

# Combining the IAP antagonist tolinapant with a DNA hypomethylating agent enhances immunogenic cell death in preclinical models of T-cell lymphoma

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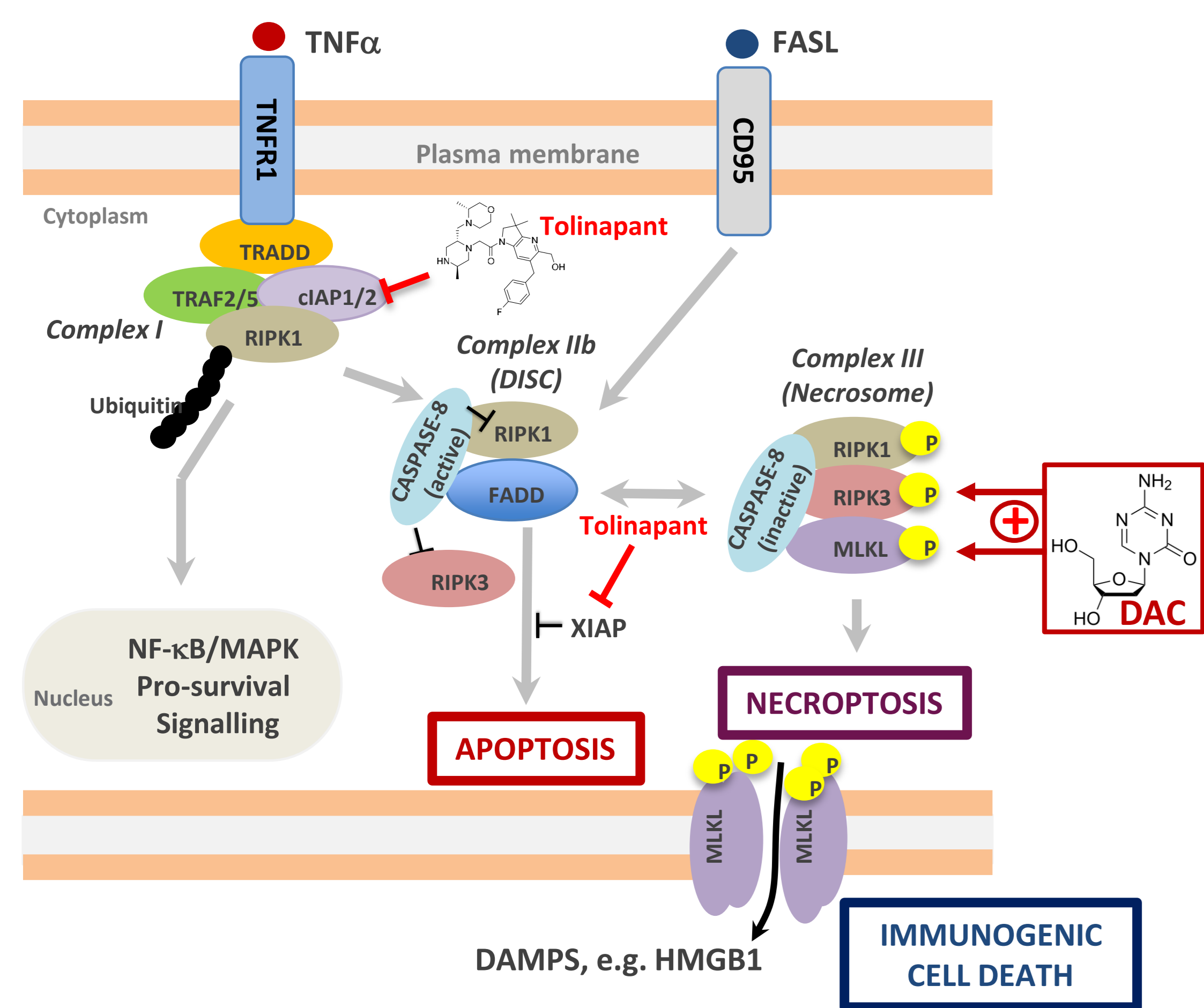
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## INTRODUCTION

Tolinapant (ASTX660) is a potent, non-peptidomimetic antagonist of cIAP1, cIAP2 and XIAP<sup>1,2</sup>, and has demonstrated immunomodulatory properties in pre-clinical models of T cell lymphoma (TCL)<sup>3</sup>. In an ongoing Phase 2 trial (NCT02503423), tolinapant has shown activity against highly pre-treated peripheral and cutaneous T-cell lymphoma<sup>4</sup>. Hypomethylating agents (HMAs) have also shown clinical responses in some subsets of PTCL<sup>5</sup>, suggesting that hypermethylation is prevalent in PTCL. Both HMAs and IAP antagonists show immunomodulatory anti-cancer potential in pre-clinical studies. A Phase 1 clinical study investigating the combination of tolinapant and ASTX727 (oral decitabine) in AML is currently in progress (NCT04155580).

