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Supplementary Materials for

Signaling from the Endoplasmic Reticulum Activates Brassinosteroid Signaling and Promotes Acclimation to Stress in *Arabidopsis*

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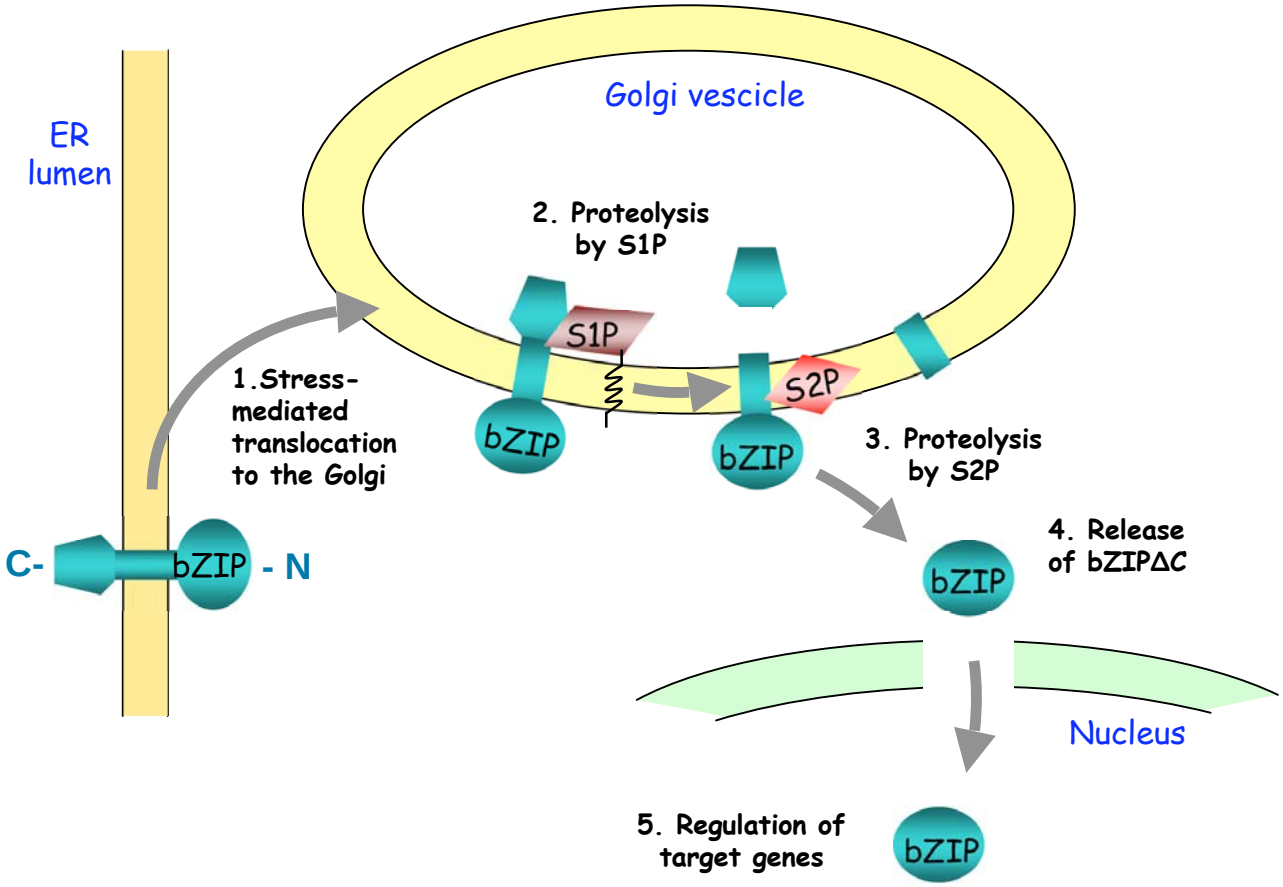

Fig. S1. A diagram of regulated intramembrane proteolysis. The details of this model are discussed in the text. In response to stresses, membrane-tethered transcription factors, such as ATF6 in human, or bZIP17 and bZIP28 in *Arabidopsis*, are translocated from the ER to the Golgi where they are released into the cytosol by sequential proteolytic cleavage by two membrane-localized proteases, S1P and S2P.

