

# Antagonism of Peroxisome Proliferator Activated Receptor Alpha (PPAR $\alpha$ ) by TPST-1120 Suppresses Tumor Growth and Stimulates Anti-Tumor Immunity

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## ABSTRACT

### Background

TPST-1120 is a first-in-class selective antagonist of human PPAR $\alpha$ , a transcription factor that induces expression of fatty acid oxidation (FAO) genes. Tumor metabolic adaptations promote its own survival and suppress tumor-specific immunity by upregulation of FAO. PPAR $\alpha$  blockade has tumor intrinsic and extrinsic effects, and inhibits tumor cell growth and induces tumor-specific immunity, demonstrated among multiple syngeneic and xenograft mouse models.

### Methods

TPST-1120 efficacy as a monotherapy or in combination with chemotherapy or anti-PD1 was evaluated in multiple syngeneic mouse models including B16 melanoma, MMTV mammary carcinoma, MC38 colon, Lewis lung carcinoma, ID8 ovarian, Panc07 pancreatic cancer, and xenograft models of CLL, melanoma, pancreatic and AML. To characterize its mechanism of anti-tumor immunity, TPST-1120 was evaluated in knock-out models of CCL2, MBL, TSP-1, STING and BatF3. Immune modulation was characterized by M2/M1 macrophage flow cytometry phenotyping and ELISA measurement of plasma and tumor matrix protein thrombospondin-1 (TSP-1), which is involved in granulocyte migration and angiogenesis.

### Results

TPST-1120 mediated PPAR $\alpha$  antagonism resulted in potent anti-tumor immune responses and significant tumor regression, either as a monotherapy or in combination with chemotherapy or anti-PD1. TPST-1120 showed anti-tumor efficacy against syngeneic models of breast, lung, colon, pancreatic and melanoma in addition to xenograft models of CLL, AML, pancreatic and melanoma cancers as a monotherapy or in combination with chemotherapy and anti-PD1. TPST-1120 demonstrated cytotoxic effect on tumor cells *in vitro*. In a pancreatic and breast cancer model, TPST-1120 combination with chemotherapies gemcitabine and eribulin, respectively, had additive effect on tumor growth. TPST-1120 combination with anti-PD1 in an ovarian orthotopic (ID8) and colon (MC38) models showed suppression of tumor growth and complete remission in some mice. Moreover, mice receiving the combination treatment conferred protection against autologous tumor re-challenge in the ID8 model, strongly suggesting immunological T cell memory against the primary tumor. Preliminary studies in genetic knock-out mice, suggest macrophages and antigen cross-presenting dendritic cells are required for TPST-1120 activity, potentially through STING activation and TSP-1. Consistent with prior reports of the involvement of PPAR $\alpha$  activation in promoting M2 macrophages, TPST-1120 skews toward an M1 effector macrophage phenotype and *in vivo* pretreated peritoneal macrophages enhance the uptake of whole tumor cells by FACS.

### Conclusions

Through its unique mechanism of restricting FAO, TPST-1120 targets a metabolic pathway that is critical for the survival of both tumor cells and of suppressive immune cell populations infiltrating the tumor microenvironment. TPST-1120 represents a promising new approach for evaluation in patients with advanced malignancies.

## INTRODUCTION

- TPST-1120 is a first-in-class, orally administered, small molecule selective antagonist of the human peroxisome proliferator-activated receptor alpha (PPAR $\alpha$ )
- PPAR $\alpha$  is a transcription factor which induces the expression of genes that regulate fatty acid oxidation (FAO) and inflammation (Figure 2)
- PPAR $\alpha$  and FAO gene signatures can be enriched in metastatic tumors<sup>4,5</sup> (Figure 1)
- FAO supports the metabolism of immune suppressive cell populations that inhibit anti-tumor immunity<sup>1,3</sup>
- The growth and progression of diverse tumor types are completely suppressed in PPAR $\alpha$ -deficient mice<sup>6,7</sup>
- TPST-1120 has significant anti-tumor activity as a monotherapy and in combination with chemotherapy or anti-PD1 antibodies
- Anti-tumor activity of TPST-1120 is mediated through: 1) direct tumor cell killing; 2) release of immune suppression; and 3) restoration of TSP-1 to homeostatic levels

