Supplementary Information for

Molecular convergence by differential domain acquisition is a hallmark of chromosomal passenger complex evolution

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https://figshare.com/articles/dataset/SI_Datasets_Movies_Figures_associated_to_pre-print_artic le_Molecular_convergence_by_differential_domain_acquisition_is_a_hallmark_chromosomal_p assenger_complex_evolution_/17840213

Or at:

10.6084/m9.figshare.17840213
Supplementary Methods

LC-MS/MS and data analysis
The obtained peptide mixtures were introduced into an LC-MS/MS system, the Ultimate 3000 RSLC nano (Dionex, Amsterdam, The Netherlands) in-line connected to a Q Exactive Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany). The sample mixture was loaded on a trapping column (made in-house, 100 μm internal diameter (I.D.) x 20 mm (length), 5 μm C18 Reprosil-HD beads, Dr. Maisch GmbH, Ammerbuch-Entringen, Germany). After back-flushing from the trapping column, the sample was loaded on a reverse-phase column (made in-house, 75 μm I.D. x 150 mm, 5 μm C18 Reprosil-HD beads, Dr. Maisch). Peptides were loaded with solvent A (0.1% trifluoroacetic acid, 2% acetonitrile), and separated with a 30 min linear gradient from 98% solvent A' (0.1% formic acid) to 50% solvent B' (0.1% formic acid and 80% acetonitrile) at a flow rate of 300 nl/min, followed by a wash step reaching 100% solvent B’. The mass spectrometer was operated in data-dependent, positive ionization mode, automatically switching between MS and MS/MS acquisition for the 5 most abundant peaks in a given MS spectrum. The source voltage was 3.6 kV, and the capillary temperature was 275°C. One MS1 scan (m/z 400–2,000, AGC target 3 × 106 ions, maximum ion injection time 80 ms), acquired at a resolution of 70,000 (at 200 m/z), was followed by up to 5 tandem MS scans (resolution 17,500 at 200 m/z) of the most intense ions fulfilling predefined selection criteria (AGC target 5 × 104 ions, maximum ion injection time 80 ms, isolation window 2 Da, fixed first mass 140 m/z, spectrum data type: centroid, intensity threshold 1.3xE4, exclusion of unassigned, 1, 5-8, >8 positively charged precursors, peptide match preferred, exclude isotopes on, dynamic exclusion time 12 s). The HCD collision energy was set to 25% Normalized Collision Energy and the polydimethylcyclosiloxane background ion at 445.120025 Da was used for internal calibration (lock mass).

Co-purified proteins were identified with Mascot (version 2.5.1, MatrixScience) using the TAIR10plus database with standard settings (1). Proteins with at least two matched high confident peptides were retained according to our standard evaluation settings. Proteins with one matched high confident peptide are added for additional information and are labeled in blue. Background proteins were filtered out based on frequency of occurrence of the co-purified proteins in a large dataset containing 543 TAP experiments using 115 different baits (1). True interactors that might have been filtered out because of their presence in the list of non-specific proteins were retained by means of semi-quantitative analysis using the average normalized spectral abundance factors (NSAF) of the identified proteins (1).
Plasmid construction
To create BORI:GFP constructs, the genomic fragment of BORI1 or BORI2 gene was amplified by PCR and cloned into pDONR221. A SmaI site was inserted in front of the stop codon of each construct. Both constructs were linearized by SmaI digestion and ligated to the monomeric GFP (mGFP) gene, followed by LR recombination reactions with the destination vector pGWB501. To create BORI_N:GFP constructs, the C-terminal region of each gene was removed from BORI:GFP constructs by inverse PCR. To create BORI<sup>R34</sup>:GFP constructs, corresponding point mutations were introduced in the BORI:GFP constructs by inverse PCR. To create the CRISPR/CAS9 construct against BORI2 gene, the BORI2 gene-specific spacer sequence was cloned into the pEn-Chimera, followed by LR recombination reaction with the destination vector pDe-CAS9. To create the amiRNA construct against the BORI2 gene, 75-bp gene-specific sequences of the BORI2 gene were synthesized and cloned into pENTR-AtMIR390a-B/c, followed by LR recombination reactions with the destination vector pGWB602. All constructs were transformed into Arabidopsis plants using the floral dip method. Primer pairs for plasmid construction are described in SI Appendix, Table S1. For the production of recombinant proteins, the attR1-attR2 Gateway cassette was amplified by PCR and cloned into pGEX6p-1, designated pGEX6p-GW. Full length BORI1 and 2 cDNAs were amplified from 10-day-old seedling RNA and full length Human Survivin cDNA was artificially synthesized. The resulting cDNA fragments were cloned into pDONR221 followed by LR recombination reactions with the destination vector pGEX6p-GW and introduced into in the Escherichia coli strain BL21 (DE3). For yeast two hybrid analysis, full length BORI1 cDNA and the C-terminus of BORI2 cDNA were amplified by PCR using gene-specific primers from cDNA made from total RNA of wild-type Arabidopsis plants, followed by PCR with universal attB primers and cloned into pDONR221. The helical region of Borealin (Human:1-76 aa, Yeast:1-73 aa) and Survivin (Human:89-142 aa, Yeast:889-954 aa) cDNA were artificially synthesized with attB site cloned into pDONR221. The truncated BORI1 constructs were created by inverse PCR. The subcloned cDNA fragments were recombined into the destination vector pGBT9 (DNA-BD) by LR reaction.

