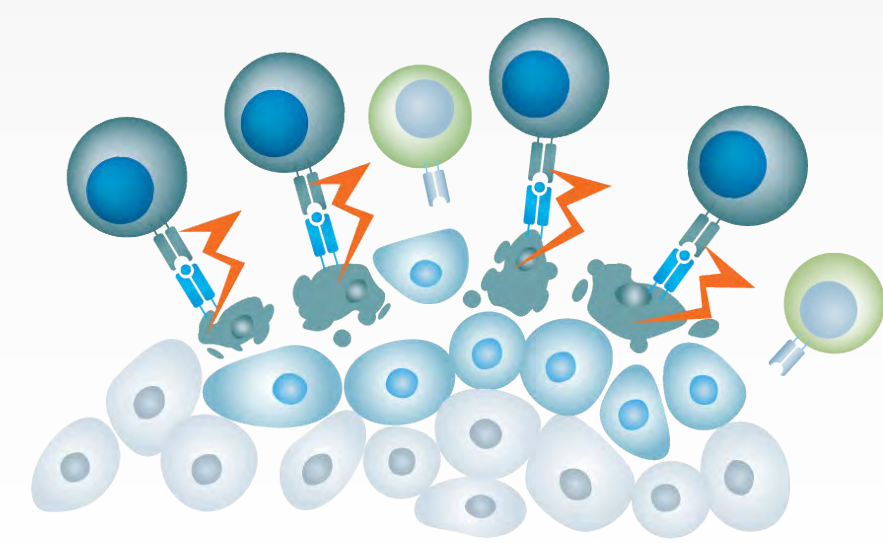


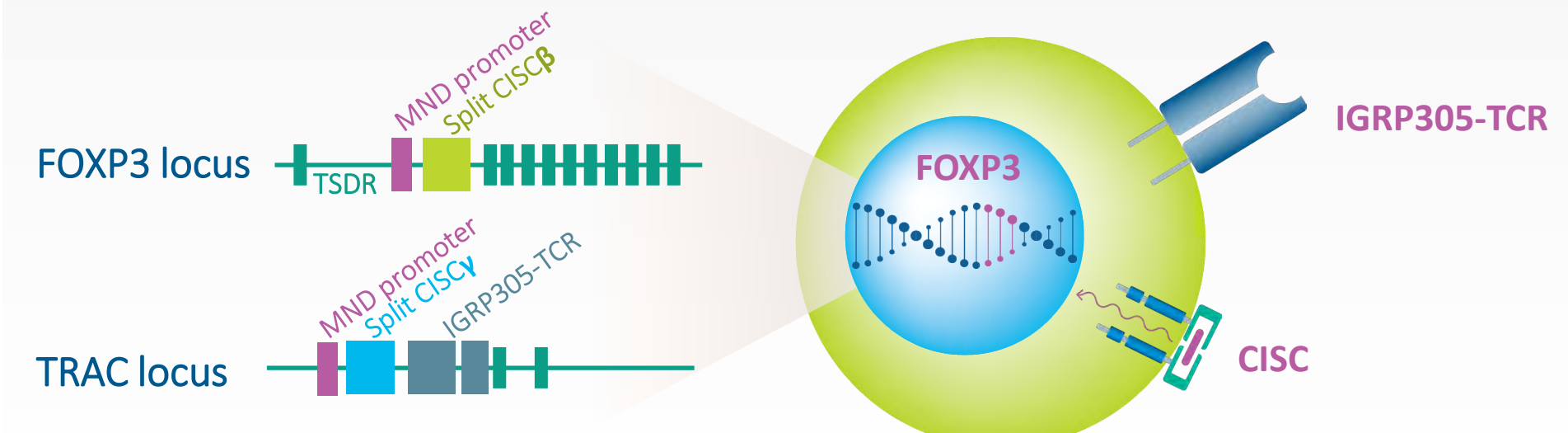
Jennifer Yam<sup>1</sup>, Hunter Kellogg<sup>1</sup>, Priya Saikumar<sup>1</sup>, Tiffany Chan<sup>1</sup>, Emma Mortensen<sup>2</sup>, Martina Hunt<sup>3</sup>, Ashley Landuyt<sup>1</sup>, Marko Repic<sup>1</sup>, Gene Uenishi<sup>1</sup>, Peter Cook<sup>3</sup>, Soo Jung Yang<sup>2</sup>, Tiffany Chen<sup>1</sup>, Jane Buckner<sup>2</sup>, David Rawlings<sup>3</sup>, Tom Wickham<sup>1</sup>, Karen Mueller<sup>1</sup>

<sup>1</sup>GentiBio, Inc., Cambridge, MA, USA, <sup>2</sup>Benaroya Research Institute, Seattle, WA, USA, <sup>3</sup>Seattle Children's Research Institute, Seattle, WA, USA

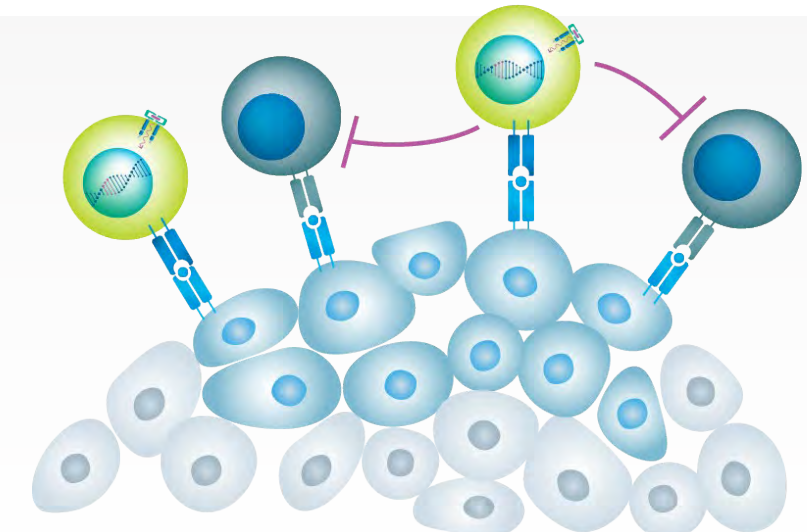
## OVERVIEW



Type 1 diabetes (T1D) is an autoimmune disease caused by autoreactive T lymphocyte-mediated destruction of insulin-producing beta cells that leads to uncontrolled hyperglycemia and life-long dependence on daily insulin administration.

