A

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AtXRCC4 366  TAAACGGCTCTCTTGGACGTTTGAGAAGGAAGGGACTAAACTTGAGTGGAGGTGGAAATG  425
             |||| |||| || ||||| |||||||| ||||| || ||  | || ||| ||||||||||
NbXRCC4 27   TAAAAGGCTTTCCTGGACATTTGAGAAAGAAGGCACAAAGTTGGAATGGCGGTGGAAATG  86

AtXRCC4 426  TAAACCATCAGATGATAGCAAGAAGATCACTGTTGGGATCTTGGATTTTCTTATGGAGGC  485
             ||| ||   |  | |||||||||| |  || |  | |||||||| ||||| ||||| ||
NbXRCC4 87   CCAACTGTCTCCTAATAGCAAGAAAACTACGGCAGACATCTTGGACTTTCTCATGGATGC  146

AtXRCC4 486  TAACATAAGGCTAAGTGAAGAAGTGGTGAACAAGACGAGATCTTTTGAGAAGATGAGAAG  545
             ||||| || || ||||| ||||| || |  || ||   ||| |||||||| | |||||
NbXRCC4 147  AAACATTAGACTTAGTGACGAAGTTGTCAGTAAAACTCAATCATTTGAGAGGCTGAGAGA  206

AtXRCC4 546  TGAAGCTGAGAGATGTCTAGCGCAAGGTGAAAAACTCTGTGACGAAAAAACAGAGTTTGA  605
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NbXRCC4 207  GGAGGCTGAAAAATGTTTAACACAAAGCGAGAAACTCAGCAAAGAGAAAGAAGAATTTGA  266

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NbXRCC4 267  ATCTGCAATATATGCAAAG     285
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B
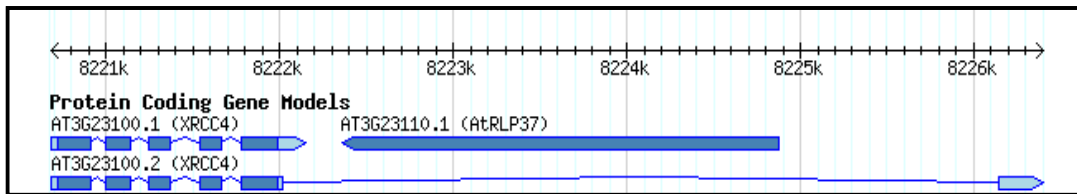
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At3G23100 139  GGCGCCGATCCAATTTTCGTCAAAGGCACTTGGCATAATTCTCGTTTCGATATCTCCGTC  198
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At3G23100 199  ACCGATGGTTCCTCCTCTTGGATTTGCAATGCGACGGAGGAGGAAGTGGCGGAGAGAGCA  258
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At1G61410 121  ACCGATGGCTCCTTCACTTGGACTTGCAATGCGACGGAGGAGGAGGTAGCGGAGAGAGCA  180

At3G23100 259  GCACAATGGGACCAGCCTGTGTCAGAGTATTTAAAGCTCGCCGAGCAATACTTAGGGTTT  318
               |  |||||||||||||||||| || ||||||| ||||||||||||||AATACTTAGGGTTT
At1G61410 181  G--CAATGGGACCAGCCTGTATCTGAGTATTTAGAGCTCGCCGAGCAATACTTAGGGTTT  238

At3G23100 319  CAACAACCTAATTCGGTCTATAGTTTCTCCGATGCTCTAGAGGGATCTAAACGGCTCTCT  378
               || || |||||||||||||||| ||||||||||||||| ||||||||||||||||||||||
At1G61410 239  CAGCAGCCTAATTCGGTCTATGGTTTCTCCGATGCTATTGAGGGATCTAAACGGCTCTCT  298

At3G23100 379  TGGACGTTTGAGAAGGAAGGGACTAAACTTGAGTGGAGGTGGAAATGTAAACCATCAGAT  438
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
At1G61410 299  TGGACGTTTGAGAAGGAAGGGACTAAACTTGAGTGGAGGTGGAAATGTAAACCATCAGAT  358

