SHANGHAI MODEL ORGANISMS

Humanized knock-in mouse models for evaluating in vivo efficacy of immuno-oncology therapies

Immune Checkpoint Humanized Mouse Models

Our vision is to provide researchers all over the world with professional, comprehensive and integrated animal model services to facilitate a simplified and highly-efficient approach to uncover the mysteries of life.

300,000

300,000 SPF mice in barrier facilities

5000+

More than 5000 GEM models developed so far

1000+

More than 1000 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 60,000 specific-pathogen free (SPF) cages that are available for 300,000 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.

Immune Checkpoint 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 100 immune checkpoint humanized mouse models, including single gene humanized, double humanized or even triple humanized models. Thanks to our unprecedentedly high R&D and production capacity, the list of available humanized models is still rapidly expanding.

Immune Checkpoint Humanized Mouse Models available at SMOC

4-1BB	*CD86*	*IL4RA*	*SIRPA*	*OX40 & CTLA4*	*PD-L1 & GITR*
4-1BBL	*CEACAM1*	*Il5*	*SLAMF7*	*PD-1 & 4-1BB*	*PD-L1 & LAG3*
APOE2	*CSF1*	*IL6R*	*TIGIT*	*PD-1 & CD3e*	*PD-L1 & OX40*
APOE3	*CSF2*	*IL7*	*TIM3*	*PD-1 & CD40*	*PD-L1 & SEMA4D*
APOE4	*CSF3*	*IL9*	*TLR7*	*PD-1 & CTLA4*	*PD-L1 & TIGIT*
BTLA	*CTLA4*	*KDR*	*TLR8*	*PD-1 & GITR*	*SIRPA & CD47*
CCR2	*CXCR2*	*KLRK1*	*TLR9*	*PD-1 & LAG3*	*TLR9 & OX40*
CD19	*DPP4*	*LAG3*	*TMEM173*	*PD-1 & OX40*	*PD-1 & PD-L1 & IDO1*
CD27	*FcRn*	*OX40*	*TNFRSF1B*	*PD-1 & PD-L1*	*PD-1 & PD-L1 & LAG3*
CD28	*GITR*	*OX40L*	*TNFRSF25*	*PD-1 & SEMA4D*	*PD-1 & PD-L1 & OX40*
CD36	*ICOS*	*PCSK9*	*VISTA*	*PD-1 & SIRPA*	*PD-1 & TIGIT & TIM3*
CD3E	*ICOSL*	*PD-1*	*VTCN1*	*PD-1 & TIGIT*	*SIRPA & CD47 & PD-1*
CD4	*IDO1*	*PD-L1*	*CD19 & CD3E*	*PD-1 & TIM3*	
CD40	*IL17A*	*PSGL-1*	*CTLA4 & 4-1BB*	*PD-1 & TLR9*	
CD47	*IL17F*	*PVR*	*ICOS & ICOSL*	*PD-L1 & 4-1BB*	
CD80	*IL23A*	*SEMA4D*	*IL6 & IL6R*	*PD-L1 & CD40*	
CD81	*IL3*	*SIGLEC15*	*LAG3 & CTLA4*	*PD-L1 & CTLA4*	

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

Humanized PD-1 Mouse

Strain Name: C57BL/6J-*Pdcd1*$^{em1(hPDCD1)/Smoc}$ Strain Background: C57BL/6

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

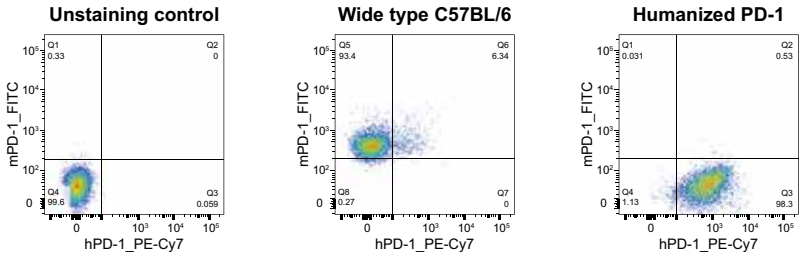

Figure 1. A complete switch from mouse to human PD-1 expression in the activated spleen lymphocytes derived from homozygous, humanized PD-1 mice was confirmed by FACS.

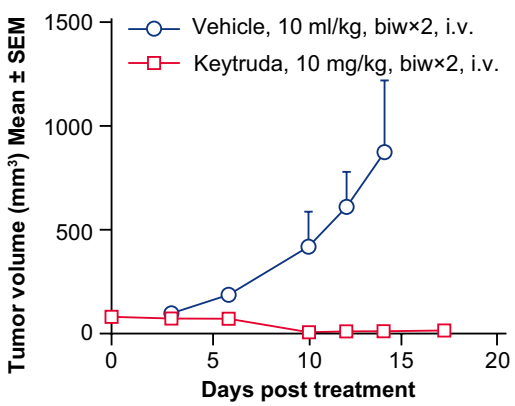

Figure 2. *In vivo* validation of humanized PD-1 mice. Homozygous humanized PD-1 mice were inoculated with MC38 colon cancer cells. When tumors reached an average volume of 100 mm^3, the animals were randomly assigned into a control group that receives placebo (blue) and a treatment group that receives Keytruda® (red). The treatment was given twice a week for a total of 4 administrations. Keytruda®, a marketed PD-1 blocking antibody manufactured by Merck & Co., exhibited a strong tumor-suppressing effect ($p<0.001$), demonstrating the utilities of humanized PD-1 mice in evaluating the efficacy of human therapeutic antibodies directed against PD-1 (In collaboration with GenScript).

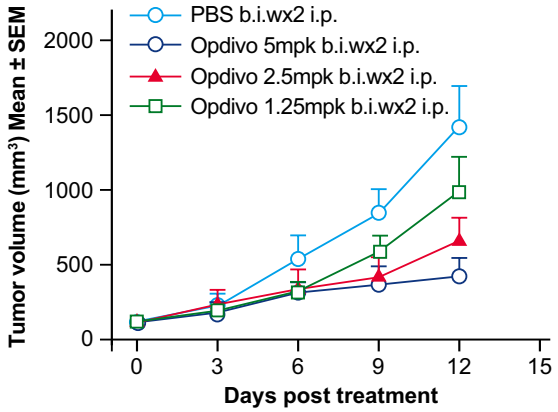

Figure 3. A similar *in vivo* validation study with the use of another FDA-approved, human PD-1 blocking antibody Opdivo®. This further demonstrates that humanzied PD-1 mice represents an ideal model for assessing the *in vivo* efficacy of therapeutic PD-1 antibody.

Humanized PD-L1 Mouse

Strain Name: C57BL/6J-*Cd274*$^{em1(hPD\text{-}L1)/Smoc}$ Strain Background: C57BL/6

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

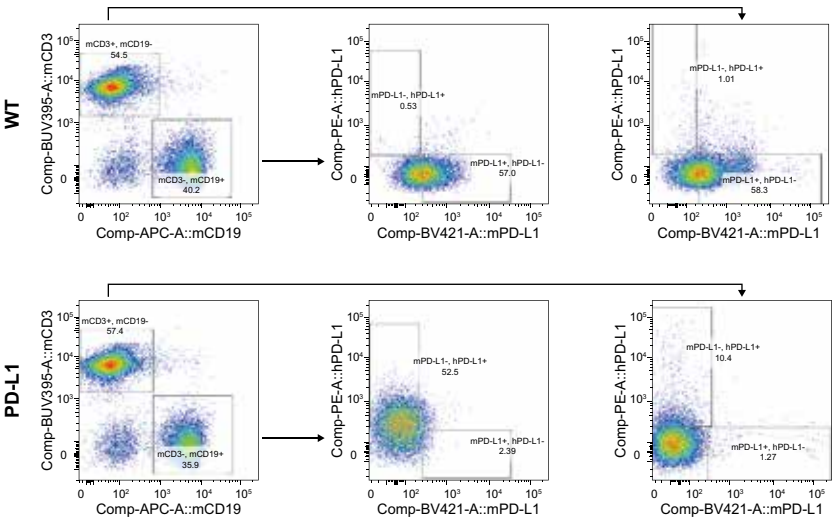

Figure 4. FACS analysis of humanized PD-L1 mice. Splenocytes from both WT C57BL/6 and homozygous, humanized PD-L1 mice were analyzed by flow cytometry. Human PD-L1 expression was confirmed in both T cells and B cells derived from humanized PD-L1 mice (In collaboration with CrownBio).

