

Figure S1. Physcomitrella genome encodes homologs of ERCC1 and XPF. The dendrograms illustrate the sequence relationships between several XPF (a), ERCC1 (b), MUS81 (c) and EME1 (d) proteins within the XPF super family of structure specific endonucleases. The branch lengths are proportional to the sequence divergence.

Accession numbers used in this analysis are, for *Physcomitrella patens*: PpXPF (XP_024403241), PpERCC1 (XP_024378954), PpMUS81 (XP_024398365), PpEME1 (XP_024376616), for *Arabidopsis thaliana*: AthXPF (NP_001031991), AthERCC1 (NP_187172), AthMUS81 (NP_194816), AthEME1a (NP_001189572), AthEME1b (NP_179804), for *Oryza sativa*: OsUVH1 (XP_015630768), OsERCC1 (XP_015612964), OsMUS81 (XP_015622535), OsEME1 (XP_015637261), for *Amborella trichopoda*: AtrXPF (XP_020519892), AtrERCC1 (XP_020525873), AtrMUS81 (XP_020522223), AtrEME1 (XP_020530544), for *Micromonas pusilla*: MpXPF (XP_003058408), MpERCC1 (XP_003056392), MpMUS81 (XP_003055357), MpEME1 (XP_003060706), for *Drosophila melanogaster*: DmMEI9 (AAC46917), DmERCC1 (NP_477468), DmMUS81 (NP_569873), DmEME1(), for *Homo sapiens*: HsXPF (NP_005227), HsERCC1 (NP_001974), HsMUS81 (AAL28065), HsEME1 (NP_689676), HsEME2 (NP_001244299), for *Saccharomyces cerevisiae*: ScRad1 (AJW05769), ScRad10 (NP_013614), ScMus81 (EDN60717), ScMms4 (CBK39173), for *Schizosaccharomyces pombe*: SpRad16 (NP_587855), ScSwi10 (NP_596115), SpMus81 (NP_001343019), SpEme1 (NP_594132).

SpombeRad16	VNTRIAGGGQLSITNEKPRVIVDLREFRSSLPSILHGNNFVIPCQLLVGDYILSPKICV	693
HomoXPF	TDTRKAGG--QEONGTQOSIVVDMREFRSELP SLIHRRGIDIEPVTLEVGDYILTPEMCV	724
PhyscoXPF	LDTRKGVG--RKQAQKQM VVDMREFGSSLP SVLHQGMKILPVTLEVGDYILSPDICV	857
AthalXPF	SLTRKAGG--RKELEKETQVIVDMREFMSSLPNVLHQGMKII PVTLEVGDYILSPSICV	766
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SpombeRad16	ERKSIRD LIQSLNNGRLYSQCEAMTEYYEIPVLLIEFEQHQSF TSPFSDLSSEIGKNDV	753
HomoXPF	ERKSISD LIGSLNNGRLYSQCISMSRYYKRPVLLIEFDPSKPFSLTSRGALFQEISSNDI	784
PhyscoXPF	ERKSIAD LFSFSSGRLYHQAE TMSRYYKYPVLLIEFSQDKSFSLQAASDIGEDIAPANI	917
AthalXPF	ERKSIQD LFQSFTSGR LHFQVEMMSRYRIPVLLIEFSQDKSFSFQSSDISDDVTPYNI	826
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SpombeRad16	QSKLVLLT LSFNLRIVWSSAYVT SII FQDLKAMEQEPDPASAASIGLEA-----GQD	807
HomoXPF	SSKL TLLTLHF PRLRILWCSPHATAELFEELKQSKPQPDAATALAITADSE-----TLP	839
PhyscoXPF	ISKLSLLVLHF PRLRIVWSRSLHATADIFMALKSNQNEPDLDRAMRVGVPTEDGLIEGDI	977
AthalXPF	ISKLSLLVLHF PRLRLLWSRSLHATAE IFTTLKSNQDEPDETRAIRVGV PSEEGIIENDI	886
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SpombeRad16	STNTYNQAPLDLLMGLPYITMKNYRNVFYGGVKDIQEASET SERKWSSELIGPEA-GRRLY	866
HomoXPF	ESEKYNPGPQDFLLKMPGVNAKNCRSLMH-HVKNIAELAALSQDELTSILGNAANAKQLY	898
PhyscoXPF	RAENFNTTAVELLRRLPGVSDANYRSLMA-GCKSIAEMALLSVDELAELMGGKQPARMLR	1036
AthalXPF	RAENYNTSAVEFLRRLPGVSDANYRSIME-KCKSLAELASLPVETLAELMGGHKVAKSLR	945
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SpombeRad16	SFFRKQLKDYE-----	877
HomoXPF	DFIHTSFAE VVSKGKGKK	916
PhyscoXPF	EFLDAKCPTLV-----	1047
AthalXPF	EFLDAKYPTLL-----	956

