Supplementary Figure 1

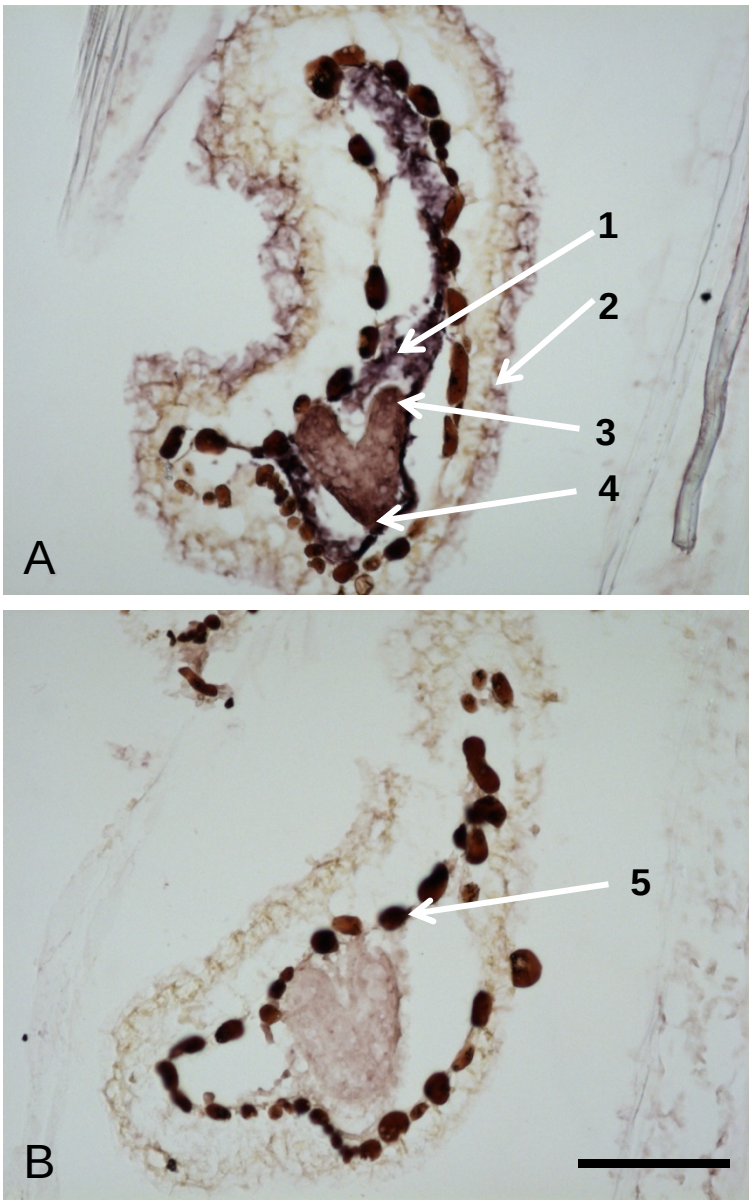
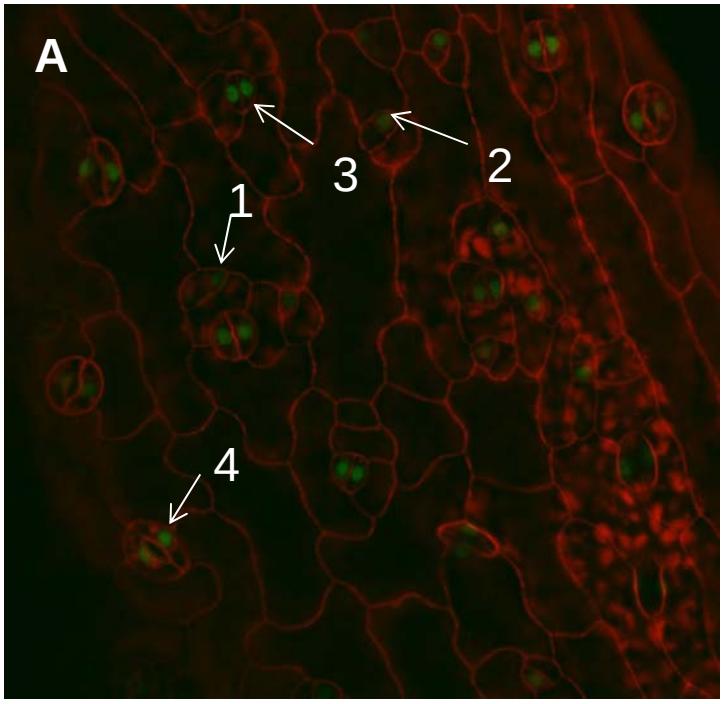
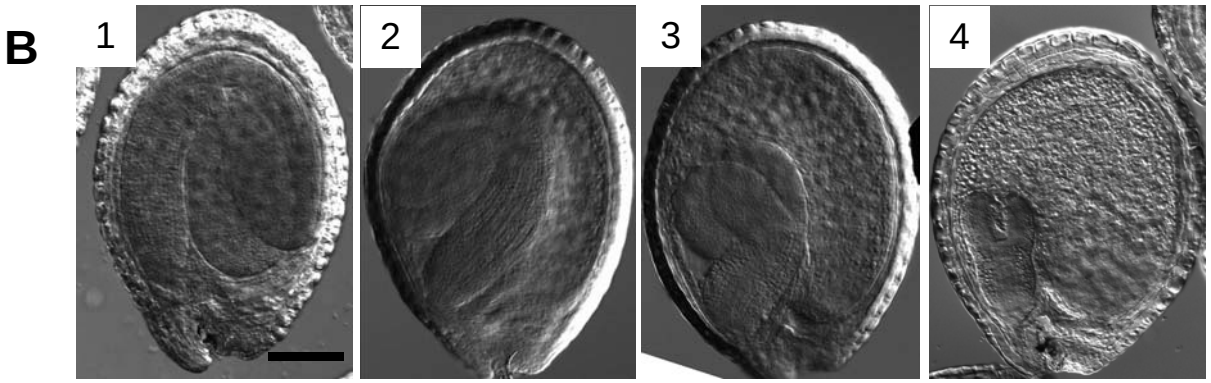


