SHANGHAI
MODEL ORGANISMS

Therapeutic Target-humanized Mouse Models for Efficacy Evaluation of Immunotherapy

Our vision is to become the leader in the field of genetically modified animal models, helping people understand the life science and improving quality of life around the world.

800K

SPF facilities able to house up to 800K mice

18K+

More than 18K GEM models developed so far

8K+

More than 8K Research-Ready GEM models

Founded in 2000, Shanghai Model Organisms Center, Inc. (SMOC) is a leading company in China to offer high-quality animal models and related services to global researchers.

SMOC strives to be the best-in-class resource center for biomedical researchers and industry partners, with its highly efficient and reliable technology platform. We have been dedicated to developing a comprehensive product portfolio, comprised of both highly customized solutions like GEM models and off-the-shelf products. Nowadays SMOC owns a rapidly expanding repository of Research-Ready models, many of which are designed for cutting-edge biomedical research like immuno-oncology studies and therapeutic antibody development.

The supply of animal models to our customers is assured by our state-of-art animal facilities. Currently SMOC operates multiple AAALAC accredited breeding facilities in Shanghai, and owns 100K cages that are available for 800K specific-pathogen free (SPF) mice. In the past decade, SMOC has established a global service network powered by our superior technical platform, talented scientific team and a group of dedicated technical support staff. We proudly work together with researchers from world-renowned academic institutes across the US, EU and APAC, as well as top pharmaceutical companies both domestically and internationally.

Therapeutic Target Humanized Mouse Models

Being recognized as the top scientific breakthroughs in 2013, cancer immunotherapy turns to be one of the most promising research areas. Although many of the immunotherapy's breakthroughs may still lie ahead, important clinical advances have been made in the past few years for some of the deadliest cancers, reaffirming immunotherapy's potential to improve outcomes for patients with many more types of cancers.

However, it is worth noting that drug candidates developed to interfere with human proteins may not comparably interact with their murine counterparts. It is therefore critical to develop humanized mouse models to enable *in vivo* efficacy evaluation of cancer immunotherapies.

Since 2015, Shanghai Model Organisms Center, Inc has generated over 5 00 immune checkpoint humanized mous e models, including single gene humanized, double humanized or even triple humanized models. Thanks to o ur unprecedentedly high R&D and production capacity, the list of available humanized models is rapidly expanding.

Therapeutic Target Humanized Mouse Models Available at SMOC

4-1BB	*CD47*	*LAIR1*	*PD-1 & OX40*	*PD-L1 & SEMA4D*	*TIM3*
4-1BBL	*CD52*	*MARCO*	*PD-1 & PD-L1*	*PD-L1 & SIGLEC15*	*TLR1*
ANGPTL3	*CD74*	*MASP2*	*PD-1 & PD-L1 & IDO1*	*PD-L1 & SIRPA*	*TLR2*
BTLA	*CD79A*	*MERTK*	*PD-1 & PD-L1 & LAG3*	*PD-L1 & TIGIT*	*TLR4*
CD147	*CD79B*	*MIF*	*PD-1 & PD-L1 & OX40*	*PD-L1 & TIM3*	*TLR7*
CD160	*CD80*	*NKG2A & CD94*	*PD-1 & PD-L1 & PD-L2*	*PD-L1 & TMEM173*	*TLR7 & TLR8*
CD19	*CD81*	*NR1I2*	*PD-1 & SEMA4D*	*PD-L1 & VISTA*	*TLR8*
CD19 & CD3E	*CD81 & OCLN*	*NT5E*	*PD-1 & SIGLEC15*	*PD-L2*	*TLR9*
CD20	*CD86*	*OX40*	*PD-1 & SIRPA*	*PSGL-1*	*TLR9 & OX40*
CD22	*CD96*	*OX40 & CTLA4*	*PD-1 & TIGIT*	*PVR*	*TMEM173*
CD24	*CTLA4*	*OX40L*	*PD-1 & TIGIT & TIM3*	*PVRL2*	*TNFRSF18*
CD27	*GITR*	*PCSK9*	*PD-1 & TIM3*	*SEMA4D*	*TNFRSF1B*
CD28	*HAVCR2*	*PD-1*	*PD-1 & TLR9*	*SIGLEC10*	*TNFRSF25*
CD30	*ICOS*	*PD-1 & CD27*	*PD-1 & TMEM173*	*SIGLEC15*	*TNFSF11*
CD33	*ICOS & ICOSL*	*PD-1 & CD28*	*PD-1 & VISTA*	*SIRPA*	*TNFSF4*
CD36	*ICOSL*	*PD-1 & CD3E*	*PD-L1*	*SIRPA & CD47*	*VISTA*
CD38	*ICOSLG*	*PD-1 & CD40*	*PD-L1 & CD40*	*SIRPA & CD47 & PD-1*	*VTCN1*
CD3E	*IDO1*	*PD-1 & CTLA4*	*PD-L1 & CTLA4*	*SIRPA & CD47 & PD-L1*	
CD3E DG	*KITLG*	*PD-1 & GITR*	*PD-L1 & GITR*	*SLAMF7*	
CD40	*KLRK1*	*PD-1 & ICOS*	*PD-L1 & KDR*	*THPO*	
CD40L	*LAG3*	*PD-1 & KDR*	*PD-L1 & LAG3*	*TIGIT*	
	LAG3 & CTLA4	*PD-1 & LAG3*	*PD-L1 & OX40*	*TIGIT & PVR*	

To get to know more about these models, visit our website **www.modelorg.us**
or contact our technical experts at **service.us@modelorg.com**

Humanized PD-1 Mouse

Strain Name: C57BL/6-*Pdcd1*$^{em1(hPDCD1)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00015

Programmed cell death protein 1, also known as PD-1 or CD279, is a cell surface receptor on activated T cells. PD-1 is an important immune checkpoint molecule that negatively modulates T cell responses upon the binding of its ligand, PD-L1. Increasing evidence indicates that the PD-L1 expression on the surface of tumor cells is up-regulated in tumor micro-environment. The binding of PD-L1 to PD-1 on activated T cells results in an apoptosis or immune disability of tumor antigen-specific T cells, thereby suppressing anti-tumor immune responses. The blockade of PD-L1 binding to PD-1 reverses T cell exhaustion and thus strengthens anti-tumor activity, which has become a classic method for enabling tumor immunotherapy.

Construction strategy

On the C57BL/6 background, the full-length coding sequence of human PDCD-1 gene was placed immediately downstream of the start codon of the mouse endogenous Pdcd1, followed by a poly(A) element. This guarantees an exclusive expression of human PD-1 in the humanized PD-1 mice.

Validation data

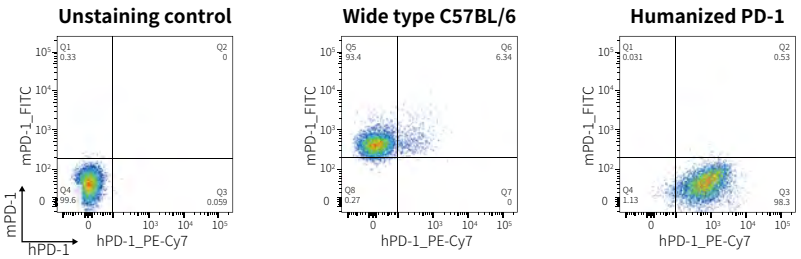


Figure 1. A complete switch from mouse to human PD-1 expression in the activated spleen lymphocytes derived from homozygous, humanized PD-1 mice was confirmed by FACS.

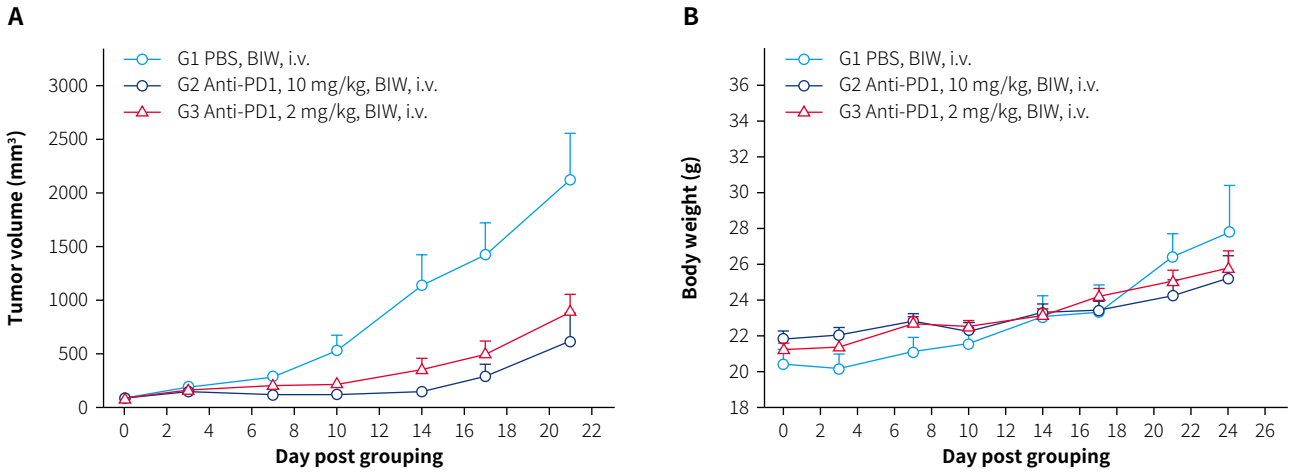


Figure 2. *In vivo* anti-tumor effect of an anti-human PD-1 antibody in a humanized mouse model of PD-1. Anti-human-PD-1 drugs significantly inhibited the growth of MC38 tumors in humanized PD-1 (hPD-1) mice, demonstrating that the hPD-1 mice can be used to assess the anti-human PD-1 antibody (In collaboration with PharmaLegacy).

Name: BALB/c-*Pdcd1* $^{em1(hPDCD1)Smoc}$ Strain Background: BALB/c Cat. NO. : NM-HU-190079

Construction strategy

The BALB/c-Pdcd1 $^{em1(hPDCD1)Smoc}$ (abbreviated as BALB/c-hPD1) mouse genetic stock was developed by first crossing BALB/c females with B6-hPD1 males.

Validation data

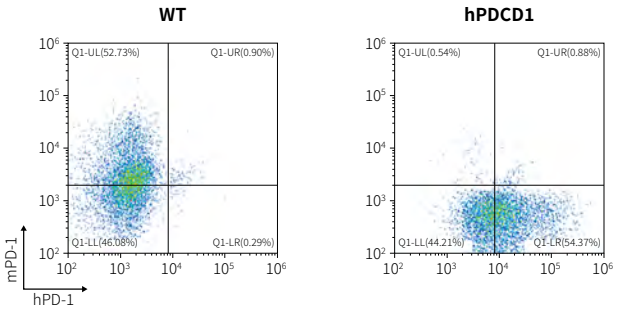


Figure 3. A complete switch from mouse to human PD-1 expression in the activated spleen lymphocytes derived from homozygous, BALB/c-hPD1 mice were confirmed by FACS.

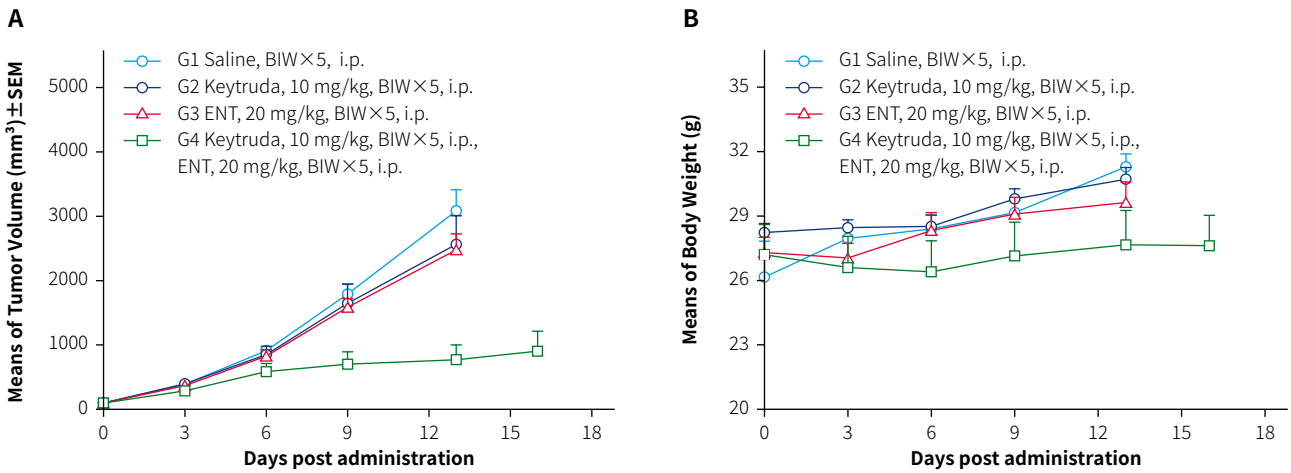


Figure 4. *In vivo* validation of homozygous BALB/c-hPD1 mice. The homozygous BALB/c-hPD1 mice were inoculated with CT26 cells, and randomly assigned to different groups (n=7) when the tumor grew to a volume of 100 mm^3. A combinatorial treatment of anti-hPD1 antibody Keytruda and Entinostat (ENT; a class I HDAC inhibitor) demonstrated a noticeable efficacy improvement compared to the same dose of single agent **(A)** without affecting the animal body weight **(B)**.

Humanized PD-L1 Mouse

Strain Name: C57BL/6-*Cd274*$^{em1(hPD\text{-}L1)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00062

Programmed cell death 1 ligand (PD-L1), also known as cluster of differentiation 274(CD274) or B7 homolog 1 (B7H1), is a 40 kDa transmembrane protein encoded by the gene CD274 in human. The binding of PD-L1 to the PD-1 receptors expressed on the surface of activated T cells transmits a negative regulatory signal. While under normal circumstances the PD-L1 pathway acts as a type of "off switch" that helps keep the T cells from attacking other cells, the high expression level of PD-L1 in the tumor microenvironment inhibits the function of tumor-infiltrating T cells, thereby allowing tumors cells to escape immune surveillance.

Construction strategy

On the C57BL/6 background, the full-length coding sequence of human CD274 gene was placed immediately downstream of the start codon of the mouse endogenous Cd274 gene, followed by a poly(A) site. This guarantees an exclusive expression of human PD-L1 in the humanized PD-L1 mice.

Validation data

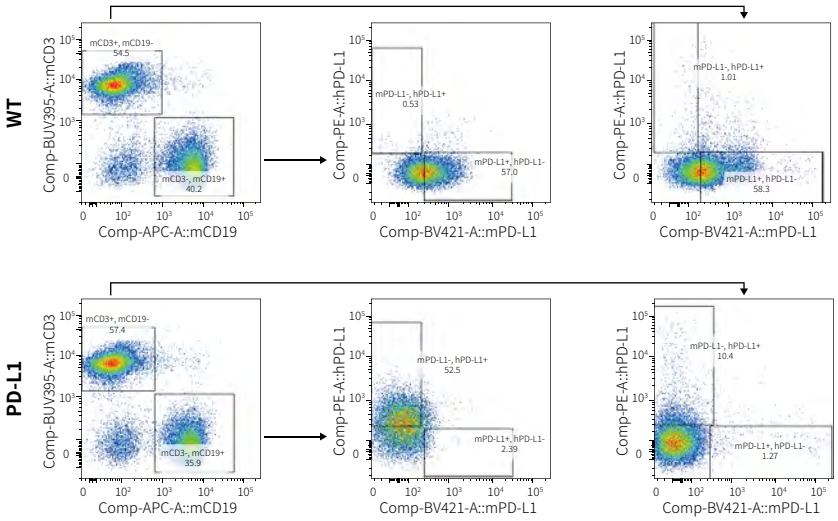


Figure 5. FACS analysis of humanized PD-L1 mice. Splenocytes from both WT C57BL/6 and homozygous, humanized PD-L1 mice were analyzed by FACS. Human PD-L1 expression was confirmed in both T cells and B cells derived from humanized PD-L1 mice (In collaboration with CrownBio).

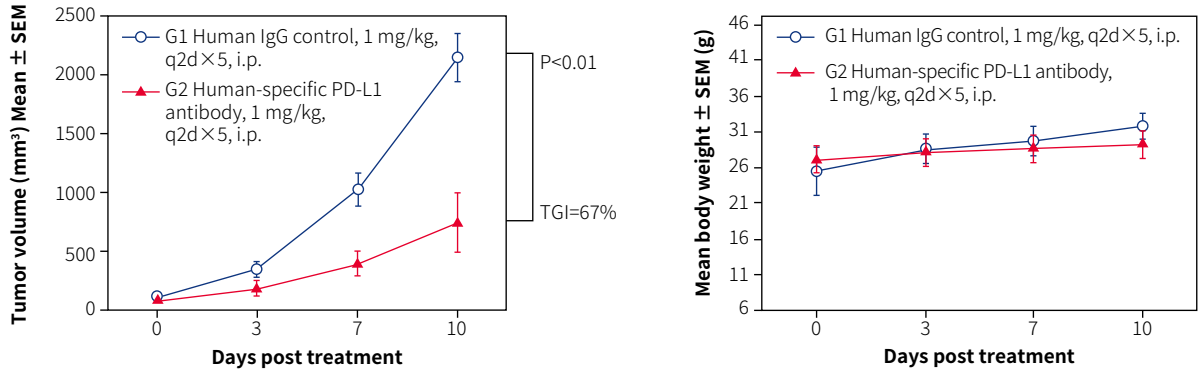


Figure 6. *In vivo* validation of humanized PD-L1 mice. (Left) Mouse colon cancer cells MC38 engineered to express human PD-L1 were implanted subcutaneously into homozygous, humanized PD-L1 mice. The mice were randomly assigned into two groups when the tumor volume reached 100 mm^3, one group receiving human IgG as a control while the other receiving a human-specific, PD-L1 antibody. The human PD-L1 blocking antibody significantly inhibited tumor growth in the homozygous humanized mice (TGI = 67%) without affecting overall body weight (Right), suggesting that humanized PD-L1 mice represents an ideal model for evaluating the efficacy of therapeutic antibodies targeting human PD-L1.

Double Humanized PD-1&PD-L1 Mouse

Strain Name: C57BL/6-*Pdcd1*$^{em1(hPDCD1)}$ *Cd274*$^{em1(hPD-L1)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00100

Checkpoint blockade is a promising immunotherapy approach to block the function of immune checkpoint proteins. In the recent years, checkpoint blockade therapies, particularly monoclonal antibodies blocking the inhibitory programmed cell death 1 (PD-1/PD-L1) pathway, have achieved significant clinical advances, leading to durable therapeutic responses and long-term remission for a growing number of solid and hematological malignancies.

