Identification and functional analysis of the ICK gene family in maize

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List of Supplementary Information

Table S1. The basic information about the identified ICK/KRP candidate genes in Maize. Length of bp* means the length of cDNA; Length of aa* shows the number of amino acid; In/ex means the number of Intron-exon; Mw is the molecular weight of the protein; Chr represents the chromosome.

Table S2. The basic information about the ICK/KRP gene family of *Arabidopsis* and *Orysa sativa*. Length of bp* means the length of cDNA; Length of aa* shows the number of amino acid; In/ex means the number of Intron-exon; Mw is the molecular weight of the protein; Chr represents the chromosome; Location shows the detail location on the chromosome.

Table S3. Primers for the main research. PCR annealing temperature is 57 ℃ to 61 ℃ for all genes based on the primers information. "Category" indicates the carriers constructed and expression analysis in research, "Gene Name" represents the name of gene in the article, "Primer name" and "Primer Sequence" represents the primer name and sequence of related gene in this article.

Table S4. The conserved motifs sequence information of ICK in maize.

Table S5. The Nuclear Location Signal (NLS) predicted information about the ICK/KRP putative proteins in Maize. The table contained putative protein sequence accession number, protein name, putative NLS, putative NLS model and certainty.

Fig. S1 Physical locations information about maize ICK genes, the grey boxes on chromosomes indicate the approximate locations of the 9 maize ICK genes. CHR is the abbreviation of chromosomes and represents as black bars. The number corresponds to the number of chromosomes.

Fig. S2 The primary sequence analysis of 40 ICK/KRP genes from *Arabidopsis thaliana* (*AtICK*), *Oryza sativa* (*OsICK*), *Brachypodium distachyon* (*BdKRP*), *Glycine max* (*GmKRP*), *Populus trichocarpa* (*PtKRP*) and *Zea mays* (*ZmICK*) contains phylogenetic analysis, gene

structure and motif analysis. The different color boxes represent the different motif in the motif analysis. The three main components in gene structure analysis represent CDS region, upstream/downstream and Intron, respectively.

Fig. S3 The results from the analysis of the cloned ZmICK genes. (A) The roots, stems and leaves were collected when maize bred line 18-599 was in the initial jointing stage of growth. (B) and (C) are differences between the cloned sequences from maize bred line 18-599 and the reference sequence of B73. ZmICK7 has a three base pair insertion and led to a single amino acid inserted. ZmICK8 contains a 109bp inserted fragment led to an early termination of translation and lost the conserved CDI region. (D) Conserved motif analysis of the cloned ZmICK proteins sequence. Different coloured boxes represent the conserved motifs, the length of the long black lines represents the amino acid number of the ICK protein sequences, the short black line above the boxes or the long black line represents the predicted nuclear localization signals, and the blue boxes indicate the conserved domain CDI regions.

Fig. S4 The protein sequence alignment between three A-type CDKs of maize and Cdc2 of S.pombe. The specific amino acid sites are marked on the sequence alignment including the conserved S-TKc domain, ATP binding site, Cyclin-binding site and T-loop region.

Fig. S5 Co-localization of C-group ZmICKs and A-type CDKs. Combinations of the ZmICK-eGFP fusion protein vectors and the CDKA-RFP fusion protein vectors were transformed into onion epidermal cell with a gene gun and visualized with an ECLIPSE 80i fluorescence microscope. A is the co-localization about control pCAMBIA2300-35S-eGFP and pCAMBIA2300-35S-RFP. B is the co-localization result of fusion protein ZmICK1-eGFP with ZmCDKA-RFP. C shows the co-localization result of fusion protein ZmICK2-eGFP with ZmCDKA-RFP. D indicates the co-localization result of fusion protein ZmICK3-eGFP with ZmCDKA-RFP. E presents the co-localization result of fusion protein ZmICK4-eGFP with ZmCDKA-RFP. The images were detected under a bright-field, RFP, or GFP view and were then merged three into one. Bar=50 μm.

Fig. S6 The results of the yeast two-hybrid analysis of ZmICKs and ZmCDKAs. SD/-Leu/-Trp indicates media lacking tryptophan (Trp) and leucine (Leu). SD/-Ade/-His/-Leu/-Trp represents the synthetic dextrose (SD) media lacking tryptophan (Trp), leucine (Leu), adenine (Ade) and histidine (His). SD/-Ade/-His/-Leu/-Trp/30mM3-AT/10uM X-a-gal indicates the synthetic dextrose (SD) media contain 30 mM 3-AT and 10 uM X-a-gal. The gray box indicates the growth condition on the same medium and every yeast plaque indicates the growth of the transformants. "+" represents the positive control of the yeast two-hybrid, the combination of pGADT7 and pGBKT7-53. "-" shows the negative control of yeast two-hybrid, the pGADT7-T co-transformed with pGBKT7-lam.

Fig. S7 Bimolecular fluorescence complementation analysis of ZmICK1, ZmICK2, ZmICK3, and ZmICK4 with ZmCDKA3, the pCAMBIA2300-eYFP served as a positive control, the pSAT6A-cEYFP and pSAT6-ZmICK-nEYFP, and pSAT6A-ZmCDKA3-cEYFP and pSAT6-nEYFP was used as a negative control. The pSAT6-ZmICK-nEYFP and the corresponding pSAT6-ZmICK-nEYFP and pSAT6A-ZmCDKA3-cEYFP images are shown side-by-side. (A1 - J1) are the detection result under bright-field. (A2-J2) present the detection under the YFP field. (A3-J3) are the merged images of bright field and YFP field. Each alphabet labelled with 1 to 3 represents the same combination. Bar=50 μm.

Fig. S8 Cytological analysis of fission yeast cells expressing C-group ZmICKs, *ZmCDKA3* and a combination of the proteins. A1 represents wild-type fission yeast cells transformed with pREP42 and pESP-3. A2 shows yeast cells expressing *ZmCDKA3* and pREP42. B1 to E1 presents the yeast cells expressing the C-group ZmICKs-pREP42 with pESP-3. B2 to E2 shows the yeast cells co-expressing C-group ZmICKs and *ZmCDKA3*. The ZmICKs-pREP42 and the corresponding ZmICKs-pREP42 with ZmCDKA3-pESP-3 images are shown side-by-side. Bars=10 μm.

Fig. S9 The averages with standard deviations of the actual cell size (um^2). Averages are the average of the transformation of the same combination between different views and standard

deviations represent the degree of dispersion of them. The X-axis represents the actual cell area size (um^2). The Y-axis shows the different transformation of combination. "**" indicates significant differences in mean values between the different treatments and controls.

Fig. S10 The 1000bp upstream sequence alignment between ZmICK6.1 and ZmICK6.2.

Supplementary Table

Table S1. The basic information about the identified ICK/KRP candidate genes in Maize.

