

Using Pharmacologically Programmed CD34⁺ Cells for the Treatment of Type 1 Diabetes

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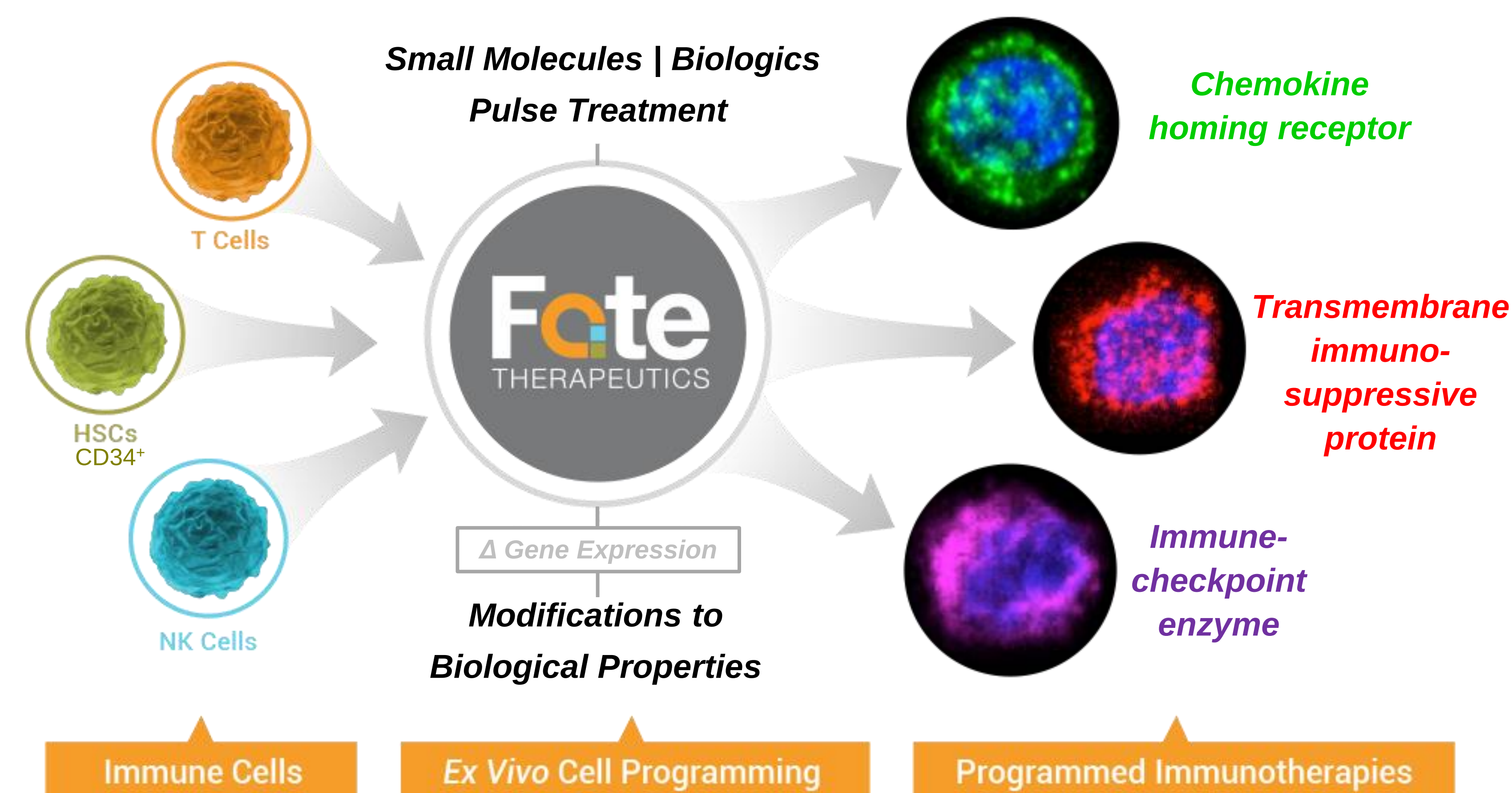


