Supplementary information for

CstF64: cell cycle regulation and functional role in 3' end processing of replication-dependent histone mRNAs

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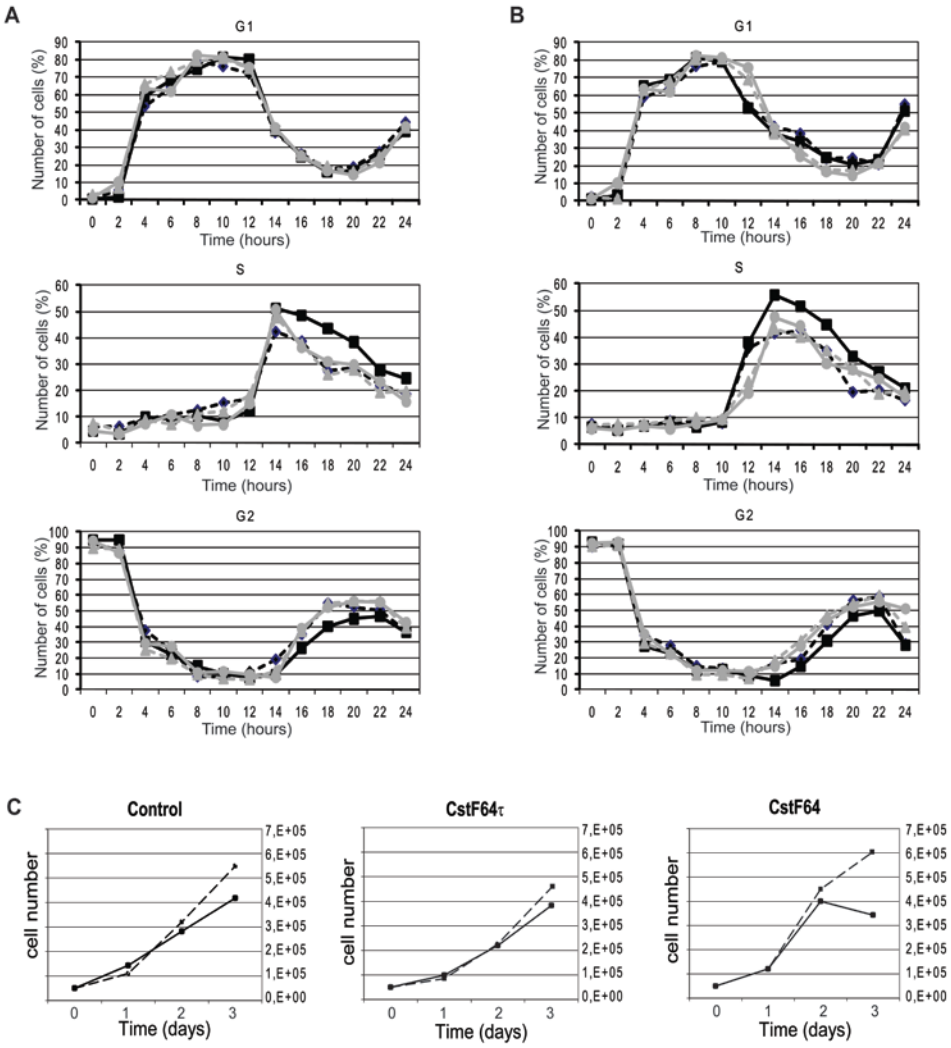
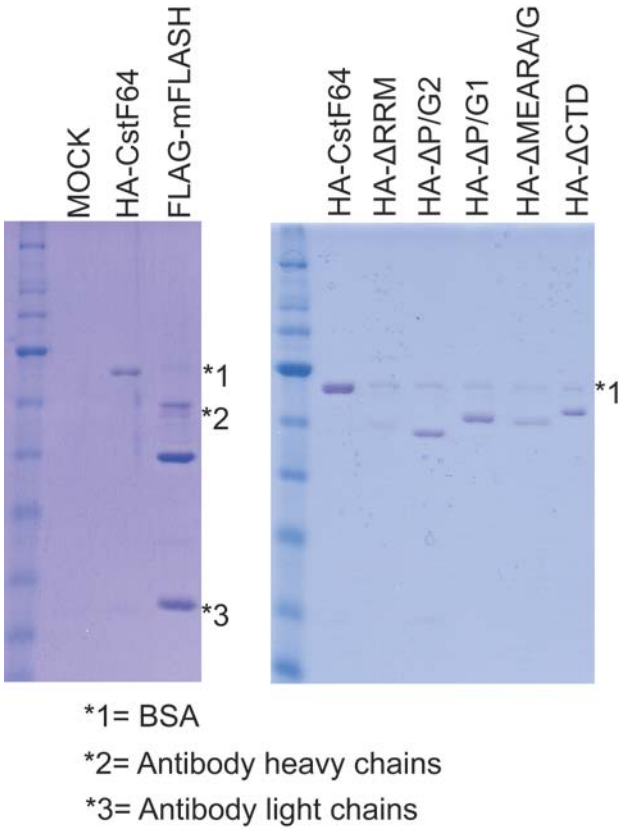


