Figures and figure supplements

The Cac1 subunit of histone chaperone CAF-1 organizes CAF-1-H3/H4 architecture and tetramerizes histones

Wallace H Liu *et al*

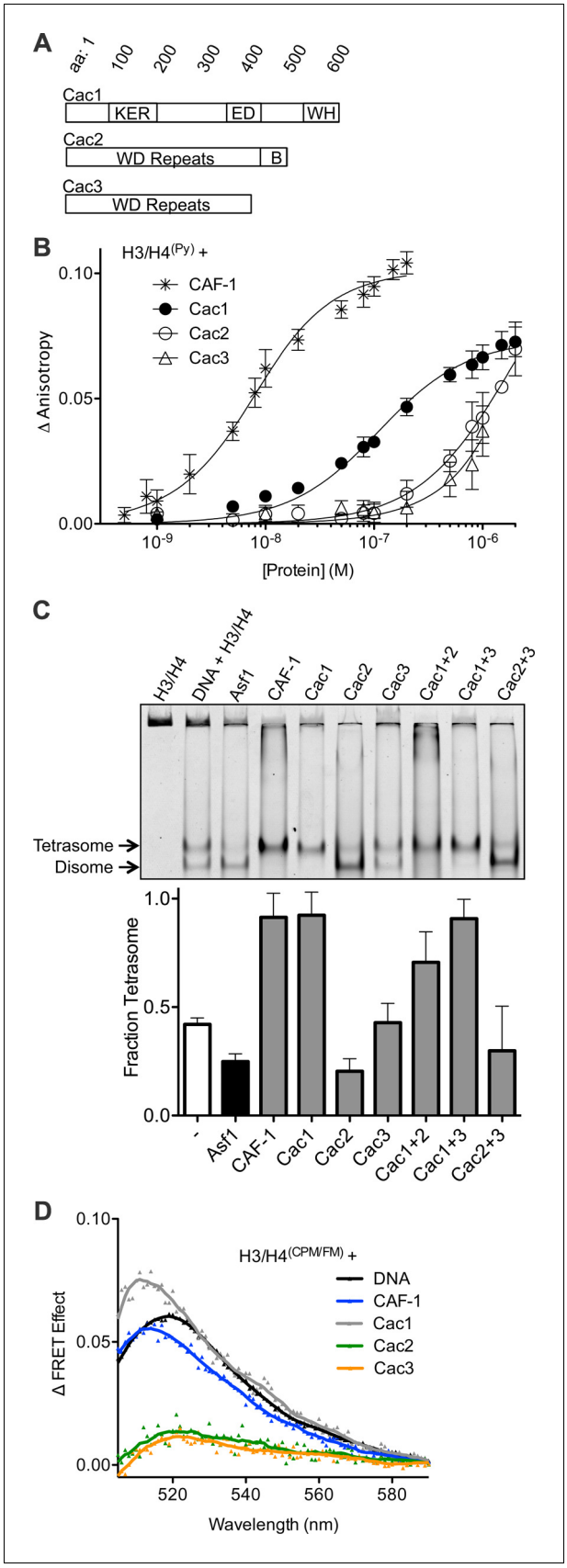
A
aa: 1
100
200
300
400
500
600
Cac1
KER
ED
WH
Cac2
WD Repeats
B
Cac3
WD Repeats
B
H3/H4(Py) +
CAF-1
Cac1
Cac2
Cac3
Δ Anisotropy
0.10
0.05
0.00
10-9
10-8
10-7
10-6
[Protein] (M)
C
H3/H4
DNA + H3/H4
Asf1
CAF-1
Cac1
Cac2
Cac3
Cac1+2
Cac1+3
Cac2+3
Tetrasome
Disome
Fraction Tetrasome
1.0
0.5
0.0
-
Asf1
CAF-1
Cac1
Cac2
Cac3
Cac1+2
Cac1+3
Cac2+3
D
H3/H4(CPM/FM) +
DNA
CAF-1
Cac1
Cac2
Cac3
Δ FRET Effect
0.10
0.05
0.00
520
540
560
580
Wavelength (nm)

Figure 1. The Cac1 subunit is sufficient for $(H3/H4)_2$ tetramerization. (**A**) Schematic of domains in the individual CAF-1 subunits. (**B**) $H3/H4^{(Py)}$ binding to individual CAF-1 subunits. Fluorescence anisotropy of 25 nM pyrene-labeled H3/H4 was monitored with titration of individually purified Cac1, Cac2, or Cac3 in Histone Buffer (H.B.: 20 mM Tris, 150 mM KCl, 2 mM $MgCl_2$, 0.5 mM TCEP, 1% Glycerol, 0.05% BRIJ-35.) The CAF-1 complex was titrated into 5 nM $H3/H4^{(Py)}$. The curves were fitted using ***Equation 3***. (**C**) A representative EMSA separating histone:DNA species as disomes or tetrasomes. 1.6 μM of the indicated histone chaperone or CAF-1 subunit was incubated with 0.2 μM $H3/H4^{(FM)}$ dimer, prior to addition of 0.4 μM 80 bp DNA. The bar graph shows the mean and standard deviation of fraction of tetrasomes formed, calculated by ***Equation 5***, from at least three independent experiments. (**D**) FRET of mixed labeled $H3/H4^{(CPM/FM)}$. Spectra were obtained for 10 nM of labeled histones incubated with 0.2 μM CAF-1 or DNA, or 1 μM CAF-1 subunit. The FRET Effect was calculated using ***Equation 4*** from at least three independent experiments.