### FIGURE 1: Upregulation of PPAR $\alpha$ and FAO Genes in Diverse Tumors

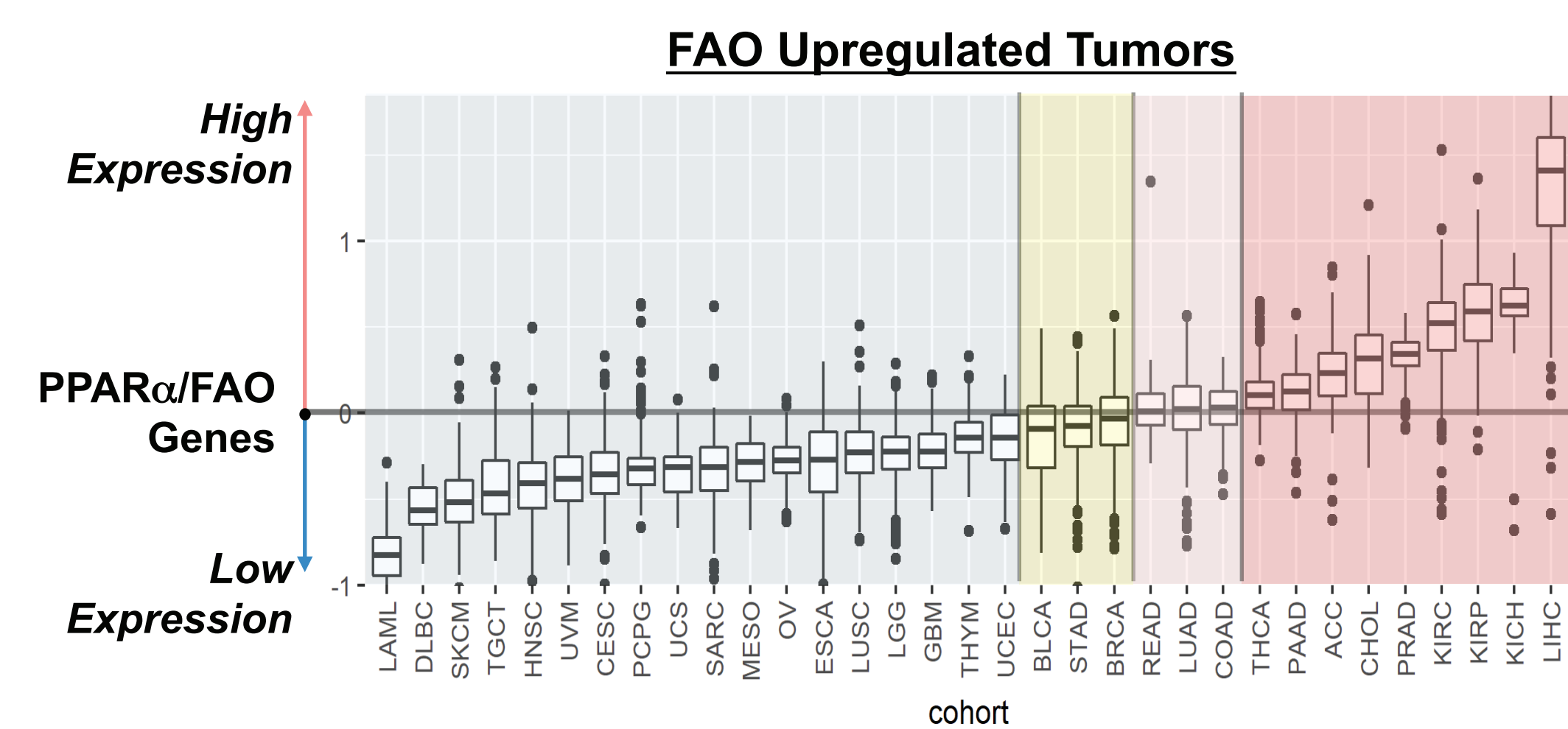


Figure 1: Enrichment of FAO associated genes including PPARA was evaluated in 10,000 unique datasets representing 33 tumor types in the TCGA (cancergenome.nih.gov).

## METHODS

- Efficacy as a monotherapy or in combination with chemotherapy or anti-PD1 was evaluated in multiple syngeneic mouse models
- TPST-1120 was evaluated in knock-out models of *Tsp-1*, *Sting* and *Batf3* to characterize its mechanism of anti-tumor immunity
- ELISA was used to measure plasma and tumor matrix protein Tsp-1 and Fgf21
- Gene expression analysis was performed by quantitative RT-PCR

