

Abstract

In order to sustain their inexorable growth, tumors have specialized reprogrammed metabolism. This metabolism creates an acidic, hypoxic and nutrient depleted tumor microenvironment (TME). Such an environment inhibits anti-tumor effector cells while promoting the differentiation and function of inhibitory cells such as T regulatory cells, Myeloid Derived Suppressor Cells (MDSC) and Tumor Associated Macrophages (TAM). We hypothesized that by targeting tumor metabolism we could alter the TME and “condition” tumors to be more susceptible to immunotherapy. To this end, along with the Johns Hopkins Drug Discovery Program we developed a novel prodrug of 6-Diazo-5-oxo-L-norleucine, inhibitor of glutamine metabolism (JHU-083). Recently, it has been shown that M2 macrophages require glutamine metabolism for differentiation and function. In light of the similarities between M2 macrophages and suppressive myeloid cells, we hypothesized that JHU-083 might inhibit the generation and function of MDSCs and TAMs. We tested this hypothesis in the 4T1 breast cancer model and 3LL lung carcinoma model. These tumors are relatively resistant to immunotherapy and are characterized by increased generation of MDSCs and distant spontaneous metastasis. JHU-083 treated mice suppressed tumor growth compared to the vehicle treated group. Immunologically, we observed markedly reduced numbers of MDSCs in circulating blood within 3 days of drug treatment compared to vehicle group, leading to favorable CD8 to MDSCs ratios. Consistently, JHU083 treated group displayed significantly decreased percentages and numbers of MDSCs in the tumor, and increased tumor infiltrating CD8 cells. Interestingly, JHU-083 treatment induced TAM reprogramming. While the TAM from the vehicle treated group displayed increased M2 markers and arginase, the JHU-083 treated tumor infiltrating cells showed increased TNF- α producing M1-like macrophage phenotypes compared to vehicle group. These TNF- α producing cells were negatively correlated with tumor sizes. Notably, JHU083 treatment not only controlled primary tumor growth but also drastically reduced spontaneous lung metastasis. The decrease of MDSCs infiltration in the lung were also observed in JHU083 treated group. Mechanistically, our data suggests that JHU-083 inhibits CSF2/CSF3 production and survival of the tumor itself as well as directly affects macrophage metabolism and signaling. Also, LC-MS based metabolites analysis from JHU-083 treated tumors revealed reduced Kynurenine:tryptophan ratios compared to the control group, indicating the metabolic modulation of the tumor microenvironment. Overall, our data support a novel role for glutamine inhibitor, JHU-083 in enhancing tumor specific immunity by targeting suppressive myeloid cells.

Introduction

- Increased monocyte derived suppressive cells are responsible for poor responses to immunotherapy and spontaneous metastasis in 4T1 (mammary) tumors
- M2 macrophages rely on glutamine metabolism for differentiation and its function
- Monocyte derived suppressive cells (MDSCs) share metabolic characteristics and markers with M2 macrophages
- Together with the Johns Hopkins Drug Discovery Program, we developed a novel prodrug of 6-Diazo-5-oxo-L-norleucine, to inhibit glutamine metabolism (JHU-083).

Objectives

We hypothesized that glutamine antagonism might enhance immunotherapy by regulation of the generation and functions of the suppressive myeloid cells

