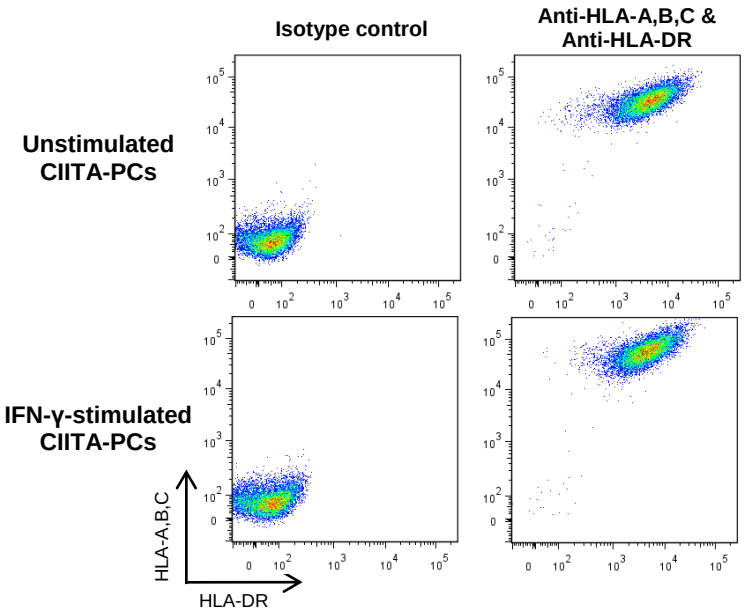
Supplemental Materials

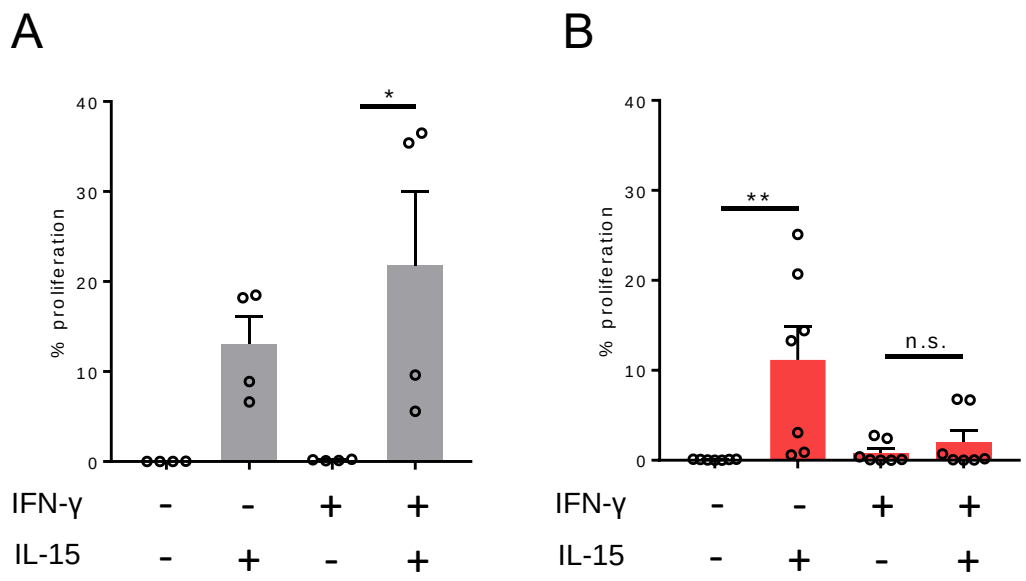
Supplementary Table I. Flow Cytometric Analysis of MHC molecule expression on same-donor human ECs and PCs

Cells	HLA-A,B,C	HLA-DR
EC	134 ± 17.93	0
γ-EC	1424 ± 43.73	1076 ± 233.4
PC	1040 ± 29.9	0
