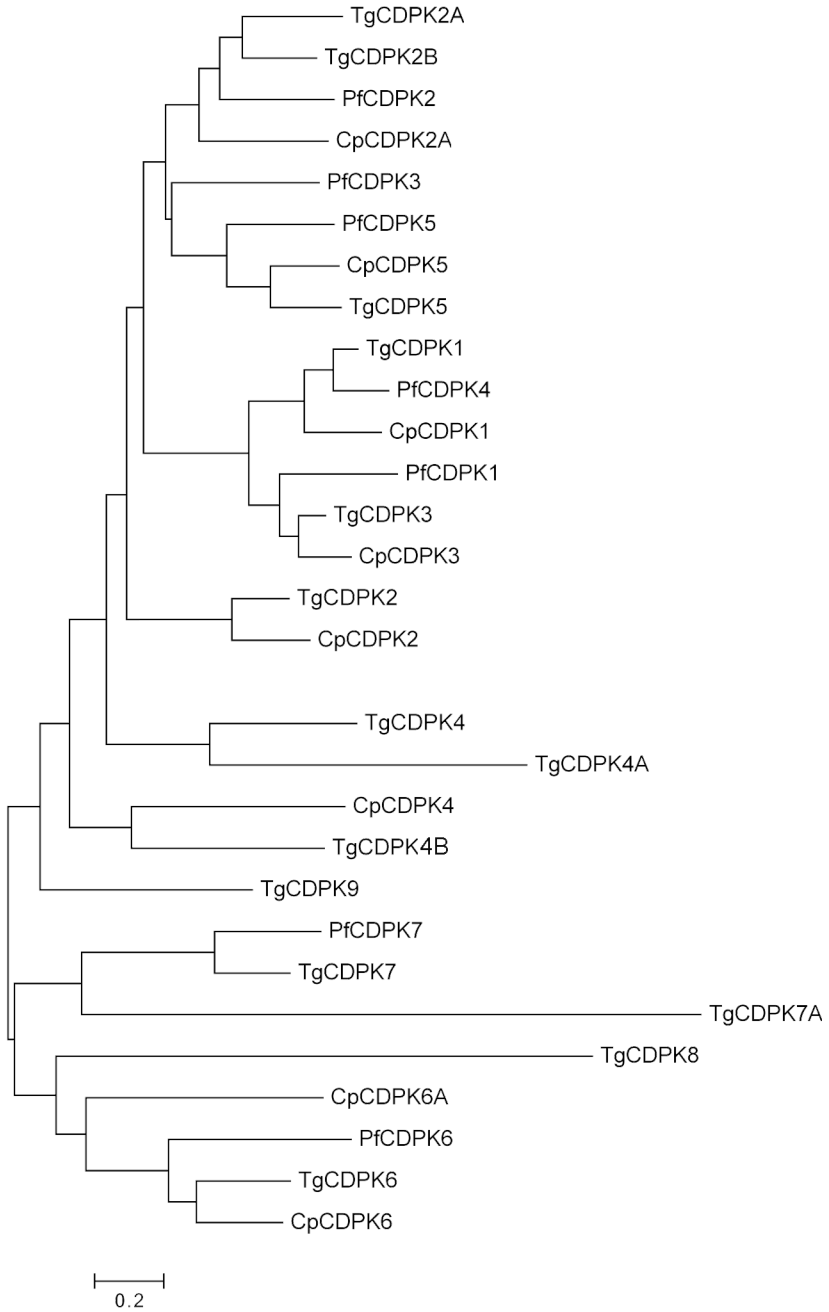

Fig. S1. Phylogenetic representation of the CDPKs in *Plasmodium falciparum* (Pf), *Toxoplasma gondii* (Tg) and *Cryptosporidium parvum* (Cp). The numbering schemes of CDPKs among different parasites are also not identical, largely for historical reasons. Protein names from (1) with inclusion of two new genes from *T. gondii*: TgDPK4B (TgME49_292055) and TgCDPK7A (TgME49_237860) (http://ToxoDB.org). Tree was generated using MEGA (6.06 MAC) using a Maximum Likelihood algorithm (2).

