

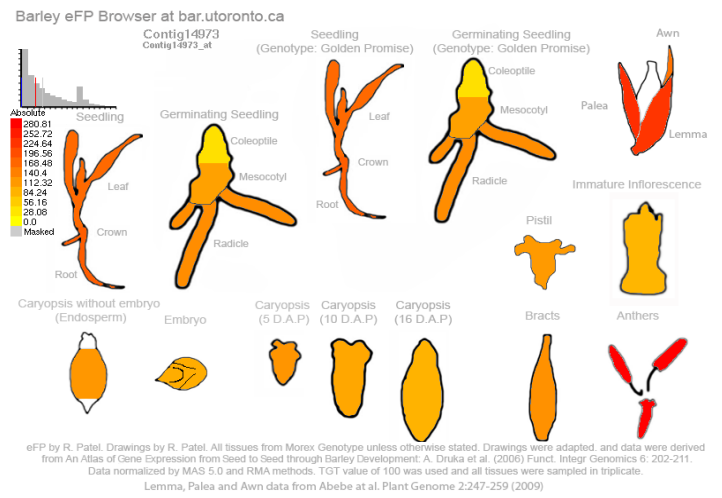
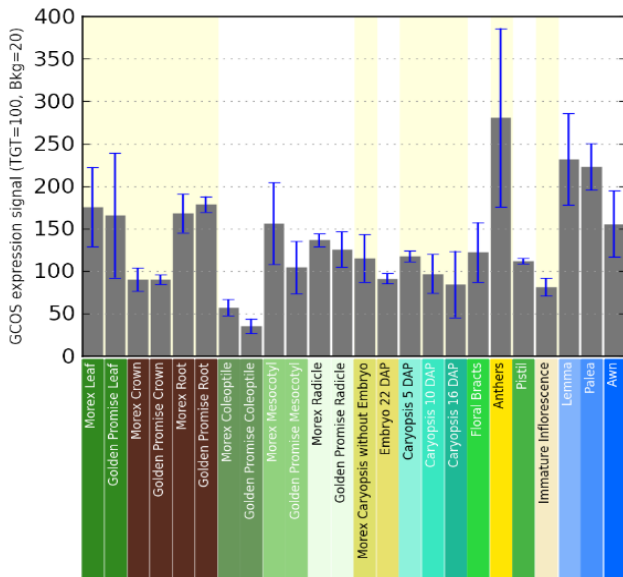
Figure S1. Alignment of *HvABCD1* and *HvABCD2* cDNA sequences, showing the region used for the RNA interference construct

The alignment was created using ClustalW2 multiple sequence alignment.

