NCG-X

Strain Name: NOD/ShiLtJGpt-*Prkdc*^{em26Cd52}*Il2rg*^{em26Cd22}kit$^{em1Cin(V831M)}$/Gpt
Strain Type: Knock-in
Strain ID: T003802
Background: NOD/ShiLtJGpt

Description

Proto-oncogene c-KIT, also known as tyrosine-protein kinase KIT, CD117 (cluster of differentiation 117) or mast/stem cell growth factor receptor (SCFR), is a receptor tyrosine kinase protein that in humans is encoded by the KIT gene. KIT is a cytokine receptor expressed on the surface of hematopoietic stem cells as well as other cell types. Altered forms of this receptor may be associated with some types of cancer. Signaling through KIT plays a role in cell survival, proliferation, and differentiation. W41 is the point mutation from Val to Met at position 831 of c-kit. The mutated mice had spontaneous anemia and their hematopoietic stem cell function was inhibited.

GemPharmatech introduced W41 point mutation in NCG immunodeficient mice to produce NCG-kit-Cas9-TM mice using Crispr/cas9 technology. The NCG-kit-Cas9-TM mice has T/B/NK cell immunodeficiency and hematopoietic stem cell function inhibition. This strain can receive human hematopoietic stem cell transplantation without receiving radiation, which is a good model of human hematopoietic stem cell transplantation.

Application

1. Human immune system development
2. Human infection studies
3. Research on autoimmune diseases

Data support

1. Detection of mRNA

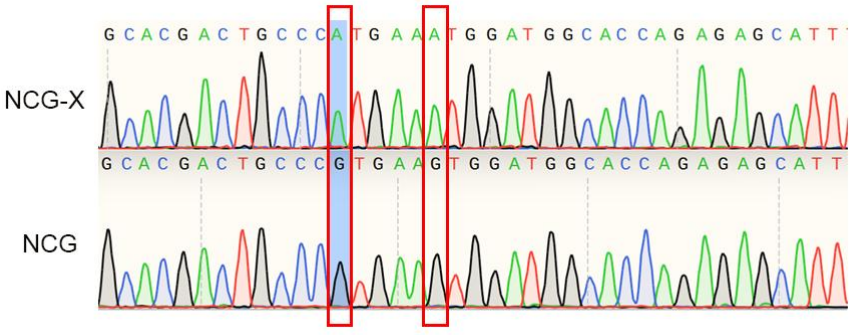

Fig 1. Detection of mRNA in NCG-X mice.

the mutation site of W41 (Left red box) and the synonymous mutation (Right red box) were introduced. The mutation site of NCG-X W41 was successful.

