BALB/c-hPD1/PDL1/hTIGIT/hCD96

Strain Name: BALB/cJGpt-*Pdcd1*$^{em1Cin(hPDCD1)}$*Cd274*$^{tm1(hCD274)}$*Tigit*$^{em1Cin(hTIGIT)}$*Cd96*$^{em1Cin(hCD96)}$*/Gpt*
Strain type: Knock-in
Strain number: T054842
Background: BALB/cJGpt

Description

PD1 (programmed death 1) is a programmed death receptor 1, an immunoglobulin superfamily type I transmembrane glycoprotein. PDL1 is the ligand of PD1 which is highly expressed in several cancers[1]. PDL1 binds to PD1 and then mediates the inhibition of activated T lymphocytes, which plays a key role in eluding the immune system during tumorigenesis. Increased expression of PDL1 on the surface of cancer cells and the resultant T cell suppression conduce to the immune escape of cancer cells[2]. Blocking PD1/PDL1 signaling pathway with antibodies has become a classic method for tumor immunotherapy.

T cell immunoglobulin and ITIM domain (TIGIT) is mainly expressed on the surface of T-cell and NK-cell. TIGIT is a type I transmembrane protein with an immunoglobulin domain in the extracellular domain and an ITIM domain in the intracellular domain. Mechanism studies have shown that TIGIT is an inhibitory receptor of T cells, because the loss of TIGIT leads to an enhanced T cell response to MOG peptide immunity. It is currently believed that TIGIT can inhibit cell activation in two ways. One is that TIGIT directly acts on cells themselves and inhibits cell activation by downstream regulating TCRα mediated signal transduction [3]. Another approach is enhancing the phosphorylation level of the signaling molecule ERK by triggering downstream signaling pathways of the PVR ligand on APC. This approach enhances the ability of APC to secrete the anti-inflammatory factor IL-10, which in turn acts on cells and inhibits T cell responses[4]. Genentech's TIGIT antibody MTIG7192A, alone or in combination with PD1 antibody Atezolizumab, have been shown to treat advanced or metastatic tumors in clinical trials.

CD96 represents a type I transmembrane glycoprotein belonging to the immunoglobulin superfamily. CD96 is expressed mainly by cells of hematopoietic origin, in particular on T and NK cells[5-7]. Upon interaction with CD155 present on target cells, CD96 was found to inhibit mouse NK cells, and absence of this interaction either by blocking with

antibody or knockout of CD96 showed profound beneficial effects in containment of tumors and metastatic spread in murine model systems. In preclinical studies, a greater reduction in metastatic burden was observed with the combination of anti-CD96 mAb with either anti-TIGIT or anti-PD1 mAbs as compared to monotherapy and this greater efficacy was dependent on NK cells. Similarly, enhanced survival and decreased metastasis was observed after treatment of mice bearing 4T1.2 mammary carcinoma with anti-CD96 mAb together with either anti-TIGIT or anti-PD1 compared to each individual agent given alone[8]. These studies have clearly demonstrated that CD96 is a promising target to combine with current immunotherapies.

GemPharmatech replaced the PD1, PDL1, TIGIT and CD96 gene extracellular domain of BALB/c mice for the corresponding fragment of human via gene editing technology, and developed BALB/c-hPD1/hPDL1/hTIGIT/hCD96 humanized model independently. Humanizing the extracellular antibody binding sites ensure endogenous intracellular signaling transduction. This mouse will be helpful for multi-drug (including CD96, TIGIT, PD1, and PDL1) combination evaluation and immunotherapy drug development.

Strategy

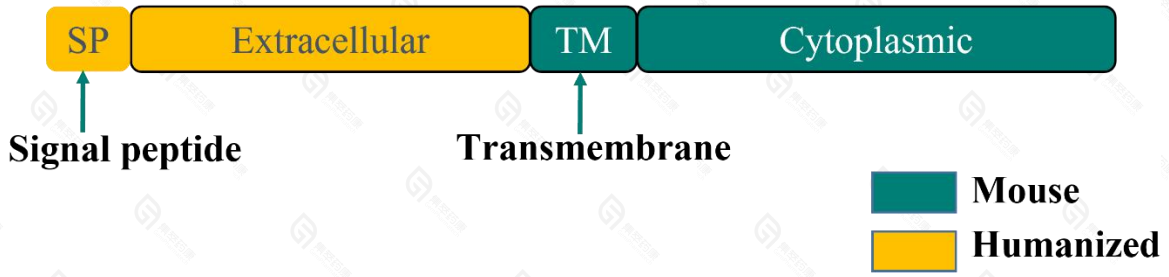

Fig.1 Schematic diagram of CD96 humanization strategy in BALB/c-hPD1/hPDL1/hTIGIT/hCD96 mice.