## RESULTS

### TPST-1120: PPAR $\alpha$ Target Validation and Target Engagement

#### FIGURE 2: PPAR $\alpha$ Induces Fatty Acid Oxidation (FAO)

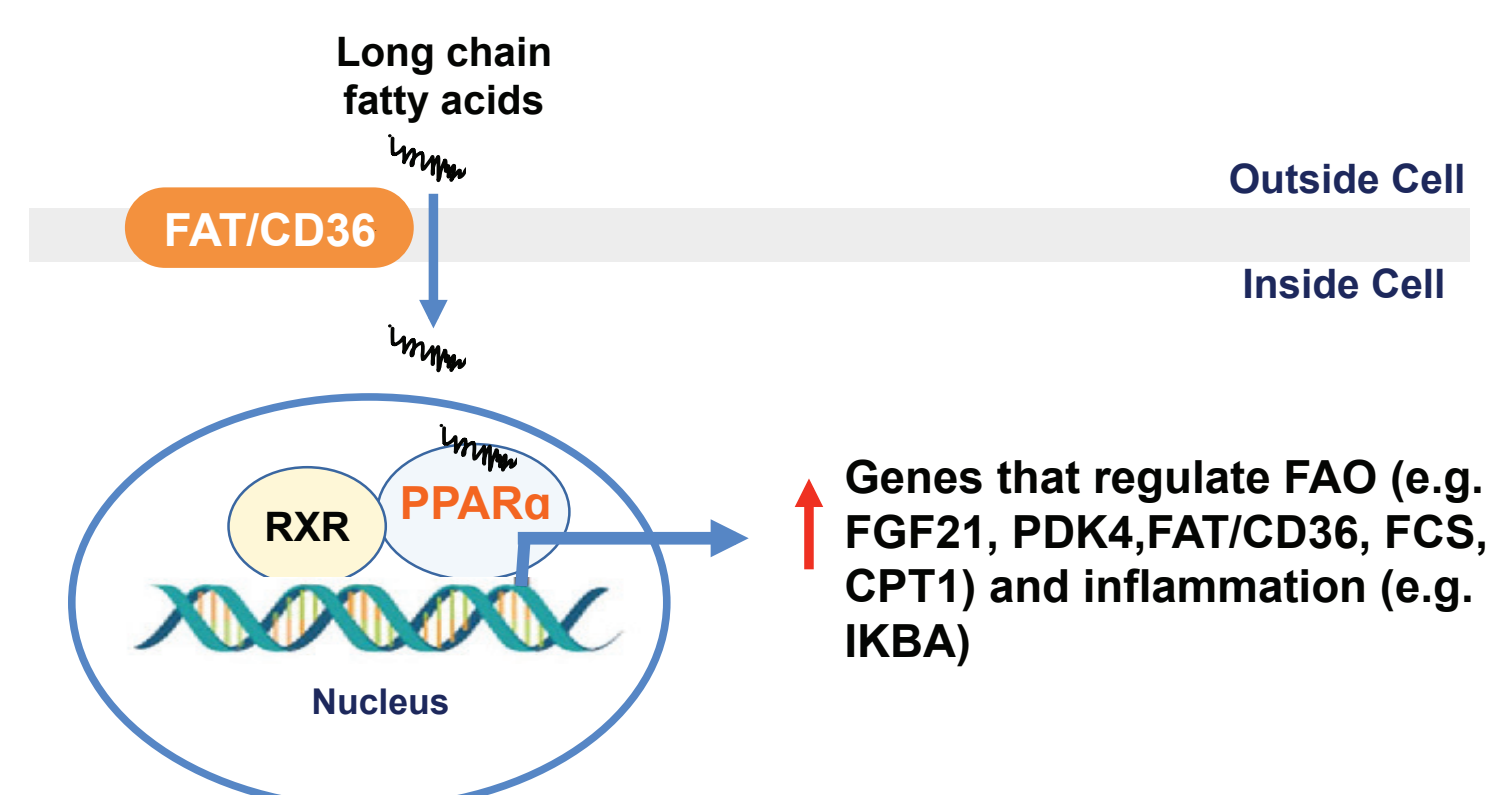
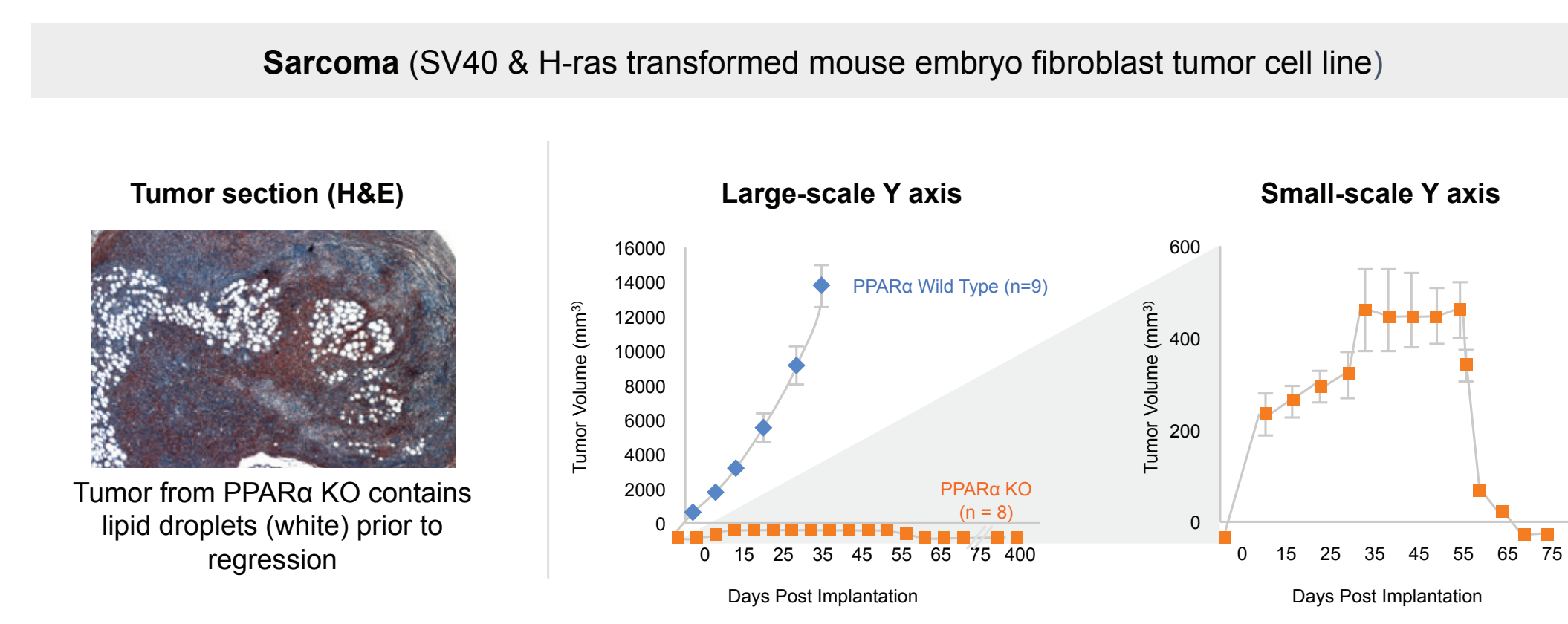


