

## Supplemental Figure 1. da3-1 Influences Cell and Organ Growth.

(A) to (C) Cotyledon area, cotyledon length and cotyledon width of 10-day-old Col-0 and da3-1 seedlings (n = 60).

(C) to (E) Petal area, petal length and petal width of Col-0 and da3-1 flowers (Stage 14) (n = 50).

Values in (A) to (E) are given as means  $\pm$  SD. \*\*P<0.01 compared with the wild type (Student's *t* test).



Supplemental Figure 2. *da3-1* Shows Increased Ploidy Levels. Flow cytometric analysis of Col-0 and *da3-1* cotyledons (A and B), petals (stage 14) (C and D) and leaves (E and F).



### Supplemental Figure 3. da3-1 Affects Plant Growth.

(A) 37-day-old plants of Col-0 (left) and da3-1 (right).

**(B)** Rosette leaves of Col-0 and *da*3-1. The top row of images shows rosette leaves of Col-0, and the bottom row of images indicates rosette leaves of *da*3-1.

(C) The average area of Col-0 and *da*3-1 rosette leaves. 37-day-old plants were used to measure leaf area. Values are given as means  $\pm$  SD (*n* = 15). \*\*P<0.01 compared with the wild type (Student's *t* test). Bars= 1 cm in (A) and (B).



#### Supplemental Figure 4. da3-1 Influences Root Growth.

(A) 7-day-old Col-0 and da3-1 seedlings.

(B) Root meristems of 7-day-old Col-0 and *da3-1* seedlings.

(C) Root length of 7-day-old Col-0 and da3-1 seedlings (n = 40).

(D) Number of cortical cells in Col-0 and da3-1 root meristems (n = 50).

(E) Length of cortical cells in Col-0 and *da*3-1 root meristems (n = 50). Values in (C) to (E) are given as means  $\pm$  SD.

\* P<0.05 and \*\* P<0.01 compared with the wild type (Student's *t* test). Bars = 1 cm in (A) and 50  $\mu$ m in (B).



Supplemental Figure 5. da3-1 Influences Cell Size and Cell Number.

(A) The average area of cells in cotyledons of Col-0 and da3-1. Thirty-five cotyledons were used to measure cotyledon cell area (n = 35).

(B) The average area of cells in petals of Col-0 and da3-1. Thirty-five petals were used to measure cotyledon cell area (n = 35).

(C) The average area of cells in first pair of leaves of Col-0 and da3-1. Twenty-eight leaves were used to measure cotyledon cell area (n = 28).

(D) The calculated number of cells in Col-0 and  $da_3$ -1 petals. Thirty-five petals were used to calculate cell number (n = 35).

(E) The calculated number of cells in Col-0 and da3-1 cotyledons. Thirty-five cotyledons were used to calculate cell number (n = 35).

(F) The calculated number of cells in the first pair of Col-0 and *da*3-1 leaves over time after emergence (DAE). Thirty leaves at each time point were used to calculate cell number (n = 30). Values in (A) to (F) are given as means  $\pm$  SD. \*\*P<0.01 compared with the wild type (Student's *t* test).



## Supplemental Figure 6. *da*3-1 Increases the Number of Trichome Branches.

Trichomes on the first pair of Col-0 and da3-1 leaves. The red arrows indicate the branches of trichomes. Bars = 50  $\mu$ m.



Supplemental Figure 7. Identification and Molecular Characterization of UBP14/DA3.

(A) Mapping of the da3-1 mutation. The da3-1 mutation was mapped in the region between markers F3H11-3 and K10D20-1. The open arrows indicate the genes in the mapping region.
(B) The DA3/UBP14 gene structure. The start codon (ATG) and the stop codon (TGA) are indicated. Closed boxes indicate the coding sequence, open boxes indicate the 5' and 3' untranslated regions, and lines between boxes indicate introns. The capital letters represent the exon sequence, and the small letters indicate the intron sequence. The mutation site (g/t) of da3-1 is shown. The mutation in da3-1 affects the splicing of UBP14, resulting in the premature stop codon (tag) (blue).

