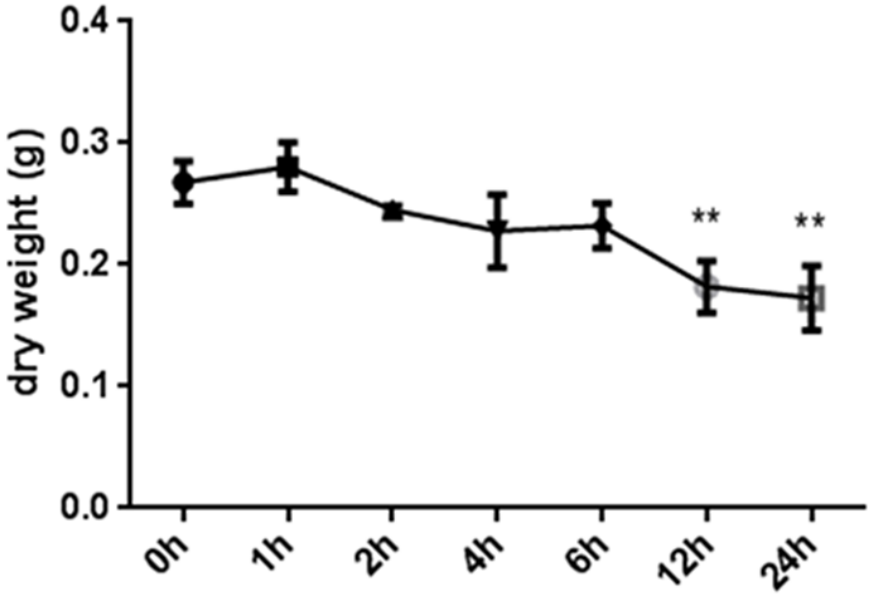

Fig. S1 HS decreased the mycelial dry weight of *G. lucidum*.

The wild-type strains were cultured on PDA liquid cultures with shaking for 5 days and then exposed to 42 ℃ for 0 to 24 h and followed until the 7th day at 28 ℃ in stationary PDA liquid cultures. The mycelial dry weight in the heat-stressed strains was measured and subjected to statistical analysis. The values are the means ± SD of three independent experiments. Asterisks indicate significant differences compared to untreated strains (Student's *t* test: $^{**}P < 0.01$).

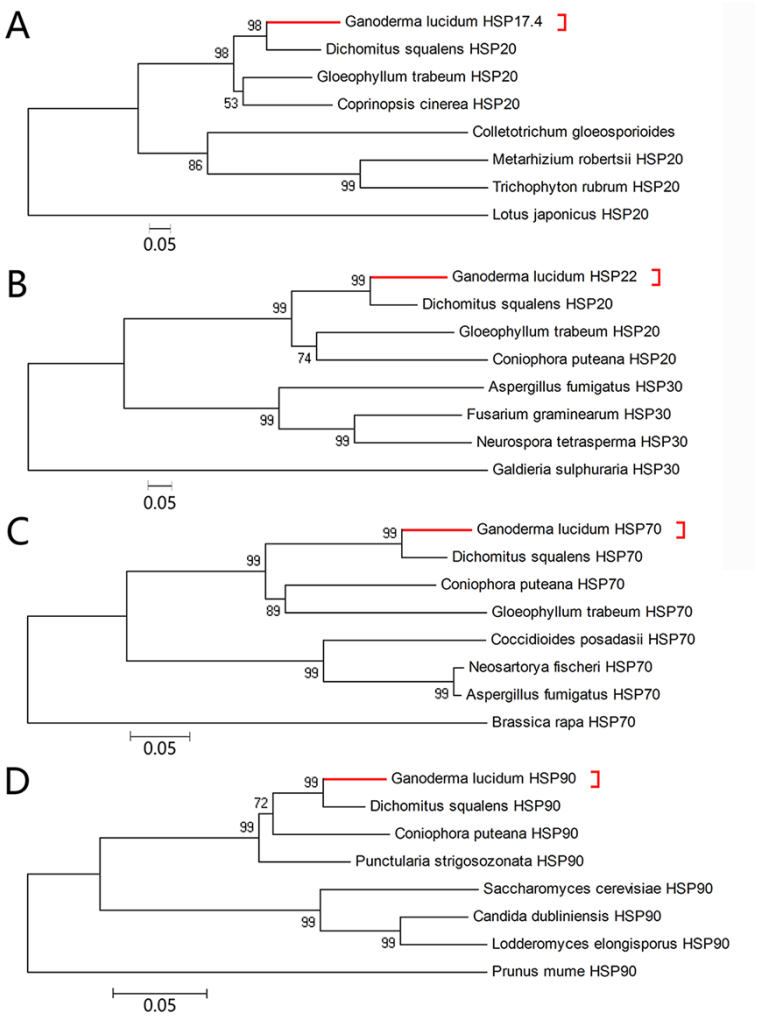

Fig. S2 Phylogenetic analysis of HSP17.4, HSP22, HSP70 and HSP90.

