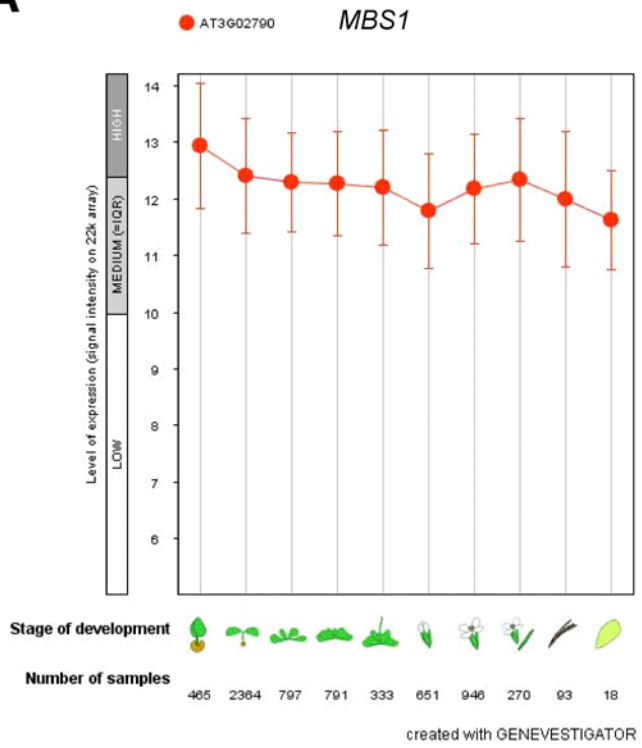
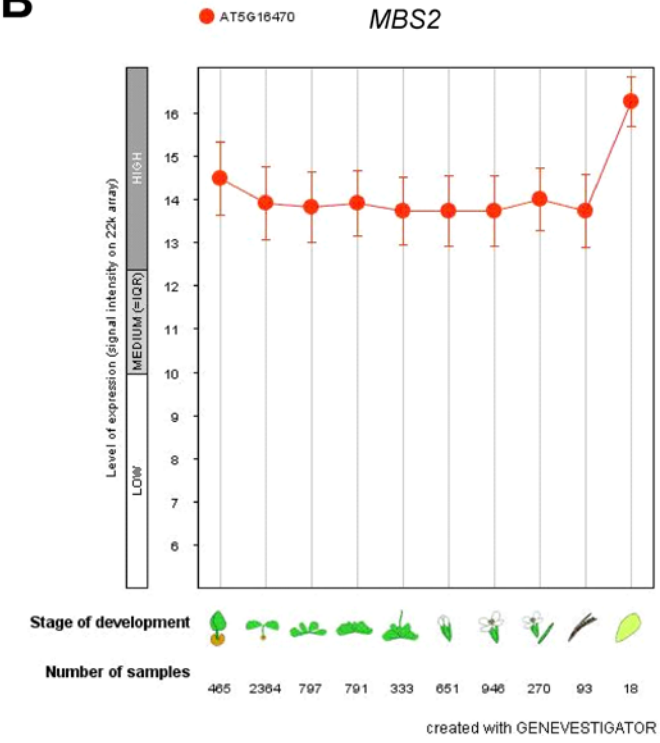
SUPPLEMENTAL DATA

