

Development of GNTI-122, a novel engineered regulatory T cell therapeutic, for future application in type 1 diabetes

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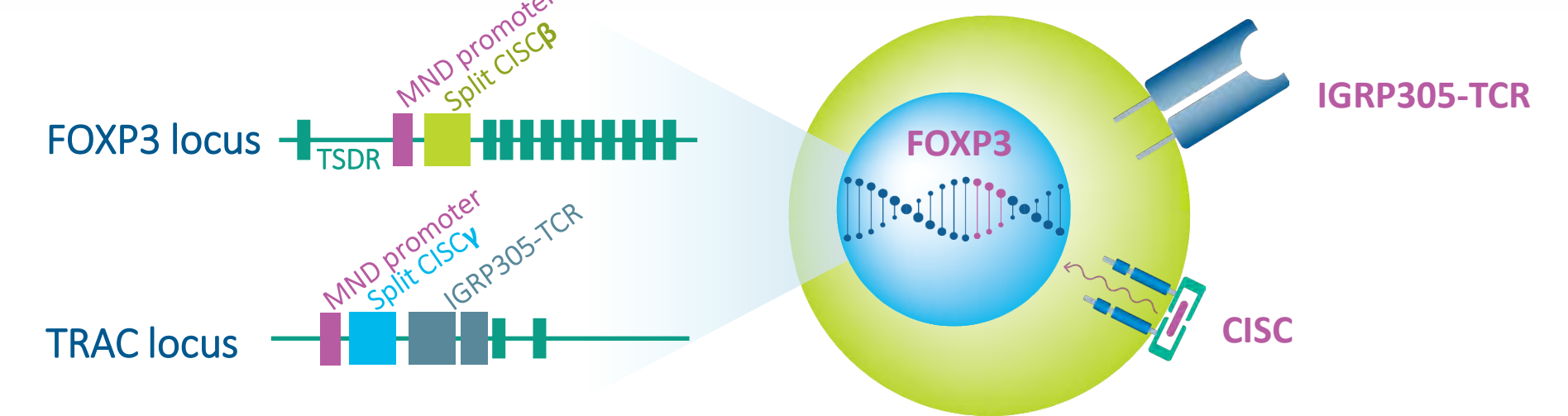
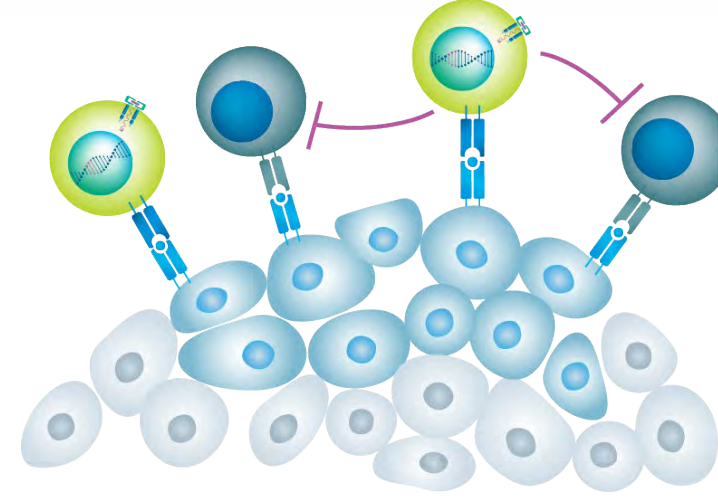
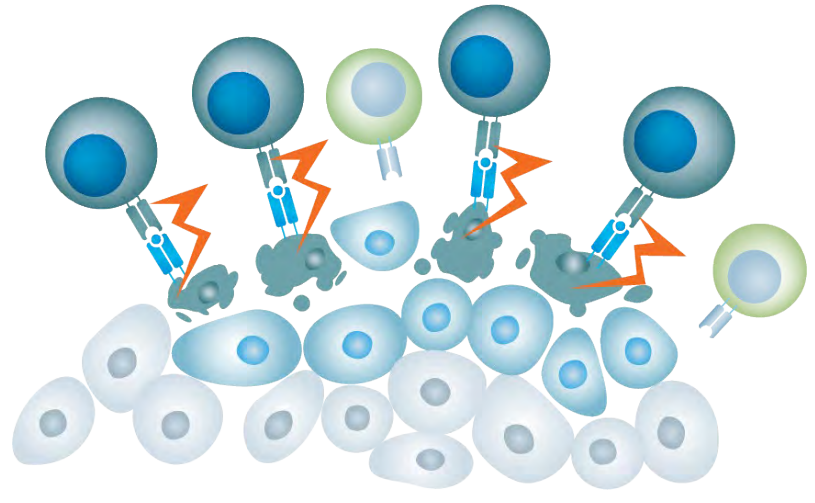
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OVERVIEW | Establish Proof-of-Concept to Support the Development of GNTI-122 Engineered Treg Therapy for the Treatment of Type 1 Diabetes