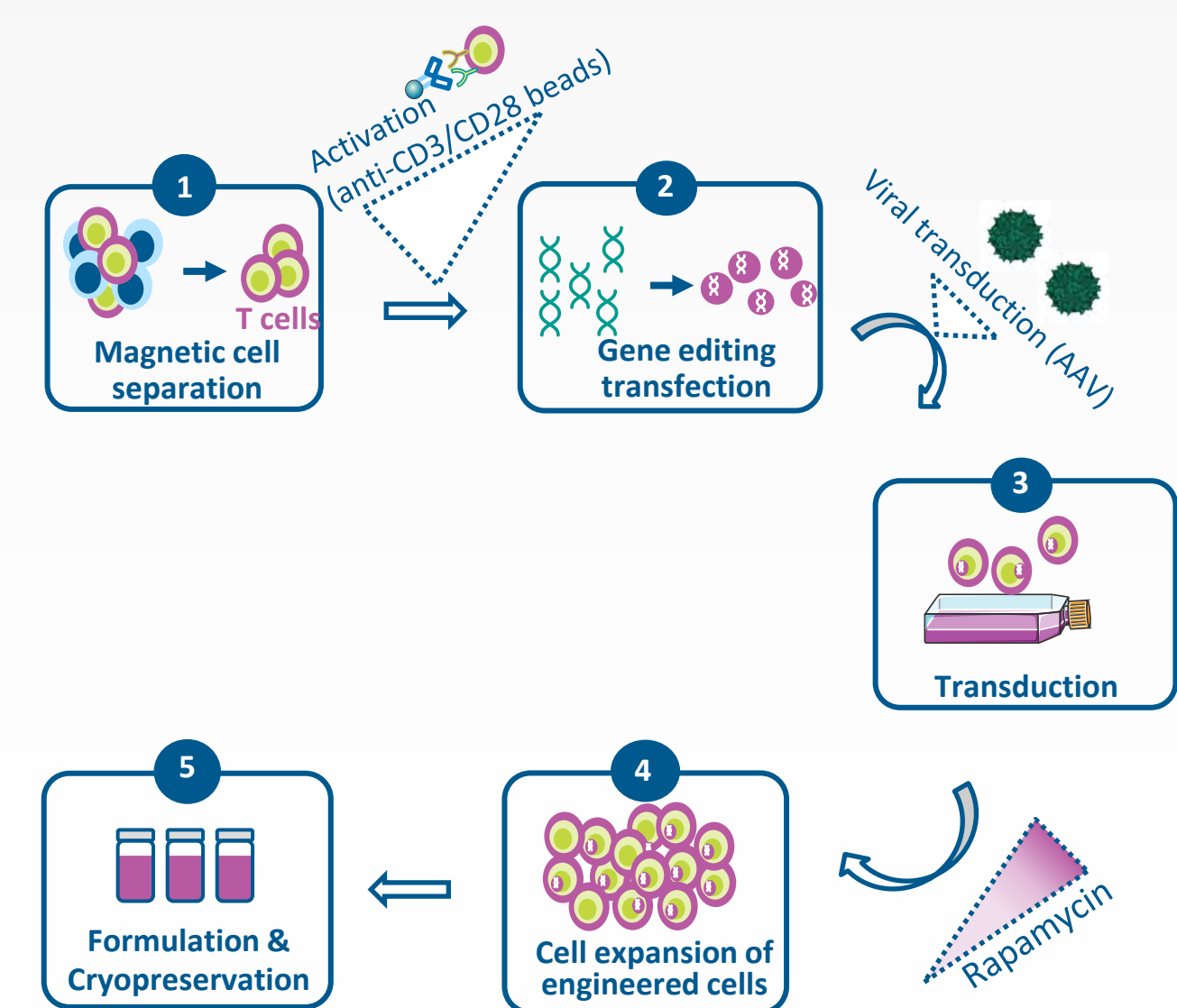


GNTI-122 is engineered from autologous CD4 T cells using HDR-mediated gene editing facilitated by RNA-guided nucleases to knock-in the following: An MND promoter into the FOXP3 gene to stabilize its expression; IGRP305-TCR, a pancreatic islet antigen-specific TCR, into the TRAC locus; and a chemically inducible signaling receptor (CISC).



GNTI-122 is designed to protect islet cells from damage by homing to the pancreas and draining lymph nodes and suppressing pathogenic effector T cells via bystander suppression and infectious tolerance. The addition of CISC enables GNTI-122 to expand in the IL-2 scarce environment of a diabetic pancreas.

## GENERATION OF GNTI-122



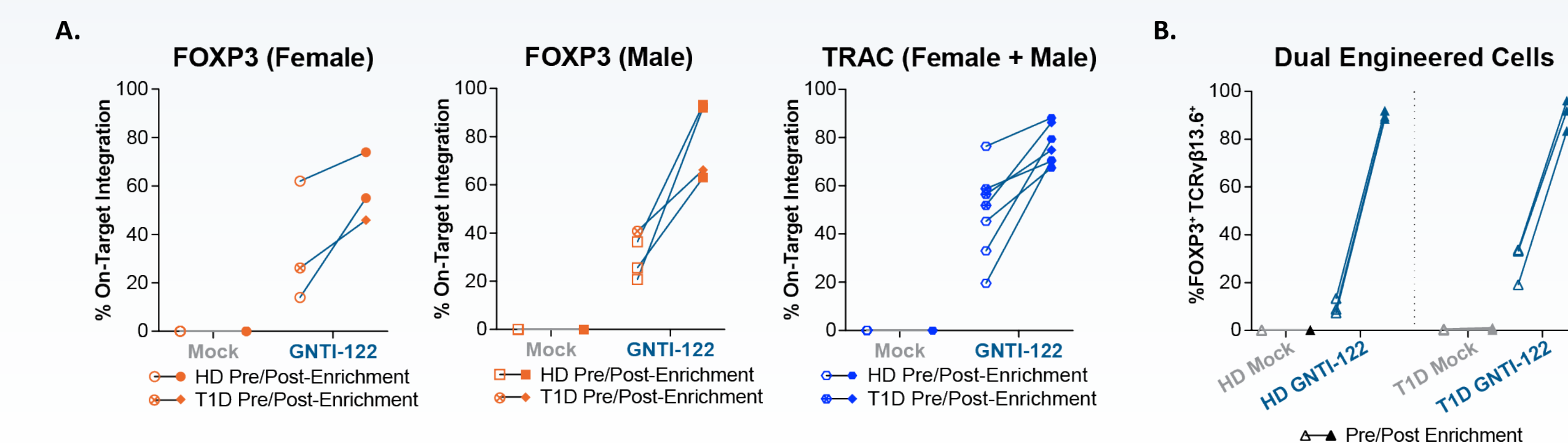
CD4<sup>+</sup> T cells were isolated via magnetic enrichment from PBMCs. The cells were then genetically modified using CRISPR-Cas9 to knock-in transgenes delivered by AAV vectors. CISC receptor enables selective expansion and enrichment of engineered Tregs in the presence of rapamycin. The expanded cells were cryopreserved at >80% purity.