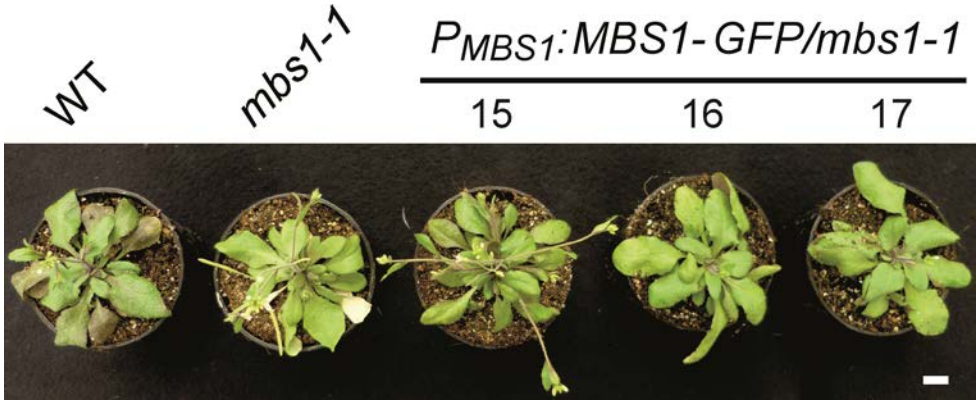
A



B

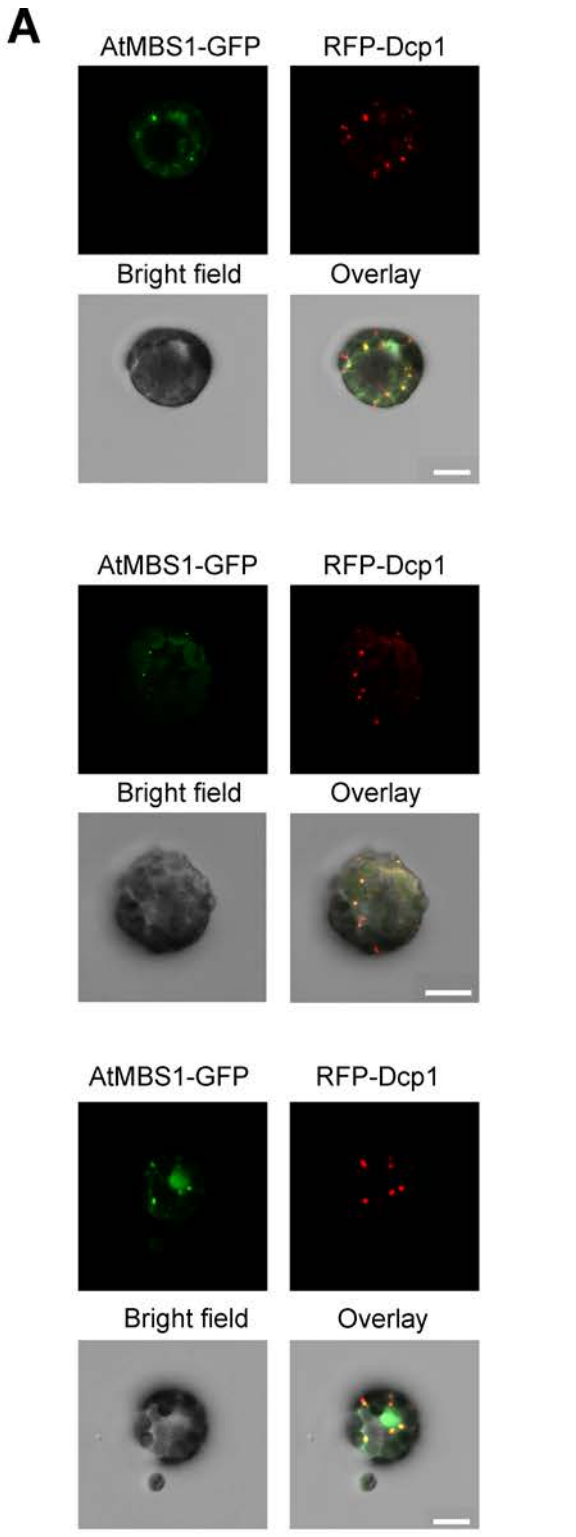


Supplemental Figure 1. Expression patterns of (**A**) *MBS1* and (**B**) *MBS2* in different tissues and developmental stages of *Arabidopsis thaliana*. The data are based on Affymetrix GeneChip data and were extracted from the Genevestigator database (https://www.genevestigator.com). The symbols representing the different tissues and developmental stages stand for (from left to right): germinated seeds, seedling, young rosette, developed rosette, bolting rosette, young flower, developed flower, flowers and siliques, mature siliques and senescent leaves.

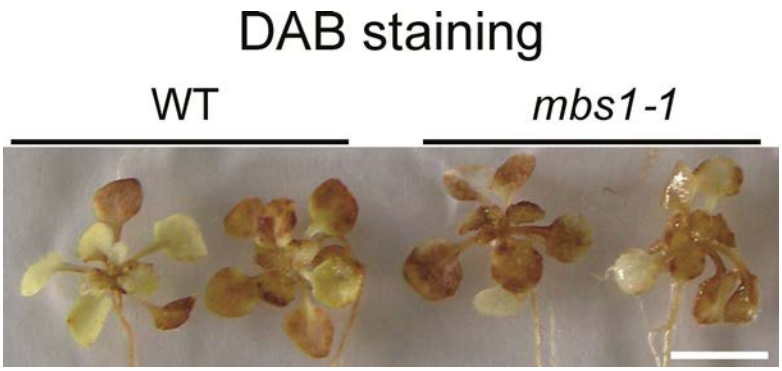


Supplemental Figure 2. Phenotypic analysis of three additional complemented lines of the *Arabidopsis mbs1-1* mutant under high light stress (cf. Figure 2C).