Construction strategy

On the C57BL/6 background, the full-length coding sequence of human CD274 gene was placed immediately downstream of the start codon of the mouse endogenous Cd274 gene, followed by a poly(A) site. This guarantees an exclusive expression of human PD-L1 in the humanized mice. A similar construction strategy was used for Pdcd-1 gene replacement.

Validation data

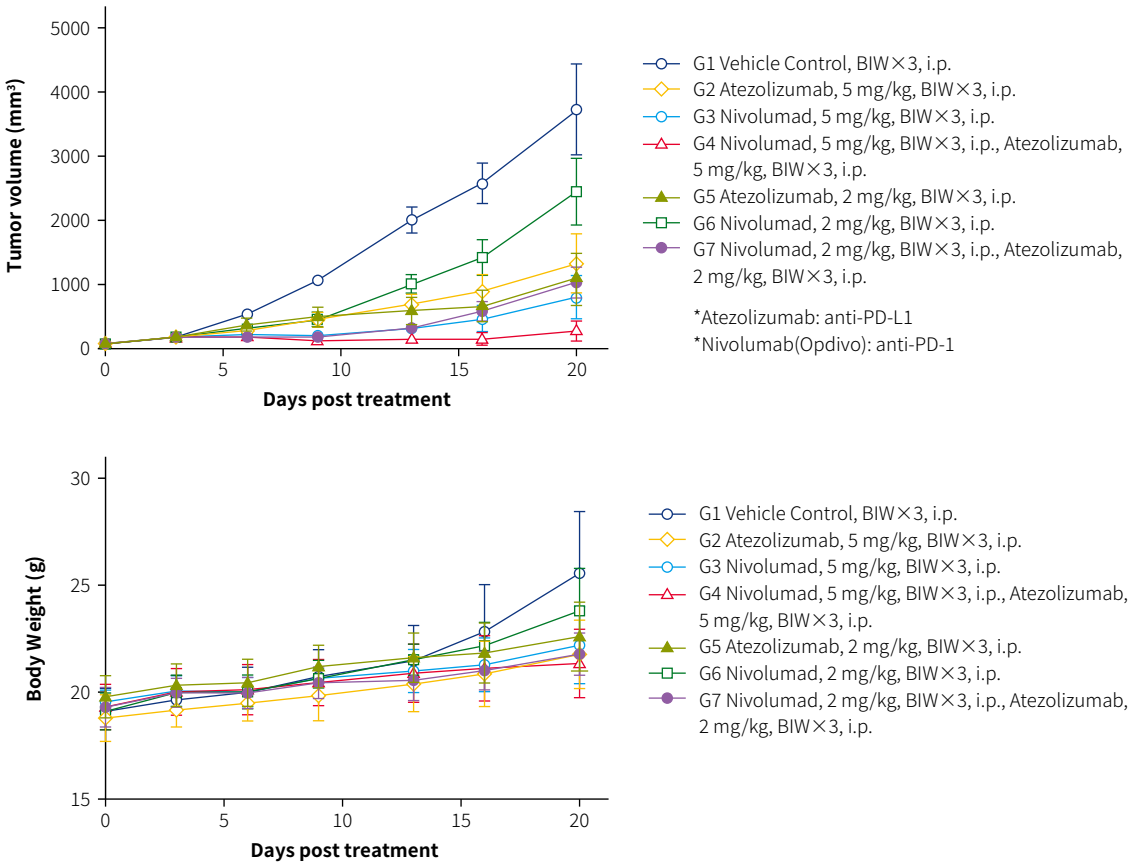


Figure 7. *In vivo* validation of double humanized PD-1&PD-L1 mice. Double humanized mice were inoculated with MC38 cells, and randomly assigned to different groups (n=8) when the tumor grew to a volume of 100 mm^3. A combinatorial treatment of anti-PD-L1 and anti-PD-1 demonstrated a noticeable efficacy improvement compared to the same dose of single agent (top) without affecting the animal body weight (Bottom).

Humanized CTLA4 Mouse

Strain Name: C57BL/6-*Ctla4* $^{em1(hCTLA4)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00014

CTLA4(cytotoxic T-lymphocyte-associated protein 4), also known as CD152, is a transmembrane glycoprotein that functions as an immune checkpoint. CTLA4 is constitutively expressed in regulatory T cells and upregulated in activated T cells. It acts as an "off" switch to downregulate immune responses upon bound to CD80 or CD86 on the surface of antigen-presenting cells (APC).

Construction strategy

Humanized CTLA4 mice were developed on the C57BL/6 genetic background. The full-length coding sequence of human CTLA4 was inserted immediately downstream of the start codon of the mouse endogenous Ctla4 gene, leading to an exclusive expression of the human CTLA4 in the humanized mice.

Validation data

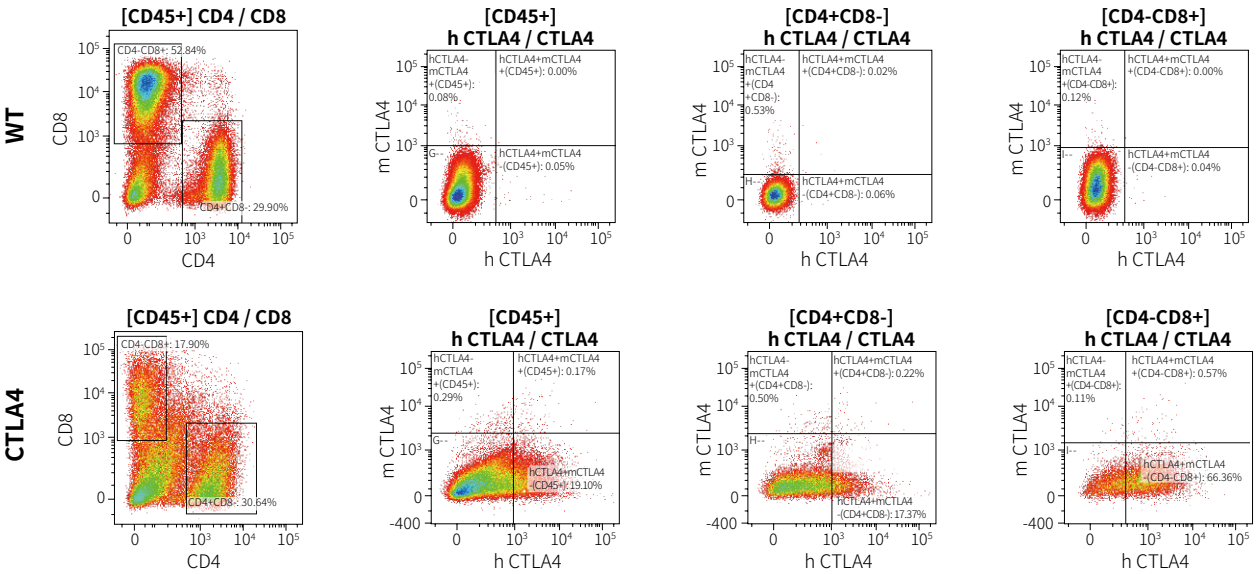


Figure 8. The expression of human CTLA4 in the splenocytes of humanized CTLA4 mice was confirmed by FACS. Spleen lymphocytes were harvested from homozygous, humanized CTLA4 mice, activated by CD3& CD28 antibodies for 48 hrs and then subjected to staining. Active expression of humanized CTLA4 can be detected in both activated $CD4^+$ and $CD8^+$ T lymphocytes derived from homozygous humanized CTLA4 mice (In collaboration with CrownBio).

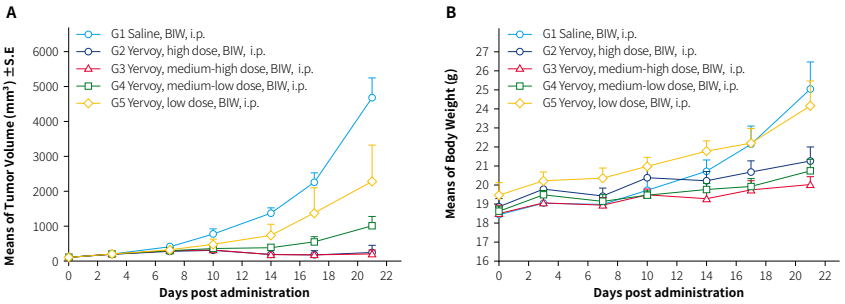


Figure 9. Efficacy evaluation of human CTLA4 blocking antibody YERVOY® in the homozygous humanized CTLA4 mice. The animals were inoculated with MC38 colon cancer cells, and randomly assigned into a control group receiving saline and some treatment groups receiving the human specific, anti-CTLA4 antibody YERVOY. The humanized CTLA4 mice responded to high or medium-high dose of YERVOY with a strong anti-tumor effect (Left) without a significant body weight change (Right), demonstrating the utility of humanized CTLA4 mice for the efficacy evaluation of therapeutic CTLA4 antibody (In collaboration with PharmaLegacy).

Strain Name: BALB/c-*Ctla4*$^{em1(hCTLA4)\ Smoc}$ Strain Background: BALB/c Cat. NO. : NM-HU-190078

Construction strategy

The BALB/c-Ctla4 $^{em1(hCTLA4)Smoc}$ (abbreviated as BALB/c-hCTLA4) mouse genetic stock was developed by first crossing BALB/c females with B6-hCTLA4 males.

Validation data

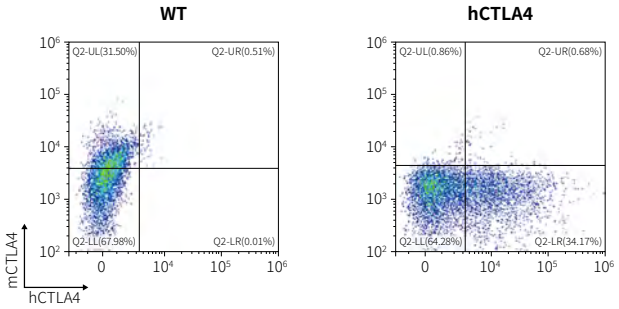


Figure 10. Expression of human CTLA4 in the activated spleen lymphocytes of homozygous, BALB/c-hCTLA4 mice were confirmed by FACS.

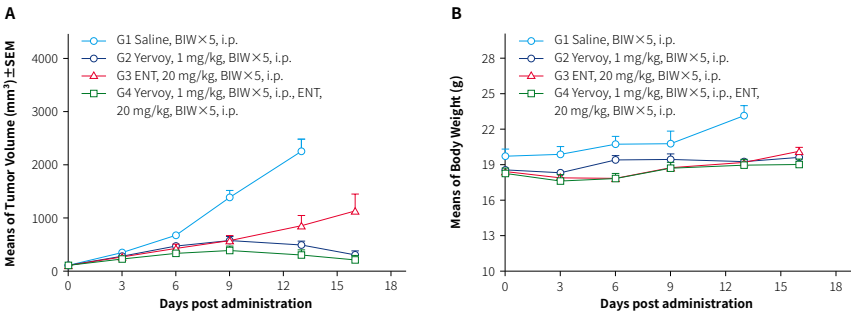


Figure 11. *In vivo* validation of homozygous BALB/c-hCTLA4 mice. The homozygous BALB/c-hCTLA4 mice were inoculated with CT26 cells, and randomly assigned to different groups (n=7) when the tumor grew to a volume of 100 mm^3. A combinatorial treatment of anti-hCTLA4 antibody Yervoy and Entinostat (ENT; a class I HDAC inhibitor) demonstrated a noticeable efficacy improvement compared to the same dose of single agent **(A)** without affecting the animal body weight **(B)**.

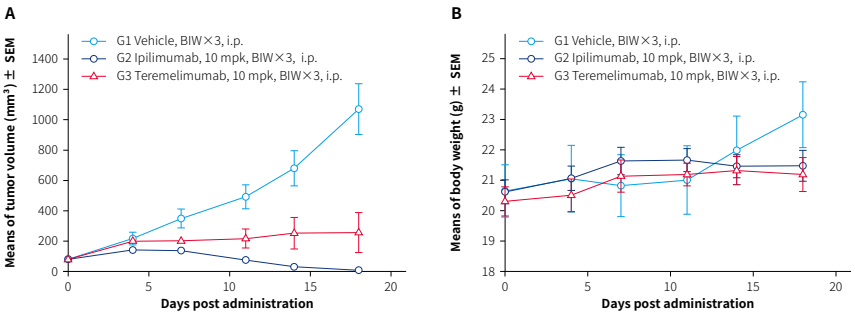


Figure 12. *In vivo* validation of homozygous BALB/c-hCTLA4 mice. The homozygous BALB/c-hCTLA4 mice were inoculated with H22 cells, and randomly assigned to different groups (n=7) when the tumor grew to a volume of 100 mm^3. Treatment of anti-hCTLA4 antibody Yervoy and Teremelimumab can effectively inhibit tumor growth in BALB/c-hCTLA4 mice **(A)** without affecting the animal body weight **(B)**.

Double Humanized PD-1&CTLA-4 Mouse

Strain Name: C57BL/6-*Pdcd1*$^{em1(hPDCD1)}$ *Ctla4*$^{em1(hCTLA4)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00079

Double humanized PD-1 and CTLA-4 mice provide a unique and valuable model for evaluating human specific, combinatorial antibody therapies.

Construction strategy

On the C57BL/6 background, the full-length coding sequence of human CTLA-4 gene was placed immediately downstream of the start codon of the mouse endogenous Ctla4 gene, followed by a poly(A) element. This guarantees an exclusive expression of human CTLA-4 in the double humanized mice. A similar construction strategy was used for the Pdcd1 gene replacement.

Validation data

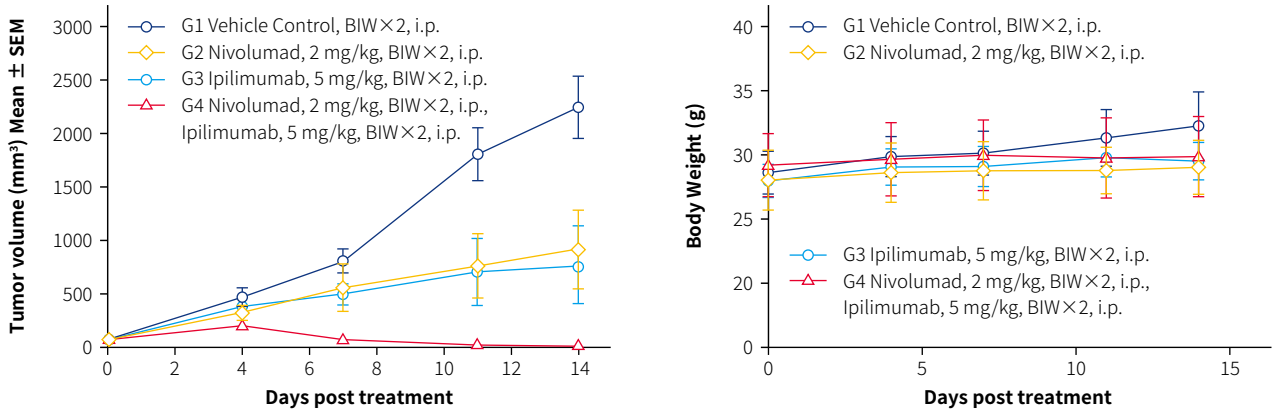


Figure 13. *In vivo* validation of double humanized PD-1&CTLA-4 mice. Double humanized mice were inoculated with MC38 cells, and randomly assigned to different groups (n=8) when the tumor grew to a volume of 100 mm^3. A combinatorial treatment of anti-CTLA-4 and anti-PD-1 demonstrated a significant efficacy improvement compared to the same dose of single agent (left) without affecting the animal body weight (right).

* Nivolumab: PD-1 inhibitor marketed as Opdivo®

* Ipilimumab:Human-specific, CTLA-4-targeting antibody marketed as Yervoy®

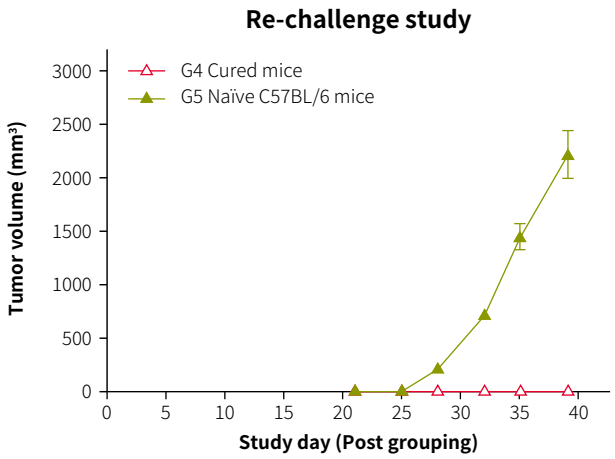


Figure 14. As shown in the previous figure, animals of group 1-4 received different doses of either single agent or combinatorial treatment. The left graph showed the progression of the tumor implanted at the right flank, implicating a systematic anti-tumor effect induced by the combinatorial antibody treatment. The cured, G4 mice were then re-grafted with MC38 cells engineered to express human PD-L1 on the opposite flank, while naïve C57BL/6 mice were used as controls (right).

Double Humanized PD-L1&CTLA4 Mouse

Strain Name: C57BL/6-*Ctla4* $^{em1(hCTLA4)}$ *Cd274* $^{em1(hPD-L1)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00102

Double humanized PD-L1 and CTLA-4 mice provide a unique and valuable model for evaluating human specifc, combinatorial antibody therapies.

Construction strategy

On the C57BL/6 background, the full-length coding sequence of human CTLA-4 gene was placed immediately downstream of the start codon of the mouse endogenous Ctla4 gene, followed by a poly(A) element. This guarantees an exclusive expression of human CTLA-4 in the double humanized mice. A similar construction strategy was used for the Cd274 gene replacement.

