Expanded View Figures

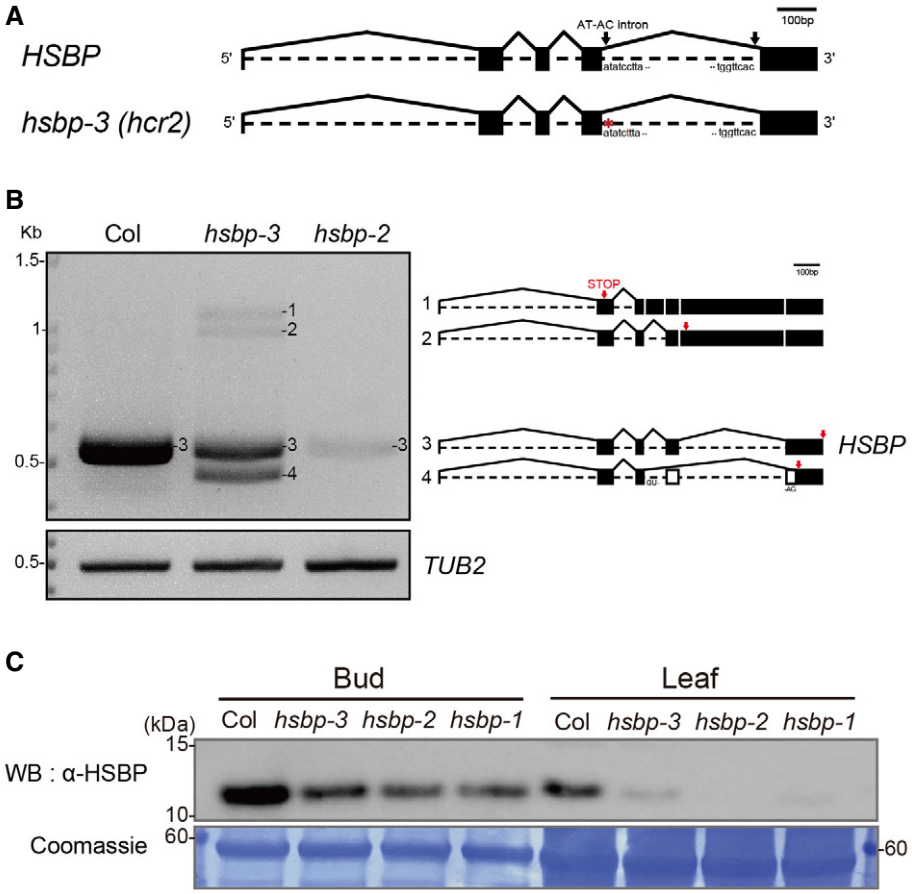

Figure EV1. Effects of *hsbp* alleles on *HSBP* transcripts and HSBP abundance.

A Schematic representation of *HSBP* transcripts and position of the *hsbp-3* mutation (red).

B Aberrant long, short splicing variants (1, 2, 4), and normal transcripts (3) of *HSBP* in *hsbp-3*. Arrows (red) indicate the positions of premature stop codons in abnormal splicing variants or the normal stop codon in Col transcripts.

C Immunoblot analysis of HSBP of Col, *hsbp-3*, *hsbp-2*, and *hsbp-1* in leaves and buds. Coomassie-stained membrane served as a loading control.

Data information: Experiments were performed at least three times (B, C).
Source data are available online for this figure.

Figure EV2. Meiosis-specific miRNA-induced gene silencing (meiMIGS) of *HSBP*.

A Schematic diagram of the *meiMIGS-HSBP* construct. Scale bar, 100 bp.

B Schematic diagram of the pipeline followed to measure crossover frequency in *meiMIGS-HSBP* lines using fluorescent *420* seed or pollen *I3bc* reporters. Scale bars: 1 mm for seeds, 0.25 mm for pollens.

C Schematic diagram of the *meiMIGS-HSBP* mode of action to generate *trans*-acting miRNAs during meiosis and silence endogenous *HSBP* transcripts.

D RT–qPCR analysis of *HSBP* transcript levels in Col and *meiMIGS-HSBP* transgenic lines. *TUB2* was used as a reference. Data points (black) represent three biological replicates and three technical repeats per replicate. Red dots and horizontal lines indicate mean ± s.d. of values (one-sided Welch's *t*-test).

