

1 **Supplementary Materials**

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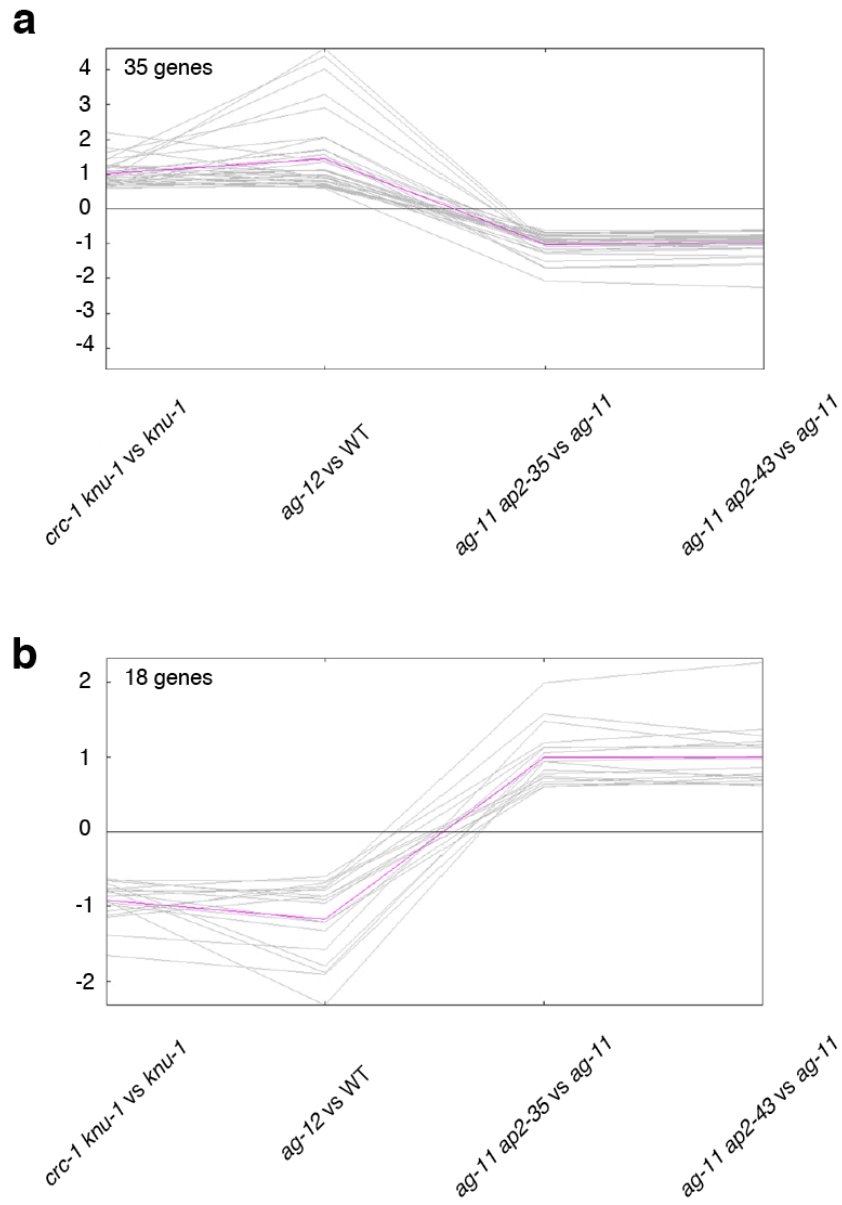
3 Chromatin-mediated feed-forward auxin biosynthesis in floral meristem determinacy

4 Yamaguchi et al.

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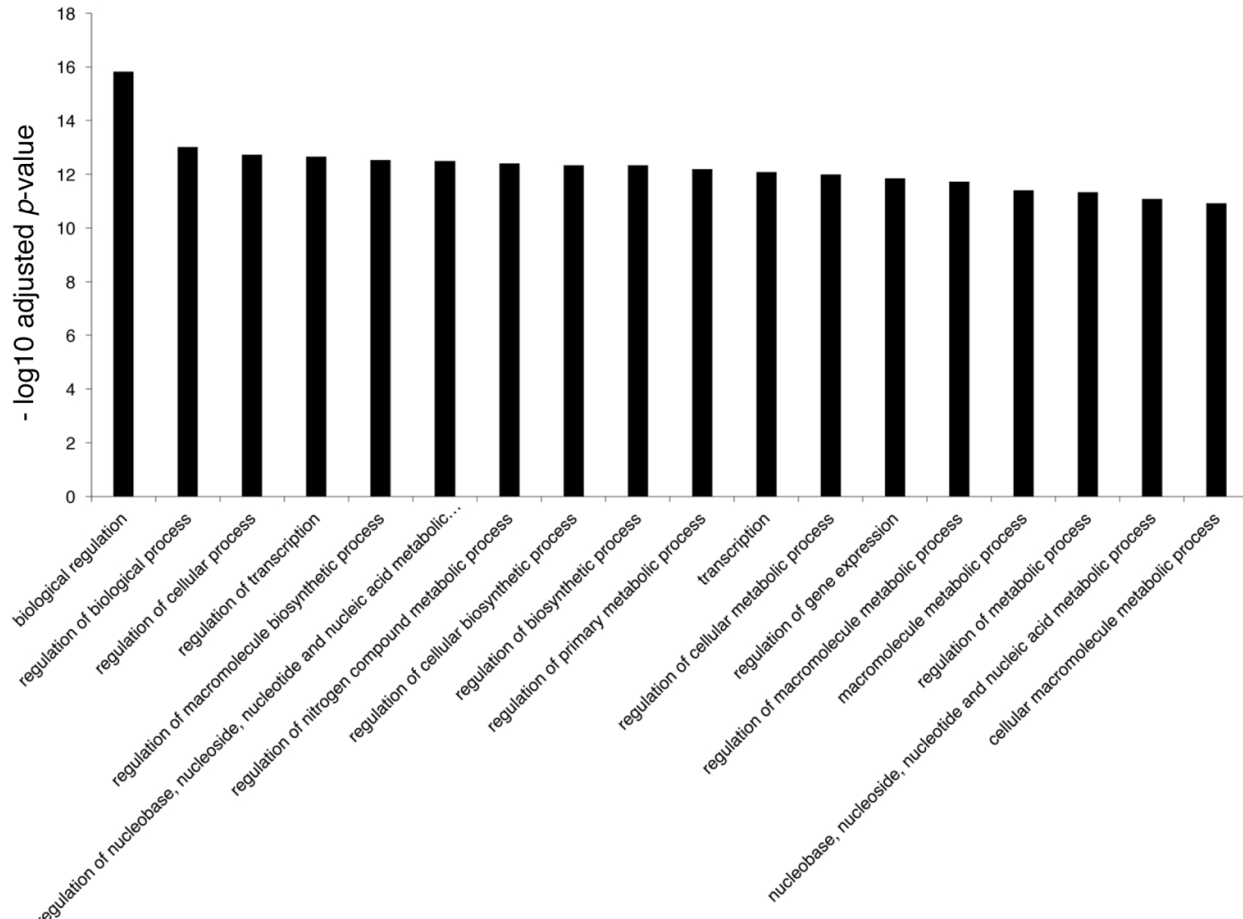
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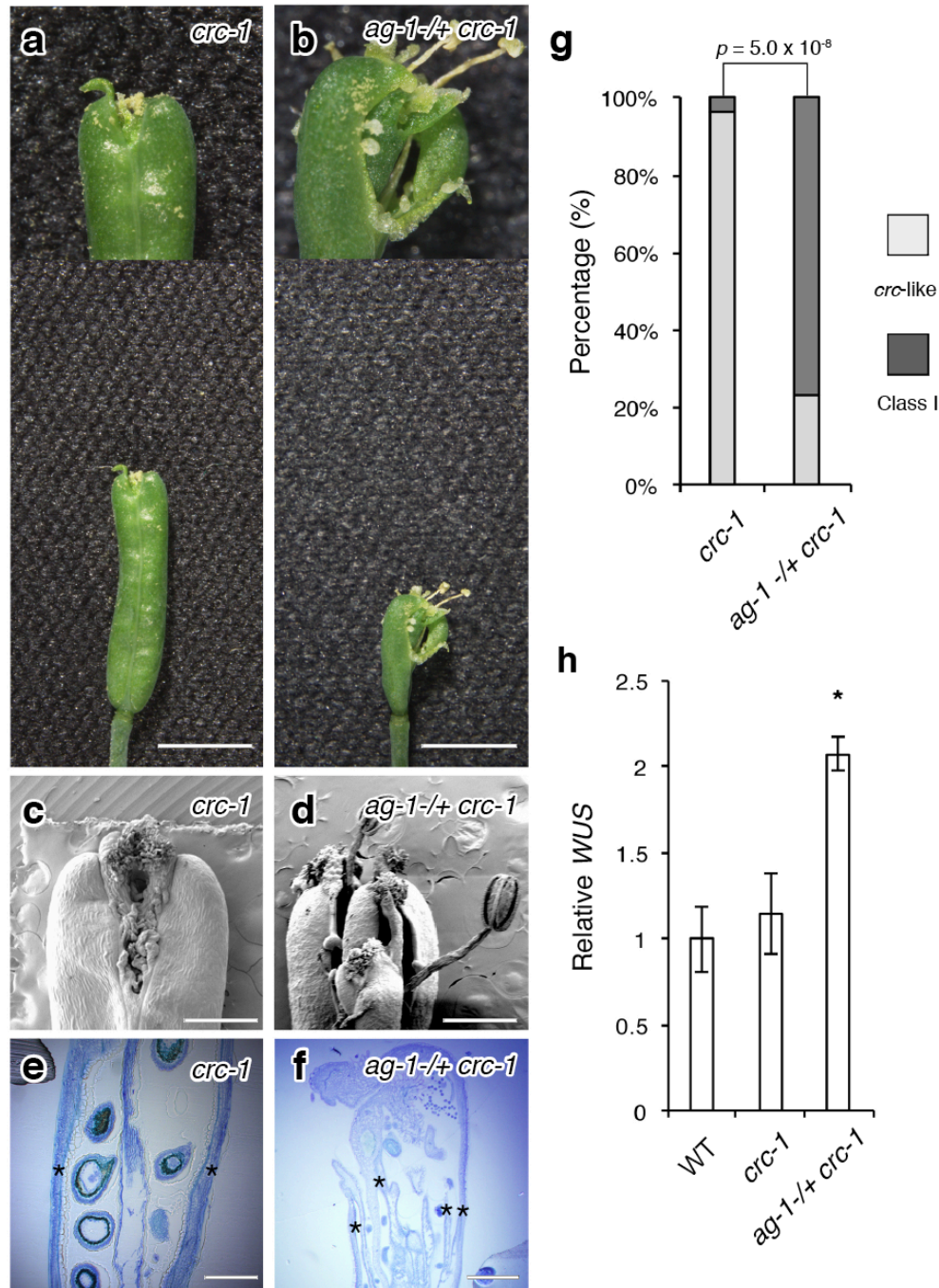


