

The Minus-End-Directed Kinesin OsDLK Shuttles to the Nucleus and Modulates the Expression of Cold-Box Factor 4

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Contents

Suppl. Fig. S1	2
Suppl. Fig. S2	3
Suppl. Fig. S3	4
Suppl. Fig. S4	6
Suppl. Table S1.....	7
Suppl. Table S2.....	8
Suppl. Table S3.....	9

Suppl. Fig. S1

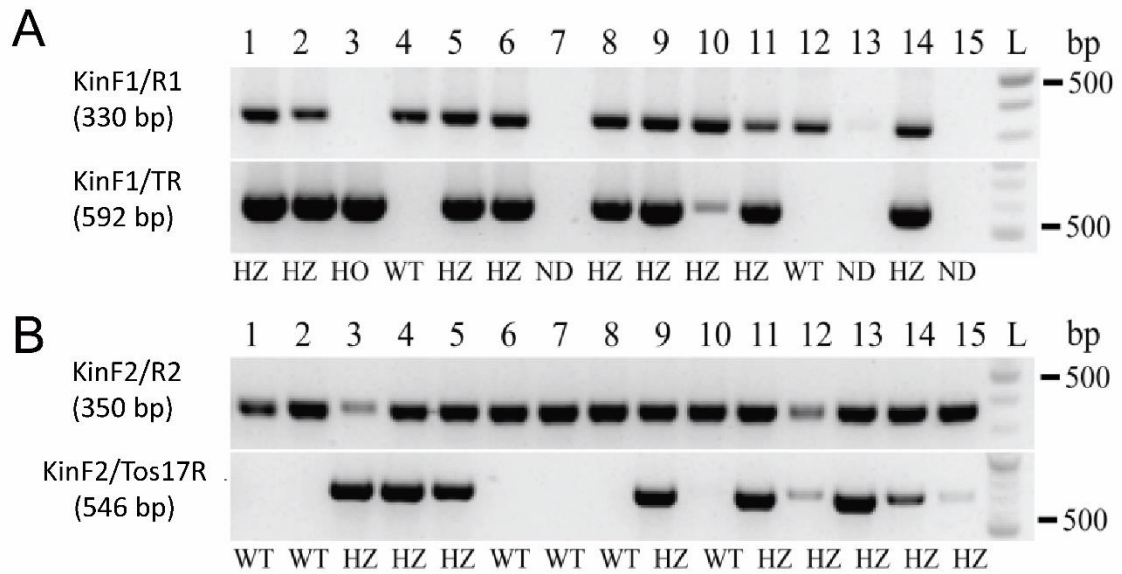


Figure S1. Representative gel electrophoresis results from PCR amplification of genotyping rice insertion lines. **A** Representative PCR genotyping results from 15 samples of genomic DNA extracted from T-DNA insertion line PFG_3A-07110.R as templates. Two rounds of PCR were carried out with two pairs of primer which were genome-specific (KinF1/R1, upper row) or insertion-specific (KinF1/TR, lower row), respectively. **B** Representative genotyping results for 16 samples of genomic DNA from Tos-17 insertion line ND4501_0_508_1 using genome specific (KinF2/R2, upper row) and insertion-specific (KinF2/Tos17R, lower row) primer combinations, respectively.

