

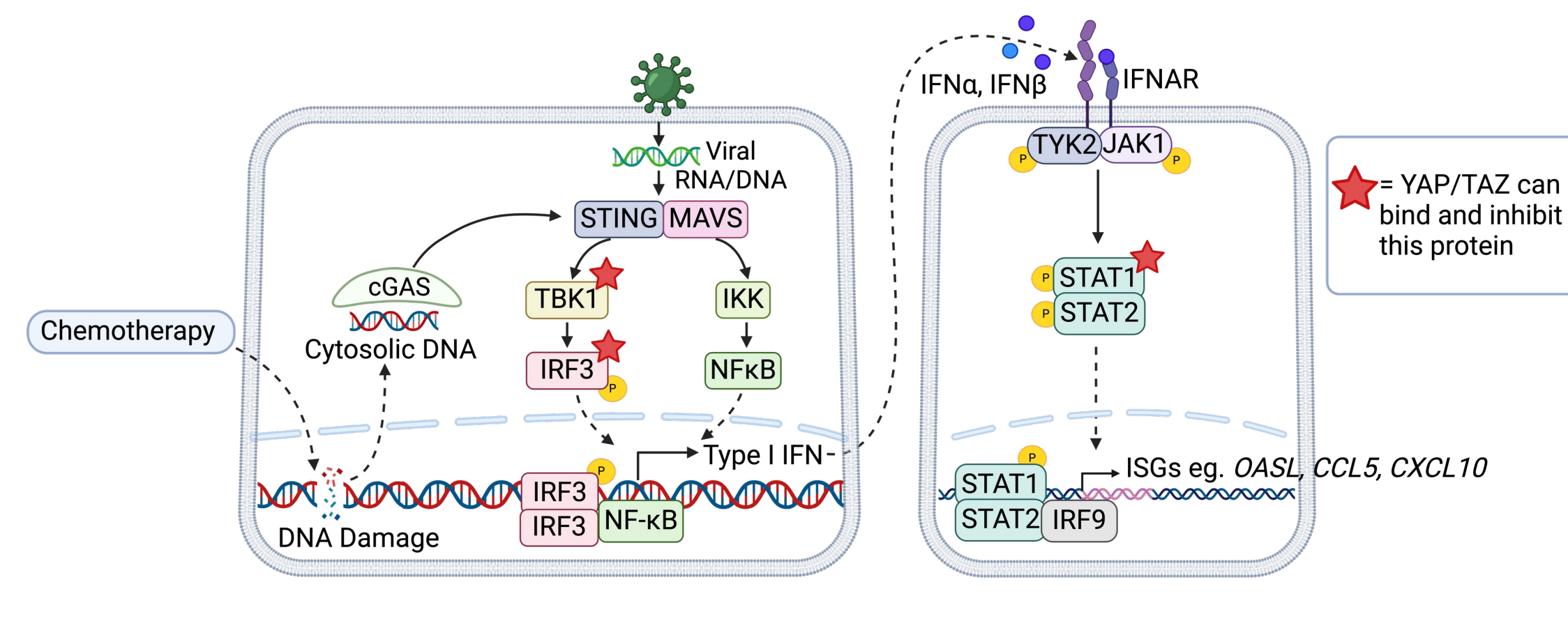
YAP/TAZ Suppression of Paclitaxel-Induced Antiviral Signaling Is a Putative Mechanism of Chemoresistance in Lung Cancer

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Introduction

- YAP/TAZ are regulated effectors of Hippo signaling which play a critical role in cell proliferation and have been implicated in the progression of cancer¹
- YAP/TAZ activation contributes to chemotherapy resistance through expression of genes that promote cell survival, drug efflux, and immune suppression¹
- The innate antiviral immune response detects cytosolic nucleic acids caused by viral infection and leads to production and secretion of type I interferons which can act in a paracrine or autocrine manner to induce interferon stimulated genes²
- Chemotherapies activate the innate antiviral signaling pathway in cancer, which enhances their anti-tumor effects³
- YAP/TAZ inhibit antiviral signaling by inhibiting TBK1, IRF3 and STAT1^{4,5}



Rationale & Objectives

Rationale

- YAP/TAZ are involved in chemotherapy resistance
- YAP/TAZ antagonize antiviral signaling
- Antiviral signaling potentiates the effects of chemotherapy

