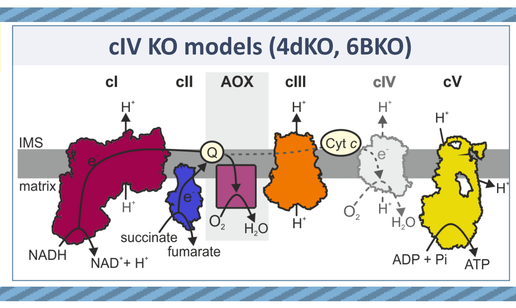
Uncovering the OXPHOS complexes interdependence mechanism.

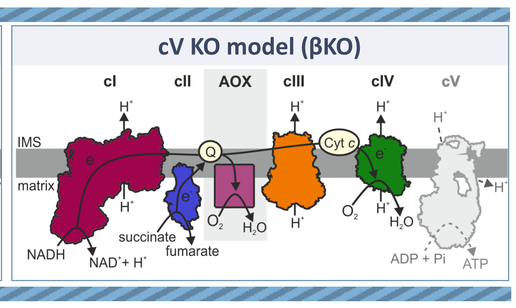
Čunátová Kristýna [1], Vrbacký Marek [1], Puertas-Frias Guillermo [1], Eliáš Jan [1], Houštěk Josef [1], Pecinová Alena [1], Mráček Tomáš [1], Pecina Petr [1]

[1] Institute of Physiology, Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic

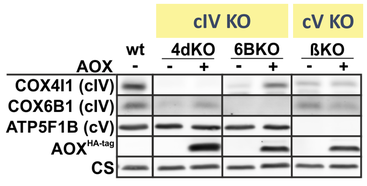
INTRODUCTION

Oxidative phosphorylation system (OXPHOS) complexes are not linked only by their function but also by the interdependence of biogenesis and maintenance of individual complexes. It was hypothesized that secondary complex deficiency takes place at the level of enzyme assembly and stability. However, we recently reported **cIV–cI interdependence** in **cIV deficient cells (COX4KO)** and ascribed it to a novel mechanism involving the **downregulation of mitochondrial protein synthesis**[1]. In the current study, we explore this mechanism of interdependence in more detail and also by using additional HEK293 cell-line-based knock-out models of **cIV (COX6BKO) and cV (βKO) deficiency**. Further, we expressed **alternative oxidase** (**AOX**, *Aspergillus nidulans*) in KO models to study the possible improvement of secondary OXPHOS deficiency.



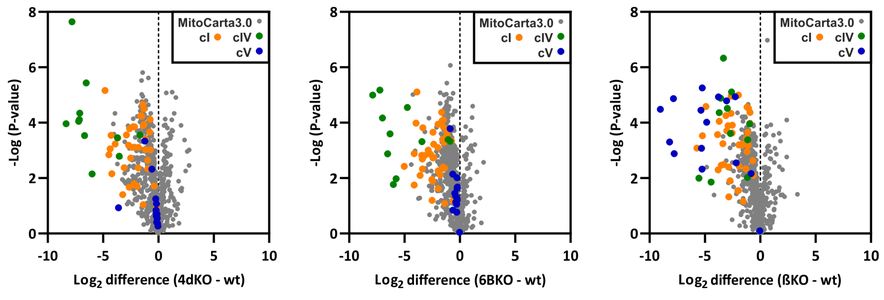


1) MODELS OF OXPHOS COMPLEXES INTERDEPENDENCE



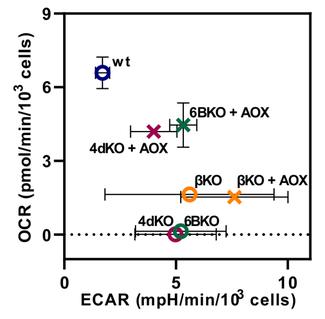
Mass spectrometry label-free quantification (MS LFQ)

- **cIV and cV KO** present with a **decrease** of subunits of the **respective targeted complex**, **secondary decrease** in the steady-state level of **cI subunits**, as well as mild overall decrease of **mitochondrial proteins** (MitoCarta 3.0)
- in addition, **cV KO** shows a **secondary decrease** in the **cIV subunits** level