Results

4T1 tumor is resistant to immune checkpoint blockade immunotherapy

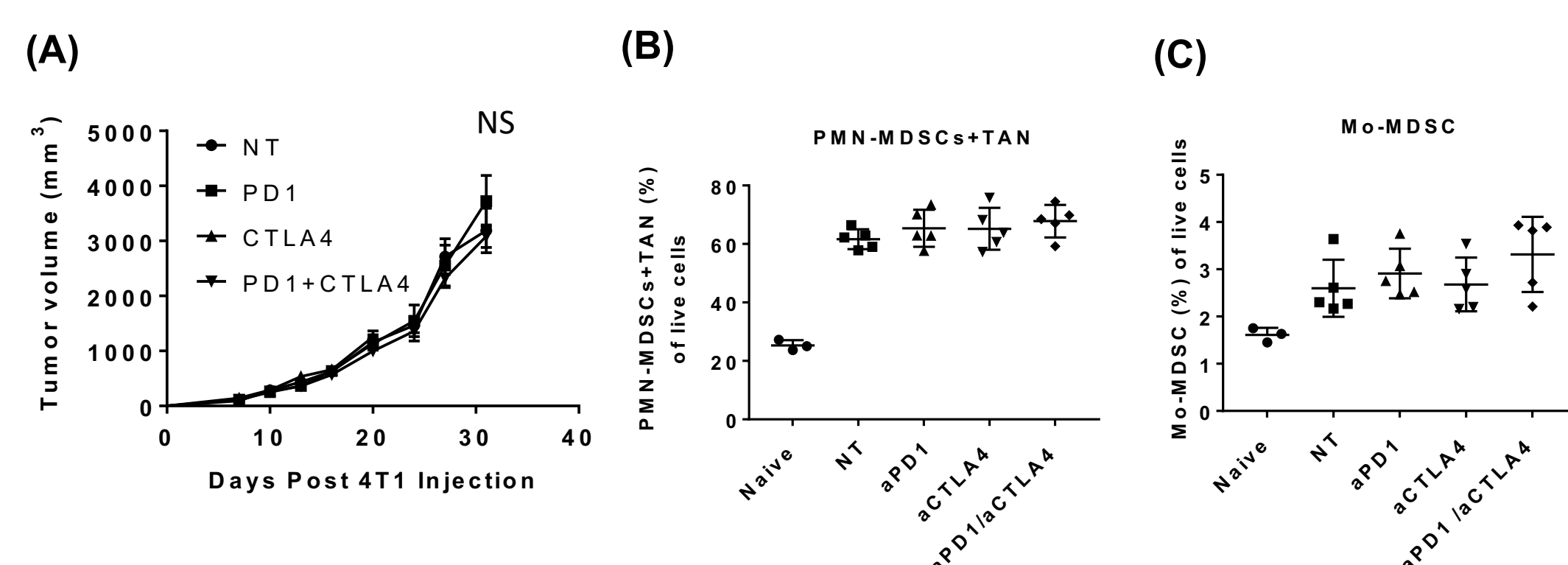


Fig 1. (A) 4T1 tumor is resistant to immune checkpoint blockade (B and C) Immune checkpoint blockade does not alter increased MDSCs in the blood from 4T1 tumor bearing mice (PMN-MDSC and TAN (polymorphonuclear-MDSCs and tumor-associated neutrophils : CD11b+F4/80-Ly6CloLy6Ghi) and Mo-MDSC (monocytic MDSC : CD11b+F4/80-Ly6GChiLy6Gneg)

Targeting glutamine using JHU-083 inhibits growth of 4T1 tumor and leads to decreased MDSCs