A wild-type plant (WT), the *mbs1-1* mutant and three independently generated complemented lines (P_{MBS1}*:MBS1-GFP*/*mbs1-1* lines 15, 16 and 17) are compared. Scale bar: 1 cm.

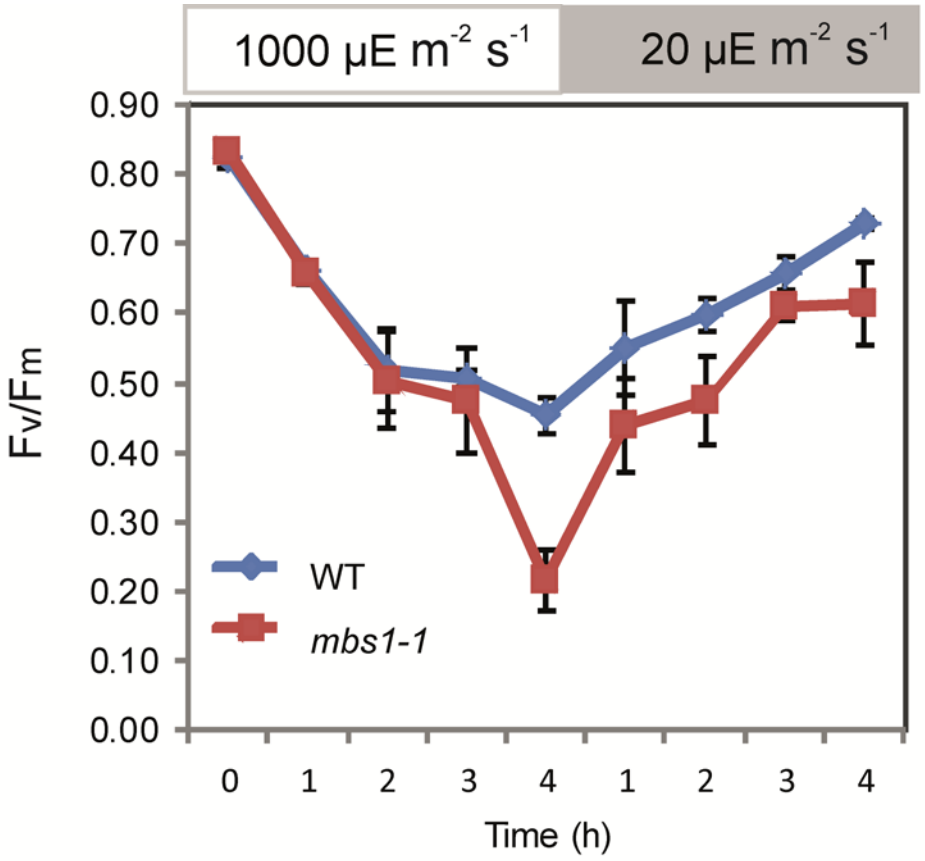

A
AtMBS1-GFP
RFP-Dcp1
Bright field
Overlay
AtMBS1-GFP
RFP-Dcp1
Bright field
Overlay
AtMBS1-GFP
RFP-Dcp1
Bright field
Overlay
B
RNase inhibitor
RNase A 10 min
MBS1-GFP

Supplemental Figure 3. AtMBS1 localization to P-bodies (PBs) under $^1O_2$ stress and RNase sensitivity of MBS-containing cytosolic granules. (**A**) Localization to PBs. The AtMBS1-GFP fusion protein was transiently co-expressed in tobacco protoplasts with the RFP-labeled PB marker Dcp1 (Weber et al., 2008). Co-localization was observed 30 min after induction of $^1O_2$ stress by exposure to high light (500 µE m^{-2} s^{-1}) in the presence of 0.5 µM MB (upper set of images), 1 µM MB (middle set) or under heat stress at 39°C (lower set). Three sets of images are shown. Scale bars: 10 µm. (**B**) Microscopic analysis of cytosolic granules prepared according to a protocol for P-body enrichment (Teixeira et al., 2005) from *P_{MBS1}:MBS1-GFP/mbs1-1* plants after high-light stress. The preparations of enriched granules were incubated in the presence of RNase inhibitor or RNase A for 10 min at room temperature and then examined for GFP fluorescence in the confocal microscope. Scale bar: 10 µm.



