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Supplemental Information

Insights into Degron Recognition by APC/C Coactivators from the Structure of an Acm1-Cdh1 Complex

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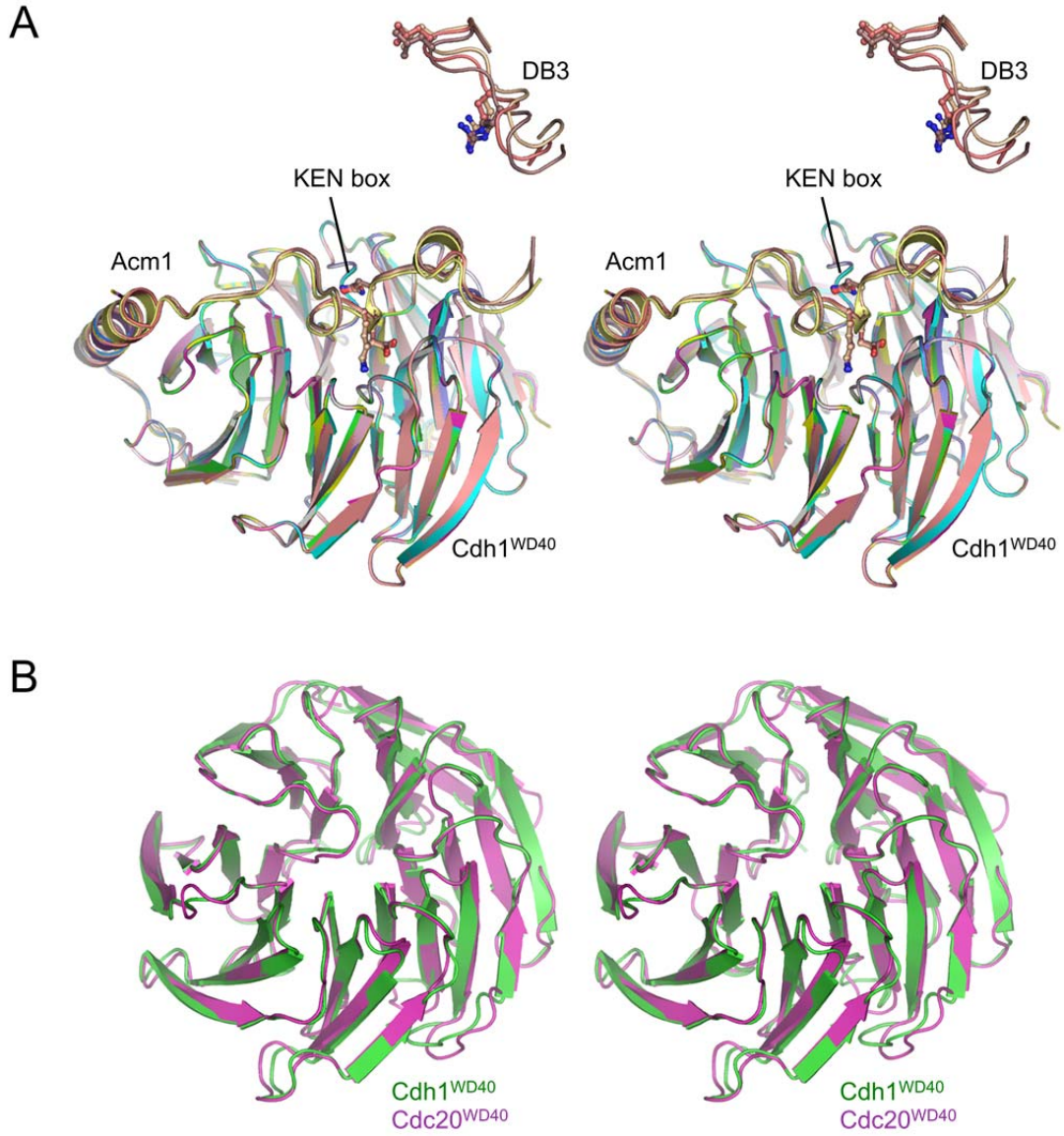

Figure S1. **Comparison of Acm1^{CIR}-Cdh1^{WD40} Complexes and Cdh1^{WD40} and Cdc20^{WD40} Structures, Related to Figure 1**

(**A**) Stereoview showing superimposition of eight Acm1^{CIR}-Cdh1^{WD40} heterodimers comprising the unit cell asymmetric. Acm1^{CIR} chain I and Cdh1^{WD40} chain A were reference structures. KEN box and DB3 (present in three Acm1 chains) are shown. (**B**) Stereoview showing superimposition of Cdh1^{WD40} (chain A) and Cdc20^{WD40} (PDB ID: 4AEZ; Chao *et al.*, (2012)).