At3G23100 439  GATAGCAAGAAGATCACTGTTGGGATCTTGGATTTTCTTATGGAGGCTAACATAAGGCTA  498
               ||||||||||||||||||||||| |||| |||||||||||||||||||||||||||||||
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At3G23100 499 AGTGAAGAAGTGGTGAACAAGACGAGATCTTTTGAGAAGATGAGAAGTGAAGCTGAGAGA 558
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At3G23100 559 TGTCTAGCGCAAGGTGAAAAACTCTGTGACGAAAAAACAGAGTTTGAGAGTGCAACTTAT 618
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At3G23100 619 GCAAAGTTTCTTTCTGTTTTAAATGCAAAGAAGGCAAAACTGAGAGCACTAAGGGACAAA 678
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At3G23100 679 GAAGATTCAGTGAGAGTAGTTGAGGAGGAAGAGTCGACAGACAAAGCTGAAAGCTTTGAG 738
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At1G61410 599 GAAGATTCAGTGAGAGCAGTTGAGGAGGAAGAGTCGACATACAAAGCTGAAAGCTTCGAG 658

At3G23100 739 AGTGGAAGAAGTGATGATGAGAAGAGCGAGGAAGAAGCCTCAAAAAAGGCAACAAGCAGC 798
              |||||||||||||||||||| |||| | ||||||||| ||| ||||||||||||||
At1G61410 659 AGTGGAAGAAGTGATGATGAGCAGAGTGGGGAAGAAGCGTCAGAAAAGGCAACAAGCAGC 718

At3G23100 799 AAAGCCCGTGGCGGGAAGAGAGCTGCACGAAGCTAAGA----GA--TTGCCCTGCCGTGA 852
              ||||||||||||||| |||||||||||||| ||||||||     ||  || ||||||| |||
At1G61410 719 AAAGCCCGTGGCGGAAAGAGAGCTGCACGGAGCTAAGACTTAGAGATTCCCCTGCCTTGA 778

At3G23100 853 GATTTTCAGG   862
              ||||| ||||
At1G61410 779 GATTT-CAGG   787
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D

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GAAGTGATGA TGAGAAGAGC GAGGAAGAAG CCTCAAAAAA GGCAACAAGC AGCAAAGCCC GTGGCGGGAA
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GTCAAACTCA TAGCTAGGAA AATTGTTTGT CAAACCTTCT TTGGTTTAAA AAAATAATTT ACAATTTGTA
TTACTTCTGA AAGTAAATGC AATAACTTAG AGATGG
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Supplemental Figure 1. *Nicotiana benthamiana* **XRCC4 Sequence Used for Virus-Induced Gene Silencing**

(A) Sequence alignment between *Arabidopsis thaliana* (At3G23100) and *N. benthamiana XRCC4* (in VIGS vector) using BLASTN function. *N. benthamiana XRCC4* shares 72% nucleotide identity with Arabidopsis *XRCC4*.

(B) Predicted gene models for *XRCC4* (At3G23100) from *Arabidopsis* genome viewer.

(C) Nucleotide sequence alignment of At3G23100 vs. At1G61410 (blastn function). Sequence in red denotes the region homologous to *N. benthamiana XRCC4* gene sequence used for VIGS.

(D) Arabidopsis *XRCC4* (At3G23100) sequence used for RNAi. Stop codon is indicated in red. The 3' UTR sequence is shown in blue.