HvABCD2	ATGTCAGGTACCATGCCGTCCCTGCAGCTGCTGCAGCTACCGAGCGTGGCCGCGGGCTG	60
RNAi-insert	-----	
HvABCD1	-----ATGTCATCTCTTCAGTTATTGCAACTAACAGAGCATGGGCGGAACCTT	48
HvABCD2	CTCGCGTCCCGAGGAGGACGCTGGCGGTTGTCTCGGGCGCGCTGGTCGCTGGCGGGGCG	120
RNAi-insert	-----	
HvABCD1	TTTTCTTCGAGAAGGAGGACGCTTGCAGTTGTTTCTGGTGCAGTCTGCTTGGTGGAACT	108
HvABCD2	TTGGCGTACGCGCGGTTCGGGCCGAGGCCAAAGGAGGCGAGGCCGTCGGGAGGCCCGCC	180
RNAi-insert	-----	
HvABCD1	TTAGCATACGCGCAGACAAGTCGACG-----GAGGAAACATCGGGAAGAAAATCCT	159
HvABCD2	GCCAATGATGGCGGCGATGCGTTGGGCAGGAATGGGAGAGGCTGGGTCACAGCGGCACG	240
RNAi-insert	-----	
HvABCD1	TGTAATGATG-CAAATGTACATAG--CAGGAGTGAAGAGAACATTAGTCAAACCGGTGTC	216
HvABCD2	GATGGCCGGTTGGCCGGGACGACGAAGCGGAGGAAGAGCGCGCTGAAGTCCTTGCAATTC	300
RNAi-insert	-----	
HvABCD1	GATGGTAAAGTG--GTAAAAACAAGGAAAAAGAAAAATTTATTGAAATCCTTACATTTT	273
HvABCD2	TTGGCCGCTATCCTGCTCAAGAAGATTGGACCGAGTGGCACGCGATACCTTCTTGGCTTG	360
RNAi-insert	-----	
HvABCD1	CTGGCTGCTATTTTACTTAAGAAGATTGGTCCAAGCGGAACAATTACCTTCTTGGCTTG	333
HvABCD2	ATGTTGACAGCCGTGCTGCGTACTGCTGTCGGGCATAGATTAGCAAAGTTCAAGGCTTT	420
RNAi-insert	-----	
HvABCD1	ATGTTAACAGCAGTGTACGTACAACCATTTGGTCACAGATTAGCAAAGTTCAAGGGTAT	393
HvABCD2	CTATTTAAAGCGGCATTTCTTCGCGGTGTTCCAACCTTTACGCGCCTAATTATAGAGAAT	480
RNAi-insert	-----	
HvABCD1	TTGTTTAAATCCGCGTTTCTTCGCGGTGTTCCGACTTTACACGCGCTAATCATTGAAAAAT	453
HvABCD2	CTGATCTTATGCTTTCTCCAATCAGCAGTATACCAGACCTCAAATACTTAACTGGGTCC	540
RNAi-insert	-----	
HvABCD1	CTTCTATTATGCTTTCTTCAGTCCACGGTATATCAGACCTCAAAGTACTTGACAGGATCC	513
HvABCD2	TTGAACTTGCCTTCAAGAAAAATTTTAAACAGACCTCGTCCATGCCGATTATTTCCAGAAT	600
RNAi-insert	-----	
HvABCD1	TTAAGCTTGCCTTCAAGAAAAATTTGACGGATCTCATCCATGCTGATTACTTCGAGAAT	573
HvABCD2	ATGGTTTACTACAAGATCTCTCATGTGGATCATCGAATATCAAATCCGGAGCAAAGGATT	660
RNAi-insert	-----	
HvABCD1	ATGGTTTACTACAAGATCTCACATGTAGATCATCGGATTTCAAACCCAGAGCAAAGGATT	633
HvABCD2	GCCAGTGATATCCAAAGTTCTCTTCTGAACTTAGTGAACCTGTACAGGATGATTTGGCT	720
RNAi-insert	-----	
HvABCD1	GCTAGTGATATTCCTAAGTTCTGCGCAGGACTAAGTGACCTTGTACAAGATGATCTGATT	693
HvABCD2	GCAGTTGCAGAAGGTTGATATATACATGGCGTCTCTGTTCTTATGCTAGTCCGAAATAC	780
RNAi-insert	-----	
HvABCD1	GCAGTTGCAGATGGGTTAATATACATCTGGCGCATCTGCTCTTATGCAAGTCCGAAATAT	753
HvABCD2	ATGCTTTGGATTCTGGCATATATACTAGTTGCTGGTGGAGCAATTAGAAAATTTCTCTCCT	840
RNAi-insert	-----	
HvABCD1	GTTCTCTGGATTCTGGCATATGTAAGTTGGTGGTGGCACAATTAGAAAATTTCTCTCCT	813
HvABCD2	GCTTTTGGGAAGATGAAATCCACAGAGCAGCACTTAGAAGGGGAGTATCGTCAACTTCAT	900
RNAi-insert	-----	
HvABCD1	TCTTTTGGGAAGTTGAAAGCCATGGAACAACAACCTAGAAGGGGAATATCGCCAGGTTTCT	873
HvABCD2	TCACGCTTAAGAACTCATGCTGAGAGTGTAGCATTTTATGGTGGTGAAGAGAGAAGAA	960
RNAi-insert	-----	
HvABCD1	TCACGACTGAGAACTCATGCTGAGAGCGTGGCATTATGGTGGTGAAGAGAGAAGAA	933

