Dose-dependent AGO1-mediated Inhibition of the miRNA 165/166 Pathway Modulates Stem Cell Maintenance in the Arabidopsis Shoot Apical Meristem

SUPPLEMENTAL FIGURES

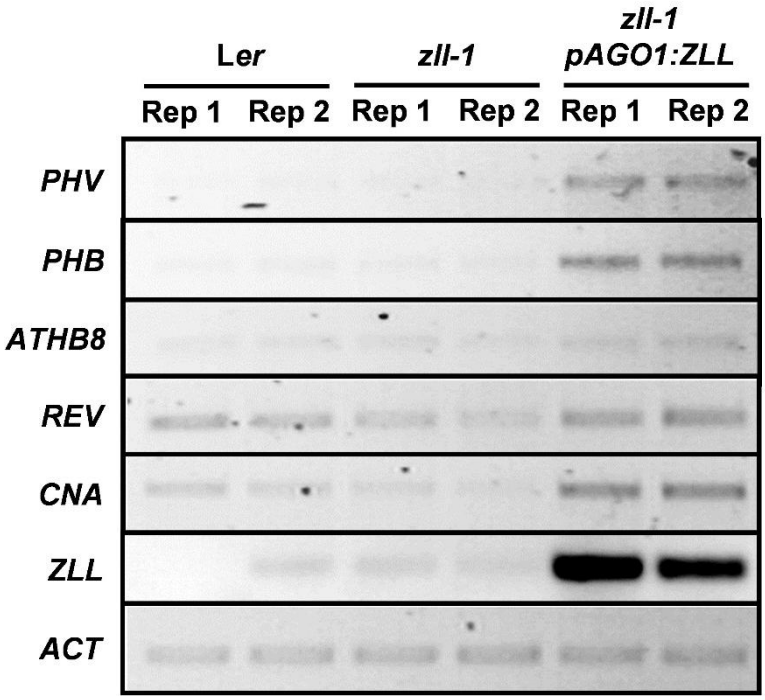

Figure S1. Overexpression of *ZLL/AGO10* increases the level of *HD-ZIP III* genes.

Semi-quantitative RT-PCR of *HD-ZIP III* mRNA levels from 14-day-old seedlings of the indicated genotypes. *ACTIN7* (*ACT*) was used as the internal control and the results of two biological replicates (Rep) are shown.

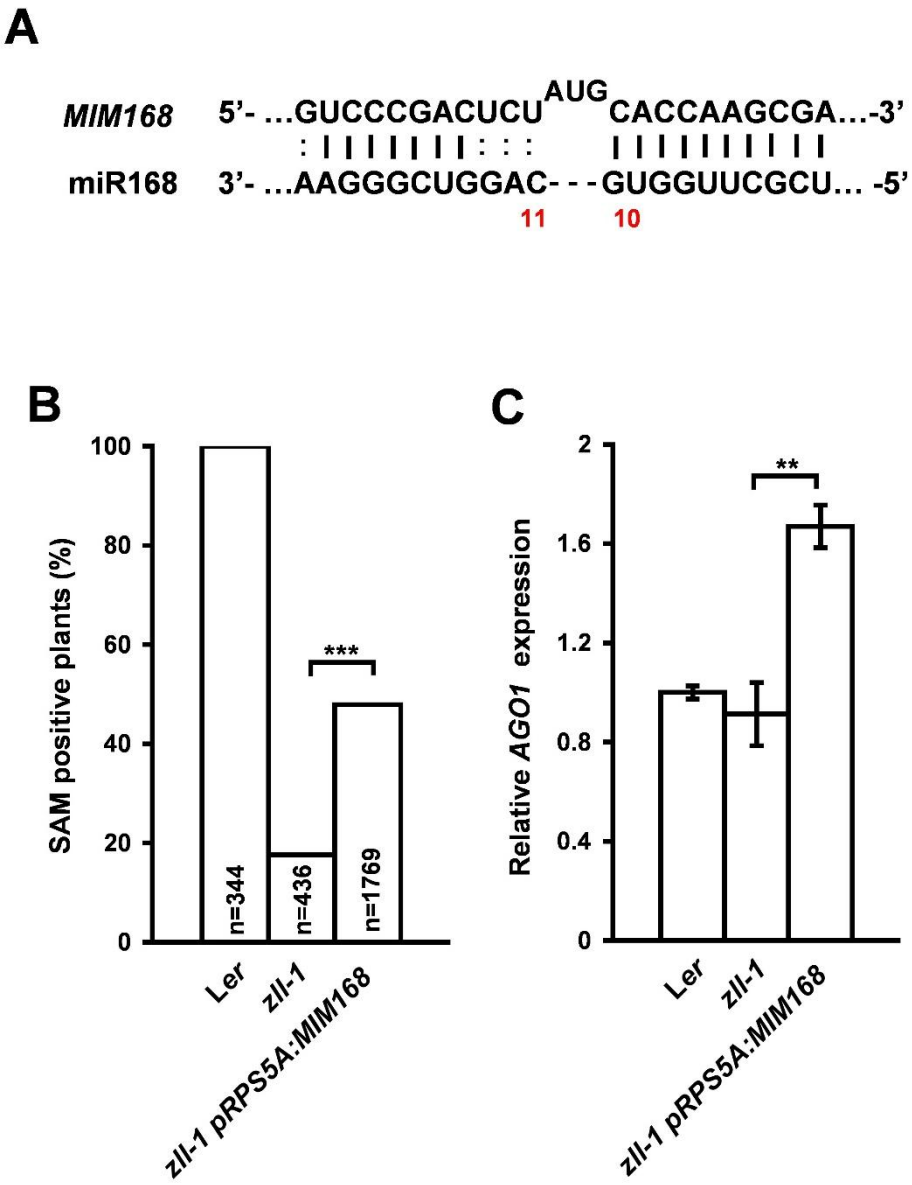

Figure S2. Blocking miR168 activity suppresses the shoot apical meristem termination in *zll-1*.

