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

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N-terminal acetylation is a highly abundant protein modification catalyzed by N-terminal acetyltransferases (NATs) NatA–NatG. The *Saccharomyces cerevisiae* protein Arl3 depends on interaction with Sys1 for its localization to the Golgi and this targeting strictly requires NatC-mediated N-terminal acetylation of Arl3. We utilized the Arl3 acetylation-dependent localization phenotype as a model system for assessing the functional conservation and *in vivo* redundancy of several human NATs. The catalytic subunit of human NatC, hNaa30 (Mak3), restored Arl3 localization in the absence of yNaa30, but only in the presence of either yeast or human Naa35 subunit (Mak10). In contrast, hNaa35 was not able to replace its yeast orthologue without the co-expression of hNaa30, suggesting co-evolution of the two NatC subunits. The most recently discovered and organellar human NAT, NatF/Naa60, restored the Golgi localization of Arl3 in the absence of yNaa30. Interestingly, this was also true for hNaa60 lacking its membrane-binding domain whereas hNaa50 did not complement NatC function. This *in vivo* redundancy reflects NatC and NatF’s overlapping *in vitro* substrate specificities. The yeast model presented here provides a robust and rapid readout of NatC and NatF activity *in vivo*, and revealed evolutionary conservation of the NatC complex and redundancy between NatC and NatF.

N-terminal (Nt) acetylation is a prevalent protein modification catalyzed by N-terminal acetyltransferases (NATs). It involves the transfer of an acetyl moiety from acetyl-coenzyme A (Ac-CoA) to the α-amino group of the first amino acid residue of a polypeptide. This is mainly considered to be a co-translational event although evidence exist that Nt-acetylation also occurs post-translationally.

In the yeast *S. cerevisiae* more than 50% of the soluble proteome is subjected to Nt-acetylation, whereas in humans this number exceeds 70% 3,5. There is an increasing number of substrates uncovered for which Nt-acetylation has significant functional roles, including global protein folding, protein–protein interaction, protein stabilization or degradation and subcellular protein targeting.

Five NATs have been identified in yeast, NatA–NatE, all of which are conserved to multicellular eukaryotes, which additionally contain NatF4,14. NatG was recently identified in the plant kingdom15. NatA and NatD target Met-cleaved N-termini whereas NatB, NatC, NatE and NatF acetylate Met-starting N-termini4,5,16,19–21. NatG is found in chloroplasts of plant cells and its activity is towards Ala-, Met-, Ser-, and Thr-starting N-termini15. NatC, NatE and NatF display overlapping substrate specificities *in vitro*, targeting Met-hydrophobic and Met-amphipathic N-termini4,22,23, whereas their *in vivo* substrates have been proposed to be distinct4,24. For instance, NatF localizes to the cytosolic surface of the Golgi apparatus and specifically Nt-acetylates membrane proteins with their N-termini facing the cytosol.

NatC is a complex consisting of the catalytic subunit Naa30 (Mak3) and the auxiliary subunits Naa35 (Mak10) and Naa38 (Mak31). In yeast, the individual deletions of NatC subunits cause growth defects on non-fermentable carbon sources (e.g. glycerol) at elevated temperatures. NatC knockdown in human cells...
results in reduced cell viability and p53-dependent apoptosis\(^2\). One of the verified NatC substrates is the yeast ADP-ribosylation factor-like 3 (Arl3) protein whose interaction with Golgi-anchored Sys1 strictly depends on NatC-mediated Nt-acetylation. In the absence of either Naa30 or Naa35, Arl3 remains unmodified and as a consequence is unable to associate with the Golgi\(^1\)\(^2\)\(^3\).

