

# A novel biparatopic TIM-3 antibody induces superior antitumor effects through multi-ligand blockade

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## Introduction

TIM-3/galectin-9 signaling contributes to T cell exhaustion and apoptosis<sup>1</sup>, dendritic cell tolerance, as well as promoting leukemic stem cell (LSC) self-renewal through Wnt signaling, and  $\beta$ -catenin accumulation in acute myeloid leukemia (AML)<sup>2</sup>; highlighting the importance of TIM-3/galectin-9 signaling blockade as a therapeutic strategy.

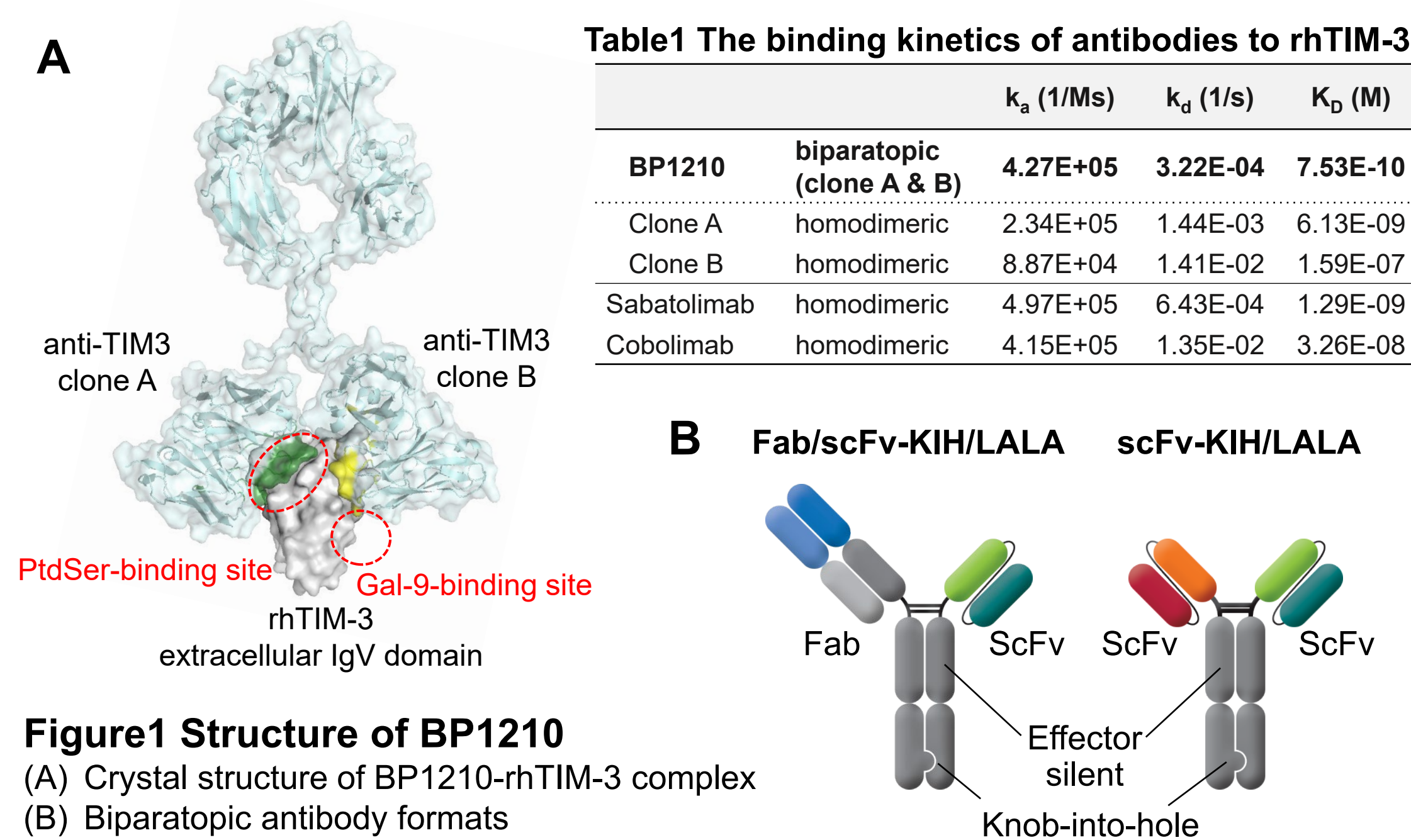
While several TIM-3 monoclonal antibodies are undergoing clinical trials for cancer treatment, these agents inhibit phosphatidylserine binding but not galectin-9 binding completely due to the location of the ligand binding sites on TIM-3.