(C) The mutation in the *da3-1* allele produces the *Apol* site that is used to develop the AT3G20630 dCAPS marker.

**(D)** RT-PCR analysis of *UBP14* in Col-0 and *da3-1* seedlings. The *da3-1* mutation causes two main transcripts, resulting in a premature stop codon at the same position (blue letter in **B**).

(E) The average area of Col-0, da3-1, gUBP14;da3-1#1 and gUBP14;da3-1#3 cotyledons (n = 50). (F) Trichome branch (br) distribution of Col-0, da3-1, gUBP14;da3-1#1 and gUBP14;da3-1#3 first pair of leaves at 15 days after germination (DAG) (n = 200).

Values in (E) are given as means  $\pm$  SD relative to the respective wild-type values, set at 100%. \*\*P<0.01 compared with the wild type (Student's t-test).



# Supplemental Figure 8. *UBP14* is Expressed in both the Proliferation and Expansion Phases during Leaf Development.

(A) Histochemical analysis of GUS activity in the developing rosette leaves of *ProUBP14: GUS* transgenic plants. (B) Histochemical analysis of GUS activity in the developing rosette leaves of *ProCYCB1;1:CDB-GUS* transgenic plants. Bars = 100  $\mu$ m in (A) and (B).



# Supplemental Figure 9. Overexpression of *GFP-DA3* Complements the Phenotypes of *da3-1*. 10-day-old seedlings of Col-0, *da3-1*, *Pro35S:GFP-UBP14;da3-1*#1

10-day-old seedlings of Col-0, *da3-1*, *Pro35S:GFP-UBP14;da3-1*#1 and *Pro35S:GFP-UBP14;da3-1*#2. Bars = 1 mm.



# Supplemental Figure 10. *UBP14* Acts Genetically with *UVI4* to Regulate Endoreduplication.

Flow cytometric analysis of Col-0, da3-1, uvi4 and uvi4 da3-1 first pair leaves.



### Supplemental Figure 11. UBP14 Modulates the Stability of CYCA2;3.

(A) Confocal images of induced CYCA2;3-GFP in Col-0 or da3-1 mutant root meristems. The top row of images shows the CYCA2;3-GFP signal in wild-type root meristems, and the bottom row of images indicates the CYCA2;3-GFP signal in da3-1 root meristems. GFP fluorescence of CYCA2;3-GFP (left), bright field (middle) and merged (right) images are shown. Bars = 50 µm.

(B) After CYCA2;3-GFP and CYCA2;3-GFP;da3-1 seedlings were induced with the inducer, expression of CYCA2;3-GFP was detected using quantitative real-time RT-PCR. Data shown are means  $\pm$  SD of three biological replicates.



Supplemental Figure 12. UBP14 Modulates the Stability of CDKB1;1. (A) Confocal images of CDKB1;1-GFP in wild-type or *da3-1*mutant root meristems. The top row of images shows the CDKB1;1-GFP signal in *CDKB1;1-GFP* root meristems, and the bottom row of images indicates the CDKB1;1-GFP signal in *CDKB1;1-GFP;da3-1* root meristems. GFP fluorescence of CDKB1;1-GFP (left), bright field (middle) and merged (right) images are shown. Bars = 50  $\mu$ m.

(B) Expression of *CDKB1;1-GFP* was detected using quantitative realtime RT-PCR. Data shown are means  $\pm$  SD of three biological replicates.



### Supplemental Figure 13. The Effect of *da3-1* on Endosperm.

(A) and (B) The endosperm in Col-0 and *da*3-1 seeds at the heart stage. The arrows indicate the nucleoli.

(C) The average area of endosperm cells in Col-0 and da3-1 seeds at the heart stage (n = 80).

**(D)** The average diameter of endosperm nucleoli in Col-0 and da3-1 seeds at the heart stage (n = 80).

Values are given as means  $\pm$  SD. \*P<0.05 and \*\*P<0.01 compared with the wild type (Student's t-test).

Bars= 50 µm in (A) and (B).



