Molecular Cell, Volume *30*

Supplemental Data

Modulation of the Mitotic Regulatory Network

by APC-Dependent Destruction

of the Cdh1 Inhibitor Acm1

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S.cerevisiae    1 --MISPSKKRTILSSKNINQKPRAVVKGNELR--SPSK----RRSQIDTD 42
S.mikatae       1 --MISPSKKRSILSSKNINQKPRAIIKGNELR--SPSK----RRSQIDTD 42
S.paradoxus     1 --MISPSRKRTILYSKNINQKPRAVVKGNELR--SPSK----RRSQIDTD 42
S.bayanus       1 --MISPSKKRTALLGKNINQKSGFIKKGNELH--SPSK----RRSQVDTD 42
S.castelli      1 --MSSPMKRRPALVGKNVNSISRRSIHKNDSTHSSPNR-----NSQL-ID 46
S.kluyveri      1 MSNSSPSKRRTALASKNVN--LRYGINKETKR--SPKKTKKSKRTRIGAL 46
S.kudriavzevii  1 --MISPSKKRTILSSKNVNQKSGAIKKGNELH--SPSKKSPAGRSRIDTE 42
                     **   *  *  ** *               **

 43 YALRRSPIKTIQIS----------KAAQFMLYEETAEERNIAVHRHN-EIYNNNNSVSNENNP 94
 43 YALRRSPIRTVQIP----------KNTQFLVYEETIEERDHIINSHKKEVCNNKIFLCNENNP 95
 43 YALRRSPIKKVQIP----------KATQFMLYEETAEERDRTIHRHNNEVYSCKNSVCNENNP 95
 43 YALRRSPIRAVKLAP--------SNPSQFLVYEETSEERDQIIHRHNENVHNIRRVACDENDP 97
 47 LALRKSPVRQVSLSPQRRTRQTEETSIPFKMFEETSEMRANILEEHQLLLTRS-VQFHDENEY 108
 47 VALQRSPIKKHETTALP-----EITDSSFSFYEETPEQRAAVLMKQMSISR---QIIHDENDY 101
 43 YALRRSPVKAVQIT----------KTPQFLVYEETPEERHEIIYRHSNELCSNRSASCDENNP 95
     **  **                    *   *** * *                    **

 95 SQVKENLSPAKICPYE---RAFLR-EGGRIALKDLSVDEFKGYIQDPLTDETIPLTLPLGDKK 153
 96 SQVKENLSPGKLCPNE---TVRLR-EGRRTALKDLSIDEFKGYVQDPLTDETIPLTLPLGTKK 154
 96 SQVKENLSPAKLHPDK---TALIR-EGKRIALKDLSVEEFKGYIQDPLTDETIPLTLPLGNKK 154
 98 LQVKENLSPTKLQSKD---IIISQREGQRTDLGDLSIDEFKGYIQDPLTEEIIPLTLPLGSKK 157
109 EDVKENVSPTKIKLMNRKGGRMNSKTSIRKPLQDLDIMEYKGYIENPQTNSRMQLTLHTN-GK 170
102 QTKKENVSPSRLKQRS----KLASDGSKRLPLADLRIDEYKGYIEYPGSKFQTPLTLHIN-HK 159
 96 SQVKENLSPTKSISNS---LALLR-EGQRTALKDLSVDEFKGYIQNPLTNETIPLTLPLGSKK 154
       *** **                  *  * **   * ***   *       ***     *

154 ISLPSFITPPRNSKISIFFTSKHQGQNPE---TKISRSTDDVSEKKVVRKLSFHVYEDE- 209
155 ITLPSFVTPPRNSKISGFFTGKHQGQNPK---TTFSRSTDDVHQNKVVRKLSFRICEDE- 210
155 ISLPSFITPPRNSKISVFFTSKHQGQNPE---TLISRSTDDVCENKVVRKLSFRICEDE- 210
158 ISLPSFVTPPRNTKISGFFSIKPQGQNPE---TIMSRSTDDVRPNKVVRKLSFRICEDN- 213
171 VHLPSFVTPPRKHKLKDYFTSNNGDHNTNLVSLHPSKTTDDIDQDKVVRKLEFKICEN-- 228
160 HVIPSFVTPPRNKMLRDFFTTRVPHNKENSSGCSRSRTTDDIPRDKVVRKLNFTIHNDCI 219
155 ISLPSFVTPPRNSKISVFFSTKTQAHNPE---TIKSRSTDDVDPNKVVRKLSFHICEDK- 210
       *** ****       *                *  ***    ****** *
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Figure S1. **Sequence alignment of Acm1 homologs from 7 closely-related *Saccharomyces* species**

Completely conserved residues are marked with an asterisk. Dark gray shading indicates Cdk consensus phosphorylation sites (S/T-P-x-K/R). Light gray shading highlights potential APC recognition motifs, including the D-box (R-x-x-L) and the KEN box. Note that only two of the three RxxL motifs in *S. cerevisiae* Acm1 are conserved in the other species.