Maize Sequence Gene ID	Gene Name	length bp*	length aa*	In/ex	pI	MW (kD)	Chr	Location	Reference	NCBI Accession No.	Synonyms
GRMZM2G116885	*ZmICK1*	1084	190	2/3	8.82	21.29	1	232,980,076-232,983,251	Coelho et al, 2005	NP_001105778	*ZmKRP1*
GRMZM2G037926	*ZmICK2*	1275	192	2/3	8.94	21.399	5	29,641,580-29,644,988	This study	NP_001168443	
GRMZM2G157510	*ZmICK3*	1446	213	2/3	7.56	23.72	1	8,442,682-8,446,230	This study	NP_001152562 NP_001183727	
GRMZM2G358931	*ZmICK4*	1343	215	2/3	8.28	23.831	9	152,252,293-152,255,757	This study	NP_001132400	
GRMZM2G101613	*ZmICK5*	1277	253	3/4	8.41	25.689	5	209,770,434-209,772,179	Coelho et al, 2005	NP_001105779 NP_001149832	*ZmKRP2*
GRMZM2G084570	*ZmICK6.1*	1267	263	3/4	8.65	26.673	4	178,774,992-178,776,727	This study	NP_001147155	
GRMZM5G854731	*ZmICK6.2*	1267	263	3/4	8.65	26.673	8	112,022,205-112,023,940	This study	NP_001147155	
GRMZM2G343769	*ZmICK7*	969	275	3/4	9.11	28.875	9	7,870,479-7,871,904	This study	KX643357	
GRMZM2G154414	*ZmICK8*	1316	216	1/2	9.09	23.09	4	5,410,267-5,411,691	This study	KX643358	

Table S2. The basic information about the ICK/KRP gene family of *Arabidopsis* and *Orysa sativa*

Gene Locus	Gene Name	length bp*	length aa*	In/ex	Chr	Location	NCBI Accession No.	Synonyms
AT2G23430	*Arath;ICK1*	950	191	3/4	2	9976746-9978021	NM_127907/NP_179924	*Arath;KRP1*
AT3G50630	*Arath;ICK2*	979	209	3/4	3	18800380-18801587	NM_114923/NP_190632	*Arath;KRP2*
AT3G24810	*Arath;ICK3*	920	189	2/3	3	9060896-9061998	NM_113393/NP_189125	*Arath;KRP5*
AT3G19150	*Arath;ICK4*	908	196	3/4	3	6616352-6617696	NM_112802/NP_188546	*Arath;KRP6*
AT1G49620	*Arath;ICK5*	789	195	3/4	1	18365491-18366559		*Arath;KRP7*
AT5G48820	*Arath;ICK6*	669	222	2/3	5	19792270-19794408	NM_124259/NP_199693	*Arath;KRP3*
AT2G32710	*Arath;ICK7*	1411	289	2/3	2	13873436-13875831	NM_128830/NP_565750	*Arath;KRP4*
OS02G0762400	Orysa;ICK1	1265	262	3/4	2	32111311-32112948	NM_001054739/NP_001048204	*Orysa;KRP1*
OS06G0213700	Orysa;ICK2	750	249	3/4	6	5794738-5795810		*Orysa;KRP2*
OS11G0614800	Orysa;ICK3	1214	214	3/4	11	23866045-23867666	NM_001074803/NP_001068271	*Orysa;KRP3*
OS09G0459900	Orysa;ICK4	917	142	2/3	9	17390977-17392630		*Orysa;KRP6*
OS03G0137800	Orysa;ICK5	1045	221	2/3	3	2088673-2091654	NM_001055440/NP_001048905	*Orysa;KRP5*
OS10G0471700	Orysa;ICK6	1025	194	2/3	10	17499846-17503202	NM_001071364/NP_001064829	*Orysa;KRP4*

Table S3. Primers for the research

Category	Gene Name	Prmier Name	Primer Sequence
Yeast two-hybrid system vector pGBKT7	*ZmICK1*	ICK1-BDF/ICK1-BDR	5'-3'CATATGATGGGCAAGTACATGCG/5'-3'GTCGACTCAGTCTAGCTTCACCCAC
	ZmICK2	ICK2-BDF/ICK2-BDR	5'-3'CATATGATGGGCAAATACATGCG/5'-3'GGTCGACTCAGTCTAGCTTCACCC
	ZmICK3	ICK3-BDF/ICK3-BDR	5'-3'CATATGATGGGGAAGTACATGCGC/5'-3'GTCGACCTAGCAGTCTAGCCTCGC
	ZmICK4	ICK4-BDF/ICK4-BDR	5'-3'CATATGATGGGGAAGTACATGC/5'-3'GTCGACCTAACAGTCTAGCCTCGC
	ZmICK5	ICK5-BDF/ICK5-BDR	5'-3'CATATGATGGGGAAGTACATGCGC/5'-3'GTCGACTCAGATGCTGACCACCG
	ZmICK6	ICK6-BDF/ICK6-BDR	5'-3'CCATATGATGGGGAAGTACATGCG/5'-3'GTCGACTCAGATGCTGACCCCTG
	ZmICK7	ICK7-BDF/ICK7-BDR	5'-3'CATATGATGGGGAAGTACATGAGG/5'-3'GTCGACTCACCAACCGCTGGC
Yeast two-hybrid system vector pGADT7	*ZmCDKA1* (M60526.1)	CDKA1-ADF/CDKA1-ADR	5'-3'GCATATGATGGAGCAGTACGAGAAGG/5'-3'TGGATCCTCACTGTACCACTTCAAGG
	ZmCDKA3(GRMZM2G174596)	CDKA3-ADF/CDKA3-ADR	5'-3'CATATGATGGACCAGTACGAGAAGGTG/5'-3'ATCCATGGATCCCTAGGCGTGCT
	ZmCDKB (GRMZM2G495626)	CDKB-ADF/CDKB-ADR	5'-3'TCGCATATGATGCCCTTGCCC/5'-3'AGTAGGATCCCTAGAACTGGGACTTGTC
	ZmCycD1 (AF351190.1)	CycD1-ADF/CycD1-ADR	5'-3'TCTGATTCATATGATGGGGGACG/5'-3'GGATCCCTACTGTCGCTGAGGTG
	ZmCycD2 (AF351189.1)	CycD2-ADF/CycD2-ADR	5'-3'CATATGATGGTGCCGGGCTATGAC/5'-3'GCGTAGGATCCTTAGAGTAGACGTCTAGTG
Cellular Location vector pC2300-35s-eGFP	*ZmICK1*	LICK1F/LICK1R	5'-3'GGTACCATGGGCAAGTACATG/5'-3'TCTAGAGTCTAGCTTCACCC
	ZmICK2	LICK2F/LICK2R	5'-3'GGTACCATGGGCAAATACATG/5'-3'TCTAGAGTCTAGCTTCACCC
	ZmICK3	LICK3F/LICK3R	5'-3'TCTAGAATGGGGAAGTACATGCG/5'-3'GTCGACGCAGTCTAGCCTCG
	ZmICK4	LICK4F/LICK4R	5'-3'GGTACCATGGGGAAGTACATGC/5'-3'TCTAGAACAGTCTAGCCTCGCCCAC
	ZmICK5	LICK5F/LICK5R	5'-3'GGTACCATGGGGAAGTACATGC/5'-3'TCTAGAGATGCTGACCACC
	ZmICK6	LICK6F/LICK6R	5'-3'GGTACCATGGGGAAGTACATGC/5'-3'TCTAGAGATGCTGACCCCT
	ZmICK7	LICK7F/LICK7R	5'-3'GGTACCATGGGGAAGTACATGAGG/5'-3'TCTAGACCAACCGCTGGCTA
Semi-quantitative RT-PCR	*ZmICK1*	qICK1F/qICK1R	5'-3'CGCAACTAAGGAGGCTGAG/5'-3'GTCATCTGGGTGTTAATCAAGC
	ZmICK2	qICK2F/qICK2R	5'-3'ACGCCATCCCAAGTTCAAC/5'-3'CTATCAGTCTAGCTTCACCCATTC
	ZmICK3	qICK3F/qICK3R	5'-3'ACTTCTGTCCCGTGAACGAC/5'-3'GCGGAGCTAGTCACATTGTG
	ZmICK4	qICK4F/qICK4R	5'-3'GCAGGATGGAAACCTCAGTC/5'-3'CTCGCCCACTCATATCGAC
	ZmICK5	qICK5F/qICK5R	5'-3'GCACGAGGTCCAGGTCC/5'-3'CAGGTGGATGTAGCAGCTTC

