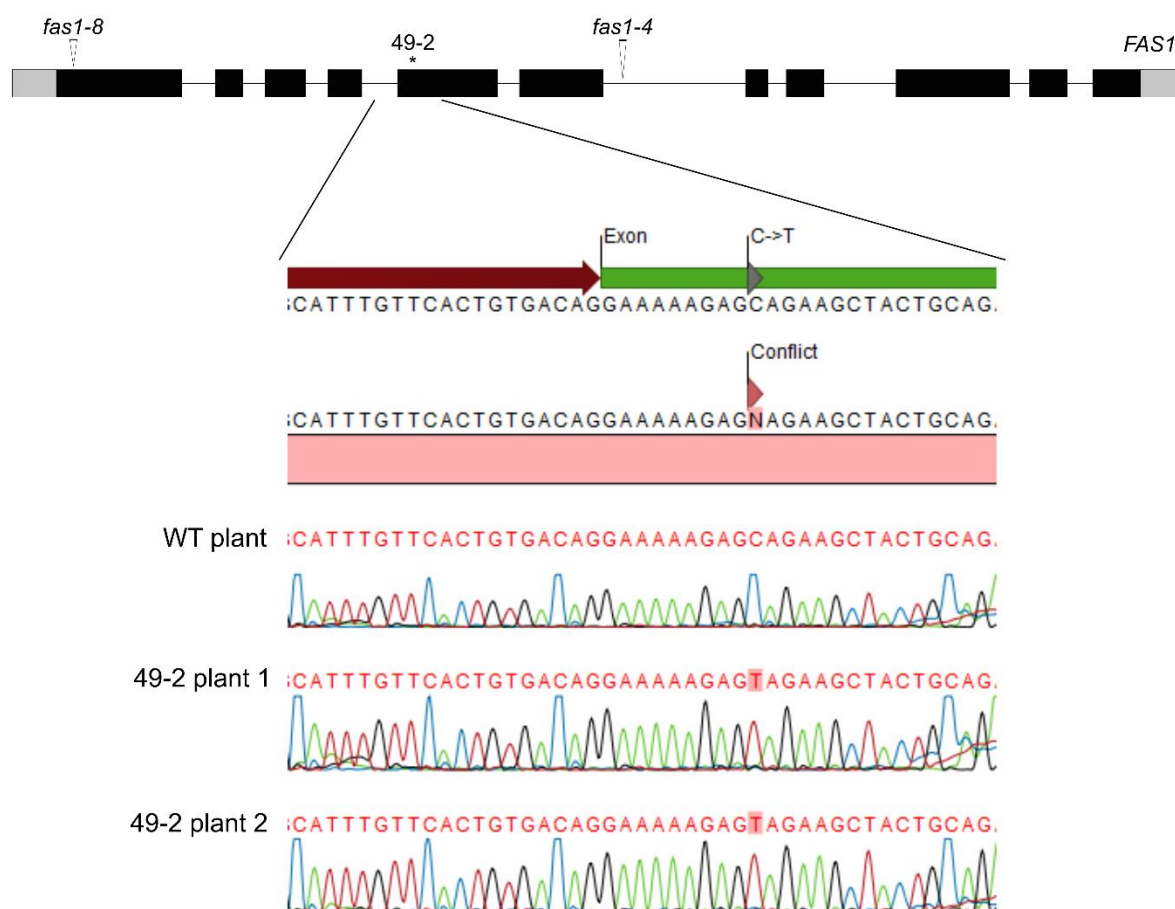


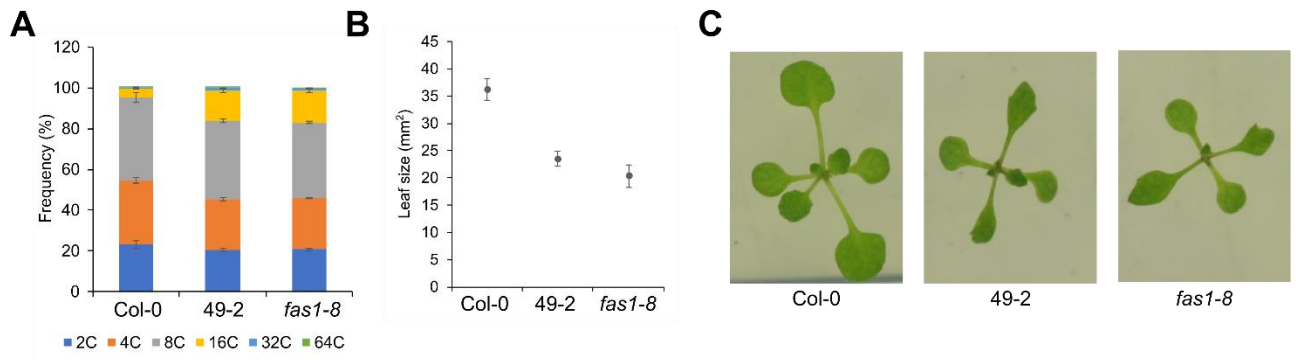
