

## Molecular Architecture of Yeast Chromatin Assembly Factor 1

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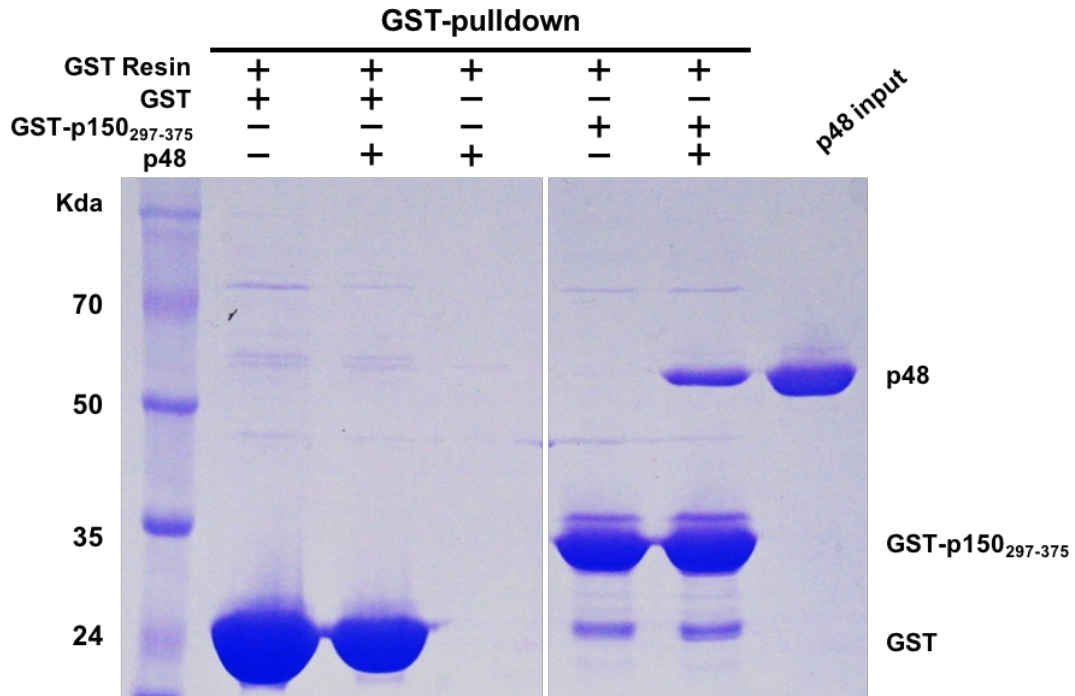
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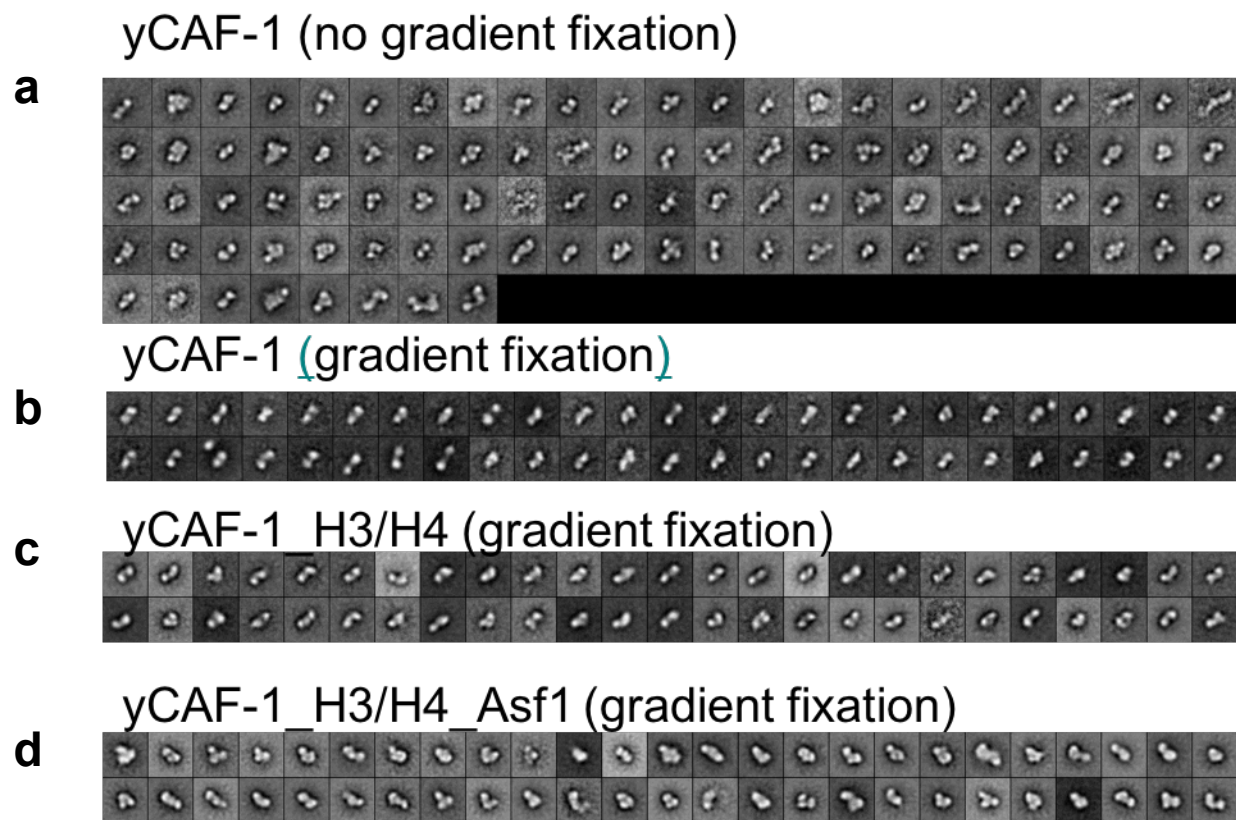
