



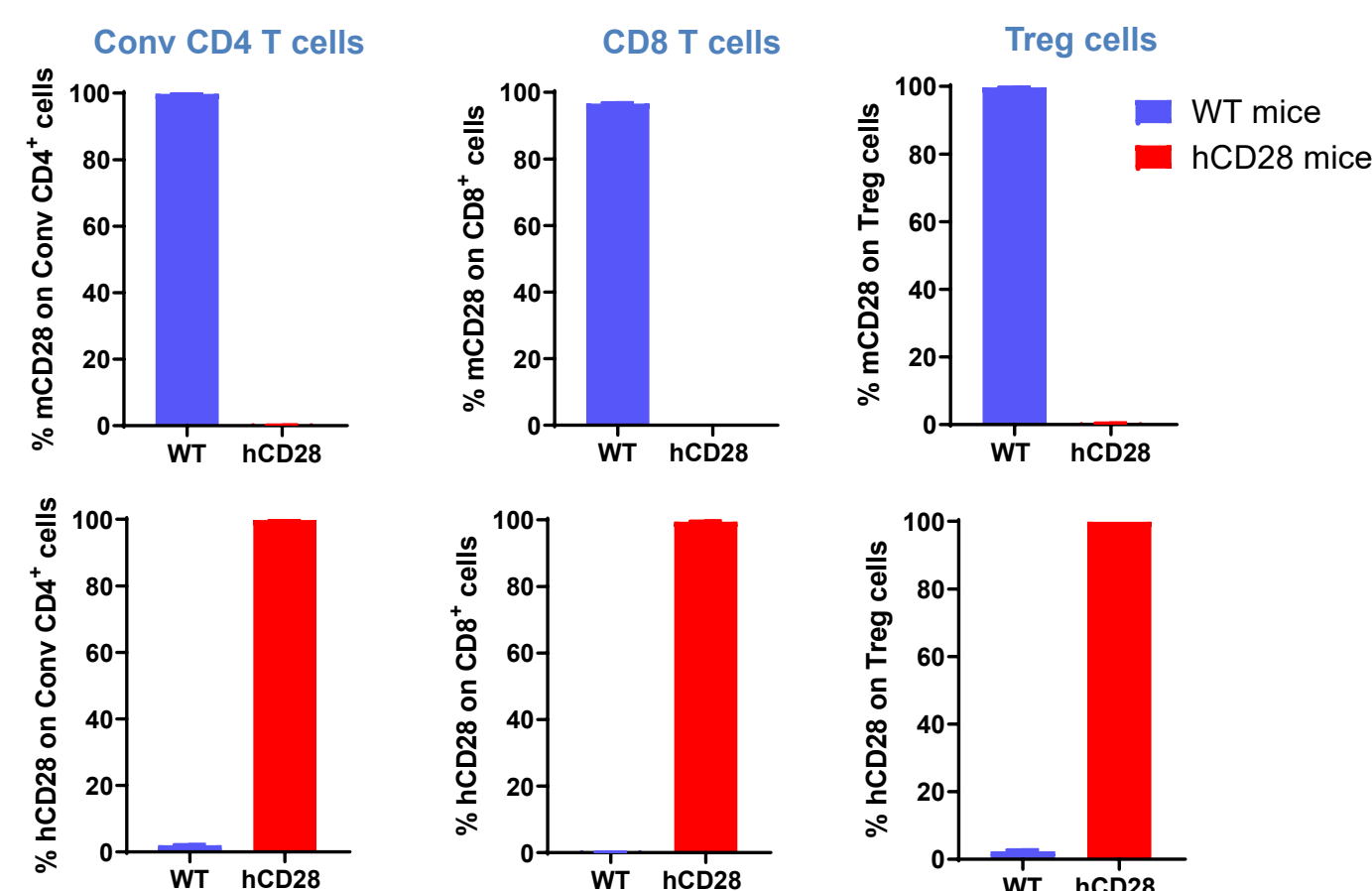
## CD28 humanized mouse model for efficacy and safety assessment of CD28-targeting therapies

