Supplemental data for

BLADE-ON-PETIOLE1 coordinates organ determinacy and axial polarity in Arabidopsis by directly activating *ASYMMETRIC LEAVES2*

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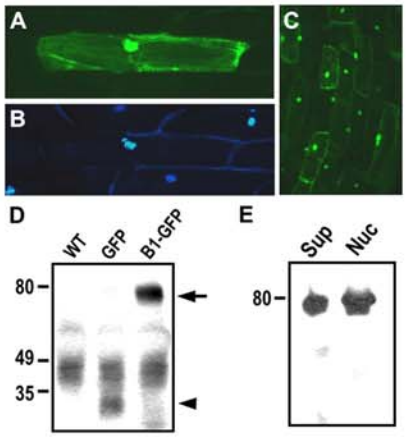
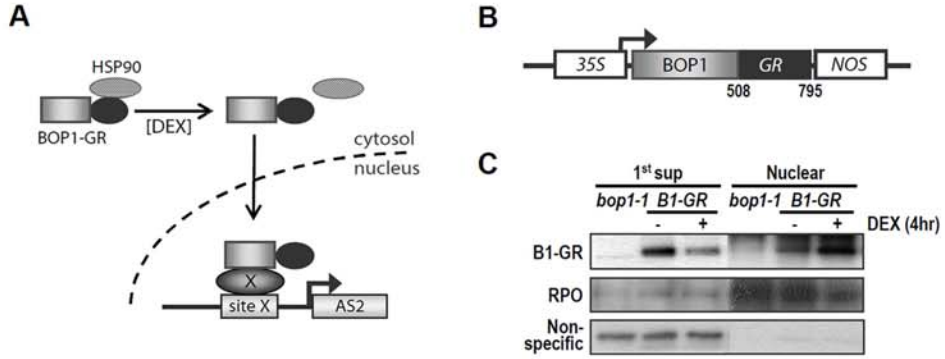
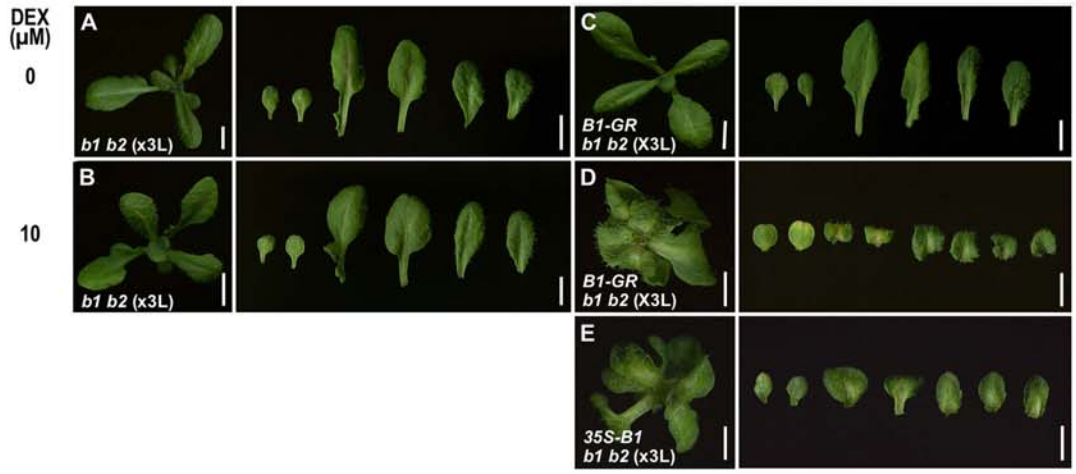


Supplemental Figure 1. Subcellular localization of BOP1 protein.
(*A*) Epidermal onion cells transiently transformed with a *35S$_{pro}$:BOP1-GFP* construct. After a 24 hour incubation the cells were observed under fluorescence using a Zeiss Axiophot microscope with BP 450/90 FT 510 LP 520 filters. (*B*) DAPI staining in the nuclei and cell wall of the onion cell in (*A*). (*C*) Confocal image of hypocotyl cells from a *35S$_{pro}$:BOP1-GFP* transgenic plant. Expression in onion cells and DAPI staining were prepared as described (Kinkema et al., 2000). (*D*) An immunoblot of seedling protein extracts probed with an anti-GFP antibody. No major degradation bands of the size of free GFP were observed. The arrow denotes the position of the band corresponding to full-length BOP1-GFP fusion protein. An arrowhead denotes the position of the band corresponding to GFP alone. WT, extract from a wild-type L*er* plant; GFP, extract from transgenic plants of *35S$_{pro}$:GFP* (Kinkema et al., 2000); B1-GFP, extract from a *35S$_{pro}$:BOP1-GFP* transgenic plant. (*E*) An immunoblot showing the distribution of BOP1-GFP fusion protein between the cytosolic (Sup) and nuclear (Nuc) fractions. Nuclei were enriched from 10-day-old *35S$_{pro}$:BOP1-GFP* seedlings.

