Background The clinical response rate to checkpoint inhibitors can vary due to disease heterogeneity and complex tumor escape mechanisms, necessitating a need for preclinical models that can be used for the assessment of new immunotherapies. Ligation of inducible T-cell costimulatory receptor (ICOS) to its ligand triggers a downstream pathway that results in the regulation of T-cell proliferation and survival, suggesting ICOS could be used as a biomarker for predicting and monitoring T-cell-mediated immunotherapy response.

Methods To address the need for a preclinical platform that can be used for efficacy assessments of novel immunotherapies, Biocytogen generated a humanized (B-hICOS) knock-in mouse model. In this model, the full-length coding sequence of human ICOS replaced murine Icos using gene editing technology. Human ICOS gene and protein expression were analyzed by RT-PCR and flow cytometry, respectively. Next, splenocytes from C57BL/6 (+/+), heterozygous B-hICOS (H/+), and homozygous B-hICOS (H/H) mice were stained using the CellTrace™ Violet Cell Proliferation Kit and incubated with either anti-mCD3ε, anti-mCD3ε and anti-mCD28, or anti-mCD3ε and human ICOSL recombinant protein for 72 hours. Mouse/human ICOS protein expression and proliferation of CD4+ and CD8+ T cells were assessed by flow cytometry. Finally, to determine the physiological function of ICOS/ICOSL, we evaluated basal serum IgG1 levels in C57BL/6 (+/+ ) and B-hICOS (H/H) mice (8-week-old, n=5). We further analyzed serum from C57BL/6 (+/+ ) and B-hICOS (H/H) mice challenged with T-cell-dependent antigen ovalbumin (OVA), and the presence of OVA-specific IgG1 and IgE levels were determined by ELISA.

Results Human ICOS mRNA and protein expression were confirmed in B-hICOS mice by RT-PCR and flow cytometry, respectively. Particularly, CD4+ and CD8+ T cells stimulated with anti-mCD3ε and anti-mCD28, or anti-mCD3ε and human ICOSL recombinant protein exhibited increased human ICOS protein expression exclusively in heterozygous and homozygous B-hICOS mice. Similarly, CD4+ and CD8+ T cell activation in heterozygous and homozygous B-hICOS mice was upregulated by anti-mCD3ε and anti-mCD28, and anti-mCD3ε and human ICOSL recombinant protein stimulation. Finally, while we observed that basal serum IgG1 levels were significantly increased in B-hICOS mice, OVA-specific IgG1 and IgE levels in B-hICOS mice were similar to those in C57BL/6 mice.

Conclusions Our data demonstrates that introduction of human ICOS in place of its mouse counterpart does not negatively impact T cell activation and that the ICOS/ICOSL pathway is functional in B-hICOS mice.

Ethics Approval All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Biocytogen Beijing Co., Ltd.