1 Supplemental figures



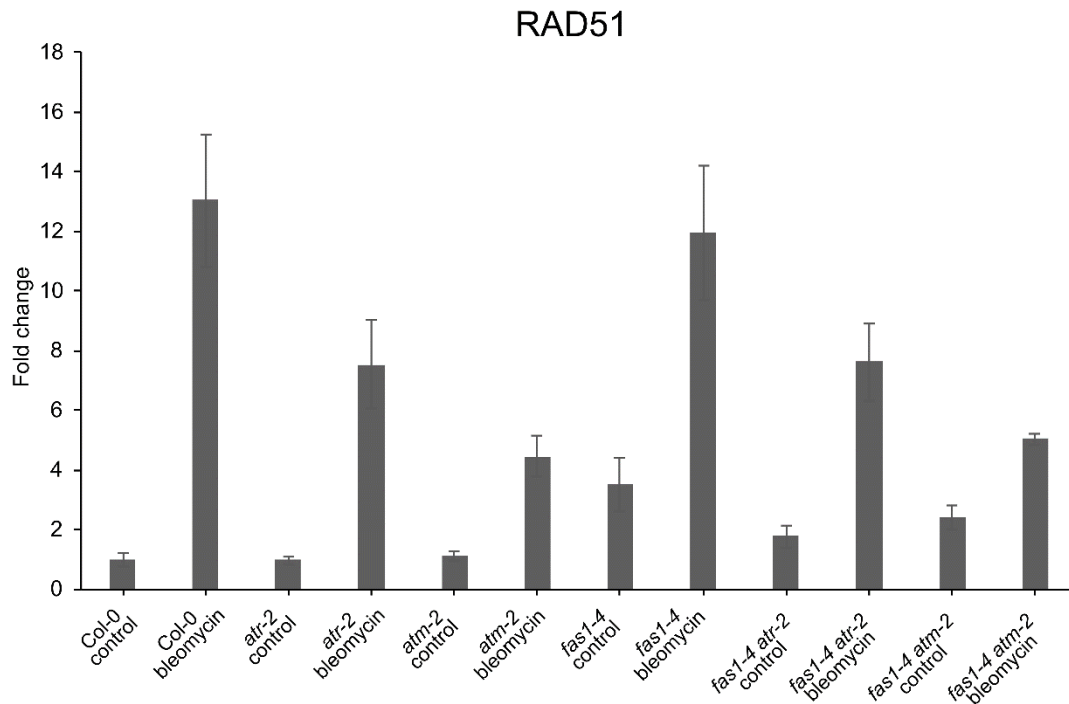
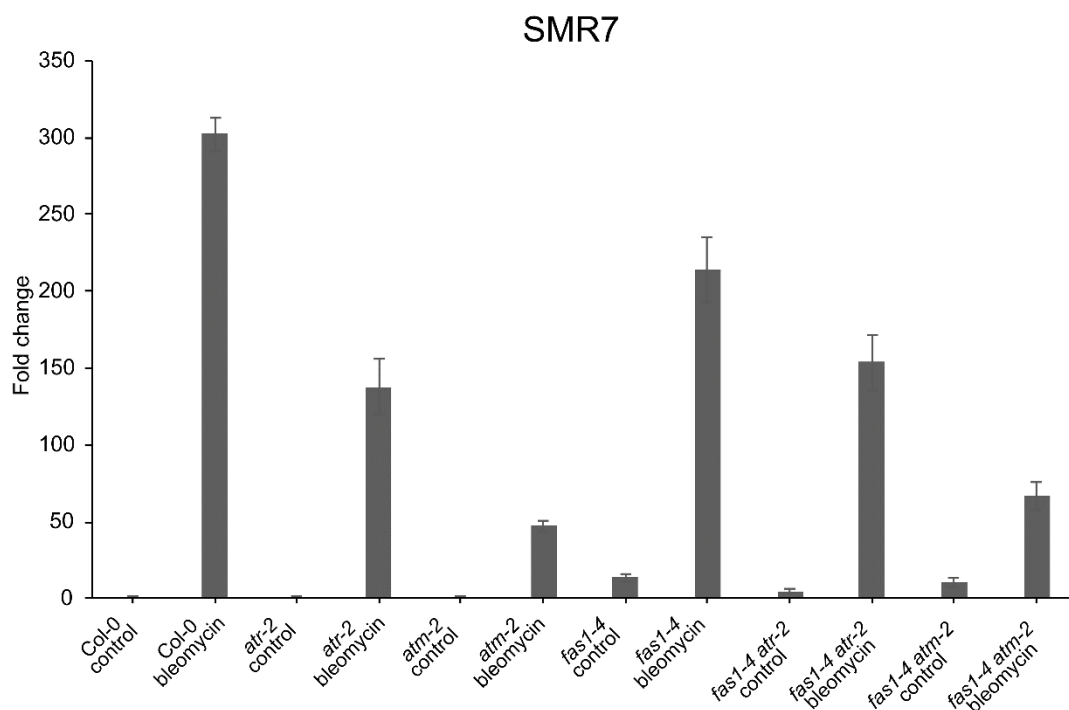
2

