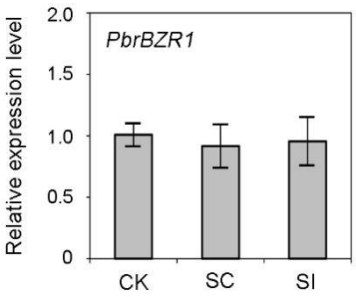
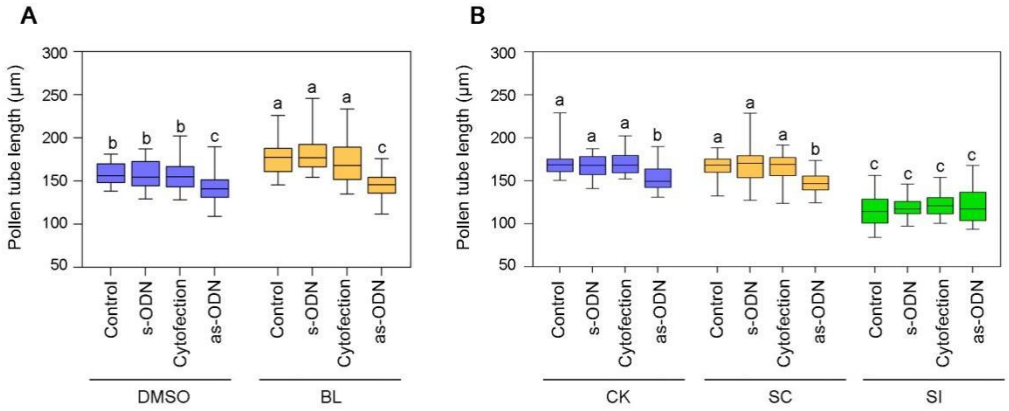
Supplemental data



Supplemental Figure S1. *PbrBZR1* does not respond to SI at the transcription level.

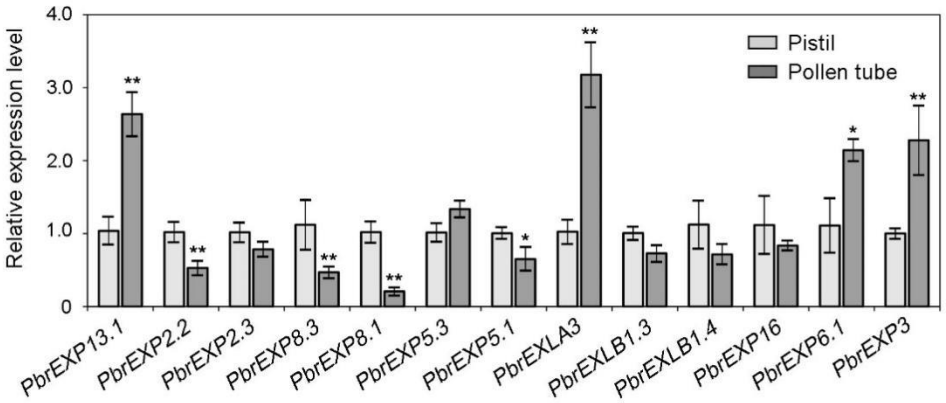
CK, liquid germination medium; SC, compatible PbrS-RNase treatment; SI, incompatible PbrS-RNase treatment. Each sample contained 0.1 g of pollens, and the data are mean ± standard deviation of three independent biological replicates.



Supplemental Figure S2. Functional verification of PbrBZR1 in BR- and SI-mediated pollen tube growth.

