

Supplementary Table 1**ITC-analysis of the NatA (Naa15-Naa10 Δ C) complex interaction with different HypK variants.**

	HypK	HypKΔN50	HypK-THB
K_d (nM)	38.3 \pm 7.3	28.6 \pm 3.3	46.9 \pm 12.6
n	0.98 \pm 0.006	0.93 \pm 0.03	1.13 \pm 0.009
ΔH (kcal mol⁻¹)	- 20.3 \pm 0.2	- 9.29 \pm 0.05	- 10.1 \pm 0.01
-TΔS (kcal mol⁻¹)	10.2	- 0.92	52.4
ΔG (kcal mol⁻¹)	- 10.1	- 10.2	42.3

Supplementary Table 2

Exact sequence of the TPRs in CtNaa15 as predicted by the TPR-prediction server¹ (<https://toolkit.tuebingen.mpg.de/tprpred>).

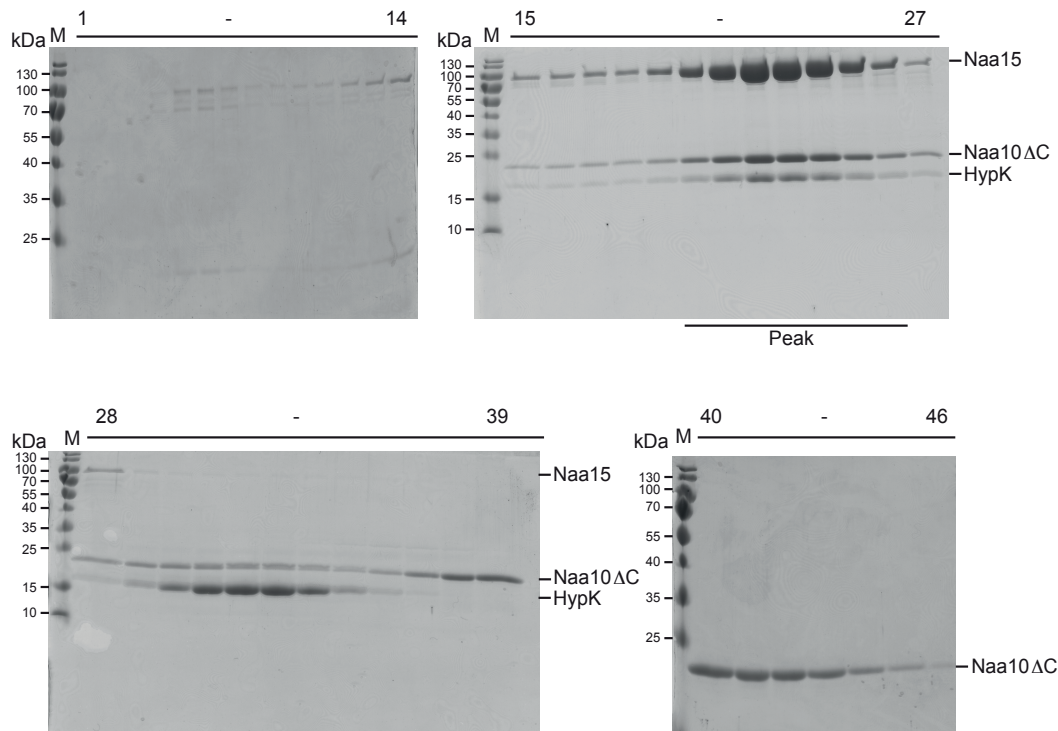
Cluster	TPR	Start residue	End residue	Sequence
TPR-N	1	9	42	EANLFRTVIRHYEDKQYKRGLKAAEQILKKNPKH
TPR-N	2	43	76	GDTMSMKALILNAQGKTEEFALAKEALTIDMKS
TPR-N	3	77	110	YICWHVYGILYRTNKNFDEAIKAYKFALKLEPES
TPR-N	4	111	144	HQIQRD LAVLQIQMRDYAGYVQSRLNMLKARPQI
TPR-N	5	145	178	RQNWTALAIAYHLEGNLEKAEHILTTYEKSLTTP
TPR-M1	6	187	220	SEALLYKNTIIAERGDIERALQHLETDCCKHCLDR
TPR-M1	7	221	254	LAVMELRASYSKLRKDEAAKAYRALLDRNPEH
TPR-M2	8	373	406	ALYYLAQHYNYYMSRDLTRALEYVEKAIELDPKN
TPR-M2	9	407	440	VDFHMTKARIFKHQGD LAKAAETMDYARSLDPKD
TPR-M2	10	443	476	INSKAAKYQLRNNENEKALATMGLFTRAETAGGP
TPR-M2	11	486	519	IWFLTEDGEAWQRRGNTALALKRYHTVFSIFDTW
TPR-C	12	638	671	KDDDPNGEKLAATKDPLGDAMKFLNYILQFSPKN
TPR-C	13	672	705	IDGQIAGFEVYIRKKKYL LALRCLKAASAIKKNH

Supplementary Table 3**Interactions between HypK-THB and Naa15 as evaluated using PISA from CCP4².**

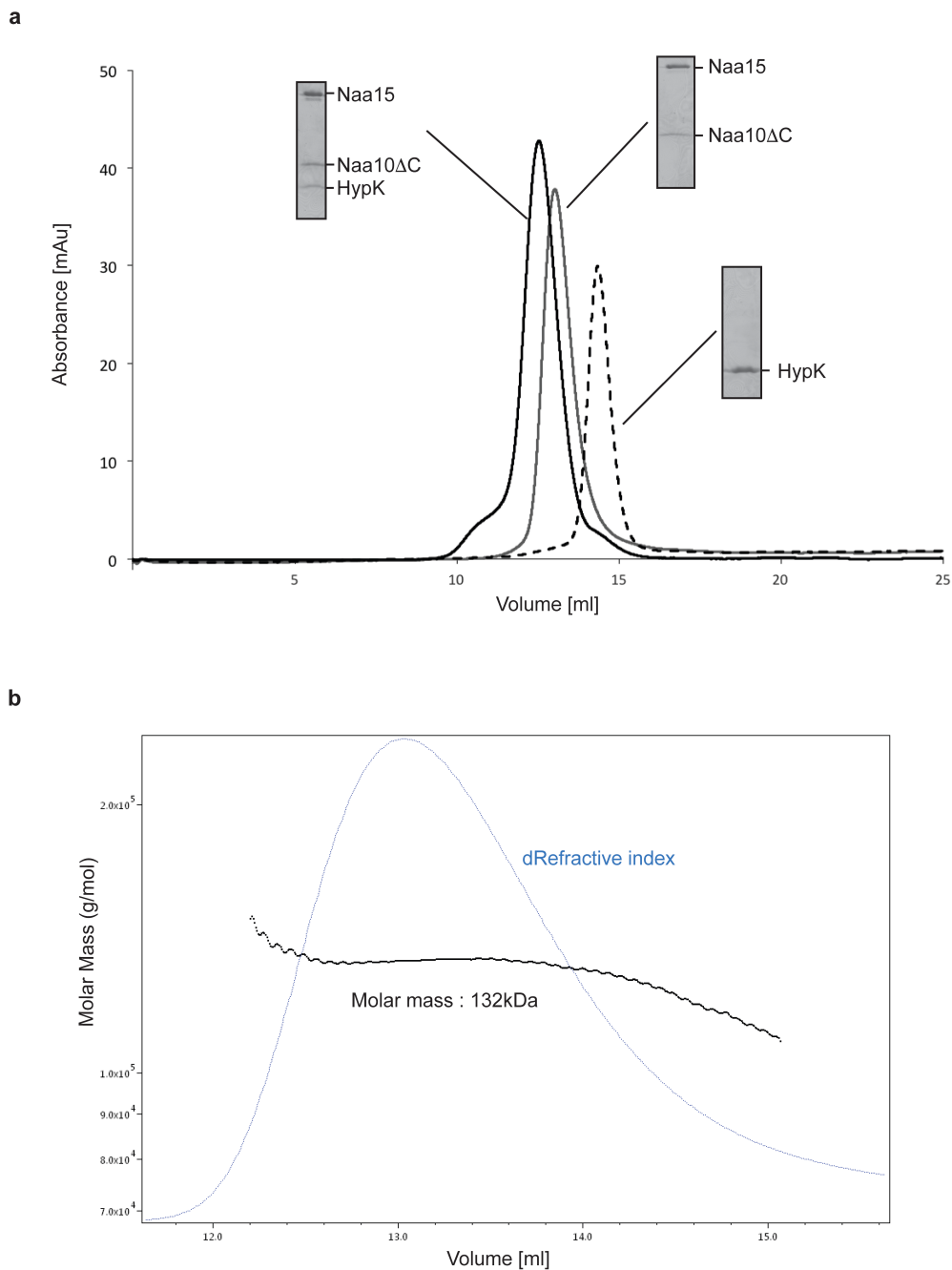
	HypK-THB	Naa15
Hydrogen bonds	α 3: Asp88	α 38: Lys729
	α 3: Glu94	loop α 35- α 36: Lys687
	α 5: Gln123	α 38: Glu737
	loop α 3- α 4: Glu97	loop α 35- α 36: Lys685
	α 5: Tyr121	α 36: Arg693
	α 5: Gln123	α 36: Arg696
Salt bridges	α 3: Asp88	α 38: Lys729
	α 3: Glu94	loop α 35- α 36: Lys687
	loop α 3- α 4: Glu97	loop α 35- α 36: Lys685
	loop α 3- α 4: Glu97	loop α 35- α 36: Lys687

