

Supplemental Figure 2

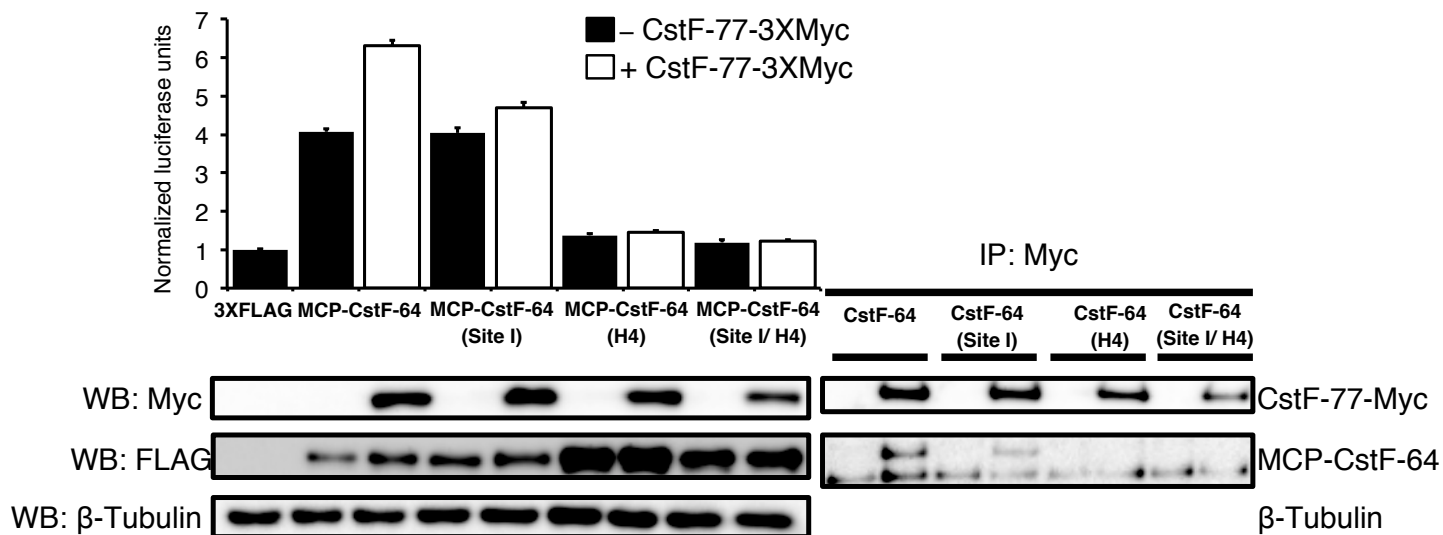
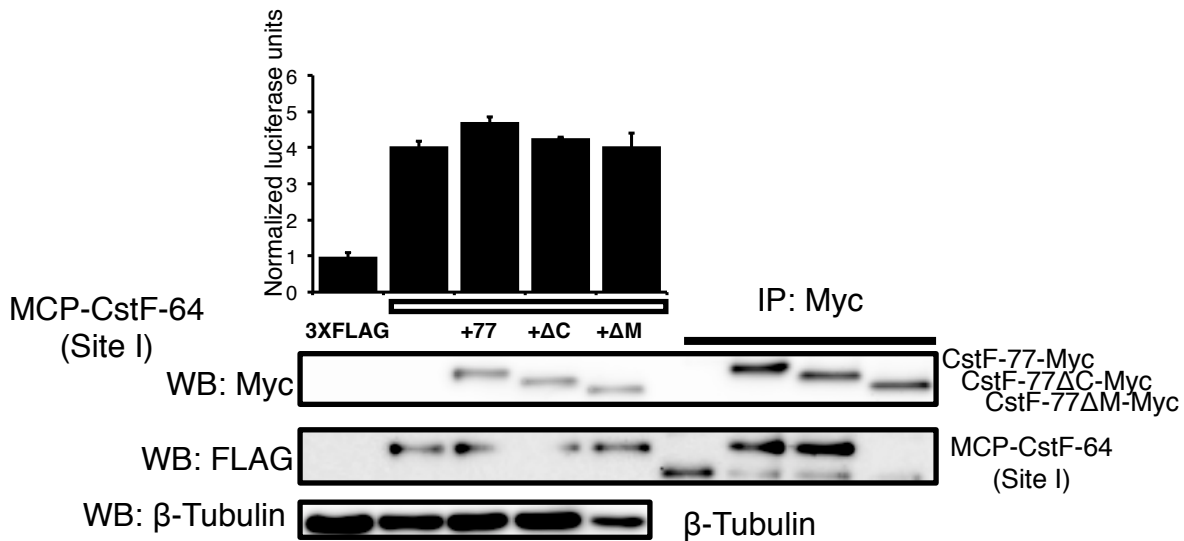