## CONCLUSIONS

- GNTI-122 engineered from CD4 T cells, from healthy donors and T1D patient donors, exhibit stable Treg phenotype and cytokine expression.
- The pancreatic islet antigen-specific TCR of GNTI-122 enables targeted direct Teff suppression in addition to bystander and polyclonal Teff suppression.
- CISC provides an IL-2-like signal and specifically expands GNTI-122 in response to rapamycin in vitro and in vivo
- GNTI-122 overcomes the key limitations of sorted Treg cell therapy supporting further evaluation of GNTI-122 in clinical trials

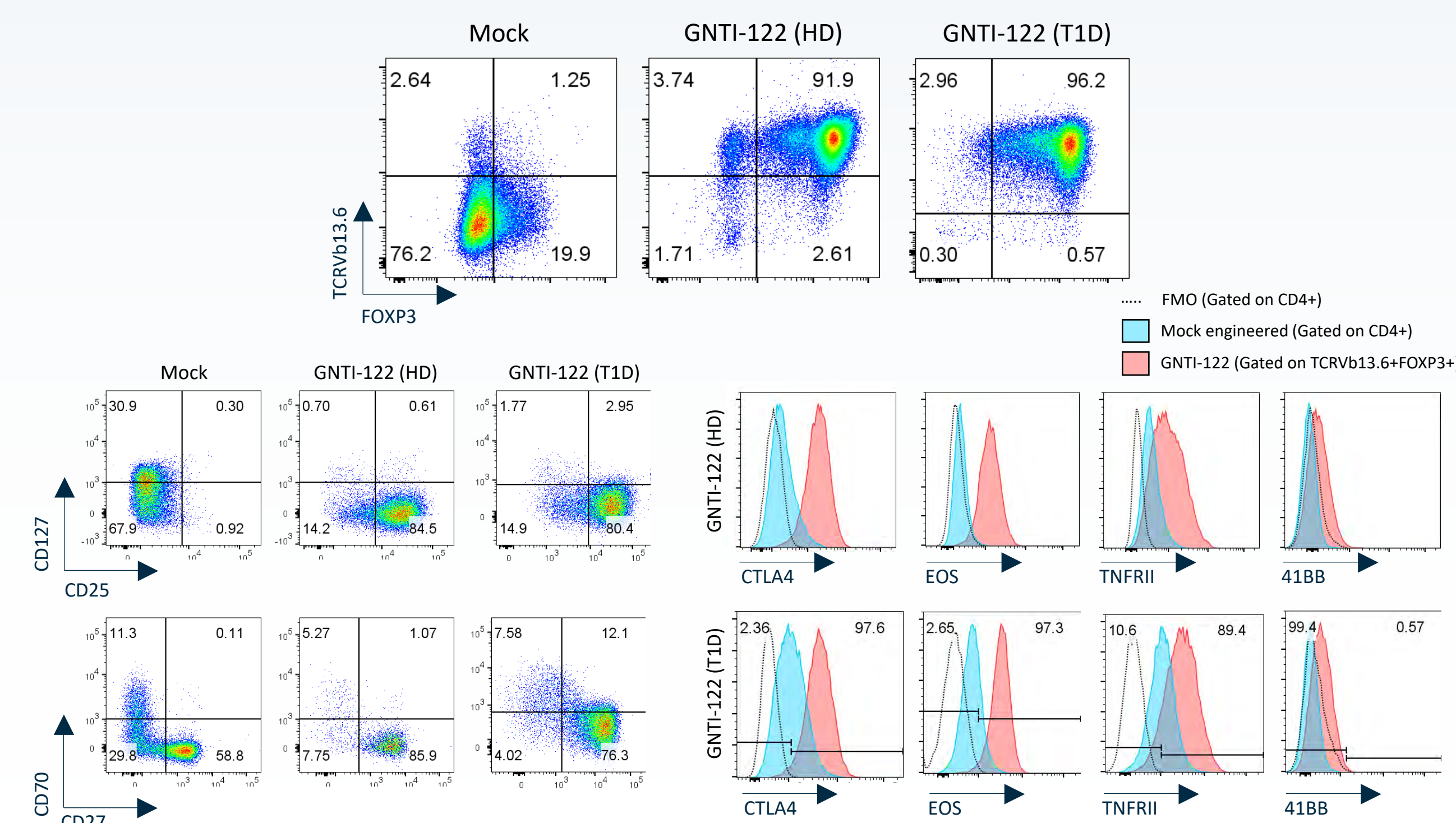
## GNTI-122 ENGINEERING IMPARTS TREG PHENOTYPE AND FUNCTION IN HEALTHY DONOR (HD) & T1D PATIENT CELLS

### Rapamycin-mediated enrichment increases frequency of dual engineered cells



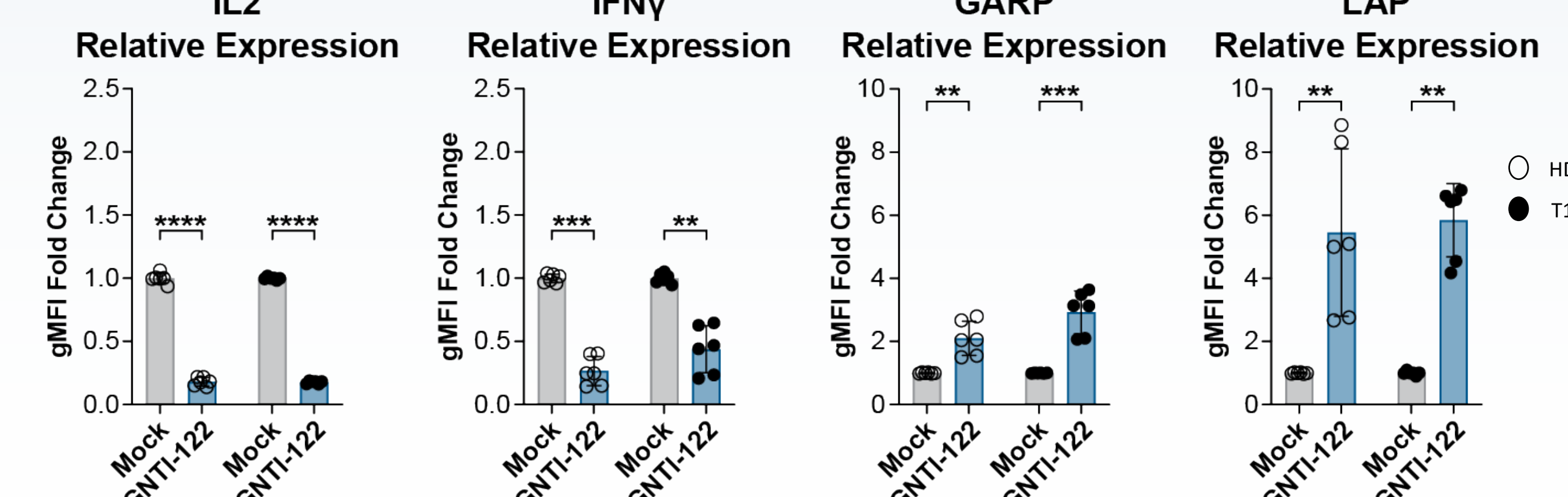
Analysis of rapamycin-mediated enrichment of dual engineered cells. The frequency of cells demonstrating expression of both FOXP3 and IGRP305-TCR significantly increases in the presence of rapamycin due to CISC activation. There are no significant differences in dual engineering frequencies or enrichment efficiency between GNTI-122 generated from healthy donors (HD) or donors with T1D. A. dPCR quantification of the frequency of engineered alleles. B. Frequency of FOXP3 and TCRβ13.6 (the TCRβ V-gene of the IGRP305-TCR) measured at single-cell resolution by flow cytometry, gated on live CD4<sup>+</sup>.

### GNTI-122 cells, from both HD & T1D donors, express Treg associated markers



GNTI-122 cells express Treg markers (FOXP3<sup>+</sup>CD25<sup>+</sup>CD127<sup>+</sup>) & increase functional (CTLA-4, 41BB) and stability (TNFR11, CD27<sup>+</sup>CD70<sup>+</sup>, EOI5) markers. Mock cells are gated on CD4<sup>+</sup> cells, and GNTI-122 cells are gated on TCR<sup>+</sup>FOXP3<sup>+</sup> cells. Reproduced across 3 independent donors of patients with T1D.