2. Splenic immune cell cluster detection

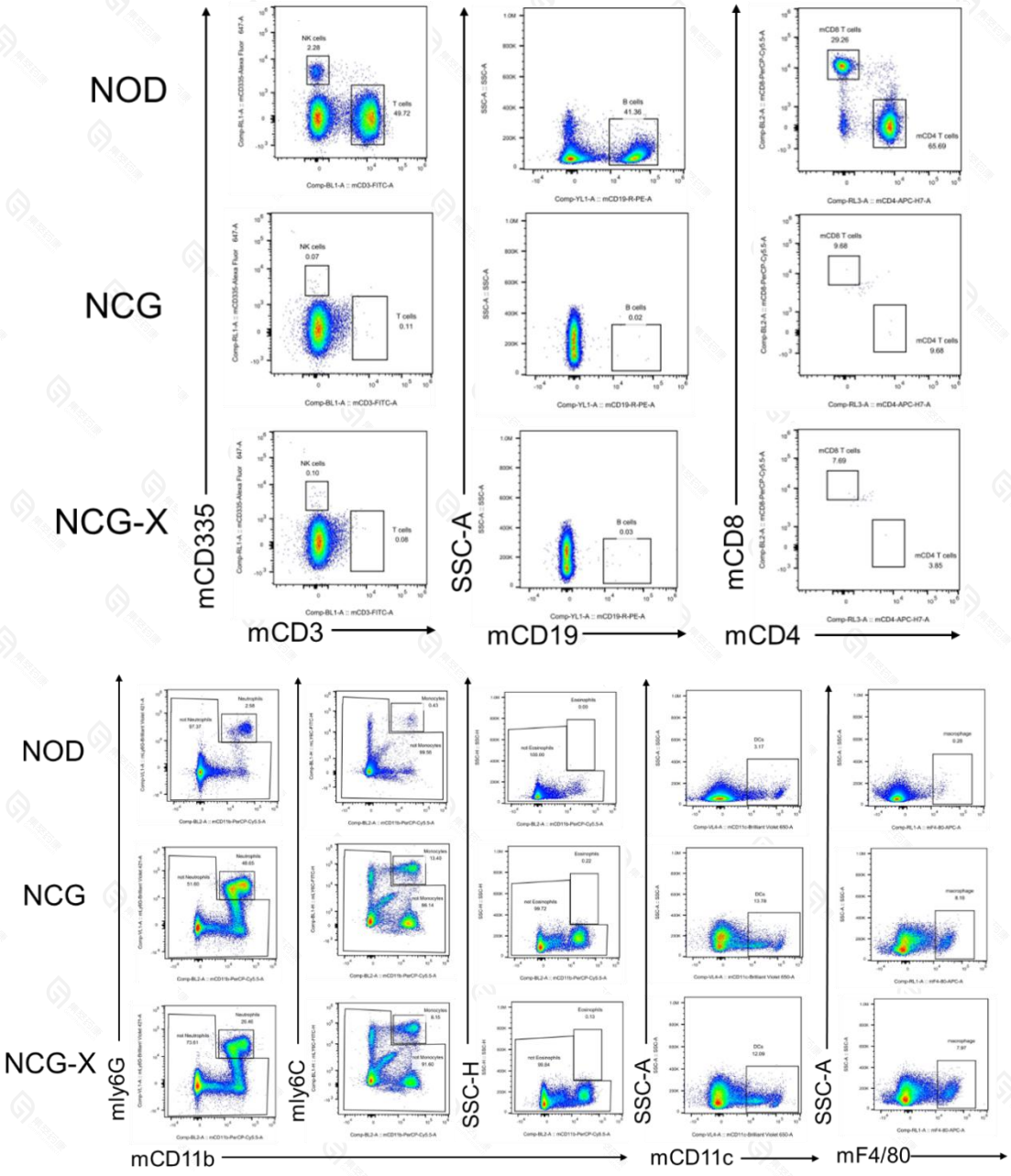

Fig 2. Detection of murine spleen immune cells

Compared with NOD mice, the spleen T/B/NK cells were absent in NGG-X mice and NCG mice, and the degree of T/B/NK cell defects in NGG-X mice and NCG mice was basically the same.

3 Detection of bone marrow cells in NGG-X mice

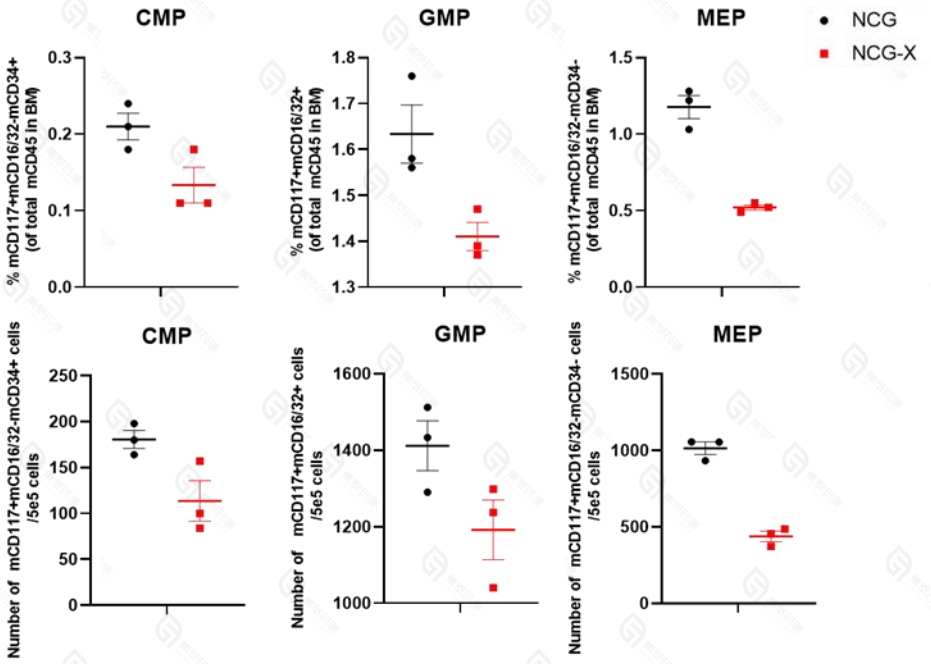

Figure 3 detection of bone marrow cells of NGG-X mice

The proportion and number of CMP, GMP and MEP in bone marrow of NCG-X mice decreased compared with NCG mice.

