Supplementary Information

SOG1 transcription factor promotes the onset of endoreduplication under salinity stress in *Arabidopsis*

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Supplementary Materials and Methods

Nuclei isolation and ploidy analysis by flowcytometry

Isolation of nuclei and subsequent analysis of the nuclear DNA content by flowcytometry were performed following previously described methods with minor modifications[1, 2]. Nuclei were isolated from root and first and second pair of rosette leaves of 7-days old wild type,*OE-1*, *sog1-6* and *sog1-1* mutant seedlings, either untreated or treated with increasing concentrations of NaCl for 12 hours by chopping method. Approximately 50–100 mg of excised plant tissue (roots or rosette leaves) was placed in a plastic 60-mm petri dish standing on a prechilled tile placed on ice in a rectangular plastic tray. Otto-I buffer [100mM citric acid and 0.5% (v/v) tween 20] was added in the proportions of 1 ml buffer per 100 mg tissue. Tissues were chopped using a new razor blade for 4-5 min. The homogenate was then filtered through a 40-µm nylon filter to remove tissue debris and then centrifuged subsequently at 500 g for 10 minutes. The nuclei pellet was resuspended in 500 µl of 1X PBS buffer (pH 7.4) with gentle tapping. The nuclear suspensions were stained with 4µg/ml DAPI and run through a FACS Calibur flowcytometer (BD) and about 10,000 flow cytometric events were recorded for each sample. The outputwas gated to eliminate signal from the debris Flowcytometry experiments were repeated at least three times for each genotype using independent biological replicates. FlowJo v. 10.0.6 (Tree Star, Inc.) was used for flow cytometric data analysis.

Study of root cell area expansion by propidium iodide staining

Roots of 7-days old wild type, *OE-1*, *sog1-6* and *sog1-1* mutant seedlings treated without or with different concentrations of NaCl for 12 hours were fixed in a solution of 50% (V/V) methanol and 10% (V/V) acetic acid and kept overnight at 4°C. On the next day, root samples were washed withsterile and incubated in 1% (W/V) periodic acid solution for 40 min. Roots were then stained with Schiff reagent containing 100 mM sodium metabisulfite, 0.15 M HCl and 10 mg/L propidium iodide. Stained samples were mounted in a chloral hydrate solution [chloral hydrate, glycerol, and water (8 g: 1 ml: 2 ml)]. Root tips were then visualized by confocal microscopy (Zeiss LSM-510 Meta). Root tip cell area and distance from the QC were measured with MBF ImageJ software by tracing cell contours.

Measurement of leaf cell area expansion

Expansion of *Arabidopsis* leaf epidermal and mesophyll cells were studied by Differential interference contrast (DIC) microscopy following the method described earlier[3, 4]. First and second pair of rosette leaves from untreated or NaCl treated 7-days old wild type, *OE-1, sog1-6* and *sog1-1* seedlings were fixed immediately in a solution of ethanol: glacial acetic acid (1:1) (v/v) for at least 12 hours following harvesting. Following incubation, leaves were dehydrated in a series of ethanol solutions (50%, 70%, 80%, 90%, and 100%) for at least 15 min in each step. After dehydration, leaves were then submerged in a clearing solution containing chloral hydrate, glycerol and water in 4:2:1 proportion. Leaves were then visualized under a Zeiss Axio Scope A1 microscope using DIC optics and images were taken using the Axiocam 503 camera attached with the microscope. Cell areas were measured in ImageJ software. Trichomes of wild type and mutant plants were observed by SEM, as previously described[5].

Total protein extraction and immunoblotting

Total protein was extracted following the procedure described previously[6] (Roy et al., 2013). About 100 mg plant tissue from wild type, *OE-1* and *sog1-1* mutant seedlings, either untreated or treated with increasing concentrations of NaCl for 12 hours, were frozen in liquid nitrogen and homogenized in 1 ml of pre-chilled protein extraction buffer (50 mMTris–HCl pH 7.5, 5 mM $MgCl_2$, 400 mM sucrose, 10% glycerol, 2.5 mM EDTA, pH 8.0, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM 2-mercaptoethanol, 5 mg/ml^{-1} leupeptin and 1 mg/ml^{-1} antipain). The homogenate was then transferred into a sterile centrifuge tube and centrifuged subsequently at 2,000 × g for 5 min at 4˚C to pellet down the debris. The supernatant was transferred to a fresh tube and concentration of protein in each sample was determined by conventional Bradford assay[7].

Western blot analysis was carried out following the method of Sambrook[8]. About 50 µg of the protein samples were separated on a 10% SDS-PAGE gel and then transferred on to a PVDF membrane (BioRad) using a Bio-Rad mini trans blot apparatus following the manufacturer's protocol. For assessment of expression of DNA damage response proteins and regulators of cell cycle and endoreduplication, we have used the respective antibodies developed in mouse system against the respective protein from mammalian system. All the proteins show considerable amount of sequence conservation between the plant and the corresponding mammalian counterpart (> 70% sequence identity). The antibodies against mammalian ATM

(SIGMA-A6093), ATR (SIGMA-c515173), CDK1(SIGMA-SAB4500050), CDK2 (SIGMA-SAB4300388), Cyclin B1(SIGMA-C8831), WEE1(SCBT, sc-5285) and E2F1(SIGMA-05-379) showed specific cross-reactivity with plant ATM, ATR, CDKB1;1, CDKB2;1, CYCB1;1 and E2Fa respectively and this is also confirmed by immunoprecipitation assays. The antibody against mammalian CCS52A1 (SIGMA-SAB2105778) detects both plant CCS52A1 and A2 counterpart. The anti-AtSOG1 polyclonal antibody was generated against the Ni^{2+}-NTA affinity resin purified full length recombinant AtSOG1 protein. His-tagged recombinant AtSOG1 protein was expressed in *E. coli* BL21 host strain and purified on nickel beads. The protein was separated by SDS-PAGE. Gel slices containing AtSOG1 protein were used to raise the antibody against the full-length AtSOG1 protein in rabbits by Abgene, Bhubaneswar, India. Titters of the antibody were assayed using ELISA. Affinity purification of immune serum was carried out using Protein A-Agarose Fast Flow resin (Sigma).

Primary antibodies were used in 1:1000 dilution and the specificity of different antibodies has been described earlier[9,10]. Alkaline phosphatase conjugated goat anti-rabbit IgG was used as secondary antibody (1:1000 dilutions). Immunospecific bands on the PVDF membrane were detected following incubation with NBT-BICP developer solution in dark for 5-10 min[8]. In case of blot analysis, for using the minimum volume of primary antibody, after the pilot experiment and determination of the position of respective specific bands, in most of the cases the blots in the relevant positions were cut and incubated with the antibody to minimize the use of excess antibodies.

Study of transcript accumulation under increasing salinity by semi-quantitative PCR

For expression analysis, total RNA was extracted from 7-days old wild type, *OE-1*, *sog1-6* and *sog1-1*mutant seedlings, treated without or with increasing concentrations of NaCl for 12 hours. Approximately 100 mg tissue was frozen immediately in liquid nitrogen after collection and then total RNA was extracted using RNeasyplant mini kit (Qiagen) following manufacturer's instructions. RNA concentration and purity were tested by spectrophotometric method. 1µg of total RNA was processed further to obtain cDNA using Superscript III Reverse Transcriptase (Invitrogen). Semi quantitative PCR was performed using the cDNA samples to examine the transcript accumulation of *AtATM*, *AtSOG1*, *AtATR*, *AtBLT*, *AtSMR5* and *AtSMR7* genes using specific expression primers (Supplementary table 2). For semi-quantitative RT-PCR analysis for

AtSOG1 transcripts, a first cycle of 2 min at 94°C, 45 s at 57°C and 1 min at 72°C was followed by 45 s at 94°C, 45 s at 57°C and 1 min at 72 °C for 22 cycles. For *AtBLT*, a first cycle of 2 min at 94°C, 45 s at 53°C and 1 min at 72°C was followed by 45 s at 94°C, 45 s at 53°C and 1 min at 72 °C for 22 cycles. For *AtATM*, a first cycle of 2 min at 94°C, 45 s at 55°C and 1 min at 72°C was followed by 45 s at 94°C, 45 s at 55°C and 1 min at 72 °C for 22 cycles. For *AtATR1*, a first cycle of 2 min at 94°C, 45 s at 57°C and 1 min at 72°C was followed by 45 s at 94°C, 45 s at 57°C and 1 min at 72 °C for 22 cycles. For *AtSMR5*, a first cycle of 2 min at 94°C, 45 s at 54°C and 1 min at 72°C was followed by 45 s at 94°C, 45 s at 54°C and 1 min at 72 °C for 22 cycles. For *AtSMR7*, a first cycle of 2 min at 94°C, 45 s at 55°C and 1 min at 72°C was followed by 45 s at 94°C, 45 s at 55°C and 1 min at 72 °C for 22 cycles. The conditions for semi-quantitative RT-PCR were chosen so that none of the mRNAs analyzed reached a plateau at the end of the amplification cycles, i.e. they were in the exponential phase of amplification, and that the two sets of primers (one set of gene specific primer and the other set for *β-tubulin* gene primers) used in each reaction did not compete with each other. Images of the RT-PCR products in ethidium bromide stained agarose gels were acquired in Bio-Rad Molecular Imager Gel Doc XR system with high resolution CCD camera and quantification of the bands was performed by Bio-Rad Image Densitometer GS-700 using Quantity One software. Band intensity was expressed as relative absorbance units. The ratio between the sample mRNA to be determined and *β-tubulin* was calculated to normalize for initial variations in sample concentration. In each set of samples, product quantity was normalized against *β-tubulin* after background subtraction. Mean values and standard deviation of all experiments performed were calculated after normalization to *β-tubulin*. Mean values from three independent trials were used to represent the relative expression levels of transcripts (normalized to *β-tubulin*) in terms of percentage. Relative expression of each gene was determined by analyzing the band intensity using the ImageJ software.

