

Abstract

Mutations in the X-linked gene WDR45 cause Beta Propeller-protein associated Neurodegeneration (BPAN). WDR45 encodes for a protein typically associated with autophagy regulators, though the protein's function is largely unknown. To explore protein function, we used a bait-BioID system to screen protein interactors with WDR45 in neuroblastoma cells. We found a strong association of WDR45 and mitochondrial proteins important for metabolism of lipids and maintaining mitochondrial membrane potential. Defects in these pathways are known to occur in neurodegenerative diseases which have significant phenotypic overlap with BPAN. To study WDR45's role in these pathways and their contribution to BPAN we have made mouse models tagging endogenous WDR45 and introducing a premature stop codon in its gene ablating protein expression. These findings reveal previously unexplored functions of WDR45 and potential causative factors of BPAN pathology.

Validation of BioID system for interactor analysis

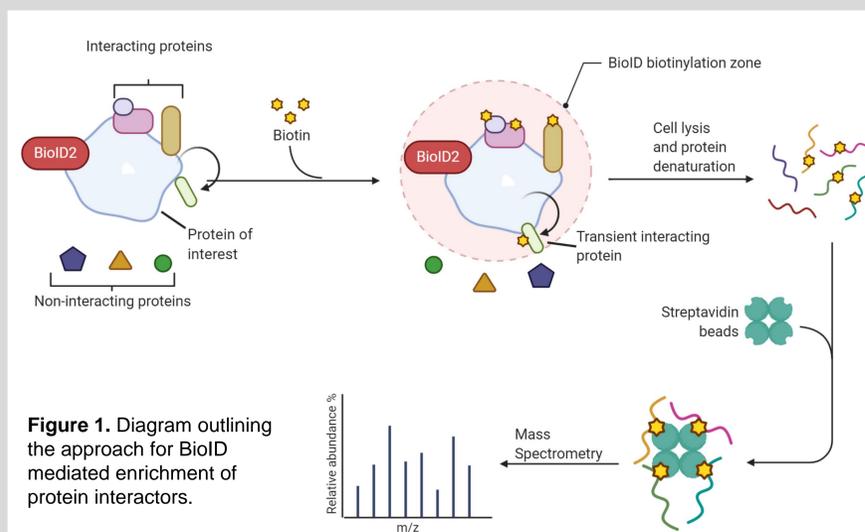


Figure 1. Diagram outlining the approach for BioID mediated enrichment of protein interactors.