γ-PC	1818 ± 196.8	527 ± 67.27

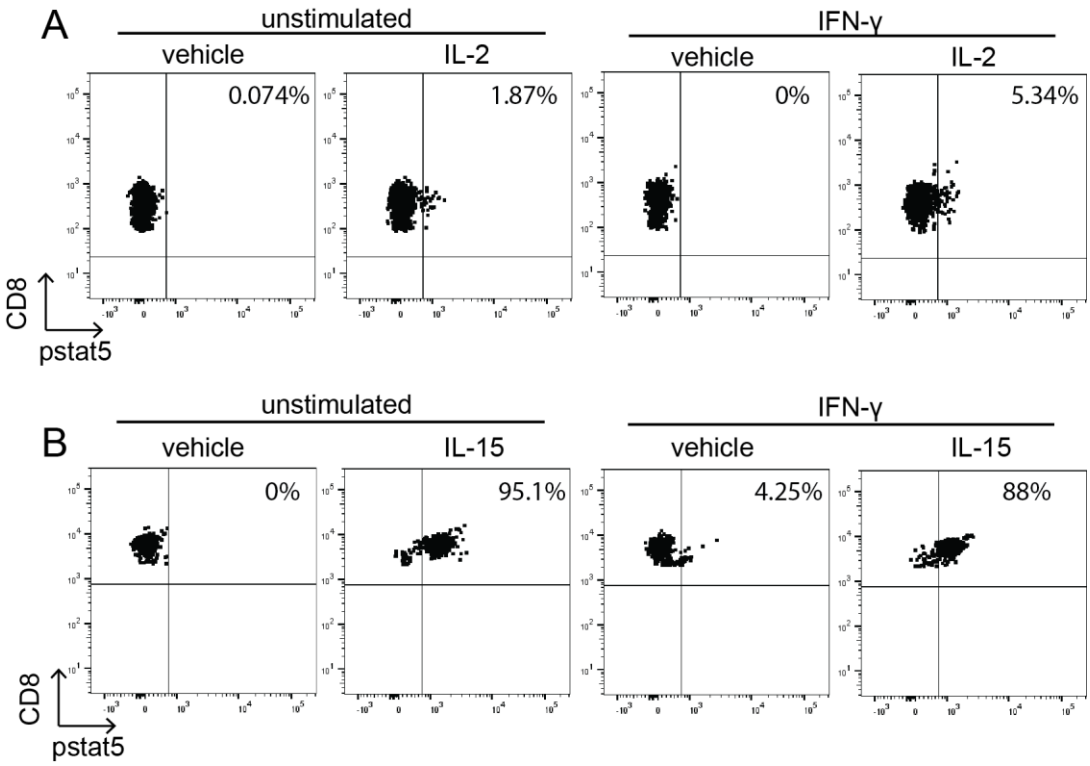
HLA-A,B,C and HLA-DR on same-donor human ECs and PCs were analyzed by flow cytometry. The results shown are the corrected median fluorescence intensity (MFI) expressed in arbitrary units of fluorescence +/-SEM [n=3 donor pairs].