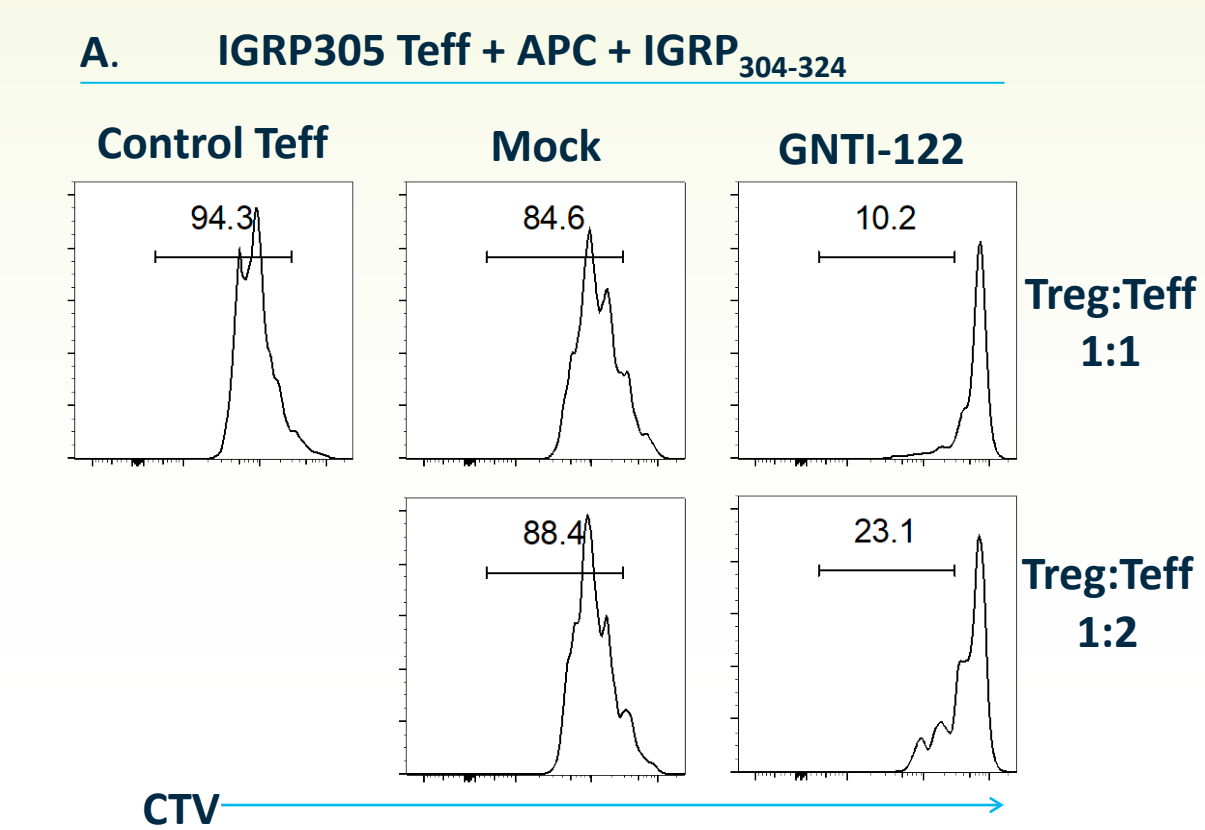
### GNTI-122 cells express TGF-β associated markers and not inflammatory cytokines



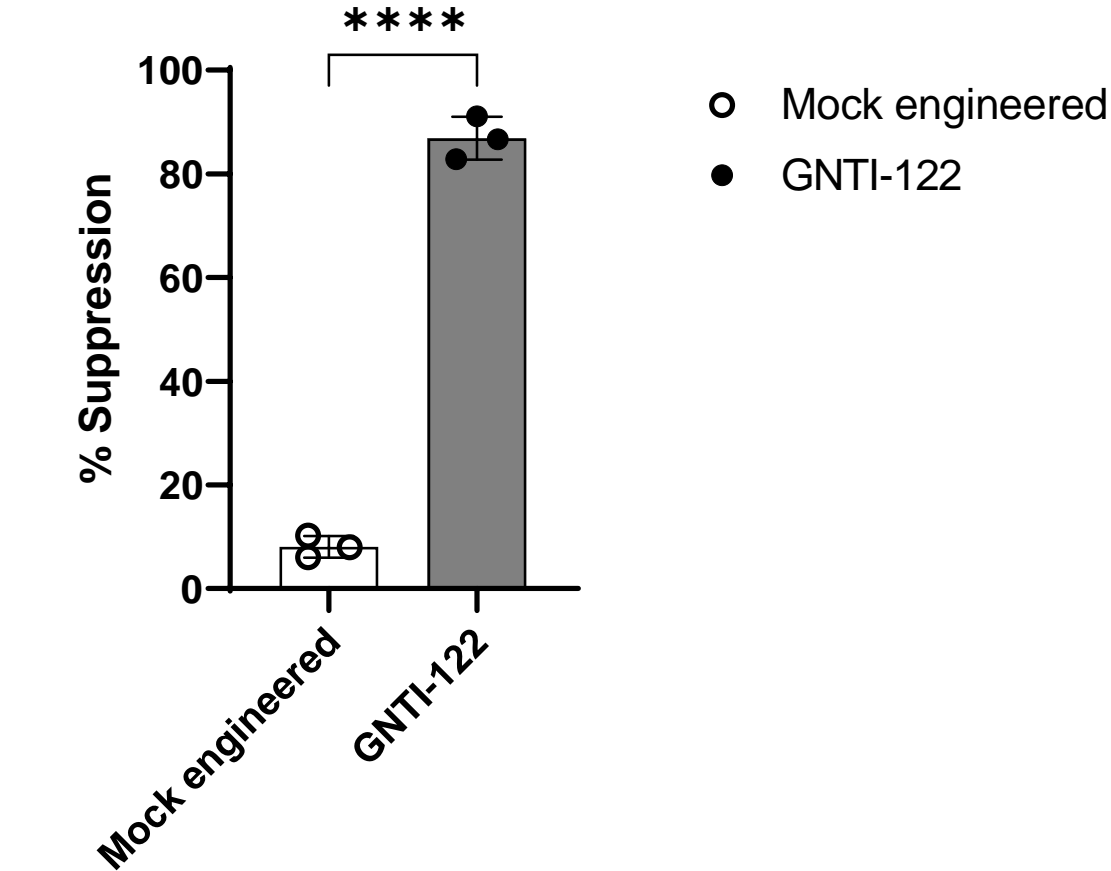
Cells were stimulated with PMA, ionomycin or with anti-CD3/CD28 beads and blocked with monensin before staining for the indicated cytokines. The relative geometric mean fluorescence intensity (gMFI) levels were normalized to mock cells. 2-way ANOVA. Representative donor data shown, reproduced across 6 independent donors.