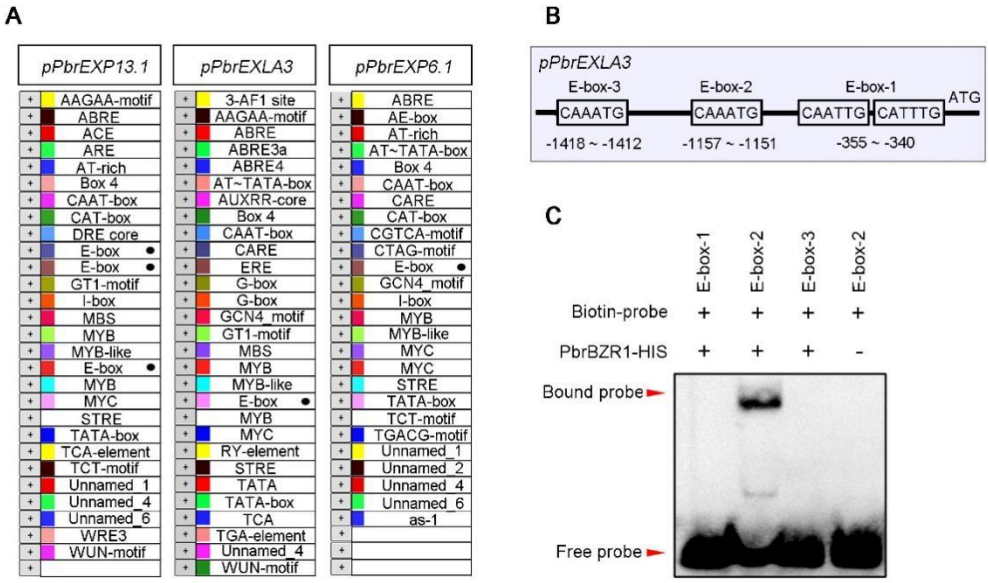
(A) Length of *PbrBZR1*-suppressed pollen tubes grown on medium containing 100 nM BL. Center line, median; Box limits, upper and lower quartiles; Whiskers, 1.5 × interquartile range; Points, outliers. DMSO, add 100 nM DMSO to the pollen medium; BL, add 100 nM BL to the pollen medium. (B) Length of *PbrBZR1*-suppressed pollen tubes grown under SC and SI

treatment. Center line, median; Box limits, upper and lower quartiles; Whiskers, 1.5 × interquartile range; Points, outliers. CK, liquid germination medium; SC, compatible PbrS-RNase treatment; SI, incompatible PbrS-RNase treatment. For (A) and (B), at least 100 pollen tubes were measured per replicate in each treatment. All treatments were performed in three independent biological replicates. Different letters indicate significant differences as determined by Tukey's HSD test ($P < 0.05$).



Supplemental Figure S3. Transcript levels of expansin genes in "Cuiguan" pear pistil and pollen.

Data are mean ± standard deviation of three independent biological replicates. Asterisks denote significant differences according to Tukey's HSD test (*$P < 0.05$, **$P< 0.01$).

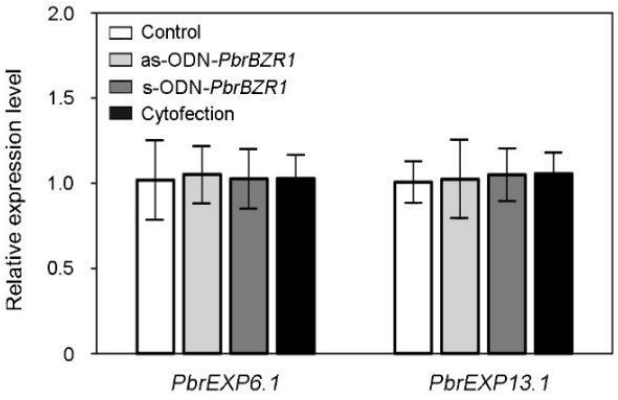


Supplemental Figure S4. PbrBZR1 binds to the promoter of *PbrEXLA3*.

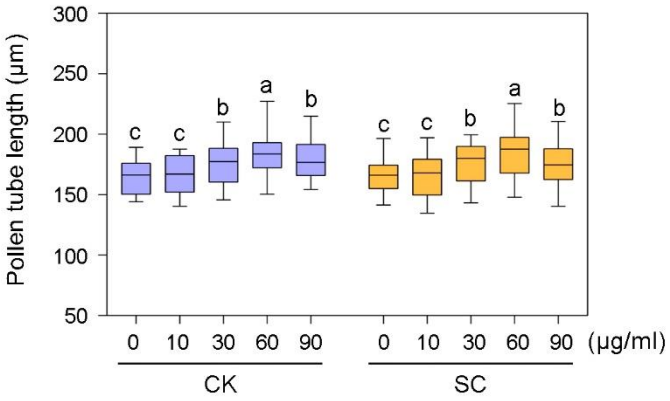
(A) Analysis of promoter elements in *PbrEXP13.1*, *PbrEXLA3*, and *PbrEXP6.1*.

