**A role for human N-terminal acetyltransferase Naa30 in maintaining mitochondrial integrity**

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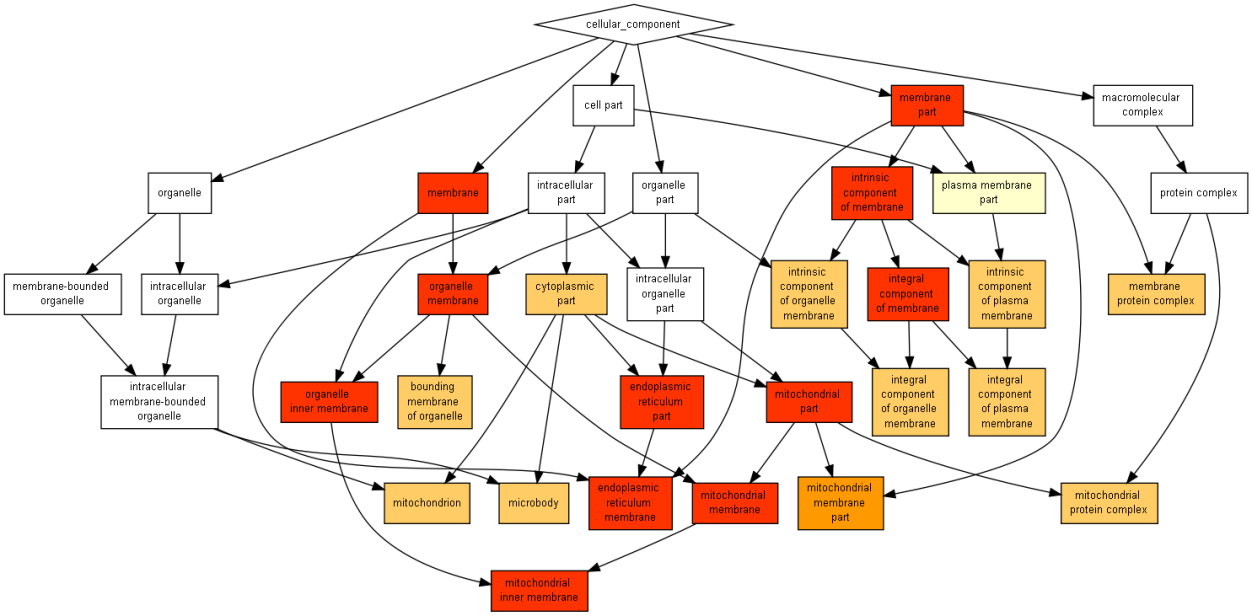
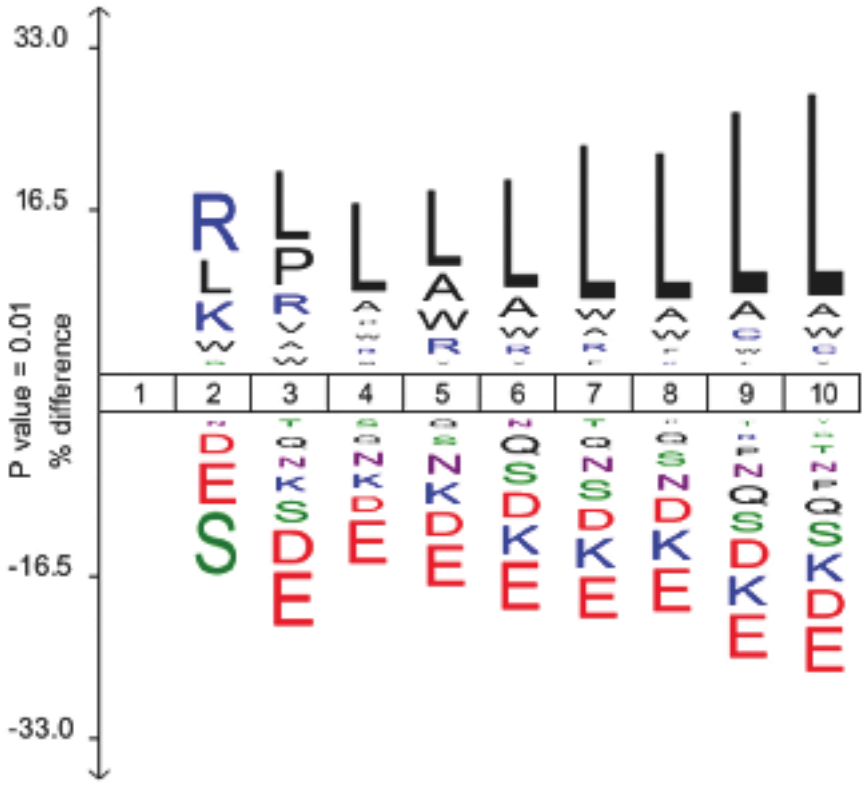
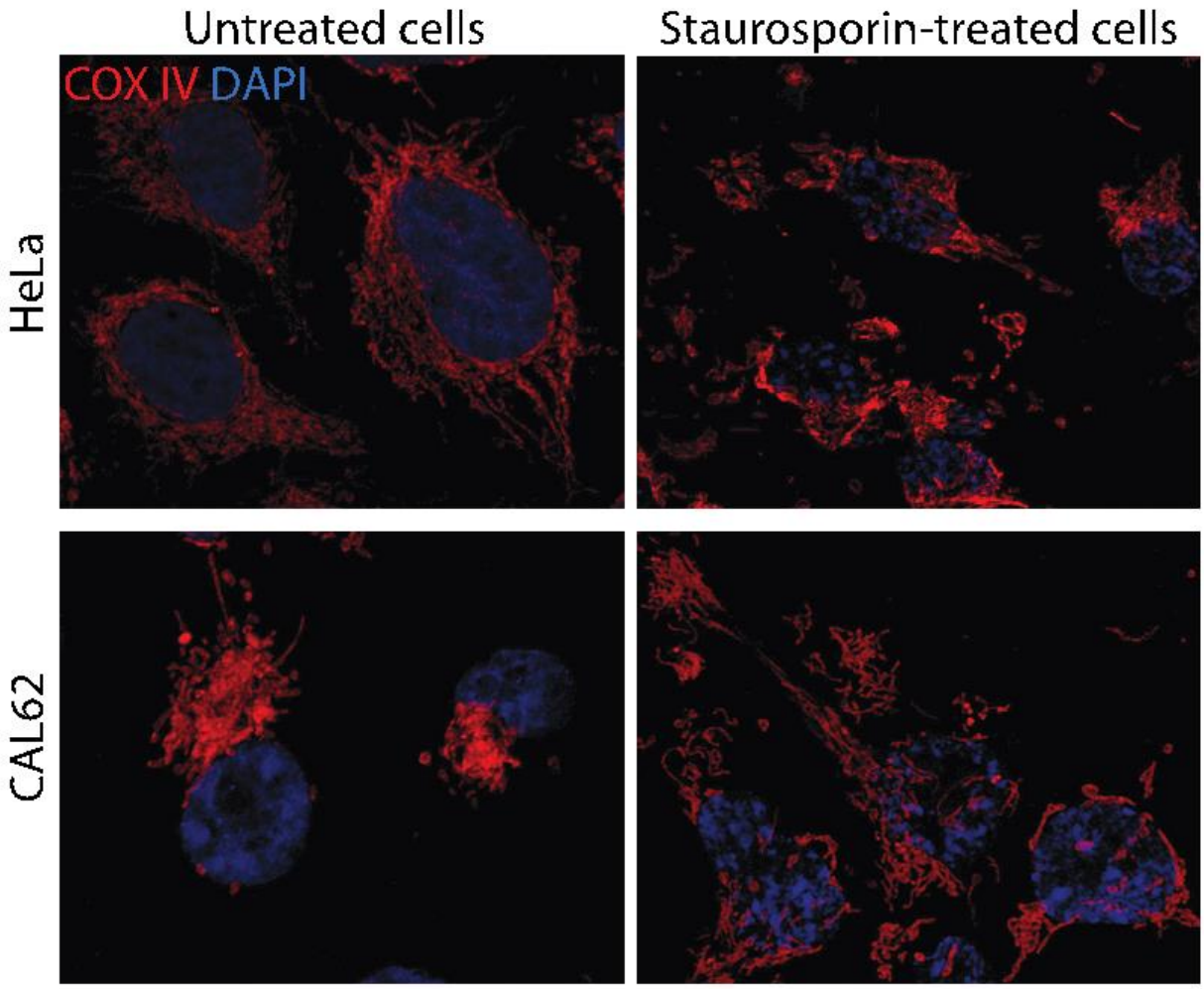
**Supplemental data**



