Title: The role of rice SOG1 and SOG1-like in DNA damage response

Short title: The role of rice SOG1 and SGL in DDR

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One sentence summary (200 characters max):

Rice transcription factor SUPPRESSOR OF GAMMA RESPONSE 1 controls DNA damage response and DNA repair through activation via phosphorylation and the direct regulation of expression of numerous genes.

22 **Abstract (250 word max)**

23 Higher plants are constantly exposed to environmental stresses, and therefore
 24 complicated defense systems, including DNA damage response (DDR) and DNA repair
 25 systems, have developed to protect plant cells. In *Arabidopsis*, the transcription factor
 26 SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1) has been reported to play a key
 27 role in DDR. Here, we focus on DDR in rice—thought to be a simpler system compared
 28 with *Arabidopsis* due to lack of induction of endocycle even under DNA damage stress.
 29 Rice SOG1 (OsSOG1) and SOG1-like (OsSGL) were identified as putative AtSOG1
 30 orthologs with complete or partial conservation of the serine-glutamine (SQ) motifs
 31 involved in activation via phosphorylation. In addition to OsSOG1- or OsSGL-knockout
 32 mutants, OsSOG1 non-phosphorylatable mutants (OsSOG1-7A) were generated by
 33 homologous recombination-mediated gene targeting. Based on DNA damage
 34 susceptibility and transcriptome analysis using these mutants, we demonstrated that
 35 OsSOG1, but not OsSGL, plays a central role in the DDR and DNA repair. OsSOG1
 36 regulated target genes via CTT (N)₇ AAG motifs reported previously as AtSOG1
 37 recognition sites. The loss of transcription activities and DNA damage tolerance of
 38 OsSOG1-7A was not complete compared with OsSOG1-knockout mutants, raising the
 39 possibility that another phosphorylation site might be involved in the activation of
 40 OsSOG1. Furthermore, our findings have highlighted differences in SOG1-mediated
 41 DDR between rice and *Arabidopsis*, especially regarding induction of cell-cycle arrest
 42 and endocycle arrest, revealing rice-specific DDR mechanisms.

43 **Introduction**

44 The sessile nature of higher plants results in constant exposure to
 45 environmental stresses; therefore, complex defense systems and/or signaling networks
 46 have been developed to protect cells from damage in response to stress. DNA damage
 47 response (DDR) and repair systems are among the most important defenses against
 48 environmental stresses such as ultraviolet B irradiation, drought, salt, heat, and other
 49 pollutants, e.g., metals. Reactive oxygen species (ROS) generated in response to
 50 environmental stresses alter bases and damage sugar residues, resulting in DNA
 51 fragmentation and strand breaks (Roldán-Arjona and Ariza, 2009). DNA double-strand
 52 breaks (DSBs) are the most severe type of damage because they lead to cell death if not
 53 repaired. DSBs are repaired by two distinct pathways: non-homologous end-joining
 54 (NHEJ), which simply ligates DNA ends, and homologous recombination (HR), which
 55 repairs DSBs precisely by copying a donor DNA molecule. The mechanisms of DDR
 56 have been well studied in mammalian cells. According to previous studies, the tumor
 57 suppressor protein p53—the “guardian of the genome”—plays a pivotal role in a variety
 58 of DDRs, including the promotion of cell cycle arrest, the induction of apoptosis, and
 59 regulating DNA repair (Ventura et al., 2007; Reinhardt and Schumacher, 2012).
 60 However, p53 orthologues are not conserved in plants (Yoshiyama et al., 2013), and
 61 DDR in plant cells remains poorly understood, except in *Arabidopsis*.

62 In *Arabidopsis*, SUPPRESSOR OF GAMMA RESPONSE 1 (SOG1), an NAC
 63 (No apical meristem, Arabidopsis transcription activation factor, Cup-shaped cotyledon)
 64 transcription factor, has been reported to regulate the expression of numerous genes
 65 relating to DNA repair, cell cycle arrest and stem cell death under DNA damage
 66 conditions. Thus, SOG1 is thought to play a key role in DDR and to be a counterpart of

67 mammalian p53 (Yoshiyama et al., 2009, 2013; Ogita et al., 2018). Similarly to p53,
68 *Arabidopsis* SOG1 (AtSOG1) is activated via phosphorylation of a serine-glutamine
69 (SQ) motif in the C-terminal region of AtSOG1 by the cellular kinases ATM
70 (ATAXIA-TELANGIECTASIA MUTATED) and ATR (ATM AND RAD3-RELATED),
71 which are involved in the sensing of DNA lesions upon treatment with DSB-inducing
72 agents (Yoshiyama et al., 2013, 2017; Sjogren et al., 2015; Yoshiyama and Kimura,
73 2018). Predicted SOG1 ortholog proteins with conserved SQ motif phosphorylated by
74 ATM and ATR are found in most land plants, from moss to angiosperm (Yoshiyama et
75 al., 2014). Wei et al. (2021) reported recently that *Arabidopsis* casein kinase (CK) 2
76 modulated AtSOG1 activity through its phosphorylation under DNA damage conditions,
77 and CK2 phosphorylation sites ([ST]XX[ED]) are conserved among putatively
78 orthologous proteins in several plant species (Wei et al., 2021). Moreover, the same
79 study also demonstrated that inhibition of CK2-dependent phosphorylation of AtSOG1
80 interfered with its phosphorylation by ATM/ATR following DNA damage (Wei et al.,
81 2021). Several recent studies have reported that SOG1 is also involved in DDR derived
82 from oxidative stress including aluminium- (Sjogren et al., 2015) and cadmium-
83 (Hendrix et al., 2020) exposure or biotic stress (Ogita et al., 2018) in *Arabidopsis*. These
84 findings suggest that *Arabidopsis* SOG1 regulates several biological processes in
85 response to the DNA damage that occurs under various environmental stress conditions
86 via its phosphorylation by ATM/ATR or CK. However, there have been hardly any
87 studies on either the transcriptional regulatory or activation mechanisms via
88 phosphorylation of SOG1 in response to DNA damage in plant species other than
89 *Arabidopsis*.

90 Even though rice is one of the most agronomically important crops, the

91 mechanisms of DDR in rice remain to be understood. Unlike in *Arabidopsis*, DDR in
 92 rice is thought to be simple, because the process of endocycle is not found in rice other
 93 than during endosperm development, even under stress conditions (Endo et al., 2012).
 94 Thus, it is expected that rice can safeguard genome integrity against environmental
 95 stresses by the repair of damaged DNA or by the induction of cell death. These
 96 differences raise interesting questions about DDR and the function of SOG1 in rice. In
 97 addition, genome editing techniques, not only clustered regularly interspaced short
 98 palindromic repeats (CRISPR)/CRISPR-associated 9 (Cas9)-mediated targeted
 99 mutagenesis for producing loss-of-function mutants by the induction of insertion or
 100 deletion mutations at CRISPR target site, but also HR-mediated gene targeting (GT) for
 101 producing gain-of-function mutants by the introduction of point mutations or the
 102 insertion of reporter gene at the desired locus, are now well-developed and make it
 103 possible to analyze precisely a characteristic feature of a target gene in rice.

104 We have identified rice SOG1 (OsSOG1, Os06g0267500), which carries the
 105 five highly conserved SQ motifs of AtSOG1, and SOG1-like (OsSGL, Os02g0594800),
 106 which partially retains SQ (or threonine-glutamine, TQ) motifs from the rice genome,
 107 by a homology search of public databases (**Supplementary Figure 1**). In *Arabidopsis*,
 108 ANAC044 and ANAC085 are identified as the closest homologs of AtSOG1, but lack
 109 SQ motifs (Takahashi et al., 2019). A total of 151 NAC genes are predicted to be present
 110 in the rice genome (Nuruzzaman et al., 2010). OsSOG1 and OsSGL were designated as
 111 ONAC091 and ONAC050, respectively, in the Rice Annotation Project (RAP) database
 112 (<https://rapdb.dna.affrc.go.jp/index.html>). However, no previous functional analyses of
 113 these transcription factors have been reported. We generated OsSOG1- and
 114 OsSGL-knockout rice plants by GT and also produced OsSOG1/OsSGL double

115 knockout mutants by CRISPR/Cas9-mediated targeted mutagenesis. In addition to these
116 knockout mutants, non-phosphorylatable and phosphomimetic OsSOG1 mutants
117 substituting the serine residues for alanine or aspartic acid, respectively, at all SQ motifs
118 of *OsSOG1* gene were generated by GT to investigate the importance of the SQ motifs
119 of OsSOG1 in DDR. These mutants, generated via GT, harbored a modified OsSOG1
120 gene with substitutions of phosphorylation sites at its endogenous locus, thereby
121 allowing the activation mechanisms via phosphorylation of OsSOG1 in response to
122 DNA damage to be assessed precisely without being affected by position effect
123 variegation.

124 In this report, we evaluated DNA damage susceptibility and transcriptome
125 analysis using a single knockout mutant of OsSOG1 or OsSGL, a double knockout
126 mutant of OsSOG1 and OsSGL, and a non-phosphorylatable mutant of OsSOG1.
127 Unfortunately, we were unable to obtain OsSOG1 phosphomimetic mutants by GT.
128 OsSOG1 was demonstrated to be a pivotal factor regulating the transcription of genes
129 involved in DNA repair and cell cycle processes through a specific motif reported
130 previously as the SOG1-binding domain in *Arabidopsis*. Based on these results, we
131 report that OsSOG1, but not OsSGL, plays a central role not only in DDR and DNA
132 repair but also in the oxidative stress response in rice. Although SQ motifs of OsSOG1
133 were important in the function of OsSOG1 in DDR, our findings suggest the existence
134 of another pathway (e.g., via CK) in the activation of OsSOG1 during DNA damage.

