Stem Cell Reports, Volume *11*

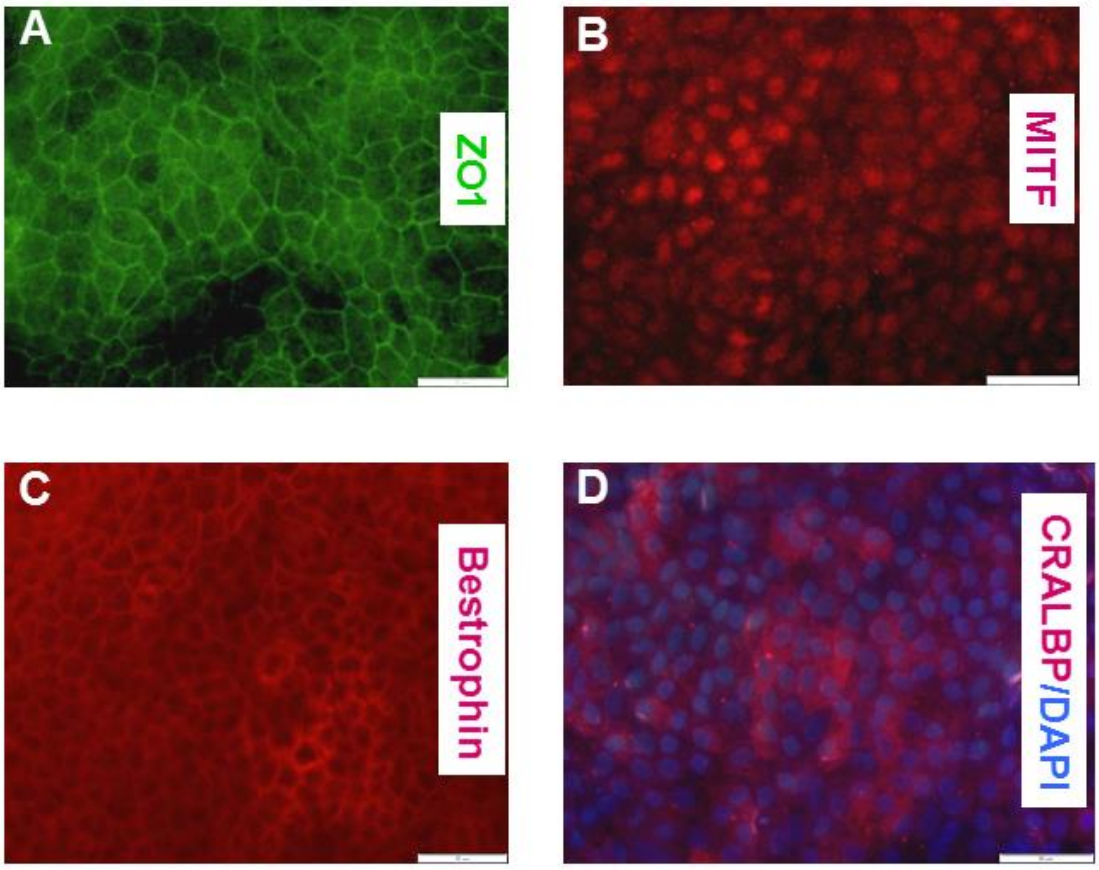
Supplemental Information

Immunological Properties of Human Embryonic Stem Cell-Derived Retinal Pigment Epithelial Cells

Masha Idelson, Ruslana Alper, Alexey Obolensky, Nurit Yachimovich-Cohen, Jacob Rachmilewitz, Ayala Ejzenberg, Ekaterina Beider, Eyal Banin, and Benjamin Reubinoff