Figure 2: PPAR $\alpha$  regulates genes that modulate FAO and inflammation. Upon ligand binding, PPAR $\alpha$  heterodimerizes with RXR, stimulates gene transcription of target genes that regulate FAO and negative modulators of inflammation.

#### FIGURE 3: Tumors Spontaneously Regress in PPAR $\alpha$ Knock-Out Mice



Source: Kaipainen et al., PLoS ONE 2007, 2(2): e260 and unpublished results  
Figure 3: Tumor growth is inhibited in PPAR $\alpha$  knock-out (KO) mice. Tumors establish but spontaneously regress in KO mice.

#### FIGURE 4: PPAR $\alpha$ Antagonist TPST-1120 Inhibits PPAR $\alpha$ -Dependent Targets

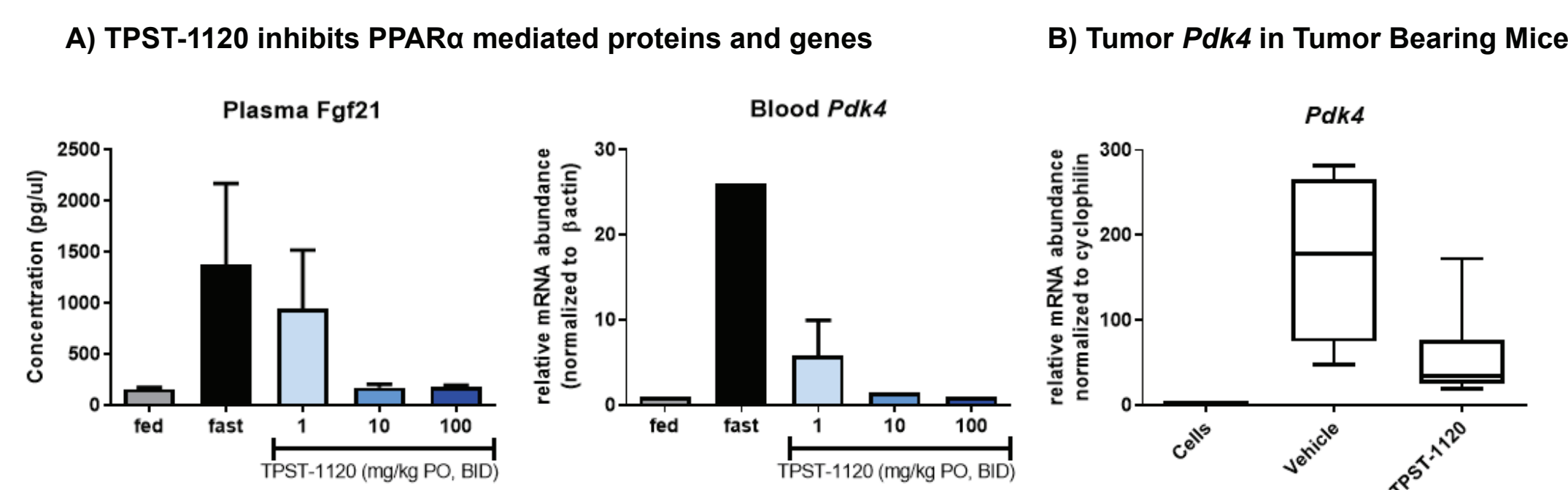


Figure 4: A) PPAR $\alpha$  induced Fgf21 protein and target gene *Pdk4* increases under fasting is inhibited by TPST-1120 at 1, 10, and 100 mg/kg PO BID in C57BL/6N mice (non-tumor bearing). B) *Pdk4* gene expression is inhibited in B16-F10 melanoma tumors in mice treated with TPST-1120 30 mg/kg PO BID compared to vehicle treated.

