



Targeting V-Domain Ig Suppressor of T Cell Activation (VISTA) in Malignant Mesothelioma

Abishek Sehgal MS*, Amber Rudenick MS*, Nithya Bhushan, Kimberly Lan BS, Jeffrey Lan BS, Blake A. Jacobson PhD, Haoran Zhang, Manish Patel DO, Robert A. Kratzke MD, Jeremy Allred MD *equal contributors

INTRODUCTION

V-domain Ig suppressor of T cell activation (VISTA) has emerged as a key immune checkpoint regulator with implications in tumor immunity. VISTA expression on T cells normally exerts inhibitory effects on T cell proliferation. The role of VISTA expression on tumors is less clear, though it has also been shown to inhibit tumor-specific T cell activity in some models. VISTA is highly upregulated in malignant mesothelioma (MM) and is a potential target for cellular therapy, but its role in modulating immune responses is unknown. In this study, we sought to identify VISTA's role and functional significance, explore its potential for modulation via small molecule inhibitors, and investigate the efficacy of a CAR T cell therapy targeting VISTA and mesothelin. This study aims to address the critical gaps in the understanding of VISTA-mediated immunoregulation within the context of MM biology. This may unveil potential therapeutic targets and strategies for improving treatment outcomes via CAR T cell therapy.

METHODS

Western blot analysis and flow cytometry were used to assess VISTA and mesothelin expression in MM cell lines. To understand and modulate key signaling pathways involved in VISTA expression, we conducted experiments treating cells with a VISTA siRNA. In addition, we treated cells with Trametinib, LY294002, Capivasertib, and Ruxolitinib. Western blot analysis was then utilized to assess the impact of the drug treatments on VISTA expression, providing comprehensive insights into the interplay between VISTA and key signaling pathways in MM pathogenesis. Concurrently, we developed VISTA- and mesothelin- targeted CAR T cells. We then performed an in vitro cytotoxicity assay using real-time fluorescence microscopy (IncuCyte) to determine cell killing of organoids made from our MM cell lines (HAY, H2373, and H2461 engineered to express GFP and Luciferase) via co-culture with the VISTA- and mesothelin-targeted CAR T cells over six days at different concentrations.

RESULTS

Western blot analysis and flow cytometry revealed the presence of VISTA expression in our MM cells, highlighting its relevance in this malignancy. After 48-hour treatment, LY294002 and Capivasertib resulted in a decrease in VISTA expression. Trametinib treatment of H2373 resulted in a marked increase in VISTA expression. 24-hour Ruxolitinib treatment resulted in a slight decrease in VISTA expression. The IncuCyte analysis showed that both VISTA- and Mesothelin-targeted CAR T cells at the lower E:T ratios of 0.5:1 and 1:1 led to potent killing of the MM cell lines HAY, H2461, and H2373.