HvABCD2	TATCATATTATGCAGCGATTTTCGAGCTCTTGTGGGCACTTGAAGCATGTACTCCATGAA	1020
RNAi-insert	-----	
HvABCD1	TCACACATTATGCAGCGGTTCCAGGCTCTTGTGCGGCACTTGAATGTTGTTCTTCATGAG	993
HvABCD2	AACTGGTGGTTCCGCATGATTCAAGATTTCTTTCTGAAGTACTTTGGGGCCACTGTAGCA	1080
RNAi-insert	-----	
HvABCD1	AACTGGTGGTTTGGCATGATTCAAGATTTCTTTCTGAAGTATCTTGGTGCCACAGTGGGA	1053
HvABCD2	GTGTGCTTATTATCGAACCGTCTTCTCCGGTGACCTTAGACCTGATTCATCTACCATA	1140
RNAi-insert	-----	
HvABCD1	GTTATCTTGATTGTGCAACCTTTCTTTGCGGGAGATCTTAAACCTGATAACATCTACTTTA	1113
HvABCD2	GGAAGGGCTGATATGTTGAGTAACCTTCGATACCACACTAGCGTGATCATAGCACTATTT	1200
RNAi-insert	-----	
HvABCD1	GGGCGGGCAGAGATGTTGAGCAATCTTAGATATCACACAAGCGTGATAATATCATTATTC	1173
HvABCD2	CAGTCTCTTGGGACCCCTCTCTATCAGCTCTAGGCGTTTGAATATCCTGAGTGGCTATGCA	1260
RNAi-insert	-----	
HvABCD1	CAGTCGCTTGGCACCCCTTCTACCAGCTCAAGACGTTTAAATCTTCTCAGTGGCTATGCA	1233
HvABCD2	GACCGGATTCGTGAGTTACTAGATGTTTACGTGAGCTGTCTGGGGTCCGTGATAGATCG	1320
RNAi-insert	-----	
HvABCD1	GACCGTATTCACGAGTTACTGGATGTTTACGTGAGCTATCTGGGGTTCGCGACAGGTCG	1293
HvABCD2	TTGAATCACAGCTCTTCTGTGGGAATTACATCAGCGAAGCAAACCATATAGAATTTTCT	1380
RNAi-insert	-----	
HvABCD1	ATGAGTCGAAATTCCTCTGCCAAAACCTATATTAGTGAGGCAAACCTATATAGAATTTTCA	1353
HvABCD2	GGTGTAAAGTGGTGACACCCGCTGGGAACGTTTGGTTGACGATTTAACTCTCCGAGTA	1440
RNAi-insert	-----	
HvABCD1	GGTGTCAAGTGGTGACACCCCTCTGGGAATGTCTTGGTTGATGATTTAACTCTCCGGGTG	1413
HvABCD2	GAAACTGGATCTAATCTTTAATCACCCGTCCCAATGGTAGTGAAAAAGCTCCCTTTT	1500
RNAi-insert	-----	
HvABCD1	GAGTCGGGTTCTAATCTTTTGATCACTGGTCCCAACGGTAGTGAAAAAGCTCTCTTTTC	1473
HvABCD2	CGGGTCTTGGAGGCCATATGGCCGCTAGTATCTGGCCATATTGTCAAACCTGGTGTGGT	1560
RNAi-insert	-----	
HvABCD1	CGTGTCTTGGGGTCTGTGGCCACTTGTATCTGGCCACATTGTCAAACCTGGTGTGGT	1533
HvABCD2	TCCGATCTTAAACAAGGAAATCTTTTATGTCCCAAAAGACCATACACAGCTGTTGGCACA	1620
RNAi-insert	-----	
HvABCD1	TCTAATCTTAAACAAGGAAATTTTATGTCCCAAGGACCATATACAGCTGTTGGAACA	1593
HvABCD2	CTTCGCGAACAACTAATCTATCCTCTTACAGCAGATCAGGAGACTGAACCCTTACCTAT	1680
RNAi-insert	-----	
HvABCD1	CTGCGCAGACCAGTTAATCTATCCACTTACAGCAGATCAGGAAACCGAACCTTAGCTAT	1653
HvABCD2	GATGGCATGGTGGACCTTCTAAAGAATGTTGATCTGGAATACTTGCTAGAACGTTATCCT	1740
RNAi-insert	-----	
HvABCD1	GGTGGTATGGTGGATCTTCTAAAGAATGTTGATTTAGAATACTTGCTAGAACGCTACCCT	1713
HvABCD2	CTTGACAAGGAAGTTAACTGGGGTGATGAGCTATCCCTTGGCGAGCAACAAAGATTAGGA	1800
RNAi-insert	-----	
HvABCD1	GTCGACAAGGAAGTTAACTGGGGCGATGAGTTGTCTCTTGGTGAGCAACAGAGATTGGGA	1773
HvABCD2	ATGGCCAGGCTGTTCTACCATAAGCCAAAATTTGCTATCTTGTGATGAGTGTACCAGTGCT	1860
RNAi-insert	-----	
HvABCD1	ATGGCCAGATTGTTCTACCATAAGCCCAAGTTTGGCCATCCTGGATGAGTGTACTAGTGCT	1833
HvABCD2	GTGACAATTGATATGGAAGAACGTTTCTGCAAAAAGGTTCCGAGCATGGGAACATCATGC	1920
RNAi-insert	-----	
HvABCD1	GTGACGACTGATATGGAAGAACGTTTCTGCAATAGGGTCCGAGCAATGGGCACGTCATGC	1893
HvABCD2	ATAACAATATCTCATCGCCAGCATTAGTTGCATTTTCATAATATTGTTTTGTCAATTGGAC	1980
RNAi-insert	-----	
HvABCD1	ATAACAATATCTCACCGACCAGCATTAGTTGCTTTTCATGATGTCGTTTTGTCTTGGAT	1953