The amino acid alignment and tree construction were performed using MEGA 5.1. A. Phylogenetic tree of HSP17.4 sequences. The GenBank accession numbers included *Ganoderma lucidum* (GL23242-R1), *Dichomitus squalens* (XP_007369368), *Gloeophyllum trabeum* (XP_007861096), *Coprinopsis cinerea* (XP_001829984), *Metarhizium robertsii* (XP_007826570), *Trichophyton rubrum* (KMQ44830), *Colletotrichum gloeosporioides* (XP_007278592) and *Lotus japonicus* (AEO22030). B. Phylogenetic tree of HSP22 sequences. The GenBank accession numbers included *Ganoderma lucidum* (GL19532), *Dichomitus squalens* (XP_007363953), *Gloeophyllum trabeum* (XP_007866791), *Coniophora puteana* (XP_007768211), *Fusarium graminearum* (XP_011316926), *Aspergillus fumigatus* (XP_754172), *Neurospora tetrasperma* (XP_009856494) and *Galdieria sulphuraria* (XP_005707918). C. Phylogenetic tree of HSP70 sequences. The GenBank accession numbers included *Ganoderma lucidum* (GL23436), *Dichomitus squalens* (XP_007362246), *Coniophora puteana* (XP_007767273), *Gloeophyllum trabeum* (XP_007864074), *Coccidioides posadasii* (XP_003070073) *Neosartorya fischeri* (XP_001264502), *Aspergillus fumigatus* (XP_752631), and *Brassica rapa* (XP_009147592). D. Phylogenetic tree of HSP90 sequences. The GenBank accession numbers included *Ganoderma lucidum* (GL24667), *Dichomitus squalens* (XP_007361188), *Coniophora puteana* (XP_007764667), *Punctularia strigosozonata* (XP_007385736), *Lodderomyces elongisporus* (XP_001523700), *Saccharomyces cerevisiae* (NP_013911), *Candida dubliniensis* (XP_002421403), and *Prunus mume* (XP_008234835). The numbers above the branches in all of the points indicate the frequency percentage of the two branches in 1000

bootstrap samples, and the distance scale length is 0.05.