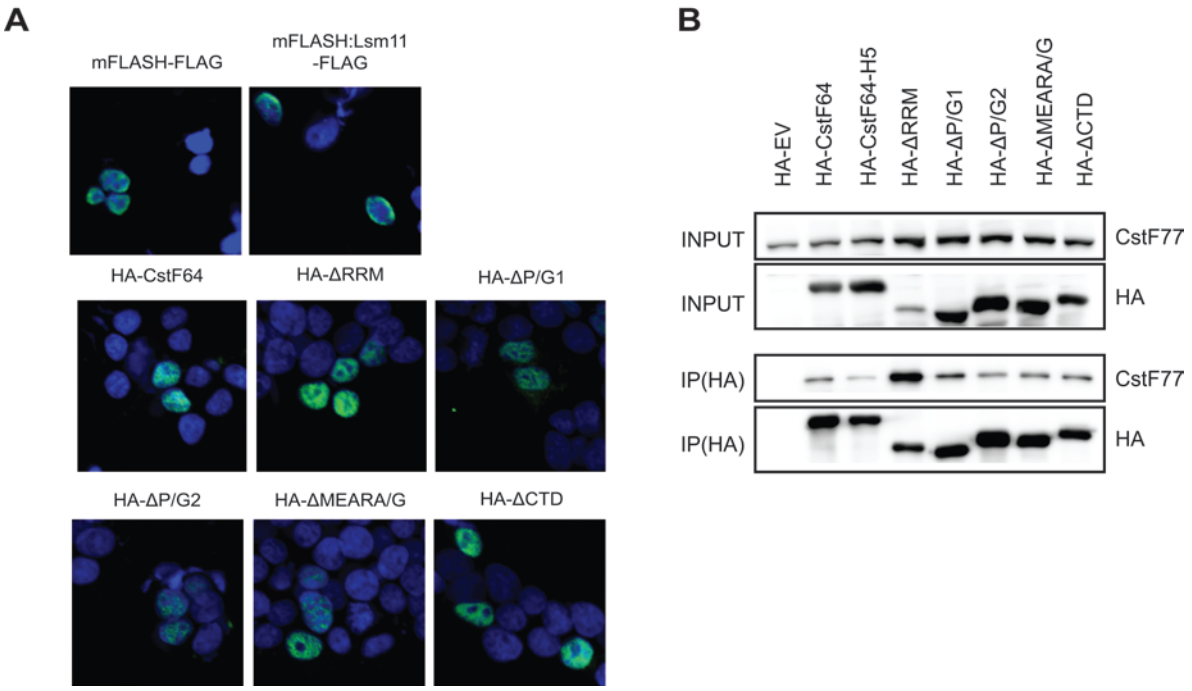
Supplementary Figure S1: CstF64 depletion impairs cell cycle progression and proliferation.

