# The relevance of ACBD3 protein in energy metabolism in various cell lines

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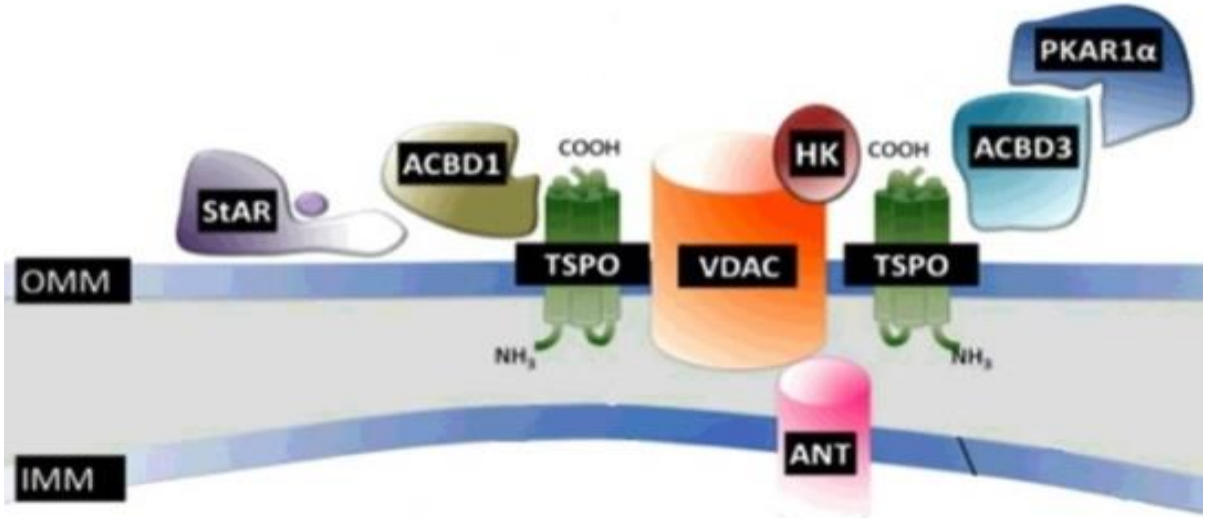
# INTRODUCTION



(Gatliff and Campanella, 2015)

## Acyl-CoA binding domain containing 3

- All human tissue
- GA, ER
- Functions: division of neuronal cells, neurodegeneration, lipid homeostasis, stress response, maintenance of GA or viral replication
- Targeting cholesterol into mitochondria
- KD ACBD3 suppresses hormone-induced steroidogenesis (Li et al. 2001)

## Patient - homozygous mutation in ACBD3

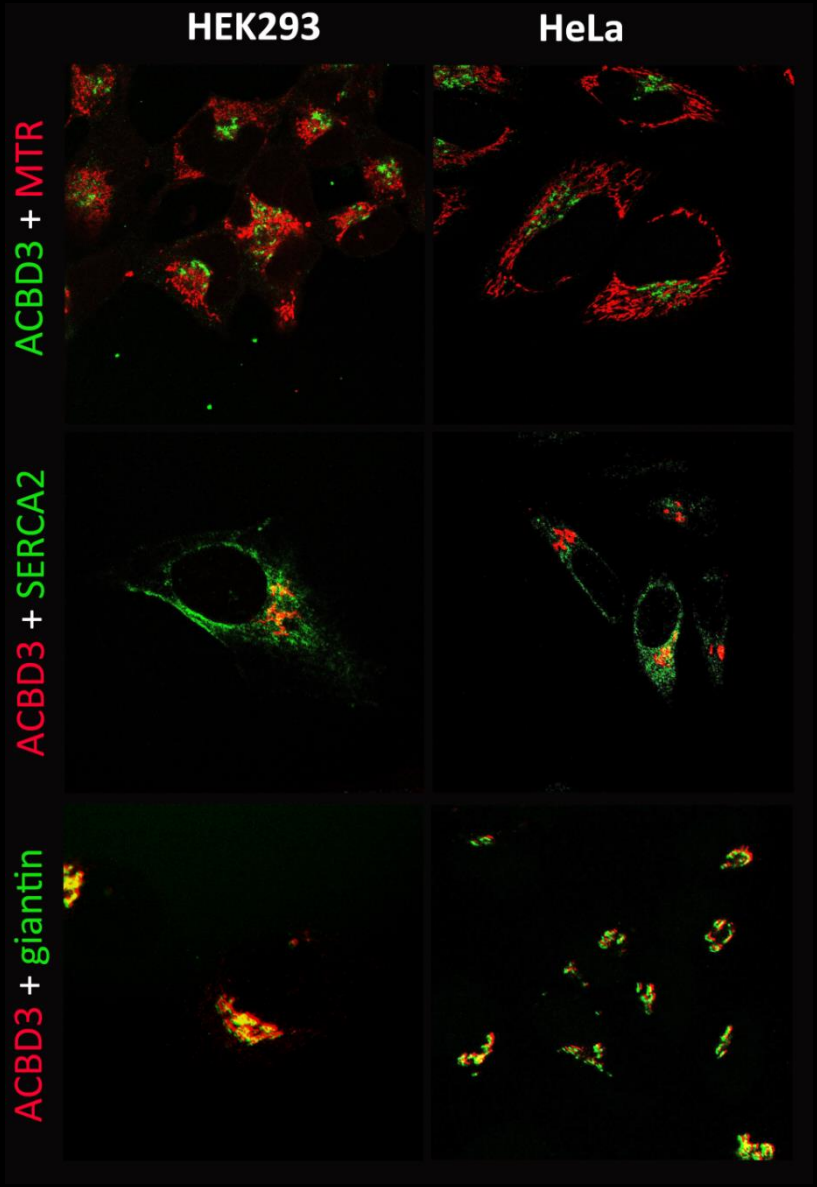
- different distribution of cholesterol in fibroblasts
- increased amount of mito. and enlarged mito. in muscle
- ↓ CI, CIII, CIV and ATP synthase