(A) The target-mimicry *MIM168* blocks miRNA activity by introducing a three-nucleotide bulge in the position where normally miRNA-guided cleavage takes place (between 10th and 11th nucleotide of miRNA sequence, indicated by red numbers).

(B) Frequencies of shoot apical meristem phenotypes of 14-day-old *zll-1* seedlings of the indicated genotypes. Individual counts from four independent transgenic lines not

significantly different by Chi-square test were combined. *** p<0.001, by two-sided, unpaired Fishes exact test. ns, not significant. n, numbers of seedlings.

(C) Levels of *AGO1* transcript in the above-ground parts of 10-day-old seedlings of the indicated genotypes and normalized to L*er*. Date represents means ± SD from three biological replicates of each genotype. Individual counts from four independent transgenic lines were pooled. *** p<0.001; ns, not significant, by two-sided. unpaired t-test.

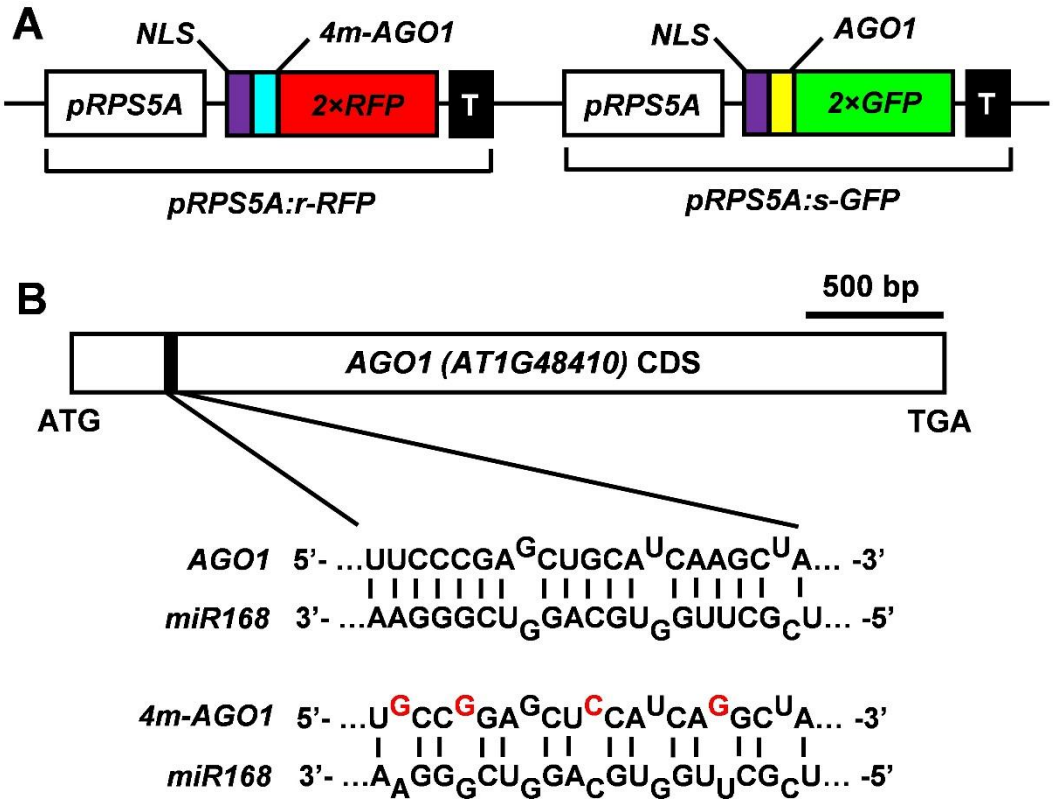

Figure S3. Structure of the miR168 tandem sensor

(A) Diagram of the miR168 tandem-sensor. Two copies of GFP are fused to a nuclear localization signal fragment from SV40 and the miR168 binding site of *AGO1*. Two copies of RFP are fused with the same nuclear localization signal fragment and the mutated miR168 binding site (*4m-AGO1*). T, NOS terminator; r-RFP, miR168-resistant RFP; s-GFP, miR168-sensitive GFP.

(B) Sequences of the miR168 binding site (black box) and mutated miR168 binding site in the coding sequence (CDS) of *AGO1*. In *4m-AGO1*, four nucleotide mutations (red) are introduced, which do not change the amino acid sequence of AGO1 protein but interfere with the binding capacity of miR168 to this site (Vaucheret et al., 2004).