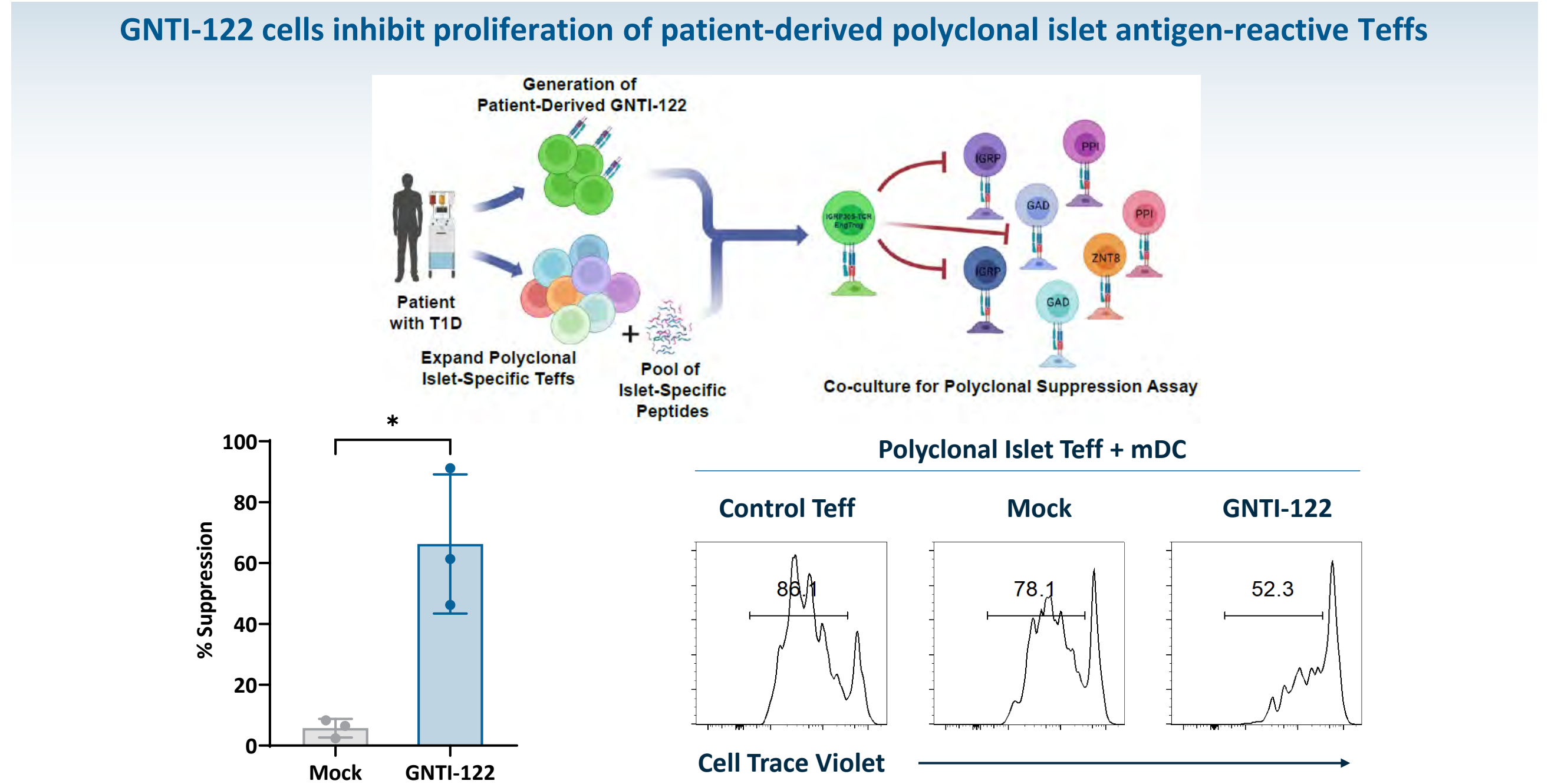
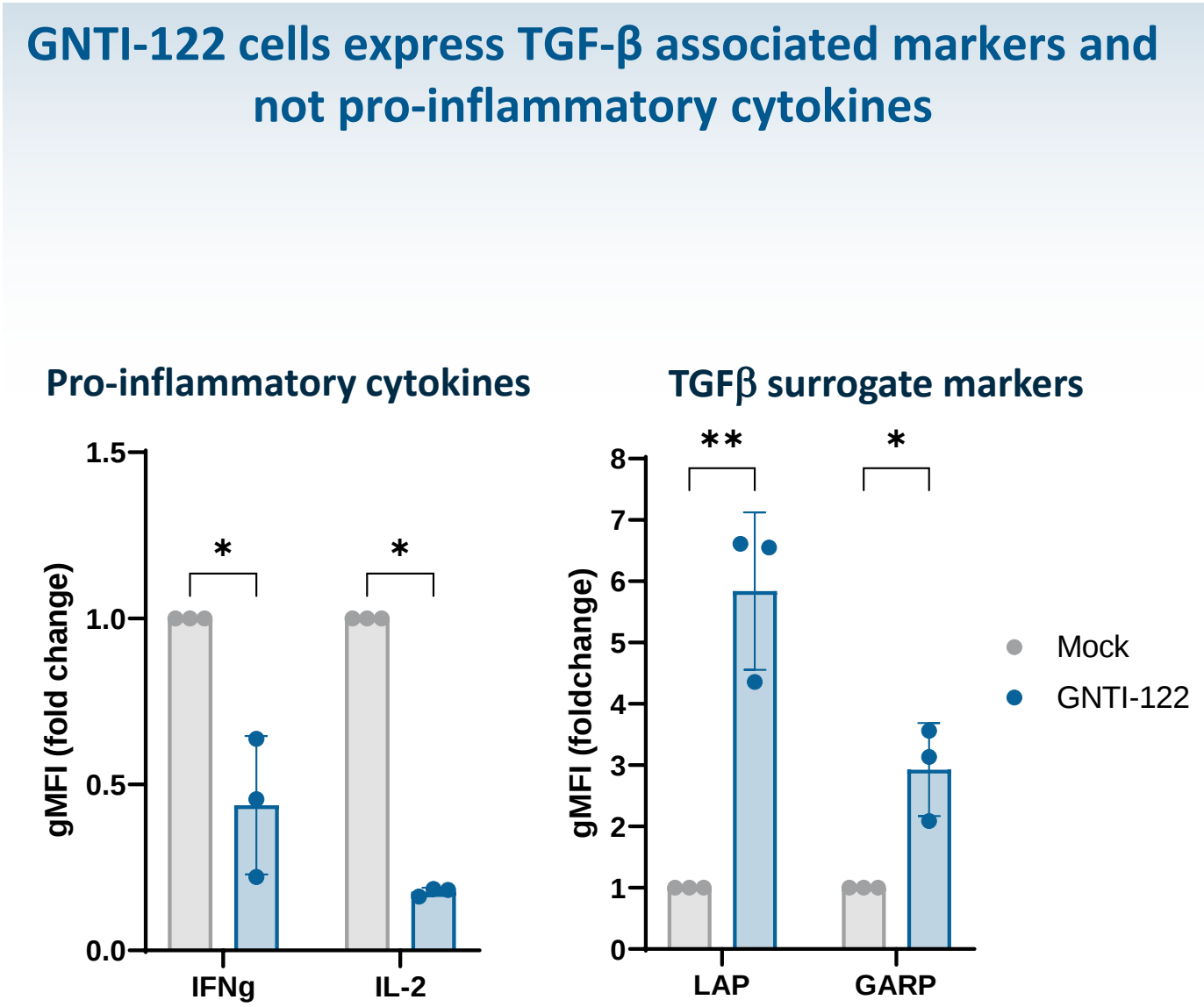
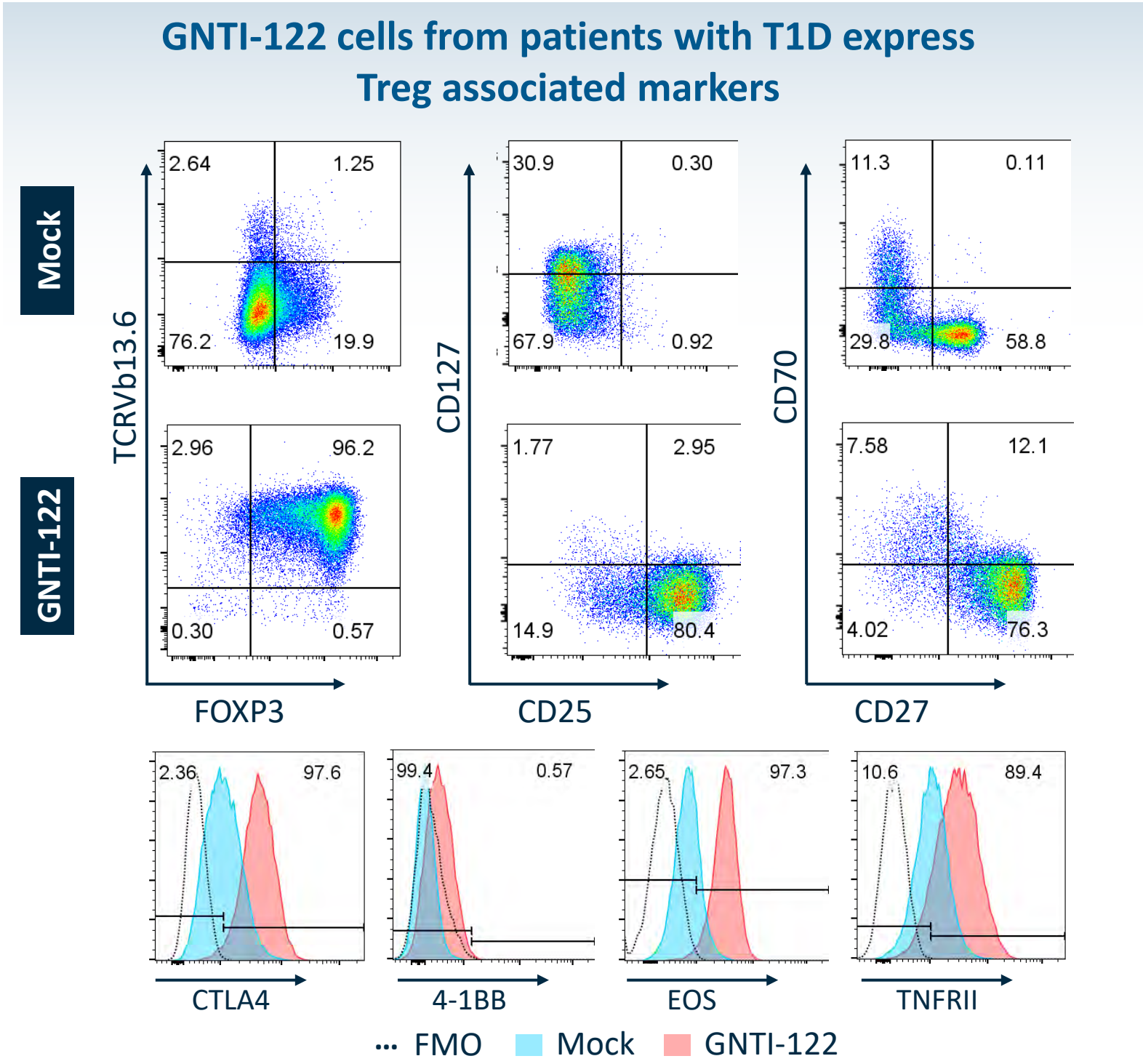
Type 1 diabetes is an autoimmune disease caused by T-lymphocyte-mediated destruction of insulin-producing beta cells that eventually leads to uncontrolled hyperglycemia and life-long dependence on daily insulin administration.

