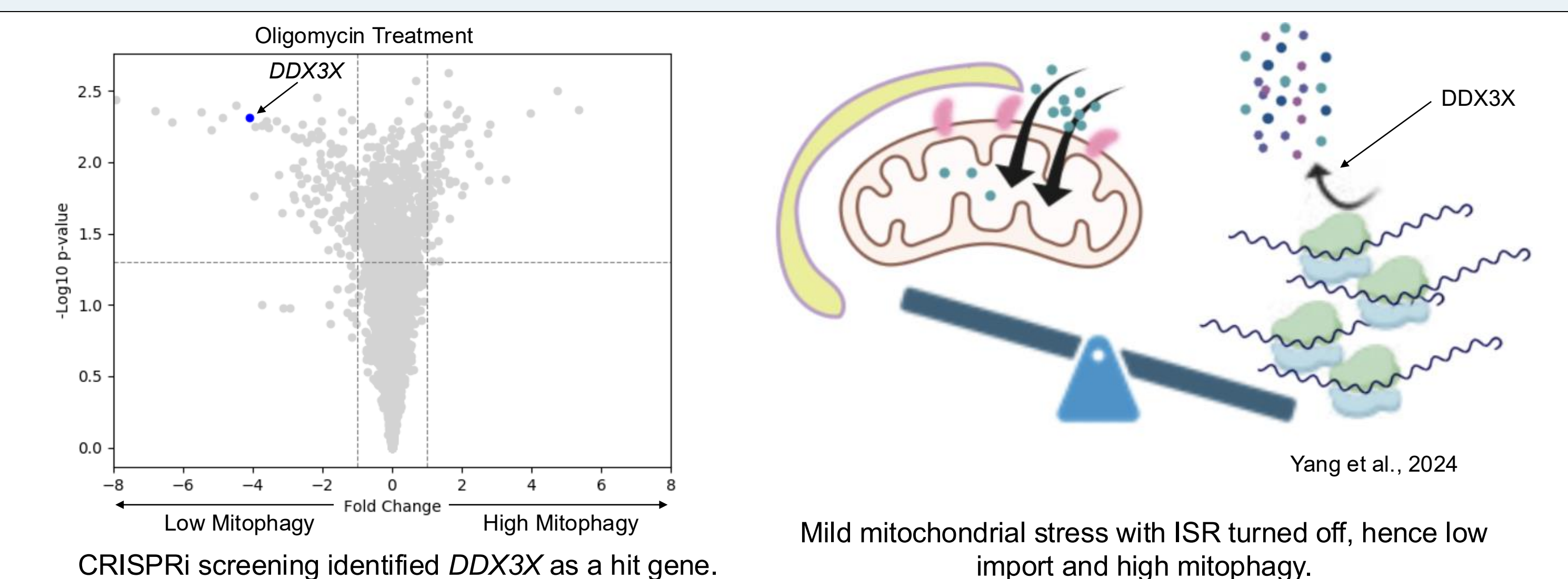


# Investigating the Role of DDX3X in the Mitochondrial Stress Response

## Background and Significance



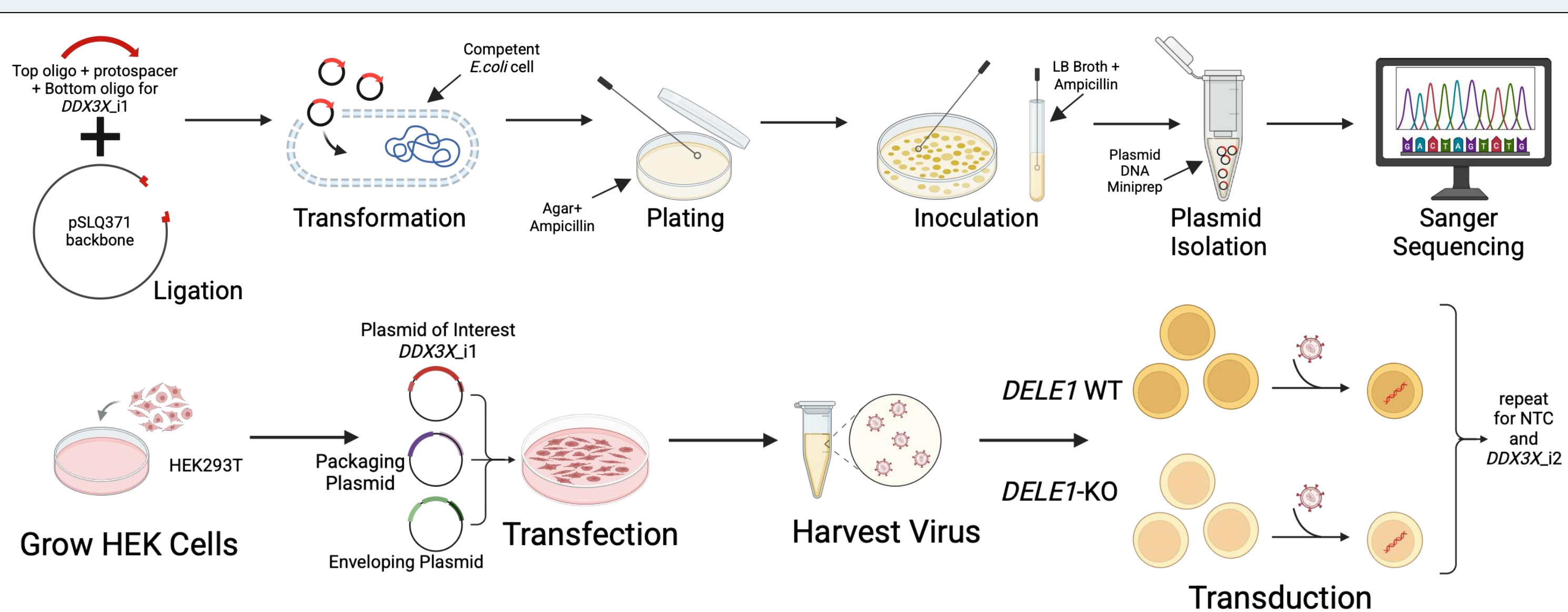
Mitochondria are highly complex organelles that are instrumental in cellular homeostasis and energy production. When mitochondria become stressed, the integrated stress response (ISR) and mitophagy are initiated. The ISR is activated by mitochondrial stress and acts as a signaling pathway to maintain a homeostatic state for the cell. *DELE1* plays an integral role in the initial activation of HRI, a kinase, that allows for the signaling process to proceed. Mitophagy, a process in which unhealthy mitochondria are selectively degraded, primarily utilize the PINK1/PARKIN pathway. Recent work from our lab showed that ISR plays a negative role in PINK1/PARKIN-mediated mitophagy.

In a recent CRISPRi screen using a mitophagy reporter, *DDX3X* appeared as a "hit gene" in which it was enriched in low mitophagy, implying that it could have a potential phenotype promoting mitophagy. *DDX3X* is a dead-box RNA helicase that plays many important roles in RNA metabolism and translation. When *DDX3X* expression is inhibited, production of essential mitochondrial translation proteins are also decreased. This can lead to an accumulation of ROS and an overall stressful state for the mitochondria. Not many studies, however, have been done to determine the role of *DDX3X* in the ISR.