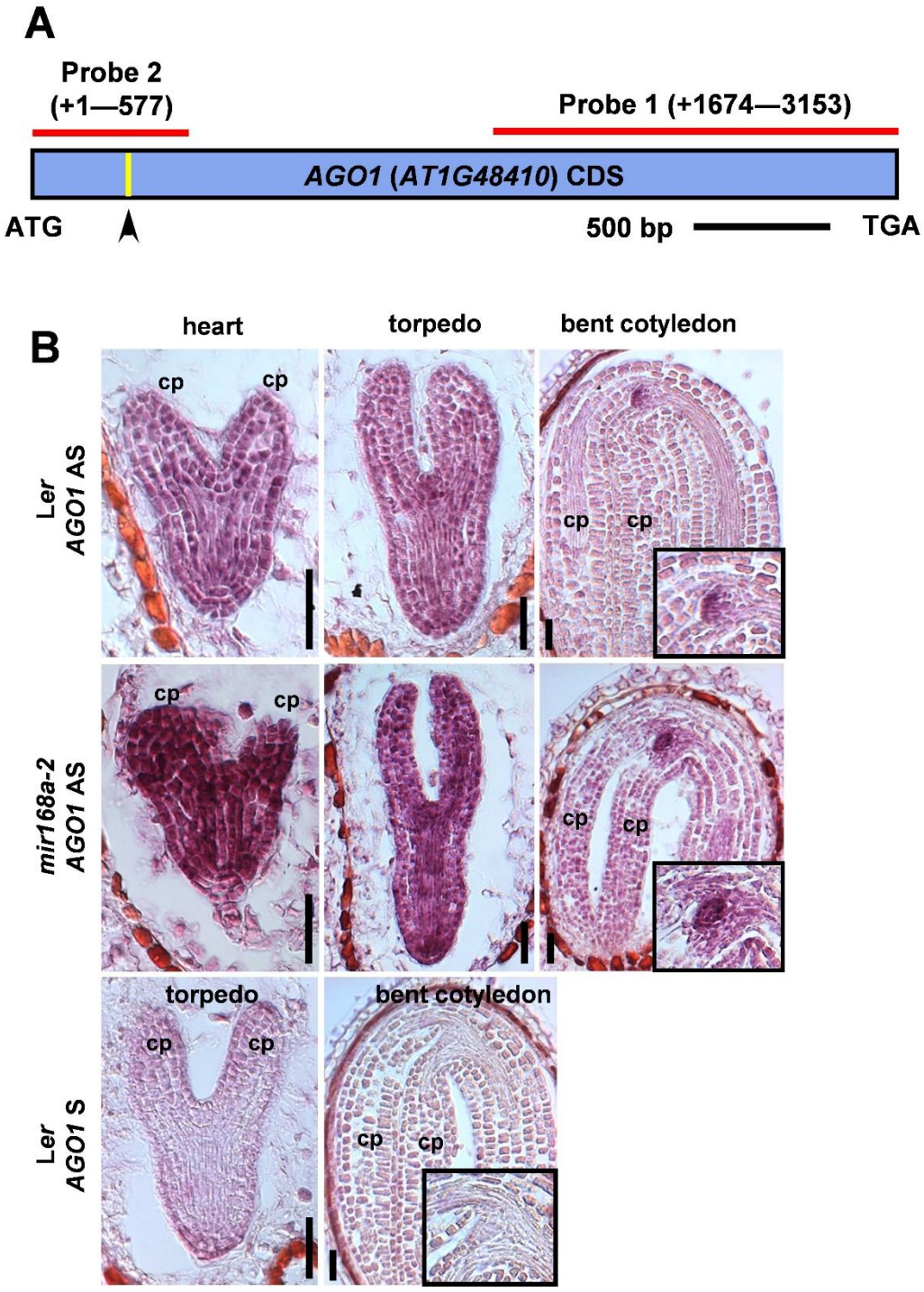

Figure S4. *In situ* hybridization of *AGO1* mRNA

(A) Diagram of the *AGO1* coding sequence. The regions used as probes for *in situ* hybridization (red lines) and the miR168 recognition site (arrowhead) are indicated.

(B) Endogenous *AGO1* mRNA patterns in wild-type L*er* and *mir168a-2* embryos at heart, torpedo and bent-cotyledon stage using antisense or sense *AGO1* probe 2, which give rise to similar patterns as using probe 1 (Figure 1C). Insets show higher magnification of the embryonic shoot apical meristems. AS, antisense; S, sense; cp, cotyledon primordium. Scale bars: 20 µm.

