

# **Harnessing natural IL-18 activity through IL-18BP blockade reshapes the tumor microenvironment for potent anti-tumor immune response**

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## **Background**

IL-18 is an inflammasome-induced proinflammatory cytokine that activates T and NK cells and stimulates IFN $\gamma$  production [1,2]. The activity of IL-18 is naturally blocked by a high affinity endogenous binding-protein (IL-18BP) [1] induced in response to IFN $\gamma$  upregulation as a negative feedback mechanism [3].

## **Methods**

By assessing total and free IL-18 we examined whether bound-IL-18 levels in the tumor are above the level required for in-vitro human T-cell activation. To unleash endogenous bound IL-18 activity, COM503, an anti-IL-18BP blocker Ab, was generated and examined in T cell-based assays. In-vivo, IL-18BP blockade was evaluated in multiple mouse tumor models. Tumor microenvironment (TME) modulation was assessed by flow cytometry, scRNA sequencing and cytokine profiling.

## **Results**

IL-18 levels were clearly elevated across 75 evaluated tumors compared to serum samples (Figure 1A-B). Results show that most of tumor IL-18 was bound to IL-18BP, and its levels were above the amount required for T-cell activation in-vitro, implying that releasing tumor IL-18 locally could lead to T cell activation (Figure 1C-D). By displacing IL-18 from IL-18:IL-18BP complex, COM503 was shown to enhance T-cell activation in an ex-vivo stimulated human CD8<sup>+</sup> tumor infiltrating lymphocytes-tumor cells co-culture assay and in human dissociated tumor cells assay (Figure 2). In addition, tumor growth inhibition was observed by anti-mouse IL-18BP Ab in multiple tumor models either alone or in combination with anti-PD-L1 (Figure 3). Furthermore, in E0771 model, IL-18BP blockade induced significant increase in functional immune-cells and striking changes in clusters of lymphocytes, including a decrease in naïve T-cells and an increase in effector T-cells and T cell clonal expansion (Figure 4). IL-18BP blockade also increased pro-inflammatory cytokine secretion and skewed cell populations of myeloid lineage to favor proinflammatory macrophages (Figure 5). In MC38OVA<sup>dim</sup> model both TME and periphery were evaluated. Anti-mouse IL-18BP Ab induced potent TME immune-modulation, including increased CD8<sup>+</sup> T-cell infiltration and IFN $\gamma$  secretion, while no increase of IFN $\gamma$  secretion, lymphocytes number or activation state was evident in the periphery (Figure 6).

## Conclusions

IL-18 is upregulated in human tumors and is mostly bound by IL-18BP. COM503, a high-affinity anti-IL-18BP Ab, induces human T-cell responses in-vitro and ex-vivo. An anti-mouse IL-18BP Ab induces potent anti-tumor responses and pronounced TME-constrained immune modulation, this in contrast to systemically administered therapeutic cytokines, which can generate systemic inflammatory responses (Figure 7) [4]. Taken together, blocking IL-18BP is a promising novel approach to harness cytokine potency for the treatment of cancer. COM503 is currently undergoing IND-enabling studies.