ABSTRACT

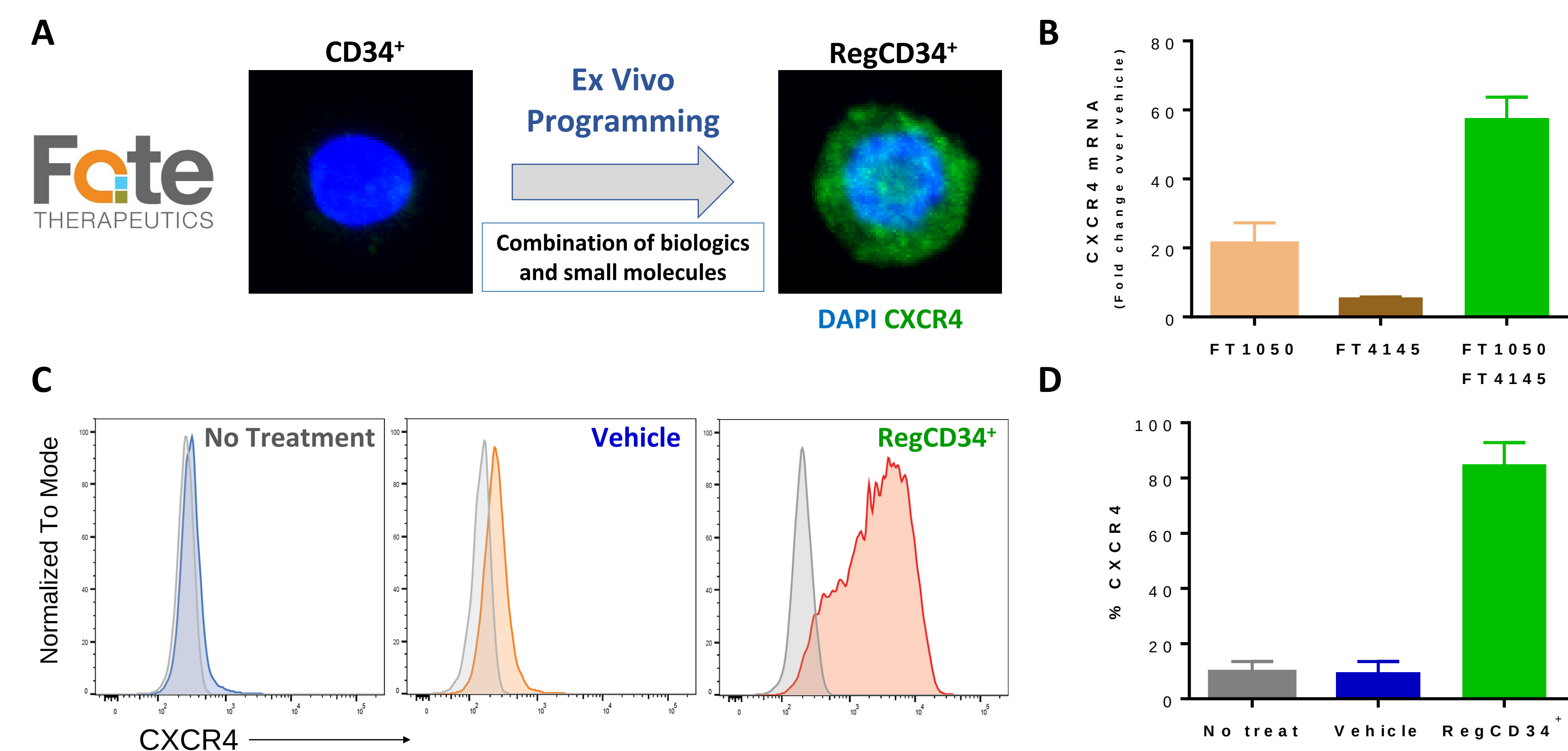
CD34⁺ cells are known to traffic to sites of inflammation via the CXCR4/SDF-1 axis. Additionally, CD34⁺ cells have been shown to possess T-cell suppressive activity mediated in part by the PD-L1 pathway. These properties suggest CD34⁺ cells might serve as potent immuno-modulatory entities for the treatment of autoimmune and inflammatory diseases, such as Type 1 diabetes (T1D). We have previously demonstrated that modulating, or programming, CD34⁺ cells with 16,16-dimethyl prostaglandin E2 (FT1050) *ex vivo* significantly increases the gene expression of CXCR4 (~20-fold) and results in improved homing and engraftment *in vivo*. In an effort to further enhance the homing and immunoregulatory properties of CD34⁺ cells, a small molecule screen was performed and we identified a combination of small molecules, dmPGE2 and dexamethasone (FT4145), that synergizes to upregulate CXCR4 mRNA by ~60-fold and PD-L1 by ~4-fold. *Ex vivo* modulation of CD34⁺ cells with this combination enhances the capacity of CD34⁺ cells to traffic, and to suppress T-cell proliferation and effector function, *in vitro* in a CXCR4-mediated migration assay. In murine and humanized T1D models, FT1050-FT4145 programmed CD34⁺ cells exhibit enhanced trafficking to the pancreas, as compared to vehicle-treated cells, and a single administration of programmed cells results in a reversion of hyperglycemia in NOD mice. We also show that programmed cells can prevent hyperglycemia when administered prior to disease onset in NOD mice. In summary, these studies provide compelling evidence that FT1050-FT4145 programmed CD34⁺ cells have enhanced trafficking and immuno-modulatory properties, and can attenuate disease in models of T1D. These results underscore the potential for *ex vivo* programmed CD34⁺ cells to be developed as a cellular immunotherapy for the treatment of autoimmunity and inflammatory diseases.