1. **Billker O, Lourido S, Sibley LD.** 2009. Calcium-dependent signaling and kinases in apicomplexan parasites. Cell Host Microbe **5:**612 - 622.
2. **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S.** 2013. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol **30:**2725-2729.

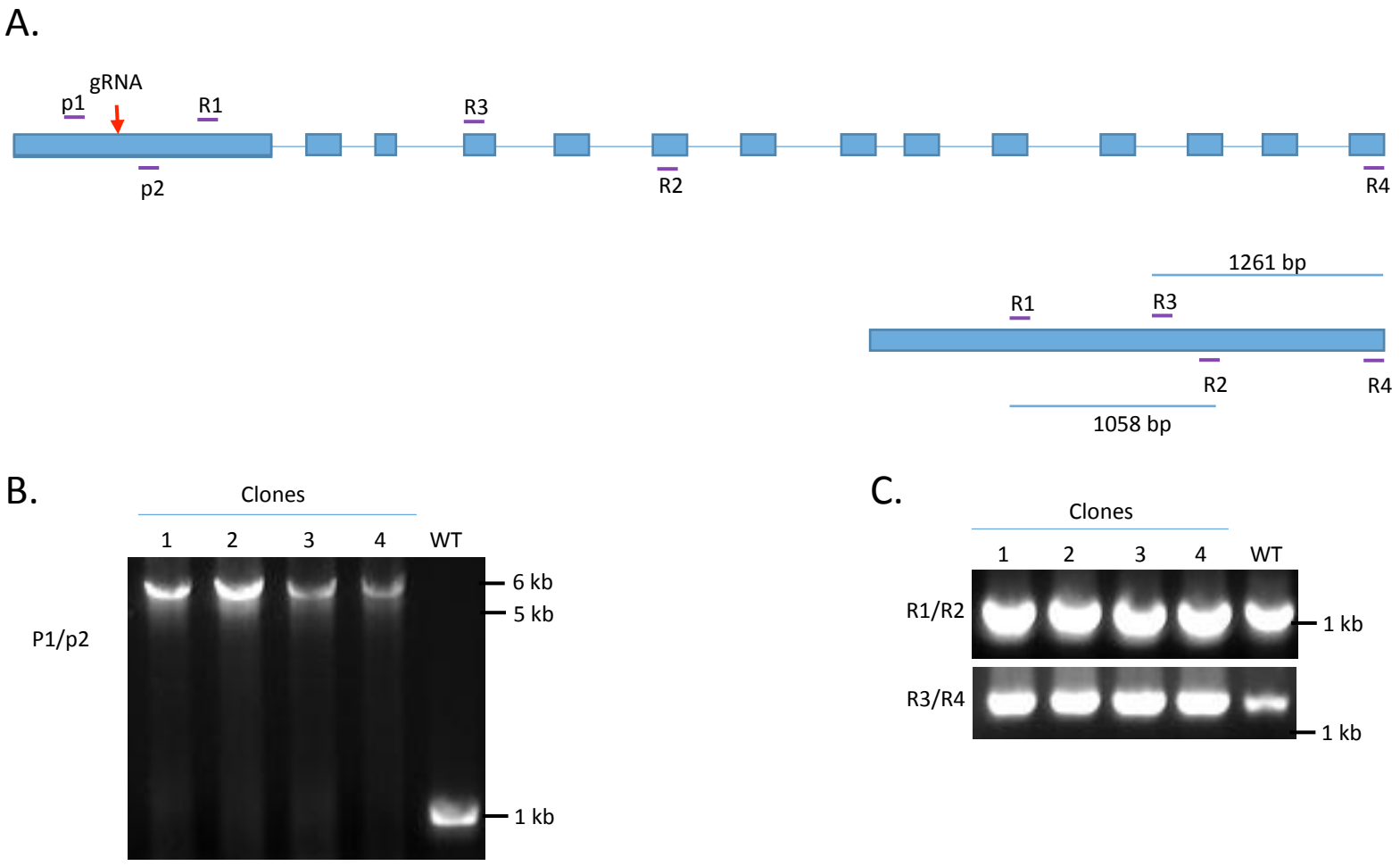

Fig. S2. Generation of a *CDPK6* disruptant and diagnostic PCR and RT-PCR. (A) a cartoon showing the CDPK6 gene structure and the gene coding sequences. The single gRNA targeting site and primers for a diagnostic PCR of gene disruption and primers for RT-PCR are shown. (B) A diagnostic PCR using primer p1 and p2. C. RT-PCR testing the expression of downstream of gene disruption site.