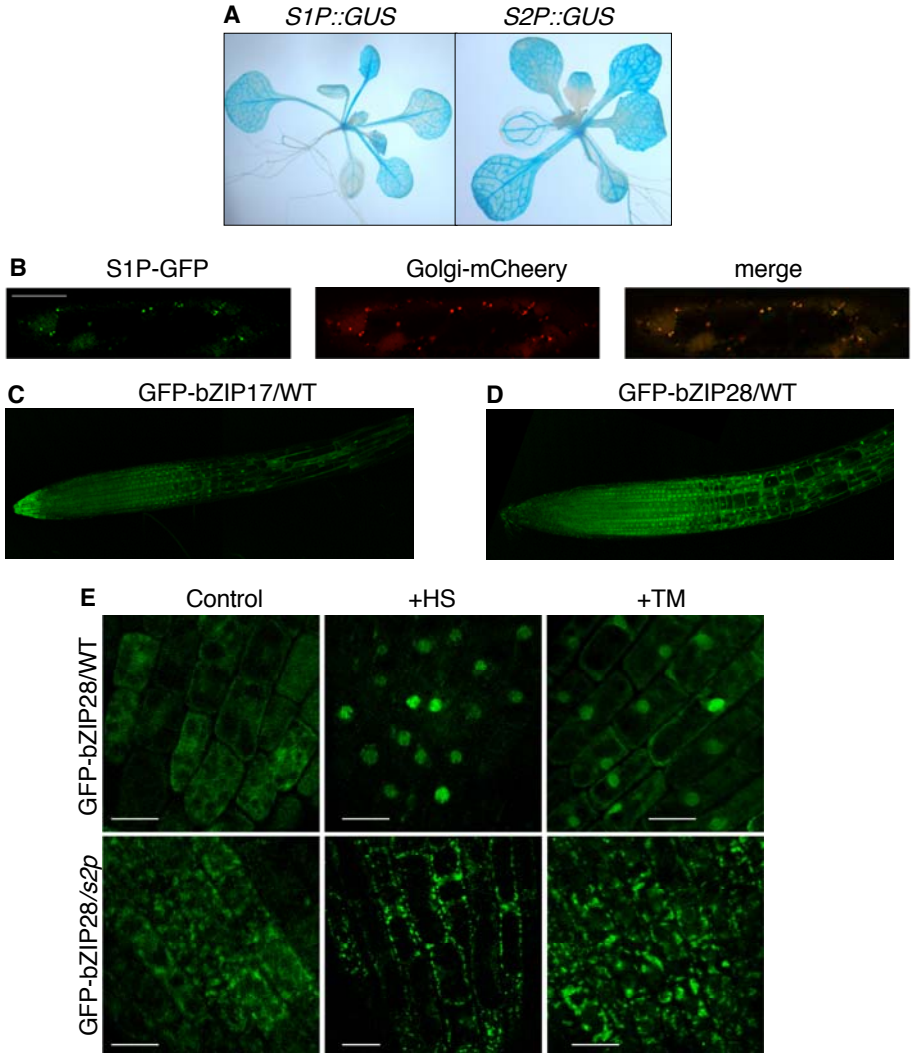

Fig. S2. Localization of S1P, S2P, and bZIP28. (**A**) Expression patterns of *S1P::GUS* and *S2P::GUS* in 11-day old *Arabidopsis* seedlings. *S1P::GUS* and *S2P::GUS* constructs were made by fusing S1P and S2P promoters to the GUS reporter gene. (**B**) Colocalization of S1P-GFP with Golgi-mCherry in onion epidermal cell by biolistic bombardment. Scale bar = 25 µm. (**C** and **D**) Fluorescence of GFP-bZIP17 and GFP-bZIP28 can be seen in the nuclei of cells in the elongation zone of roots without applied stress treatment. (**E**) Localization of bZIP28 without applied stress and in response to 60 min heat shock (HS) and 4 h tunicamycin (TM) treatment in wild-type and *s2p* plants stably transformed with GFP-bZIP28. Scale bars = 12.5 µm.

