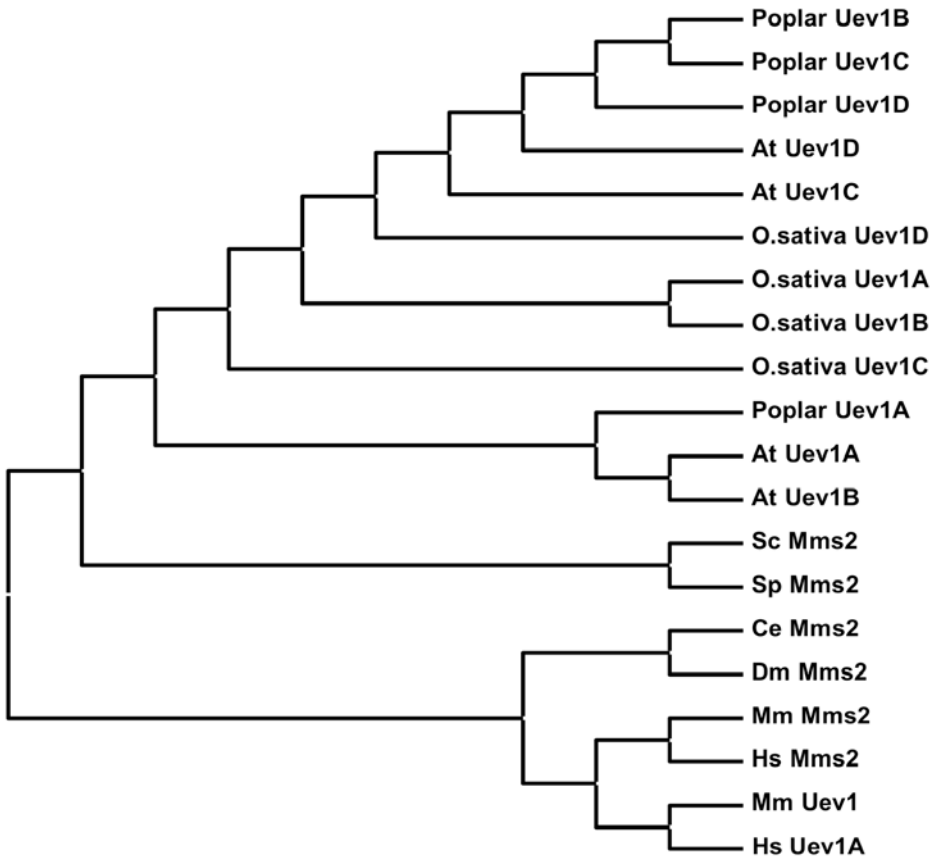
Supplemental Data. Wen et al. (2008). *Arabidopsis UEV1D* promotes Lys63-linked polyubiquitination and is involved in DNA damage response

Supplemental Table 1. *Saccharomyces cerevisiae* strains

Strain	Genotype	Source
PJ69-4A	*MAT**a** trp1-901 leu2-3,112 ura3-52 his3-200 gal4*Δ *gal80*Δ *Met2::GAL7-lacZ LYS2::GAL1-HIS3 GAL2-ADE2*	P. James
HK580-10D	*MAT*α *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1*	H. Klein
WXY942	HK580-10D with *mms2*Δ*::HIS3*	Lab stock
WXY955	HK580-10D with *mms2*Δ*::HIS3 ubc13*Δ*::hisG-URA3-hisG*	This study
DBY747	*MAT**a** his3-1 leu2-3,112 trp1-289 ura3-52*	D. Botstein
WXY642	DBY747 with *mms2*Δ*::HIS3*	Lab stock