Figure S2. Physcomitrella genome encodes an XPF homolog. Clustal Omega alignment of the 250 C terminal amino acids of XPF proteins from *P. patens*, *A. thaliana*, *S. pombe* and *H. sapiens*. The restriction endonuclease type II-like domain or ERCC4 domain ([pfam02732](#) (819-946) and the RuvA 2-like domain (978-1042) or HhH domain (SMART [HHH_5](#) 987-1040) are indicated with green and blue lines, respectively. Green box contains the residues required for nuclease activity. Accession numbers used in this analysis are as follows: PpXPF (P_024403241), AtXPF (NP_001031991), SpRAD16 (NP_587855) and HsXPF (NP_005227).

SpomSWI10	-----	0
HomoERCC1	MD----PGKDKE-----GVPQPSGPPARKKFKVIPLDEDEVPPGVAKPLFRSTQSL	46
PpatERCC1	MAAREVDSQAGN-----GMKSFHLHIPSHAEVLEERSRP-QAPPSFFRPSSSAAPPV	49
AthalERCC1	MANEDDDGKSRSLHQIARKPKTQIVIGVPSYQEVLESSQTKSTPPSLFKP-----	52
SpomSWI10	-----MSDIDDEEF-----	9
HomoERCC1	-----PTVDTSA-----Q---AAPQT-	59
PpatERCC1	EAVGAASVSSASQQQQSLGRAGVPGSFGAEFSFVRQTEFYTPPPAAPTPTVPVAPSEG	109
AthalERCC1	-----SQSFSQAFAFVKSSDVYSPPPSSAAASSSQPSGA	87
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SpomSWI10	-----EQLAVSALEEVEKKAGFAQQPTPQKVSRTAHSILVNPRQKGNPLLPHV	58
HomoERCC1	-YAEYAI SQPLEGAGATCPTGSEPLA--GETPNQALKPGAKSNSIIVSPRQRGNPVLKQV	116
PpatERCC1	-----VPGNGVG-----SRS----QEALPTVNSQARNAILVNRRTGNPVLKQI	149
AthalERCC1	SQVPHSSSQTHQTDGASS--STPVA----TGSVPSNTTQNRNAILVSHRQKGNPLPKHI	141
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SpomSWI10	RNVPEWYTDIVPDFVMGTGICSLFSLKYHHLHPEYIYSRISKLGKSYNLRILLIIVDVE	118
HomoERCC1	RNVPEWFGDVI PDYVLGQSTCALFSLRYHNLHPDIHGRLQSLGKNFALRVLLVQVDVK	176
PpatERCC1	RNVRVWFGDIVPDYLLGQSTCALYISLRYHLLHPDYLYFRIRELQKGFRLRVVLCHVDLE	209
AthalERCC1	RNVKWFVSDIIPDYVLGQNSCALYLSLRYHLLHPDYLYFRIRELQKNFKLSVVLCHVDVE	201
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SpomSWI10	NHQASIQELVKTSIVNQYTLILAWSSEEAAARYLETYKAYENMSPALIMEKPKSTDYLSQVQ	178
HomoERCC1	DPQQALKEKELAKMCI LADCTLILAWSPEEAGRYLETYKAYEQKPADLLMEKLEQDFVSRVT	236
PpatERCC1	DVIKPLHEVIKTSLLHDCSLLCAWSLEECARYLET LKTYEHKPADNIQERTDNDYISRMT	269
AthalERCC1	DTVKPLLEVTKTALLHDCTLLCAWSMTECARYLETIKVYENKPADLIQGMDDTDYLSRLN	261
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SpomSWI10	SFLTSIRGINKSDSLSLLSKFGSTERALVASRDELEQLEGWGPVKVNFLEAVQQPFMSH	238
HomoERCC1	ECLTIVKSVNKTDSQTLTTFGSLAQLIAASREDLALCPGIGPQKARFI FFDVLHEPFLKV	296
PpatERCC1	SALTTVRHVNKTDVLTGSTFETLTGIFASMEDLARCPIGERKVKFIYDAFHEPFRRRT	329
AthalERCC1	HSLTSIRHVNKSDVVTGSTFGSLAHIIDASMEDLARCPIGERKVKFIYDTFHEPFKRA	321
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SpomSWI10	STIKRPEAINLKQT-----	252
HomoERCC1	P-----	297
PpatERCC1	TKQLQLDNMLTKVSQSVTAES--NTTLDSSTMQIDTVDTAVGASEPNADIPELSVRQALN	387
AthalERCC1	TSSYPSVVEPPIPEAPVEKDVNSEEPVEED---EDFVED-SRKRKKKEPEPEKTVKTALS	377
SpomSWI10	-----	252
HomoERCC1	-----	297
PpatERCC1	TAYAKVVERRRGSRTETSMKDMSTDCRHTPLVNVEVIDIT	427
AthalERCC1	AVFARYSDRLSKKKEKQKEKDTTASDAETHQN-----	410