A



B

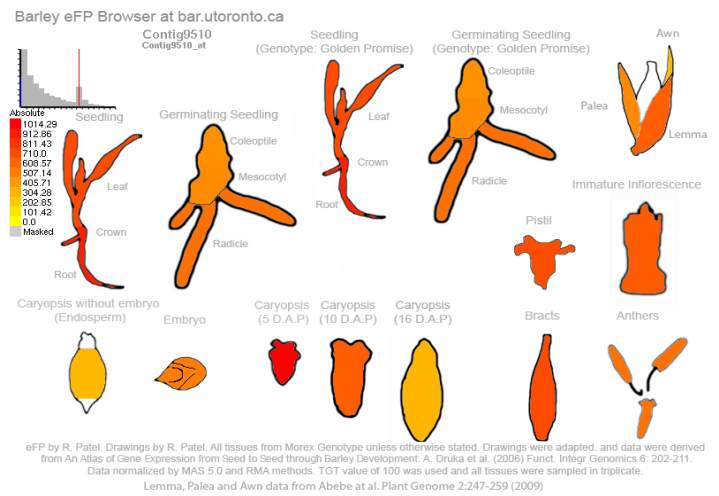
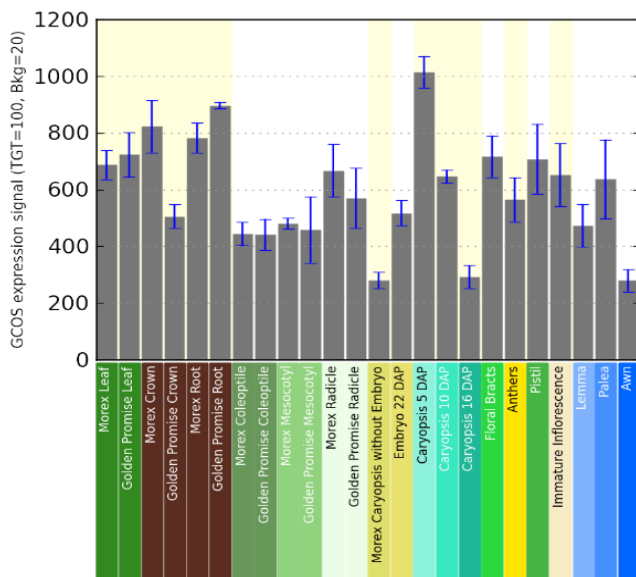