E Correlation between *420* genetic distances (*y*-axis, in cM) and *HSBP* transcript levels in floral buds of Col and *meiMIGS-HSBP* lines. The *x*-axis indicates fold-enrichment of *HSBP* transcript levels compared to those in Col, as determined by RT–qPCR. *DMC1* was used as a meiotic gene for normalization. Mean values of triplicate RT–qPCR in Col plants and transgenic lines were used. Col and *meiMIGS-HSBP* plants are shown as black and red dots, respectively.

F Crossover frequency (cM) of *I3bc* in wild-type Col (blue) and *meiMIGS-HSBP* T_1 transgenic plants using the meiotic promoters from the genes *DMC1* (red), *HEI10* (orange), and *ASY1* (purple) to drive *MIGS-HSBP* expression. Data points (color) indicate cM values from individual plants. Black dots and horizontal lines indicate mean ± s.d. of cM values (one-sided Welch's *t*-test). $n \geq 5$ plants of biological replicates.

G Immunoblot analysis of HSBP abundance in Col, *hsbp-3*, *hsbp-2*, *hsbp-1*, and three *meiMIGS-HSBP* T_2 transgenic lines in buds. Coomassie-stained membrane served as a loading control. Experiments were performed at least three times.

Source data are available online for this figure.

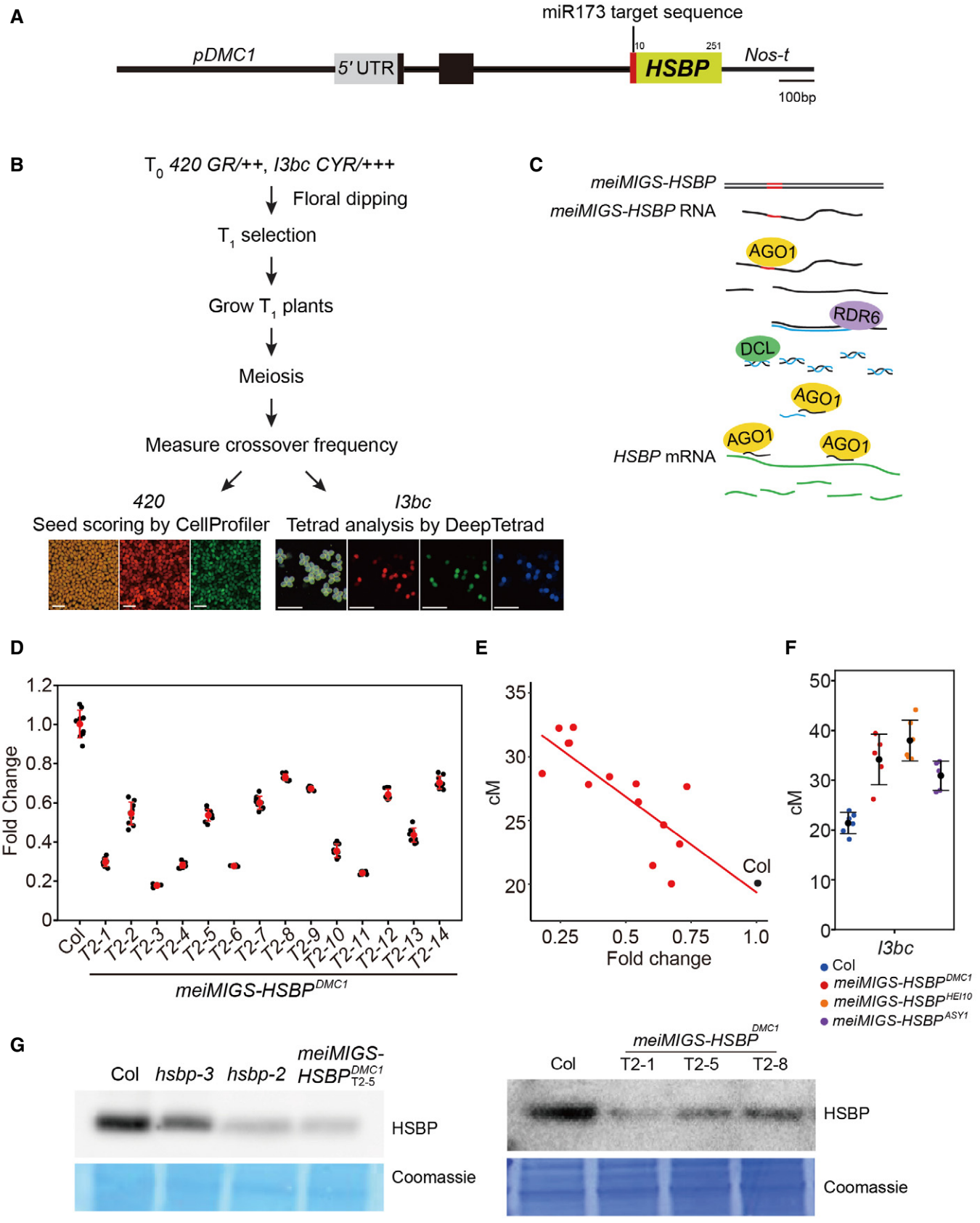

Figure EV2.

Figure EV3. HSBP represses *HEI10* transcription by inhibiting HSFs.

A Heatmap representation of transcript levels in Col-0 and *hsbp-3* for *Arabidopsis* class A *HSF* genes in seedlings and buds from RNA-seq data.
B RT–qPCR analysis of relative *HEI10* transcript levels following transient transfection of *HSFA1a* and *HSBP* effector constructs in *Arabidopsis* protoplasts and after heat treatment (40°C for 1 h). Immunoblots of HSF1a-HA and HSBP-Myc in transfected protoplasts. Coomassie-stained membrane served as a loading control.
C As in B, for *HSFA7a* and *HSBP*.
D DAP-seq data of HSFs at *HEI10* regulatory regions. Candidate HSEs (*HEI10*_1 to 5) around *HEI10* are shown.
