

Supplementary Table S1
Primers used in this study

Purpose	Primer name	Sequence (5'-3')
CRISPR		
CRISPR for BORR	BORR_CRISPR_F	attgGCTTCGTTTCGATTTTACTG
	BORR_CRISPR_R	aaacCAGTAAAATACGAACGAAGC
Genotyping		
1st PCR for <i>borr-1</i>	BORR_dCAPS_F	GAGATTGAACAAATGTAACTGCATTGCGGTTGCTTCGTTTCGTAATTA
	BORR_check_R	CACATATGAGTGCATGATCATGATGTACG
2nd PCR for <i>borr-1</i>	BORR_dCAPS_F	GAGATTGAACAAATGTAACTGCATTGCGGTTGCTTCGTTTCGTAATTA
	BORR_dCAPS_R	TCATAGCCACCAAGAGATGACCTACTG
T-DNA of <i>wyr-2</i>	INCENP_T-DNA check_F	AGATAGAGAGAAAACGACATCGTATG
	GABI_LB	ATATTGACCATCATACTCATTGC
WT of <i>wyr-2</i>	INCENP_WT check_F	GCTACCTTGGTTTACCTGTTTCAGGGATC
	INCENP_WT check_R	TTGTGTGAGAGTTGTGTGTC
GFP	GFP_check_F	GAAGGGCATCGACTTCAAGG
	GFP_check_R	CTTGATACAGCTCGTCCATGCCGTG
RT-qPCR		
RT-qPCR for BORR	BORR_qRT_F	GTAGGTCATCTCTTGGTGGCTATG
	BORR_qRT_R	GGAAGCATTGGTTCTCAGATGTC
RT-qPCR for PP2AA3	PP2AA3_qRT_F	GCCAAAATGATGCAATCTCTC
	PP2AA3_qRT_R	CCGTATCATGTTCTCCACAAC
Cloning of GUS or GFP fusion constructs		
BORR	BORR_F	GAACGCTACTGATAAGGCTCCTTCTTC
	BORR_R	GTTACTGTGGCTCTGTTTCCAGAGCTAG
	pENTR2B for BORR_F	GCTCTGGAAACAGAGCCACAGTAACCGGCCGCACTCGAGATATCTAG
	pENTR2B for BORR_R	AAAGGAGCCTTATCAGTAGGCGTTTCGGATCCAGTCCGACTGAATTGGTTC
	Nter_SmaI_BORR_F	gggATGCCGAAGCGAAAAGCAAAGGAGTG
	Nter_SmaI_BORR_R	gggTATCGATTTCTTTCGAAGAATTAGATGC
	Cter_SmaI_BORR_F	gggTGAAAAGCTTCAACGATTCAGACAC
BORR ^{S214A}	Cter_SmaI_BORR_R	gggACTGTTTTCTCTGCAAAATC
	BORR_S214A_F	gCTCCTTTGGGGGTCTACAAAGAAG
BORR ^{S214D}	BORR_S214A_R	GCCATGCACAGAGAGCATCATCTC
	BORR_S214D_F	gaTCCTTTGGGGGTCTACAAAGAAG
