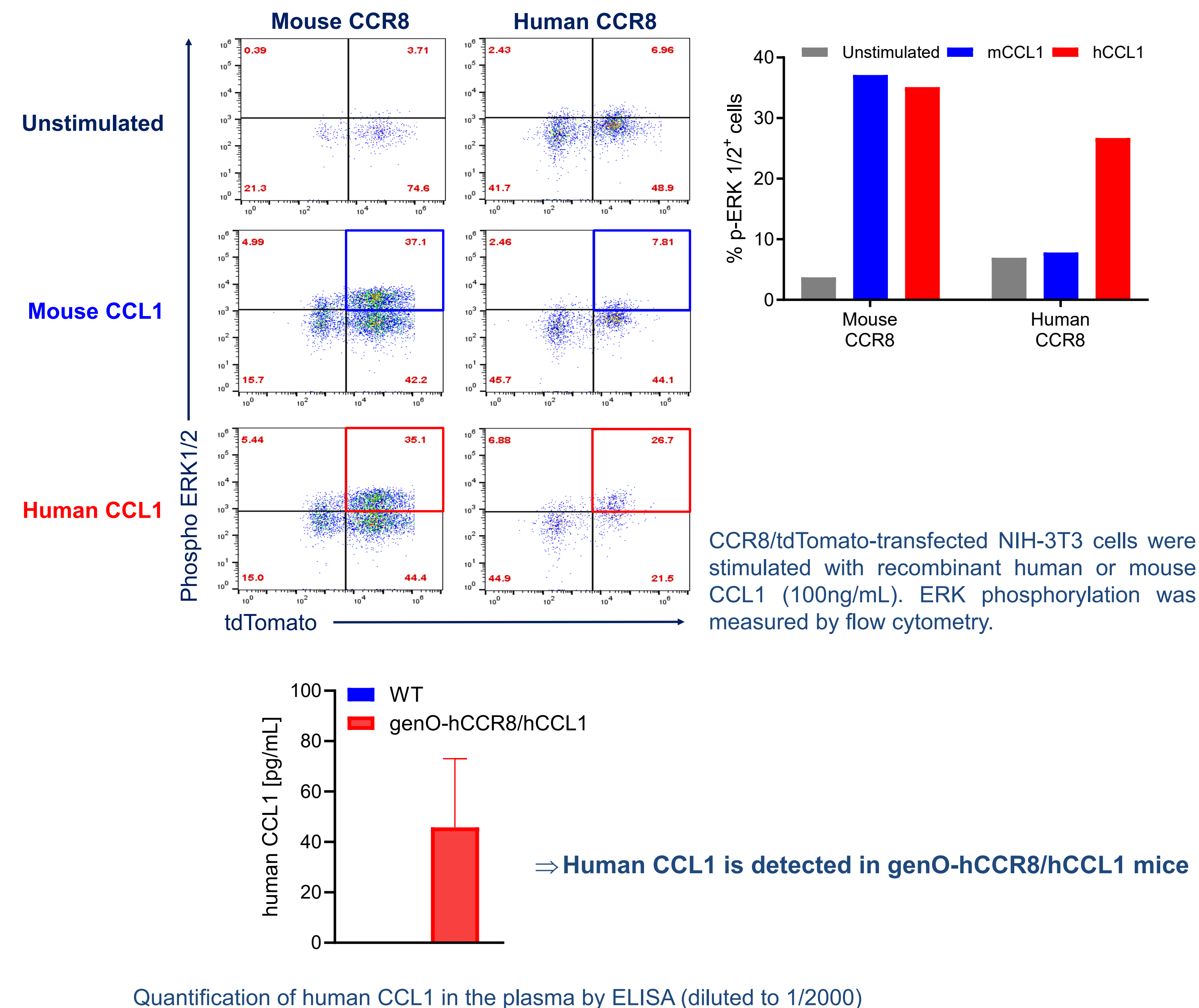


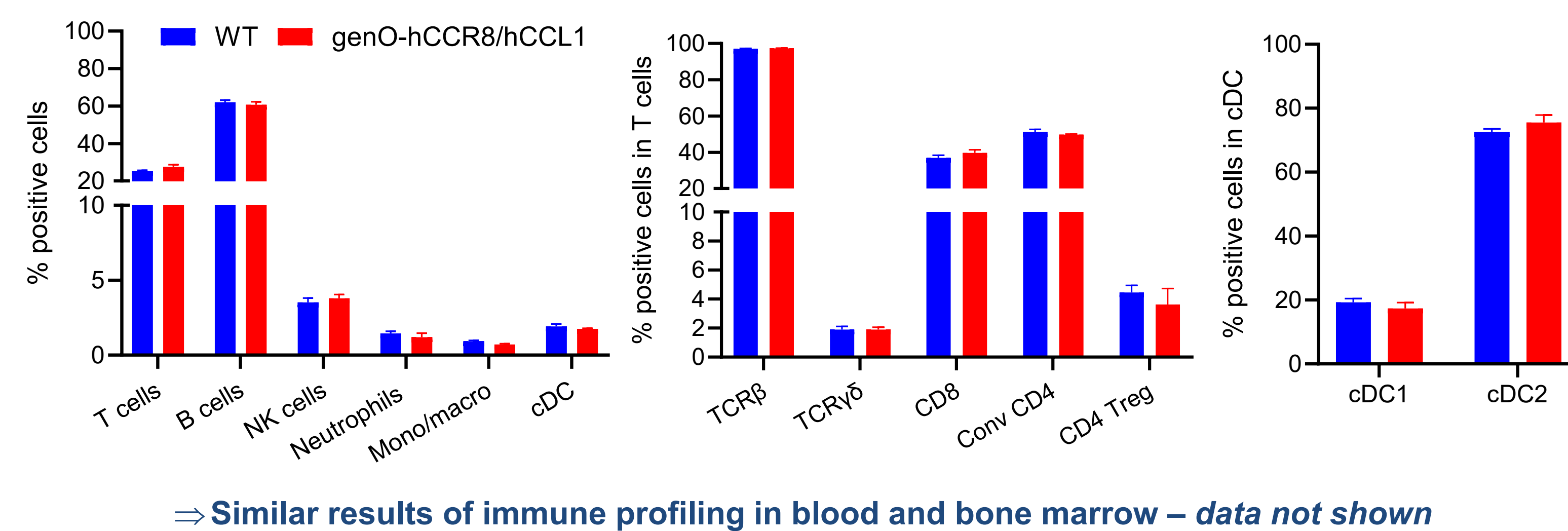
ULTIMATE PREDICTABILITY

Background: CCR8 is a potential therapeutic target to treat cancer due to its role in the immunosuppression induced by regulatory T cells (Treg). The development of a physiologically relevant model to assess CCR8-targeting therapies depends, among others, on the maintenance of a proper interaction of the receptor and ligands. CCR8 has 2 ligands: CCL1 and CCL8. Mouse CCL8 induces specific calcium flux in human CCR8-transfected mouse cells⁽¹⁾, suggesting that humanization of CCL8 is not mandatory. However, *in vitro* studies showed that murine CCL1 was unable to interact with human CCR8. Lack of functional CCR8/CCL1 axis could have an impact on the suppressive activity of Treg cells, thus requiring the humanization of both CCR8 and CCL1. Here we describe two humanized models, genO-hCCR8/hCCL1 and genO-BRGSF-HIS^(2,3) suitable for the assessment of efficacy and safety of CCR8-targeting compounds.

