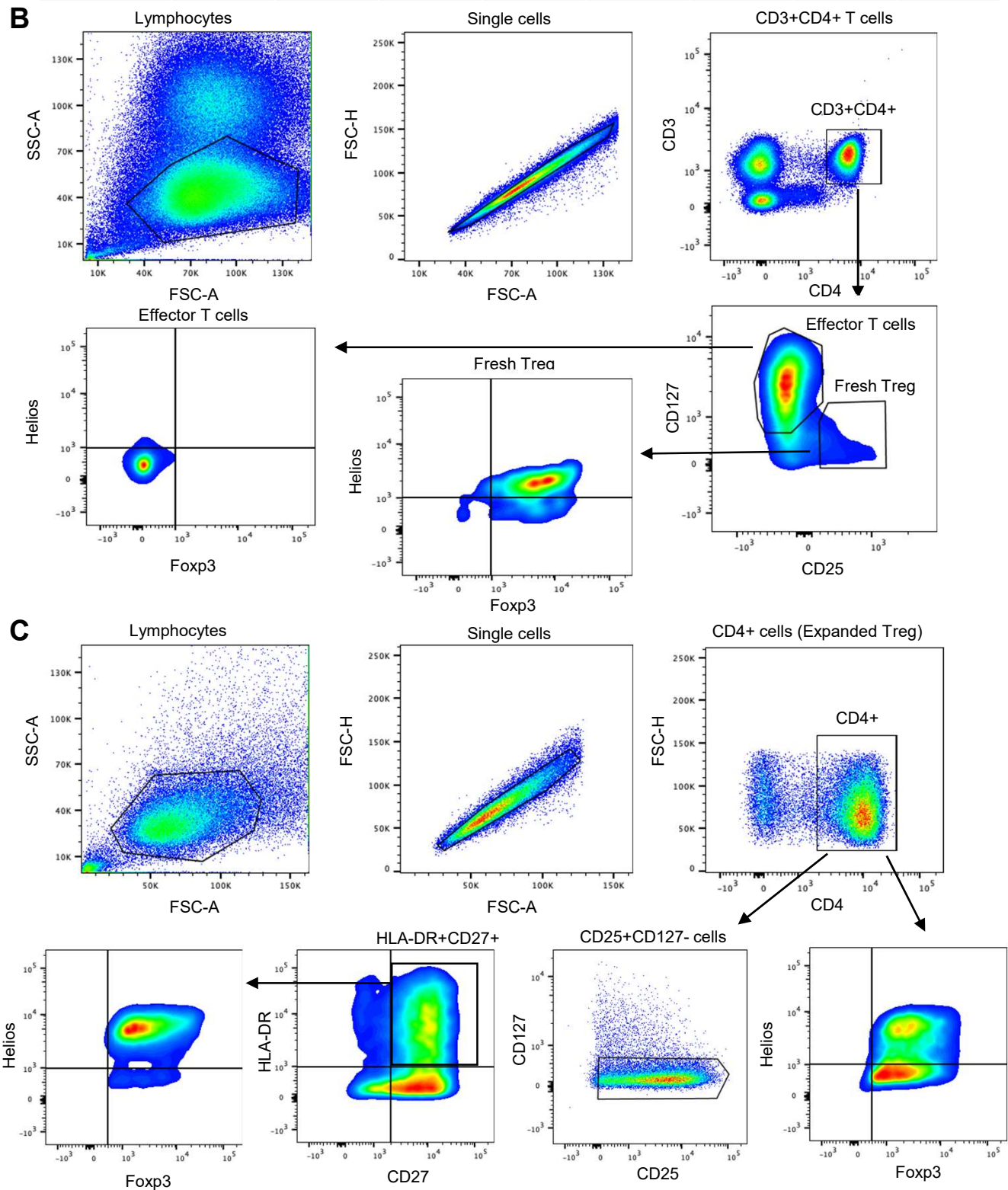
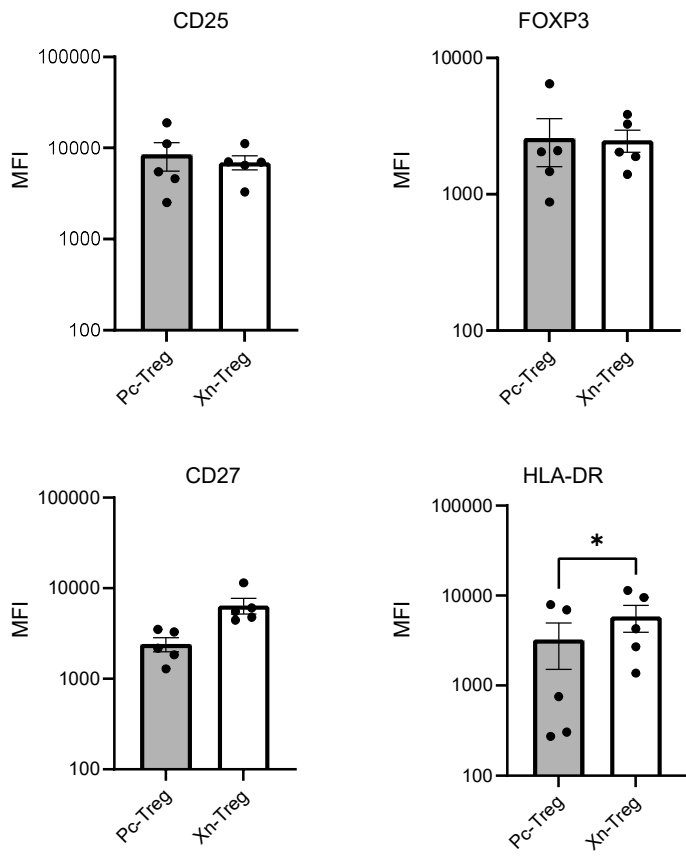