Although NatC has been deemed evolutionarily conserved in several aspects - such as in \textit{in vitro} substrate specificity, ribosome association and subunit composition\(^2\)\(^2\)\(^7\)\(^-\)\(^9\)\(^1\)\(^0\)\(^1\)\(^1\) - its conservation in terms of \textit{in vivo} protein function remains unknown. In this study, we applied the yeast Arl3 localization phenotype as an \textit{in vivo} system to study the functional conservation between yeast and human NatC. We found that the human Naa30-Naa35 NatC complex could functionally complement yeast Nt-acetylation, as indicated by its ability to restore the punctate Golgi localization of Arl3. Moreover, the possible NatC-redundancy of NatE and NatF was challenged in the Arl3 complementation assay to enlighten a potential \textit{in vivo} redundancy. The organelle associated human NatF/Naa60, but not NatE/Naa50, was able to restore normal Arl3 distribution pattern in yeast. Surprisingly, also a truncated cytosolic hNaa60 complemented the Arl3 localization phenotype in the absence of active NatC complex. These results enlightened a functional difference \textit{in vivo} among NatC, NatE and NatF, which are known to have partially overlapping \textit{in vitro} substrate specificities.

**Results and Discussion**

**Functional conservation of Naa30 revealed by Arl3 localization complementation assay.** The dependency of NatC-mediated Nt-acetylation of Arl3 for correct subcellular localization has previously been shown by fluorescence microscopy and mass spectrometry\(^1\)\(^2\)\(^3\)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\(^)\[^]
Figure 1. Arl3-GFP punctate localization as a model for active NatC revealed functional conservation of the NatC complex. (A–C) Live-cell microscopy of endogenous Arl3-GFP in yeast cells with the indicated genotypes. A corresponding bar graph below the fluorescent images shows the number of cells (n > 500 cells) harbouring a punctate localization pattern of Arl3-GFP. Scale bar, 5 μm. (A) WT, naa30Δ, naa35Δ and naa38Δ yeast. (B) Human Naa30 exogenously expressed in naa30Δ and naa35ΔΔ cells, whereas hNaa35 was exogenously expressed in naa35Δ and naa35Δnaa38Δ yeast. (C) Exogenous expression of both human Naa30 and Naa35 in naa35Δ, naa30Δnaa35Δ and NatCΔ cells. (D) Western blot analysis using anti-HA and anti-FLAG antibody verified the exogenous expression of hNaa30 and hNaa35, respectively, in different yeast strains. Asterisks represent undetermined band detected by anti-FLAG or anti-HA (Sigma-Aldrich) antibodies. Abbreviations: 30Δ, naa30Δ; 35Δ, naa35Δ; 38Δ, naa38Δ; NatCΔ, naa30Δnaa35Δnaa38Δ; h30, HA-hNaa30; h35, hNaa35-FLAG.
in vivo substrate profiles when expressed in yeast4,24. All three enzymes recognize Met-starting N-termini containing a hydrophobic or amphipathic amino acid residue in the second position. Arl3 has a Met-Phe-starting N-terminus and thus may be a putative target of these enzymes. To challenge the suggested substrate redundancy between human NatC, NatE and NatF in vivo, we again utilized the Arl3 complementation model (Fig. 4). Here, the human catalytic subunits Naa30, Naa50 and Naa60 were individually expressed in Arl3-GFP nna30Δ cells. Human Naa50, which in human cells is cytosolic and partially ribosome-associated23,28, was not able to

Figure 2. Untagged human Naa35 behaved similarly to hNaa35-FLAG. (A) Fluorescence microscopy of Arl3-GFP in different yeast strains. Untagged hNaa35 was exogenously expressed in nna35Δ and nna35Δ nna38Δ cells as well as together with hNaa30 in NatCΔ yeast. The Arl3 mislocalization phenotype was demonstrated in nna35Δnna38Δ cells. Scale bar, 5 μm. (B) Western blot analysis of cell lysates revealed the presence of hNaa30 and hNaa35 (untagged) in the three yeast strains, represented by two individual clones. Asterisk indicates an undetermined band achieved by anti-FLAG. Abbreviations: 35Δ, nna35Δ; 38Δ, nna38Δ; NatCΔ, nna30Δnna35Δnna38Δ; h30, HA-hNaa30; h35, hNaa35.

