

Supplementary Information for

**Conserved patterns hidden within group A *Streptococcus*
M protein hypervariability are responsible for recognition
of human C4b-binding protein**

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Supplementary Table S1. Data collection, phasing and refinement statistics for native and SAD (SeMet) structures

	M2-C4BP	M2-C4BP (SeMet L46M/ L71M)	M2-C4BP (SeMet L29M/ L46M)	M28-C4BP	M22-C4BP	M49-C4BP (SeMet L29M/ L46M)	M2 (K65A/ N66A)- C4BP
PDB ID	5HYU			5HYP	5HYT	5HZP	5IQQ
Data collection							
Space group	P 4 ₃ 3 2	P 4 ₃ 3 2	P 4 ₃ 3 2	P 4 ₃ 3 2	P 2 ₁ 2 ₁ 2 ₁	P 4 ₃ 2 ₁ 2	P 4 ₃ 3 2
Cell dimensions							
<i>a</i> , <i>b</i> , <i>c</i> (Å)	148.3	148.7	148.3	133.7	68.08	78.1	148.6
	148.3	148.7	148.3	133.7	80.35	78.1	148.6
	148.3	148.7	148.3	133.7	152.9	345.3	148.6
α , β , γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Wavelength	0.984 Å	0.979 Å	0.979 Å	0.979 Å	0.979 Å	0.979 Å	0.979 Å
Resolution (Å)	50.00- 2.56(2.60- 2.56) ^a	50.00- 2.90(2.95- 2.90) ^a	50.00- 3.00(3.05- 3.00) ^a	50.00- 3.02(3.07- 3.02) ^a	80.40- 2.54(2.68- 2.54) ^a	86.40- 2.74(2.89- 2.74) ^a	50.00- 2.29(2.37- 2.29) ^a
<i>R</i> _{merge}	0.18(1.00)	0.18(1.00)	0.15(1.00)	0.14(1.00)	0.14(1.00)	0.23(1.00)	0.13(1.00)
<i>I</i> / σ _{<i>I</i>}	67.3(4.17)	21.3(2.17)	22.4(3.44)	13.2(0.81)	10.7(1.2)	11.3(1.00)	46.4(3.22)
Completeness (%)	100(100)	99.9(100)	100(100)	99.8(100)	99.8(99.9)	99.8(99.1)	100(100)
Redundancy	42.8(43.1)	40.6(40.7)	22.9(22.6)	9.5(9.7)	6.6(6.7)	9.4(9.6)	40.5(32.6)
cc _{1/2}	1.00(0.86)	0.99(0.86)	0.99(0.86)	0.99(0.48)	1.00(0.60)	1.00(0.61)	0.99(0.86)
Refinement							
Resolution (Å)	35.97- 2.56(2.65- 2.56)			44.40- 3.02(3.21- 3.02)	76.50- 2.54(2.60- 2.54)	71.16- 2.74(2.84- 2.74)	49.54- 2.29(2.37- 2.29)
No. reflections	18514 (1808)			7741 (1084)	28328 (1975)	28700 (2449)	47375 (4655)
<i>R</i> _{work} / <i>R</i> _{free}	0.21(0.27)/ 0.22(0.28)			0.25(0.33)/ 0.29(0.39)	0.21(0.34)/ 0.27(0.42)	0.25(0.42)/ 0.31(0.43)	0.20(0.26)/ 0.22(0.29)
No. atoms							
Protein	1259			1197	4844	3146	1252
Ligand/ion	0			0	0	25	0
Water	76			0	70	20	132
<i>B</i> -factors							
Protein	76.7			110.9	70.6	106.7	53.4
Ligand/ion						145.2	
Water	103.8				54.6	68.2	67.0
R.m.s deviations							
Bond lengths (Å)	0.01			0.01	0.01	0.01	0.01
Bond angles (°)	1.35			1.31	1.48	1.21	1.35
MolProbity score	3.03[44 th] ^b			3.33[52 nd] ^b	2.49[78 th] ^b	3.03[53 rd] ^b	2.80[37 th] ^b
Ramachandran							
% preferred	90.3			86.7	92.6	90.4	92.9
% allowed	8.4			11.3	4.2	7.8	6.4
% disallowed	1.3			2.0	3.2	1.8	0.7
Clashscore	18.23[81 st]			16.4[96 th]	9.81[97 th]	11.69[97 th]	9.78[94 th]

^aHighest resolution bin in parentheses here and other rows.^bPercentile in brackets here and other rows.

Supplementary Table S2. Ionic Interaction Pair Occupancy in C4BP α 2 for Quadrilateral Residues of M2 (%)

C4BP-M2	M2 Wild-type	M2 (K65A)	M2 (N66D)	M2 (N66A)	M2 (K65A/N66A)
H67-D62	64.3/94.4 ^a	96.5/100	93.4/88.6	75.8/77.5	99.0/98.4
R64-E68	73.6/44.2	33.8/41.8	50.1/73.0	61.2/35.8	56.2/28.0
R66-N/D/A66	5.70/4.70	59.0/44.4	45.7/26.0	—	—

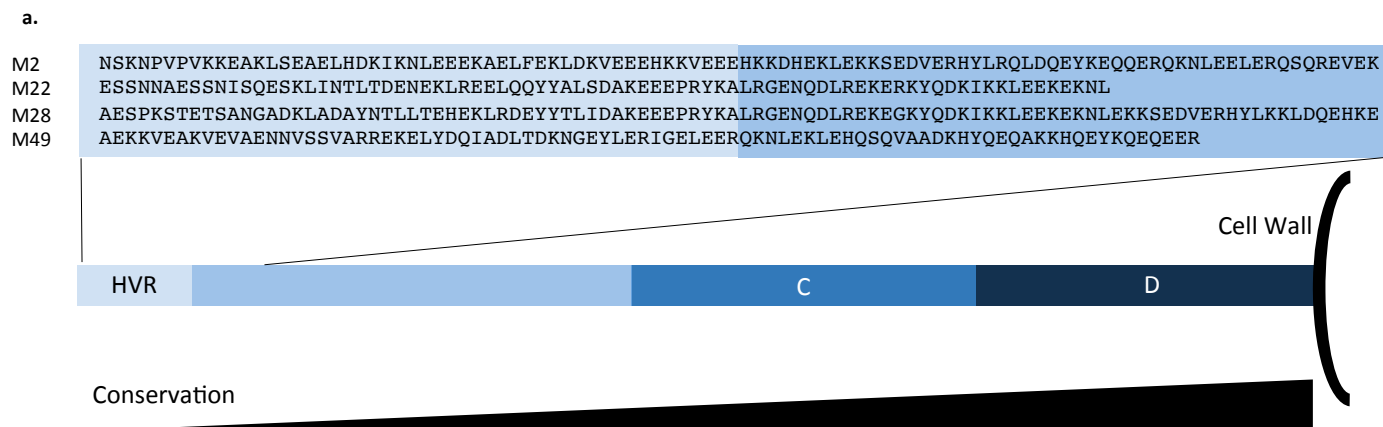
^aThe two percentages reflect the two binding sites in the 2:2 C4BP-M protein complex.

Supplementary Table S3. Ionic Interaction Pair Occupancy in Quadrilateral for residues of M49, M22, and M28 (%)

C4BP-M49		C4BP-M22		C4BP-M28	
H67-D69	75.3/90.4 ^a	H67-N60	100/100	H67-N63	100/100
		R64-E65	71.4/55.7	R64-E68	52.4/30.2
R66-D73	54.1/43.3	R66-D64	95.6/96.0	R66-E70	21.1/28.8

^aThe two percentages reflect the two binding sites in the 2:2 C4BP-M protein complex.

Supplementary Figure S1



b.

