Title: The AtRAD21.1 and AtRAD21.3 Arabidopsis cohesins play a synergistic role in somatic DNA double strand break damage repair.

Authors: da Costa-Nunes JA, Capitão C, Kozak J, Costa-Nunes P, Ducasa GM, Pontes O, Angelis KJ

Additional file: Materials and Methods

Plant material

*Arabidopsis thaliana* accession Columbia-0 (Col) was obtained from the Nottingham Arabidopsis Stock Centre. The accession Wassilewskija-1 (Ws) and the *atku80* mutant were kindly provided by Dr. C.E. West (Faculty of Biological Sciences, Leeds University, UK) [1]. The mutants *atrad21.1* (salk_044851), *atrad21.3* (salk_076116) and the double mutant *atrad21.1 atrad21.3* have been previously described [2]. The double mutant *atku80 atrad21.1* was obtained via controlled cross-pollination.

qRT-PCR analysis

Rosette leaves from non-irradiated and irradiated four weeks old Col plants grown in GM medium, were harvested and immediately frozen in liquid nitrogen, 5, 15, 30 and 45 minutes, and 1, 2, 4, 6, 8, 10, 24 and 48 hours after the 316 Gy irradiation sessions (2.65 Gy/minute; source: Co60). Three independent biological replicas (irradiated and non-irradiated) of four weeks old Col plants were obtained. Total RNA was extracted using the RNeasy Plant kit (Qiagen). The quality, quantity and integrity of all the RNA samples was assessed before being individually processed with the Turbo-DNA-free kit (Ambion). 1μg of total RNA from each individual sample was used for cDNA synthesis (using SuperScript III/RNaseOUT enzyme mix and Oligo(dt)20).
qRT-PCR reaction was performed in the iQ™5 Real-Time PCR Detection System, in a 20μl reaction mix containing cDNA corresponding to 6.1ng of total RNA, 250nM of each primer and 10 μl of iQ™ SYBR Green Supermix (Bio-Rad). Quantification of gene expression was carried out after PCR amplification (1 cycle, 95°C for 3 minutes; 45 Cycles, 95°C for 10 seconds, 60°C for 10 seconds, 72°C for 10 seconds (with plate read); 1 Cycle, Melting curve from 55°C to 95.5°C, reading every 0.5°C, hold 10 seconds). Three different qRT-PCR reactions from each of the three cDNA replicas were carried out and quantified. For a given time point after the irradiation, the expression value attributed to each gene is the average from the three independent biological replicas. For each gene, the data was normalised using the expression level of non-irradiated samples as reference (arbitrary value of 1). Actin2 [3] and AtEF1αA4 [4] were used as reference genes. Relative quantification of transcript accumulation of the genes of interest, using Actin2 and AtEF1αA4 as reference genes, was obtained using the Pfaffl method [5]. Reference values for inter-plate calibration were obtained by amplifying, with the Actin2 primers, a dilution series of a bulk mix of all cDNA samples. The quantification cycle (Cq) was determined using Bio-Rad iQ5 Optical System Software, Version 2.0, Standard Edition.

The primers (with a calculated Tm of approximately 60°C) were designed with the aid of the Netprimer program. Primers were empirically tested; the melting curve was analysed (iQ™5 Real-Time PCR) and the size and number of the PCR products assessed (not shown) in agarose gels. All the primer pairs promote PCR amplification of single PCR products with efficiency between 90% and 106%. Primer pairs sequence and additional information is provided here in, in Additional file Table S3. Each of the primers qRTmR21-1L, qRTR21-1R, qRTR21-2Rz, qRTR21-3Lx, qRTR21-3Rx, qRTR51-L and rtATM-R spans two adjacent exons. The primer qRTef1-Lx spans the 5’UTR and the 1st exon. The two qRT-PCR amplicons from AtRAD21.1 span the 9th and 10th exons (87bp), and the 12th and 13th exons (110bp). The amplicon from AtRAD21.2 spans the 7th and 8th exons (108bp), and the one from AtRAD21.3 spans the 12th, 13th and 14th exons (132bp). The amplicon from AtRAD51 spans the 1st and 2nd exons, and that of the AtATM spans the 45th, 46th and 47th exons. Both the qRT-PCR amplicon
from Actin2 and that from AtEF1αA4 span the 5’UTRs and the 1st exon. The regions of the AtRAD21 genes that are amplified (and the respective amplicon length) with the qRT-PCR primer pairs are depicted in Additional file Figure S1f).

**Comet assay**

Seeds were germinated under a 16 hours of light (at 22° C) followed by 8 hours of darkness (at 18° C) cycle, on Petri dishes containing ½MS media with Gamborg B5 Vitamins (Duchefa, Haarlem, The Netherlands) solidified with 0.8% Plant agar (Duchefa, Haarlem, The Netherlands) and overlaid with cellophane to facilitate collection of seedlings. Prior treatment, seedlings were gently transferred from cellophane to liquid ½MS media, to avoid dehydration.

Nuclear DNA fragmentation was measured in 10-days-old seedlings untreated and treated with 10 µg/ml or 30 µg/ml Bleomycin Sulfate (Bleomedac; Hamburg, Germany) for 1 hour in liquid ½MS. After Bleomycin treatment, seedlings were thoroughly rinsed in H2O, blotted on filter paper and either immediately flash frozen in liquid nitrogen (t = 0) or left to recover in ½MS for the indicated repair times, before being frozen. DNA double strand breaks (dsb) were assayed using a neutral comet assay [6,7]. Plant material processing and data acquisition was carried out as described in previous reports [7]. In brief 70 µl of nuclear suspension obtained by chopping seedlings with a razor blade were dispersed in 280 µl of melted 0.7% LMT agarose (GibcoBRL, Gaithersburg, USA) and used to cast four gels on two microscopic slides per sample analysed. Comets were viewed in epifluorescence with a Nikon Eclipse 800 microscope after staining with SYBR Gold stain (Molecular Probes/Invitrogen, Eugene, USA) and evaluated by the Comet module of the LUCIA cytogenetics software suite (LIM, Praha, Czech Republic). The incidence of DNA dsb damage was measured as the fraction of fragmented DNA that moved from the comet head to the comet tail (% tail-DNA). The calculated percentage of damage remaining for each given repair time t_x is defined as:

\[
K(t_x) = \frac{\text{mean } \% \text{tail-DNA(t)} - \text{mean } \% \text{tail-DNA(control)}}{\text{mean } \% \text{tail-DNA(0)} - \text{mean } \% \text{tail-DNA(control)}} \times 100
\]

Data for Arabidopsis Col and the mutant lines (atrad21.1, atrad21.3, atrad21.1 atrad21.3) analysed in this study were measured in three independent
experiments and compiled. DNA dsb in these Arabidopsis mutants and Col were measured as the % tail-DNA in control seedlings and all mutated lines without a treatment, and in the following seven time-points: 0, 3, 5, 10, 20, 60 and 180 minutes after the treatment. 25 evaluated comets per independent gel replica, total in, at least, 300 comets analysed per experimental point. Microscopic slides were coded, and blind measured and evaluated.