4 Erythrocyte detection

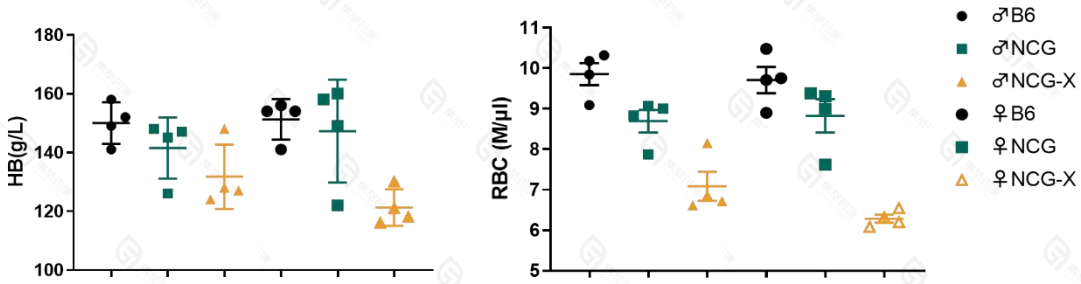

Fig 4. Ersythrocyte test of NCG-X mice.

Compared with B6 and NCG mice, NCG-X mice had significantly reduced red blood cell and hemoglobin (8 weeks old).

5 Weight detection of NGG-X mice

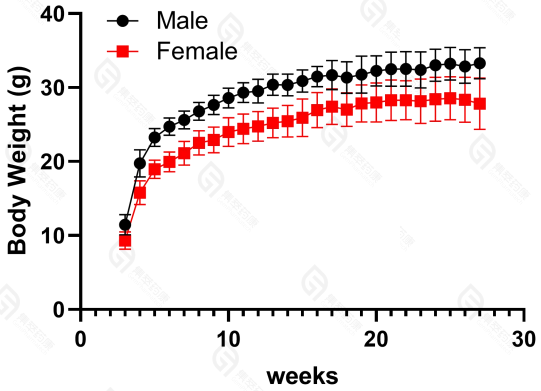

Figure 5 Weight detection of NGG-X mice

NCG-X male mice grew faster than female mice.

6 Survival rate detection of NGG-X mice

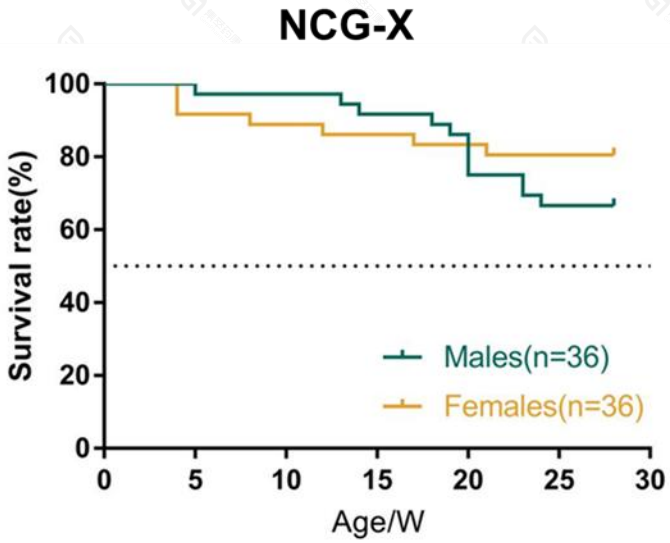

Figure 6 Survival rate of NGG-X mice

Female NGG-X mice had a higher survival rate than male mice. Slight death occurs over time.