Figure 3. Partial co-localization was observed between Arl3 and two individual Golgi marker proteins. (A) Imaging of live WT yeast expressing endogenous Arl3-GFP and exogenous Sec7-mRFP. (B) Endogenous Arl3-GFP and exogenous mCherry-Sed5 were imaged in live WT cells and in nna30Δ cells exogenously expressing HA-hNaa30. Scale bars, 2 μm. Abbreviations: 30Δ, nna30Δ; h30, HA-hNaa30.
complement yNaa30 whereas hNaa60 almost completely reinstated the punctate Arl3 localization, similar to hNaa30 (Fig. 4A). The same was true for hNaa60 expressed in \( \text{naa35} \Delta \) cells (Fig. 4C), indicating that hNaa60 may operate independently of any of the yNatC subunits, unlike hNaa30, which requires the assumed ribosome binding subunit Naa35.

Naa60 was recently described in human cells as the first organellar NAT and its catalytic activity is facing towards the cytosol\(^5\). It might be possible that the ability of human Naa60 to complement the NatC activity on Arl3 relates to Naa60’s association with the yeast Golgi membrane. However, upon expression of truncated Naa60\(_{1-184}\) (h60del-MEM) were exogenously expressed in \( \text{naa35} \Delta \) and \( \text{naa30} \Delta \) cells, respectively. (D) Amount of cells for which the Arl3-GFP punctate localization was observed in the indicated yeast strains. The number from \( \text{naa30} \Delta \) yeast expressing hNaa30 is a re-representation of data in Fig. 1B as a positive control reference (n > 500 cells). Abbreviations: \( \text{30} \Delta \), \( \text{naa30} \Delta \); \( \text{35} \Delta \), \( \text{naa35} \Delta \); h30, HA-hNaa30; h50, hNaa50; h60, hNaa60; h60del-MEM, hNaa60 without membrane binding region.

Figure 4. Human Naa60 has in vivo redundancy with Naa30 that is independent of its organellar localization. (A) Live-cell microscopy of Arl3-GFP in yeast genetically modified as indicated. Restoration of Arl3-GFP localization by hNaa30 is shown as a positive control. (A, C) Scale bar, 5\( \mu \)m. (B) Western blot analysis of cell lysates verified the expression of exogenous human Naa30, Naa50 or Naa60 in yeast strains shown in (A). (C) Detection of endogenous Arl3-GFP in live yeast. Human Naa60 and a C-terminally truncated variant Naa60\(_{1-184}\) (h60del-MEM) were exogenously expressed in \( \text{naa35} \Delta \) and \( \text{naa30} \Delta \) cells, respectively. (D) Amount of cells for which the Arl3-GFP punctate localization was observed in the indicated yeast strains. The number from \( \text{naa30} \Delta \) yeast expressing hNaa30 is a re-representation of data in Fig. 1B as a positive control reference (n > 500 cells). Abbreviations: \( \text{30} \Delta \), \( \text{naa30} \Delta \); \( \text{35} \Delta \), \( \text{naa35} \Delta \); h30, HA-hNaa30; h50, hNaa50; h60, hNaa60; h60del-MEM, hNaa60 without membrane binding region.

From these results, it is clear that human Naa60 and Naa60\(_{1-184}\) are able to rescue the Arl3 localization phenotype, thus enlightening an interesting Nt-acetylation performance of NatF as well as functional
redundancy with NatC. Moreover, human Naa50 acts as a negative control in the assay for Arl3 localization, excluding mere NAT overexpression to be sufficient for phenotype rescue.

Conclusions
We applied *S. cerevisiae* as a model system to study the *in vivo* function of human NATs. The NatC-dependent subcellular localization of yeast Arl3 proved to be a suitable model for studying functional conservation among NatC orthologues as well as diverging *in vivo* function among NatC, NatE and NatF harbouring overlapping *in vitro* substrate specificities (Fig. 6). Naa30 was defined as functionally conserved between yeast and humans through its ability to complement Arl3 mislocalization, which interestingly required the presence of either yeast or human Naa35; accordingly we propose that NatC complex formation occurred (Fig. 6A). Human Naa35 on the other hand, could not complement its yeast orthologue and we suggest this to be due to insufficient binding between yNaa30 and hNaa35 or formation of an impaired complex (Fig. 6B). Taken together, these results indicate that the two subunits Naa30 and Naa35 have co-evolved.