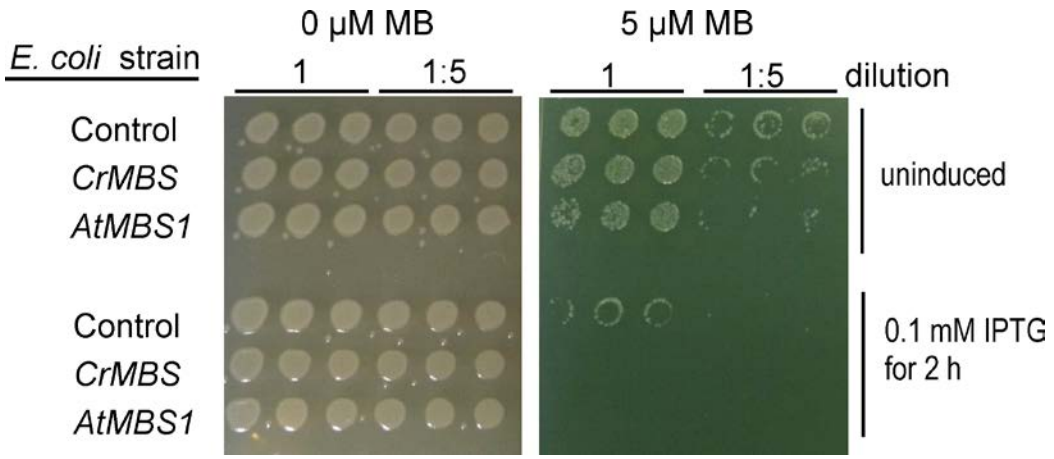
Supplemental Figure 4. Production of hydrogen peroxide (H_2O_2) in response to high-light stress in *Arabidopsis* wild type (WT) seedlings and the *mbs1-1* mutant.

20 day-old seedlings pretreated in high light (HL, 900 µE m^{-2} s^{-1}) for 2 days were stained with DAB for 3 h in the dark and then exposed to HL for 1 h. To visualize the staining, the leaf pigments were extracted with ethanol. Scale bar: 0.5 cm.