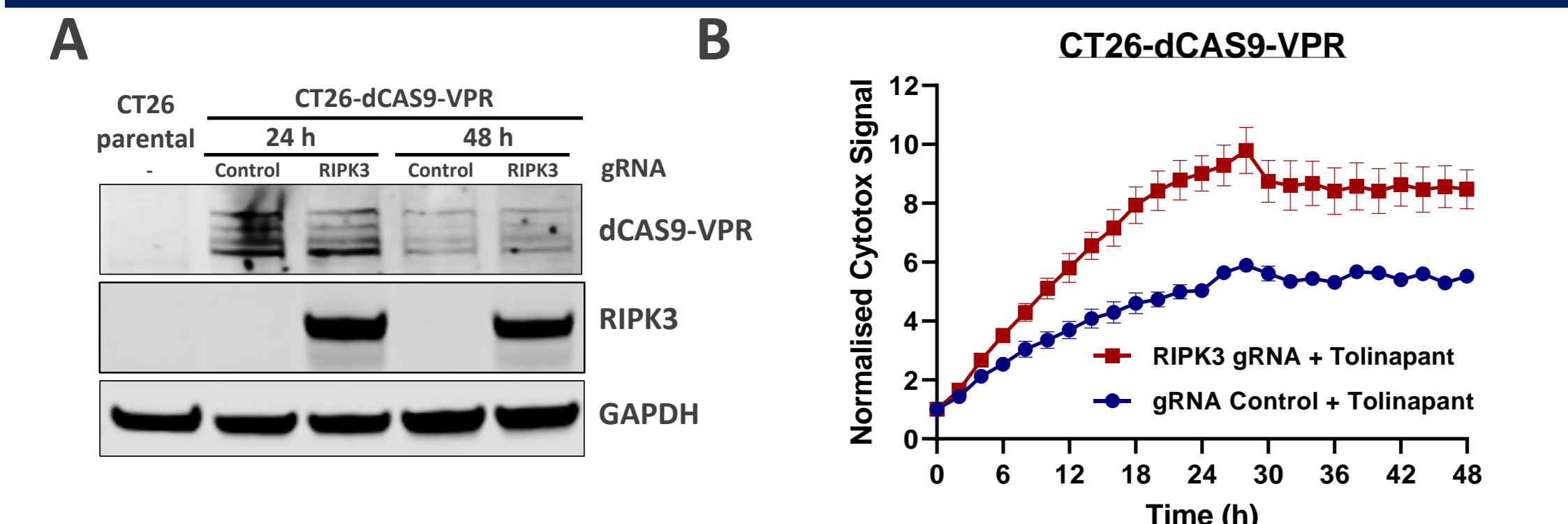
Here we have investigated the potential for HMA-induced reversal of epigenetic silencing or altered cell signalling to promote the induction of immunogenic forms of cell death (ICD), such as necroptosis, driven by tolinapant treatment in pre-clinical models of T-cell lymphoma (TCL).

FIGURE 1 COMBINATION MECHANISM OF ACTION



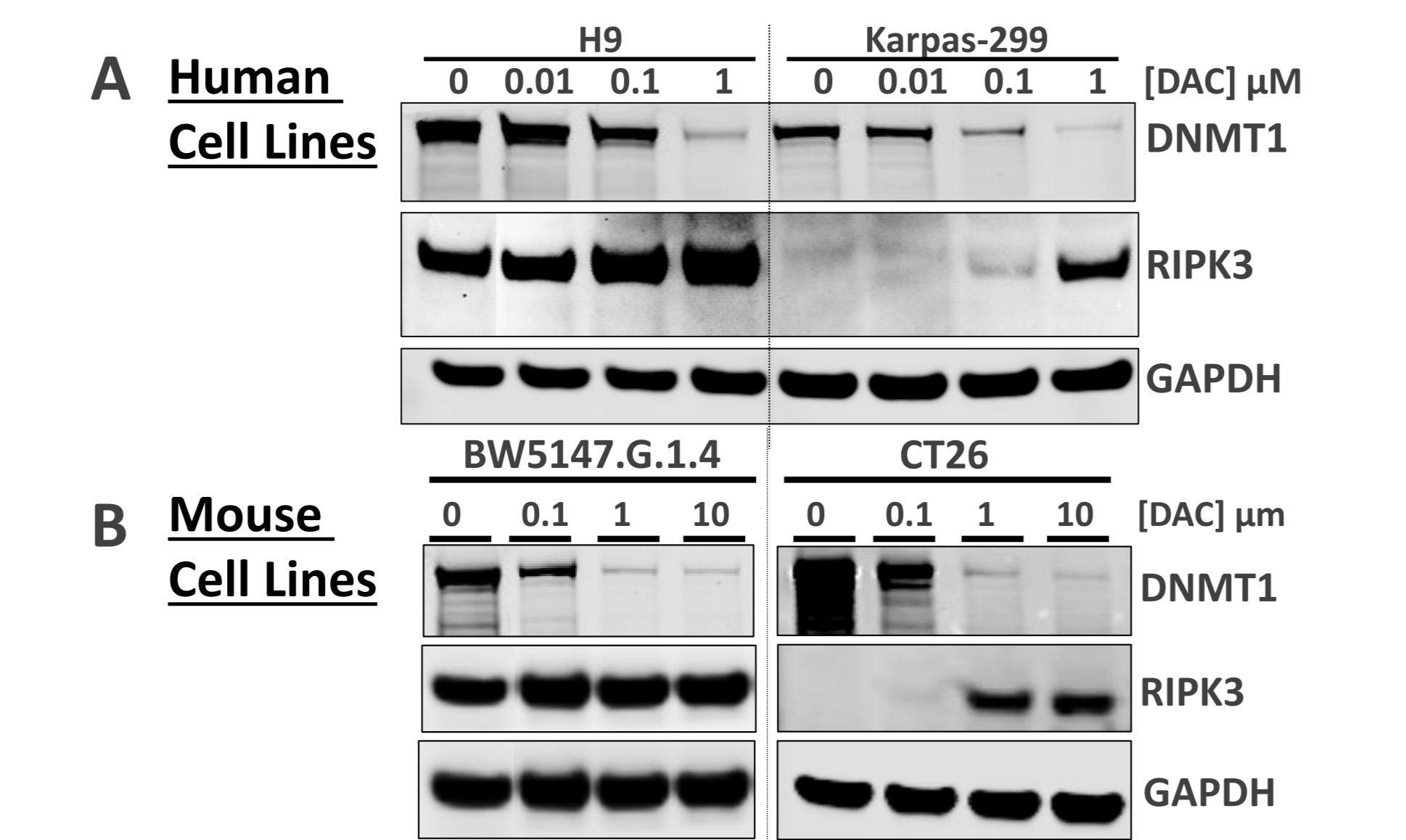
- The combination of tolinapant and decitabine (DAC) can drive an immunogenic form of cell death in TCL (necroptosis).
- Decitabine treatment leads to upregulation of key necroptosis biomarkers within the necrosome by direct promoter demethylation or altered interferon signalling.