A

B
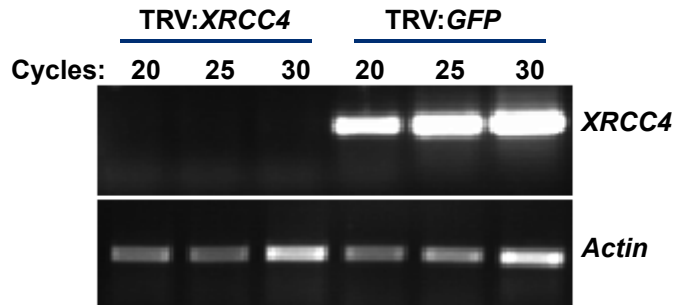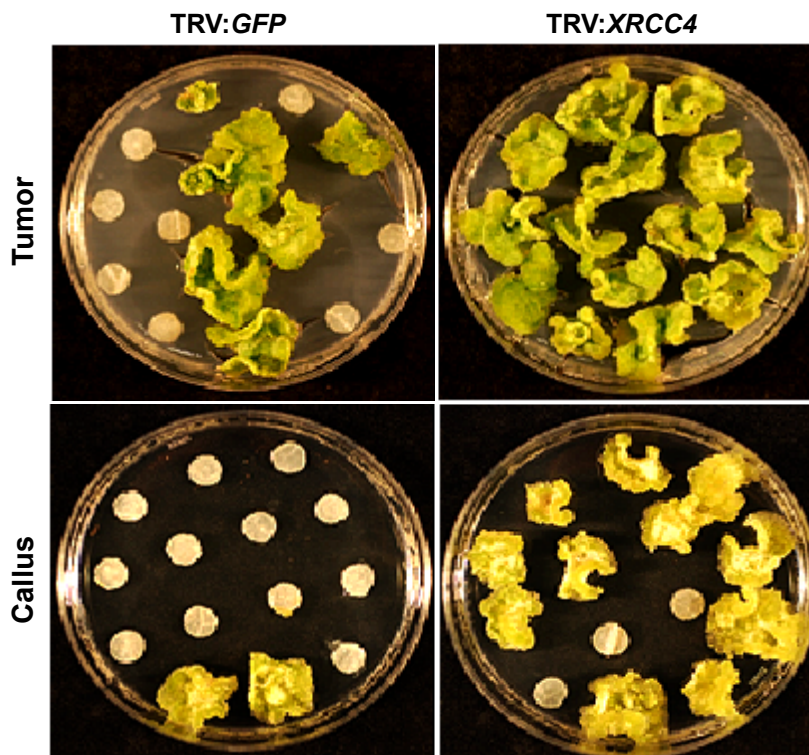
Supplemental Figure 2. Effect of *XRCC4* Down-regulation on Stable Transformation in *Nicotiana benthamiana*
(A) RT-PCR showing VIGS-mediated reduction of *XRCC4* transcript in silenced plants. *Actin* was used as the loading control.
(B) Representative plates from tumor and callus stable transformation assays performed on leaf discs obtained from TRV:*XRCC4*-silenced and TRV:*GFP* infected control plants. Leaf discs were inoculated with either tumorigenic *A. tumefaciens* strain A348 (tumor assay) or disarmed *A. tumefaciens* GV2260 harboring *pCAS1* plasmid (callus assay). Photographs were taken after 30 days.
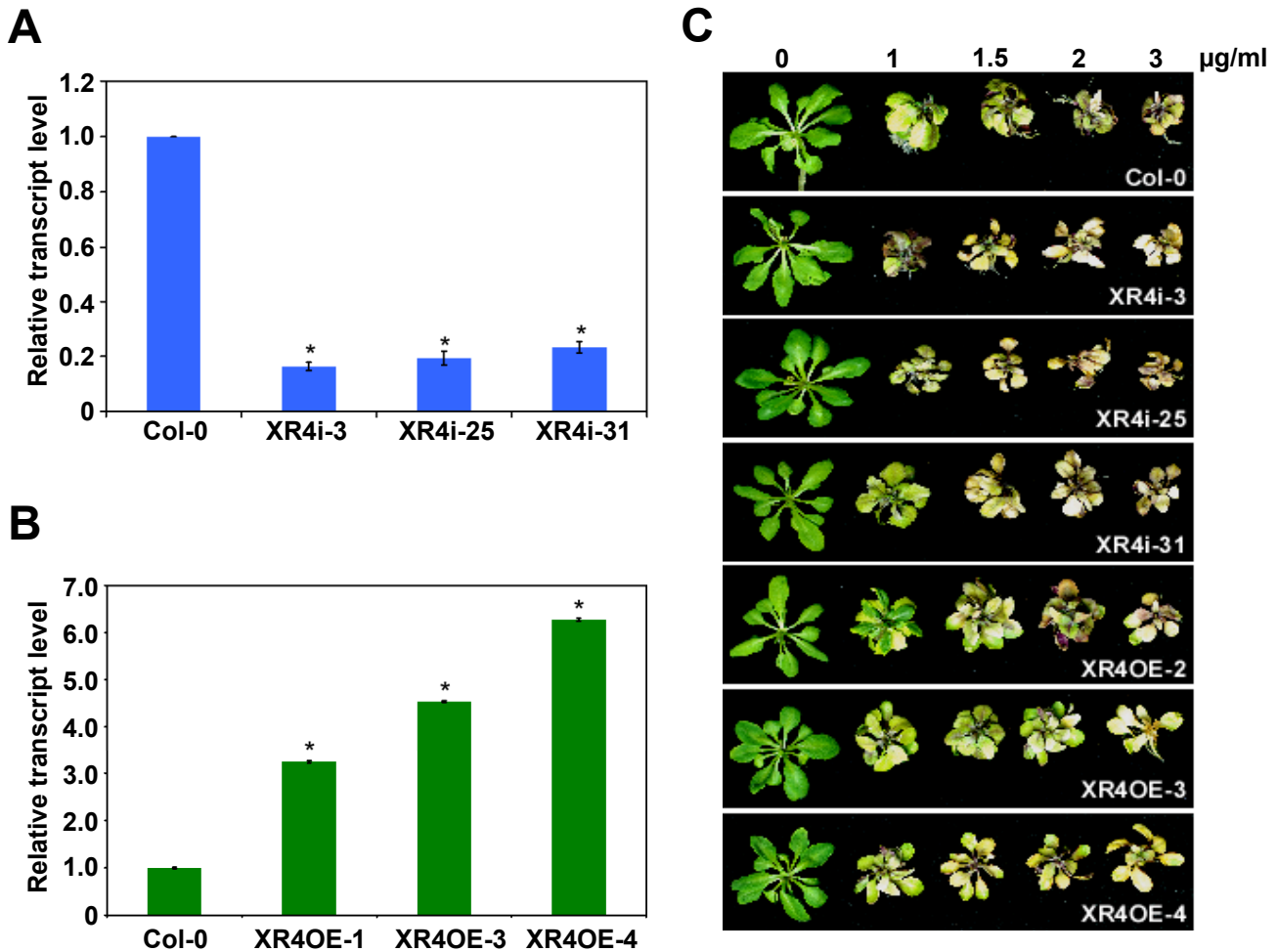
Supplemental Figure 3. Estimation of Arabidopsis *XRCC4* Transcript Level by Quantitative RT-PCR in RNAi and Over-Expression Lines and Sensitivity of these Lines to Bleomycin