BORR ^{S214D}	BORR_S214D_R	same as BORR_S214A_R
	BORR_S214D_F	same as BORR_S214A_R
INCENP	INCENP_F	GCAGAAAGATAGCAGTAAATCCGAGCCA
	INCENP_R	CGACATGGATAGAGCTGTTATCCATGTC
	pENTR2B for INCENP_F	ATGGATAACAGCTCTATCCATGTCGGCGGCCGCACTCGAGATATCTAG
	pENTR2B for INCENP_R	CTCGGATTTACTGCTATCTTTCTGCGGATCCAGTCCGACTGAATTGGTTC
	Nter_SmaI_INCENP_F	gggATGTTTTCCGTCAAGGAGAATCC
INCENP	Nter_SmaI_INCENP_R	gggCGCCGACGACCTACTCTAGAGATAG
Yeast two-hybrid assay		
Universal attB primers	attB1 adapter	GGGGACAAGTTTGTACAAAAAAGCAGGCT
	attB2 adapter	GGGGACCACTTTGTACAAGAAAGCTGGGT
AUR1	cDNA_AUR1_F	CAAAAAAGCAGGCTCCACCATGGCGATCCCTACGGAGACACAAC
	cDNA_AUR1_R	CAAGAAAGCTGGGTTTAAACTCTGTAGATTCCAGAAGGATC
AUR2	cDNA_AUR2_F	CAAAAAAGCAGGCTCCACCATGGGGATTCTACAGAGACGCAGC
	cDNA_AUR2_R	CAAGAAAGCTGGGTTTCATCCTCTGTAAAGGCCTGATGGGTC
AUR3	cDNA_AUR3_F	CAAAAAAGCAGGCTCCACCATGAGTAAGAAATCGACAGAATCTGAC
	cDNA_AUR3_R	CAAGAAAGCTGGGTTTCAAATATCAATTGAGGCACACACACC
BORR	cDNA_BORR_F	CAAAAAAGCAGGCTCCACCATGCCGAAGCGAAAAGCAAAGGAGTG
	cDNA_BORR_R	CAAGAAAGCTGGGTTACTGTTTTCCTCATTATTGCTCCC
INCENP_Full	cDNA_INCENP_F	CAAAAAAGCAGGCTCCACCATGTTTTCCGTCAAGGAGAATCCGAGG
	cDNA_INCENP_R	CAAGAAAGCTGGGTTCTATCTCGACTGGAACCTTTCGCGGCAAAAG
INCENP_N	INCENP_N_F	same as cDNA_INCENP_F
	INCENP_N_R	CAAGAAAGCTGGGTTTAAATGTTGAGGTCTCTTTGTCAATGCATC
INCENP_C	INCENP_C_F	CAAAAAAGCAGGCTCCACCATGGAAACATCCTCTGAAAAAGCTGTTC
	INCENP_C_R	same as cDNA_INCENP_R
INCENP C ^{W1723G}	INCENP_C_W1723G_F	GGGCCAGCAAGAGCAATGTACG
	INCENP_C_W1723G_R	cAGTAGGAGCGAATTTTTTGTGGAC
INCENP C ^{F1745A}	INCENP_C_F1745A_F	gcTCCTGCAAAAAAGCGCCTGTG
	INCENP_C_F1745A_R	AGTAACATCGGGATCAATGTTTTGTGG