RESULTS

Programming cell function: Enhancing the therapeutic function of immunotherapies



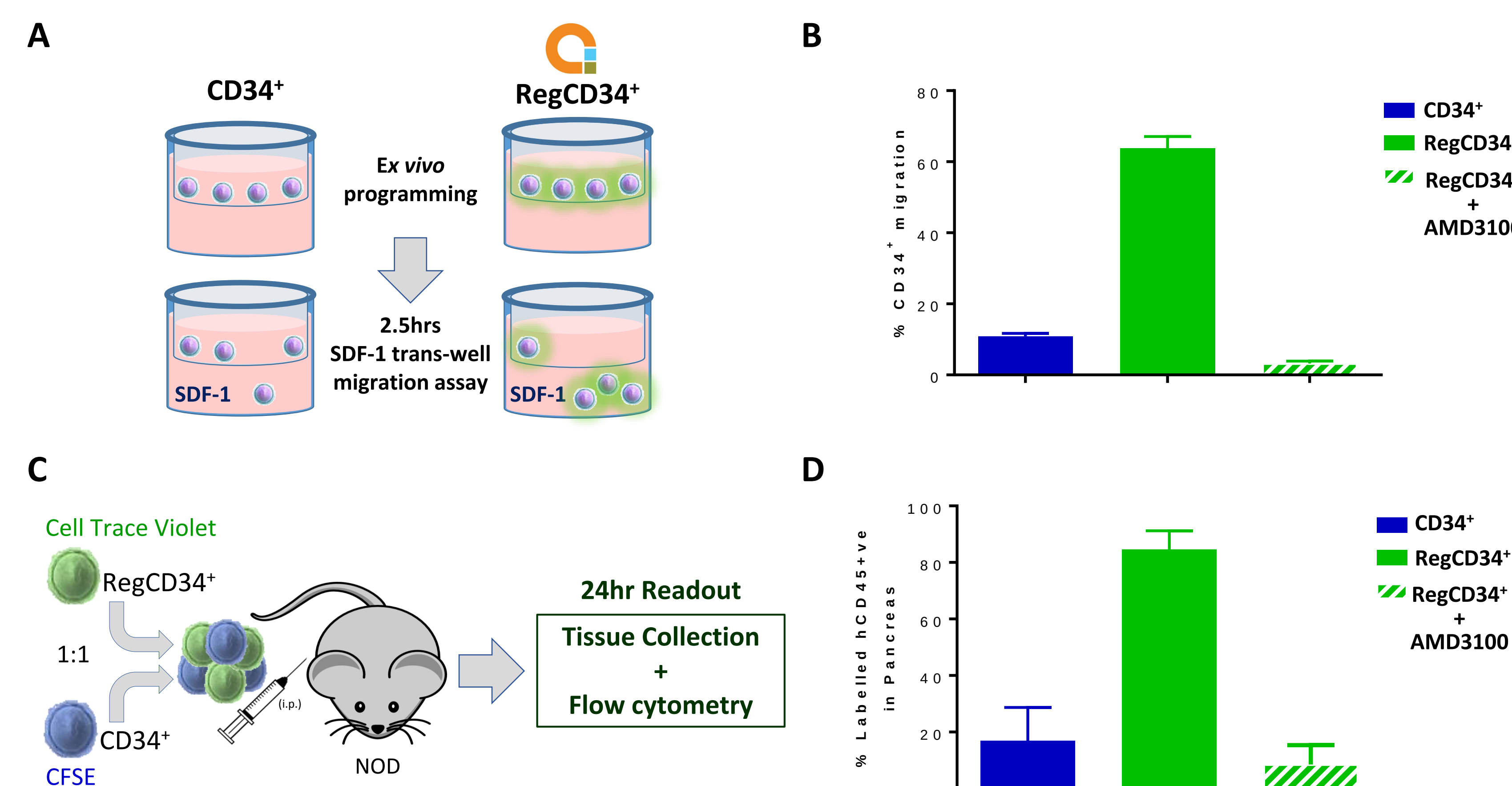
Enhancing the function of CD34⁺ cells using *ex vivo* programming