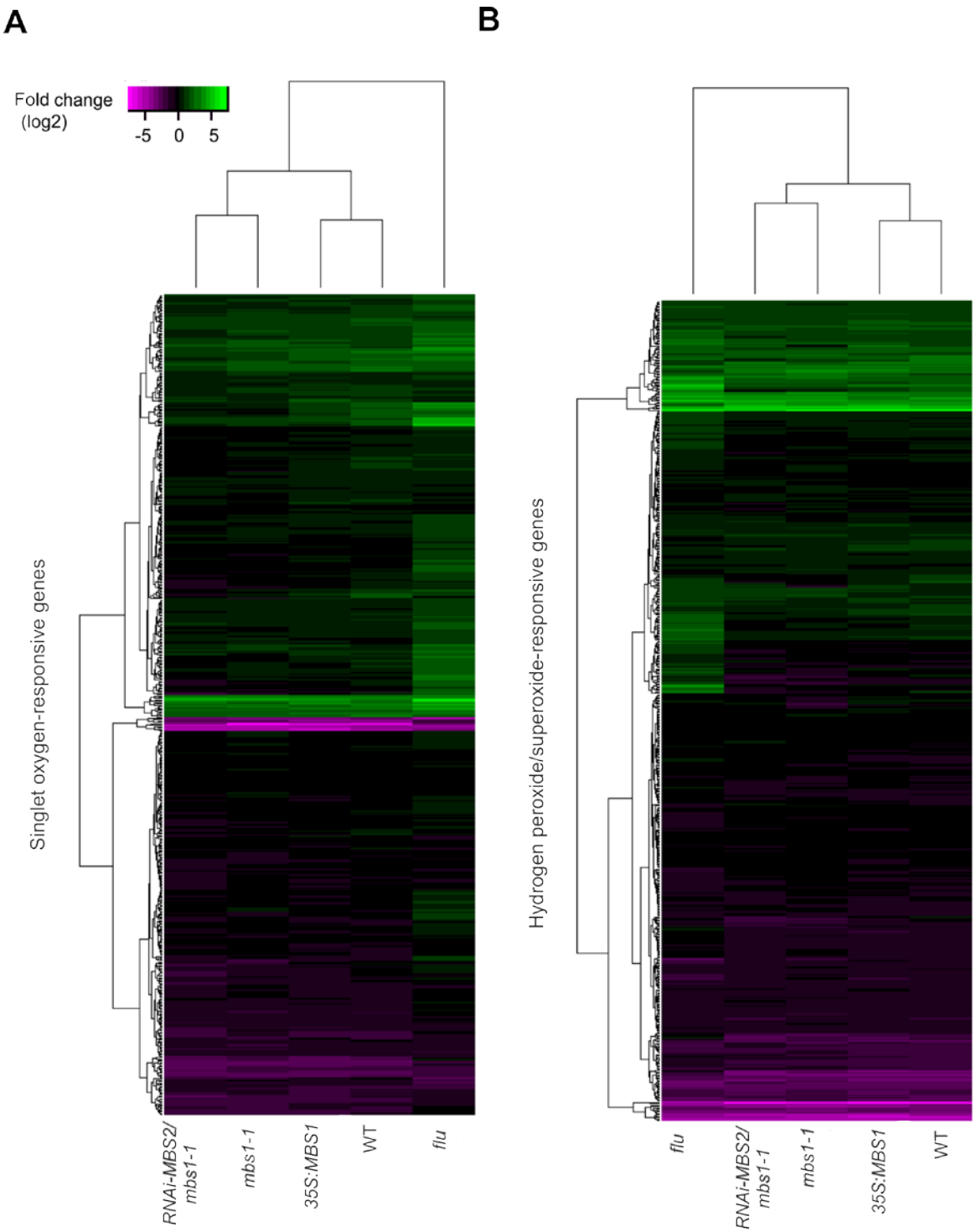


Supplemental Figure 5. Photosystem II (PSII) photoinhibition in leaves of *Arabidopsis* wild-type plants (WT) and *mbs1-1* mutant plants exposed to high-light stress.

PSII photoinhibition was measured as the decrease in the chlorophyll fluorescence ratio F_v/F_m. Leaves were exposed to 1000 µE m^{-2} s^{-1} for 4 h, and then allowed to recover at low light intensity (20 µE m^{-2} s^{-1}) for 4 h. The values represent the means of three biological replicates, error bars indicate the standard deviation.



Supplemental Figure 6. Test for a possible $^1O_2$-scavenging function of MBS proteins in *E. coli*. The *CrMBS* and At*MBS1* genes were overexpressed from pET vectors and compared to a control strain overexpressing an ovalbumin protein fragment. Protein expression was induced by addition of IPTG. Spot tests of two different concentrations of bacterial cells are shown. $^1O_2$ stress was applied by growth on LB medium containing 5 µM MB in the light. Each sample was analyzed in triplicate. Note that tolerance to $^1O_2$ stress in the MBS-expressing strains is not elevated compared to the control strain. It may even be slightly lower (as evidenced by some growth of the undiluted control strain in the presence of MB), presumably due to the metabolic burden imposed by massive overexpression of the MBS proteins.

