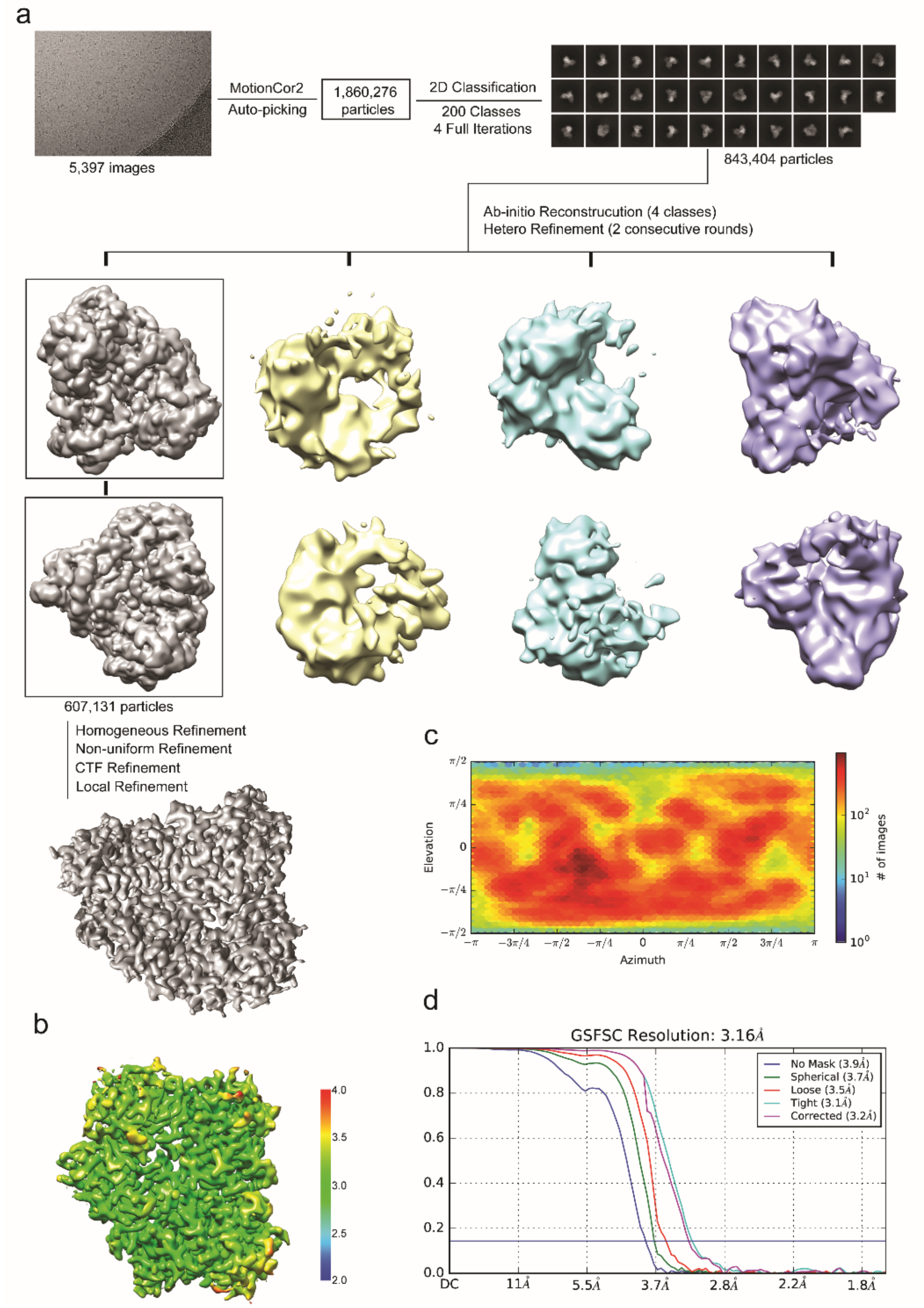
Supplementary information

Molecular mechanism of N-terminal acetylation by the ternary NatC complex

Sunbin Deng, Leah Gottlieb, Buyan Pan, Julianna Supplee, Xuepeng Wei, E James Petersson, Ronen Marmorstein