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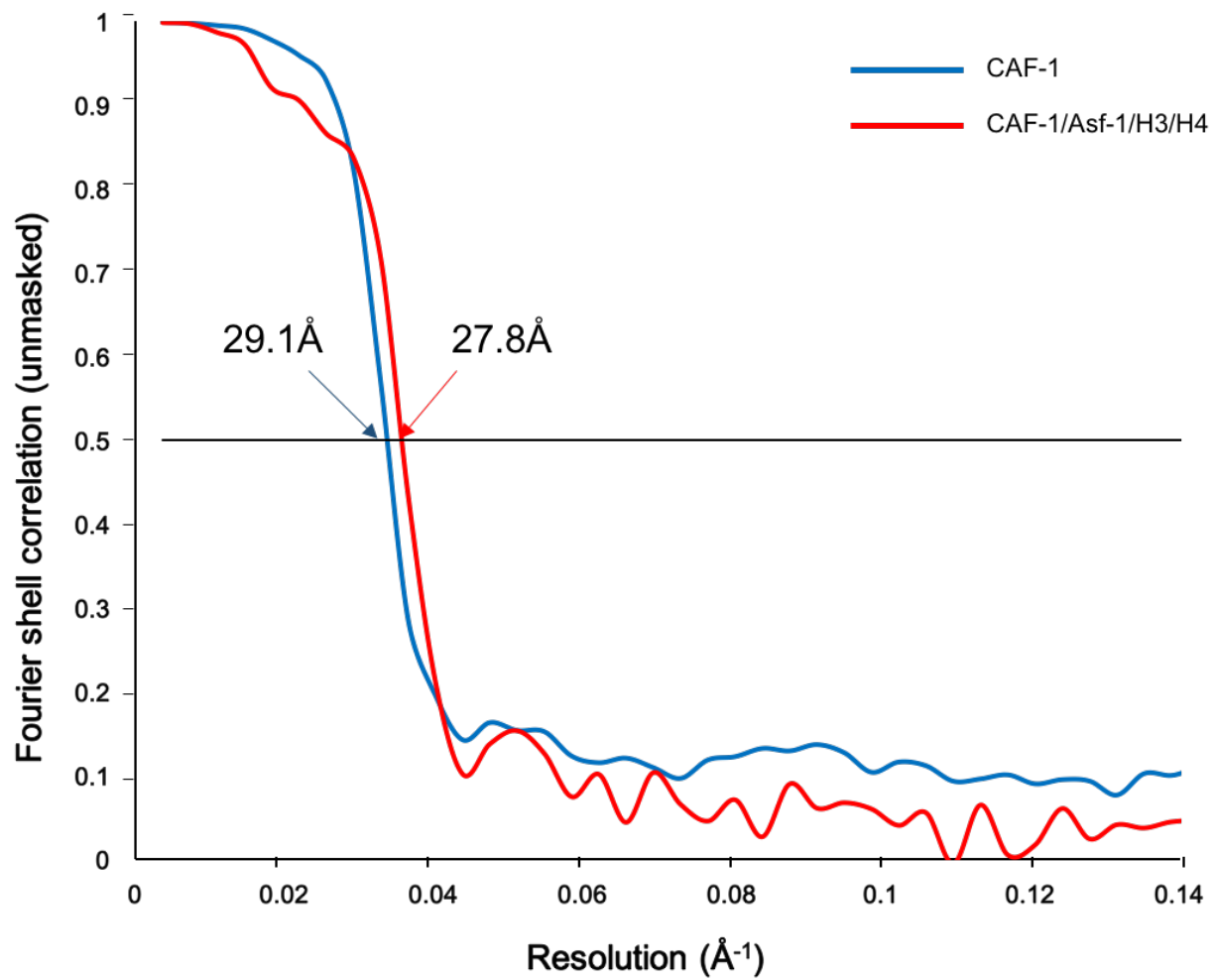
**Supplementary Figure 1. GST-pull down experiments showing that the middle region of the largest subunit interacts with p48 of Caf-1.**