We have developed a humanized biparatopic antibody, BP1210, capable of simultaneously blocking both phosphatidylserine and galectin-9 binding to TIM-3. This antibody potentially improves the efficacy of TIM-3 blockade.

<sup>1</sup>Nat Immunol. 2005 Dec;6(12):1245-52.

<sup>2</sup>Blood Adv. 2023 May 23;7(10):2053-2065.

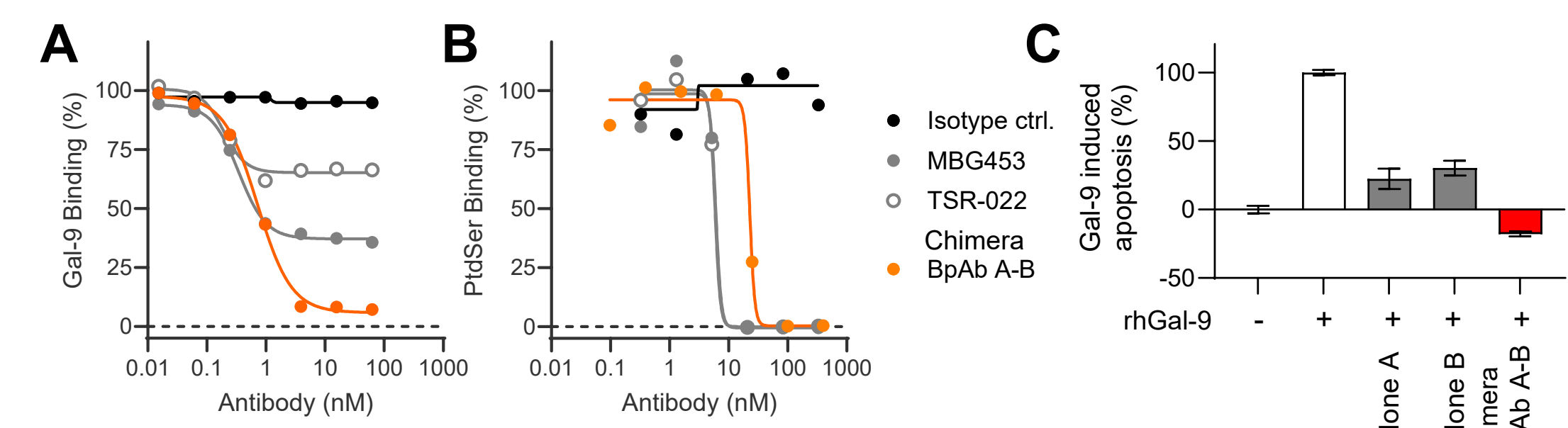
## Structure and binding affinity of BP1210