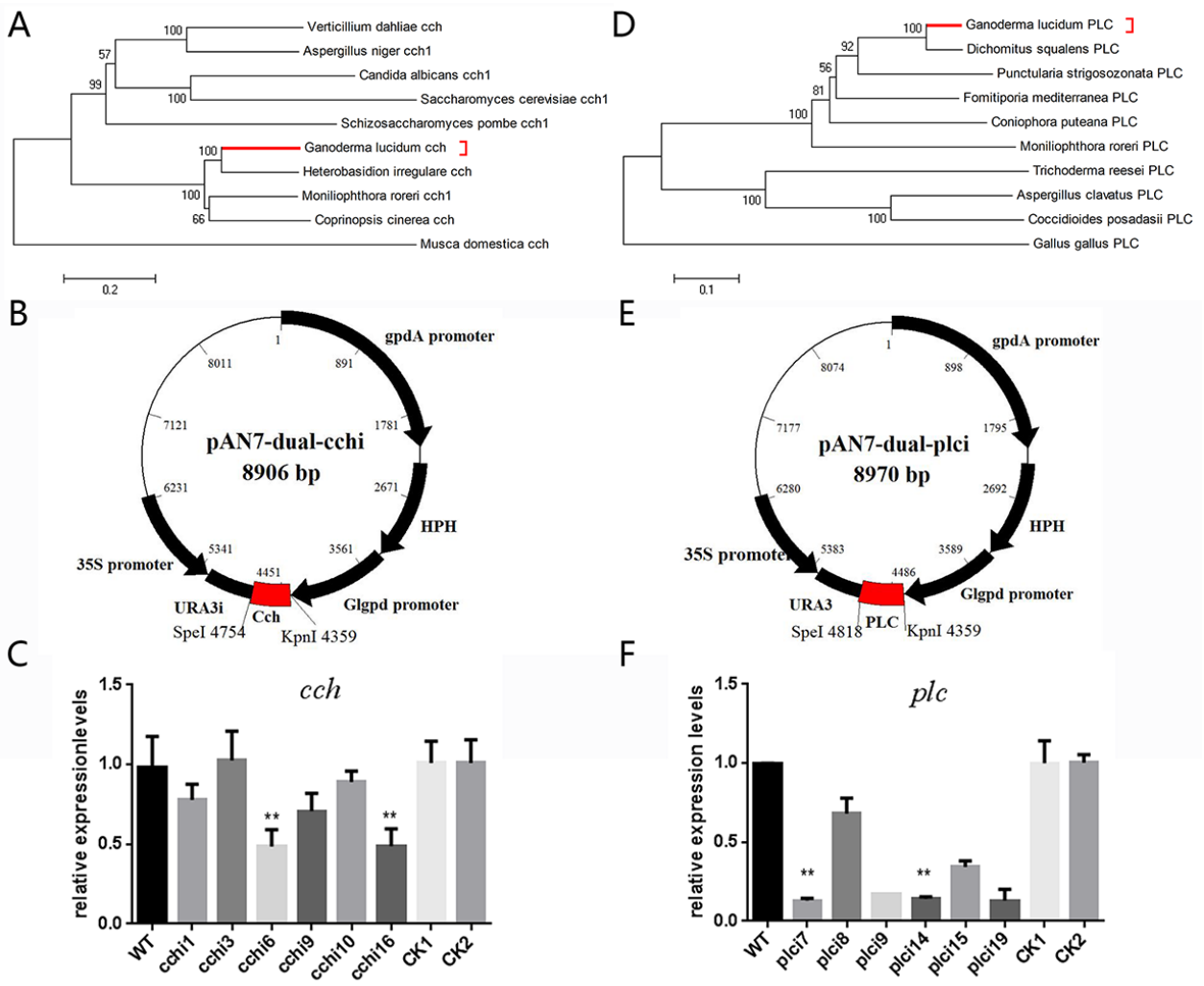

Fig. S3 Characterization of the *G. lucidum cchi* and *plci* strains. A. Phylogenetic tree of *cch* sequences.

The GenBank accession numbers for the *cch* sequences were as follows: *Ganoderma lucidum* (GL23103), *Moniliophthora roreri* (XP_007848898), *Heterobasidion irregulare* (XP_009551146), *Coprinopsis cinerea* (XP_001838182), *Aspergillus niger* (XP_001392456), *Candida albicans* (XP_718121), *Saccharomyces cerevisiae* (NP_011733) and *Musca domestica* (XP_005178247). The numbers above the branches in all of the points indicate the frequency percentage of the two branches in 1000 bootstrap samples, and the distance scale length is 0.1. B. Construction of *cch* RNAi expression cassette plasmids. The *cch* fragment was double-digested with the restriction sites *Kpn*I and *Spe*I and inserted into the plasmid pAN7-dual after *Kpn*I/*Spe*I restriction digestion. In the plasmid, *ura3* transcription and *cch* are driven by the 35S promoter and the *gpd* promoter. C. The relative mRNA levels of *cch* in *G. lucidum*. The expression level of the *cch* gene in the WT strain was arbitrarily set to 1. D. Phylogenetic tree of *plc* sequences. The GenBank accession numbers for the *plc* sequences were as follows: included *Ganoderma lucidum* (GL20551), *Dichomitus squalens* (XP_007367392), *Fomitiporia mediterranea* (XP_007267331), *Gloeophyllum trabeum* (XP_007870641), *Aspergillus niger* (XP_001392747), *Sclerotinia sclerotiorum* (XP_001592832), *Neosartorya fischeri* (XP_001264434) and *Falco cherrug* (XP_005442881). The numbers above the branches in all of the points indicate the frequency percentage of the two branches in 1000 bootstrap samples, and the distance scale length is 0.1. E. Construction of *plc* RNAi expression cassette plasmids. The *plc* fragment was double-digested with the restriction sites *Kpn*I and *Spe*I and inserted into the plasmid pAN7-dual after *Kpn*I/*Spe*I restriction digestion. In the

plasmids, *ura3* transcription and *plc* are driven by the 35S promoter and the *gpd* promoter. F. The relative mRNA levels of *plc* in *G. lucidum*. The expression level of the *plc* gene in the WT strain was arbitrarily set to 1. The values are the means ± SD of three independent experiments. Asterisks indicate significant differences compared to WT strains (Student's t test: $^{**}P < 0.01$).