#### FIGURE 5: TPST-1120 Inhibits Proliferation of Primary CLL Tumor Cells

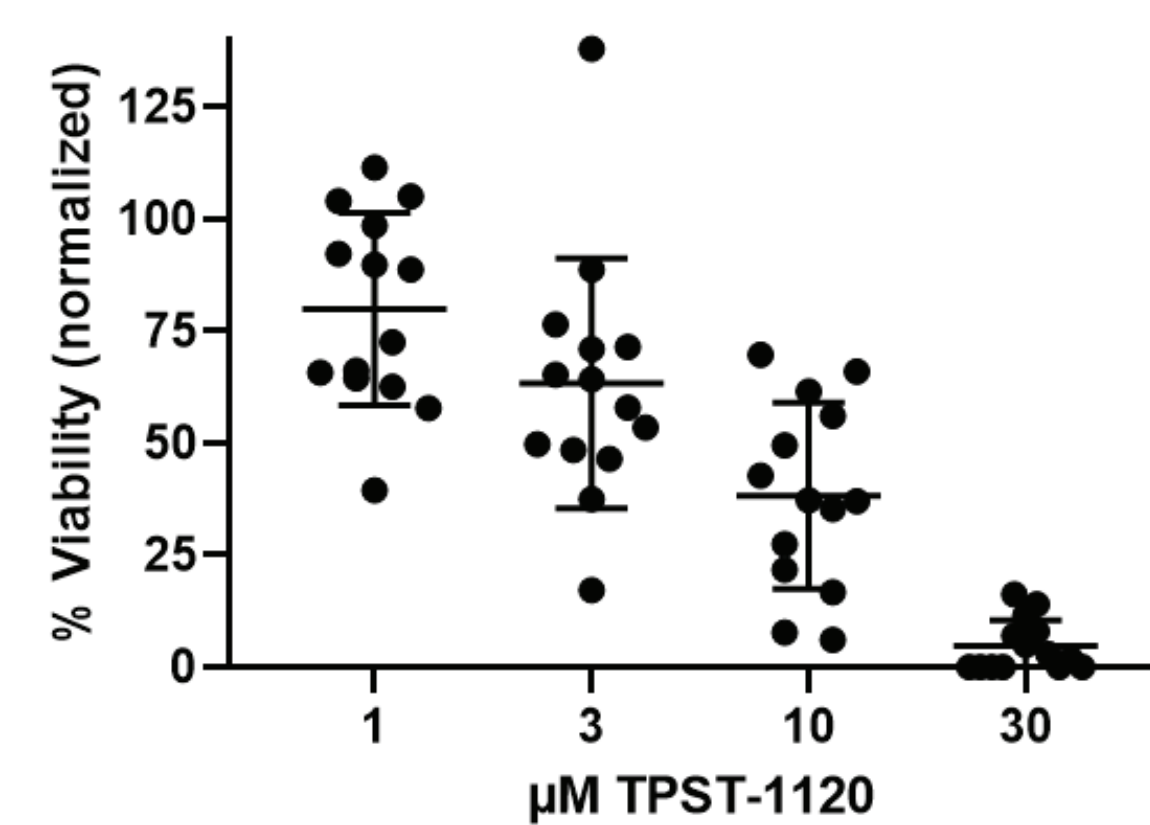


Figure 5: TPST-1120 directly inhibits proliferation of primary CLL tumor cells in a dose dependent manner after 48 hours of incubation. Cells were obtained from consented patients (n=14).