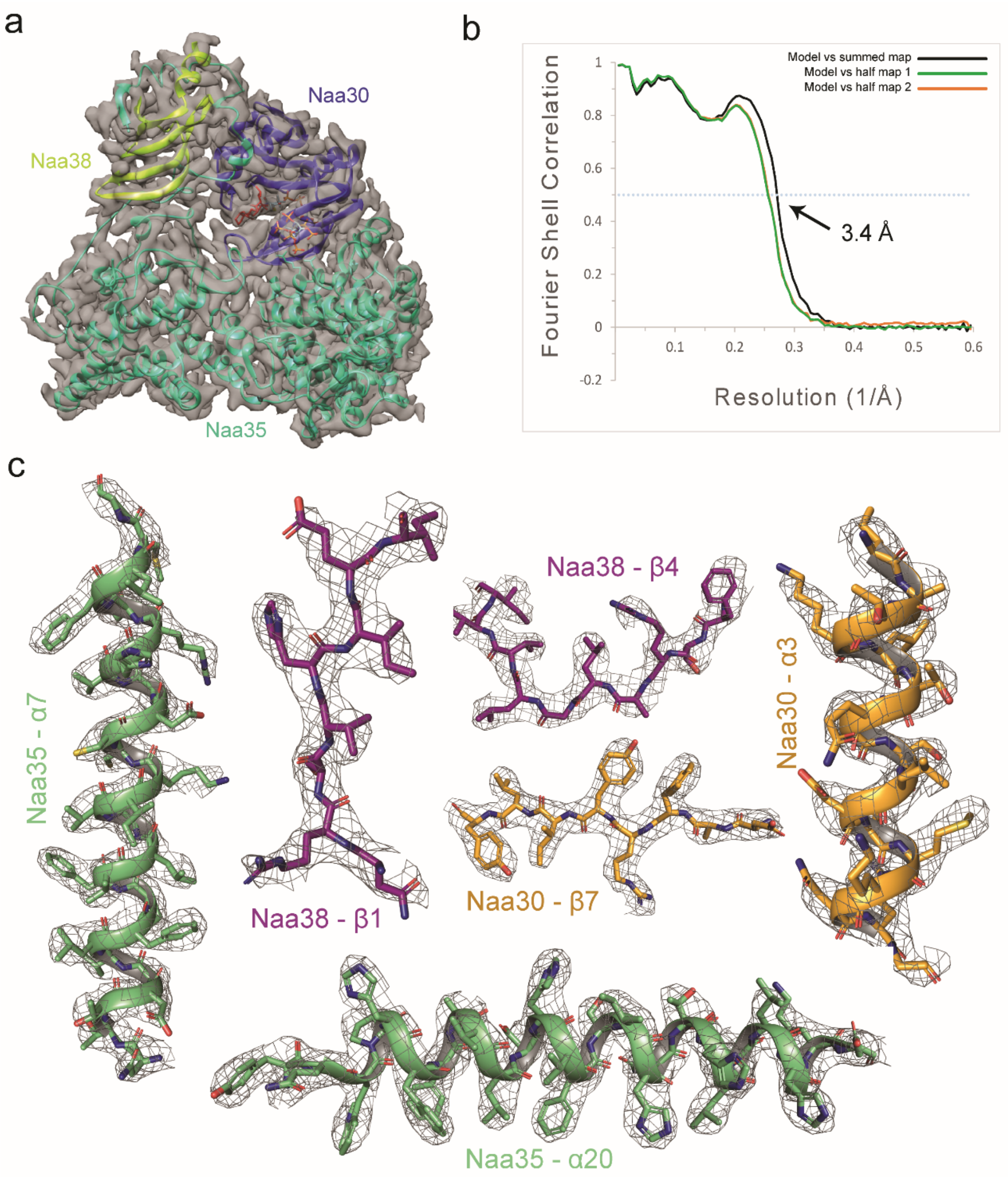
Supplementary Table 1. Sequence of primers for preparing mutations, Related to STAR Methods section SpNatC Complex Expression and Purification