Validation data

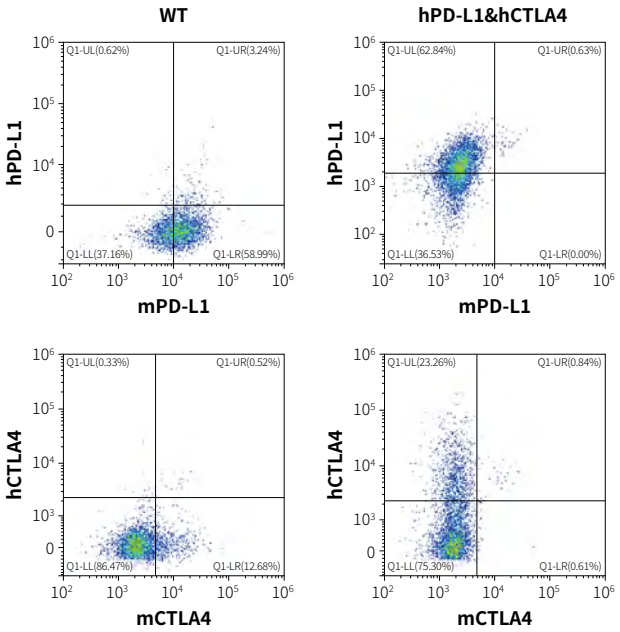


Figure 15. Splenocytes from homozygous hPDL1/hCTLA4 mice were analyzed by flow cytometry after activation. mPDL1$^+$ and mCTLA4$^+$ cells were detectable in wild type (WT) C57BL/6, while hPDL1$^+$ and hCTLA4$^+$ cells were detectable in the homozygous hPDL1/hCTLA4 mice.

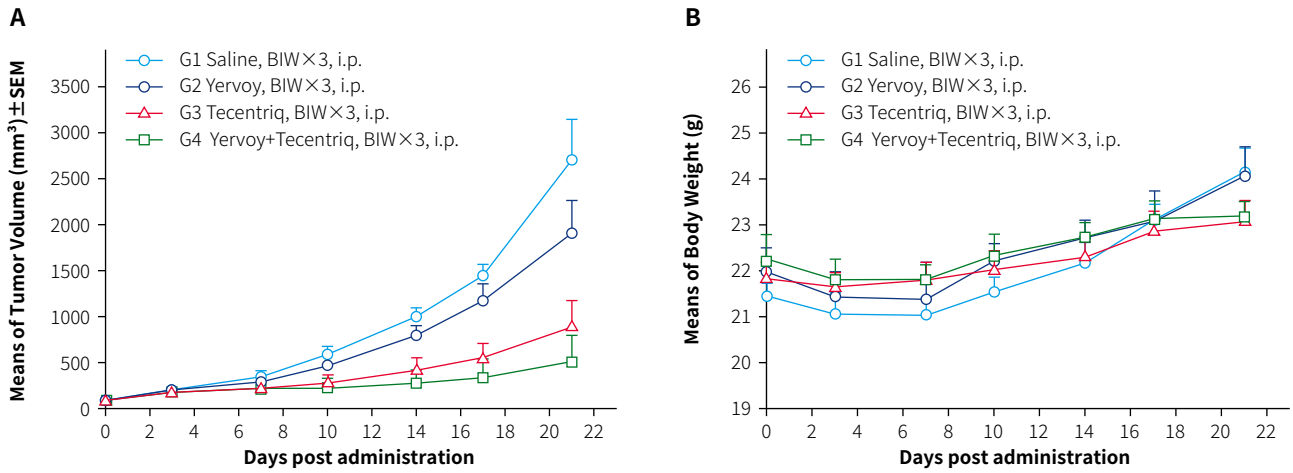


Figure 16. *In vivo* validation of double humanized PD-L1&CTLA4 mice. Double humanized mice were inoculated with MC38 cells, and randomly assigned to different groups when the tumor grew to a volume of 100 mm^3. A combinatorial treatment of anti-PD-L1 and anti-CTLA4 demonstrated a noticeable efficacy improvement compared to single-agent therapy (top) without affecting the animal body weight (Bottom).

Humanized TIGIT Mouse

Strain Name: C57BL/6-*Tigit*$^{em1(hTIGIT)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00053

TIGIT (T-cell immunoreceptor with Ig and ITIM domains) is identified as a co-inhibitory molecule in the PVR family of immunoglobulin (Ig) proteins. Upon the interaction of TIGIT with its ligand, TIGIT can inhibit the functions of anti-tumor immune cells at multiple steps. The blockade of TIGIT has been shown to restore the cytotoxicity of NK cells, thereby facilitating the elimination of tumor cells.

Construction strategy

The humanized TIGIT mouse model was developed on the C57BL/6 background. A chimeric expression cassette that encodes the extracellular domain of human TIGIT as well as the transmembrane and intracellular domains of murine Tigit was inserted immediately downstream of the start codon of the mouse endogenous Tigit gene, followed by a poly(A) signal. Thereby, the extracellular domain of the mouse Tigit was replaced by its human counterpart while the rest of the mouse gene was retained.

Validation data

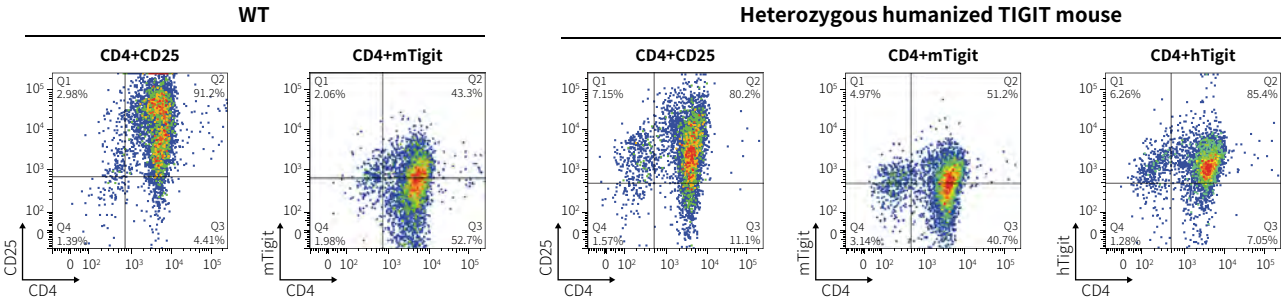


Figure 17. The expression of human TIGIT in the polarized CD4$^+$ T cells derived from humanized TIGIT mice was confirmed by FACS. Naive spleen CD4$^+$ T cells were isolated from heterozygous humanized TIGIT mice. After *in vitro* stimulation, activation and expansion by cytokines and antibodies, the CD4$^+$ T cells were re-stimulated with PMA/ionomycin, followed by the measurement of human TIGIT expression in the polarized CD4$^+$ T cells by FACS. The results demonstrated an active expression of human TIGIT in polarized CD4$^+$ T cells derived from heterozygous humanized mice, with a comparable expression level to the endogenous mouse TIGIT.

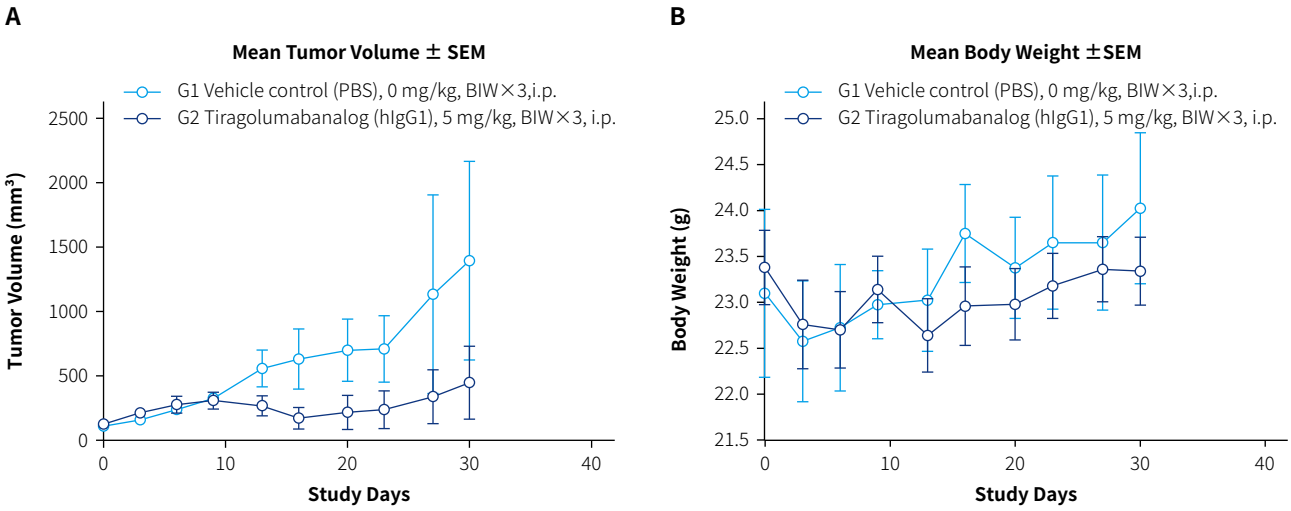


Figure 18. *In vivo* validation of anti-tumor efficacy in a Hepa1-6 tumor-bearing model of humanized TIGIT mice. Homozygous humanized TIGIT mice were inoculated with Hepa1-6 cells. After the tumors grew to 110 mm^3, the animals were randomly assigned into a control group and a treatment group. The results showed a significant anti-tumor effect was observed when the antibody targeting human TIGIT (In collaboration with CrownBio).

Double Humanized TIGIT&PVR Mouse

Strain Name: C57BL/6-*Tigit*$^{em1(hTIGIT)}$ *Pvr*$^{em1(hPVR)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-2000091

TIGIT and its ligand PVR (poliovirus receptor, CD155) have recently emerged as promising targets in immunotherapy. PVR is a member of the nectin-like family of proteins. Similar to other members of the family, PVR is involved in important cellular processes, such as adhesion, contact inhibition, migration, proliferation, and the immune response.

Construction strategy

The humanized TIGIT&PVR mouse model was developed on the C57BL/6 background. A chimeric expression cassette that encodes the extracellular domain of human PVR as well as the transmembrane and intracellular domains of murine PVR was inserted immediately downstream of the start codon of the mouse endogenous Pvr gene, followed by a poly(A) signal. Thereby, the extracellular domain of the mouse Pvr was replaced by its human counterpart while the rest of the mouse gene was retained. A similar construction strategy was used for Tigit gene replacement.

Validation data

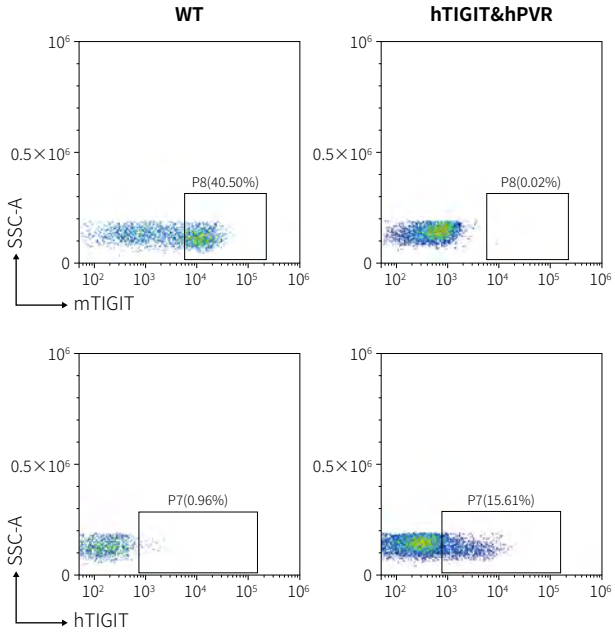


Figure 19. Splenocytes from homozygous TIGIT&PVR mice were analyzed by flow cytometry after activation. Active expression of human TIGIT can be detected in lymphocytes collected from homozygous TIGIT&PVR mice.

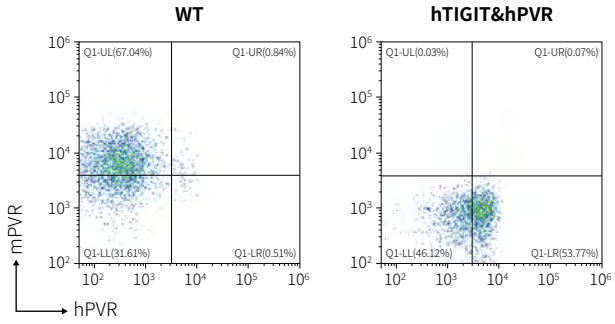


Figure 20. Splenocytes from homozygous TIGIT&PVR mice were analyzed by flow cytometry after activation. Active expression of human PVR can be detected in lymphocytes collected from homozygous TIGIT&PVR mice.

Humanized CD40 Mouse

Strain Name: C57BL/6-*Cd40*$^{em1(hCD40)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00076

CD40 is a member of the tumor necrosis factor (TNF) receptor family and is also known as TNFRSF5. CD40 is expressed on antigen presenting cells (APC) such as B cells, dendritic cells (DC), and monocytes as well as many non-immune cells and various types of cancer cells. Upon binding to its ligand CD40L expressed on the antigen presenting cells (APCs), the CD40-CD40L complex plays a vital role in the helper T cells function to prime $CD8^+$ T cells. It has been demonstrated *in vitro* that agonistic CD40 mAb can improve the ability of APCs to cross-prime naive T cells to tumor antigens.

Construction strategy

The humanized CD40 mice were developed on the C57BL/6 background. The coding sequence for the extracellular domain of the mouse endogenous Cd40 was completely replaced with the human-derived sequence, resulting in the expression of a humanized, chimeric CD40 gene.

Validation data

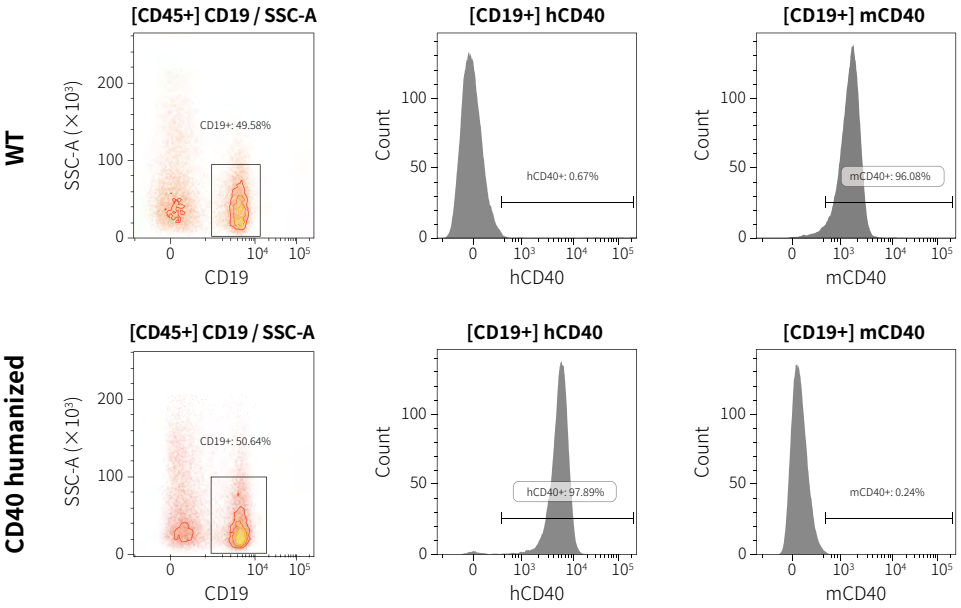


Figure 21. The expression of human CD40 in the peripheral blood cells derived from the humanized CD40 mice was confirmed by FACS. An active expression of human CD40 was detected in $CD19^+$ cells derived from homozygous humanized CD40 mice, with a comparable expression level to that of mouse CD40 in WT mice (In collaboration with CrownBio).

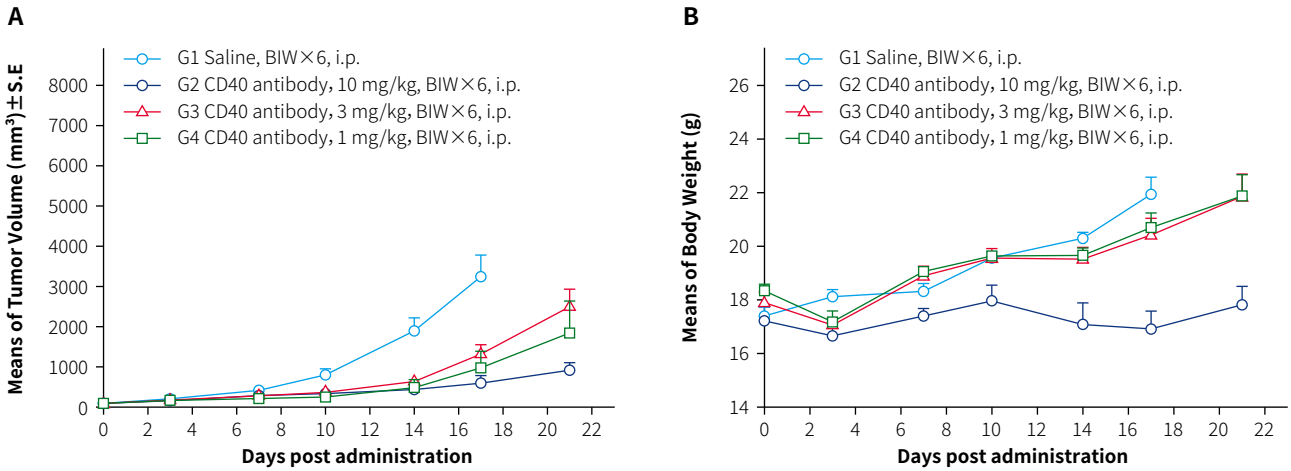
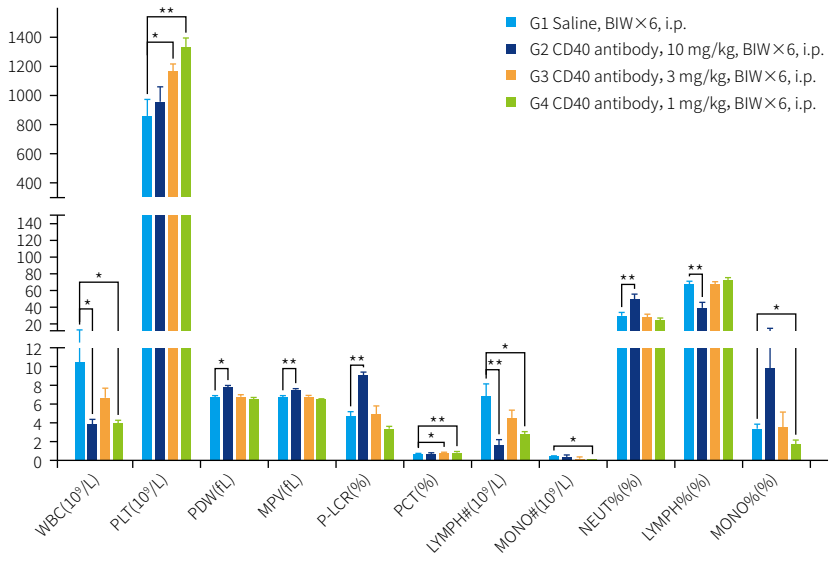
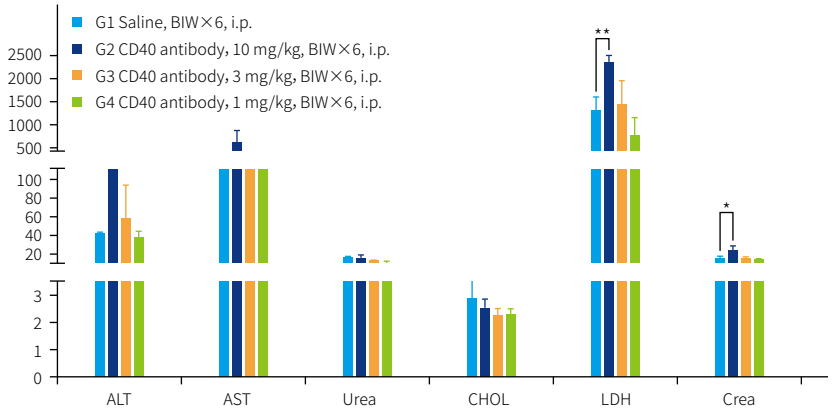


Figure 22. *In vivo* validation of anti-tumor efficacy in a MC38 tumor-bearing model of humanized CD40 mice. Homozygous humanized CD40 mice were inoculated with MC38 colon cancer cells. The results showed that an anti-human CD40 antibody exerted a very significant anti-tumor effect, demonstrating that the humanized CD40 mouse model is a good *in vivo* model for validating the efficacy of antibodies targeting human CD40. Mean volume ± SEM of tumor tissues **(A)** Mean body weight ± SEM of mice **(B)**.