Figure S2. Expression analysis of *HvABCD1* and *2*

Histograms and and electronic fluorescent pictographs for (A) *HvABCD1* and (B) *HvABCD2* were obtained by interrogating the Bio-Array Resource barley eFP browser.

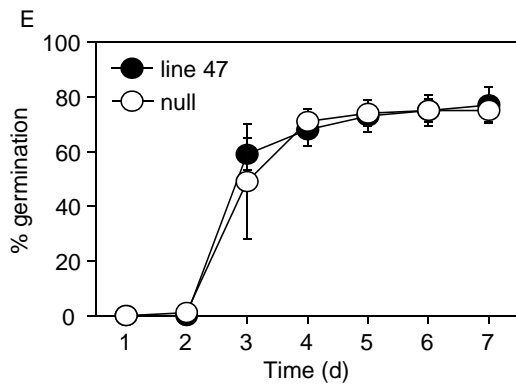
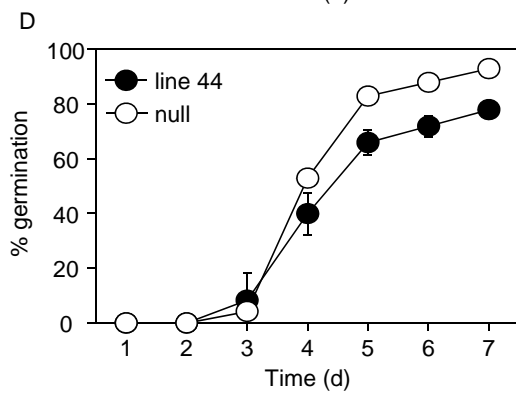
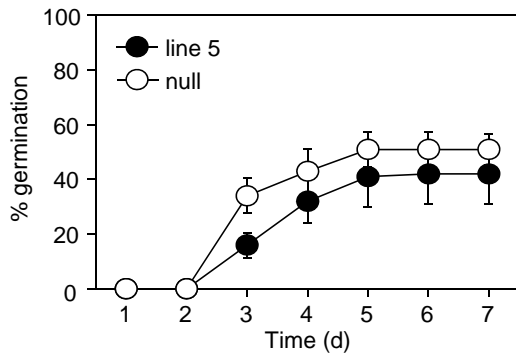


Figure S3. Germination of RNAi lines and corresponding nulls over 7 d.

Values are means \pm SE (n=3; 50 seeds per replicate).

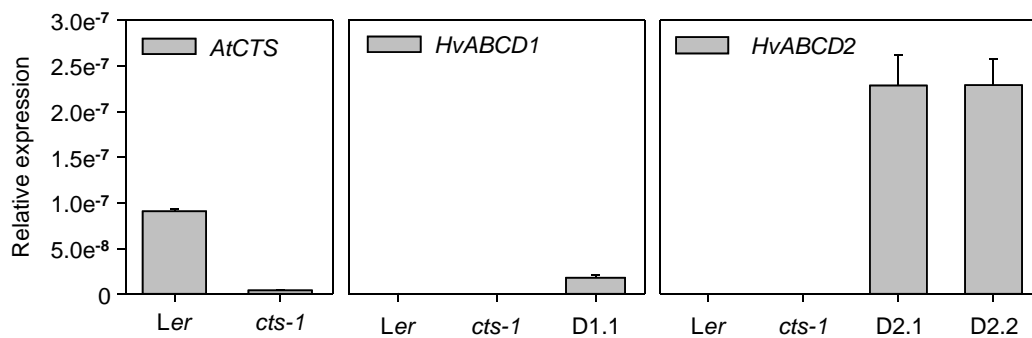


Figure. S4. Q-PCR analysis of gene expression in Arabidopsis transgenic lines and controls.

