Supplementary Information

Aim-less translation: loss of *Saccharomyces cerevisiae* mitochondrial translation initiation factor mIF3/Aim23 leads to unbalanced protein synthesis

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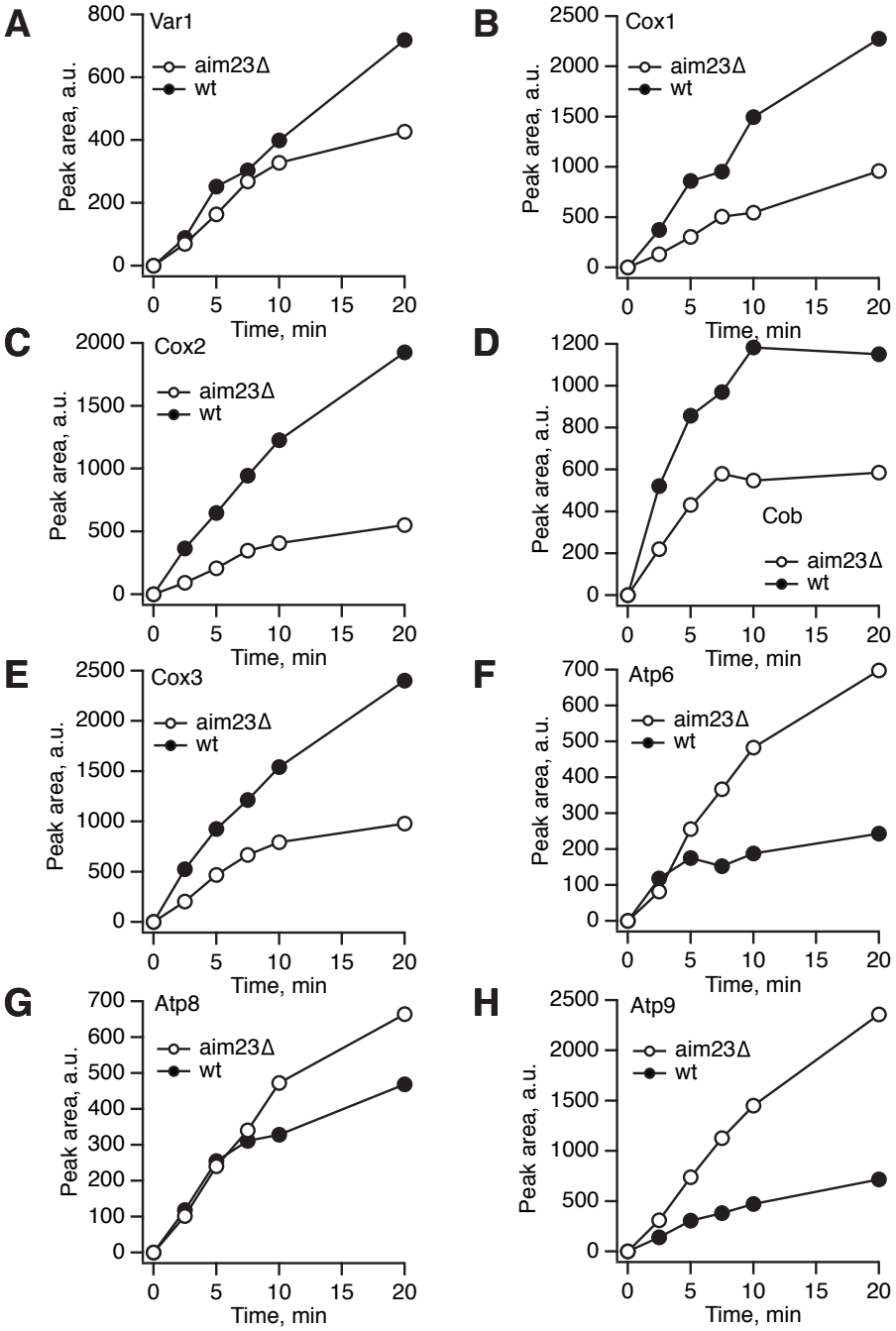
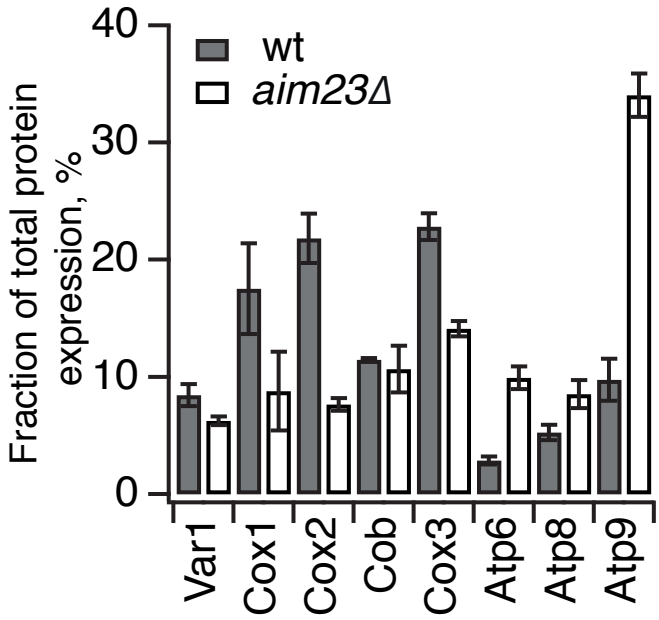
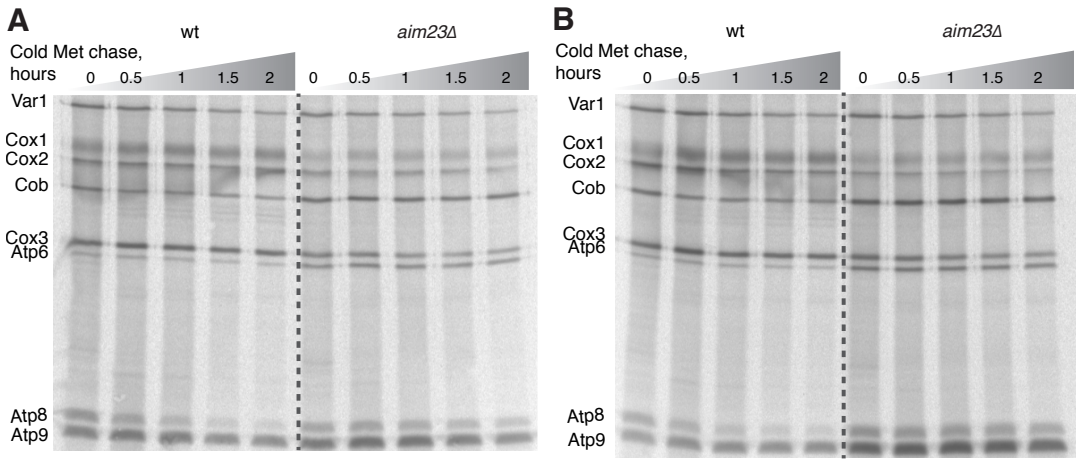