## References

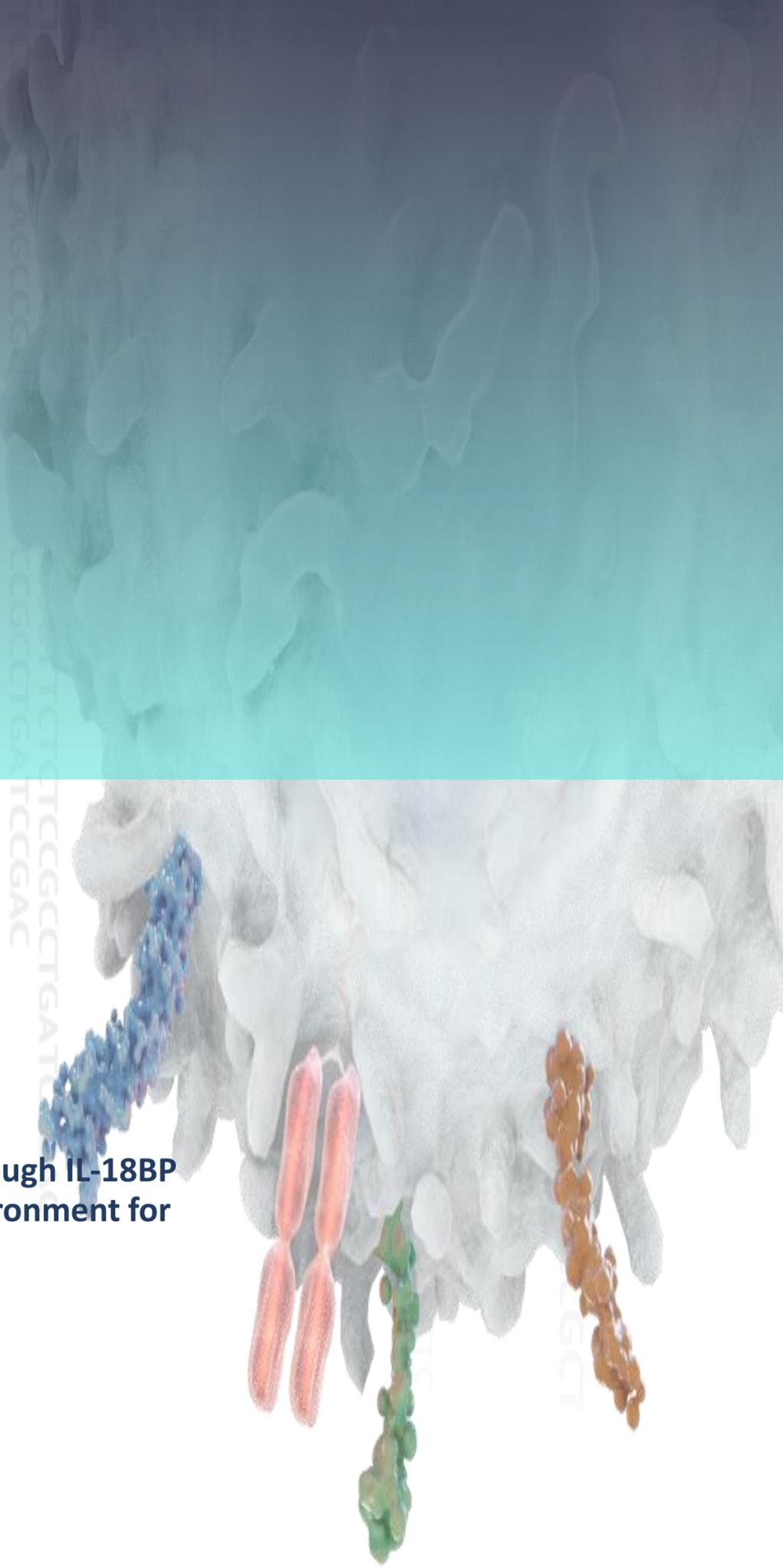
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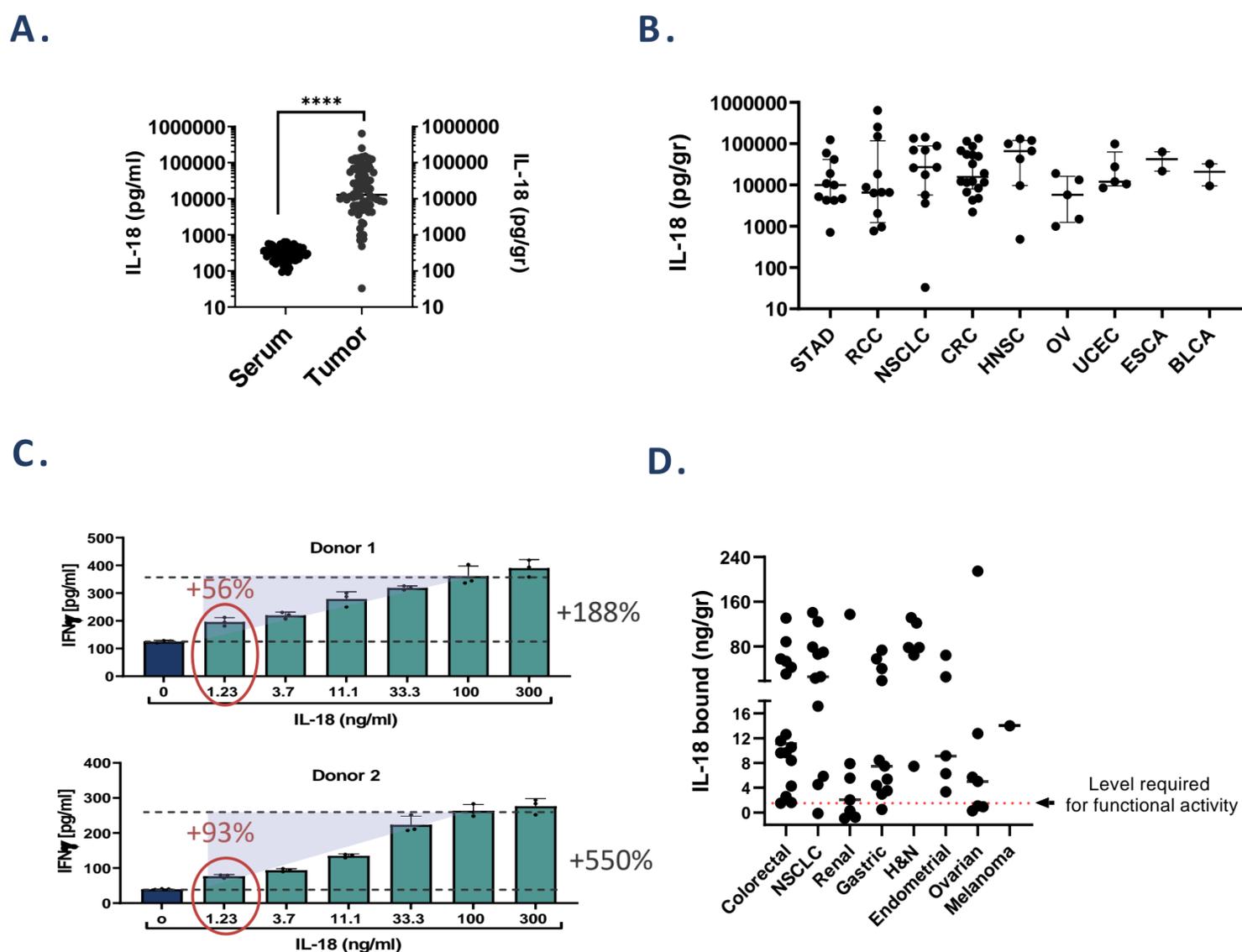


