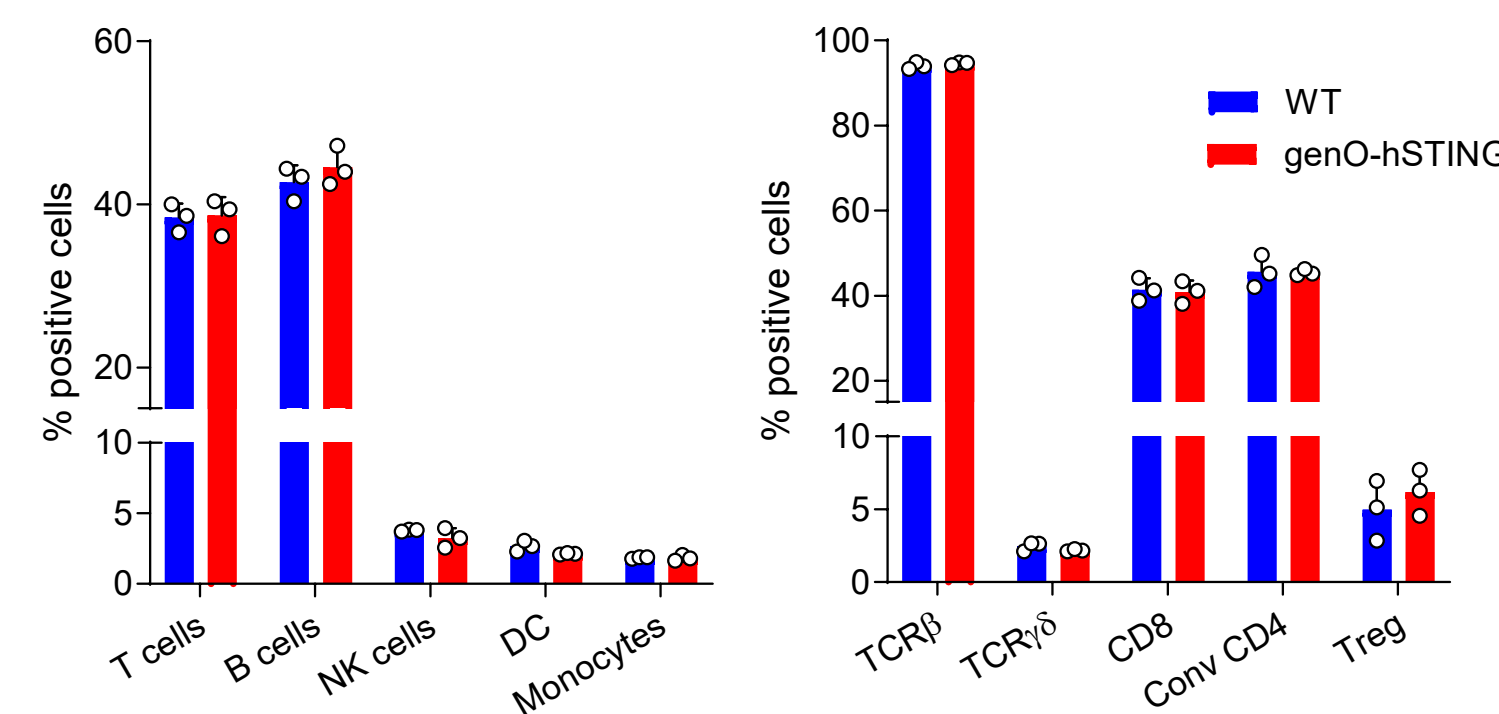


Background: The cyclic GMP–AMP synthase–stimulator of interferon genes (cGAS–STING) signaling axis represents a pivotal immunostimulatory pathway and an attractive pharmacological target in oncology. Its activation within the tumor microenvironment promotes cross-priming of tumor-associated antigens and enhances infiltration of effector T lymphocytes. Owing to its potent antitumor activity, the cGAS–STING pathway offers significant promise for the development of cancer vaccines, immunotherapeutic strategies such as antibody-drug conjugates, and interventions against virus-driven malignancies. However, translating preclinical findings to clinical applications has been challenging due to species-specific differences between human and mouse cGAS and STING. A model expressing human STING only has been previously reported and showed to be a valuable tool to investigate the activity of STING agonist in different tumor types. Herein, we report an immunocompetent mouse model expressing both human cGAS and human STING (genO-hcGAS/hSTING).