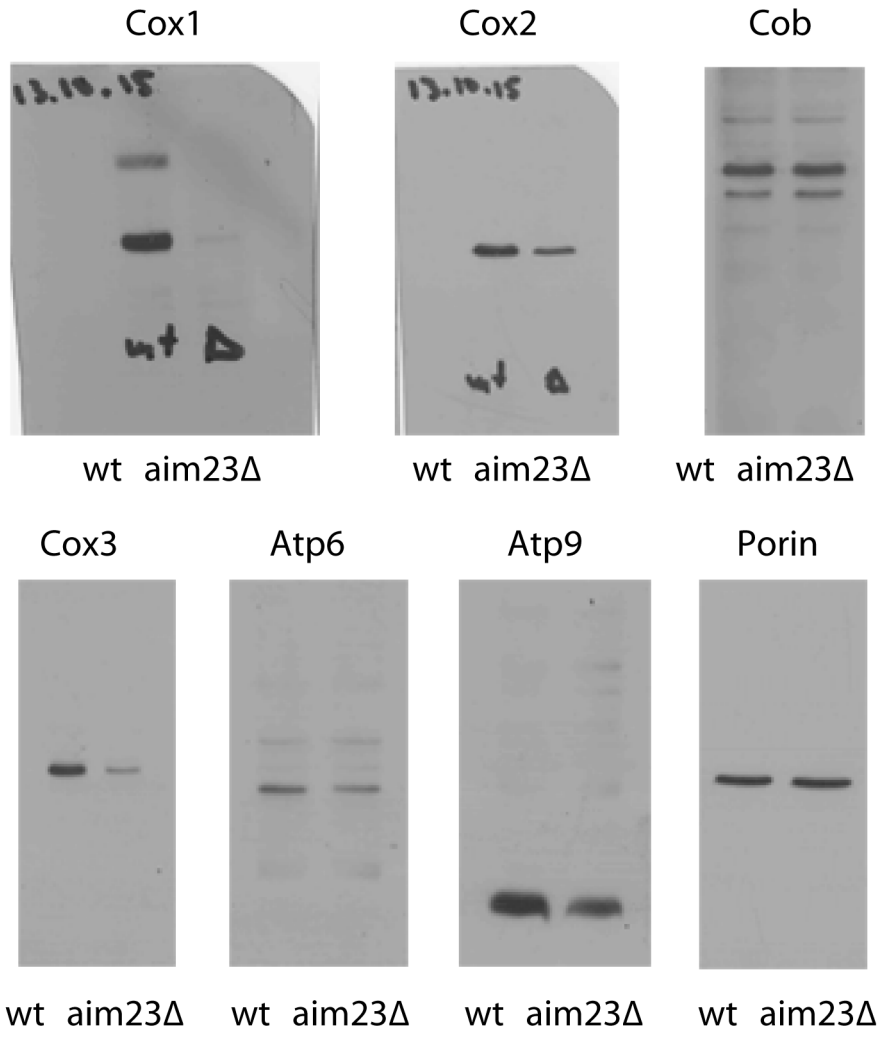
Supplementary Figure 1 | Kinetics of ^{35}S-methionine incorporation in newly synthesized mitochondrial proteins. The experiments were performed using a congenic set of wild type and *aim23Δ* cells in a BY4741 background. The raw data are presented in **Figure 3A**. ^{35}S-methionine pulse labeling was carried out as per Gouget and colleagues[1]



Supplementary Figure 2 | Levels of mitochondrially-encoded proteins after 20 min labeling with ^{35}S-methionine. The relative expression is normalized to total expression of mitochondrially encoded protein genes.



Supplementary Figure 3 | Turnover of mitochondrially synthesised proteins in wild type and *aim23Δ* strains. After 15 minutes of ^{35}S methionine pulse labeling was carried out as per Gouget and colleagues[1], the labeling reaction was stopped by the addition of cold methionine (final concentration of 80 mM) and puromycin (final concentration of 4 µg/ml). Samples were collected after the indicated time points, proteins were resolved on SDS PAGE and visualized by radioautography. Panels (**A**) and (**B**) represent two biological replicates.



Supplementary Figure 4 | Unprocessed pictures of Western blots used in this work (see Figure 3D).

Supplementary References:

1. Gouget, K., Verde, F. & Barrientos, A. In vivo labeling and analysis of mitochondrial translation products in budding and in fission yeasts. *Methods Mol Biol* **457**, 113-24 (2008).