## Supplemental Figure 14. The da3-1 Mutation Influences the Levels of UVI4 Proteins.

(A) Confocal images of UVI4-GFP in ProUVI4:UVI4-GFP#1 and ProUVI4:UVI4-GFP#1;da3-1 root meristems. The top row of images shows the UVI4-GFP signal in *ProUVI4:UVI4-GFP#1* root meristems, and the bottom row of images indicates the UVI4-GFP signal in ProUVI4:UVI4-GFP#1;da3-1 root meristems. GFP fluorescence of UVI4-GFP (left), bright field (middle) and merged (right) images are shown. (B) Confocal images of UVI4-GFP in ProUVI4:UVI4-GFP#3 and ProUVI4:UVI4-GFP#3;da3-1 root meristems. The top row of images shows the UVI4-GFP signal in *ProUVI4:UVI4-GFP#3* root meristems, and the bottom row of images indicates the UVI4-GFP signal in ProUVI4:UVI4-GFP#3;da3-1 root meristems. GFP fluorescence of UVI4-GFP (left), bright field (middle) and merged (right) images are shown. (C) Confocal images of UVI4-GFP in ProUVI4:UVI4-GFP#4 and ProUVI4:UVI4-GFP#4;da3-1 root meristems. The top row of images shows the UVI4-GFP signal in *ProUVI4:UVI4-GFP#4* root meristems, and the bottom row of images indicates the UVI4-GFP signal in ProUVI4:UVI4-GFP#4;da3-1 root meristems. GFP

fluorescence of UVI4-GFP (left), bright field (middle) and merged (right) images are shown.

(D) The da3-1 mutation increases the level of UVI4-GFP. Total proteins from ProUVI4:UVI4-GFP#4 (left lane) and ProUVI4:UVI4-GFP#4;da3-1 (right lane) seedlings were subjected to immunoblot assays using anti-GFP and anti-Actin (as loading control) antibodies. Bars = 50  $\mu$ m in (A) to (C).



### Supplemental Figure 15. UBP14 is Expressed in Roots.

(A) and (B) UBP14 expression was monitored by *ProUBP14:UBP14-GFP* transgene expression. GFP fluorescence of UBP14-GFP in root meristem (A) and differentiated regions (B) of *ProUBP14:UBP14-GFP* transgenic plants. GFP fluorescence of UBP14-GFP (left), bright field (middle) and merged (right) images are shown

Bars = 50  $\mu$ m in (A) and (B).

Primer Name	Primers	
PCR-based markers	s developed for <i>DA3/UBP14</i> mapping in this study	
GAPAB-F	TCCTGAGAATTCAGTGAAACCC	
GAPAB-R	CACCATGGCTTCGGTTACTT	
MXL8-F	GTAGCCCAAAGCCGTACAG	
MXL8-R	GAGATGCGTTTCACCTACAA	
MQC12-1F	GCCTACAGAAAAACGAACAGG	
MQC12-1R	CAATTTCTCCGGGAAAGGTA	
MOE17-1F	CGGGTGAAATCCTACATATAACAA	
MOE17-1R	AAGATGGCTATCACCAATATGAAA	
K10D20-1F	GAATACACCGCTCACAGCAG	
K10D20-1R	ATCCATGCGGGTATGAAGAC	
F3H11-3F	CATGATGATCTCCCATAGCTTC	
F3H11-3R	CCACAATCTGAGAAGACACAACA	
dCAPS marker for DA3/UBP14		
DA3d-F2	ACTCCGGCTCAAAGTGGATTACCAGATGGAGGAGGAA	
DA3d-R2	GAGTTTTATTCATACTGCTTTGTGGA	
PCR products were d	ligested with ApoI.	
Primers for T-DNA identification		
SALK_083656-LP	AAGACTCGGTATGTGTGACCG	
SALK_083656-RP	CGTGGACTGTAGGATTCTTGC	
SALK_086463-LP	CTCACGATCTGTTCGTTTTCC	
SALK_086463-RP	AGAAGCTGATCTCCCAAGAGG	
Lba1	TGGTTCACGTAGTGGGCCATCG	
Primers for constructs		
UBP14genome-F	GTGTGCAGACGAGTGTTCCGTTCC	
UBP14genome-R	ATCAAGCCGCTGAAAGAAGTAGACATCA	
UBP14CDS-F	ATGGAGCTCCTCCGATCCAAC	
UBP14CDS-R	TCAATCAAGC CGCTGAAAG	
UBP14pro-F	GTGTGCAGACGAGTGTTCCGTTCC	
UBP14pro-R	AGGGTTTTTGCGAAATCGGCGAAA	
UBP14CDSM-R	ACTCCCTCCATCTGGTAATCCA	
UBP14MBP-F	TAC GTA GGA TCCATGGAGCTCCTCCGATCCAAC	
UBP14MBP-R	CTG CAG GTC GACTCAATCAAGC CGCTGAAAG	
UBP14MBP-MR	CTGCAGGTC GACACTCCCTCCATCTGGTAATCCA	
UBP14UMBP-F	TACGTAGGA TCCAGCCACATGCGTAGCAAAGGACTC	
UBP14UMBP-R	CTGCAG GTCGACTCATTTCCCTCCTCCATCTGGT	
UBP14dUMBP-F	GCGCAGCCCGTGGCAAACCCTAATGCATCT	
UBP14dUMBP-R	AGATGCATTAGGGTTTGCCACGGGCTGCGC	
UBQ14-F	ATGCAGATCTTTGTTAAGACTCTCAC	