Supplementary Table 4**Primers used in this study.**

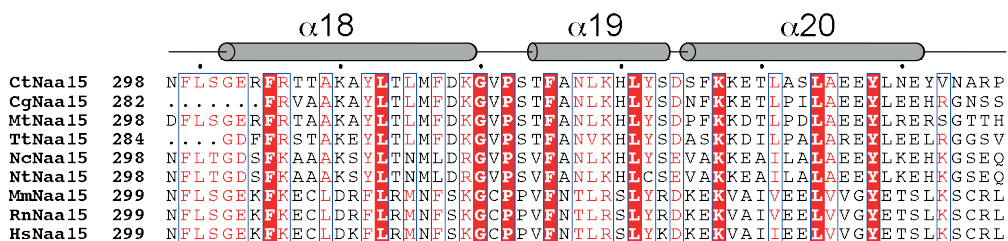
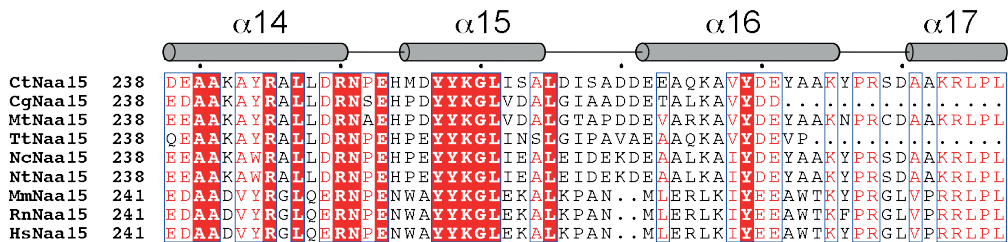
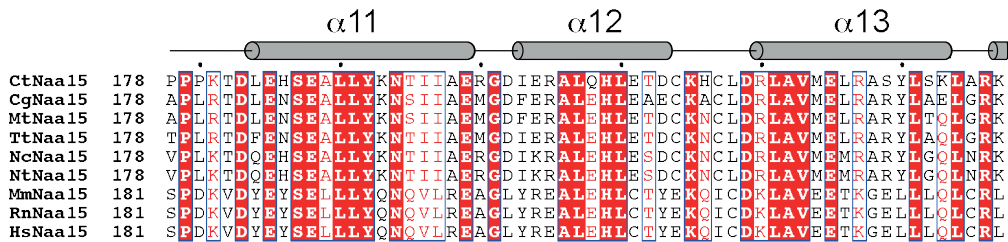
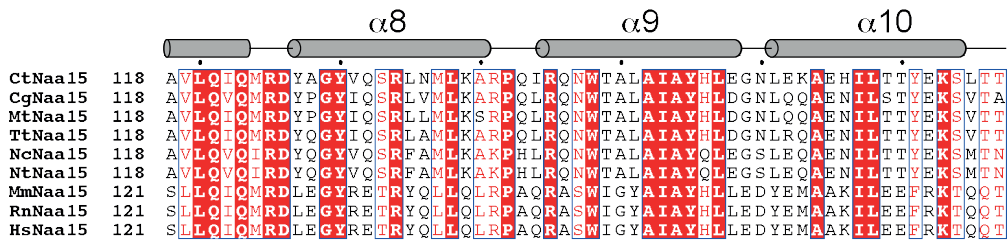
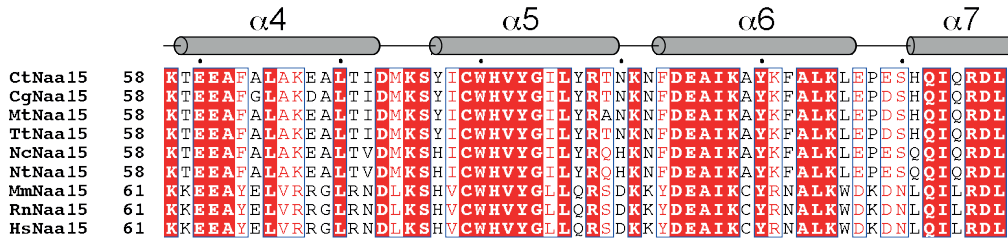
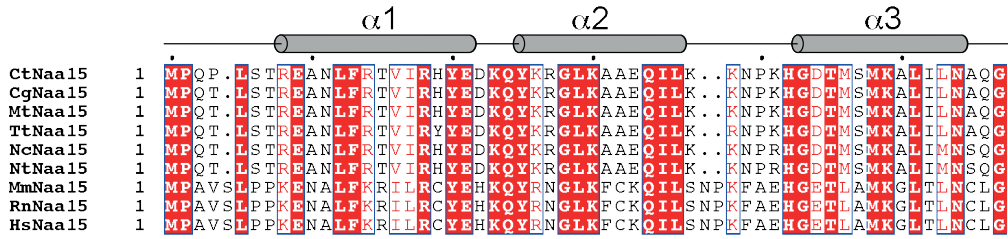
Name of primer	Sequence	5'	3'
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ct-Nat1-R	CTAGGCGCCAGGCACCA		
Nat-dNco-A-F	GGCTGCAGAACTATGGACTATGCGCGG		
Nat-dNco-A-R	CCGCGCATAGTCCATAGTTTCTGCAGCC		
Nat-dNco-B-F	GCCCTTGCCACTATGGGCTTGTTCACTC		
Nat-dNco-B-R	GAGTGAACAAGCCCATAGTGGCAAGGGC		
Nat1-NcoI-oH-F	TTAACCATGGGCCCCGAGCCACTGAGTAC		
Nat-Nco-H6-F	TTAACCATGGGCCACCATCACCATCACCATCCGCAGCCACT GAGTAC		
Nat1-Bam-R	TTAAGGATCCTTAGGCGCCAGGCACACCAAC		
Ard1-delNco-F	GAGCCAAGTAGCAATGGTAGAAACCTAC		
Ard1-delNco-R	GTAGGTTTCTACCATTGCTAGTTGGCTC		
Ard1-Nco-F	TTAACCATGGACATCCGCCTCCTCCG		
Ard1-6H-Bam-R	TTAAGGATCCTTAATGGTGATGGTGATGGTGTGTATGCTTA CTCTCATCC		
Ard1-Bam-R	TTAAGGATCCTTATGTATGCTTACTCTCATCCC		
Ard1-dC54-R	TTAAGGATCCTTAATGGTGATGGTGATGGTGATTAACGCCA TTCGAACCTGCAGC		
HypK-NcoI-6H-F	TTAACCATGGGCCACCATCACCATCACCATGCTG CCGAGGACAGACAGCCC		
HypK_Nco_F	TTAACCATGGGCGCTGCCGAGGACAGACAGCCC		
HypK-BamHI-R	TTAAGGATCCTTAAAAAGCAGGCTGGATATAAGCCTTCA		
ctHypKDN1CStrep_F	GTCTGTCTCGGCAGCCTGAAAATAAAGATTCTCAG		
ctHypKDN1CStrep_R	CTGAGAATCTTTATTTTTCAGGCTGCCGAGGACAGAC		
ctHypKDN14CStrep_F	CTGAGAATCTTTATTTTTCAGGCCACCGCAGGCGATG		
ctHypKDN14CStrep_R	CATCGCCTGCGGTGGCCTGAAAATAAAGATTCTCAG		
ctHypKDN25CStrep_F	GAGAATCTTTATTTTTCAGGCCCGGCCGCAAG		
ctHypKDN25CStrep_R	CTTGGCGGCCGGGCTGAAAATAAAGATTCTC		
HypKDC44_Ba_R	AATTAAGGATCCTTACTTCTTGACCTCCTTCTTCTCC		
HypKdN50_Nco_F	TTAATTCCATGGCCCCGGCCCCGGAAG		
HypK_THB_Nco_F	TTAATTCCATGGGGAAGGTCGACCCGGCTGATGT		
ctHypK_CStrepII_R	TTAATTAAGCTTTTATTTTTCGAACTGCGGGTGGCTCCAGC TACCACTACCGGATCCAAAAGCAGGCTGGATATAAGCCTT		



Supplementary Figure 1: Purification of the HypK - NatA complex. Uncropped SDS-PAGE showing all SEC fractions of **Fig. 1b**. M: protein ladder. Peak: fractions from the SEC peak (**Fig. 1b**).



Supplementary Figure 2: Analytical size exclusion chromatography and SEC-MALS of the NatA - HypK complex. (a) Sequential runs of NatA (Naa15-Naa Δ C, gray line), HypK (black dotted line) and NatA-HypK (black line) on a Superdex S200 10/300. The peak was analyzed by SDS-PAGE (shown as an inset). (b) SEC-MALS analysis of the NatA - HypK complex (theoretical calculated molar mass: 122 kDa). The differential refractive index is shown as blue line, the absolute molecular mass as black dotted line.