Objectives

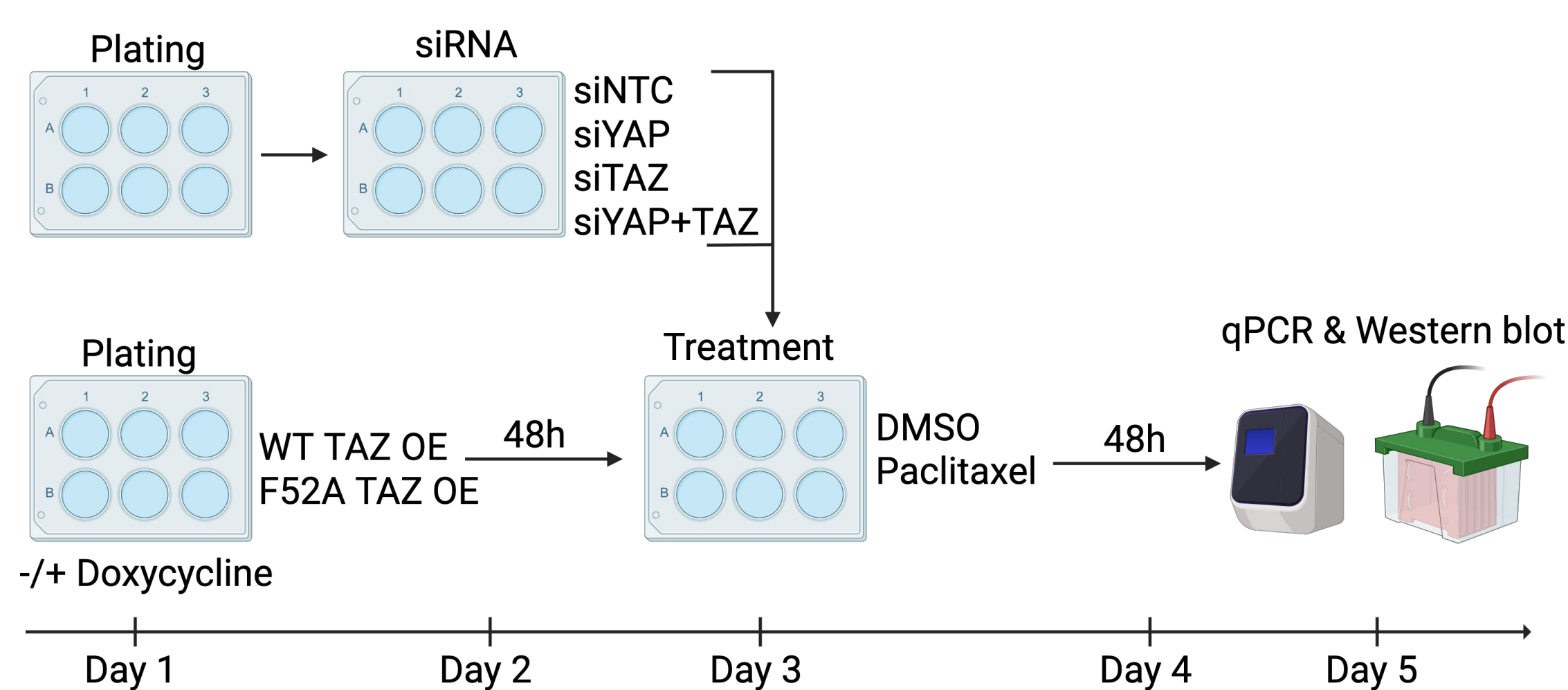
- Establish if paclitaxel influences antiviral signaling in lung adenocarcinoma cells (LUAD)
- Determine whether YAP/TAZ manipulation affects paclitaxel-induced IFN signaling

Hypothesis & Experimental Design

Hypothesis

- YAP/TAZ contribute to chemotherapy resistance in non-small cell lung cancer by suppressing antiviral signaling

Experimental Design



Paclitaxel induces phospho-TBK1, type I interferons and interferon stimulated genes (ISGs) in LUAD cell lines