Figure S3. Physcomitrella genome encodes an ERCC1 homolog. Clustal Omega alignment of ERCC1 sequences from moss (Ppat), Arabidopsis (Athal), Human (homo) and fission yeast (Spom). Restriction endonuclease type II-like domain (132-257), RuvA 2-like domain (259-328) and Helix-hairpin-helix DNA-binding motif (305-321) are indicated with green, blue and red lines respectively. Hydrophobic amino acids critical for the formation of the HhH motifs are indicated with brown boxes. Accession numbers used in this analysis are as follows: PpatERCC1 (XP_024378954), AtERCC1 (NP_187172), Spombe SWI10 (NP_596115) and HsERCC1 (NP_005227).

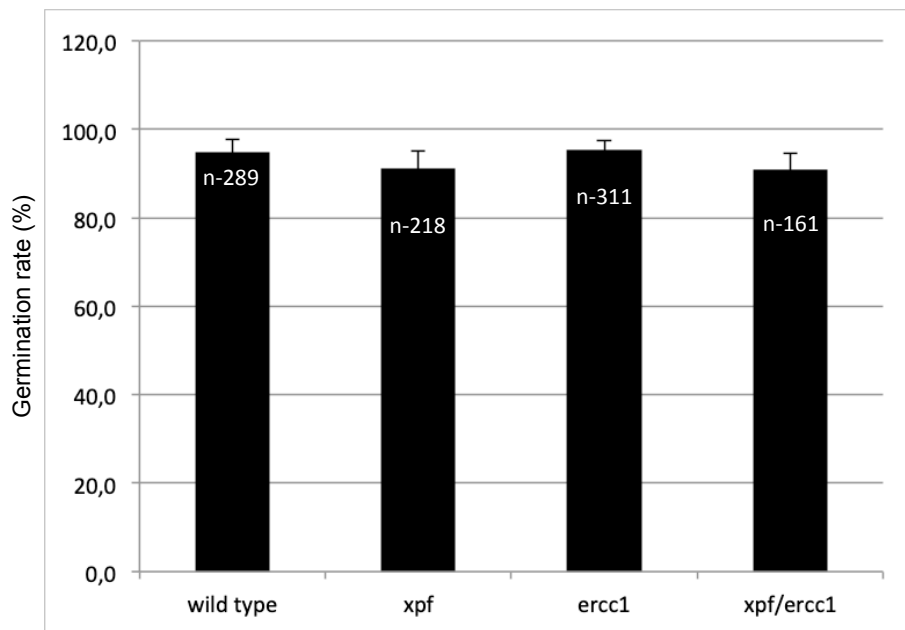


Figure S4. Germination rate of wild type and *xpf*, *ercc1* and *xpf/ercc1* mutants spores. Freshly harvested spores from wild type and *xpf*, *ercc1* and *xpf/ercc1* capsules were sown on PpNH4 medium. Percentages of germination were calculated three days after sowing. Error bars indicate SD based on three independent experiments.