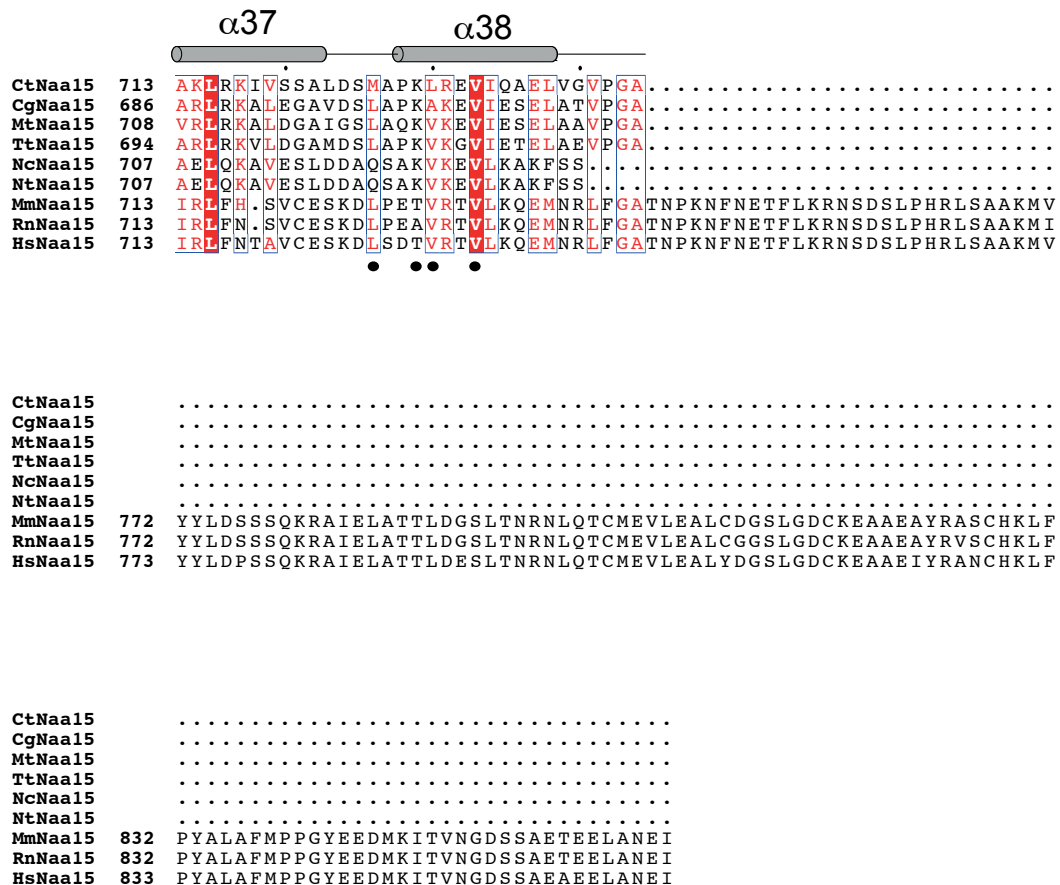


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CgNaa15	335	...	A	N	G	D	S	.	.	.	K	G	E	G	A	A	L	Y	F	L	A	O	H	Y	N	Y	S	R	D	L	S	R	A	T	E	V	V	E	K	A	I	E	L	D	P	K	N	V	D	F	H	M	T	K	A	R
MtNaa15	358	TDGQTN	G	D	S	.	.	.	K	G	E	G	A	A	L	Y	F	L	A	O	H	Y	N	Y	S	R	D	L	S	R	A	T	E	V	V	E	K	A	I	E	L	D	P	K	N	V	D	F	H	M	T	K	A	R		
TtNaa15	340	ARDQAN	G	D	S	.	.	.	K	G	E	G	A	A	L	Y	F	L	A	O	H	Y	D	Y	L	S	R	D	L	A	R	A	M	E	V	V	E	K	A	I	E	L	D	P	K	S	V	D	F	H	M	T	R	A	R	
NcNaa15	358	...	A	N	G	D	S	.	.	.	K	G	V	G	A	L	Y	F	L	A	O	H	Y	N	Y	S	R	D	L	A	K	A	M	E	V	V	E	Q	A	I	E	L	D	P	K	N	V	D	F	H	M	T	K	A	R	
NtNaa15	358	...	A	N	G	D	S	.	.	.	K	G	V	G	A	L	Y	F	L	A	O	H	Y	N	Y	S	R	D	L	A	K	A	M	E	V	V	E	Q	A	I	E	L	D	P	K	N	V	D	F	H	M	T	K	A	R	
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RnNaa15	359	FNPNDG	G	K	E	E	P	P	T	T	L	L	W	V	Q	Y	L	A	O	H	Y	D	K	I	G	.	.	P	S	I	A	L	E	V	I	N	T	A	I	E	S	T	P	T	L	I	E	L	F	L	V	K	A	K		
HsNaa15	359	FNPNDG	G	K	E	E	P	P	T	T	L	L	W	V	Q	Y	L	A	O	H	Y	D	K	I	G	.	.	P	S	I	A	L	E	V	I	N	T	A	I	E	S	T	P	T	L	I	E	L	F	L	V	K	A	K		

CtNaa15	416	I	F	K	H	Q	G	D	L	A	K	A	E	T	M	D	Y	A	R	S	L	D	P	K	D	R	Y	I	N	S	K	A	A	K	Y	Q	L	R	N	N	E	N	E	K	A	L	A	T	M	G	L	F	T	R	A	E	T	A	G	
CgNaa15	389	I	F	K	H	Q	G	E	I	A	K	A	E	A	M	D	H	A	R	S	L	D	T	R	D	R	Y	I	N	S	K	A	A	K	Y	Q	L	R	N	N	E	N	E	K	A	L	A	T	M	G	L	F	T	R	A	E	T	V	G	
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NcNaa15	412	I	F	K	H	Q	G	D	T	A	K	A	S	E	T	M	D	R	A	R	S	L	D	T	K	D	R	Y	I	N	S	K	A	A	K	Y	Q	L	R	N	N	E	N	E	K	A	L	E	T	M	G	L	F	T	R	A	E	T	V	G
NtNaa15	412	I	F	K	H	Q	G	D	T	A	K	A	S	E	T	M	D	R	A	R	S	L	D	T	K	D	R	Y	I	N	S	K	A	A	K	Y	Q	L	R	N	N	E	N	E	K	A	L	E	T	M	G	L	F	T	R	A	E	T	V	G
MmNaa15	417	I	Y	K	H	A	G	N	I	K	E	A	A	R	W	M	D	E	A	Q	A	L	D	T	A	D	R	F	I	N	S	K	C	A	K	Y	M	L	K	A	N	L	I	K	E	A	E	M	C	S	K	F	T	R	E	G	T	S	..	
RnNaa15	417	I	Y	K	H	A	G	N	I	K	E	A	A	R	W	M	D	E	A	Q	A	L	D	T	A	D	R	F	I	N	S	K	C	A	K	Y	M	L	K	A	N	L	I	K	E	A	E	M	C	S	K	F	T	R	E	G	T	S	..	
HsNaa15	417	I	Y	K	H	A	G	N	I	K	E	A	A	R	W	M	D	E	A	Q	A	L	D	T	A	D	R	F	I	N	S	K	C	A	K	Y	M	L	K	A	N	L	I	K	E	A	E	M	C	S	K	F	T	R	E	G	T	S	..	