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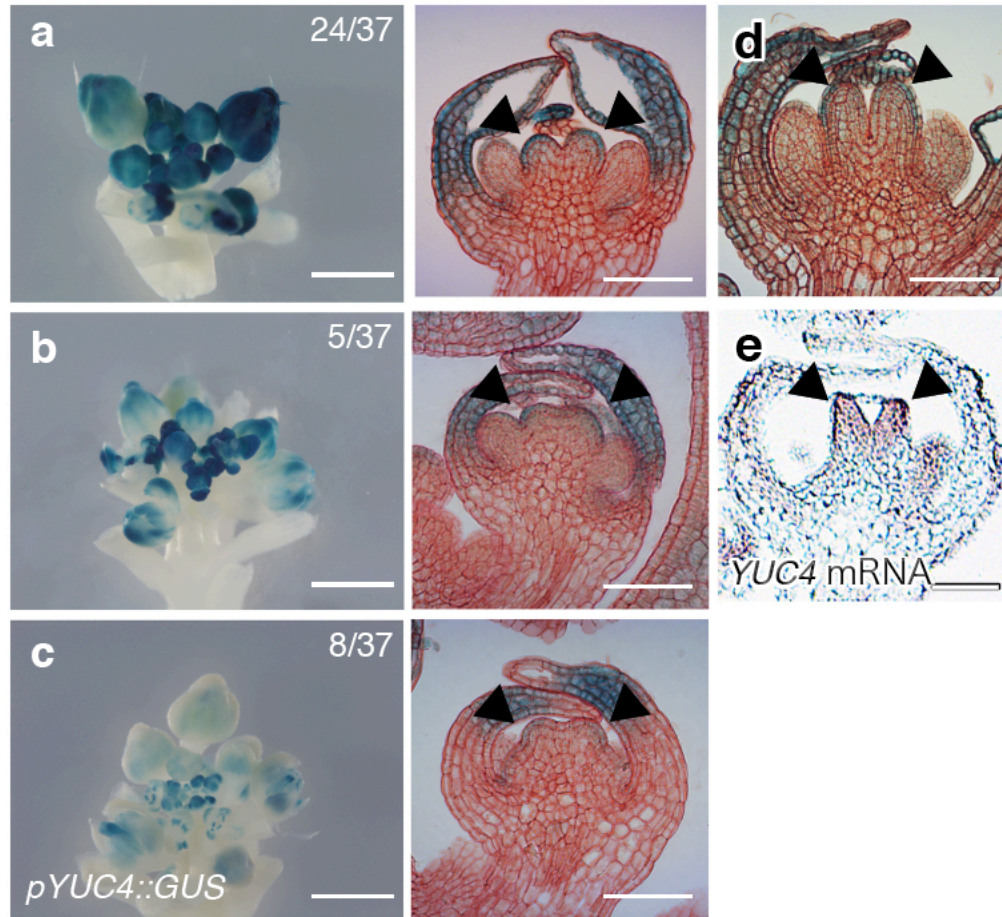
4 **Supplementary Figure 1** Gene expression changes of 53 genes downstream of AG and CRC. **a**
5 Graphs display log₂ expression changes of the 53 targets based on four public transcriptome
6 datasets. One cluster contains the 35 genes that are upregulated in *crc-1 knu-1* compared to
7 *knu-1*. **b** The other cluster contains the 18 genes that are downregulated in *crc-1 knu-1*
8 compared to *knu-1*.



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 2 **Supplementary Figure 2** GO term enrichment analysis of the 53 genes. Graphs display $-\log_{10}$
 3 adjusted p -values of the 53 targets based on agriGO followed by REVIGO. The false discovery
 4 rate (FDR) was lower than 1.7×10^{-10} .