#### FIGURE 6: TPST-110 Inhibits the Outgrowth of Syngeneic Tumors

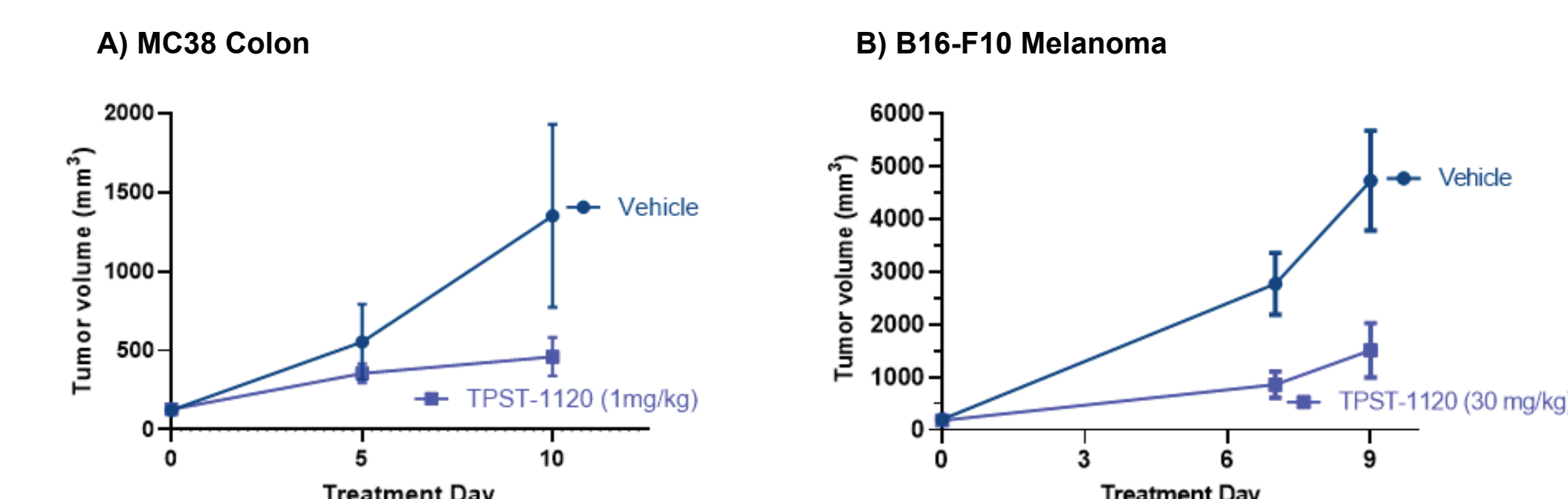


Figure 6: Tumor growth is suppressed compared to vehicle control by TPST-1120 in A) MC38 colon model at 1 mg/kg, and B) in B16F10 melanoma at 30 mg/kg BID PO.

#### FIGURE 7: TPST-1120 Significantly Enhances Benefit of Chemotherapy

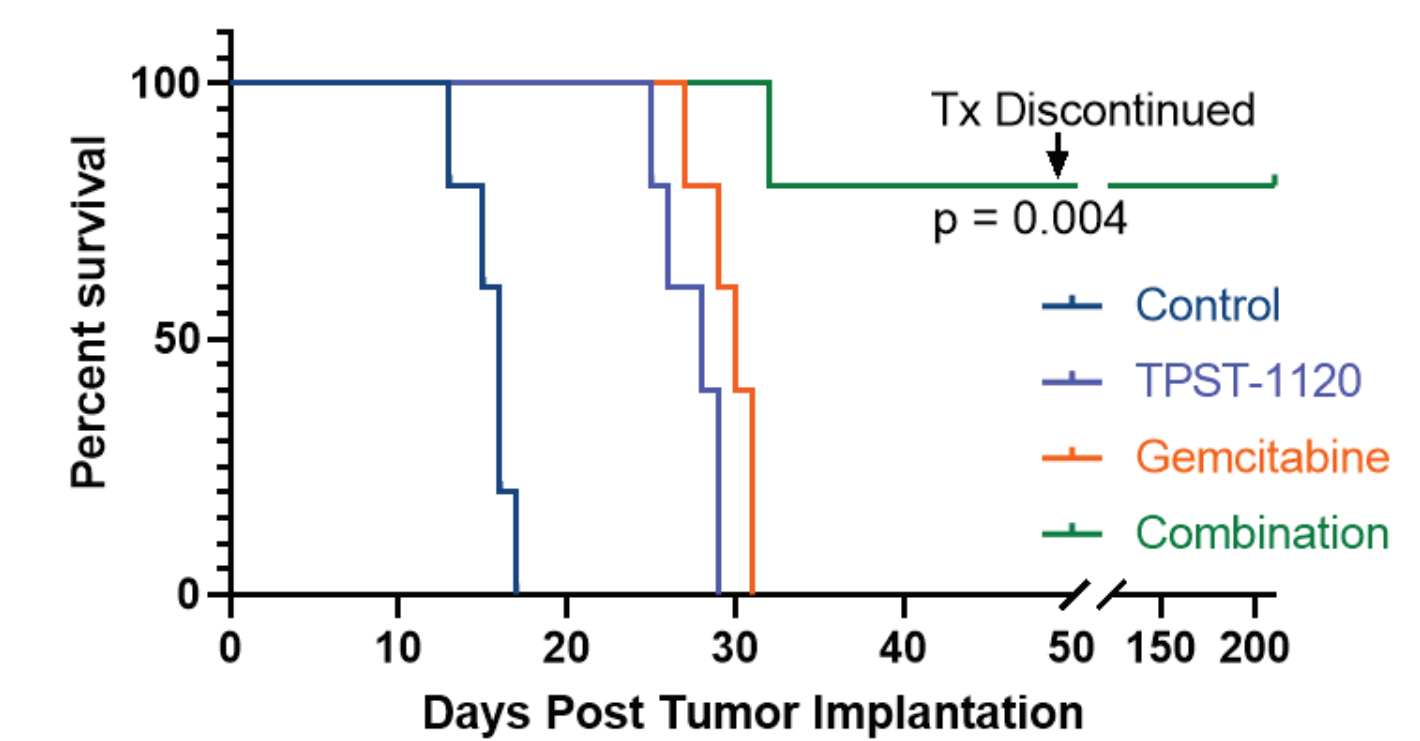


Figure 7: TPST-1120 (30 mg/kg PO BID) response against Panc07 tumors was improved by combination with gemcitabine 50 mg/kg IP q4d (N=5/group) in Panc07. 4/5 mice in the combo group were alive at day 210 (treatment discontinued at day 48).