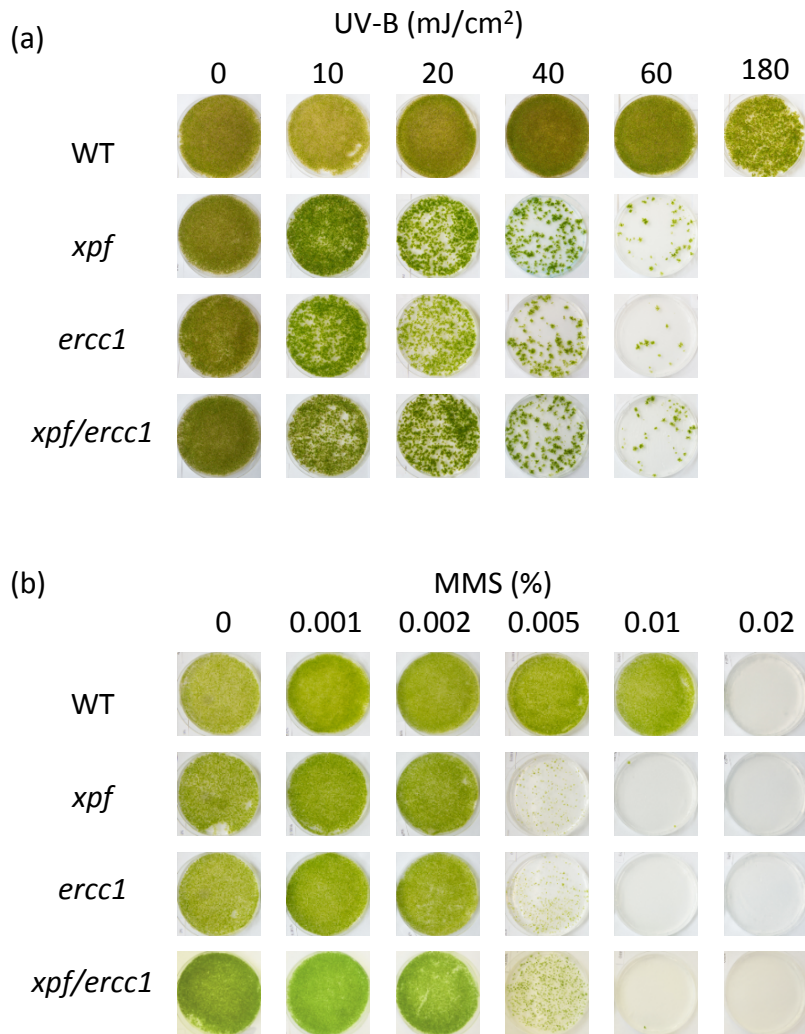


Figure S5. Sensitivity of the wild-type (WT) and of the *xpf*, *ercc1* and *xpf/ercc1* mutants towards genotoxic agents. 3 weeks old plates of WT and the 3 mutants exposed to low doses of UV-B light B (a) or to increasing doses of MMS (b).