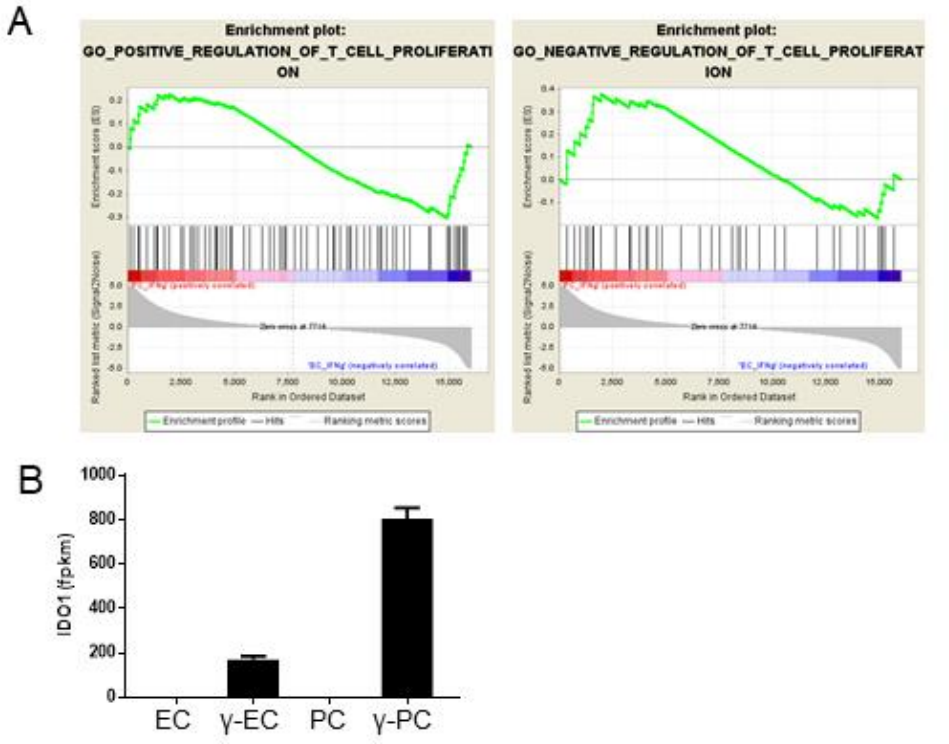
Supplementary Figure 1. MHC Class I and Class II expression by unstimulated or IFN-γ-stimulated CIITA-PCs. CIITA-PCs were stimulated with vehicle or 50 ng/mL of IFN-γ for 72 hours, and surface expression level of HLA-A,B,C and HLA-DR by these CIITA-PCs were assessed by flow cytometry. Representative results from one of the three PC donors were shown. Corrected median fluorescence intensity (MFI, expressed in arbitrary units of fluorescence) +/- SEM for HLA-A,B,C expression by unstimulated CIITA-PCs and IFN-γ-stimulated CIITA-PCs are 34018 ± 1954 and 63964 ± 