Supplementary References

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Supplementary table 1: Primer sequences used for PCR genotyping

Primer	**Sequence 5' ……3'**
LP (SALK_067631C)	TGGTCCTTGGTTCTTTTTCAG
RP (SALK_067631C)	TGTCGCGTCTGCTTTAGGTAG
LBb1.3	ATTTTGCCGATTTCGGAAC

Supplementary table 2: Primer sequences used for transcript profile analysis by semi-quantitative RT-PCR

Primer	**Sequence 5'------► 3'**	**T_m (°C)**
AtBLT FP	GAGGAAGAATGATTGAAGCTGGT	58
AtBLT RP	ATCATCAGCTCTGCCCGTCT	61
AtSOG1 FP	GCTGATCGATAGCAACCGGA	62
AtSOG1 RP	CTGTTGTGGCTGCTGGTAGA	62
AtATM FP	GAAGGCGGAGCAATCTGACT	62
AtATM RP	TCTAGCAAGTCCGATGCCAA	60
AtATR FP	TGCCATTTATGTTAATACAGCTC	62
AtATR RP	TTCTAGCAGCAAACGTGCCT	60
AtSMR5 FP	GCCTACACGTGATGATTGCC	59
AtSMR5 RP	CTTCGGTGGTTCCCTCTTCT	59
*AtSMR7*FP	CGAGAGGAGCAAAGACTCCG	60
*AtSMR7*RP	CCTATCGGATCGGGAAGTCG	60
Atβ-tubulin FP	CCTGATAACTTCGTCTTTGG	58
Atβ-tubulin RP	GTCAACTCCATCTCGTCCAT	58

Supplementary Figures

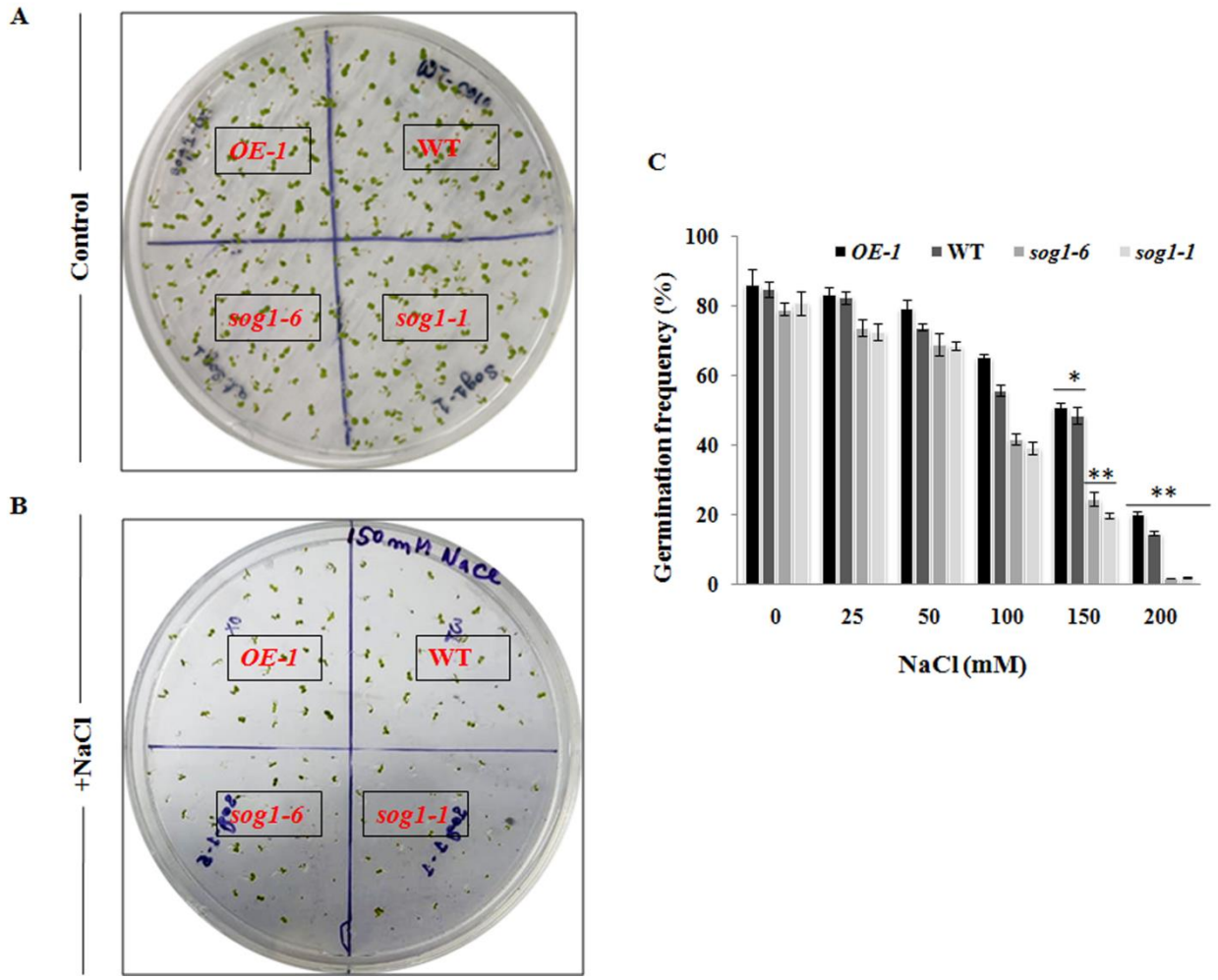

Fig. S1: Determination of germination performance. (A-C) Analysis of germination frequencies in 3-days old SOG1 overexpressor line *OE-1*, wild-type, *sog1-6* and *sog1-1* mutant lines grown in absence or presence of increasing concentrations of NaCl. Three independent biological replicates for each genotype, each containing at least 30 seeds, were analysed. The mean value of germination frequency of individual experiment was determined and averaged subsequently for the three biological replicates. Error bar in the graphs represents ±SD. Asterisks represent statistically significant differences within a 5 and 1% confidence interval (*$p < 0.05$, **$p < 0.01$), respectively based on one-way ANOVA factorial analysis indicating the genotype that differs significantly in germination performance.