Fig. S1. *ICE1* antisense and sense probe hybridization to wild-type seed sections.
Expression of *ICE1* during early wild-type seed development A) Antisense probe, showing strong staining in the embryo surrounding endosperm (1), testa (2) and some staining in the embryonic cotyledons (3) and root pole.(4). B) Sense probe, showing only artefactual dark colouration of the endothelium (5). Scale bar = 100μm.

Supplementary Figure 2





ice1-2/ICE1	**92**	**0**	**0**	**28**
***ice1-2/ICE1* (36-A)**	**97**	**0**	**1**	**6**
***ice1-2/ICE1* (36-D)**	**87**	**7**	**9**	***5***
***ice1-2/ICE1* (32-8)**	**102**	**0**	**2**	**8**

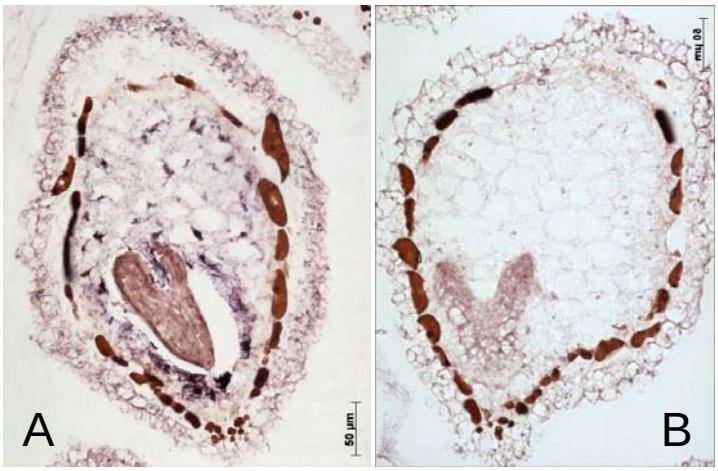
Fig. S2. The *ice1-2* endosperm persitence phenotype is complemented by an ICE1:GFP fusion protein. The promoter of *ICE1*, including the 5'UTR, was amplified with the primers *pICE1F* and *pICE1R* and then cloned into pENTR5'-MCS, an entry vector containing a multiple cloning site, as a BamH1 Not1 fragment (Lee et al., 2006). The *ICE1* ORF without the stop codon was amplified with *ICE1ORFF* and *ICE1ORFR* and cloned into pENTR/D-TOPO (Invitrogen). A triple LR recombination using the resulting two plasmids and pENTR R2-L3-GFP S65C+stop (as described in (Fobis-Loisy et al., 2007; Heim et al., 1995)) was then carried out into the destination vector pART27 (Gleave et al., 1992), to give *pICE1:ICE1-GFP*. Five independent homozygous transgenic lines carrying one copy of the transgene were generated, and expression of the transgene was monitored in the stomatal lineage by confocal microscopy (Supplemental Figure 2). The lines showing the strongest expression levels were crossed to *ice1-2*, and the seeds of T1 individuals carrying the transgene and heterozygous for *ice1-2* and were scored for the ice1-2 seed shrivelling phenotype.