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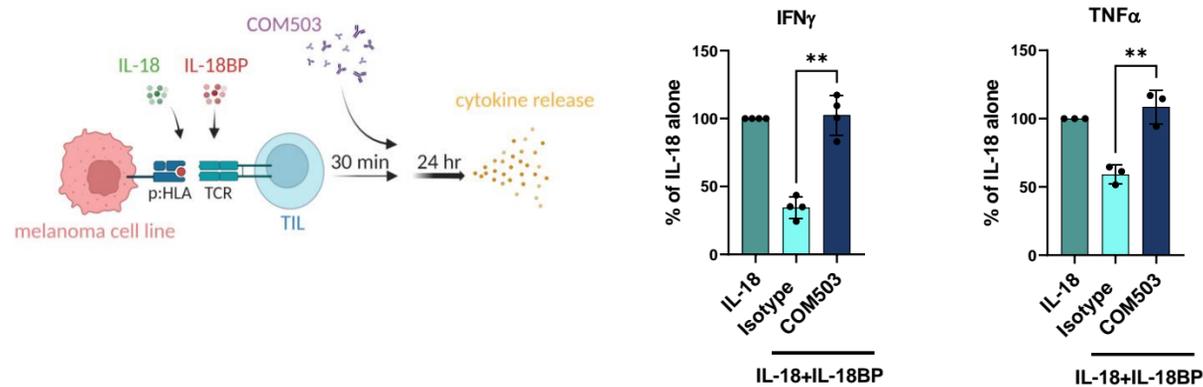
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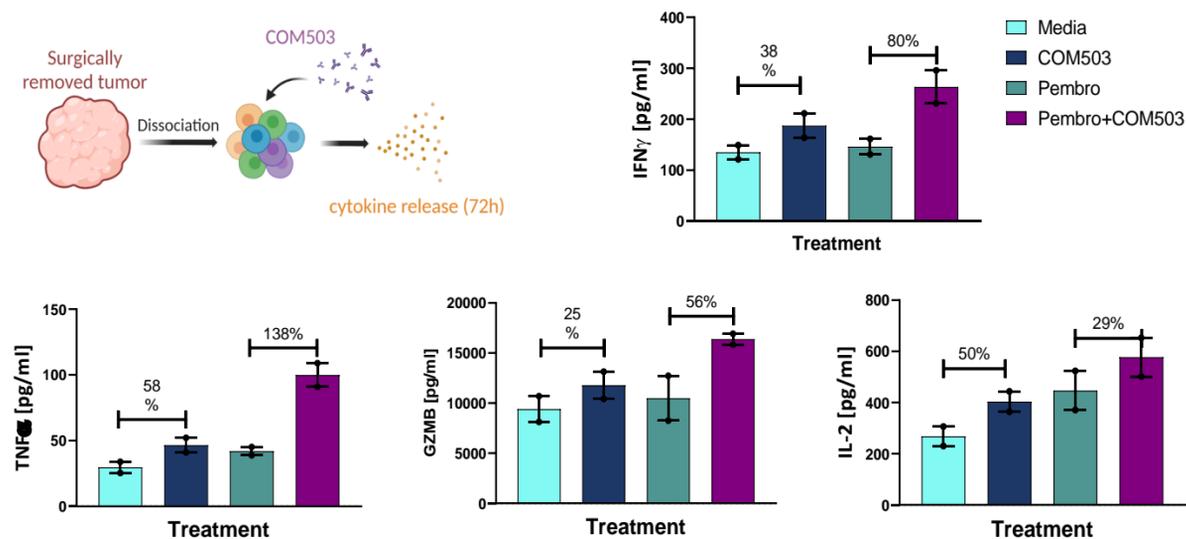


**Figure 1. IL-18 is upregulated in the TME and IL-18BP-bound IL-18 level is above the amount required for T cell activation in vitro.** **A.** IL-18 levels are significantly higher in the tumor compared to serum. Serum samples and tumor biopsies were collected from cancer patients. Tumor biopsies were dissociated, and supernatants were collected. IL-18 expression was analyzed in serum and tumor derived supernatants (TDS) using ELISA assay. **B.** IL-18 expression in TDS samples from individual patients across different indications. **C.** Recombinant (r) IL-18 increased IFN $\gamma$  release by stimulated CD8 $^{+}$  tumor infiltrated lymphocytes (TILs) in TILs-tumor cells co-culture assay in a dose-dependent manner. MEL624 cells and TILs were seeded and treated with rIL-18 (0-100ng/ml). Plates were incubated for 24 hours, after which the supernatant was collected for cytokine secretion evaluation. **D.** Levels of bound IL-18 in TDS across indications are above the level required for in vitro T cell activation. Tumor biopsies were dissociated, supernatants were collected and analyzed using free and total IL-18 ELISA assays. IL-18BP bound IL-18 levels were calculated by deducting free IL-18 from total IL-18 measured for each sample by two separate ELISA kits. Dashed red line represent the level required for functional activity (1.5ng/gr).