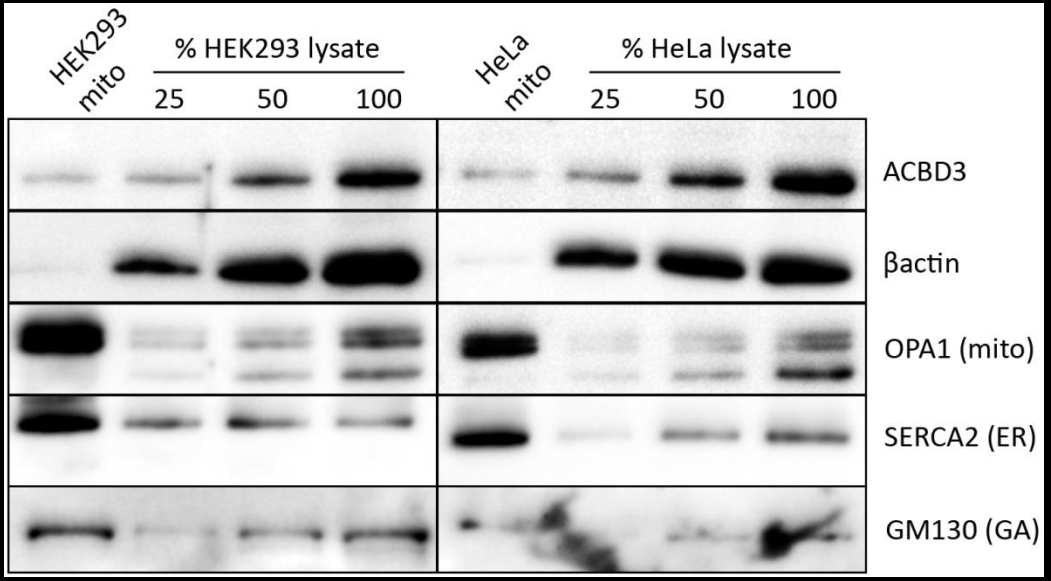
## The aim

- Role of ACBD3 in mitochondrial energetic metabolism in HEK293 and HeLa cell lines