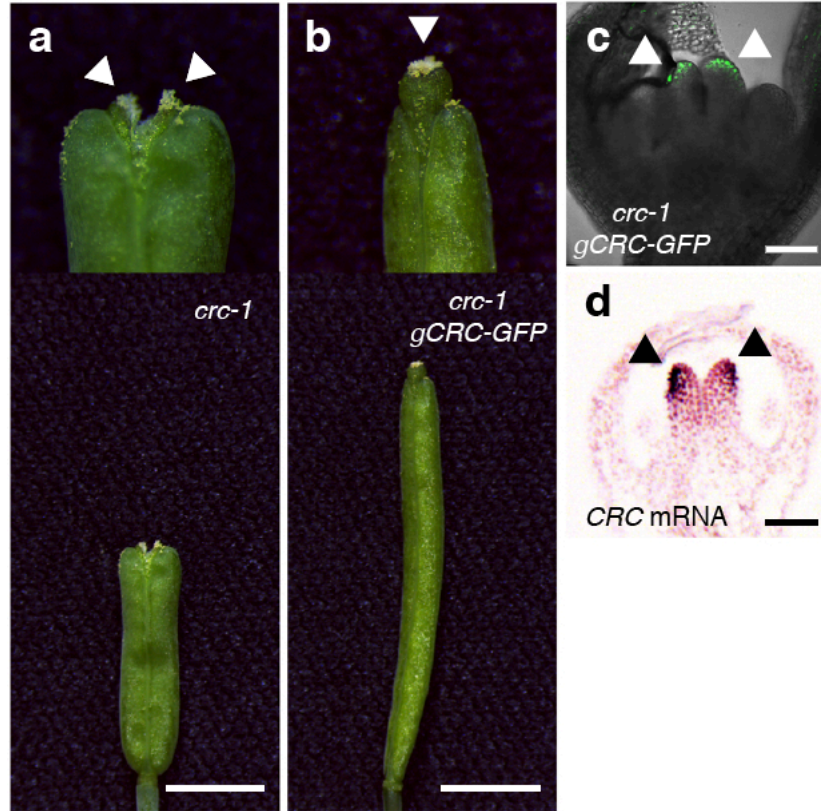


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2 **Supplementary Figure 3** Indeterminate phenotype of *ag-1 -/+ crc-1*. **a, b** Morphology of *crc-1*
3 (**a**) and *ag-1 -/+ crc-1* (**b**) fruits. Above: Close-up views of fruit tips. Below: Whole fruits.
4 Arrowheads indicate stigma structures. **c, d** Scanning electron micrograph of *crc-1* (**c**) and
5 *ag-1 -/+ crc-1* (**d**) fruits. **e, f** Longitudinal section of *crc-1* (**e**) and *ag-1 -/+ crc-1* (**f**) fruits.
6 Asterisks indicate carpels. **g** Quantification of the mutant phenotype. *p*-values were calculated
7 with a Chi-Square test. **h** mRNA abundance of the stem cell marker *WUS* in wild-type, *crc-1*,
8 and *ag-1 -/+ crc-1* flowers. The values are represented as the means \pm SEMs. *p*-values were
9 calculated with a Student's *t*-test. Bars = 1 cm in **a, b**; 1 mm in **c, d**; 500 μ m in **e, f**.



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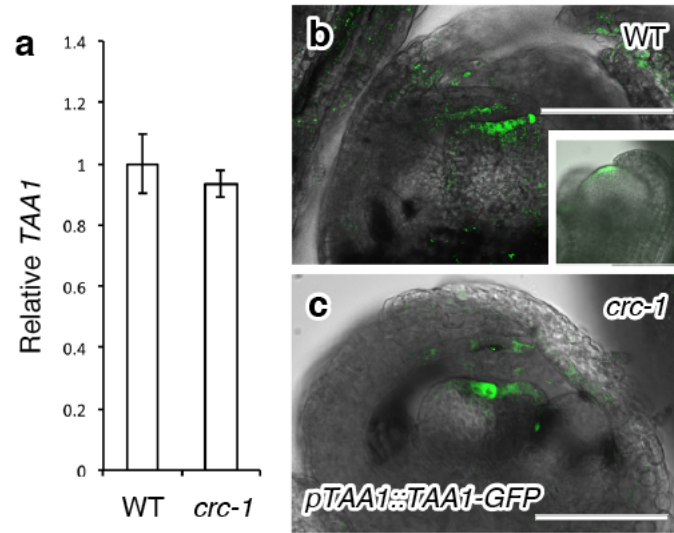
Supplementary Figure 4 *YUC4* expression in floral buds. **a-c** GUS staining of *pYUC4::GUS* in the inflorescences (left) and stage 6 floral buds (right). Independent T1 lines were categorized in three difference classes depending on expression levels: lines with strong (**a**), intermediate (**b**), and weak (**c**) signals. Specifically, 24, 5, and 8 out of 37 lines showed strong, intermediate, and weak expression, respectively. The representative line from the strong category were shown. **d** GUS staining of *pYUC4::GUS* in the stage 7 floral buds. **e** *YUC4* expression pattern, as determined by *in situ* hybridization. Arrowheads indicate abaxial side of epidermal cells in carpels at stage 6. Bars = 3 mm in **a-c** (left); 50 μ m in **a-c** (right), **d**, **e**.



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Supplementary Figure 5 Expression of *gCRC-GFP* and *CRC* mRNA. **a, b** Phenotypic rescue of the *crc-1* mutant phenotype by *gCRC-GFP* expression. Morphology of *crc-1* (**a**) and *crc-1 gCRC-GFP* (**b**) plants. Top: Close-up views of fruit tips. Bottom: Whole fruits. Arrowheads indicate stigma structures. **c** *gCRC-GFP* expression in wild-type stage 6 floral buds. **d** *In situ* hybridization of *CRC* mRNA in wild-type stage 6 floral buds. Arrowheads indicate the abaxial side of carpel primordia. Bars = 5 mm in **a, b**; 50 μ m in **c, d**.

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4 **Supplementary Figure 6** *TAA1* expression in the *crc-1* mutant. **a** *TAA1* expression in wild-type,
5 and *crc-1*. The values are represented as the means \pm SEMs. **b**, **c** *TAA1*-GFP in longitudinal
6 sections of wild-type (**b**), and *crc-1* (**c**) stage 6 floral buds. Inset was *TAA1*-GFP expression in
7 stage 4 floral buds. Bars = 50 μ m in **b**, **c**.

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BnapusYUC41:12408-12873 TGTGTGGTCATGTAACATATGATCTATGATGAGACAAAATGAGGGAGAAATTCAGCCTGA
BrapaYUC4:18227-18692 TGTGTGGTCATGTAACATATGATCTATGATGAGACAAAATGAGGGAGAAATTCAGCCTGA
LalabamicaYUC4:10757-11228 AATGTGGTCATGTAACATATGTTGA--GACAAATCGAGAGTGAGGGCAAATTCAGCCTGA
AthaYUC4:1570-2042 AGTGTGGTCATGTAACATATGGTGA--GAAAAATTGAGGTTGAGGGCAAATTCAGCCTGA
AlyraYUC4:240239-240713 AGTGTGGTCATGTAACATATGGTFA--GACAAATGAGGTTGAGGGCAAATTCAGCCTGA
CrubellaYUC4:86652-87138 AGTGTGGTCATGTAACATATGGTGA--GACAAATGAGGCTGAGGGCAAATTCAGCCTGA
CsativaYUC4:41129-41606 AGTGTGGTCATGTAACATAT-----GGTATTGAGGTTGAGGGCAAATTCAGCCTGA
* * * * *

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BrapaYUC4:18227-18692 CAGAAAAATCCAAAGAGCACTGAGTCGGGAGGAT-----GCAGAATGAGAA--AATTC
LalabamicaYUC4:10757-11228 CAGAAAAATCCAAAGGCATTTGCTCGGGTTAATG-----CATAAGAAGAAAATAGA
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AlyraYUC4:240239-240713 CAGAGAAAATCCAAAGAGC-----AGTGTAGGATAATGCAGAAGGAGAA--AATGG
CrubellaYUC4:86652-87138 CAGAAAAATCCAAAGAGAAAT-----ACTGAGTCGGATAATGCAGAAGGAGAA--AATGG
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**** * * ***** ** * * * * *

