Fn14 ameliorates cell death in cisplatin induced acute kidney injury (AKI)

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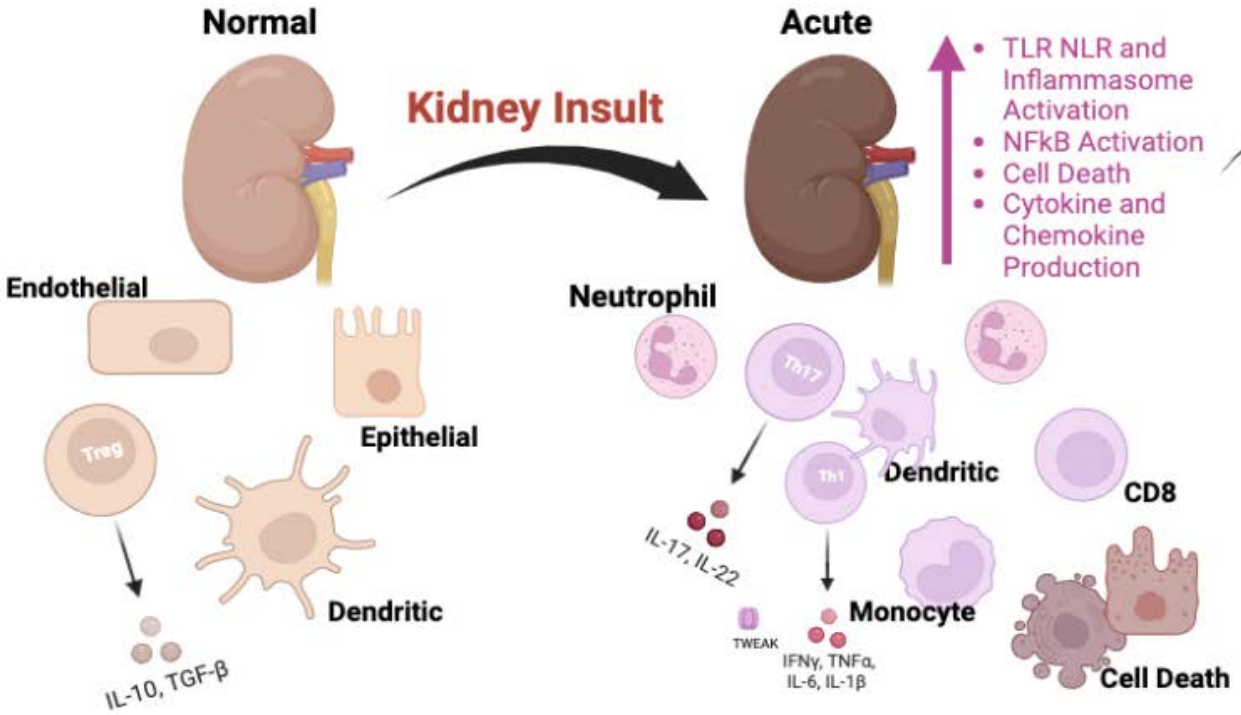
Kidney Injury Response Mechanisms

