# The beta-carboline Harmine sensitives microsatellite stable colorectal cancer mouse model to anti-PD-1 through upregulating MHC-I dependent antigen presentation machinery and improving the infiltration of CD8 T cells into the tumor microenvironment


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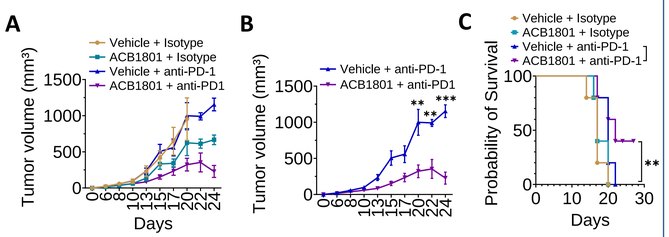
## ABSTRACT

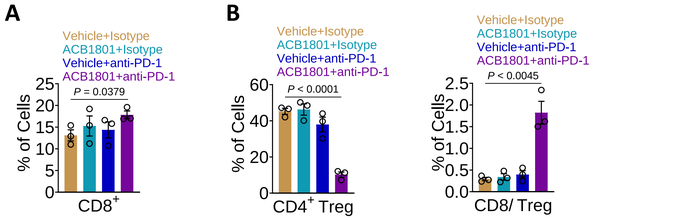

We investigated the effect of beta-carboline Harmine (ACB1801) to improve the effectiveness of anti-PD-1 therapy in a mouse model of microsatellite stable colorectal cancer (MSS-CRC). Our results show that combining ACB1801 with anti-PD-1 significantly improves treatment outcomes in MSS-CRC mice. This improvement is related to increased infiltration of CD8+ T cells and decreased infiltration of regulatory T cells (Tregs) in the tumor microenvironment. ACB1801 also triggers the expression of CXCL10, a proinflammatory chemokine, potentially attracting CD8+ T cells to the tumor site. Furthermore, ACB1801 enhances the expression of MHC-I genes, improving antigen presentation on CRC cells. Overall, our findings suggest that combining ACB1801 with anti-PD-1 therapy could convert MSS CRC into an "immune hot" tumor, offering a promising treatment approach for this colorectal cancer subtype.


## Results 1: ACB1801 enhances the therapeutic benefit of anti-PD-1



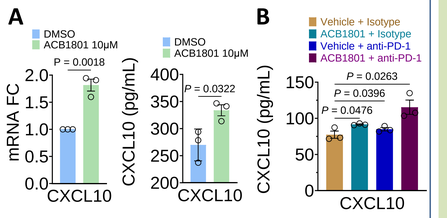

Combining ACB1801 improves the therapeutic benefit of anti-PD-1 in the CT26 MSS CRC mouse model. Mice treated with the combination of ACB1801/anti-PD-1 exhibited significant tumor growth inhibition **(A and B)** and enhanced survival **(C).**

## Results 2: The combination of ACB1801 and anti-PD-1 increases infiltration of CD8+ T cells and decreases infiltration of Tregs into tumors.



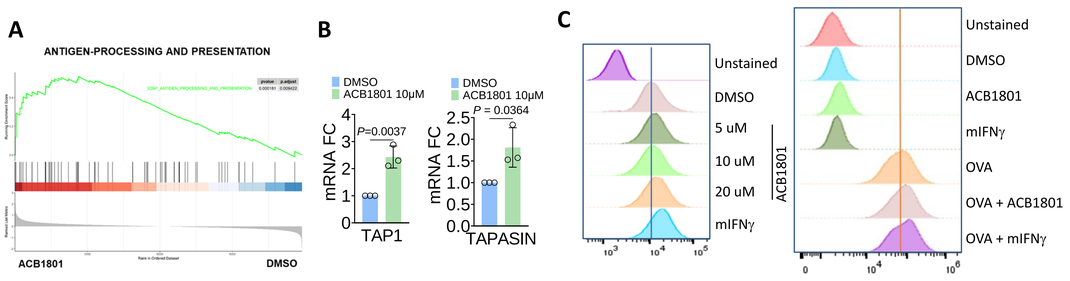

In CT26 tumors, treatment with the ACB1801/anti-PD-1 combination results in elevated infiltration of CD8+ T cells **(A)** and reduced presence of Tregs (**B**). This leads to a significantly increased CD8/Treg ratio in tumors subjected to combination therapy **(C)**.

