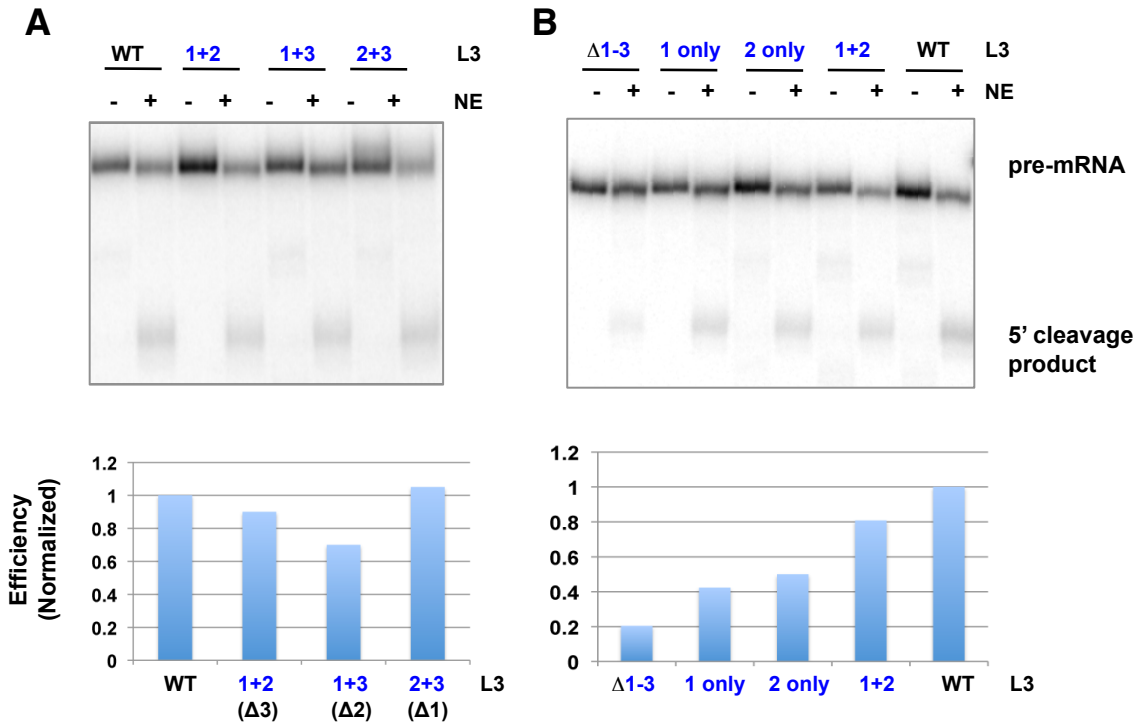
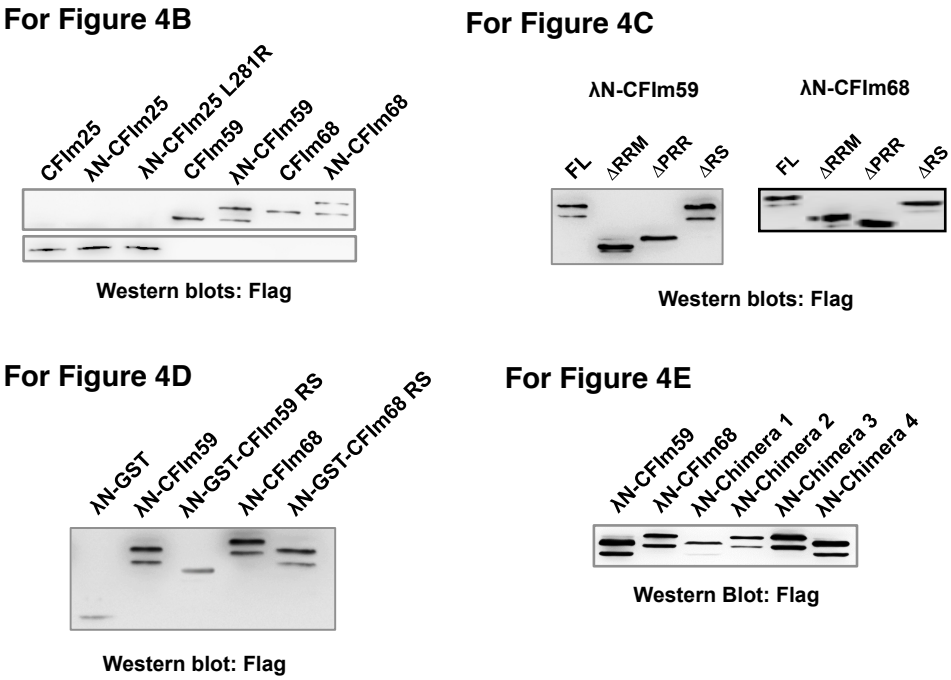