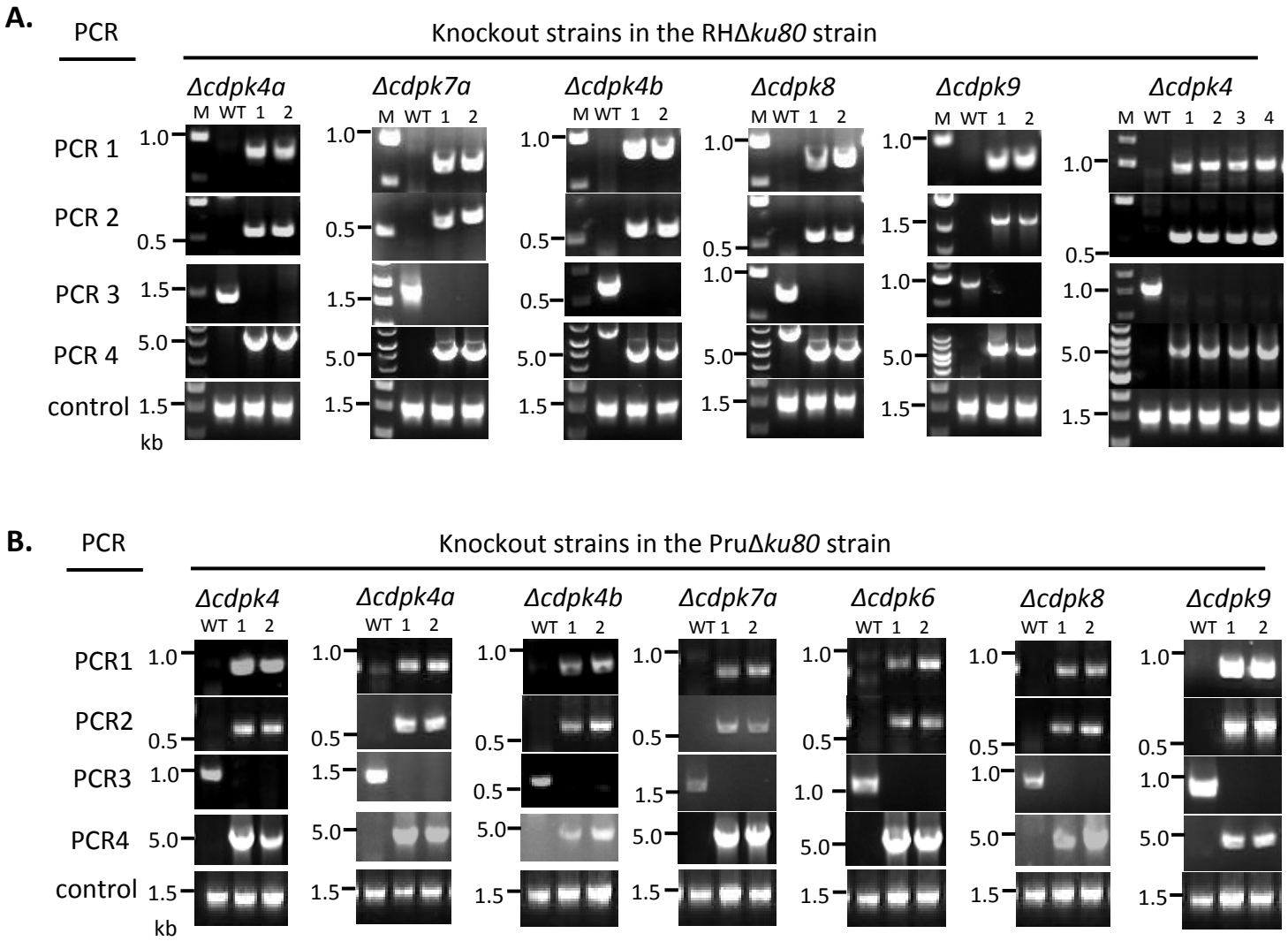

Fig. S3. Diagnostic PCR to confirm single *CDPK* gene knockouts in the RHΔ*ku80* (A) and PruΔ*ku80* (B) strains. Diagnostic PCRs were designed as illustrated in Fig. 2A.