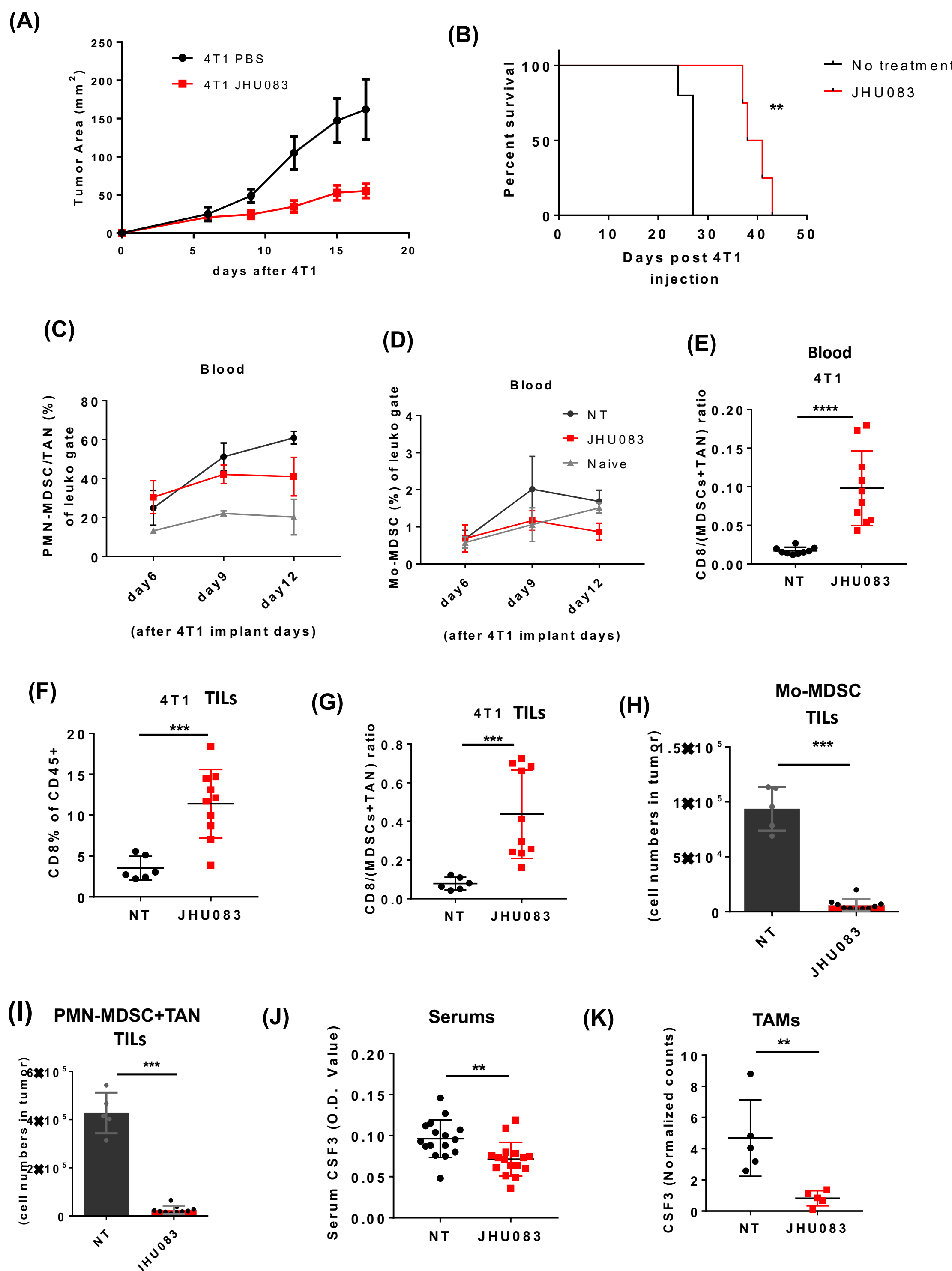


Fig 2. Targeting glutamine using JHU-083 in the 4T1 tumor bearing mice showed (A) delayed 4T1 tumor growth (B) increases survival (C) decreased % of PMN-MDSCs and TANs and (D) Mo-MDSCs in blood (E) increased ratio of CD8+ to MDSCs and TANs in the blood (F) increased % of CD8+ in TIL (tumor-infiltrating leukocytes) (G) increased ratio of CD8+ to MDSCs and TANs in TIL (H) reduced the relative numbers of Mo-MDSCs and (I) PMN-MDSCs and TANs (J) decreased CSF3 in the serum (K) decreased CSF3 mRNA in TAMs