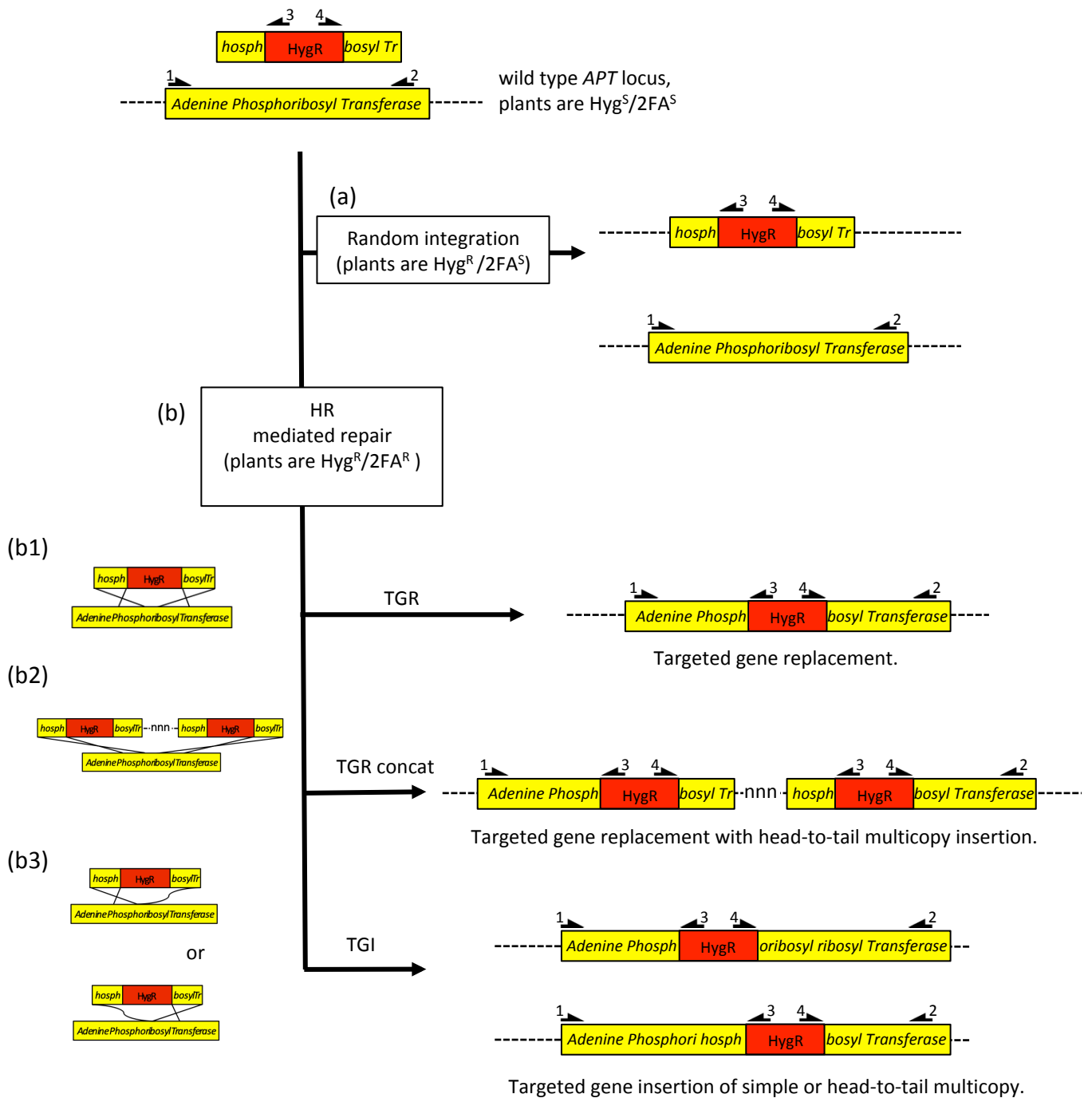


Figure S6. Different outputs of repair of a CRISPR-Cas9 induced DSB in presence of a donor template. Integration of the Donor DNA can be random (a) and in this case plants are resistant to hygromycin but sensitive to 2FA, or via gene targeting (b). As targeting of the *APT* gene leads to resistance to 2FA, selection of hygromycin resistant plants on this compound will permit selection of clones that experienced an HR mediated integration of the donor DNA at the targeted locus. HR mediated integration of the donor template can lead to TGR (b1), TGR with concatemers (b2) or TGI (b3). For a given 2FA and Hygromycin resistant clone, analysis of PCR products 1+3 and 4+2 will permit to determine whether it results from a TGR or TGI integration. Furthermore analysis of PCR products 1+2 will permit to determine whether it is or it is not a monocopy insertions. In this study primers 1, 2, 3 and 4 correspond to PpAPT#2, PpAPT#20, ProRev and TerFwd respectively (Table S1).