Supplemental Tables

Table S1. Oligonucleotides for the construction of BiFC assay vectors.

The oligonucleotides were designed for PCR amplification of cDNAs by adding the appropriate restriction sites for cloning into the vectors containing the N-terminal or the C-terminal ends of the YFP protein, namely NYFP and CYFP, respectively.

Table S1. Oligonucleotides for BiFC assay	
C*c* (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatgggtgatgttgagaaaggcaag-3'
	Rv: 5'-CGAGCGGccgctcattagtagctttttgagat-3'
CSNKIIβ (*Bam*HI/*Not*I)	Fw: 5'-CTCGGAtccatgagcagctcagaggaggtgtcc-3‘
	Rv: 5'-CGAGCGGccgctcgcgaatcgtcttgactgggc-3'
CORO1A (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatgagccggcaggtggtccgctcc-3'
	Rv: 5'-CGAGCGGccgcctcttggcctggactgtctcct-3'
eIF2α (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatgccgggtctaagttgtagattt-3'
	Rv: 5'-CGAGCGGccgcaagtatctttcagctttggctt-3'
ALDOA (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatgccctaccaatatccagcactg-3'
	Rv: 5'-CGAGCGGccgcttaaaggcgtggttagagacga-3'
TUBB (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatgagggaaatcgtgcacatccag-3'
	Rv: 5'-CGAGCGGccgcgcctcctcttcggcctcctcac-3'
YWHAE (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatggatgatcgagaggatctggtg-3'
	Rv: 5'-CGAGCGGccgctcctgattttcgtcttccacgt-3'
hnRNP C1/C2 (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatggccagcaacgttaccaacaag-3'
	Rv: 5'-CGAGCGGccgcaaagagtcatcctcgccattgg-3'
MCM6 (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatggacctcgcggcggcagcggag-3'
	Rv: 5'-CGAGCGGccgctcaacttcgagcaagtagttag-3'
SET (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatgtcggcgccggcggccaaagtc-3'
	Rv: 5'-CGAGCGGccgcttgtcatcttctccttcatcct-3'
ANP32B (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatggacatgaagaggaggatccac-3'
	Rv: 5'-CGAGCGGccgctcatcatcttctccttcatcat-3'
MCM7 (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatggcactgaaggactacgcgcta-3'
	Rv: 5'-CGAGCGGccgctcgacaaaagtgatccgtgtcc-3'
NCL (*Eco*RI/*Not*I)	Fw: 5'-GTGGAAttcatggtgaagctcgcgaaggcaggt-3'
	Rv: 5'-CGAGCGGccgcctttcaaacttcgtcttctttc-3'
STRAP (*Bam*HI/*Not*I)	Fw: 5'-CTCGGAtccatggcaatgagacagacgccgctc-3'
	Rv: 5'-CGAGCGGccgctcggccttaacatcaggagctg-3'
HSPA5 (*Bam*HI/*Not*I)	Fw: 5'-CTCGGAtccatgaagctctccctggtggccgcg-3'
	Rv: 5'-CGAGCGGccgccacaactcatctttttctgctg-3'

Table S2. Human Cc novel targets identified by affinity chromatography and MALDI-TOF MS/MS

Spot	Protein name	Gene	Accession #	Score	Cell location[#]	MW(kDa)/*p*I
1	Casein kinase II subunit beta	*CSNKIIβ*	P67870	81		25.2/5.33
2	Chaperonin containing TCP1 subunit 2	*CCT2*	P78371	119		53.0/6.00
3	Coronin-like protein	*CORO1A*	P31146	118		51.0/6.12
4	Eukaryotic translation initiation factor 2 alpha	*eIF2α*	P05198	109	CYTOPLASM	36.4/5.02
5	Fructose 1,6-bisphosphate aldolase A	*ALDOA*	P00883	190		39.7/8.39
6	Ribosomal protein S7	*RPS7*	P62081	107		22.1/10.09
7	Tubulin beta chain	*TUBB*	P07437	74		50.1/4.78
8	Tumor rejection antigen 1	*HSP90B1*	P14625	127		92.7/4.76
9	14-3-3 epsilon	*YWHAE*	P62258	111		29.3/4.63
10	Heterogeneus nuclear ribonucleoprotein C1/C2	*hnRNPC1/C2*	P07910	146		33.7/4.95
11	Histone-binding protein RBBP7	*RBBP7*	Q16576	139		48.1/4.89
12	Minichromosome maintenance complex 6	*MCM6*	Q14566	95	NUCLEUS	93.0/5.32
13	Minichromosome maintenance complex 7	*MCM7*	P33993	119		81.3/5.98
14	SET nuclear oncogene	*SET*	Q01105	82		33.5/4.22
15	Acidic nuclear phosphoprotein 32B	*ANP32B*	Q92688	103		28.9/3.94
16	Heterogeneus nuclear ribonucleoprotein L	*hnRNPL*	P14866	72		60.1/6.81
17	Nucleolin	*NCL*	P19338	82	NUCLEUS, CYTOPLASM	58.5/4.57
18	Nucleosome assembly protein 1-like 4	*NAP1L4*	Q99733	73		42.9/4.60
19	Ser/Thr kinase receptor associated protein	*STRAP*	Q9Y3F4	90		38.7/4.98
20	ATP synthase subunit beta	*ATP5β*	P06576	108	MITOCHONDRIA	56.5/5.26
21	Heat shock 70 kDa protein	*HSPA5*	P11021	164	ER, CYTOPLASM	72.2/5.03