FIGURE 2 RIPK3 EXPRESSION INCREASES TOLINAPANT SENSITIVITY



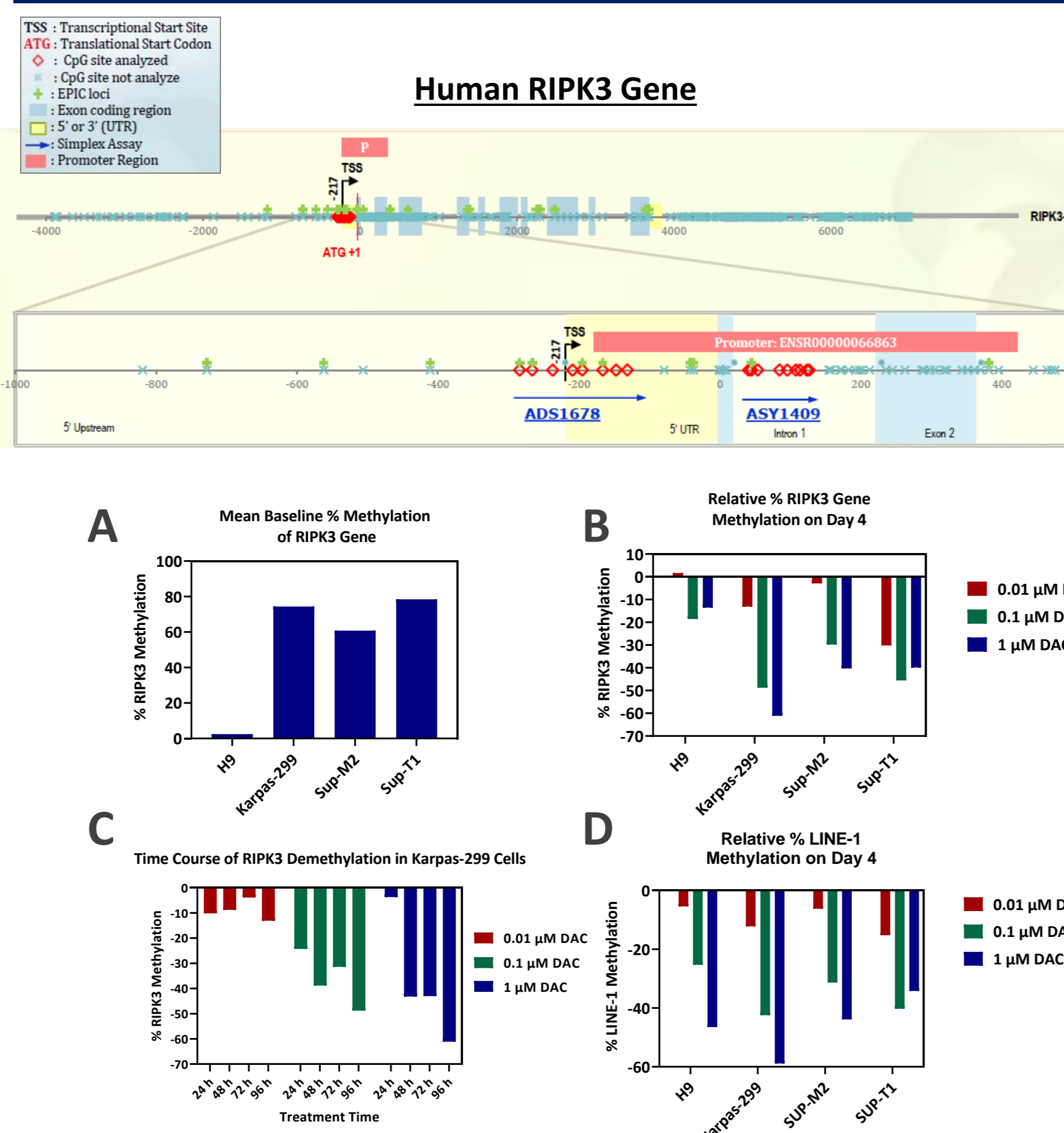
- Re-expression of RIPK3 in CT26 cells by CRISPRa (A) increases lytic cell death (cytotox-NIR staining) on treatment with tolinapant by real-time microscopy (B).

