

## Supplementary Information

**Title:** Microscopy-based *Saccharomyces cerevisiae* complementation model reveals functional conservation and redundancy of N-terminal acetyltransferases

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## Supplementary tables

**Table S1. Yeast strains of this study.**

yTA #	Short name	Genotype	pTA #	Use	Source
36	WT	BY4742		SG	EUROSCARF (#Y10000)
44	30Δ	BY4742; <i>naa30Δ::kanMX4</i>		SG	EUROSCARF (#Y15470)
59	WT	BY4741		SG	EUROSCARF (#Y00000)
256	Arl3-GFP, WT	BY4741; <i>ARL3::GFP-HIS3MX6</i>		Fig. 1A,D, SG	Invitrogen (GFP-collect.)
266	Arl3-GFP, 30Δ	BY4741; <i>ARL3::GFP-HIS3MX6, naa30Δ::kanMX4</i>		Fig. 1A,D, SG	<sup>1</sup>
480	Arl3-GFP, 35Δ	BY4741; <i>ARL3::GFP-HIS3MX6; naa35Δ::kanMX4</i>		Fig. 1A,D, SG	This study
485	Arl3-GFP, 38Δ	BY4741; <i>ARL3::GFP-HIS3MX6; naa38Δ::kanMX4</i>		Fig. 1A,D	This study
375	Arl3-GFP, 30Δ, h30	BY4741; <i>ARL3::GFP-HIS3MX6; naa30Δ::kanMX4; [pBEVY-U-HA-hNAA30]</i>	231	Fig. 1B,D, Fig. 4	This study
728	Arl3-GFP, 35Δ, h30	BY4741; <i>ARL3::GFP-HIS3MX6; naa35Δ::kanMX4; [pBEVY-U-HA-hNAA30]</i>	231	Fig. 1B,D	This study
730	Arl3-GFP, 35Δ, h35	BY4741; <i>ARL3::GFP-HIS3MX6; naa35Δ::kanMX4; [pBEVY-L-hNAA35-FLAG]</i>	226	Fig. 1B,D	This study
772	Arl3-GFP, 35Δ38Δ, h35	BY4741; <i>ARL3::GFP-HIS3MX6; naa35Δ::kanMX4; naa38Δ::hphNT1; [pBEVY-L-hNAA35]</i>	226	Fig. 1B,D	This study
732	Arl3-GFP, 35Δ, h30, h35	BY4741; <i>ARL3::GFP-HIS3MX6; naa35Δ::kanMX4; [pBEVY-U-HA-hNAA30]; [pBEVY-L-hNAA35-FLAG]</i>	226, 231	Fig. 1C,D	This study
698	Arl3-GFP, 30Δ35Δ, h30, h35	BY4741; <i>ARL3::GFP-HIS3MX6; naa30Δ::kanMX4; naa35Δ::natNT2; [pBEVY-U-HA-hNAA30]; [pBEVY-L-hNAA35]</i>	226, 231	Fig. 1C,D	This study

