BALB/c-hKLRB1

Strain Name: BALB/cJGpt-*Klrb1*$^{em1Cin(hKLRB1)}$/Gpt
Strain Type: Knock-in
Strain Number: T054645
Background: BALB/cJGpt

Description

KLRB1(Killer cell lectin-like receptor subfamily B, member 1) is also known as NKR-P1A or CD161. The KLRB1/NKR-P1 family of proteins are type-II-transmembrane C-type lectin receptors. NKRP1A is present on a subset of human NK cells and on approximately 25% of peripheral blood T cells.

In the human gene family, a single homologue has been designated KLRB1, NKR-P1A, or CD161. In human, the NKR-P1A receptor binds a Clr orthologue termed lectin-like transcript 1(LLT1), which is able to specifically inhibit NK cytolytic activity and cytokine production. But there are several *Klrb1 (Nkrp1)* genes in mouse, with *Klrb1a*, *Klrb1c*, and *Klrb1f* taking activating forms and *Klrb1b*, *Klrb1g* and *Klrb1d* being inhibitory[1]. Among these, *Klrb1g* (NKR-P1G) has been reported more closely represent a hNKR-P1A homologue[1].

Recent studies have showed, genetic inactivation of KLRB1 or antibody-mediated CD161 blockade enhances T cell-mediated killing of glioma cells in vitro, and their anti-tumor function in vivo is also confirmed[2]. In chronic HBV infection, $CD161^{+}CD4^{+}$ T cells were found to accumulate in the circulation of HBV cohorts, played anti-viral, pro-inflammatory and pro-fibrogenic roles, and showed a significant correlation with the clinical parameters of disease progression[3]. In Crohn's disease patients with fistulizing disease, there is an accumulation of CD161+ T helper lymphocytes[4].

The BALB/c-hKLRB1 mice was created at GemPharmatech using gene editing technology whereby the coding sequence of extracellular domain of NKR-P1G was replaced with the human NKR-P1A counterpart on BALB/c background. The transmembrane and cytoplasmic regions of murine NKR-P1G were completely retained and physiological cytoplasmic signal transduction was confirmed. This strain will facilitate cancer related research and drug development.

Applications

1. Targeted drug test (screening human KLRB1 neutralizing antibodies or small molecule drugs that inhibit human KLRB1 activity)

2. Anti-tumor drug screening and efficacy test
3. Studies on autoimmune diseases

Strategy

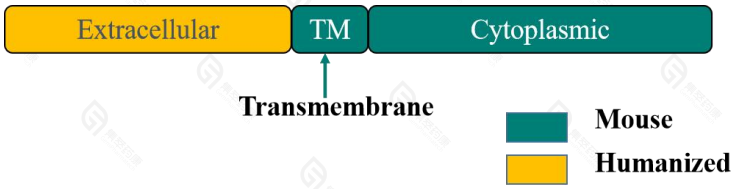

Fig 1. Schematic diagram of KLRB1 humanization strategy on BALB/c-hKLRB1 mice.

Data support

1. Detection of KLRB1 mRNA expression

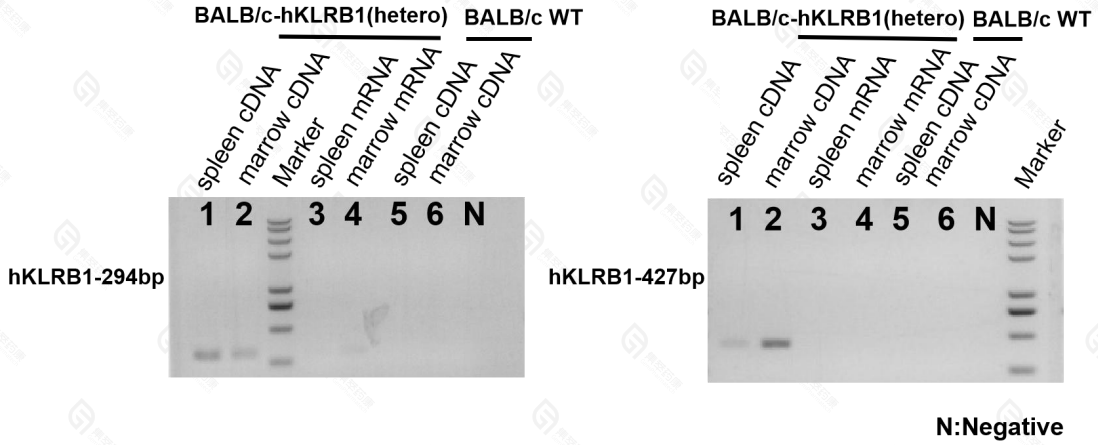

Fig 2. Detection of hKLRB1 expression on BALB/c-hKLRB1mice.