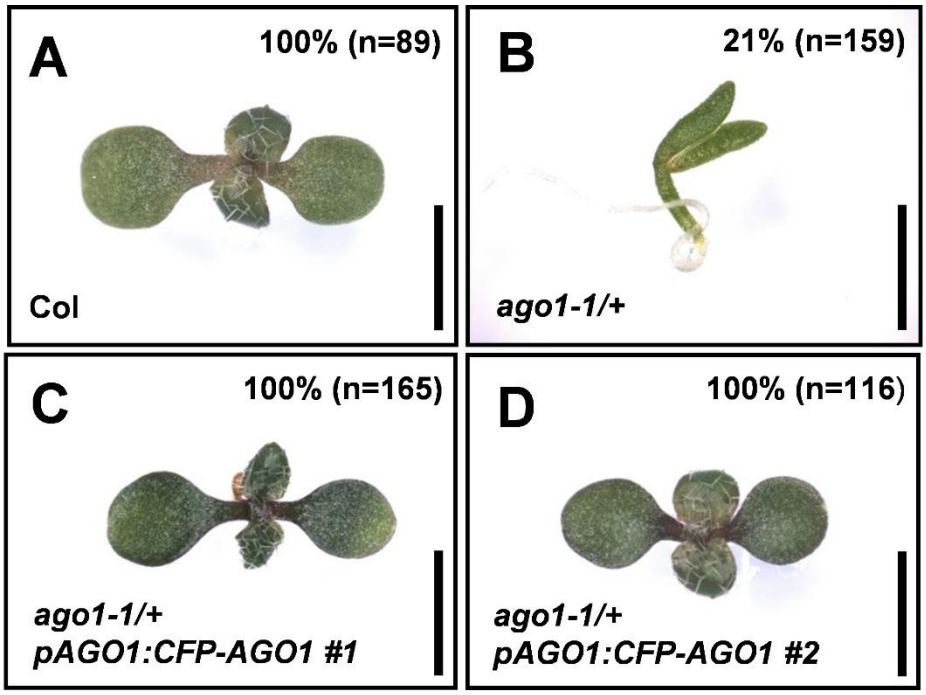

Figure S5. *pAGO1:CFP-AGO1* fully complements seedling phenotypes in *ago1-1*.

(A-D) Phenotypes of 10-day-old seedlings of Col, *ago1-1* mutant and two independent transgenic lines that complement the defect of *ago1-1* mutant. The genotype of the mother plant and the percentage of corresponding progenies displaying the given phenotype are indicated. Scale Bars, 2 mm.

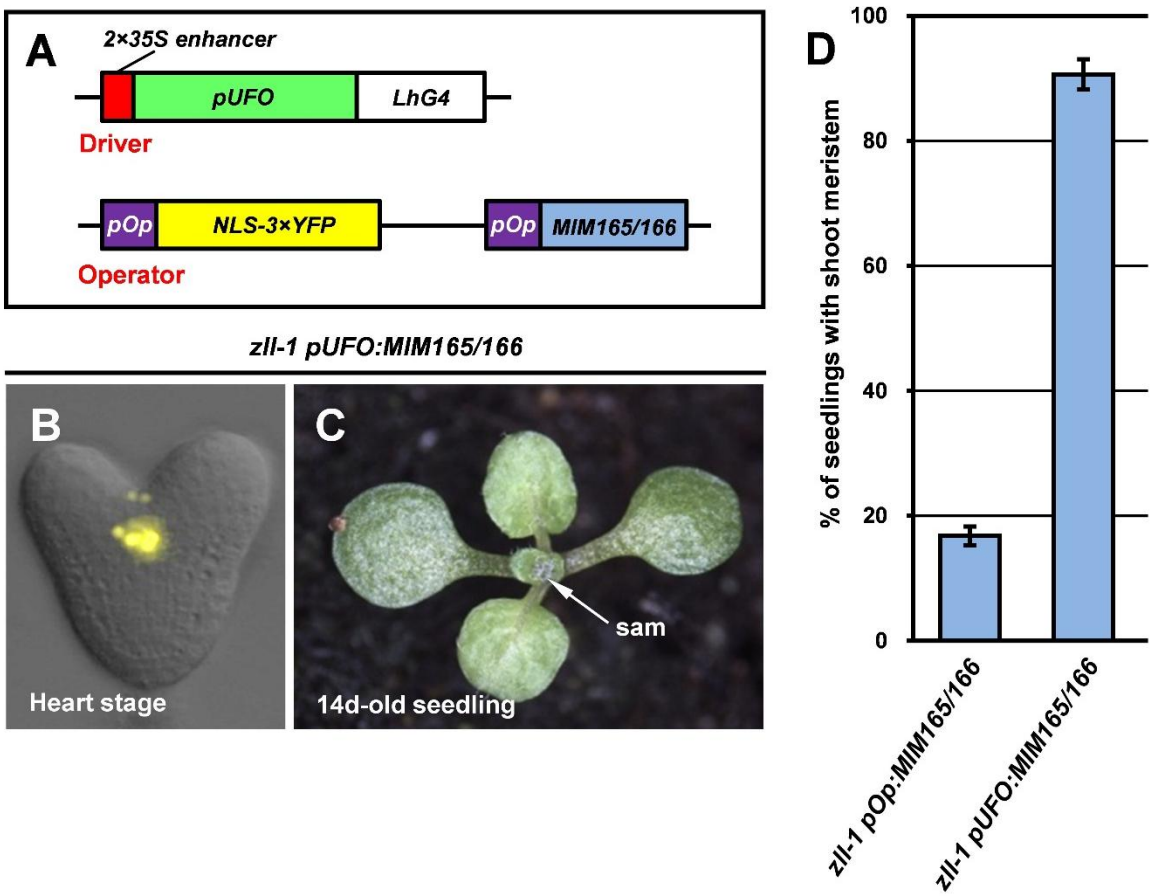

Figure S6. Expression of *MIM165/166* in the meristem region is sufficient to restore shoot meristem development in *zll-1*

(A) Transgenic plant expressing *pUFO:MIM165/166* were created by crossing a driver line with an operator line based on the *LhG4-pOp* system, as indicated. The tandem *YFP* reporter was used as an expression domain indicator.