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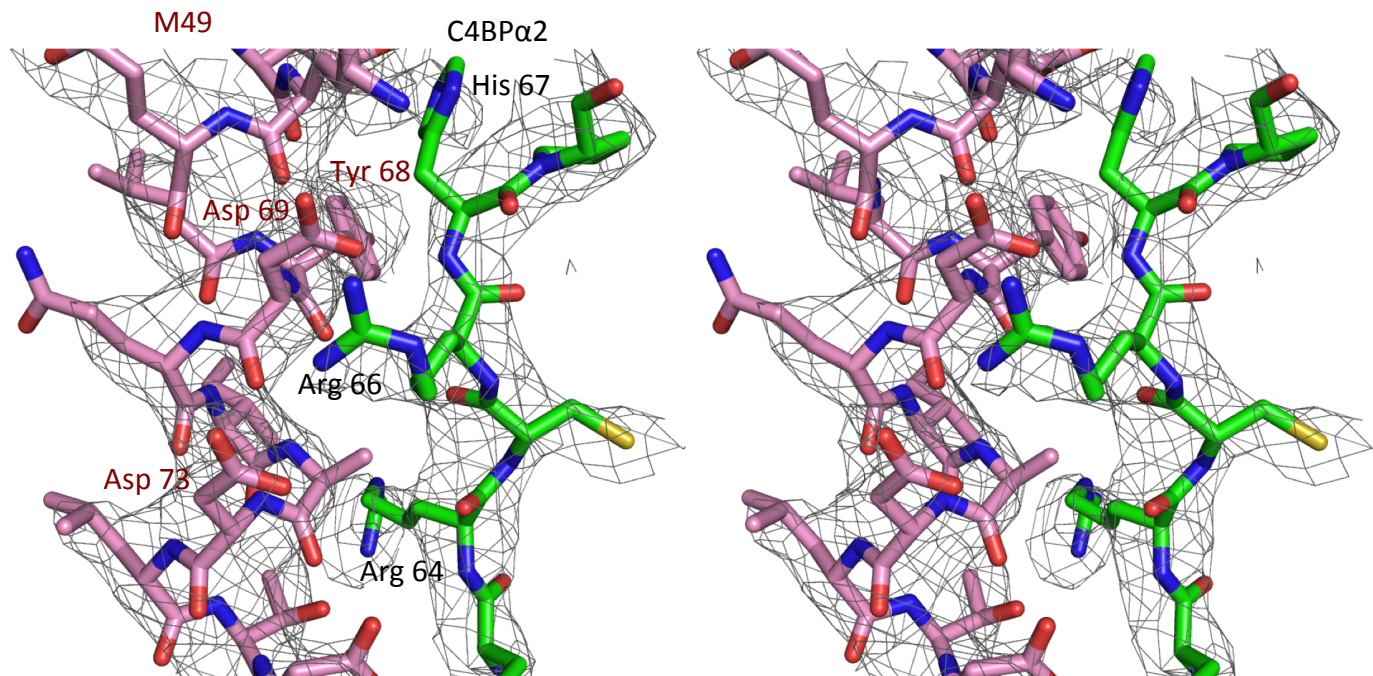
M2      -----NSKNPVPVKKEAKLSEA--ELHDKIKNLEEEKAELFEKLDKVEEEHKKVVEE
M22     -----ESSNNAESSNISQESKLINTLTDENEKLRREELQYYALSDAKEEPRYKA--
M28     AES--PKSTETSANGADKLADAYNTLLTEHEKLRD---EYYTLIDAKEEPRYKA--
M49     AEKKVEAKVEVAENNVSSVARREKELYDQIADLTDKNGEYLERIGELEER-----
          . . . . : * : .* : : . **
  
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Supplementary Figure S1. Schematic of M protein domains.

a. The sequences of M2, M22, M28, and M49 co-crystallized with C4BP α 1-2 are depicted, with the HVR in light blue and other regions of the protein in darker blue or black. M proteins are hypervariable at their N-termini, with conservation increasing towards their C-termini.

b. Multiple sequence alignment of the M2, M22, M28, and M49 HVRs is shown, as carried out using MUSCLE¹. The asterisks indicate identical amino acids, the colons indicate amino acids with strongly similar properties, and the periods indicate amino acids with weakly similar properties.

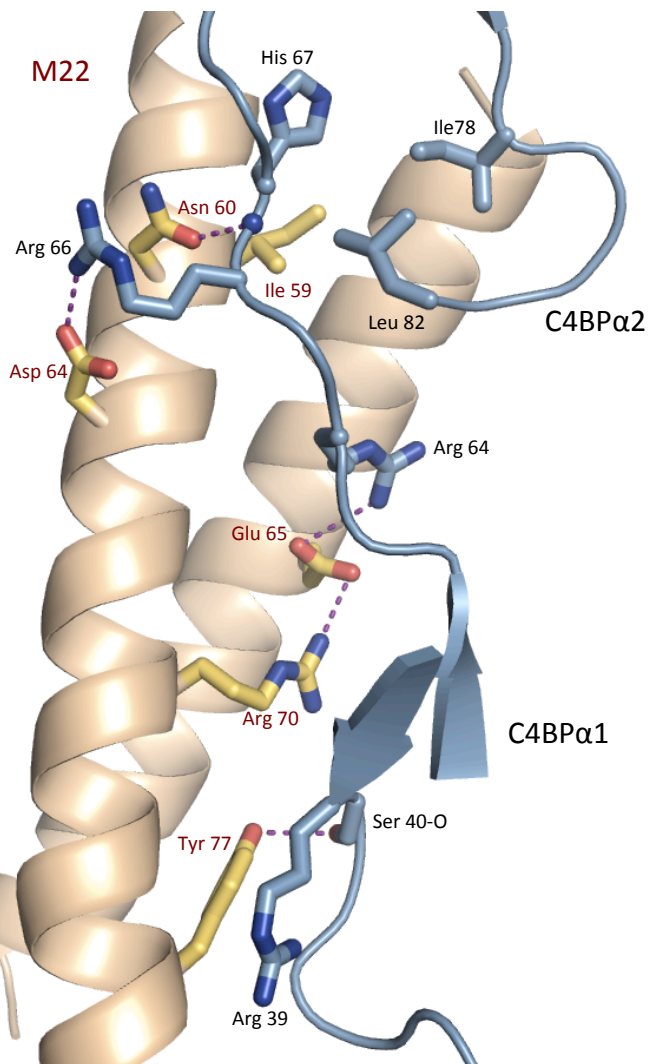
Supplementary Figure S2



Supplementary Figure S2. Electron density for the M49 HVR-C4BPα1-2 complex.

Electron density from a simulated annealing 2Fo-Fc omit map (contoured at 1σ) for the M49 HVR-C4BPα1-2 structure. Residues M49 68-75 and C4BPα1-2 64-67 and 77-82 were excluded along with a 3.5 Å sphere around these residues, prior to performing simulated annealing (2500 K) and phase calculation. The final model is overlaid in bonds representation. M49 is depicted with pink carbons, and C4BP with green carbons; nitrogens and oxygens are blue and red, respectively, for both. The numbering of M proteins is such that the initiator Met is residue 1.