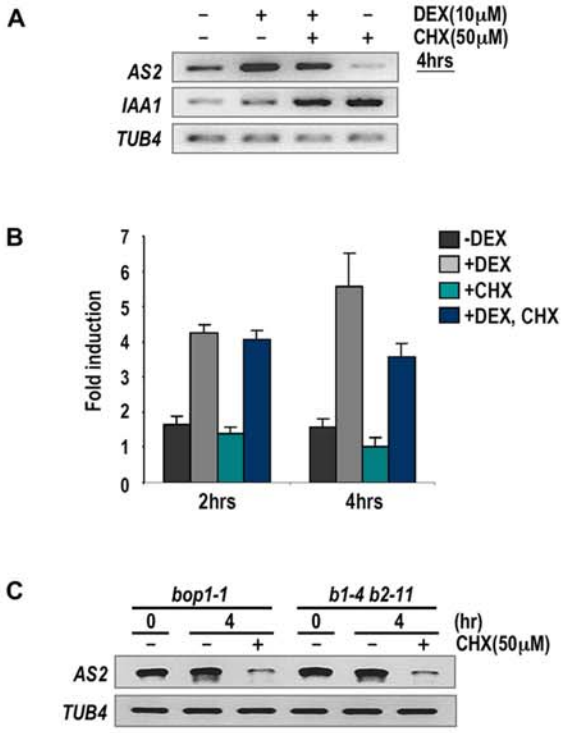


Supplemental Figure 2. Dex-dependent enrichment of BOP1-GR in the nuclear fraction.
(*A*) Strategy used to control the nuclear localization of BOP1 and working model for the regulation of the *AS2* locus by BOP1. Without Dex treatment, BOP1-GR protein is retained in the cytoplasm through an association with the heat shock protein HSP90. Because BOP1 is recruited to the *AS2* promoter upon Dex treatment, although it does not contain a known DNA binding domain, we hypothesize that BOP1 is recruited by an unknown protein (protein X) to a specific DNA sequence motif (site X) to function as a transcriptional coactivator. Alternatively, BOP1 may bind directly to the *AS2* promoter via an unidentified DNA binding domain. (*B*) Schematic representation of the $35S_{pro}$*:BOP1-GR* construct. GR, glucocorticoid receptor; 35S, CaMV 35S promoter; NOS, nos terminator. (*C*) An immunoblot showing the distribution of BOP1-GR fusion protein between the cytosolic (1st sup) and nuclear (Nuclear) fractions. Nuclei were enriched as described (Huq et al., 2003) from 15-day-old *bop1-1* and $35S_{pro}$*:BOP1-GR bop1-1* seedlings after incubation in the presence (+) or absence (-) of 10 μM Dex for 4 hours. Quantitative recovery and equivalent loading of nuclear-derived proteins were verified by probing same blot with an anti-RNA polymerase α-subunit (RPO) antibody (Covance). Non-specific protein detection by an anti-GR antibody (Santa Cruz Biotechnology) was used as a loading control for the cytosolic fraction.

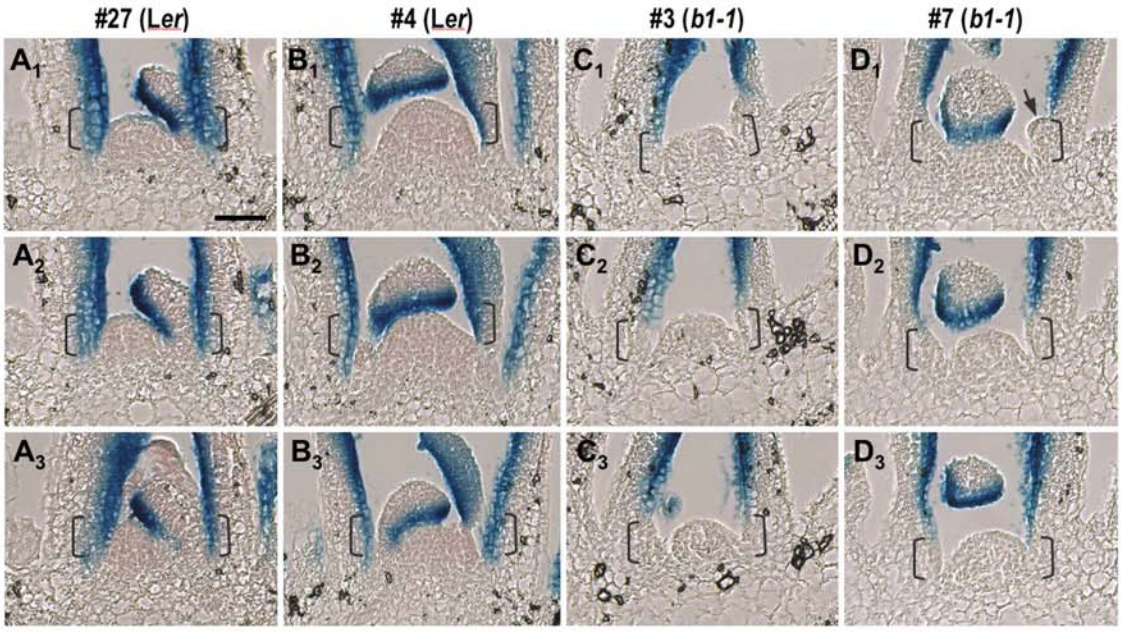
Supplemental Figure 3. Phenotypes of L*er* and *bop1-1* plants after Dex treatment.
(*A*) L*er*. (*B*) *bop1-1*. Representative 16-day-old whole plants (left panel) and heteroblastic leaf series (right panel) from cotyledon to leaf number two or four are shown for each line. Plants were grown on MS media containing 10 μM Dex under continuous light conditions. Bars: 5 mm.