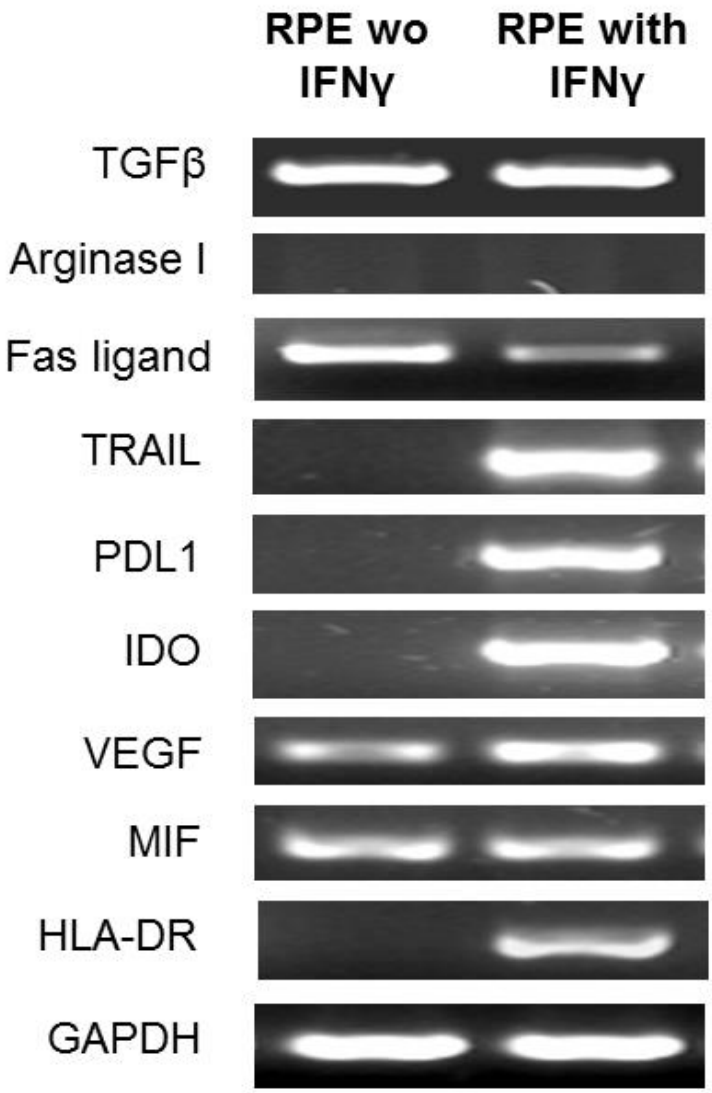
SUPPLEMENTAL INFORMATION

Figure S1



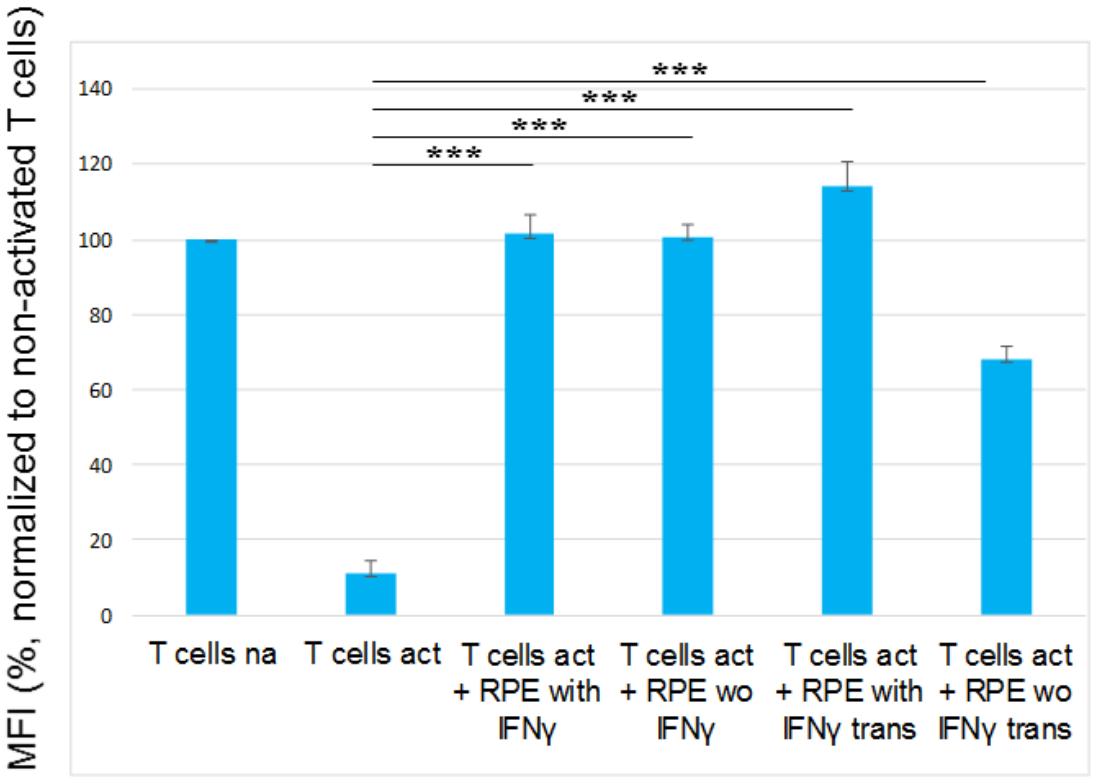
Human ESC - derived pigmented cells are putative RPE cells
Immunostaining showing the expression of markers of early (MiTF (B)) and mature (ZO-1(A), CRALBP (D), Bestrophin (C)) RPE cells. Scale bars: (A-D) 50μm.

