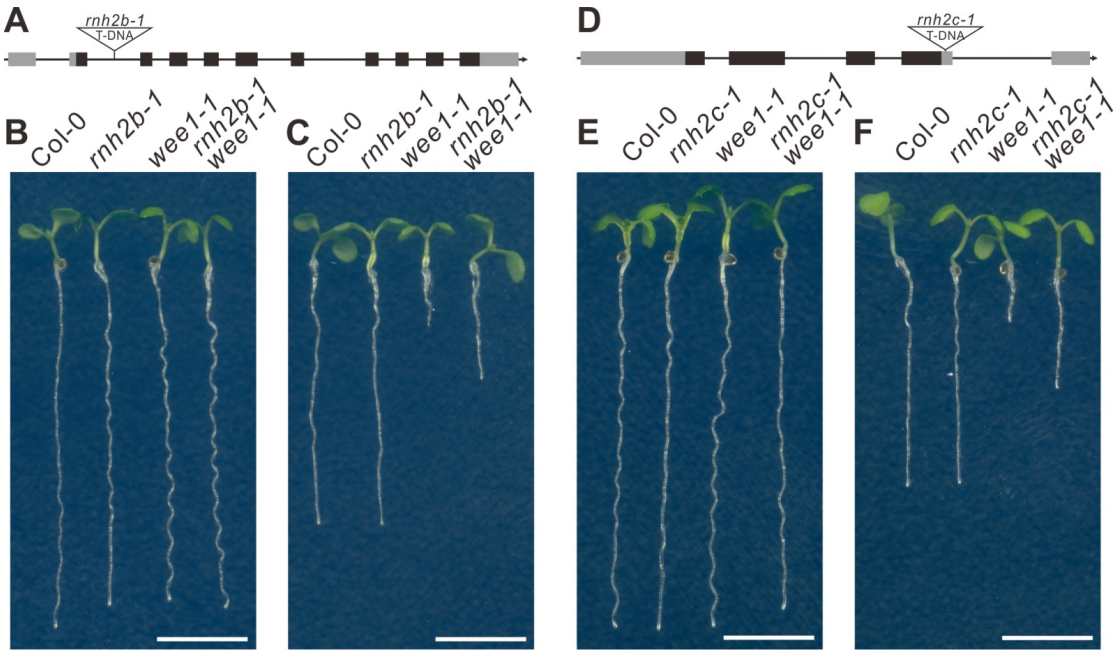
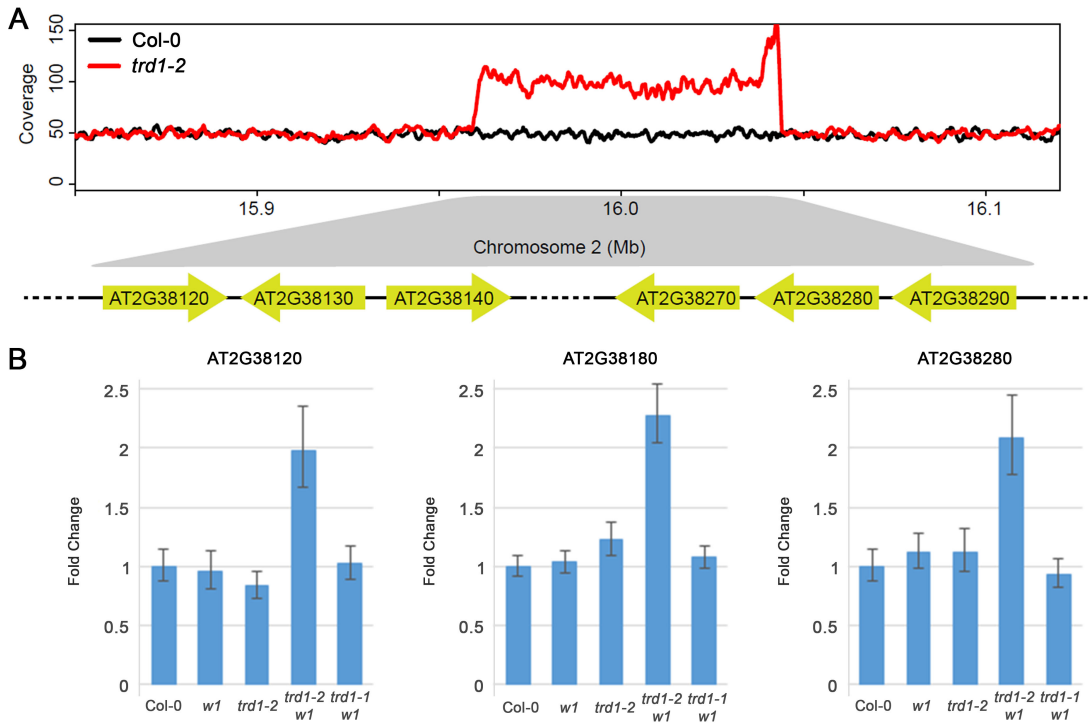