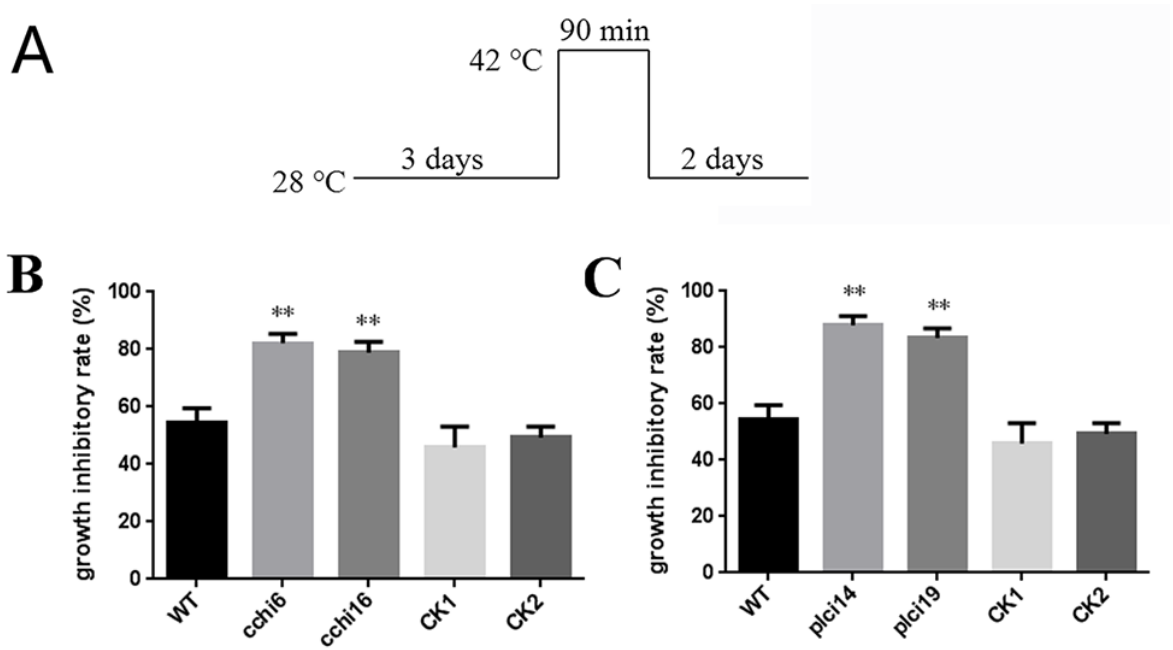

Fig. S4 *Cch*-silenced and *plc*-silenced strains displayed thermosensitive phenotypes compared with the wild-type (WT) and empty vector (CK) strains after HS.

WT, CK1, CK2, *cch*-silenced strains (cchi6, cchi16) and *plc*-silenced strains (plci14, plci19) were cultured on PDA plates for 3 days and then exposed to 42 ℃ for 0 min to 120 min followed by 2 days at 28 ℃. B. The mycelial growth rate of the WT, CK1, CK2 and *cch*-silenced strains (cchi6, cchi16) in cm day^{-1} was measured following 2 days of 28 ℃ after HS and was subjected to statistical analysis. C. The mycelial growth

rate of the WT, CK1, CK2 and *plc*-silenced strains (plci14, plci19) in cm day^{-1} was measured following 2 days of 28 ℃ after HS and was subjected to statistical analysis. The values are the means ± SD of three independent experiments. Asterisks indicate significant differences compared to WT strains (Student's t test: $^{**}P < 0.01$).