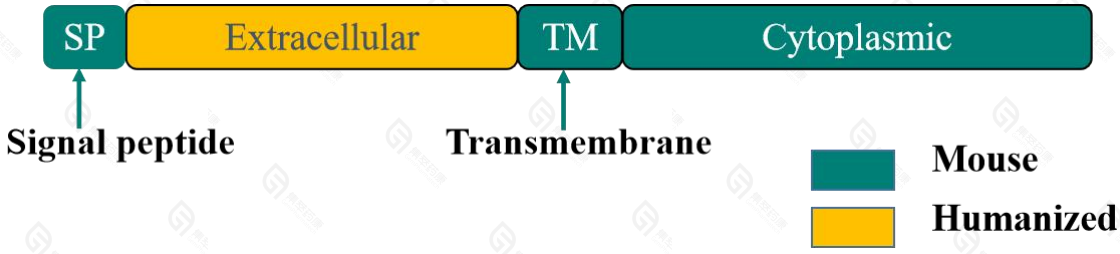

Fig.2 Schematic diagram of TIGIT humanization strategy in BALB/c-hPD1/hPDL1/hTIGIT/hCD96 mice.

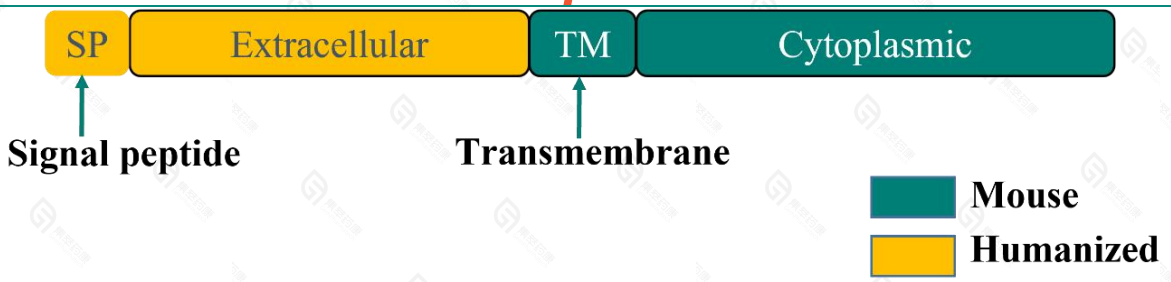

Fig.3 Schematic diagram of PDL1 humanization strategy in BALB/c-hPD1/hPDL1/hTIGIT/hCD96 mice.

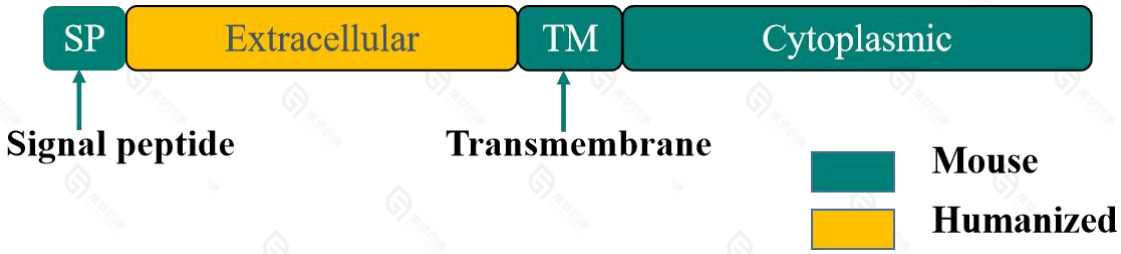

Fig.4 Schematic diagram of PD1 humanization strategy in BALB/c-hPD1/hPDL1/hTIGIT/hCD96 mice.