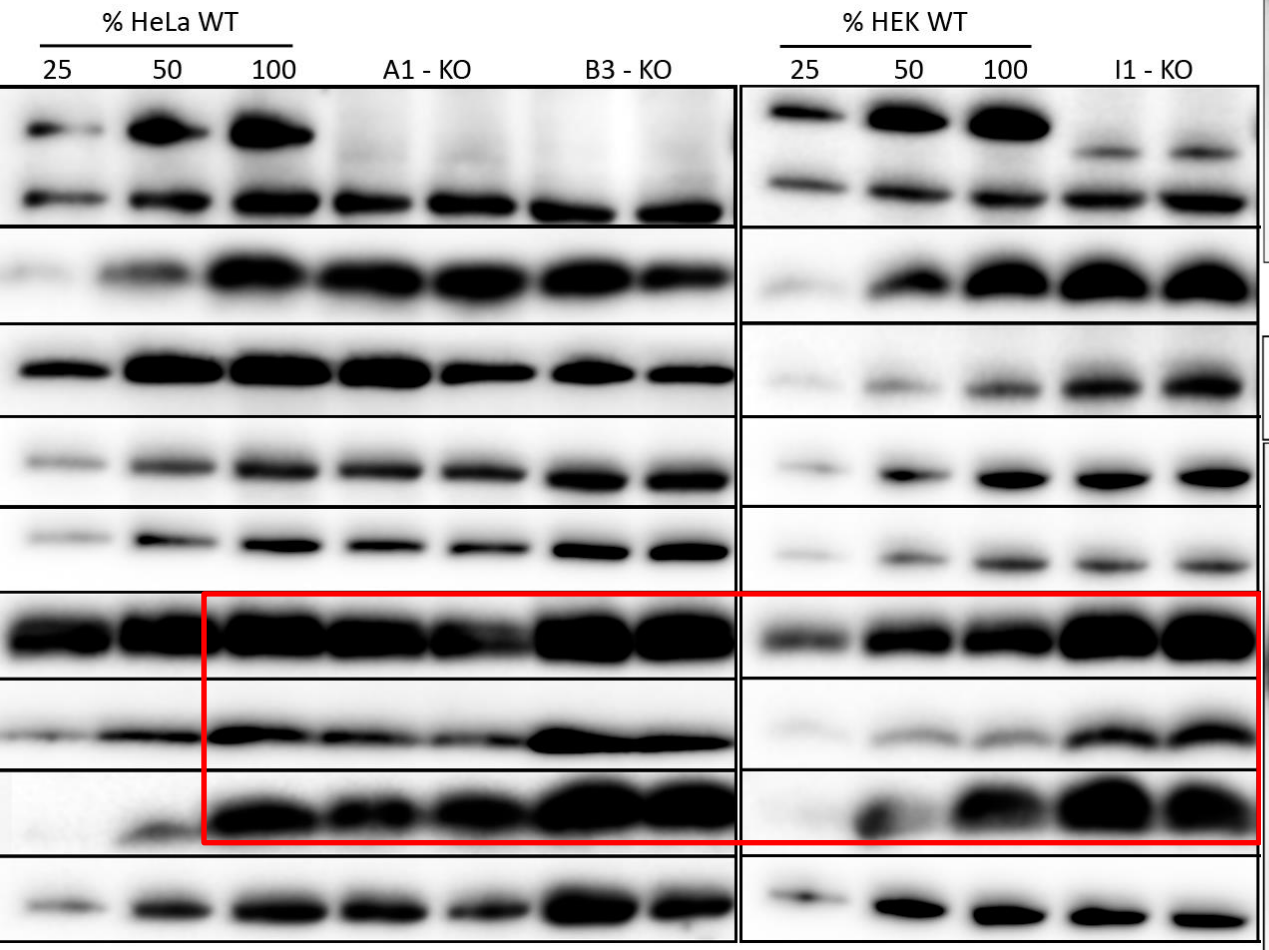
# RESULTS A:

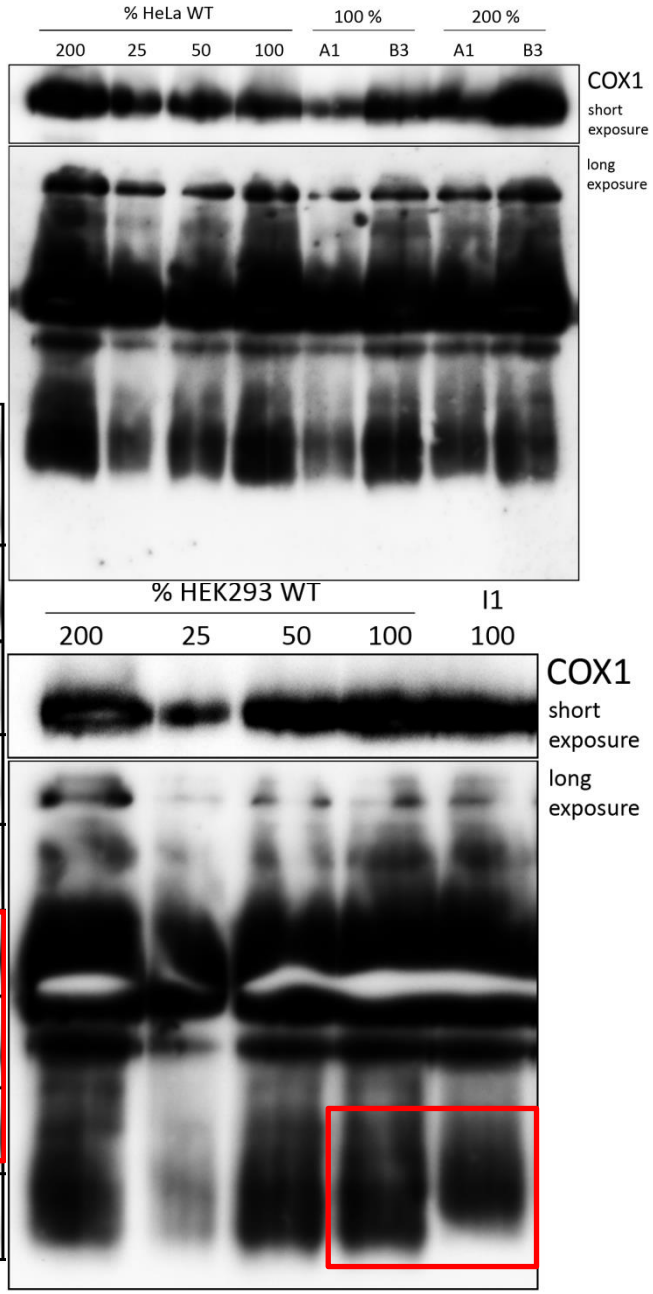
## Localization of ACBD3 in WT HEK293 and HeLa