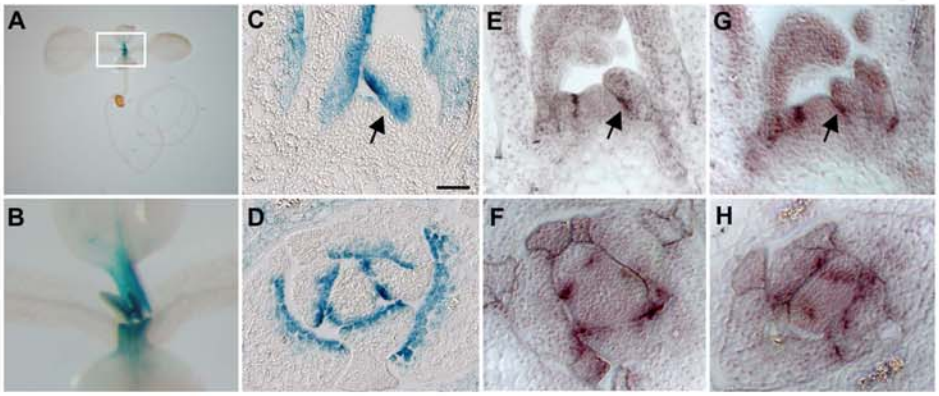
Supplemental Figure 4. Phenotypes of *35S$_{pro}$:BOP1-GR bop1-4 bop2-11* plants.
(*A*, *B*) *bop1-4 bop2-11* plants. (*C*, *D*) *35S$_{pro}$:BOP1-GR bop1-4 bop2-11* plants (*E*) *35S$_{pro}$:BOP1 bop1-4 bop2-11* plants. (*A-E*). Complementation of the *bop1-4 bop2-11* phenotype with the BOP1-GR fusion protein in a Dex-dependent manner, using *bop1-4 bop2-11* plants backcrossed three times into L*er* (X3L). Representative 16-day-old whole plants (left panel) and heteroblastic leaf series (right panel) from cotyledon to leaf number two or four are shown for each line. Plants were grown on MS media containing 0.1% EtOH (mock treatment) or 10 μM Dex under continuous light conditions. Bars: *A-C* 10 mm; *D, E* 5 mm.



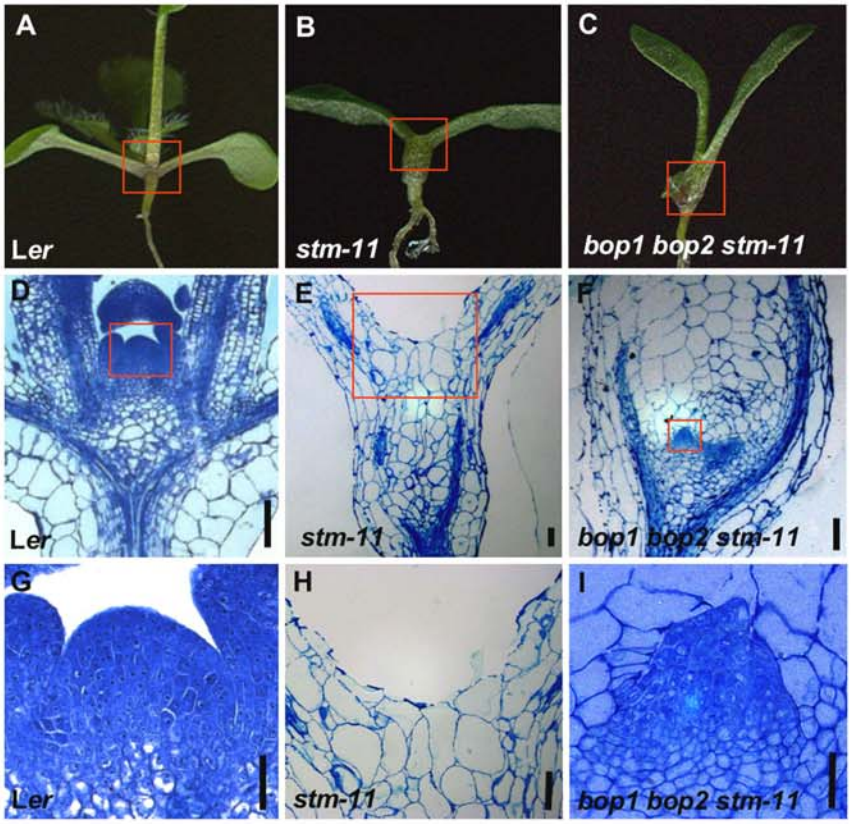
Supplemental Figure 5. Direct regulation of *AS2* expression by BOP1.
(*A*) RT-PCR analysis of *AS2* and *IAA1* expression in 11-day-old $35S_{pro}$:*BOP1-GR* plants 2 or 4 hours after mock, Dex, CHX or Dex+CHX treatment. *TUB4* was used as a control. (*B*) Real-time qRT-PCR analysis of *AS2* in 11-day-old $35S_{pro}$:*BOP1-GR* plants after 2 or 4 hours of mock (-Dex), Dex, CHX or Dex+CHX treatment. Expression values are normalized to *TUB4*. Mean transcript levels were determined by quantitative real-time RT-PCR analyses of three biological replicates. Error bars indicate s.d. (*C*) RT-PCR analysis of *AS2* expression in 11-day-old *bop1-1* and *bop1-4 bop2-11* plants after 4 hours of mock or CHX treatment. *TUB4* was used as a control. *AS2* transcript levels showed the same response to the addition of CHX alone in Dex-treated or non-treated BOP1-GR samples as they did in untransformed *bop* plants, indicating that this CHX effect appears to be independent of BOP1 function. In (A) and (C) the PCR products were visualized by EtBr staining in agarose gels.