1. West CE, Waterworth WM, Story GW, Sunderland PA, Jiang Q, Bray CM: Disruption of the Arabidopsis AtKu80 gene demonstrates an essential role for AtKu80 protein in efficient repair of DNA double-strand breaks in vivo. Plant J 2002, 31(4):517-528.

2. da Costa-Nunes JA, Bhatt AM, O’Shea S, West CE, Bray CM, Grossniklaus U, Dickinson HG: Characterization of the three Arabidopsis thaliana RAD21 cohesins reveals differential responses to ionizing radiation. J Exp Bot 2006, 57(4):971-983.

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5. Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001, 29(9):e45.

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7. Kozak J, West CE, White C, da Costa-Nunes JA, Angelis KJ: Rapid repair of DNA double strand breaks in Arabidopsis thaliana is dependent on proteins involved in chromosome structure maintenance. DNA Repair 2009, 8(3):413-419.
Additional file:
Figure S1

*AtRAD21.1-GFP-6xHis* transcript detection, and gene schematic representation.
Figure S1 legend:

The complementation line (Comp) expresses the \textit{AtRAD21.1-GFP-6xHis} transcript.

\textit{AtRAD21.1-GFP-6xHis} gene expression is unequivocally detected in the complementation line (Comp); \textit{a) \text{\textdollar\texttextdollar}} (primer pair CR1 and GFPOUT); \textit{c) \text{\textdollar\texttextdollar\texttextdollar}} (primer pair 3HOM6 and GFPOUT). High levels of \textit{AtRAD21.1} and \textit{AtRAD21.1-GFP-6xHis} gene expression (\textit{a, b, c}) are detected in samples (Het and Comp) exposed to ionising radiation (\textit{+ \textdollar\texttextdollar}) (a DNA dsb inducing agent). The higher levels of expression of the \textit{AtRAD51} gene in the Het and Comp samples confirm that these samples were exposed to ionising radiation (\textit{+ \textdollar\texttextdollar\texttextdollar} \textdollar\texttextdollar\textdollar) \textdollar\texttextdollar\textdollar) \textdollar\texttextdollar\textdollar\textdollar) [1]. \textit{Beta Tubulin} gene family expression data was used as control, showing that the detected differences in gene expression levels are not due to differences in cDNA template input (\textit{e}) \textdollar\texttextdollar\textdollar\textdollar\textdollar\textdollar) [2]. Samples non-exposed to DNA dsb inducing agents (Col - ) have low/undetectable \textit{AtRAD21.1} transcript content [3].

\textbf{Genotypes:}

Col - wild-type Columbia-0 plant
Het - \textit{AtRAD21.1 atrad21.1} heterozygous plant
Comp - \textit{atrad21.1} homozygous mutant containing the complementation construct.

RAD: ( - ) non-irradiated sample; ( + ) samples harvested 1 hour after the exposure to ionising radiation (150 Gy; 3.25 Gy/minutes; source: Cs137).

Comp and Het were irradiated to increase the \textit{AtRAD21.1} (and expecting to increase \textit{AtRAD21.1-GFP-6xHis}) transcript content, to facilitate the detection of the transcript by RT-PCR.

Samples Col and Het were harvested from rosette leaves; samples from Comp were harvested from seedlings. According to previous reports [3], both tissues (seedlings and rosette leaves) have undetectable or low transcript levels of \textit{AtRAD21.1} transcript in non-irradiated samples, but both accumulate \textit{AtRAD21.1} transcripts after exposure to ionising radiation. In contrast, the \textit{atrad21.1} mutant allele does not exhibit this increase in gene expression after exposure to ionising radiation.
PCR products (a to e) were amplified with the primers: a): CR1, GFPOUT and CR1, CR11; b): 3HOM6, CR11; c): 3HOM6, GFPOUT; d): atrad51cU, atrad51cD; e): TUB-L, TUB-R.

The primer pairs CR1 and CR11, and 3HOM6 and CR11, amplify the cDNA from both the native AtRAD21.1 transcript and the AtRAD21.1-GFP-6xHis transcript (a, b). The PCR products obtained with the GFPOUT and CR1, and GFPOUT and 3HOM6 primer pairs amplify solely the complementation construct’s cDNA (a, c); these primer pairs do not amplify spurious products from the AtRAD21.1 wild-type allele (in Col and Het) nor from the atrad21.1 alleles (in heterozygous (Het) AtRAD21.1 atrad21.1) in plants exposed and non-exposed to γ-rays. Primer pair atrad51cU and atrad51cD attests which samples were non-exposed (low expression) and exposed (high expression) to DNA dsb ionising radiation (d). TUB-L - TUB-R PCR products were used to certify that the cDNA input was identical in all PCR reactions (e).

Vertical line (in image A) separates two different PCR products photographed in the same gel.

(f) Schematic representation of the pAtRAD21.1-AtRAD21.1-GFP-6xHis complementation construct and the AtRAD21 genes, depicting primers position (black triangles), gene structure (adapted from [3]), the qRT-PCR amplicons (light grey rectangles), the amplicons’ length (87, 110, 108, 132bp), and their respective names (1, 1m, z, 3).

Exons (open boxes), introns (black lines), and 3’ and 5’ UTR of the three Arabidopsis RAD21 paralogs (grey lines). Grey arrowhead (ATG codon); Inverted open triangle (T-DNA). The grey rectangles (qRT-PCR amplicons) span over more then one exon.

The upstream genomic sequence of AtRAD21.1 (AtRAD21.1 promoter; pAtRAD21.1) is represented by the dotted horizontal line; this is not represented in the same scale used for the AtRAD21 genes (scale bar: 50bp).
1. Klimyuk VI, Jones JDG: *AtDMC1, the Arabidopsis homologue of yeast DMC1* gene: characterization, transposon-induced allelic variation and meiosis-associated expression. *Plant J* 1997, 11(1):1-14.

2. Knight H, Veale E, Warren GJ, Knight MR: The *sfr6* mutation in *Arabidopsis* suppresses low-temperature induction of genes dependent on the CRT/DRE sequence motif. *Plant Cell* 1999, 11(5):875-886.

3. da Costa-Nunes JA, Bhatt AM, O’Shea S, West CE, Bray CM, Grossniklaus U, Dickinson HG: Characterization of the three *Arabidopsis thaliana RAD21* cohesins reveals differential responses to ionizing radiation. *J Exp Bot* 2006, 57(4):971-983.
Additional file:
Figure S2

Relative variation of steady-state transcript levels during the 48 hours after
the exposure to ionising radiation

(A)
Figure S2

(B)

Figure S2 legend:

(A) - Relative variation of steady-state transcript levels, in four weeks old Col rosette leaves irradiated with 316 Gy (2.65 Gy/minute; source: Co60), during the 48 hours (2880 minutes) (left column), and detail of only the first 2 hours (120 minutes) (right column), after the exposure to ionising radiation. A sharp increase in \textit{AtRAD21.1} transcript steady state levels is detected as early as 5 minutes AEI (right column); data obtained using two different primer pairs that amplify two different amplicons (1 and 1m). \textit{AtRAD21.1} and \textit{AtRAD51} (51) steady-state transcript level variation patterns are similar (left column). In comparison, relative variation in \textit{AtRAD21.2} (z), \textit{AtRAD21.3} (3) and \textit{AtATM} (at) steady-state transcript level is not as dramatic. Error bars represent the standard deviation.