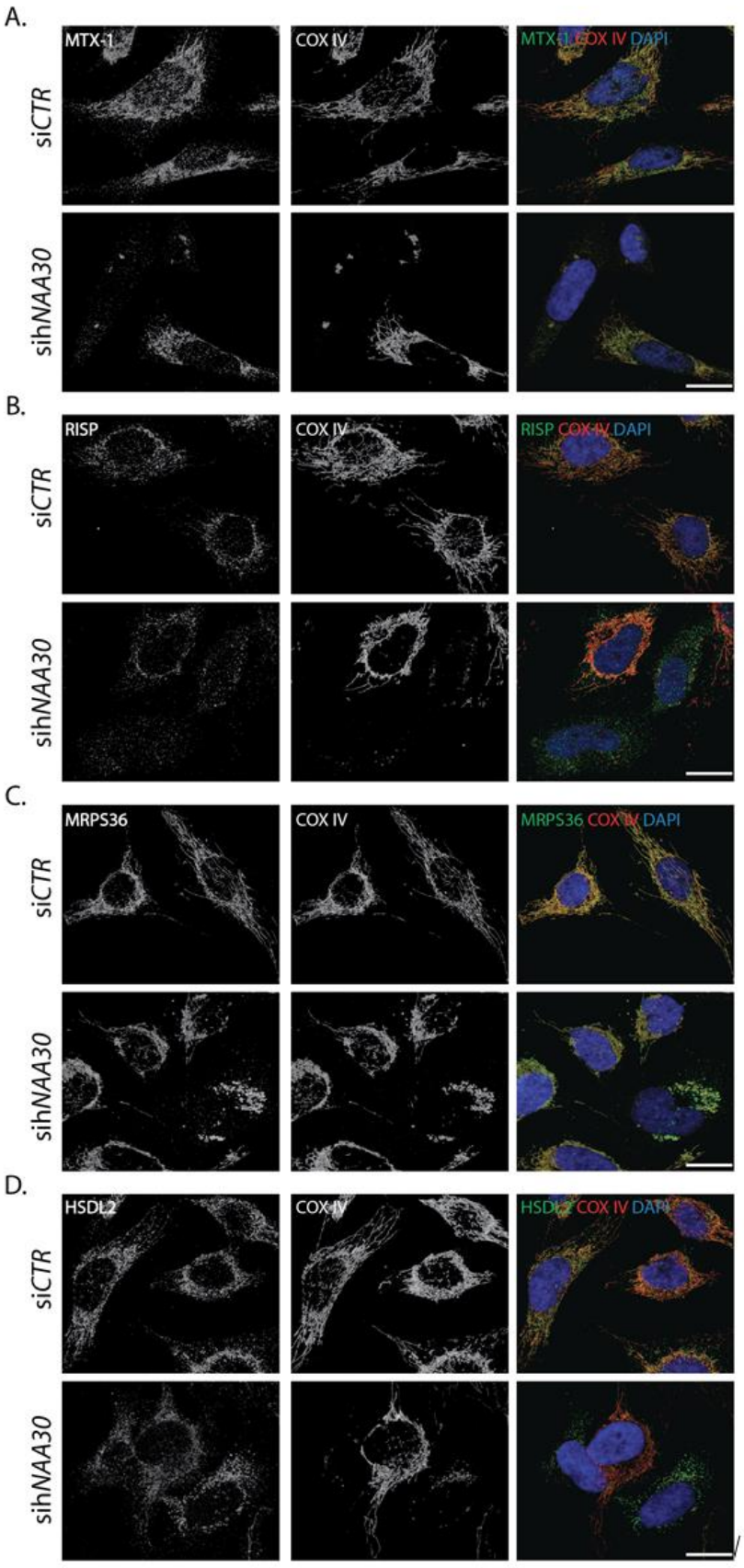

**Supplementary Figure S1| Enriched GO-terms in organelle versus cytosolic enriched fractions.** GOrilla (1) was used to search for enriched GO terms indicative of protein subcellular localization. The proteins exclusively identified in the organelle-enriched fraction (n=209) were used as input protein list against a background list consisting of proteins exclusively identified in the cytosolic fraction (n=623). The resulting enriched GO terms are visualized using a DAG graphical representation with color coding reflecting their degree of enrichment as described in (1). Only GO terms with a p-value > 0.0001 are reported.