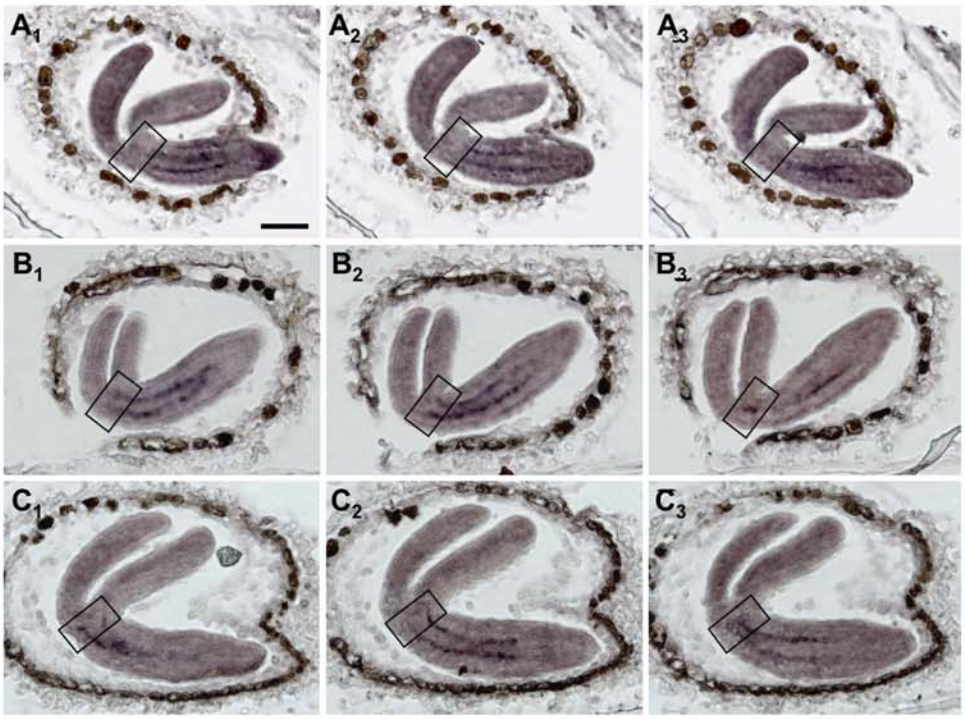
Supplemental Figure 6. Polar regulation of *AS2* expression by BOP in seedlings.
(*A, B*) Serial sections of a 10-day-old $AS2_{pro}$*:GUS* L*er* seedling shoot apex. (*C, D*) Serial sections of a 10-day-old $AS2_{pro}$*:GUS bop1-1* seedling shoot apex. Brackets denote the proximal region of the rosette leaves. The numbers at the top indicate independently transformed lines. The arrow indicates an ectopic outgrowth in the proximal region of a developing $AS2_{pro}$*:GUS bop1-1* rosette leaf. Bar: 100 μm.