BALB/ c-hKLRB1 mice can successfully express hKLRB1 mRNA.

2. Humanized KLRB1 protein expression analysis

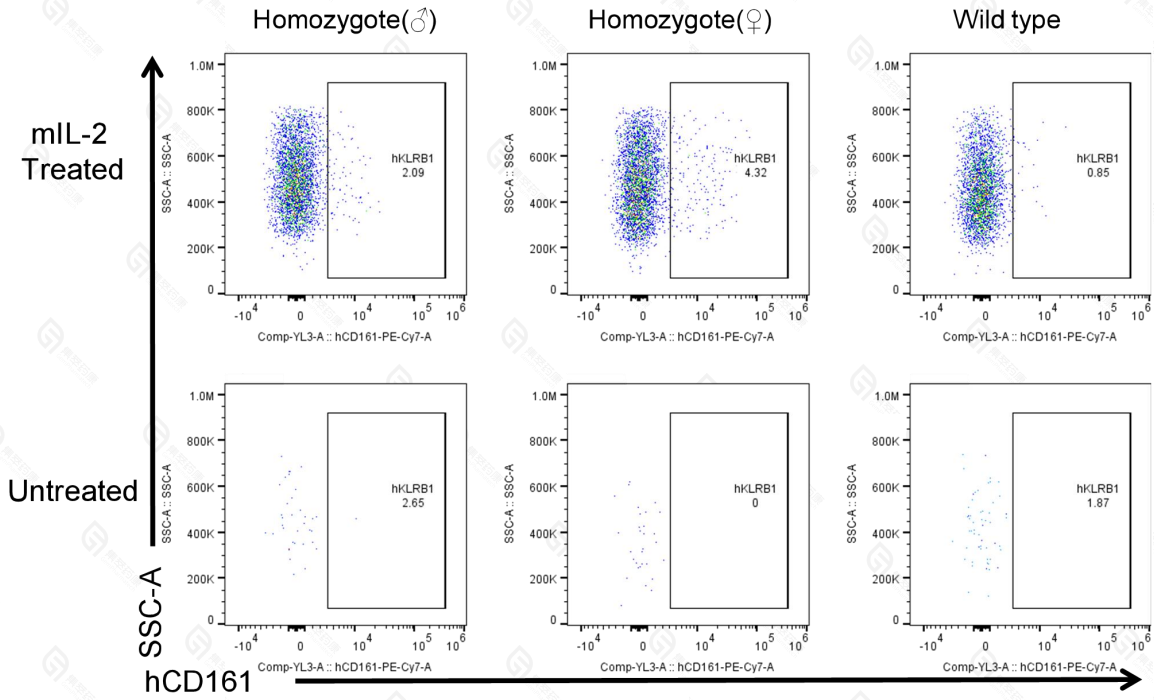

Fig 3. Detection of KLRB1 expression after stimulation.

The spleen cells of BALB/c-hKLRB1 mice were cultured in vitro and treated with mouse IL-2 for 5 days, and the expression of humanized KLRB1 on the surface of NK cells was detected (Single live $CD45^{+}CD3^{-}CD335^{+}$ cells were gated for further analysis).

Expression of hKLRB1 were confirmed on the surface of NK cells of BALB/c-hKLRB1 humanized mice, after stimulation with IL-2, and could not been detected in wild type.