Targeting glutamine using JHU-083 markedly reduces kynurenine

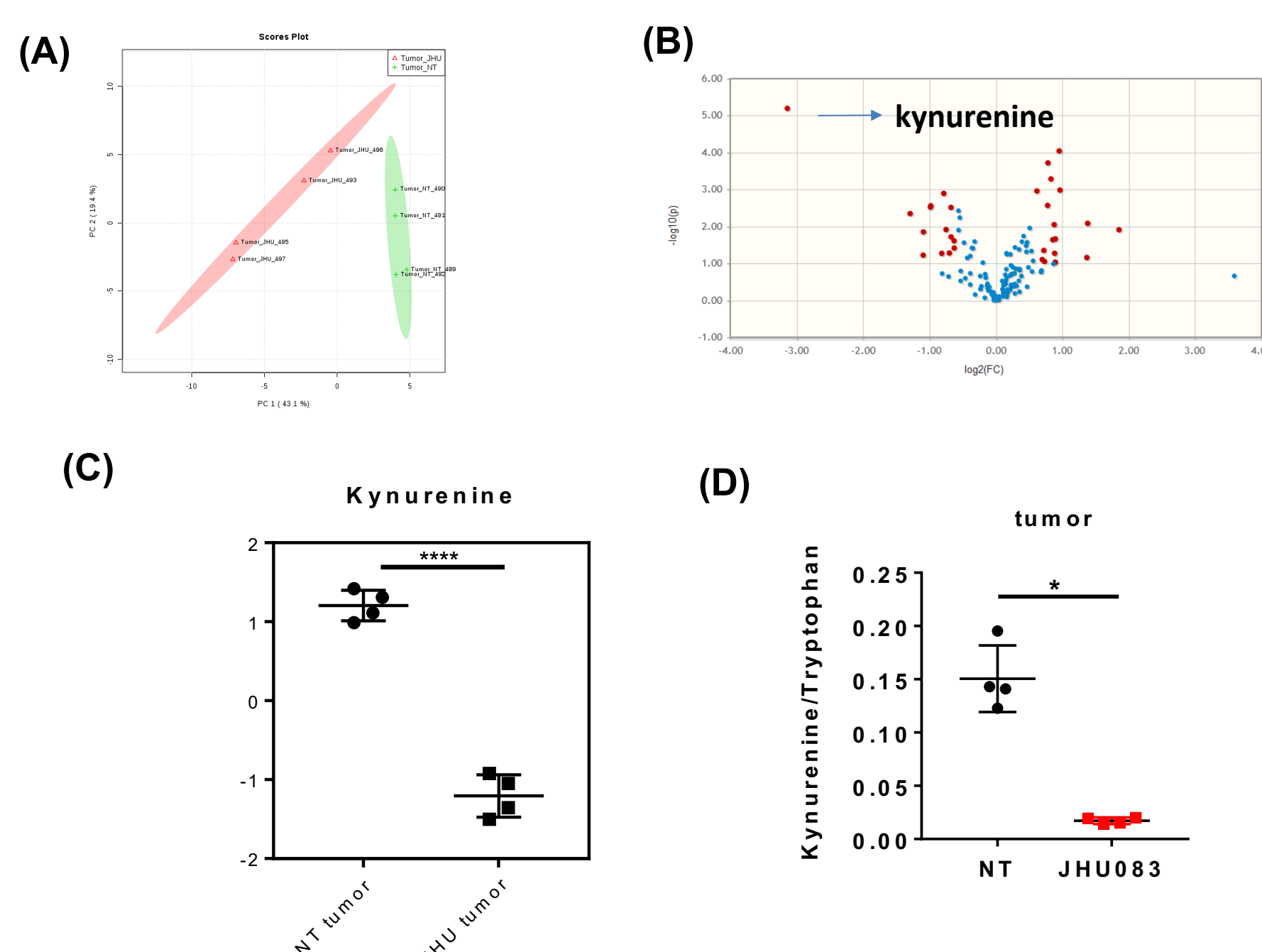


Fig 3. LC-Mass spec of metabolites of tumors from the NT and glutamine antagonist JHU-083 groups revealed (A) two distinct metabolic clusters correlated between experimental groups (B and C) markedly reduced kynurenine in volcano plot of metabolites (D) decreased ratio of kynurenine to tryptophan