Despite human Naa30, Naa50 and Naa60's shared catalytic activity towards specific N-terminal sequences, we found opposite complementation patterns of NatE/Naa50 and NatF/Naa60 for the NatC-deficient phenotype of Arl3. This clearly hints to unique *in vivo* functions of the two. Furthermore, in light of the different subcellular localizations of Naa60 and Naa60*del-MEM* in human cells and in yeast cells, both being able to rescue the Arl3 localization (Fig. 6C), the difference between Naa50 and Naa60 may reflect differences in the substrate binding site rather than differences in subcellular localization. This assumption is also based on the crystal structures and catalytic efficiencies of human Naa50 and Naa60 suggesting that Naa60 may accommodate larger and more bulky substrate side chains as compared to Naa50.

The yeast model presented here may act as a useful and rapid *in vivo* setup to assess the functionality of various NAT subunits, meaning isoforms of human Naa30, Naa35 and Naa60. For a Naa10 mutant causing the Ogden syndrome, a yeast model provided useful insights into the impaired functionality of NatA. Similarly, the yeast model described here may be used in any future studies of pathological mutants of NatC or NatF.

Methods

Yeast Cultivation. The yeast strains used in this study (see Supplementary Table S1) are derived from one of the two parental strains BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) and BY4742 (*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*). According to their genotype and required selection, the cells were either grown in YPD (Sigma-Aldrich, YPD Broth #Y1375 and YPD Agar #Y1500) or appropriate synthetic defined dropout media (Sunrise

Figure 5. Human Naa60-rescued Arl3 partially co-localizes with the *cis*-Golgi marker Sed5. Yeast cells of the indicated genotypes were imaged for the detection of endogenous Arl3-GFP and exogenous mCherry-Sed5. Scale bar, 2 μm. (B) Subcellular localization of C-terminally EGFP-tagged hNaa60 and hNaa60*del-MEM* in live yeast cells. Scale bar, 5 μm. Abbreviations: 30Δ, naa30Δ; h60, hNaa60; h60del-MEM, hNaa60 without membrane binding region.
**Figure 6.** Putative model of the NatC, NatE and NatF subunits and their effect on Arl3 localization. In WT yeast cells Arl3 is N-terminally acetylated by the NatC complex (Naa30 and Naa35, whereas Naa38 is dispensable for Arl3-acetylation) and targeted to the Golgi transmembrane protein Sys1, mediating Arl3 punctate Golgi localization. However, in the absence of a functional NatC complex Arl3 remains unacetylated after protein synthesis and the targeting is abrogated, leading to Arl3 cytosolic mislocalization. (A) Human Naa30 is able to function in complex with yNaa35 (and possibly yNaa38) or hNaa35, indicating a functionally conserved catalytic subunit between yeast and humans. WT yNatC is depicted framed in dotted line. (B) No
Nt-acetylation activity of hNaa30 is observed in the absence of yeast or human Naa35, which is thought to be the ribosome-binding subunit. Human Naa35 cannot replace its yeast orthologue and function together with yNaa30, neither in the presence nor absence of yNaa38. (C) Naa30 and Naa60 exhibit in vivo redundancy. The Arl3 localization phenotype is complemented by human Naa60 and Naa60del-MEM in both nnaa30Δ and nnaa35Δ yeast. Based on the subcellular localizations of human Naa60 and Naa60del-MEM, a possible post-translational NAT activity at the cytosolic side of Golgi or freely in the cytosol is suggested. Abbreviations: y30, yNaa30; y35, yNaa35; y38, yNaa38; h30, hNaa30; h35, hNaa35; h50, hNaa50; h60, hNaa60; h60del-MEM, hNaa60 without membrane binding region; Ac-CoA, acetyl-coenzyme A.

Plasmid construction. A record of all plasmids and primers used is found in Supplementary Table S2 and Table S3, respectively. Human NAA35 (Gene ID 105606) was sub-cloned from pRS315-HA-hMAK3, which was a kind gift from Professor Sean Munro, MRC, Cambridge, into the pBEVY-U yeast vector downstream of the ADH1 promoter using restriction enzyme sites XmaI (5’) and EcoRI (3’). The N-terminal HA-tag was retained from the original gene construct.