(A) RNAi-mediated down-regulation of *XRCC4* gene expression in T_0 *XRCC4* RNAi (XR4i) events. The data represent the average of three technical replicates with SD values shown as error bars. Asterisks denote significant difference in the *XRCC4* transcript level between RNAi lines versus Col-0 as determined by student's t-test ($p < 0.05$).

(B) Over-expression (OE) of *XRCC4* in selected transgenic lines (T_0) as determined by Quantitative RT-PCR. *EF1-α* amplification was used as internal control. Asterisks denote significant difference in the *XRCC4* transcript level between over-expression lines versus Col-0 as determined by student's t-test ($p < 0.05$). Error bars denote standard deviation of three technical repeats in each of the treatments.

(C) Response of representative samples of Col-0, *XRCC4* RNAi (XR4i) and over-expression (XR4OE) lines after exposure to bleomycin at noted concentrations. Photographs were taken at 25 days after exposure.

A

B
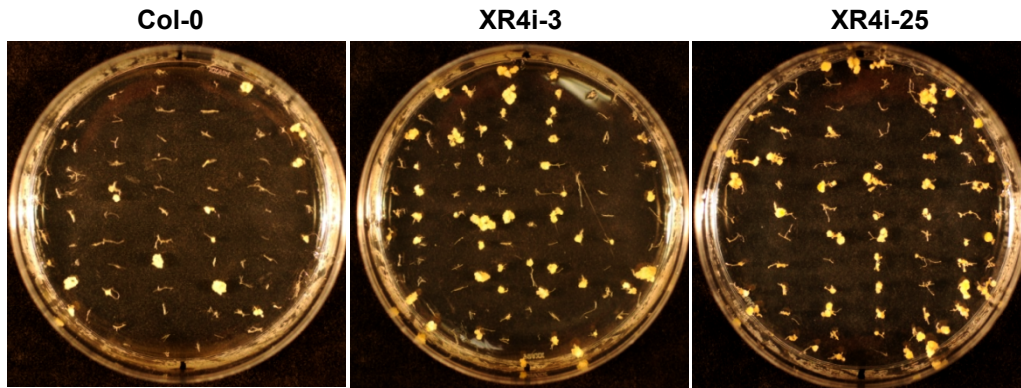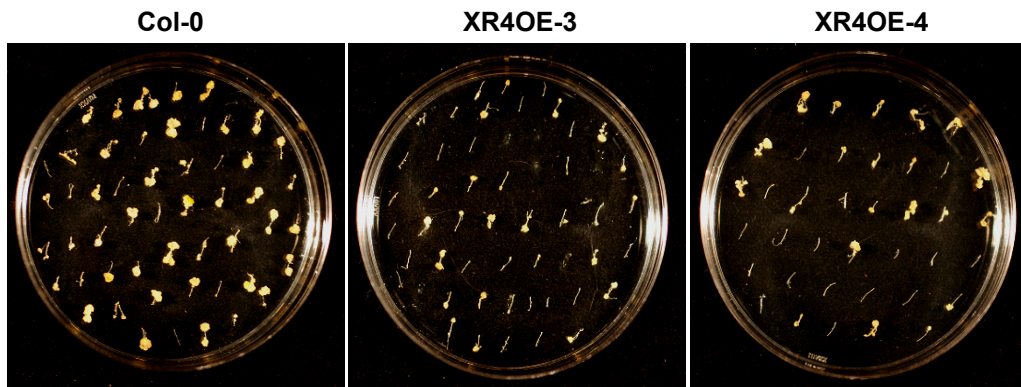
Supplemental Figure 4. Root Callus Assay to Determine the Effect of Arabidopsis *XRCC4* Down-Regulation and Over-Expression on Stable Transformation