	ZmICK6	qICK6F/qICK6R	5'-3'AAGACTGGCTGCTCGTCGTC/5'-3'CGAAGTCGAAGTTGTACTTGGAAG
	ZmICK7	qICK7F/qICK7R	5'-3'GGCAGTGACTGGGAACACCAGC/5'-3'AGTCGTCGTCGTCGTTGCGTG
	ZmCDKA1 (M60526.1)	qCDKA1F/qCDKA1R	5'-3'CCTCGCCACTTAGTTCGTTCC/5'-3'TTGCCGTGGTTCATCTCCTT
	ZmCDKA3(GRMZM2G174596)	qCDKA3F/qCDKA3R	5'-3'CTCCCCTGCTCCCTCTGTAA/5'-3'CTCTCTCCAGCCATCATACAGG
	ZmCDKB (GRMZM2G495626)	qCDKBF/qCDKBR	5'-3'AGGCTCTCTTTCCTGGTGACTC/5'-3'TCCAGGCCACTGTTCCTCAG
	ZmCycD1 (AF351190.1)	qCycD1F/qCycD1R	5'-3'TTCACTTTTCTTCCCTTTCCC/5'-3'TTCATCCAGTCCTCCATTTCC
	ZmCycD2 (AF351189.1)	qCycD2F/qCycD2R	5'-3'TGTATTGATTGCTCGCTGCTC/5'-3'GGAACTCCGTCAAAATCCCAT
	Actin	ActinF/ActinR	5'-3'TCACTACGACTGCCGAGCGAG/5'-3'GAGCCACCACTGAGGACAACATTAC
	ZmCDKA1 (M60526.1)	CDKA1BiFCF/CDKA1BiFCR	5'-3'GAATTCATGGAGCAGTACGAGAAG/5'-3'CCCGGGCTGTACCACTTCAAGGTC
	ZmCDKA3(GRMZM2G174596)	CDKA3BiFCF/CDKA3BiFCR	5'-3'GAATTCATGGACCAGTACGAGAAG/5'-3'CCCGGGGGCGTGCTCGAGGTCCCT
Bimolecular Fluorescence	*ZmICK1*	ZmICK1BiFCF/ZmICK1BiFCR	5'-3'GAATTCATGGGCAAGTACATGCGCA/5'-3'CCCGGGGTCTAGCTTCACCCACTCA
Complementation	*ZmICK2*	ZmICK2BiFCF/ZmICK2BiFCR	5'-3'GAATTCATGGGCAAATACATGCGCA/5'-3'CCCGGGGTCTAGCTTCACCCATTC
	ZmICK3	ZmICK3BiFCF/ZmICK3BiFCR	5'-3'GAATTCATGGGGAAGTACATGCGCA/5'-3'CCCGGGGCAGTCTAGCCTCGCCCA
	ZmICK4	ZmICK4BiFCF/ZmICK4BiFCR	5'-3'GAATTCATGGGGAAGTACATGCGCA/5'-3'CCCGGGACAGTCTAGCCTCGCCCAC
	ZmCDKA1 (M60526.1)	CDKA1-ADF/CDKA1-ADR	5'-3'CATATGATGGAGCAGTACGAGAAGG/5'-3'TGGATCCTCACTGTACCACTTCAAGG
	ZmCDKA3(GRMZM2G174596)	CDKA3-ADF/CDKA3-ADR	5'-3'CATATGATGGACCAGTACGAGAAGGTG/5'-3'ATCCATGGATCCCTAGGCGTGCT
Co-expressing the gene in	*ZmICK1*	ICK1-BDF/ICK1-BDR	5'-3'CATATGATGGGCAAGTACATGCG/5'-3'GTCGACTCAGTCTAGCTTCACCCAC
the fission yeast cell	*ZmICK2*	ICK2-BDF/ICK2-BDR	5'-3'CATATGATGGGCAAATACATGCG/5'-3'GGTCGACTCAGTCTAGCTTCACCC
	ZmICK3	ICK3-BDF/ICK3-BDR	5'-3'CATATGATGGGGAAGTACATGCGC/5'-3'GTCGACCTAGCAGTCTAGCCTCGC
	ZmICK4	ICK4-BDF/ICK4-BDR	5'-3'CATATGATGGGGAAGTACATGC/5'-3'GTCGACCTAACAGTCTAGCCTCGC

PCR annealing temperature is 57 ℃ to 61 ℃ for all genes based on the primers information

Table S4. The conserved motifs sequence information of ZmICKs

Protein Name	Motif 1	Motif 2	Motif 3	Motif 4	Motif 5
ZmICK1	HAVPSSREMNEYFAAEQRRQQQDFIDKYNFDPANDCPL	MGKYMRKAKASSEVVIMDVA	QTQWEEGAGGEYLELRNRRLEKLPPPPAT	MERITRETTPCSLIN	GRFEWVKL
ZmICK2	HAIPSSTEMNEYFAAEQRRQQQAFIDKYNFDPVNDCPL	MGKYMRKVKVSSEVAIMDVA	QVQQEEGCGGEYLELRSRRLEKLPPQVAS	MGRNTRETTPCSLIN	GRFEWVKL
ZmICK3	RFIPSSLEMEEFFSAAEQQEQHSFREKYNFCPVNDCPL	MGKYMRKGKVSGEVAVMEVP	DKGDAEDAAAEYLELRSRRLEKPHKEHPS	MERSTRETTPCSLIR	GRYEWARL
ZmICK4	RFIPSSLEMEEFFSAAEQQEQHNFREKYNFCPVNDCPL	MGKYMRKGKMSGEVAVMEVP	DKGDADDAAGQYLELRSRRLEKPHKEHQP	MERSTRETTPCSLIR	
ZmICK7	RATPPAEEIEQFFAAAEKAQAERFAAKYNFDVARGLPL	MGKYMRKRKRNVGRTTPAAE	NTSGAAAPGSCYLHLRTRRLQFLPGGEGE	CRRDRRETTPSSQAR	GRYEWTPV
ZmICK5	LIVPPAHEIQEFFAAAEAAQAKRFASKYNFDFVRGVPL	MGKYMRKCRGAAGAEVAAVE	SVGAGGDGGSCYIHLRSRMLFMAPPQPQP	PDRERRETTPSSRAH	GRFEWAPV
ZmICK6.1	LIVPPAQEIQEFFAAAEAAHAKRFASKYNFDFVRGVPL	MGKYMRKRRGAAGEGVAAVE	GAGGGDGGSCCYIHLRSRMLFMAAPQQQP	PDRERRETTPSSSRA	GRFEWTPG
ZmICK6.2	LIVPPAQEIQEFFAAAEAAHAKRFASKYNFDFVRGVPL	MGKYMRKRRGAAGEGVAAVE	GAGGGDGGSCCYIHLRSRMLFMAAPQQQP	PDRERRETTPSSSRA	GRFEWTPG
ZmICK8	PPPPTETEIEAFFADAELAERRRFAEAYNYDVALDRPL				GRFEWVPL