(A-B) Biological replicates of cell cycle experiment performed in Figure 2A. HeLa cell lines carrying doxycyclin-inducible shRNA expression cassettes for the depletion of either CstF64 or CstF64τ were generated by lentiviral transduction. After induction of shRNA expression by doxycyclin, the cells were arrested in G2/M by nocodazole treatment. After release, the cell cycle progression was monitored by flow cytometry (PI DNA staining) and compared with identically synchronised doxycyclin-free cells. The three panels from top to bottom show the subpopulations of G1, S and G2 phase cells. Shown in grey are cells containing shCstF64τ without (triangles, dashed lines) or with doxicyclin induction (discs, solid lines). Cells containing shCstF64 without (diamonds, dashed lines) or with doxycyclin induction (squares, solid lines) are shown in black. Note that CstF64 depletion appears to delay passage through S phase, resulting in an accumulation of cells in S and delayed entry into G2. **(C)** Growth curves (cumulative cell counting) of HeLa cells depleted of CstF64 (right panel) or CstF64τ (middle) by doxycycline induction of shRNAs (solid lines), compared to their controls without shRNA induction (dashed lines). As additional control, HeLa cells with inducible depletion of TCRβ were included (left pan-el). Cells were seeded in equal numbers, and depletion was induced on day 1. CstF64-depleted cells showed a growth defect after the second day of depletion (right panel, solid line).



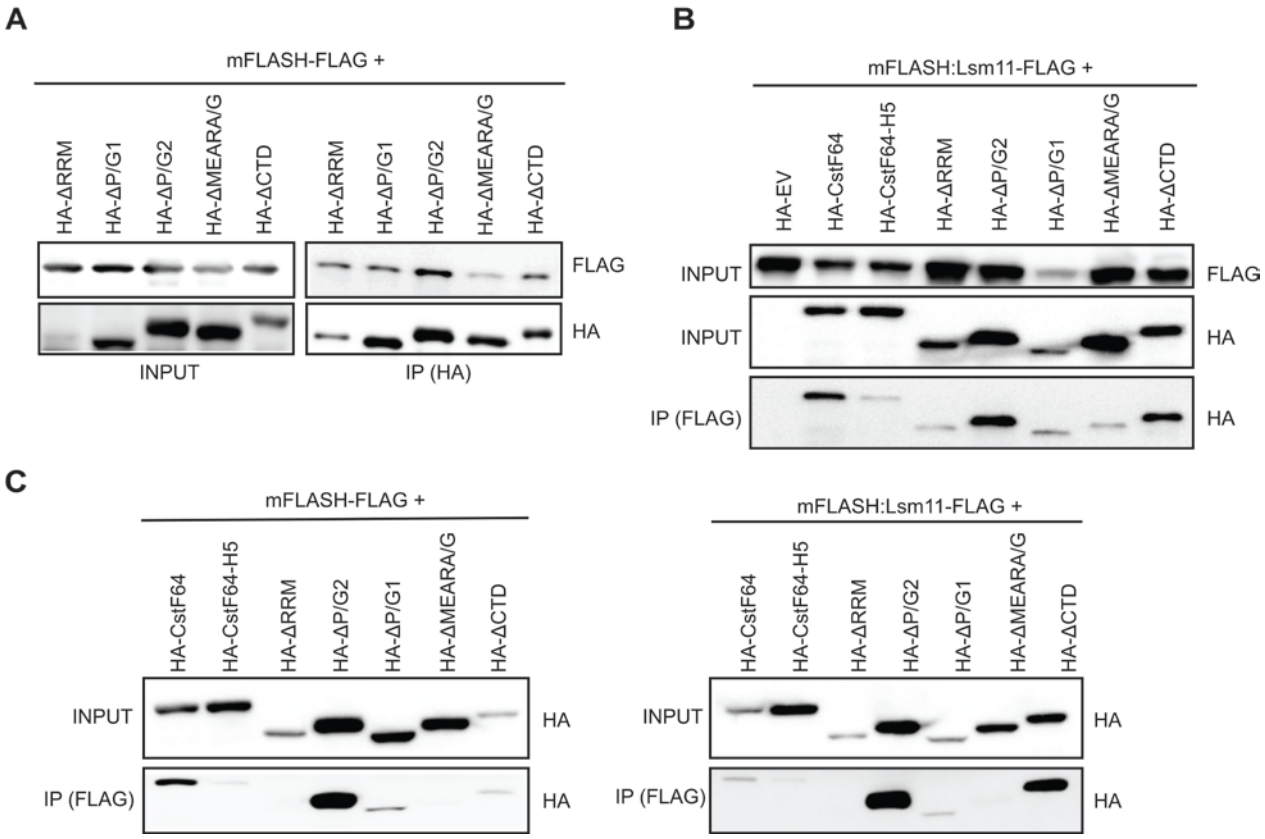
Supplementary Figure S2: Quality control of recombinant proteins used for *in vitro* interaction assays.