(A) Representative plates of callus transformation assay from Col-0 versus *XRCC4* RNAi lines. Root segments of wild-type Col-0 and *XRCC4* RNAi (XR4i) were infected with disarmed A. tumefaciens strain GV3101 harboring the binary vector *pCAS1* that contains bar gene as plant selection marker. Phosphinothricin-resistant calli developing from root segments were visualized and scored four weeks after infection.

(B) Root callus assay on Col-0 and *XRCC4* over-expression (XR4OE) lines. Data on formation of phosphinothricin-resistant calli was recorded and photograph was taken four weeks after infection.
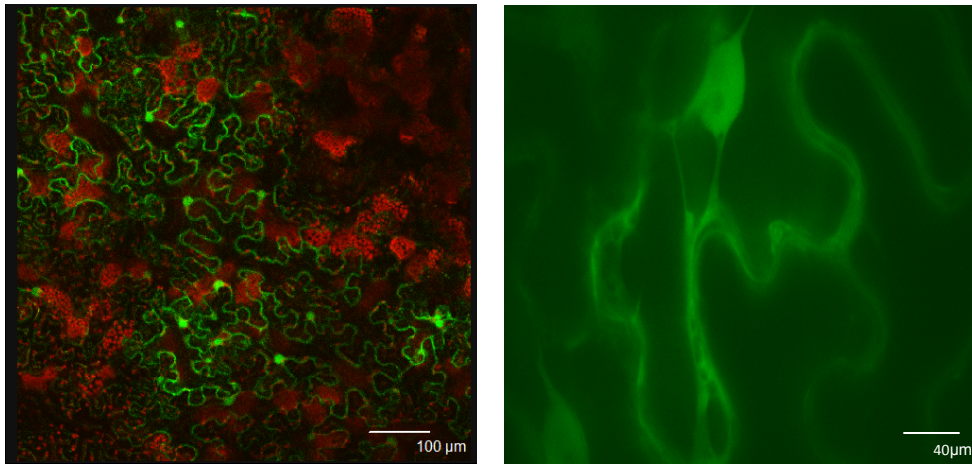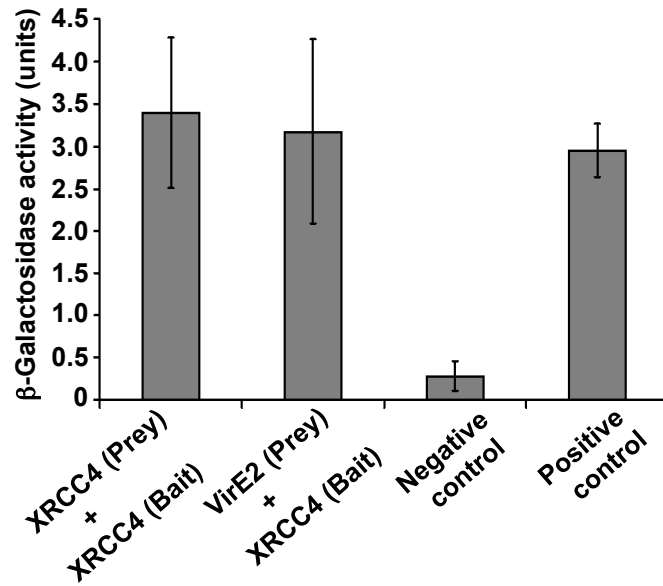
Supplemental Figure 5. Localization of Arabidopsis XRCC4
Nucleo-cytoplasmic localization pattern of *GFP-XRCC4* fusion observed in agroinfiltrated *N. benthamiana* leaves 48 hr post- infiltration. Pictures were taken at 20X view (left panel) and 60X view (right panel).
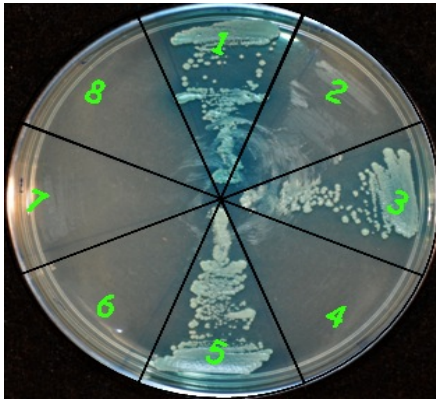
1-XRCC4 (Bait) + XRCC4 (Prey)
2- XRCC4 (bait) only
3- XRCC4 (Bait) + VirE2 (Prey)
4- VirE2 (prey) only
5- positive control
6 -negative control
7- XRCC4 (bait) + Rec7 empty
8- VirE2 (prey) + LamC