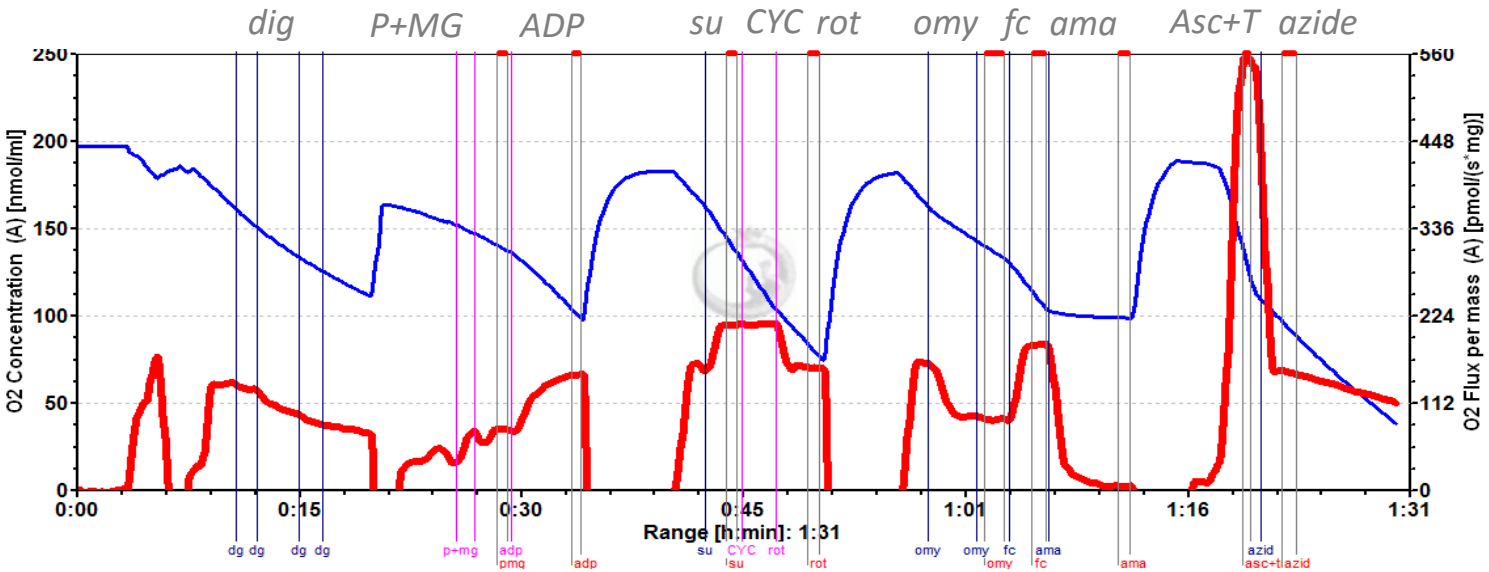



# Results B: SDS-PAGE/WB and BN-PAGE

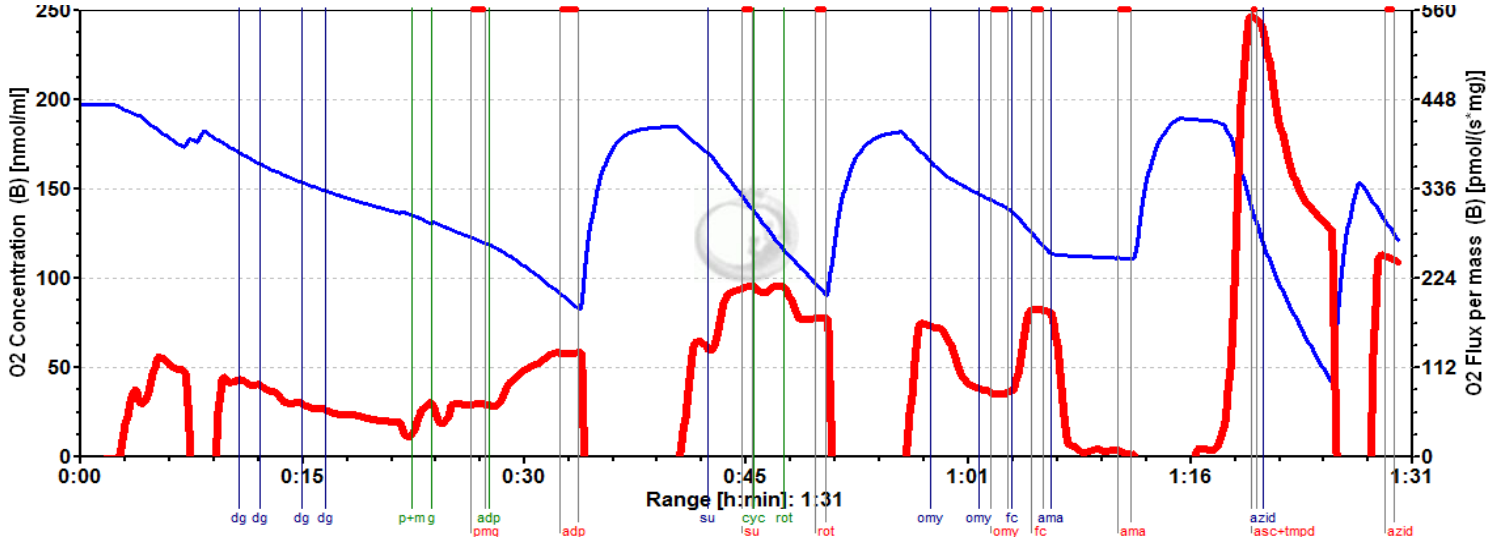


# Results C:Mitochondrial respiration (Oxygraph-2k)




HEK 293 WT

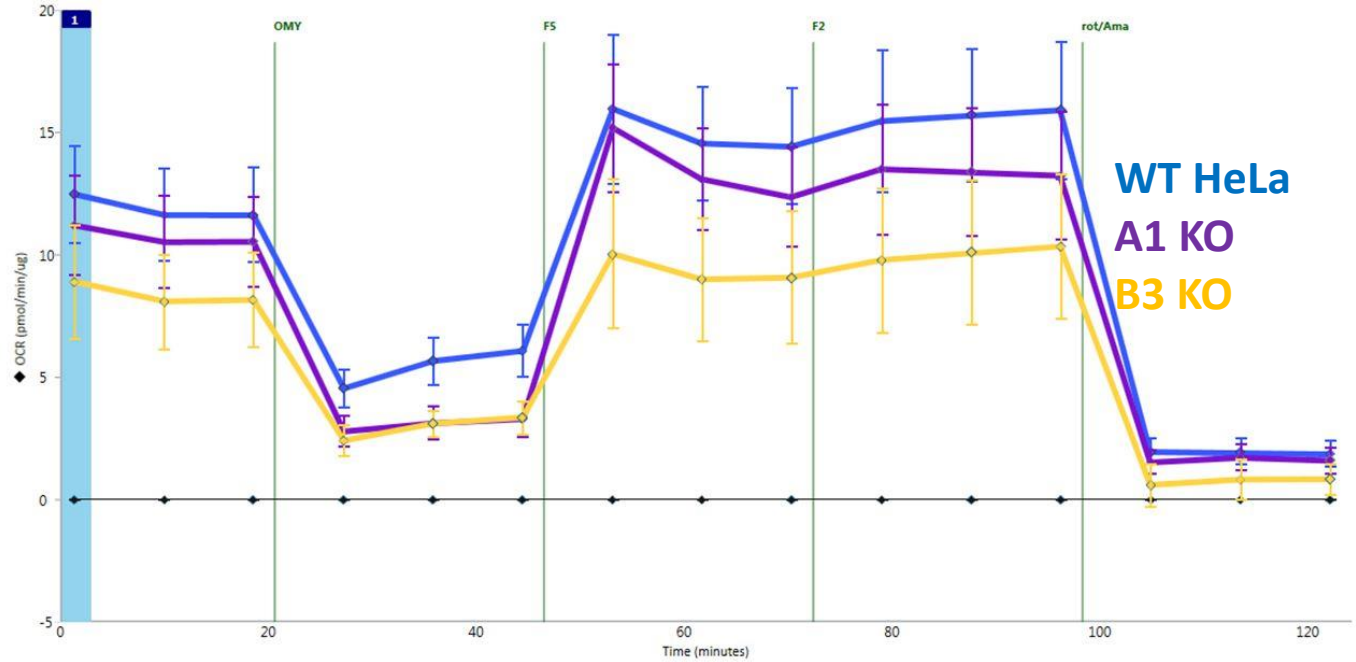


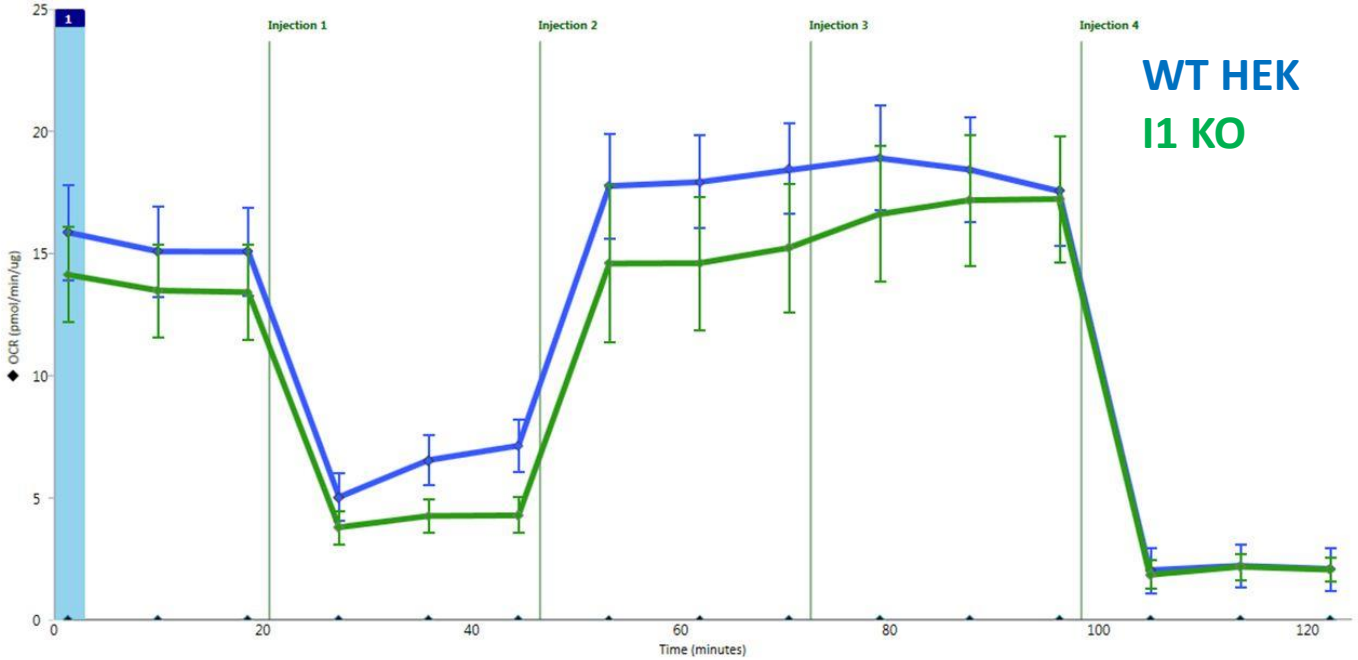