Human NAA35 (Gene ID 105606) was amplified from pCMV6-AC-NAA35-GFP (Origene, #RG213022) with the insertion of XbaI (5’) and PstI (3’) restriction enzyme sites as well as a C-terminal FLAG-tag and ligated into pBEVY-L after the GPD promoter. Additionally, pBEVY-L-hNAA35 was generated by introducing a stop codon downstream of the NAA35 ORF by mutagenesis (Agilent Technologies, #210514).

A plasmid containing human Naa601-184 (Naa60del-MEM) was generated by mutagenesis of pBEVY-U-hNAA604, introducing a stop codon after the codon representing amino acid 184 of Naa60. Moreover, pBEVY-U-hNAA60-EGFP and pBEVY-U-hNAA601-184-EGFP (Naa60del-MEM) were generated for subcellular localization studies by subcloning from mammalian pEGFP-N1 vectors in which the respective NAA60 sequences were inserted between NheI and KpnI sites. The C-terminally EGFP-tagged NAA60 constructs were inserted between XbaI (5’) and Sall (3’) restriction enzymes sites in the pBEVY-U vector after the GPD promoter.

Plasmid p415MET25-mRFP-SED5 was kindly provided by Professor Bianche Schwappach, UMG, Göttingen and pRS316-SEC7-mRFP was from RIKEN BRC DNA BANK (#RD808663). Yeast transformation. Yeast transformation was based on a method developed by Gietz and Schiestl. Exponentially growing yeast cells (10 ml culture, OD600 1.0) were harvested, washed twice with water and resuspended in 240 μl 50% PEG-3350 before addition of 36 μl 1 M lithium acetate, 100 μg salmon sperm ssDNA, DNA template (10 μg gene deletion cassette or 5 μg plasmid), and water up to 360 μl. After a 40 min heat-shock at 42°C the cells were washed four times with water, before plating or incubation in YPD, depending on selection.

Yeast gene deletions were performed by homologous recombination and a complete overview of the primers used to amplify the respective deletion cassettes as well as the PCR-amplified screen for positive mutants are listed in Supplementary Table S3.

Protein extraction and Western blot analysis. Protein extraction was done according to Kushnir et al. with modifications. Exponentially growing yeast cells (4 ml yeast culture, OD600 1.0) were harvested, washed twice with water and resuspended in 240 μl 50% PEG-3350 before addition of 36 μl 1 M lithium acetate, 100 μg salmon sperm ssDNA, DNA template (10 μg gene deletion cassette or 5 μg plasmid), and water up to 360 μl. After a 40 min heat-shock at 42°C the cells were washed four times with water, before plating or incubation in YPD, depending on selection.

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Fluorescence microscopy. Cells in exponential growth phase (OD600 0.8–1.2) were harvested and washed three times in 1x PBS, 2% glucose. Fluorescence images were acquired in the washing solution on a Leica DMi6000 B widefield microscope as previously described. Recorded images were processed using Photoshop CS5 (Adobe Systems, San Jose, CA, USA). Arl3-GFP subcellular localization was characterized as normal (punctate) or mislocalization (other).

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We thank N. Glomnes for technical assistance and Professor S. Munro and Professor B. Schwappach for kindly providing plasmids. This work was supported by the Research Council of Norway (grants 197136 and 230865).
to TA), the Western Norway Regional Health Authority (grant 911883 to TA and grant 911761 to CO), the Norwegian Cancer Society (to TA), and the Bergen Research Foundation (to TA).

Author Contributions
C.O. wrote the manuscript, generated most of the plasmids and yeast strains for this study, conceived and performed most of the experimental work; H.A. generated plasmids and yeast strains, supervised the project and worked on the manuscript; S.N. generated yeast strains and performed fluorescence microscopy; M.M. generated Figures 3 and 5A based on data from C.O. and worked on the manuscript; T.A. initiated, conceived and supervised the project as well as prepared the manuscript.

Additional Information
Supplementary information accompanies this paper at http://www.nature.com/srep

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Osberg, C. et al. Microscopy-based Saccharomyces cerevisiae complementation model reveals functional conservation and redundancy of N-terminal acetyltransferases. Sci. Rep. 6, 31627; doi: 10.1038/srep31627 (2016).

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