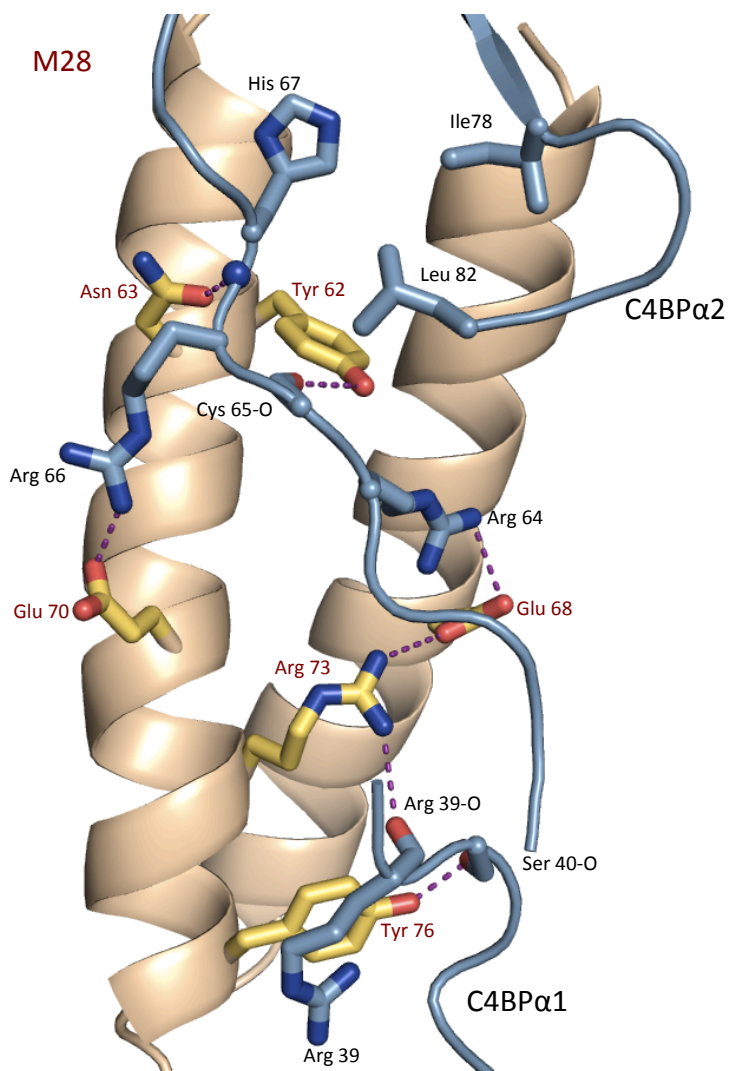
Supplementary Figure S3



Supplementary Figure S3. Structure of M2-C4BP.

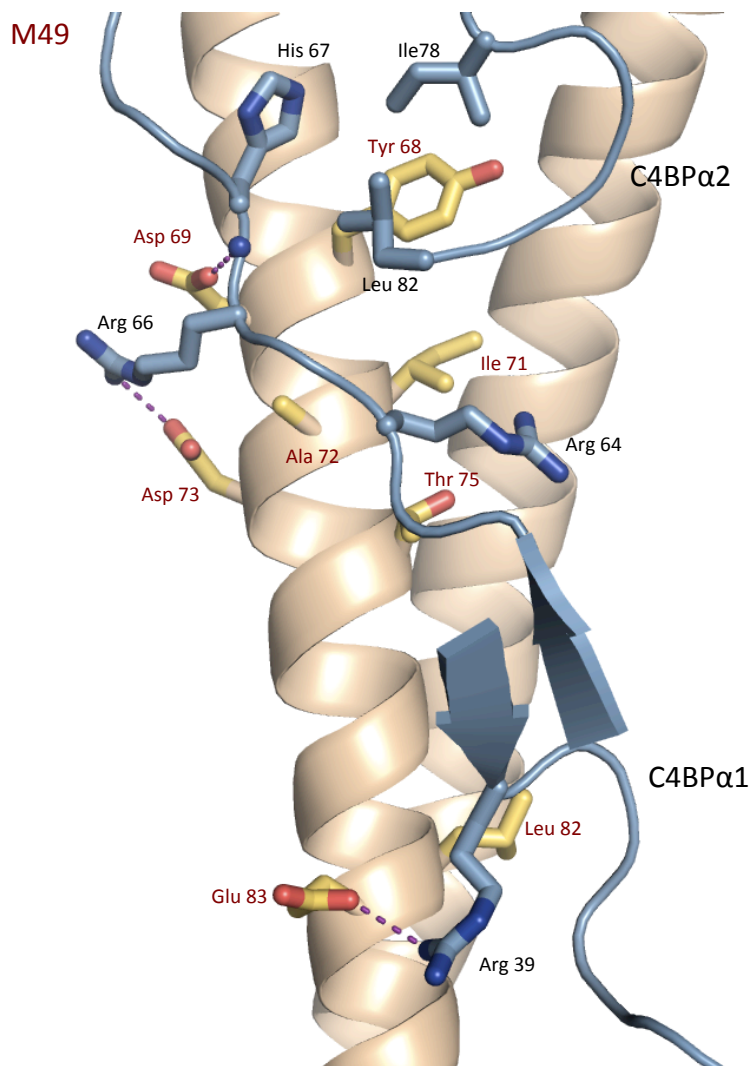
M2 is in gray ribbon representation with key side chains in bonds representation, in which carbons are yellow, oxygens red, and nitrogens blue. C4BPα1-2 is in cyan ribbon representation, with key side chains in bonds representation, in which carbons are cyan, oxygens red, and nitrogens blue. Hydrogen bonds and salt bridges depicted by dashed magenta lines.

Supplementary Figure S4



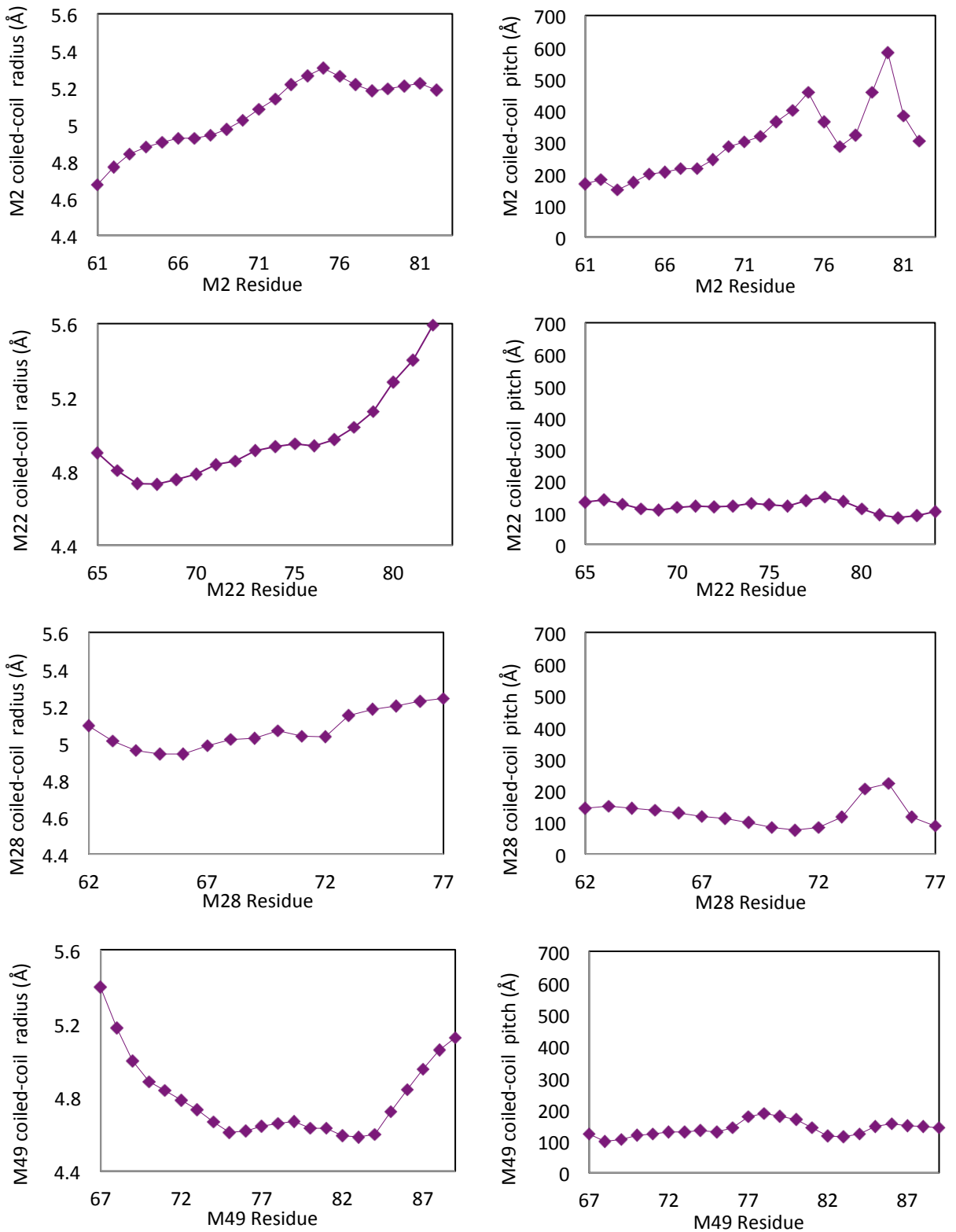
Supplementary Figure S4. Structure of M28-C4BP.
The depiction is as in Supplementary Figure S3.