I1 KO

Abbreviations: dig - digitonin (8mM, 1-2 µl); P - pyruvate (1M, 20 µl), M - malate (1M, 6 µl), G - glutamate (1M, 20 µl), ADP + $Mg^{2+}$ (0,5M, 4 µl), su - succinate (1M, 20 µl), CYC - cytochrome C (2mM, 5 µl), rot - rotenone (0,5 mM, 1 µl), omy - oligomycine (2,5 mM, 0,2-0,6 µl), fc - FCCP (1mM, 0,2-0,6 µl), ama - antimycyne A (1mM, 1 µl), asc - ascorbate (0,2M, 25 µl), T - TMPD (40mM, 45 µl), azide (4M, 5 µl)

# Results D:

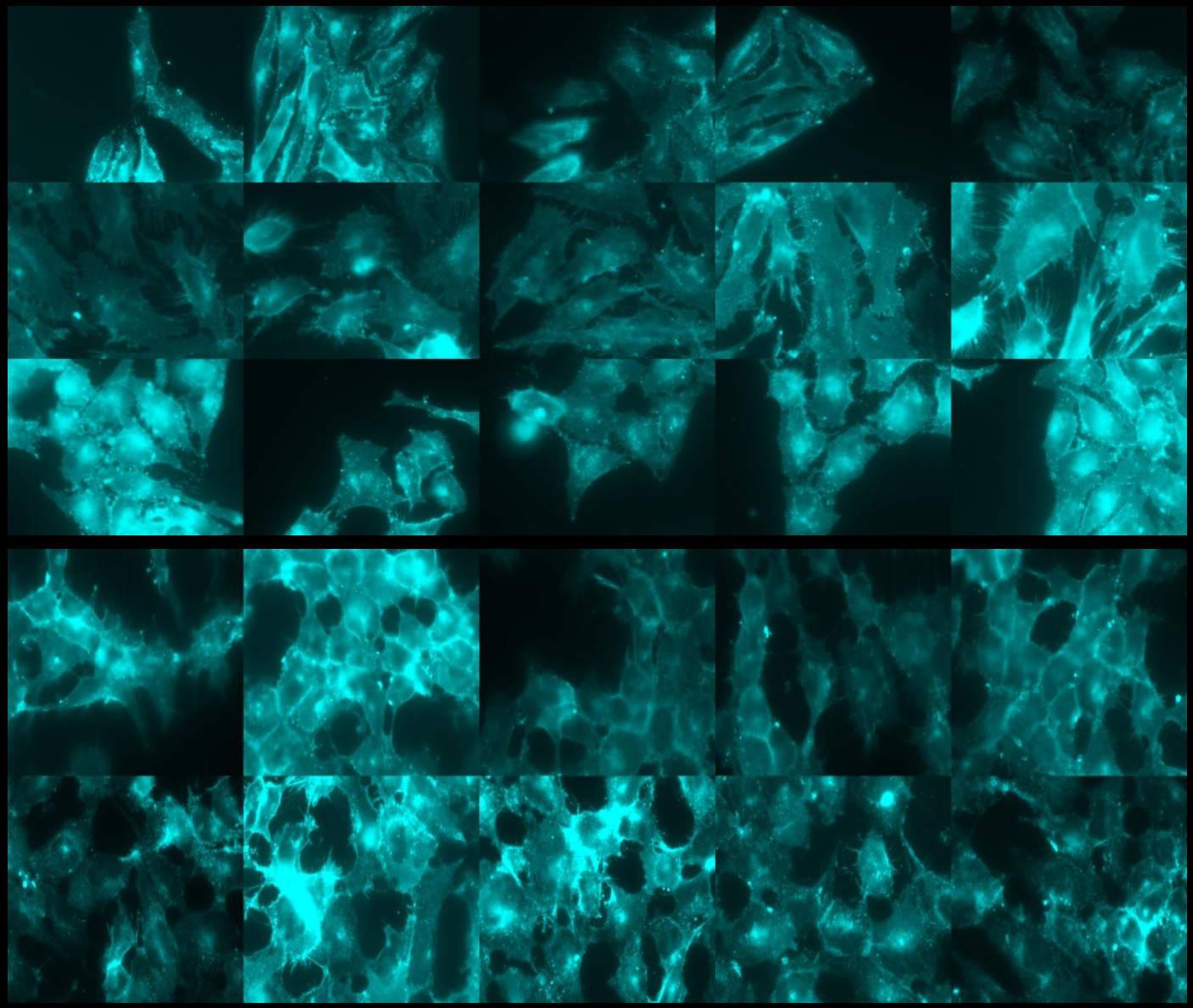
## according: Seahorse XF Cell Mito Stress Test Kit