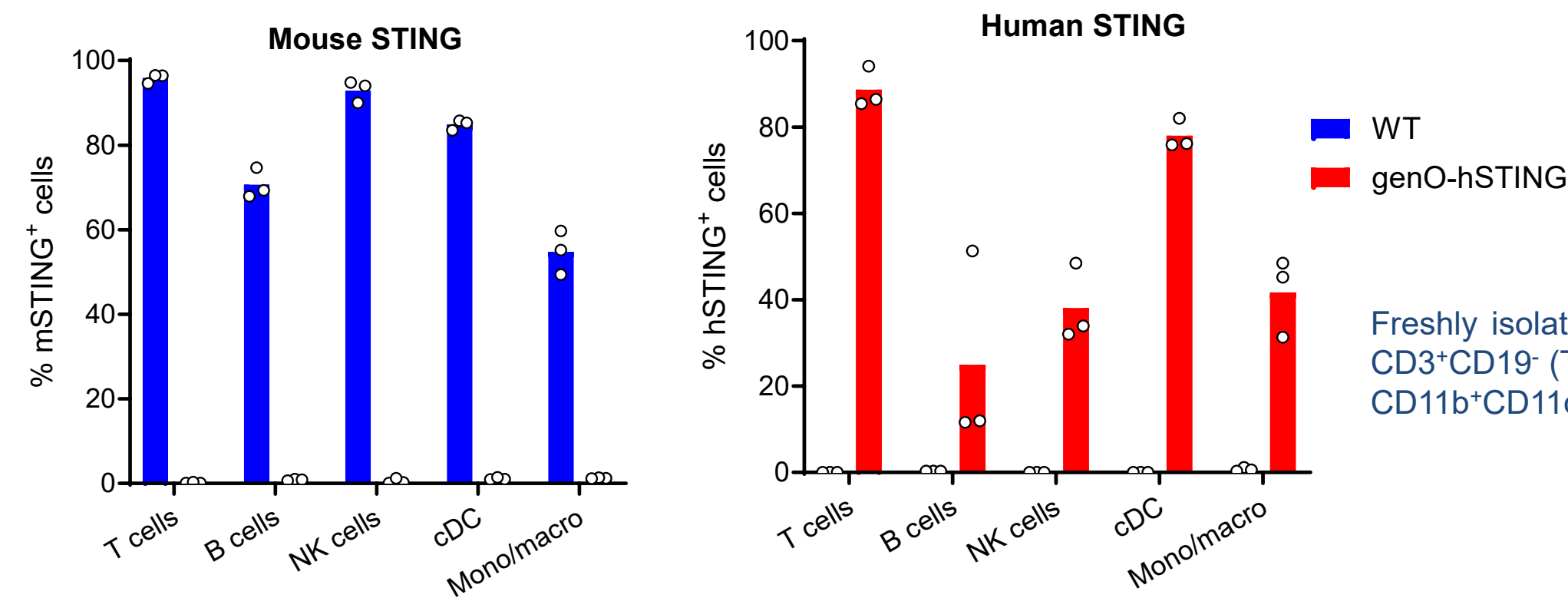
1. genO-hSTING mouse model

a) Humanization of STING does not alter immune cell distribution



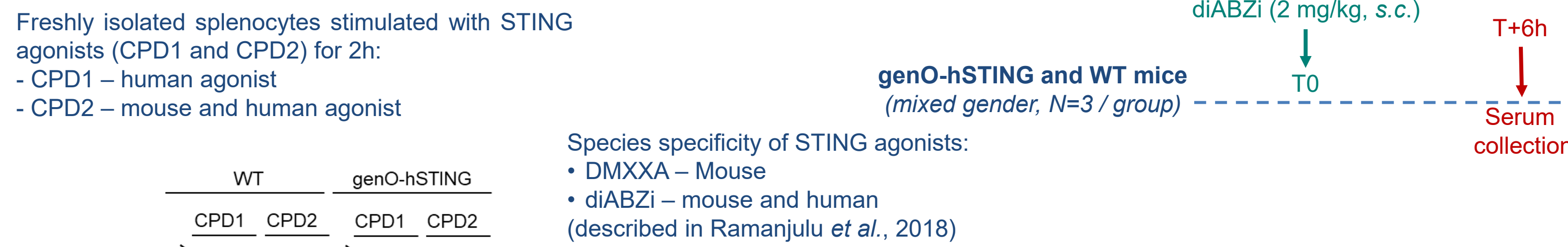
Freshly isolated splenocytes gated on live CD3⁺CD19⁻ (T cells) CD3⁺CD19⁺ (B cells) CD3⁺CD19⁻Nkp46⁺ (NK cells), CD3⁺CD19⁻CD11b⁺CD11c⁺ (DC) and CD3⁺CD19⁻CD11b⁺CD11c⁻ (Monocytes). T cells subsets gated on live CD3⁺, CD4⁺CD25⁻ (conv CD4) and CD4⁺CD25⁺FoxP3⁺ (Treg).