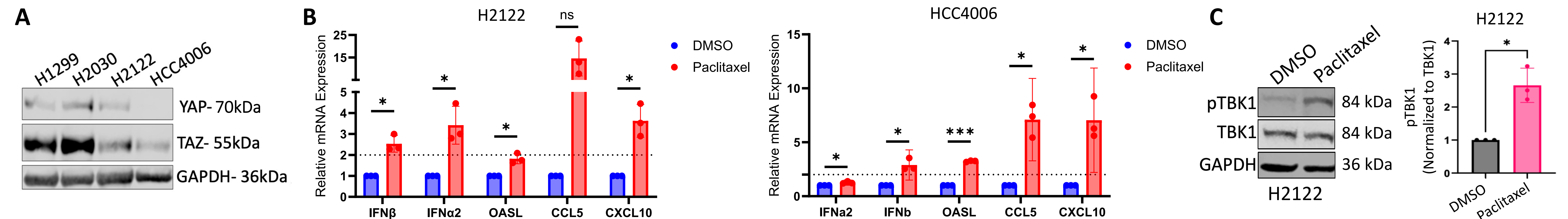


Figure 1: Type I interferons, interferon stimulated genes and pTBK1 expression following paclitaxel treatment in H2122 and HCC4006 cells (N=3). A) YAP/TAZ protein expression in LUAD cell lines. B) H2122 and HCC4006 cells were treated with 10nM paclitaxel for 48hr. mRNA expression in paclitaxel treated cells relative to DMSO control cells was calculated by $\Delta\Delta Ct$ analysis and normalized to HPRT1 endogenous control. C) H2122 cells were treated with 10nM paclitaxel for 48hr and pTBK1 was measured by western blot and quantified relative to the total TBK1. One sample t-test was performed * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$.

YAP/TAZ knockdown potentiates ISGs, while overexpression of wild type (WT) and the transcriptionally inactive TAZ mitigates paclitaxel induction of IFNβ and ISGs