Ex vivo programming increases levels of the SDF-1 chemokine receptor CXCR4 **A**, CD34⁺ cells from mobilized peripheral blood are programmed *ex vivo* with a proprietary combination of biologics and small molecules identified using high throughput gene expression screening of chemical space for enhancers of genes with therapeutic potential. Programming increases the surface expression of CXCR4 as shown by the representative confocal images of CD34⁺ and programmed CD34⁺ cells (RegCD34⁺) stained with anti-CXCR4. **B**, Synergistic effect of FT1050 and FT4145 programming on increasing the expression of CXCR4 in CD34⁺ cells. FT1050/FT4145 combination increases the expression of PD-L1 by 5.5 ± SD 3.25 fold over vehicle. **C**, Representative histograms of untreated, vehicle treated CD34⁺ and RegCD34⁺ cells analyzed by flow cytometry for surface expression of CXCR4. **D**, Quantification of data in C averaged across 3 separate donors of mobilized peripheral blood CD34⁺ cells.

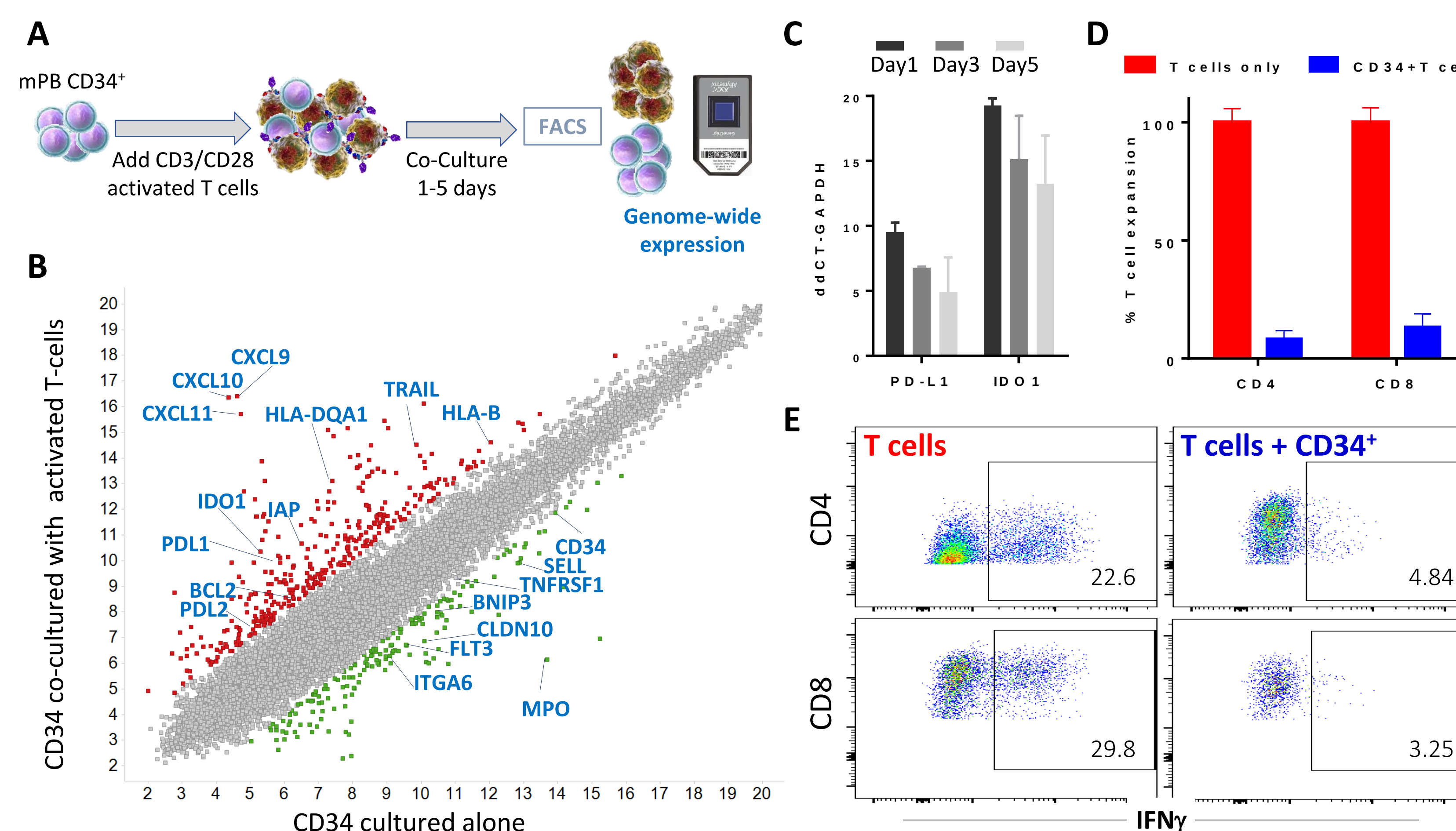
RESULTS

Programming enhances the trafficking properties of CD34⁺ cells



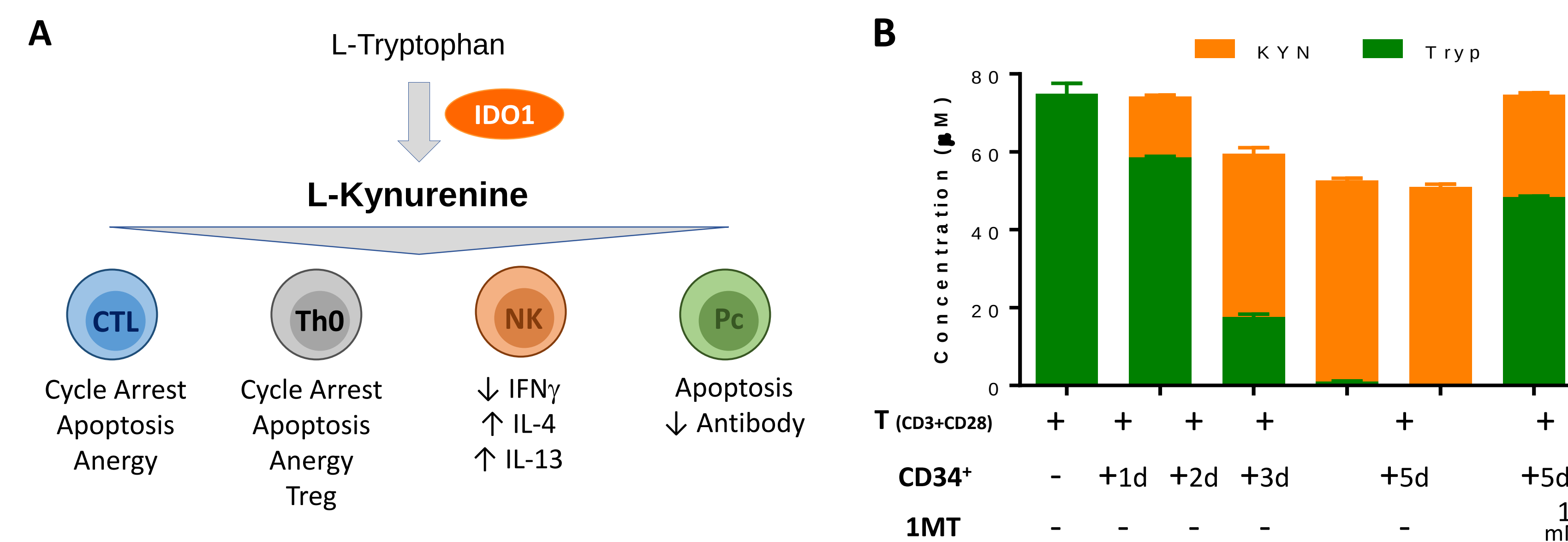
Programming of CD34⁺ cells enhances SDF-1 chemotaxis *in vitro* and homing to the pancreas in a mouse model of Type 1 Diabetes. **A**, Schematic of SDF-1 trans-well migration assay. **B**, SDF-1 chemotaxis of RegCD34⁺ cells in the trans-well assay described in A, is significantly enhanced compared to vehicle treated cells and is reversed by a CXCR4 antagonist (AMD3100). **C**, Schematic of competitive *in vivo* homing assay in which RegCD34⁺ and CD34⁺ are labelled with Cell Trace Violet and CFSE respectively and administered together, via i.p. injection, into the same NOD T1D animal. **D**, Homing of RegCD34⁺ to the pancreas is significantly increased compared to that of vehicle treated CD34⁺ cells and is dependent on CXCR4 mediated trafficking.

