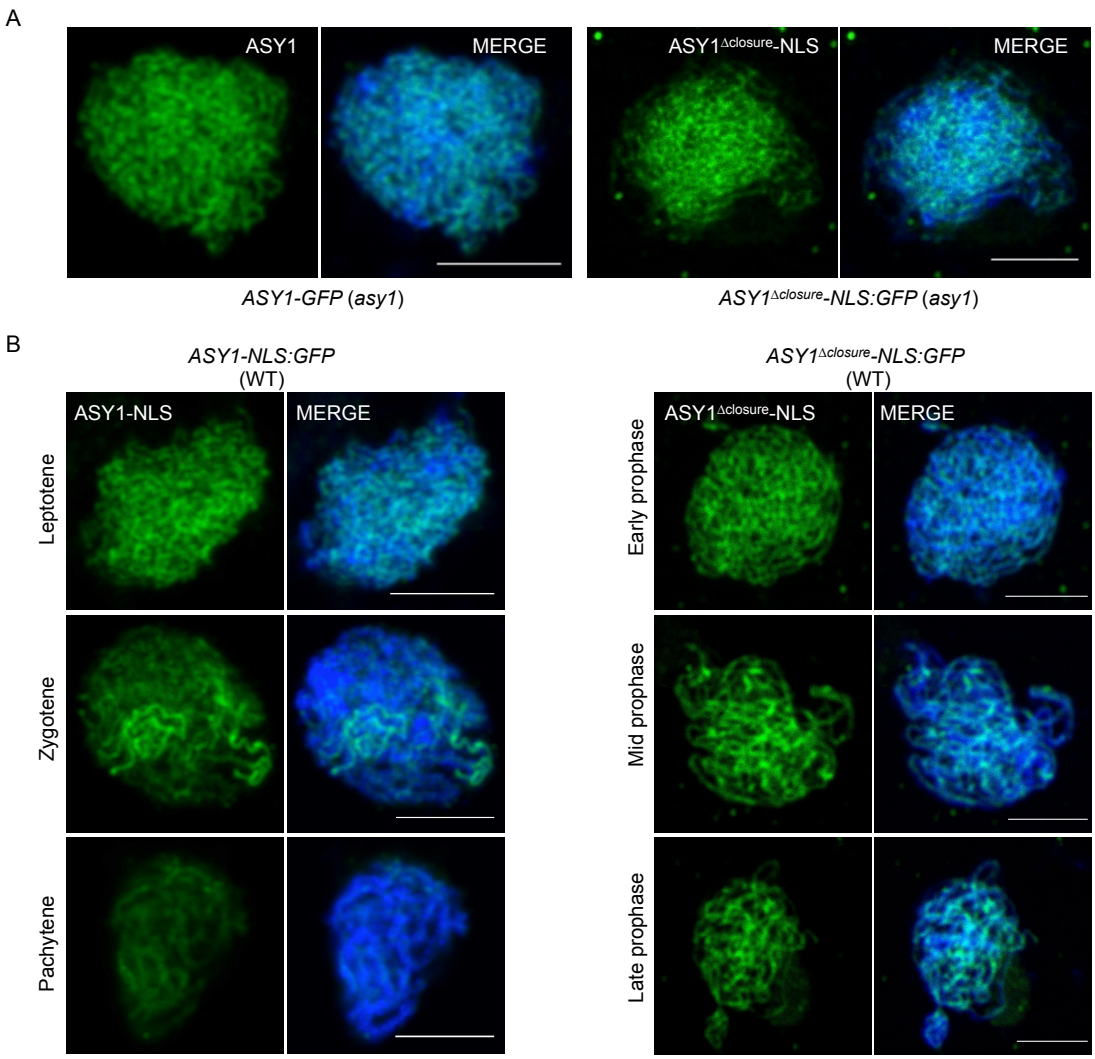
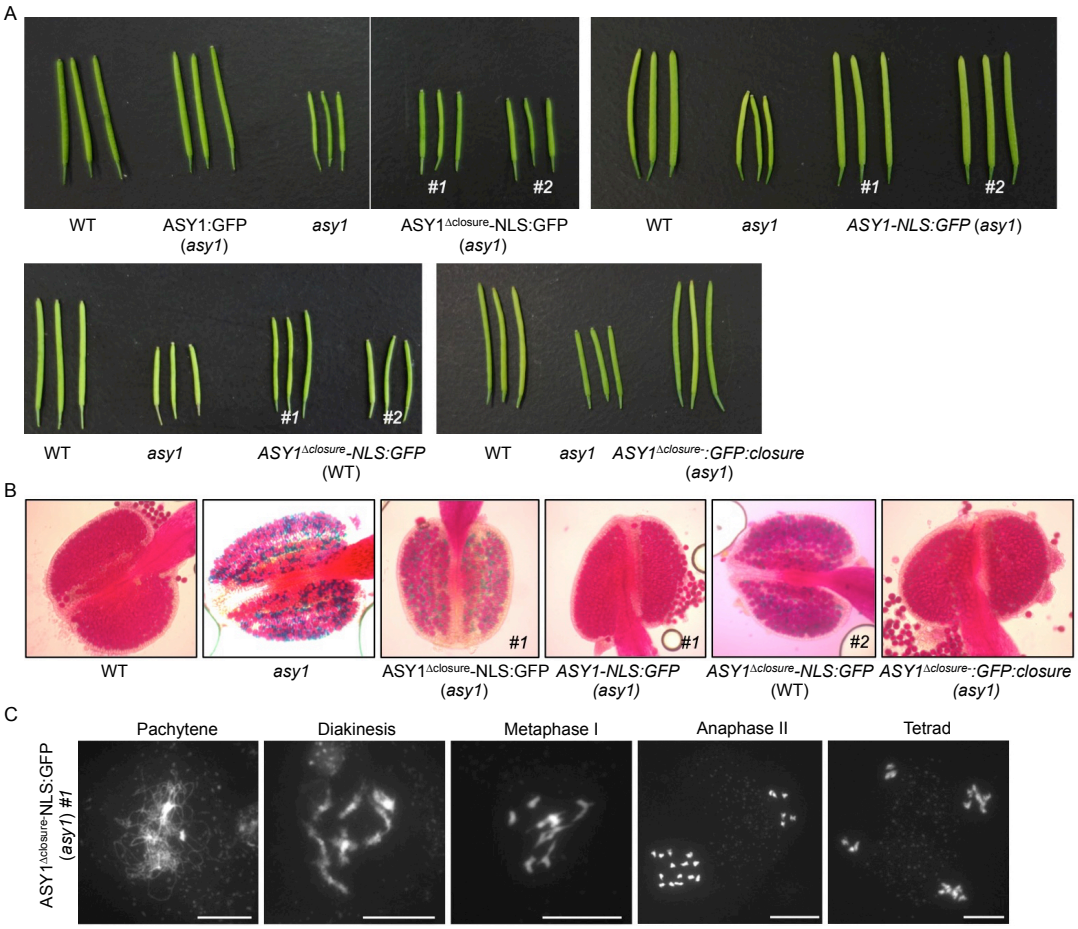
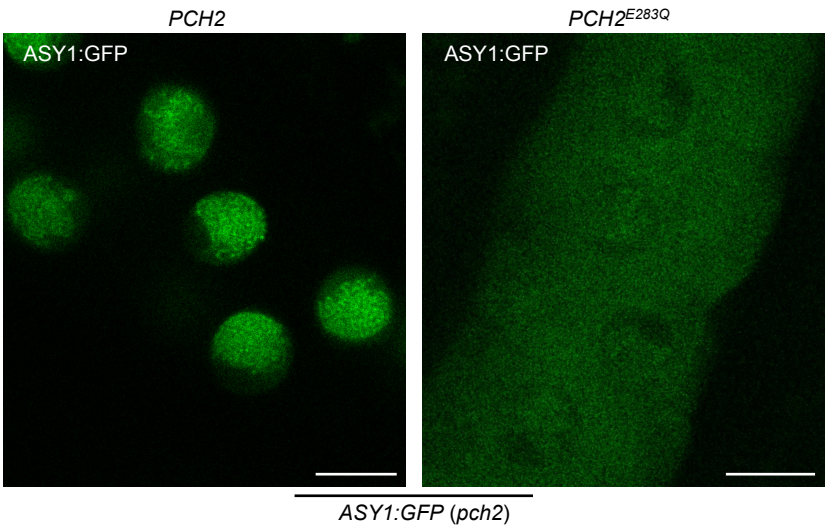


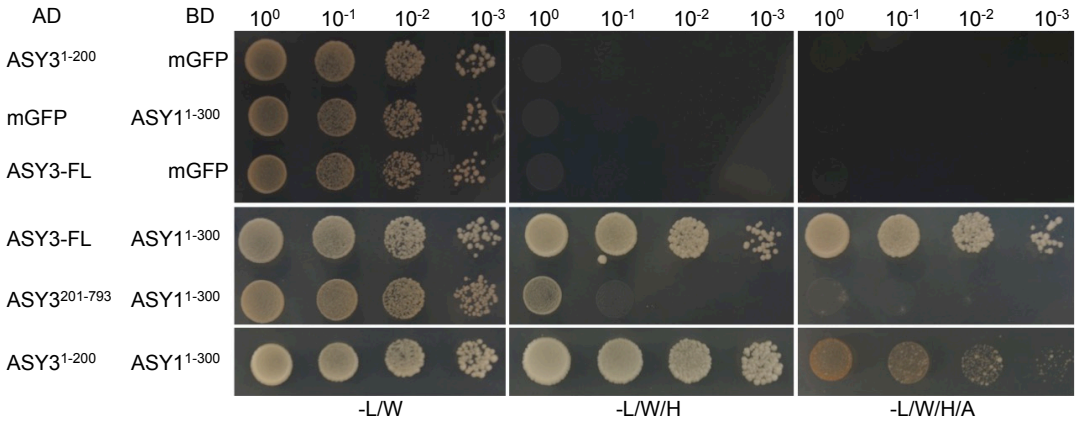
Supplemental Figure 1. The closure motif of ASY1 is not required for its chromosomal localization. (A) Immunolocalization of ASY1:GFP and ASY1$^{\Delta closure}$-NLS:GFP at early prophase in male meiocytes of *asy1* mutants using antibody against GFP. MERGE shows the overlay of ASY1 signal and DAPI-stained DNA. Bar: 5 µm. (B) Immunolocalization of ASY1-NLS:GFP and ASY1$^{\Delta closure}$-NLS:GFP at different prophase stages in male meiocytes of wild-type (WT) plants. Bar: 5 µm.



Supplemental Figure 2. Phenotypic analysis of different *ASY1* alleles. (A) Siliques of wild-type (WT), *asy1* mutant, *ASY1:GFP*(*asy1*), *ASY1$^{\Delta closure}$-NLS:GFP*(*asy1*), *ASY1-NLS:GFP*(*asy1*), *ASY1$^{\Delta closure}$-NLS:GFP*(WT), and *ASY1$^{\Delta closure}$:GFP:closure*(*asy1*) plants. (B) Peterson staining for the pollens from the *ASY1* alleles shown in (A). Blue indicates aborted pollen grains. (C) Chromosome spread analysis of *asy1* mutants harboring *ASY1$^{\Delta closure}$-NLS:GFP* at different meiotic stages. Bar: 10 μm.