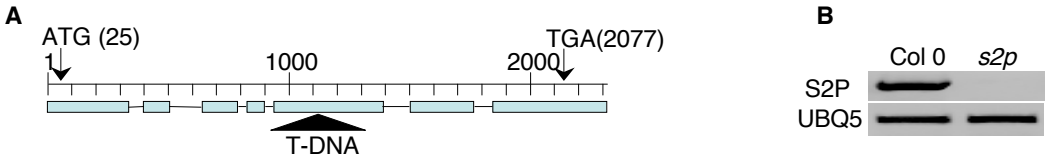

Fig. S3. Characterization of *s2p* mutant. (**A**) Schematic representation of the *S2P* gene (At4g20310). Shaded boxes represent exons and lines represent introns. The location of the T-DNA insertion in *s2p* mutant is shown by the triangle. (**B**) RT-PCR analysis of *S2P* transcripts in RNA extracted from 7-day-old Columbia wild-type and *s2p* seedlings and confirmed it to be a null *s2p* mutant.

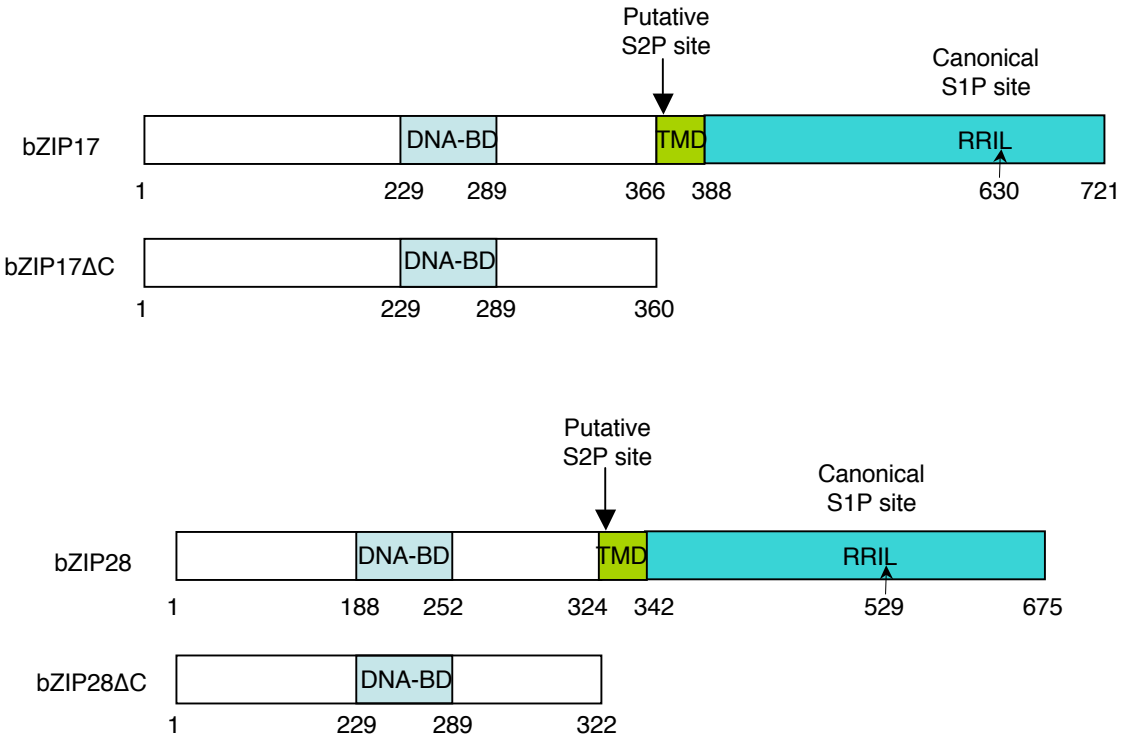

Fig. S4. Structures of full-length and truncated bZIPs. The positions of the DNA binding domain (DNA-BD), transmembrane domain (TMD), putative S1P and S2P sites, and the truncated bZIP structures are indicated. Numbers represent amino acid residue positions.