**Figure1 Structure of BP1210**

(A) Crystal structure of BP1210-rhTIM-3 complex (B) Biparatopic antibody formats

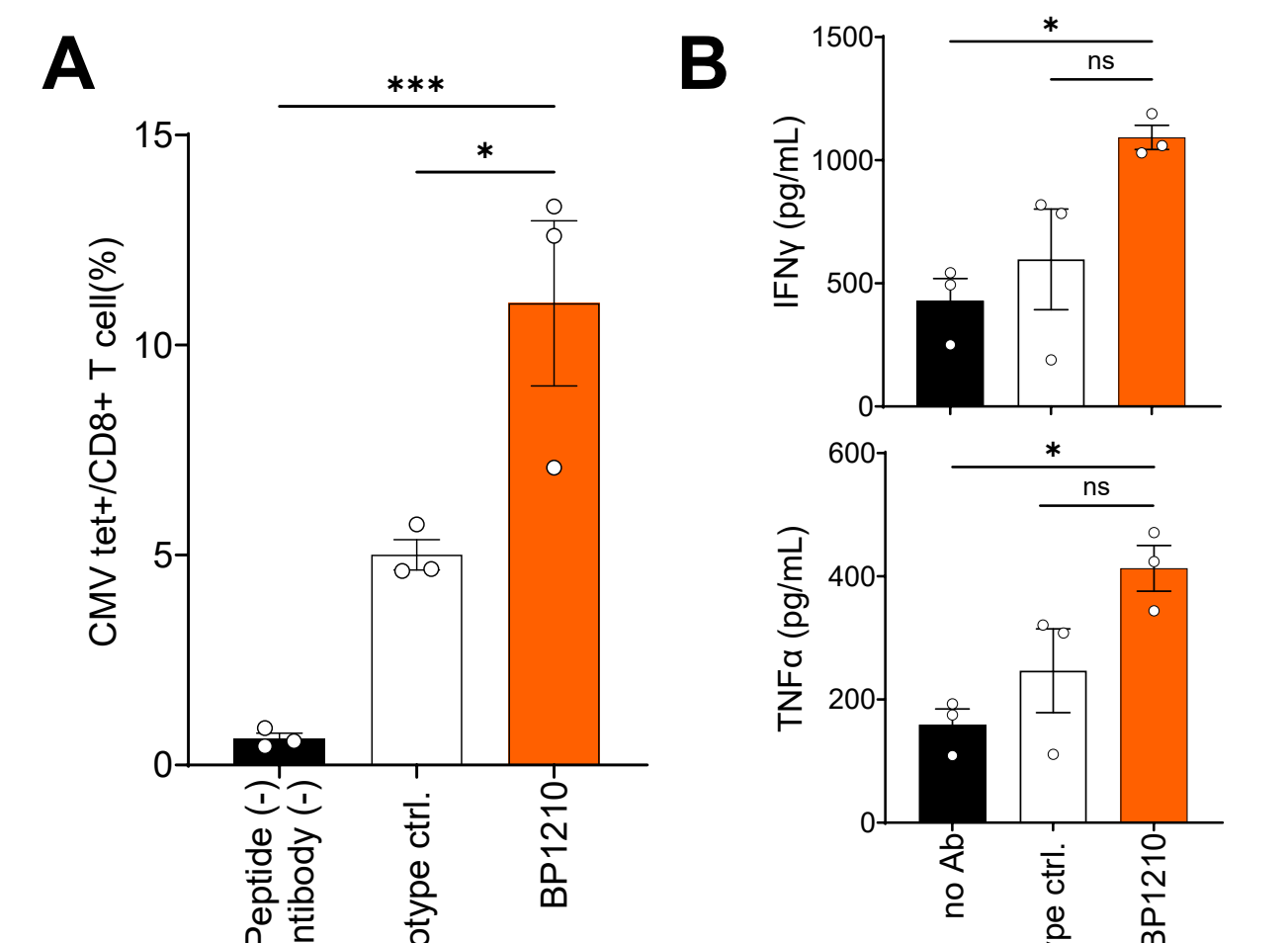
## Inhibition of the ligand binding



**Figure2 Inhibition of the ligand binding to TIM-3 by BP1210**

(A) Inhibition of Galectin-9 (Gal-9) binding to rhTIM-3-Fc via the antibodies was quantified by ELISA. (B) Inhibition of Phosphatidylserine (PtdSer) binding to rhTIM-3-Fc via the antibodies was analyzed by FACS. (C) Inhibition of T cell apoptosis induced by rhGal-9 was analyzed by FACS. Annexin V positive cells were normalized to rhGal-9(+)/Ab(-) as 100% and rhGal-9(-)/Ab(-) as 0%. MBG453 : Patent No. US9605070B2, TSR-022 : Patent No. WO2016161270