| Marker | CD4 | CD25 | FOXP3 | CD127 | HLA-DR | CD3 | CD27 | Helios |
|--------------------|---------|---------|----------|---------|---------|---------|---------|--------------|
| Fuorochrome | APC-H7 | APC | PE-CF594 | PE | FITC | PerCP | BV711 | Pacific Blue |
| Laser (nm) | 637 | 637 | 561 | 561 | 488 | 488 | 406 | 406 |
| Power (mw) | 140 | 140 | 150 | 150 | 200/150 | 200/150 | 200 | 200 |
| Detector | R820_60 | R670_30 | Y610_20 | Y586_15 | B515_30 | B670_30 | V710_50 | V474_25 |

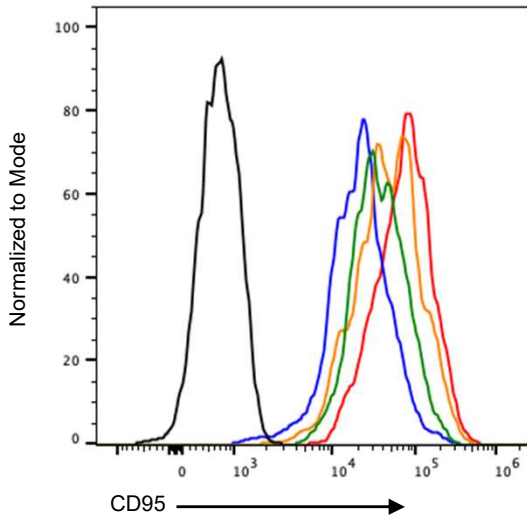


Supplemental Figure 1. Gating strategies for FOXP3 and Helios co-expression. (A) Multicolor flow cytometry panel performed on BD FACSymphony Cell Analyzer for phenotypic analysis of co-expression of FOXP3 and Helios on different types of Treg. (B) Gating strategies for FOXP3 and Helios co-expression on effector T cells and Fresh-Tregs and (C) polyclonal stimulated Tregs (Pc-Treg), xenantigen expanded Treg (Xn-Treg) and HLA-DR+CD27+ DP-Enriched Xn-Treg. Series of gates used to distinguish effector T cells and Fresh-Tregs from human PBMC for identifying the proportion of FOXP3+Helios+ cells. These are the gates for lymphocyte proportion, excluding doublets and CD4+CD3+ T cells, then CD25-CD127+/low and CD25+CD127-/low on CD25 vs. CD127 flow cytometric plots (B) (data shown in Figure 1B). After three stimulation rounds, ex-vivo expanded Xn-Tregs and Pc-Tregs were gated on CD4+ and the proportion of FOXP3+Helios+ cells on Pc-Treg and Xn-Treg was assessed (C) (data shown in Figure 1B). A further gate on HLA-DR vs. CD27 flow cytometric plots allowed the identification of FOXP3+Helios+ cells for HLA-DR+CD27+ DP-Enriched Xn-Treg (data shown in Figure 2C).