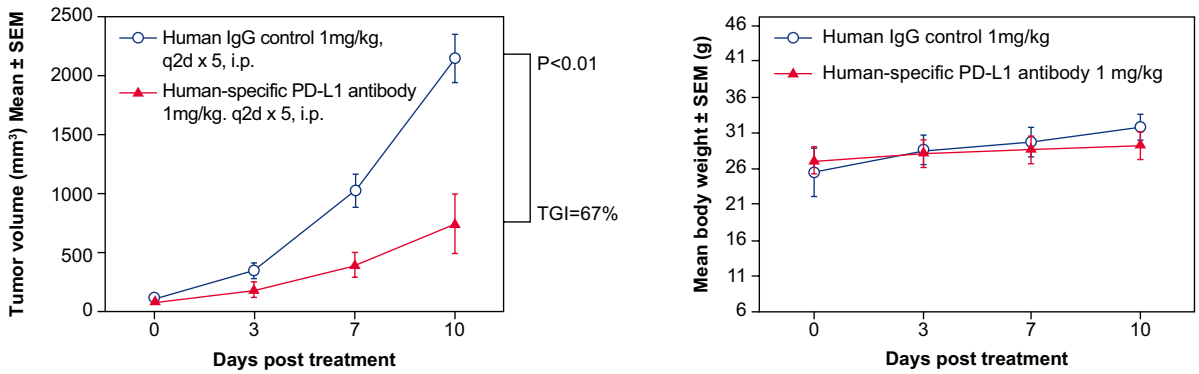

Figure 5. *In vivo* validation of humanized PD-L1 mice. (Left) Mouse colon cancer cells MC38 engineered to express human PD-L1 were implanted subcutaneously into homozygous, humanized PD-L1 mice. The mice were randomly assigned into two groups when the tumor volume reached 100 mm^3, one group receiving 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.

Humanized CTLA4 Mouse

Strain Name: C57BL/6J-*Ctla4* $^{em1(hCTLA4)/Smoc}$ Strain Background: C57BL/6

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

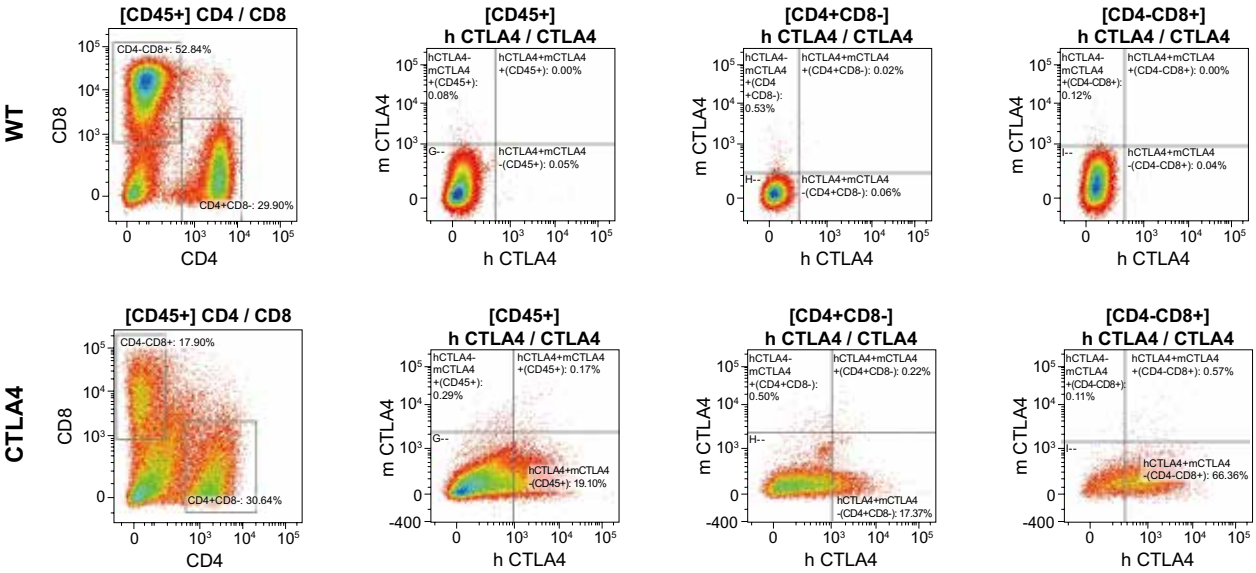

Figure 6. The expression of human CTLA4 in the splenocytes of humanized CTLA4 mice was confirmed by FACS. Spleen lymphocytes were harvested from homozygous, humanized CTLA4 mice, activated by CD3& CD28 antibodies for 48 hrs and then subjected to staining. Active expression of humanized CTLA4 can be detected in both activated $CD4^+$ and $CD8^+$ T lymphocytes derived from homozygous humanized CTLA4 mice. (In collaboration with CrownBio)

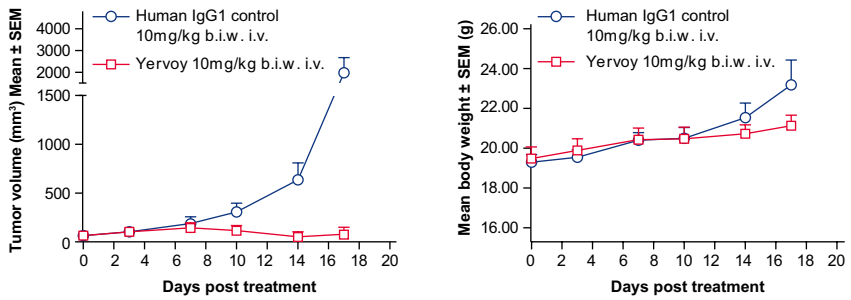

Figure 7. Efficacy evaluation of human CTLA4 blocking antibody YERVOY® in the homozygous humanized CTLA4 mice. The animals were inoculated with MC38 colon cancer cells, and randomly assigned into a control group receiving human IgG1 and a treatment group receiving the human specific, anti-CTLA4 antibody YERVOY (n=8) when the tumors grew to 100 mm^3. The humanized CTLA4 mice responded to YERVOY with a strong antitumor 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) YERVOY (Ipilimumab): A FDA-approved monoclonal antibody targeting human CTLA-4, marketed by Bristol-Myers Squibb.

Humanized OX40 Mouse

Strain Name: C57BL/6J-*Tnfrsf4*$^{em1(hTNFRSF4)Smoc}$ Strain Background: C57BL/6

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 murine 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

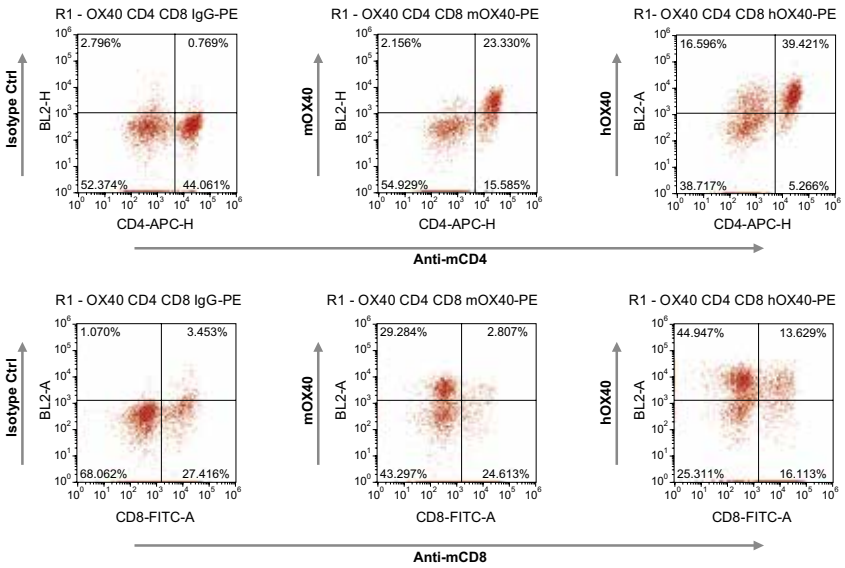

Figure 8. The expression of human OX40 in the splenocytes of humanized OX40 mice was confirmed by FACS. Spleen lymphocytes were collected from heterozygous, humanized OX40 mice, activated by CD3& CD28 antibodies for 48 hrs and then subjected to staining. Active expression of human OX40 can be detected in both activated $CD4^+$ and $CD8^+$ T lymphocytes collected from heterozygous humanized OX40 mice.