Protein Name	Motif 6	Motif 7	Motif 8	Motif 9	Motif 10
ZmICK1	LGVRTRARALALQR	MTSTPGSTRSGHSCH	SYGENMLELE		TRRSGGRK
ZmICK2	LGVRTRARALALQR	MISTPGSTTRSSHSS	SYGENMLESE		VRRCGGRK
ZmICK3	LGVRTRSRTLALQR	MISTPGSTTKSKTSS	SFGDNVLDLD		TKRGAGRK
ZmICK4	LGVRTRSRTLALQR	MISTPGSTTKSKTSN	SFGENVLDLD	SRRRMETSVCR	AKRGAGRK
ZmICK7	HGVRTRAQSDAVAS	PVEGAGSSRCSSTAS		EARNDDDDYCG	TRHRRRRK
ZmICK5	VGVRTRSRSAAATG	AALAAGLSRCSSTAS	AGGDHVLVDV	ELSDLESDLAG	KVVAPRRK
ZmICK6.1	VGVRTRSRSAAATG	VALAAGLSRCSSTAS	VDGDHVPDVV	ELSDLESDLVG	KVAPPRRK
ZmICK6.2	VGVRTRSRSAAATG	VALAAGLSRCSSTAS	VDGDHVPDVV	ELSDLESDLVG	KVAPPRRK
ZmICK8	LGVGARQQQLCADD			KANKHENDECG	TRSRPRRH

Table S5. The information about the NLS prediction of ICK/KRP putative proteins in Maize

ACCESSION NO.	Protein Name	Putative NLS	Model	Certainty
GRMZM2G116885-T01	ZmICK1	no detect	n/a	Not Clear
GRMZM2G037926-T01	ZmICK2	no detect	n/a	Not Clear
GRMZM2G157510-T01	ZmICK3	no detect	n/a	Not Clear
GRMZM2G358931-T02	ZmICK4	no detect	n/a	Not Clear
GRMZM2G101613-T01	ZmICK5	52 (5) RRKR	[K/R]4	0.6
GRMZM2G084570-T01	ZmICK6.1	53 (5) RRKK	[K/R]4	0.7
GRMZM5G854731-T01	ZmICK6.2	53 (5) RRKK	[K/R]4	0.7
GRMZM2G343769-T01	ZmICK7	12 (5) KRKR	[K/R]4	0.7

Supplementary Figures

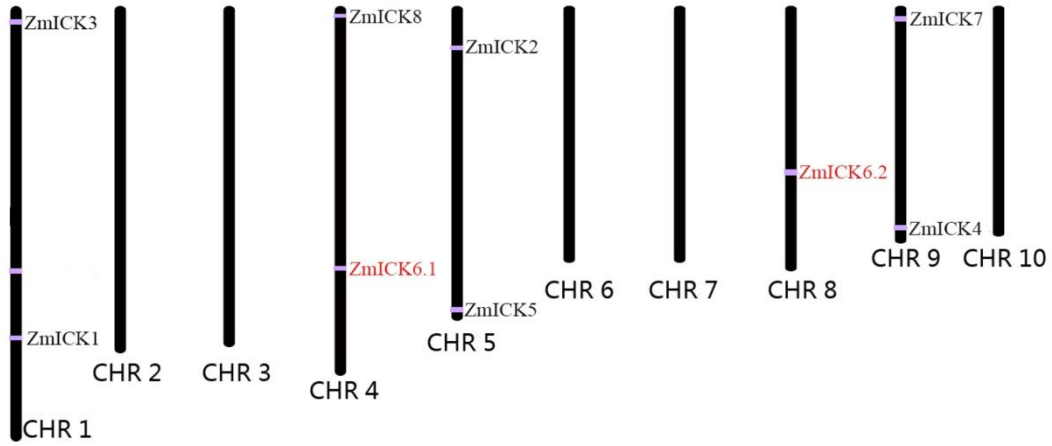

Figure S1 Physical locations information about maize ICK genes, the grey boxes on chromosomes indicate the approximate locations of the 9 maize ICK genes. CHR is the abbreviation of chromosomes and represents as black bars. The number corresponds to the number of chromosomes.

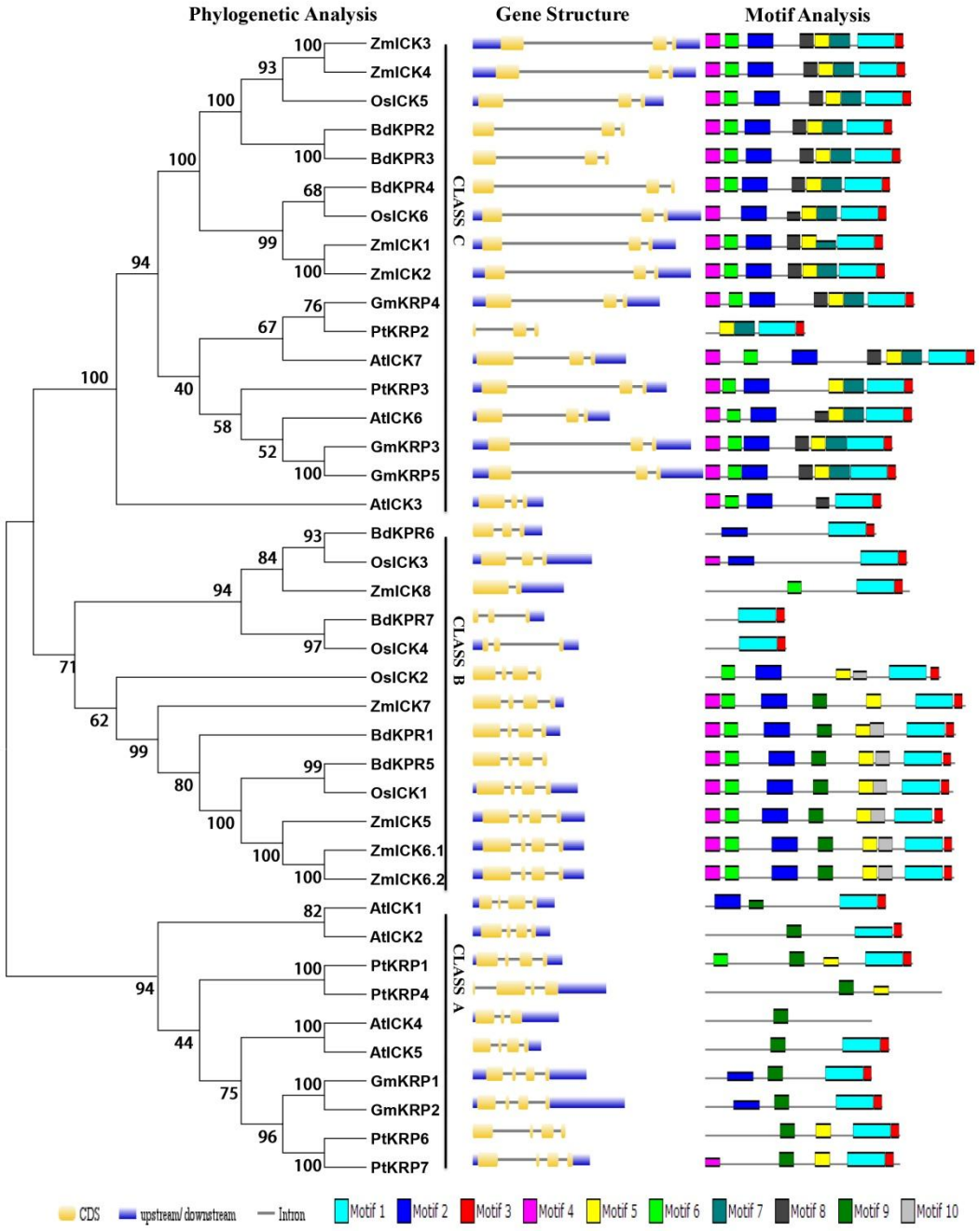

Figure S2 The primary sequence analysis of 40 ICK/KRP genes from *Arabidopsis thaliana* (*AtICK*), *Oryza sativa* (*OsICK*), *Brachypodium distachyon* (*BdKRP*), *Glycine max* (*GmKRP*), *Populus tric*hocarpa (PtKRP) and Zea mays (ZmICK) contains phylogenetic analysis, gene structure and motif analysis. The different color boxes represent the different motif in the motif analysis. The three main components in gene structure analysis represent CDS region, upstream/downstream and Intron, respectively.