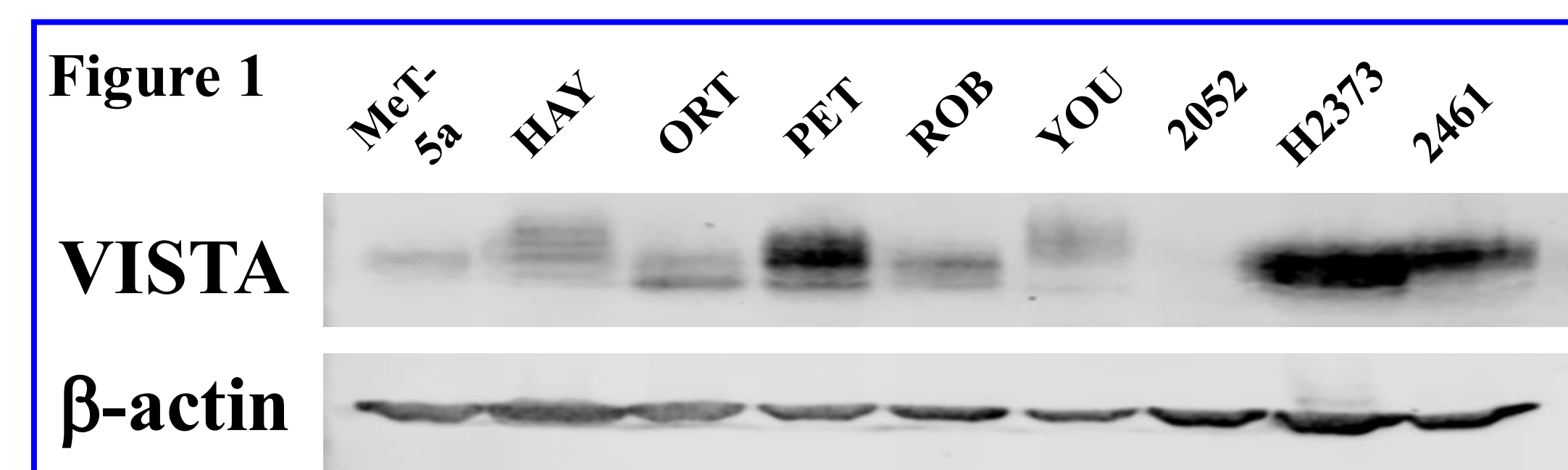


Figure 1. MM expresses VISTA. Immunoblots 8 human (HAY, ORT, PET, ROB, YOU, 2052, H2373, and 2461) MM cell lines, and an immortalized mesothelial cell line (MeT-5a) at steady-state growth for VISTA are shown. β -Actin serves as a loading control.