3 **Supplemental figure S1. Confirmation of the mutation in *FAS1* in line 49-2 by Sanger**
 4 **sequencing.** Schematic representation of mutation sites in the *FAS1* gene, with a focus on the
 5 sequence surrounding the EMS mutation site. Genomic DNA from a WT plant and two
 6 individual 49-2 plants was sequenced for the locus in *FAS1* containing the EMS mutation.

7

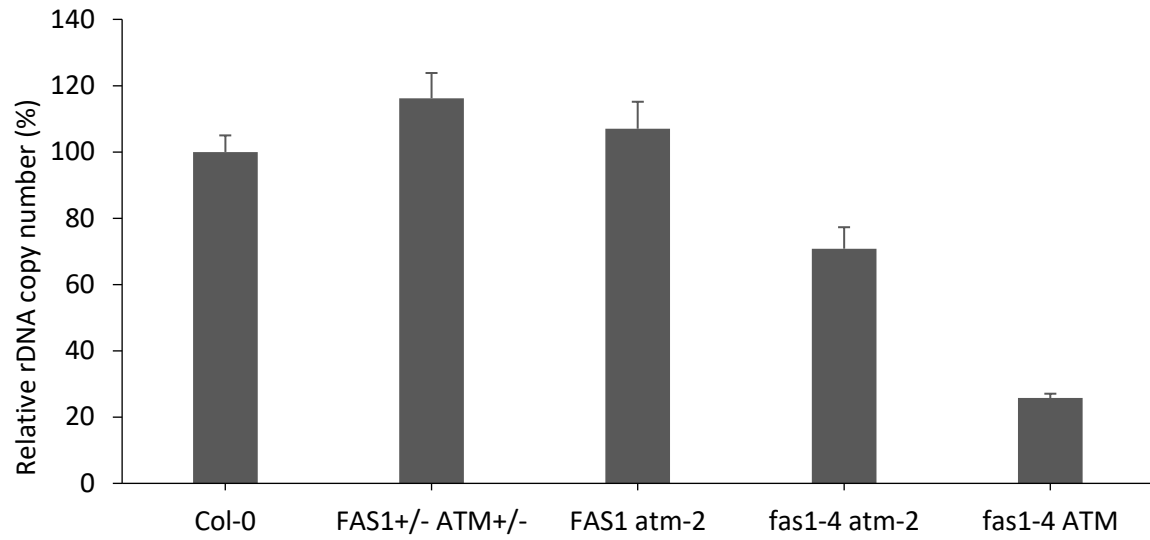


Supplemental figure S2. 49-2 revertant phenocopies the *fas1-8* knockout line. (A) Ploidy profile of first leaf of plants of line 49-2 and *fas1-8* at 21 DAS. (B) Leaf size of the first leaf of plants of line 49-2 and *fas1-8* at 21 DAS (n = 27, 3 biological repeats). (C) Representative rosette phenotype of line 49-2 and *fas1-8* at 15 DAS.

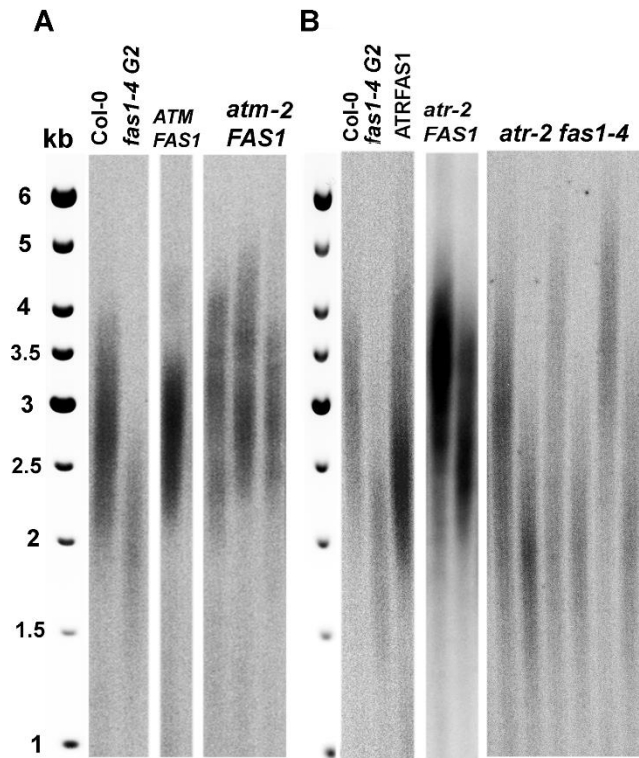
A**B**

14

15 **Supplemental figure S3. Treatment with a DNA damaging agent induces DDR genes to a**
 16 **similar extent in *fas1* background as in WT.** Relative expression levels in root tips of 7-d-
 17 old WT (Col-0), *atr-2*, *atm-2*, *fas1-4*, *fas1-4 atr-2* and *fas1-4 atm-2* mutant plants under control
 18 conditions or treated with 0.6 μ g/ml bleomycin for 24h, as determined by qRT-PCR. Data
 19 represent mean $\pm$ SEM, normalized to WT levels under control conditions that were arbitrarily
 20 set to one (n = 3).



Supplemental figure S4. rDNA copy number of plants segregated from heterozygous *FAS1/fas1 ATM/atm* plants. The relative rDNA copy number was determined by qPCR using the $2^{-\Delta\Delta CT}$ method and 18S primers. *UBQ10* was used as a reference.



Supplemental figure S5. Terminal restriction fragment analysis of additional lines. TRF analysis in *FAS1 atm-2* (segregated from *FAS1/fas1 ATM/atm* plants), *FAS1 atr-2* (segregated from *FAS1/fas1 ATR/atr* plants) and *fas1-4 atr-2* mutants (additional lines showing increased heterogeneity among individual mutant lines). Molecular markers (kb) are shown on the left

Supplemental tables

Supplemental table S1. Cell cycle parameters of Col-0, *wee1-1*, *fas1-8* and *fas1-8 wee1-1* under control conditions.