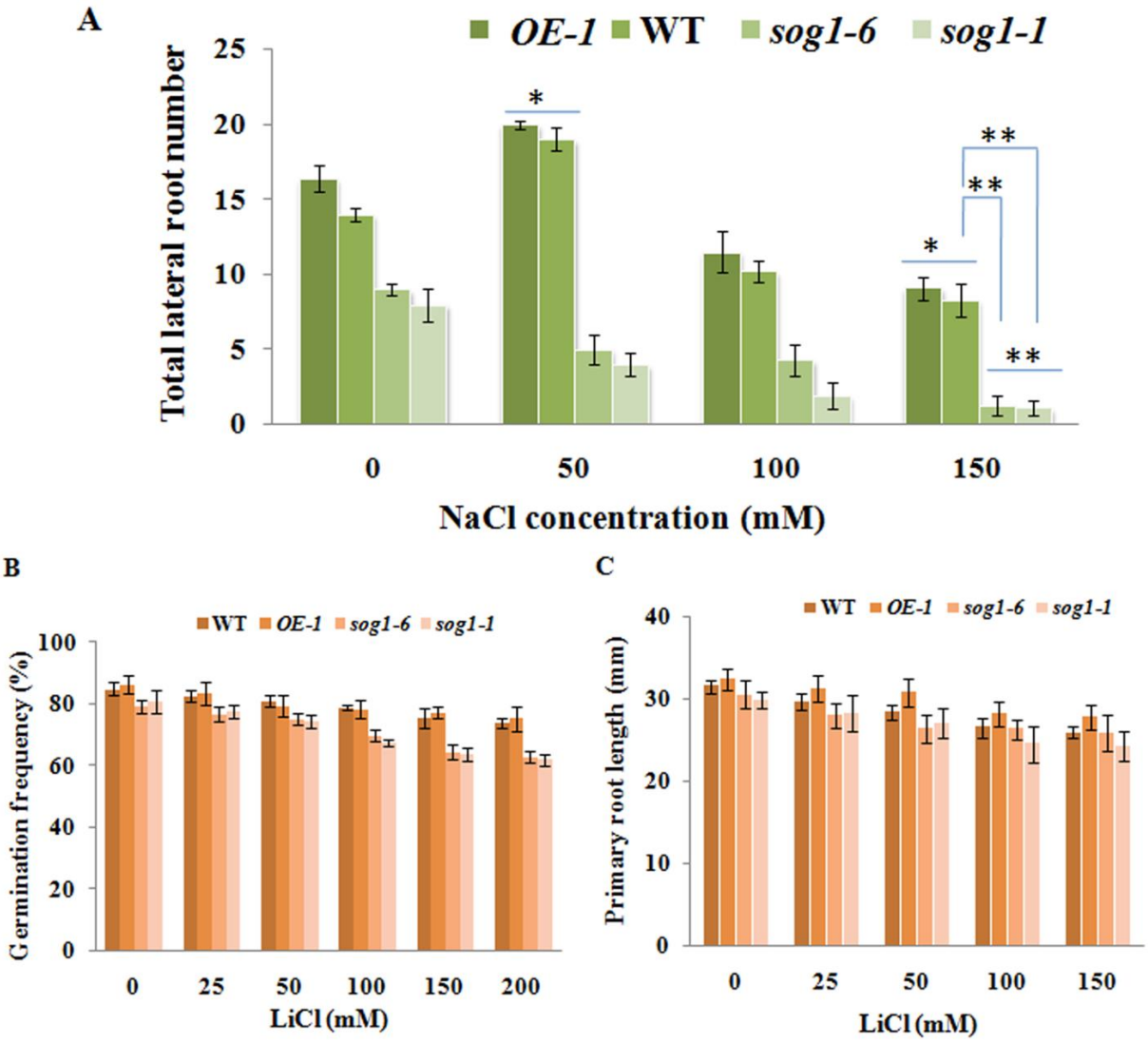

Fig. S2: Change in number of lateral roots under increasing salinity and phenotypic response in presence of an alternate chloride salt. (A) Number of lateral roots in 12-days old *OE-1*, wild-type, *sog1-6* and *sog1-1* mutant lines under untreated conditions and in response to increasing salinity. (B) Determination of germination percentages in wild-type, *OE-1*, *sog1-6* and *sog1-1* mutant lines germinated either on control plates or supplemented with increasing concentrations of LiCl. (C) Primary root growth in 12-days old wild-type, *OE-1*, *sog1-6* and *sog1-1* mutant lines in the presence of increasing concentrations of LiCl. Each bar represents the mean value of three independent replicates. Error bar indicates standard deviation. Asterisks indicate significant statistical differences (*$p < 0.05$ or **$p < 0.01$) from control (without NaCl treatment) using one-way ANOVA factorial analysis.

A

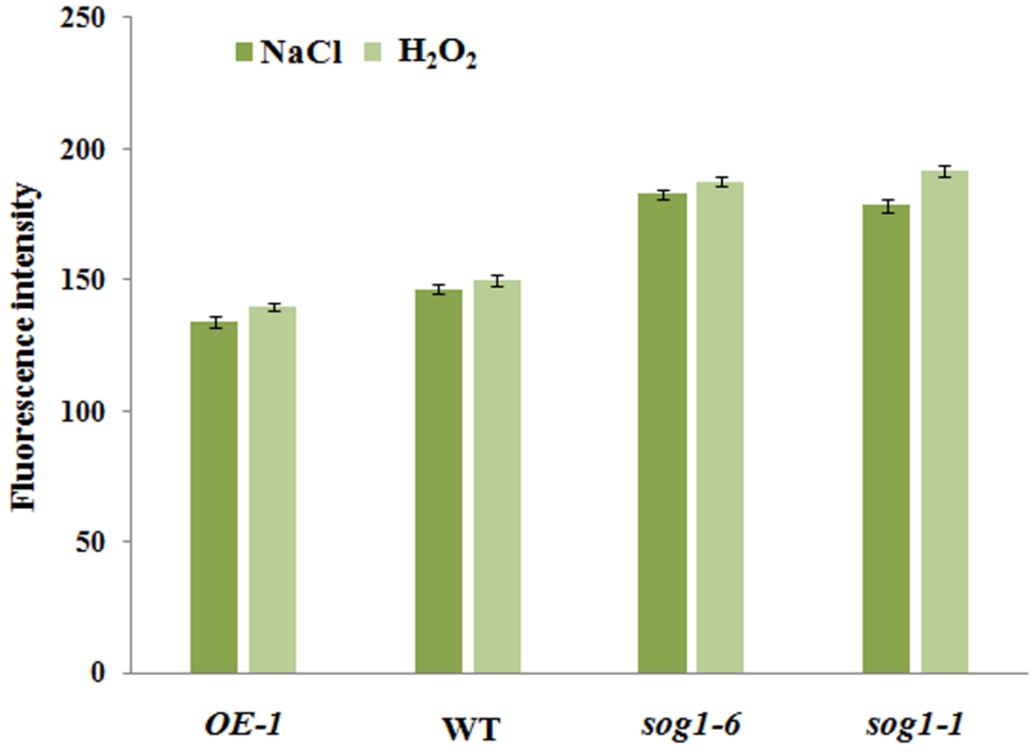

Fig. S3: Total ROS production in presence of H_2O_2. (A) Quantitative estimation of total ROS in the roots of 7-days old *OE-1*, wild-type, *sog1-6* and *sog1-1*mutant line seedlings, treated with either NaCl (150 mM) or H_2O_2 (20 mM). Quantitative data has been represented as the mean value of three independent biological replicates. Error bars represent standard deviation.

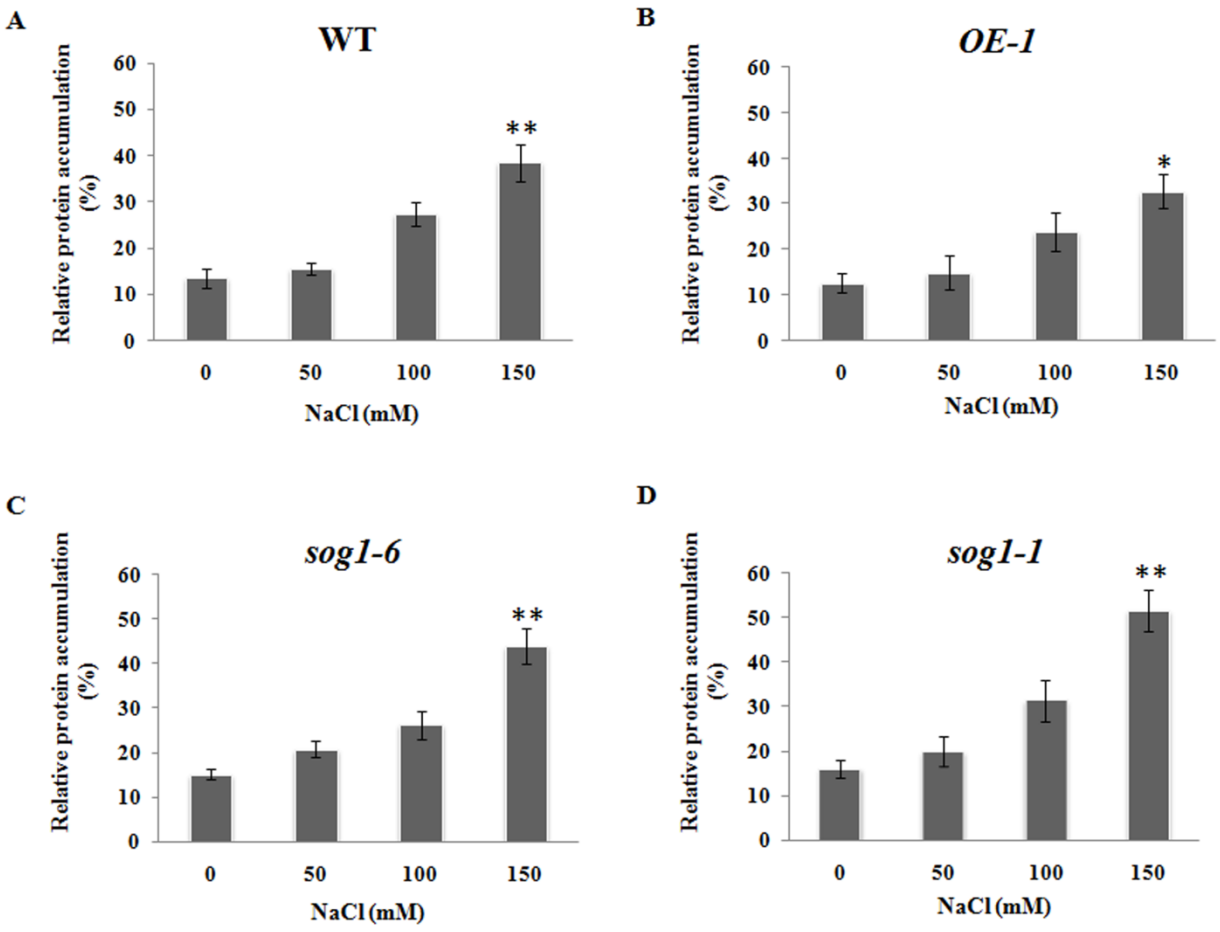

Fig. S4: Quantification of γ-H2AX accumulation from band intensity. (A-D) Quantification of the data related to accumulation level of DSB marker, histone variant γ-H2AX protein in wild-type (A), *OE-1* (B), *sog1-6* (C) and *sog1-1* (D) seedlings, as detected by immunoblotting using protein extracts from 7-days old seedlings grown in absence or presence of different concentrations of NaCl for 12 hours as mentioned under 'Materials and methods' section. Densitometry analysis was carried out by ImageJ software (NIH). Asterisks indicate significant statistical differences (*$p < 0.05$ or **$p < 0.01$) from control (without NaCl treatment) using one-way ANOVA factorial analysis.