CD34⁺ cells have potent immuno-regulatory functions

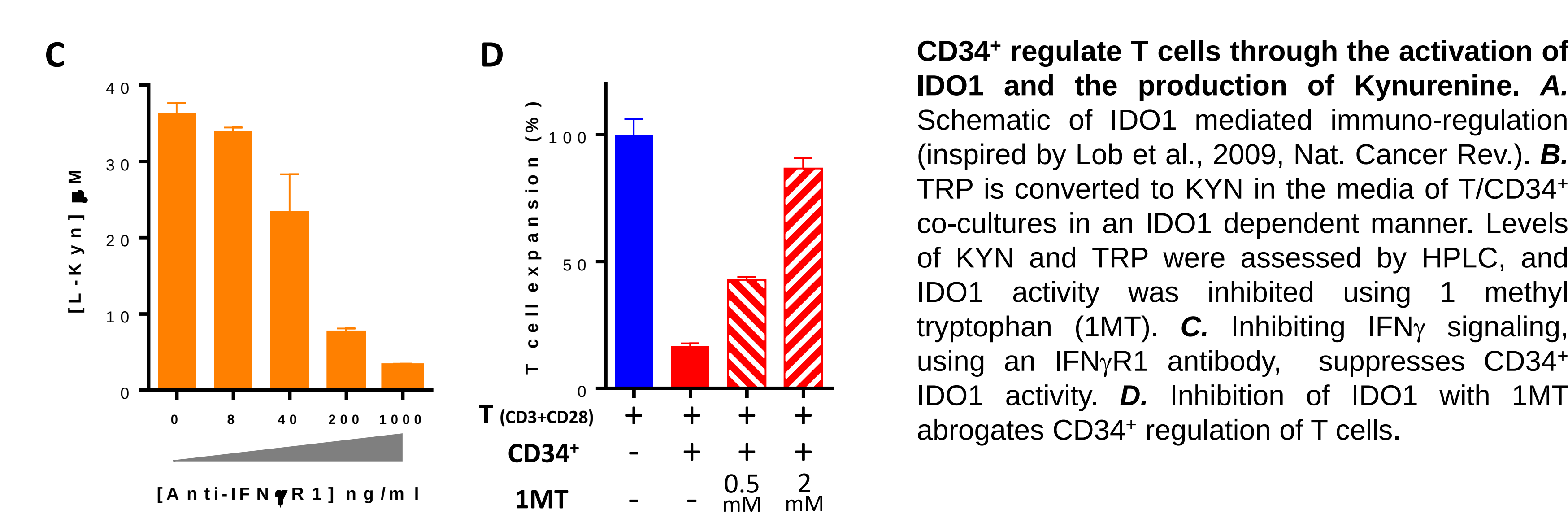


CD34⁺ cells are potent immuno-regulators of T cell proliferation and effector function. **A**, Schematic of *in vitro* T cell suppression assay. **B**, Gene expression profiling of CD34⁺ cells co-cultured with activated T cells for 24hrs compared to CD34⁺ cells cultured alone shows a significant up regulation of key immune checkpoint genes. **C**, The expression of IDO1 and PD-L1 is increased in CD34⁺ cells within 24hrs of co-culture with activated T cells. **D**, CD34⁺ cells suppress the expansion of both CD4⁺ and CD8⁺ T cells at day 5 in the *in vitro* T cell suppression assay described in A. **E**, CD34⁺ cells suppress pro-inflammatory cytokine production in both CD4⁺ and CD8⁺ T cells.