1. Superimposition of eight Cdh1 subunits of the asymmetric unit. Superimposition based on Cα-atoms. Superscript refers to the chain identification. For Figure S1A.

Subunit	Residues	Cdh1-A^{B}	Cdh1-A^{C}	Cdh1-A^{D}	Cdh1-B^{E}	Cdh1-B^{F}	Cdh1-B^{G}	Cdh1-B^{H}
Cdh1-A^{A}	245-548	0.12 Å	0.12 Å	0.11 Å	0.35 Å	0.17 Å	0.14 Å	0.09 Å
Cdh1-A^{B}	245-548	-	0.11 Å	0.14 Å	0.32 Å	0.20 Å	0.09 Å	0.06 Å
Cdh1-A^{C}	245-548	-	-	0.17 Å	0.37 Å	0.14 Å	0.14 Å	0.05 Å
Cdh1-A^{D}	244-548	-	-	-	0.32 Å	0.20 Å	0.09 Å	0.05 Å
Cdh1-B^{E}	246-548	-	-	-	-	0.37 Å	0.15 Å	0.05 Å
Cdh1-B^{F}	245-547	-	-	-	-	-	0.11 Å	0.05 Å
Cdh1-B^{G}	245-546	-	-	-	-	-	-	0.05 Å
Cdh1-B^{H}	248-546	-	-	-	-	-	-	-

2. Superimposition of eight Acm1 subunits of the asymmetric unit. Superimposition based on Cα-atoms. Superscript refers to the chain identification. For Figure S1A.

Subunit	Residues	Acm1-A^{J}	Acm1-A^{K}	Acm1-A^{L}	Acm1-B^{M}	Acm1-B^{N}	Acm1-B^{O}	Acm1-B^{P}
Acm1-A^{I}	60-125	0.94 Å	0.94 Å	0.06 Å	0.92 Å	0.63 Å	0.60 Å	0.86 Å
Acm1-A^{J}	60-109	-	0.51 Å	0.08 Å	0.91 Å	0.63 Å	0.60 Å	0.86 Å
Acm1-A^{K}	60-125	-	-	0.07 Å	0.92 Å	0.63 Å	0.60 Å	0.87 Å
Acm1-A^{L}	60-125	-	-	-	0.91 Å	0.62 Å	0.59 Å	0.86 Å
Acm1-B^{M}	60-80	-	-	-	-	0.12 Å	0.15 Å	0.21 Å
Acm1-B^{N}	60-79	-	-	-	-	-	0.24 Å	0.21 Å
Acm1-B^{O}	60-79	-	-	-	-	-	-	0.22 Å
Acm1-B^{P}	60-80	-	-	-	-	-	-	-

3. Summary of superimposition of Cdh1 and Acm1 subunits of the asymmetric unit. Superimposition based on Cα-atoms.

Subunits superimposed	Subunits (N)	Mean RMSD (Å)	RMSD range (Å)
Cdh1-A to Cdh1-A	6	0.13	0.11 to 0.17
Cdh1-B to Cdh1-B	6	0.13	0.05 to 0.37
Cdh1-A to Cdh1-B	16	0.16	0.05 to 0.35
All Cdh1 to Cdh1	28	0.15	0.05 to 0.37
Acm1-A to Acm1-A	6	0.43	0.07 to 0.94
Acm1-B to Acm1-B	6	0.11	0.12 to 0.24
Acm1-A to Acm1-B	16	0.32	0.59 to 0.92
All Acm1 to Acm1	28	0.30	0.07 to 0.94

4. Superimposition of *S. cerevisiae* Cdh1^{WD40}-A^{A} to *S. pombe* Cdc20^{WD40} (PDB: 4AEZ, Chao et al., (2012). RMSD = 0.9 Å for 314 equivalent Cα-atoms. For Figure S1B.