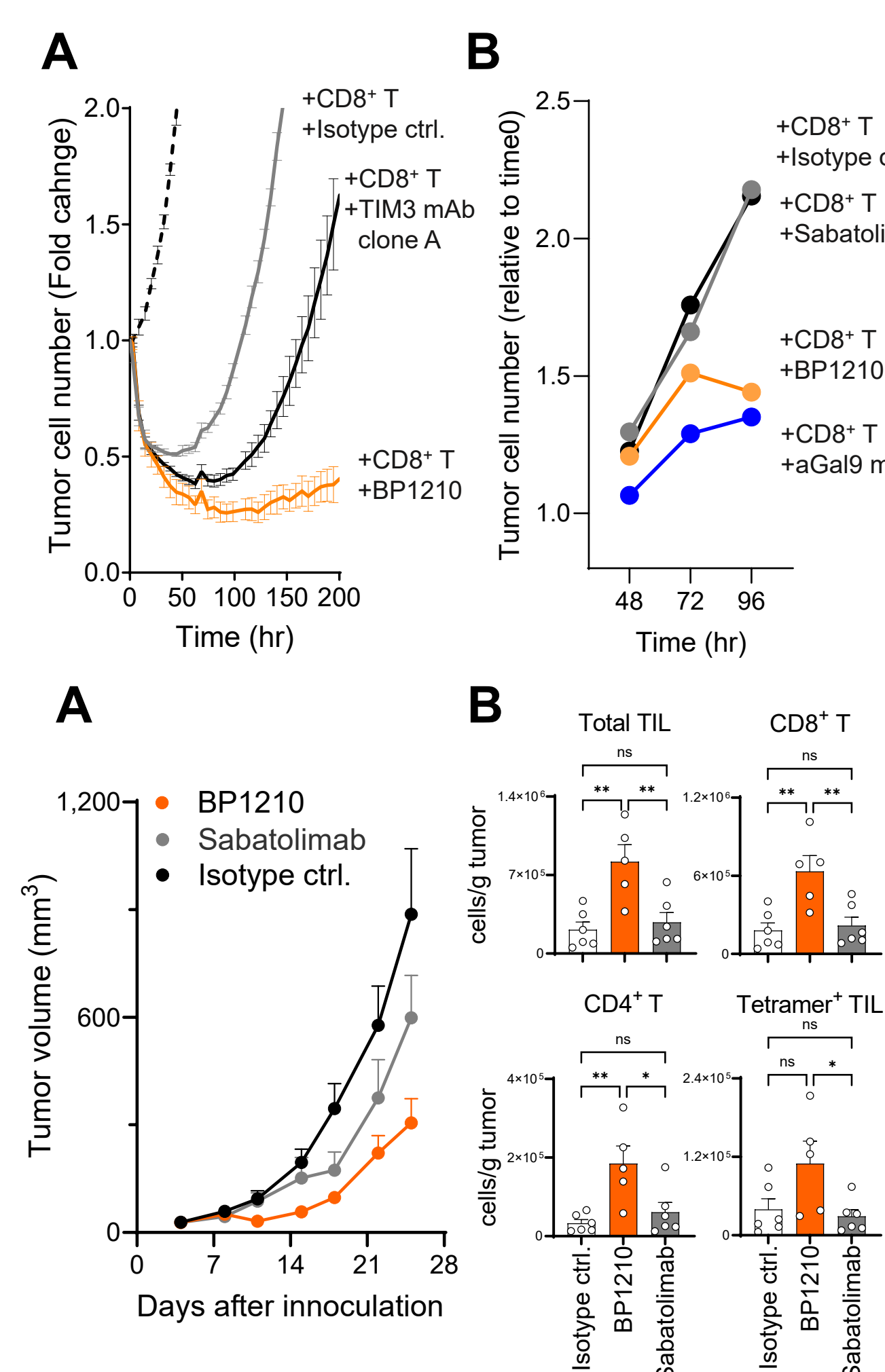
## Expansion and activation of CTL



**Figure3 T cell expansion and cytokine production are enhanced by BP1210**

PBMCs from healthy donor were cultured *in vitro* with or w/o BP1210. (A) BP1210 increased CMV-tetramer<sup>+</sup> T cells in PBMC stimulated by CMV pp65 class I peptide. (B) BP1210 increased IFN $\gamma$  and TNF $\alpha$  concentration in the culture supernatant. Data are represented as mean  $\pm$  SEM. Tukey's multiple comparison following one-way ANOVA was performed (\*P<0.05, \*\*\*P<0.001).