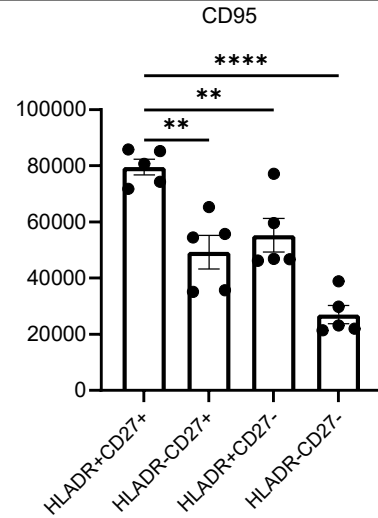


Supplemental Figure 2. Phenotypical characterization of Xn-Treg and Pc-Treg. Expression level (MFI) of CD25, FOXP3, CD27 and HLA-DR in polyclonal stimulated Tregs (Pc-Treg) and xenoantigen expanded Treg (Xn-Treg) after 3 rounds of stimulation. Data represented as mean \pm SEM of three independent experiments with Treg from five individual donors. P value (Paired t test) (2-tailed): *P < 0.05.

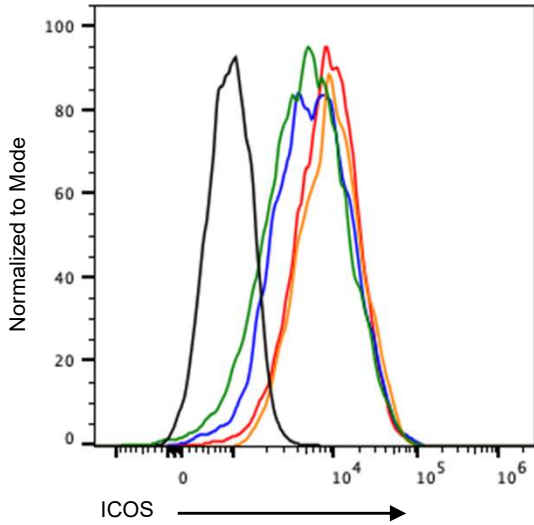
A



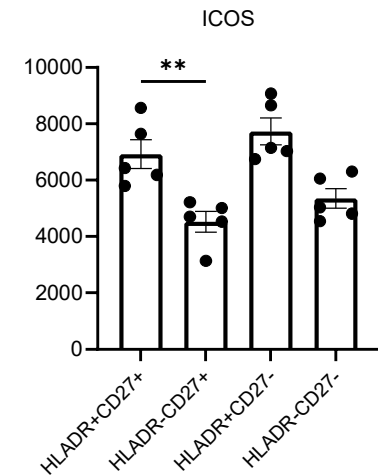
Legend:
 HLADR+CD27+
 HLADR+CD27-
 HLADR-CD27-
 HLADR-CD27+
 Unstained



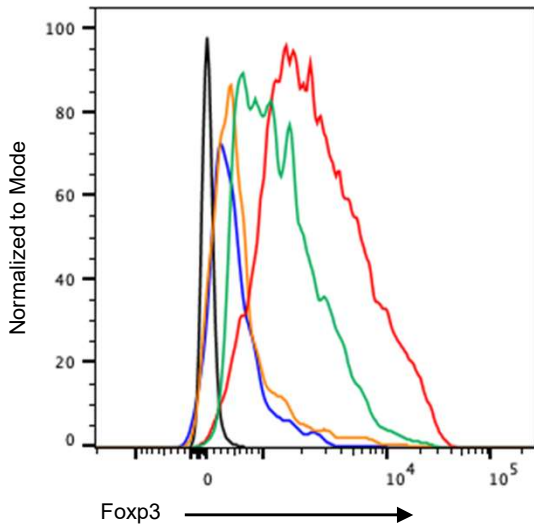
B



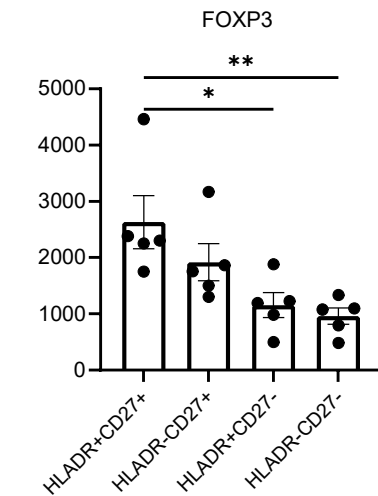
Legend:
 HLADR+CD27+
 HLADR+CD27-
 HLADR-CD27-
 HLADR-CD27+
 Unstained