Abbreviation	Stand for
WBC	White blood cell count
PDW	Platelet distribution width
MPV	Mean platelet volume
PLCR	Platelet larger cell ratio
PLT	Platelet
PCT	Thrombocytocrit
LYMPH#	Lymphocyte count
MONO#	Monocyte count
NEUT%	Neutrophil count
LYMPH%	Lymphocyte ratio
MONO%	Monocyte ratio

Figure 23. Complete blood count (CBC) of anti-human CD40-antibody treated hCD40 mice.



Abbreviation	Stand for
ALT	Alanine transaminase
AST	Aspartate transaminase
Urea	Urea
CHOL	Cholesterol
LDH	Lactate dehydrogenase
Crea	Creatinine

Figure 24. Blood chemistry of anti-human CD40-antibody treated hCD40 mice.

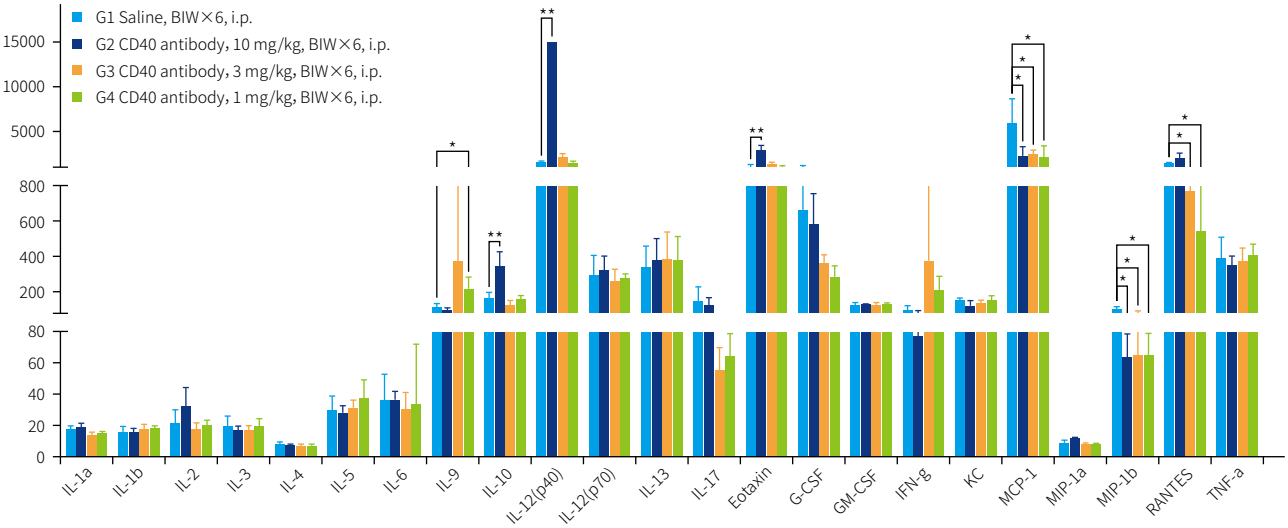


Figure 25. Cytokine analysis of anti-human CD40 antibody treated MC38 tumor-bearing hCD40 mice. Anti-human CD40 antibody treatment led to significant increase of several cytokines including IL-12(p40), Eotaxin, etc.

Humanized TNFR2 Mouse

Strain Name: C57BL/6-*Tnfrsf1b*$^{em2(hTNFRSF1B)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-190010

Tumor necrosis factor receptor 2 (TNFR2), also known as tumor necrosis factor receptor superfamily member 1B (TNFRSF1B), and CD120b, is one of major receptors of the cytokines, TNF and lymphotoxin-α. TNFR2 has proinflammatory effects too, but it also elicits strong anti-inflammatory activities and has protective effects on oligodendrocytes, cardiomyocytes, and keratinocytes.

Construction strategy

On the C57BL/6 background, the full-length coding sequence of human TNFR2 gene was placed immediately downstream of the start codon of the mouse endogenous Tnfr2 gene, followed by a poly(A) element gene via CRISPR/Cas9 mediated recombination. This guarantees an exclusive expression of human TNFR2 in the hTNFR2 mice.

Validation data

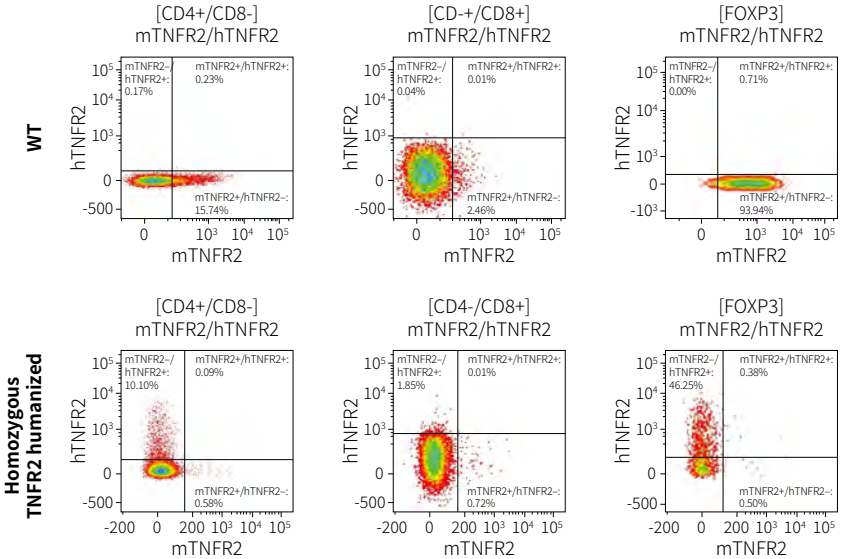


Figure 26. The expression of human TNFRSF1B (TNFR2) in humanized TNFR2 mice was confirmed by FACS. In humanized TNFR2 mice, active expression of human TNFR2 was detected in CD4$^+$ T cells and FOXP3$^+$ Treg cells isolated from spleen.

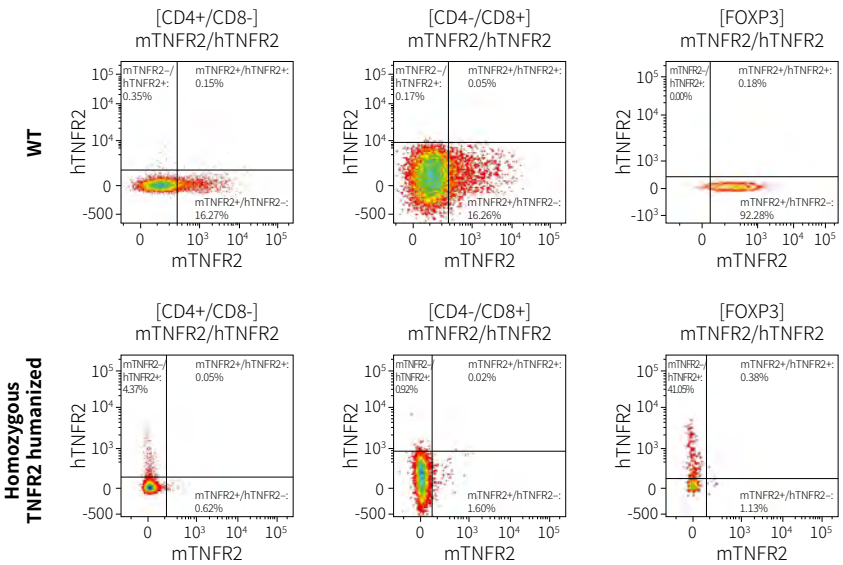


Figure 27. The expression of human TNFR2 in humanized TNFR2 mice was confirmed by FACS. In humanized TNFR2 mice, active expression of human TNFR2 was detected in CD4$^+$ T cells and FOXP3$^+$ Treg cells isolated from peripheral blood cells.

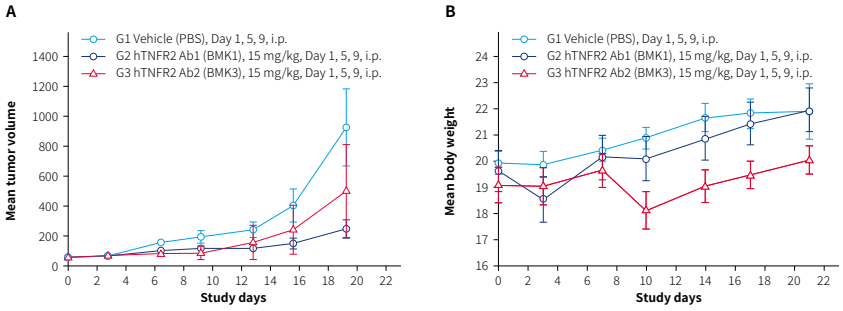


Figure 28. The expression of human TNFR2 in humanized TNFR2 mice was confirmed by FACS. In heterozygous humanized TNFR2 mice, active expression of human TNFR2 was detected in Treg cells isolated from Lymph node.

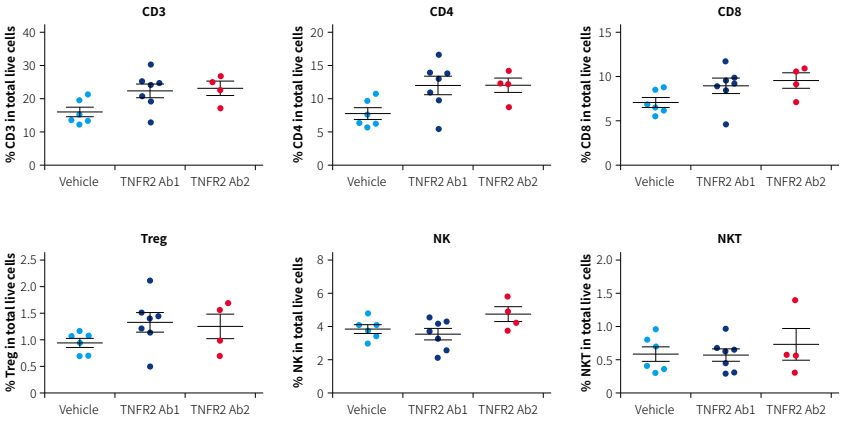


Figure 29. FACS for blood upon TNFR2 antagonist treatment. Addition of antagonistic hTNFR2 antibody can impair hTNF-induced Treg cells proliferation, while the agonistic hTNFR2 antibody alone can induce a modest proliferative response.

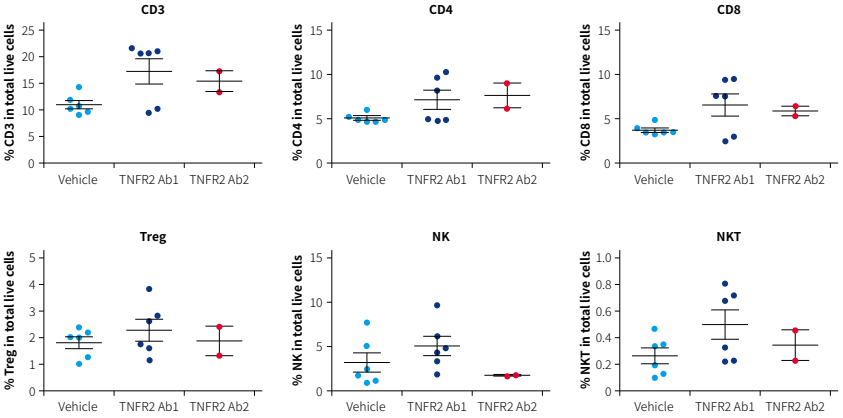


Figure 30. TIL analysis upon TNFR2 antagonist treatment. One tumor in hTNFR2 Ab1 treatment group decreased (1/7, TV=0), while two tumors in hTNFR2 Ab2 treatment group decreased (2/7, TV=0; 3/7 died during dosing period).

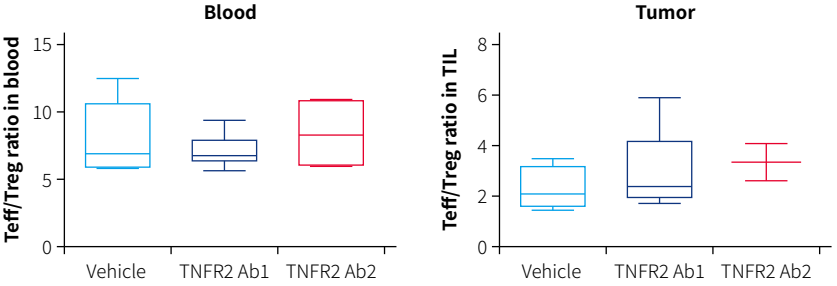


Figure 31. TNFR2 blockade increases the Teff/Treg ratios in tumor microenvironment.

Humanized CD3E Mouse

Strain Name: C57BL/6-*Cd3e*$^{em1(hCD3E)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00114

CD3E, also known as CD3-epsilon molecule, is a type I membrane glycoprotein on T cell surface. CD3E, together with many other molecules, form the T cell receptor CD3 complex, which performs a critical role in the T cell antigen receptor (TCR) signaling response. CD3E has become an emerging target for the development of new immune-modulating agents.

Construction strategy

The humanized CD3E mice were developed on the C57BL/6 background. The coding sequence for the extracellular domain of the mouse endogenous Cd3e gene was completely replaced by the human sequence, resulting in the expression of a humanized, chimeric protein.

Validation data

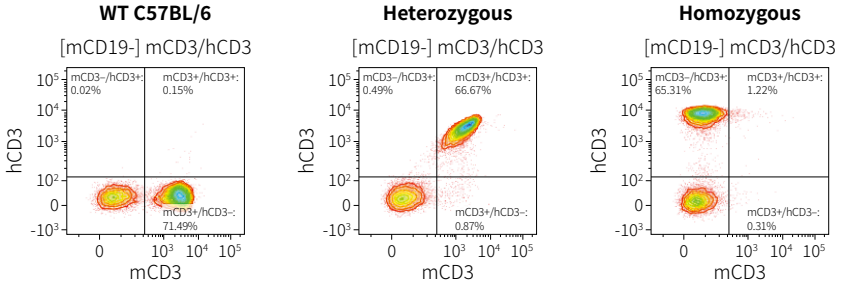


Figure 32. The expression of human CD3E in the peripheral blood cells derived from heterozygous or homozygous humanized mice was confirmed by FACS.

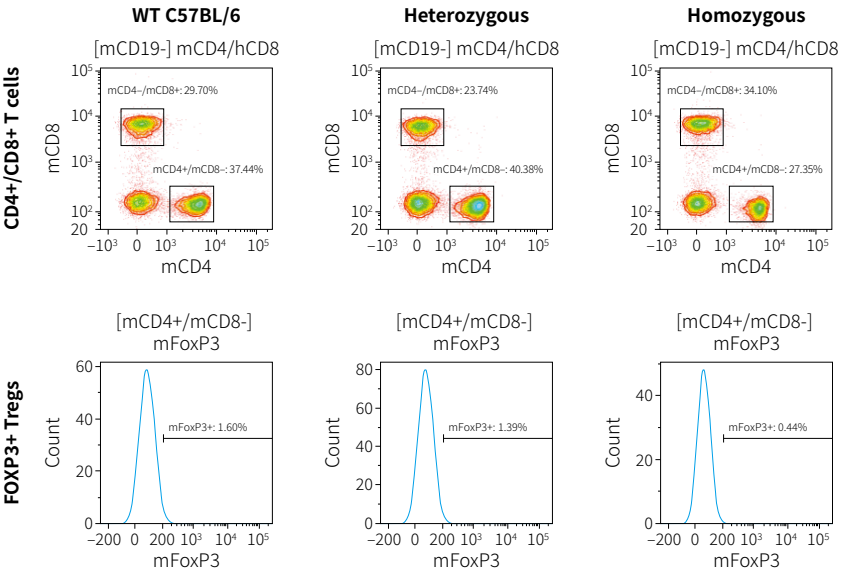


Figure 33. Detection of T cell subsets in the peripheral blood cells derived from heterozygous or homozygous CD3E humanized mice.

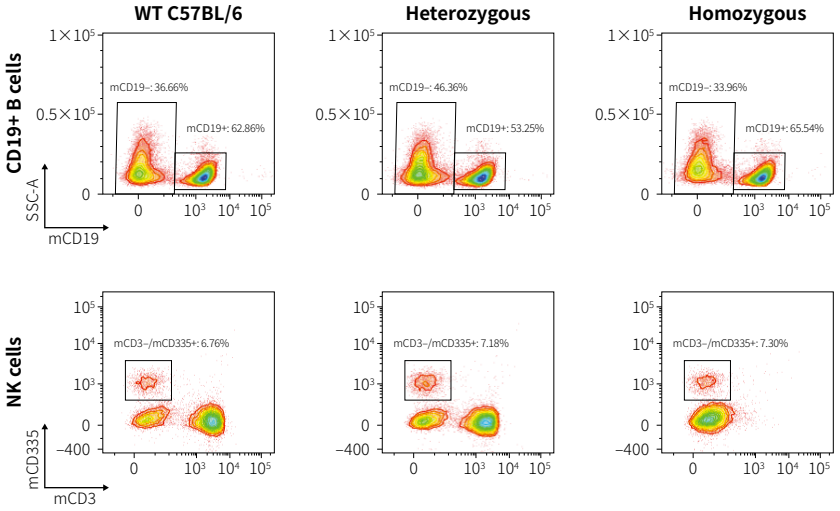


Figure 34. Detection of B and NK cells in the peripheral blood cells derived from heterozygous or homozygous CD3E humanized mice.