GNTI-122, a novel engineered T regulatory cell in development for the treatment of recently diagnosed T1D, is designed to protect islet cells from damage by homing to the pancreas and draining lymph nodes and suppressing pathogenic effector T cells via the mechanisms of bystander suppression and infectious tolerance.

GNTI-122 is engineered from autologous CD4 T cells with an RNA-guided nuclease gene editing to knock-in an MND promoter into the FOXP3 gene to stabilize its expression, a pancreatic islet antigen-specific TCR into the TRAC locus, and a rapamycin-mediated IL-2 signaling complex (CISC).



RESULTS | GNTI-122 Cells Have a Treg Phenotype and Suppress Islet Antigen-Reactive T Effector Cells from Donors with T1D

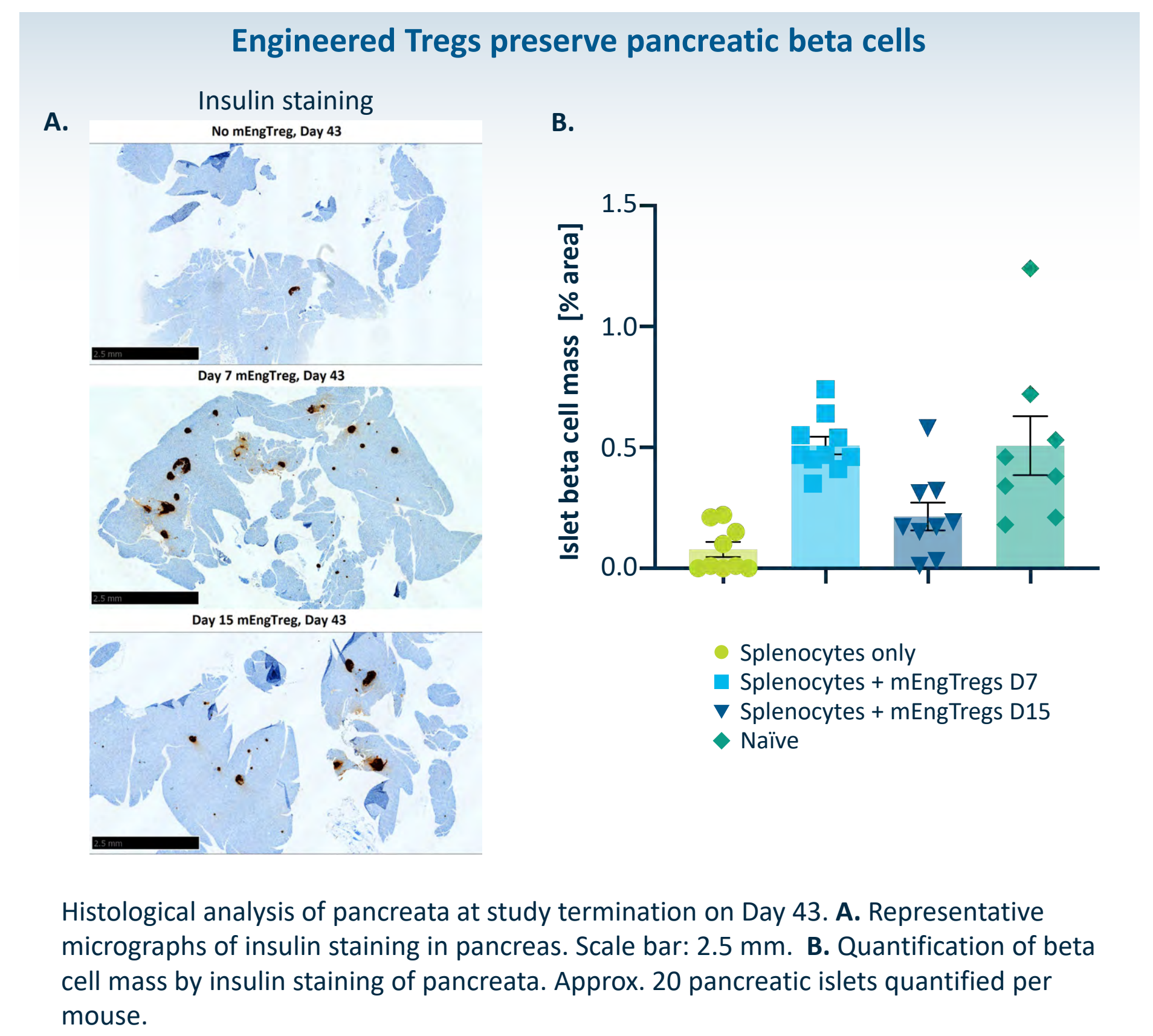
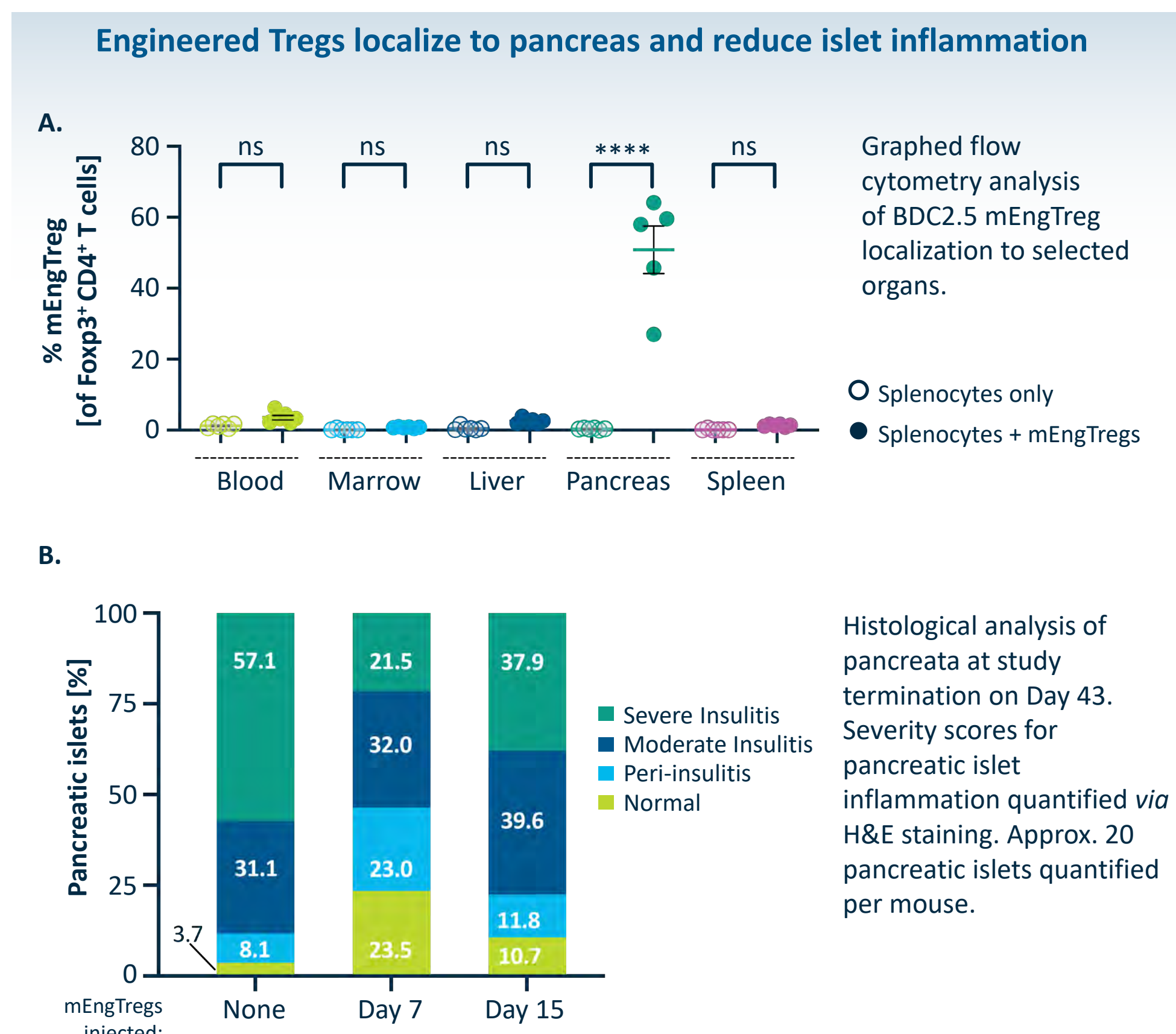
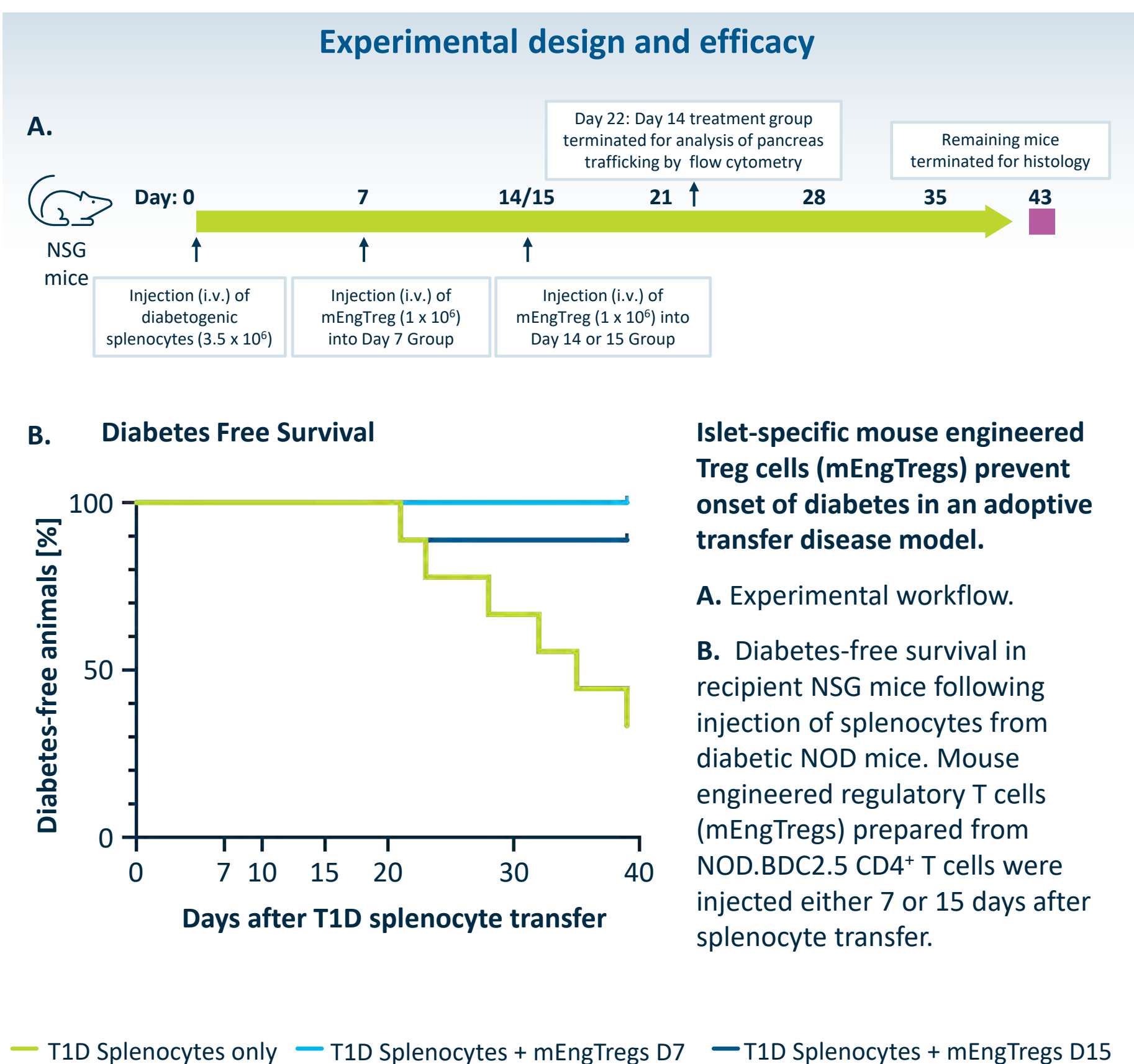