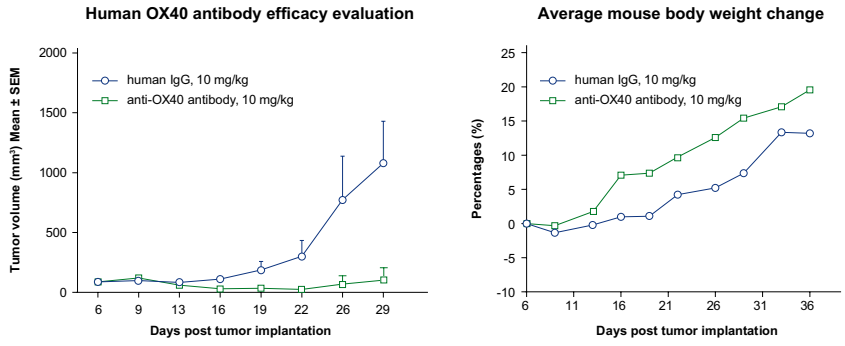

Figure 9. Evaluation of human-specific, OX40 antibody in the heterozygous humanized OX40 mice. The animals were inoculated with MC38 colon cancer cells, and randomly assigned into a control group and a treatment group (n=8) when the tumors grew to 100 mm^3. The humanized OX40 mice responded to human anti-OX40 (Left) without a significant body weight change at late time points (Right).

Humanized LAG3 Mouse

Strain Name: B6.129-*Lag3*$^{tm1(hLAG3)/Smoc}$ Strain Background: B6.129

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

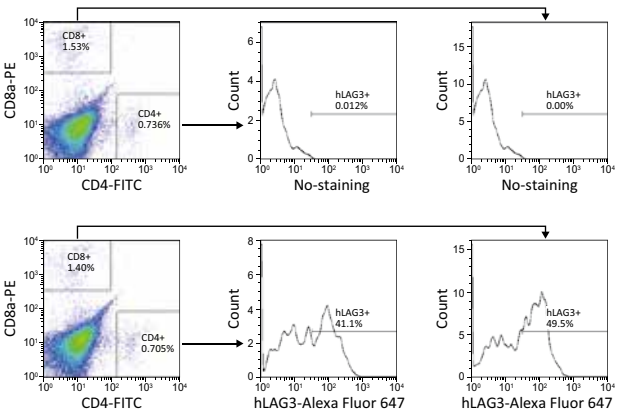

Figure 10. Human LAG3 expression in tumor-infiltrating lymphocytes collected from humanized LAG3 mice was confirmed by FACS. Homozygous, humanized LAG3 mice were inoculated with MC38 colon cancer cells. When the tumors grew to an average volume of 100 mm^3, tumor-infiltrating lymphocytes were collected and subjected to staining (In collaboration with CrownBio).

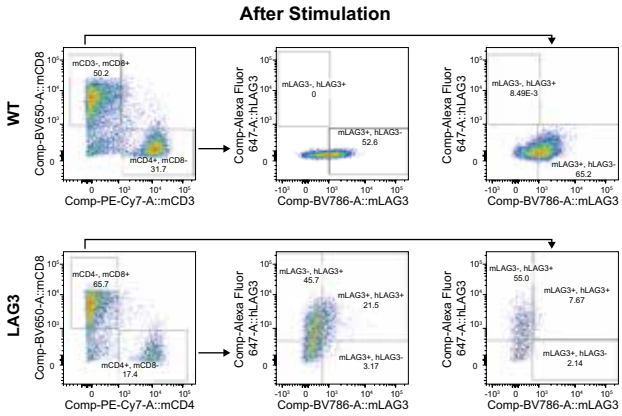

Figure 11. Human LAG3 expression in activated splenocytes from humanized LAG3 mice was measured by FACS. The results showed an active expression of human LAG3 in both activated CD4$^+$ and CD8$^+$ T lymphocytes derived from homozygous, humanized LAG3 mice (In collaboration with CrownBio).

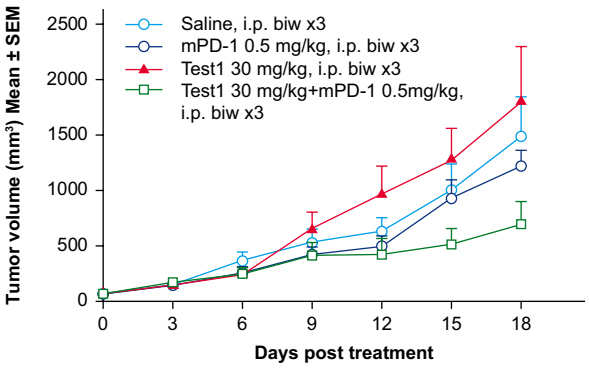

Figure 12. *In vivo* validation of humanized LAG3 mice. Homozygous humanized LAG3 mice were firstly inoculated with MC38 colon cancer cells, and then randomly assigned into different groups (n=5) when the tumors grew to an average volume of 50 mm^3. A significant anti-tumor effect was observed when the human LAG3-targeting antibody Test1 was administered along with the mouse PD-1 antibody.

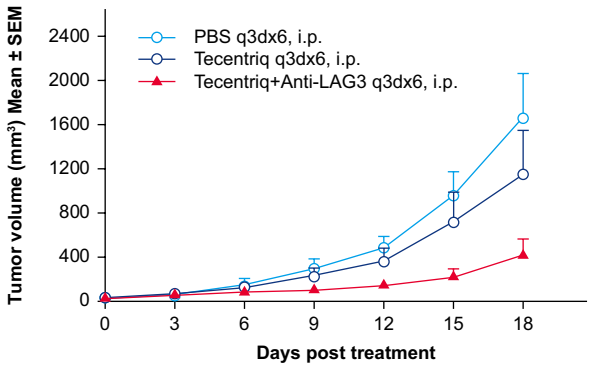

Figure 13. Homozygous humanized LAG3 mice were inoculated with MC38 colon cancer cells, and randomly assigned to different groups (n=8) when the tumors grew to 70-80 mm^3. Similar to the previous figure, a significant anti-tumor effect was observed when the human LAG3 antibody was administered together with TENCENTRIQ®. TENCENTRIQ (atezolizumab): A monoclonal antibody of IgG1 isotype against human PD-L1 marketed by Roche.

Humanized CD40 Mouse

Strain Name: C57BL/6J-*Cd40*$^{em1(hCD40)Smoc}$ Strain Background: C57BL/6

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

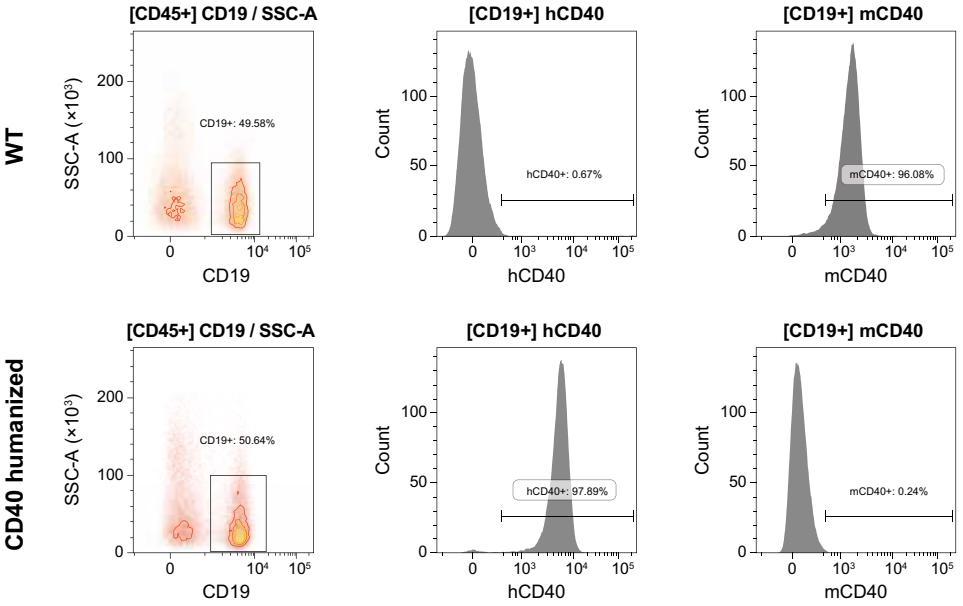

Figure 14. The expression of human CD40 in the peripheral blood cells derived from the humanized CD40 mice was measured by FACS. An active expression of human CD40 was found on $CD19^+$ cells derived from homozygous humanized CD40 mice, with a comparable expression level to that of mouse Cd40 in WT mice (In collaboration with CrownBio).