Detection and definition of the BORI/Survivin gene family in eukaryotes
To detect homologs of BORIs and Survivin in eukaryotes, we optimized multiple profile Hidden Markov Models (HMMs) based on iterative reciprocal similarity searches using various tools from the HMMER package version 3.1b2 (2), similar to a strategy used in our previous work (3). Iterative searches with ‘jackhammer’ were executed with standard inclusion thresholds (E>0.01, bitscore>25) until no new candidate homologs could be included, or as otherwise stated. HMMs were constructed using ‘hmmbuild’ based on multiple sequence alignments of curated homologs. We used both full-length and
subdomain (BIR, FHA and helix) HMMs as seeds for reciprocal iterative sequence searches using 'hmmrsearch'. Our search protocol was based on the following steps/considerations: (I) To limit the amount of homologs to be queried, when searching the widely present BIR and FHA domains and full-length sequences, we used stringent bitscore inclusion cut-offs of 60 up to 70 (--incT 60-70 and --incdomT 60-70). (II) We only considered sequences as candidate Survivin/BORI orthologs if they harbored both a phosphate-binding domain (FHA/BIR) and/or a conserved helix, on the condition that the helix alone should yield reciprocal best hits (phmmer) and/or reciprocal iterative (jackhammer) hits with bona fide homologs. (III) Putative candidates that contained a single short helical domain were only included in case reciprocal similarity searches yielded phosphate-binding domain containing candidates found in other eukaryotic lineages, and when a particular species or lineage did not yet contain a Survivin/BORI homolog (i.e. putative candidates in plants, animals and fungi were excluded). (IV) Were possible, we aimed to optimize one single HMM of the conserved helical domain to capture all Survivin/BORI orthologs (see SI Appendix, Dataset S5). We therefore trained an HMM of the gene family-defining feature, the conserved helical domain, on large eukaryotic sequence databases, including our in-house dataset (3), EukProt (4), and UniProt (5). Sequences of orthologs and presence-absence patterns of the Survivin/BORI gene family in a subset of representative eukaryotes can be found in separate text files in SI Appendix, Dataset S5. Clade-specific HMMs can be found in SI Appendix, Dataset S6.

Phylogenetic analyses of FHA domains found in BORI-like homologs

To prevent the inclusion of a high number of potential FHA domain-based BORI homologs to consider for phylogenetic analysis, we used a high bitscore cut-off (bitscore>70; see above). Candidate homologs were found in Viridiplantae (Chlorophyta and Streptophyta) and all contained a C-terminal helix (Fig. 1), which strongly suggested that these were orthologous to the BORIs found in Arabidopsis. To find the closest FHA domain to that of BORI, we aligned all Viridiplantae BORI orthologs and generated an HMM of the FHA domain using hmmbuild v3.1b2 (2). Using a similar HMM-based approach, but now with a bitscore cut-off of 60 (--incT 60 --incdomT 60), we found the FHA domains of the PP2C phosphatase KAPP orthologs to be the closest to those of BORI. Subsequent iterations with lower bitscore cut-offs (>50) revealed many non-BORI/KAPP FHA domains to have a roughly similar bitscore, therefore no clear outgroup for BORI and KAPP could be defined in eukaryotes. We therefore searched the Uniprot database for putative prokaryotic homologs. Indeed, Deltaproteobacterial FHA domain-containing sequences were found to be more similar compared to other eukaryotic sequences.