5039, respectively. Corrected MFI for HLA-DR expression by unstimulated CIITA-PCs and IFN-γ-stimulated CIITA-PCs are 5153 ± 131 and 5458 ± 104.4, respectively.



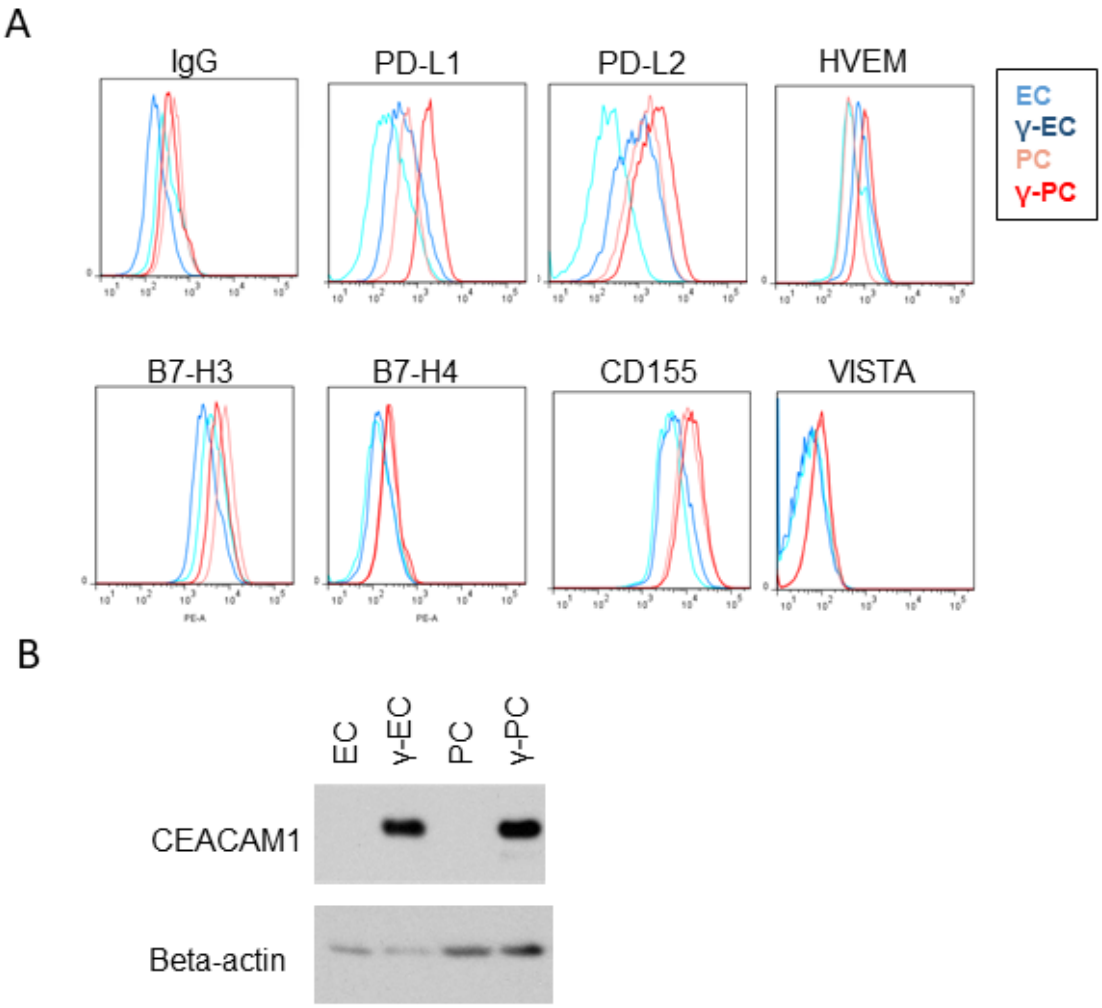
Supplementary Figure 2. γ-PCs potently inhibit IL-15-induced CD8$^+$ T$_{EM}$ proliferation. Proliferation of CFSE-labelled CD8$^+$ T$_{EM}$ after co-cultured with unstimulated or IFN-γ pre-stimulated ECs (A) or PCs (B) for 7 days. 10ng/mL of recombinant IL-15 was added on day 3 of co-culture where indicated (n = 4–7, one-way ANOVA, SEM). *p < 0.05, **p < 0.01.



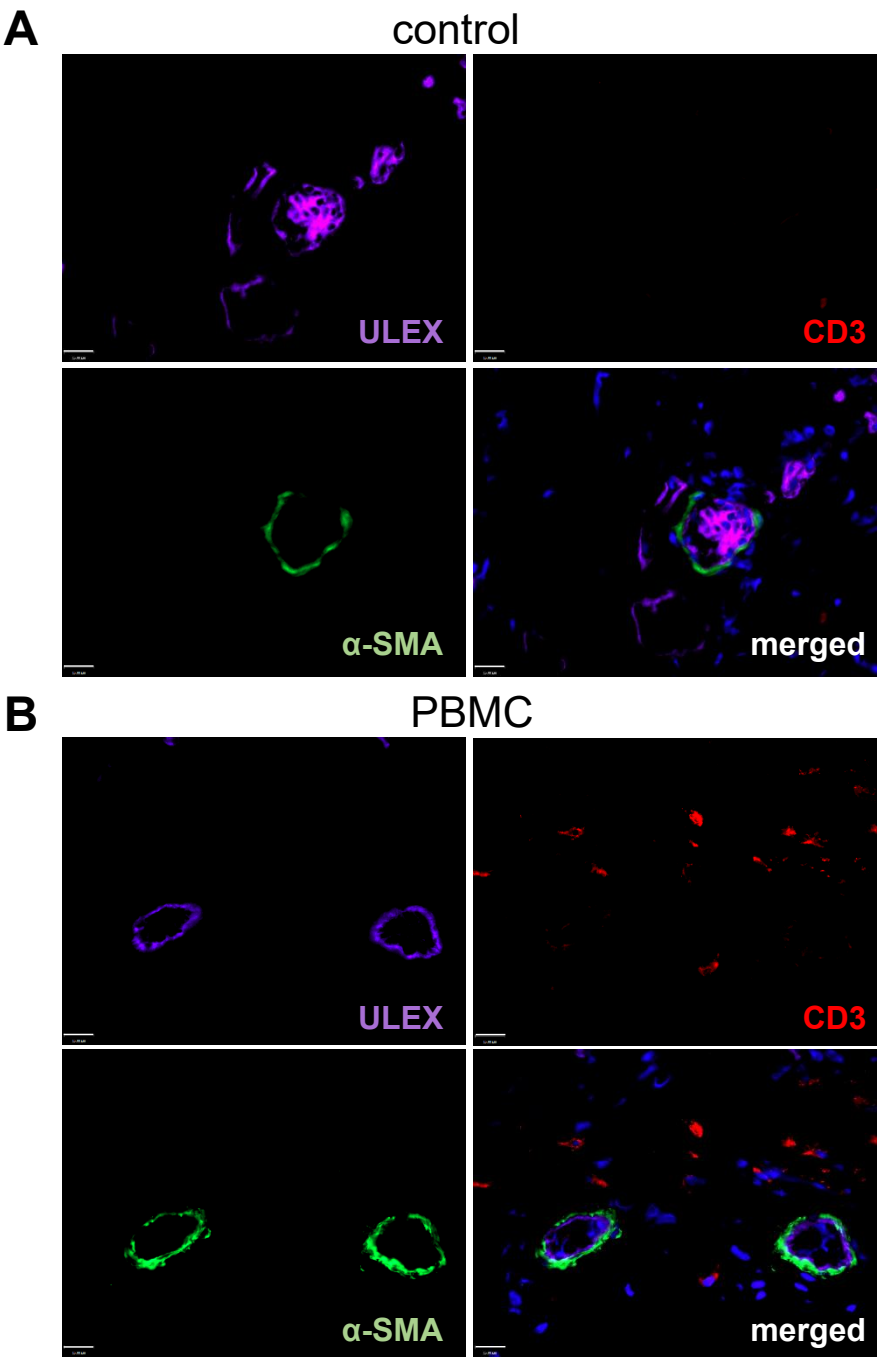
Supplementary Figure 3. $CD8^+$ T_{EM} co-cultured with γ-PCs phosphorylates STAT5 in response to common γ chain cytokines. A) $CD8^+$ T_{EM} were co-cultured with PCs or γ-PCs for 3 days, and then were stimulated with recombinant IL-2 (25u/mL) for 25 minutes before the status of STAT5 phosphorylation was assessed by flow cytometry. B) $CD8^+$ T_{EM} were co-cultured with PCs or γ-PCs for 3 days, and then were stimulated with recombinant IL-15 (10ng/mL) for 25 minutes before the status of STAT5 phosphorylation was assessed by flow cytometry. Representative results from one of the three independent experiments were shown.