Plasmids encoding either a FLAG-tagged version of mFLASH (a minimal 314 amino acids domain of FLASH that is functional in histone RNA processing (1, 2) or HA-tagged versions of wild-type or mutant CstF64 were singularly transfected into HEK293T cells. The transfected cells were synchronised in S phase with aphidicolin and harvested 4 hrs after release of the block. mFLASH-FLAG as well as HA-CstF64 full length and deletion mutants were purified by immunoprecipitation under stringent conditions. The obtained proteins were tested by SDS-PAGE and Coomassie staining to verify the absence of contaminants that might interfere with the subsequent assay. Notably, also a Western blot analysis was performed to verify that the band labelled as *1 is not endogenous CstF64 or CstF64τ.



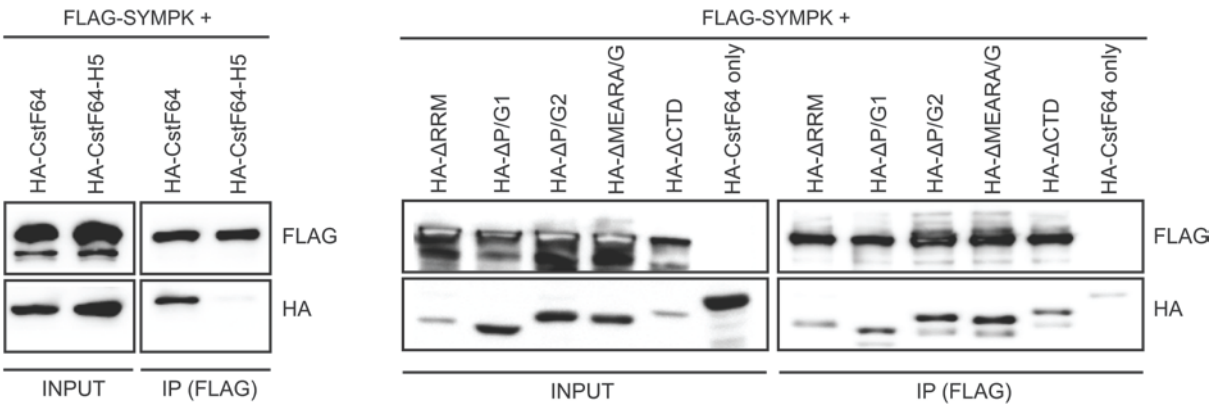
Supplementary Figure S3: Subcellular localization of HA-CstF64 deletion mutants.