Supplementary Table S2

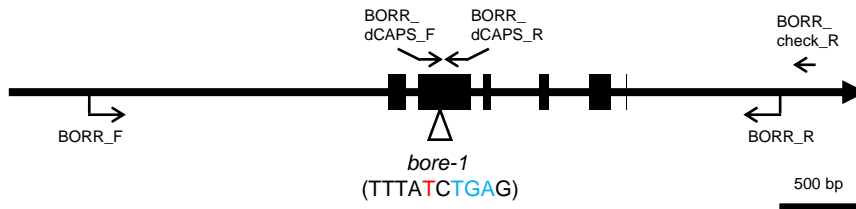
Synthesized oligonucleotides used in this study.

Purpose	Sequence (5'-3')
artificial microRNA	GGGGACAAGT T TGTACAAAAAAGCAGGC tctcgag TATAGGGGGGAA AAAAAGGTAGTCATCAGATATATATTTTGGTAAGAAAATATAGAAAT GAATAATTTACGTTTAAACGAAGAGGAGATGACGTGTGTTTCCTTCG AACCCGAGTTTTGTTCGTCTATAAATAGCACCTTCTCTTCTCCTTCTT CCTCACTTCCATCTTTTTAGCTTCACTATCTCTCTATAATCGGTTTTAT CTTTCTCTAAGTCACAACCCAAAAAACAAGTAGAGAAGAATCTG amiBORR1 <u>TATTCGTCTCTCCACTTTAACTGATGATGATCACATTCGTTATCTATTT</u> <u>TTTCAGTTAAAGTTGAGAGACGAAACATTGGCTCTTCTTACTACAATG</u> AAAAAGGCCGAGGCAAACGCCTAAAATCACTTGAGAATCAATTCT TTTTACTGTCCATTTAAGCTATCTTTTATAAACGTGTCTTATTTTCTAT CTCTTTTGTTTAAACTAAGAACTATAGTATTTTGTCTAAAACAAAA CATGAAAGAACAGATTAGATCTCATCTTTAGTCTCactagtACCCAGCT TTCTTGTACAAAGTGGTCCCC GGGGACAAGT T TGTACAAAAAAGCAGGC tctcgag TATAGGGGGGAA AAAAAGGTAGTCATCAGATATATATTTTGGTAAGAAAATATAGAAAT GAATAATTTACGTTTAAACGAAGAGGAGATGACGTGTGTTTCCTTCG AACCCGAGTTTTGTTCGTCTATAAATAGCACCTTCTCTTCTCCTTCTT CCTCACTTCCATCTTTTTAGCTTCACTATCTCTCTATAATCGGTTTTAT CTTTCTCTAAGTCACAACCCAAAAAACAAGTAGAGAAGAATCTG amiBORR2 <u>TATATCGAGCTGTGGGTATCACATGATGATCACATTCGTTATCTATTT</u> <u>TTTGTGATAACCCCAGCTCGATACATTGGCTCTTCTTACTACAATGA</u> AAAAGGCCGAGGCAAACGCCTAAAATCACTTGAGAATCAATTCTT TTACTGTCCATTTAAGCTATCTTTTATAAACGTGTCTTATTTTCTATC TCTTTTGTTTAAACTAAGAACTATAGTATTTTGTCTAAAACAAAAAC ATGAAAGAACAGATTAGATCTCATCTTTAGTCTCactagtACCCAGCTTT CTTGTACAAAGTGGTCCCC
	Underline: BORR target site with AtMIR390-distal loop sequence

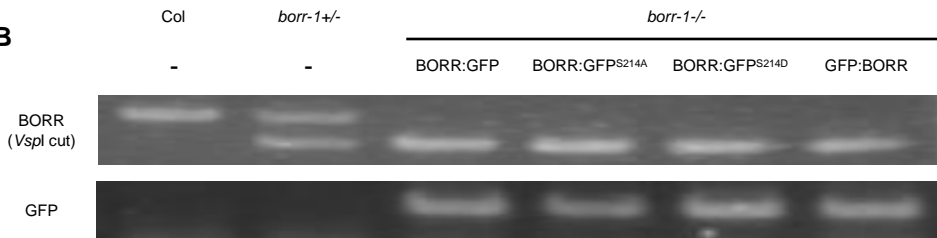
Supplemental Figure S1

A

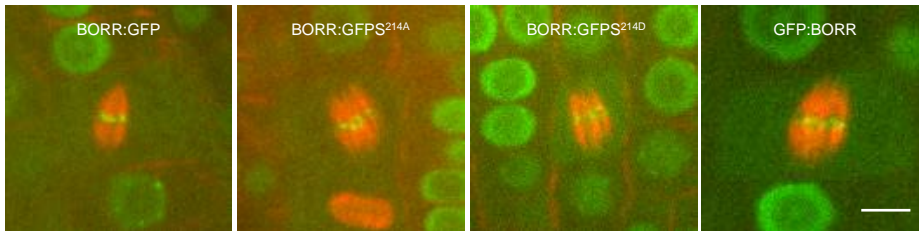
AT4G39630 (*BORR*)



