

Supplementary materials

Table S1 The list of primers used in this study

Primer name	Sequence (5'–3')	Used for
HTA3-CDS	F: ATGAGTTCCGGCGCCG R: TTAAACTCTTGAGAAGCAGATCCG	Gene cloning
HTA5-CDS	F: ATGAGTACAGGCGCAGGAA R: TCAGAACTCCTGAGAAGCAGAT	Gene cloning
HTA3-Promoter	F: gactcttgaccatggCGAGGGTGAAGGACCCATT R: tcagatctaccatggTTTTGTTTATCGGGAAATTTGA	GUS staining
HTA5-promoter	F: gactcttgaccatggTGCACCTAGTCATAGATTGAAAA R: tcagatctaccatggCGTCTTCTTCGAAATTTAGAACA	GUS staining
HTA3-Com	F: catgattacgaattcCGAGGGTGAAGGACCCATT R: gtcgtcctttagtcAAACTCTTGAGAAGCAGATCCG	Complementation lines
HTA5-Com	F: catgattacgaattcTGCACCTAGTCATAGATTGAAAA R: gtcgtcctttagtcGAACTCCTGAGAAGCAGAT	Complementation lines
HTA3-GFP	F: acgggggactctagaATGAGTTCCGGCGCCG R: gctcaccatggatccAAACTCTTGAGAAGCAGATCCG	Subcellular localization
HTA5-GFP	F: acgggggactctagaATGAGTACAGGCGCAGGAA R: gctcaccatggatccGAACTCCTGAGAAGCAGAT	Subcellular localization
HTA3-CR-Target	F: attgGCAGTGGAACAATAAAGG R: aaacCCTTTAGTTGTCCACTGC	CRISPR/Cas9
HTA5-CR-Target	F: attgATGAGTACAGGCGCAGGAAG R: aaacCTTCCTGCGCCTGTACTCAT	CRISPR/Cas9
HTA3-CR-Check	F: CACTCACAAAATCCTCAGCCATC R: CTTCAAACCGAATTCATTACTGGA	Mutation sequencing
HTA5-CR-Check	F: AACAAATCACAATCAATCTTAGTAGCC R: TCAGTTCCATTGATACATGATTCTGT	Mutation sequencing
Cas9-Check	F: CCACTCCATCAAGAAGAACCTC R: TTGGACTTGAAGTTAGGGGT	Cas9-free isolation
<i>atr</i> -LP <i>atr</i> -RP LBb1.3	F: GCAGCAAAAATTTCTTGTTG R: ACTTCAAGGGTTCCGATGTTC ATTTTGCCGATTTCGGAAC	T-DNA genotyping
HTA3-qPCR	F: CTCACAAAATCCTCAGCCATCTC R: CTTTCCTCTGCCACCTTTAGTTG	RT-qPCR
HTA5-qPCR	F: AACAAATCACAATCAATCTTAGTAGCC R: CTGCGCCTGTACTCATCGTCT	RT-qPCR
BRCA1-qPCR	F: GTTACGTGTGCAAACTCATACCAGAATG R: GATACTTGTTTAGGCTGAGAGTGCAGTGG	RT-qPCR

RAD51-qPCR	F: CTCCGAGGAAGGATCTCTTGCAG R: GCTCGCACTAGTGAACCCCAGAGG	RT-qPCR
WEE1-qPCR	F: TCATCGGCGTTTCAGGGT R: CTAAGCCGCTCATCAAGTTCTG	RT-qPCR
TSO2-qPCR	F: GAACGAATCATCGCTTTCGCTTGC R: CGTCACGTGAGATCAATTCGTTTGAG	RT-qPCR
CYCD1;1-qPCR	F: CCTGGTGATTCTATCGCTTGTTTA R: CGCTTGACCTTGAGAATCCATG	RT-qPCR
ABI1-qPCR	F: CGTCTCACATCTTCGTCGCT R: TCAATCCTCGCAGCTTCATC	RT-qPCR
ABI2-qPCR	F: CGGTTCTCAGGTAGCGAATTATT R: AGCCTTCTTCCACTTCTCTTGC	RT-qPCR
ABI3-qPCR	F: TTTGGTTGCGGCGAAGG R: GGTGTCAAAGAACTCGTTGCTATC	RT-qPCR
ABI4-qPCR	F: CGTTAGGGCAGGAACAAGGA R: GGATCCAGACCCATAGAACATACC	RT-qPCR
ABI5-qPCR	F: ATGGGAGGGCTAAGGGGAA R: ACTCCGCCAATGCATGTTTTA	RT-qPCR
RD29A-qPCR	F: ACACACCAGCAGCACCCAGA R: TGATCTTGCTCATGCTCATTGC	RT-qPCR
GAPDH-qPCR	F: TTGGTGACAACAGGTCAAGCA R: AAACCTTGTCGCTCAATGCAATC	RT-qPCR