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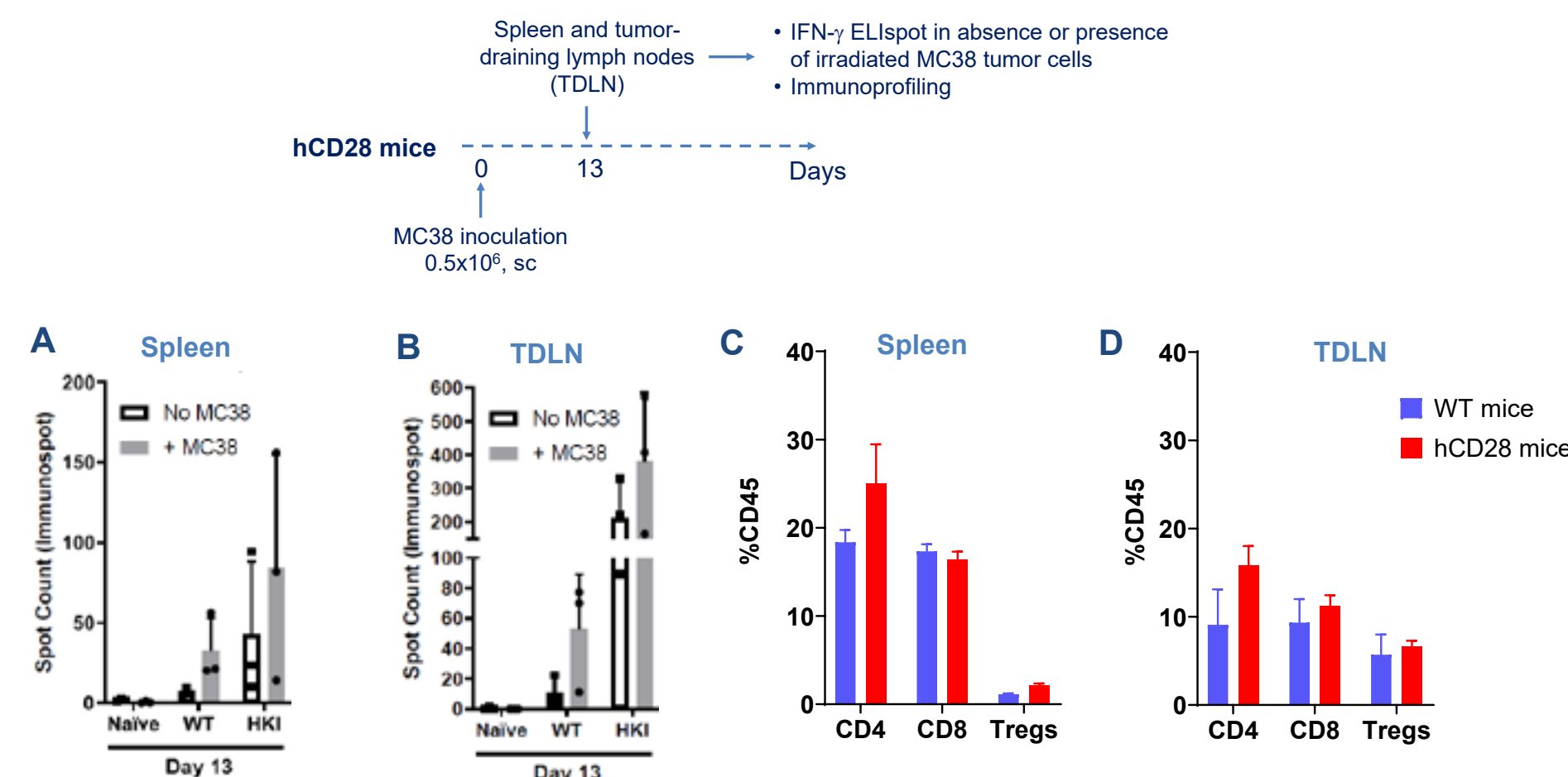
**Background:** Agonist antibodies targeting CD28 have proven to be effective against cancer, but also faced challenges due to severe adverse events triggered by its activation. Human and mouse CD28 (hCD28 and mCD28) have different signaling responses, with CD28 ligands and superagonists inducing pro-inflammatory cytokines upon stimulation in absence of TCR ligation in humans, but not in mice. Expression of CD28i amplifier isoform, which is thought to enhanced the production of cytokines in humans, could partially explain this difference, as it is not expressed in mice. In addition, evidence suggests that the different signaling between hCD28 and mCD28 relies on one amino acid change in the intracellular domain (ICD) [1]. Herein, we describe a CD28 humanized mouse model for assessment of CD28-targeting agents. To improve translatability, we decided to keep the expression of both canonical and CD28i human isoforms to avoid undermining the biological effects of the testing agents.