Supplementary Figure S5



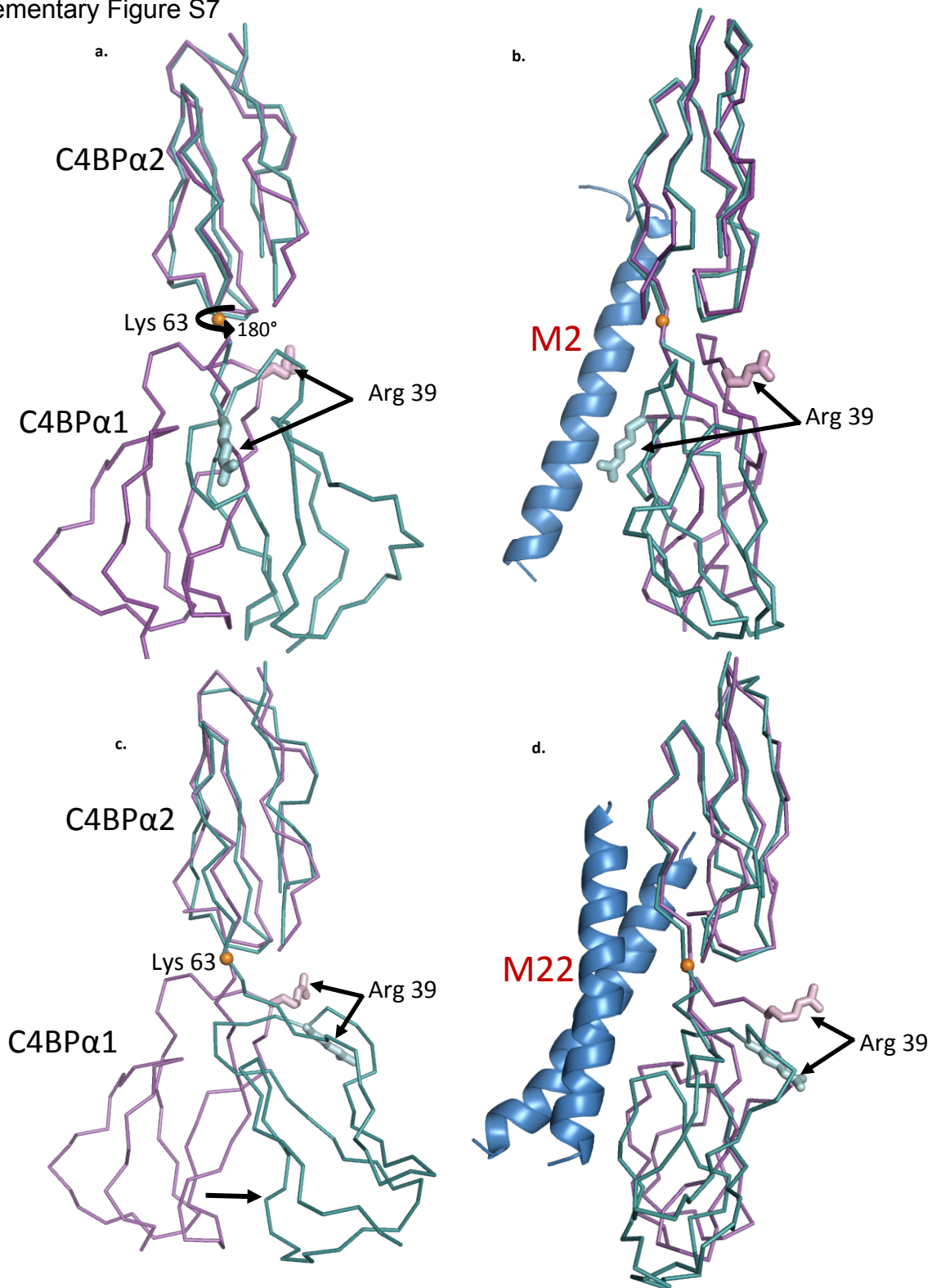
Supplementary Figure S5. Structure of M49-C4BP.
The depiction is as in Supplementary Figure S3.

Supplementary Figure S6



Supplementary Figure S6. Coiled coil parameters of M proteins. Radius and pitch of the M protein HVR α -helical coiled coils, reporting on the residues that are at the interface with C4BP α 1-2.

Supplementary Figure S7



Supplementary Figure S7. Rotation of C4BP α 1-2.

a. Superposition of free (magenta) and M protein-bound C4BP α 1-2 (cyan) based on the C4BP α 2 domain, showing the 180° rotation of C4BP α 1 around Lys 63 (indicated

Supplementary Figure S7 Continued.

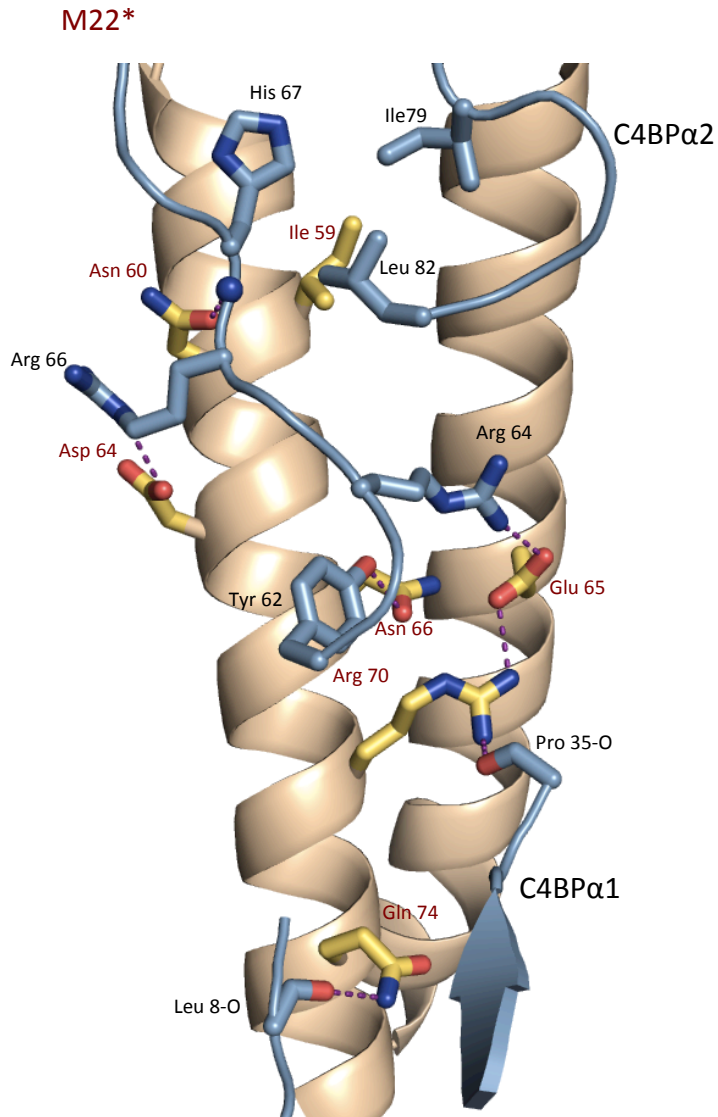
by golden ball). The molecules are depicted as C α chain traces. The position of C4BP Arg 39 is shown in bonds representation. The same depiction is used in the following panels.

b. A 90° rotated view of the superposition shown in panel a, with one α -helix of the M2 coiled coil shown as a blue ribbon.

c. One of the C4BP α 1-2 molecules bound to M22 that is restricted from undergoing a 180° rotation by a crystal contact is depicted. Superposition of free (magenta) and the M22 protein-bound C4BP α 1-2 (cyan) is based on the C4BP α 2 domain. The bound C4BP α 1 domain is related to free C4BP α 1-2 by a tilt rather than a rotation.

d. A 90° rotated view of the superposition shown in panel c, with the M22 coiled coil shown as a blue ribbon.