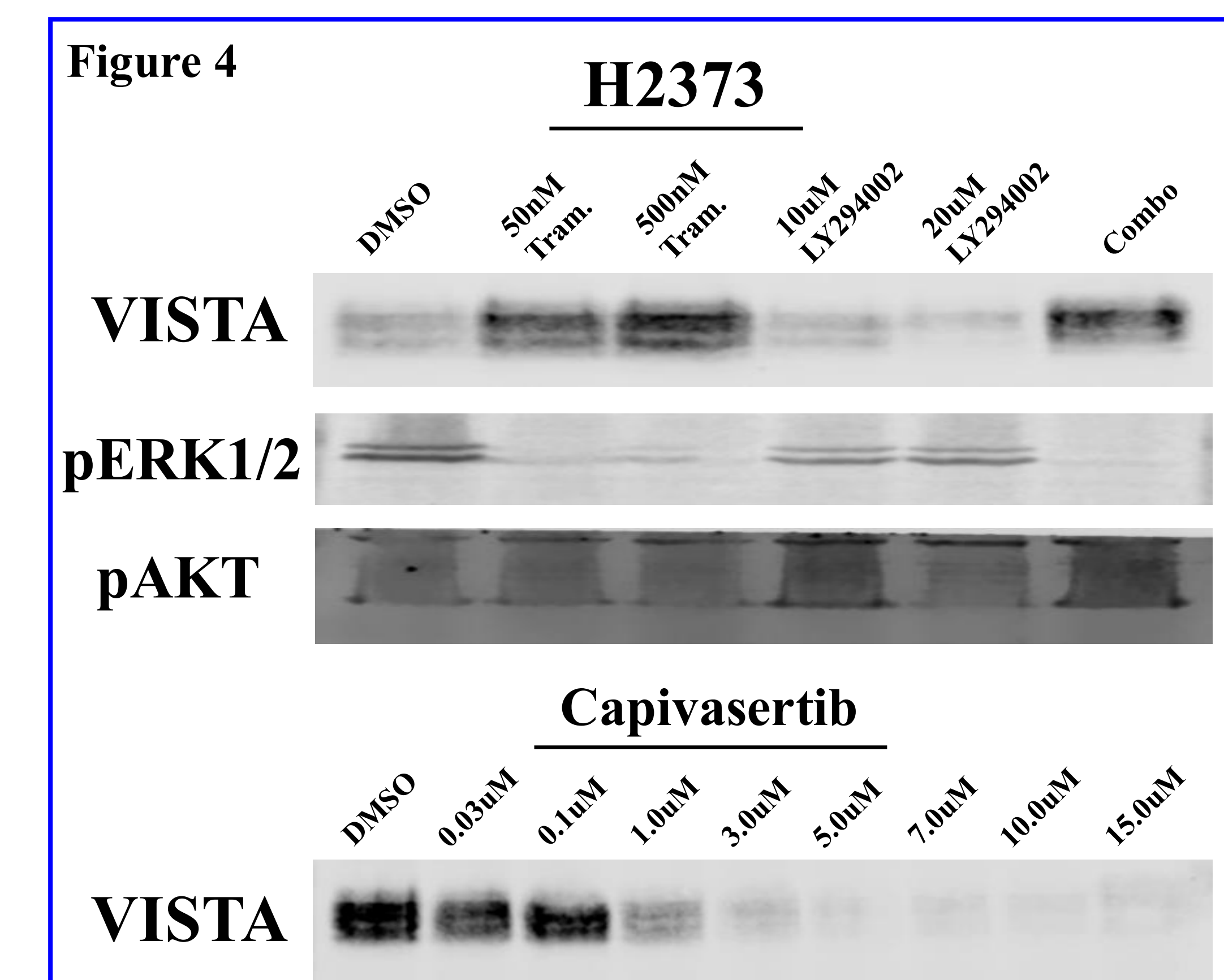


Figure 4. Human 2373 cells (Sarcomatoid MM) after 48-hour treatment of MEK inhibitor Trametinib, PI3K inhibitor LY294002, and AKT inhibitor Capivasertib. VISTA showed a marked decrease in expression after PI3K and AKT inhibition.