FIGURE 3 DAC TREATMENT INDUCES RIPK3 EXPRESSION IN TCL CELLS



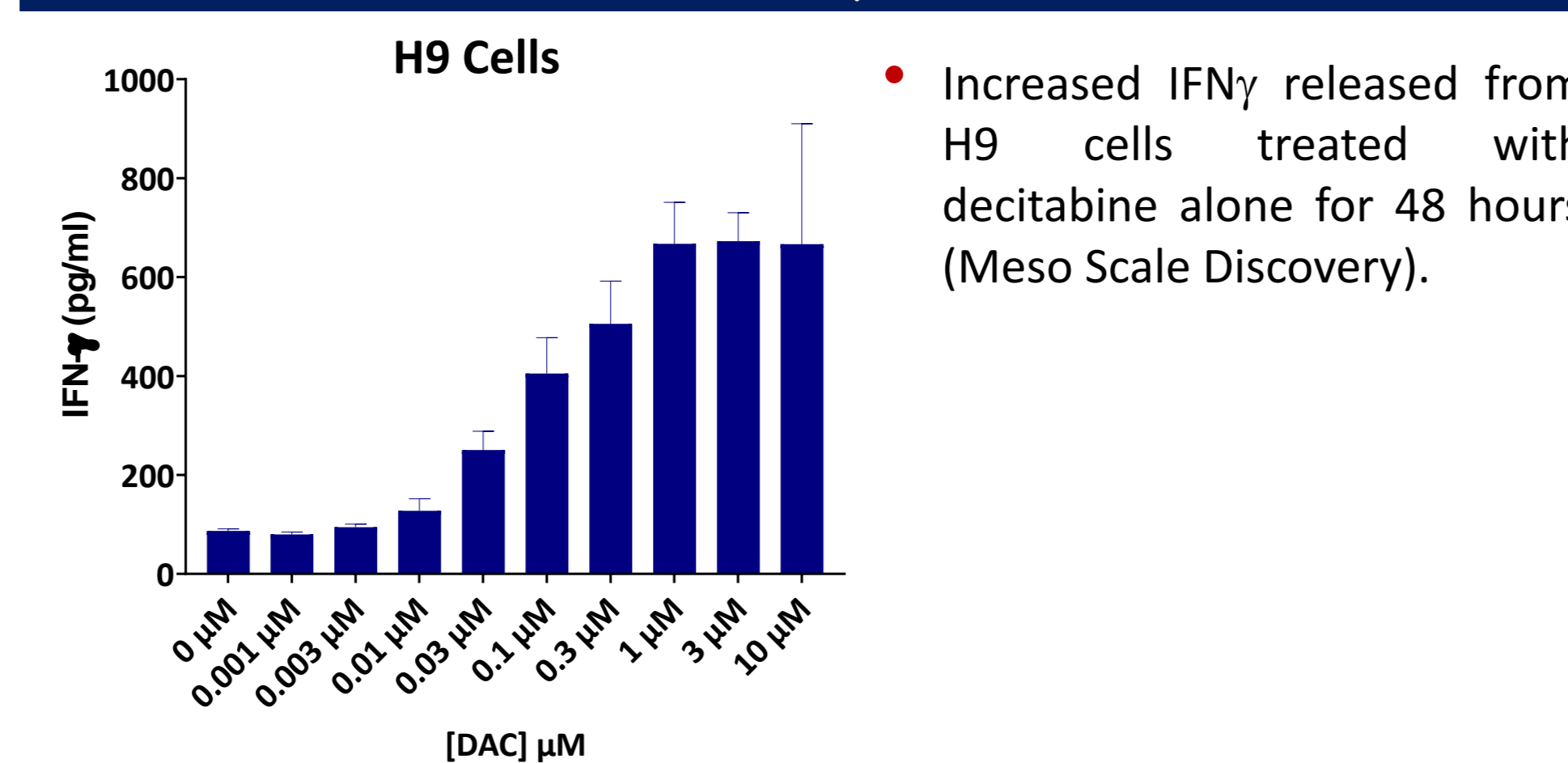
- Human (A) and mouse (B) cell lines were treated with DAC for 4 or 2 days, respectively. RIPK3 was detected in DAC-treated Karpas-299 (A) or CT-26 cells (B) in which RIPK3 is normally silenced; whilst H9 (A) and BW5147.G.1.4 cells (B) have high RIPK3 basal expression.

FIGURE 4 DAC REDUCES HUMAN RIPK3 PROMOTER METHYLATION



- Human H9 cells have low basal methylation of the RIPK3 gene promoter (A).
- Decitabine treatment of human TCL cell lines leads to RIPK3 gene promoter (B & C) and LINE-1 (D) demethylation by pyrosequencing (EpigenDX).