Supplementary Figure S8



Supplementary Figure S8. Structure of the M22-C4BP interaction in which C4BPα1 is tilted rather than rotated.

The depiction is as in Supplementary Figure S3. M22 is denoted with an asterisk to indicate that in this complex, the C4BPα1 domain is tilted rather than rotated.

Supplementary Figure S9

abcdefghijklmnopabcdefghijklmnopabcdefghijklmnopabcdefghijklmnop

M2 KKEAKLSEAELHDKIKNLEEEKAELFEKLDKVEEE
M49 VSSVARREKELYDQIADLTDKNGEYLERIGELEER
M114 KEATKLSEAELYNKIQELEGKAELFDKLEKVEEE
M73 KEAKLNEAELYNKIQELEGKAELFDKLEKVEEE
M77 EGVSVGSDASLHNRI TDLEEREKLLNKLDKVEEE
M84 ASVKKNNEEELHNKIADLLDQNEEYLNKIDELKEG
M89 SVSVKDNEKELHNKIADLEERGEHLDKIDELKEE
M97 GPVPRSLWLREYDKNQELTKKLTEFEEKLLQN
M102.1 SSVPVKKAELYDKIKELEEGREELLNLDKVKED
M106 QKQNVSSNGRIYEIYDELQTKYDELQTKHEELLGE
M112 SSVSVKNEVKLHNEIAALQEEKEKLLNELDVKEEH
M118 ADSNASSVAKLYNQIADLTDKNGEYLERIEELEER
M124 ATKSKLSEAELHDKIKNLEEEKAELFEKLDKVEEE

Supplementary Figure S9. Sequence alignment of C4BP-binding M protein HVRs of the M2/M49 pattern.

Residues that contact (first two lines) or are predicted to contact C4BP are in red, and the heptad register is indicated above. Residues observed (first two lines) or predicted to be at core *d* positions of the heptad register are highlighted in blue for visual reference.

Supplementary Figure S10

Abcdefgabcdefgabcdefgabcdefgabcdefg

M22 ISQESKLINTLTDENEKLREELQQYYALSDAKEEEE

M28 ADKLADAYNTLLTEHEKLRDEYYTLIDAKEEEEPY

M4 AAWNWPKEYNALLKENEELKVEREKYLSYADDKEKD

M4.1 AAWNWPKEYNALLKENEELKVEREKYLSYADDKEKD

Prth NAKLVEVVEVETTSLENEKLSSENEENKKNLDDKLSKD

M8 NEQLINELNLI EENNDLKDKLARNL DLLDNTREK

M9.2 LSVPKTEYDKLYDDYDKLQEKSAEYLERIGELEER

M11 TNVSADLYNSLW DENKTLREKQEEYITKIQNEETK

M15.1 WKLTIEEYNKLLDENEKLKEKNEEYLEKIGEQEER

M25 AKAAEAKVDKLEKQLEGYKKLEEDYFNLEKR

M42 KVKLEVLYNSLW EENKTLREEQEEYIAKIDKLDEK

M44 GSVSLELYDKLS DEN DILREKQDEYLT KIDGLDKE

M48.1 ELPPEAR YKAWKSE NDELRENHRKILD KFNAEQNK

M55 LYQERQRLQDLKSKFQDLKNRSEGYIQQYYDEEKN

M59 ELTLQQKYDAL T NENKSLRRERDNYL NYLYEKEEL

M60 ISKERELINTLV DEN NKLMEERARHLDLIDNI

M61 GSVSLELYDKLS DEN DILREKQDEYLT KIDGLDKE

M63.4 KLTLEHKYNALT NENKSLIRREKDKYLYEKEELEK

M66 QNTWEKRYQKLS DDHTLLQDAIEEISSENEK LKSE

M67 GGVRLDLYDKLSKENDILREKQDEYLT KIDELGK

M69.1 GGVSLDLYSKLLNENDILRDKQDDYLT KIDELTEK

M76 SNVSINLYNELQAEHDKLQTKHEELLA EHDALKEK

M78 SITNEQLIDKLV EENNDLKEERAKYLDLLDNREKD

M81 ENVPKQQYNALW EENEDLRGRERKYIAKLEKEEIQ

M85 TSVSADLYNSLW DENKTLREKQGEYITKIQNEETK

M88.1 ISNNERLINELT DENNELKDKLARS DLLDNTREK

M92 SGSVSTPYNNLLNEYD DLLAKHGELLSEYDALKEK

M96 LDQFGRDYDELQKKYDKLDKENKEYASQLGK

M109 ADNLAKEYNTLLTENEK LREELQQYYALIDAKEEEE

M110 HEELWKEYDILKEKLDKDQEEREKIELNYLK

M111.1 VTAPAHFWENQRREIEK LKGEIDQLKLLLGKS

M117 GSVSIDRYNELSGEYNKLLDQNGNLLDENEILREK

Supplementary Figure S10. Sequence alignment of C4BP-binding M protein HVRs of the M22/M28 pattern.

Residues that contact (first two lines) or are predicted to contact C4BP are in red, and the heptad register is indicated above. Residues observed (first two lines) or predicted to be at core *d* positions of the heptad register are highlighted in blue for visual reference.