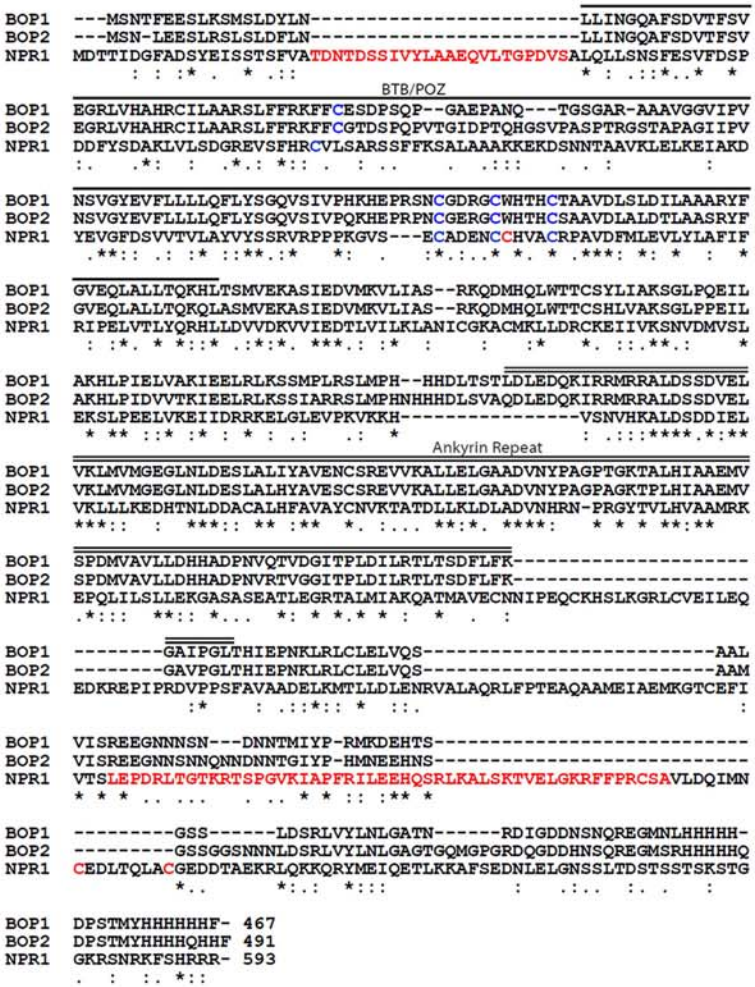
Supplemental Figure 7. Comparison of *AS2*, *BOP1* and *BOP2* expression patterns.
(*A*) GUS activity in 9-day-old $AS2_{pro}$:*GUS* L*er* seedlings. (*B*) Magnified view of the region boxed in (*A*). (*C*) Longitudinal section of a 9-day-old $AS2_{pro}$:*GUS* L*er* shoot apex. (*D*) Transverse section of a 9-day-old $AS2_{pro}$:*GUS* L*er* shoot apex. (*E, F*) RNA in situ hybridization of *BOP1* expression in a 9-day-old L*er* seedling. (*G, H*) RNA in situ hybridization of *BOP2* expression in a 9-day-old L*er* seedling. Arrows indicate where *BOP1* and *BOP2* expression overlaps with *AS2* expression in the proximal region of developing leaf primordia. Bars: *C*-*H* 100 μm.



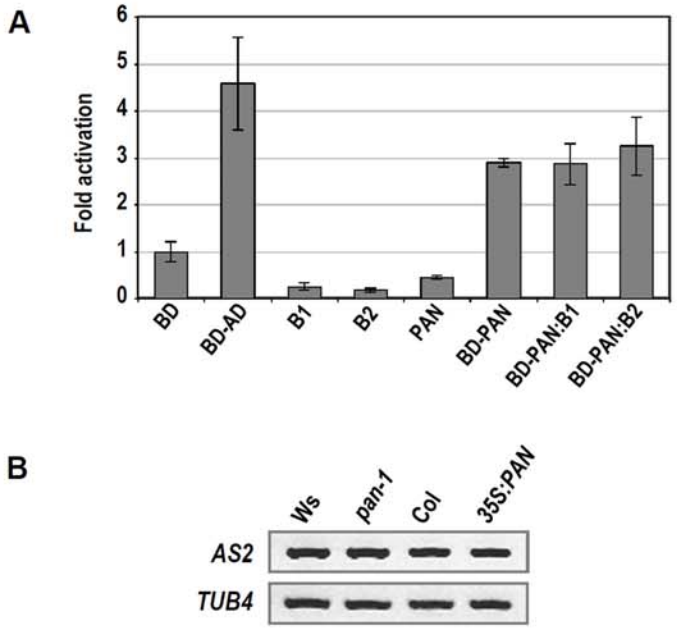
Supplemental Figure 8. Ectopic shoot meristem formation in rescued *stm-11* plants.
(*A*) A 10-day-old L*er* seedling. (*B*) A 10-day-old *stm-11* seedling. (*C*) A 10-day-old *bop1-4 bop2-11 stm-11* seedling. (*D, G*) Longitudinal section of (*A*). (*E, H*) Longitudinal section of (*B*). (*F, I*) Longitudinal section of (*C*). Boxes in (*A-C*) indicate the sectioned region in (*D-F*). (*G-I*) are magnified views of boxed regions in (*D-F*). Bars: *D-F* and *H* 100 μm; *G*, *I* 20 μm.



Supplemental Figure 9. Regulation of embryonic *BP* expression by BOP1 and BOP2.
(*A*) *BP* expression in serial sections of a bent-cotyledon stage L*er* embryo. (*B*) *BP* expression in serial sections of a bent-cotyledon stage *bop1-4 bop2-11* embryo. (*C*) *BP* expression in serial sections of a bent-cotyledon stage *bop1-1* embryo. Boxes denote the junction between the hypocotyl and base of the cotyledons. Bars: 100 μm.