### 1. Human CD28 expression pattern recapitulates mCD28



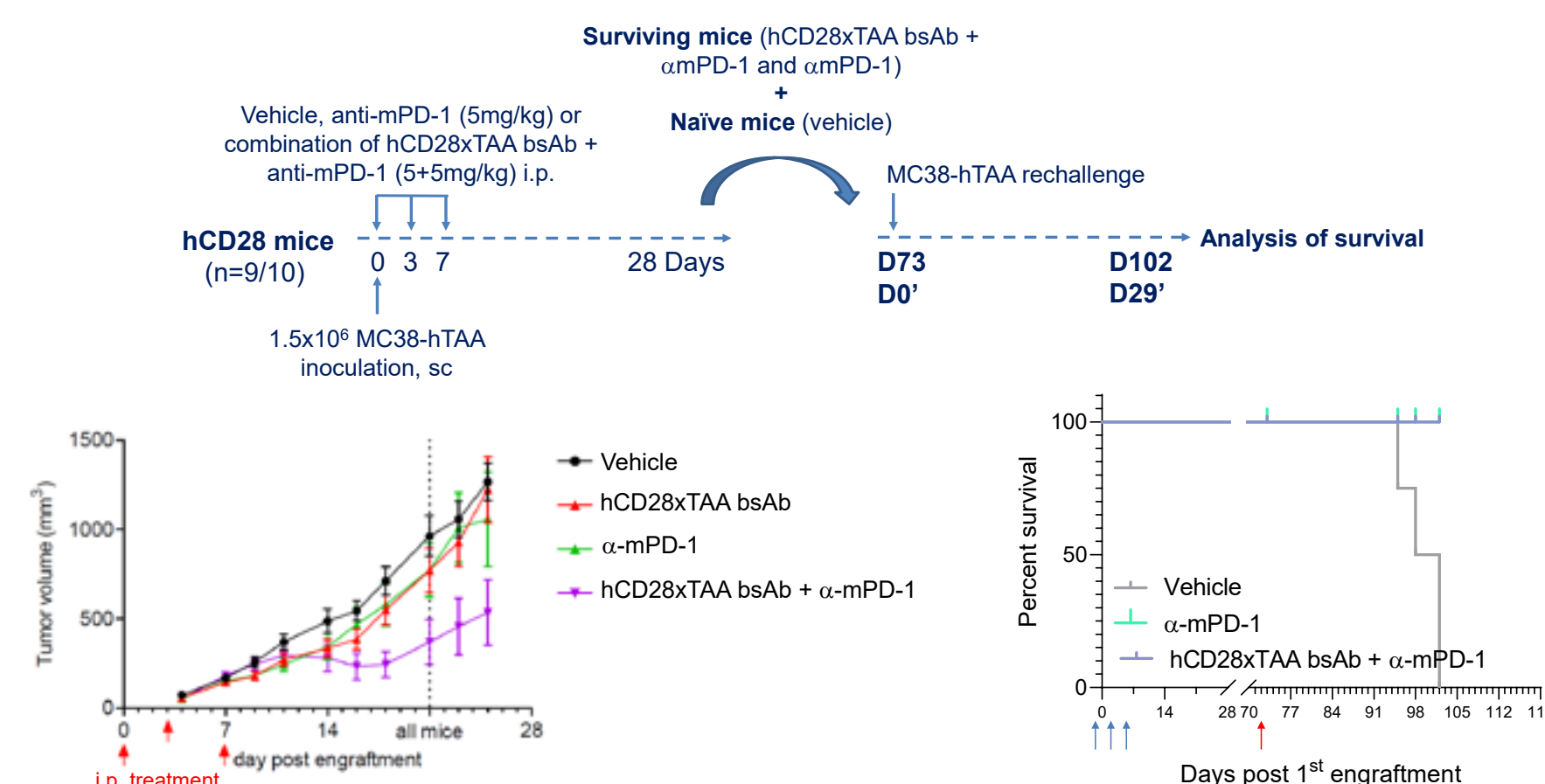
### 3. Ex vivo MC38 rechallenge of cells isolated from MC38-bearing mice induces IFN- $\gamma$ secretion