Application

1. Efficacy evaluation and safety of single or combination drugs of human PD1, PDL1, TIGIT and CD96
2. Anticancer drug research and development
3. Research on autoimmune diseases

Supporting data

1. hCD96 mRNA expression analysis

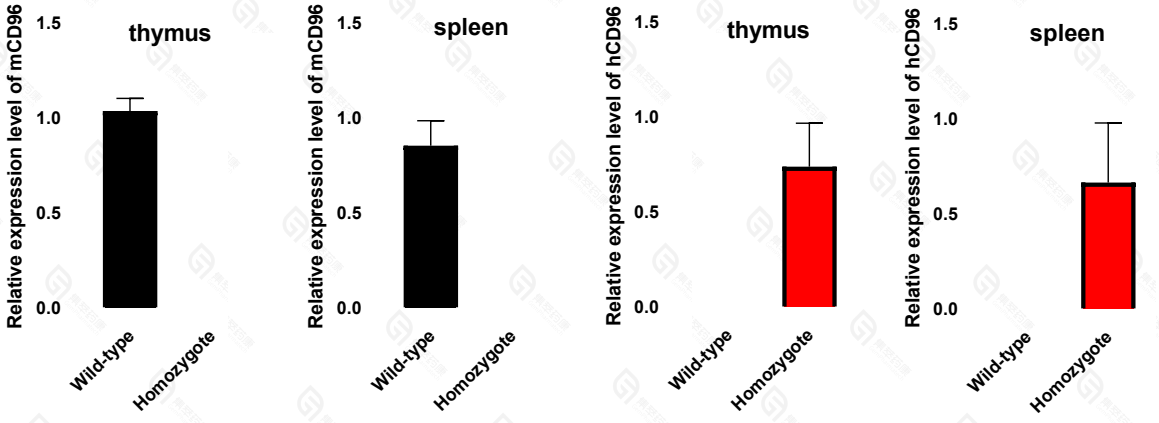

Fig.5 Quantitative PCR analysis of hCD96 mRNA expression in BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice.

Total RNA was isolated from spleen and thymus of wild-type mice and homozygous BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice, qPCR method was used to detect the mRNA expression of CD96. The

results showed that the expression of mCD96 mRNA was detectable in wild-type mice. The expression of hCD96 mRNA was only detectable in homozygous BALB/c-hPD1/PDL1/hTIGIT/hCD96.

2. hCD96 Protein expression analysis

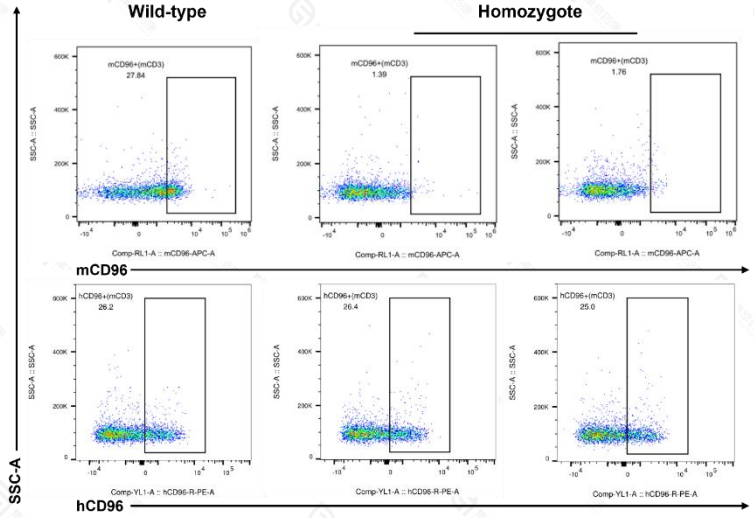

Fig.6 Detection of CD96 expression in BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice.

Mouse CD96 was detectable in wild-type mice. Human CD96 was detectable in both homozygous BALB/c-hPD1/PDL1/hTIGIT/hCD96 and wild-type mice due to the cross-reactivity of the antibody.

3. hPD1 Protein expression analysis

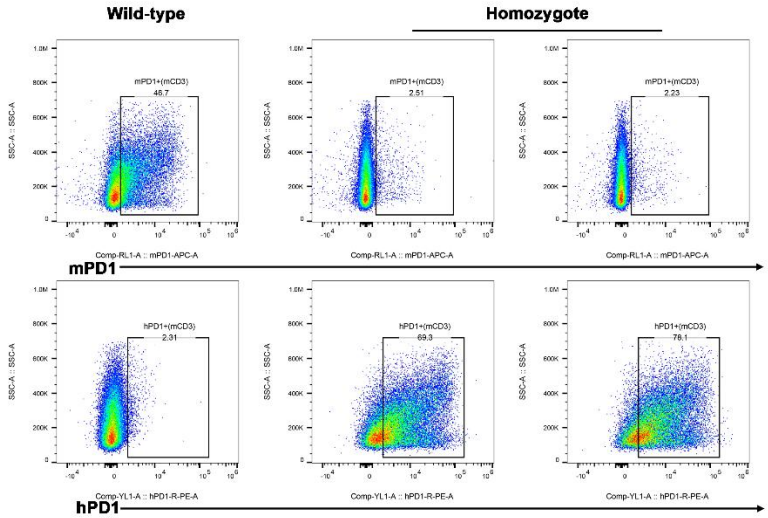

Fig.7 Detection of PD1 expression in BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice.