Figure S2. The interaction between the hinge of CstF-64 and the monkeytail of CstF-77 is needed for the stimulatory effect of CstF-77, related to Figure 5. A double mutant containing the H4 and Site I mutations in CstF-64 does not provide a stimulatory effect because of CstF-77. Normalized SLAP of the wild type, Site I, H4 and Site I/H4 double mutants of CstF-64 alone and co-expressed with CstF-77-Myc. Western blots were performed to show expression of MCP-CstF-64 constructs (WB: FLAG) and CstF-77-Myc (WB: Myc). β -tubulin was used as a loading control. Immunoprecipitation with an antibody against Myc tag: vector plasmid, followed by CstF-77-Myc combined with MCP-CstF-64, MCP-CstF-64 (site I), MCP-CstF-64 (H4), and MCP-CstF-64 (Site I/H4) double mutant.

Supplemental Figure 4A



Supplemental Figure 4B

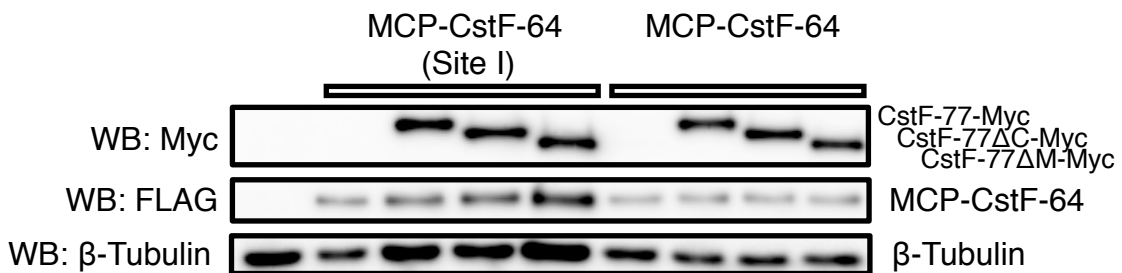


Figure S4. Co-expression of MCP-CstF-64 (Site I) with CstF-77-Myc, CstF-77C-Myc, CstF-77M-Myc completely eliminates the stimulatory effect of CstF-77 in SLAP, related to Figure 6. **(A)** Normalized SLAP of the MCP-CstF-64 (Site I) mutant of the RRM of CstF-64 co-expressed with vector, CstF-77-Myc, CstF-77ΔC-Myc, CstF-77ΔM-Myc. Expression of the MCP-CstF-64 (Site I) is shown by Western blot with FLAG antibody (WB: FLAG). Expression of the CstF-77-Myc, CstF-77ΔC-Myc, CstF-77ΔM-Myc is shown by Western blot with anti-Myc antibody (WB: Myc). -tubulin was used as a loading control. MCP-CstF-64 (site I) mutant interacts with CstF-77ΔC-Myc as shown by co-immunoprecipitation with an antibody against the Myc tag. **(B)** MCP-CstF-64 and MCP-CstF-64 (Site I) are expressed in relatively equal amounts. Expression of MCP-CstF-64 (Site I) and MCP-CstF-64 (WB: FLAG) without or with co-expression of CstF-77-Myc, CstF-77ΔC-Myc, CstF-77ΔM-Myc (WB: Myc) and β-tubulin was used as a loading control.