Basal respiration vs. glycolytic rate

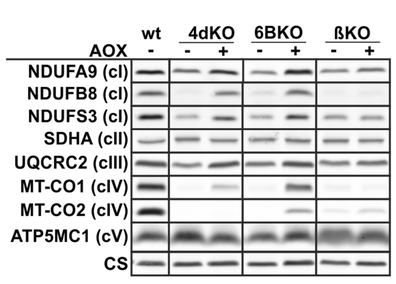
- **cIV KO** models show **absence of mitochondrial respiratory chain activity** (**OCR**), which is compensated by **increased glycolytic rate** (**ECAR**)
- **AOX** introduction **partially restores electron flux** in the **cIV KO** model
- the already **diminished respiration** in **cV KO** model is **not influenced by AOX** expression

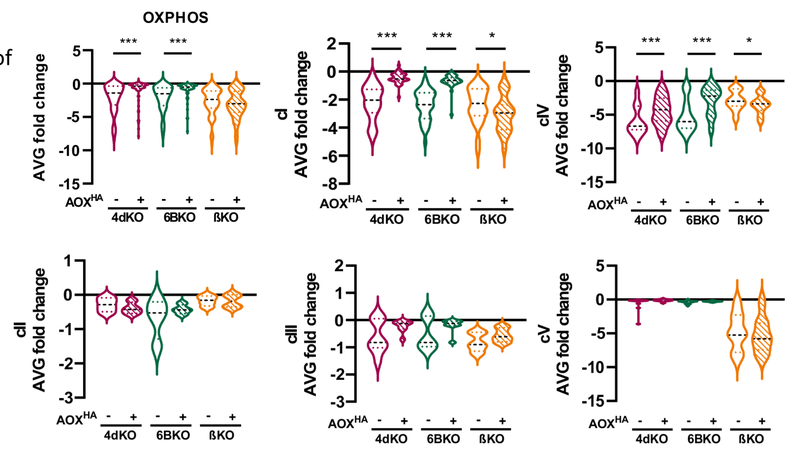


2) AOX EXPRESSION REINSTATES cI LEVEL IN cIV KOs

MS LFQ, SDS-PAGE/WB

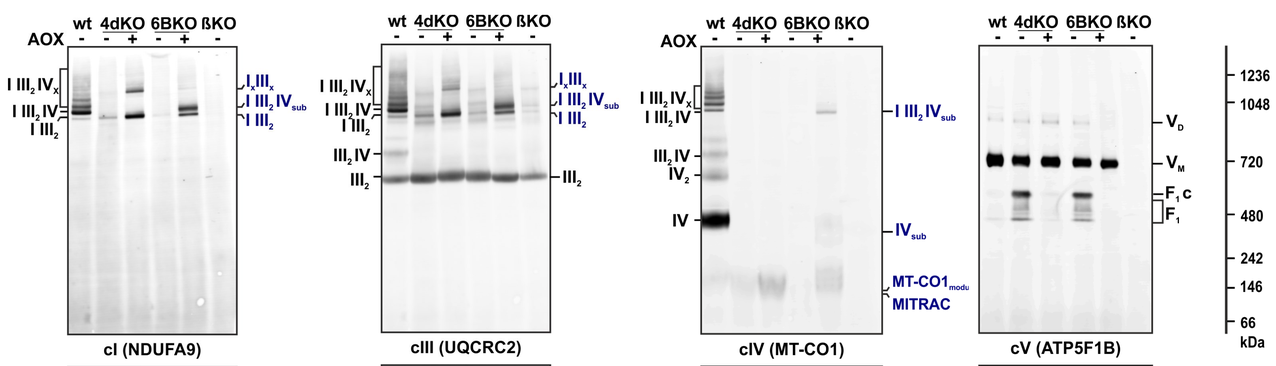
- **AOX expression reinstates** steady-state level of **cI and cIV** subunits **only in cIV deficient cells**, not in cV KO
- *average fold changes (Log_2, KO model - wt)*