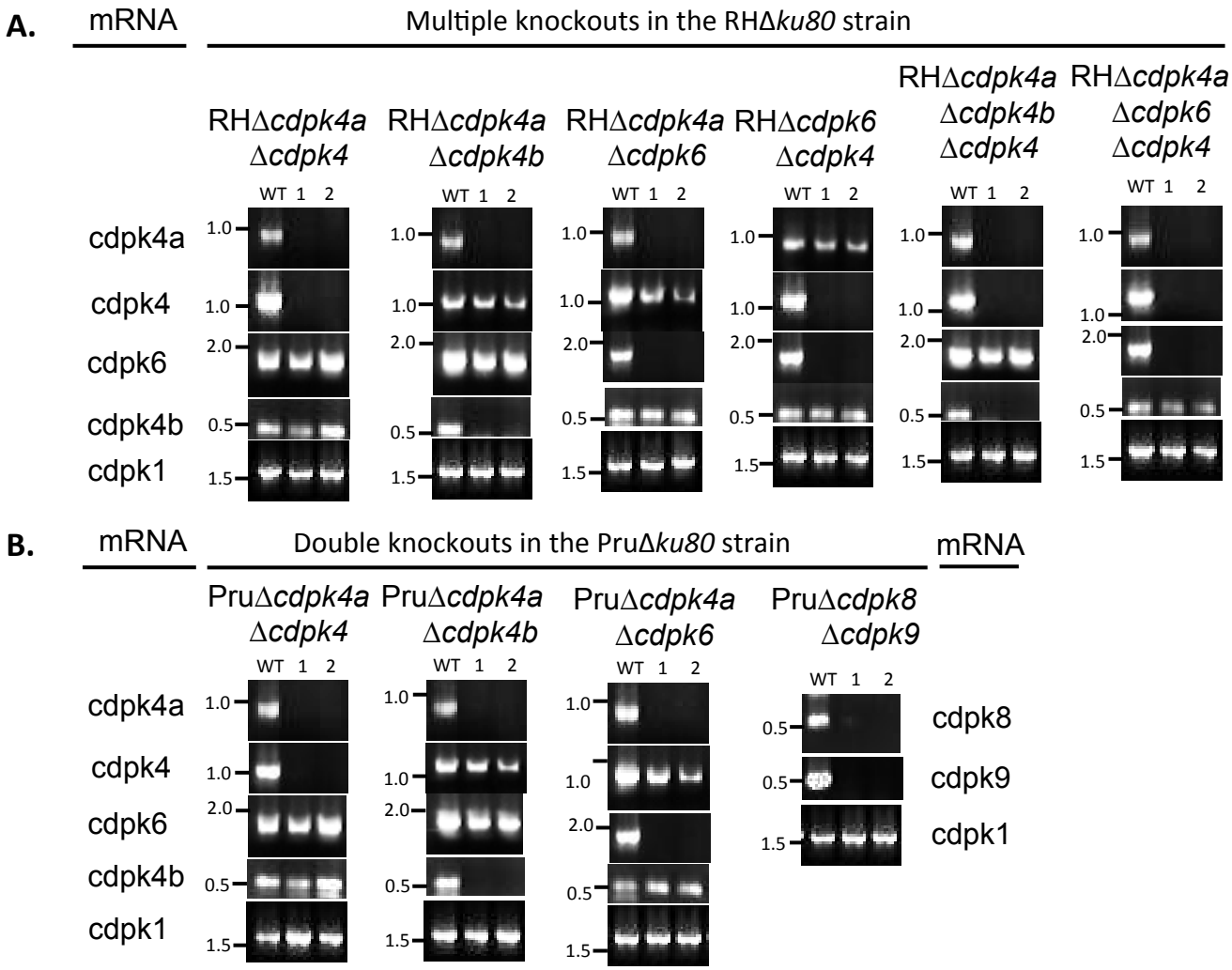

Fig. S4. RT-PCR confirmation of multiple *CDPK* gene knockouts in the RHΔ*ku80* and PruΔ*ku80* strains. Multiple knockouts both in the RHΔ*ku80* strain (A) and PruΔ*ku80* strains (B) were generated using the same strategy used for the single gene knockouts, after excision of the DHFR-mCherry cassette by transfection of a Cre-GFP plasmid. *CDPK1* served as a positive control for RNA template quality in all the cDNA samples.

Table S1. primers for a *CDPK6* disruptant using a single gRNA strategy

Names	Primer Sequence (5'-3')
K6sgRNA	GCCTCGAACGTACGTGCCAG GTTTTAGAGCTAGAAATAGC
K6HR1	GTTTTTGTGGTGTGCGCCGGCACCTGGCGAGCC CGACGGCCAGTGAATTCG
K6HR2	CAAGTTTCGGAAATCCCAGTCTTCCAGCCCCT ATACGACTCACTATAGGG
p1	CAGGAAGCCACAGGGAAAAGAG
p2	GAAACGCGAGATTCGACGC
R1	CCTCGACTCCAAGAGGCAAG
R2	TTGCTTCATCAACTCAGCAAC
R3	TAGTGGTTTGGAGCGTGTAATC
R4	TTAGTCGTGGCGCATATACGC

Table S2. Primers for generation of CRISPR-Cas9 plasmids targeting to the non-canonical *CDPKs* in *T.gondii*

Genes	Names	Primer Sequence (5'-3')	CRISPR-Cas9 plasmids	double CRISPR-Cas9 plasmids
CDPK4	4g1	GGCTCAAGCGGCGAGGCCTG**GTTTTAGAGCTAGAAATAGC**	pCAS9-gRNA1/*CDPK4*	pCAS9-gRNA1-2/*CDPK4*
	4g2	GACATCGCGGCTACACCATCGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA2/*CDPK4*	
CDPK4A	4ag1	GTGGTGGGGCAGGGGGCGAGGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA1/*CDPK4A*	pCAS9-gRNA1-2/*CDPK4A*