Results

Targeting glutamine increases pro-inflammatory TAMs

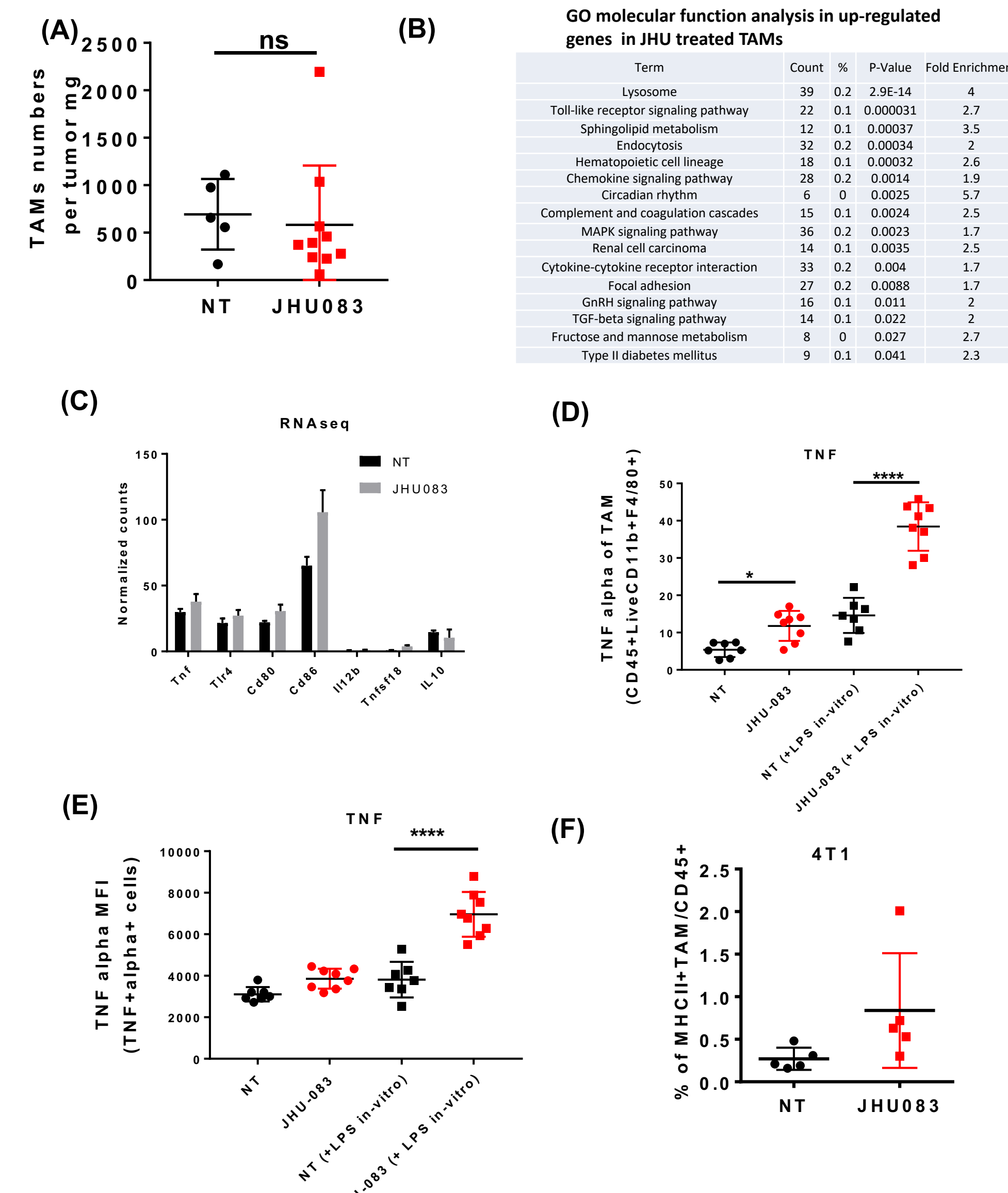


Fig 4. (A) Targeting glutamine using JHU-083 in the 4T1 tumor does not alter the numbers of tumor-associated macrophages (TAMs) (B and C) Increased gene expressions related to lysosome and TLR signaling in TAMs (D) increased % of TNF+ TAMs (E) increased amounts of TNF secretion in TAMs (F) increased MHCII+ TAMs from the glutamine antagonist JHU-083 treated mice