Suppl. Fig. S2

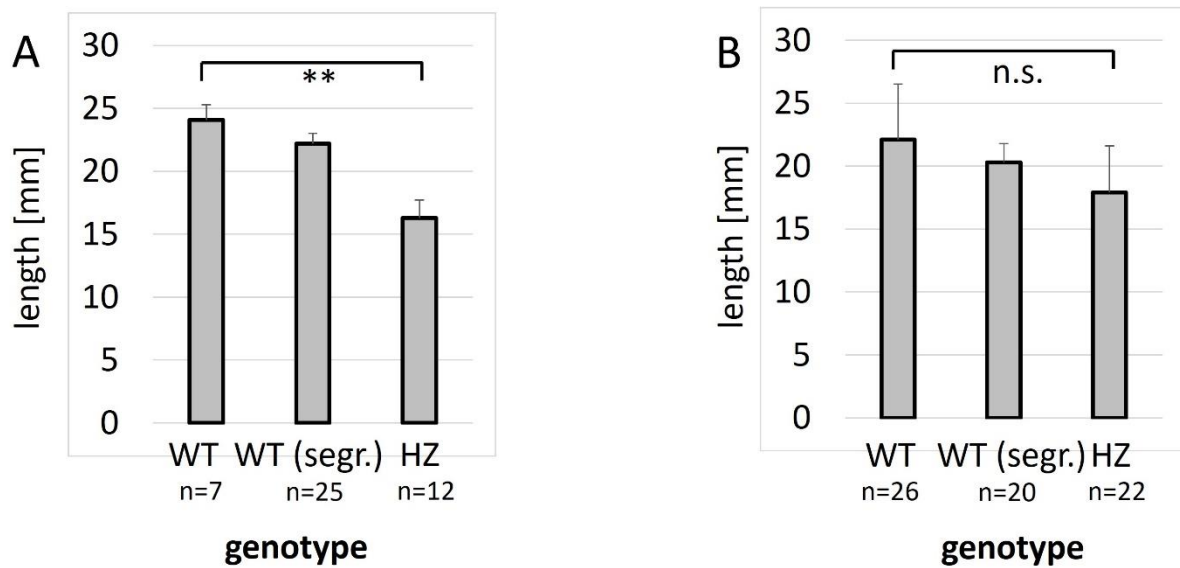


Figure S2. Relationship between DLK and elongation of the fully expanded second leaf sheath. Mean length of fully expanded etiolated coleoptiles in the Tos-17 insertion line ND4501_0_508_1A (**A**) and the T-DNA insertion line PFG_3A-07110.R (**B**). WT gives the values for the respective background (‘Nipponbare’ in **A**, ‘Dongjin’ in **B**), WT segr. for segregating plants genotyped as WT, HZ for the genotyped heterozygotes. No homozygous mutants were recovered at this stage. Error bars represent SE, ns not significant at $P < 5\%$, ** significant at $P < 1\%$ based on a homoskedastic t-test. The number of individuals per set is indicated below the respective bar.

Suppl. Fig. S3

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AthCBF4 B2BJ26  MNPFYSTFPDS---FLSISDHRSP---VSDSSECSPKLASSCPKKRAGRKKFRETRHPIY  54
AthCBF1 B6DTR3  MNSF-SAFSEM-----FGSDYEP-----QGGDYCPTLATSCPKKPAGRKKFRETRHPIY  48
AthCBF2 B2BIW9  MNSF-SAFSEM-----FGSDYESP---VSSGGDYSPKLATSCPKKPAGRKKFRETRHPIY  51
AthCBF3 B2BIZ3  MNSF-SAFSEM-----FGSDYESS---VSSGGDYIPTLASSCPKKPAGRKKFRETRHPIY  51
OsDREB1F Q8S9Z5 MDT-----EDTSSASSSSVSPSSPGGG---HHHRLPPKRRAGRKKFRETRHPVY  47
NtAvr9 Q9FQZ8  MDIFRSYSDPLAEYSSISDSSSSSCNRANHSDEEVMLASNNPKKRAGRKKFRETRHPVY  60
      *:              :.              .              **: *****:*

AthCBF4 B2BJ26  RGVQRNS-GKWVCEVREPNNKSRIWLGTFTVEMAARAHDAALALRGR-SACLNFADS  112
AthCBF1 B6DTR3  RGVQRNS-GKWVSEVREPNNKTRIWLGTFTQTAEMAARAHDAALALRGR-SACLNFADS  106
AthCBF2 B2BIW9  RGVQRNS-GKWVCELREPNNKTRIWLGTFTQTAEMAARAHDAALALRGR-SACLNFADS  109
AthCBF3 B2BIZ3  RGVRRNS-GKWVCEVREPNNKTRIWLGTFTQTAEMAARAHDAALALRGR-SACLNFADS  109
OsDREB1F Q8S9Z5 RGVRRAGGSRWVCEVREPQAQARIWLGTYPTEMAARAHDAALALRGERGAELNFPDS  107
NtAvr9 Q9FQZ8  RGVKRNS-DKWVCELREPNNKSRIWLGTFPSAEMAARAHDAALALRGR-SACLNFADS  118
      **** * . .:*.*:***: ::*****: : *****:****. .* *** **