(B) - Compiled data from the six charts shown in Fig. S2 (A), to illustrate the broad difference in relative transcript content variation, detected by qRT-PCR, of the \textit{AtRAD21.1} gene (1 and 1m) and \textit{AtRAD51} (51), in contrast with that of
AtRAD21.2 (z), AtRAD21.3 (3) and AtATM (at), during the first 48 hours after exposure to ionising radiation. The steady-state transcript levels of non-irradiated samples was used as reference (with the arbitrary value: 1). 0- non-irradiated samples.

Samples (rosette leaves) were harvested 5, 15, 30, and 45 minutes, and 60, 120, 240, 360, 480, 600, 1440 and 2880 minutes (i.e. 1, 2, 4, 6, 8, 10, 24 and 48 hours, respectively) after the end of irradiation.

Sequence of the primer pairs amplifying the qRT-PCR amplicons (51, 1, 1m, z, 3 and at) is provided in Additional file Table S3, and qRT-PCR data is provided in Additional file Table S2.

Col - wild-type Columbia-0
min. -minutes
Additional file:
Figure S3

Frequency of seedlings with different numbers of true leaves, in different genotypes, before and after exposure to ionising radiation

- **x axis**: genotypes and the number of true leaves per seedling (from 0 to 7).
- **y axis**: frequency of seedlings with 0, 1, 2, 3, 4, 5, 6 or 7 true leaves; the sum of frequencies equals 1.
Figure S3 legend:

The *atrad21.1 atrad21.3* double mutant is the *atrad21* mutant with the highest hypersensitivity to ionising radiation (as assessed via the number of true leaves per seedling). The *atrad21.1 atrad21.3* double mutant requires a lower dose of ionising radiation (100 Gy) to exhibit a higher frequency of seedlings with none or one true leaf. In contrast, under the experimental conditions used, the *atrad21.1* and *atrad21.3* single mutants only exhibit a high frequency of seedlings with few true leaves (0 and 1) when exposed to higher doses of ionising radiation (γ-rays; 150 Gy). At 150 Gy, the *atrad21.1* mutant has a higher incidence of seedlings with 0 and 1 true leaves than the *atrad21.3* mutant.

The total number of seedlings used in the calculation of the frequencies was the following at 0 Gy: Col (79), *atrad21.1* (87), *atrad21.3* (94), *atrad21.1 atrad21.3* (72). At 100 Gy: Col (63), *atrad21.1* (87), *atrad21.3* (101), *atrad21.1 atrad21.3* (83). At 150 Gy: Col (124), *atrad21.1* (158), *atrad21.3* (117), *atrad21.1 atrad21.3* (112). The total number of seedlings is shown between brackets. 0 Gy - non-exposed to ionising radiation. 100 Gy, 150 Gy - exposed to 100 Gy (or 150 Gy) of ionising radiation (γ-rays; 3.25 Gy/minute; source: Cs137). Col - Wild type Col-0
Additional file:

Figure S4

Mean of the number of true leaves per seedling after exposure to ionising radiation
Figure S4 legend:

Both the median (Figure 4) and the mean (Additional files Figure S4 and Table S5) illustrate the dramatic increase in the number of seedlings which either do not have any true leaf, or only have one or two true leaves per seedling, 15 days after the exposure to radiation. The mean illustrates less clearly the differences between the wild type and the atrad21 mutants, most likely due to the effect of outlier values that distort the value of the mean in skewed populations (in particular in the atrad21.1 atrad21.3 double mutant at 100 Gy and 150 Gy, and in the atrad21 mutant at 150 Gy).

The reduced number of true leaves per seedling, that is characteristic to plants exposed to DNA damage inducing ionising radiation, is illustrated in Figure 3 as well as in other sources [1,2]. Error bars represent the standard deviation of the data above and below the mean. Black asterisk denotes significant difference between wild type Columbia-0 (Col) and atrad21.1, 15 days after exposure to 100 Gy (Mann-Whitney U=2026; p value (p)=0.00652). Grey asterisk denotes significant difference (p=0) between the atrad21.1 atrad21.3 mutant and Col at 100 Gy (Mann-Whitney U=726.5; p=0), and between atrad21 mutants and Col 15 days after exposure to 150 Gy (Col versus atrad21.1; Mann-Whitney U=5278.5; p=0), (Col versus atrad21.3; Mann-Whitney U=4712; p=0), (Col versus atrad21.1 atrad21.3; Mann-Whitney U=2920.5; p=0). At 0 Gy, none of the atrad21 single and double mutants is significantly different from Col (Col versus atrad21.1; Mann-Whitney U=2769; p=0.03078), (Col versus atrad21.3; Mann-Whitney U=3297; p=0.20408), (Col versus atrad21.1 atrad21.3; Mann-Whitney U=2346.5; p=0.06432). Statistical analysis was carried out using the Mann-Whitney non-parametric U-test (p<0.01, 2-tailed hypothesis).

The total number of seedlings used in the calculation of the means was the following at 0 Gy: Col (79), atrad21.1 (87), atrad21.3 (94), atrad21.1 atrad21.3 (72). At 100 Gy: Col (63), atrad21.1 (87), atrad21.3 (101), atrad21.1 atrad21.3 (83). At 150 Gy: Col (124), atrad21.1 (158), atrad21.3 (117), atrad21.1 atrad21.3 (112). The total number of seedlings is shown between brackets. 0 Gy - non-exposed to ionising radiation. 100 Gy, 150 Gy - exposed to 100 Gy (or 150 Gy) of ionising radiation (γ-rays; 3.25 Gy/minute; source: Cs137). Col - Wild type Col-0
1. Friesner J, Britt AB: *Ku80*- and *DNA ligase IV*-deficient plants are sensitive to ionizing radiation and defective in T-DNA integration. *Plant J* 2003, 34(4):427-440.

2. da Costa-Nunes JA, Bhatt AM, O'Shea S, West CE, Bray CM, Grossniklaus U, Dickinson HG: Characterization of the three *Arabidopsis thaliana RAD21* cohesins reveals differential responses to ionizing radiation. *J Exp Bot* 2006, 57(4):971-983.
Comet assay – significant differences
DNA dsb damage induction and repair
(after DNA dsb damage induction with 30µg Bleomycin)

% of damaged (dsb) DNA in *nuclei* of *atrad21* mutants and Col
after induction of DNA dsb (0 to 180 minutes)
Graphics illustrating the significant differences in nuclear DNA dsb fragments content between two different genotypes, at 10, 20 and 60 minutes after the induction of DNA dsb damage.