Supplemental Figure 6. Interaction of Arabidopsis XRCC4 with *Agrobacterium* VirE2 protein
Yeast two-hybrid assay showing XRCC4 dimerization and interaction with VirE2 (left panel) and quantification of interaction using beta-galactosidase activity assay (right panel). Error bars indicate standard deviation among three biological and three technical replicates.
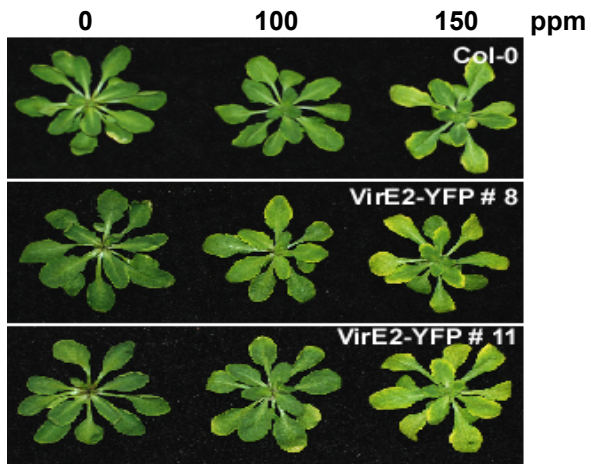
Supplemental Figure 7. Methyl methanesulfonate (MMS) sensitivity assay for *VirE2-YFP* expressing lines.
One-week-old healthy seedlings of Col-0 and *VirE2-YFP* expressing lines were transferred to MS medium containing MMS at 100 and 150 ppm. Photographs were taken after 21 days.

Supplemental Table 1. List of Primers Used in VIGS and Arabidopsis Studies

Gene	Forward Primer (5′- 3′)	Reverse Primer (5′- 3′)
Nb-*XRCC4**	ggggacaagtttgtacaaaaaagcaggctCACTCCATTGCGGTGGCTGCCGCT	ggggaccactttgtacaagaaagctgggtAGTGCCAACATCATCATTCTCCAC
At-*XRCC4* RNAi*	ggggacaagtttgtacaaaaaagcaggctGAACTGATGATGAGAAGAGCGAGGA	ggggaccactttgtacaagaaagctgggtCCATCTCTAAGTTATTGCATTTAC
At-*XRCC4* OE*	ggggacaagtttgtacaaaaaagcaggctATGATCGGAGTTGACTCAAAATCT	ggggaccactttgtacaagaaagctgggtCCATCTCTAAGTTATTGCATTTAC
At-*XRCC4* Realtime	GGCACTTGGCATAATTCTCGTT	TCGCATTGCAAATCCAAGAGGA

* The sequences in lower case correspond to the attB1 and attB2 adapters at 5' end
of the forward and reverse primers, respectively for GATEWAY cloning.