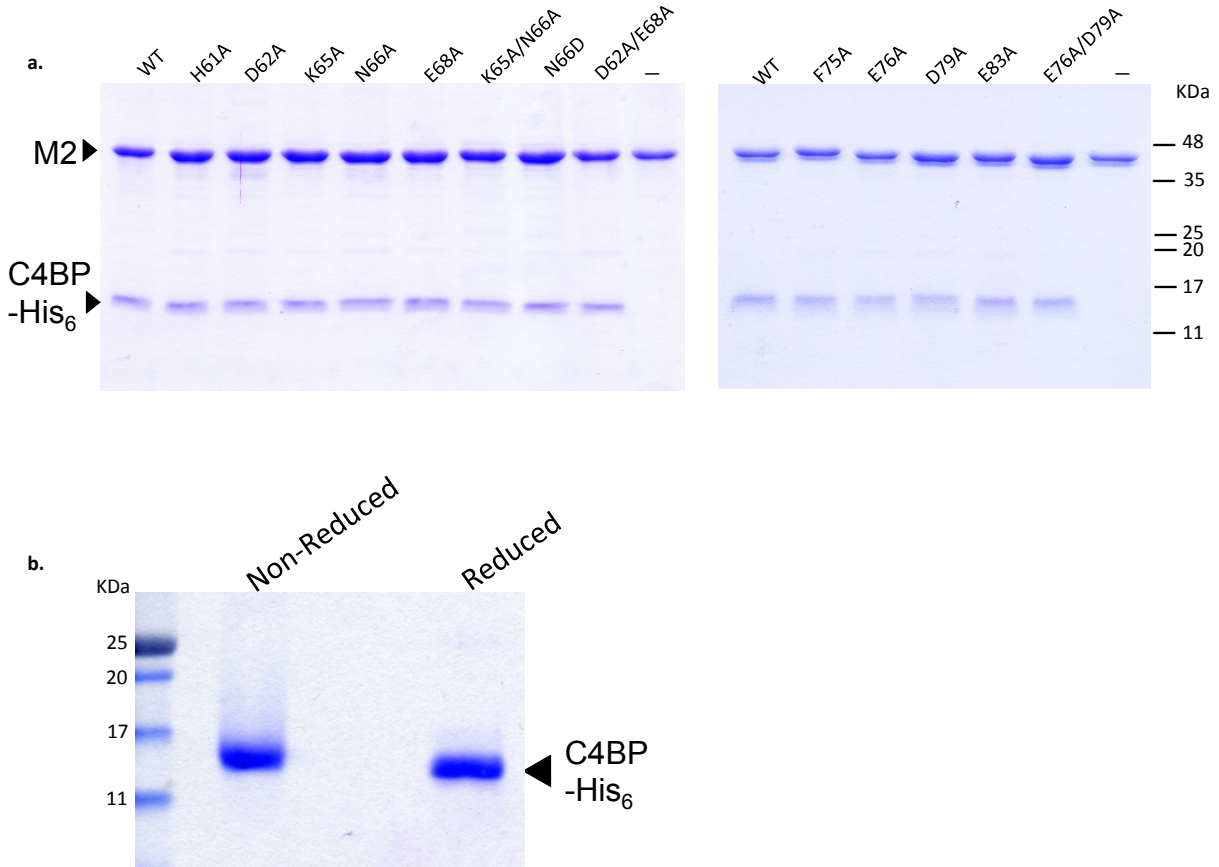
Supplementary Figure S11

M14.5 DRVSRMSRDDL LNRAQNLEAKNHGLEHQNTK LSTENKTLQEQAEARQKE
M29.2 RVYITRRMTKEDVEKI ANDLDTENHGLKQQNEQLSTEKQGLEEQNKQLST
M38 EGEPREVSEELVNSNPVLLNKKIAKKEELANKEQESKESKEAIDALNNI
M54 EVLTRRQSQDPKYVTQRISDLEVKNHDL ENKNEKLTSENQNLKNKTTELE
M62 EEAGASRTITSSENISKLYDENSKLIEERADLLGKLEEKEDKLESVERQY
M90 EGKAAAVSRSNSEQNNSEQNNLEKRYRKLSDDYTVLQEAIEG ISSENEKL
M94 EEASNNGQLTLQHKNNAL TSENESLRREKEELEKKNKELDSQVAGLIGVV
M99 DGERVPKNNRLSKKYSELSEKYGALSEKYGALLDKQGALLDKQEELEKEN
M105 EVNTRSRAQDAGYQKGRADKLETENHGLKFQNEK LQNQNNDLKTQTATLT
M18.6 APLTRATADNKDEL IKRANDYEIQNHQLTVENRKLKTDKEQLTKENDDLK
M32 KAVTRGTVSDPETARQ TIDKYDIKNHQLTQENEKLTKEKEELTQENEKLT
M36 KALTRSTASNSETARQ TINDYEIKNHNL TQENEKLT EQNKELTSEKEKLT
M46 AAVTRHMSTEQLKQRVREYDIENHKLKTDKARLEAEKGQLETKKNELEAK
M71 RAITRATSDDPAKLLKQMV EGYELENHTLKN DKEKLT TENSALTTEKNRLT
M100.1 RVTTRSQAQDAAGLKEKADQYEV RNHELEHNNEK LKTENS DLKTENSKLT
M115 KAVTRSTASDPEKARQ TINEYEVKNHKL TQDNERLAQEKGLTQNNERLT
M58 DSSREVTNELTASMWKAQADS AKAKAKELEKQVEEYKKNYETLEKGYDDL
M79.1 DSRDITGTLPATM WKQAEAAKAKASNLEKQLEEAR KDYSQIEEKLEQFG
M87 ESPREVTNELAASVWKKKV EEAKEKASKLEKQLEEAQ KDYSEIEGKLEQF
M103 DSPRDVTSDLT TSMWKKKAEAAEAKASKFEKQLEDYKKAQKDYEIEEKL
M104 EGVNRHNS EQNTWEKRYRELS EDHALLEATIDD ISLENEK LKSENKKNLE
M33 EEHEKVTQAREAV IREMQRGTNF GPLLASTMRDNHNLKETLDKTKKEID
M41.2 EGNARLAQAQEEALRDV LNNTPHNQLRDAYAGAFRRNNELEKIIQEKERE
M43.2 EEHPDVVAARES VLNNVRVPGTLWLRQKEENDK LKSEKKGLETTELQEQ
M52 DQPVDDHRYTEANDAVLQGR TV SARALLHEINKNGQLRSENEELKADLQK
M64 DRLHPGYTAANNAARNEFLVPAGAVLHEREKNDELRLKNEELKADLQKKE
M68.4 EEVKKAEVKKAAE ESESKSAKMWENMYKELDRDYSLEKTVENMSLENM
M70 EEHESVTRAREAAI REMMRQGRGDFAPLLANAIRDNNNL TETLDKTKKEI
M72 NRADDARREVLRGQFVEAELWHHQIQENDQLKLEKEELKSDLQKKEQELK
M74 FTVTRSMTRDYLA KVVDFTKNHELETHNSELSATNQTLQGOVEAEQKK
M75 EEERTFTELPYEAR YKAWKSENDELRENYRR TLDKFNTEQGKTTRLEEQN
M80 HQLADAARREVLKGETVPAHLWYYQKEENDK LKSANEELETTLQKKEQEL
M82 DSSSRDITEAGVSKFWKSKFDAEQNRANELEKKLSGYEKDYKTLEQEYEN
M83.1 DNPRYTDHNAV TQGRTVPLQNL LH EMDKNGKLRSENEELKADLQKKEQE
M86 DNVGRVDVDKIREEALHQAIGGMTNVQLRNTLAGSFRMNDELKKA IQEKE
M91 ADDHPGAVAARNDVLSGF SVPGNVWYRQHQEIGK LKSEKEELETTELQEQE
M93 ENNTRYNEAYEQALREVLGGMNNVQQLRGALAGSFHRNNELOKTIQEKER
M98 DRYTDHNAV TQGRTVPLRNL LLEMDKNSKLRSENEELQAGLQEQERELE
M101 ADHPSYTA AKDEVLSKFSVPGHVWAHEREKNDKLSSENEGLKAGLQEQEQ
M107 AEAQAQAQAEAKAEAKAPAPAKAPAKAQ TREKQLLLLEEYRKL EEGYFNL
M108 KEHESVTRAREAAIRQMMQGG RDFAPLLADTIRDNNNL RETLDETKKEI
M116.2 DHPLYTAANNAVRNGLSPSDRAVLAEIDKNDKLRLENKELKAGLQEQEQE
M119.2 DQPNHPRYTDANNAVRNGLSPRDRAVLAEIDKNDKLRLENEK LKAGLEEL
M120 DDNPRYTAAQDEVLRELPGQAQAFSRAFLYERQKNGELRLENEGLKTALQ
M121 DQPNHPGYTEANNAV LINGYSVPLRYWAHEREKNDKLSSENEELKAGLQKK
M123 AENHPLAESARRQVLGE STVPASAWYYQKEENDK LKSENEGLKTDLQKQE

Supplementary Figure S11. C4BP-binding M protein HVRs that cannot be classified as belonging to either M2/M49 or M22/M28 patterns.

Residues predicted to be at core *d* positions of the heptad register are highlighted in blue.