## Antitumor effect *in vitro* and *in vivo*



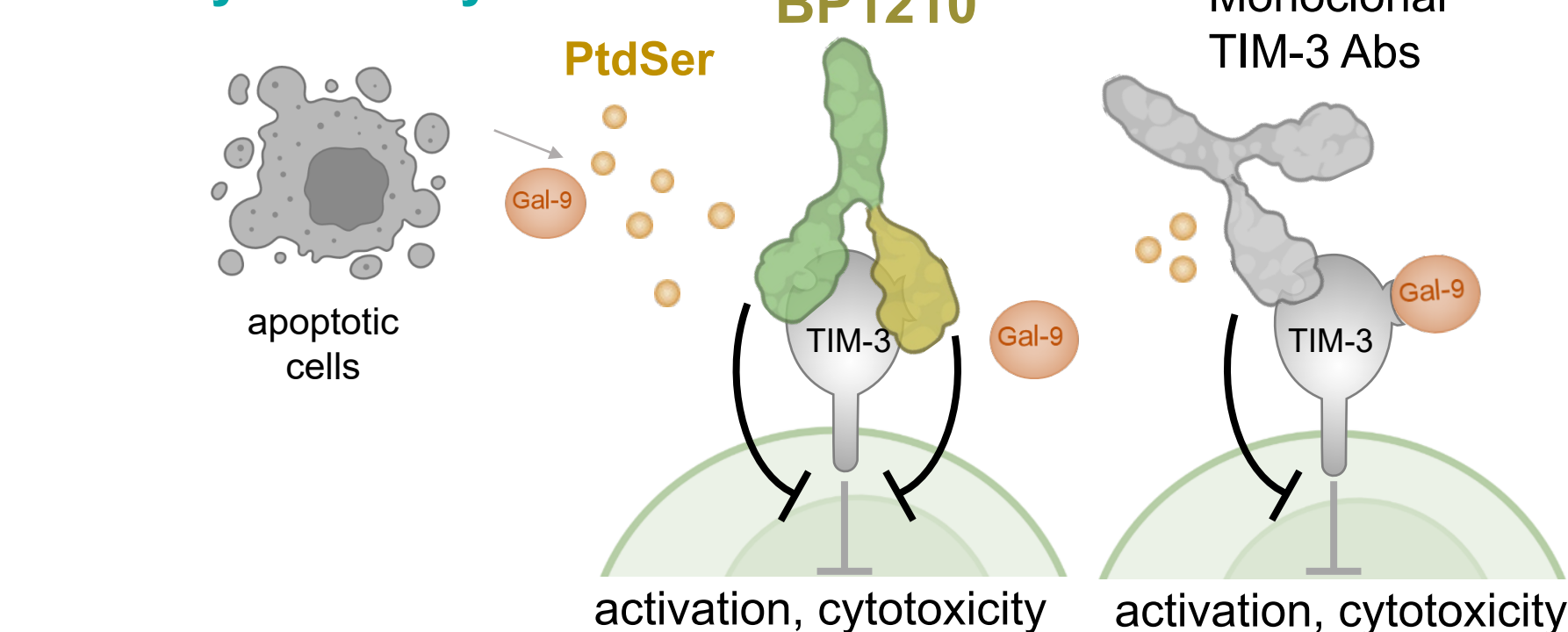
**Figure4 *in vitro* cytotoxicity of CD8<sup>+</sup> T cells is enhanced by BP1210**

SW620/CMVpp65 cells were co-cultured with CMV peptide-stimulated PBMCs. (A) Addition of BP1210 significantly enhanced the anti-tumor effect of CTLs compared to an anti-TIM-3 monoclonal antibody. (B) The antitumor activity attenuated via rhGal-9 was restored by BP1210, but not by Sabatolimab.