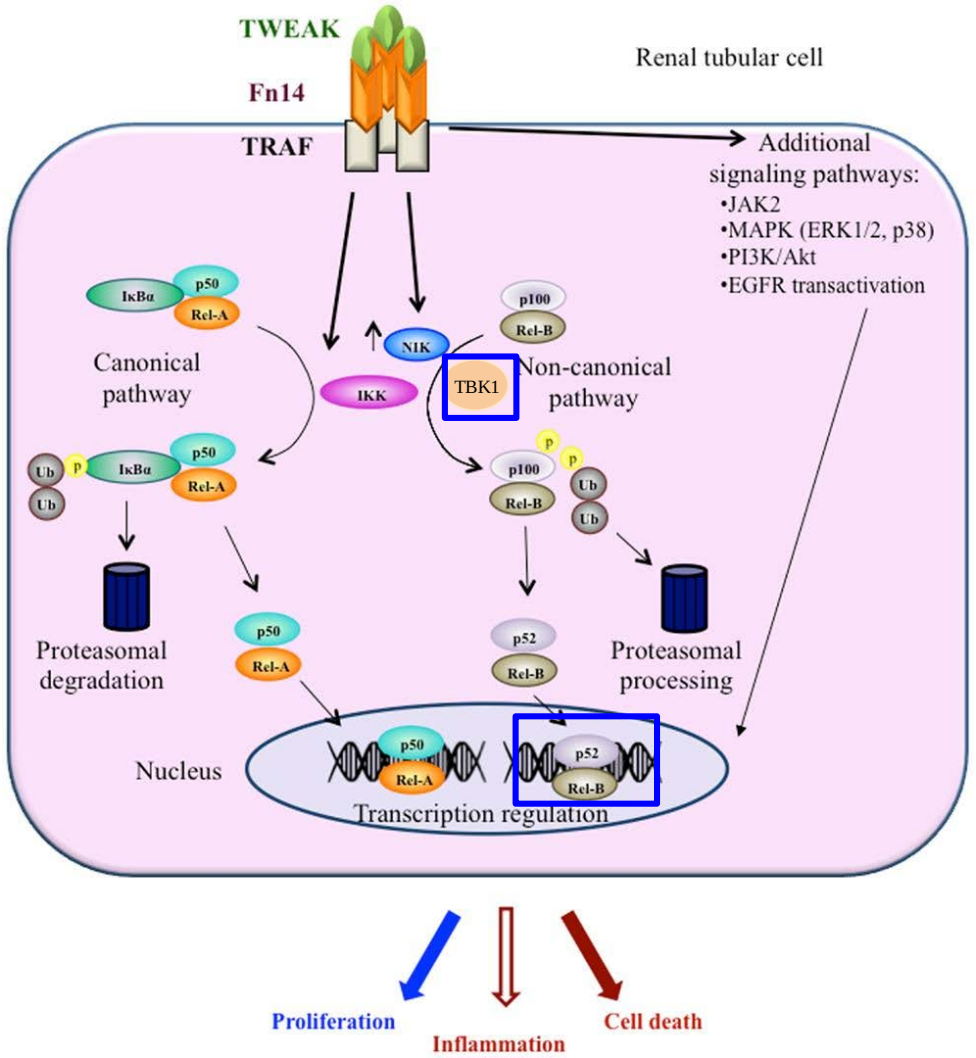


- ~15% of US adults (37 million) are estimated to have CKD
- Many unaware of having CKD due to absence of symptoms in early stages.
- Diabetes in Hawaii exacerbates occurrence of CKD and can cause diabetes-induced ESRD

(Andrade-Oliveira, et al., *Front. Pharmacol.*, 2019)

Non-canonical NFκB Signaling Pathway: Role of TWEAK + Fn14

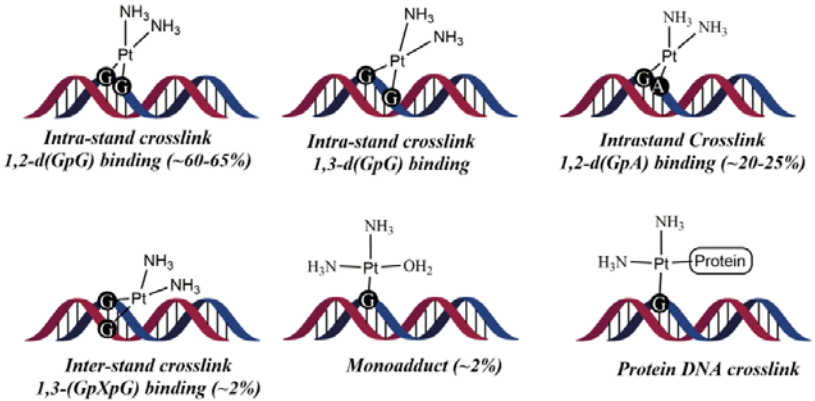
- NFκB pathway controls gene expression, cytokine production, cell survival
- Fn14, a TNF receptor, is one of the few known activators of the non-canonical NFκB pathway
- Fn14 is highly upregulated in response to kidney injury or in renal disease