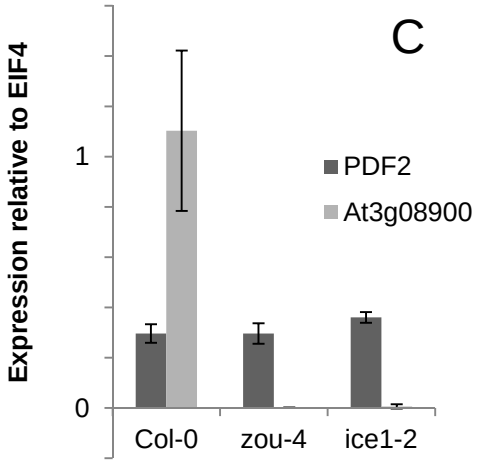
A) Analysis of fusion protein expression from the *pICE1-ICE1-GFP* construct used in complementation studies showing nuclear localized GFP signal in 1) meristemoids, 2) guard mother cells (GMC), 3) newly divided GMCs and 4) newly differentiated stomata in the epidermal cell layer of a young leaf. This expression pattern reflects exactly that shown in (Kanaoka et al. 2008).
B) Complementation of the *ice1-2* seed phenotype by the introduction of *pICE1-ICE1-GFP* construct. Siliques from three independent F1 progeny of crosses between *ice1-2* homozygotes and either independent homozygous *pICE1-ICE1-GFP* transgenic lines or Col-0 were cleared and pooled for each experiment. Seeds were divided into four phenotypic classes 1) Wild-type, 2 and 3) intermediate and 4) ice1. Results are consistent with complementation by 36-A and 32-8, and partial complementation by 36-D. (Fisher's exact test confidence >99.0% in each case). Scale bar = 100μm.

References

Fobis-Loisy, I., Chambrier, P. and Gaude, T. (2007). Genetic transformation of Arabidopsis lyrata: specific expression of the green fluorescent protein (GFP) in pistil tissues. *Plant Cell Rep* 26, 745-53.
Gleave, A. P. (1992). A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome. *Plant Mol Biol* 20, 1203-7.
Heim, R., Cubitt, A. B. and Tsien, R. Y. (1995). Improved green fluorescence. *Nature* 373, 663-4.
Lee, J. Y., Colinas, J., Wang, J. Y., Mace, D., Ohler, U. and Benfey, P. N. (2006). Transcriptional and posttranscriptional regulation of transcription factor expression in Arabidopsis roots. *Proc Natl Acad Sci U S A* 103, 6055-60.

Supplementary Figure 3





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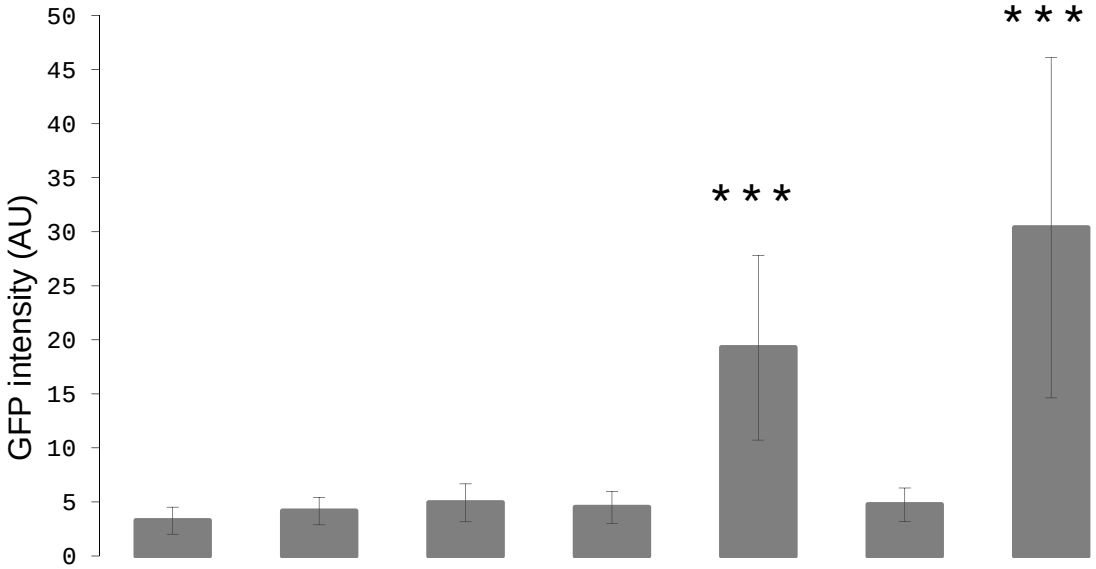

protoplasts	+	+	+	+	+	+	+
p*At3g08900:*GFP	-	+	+	+	+	+	+
proOsActin: AtZOU	-	-	+	-	+	-	+
proOsActin: AtICE1	-	-	-	+	+	-	-
proOsActin: SCRM-D	-	-	-	-	-	+	+