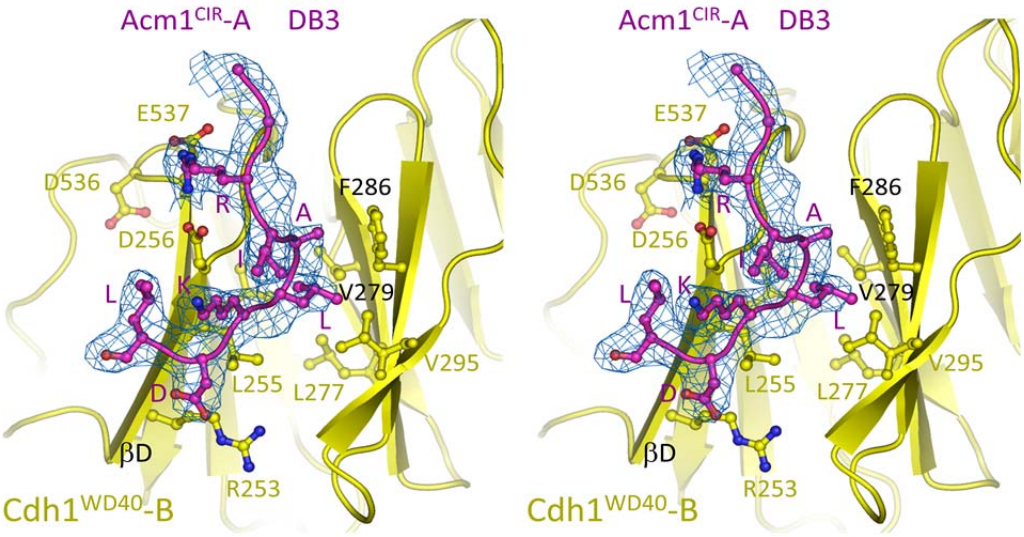

Figure S2. **Structure of the D Box Receptor, Related to Figure 4**

Stereoview showing electron density of DB3 of *S. cerevisiae* Acm1-A (residues 119-127) bound to the WD40 domain of Cdh1-B. Shown are: **R**I**A**L**L**KDL of Acm1^{CIR}-A DB3. Map shown is 2Fo-Fc map contoured at 1σ.