Figure S1. Test the activities of UGUAs in L3 individually and in combination; related to Figure 1.

The indicated L3 and L3 derived PAS RNAs were used for in vitro cleavage assays. The pre-mRNAs and 5' cleavage products are marked. Processing efficiency was calculated as % processed (5' cleavage product/pre-mRNAx100), normalized by the WT, and plotted below.

A



B

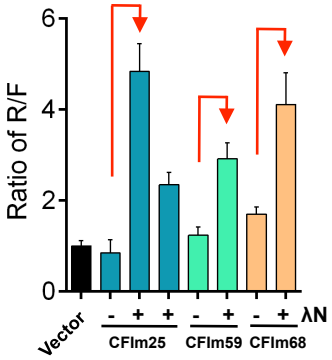


Figure S2. Related to Figure. 4.

(A) Western blotting analyses of the over-expressed proteins in reporter assays using an anti-Flag antibody. All expressed proteins had an N-terminal Flag tag. (B) qRT-PCR verification of the APA changes in tethering assays**.** For all the samples shown in Fig. 4B, total RNAs were extracted and qRT-PCR was performed using two sets of primers, one for Renilla luciferase and another for Firefly luciferase. The ratio between Renilla and Firefly was plotted. The increases in R/F ratio indicate that tethering CFIm subunits promoted the usage of the L3-2xBoxB PAS and the production of the shorter mRNA isoform.

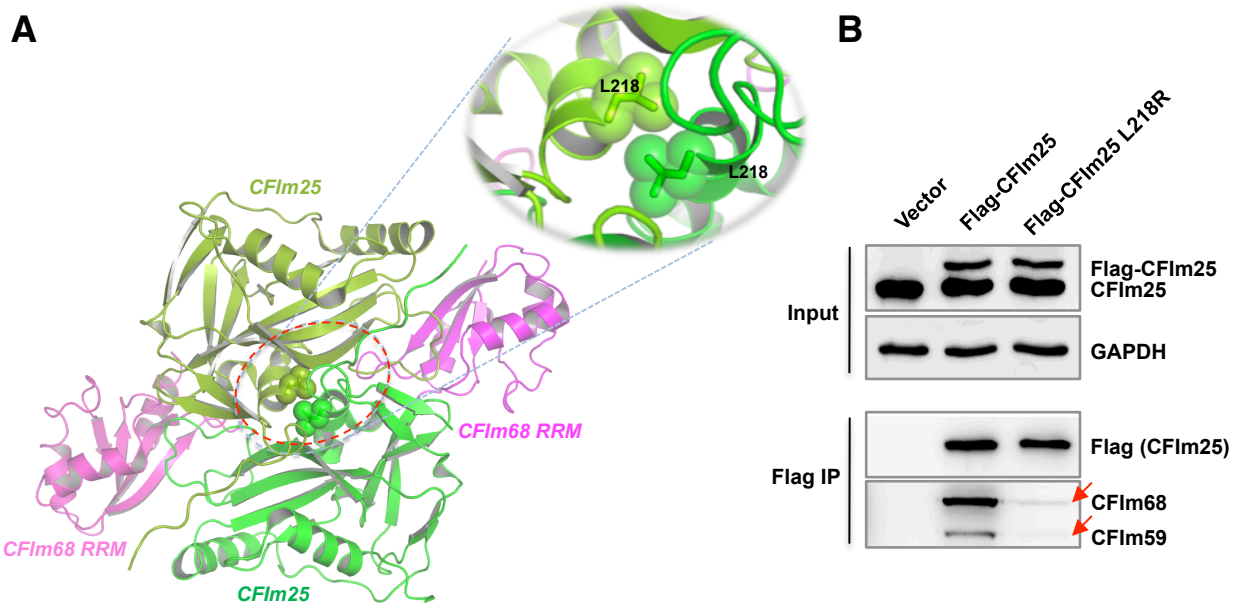


Figure S3. The point mutation L218R disrupts interactions between CFIm25 and CFIm68/59; related to Figure 4.