**Final concentration of substrate/inhibitor per well:**
oligomycine (2µM), FCCP (0,5µM), FCCP (0,2µM), antimycine A (1µM), rotenone (2µM)

# Results E: Filipin test



HeLa WT

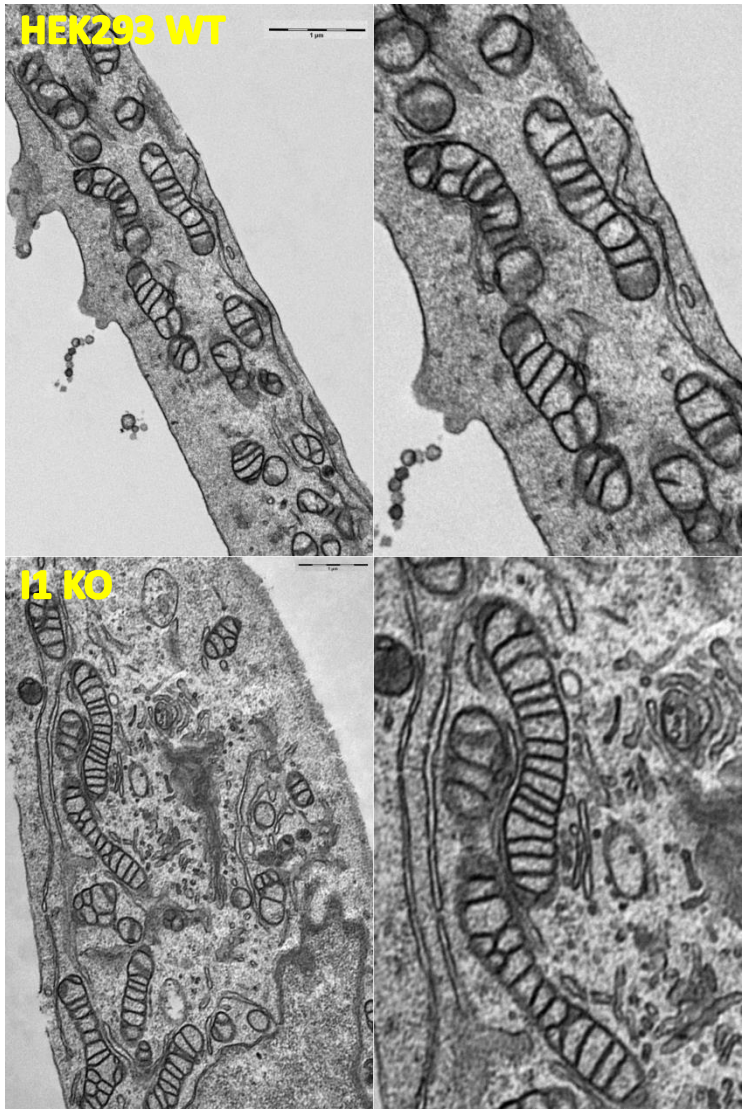
A1 KO

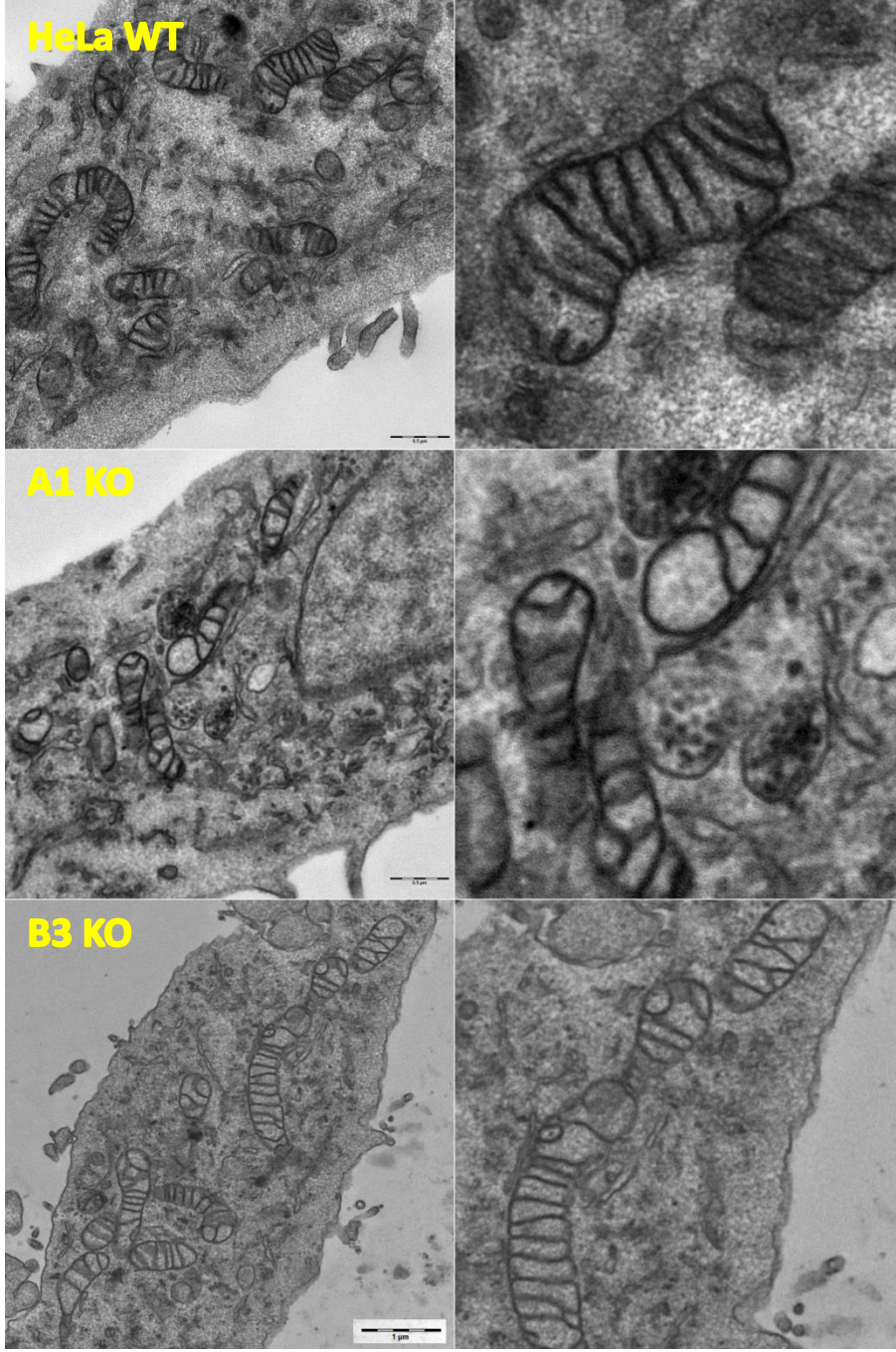
B3 KO

HEK293 WT

I1 KO

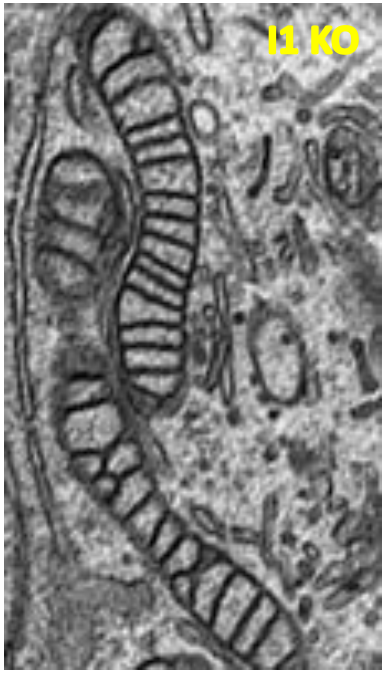
# Results F: Electron microscopy



# SUMMARY AND PLAN

- ACBD3 is mainly localized in GA in HEK293 and HeLa cells
- ACBD3 KO cells has
  - altered amount of COX subunits
  - slightly decreased mitochondrial respiration
  - Unchanged distribution of cholesterol
  - Unchanged structure of mitochondria




We need to perform further analysis to characterize impact of ACBD3 in mitochondrial metabolism

- BN-PAGE; MS analysis; structure of Golgi on electron/confocal microscopy; study of trasduceosome on BN-PAGE

# ACKNOWLEDGMENT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Thank you for your attention

mitoLab PRAGUE