% of DNA that is damaged (dsb) in the nuclei of

| genotype | minutes after DNA dsb damage induction treatment |
|----------|--------------------------------------------------|
| atrad21.1 | 0 3 5 10 20 30 40 50 60 60 70 80 90 control |
| Col      | 0 3 5 10 20 30 40 50 60 60 70 80 90 control |

% of DNA that is damaged (dsb) in the nuclei of

| genotype | minutes after DNA dsb damage induction treatment |
|----------|--------------------------------------------------|
| atrad21.3 | 0 3 5 10 20 30 40 50 60 60 70 80 90 control |
| Col      | 0 3 5 10 20 30 40 50 60 60 70 80 90 control |
Graphic illustrating the significant differences in nuclear DNA dsb fragments content between two different genotypes, at 10, 20 and 60 minutes after the induction of DNA dsb damage.

% of DNA that is damaged (dsb) in the nuclei of *atrad21.1* and *atrad21.1 atrad21.3*.

% of Tail DNA

minutes after DNA dsb damage induction treatment

control

genotype

* I

* D

0, 3, 5, 10, 20, 60, 180
Graphics illustrating no significant differences in nuclear DNA dsb fragments content between two different genotypes.
Graphic illustrating no significant differences in nuclear DNA dsb fragments content between two different genotypes

Figure S5 legend:

dsb - double strand break

% Tail DNA - % of dsb damaged DNA in the nucleus ; ( Y-axis )
error bars represent the standard error (SE)

Genotype:

1 - atrad21.1 homozygous mutant
3 - atrad21.3 homozygous mutant
D - atrad21.1 atrad21.3 double homozygous mutant
C - Col ; wild-type Columbia-0

Data (% Tail DNA; SE) is provided in Additional file Table S6.
Figure S6 legend:

Top row:
Genotyping of Col, and atrad21.1, atrad21.1 atrad21.3 and atrad21.3 mutants using the primer mix (51L+ 51R + LBa1) and the primer mix (16L + 16 R + LBa1).

The amplicon (AtRAD21.1 allele specific) amplified with the primers 51L and 51R has a higher molecular weight than the amplicon (atrad21.1 allele specific) amplified with the 51L and LBa1 primers.

Only one AtRAD21.3 allele specific amplicon (16L and 16R) is amplified with the primer mix 16L, 16R and LBa1. The combination of the three primers 16L, 16R and LBa1 (that targets the atrad21.3 allele) yields two amplicons with different molecular weights.
Schematic drawing of gene structure (AtRAD21.1 and AtRAD21.3) and primers relative position is depicted in Additional file Figure S1f).

Bottom row:
Genotyping of atku80 atrad21.1 double homozygous mutant, atku80 single homozygous mutant and Col using the primer pairs 51L and 51R (that target the AtRAD21.1 allele), 51L and LBa1 (that target the atrad21.1 allele), K6 and KR (that target the AtKu80 allele) and K6 and LBFel (LBF) (that target the atku80 allele).
Black vertical line in the atku80 and Col gel separates two sections of the same agarose gel.
Primers sequence is provided in Additional file Table S7.
Col - wild-type Columbia-0
Figure S7 legend:
Bolting phenotype of the *atrad21* mutants. Vegetative growth of six weeks old *atrad21* mutants and Col plants is comparable. While bolting time in *atrad21.3* is delayed, and in *atrad21.1* it is wild type-like (Col) [1], the *atrad21.1 atrad21.3* double mutant exhibits an intermediate phenotype. This observation was carried out several times, with all four genotypes being sown and grown simultaneously in soil (in the same substrate), in different growth chambers. These plants were not exposed to ionising radiation. Col - wild-type Columbia-0

1. da Costa-Nunes JA, Bhatt AM, O’Shea S, West CE, Bray CM, Grossniklaus U, Dickinson HG: **Characterization of the three Arabidopsis thaliana RAD21 cohesins reveals differential responses to ionizing radiation.** *J Exp Bot* 2006, **57**(4):971-983.
**Primers to monitor gene expression (RT-PCR)**

| Gene   | Primer | Primer sequence          | PCR conditions                                      |
|--------|--------|--------------------------|-----------------------------------------------------|
| **AtRAD21.1**                              | CR1     | ATGTTTTACTCGCATTGTCTAG   | Annealing: 55°C, 45'' extension: 72°C, 3’ 37 extension cycles |
|        | CR11   | CAAGCTTTTTGTGGTCTGGA     | amplifies wild type allele and Comp.                |
| **At5g40840**                              |         |                          |                                                     |
| **AtRAD21.1**                              | CR1     | See above                | Annealing: 55°C, 45'' extension: 72°C, 3’ 37 extension cycles |
|        | GFPOUT + | GTATGTTGCATCACCTTCAC     | amplifies Comp. only                                |
| **AtRAD21.1**                              | 3HOM6 + | GTAACGTGGTTTCGGTTGAG     | Annealing: 54°C, 45'' extension: 72°C, 1’ 35 extension cycles |
|        | CR11   | See above                | amplifies wild type allele and Comp.                |
| **AtRAD21.1**                              | 3HOM6 + | See above                | Annealing: 56°C, 45'' extension: 72°C, 1’ 35 extension cycles |
|        | GFPOUT + | See above                | amplifies Comp. only                                |
| **AtRAD51**                                | Atrad51 cU + | AGCCATGATATTTCCCACCAATC | Annealing: 54°C, 45'' extension: 72°C, 1’ 35 extension cycles |
| **At5g20850**                              | Atrad51 cD + | GACTTGTCACACCTCCCATGG   | amplifies wild type allele                          |
| gene family | TUB-L [2] | TUB-R [2] |  |
|-------------|-----------|-----------|------------------------|
| $\beta$-tubulin | CCTGATAACTCGTCTTTGG | GTGAACCTCCATCTCGCCCAT | Annealing: 60°C, 45'', Extension: 72°C, 1.5', 25 extension cycles, amplifies wild type allele |

+ primers designed for this study
Comp - complementation construct: gDNA $pARAD21.1$-$AtRAD21.1$-$GFP$-$6xHis

1. da Costa-Nunes JA, Bhatt AM, O'Shea S, West CE, Bray CM, Grossniklaus U, Dickinson HG: **Characterization of the three Arabidopsis thaliana RAD21 cohesins reveals differential responses to ionizing radiation.** *J Exp Bot* 2006, **57**(4):971-983.
2. Knight H, Veale E, Warren GJ, Knight MR: **The sfr6 mutation in Arabidopsis suppresses low-temperature induction of genes dependent on the CRT/DRE sequence motif.** *Plant Cell* 1999, **11**(5):875-886.
Additional file:

Table S2

Relative variation of transcript steady-state content in Col, after the induction of DNA dsb damage.