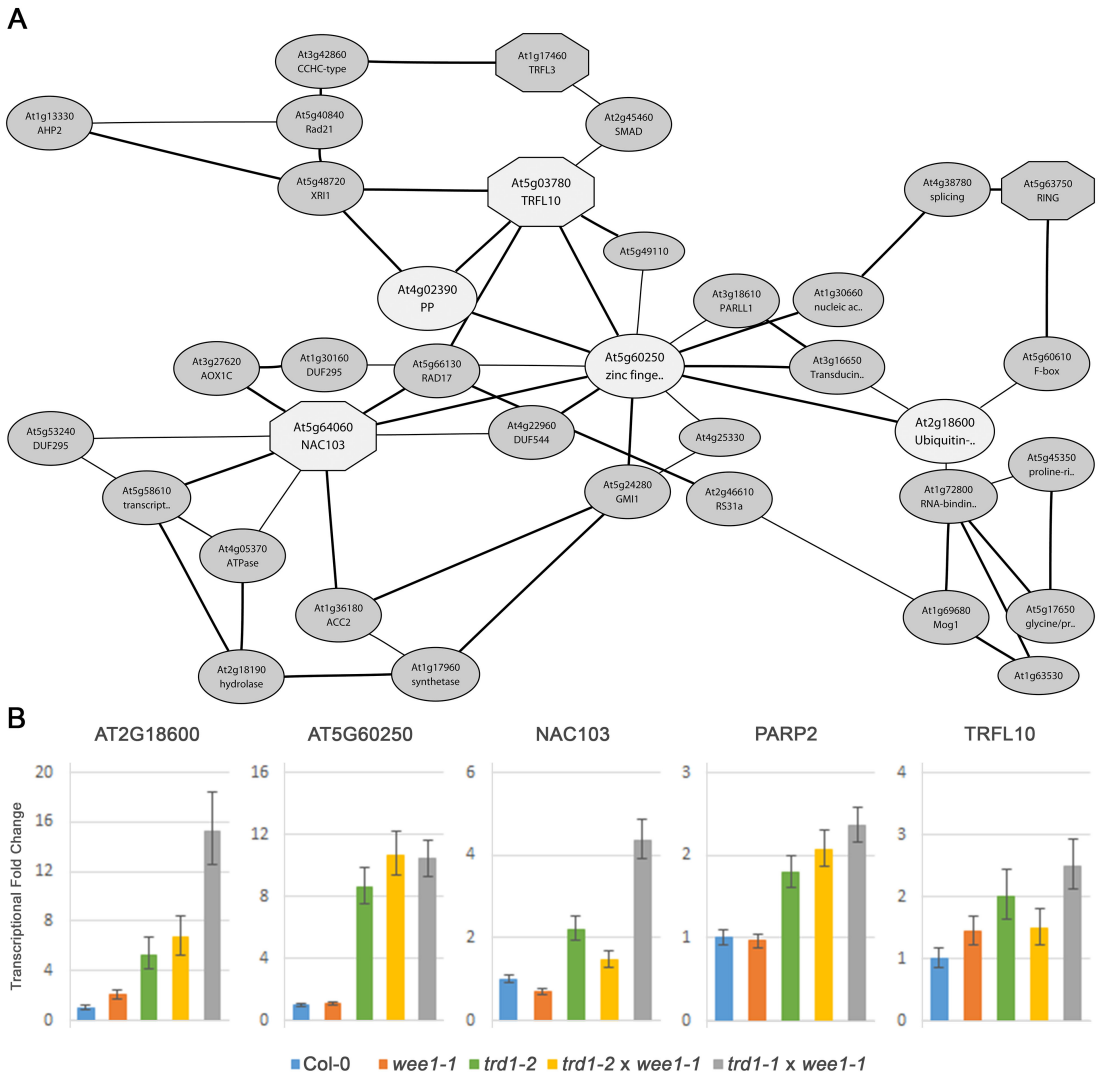


