Humanized OX40 Mouse

**Strain Name:** C57BL/6-Tnfrsf4<sup>em1(hTNFRSF4)Smoc</sup>  **Strain Background:** C57BL/6  **Cat. No.:** IT-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<sup>+</sup> and CD8<sup>+</sup> 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**

- Flow cytometry (FACS) analysis data of lymph node T-cells collected from humanized OX40 mice
Figure 1. Expression of OX40 in the spleen lymphocytes of humanized OX40 mice is detected by FACS.

The spleen lymphocytes of heterozygous humanized OX40 mice were activated by anti-CD3 and anti-CD28 for 48 hours, and then collected for staining. Along with a group undergoing no stimulation, the expression of murine and human OX40 was detected by FACS. The results showed that the active expression of human OX40 can be detected in both activated CD4+ and CD8+ T lymphocytes collected from heterozygous humanized OX40 mice, and the expression trend of human OX40 and murine Ox40 was similar.

- In vivo validation in a MC38 tumor-bearing model of humanized OX40 mouse.

○ Case study 1

![Graph showing tumor volume over time for different groups](image-url)
Figure 2. OX40 antibody showed dose-dependent anti-tumor activity in human OX40 knock-in mice bearing MC38 tumors. The OX40 antibody was obtained from Innoventbio.
Case study 2
Figure 3. IBI101 showed dose-dependent anti-tumor activity and enhanced tumor-specific CD8+ T cell response in human OX40 knock-in mice bearing MC38 tumors. a Tumor growth curve of mice treated with different doses of IBI101 alone or in combination with anti-mouse PD-1 antibody. Different doses of IBI101 and anti-mouse PD-1 antibody were administrated as indicated by the arrow heads after MC38 cells implantation. b Animal body weights were measured during the time course of the experiment. c Mice were injected with h-IgG (10 mg/kg), IBI101 (10 mg/kg), anti-PD-1 (0.5 mg/kg) alone or IBI101 (10 mg/kg)+anti-PD-1 (0.5 mg/kg) at day 10 and 14 post tumor cell implantation. At day 17, tumor and spleen were collected and analyzed by flow cytometry for the absolute counts of the indicated cell subsets in tumor and d proportions of indicated cell subsets in CD45+ splenocytes. Flow cytometry results showing the proportions of cytokine-secreting cancer-specific CD8+ and CD4+ T cells from tumor (e) and spleen (f) (n≥5) (In collaboration with Innoventbio)
Humanized CTLA4 Mouse

**Strain Name:** C57BL/6-Ctla4<sup>em1(hCTLA4)/Smoc</sup>  **Strain Background:** C57BL/6  **Cat. No.:** IT-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**

- Flow cytometry (FACS) analysis data of humanized CTLA4 mouse

![Flow cytometry analysis](image)

Figure 1. Expression of CTLA4 in the activated spleen lymphocytes of humanized CTLA4 mice is detected by FACS. The spleen lymphocytes of homozygous humanized CTLA4 mice were activated by anti-CD3 and anti-CD28 for 72 hours, and then collected for staining. The expression of humanized CTLA4 was detected by FACS. The results showed that the active expression of humanized CTLA4 can be detected in both activated CD4<sup>+</sup> and CD8<sup>+</sup> T lymphocytes collected from homozygous humanized CTLA4 mice. (Completed in collaboration with CrownBio).
- In vivo validation in a MC38 tumor-bearing model of humanized CTLA4 mouse
Figure 2. In vivo anti-tumor effect of Ipilimumab, a humanized anti-CTLA4 antibody, in a humanized mouse model of CTLA4

In vivo validation of anti-tumor efficacy in a MC38 tumor-bearing model of humanized CTLA4 mice. Homozygous humanized CTLA4 mice were inoculated with MC38 colon cancer cells. The results showed: Yervoy, a drug targeting human CTLA4, showed a very significant anti-tumor effect ($p<0.001$), demonstrating that the humanized CTLA4 mice are a good in vivo model for validating the efficacy of antibodies targeting human CTLA4.
**Strain Name:** BALB/c-Ctla4\(^{em1(hCTLA4)Smoc}\)  
**Strain Background:** BALB/c  
**Cat. NO.:** IT-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 1. Expression of CTLA4 in the activated spleen lymphocytes of homozygous humanized CTLA4 BALB/c KI mice is detected by FACS.
Figure 2. 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 3. 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 mm3. Treatment of anti-hCTLA4 antibody Yervoy and Teremelimumab can effectively control tumor growth in BALB/c-hCTLA4 mice (A) without affecting the animal body weight (B).
## Immune Checkpoint Humanized Mouse Models

Being recognized as a top scientific breakthrough in 2013, cancer immunotherapy is predicted to be one of the most promising research areas for improving patient outcomes. Although many immunotherapy breakthroughs may still lie ahead, important clinical advances have been made in the past few years for some of the deadliest cancers, reaffirming the potential of immunotherapy for many types of patients.

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.

### Immune Checkpoint Humanized Mouse Models available at ingenious targeting laboratory

| 4-1BB | PD-1/PD-L1 |
| CD40  | PD-1/TIGIT |
| CD47  | PD-1/TIM3 |
| CD73 (NT5E) | PD-L1 |
| CTLA4 (C57BL/6) | PD-L1/CTLA4 |
| CTLA4 (BALB/c) | PD-L1/LAG3 |
| KDR   | PD-L1/OX40 |
| LAG3  | PD-L1/TIGIT |
| OX40  | SIRPA |
| OX40/CTLA4 | SIRPA/CD47 |
| PD-1 (C57BL/6) | TIGIT |
| PD-1 (BALB/c) | TIM3 (C57BL/6) |
| PD-1/4-1BB | TIM3 (BALB/c) |
| PD-1/CD40 | TNFRSF1B |
| PD-1/CTLA4 | And more to come! |
| PD-1/LAG3 | |
| PD-1/OX40 | |

To get to know more about these models, visit our website [www.genetargeting.com](http://www.genetargeting.com) or contact our scientific experts at [inquiry@genetargeting.com](mailto:inquiry@genetargeting.com)
About ingenious targeting laboratory

ingenious targeting laboratory (ingenious) has been a leading global provider of custom genetically modified mouse, rat, and rabbit models for over 20 years. As one of the very first mouse gene targeting companies, our trusted service is built on two decades’ worth of successful animal model creation for investigators, organizations, and companies worldwide. Our models have been published in hundreds of journals including Science, Nature, and Cell, making us one of the most validated and respected production companies in the industry. We are excited to add catalog mouse models to our service repertoire by means of our collaboration with Shanghai Model Organisms Center (SMOC).