135 In addition, our findings suggest that monocotyledonous rice might overcome
136 DNA damage using different mechanisms from dicotyledonous *Arabidopsis*, e.g.
137 cell-cycle arrest. Knowledge gained from our studies could help elucidate mechanisms
138 of DDR in rice—one of our most important agricultural crops—and has significant

139 implications with respect to the development of genome editing techniques that exploit

140 DNA repair pathways for crops.

141

142 **Results**

143 **Generation of OsSOG1 and OsSGL mutant rice by HR-mediated GT and/or** 144 **CRISPR/Cas9-mediated mutagenesis**

145 To investigate the function of OsSOG1 and OsSOG1-like (OsSGL) in DDR
146 and DNA repair, we generated several types of OsSOG1 and OsSGL mutant rice plants.
147 OsSOG1 promoter:: β -glucuronidase (Psog1-GUS) and OsSGL promoter::GUS
148 (Psgl-GUS) knock-in (simultaneous knock-out of OsSOG1 and OsSGL, respectively)
149 rice mutants were generated by the introduction of a GUS::terminator construct
150 in-frame with the ATG codon of *OsSOG1* or *OsSGL* by HR-mediated GT with
151 positive-negative selection (Terada et al., 2002; Nishizawa-Yokoi et al., 2015) (**Figure**
152 **1A and Figure S2A**). Two independent lines each of Psog1-GUS (#1 and #2) and
153 Psgl-GUS (#1 and #2) were obtained, and their homozygous mutant plants in the T₂ or
154 T₃ generation were subjected to further analysis (**Figures 1 B, C and S2B, C**). No
155 full-length transcripts of OsSOG1 and OsSGL were detected in Psog1-GUS and
156 Psgl-GUS homozygous mutant plants, respectively, by reverse transcriptase-PCR
157 (RT-PCR) (**Figure S3**).

158 A previous study demonstrated that ATM-mediated phosphorylation of the SQ
159 motif in the C-terminal region of AtSOG1 plays a pivotal role in DDR in Arabidopsis
160 (Yoshiyama et al., 2013). Yoshiyama et al. (2017) also revealed that the extent of SQ
161 phosphorylation in SOG1 regulates gene expression levels and determines the strength
162 of DDR (Yoshiyama et al., 2017). Therefore, we substituted the serine residues with
163 alanine (non-phosphorylatable mutations) at all seven SQ motifs of the *OsSOG1* gene
164 via GT with positive-negative selection and subsequent marker-excision by *piggyBac*
165 transposition (Nishizawa-Yokoi et al., 2015) (**Figure 2A**). These mutant lines were

166 called mutants OsSOG1-7A. After marker excision, seven amino acid substitutions
 167 (S360A, S316A, S322A, S338A, S340A, S398A, and S403A) and a silent mutation for
 168 the production of a *HpaI* restriction enzyme recognition site used for the insertion of
 169 *piggyBac* transposon with the positive selection marker were left in the *OsSOG1* gene
 170 locus (**Figure 2B**). The *piggyBac* transposase expression cassette was transformed into
 171 a GT callus line for marker excision from the *OsSOG1* locus; thus, T₁ progeny plants
 172 with the desired mutations in the *OsSOG1* locus in the homozygous state and without
 173 transgene were selected by PCR. Their homozygous mutant progenies in the T₂ or T₃
 174 generation were subjected to further analysis (**Figure 2C**). RT-PCR revealed transcript
 175 levels of the *OsSOG1* gene in OsSOG1-7A mutants to be comparable with those of
 176 wild-type (**Figure S3**). We also attempted to introduce substitution of the serine residues
 177 to aspartic acid (phosphomimetic mutations) at all seven SQ motifs of OsSOG1 by
 178 CRISPR/Cas9-mediated GT in rice (Nishizawa-Yokoi et al., 2020).
 179 Hygromycin-resistant callus lines propagated clonally from calli transformed with GT
 180 vector were subjected to cleaved amplified polymorphic sequence (CAPS) screening for
 181 isolation of GT calli (**Figure S4A**), since the additional silent mutation was introduced
 182 together with amino acid substitutions into the 5th exon of *OsSOG1* via GT to produce a
 183 *PstI* restriction enzyme site (**Figure S4B**). Among the total of 2,767
 184 hygromycin-resistant callus lines analyzed, no CAPS positive callus lines were
 185 obtained.

186 The *OsSOG1* and *OsSGL* double mutant rice (termed *sog1sgl* mutant) were
 187 generated by CRISPR/Cas9-mediated mutagenesis to target the fourth exon of the
 188 *OsSOG1* and *OsSGL* genes using a gene-specific guide RNA (gRNA) (**Figure 3 A–C**).
 189 We selected two independent lines, *sog1sgl* #1, which contained a homozygous 25 bp

190 deletion and homozygous 1 bp insertion in the *OsSOG1* and *OsSGL* genes, respectively,
191 and #2, which carried a homozygous 1 bp deletion and homozygous 1 bp insertion in
192 the *OsSOG1* and *OsSGL* genes, respectively, in the T₁ generation (**Figures 3D and 3E**).
193 These frameshift mutations in *sog1sgl* #1 and #2 mutants led to an early stop codon
194 prior to the conserved SQ motifs in the C-terminal region of *OsSOG1* and *OsSGL* genes
195 (**Figures 3D,E and S1**). In *sog1sgl* #1, CRISPR/Cas9-mediated mutations at the
196 *OsSOG1* gene did not affect transcript levels, while full-length transcripts of *OsSGL*
197 were barely detectable by RT-PCR, probably due to nonsense-mediated mRNA decay
198 (**Figure S3**). In addition, we confirmed by PCR analysis that these mutants lacked
199 T-DNA. Their T₂ or T₃ progenies were subjected to further analysis.

200

201 **OsSOG1 mutant and *sog1sgl* double mutant plants, but not OsSGL mutants, are** 202 **highly sensitive to DNA damage agents**

203 To elucidate the role of OsSOG1 and OsSGL in DDR and DNA repair in rice,
204 we assessed the sensitivity of OsSOG1 and OsSGL mutants to the DNA damage agent
205 bleomycin. Seeds of Psog1-GUS, Psgl-GUS, OsSOG1-7A mutants, and *sog1sgl* double
206 mutants were sown on medium containing various concentrations of bleomycin and
207 cultured for 1 week. Compared with that of wild-type plants, root growth of Psog1-GUS
208 mutants was significantly inhibited by 10 μM bleomycin treatment (**Figure 4A and 4B**).
209 However, the sensitivity of Psgl-GUS mutant plants was comparable with that of
210 wild-type plants even under treatment with high concentration bleomycin. Although
211 there was no statistically significant difference between the DNA damage susceptibility
212 of OsSOG1-7A mutants and wild-type plants under treatment with 10 μM bleomycin,
213 OsSOG1-7A mutants appeared to be more sensitive to DNA damage than wild-type or

214 Psgl-GUS mutant plants. Inhibition of root growth of *sog1sgl* double mutants was
215 observed at low concentrations of bleomycin, indicating that *sog1sgl* double mutants
216 revealed the highest sensitivity among mutant plants used in this study. These results
217 suggest that OsSOG1, but not OsSGL, works in DNA repair and/or DDR in rice, and its
218 functions are partially regulated by ATM- and/or ATR-mediated phosphorylation of SQ
219 motifs.

220

221 **Analysis of DNA damage-response and tissue-specific expression of OsSOG1 and** 222 **OsSGL in rice**

223 Next, we analyzed transcript levels of *OsSOG1* and *OsSGL* genes in wild-type
224 rice plants treated with bleomycin using quantitative RT-PCR (qRT-PCR). Transcript
225 levels of OsSOG1 were barely affected by bleomycin treatment in either roots or shoots
226 (**Figure S5A**) whereas transcript levels of *OsSGL* and *OsRad51A2*—well-known as
227 DNA damage-response genes—increased significantly in response to bleomycin
228 treatment, especially in roots (**Figure S5A**). Consistent with these data, GUS gene
229 expression was detected in root tips of Psgl-GUS mutant plants upon treatment with
230 bleomycin (**Figure S5B,C**). However, Psog1-GUS mutant plants displayed no
231 detectable GUS expression either with or without bleomycin treatment. Based on these
232 results, microarray analysis was performed with roots to evaluate the effect of mutation
233 of OsSOG1 on the expression of genes responsive to DNA damage .

234

235 **Transcriptome analysis in OsSOG1 and OsSGL mutants under treatment with a** 236 **DNA damage agent**

237 To identify target genes regulated by OsSOG1 and/or OsSGL under DNA damage

238 conditions, 1-week-old seedlings of wild-type and Psog1-GUS, Psog1-GUS, OsSOG1-7A
239 mutants, and the *sog1sog1* double mutant were treated with or without 10 μ M bleomycin
240 for 8 h and harvested for microarray analysis. Total RNA extracted from roots (about 2
241 cm area from the root tip) was subjected to microarray analysis. Microarray analysis
242 using wild-type with and without bleomycin treatment showed that the transcript levels
243 of 1,068 probes were induced (**Table S1**) and 608 probes were reduced by more than
244 two-fold ($P < 0.01$) in response to bleomycin treatment (**Table S2**). In Psog1-GUS,
245 OsSOG1-7A and *sog1sog1* mutants, the number of probes upregulated in response to
246 DNA damage was only about half of that in wild-type (**Table 1**). On the other hand,
247 mutation of *OsSGL* was thought not likely to affect gene expression profiles in response
248 to DNA damage.