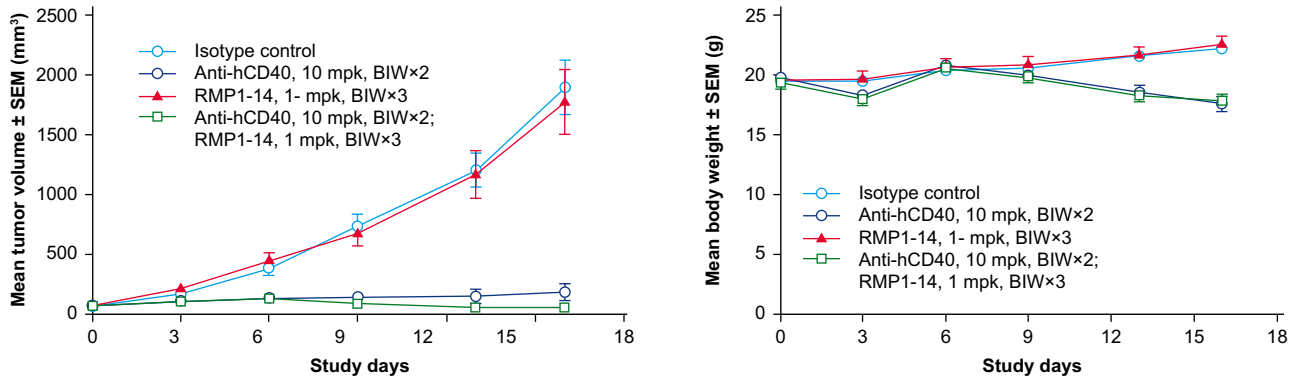

Figure 15. *In vivo* validation of humanized CD40 mice. Humanized mice were inoculated with MC38 colon cancer cells, and randomly assigned into different groups (n=8) when the tumors grew to 100 mm^3. As expected, human CD40-targeting antibodies demonstrated a significant anti-tumor effect ($p<0.001$). Plus, a combinatorial treatment of CD40 and PD-1 showed a marginally improved anti-tumor efficacy. *RMP1-14: a monoclonal antibody against mouse PD-1.

Humanized 4-1BB Mouse

Strain Name: C57BL/6J-*Tnfrsf9*$^{em1(hTNFRSF9)Smoc}$ **Strain Background: C57BL/6**

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

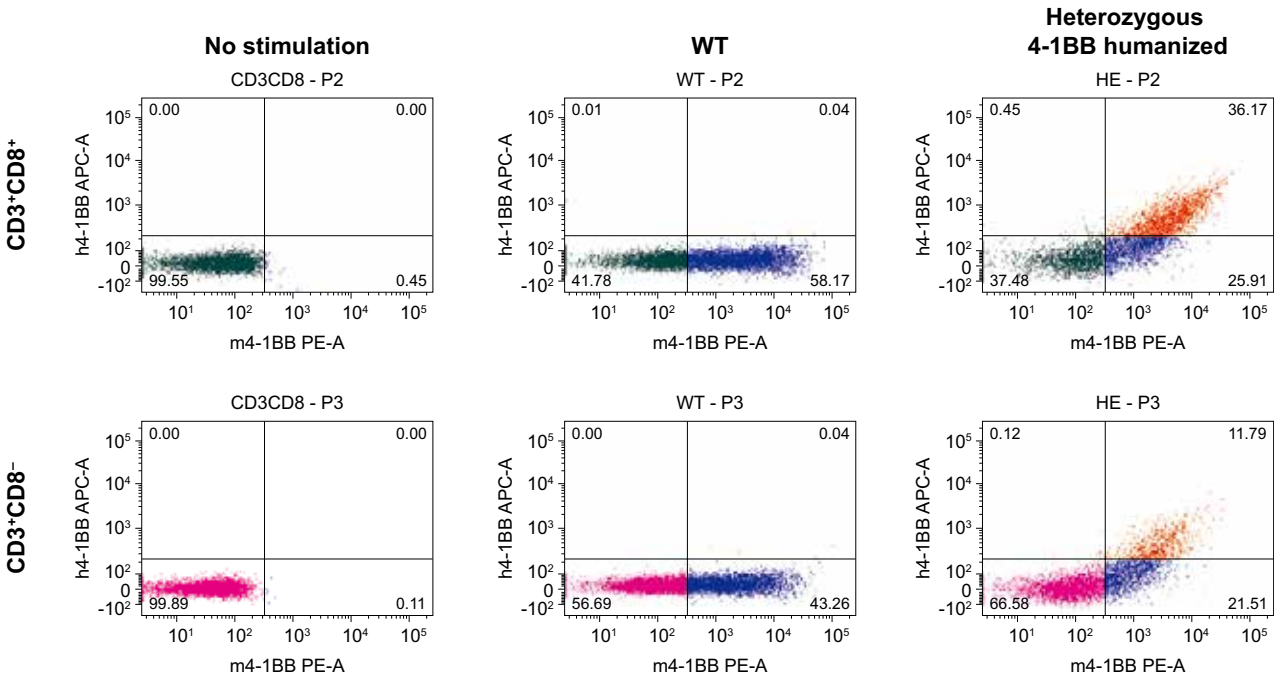

Figure 16. Human 4-1BB expression in the activated splenocytes derived from humanized 4-1BB mice was confirmed by FACS. Spleen lymphocytes were harvested from heterozygous humanized 4-1BB mice, activated by anti-CD3 and anti-CD28 for 48 hrs, and then subjected to staining.

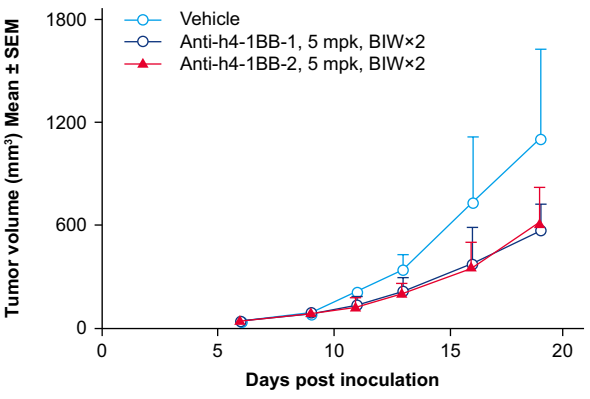

Figure 17. *In vivo* validation of humanized 4-1BB mice. Humanized 4-1BB mice were inoculated with MC38 colon cancer cells, and were randomly assigned into different groups (n=8) when the tumors grew to a volume of 100 mm^3. Human-specific, 4-1BB antibodies were given biweekly for two weeks.

Double humanized PD-1&PD-L1 mouse

Strain Name: C57BL/6J-*Pdcd1*$^{em1(hPDCD1)}$ *Cd274*$^{em1(hPD-L1)/Smoc}$ Strain Background: C57BL/6

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

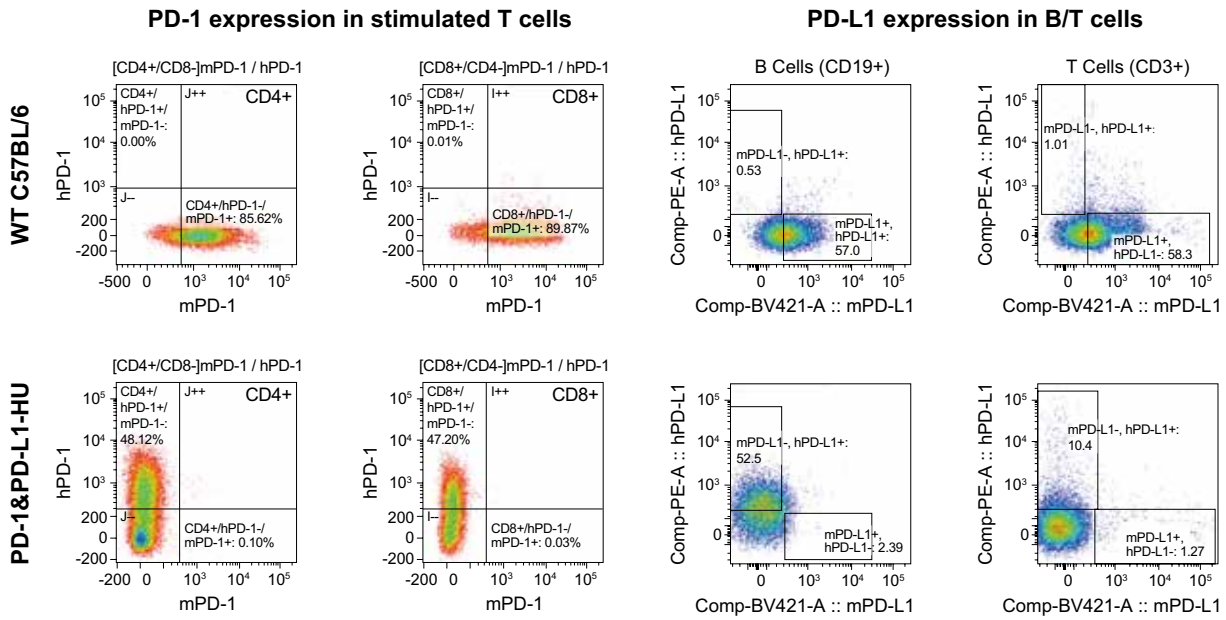

Figure 18. The expression of human PD-1 and PD-L1 in double humanized PD-1&PD-L1 mice was confirmed by FACS.