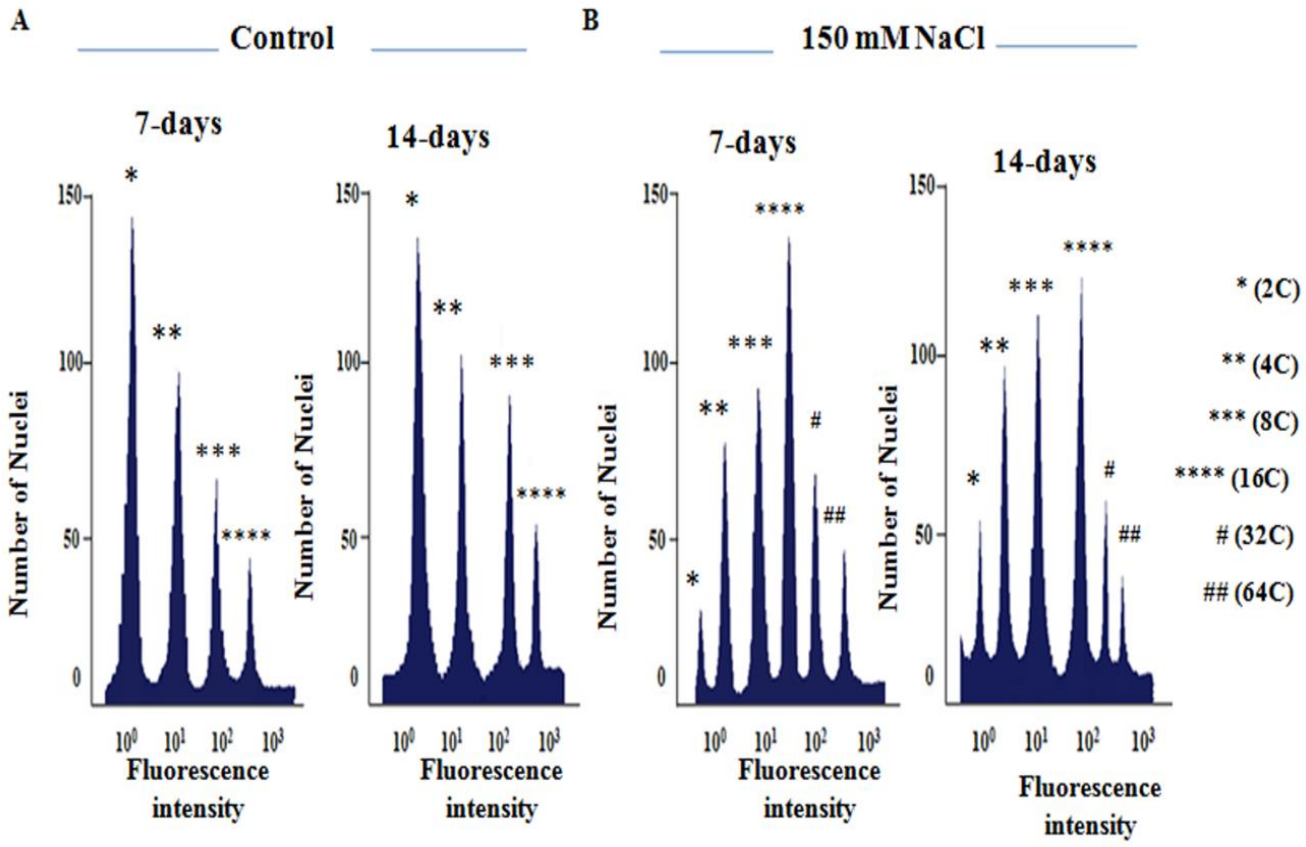

Fig. S5: Retention of endopolyploidy after 14 days. (A and B) Flowcytometric analysis of ploidy level in the DAPI-stained root cell nuclei of 7 and 14-days old wild-type *Arabidopsis* seedlings either untreated (A) or treated with 150 mM NaCl (B).

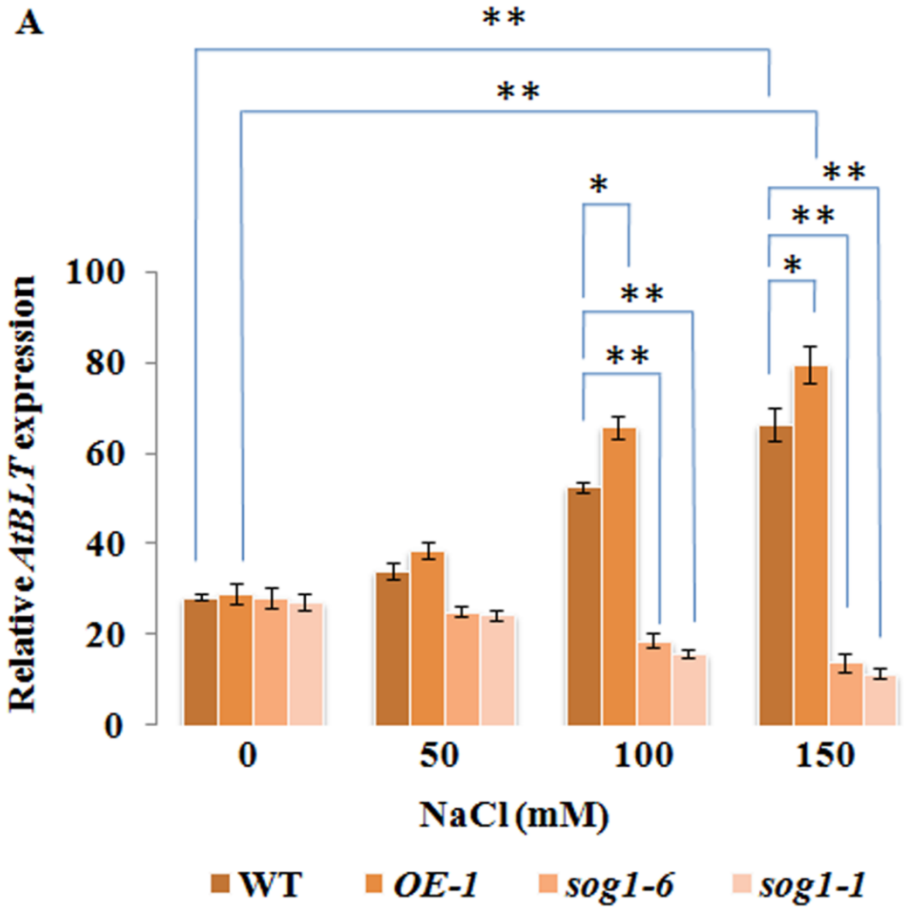

Fig. S6: Quantification of *AtBLT* gene expression from band intensity. (A) Relative expression of *AtBLT* gene as obtained from semi-quantitative RT-PCR in 7-days old wild-type, *OE-1*, *sog1-6* and *sog1-1* mutant seedlings grown in absence or presence of different concentrations of NaCl. Densitometry analysis was carried out by ImageJ software (NIH). Each bar represents the mean value of three independent replicates. Error bar indicates standard deviation. Asterisks indicate significant statistical differences (*$p < 0.05$ or **$p < 0.01$) from control (without NaCl treatment) using one-way ANOVA factorial analysis.

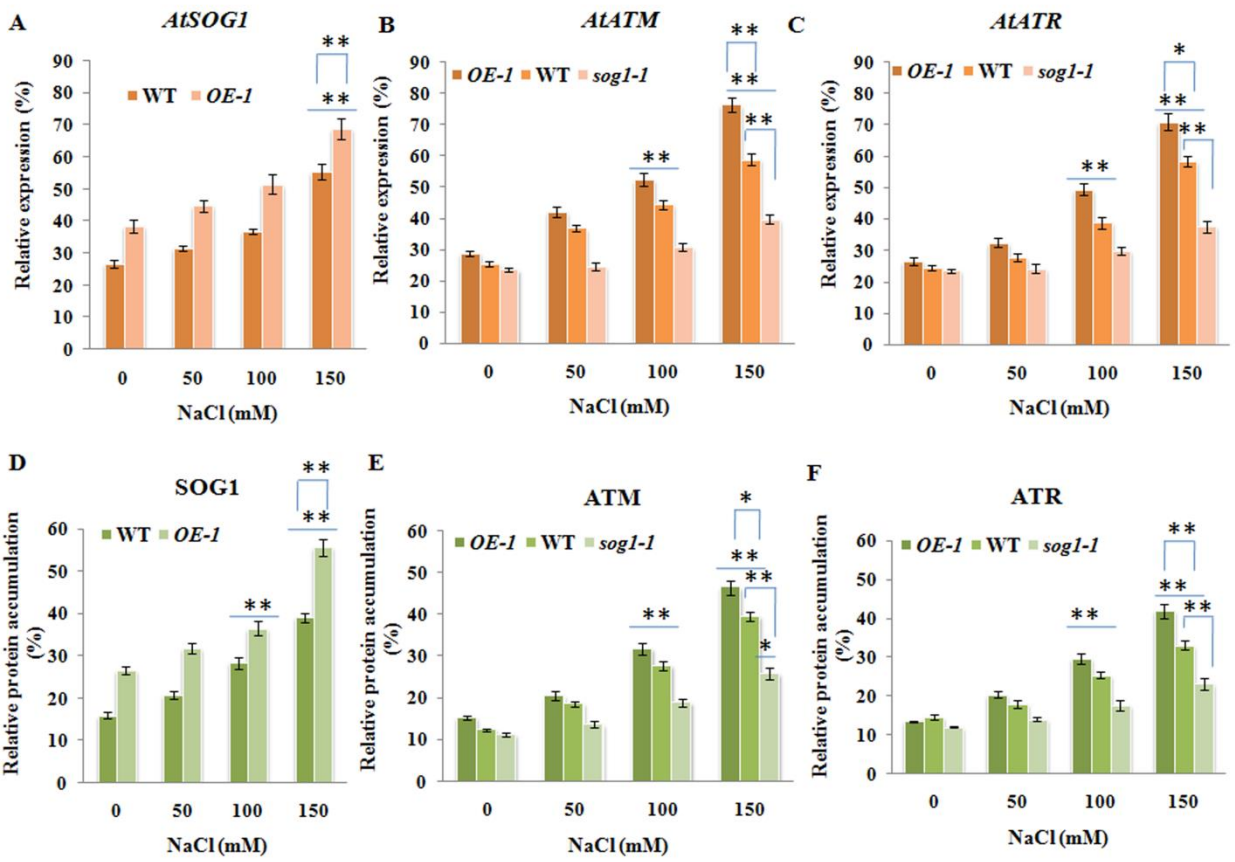

Fig. S7: Quantification of transcript and protein accumulation from band intensity. (A-C) Quantification of relative expression of various DNA damage response genes including SOG1 (A), ATM (B) and ATR (C) as obtained from semi-quantitative RT-PCR in 7-days old wild-type, *OE-1*, *sog1-6* and *sog1-1* mutant seedlings grown in absence or presence of different concentrations of NaCl. (D-F) Quantification of the data related to accumulation level of SOG1(D), ATM (E), and ATR (F), proteins as detected by immunoblotting using protein extracts from 7-days old wild-type, *OE-1*, *sog1-6* and *sog1-1* mutant seedlings grown in absence or presence of different concentrations of NaCl for 12 hours as mentioned under 'Materials and methods' section. Densitometry analysis was carried out by ImageJ software (NIH). Each bar represents the mean value of three independent replicates. Error bar indicates standard deviation. Asterisks indicate significant statistical differences (*$p < 0.05$ or **$p < 0.01$) from control (without NaCl treatment) using one-way ANOVA factorial analysis.

