Supporting Information

Supplementary Figure 1. Sequence alignment of residues 551-600 CstF-77 across representative organisms. The CstF-77 orthologs belong to Human (Homo sapiens), Mouse (Mus musculus), Bird (Taeniopygia guttata), Frog (Xenopus tropicalis), Fish (Danio rerio), Fly (Drosophila melanogaster) and Worm (Caenorhabditis elegans). Residues 551-580 diverge, while 581-600 are conserved (grey shade).

Human 551RAKLAA---IIPDPVV---------APSIVPVLKDEVDRKPEYPKPDTQQMIIFQPVRHLAPP600
Mouse 551RAKLAA---IIPDPVV---------APSIVPVLKDEVDRKPEYPKPDTQQMIIFQPVRHLAPP600
Bird 552RAKLAA---IIPDPVV---------APSIVPVLKDEVDRKPEYPKPDTQQMIIFQPVRHLAPP601
Frog 552RAKLAA---LIPDPVIL---------APSIALPSLKDVDVRKPEYPKPDTSQMIIFQPVRHLAPP601
Fish 551RAKYAS---LIPEAVV---------APS-TPALKDEADRKPEYPKPDTQQMIIFQPVRHLAPP599
Fly 549YAENVG---IILNKV--------GGGAQSQNTGNTVEDSEOATPLPRDFSQMIIFKPKRPAHP601
Worm 542GESIAGPSFVGSKNVPTHGPQAAASAYMGAGGADVARYCPRPDSIQMIIFKPVRNCTA602
Supplementary Figure 2. Electron density map (fo-fc) of CstF-77 peptide with refined model fitted in. The side chains of D585, Q587, Q588 and Q593 exhibit poor electron density, and make no direct contact with CstF-50.
Supplementary Figure 3. ITC data used to generate the quantitative binding parameters reported in Figure 4A. The protein and RNA used in each titration are listed above the figure. Raw injection heats are shown in the upper panels and the corresponding integrated heat changes are shown in the bottom panels versus the molar ratio of RNA to protein.
E  Protein: CstF  
RNA: GUGUGAUGUGU  

F  Protein: CstF  
RNA: GUGUGAAUGUGU  

Protein: CstF-77ΔN/CstF-64RH  
RNA: GUGUGAUGUGU  

Protein: CstF-77ΔN/CstF-64RH  
RNA: GUGUGAAUGUGU
G  Protein: CstF  
RNA: GUGUGAAAAUGUGU  

H  Protein: CstF  
RNA: GUGUGA10UGUGU  

G  Protein: CstF-77ΔN/CstF-64RH  
RNA: GUGUGAAAAUGUGU  

H  Protein: CstF-77ΔN/CstF-64RH  
RNA: GUGUGA10UGUGU
Protein: CstF
RNA: ACACACACACACAC

Protein: CstF-64RRM
RNA: GUGUGUGUGUGUGU

N=1.69
K_s=2.45±0.18μM
Supplementary Figure 4. A new binding surface identified on the circumference of the WD40 domain of CstF-50. We performed a Dali search to investigate structural similarities between our complex and other WD40-peptide complexes. As expected, the structure of CstF-50 closely resembles WD40 repeat proteins with a root-mean-square deviation (r.m.s.d.) of less than 3Å over the aligned Cα atoms. WDR5 shares the highest similarity with CstF-50 (r.m.s.d. 1.7 Å over 304 residues), but the peptide (histone H3) recognition site on WDR5 is located on the top surface of the propeller (Fig. S6B) and close to the central channel (1), which marks the classic peptide binding site identified in most WD40-peptide complex (2). The circumference of the WD40 domain is another site capable of binding peptides with high affinity, as demonstrated by structures such as BRCA2, which binds in a pocket formed by the tips of blades 4 and 5 of PALB2 (Fig. S6C) (3); MTA1, which sits into an acidic groove formed between the N-terminal α-helix and blade 7 of RbAp48 (Fig. S6D) (4); and clathrin, which recognizes the peptide from β-arrestin 2 through a cleft between blades 1 and 2 (5). Other reported examples of non-canonical peptide binding sites are also distinct from that observed for the CstF-50/CstF-77 complex (Fig. S6E-H) (2). WD40 domain proteins form ideal platforms for subunit recruitment and complex assembly using a diverse set of interacting surfaces and our study thus identifies a new binding surface on the circumference of the β-propeller.
**Supplementary Figure 5. CstF-77 peptide binds to a conserved surface on CstF-50.**
Amino acid conservation of CstF-50 among 7 orthologs (*Homo sapiens, Mus musculus, Taeniopygia guttata, Xenopus tropicalis, Danio rerio, Drosophila melanogaster and Caenorhabditis elegans*) is mapped onto the surface with residues colored from most (magenta) to least (cyan) conserved. The CstF-77 peptide is shown in a cartoon representation and colored in yellow. The electrostatic surface potential of CstF-50 is also shown on the right panel.
Supplementary Figure 6. Sequence alignment of CstF-77. The orange box represents the binding site for CstF-50 that is absent in yeast homologues, while the HAT domain (grey shade) and CstF-64 binding sites (green box) are retained in yeast CstF-77.
References