(B) Localization of YFP in a heart stage embryo.

(C) Recovered shoot apical meristem (sam) of a 14-day-old *zll-1* seedling expressing *pUFO:MIM165/166*.

(D) Frequencies of shoot apical meristems of 14-day-old *zll-1* seedlings expressing *pUFO:MIM165/166*. Transgenic lines containing only the operator were used as the control. At least three independent lines with homozygous transgenes in F4 generation were analyzed, and the mean ± SD values are indicated.

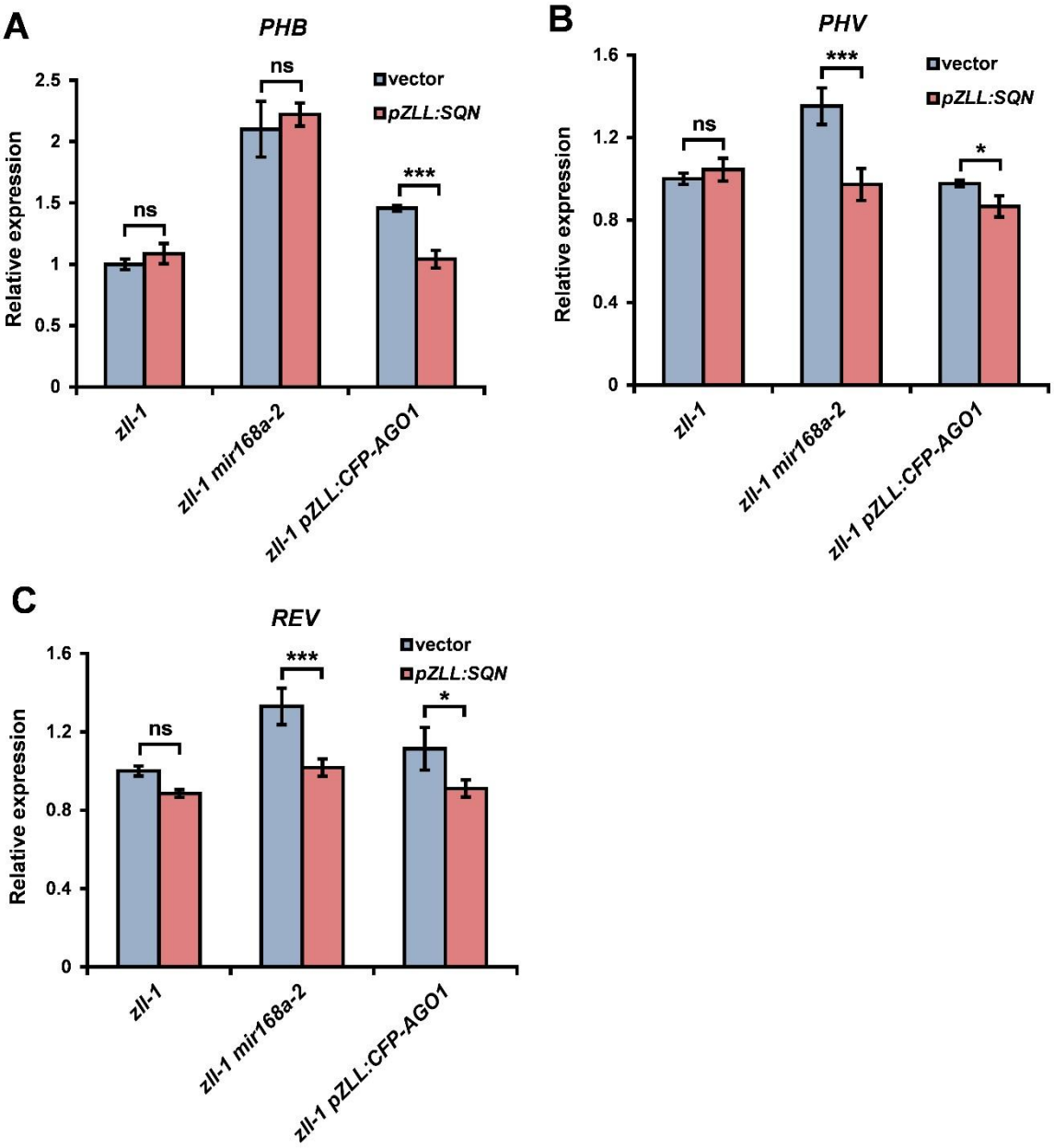

Figure S7. Expression levels of *HD-ZIP IIIs* when the co-factor *SQN* is expressed

(A-C) Expression levels of the indicated *HD-ZIP III* mRNAs from dissected shoot apical meristems of 14-day-old seedlings of *zll-1*, *zll-1 mir168a-2* and *zll-1 pZLL:CFP-AGO1* with empty vector or *SQN* expression. Expression levels of *PHB* (A) *PHV* (B) and *REV* (C) are shown. Data represents mean values ± SD of three biological replicates by quantitative RT-PCR. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns, not significant, by two-sided, unpaired t-test.