To provide an outgroup, we added the seed sequences for the FHA PFAM model (PF00498, see SI appendix, Dataset S3). FHA domains were aligned using MAFFT (option g-ins-i) (6). For the
phylogenetic analysis in Figure 8C, FHA domain-containing proteins were added that were significantly similar to BORI-like homologs found amongst Stramenopila, Haptista and Cryptista in the EukProt database (4), with a bitscore cut-off (>60). Maximum-likelihood phylogenetic analyses (for analysis files and further description see SI Appendix, Supplementary text, Dataset S3 and Dataset S4) were performed with the IQ-Tree webserver (version 1.6.12) using standard settings for model selection, including the assessment of all mixture models, 1000 Ultrafast bootstrap and SH-like approximate likelihood ratio test replicates (7). Parameters for the final phylogenetic analyses are as follows: analysis Fig. 1C (see SI Appendix, Dataset S3 – settings: -m LG4X+F -bb 1000 -alrt 1000 -pers 0.3 -numstop 410; 65 positions, 194 sequences with at least 70% occupancy per column, model: LG4X); Fig. 8C (see SI Appendix, Dataset S4 – settings: -m LG4X+F -bb 1000 -alrt 1000 -pers 0.4 -numstop 300; 64 positions, 408 sequences with at least 70% occupancy per column, model LG4X). Trees were visualized and annotated using FigTree v1.4.4 (8), and/or itol (9).

Accession Numbers
Sequence data from this article can be found in the Arabidopsis Information Resource database under the following accession numbers: AT2G45490 (AUR3), AT3G02400 (BORI1), AT4G14490 (BORI2), AT4G39630 (BRR), AT5G55820 (INCENP/WYRD), and AT5G19280 (KAPP). Sequences of homologs, and presence-absence patterns of Aurora kinase, INCENP, and Borealin in a subset of representative eukaryotes (see also (10)), can be found in separate text files in SI Appendix, Dataset S5.