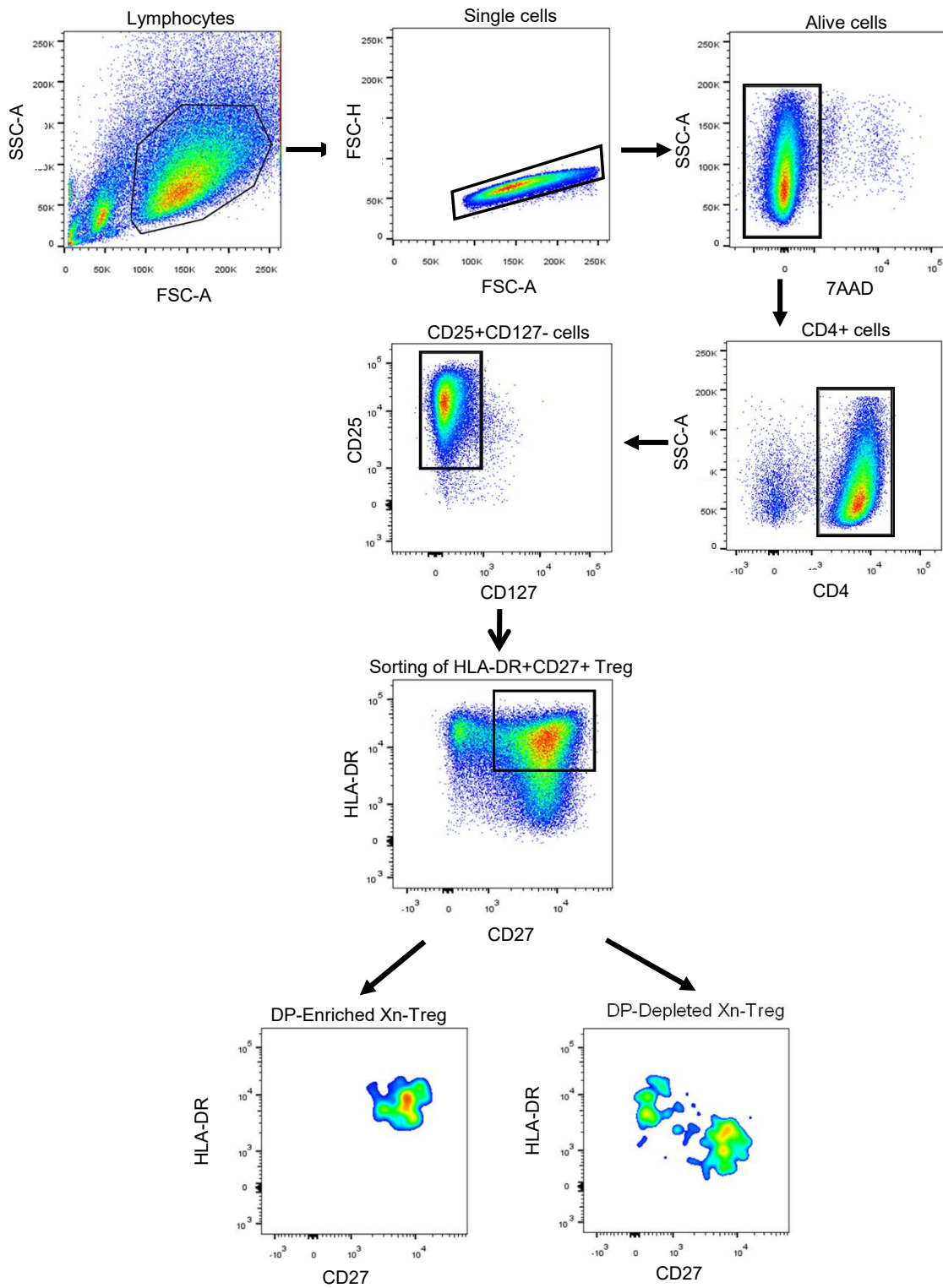
C



Legend:
 HLADR+CD27+
 HLADR+CD27-
 HLADR-CD27-
 HLADR-CD27+
 Unstained

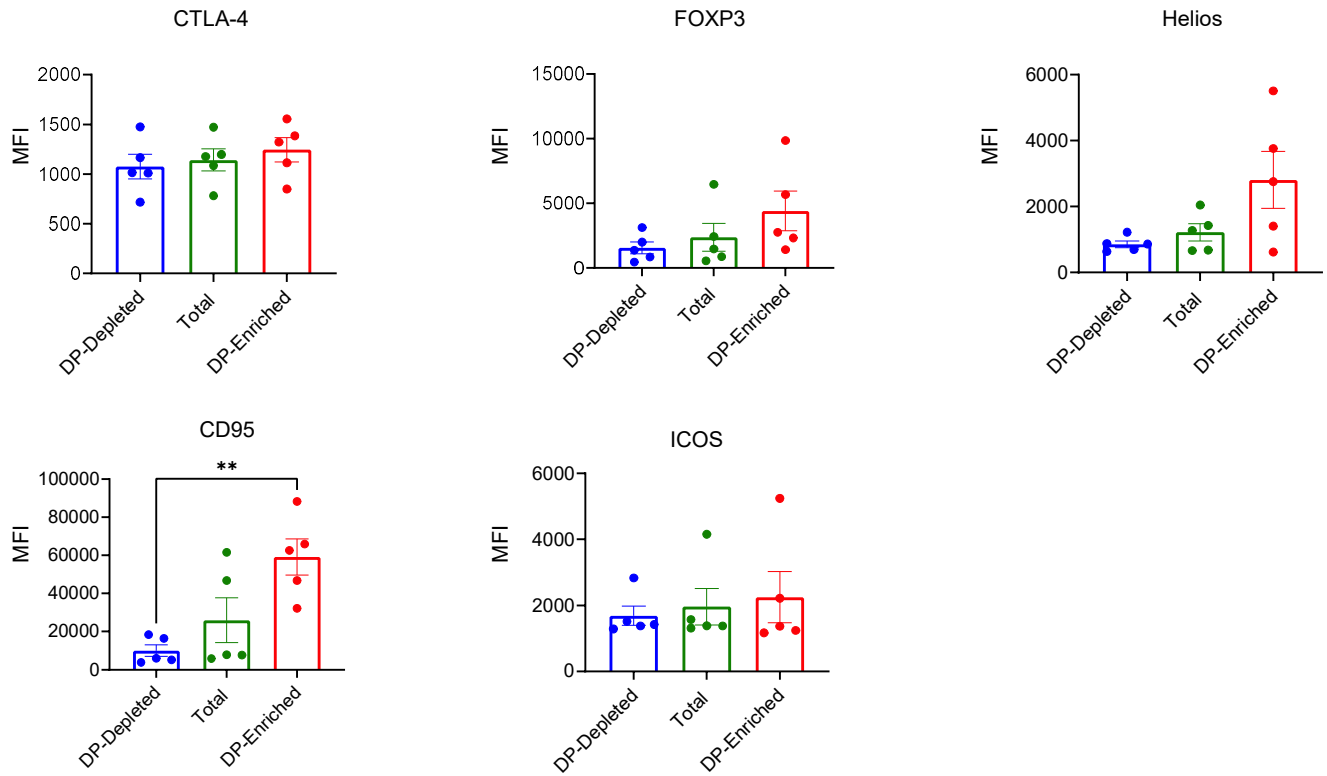


Supplemental Figure 3. The expression of CD95, ICOS, and FOXP3 was associated with HLA-DR and CD27 expression within Xn-Treg. Representative histograms of the expression level of CD95 (A), ICOS (B) and FOXP3 (C) shown as MFI for four subpopulations within Xn-Treg, based on their expression of HLA-DR and/or CD27. Data represents three independent experiments with Xn-Treg from 5 individual donors. Error bars indicate the mean \pm SEM. P value (1-way ANOVA): *P<0.05, **P< 0.01 and ****P< 0.0001.