yTA #	Short name	Genotype	pTA #	Use	Source
751	Arl3-GFP, NatCΔ, pBEVY-U, pBEVY-L	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; <i>naa38Δ::hphNT1</i> ; [pBEVY-U]; [pBEVY-L]	53, 224	Fig. 1C,D	This study
753	Arl3-GFP, NatCΔ, h30, h35	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; <i>naa38Δ::hphNT1</i> ; [pBEVY-U-HA-hNAA30]; [pBEVY-L-hNAA35-FLAG]	226, 231	Fig. 1C,D	This study
692	Arl3-GFP, 30Δ35Δ, pBEVY-U, pBEVY-L	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; [pBEVY-U]; [pBEVY-L]	53, 224	Fig. 1D	This study
696	Arl3-GFP, 30Δ35Δ, h30, pBEVY-L	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; [pBEVY-U-HA-hNAA30]; [pBEVY-L]	224, 231	Fig. 1D	This study
694	Arl3-GFP, 30Δ35Δ, pBEVY-U, h35	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; [pBEVY-U]; [pBEVY-L-hNAA35]	53, 226	Fig. 1D	This study
673	Arl3-GFP, pBEVY-L, pBEVY-U	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; [pBEVY-L]; [pBEVY-U]	53, 224	Fig. 1D (Ctrl)	This study
770	Arl3-GFP, 35Δ38Δ, pBEVY-L	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa35Δ::kanMX4</i> ; <i>naa38Δ::hphNT1</i> ; [pBEVY-L]	224	Fig. 2	This study
858	Arl3-GFP, 35Δ, h35 (n.t.)	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa35Δ::kanMX4</i> ; [pBEVY-L-hNAA35]	342	Fig. 2	This study
860	Arl3-GFP, 35Δ38Δ, h35 (n.t.)	BY4741; <i>ARL3::GFP-HIS3MX6</i> , <i>naa35Δ::kanMX4</i> ; <i>naa38Δ::hphNT1</i> ; [pBEVY-L-hNAA35]	342	Fig. 2	This study
862	Arl3-GFP, NatCΔ, h30, h35 (n.t.)	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; <i>naa38Δ::hphNT1</i> ; [pBEVY-U-HA-hNAA30]; [pBEVY-L-hNAA35]	231, 342	Fig. 2	This study
771	Arl3-GFP, 35Δ38Δ, pBEVY-L	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa35Δ::kanMX4</i> ; <i>naa38Δ::hphNT1</i> ; [pBEVY-L]; clone 2	224	Fig. 2B	This study
859	Arl3-GFP, 35Δ, h35 (n.t.)	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa35Δ::kanMX4</i> ; [pBEVY-L-hNAA35]; clone 2	342	Fig. 2B	This study

yTA #	Short name	Genotype	pTA #	Use	Source
861	Arl3-GFP, 35Δ38Δ, h35 (n.t.)	BY4741; <i>ARL3::GFP-HIS3MX6</i> , <i>naa35Δ::kanMX4</i> ; <i>naa38Δ::hphNT1</i> ; [ <i>pBEVY-L-hNAA35</i> ]; clone 2	342	Fig. 2B	This study
863	Arl3-GFP, NatCΔ, h30, h35 (n.t.)	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; <i>naa38Δ::hphNT1</i> ; [ <i>pBEVY-U-HA-hNAA30</i> ]; [ <i>pBEVY-L-hNAA35</i> ]; clone 2	231, 342	Fig. 2B	This study
855	Arl3-GFP, Sec7-mRFP	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; [ <i>316-Sec7-mRFP</i> ]	321	Fig. 3A	This study
830	Arl3-GFP, mCherry-Sed5	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; [ <i>p415Met25-mCherry-Sed5</i> ]	340	Fig. 3B	This study
832	Arl3-GFP, 30Δ, h30, mCherry-Sed5	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; [ <i>pBEVY-U-HA-hNAA30</i> ]; [ <i>p415Met25-mCherry-Sed5</i> ]	231, 340	Fig. 3B	This study
371	Arl3-GFP, pBEVY-U	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; [ <i>pBEVY-U</i> ]	53	Fig. 4	This study
373	Arl3-GFP, 30Δ, pBEVY-U	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; [ <i>pBEVY-U</i> ]	53	Fig. 4	This study
377	Arl3-GFP, 30Δ, h50	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; [ <i>pBEVY-U-hNA50</i> ]	285	Fig. 4A,B,D	This study
379	Arl3-GFP, 30Δ, h60	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; [ <i>pBEVY-U-hNAA60</i> ]	286	Fig. 4A,B,D	This study
954	Arl3-GFP, 30Δ, h60 <sub>1-184</sub>	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; [ <i>pBEVY-U-hNAA60<sub>1-184</sub></i> ]	378	Fig. 4C,D, SG	This study
509	Arl3-GFP, 35Δ, pBEVY-U	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa35Δ::kanMX4</i> ; [ <i>pBEVY-U</i> ]	53	Fig. 4C,D	This study
532	Arl3-GFP, 35Δ, h60	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa35Δ::kanMX4</i> ; [ <i>pBEVY-U-hNAA60</i> ]	286	Fig. 4C,D	This study
1010	Arl3-GFP, pBEVY-U, mCherry-Sed5	BY4741; [ <i>pBEVY-U</i> ]; [ <i>p415Met25-mCherry-Sed5</i> ]	53, 340	Fig. 5A	This study
1012	Arl3-GFP, 30Δ, mCherry-Sed5	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; [ <i>pBEVY-U</i> ]; [ <i>p415Met25-mCherry-Sed5</i> ]	53, 340	Fig. 5A	This study