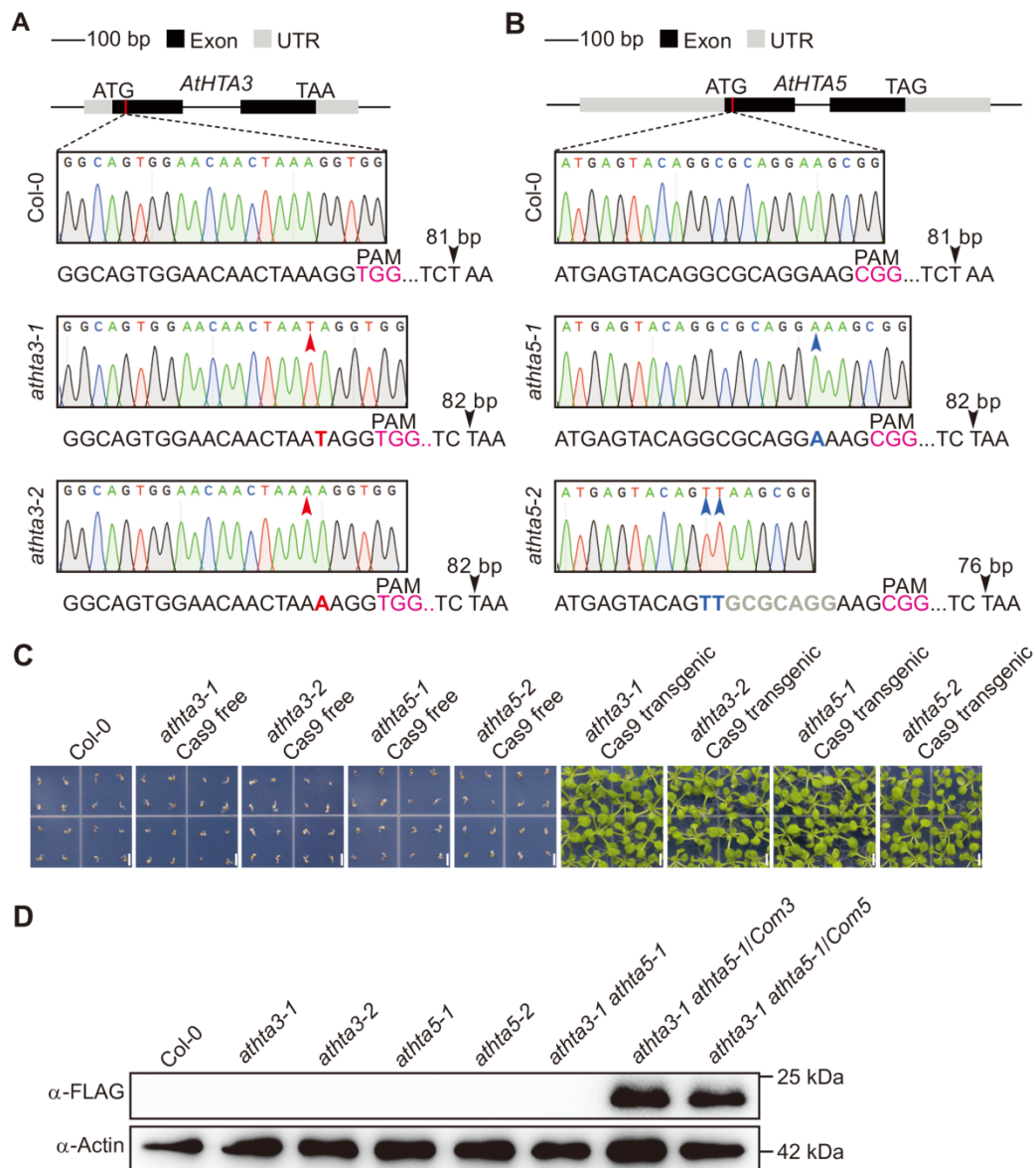


Figure S1. Generation of *ath2a.x* mutants and complementation lines. **(A and B)** Sequence chromatograms of Sanger sequencing showing the mutations of *AtHTA3* or *AtHTA5* generated by CRISPR/Cas9 technology. Red bar: target site. Red and blue arrows indicates the mutation sites. Protospacer adjacent motif (PAM) sites are highlighted in magenta. **(C)** Isolation of *Cas9*-free mutants. Different genotypes were grown on solid media supplemented with hygromycin (50 mg/L). Photos were taken 2 weeks after sowing. White bars = 5 mm. **(D)** Immunoblot analysis of *AtHTA3_{pro}:gAtHTA3-2×FLAG* and *AtHTA5_{pro}:gAtHTA5-2×FLAG* in complementation lines using anti-FLAG antibody. Actin2 was used as a loading control.