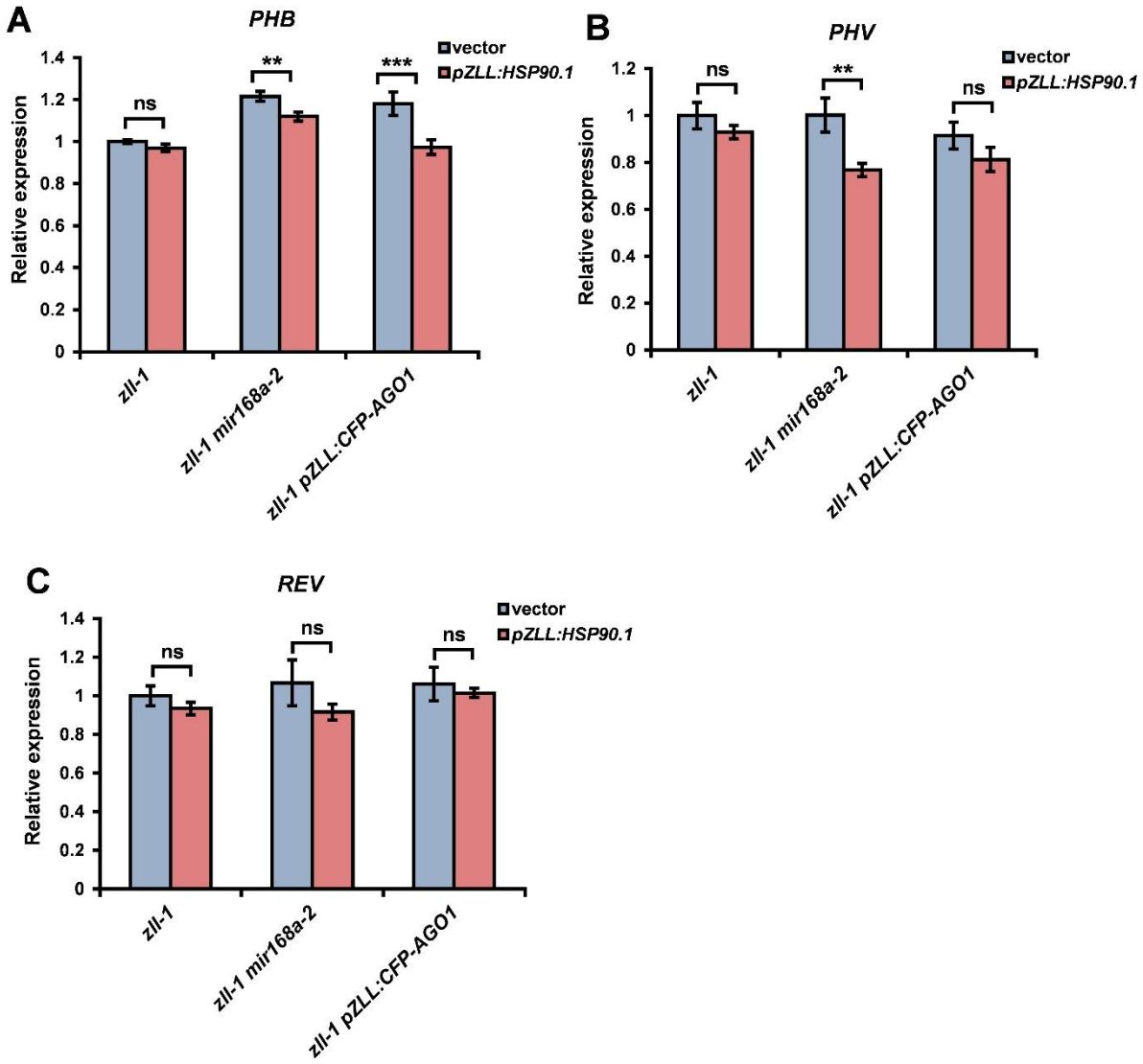

Figure S8. Expression levels of *HD-ZIP IIIs* when the co-factor *HSP90.1* is expressed

(A-C) Expression levels of *HD-ZIP III* mRNAs from dissected shoot apical meristems of 14-day-old seedlings of *zll-1*, *zll-1 mir168a-2* and *zll-1 pZLL:CFP-AGO1* with empty vector or *HSP90.1* expression. Expression levels of *PHB* (A) *PHV* (B) and *REV* (C) are shown. Data represents mean values ± SD of three biological replicates by quantitative RT-PCR. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns, not significant, by two-sided, unpaired t-test.

SUPPLEMENTAL TABLES

Table S1. Recovery of the shoot meristem in *zll-1* by additional *AGO1* copies

Genotype	n	shoot meristem (%)
zll-1	158	28.5
zll-1/Col-AGO1#1	368	98.0
zll-1/Col-AGO1#3	398	98.5
zll-1/Col-AGO1#4	320	92.2
zll-1 mir168a-2	481	96.1

Frequencies of wild-type like shoot meristems at the seedling stage in mutants and three independent transformants are indicated. The transformed transgene is homozygous. n, number.