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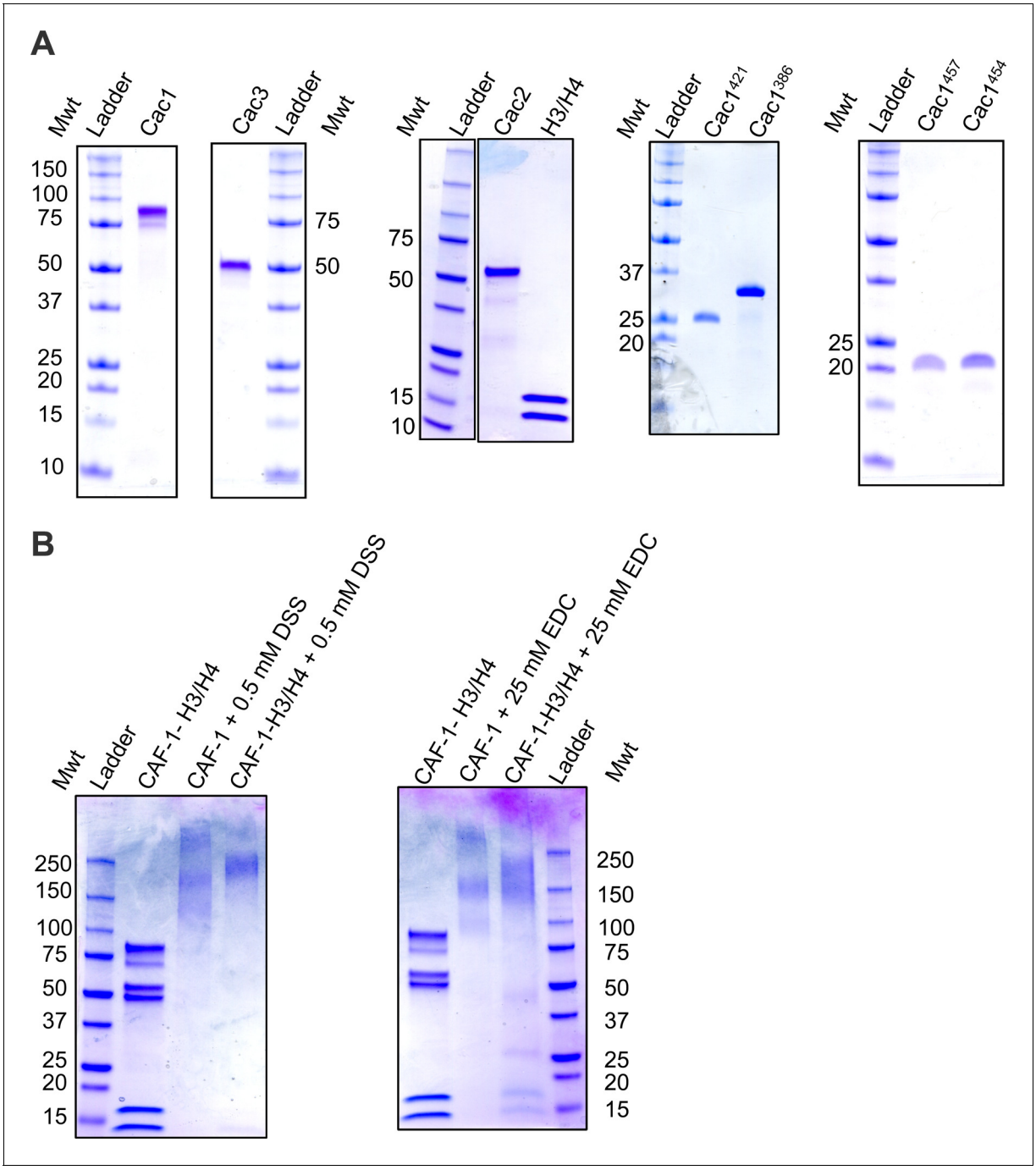