(B) Schematic showing distribution of E-box elements in *PbrEXLA3* promoter.

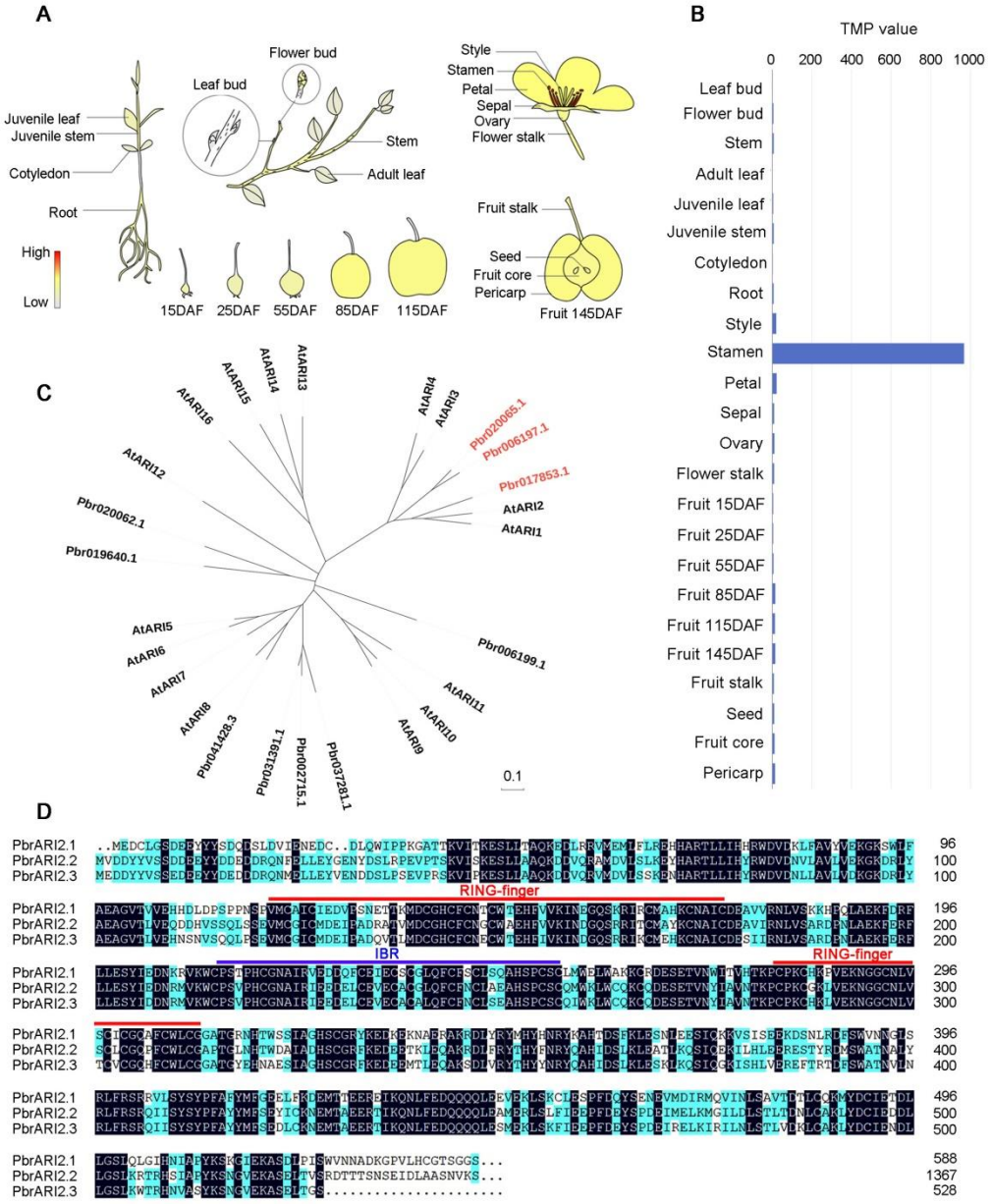
(C) Binding of PbrBZR1 protein to E-box element in the *PbrEXLA3* promoter as detected by EMSA.