The middle region of the largest subunit of human CAF-1 (p150<sub>297-375</sub>) was generated in GST fusion, and incubated with p48. p48 specifically interact with the middle region of p150 but not GST.



**Supplementary Figure 2. Galleries of yCAF-1 class averages from 2D negative stain EM analysis.**

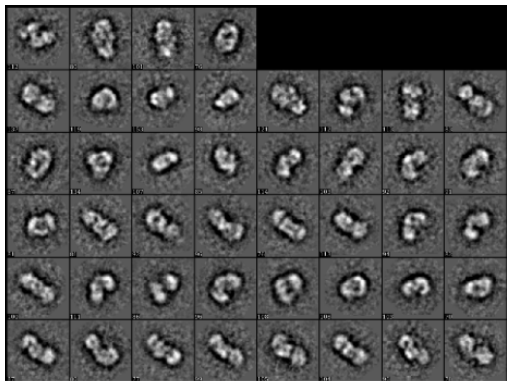
(a) yCAF-1 complex prepared without gradient fixation stabilization (4,398 particles). Side length of all class averages shown is 374 Å. (b,c,d) yCAF-1 (4,260 particles), yCAF-1\_H3/H4 (5,044 particles), and yCAF-1\_H3/H4\_Asf1 complexes (8,735 particles) prepared with gradient fixation stabilization. Side length for these class averages is 336 Å.



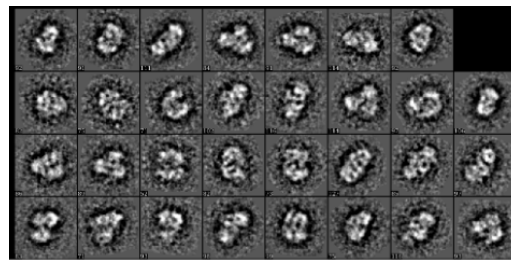
**Supplementary Figure 3. Fourier Shell Correlation Curves.**

FSC for CAF-1 (Blue) and CAF-1\_H3/H4\_Asf-1 (Red) indicates the resolution for the 3D reconstructions with 29.1  $\text{\AA}$  and 27.8  $\text{\AA}$  respectively