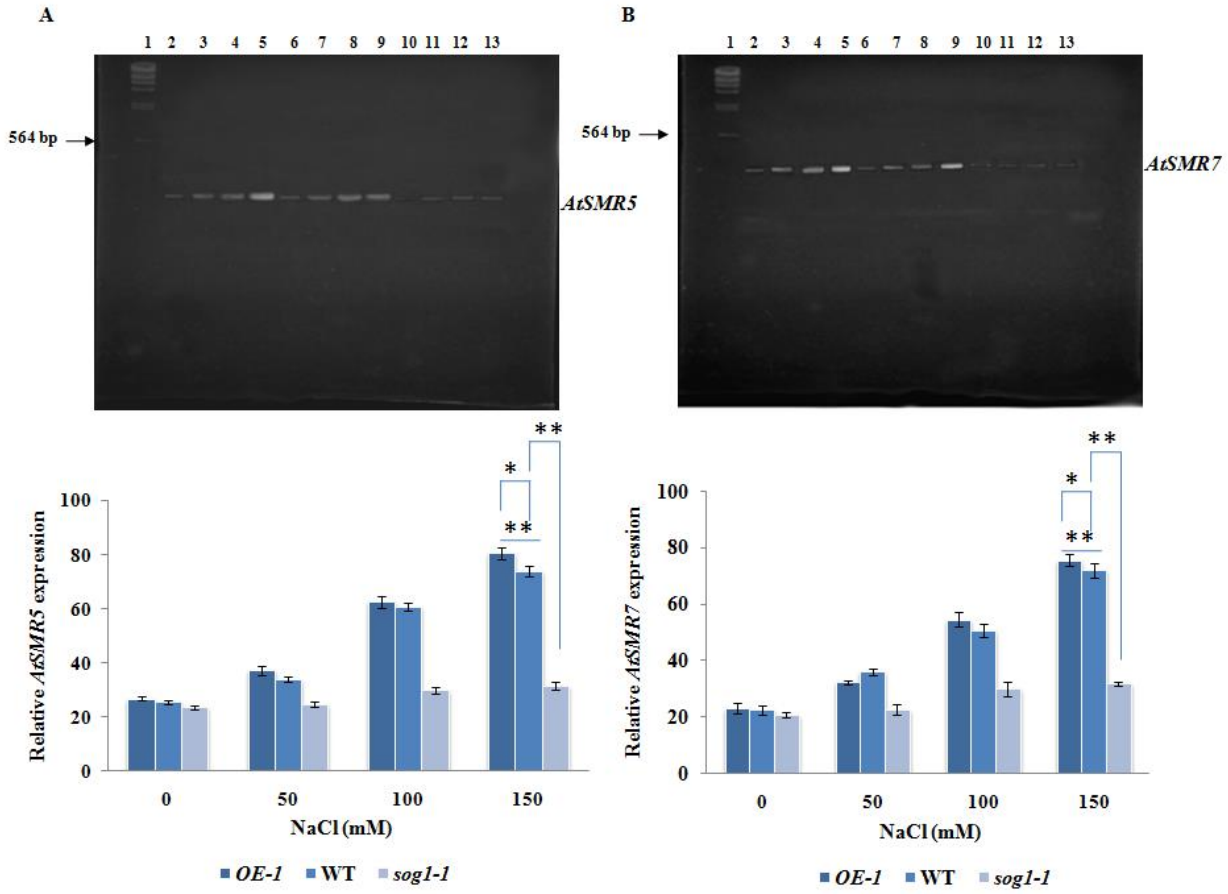

Fig. S8: SOG1 mediated induction of *AtSMR5* and *AtSMR7* genes in response to increasing salinity. (A and B) Semi-quantitative RT-PCR showing expression levels of *AtSMR5* and *AtSMR7* genes in *OE-1*, wild-type and *sog1-1* seedlings in response to increasing NaCl concentrations. Each bar represents the mean value of three independent replicates. Lane1: Marker; Lane 2-5 (*OE-1*): 0, 50, 100 and 150 mM NaCl, respectively; Lane 6-9 (wild-type): 0, 50, 100 and 150 mM NaCl, respectively; Lane 10-13 (*sog1-1*): 0, 50, 100 and 150 mM NaCl, respectively. Error bar indicates standard deviation. Asterisks indicate significant statistical differences (*$p < 0.05$ or **$p < 0.01$) from control (without NaCl treatment) using one-way ANOVA factorial analysis.

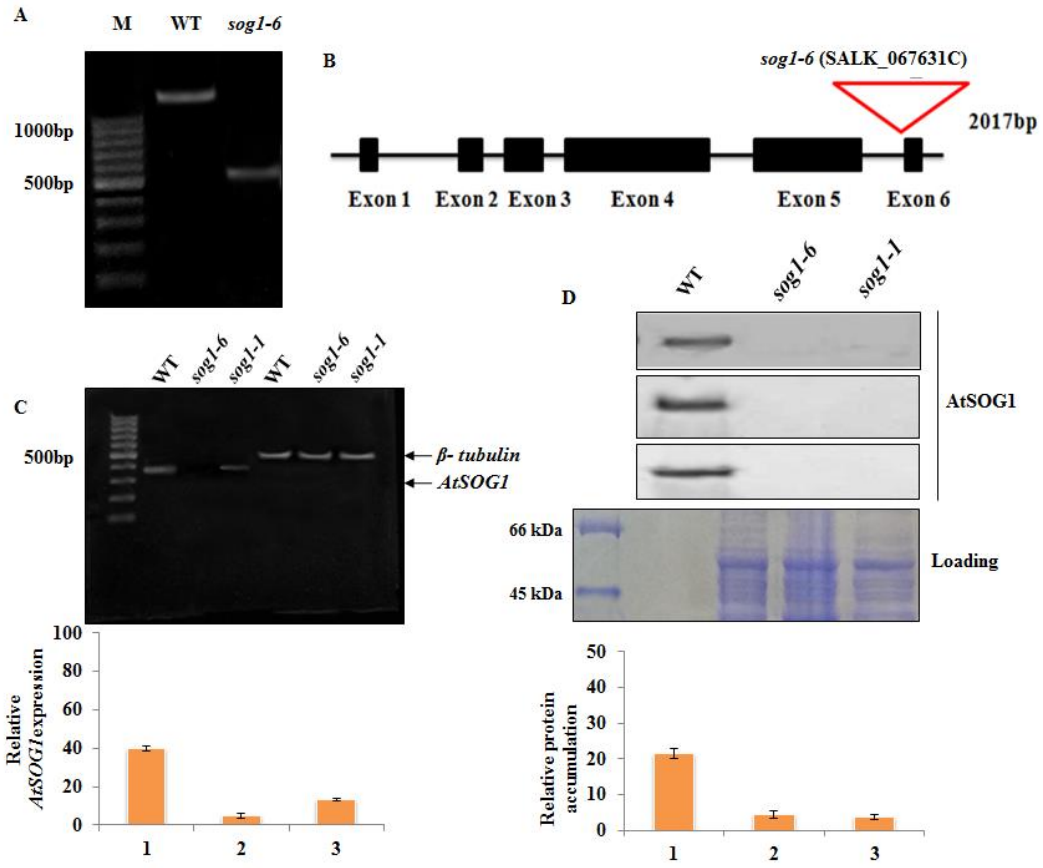

Fig. S9: Characterization of loss-of-function mutant lines. (A) Leaf PCR genotyping for determining homozygosity of the *sog1* mutant line SALK_067631C (B) *AtSOG1* gene is comprised of 6 exons indicated by black rectangles and red triangle showing the insertion of T-DNA at exon 6 and the mutant is designated as *sog1-6*. (C) Semi quantitative RT- PCR using total RNA isolated from 7-days old wild-type, *sog1-6* and *sog1-1* mutant seedlings. Transcript level of *β-tubulin* has been shown as the internal control. (D) Representative triplicates of SOG1 protein gel blots using total protein extracted from 7-days old wild-type, *sog1-6* and *sog1-1* mutant seedlings. Affinity purified custom made anti-SOG1 polyclonal antibody (1:250 dilutions) was used to detect the SOG1 protein which is detected in WT and has been mentioned on the right-hand side of the gel image. Portion of the blots containing the specific bands of interest were cut prior to incubation with the primary antibody. Respective loading control of total protein extracts from wild-type, *sog1-6*, and *sog1-1* seedlings, respectively has been presented below the blot image. Representative gel blot images from at least three independent experiments are shown.