Supplemental Figure S5. Transcript levels of *PbrEXP6.1* and *PbrEXP13.1* in *PbrBZR1*-suppressed pollen tubes. Each sample contained 0.1 g of pollens, and the data are mean ± standard deviation of three independent biological replicates.



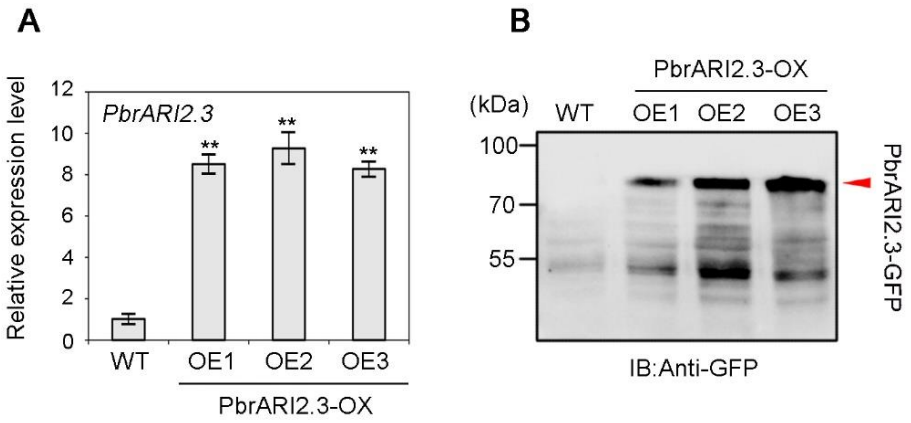
Supplemental Figure S6. Length of SC-treated pollen tubes grown on medium containing different concentrations of PbrEXLA3-HIS fusion protein. Center line, median; Box limits, upper and lower quartiles; Whiskers, 1.5 × interquartile range; Points, outliers. CK, liquid germination medium; SC, compatible PbrS-RNase treatment. At least 100 pollen tubes were measured per replicate in each treatment. All treatments were performed in three independent biological replicates. Different letters indicate significant differences as determined by Tukey's HSD test ($P < 0.05$).



Supplemental Figure S7. Identification of *PbrARI2.3* in pear.

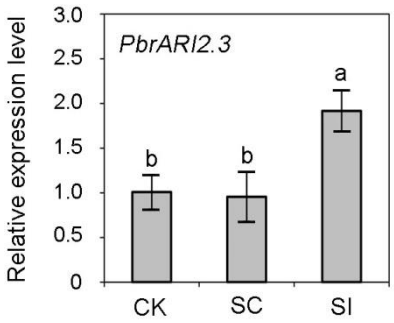
(A) Heatmap and (B) histogram showing *PbrARI2.3* transcript levels in different organs of pear. Data were obtained from Pear Expression Database (http://www.peardb.org.cn/). (C) Phylogenetic analysis of ARI proteins from *Arabidopsis* and pear, generated in MEGA 7 with the neighbor-joining method. The scale bar indicates the branch length. (D) Aligned amino acid sequences of three PbrARI proteins containing an IBR domain (215–257 aa) between two

RING-finger motifs (124–168 aa; 284–312 aa).



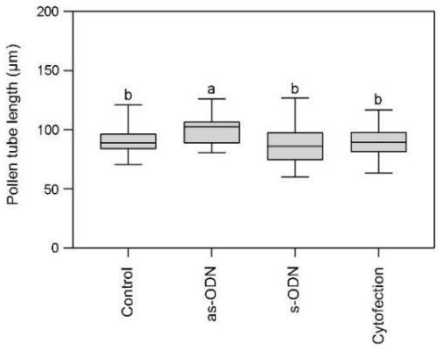
Supplemental Figure S8. Identification of *PbrARI2.3*-overexpressing transgenic pear calli.

(A) *PbrARI2.3* transcript levels in WT and three lines of *PbrARI2.3*-overexpressing (OE1, OE2, and OE3) as determined by RT-qPCR analyses. Data are mean ± standard deviation of three independent biological replicates. Asterisks denote significant differences according to Tukey's HSD test (**P< 0.01). (B) Presence of the transgene in *PbrARI2.3*-overexpressing calli as confirmed by western blotting with anti-GFP. The experiments were performed three times with similar results, and a representative picture is shown.