249 To define the downstream genes of OsSOG1-mediated DDR in rice, we
250 identified the probes upregulated or downregulated more than two-fold in wild-type
251 treated with bleomycin compared with non-treated controls, as well as transcriptional
252 changes reduced to less than half of wild-type in *sog1* mutant plants even under DNA
253 damage conditions. Within the 1,068 bleomycin-induced probes in wild-type, induction
254 of 758 and 738 probes was canceled in both of two independent lines of Psog1-GUS #1
255 and #2, or OsSOG1-7A #1 and #2, respectively (**Table S1**). Among these, 608 probes
256 were consistently not induced in four lines of Psog1-GUS#1, #2, OsSOG1-7A#1, and
257 #2 compared with wild-type (**Figure 5A**). The average fold changes of 60% (362/608)
258 probes were higher in OsSOG1-7A#1, #2 than in Psog1-GUS#1, #2, suggesting the
259 persistence of transcriptional activity of the OsSOG1-7A mutant. The induction of 908
260 probes in response to bleomycin treatment was impaired in the *sog1sog1* mutant (**Figure**
261 **5A**). Loss of the *OsSGL* gene led to a decrease in the 278 probes responsive to DNA

262 damage in Psogl-GUS mutants #1 and #2 (**Table S1 and Figure 5B**). Among 608
263 downregulated probes in response to bleomycin in wild-type, no reduction in the levels
264 of 237, 417, and 192 probes was found in either of two independent lines of Psogl-GUS,
265 OsSOG1-7A, or Psogl-GUS, respectively (**Table S2, Figure 5C, and D**). The
266 downregulated 501 probes in wild-type treatment with bleomycin were not reduced in
267 *sog1sgl* mutant following treatment with bleomycin.

268 The bleomycin-induced 566 and bleomycin-reduced 163 probes in wild-type
269 were consistently not reduced in Psogl-GUS#1, #2, OsSOG1-7A#1, #2, and *sog1sgl*
270 mutant plants (**Figure 5A and 5C**). However, it is thought that OsSOG1-7A mutants led
271 to partial disruption of OsSOG1 function based on the persistence of transcriptional
272 activity and DNA damage susceptibility of OsSOG1-7A mutants; thus, we defined 690
273 bleomycin-induced and 216 bleomycin-reduced probes in which expression levels were
274 not changed in Psogl-GUS and *sog1sgl* mutant plants as candidate target genes of
275 OsSOG1-mediated DDR (**Table S1**). Gene ontology (GO) enrichment analysis with
276 ShinyGO (v0.66, <http://bioinformatics.sdstate.edu/go/>) revealed that “Glutathione
277 metabolic process”, “Meiotic cell cycle process”, “Double-strand break repair”,
278 “Homologous recombination”, and “Jasmonic acid metabolic process” etc., were
279 enriched in upregulated OsSOG1-target candidate genes in response to bleomycin
280 treatment (**Table S3**). Functional categories regarding chromatin organization were
281 concentrated in the bleomycin-reduced OsSOG1-target candidate gene (**Table S4**).

282 The OsSOG1-target candidate contained some transcription factors including
283 OsSGL, indicating that these genes comprised genes regulated directly and indirectly by
284 OsSOG1. It has been reported previously that *Arabidopsis* SOG1 binds to the CTT (N)₇
285 AAG motif on the promoter region of AtSOG1 target genes (Ogita et al., 2018). We

286 validated whether CTT (N)₇ AAG motifs were located within 1 kb upstream of the start
287 site of the protein-coding region (CDS) of upregulated and downregulated transcripts in
288 wild-type in response to treatment with bleomycin. As a result of the alignment between
289 the microarray probe sequences and the reference genome sequence (the Nipponbare
290 IRGSP-1.0 reference genome), the 785 upregulated- and 477 downregulated-transcripts
291 among 1,068 upregulated- and 608 downregulated-probes in wild-type treated with
292 bleomycin, respectively, were used to search for the AtSOG1 binding motif in the
293 promoter region. AtSOG1 binding motifs with the sequence CTT (N)₇ AAG were found
294 in the 1-kb region upstream of the start site of the CDS of 337 bleomycin-induced
295 (337/785, 42.9%, **Table S5**) and 87 bleomycin-reduced transcripts (87/477, 18.2%,
296 **Table S6**). Among them, 74.8% (252/337, **Table S5**) and 28.7% (25/87, **Table S6**)
297 bleomycin-induced and reduced transcripts were contained in the OsSOG1-target
298 candidate, indicating that these are regulated directly by OsSOG1.

299

300 **OsSOG1 regulated the expression of DNA repair-related genes directly in response** 301 **to DNA damage**

302 Microarray analysis showed that the bleomycin-induced OsSOG1-target
303 candidates included DNA repair-related genes, such as OsRad51A2, OsMSH4,
304 OsMSH5, OsDMC1A, DMC1B, Tyrosyl-DNA phosphodiesterase family protein,
305 Regulator of telomere elongation helicase, Rad21/Rec8 family protein, and Kleisin
306 Nse4 domain-containing protein, etc. To confirm these data, we analyzed the expression
307 levels of representative DNA repair-related genes by qRT-PCR. The expression levels of
308 all genes induced by treatment with bleomycin (Rad51A2, PARP2A, DMC1A, DMC1B,
309 MSH4, MSH5, Rad21-2, Rad21-4, and BRCA1) were suppressed completely in

310 Psog1-GUS and *sog1sgl* mutants (**Figure 6**). In Sog1-7A mutants, the expression levels
311 of these DNA repair-related genes were also depressed, even upon treatment with
312 bleomycin, although often not completely. These results provided support for the data
313 that SOG1-7A mutation has a partial transcriptional activity revealed by the microarray
314 analysis whereas mutation of OsSGL did not affect induction of these DNA
315 repair-related genes.

316 AtSOG1 binding motifs [CTT (N)₇ AAG] were found on the promoter of
317 almost all DNA repair-related genes that were upregulated in response to DNA damage
318 treatment (**Table S5**). To assess whether the AtSOG1 binding motif mediated the
319 upregulation of these DNA repair-related genes in response to DNA damage, we
320 introduced mutations in the AtSOG1 binding motif on the promoter of OsRad51A2 and
321 OsDMC1A via CRISPR/Cas9-based mutagenesis (**Figure 7A,B**). Wild-type rice calli
322 were transformed with expression vectors of CRISPR/Cas9 with a gRNA expression
323 cassette targeting the CTT(N)₇AAG motif on the promoter of these genes via
324 *Agrobacterium*-mediated transformation. Transgenic calli were propagated clonally and
325 subjected to DNA heteroduplex mobility assay (HMA) to detect
326 CRISPR/Cas9-mediated targeted mutagenesis at the target site. Transgenic plants were
327 regenerated from callus lines harboring mutations at high and low frequency at the
328 target site, and plants with and without CRISPR/Cas9-mediated targeted mutations were
329 then isolated using sequencing analysis. Plants with or without mutations at the CTT(N)
330 ₇AAG motif on the promoter of OsRad51A2 or OsDMC1A were treated with bleomycin
331 and collected for analysis of the bleomycin-inducible expression of these genes. The
332 expression levels of OsRad51A2 or OsDMC1A were significantly induced in response
333 to bleomycin treatment in control plants [without mutations by CRISPR/Cas9 at

CTT(N)₇AAG motif]; however, the induction of bleomycin-responsible expression was impaired in mutant plants. These results suggest that the DNA damage-inducible expression of DNA repair-related genes, OsRad51A2 and OsDMC1A, was regulated via the CTT(N)₇AAG motif in rice as in Arabidopsis.

Some cell cycle-related genes are regulated by OsSOG1

A previous study demonstrated that AtSOG1 causes G2 arrest in the meristem through regulation of CDK inhibitor expression under DNA damage conditions (Adachi et al., 2011). Therefore, we analyzed the expression levels of cell cycle-related genes and revealed significant expression changes in response to DNA damage in microarray using qRT-PCR. The expression level of EL2—a cyclin-dependent kinase inhibitor—was induced significantly in response to bleomycin treatment in wild-type; however, its induction was cancelled in Psog1-GUS mutants (**Figure S6**). Bleomycin treatment led to downregulation of CDKB2;1 and CYCB2;1 expression in wild-type but not in Psog1-GUS mutants, whereas there was no significant difference in the expression of KNOLLE, CYCA1;1, CYCA1;2, CYCA3;2, CYCB1;1, CYCB1;2 and CYCB2;2 between wild-type and Psog1-GUS mutants (**Figure S6**).

OsSOG1 plays an important role not only in the DNA damage but also the oxidative stress response

In Arabidopsis, AtSOG1 is involved not only in DDR but also in the oxidative stress response under abiotic stress conditions such as exposure to cadmium, aluminium, and UV-B light. Treatment with bleomycin induced the expression of ROS homeostasis-related genes (e.g., glutathione-S-transferase and dehydroascorbate

358 reductase) in rice, and its bleomycin-inducible expression was suppressed in the
 359 OsSOG1 mutant. To test the hypothesis that OsSOG1 can contribute to oxidative stress
 360 tolerance by regulation of expression of ROS homeostasis-related genes, we evaluated
 361 the sensitivity of OsSOG1 mutant plants to the herbicide methyl viologen (MV), which
 362 produces ROS in the chloroplast under illumination. All *sog1* mutant plants
 363 (*Psog1*-GUS, *SOG1*-7A, and *sog1sgl* mutant) showed increased susceptibility to MV
 364 compared with wild-type plants (**Figure S7**).

366 Discussion

367 A database search identified OsSOG1 and OsSGL as potential rice orthologs of
368 the Arabidopsis DDR protein AtSOG1. The functional SQ motif of AtSOG1 was
369 conserved completely or partially [Aligned score: 47.1292 (AtSOG1 vs OsSOG1) and
370 39.1852 (AtSOG1 vs OsSGL)] and DNA damage susceptibility tests and transcriptome
371 analyses using OsSOG1 and OsSGL mutant rice demonstrated that deficiency of
372 OsSOG1, but not of OsSGL, led to significantly increased sensitivity to DNA damage
373 and inhibition of the induction of gene expression in response to DNA damage. Because
374 induction of OsSGL expression in response to DNA damage was repressed in OsSOG1
375 mutants, and a SOG1 binding motif was identified in the promoter region of OsSGL,
376 OsSGL was thought to be regulated directly by OsSOG1. However, *sog1sgl* double
377 mutant plants showed a more severe phenotype to DNA damage and a stricter
378 suppression of DDR gene expression than that of the *OsSOG1* single mutant
379 Psog1-GUS. Thus, OsSGL might have a role not only as a downstream target of
380 OsSOG1 but also as a member of another signal transduction pathway.