(A) Expression and nuclear localisation of recombinant proteins used for interaction studies. Immunofluorescence staining of HeLa cells transiently transfected with HA-tagged CstF64 mutants or FLAG-tagged FLASH or FLASH:Lsm11. Briefly: 0.5 µg DNA was transfected into HeLa cells previously seeded on glass coverslips. 24 hours post-transfection cells were fixed with 3% paraformaldehyde solution, stained with rabbit anti-HA sc-805 or anti-OCTA probe sc-807 (as reported in Supplementary Table S3). Secondary antibody donkey anti-rabbit AlexaFluor 488 (Invitrogen) was then applied. Images were analysed with OLYMPUS Fluoview 1000-BX61 (OLYMPUS-Tokio). Scale bar 10µm. **(B)** Western blot analysis of immunoprecipitated material showing interactions between CstF64 mutants and endogenous CstF77. HeLa cells transfected with HA-tagged forms of CstF64 were synchronised in S phase by aphidicolin treatment and harvested in S phase. Whole cell lysate was immunoprecipitated with anti-HA-coated magnetic beads. INPUT (10% of total) and immunoprecipitated material (IP) were probed by Western blot with anti-CstF77 (Bethyl A301-096A) and anti-HA (sc-805).



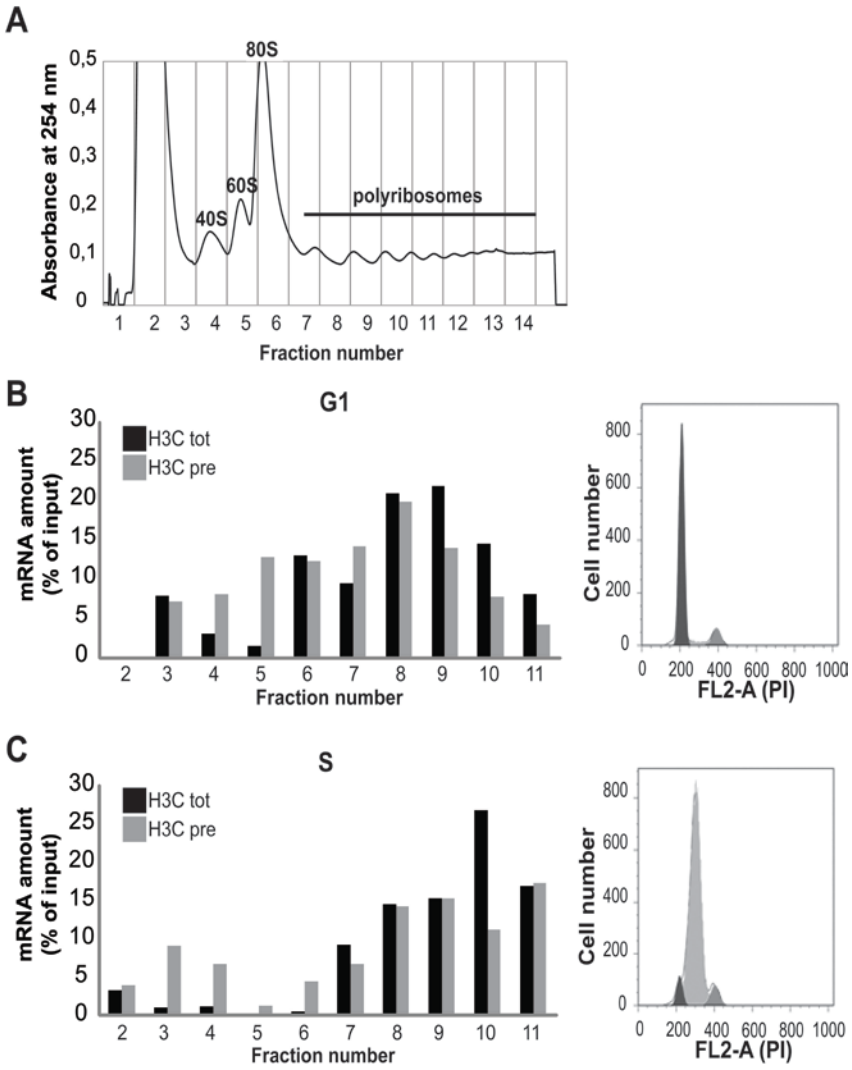
Supplementary Figure S4: Mapping CstF64 interactions with mFLASH or mFLASH:Lsm11.