yTA #	Short name	Genotype	pTA #	Use	Source
834	Arl3-GFP, 30Δ, h60, mCherry-Sed5	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; [ <i>pBEVY-U-hNAA60</i> ]; [ <i>p415Met25-mCherry-Sed5</i> ]	286, 340	Fig. 5A	This study
1014	Arl3-GFP, 30Δ, h60 <sub>1-184</sub> , mCherry-Sed5	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; [ <i>pBEVY-U-hNAA60<sub>1-184</sub></i> ]; [ <i>p415Met25-mCherry-Sed5</i> ]	340, 378	Fig. 5A	This study
1001	h60-EGFP	BY4741; [ <i>pBEVY-U-hNAA60-EGFP</i> ]	452	Fig. 5B	This study
1006	h60 <sub>1-182</sub> -EGFP	BY4742; [ <i>pBEVY-U-hNAA60<sub>1-182</sub>-EGFP</i> ]	453	Fig. 5B	This study
639	Arl3-GFP, pBEVY-L	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; [ <i>pBEVY-L</i> ]	224	SG	This study
683	Arl3-GFP, 30Δ35Δ	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i>		SG	This study
767	Arl3-GFP, 35Δ38Δ	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa35Δ::kanMX4</i> ; <i>naa38Δ::hphNT1</i>		SG	This study
748	Arl3-GFP, NatCΔ	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; <i>naa38Δ::hphNT1</i>		SG	This study
686	Arl3-GFP, 30Δ35Δ, pBEVY-U	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; [ <i>pBEVY-U</i> ]	53	SG	This study
688	Arl3-GFP, 30Δ35Δ, h30	BY4741; <i>ARL3::GFP-HIS3MX6</i> ; <i>naa30Δ::kanMX4</i> ; <i>naa35Δ::natNT2</i> ; [ <i>pBEVY-U-HA-hNAA30</i> ]	231	SG	This study

Abbreviations: 30/35/38 Δ; *naa30/35/38 Δ*; h30/35/50/60; hNaa30/35/50/60; n.t., not tagged; p, plasmid; SG, strain generation; yTA#, internal yeast strain number; pTA#, internal plasmid number; TA, Thomas Arnesen lab; yTA, internal yeast strain number.

**Table S2. The plasmids of this study.**

<b>pTA #</b>	<b>Name</b>	<b>Source</b>
53	pBEVY-U	<sup>2</sup>
75	pCDNA3.1- <i>hNAA60-V5-His</i>	<sup>3</sup>
185	pCMV6-AC- <i>NAA35-GFP</i>	OriGene
224	pBEVY-L	<sup>2</sup>
226	pBEVY-L- <i>hNAA35-FLAG</i>	This study
229	pFA6a- <i>natNT2</i>	EUROSCARF
231	pBEVY-U- <i>HA-hNAA30</i>	This study
250	pFA6a- <i>hphNT1</i>	EUROSCARF
285	pBEVY-U- <i>hNAA50</i>	<sup>4</sup>
286	pBEVY-U- <i>hNAA60</i>	<sup>3</sup>
378	pBEVY-U- <i>hNAA60<sub>1-184</sub></i>	This study
321	316- <i>Sec7-mRFP</i>	RIKEN BRC DNA BANK
340	p415Met25- <i>mCherry-Sed5</i>	<sup>5</sup>
342	pBEVY-L- <i>hNAA35</i>	This study
407	<i>phNAA60-EGFP-N1</i>	This study
439	<i>phNAA60<sub>1-182</sub>-EGFP-N1</i>	This study
452	pBEVY-U- <i>hNAA60-EGFP</i>	This study
453	pBEVY-U- <i>hNAA60<sub>1-182</sub>-EGFP</i>	This study