#### The anti-tumor effects of PPAR $\alpha$ antagonism is dependent upon immune modulation

#### FIGURE 8: TPST-1120 Anti-Tumor Activity Requires Innate and Adaptive Immunity

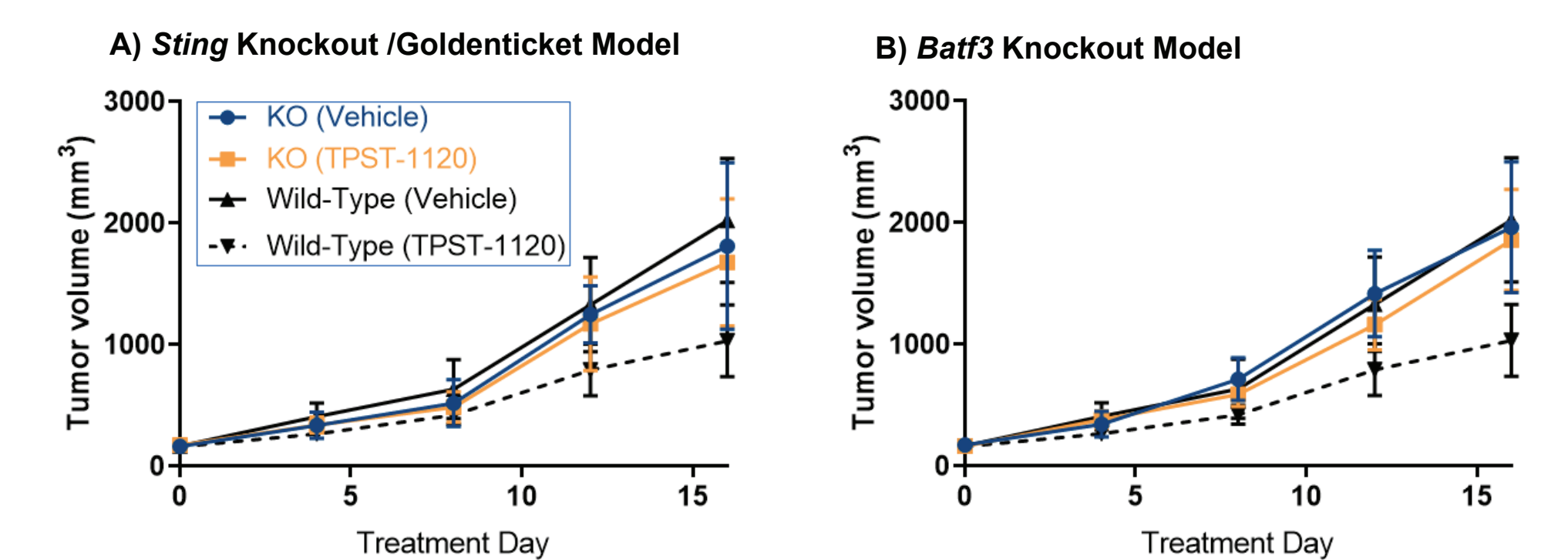


Figure 8: A) TPST-1120 at 30 mg/kg PO BID had no effect in *Sting* or *Batf3* knockout mice (n=10) compared to wild type control (n=5).