CAF-1

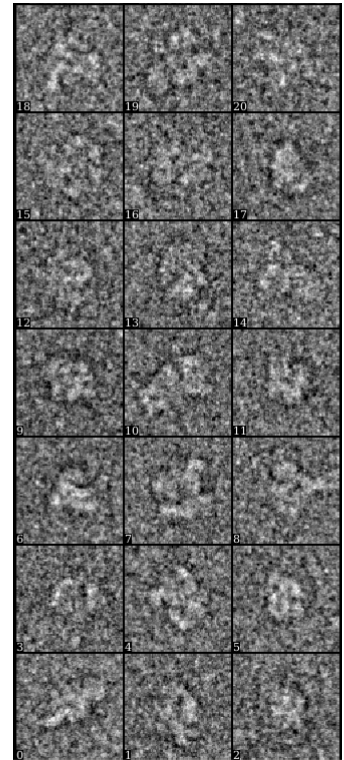
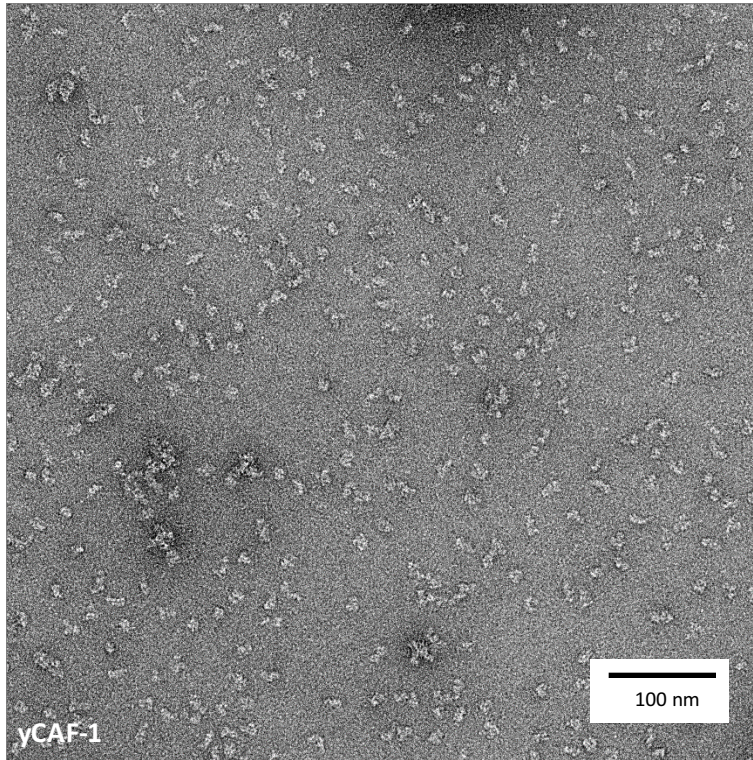


CAF-1/Asf-1/H3/H4

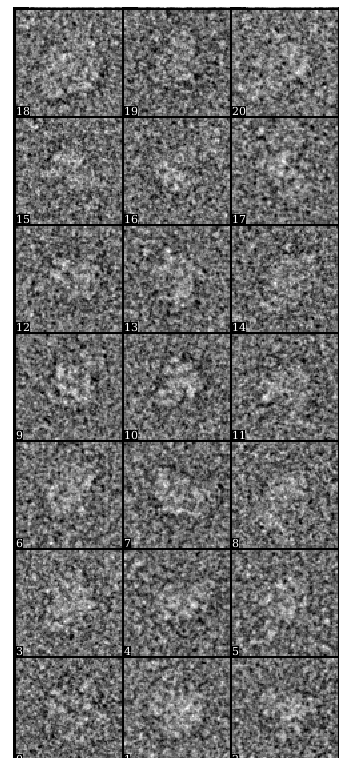
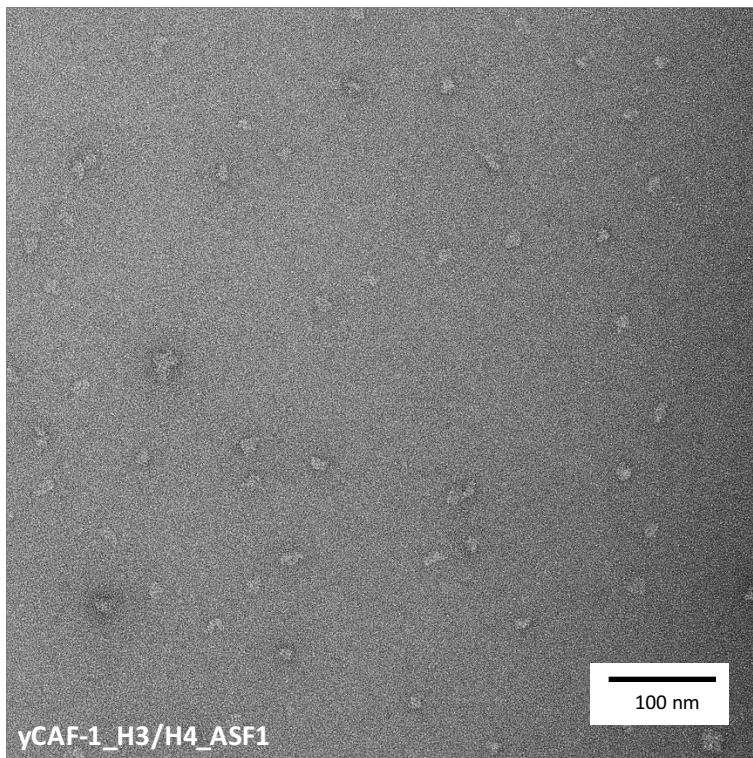


