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SHANGHAI
MODEL ORGANISMS

hCD3EDG triple knockin humanized mouse empowers the efficacy and safety evaluation of CD3 bispecifics in precinical trials

1. Why we develop hCD3EDG triple knockin mice?

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- Evaluate **human-specific** biological therapies when no mouse ortholog is available
- Amenable to *in vivo* **efficacy testing** in a fully immunocompetent environment when engrafted with a syngeneic murine cancer cell line
- **Target humanized mice** can be combined with Target/TAA humanized syngeneic cancer cells to **recreate a fully humanized ligand-receptor complex** between the tumor and its microenvironment
- Reduce donor-to-donor variability of *in vitro* immunotherapy assessment and GvHD *in vivo* by using immune cells expressing humanized targets from **target humanized mice** as an alternative to human PBMCs.
- Double and triple knockin models also available to **evaluate combination therapies**
- **Robust** humanized target models

1. hCD3EDG and hCD3EDG-derived lines at SMOC

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目录号（Cat. No.）	小鼠品系（Strain）	小鼠背景（Background）
NM-HU-220120	hCD3EDG	C57BL/6
NM-HU-220122	hCD3EDG	BALB/c
NM-HU-220109	hCD3EDG/hCD19	C57BL/6
NM-HU-220110	hCD3EDG/h4-1BB(2)	C57BL/6
NM-HU-220111	hCD3EDG/hTPBG	C57BL/6
NM-HU-220112	hCD3EDG/hPD-L1	C57BL/6
NM-HU-220113	hCD3EDG/hCD28	C57BL/6
NM-HU-220114	hCD3EDG/hCD38(2)	C57BL/6
NM-HU-220115	hCD3EDG/hCD28/hCD38(2)	C57BL/6
NM-HU-220116	hCD3EDG/hCD40	C57BL/6
NM-HU-220117	hCD3EDG/hCD40/h4-1BB(2)	C57BL/6
NM-HU-220119	hCD3EDG/hCD20(2)	C57BL/6
NM-HU-220123	hCD3EDG/hEpCAM	C57BL/6

2. Lymphocytes Lineage Characterization in Blood in Heterozygous hCD3EDG Mice

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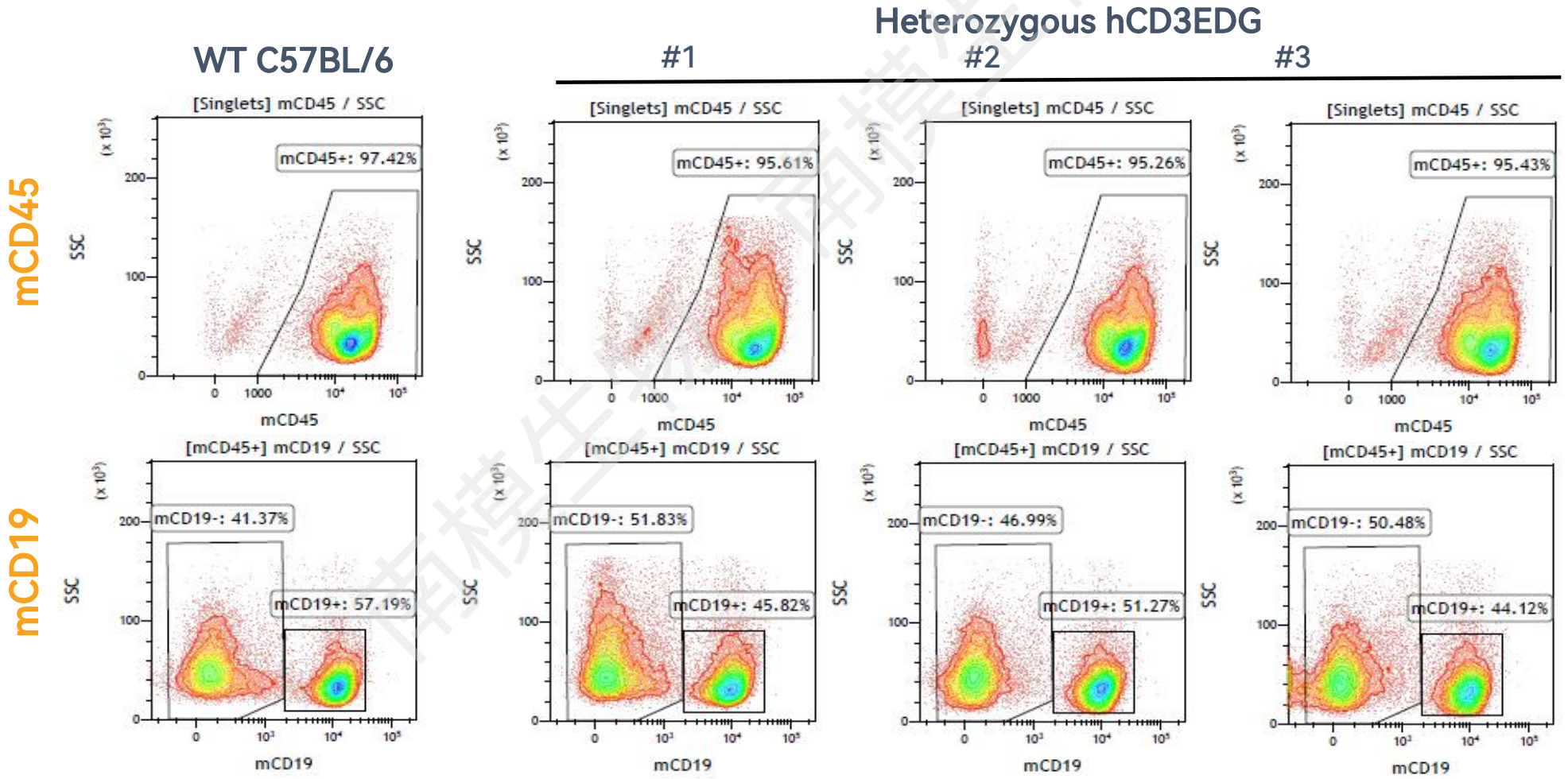
- Heterozygous hCD3EDG mice have comparable levels of B cell and lymphocytes development as WT mice.



2. Lymphocytes Lineage Characterization in Blood in Heterozygous hCD3EDG Mice

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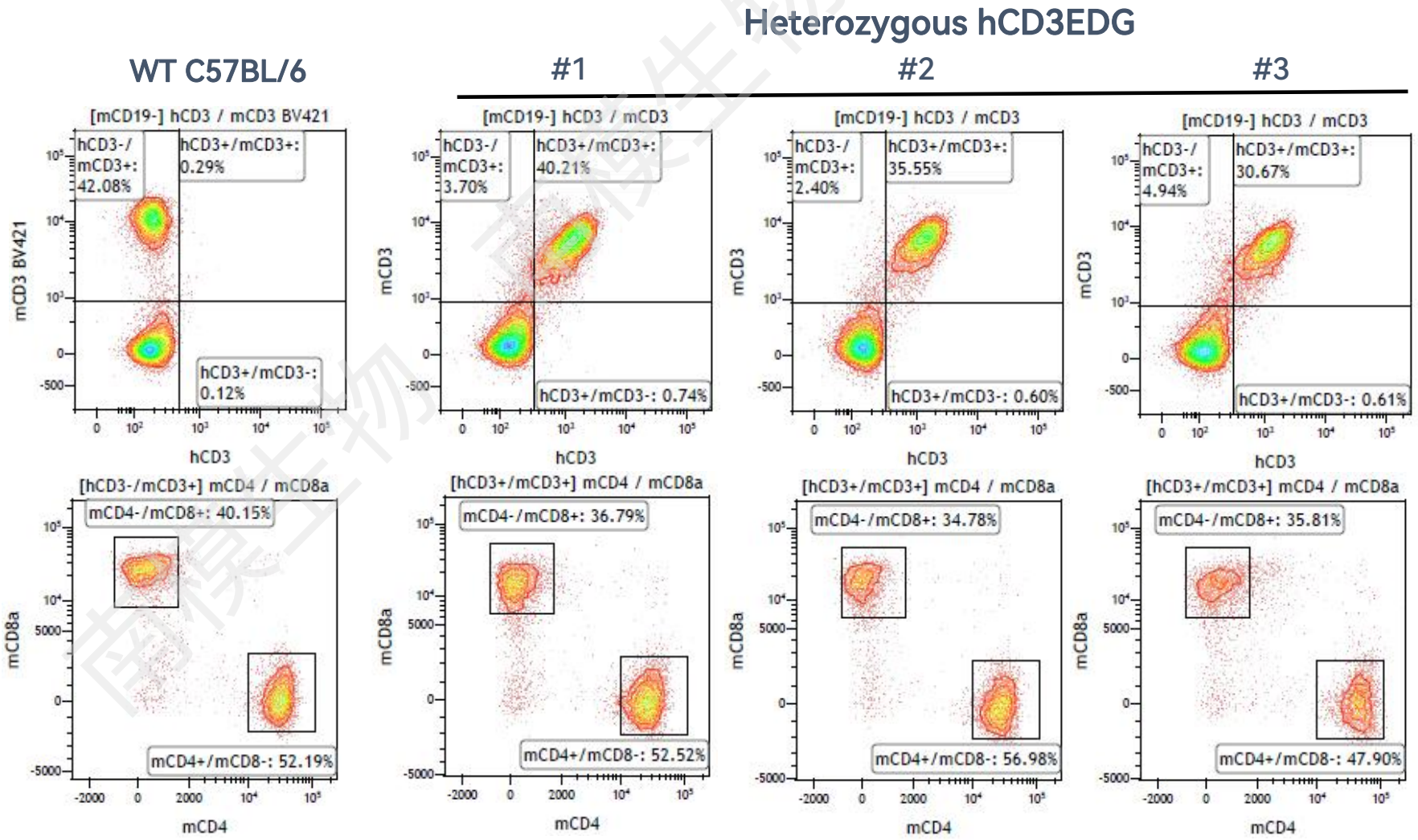
- >80% of T cells (CD3) express human and mouse CD3 T cells
- Heterozygous hCD3EDG mice have comparable levels of CD4+/CD8+ T cells as WT



2. Lymphocytes Lineage Characterization in Blood in Heterozygous hCD3EDG mice

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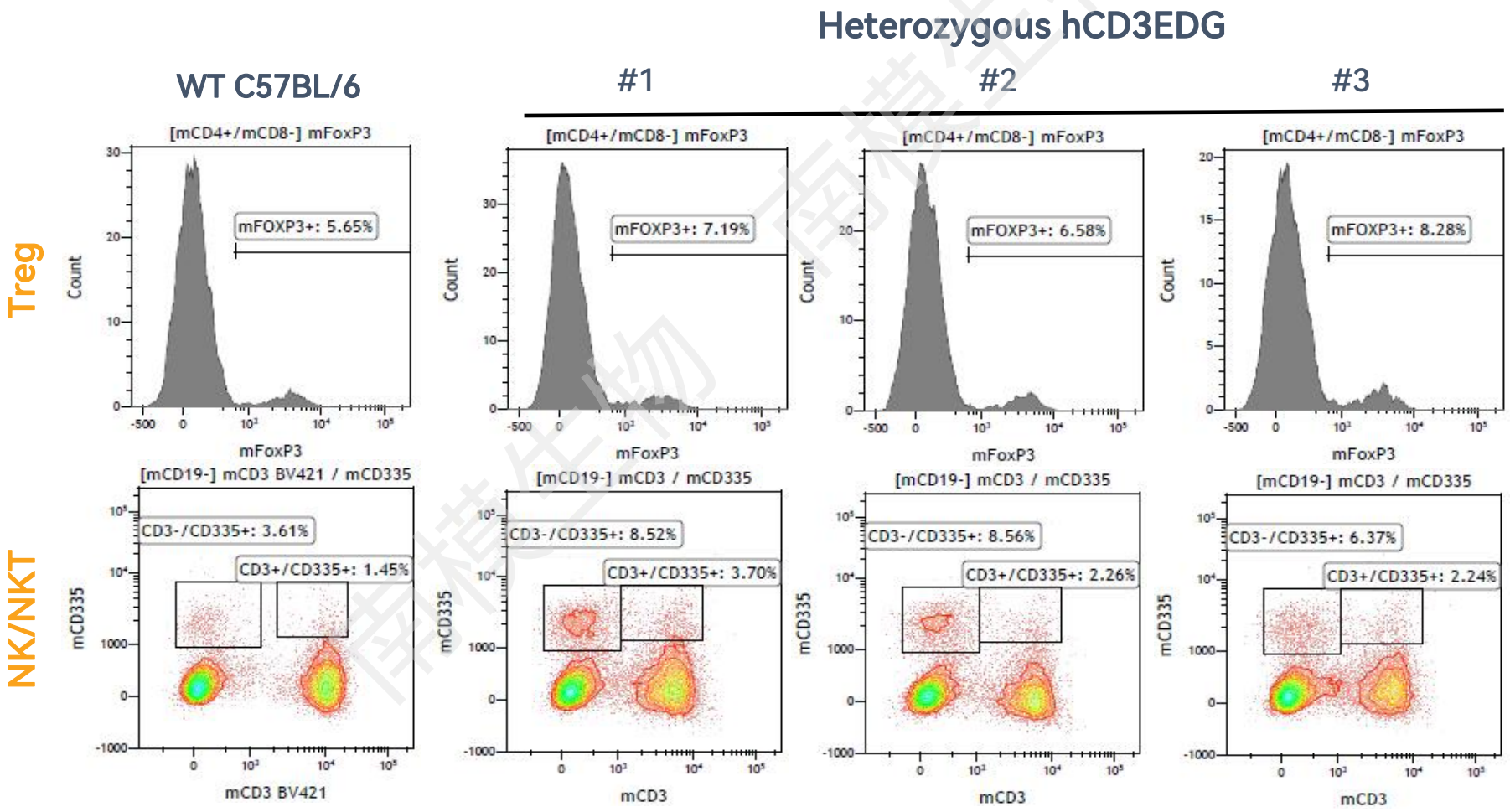
- Heterozygous hCD3EDG Mice have comparable levels of NK cells and Tregs as WT mice.