Graphics and other software
Plots and alignments were manually compiled into figures using the open-source scalable vector graphics editor Inkscape 1.0rc1 for macOS (Inkscape Project 2020, retrieved from https://inkscape.org). 3D protein structures were visualized using Pymol v2.5. Alignments were manipulated using Jalview (11).
Figure S1. Immunoblot analysis of GAL4-BD fusion constructs. Proteins extracted from transformed yeast cells shown in Fig.2B were subjected to immunoblotting with an anti-GAL4-BD antibody. Proteins extracted from non-transformed yeast were used as a negative control. Arrowheads indicate the corresponding bands for GAL4-BD fusion proteins.
| Species                      | Genus                  | Species                  |
|------------------------------|------------------------|--------------------------|
| Homo sapiens                 | ARX5 - TGG - AGP90 - LA|
| Xenopus tropicalis           | ARX5 - TGG - AGP90 - LA|
| Danio rerio                  | ARX5 - TGG - AGP90 - LA|
| Branchiostoma floridanum     | ARX5 - TGG - AGP90 - LA|
| Strongylocentrotus purpuratus| ARX5 - TGG - AGP90 - LA|
| Drosophila melanogaster      | ARX5 - TGG - AGP90 - LA|
| Anopheles gambiae            | ARX5 - TGG - AGP90 - LA|
| Bombyx mori                  | ARX5 - TGG - AGP90 - LA|
| Nasonia vitripennis          | ARX5 - TGG - AGP90 - LA|
| Caenohabditis elegans       | ARX5 - TGG - AGP90 - LA|
| Brugia malayi                | ARX5 - TGG - AGP90 - LA|
| Schistosoma mansoni          | ARX5 - TGG - AGP90 - LA|
| Necator americanus           | ARX5 - TGG - AGP90 - LA|
| Trichoplax adhaerens         | ARX5 - TGG - AGP90 - LA|
| Monosiga brevicollis         | ARX5 - TGG - AGP90 - LA|
| Salpingoea rosetta           | ARX5 - TGG - AGP90 - LA|
| Capsaeraria ovicarcazi       | ARX5 - TGG - AGP90 - LA|
| Creolimax fragilissima       | ARX5 - TGG - AGP90 - LA|
| Saccharomyces cerevisiae     | ARX5 - TGG - AGP90 - LA|
| Debaryomyces hansenii        | ARX5 - TGG - AGP90 - LA|
| Yarrowia lipolytica          | ARX5 - TGG - AGP90 - LA|
| Aspergillus fumigatus        | ARX5 - TGG - AGP90 - LA|
| Neospora caninum             | ARX5 - TGG - AGP90 - LA|
| Schizosaccharomyces pombe    | ARX5 - TGG - AGP90 - LA|
| Ustilago maydis              | ARX5 - TGG - AGP90 - LA|
| Cryptococcus neoformans      | ARX5 - TGG - AGP90 - LA|
| Acremonium circinellum       | ARX5 - TGG - AGP90 - LA|
| Mortierella vermifera        | ARX5 - TGG - AGP90 - LA|
| Rhizopus nigricans           | ARX5 - TGG - AGP90 - LA|
| Aspergillus niger            | ARX5 - TGG - AGP90 - LA|
| Coniothyrium carbonum        | ARX5 - TGG - AGP90 - LA|
| Atractomyces flavus          | ARX5 - TGG - AGP90 - LA|
| Brevicrombomyces drosophila  | ARX5 - TGG - AGP90 - LA|
| Encephalitozoon intestinalis| ARX5 - TGG - AGP90 - LA|
| Trypanosoma brucei          | ARX5 - TGG - AGP90 - LA|
| T. cruzi                     | ARX5 - TGG - AGP90 - LA|
| Trypanosoma cruzi            | ARX5 - TGG - AGP90 - LA|
| Trypanosoma brucei           | ARX5 - TGG - AGP90 - LA|
| Neoplasia gruberi            | ARX5 - TGG - AGP90 - LA|
| Hymenolepis diminuta         | ARX5 - TGG - AGP90 - LA|
| Namnochoria gauthiera R-31   | ARX5 - TGG - AGP90 - LA|
| Ectocarpus siliculosus       | ARX5 - TGG - AGP90 - LA|
| Aureococcus anophageferrensis| ARX5 - TGG - AGP90 - LA|
| Phaeodactylum tricornutum    | ARX5 - TGG - AGP90 - LA|
| Thalassiosira pseudonana     | ARX5 - TGG - AGP90 - LA|
| Physodiora niitonda          | ARX5 - TGG - AGP90 - LA|
| Albugo abaculi            No.14| ARX5 - TGG - AGP90 - LA|
| Saprolegnia parasitica CB5  223-65| ARX5 - TGG - AGP90 - LA|
| Aurantiochyclus limacinum ATCC 15812| ARX5 - TGG - AGP90 - LA|
| Blastocystis hominis         | ARX5 - TGG - AGP90 - LA|
| Brevicrombomyces drosophila  | ARX5 - TGG - AGP90 - LA|
| Plasmcidium tachyurus 30838  | ARX5 - TGG - AGP90 - LA|
| Plasmcidium tachyurus 30838  | ARX5 - TGG - AGP90 - LA|
| Toxoplasma gondii ME49       | ARX5 - TGG - AGP90 - LA|
| Tetrahymena thermophila      | ARX5 - TGG - AGP90 - LA|
| Gyotricha triflax             | ARX5 - TGG - AGP90 - LA|
| Bigelowiella nolens, CCMP2755| ARX5 - TGG - AGP90 - LA|
| Plasmcidium brassicae        | ARX5 - TGG - AGP90 - LA|
| Chytridium chironomus CTCC291| ARX5 - TGG - AGP90 - LA|
| Eumiais, uxei, CCMP1016      | ARX5 - TGG - AGP90 - LA|
| Arabidopsis thaliana         | ARX5 - TGG - AGP90 - LA|
| Oryza sativa japonica       | ARX5 - TGG - AGP90 - LA|
| Amborella trichopoda         | ARX5 - TGG - AGP90 - LA|
| Solanum meloformi            | ARX5 - TGG - AGP90 - LA|
| Physcomitrella patens subsp. | ARX5 - TGG - AGP90 - LA|
| Kobsonium nitens             | ARX5 - TGG - AGP90 - LA|
| Chloridion moniliferum       | ARX5 - TGG - AGP90 - LA|
| Chlorotrichia variabilis NOS 4A| ARX5 - TGG - AGP90 - LA|
| Ostreococcus lucimarinus OE901| ARX5 - TGG - AGP90 - LA|
| Micromonas sps              | ARX5 - TGG - AGP90 - LA|
| Cyanophora paradoxa          | ARX5 - TGG - AGP90 - LA|