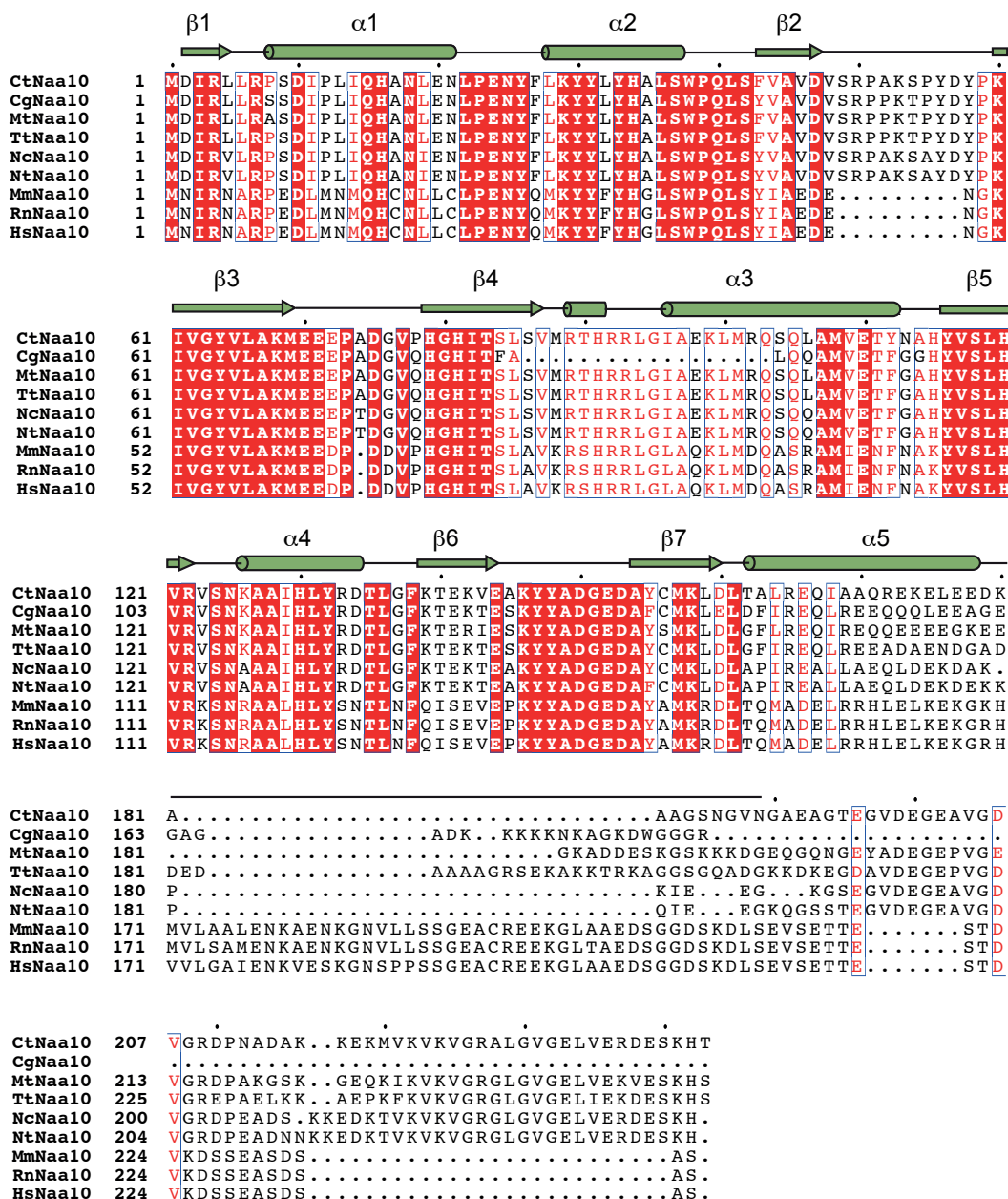
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CgNaa15	449	P	L	V	D	L	T	D	M	Q	C	I	W	F	L	T	E	D	G	E	A	W	Q	R	R	G	N	I	G	L	A	L	K	R	Y	H	T	I	F	N	I	F	D	I	W	Q	E	D	Q	F	D	F	H	S	F	S	L	R	K	G
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RnNaa15	475	A	V	E	N	L	N	E	M	Q	C	M	W	F	Q	T	E	C	A	Q	A	Y	K	A	M	N	K	F	G	E	A	L	K	K	C	H	E	I	E	R	H	F	E	I	T	D	D	Q	F	D	F	H	Y	C	M	R	K	I		
HsNaa15	475	A	V	E	N	L	N	E	M	Q	C	M	W	F	Q	T	E	C	A	Q	A	Y	K	A	M	N	K	F	G	E	A	L	K	K	C	H	E	I	E	R	H	F	E	I	T	D	D	Q	F	D	F	H	Y	C	M	R	K	I		

CtNaa15	536	I	R	A	Y	V	D	M	V	R	W	E	D	R	L	R	E	H	P	F	Y	F	R	A	L	D	A	V	N	L	Y	L	S	M	Y	D	K	P	Q	S	.	A	N	G	A	N	G	T	E	A	A	N	P	N	G	.	E	D	A
CgNaa15	509	I	R	A	Y	I	D	L	V	R	W	E	D	R	L	R	E	H	P	F	Y	F	R	A	L	D	A	V	N	L	Y	L	S	M	Y	D	K	P	Q	.	.	.	N	G	A	N	G	V	E	G	S	H	T	N	G	E	D	A	
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NcNaa15	532	I	R	A	Y	V	D	M	I	R	W	E	D	S	I	R	E	H	P	F	Y	F	R	A	L	D	A	V	N	L	Y	L	S	M	H	D	K	P	Q	.	.	.	N	G	T	N	G	T	E	A	T	N	G	.	E	D	A		
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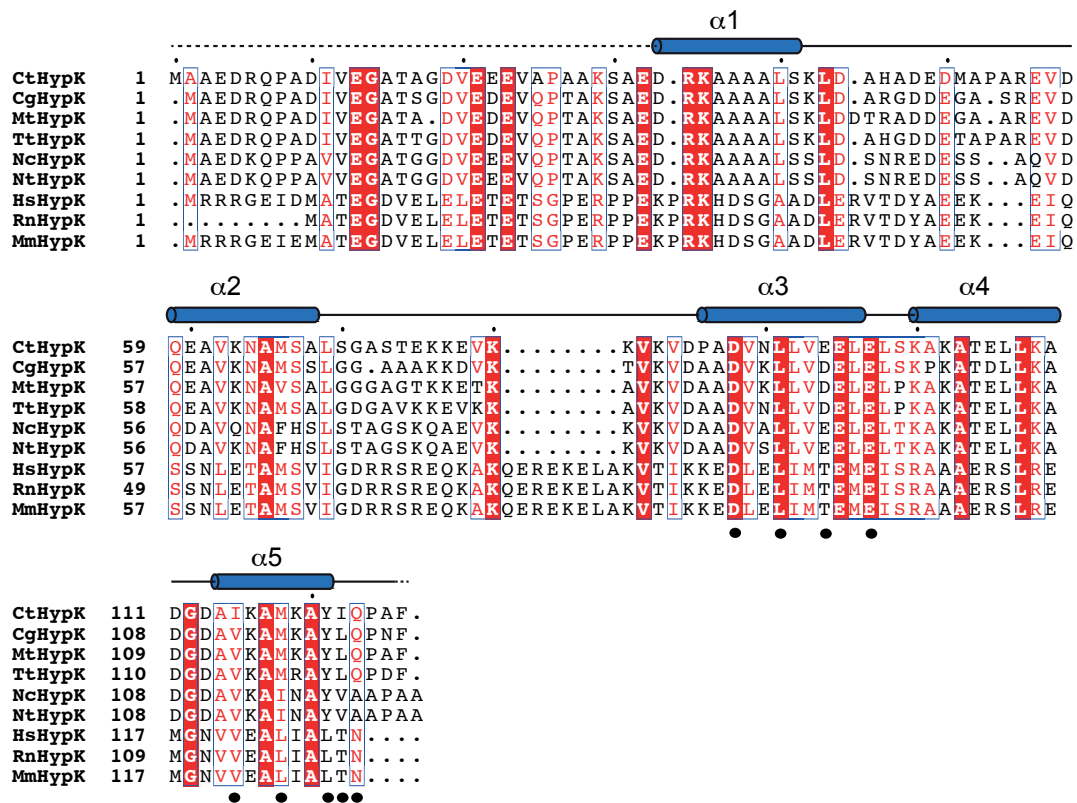
Supplementary Figure 3: Multiple sequence alignment of Naa15 homologs.

Highly conserved residues are marked with red boxes. Ct: *Chaetomium thermophilum*, Cg: *Chaetomium globosum*, Mt: *Myceliophthora thermophila*, Tt: *Thielavia terrestris*, Nc: *Neurospora crassa*, Nt: *Neurospora tetrasperma*, Mm: *Mus musculus*, Rn: *Rattus norvegicus* and Hs: *Homo sapiens*. Multiple sequence alignments were generated using ClustalW2 (ref. 3) and visualized using the ESPript server⁴ (<http://esript.ibcp.fr>). Residues involved in the interaction with HypK are highlighted with a black dot. Secondary structure elements are based on the crystal structure and are represented at the top.

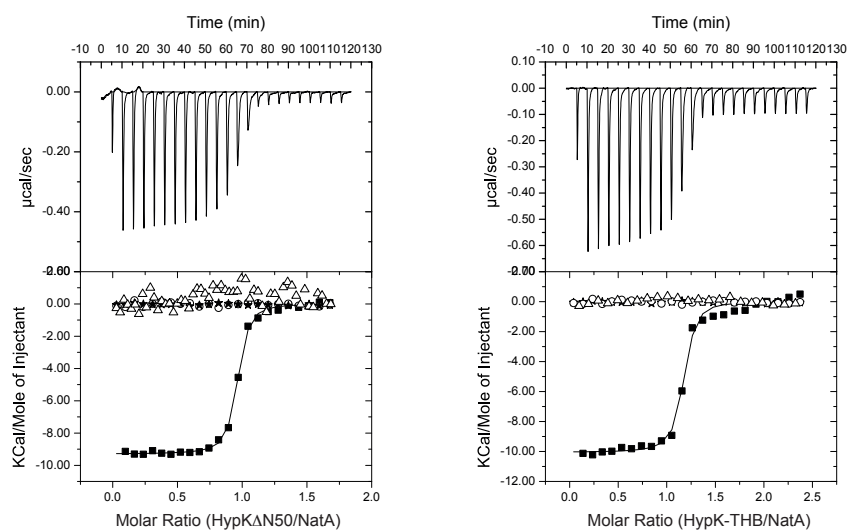


Supplementary Figure 4: Multiple sequence alignment of Naa10 homologs.