## Aims

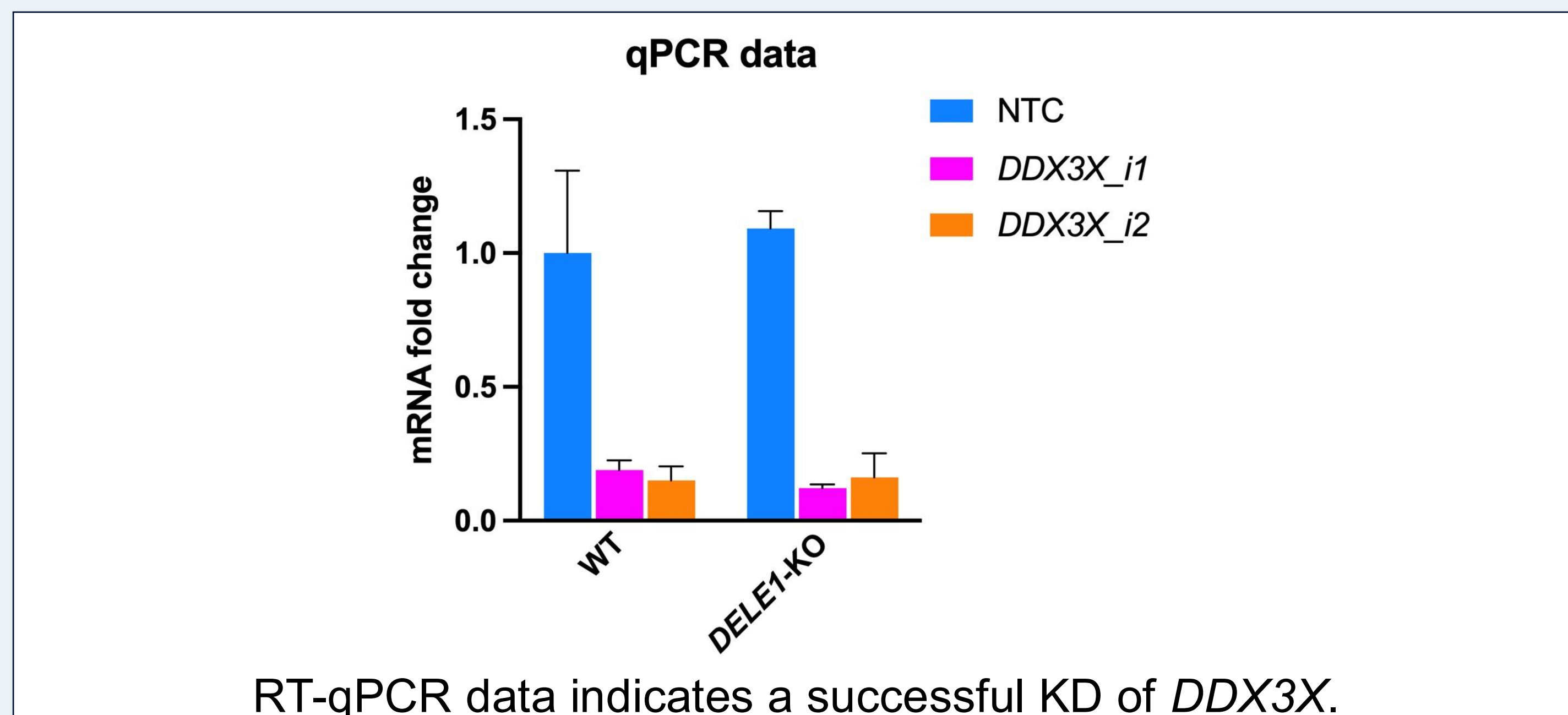
1. Utilize CRISPRi to knockdown the *DDX3X* gene (validate through western blotting and qPCR) to assess the change in mitophagy.
2. Determine how inhibition of *DDX3X* expression affects a *DELE1*-KO cell line compared to a *DELE1*-WT cell line in terms of mitophagy levels.