## GNTI-122 REDUCES PROLIFERATION OF PATHOGENIC TEFFS VIA ANTIGEN-SPECIFIC STIMULATION

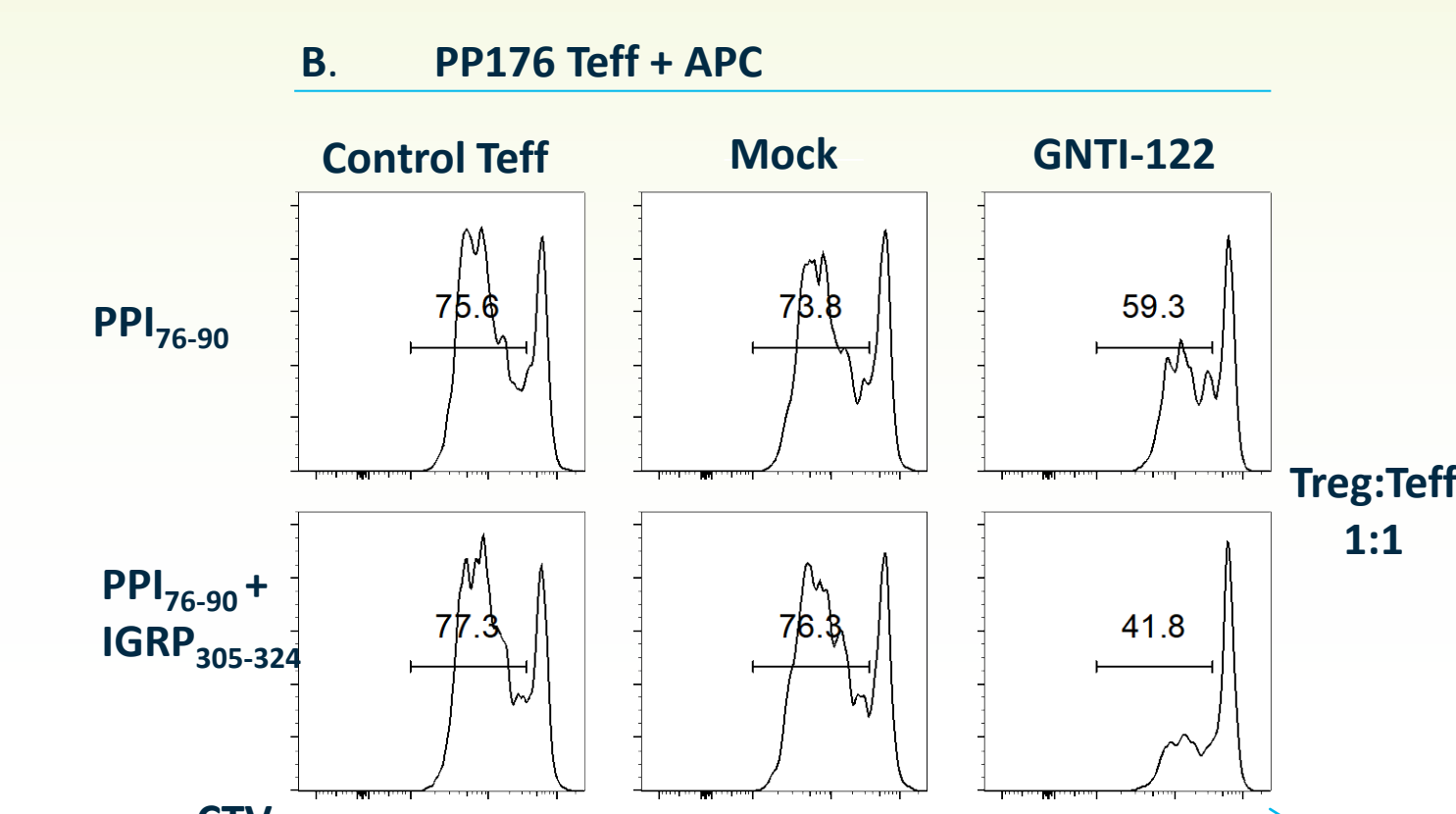
### GNTI-122 cells inhibit the proliferation of Teff cells via direct suppression