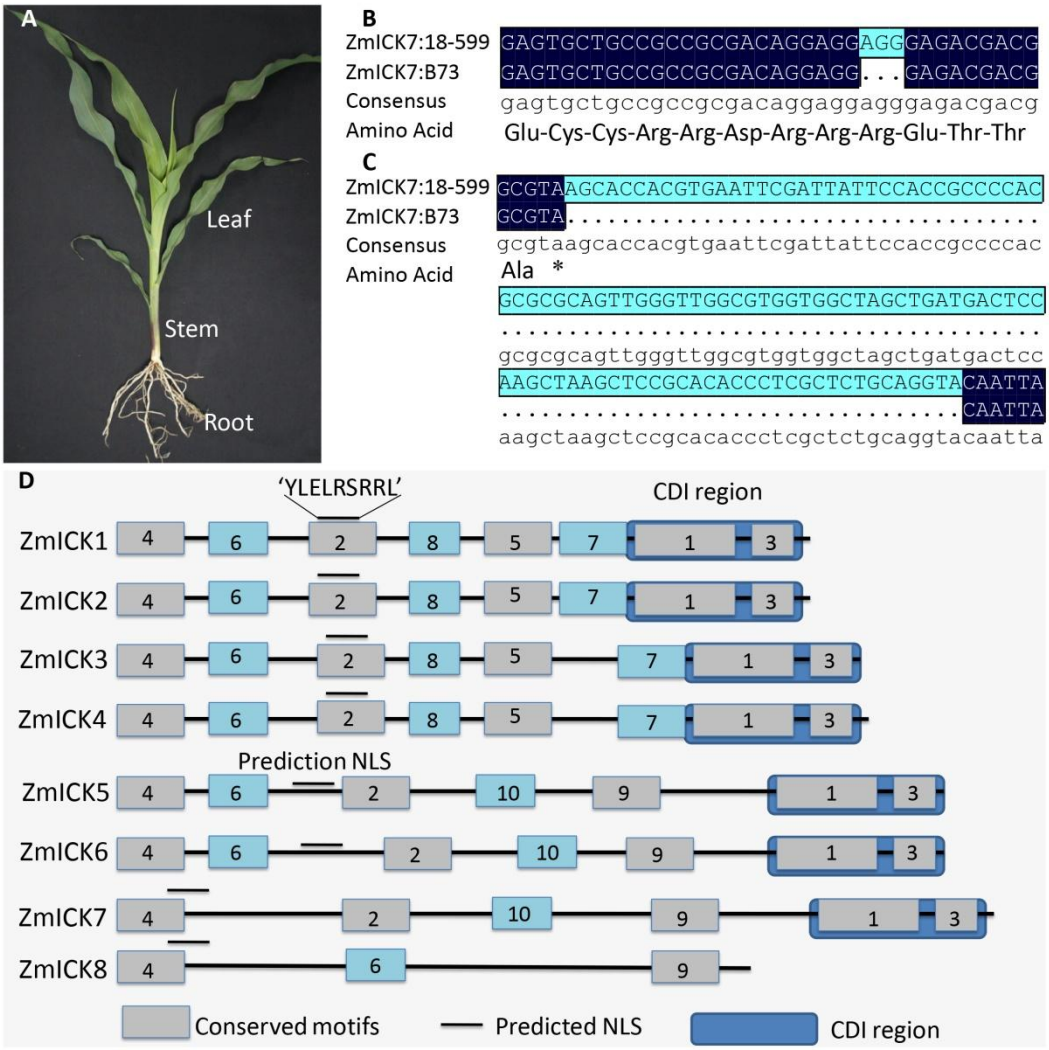

Figure S3 The results from the analysis of the cloned ZmICK genes. (A) The roots, stems and leaves were collected when maize bred line 18-599 was in the initial jointing stage of growth. (B) and (C) are differences between the cloned sequences from maize bred line 18-599 and the reference sequence of B73. ZmICK7 has a three base pair insertion and led to a single amino acid inserted. ZmICK8 contains a 109bp inserted fragment led to an early termination of translation and lost the conserved CDI region. (D) Conserved motif analysis of the cloned ZmICK proteins sequence. Different coloured boxes represent the conserved motifs, the length of the long black lines represents the amino acid number of the ICK protein sequences, the short black line above the boxes or the long black line represents the predicted nuclear localization signals, and the blue boxes indicate the conserved domain CDI regions.

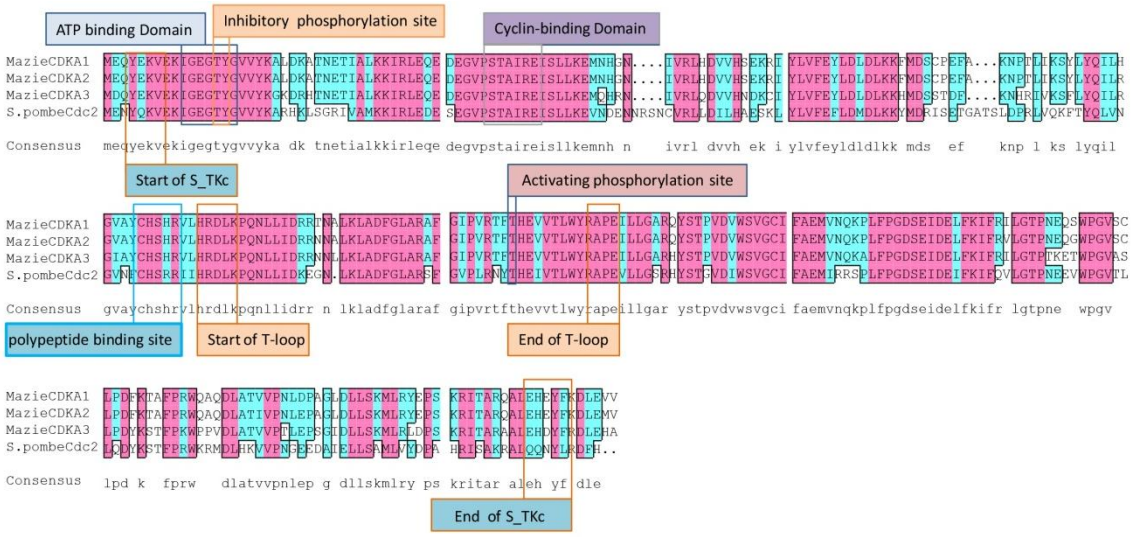

Figure S4 The protein sequence alignment among three A-type CDKs of maize and Cdc2 of *Schizosaccharomyces pombe*. The specific amino acid sites are marked on the sequence alignment including the conserved S-TKc domain, ATP binding site, Cyclin-binding site and T-loop region.

