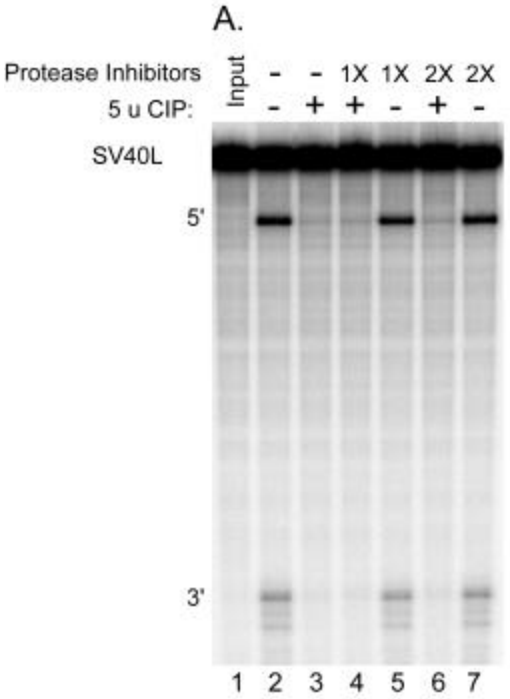
Figure S1 Control for proteases in CIP preparation



B.

Tested for protease activity w/ Azocoll	OD_{516}
Buffer blank	0.069 ± 0.014
90 u CIP	0.070 ± 0.012
0.2 ng Proteinase K	0.131
2 ng Proteinase K	.215

Figure S1 Protease activity is not detectable in the CIP preparation. (*A)* **Protease inhibitors** : HeLa cell nuclear extract was pre-treated with 5 u CIP and 1 mM $MgCl_2$ with or without the following protease inhibitors, as indicated: (1X) leupeptin (1 μg/ml), pepstatin (0.7 μg/ml), aprotinin (1.8 μg/ml), phosphoramidon (50 μg/ml). (2X, inhibitor concentrations doubled). SV40L pre-mRNA was then added to initiate processing. (B) **Azocoll protease detection assay** – To a 10 mg sample of Azocoll (< 50 mesh, Calbiochem) suspended in 1 ml Buffer D with a 1 mM $MgCl_2$ excess over the EDTA in Buffer D was added 4.5 μl (90 U) of CIP (Promega). The reaction was agitated at 30 °C for 3.5 h, the reaction tube was centrifuged and the supernatant's absorbance at 516 nm recorded. Proteinase K was used as a positive control. Average of triplicate runs ± SD.

Figure S2 Confirmation of DEAE separated cleavage factor identity.

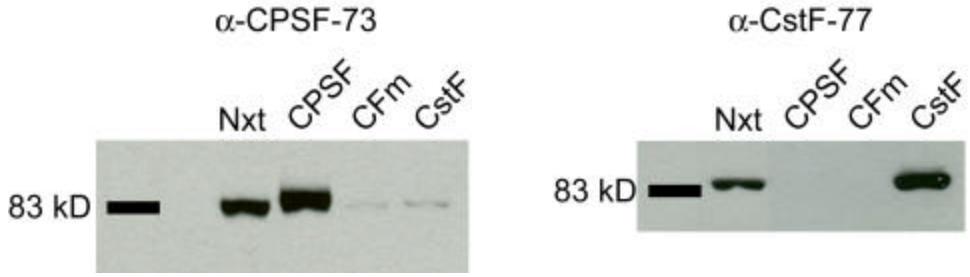


Figure S2 Western blots of the DEAE-sepharose fractions used for in vitro cleavage. HeLa nuclear extract (Nxt, ~7 μg) and the indicated protein fractions (~3.5 μg) were resolved on a 10% SDS-acrylamide gel and Western blotted using the indicated antibodies (CPSF-73, Novus H00051692-M01; CstF-77, Novus H00001479-M01). MW marker: NEB P7708S.