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LalabamicaYUC4:10757-11228 AATAAGAGTCCAAAGG---AAGATGATGTTT--TTGTAGGACATGATACTTTTGTCTGC
AthaYUC4:1570-2042 AAGAAGAGGCCAGAGC---AAGATGACGTTTTTATGGGACCCAAAATCTTTTGTCTAC
AlyraYUC4:240239-240713 AAGAAGATGCCAGAGG---AAGATGACGTTTTTATGGGACCCAAAATCTTTTGTCTAC
CrubellaYUC4:86652-87138 AAGAAGAGGCCAGAGG---AAGATGACGTTTTTATGGGACCGTTAAATCTTTTGTCTAC
CsativaYUC4:41129-41606 TAGAAGAGGCCAGAGG---AAGATGACGTTTTTATGGGACCCAAAATCTTTTGTCTAC
* * * * * * * * * * * * * * * * * * * * *

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BrapaYUC4:18227-18692 GTATATAATATTCTTGTTTTGGCCAT-----
LalabamicaYUC4:10757-11228 ATTGTAATATTCAATTTTACCAT-----
AthaYUC4:1570-2042 ATTGTAATATTCAATTTTGGCCAT-----
AlyraYUC4:240239-240713 ATTGTAATATTCAATTTTGGCCAT-----
CrubellaYUC4:86652-87138 ATTGTAATATTCAATTTTGGCCAT-----
CsativaYUC4:41129-41606 ATTGTAATATTCAATTTTGGCCAT-----
* * * * * * * * * *

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BrapaYUC4:18227-18692 -----
LalabamicaYUC4:10757-11228 -----
AthaYUC4:1570-2042 -----
AlyraYUC4:240239-240713 -----
CrubellaYUC4:86652-87138 -----
CsativaYUC4:41129-41606 -----

TarvenseYUC4:58907-59887 AGATGATAACCTATTCTCAATTTAAAGATTTTGGATCCATGTGAAAATCCTTAACATAA
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BrapaYUC4:18227-18692 -----
LalabamicaYUC4:10757-11228 -----
AthaYUC4:1570-2042 -----
AlyraYUC4:240239-240713 -----
CrubellaYUC4:86652-87138 -----
CsativaYUC4:41129-41606 -----

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BrapaYUC4:18227-18692 -----
LalabamicaYUC4:10757-11228 -----
AthaYUC4:1570-2042 -----
AlyraYUC4:240239-240713 -----
CrubellaYUC4:86652-87138 -----
CsativaYUC4:41129-41606 -----

TarvenseYUC4:58907-59887 TAGATCAAAAACACAATAATTTAGAATATTAATTTAAAAATTGTATGTTGTAATATATATT
BnapusYUC41:12408-12873 -----
BrapaYUC4:18227-18692 -----
LalabamicaYUC4:10757-11228 -----
AthaYUC4:1570-2042 -----
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CrubellaYUC4:86652-87138 -----
CsativaYUC4:41129-41606 -----

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LalabamicaYUC4:10757-11228 -----
AthaYUC4:1570-2042 -----
AlyraYUC4:240239-240713 -----
CrubellaYUC4:86652-87138 -----
CsativaYUC4:41129-41606 -----

TarvenseYUC4:58907-59887 TATCAAAGTCTGTTATGCATAAGTTAAATTATATACTAATATCATTTTCAATTGGTATT
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LalabamicaYUC4:10757-11228 -----
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LalabamicaYUC4:10757-11228 -----
AthaYUC4:1570-2042 -----
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CsativaYUC4:41129-41606 -----

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LalabamicaYUC4:10757-11228 -----
AthaYUC4:1570-2042 -----
AlyraYUC4:240239-240713 -----
CrubellaYUC4:86652-87138 -----
CsativaYUC4:41129-41606 -----

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BrapaYUC4:18227-18692 -----
LalabamicaYUC4:10757-11228 -----
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LalabamicaYUC4:10757-11228 ATTCCTCGTCTAACCAAAGGGACCATGATGAATACATATATATGTAACATGCATATAAAGT
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* * * * *

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BrapaYUC4:18227-18692 --ATTACTAGTAGCTGATACACTTGCATAG----CTTGT----AATTTCTCCTAGATT
LalabamicaYUC4:10757-11228 ATTTTACTAGTAGCTGATACACTTGTAT-----TTTTCTCCAGCTT
AthaYUC4:1570-2042 AGATTAATAGTATAGCCGA---TACACTGTATAACTTGTAT----TTTTCTCCAGCTT
AlyraYUC4:240239-240713 AGATT--AATAGTAGCTGA---TACACTGTATAACTTGTAT----TTTTCTCCAGCTT
CrubellaYUC4:86652-87138 AGATTAGTAGTAGCTGATACACTTGTATTTGTATAACTTGTATTTTTTCTCCAGCAGCTT
CsativaYUC4:41129-41606 ---TTAATAGTAGCTGATACACTTGTATAACTT----GTATTTTCTTACCAGCTAGCTT
* * * * *

- 1 **Supplementary Figure 7** DNA sequence alignment of the CRM1 region in the *YUC4* promoter.
- 2 DNA sequence information for all eight species was obtained from GenBank. No potential
- 3 YABBY binding sites were found in this region.
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CRM2

| | |
|----------------------------------|---|
| <u>BnapusYUC4</u> :14178-14351 | ----TAGTATGTTTAAATAGTATTATATAGCAATCTCAAAGGTTTCGTAGGGGAAGTGGAG |
| <u>BrapaYUC4</u> :16831-17018 | ----TAGTATGTTTAAATAGTATTATATAGCAACCTCAAAGGTTTCGTAGGGGAAGTGGAG |
| <u>CrubellaYUC4</u> :85613-85792 | AAAATATGTTAAATATTGATAATGCAAACCGCAGAGGAGATGATATAGGGGAAGTGGAG |
| <u>AthaYUC4</u> :3184-3344 | -AAA-----TATGTTAAATATTGATATTGCAACCTTATAGAGATCATAGGGGAAGTGGAG |
| <u>TarvenseYUC4</u> :57715-57890 | -AACTATGCTAAGTATTTAGTATATTTCAACCTCAAAGGAGGATCATAGGGGAAGTGGAG |
| <u>AlyraYUC4</u> :238353-239045 | -AAATATGTTAA--ATATTGATATTGCAACCTCAGAGAAGAGATTATAGGGGAAGTGGAG |
| <u>CsativaYUC4</u> :42737-42908 | -AGATATGCTGA--AAATTGATATTGCAACCTC--AGAGGAGATCATAGGGGAAGTGGAG |