Transcript abundance is shown relative to housekeeping genes. RNA was isolated from seedlings grown on 0.5 x MS containing 0.5 % sucrose. The testae of *cts-1* and line A19 seeds were mechanically disrupted to induce germination. Values are means \pm SE (n=3)

Supplementary Data

Supplementary Table 1 Primers used in this study

Name	Sequence (5'-3')
CTS-a start F	CAATGTCATCTCTTCAGTTATTGC
Hv14973 R2	GGTGCTTCCTCTGTTTGTTG
HvABCD1 (CTSa) F	AGTGTGCCATCTGAACAAAC
HvABCD1 (CTSa) R	AATGGAACTTTTGCCACTGC
HvABCD2 (CTSb) F	CAGCCTCTCCCCATTCT
HvABCD2 (CTSb) R	AGTGCAGCCTCAGACGAAAT
α -tub F	AGTGCCTGTCCACCCACTC
α -tub R	AGCATGAAGTGGATCCTTGG
PM19F	GGAGGCGTTCGTCATCATC
PM19R	TCCTATTTAGACCCTTGCCAC
HvABCD RNAi F	TTCCTGAGAGCTATATCCCCTGA
HvABCD RNAi R	GGCACTGGGGTATCCTTTTGAG
HvELF1F	CCTGCCCAAGTTCCTCAA
HvELF1R	GGCTTGGTGGGAATCATCT
HvTubulinF	ACACCATTGGCAAGGAGATT
HvTubulinR	TGTCGGACAGCTTCCTGATAC
HvJIP2 F	ACCTTCATGACGGCAACTG
HvJIP2 R	CTCCCGGTGTTCTTTTCATC
HvJRG1.2 F	GAAATGGAGGGGGTGATAAAG
HvJRG1.2 R	CGCTGCTAACAGTGATGCTC
AtCTS F	GAGATTAGGCATGGCACGTT
AtCTS R	GTCGCATTTGTGCATTCATC
AtCTL1 F	AGTGGAGAGGCTGCAGAAGA
AtCTL1 R	CTCGGGTAGCACGAGCTTTA
AtCTL3 F	GAAGTGTCTCGACAAAGGTCGT
AtCTL3 R	CCTTTTGGCACTTCTGGTG
AtCTSp5'Nco	TAACCATGGACTTGGAAGAAGGCGGTGA
AtCTSp3'Kpn	TAAGGTACCTGACATGGCCTGGAATTGAGTATA
HvCTSa Kpn5'	TTAGGTACCATGTCATCTCTTCAGTTATTGCAA
HvCTSa Not3'	TTTGCGGCCGCTGGTGCTTCTCTGTTTGTTG
CTSb Kpn5'	TTAGGTACCATGCCGTCCCTGCAGCTG
CTSb Not I-Bgl II R	TTAGCGGCCGCATCACTGGCAATCCTTTGCTC
CTSb Bgl IIF	ACTGCTGTCCGGCATAGATT
CTSb Not3'	ATTGCGGCCGCCGACCCGGTATGAACAACCTT
FT206	CCCAATGGTACCAAATGTCATCTCTTCAG
FT207	CGAAGGCTGCAGTCAAGCGTAATCTGGAACATCGTATGGGTAGTGTTG ATTGAACAC
FT208	CCCAATGGATCCAAAATGCCGTCCCTGCAGC
FT209	CGAAGGGTCGACTCAAGCGTAATCTGGAACATCGTATGGGTAATGTTGG

ATCTCGCATAGC

Barley accession numbers and Arabidopsis gene identifiers: HvJIP2, CAA47017; HvJIP37, CAA58110; HvJRG1.2, AAB72097; AtCTS, AT4G39850; AtCTL1, At1g04850; AtCTL3, At5g18800.