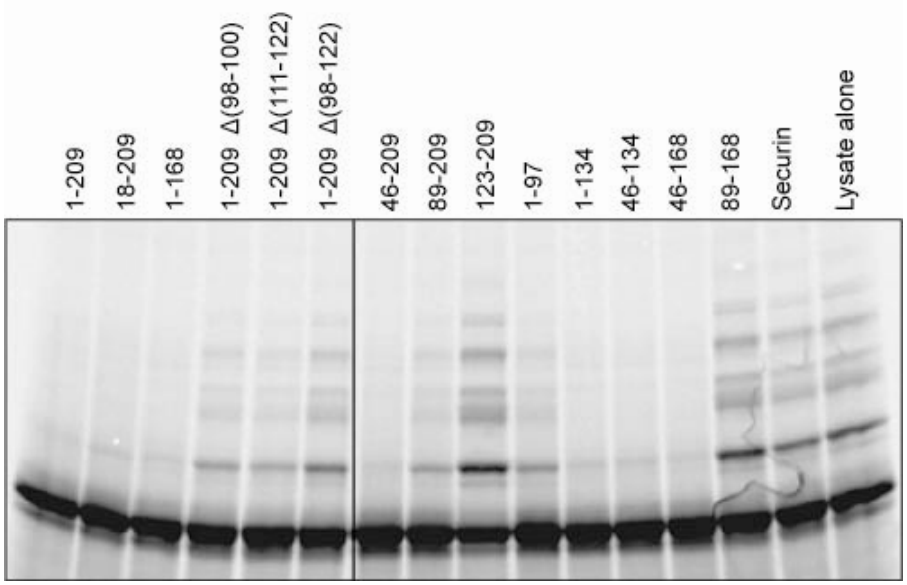

Figure S2. Inhibition of APC^{Cdh1} activity *in vitro* by wild-type and mutant Acm1 proteins

The indicated Acm1 proteins, with residue numbers as shown in Figure 2, were translated unlabeled *in vitro* and added to APC^{Cdh1} reactions, using ^{35}S-methionine-labeled securin as substrate. Translation lysate lacking Acm1 was added as a control (far right). As an additional control, unlabeled securin was translated *in vitro* and added to an APC^{Cdh1} reaction to demonstrate that activity is not significantly affected by addition of excess substrate. These data, as well as data from similar experiments with other Acm1 mutants, are summarized qualitatively in Figure 2 (column A).