Figure S2



Expression of immunomodulatory molecules by hESC-RPE cells
RT-PCR analysis of the expression of immunomodulatory molecules, TGFβ, Arginase I, Fas ligand, TRAIL, PDL1, IDO, VEGF, MIF and HLA-DR by RPE cells cultured for 3 days in the presence or absence of IFNγ.

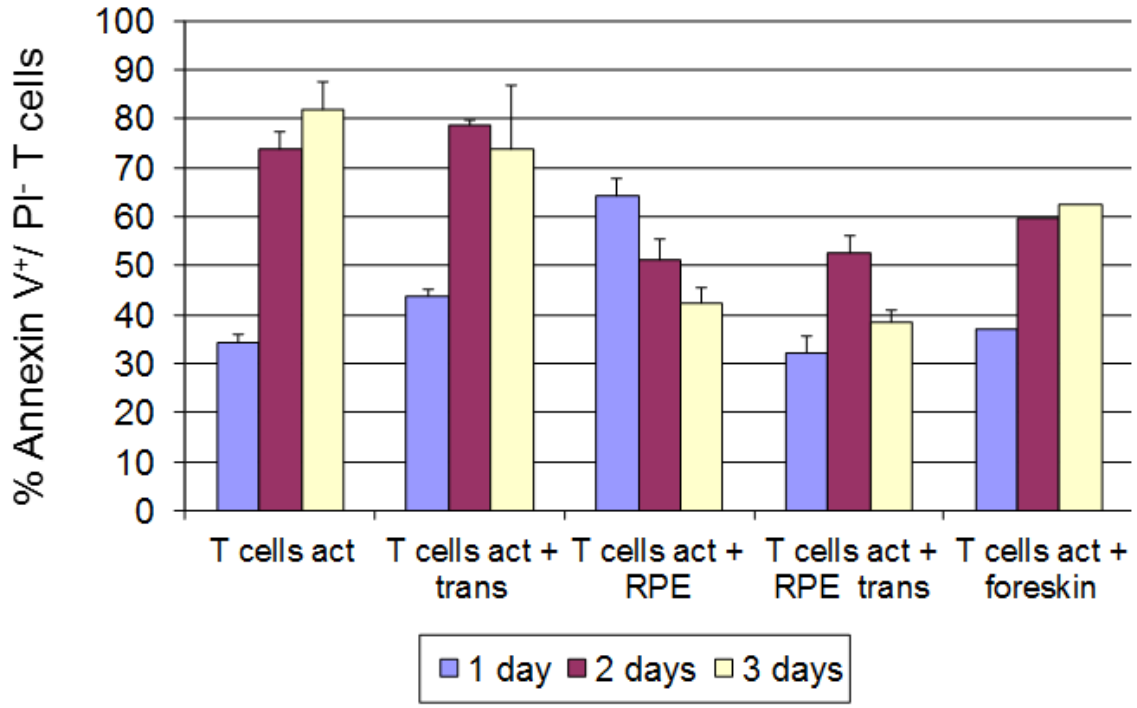
Figure S3



T cell proliferation was highly attenuated by co-culture with hESC-RPE cells

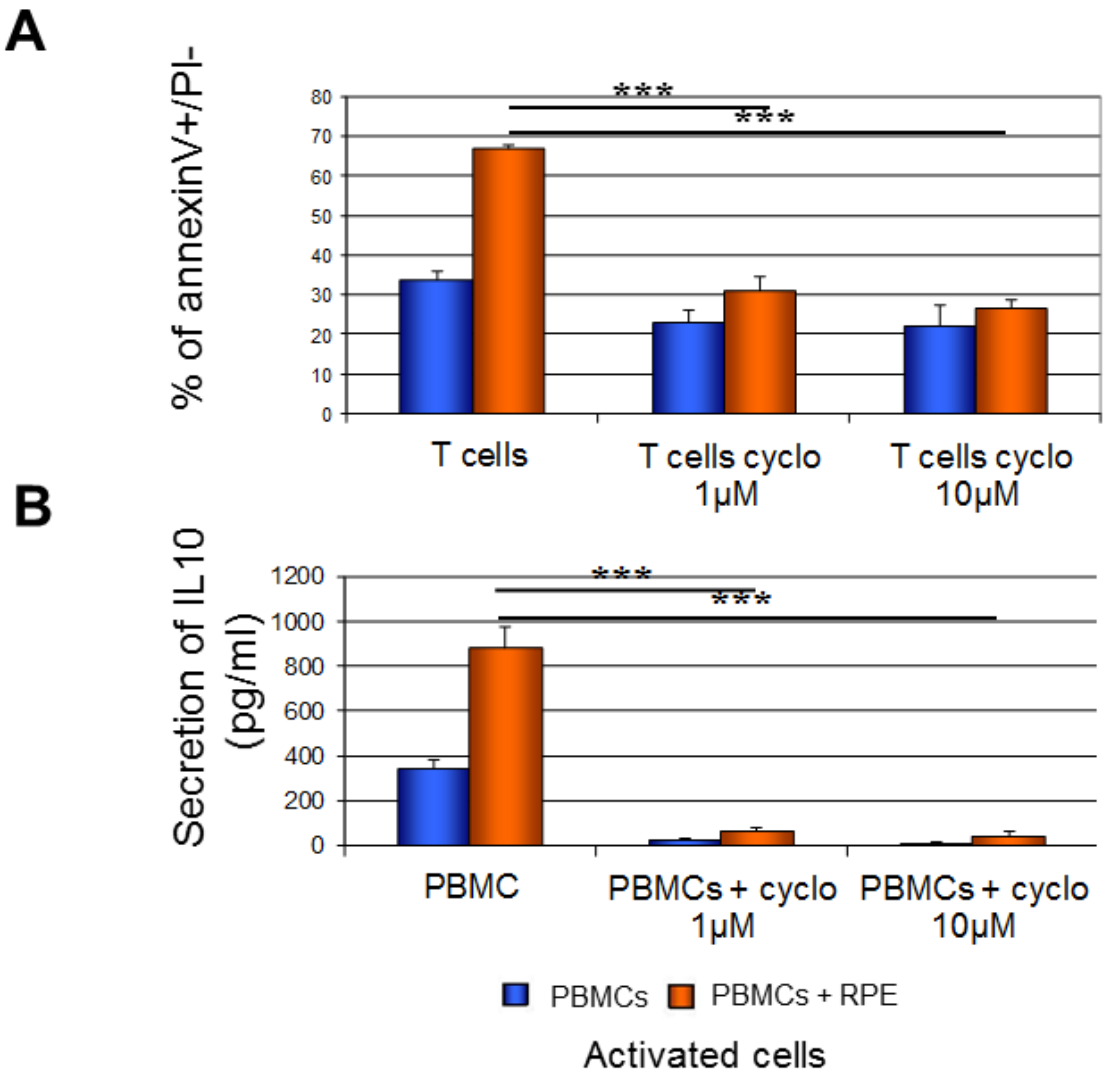
PBMCs were labeled with CFSE. The cells were then stimulated with anti-CD3/anti-CD28 antibodies and co-cultured for 4 days with RPE cells that were pre-treated or not with IFNγ. The level of proliferation was analyzed within the $CD3^+$ gate. PBMCs were co-cultured with RPE cells directly or using transwells (**trans**). The level of staining with CFSE in the non-activated $CD3^+$ T cells that didn't proliferate was set as 100%. *** $p \leq 0.001$