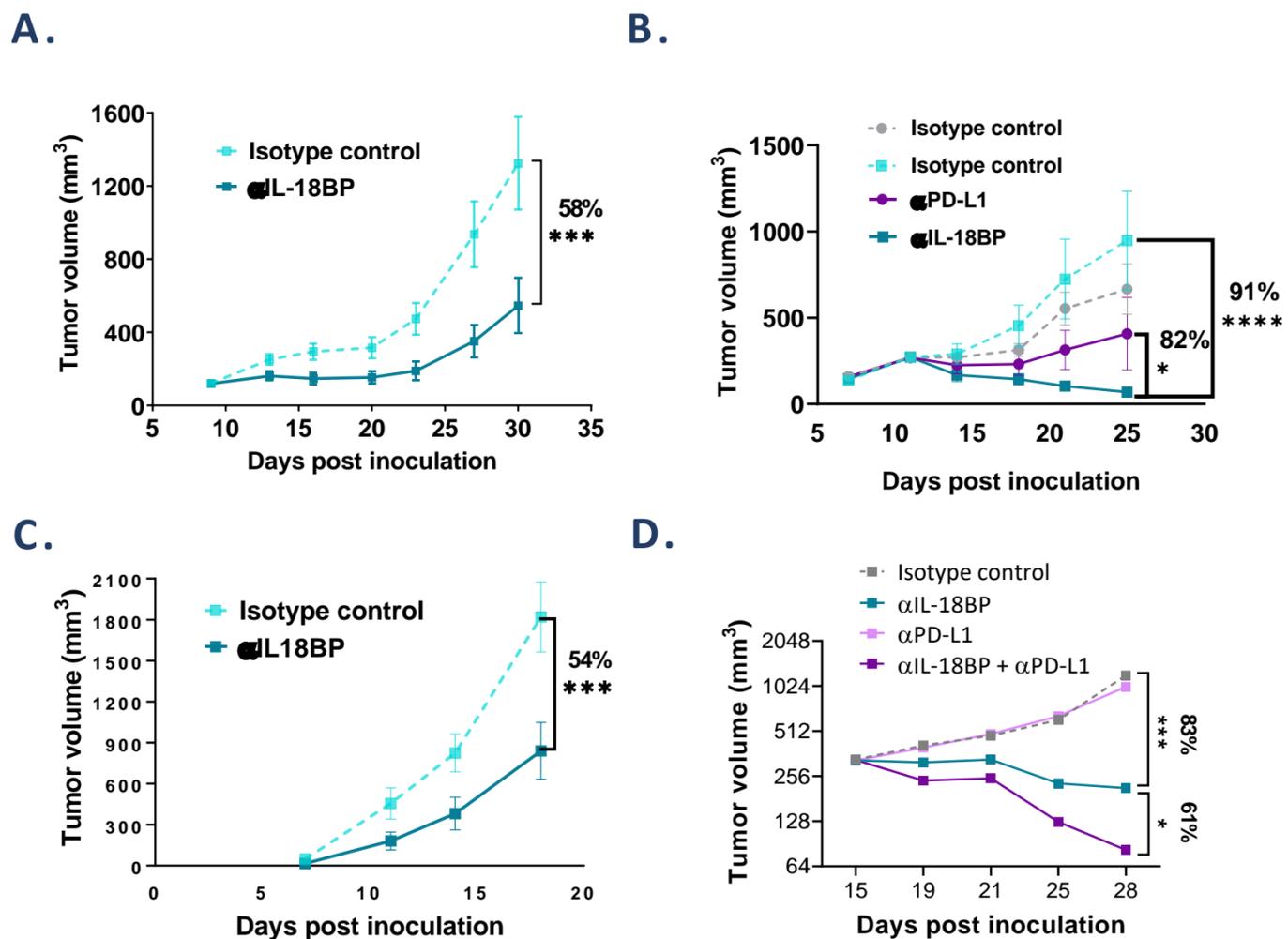
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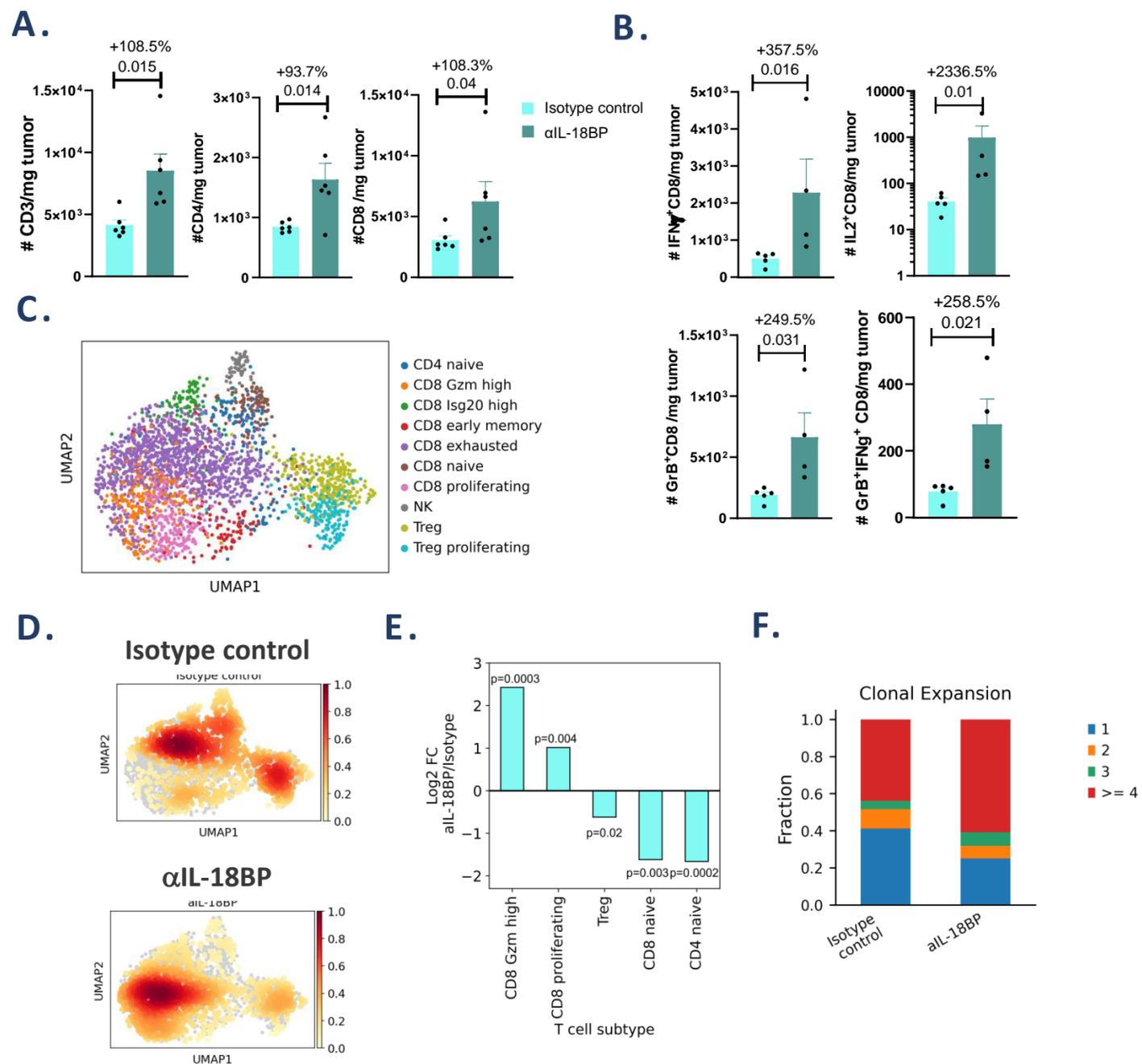
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**Figure 2. COM503, high affinity Ab against IL-18BP, released IL-18 to enhance T cell activity in vitro.** A. COM503 (10ug/ml) displaced IL-18 from a preformed IL-18:IL-18BP complex to increase IFN $\gamma$  and TNF $\alpha$  release from stimulated human CD8+TILs (N=3-4) in TILs-tumor cells co-culture assay in the presence of rIL-18BP and rIL-18 B. COM503 increased IFN $\gamma$ , TNF $\alpha$ , GZMB, and IL-2 release by human tumor dissociated cells. Resected cancer specimens dissociated to single-cell suspension were cultured with anti-CD3/anti-CD28 mAbs for T-cell stimulation and treated with COM503, anti-PD1 Ab (pembrolizumab) or with combination of COM503+Pembrolizumab (10 ug/ml). After 3 days, cytokines and Granzyme secretion were measured in supernatants. Representative example from a human ovarian TDC sample is shown.

