

(B) Detection of ubiquitination levels of HEI10 and HEI10^{S70F} immunoprecipitated in *Arabidopsis* central inflorescence by anti-Flag and anti-UBQ11 antibodies. The Col-0 sample is used as negative control.

(C) Tube2 IP detects the enrichment of ubiquitin-modified HEI10 proteins in tobacco cells. GFP-flag is used as the negative control.

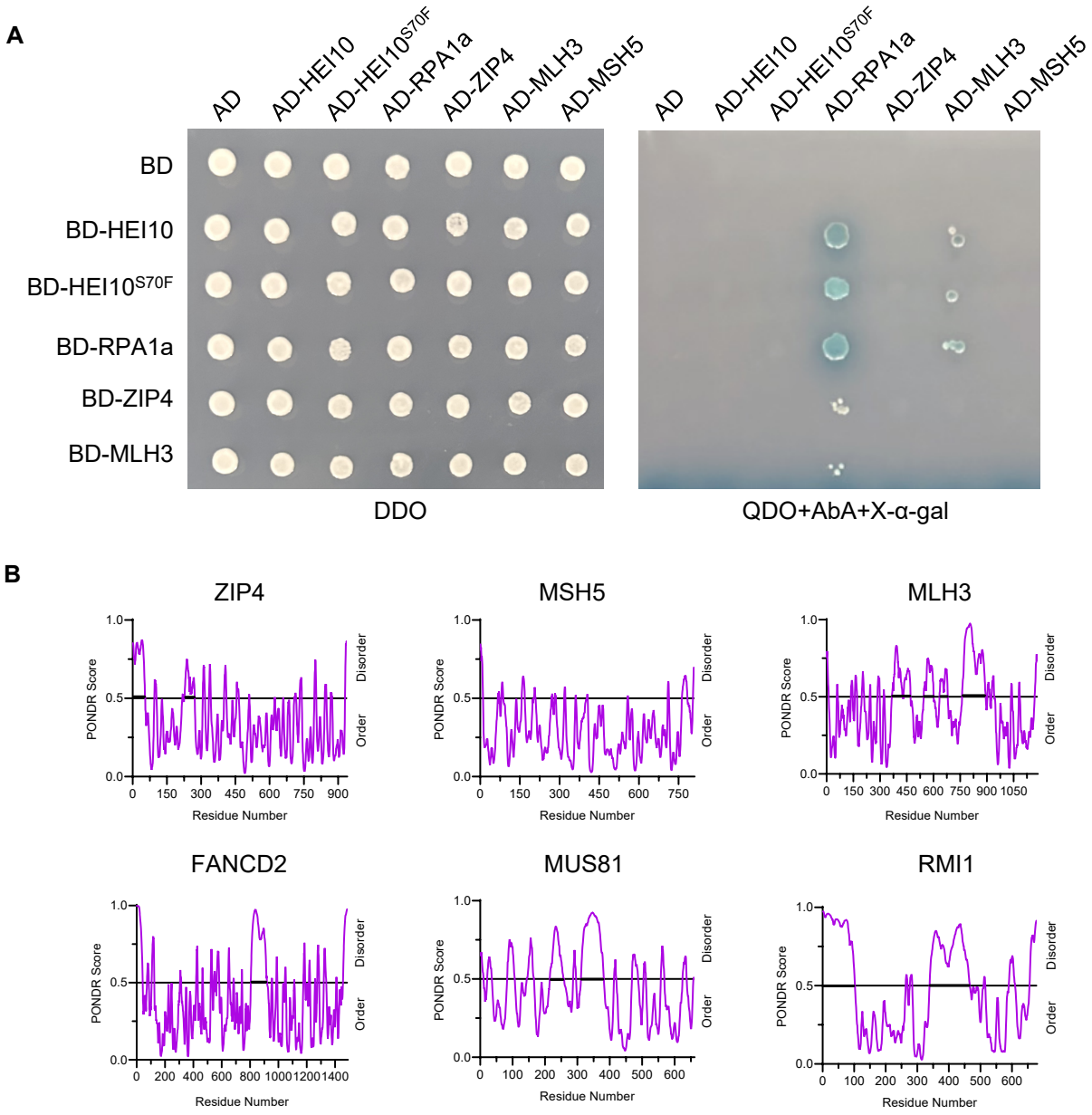

Fig. S6. Interaction of HEI10 with Class I CO proteins and prediction of meiotic recombination factors' IDRs
(A) Interaction of HEI10 with RPA1a, ZIP4, MSH5 and MLH3 in yeast two-hybrid assay. The initial concentration is "OD_{600}≈0.5", spotted on DDO (synthetic dropout media lacking leucine and tryptophan) and QDO (synthetic dropout media lacking leucine, tryptophan, histidine and adenine) for 5 days.
(B) Prediction of IDRs for other meiotic recombination factors by PONDR.