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| <u>BnapusYUC4</u> :14178-14351 | GTAGGCTGCATTAGTTGACATTTTACCGAAGGAGTGGTCAGTCATGCGCAACGCAATTAC |
| <u>BrapaYUC4</u> :16831-17018 | GTAGGCTGCATTAGTTGACATTTTACCGAAGGAGTGGTCAGTCATGCGCAACGCAATTAC |
| <u>CrubellaYUC4</u> :85613-85792 | GTCGGCTGCATAAGTTGACATTTAA-CGAAGGAGTGGTCAGTCATGCGCAACTCAGTTA |
| <u>AthaYUC4</u> :3184-3344 | GTCGGCTGCATTAGTTGACATTTAA-CGAAGGAGTGGCAAGTCAAATFACATTTTCCACA |
| <u>TarvenseYUC4</u> :57715-57890 | GTCGGCTGCATTAGTTGACATTTAA-CGAAGAAGTGGCCAGTCATGCGCAACACAGATAC |
| <u>AlyraYUC4</u> :238353-239045 | GTCGGCTGCATTAGTTGACATTTAA-CGAAGGAGCGGTCAGTCATGCGCAACTCAATTAC |
| <u>CsativaYUC4</u> :42737-42908 | GTCGGCTGCATTAGTTGACATTTAA-CGAAGGAGTGGTCAGTCATGCGCAACTCAATTAC |

| | |
|----------------------------------|--|
| <u>BnapusYUC4</u> :14178-14351 | ATTTTCC---ACATATCTACTTATTAATTATGTGTTTGGTACAATCCTCATTAAAT---A |
| <u>BrapaYUC4</u> :16831-17018 | ATTTTCC---ACATATCTACTTACTAATATTAATTATGTGTTTGGTACAATCCTCATTAA |
| <u>CrubellaYUC4</u> :85613-85792 | CATTTTCCACATACATCTACTTATTAATTATGTGTTTGGTGCATCCTCATTAAATGTGT |
| <u>AthaYUC4</u> :3184-3344 | -----TATCCAC----- |
| <u>TarvenseYUC4</u> :57715-57890 | ATTTTCC---ACATATCTATTTATTACTTATGTGTTTGGTACAATCCTCGTTAATTGTTC |
| <u>AlyraYUC4</u> :238353-239045 | ATTTCCA---CATATCTACTTATTAATTATGTGTTTGGACCCTGTPTTGAA |
| <u>CsativaYUC4</u> :42737-42908 | ATTTTCC---ACATATCTACTTATTAATTATGTGTTTGGTACAATCCTCATTAAATGTGC |

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CRM3

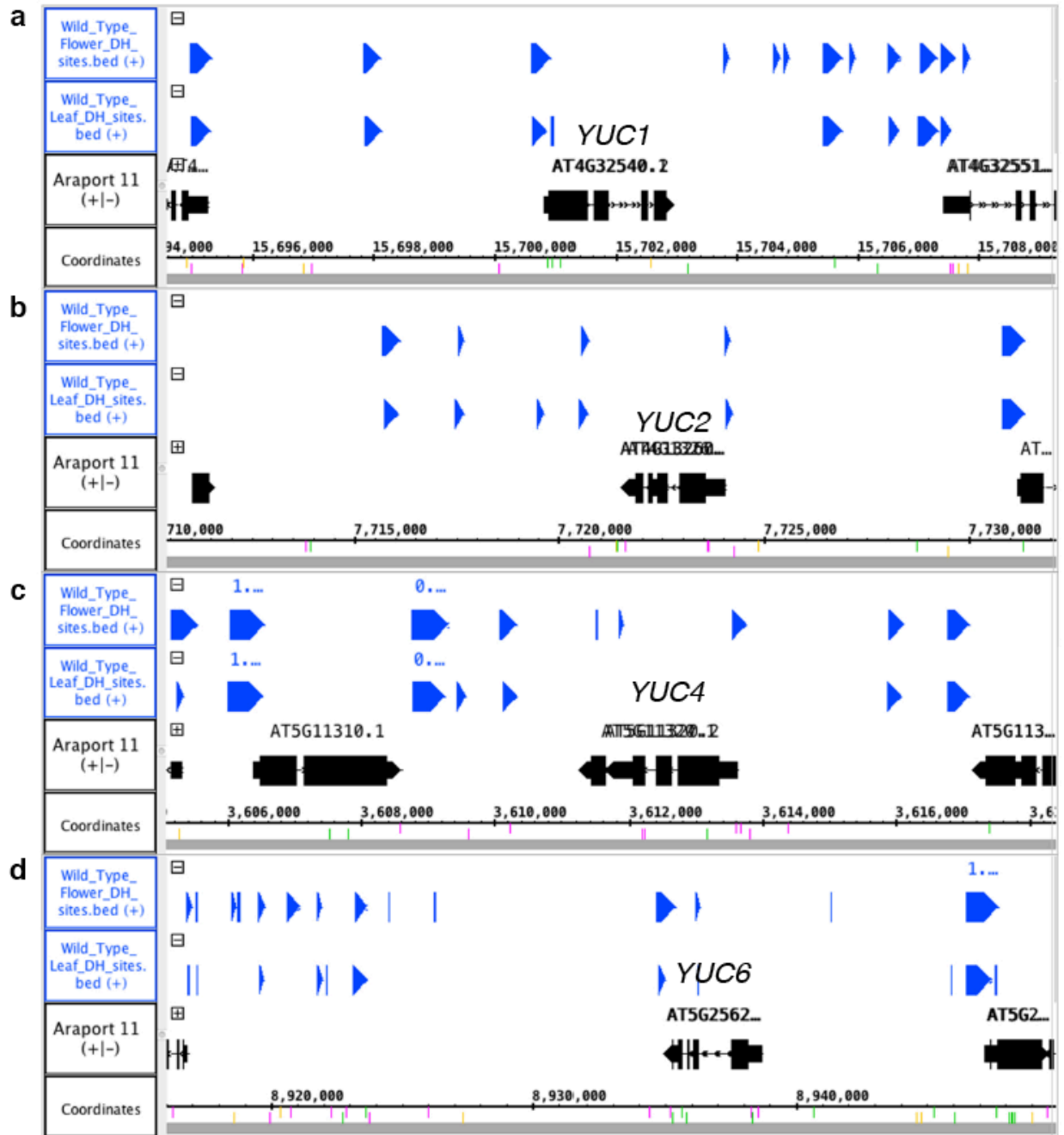
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| <u>TarvenseYUC4</u> :57093-57154 | GCATTTGTTAAAGCAAGAAATGTTAA--TTATAGGCATTTTATTACATGAATCTTCTATTTTC |
| <u>BnapusYUC4</u> :14668-14728 | ACCTTTGTTTAAAGTAAGAAATGTTAA--TTAGTGGGAAGTTAATTACCTGAGTCTTCTATAAT- |
| <u>CsativaYUC4</u> :43331-43392 | ACCTTTGTTAAAGTAAACAATATTTGAGTATTAGACATTTA-TTTAAATAAATCTTCCGTTT- |
| <u>AthaYUC4</u> :3507-3569 | ACCTTTGTTAAAGTAAACAATATTTGATTAATTGATAAATTAATGACATGAATCTTCTGTTTC- |
| <u>AlyraYUC4</u> :238044-238106 | ACCTTTGTTAAAGTAACAGTATTTGATTAATTAGGCAATTAATTACATGAATCTTCTGTTTC- |