Abbreviations: pTA#, internal plasmid number; TA, Thomas Arnesen lab

**Table S3. Primers used for amplification of yeast and human genes.**

oyTA #	Sequence (5' to 3')	Use	Source
49	CATGAAGAACAAAGTTTCAC	Amplify <i>kanMX4</i> with <i>yNAA30</i> flanks from genomic DNA of yTA44	1
50	CCTCTTTTCTCTACTGCC		
211	CTGTGCTAGTGTAGAGCAACTTTGATAATAGCTGGT ACGTTTCCGAGAATGGAAGTAGACAGTATATTAGGC GGATCCCCGGGTTAATTAA	Amplify <i>natNT2</i> with <i>yNAA35</i> flanks from pTA229	This study
212	CCTTTATAATAATTAATAGCGGGTATATAATGCTAA ATATATTTTGTACAGTTAGGGTAAATCCAGATTATT TATAGCGGGAATTCGAGCTCGATTACAA		
148	CTCCCACTTTAAAGTAAATCCGGAGATTCCC	Amplify <i>natNT2</i> with <i>yNAA35</i> flanks from genomic DNA of yTA683	This study
149	GGGGGAACGCACTGCATTTTACC		
244	GGGTGAAGATAGTCTTCAACAACTTAGTAAGGGGC ACCAAAGCGTCATGCGTACGCTGCAGGTCGAC	Amplify <i>hphNT1</i> with <i>yNAA38</i> flanks from pTA250	This study
245	GCCAAGCAATTGTTACTATGGAACTATTAGAACCAT ATGCGCTAATCGATGAATTCGAGCTCG		
51	CTACCAAGAAACCGGGTAGC	PCR screen for <i>naa30Δ</i>	1
52	GAAACTTGCTTTATTATCTCTC		
146	CGTAGCTTCCCAACTTCACCAG	PCR screen for <i>naa35Δ</i>	This study
147	CGAACTGTAACCTCAAAGAAGCCTTGAAATTTACC		
9	CTCGGAAGTGCATGAGTTCTGAC	PCR screen for <i>naa38Δ</i>	This study
11	G TTCAGTCCAGTTCATATTCG		
oTA #	Sequence (5' to 3')	Use	Source
99	CATTCTAGACTCGAGACCATGGTTATGAAAGCTTCTG	Amplify <i>hNAA35-FLAG</i> from pTA185	This study
98	CATCTGCAGTCACTTGTATCGTCGTCCTTGTAATCA ACAAGTTTCACAACAGG		
420	GTCGTCCTTGTAATCTCAAACAAGTTTCACAAC	Mutagenesis of pTA226, generating pTA342	This study
490	CCTATGTCCTCTACATCAACGGCGGCTGACCTCCCTG GACGATTTTGG	Mutagenesis of pTA286, generating pTA378	This study
17	GCGGGCTAGCATGACAGAGGTGGTGCCATCC	Amplify <i>hNAA60<sub>1-182</sub></i> from pTA75	This study
463	GGTACCGGGTTGATGTAGAGGACATAGG		
18	GCGGGGTACCCCATGGTCCGGCTGTACTCG	Amplify <i>hNAA60</i> from	This study

		pTA75 (together with oTA17)	
528	GATCATTCTAGAGAGAATGACAGAGGTGGTGC	Amplify <i>hNAA60-EGFP</i> from pTA407 and <i>hNAA60<sub>1-182</sub>-EGFP</i> from pTA439	This study
529	GATCATGTCGACTTACTTGTACAGCTCGTCC		

Abbreviations: oTA, internal oligo number; oyTA, internal yeast oligo number; pTA#, internal plasmid number; TA, Thomas Arnesen lab



## References Supplementary Tables

- 1 Aksnes, H., Osberg, C. & Arnesen, T. N-terminal acetylation by NatC is not a general determinant for substrate subcellular localization in *Saccharomyces cerevisiae*. *PLoS One* **8**, e61012,(2013).
- 2 Miller, C. A., Martinat, M. A. & Hyman, L. E. Assessment of aryl hydrocarbon receptor complex interactions using pBEVY plasmids: expression vectors with bi-directional promoters for use in *Saccharomyces cerevisiae*. *Nucleic Acids Research* **26**, 3577-3583,(1998).
- 3 Van Damme, P. *et al.* NatF contributes to an evolutionary shift in protein N-terminal acetylation and is important for normal chromosome segregation. *PLoS Genet* **7**, e1002169,(2011).
- 4 Van Damme, P., Hole, K., Gevaert, K. & Arnesen, T. N-terminal acetylome analysis reveals the specificity of Naa50 (Nat5) and suggests a kinetic competition between N-terminal acetyltransferases and methionine aminopeptidases. *Proteomics* **15**, 2436-2446,(2015).
- 5 Powis, K. *et al.* Get3 is a holdase chaperone and moves to deposition sites for aggregated proteins when membrane targeting is blocked. *Journal of Cell Science* **126**, 473-483,(2013).