(A) Association of mFLASH-FLAG with wild-type or mutant CstF64 *in vivo*. Inversely as in Figure 4D of the main paper, lysates from cells transfected with pairwise combinations of expression constructs were subjected to immunoprecipitation with HA-coated magnetic beads. Western blots from INPUT samples (10% of the total) were probed with anti-HA and anti-FLAG antibodies to show expression of the various proteins. The immunoprecipitated material (IP) was analysed in the same way. **(B)** Association of mFLASH:Lsm11-FLAG with wild-type or mutant CstF64. HeLa cells were co-transfected with mutant forms of CstF64 and FLAG-tagged fusions of a minimal FLASH fragment with the N-terminus of Lsm11 (see main text and Materials and Methods for details). Cell lysates were immunoprecipitated with anti-FLAG-coated magnetic beads. Protein expression in INPUT (10% of the total) and immunoprecipitated material (IP) was analysed by Western blot, by probing with anti-HA and anti-FLAG antibodies. **(C)** Analysis of the association of mFLASH-FLAG and mFLASH:Lsm11-FLAG with wild-type or mutant CstF64 in presence of 200µg/mL RNAseA. Western blots were probed with anti-HA antibody as decribed for panel B.



Supplementary Figure S5: Analysis of interactions of symplekin with CstF64 mutants.

Association of HA-tagged mutant forms of CstF64 with FLAG-symplekin *in vivo*. Lysates from cells transfected with pairwise combinations of expression constructs were subjected to immunoprecipitation with anti-FLAG-coated magnetic beads. INPUT (10%) and IP material were probed by Western blot with anti-HA and anti-FLAG antibodies. Left panel: control co-transfection of FLAG-symplekin and either wild-type CstF64 or the hinge helix 5 mutant. Right panel: co-transfection of FLAG-symplekin with HA-tagged CstF64 deletion mutants.



Supplementary Figure S6: H3C precursor and total RNA profiles in sucrose gradients for G1 and S phase synchronised HeLa cells.

Extracts from G1 and S phase-synchronised HeLa cells were analysed by centrifugation on linear 15%-45% sucrose gradients. H3C precursor and total transcripts were measured over the fractions collected. **(A)** Exemplary UV absorption profile of the used sucrose gradients: fractions 1 to 6 represent pre-polysome fractions, from fraction 7 onwards polysomes are present. **(B)** Gradient distribution of H3C precursor (pre, grey bars) and total (tot, black bars) transcripts in G1 phase-synchronised HeLa cells. G1-synchronised cells were obtained by nocodazole block and collected 8 hours post-release. **(C)** Gradient distribution of H3C precursor and total transcripts in S phase-synchronised HeLa cells. S phase synchronisation was achived by aphidicolin block, and cells were collected 4 hours after release. B and C right panels show cytofluorometric analyses of the DNA content as detected by propidium iodide staining.

References

1. **Burch, B. D., A. C. Godfrey, P. Y. Gasdaska, H. R. Salzler, R. J. Duronio, W. F. Marzluff, and Z. Dominski.** 2011. Interaction between FLASH and Lsm11 is essential for histone pre-mRNA processing in vivo in Drosophila. RNA **17:**1132-1147.

2. **Yang, X. C., B. Xu, I. Sabath, L. Kunduru, B. D. Burch, W. F. Marzluff, and Z. Dominski.** 2011. FLASH is required for the endonucleolytic cleavage of histone pre-mRNAs but is dispensable for the 5' exonucleolytic degradation of the downstream cleavage product. Molecular and Cellular Biology **31:**1492-1502.