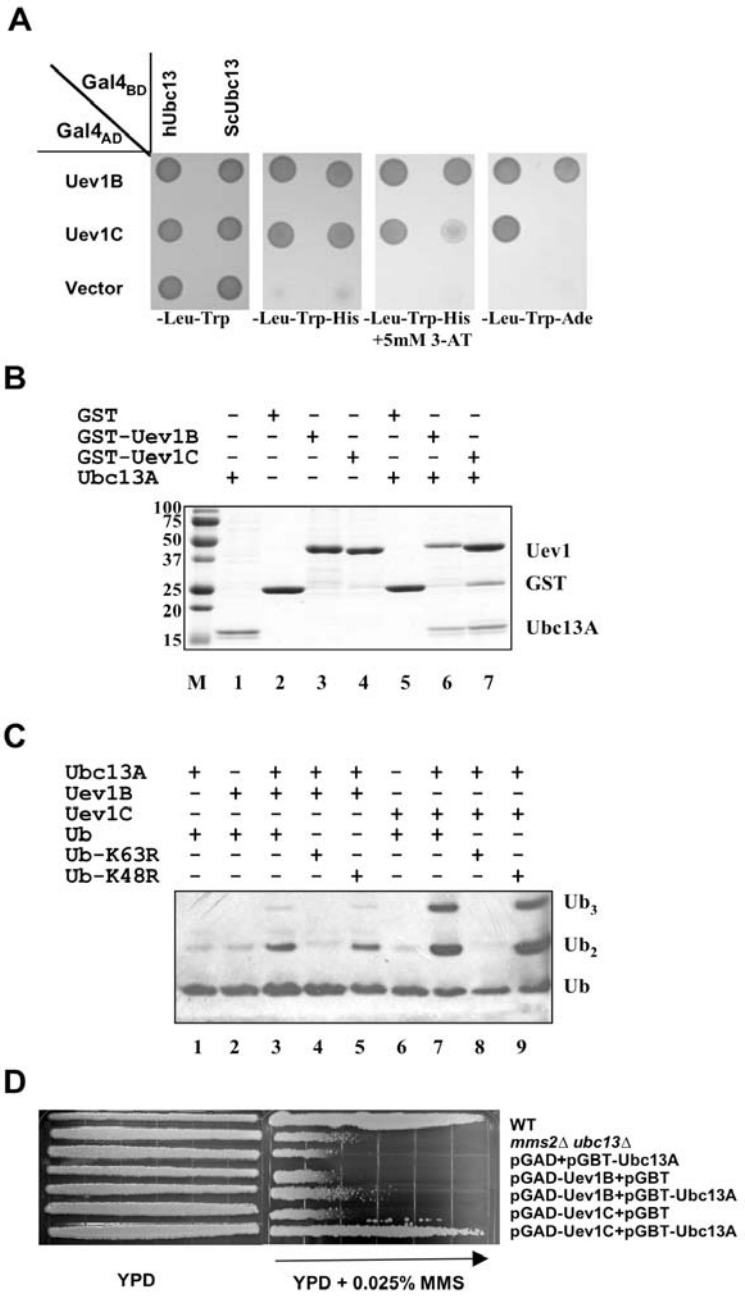
Supplemental Table 2. Oligonucleotide sequences

Primer	Sequence
UEV1A-1	5’-cccgtcgacaATGAGTTCGGAGGAAGCCAAG-3’
UEV1A-2	5’-cccgtcgacTCACATCACACAACATTTAGC-3’
UEV1B-1	5’-cccgtcgacaATGGGTTCGGAAGAAGAGAAG-3’
UEV1B-2	5’-cccgtcgacTCACATCACGCAACATTTCACCAC-3’
UEV1C-1	5’-cccgtcgacaATGACTCTTGGCTCAGGATCG-3’
UEV1C-2	5’-cccgtcgacTTAGAAGAAAGTTCCTTCGGG-3’
UEV1D-1	5’-cccgtcgacaATGACTCTTGGCTCAGGAGG-3’
UEV1D-2	5’-cccgtcgacCTAGAAGCAAGTACCTTCCGG-3’
UBQ11-1	5’-CAGATTTTTGTTAAAACCCTA-3’
UBQ11-2	5’-CTTCTGAATGTTGTAATCC-3’
LB1	5’-GCGTGGACCGCTTGCTGCAACT-3’
4g33380-F	5’-ATGAGAAGCTGGAGGAAGC-3’
4g33380-R	5’-TCAAGCCGTTACAACACC-3’



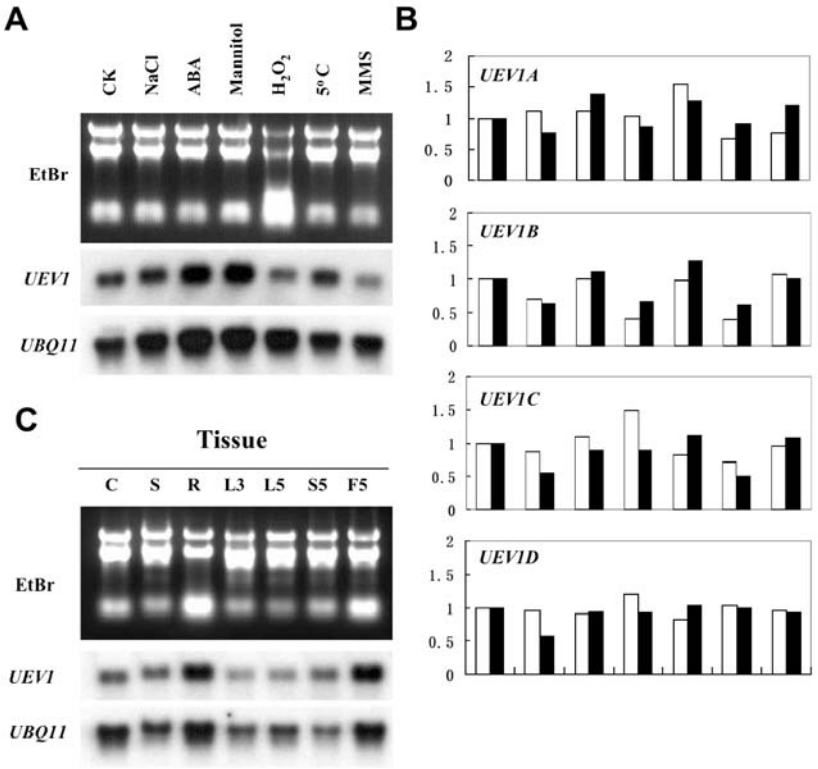
Supplemental Figure 1. Phylogenetic analyses of hypothetical Uev family proteins from different organisms.