	4ag2	ATGGTAAGGGTCGCTCTTCGGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA2/*CDPK4A*	
CDPK4B	4bg1	GTCATGGAGCTCTGCCGAGGGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA1/*CDPK4B*	pCAS9-gRNA1-2/*CDPK4B*
	4bg2	CTCGGAGCACCGTAACGAAAGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA2/*CDPK4B*	
CDPK6	6g1	GCCTCGAACGTACGTGCCAG**GTTTTAGAGCTAGAAATAGC**	pCAS9-gRNA1/*CDPK6*	pCAS9-gRNA1-2/*CDPK6*
	6g2	TCTTGAAATGCGTTTAGTCGGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA2/*CDPK6*	
CDPK7A	7ag1	TCAATCTgtaaggttgtcgaGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA1/*CDPK7A*	pCAS9-gRNA1-2/*CDPK7A*
	7ag2	GAATCGCTGGGAGCTGGTTTGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA2/*CDPK7A*	
CDPK8	8g1	GACGACGAGGCTCGCCCAGCGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA1/*CDPK8*	pCAS9-gRNA1-2/*CDPK8*
	8g2	GCGATTCTCTCTATCACATGGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA2/*CDPK8*	
CDPK9	9g1	GATATGCAGAGCGTAAGCTGGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA1/*CDPK9*	pCAS9-gRNA1-2/*CDPK9*
	9g2	GAAAACAGAGAGCAGCTAAGGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA2/*CDPK9*	
CDPK1	1g1	GCGGGGACCAGCGGTGGACCGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA1/*CDPK1*	
CDPK7	7g1	GGGCGTCCAGTCGACCCGCGGTTTTAGAGCTAGAAATAGC	pCAS9-gRNA1/*CDPK7*	