Supplemental Figure 10. Multiple sequence alignment of the BOP1, BOP2 and NPR1 proteins. The alignment was obtained using the ClustalW 1.82 program. Periods, colons and asterisks mark partial to full amino acid conservation. The BTB/POZ domain and ankyrin repeat region are indicated with single and double lines, respectively. The two transactivation repression domains (amino acids 22 to 44 and 463 to 513) in NPR1 are shaded in red. The two oxidized cysteine residues (amino acids 521 and 529) in NPR1 that are involved in modulation of transactivation in SA-stimulated cells, as well as another cysteine residue (amino acid 156) that is required for both GSNO-triggered S-nitrosylation and oligomerization, are colored in red. Four other conserved cysteine residues (amino acids 82, 150, 155 and 160) in NPR1 that are required for oligomer-monomer exchange are colored in blue.



Supplemental Figure 11. Analysis of the relationship between BOP, AS2 and PAN.
(*A*) Transcription activation assays of BOP1, BOP2 and PAN. Gal4 BD-AD and BD were used as positive and negative controls, respectively. BD-PAN protein was mixed with BOP1 (BD-PAN:B1) or BOP2 (BD-PAN:B2) protein at a 1:1 ratio. Fold activation represents the relative luciferase units obtained for the given construct(s) divided by those obtained with the unfused Gal4 BD construct alone (n=9). Error bars represent the s.d. AD:GAL4 activation domain; BD:GAL4 DNA binding domain; B1:BOP1; B2:BOP2.
(*B*) RT-PCR analysis of *AS2* transcription in 11-day-old wild-type (Ws or Col), *pan-1* and $35S_{pro}$*:PAN* plants. The PCR products were visualized by EtBr staining in agarose gels and *TUB4* expression was used as a control.

Supplemental Table 1. Rescue of leaf phenotypes by the *BOP1$_{pro}$:AS2* construct.

Genotype	T_1 plants[a]	Rescued plants[b]	Extent of rescue	
			Complete	Partial[c]
bop1-4 bop2-11	230	12 (5.2%)	0	12
bop1-1	37	4 (10.8%)	0	4
as2-1	93	37 (39.8%)	0	37

[a] Total number of T_1 transgenic plants analyzed.
[b] Total number of T_1 transgenic plants that showed leaf development from ectopic SAM. The percentage of rescued plants is shown in parenthesis.
[c] Partially rescued plants displayed several complete suppression of the ectopic organ outgrowth *(bop)* or lobed leaf *(as2)* phenotype in more than one rosette leaf per plant, and in the remaining rosette leaves the extent of ectopic organ outgrowth or leaf lobing was reduced compared to untransformed control plants.

Supplemental Table 2. *$AS2_{pro}$:GUS* expression in wild-type, *stm* and *stm bop1 bop2* embryos.

Genotype	*$AS2_{pro}$:GUS* pattern	
	WT	Ectopic expression in shoot apex
L*er*	311	0
stm-11/+	458	140
bop1-4 bop2-11 stm-11/+	430	0

GUS activity was scored in bent cotyledon-stage embryos after genotyping the parental plants. WT, wild-type L*er*.

Supplemental Table 3. Oligonucleotides used in this study.