Monitoring (qRT-PCR) the first 48 hours (0 to 2880 minutes) after exposure to ionising radiation.

| Amplification | Minutes after exposure to radiation |
|---------------|-------------------------------------|
|               | 0        | 5        | 15       | 30       | 45       | 60       | 120      | 240      | 360      | 480      | 600      | 1440     | 2880     |
| Fold Var      | 51       | 1        | 1.166702 | 0.047643 | 0.192384 | 0.563913 | 0.135279 | 15.06317 | 1440     | 1.198238 | 0.599464 | 1.534173 | 204.8426 |
| St Dev        | 51       | 1        | 0.047643 | 0.192384 | 0.563913 | 0.135279 | 15.06317 | 1440     | 1.198238 | 0.599464 | 1.534173 | 204.8426 |
|               | 1        | 0.047643 | 0.192384 | 0.563913 | 0.135279 | 15.06317 | 1440     | 1.198238 | 0.599464 | 1.534173 | 204.8426 |
|               | 3        | 0.047643 | 0.192384 | 0.563913 | 0.135279 | 15.06317 | 1440     | 1.198238 | 0.599464 | 1.534173 | 204.8426 |
|               | 1m       | 0.047643 | 0.192384 | 0.563913 | 0.135279 | 15.06317 | 1440     | 1.198238 | 0.599464 | 1.534173 | 204.8426 |
|               | at       | 0.047643 | 0.192384 | 0.563913 | 0.135279 | 15.06317 | 1440     | 1.198238 | 0.599464 | 1.534173 | 204.8426 |

Table S2 Additional file:

Monitoring (qRT-PCR) the first 48 hours (0 to 2880 minutes) after exposure to ionising radiation.

AtrAD21.1 qRT-PCR products amplified with two different primer pairs …………. (1m and 1)
AtrAD21.2 qRT-PCR product amplified with one primer pair …………………….. (z)
AtrAD21.3 qRT-PCR product amplified with one primer pair …………………….. (3)

Controls:
- positive control: AtrAD51 qRT-PCR product amplified with one primer pair …………. (51)
- control non-responsive to radiation induced expression: AtrATM qRT-PCR product amplified with one primer pair ………………………………… (at)
- reference control: Actin2 qRT-PCR product amplified with one primer pair ………….. (ac)
- reference control: AtrEF1 a44 qRT-PCR product amplified with one primer pair …….. (f1)

| Samples harvested at the end of the irradiation |
|-----------------------------------------------|
| Samples harvested 0 minutes after the end of the exposure to ionising radiation (non-irradiated) …………. (0) |
| Samples harvested 5 minutes after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) ……… (5) |
| Samples harvested 15 minutes after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) …… (15) |
| Samples harvested 30 minutes after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) ….. (30) |
| Samples harvested 45 minutes after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) ….. (45) |
| Samples harvested 1 hour after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) …….. (60) |
| Samples harvested 2 hours after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) …….. (120) |
| Samples harvested 4 hours after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) ……. (240) |
| Samples harvested 6 hours after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) …… (360) |
| Samples harvested 8 hours after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) …… (480) |
| Samples harvested 10 hours after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) …… (600) |
| Samples harvested 24 hours after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) ….. (1440) |
| Samples harvested 48 hours after the end of the exposure to ionising radiation (316 Gy; 2.65 Gy/minute) ….. (2880) |

| Minutes after exposure to irradiation |
|--------------------------------------|
| 0   | 5   | 15  | 30  | 45  | 60  | 120 | 240 | 360 | 480 | 600 | 1440 | 2880 |
| 0   | 5   | 15  | 30  | 45  | 60  | 120 | 240 | 360 | 480 | 600 | 1440 | 2880 |
The sequences of primer pairs amplifying the qRT-PCR amplicons (1m, 1, z, 3, 51 at) are provided in Additional file Table S3.

Fold Var - Relative variation of transcript content
St Dev - Standard deviation
Col - wild-type Columbia-0

Non-irradiated samples (0 minutes) were used as the reference value (with the arbitrary value: 1). Irradiated samples were exposure to ionising radiation: 316 Gy; 2.65 Gy/minute; source: Co60.
Additional file:

Table S3

Primers for qRT-PCR quantification of *AtRAD21* transcript steady state levels variation after exposure to ionising radiation.

| Gene       | primer  | Primer sequence               | PCR product; amplicon length (and name) | Efficiency (%) and $R^2$ |
|------------|---------|-------------------------------|-----------------------------------------|--------------------------|
| *AtRAD21.1*<br> *At5g40840*<br> qRTmR21-1L |         | CTTTCATCTGCATTACCTCATC        | 87 bp (1m)                             | 99.1% $R^2 = 0.986$     |
|            | qRTmR21-1R | CTTGAGGAGCACCGTCTG            |                                        |                          |
| *AtRAD21.1*<br> *At5g40840*<br> qRTR21-1L |         | GAACAGAGGGGAAAGAGAGAAGAG   | 110 bp (1)                             | 91.9% $R^2 = 0.993$     |
|            | qRTR21-1R  | GTTTCAACACAAAGTCTCAG         |                                        |                          |
| *AtRAD21.2*<br> *At3g59550*<br> qRTR21-2Lz |         | CTGTGTCCTCCATCTCCTC          | 108 bp (z)                             | 94.8% $R^2 = 0.994$     |
|            | qRTR21-2Rz | AAGCCTTCACCTTTTCTTTG         |                                        |                          |
| *AtRAD21.3*<br> *At5g16270*<br> qRTR21-3Lx |         | CACATGACACAGGATTTTGAGCG     | 132 bp (3)                             | 100% $R^2 = 0.989$      |
|            | qRTR21-3Rx | CCAGCCTACTAGTACGAGAAC       |                                        |                          |
| *AtRAD51*<br> *At5g20850*<br> qRTR51-L |         | TCGAACAGCTCCAGGCAGCAG       | 102 bp (51)                            | 102.2% $R^2 = 0.998$    |
|            | qRTR51-R  | CCTTTCTCGAGTATAAGCAACAC     |                                        |                          |
| *AtATM*<br> *At3g48190*<br> rtATM-L |         | GATGTCAGCGTGCCCATAACT       | 95 bp (at)                             | 105.8% $R^2 = 0.998$    |
|            | rtATM-R  | TGCACCACAAACTACTGCTGA       |                                        |                          |
| *Actin2*<br> *At3g18780*<br> qRTAC2-L |         | GAGAGAAAGTAAAGGATAATCCAGGAG | 101 bp                                | 94% $R^2 = 0.998$       |
|            | qRTAC2-R  | GCCATTTTTATAGCTTTGCCAGAAG  |                                        |                          |
| *AtEF1αA4*<br> *At5g60390*<br> qRTEF1-Lx |         | CTTACTTGAGCTATGGGTAAGAAG    | 111 bp                                 | 103.2% $R^2 = 0.998$    |
| 1 and .2   | qRTEF1-Rx | CCAGCCTTGAGTACGAGTGAC       |                                        |                          |
All primers were specifically designed for this study.
Additional file:
Table S4  Number of true leaves per seedling
15 days after irradiation

0 Gy - 1st assay

| Total number of seedlings | Genotype   | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|---------------------------|------------|----|----|----|----|----|----|----|----|
| 19                        | Col        | 0  | 0  | 2  | 6  | 7  | 3  | 1  | 0  |
| 53                        | atrad21.1  | 5  | 0  | 5  | 9  | 17 | 13 | 4  | 0  |
| 38                        | atrad21.3  | 0  | 4  | 9  | 6  | 15 | 4  | 0  | 0  |
| 27                        | atrad21.1  | 4  | 0  | 3  | 4  | 10 | 4  | 1  | 1  |
| 26                        | Ws         | 1  | 0  | 3  | 6  | 12 | 4  | 0  | 0  |
| 35                        | atku80     | 0  | 0  | 2  | 10 | 18 | 4  | 0  | 1  |
| 55                        | atku80     | 0  | 1  | 6  | 7  | 21 | 18 | 2  | 0  |