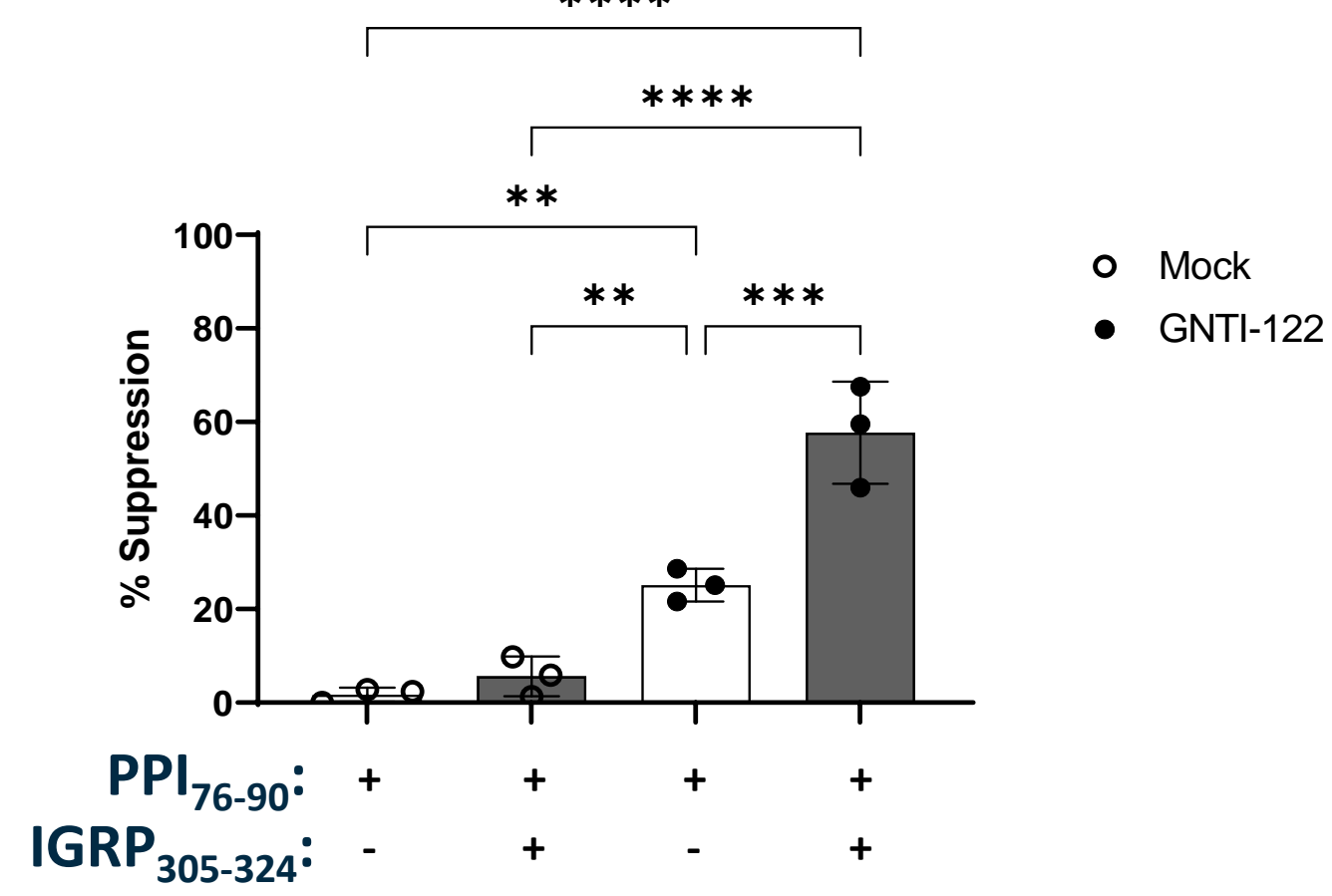
#### Direct Suppression



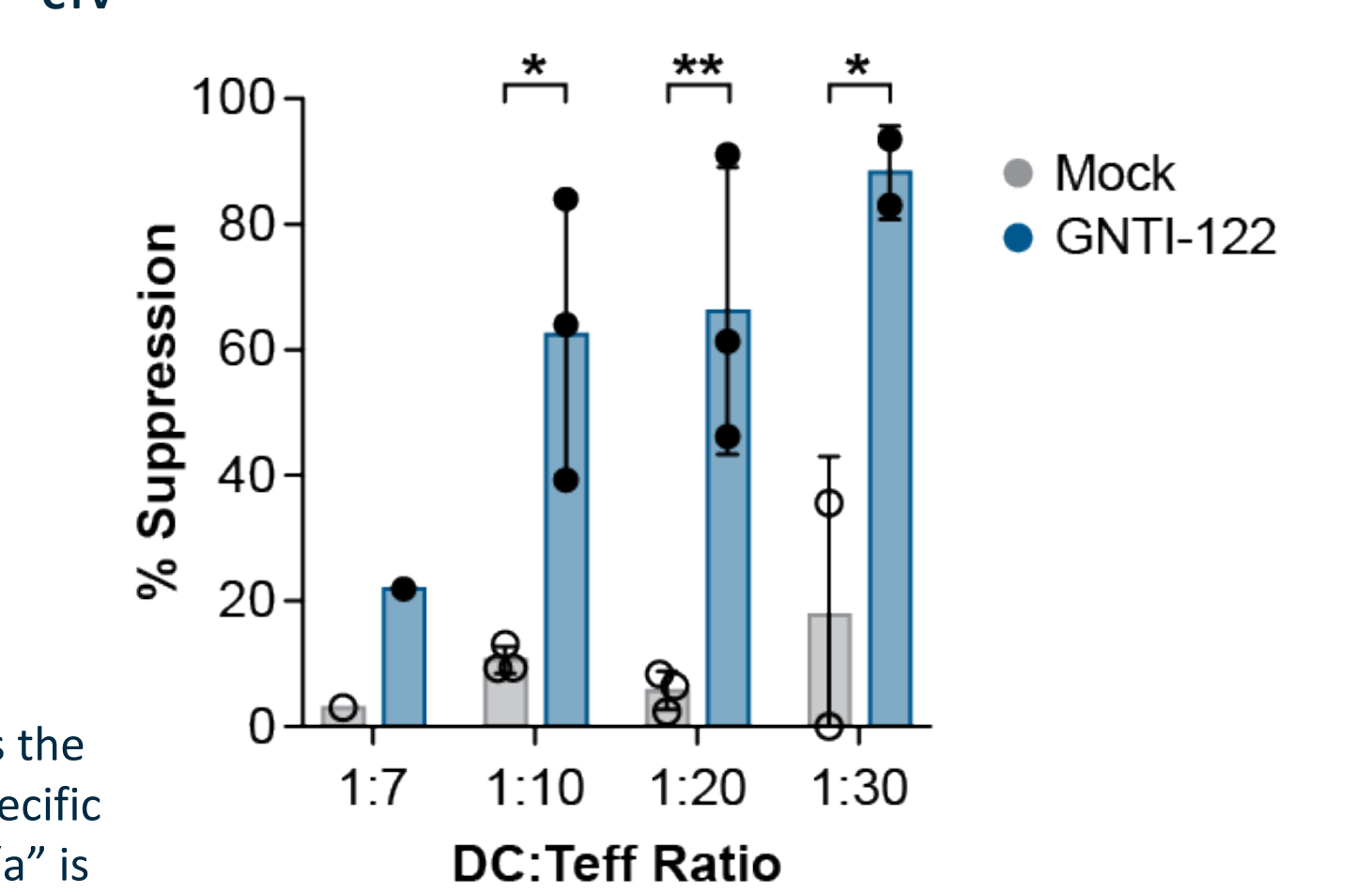
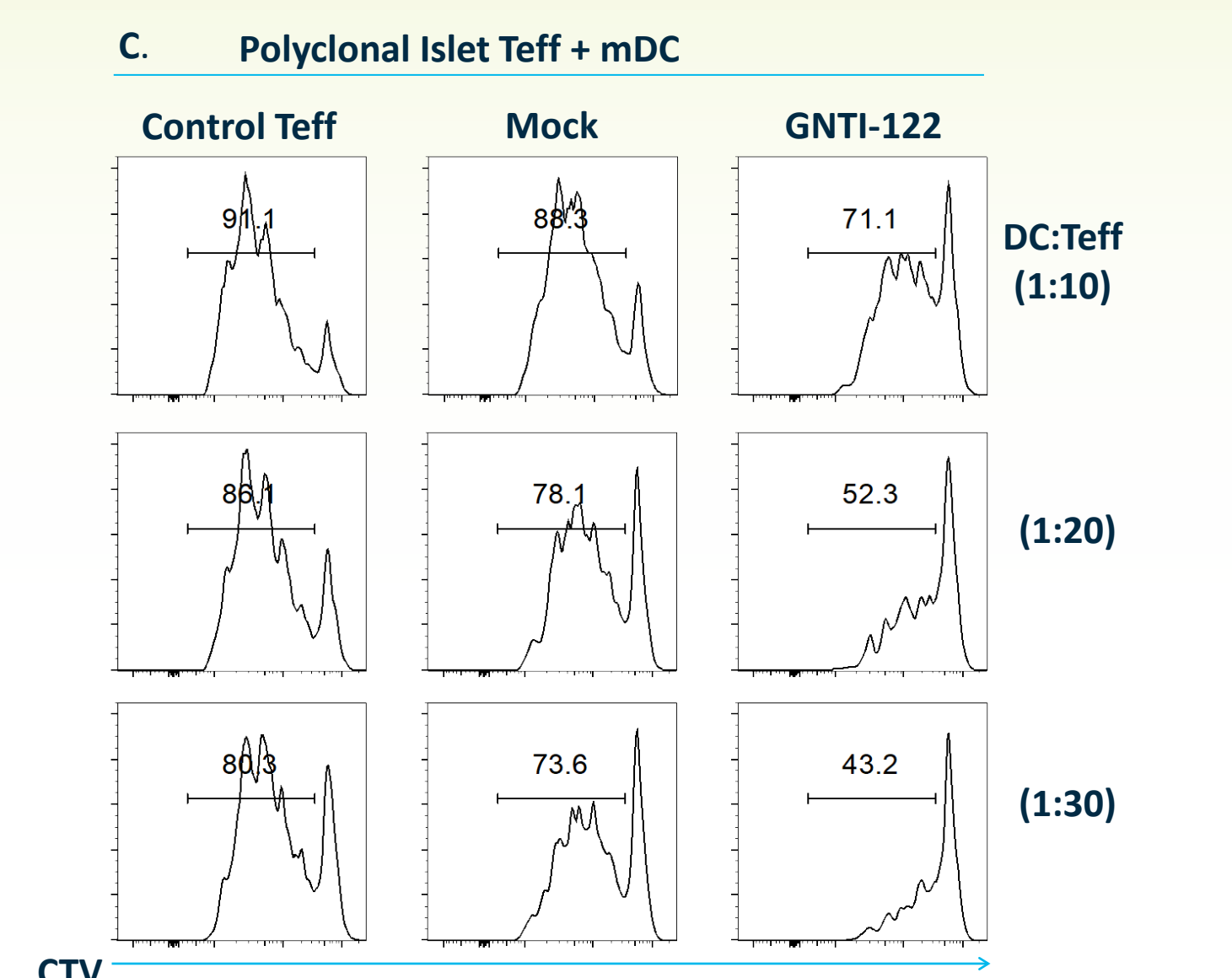
### GNTI-122 cells inhibit the proliferation of Teff via bystander suppression