381 Phosphorylation of AtSOG1 via ATM is reported to be required for the DDR in
382 Arabidopsis (Yoshiyama et al., 2013, 2017). Expression of AtSOG1 with
383 serine-to-alanine substitutions (that is, non-phosphorylatable mutations) at all five SQ
384 motifs in the *Arabidopsis sog1* mutant does not complement the *sog1* mutant phenotype
385 at all (Yoshiyama et al., 2017), whereas a recent study demonstrated that casein kinase 2
386 (CK2) also regulated AtSOG1 activity through phosphorylation in response to DNA
387 damage conditions (Wei et al., 2021). In the present study, we used GT to generate
388 OsSOG1-7A mutants, which carried substitutions of serine to alanine at all seven SQ
389 motifs of OsSOG1, and observed that these mutants were less sensitive to bleomycin

390 than Psog1-GUS and *sog1sgl* mutant plants (**Figure 4**), indicating that the substitution
 391 SQ motif mutations of OsSOG1 inhibited OsSOG1 activity, but not completely. The
 392 OsSOG1 protein has nine potential CK2 phosphorylation sites [(S/T)XX(E/D)], raising
 393 the possibility that OsSOG1 is also regulated via CK-mediated signaling in a DDR
 394 pathway. Although we attempted to introduce phosphor-mimetic mutations (the
 395 substitution of serine with aspartic acid) at SQ motifs of OsSOG1 by
 396 CRISPR/Cas9-mediated GT (developed in rice by Nishizawa-Yokoi et al., 2020),
 397 mutant plants carrying desired mutations in *OsSOG* (OsSOG1-7D mutants) were not
 398 obtained using this method (**Figure S4**). Unlike GT with positive-negative selection,
 399 DSBs were induced at the *OsSOG1* locus via CRISPR/Cas9 using
 400 CRISPR/Cas9-mediated GT; therefore, one allele was disrupted by the introduction of
 401 insertion or deletion mutations at the CRISPR-target site even if GT had occurred on
 402 another allele. This could explain why we could not obtain phospho-mimetic mutants
 403 (OsSOG1-7D) rice. There is much room for examination regarding the mechanisms of
 404 OsSOG1 activation in response to DNA damage.

405 Microarray analysis and qRT-PCR revealed that DNA repair-related genes were
 406 regulated by OsSOG1 in response to DNA damage in a manner similar to that of
 407 AtSOG1 reported previously (Yoshiyama et al., 2009; Ogita et al., 2018). Rad51,
 408 BRCA1, and PARP2—representative DNA damage-response genes—were identified as
 409 SOG1 target genes in both Arabidopsis and rice. However, OsMSH4, OsMSH5,
 410 OsDMC1A, OsDMC1B, OsRad21-2, and OsRad21-4 genes were isolated as
 411 OsSOG1-specific target genes (**Table S1 and Figure 6**). MSH4 and MSH5 encode a
 412 meiosis-specific member of the MutS-homologous family of genes, and these proteins
 413 have been revealed to interact with each other and to promote the formation of

414 crossovers between homologous chromosomes in meiosis both in *Arabidopsis* and rice
 415 (Higgins et al., 2008; Luo et al., 2013; Zhang et al., 2014). Similar to Rad51, DMC1 is a
 416 eukaryotic RecA homolog. It has been reported that both Rad51 and DMC1 bind to the
 417 single-stranded DNA processed from DSBs and promote single-strand invasion to
 418 search for homologous sequences during HR in many species, including *Arabidopsis*
 419 and rice (Bishop et al., 1992; Shinohara et al., 1997; Kobayashi et al., 2018). In various
 420 eukaryotes, it is thought that Rad51 is involved in both mitotic and meiotic HR, whereas
 421 DMC1 catalyzes specifically meiotic HR (Shinohara et al., 1997; Kurzbauer et al.,
 422 2012; Pradillo et al., 2012; Da Ines et al., 2013). Wang et al. (2016) reported that
 423 *Osdmc1a Osdmc1b* double mutant rice plants were completely sterile because of
 424 meiotic defects (Wang et al., 2016). Rad21, and its meiosis-specific variant Rec8, have
 425 been reported to play a significant role in sister chromatid cohesion and segregation for
 426 mitosis and meiosis, respectively, in yeast (Watanabe and Nurse, 1999). Among four
 427 Rad21/Rec8 homologs encoded in the rice genome (Zhang et al., 2006), at least
 428 OsRad21–4 functions in homologous pairing of chromosome and telomere bouquet
 429 formation during the meiotic processes (Shao et al., 2011). Considering that these
 430 factors were upregulated via OsSOG1 in somatic cells under DNA damage conditions, it
 431 seems likely that they are involved in DNA repair, especially HR, during mitosis.

432 It has been reported that DNA damage inhibits plant cell division and induces
 433 endoreplication, in which cells increase their genomic DNA content without dividing, in
 434 *Arabidopsis* (Adachi et al., 2011). *Arabidopsis* SOG1 has been reported to be involved
 435 in both inhibition of the cell cycle and induction of the endocycle during DNA damage
 436 (Yoshiyama et al., 2009; Adachi et al., 2011; Yoshiyama, 2016; Ogita et al., 2018). In
 437 contrast to *Arabidopsis*, endocycle has not been found in rice other than during

438 endosperm development, even under stress conditions (Endo et al., 2012). Cell cycle
439 arrest occurs via transcriptional activation of SIAMESE/SIAMESE-RELATED 5 and 7
440 (SMR5 and SMR7), cyclin-dependent kinase inhibitors, with a subsequent decrease in
441 the expression of cyclin A (CYCA) and B (CYCB) genes, which are essential for CDK
442 activation at the G2/M transition of the mitotic cell cycle, and KNOLLE, which is
443 required for cytokinesis, depends on SOG1 in Arabidopsis (Adachi et al., 2011; Yi et al.,
444 2014; Chen et al., 2017, 2019). Thus, root elongation of Arabidopsis *sog1* mutants was
445 not inhibited on medium containing DNA damage agents, resulting in a more tolerant
446 phenotype of Arabidopsis *sog1* mutants to DNA damage compared with wild-type
447 (Yoshiyama et al., 2009, 2014; Sjogren et al., 2015). However, in the present study, root
448 growth of the rice *sog1* mutant was suppressed on medium containing bleomycin at any
449 concentration in comparison to wild-type (**Figure 4**). Microarray analysis and qRT-PCR
450 revealed that expression of EL2, a cyclin-dependent kinase inhibitor, increased
451 significantly in response to DNA damage in wild-type, but this induction was lost in rice
452 *sog1* mutants (**Figure S6**). In addition, downregulation of OsCDKB2;1 and
453 OsCYCB2;1 in response to DNA damage likely depended on OsSOG1; however, the
454 regulation of expression of many other cell cycle-related genes (e.g. OsKNOLLE,
455 OsCYCB1;1 and OsCYCB1;2) was independent of OsSOG1 (**Figure S6**). Surprisingly,
456 no transcriptional change of rice cyclin-dependent kinase inhibitors, other than EL2,
457 was found even after bleomycin treatment in wild-type.

458 These results suggest that the mechanisms by which cells prevent DNA damage
459 in rice, especially regarding the induction of cell-cycle arrest and endocycle, might
460 differ from those of *Arabidopsis*. Such differences could be attributed to the distribution
461 of dividing cells within the body of the plant. In contrast to Arabidopsis, which has

462 dividing cells throughout its whole structure, dividing cells exist only in meristematic
463 tissue in rice. As mentioned above, OsSOG1-targeted DNA repair factors, e.g.
464 OsDMC1s and OsMSH4/5, which were thought to be meiosis-specific recombination
465 proteins in many organisms, might be involved in somatic HR during DNA damage in
466 rice, raising the possibility that DNA repair, especially HR, is strengthened in somatic
467 cells under DNA damage conditions. This phenomenon is assumed to be a rice-specific
468 DDR. Further investigations are required to clarify the mechanisms of DDR in rice.

469 In Arabidopsis, genes involved in defense responses, to not only abiotic stress
470 but also biotic stress, include SOG1 target genes. In addition, *sog1* mutants exhibit
471 decreased resistance to infection with certain types of fungus in Arabidopsis (Ogita et
472 al., 2018). Furthermore, the flavin-containing monooxygenase (FMO1) gene related to
473 activation of systemic acquired resistance through salicylate acid signaling was reported
474 to be regulated directly by Arabidopsis SOG1 (Chen and Umeda, 2015). Under DNA
475 damage conditions, OsSOG1 was shown by microarray analysis to regulate the
476 expression of a large number of detoxifying enzymes, including
477 glutathione-S-transferases (GSTs) and cytochrome P450 (CYPs). The DNA
478 damage-inducible expression of ROS homeostasis-related genes and the content of
479 glutathione in shoots and roots under DNA damage conditions were also affected by
480 OsSOG1 mutation. In addition, microarray analysis revealed that jasmonic acid (JA)
481 metabolic process-related genes were enriched in the OsSOG1 target. Many previous
482 studies have reported that JA plays a crucial role in rice immunity against bacterial and
483 fungal infection (Yamada et al., 2012; Ranjan et al., 2015; Ghorbel et al., 2021). These
484 results suggested that OsSOG1 induces multiple defense pathways in response to DNA
485 damage and plays a key role in acquiring resistance against DNA damage, as in

486 Arabidopsis.

487 Recently developed genome editing technologies have become an essential tool
 488 not only for basic plant science but also for plant breeding. Although these techniques
 489 were categorized as targeted mutagenesis and HR-mediated GT, these mechanisms
 490 introduce mutations via the DSB repair pathways of NHEJ, and HR, respectively.
 491 Therefore, extending our knowledge of the response to DNA damage and the regulation
 492 mechanisms of DNA repair pathways is important for the establishment of efficient
 493 plant genome editing technologies. Elucidation of the regulatory mechanisms of
 494 OsSOG1-mediated DDR and DNA repair will contribute to the development of
 495 efficient and widely applicable genome editing methods via regulation of OsSOG1
 496 activity in the future.