Table S2. Information and genotyping methods of the mutant alleles used in this study

Genotype	Resistance	Mutation type	Genotyping method	Reference
zll-1		EMS	oSB252/MT14+AciI, WT: 300+170 bp; Mutant: 470 bp	(Tucker et al., 2008)
zll-15		EMS	oFD909/910+Hpy188III, WT: 263+274 bp; Mutant: 537 bp	(Moussian et al., 1998)
mir168a-2	Kanamycin	Transposon insertion	oFD905/906, WT: no amplification; Mutant: 140 bp oFD907/906, WT: 390 bp; Mutant: no amplification	(Vaucheret, 2009)
ago1-1	Hygromycin	T-DNA insertion	oET39/40, WT: no amplification; Mutant: 500 bp A13/14, WT: 500 bp; Mutant: no amplification	(Bohmert et al., 1998)

Table S3. Primer sequences for mutant genotyping.

Genotype	Primer sequence
zll-1	oSB252: ATGGTTCTTTGTGAGTTAGAACTTAGA MT14 : CATGCCTAAGACTTCACACATCTGA
zll-15	oFD909: GAAAGCTCTAAAGCATGTTTATCACAC oFD910: CAACAGCAGCGATTGAAGGG
mir168a-2	oFD905: ATTGGCTTAGCTCACTGGATTTTG oFD906: TTCCCGACCTGCACCAAGCGA oFD907: CGCTCACGTGGTTTACGAGCG
ago1-1	oET39: ATGAAAAAGCCTGAACTCACCG oET40: CGTCCATCACAGTTTGCCAGT A13: ATGAATGGCTTAAAAACCTG A14: CCTTATTCATCATGTTCCAT

Table S4. Primer sequences for plasmid construction. (F: forward primer; R: reverse primer)

Primer ID	Sequence (5'→3')	Restriction	Usage
oFD653	TGGCGCGCCATGGTGAG CAAGGGCGAGGAG	AscI	Amplify CFP-AGO1, F Amplify CFP-NOS terminator, F
oFD654	GTTAATTAAGCCCCCCCT CGAGTATGTGGT	PacI	Amplify CFP-AGO1, R
oFD717	TGGATCCATCCACTGTGG AATTCGCCCTTGAG	BamHI	Amplify CFP-NOS terminator, R
oFD894	TGGCGCGCCAATCGTGG TTGAGTCAACCAGGTG	AscI	Amplify genomic *MIR168A*, F
oFD930	TCCCGGGAGATGGTTCA AAACTGATCGCTGG	XmaI	Amplify genomic *MIR168A*, R
oFD971	TGGCGCGCCAAGAAAAA TGGCCATCCCCTAGC	AscI	Amplify genomic *IPS1*, F
oFD972	TCTCGAGGAGGAATTCA CTATAAAGAGAATCG	XhoI	Amplify genomic *IPS1*, R
oFD899	TGTCGACATGGCTCCAA AGAAGAAGAGAAAGGT CGTTCCCGAGCTGCATC AAGCTACCATGGTGAGC AAGGGCGAGGAGCTG	SalI	Amplify NLS-miR168 site-1×GFP-Nos T, F
oFD900	TCCCGGGGAACAAAAGC TGGAGCTCCACCGC	XmaI	Amplify NLS-miR168 site-1×GFP-Nos T, R

oFD915	TCTCGAGGGATCCCAGGGCGCCGGTG	XhoI	Amplify NLS-miR168-4m site-1×RFP no stop, R
oFD916	TGGTACCATGGCTCCAAAGAAGAAGAGAAAGGTCGTGCCGGAGCTCCATCAGGCTACCATGGCCTCCTCCGAGGACGTCATC	KpnI	Amplify NLS-miR168-4m site-1×RFP no stop, F
oFD1134	TGGCGCGCCATGGGTAGGTCAAAGTGTTTCATGG	AscI	Amplify full length SQN CDS, F
oFD1135	TGCGGCCGCCTATACGAACATTTTGCGGTACTGC	NotI	Amplify full length SQN CDS, R
oFD1136	TGGCGCGCCATGGCGGACGCTGAAACCTTTGCT	AscI	Amplify full length HSP90.2 CDS, F
oFD1137	TGCGGCCGCTTAGTCGACTTCCTCCATCTTGCTAC	NotI	Amplify full length HSP90.2 CDS, R
oFD1138	TGGCGCGCCATGGCGGACGCAGAAACCTTTGCTT	AscI	Amplify full length HSP90.3 CDS, F
oFD1139	TGCGGCCGCTTAGTCAACTTCCTCCATCTTGCTAC	NotI	Amplify full length HSP90.3 CDS, R
oFD1144	TGGCGCGCCATGGCGGATGTTCAGATGGCTGATG	AscI	Amplify full length gHSP90.1, F
oFD1145	TGCGGCCGCTTAGTCGACTTCCTCCATCTTGCTC	NotI	Amplify full length gHSP90.1, R

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Table S5. Constructs used for modification.