Splenocytes were collected from wild-type BALB/c and homozygote BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice stimulated with anti-CD3ε in vivo. Mouse PD1 was exclusively detectable in wild-type mice. Human PD1 was exclusively detectable in homozygous BALB/c-hPD1/PDL1/hTIGIT/hCD96.

4. hPDL1 Protein expression analysis

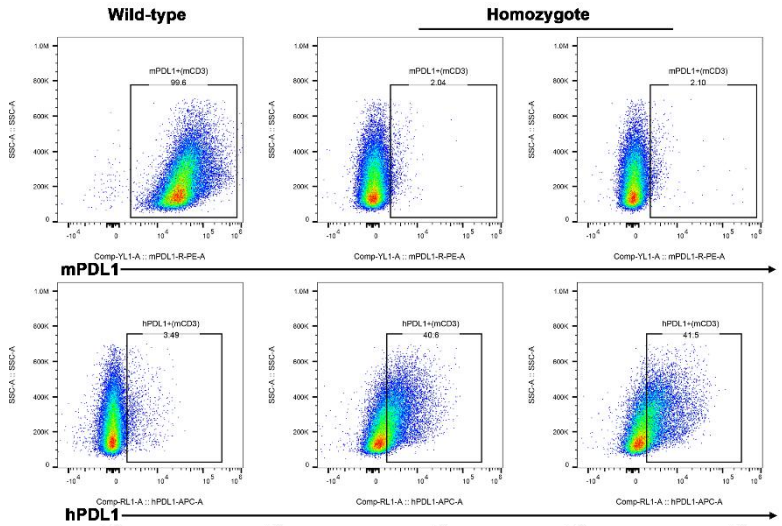

Fig.8 Detection of PDL1 expression in BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice.

Splenocytes were collected from wild-type BALB/c and homozygote BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice stimulated with anti-CD3ε in vivo. Mouse PDL1 was exclusively detectable in wild-type mice. Human PDL1 was exclusively detectable in homozygous BALB/c-hPD1/PDL1/hTIGIT/hCD96.

5. hTIGIT Protein expression analysis

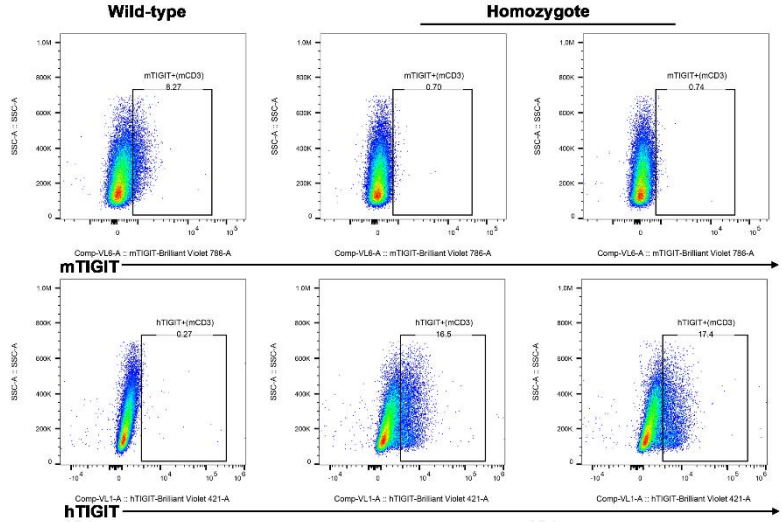

Fig.9 Detection of TIGIT expression in BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice.

Splenocytes were collected from wild-type BALB/c and homozygote BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice stimulated with anti-CD3ε in vivo. Mouse TIGIT was exclusively detectable in wild-type mice. Human TIGIT was exclusively detectable in homozygous BALB/c-hPD1/PDL1/hTIGIT/hCD96.