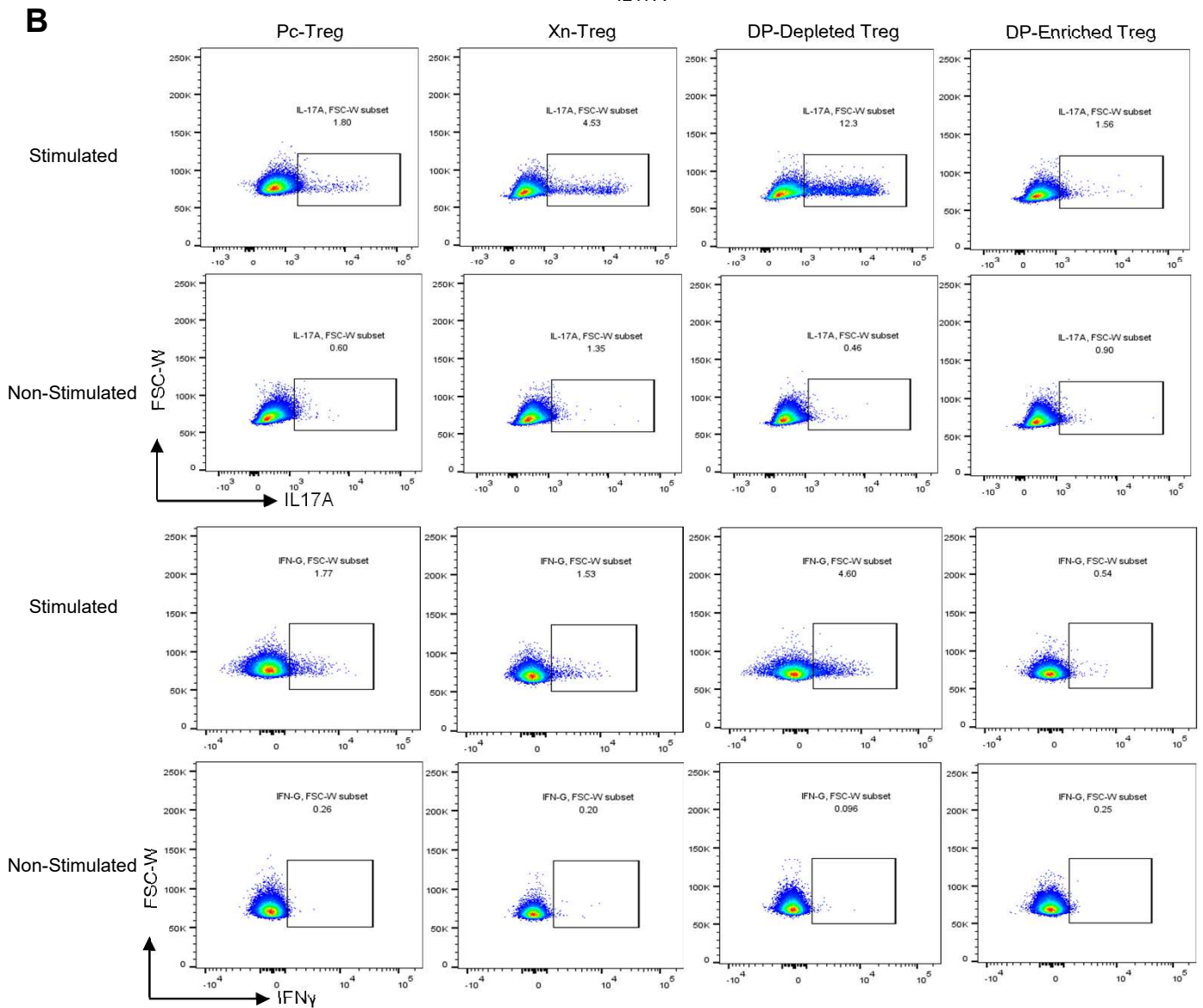
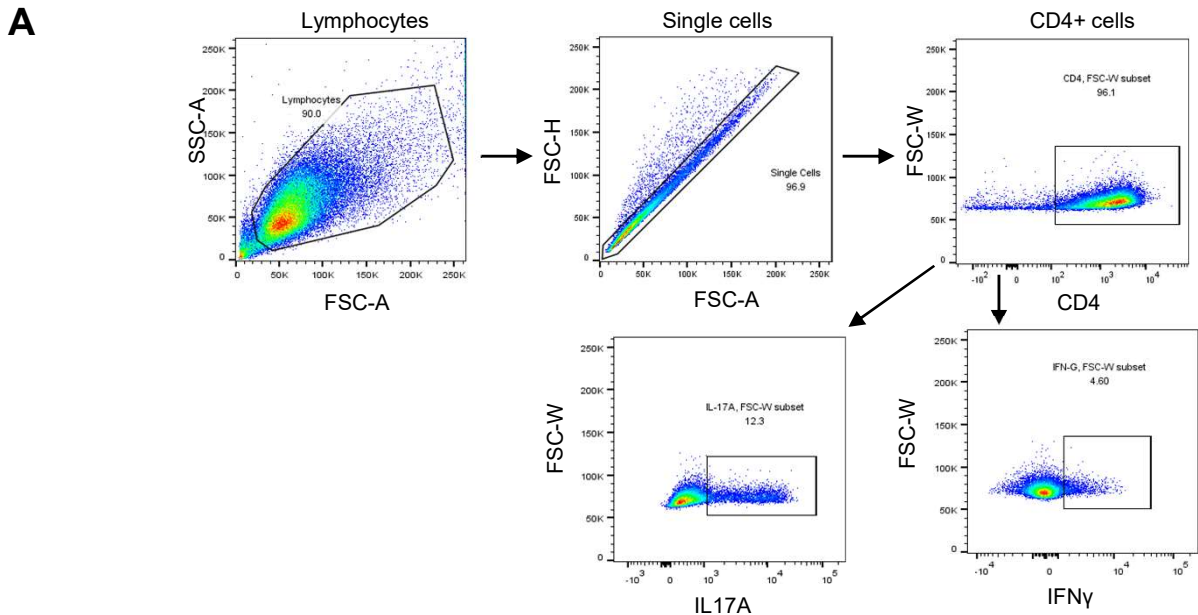


Supplemental Figure 4. Cell sorting strategy for isolation of HLA-DR+CD27+ DP Enriched Xn-Treg subset. After expansion with xenoantigen stimulation for three cycles, Xn-Treg were collected for cell sorting of HLA-DR+CD27+ DP-Enriched Treg. A series of cell gating was applied to deplete porcine cells, dead cells and CD4+CD25-CD127+ cells as indicated. The remaining cells (CD4+CD25+CD127-) were gated on HLA-DR+CD27+ cells to acquire HLA-DR+CD27+ DP-Enriched Xn-Treg and DP-Depleted Xn-Treg subsets.