AthCBF4 B2BJ26  AWRLRIPETTCPKEIQKAASEAAMAFQNETTTEGSKTA-AEAEAAAGEGVREGERRAEQ  171
AthCBF1 B6DTR3  AWRLRIPESTCAKDIQKAAEAALAFQDETCDTTTNHGLDMEETM-----VEAIYTPEQ  161
AthCBF2 B2BIW9  AWRLRIPESTCAKEIQKAAEAALNFQDEMCHMTTDAHGLDMEETL-----VEAIYTPEQ  164
AthCBF3 B2BIZ3  AWRLRIPESTCAKDIQKAAEAALAFQDEMCDATT-DHGFDMETL-----VEAIYTAEQ  163
OsDREB1F Q8S9Z5 PSTLPRARTASPEDIRLAAAQAAELYRRPPPPLA-----LPEDPQEGTSGGGATATSG  160
NtAvr9 Q9FQZ8  AWKLPIPASTDAKDIQKAAEAEEAFRSSEAENMPEYSGEDTK-----EVNST  166
      *      ::  ::*: **:***  ::              . .

AthCBF4 B2BJ26  NGGVFYMDDEALLGMPNFFENMAEGMLLPPEVGWNHNDF--DGV-----GDVSLWSFD  223
AthCBF1 B6DTR3  SEGAFYMDEETMFGMPTLLDNMAEGMLLPSPSVQWNHNYDGED-----GDVSLWSY-  213
AthCBF2 B2BIW9  SQDAFYMDDEAMLGMSLLDNMAEGMLLPSPSVQWNHNYDVEGD-----DDVSLWSY-  216
AthCBF3 B2BIZ3  SENAFYMHDEAMFEMPSLLANMAEGMLLPSPSVQWNHNEVDGDD-----DDVSLWSY-  216
OsDREB1F Q8S9Z5 RPAAVFVDEDAIFDMPLGIDDARGMMLTPPAIGRSLDDWAAIDDDDDHYHMDYKLWMD-  219
NtAvr9 Q9FQZ8  PENMFYMDEEALFFMPGLLVNMAEGLMLPPPQCSQIGDHM--EAD-----VDMPLWSYS  218
      .:.:.:.:.: *  :: :*.*::*  *      :              *  **

AthCBF4 B2BJ26  E 224
AthCBF1 B6DTR3  -
AthCBF2 B2BIW9  -
AthCBF3 B2BIZ3  -
OsDREB1F Q8S9Z5  -
NtAvr9 Q9FQZ8  I 219

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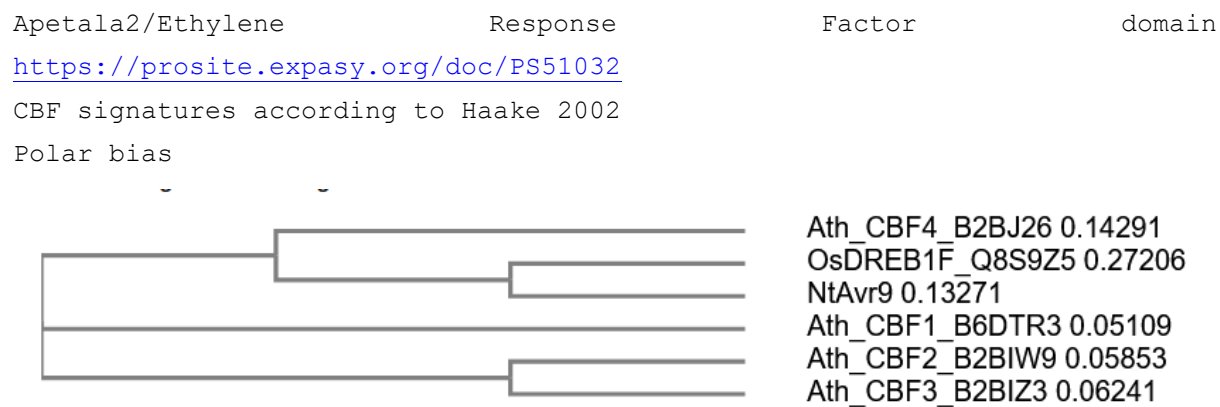


Figure S3. Sequence analysis of NtCf9 and other C-repeat-binding factors (CBFs).