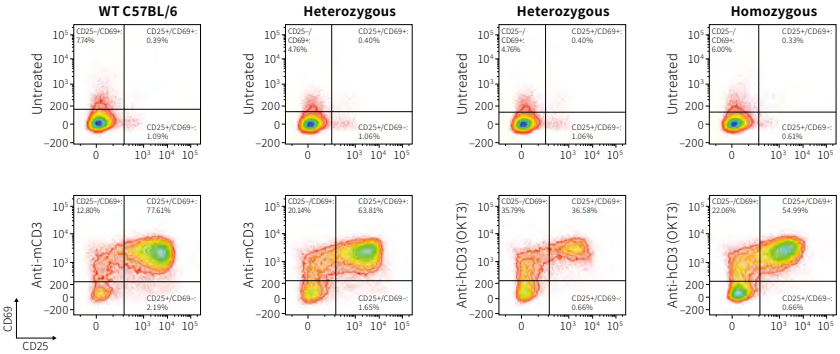


Figure 35. *In vitro* T cell activation with heterozygous or homozygous CD3E humanized mice.

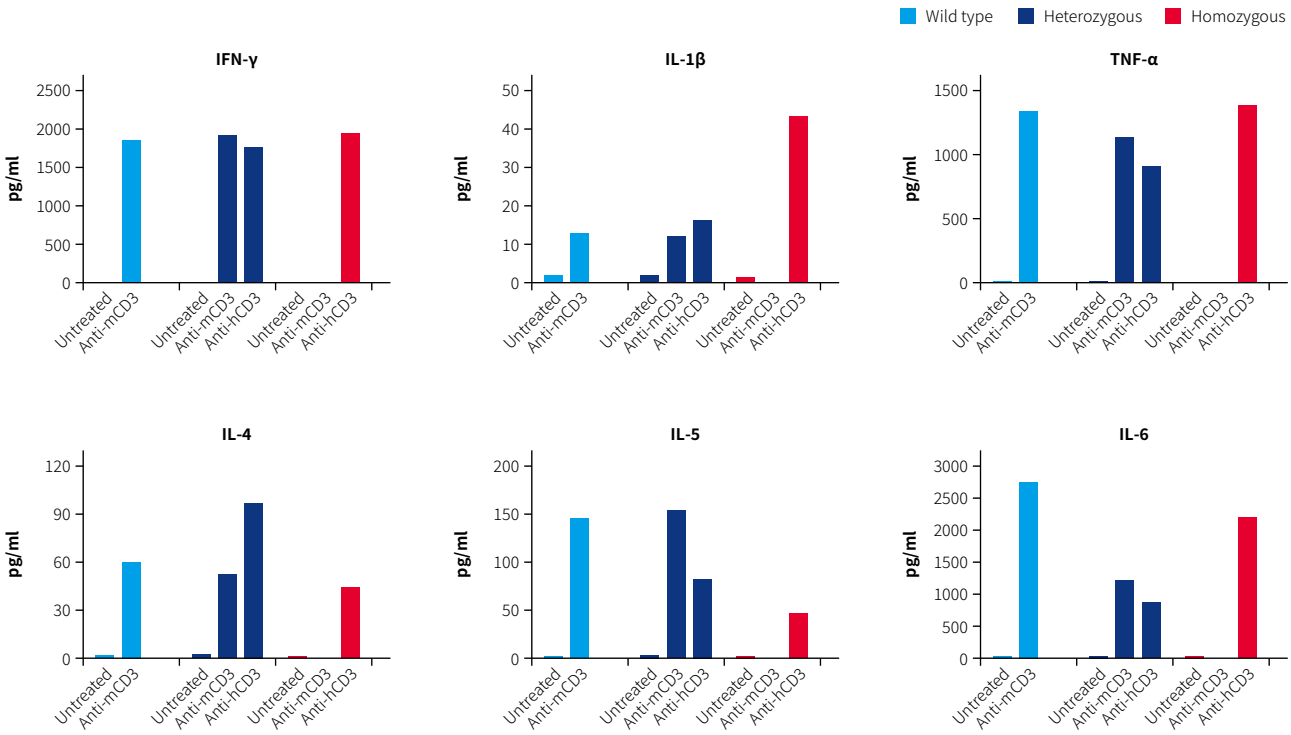


Figure 36. Cytokine analysis of anti-human CD3E antibody treated hCD3E mice. The data is provided by Crownbio.

Humanized STING Mouse

Strain Name: C57BL/6-*Tmem173*$^{tm2(hTMEM173)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-190036

STING is encoded by the TMEM173 gene，it is expressed in hematopoietic cells in peripheral lymphoid tissues, including T lymphocytes, NK cells, myeloid cells and monocytes. STING plays an important role in innate immunity. STING induces type I interferon production when cells are infected with intracellular pathogens, such as viruses, mycobacteria and intracellular parasites.

Construction strategy

Humanized STING mice were developed on the C57BL/6 background. Via homologous recombination-mediated ES cell targeting, the full-length coding sequence for the mouse Tmem173 gene was replaced by the human counterpart, leading to an exclusive expression of human-derived STING.

Validation data

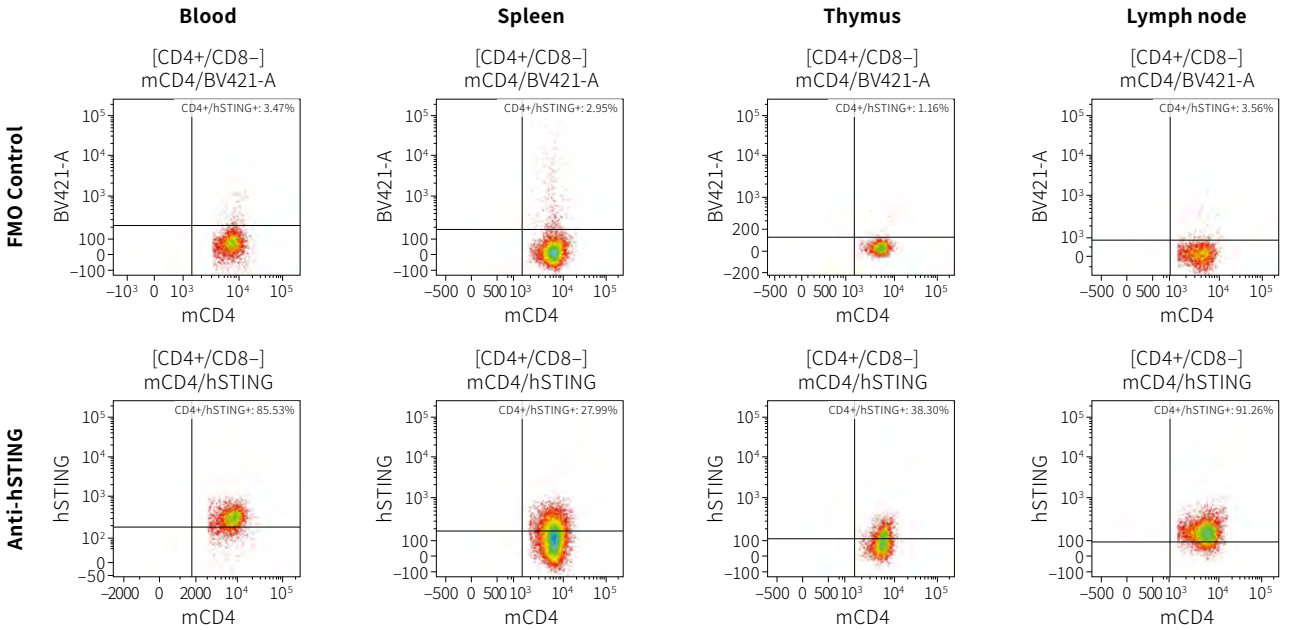


Figure 37. The expression of human STING in CD4$^+$ T lymphocytes of humanized STING mice was confirmed by FACS. In heterozygous humanized STING mice, active expression of human STING was detected in CD4$^+$ T lymphocytes derived from blood, spleen, thymus and lymph node.

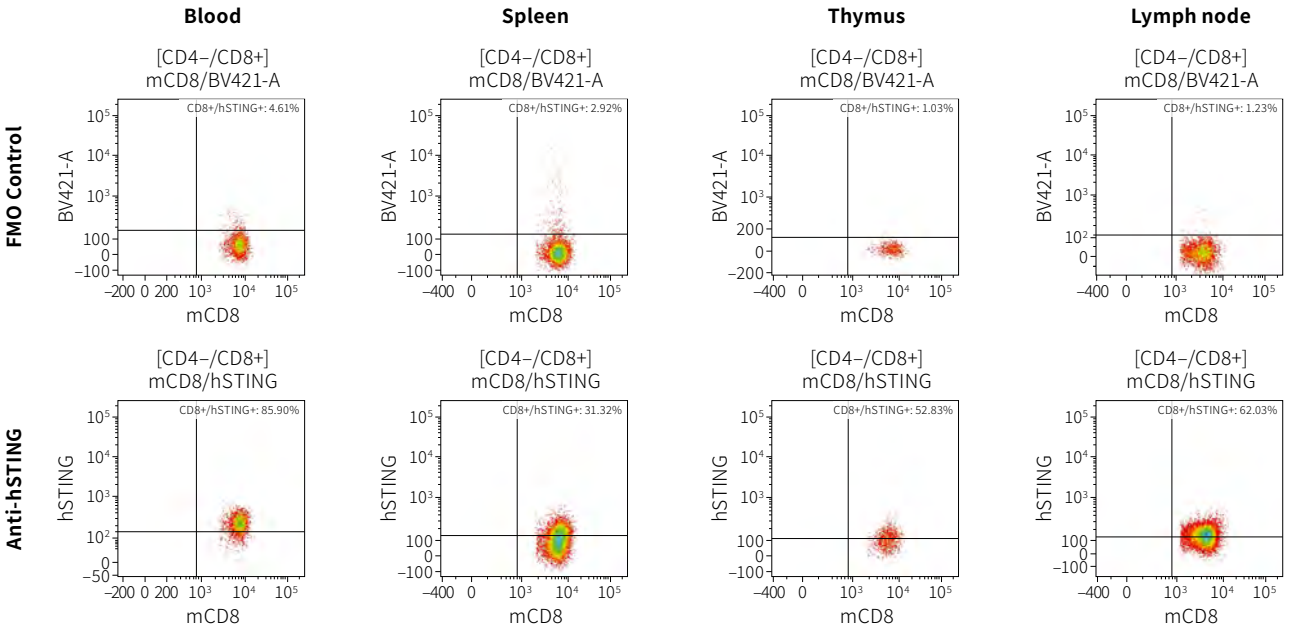


Figure 38. The expression of human STING in CD8$^+$ T lymphocytes of humanized STING mice was confirmed by FACS. In heterozygous humanized STING mice, active expression of human STING was detected in CD8$^+$ T lymphocytes derived from blood, spleen, thymus and lymph node.

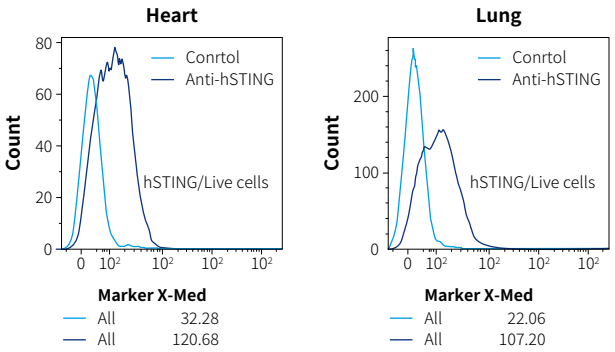


Figure 39. The expression of human STING in the heart and lung cells was confirmed by FACS. In heterozygous humanized STING mice, active expression of human STING was detected in the heart and lung cells.

Humanized SIRPA Mouse

Strain Name: C57BL/6-*Sirpa*$^{tm2(hSIRPA)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-18015

Construction strategy

Humanized SIRPA mice were developed on the C57BL/6 background. Via homologous recombination-mediated ES cell targeting, the full-length coding sequence for the mouse Sirpa gene was replaced by the human counterpart, leading to an exclusive expression of human-derived SIRPA.

Validation data

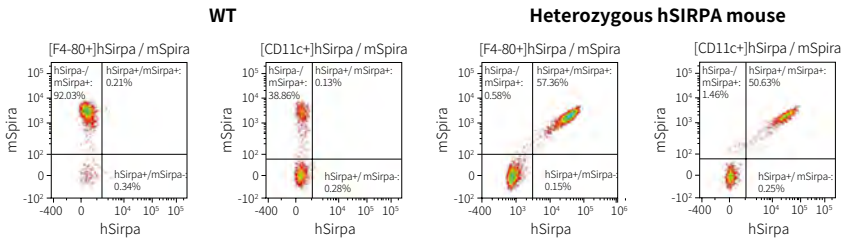


Figure 40. The expression of human SIRPA in humanized SIRPA mice was measured by FACS. In heterozygous humanized SIRPA mice, active expression of human SIRPA was detected in both B cells and macrophages isolated from peripheral blood cells.

Humanized CD47 Mouse

C57BL/6-*Cd47*$^{em1(hCD47)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00050

Construction strategy

Humanized CD47 mice were developed on the C57BL/6 background. The coding sequence for the first extracellular domain of the mouse endogenous Cd47 gene was replaced by the human sequence, resulting in the expression of a humanized, chimeric protein.

Validation data

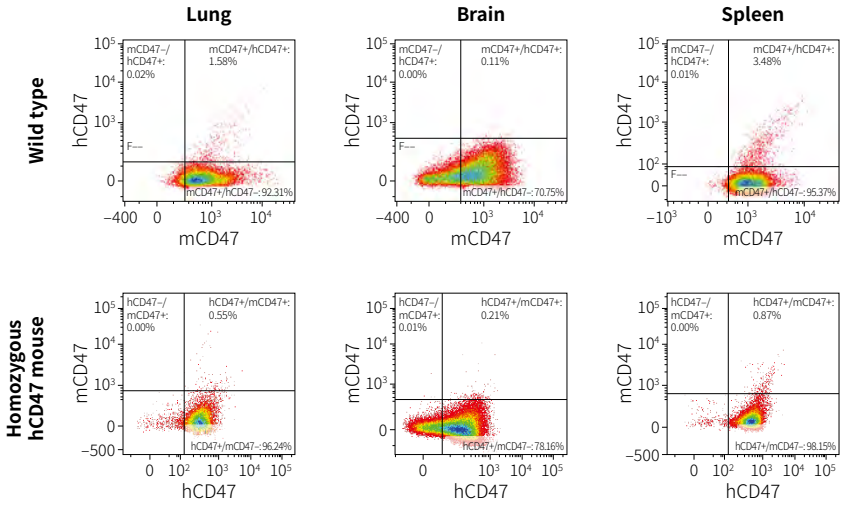


Figure 41. Detection of CD47 expression in live cells from lung, brain and spleen of humanized CD47 mice. The FACS results showed that the high expression of human CD47 was detected in the live cells from lung, brain and spleen of Homozygous humanized CD47 mice.

Double Humanized SIRPA&CD47 Mouse

Strain Name: C57BL/6-*Sirpa*$^{tm2(hSIRPA)}$ *Cd47*$^{em1(hCD47)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-190019

SIRPA is an immunoglobulin superfamily transmembrane protein with intracellular docking sites for two Src homology domain containing tyrosine phosphatases, and expressed on all myeloid cells, including monocytes, macrophage, granulocytes and myeloid dendritic cells. SIRPA is a critical immune inhibitory receptor on macrophages. CD47 is a ligand for SIRPA, and CD47 interaction with SIRPA serves as a 'self-recognition' that prevents phagocytosis of the cells expressing CD47.

Construction strategy

Humanized SIRPA&CD47 mice were developed on the C57BL/6 background. Via homologous recombination-mediated ES cell targeting, the full-length coding sequence for the mouse Sirpa gene was replaced by the human counterpart, leading to an exclusive expression of human-derived SIRPA. The coding sequence for the first extracellular domain of the mouse endogenous Cd47 gene was replaced by the human sequence, resulting in the expression of a humanized, chimeric protein.

Validation data

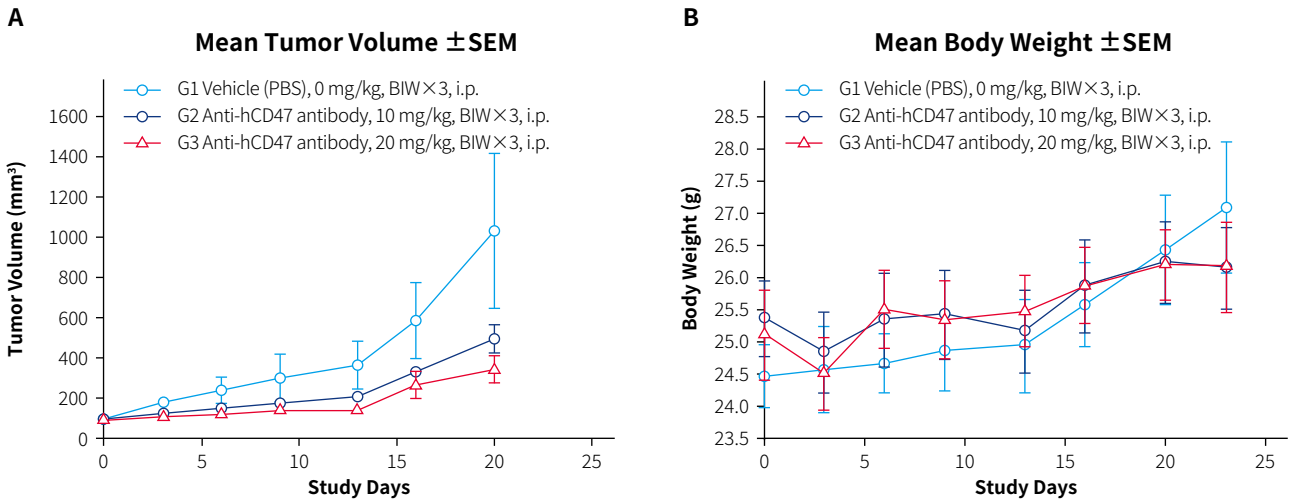


Figure 42. Evaluation of human-specifc, CD47 antibody in the double humanized SIRPA&CD47 mice. The animals were inoculated with MC38 colon cancer cells, and randomly assigned into a control group and two treatment groups (n=6) when the tumors grew to 100 mm^3. The humanized SIRPA&CD47 mice responded to human anti-CD47 (Left) without a significant body weight change at late time points (Right).