Pc-Tregs

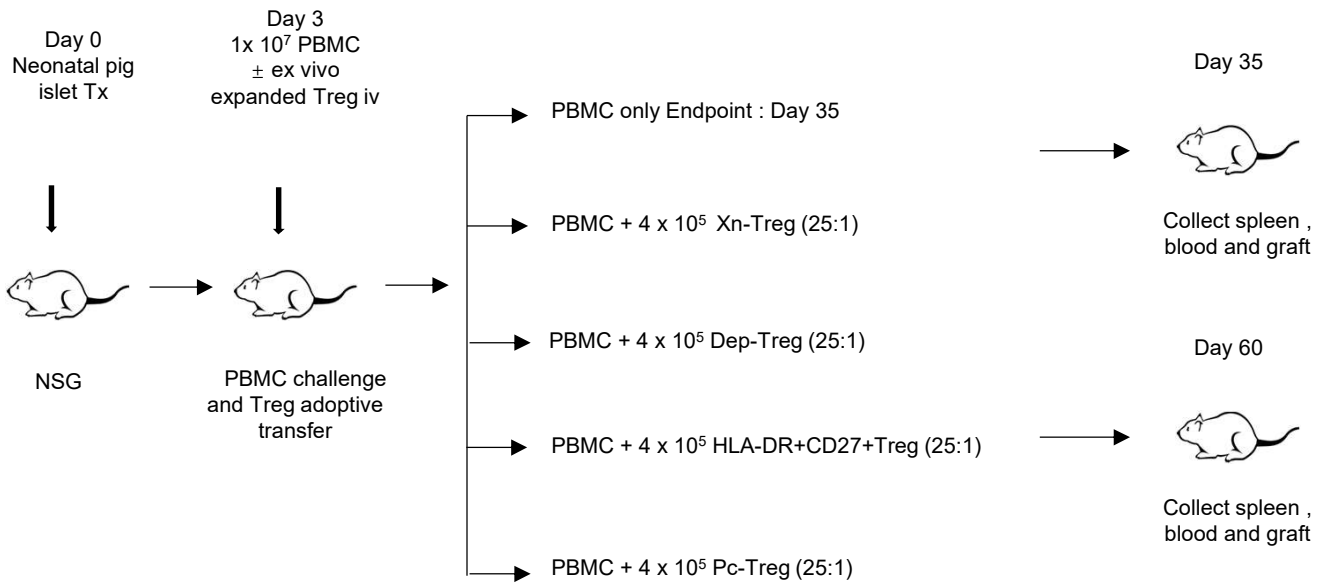


Supplemental Figure 5. Phenotyping of HLA-DR+CD27+ Treg subset analyzed on Pc-Treg. Expression level of CTLA-4 (surface), FOXP3 (intracellular), Helios (intracellular), CD95 (intracellular) and ICOS (surface) shown by the MFI in different types of Pc-Treg including total Pc-Treg (Total), HLA-DR+CD27+ DP-Enriched Pc-Treg (DP-Enriched) and DP-Depleted Pc-Treg (DP-Depleted). Data represents 3 independent experiments with Treg from 5 individual donors. P value (1-way ANOVA): **P< 0.01. Error bars indicate the mean ± SEM



Supplemental Figure 6. Detecting different types of Treg cells expressing IL17 or IFN γ under pro-inflammatory conditions. (A) Gating strategy for detecting Treg cells expressing IL17 or IFN γ . A series of gates was applied including gates for lymphocyte proportion, excluding doublets then gated on CD4+ cells for further identifying the proportion of IL17 or IFN γ expressing Treg in different types of expanded Tregs under pro-inflammatory conditions. **(B)** Representative flow cytometric plots showing the proportion of IL17 or IFN γ expressing Treg in different types of expanded Tregs under pro-inflammatory conditions. These types of Treg included Pc-Treg, HLA-DR+CD27+ DP-Enriched Xn-Treg and DP-Depleted Xn-Treg, and total Xn-Treg that were stimulated with a combination of pro-inflammatory cytokines (IL1 β , IL6, IL21, IL23, TGF β) and IL2 for 6 days (Stimulated). Control samples were Treg subsets with IL2 only (Non-Stimulated).