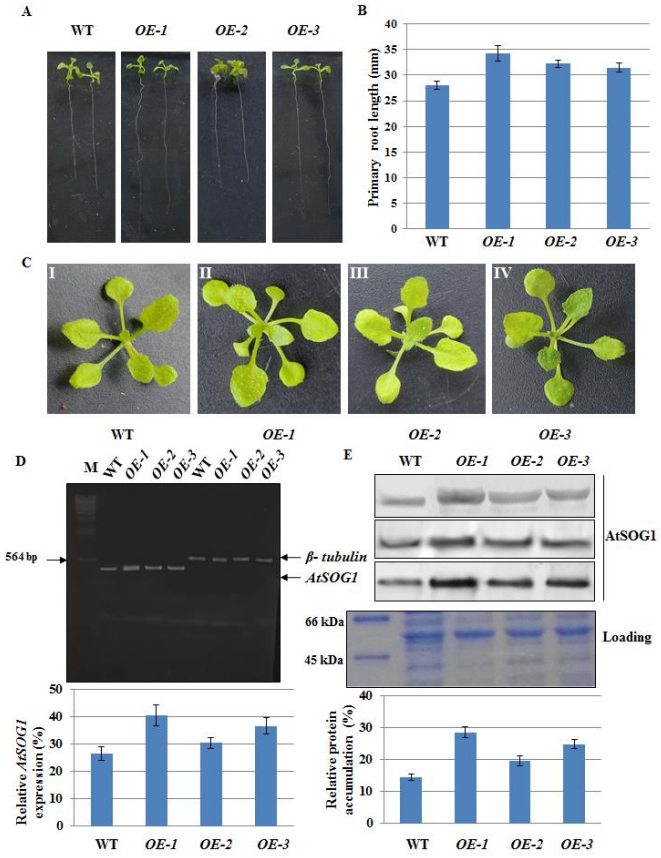

Fig. S10: Selection of transgenic line overexpressing SOG1. (A) Growth responses of wild-type and transgenic lines overexpressing SOG1 (*OE-1*, *OE-2* and *OE-3*). (B) Measurement of primary root length of 12-days old wild type and SOG1 overexpression line (*OE-1*, *OE-2* and *OE-3*) seedlings. Each column represents the mean value of three independent replicates. Error bar indicates standard deviation. (C) Phenotypes associated with SOG1 overexpressor lines, 21 days past germination, I, wild-type; II, *OE-1*; III, *OE-2*; IV, *OE-3*. (D) Semi-quantitative RT-PCR using total RNA isolated from 7-days old wild-type, *OE-1*, *OE-2* and *OE-3* seedlings. Transcript level of *β-tubulin* has been shown as the internal control. (E) Representative triplicates of SOG1 protein gel blots using total protein extracted from 7-days old wild-type, *OE-1*, *OE-2* and *OE-3* seedlings. Affinity purified custom made anti-SOG1 polyclonal antibody (1:250 dilutions) was used to detect the SOG1 protein which is detected in wild-type and has been mentioned on the right-hand side of the gel image. The blots were cut prior to hybridization with the primary antibody. Respective loading control of total protein extracts from wild-type, *OE-1*, *OE-2* and *OE-3* seedlings respectively has been presented below the blot image. Representative gel blot images from at least three independent experiments are shown.

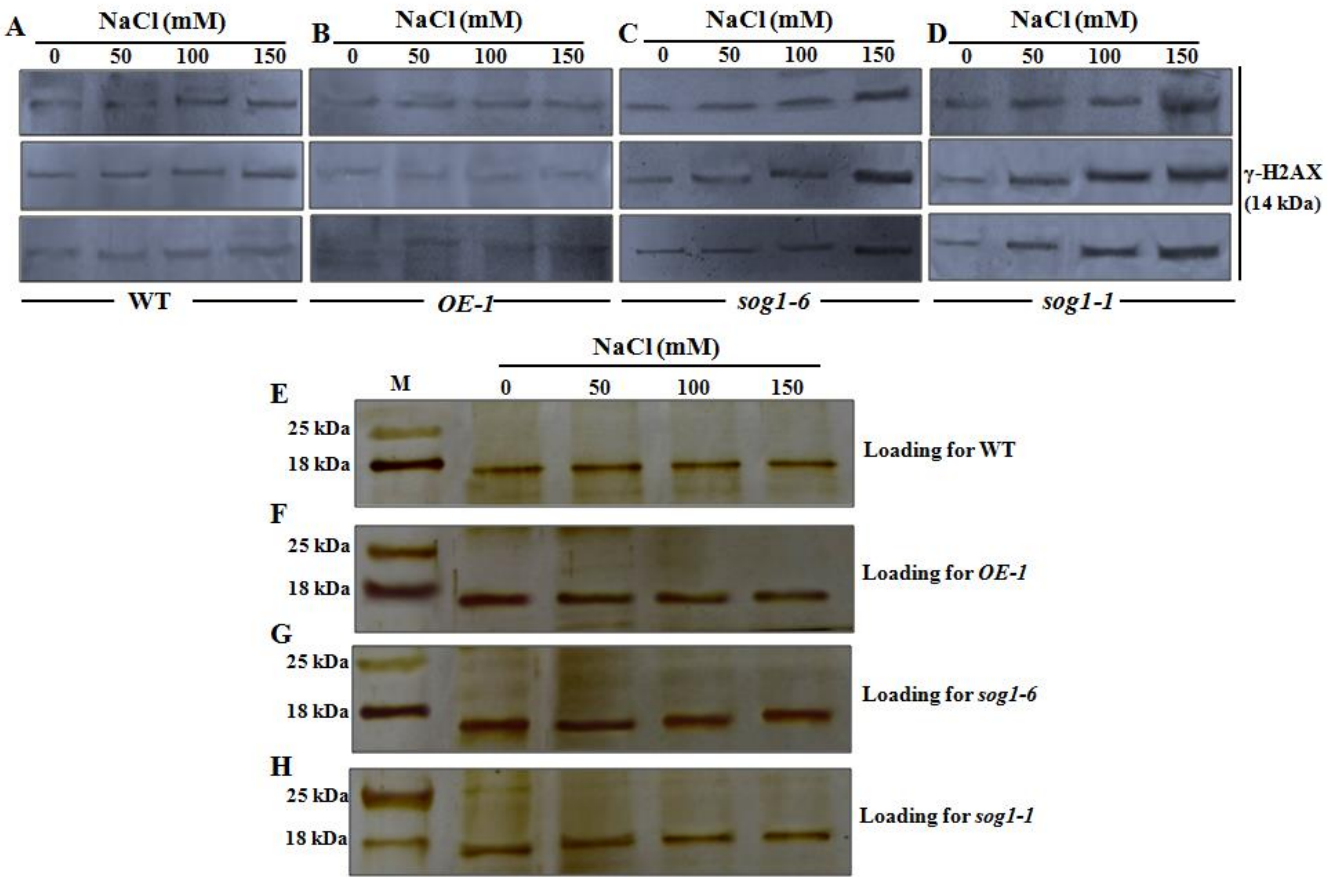

Fig. S11: Replicates of γ-H2AX gel blots. (A-D) Representative triplicates of γ-H2AX gel blots using the total histone protein isolated from untreated and NaCl treated 7-days old wild-type, *OE-1*, *sog1-6* and *sog1-1*mutant plants, respectively. (E-H) Respective loading controls from the four genotypes.