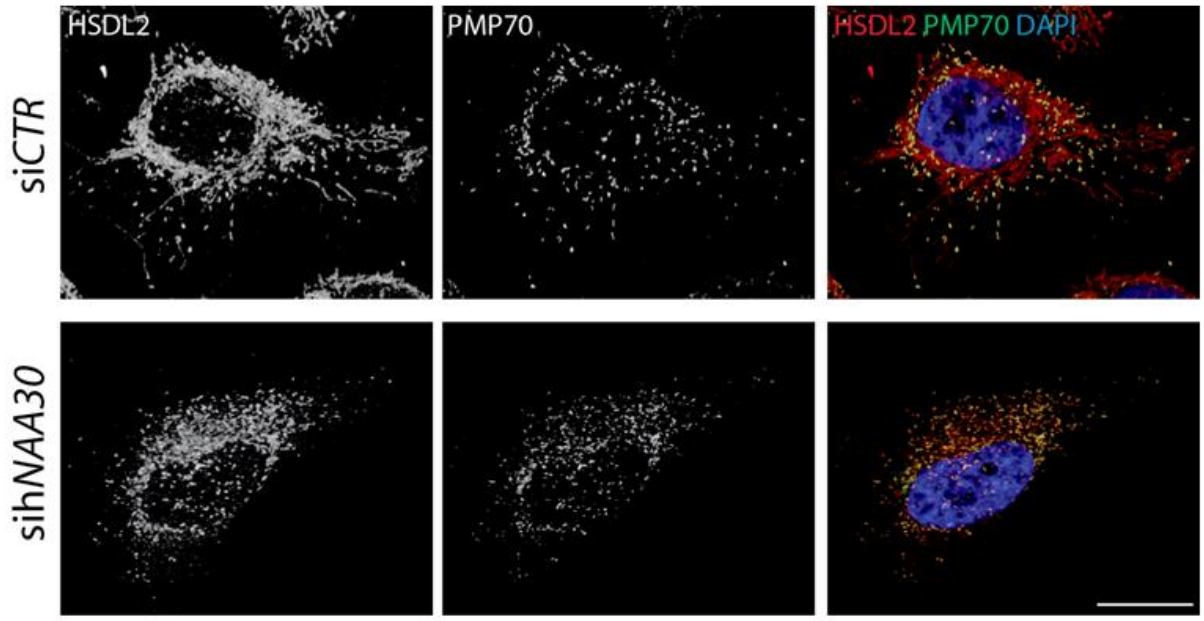

**Supplementary Figure S2| N-terminal amino acid composition of signal sequence containing proteins**. A differential IceLogo representation was created of all human iMet-starting Swiss-Prot N-termini harboring a transit/signal sequence (3930 of 20246 Swiss-Prot entries) versus those lacking an annotated signal/transit sequence. Multiple sequence alignments of peptide substrate motifs are given as 1 to 10, corresponding to the first 10 N-terminal amino acids. Numbering is such that the initiator methionine is at position 1. Statistically significant residues (p-value ≤ 0.01) are plotted with the size of the amino acids proportional to the difference in occurrence between both datasets (1).




**Supplementary Figure S3| Characterization of the mitochondrial network in cells during apoptosis.** Confocal micrographs show HeLa and CAL-62 cells immunostained for the mitochondrial marker COX IV-rabbit (red), in both a normal state and during apoptosis. Apoptosis was induced by treating the cells with 2 µM staurosporin for 6 hours. DAPI (blue) staining was used to visualize nuclei.