1. Mouse CCL1 does not induce signaling through human CCR8, requiring the humanization of both CCR8 and CCL1 to maintain functional CCR8/CCL1 axis

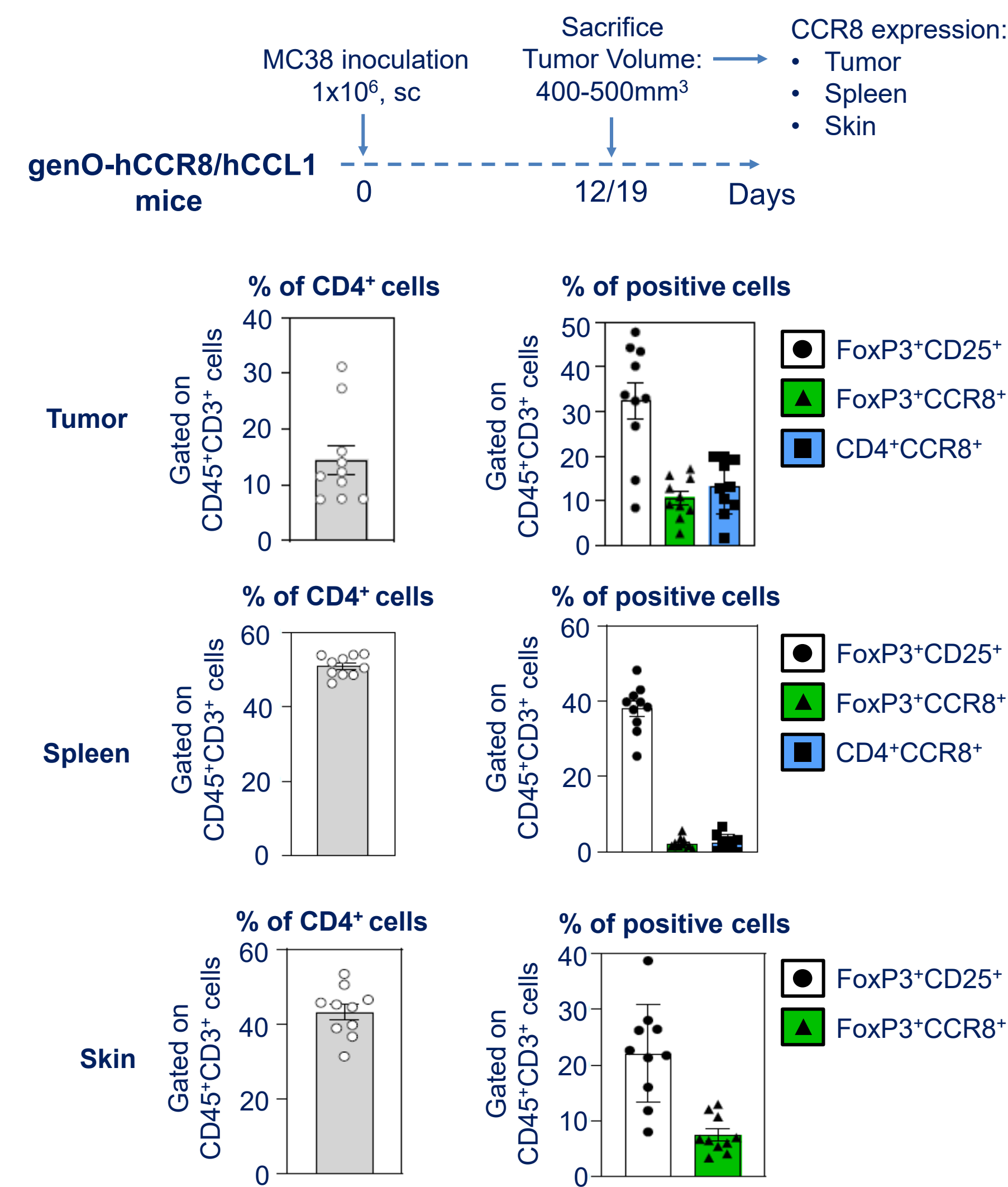


2. Humanization of both CCR8 and CCL1 does not alter immune cell distribution in genO-hCCR8/hCCL1 compared to WT mice

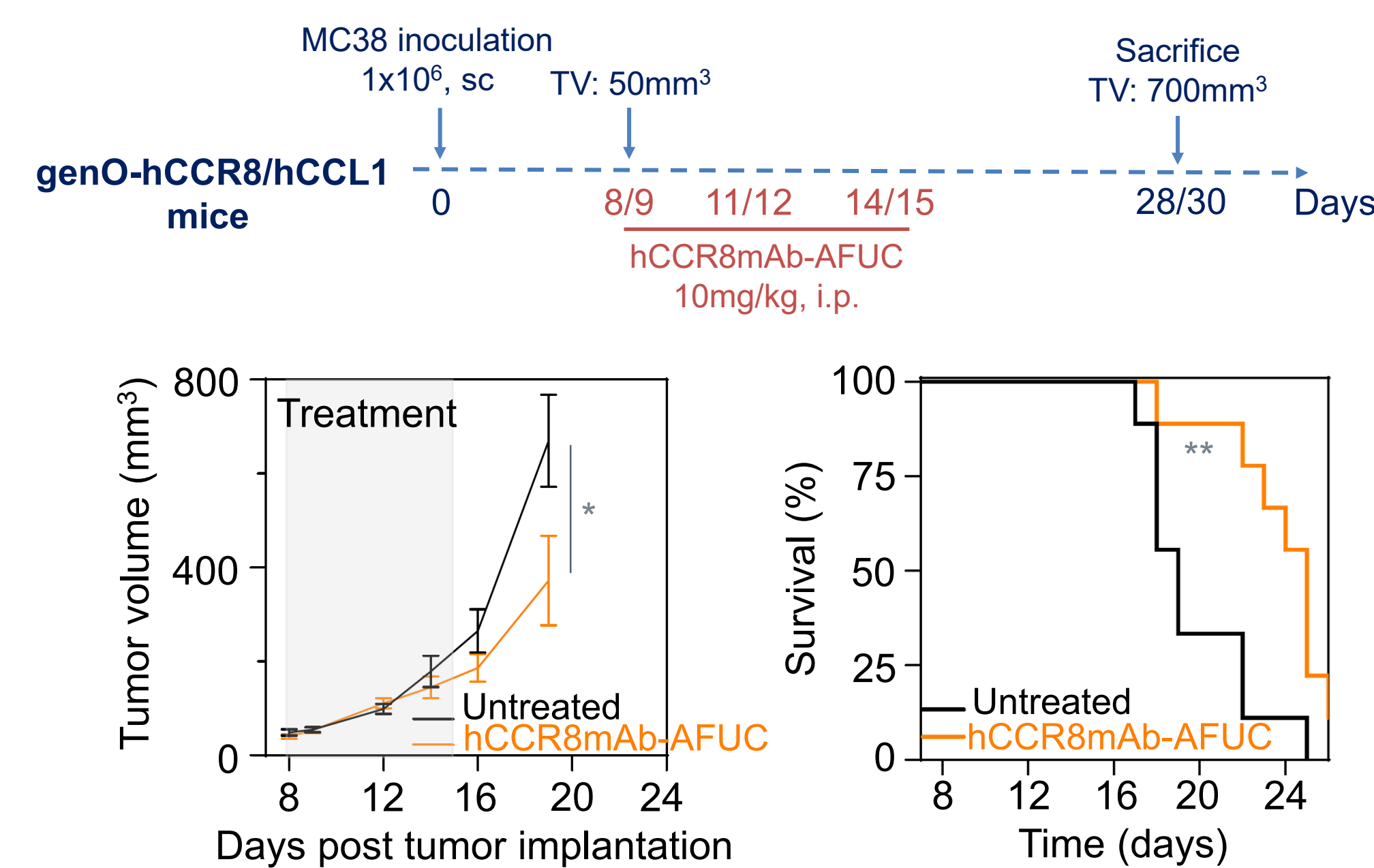


Freshly isolated splenocytes, gated on live CD3⁺ CD19⁻ (T cells) and CD3⁻ CD19⁺ (B cells), in CD3⁻ CD19⁻ cells: NKp46⁺ (NK cells), CD317⁺ (pDC), Ly-6G⁺ (Neutrophils), Ly-6G⁻ CD11c⁺ MHC-II⁺ (cDC) and Ly-6G⁻ Ly-6C⁺ CD11b⁺ (Monocytes/macrophages). In cDC: CD8⁺ CD11b⁻ (cDC1) and CD8⁺ CD11b⁺ (cDC2). T cell subsets gated on CD3⁺ TCRβ⁺ TCRγδ⁻: CD4⁺ CD8⁺ (CD8), CD4⁺ CD25⁺ FoxP3⁺ (convCD4) and CD4⁺ CD25⁺ FoxP3⁺ (Treg).