0 Gy - 2nd assay

| Total number of seedlings | Genotype   | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|---------------------------|------------|----|----|----|----|----|----|----|----|
| 60                        | Col        | 2  | 0  | 9  | 9  | 32 | 7  | 1  | 0  |
| 34                        | atrad21.1  | 0  | 0  | 3  | 4  | 10 | 14 | 3  | 0  |
| 56                        | atrad21.3  | 7  | 4  | 8  | 9  | 9  | 12 | 6  | 1  |
| 45                        | atrad21.1  | 0  | 0  | 8  | 6  | 8  | 8  | 11 | 4  |
| 29                        | Ws         | 0  | 0  | 5  | 7  | 10 | 7  | 0  | 0  |
| 21                        | atku80     | 0  | 0  | 5  | 10 | 6  | 0  | 0  | 0  |
| 43                        | atku80     | 0  | 0  | 6  | 8  | 15 | 14 | 0  | 0  |

0 Gy - Compiled data from both assays

| Total number of seedlings | Genotype   | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
|---------------------------|------------|----|----|----|----|----|----|----|----|
| 79                        | Col        | 2  | 0  | 11 | 15 | 39 | 10 | 2  | 0  |
| 87                        | atrad21.1  | 5  | 0  | 8  | 13 | 27 | 27 | 7  | 0  |
| 94                        | atrad21.3  | 7  | 8  | 17 | 15 | 24 | 16 | 6  | 1  |
| 72                        | atrad21.1  | 4  | 0  | 11 | 10 | 18 | 12 | 12 | 5  |
| 55                        | Ws         | 1  | 0  | 8  | 13 | 22 | 11 | 0  | 0  |
| 56                        | atku80     | 0  | 0  | 2  | 15 | 28 | 10 | 0  | 1  |
| 98                        | atku80     | 0  | 1  | 12 | 15 | 36 | 32 | 2  | 0  |
15 days after irradiation

100 Gy - 1st assay

| Total number of seedlings | genotype   | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---------------------------|------------|---|---|---|---|---|---|---|---|
| 39 | Col         | 4 | 3 | 3 | 7 | 10| 6 | 5 | 1 |
| 58 | atrad21.1   | 15| 12| 10| 9 | 5 | 7 | 0 | 0 |
| 55 | atrad21.3   | 10| 6 | 16| 7 | 10| 2 | 4 | 0 |
| 36 | atrad21.1 atrad21.3 | 20| 10| 5 | 1 | 0 | 0 | 0 | 0 |
| 54 | Ws          | 2 | 4 | 15| 22| 9 | 1 | 1 | 0 |
| 161| atku80      | 134|26| 1 | 0 | 0 | 0 | 0 | 0 |
| 165| atku80 atrad21.1 | 150|13| 2 | 0 | 0 | 0 | 0 | 0 |

100 Gy - 2nd assay

| Total number of seedlings | genotype         | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---------------------------|------------------|---|---|---|---|---|---|---|---|
| 24 | Col             | 1 | 5 | 6 | 2 | 7 | 2 | 1 | 0 |
| 29 | atrad21.1       | 1 | 2 | 9 | 2 | 9 | 5 | 1 | 0 |
| 46 | atrad21.3       | 0 | 8 | 8 | 11| 12| 5 | 2 | 0 |
| 47 | atrad21.1 atrad21.3 | 23| 13| 5 | 3 | 2 | 1 | 0 | 0 |
| 32 | Ws              | 1 | 0 | 3 | 14| 13| 1 | 0 | 0 |
| 129| atku80          | 117|12| 0 | 0 | 0 | 0 | 0 | 0 |
| 154| atku80 atrad21.1 | 151|3 | 0 | 0 | 0 | 0 | 0 | 0 |

100 Gy - Compiled data from both assays

| Total number of seedlings | genotype         | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---------------------------|------------------|---|---|---|---|---|---|---|---|
| 63 | Col             | 5 | 8 | 9 | 9 | 17| 8 | 6 | 1 |
| 87 | atrad21.1       | 16| 14| 19| 11| 14| 12| 1 | 0 |
| 101| atrad21.3       | 10| 14| 24| 18| 22| 7 | 6 | 0 |
| 83 | atrad21.1 atrad21.3 | 43| 23| 10| 4 | 2 | 1 | 0 | 0 |
| 86 | Ws              | 3 | 4 | 18| 36| 22| 2 | 1 | 0 |
| 290| atku80          | 251|38| 1 | 0 | 0 | 0 | 0 | 0 |
| 319| atku80 atrad21.1 | 301|16| 2 | 0 | 0 | 0 | 0 | 0 |
15 days after irradiation

150 Gy - 1st assay

| Total number of seedlings | genotype  | Number of seedlings with 0, 1, 2, 3, 4, 5, 6 and 7 true leaves |
|--------------------------|-----------|---------------------------------------------------------------|
|                          |           | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
| 61                       | Col       | 17 | 13 | 6  | 10 | 11 | 3  | 1  | 0  |
| 72                       | atrad21.1 | 32 | 15 | 7  | 12 | 6  | 0  | 0  | 0  |
| 68                       | atrad21.3 | 28 | 23 | 6  | 7  | 4  | 0  | 0  | 0  |
| 6                        | atrad21.1 | 5  | 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| 68                       | Ws        | 18 | 26 | 14 | 9  | 0  | 0  | 1  | 0  |
| 204                      | atru80    | 203| 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| 283                      | atru80    | 271| 5  | 6  | 1  | 0  | 0  | 0  | 0  |

150 Gy - 2nd assay

| Total number of seedlings | genotype  | Number of seedlings with 0, 1, 2, 3, 4, 5, 6 and 7 true leaves |
|--------------------------|-----------|---------------------------------------------------------------|
|                          |           | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
| 63                       | Col       | 6  | 6  | 12 | 15 | 14 | 10 | 0  | 0  |
| 86                       | atrad21.1 | 47 | 18 | 12 | 3  | 3  | 1  | 2  | 0  |
| 49                       | atrad21.3 | 11 | 15 | 7  | 7  | 5  | 3  | 1  | 0  |
| 106                      | atrad21.1 | 62 | 20 | 15 | 7  | 2  | 0  | 0  | 0  |
| 106                      | atrad21.3 | 62 | 20 | 15 | 7  | 2  | 0  | 0  | 0  |
| 62                       | Ws        | 13 | 26 | 15 | 7  | 1  | 0  | 0  | 0  |
| 103                      | atru80    | 103| 0  | 0  | 0  | 0  | 0  | 0  | 0  |
| 138                      | atru80    | 136| 2  | 0  | 0  | 0  | 0  | 0  | 0  |