## Results 3: ACB1801 increases the expression of CXCL10 by tumor cells




ACB1801 enhances both the expression and release of CXCL10 in CT26 tumor cells **(A)** and within the tumor microenvironment of CT26 **(C)**.

## Results 4: Results: ACB1801 upregulates the expression of functional MHC-I on the cell surface

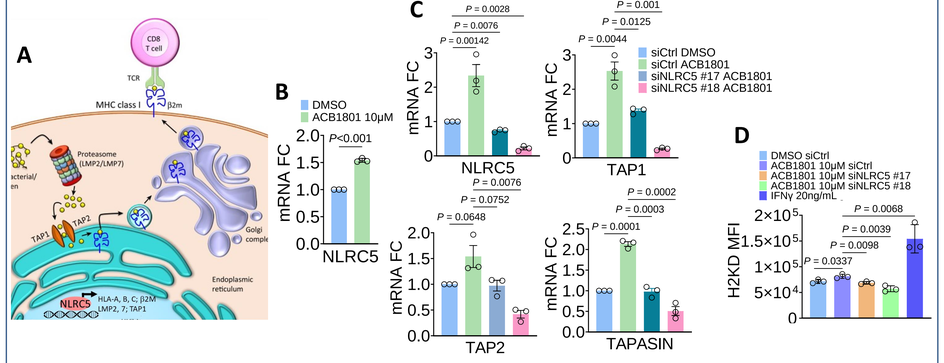



**A:** Bulk RNAseq analysis of untreated and ACB1801-treated CT26 cells indicates an enrichment in genes associated with the antigen-processing and presentation machinery.

**B:** ACB1801 upregulates the expression of TAP1 and TAPASIN, two crucial proteins involved in MHC-I, within CT26 cells. (FC: Fold change)

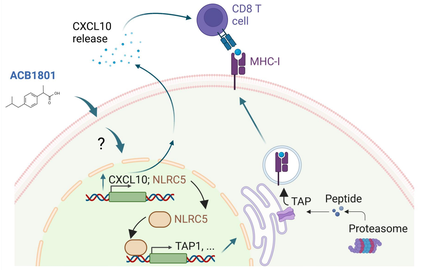
**C: Left:** ACB1801 enhances the cell surface expression of MHC-I in CT26 CRC cells. **Right:** ACB1801 enhances the loading of ovalbumin (OVA) antigen peptides onto MHC I-peptide complexes (H2-Kb) in MC38 CRC cells. Antibody used: APC anti-mouse H-2Kb bound to SIINFEKL Antibody. Mouse Interferon gamma (mIFNg) is used as a control.

## Results 5: ACB1801 operates through the upregulation of NLRC5




**A:** NOD-like receptor family CARD domain-containing 5 (NLRC5) regulates MHC-I genes. **B:** ACB1801 treatment increases NLRC5 expression in CT26 cells. **C and D:** Silencing NLRC5 with siRNA (siNLRC5 #17 and #18) in CT26 cells abolishes ACB1801's ability to enhance TAP1, TAP2, and Tapasin expression **(C)** and decreases H-2KB expression on MC38 tumor cell surfaces **(D).**

## CONCLUSION




ACB1801 exhibits a potent synergistic effect with anti-PD-1 by enhancing the therapeutic efficacy through two key mechanisms: augmenting the expression of functional MHC-I and increasing the release of CXCL10 in an NLRC5-dependent manner. This dual action facilitates the recruitment of CD8 T cells into the tumor microenvironment, thereby increasing the anti-tumor response. While the precise mechanism underlying ACB1801's modulation of NLRC5/CXCL10/MHC-I axis warrants further investigation, our preclinical findings substantiate the potential of ACB1801/anti-PD-1 combination therapy, particularly for MSS CRC patients.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