7 Immune reconstitution in peripheral blood of huHSC-NCG-X mice

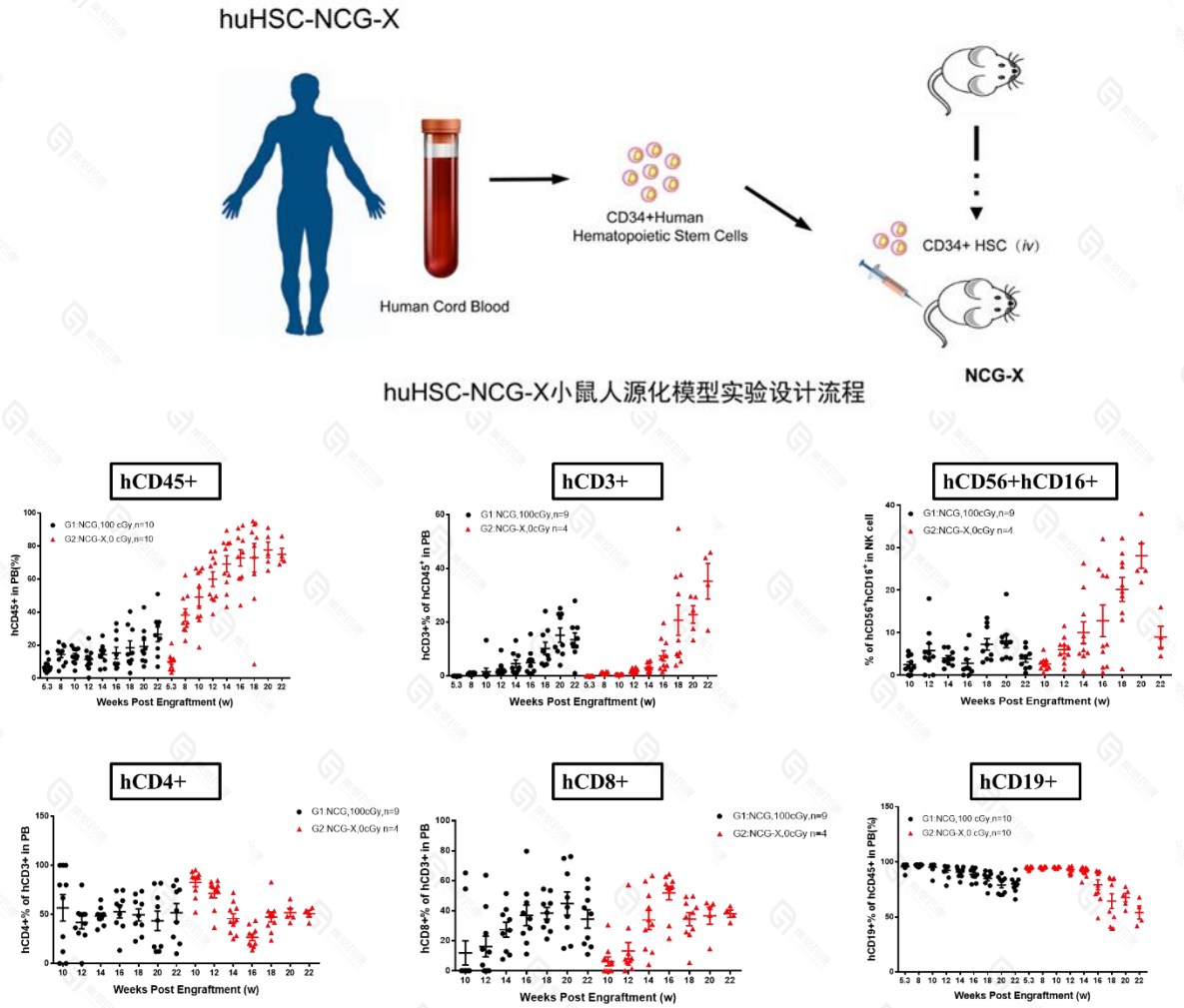

Fig 7. human HSC reconstitution in NCG-X mice.

Compared with irradiated NCG mice, NCG-X mice were able to efficiently rebuild the human immune system without irradiation.