A

D box

UniProt	D-box sequence	Protein name, species and ref
P07818	QKPAQRAALGNISNVVRTA	(*A.p.* G2/mitotic cyclin B) [1,2]
P10815	NVPKKRHALDDVSNFHNKE	(*S.p.* Cdc13) [3]
P20248	QQPRTRAALAVLKSGNPRG	(*H.s.* Cyclin A2) [4]
P14635	PGLRPRTALGDIGNKVSEQ	(*H.s.* Cyclin B1) [5]
P24869	LRNVQRLALNNVTNTTFQK	(*S.c.* Clb2) [6]
P30283	QDSKPRRALTDVPVNNNPL	(*S.c.* Clb5) [7]
P13350	PGLRPRTALGDIGNKAEVK	(*X.l.* G2/mitotic cyclin-B1) [1,2]
P18606	PNLPQRTVLGVIGDNEQRR	(*X.l.* G2/mitotic cyclin-A1) [1,2,8,9]
P36630	INLPRRTVLSDVSNVGKNN	(*S.p.* Cig2 (cyclin) [10]
P21135	GAGSKRAPLGSTKQSNAPS	(*S.p.* Cut2) [11]
P21135	SVTVPRTVLGGKSTNISKF	(*S.p.* Cut2) [11]
P34244	EQKPKRAALSDITNSFNKM	(*S.c.* Hsl1) [12]
P40316	AQQQGRLPLAAKDNNRSKS	(*S.c.* Pds1) [13]
O95997	LPKATRKALGTVNRATEKS	(*H.s.* PTTG1) [14]
O00429	VTCLLRKRLPVTNEMVHNL	(*H.s.* Dynamin) [15]
O75496	NSSVPRRTLKMIQPSASGS	(*H.s.* Geminin) [16]
P03116	SDSNARAFLATNSQAKHVK	(*B. papillomavirus* E1) [17]
P12757	VGGEKRLCLPQVLNSVLRE	(*H.s.* SnoN) [18]
P15036	VANSYRGTLKRQPAFDTFD	(*H.s.* ETS family) [19]
P24699	ATMRERRRLKKVNQAFETL	(*M.m.* Myf5) [17]
P32325	KRSLERLELQQQQHLHEKK	(*S.c.* Dbf4) [20]
P33981	AEIGYRNSLRQTNKTKQSC	(*H.s.* Mps1) [21]
P38936	GPRRGRDELGGGRRPGTSP	(*H.s.* CDKN1A) [22]
P39001	HNKTSRATLMNSSQDGKKH	(*S.c.* Ume6) [23]
P41005	RRPLQRRPLQELSIELVKP	(*S.p.* Mes1) [24]
P41134	RGLPVRAPLSTLNGEISAL	(*H.s.* ID1) [25]
P42261	EHAAFRFALSQLTEPPKLL	(*H.s.* GluR1) [26]
P42261	FVLQLRPELQDALISIIDH	(*H.s.* GluR1) [26]
P47074	RPKISRKALVSKSLTPSNQ	(*S.c.* Mad3) [27]
P51955	SLLKERKFLSLASNPELLN	(*H.s.* Nek2A) [28]
P53350	LDPSNRKPLTVLNKGLENP	(*H.s.* Plk1) [29]
P32562	KQLNTRSKLVHTPIKGNTA	(*S.c.* Cdc5) [30]
P32562	AQKKKREKLSALCKTPPSL	(*S.c.* Cdc5) [30]
P53718	SIMKQRLPLGEFSSSKINK	(*S.c.* NRM1) [31]
P54792	LEIRDRMWLKITIANAVIG	(*H.s.* DVL-1) [32]
Q02363	QNQASRTPLTTLNTDISIL	(*H.s.* ID2) [25]
Q04116	FNAYERKPLGEVDLNSFKN	(*S.c.* YHP1) [31]
Q07820	SGATSRKALETLRRVGDGV	(*H.s.* Mcl-1) [33]
Q08050	MKTSPRRPLILKRRRLPLP	(*H.s.* FoxM1) [34]
Q08050	ILKRRRLPLPVQNAPSETS	(*H.s.* FoxM1) [34]
Q08981	SPSKKRTILSSKNINQKPR	(*S.c.* Acm1-DB1 (Cdc20))‡ [35]
Q08981	LREGGRIALKDLSVDEFKG	(*S.c.* Acm1-DB3) [35,36,37]
Q13309	MHRKHLQEIPDLSSNV	(*H.s.* Skp2) [38,39]
Q15303	IQGDDRMKLPSPNDSKFFQ	(*H.s.* ErbB4) [40]
Q15784	LQLNSRNFLTEQGADGAGR	(*H.s.* NEUROD2) [41]
Q5FBB7	TQQSPHLSLKDITNVSLYP	(*H.s.* Shugosin-like) [42]
Q9CXH7	TQESPRCSLKDVTNILQCP	(*M.m.* Shugosin) [42]
Q91820	HNPNHRLPLKGVLEHPWII	(*X.l.* Aurora A) [43,44]
Q96GD4	HNPSERLPLAQVSAHPWVR	(*H.s.* Aurora B) [45]
Q969U6	AKEGLRHFLDRVLEGRAQP	(*H.s.* FboxW5) [46]
Q96RU7	FADRERKKLVLENLEDSCV	(*H.s.* TRIB3) [47]
Q99741	LPLSPRKRLGDDNLCNTPH	(*H.s.* Cdc6) [48]
Q9BSJ6	FQFQRRLPLRAVNLNLRAG	(*H.s.* Fam64A/RCS1) [49]
Q9D1C1	ALYDVRTILLSIQSLLGEP	(*M.m.* UbcH10) [50]
Q9LGE3	FQFHKRGSLDLISLPADFS	(*O.s.* FPF1-like protein) [51]
Q9NQW6	HAKRARQPLSEASNQQPLS	(*H.s.* ANLN) [52]