E As in (B), but showing ChIP–qPCR analysis of HSF7a at *HEI10* regulatory regions in protoplasts expressing *HSF7a-HA*. IgG was used as a negative control.
F As in (B), but showing HSBP ChIP–qPCR analysis in heat-treated seedlings using anti-HSBP antibody. Seedlings (10-day) were incubated at 37°C for 3 h and used for ChIP analysis.
G RT–qPCR analysis of relative *HEI10* transcript levels in Col and *hsbp-3* after heat treatment. Seedlings (10-day-old) were incubated at 37°C for 4 h. Data points (color) indicate values of replicates. Black dots and horizontal lines mean ± s.d. values (one-sided Welch's *t*-test). $n \geq 6$ two or three technical duplicates of three biological replicates.
H Co-localization of the fluorescent fusion proteins HSBP-RFP and HSFA1a-GFP in protoplasts. Heat indicates incubation of transfected protoplasts at 40°C for 1 h. Scale bars: 5 μm.
I As in (H), but showing HSBP-CFP and HSFA1a-GFP (left), and HSBP-GFP and nucleus marker (ARR2-mRFP) (right) after H_2O_2 treatment. Scale bars: 5 μm.
J Nuclear location of HSBP in male meiocytes of *HSBPpro:HSBP-YFP* plants. Nuclei spreads were stained with DAPI. Scale bars: 10 μm.
K Immunoblot analysis of HSBP and histone H3 levels using total proteins and nuclear extracts of Col seedlings. Seedlings (10 days old) were incubated at 37°C for 3 h. The Coomassie-stained membrane was used as a loading control. Experiments were performed at least three times.
L Co-immunoprecipitation analysis of Myc-HSBP with HSF7a-HA. IB, immunoblot; IP, immunoprecipitation. Ponceau S staining of the membrane, a loading control. Experiments were performed at least three times.

Data information: (B, C, E, F) Experiments were performed three times. Data points (black) indicate three technical duplicates of three biological replicates. Red dots and horizontal lines indicate mean ± s.d. values (one-sided Welch's *t*-test).
Source data are available online for this figure.