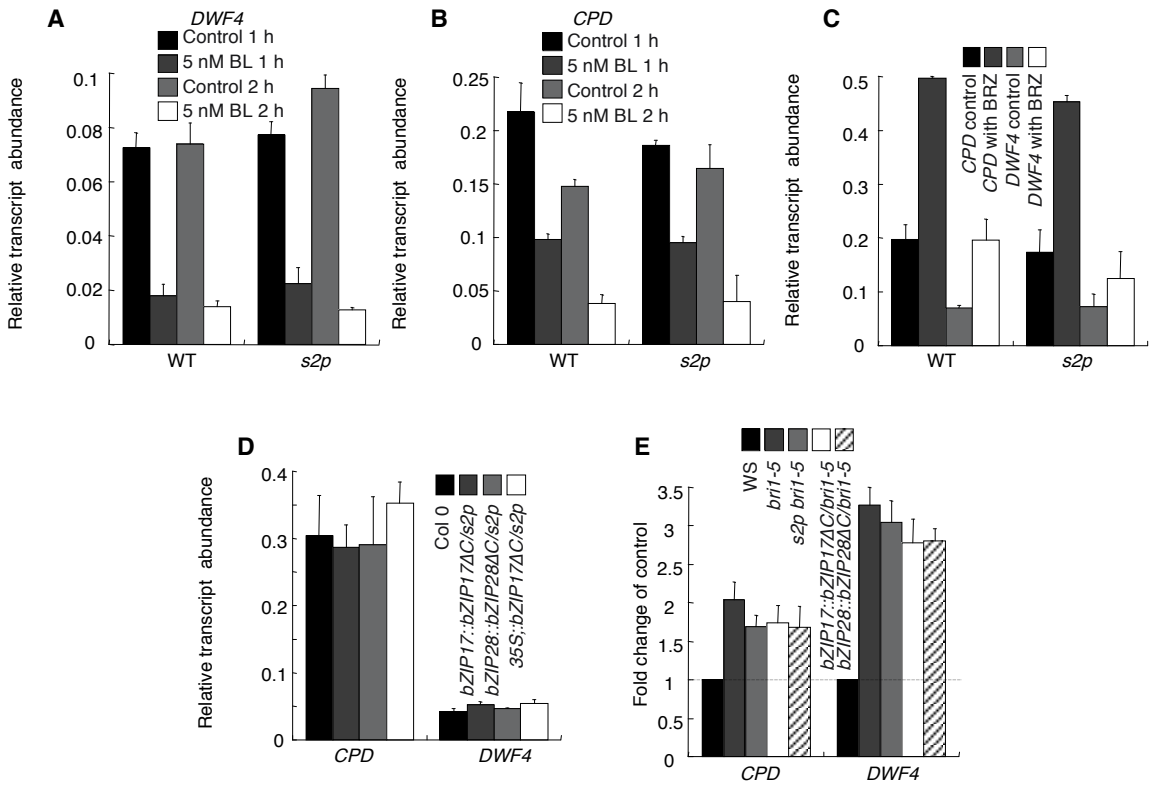

Fig. S5. S2P-RIP does not affect BR biosynthesis gene expression. (**A**-**C**) qPCR analysis of *DWF4* and *CPD* expression in Arabidopsis roots treated with 5 nM BL (**A** and **B**) for 1 or 2 h or 3 μM BRZ for 4 h (**C**). (**D** and **E**) The expression of *DWF4* and *CPD* is not affected by bZIPΔCs in the background of *s2p* (**D**) and *bri1-5* (**E**). Error bars indicate SE (n=3).