Humanized OX40 Mouse

Strain Name: C57BL/6-*Tnfrsf4*$^{em1(hTNFRSF4)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00041

OX40 is a co-stimulatory molecule expressed on the surface of activated cytotoxic T cells and regulatory T cells. Administration of agonistic, anti-OX40 antibody increases proliferation of peripheral blood $CD4^+$ and $CD8^+$ T cells, thereby creating a tumor microenvironment that is more favorable to anti-tumor immune responses. Accumulating preclinical evidence supports the application value of anti-OX40 antibodies in cancer therapy, and several such agonistic antibodies are now tested in early stage of clinical trials. The humanized OX40 mice developed by SMOC provide a translational model that enables the *in vivo* efficacy evaluation of human-specific therapeutic OX40 antibodies.

Construction strategy

The humanized OX40 mouse model was developed on the C57BL/6 background. A chimeric expression cassette that encodes the extracellular domain of human OX40 as well as the transmembrane and intracellular domains of mouse OX40 was placed immediately downstream of the start codon of the mouse endogenous OX40 gene, followed by a poly(A) signal. Thereby, the extracellular domain of the mouse OX40 was replaced by its human counterpart while the rest of the mouse gene remained untouched.

Validation data

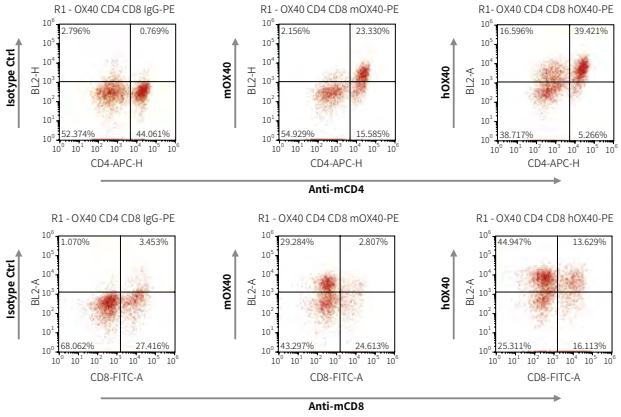


Figure 43. The expression of human OX40 in the splenocytes of humanized OX40 mice was confirmed by FACS. Spleen lymphocytes were collected from heterozygous, humanized OX40 mice, activated by CD3& CD28 antibodies for 48 hrs and then subjected to staining. Active expression of human OX40 can be detected in both activated $CD4^+$ and $CD8^+$ T lymphocytes collected from heterozygous humanized OX40 mice.

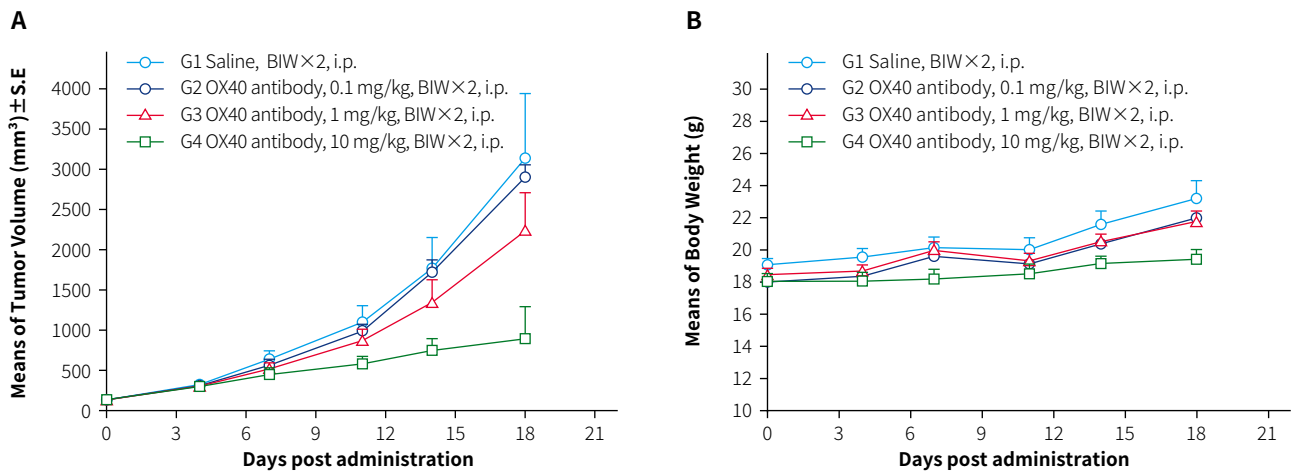


Figure 44. OX40 antibody showed dose-dependent anti-tumor activity in humanized OX40 mice. The animals were inoculated with MC38 colon cancer cells, and randomly assigned into a control group and three treatment groups when the tumors grew to 100 mm^3. The humanized OX40 mice responded to human anti-OX40 (Left) without a significant body weight change at late time points (Right). The OX40 antibody was obtained from Innoventbio.

Humanized LAG3 Mouse

Strain Name: B6.129-*Lag3*$^{tm1(hLAG3)/Smoc}$ Strain Background: B6.129 Cat. No. : NM-HU-00049

LAG3 (lymphocyte activating 3, also known as CD223) has been shown to act as a co-inhibitory molecule expressed on activated T cells, NK cells, B cells, and plasmacytoid dendritic cells. LAG3 is an immune checkpoint receptor that binds to the antigen-MHC complex to present antigens to T cells. Experiments have shown that LAG3 negatively regulates T cell proliferation as well as the development of lasting memory T cells.

Construction strategy

The coding sequence for the extracellular domain of mouse endogenous LAG3 was completely replaced by the human LAG3 counterpart, leading to the expression of a chimeric LAG3 protein.

Validation data

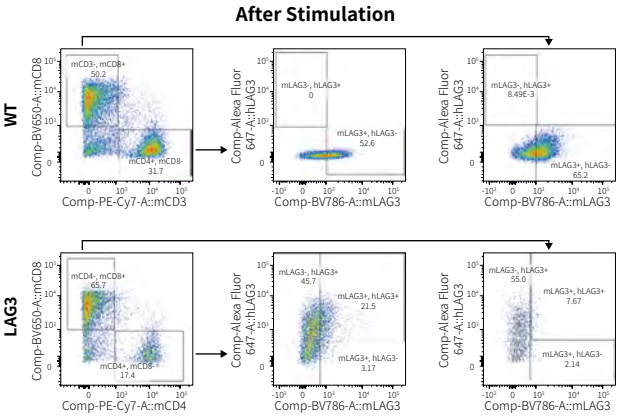


Figure 45. Human LAG3 expression in activated splenocytes from humanized LAG3 mice was measured by FACS. The results showed an active expression of human LAG3 in both activated CD4$^+$ and CD8$^+$ T lymphocytes derived from homozygous, humanized LAG3 mice (In collaboration with CrownBio).

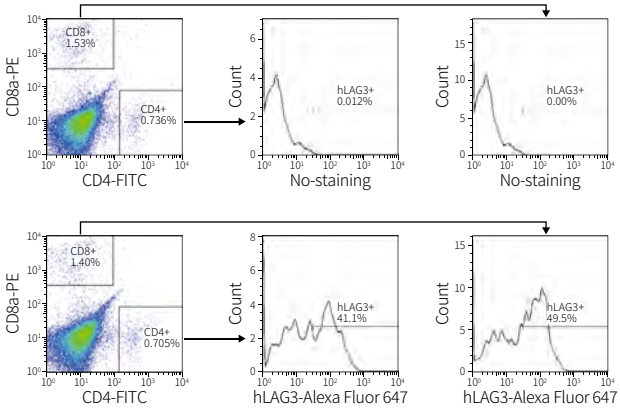


Figure 46. Human LAG3 expression in tumor-infiltrating lymphocytes collected from humanized LAG3 mice was confirmed by FACS. Homozygous, humanized LAG3 mice were inoculated with MC38 colon cancer cells. When the tumors grew to an average volume of 100 mm^3, tumor-infiltrating lymphocytes were collected and subjected to staining (In collaboration with CrownBio).

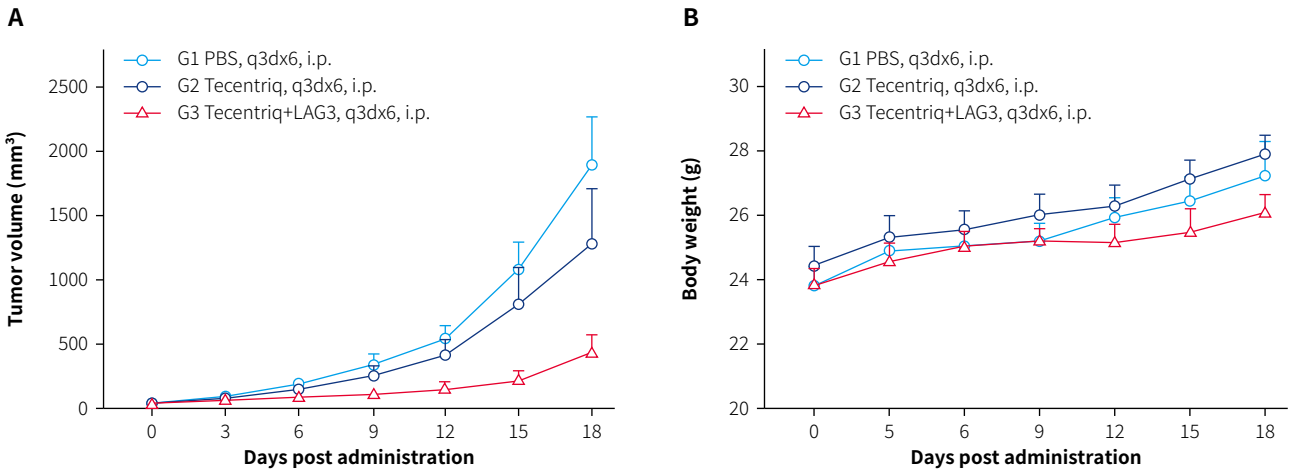


Figure 47. Homozygous humanized LAG3 mice were inoculated with MC38 colon cancer cells, and randomly assigned to different groups (n=8) when the tumors grew to 70–80 mm^3. Similar to the previous figure, a significant anti-tumor effect was observed when the human LAG3 antibody was administered together with TENCENTRIQ®. TENCENTRIQ (Atezolizumab): A monoclonal antibody of IgG1 isotype against human PD-L1 marketed by Roche.

Humanized 4-1BB Mouse

Strain Name: C57BL/6-*Tnfrsf9*$^{em1(hTNFRSF9)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-190077

4-1BB (also known as TNFRSF9 and CD137), a member of the tumor necrosis factor (TNF) receptor family, is mainly expressed on activated T cells. Upon the binding of its agonistic antibodies or its natural ligand, 4-1BB delivers a co-stimulatory signal to T cells, which synergizes with the primary TCR signal to enhance cell proliferation and induce cytokine secretion.

Construction strategy

The humanized 4-1BB mouse model was developed on the C57BL/6 background. A chimeric expression cassette that encodes the extracellular domain of human 4-1BB as well as the transmembrane and intracellular domains of murine 4-1bb was inserted immediately downstream of the start codon of the mouse endogenous 4-1bb gene, followed by a poly(A) element. Thereby, the extracellular domain of the mouse 4-1bb was replaced by its human counterpart while the rest of the mouse gene was retained.

Validation data

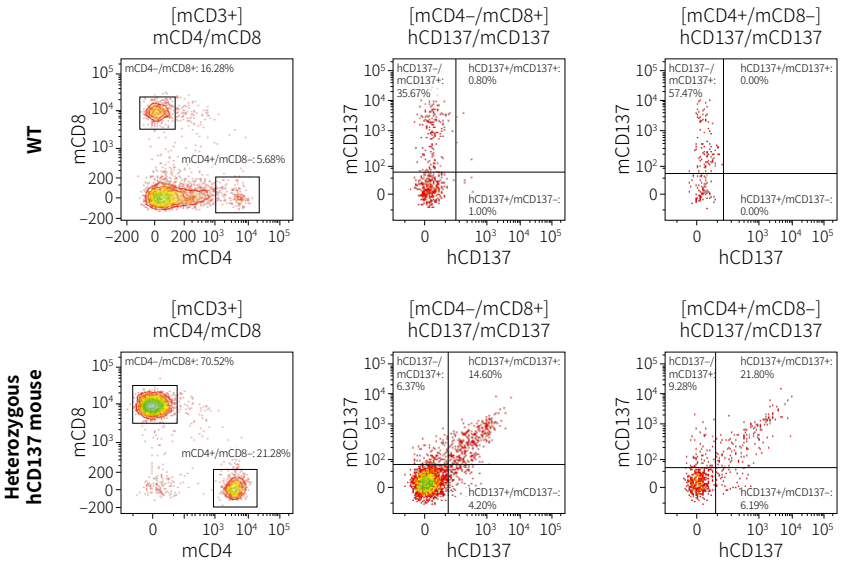


Figure 48. Expression of humanized CD137(4-1BB) in the activated spleen lymphocytes of heterozygous humanized 4-1BB mice is detected by FACS. The spleen lymphocytes of heterozygous humanized 4-1BB mice were activated by anti-CD3 and anti-CD28 for 48 hours, and then collected for staining. FACS results showed that the active expression of humanized 4-1BB can be detected in both activated $CD4^+$ and $CD8^+$ T lymphocytes collected from heterozygous humanized 4-1BB mice.

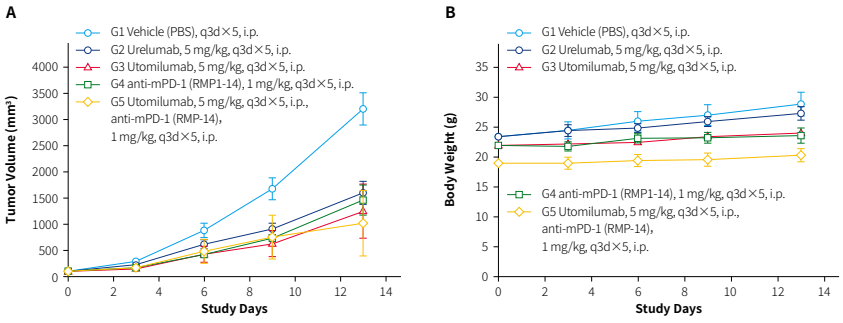


Figure 49. *In vivo* validation of humanized 4-1BB mice. Humanized 4-1BB mice were inoculated with MC38 colon cancer cells, and were randomly assigned into different groups (n=6) when the tumors grew to a volume of 100 mm^3. Human-specifc, 4-1BB antibody (Urelumab or Utomilumab) was given every three days (Q3D) for two weeks (In collaboration with Crownbio).

Humanized PCSK9 Mouse

Strain Name: C57BL/6-*Pcsk9*$^{em2(hPCSK9)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00075

Proprotein convertase subtilisin/kexin 9 (PCSK9) is the ninth member of the secretory serine protease family. It binds to low-density lipoprotein receptor (LDLR) for endocytosis and lysosome degradation in the liver, resulting in an increasing in circulating LDL-cholesterol (LDL-c) level. Since a PCSK9 induced increase in plasma LDL-c contributes to atherosclerosis, PCSK9 inhibition has become a new strategy in preventing and treating atherosclerosis.

Construction strategy

The humanized PCSK9 mice were developed on the C57BL/6 background. The signal peptide of murine Pcsk9 was retained, while the rest of the mouse gene was replaced by its human counterpart.

Validation data

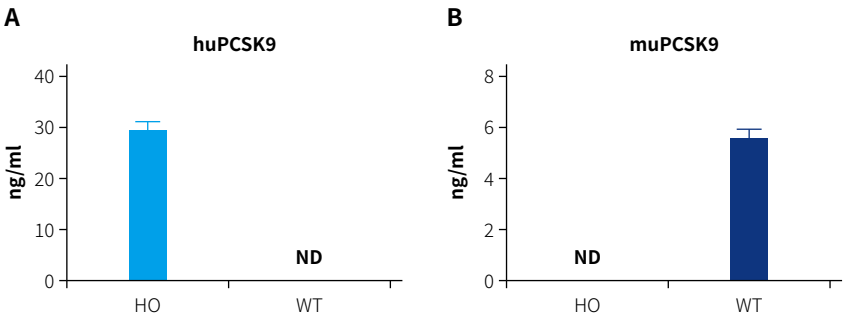


Figure 50. Expression of PCSK9 in the serum of humanized PCSK9 homozygous mice is detected by Elisa. **A.** The expression of human PCSK9 can be detected in the serum collected from homozygous mice. **B.** The expression of mouse PCSK9 can not be detected in the serum collected from homozygous mice.

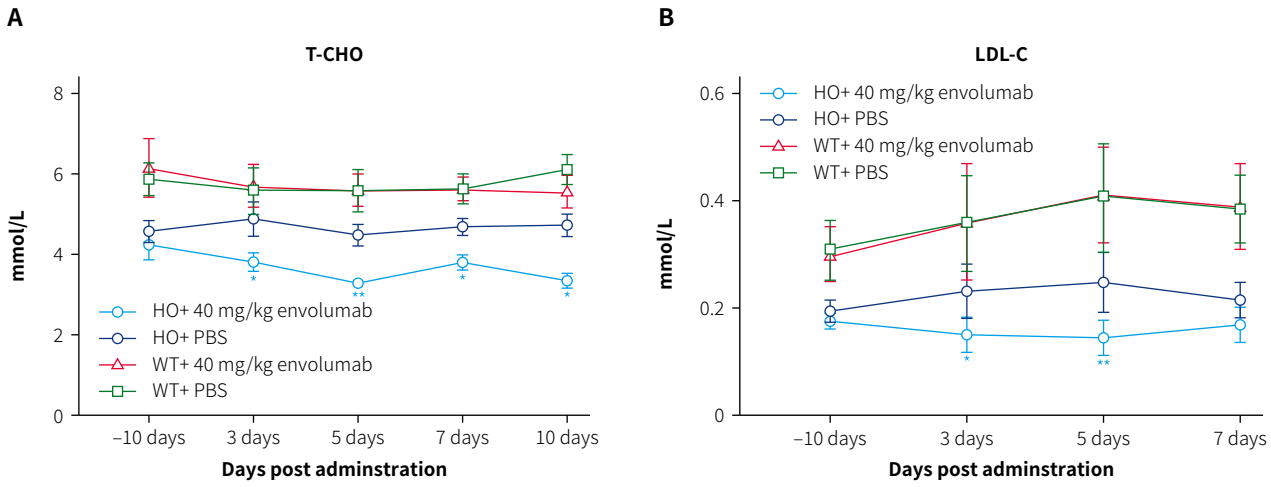


Figure 51. Impact of evolocumab treatment on CHOL and LDL-C levels in humanized PCSK9 homozygous mice. A single intravenous injection of evolocumab reduced CHOL(a) and LDL-C(b) in humanized PCSK9 homozygous mice after 16 weeks of high-fat diet feeding. Average + SD; n = 6 to 7 mice per condition; *p<0.05, **p<0.01 (Student's t test).