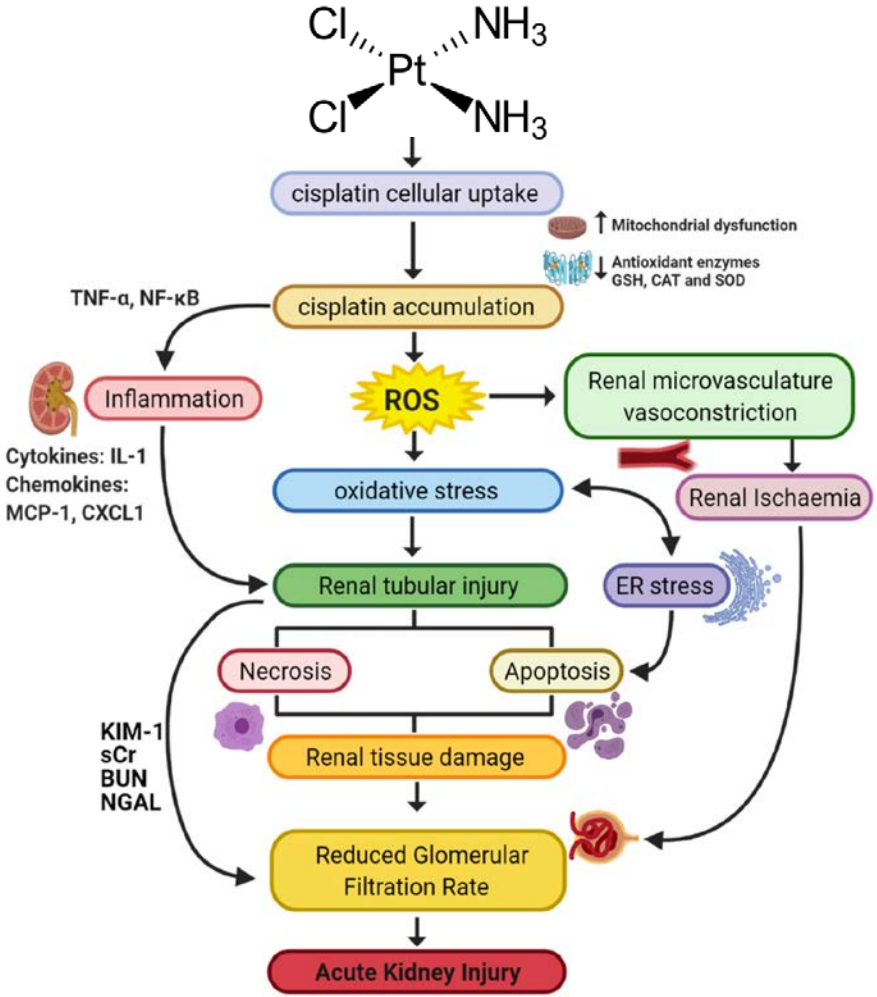
(Poveda, et al. *Front. Immunol.,* 2013)

Cisplatin

- Metal-based chemotherapeutic
  - Intercalating agent to gDNA and mtDNA via purine bases
- **Nephrotoxicity** = dose-limiting factor
- CI-AKI murine models → introducing DNA damage
  - Fn14 ameliorates cell death in CI-AKI



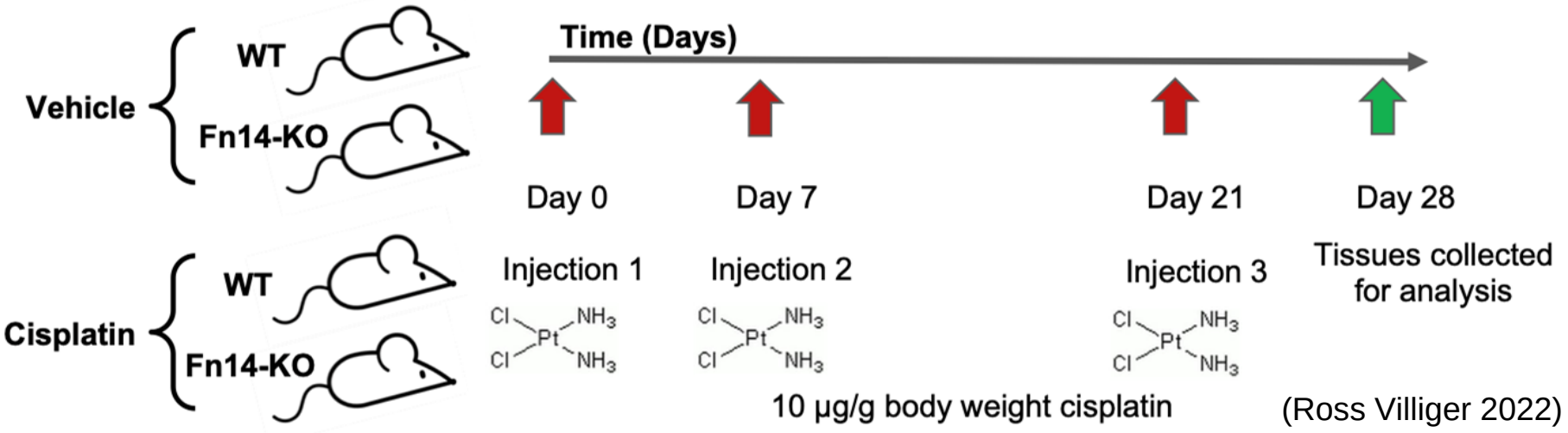
(Gosh, *Bioorganic Chemistry*, 2019)



(McSweeney, et al., *Cancers*, 2021)