2. Surface Expression Detection of Human CD3E/CD3D/CD3G in Homozygous hCD3EDG Mice

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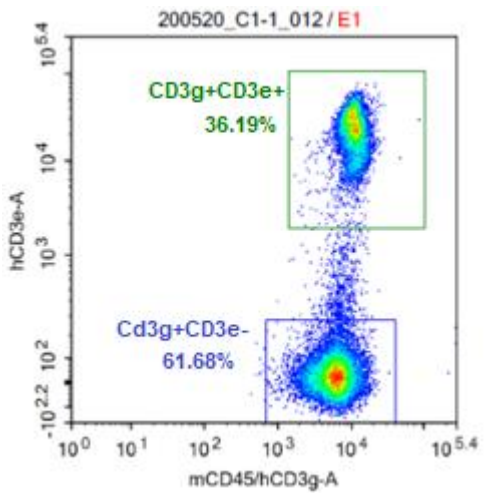
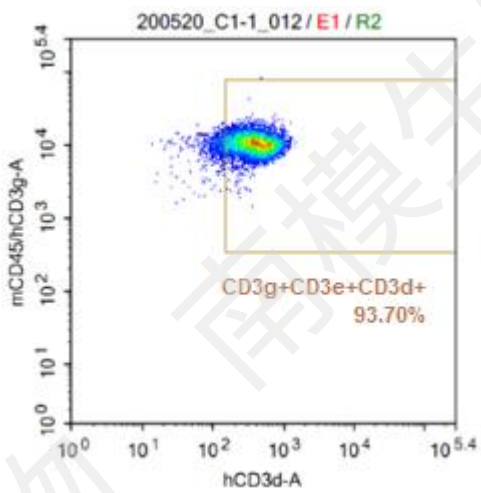
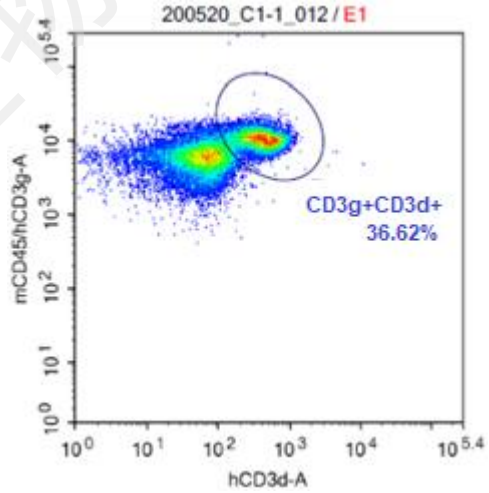
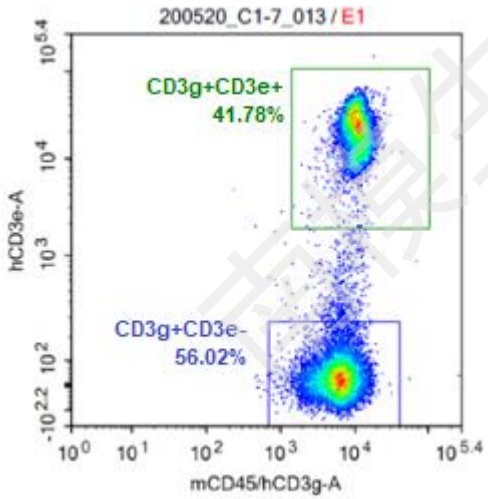
Homozygous hCD3EGD #1

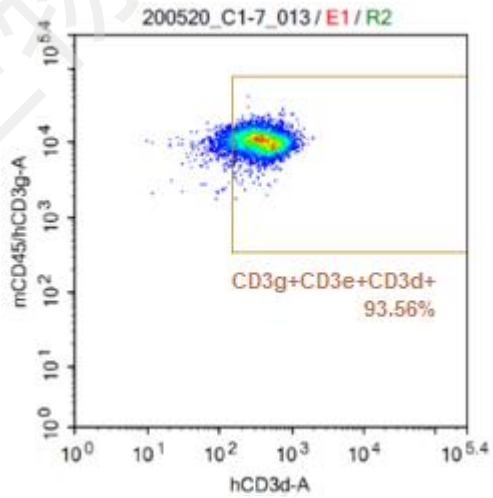


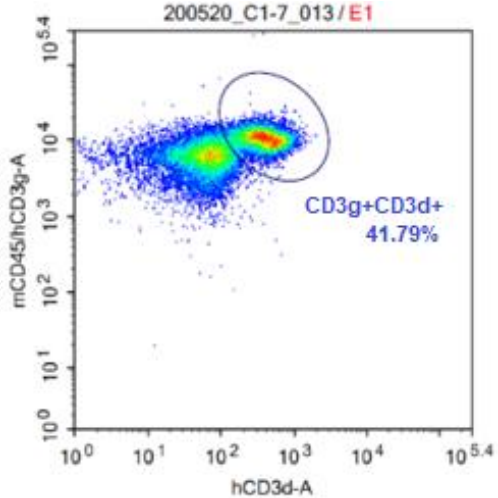




Homozygous hCD3EGD #2







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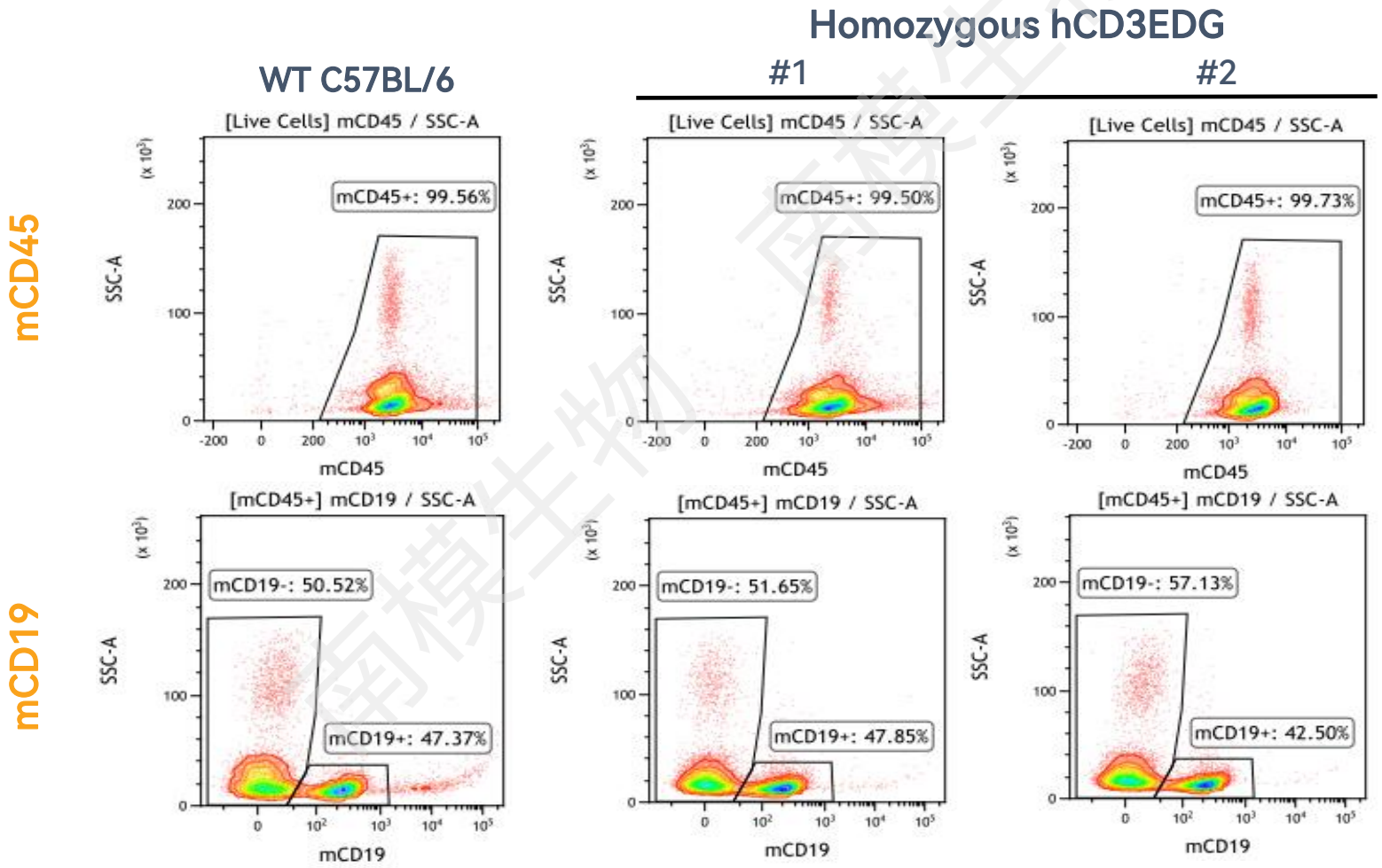

2. Lymphocyte Lineage Characterization in Blood in Homozygous hCD3EDG Mice

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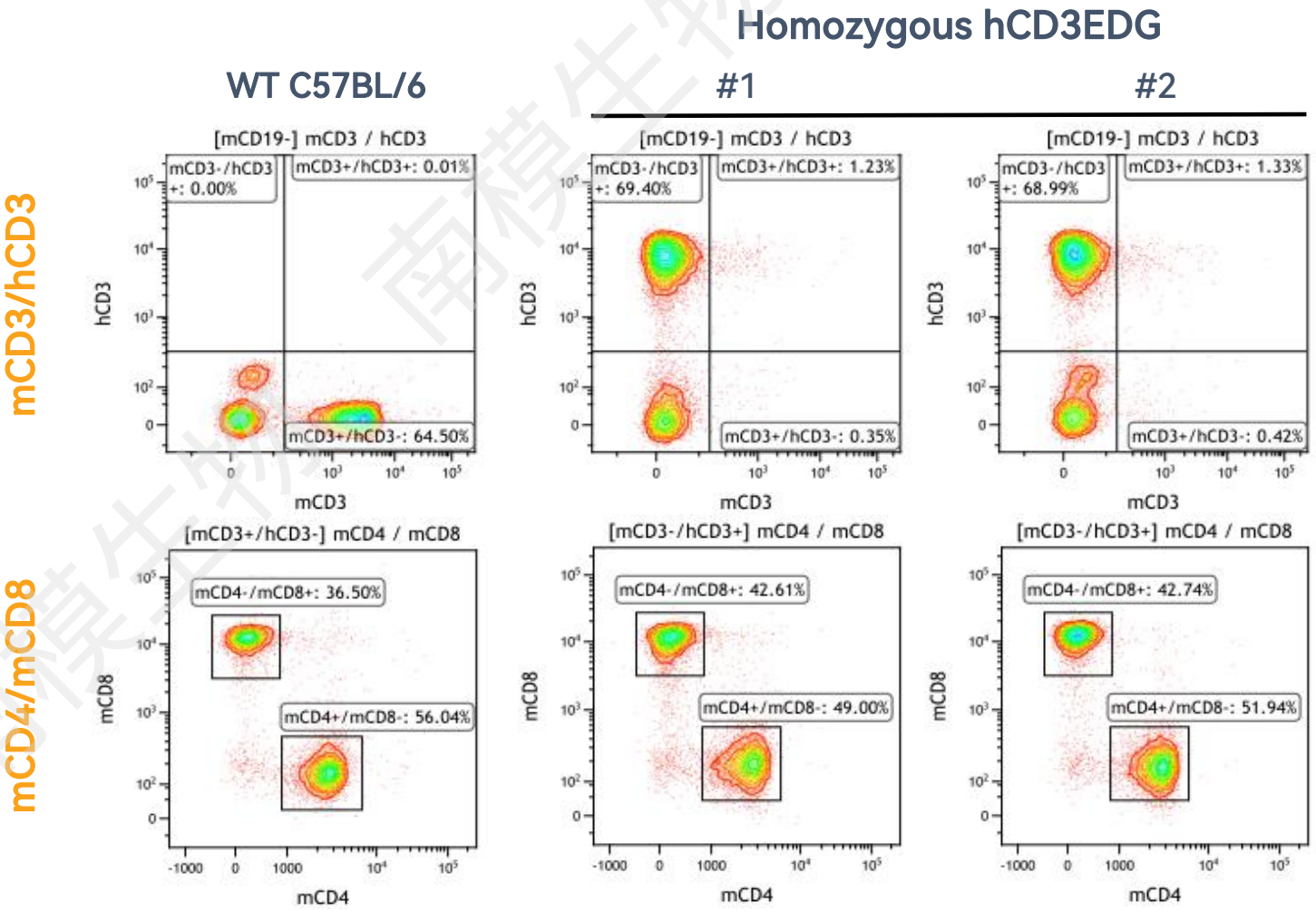
- >65% of T cells express human CD3
- Homozygous hCD3EDG have comparable levels of CD4+/CD8+ T cells as WT