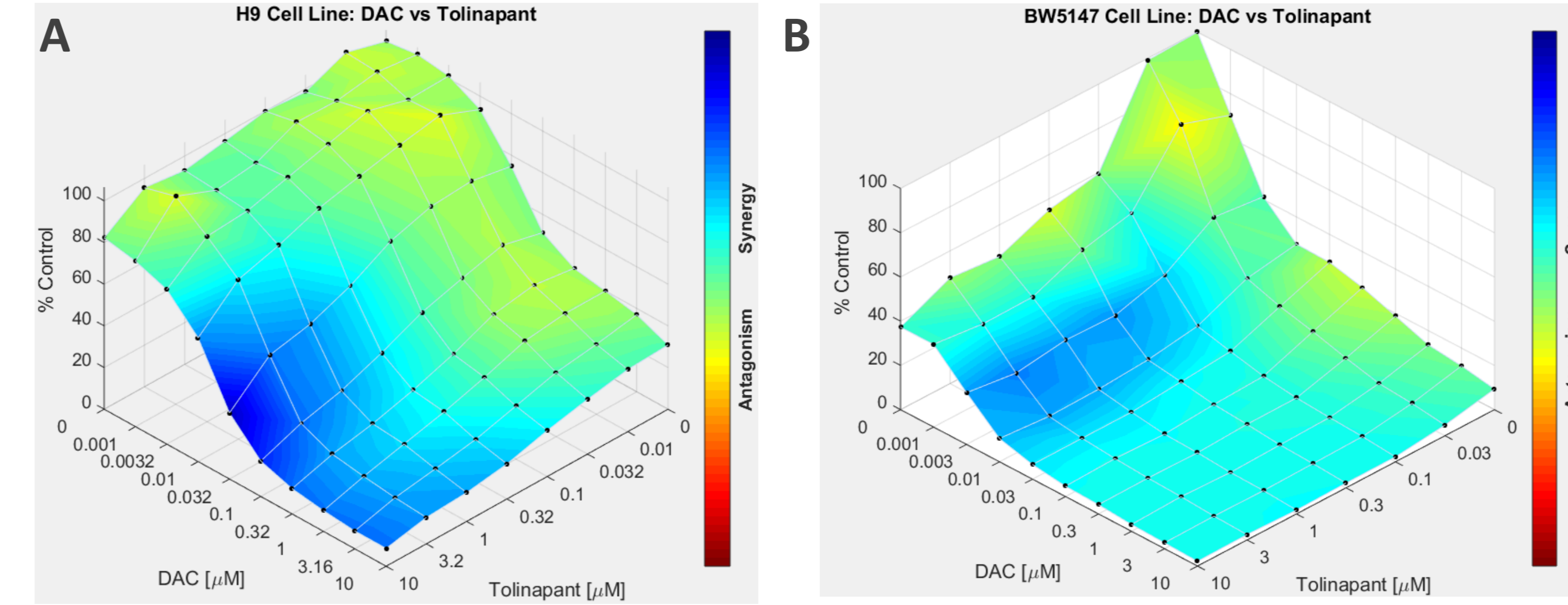
FIGURE 5 DAC INCREASES IFNγ RELEASE FROM H9 CELLS



- Increased IFNγ released from H9 cells treated with decitabine alone for 48 hours (Meso Scale Discovery).