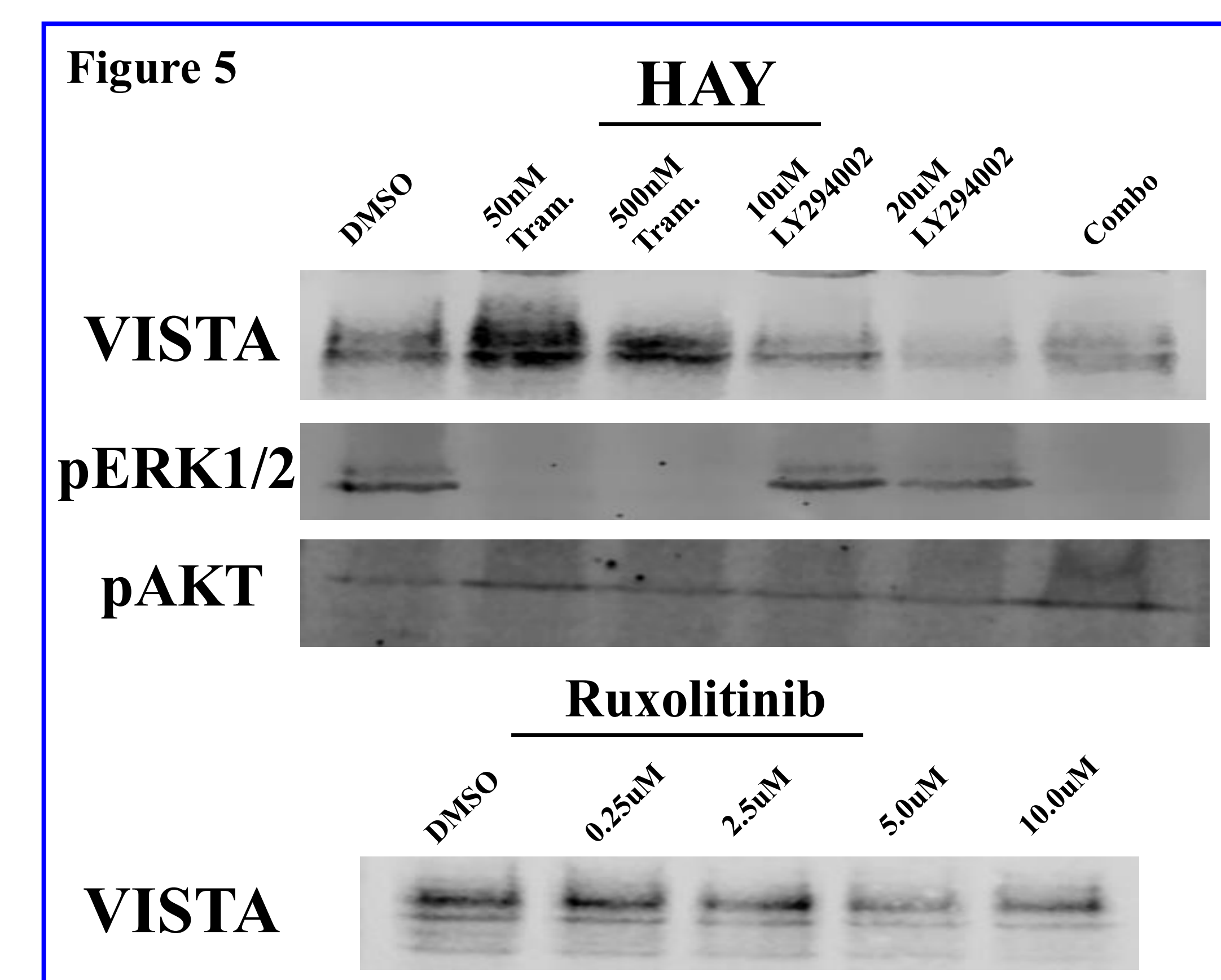


Figure 5. Human HAY cells (Epithelioid MM) after 48-hour treatment of MEK inhibitor Trametinib, PI3K inhibitor LY294002, and JAK/STAT inhibitor Ruxolitinib. VISTA showed a decrease in expression after PI3K inhibition and a slight decrease in expression after JAK/STAT inhibition.