b) Human STING is expressed on immune cells from genO-hSTING mice



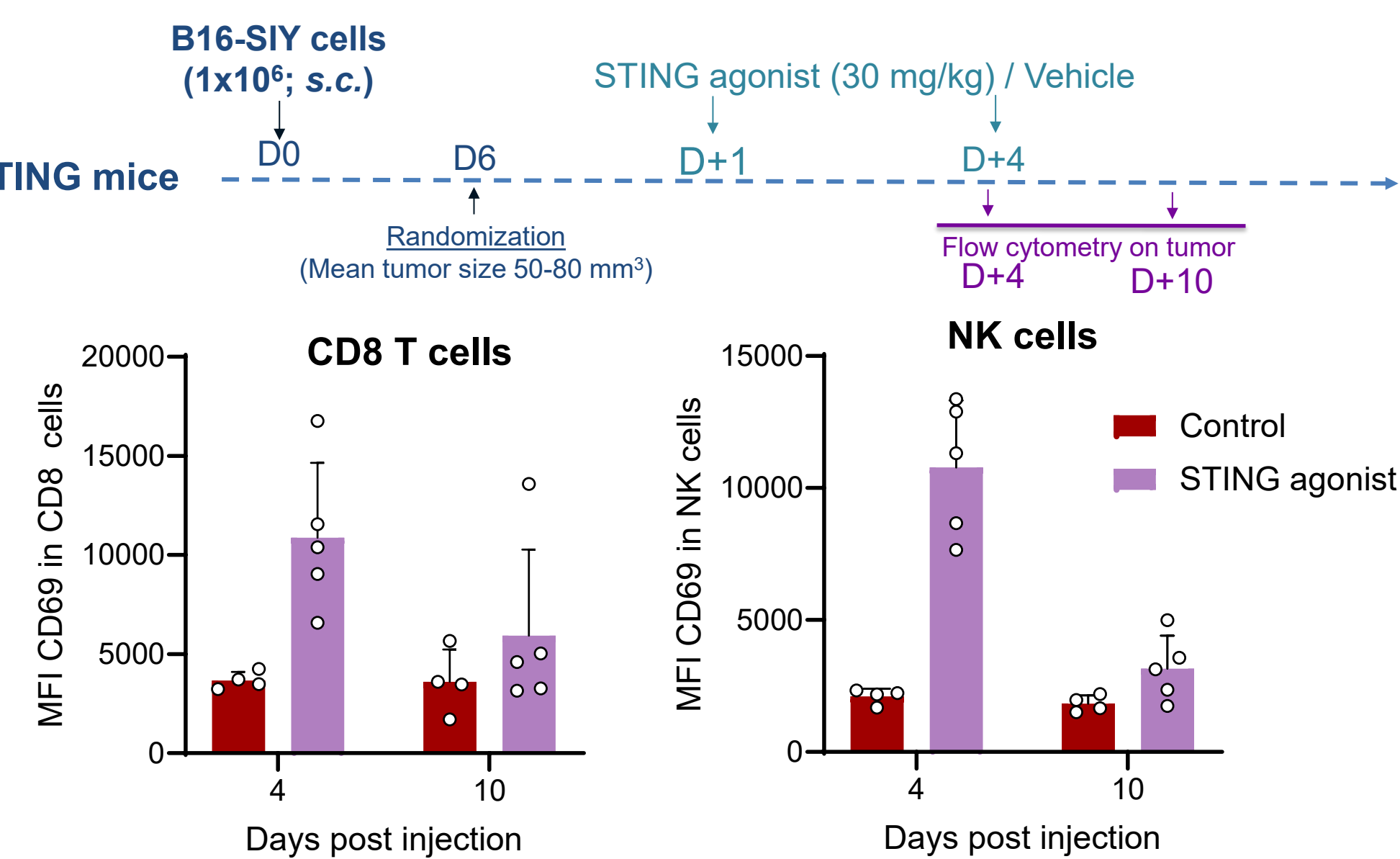
Freshly isolated splenocytes, gated on live: CD3⁺CD19⁻ (B cells), CD3⁺CD19⁺ (T cells), CD3⁺CD19⁻Nkp46⁺ (NK cells), CD3⁺CD19⁻CD11b⁺CD11c⁺ (DCs), CD3⁺CD19⁻CD11b⁺CD11c⁻ (Monocytes), CD3⁺CD19⁻CD335⁺ (NK cells)