Figure 1—figure supplement 1. Purified proteins used in this study. Coomassie-stained SDS PAGE of the (**A**) individual, purified CAF-1 subunits, Cac1 truncations, and (**B**) CAF-1 and CAF-1-H3/H4 complexes cross-linked by DSS or EDC.
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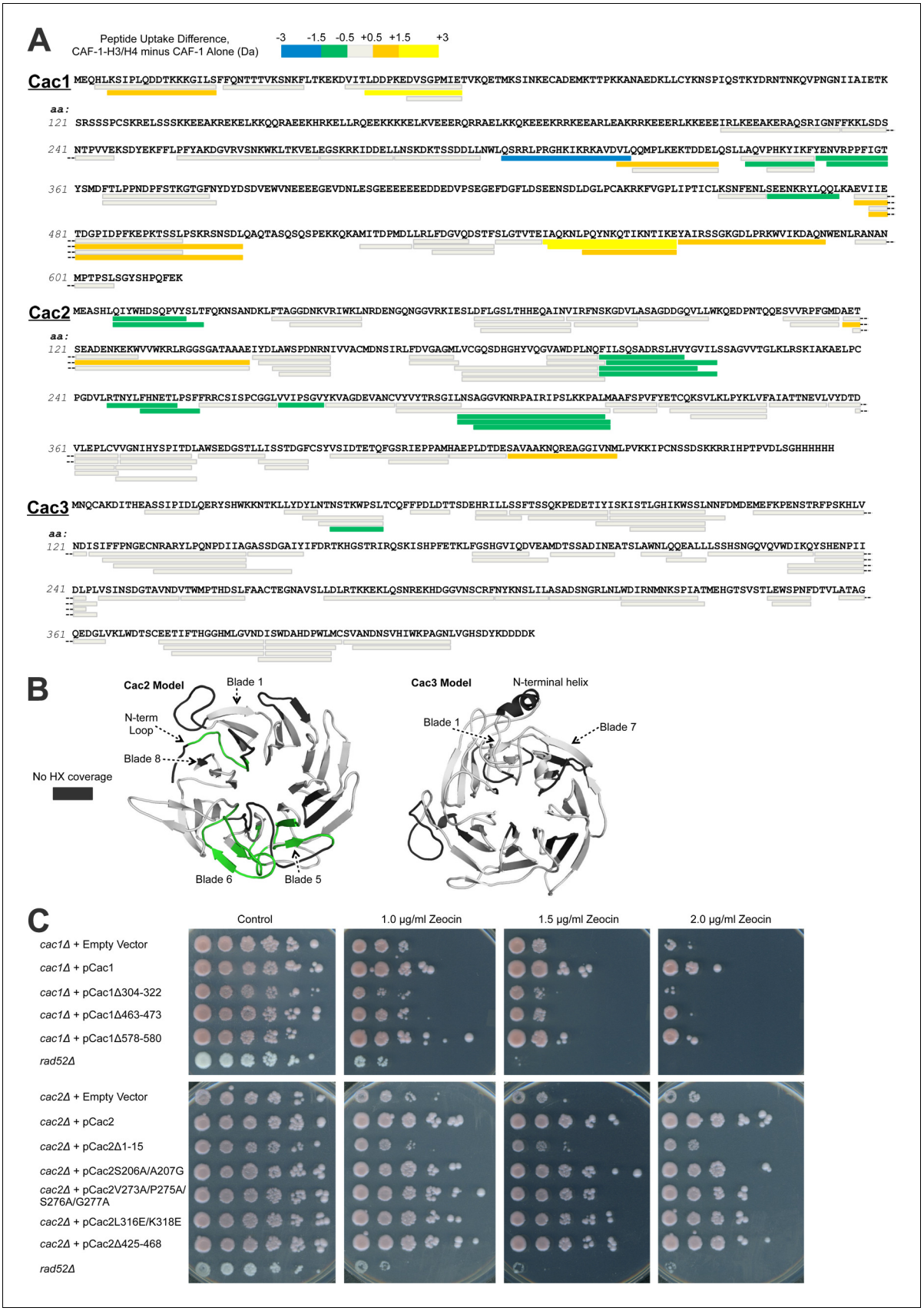
A
Peptide Uptake Difference, CAF-1-H3/H4 minus CAF-1 Alone (Da)
-3 -1.5 -0.5 +0.5 +1.5 +3
Cac1
MEQHLKSIPLQDDTKKKGILSFFQNTTTVKSNKFLTKEKDVITLDDPKEDVSGPMIETVKQETMKSINKECADEMKTTPKKANAEDKLLCYKNSPIQSTKYDRNTNKQVPNGNIIAIETK
aa:
121 SRSSSPCSKRELSSSKKEEAKREKELKKQQRAEEKHRKELLRQEEKKKKELKVEEERQRRAELKKQKEEEKRRKEEARLEAKRRKEEERLKKEEEIRLKEEAKERAQSRIGNFFKKLSDS
241 NTPVVEKSDYEKFFLPFYAKDGVRVSNKWKLTKVELEGSKRKIDDELLNSKDKTSSDDLLNWLQSRRLPRGHKIKRKAVDVLQQMPLKEKTDDELQSLLAQVPHKYIKFYENVRPPFIGT
361 YSMDFTLPPNDPFSTKGTGFNYDYDSDVEWVNEEEEGEVDNLESGEEEEEEEDDEDVPSEGEFDGFLDSEENSDLDGLPCAKRKFVGPLIPTICLKSNFENLSEENKRYLQQLKAEVIIE
481 TDGPIDPFKEPKTSSLPSKRSNSDLQAQTASQSQSPEKKQKAMITDPMDLLRLFDGVQDSTFSLGTVTEIAQKNLPQYNKQTIKNTIKEYAIRSSGKGDLPRKWVIKDAQNWENLRANAN
601 MPTPSLSGYSHPQFEK
Cac2
MEASHLQIYWHDSQPVYSLTFQKNSANDKLFTAGGDNKVRIWKLNRDENGQNGGVRKIESLDFLGSLTHHEQAINVIRFNSKGDVLASAGDDGQVLLWKQEDPNTQQESVVRPFGMDAET
aa:
121 SEADENKEKWVVWKRLRGGSGATAAAEIYDLAWSPDNRNIVVACMDNSIRLFDVGAGMLVCGQSDHGHYVQGVAWDPLNQFILSQSADRSLHVYGVILSSAGVVTGLKLRSKIAKAELPC
241 PGDVLRTNYLFHNETLPSFFRRCSISPCGGLVVIPSGVYKVAGDEVANCVYVYTRSGILNSAGGVKNRPAIRIPSLKKPALMAAFSPVFYETCQKSVLKLPYKLVFAIATTNEVLVYDTD
361 VLEPLCVVGNIHYSPITDLAWSEDGSTLLISSTDGFCSYVSIDTETQFGSRIEPPAMHAEPLDTDESAVAAKNQREAGGIVNMLPVKKIPCNSSDSKKRRIHPTPVDLSGHHHHHH
Cac3
MNQCAKDITHEASSIPIDLQERYSHWKKNTKLLYDYLNTNSTKWPSLTCQFFPDLDTTSDEHRILLSSFTSSQKPEDETIYISKISTLGHIKWSSLNNFDMDEMEFKPENSTRFPSKHLV
aa:
121 NDISIFFPNGECNRARYLPQNPDIIAGASSDGAIYIFDRTKHGSTRIRQSKISHPFETKLFGSHGVIQDVEAMDTSSADINEATSLAWNLQQEALLLSSHSNGQVQVWDIKQYSHENPII
241 DLPLVSINSDGTAVNDVTWMPTHDSLFAACTEGNAVSLLDLRTKKEKLQSNREKHDGGVNSCRFNYKNSLILASADSNGRLNLWDIRNMNKSPIATMEHGTSVSTLEWSPNFDTVLATAG
361 QEDGLVKLWDTSCEETIFTHGGHMLGVNDISWDAHDPWLMCSVANDNSVHIWKPAGNLVGHSDYKDDDDK
B
Cac2 Model
Blade 1
N-term Loop
Blade 8
No HX coverage
Blade 6
Blade 5
Cac3 Model
N-terminal helix
Blade 1
Blade 7
C
Control
1.0 µg/ml Zeocin
1.5 µg/ml Zeocin
2.0 µg/ml Zeocin
cac1Δ + Empty Vector
cac1Δ + pCac1
cac1Δ + pCac1Δ304-322
cac1Δ + pCac1Δ463-473
cac1Δ + pCac1Δ578-580
rad52Δ
cac2Δ + Empty Vector
cac2Δ + pCac2
cac2Δ + pCac2Δ1-15
cac2Δ + pCac2S206A/A207G
cac2Δ + pCac2V273A/P275A/S276A/G277A
cac2Δ + pCac2L316E/K318E
cac2Δ + pCac2Δ425-468
rad52Δ

Figure 2. Hydrogen/deuterium exchange of CAF-1, and CAF-1-H3/H4 complexes. (**A**) The sequences of the three individual CAF-1 subunits are shown. Each bar represents an individual identical peptide observed in the protein between the compared samples, plotted as the difference in deuteron uptake between the CAF-1 and CAF-1-H3/H4 samples (i.e., difference = CAF-1-H3/H4 – CAF-1 only). The differences in deuteron uptake at 60' are colored according to the legend. The 'cooler' colors (green, blue, and purple) represent an increase in apparent protection for the peptide in CAF-1-H3/H4 compared to the CAF-1 sample, whereas the 'warmer' colors (orange, yellow, and red) represent decreased apparent protection. Peptide coverage was approximately 60%, 80%, and 80% for Cac1, Cac2 and Cac3, respectively. (**B**) Differences in HX at 60' were mapped on PHYRE2 models of Cac2 and Cac3. The coloring scheme is the same as for A, but amino acids with no coverage are colored dark gray to distinguish these residues from those that have coverage but did not exchange significantly. (**C**) The top panel shows five fold serial dilution analysis of strain CFY53 (*cac1*) with the vector pCac1 introduced that was either empty, expressed wild type Cac1 or Cac1 with the indicated amino acid changes. The bottom shows five fold serial dilution analysis of strain CFY54 (*cac2*) with the vector pCac2 introduced that was either empty, expressed wild type Cac2 or Cac2 with the indicated amino acid changes.