(A) Crystal structure of the tetramer formed by two copies of CFIm25 (green) and CFIm68 RRM domains (purple). L218 is shown in ball-and-stick model while the rest of the proteins in ribbon models. A close-up image of the area within the oval of red dotted line is shown separately to emphasize that L218 is located at the interface between the two CFIm25 molecules. **(B)** The Flag-tagged wild type or L218R CFIm25 were expressed in HEK293T cells. The input panel shows that similar levels of Flag-CFIm25 and Flag-CFIm25(L218R) were expressed. The cell lysates from these cells were used for immunoprecipitation with an anti-Flag antibody. The Flag IP panel shows that the same amounts of Flag-CFIm25 and Flag-CFIm25(L218R) were immunoprecipitated. However, lower levels of CFIm68 and CFIm59 were co-precipitated with Flag-CFIm25(L218R).

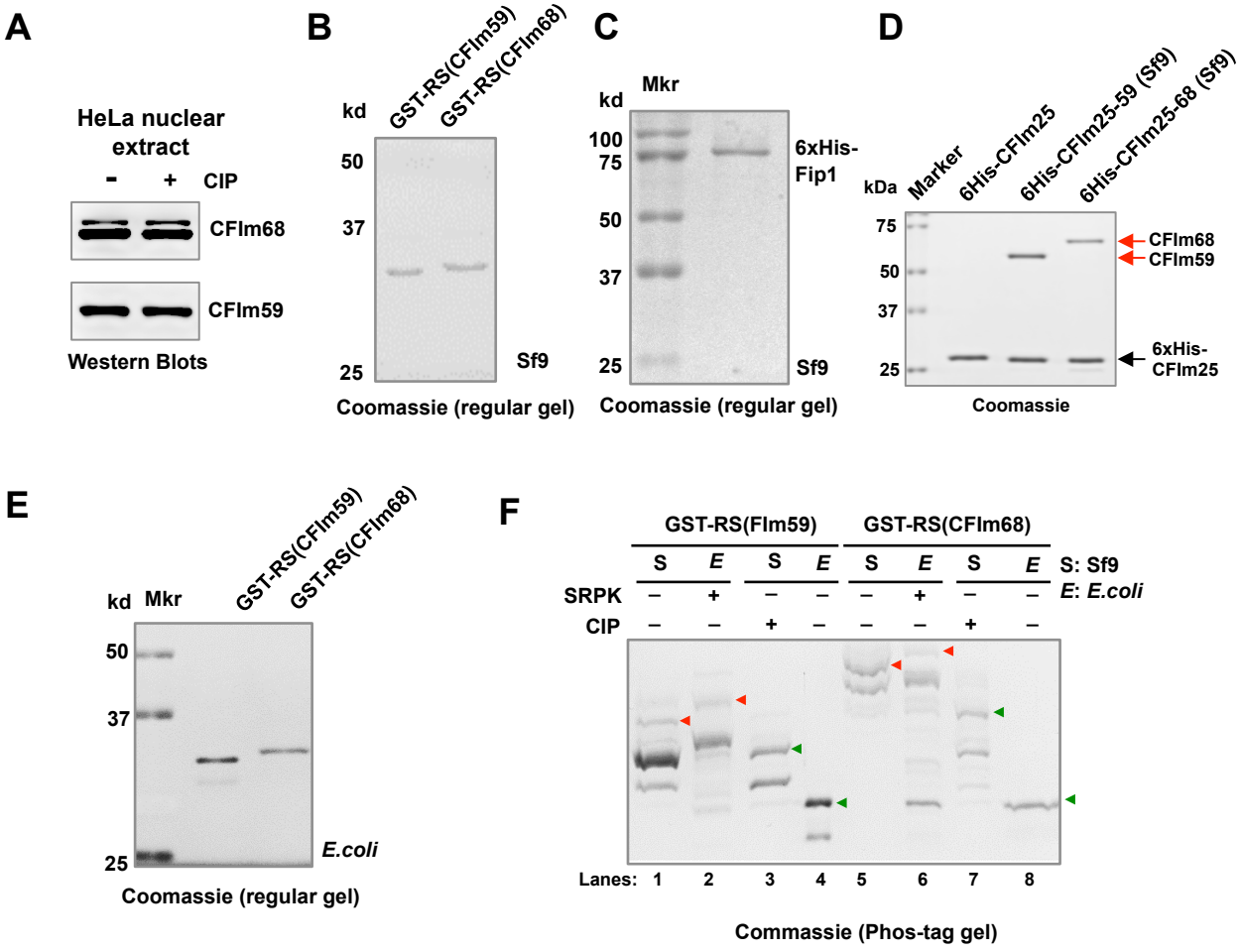


Figure S4. Related to Figure 5. (A) CIP (alkaline phosphatase) treatment of HeLa nuclear extract failed to induce visible mobility shift for CFIm68 and CFIm59 on regular SDS-PAGE **(B)** GST-RS(CFIm59) and GST-RS(CFIm68) expressed in Sf9 cells, used in Fig. 5C, D, F. **(C)** Purified 6xHis-Fip1 (Sf9) used in Fig. 5C. **(D)** Purified 6xHis-CFIm25 (*E.coli*), 6xHis-CFIm25-CFIm59 (Sf9), and 6xHis-CFIm25-CFIm68 (Sf9), used in Fig. 5E. **(E)** GST-RS(CFIm59) and GST-RS(CFIm68) expressed in *E. coli*, used in Fig. 5F. **(F)** The purified proteins shown in (B) and (E) were treated with CIP or SRPK1, repurified, and resolved on Phos-tag gels and visualized by Commassie staining. Although all proteins appeared as a single band on regular SDS-PAGE (shown in (B) and (E)), they appeared as a doublet on Phos-tag gels. The arrowheads mark the highest band for each protein. Red arrowheads indicate phosphorylated proteins and green arrowheads mark un/de-phosphorylated proteins. SRPK1-treated GST-RS had the highest phosphorylation levels (lanes 2 and 6) and were designated as hyperphosphorylation. GST-RS expressed in Sf9 cells had intermediate levels of phosphorylation. GST-RS expressed in E. coli were completely unphosphorylated.