2. Lymphocyte Lineage Characterization in Blood in Homozygous hCD3EDG Mice

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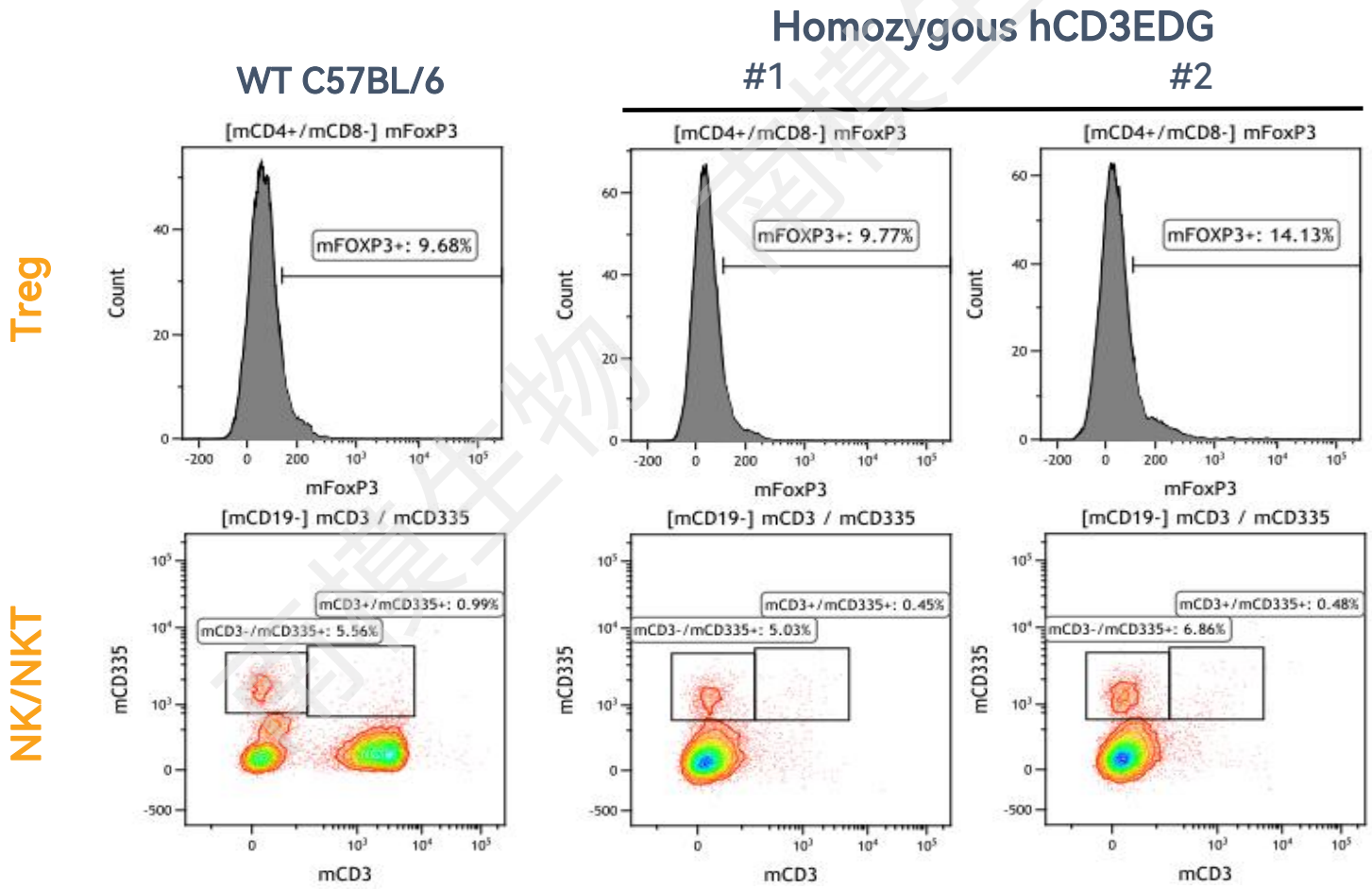
- Homozygous hCD3EDG mice have comparable levels of NK cells and Tregs as WT mice.



3. *In Vitro* Evaluation of Different Anti-CD3 Antibodies

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- *In vitro* binding assays
  - Antibodies were labeled with biotin for binding assay by FACS using both hCD3EDG-derived splenocytes and human PBMCs
  - Differential binding between the antibodies and CD4+ and CD8+ T cells were observed
- *In vitro* T cell activation
  - These antibodies were further investigated for T cell activation via FACS and ELISA in comparison to OKT3 (monoclonal anti-hCD3 antibody) and an anti-mCD3 antibody at 3 different timepoints
  - Varying levels of cytokine release (IFN-γ, TNF-α, IL-6, IL-10 and IL-2) was observed following T cell activation

Test Ab	Description
Anti-huCD3#1	conformational
Anti-huCD3#2	conformational
Anti-huCD3#3	linear epitope
Anti-huCD3#4	conformational

3. Binding Assay of Biotin-Labeled Anti-Human CD3 #1

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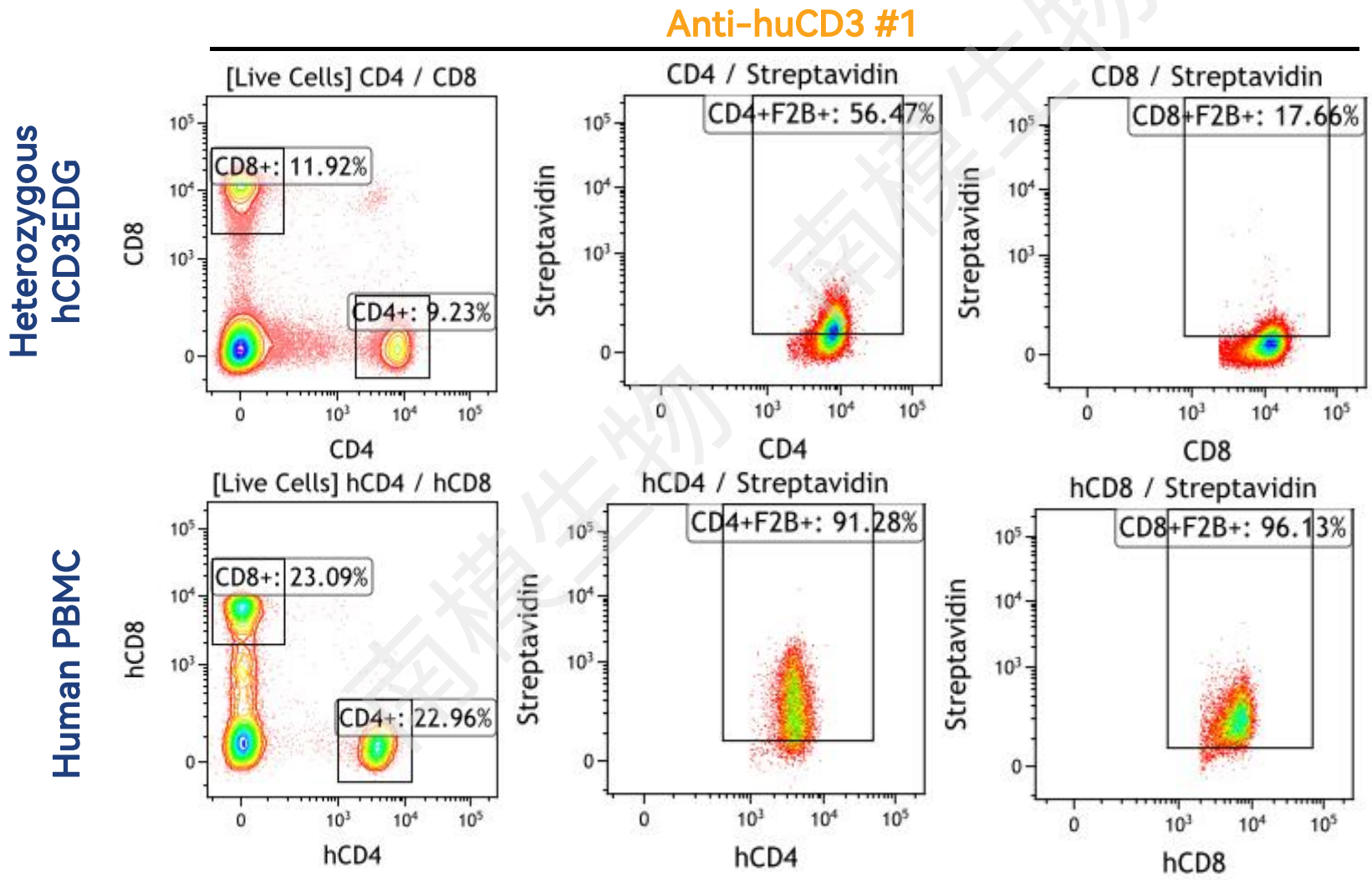

3. Binding Assay of Biotin-Labeled Anti-Human CD3 #2

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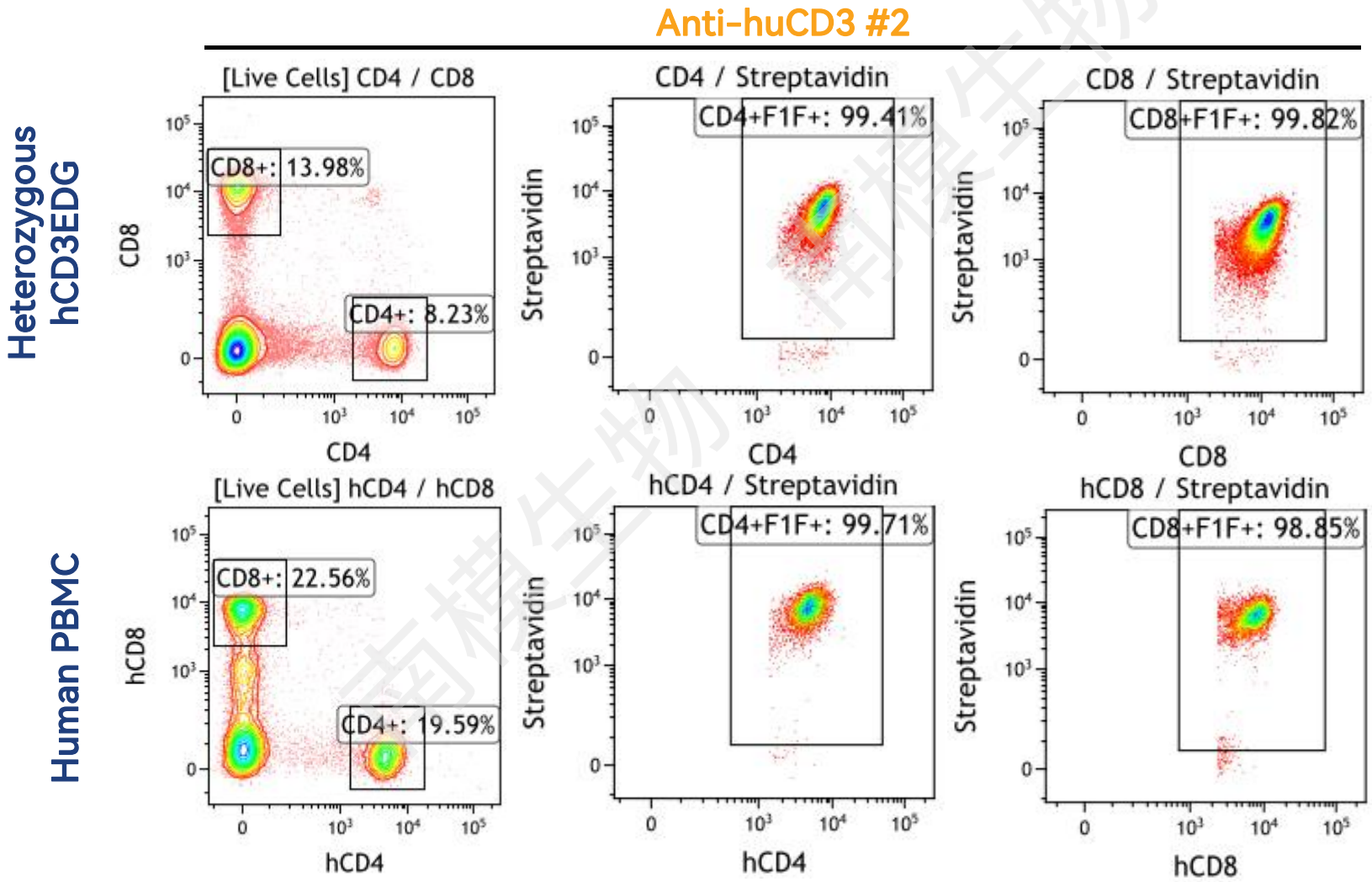

3. Binding Assay of Biotin-Labeled Anti-Human CD3 #3

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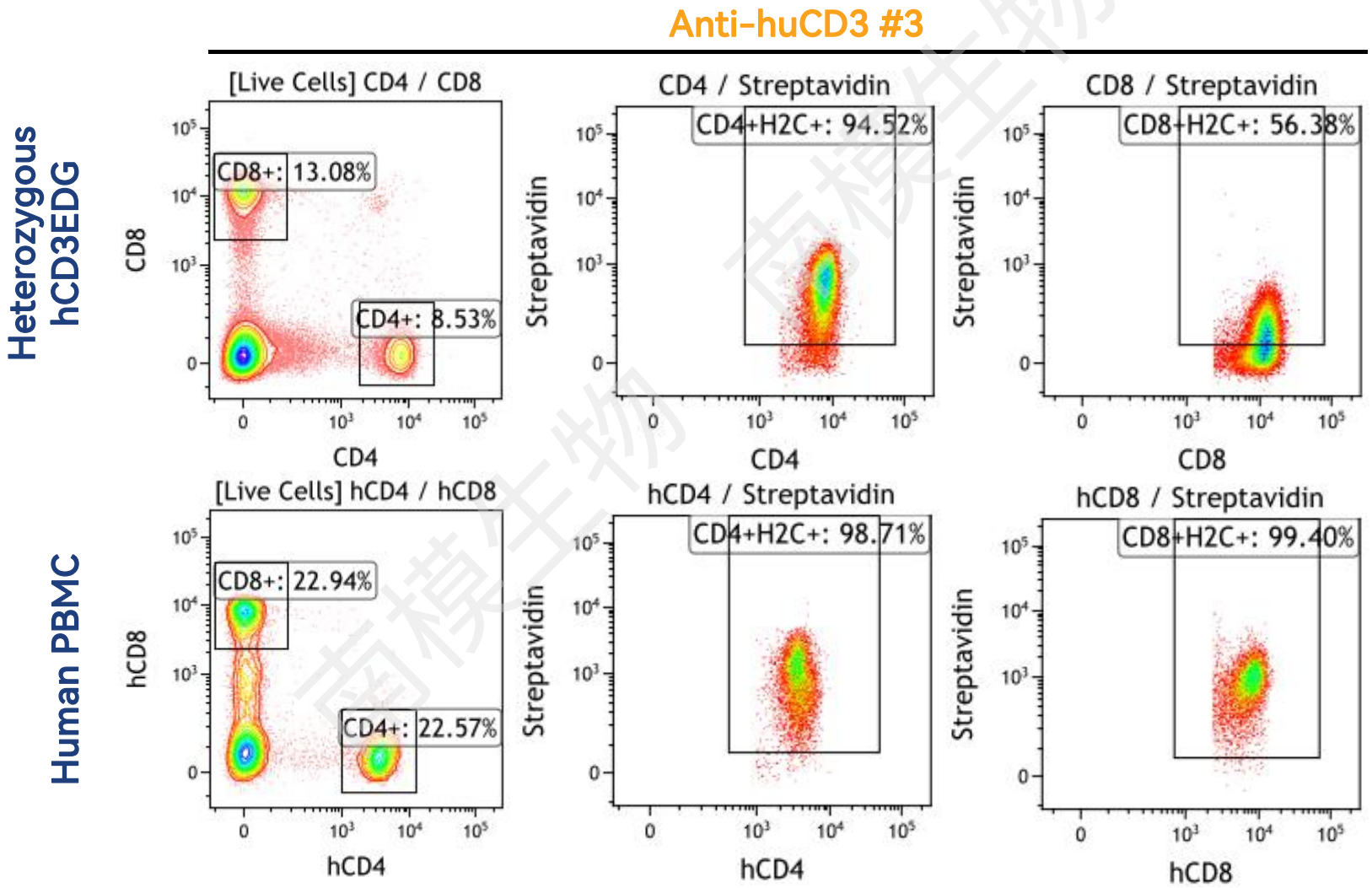