- **8 Reconstitution of bone marrow and peripheral blood erythrocytes in HuhSC-NGG-X mice**

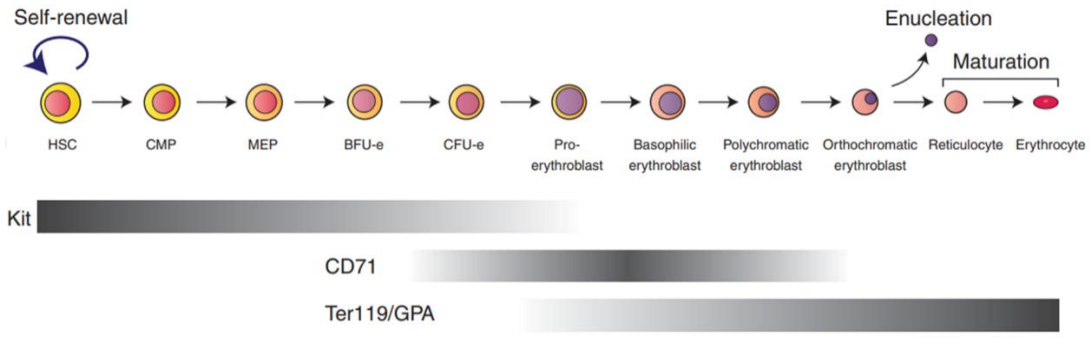

Fig 8. Erythroid development in mouse bone marrow

Red blood cell maturation occurs primarily in the bone marrow, First, hematopoietic stem cells differentiated into myeloid progenitor cells (CMP), and then into megakaryocyte-erythroid progenitor cells (MEPs), erythroid burst forming units (BFU-e), erythroid colony forming units (CFU-e), pro-erythroblast, Basophilic erythroblast, Polychromatic erythroblast, Orthochromatic erythroblast, Reticulocytes, and mature erythrocytes. Primitive red blood cells are nucleated cells. During maturation, their nuclei gradually disappear, cytoskeleton changes, and mature red blood cells in the shape of double concave disks are gradually released into peripheral blood.

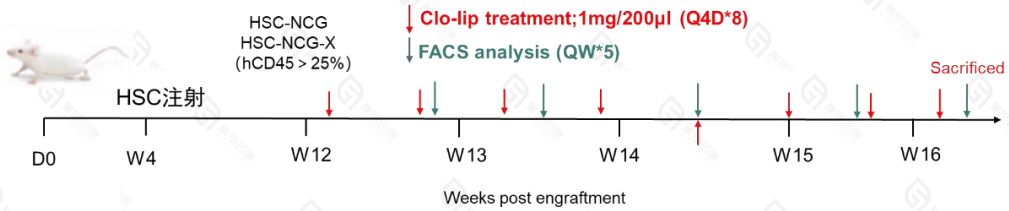

Fig 9 Experimental scheme of erythrocyte reconstitution in mice

Two different donor HSC CD34+ were transplanted into NCG and NCG-X mice. At the 11th week after reconstitution, peripheral blood of mice was collected to test the reconstitution efficiency, and the mice were randomly grouped according to the hCD45 reconstitution efficiency of peripheral blood of huHSC-NCG, HuHSC-NSC-X and the weight of mice. The injection of disodium-chlorophosphate liposome (Clo-lip, 40337ES10) was started at the 12th week, and the experimental endpoint was at the 16th week. Mouse macrophages have phagocytic effect on human red blood cells, and intraperitoneal injection of Clo-lip can deplete macrophages, and mature red blood cells developed in bone marrow can enter the peripheral blood, so as to reconstitute human mature red blood cells in the peripheral blood.