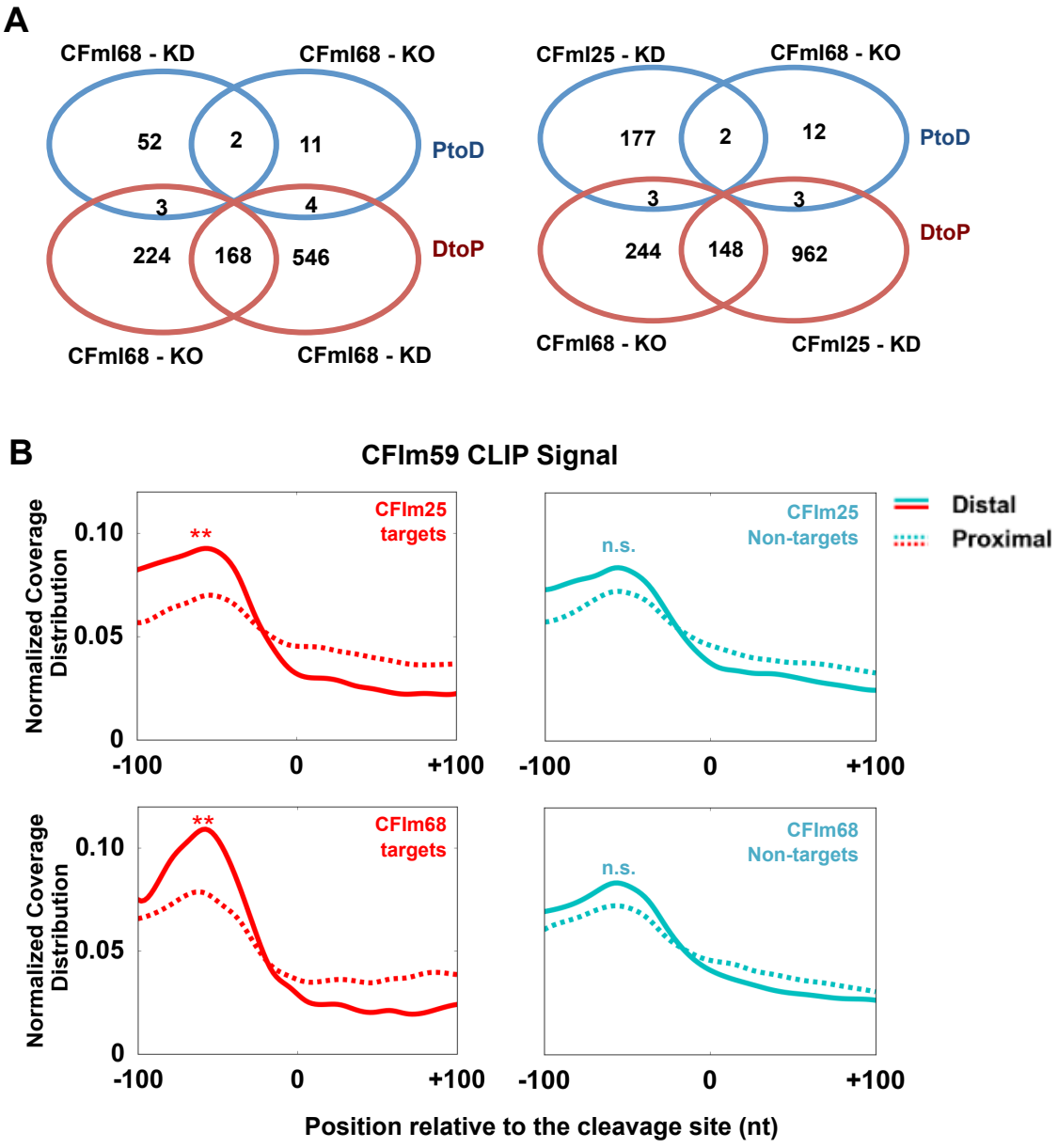


Figure S5. Related to **Figure 6**. **(A)** Comparison of the APA profiles of CFIm68 KO cells and CFIm68 knockdown (KD) (left panel) and CFIm25 KD cells; Red ovals contain genes with distal-to-proximal (DtoP) APA changes while blue ovals proximal-to-distal (PtoD). The number of common genes are shown in the intersections. **(B)** CFIm59 PAR-CLIP signals at CFm25 or CFIm68 target and non-target genes. The formats are identical to Fig. 6D. The CLIP signal curves at proximal and distal were compared and ** indicates p value <0.05 (K-S test) and n.s. indicates not significant.

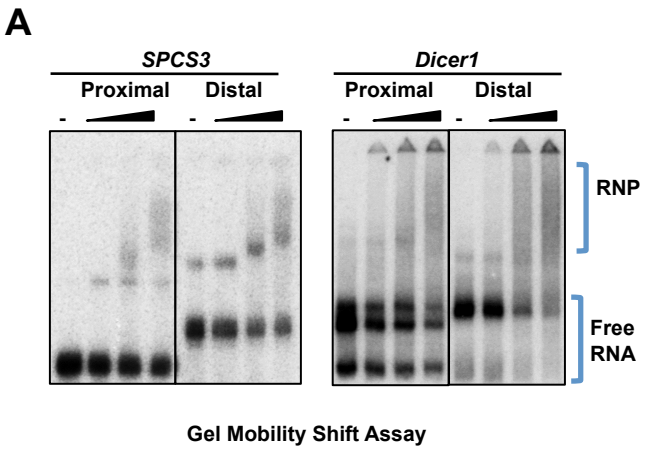


Figure S6. Related to Figure 6. (A) CFIm25-68 preferentially binds to distal PASs. Gel mobility shift assays were performed as described in Fig. 6E. **(B)** Expression levels of transfected genes in Fig. 6F.