Suppl. Fig. S4

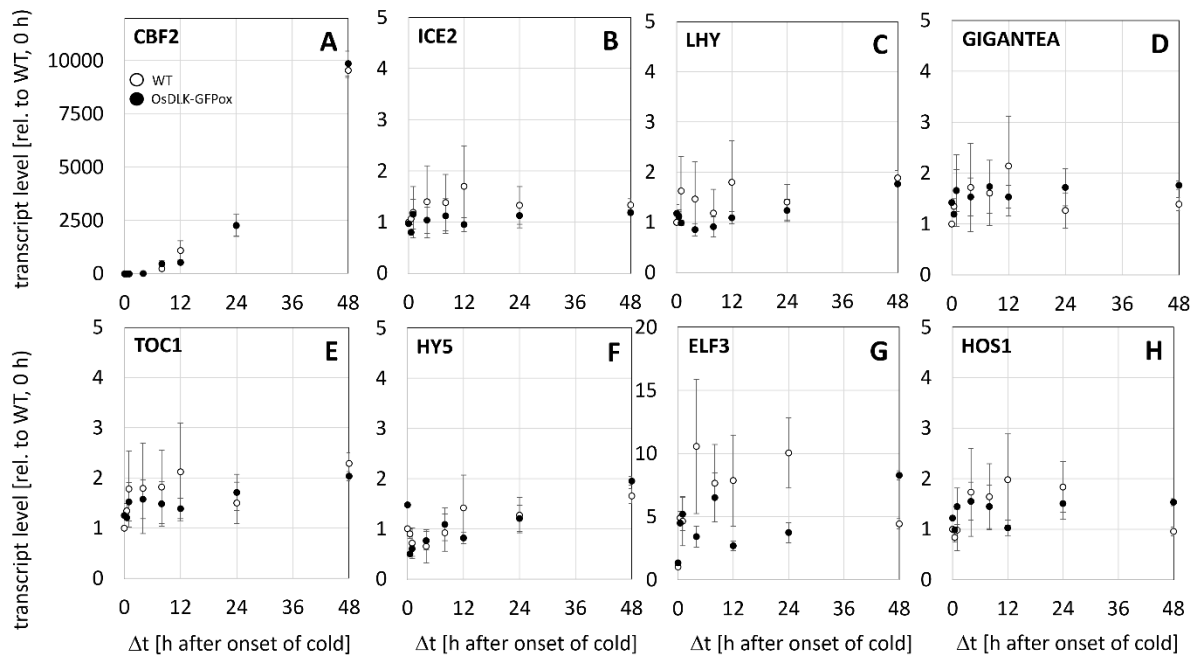


Figure S4. Time course of steady-state transcript levels under continuous cold stress (0°C) for different genes involved in the regulation of cold responses in non-transformed tobacco BY-2 cells (WT, open circles) and in cells expressing OsDLK-GFP under control of the CaMV 35S promoter (OsDLK-GFPox, closed circles). Data represent means and standard error from two independent experimental series with three technical replications per set. The transcripts were A Cold Box Factor 2 (CBF2), a transcriptional activator of cold-responsive genes, B Inducer of CBF expression (ICE2), the master switch for CBFs, C Late Elongated Hypocotyl, a regulator of CBFs acting in parallel of ICE, D Gigantea, a positive regulator of freezing tolerance acting independently of CBFs, E Timing of Cab Expression 1 (TOC1), a phytochrome dependent repressor of CBF expression, F Hypocotyl 5 (HY5), a light-dependent regulator of cold acclimation acting independently of CBFs, G Early Flowering 3 (ELF3) a phytochrome dependent regulator of CBFs H High Expression of Osmotically Responsive Genes (HOS1) a negative regulator of CBFs. Data are normalised to the resting level in the wild type.