*Sp*Naa30- E25A	**Forward:** *GATTTGAGTGCGCCATATAG* **Reverse:** *CTATATGGCGCACTCAAATC*
*Sp*Naa30- Y27A	**Forward:** *GAGTGAGCCAGCTAGTAAATATGTG* **Reverse:** *CACATATTTACTAGCTGGCTCACTC*
*Sp*Naa30-S24A	**Forward:** *CAAAAAGATTTGGCTGAGCCATATAG* **Reverse:** *CTATATGGCTCAGCCAAATCTTTTTG*
*Sp*Naa30-Y71A	**Forward:** *CGCTTAGAGGTGCTATAGCAATGCTGG* **Reverse:** *CCAGCATTGCTATAGCACCTCTAAGCG*
*Sp*Naa30-Y71F	**Forward:** *CGCTTAGAGGTTTTATAGCAATGCTGG* **Reverse:** *CCAGCATTGCTATAAAACCTCTAAGCG*
*Sp*Naa30-M74A	**Forward:** *GAGGTTATATAGCAGCGCTGGCCATAG* **Reverse:** *CTATGGCCAGCGCTGCTATATAACCTC*
*Sp*Naa30-E109A	**Forward:** *GGAAATTGTTTTGGCAACAGAGGTGG* **Reverse:** *CCACCTCTGTTGCCAAAACAATTTCC*
*Sp*Naa30-E109Q	**Forward:** *GGAAATTGTTTTGCAAACAGAGGTGG* **Reverse:** *CCACCTCTGTTTGCAAAACAATTTCC*
*Sp*Naa30-N114A	**Forward:** *CAGAGGTGGATGCTGAGGCAGCCATG* **Reverse:** *CATGGCTGCCTCAGCATCCACCTCTG*
*Sp*Naa30-Y121F	**Forward:** *GCCATGTCTTTTTTTGAAAGACTCGGG* **Reverse:** CCCGAGTCTTTCAAAAAAAGACATGGC
*Sp*Naa30-Y121A	**Forward:** *GCCATGTCTTTTGCTGAAAGACTCGGG* **Reverse:** *CCCGAGTCTTTCAGCAAAAGACATGGC*
*Sp*Naa30-Y135A	**Forward:** *CGTCTTTATCGTGCTTATTTGAACGGC* **Reverse:** *GCCGTTCAAATAAGCACGATAAAGACG*
*Sp*Naa30-Y136A	**Forward:** *CTTTATCGTTATGCTTTGAACGGCAC* **Reverse:** *GTGCCGTTCAAAGCATAACGATAAAG*



Supplementary figure 1. Cryo-EM workflow and resolution of *Sp*NatC EM map, Related to Figure 2.

(**a**) 2D and 3D classification scheme and workflow for *Sp*NatC EM map determination. (**b**) Local resolution of the final *Sp*NatC EM map. (**c**) Viewing direction distribution of *Sp*NatC final EM map 3D reconstruction generated by cryoSPARC v2. (**d**) Gold standard Fourier Shell Correlation (FSC) curves of *Sp*NatC EM map 3D reconstruction, generated by cryoSPARC v2.