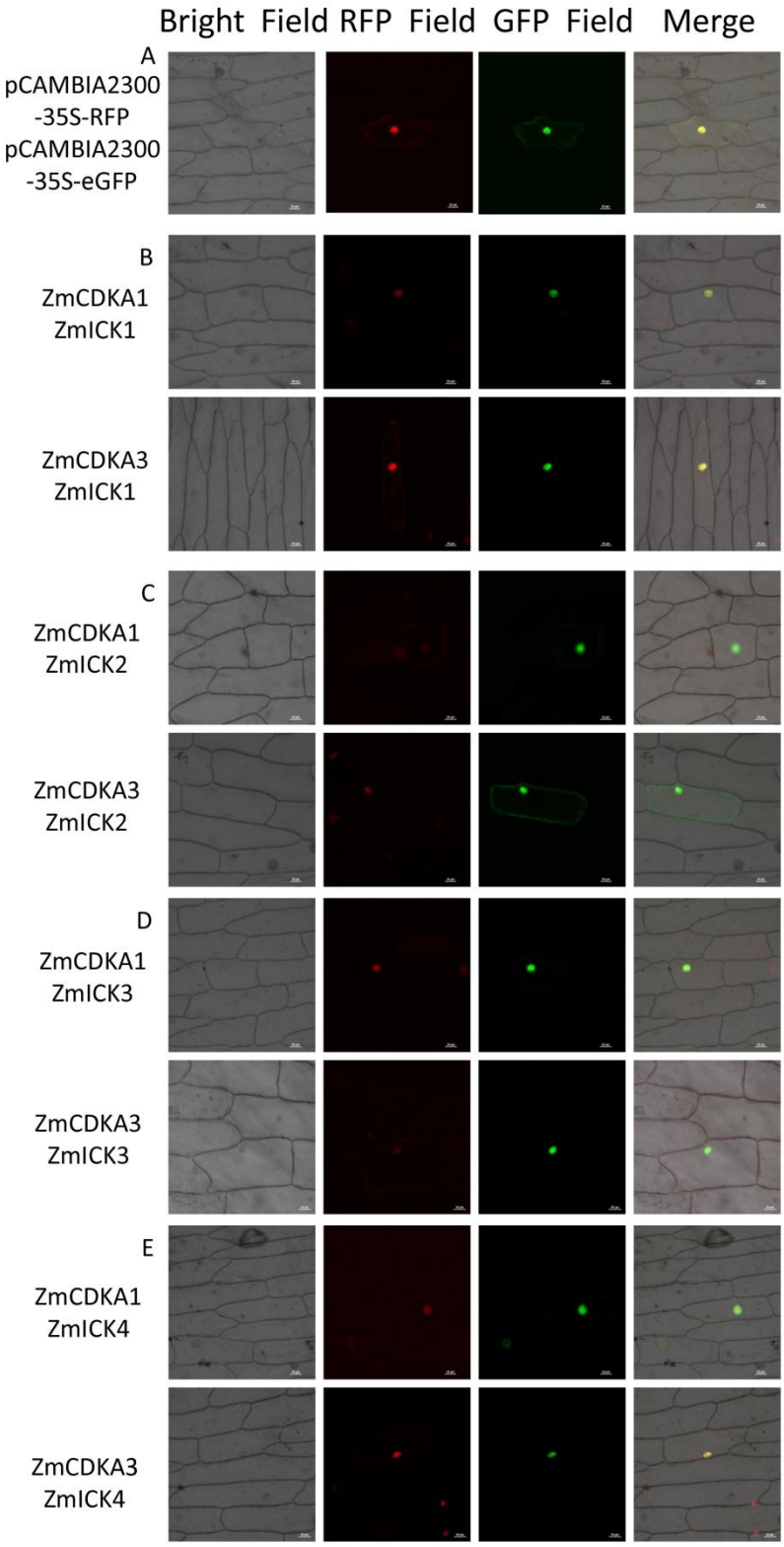

Figure S5 Co-localization of C-group ZmICKs and A-type CDKs. Combinations of the ZmICK-eGFP fusion protein vectors and the CDKA-RFP fusion protein vectors were transformed into onion epidermal cell with a gene gun and visualized with an ECLIPSE 80i fluorescence microscope. A is the co-localization about control pCAMBIA2300-35S-eGFP

and pCAMBIA2300-35S-RFP. B is the co-localization result of fusion protein ZmICK1-eGFP with ZmCDKA-RFP. C shows the co-localization result of fusion protein ZmICK2-eGFP with ZmCDKA-RFP. D indicates the co-localization result of fusion protein ZmICK3-eGFP with ZmCDKA-RFP. E presents the co-localization result of fusion protein ZmICK4-eGFP with ZmCDKA-RFP. The images were detected under a bright-field, RFP, or GFP view and were then merged three into one. Bar=50 μm.

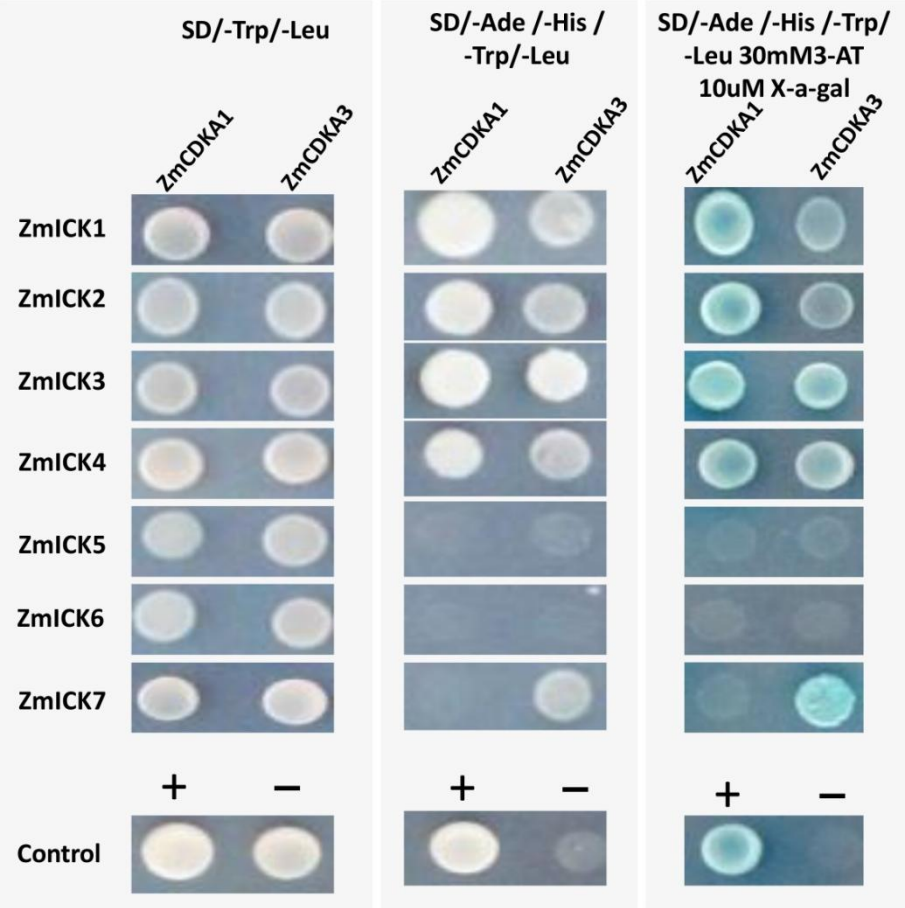

Figure S6 The results of the yeast two-hybrid analysis of ZmICKs and ZmCDKAs. SD/-Leu/-Trp indicates media lacking tryptophan (Trp) and leucine (Leu). SD/-Ade/-His/-Leu/-Trp represents the synthetic dextrose (SD) media lacking tryptophan (Trp), leucine (Leu), adenine (Ade) and histidine (His). SD/-Ade/-His/-Leu/-Trp/30mM3-AT/10uM X-α-gal indicates the synthetic dextrose (SD) media contain 30 mM 3-AT and 10 uM X-α-gal. The gray box indicates the growth condition on the same medium and every yeast plaque indicates the growth of the transformants. "+" represents the positive control of the yeast two-hybrid, the combination of pGADT7 and pGBKT7-53. "-" shows the negative control of yeast two-hybrid, the pGADT7-T co-transformed with pGBKT7-lam.