**Supplementary Figure 4. 2D class averages for yCAF-1 and yCAF-1\_H3/H4\_Asf-1 used for 3D reconstruction.**

**a**



**b**



**Supplementary Figure 5**

Representative micrographs and particles of yCAF-1 (a) and yCAF-1\_H3/H4\_ASF1 complex (b)

**Supplementary Table 1. Cross-linked peptides of yeast CAF-1**

<b>Cross-linked Peptide ID</b>	<b>Protein1</b>	<b>Protein2</b>	<b>Position1</b>	<b>Position2</b>
GILSFFQNTTTVKSNK-KEKLQSNR-a13-b3	CAC1	CAC3	30	287
LLCYKNSPIQSTK-LFTAGGDNKVR-a5-b9	CAC1	CAC2	92	38
KLSDSNTPVVEK-LFTAGGDNKVR-a1-b9	CAC1	CAC2	236	38
KLSDSNTPVVEK-TKHGSTR-a1-b2	CAC1	CAC3	236	161
KLSDSNTPVVEK-EKLQSNR-a1-b2	CAC1	CAC3	236	287
KLSDSNTPVVEK-QSKISHPFETK-a1-b3	CAC1	CAC3	236	171
SDYEKFFLPFYAK-QSKISHPFETK-a5-b3	CAC1	CAC3	252	171
IDDELLNSKDK-EKLQSNR-a9-b2	CAC1	CAC3	291	287
KIDDELLNSKDK-EKLQSNR-a10-b2	CAC1	CAC3	291	287
DKTSSDLLNWLQSR-EKHDGGVNSCR-a2-b2	CAC1	CAC3	293	294
DKTSSDLLNWLQSR-EKLQSNR-a2-b2	CAC1	CAC3	293	287
KAVDVLQQMPLK-LFTAGGDNKVR-a1-b9	CAC1	CAC2	317	38
RKAVDVLQQMPLK-LFTAGGDNKVR-a2-b9	CAC1	CAC2	317	38
EKTDELQSLLAQVPHK-NTKLLYDYLNTNSTK-a2-b3	CAC1	CAC3	330	31
KFVGPLIPTICLK-LFTAGGDNKVR-a1-b9	CAC1	CAC2	444	38
AEVIIETDGPIDPFKEPK-SVLKLPYK-a15-b4	CAC1	CAC2	489	339
SNSDLQAQTASQSQSPEKK-IAKAELPCPGDVLR-a18-b3	CAC1	CAC2	518	235
LFDGVQDSTFSLGTVEIAQKNLPQYNK-IAKAELPCPGDVLR-a21-b3	CAC1	CAC2	553	235
SSGKGDLPV-SVLKLPYK-a4-b4	CAC1	CAC2	577	339
LFTAGGDNKVR-LKKEEEIR-a9-b3	CAC2	CAC1	38	212
IAKAELPCPGDVLR-SSGKGDLPV-a3-b4	CAC2	CAC1	235	577
IAKAELPCPGDVLR-NTIKEYAIR-a3-b4	CAC2	CAC1	235	568
IAKAELPCPGDVLR-QTIKNTIK-a3-b4	CAC2	CAC1	235	564
IAKAELPCPGDVLR-QKAMITDPMDLLR-a3-b2	CAC2	CAC1	235	521
IAKAELPCPGDVLR-NLPQYNKQTIK-a3-b7	CAC2	CAC1	235	560