Humanized ICOS Mouse

Strain Name: C57BL/6-*Icos*$^{em1(hICOS)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00052

ICOS (Inducible T-cell co-stimulator), also known as CD278, is a co-stimulatory molecule expressed on activated T cells and regulatory T cells. ICOS forms homodimers and plays a critical role in regulating the survival and functions of T cells. Upon the interaction with its ligand ICOSL, ICOS mediates the interaction between tumor-infiltrating $CD4^+$ T cells and plasmacytoid dendritic cells, leading to the amplification of Tregs and IL-10 secretion. ICOS expression is induced rapidly after T cell activation, and it is reported that administration of anti-ICOS mAb during the anti-CTLA4 therapy results in an improved anti-tumor efficacy.

Construction strategy

The humanized ICOS mouse model was developed on the C57BL/6 background. A chimeric expression cassette that encodes the extracellular domain of human ICOS as well as the transmembrane and intracellular domains of murine ICOS was inserted immediately downstream of the start codon of the mouse endogenous Icos gene, followed by a poly(A) element. Thereby, the extracellular domain of the mouse Icos was replaced by its human counterpart while the rest of the mouse gene was retained.

Validation data

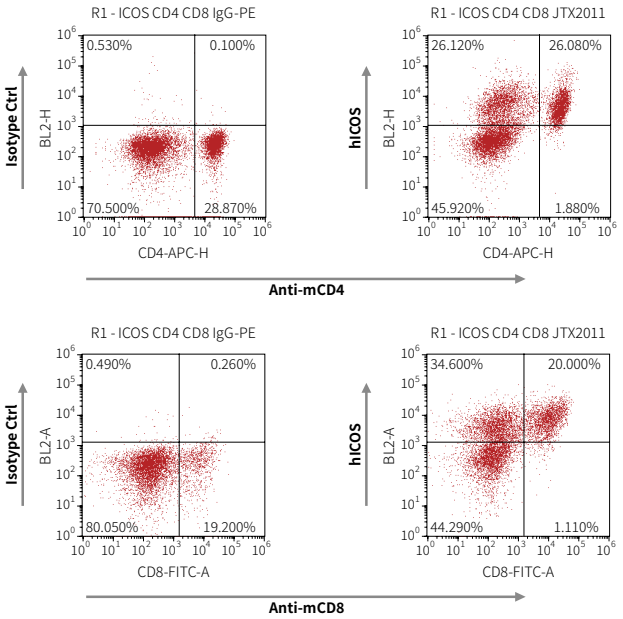


Figure 52. The expression of human ICOS in the splenocytes of humanized ICOS mice was confirmed by FACS. Spleen lymphocytes were collected from heterozygous, humanized ICOS mice, activated by CD3& CD28 antibodies for 48 hrs and then subjected to staining. The results showed that the active expression of human ICOS can be detected in both activated $CD4^+$ and $CD8^+$ T lymphocytes derived from humanized ICOS mice.

Humanized TIM3 Mouse

Strain Name: C57BL/6-*Havcr2*$^{em1(hHAVCR2)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00054

As an inhibitory receptor on T cells, TIM3 (T-cell immunoglobulin and mucin-domain containing-3，also known as HAVCR2) is expressed on Th1, Th17, and CD8$^+$ T cells. Thanks to its demonstrated success in multiple preclinical studies, TIM3 exhibits unique features that make it an intriguing candidate for the next wave of immune checkpoint therapies.

Construction strategy

The humanized TIM3 mouse model was developed on the C57BL/6 background. A chimeric expression cassette that encodes the extracellular domain of human TIM3 as well as the transmembrane and intracellular domains of murine Tim3 was inserted immediately downstream of the start codon of the mouse endogenous Tim3 gene, followed by a poly(A) site. Thereby, the extracellular domain of the mouse Tim3 was replaced by its human counterpart while the rest of the mouse gene remained unchanged.

Validation data

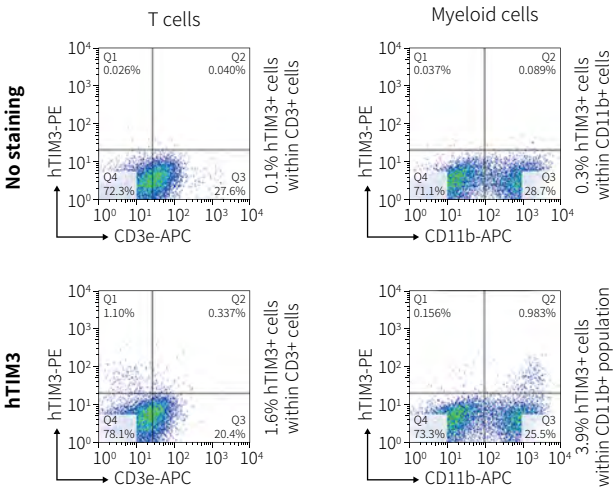


Figure 53. Human TIM3 expression in tumor infiltrating lymphocytes collected from humanized TIM3 mice was confirmed by FACS. Homozygous humanized TIM3 mice were inoculated with MC38 colon cancer cells. When the tumors grew to an average volume of 100 mm^3, tumor infiltrating lymphocytes were isolated and subjected to staining. The results showed a positive expression of human TIM3 in the tumor infiltrating lymphocytes collected from humanized TIM3 mice (In collaboration with GenScript).

Humanized GITR Mouse

Strain Name: C57BL/6-*Tnfrsf18*$^{em1(hTNFRSF18)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00096

GITR (also known as TNFRSF18 and CD357), a member of the tumor necrosis factor (TNF) receptor family, plays a key role in immunological self-tolerance maintained by regulatory T cells. GITR has been shown to be upregulated upon T cell activation. This molecule is currently of interest to research community as a co-stimulatory immune checkpoint molecule.

Construction strategy

The humanized GITR mouse model was developed on the C57BL/6 background. A chimeric expression cassette that encodes the extracellular domain of human GITR as well as the transmembrane and intracellular domains of murine Gitr was inserted immediately downstream of the start codon of the mouse endogenous Gitr gene, followed by a poly(A) signal. Thereby, the extracellular domain of the mouse Gitr was replaced by its human counterpart while the rest of the mouse gene remained unchanged.

Validation data

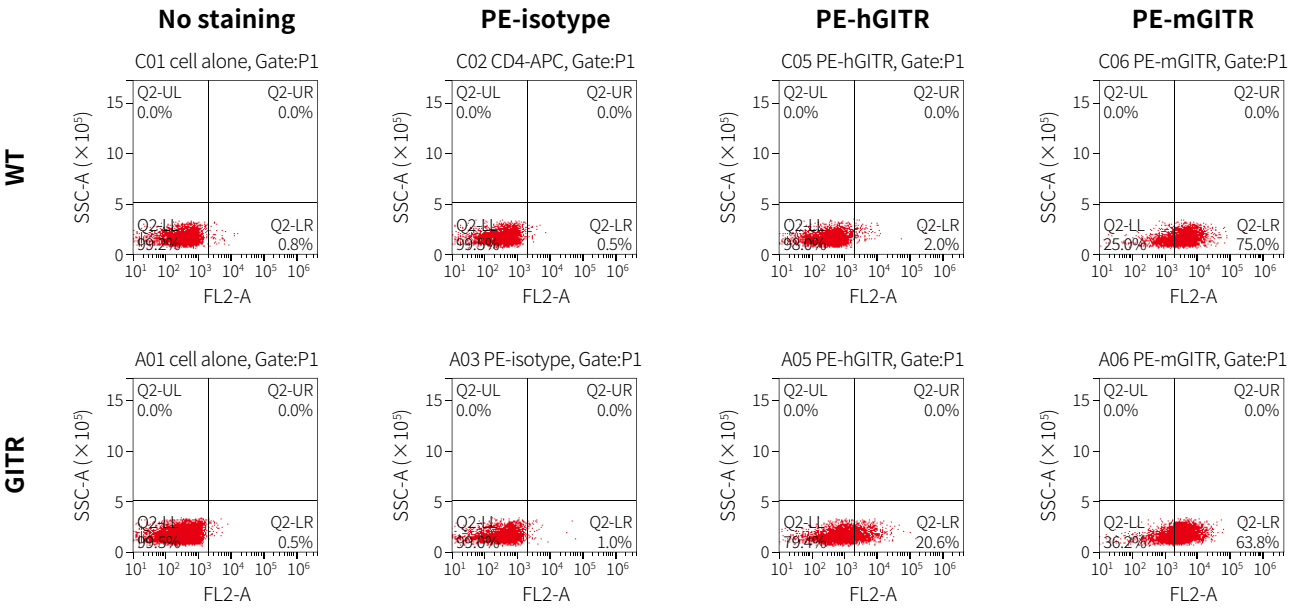


Figure 54. The expression of human GITR in the splenocytes collected from heterozygous humanized GITR mice was measured by FACS.

Humanized PD-L2 Mouse

Strain Name: C57BL/6-*Pdcd1lg2*$^{em1(hPDCD1LG2)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-190050

PD-L1 and PD-L2 are both ligands of PD-1, and these interactions transduce co-inhibitory signals for T cell activation, suppress T cell function, which is called T cell exhaustion, and ultimately promote tumor evasion of the immune system. PD-1, PD-L1, and PD-L2 have different prognostic roles in various tumors. The combination between PD-L2 and PD-1 also has a negative effect on T cell activation, which may be an important reason why some tumors express little to no PD-L1 yet still respond to PD-1 immunotherapy.

Construction strategy

The humanized PD-L2 mice were developed on the C57BL/6 background. The coding sequence for the extracellular domain of the mouse endogenous Pdcd1lg2 gene was completely replaced by the human sequence, resulting in the expression of a humanized, chimeric protein.

Validation data

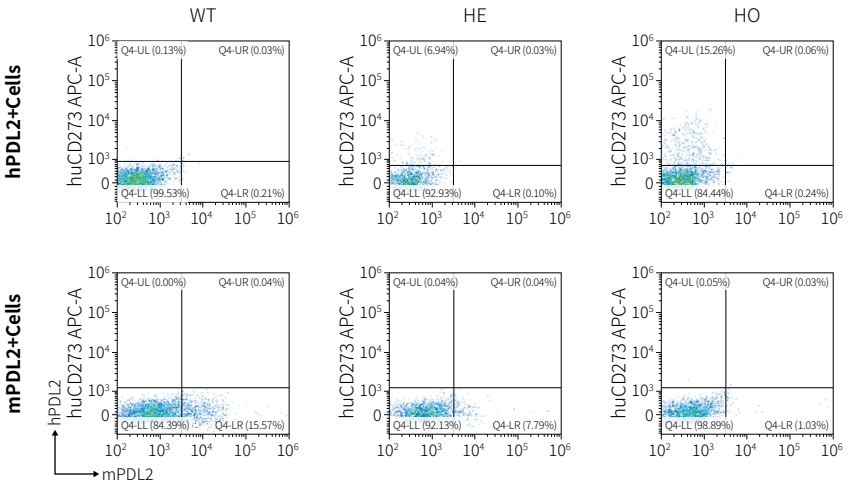


Figure 55. Splenocytes from humanized PD-L2 mice were analyzed by flow cytometry after activation. hPD-L2$^+$ (but not mPD-L2$^+$) cells were detectable in the homozygous hPD-L2 mice, while hPD-L2$^+$ and mPD-L2$^+$ cells were both detectable in heterozygous humanized PD-L2 mice.

Humanized SEMA4D Mouse

Strain Name: C57BL/6-*Sema4d*$^{em1(hSEMA4D)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00117

Semaphorin 4D (SEMA4D or CD100) is a member of the semaphorin family of proteins and an important mediator of the movement and differentiation of multiple cell types, including those of the immune, vascular, and nervous systems. Blocking the binding of SEMA4D to its receptors can result in physiologic changes that may have implications in cancer, autoimmune, and neurological disease.

Construction strategy

The humanized SEMA4D mice were developed on the C57BL/6 background. A chimeric expression cassette that encodes the extracellular domain of human SEMA4D as well as the transmembrane and intracellular domains of mouse SEMA4D was placed immediately downstream of the start codon of the mouse endogenous SEMA4D gene, followed by a poly (A) signal. Thereby, the extracellular domain of the mouse SEMA4D was replaced by its human counterpart while the rest of the mouse gene remained untouched.

Validation data

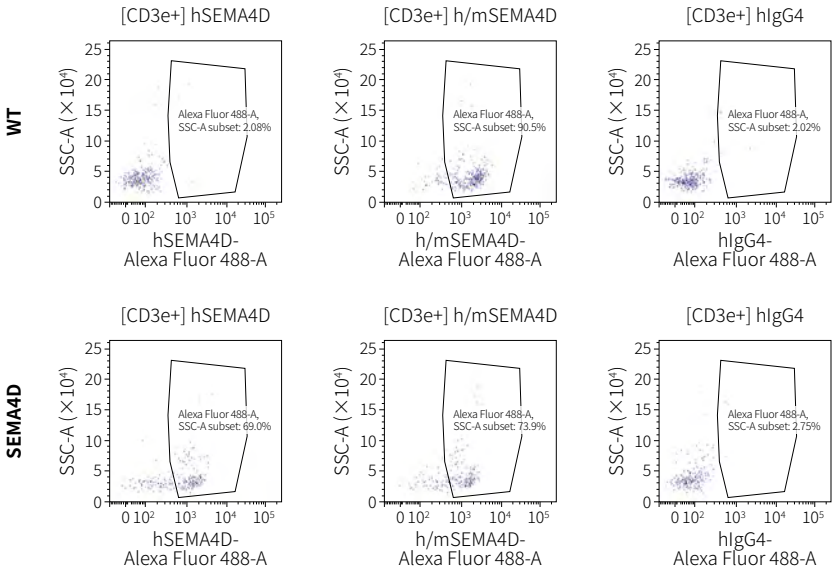


Figure 56. Expression of SEMA4D in the PBMC of homozygous humanized SEMA4D mice was confirmed by FACS. The results showed that the expression of human SEMA4D can be detected in T cells collected from the peripheral blood of homozygous humanized SEMA4D mice.

Humanized VISTA Mouse

Strain Name: C57BL/6-*Vsir*$^{em1(hVSIR)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00118

VISTA, V domain immunoglobulin suppressor of T cell activation, is an inhibitory B7 family immune checkpoint molecule. Similar to PD-L1, VISTA potently suppresses T cell activation and plays critical roles in maintaining peripheral tolerance and controlling immune responses against self and foreign antigens.

Construction strategy

On the C57BL/6 background, the exons 2 and 3 of the mouse endogenous Vsir gene was replaced by the human counterparts, leading to the expression of a humanized, chimeric protein.

Validation data

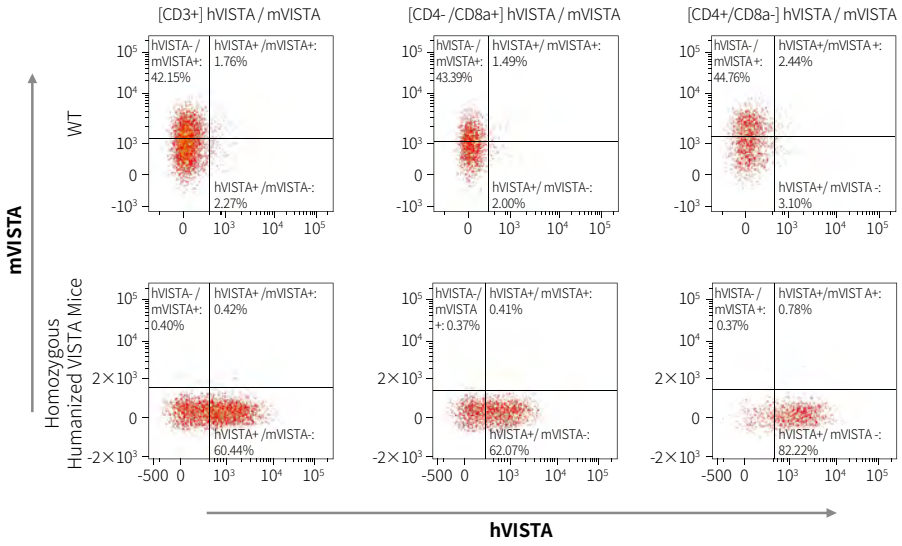


Figure 57. The expression of human VISTA in peripheral T cells derived from homozygous, humanized VISTA mice was confirmed by FACS.

Humanized CCR2 mouse

Strain Name: C57BL/6-*Ccr2*$^{em2(hCCR2)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-18026

CCR2 is a receptor for monocyte chemoattractant protein-1 (MCP-1), a chemokine that plays a role in monocyte infiltration in inflammatory responses to rheumatoid arthritis and tumours.

Construction strategy

The humanized CCR2 mice were developed on the C57BL/6 background. The coding sequence for the entire mouse Ccr2 gene was replaced by its human counterpart.