#### FIGURE 9: TPST-1120 Anti-Tumor Efficacy is Dependent on TSP-1

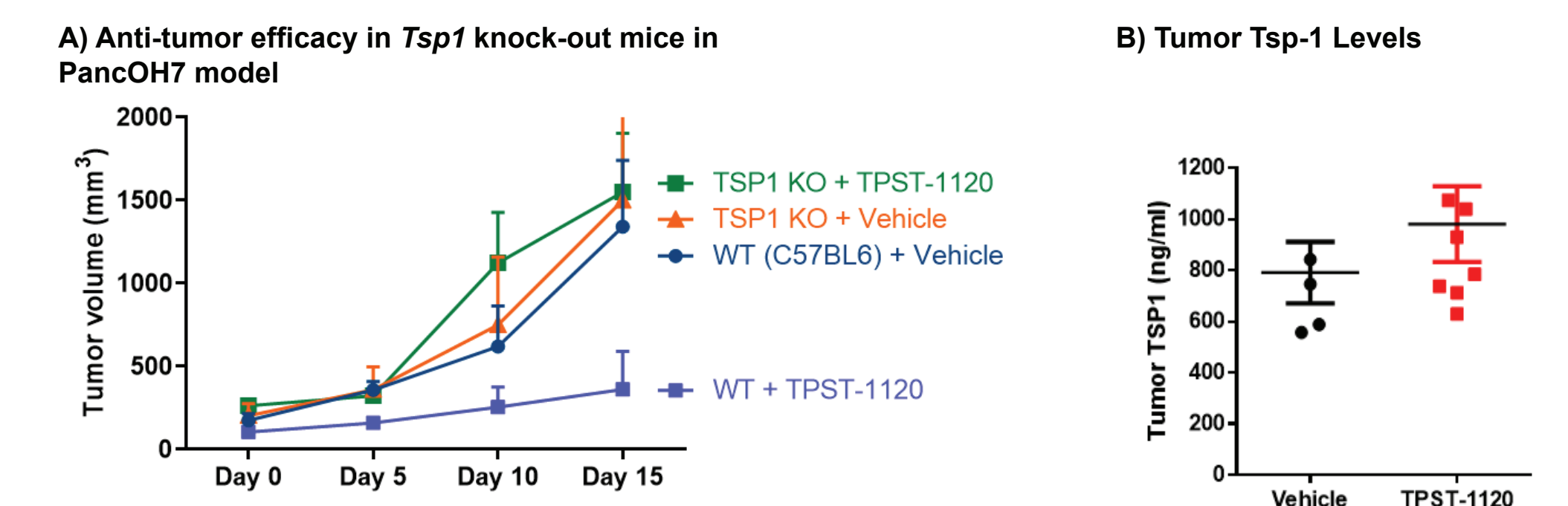


Figure 9: A) TPST-1120 has potent anti-tumor monotherapy activity in WT mice but efficacy was lost when Panc07 tumors were implanted in Tsp-1 knock-out mice. B) Anti-tumor efficacy is associated with increased Tsp-1 levels in the TME

#### FIGURE 10: TPST-1120 Combination with Anti-PD1 Induces Protective Immunity

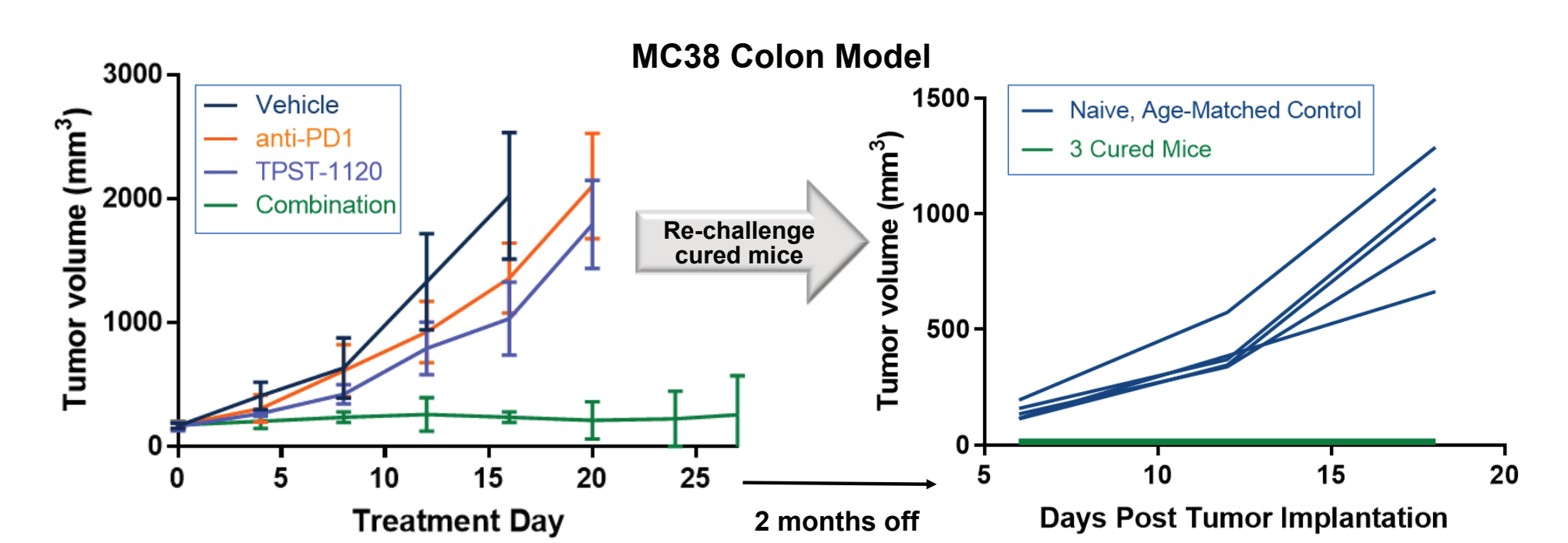


Figure 10: Established MC38 tumors (100-150mm<sup>3</sup>) treated with vehicle (n=10), TPST-1120 (n=7), anti-PD1 alone (n=10), or TPST-1120 + anti-PD1 (n=15) were monitored for tumor growth. By day 24 only animals in the combo group remained alive. Three were tumor free at day 60 post removal of therapy and were tumor-free upon autologous tumor re-challenge while aggressive tumor growth occurred in naive mice.

## TPST-1120 CONCLUSIONS

- A first-in-class PPAR $\alpha$  antagonist provides anti-tumor efficacy as a monotherapy or in combination with chemotherapy or anti-PD1
- Directly inhibits tumor proliferation
- Requires both innate and adaptive immunity for anti-tumor efficacy
- Anti-tumor efficacy is TSP-1 dependent
- A Phase 1/1b open-label, dose-escalation and dose-expansion study of TPST-1120 as a single agent or in combination with systemic anti-cancer therapies in subjects with advanced solid tumors is planned to initiate in early 2019

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