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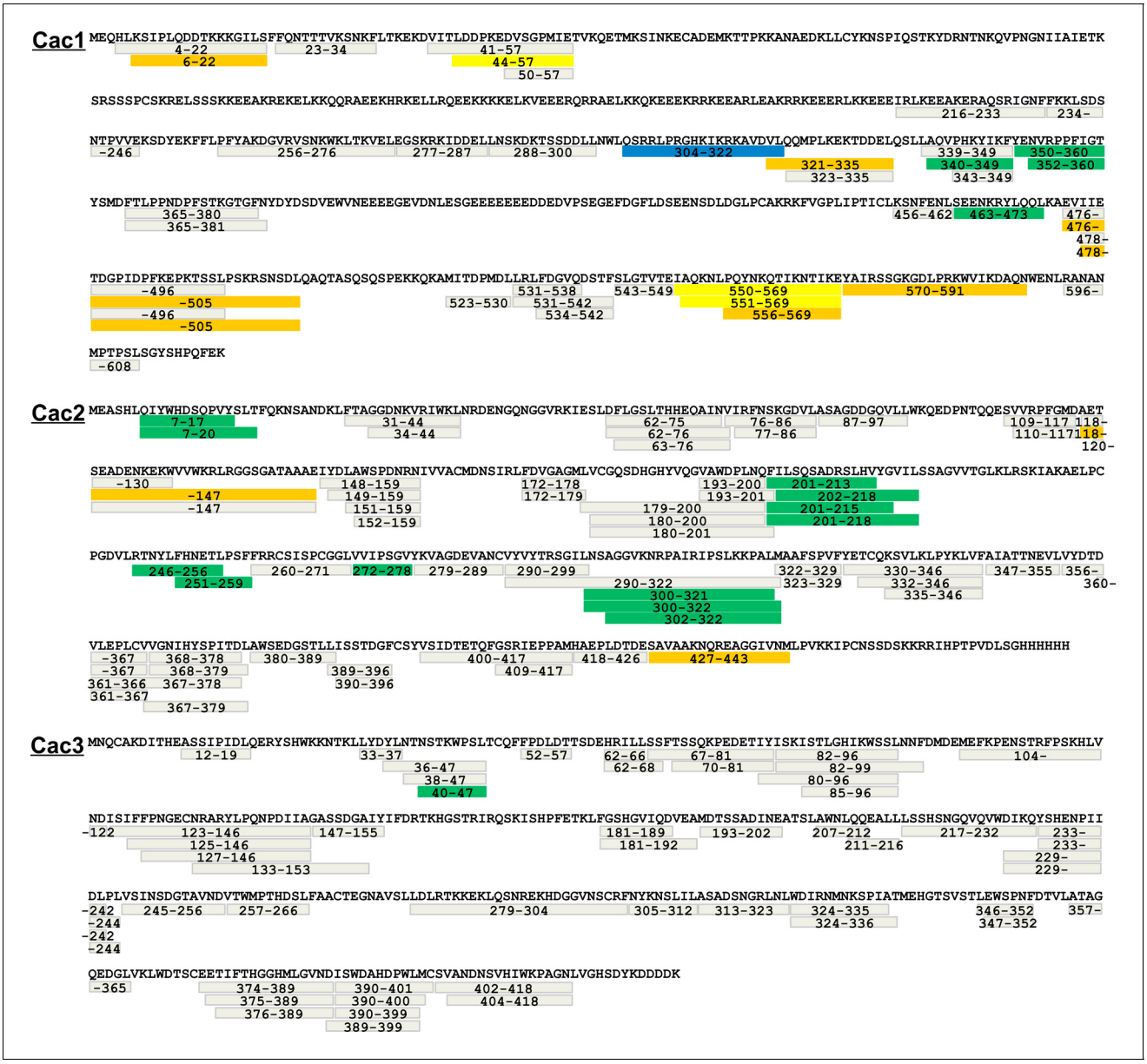

Figure 2—figure supplement 1. Peptide coverage in HX-MS. Coverage maps of Cac1, Cac2, and Cac3, from side-to-side comparisons between CAF-1 and CAF-1-H3/H4 samples in the HX study. All identical peptides between multiple samples were calculated for differences in deuteron uptake at 60' and colored accordingly, using the same scheme as in ***Figure 2B***.

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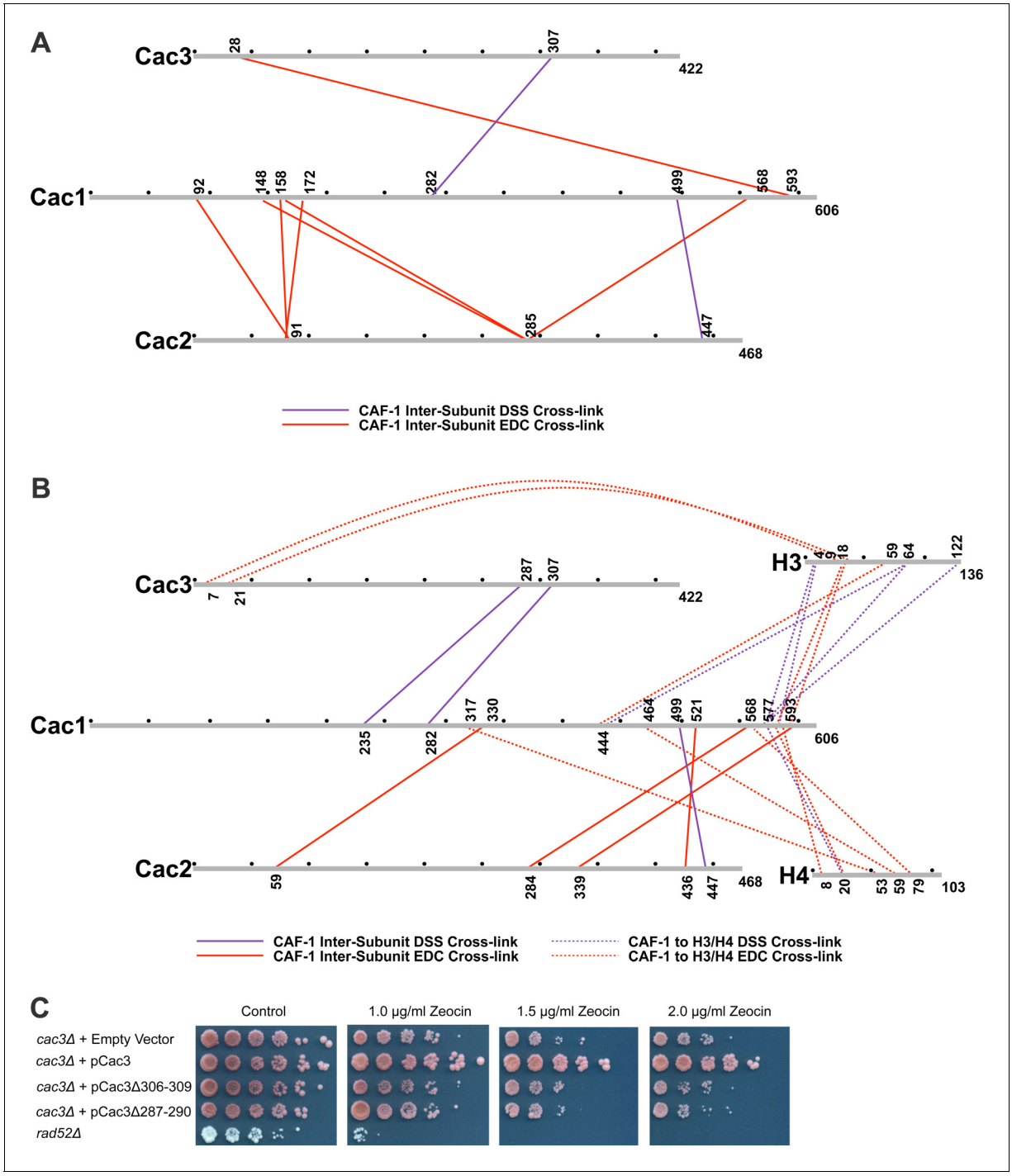