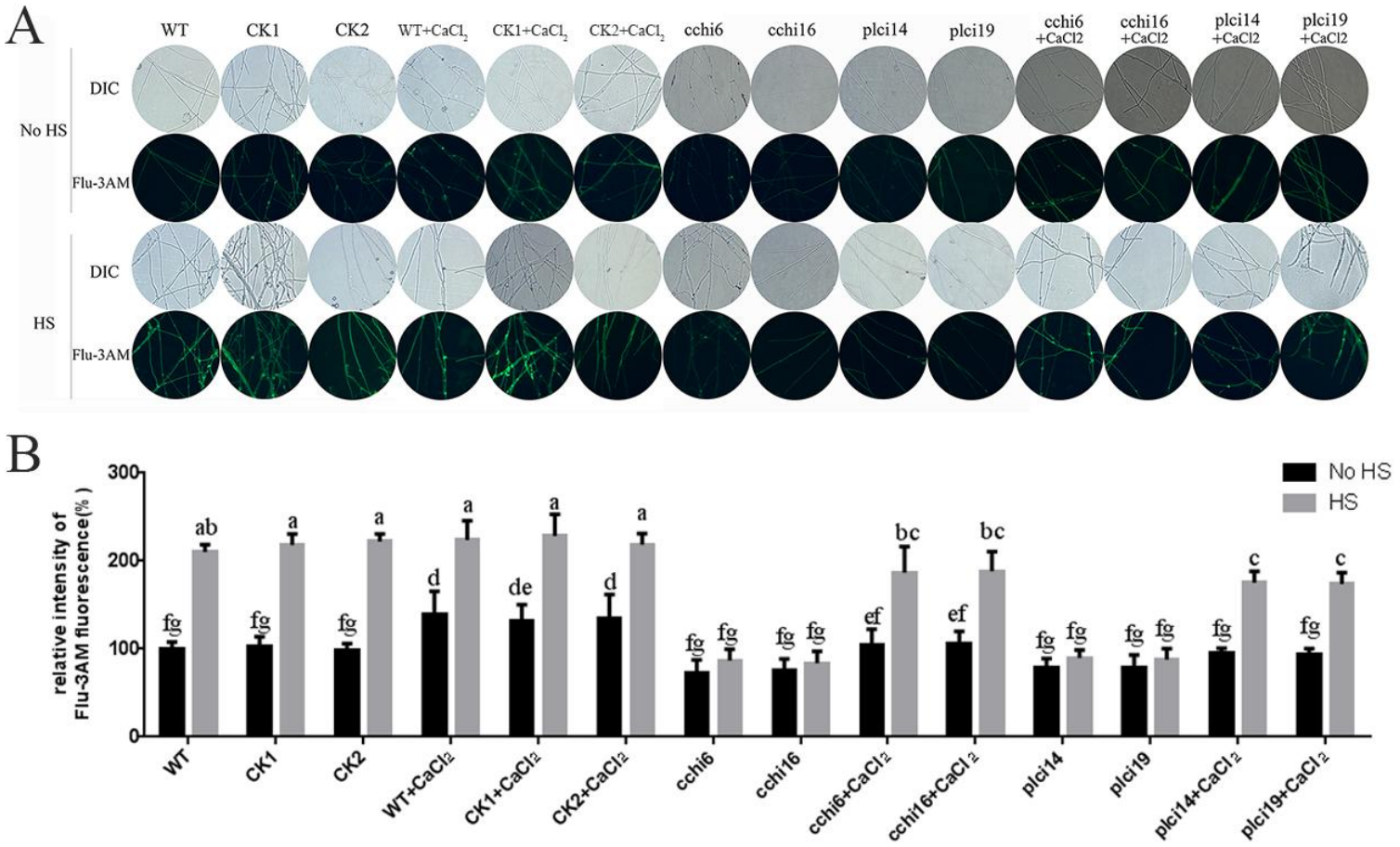

Fig. S5 Cch and plc participate in regulating cytosolic Ca^{2+} concentrations under HS.

A. Wild-type (WT), CK1, CK2 (transformed with the empty plasmid), *cch*-silenced strains (cchi6, cchi16) and *plc*-silenced strains (plci14,

plci19) were cultured on PDA plates with or without 2 mM $CaCl_2$ for 5 days and then exposed to 42 ℃ for 20 min. To detect cytosolic Ca^{2+} concentrations, 50 μM Flu-3AM, a Ca^{2+} fluorescent probe, was used, and the intensity was monitored using CLSM. Green fluorescence represents free cytosolic Ca^{2+}. B. Changes in the Ca^{2+} fluorescence ratio in the heat-stressed strains. The Y-axis is the Ca^{2+} fluorescence ratio measured by CLSM, and the X-axis represents the different treatments. The values are the means ± SD of three independent experiments. Different letters indicate significant differences between the lines ($P< 0.05$, according to Duncan's multiple range test).