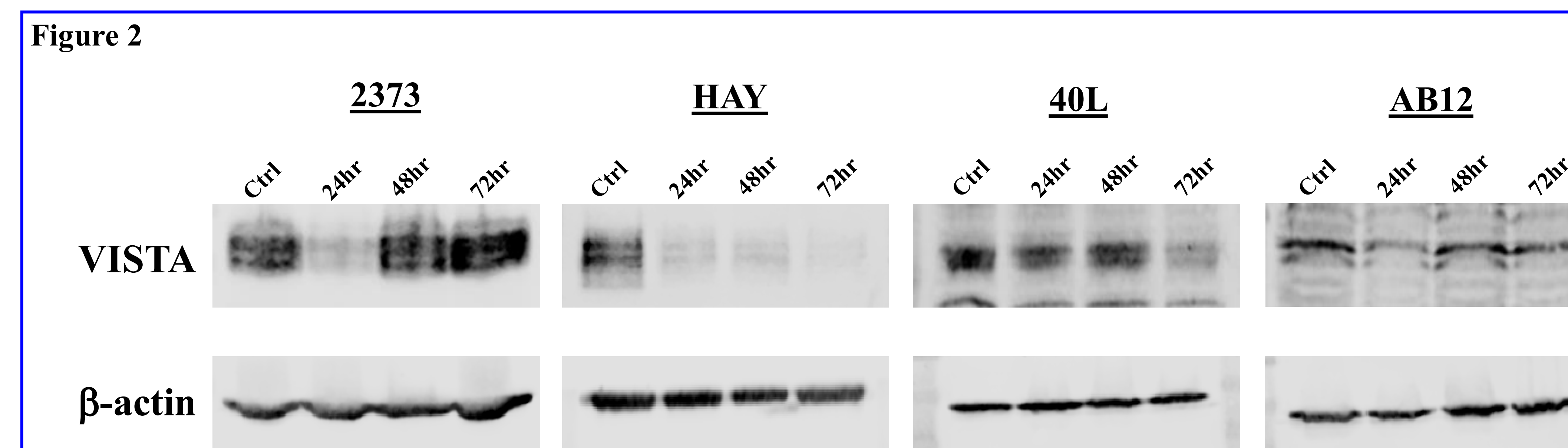


Figure 2. Immunoblotting was used to assess VISTA expression in human (HAY and 2373) and murine (40L and AB12) MM cells treated with 100nM VISTA siRNA for 24, 48, and 72 hours. VISTA expression was knocked down in all cells.

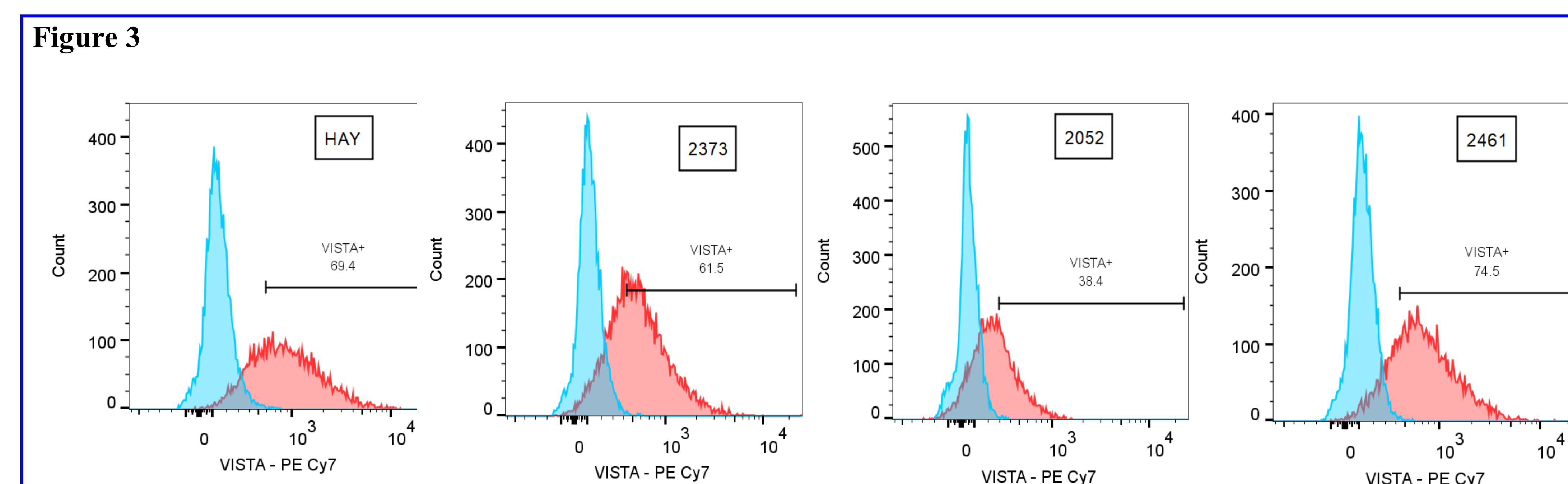


Figure 3. Flow Cytometry was used to analyze the amount of VISTA expressed on human MM cells. 2461 expressed the highest amount of VISTA on the cell surface. 2052 expressed the least amount of VISTA on the cell surface.