Figure 3. Chemical cross-linking of CAF-1 and CAF-1-H3/H4 complexes. (**A**) CAF-1 or (**B**) CAF-1-H3/H4 complexes were covalently cross-linked with DSS or EDC, then digested and run on an LTQ-Orbitrap. Cross-linked peptides were analyzed using Protein Prospector. The primary sequences are depicted in gray bars, with each gray circle marking 50 amino acid segments. DSS cross-links are shown in purple and EDC cross-links are in red. DSS leaves a 11.4 Å spacer arm between covalently-linked amine groups. EDC treatment results in a zero length cross-link between amine and carboxyl groups. The inter-subunit cross-links are represented as solid lines and cross-links to H3 and H4 are shown as dotted lines. (**C**) Analysis of Cac3 mutants in yeast. Cac3 mutants were subjected to zeocin-induced DNA damage response in vivo. The panel shows five fold serial dilution analysis of strain CFY58 (cac3) with the vector pCac3 introduced that was either empty (EV), expressed wild type Cac1, or Cac1 with the indicated amino acid changes.
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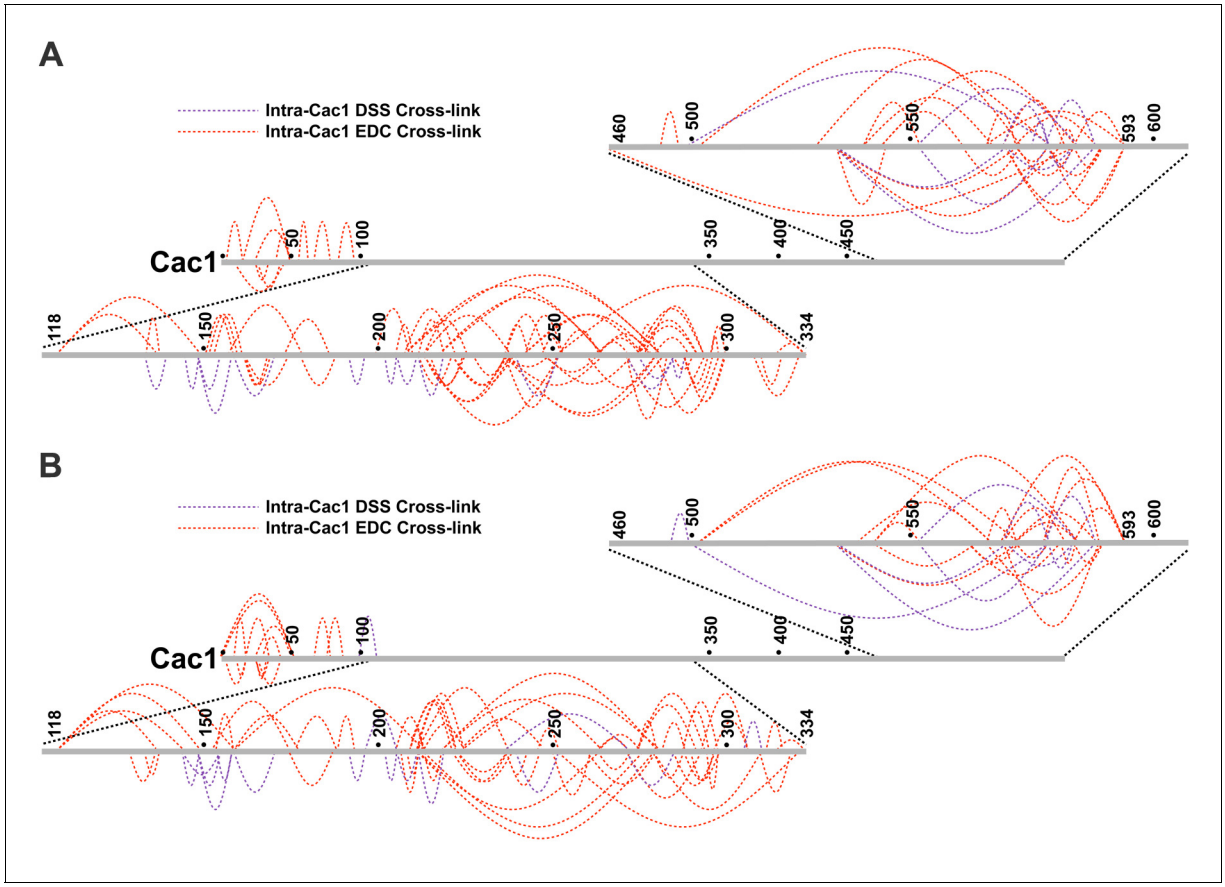

Figure 3—figure supplement 1. Intra-Cac1 cross-links. (**A**) DSS (purple) and EDC (red) cross-links detected within the Cac1 protein in the CAF-1 complex and (**B**) CAF-1-H3/H4 complex.
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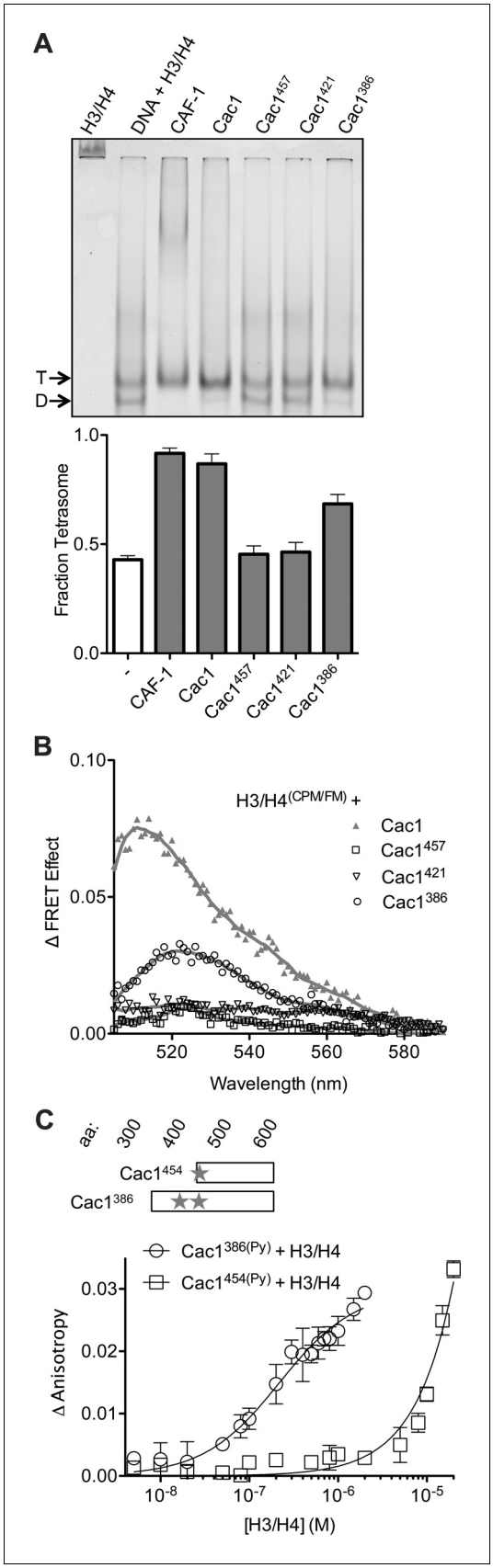
A
H3/H4
DNA + H3/H4
CAF-1
Cac1
Cac1457
Cac1421
Cac1386
T
D
Fraction Tetrasome
1.0
0.5
0.0
-
CAF-1
Cac1
Cac1457
Cac1421
Cac1386
B
H3/H4(CPM/FM) +
Cac1
Cac1457
Cac1421
Cac1386
Δ FRET Effect
0.10
0.05
0.00
520
540
560
580
Wavelength (nm)
C
aa:
300
400
500
600
Cac1454
Cac1386
Cac1386(Py) + H3/H4
Cac1454(Py) + H3/H4
Δ Anisotropy
0.03
0.02
0.01
0.00
10-8
10-7
10-6
10-5
[H3/H4] (M)