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AAATATGTTAAATATTGATATTGCAACCTTATAGAGATCATAGGGGAAGTGGAGGTCGG
 CTGCATTAGTTGACATTTAACGAAGGAGTGGCAAGTCAAATTACATTTTCCACATATCC
 ACTTATTAATTATGTGTTTGGTGAATTTCTCATTAAATTGTGCTTGAATATATCATCCCT
 CCAGCTACCTCAAATCAAAGCGTAGAAGTAGTTATCTTTTGATTATGTAT**CTTCTTT**
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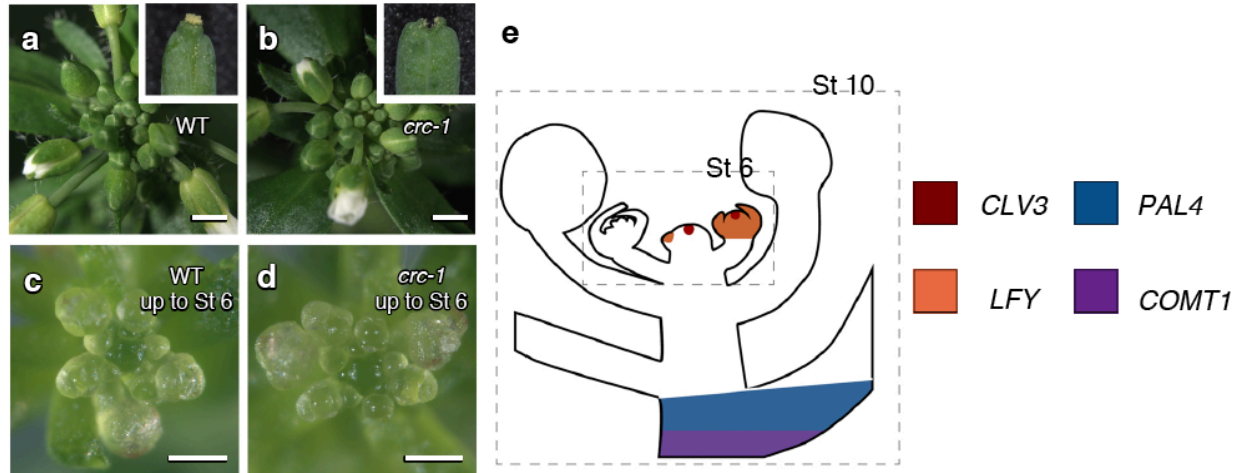
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Supplementary Figure 8 DNA sequence alignment of the CRM2 and CRM3 regions in the *YUC4* promoter. **a**, **b** DNA sequence alignment of the CRM2 (**a**) and CRM3 regions (**b**). DNA sequence information for all eight species was obtained from GenBank. **c** DNA sequence between CRM2 and CRM3 in the *Arabidopsis thaliana YUC4* promoter where a potential YABBY binding site (bold) was identified.



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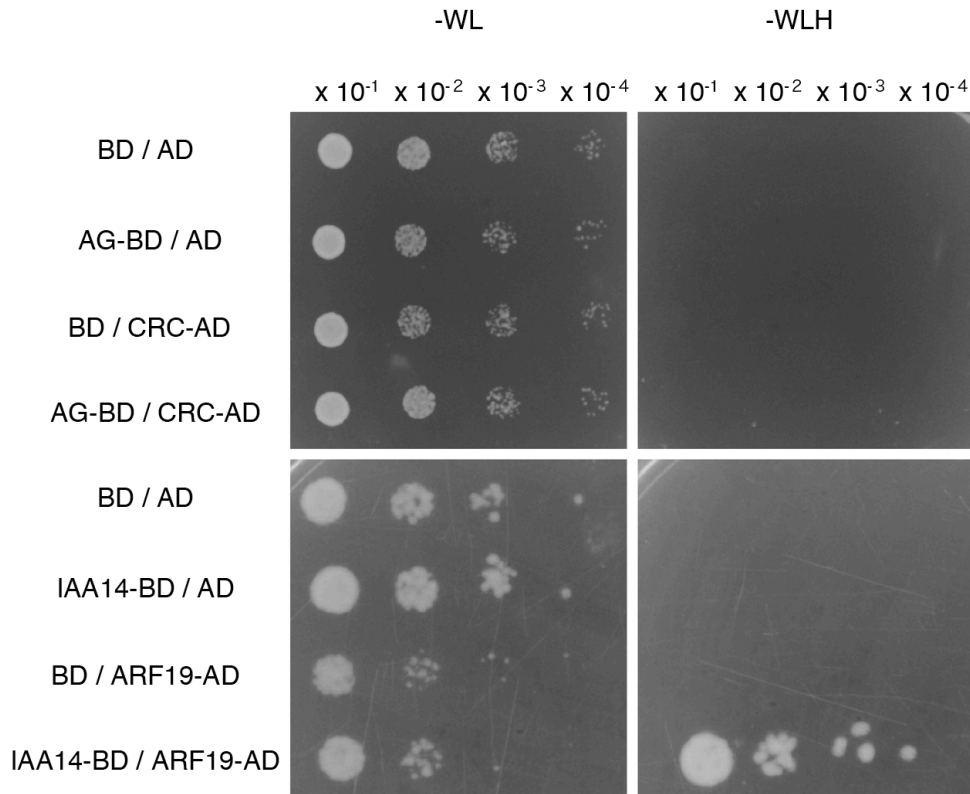
Supplementary Figure 10 Regulatory region of the *YUC* genes. **a-d** Identification of the YABBY-binding sites by a DNase I HS assay and sequences of potential YABBY-binding sites in the *YUC1* (**a**), *YUC2* (**b**), *YUC4* (**c**), and *YUC6* (**d**) genes. Pink, GA[GA]AGAAA; Green, CC[CA][TC]C[TA][CT]C; Yellow, CCCCAC. Flower-specific DNase I HS sites, which contain four potential YABBY-binding sites were specifically observed in the 5' region of the *YUC4* promoter.



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Supplementary Figure 11 Trimming of wild-type and *crc* inflorescences for auxin quantification. **a, b** Top view of wild-type (**a**), and *crc-1* (**b**) shoot apices. Fruit tips were shown in insets. **c, d** Top view of wild-type (**c**) and *crc-1* (**d**) shoot apices after removal of flowers from plants at stage 7 or later. Bars = 1 mm in **a, b**; 200 μ m in **c, d**. **e** Schematic diagram of spatial *CLV3*, *LFY*, *PAL4*, and *COMT1* expression patterns.