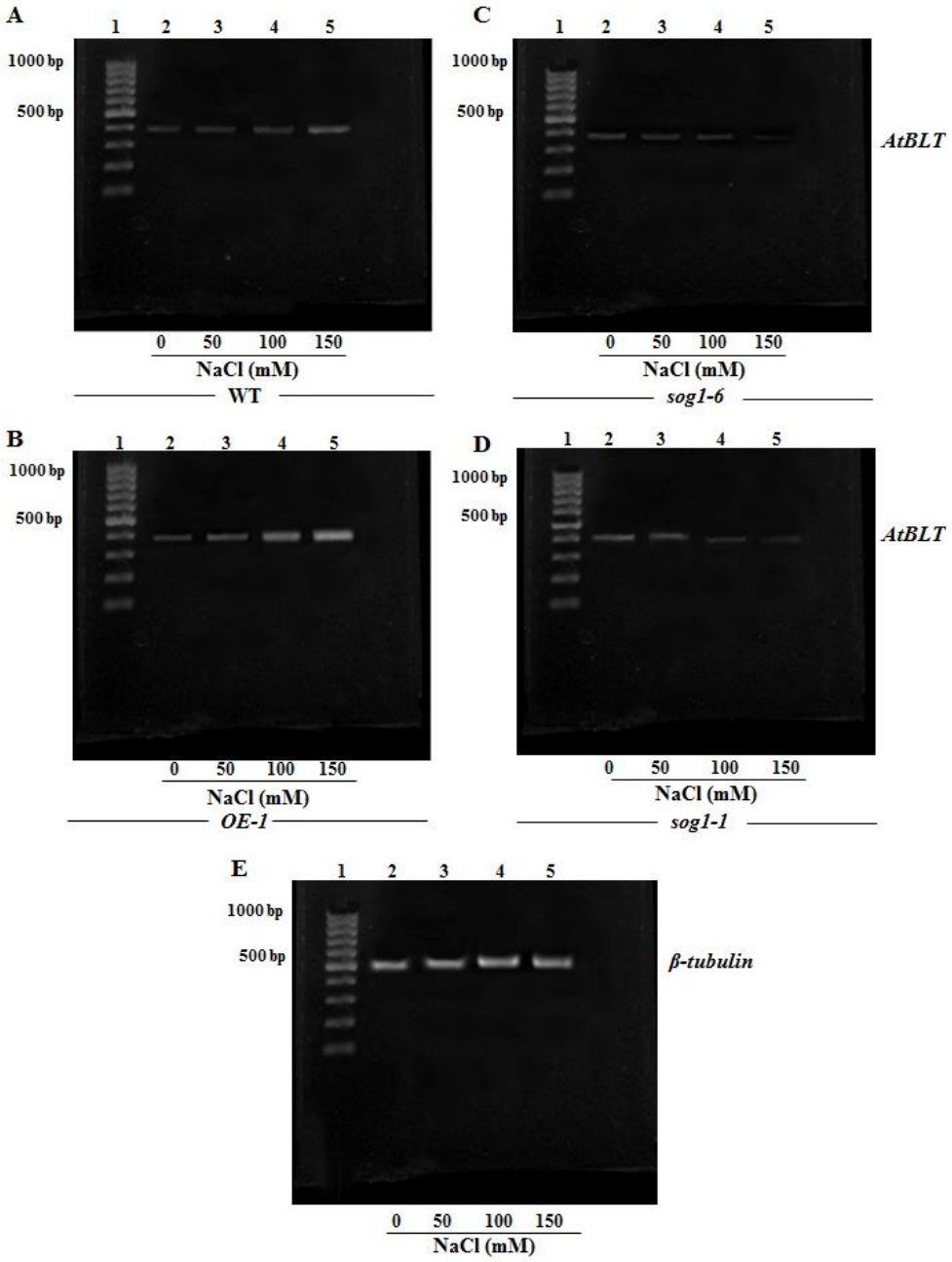

Fig. S12: Full length gel images of *AtBLT* expression. (A-D) Representative full length semi quantitative RT-PCR gel images showing expression level of *AtBLT* gene in 7-days old wild-type, *OE-1*, *sog1-6* and *sog1-1*mutant plants, respectively in response to increasing NaCl concentrations. (E) Full length gel image of *β-tubulin* gene expression. Lane1: Marker; Lane 2-5: 0, 50, 100 and 150 mM NaCl, respectively.

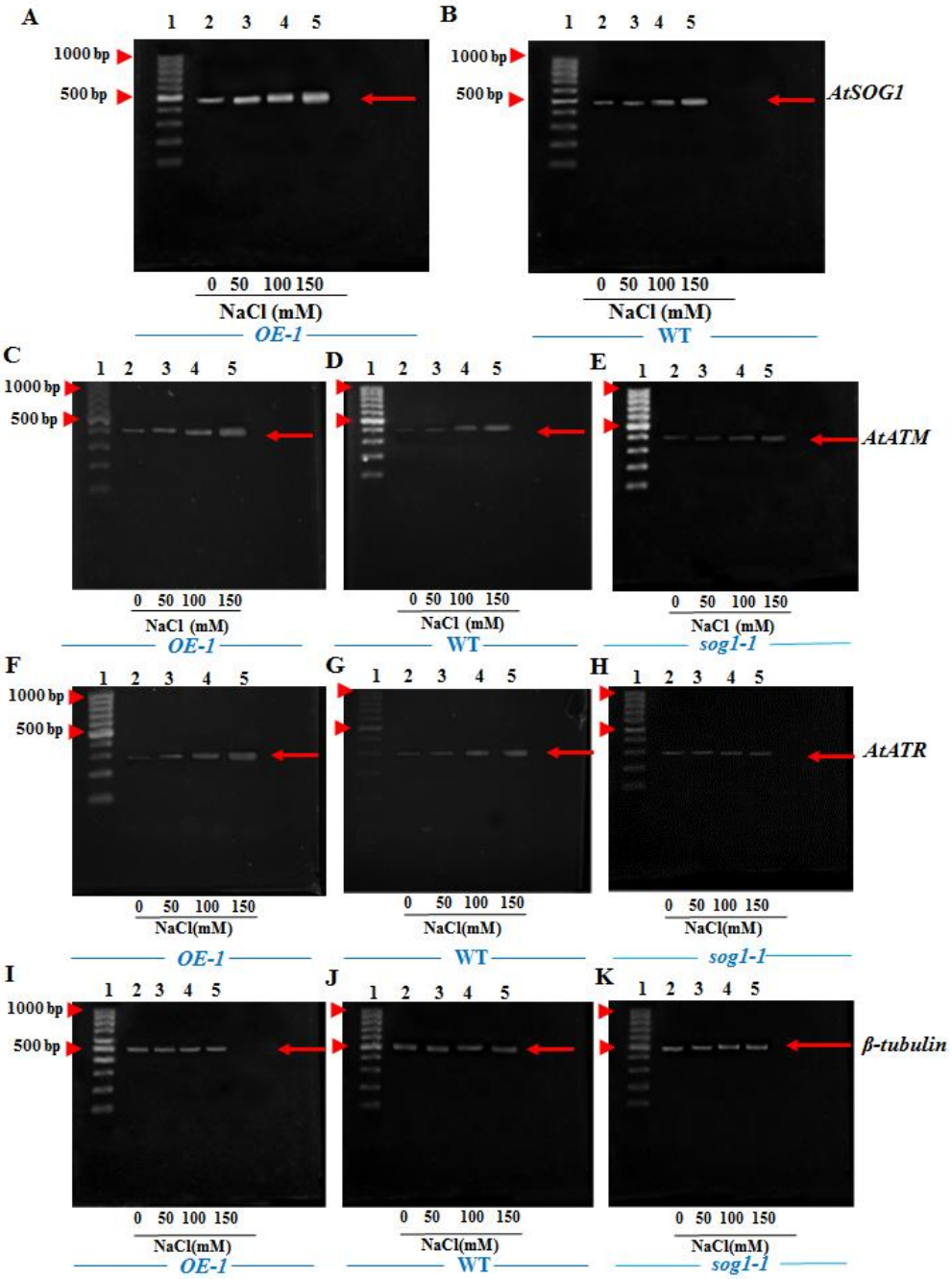

Fig. S13: Full length gel images of *AtSOG1*, *AtATM*, *AtATR* and *β-tubulin* gene expression. (A and B) Representative full-length semi-quantitative RT-PCR gel images showing expression level of *AtSOG1* gene in 7-days old *OE-1* (A) and wild-type (B) seedlings in response to increasing salinity. (C-K) Representative full length semi quantitative RT-PCR gel images showing expression level of *AtATM* (C-E), *AtATR* (F-H) and *β-tubulin* (I-K) genes in *OE-1*, wild-type and *sog1-1* mutant plants, respectively. Lane1: Marker; Lane 2-5: 0, 50, 100 and 150 mM NaCl, respectively.

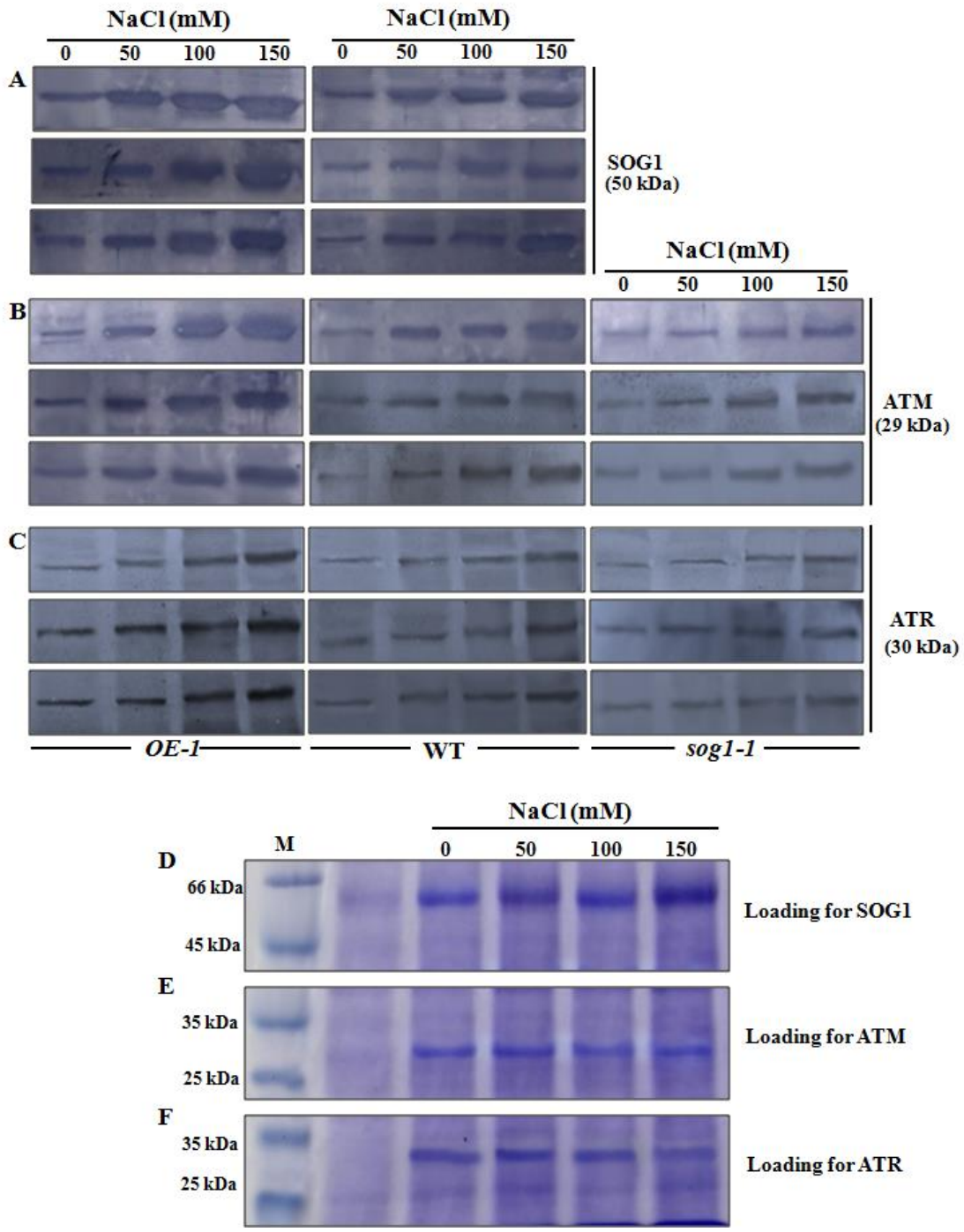

Fig. S14: Replicates of SOG1, ATM and ATR gel blots. (A) Representative triplicates of SOG1 protein gel blots using the total protein isolated from untreated and NaCl treated of 7-days old *OE-1* and wild-type plants, respectively under increasing salinity. (B and C) Representative triplicates of ATM and ATR protein gel blots, respectively using the total protein isolated from 7-days old *OE-1*, wild-type and *sog1-1*mutant plants respectively in response to increasing NaCl. (D-F) Full length gel images of respective loading controls.

