Background Human immune cell reconstitution (PBMC or CD34+ hematopoietic stem cell; HSC) is commonly done in immunodeficient mouse lines such as NCG, NSG or NOG. The use of PBMC over CD34+ HSC is preferred for multiple reasons, including faster engraftment, lower cost, and the presence of mature immune cell populations. One major limitation of human PBMC reconstituted models is the acute Graft-versus-Host Disease (GvHD) that sets in within weeks of PBMC injection, leading to a high mortality rate. While donor-to-donor variations exist and specific donors can be selected, the symptoms of GvHD typically occur within 4 weeks. This significantly limits the study window required for appropriate evaluation of the agents, such as cancer immunotherapies. GvHD is an immune reaction triggered mainly by donor T cells identifying the mouse MHC class I and class II as foreign and attacking the host cells and organs. Removing MHC molecules from the host seems a viable approach to avoiding GvHD.

Methods We used CRISPR-Cas9 to knockout the mouse H2-K1, H2-D1 and H2-Ab1 genes. We first generated two KO strains for H2-K1 and H2-Ab1 respectively. After identifying the correctly engineered strain targeting the H2-K1 gene, we generated H2-K1 and H2-D1 double KO strains by knocking out the H2-D1 gene in the embryos of the H2-K1 KO strain. In the final step, we generated the triple KO strain by cross-breeding the H2-K1 and H2-D1 double KO strain with the H2-Ab1 KO strain.

Results While the deletion of β2M (NCG-β2M-KO) reduces the occurrence of GvHD, this model knocked out not only the MHC class I subunit but also the FcRn subunit, which shortens the half-life of IgG, making it unsuitable for IgG antibody agent evaluation. In addition, deletion of MHC class I or class II alone resulted in low or high CD4/CD8. Compared with the existing NCG-β2M or other β2m null mouse models, the NCG-MHC dKO significantly prolonged survival and reduced GvHD occurrences when reconstituted with PBMC.

Conclusions Based on our preliminary data, the NCG-SSC1 - MHC dKO is a promising model for antibody and cell therapy agent evaluation, determining therapeutic-related cytokine release syndrome, and long-term toxicity evaluation of cell therapeutics.