SGILNSAGGVKNRPAIR-QKAMITDPMDLLR-a11-b2	CAC2	CAC1	306	521
SGILNSAGGVKNRPAIR-SSGKGDLPK-a11-b4	CAC2	CAC1	306	577
SGILNSAGGVKNRPAIR-NTIKEYAIR-a11-b4	CAC2	CAC1	306	568
SGILNSAGGVKNRPAIR-SSGKGDLPK-a11-b4	CAC2	CAC1	306	577
KPALMAAFSPVFYETCQK-KFVGPLIPTICLK-a1-b1	CAC2	CAC1	318	444
KPALMAAFSPVFYETCQK-SSGKGDLPK-a1-b4	CAC2	CAC1	318	577
KPALMAAFSPVFYETCQK-RKFGVPLIPTICLK-a1-b2	CAC2	CAC1	318	444
SVLKLPHYK-TSSLPSKR-a4-b7	CAC2	CAC1	339	499
EAGGIVNMLPVKK-TSSLPSKR-a12-b7	CAC2	CAC1	447	499
NTKLLYDYLNTNSTK-KAVDVLQQMPLK-a3-b1	CAC3	CAC1	31	317
NTKLLYDYLNTNSTK-AVDVLQQMPLKEK-a3-b11	CAC3	CAC1	31	328
QSKISHPFETK-LKEEAK-a3-b2	CAC3	CAC1	171	219
QSKISHPFETK-LKEEAKER-a3-b6	CAC3	CAC1	171	223
QSKISHPFETK-IGNFFKK-a3-b6	CAC3	CAC1	171	235
QSKISHPFETK-LKKEEEIR-a3-b3	CAC3	CAC1	171	212
EKHDGGVNSCR-LKKEEEIR-a2-b2	CAC3	CAC1	294	211
EKHDGGVNSCR-LKKEEEIR-a2-b3	CAC3	CAC1	294	212
EKHDGGVNSCR-ELKVEEER-a2-b3	CAC3	CAC1	294	172
FNYKNSLILASADSNR-KIDDELLNSK-a4-b1	CAC3	CAC1	307	282
FNYKNSLILASADSNR-RKIDDELLNSK-a4-b2	CAC3	CAC1	307	282



**Supplementary Table 2. Cross-linked peptides of yeast CAF-1\_HistoneH3/H4**

<b>Cross-linked Peptide ID</b>	<b>Protein1</b>	<b>Protein2</b>	<b>Position1</b>	<b>Position2</b>
KLSDSNTPVVEK-TKHGSTR-a1-b2	CAC1	CAC3	236	161
KLSDSNTPVVEK-EKLQSNR-a1-b2	CAC1	CAC3	236	287
KLSDSNTPVVEKSDYEK-QSKISHPFETK-a12-b3	CAC1	CAC3	247	171
SDYEKFFLPFYAK-QSKISHPFETK-a5-b3	CAC1	CAC3	252	171
FFLPFYAKDGVR-YQKSTELLIR-a8-b3	CAC1	H3	260	57
IDDELLNSKDK-EKLQSNR-a9-b2	CAC1	CAC3	291	287
KIDDELLNSKDK-EKLQSNR-a10-b2	CAC1	CAC3	291	287
DKTSSDLLNWLQSR-EKHDGGVNSCR-a2-b2	CAC1	CAC3	293	294
AVDVLQQMPLKEK-YQKSTELLIR-a11-b3	CAC1	H3	328	57
AVDVLQQMPLKEK-RYQKSTELLIR-a11-b4	CAC1	H3	328	57
AVDVLQQMPLKEK-LFTAGGDNKVR-a11-b9	CAC1	CAC2	328	38
EKTDELQSLLAQVPHK-YQKSTELLIR-a2-b3	CAC1	H3	330	57
EKTDELQSLLAQVPHK-RYQKSTELLIR-a2-b4	CAC1	H3	330	57
EKTDELQSLLAQVPHK-NTKLLYDYLNTNSTK-a2-b3	CAC1	CAC3	330	31
KFVGPLIPTICLK-YQKSTELLIR-a1-b3	CAC1	H3	444	57
RKFVGPLIPTICLK-KLPFQR-a2-b1	CAC1	H3	444	65
KFVGPLIPTICLK-KLPFQR-a1-b1	CAC1	H3	444	65
RKFVGPLIPTICLK-YQKSTELLIR-a2-b3	CAC1	H3	444	57
AEVIIETDGPIDPFKEPK-SVLKLPYK-a15-b4	CAC1	CAC2	489	339
AEVIIETDGPIDPFKEPKTSSLPSK-SVLKLPYK-a15-b4	CAC1	CAC2	489	339
AEVIIETDGPIDPFKEPK-EAGGIVNMLPVKK-a15-b12	CAC1	CAC2	489	447
AEVIIETDGPIDPFKEPK-KIPCNSSDSKK-a15-b10	CAC1	CAC2	489	457
SNSDLQAQTASQSQSPEKK-IAKAELPCGDVLR-a18-b3	CAC1	CAC2	518	235
LFDGVQDSTFSLGTVEIAQKNLPQYNK-IAKAELPCGDVLR-a21-b3	CAC1	CAC2	553	235
LFDGVQDSTFSLGTVEIAQKNLPQYNK-EAGGIVNMLPVKK-a21-b12	CAC1	CAC2	553	447