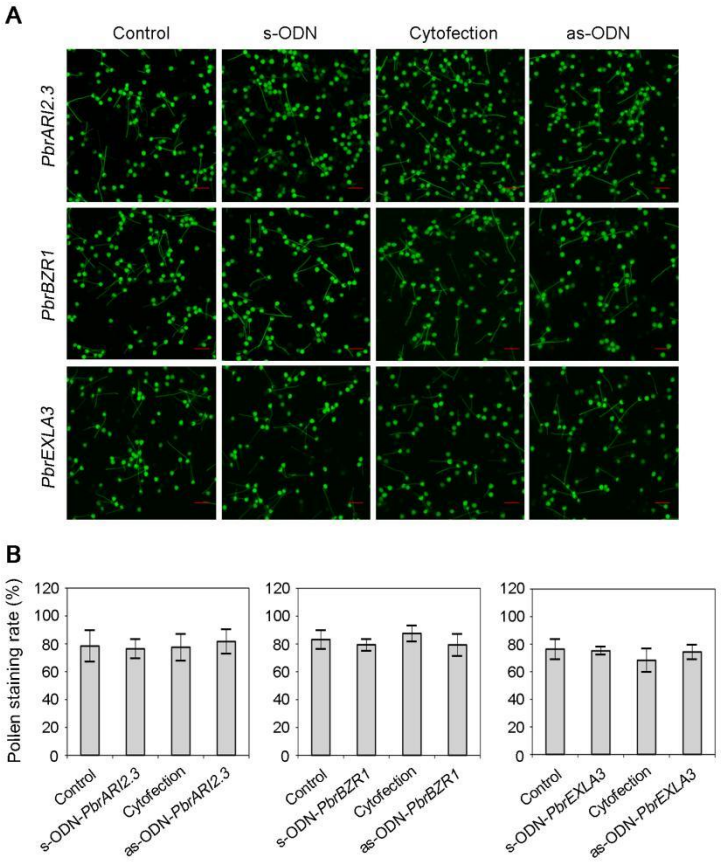


Supplemental Figure S9. *PbrARI2.3* responds to SI at the transcriptional level. CK, liquid germination medium; SC, compatible PbrS-RNase treatment;

SI, incompatible PbrS-RNase treatment. Data are mean ± standard deviation of three independent biological replicates. Different letters indicate significant differences as determined by Tukey's HSD test ($P < 0.05$).



Supplemental Figure S10. Length of *PbrARI2.3*-suppressed pollen tubes grown under SC treatment. Center line, median; Box limits, upper and lower quartiles; Whiskers, 1.5 × interquartile range; Points, outliers. At least 100 pollen tubes were measured per replicate in each treatment. All treatments were performed in three independent biological replicates. Different letters indicate significant differences as determined by Tukey's HSD test ($P < 0.05$).



Supplemental Figure S11. Detection of pollen viability after as-ODN treatments.

(A) FDA staining of as-ODN-*PbrARI2.3*, as-ODN-*PbrBZR1*, and as-ODN-*PbrEXLA3* treated pear pollen at 2.5 h. Bars = 100 μm. (B) Statistics of pollen staining rate. At least 100 pollen tubes were measured per replicate in each treatment. Data are mean ± standard deviation of three independent biological replicates.

Supplemental Table S1. Differentially expressed expansin genes after pollination.

GeneID	**FPKM values**					
	CP24h	CP48h	CP72h	SP24h	SP48h	SP72h
Pbr012636.1	6.299794	6.711144	3.914175	4.070464	5.758334	3.059617

Pbr013129.1	105.0993	94.79531	63.31297	132.4519	121.6789	69.2321
Pbr017279.1	232.338	249.9839	283.1354	239.5693	425.081	231.7547
Pbr002929.1	32.29454	64.5802	62.93233	75.16617	51.27522	68.91439
Pbr003748.1	104.1606	153.87	123.5764	116.0598	178.3124	176.3387
Pbr019282.1	11.02273	12.95697	3.592858	7.984577	7.571882	1.007222
Pbr039073.1	241.7725	328.3371	208.3292	246.2742	313.2665	166.4599
Pbr033313.1	17.45234	15.50991	11.73893	10.44614	7.241533	6.01985
Pbr013752.1	5.711068	3.939028	1.949116	2.606304	1.548849	1.427734
Pbr022160.1	9.768462	9.348018	9.981355	5.256199	4.139792	3.291827
Pbr009385.1	0.587197	2.604321	1.063963	2.553364	7.059518	7.928769
Pbr003883.1	420.2582	383.0673	111.8454	318.4312	218.4479	149.1841
Pbr033539.1	50.31034	45.08289	24.03397	29.48638	33.13551	15.28466

Supplemental Table S2. Primers used to analyze gene expression and construct vectors.