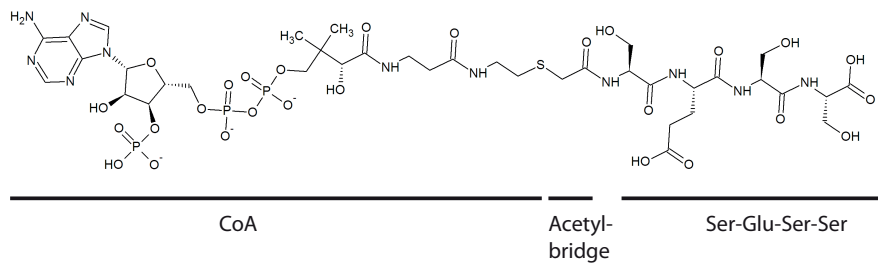
Highly conserved residues are marked with red boxes. Ct: *Chaetomium thermophilum*, Cg: *Chaetomium globosum*, Mt: *Myceliophthora thermophila*, Tt: *Thielavia terrestris*, Nc: *Neurospora crassa*, Nt: *Neurospora tetrasperma*, Mm: *Mus musculus*, Rn: *Rattus norvegicus* and Hs: *Homo sapiens*. Multiple sequence alignments were generated using ClustalW2 (ref. 3) and visualized using the ESPrnt server⁴ (<http://esprnt.ibcp.fr>). Secondary structure elements are based on the crystal structure (this study) and are represented at the top.



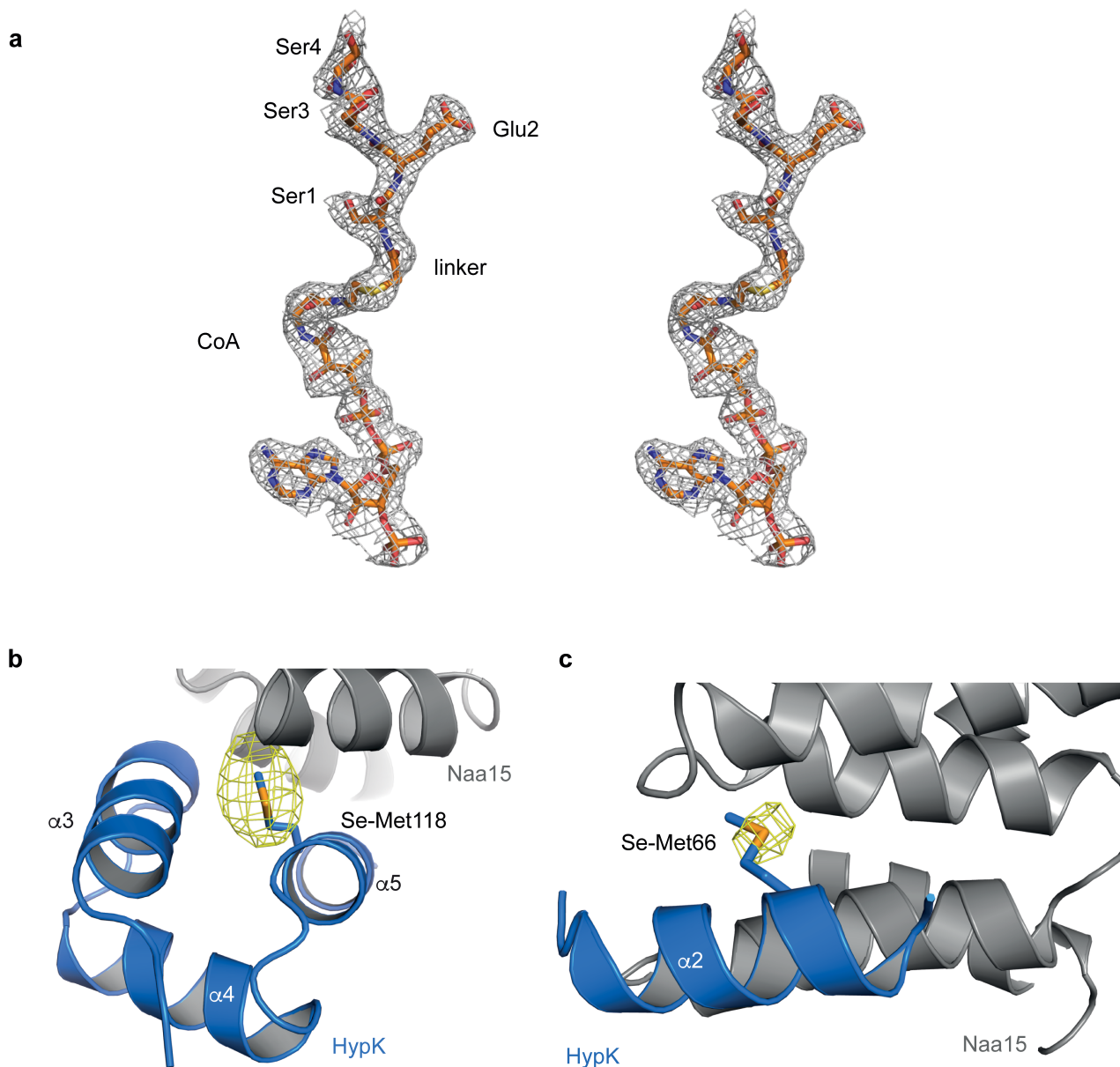
Supplementary Figure 5: Multiple sequence alignment of HypK homologs. Highly conserved residues are marked with red boxes. Ct: *Chaetomium thermophilum*, Cg: *Chaetomium globosum*, Mt: *Myceliophthora thermophila*, Tt: *Thielavia terrestris*, Nc: *Neurospora crassa*, Nt: *Neurospora tetrasperma*, Hs: *Homo sapiens*, Rn: *Rattus norvegicus* and Mm: *Mus musculus*. Multiple sequence alignments were generated using ClustalW2 (ref. 3) and visualized using the ESPript server⁴ (<http://esript.ibcp.fr>). Residues involved in the interaction with Naa15 are highlighted with a black dot. Secondary structure elements are based on the crystal structure (this study) and are represented at the top.



Supplementary Figure 6: Characterization of the interaction between the NatA complex and different HypK-deletion variants by isothermal titration calorimetry. Titration of the NatA complex with HypK Δ N50 or HypK-THB (black square). The heats of dilution of the buffer-buffer (black star), buffer-NatA (empty circle) and HypK variants-buffer (empty triangle) are represented.

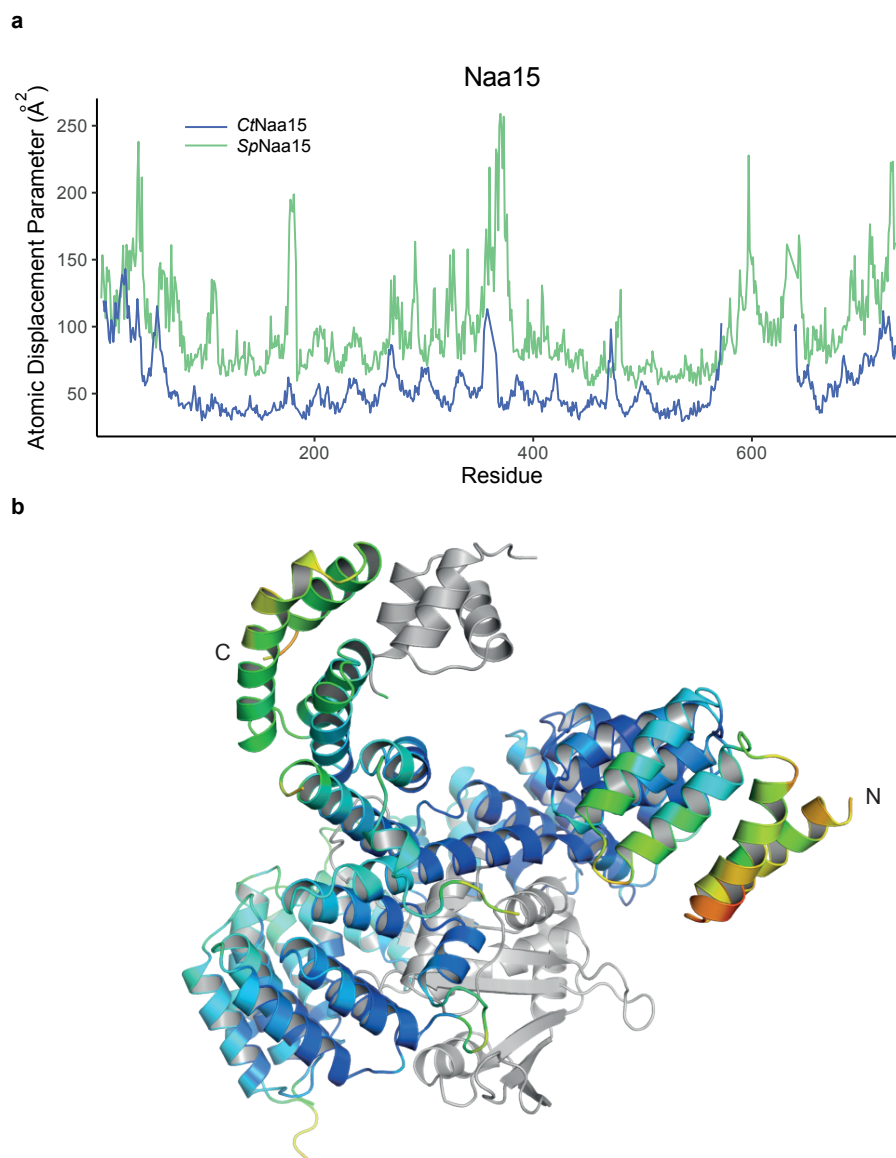


Supplementary Figure 8: Structure of the CoA-SESS bi-substrate⁶.