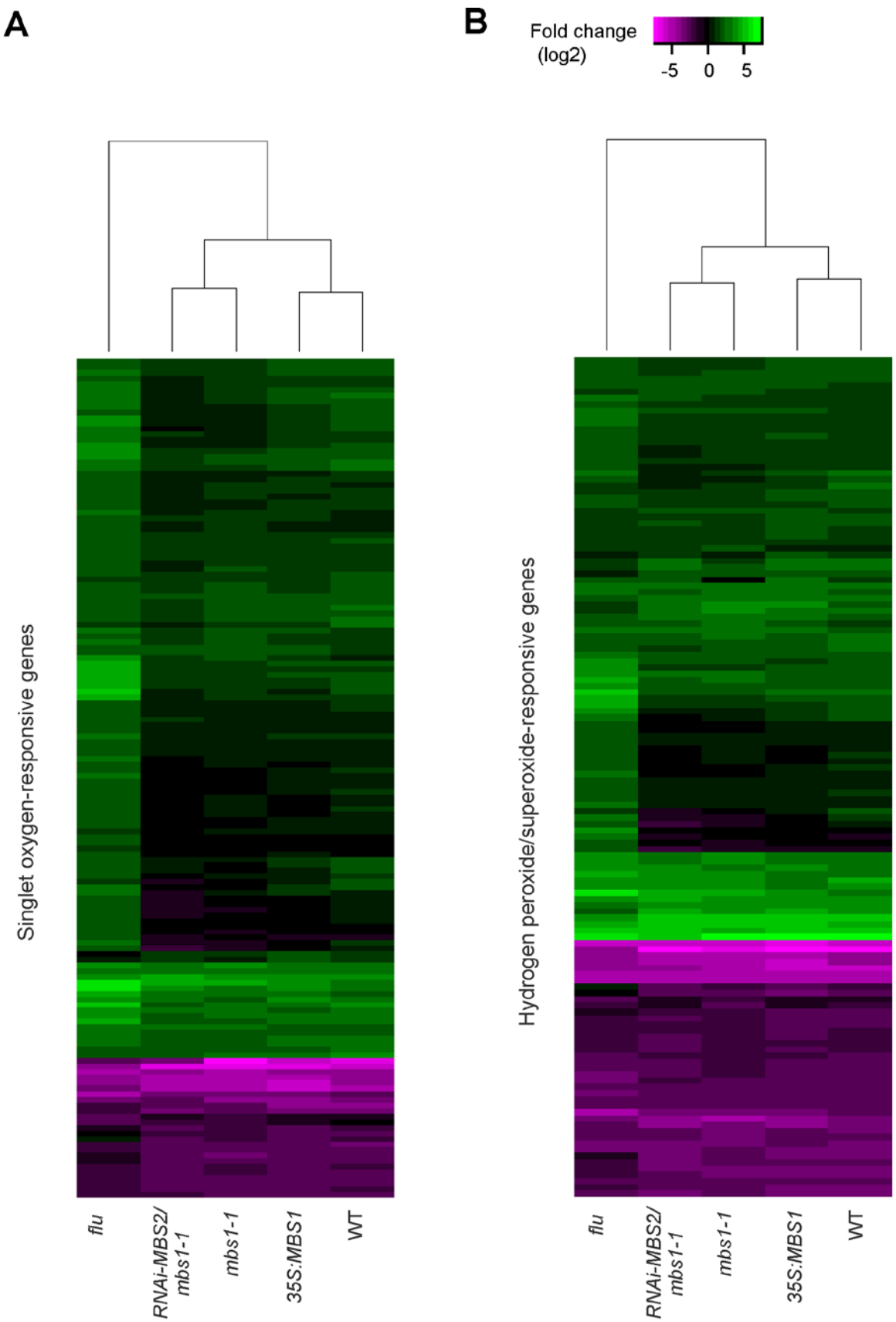


Supplemental Figure 7. Light stress response of genes previously reported as responsive to reactive oxygen species. Heat maps of microarray data are shown for the wild type (WT), the

mbs1-1 mutant, the *RNAi-MBS2/mbs1-1* double mutant, the *35S:MBS1* overexpression line and the *flu* mutant.

(**A**) Heat map of $^1O_2$-responsive genes (op den Camp et al., 2003; Gadjev et al., 2006).

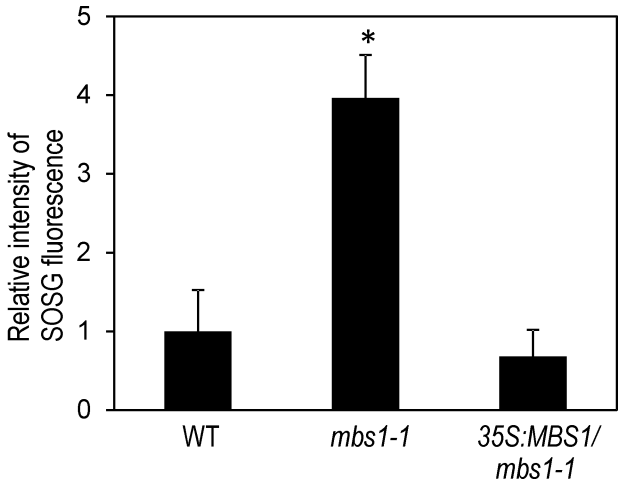
(**B**) Heat map of H_2O_2/O_2^--responsive genes (Gadjev et al., 2006; Supplemental Dataset 1 online). For each gene and plant line, the ratio of the expression value prior to the high-light stress to the expression value 3 h after the onset of the high-light stress was calculated. Red indicates up-regulation and green indicates down-regulation (cf. Figure 7).


A
B
Fold change
(log2)
-5
0
5
Singlet oxygen-responsive genes
Hydrogen peroxide/superoxide-responsive genes
flu
RNAi-MBS2/
mbs1-1
mbs1-1
35S:MBS1
WT

Supplemental Figure 8. ROS-responsive genes that are significantly changed in their expression under light stress (*p*-value threshold: 0.05, Student's t-test with FDR correction and |log2-fold change|≥2) in at least one of the plant lines analyzed (WT = wild type, *mbs1-1*, *RNAi-MBS2/mbs1-1*, *35S:MBS1* and *flu*).