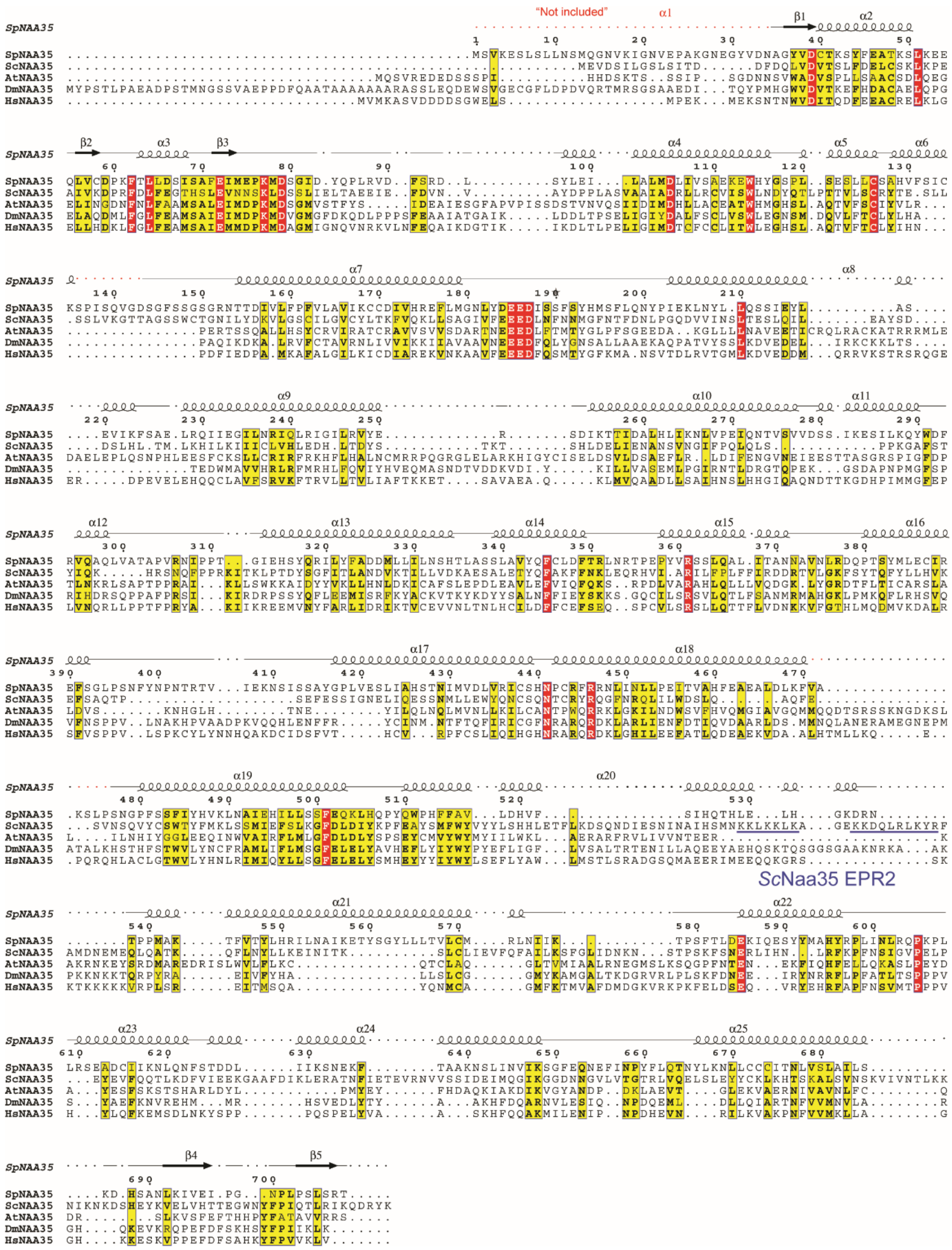


Supplementary figure 2. Correlation between Cryo-EM map of *Sp*NatC EM and its atomic model, Related to Figure 2.

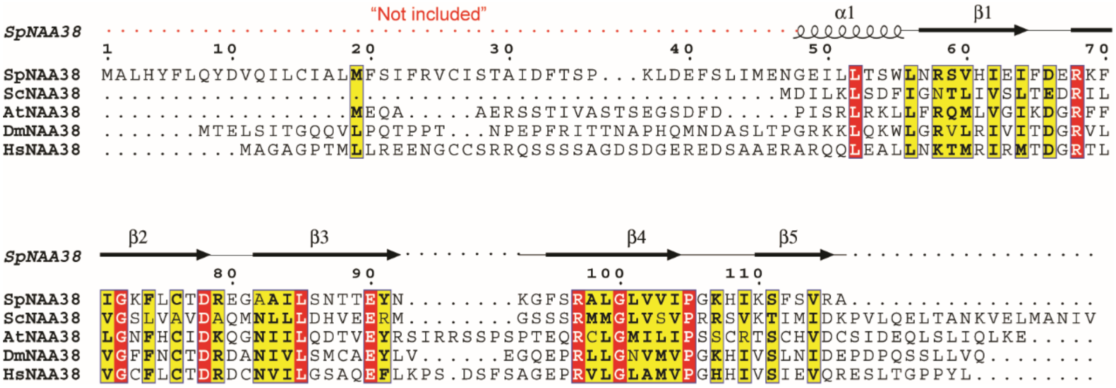
(a) Atomic model of *Sp*NatC fitted into the Cryo-EM map.

(b) FSC curves of the refined model versus the overall map that it was refined against (black); of the model refined in the first of the two independent maps used for the gold-standard FSC versus the same map (green); and of the model refined in the first of the two independent maps versus the second independent map (orange).

(c) The fit of several helical segments or β-strands from all three subunits of NatC in the EM density. The contour level is 5 sigma.