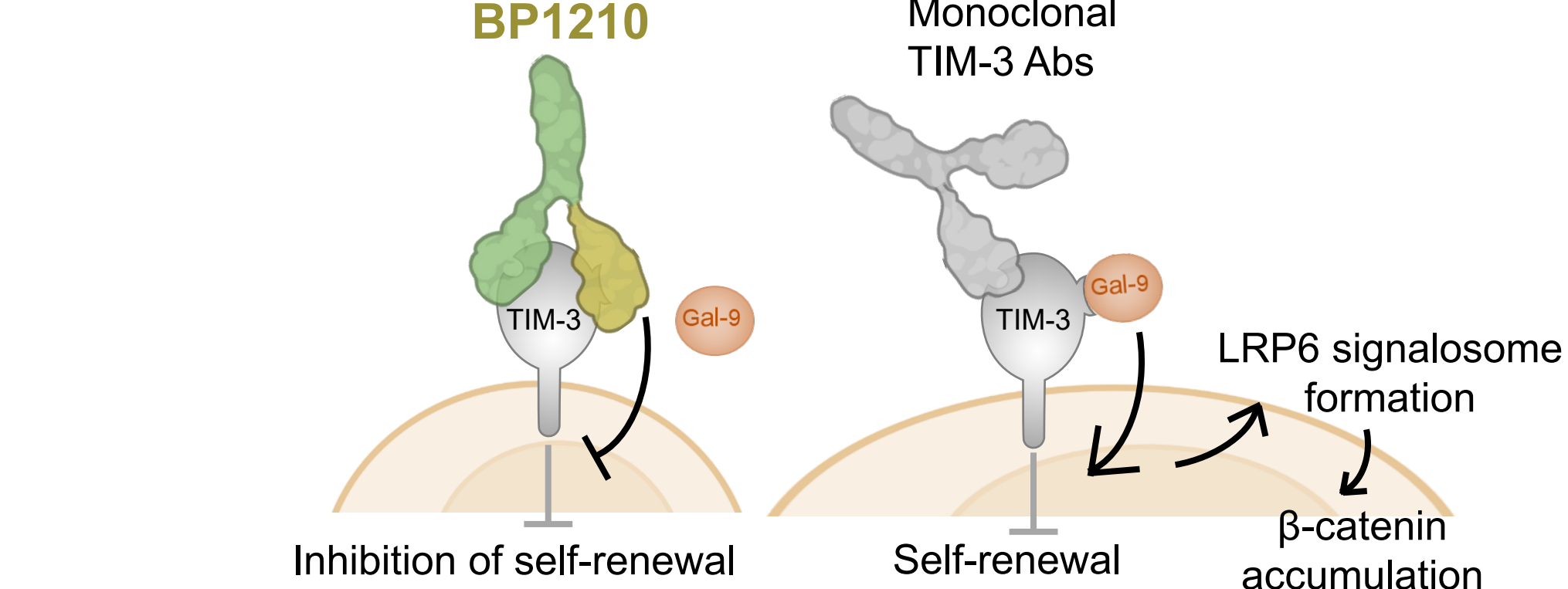
**Figure5 *in vivo* cytotoxicity of TILs is enhanced by BP1210**

(A) Mice bearing MC-38/OVA cells were treated with BP1210 or Sabatolimab (BIW x5). BP1210 delayed tumor growth more potently than Sabatolimab. (B) Mice were euthanized at the study endpoint and analyzed for TIL. The numbers of total T cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and OVA tetramer<sup>+</sup>CD8<sup>+</sup> T cells were significantly higher in BP1210-treated mice than in Sabatolimab-treated mice. Data are represented as mean  $\pm$  SEM. Tukey's multiple comparison following one-way ANOVA was performed (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001).