Supplemental Figure Legends

Figure S1. Apoptotic hallmarks in Jurkat T cell cultures treated with CPT.

(A) DNA laddering assay. DNA samples were obtained at varying times following CPT treatment.

(B) Changes in the nuclear morphology of CPT-treated cells analyzed by DAPI nuclear staining. Cells were observed by fluorescence (Upper Panels) and bright-field (Lower Panels) microscopy. Left and right panels correspond to 0 and 24 h of CPT treatment, respectively. Apoptotic nuclei are indicated by arrows.

(C) Percentage of apoptotic cells. Cell death at different times following treatment with 10 μM CPT was analyzed using trypan blue dye exclusion assay (grey bars) and flow cytometry (black bars). Trypan blue dye exclusion assay is the average of three independent experiments. Flow cytometry data was the result of counting 10,000 cells.

Figure S2. Flow cytometry of Jurkat T cells at varying times after CPT treatment.

Cells were set in 10 % ethanol and the nuclei were stained with propidium iodide. M1 region corresponds to apoptotic cells with a sub-G1 DNA content, whereas M2 corresponds to viable cells.

Figure S3. 2D SDS-PAGE from Jurkat T cell extracts obtained under different experimental conditions.

(A) Untreated cell extracts eluted from a Blank TS-4B column devoid of C*c*.

(B) Cell extracts treated with 10 μM CPT for 6 h and eluted from a Blank TS-4B column devoid of C*c.*

(C) Untreated cell extracts eluted from a C*c*-bound TS-4B column.

(D) Cell extracts treated with 10 µM CPT during 6 h and eluted from a C*c*-bound TS-4B column. Red circles in (C) and (D) refer to proteins that were further analyzed.

Figure S4. Design of vectors for BiFC assays.

Scheme of the cDNA cloning into the BiFC vectors. Whereas the C*c*-coding cDNA was fused with the cYFP domain, the novel C*c* targets were cloned into the vector containing the nYFP domain. These targets were classified into two categories: type I, which includes the targets cloned with the *Bam*HI/*Not*I restriction sites; and type II, which comprises the targets cloned with the *Eco*RI/*Not*I restriction sites. MCS, Multicloning site; pCMV, Cytomegalovirus promoter.

Figure S5. Homology model and BiFC assays using *Arabidopsis thaliana* C*c*.

(A) Electrostatic potential surfaces of human C*c* (1J3S.pdb) and the homology model of *Arabidopsis thaliana* C*c*, which was built using the structure of rice C*c* (1CCR.pdb; Ochi *et al*. 1983, *J. Mol. Biol.* **166**:407-418) as a template into Modeller software (Fiser & Sali 2003, *Meth. Enzymol.* **374**:461–491). The electrostatic potential surfaces were created in Chimera (Pettersen *et al*. 2004, *J. Comput. Chem.* **25**:1605-1612) using a color ramp for positive (blue) and negative (red) potentials. Each view is rotated 180º around the vertical axis.

(B) BiFC assays showing the *in vivo* interaction of *Arabidopsis thaliana* C*c* with the potential human C*c* protein partners. HEK293T cells were transfected with the plant C*c*-cYFP vector, along with another vector containing the N-terminal YFP fragment (nYFP) bound to each human C*c* protein interaction partner. Images were captured 24 h after transient transfection with Lipofectamine 2000 (Invitrogen) and after 6 h of treatment with 10 µM CPT. Reconstruction of eYFP leads to the obtainment of fluorescence signal emission, indicative of interaction between plant C*c* and the human C*c* protein partners. Scale bar is 5 µm.

Supplemental Data 1. Peptides identified by MALDI-TOF for the novel 21 C*c*-targets.