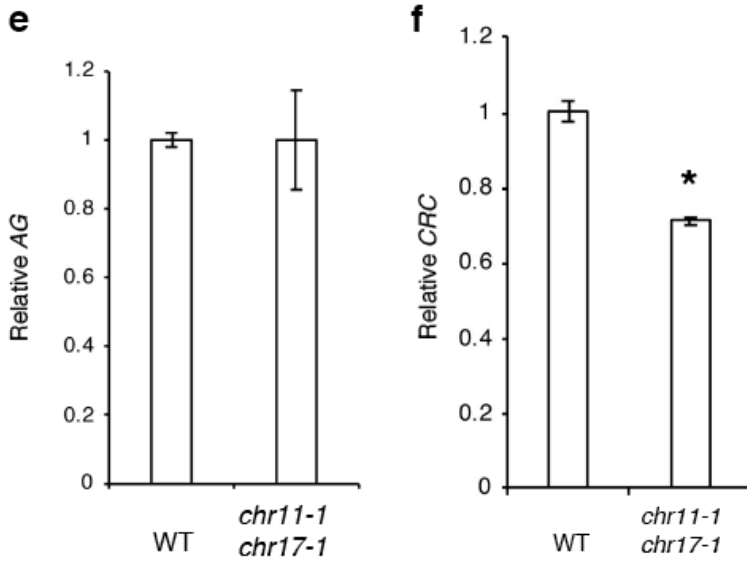
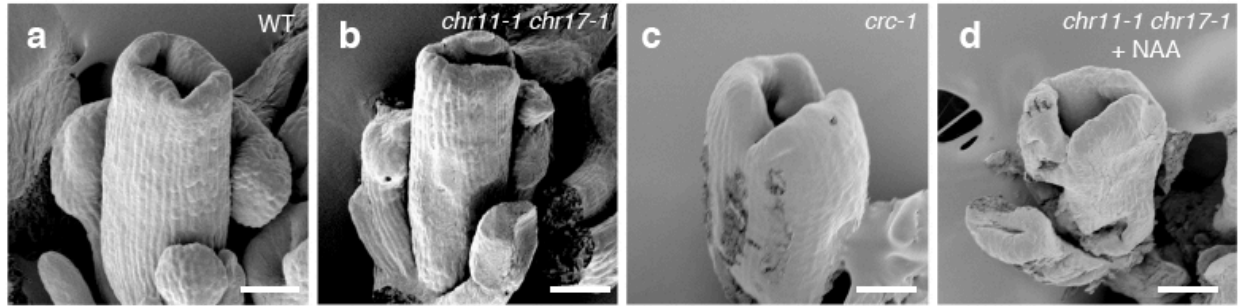
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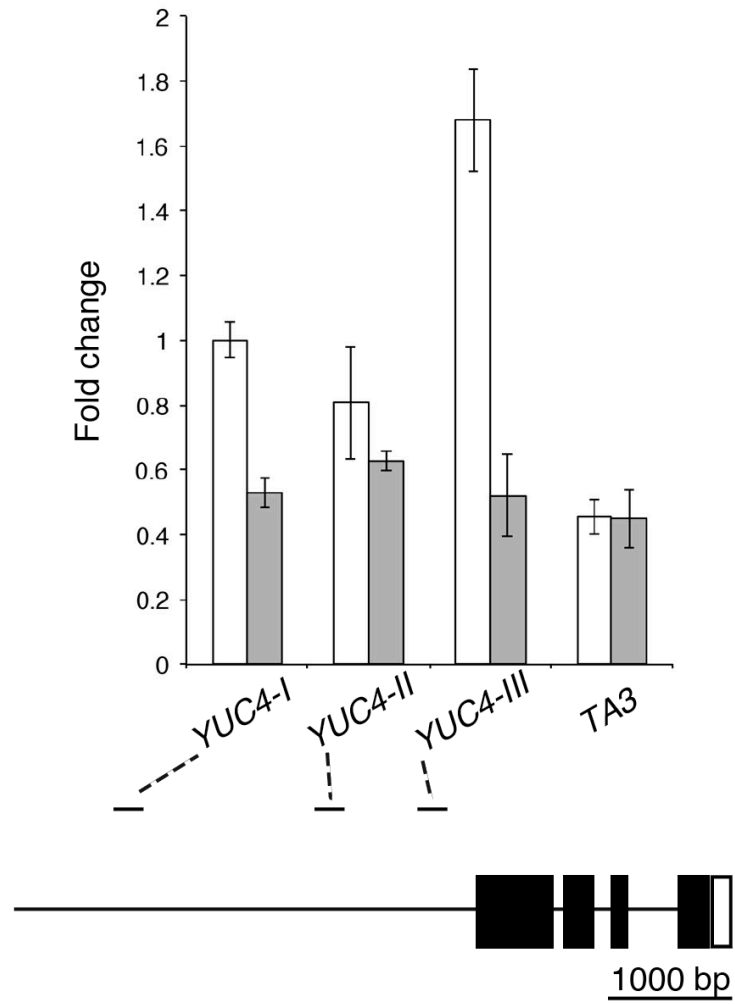
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4 **Supplementary Figure 12** Yeast-two hybrid test for an AG-CRC interaction. Left; Yeast grown
5 under nonselective conditions (-WL). Right; Yeast grown under selective conditions (-WLH).
6 Double transformants carrying both the *pDEST22* and *pDEST32* empty vectors were used as a
7 negative control. A positive AUXIN RESPONSE FACTOR19 and IAA14 interaction was
8 detected under our experimental conditions¹.

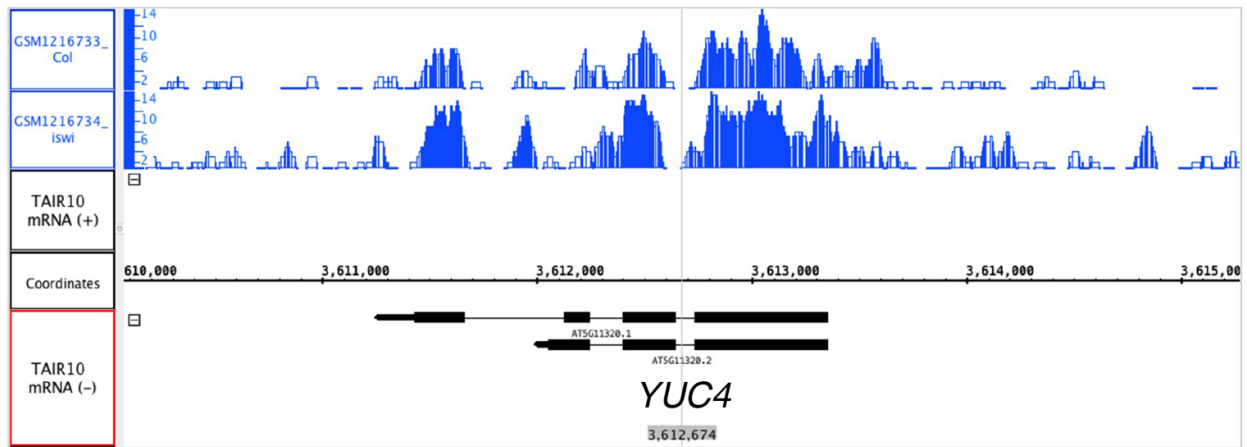


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Supplementary Figure 13 Characterization of the *chr11-1 chr17-1* double mutants. **a-d** Scanning electron micrograph of the wild-type (**a**), *chr11-1 chr17-1* (**b**), *crc-1* (**c**), and auxin-treated *chr11-1 chr17-1* (**d**) gynoecia. **e, f** *AG* (**e**) and *CRC* (**f**) mRNA levels in the wild-type and *chr11-1 chr17-1* floral buds. The values are represented as the means \pm SEMs. *p*-values were calculated using a Student's *t*-test. *: *p* < 0.05 compared to the wild type. Bars = 60 μ m in **a-d**.



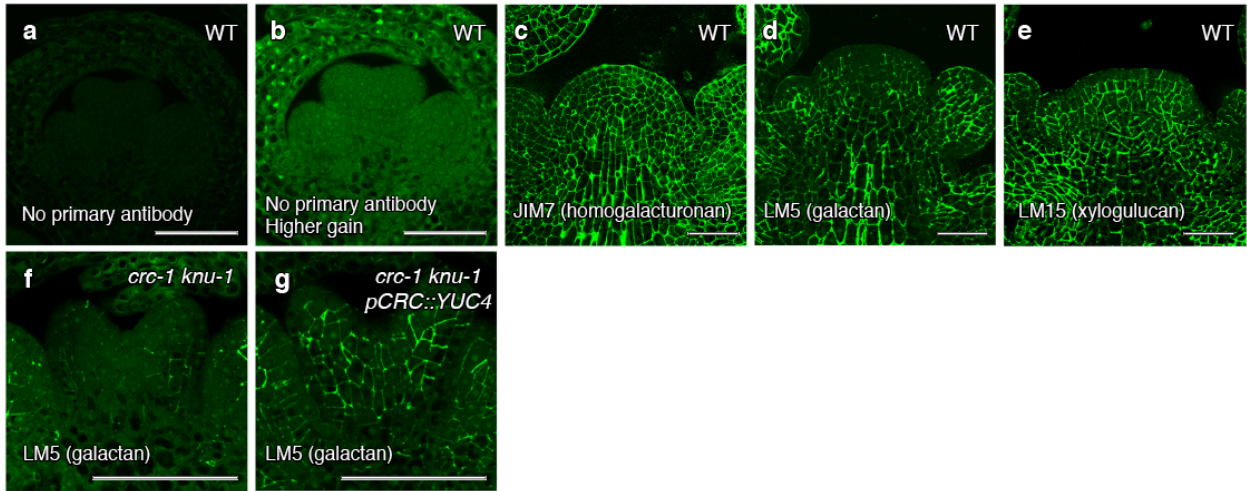
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2 **Supplementary Figure 14** AG ChIP enrichment at the *YUC4* locus. Top: Anti AG ChIP to test
3 AG occupancy at the *YUC4* locus. The values are represented as the means \pm SEMs. Bottom:
4 Diagram of the ChIP-qPCR amplicons tested.
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Supplementary Figure 15 Published MNase-seq data in wild-type and *chr11-1 chr17-1* plants. Nucleosome positioning identified by MNase-seq. Blue bars: Nucleosome occupancy. Black square: exon. Black line: intron.