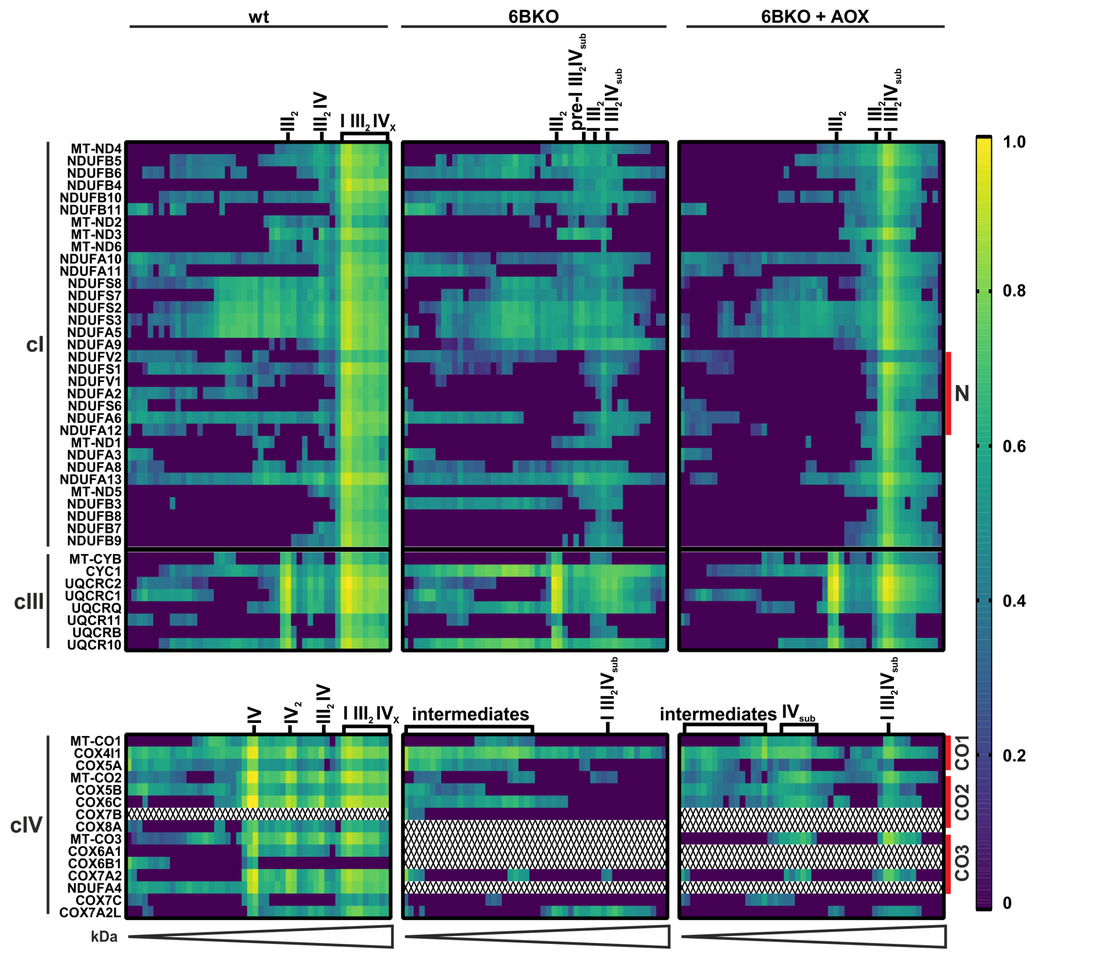


BN-PAGE/WB

- cIV holoenzyme is completely absent in cIV KO models, as well as cV in the cV KO model
- content of cI is severely diminished in all KO models, the preserved portion is mostly present in association with cIII dimer
- levels of complex III are not significantly changed, but its migration shows redistribution from supercomplexes into dimers and to a lesser extent to I III_2 supercomplex
- **AOX expression reinstates native cI level** (in cIV KO models)
- interestingly, in the **6BKO** model, **AOX expression** allows the formation of **I III_2 IV_{sub}** supercomplex not observed elsewhere



3) AOX EXPRESSION STABILIZES cIV IN 6BKO

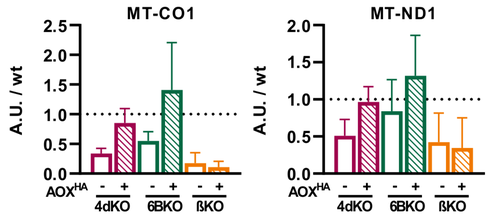


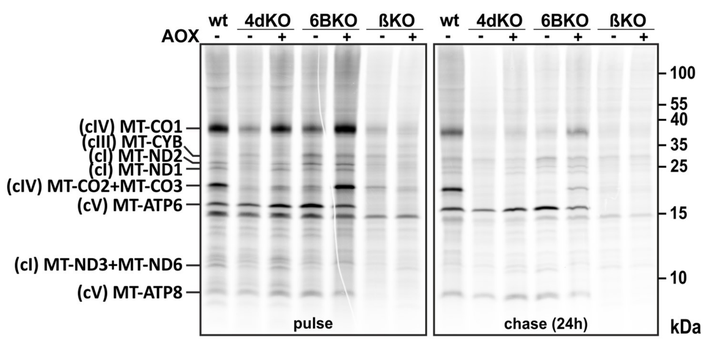
Complexome profiling analysis

- *iBAQ values of individual subunits of cI, cIII, and cIV, organized according to the increasing molecular weight (kDa)*
- in 6BKO only a few **cIV subunits are present in SC (I III_2IV_{sub})**, after AOX expression IV_{sub} in SC gets stabilised
- besides the early cIV intermediates in **6BKO with AOX**, IV_{sub} subassembly is also detected
- cI assembly intermediate in association with cIII and cIV subunits COX4I1, COX5A, and COX7A2L (**pre-I III_2IV_{sub}**) lacking matrix facing domain necessary for its catalytic function (N-module) is present **in 6BKO**