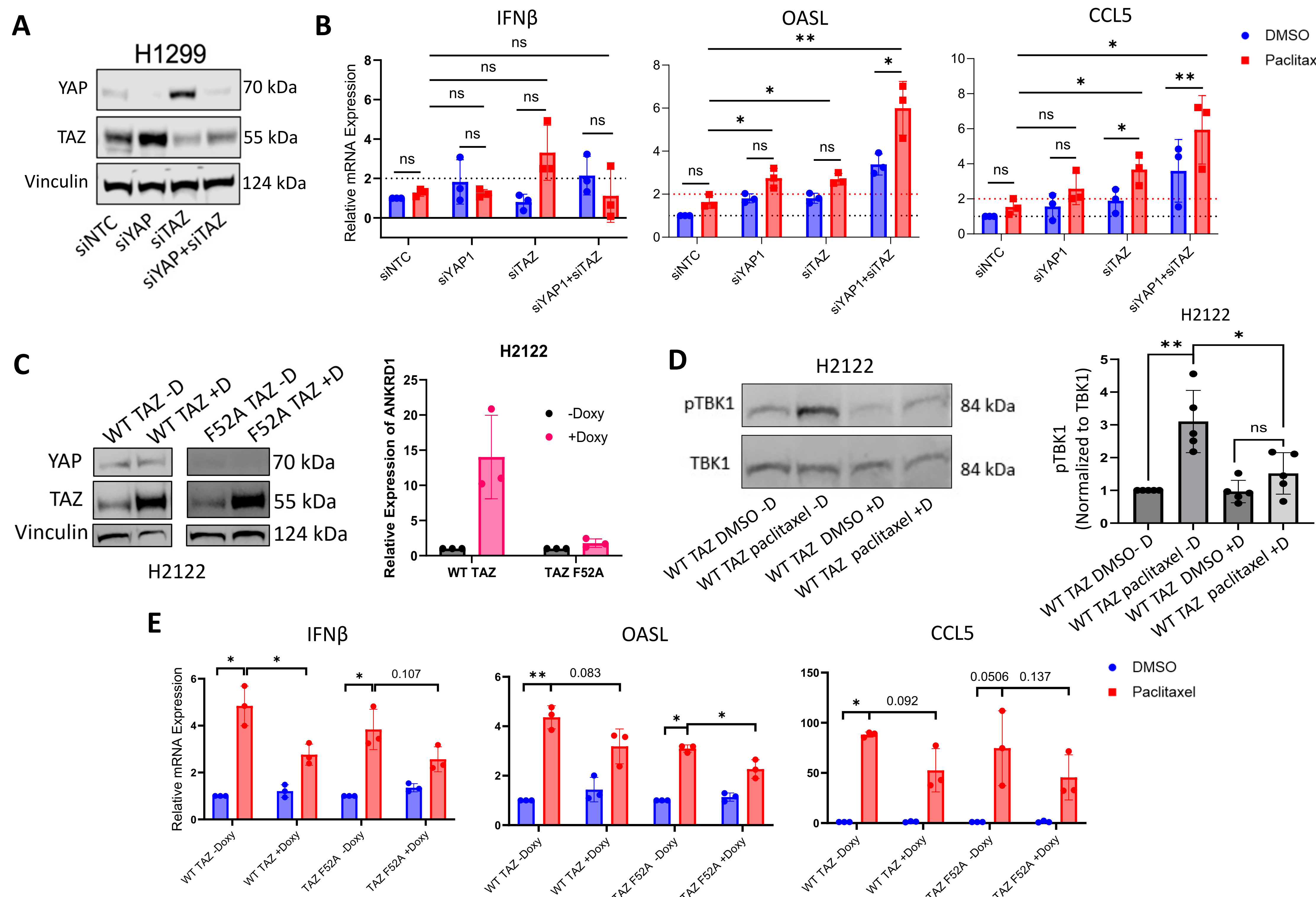


Figure 2: Gene expression changes induced by YAP/TAZ knockdown with paclitaxel treatment in H1299 cells and phospho-TBK1 and gene expression changes in WT TAZ or transcriptionally inactive TAZ F52A overexpression with paclitaxel treatment in H2122 cells. A) YAP/TAZ knockdown (KD) was confirmed by western blot 48hr post transfection in H1299 cells. B) Cells with confirmed YAP/TAZ KD were treated with 10nM paclitaxel and harvested 48hr after treatment. *IFNβ*, *CCL5* and *OASL* expression was detected by RT-qPCR (N=3). *IFNβ*, *CCL5* and *OASL* mRNA expression in paclitaxel treated cells relative to siNTC was calculated by $\Delta\Delta Ct$ analysis and normalized to HPRT1 endogenous control. C) Doxy-induced WT TAZ and TAZ F52A overexpression (OE) was confirmed in cells treated with 100ng/ml doxycycline (doxy) for 48hr by western blot. Transcriptional activity of WT TAZ and TAZ F52A was measured by mRNA expression of *ANKRD1* using RT-qPCR. D) pTBK1 was measured by western blot in cells with confirm OE of WT TAZ and normalized to total TBK1 (N=5). E) Cells with confirmed WT TAZ or F52A TAZ OE were treated with 10nM paclitaxel and harvested 48hr after treatment. *IFNβ*, *CCL5* and *OASL* expression was detected by RT-qPCR (N=3). *IFNβ*, *CCL5* and *OASL* mRNA expression in paclitaxel treated cells relative to -doxy DMSO control was calculated by $\Delta\Delta Ct$ analysis and normalized to HPRT1 endogenous control. Paired and one sample t-tests were performed * $p < 0.05$, ** $p < 0.005$.

Conclusions & Future Directions

Conclusions

- Paclitaxel induces pTBK1, *IFNβ* and interferon stimulated genes in LUAD cells
- Individual and combined knockdown of YAP/TAZ upregulates paclitaxel-induced expression of ISGs, *CCL5* and *OASL* however this is not observed for *IFNβ* which may be upregulated earlier
- WT TAZ suppresses paclitaxel-induced phospho-TBK1 induction
- A trend towards suppression of paclitaxel induced *IFNβ*, *OASL* and *CCL5* expression is evident upon overexpression of WT and transcriptionally inactive (F52A) TAZ in H2122 cells; repeat experiments are ongoing

Future Directions

- Confirm YAP/TAZ-mediated suppression of antiviral signaling induced by paclitaxel is transcription-independent
- Determine whether YAP/TAZ-mediated suppression of antiviral signaling influences the efficacy of paclitaxel in LUAD models

References & Acknowledgments

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