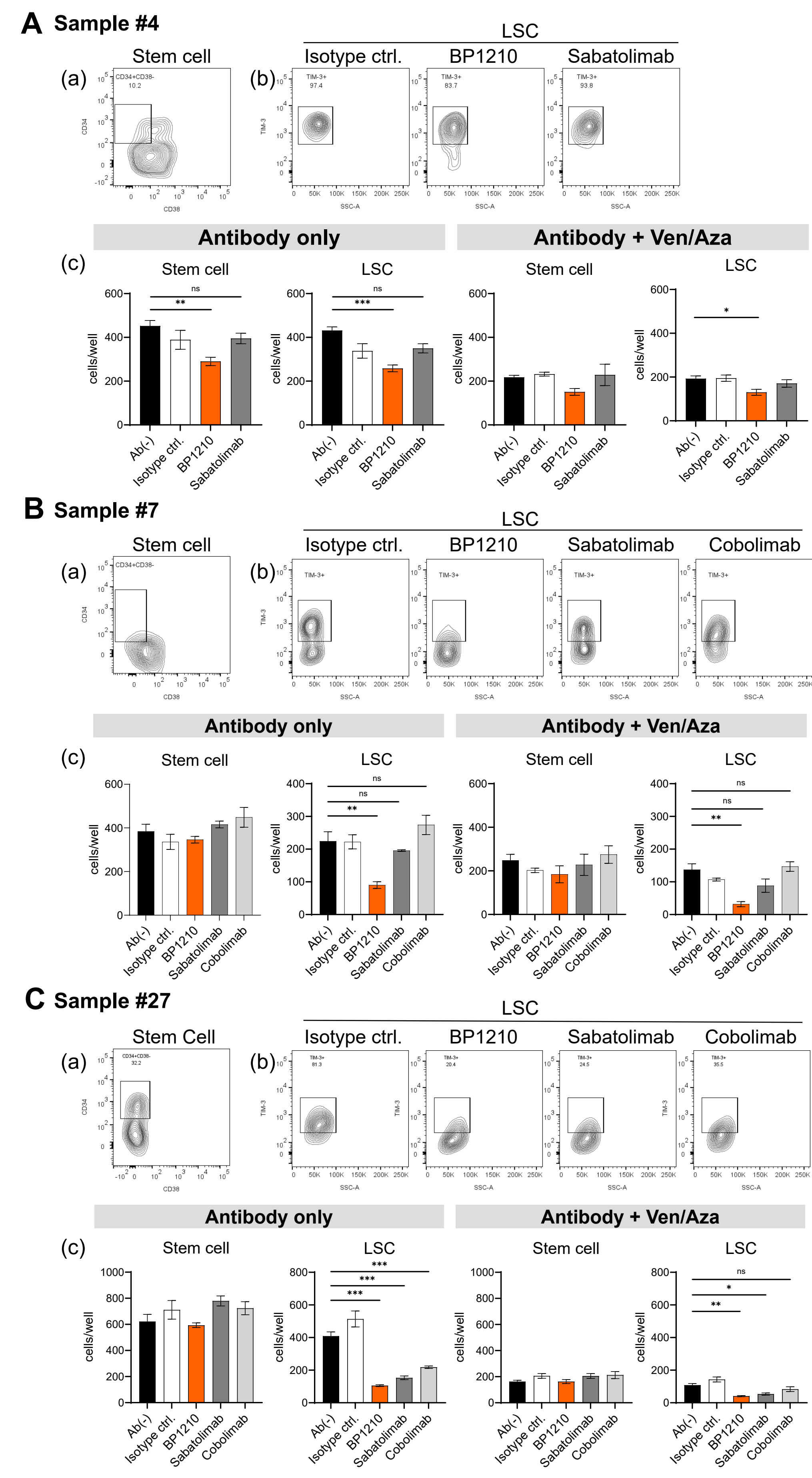
## T cell cytotoxicity



## LSC self-renewal



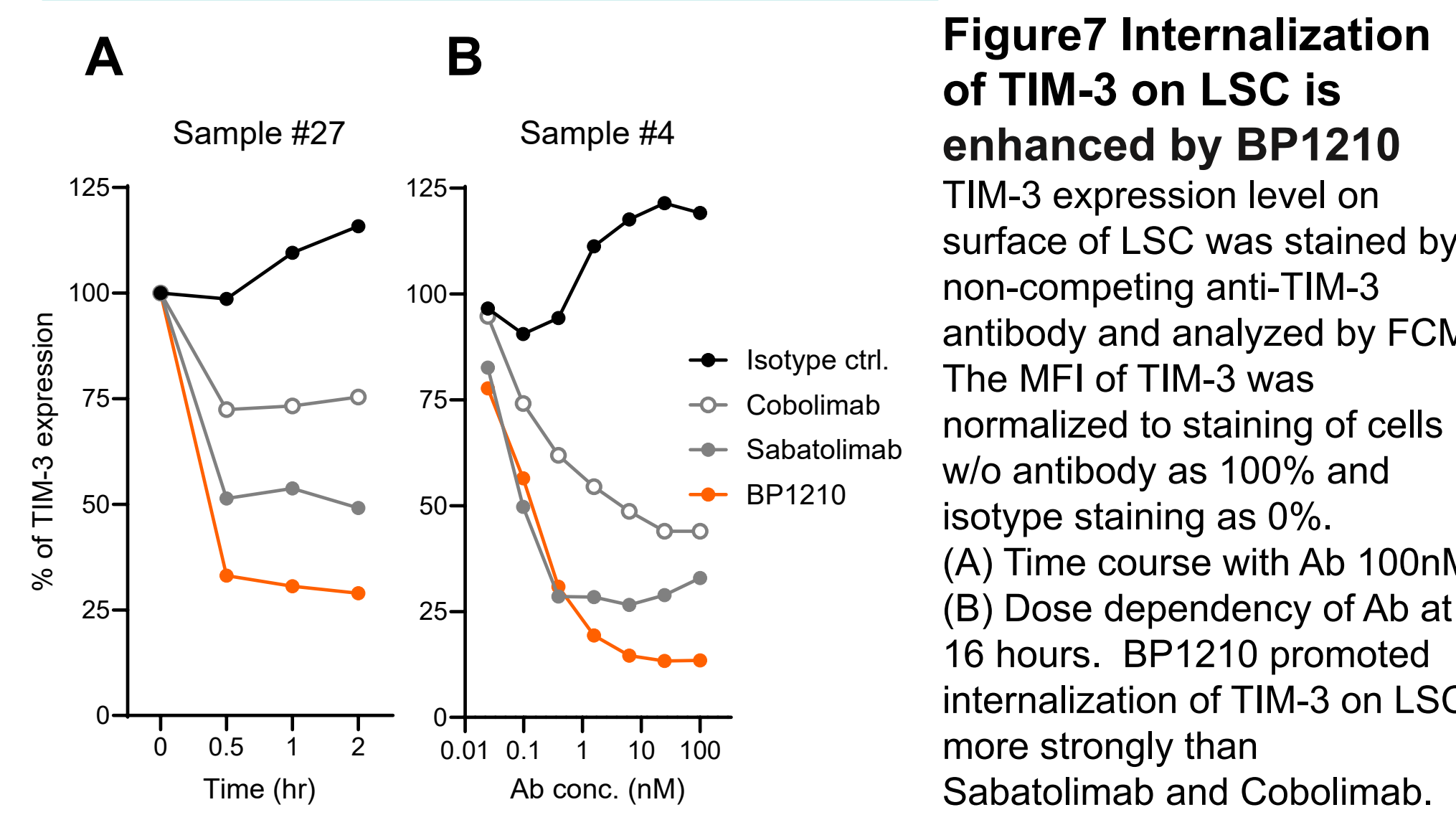
## Inhibition of LSC proliferation



**Figure6 Proliferation of LSC is inhibited by BP1210**

CD34 positive cells were isolated from BMBC of AML patients, then cultured in medium for LSC preparation (Leukemic cell culture kit, STEMCELL technologies) for 6-7days. LSC were cultured with 100nM antibody, and with or w/o 150nM Venetoclax(Ven), and 50nM Azacitidine(Aza) for 7days. Stem cell and LSC were counted by FCM analysis with counting beads. (a) FCM counter plot of Stem-cell ( $SSC^{low}CD45^{dim}CD34^{+}CD38^{-}$ ) (b) FCM counter plot of LSC ( $SSC^{low}CD45^{dim}CD34^{+}CD38^{+}Tim3^{+}$ ) (c) Cell number stem cell and LSC with or without Ven/Aza (A-C) BP1210 inhibited proliferation of each LSC more potently than Sabatolimab and Cobolimab. Data are represented as mean  $\pm$  SEM. Tukey's multiple comparison following one-way ANOVA was performed (\*P<0.05, \*\*P<0.01, \*\*\*P<0.001).