## Generate *DDX3X* KD Cell Lines

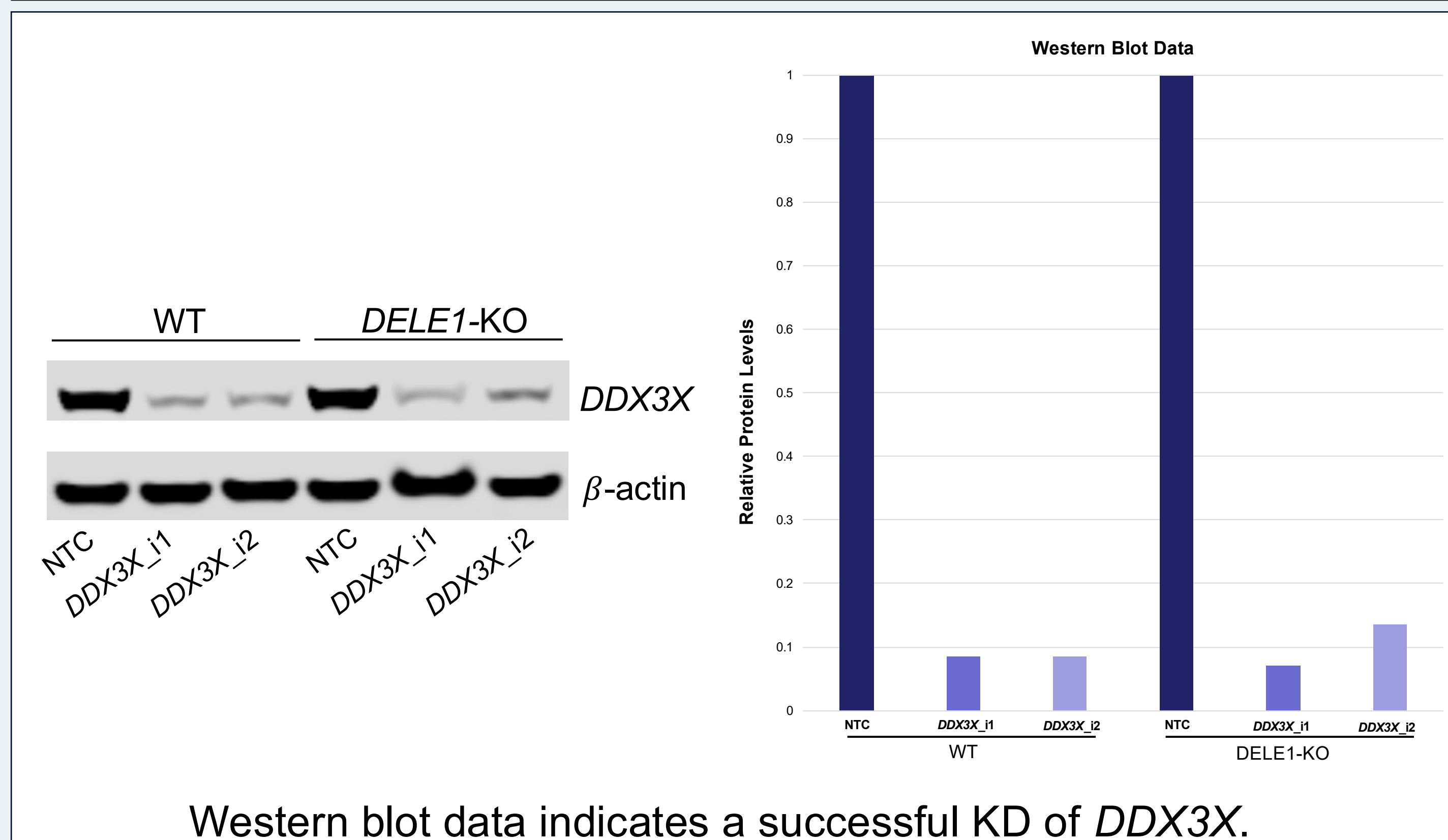


The sgRNA plasmid has a BFP fluorescence marker. (A) BFP+ cells are indicative of a