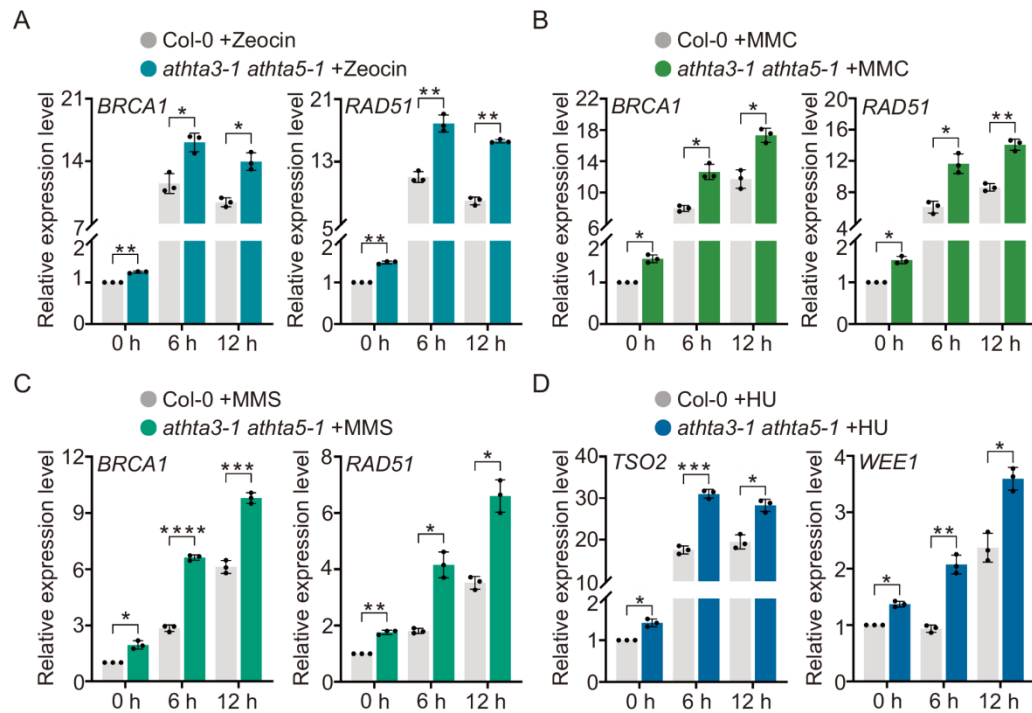


Figure S2. RT-qPCR validation analyses of DNA damage response-related marker genes. (A–D) The wild-type Col-0 and the *athta3-1 athta5-1* double mutants were separately treated under 25 μ M Zeocin, 10 μ M mitomycin C (MMC), 100 p.p.m. methyl methanesulfonate (MMS), or 1 mM hydroxyurea (HU) for 0 h, 6 h and 12 h. *GAPDH* was used as an internal control. Error bars indicate mean \pm SD of three biological replicates, each with 20 plants. Each black point on the plot indicated one independent replicate. Statistical analyses were performed by Student's test (* indicates $p < 0.05$; ** indicates $p < 0.01$; *** indicates $p < 0.001$; **** indicates $p < 0.0001$).