497

498

500 **Methods**

501 **Plant materials**

502 *Oryza sativa* L. cv. Nipponbare was used in this study. Rice calli were grown on N6D
503 medium at 33°C under continuous light, and plants were regenerated from calli on ReIII
504 medium or germinated from seeds grown at 28°C under 16 h light:8 h dark conditions.

505

506 **Vector construction**

507 The GT vectors, pKOD4/Psog1-GUS_HPT (**Figure 1A**) and
508 pKOD4/Psgl-GUS_HPT (**Figure S2A**) used to generate Psog1-GUS and Psgl-GUS
509 mutant rice using positive-negative selection were constructed as follows. The
510 hygromycin phosphotransferase (hpt) expression cassette containing the CaMV 35S
511 terminator (T35S)::nopaline synthase terminator (Tnos)::CaMV35S promoter
512 (P35S)::hpt gene::rice heat shock protein 17.3 terminator (Thsp17.3) was cloned into
513 *AscI/PacI* site between attL1 and attL2 sequence in pENTR(L1-L2) (Thermo Fisher
514 Scientific), yielding pE(L1-L2)hpt. The promoter regions (3 kb upstream from ATG) of
515 the OsSOG1 and OsSGL genes and the GUS gene coding region were amplified by
516 PCR from rice genomic DNA or pENTR-gus plasmid (Thermo Fisher Scientific),
517 respectively, using the primer sets listed in **Table S7**. PCR fragments representing either
518 the OsSOG1 promoter+GUS gene or OsSGL promoter+GUS gene were inserted into
519 the *AscI/AvrII* site using an In-Fusion HD cloning kit (Takara) between attL1 and T35S
520 in pE(L1-L2)hpt, yielding pE(L1-L2)Psog1-GUS_hpt and pE(L1-L2)Psgl-GUS_hpt,
521 respectively. The 3-kb DNA fragments with *OsSOG1* or *OsSGL* coding region were
522 amplified by PCR from rice genomic DNA and cloned into the *PacI* site between
523 Thsp17.3 and attL2 in pE(L1-L2)Psog1-GUS_hpt and pE(L1-L2)Psgl-GUS_hpt,

524 respectively. Finally, DNA fragments of the OsSOG1
525 promoter::GUS::T35S::Tnos::P35S::hpt::Thsp17.3::OsSOG1 coding region and OsSGL
526 promoter::GUS::T35S::Tnos::P35S::hpt::Thsp17.3::OsSGL coding region were cloned
527 into pKOD4 (Nishizawa-Yokoi et al., 2015) with two *DT-A* gene expression cassettes
528 (maize polyubiquitin 1 promoter + *DT-A* + rice hsp 16.9a terminator and rice elongation
529 factor-1a promoter + *DT-A* + rice hsp 16.9b terminator) by a Gateway LR clonase II
530 reaction (Thermo Fisher Scientific), yielding pKOD4/Psog1-GUS_HPT (**Figure 1A**)
531 and pKOD4/Psgl-GUS_HPT (**Figure S2A**), respectively.

532 The GT vector pKOD4/SOG1_7ApHPTb used to produce OsSOG1-7A
533 mutants carrying a serine to alanine substitution at all seven SQ motifs of the OsSOG1
534 gene by positive-negative selection was constructed as follows. A 0.6 kb artificially
535 synthesized fragment from *Bsr*GI to *Bsr*GI (Thermo Fisher Scientific) of the *OsSOG1*
536 gene containing the 5th exon (with S306A/S316A/S322A/S338A/S340A/S398A
537 mutations), 5th intron (with silent mutation producing *Hpa*I site, gttaaa to gttaac), 6th
538 exon (with S403A mutation), and 3' UTR region was cloned into the *Bam*HI/*Xho*I site
539 of pBluescript KS- using an In-Fusion HD cloning kit (Takara), yielding pOsSOG1_7A.
540 The *piggyBac* inverted-repeat transposable element harboring the meganuclease I-*Sce*I
541 site (Nishizawa-Yokoi et al., 2014) was cloned into a *Hpa*I site in the 5th intron of
542 *OsSOG1* in pOsSOG1_7A, yielding pOsSOG1_7Apb. A 2.6 kb upstream and 2.9 kb
543 downstream fragments from two *Bsr*GI sites at the 4th intron and 3' UTR region of the
544 OsSOG1 gene were amplified by PCR from rice genomic DNA and inserted together
545 with the OsSOG1_7Apb fragment digested with *Bsr*GI into the *Asc*I/*Pac*I site of
546 pENTR(L1-L2) (Thermo Fisher Scientific) using an In-Fusion HD cloning kit (Takara),
547 yielding pE(L1-L2)OsSOG1_7Apb. A 4.3 kb positive selection marker expression

548 cassette containing the rice actin terminator, P35S, hpt and Thsp17.3 was digested with
549 I-SceI and integrated into the I-SceI site within the *piggyBac* inverted repeat of
550 pE(L1-L2)OsSOG1_7Apb, yielding pE(L1-L2)OsSOG1_7ApHPTb. The
551 OsSOG1_7ApHPTb fragment was re-cloned into the GT binary vector pKOD4 using a
552 Gateway LR clonase II reaction (Thermo Fisher Scientific), yielding pKOD4/
553 SOG1_7ApHPTb (**Figure 2A**).

554 To construct the CRISPR/Cas9 expression vector targeting *OsSOG1* and
555 *OsSGL* genes, 20-nt annealed oligonucleotide pairs for the target sequences shown in
556 **Table S7** were cloned into the *BbsI* site of the sgRNA expression vector described as
557 pU6gRNA-oligo (Mikami et al., 2015). The sgRNA expression cassette targeting
558 OsSGL (OsU6::sgOsSGL) was digested with *PvuII/AscI* and ligated into the
559 *EcoRV/AscI* sites in pU6::sgOsSOG1 with the sgRNA expression cassette targeting the
560 OsSOG1 gene to connect these two sgRNA expression cassettes in tandem. The
561 fragment with two sgRNA expression cassettes (OsU6::sgOsSOG1 and
562 OsU6::sgOsSGL) was digested with *AscI/PacI* and introduced into
563 pZH_OsU6sgRNA_MM Cas9 (Mikami et al., 2015). The
564 CRISPR/Cas9-mediated GT vector used to introduce phosphomimetic mutations
565 (substitutions of serine to aspartic acid) at all seven SQ motifs of *OsSOG1* gene was
566 constructed as follows. A 0.9 kb artificially synthesized DNA fragment (SOG1-7D GT
567 template) that contained the *OsSOG1* coding region containing the 4th intron, 5th exon
568 (with S306D/S316D/S322D/S338D/S340D/S398D mutations and silent mutation
569 producing an artificial *PstI* site, cttcag to ctgcag), 5th intron, 6th exon (with S403D
570 mutation), and 3' UTR region was cloned into the *PacI* site in
571 pZH_OsU6sgRNA_SpCas-wt (Endo et al., 2019) using an In-Fusion HD cloning kit

(Takara). Two pairs of 20-nt annealed oligonucleotides targeting exon 4 of the *OsSOG1* gene were cloned into the *Bbs*I site of the pU6gRNA-oligo (pU6::sgOsSOG1-2 and pU6::sgOsSOG1-3) (Mikami et al., 2015), respectively. These sgRNA expression cassettes (OsU6::sgOsSOG1-2 and OsU6::sgOsSOG1-3) were amplified with the primer sets listed in **Table S7**, combined in tandem and integrated into the *Asc*I site in pZH_OsU6sgRNA_SpCas-wt harboring SOG1-7D GT template by an In-Fusion reaction (Takara).

To introduce mutations in the SOG1 binding motif in the promoter region of *OsRad51A2* and *OsDMC1* via CRISPR/Cas9-mediated targeted mutagenesis, the sgRNA expression vector targeting the SOG1 binding motif on the *OsRad51A2* and *OsDMC1A* promoters were constructed as detailed above. These expression cassettes (OsU6::sgOsRad51A2 pro and OsU6::sgOsDMC1A pro) were digested with *Asc*I/*Pac*I and each ligated into pZH_OsU6sgRNA_MM Cas9 (Mikami et al., 2015).

Binary vectors were transformed into *Agrobacterium tumefaciens* EHA105 (Hood et al., 1993) by electroporation (*Escherichia coli* Pulser, Bio-Rad).

587

588 **Generation of Psog1-GUS, Sog1-7A, Sog1-7D and Psogl-GUS mutant rice via GT**

The GT procedure with positive-negative selection using hpt as a positive selection marker and DT-A as a negative selection marker and subsequent marker excision via *piggyBac* transposition was performed as described previously (Nishizawa-Yokoi et al., 2015). Four-week-old rice calli transformed with *Agrobacterium* harboring the GT vector were selected on callus induction medium (N6D) containing 50 mg/l hygromycin B and 25 mg/l meropenem (Wako Pure Chemical Industries) for 4 weeks. Hygromycin-resistant calli were transferred to N6D

medium with hygromycin and meropenem and cultured for a further 2 weeks. Genomic DNA was extracted from hygromycin-resistant calli using Agencourt chloropure (Beckman Coulter) according to the manufacturer's protocol and subjected to PCR analysis with KOD FX neo or KOD one (TOYOBO) using primer sets 2 and 3 (**Table S7**) shown in **Figures 1, 2** and **S2**. The PCR amplicon from primer set 2 or 3 was sequenced using the primers listed in Table S1 to confirm the junction sequence between genomic sequence and the GUS gene or positive selection marker and the amino acid substitutions in SOG1-7A mutant introduced via GT. GT callus lines that produced PCR fragments with both primer sets 2 and 3 were transferred to regeneration medium (ReIII) containing 25 mg/l meropenem without antibiotics. Shoots regenerated from callus were transferred to Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) without phytohormones. GT plants carrying the insertion of the positive selection marker in the *OsSOG1* or *OsSGL* gene were identified by PCR analysis and grown in a greenhouse. T₁ progeny plants with homozygous insertion of the positive selection marker via GT in the *OsSOG1* or *OsSGL* gene were obtained from self-pollinating T₀ plants, and T₂ plants obtained from T₁ plants were subjected to further analysis.