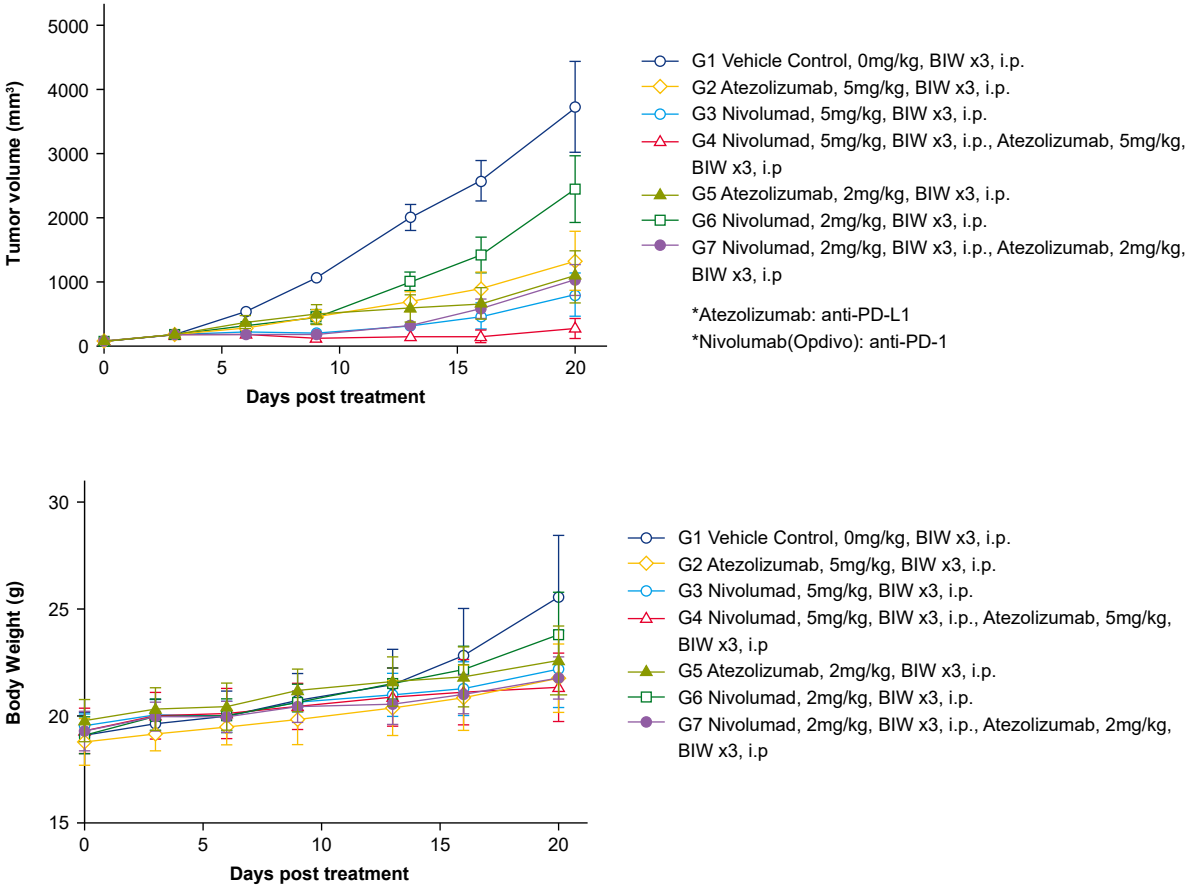

Figure 19. *In vivo* validation of double humanized PD-1&PD-L1 mice. Double humanized mice were inoculated with MC38 cells, and randomly assigned to different groups (n=8) when the tumor grew to a volume of 100 mm^3. A combinatorial treatment of anti-PD-L1 and anti-PD-1 demonstrated a noticeable efficacy improvement compared to the same dose of single agent (top) without affecting the animal body weight (Bottom).

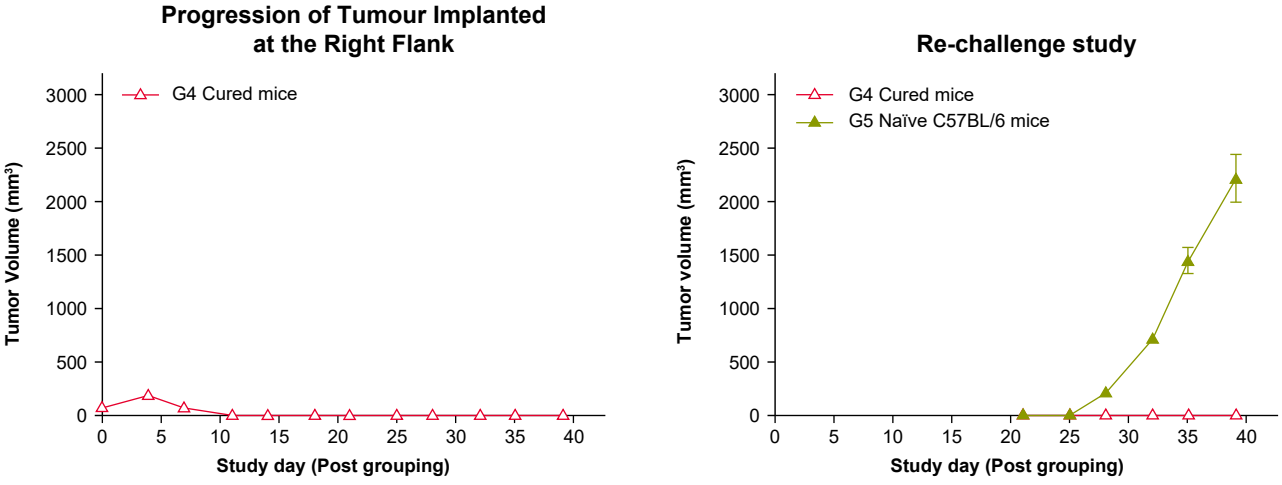

Figure 20. As shown in the previous figure, animals of group 1-4 received different doses of either single agent or combinatorial treatment. The left graph showed the progression of the tumor implanted at the right flank, implicating a systematic anti-tumor effect induced by the combinatorial antibody 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-1&CTLA-4 mouse

Strain Name: C57BL/6J-*Pdcd1*$^{em1(hPDCD1)}$ *Ctla4*$^{em1(hCTLA4)/Smoc}$ Strain Background: C57BL/6

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

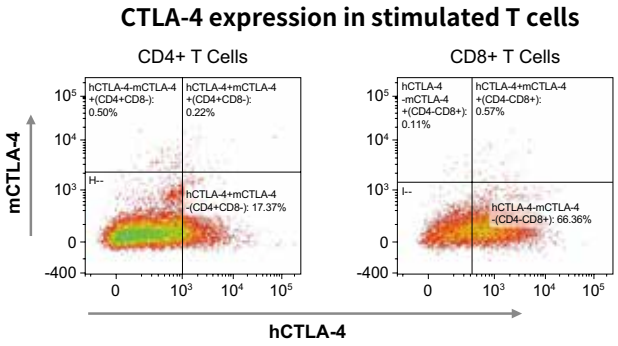

Figure 21. The expression of human CTLA-4 in double humanized PD-1&CTLA-4 mice was confirmed by FACS.