Figure 4. The C-terminus of Cac1 binds and tetramerizes H3/H4. (**A**) EMSA evaluating tetrasome formation by Cac1 N-terminal truncations $Cac1^{386}$, $Cac1^{421}$ and $Cac1^{457}$ in H.B. The graph shows the mean and standard deviation from at least three independent experiments. Arrows point to complexes of DNA bound to H3/H4 dimers (D) or tetramers (T), respectively. (**B**) Change in FRET Effect of $H3/H4^{(CPM/FM)}$ induced by 2 μM $Cac1^{386}$, $Cac1^{421}$ or $Cac1^{457}$. The Cac1 spectrum is included from ***Figure 2B*** for reference. (**C**) Fluorescence anisotropy of $Cac1^{386(Py)}$ or $Cac1^{454(Py)}$ titrated with H3/H4 in H.B. The schematic indicates two labeled residues on $Cac1^{386}$ (cysteines 440 and 454), and one on $Cac1^{454}$.

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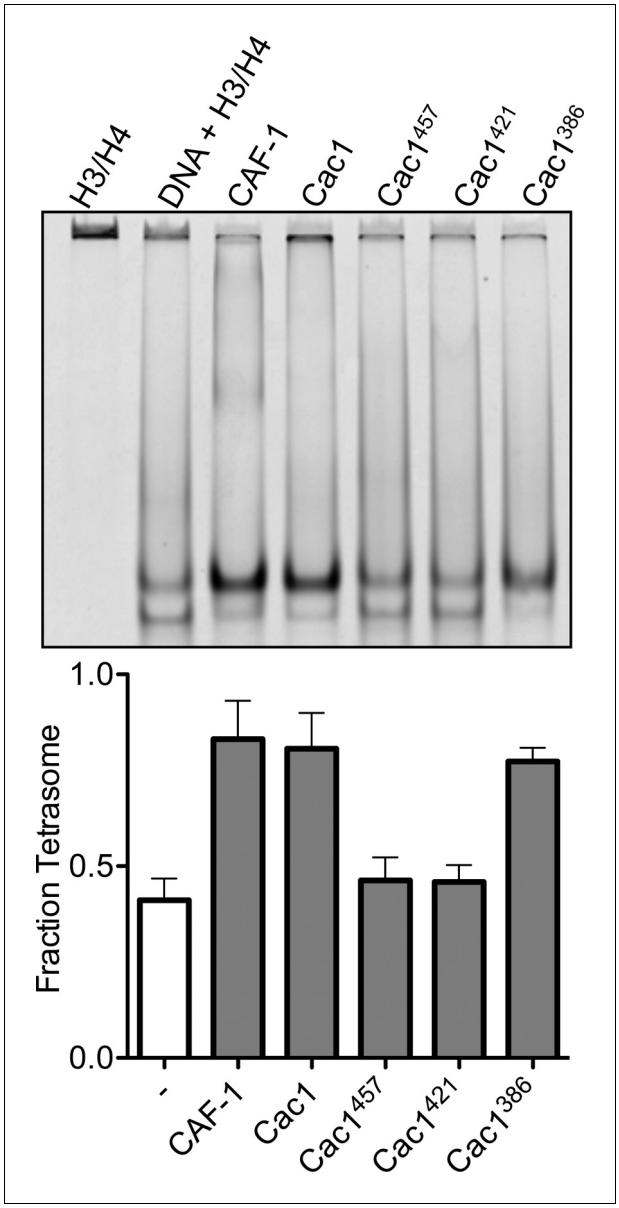

Figure 4—figure supplement 1. Histone deposition assay of Cac1 truncations in Minimal Buffer (M.B.). 1.6 μM of each Cac1 truncation was incubated with 0.2 μM $H3/H4^{FM}$, then allowed to interact with 0.4 μM 80 bp DNA. The EMSA (upper panel) is representative of at least four independent experiments that were used for comparisons in the bar graph (lower panel).