For marker elimination in SOG1-7A mutant calli, a portion of GT callus was transferred to N6D medium without hygromycin and meropenem and cultured for 2 weeks, before being transformed with *Agrobacterium* harboring the hyPBase expression vector pPN/hyPBase (Nishizawa-Yokoi et al., 2014). Calli transformed with the hyPBase expression cassette were selected on N6D medium containing 35 mg/l geneticin (Nacalai tesque) and 25 mg/l meropenem. Geneticin-resistant callus lines were transferred to ReIII medium containing 25 mg/l meropenem to regenerate shoots from

620 calli. To determine whether the positive selection marker was excised by hyPBase
 621 expression from the SOG1 locus with GT-mediated amino acid mutations at 7 SQ
 622 motifs, CAPS analysis combining PCR amplification and restriction digestion was
 623 performed with genomic DNA extracted from regenerated plants. PCR fragments
 624 amplified with primer set 1 (**Figure 2A**) were digested with the *HpaI* remaining in the
 625 5th intron of *OsSOG1* after *piggyBac* excision and sequenced with the primers shown in
 626 **Table S7** to check the presence of the desired mutations by GT and the absence of a
 627 footprint at *piggyBac* excision sites in the *OsSOG1* gene. To detect re-integration of the
 628 *piggyBac* transposon, PCR analysis was also conducted using hpt-specific primers
 629 (**Table S7**). Marker-free GT plants harboring *HpaI*-digested PCR fragments but not
 630 hpt-specific PCR fragments were grown in a greenhouse to obtain T₁ progenies. T₁
 631 plants with homozygous point mutations via GT and segregated out hyPBase expression
 632 cassette were selected by PCR, and T₂ progenies obtained from self-pollinating T₁
 633 plants were subjected to further analysis.

634 To generate SOG1-7D mutant rice by CRISPR/Cas9-mediated GT, a GT vector
 635 harboring CRISPR/Cas9, OsU6::sgSOG1-2/3, hpt, and a SOG1-7D GT template were
 636 introduced into 4-week-old rice calli by *Agrobacterium*-mediated transformation.
 637 Following a 4-week hygromycin selection, hygromycin-resistant callus lines were
 638 subjected to DNA extraction using a one-step DNA extraction protocol (Kasajima et al.,
 639 2004). PCR fragments amplified with primers shown in **Figure S4 (Table S7)** were
 640 digested with the *PstI* site introduced into 5th exon of *OsSOG1* gene via GT.

641

642 **Generation of mutant rice plants by the CRISPR/Cas9 system**

643 Four-week-old rice calli transformed with *Agrobacterium* harboring a

644 CRISPR/Cas9 expression vector were selected for 2 weeks on N6D medium containing
645 50 mg/l hygromycin B and 25 mg/l meropenem. Hygromycin-resistant calli were
646 transferred to N6D medium with hygromycin and meropenem and cultured for a further
647 2 weeks. Genomic DNA was extracted from hygromycin-resistant calli using a one-step
648 DNA extraction protocol (Kasajima et al., 2004).

649 CRISPR/Cas9-mediated mutations in the *OsSOG1* and *OsSGL* genes were
650 detected in transgenic calli by CAPS analysis. The target region on the *OsSOG1* or
651 *OsSGL* gene was amplified using the primers shown in **Table S7**. PCR products were
652 digested with *PmeI* and *AvaII* for the detection of mutations in *OsSOG1* and *OsSGL*
653 genes, respectively, and then analyzed by agarose gel electrophoresis. Targeted
654 mutations via CRISPR/Cas9 at SOG1 binding motifs in *OsRad51A2* and *OsDMC1A*
655 were analyzed in transgenic calli by HMA. The target region on the *OsRad51A2* or
656 *OsDMC1A* promoter was amplified using the primers shown in **Table S7**. PCR products
657 were analyzed using MCE-202 MultiNA with a DNA-500 kit (Shimadzu).

658 Transgenic calli carrying targeted mutations via CRISPR/Cas9 at high
659 frequency were transferred to ReIII medium containing 25 mg/l meropenem.
660 Regenerated plants were subjected to sequencing analysis using the primers listed in
661 **Table S7** to examine the mutation pattern at the target site. Regarding the *sog1sgl*
662 double mutant, T₁ plants obtained from self-pollinating T₀ plants were subjected to PCR
663 analysis using hpt-specific primers (**Table S7**) to select double mutant plants in which
664 the CRISPR/Cas9 vector had segregated out. T₂ progenies obtained from
665 self-pollinating CRISPR/Cas9 cassette-free T₁ plants were subjected to further analysis.

666

667 Southern blot analysis

668 Genomic DNA was extracted from GT plants using the Nucleon Phytopure
669 Extraction Kit (Cytiva) according to the manufacturer's protocol. Genomic DNA (2 µg)
670 was digested with *Xba*I (for Psog1-GUS mutant), *Hind*III (for Psgl-GUS mutant), or
671 *Xho*I (SOG1-7A mutant) and fractionated in a 0.7% (w/v) agarose gel. Southern-blot
672 analysis was performed according to the Digoxigenin Application Manual (Roche
673 Diagnostics). Specific DNA probes for hpt and *OsSOG1* were synthesized with a PCR
674 Digoxigenin Probe Synthesis Kit (Roche Diagnostics) using the primers shown in **Table**
675 **S7**.

676

677 **Histochemical analysis of GUS gene expression**

678 Psog1-GUS and Psgl-GUS mutant plants were grown at 28°C under 16 h
679 light:8 h dark on 1/2 MS medium (pH 5.8) for 1 week. Seedlings were transferred to 1/2
680 MS liquid medium with or without 10 µM bleomycin and cultured for 8 h at 28°C under
681 normal light conditions. Histochemical analysis of GUS activities in Psog1-GUS and
682 Psgl-GUS mutant plants was performed by incubating whole seedlings in
683 5-bromo-4-chloro-3-indolyl glucuronide (X-gluc) buffer [100 mM sodium phosphate
684 buffer, pH 7.0, 0.5 mM K₃Fe(CN)₆, 0.5 mM K₄Fe(CN)₆, 0.3% Triton X-100, 20%
685 methanol, 1.9 mM X-gluc] at 37 °C overnight. The seedlings were washed with 70%
686 ethanol until they were cleared of chlorophyll, then observed using Leica MZFIII
687 microscope and DFC310 FX Digital color camera (Leica).

688

689 **DNA damage and oxidative stress susceptibility test**

690 For the DNA damage susceptibility test, wild-type, Psog1-GUS, SOG1-7A,
691 Psgl-GUS, *sog1sgl* mutant seeds were sown on 1/2 MS medium containing bleomycin

692 at various concentrations and were cultured for 1 week, and their sensitivity evaluated
693 by measuring root length. For the oxidative stress susceptibility test, wild-type,
694 Psog1-GUS, SOG1-7A and sog1sgl mutant seeds were sown on 1/2 MS medium with or
695 without 0.05 μ M MV and cultured for 1-week.

696

697 RNA extraction and microarray analysis

698 One-week-old wild-type, Psog1-GUS, SOG1-7A, Psog1-GUS, *sog1sgl* mutants
699 were transferred to 1/2 MS liquid medium with or without 10 μ M bleomycin and were
700 cultured for 8 h at 28°C under normal light conditions. The shoots and roots were
701 harvested separately and frozen immediately in liquid nitrogen. Total RNA was isolated
702 from roots treated with or without bleomycin using an RNeasy Plant Mini Kit (Qiagen),
703 labeled using a Low Input Quick-Amp Labeling Kit (Agilent Technologies), and
704 hybridized to a rice 4 $\times$ 44 K custom oligo DNA microarray (Agilent Technologies)
705 according to the manufacturer's instructions. Hybridization microarray slides were
706 scanned with a DNA microarray scanner G2505C (Agilent Technologies). The images
707 generated were analyzed using Feature Extraction software version 11.5.1.1 (Agilent
708 Technologies) applying standard normalization procedures.

709

710 RT-PCR and qRT-PCR

711 Total RNA isolated from shoots and roots treated with or without bleomycin
712 using an RNeasy Plant Mini Kit (Qiagen) was treated with DNase I (Qiagen) to
713 eliminate any contaminating DNA. First-strand cDNA was synthesized using ReverTra
714 Ace (TOYOBO) with an oligo (dT)₂₀ primer. RT-PCR was performed with PrimeSTAR
715 GXL DNA polymerase (Takara) using gene-specific primers (**Table S7**). Quantitative

716 RT-PCR was conducted with a Power SYBR Green PCR Master Mix (Thermo Fisher)
717 and Applied Biosystems ViiA 7 Real-Time PCR System (Thermo Fisher) and using
718 gene-specific primers (**Table S8**).

719

720 **Search for SOG1 binding motif in the promoter region of DDR genes**

721 We extracted the 1 kb regions upstream of the translation start site of genes and
722 searched for the possible SOG1 promoter motif "CAA(N)₇TTG" that was reported as a
723 binding motif of Arabidopsis SOG1 using a Perl script for pattern matching.