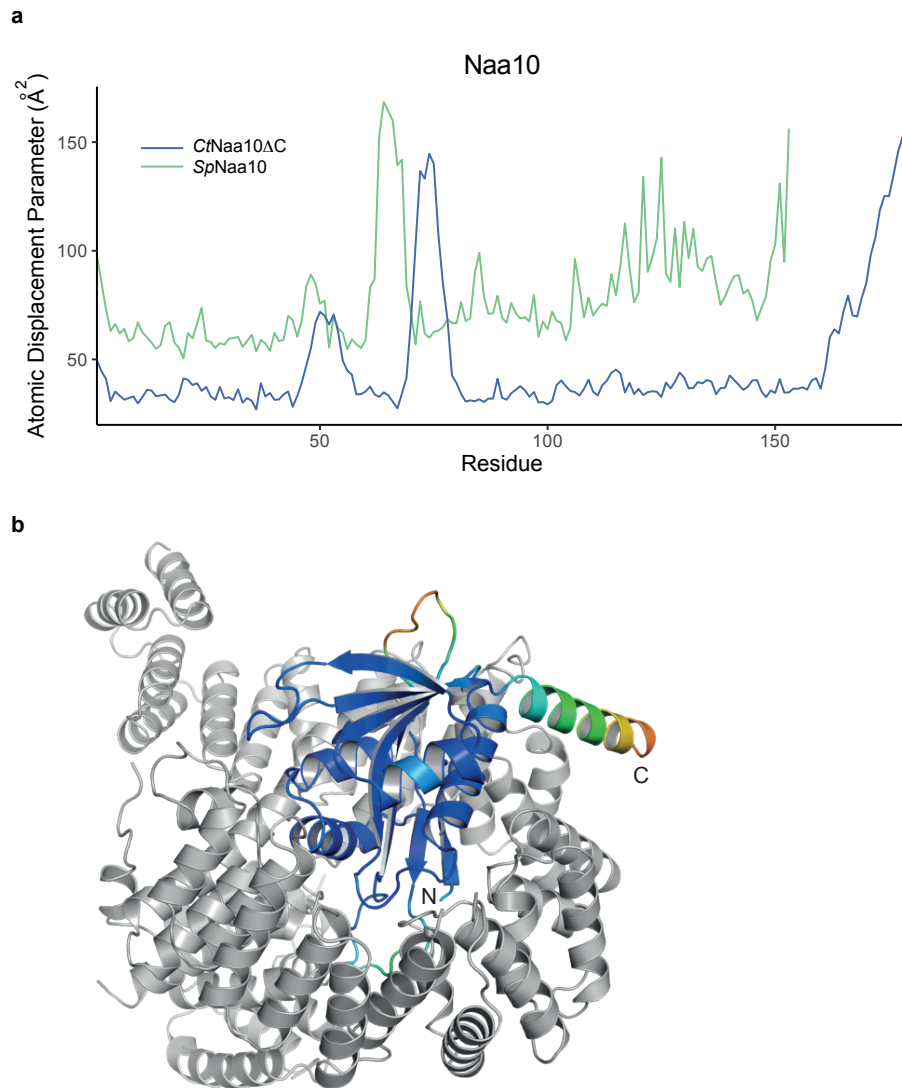


Supplementary Figure 9: Electron density maps of selected regions of HypK.

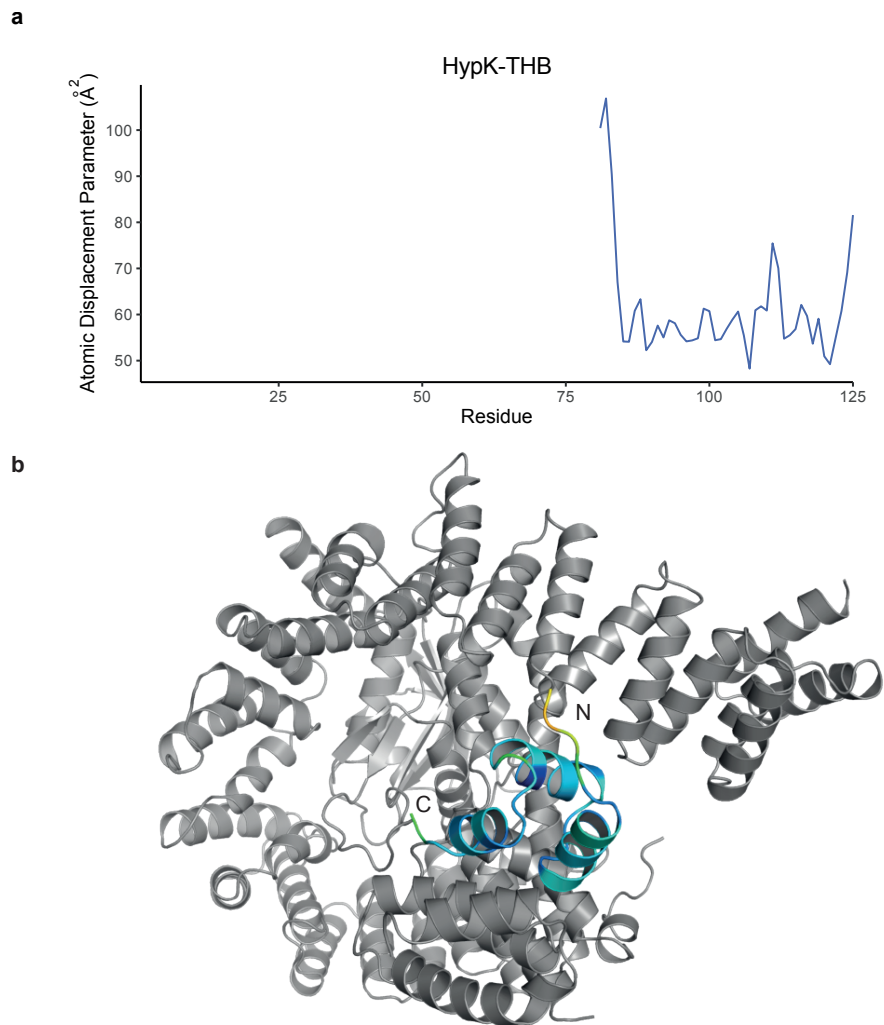
(a) Stereo view of the bi-substrate analog CoA-SESS, shown as orange sticks with corresponding electron density at contour level of 1σ (gray mesh) of a 2mFo-DFc simulated annealing composite omit map. The two CoA-SESS molecules were removed from the model prior to map calculation. The 2mFo-DFc composite omit map was calculated in Phenix⁷ using the torsion angle simulated annealing option with an omit fraction of 5% at a time. (b-c) Anomalous density map of the selenomethionine residue Se-Met118 (b) and Se-Met66 (c) used to assign the sequence is shown at contour level of 5σ (yellow mesh). HypK, blue; Naa15, gray.