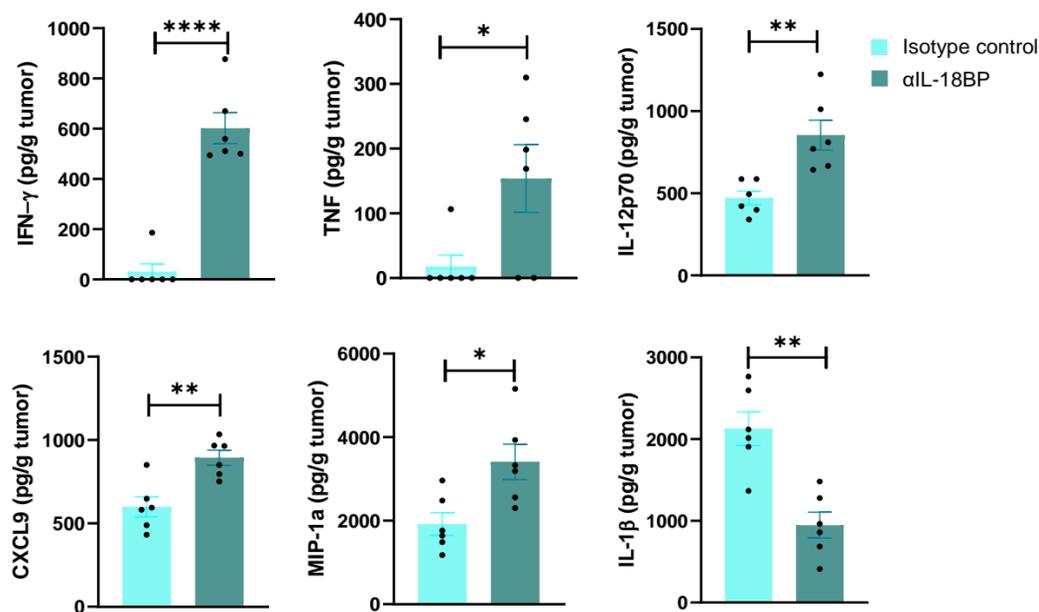


**Figure 3. Anti-mouse IL-18BP Ab inhibited tumor growth across murine syngeneic tumor models as a single agent and in combination with anti-PD-L1. A-C.** anti-mouse IL-18BP Ab (15mg/kg) inhibited tumor growth as a single agent in MC38OVA<sup>dim</sup> (Treatment initiated in 130-260 mm<sup>3</sup> tumor volume) (A), E0771 (Treatment initiated in 250-270 mm<sup>3</sup> tumor volume) (B), and B16F10-hmgp100 (Treatment initiated on day 4 post tumor inoculation) (C) mouse tumor models compared to isotype control. Tumors were inoculated in C57Bl/6 mice; mice (N=10) were treated twice a week for total of 6 treatments. **D.** Anti-mouse IL-18BP (15mg/kg) synergized with anti PD-L1 Ab (5mg/kg) to inhibit tumor growth in E0771 tumor model. Treatment initiated in established tumor (330mm<sup>3</sup>, N=10) and was given twice a week for a total of 6 treatments. Tumor volumes are represented as the Mean volume + SEM.

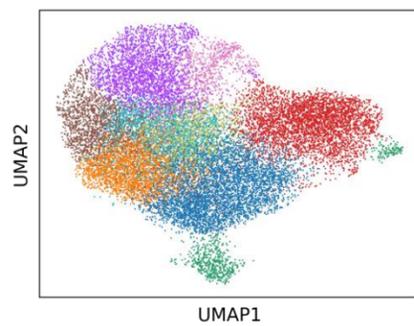


**Figure 4. IL-18BP blockade increased T cell effector state and clonal expansion in E0771 mouse tumor model.** C57BL/6 mice were orthotopically inoculated with E0771 cells and treated with anti-IL-18BP Ab or isotype control (15mg/kg) at tumor volume of 330mm<sup>3</sup> twice a week. Tumors were collected 24hr post the third treatment and dissociated. Immune modulation was assessed by flow and scRNA sequencing. **A.** Anti IL-18BP Ab increased CD3<sup>+</sup>, CD8<sup>+</sup> and CD4<sup>+</sup> T cells infiltration into the tumor. **B.** Anti IL-18BP Ab increased T cell polyfunctionality as evident by increase in IFN $\gamma$ <sup>+</sup>, IL-2<sup>+</sup>, GrB<sup>+</sup> and GrB<sup>+</sup>IFN $\gamma$ <sup>+</sup> CD8<sup>+</sup> T cells **C.** UMAP projection showing T and NK cells present in E0771 tumors treated with anti-IL-18BP or isotype control. **D.** Visualization of the average cell density within the anti-IL-18BP (bottom) and Isotype control (top) group, using embedding density estimation on T/NK UMAP. Darker colors correspond to denser regions. **E.** Log<sub>2</sub> fold change of T cell subpopulations comparing anti-IL-18BP Ab treatment to the control group. Only populations with significant changes are depicted. **F.** Quantification of clonal expansion frequencies in anti-IL-18BP Ab treatment compared to the control group.

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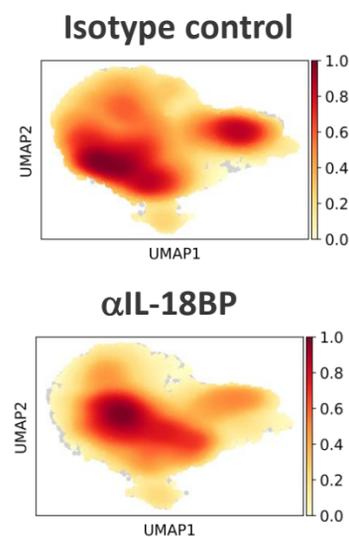