Supplementary Table S1: shRNAs

Name	Target mRNA	Comments
CstF64-1	CSTF2 NM_001325.2	Ruepp et al., 2011
CstF64-2	CSTF2 NM_001325.2	Ruepp et al., 2011
CstF64T-1	CSTF2T NM_015235.2	Ruepp et al., 2011
CstF64T-2	CSTF2T NM_015235.2	Ruepp et al., 2011
scrambled	No target	Ruepp MD. Sequence: AGGUAGUGUAAUCGCCUUGdtdt
Rrp44	DIS3 NM_001128226.2	kindly provided by Dr. P. Nicholson (Mühlemann Group, Eberle A. 2007)

Supplementary Table S2: Primers and Probes

Name	Sequence	Target sequence	Amplicon (bp)	Comments
H3 pre *Taq*Man probe	FAM-CACCCACATCAGCACTT-TAMRA	HIST2H3C NM_021059.2		*Taq*Man
H3 pre forward primer	TTCCATCGTATCCAAAAGGCTCTT	HIST2H3C NM_021059.2		*Taq*Man
H3 pre reverse primer	CAAGCGGTACAGCTTCTTCC	HIST2H3C NM_021059.2	69	*Taq*Man
H3 tot *Taq*Man probe	FAM-TCGCTATGGCCCGTACTAA-TAMRA	HIST2H3C NM_021059.2		*Taq*Man
H3 tot forward primer	GCTGGTAAGCCTGTGTTTTGG	HIST2H3C NM_021059.2		*Taq*Man
H3 tot reverse primer	GCCGCCGGTCGACTT	HIST2H3C NM_021059.2	69	*Taq*Man
b-Actin pre *Taq*Man probe	ACTTGGCCTCATTTTTAAGG	ACTB NM_001101.3		*Taq*Man
b-Actin pre forward primer	GTACACTGACTTGAGACCAGTTGAA	ACTB NM_001101.3		*Taq*Man
b-Actin pre reverse primer	CCCAGCCACACCACAAAGT	ACTB NM_001101.3	79	*Taq*Man
b-Actin tot *Taq*Man probe and primers	Applied Biosystems: Hs99999903_m1	ACTB NM_001101.3		*Taq*Man
SybrCstF2-Fw	CCACTGGAGCACCTTGATAG	CSTF2 NM_001325.2		SYBR
SybrCstF2-Rev	CCACCTGGCACTGATTTG	CSTF2 NM_001325.2	131	SYBR
qhCSTF2T_fw	GGAATAATCAAGGCCCAGGT	CSTF2T NM_015235.2		SYBR
qhCSTF2T_rv	GACTAAATGGCTGTATTCCATGC	CSTF2T NM_015235.2	102	SYBR
DIS3_RTPCR_Fw	AGCAGGGAAAATTACTTGGAAGC	DIS3 NM_001128226.2		SYBR
DIS3_RTPCR_Rev	AGCTCCACAGCCACAATATCT	DIS3 NM_001128226.2	128	SYBR

Supplementary Table S3: Antibodies

Name	Code	Provider	Dilution used	Host
CstF64/CstF64T	sc-28201	SantaCruz Biotechnology	1:1000	Rabbit
CstF77	A301-096A	Bethyl	1:5000	Rabbit
CPSF2 (CPSF100)	sc-165983	SantaCruz Biotechnology	1:1000	Mouse
CPSF73	A301-091A	Bethyl	1:3000	Rabbit
SYMPK	A301-465A	Bethyl	1:3000	Rabbit
β-Actin	A 5060	Sigma-Aldrich	1:5000	Rabbit
Lamin A/C	sc-7293	SantaCruz Biotechnology	1:3000	Mouse
α-Tubulin	T-5168	Sigma-Aldrich	1:5000	Mouse
HA	sc-805	SantaCruz Biotechnology	1:1000	Rabbit
OCTA probe (FLAG)	sc-807	SantaCruz Biotechnology	1:1000	Rabbit
anti-Rabbit-HRP	sc-2004	SantaCruz Biotechnology	1:5000-10000	Goat
anti-Mouse-HRP	sc-2005	SantaCruz Biotechnology	1:5000-10000	Goat