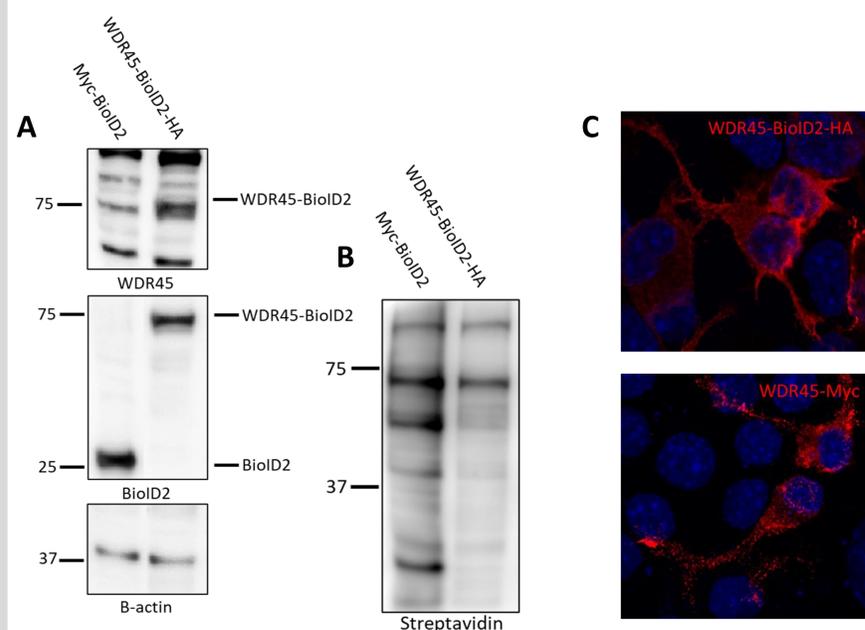
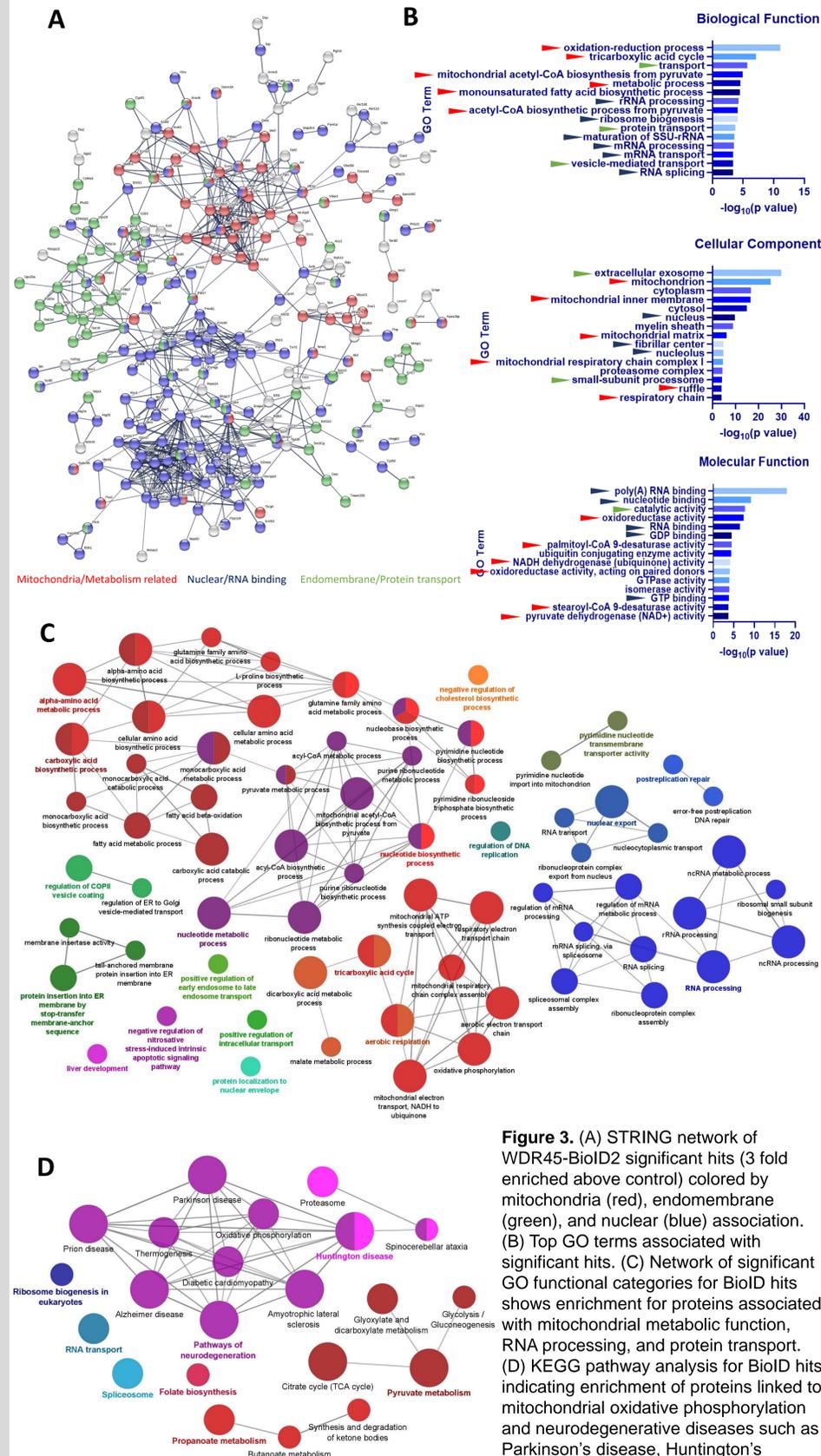
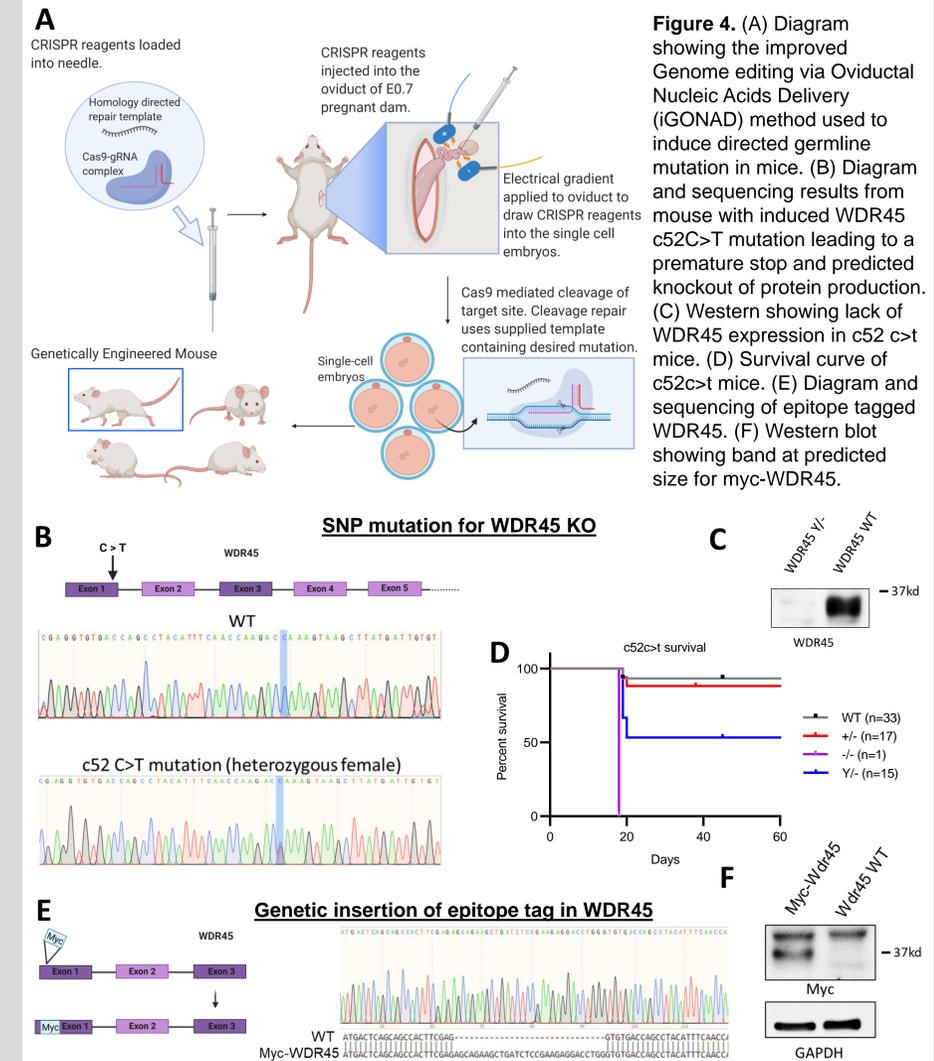


Figure 2. (A) Western blot validation of WDR45-BioID2 fusion protein production in Neuro2a neuroblastoma cells. (B) Streptavidin probed protein blot visualizing biotinylated proteins. (C) Immunocytochemistry of WDR45-BioID2 and epitope tagged WDR45 indicating BioID fusion protein properly localizes within the cell.

BioID results indicate WDR45's role in mitochondrial function



Engineered mouse models to explore WDR45 function in-vivo



Summary

- Generation of Neuro2a cell line with stable expression of WDR45-BioID2 show proper expression of fusion protein and active biotinylation of protein.
- WDR45 shows no change in localization with large BioID fusion relative to small epitope tagging.
- Significant interactors showed clustering and functional annotations associated with mitochondrial function, RNA binding, and the endoplasmic reticulum membrane.
- Pathway analysis of WDR45-BioID interactors shows enrichment for neurodegeneration associated proteins.
- Pathway and functional analyses indicate a possible role of mitochondrial dysfunction leading to BPAN pathology.
- Generation of mouse models tagging and ablating WDR45 protein will give tools for studying this protein in-vivo and understanding the pathology which underlies BPAN

Acknowledgements

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