GNTI-122 cells express Treg markers (FOXP3⁺CD25⁺CD127⁺) and increase functional (CTLA-4, 4-1BB) and stability (TNFR1, CD27⁺CD70⁺, EOS) markers. Mock cells are gated on CD4⁺ cells, and GNTI-122 cells are gated on TCRβ⁺FOXP3⁺ cells. Representative donor data shown. Reproduced in 3 independent donors with T1D.

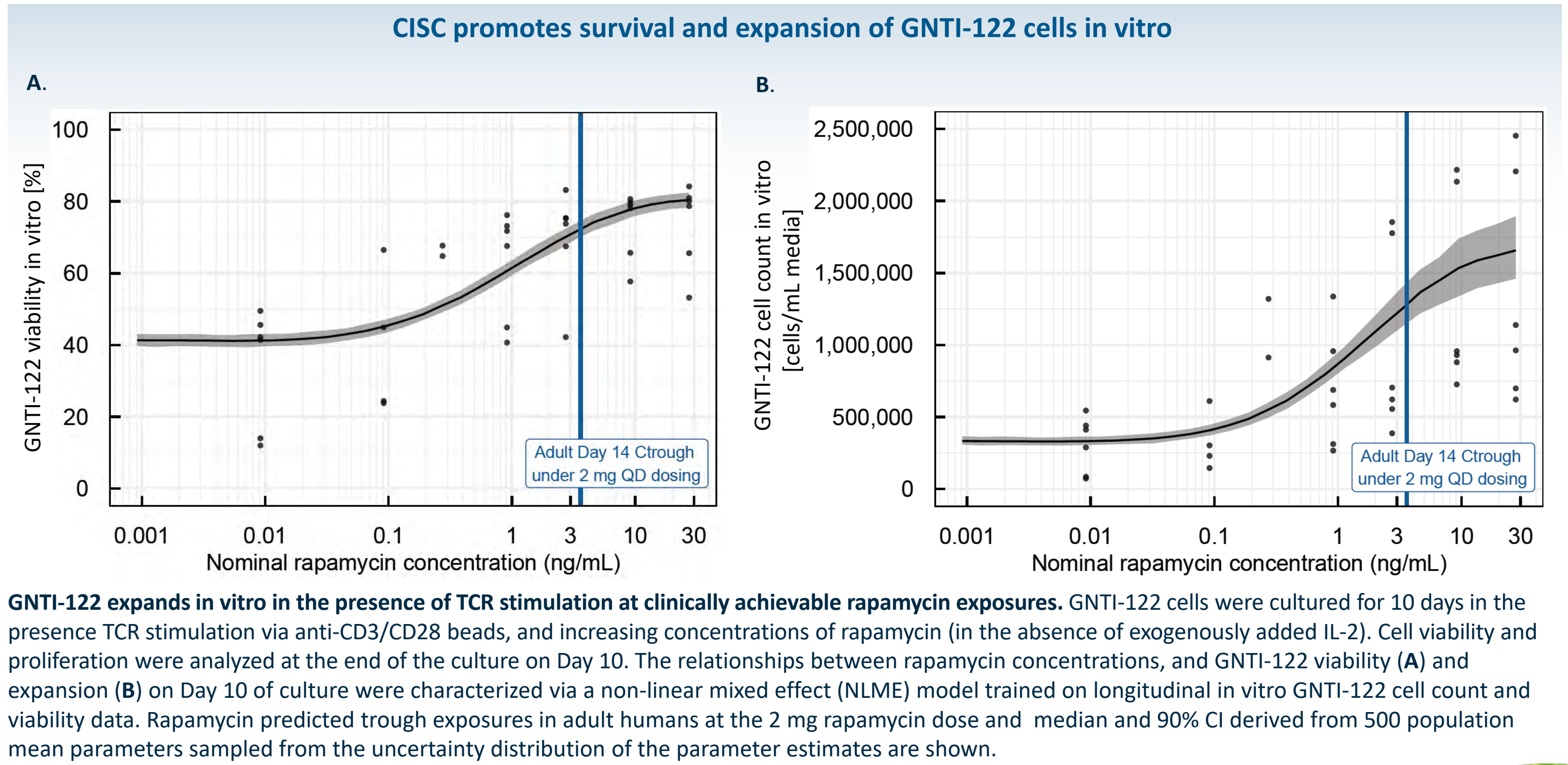