Q9NYD6	APFEQRASLNPRAEHLESP	(*H.s.* HOXC10) [53]
Q9NYD6	WFQNRRMKLKKMNRENRIR	(*H.s.* HOXC10) [53]
Q9UKT4	EYVMFRTPLASVQKSAAQT	(*H.s.* Fbxo5 (Emi1)) [54]
Q99618	SKVLGRSPLTILQDDNSPG	(*H.s.* Tome-1) [55]
Q96RL1	SGDTSRHCLPTLADAKGLQ	(*H.s.* RAP80) [56]
Q03898	MSNKSNRRSLRDIGNTIGRN	(*S.c.* Fin1) [57]
Q9NQW6	HAKRARQPLSEASNQQPLS	(*H.s.* Anillin) [52]
P50275	HAVKPRQLFPIPLNKVDTK	(*S.c.* Ase1) [58]
Q9Y2M0	DKKRPRRSLSISKNKKKAS	(H.s. Fan1) [59]

‡D box selective for Cdc20 (Ref. 35)

Text version of **Figure 5A**.

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PRLPLGDVSNN   (most frequent)
KHKAFSEINLS
E TS KTLTR
  RK TSAQI
  AT PQTLE
   F AVKGD
   V   QPA    (least frequent)
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B

KEN box

UniProt	KEN-box sequence	Protein name, species and ref
O59767	DVIEQSKENIEPRKAGHS	(*S.p.* Mad3) [27,60]
O60566	DEWELSKENVQPLRQGRI	(*H.s.* Bub1b) [61]
O43683	VFEDGNKENYGLPQPKNK	(*H.s.* Bub1) [62]
O43683	SVILEDKENVVAKQCTQA	(*H.s.* Bub1) [62]
O76755	MDFDNAKENIQPLASGRN	(*D.m.* BubR1) [27]
P47074	EEIETQKENILPLKEGRS	(*S.c.* MAD3-1) [60]
Q08981	NNPSQVKENLSPAKICPY	(*S.c.* ACM1) [35,36,37]
O95997	MATLIYVDKENGEPGTRVVA	(*H.s.* Securin) [63]
P21135	TMFSYGKENAFPVTPISN	(*S.p.* Cut2) [11]
P40316	MPANEDKENNIVYTGNES	(*S.c.* Pds1) [13]
Q24454	MDQILNKENTGINLPANP	(*D.s.* Pim (securin)) [64]
P41410	TVLSKNKENVPGKLFKKF	(*S.p.* Rhp54) [65]
P49454	SPLSLGKENLAESSKPTA	(*H.s.* CENPF) [66]
Q563C3	ISPKIQKENAFSEQSQIV	(*X.l.* Sororin) [67]
P51955	HFSGESKENIMRSENSES	(*H.s.* Nek2A) [28]
P13864	KYDKEDKENAMKRRRCGV	(*M.m.* DNA methyltransferase) [68]
P27895	MKNYGNKENATKDEMIEN	(*S.c.* Cin8) [69]
P04183	PAGPDNKENCPVPGKPGE	(*H.s.* Thymidine kinase) [70]
P0c2x8	WESSLNKENAEYGHSNS-	(*X.l.* Cdca3) [55]
Q08050	KQEMEYKENCHLEQRQVK	(*H.s.* FoxM1) [34]
Q4KLP7	MDDLTEKENVGASPLKSS	(*X.l.* Esco2) [71]
Q8WWK9	KTKMADKENMKRPAESKN	(*H.s.* CKAP2) [72]
Q99741	PPKQGKKENGPPHSHTLK	(*H.s.* Cdc6) [48]
Q9ULW0	YYKEAEKENLVEQSIPSN	(*H.s.* Tpx2) [73]
O94925	GDSDNGKENQTVHKNLDG	(*H.s.* Glutaminase) [74]
P03116	AECESDKENEEPGAGVEL	(*B. papillomavirus* E1) [17]
P24869	NNLLDDKENQDPSSQQFG	(*S.c.* Clb2) [75]
P34244	SGVSTNKENEGPEYPTKI	(*S.c.* Hsl1) [12]
P41005	MVNTDNKENEPPNMEKAH	(*S.p.* Mes1) [24]
P11157	RLTLADKENTPPTLSSTR	(*M.m.* Ribonucleoside-red M2) [76]
Q14807	AQKAEEKENHCPTMLRPL	(*H.s.* Kif22) [77]
Q96GD4	MAQKENSYPWPYGRQ	(H.s. Aurora B) [78]