(**A**) Heat map of $^1O_2$-responsive genes.

(**B**) Heat map of H_2O_2/O_2^--responsive genes genes (Gadjev et al., 2006; Supplemental Dataset 1 online). For each gene and plant line, the ratio of the expression value prior to the high-light stress to the expression value 3 h after the onset of the high-light stress was calculated. Red indicates up-regulation and green indicates down-regulation (cf. Supplemental Dataset 2 online; Figure 7).



Supplemental Figure 9. Quantitation of $^1O_2$ accumulation in leaves of the *Arabidopsis* wild type (WT), the *mbs1-1* mutant and the *35S:MBS1/mbs1-1* transgenic line under light stress. Seedlings were stained with singlet oxygen sensor green (SOSG) and the fluorescence intensities were quantified using the software Image J. Fluorescence intensities are displayed as relative values to the WT after the HL treatment, to which a value of 1 was assigned. Data represent mean values of ten biological replicates. The standard deviation is indicated by error bars. Significant differences are indicated by asterisks ($p < 0.01$).

Supplemental Table 1. Oligonucleotides used for *Chlamydomonas*. Restriction sites are underlined, lowercase letters indicate extra nucleotides introduced at the 5' end.

Primer	**Sequence 5'→3'**	**Gene**
LMS-Hyg-f	CTGCACGACTTCGAGGTGTT	*aph7"*
LMS-Hyg-r	TCCGAATCAATACGGTCGAG	
Inv-Hyg-f	GAACTGCTCGCCTTCACCTTC	*aph7"*
Inv-Hyg-r	CTCCCAGAATTCCTGGTCG	
NdeI-ZF	ggaattcCATATGGGAAAGGCTAAGCCC	Cr-*MBS*
ZF-EcoRI	gGAATTCTCACTTCTTGGTAGATCCGC	
NdeHpa-ZF	ggaattcCATATGGTTAACGGAAAGGCTAAGCCC	Cr-*MBS*
ZF-SnaBEcoRI	gGAATTCTCATACGTACTTCTTGGTAGATCCGC	
HpaI-YFP	GTTAACGTGAGCAAGGGCGAGG	*YFP*
YFP-SnaB	TACGTACTTGATCAGCTCGTCCATGC	
YFP-TGA-EcoRI	gGAATTCTCACTTGATCAGCTCGTCCATGC	*YFP*
SnaB-YFP	TACGTAGTGAGCAAGGGCGAGG	

Supplemental Table 2. Oligonucleotides for genotyping of T-DNA insertion lines.

Primer	**Sequence 5'→3'**	**Gene**	**AGI code**
At3g02790-I-f	TTCCATTTTCTCACCGACCAA	*MBS1*	At3g02790
At3g02790-I-r	TTCTTCAAGCTTCCCCTGAT		
at5g16470-I-f	CGGTTTCTGTTTTATGAAATG	*MBS2*	At5g16470
at5g16470-I-r	TAAGGCTTCCTCTGATTCCA		
LB-SALK-b1.3	ATTTTGCCGATTTCGGAAC		
LB-Wisc	AACGTCCGCAATGTGTTATTAAGTTGTC		
LB-SAIL	GCCTTTTCAGAAATGGATAAATAGCCTTGCTTCC		
At3g02790-ATG	ATGACTGGTAAGGCCAAGC	*MBS1*	At3g02790
At5g16470-ATG	ATGACAGGCAAAGCAAAG	*MBS2*	At5g16470

Supplemental Table 3. Transformation vectors for the generation of RNAi lines and their gene-specific tags (GST).

The sizes of the coding regions (CDS) of the wild-type alleles and the region of each locus covered by the RNAi construct are also given.

Gene	AGI code	Vector	GST (bp)	CDS (bp)	Region covered
MBS1	At3g02790	pENTR207_CATMA3a01730	180	318	252-431
MBS2	At5g16470	pENTR207_CATMA5a14770	300	315	120- 419

Supplemental Table 4. Oligonucleotides for transgenic constructs in *Arabidopsis*.

Restriction sites are underlined, lowercase letters indicate extra nucleotides introduced at the 5' end.