CD34⁺ regulate T cells through IFN γ dependent activation of IDO1

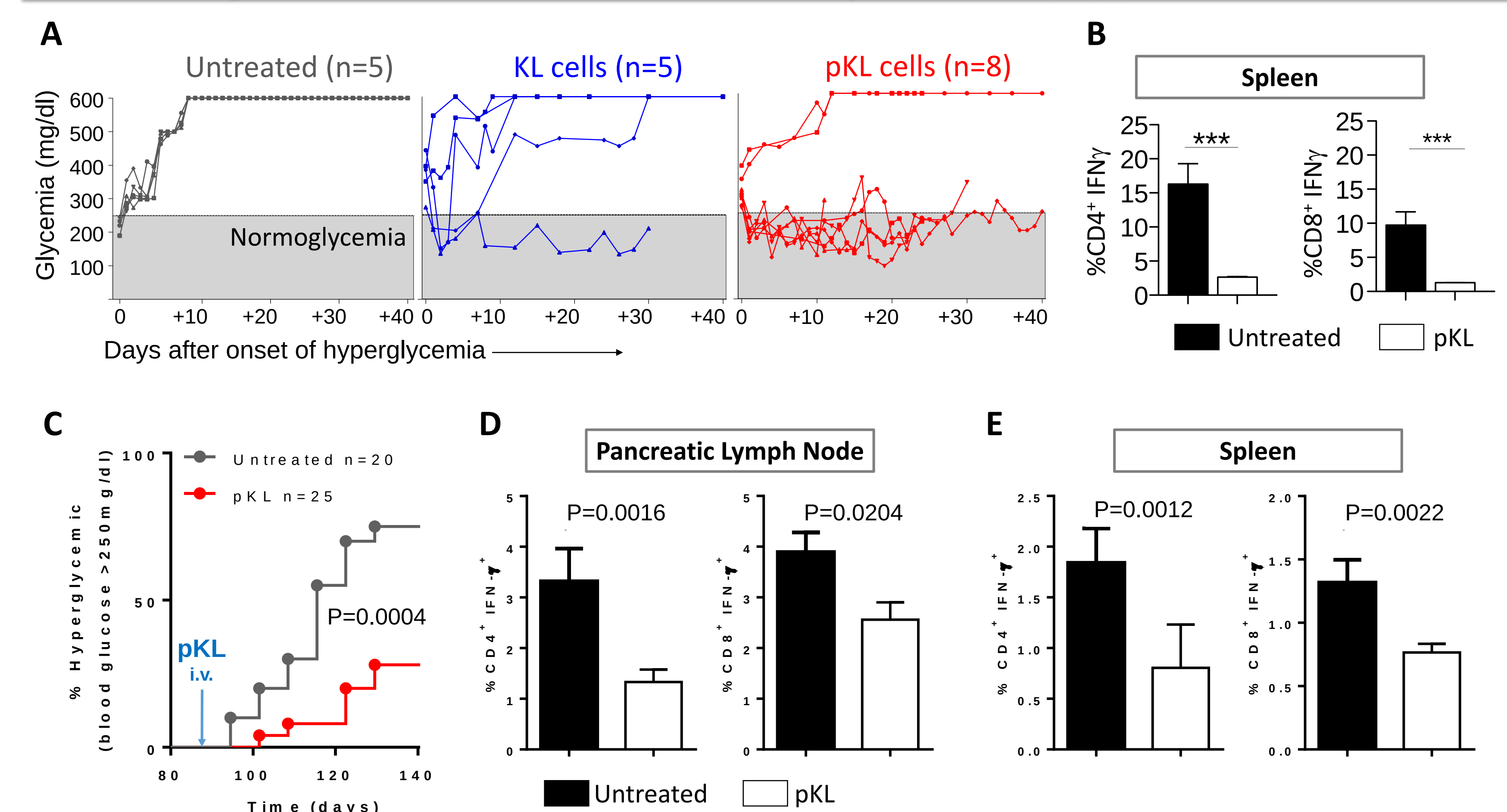


CD34⁺ regulate T cells through IFN γ dependent activation of IDO1 (continued)