c) Human STING activation leads to downstream signaling and cytokine release



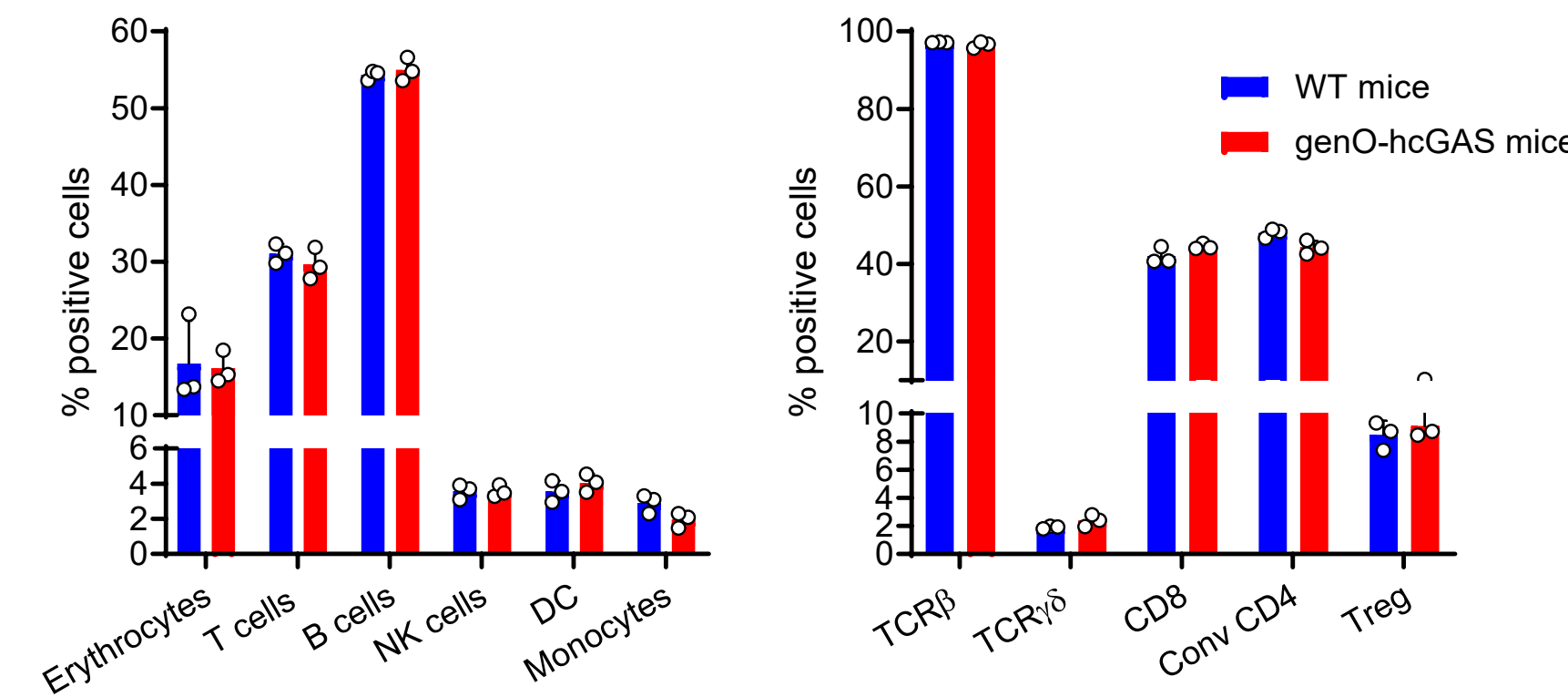
Species specificity of STING agonists:
• DMXAA – Mouse
• diABZI – mouse and human
(described in Ramanjulu *et al.*, 2018)

d) Human STING agonist, induces activation of T and NK cells in the TME of hSTING mice