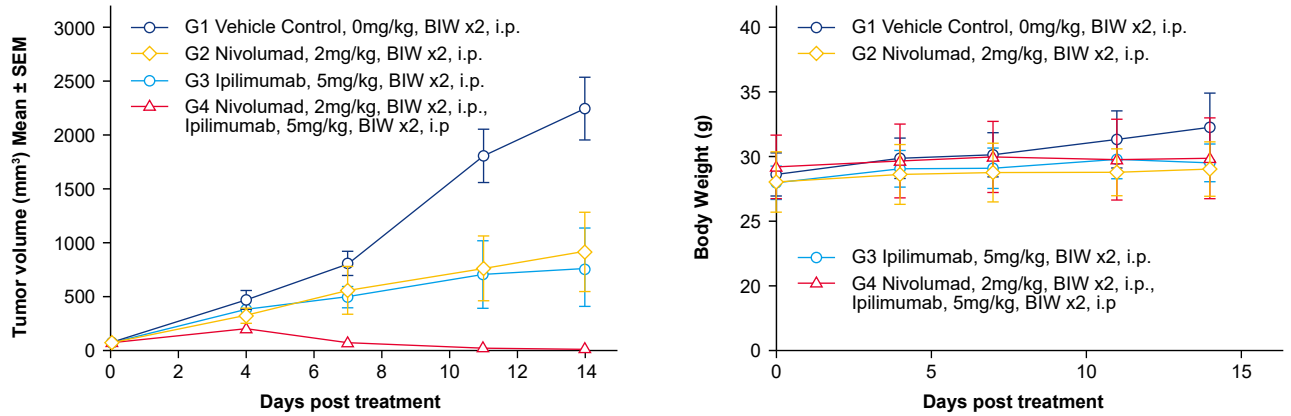

Figure 22. *In vivo* validation of double humanized PD-1&CTLA-4 mice. Double humanized mice were inoculated with MC38 cells, and randomly assigned to different groups (n=8) when the tumor grew to a volume of 100 mm^3. A combinatorial treatment of anti-CTLA-4 and anti-PD-1 demonstrated a significant efficacy 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®

Humanized ICOS Mouse

Strain Name: C57BL/6J-*Icos*$^{em1(hICOS)Smoc}$ Strain Background: C57BL/6

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

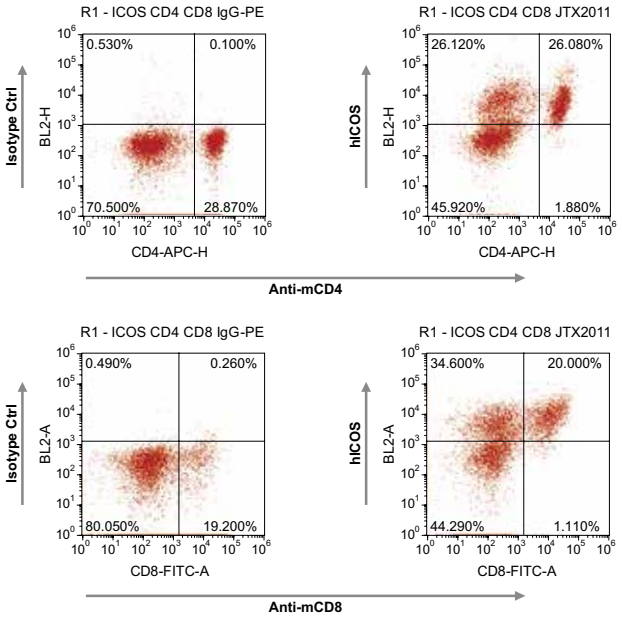

Figure 23. The expression of human ICOS in the splenocytes of humanized ICOS mice was confirmed by FACS. Spleen lymphocytes were collected from heterozygous, humanized ICOS mice, activated by CD3& CD28 antibodies for 48 hrs and then subjected to staining. The results showed that the active expression 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/6J-*Havcr2*$^{em1(hHAVCR2)/Smoc}$ Strain Background: C57BL/6

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

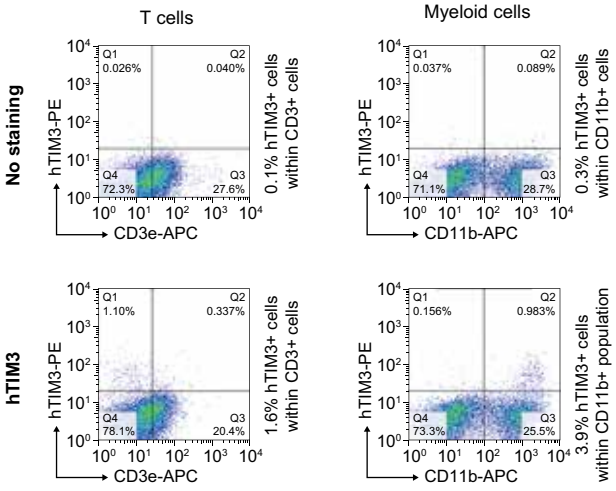

Figure 24. Human TIM3 expression in tumor infiltrating lymphocytes collected from humanized TIM3 mice was confirmed by FACS. Homozygous humanized TIM3 mice were inoculated with MC38 colon cancer cells. When the tumors grew to an average volume of 100 mm^3, tumor infiltrating lymphocytes were isolated 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 TIGIT Mouse

Strain Name: C57BL/6J-*Tigit*$^{em1(hTIGIT)Smoc}$ Strain Background: C57BL/6J

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

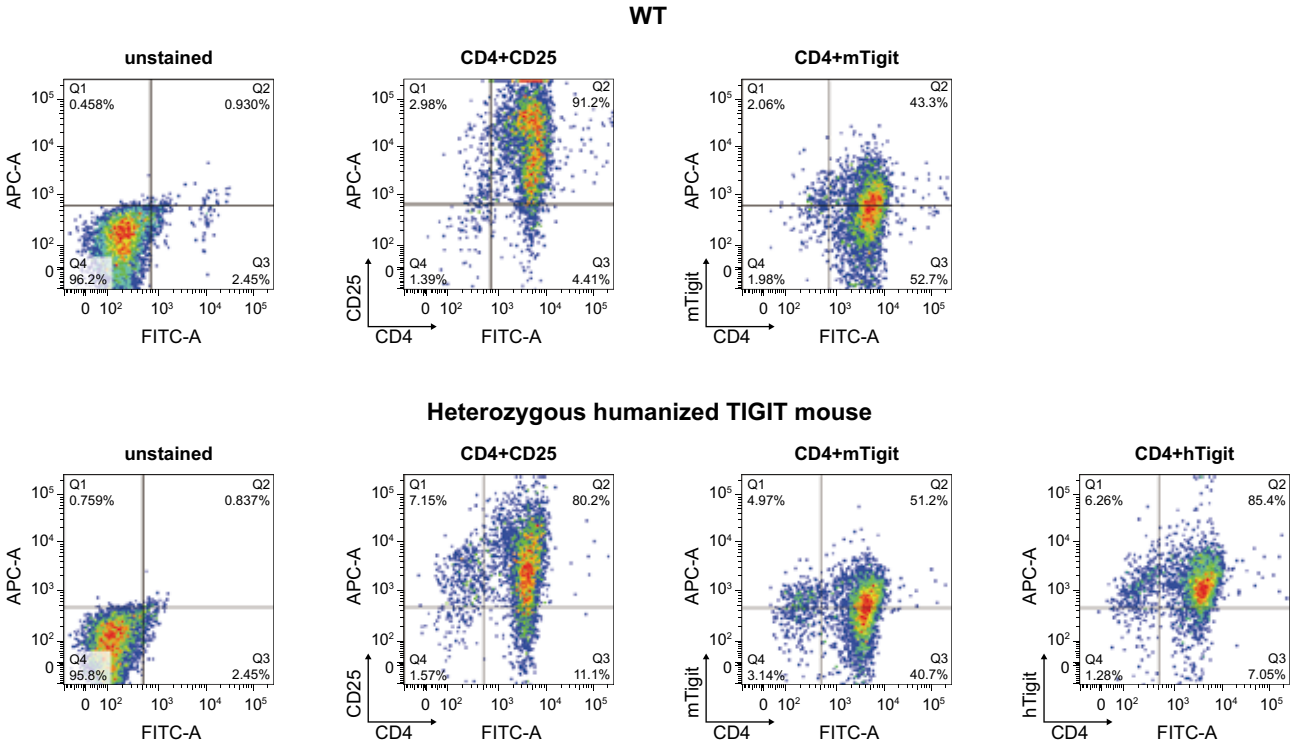

Figure 25. The expression of human TIGIT in the polarized CD4$^+$ T cells derived from humanized TIGIT mice was confirmed by FACS. Naive spleen CD4$^+$ T cells were isolated from heterozygous humanized TIGIT mice. After *in vitro* stimulation, activation and expansion by cytokines and antibodies, the 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 murine Tigit.