3. Analysis of blood immune cell sub-population

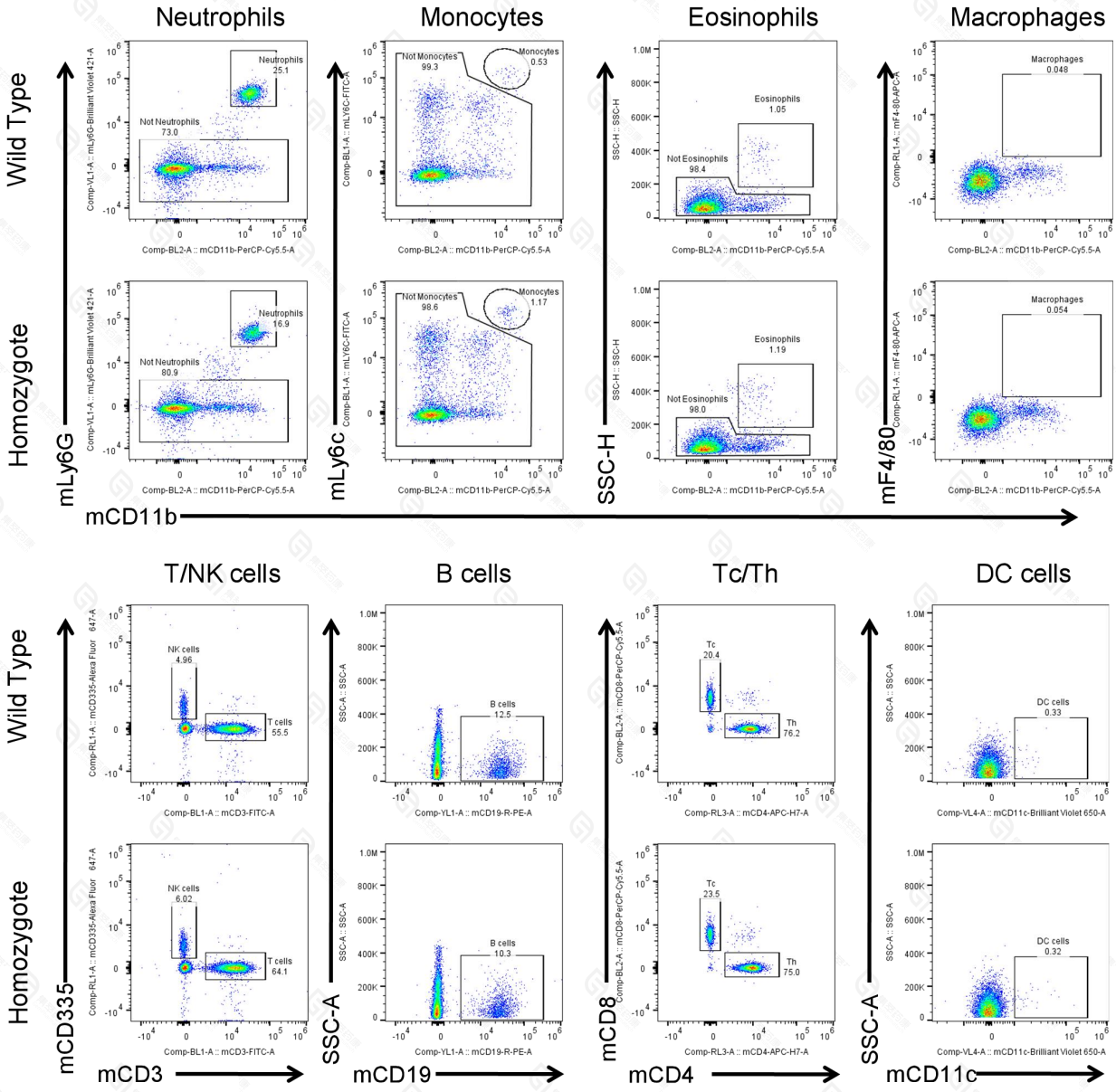

Figure 4 Blood immune sub-population ratio assay

Blood was isolated from female BALB/c (wild type) and BALB/c-hKLRB1 mice (homozygote) for flow cytometric analysis to assess immune sub-populations. As shown in Figure 4, the percentages of T cells, NK cells, B cells, neutrophils, monocytes, and dendritic cells in BALB/c-hKLRB1 mice were similar to those in BALB/c, indicating that the replacement of mNKR-P1G by hNKR-P1A did not alter the development, differentiation, and distribution of these cells in blood.