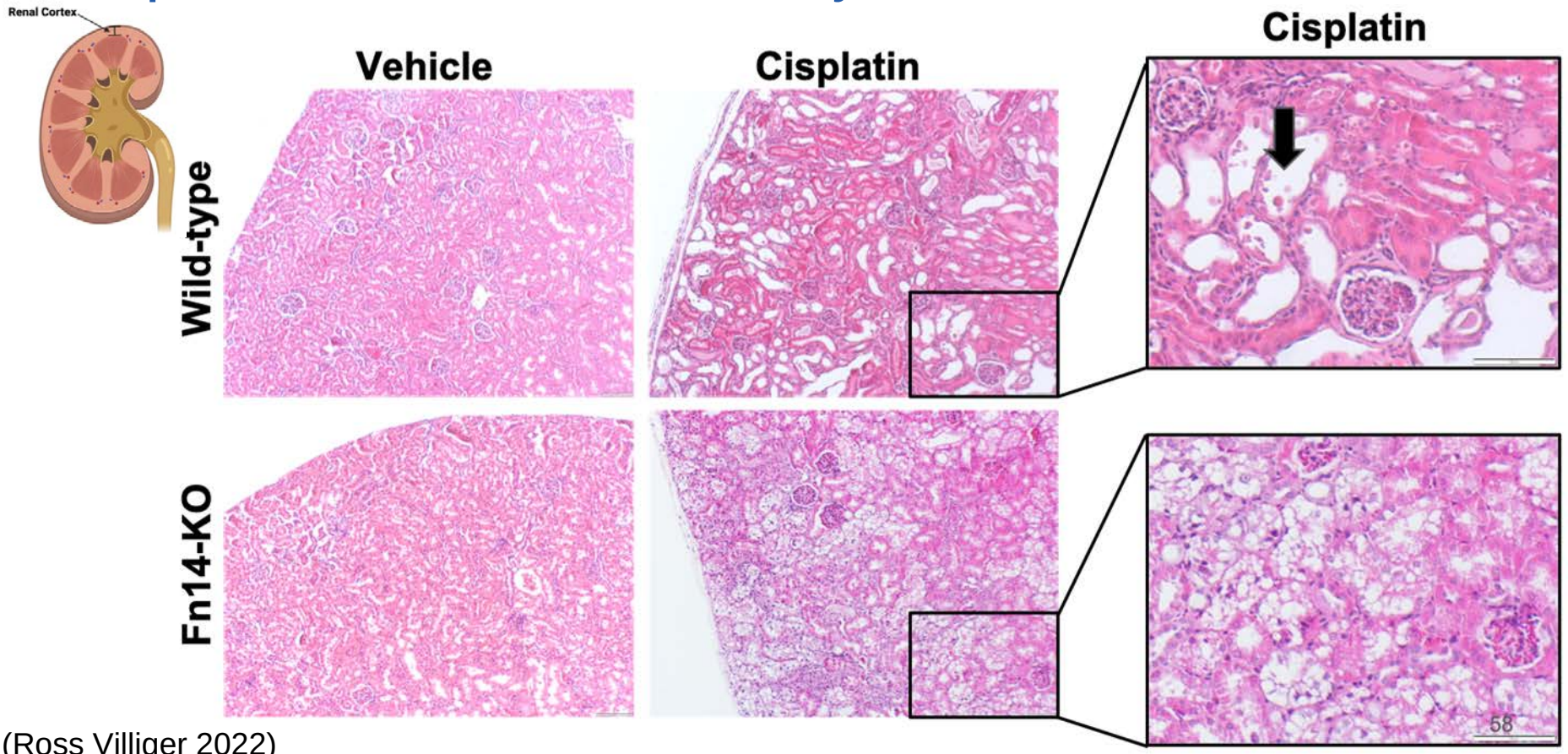
Is TWEAK-Fn14 signaling axis a stress response that contributes to renal and urological diseases?

- Interstitial cystitis/Bladder Pain Syndrome (IC/BPS)
  - Repeated dose of cyclophosphamide (CYP)
- **Chronic Kidney Disease (CKD)**
  - **Repeated low-dose of cisplatin** by intraperitoneal injection (IP)



(Ross Villiger 2022)

Cisplatin-treated WT kidneys had dying proximal tubule cells, cisplatin-treated Fn14-KO kidney had a vesicle accumulation



(Ross Villiger 2022)