Humanized GITR Mouse

Strain Name: C57BL/6J-*Tnfrsf18*$^{em1\ (hTNFRSF18)\ Smoc}$ **Strain Background: C57BL/6**

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

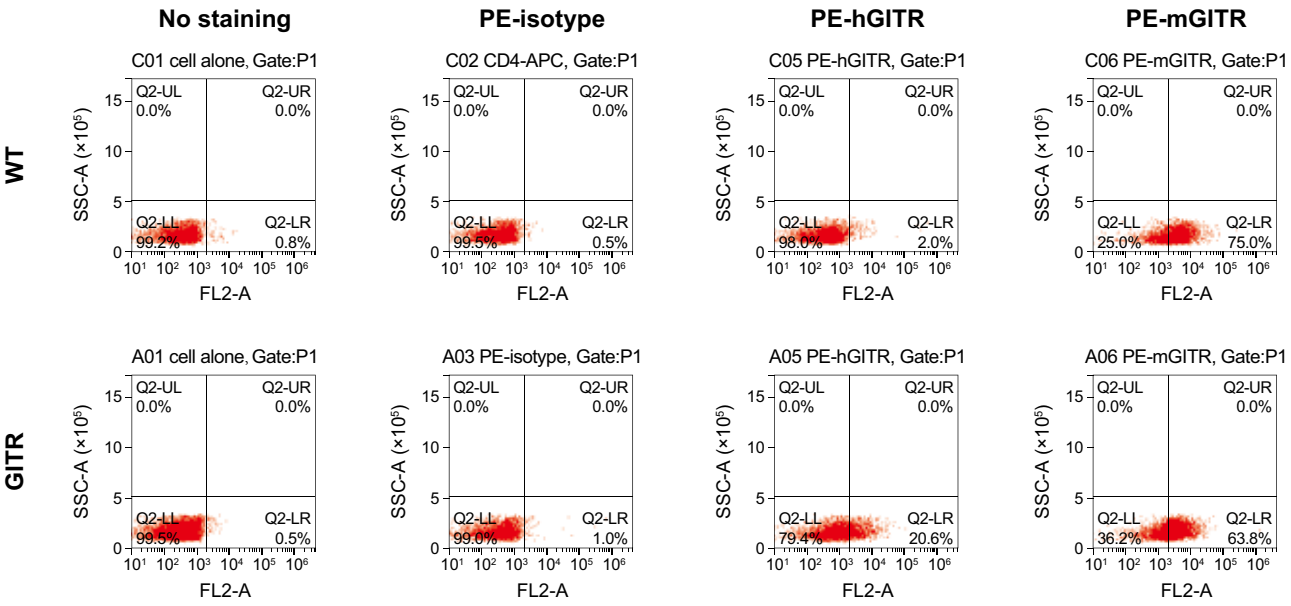

Figure 26. The expression of human GITR in the splenocytes collected from heterozygous humanized GITR mice was measured by FACS.

Humanized CD3E Mouse

Strain Name: C57BL/6J-*Cd3e*$^{em1(hCD3E)Smoc}$ Strain Background: C57BL/6

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

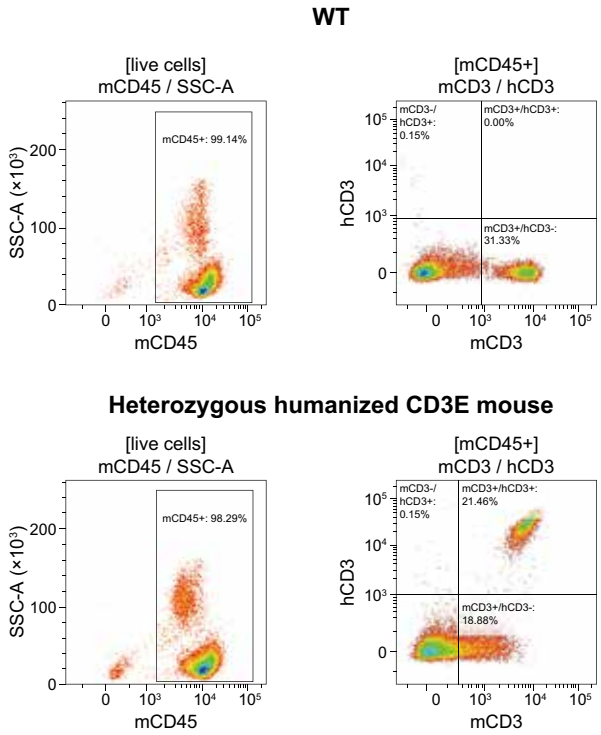

Figure 27. The expression of human CD3E in the PBMC derived from heterozygous humanized CD3E mice was confirmed by FACS.

Humanized CD19 mouse

Strain Name: C57BL/6J-*Cd19*$^{em1(hCD19)Smoc}$ Strain Background: C57BL/6

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 entire mouse Cd19 gene was replaced by its human counterpart.

Validation data

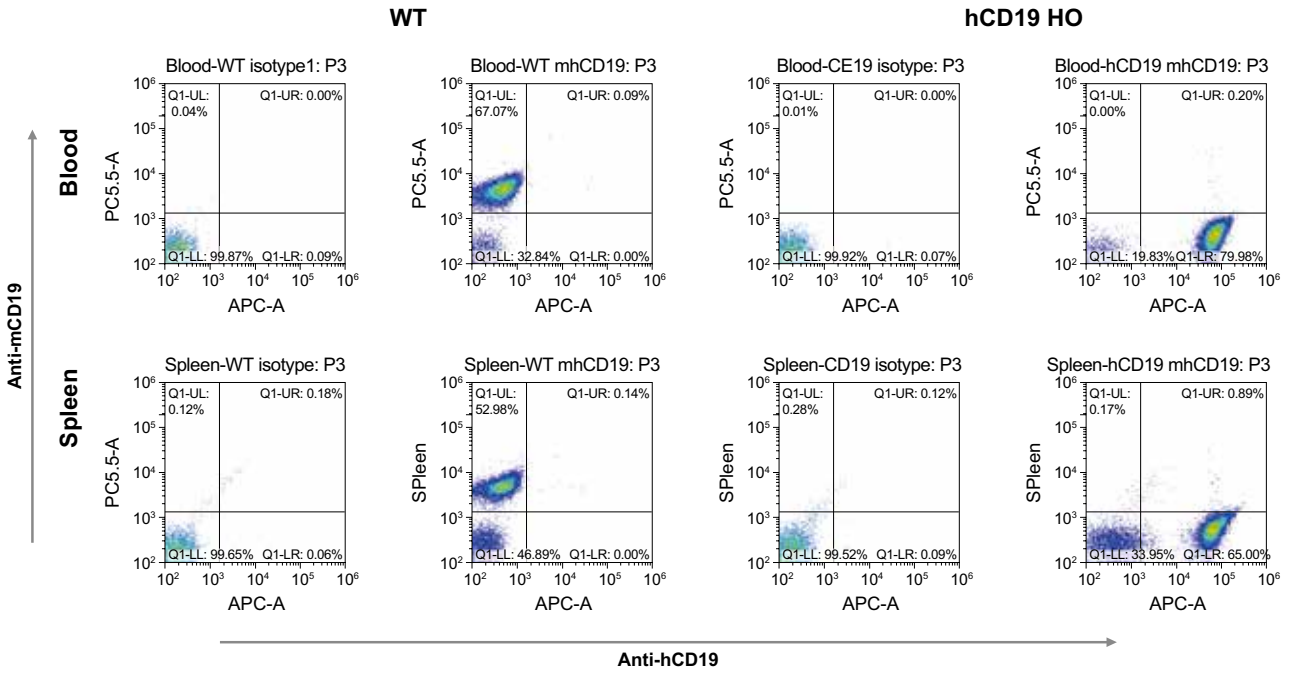

Figure 28. The active expression of human CD19 was confirmed in both peripheral blood cells and spleen lymphocytes derived from homozygous, humanized CD19 mice.

Humanized SIRPA mouse

Strain Name: C57BL/6J-*Sirpa*$^{tm2(hSIRPA)Smoc}$ Strain Background: C57BL/6

Signal regulatory proteins (SIRP) are a multigene family of immuno-receptors. In human and rodents, the major ligand identified for SIRPα is the surface glycoprotein CD47. SIRPα is expressed on all myeloid cells, including monocytes, macrophage, granulocytes and myeloid dendritic cells. In a wide variety of preclinical studies, therapies that block the CD47/SIRPα axis stimulate phagocytosis of cancer cells *in vitro* and anti-tumor immune responses *in vivo*.