| Chondrus crispus             | ARX5 - TGG - AGP90 - LA|
| Porphyridium purpureum       | ARX5 - TGG - AGP90 - LA|
| Cyanobacterium merolae 1D   | ARX5 - TGG - AGP90 - LA|
| Galderia sulphuraria         | ARX5 - TGG - AGP90 - LA|
| Guillardia theta, CCMP2712   | ARX5 - TGG - AGP90 - LA|
| Gonionomas avorius          | ARX5 - TGG - AGP90 - LA|
Figure S2. Multiple sequence alignment of the N-terminal tail (1-22) of Histone H3 variants across the eukaryotic kingdom. A subset of species is shown that is representative of the diversity found amongst the eukaryotic tree of life. Per species, only one canonical Histone H3 variant is shown, that harbor a conserved H3T3 position. The position of the T3 (Haspin phosphosite), S10 (mitotic Aurora phosphosite) and T11 (Haspin phosphosite) are indicated below the alignment. On the right: lineages are grouped by phylogenetic (super) groups. TSAR (Telonema, Stramenopila, Alveolata, Rhizaria). A subset of this multiple sequence alignment is shown in Figure 3A.
Figure S3. Molecular characterization of bori mutants and knock-down lines. (A) Gene structures of BORI genes in Arabidopsis thaliana. Arrowheads indicate the position of the here-presented mutations. Red color indicates the deleted nucleotides and blue color indicates the premature stop codon in bori2-1 mutant. Rescue constructs include the region that is amplified by BORI1_F and BORI1_R or BORI2_F and BORI2_R primers. (B) Genotyping of bori mutants and BORI complementation lines by PCR. Only the PCR product from WT BORI2 is digested by the Ddel restriction enzyme. ACT8 is used as a control. (C) Expression analysis of BORI1 in bori1 mutants by RT-PCR using ACT8 as control. (D) Relative expression level of BORI2 in the amiBORI2 plants was confirmed by RT-qPCR analysis with three biological replicates. Error bars represent means ± SD. (E) Relative expression level of AUR3 in the amiAUR3 plants was assessed by RT-qPCR analysis with three biological replicates. Error bars represent means ± SD. (F) Relative expression level of BORR in the amiBORR plants was determined by RT-qPCR analysis with three biological replicates. Error bars represent means ± SD.
Figure S4. Phenotypes of *bori* and *aur3* mutants. (A) 3-week-old plants. Scale bar, 1 cm. (B) 30-day-old plants. Scale bar, 5 cm. (C) Inflorescences of 5-week-old plants. Scale bar, 1 cm. (D) 3-week-old plants expressing *BORI1*<sup>R3A</sup>:GFP. Scale bar, 1 cm. (B) 30-day-old plants expressing *BORI1*<sup>R3A</sup>:GFP. Scale bar, 5 cm. (C) Inflorescences of 5-week-old plants expressing *BORI1*<sup>R3A</sup>:GFP. Scale bar, 1 cm.
Figure S5. Subcellular localization of BORIs. (A and B) Subcellular localization of BORI2:GFP (A) or BORI2R3A:GFP (B) during the cell cycle. Microtubule structures were visualized by RFP:TUA5. Scale bar, 10 μm. (C) Subcellular localization of BORI1:GFP in amiBORR mutant background during the cell cycle. Microtubule structures were visualized by RFP:TUA5. Scale bar, 10 μm. (D-F) Subcellular localization of BORI1:GFP in WT (D), BORI1:GFP in amiBORR (E), or BORI1_N:GFP in WT (F) in anaphase cells. Merged pictures of D-F correspond with Fig. 6A; anaphase, Fig. S4C; anaphase, and
Fig. 6C; anaphase, respectively. The yellow dotted line indicates the positions where the line profiles were obtained. Arrowhead indicates the lagging chromosome. Scale bar, 10 μm. (G) Co-localization of BORI1:GFP and the inner kinetochore marker RFP:CENH3. Scale bar, 5 μm. (H) Subcellular localization of BORI1:GFP in metaphase cells. 5-day-old seedlings were treated without 5-ITu (0.05% DMSO control) or with 10 μM 5-ITu for 90 min. Microtubule structures were visualized by RFP:TUA5. Scale bar, 10 μm. (I and J) Colocalization of Histone H3:RFP and BORI1:GFP (I) or BORI1R3A:GFP (J) in metaphase cells. The yellow dotted line indicates the positions where the line profiles were obtained. Pictures of I and J correspond with Fig. 6E and F, respectively. Scale bar, 10 μm.
| Table S1. Primers used in this study. |
|--------------------------------------|
| **Purpose**                          | **Primer name**                  | **Sequence (5’-3’)**                        | **Template** |
| CRISPR                              | BORI2_CRISPR_F                   | attgGATATCAGTAGGAGAGAGAA                    | Genomic DNA  |
|                                     | BORI2_CRISPR_R                   | aaccTTTTCCTCAGTGATATAC                     | Genomic DNA  |
| Genotyping                          | BORI1_T-DNA_check_F              | AAAATTCATATTTCATGACTACAAAG                 | Genomic DNA  |
|                                     | SALK_Lbb1.3                      | ATTTGCGGATTCCGAA                           | Genomic DNA  |
|                                     | BORI1_WT_check_F                 | AATGGTGTTGGTGAGGAGGATGAGG                 | Genomic DNA  |
|                                     | BORI1_WT_check_R                 | GCTCTATTTTTTCTAATCTCTCTCAAC               | Genomic DNA  |