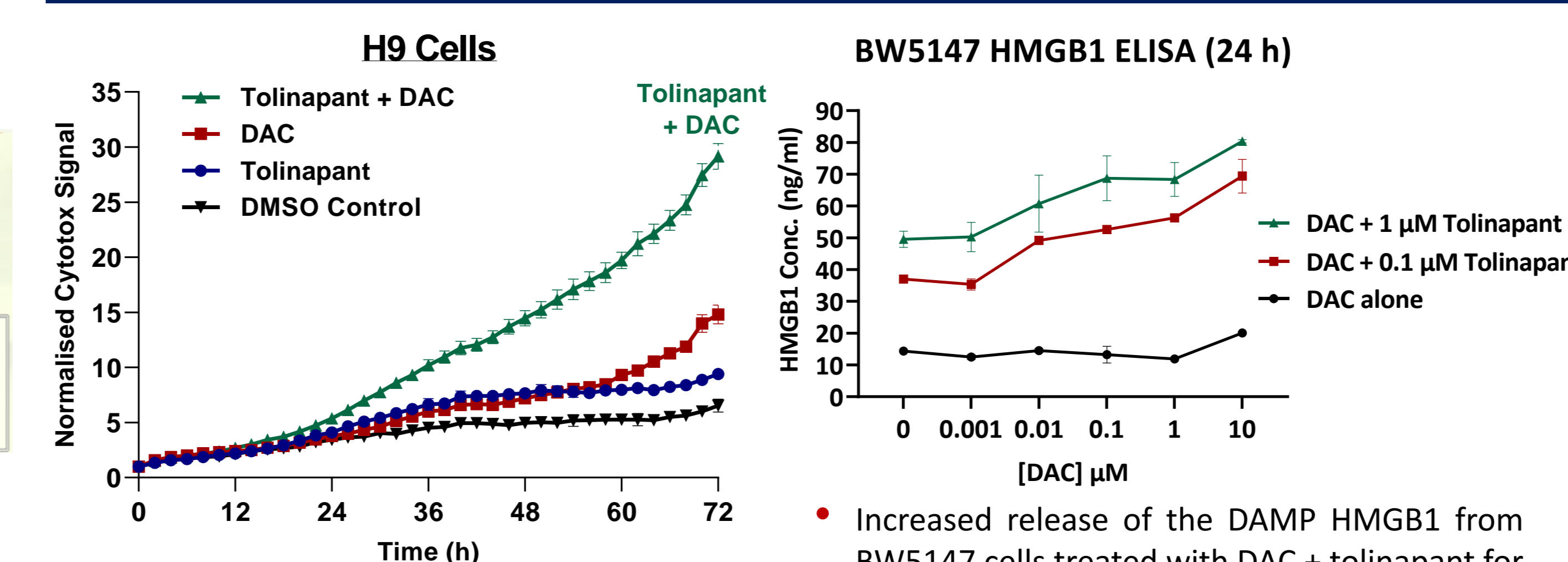
## IN VITRO ACTIVITY OF TOLINAPANT AND DECITABINE IN TCL CELL LINES

FIGURE 6 DAC + TOLINAPANT COMBINATION SYNERGISTICALLY REDUCES CELL VIABILITY



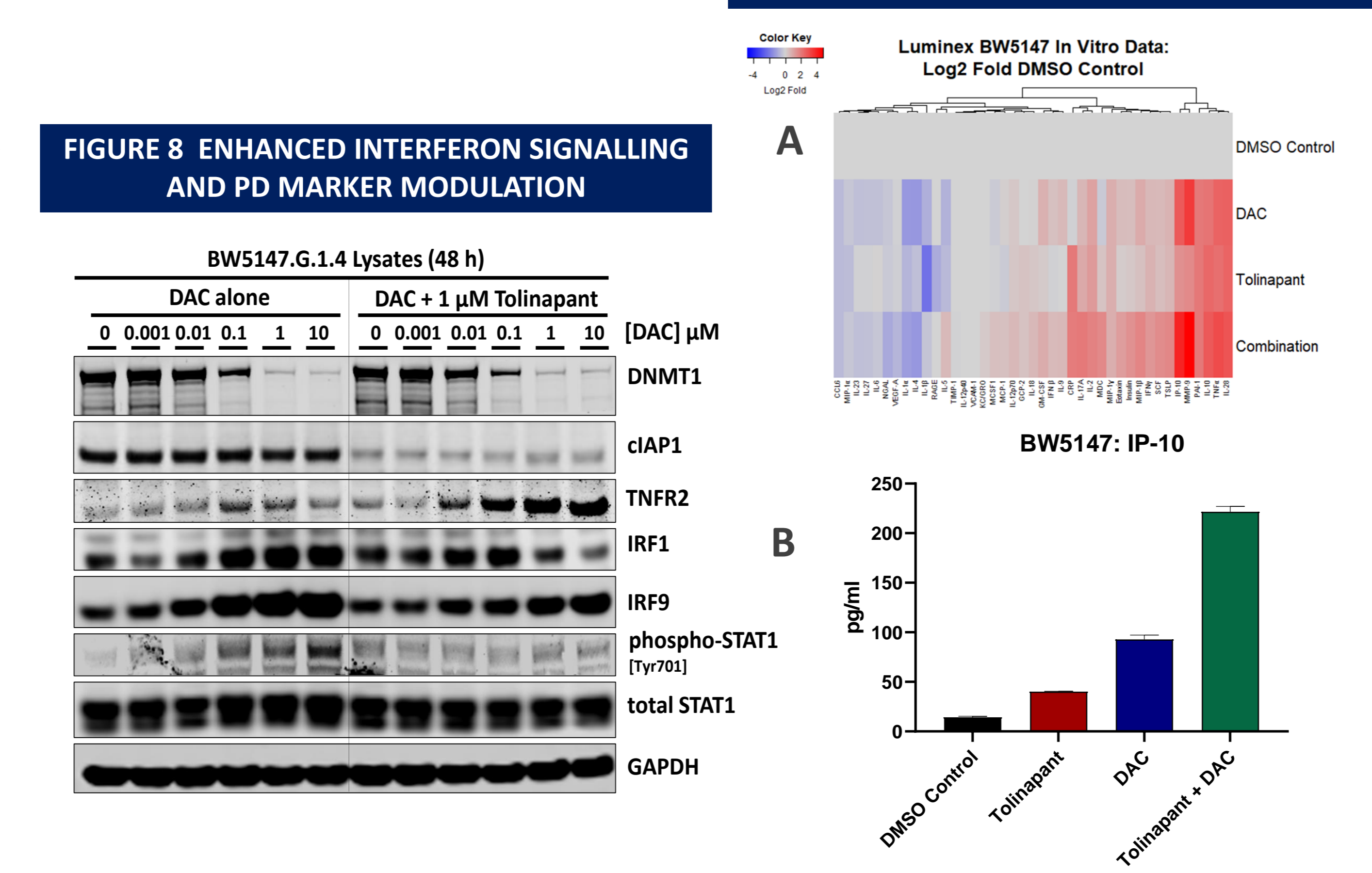
- H9 (A) and BW5147 (B) cell viability is reduced on combination of tolinapant and DAC treatment (72 h proliferation assay). Synergy assessed by HSA model (Combeneft).