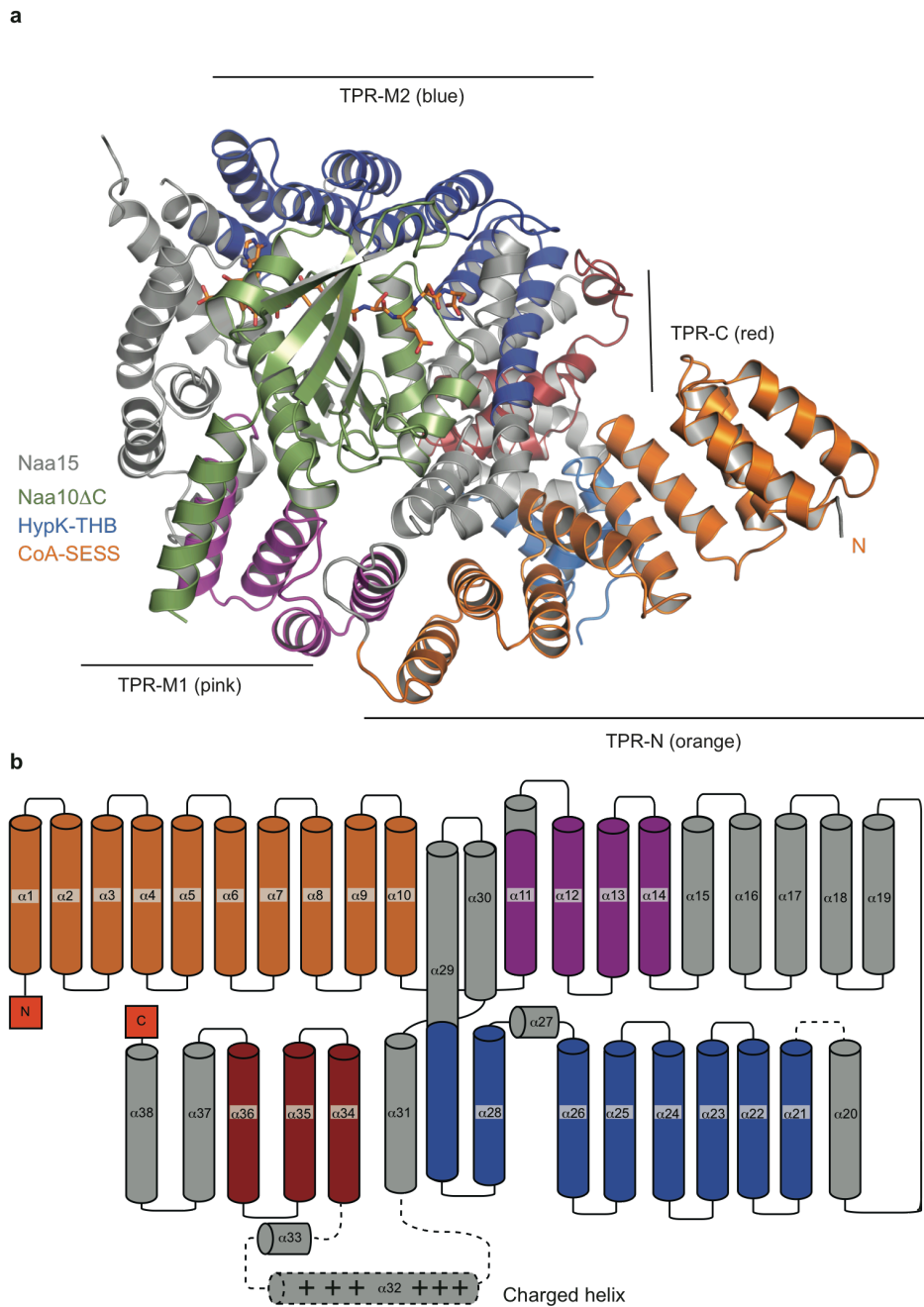
Supplementary Figure 10: B-factor plot for the Naa15 structure. (a) Atomic displacement parameter derived dynamics for *CtNaa15* (this study) and *SpNaa15* (pdb: 4KVM (ref. 8)). The atomic displacement parameters (residue average, calculated with BAVEGAGE in CCP4 (ref. 9)) are plotted as a function of the residue number for chain A. (b) Structure of HypK-THB - NatA complex. Naa15 is colored according to atomic displacement parameters (residue average), scaled from 20 (blue) to 80 (red) \AA^2 .



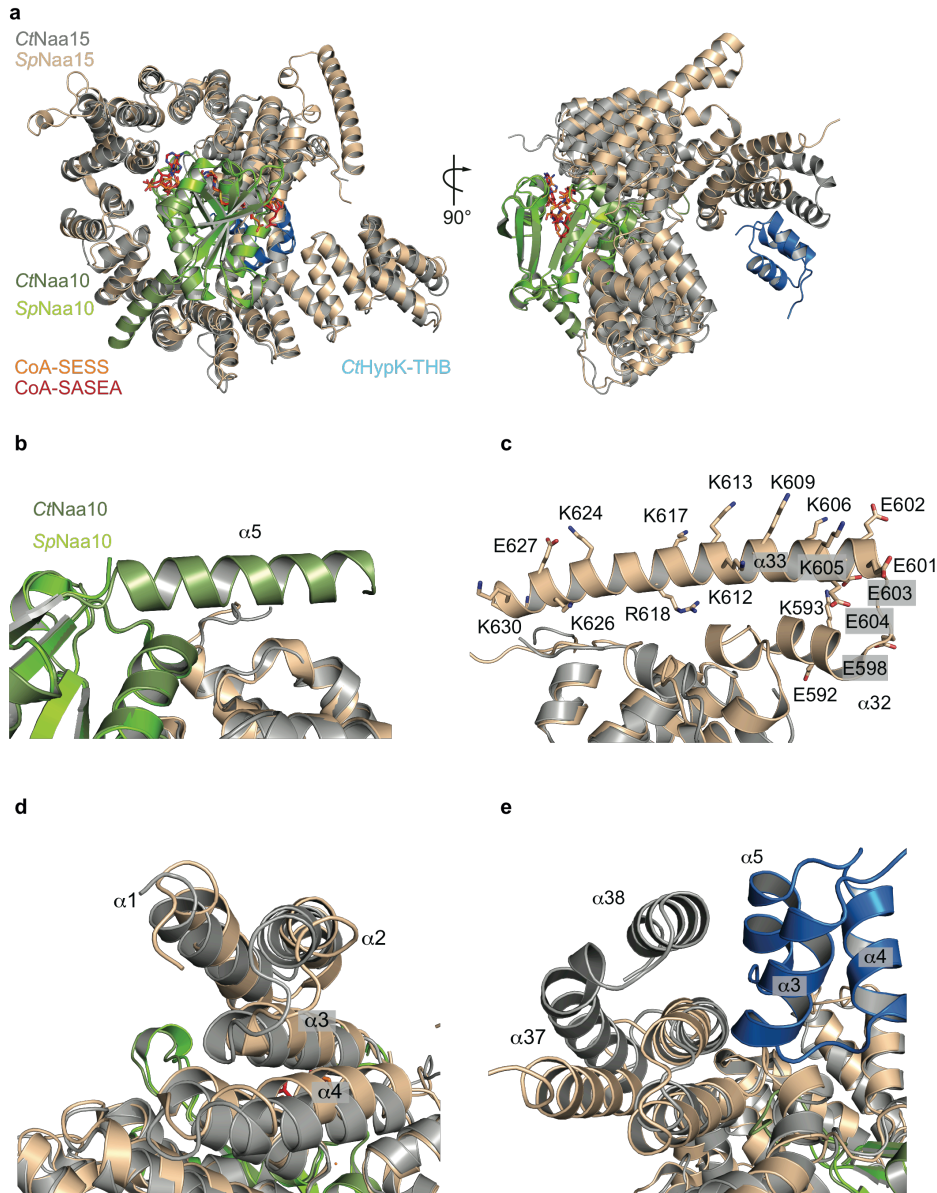
Supplementary Figure 11: B-factor plot for the Naa10 structure. (a) Atomic displacement parameter derived dynamics for *CtNaa10* (this study) and *SpNaa10* (pdb: 4KVM (ref. 8)). The atomic displacement parameters (residue average, calculated with BAVEGAGE in CCP4 (ref. 9)) are plotted as a function of the residue number for chain B. (b) Structure of HypK-THB - NatA complex. Naa10 is colored according to atomic displacement parameters (residue average), scaled from 20 (blue) to 80 (red) \AA^2 .



Supplementary Figure 12: B-factor plot for the HypK-THB structure. (a) Atomic displacement parameter derived dynamics for HypK-THB. The atomic displacement parameters (residue average, calculated with BAVEGAGE in CCP4 (ref. 9)) are plotted as a function of the residue number for chain C. (b) Structure of HypK-THB - NatA complex. HypK-THB is colored according to atomic displacement parameters (residue average), scaled from 20 (blue) to 80 (red) Å².

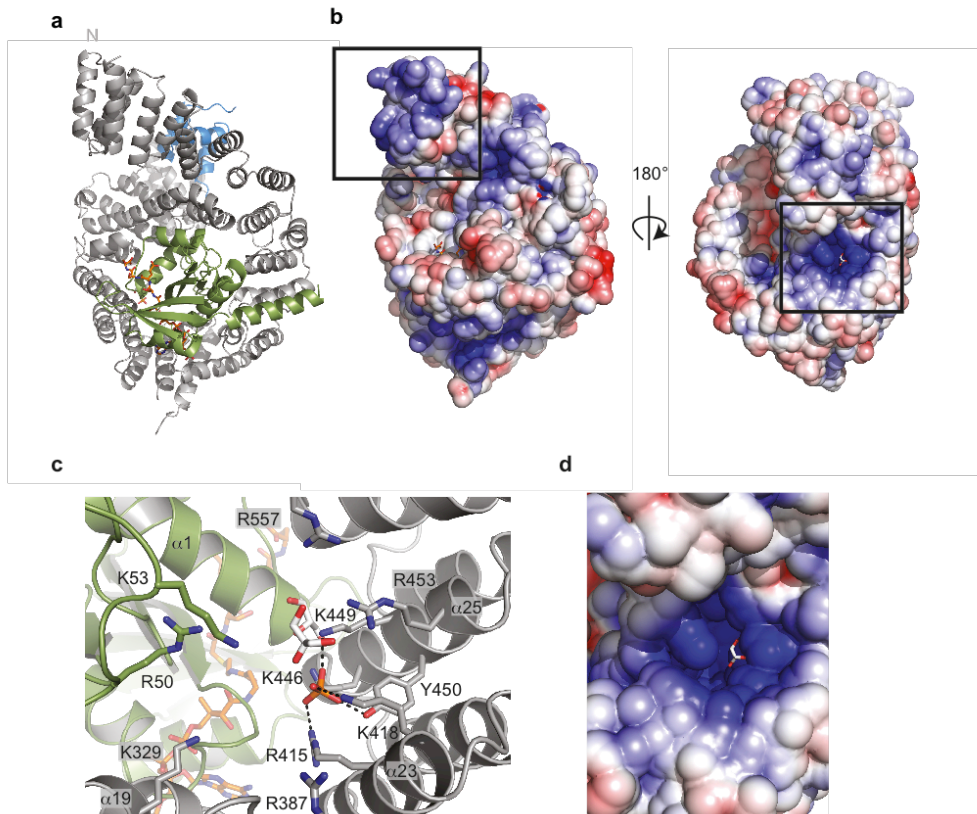


Supplementary Figure 13: Naa15 contains 13 conserved TPR repeats. (a) Ribbon representation of the Naa15 (orange, pink, blue, red and gray), Naa10ΔC (green), HypK-THB (light blue) and CoA-SESS (orange sticks) complex structure. The 13 TPR repeats of Naa15 are grouped in four clusters, the N-terminal cluster (TPR-N, orange), the two middle clusters (TPR-M1, pink; TPR-M2, blue) and the C-terminal cluster (TPR-C, red). (b) Schematic representation of the topology of Naa15. The TPR-repeats are colored according to (a).

