RuvBL1 is required for mitochondrial integrity and supports the metabolic reprogramming of HCC cells.

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1 Introduction

RuvBL1 is a AAA+ ATPase involved in multiple cellular activities, including proliferation, chromatin remodeling, DNA repair, transcription/translation and mTOR pathway activity. High RuvBL1 expression in hepatocellular carcinoma (HCC) correlates with poor prognosis.

2 Aim

We have previously shown that hepatic RuvBL1 haploinsufficiency impairs mTORC1 signaling thereby altering liver metabolism and glucose homeostasis, suggesting that RuvBL1 overexpression may support the metabolic rewiring of HCC. We therefore aimed at dissecting RuvBL1 role in HCC cell metabolism.

3 Method

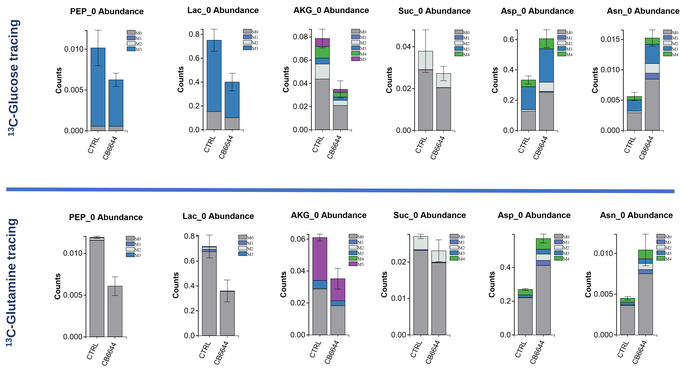
Cell lines: HepG2, Hep3B, Huh7 (human HCC), Hepa1-6 (mouse HCC), AML-12 (mouse hepatocytic cells) and mouse freshly isolated hepatocytes. RuvBL1 was targeted by RNAi or by inhibitor CB-6644. Metabolomics analysis was performed by untargeted GC/MS or by 13C-glucose and 13C-glutamine metabolic tracing. OXPHOS was evaluated by Seahorse. analyzer. RuvBL1 localization was assessed via STED microscopy and immunogold labelling/TEM. RuvBL1 mitochondrial interactome was evaluated by co-immunoprecipitation coupled with MS. In silico analysis in human HCC samples was performed on the TCGA_LIHC dataset.

4 Results

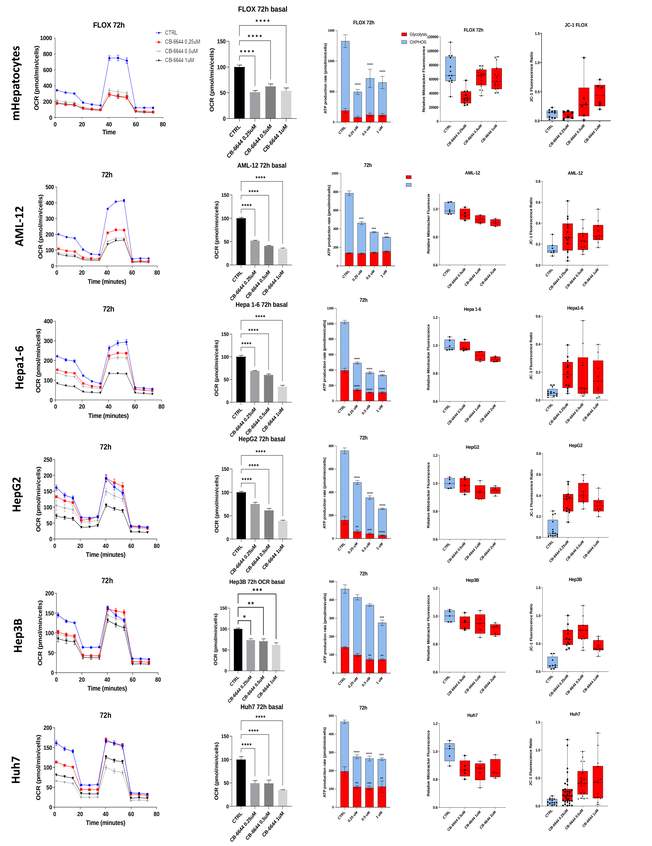
RuvBL1 silencing affects pathways involved in cancer metabolic reprogramming.