#### Bystander Suppression



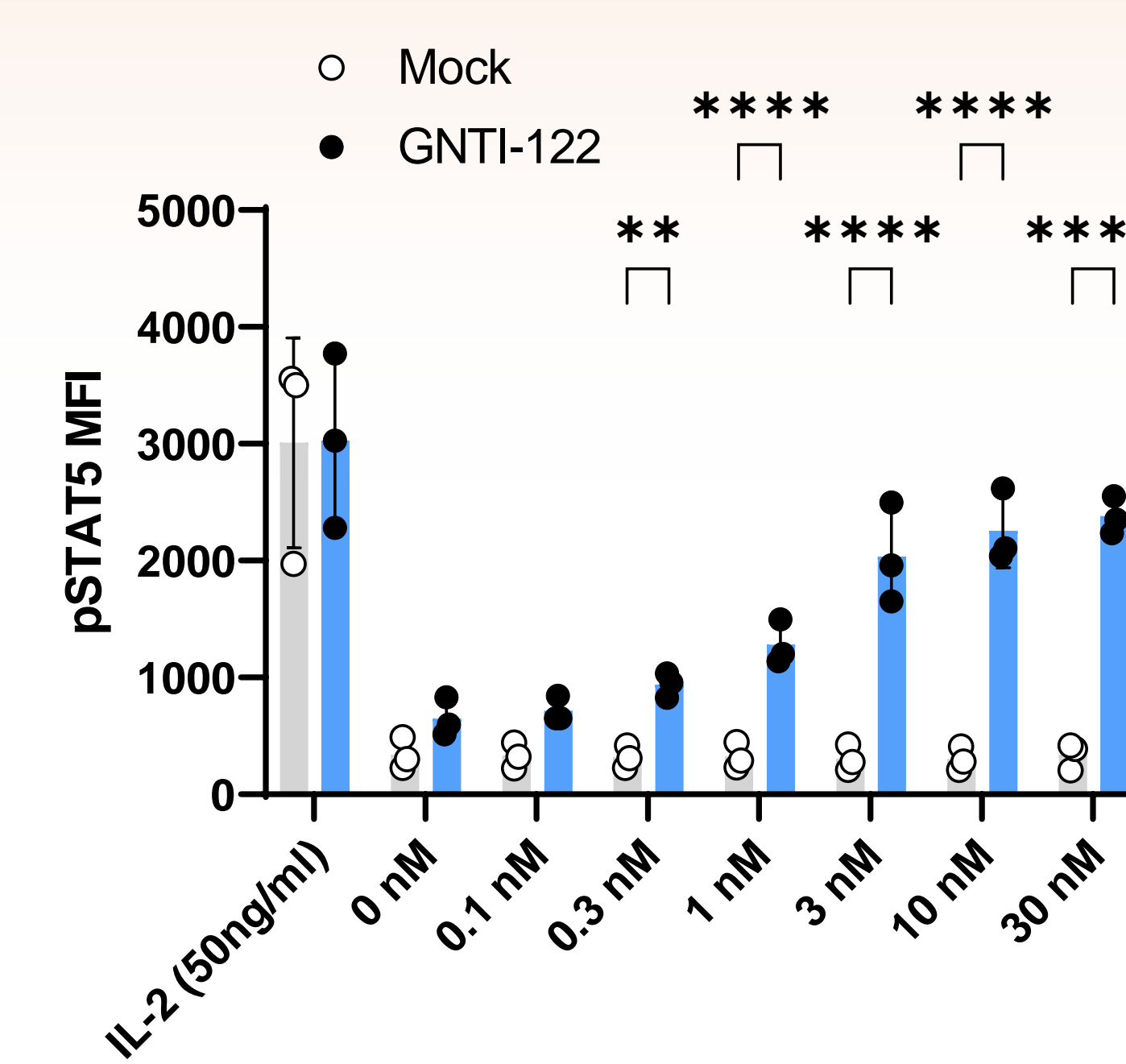
### GNTI-122 cells inhibit the proliferation of polyclonal islet antigen-specific Teffs



GNTI-122 cells are cocultured with autologous Teffs from patient donors with T1D, and monocyte-derived dendritic cells as antigen-presenting cells (APCs). A. The Teffs express the same TCR, and APCs were loaded with their cognate peptide. B. The Teffs express a different TCR, and the APCs are loaded with corresponding cognate peptide. C. The Teffs specific to 9 different cognate peptides were isolated and APCs were loaded with their cognate peptides. Suppression was calculated as follows: % suppression = ((a-b)/a)x100, 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.