ID	Genotype	Usage
A53	pZLL:CFP-AGO1	CFP-AGO1 amplification
ALH007	CFP-NOSt with GAGA-Linker	CFP-NOSt amplification
SB225	pOp:NLS-3×YFP-35St	Generation of the operator construct
SB229	pOp:MIM165/166-35St	Generation of the operator construct
STK075	pZLL AscI flanked	Introducing ZLL promoter to CFP-AGO1
STK078	pAS2 AscI flanked	Introducing AS2 promoter to CFP-AGO1
STK079	pAS1 AscI flanked	Introducing AS1 promoter to CFP-AGO1
STK082	pATHB8 AscI flanked	Introducing ATHB8 promoter to CFP-AGO1
STK137	pRPS5A AscI flanked	Introducing RPS5A promoter to CFP-NOSt
STK164b	2×35Senhancer:_AscI_LhG4	Introducing *UFO* promoter to generate the driver construct
STK195	pUFO AscI flanked	Introducing UFO promoter to CFP-AGO1
STK198	pAGO1 AscI flanked	Introducing AGO1 promoter to CFP-AGO1
STK157	pRPS5A:NLS-miR394 site-2×GFP	NLS-miR168 site-1×GFP-NOSt amplification
MU594	pATML1ΔB:NLS-3×GFP	Introducing GFP
STK285	NLS-miR394 site-4m-RFP no stop	NLS-miR168-4m site-1×RFP amplification
STK286	RFP stop BamHI flanked	Introducing RFP

Table S6. Primer sequences for the preparation of *in situ* hybridization (ISH) probes. (F: forward primer; R: reverse primer)

Primer ID	Sequence (5'→3')	Usage
CNA-ISH-F	GGATTTGGAGGCTTGTAGCGTGC	CNA in situ probe
CNA-ISH-R	TCACACAAAGGACCAATTGATGAAC	CNA in situ probe
ATHB8-ISH-F	ATCTTGAGCCATGGAGTGTGCC	ATHB8 in situ probe
ATHB8-ISH-R	CCAGTTGAGGAACATGAAGCAG	ATHB8 in situ probe
AGO1-ISH-F1	TTCTCTGGCTTCTGTTGAGGCTC	AGO1 in situ probe 1
AGO1-ISH-R1	TCAGCAGTAGAACATGACACGCT	AGO1 in situ probe 1
AGO1-ISH-F2	ATGGTGAGAAAGAGAAGAACGG	AGO1 in situ probe 2
AGO1-ISH-R2	ACGCTTTCCACTCTGTCCTTTAC	AGO1 in situ probe 2
PHB-ISH-F	ACAGAAATCTACTCCGAACGGTGC	PHB in situ probe (Smith and Long, 2010)
PHB-ISH-R	TGCCTGCTCGTAAGATACCATC	PHB in situ probe (Smith and Long, 2010)
PHV-ISH-F	ACAAATCCGAATGATCATCAAT	PHV in situ probe (Smith and Long, 2010)
PHV-ISH-R	TCGCTTGCTCATACGAAACCG	PHV in situ probe (Smith and Long, 2010)

REV-ISH-F	GTAGGAGCCTTGAAGTTTTCAC	REV in situ probe (Smith and Long, 2010)
REV-ISH-R	AGCTTGTTCATAACTCACATGTC	REV in situ probe (Smith and Long, 2010)

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Table S7. Primers for RT-PCR and qRT-PCR. (F: forward primer; R: reverse primer)

Primer ID	Sequence (5'→3')	Usage
PHB-RT-F	GAGATATGATGAACAGAGAGTCGCC	RT-PCR
PHB-RT-R	ACCAAACTTCCCAGGGGACA	RT-PCR
PHV-RT-F	CCATGGACGATAGAGACTCTCC	RT-PCR
PHV-RT-R	ACCACTTCCAAAACTTGGAAGA	RT-PCR
REV-RT-F	AACCACCGTGAGAGAAGCAGT	RT-PCR
REV-RT-R	CCGGGAACATAGTGAAAACTTC	RT-PCR
CNA-RT-F	TCTTGCAAGGATGGTAAGTTGG	RT-PCR
CNA-RT-R	CTATTAGTCTGAGTAACCTCCTGAGC	RT-PCR
ATHB8-RT-F	AGGAAGCAATAATAGTCACAATATGG	RT-PCR
ATHB8-RT-R	ATACTTGGCCCGTTTTGTGTATT	RT-PCR
ZLL-RT-F	GGAATTCCAGAGAACGGGGAAGAGTCA	RT-PCR
ZLL-RT-R	GGAATTCTTCCGCTTCGTCAGTACTG	RT-PCR
ACTIN7-RT-F	GGTGAGGATATTCAGCCACTTGTCTG	RT-PCR
ACTIN7-RT-R	TGTGAGATCCCGACCCGCAAGATC	RT-PCR
AGO1-qRT-F	GGACCACCGCAGAGACAATCAG	qRT-PCR
AGO1-qRT-R	GGGAGCTCCTTGTTCAACAGAG	qRT-PCR
PHB-qRT-F	ATGCAACAGGGCTATGCTC	qRT-PCR
PHB-qRT-R	CATCCTTCCCATGCTTGAC	qRT-PCR
PHV-qRT-F	TGCAGCAGGGATATGCGAATCTTC	qRT-PCR
PHV-qRT-R	ACCGTCGCTTGCTCAT ACGAAAC	qRT-PCR
REV-qRT-F	CGCCAAGCTAATGCAACAGGGATT	qRT-PCR
REV-qRT-R	TGTCTTCCCATCGTTGACACACAG	qRT-PCR

PP2AA3-qRT-F	TAACGTGGCCAAAATGATGC	qRT-PCR
PP2AA3-qRT-R	GTTCTCCACAACCGCTTGGT	qRT-PCR

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