B

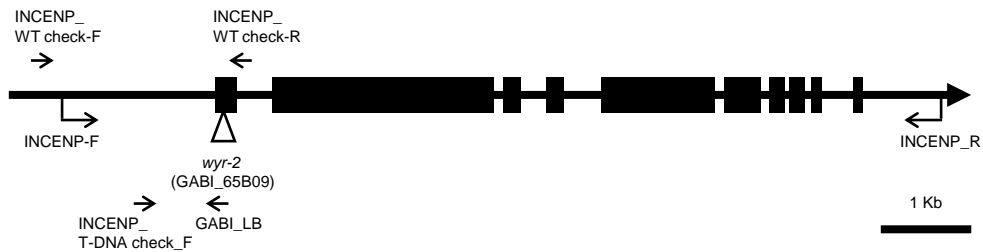


C

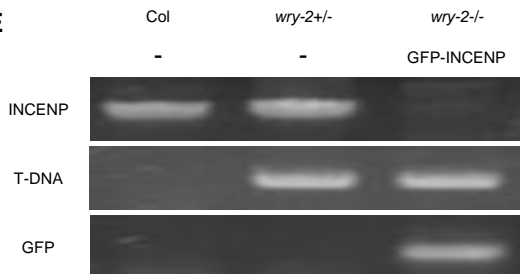


D

AT5G55820 (*INCENP/WYRD*)