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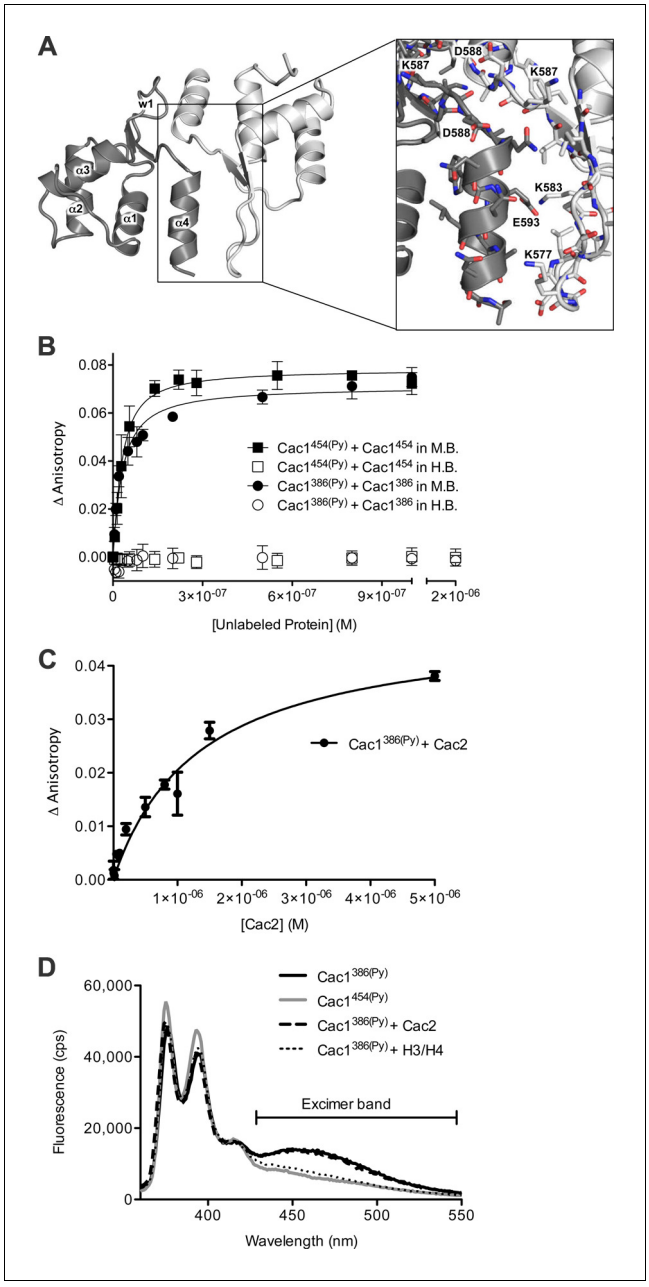

Figure 5. The Cac1 C-terminal winged helix (WH) domain can form a homodimer. (**A**) Crystal structure of amino acids 520–600 at a resolution of 2.9 Å (PDB ID 5JBM), shown as two crystallographically related monomers colored separately (light gray and dark gray). The inset shows major interacting residues buried in half of the homodimer interface, which is arranged in a head-to-tail symmetry with identical interactions on both halves. (**B**) Homo-dimerization of the Cac1 C-terminus quantified by titrating unlabeled $Cac1^{386}$ or $Cac1^{454}$ into 10 nM of labeled $Cac1^{386(Py)}$ or $Cac1^{454(Py)}$, respectively. The pyrene anisotropy of $Cac1^{386(Py)}$ or $Cac1^{454(Py)}$ increases in Minimal Buffer (M.B.: 20 mM HEPES, 150 mM NaCl, 1 mM DTT, pH 7.5), but homo-dimerization does not occur in H.B. (**C**) Binding affinity of the $Cac1^{386(Py)}$-Cac2 interaction. Pyrene fluorescence anisotropy of 10 nM $Cac1^{386(Py)}$ titrated with increasing concentration of Cac2 in Histone Buffer (H.B.). The K_D was determined to be 1.3 μM (***Table 1***). (**D**) Pyrene fluorescence spectra of $Cac1^{386(Py)}$ alone, $Cac1^{454(Py)}$ alone, and $Cac1^{386(Py)}$ bound to 2 μM H3/H4 or 13 μM Cac2. The excimer band that peaks at 465 nm is indicated.

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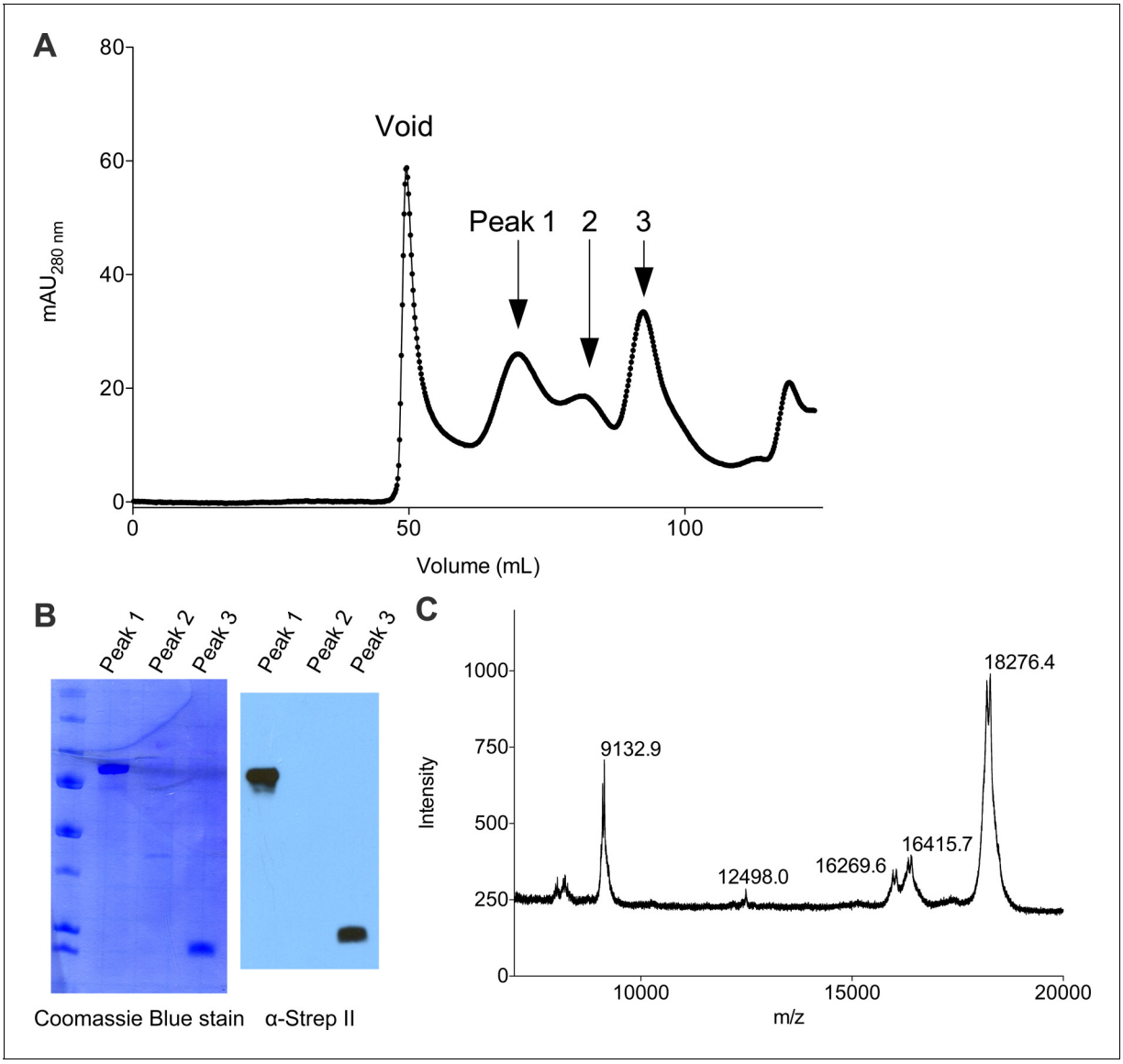