2. genO-hcGAS mouse model

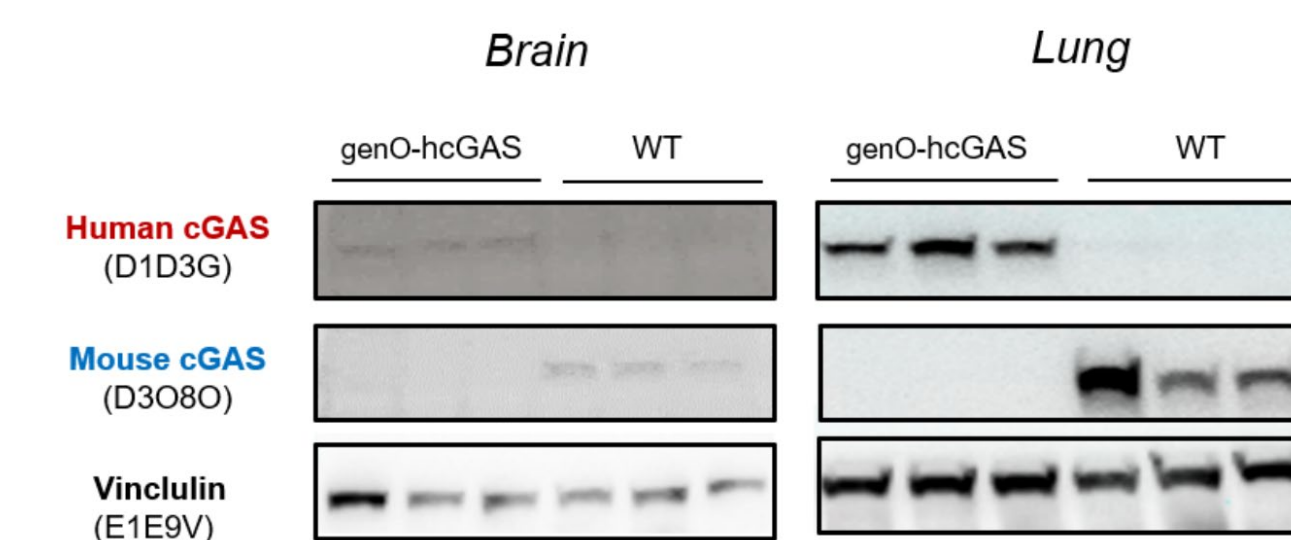
a) Humanization of cGAS did not alter immune cell distribution



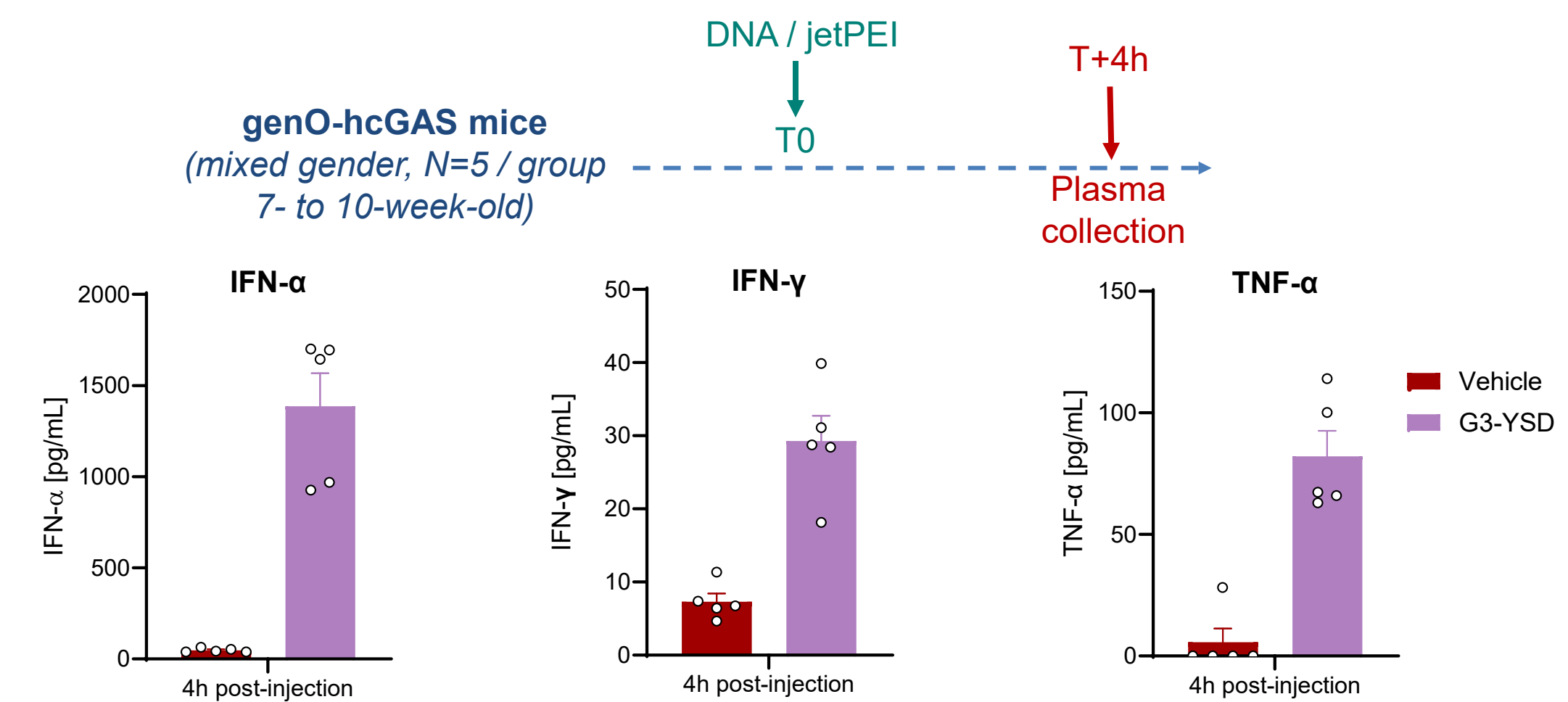
Freshly isolated splenocytes gated on live CD3⁺CD19⁻ (T cells) CD3⁺CD19⁺ (B cells) CD3⁺CD19⁻Nkp46⁺ (NK cells), CD3⁺CD19⁻CD11b⁺CD11c⁺ (DC) and CD3⁺CD19⁻CD11b⁺CD11c⁻ (Monocytes). T cells subsets gated on live CD3⁺, CD4⁺CD25⁻ (conv CD4) and CD4⁺CD25⁺FoxP3⁺ (Treg).