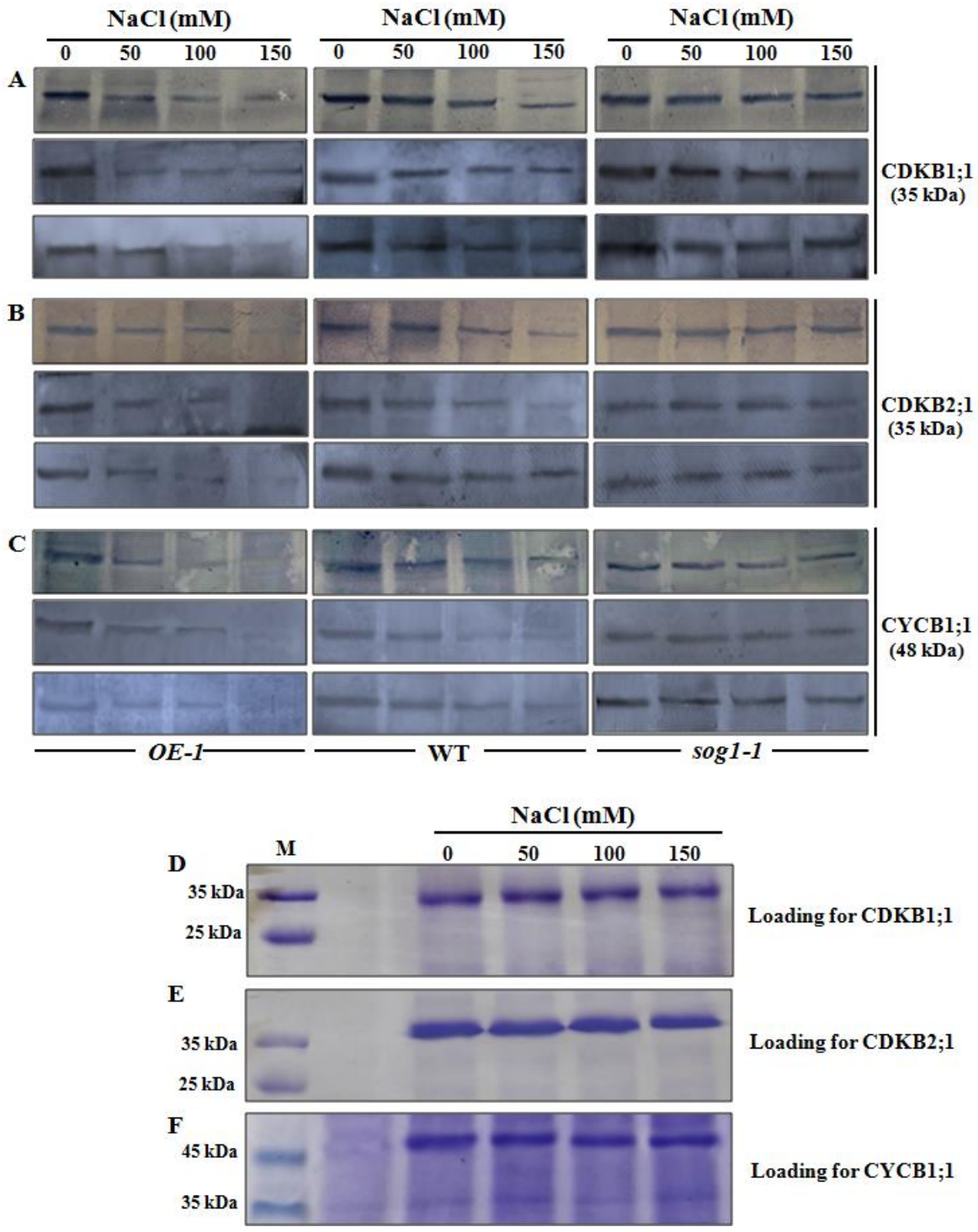

Fig. S15: Replicates of cell cycle regulatory protein gel blots. (A-C) Representative triplicates of CDKB1;1, CDKB2;1 and CYCB1;1 protein gel blot, respectively using the total protein isolated from 7-days old *OE-1*, wild-type and *sog1-1*mutant plants respectively in the presence of increasing salinity. (D-F) Full length gel images of respective loading controls.

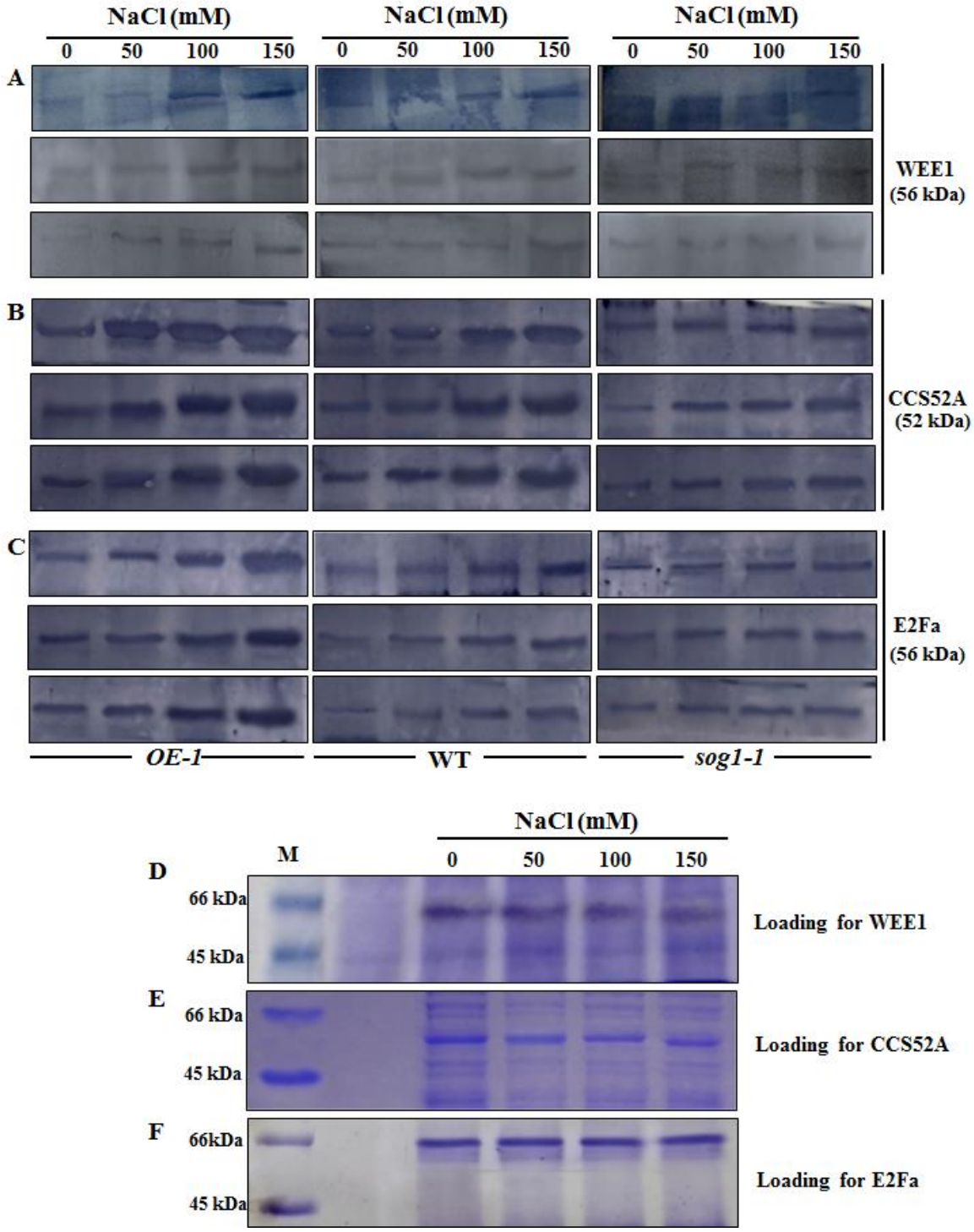

Fig. S16: Replicates of cell cycle and endocycle regulatory protein gel blots. (A-C) Representative triplicates of WEE1; CCS52A and E2Fa protein gel blot, respectively using the total protein isolated from 7-days old *OE-1*, wild-type and *sog1-1*mutant plants respectively in the presence of increasing salinity. (D-F) Full length gel images of respective loading controls.