Table S1. List of meiotic genes identified by IP-MS of HEI10

Identified gene	Description	Sum PEP Score		
		Rep1	Rep2	Average
AT4G01370	ATMPK4	6.976	75.69	41.333
AT5G03470	Protein phosphatase 2A regulatory B subunit family protein	3.116	73.783	38.4495
AT3G48750	CDC2, CDC2A, CDC2AAT, CDK2, CDKA1, CDKA;1, CELL DIVISION CONTROL 2	23.655	45.75	34.7025
AT2G47980	SCC3	3.362	43.982	23.672
AT1G75950	ASK1/SKP1/UIP1	11.96	29.697	20.8285
AT3G12280	ATRBR1	5.126	19.186	12.156
AT2G27170	SMC3	2.72	18.261	10.4905
AT5G22010	ATRFC1	9.668	5.088	7.378
AT3G54670	ATSMC1	4.931	9.571	7.251
AT2G06510	ATRPA1A	5.252	8.99	7.121
AT5G19400	SMG7	2.586	3.84	3.213

Table S2. List of primers used in this study

Primers for transgene expression	
35S/Act7-HEI10-Flag-BamHI-F	GAGCTCGGTACCCGGGGATCCATGAGATGCAACGCGTGTT
35S/Act7-HEI10-Flag-SalI-R	GATCCAAGGGCGAATTGGTCGACTAGCGTGAACAGCTGAGGG
35S-RPA1a-Flag-KpnI-F	CGGGGGACGAGCTCGGTACCATGCCGGTGAGTTTGACTCC
35S-RPA1a-Flag-BamHI-R	TGGTCGACTCTAGAGGATCCCCTTACGAGCAAATCAAGCA
35S-RPA1a-Myc-SacI-F	AGAACACGGGGGACGAGCTCATGCCGGTGAGTTTGACTCC
35S-RPA1a-Myc-SalI-R	ATGAGCTTTTGCTCGTCGACCCTTACGAGCAAATCAAGCA
35S-RPA1a-GFP-KpnI-F	CGGGGGACGAGCTCGGTACCATGCCGGTGAGTTTGACTCC
35S-RPA1a-GFP-BamHI-R	ATGTCGACTCTAGAGGATCCCCTTACGAGCAAATCAAGCA
35S-GFP-Flag-BamHI-F	AGCTCGGTACCCGGGGATCCATGGTGAGCAAGGGCGAGGA
35S-GFP-Flag-SalI-R	CCAAGGGCGAATTGGTCGACCTTGTACAGCTCGTCCATGCC
35S-ZIP4-GFP-KpnI-F	CGGGGGACGAGCTCGGTACCATGAGAATCGCCGAGATCAC
35S-ZIP4-GFP-BamHI-R	ATGTCGACTCTAGAGGATCCAGCAGAAGAAACTTTGGTCT
35S-MSH5-GFP-KpnI-F	CGGGGGACGAGCTCGGTACCATGGAGGAAATGGAAGACAC
35S-MSH5-GFP-BamHI-R	ATGTCGACTCTAGAGGATCCGGAAGTGAAGATATCTTGAA
35S-MLH3-GFP-KpnI-F	CGGGGGACGAGCTCGGTACCATGAAGACGATCAAGCCCTT
35S-MLH3-GFP-BamHI-R	ATGTCGACTCTAGAGGATCCACTTTTAGCGTTGTCTAAGC
35S-FANCD2-GFP-KpnI-F	CGGGGGACGAGCTCGGTACCATGGTGTTTCTCTCTCGCAA
35S-FANCD2-GFP-BamHI-R	ATGTCGACTCTAGAGGATCCAGGTGTCAATGGAAGTTCAT
35S-MUS81-GFP-KpnI-F	CGGGGGACGAGCTCGGTACCATGGATGACGAGAGACGGGT
35S-MUS81-GFP-BamHI-R	ATGTCGACTCTAGAGGATCCTTCACCCCAAACTAACTTGA
35S-RMI1-GFP-KpnI-F	CGGGGGACGAGCTCGGTACCATGCGTAGACGGCGCCTGCA