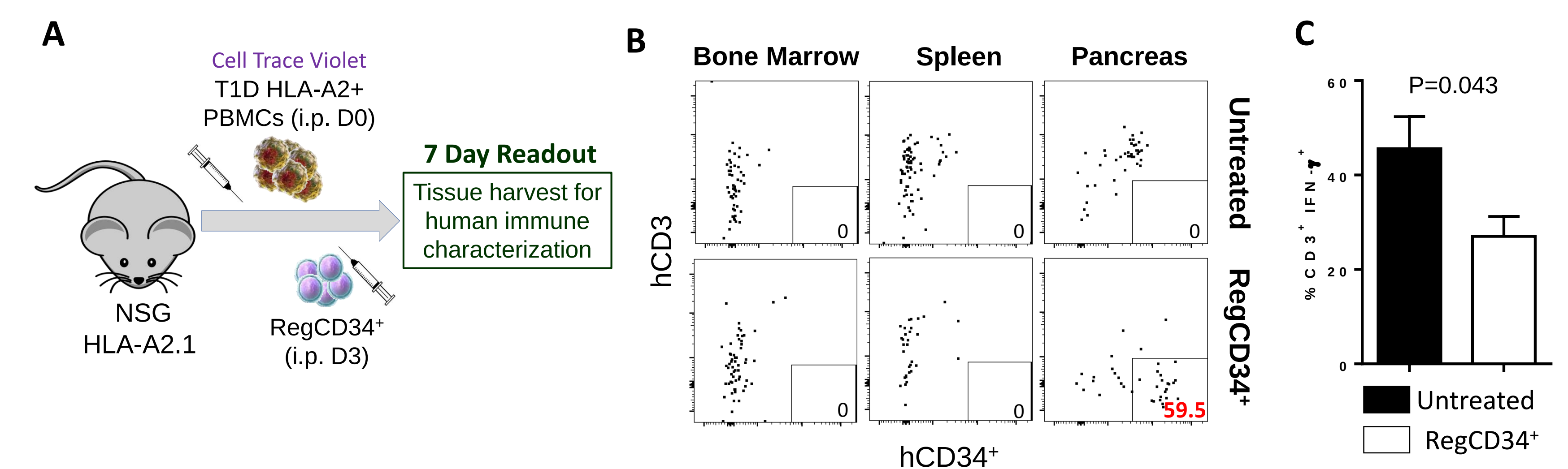
CD34⁺ regulate T cells through the activation of IDO1 and the production of Kynurenine. **A**, Schematic of IDO1 mediated immuno-regulation (inspired by Lob et al., 2009, Nat. Cancer Rev.). **B**, TRP is converted to KYN in the media of T/CD34⁺ co-cultures in an IDO1 dependent manner. Levels of KYN and TRP were assessed by HPLC, and IDO1 activity was inhibited using 1 methyl tryptophan (1MT). **C**, Inhibiting IFN γ signaling, using an IFN γ R1 antibody, suppresses CD34⁺ IDO1 activity. **D**, Inhibition of IDO1 with 1MT abrogates CD34⁺ regulation of T cells.

Programmed mouse HSPCs revert/delay disease course in NOD mice



Murine HSPC (Lineage: c-Kit⁺; aka KL) cells revert/delay hyperglycemia in NOD mice. **A**, Programmed KL cells (pKLs) reverse hyperglycemia in NOD mice following a single injection of 1 million cells upon onset of diabetes. **B**, Pro-inflammatory cytokine production by T cells is diminished in animals injected with pKLs (14 days post onset of diabetes and KL cell injection). **C**, Administration of pKLs (1e6 cells) into NOD mice at 12wk of age delays hyperglycemia onset. **D**, and **E**, Pro-inflammatory cytokine production by T cells from the draining lymph node (D) and the spleen (E) is reduced in animals injected with pKLs 8 weeks post administration.

RegCD34⁺ cells regulate pancreatic T cells in a humanized model of T1D



RegCD34⁺ cells home to the pancreas and suppress T cells in a humanized model of T1D. **A**, Schematic of humanized T1D *in vivo* model. **B**, RegCD34⁺ cells were detected in the pancreas of mice following injection with T1D PBMCs and programmed CD34⁺ cells and not in the spleen or bone marrow. Numbers of hCD3⁺ cells detected in the pancreas of RegCD34⁺ injected mice were lower than in the pancreas from untreated mice. **C**, Pro-inflammatory cytokine production by T cells from the pancreas is reduced in animals injected with RegCD34⁺ cells.

CONCLUSIONS

RegCD34⁺ have enhanced trafficking and immuno-modulatory properties, and can attenuate disease in models of Type 1 Diabetes. These results underscore the promise for *ex vivo* programmed CD34⁺ cells as a new therapeutic approach with disease-modifying potential for patients with Type 1 Diabetes.