Supplementary figure 3. Sequence alignment of Naa35 orthologs, Related to Figure 2. Sequences are from *Schizosaccharomyces pombe* (Sp), *Saccharomyces cerevisiae (*Sc), *Arabidopsis thaliana* (At), *Drosophila melanogaster (*Dm), and *Homo sapiens* (Hs). Numbering and secondary structure elements for *Sp*Naa30 are indicated above the sequence alignment. Residues truncated from the *Sp*Naa35 protein construct used in this study are indicated above in red ("Not included"). EPR2 (**Figure 5a)** from *Sc*Naa35 is annotated in blue.



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SpNAA38 MALHYFLQYDVQILCIALMFSIFRVCISTAIDFTSP...KLDEFSLIMENGEILLTSWLNRSVHIEIFDERKF
ScNAA38 .................................................MDILKLSDFIGNTLIVSLTEDRIL
AtNAA38 ..................MEQA.....AERSSTIVASTSEGSDFD.....PISRLRKLLFRQMLVGIKDGRFF
DmNAA38 .......MTELSITGQQVLPQTPPT...NPEPFRITTNAPHQMNDASLTPGRKKLQKWLGRVLRIVITDGRVL
HsNAA38 ..........MAGAGPTMLLREENGCCSRRQSSSSAGDSDGEREDSAAERARQQLEALLNKTMRIRMTDGRTL