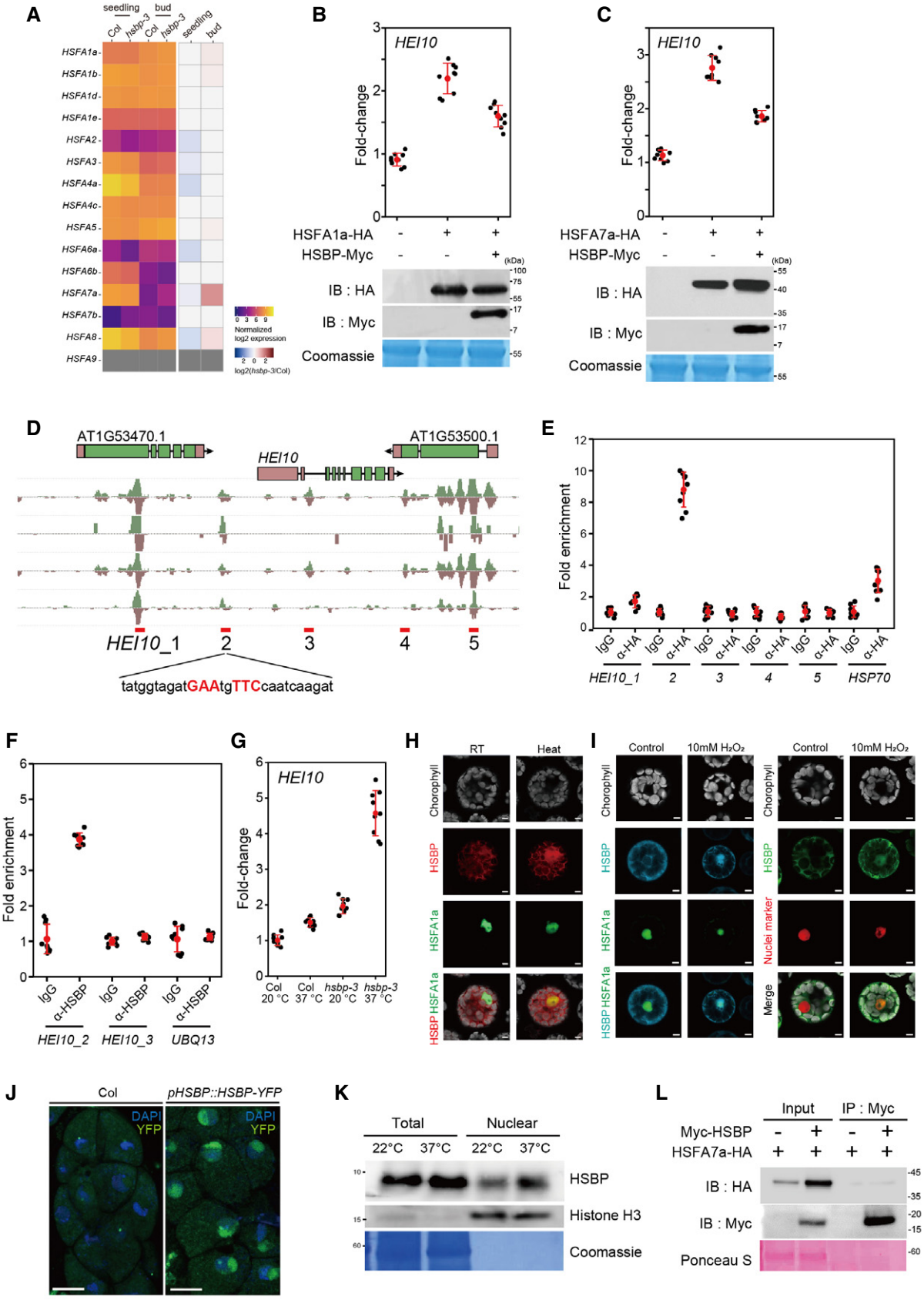

Figure EV3.

Figure EV4. Representative images of HSBP immunostaining in wild-type Col, *hsbp-3*, and *hsbp-2* during meiosis.

Nuclei spreads were stained with DAPI (white). HSBP signals (green) were reduced in *hsbp-3* and rarely detected in *hsbp-2*. Scale bars: 5 μm. Images representative of three biological replicates.