Suppl. Table S1

Table S1. Oligonucleotid primers used for genotyping of rice mutants bearing T-DNA and Tos-17 insertion in the OsDLK locus.

name	sequence	target
TR	5' -CATCGAAACGCAGCACGATACGC-3'	insert-specific for T-DNA insertion line PFG_3A-07110.R
Tos17R	5' -CTGTATAGTTGGCCCATGTCCAG-3'	insert-specific for Tos-17 insertion line, ND4501_0_508_1A
KinF1	5' -ATCTGCTAGCCACTAATCGCAC-3'	upstream for T-DNA insertion line PFG_3A-07110.R
KinR1	5' -TTATGCTTCTAACTTCACTTCAGGC-3'	downstream for T-DNA insertion line PFG_3A-07110.R
KinF2	5' -GATTTTGTTCCTTGGACA-3'	upstream for Tos-17 insertion line ND4501_0_508_1A
KinR2	5' -GCTCGTACAATCAACAAAGCC-3'	downstream for Tos-17 insertion line ND4501_0_508_1A

Suppl. Table S2

Table S2. Oligonucleotid primers used to measure expression of OsDLK in rice plants.

Annealing temperature was 60°C throughout.

designation	sequence
Ubiquitin 10 fw	5' -GAGCCTCTGTTCGTCAAGTA-3'
Ubiquitin 10 rv	5' -ACTCGATGGTCCATTAAACC-3'
GAPDH fw	5' -CTGATGATATGGACCTGAGTCTACTTTT-3'
GAPDH rv	5' -CAACTGCACTGGACGGCTTA-3'
qDLK fw	5' -AGATTTCCCAACTCATCCAA-3'
qDLK rv	5' -ATCCTTTCTGGTCATGCAAT-3'

Suppl. Table S3

Table S3. Oligonucleotid primers for cold-stress related genes expression in tobacco BY-2 cells. Annealing temperature was 58°C throughout [16].

name	sequence	target
L25 fw	5' -GTTGCCAAGGCTGTCAAGTCAGG-3'	housekeeping gene L25
L25 rv	5' -GCACTAATACGAGGGTACTTGGGG-3'	housekeeping gene L25
EF1 α fw	5' -TGAGATGCACCACGAAGCTCTTC-3'	housekeeping gene EF1 α
EF1 α rv	5' -GCTGAAGCACCCATTGCTGGG-3'	housekeeping gene EF1 α
CBF2 fw	5' -CTCTACTAGCATCAGAAAGTGT-3'	tobacco CBF2
CBF2 rv	5' -ACTTGCCTAACCAAGTCAT-3'	tobacco CBF2
Cf9 fw	5' -AAGAGGAATTCAGACAAGTG-3'	tobacco CBF4
Cf9 rv	5' -AAAGTTCAAGCAAGCAGAAC-3'	tobacco CBF4
TOC1 fw	5' -AAGAAATCCTCTGCTCTCAC-3'	tobacco TOC1
TOC1 rv	5' -CGATTAACCTTCTCCGGTCCA-3'	tobacco TOC1
HY5 fw	5' -TGTAGGTAAGGCCGAGAT-3'	tobacco HY5
HY5 rv	5' -ATCACTCTCCATACCTTCACA-3'	tobacco HY5
G1 fw	5' -ACAGCTAGAGCAGTACAAC-3'	tobacco G1
G1 rv	5' -CGAACTGTGGCTGGTAAG-3'	tobacco G1
LHY fw	5' -TGGAGATGCTGGGAATCG-3'	tobacco LHY
LHY rv	5' -GGCAACTTCTCTCTGGTG-3'	tobacco LHY
HOS1 fw	5' -TGAGATTAGCGATTTGAGGC-3'	tobacco HOS1
HOS1 rv	5' -ATCAAGGTCAGTTTACGCA-3'	tobacco HOS1
ELF3 fw	5' -TCCTTCTCAACCACACAGTTTA-3'	tobacco ELF3
ELF3 rv	5' -GTAGTTCAAACACTTGTATCGC-3'	tobacco ELF3