Fig. S3. ZOU and ICE1 form heterodimers to activate target gene expression.
Expression of *At3g08900* in the endosperm of a wild-type seed (A) and *zou-4* mutant seed (B). C) qRT-PCR expression data for *PDF2* (control) and *At3g08900* in the developing siliques of wild-type, *zou-4* and *ice1-2* mutants. D) Study of *At3g08900* promoter activities.The *ZOU* ORF, and the *ICE1* ORF or *SCRM-D* ORF were amplified from cDNA prepared from either wild-type or *SCRM-D* siliques using the primers AtZouF and AtZouR , or AtIce1F and AtIce1R respectively, and recombined directly into pDONOR221 (Invitrogen) using Gateway ™ technology. ORFs were then recombined into the destination vector *pBS TPp-A* (Thévenin et al., 2012) which allows constitutive expression in protoplasts under a rice actin gene promoter. The promoter of *At3g08900* was amplified from wild-type genomic DNA with the primers *p At3g08900F and p At3g08900R* and recombined into pDONOR221 (Invitrogen) using Gateway ™ technology. This was then recombined into the plasmid *pBS TPp-B (Thévenin et al., 2012)*, upstream of a promoterless GFP-encoding ORF. Different combinations of purified plasmids were transformed into protoplasts, and resulting promoter activity was quantified using flow cytometry as described in Thévenin et al., 2012. Green fluorescent protein (GFP) intensities (arbitrary units) measured in *P.patens* protoplasts co-transfected with 5µg of each vector alone or in combination, compared with nontransfected protoplasts, are presented. Transactivation activities were monitored from three biological repetitions by GFP fluorescence quantification using flow cytometry. t-test significance: ***, $P < 0.001$. Error bars indicate Standard Deviation. Scale bar = 50µm.

Table S1. Primers used in this study

Primer	Sequence
ICE T-DNA F	AATCTGATGGCTGAGAGGAGAA
ICE T-DNA R	TTCATGGTAGCGAGCAACAGAC
SALK LB	ATTTGCCGATTTCGGAAC
1g71250cdsFor	CACCATGAACACAAACAGAAAGAAGATG
1g71250cdsRev	GTGAAGGAGTGTCATCTGTTGG
2g43870cdsFor	CACCATGGCTTCACTTCTTGTCCTC
2g43870cdsRev	TAGACAATTTGGCTGAAAAAATC
At3g38000cdsFor	CACCTTTATAACTCATGATGACGTCATCCCATCA
At3g38000cdsRev	TGATAATTGATAATTAGAGCAGAGCATTATTATTAGCATTA
ICE1-Q-L	GTTTGCCTTGGATGTTTTCC
ICE1-Q-R	GCTTTGATTTGATCAGGCAGT
ICE 5' YEAST	TGGCCATTACGGCCATGGGTCTTGACGGAAACAATGG
ICE 3' YEAST BAIT	CGACATGGCCGAGGCGGCCAAGATCATACCAGCATACCCTGC
ICE 3' YEAST PREY	CGAGAGGCCGAGGCGGCCGGTACATACCAGCATACCCTGC
ICE-SHORT YEAST 5'	TGGCCATTACGGCCGGGGGAGATATGGATGAGACT
ZOU 5' YEAST	TGGCCATTACGGCCATGACTAATGCTCAAGAGTTGGG
ZOU-SHORT YEAST 5'	TGGCCATTACGGCCATATCAAACCATCCCATAGACGC
ZOU 3' YEAST BAIT	CGACATGGCCGAGGCGGCCAATAGAGATGAAAAATATAACACCAG
ZOU 3' YEAST PREY	CGAGAGGCCGAGGCGGCCAATAGAGATGAAAAATATAACACCAG
AtZouF	GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGACTAATGCTCAAGAGT
AtZouR	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTATAGAGATGAAAAATATA
AtIce1F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTTATGGGTCTTGACGGAAACAA
AtIce1R	GGGGACCACTTTGTACAAGAAAGCTGGGTTTCAGATCATACCAGCATAC
p At3g08900F	GGGGACAAGTTTGTACAAAAAAGCAGGCTTTGGAAAGCTGCCCCTATTGAGA
p At3g08900R	GGGGACCACTTTGTACAAGAAAGCTGGGTTTTGAAACGACAAGGAAGA
pICE1F	AAGGAAAAAAGCGGCCGCAAAGCAAATTAAGTGGTTCTA
pICE1R	CGCGGATCCCGCCAAAGTTGACACCTTTAC

ICE1ORFF	CACCATGGGTCTTGACGGAAACAAT
ICE1ORFR	GCAGGGTATGCTGGTATGATC