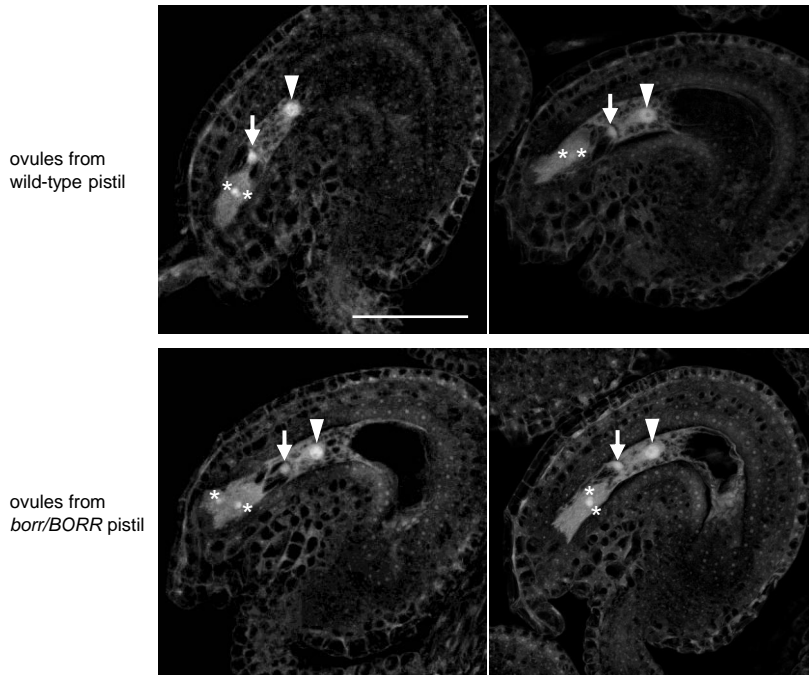


E



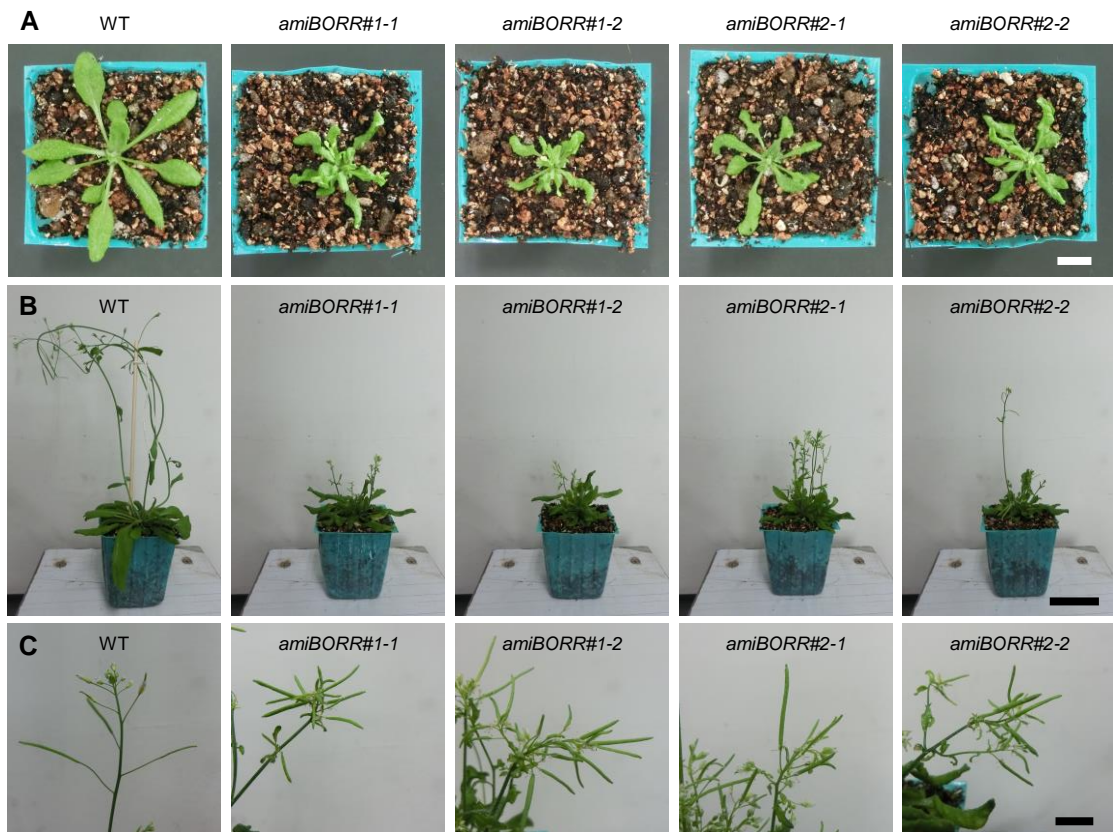
Complementation test of *borr* and *incenp* mutants. A, Gene structure of the *BORR* gene in Arabidopsis. Arrow head marks the position of the mutation position in *borr-1*. Red color indicates the inserted nucleotide and blue color indicates the premature stop codon. All rescue constructs include the region that is amplified by *BORR_F* and *BORR_R* primers. B, Complementation test of *borr-1* by dCAPS marker and PCR. C, Subcellular localization of *BORR* tagged with GFP. The mutation at S214 does not affect the *BORR* localization. Scale bar: 10 μ m. D, Gene structure of *INCENP/WYRD* gene in Arabidopsis. Arrow head shows the T-DNA position in *wyr*. The rescue construct includes the region which are amplified by *INCENP-F* and *INCENP-R* primers. E, Complementation check of *wyr* by PCR.