Validation data

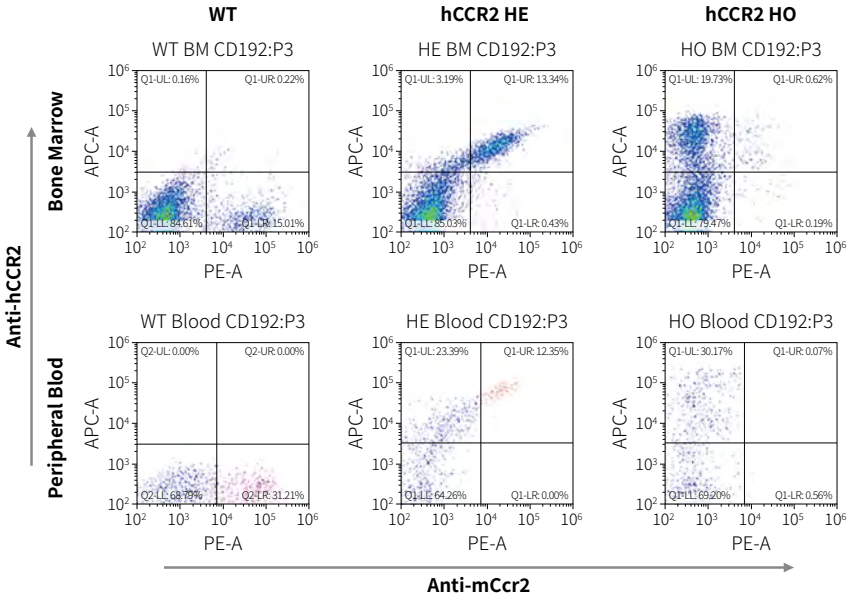


Figure 58. The expression of human CCR2 in humanized CCR2 mice was confirmed by FACS. In heterozygous or homozygous humanized CCR2 mice, active expression of human CCR2 was detected in bone marrow and peripheral blood cells.

Humanized CD19 mouse

Strain Name: C57BL/6-*Cd19*$^{em1(hCD19)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-00110

As a marker of B cells, CD19 (Cluster of Differentiation 19), also known as B-lymphocyte antigen CD19, is expressed in nearly all B lineage cells in human. It acts as an adaptor protein to recruit cytoplasmic signaling proteins to the membrane and plays an essential role during B cell receptor signaling pathways. CD19-targeted therapies based on T cells that express CD19-specific chimeric antigen receptors (CARs) have been widely utilized in patients with CD19^{+} lymphoma and leukemia.

Construction strategy

The humanized CD19 mice were developed on the C57BL/6 background. The coding sequence for the extracellular domain of the mouse endogenous Cd19 gene was replaced by the human sequence, resulting in the expression of a humanized, chimeric protein.

Validation data

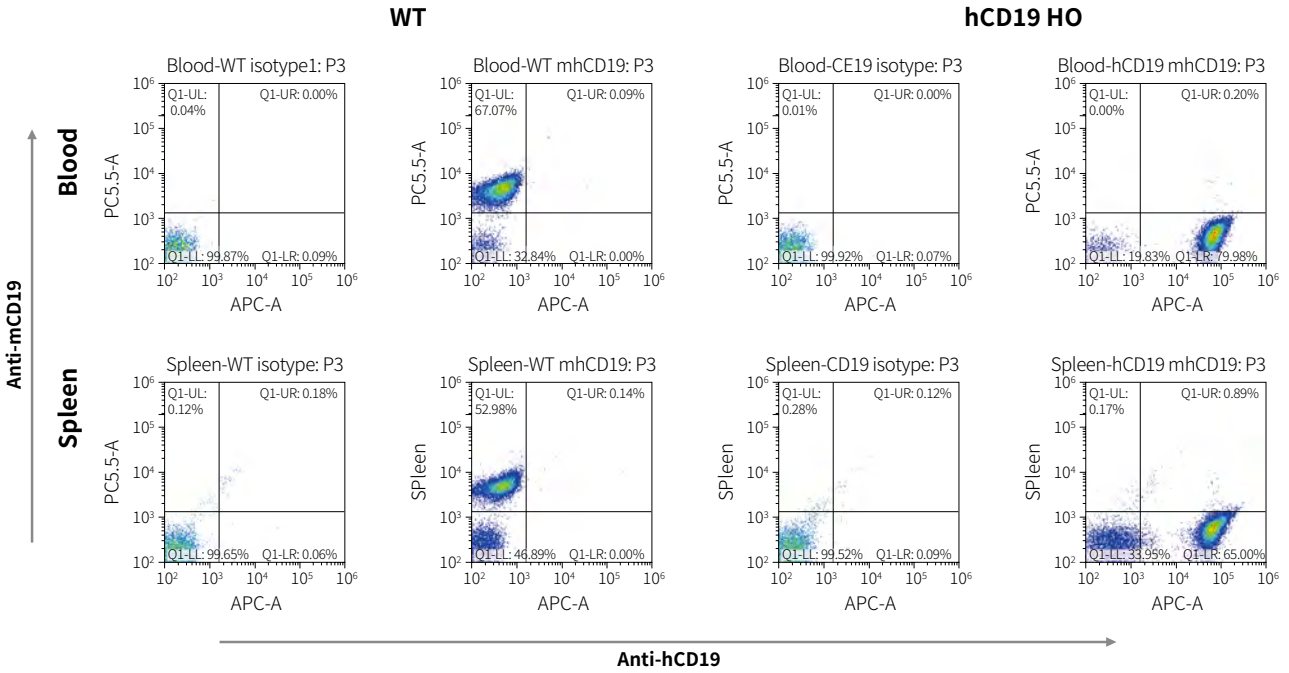


Figure 59. The active expression of human CD19 was confirmed in both peripheral blood cells and spleen lymphocytes derived from homozygous, humanized CD19 mice.

Humanized CD27 Mouse

Strain Name: C57BL/6-*Cd27*$^{tm3(hCD27)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-190035

CD27, also known as TNFRSF7, belongs to the tumor necrosis factor receptor superfamily. CD27 promotes the expansion of antigen-specific T cells, and is required for the generation of T cell memory. CD27 agonism is expected to be more successful when used in combination with other forms of immunotherapies.

Construction strategy

On the C57BL/6 background, the coding sequence for the extracellular domain of the mouse endogenous Cd27 gene was replaced by the human sequence, resulting in the expression of a humanized, chimeric protein.

Validation data

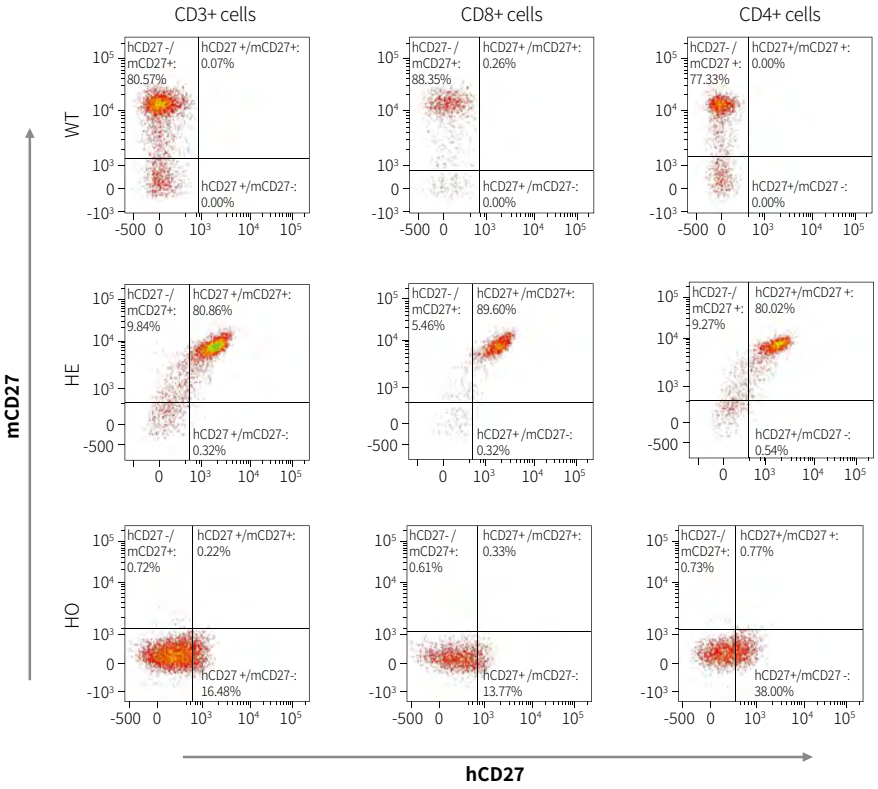


Figure 60. The expression of human CD27 in peripheral T cells derived from heterozygous or homozygous humanized mice was confirmed by FACS.

Humanized CD28 Mouse

Strain Name: C57BL/6-*Cd28*$^{em2(hCD28)/Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-190011

CD28 (the cluster of differentiation 28) is a transmembrane protein that belongs to the immunoglobulin gene superfamily containing an extracellular "V-like" domain. CD28 has co-stimulatory functions on T cell activation and survival. The importance of CD28 costimulatory signaling pathway makes it an appealing target for new drug development to modulate T cell functions.

Construction strategy

On the C57BL/6 background, the mouse endogenous Cd28 gene was replaced by the human ortholog, which results in an exclusive expression of the human CD28 gene.

Validation data

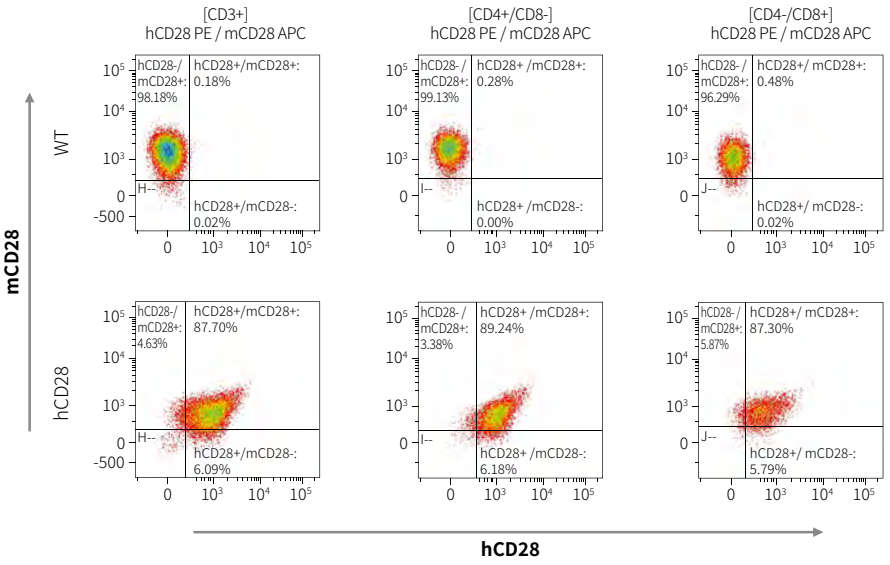


Figure 61. The expression of human CD28 in peripheral blood mononuclear cells derived from the heterozygous, humanized mice was confirmed by FACS.

Humanized CD38 Mouse

Strain Name: C57BL/6-*Cd38* $^{em2(CD38)Smoc}$ Strain Background: C57BL/6 Cat. No. : NM-HU-190059

CD38 is a glycoprotein found on the surface of many immune cells, including CD4+, CD8+, B lymphocytes and natural killer cells. It has been connected to HIV infection, leukemias, myelomas, solid tumors, type II diabetes mellitus and bone metabolis m, as well as some genetically determined conditions. CD38 has been used as a target in treating multiple myelomaby using d aratumumab.

Construction strategy

The humanized CD38 mice were developed on the C57BL/6 background. The coding sequence for the extracellular domain of the mouse endogenous Cd38 gene was completely replaced by the human sequence, resulting in the expression of a humanized, chimeric protein.

Validation data

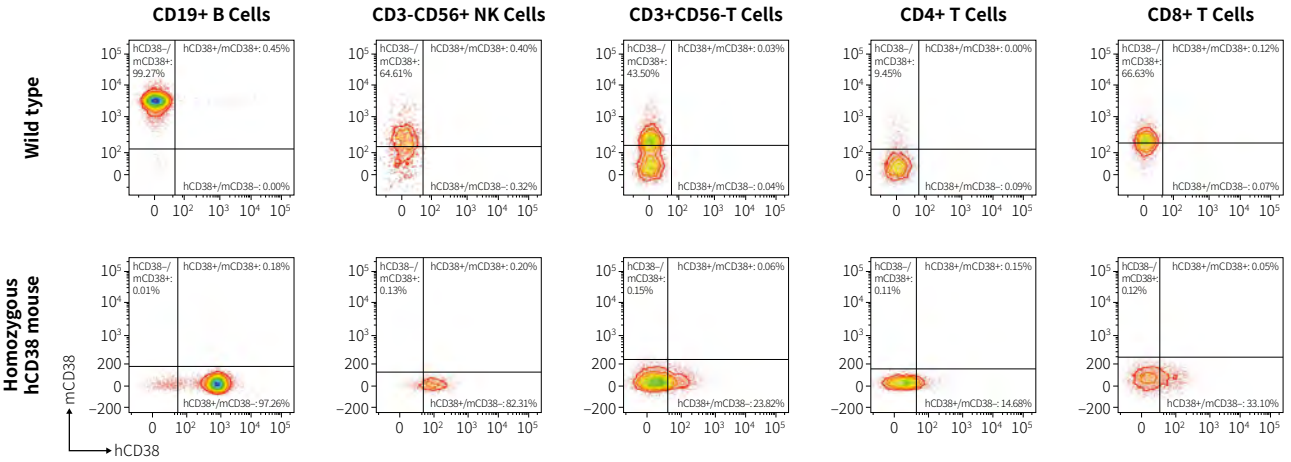


Figure 62. Detection of CD38 expression in live cells from peripheral blood of humanized CD38 mice. The FACS results showed that the high expression of humanized CD38 was detected in B, T, NK cells from peripheral blood of homozygous humanized CD38 mice.

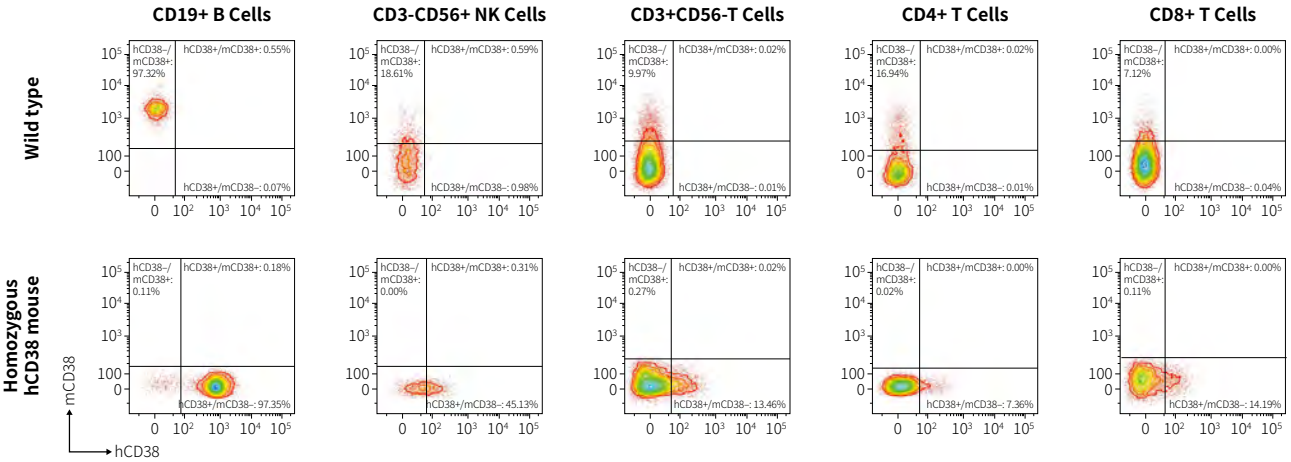


Figure 63. Detection of CD38 expression in live cells from the spleen of humanized CD38 mice. The FACS results showed that the high expression of humanized CD38 was detected in B, T, NK cells from the spleen of homozygous humanized CD38 mice.

Humanized CD79B Mouse

Strain Name: C57BL/*6-Cd79b* $^{em2(hCD79B)Smoc}$ Strain Background: C57BL/6 Cat. NO. : NM-HU-2000012

CD79B (also known as CD79b molecule, immunoglobulin-associated beta) is a 37-39 kDa member of the Ig-Superfamily. It is expressed on B cells, and required in cooperation with CD79A for initiation of the signal transduction cascade activated by the B-cell antigen receptor complex (BCR) which leads to internalization of the complex, trafficking to late endosomes and antigen presentation. CD79B is also required for formation of pre-B cells during B cell development.

Construction strategy

The humanized CD79B mice were developed on the C57BL/6 background. The coding sequence for the extracellular domain of the mouse endogenous CD79B was replaced with the human-derived sequence, resulting in the expression of a humanized, chimeric CD79B gene.

Validation data

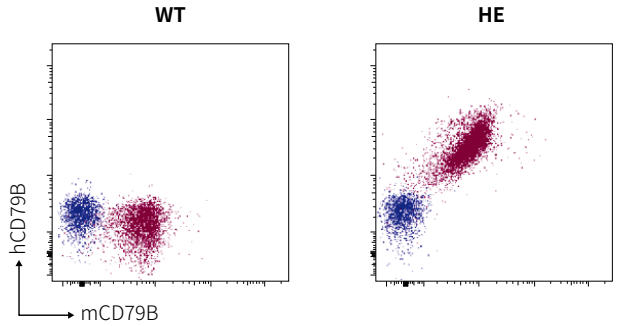


Figure 64. Expression of human CD79B in B lymphocytes (red) of heterozygous humanized CD79B mice was confirmed by FACS (In collaboration with InnoventBio).

Humanized CD147 Mouse

Strain Name: C57BL/*6-Bsg* $^{tm1(hBSG)/Smoc}$ Strain Background: C57BL/6 Cat. NO. : NM-HU-2000021

CD147 also known as Basigin (BSG) is a member of the immunoglobulin superfamily, with a structure related to the putative primordial form of the family. As members of the immunoglobulin superfamily play fundamental roles in intercellular recognition involved in various immunologic phenomena, differentiation, and development, CD147 is thought also to play a role in intercellular recognition. Recently, CD147 was identified as a novel receptor for SARS-CoV-2 invasion.

Construction strategy

The humanized CD147 mice were developed on the C57BL/6 background. The coding sequence for the extracellular domain of the mouse endogenous CD147 was replaced with the human-derived sequence, resulting in the expression of a humanized, chimeric CD147 gene.

Validation data

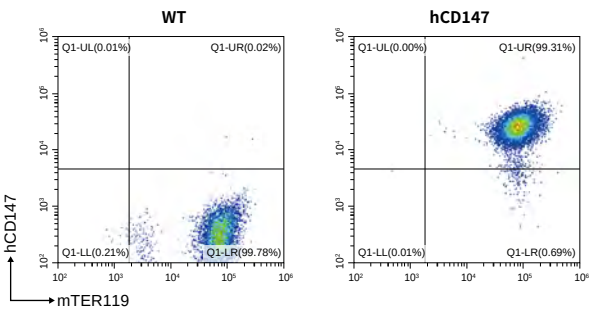


Figure 65. Expression of human CD147 in the red blood cells of humanized CD147 mice was confirmed by FACS.

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