FIGURE 7 COMBINATION TREATMENT INCREASES LYTIC CELL DEATH



- Combining DAC and tolinapant leads to increased lytic cell death (Cytotox staining) of H9 human TCL cells by real-time microscopy.
- Increased release of the DAMP HMGB1 from BW5147 cells treated with DAC + tolinapant for 24 hours, indicating enhanced lytic cell death.

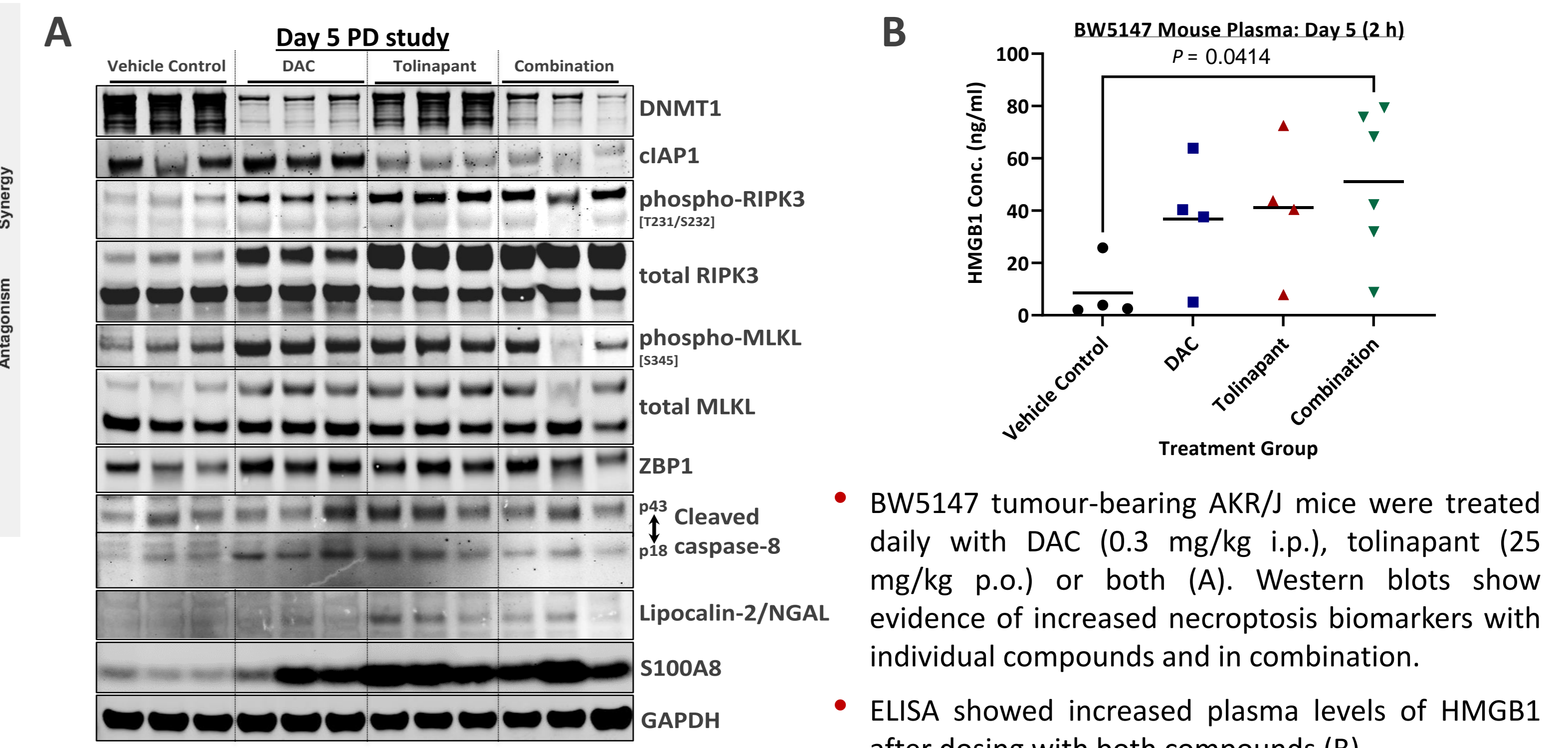
FIGURE 9 IN VITRO RELEASE OF CYTOKINES AND CHEMOKINES



- BW5147 cells treated for 48 h with DAC or DAC + 1 μM tolinapant show increased interferon signalling and PD marker modulation by Western blotting.
- Elevated cytokines/chemokines secreted *in vitro* after treatment of BW5147 cells with the combination of tolinapant and decitabine for 48 hours (A & B).