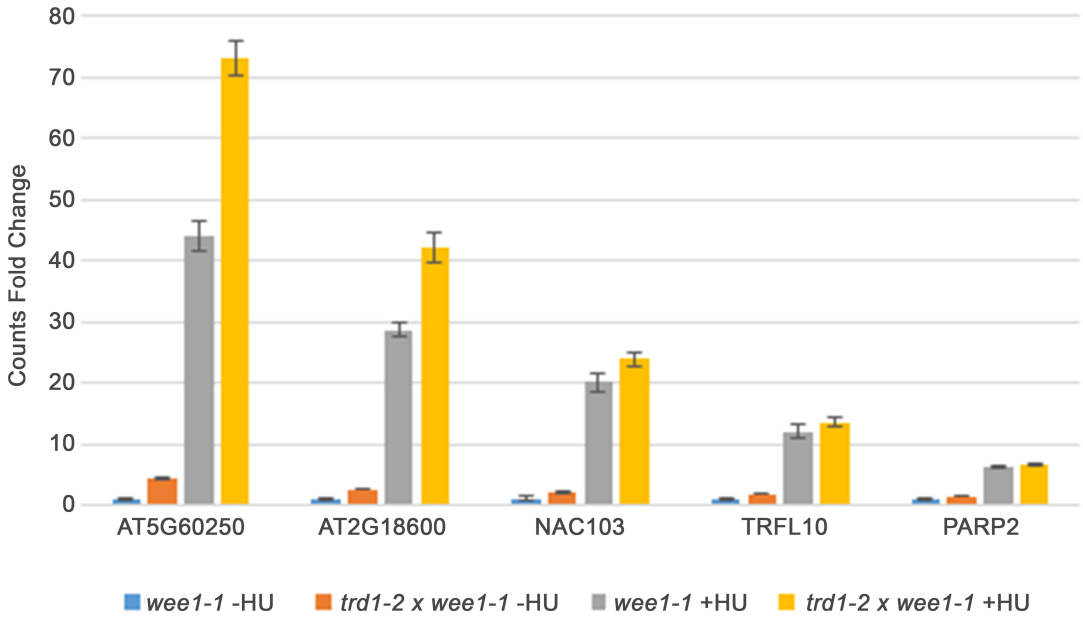

**Supplemental Figure 1.** Mutations in the regulatory subunits of the RNase H2 complex partially rescue the HU hypersensitivity phenotype of *WEE1*$^{KO}$ plants. (**A**) Intron-exon organization of the *RNase H2* subunit B *(RNH2B)*. Black and grey boxes represent exons and untranslated regions, respectively. The T-DNA insertion site is indicated. (**B-C**) Root growth of 7-day-old wild-type (Col-0) and *rnh2b-1*, *wee1-1*, and *rnh2b-1 wee1-1* plants grown on control medium (B) or medium supplemented with 0.75 mM HU (C). Scale bar = 0.5 cm (**D**) Intron-exon organization of the *RNase H2* subunit C *(RNH2C)*. Black and grey boxes represent exons and untranslated regions, respectively. The T-DNA insertion site is indicated. (**E-F**) Root growth of 7-day-old wild-type (Col-0) and *rnh2c-1*, *wee1-1*, and *rnh2c-1 wee1-1* plants grown on control medium (E) or medium supplemented with 0.75 mM HU (F). Scale bar = 0.5 cm.