WT and hCD28 mice were inoculated with MC38 cells. (A) Spleens and (B) tumor-draining lymph nodes (TDLNs) harvested on day 13 post-implantation. IFN- $\gamma$  ELISpot performed in absence or presence of irradiated MC38 tumor cells. (C) Immunoprofiling on spleen and (D) tumor was also performed to determine the frequency of CD4 (CD45<sup>+</sup> CD4<sup>+</sup> CD25<sup>-</sup> FoxP3<sup>-</sup>), CD8 (CD45<sup>+</sup> CD8<sup>+</sup>) and Treg cells (CD45<sup>+</sup> CD4<sup>+</sup> CD25<sup>+</sup> FoxP3<sup>+</sup>). Results are expressed as mean  $\pm$  SEM (n=3)

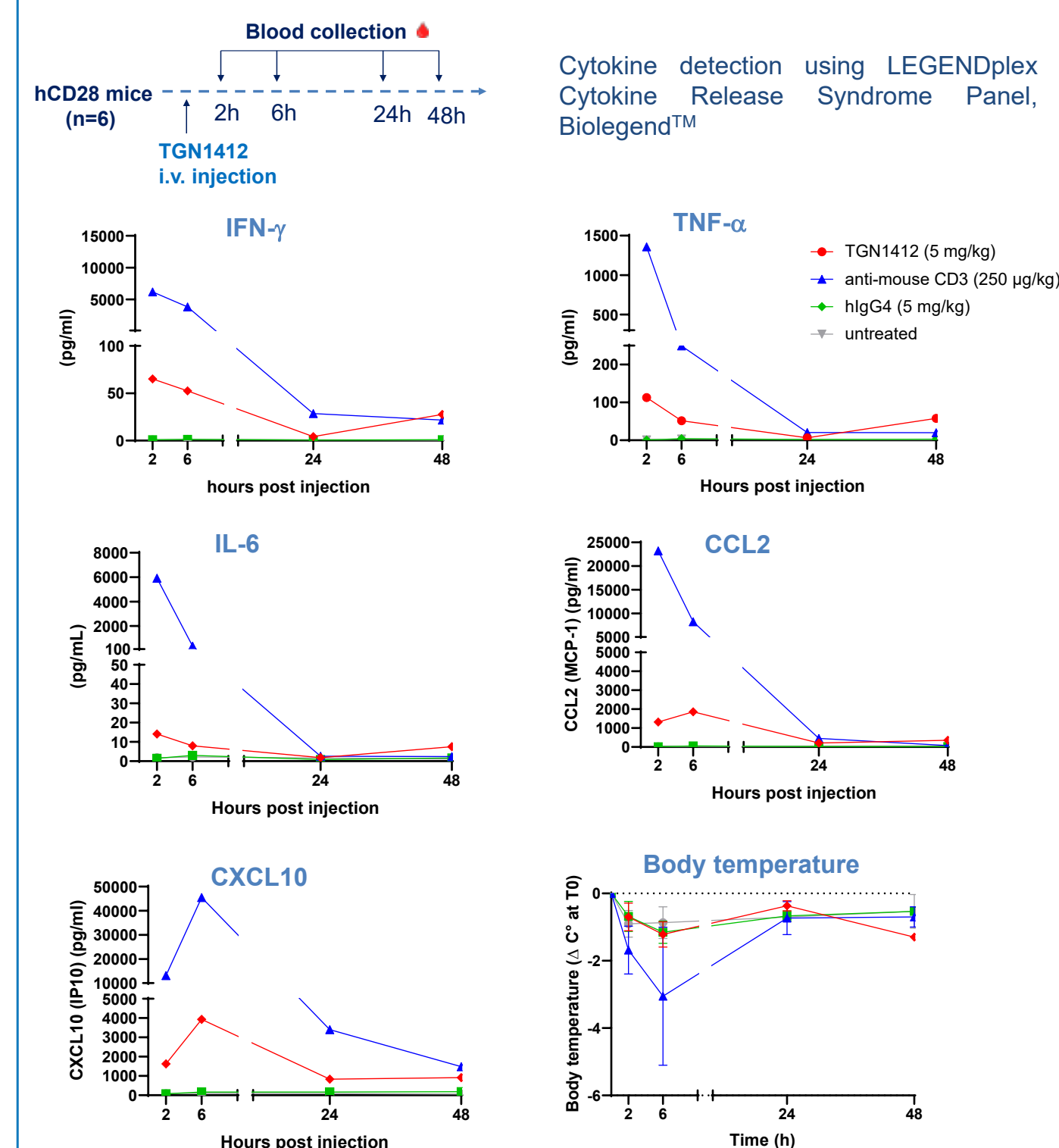


⇒ IFN- $\gamma$  levels are higher in hCD28 than in WT mice  
⇒ Since the frequency of CD3 cells (CD4<sup>+</sup>, CD8<sup>+</sup> and Treg) is comparable to WT mice, this could be explained by the expression of the amplifier CD28i human in hCD28 mice (equivalent isoform is absent in WT mice).