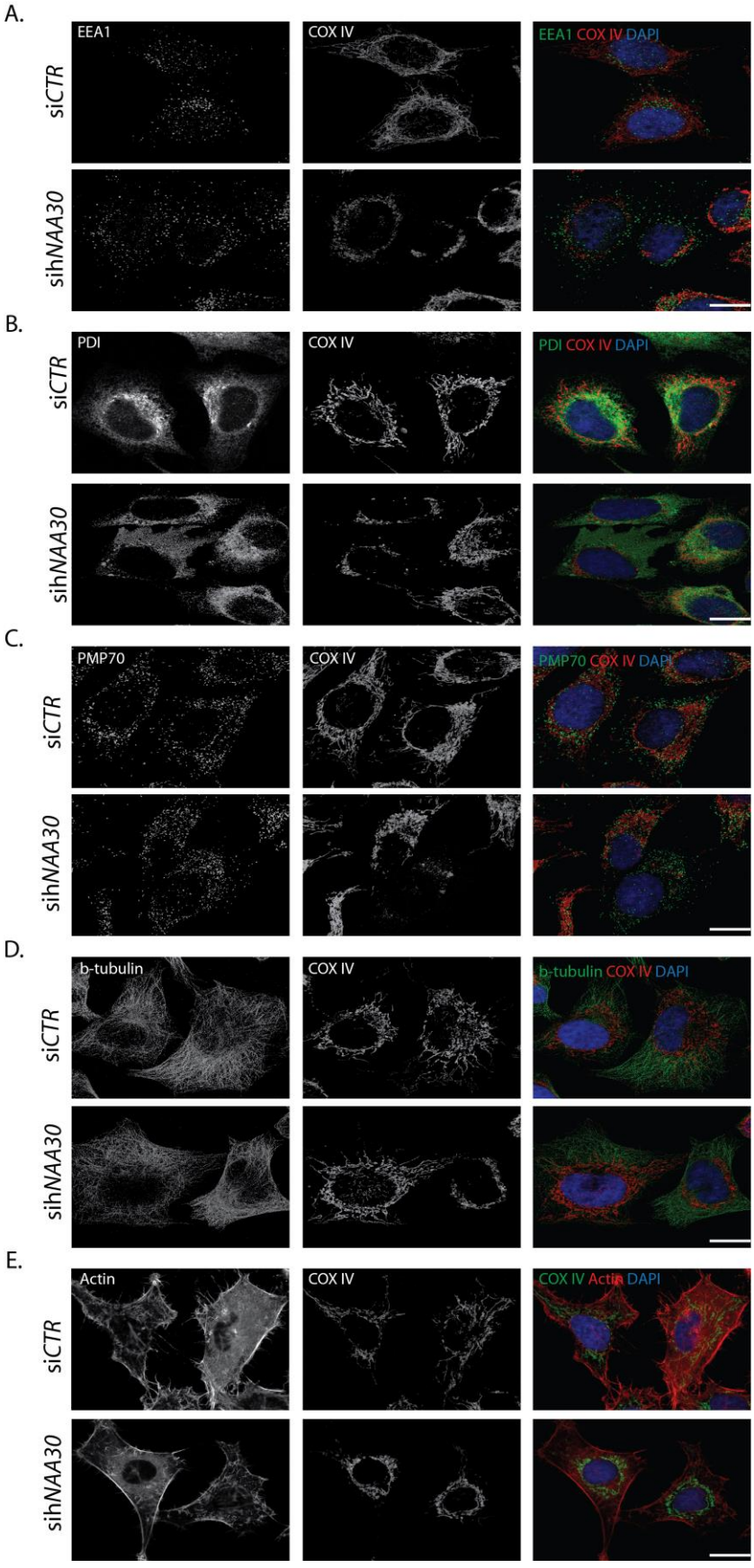

A.
MTX-1
COX IV
MTX-1 COX IV DAPI
siCTR
sihNAA30
B.
RISP
COX IV
RISP COX IV DAPI
siCTR
sihNAA30
C.
MRPS36
COX IV
MRPS36 COX IV DAPI
siCTR
sihNAA30
D.
HSDL2
COX IV
HSDL2 COX IV DAPI
siCTR
sihNAA30

**Supplementary Figure S4| Subcellular localization of selected human NatC mitochondrial substrates upon h*NAA30* knockdown.** Confocal micrographs of siRNA treated HeLa cells immunolabelled with **(A)** anti-MTX-1, **(B)** anti-UCRI, **(C)** anti-MRPS36 or **(D)** anti-HSDL2 was co-stained with anti-COX IV. DAPI staining was used to visualize nuclei. White bars correspond to 10 µm.




**Supplementary Figure S5| Subcellular localization of HSDL2 in siCTR and sihNAA30 treated HeLa cells.**

Micrographs show HeLa cells immunostained with anti-HSDL2 (red) and peroxisomal marker anti-PMP70 (green). DAPI staining was used to visualize nuclei. White bar correspond to 10 µm.


A.
EEA1
COX IV
EEA1 COX IV DAPI
siCTR
sihNAA30
B.
PDI
COX IV
PDI COX IV DAPI
siCTR
sihNAA30
C.
PMP70
COX IV
PMP70 COX IV DAPI
siCTR
sihNAA30
D.
b-tubulin
COX IV
b-tubulin COX IV DAPI
siCTR
sihNAA30
E.
Actin
COX IV
COX IV Actin DAPI
siCTR
sihNAA30

**Supplementary Figure S6: Naa30 depletion does not affect the morphology or distribution of endosomes, ER, peroxisomes, microtubules or the actin cytoskeleton.** Confocal micrographs of siRNA treated HeLa cells immunostained for **(A)** EEA1 probing early endosomes, **(B)** PDI probing the endoplasmatic reticulum, **(C)** PMP70 probing peroxiomes, **(D)** β-tubulin probing microtubules and **(E)** Rhodamine Phalloidin probing F-actin. DAPI staining was used to visualize nuclei. White bars correspond to 10 μm.

**References**

1. Eden, E., Navon, R., Steinfeld, I., Lipson, D., and Yakhini, Z. (2009) GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC bioinformatics* 10, 48