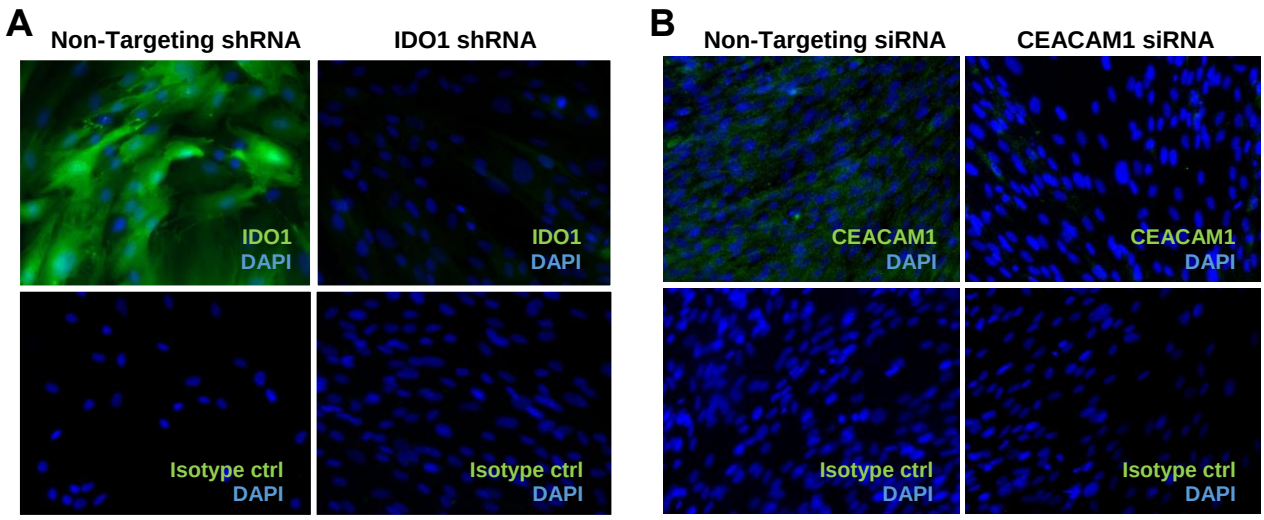
Supplementary Figure 4. RNAseq analysis comparing transcriptome signatures of ECs, γ-ECs, PCs, and γ-PCs revealed distinct action of IFN-γ on ECs versus PCs. A) Gene set enrichment analysis using two selected gene sets relevant to T cell proliferation (*Positive Regulation of T cell Proliferation and Negative Regulation of T cell Proliferation*), showing their enrichment scores, where a positive score indicates enrichment (higher expression) in γ-PCs relative to γ-ECs. FDR=0.10 for both analyses. B) IDO1 transcript level expressed by EC, γ-EC, PC, and γ-PC. Data derived from the RNAseq analysis (bar graphs show mean +/- SEM of 3 replicates for each condition).



Supplementary Figure 5. IFN-γ induces expression of PD-1 ligands and CEACAM1 on ECs and PCs. A) Representative FACS plot showing expression level of PD-L1, PD-L2, HVEM, B7-H3, B7-H4, CD155, and VISTA by unstimulated or IFN-γ-stimulated ECs or PCs assessed by flow cytometry (n=3). B) Immunoblotting of CEACAM1 expressed by unstimulated or IFN-γ-stimulated ECs or PCs (n=3).



Supplementary Figure 6. Perivascular infiltration of allogeneic human T cells in vivo in a skin allograft rejection humanized mouse model. Immunofluorescence analysis of human skin allograft implanted on immunodeficient mice receiving control saline (A) or human PBMCs (B). Skin grafts were harvested on day 7 after injection. Freshly frozen tissues were co-stained for Ulex Europaeus Agglutinin I (ULEX), alpha-smooth muscle actin (α-SMA), and CD3. Results shown are representative of three skin grafts. Scale bars = 16 μm.



Supplementary Figure 7. Validation of IDO1 and CEACAM1 antibody specificities for immunofluorescence staining. A) Non-targeting or IDO1 shRNA PCs were stimulated with IFN-γ (50ng/mL) for 72 hours, and then stained using IDO1 or rabbit IgG isotype control. B) Non-targeting or CEACAM1 siRNA PCs were stimulated with IFN-γ (50ng/mL) for 72 hours, and then stained using CEACAM1 or mouse IgG isotype control.