Purpose	Construct/ Gene	Name	Sequence
Cloning			
GUS reporter	*AS2$_{pro}$:GUS*	AS2$_{pro}$(1.0)	CACCGATGAGCTTTGCCCATCGA
		AS2$_{pro}$(2.1)	CACCATAGGGTTACTAATCATGGGT
		AS2$_{pro}$(2.6)	CACCCTGCTAGTACATAAGGTAAATG
		AS2$_{pro}$(3.2)	CACCTGAAAACGATATCAGCCAAACA
		AS2$_{pro}$(4.0)	CACCGCGGTAAATTGGTCTTCGCT
		AS2$_{pro}$-F1	CACCTAATGATCGGTGAGAGGGATT
		AS2$_{pro}$-R	TTTAATGACTTGAAAATGGAGTTT
		AS2$_{pro}$(2.6)-R	CATTTACCTTATGTACTAGCAG
		35$_{pro}$(-46)-PstI-F	AACTGCAGGCAAGACCCTTCCTCTATATG CAAGACCCTTCCTCTATATAAGGAAGTTC ATTTCATTTGGAGAGGA
		GUS(stop)-BstEII-R	GGGTAACCTCATTGTTTGCCTCCCTGCTTC ATTGTTTGCCTCCCTGCTGCGGTTTTTCAC CGAAGTTCATGCCAG
	BOP1$_{pro}$:GUS	BOP1$_{pro}$-6F	CACCTAACTCTTTGAAGCTGTATGT
		pBOP1$_{pro}$-R	TGAGTGATTCTTCGAAAGTATTG
Inducible BOP1 activation	*BOP1-GR*	FBN(3)	GGATCCGCGGCCGCTGCCGCAGCGGCAG CGGCAGCGAAATGGTGGTGGTGGTGATG
		FBN(1)	ATGAGCAATACTTTCCAACAA
Localization	*BOP1-GFP*	FKN(1)	ACGGTACCTATCAAAGAAATCAACAAAG GAGCT
		FBN(1)	CCGGATCCGCGAAATGGTGGTGGTGGTGA TG
Transactivation assay	*BD-BOP1*	TR-B1-T	GGATCCAGCAATACTTTCGAAGAATCACT
		TR-B1-R	GGTACCCTAGAAATGGTGGTGGTGGTGAT
	BD-BOP2	TR-B2-F	GGATCCAGCAATCTTGAAGAATCTTTGAG A
		TR-B2-R	GGTACCCTAGAAGTGATGTTGATGATGGT
	BD-bop1-1	TR-B1-T	GGATCCAGCAATACTTTCGAAGAATCACT
		TR-mb1-R	GGTACCCTACAAAAAGACGTAGAAATGG T
	BD-PAN	PAN-SmaI-F	CCCGGGATGCAGAGCAGCTTCAAAAC
		PAN-KpnI-R	GGTACCTTAGTCTCTAGGTCTGGCTA
Yeast two hybrid	*AD/BD-BOP1*	BOP1-Y2H-F	CACCAGCAATACTTTCGAAGAATCA
		BOP1-Y2H-R	CTAGAAATGGTGGTGGTGGT
	AD/BD-BOP2	BOP2-F(g)1	CACCAGCAATCTTGAAGAATCTTTGA
		BOP2-R(g)	CTAGAAGTGATGTTGATGAT
	AD/BD-bop1-1	BOP1-Y2H-F	CACCAGCAATACTTTCGAAGAATCA
		mbop1-Y2H-R	CTACAAAAAGACGTAGAAATGGT
Complementation	*BOP1$_{pro}$:AS2*	BOP1$_{pro}$-6-EcoRI-F	GGAATTCCTAACTCTTTGAAGCTGTATGT
		BOP1$_{pro}$-PstI-R	AACTGCAGCAGCTCCTTTGTTGATTTCTTT GA
		AS2-PstI-F	AACTGCAGCATGGCATCTTCTTCAACAAA CT
		AS2-BstEII-R	GGGTAACCCTCAAGACGGATCAACAGTA C
In situ probe	*BOP1*	IF(5)	ATAGTCTCTCTCTCTCTTCTT
		IF(3)	ACCATAACAACAATTTAATTAGATATTG
	BOP2	IIF(5)	CTTTCAGAGAGGAGGAGCAA
		IIF(3)	TAAAGATAAGATATTAATCGATGGCA
Expression analysis			
RT-PCR	*AS1*	AS1-RTF	AAACTTGGTGAGTCTGATATGCC
		AS1-RTR	AAGAGAGACAACGTTGGAGTGG
	AS2	AS2-RTF	TCAAGACGGATCAACAGTACGG
		AS2-RTR	CTTCTTCAACAAACTCACCATGC
	LOB/ASL4	LOB R	AAGATTTTGTGGACGTTGGC
		LOB F	TTGGAAGCGAAATTCAAAGG
	LBD36/ASL1	LBD36-F	TGCGCAGCTTGTAAATTCTTGA
		LBD36-R	TTGCGGTTGATGATGATGGTGT
	BP	KNAT1-F	GATGATCCCATATTGTCACTCTTCCC
		KNAT1-R	ATGGAAGAATACCAGCATGACAAC