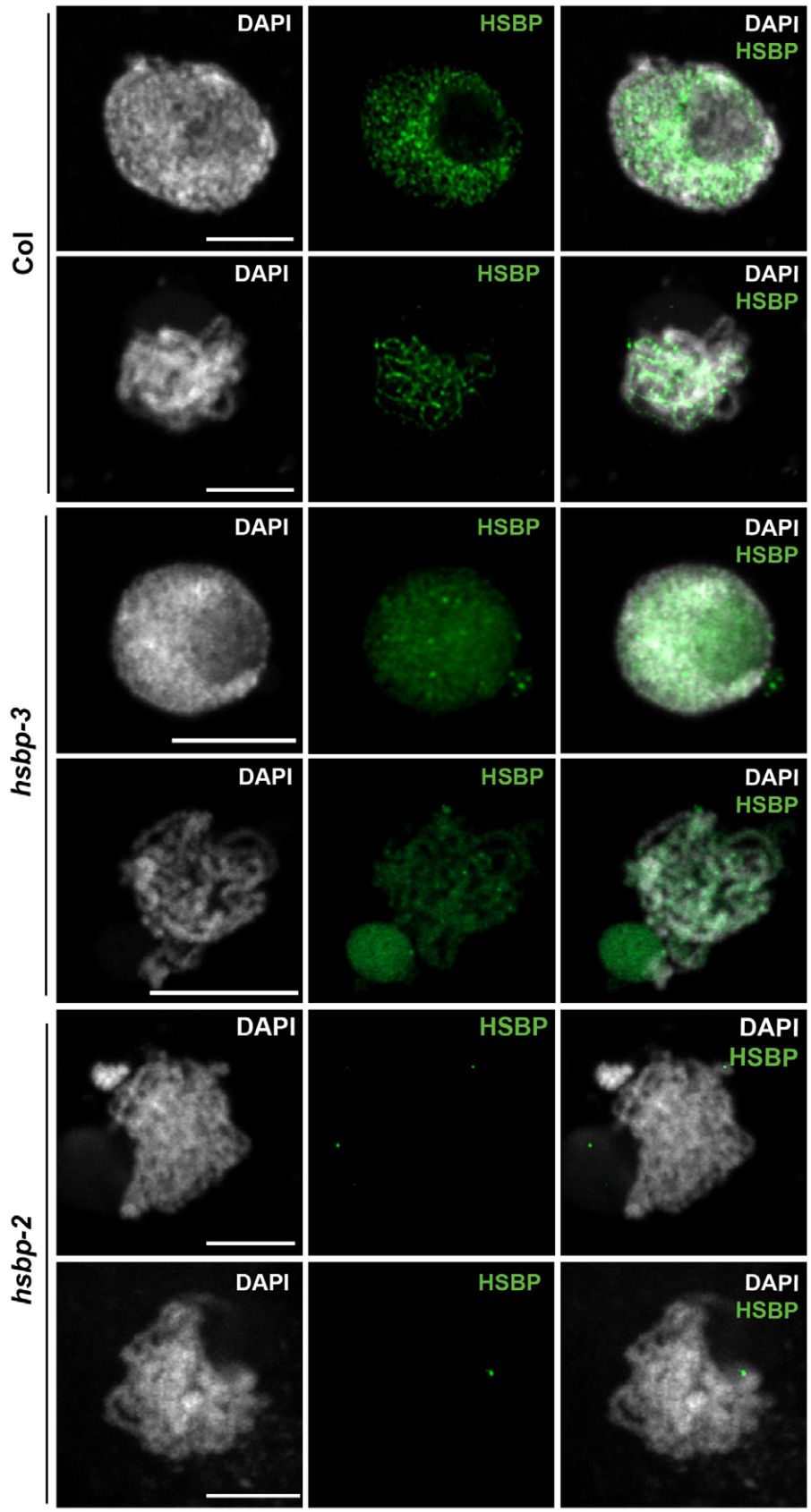

Figure EV4.