Glutamine antagonist JHU-083 inhibits spontaneous lung metastasis

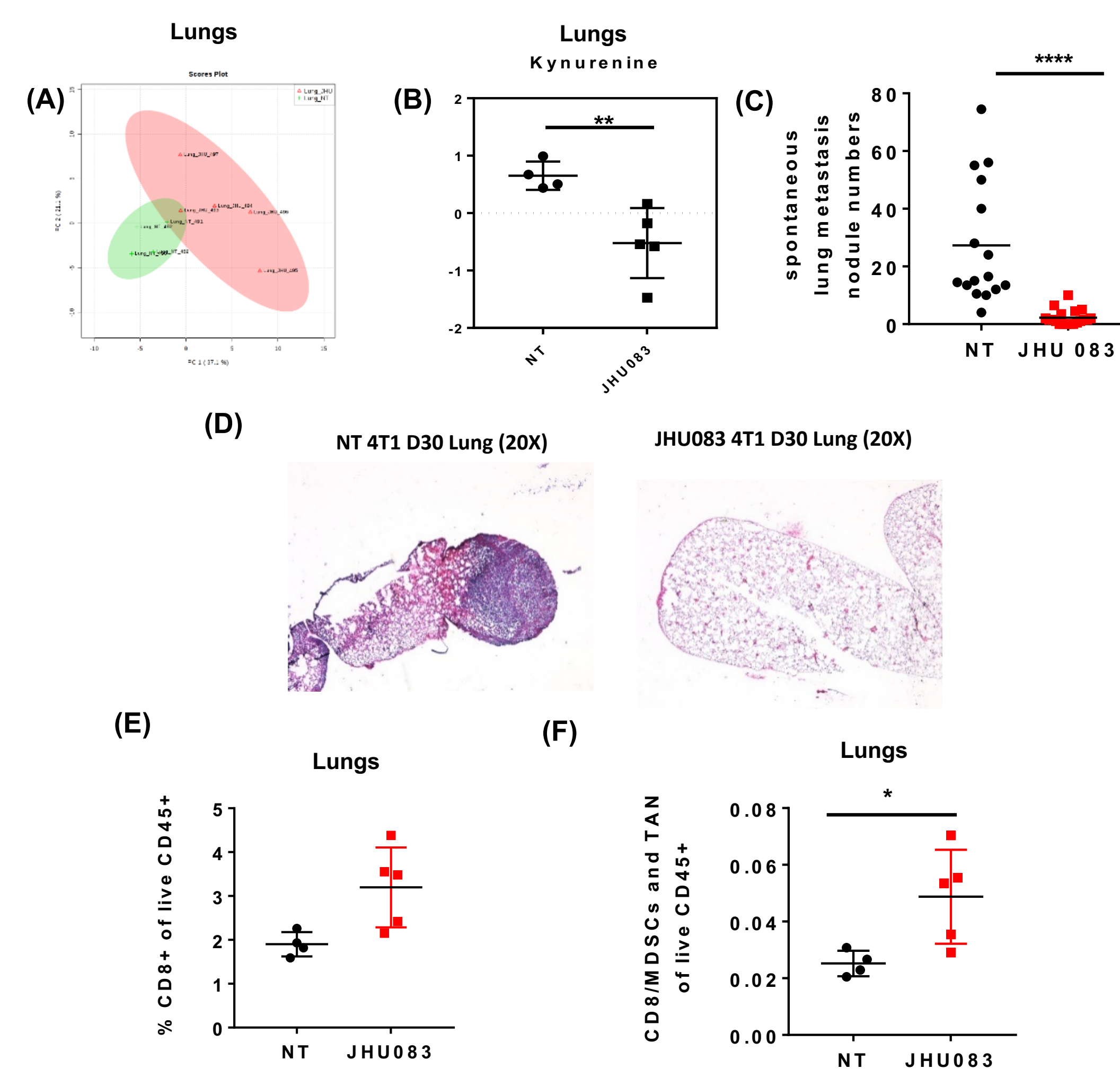


Fig 5. (A) LC-Mass spec of metabolites of spontaneous metastatic site (lung) from the NT and glutamine antagonist JHU-083 groups revealed two distinct metabolic clusters (B) markedly reduced kynurenine in volcano plot of metabolites. (C and D) Reduced spontaneous lung metastasis (E) increased % of CD8+ in TIL (tumor-infiltrating leukocytes) (F) increased ratio of CD8+ to MDSCs and TANs in cells from lung of 4T1 tumor bearing mice

Glutamine antagonism enhances priming of tumor antigen

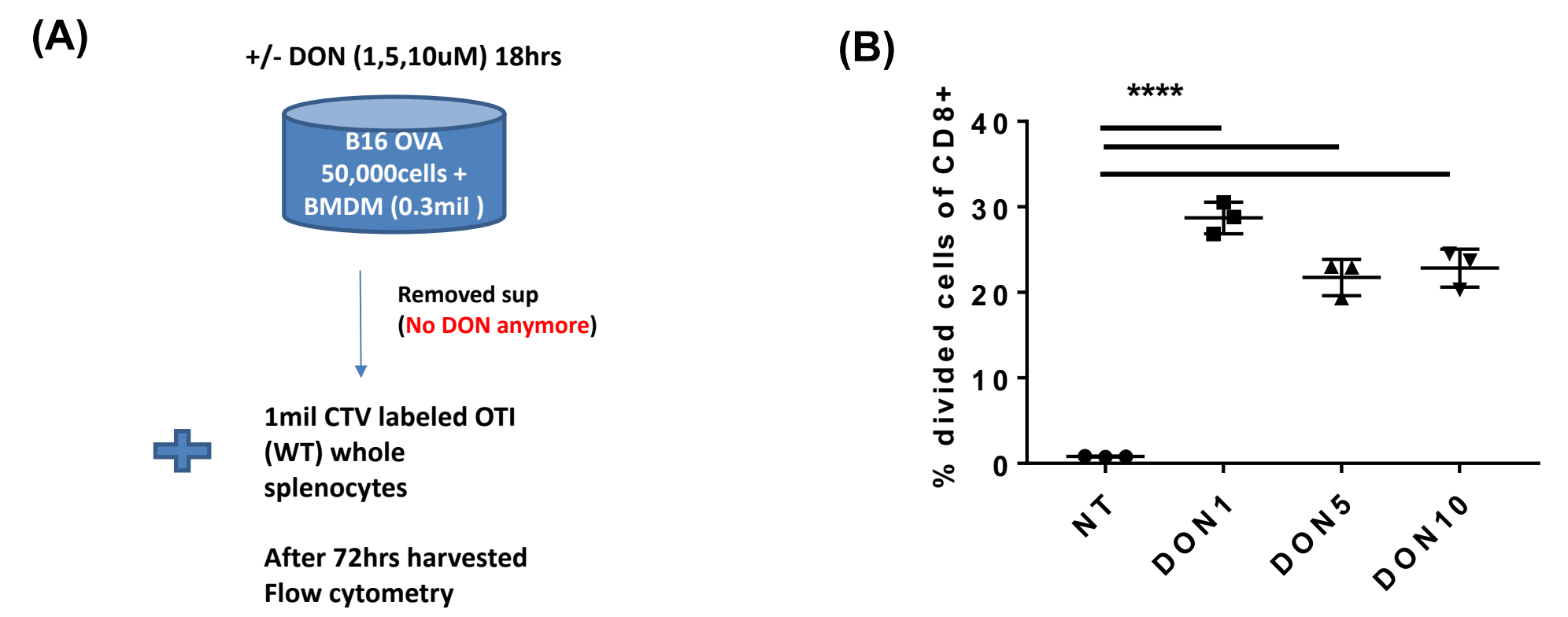


Fig 6. (A) Scheme of T cell priming experiment (B) Increased tumor antigen specific T cell priming