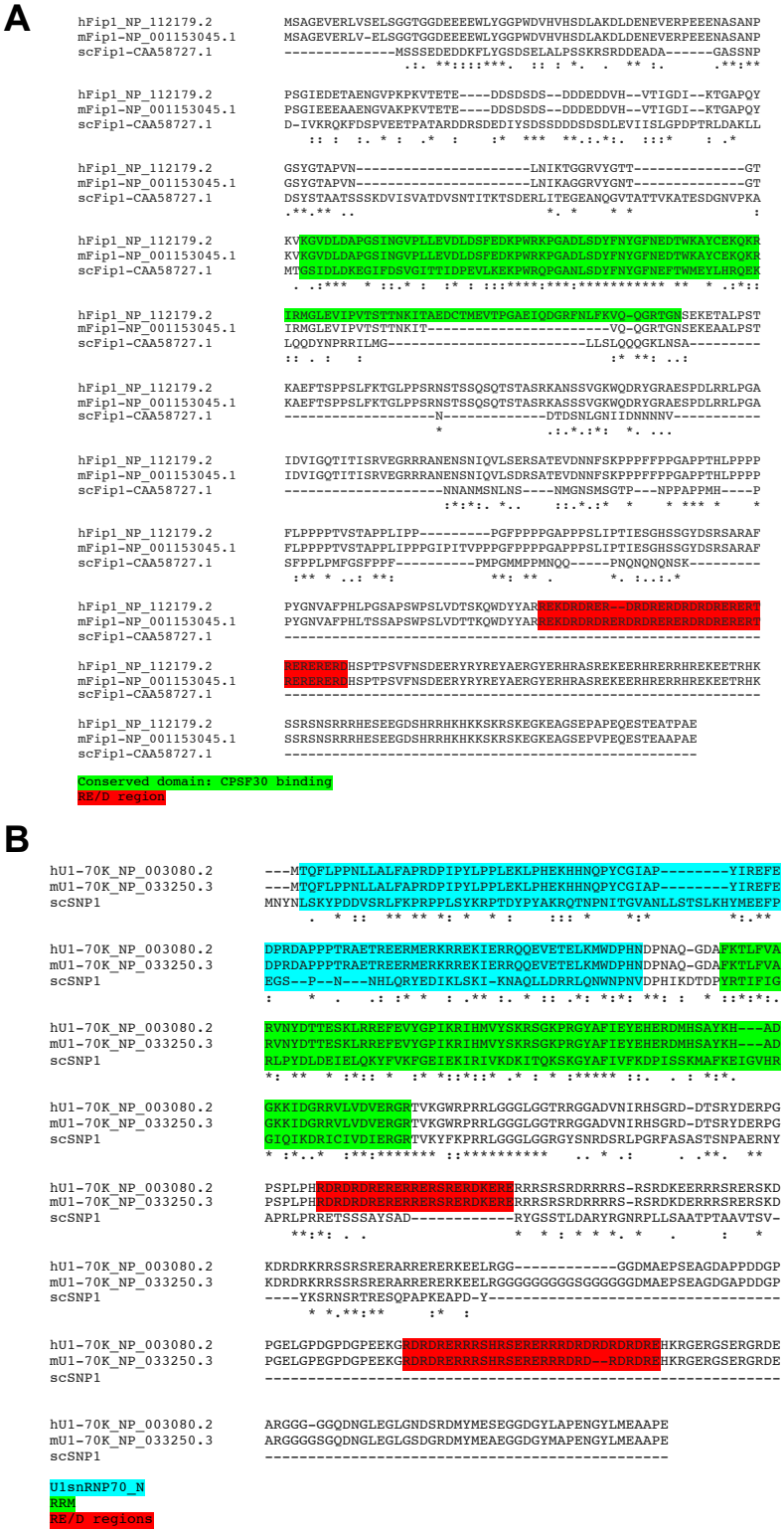


Figure S7. Sequence comparison of Fip1 and U1-70K; related to Figure 7. Sequence alignment was generated for human (h), mouse (m) and budding yeast (sc) using Clustal W. The RE/D region is shaded in red and is missing in yeast.