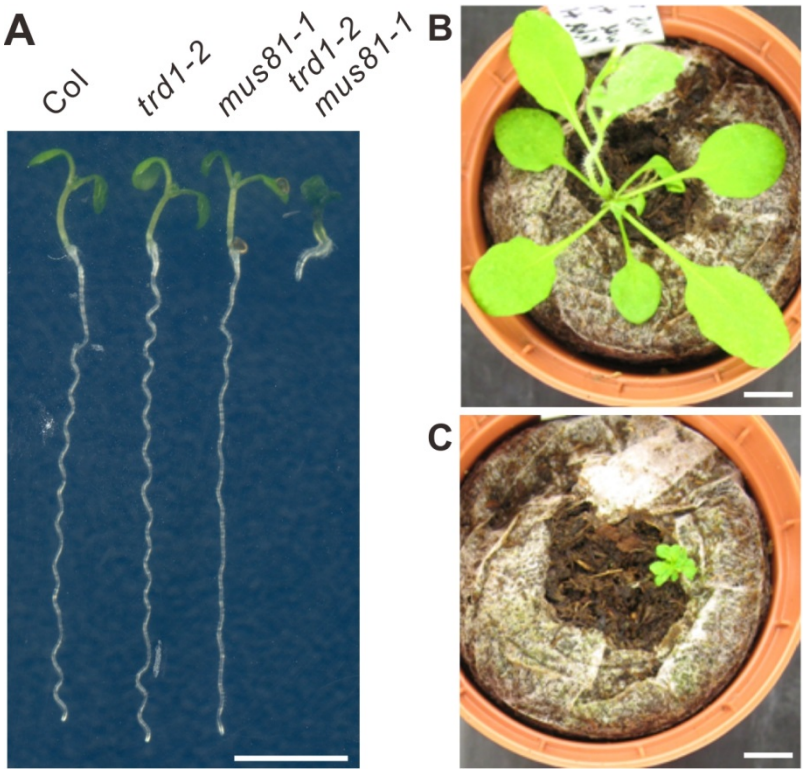

**Supplemental Figure 2.** The *trd1-2* mutant holds a large DNA duplication. **(A)** Whole genome sequencing indicates a potentially duplicated region in *trd1-2* (red), as seen by a 100% increase in read coverage relative to genome wide mean. **(B)** Relative expression levels of three genes within the duplicated region in 5-day-old wild-type (Col-0), *wee1-1* (w1), *trd1-2* (without genome duplication), *trd1-2 wee1-1*, and *trd1-1 wee1-1* root tips. Expression levels in wild-type were arbitrarily set to one. Data represent least square means ± SE, normalized to wild-type levels that were arbitrarily set to one (n = 2-3, *P value < 0.01).



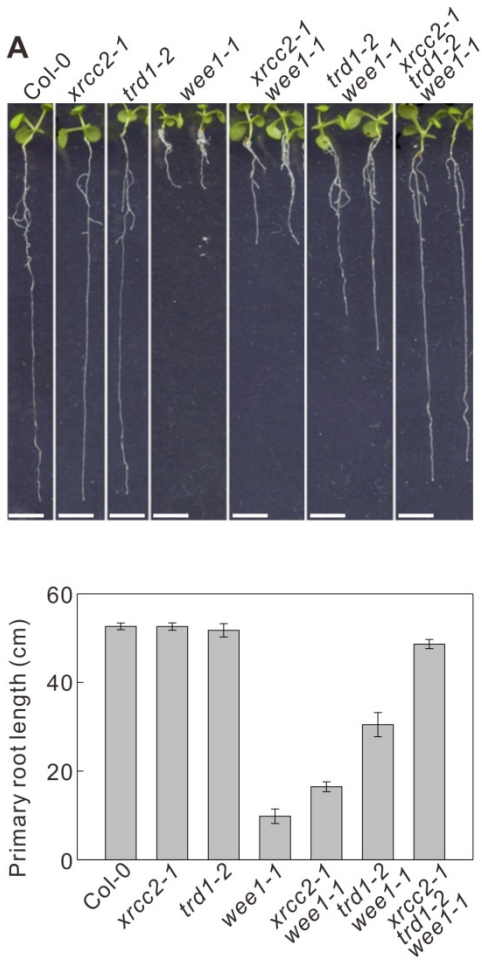

**Supplemental Figure 3.** Absence of RNase H2 activates a DNA repair co-expression cluster. (**A**) Co-expression cluster of genes induced in absence of RNase H2 (seed genes indicated in light grey). (**B**) Confirmation of transcriptional induction of DNA repair genes in RNase H2 deficient plants. Relative expression levels of the indicated gene in 5-day-old wild-type (Col-0), *wee1-1*, *trd1-2* (without genome duplication), *trd1-2 wee1-1*, and *trd1-1 wee1-1* root tips. Expression levels in wild-type were arbitrarily set to one. Data represent least square means ± SE (n = 2-3).