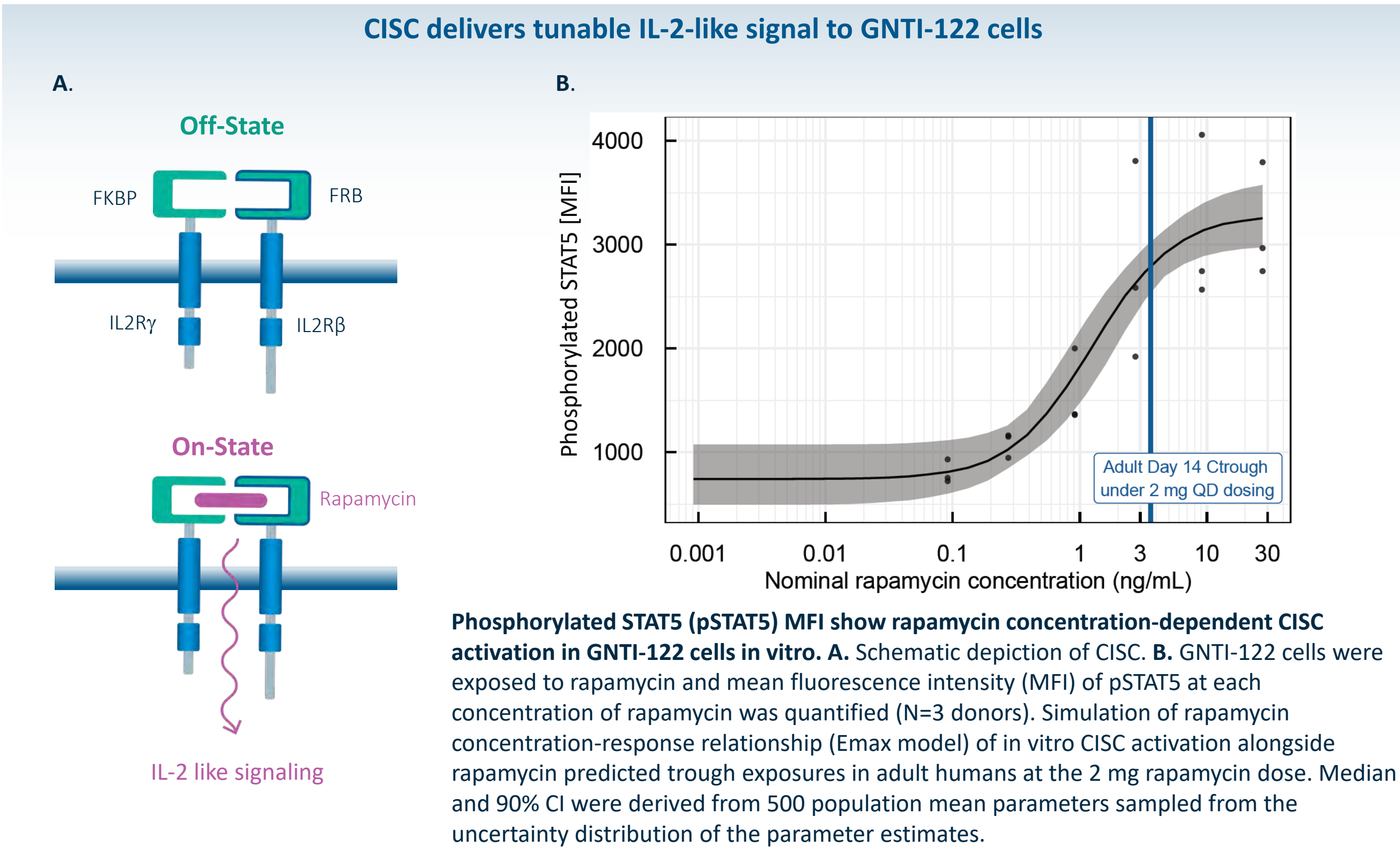
Cells were stimulated with PMA, ionomycin or with anti-CD3/CD28 beads and blocked with monensin before staining for the indicated cytokines. The relative geometric mean fluorescence intensity (gMFI) levels were normalized to Mock cells. Data from 3 donors with T1D. One-sample t-test (* p<0.05; ** p<0.01).