successful transduction. (B) serves as a control where no transduction was performed.

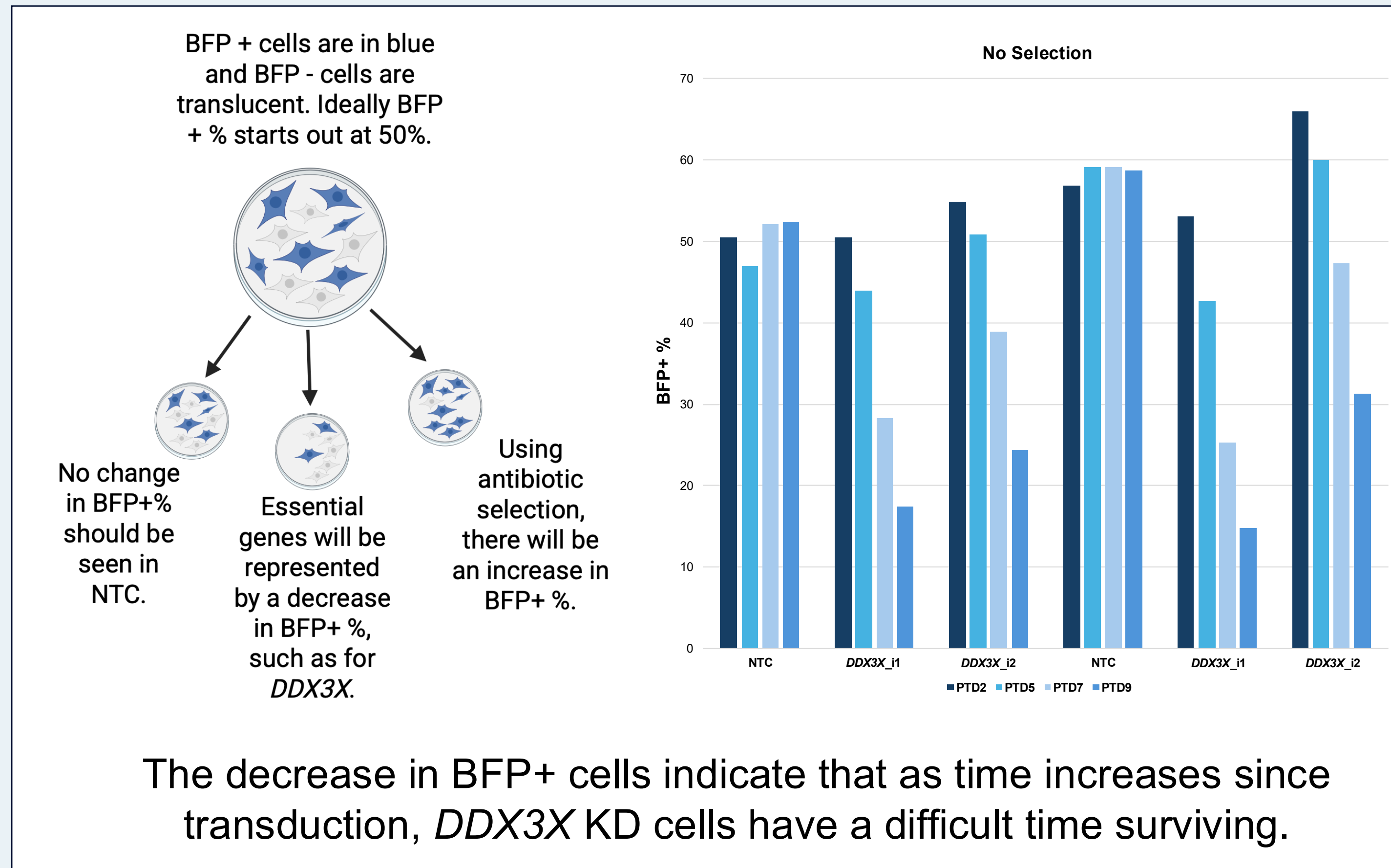
## *DDX3X* Knockdown Validation: RT-qPCR