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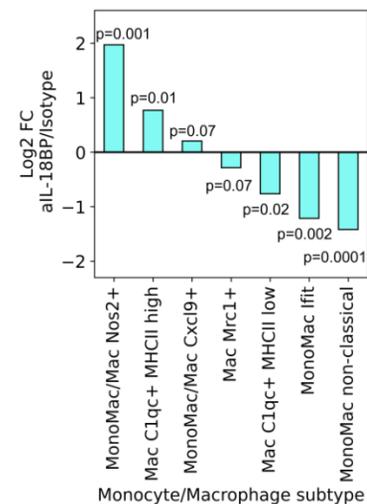


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- Mac C1qc+ MHCII low
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- MonoMac
- MonoMac lfit
- MonoMac non-classical
- MonoMac/Mac Cxcl9+
- MonoMac/Mac Nos2+

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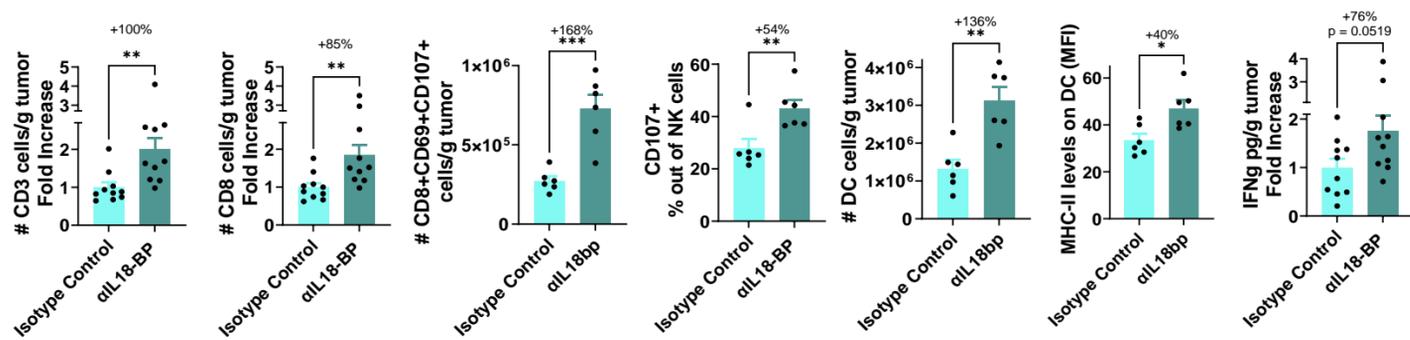


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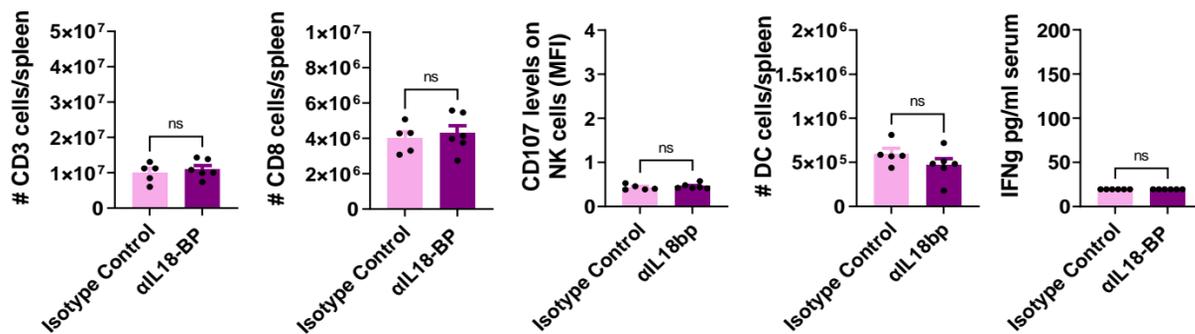


**Figure 5. IL-18BP blockade increased proinflammatory cytokine secretion and skewed myeloid cells to favor proinflammatory state in E0771 mouse tumor model.** C57BL/6 mice were orthotopically inoculated with E0771 cells and treated with anti-IL-18BP Ab or isotype control (15mg/kg) at tumor volume of 330mm<sup>3</sup> twice a week. Tumors were collected 24hr post the third treatment and dissociated. Immune modulation was assessed by cytokine profiling and scRNA sequencing. **A.** Anti IL-18BP Ab increased IFN $\gamma$ , TNF $\alpha$ , IL-12p70, CXCL9 and MIP-1 $\alpha$  secretion and decreased IL-1 $\beta$  secretion. **B.** UMAP projection showing tumor-associated monocyte and macrophage subpopulations present in E0771 tumors treated with anti-IL-18BP or isotype control. **C.** Visualization of the average cell density within the anti-IL-18BP (bottom) and Isotype control (top) group, using embedding density estimation on tumor-associated monocyte and macrophage UMAP. Darker colors correspond to denser regions. **D.** Log2 fold change of monocyte and macrophage subpopulations comparing anti-IL-18BP Ab treatment to the control group. Only populations with notable changes are depicted.