## Internalization of TIM-3 on LSC



**Figure7 Internalization of TIM-3 on LSC is enhanced by BP1210**

TIM-3 expression level on surface of LSC was stained by non-competing anti-TIM-3 antibody and analyzed by FCM. The MFI of TIM-3 was normalized to staining of cells w/o antibody as 100% and isotype staining as 0%. (A) Time course with Ab 100nM. (B) Dose dependency of Ab at 16 hours. BP1210 promoted internalization of TIM-3 on LSC more strongly than Sabatolimab and Cobolimab.

## Conclusion

- ◆ BP1210 completely inhibited the ligand-binding of both TIM-3 ligand, galectin-9 and phosphatidylserine.
- ◆ BP1210 enhanced T cell proliferation and cytokine production, demonstrated superior antitumor activity compared with the TIM-3 monoclonal antibodies.
- ◆ BP1210 potently inhibited proliferation of LSC and enhanced internalization of TIM-3 on LSC derived from AML patients compared to the TIM-3 monoclonal antibodies.

- BP1210 may offer better anti-tumor efficacy compared to the TIM-3 monoclonal antibodies that are or have been in clinical trials.
- BP1210 is a promising therapeutic candidate for AML with its dual mechanism of action enhancing antitumor immunity and inhibiting LSC self-renewal
- BP1210 has strong TIM-3 internalization ability and is a promising candidate of antibody-drug conjugate targeting LSC.