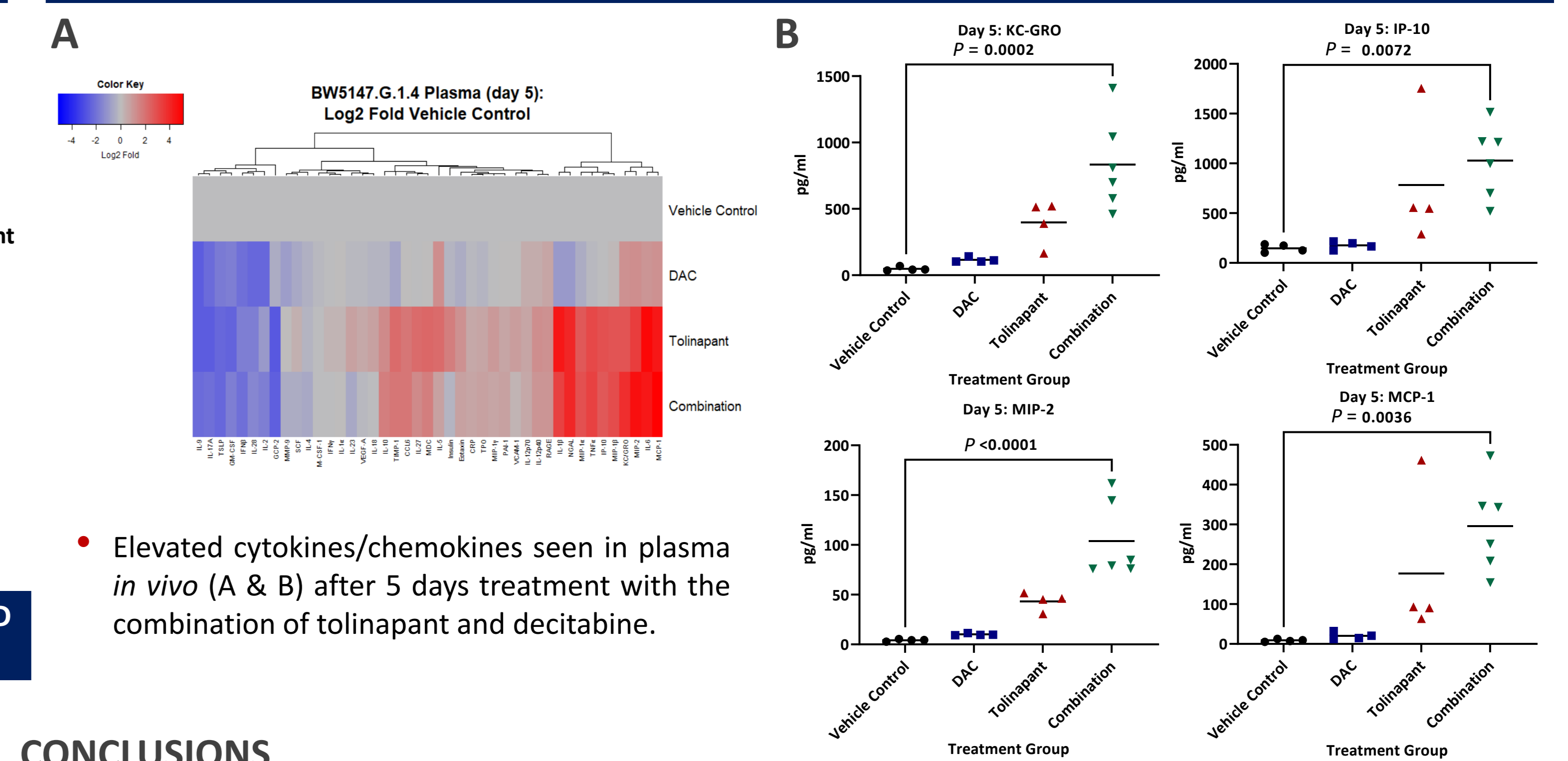
## IN VIVO COMBINATION ACTIVITY IN A MOUSE SYNGENEIC TCL MODEL

FIGURE 10 INDUCTION OF NECROPTOSIS IN SYNGENEIC MOUSE TCL TUMOURS



- BW5147 tumour-bearing AKR/J mice were treated daily with DAC (0.3 mg/kg i.p.), tolinapant (25 mg/kg p.o.) or both (A). Western blots show evidence of increased necroptosis biomarkers with individual compounds and in combination.
- ELISA showed increased plasma levels of HMGB1 after dosing with both compounds (B).

FIGURE 11 IN VIVO RELEASE OF CYTOKINES/CHEMOKINES



- Elevated cytokines/chemokines seen in plasma *in vivo* (A & B) after 5 days treatment with the combination of tolinapant and decitabine.

## CONCLUSIONS

- Re-expression of RIPK3 using CRISPR activation in the CT26 cell line led to increased cell death after treatment with tolinapant. This not only highlights the importance of RIPK3 in tolinapant-driven cell death, but also provides rationale for combining tolinapant with agents that can increase RIPK3 expression.
- RIPK3 is known to be silenced by methylation<sup>6</sup> and we confirmed that re-expression of RIPK3 in TCL cell lines can be achieved by decitabine (hypomethylating agent) treatment of TCL cell lines.
- Upregulation of interferon signalling and enhancement of chemokines and cytokines demonstrates potential of HMA for driving immunomodulatory activity in the tumor microenvironment<sup>7</sup>.
- The combination of tolinapant and decitabine synergistically reduced viability in human (H9) and mouse (BW5147) T-cell lymphoma cell line proliferation assays.
- Necroptosis was induced by decitabine and tolinapant alone and the combination of compounds in a mouse TCL syngeneic model *in vivo*, with robust activation of RIPK3 and MLKL.
- Collectively, the data presented here suggest a mechanistic rationale for testing the combination of tolinapant and ASTX727 (oral decitabine) in TCL.

## REFERENCES

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