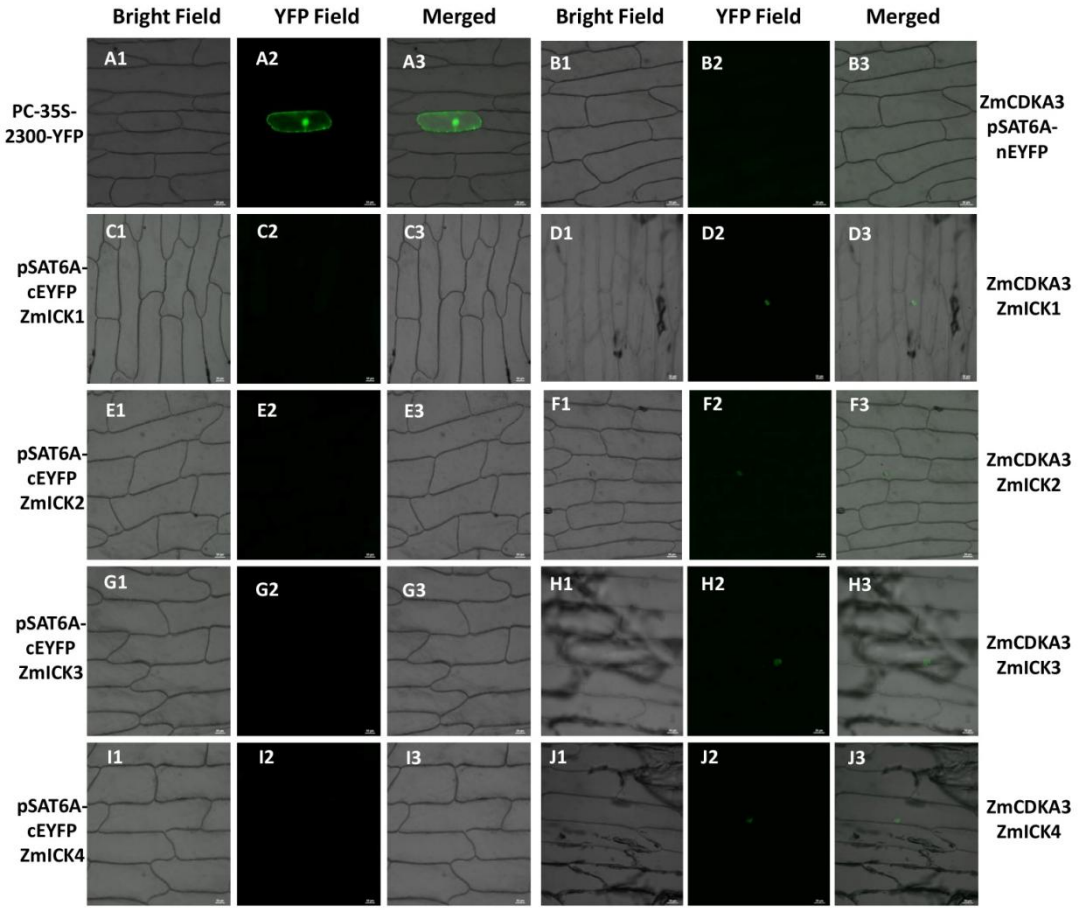

Figure S7 Bimolecular fluorescence complementation analysis of ZmICK1, ZmICK2, ZmICK3, and ZmICK4 with ZmCDKA3, the pC-35S-2300-eYFP served as a positive control, the pSAT6A-cEYFP and pSAT6-ZmICK-nEYFP, and pSAT6A-ZmCDKA3-cEYFP and pSAT6-nEYFP was used as a negative control. The pSAT6-ZmICK-nEYFP and the corresponding pSAT6-ZmICK-nEYFP and pSAT6A-ZmCDKA3-cEYFP images are shown side-by-side. (A1 - J1) are the detection result under bright-field. (A2-J2) present the detection under the YFP field. (A3-J3) are the merged images of bright field and YFP field. Each alphabet labelled with 1 to 3 represents the same combination. Bar=50 μm.