The similarity clustering was conducted by using MEGA version 3 (Kumar et al., 2004). High similarity is indicated by the short branch length between any two sequences. Source of sequences: *O. sativa* Uev1 - NP_001062804, Uev2 - NP_001067224, Uev3 - NP_001051063 and Uev4 - NP_001062804. Poplar (*Populus trichocarpa*) Uev1 - CX655441, Uev2 - AJ767274, Uev3 - DT525203 and Uev4 - BU894366. The above gene names are arbitrary for the purpose of comparison only.



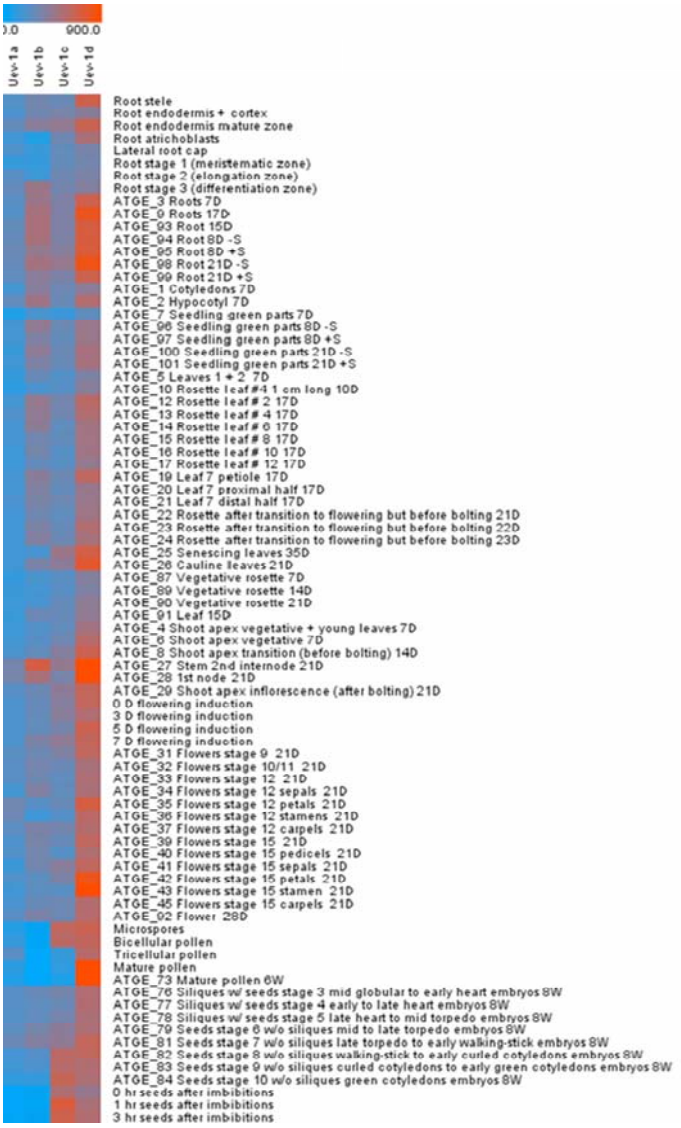
Supplemental Figure 2. Characterization of Arabidopsis *UEV1B* and *UEV1C.*

(A) Physical interactions between Uev1B/C and Ubc13s from yeast or human in a yeast two-hybrid assay. Experimental conditions were as in Figure 2A. **(B)** Protein interactions between Uev1B/C and Ubc13 by an affinity pull-down assay. Experimental conditions were as in Figure 2C. **(C)** Ubiquitin conjugation by Ubc13 and Uev1B/C. Experimental conditions were as described in Figure 2D. (D) Complementation of the *mms2 ubc13* double mutant by *UEV1B/C* and *UBC13*. Experimental conditions were as in Figure 3B.