RT-qPCR assays	
PbrBZR1-F:	TTCCCAACTGGGACTCCATC
PbrBZR1-R:	GGCAGCTGGAGGATGAGTAT
PbrEXP13.1-F:	GGCTACGGAGACCTCGAGAA
PbrEXP13.1-R:	GTTGGTCGCCGTCACGATAA
PbrEXP2.2-F:	ACAATGGCGGATGGTGCAAT
PbrEXP2.2-R:	TAGGAGTGGCCGTTGATGGT
PbrEXP2.3-F:	ACAATGGCGGATGGTGCAAT
PbrEXP2.3-F:	TAGGAGTGGCCGTTGATGGT
PbrEXP8.3-F:	CACAAACGTGGGAGGAGCAG
PbrEXP8.3-R:	CGTCGCTGGTAGTGACTTGG
PbrEXP8.1-F:	TGATCACGAACGTCGGAGGA
PbrEXP8.1-R:	TGTTGGCGGTGAGAGTTCTG
PbrEXP5.3-F:	ACAGCCAGGGTTATGGGACT
PbrEXP5.3-R:	GGGCAGAAGTTTGTGGCAGT

PbrEXP5.1-F:	CCGGTGATGTGCACTCTGTT	
PbrEXP5.1-R:	AGGTCTGCCCAAAGGACCAT	
PbrEXLA3-F:	TGCAACTGCTTGTGATCGCT	
PbrEXLA3-R:	CCACAACCAGCTCCACCTTT	
PbrEXLB1.3-F:	CGCTTGCAGGTTCTCTGGTT	
PbrEXLB1.3-R:	CTCCATTGCTCGCGATCCTC	
PbrEXLB1.4-F:	ACCAATCCAGCTTCGTCTGC	
PbrEXLB1.4-R:	TCCTGCCAAACCTCAACAGC	
PbrEXP16-F:	TCCTGCCAAACCTCAACAGC	
PbrEXP16-R:	GGACTGCCAGATTTGCACCA	
PbrEXP6.1-F:	GGGATCGTCCCTGTTGCCTA	
PbrEXP6.1-R:	ATGCTCATCCAGCCAGTCCT	
PbrEXP3-F:	TGCGGGTACGGAAACCTCTA	
PbrEXP3-R:	TGGGCTGAGCGAAGTTAGGA	
Y1H assays		
pHIS2-PbrEXP13.1-F	TACGACTCACTATAGGGCGAATTCTACATTGCATGGTAAGATAC	pHIS2 vector restriction enzyme sites: EcoRI, SacI. Plasmid construction by homologous recombination.
pHIS2-PbrEXP13.1-R	GATCGATTCGCGAACGCGTGAGCTCTTCTGGGCTTGTTTGTTTG	
pHIS2-PbrEXLA3-F	TACGACTCACTATAGGGCGAATTCCCGCGGAATATCTTGTTGAT	pHIS2 vector restriction enzyme sites: EcoRI, SacI. Plasmid construction by homologous recombination.
pHIS2-PbrEXLA3-R	GATCGATTCGCGAACGCGTGAGCTCAAACTTTTTGAACTTTTAGGAC	
pHIS2-PbrEXP6.1-F	TACGACTCACTATAGGGCGAATTCAGTGTTACTATTTTACCCAA	pHIS2 vector restriction enzyme sites: EcoRI, SacI. Plasmid construction by homologous recombination.
pHIS2-PbrEXP6.1-R	GATCGATTCGCGAACGCGTGAGCTCTAGCGAGATTAGTAGAGT	
Dual-luciferase reporter assays		
pGreenII-pPbrEXLA3-F	CTATAGGGCGAATTGGGTACCCCGCGGAATATCTTGTTGAT	pGreenII 0800-LUC vector restriction enzyme sites: KpnI, HindIII. Plasmid construction by homologous recombination.
pGreenII-pPbrEXLA3-R	CAGGAATTCGATATCAAGCTTAAACTTTTTGAACTTTTAGGAC	