### 4. Combination of CD28xTAA bsAb and anti-mouse PD-1 induces tumor growth inhibition and long term immune response



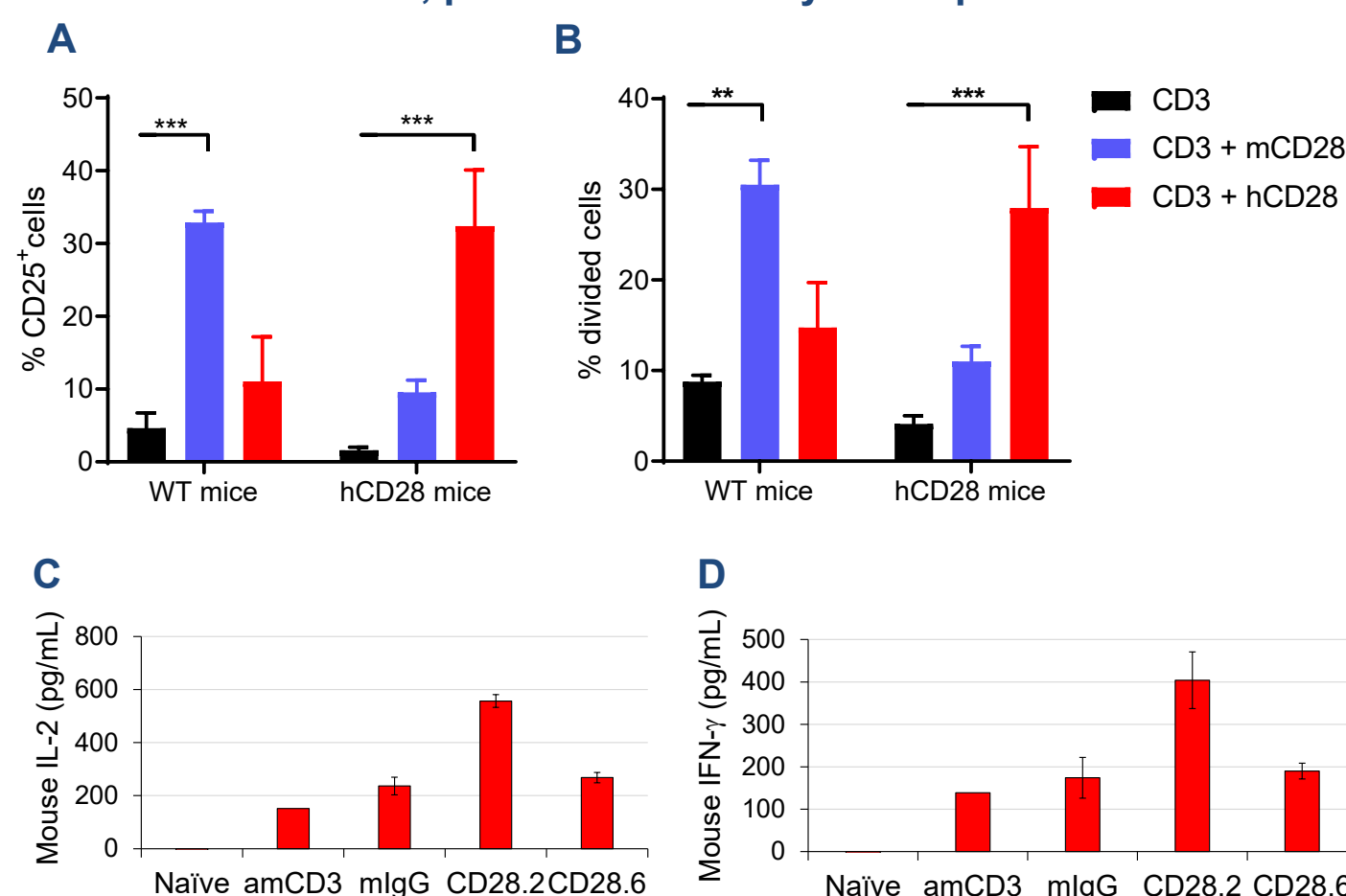
### 5. TGN1412 induces early cytokine release in hCD28 mice



**Conclusion:** Altogether, data suggest that hCD28 model enables assessment of efficacy and safety of CD28-targeting agents. The hCD28 model was intercrossed with CD3 humanized models to enable assessment of combination therapies and bispecific antibodies targeting both CD3 and CD28.

**References:**  
[1] Porciello N, Grazioli P, Campese AF, et al. A non-conserved amino acid variant regulates differential signalling between human and mouse CD28. Nat Commun 2018; 9:1–16

### 2. hCD28 is functional: $\alpha$ -human CD28 co-stimulation induces T cell activation, proliferation and cytokine production



Isolated T cells were labeled with CTV and activated with  $\alpha$ CD3 and soluble  $\alpha$ CD28 (anti-mouse CD28 clone 37.51, anti-human CD28 clone CD28.2) for 4 days. Representative FACS bar graph indicating (A) CD25 expression and (B) CTV dilution (as a measure of proliferation gated on live T cells). Results are expressed as mean  $\pm$  SD. Unpaired T test analyses (\*\*p<0.01, \*\*\*p<0.001).  
(C) IL-2 and IFN- $\gamma$  production were measured by ELISA in the supernatant of splenocytes activated with  $\alpha$ CD3 and soluble human  $\alpha$ CD28 (CD28.2: agonist mAb or CD28.6: blocking mAb).