3. CCR8 is expressed on tumor infiltrating Treg cells but not in the spleen



4. Blocking of human CCR8 efficiently inhibits tumor growth



⇒ CCR8-targeting therapies can be assessed in genO-hCCR8/hCCL1 model

⇒ However, CCR8 expression pattern differs between human and mouse, as, in humans CCR8 is expressed on both infiltrating and peripheral Tregs

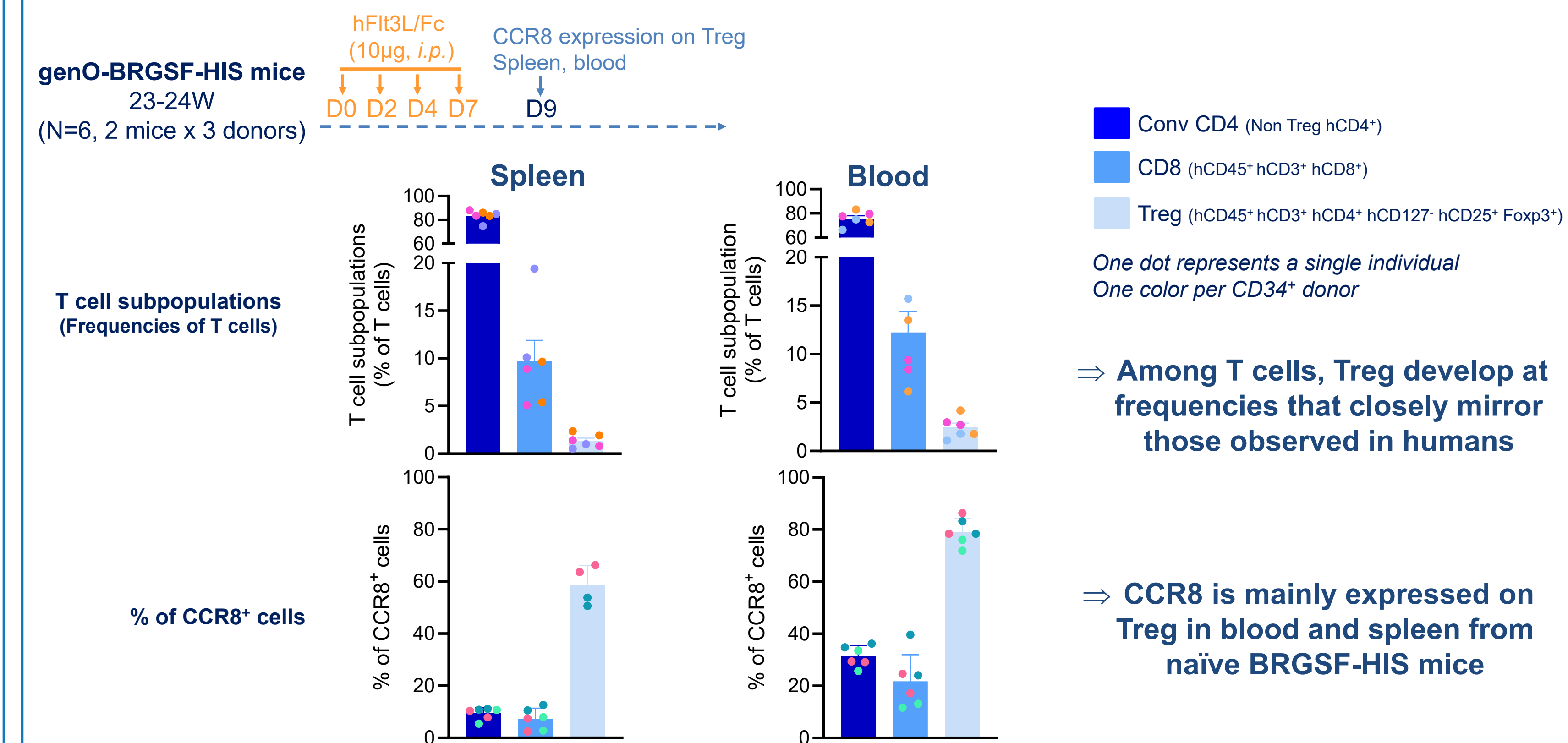
⇒ Safety assessment requires a model with a human-like expression of CCR8