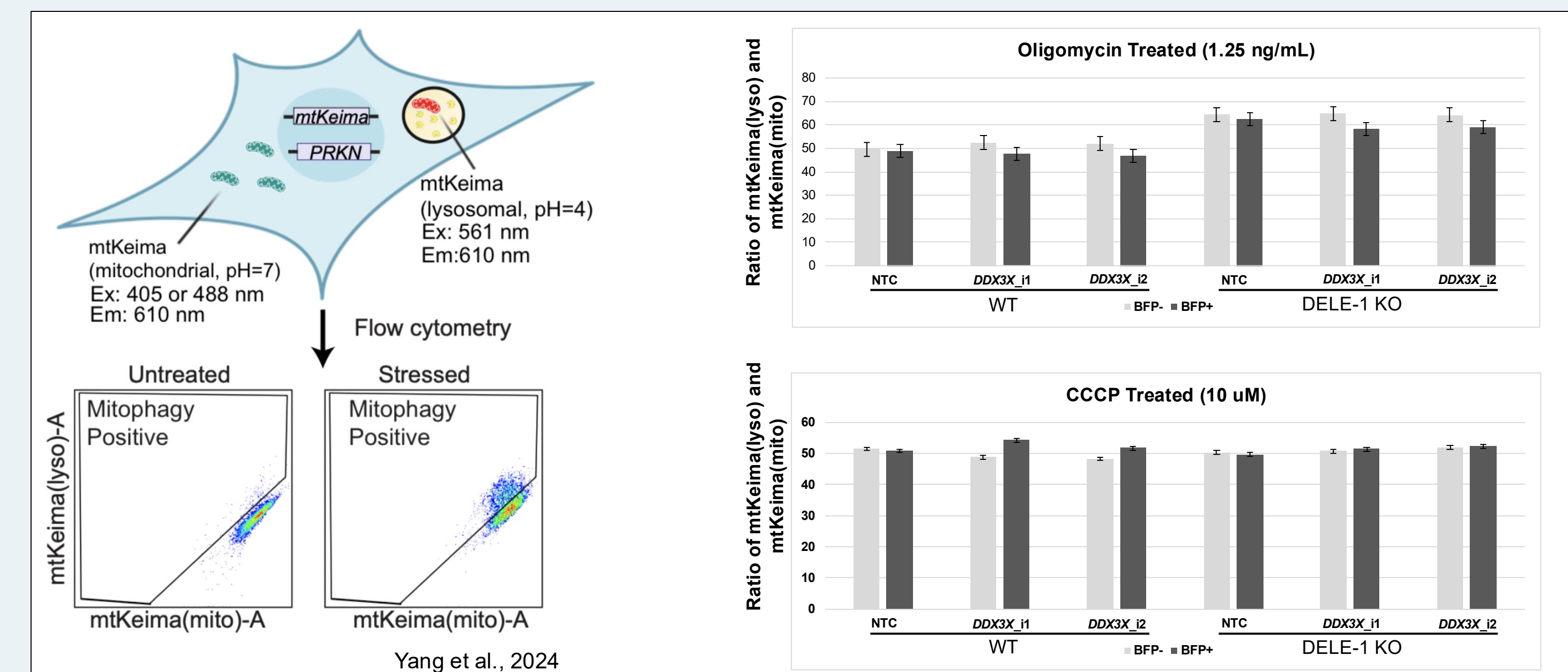
## *DDX3X* Knockdown Validation: Western Blot