LFTAGGDNKVRIWK-SRSSPCSKR-a9-b9	CAC2	CAC1	38	129
KIESLDFGLSLTHHEQAINVIR-AVDVLQQMPLKEK-a1-b11	CAC2	CAC1	57	328
IAKAELPCPGDVLVLR-SSGKGDLPVLR-a3-b4	CAC2	CAC1	235	577
IAKAELPCPGDVLVLR-NTIKEYAIR-a3-b4	CAC2	CAC1	235	568
IAKAELPCPGDVLVLR-QKAMITDPMDLLR-a3-b2	CAC2	CAC1	235	521
IAKAELPCPGDVLVLR-DAVITYTEHAKRK-a3-b10	CAC2	H4	235	78
SGILNSAGGVKNRPAIR-SSGKGDLPVLR-a11-b4	CAC2	CAC1	306	577
SGILNSAGGVKNRPAIR-NTIKEYAIR-a11-b4	CAC2	CAC1	306	568
KPALMAAFSPVIFYETCQK-KFVGPLIPTICLK-a1-b1	CAC2	CAC1	318	444
KPALMAAFSPVIFYETCQK-RKFVGPLIPTICLK-a1-b2	CAC2	CAC1	318	444
KPALMAAFSPVIFYETCQKSVLK-SSGKGDLPVLR-a18-b4	CAC2	CAC1	335	577
KPALMAAFSPVIFYETCQKSVLK-TSSLPSKR-a18-b7	CAC2	CAC1	335	499
KPALMAAFSPVIFYETCQKSVLK-QKAMITDPMDLLR-a18-b2	CAC2	CAC1	335	521
IEPPAMHAELDTDESAAKNQR-SSGKGDLPVLR-a21-b4	CAC2	CAC1	432	577
ISTLGHIKWSSLNFDMDMEFEKPENSTR-YQKSTELLIR-a8-b3	CAC3	H3	92	57
QSKISHPFETK-IGNFFKK-a3-b6	CAC3	CAC1	171	235
IRQSKISHPFETK-KLSDSNTPVVEK-a5-b1	CAC3	CAC1	171	236
QSKISHPFETK-LKKEEEIR-a3-b3	CAC3	CAC1	171	212
QSKISHPFETK-LKEEAK-a3-b2	CAC3	CAC1	171	219
QSKISHPFETK-LKKEEEIR-a3-b2	CAC3	CAC1	171	211
QSKISHPFETK-RKEEER-a3-b2	CAC3	CAC1	171	205
EKHDGGVNSCR-ELKVEEER-a2-b3	CAC3	CAC1	294	172
EKHDGGVNSCR-ELLRQEEKK-a2-b8	CAC3	CAC1	294	166
FNYKNSLILASADSNR-RKIDDELLNSK-a4-b2	CAC3	CAC1	307	282
FNYKNSLILASADSNR-KIDDELLNSK-a4-b1	CAC3	CAC1	307	282
KLPFQRLVLR-QEEKKK-a1-b4	H3	CAC1	65	166
DNIQGITKPAIR-ELKVEEER-a8-b3	H4	CAC1	32	172
GLGKGGAK-HRKELLR-a4-b3	H4	CAC1	13	158