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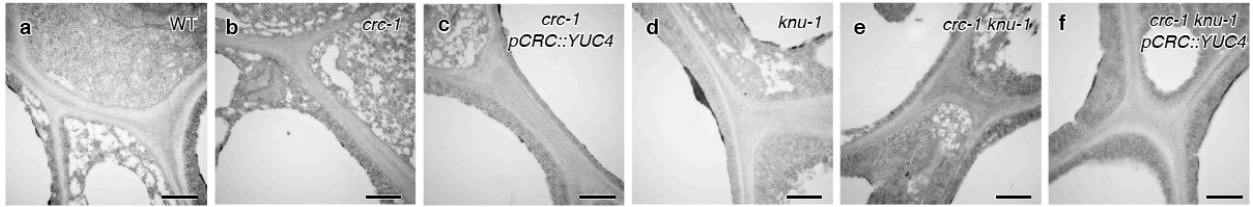


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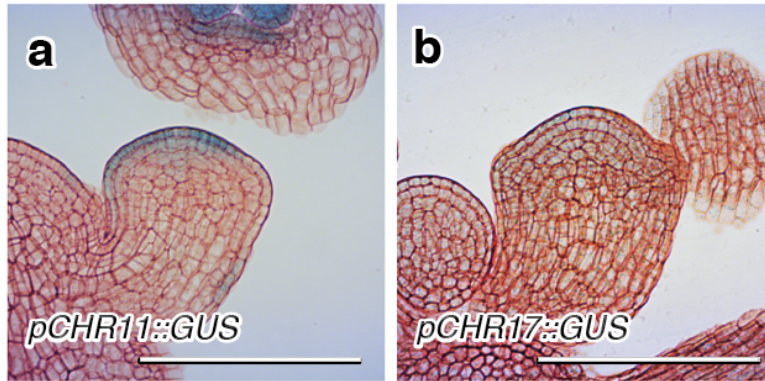
4 **Supplementary Figure 16** Immunolocalization of cell wall components in the inflorescence
5 meristem. **a, b** Immunolocalization using longitudinal sections of wild-type stage 6 flowers
6 without the primary antibody. Without primary antibody (**a**). Without primary antibody when
7 images were taken at higher gain settings. (**b**). Cell wall specific-fluorescence was not
8 observed without primary antibody. The same section was used for **a** and **b**. **c**
9 Immunolocalization of homogalacturonan epitopes was detected with JIM7 in longitudinal
10 sections of the wild-type inflorescence meristem. **d** Immunolocalization of (1-4)- β -D-galactan
11 epitopes was detected with LM5 in longitudinal sections of the wild-type inflorescence
12 meristem. **e** Immunolocalization of xyloglucan epitopes was detected with LM15 using
13 longitudinal sections of the wild-type inflorescence meristem. **f, g** Immunolocalization of
14 (1-4)- β -D-galactan epitopes was detected with LM5 in longitudinal sections of the *crc-1*
15 *knu-1* (**f**) and *crc-1 knu-1 pCRC::YUC4* (**g**) terminating floral meristem. Half-strength of
16 antibody was used. Bars = 50 μ m in **a-g**.

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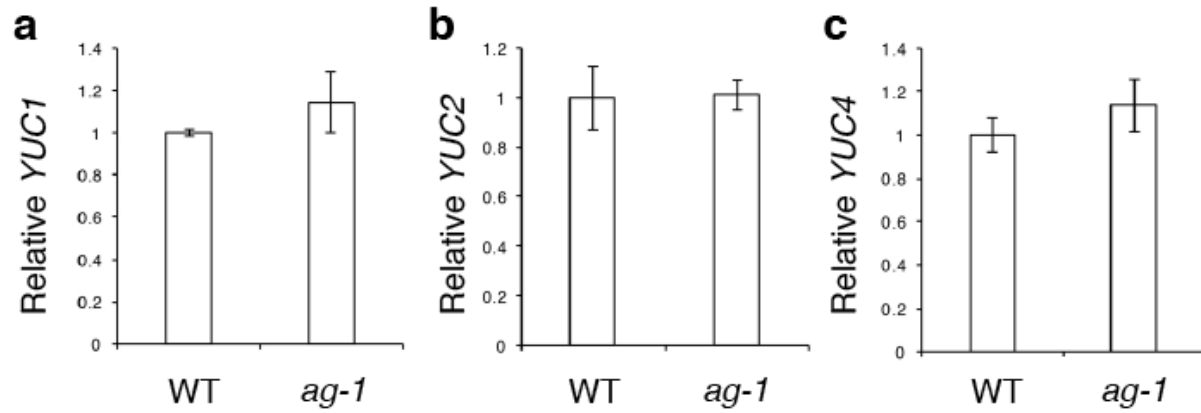
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Supplementary Figure 17 TEM image of stage 6 terminating floral meristem cells. **a-f** TEM images of longitudinal sections from wild-type (**a**), *crc-1* (**b**), *crc-1 pCRC::YUC4* (**c**), *knu-1* (**d**), *crc-1 knu-1* (**e**), and *crc-1 knu-1 pCRC::YUC4* (**f**) terminating floral meristem at flower development stage 6. All images are shown at the same magnification. Bars = 500 nm in **a-f**.



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Supplementary Figure 18 Expression of *pCHR11::GUS* and *pCHR17::GUS* in stage 3 floral buds.
a, b GUS staining of *pCHR11::GUS* (**a**) and *pCHR17::GUS* (**b**) in stage 3 floral meristems. Bars =
50 μ m in **a, b**.



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Supplementary Figure 19 Expression of *YUC* family genes in the wild type and *ag-1* mutant. **a-c** mRNA abundance of *YUC1* (a), *YUC2* (b), and *YUC6* (c) in wild-type and *ag-1* plants. The values are represented as the means \pm SEMs.

1 **Supplementary Table 1** Summary of transcriptomic datasets used to identify genes regulated
 2 by AG and CRC.
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| Dataset | Developmental stage/tissue | Number of genes | Annotation | Data References |
|--------------------------------|---|-----------------|------------|-----------------|
| WT vs <i>ag-12*</i> | More than 3 weeks old (flowers at stage 12) | 6673 | TIGR 5.0 | 2 |
| <i>knu-1 vs crc-1 knu-1*</i> | Floral bud clusters up to stage 10 | 8150 | TAIR 7 | 3 |
| <i>ag-11 ap2-35 vs ag-11**</i> | Floral bud clusters up to stage 7 | 2093 | TAIR 10 | 4 |
| <i>ag-11 ap2-43 vs ag-11**</i> | Floral bud clusters up to stage 7 | 2026 | TAIR 10 | 4 |
| AG ChIP | Synchronized floral bud clusters at stage 5 | 1421 | TAIR10 | 5 |

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 6 * Array data were downloaded from TAIR (ATGE_58A-C, ATGE_92A-C) and GEO (GSE88969) and reanalyzed.
 7 Genes showing at least a 1.5-fold change in expression were considered to be differentially expressed.
 8 ** The data were obtained from Huang et al., 2016⁴. Genes showing a 1.5-fold change in expression were
 9 considered to be differentially expressed, as written in Huang et al., 2016⁴.

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