150 Gy - Compiled data from both assays

| Total number of seedlings | genotype  | Number of seedlings with 0, 1, 2, 3, 4, 5, 6 and 7 true leaves |
|--------------------------|-----------|---------------------------------------------------------------|
|                          |           | 0  | 1  | 2  | 3  | 4  | 5  | 6  | 7  |
| 124                      | Col       | 23 | 19 | 18 | 25 | 25 | 13 | 1  | 0  |
| 158                      | atrad21.1 | 79 | 33 | 19 | 15 | 9  | 1  | 2  | 0  |
| 117                      | atrad21.3 | 39 | 38 | 13 | 14 | 9  | 3  | 1  | 0  |
| 112                      | atrad21.1 | 67 | 21 | 15 | 7  | 2  | 0  | 0  | 0  |
| 130                      | Ws        | 31 | 52 | 29 | 16 | 1  | 0  | 1  | 0  |
| 307                      | atru80    | 306| 1  | 0  | 0  | 0  | 0  | 0  | 0  |
| 421                      | atru80    | 407| 7  | 6  | 1  | 0  | 0  | 0  | 0  |
Table S4 legend:

Number of emerging and fully expanded true leaves in seedlings (Col, Ws, atrad2.1, atrad21.3, atrad21.1 atrad21.3, atku80, atku80 atrad21.1) non-exposed to ionising radiation (0 Gy) and exposed to 100 Gy and 150 Gy of ionising radiation (γ-rays; 3.25 Gy/minute; source: Cs137).

Tables show the number of seedlings with 0, 1, 2, 3, 4, 5, 6 or 7 true leaves observed 15 days after irradiation (15 DAI) of pre-imbibed seed, in two independent biological replicas (assays). Col- Wild type Col-0. Ws- Wild type Wassilewskija-1
Additional file:

Table S5  **Mean, Mode and Median (true leaves per seedling)**  
15 days after irradiation

|             | Mean | σ up (xi ≥ mean) | σ down (xi < mean) | Mode | Median | σ up (xi ≥median) | σ down (xi<median) |
|-------------|------|------------------|---------------------|------|--------|------------------|-------------------|
|             | Col  | 3.608            | 0.84992             | 1.49065 | 4      | 4                | 0.67505           | 1.53741          |
|             | atrad21.1 | 3.908          | 1.01671             | 2.156  | 4, 5   | 4                | 1.12444           | 1.71499          |
|             | atrad21.3 | 3.255          | 1.60607             | 1.76092 | 4      | 3.5              | 1.39719           | 1.96574          |
|             | atrad21.1 | 4.042          | 1.86437             | 1.70486 | 4      | 4                | 1.67083           | 1.83615          |
|             | atrad21.3 | 3.6  | 0.87178             | 1.34731 | 4      | 4                | 0.63246           | 1.5172           |
|             | Ws   | 3.893            | 0.75504             | 1.09327 | 4      | 4                | 0.82375           | 0.92296          |
|             | atku80 | 3.939           | 0.79884             | 1.57483 | 4      | 4                | 0.90351           | 1.22474          |

|             | Mean | σ up (xi ≥ mean) | σ down (xi < mean) | Mode | Median | σ up (xi ≥median) | σ down (xi<median) |
|-------------|------|------------------|---------------------|------|--------|------------------|-------------------|
|             | Col  | 3.238            | 1.71959             | 1.88684 | 4      | 4                | 1.14087           | 2.54146          |
|             | atrad21.1 | 2.379          | 1.89554             | 1.58079 | 2      | 2                | 2.09542           | 1.35473          |
|             | atrad21.3 | 2.723           | 1.6137              | 1.65172 | 2      | 3                | 1.43506           | 1.8532           |
|             | atrad21.1 | 0.819           | 1.33629             | 0.82897 | 0      | 0                | 1.93882           | 0                |
|             | atrad21.3 | 2.930           | 0.84294             | 1.53077 | 3      | 3                | 0.95235           | 1.20515          |
|             | Ws   | 0.138            | 0.90167             | 0.13821 | 0      | 0                | 0.5382            | 0                |
|             | atku80 | 0.0627          | 1.0945              | 0.0628  | 0      | 0                | 0.3879            | 0                |

|             | Mean | σ up (xi ≥ mean) | σ down (xi < mean) | Mode | Median | σ up (xi ≥median) | σ down (xi<median) |
|-------------|------|------------------|---------------------|------|--------|------------------|-------------------|
|             | Col  | 2.427            | 1.62414             | 1.73462 | 3, 4   | 3                | 1.17775           | 2.22136          |
|             | atrad21.1 | 1.069         | 2.15533             | 0.90316 | 0      | 0.5              | 2.06155           | 0.50319          |
|             | atrad21.3 | 1.393           | 2.01461             | 1.03599 | 0      | 1                | 1.95243           | 0.82357          |
|             | atrad21.1 | 0.714           | 1.37173             | 0.71968 | 0      | 0                | 1.77281           | 0                |
|             | atrad21.3 | 1.292           | 1.38899             | 0.82798 | 1      | 1                | 1.3978            | 0.69597          |
|             | Ws   | 0.004            | 0.99674             | 0.00326 | 0      | 0                | 0.08071           | 0                |
|             | atku80 | 0.0523          | 1.64184             | 0.05232 | 0      | 0                | 0.43592           | 0                |

|             | Mean | σ up (xi ≥ mean) | σ down (xi < mean) | Mode | Median | σ up (xi ≥median) | σ down (xi<median) |
|-------------|------|------------------|---------------------|------|--------|------------------|-------------------|
|             | Col  | 2.930            | 0.84294             | 1.53077 | 3      | 3                | 0.95235           | 1.20515          |
|             | Ws   | 0.138            | 0.90167             | 0.13821 | 0      | 0                | 0.5382            | 0                |
|             | atku80 | 0.0627         | 1.0945              | 0.0628  | 0      | 0                | 0.3879            | 0                |
Table S5 legend:

Mean, Mode and Median of the number of emerging and fully expanded true leaves in seedlings (Col, Ws, atrad2.1, atrad21.3, atrad21.1 atrad21.3, atku80, atku80 atrad21.1) non-exposed to ionising radiation (0 Gy) and exposed to 100 Gy and 150 Gy of ionising radiation.

Due to the detection of skewed data distributions at 100 Gy and 150 Gy, the values of the Median and Mode are shown to illustrate better the higher incidence of seedlings with 0 true leaves, 15 days after the irradiation, in the genotypes that are more hypersensitive to ionising radiation exposure. The atrad21.1 atrad21.3 and the atku80 atrad21.1 double mutants and the atku80 single mutant have a particularly high incidence of seedlings with none or few true leaves at 100 Gy, and also at 150 Gy, illustrating their clear hypersensitivity to ionising radiation exposure.

Mean, Median and Mode obtained from the combined data (Additional file Table S4) from both independent biological replicas (assays) combined. σ up - standard deviation of data with equal or higher value than the mean (or median); σ down - standard deviation of data with lower value than the mean (or median). 0 Gy: non-exposed to ionising radiation. 100 Gy, 150 Gy: exposed to 100 Gy (or 150 Gy) of ionising radiation (γ-rays; 3.25 Gy/minute; source: Cs137 ). Col-Wild type Col-0. Ws- Wild type Wassilewskija-1
**Additional file:**

**Table S6**

**Comet assay data**

DNA dsb repair during the first 3 hours (0 to 180 minutes (tx)) after DNA dsb induction with 30 µg/ml Bleomycin (in Col, atrad21.1, atrad21.3 and atrad21.1 atrad21.3 mutants).