Table S1 Oligonucleotide primers used

Primer	Sequence (5′ to 3′)	Description
HSP17.4RT-F	GCCGAGCTTCCTGGTGTCA	Detects the *hsp17.4* expression
HSP17.4RT-R	GCTGCCGTAGTTTGCGTTT	
HSP22RT-F	AGCCCTTCTACACCCTCTC	Detects *hsp22* expression
HSP22RT-R	TTCTGGTCCCTGTCCTCAT	
HSP70RT-F	GCGTTCGTCTGGGGTCATA	Detects the *hsp70* expression
HSP70RT-R	TCTTCCGTTCCTCAGTGTC	
HSP90RT-F	GTTCATCTCTTACCCCATCC	Detects *hsp90* expression
HSP90RT-R	CTCTTCGTCCTCGACTTCC	
SqsRT-F	CTGCTTATTCTACCTGGTGCTACG	Detects the *Sqs* expression
SqsRT-R	GGCTTCACGGCGAGTTTGT	
OscRT-F	AGGGAGAACCCGAAGCATT	Detects the Osc expression
OscRT-R	CGTCCACAGCGTCGCATAAC	
HmgrRT-F	GTCATCCTCCTATGCCAAAC	Detects the Hmgr expression
HmgrRT-R	GGGCGTAGTCGTAGTCCTTC	
PlcRT-F:	CAACTTTGACGACGTAGAGC	Detects the *Phospholipase C* expression
PlcRT-R:	GGCGTGCCTTGAGGGACTT	
CchRT-F:	GCTGGTGCTGGGTAGGA	Detects the *cch* expression

CchRT-R:	TCCAGTCAGGCAAATAGG	
Cchi-F	ACTGgttaccGGAGAACAGCGACAGCCCTAA	Get the fragment of *G. lucidum* cch gene
Cchi-R	ACTGactagtGCAAATCCTACCCAGCACCAG	
Plci-F	GTACggtaccAAGAATCGTGGCTACCT	Get the fragment of *G. lucidum* Phospholipase C gene
Plci-R	GTACactagtCGGAGTTATCTGCTGAAA	
MidRT-F	AACTTGTCCTTGCCATCACC	Detects the *mid* expression
MidRT-R	CCAGCCATCCGTATTCTTC	
YvcRT-F	ATTTGTCGTCCTTATGGTTATCG	Detects the yvc expression
YvcRT-R	GGGAAACTCGTGAGCCTGAT	
Ca^{2+} exchanger(26136)RT-F	TTGTTGAGTATCAGCGTAGGG	Detects the Ca^{2+} exchanger expression
Ca^{2+} exchanger(26136)RT-R	TGCGTATGGGACCAGAGTT	
Ca^{2+} exchanger(16447)RT-F	GGGGACCTGGTTGTTGTTAC	Detects the Ca^{2+} exchanger expression
Ca^{2+} exchanger(16447)RT-R	TTCAGTTCAGTTCACTATGGA	
Ca^{2+} exchanger(26055)RT-F	GGCAAGATCAGCAAGGAGT	Detects the Ca^{2+} exchanger expression
Ca^{2+} exchanger(26055)RT-R	CCAGGGATAGCGTGAGTTTA	
Ca^{2+} exchanger(27830)RT-F	CCAACGCACCAGGCACAAT	Detects the Ca^{2+} exchanger expression
Ca^{2+} exchanger(27830)RT-R	ACCCGAAGAGCACAAGCAAAG	
Ca^{2+} exchanger(24479)RT-F	AGGGCCTCAAGGTTATCTC	Detects the Ca^{2+} exchanger expression
Ca^{2+} exchanger(24479)RT-R	TCACGGTGACCAGGAGTAG	