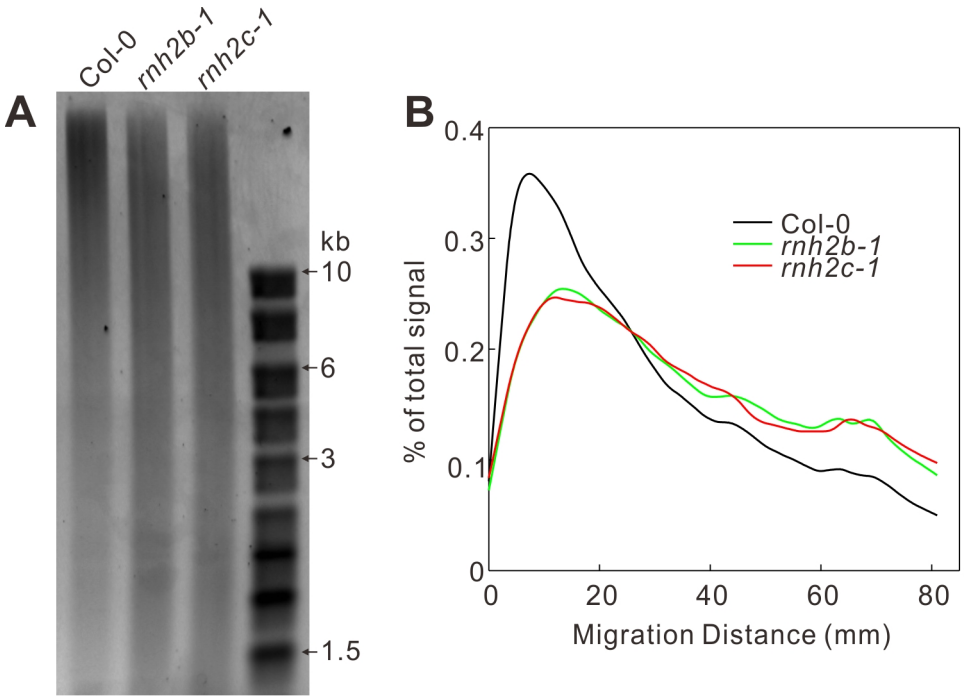

**Supplemental Figure 4.** Transcriptional induction of *trd1-2* differentially expressed genes by HU. Normalized counts of transcript after RNA sequencing of the indicated genes in 5-day-old *wee1-1* versus *wee1-1 trd1-2* root tips grown in the absence (-HU) or presence of HU (+HU). Expression levels in *wee1-1* plants grown under control conditions were arbitrarily set to one. Data represent mean ± SE (n = 3).




**Supplemental Figure 5.** The *trd1-2* mutant is synthetically lethal in a *mus81-1* mutant background. (**A**) Root growth of 7-day-old wild-type (Col-0) and *trd1-2*, *mus81-1*, and *trd1-2 mus81-1* plants. Scale bar = 0.5 cm. (**B-C**) Three-week-old wild-type (B) and *trd1-2 mus81-1* (C) plants. Scale bar = 1 cm.