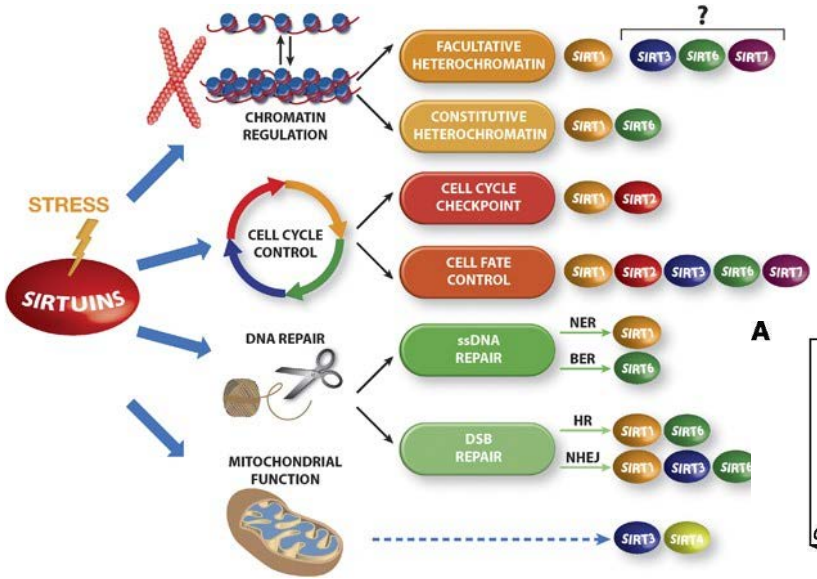
Overall Goal

To advance understanding of cellular and **molecular mechanisms involved in NFκB signaling** promotion of cell death and inflammation in kidneys to **prevent progression from AKI to CKD**.

Central Hypothesis

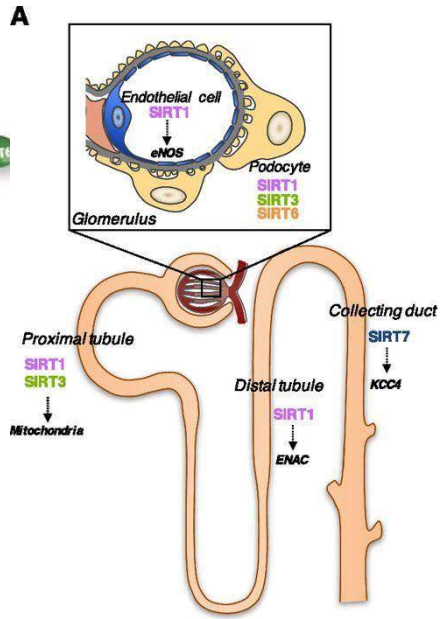
Utilization of **siRNA knockdowns of targeted genes** leads to **difference in damage response** between untreated and cisplatin-treated cells.

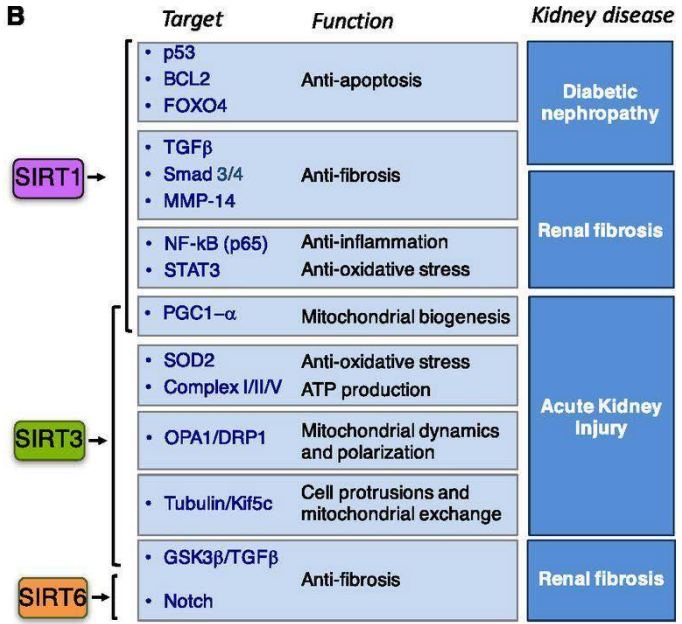
Sirtuins: Critical Stress Adaptors + DNA Damage Response



(Bosch-Presegué, et al., Oncogene, 2014)

(Morigi et al. *JASN* 2018)