The red sequence in the primers indicate sequence matching to the gRNA scaffold right after the gRNA in the previously reported CRISPR CAS9-gRNA plasmid .

Table S3. Primer sets for constructing pLoxP-DHFR-mCherry

Primer sets	Sequence (5'-3')	Fragments
Set 1	TGAATTCGAGCTCGGTACCCataacttcgtatagcatacattatacgaagttataagcttcgccaggctgtaaatc acttccacctccaccgacagccatctccatctggatt	LoxP-DHFR 5'UTR-DHFR-TS
Set 2	ggtggaggtggaagtatggccatcatcaaggagttcatg GTCGACTCTAGAGGATCCCCataacttcgtataatgtatgctatacgaagttatgatattgacccatgtggcgcgt	mCherry-SAG1 3'UTR-LoxP
Set 3	GGGGATCCTCTAGAGTCGAC GGGTACCGAGCTCGAATTCA	plasmid backbone
Set 4	tGTACAAGtaagctgagcataacttcgtatagcatacattatacgaagttatCGGAAATACAGAAGCTGCCCGTCTC GGTCGACTCTAGATCACCAGTTGATTTGTTCGGTCAC	LoxP-DHFR 3'UTR

Table S4. Primers for generation of LoxP-DHFR-mCherry amplicoms for integration into targeted non-canonical *CDPKs*

Genes	HR1 F/HR2 R	Sequence (5'-3')
CDPK4	4HR1 F	GCGGTTTCTGCTGGCTTCCCCAGAGAGAATGCGGAGCCACAGGTAAAACGACGGCCAGTG
	4HR2 R	GCATTGTTGCAAGCGTCTGGCACCGCCATAAACTTCCCAGATAATACGACTCACTATAGG
CDPK4A	4aHR1 F	CAAGGACTACGACGTGTCTTGTCGGGTGGTGGGGCAGGGGGCGTAAAACGACGGCCAGTG
	4aHR2 R	CGAACGGAGGAGACACTGGAAAGCGGCGCAAAAAGACCACGA AATACGACTCACTATAGG
CDPK4B	4bHR1 F	TATCGGTCTGACTTCTTCGCTACAGGTCATGGAGCTCTGCCGGTAAAACGACGGCCAGTG
	4bHR2 R	CTACCCTGAACTGCCAGGCGAGGATGATGAAGACGTCCGTTTAATACGACTCACTATAGG
CDPK6	6HR1 F	GGTGTGCGCCGGCACCTGGCGAGCCGCCTCGAACGTACGTGCGTAAAACGACGGCCAGTG
	6HR2 R	AGCGACGAAAGGAAAAAGGCTCGAATCTTGAAATGCGTTTAGAATACGACTCACTATAGG
CDPK7A	7aHR1 F	GAAGGAAAACTCCTCCGTTTCCTCTTCAATCTgtaaggttgtGTAAAACGACGGCCAGTG
	7aHR2 R	GTGTATGTAGTCTGGGGATTCAATGAATCGCTGGGAGCTGGAATACGACTCACTATAGG
CDPK8	8HR1 F	GCGCCAACGGGCCGGGCAGCAGCCAGACGACGAGGCTCGCCCGTAAAACGACGGCCAGTG
	8HR2 R	GTCTATGTTCCACTGCGGCTCTTCCGCGATTCTCTCTATCACAATACGACTCACTATAGG
CDPK9	9HR1 F	AACATGCCGGAGACAAAGGGAGGGGGATATGCAGAGCGTAAGGTAAAACGACGGCCAGTG
	9HR2 R	CCTGGTCACCGGCCAGACCCTGCCGGAAAACAGAGAGCAGCTAATACGACTCACTATAGG