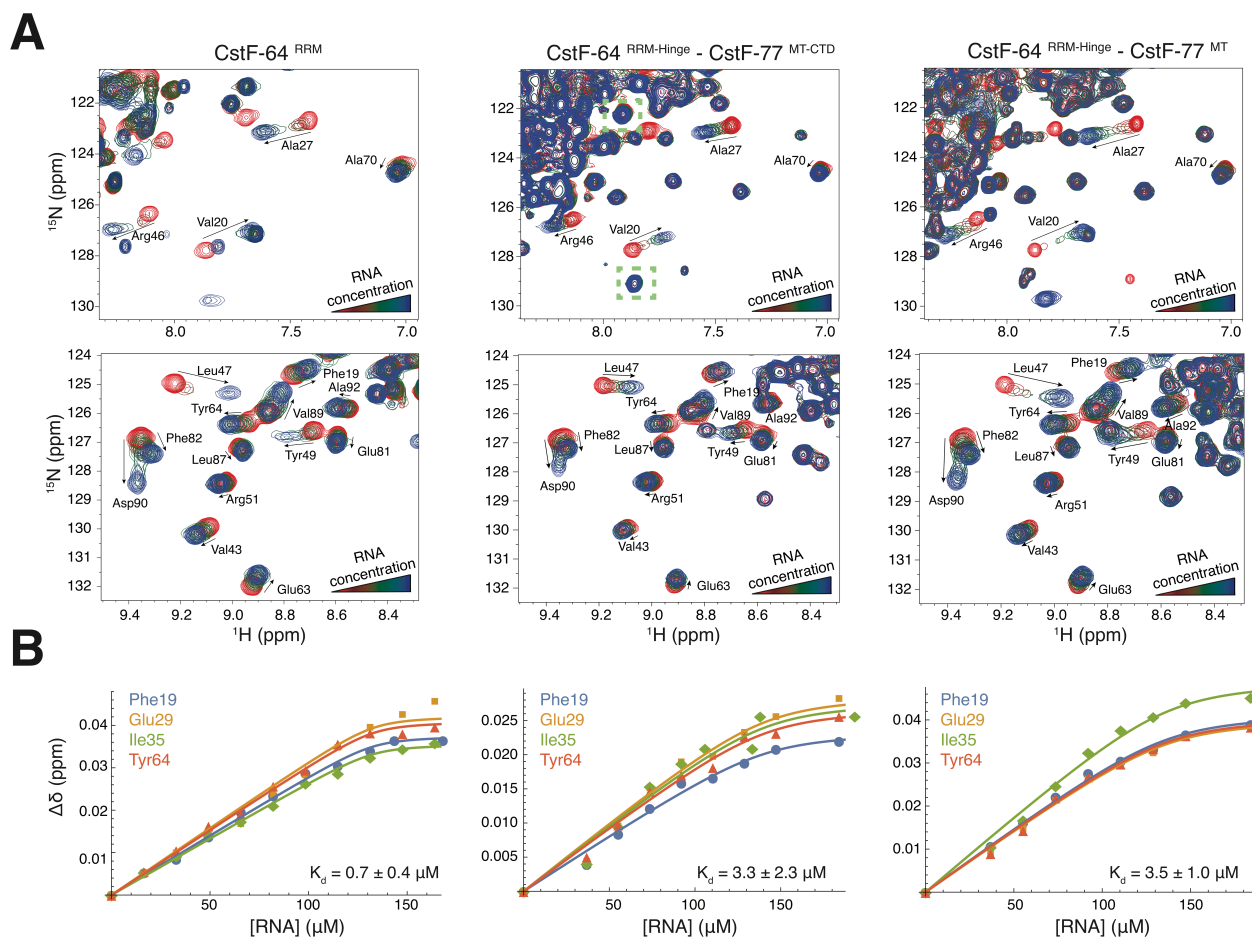


Figure S6. CstF-77^{CTD} affects SVL RNA binding, related to Figure 7. **(A)** Overlay of 2D ^{15}N - ^1H HSQC spectra for titrations of CstF-64^{RRM} (*left*), CstF-64^{RRM-Hinge}-CstF-77^{MT-CTD} (*middle*), and CstF-64^{RRM-Hinge}-CstF-77^{MT} (*right*). The red-green-blue contour gradient represents increasing concentrations of the SVL RNA. Two CstF-77^{CTD} peaks are highlighted by green dashed boxes in the *middle* panel. **(B)** Representative plots of the amide CSPs vs [RNA] used to determine K_d values at 30°C. For each construct, data are shown for Phe19 (blue circles), Glu29 (yellow squares), Ile35 (green diamonds), and Tyr64 (orange triangles).