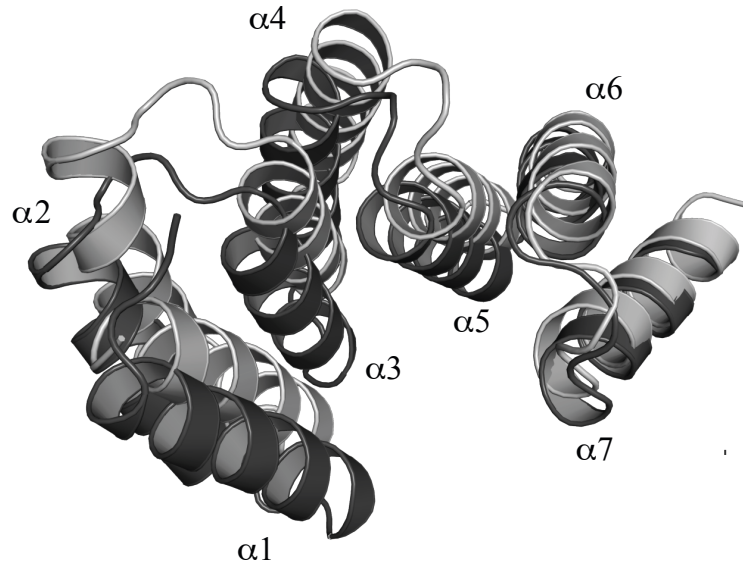


Supplementary Figure 14: Structural comparison between CtNatA and SpNatA.

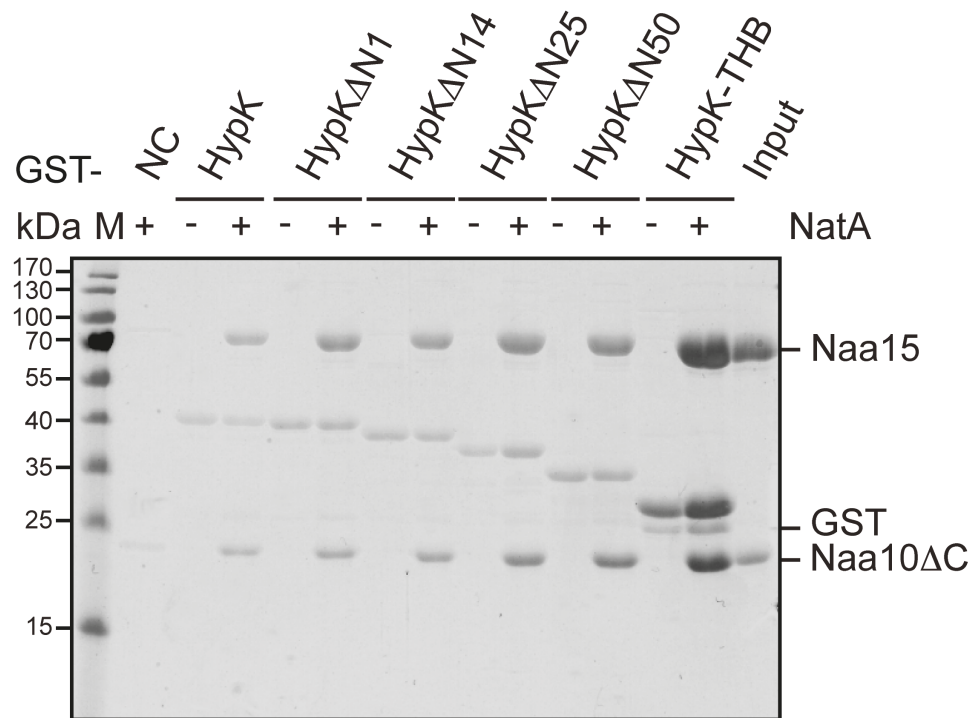
(a) Superimposition of the CtNatA - HypK-THB (Naa15 in gray, Naa10 in green, HypK-THB in light blue and CoA-SESS in orange) with the SpNatA homolog (Naa15 in wheat, Naa10 in mint and CoA-SASEA in red, pdb: 4KVM (ref. 8)). (b) Close-up view on the helix $\alpha 5$ of CtNatA structure, omitted in the construct used for SpNatA (pdb: 4KVM (ref. 8)). (c) Close up view on the helices $\alpha 32$ and $\alpha 33$. The highly mobile helices $\alpha 32$ and $\alpha 33$ are not resolved in the CtNatA structure, but in the SpNatA structure. The positions of the charged residues are labeled. (d) Close up view on the N-terminal rearrangement of Naa15. Helix $\alpha 1$ - $\alpha 4$ of CtNaa15 are rearranged in comparison with the structure of SpNaa15. (e) Close up view on the C-terminal rearrangement of Naa15. The additional helix $\alpha 38$ of CtNaa15 is visible.



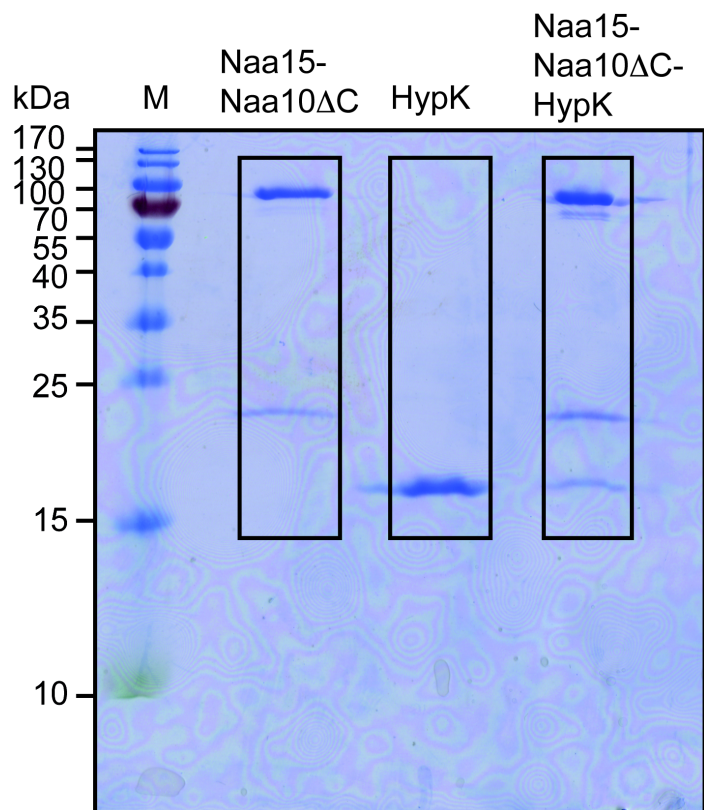
Supplementary Figure 15: Surface charge distribution of the HypK-THB - Naa15-Naa10 Δ C complex. (a) Cartoon representation of the heterotrimeric complex. Naa15 in gray, Naa10 Δ C in green, HypK-THB in blue and CoA-SESS (orange sticks). (b) Electrostatic surface potential of the HypK-THB - Naa15-Naa10 Δ C complex. The highly charged patch at the N-terminus of Naa15 is highlighted with a black box. The two glycerol molecules (white sticks) and phosphate ion (orange sticks) are shown. The surface charge spans from -5 kT/e (deep red) to +5 kT/e (deep blue). (c) Close up view of the charged cavity formed by lysine and arginine residues of Naa15 and Naa10. Residues forming the cavity are labeled and the coordination of the phosphate ion is shown as black lines. (d) Close up view of the charged cavity as electrostatic surface potential.



Supplementary Figure 16: Superimposition of Naa15 structures. Cartoon representation of the Naa15 N-terminal region (helix $\alpha 1-7$) of the HypK-THB - NatA complex (light gray) superimposed on HypK as part of the full-length HypK - NatA complex (dark gray).



Supplementary Figure 17: Uncropped image of Fig. 1a.



Supplementary Figure 18: Uncropped image of Supplementary Fig. 2.

Supplementary References

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