35S-RMI1-GFP-BamHI-R	ATGTCGACTCTAGAGGATCCAGGGGACAGAACAACAACTG
Primers for Yeast Two Hybrid	
AD-RPA1a-F	GTACCAGATTACGCTCATATGATGCCGGTGAGTTTGACTCC
AD-RPA1a-R	CAGCTCGAGCTCGATGGATCCTTACCTTACGAGCAAATCAAGCATGT
BD-RPA1a-F	TCAGAGGAGGACCTGCATATGATGCCGGTGAGTTTGACTCC
BD-RPA1a-R	CCGCTGCAGGTCGACGGATCCTTACCTTACGAGCAAATCAAGCATGT
AD-HEI10-F	GTACCAGATTACGCTCATATGATGAGATGCAACGCGTG
AD-HEI10-R	CAGCTCGAGCTCGATGGATCCCTATAGCGTGAACAGCTGAGG
BD-HEI10-F	TCAGAGGAGGACCTGCATATGATGAGATGCAACGCGTG
BD-HEI10-R	CCGCTGCAGGTCGACGGATCCCTATAGCGTGAACAGCTGAGG
AD-ZIP4-F	TACCAGATTACGCTCATATGATGAGAATCGCCGAGATCAC
AD-ZIP4-R	AGCTCGAGCTCGATGGATCCAGCAGAAGAAACTTTGGTCT
BD-ZIP4-F	CAGAGGAGGACCTGCATATGATGAGAATCGCCGAGATCAC
BD-ZIP4-R	CGCTGCAGGTCGACGGATCCAGCAGAAGAAACTTTGGTCT
BD-MLH3-F	CAGAGGAGGACCTGCATATGATGAAGACGATCAAGCCCTT
BD-MLH3-R	CGCTGCAGGTCGACGGATCCACTTTTAGCGTTGTCTAAGC
AD-MLH3-F	TACCAGATTACGCTCATATGATGAAGACGATCAAGCCCTT
AD-MLH3-R	AGCTCGAGCTCGATGGATCCACTTTTAGCGTTGTCTAAGC
AD-MSH5-F	TACCAGATTACGCTCATATGATGGAGGAAATGGAAGACAC
AD-MSH5-R	AGCTCGAGCTCGATGGATCCGGAAGTGAAGATATCTTGAA
Primers for recombinant protein expression	
His-Sumo-HEI10-F	ACAGAGAACAGATTGGTGGATCCATGAGATGCAACGCGTG
His-Sumo-HEI10-R	GCGGCCGCAAGCTTGTCGACTAGCGTGAACAGCT

	GAGG
GST-RPA1a-F	ATCTGGTTCCGCGTGGATCCATGCCGGTGAGTTTG ACTCC
GST-RPA1a-R	GATGCGGCCGCTCGAGTCGACTTACCTTACGAGCA AATCAAGC
His-Sumo-RPA1a-F	ACAGAGAACAGATTGGTGGATCCATGCCGGTGAGT TTGACTCC
His-Sumo-RPA1a-R	GCGGCCGCAAGCTTGTCGACCCTTACGAGCAAATC AAGCAT
His-NusA-mRFP-F	TCCTCTTTCAGGGACCCGGGATGGCCTCCTCCGA GGACGT
His-NusA-mRFP-R	TAGGTTAATTAAGCCTCGAGGGCGCCGGTGGAGTG GCGGC
mRFP-linker-HEI10-F	GGTCCGTCTGGACCGAGCGGCCCGTCAGGTCCGA GTATGAGATGCAACGCGTGTTG
mRFP-linker-HEI10-R	ACTCGGACCTGACGGGCCGCTCGGTCCAGACGGA CCGGCGCCGGTGGAGTGGCGGC
His-NusA-mRFP-HEI10-R	TAGGTTAATTAAGCCTCGAGTAGCGTGAACAGCTGA GGGC
Primers for genotyping mutant alleles	
hei10-2-LP	GCAAGGAGTTCCCAGAGATG
hei10-2-RP	CCAAGAACCCGACTTTTTCTC
rpa1a-1-LP	GCCAGGAGAGGTATCGTTTC
rpa1a-1-RP	TCGACCTTGGTATGGATTGAG
LBb1.3	ATTTTGCCGATTTCGGAAC