|                                     | BORI2_CAPS_F                     | AATCTCGGAGATGGAGGAGGATGTTATC              | Genomic DNA  |
|                                     | BORI2_check_R                     | TGGACACTTTTGTCAAACTGAACACATAC              | Genomic DNA  |
|                                     | BORI2_CAPS_R                     | CTTCTACCTTCTACATTAAACAC                   | Genomic DNA  |
|                                     | BORI1_check_F                     | GCTTGATGATCATAGGGTGAATACGTCACGTC           | Genomic DNA  |
|                                     | BORI1_check_R                     | TTAATCAGATGGAGGTCTCCAGC                   | Genomic DNA  |
|                                     | ACT8_F                           | AATGAAATAGGTCGTCGGCA                      | Genomic DNA  |
|                                     | ACT8_R                           | TCCGAGTTTGAAGAGGCTAC                      | Genomic DNA  |
| RT-qPCR                             | BORI1_RT_F                        | ATGTGTCGCCCCATGCTGAGGCTGAC                | Genomic DNA  |
|                                     | ACT8_RT_F                        | Same as ACT8_F                           | Genomic DNA  |
|                                     | ACT8_RT_R                        | Same as ACT8_R                           | Genomic DNA  |
| RT-qPCR                             | BORI2_qRT_F                      | ATGTGTCGCCCCATGCTGAGGCTGAC                | Genomic DNA  |
|                                     | BORI2_qRT_R                      | CAAACATCGATGCGCATCTGC                    | Genomic DNA  |
| RT-qPCR                             | AUR3_qRT_F                       | GACAGAATCTGGCAAGGCCA0CCTCA                 | Genomic DNA  |
|                                     | AUR3_qRT_R                       | CCAATCTGGCTCAGGACCTC                     | Genomic DNA  |
| RT-qPCR                             | BORR_qRT_F                       | GTAGGATCCATCTTGAGGCTATG                   | Genomic DNA  |
|                                     | BORR_qRT_R                       | GGAAGCTATGGTCTCGAGATGTC                   | Genomic DNA  |
| RT-qPCR                             | PP2AA3_qRT_F                     | GCCAAATGATGACTCACTCTC                     | Genomic DNA  |
|                                     | PP2AA3_qRT_R                     | CCGTACATGCTCTCCACAC                      | Genomic DNA  |
| Cloning of FP fusion constructs     | attB1 adapter                     | GGSSGACAAGTTTGTGACAAAAAGGCCAGGCT         | attB1 adapter |
|                                     | attB2 adapter                     | GGGGACCACCTTTGTCAGAAAGAAGGCTGGT           | attB2 adapter |
|                                     | BORI1_GFP                         | caaaaagagccgctacgcgtCTCATCGAGGCAAGGCTAACTAG | Genomic DNA  |
|                                     | BORI1_R                           | caagaaagctgggtAGAGGAGGAAACTAAAGCTCGATTACG | Genomic DNA  |
|                                     | Cter_Smal_BORI1_F                | gggTAAATCTTTGTTGATGCTGCC                 | Genomic DNA  |
|                                     | Cter_Smal_BORI1_R                | gggTACAGAATCTTTGCTCTGCC                   | Genomic DNA  |
|                                     | BORI1_N_GFP                       | CCGGGGACCGttcgctactacgcgtCAGGGAAGGATGGAAAGTTAGTAG | Genomic DNA  |
|                                     | BORI1_N_GFP_R                    | TCCATCAACCGAAATCTTTGCTCCACAC             | Genomic DNA  |
|                                     | BORI11ساـ1-GFP_1stPCR            | ggaAGTCTGGCAATCTTCAGGTTTAAACC             | 1st PCR product. |
|                                     | BORI11ساـ1-GFP_2ndPCR            | GGCTACCCTGAGGCTAGGAGGCT                   | 1st PCR product. |
|                                     | BORI21ساـ1-GFP_1stPCR            | ggaAGTCTGGCAATCTTCAGGTTTAAACC             | 1st PCR product. |
|                                     | BORI21ساـ1-GFP_2ndPCR            | GGCTACCCTGAGGCTAGGAGGCT                   | 1st PCR product. |
|                                     | BORI22ساـ1-GFP_1stPCR            | ggaAGTCTGGCAATCTTCAGGTTTAAACC             | 1st PCR product. |
|                                     | BORI22ساـ1-GFP_2ndPCR            | GGCTACCCTGAGGCTAGGAGGCT                   | 1st PCR product. |
|                                     | Histone H3.1-RFP                  | GACTGGATCATTGGAGGTCTTCTTCCAGGGACAT        | Genomic DNA  |
|                                     | Histone H3.1_R                    | GGGAAGGCGCCGCTGAGGCTAGGTTAGGGAGGATGAGGAA | Genomic DNA  |
|                                     | pENTR2B for Histone H3.1_F       | TCACTCCACCTCTCTCCGGCGCGCGCATGAGATCATC    | pENTR2B      |
|                                     | pENTR2B for Histone H3.1_R       | TCACTCCACCTCTCTCCGGCGCGCGCATGAGATCATC    | pENTR2B      |
|                                     | Cter_Smal_Histone H3.1_F         | ggaAGTATTGAAGAAGGACCTAGGGAAGGATGAAAGGAA | pENTR2B      |