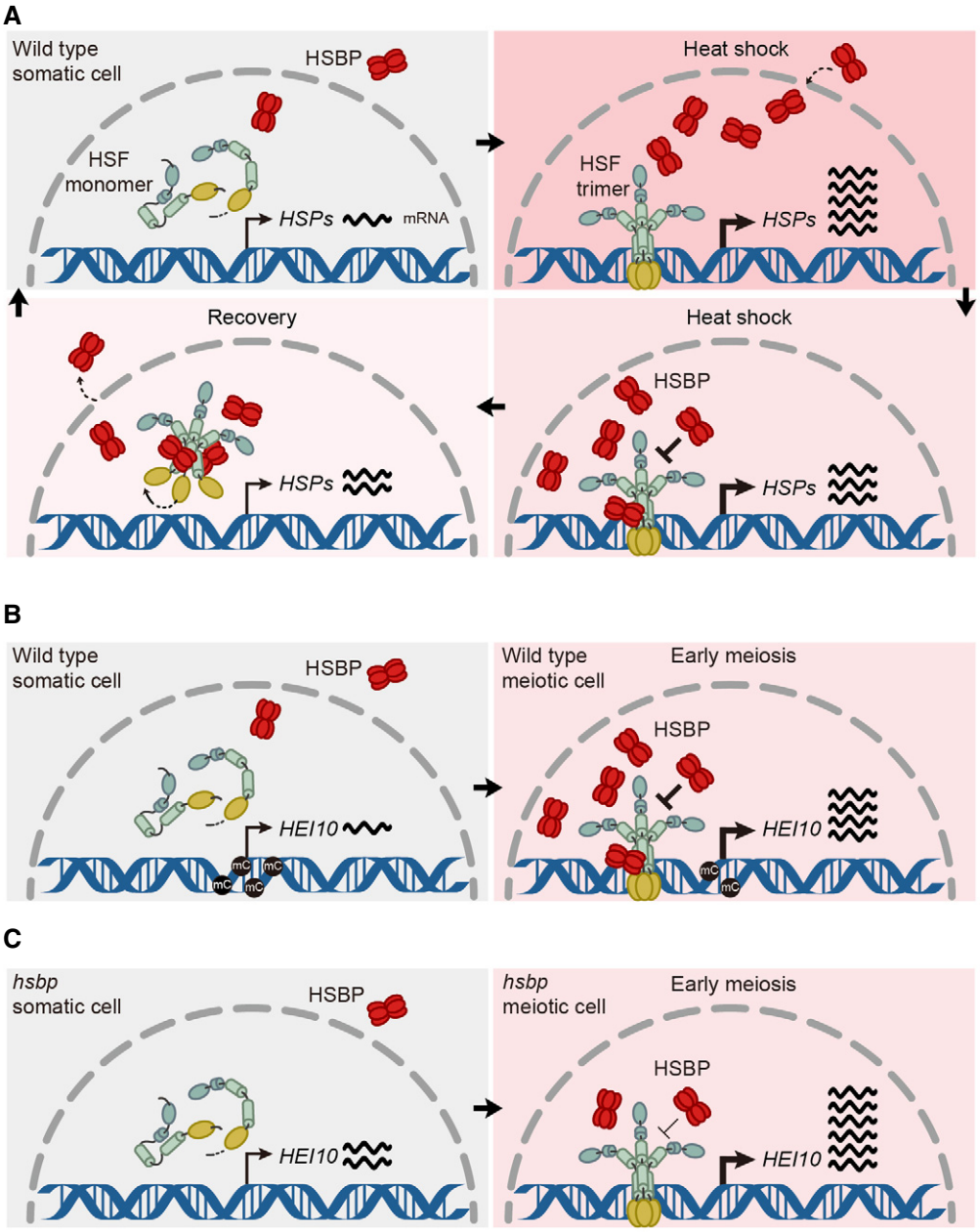

Figure EV5. Proposed model of HSBP for control of *HEI10* transcription.

A In somatic cells during heat shock, HSBPs translocate from the cytosol to the nucleus to attenuate the activity of the HSF (heat shock factor) trimer. The activation-attenuation cycle of HSF-HSBP controls the transcription of heat shock protein genes (*HSPs*) and many other genes including *HEI10*.

B In meiotic cells, during early meiosis, HSFs are highly expressed and activated by unknown developmental factors and signals, potentially including reactive oxygen species (ROS). Plant meiocytes are surrounded by multi-layered cells and thick callose cell walls, which may induce hypoxia and ROS. Active HSFs contribute to the transcriptional induction of *HEI10*, in addition to many other genes. Simultaneously or subsequently, HSBP accumulates to high levels and moves to the nucleus during early meiosis. HSBP hexamers bind to HSF trimers and attenuate HSF activity, which decreases *HEI10* transcription. The protein levels and activities of HSFs and HSBP are likely important for determining transcript levels of *HEI10* during early meiosis.

C In *hsbp-3* meiotic cells, HSBP levels are lower than in wild type during early meiosis, which leads to higher *HEI10* transcripts, HEI10 protein levels, and class I crossovers. The lack of DNA methylation at the *HEI10* 5′ UTR contributes to the initiation of meiosis with higher *HEI10* transcript levels in *hsbp-3*.