⇒ Similar results in blood and bone marrow – data not shown

b) Human cGAS is detected in brain and lung tissues of genO-hcGAS mice



c) Cytokine responses in WT and genO-hcGAS mice after G3-YSD stimulation, a human cGAS agonist

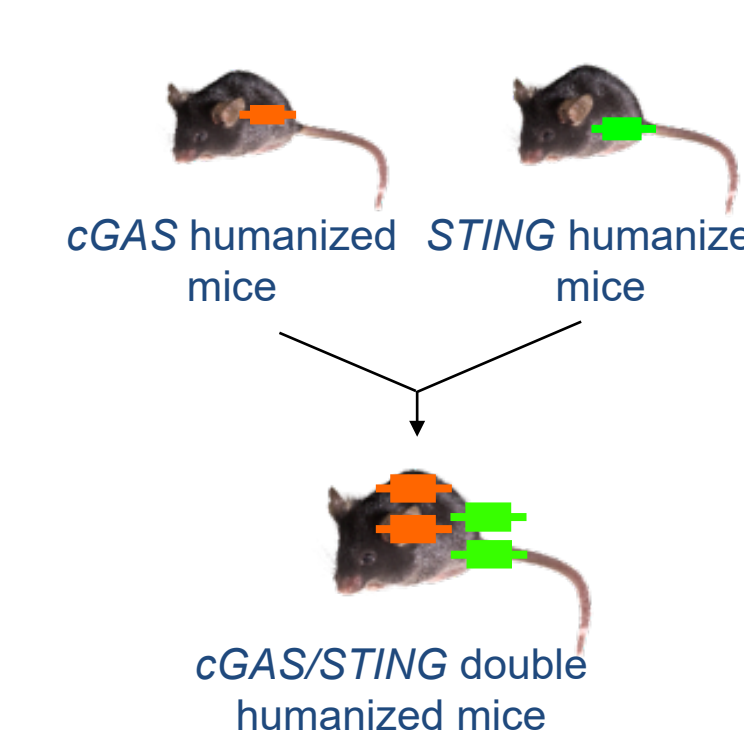


⇒ G3-YSD induced the secretion of IFN-α, IFN-γ and TNF-α: genO-hcGAS is fully functional