5. genO-BRGSF-HIS model for safety assessment of CCR8/CCL1-targeting therapies

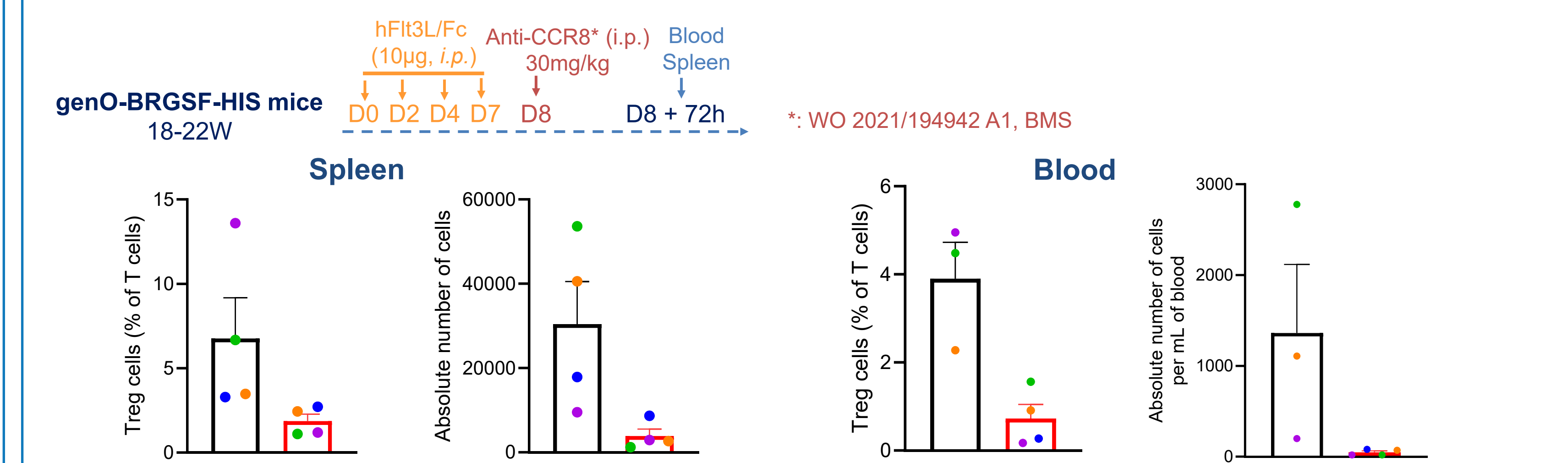
a. genO-BRGSF-HIS model features

- BRGSF (BALB/c Rag2^{-/-}, IL2Rγ^{-/-}, SIRPα^{NOD}, Flt3^{-/-}):
- Highly immunodeficient with reduced murine myeloid cells
 - Normal lifespan (no anemia, no weight loss, normal fur texture & integrity, normal activity & posture)
 - Upon CD34⁺ cells injection:
 - Lymphoid and Myeloid compartment development

b. Treg develop in genO-BRGSF-HIS and express CCR8



c. Anti-CCR8 depletes Treg in spleen and blood of genO-BRGSF-HIS



Conclusion: Altogether, data suggest that genO-hCCR8/hCCL1 humanized model is a valuable tool for CCR8-targeting agents efficacy assessment, enabling the investigation of immunomodulatory mechanisms at the tumor microenvironment. Nevertheless, this model has limitations to assess safety of CCR8-targeting compounds as the pattern of expression differs between human and mouse. The human-like expression pattern of CCR8 in naïve Treg from genO-BRGSF-HIS model suggests it could be a more suitable model for safety and PD assessment.