<u>Supplemental Table 1</u>. Primers Used in this Study.

UBQ14-R	TTAGAAACCA CCACGGAGCC TTAGC
UBQ10-F	CGCGGATCC ATGCAGATCT TTGTTAAGAC TCTC
UBQ10-R	CGCAAGCTTG TTAGAAACCACCACGAAGACGCA
CCS52A1GST-F	GAATTCCCGGGTATGGAAGAAGAAGAAGATCCTACAGCA
CCS52A1GST-R	CGA TGC GGCCGCTCACCGAATTGTTGTTCTACCAAA
UVI4GST-F	CCGGAATTCATGCCAGAAGCACGAGATCGAATAG
UVI4GST-R	CGCGTCGACTCATCGCATCGACATTAGCGTCCTAAC
CCS52A1MBP-F	TGCTCTAGAATGGAAGAAGAAGATCCTACAGCAA
CCS52A1MBP -R	CCCAAGCTTTCACCGAATT GTTGTTCTAC CAAA
Myc-CCS52A1-F	CGGGGTACCCATGGAAGAAG AAGATCCTAC AG
Myc-CCS52A1-R	ATCGAGCTCTCACCGAATT GTTGTTCTAC CAAAG
Myc-UVI4-F	CGGGGTACCCATGCCAGAAGCACGAGATCGAAT
Myc-UVI4-R	CGCGGATCCTCATCGCATCGACATTAGCGTCC
CCS52A1CDS-F	ATGGAAGAAG AAGATCCTACAG
CCS52A1CDS-R	TCACCGAATT GTTGTTCTAC CAAAG
UVI4CDS-F	ATGCCAGAAGCACGAGATCGAAT
UVI4CDS-R	TCATCGCATCGACATTAGCGTCC
UVI4107-F	CAAGAATTAAATCAATGAAAC
UVI4107-R	TCGCATCGACATTAGCGTCCTAAC
Primers for RT-PCR and QRT-PCR	
ACTIN2-F	GAAATCACAGCACTTGCACC
ACTIN2-R	AAGCCTTTGATCTTGAGAGC
QRTUBP14N-F	AAGTTAATTAGTGAACATGC
QRTUBP14N-R	TTTCTTCCACAGAGAATC
RTUBP14-F	ACCAATCTCCCACCAGACATCTG
RTUBP14-R	TCAATCAAGCCGCTGAAAGAAG
GFP-F	ACGCCGCCGTCTTCGATGTTGT
GFP-R	GTGCTTCTCCCGTTACCCTGAT