DNIQGITKPAIRR-EKELKK-a8-b2	H4	CAC1	32	144
DAVTYTEHAKR-EIAQDFKTDLR-a10-b7	H4	H3	78	80

**Supplementary Table 3. Cross-linked peptides of yeast CAF-1\_HistoneH3/H4\_Asf1**

<b>Cross-linked Peptide ID</b>	<b>Protein1</b>	<b>Protein2</b>	<b>Position1</b>	<b>Position2</b>
FLTKEKDVITLDDPK-MSGRGKGGK-a6-b6	CAC1	H4	39	6
LKKEEEIR-EKLQSNR-a2-b2	CAC1	CAC3	211	287
KLSDSNTPVVEK-EKLQSNR-a1-b2	CAC1	CAC3	236	287
KLSDSNTPVVEK-QSKISHPFETK-a1-b3	CAC1	CAC3	236	171
SDYEKFFLPFYAK-TKHGSTR-a5-b2	CAC1	CAC3	252	161
SDYEKFFLPFYAK-QSKISHPFETK-a5-b3	CAC1	CAC3	252	171
KIDDELLNSKDK-EKLQSNR-a10-b2	CAC1	CAC3	291	287
IDDELLNSKDK-EKLQSNR-a9-b2	CAC1	CAC3	291	287
DKTSSDLLNWLQSR-EKHDGGVNSCR-a2-b2	CAC1	CAC3	293	294
RKAVDVLQQMPLK-LFTAGGDNKVR-a2-b9	CAC1	CAC2	317	38
EKTDELQSLLAQVPHK-NTKLLYDYLNTNSTK-a2-b3	CAC1	CAC3	330	31
EKTDELQSLLAQVPHK-EKHDGGVNSCR-a2-b2	CAC1	CAC3	330	294
KFVGPLIPTICLK-KLPFQR-a1-b1	CAC1	H3	444	65
KFVGPLIPTICLK-YQKSTELLIR-a1-b3	CAC1	H3	444	57
AEVIIETDGPIDPFKEPK-SVLKLPYK-a15-b4	CAC1	CAC2	489	339
AEVIIETDGPIDPFKEPK-SGILNSAGGVKNRPAIR-a15-b11	CAC1	CAC2	489	306
AEVIIETDGPIDPFKEPKTSSLPSK-SVLKLPYK-a18-b4	CAC1	CAC2	492	339
RSNSDLQAQTASQSQSPEKK-IAKAELPCPGDVLR-a19-b3	CAC1	CAC2	518	235
LFTAGGDNKVR-YQKSTELLIR-a9-b3	CAC2	H3	38	57
IAKAELPCPGDVLR-NTIKEYAIR-a3-b4	CAC2	CAC1	235	568
IAKAELPCPGDVLR-SSGKGDLPK-a3-b4	CAC2	CAC1	235	577
IAKAELPCPGDVLR-DAVITYTEHAQR-a3-b10	CAC2	H4	235	78
IAKAELPCPGDVLR-QKAMITDPMDLLR-a3-b2	CAC2	CAC1	235	521
KPALMAAFSPVYETCQK-KFVGPLIPTICLK-a1-b1	CAC2	CAC1	318	444

KPALMAAFSPVIFYETCQK-RKFVGPLIPTICLK-a1-b2	CAC2	CAC1	318	444
KPALMAAFSPVIFYETCQKSVLK-KEEEIRLK-a18-b1	CAC2	CAC1	335	212
KPALMAAFSPVIFYETCQKSVLK-TSSLPSKR-a18-b7	CAC2	CAC1	335	499
KPALMAAFSPVIFYETCQKSVLK-QTIKNTIK-a18-b4	CAC2	CAC1	335	564
IEPPAMHAEPLDDESAVAAKNQR-AEVIIETDGPIDPFKEPK-a21-b15	CAC2	CAC1	432	489
NTKLLYDYLNTNSTK-AVDVLQQMPLKEK-a3-b11	CAC3	CAC1	31	328
QSKISHPFETK-LKEEAKER-a3-b6	CAC3	CAC1	171	223
QSKISHPFETK-LKEEAK-a3-b2	CAC3	CAC1	171	219
QSKISHPFETK-LKKEEEIR-a3-b3	CAC3	CAC1	171	212
QSKISHPFETK-RAELKK-a3-b5	CAC3	CAC1	171	184
FNYKNSLILASADSNR-RKIDDELLNSK-a4-b2	CAC3	CAC1	307	282
FNYKNSLILASADSNR-KIDDELLNSK-a4-b1	CAC3	CAC1	307	282
KLPFQRLVR-QEEKKK-a1-b4	H3	CAC1	65	166
KLPFQRLVR-QEEKKK-a1-b5	H3	CAC1	65	167