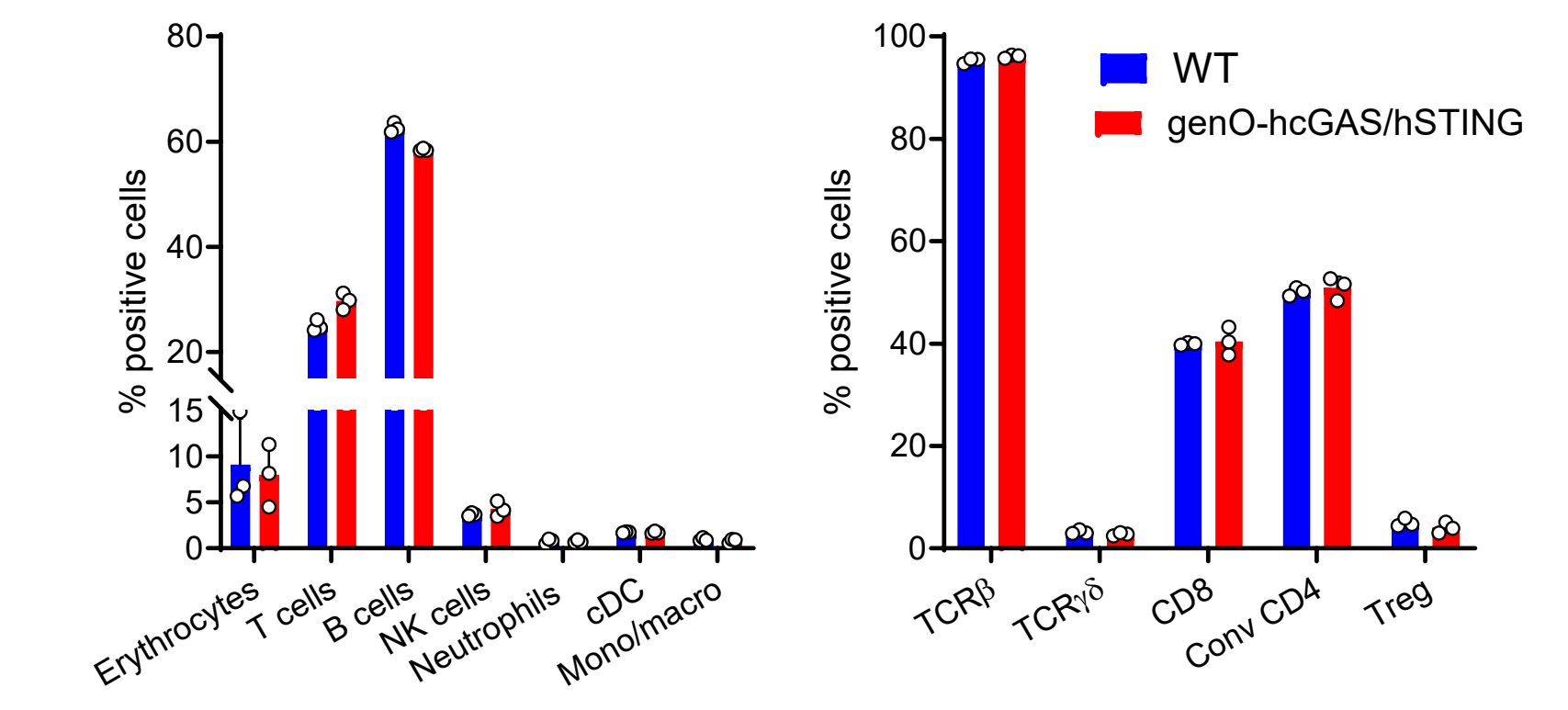


3. genO-hcGAS/hSTING mouse model

a) Double-humanized cGAS/STING model:

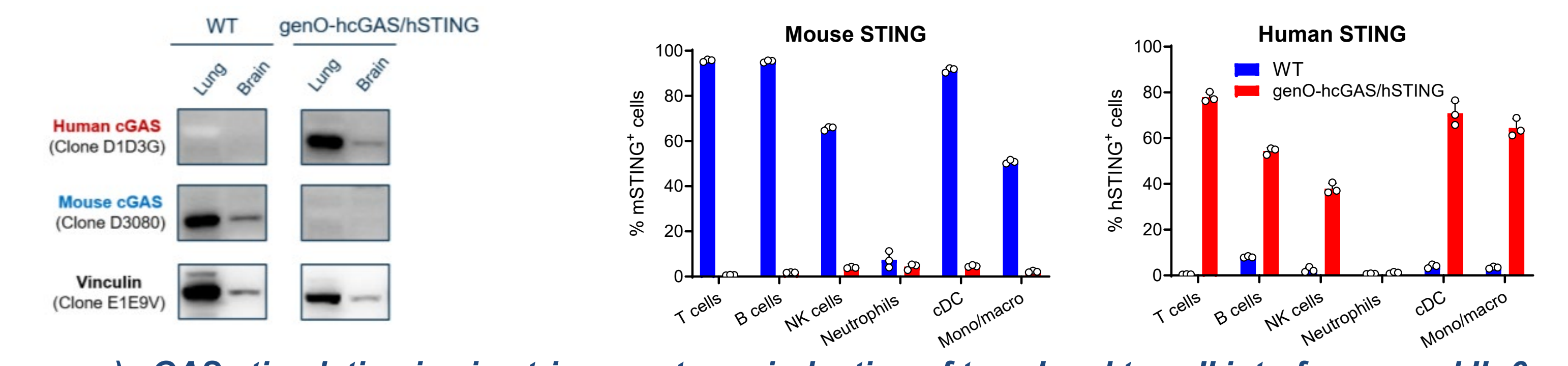


b) Humanization of STING and cGAS does not alter immune cell distribution



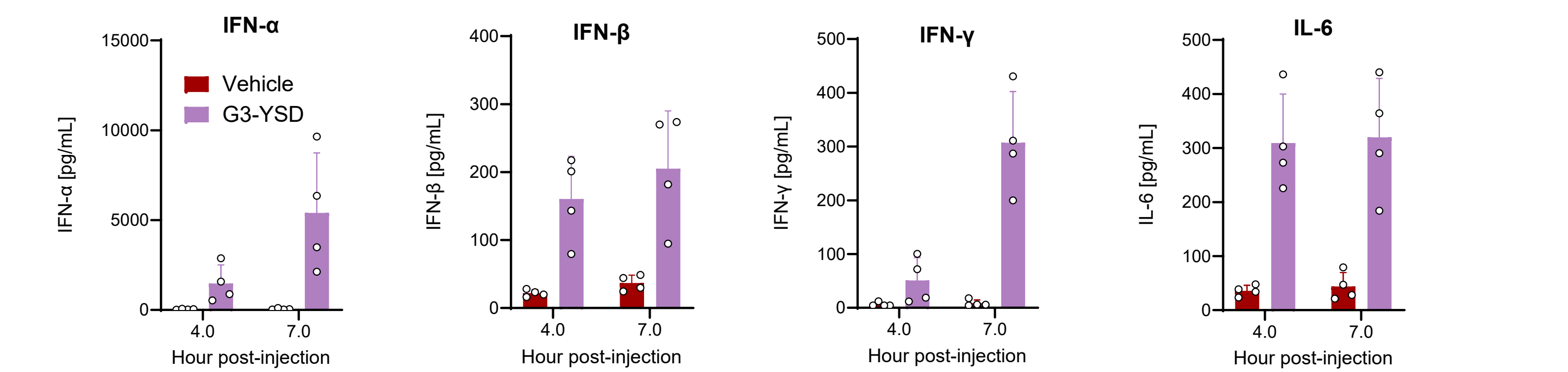
Freshly isolated splenocytes, gated on single live CD3⁺CD19⁻ (T cells) and CD3⁺CD19⁺ (B cells), in CD3⁺CD19⁻ cells: Nkp46⁺ (NK cells), Ly-6G⁺ (Neutrophils), Ly-6G⁺CD11c⁺MHC-II⁺ (cDC) and Ly-6G⁺Ly-6C⁺CD11b⁺ (Monocytes/macrophages). T cell subsets gated on CD3⁺, TCRβ⁺TCRγδ⁻: CD4⁺CD8⁺ (CD8), CD4⁺CD25⁻FoxP3⁺ (convCD4) and CD4⁺CD25⁺FoxP3⁺ (Treg). In cDC: CD8⁺CD11b⁻ (cDC1) and CD8⁺CD11b⁺ (cDC2).

b) hcGAS and hSTING expression is confirmed in double-humanized model

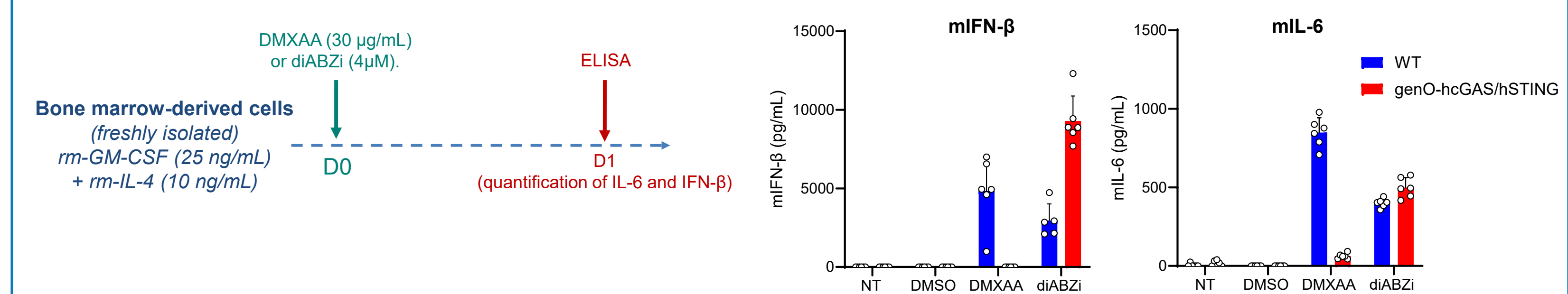


c) cGAS stimulation in vivo triggers strong induction of type I and type II interferons and IL-6

G3-YSD (50 μg) + jetPEI in 5% glucose *i.v.* or jetPEI in 5% glucose *i.v.* (vehicle) +4h +7h
Plasma collection: cytokine secretion quantification by LEGENDplex



d) STING agonist induce cytokine release in both genO-hcGAS/hSTING and WT cells, whereas DMXAA triggers cytokine release only in WT cells



Conclusion: These data indicate that human STING and cGAS expressed in genO-hcGAS/hSTING mice are functional and their mouse counterpart is no longer expressed in these mice. These humanized models provide valuable tools for assessing the efficacy and specificity of compounds targeting human cGAS/STING pathway.