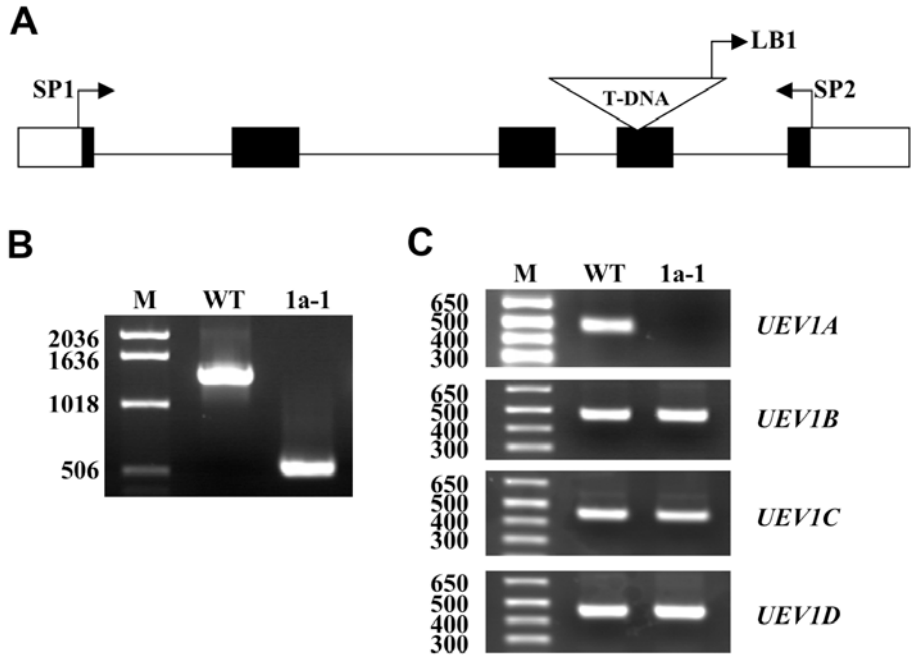
Supplemental Figure 3. *UEV1* expression profiles.

(A) Northern hybridization measuring *UEV1* transcript levels from cell suspension culture under 24-h stress. Each lane contains 15 μg of total RNA from different samples. The treatments were: CK, control; NaCl, 300 mM; ABA, 20 μM (+)-abscisic acid; mannitol, 400 mM; H_2O_2, 20 mM; 5 ºC, low temperature; and MMS, 0.01%. Abnormal rRNA profile after H_2O_2 treatment was repeatedly observed (Wen et al., 2006). **(B)** Graphic presentation of data extracted from microarray analyses in www.arabidopsis.org and expressed as relative levels to the untreated control. Tissue sources (except ABA): open bars, shoots; solid bars, roots. The conditions for the treatments and source of information (TAIR accession No.) were: NaCl, 150 mM (1007966888); ABA, 10 μM (1007964750); mannitol, 300 mM (1007966835) for osmotic stress; methyl viologen, 10 μM (1007966941) for oxidative stress; 5 ºC (1007966553) for cold stress; and bleomycin (1.5 μg/ml) plus mitomycin (22 μg/ml) (1007966782) for genotoxic treatment. All treatments were for 24 h except for ABA treatments, which were seedlings treated for 1 h (open bars) and 3 h (solid bars). **(C)** Measuring *UEV1* transcript levels from different tissues by Northern hybridization. Each lane contains 20 μg of total RNA from different tissues: C, cell suspension culture; S, shoot of 13-day seedlings; R, root of 13-day seedlings; L3, leaves of 3-week plants; L5, leaves of 5-week plants; S5, stems of 5-week plants; and F5, floral tissues of 5-week plants.



Supplemental Figure 4. Heat map showing the transcript levels of Arabidopsis *UEV1* genes in different tissues and developmental stages.

The expression of *UEV1* genes is shown in a colored representation with red and blue for high and low levels of expression, respectively. Note that *UEV1D* expression in most databases is higher than that of the other three *UEV1* genes.



Supplemental Figure 5. Confirmation of the *uev1a-1* T-DNA insertion mutant.
(A) Genomic structure showing the position of T-DNA insertion in *UEV1A*. Boxes represent exons, solid boxes represent *UEV1A* ORF and lines represents introns. SP1, 5' gene-specific primer UEV1A-1; SP2: 3' gene-specific primer UEV1A-2; LB1, T-DNA left border primer. **(B)** Genomic DNA PCR to confirm *uev1a-1*. The fragment was amplified by using three primers (SP1, SP2 and LB1) in each reaction and genomic DNA from WS-4 (WT) or *uev1a-1* (1a-1) as a template. **(C)** RT-PCR detecting each of the *UEV1* transcripts. *UEV1* gene specific primers were used for an RT-PCR reaction against total RNA extracted from WS-4 (WT) and *uev1a-1* (1a-1) lines.