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P30304    IDPDENKENEAFEFKKPV   (H.s. Cdc25A) [79]
P30305    SSSGEDKENDGFVFKMPW   (H.s. Cdc25B) [80]
P30307    CSSSANKENDNGNLVDSE   (H.s. Cdc25C) [81]
Q04116    IKSPKGKENRLKFNAYER   (S.c. YHP1) [31]
Q5FBB7    KRMSKYKENKSENKKTVP   (H.s. Shugoshin-like) [42]
Q9BXS6    KPWGQSKENNYLNQHVNR   (H.s. Nusap1) [82]
Q99618    QGQDHDKENQHFPLVES-   (H.s. Tome-1) [55]
Q12834    ASFLLSKENQPENSQTPT   (H.s. Cdc20) [83]
Q15004    SPKDSEKENQIPEEAGSS   (H.s. PAF15) [84]
Q8IWQ3    EGGEAQKENGIYSVTFTL   (H.s. BRSK2) [85]
Q9URT4    DFMCTDKENVAFPLKTTT   (S.p. Ams2)[86]
Q9URT4    PVCDSEKENDDLECYFRT   (S.p. Ams2)[86]
Q9Y2M0    LENSSQKENVFKCDSLKE   (H.s. Fan1) [59]
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Text version of **Figure 5B**.

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SEDKENVPP    (most frequent)
DAN   Q L
ELE   A K
LTS   I F
MGQ     E    (least frequent)
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C

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Figure S3. Sequence Alignment of Experimentally Validated D Box and KEN Box Motifs, Related to Figures 5A and 5B

(**A**) Multiple sequence alignment of D box motifs validated by mutagenesis and used to generate the D box consensus sequence (**Figure 5A**). Conserved Arg (P1) and Leu (P4) residues indicated in red, conserved residues at P(6,7,8,9) are in green. Uniprot ID and primary citation are given.

(B) Multiple sequence alignment of KEN box motifs validated by mutagenesis and used to generate the KEN box consensus sequence (**Figure 5B**). Conserved Lys (P1), Glu (P2) and Asn (P3) residues indicated in red, conserved residues at P-1 and C-terminal prolines (P+2 and P+3) are in green. Uniprot ID and primary citation are given.

(**C**) References for Figure S3.