GNTI-122 cells are cocultured with autologous Teffs expanded from patient donors with T1D, and monocyte-derived dendritic cells (DC) as antigen-presenting cells. The Teffs specific to 9 different cognate peptides were isolated and DCs were loaded with their cognate peptides. Suppression was calculated as follows: % suppression = ((a-b)/a)×100, where "a" is the percentage of Teff proliferation in the absence of Tregs and "b" is the percentage of Teff proliferation in the presence of Tregs. Welch t-test (* p<0.05)

RESULTS | Mouse Engineered Tregs Protect Pancreatic Beta Cells in an Adoptive Transfer Model of Type 1 Diabetes



RESULTS | CISC Activation with Rapamycin Emulates IL-2 Signaling and Promotes GNTI-122 Expansion in the Absence of IL-2



CONCLUSIONS

- GNTI-122 engineered from donors with T1D display Treg phenotype, cytokine profile, and polyclonal suppression of islet antigen-specific Teff cells
- Mouse engineered islet-specific Treg cells localize to pancreas to suppress inflammation, preserve pancreatic islets and prevent diabetes
- CISC provides on-demand IL-2-like signaling controllable by subtherapeutic rapamycin exposure levels to promote GNTI-122 viability and expansion in the absence of exogenous IL-2
- GNTI-122 represents a novel therapeutic modality with potential to restore immune tolerance in T1D

ACKNOWLEDGEMENT: The laboratory of Dr. David Rawlings at Seattle Children's Hospital pioneered the gene editing approach to produce engineered Tregs.

We make Tregs. Better.