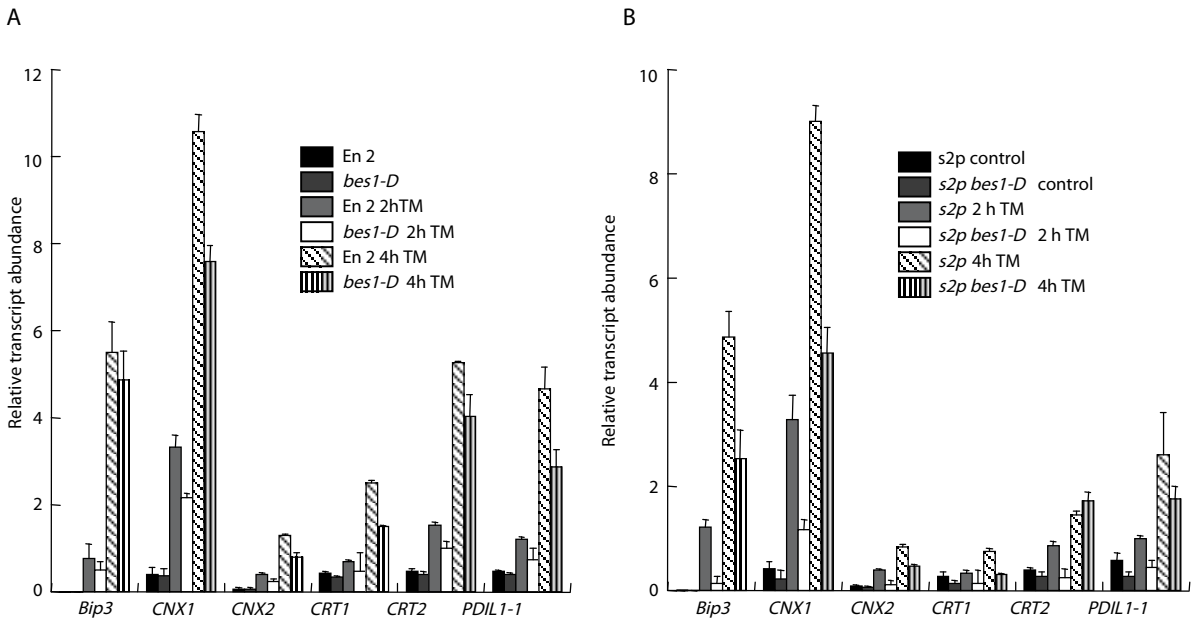

Fig. S6. BES1 does not induce chaperone gene expression in wild-type or *s2p* backgrounds. qRT-PCR analysis of chaperone gene expression in wild type (**A**) and *s2p* (**B**) under the treatment of 5 μg/ml TM for the times indicated.

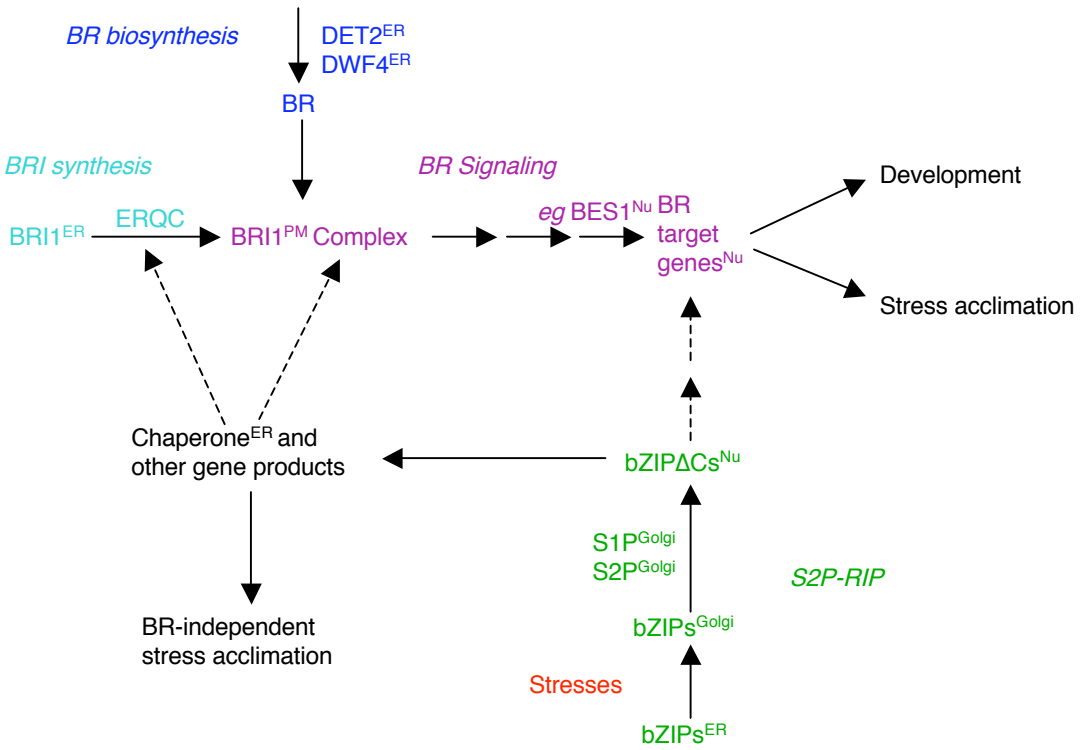

Fig. S7. Possible interactions of stress-induced S2P-RIP with BR signaling pathway. S2P-RIP is proposed to induce BR signaling through bZIP-directed synthesis of chaperones or more directly by activation of BR-responsive genes (dashed lines). Chaperones could facilitate translocation of BRI1 from the ER to the plasma membrane or its interaction with other components of the BR signaling pathway. Superscripts indicate the subcellular localization of the corresponding proteins. PM, plasma membrane; Nu, nucleus. ERQC refers to the ER Quality Control system. BRI1^{PM}Complex represents the association of BRI1 with protein kinases BAK1and BSK1 on the plasma membrane.