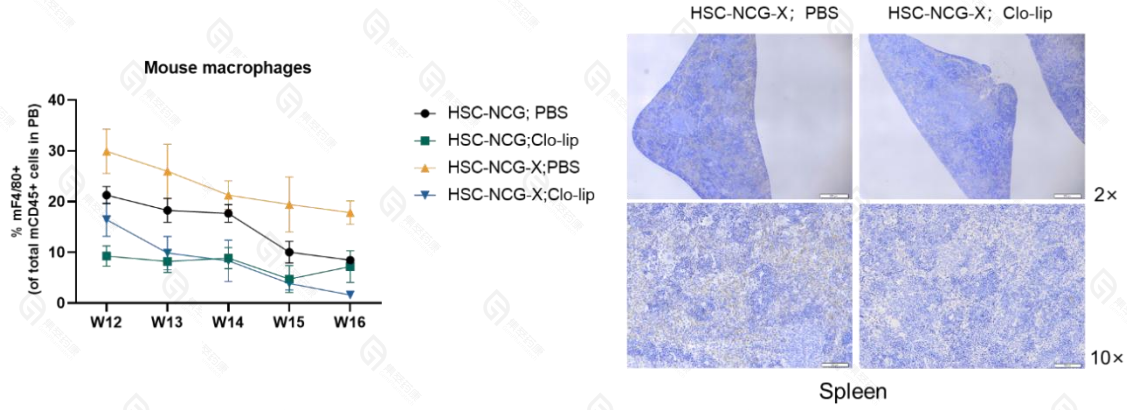

Fig 10. Detection of mouse macrophages

Compared with PBS group, murine macrophages of huHSC-NCG/NCG-X mice gradually decreased after Clo-lip treatment in peripheral blood. Mouse spleen tissues were collected and immunohistochemical analysis was performed with anti-mouse F4/80 antibody. The results showed that huHSC-NCG-X spleen murine macrophage infiltration decreased.

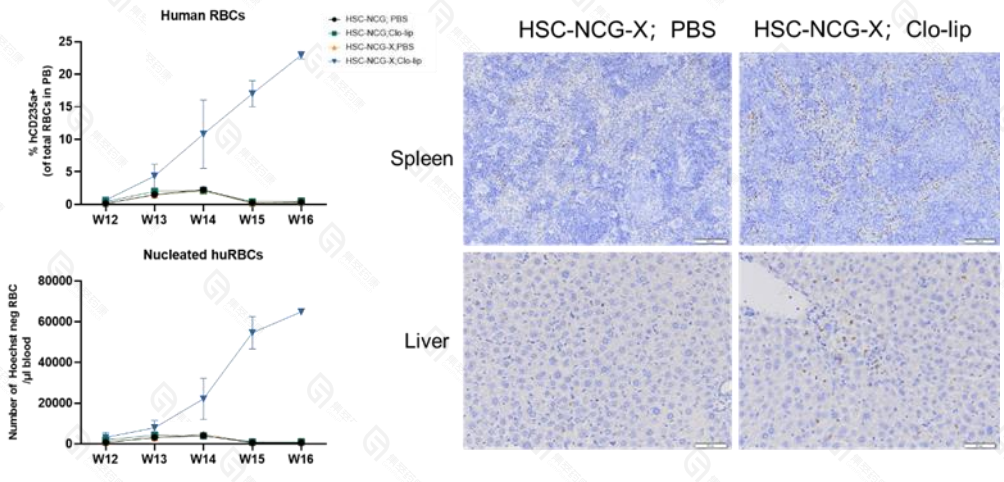

Fig 11. Detection of human mature erythrocytes in mice

Compared with PBS group, the reconstitution erythrocyte in peripheral blood of huHSC-NCG-X were mature erythrocytes after receiving Clo-lip. The spleen and liver tissues of mice were collected for immunohistochemical analysis with human CD235a+ antibody. The results showed that huHSC-NCG-X had a large number of human erythrocyte infiltration in the spleen and liver.

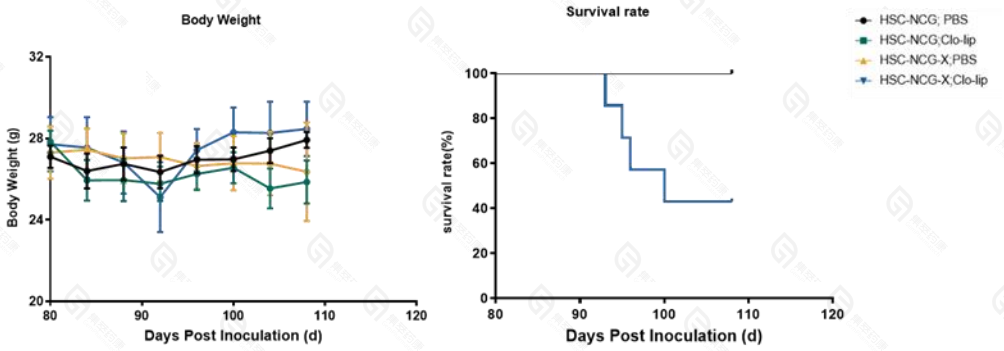

Fig 13. Body weight and survival curves of mice treated with Clo-lip

The mortality of huHSC-NCG-X mice increased after Clo-lip treatment, which may be related to the increased efficiency of erythrocyte reconstitution and the occurrence of anemia in mice.

References

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