Supplementary Figure S12

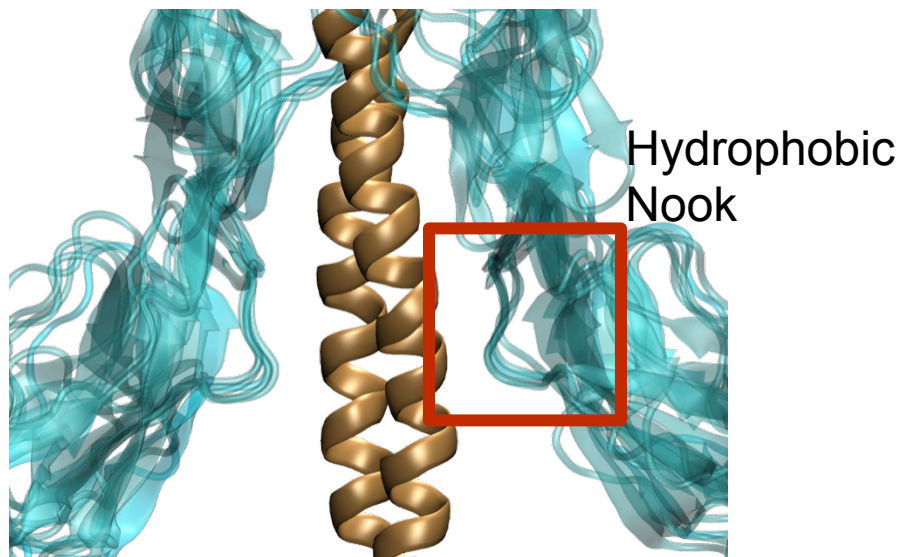


Supplementary Figure S12. C4BP-M2 interaction.

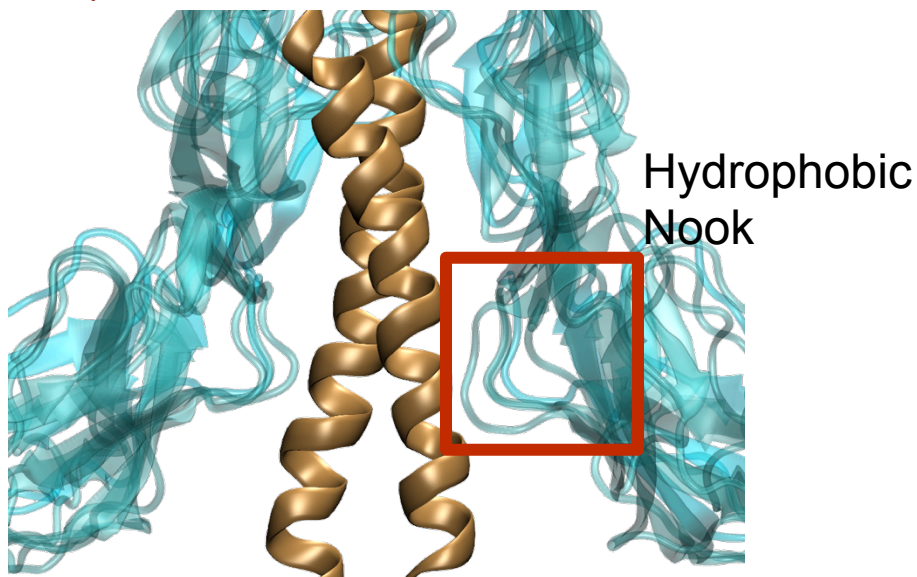
a. Input samples from the experiment shown in Figure 4a visualized by non-reducing, Coomassie-stained SDS-PAGE. Molecular mass markers were not run on these particular gels; their positions are based on measurements from equivalent gels.

b. C4BPα1-2 visualized on Coomassie-stained SDS-PAGE under non-reducing and reducing conditions, showing that C4BPα1-2 used in these experiments is intact and not nicked. Molecular mass markers are in the leftmost lane.

M2-C4BP α 1



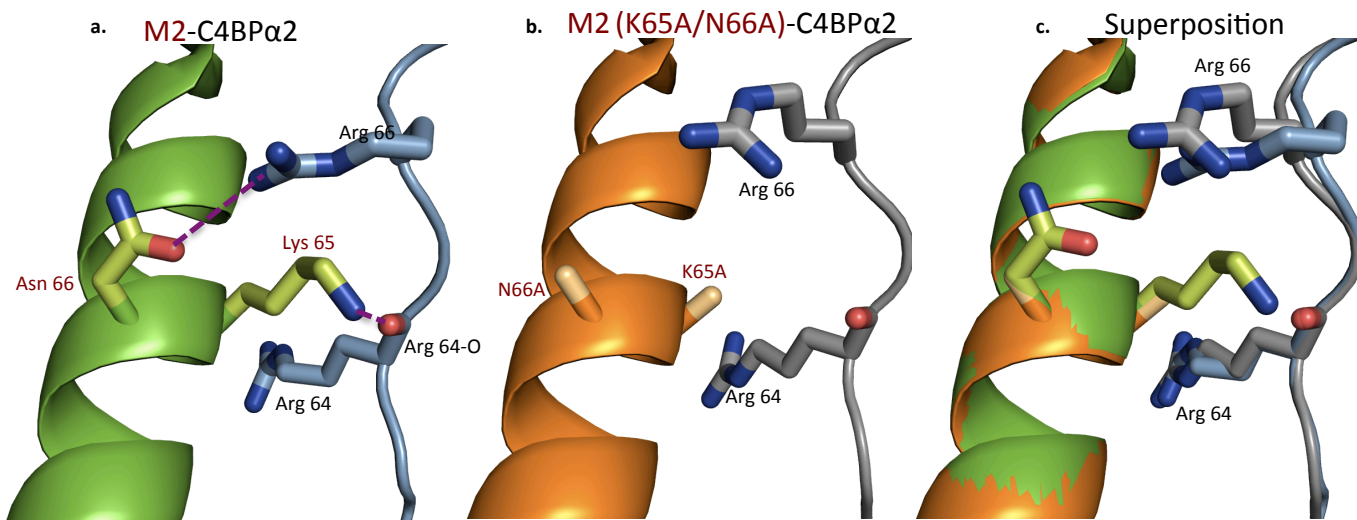
M2 (F75A)-C4BP α 1



Supplementary Figure S13. Molecular dynamics simulation of the Arg39 'hydrophobic nook' interaction with wild-type M2 and M2 F75A.

Five overlaid replicates of simulated interactions between C4BP α 1-2 (cyan, ribbon representation) and wild-type M2 (upper panel) or M2 F75A (lower panel); C4BP α 1-2 is in cyan ribbon representation, and M2 in gold ribbon representation. The simulated coordinates of the five replicates were aligned on M2 protein. The images highlight the hydrophobic C4BP α 1 Arg39 'hydrophobic nook'.

Supplementary Figure S14



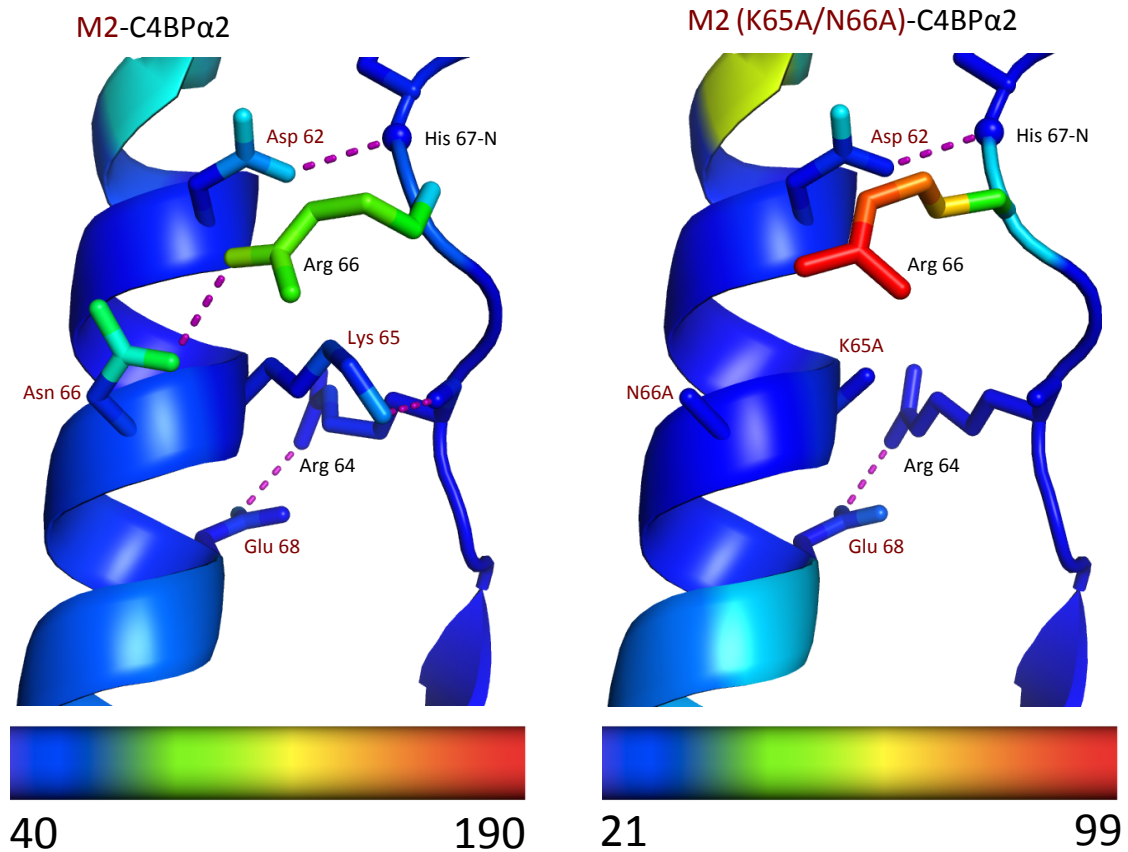
Supplementary Figure S14. Interactions of M2 and M2 (K65A/N66A) with C4BPα2.