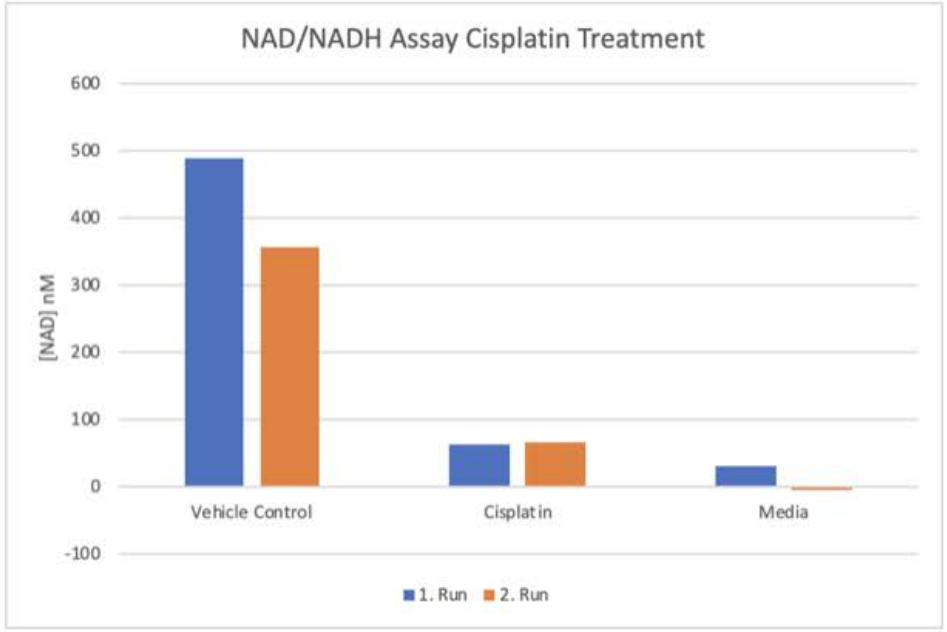


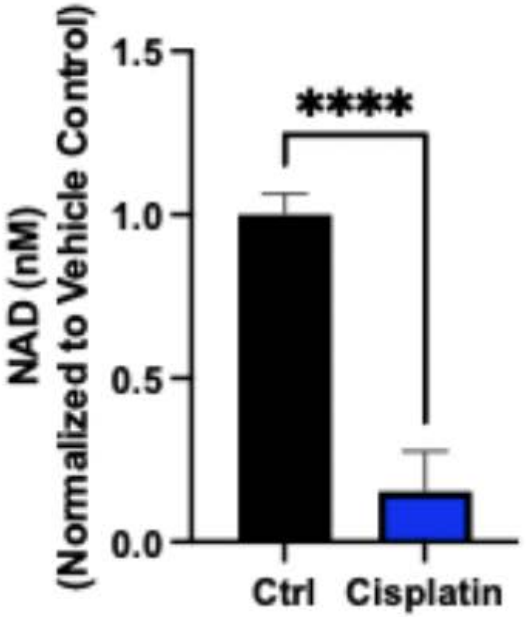


Summer Research Aim

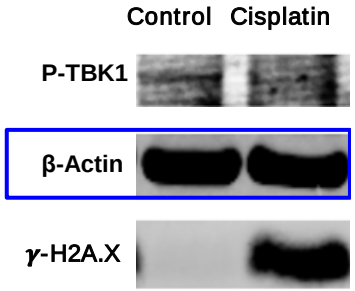
To test the hypothesis that loss of function of target genes (SIRT1, SIRT2, SIRT6, TBK1) leads to difference in damage response, post-modificational status, and cellular behavior

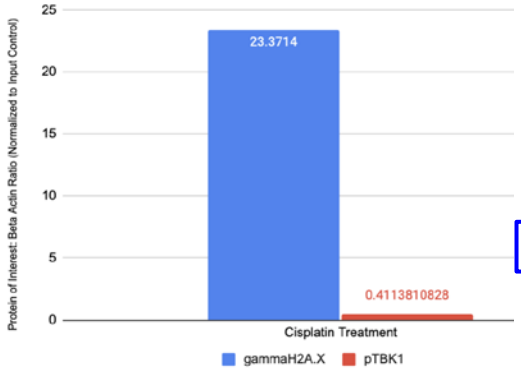
40µM cisplatin treatment leads to 5-fold decrease in NAD levels

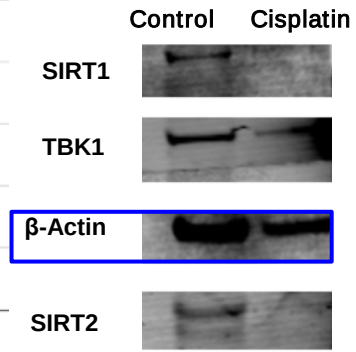


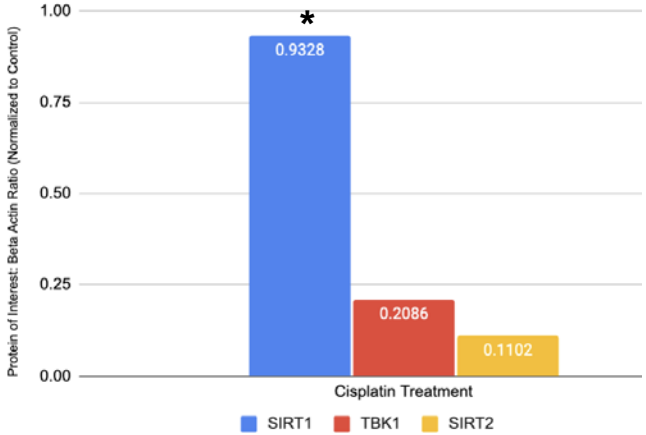


40μM cisplatin treatment causes sufficient increase of DSB in DNA and decreases in amount of specific proteins