## *DDX3X* is an essential gene



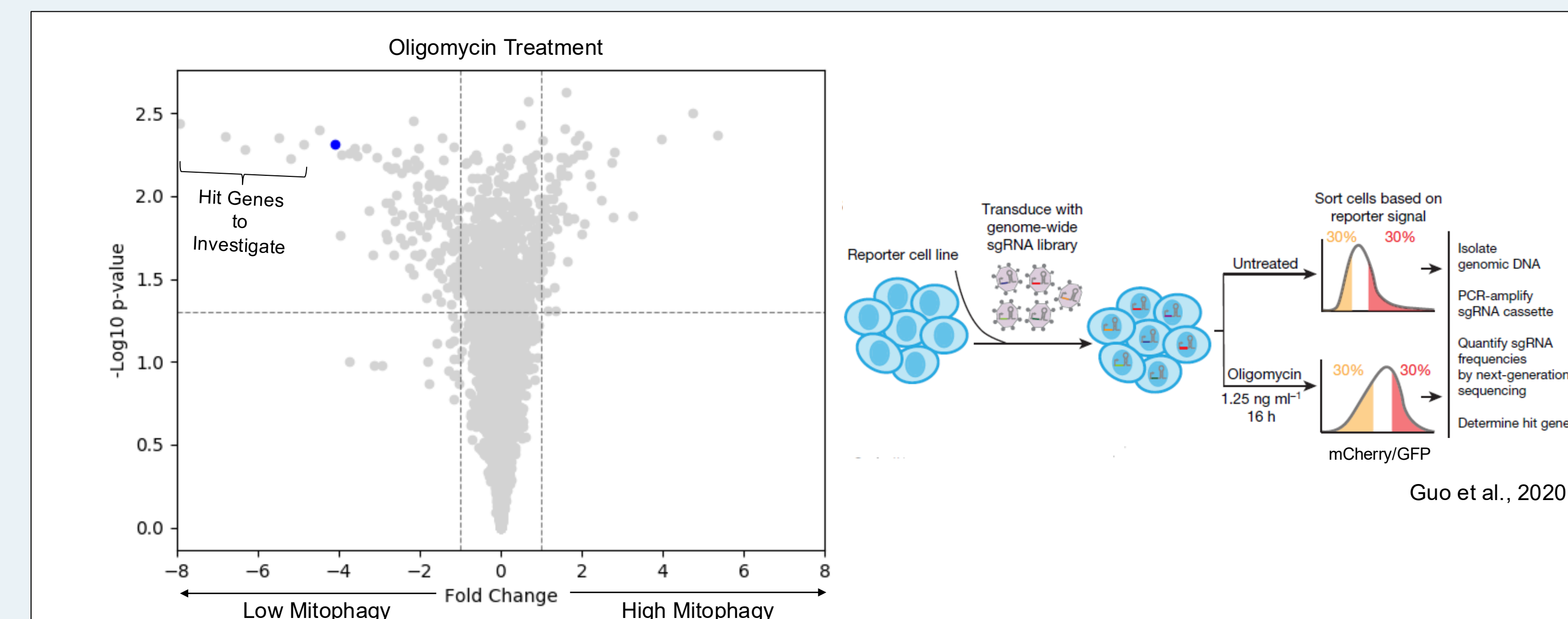
## Mitophagy Measurement with Flow Cytometry



## Conclusions

1. *DDX3X* KD inhibits mitophagy induced by oligomycin treatment in both WT cells and *DELE1*-KO cells.
2. *DDX3X* KD promotes mitophagy induced by CCCP treatment in WT cells.
3. *DDX3X* is an essential gene.

## Future Directions



Research other hit genes from current CRISPRi screening and complete genome wide CRISPRi screening. Investigate why oligomycin and CCCP treatment have opposite effects on mitophagy levels with *DDX3X* KD.

## References

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