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### Yeast two-hybrid assay

| Gene    | Forward Sequence | Reverse Sequence | Source                  |
|---------|------------------|------------------|-------------------------|
| BORI1_1-585 | caaaaagcaggctccaccATGGTTGCGCCATTTGCTGAAGGCTGAC | caagaaagctggttTTAGTCGCCCTTTTCTCTTGTGCTGAC | genomic DNA |
| BORI1_1-293 | Same as BORI1_1-585_F | Same as BORI1_1-585_F | genomic DNA |
| BORI1_294-585 | GTCAAAGATGAGAAGAGACTCAAGAAG | Same as BORI1_1-293_R | pDONR221/BORI1_1-585 |
| BORI1_535-585 | TTTAGAAAAATGACACTCAAGAAGACT | Same as BORI1_294-585_R | pDONR221/BORI1_1-585 |
| BORI1_294-534 | Same as BORI1_1-293_F | Same as BORI1_1-293_F | pDONR221/BORI1_294-534 |
| BORI1_N | Same as BORI1_1-585_F | Same as BORI1_1-585_F | pDONR221/BORI1_1-585-GFP |
| BORI2_N | Same as BORI1_1-585_F | Same as BORI1_1-585_F | pDONR221/BORI2_N-GFP |
| amiBORI2 | TGTATTGTAATCTAGAGCATCGCAATGATGACCATACATTTCATTTTTTTGCGATGCTATAGATTACAA | AATGTTTGAACTCATCTGAGAAGAATGATGACCATACATTTCATTTTTTTGCGATGCTATAGATTACAA |
| amiAUR3 | TGTATTGCAAGCGGCTCTGTGAGAATGATGACCATACATTTCATTTTTTTGCGATGCTATAGATTACAA | AATGTTTGAACTCATCTGAGAAGAATGATGACCATACATTTCATTTTTTTGCGATGCTATAGATTACAA |