The green sequences in primers match to the M13F sequence in pLoxP-DHFR-mCherry, and the red sequences in primers match to the T7 promoter in the plasmid.

Table S5. Primers for diagnostic PCRs

Genes	Names	Sequences (5'-3')
CDPK4	p1	CGAACCTCCCGCTTTCTCAC
	p2	TTGCCTCGTCACTTTCTCTAGC
	p3	TTGCCGGTGCACAGTTCCAT
	p4	tgtgttcttgctcgcgtgc
CDPK4A	p1	acctaggccacaaccttacg
	p2	CTAGATCAGGGCGTCCGTTC
	p3	tacaggtcgatgcagaagcag
	p4	ttcgtacagaagccggagat
CDPK4B	p1	cgcggttccacaaaaggaag
	p2	TACAGGGAGGAGAGACAAGG
	p3	TGAAAACAGAGGCAACAACG
	p4	TCTTGTCTCGCGAAGATCC
CDPK6	p1	GAGGTCTACTGCCCGGAACTG
	p2	CTGGCTTGGGCAGAGCGACC
	p3	TAGTGGTCTGGAGCGTGTAATC
	p4	TTGCTTCATCAACTCAGCAAC
CDPK7A	p1	GAGCGGTTCTTCGATCTTCTCG
	p2	CACACATTCCCTCACATGC
	p3	TCTTCAGCTTCGTGCTCCTC
	p4	AGAAGGCGAGAAACAAAGTCG
CDPK8	p1	AAAGGAAATTGCACTTTGTCG
	p2	CTCGACGTGTACTGAGAGGC
	p3	GGGGAGAGAAGACCTTTTGG
	p4	CACCAAGCCGTCAAAACTACG
CDPK9	p1	CCGAACCACACCCTGACTGTC
	p2	ACCGCACAGATCGGTGCTC
	p3	CCGAAAAAGCGCCTTAGTGCTG
	p4	GCCCATATCGAGAGCCTTTGAG

Table S6. Primers for RT-PCRs

Genes	Names	Sequence (5'-3')
CDPK4	p7	GACGTCAGCAACCACGTTCTG
	p8	CGATACTCCAGAGGTCACATTTTTC
CDPK4A	p9	GACGTGTCTTGTCGGGTGGTG
	p10	AACGTGGTTTAGAGCCTCGTTG
CDPK4B	p11	TGAAAACAGAGGCAACAACG
	p12	TCTTGTCTCGCGAAGATCC
CDPK6	p13	CCTCGACTCCAAGAGGCAAG
	p14	TTGCTTCATCAACTCAGCAAC
CDPK7A	p15	TCTTCAGCTTCGTGCTCCTC
	p16	AGAAGGCGAGAAACAAAGTCG
CDPK8	p17	GGGGAGAGAAGACCTTTTGG
	p18	CACCAAGCCGTCAAAACTACG
CDPK9	p19	CCGAAAAAGCGCCTTAGTGCTG
	p20	GCCCATATCGAGAGCCTTTGAG