3. Binding Assay of Biotin-Labeled Anti-Human CD3 #4

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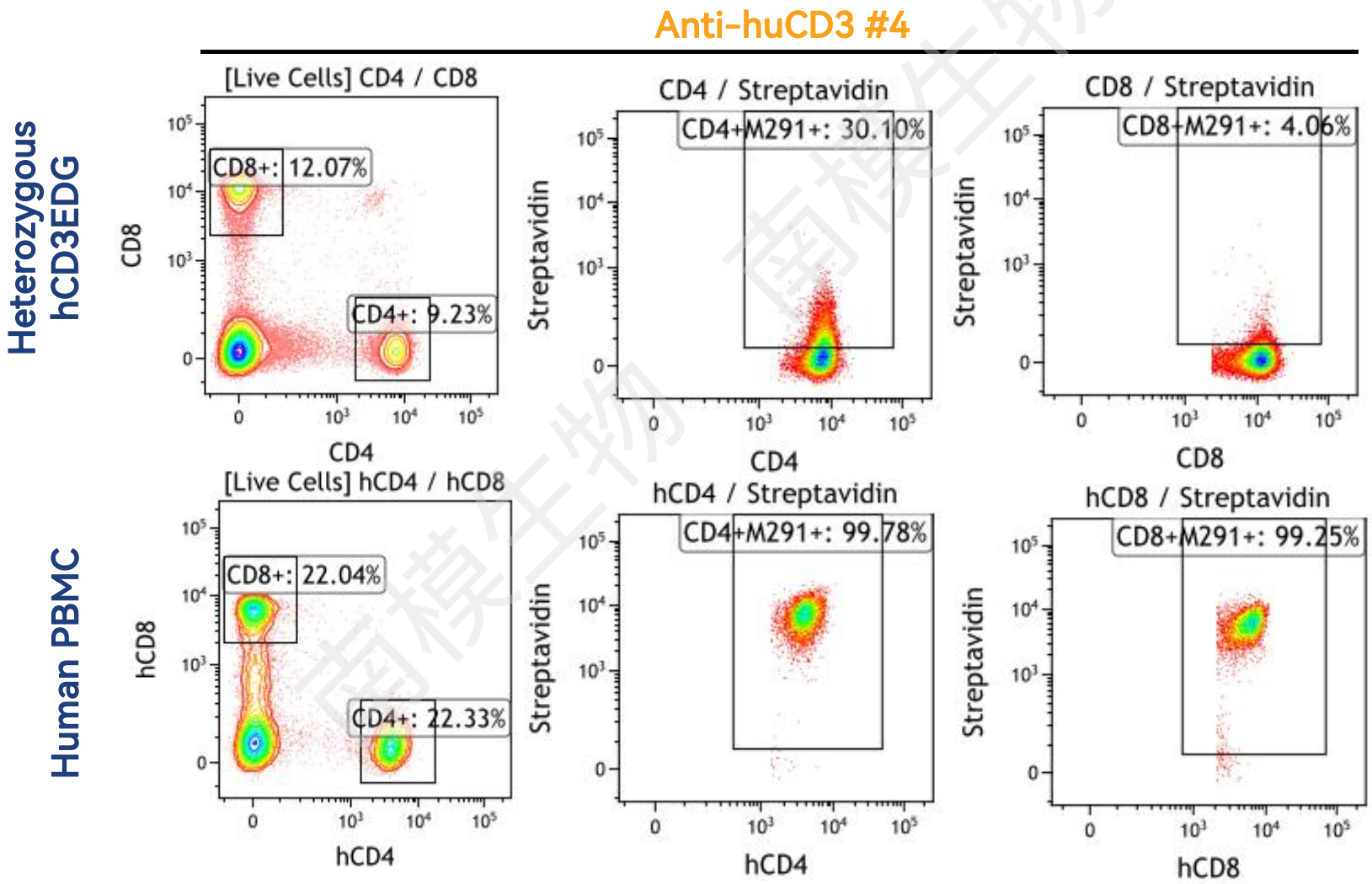

3. *In Vitro* T Cell Activation with Heterozygous hCD3EDG Mice

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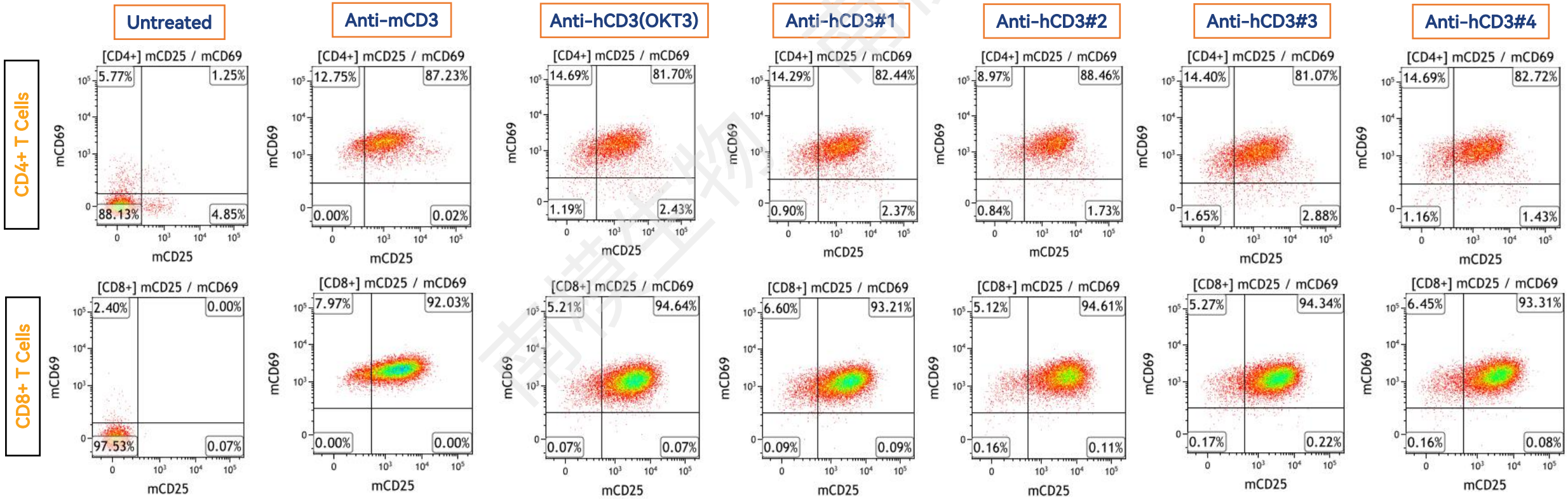
- The expression of T cell activation markers CD25, CD69, on CD4+ and CD8+ T cells were determined by flow cytometry
- CD25 and CD69 expression increased on T cells derived from hCD3EDG mice treated with anti-CD3 Ab



3. Cytokine Release Detection by MSD Upon Stimulation *In Vitro* with Various Anti-CD3 Antibodies

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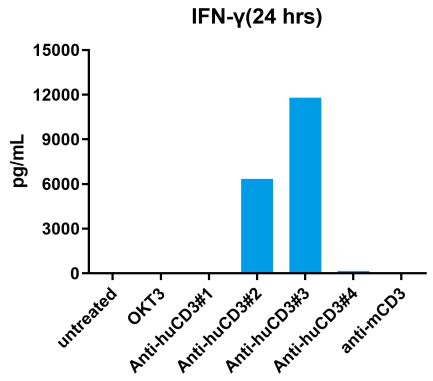
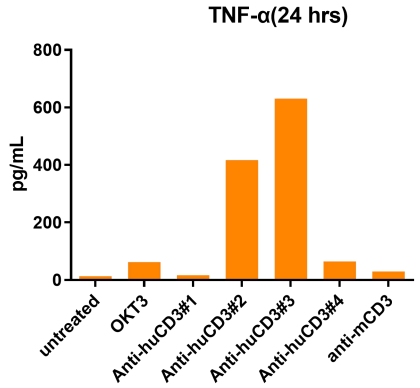
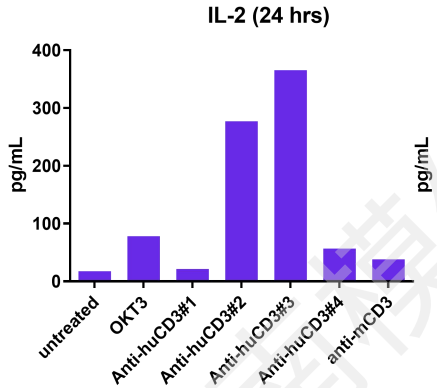
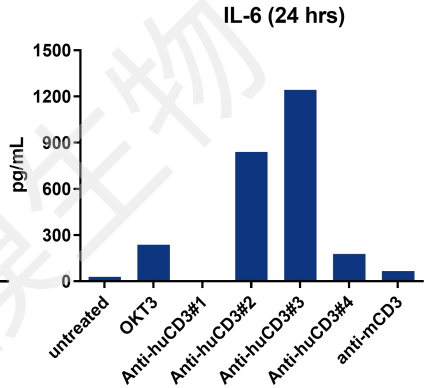
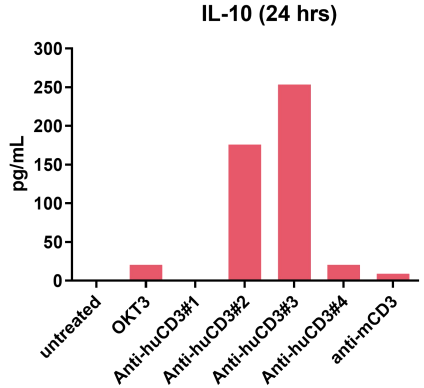
24 hours



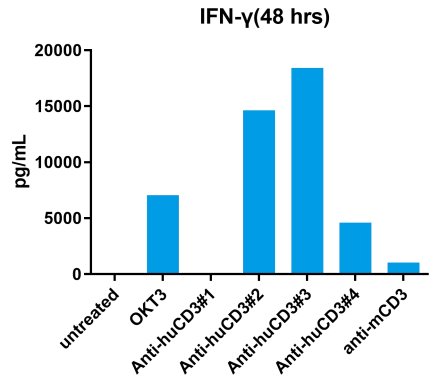


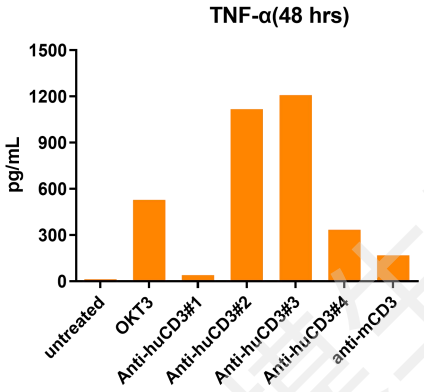


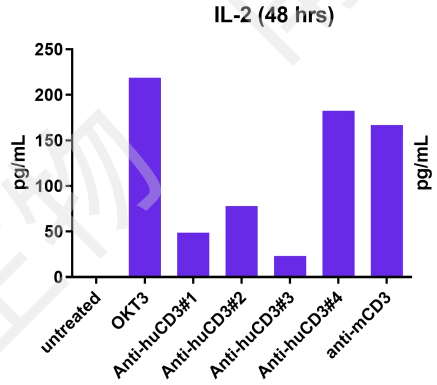


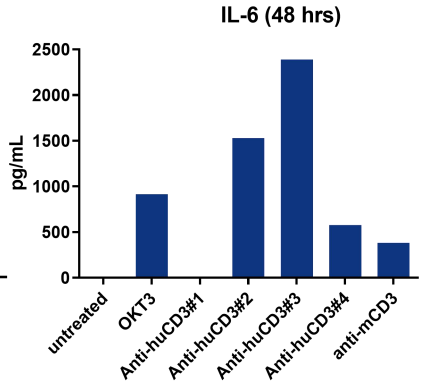


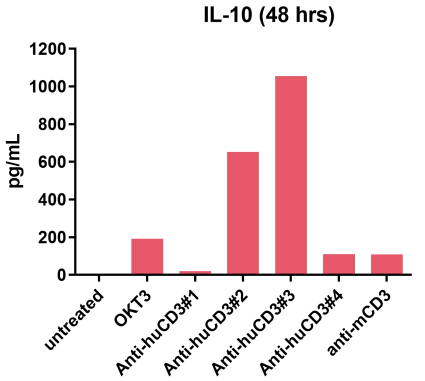
48 hours



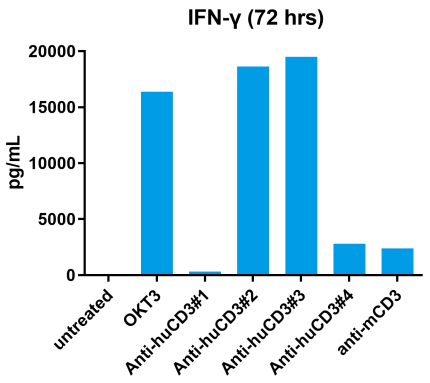


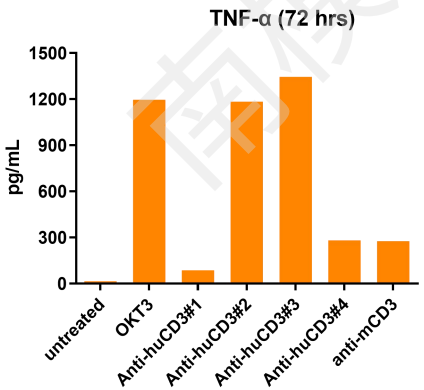


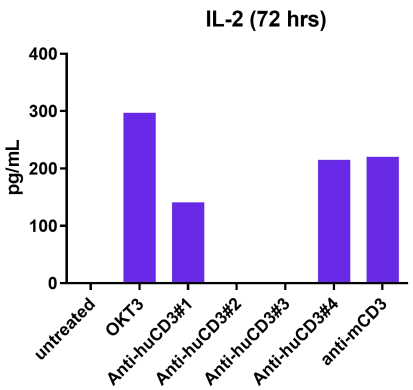


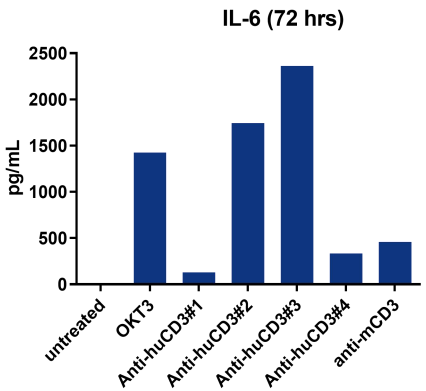


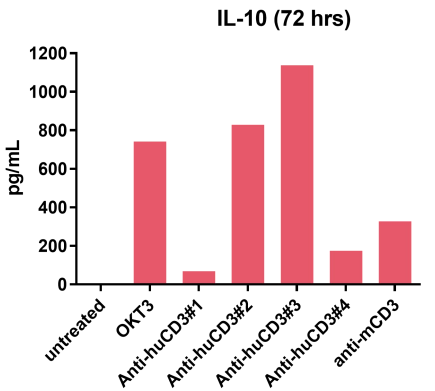
72 hours











4. *In Vivo* Response with Bispecific T Cell Engager Antibodies in Heterozygous hCD3EDG Mice

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- The objective of this study was to evaluate a bispecific T cell engager antibodies in the heterozygous hCD3EDG mice.
- MC38-hEpCAM syngeneic tumors were engrafted in heterozygous hCD3EDG mice.
- Mice were treated with anti-hEpCAM/CD3 bispecific antibodies at different doses.
- Increase in TGI was observed with bispecific T cell engager antibodies in comparison to controls in a dose dependent way.