Supplemental Figure S2



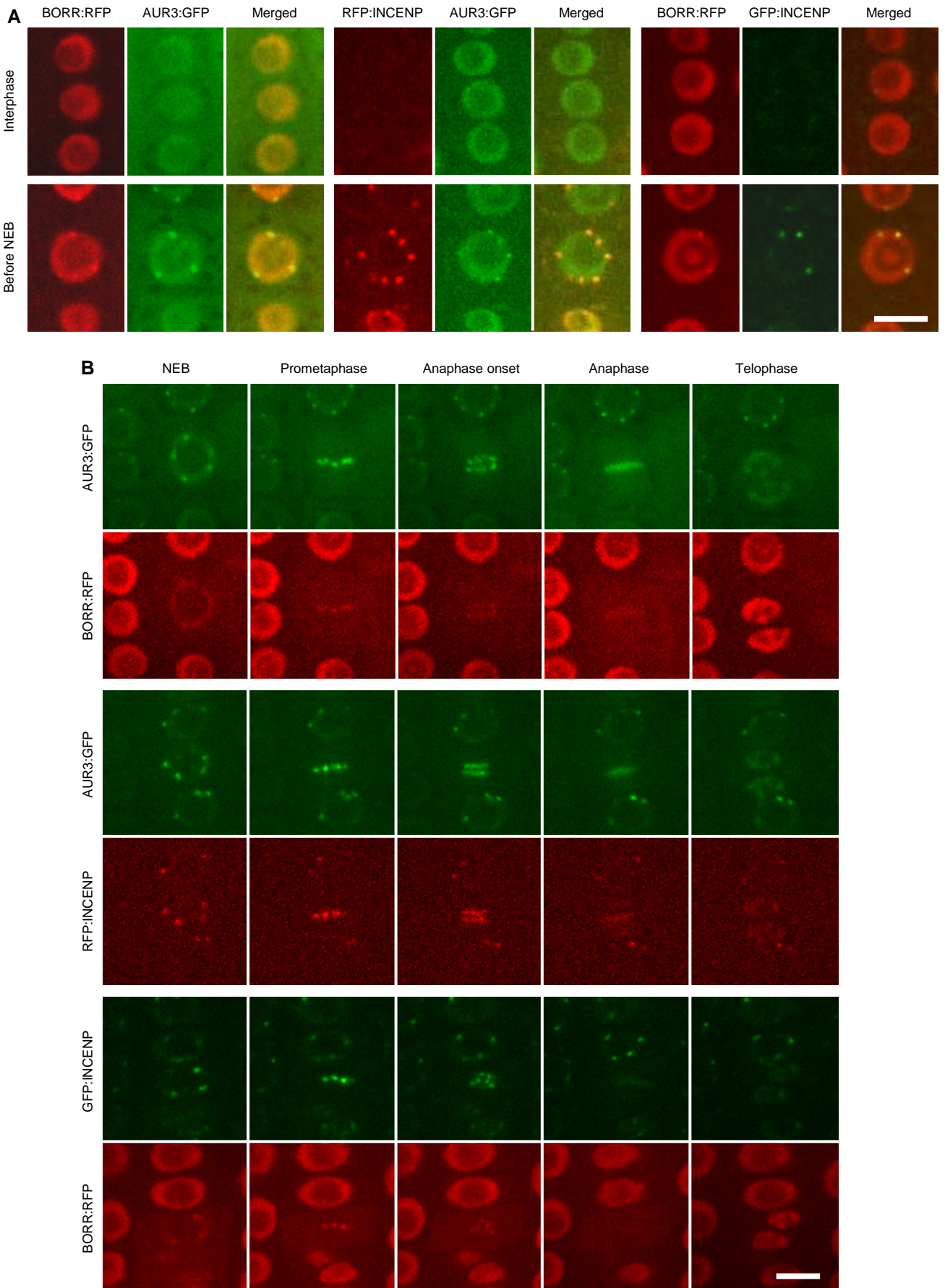
The morphology of the female gametophyte of *borr* mutants is indistinguishable from the WT. Analysis of the morphology of the female gametophyte in mature ovules of *borr* heterozygous mutants by confocal laser scanning microscopy. Two representative ovules from each of WT (above row) and heterozygous *borr* mutant pistils (below row) are shown. Morphology and nuclear positioning of the egg cell (arrow), central cell (arrowhead), and two synergid cells (asterisks) were indistinguishable from each other ($n > 50$). Scale bar: 50 μm .