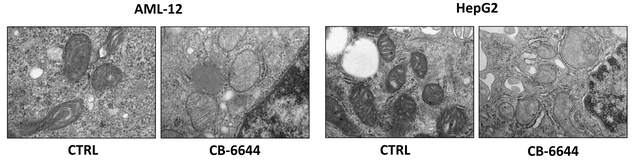
Pathway name	Pathway source	P metabolites	Q metabolites
Amino Acid metabolism	Wikipathways	1,52E-15	2,54E-12
Central carbon metabolism in cancer - Homo sapiens	KEGG	7,34E-16	2,54E-12
Aminoacyl-tRNA biosynthesis - Homo sapiens	KEGG	3,56E-12	1,52E-09
tRNA charging	HumanCyc	3,56E-12	1,52E-09
Glutamate Glutamine metabolism	INOH	1,54E-09	4,11E-07
Glucose Homeostasis	Wikipathways	6,72E-09	1,20E-06
Metabolism of amino acids and derivatives	Reactome	4,02E-08	6,13E-06
Metabolic reprogramming in colon cancer	Wikipathways	8,63E-08	9,70E-06
Glutaminolysis and Cancer	SMPDB	7,70E-07	7,65E-05
Glycolysis Gluconeogenesis	INOH	4,83E-06	2,64E-04
Warburg Effect	SMPDB	6,96E-05	2,08E-03
Citrate cycle	INOH	2,34E-03	3,13E-02

RuvBL1 inhibition by CB-6644 affects ^{13}C-glucose and ^{13}C-glutamine metabolism



RuvBL1 ATPase activity is required for mitochondria function and structure





6 Acknowledgements

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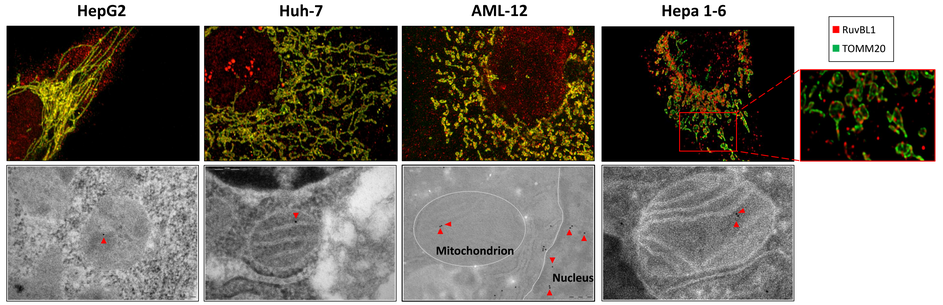
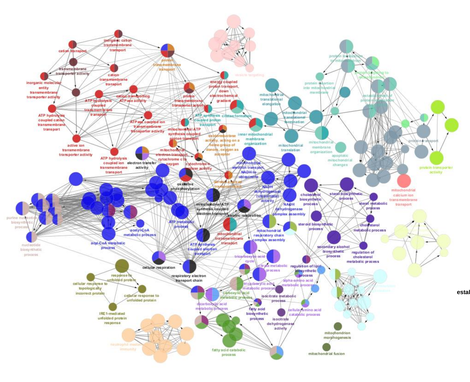
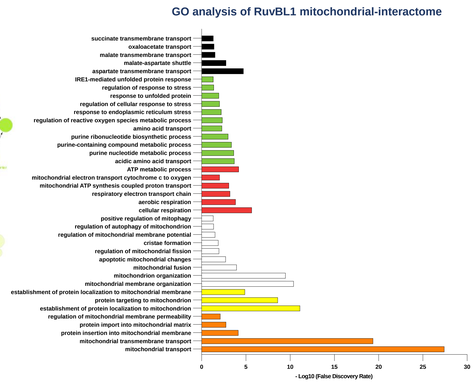
Super-resolution STED and immunogold TEM show RuvBL1 localization in mitochondria

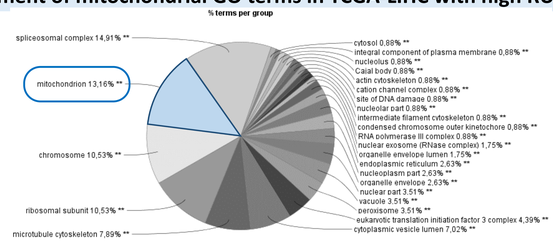


GO analysis of co-IP RuvBL1- interacting partners in purified mitochondria





Enrichment of mitochondrial GO terms in TCGA-LIHC with high RUVBL1.



5 Conclusions

Our data uncover a novel localization and function of RuvBL1 in mitochondria, suggesting that RuvBL1 overexpression is exploited in HCC to support mitochondria-related processes.

7 Contact information

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