4. *In Vivo* Response with Bispecific T Cell Engager Antibodies in Heterozygous hCD3EDG Mice

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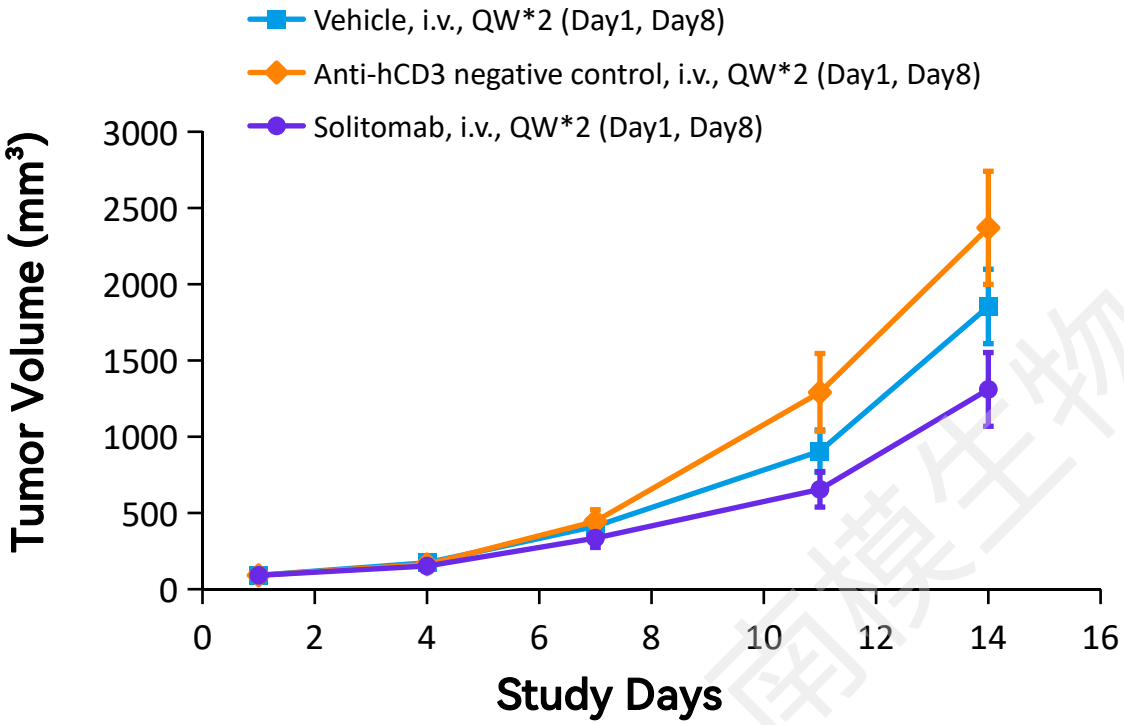
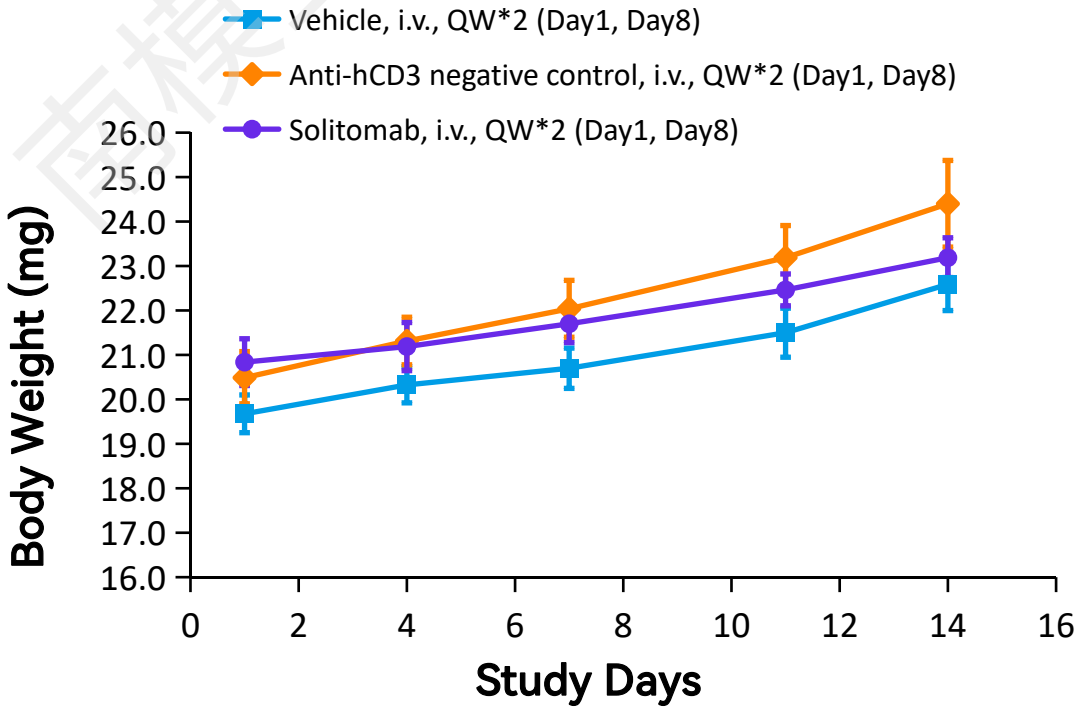
- Heterozygous hCD3EDG mice were engrafted with MC38-hEpCAM to evaluate the *in vivo* efficacy of anti-CD3×EpCAM bispecific antibody.





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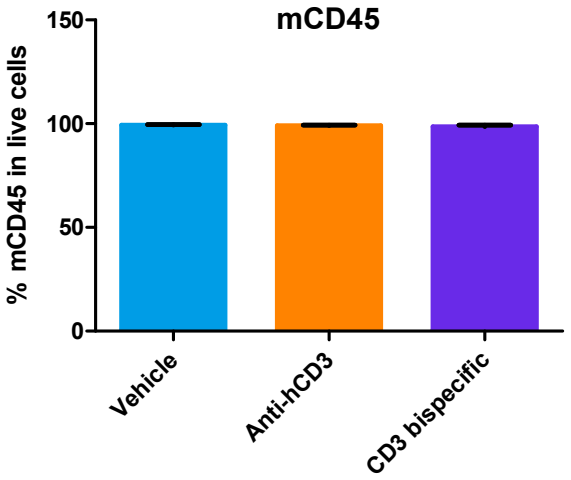
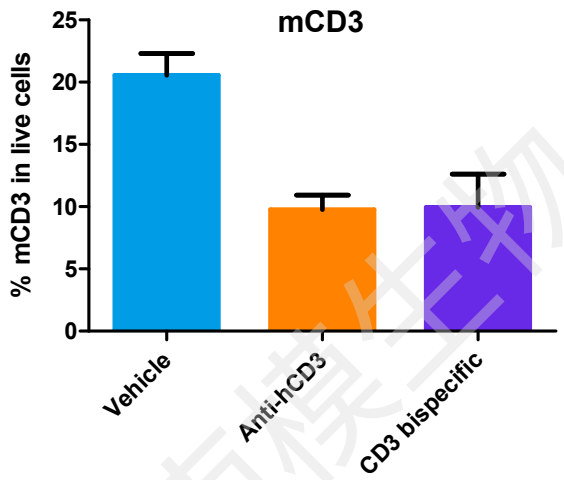
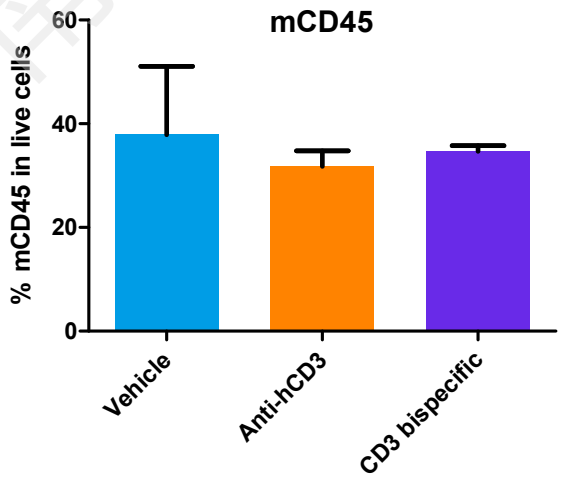
- Tumors and spleens were collected 7 days post final dose for CD45 and CD3 analysis by FACS

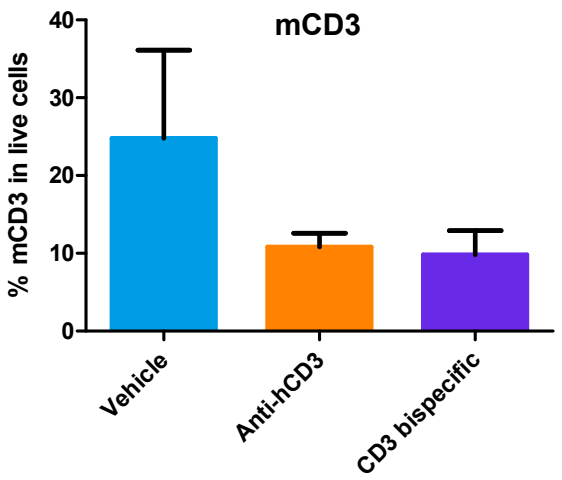
Tumor





Spleen





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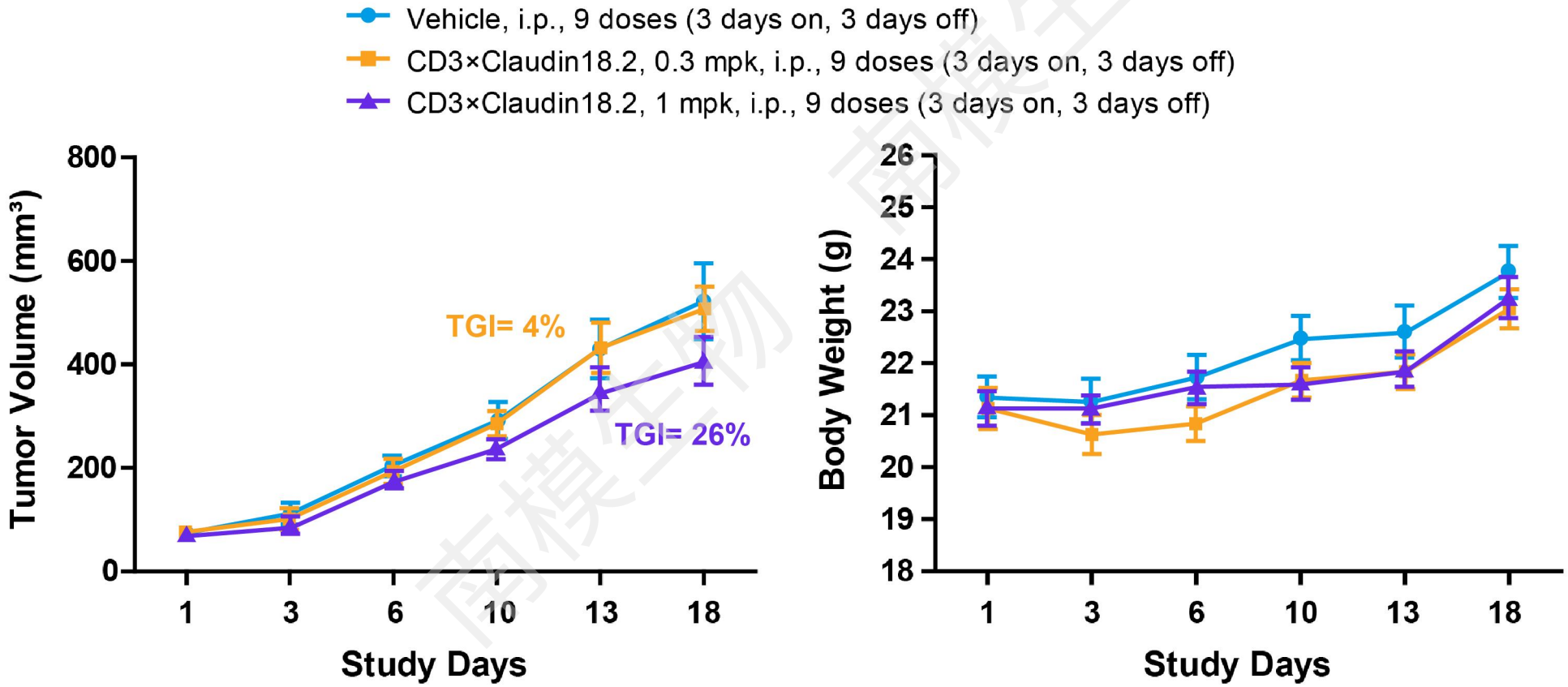
- Heterozygous hCD3EDG mice were engrafted with MC38-hCLDN18.2 to evaluate the *in vivo* efficacy of anti-CD3×Claudin18.2 bispecific antibody in parallel with homozygous hCD3EDG mice.