Supplemental Figure 3. Localization of ASY1:GFP at early prophase in the male meiocytes of *pch2* mutants harboring the wild-type version of *PCH2* or the *PCH2*^{E283Q}. Bar: 10 μm.



Supplemental Figure 4. Yeast two-hybrid assay for the interaction of ASY1 HORMA domain with different versions of ASY3. Yeast cells co-transformed with the relevant AD- and BD-tagged proteins were grown on plates of double (-L/W), triple (-L/W/H) and quadruple (-L/W/H/A) dropout medium with different dilutions for 3 days.

Supplemental table 1. Primers used in this research.

Primer name	Sequnence (5' to 3')
gASY1 1-570aa-R	CTGTGAGGCTTGGCTACAGTTGACTGTC
mGFP-F	CCCGGGGGTGGCatggtgagcaagggcgaggagc
gASY1 1-570aa-R+NLS	CACCTTTCTCTTCTTCTTTGGCTGTGAGGCTTGGCTACAGTTGACTGTC
gASY1-promoterATG-R	CATttttgcagaagtgtgaaacgaataacgag
gASY1-NLS-571aa-F	CCAAAGAAGAAGAGAAAGGTGGACAGACGTGGCAGGAAAACCAGC
gASY1-R+NLS	CACCTTTCTCTTCTTCTTTGGATTAGCTTGAGATTTCTGACGCTTGG
gASY1-11aa-F	GAGATCACTGAGCAGGACTCGCTTCTTCTGg
gASY1-intron1-R	ctggaggtaagaaacaaaacgaaacgctaaaatcagactg
ASY1-intron2-F	gtaagctacgccgatcatcgagcttttgagtttttgtttc
ASY1-TGA-F	AAATCTCAAGCTAATTGAagacaccacctctatcagacaccataaccacc
mGFP-SmaI-R	CCTGCCACGTCTGTCCCCGGGTCCACCTCCcttgtacagctcgtccatg
ASY1 571-596aa-F	GGAGGTGGACCCGGGGACAGACGTGGCAGGAAAACCAGCATGGTGAGGGAGCCTATTCTGCAGTACT CCAAGCGTCAGAAATCTCAAGCTAATTGAagacaccacctc
ASY1 571-596aa-R	gaggtggtgtctTCAATTAGCTTGAGATTTCTGACGCTTGGAGTACTGCAGAATAGGCTCCCTCACCATG CTGGTTTTCCTGCCACGTCTGTCCCCGGGTCCACCTCC
NLS-SmaI-F	CCAAAGAAGAAGAGAAAGGTGCCCGGGGGTGGC
ASY3-SLICE-F	AGTCAACTGTAGCCAAGCCTCACAGATGAGCGACTATAGAAGCTTCGGCA
ASY3-SLICE-50aa-R	CGGGCACCTTTCTCTTCTTCTTTGGCAACTTTTCTACTCTAGCAATAAC
ASY3-SLICE-100aa-R	CGGGCACCTTTCTCTTCTTCTTTGGCTCAAGAGTCCCTAATTTCCGATG
ASY1 T184G CDS-F	GGGCCACCAGATTACGAGCCACCTT
ASY1 T184-R	CACATCATCGTAGTACAGAAGCTTC
ASY1-11aa-F	GAGATCACTGAGCAGGACTCGCTTCTTCTG
ATG-attL1-R2	CATGAAGCCTGCTTTTTTGTACAAAGTTGG
ASY1-21aa-F	ACTAGAAATTTGCTTCGTATTGCTATCTTC
ASY3-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGAGCGACTATAGAAGCTTCGGCA
ASY3-200aa-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCACAGTTTTGATCTCAAAACATCAGT
ASY3-201aa-attB1-F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTCTGGGAGATATTGGGAAAAGCTTCAC
ASY3-attB2-R	GGGGACCACTTTGTACAAGAAAGCTGGGTTATCATCCCTCAAACATTCTGCGACA