Figure 5—figure supplement 1. Purification of full-length Cac1 and Cac1^{457} from baculovirus-infected Sf9 cells. (**A**) Cac1 elutes from a 120 mL Sephadex 200 column in 3 peaks. (**B**) Western blotting for the Strep II epitope present on the Cac1 C-terminus. Peak 1 is full-length Cac1, whereas Peak 3 is truncated from the N-terminus. (**C**) MALDI identified the C-terminal regions as residues 457–606 (expected mass 18285.3; observed mass 18276.4).
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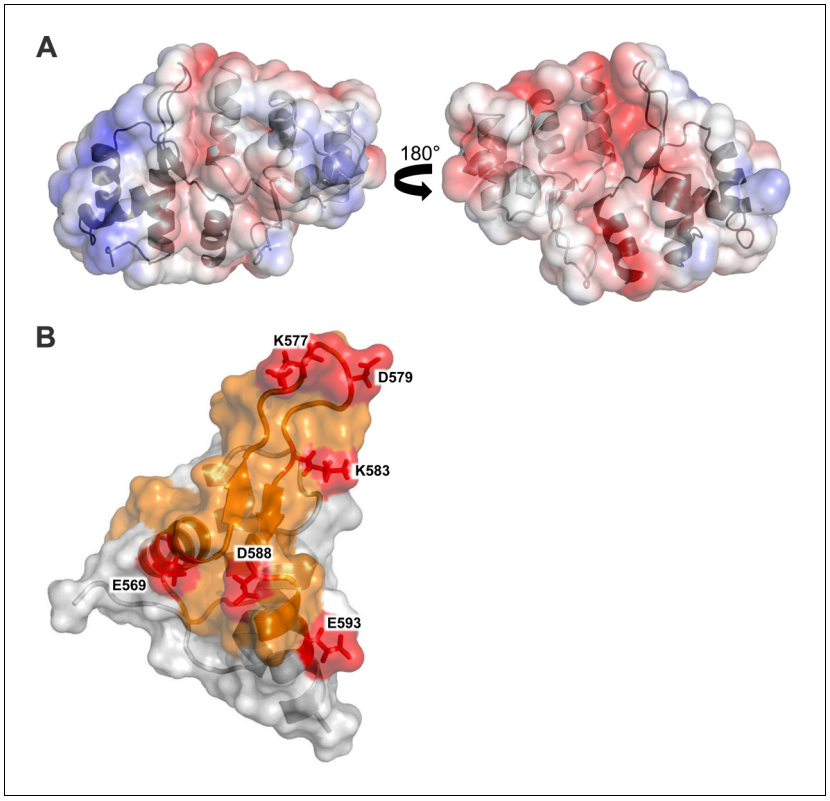

Figure 5—figure supplement 2. Structural analysis of the Cac1^{457} WH domain. (**A**) The Cac1 WH domain monomer and one of the symmetry mates are depicted in both a ribbon and surface representation, in two orientations. The Cac1 monomers are colored light gray and dark gray, respectively, with the electrostatic potential shown mapped onto the surface, colored from red to blue, indicating negatively charged to positively charged regions. (**B**) The Cac1 WH domain monomer is depicted in both a ribbon and surface representation. The putative dimerization interface faces to the right. HX changes are colored in orange to represent an increase in HX with H3/H4 bound to CAF-1 (***Figure 3***). Amino acids that cross-link to H3/H4 are labeled and colored in red.
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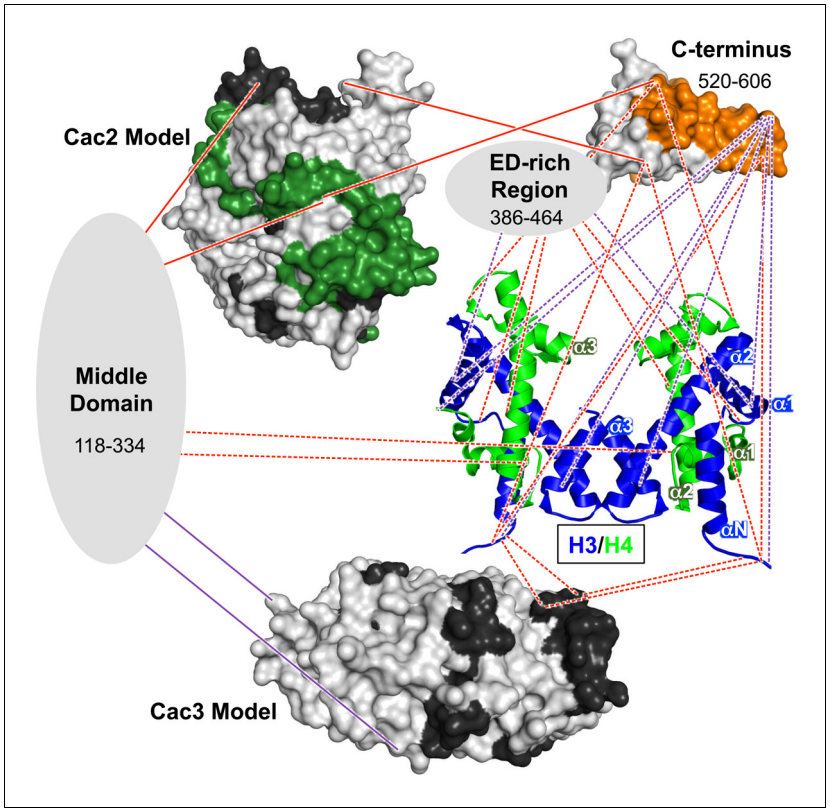

Figure 6. Architectural model of the CAF-1-H3/H4 complex. Cac2 and Cac3 are presented using PHYRE2 models. Cac1 is presented with respect to the domains observed in this study: the 'Middle Domain,' which consists of amino acids 118–334 and includes the KER region; the 'ED-rich Region' that includes the ED domain and the adjacent amino acids; and the 'C-terminus' that includes the WH domain. The nucleosomal $(H3/H4)_2$ tetramer is shown (1ID3.pdb) with H3 (blue) and H4 (bright green) colored to distinguish the histones. CAF-1 proteins are colored according to the 60' HX data and coloring scheme in ***Figure 2B***. The cross-linking data is incorporated using the same coloring and line schemes as in ***Figure 3B***.

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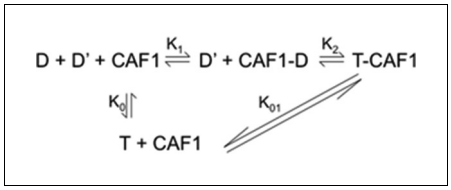

Figure 7. Potential equilibrium for CAF-1 association with H3/H4. D and T indicate dimers or tetramers of H3/H4, respectively.
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