pGreenII -PbrBZR1-F	GCGGCCGCTCTAGAACTAGTGGATCCATGACGTCGGATGGGGCAAC	pGreenII 62-SK vector restriction enzyme sites: BamHI, HindIII. Plasmid construction by homologous recombination.
pGreenII -PbrBZR1-R	CTCGAGGTCGACGGTATCGATAAGCTTAATCCGAGCCTTACCATTTCCA	
Y2H assays		
pGAD-PbrARI2.1-F	GCCATGGAGGCCAGTGAATTCATGGAGGATTGCTTGGG	pGADT7 vector restriction enzyme sites: EcoRI, BamHI. Plasmid construction by homologous recombination.
pGAD-PbrARI2.1-R	CAGCTCGAGCTCGATGGATCCTGAATCAGATCTGTCAACCTCT	
pGAD-PbrARI2.2-F	GCCATGGAGGCCAGTGAATTCATGGTGGACGATTATTACG	pGADT7 vector restriction enzyme sites: EcoRI, BamHI. Plasmid construction by homologous recombination.
pGAD-PbrARI2.2-R	CAGCTCGAGCTCGATGGATCCTCATGGTAGCCAAAAGGTCTCTC	
pGAD-PbrARI2.3-F	GCCATGGAGGCCAGTGAATTCATGGAGGACGATTATTACG	pGADT7 vector restriction enzyme sites: EcoRI, BamHI. Plasmid construction by homologous recombination.
pGAD-PbrARI2.3-R	CAGCTCGAGCTCGATGGATCCTGGAACCGGTAAGTTCTGAAGC	
pGBD-PbrBZR1-F	ATGGCCATGGAGGCCGAATTCATGACGTCGGATGGGGCAAC	pGBKT7 vector restriction enzyme sites: EcoRI, BamHI. Plasmid construction by homologous recombination.
pGBD-PbrBZR1-R	CCGCTGCAGGTCGACGGATCCAATCCGAGCCTTACCATTTCCA	
BIFC assays		
YFPN-PbrBZR1-F	GAGAACACGGGGGACTCTAGAATGACGTCGGATGGGGCAAC	pSPYNE vector restriction enzyme sites: XbaI, BamHI. Plasmid construction by homologous recombination.
YFPN-PbrBZR1-R	GACAGTACTATCGATGGATCCAATCCGAGCCTTACCATTTCCA	
YFPC-PbrARI2.3-F	GAGAACACGGGGGACTCTAGAATGGAGGACGATTATTACG	pSPYCE vector restriction enzyme sites: XbaI, BamHI. Plasmid construction by homologous recombination.
YFPC-PbrARI2.3-R	GACAGTACTATCGATGGATCCTGGAACCGGTAAGTTCTGAAGC	