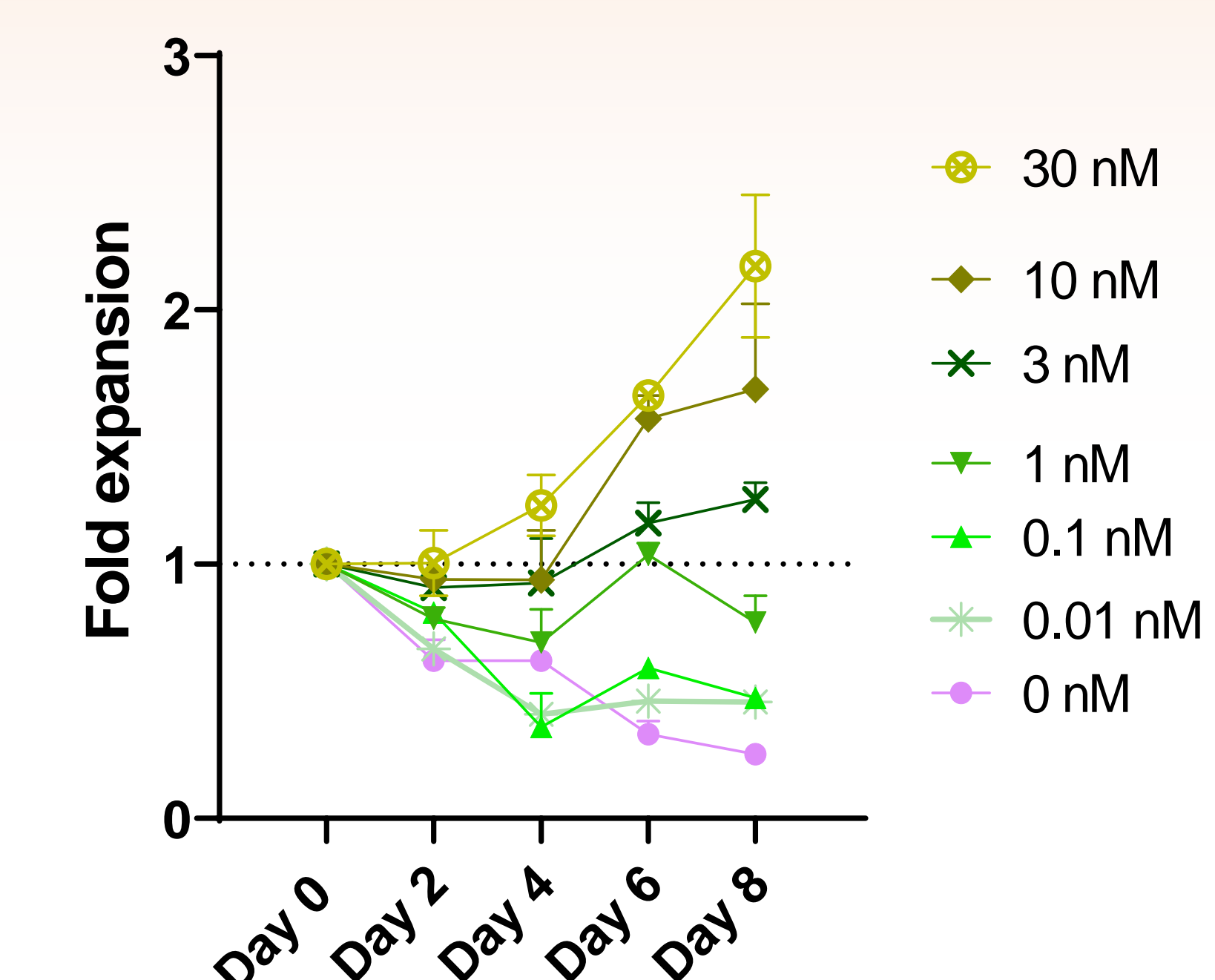
## RAPAMYCIN MEDIATES IL-2 LIKE SIGNAL THROUGH CISC ACTIVATION IN VITRO AND IN VIVO

### Rapamycin promotes cell proliferation by mediating STAT5 phosphorylation



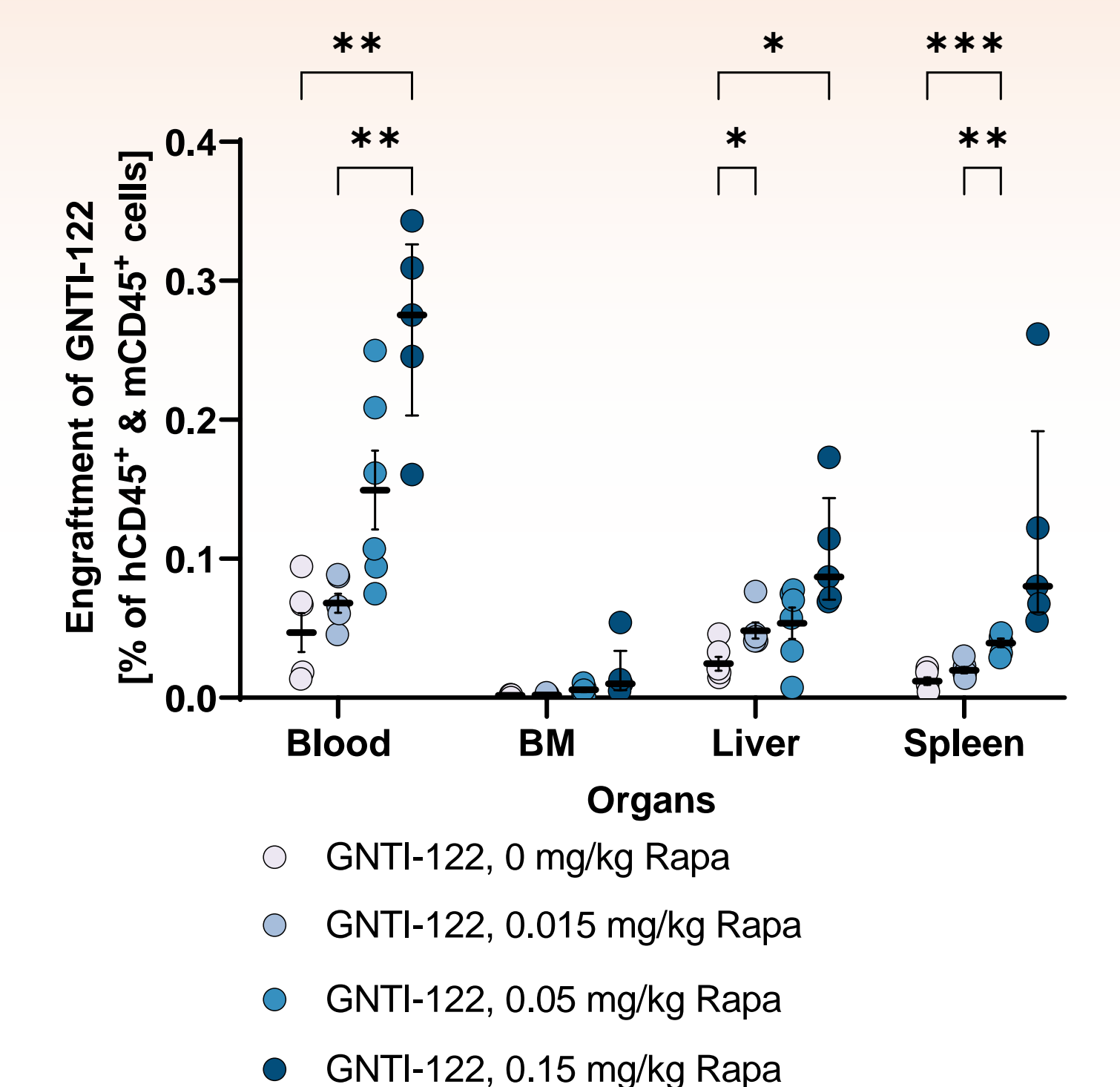
Phosphorylated STAT5 (pSTAT5) MFI show dose-specific response with rapamycin in GNTI-122 cells in culture. Quantification of mean fluorescence intensity (MFI) of pSTAT5 at each concentration of rapamycin. Repeated measures ANOVA with Sidak's multiple comparison tests at each dose (\*\*p<0.01, \*\*\*\*p<0.0001). The errors bars represent mean +/- SEM, N=3 donors.

### GNTI-122 expands in a rapamycin concentration-dependent manner



CISC supports GNTI-122 expansion in a rapamycin dose-dependant manner. Fold expansion of representative donor across 7 concentrations of rapamycin over 8 days. All samples are TCR stimulated. Data represents the mean +/- SEM of duplicates.

### Rapamycin improves engraftment of GNTI-122 in vivo



GNTI-122 engrafts in response to rapamycin dose in vivo. GNTI-122 cells were injected into irradiated NSG mice at a dose of 5x10<sup>6</sup> cells/mouse, and were administered with rapamycin every other day intraperitoneally for 17 days post cell injection. On day 19, mice were sacrificed and engraftment levels were checked in each specified organ. 2-way ANOVA with Tukey's multiple comparison test, significance displayed (\*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001)