6. Analysis of blood immune cell subpopulations in BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice

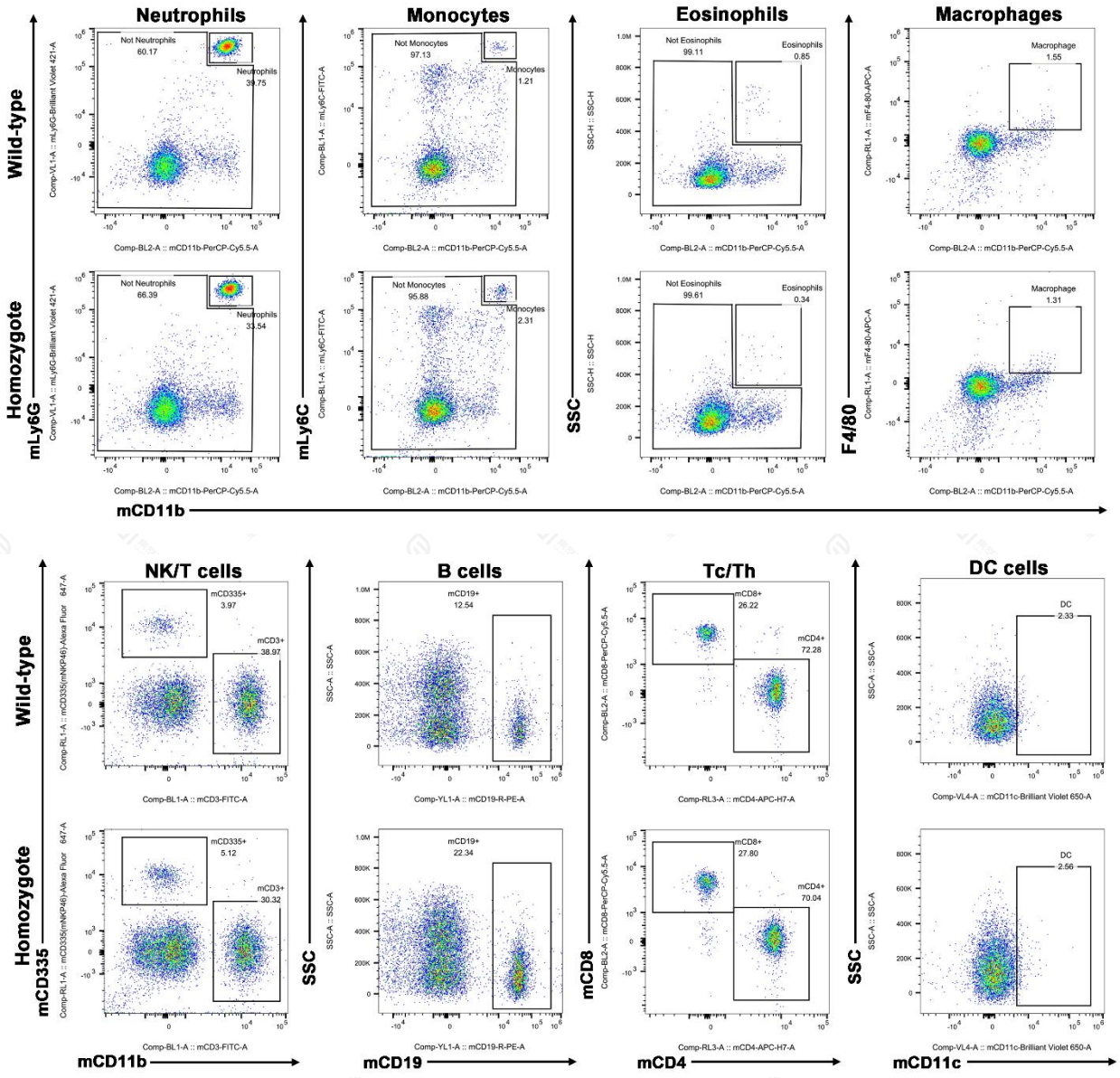

Fig.10 Analysis of blood immune cell subpopulations in BALB/c and BALB/c-hPD1/PDL1/hTIGIT/hCD96

Blood was taken from BALB/c and BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice for flow cytometry analysis to assess immune cell subpopulations. As shown in Figure 10, the percentages of T cells, NK cells, eosinophils, monocytes, macrophages and dendritic cells in BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice were similar to those in BALB/c.