4) AOX EXPRESSION RESTORES MITOCHONDRIAL TRANSLATION IN cIV KOs

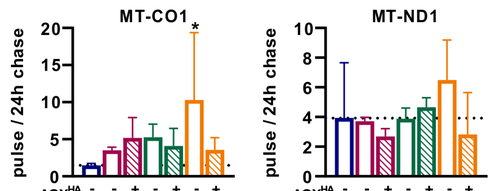
Pulse intensity relative to controls



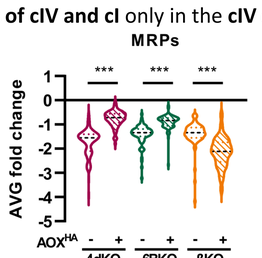


- *^{35}S in vivo labelling – Met + Cys*
- **cIV KO** models (4dKO, 6BKO) present mainly with the **decrease in pulse signal of cIV and cI mtDNA-encoded subunits**
- **cV KO** shows an even more pronounced **decrease in the pulse of cI and cIV** with an additional significant decrease of **cV subunits**
- increased pulse level of MT-CO2+MT-CO3 in 6BKO after AOX expression reflects the stabilization of the MT-CO2 subunit in subcomplexes and supercomplex (Part 3)
- **AOX expression** results in an **increase of newly synthesized mtDNA-encoded proteins of cIV and cI** only in the **cIV deficient cells**

Pulse/chase ratio



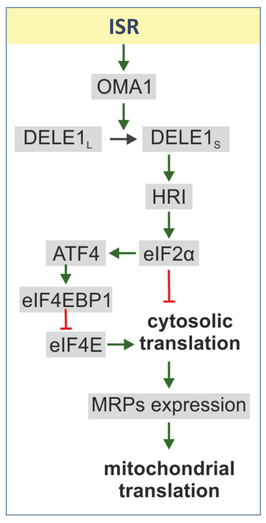
- **cIV subunits** are not only synthesized at a slower pace but also have a **faster turnover**
- **cI mtDNA-encoded subunits** level decrease in cIV and cV deficient models is not caused by a faster turnover but is rather **explained by the lower mitochondrial protein synthesis rate**



MS LFQ

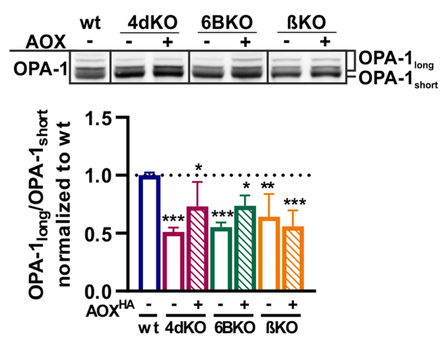
- mitochondrial ribosomal proteins (**MRPs**) steady-state level is **decreased in KO models**
- this decrease is partially **reverted by AOX** expression only in **cIV KO models**
- *(log2 difference (model-wt))*

5) INTEGRATED STRESS RESPONSE (ISR) ACTIVATION IN KO MODELS



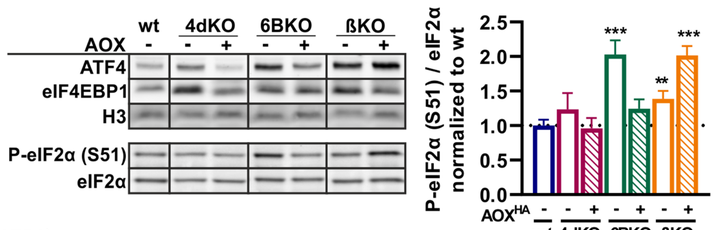
ISR

- *ISR represents a possible signaling pathway interconnecting primary OXPHOS deficiency with the mitochondrial protein synthesis attenuation, resulting in a secondary OXPHOS complex deficiency*