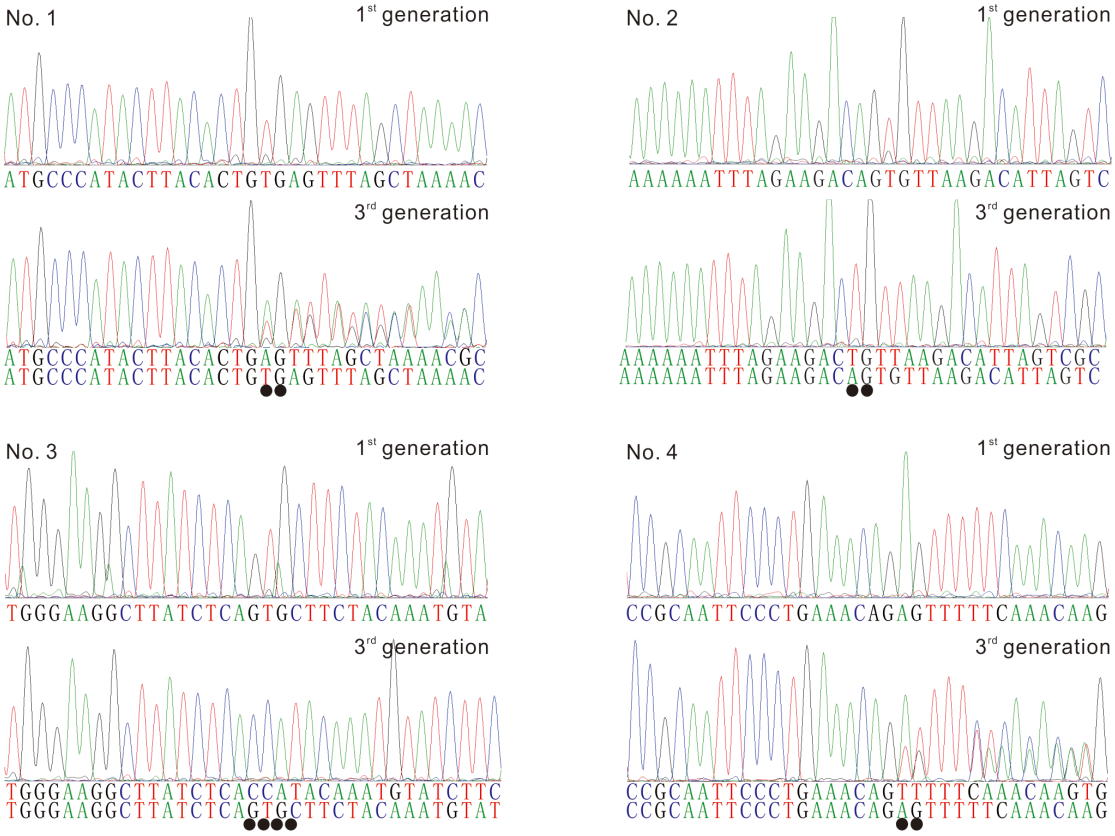



**Supplemental Figure 6.** Simultaneous knockout of *XRCC2* and *TRD1* rescues the HU hypersensitivity phenotype of *WEE1*$^{KO}$ plants completely. (**A**) Root growth of 15-day-old wild-type (Col-0) and *xrcc2-1*, *trd1-2*, *wee1-1*, *xrcc2-1 wee1-1, trd1-2 wee1-1* and *xrcc2-1 trd1-2 wee1-1* plants grown on medium supplemented with 0.75 mM HU. Scale bar = 5 cm. (**B**) Quantification of the root length of plants shown in (A). Data represent ± S.D. (n > 5).

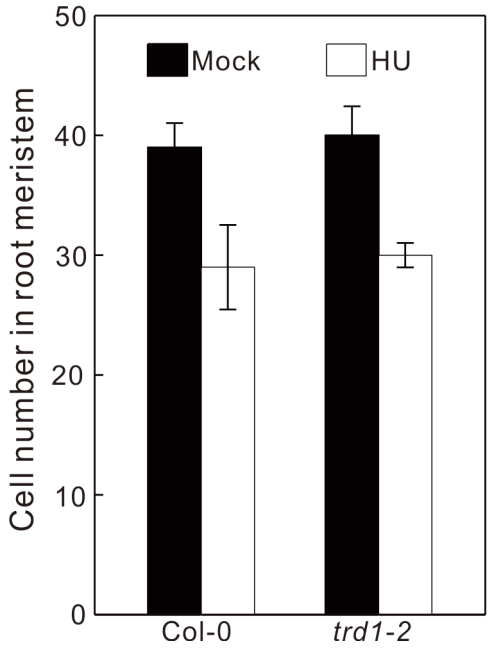



**Supplemental Figure 7.** *RNase H2* mutant plants accumulate rNMPs in DNA. (**A**) Alkaline cleavage products of genomic DNA extracted form 7-day-old wild type (Col-0), *rnah2b-1*, and *rnah2c-1* seedlings. (**B**) Densitometry plot of lanes in (A).

| Sequence read | Genotype | Chromsome position | Deletion length(bp) |
|---|---|---|---|
| No. 1 | *trd1-2* | Chr2_18615227-18615228 | 2 |
| No. 2 | *trd1-2* | Chr2_15395489-15395492 | 2 |
| No. 3 | *trd1-2* | Chr5_14232986-14232989 | 4 |
| No. 4 | *trd1-2wee1-1* | Chr4_4311376-4311377 | 2 |




**Supplemental Figure 8.** Confirmation of small base pair deletions in *RNase H2* deficient plants grown in the presence of HU. Sequencing reads of mutant loci in first versus third generation plants. Deleted base-pairs (indicated by dark circles) result in dual sequence reads. Deletions No. 2 and No. 3 in the third generation plants are homozygous.




**Supplemental Figure 9.** Number of meristematic cortex cells in wild type (Col-0) and *trd1-2* roots. Data represent mean ±SD (n > 10).