Table S3. Oligonucleotides used for PCR, RT-PCR, and qRT-PCR

Gene name	Accession	Forward primer	Reverse primer
Actin-2	At3g18780	CTTGCACCAAGCAGCATGAA	CCGATCCAGACACTGTACTTCCTT
Bip1,2	At5g28540 At5g42020	TCACTTGGGAGGTGAGGACTTT	CTCACATTCCCTTCGGAGCTTA
Bip3	At1g09080	CACGGTTCCAGCGTATTTCAA T	ATAAGCTATGGCAGCACCCGTT
PDIL1-1	At1g21750	CTCGTGAAGCTGAGGGTATTG	TGTGCGAAATCTAACTCAGAG
CRT1	At1g56340	AGACCTTAGTCTTCCAATTCTC	CCATTGTAAGTAAGGATAGCATG
CRT2	At1g09210	GGGAGGCTCCATTGATTGACAAC	CCTGATTTCACCTGCCACAATTCG
CNX1	At5g61790	ATGAGACAACGGCAACTATTTTCC	CCATAATCCTCATGTCCTTCACT
CNX2	At2g31955	TTTCGCTGCTTCTTGTAGCTTTGCT	CGACCATCAAAAGGCTCGTCAAAC
EXP8	At2g40610	GCATTGTTCCTGTCTCTTTCCGAAG	TTGAGACGGCGTGTACGTCTCCTG
EXPB1	At2g20750	CATTGGCTCTATGCACATTCGTCAA	AGTTGTGAGCTTCACGGAGAATGGT
CPD	At5g05690	TTACCGCAAAGCCATCCAA	TCATCACCACCACCGTCAAC
DWF4	At3g50660	GTTGGCCATTTCTTGGTGAAA	TGGCGGTGTACGGTTTAAGA
UBQ5	At3G62250	TTGAAGACGGCCGTACCCTC	CGCTGAACCTTTCAAGATCCATCG
S2P rt	At4g20310	ATGGAAATTTCAGGACGGCGAATGA	TAATGATACACGCGTGGAAGGAGAG
S2P TDNA	At4g20310	CGTGTATCATTATCAGGCATTGCCT	CAAACAAACAGATACCTCTCTT

Table S4. Oligonucleotides used for plasmid constructs.

Small letters indicate linker DNA with restriction sites used for cloning

Construct name	Forward primer	Reverse primer
bZIP17::bZIP17ΔC	cccctgcagGGCTTGTGTCTTTCAAAACTCTCGT	atggatccTCAAGCTTCACTCTTCTTACTCTCG
bZIP28::bZIP28ΔC	cccaagcTTATTTCGACCATTCAGCTTCACGGGT	atggatccTCAAACCTTCTTGAGCTTACTTTTA
S1P::GUS	cccctgcagGACAGAAGGAACTATACAGATTCAA	atggatccGGCGATGGTTGAATTAGGGTTTGAT
S2P::GUS	cccctgcagATATAAATACACCGATACAAAGTAAT	atggatccTCTTACAAGATTATGGTTTAAATTAAAG
S2P::S2P	cccctgcagATATAAATACACCGATACAAAGTAAT	atcccgggCCTCAATAAGTATATTACTAGAATTCA
35S::GFP-bZIP17	atggatccATGGCTGAACCAATCACCAAGG	ccgcggccgcTCAAGTGGTCACAAGATGAGGAGC
35S::GFP-bZIP28	atggatccATGACGGAATCAACATCCGTGG	ccgcggccgcTCAGGTGGCTACGAGATGGA
35S::S2P-GFP	gcatcgaattcATGGAAATTTCAGGACGGCGAATGA	gcatcggatccCCATCGGCGGCTCAGAGGTAAACC
35S::S1P-GFP	gcatcgaattcATGAAGGTGCTCGGAGAAGCTTCTTC	gcatcggatccGGCTAATCGATTCGACCCTGATGCTC
35S::bZIP17	tagcggcgcgccATGGCTGAACCAATCACCAA	gactagtAGCTTCACTCTTCTTACTCT