In Vivo Study Design



Supplemental Figure 7. A schematic representation of the in vivo humanized mouse model. NSG mice were transplanted with NICC xenografts and, 3 days after transplantation, were reconstituted with 10×7 human PBMC (CD4+CD25+CD127-/low Treg depleted) alone or PBMC co-transferred with 4×10^5 of different individual Treg subsets at a 25:1 ratio of PBMC:Treg. Blood, spleen and graft samples were taken at predetermined time points days 35 and 60 post transplantation or human cell transfer from recipient mice adoptively transferred with human PBMC alone or together with different individual Treg subsets, respectively for the subsequent experiments as performed in Figures 5, 6 and 7, and Table 1.

Supplemental Table 1. Antibodies for flow cytometric analysis and cell sorting

| Antibodies Flow Cytometric Analysis and Cell sorting | | | |
|--|--------------|------------------|--------------------------------------|
| <i>Antibody//Target</i> | <i>Clone</i> | <i>Conjugate</i> | <i>Catalogue number, Supplier</i> |
| CD3 | UCHT-1 | PE | 555333, BD Pharmingen |
| CD3 | SP34-2 | PerCP | 552851, BD Pharmingen |
| CD4 | RPA-T4 | APC-H7 | 560158, BD Biosciences |
| CD4 | SK3 | PE-Cy7 | 348789, BD Pharmingen |
| CD8 | RPA-T8 | PE | 555367, BD Pharmingen |
| CD25 | BC96 | APC | 17-0259-42, eBioscience (Invitrogen) |
| CD27 | L128 | BV711 | 563167, BD Biosciences |
| CD39 | TU66 | BUV737 | 612852, BD Biosciences |
| CD45 | 2D1 | FITC | 347463, BD Biosciences |
| CD62L | DREG-56 | BV650 | 563808, BD Horizon |
| CD62L | DREG-56 | PE | 555544, BD Pharmingen |
| CD95 | DX2 | BV421 | 305623, BioLegend |
| CD127 | hIL-7RM21 | PE | 557938, BD Pharmingen |
| CTLA-4 (CD152) | BN13(BNI3) | BV605 | 369609, BioLegend |
| CTLA-4 (CD152) | BNI3 | PE | 557301, BD Biosciences |
| ICOS (CD278) | DX29 | BV421 | 562901, BD Horizon |
| FOXP3 | 259D/C7 | PECF-594 | 562421, BD Biosciences |
| FOXP3 | PCH101 | PE | 12-4776-42, eBioscience (Invitrogen) |
| GITR (CD357) | 110416 | PE | FAB689P, R&D systems |
| Helios | 22F6 | Pacific Blue | 137210, BioLegend |
| HLA-DR | L243 | FITC | 347363, BD Pharmingen |
| IL-17A | N49-653 | BV421 | 562933, BD Biosciences |
| IFN- γ | B27 | BV711 | 564039, BD Horizon |
| 7-AAD | | | 559925, BD Pharmingen |
| Human Fc Block | | | 564220, BD Pharmingen |

Supplemental Table 2: Immunohistochemistry and immunofluorescence staining antibodies

| Primary Antibodies | | |
|--|-------------------------|--------------------------|
| <i>Antibody/Clone</i> | <i>Catalogue number</i> | <i>Supplier</i> |
| Guinea pig Anti-Insulin (polyclonal) | IR00261-2 | Agilent Dako |
| Rabbit Anti-CD4 (polyclonal) | ab231460 | Abcam |
| Mouse Anti-CD8 (C8/144B) | ab17147 | Abcam |
| Mouse Anti-FOXP3 (236A/E7) | ab20034 | Abcam |
| Guinea pig anti-porcine glucagon | LS-C202759 | LS Bio |
| Goat anti-Somatostatin (polyclonal) | Sc-7819 | Santa Cruz Biotechnology |
| Secondary Antibodies and Counterstain | | |
| Rabbit Anti-Guinea pig immunoglobulins/HRP | P0141 | Agilent Dako |
| Donkey anti-goat immunoglobulins/HRP | A15999 | ThermoFisher |
| Goat Anti-Rabbit IgG H&L (Alexa Fluor 488) | Ab150081 | Abcam |
| Goat Anti-Mouse IgG H&L (Alexa Fluor 594) | Ab150120 | Abcam |
| Goat Anti-Guinea pig IgG H&L (Alexa Fluor 647) | Ab150187 | Abcam |
| DAPI (Vectashield Mounting Medium) | H-1200-10 | Vector Laboratories |