4. Analysis of spleen immune cell sub-population

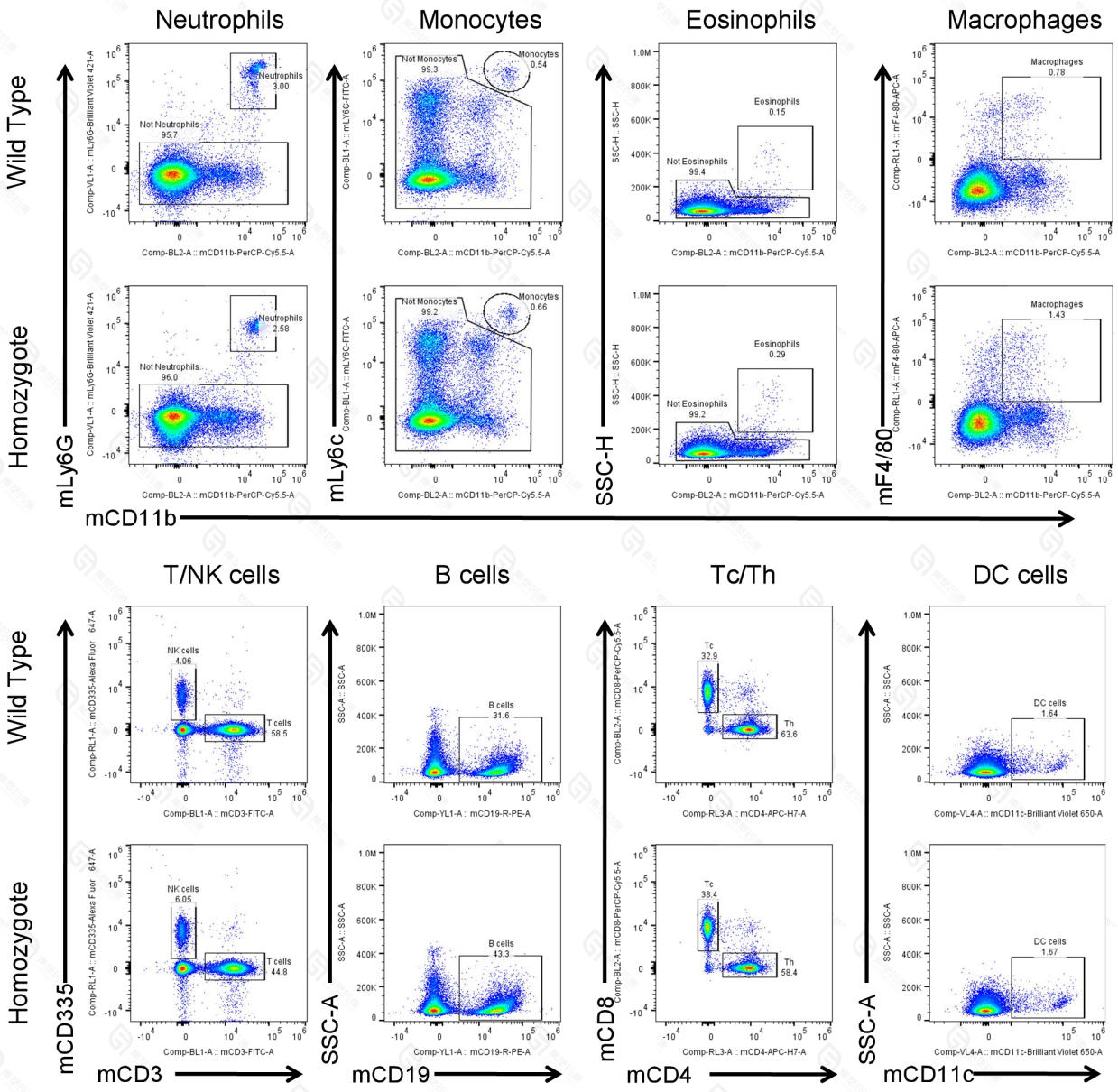

Figure 5 Spleen leukocyte sub-population ratio assay

Splenocytes were isolated from female BALB/c (wild type) and BALB/c-hKLRB1 mice (homozygote) for flow cytometric analysis to assess immune sub-populations. As shown in Figure 5, the percentages of T cells, NK cells, B cells, neutrophils, monocytes, and dendritic cells in BALB/c-hKLRB1 mice were similar to those in BALB/c, indicating that the replacement of mNKR-P1G by hNKR-P1A did not alter the development, differentiation, and distribution of these cells in spleen.

References

1. Kelley, J., L. Walter, and J. Trowsdale, *Comparative Genomics of Natural Killer Cell Receptor Gene Clusters.* PLOS Genetics, 2005. **1**(2): p. e27.
2. Mathewson, N.D., et al., *Inhibitory CD161 receptor identified in glioma-infiltrating T cells by single-cell analysis.* Cell, 2021. **184**(5): p. 1281-1298.e26.
3. Li, J., et al., *IFN-γ facilitates liver fibrogenesis by CD161(+)CD4(+) T cells through a regenerative IL-23/IL-17 axis in chronic hepatitis B virus infection.* Clin Transl Immunology, 2021. **10**(11): p. e1353.
4. Maggi, L., et al., *CD4+CD161+ T lymphocytes infiltrate Crohn's disease-associated perianal fistulas and are reduced by anti-TNF-α local therapy.* Int Arch Allergy Immunol, 2013. **161**(1): p. 81-6.