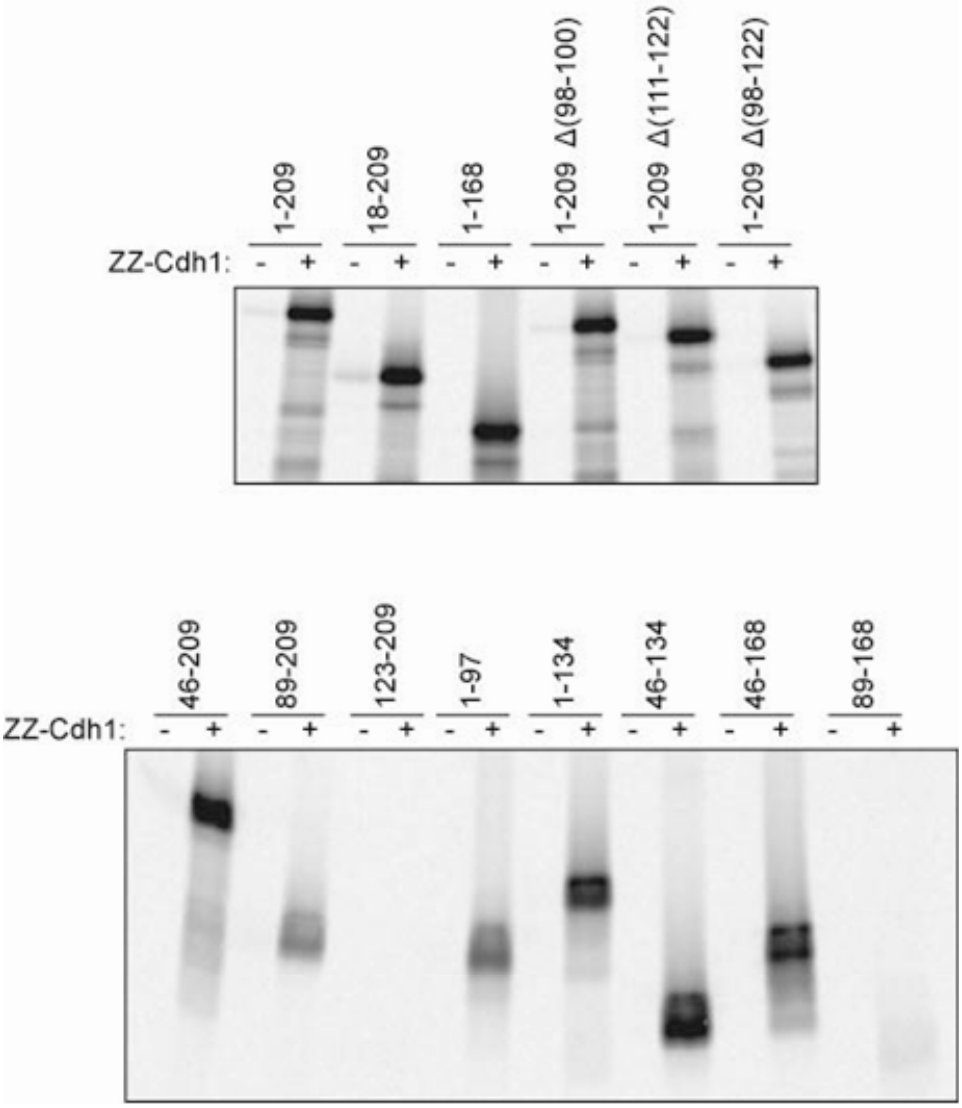

Figure S3. Cdh1 binding *in vitro* by wild-type and mutant Acm1 proteins
The indicated Acm1 proteins, with residue numbers as shown in Figure 2, were translated *in vitro* with ^{35}S-methionine. ZZ-tagged Cdh1 was translated separately in the absence of ^{35}S-methionine. Acm1 proteins were then mixed with IgG beads either with or without ZZ-Cdh1 as indicated. After incubation for 1 h, the beads were washed and the binding of the Acm1 mutants was analyzed by SDS-PAGE. These data, as well as data from similar experiments with other Acm1 mutants, are summarized qualitatively in Figure 2 (column C).

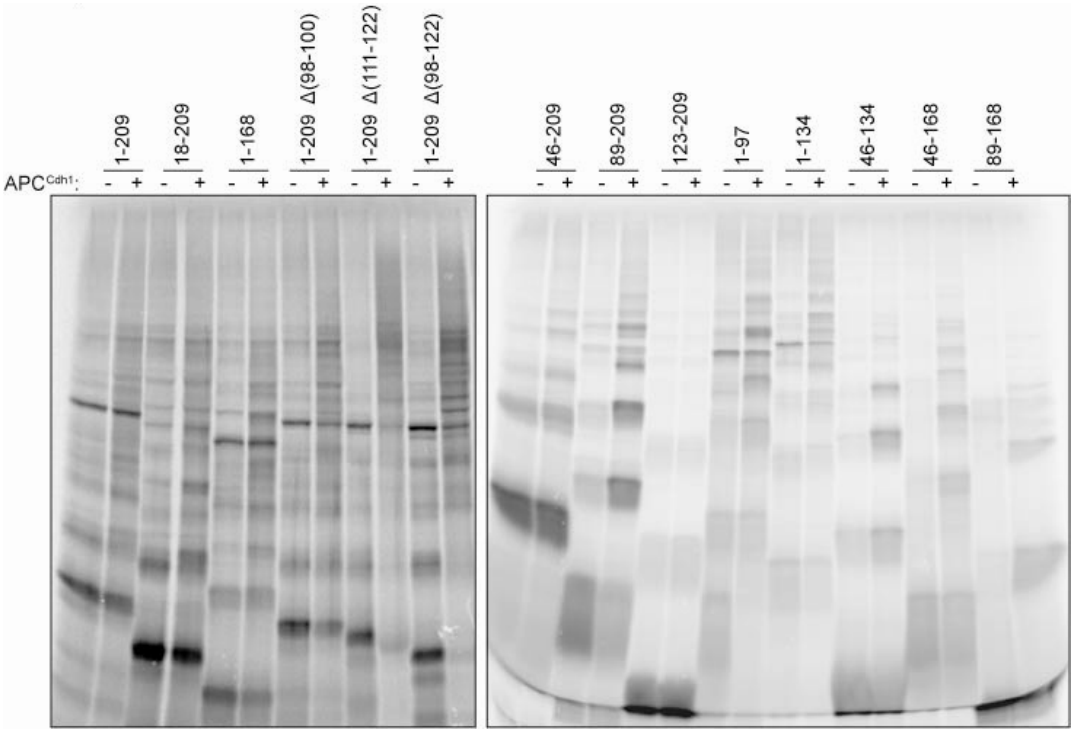

Figure S4. Ubiquitination of wild-type and mutant Acm1 proteins by APC^{Cdh1} *in vitro*.

The indicated Acm1 proteins, with residue numbers as shown in Figure 2, were translated *in vitro* with ^{35}S-methionine and then incubated with or without complete APC^{Cdh1} reactions. Reaction products were analyzed by SDS-PAGE. Note that APC^{Cdh1} activity is assessed not just by the formation of diffuse multi-ubiquitinated species above the substrate but also by the depletion of the unmodified substrate band, which is generally the lowest molecular weight band and varies greatly in size for the various mutants. These data, as well as data from similar experiments with other Acm1 mutants, are summarized qualitatively in Figure 2 (Column D).