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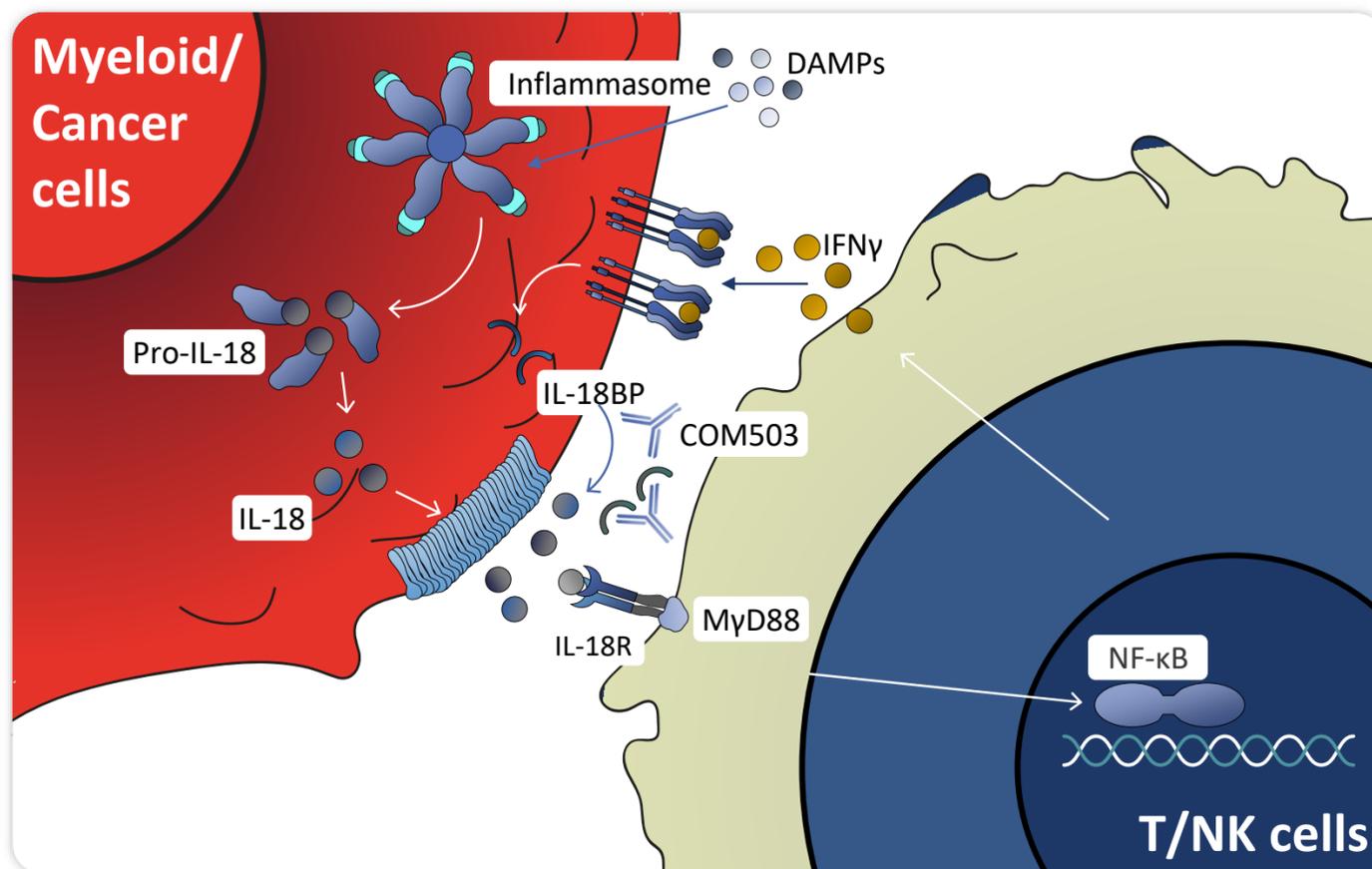


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**Figure 6. Anti-mouse IL-18BP Ab alters the immune infiltrate composition of MC38OVA<sup>dim</sup> tumors without affecting the periphery.** MC38OVA<sup>dim</sup> tumors were inoculated in C57Bl/6 mice. At tumor volume of 120 mm<sup>3</sup> mice were randomized and treated either with anti-IL-18BP Ab or with isotype control (15mg/kg) twice a week for a total of 4 treatments. Tumors and spleen were harvested 24 hours after the 4<sup>th</sup> treatment and immune composition was examined. Tumor supernatants and blood serum were collected at the same timepoint and analyzed for cytokine concentrations. **A-C.** Anti-mouse IL-18BP Ab affected immune composition and cytokine concentrations in the TME (**A**), but not in the spleen (**B**) and in the serum (**C**).



**Figure 7. COM503, a potential first-in-class anti-IL-18BP blocker antibody that unleashes endogenous IL-18 in the TME.** IL-18 is an effector cytokine that is upregulated in the TME and secreted upon inflammasome activation. IL-18BP is secreted via an IL-18 negative feedback mechanism, Binds IL-18, and blocks its immune stimulatory activity. COM503, high affinity IL-18BP blocker Ab has the potential to Induce potent anti-tumor responses and pronounced TME-constrained immune modulation