Figure S4



T cell apoptosis was increased at day 1 after co-culture with hESC-RPE cells
PBMCs were stimulated with anti-CD3/anti-CD28 antibodies and co-cultured for 1-3 days with RPE cells. PBMCs were co-cultured with RPE cells directly or using transwells (trans). As a control, PBMCs were incubated with foreskin cells. PBMCs were labeled with annexin V and PI and the percentage of apoptotic annexin V $^{+}$/ PI^{-} cells was analyzed. The level of apoptosis was analyzed within the population of CD3^{+} T cells; na - non-activated T cells, act - cells activated with anti-CD3/anti-CD28 antibodies.

Figure S5



Cyclosporine significantly reduces the RPE cells-induced T cell apoptosis and secretion of IL10

(A) PBMCs were stimulated with anti-CD3/anti-CD28 antibodies and co-cultured for 1 day with RPE cells in the presence or absence of cyclosporine (**cyclo**). The PBMCs were then labeled with annexin V and PI. The population of $CD3^+$ T cells was analyzed for the percentage of apoptotic (annexin V^+/ PI^-) cells. (B) ELISA analysis of the secretion of IL10 by PBMCs that were stimulated with anti-CD3/anti-CD28 antibodies and co-cultured for 1 day with RPE cells in the presence or absence of cyclosporine (**cyclo**). *** $p \leq 0.001$

Supplemental Experimental Procedures

List of human primer sequences (forward and reverse 5'–3') and the length of PCR products:
TRAIL (CCCAATGACGAAGAGAGTATGA, GGAATAGATGTAGTAAAACCCT; 359bp)
PDL1 (AAACAATTAGACCTGGCTG, TCTTACCACTCAGGACTTG; 399bp)
Fas ligand (GGTCCATGCCTCTGGAATGG, CACATCTGCCCAGTAGTGCA; 249bp)
IDO (GCAAATGCAAGAACGGGACACT; TCAGGGAGACCAGAGCTTTCACAC; 463 bp)
IL6 (ATGAACTCCTTCTCCACAAGC, GAAGAGCCCTCAGGCTGGACT; 628bp)
IL18 (GCTTGAATCTAAATTATCAGTC, CAAATTGCATCTTATTATCATG; 334bp)
IL15 (GATTTACCGTGGCTTTGAGTAATGAG, GAATCAATTGCAATCAAGAAGTG; 522bp)
IL12A (TTCACCACTCCCAAAACCTGC, GAGGCCAGGCAACTCCCATTAG; 225bp)
HLA-DR (TGGGACCATCTTCATCATCAAGG, GGGCATTCCATAGCAGAGACAGAC; 379bp)
Arginase I (TGGAAACTTGCATGGACA, AAGTCCGAAACAAGCCAA; 253 bp)
MIF (GTTCCTCTCCGAGCTCACCCAGCAGC, GCAGCTTGCTGTAGGAGCGGTTCTG; 185bp)
VEGF (ACATTGTTGGAAGAAGCAGC, ATCCTGCCCTGTCTCTCTGT; 231 bp)
TGFβ1 (CGGAGTTGTGCGGCAGTGGTTGA, GCGCCCGGGTTATGCTGGTTGTA; 450bp)
GAPDH (AGCCACATCGCTCAGACACC, GTACTCAGCGCCAGCATCG; 301bp)