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

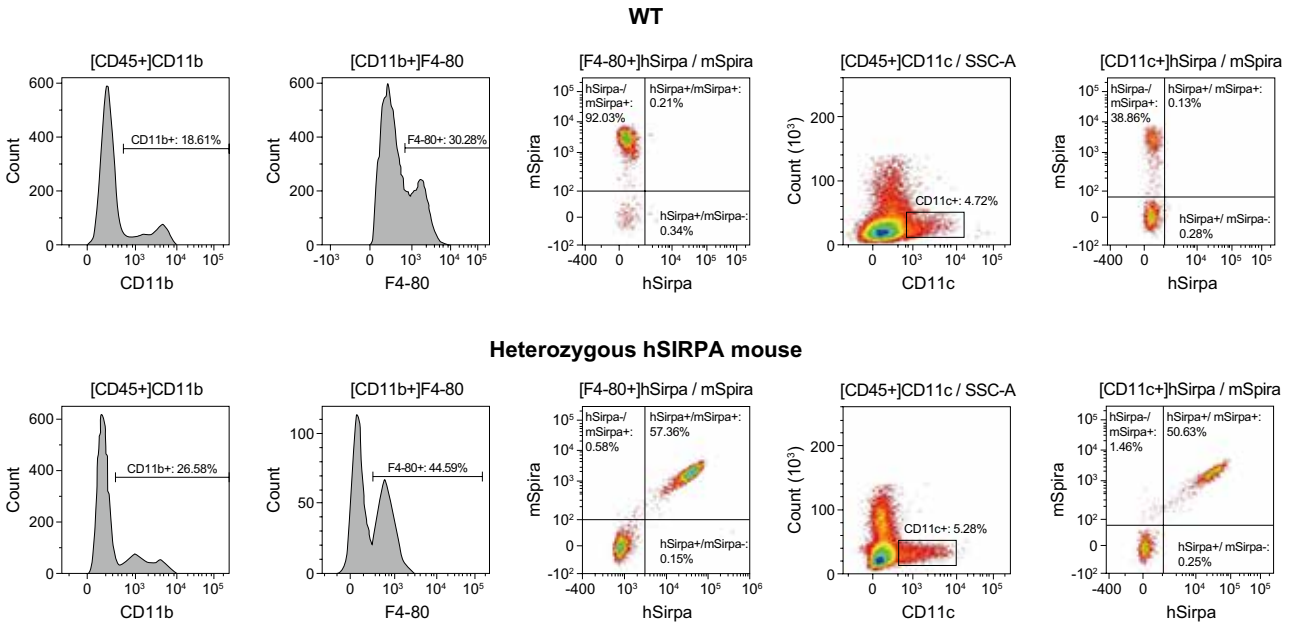

Figure 29. The expression of human SIRPA in humanized SIRPA mice was measured by FACS. In heterozygous humanized SIRPA mice, active expression of human SIRPA was detected in both B cells and macrophages isolated from peripheral blood cells.

Humanized CD27 mouse

Strain Name: C57BL/6J-*Cd27*$^{tm3(hCD27)Smoc}$ Strain Background: C57BL/6

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 full-length coding sequence of human CD27 gene was placed immediately downstream of the start codon of the mouse endogenous Cd27 gene, followed by a poly(A) site. This guarantees an exclusive expression of human CD27 in the humanized mice.

Validation data

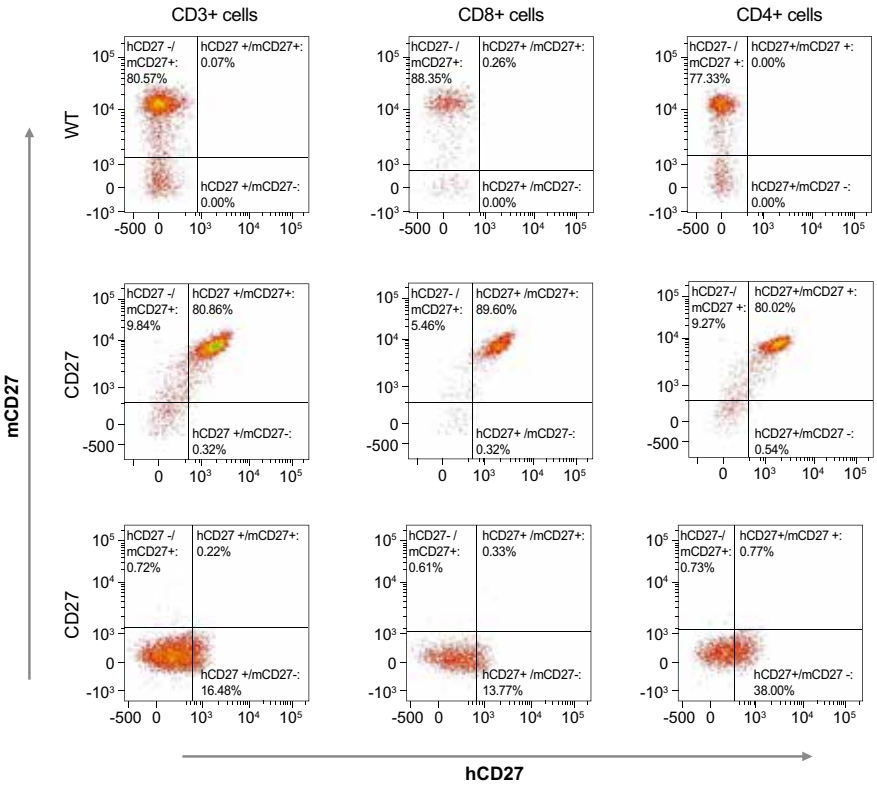

Figure 30. The expression of human CD27 in peripheral T cells derived from heterozygous or homozygous humanized mice was confirmed by FACS.

Humanized VISTA mouse

Strain Name: C57BL/6J-*Vsir*$^{em1(hVSIR)/Smoc}$ Strain Background: C57BL/6

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

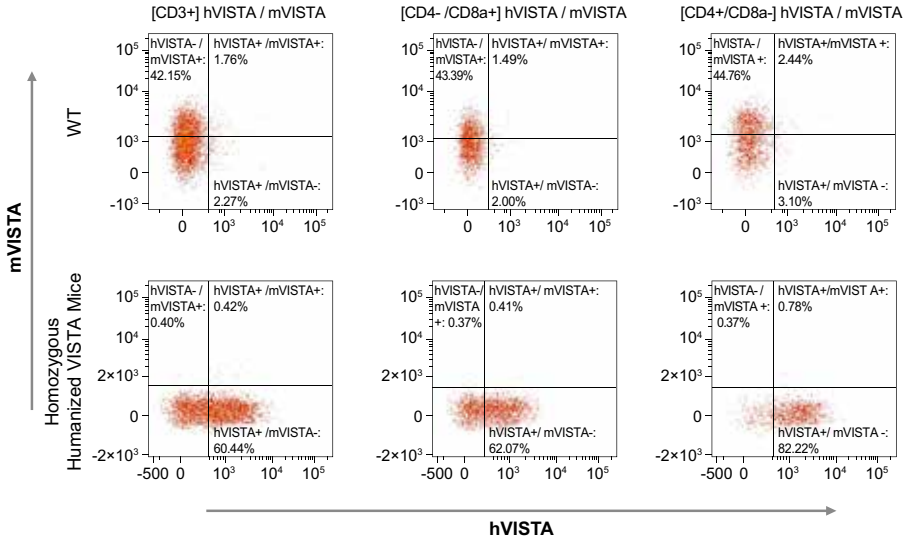

Figure 31. The expression of human VISTA in peripheral T cells derived from homozygous, humanized VISTA mice was confirmed by FACS.

Humanized CD28 mouse

Strain Name: C57BL/6J-*Cd28*$^{em2(hCD28)/Smoc}$ Strain Background: C57BL/6

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

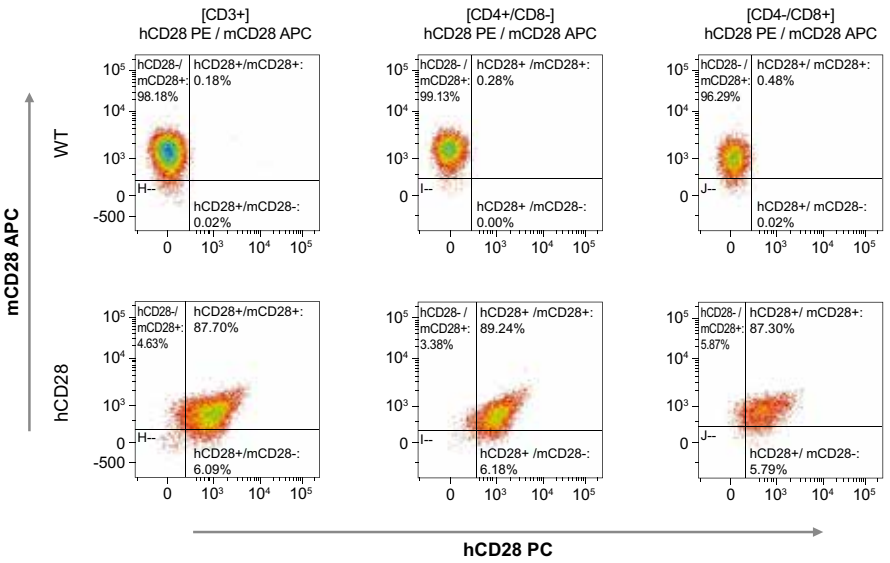

Figure 32. The expression of human CD28 in peripheral blood mononuclear cells derived from the heterozygous, humanized mice was confirmed by FACS.

Everything you need for model organisms

- Model customization
- Research-Ready models
- Breeding
- Phenotyping
- Drug screening

Shanghai Model Organisms Center, Inc.

1820, 29th Ave, San Francisco, CA94122

www.modelorg.com • service.us@modelorg.com