a. M2 is depicted as a green ribbon, with Lys 65 and Asn 66 in bonds representation (carbons are green, oxygens red, and nitrogens blue). C4BPα2 is in cyan ribbon representation, with Arg 64 and its main chain carbonyl and Arg 66 in bonds representation (carbons are cyan, oxygens red, and nitrogens blue). Hydrogen bonds are depicted as red dashed lines.

b. M2 (K65A/N66A) is depicted as a gold ribbon, with Ala 65 and Ala 66 in bonds representation (carbons are light gold). C4BPα2 is in gray ribbon representation, with the same groups as in panel a shown.

c. Superposition of the structures shown in panels a and b.

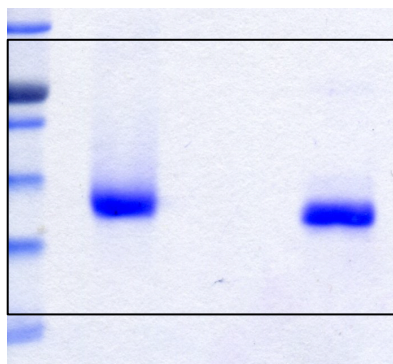
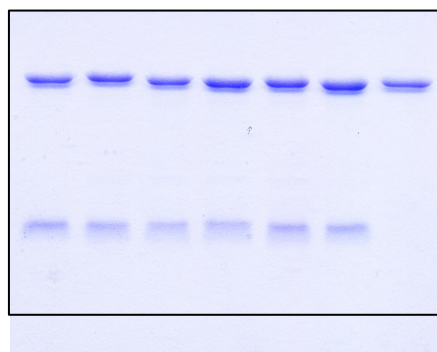
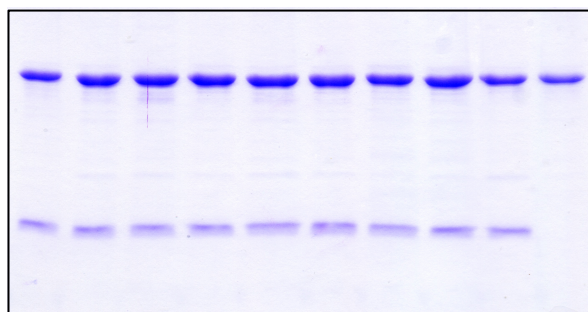
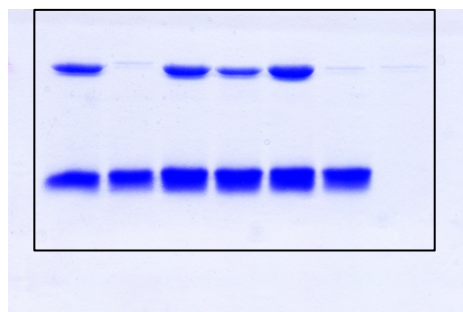
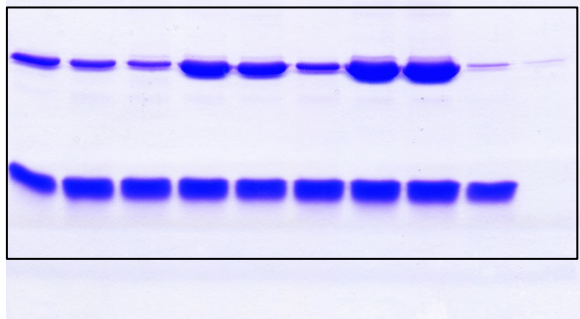
Supplementary Figure S15



Supplementary Figure S15. B-factors of C4BPα2 bound to M2 or M2 (K65A/N66A).

B-factors in the M2-C4BPα1-2 (left) and M2 (K65A/N66A)-C4BPα1-2 (right) structures represented by a color spectrum. The coloring of the M2-C4BPα1-2 complex is from the lowest to highest B-factor in the structure. The scale for the M2 (K65A/N66A)-C4BPα1-2 complex was set equivalent to that of the M2-C4BPα1-2 complex, but adjusted to account for the difference in Wilson B-factors between these two structures. This was done by dividing the B-factors of the M2-C4BPα1-2 structure by the ~2-fold lower Wilson B-factor of the M2 (K65A, N66A)-C4BPα1-2 structure (30.9 vs 59.7 Å²).

Supplementary Figure S16



Supplementary Figure S16. Uncropped gels from Figures 4a (top two) and Supplementary Figure S12 (bottom three).

Supplementary Video Legends

Supplementary Video S1. R39 nook in M2-C4BP α 1-2.

Portion of the molecular dynamics simulation of the M2-C4BP α 1-2 complex focusing on the R39 'hydrophobic nook'. M2 is in gold ribbon representation, and C4BP in cyan ribbon representation. Individual side chains are shown in bonds representation, with carbons cyans, nitrogens blue, and oxygens red. Water molecules are shown as purple spheres. Of particular note is the lack of water molecules at the M2-C4BP α 1 interface, and the stability of the loop on which C4BP R39 is located.

Supplementary Video S2. R39 nook in M2 (F75A)-C4BP α 1-2.

Portion of the molecular dynamics simulation of the M2 (F75A)-C4BP α 1-2 complex. The depiction is the same as in Supplementary Video S1. Of particular note is the increased number of water molecules at the M2 (F75A)-C4BP α 1 interface, and the instability of the loop on which C4BP R39 is located.

Supplementary Video S3. C4BP α 2 contacts in M2-C4BP α 1-2.

Portion of the molecular dynamics simulation of the M2-C4BP α 1-2 complex. The depiction is the same as in Supplementary Video S1. Of particular note is the infrequent interaction between C4BP α 2 R66 and M2 N66.

Supplementary Video S4. C4BP α 2 contacts in M2 (K65A)-C4BP α 1-2.

Portion of the molecular dynamics simulation of the M2 (K65A)-C4BP α 1-2 complex. The depiction is the same as in Supplementary Video S1. Of particular note is the increased frequency of interaction between C4BP α 2 R66 and M2 N66.

Supplementary Video S5. C4BP α 2 contacts in M2 (N66D)-C4BP α 1-2.

Portion of the molecular dynamics simulation of the M2 (N66D)-C4BP α 1-2 complex. The depiction is the same as in Supplementary Video S1. Of particular note is the increased frequency of interaction between C4BP α 2 R66 and M2 N66D.

Supplementary References

1. Edgar, R. C. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**, 1792-1797, (2004).