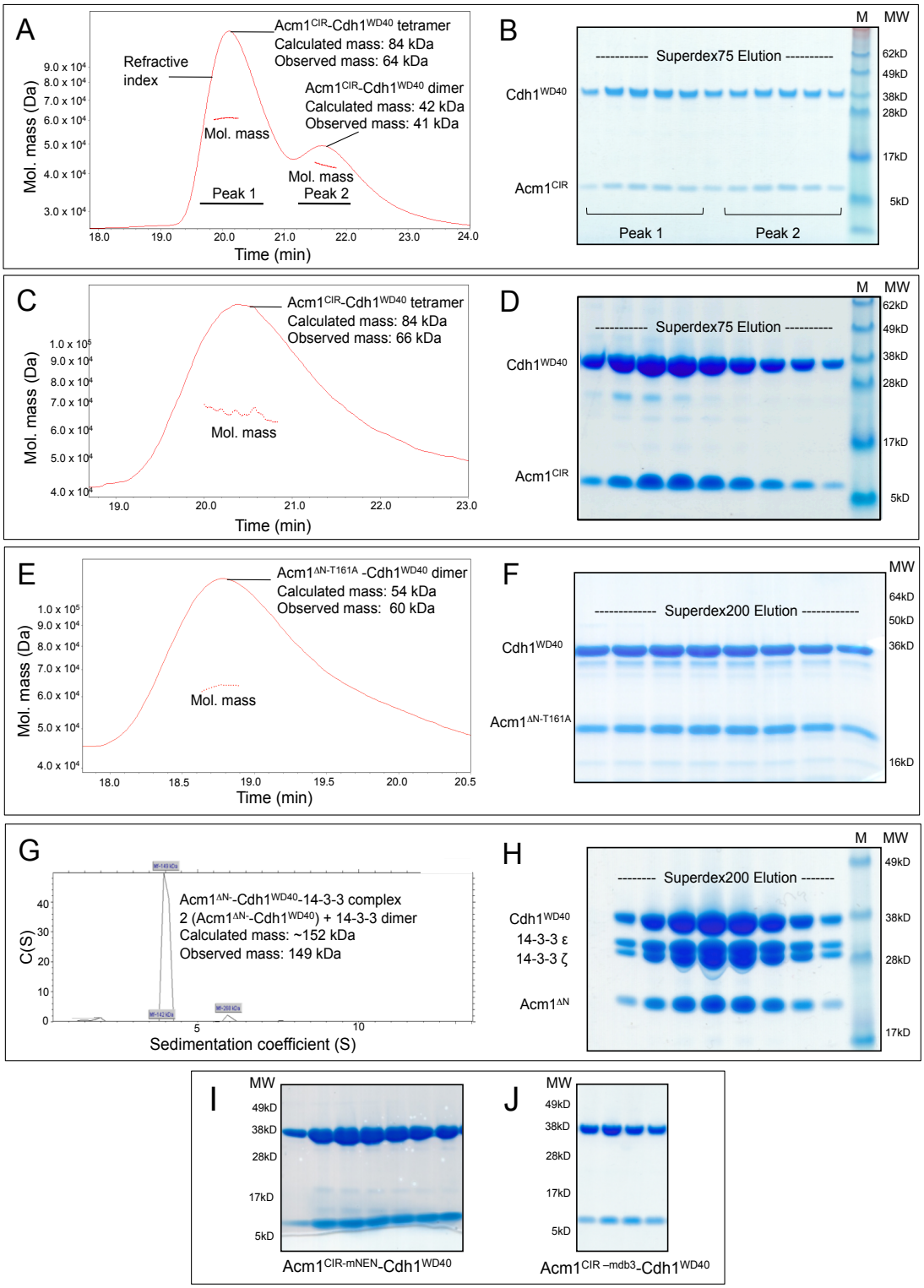

Figure S4. Oligomeric states of Acm1-Cdh1 complexes. Related to Figure 6.

(**A)** MALS analysis shows that Acm1^{CIR}-Cdh1^{WD40} forms both heterotetrameric (Peak 1) and heterodimeric (Peak 2) species at ~1 mg/ml without glycerol. (**B**) SDS PAGE gel of

Acm1^{CIR}-Cdh1^{WD40}. Shown is the eluate from an equivalent Superdex 75 size exclusion column to that used for the MALS experiment. Peak 1 corresponds to the heterotetrameric state of the Acm1^{CIR}-Cdh1^{WD40} complex and Peak 2 corresponds to the heterodimer. Associated size exclusion chromatograph is shown in **Figure 6C.** (**C**) MALS analysis shows that Acm1^{CIR}-Cdh1^{WD40} in 10% (v/v) glycerol exists only as a heterotetrameric species at ~1 mg/ml. (**D**) SDS PAGE gel of Acm1^{CIR}-Cdh1^{WD40} in 10% glycerol. Shown is the eluate from an equivalent Superdex 75 size exclusion column to that used for the MALS experiment. Associated size exclusion chromatograph is shown in **Figure 6C**. **(E**) MALS data of Acm1$^{DN\text{-}T161A}$-Cdh1^{WD40} shows a heterodimer at ~1 mg/ml in 10%(v/v) glycerol. (**F**) SDS PAGE gel showing eluate from an equivalent Superdex 200 size exclsion column to that used for the MALS experiment. (**G**) AUC data showing that the Acm1^{DN}-Cdh1^{WD40}-14-3-3 complex (1.5 mg/ml and 10% (v/v) glycerol) is consistent with two molecules each of Acm1^{DN} and Cdh1^{WD40} and a 14-3-3 dimer of a 1:1 ratio of 14-3-3 ε and 14-3-3 ζ based on *Bombyx mori* sequence similarity. Calculated molecular mass of the insect cell 14-3-3 dimer was estimated based on *Bombyx mori* 14-3-3 ε and 14-3-3 ζ sequences. (**H**) SDS PAGE gel for Acm1^{DN}-Cdh1^{WD40}-14-3-3 complex. The 14-3-3 ε and 14-3-3 ζ subunits were identified by mass spectrometry as being related to *Bombyx mori* 14-3-3 ε and 14-3-3 ζ proteins. (**I**) SDS PAGE gel for Acm1$^{CIR\text{-}mNEN}$-Cdh1^{WD40} complex. (**J**) SDS PAGE gel for Acm1$^{CIR\text{-}mdb3}$-Cdh1^{WD40} complex.