Amount of DNA in comet tail (% Tail DNA) decreases as a result of DNA dsb damage repair.

**Kinetics (K) of DNA dsb damage repair**

|       | % Tail DNA | SE  | C(tx) | K(tx) | tx     |
|-------|------------|-----|-------|-------|--------|
| **1 - atrad21.1** |            |     |       |       |        |
|       | 65.5       | 4.0 | 38.6  | 100.0 | 0      |
|       | 63.4       | 8.7 | 36.5  | 94.6  | 3      |
|       | 59.0       | 8.5 | 32.2  | 83.2  | 5      |
|       | 50.8       | 5.2 | 24.0  | 62.1  | 10     |
|       | 48.2       | 7.7 | 21.4  | 55.4  | 20     |
|       | 35.4       | 2.4 | 8.5   | 22.1  | 60     |
|       | 28.7       | 1.6 | 1.8   | 4.7   | 180    |
|       | 26.8       | 1.3 |       |       | control|
| **3 - atrad21.3** |            |     |       |       |        |
|       | 64.8       | 4.8 | 39.0  | 100.0 | 0      |
|       | 64.3       | 9.9 | 38.6  | 98.9  | 3      |
|       | 56.0       | 7.8 | 30.3  | 77.5  | 5      |
|       | 53.9       | 8.8 | 28.2  | 72.2  | 10     |
|       | 49.5       | 8.0 | 23.8  | 60.9  | 20     |
|       | 33.7       | 3.0 | 7.9   | 20.3  | 60     |
|       | 29.3       | 1.7 | 3.5   | 9.0   | 180    |
|       | 25.7       | 1.8 |       |       | control|
| **D - atrad21.1 atrad21.3** |            |     |       |       |        |
|       | 71.5       | 7.2 | 45.6  | 100.0 | 0      |
|       | 69.8       | 5.2 | 43.9  | 96.4  | 3      |
|       | 55.1       | 4.2 | 29.3  | 64.2  | 5      |
|       | 43.4       | 2.1 | 17.5  | 38.4  | 10     |
|       | 40.1       | 3.3 | 14.3  | 31.3  | 20     |
|       | 32.0       | 1.5 | 6.1   | 13.3  | 60     |
|       | 28.1       | 1.4 | 2.2   | 4.8   | 180    |
|       | 25.9       | 1.6 |       |       | control|
| **C - Col** |            |     |       |       |        |
|       | 72.3       | 6.3 | 52.9  | 100.0 | 0      |
|       | 65.2       | 7.1 | 45.8  | 86.6  | 3      |
|       | 55.9       | 7.3 | 36.5  | 68.9  | 5      |
|       | 40.6       | 3.0 | 21.2  | 40.2  | 10     |
|       | 36.0       | 2.3 | 16.6  | 31.3  | 20     |
|       | 28.5       | 1.1 | 9.1   | 17.1  | 60     |
|       | 26.3       | 1.1 | 6.9   | 13.0  | 180    |
|       | 19.4       | 1.2 |       |       | control|
Table S6 legend:
dsb - double strand break

% Tail DNA - percentage of total nuclear that has dsb fragments (tail of the comet)
SE - standard error
C(tx) - Increment of the amount of DNA dsb fragments (DNA in tail) in comparison to samples not exposed to DNA induction
C(tx) = % Tail DNA (tx) - % Tail DNA (control)

K(tx) - DNA dsb repair kinetics

K(tx) = C(tx) / C(t0) x 100

tx - time (minutes) after the DNA dsb induction treatment
t0 - 0 minutes (minutes) after the DNA dsb induction treatment

control - samples not treated with Bleomycin

Genotype:

1 - atrad21.1 homozygous mutant
3 - atrad21.3 homozygous mutant
D - atrad21.1 atrad21.3 double homozygous mutant
C - Col; wild-type Columbia-0
### Additional file:

**Table S7**

| Gene / mutant / construct | Primer name | PCR product | Primer sequence | Observations |
|---------------------------|-------------|-------------|-----------------|--------------|
| *atrad21.1*               | LBa1 [1]    | amplifies   | TGGTTCACGTAGTG GCCCATCG | Annealing: 55°C, 45’ |
|                           | 51L [2]     | mutant      |                 | extention: 72°C, 1.5’ |
|                           |             | gDNA only   | GAGATGGTCACACAG AGAATTTAG | |
| *AtRAD21.1*               | 51L [2]     | amplifies   |                 | Annealing: 53°C, 45’ |
| *At5g40840*               |             | wild type   |                 | extention: 72°C, 1.5’ |
|                           | 51R [2]     | gDNA only   | CT CCTCTCAGGACAG TCAGTATG | |
| *atrad21.3*               | LBa1 [1]    | amplifies   |                 | Annealing: 58°C, 30’ |
| *Salk_076116*             |             | mutant      | see above       | extention: 72°C, 1.5’ |
|                           | 16L [2]     | gDNA only   | CT GTGT CATG TG CATTTTCCATGG | |
| *atrad21.3*               | LBa1 [1]    | amplifies   |                 | Annealing: 58°C, 30’ |
| *Salk_076116*             |             | mutant      | see above       | extention: 72°C, 1.5’ |
|                           | 16R [2]     | gDNA only   | CCGTGT AGAG AG TACAGGTG | |
| *AtRAD21.3*               | 16L [2]     | amplifies   |                 | Annealing: 58°C, 30’ |
| *At5g16270*               |             | wild type   | see above       | extention: 72°C, 1.5’ |
|                           | 16R [2]     | gDNA only   | see above       | |
| *atku80*                  | LB Fel [3]  | amplifies   | GATTCTTTTTATGC ATAGATGCAC | Annealing: 56°C, 45’ |
| *West et al., 2002*       | K6 [3]      | mutant      | CTCAAGACGCAGC GCTTTAC | extention: 72°C, 2’ |
|                           |             | gDNA only   |                 | |
| *atku80*                  | RB Fel +    | amplifies   | TCCGCCTTCGGTTCCACC | Annealing: 56°C, 45’ |
| *West et al., 2002*       | KR +        | mutant      | CGTATCTGCTATTGCAAGAGA | extention: 72°C, 2’ |
|   | AtKU80 | At1g48050 |
|---|--------|-----------|
|   | K6     | [3]       |
|   | KR     | +         |
|   | amplifies | wild type |
|   | gDNA only | see above |
|   | see above |           |
|   | Annealing: | 56°C, 45’’ |
|   | extention: | 72°C, 2’  |
| Comp | GFPOUT + | amplifies |
|      | Comp only | GTATGTTGCATCACCTTCAC |
|      | 3HOM6 +  | GTAACGTGGTTTCGTTGAG |
|      | Annealing: | 56°C, 45’’ |
|      | extention: | 72°C, 2.5’ |

+ primers designed for this study

Comp - complementation line and complementation construct gDNA
pARAD21.1-AtRAD21.1-GFP-6xHis

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