LCI assays		
Nluc-PbrBZR1-F	CTCGGTACCCGGGGATCCATGACGTCGGATGGGGCAAC	pCAMBIA1300-NLuc vector restriction enzyme sites: BamHI, SalI. Plasmid construction by homologous recombination.
Nluc-PbrBZR1-R	GTACGAGATCTGGTCGACAATCCGAGCCTTACCATTTCCA	
Cluc-PbrARI2.3-F	GCGTCCCGGGGCGGTACCATGGAGGACGATTATTACG	pCAMBIA1300-CLuc vector restriction enzyme sites:KpnI, BamHI. Plasmid construction by homologous recombination.
Cluc-PbrARI2.3-R	AGTCCATTTGTTGGATCCGGAACCGGTAAGTTCTGAAGC	
Co-IP assays		
pEarlyGate-PbrBZR1-F	CTTAATTAATCTAGAGAATTCATGACGTCGGATGGGGCAAC	pEarlyGate201-HA vector restriction enzyme sites:EcoRI, BamHI. Plasmid construction by homologous recombination.
pEarlyGate-PbrBZR1-R	ATGGTAACCTGCCATGGATCCAATCCGAGCCTTACCATTTCCA	
pEarlyGate-PbrARI2.3-F	CGCTCTAGAACTAGTGGATCCATGGAGGACGATTATTACG	pEarlyGate202-FLAG vector restriction enzyme sites: BamHI, HindIII. Plasmid construction by homologous recombination.
pEarlyGate-PbARI2.3-R	GTCGACGGTATCGATAAGCTTGGAACCGGTAAGTTCTGAAGC	
Prokaryotic expression		
pET-32a-PbrBZR1-F	CAAGGCCATGGCTGATATCGGATCCATGACGTCGGATGGGGCAAC	pET-32a vector restriction enzyme sites: BamHI, EcoRI. Plasmid construction by homologous recombination.
pET-32a-PbrBZR1-R	TGTCGACGGAGCTCGAATTCAATCCGAGCCTTACCATTTCCA	
pET-32a-PbrEXLA3-F	CAAGGCCATGGCTGATATCGGATCCATGTCTTTGTTTATTTGCTTCCTCC	pET-32a vector restriction enzyme sites: BamHI, EcoRI. Plasmid construction by homologous recombination.
pET-32a-PbrEXLA3-R	TGTCGACGGAGCTCGAATTCTATCCAGCTCCCATCATCACAG	
pGEX-4T-PbrARI2.3-F	CTGGTTCCGCGTGGATCCATGGAGGACGATTATTACG	pGEX-4T-1 vector restriction enzyme sites: BamHI, EcoRI. Plasmid construction by homologous recombination.
pGEX-4T-PbrARI2.3-R	GAGTCGACCCGGGAATTCGGAACCGGTAAGTTCTGAAGC	

pET-32a-PbrS$_3$-RNase-F	GCCATGGCTGATATCGGATCCATGCAATTTACGCAGCAATATCA	pET-32a vector restriction enzyme sites: BamHI, EcoRI. Plasmid construction by homologous recombination.
pET-32a-PbrS$_3$-RNase-R	TTGTCGACGGAGCTCGAATTCATACTTGATATTGTTGGTGGGGC	
pET-32a-PbrS$_5$-RNase-F	GCCATGGCTGATATCGGATCCATGTATTTTCAATTTACGCAGCAAT	pET-32a vector restriction enzyme sites: BamHI, EcoRI. Plasmid construction by homologous recombination.
pET-32a-PbrS$_5$-RNase-R	TTGTCGACGGAGCTCGAATTCATACTTGATATTGTTGGTGGGGC	
pET-32a-PbrS$_7$-RNase-F	GCCATGGCTGATATCGGATCCATGTACGATTATTTTCAATTTACGCA	pET-32a vector restriction enzyme sites: BamHI, EcoRI. Plasmid construction by homologous recombination.
pET-32a-PbrS$_7$-RNase-R	TTGTCGACGGAGCTCGAATTCATACTTAACATCGGCCGGGC	
pET-32a-PbrS$_{34}$-RNase-F	GCCATGGCTGATATCGGATCCATGTACGATTATTTTCAATTTACGCA	pET-32a vector restriction enzyme sites: BamHI, EcoRI. Plasmid construction by homologous recombination.
pET-32a-PbrS$_{34}$-RNase-R	TTGTCGACGGAGCTCGAATTCATACTGAATACTATTGTTTGGGCAAAA	
Genetic transformation		
pRI101-PbrARI2.3-F	GTTGATACATATGCCCGTCGACATGGAGGACGATTATTACG	pRI101 vector restriction enzyme sites: SalI, SmaI. Plasmid construction by homologous recombination.
pRI101-PbrARI2.3-R	TCAGAATTCGGTACCCCCGGGGGAACCGGTAAGTTCTGAAGC	
Phosphorothioated ODNs sequences		
PbrBZR1	GAAGCAAACCCATTGGCCGGAATCG	
PbrEXLA3	GTGATCTGAACCTTTGTGTCATATA	
PbrARI2.3	TTGCATAGATCTTCGCTAAACATGT	

Supplemental Table S3. Primers used to synthesize EMSA probes.

EMSA	
E-box-1	AACTTCTTGTTTCATTTGAGTCATTTGAATTCCAAA
E-box-2	TCCAGAATTTTGGCACATGCGCATGGTCCATACG
E-box-3	CTGAGTTTGCTACACATGTATTTACACATAAAA
E-box-2(mut)	TCCAGAATTTTGGAAAAAACGCATGGTCCATACG