	KNAT2	KNAT2-F	CCGAAGGCTTCCAATGGCG
		KNAT2-F	GCGGCGATCACTGATCGTATC
	KNAT6	KNAT6-F	TCATTCCTCGGTAAAGAATGATCCACTAG
		KNAT6-R	ATCTACAATTTCCATTCGGCCGGTG
	PHB	PHB-5	TGATGGTCCATTCGATGAGC
		PHB-3	TCTAAACTCACGAGGCCGCA
	FIL	FIL-3	GCTATGTCCAATGCAACTTT
		FIL-4	TTCTTGGCAGCAGCACTAAA
	IAA1	IAA1-F	ATGGAAGTCACCAATGGGC
		IAA1-R	TCATAAGGCAGTAGGAGCTTCGGATCC
	TUB4	TUB4-F	CCTCTTCTTCCTCCTCGTAC
		TUB4-R	AGAGGTTGACGAGCAGATGA
	EF1α	EF1-F	CAGGCTGATTGTGCTGTTCTTATCAT
		EF1-R	CTTGTAGACATCCTGAAGTGGAAGA
qRT-PCR	*AS2*	AS2-q-F1	ATCAACTTACTCGCCGGAGC
		AS2-q-R1	GTCCAATGGCAAGAATCCCA
	LOB/ASL4	LOB-q-F1	GAAGAAAAACAAATGGCGTCG
		LOB-q-R1	AGATTTTGTGGACGTTGGCG
	TUB4	TUB-q-F1	GGTCAATACGTCGGCGATTC
		TUB-q-R1	TCTGACCGAACGGACCAGAT
Chromatin IP			
qPCR	*AS2*	$AS2_{pro}$-4.2-qF	TCAACAAAACTCGGGCTACTTG
		$AS2_{pro}$-4.2-qR	GGACGTCAATCACACCAACAAA
		$AS2_{pro}$-3.0-qF1	TTGCAGACTCTGCTCCAACAGT
		$AS2_{pro}$-3.0-qR1	CGAAGAAGCGTGCAAAGAGAA
		AS2-q-F1	ATCAACTTACTCGCCGGAGC
		AS2-q-R1	GTCCAATGGCAAGAATCCCA
PCR	*AS2*	C-AS2-4.4-F	CGAATATAACTATATGTCCTATGT
		C-AS2-4.15-R	ATCAAATCCTCCCTATCAGA
		C-AS2-4.0-F	TCTTACCGTCTATAGTTTCCC
		C-AS2-3.7-R	TGAGTACATTATCACAATCCTC
		C-AS2-3.7-F	GAGGATTGTGATAATGTACTCA
		C-AS2-3.4-R	ACGATTAAGTAAAATCATCTCCA
		C-AS2-3.4-F	TGGAGATGATTTTACTTAATCGT
		C-AS2-3.2-R	TGTTTGGCTGATATCGTTTTCA
		C-AS2-3.0-F	CAACAATGCAATATGCAACCA
		C-AS2-2.7-R	CTTTGGGTTAAGCGATTGTC
		C-AS2-2.5-F	TACAAAAATGGCACACTTTATG
		C-AS2-2.2-R	CCATGATTAGTAACCCTATGA
		C-AS2-2.2-F	TCATAGGGTTACTAATCATGG
		C-AS2-1.9-R	AGTTTAGGATCAGTACTCCCTA
		C-AS2-1.6-F	AGCTTTTGTCAAACCGTATCA
		C-AS2-1.3-R	ACCCTAGCATAGACTCTAACA
		C-AS2-1.0-F	AGATGAGCTTTGCCCATCGA
		C-AS2-0.7-R	CAGACCTAGAGATATGTGTAG
		C-AS2-0.7-F	CTACACATATCTCTAGGTCTG
		C-AS2-0.4-R	TCTGCTTTCTTTAAGCTGCT
Genotyping			
Mutant isolation	*STM*	stm-11-F1	AGGTTGGAGCACCACCGGA
		stm-11-R1	AACCGGAGAAAGAGGAAGGT
	BP	bp-F1	GCATTACAAGTGGCCATATC
		bp-R1	CTTCTCTGACTCCTGCCACA

Supplemental Methods

Protein detection

Protein was extracted from 10 to 15-day-old seedlings as described (Huq et al., 2003). Forty micrograms of protein from the first supernatant and 20 μg protein from the nuclear fractions were separated on a 6-8% SDS-PAGE gel, blotted and probed with an anti-GFP antibody (Santa Cruz Biotechnology).

Construction of transgenic plants

The *BOP1-GFP* fusion construct was generated in the pEZS vector (Carnegie Institution) carrying 35S-MCS-$(Ala)_{10}$-EGFP using the KpnI and BamHI restriction sites, and the Not I cassette fragment transferred into pART27 (Gleave, 1992). Primer sequences are listed in Supplemental Table 3.

Expression analysis

For GUS staining, whole-mount embryo clearing was performed in Hoyer's medium (Liu and Meinke, 1998) and the samples visualized using a Zeiss Axiophot microscope equipped with Nomarski optics. For RT-PCR, cDNA was synthesized from 5 mg of total RNA using an oligo(dT_{18}) primer and SuperScript III reverse transcriptase (Invitrogen). For RT-PCR and real-time RT-PCR analysis, one and 0.1 microliter of the first-strand cDNA reaction was used as a template, respectively. The annealing temperature for RT-PCR was 55^{o}C for all primer pairs and the number of PCR cycles was *EF1α*: 24 cycles; *TUB4*: 26 cycles; *AS1*, *AS2*, *IAA1*: 27 cycles; *BP*, *KNAT2*, *KNAT6, FIL, PHB*: 30 cycles; *LOB*: 36 cycles; *LBD36*: 39 cycles. Primer sequences are listed in Supplemental Table 3.

Histological analysis

Seedling samples were fixed and prepared as described previously (Ha et al., 2003). Tissue sections (2 μm thick) were cut using a rotary microtome (MICROM International) and stained with methylene blue.

Transactivation assays

For the Gal4 DNA binding domain (BD) fusion, the *PAN* coding sequence was amplified and cloned into the SmaI and KpnI restriction sites in frame to the BD in the pMN6 plasmid (Huq et al., 2004). Primer sequences are listed in Supplemental Table 3. Up to 5 μg of each effector and reporter plasmid, as well as 0.2 μg of internal control plasmid, were delivered to the tissues by particle bombardment. Transcription activity was measured using the dual-Luciferase system (Promega) from three bombardments repeated three times.

Supplemental References

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