7. Analysis of spleen immune cell subpopulations in BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice

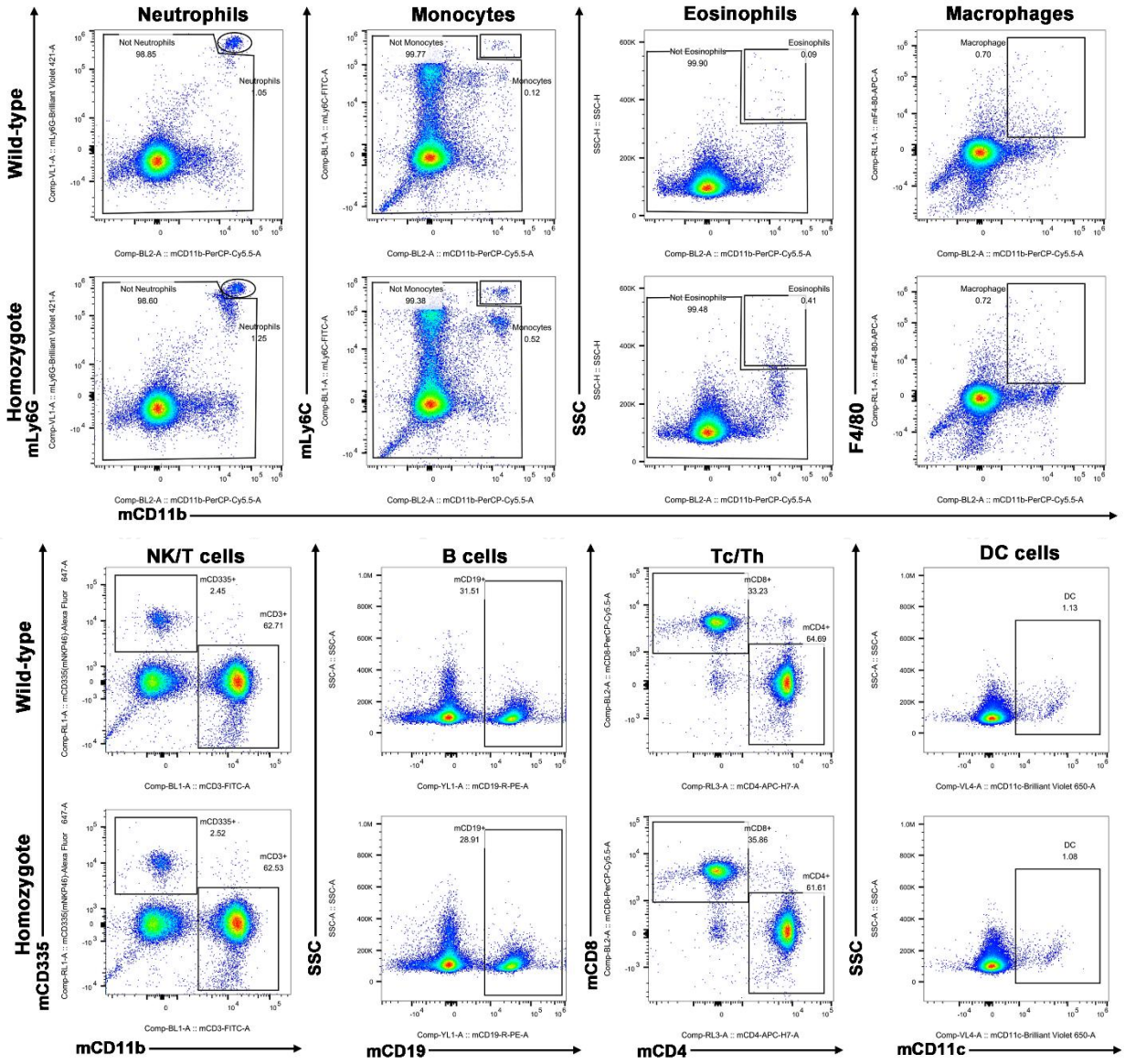

Fig.11 Analysis of spleen immune cell subpopulations in BALB/c and BALB/c-hPD1/PDL1/hTIGIT/hCD96

Splenocytes were taken from BALB/c and BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice for flow cytometry analysis to assess immune cell subpopulations. As shown in Figure 11, the percentages of T cells, NK cells, B cells, neutrophils, monocytes, macrophages and dendritic cells in BALB/c-hPD1/PDL1/hTIGIT/hCD96 mice were similar to those in BALB/c.

References

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