Experiment	Genotype	Cell cycle length (h)	Confidence interval	S-pase length (h)	Confidence interval
1	Col-0	20.6	[19.1-22.0]	3.7	[2.8-4.5]
	<i>wee1-1</i>	21.3 n.s	[19.6-23.3]	3.9 n.s	[2.9-5.0]
	<i>fas1-8</i>	31.9 ***	[29.1-35.1]	3.9 n.s	[2.8-5.0]
	<i>fas1-8 wee1-1</i>	36.8 ***	[32.5-42.4]	5.8 **	[4.2-7.8]
2	Col-0	20.7	[19.5-21.9]	3.4	[2.8-4.0]
	<i>wee1-1</i>	21.3 n.s	[19.4-23.8]	2.9 n.s	[2.0-4.2]
	<i>fas1-8</i>	29.7 ***	[27.1-32.6]	4.2 n.s	[3.1-5.3]
	<i>fas1-8 wee1-1</i>	34.6 ***	[30.9-39.6]	6.1 ***	[4.5-8.0]

Parameters were measured using a time course of EdU staining according to the protocol of Hayashi et al. (2013). Data show results of two independent experiments. Asterisks indicate statistically significant differences (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; n.s. = not significant).

38 **Supplemental table S2. Cell cycle parameters of Col-0, *wee1-1*, *fas1-8* and *fas1-8 wee1-1* under replication stress.**

Experiment	Genotype	Cell cycle length (h)	Confidence interval	S-phase length (h)	Confidence interval
1	Col-0	27.4 a ^{***}	[23.7-30.8]	4.8 a [*]	[3.0-6.3]
	<i>wee1-1</i>	27.8 a ^{***} b ^(n.s)	[24.4-31.0]	4.3 a ^(n.s) b ^(n.s)	[2.9-5.7]
	<i>fas1-8</i>	40.2 a ^{**} b ^{***}	[36.3-45.3]	7.2 a ^{**} b ^{**}	[5.8-9.1]
	<i>fas1-8 wee1-1</i>	38.0 a ^(n.s) b ^{***}	[34.6-43.3]	5.6 a ^(n.s) b ^(n.s)	[4.2-7.2]
2	Col-0	29.2 a ^{***}	[26.7-32.7]	5.0 a [*]	[3.8-6.5]
	<i>wee1-1</i>	32.2 a ^{***} b ^(n.s)	[27.9-34.3]	3.6 a ^(n.s) b ^(n.s)	[2.2-4.6]
	<i>fas1-8</i>	41.7 a ^{***} b ^{***}	[37.5-45.8]	7.0 a ^{***} b [*]	[5.5-8.4]
	<i>fas1-8 wee1-1</i>	36.7 a ^(n.s) b ^{***}	[32.8-41.1]	6.4 a ^(n.s) b ^(n.s)	[4.9-8.1]

39 Parameters were measured using a time course of EdU staining according to the protocol of Hayashi et al. (2013). Data show results of two
40 independent experiments. Asterisks indicate statistically significant differences (* p<0,05; ** p<0,01; *** p<0,001; n.s. = not significant; a:
41 comparison with values of untreated plants; b: comparison with values of Col-0).

42 **Supplemental table S3. Cell cycle parameters of Col-0, *fas1-4*, *fas1-4 atm-2*, *fas1-4 atr-2*, *atr-2* and *atm-2* under control conditions.**

