nuclear extract (NE) containing RFX5 (panel a) & USF-1 (b) or cytosolic extract (CE) containing Puma (c) and keratin 8 (d) were used as substrates to mix with the enzyme source (either L2S100 or various GST-CPAF fusion proteins) and the mixtures were incubated for 1 h at 37 ^{o}C. The entire mixture from each reaction was loaded into the corresponding lanes as indicated on top of the figure. After electrophoresis, the resolved protein bands were blotted onto nitrocellulose membrane for corresponding antibody detection as indicated along the left side of the figure. One independent set of enzyme/substrate reactions was used for detecting each substrate. The intact full-length keratin 8 has a molecular weight of 52 kDa and CPAF is known to cleave keratin 8 into 38 kDa fragments [17].

Figure 5 Detection of CPAF processing using a Western blot. The various GST-CPAF fusion proteins were loaded onto a SDS PAGE gel as described in the legend to **Figure 3**. After electrophoresis, the proteins bands were transferred onto nitrocellulose membrane for detection with the anti-CPAFc mAb 100a. All CPAF preps displayed the full-length GST-CPAF fusion protein along with fragments or varying length. However, only the Wt (lanes 2-4) and the unrelated CPAF mutant K540A (lane 17) displayed a protein band migrating at the position similar to band of CPAFc from the L2S100 sample (lanes 1 & 14).

Supplementary Figure 1 Intervening sequences in CPAF not found in the catalytic domains of tricorn protease. These intervening sequences, corresponding to CPAF residues 159-183, 217-249, 274-321, 335-345, and 401-474, are shown in extended conformation (see text).