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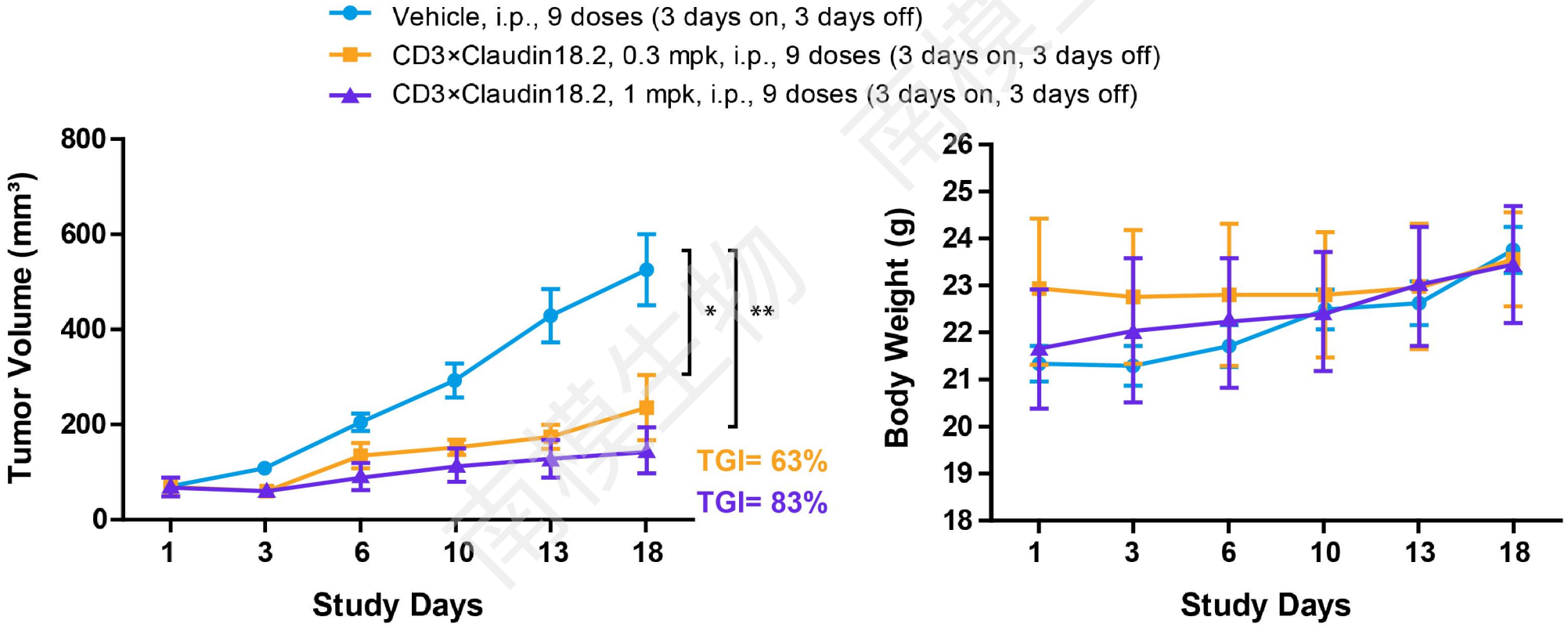

4. *In Vivo* Response with PD-1 Antibodies in Homozygous hCD3EDG Mice

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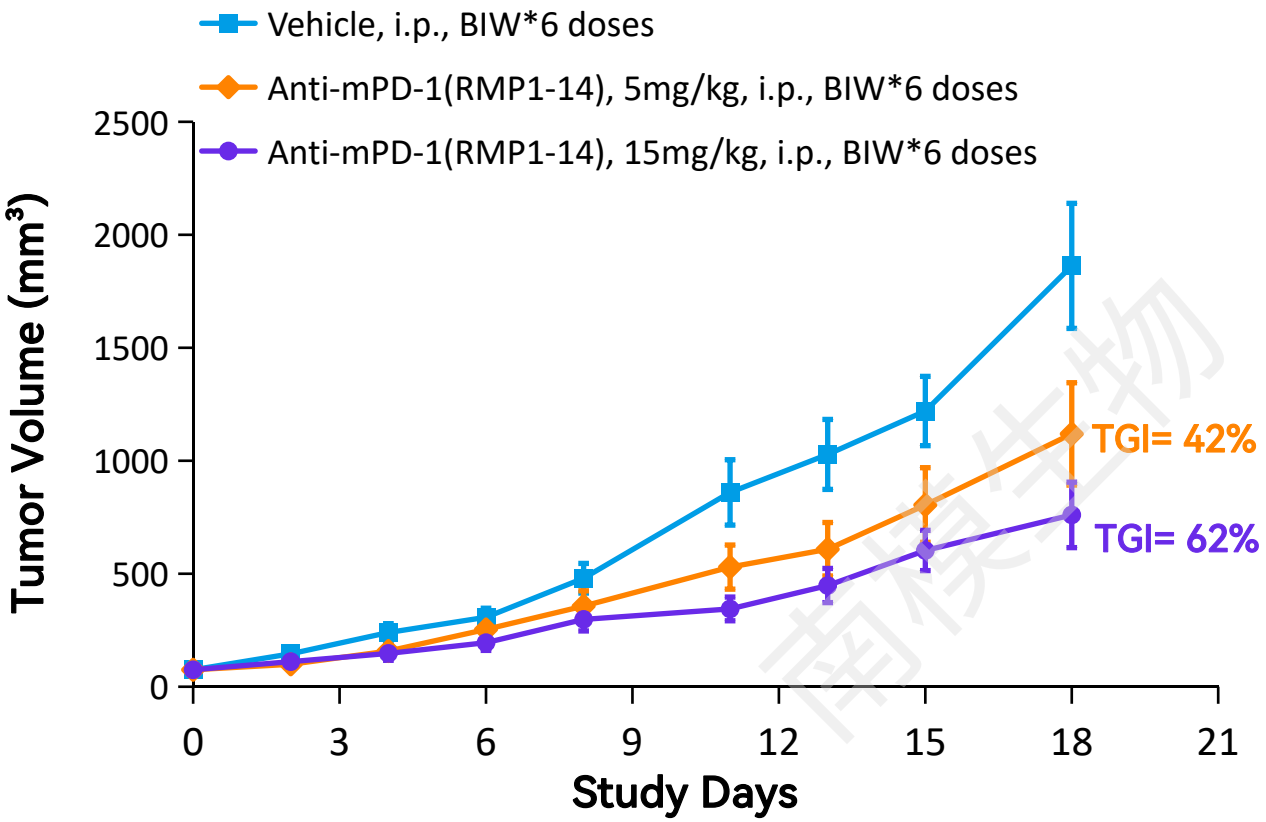
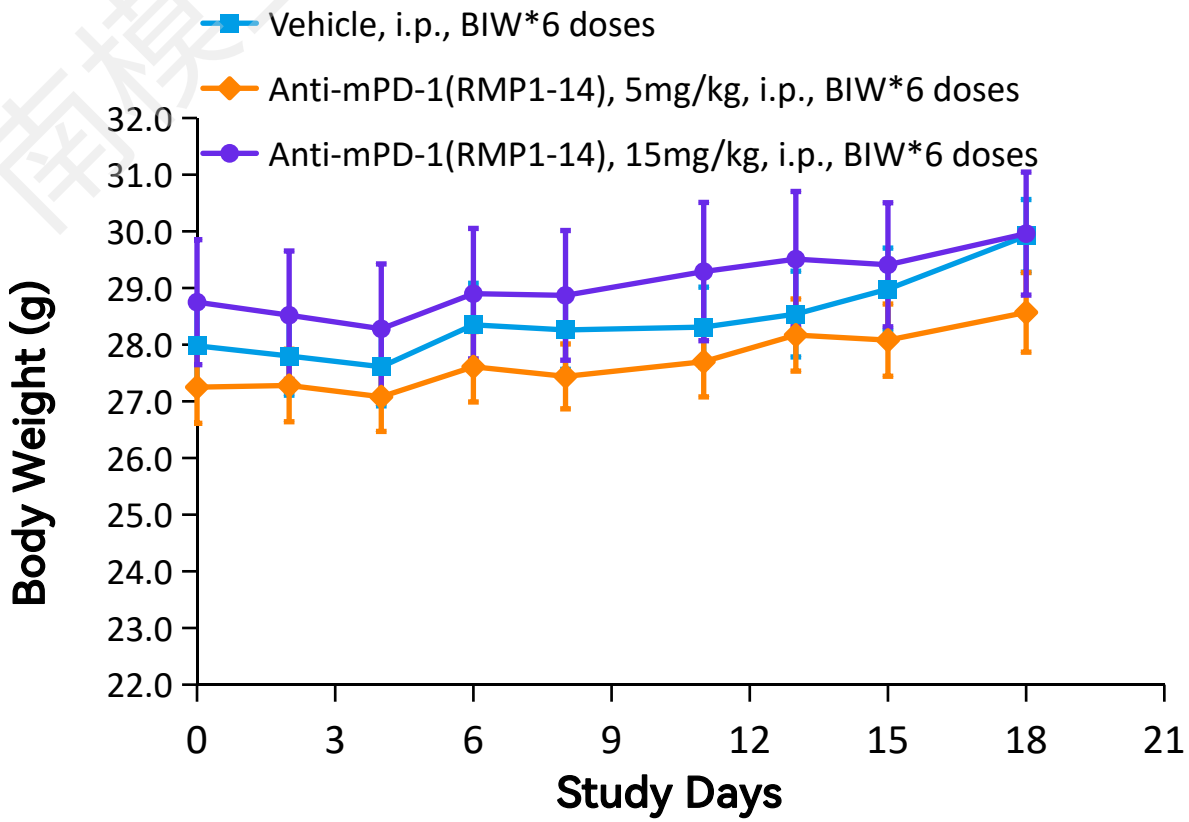
- The anti-tumor response of anti-mPD-1 was evaluated in homozygous hCD3EDG mice bearing MC38 syngeneic tumor model.





4. *In Vivo* Response with PD-1 Antibodies in Homozygous hCD3EDG Mice

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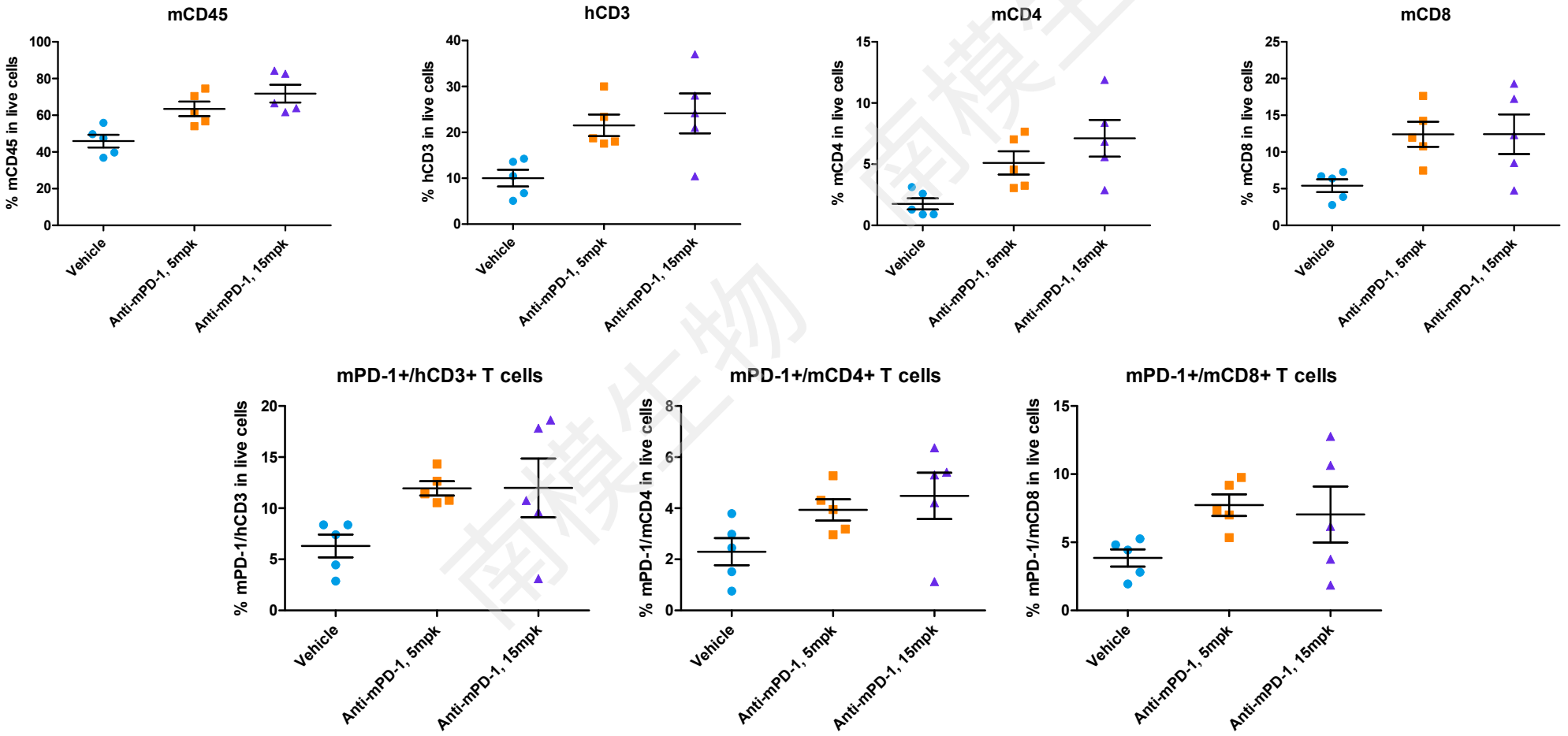
- Analysis of tumor infiltrating lymphocytes upon treatment of anti-mPD-1 in homozygous hCD3EDG.



5. *In vivo* AICD and irAE Assessment of CD3 Bispecific Antibody with Homozygous hCD3EDG Mice

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- Homozygous hCD3EDG mice were engrafted with MC38-hEpCAM to eveluate the AICD and irAE of OKT3 and anti-CD3×EpCAM bispecific antibody *in vivo*.