Experiment		Cell cycle length (h)	Confidence interval	S-pase length (h)	Confidence interval
1	Col-0	20.7 b ^{***}	[19.0-22.8]	3.05 b ^(n.s)	[2.1-4.1]
	<i>fas1-4</i>	29.9 a ^{***}	[27.1-33.3]	3.3 a ^(n.s)	[2.2-4.5]
	<i>fas1-4 atm-2</i>	24.4 a [*] b ^{**}	[22.9-26.3]	4.6 a [*] b [*]	[3.7-5.5]
	<i>fas1-4 atr-2</i>	24.6 a ^{**} b ^{**}	[22.6-26.8]	3.6 a ^(n.s) b ^(n.s)	[2.8-4.8]
	<i>atr-2</i>	20.2 a ^(n.s) b ^{***}	[17.9-23.1]	2.5 a ^(n.s) b ^(n.s)	[1.3-4.0]
	<i>atm-2</i>	19.3 a ^(n.s) b ^{***}	[17.7-21.2]	2.5 a ^(n.s) b ^(n.s)	[1.7-3.6]
2	Col-0	19.9 b ^{***}	[18.5-21.6]	2.8 b ^(n.s)	[2.0-3.7]
	<i>fas1-4</i>	30.3 a ^{***}	[27.8-33.5]	3.6 a ^(n.s)	[2.6-4.8]
	<i>fas1-4 atm-2</i>	26.3 a ^{***} b [*]	[24.2-29.0]	5.2 a ^{**} b [*]	[4.1-6.5]
	<i>fas1-4 atr-2</i>	24.7 a ^{**} b ^{**}	[22.5-27.5]	3.5 a ^(n.s) b ^(n.s)	[2.5-4.8]
	<i>atr-2</i>	20.5 a ^(n.s) b ^{***}	[18.2-23.5]	2.7 a ^(n.s) b ^(n.s)	[1.5-4.2]
	<i>atm-2</i>	20.2 a ^(n.s) b ^{***}	[18.2-22.7]	2.8 a ^(n.s) b ^(n.s)	[1.7-4.2]

43 Parameters were measured using a time course of EdU staining according to the protocol of Hayashi et al. (2013). Data show results of two
44 independent experiments. Asterisks indicate statistically significant differences (* p<0,05; ** p<0,01; *** p<0,001; n.s. = not significant; a:
45 comparison with values of Col-0; b: comparison with values of *fas1-4*).

46 **Supplemental table S4. List of primers used for genotyping and qRT-PCR.**

Primer	Sequence
GABI_WEE1_FW	TCAATAAGGCTTGGTTTCTTCAGT
GABI_WEE1_REV	CCCTACGAACAAAACAAGATAAAAAC
GABI_TDNA_REV	ATAATAACGCTGCGGACATCTACATTTT
GABI_FAS1_FW	CCTTCATCTTCTCCAAAACCTTGAG
GABI_FAS1_REV	GACCAACAGAAACAACAGCACT
fas1-4_FW	TAACATGGAGAAGCAAGGGG
fas1-4_REV	GATCCTTTTGCAGAGGACGA
fas1-4_SALK_FW	TTAAAAACGTCCGCAATGTG
atm-2-genotype_FW	GGTTGGGCAGTTCCAAAGATGA
atm-2-genotype_REV	TCTCTCCTTGTTTCAAGCTCTG
atr-2-genotype_FW	CAAGGGTTCCGATGTTCAAAGTG
atr-2-genotype_REV	CAATCAGCAGGAAAAGACAAATC
SALK_TDNA_FW	ATTTTGCCGATTTCGGAAC
EMB2386_RT_FW	CTCTCGTTCCAGAGCTCGCAAAA
EMB2386_RT_REV	AAGAACACGCATCCTACGCATCC
PAC1_RT_FW	TCTCTTTGCAGGATGGGACAAGC
PAC1_RT_REV	AGACTGAGCCGCCTGATTGTTTG
RPS26C_RT_FW	GACTTTCAAGCGCAGGAATGGTG
RPS26C_RT_REV	CCTTGTCCTTGGGGCAACACTTT
RAD51B_qRT_FW	TGGTCCTTGAAGCCAAGTCAGGTC
RAD51B_qRT_REV	AGGCTAAAGGAGGCGACATAGGAG
RAD51_qRT_FW	TTCCGCTCTGGAAAGACTCAGC

RAD51_qRT_REV	ACCTCCTTGATCCATGGGAAGTTG
SMR7_qRT_FW	TTCATAAAGCCGGTGAAGACG
SMR7_qRT_REV	CGCCGTGGGAGTGATACAAATTC
SMR5_qRT_FW	AAACTACGACGACGGAGATACG
SMR5_qRT_REV	GCTACCACCGAGAAGAACAAGT
NAC044_qRT_FW	GAGCGCTAGAAAGGGAACGA
NAC044_qRT_REV	CCCCGGAAGTACTCTCACCTTC
NAC085_qRT_FW	AGCACACCGAAAAGTAGTAC
NAC085_qRT_REV	CTTCAATAAACTCACATTCCC

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