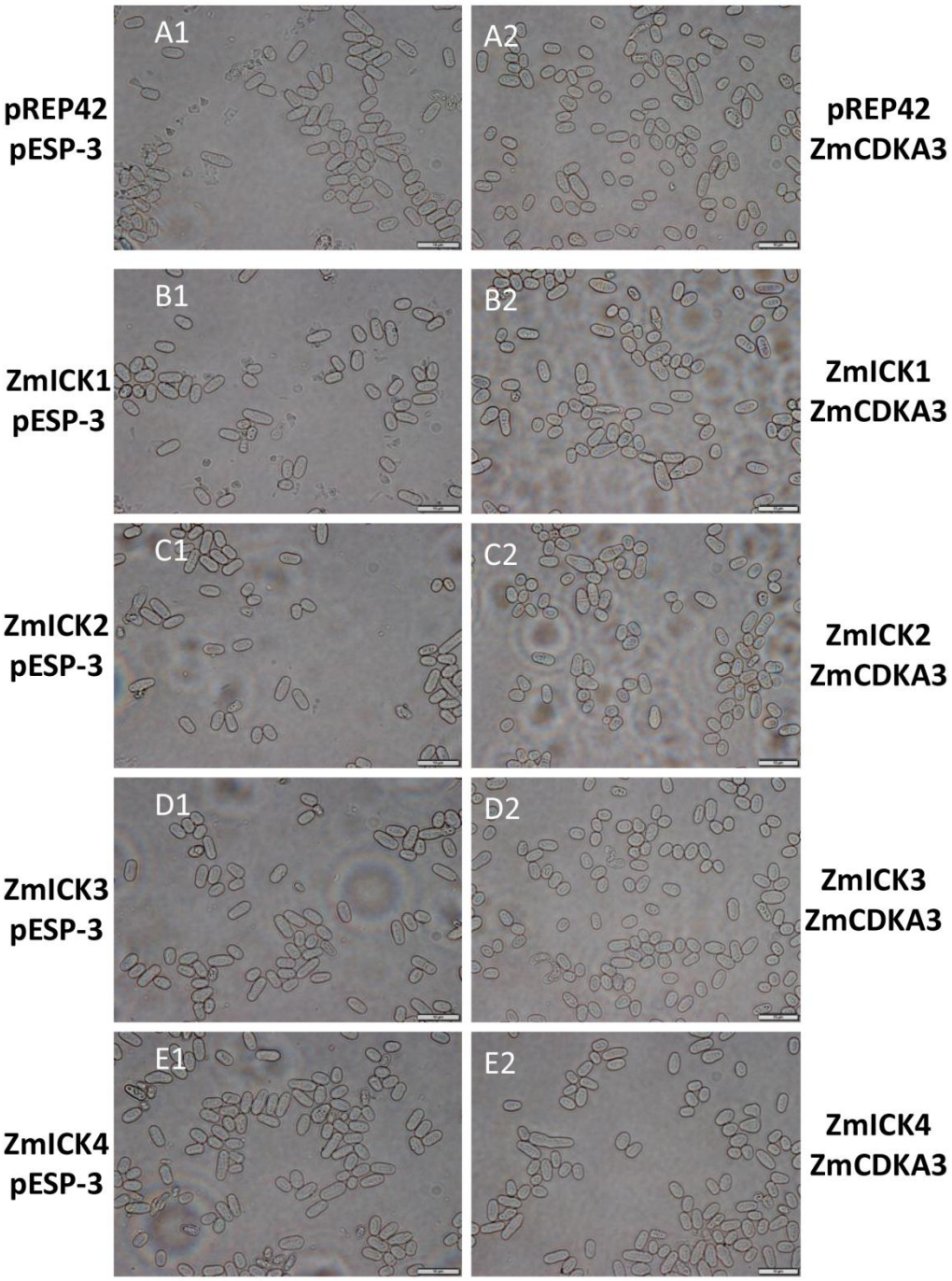

Figure S8 Cytological analysis of fission yeast cells expressing C-group ZmICKs, ZmCDKA3 and a combination of the proteins. A1 represents wild-type fission yeast cells transformed with pREP42 and pESP-3. A2 shows yeast cells expressing ZmCDKA3 and pREP42. B1 to E1 presents the yeast cells expressing the C-group ZmICKs-pREP42 with pESP-3. B2 to E2 shows the yeast cells co-expressing C-group ZmICKs and ZmCDKA3. The ZmICKs-pREP42 and the corresponding ZmICKs-pREP42 with ZmCDKA3-pESP-3 images are shown side-by-side. Bars=10 μm.

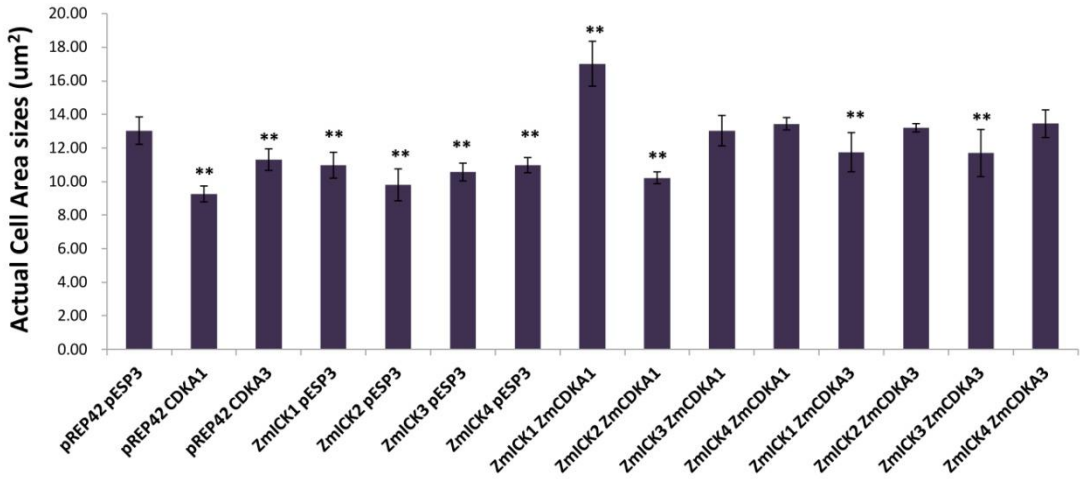

Figure S9 The averages with standard deviations of the actual cell size (um^2). Averages are the average of the transformation of the same combination between different views and standard deviations represent the degree of dispersion of them. The X-axis represents the actual cell area size (um^2). The Y-axis shows the different transformation of combination. "**" indicates significant differences in mean values between the different treatments and controls.

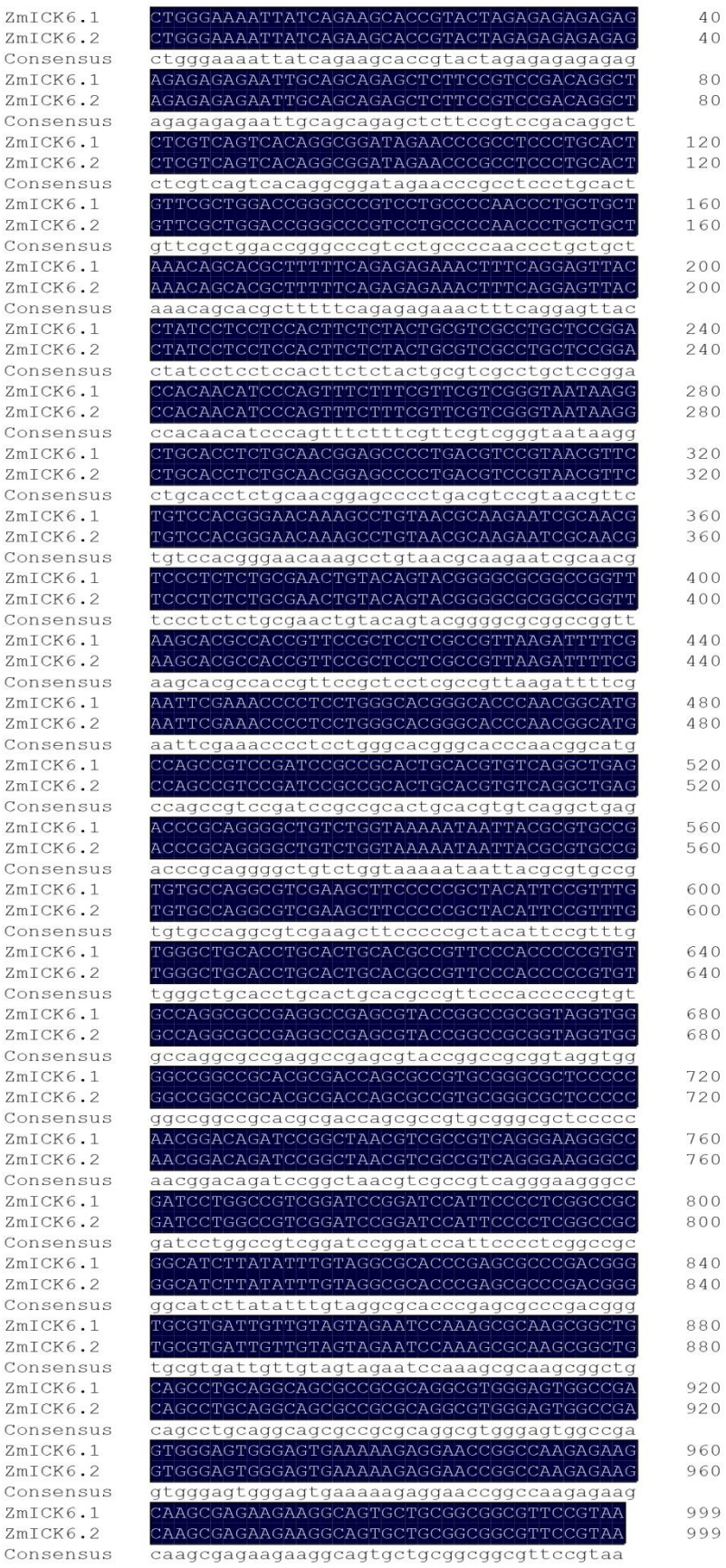

Figure S10 The 1000bp upstream sequence alignment between ZmICK6.1 and ZmICK6.2