Group	N	Treatment group	Dose Level	Dosing route	Dose volume	Dosing frequency
1	10	Vehicle	-	*i.v.*	5 uL/g	Day 0, 1, 7, 14, 18
2	10	Anti-hCD3 (OKT3)	0.5 mg/kg	*i.v.*	5 uL/g	Day 0, 1, 7, 14, 18
3	10	Anti-hCD3×hEpCAM	0.5 mg/kg	*i.v.*	5 uL/g	Day 0, 1, 2, 6, 7, 8, 12, 13, 14, 18

5. *In vivo* AICD and irAE Assessment of CD3 Bispecific Antibody with Homozgyous hCD3EDG Mice

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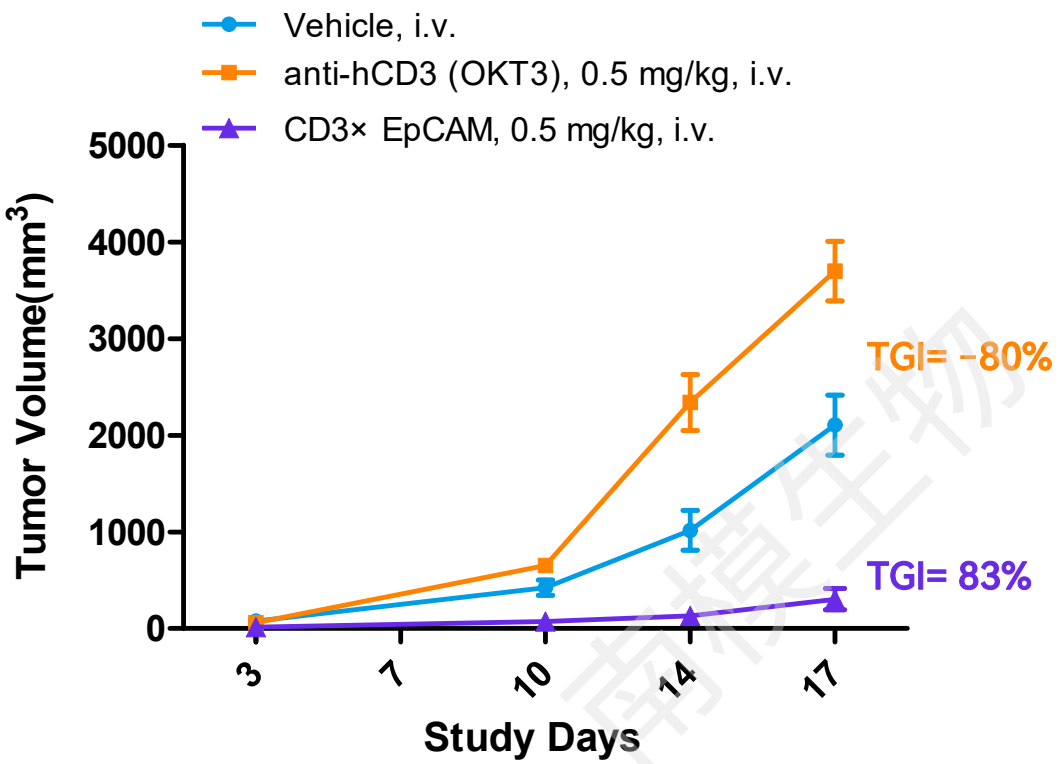

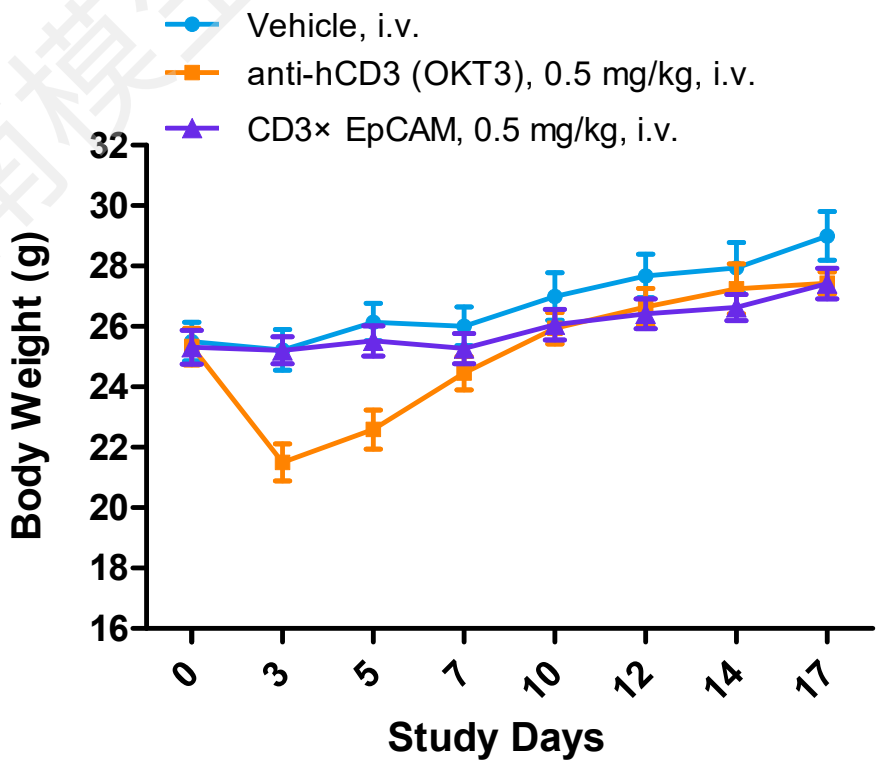

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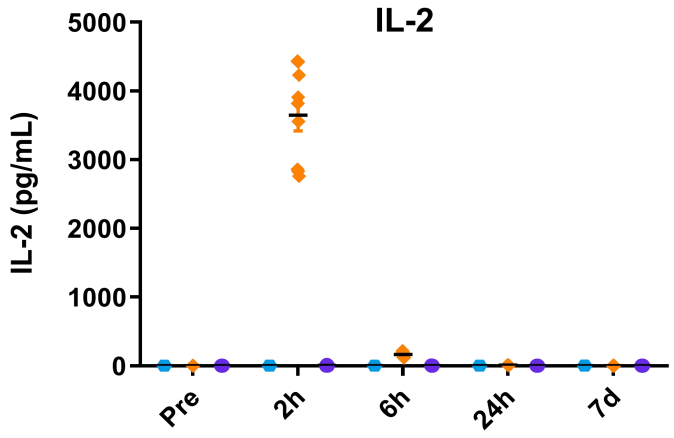