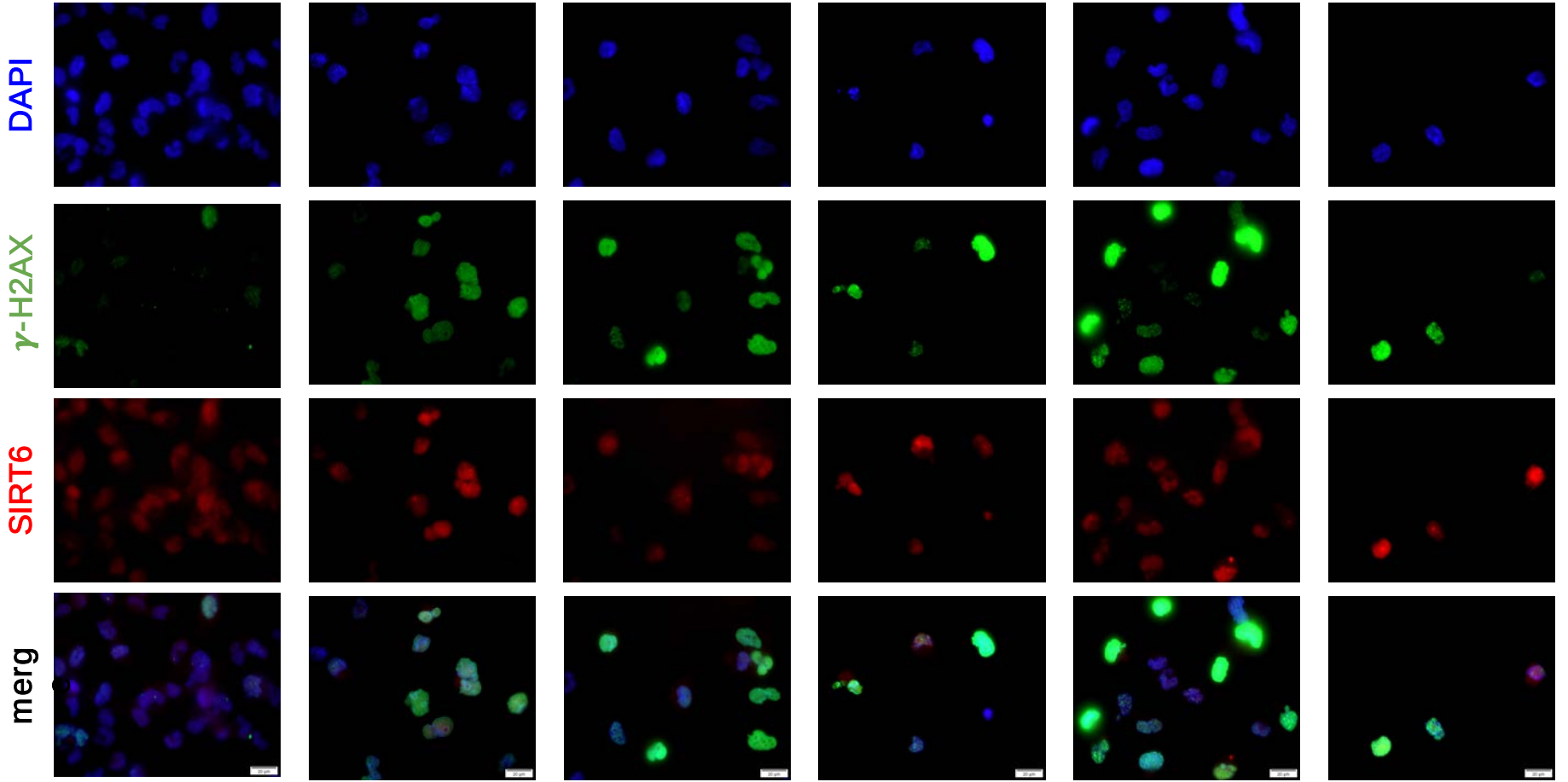




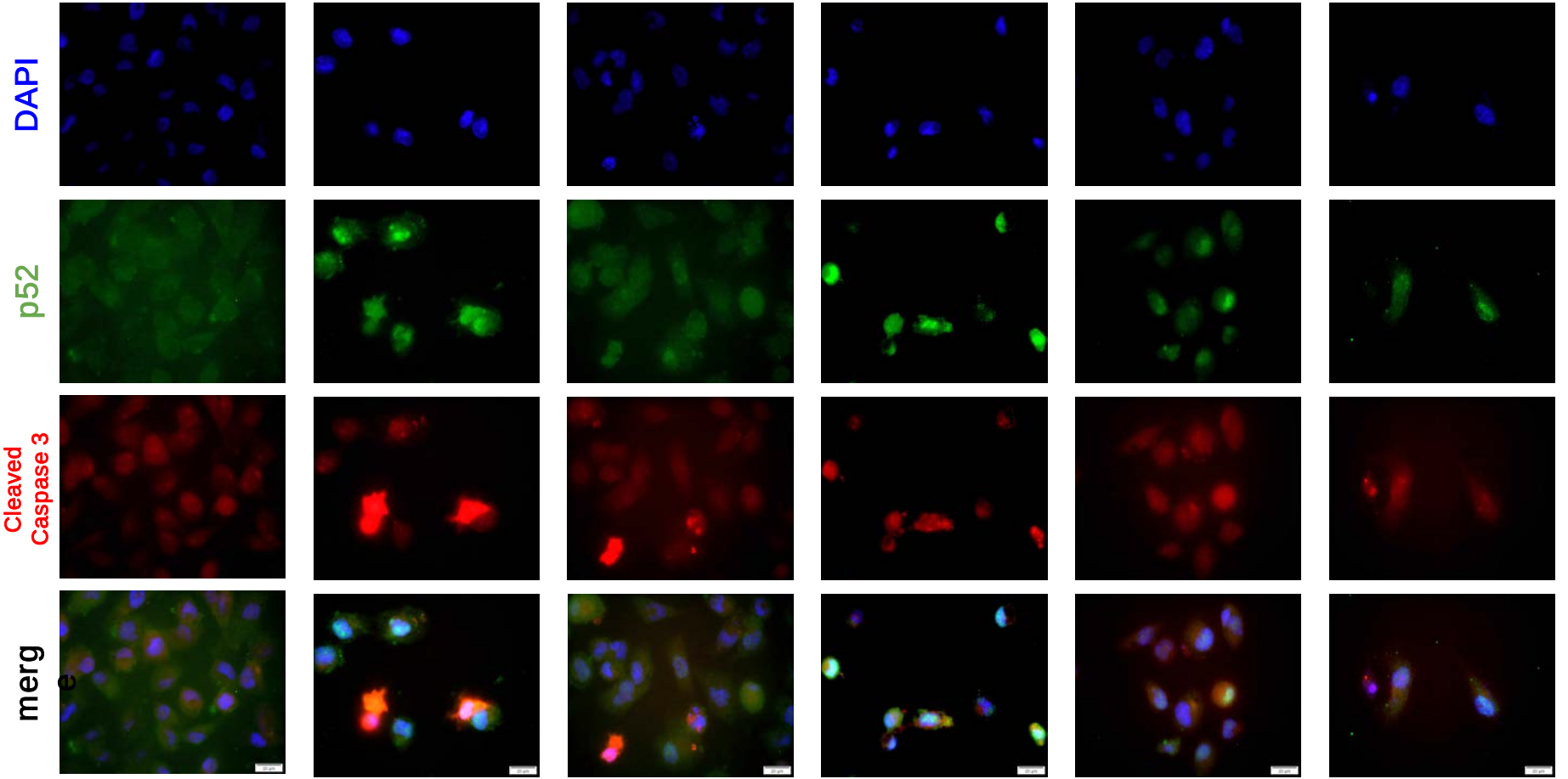




Damage from cisplatin treatment causes an upregulation of DSBs



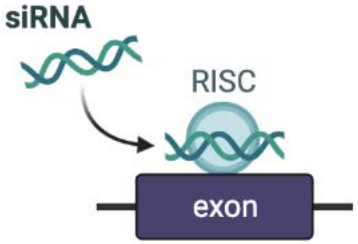
Repeated injuries increases levels of apoptosis and activation of NFκB signaling



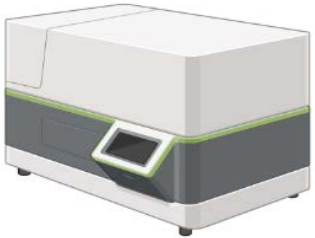
Conclusions

1. 40μM cisplatin treatment decreases expression of SIRT1 and SIRT2. Further validation is needed to understand the mechanism.
2. Staggered cisplatin treatment (12+12) activates non-canonical NFκB signaling, while canonical activation requires presence of cytokines.

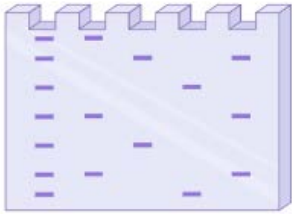
Future Directions



Verifying successful KD



nCounter analysis → high-throughput direct total gene counts to detect up- and down-regulated genes & spatial transcriptomics (RNAseq)



Continuing to study protein interactions + behaviors via IP and WB

Collected media serum to quantify released cytokine expression during cell treatments

How cells respond to damage in presence of different cytokine cocktails (TWEAK, TNFα , IL6)

Start an Fn14 KO line; repeat NAD/NADH assay

Acknowledgements

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Mahalo!

Questions?

Any lingering questions? Email: nicole_m_wong@brown.edu