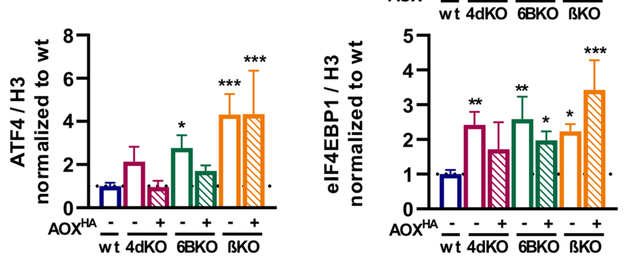
SDS-PAGE/WB quantification

- analysis of OPA-1 cleavage revealed a higher proportion of short OPA-1 forms in KO models, decreasing after AOX expression in cIV KO models, i.e. higher OMA1 activity

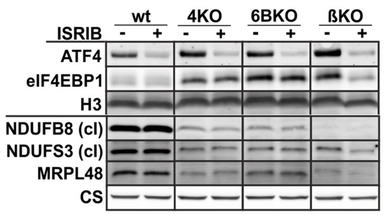


SDS-PAGE/WB quantification

- activating transcription factor 4 (**ATF4**) steady-state level is **increased in KO models**, **drops after AOX expression in cIV KO models**, yet not in cV KO
- interestingly, **eIF4EBP1** protein has a similar pattern as ATF4 across the models

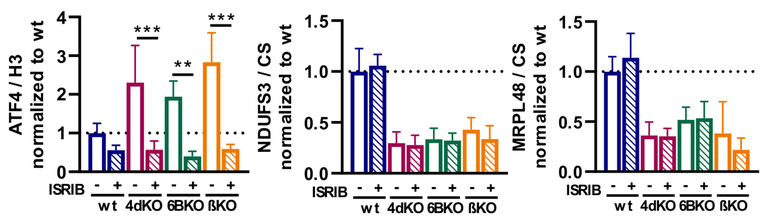


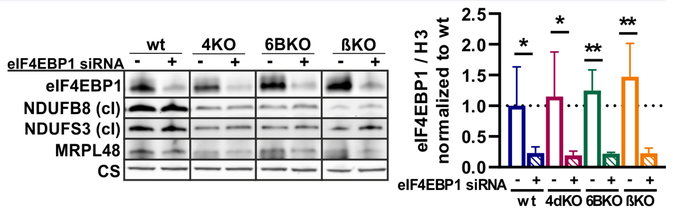
6) ACTIVATION OF ISR DOES NOT DETERMINE OXPHOS COMPLEXES INTERDEPENDENCE

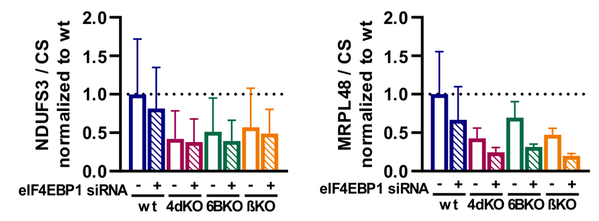


SDS-PAGE/WB quantification

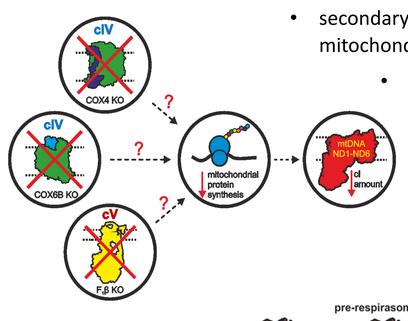
- neither ISR inhibition by **ISRIB** (for 3 days) nor **eIF4EBP1 knock-down** increase steady-state levels of **cI and MRP subunits** in **cIV** and **cV KO** models







CONCLUSIONS



- secondary decrease of cI, induced by cIV/cV severe defect, is coupled to the mitochondrial protein synthesis attenuation
  - mitochondrial protein synthesis attenuation observed in cIV and cV deficient models implicates cI biogenesis rather than turn-over alteration
    - partial restoration of the electron flux by AOX introduction improves the phenotype of cIV deficient models only
- **mitochondrial ISR is triggered by cIV/cV severe deficiency, yet it does not explain the secondary deficiency of cI**
- **COX6B plays a role in early steps of cIV assembly**
- **COX6B lacking cells support the hypothesis of cooperative cIV assembly[2] into the respirasome**

petr.pecina@fgu.cas.cz

[1] K. Čunátová *et al.*, Loss of COX4I1 Leads to Combined Respiratory Chain Deficiency and Impaired Mitochondrial Protein Synthesis, Cells, 10 (2021).

[2] E. Fernández-Vizarra, C. Ugalde, Cooperative assembly of the mitochondrial respiratory chain, Trends Biochem Sci. (2022).