724

725

726 **Author contributions**

727 AN-Y and ST designed the research, AN-Y, AM, KI conducted the experiments,
728 RM performed microarray analysis. TT conducted in silico analysis, AN-Y wrote the
729 manuscript, ST edited the manuscript.

730

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747

748

749

750

Figure 1 The generation of Psog1-GUS mutants by knock-in of the GUS gene downstream of the *OsSOG1* promoter via GT.

A, Strategy for the knock-in of a GUS::terminator construct in-frame with the translational start codon (ATG) of the *OsSOG1* gene by GT with positive-negative selection. The top line indicates the genomic structure of the wild-type *OsSOG1* gene region. Gray and white boxes show untranslated regions and exons, respectively. The middle line shows a schematic of the GT vector carrying two expression cassettes of diphtheria toxin A subunit gene (*DT-A*) under the control of the maize polyubiquitin 1 promoter (P_{ubi}) or rice elongation factor 1 α promoter (P_{ef}) located on both ends as a negative selection marker, a GUS gene::terminator cassette, and the cauliflower mosaic virus 35S promoter (P_{35S})::hpt::terminator expression cassette as a positive selection marker within the sequence homologous to the *OsSOG1* locus (promoter region, 3.0 kb; coding region, 3.1 kb). LB, left border; RB, right border. The bottom line represents the Psog1-GUS mutant locus resulting from HR between the GT vector and wild-type locus. Primer sets 1, 2, and 3 were used for PCR analysis to identify the Psog1-GT mutants. *XbaI* was the restriction enzyme used for Southern blot analysis.

B, PCR analysis with wild-type and two independent lines of Psog1-GUS T2 homozygous mutants (#1 and #2) using primer sets 1, 2, and 3 shown in A. C, Southern blot analysis with the hpt-specific probe shown in A using genomic DNA digested with *XbaI*.

Figure 2 Generation of *OsSOG1*-7A mutants via GT and subsequent marker elimination using *piggyBac* transposon.

A, Strategy for the introduction of point mutations into the *OsSOG1* locus via GT with positive-negative selection. The top line indicates the genomic structure of the wild-type *OsSOG1* locus. Gray and white boxes show untranslated regions and exons, respectively. The bottom line shows a schematic of the GT vector carrying the *DT-A* and *hpt* expression cassettes as a negative and positive selection markers, respectively. A rice actin terminator and *hpt* expression cassette ($P_{35S}::hpt::ter$) were inserted within *piggyBac* transposon inverted repeats (black arrowheads) and introduced into the artificially synthesized *HpaI* site at the 5th intron of the sequence homologous to the *OsSOG1* locus on the GT vector carried a substitution of seven amino acids [S306A/S316A/S322A/S338A/S340A/S398A mutations at the 5th exon (*) and S403A mutation at the 6th exon (**)]. LB, left border; RB, right border. **B**, Strategy for marker excision from the GT locus via *piggyBac* transposition. The top line represents a schematic of the GT locus resulting from HR between the GT vector and wild-type locus. The bottom line shows the genomic structure of the *OsSOG1*-7A mutant locus containing the substitutions of seven amino acids and silent mutation (*HpaI* site) resulting from precise marker elimination by *piggyBac* transposition. Primer set 1 was used for CAPS analysis to check the marker excision from the GT locus. **C**, Southern blot analysis with wild-type, *OsSOG1*-7A T0 plants transformed with (hyPBBase-1, -2, and -3) or without (w/o) *piggyBac* transposase (hyPBBase) expression cassette. Genomic DNA was digested with *XhoI*. Upper and lower panels were detected with probes 1 and 2, respectively, shown in **A** and **B**. **D**, Sequencing chromatograms of the substitution mutations of seven amino acids and the marker excision (*HpaI*) site in wild-type (upper) and *OsSOG1*-7A T2 homozygous mutant (lower) plants.

Figure 3 CRISPR/Cas9 target sites at OsSOG1 and OsSGL genes in *sog1sgl* double mutants.

A, Construction of a CRISPR/Cas9 expression vector carrying the expression cassettes of the single gRNA targeting the *OsSOG1* (U6:gSOG1) and *OsSGL* (U6:gSGL) genes. B and C, Target sites of CRISPR/Cas9-mediated target mutagenesis in the *OsSOG1* (B) and *OsSGL* (C) genes. Gray and white boxes show untranslated region and exons, respectively. D and E, Mutation patterns induced via CRISPR/Cas9 system at *OsSOG1* (D) and *OsSGL* (E) gene in *sog1sgl* double mutants #1 and #2, respectively. *PmeI* (D) and *AvaII* (E) sites are shown in italics. Gray highlights indicate the deduced amino acid sequences in these mutants.

A **B**

Figure 4 The effect of mutation in *OsSOG1* and/or *OsSGL* on sensitivity to the DNA-damaging agent Bleomycin.

A, Representative morphology of 1-week-old wild-type, Psog1-GUS #1, Sog1-7A #1, and Psgl-GUS #1 sog1sgl #1 mutant rice plants grown with or without the indicated concentration of bleomycin. Bar=2cm. B, Quantification of the root length of plants grown with or without various concentrations of bleomycin. Error bars represent SE. T-test *, $P < 0.01$.

Figure 5 Identification of *OsSOG1* and *OsSGL* target genes by microarray analysis.

A and B, Venn diagrams showing the overlap of microarray probes that were repressed in Psog1-GUSs/*OsSOG1*-7As/*sog1sgl*s (A), and in *sog1sgl*s/Psogl-GUSs (B), respectively, among the bleomycin-induced 1,068 probes in wild-type. C and D, Venn diagrams representing the overlap of microarray probes that were not reduced in Psog1-GUSs/*OsSOG1*-7As/*sog1sgl*s (C), and in *sog1sgl*s/Psogl-GUSs (D), respectively, among 608 probes downregulated in response to bleomycin in wild-type rice.

Figure 6 Transcript levels of DNA repair-related genes in response to DNA damage in wild-type, *Psog1-GUS*, *Sog1-7A*, *Psgl-GUS*, and *sog1sgl* mutant plants.

Plants were treated with 10 μ M bleomycin for 8 h, and roots were harvested and subjected to RNA extraction and qRT-PCR analysis.

Figure 7 CTT(N)₇AAG motif-mediated regulation of the *OsDMC1A* and *OsRad51A2* genes by *OsSOG1*.
 A and B, Mutation patterns induced via the CRISPR/Cas9 system at CTT(N)₇AAG motifs (uppercase letters) on the promoter region of *OsDMC1A* (A) and *OsRad51A2* (B) genes in *osdmc1a* and *osrad51a2* mutant plants. The top lines indicate the genomic structures of *OsDMC1A* (A) and *OsRad51A2* (B) genes, respectively. Solid and open boxes represent CTT(N)₇AAG motifs and open reading frames, respectively. The underlined sequence indicates the 20 bp target sequence for the sgRNA, and bold letters indicate the PAM sequence. The dashes and red letters show deletion and insertion mutations. The *osdmc1a* #1 was used as control that did not have mutations at CTT(N)₇AAG motifs even with the CRISPR/Cas9 vector. C and D, Transcript levels of *OsDMC1A* and *OsRad51A2* in wild-type, *osdmc1a*, and *osrad51a2* mutant plants treated with or without 10 μM bleomycin.

Parsed Citations

Adachi, S., Minamisawa, K., Okushima, Y., Inagaki, S., Yoshiyama, K., Kondou, Y., Kaminuma, E., Kawashima, M., Toyoda, T., Matsui, M., Kurihara, D., Matsunaga, S., and Umeda, M. (2011). Programmed induction of endoreduplication by DNA double-strand breaks in Arabidopsis. Proc Natl Acad Sci U S A 108, 10004-10009.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Bishop, D.K., Park, D., Xu, L., and Kleckner, N. (1992). DMC1: a meiosis-specific yeast homolog of E. coli recA required for recombination, synaptonemal complex formation, and cell cycle progression. Cell 69, 439-456.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Chen, P., and Umeda, M. (2015). DNA double-strand breaks induce the expression of flavin-containing monooxygenase and reduce root meristem size in Arabidopsis thaliana. Genes to cells : devoted to molecular & cellular mechanisms 20, 636-646.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Chen, P., Sjogren, C.A., Larsen, P.B., and Schnittger, A. (2019). A multi-level response to DNA damage induced by aluminium. Plant J 98, 479-491.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Chen, P., Takatsuka, H., Takahashi, N., Kurata, R., Fukao, Y., Kobayashi, K., Ito, M., and Umeda, M. (2017). Arabidopsis R1R2R3-Myb proteins are essential for inhibiting cell division in response to DNA damage. Nature communications 8, 1-12.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Da Ines, O., Degroote, F., Goubely, C., Amiard, S., Gallego, M.E., and White, C.I. (2013). Meiotic recombination in Arabidopsis is catalysed by DMC1, with RAD51 playing a supporting role. PLoS genetics 9, e1003787.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Endo, M., Nakayama, S., Umeda-Hara, C., Ohtsuki, N., Saika, H., Umeda, M., and Toki, S. (2012). CDKB2 is involved in mitosis and DNA damage response in rice. Plant J 69, 967-977.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Endo, M., Mikami, M., Endo, A., Kaya, H., Itoh, T., Nishimasu, H., Nureki, O., and Toki, S. (2019). Genome editing in plants by engineered CRISPR-Cas9 recognizing NG PAM. Nat Plants 5, 14-17.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Ghorbel, M., Brini, F., Sharma, A., and Landi, M. (2021). Role of jasmonic acid in plants: the molecular point of view. Plant Cell Reports 40, 1471-1494.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hendrix, S., Iven, V., Eekhout, T., Huybrechts, M., Pecqueur, I., Horemans, N., Keunen, E., De Veylder, L., Vangronsveld, J., and Cuyper, A. (2020). Suppressor of Gamma Response 1 Modulates the DNA Damage Response and Oxidative Stress Response in Leaves of Cadmium-Exposed Arabidopsis thaliana. Front Plant Sci 11, 366.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Higgins, J.D., Vignard, J., Mercier, R., Pugh, A.G., Franklin, F.C., and Jones, G.H. (2008). AtMSH5 partners AtMSH4 in the class I meiotic crossover pathway in Arabidopsis thaliana, but is not required for synapsis. Plant J 55, 28-39.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Hood, E.E., Gelvin, S.B., Melchers, L.S., and Hoekema, A. (1993). New Agrobacterium helper plasmids for gene transfer to plants. Transgenic research 2, 208-218.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kasajima, I., Ide, Y., Ohkama-Ohtsu, N., Hayashi, H., Yoneyama, T., and Fujiwara, T. (2004). A protocol for rapid DNA extraction from Arabidopsis thaliana for PCR analysis. Plant Molecular Biology Reporter 22, 49-52.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kobayashi, W., Liu, E., Ishii, H., Matsunaga, S., Schlögelhofer, P., and Kurumizaka, H. (2018). Homologous pairing activities of Arabidopsis thaliana RAD51 and DMC1. The Journal of Biochemistry 165, 289-295.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Kurzbauer, M.T., Uanschou, C., Chen, D., and Schlögelhofer, P. (2012). The recombinases DMC1 and RAD51 are functionally and spatially separated during meiosis in Arabidopsis. Plant Cell 24, 2058-2070.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Luo, Q., Tang, D., Wang, M., Luo, W., Zhang, L., Qin, B., Shen, Y., Wang, K., Li, Y., and Cheng, Z. (2013). The Role of OsMSH5 in Crossover Formation during Rice Meiosis. Molecular plant 6, 729-742.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Mikami, M., Toki, S., and Endo, M. (2015). Comparison of CRISPR/Cas9 expression constructs for efficient targeted mutagenesis in rice. Plant Mol Biol 88, 561-572.

- Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Nishizawa-Yokoi, A., Mikami, M., and Toki, S. (2020). A Universal System of CRISPR/Cas9-Mediated Gene Targeting Using All-in-One Vector in Plants. *Frontiers in Genome Editing* 2.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Nishizawa-Yokoi, A., Endo, M., Osakabe, K., Saika, H., and Toki, S. (2014). Precise marker excision system using an animal-derived piggyBac transposon in plants. *Plant J* 77, 454-463.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Nishizawa-Yokoi, A., Endo, M., Ohtsuki, N., Saika, H., and Toki, S. (2015). Precision genome editing in plants via gene targeting and piggyBac-mediated marker excision. *Plant J* 81, 160-168.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Nuruzzaman, M., Manimekalai, R., Sharoni, A.M., Satoh, K., Kondoh, H., Ooka, H., and Kikuchi, S. (2010). Genome-wide analysis of NAC transcription factor family in rice. *Gene* 465, 30-44.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Ogita, N., Okushima, Y., Tokizawa, M., Yamamoto, Y.Y., Tanaka, M., Seki, M., Makita, Y., Matsui, M., Okamoto-Yoshiyama, K., Sakamoto, T., Kurata, T., Hiruma, K., Saijo, Y., Takahashi, N., and Umeda, M. (2018). Identifying the target genes of SUPPRESSOR OF GAMMA RESPONSE 1, a master transcription factor controlling DNA damage response in Arabidopsis. *Plant J* 94, 439-453.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Pradillo, M., López, E., Linacero, R., Romero, C., Cuñado, N., Sánchez-Morán, E., and Santos, J.L. (2012). Together yes, but not coupled: new insights into the roles of RAD51 and DMC1 in plant meiotic recombination. *The Plant journal : for cell and molecular biology* 69, 921-933.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Ranjan, A., Vadassery, J., Patel, H.K., Pandey, A., Palaparthi, R., Mithöfer, A., and Sonti, R.V. (2015). Upregulation of jasmonate biosynthesis and jasmonate-responsive genes in rice leaves in response to a bacterial pathogen mimic. *Functional & Integrative Genomics* 15, 363-373.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Reinhardt, H.C., and Schumacher, B. (2012). The p53 network: cellular and systemic DNA damage responses in aging and cancer. *Trends in Genetics* 28, 128-136.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Roldán-Arjona, T., and Ariza, R.R. (2009). Repair and tolerance of oxidative DNA damage in plants. *Mutation Research/Reviews in Mutation Research* 681, 169-179.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Shao, T., Tang, D., Wang, K., Wang, M., Che, L., Qin, B., Yu, H., Li, M., Gu, M., and Cheng, Z. (2011). OsREC8 Is Essential for Chromatid Cohesion and Metaphase I Monopolar Orientation in Rice Meiosis. *Plant Physiology* 156, 1386-1396.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Shinohara, A., Gasior, S., Ogawa, T., Kleckner, N., and Bishop, D.K. (1997). *Saccharomyces cerevisiae* recA homologues RAD51 and DMC1 have both distinct and overlapping roles in meiotic recombination. *Genes to cells : devoted to molecular & cellular mechanisms* 2, 615-629.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Sjogren, C.A., Bolaris, S.C., and Larsen, P.B. (2015). Aluminum-Dependent Terminal Differentiation of the Arabidopsis Root Tip Is Mediated through an ATR-, ALT2-, and SOG1-Regulated Transcriptional Response. *Plant Cell* 27, 2501-2515.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Takahashi, N., Ogita, N., Takahashi, T., Taniguchi, S., Tanaka, M., Seki, M., and Umeda, M. (2019). A regulatory module controlling stress-induced cell cycle arrest in Arabidopsis. *eLife* 8, e43944.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Terada, R., Urawa, H., Inagaki, Y., Tsugane, K., and Iida, S. (2002). Efficient gene targeting by homologous recombination in rice. *Nat Biotechnol* 20, 1030-1034.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Ventura, A., Kirsch, D.G., McLaughlin, M.E., Tuveson, D.A., Grimm, J., Lintault, L., Newman, J., Reczek, E.E., Weissleder, R., and Jacks, T. (2007). Restoration of p53 function leads to tumour regression in vivo. *Nature* 445, 661-665.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Wang, H., Hu, Q., Tang, D., Liu, X., Du, G., Shen, Y., Li, Y., and Cheng, Z. (2016). OsDMC1 Is Not Required for Homologous Pairing in Rice Meiosis. *Plant Physiol* 171, 230-241.**
Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)
- Watanabe, Y., and Nurse, P. (1999). Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature* 400,**

461-464.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Wei, P., Demulder, M., David, P., Eekhout, T., Yoshiyama, K.O., Nguyen, L., Vercauteren, I., Eekhout, D., Galle, M., De Jaeger, G., Larsen, P., Audenaert, D., Desnos, T., Nussaume, L., Loris, R., and De Veylder, L. (2021). Arabidopsis casein kinase 2 triggers stem cell exhaustion under AI toxicity and phosphate deficiency through activating the DNA damage response pathway. Plant Cell.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yamada, S., Kano, A., Tamaoki, D., Miyamoto, A., Shishido, H., Miyoshi, S., Taniguchi, S., Akimitsu, K., and Gomi, K. (2012). Involvement of OsJAZ8 in jasmonate-induced resistance to bacterial blight in rice. Plant & cell physiology 53 12, 2060-2072.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yi, D., Alvim Kamei, C.L., Cools, T., Vanderauwera, S., Takahashi, N., Okushima, Y., Eekhout, T., Yoshiyama, K.O., Larkin, J., Van den Daele, H., Conklin, P., Britt, A., Umeda, M., and De Veylder, L. (2014). The Arabidopsis SIAMESE-RELATED cyclin-dependent kinase inhibitors SMR5 and SMR7 regulate the DNA damage checkpoint in response to reactive oxygen species. Plant Cell 26, 296-309.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yoshiyama, K., Conklin, P.A., Huefner, N.D., and Britt, A.B. (2009). Suppressor of gamma response 1 (SOG1) encodes a putative transcription factor governing multiple responses to DNA damage. Proc Natl Acad Sci U S A 106, 12843-12848.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yoshiyama, K.O. (2016). SOG1: a master regulator of the DNA damage response in plants. Genes & genetic systems 90, 209-216.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yoshiyama, K.O., and Kimura, S. (2018). Ser-Gln sites of SOG1 are rapidly hyperphosphorylated in response to DNA double-strand breaks. Plant signaling & behavior 13, e1477904.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yoshiyama, K.O., Kaminoyama, K., Sakamoto, T., and Kimura, S. (2017). Increased Phosphorylation of Ser-Gln Sites on SUPPRESSOR OF GAMMA RESPONSE1 Strengthens the DNA Damage Response in Arabidopsis thaliana. Plant Cell 29, 3255-3268.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yoshiyama, K.O., Kimura, S., Maki, H., Britt, A.B., and Umeda, M. (2014). The role of SOG1, a plant-specific transcriptional regulator, in the DNA damage response. Plant signaling & behavior 9, e28889.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Yoshiyama, K.O., Kobayashi, J., Ogita, N., Ueda, M., Kimura, S., Maki, H., and Umeda, M. (2013). ATM-mediated phosphorylation of SOG1 is essential for the DNA damage response in Arabidopsis. EMBO reports 14, 817-822.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zhang, L., Tao, J., Wang, S., Chong, K., and Wang, T. (2006). The rice OsRad21-4, an orthologue of yeast Rec8 protein, is required for efficient meiosis. Plant Mol Biol 60, 533-554.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)

Zhang, L., Tang, D., Luo, Q., Chen, X., Wang, H., Li, Y., and Cheng, Z. (2014). Crossover formation during rice meiosis relies on interaction of OsMSH4 and OsMSH5. Genetics 198, 1447-1456.

Google Scholar: [Author Only](#) [Title Only](#) [Author and Title](#)