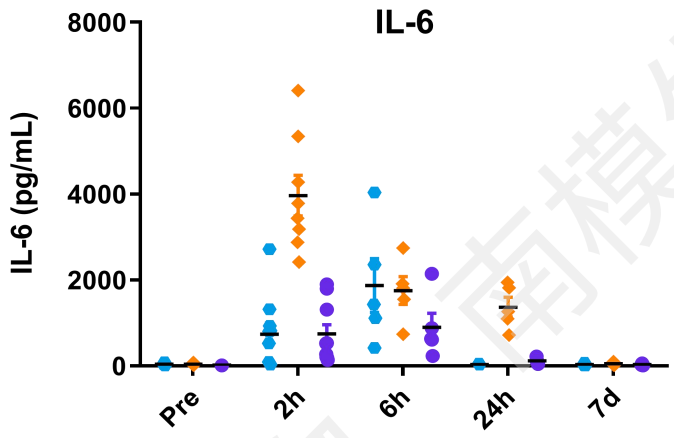

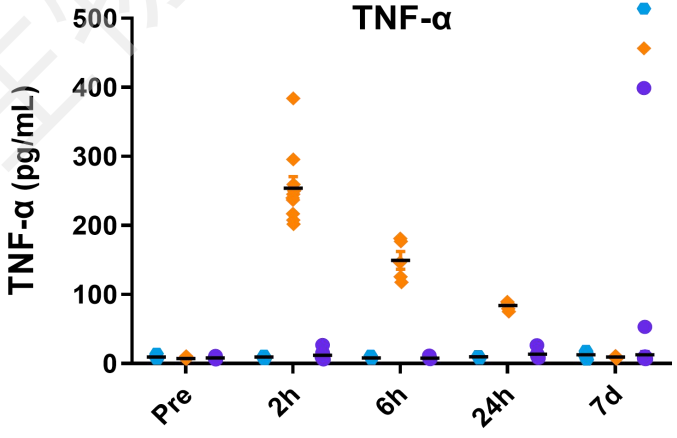

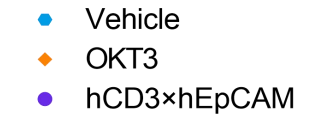

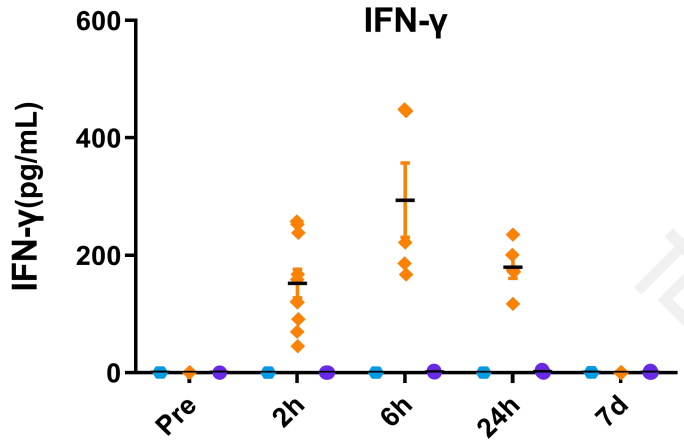

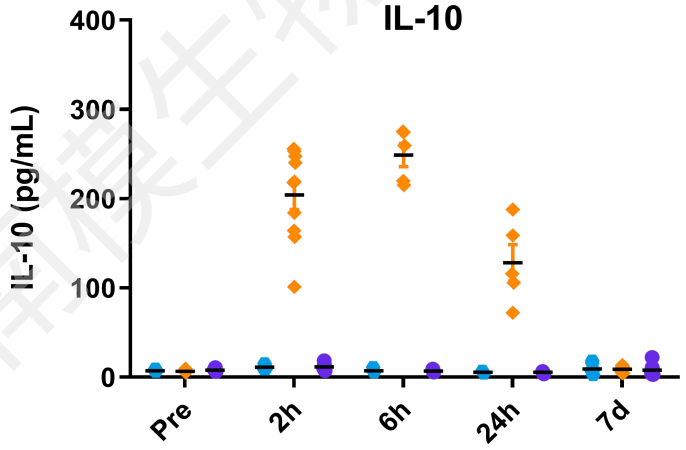

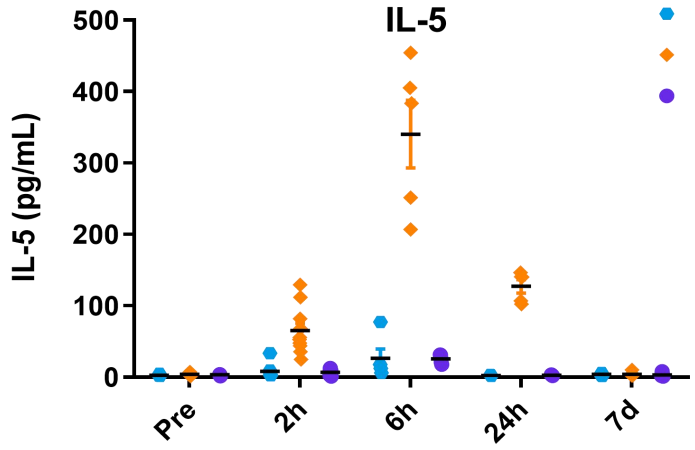

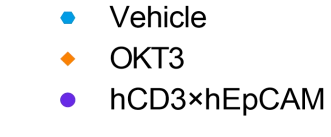

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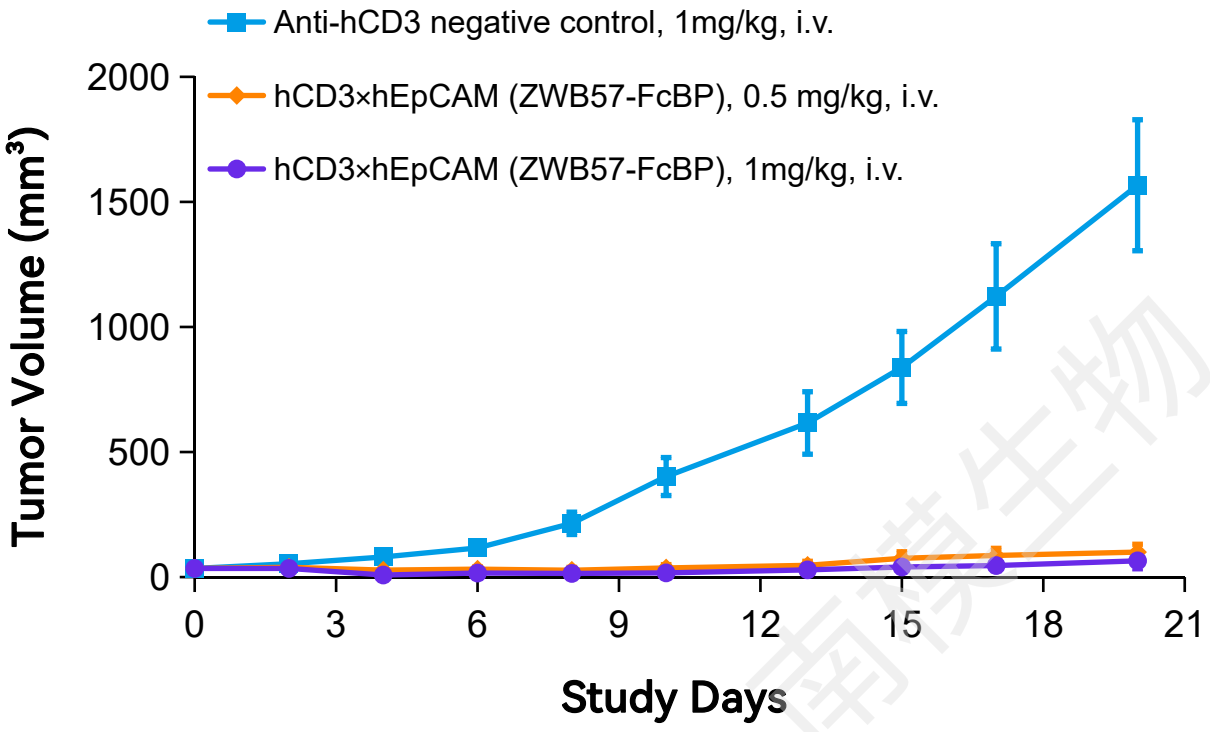
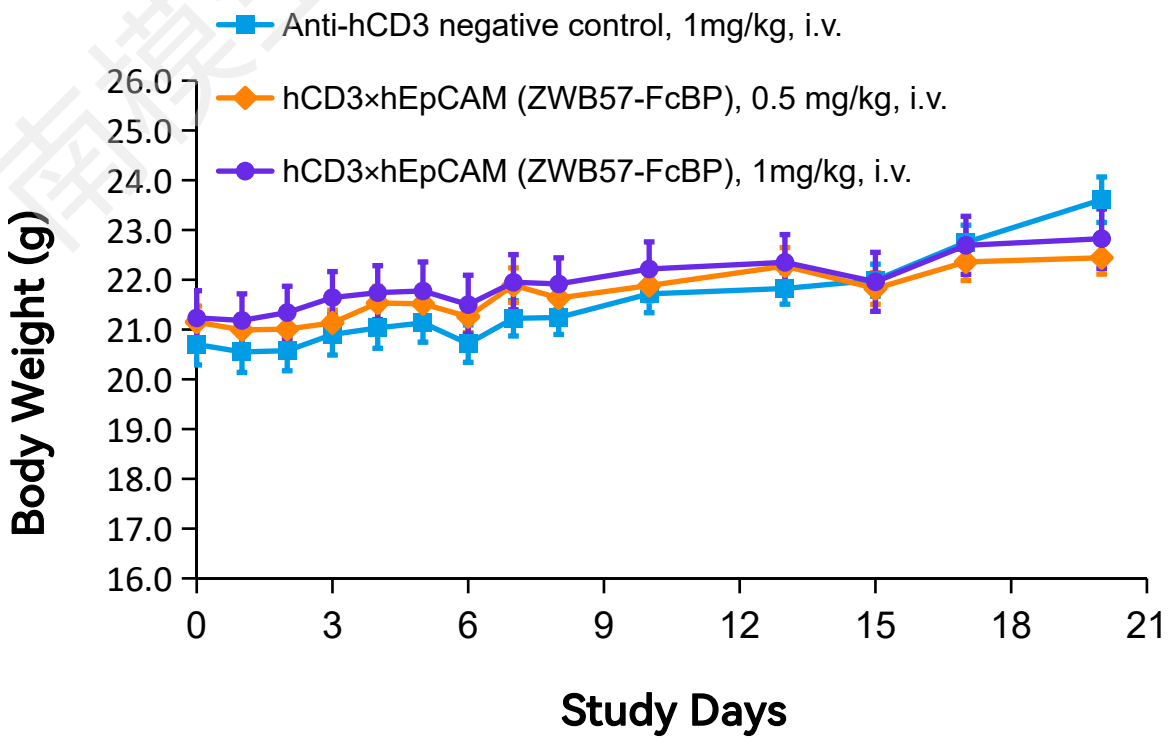
- Homozygous hCD3EDG mice were engrafted with MC38-hEpCAM to eveluate the AICD and irAE of anti-CD3×EpCAM bispecific antibody at low and high dose levels *in vivo*.





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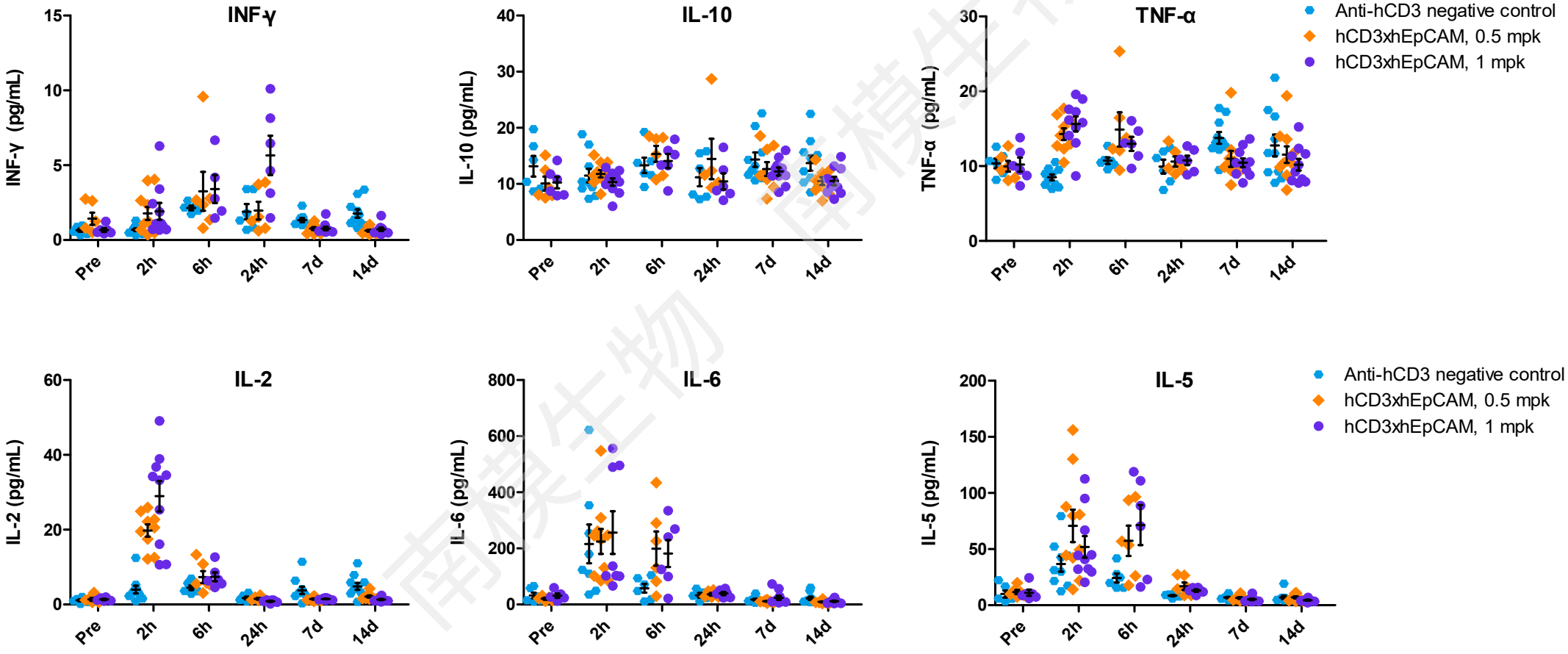

In Vivo Response with Bispecific T Cell Engager Antibodies

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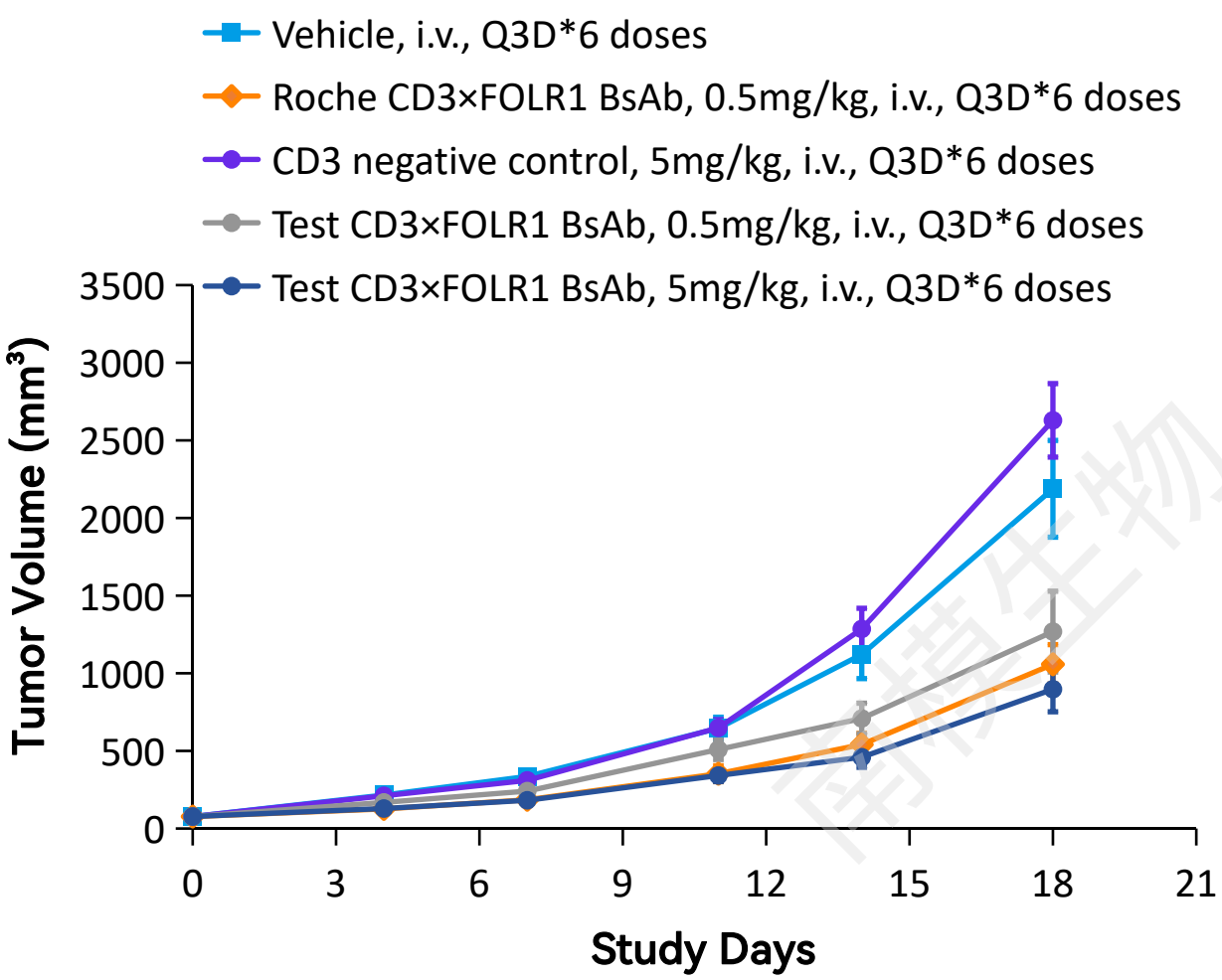

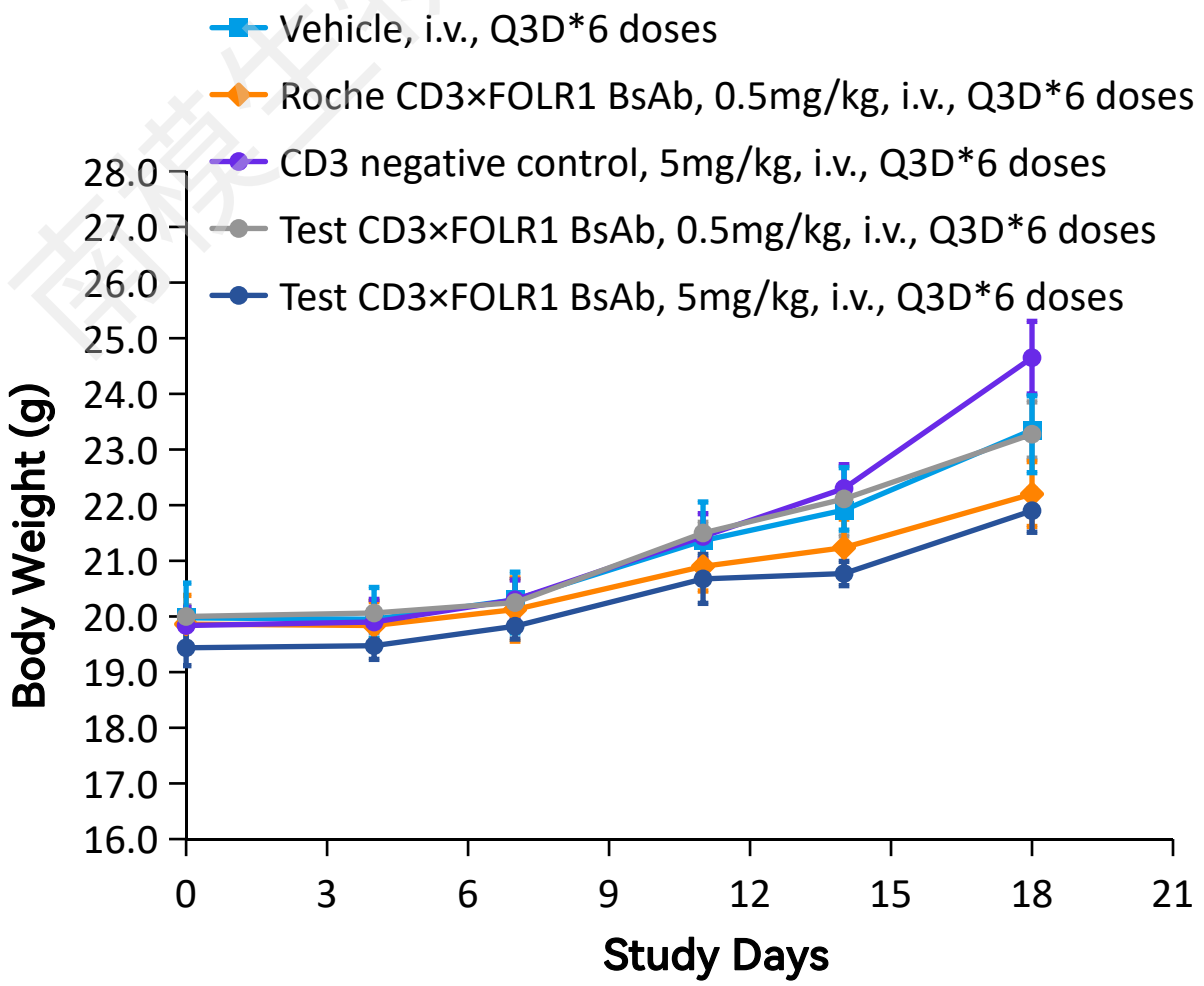

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Thanks!