### Peptide-binding assay

| Gene    | Forward Sequence | Reverse Sequence | Source                  |
|---------|------------------|------------------|-------------------------|
| BORI1_N | caaaaagcaggctccaccATGGTTGCGCCATTTGCTGAAGGCTGAC | caagaaagctggttTTAGTCGCCCTTTTCTCTTGTGCTGAC | genomic DNA |
| BORI1_N | Same as BORI1_1-293_R | Same as BORI1_1-293_R | genomic DNA |
| BORI2_N | Same as BORI1_1-293_F | Same as BORI1_1-293_F | pDONR221/BORI1_N-GFP |
| BORI2_N | caaaaagcaggctccaccATGGTTGCGCCATTTGCTGAAGGCTGAC | caagaaagctggttTTAGTCGCCCTTTTCTCTTGTGCTGAC | genomic DNA |
| BORI2_N | Same as BORI1_1-293_F | Same as BORI1_1-293_F | pDONR221/BORI2_N-GFP |

### artificial microRNA

| Gene    | Forward Sequence | Reverse Sequence | Source                  |
|---------|------------------|------------------|-------------------------|
| amiBORI2 | TGTATTGTAATCTAGAGCATCGCAATGATGACCATACATTTCATTTTTTTGCGATGCTATAGATTACAA | AATGTTTGAACTCATCTGAGAAGAATGATGACCATACATTTCATTTTTTTGCGATGCTATAGATTACAA |
| amiAUR3 | TGTATTGCAAGCGGCTCTGTGAGAATGATGACCATACATTTCATTTTTTTGCGATGCTATAGATTACAA | AATGTTTGAACTCATCTGAGAAGAATGATGACCATACATTTCATTTTTTTGCGATGCTATAGATTACAA |
**Supplementary Movies**

**Movie S1**
Subcellular localization of BORI1:GFP during mitosis.

**Movie S2**
Subcellular localization of BORI2:GFP during mitosis.

**Movie S3**
Co-localization of BORI1:GFP and BORR:RFP during mitosis.

**Movie S4**
Subcellular localization of BORI1_N:GFP during mitosis.

**Movie S5**
Subcellular localization of BORI1:GFP in *amiBORR* during mitosis.

**Movie S6**
Subcellular localization of BORI1^{R3A}:GFP during mitosis.

**Movie S7**
Subcellular localization of BORI2^{R3A}:GFP during mitosis.

**Movie S8**
3D projection of *bori1 bori2* root cells complemented with *BORI1:GFP*. Both cells have 10-dotted-GFP signals. Left cell is shown in Fig. 7E.

**Movie S9**
3D projection of *bori1 bori2* root cells complemented with *BORI1^{R3A}:GFP*. The cell has 9-dotted-GFP signals.

**Movie S10**
3D projection of *bori1 bori2* root cells complemented with *BORI1^{R3A}:GFP*. Both cells have 11-dotted-GFP signals. Left cell is shown in Fig. 7F.
Supplementary Datasets

Supplementary Datasets S2 to S6 can be found at:

https://figshare.com/articles/dataset/SI_Datasets_Movies_Figures_associated_to_pre-print_article_Molecular_convergence_by_differential_domain_acquisition_is_a_hallmark_chromosomal_passenger_complex_evolution_/17840213

Or at:

10.6084/m9.figshare.17840213

Dataset S1
Protein Identification details obtained by mass spectrometry.

Dataset S2
Separate .pdb files of predicted 3D structures of AthaBORI 1 and 2, and a pymol session file with BORI1 and 2 aligned corresponding to the structures shown in Figure 1D. BORI1: At3g02400 (uniprot-ID:Q9M8A0); BORI2: At4g14490 (uniprot-ID:O23305) - downloaded from the AF2 repository https://alphafold.ebi.ac.uk/.

Dataset S3
Text files and multiple sequence alignment used for IQ-Tree maximum-likelihood phylogenetic analysis found in Figure 1D.

Dataset S4
Text files and multiple sequence alignment used for IQ-Tree maximum-likelihood phylogenetic analysis found in Figure 8C.

Dataset S5
Text files, including: (1) .xlsx table with genome/transcriptome sources, presences and absences of Aurora kinase, INCENP, Borealin, Survivin/BORI and the H3 N-terminal tail (1-21), (2) sequences of homologs in separate text files, and (3) including separate domain annotations for the Survivin/BORI gene family + Hidden Markov models used for these annotations.

Dataset S6
Hidden Markov Models and multiple sequence alignment files for the detection of both N- and C-terminal helices in Survivin/BORI orthologs (EukProt sequence database).
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