Ca^{2+} exchanger(23942)RT-F	CCATTGTCCCGACGCTGTT	Detects the Ca^{2+} exchanger expression
Ca^{2+} exchanger(23942)RT-R	GGGTCAGGGTGCGTGTAAT	
Ca^{2+} pump(21462)RT-F	TGTCACCCACGAACCCTTAT	Detects the Ca^{2+} pump expression
Ca^{2+} pump(21462)RT-R	GGAGGACCTTTGCTAGACG	
Ca^{2+} pump(28993)RT-F	CGCGAATGTCCTCCCTACT	Detects the Ca^{2+} pump expression
Ca^{2+} pump(28993)RT-R	GCAACACCTTCCACCCAAT	
Ca^{2+} pump(21619RT-F	TGACGAAGAACGAGCAGAC	Detects the Ca^{2+} pump expression
Ca^{2+} pump(21619)RT-R	TGTTGCAGACCGAGCCTATC	
Ca^{2+} pump(28969)RT-F	CAGAACCACGCAGAAGCAC	Detects the Ca^{2+} pump expression
Ca^{2+} pump(28969)RT-R	CATTCGCGGCCATAGGAGT	
Ca^{2+} pump(24243)RT-F	CTCGCTATCGCCCGTCAGGT	Detects the Ca^{2+} pump expression
Ca^{2+} pump(24243)RT-R	CCGTCCTCCTCTTTGGGTT	
Ca^{2+} pump(25555)RT-F	CTTTCTGCCAGATGGATTGC	Detects the Ca^{2+} pump expression
Ca^{2+} pump(25555)RT-R	GGGTCAGAGTGCCTGTTTT	
Ca^{2+} pump(29594)RT-F	GACGGCAGGTTGGTCTTTG	Detects the Ca^{2+} pump expression
Ca^{2+} pump(29594)RT-R	GGCTTTGCTTGGCGGCTTCT	
Ca^{2+} pump(22885)RT-F	ACAAGGGAAAGGGAGAACA	Detects the Ca^{2+} pump expression
Ca^{2+} pump(22885)RT-R	TGGGCAGCCATACGAGTAG	
Ca^{2+} pump(22533)RT-F	GTCGCTATTGCCCTTTCCA	Detects the Ca^{2+} pump expression

Ca^{2+} pump(22533)RT-R	AGCCAGGGAGTCCATGAGA	
Ca^{2+} pump(30508)RT-F	CGCTCAGCAATCACAAAGT	Detects the Ca^{2+} pump expression
Ca^{2+} pump(30508)RT-R	TATGCGGAGGGTGAGAAAA	
Ca^{2+} pump(25712)RT-F	TATTCCCTCATCCAGTTCACC	Detects the Ca^{2+} pump expression
Ca^{2+} pump(25712)RT-R	CGATACTTGCCAGGACTTTCT	
Ca^{2+} pump(27118)RT-F	TACAGGCATACTTCATCTC	Detects the Ca^{2+} pump expression
Ca^{2+} pump(27118)RT-R	ATACCATTGACGGAGCACTT	
CrzRT-F	ACGCCCCTTCCTATGCGAGTG	Detects the Calcineurin-responsive zinc finger tanscription factor expression
CrzRT-R	AGGAAACGGCGTCAGTAGC	
CNA1RT-F	AAGTGTACGATGCGTGTATCAAGTCC	Detects the *CNA1* expression
CNA1RT-R	CTGGTTCCTCAAAGCGGTTTATGT	
CNA2RT-F	GCTCTGCTCGCTTGCCATAC	Detects the *CNA2* expression
CNA2RT-R	CCCTTGCCCTCTTCGGATT	
CNBRT-F	ATGGAGGAGGCACGGTAGA	Detects the *CNB* expression
CNBRT-R	CTTTAGGACGAGGAACAGC	
CamRT-F	CCCCGAGTTCCTGACGATG	Detects the proteins that undergoes conformational change upon binding to Ca^{2+} expression
CamRT-R	AGCTTCTCGCCGAGGTTGG	
CamK1RT-F	GTCGATATGTGGTCAACAGGGATT	Detects the *CamK1* expression
CamK1RT-R	ACGGTCGGTGGGAAGTCAGC	

CamK2RT-F	GAAGTTCCACCTCTTGACGG	Detects the *CamK2* expression
CamK2RT-R	TCACCACGGATGATAGCC	
CamK3RT-F	TATCAAGCCCGAGAACCTG	Detects the *CamK3* expression
CamK3RT-R	TGTAGTGTCCACGAGCAACC	
CABPRT-F	AGATGCTTCAGATCGTCCAGTC	Detects the *CABP* expression
CABPRT-R	CTTCCCTCCACAAACTCCTCGT	
calreticutinRT-F	TAGACGAGCCCATTCACAA	Detects the *calreticutin* expression
calreticutinRT-R	CTTTCACAGGAGCAACGATA	