Glutamine inhibition enhances immunotherapy

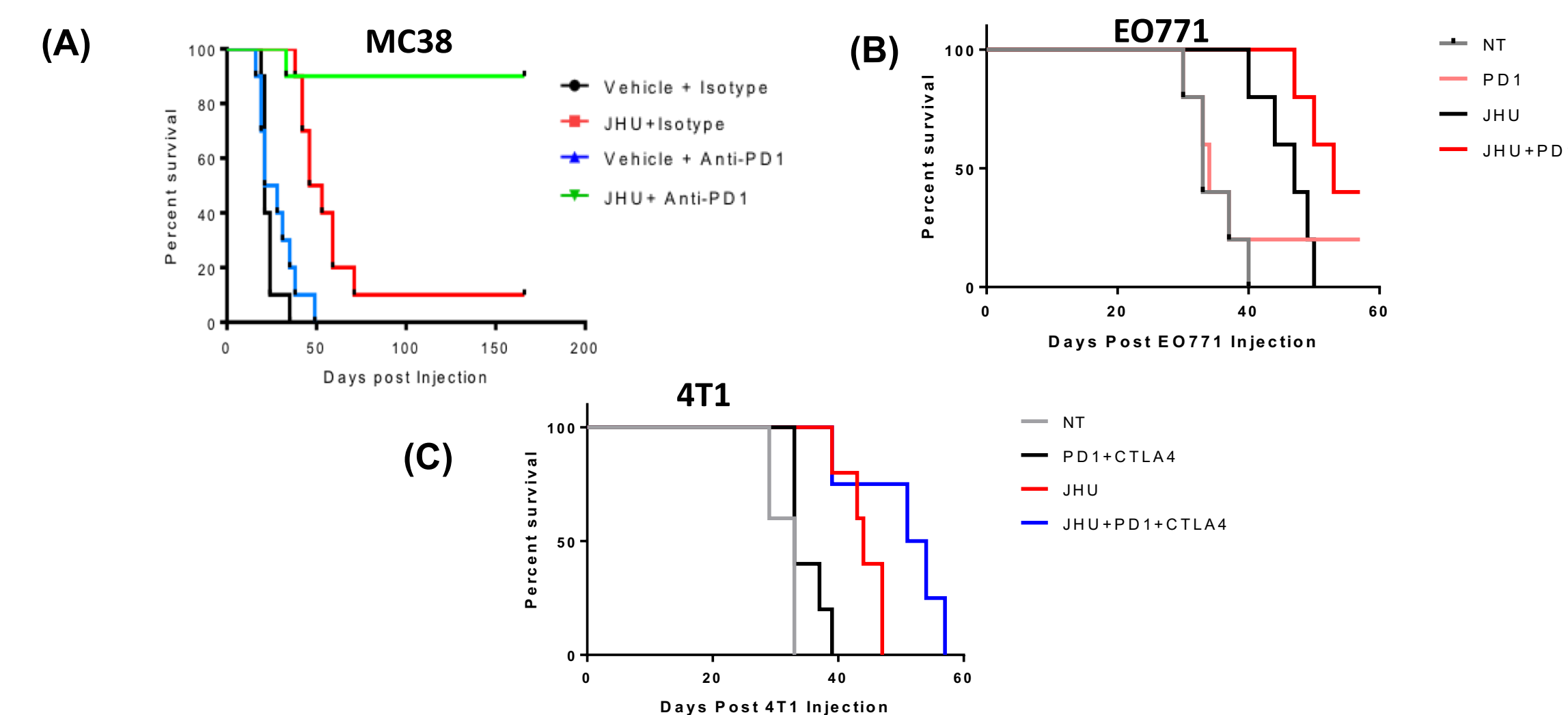
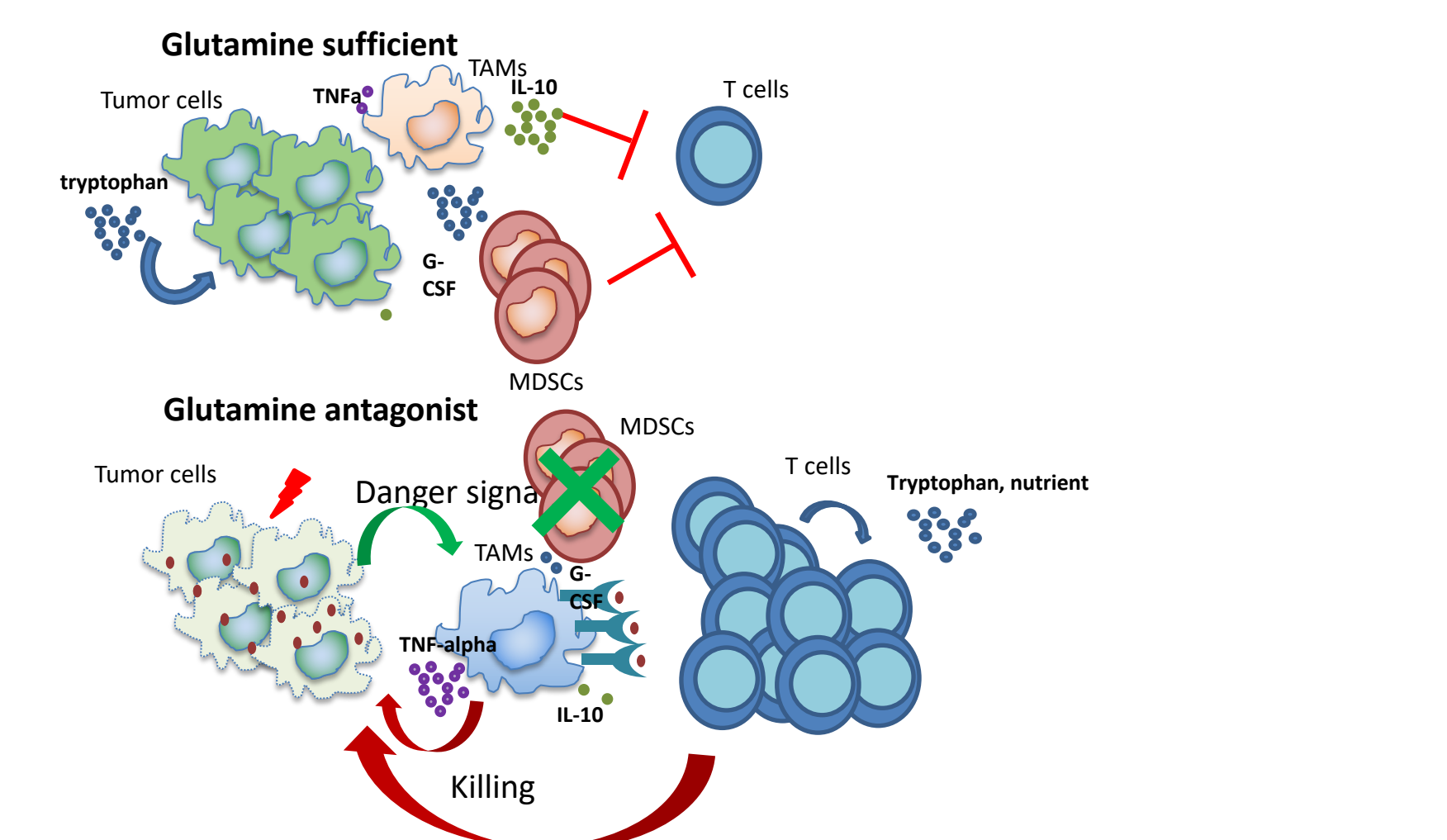


Fig 7. (A) glutamine inhibition enhances (A) aPD1 therapy in MC38 tumor model, susceptible tumors to immune checkpoint blockade (B) aPD1 therapy in EO771 tumor model, mildly susceptible to immune checkpoint blockade (C) aPD1 and aCTLA4 therapy in 4T1 tumor model resistant to immune checkpoint blockade

Summary & Conclusion

Targeting glutamine metabolism using JHU-083 enhances immunotherapy for cancer



- Inhibition of glutamine metabolism leads to reduced MDSCs
- In part, this decrease is due to decreased CSF3 secretion from TAMs
- Inhibition of glutamine metabolism leads to markedly decreased kynurenine in both primary tumors and metastatic sites, correlating with reduced MDSCs in the lung
- Consequently, JHU-083 markedly inhibits lung metastasis
- Treatment with JHU-083 enhances inflammatory TAMs
- Treatment with JHU-083 leads to enhanced immunologic cell death resulting in enhanced tumor antigen presentation and activation
- Treatment with JHU-083 is able to enhance immunotherapy in immune checkpoint blockade resistant tumor