Primer	**Sequence 5'→3'**	**Gene**	**AGI code**
BamH-3g02790	cgGGATCCATGACTGGTAAGGCCAAGC	*MBS1*	At3g02790
At3g02790-XbaI	gcTCTAGAACCTTCTTCAAGCTTCCCCTGATAC		
SacI-3g02790	cGAGCTCTAACCTCCCATATTTTTG	*MBS1*	At3g02790
At3g02790-NcoI	ttCCATGGCCTTCTTCAAGCTTCCCCTGATAC		
XhoI-At5g16470	ttCTCGAGATGACAGGCAAAGCAAAGCC	*MBS2*	At5g16470
At5g16470-NcoI	ttCCATGGCTTCTTAAGGCTTCCTCTGATTC		
XhoI-At3g02790	ttCTCGAGATGACTGGTAAGGCCAAGCCA	*MBS1*	At3g02790
At3g02790-NcoI	ttCCATGGCCTTCTTCAAGCTTCCCCTGATAC		
NdeI-At3g02790	ggaattcCATATGACTGGTAAGGCCAAGC	*MBS1*	At3g02790
At3g02790 EcoRI	gGAATTCTTACTTCTTCAAGCTTCCCC		

Supplemental Table 5. Oligonucleotides for RT-PCR.

Primer	**Sequence 5'→3'**	**Gene**	**AGI code**
Actin2-F	TCTTCTTCCGCTCTTTCTTTCC	*Actin2*	AT3G18780
Actin2-R	TCTTACAATTTCCCGCTCTGC		
Ubiq RT-f	GATCTTTGCCGGAAAACAATTGGAGGATGGT	*Ubi10*	At4G05320
Ubiq RT-r	CGACTTGTCATTAGAAAGAAAGAGATAACAGG		
NdeI-ZF	GGAATTCCATATGGGAAAGGCTAAGCCC	Cr-*MBS*	-
ZF-EcoRI	GGAATTCTCACTTCTTGGTAGATCCGC		
At3g02790-ATG	ATGACTGGTAAGGCCAAGC	*At-MBS1*	At3g02790
At3g02790-I-r	TTCTTCAAGCTTCCCCTGAT		
at5g16470-ATG	ATGACAGGCAAAGCAAAG	*At-MBS2*	At5g16470
at5g16470-I-r	TAAGGCTTCCTCTGATTCCA		

Supplemental Table 6. Oligonucleotides for quantitative real-time PCR.

Primer	Sequence 5'→3'	Gene	AGI code
PROFILIN 1-r	AGAGCGCCAAATTTCCTCAG	*PROFILIN1*	At2g19760
PROFILIN 1-f	CCTCCAGGTCCCTTCTTCC		
aaa-f	GGCAATCTTTCTCGTTTTACCC	*AAA-ATPase*	At3g28580
aaa-r	GCTCTATCGTCTTCCCTTCTCTC		
BAP1-f	GTGGGATCGTCAATCTTTCG	*BAP1*	At3g61190
BAP1-r	GGCCACCGTATCCATCAATC		
APX1-f	TGCCTTTTTCGCTGATTACG	*APX1*	At1g07890
APX1-r	CAAAAACAGCCATGACTCTCG		
ZAT12-f	TGCGAGTCACAAGAAGCCTA	*ZAT12*	At5g59820
ZAT12-r	GTGTCCTCCCAAAGCTTGTC		
CAT2qRT-f	AACTCCGCCTGCTGTCTG	*CAT2*	AT4g35090
CAT2qRT-r	ATAGGGCATCAATCCATC		
NOD-f	GAGGTCTTCAAAGGAACAAAGGAG	*NOD2*	At5g64870
NOD-r	CTGCTTCACATTGGCGTTG		
WRKY40-f	TCTCACTATTGGCGTTACTCG	*WRKY40*	At1g80840
WRKY40-r	CGAGAGCTTCTTGTTCTCAGC		
At3g50970f	CAAACAGGCGATCAAAGGAT	*Toll-IL-resistance domain protein*	At3g50970
At3g50970r	CAACACCACGAAGAAGCGTA		
At3gqRT3'UTR f	AAAGCTCGTGAATCTCCATGCTGT	*MBS1*	At3g02790
At3gqRT3'UTR r	GCACATTACACTCTTTAACATCAAA		
qSIB1P1	GACGACCACAACCTTTCTCCA	*SIB1*	At3g56710
qSIB1P2	CGCATTCAACGGCTCATAAC		
CYP5_qf	TCTTCCTCTTCGGAGCCATA	*CYP5*	At2g29960
CYP5_qr	AAGCTGGGAATGATTCGATG		