Supplemental Figure S3



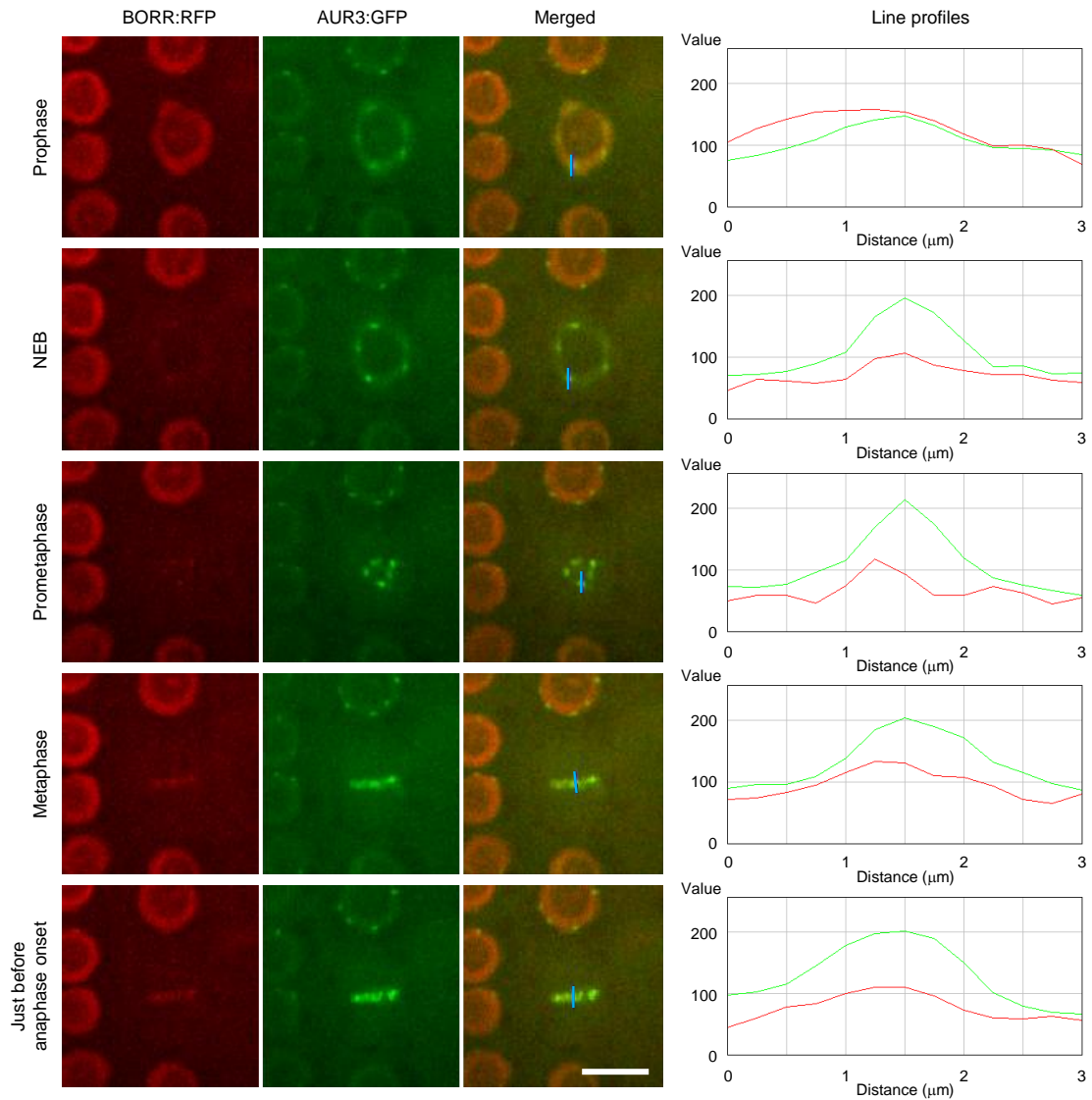
Plant growth phenotypes of WT and BORR knockdown plants. A, 3-week-old plants of WT and *amiBORR* plants. Scale bar: 1 cm. B, 35-day-old plants of WT and *amiBORR* plants. Scale bar: 5 cm. C, 5-week-old plants of WT and *amiBORR* plants. *amiBORR* plants exhibited the BONSAI phenotype. Scale bar: 1 cm.

Supplemental Figure S4



Co-localization of CPC components. A, Co-localization of GFP:INCENP, BORR:GFP, and AUR3:GFP in interphase (upper panels) and before NEB (lower panels). Root tips of 5-day-old seedlings were used. Scale bar: 10 μ m. B, Shown are the GFP channel and the RFP channel of Fig. 5H. Scale bar: 10 μ m.

Supplemental Figure S5



Both BORR and AUR3 localize to inner centromeres. Localization of BORR and AUR3 from prophase to just prior to anaphase onset. For live imaging, root tips of 5-day-old seedlings were used. Blue bar indicates the position where the line profiles were observed. Scale bar: 10 μm .