SpNAA38 IGKFLCTDREGAAILSNTTEYN........KGFSRALGLVVIPGKHIKSFSVRA..................
ScNAA38 VGSLVAVDAQMNLLLDHVEERM........GSSSRMMGLVSVPRRSVKTIMIDKPVLQELTANKVELMANIV
AtNAA38 LGNFHCIDKQGNIILQDTVEYRSIRRSSPSPTEQRCLGMILIPSSCRTSCHVDCSIDEQLSLIQLKE.....
DmNAA38 VGFFNCTDRDANIVLSMCAEYLV......EGQEPRLLGNVMVPGKHIVSLNIDEPDPQSSLLVQ........
HsNAA38 VGCFLCTDRDCNVILGSAQEFLKPS.DSFSAGEPRVLGLAMVPGHHIVSIEVQRESLTGPPYL.........
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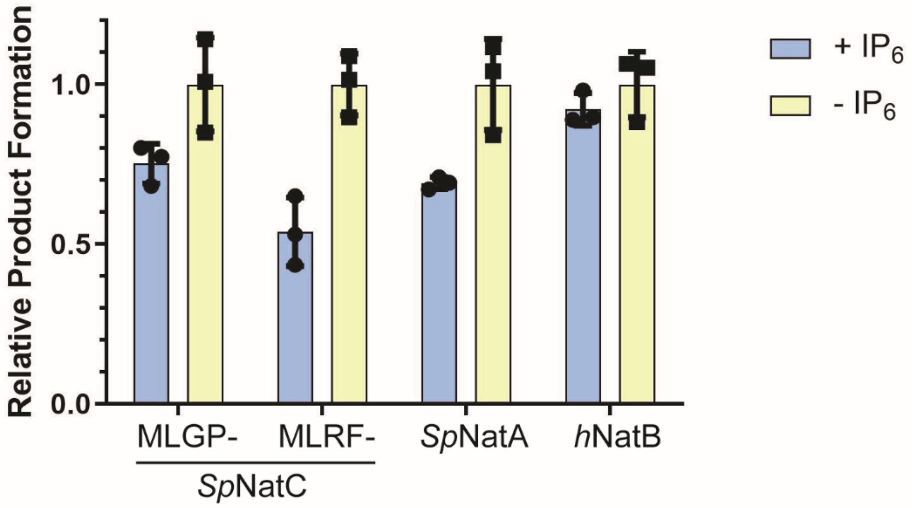
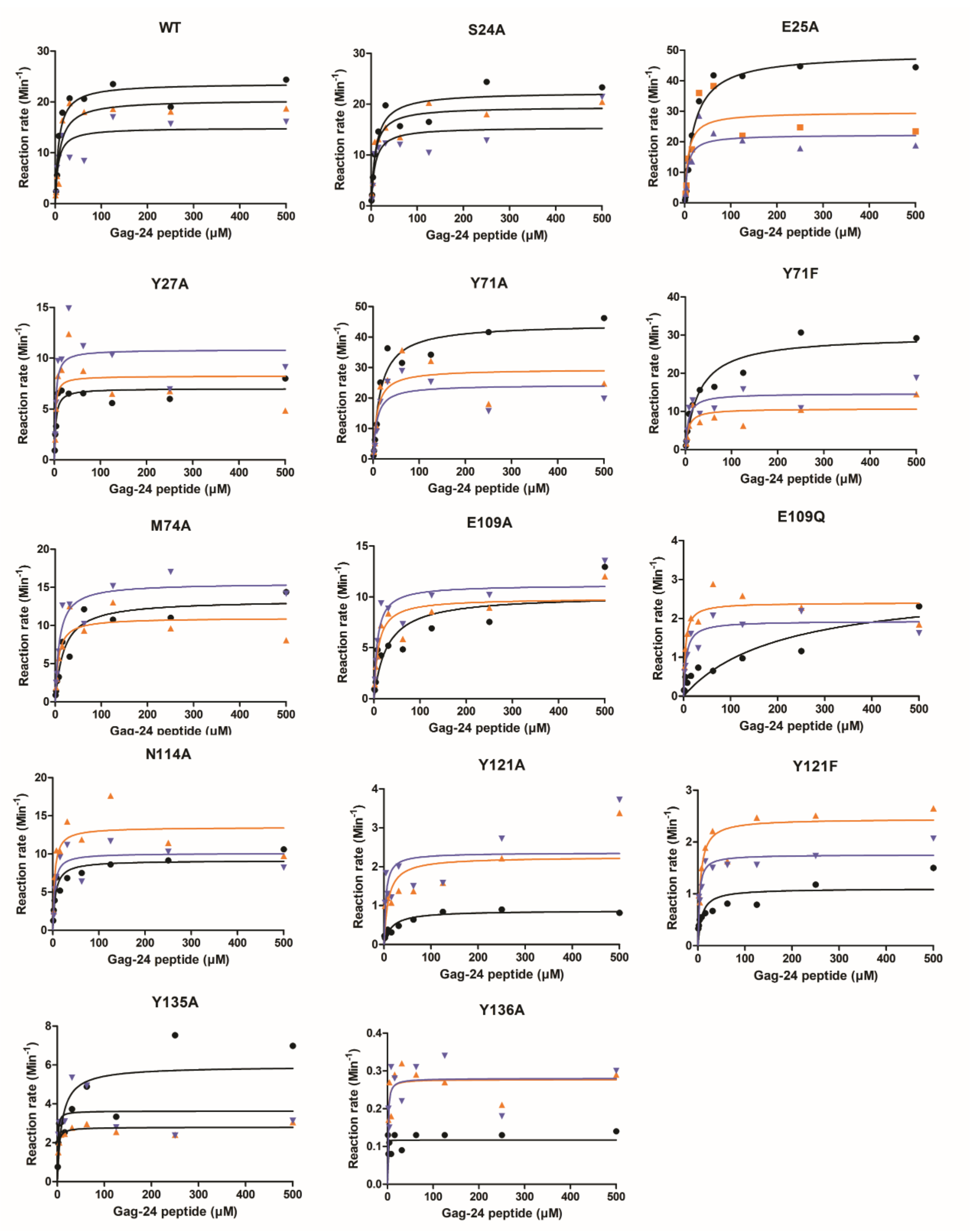
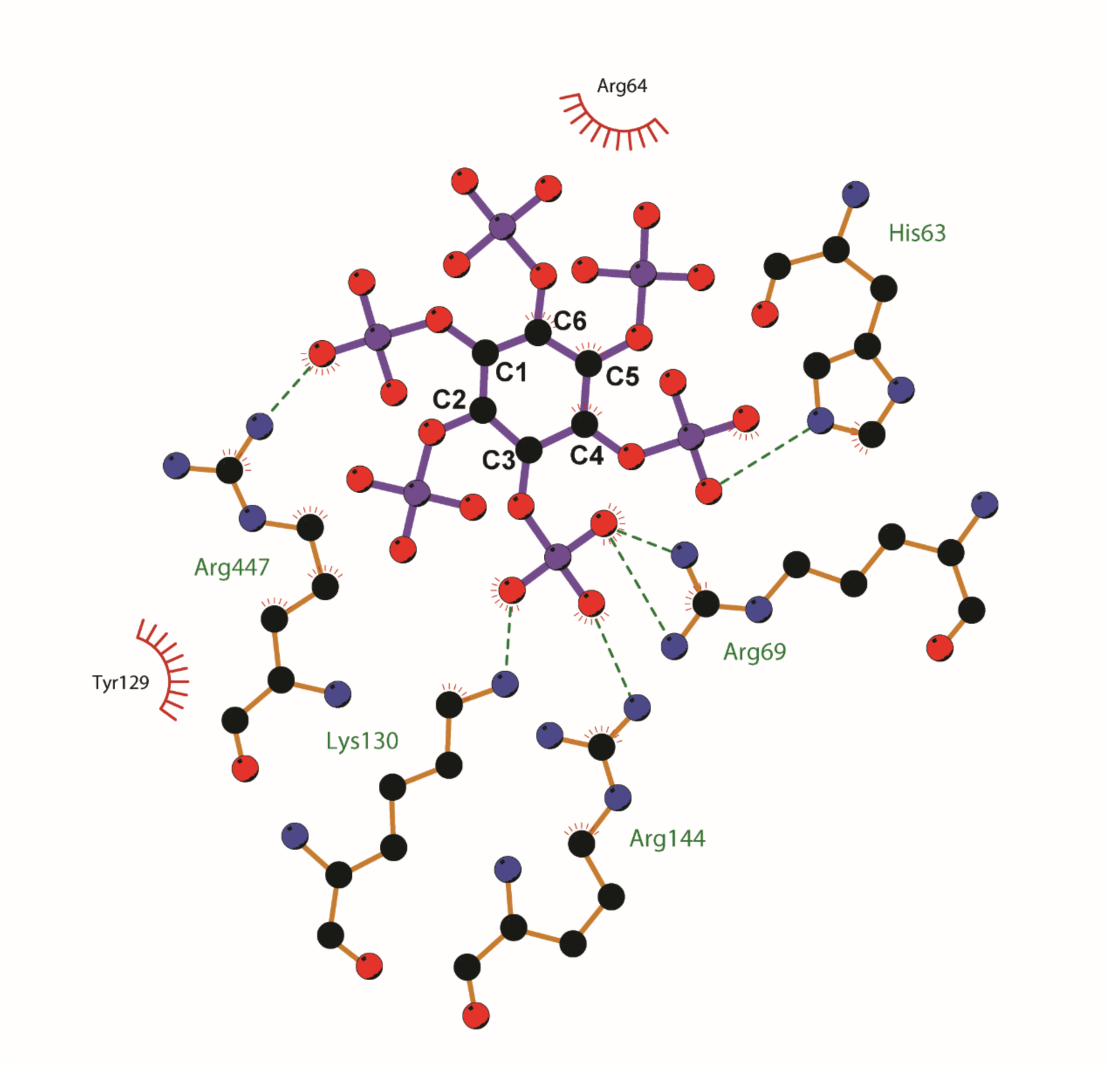
Supplementary figure 4. Sequence alignment of Naa38 orthologs, Related to Figure 2. Sequences are from *Schizosaccharomyces pombe* (Sp), *Saccharomyces cerevisiae (*Sc), *Arabidopsis thaliana* (At), *Drosophila melanogaster (*Dm), and *Homo sapiens* (Hs). Numbering and secondary structure elements for *Sp*Naa38 are indicated above the sequence alignment. Residues truncated from the *Sp*Naa38 protein construct used in this study are indicated above in red ("Not included").



Supplementary figure 5. The activity impacts of IP_6 on selective NATs, Related to Figure 2. 50 nM of either *Sp*NatC, *Sp*NatA, and hNatB was mixed with 2 μM or no IP_6 for measuring product formation. Data was normalized to NATs activity without IP_6. Error bars indicates Mean with SD, n=3.



Supplementary figure 6. Michaelis–Menten kinetic curves of WT and mutant *Sp*NatC complexes, Related to Table 2. The data is summarized in **Table 2**.



Supplementary figure 7. Interaction between IP$_6$ and *Sp*NatC, Related to Figure 2. Residues that mediate IP$_6$ hydrogen bonds and van der Walls interaction are shown. The figure was generated with LIGPLOT(Laskowski R A, Swindells M B (2011).