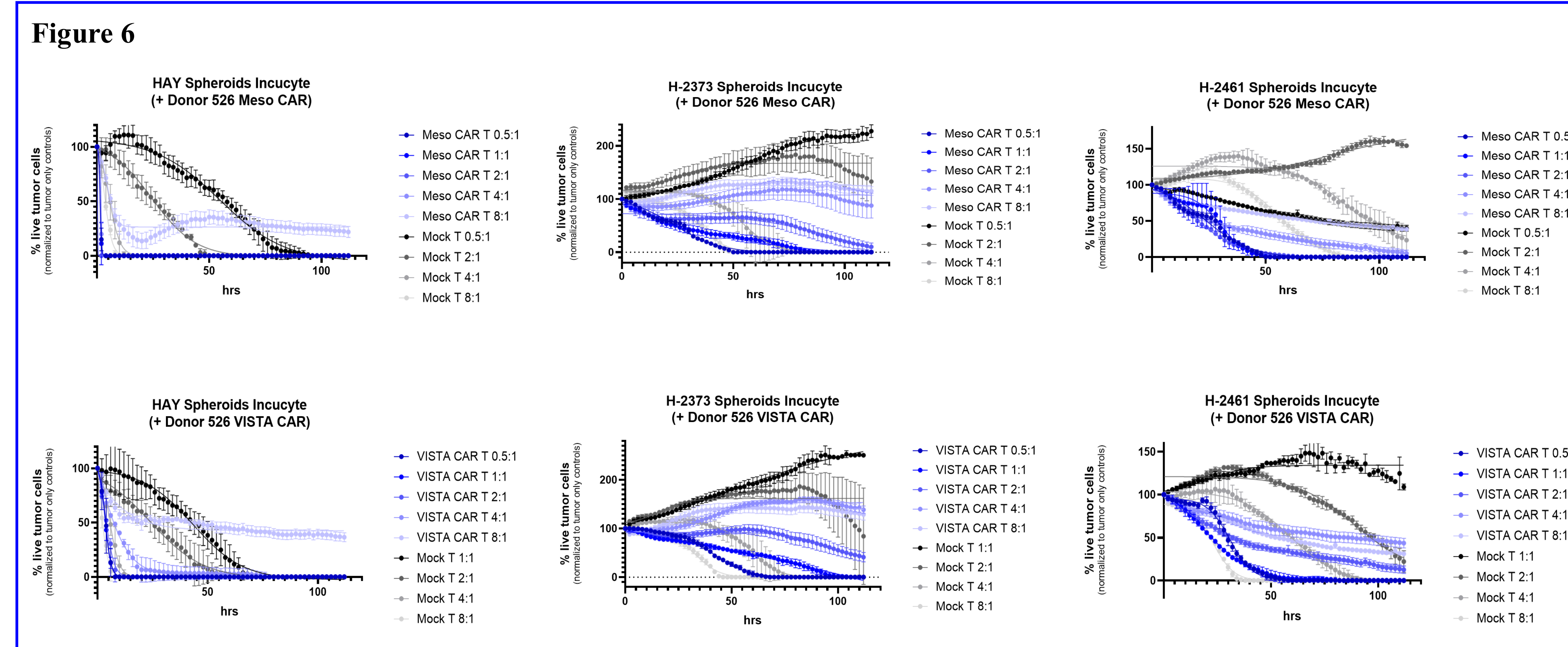


Figure 6. Cytotoxicity assay using real-time fluorescence microscopy (IncuCyte) via co-culture with VISTA- and mesothelin-targeted CAR T cells showed that killing of MM cell lines were better/faster at lower E:T ratios.

CONCLUSIONS

- 1) There is an interaction between VISTA expression and ERK, AKT, and JAK/STAT signaling pathways.
- 2) Inhibition of the AKT signaling pathways leads to decreased VISTA expression, while inhibition of MEK signaling pathways leads to increased VISTA expression in sarcomatoid mesothelioma (H2373).
- 3) In epithelioid mesothelioma (HAY), AKT and JAK/STAT inhibition lead to decreased VISTA expression.
- 4) VISTA- and mesothelin-targeted CAR T cells show increased killing at lower E:T ratios.