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Supplemental Information

Lack of T Cell Response to iPSC-Derived Retinal Pigment Epithelial Cells from HLA Homozygous Donors

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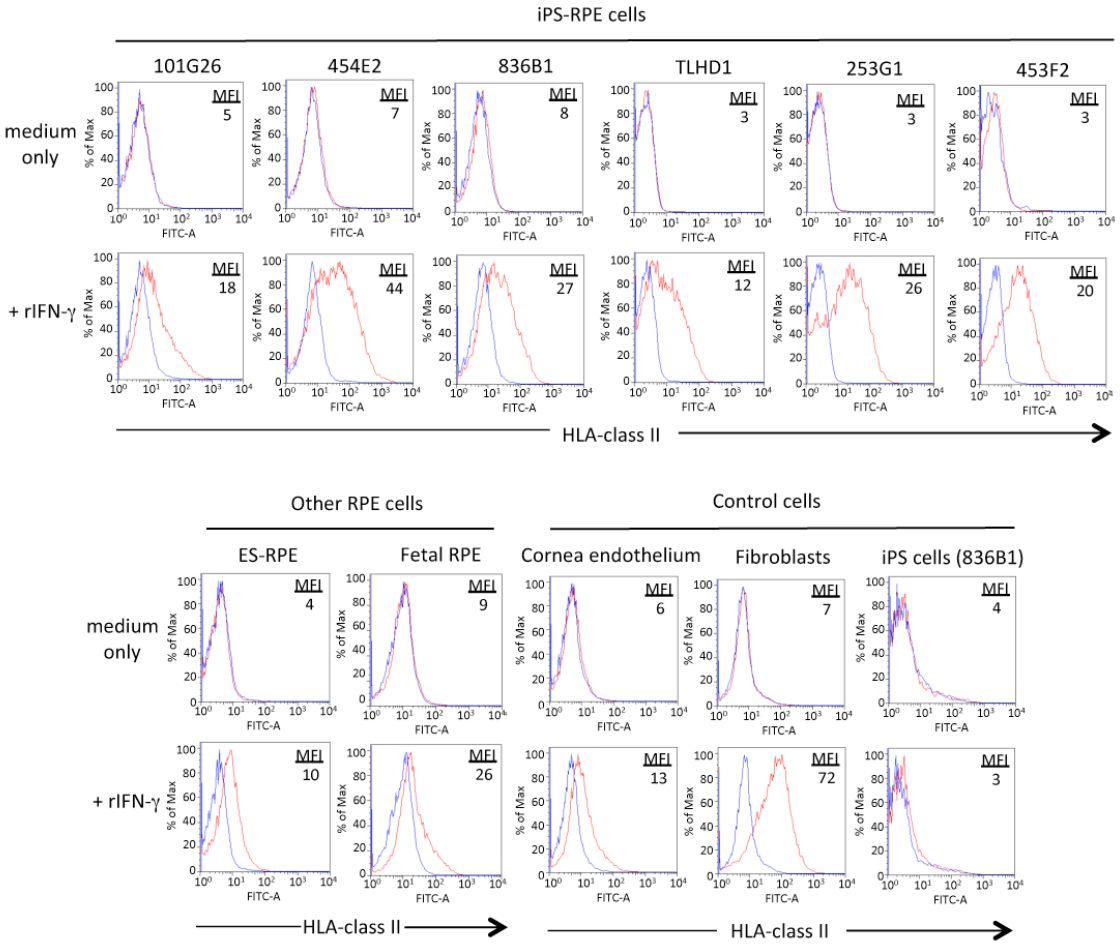
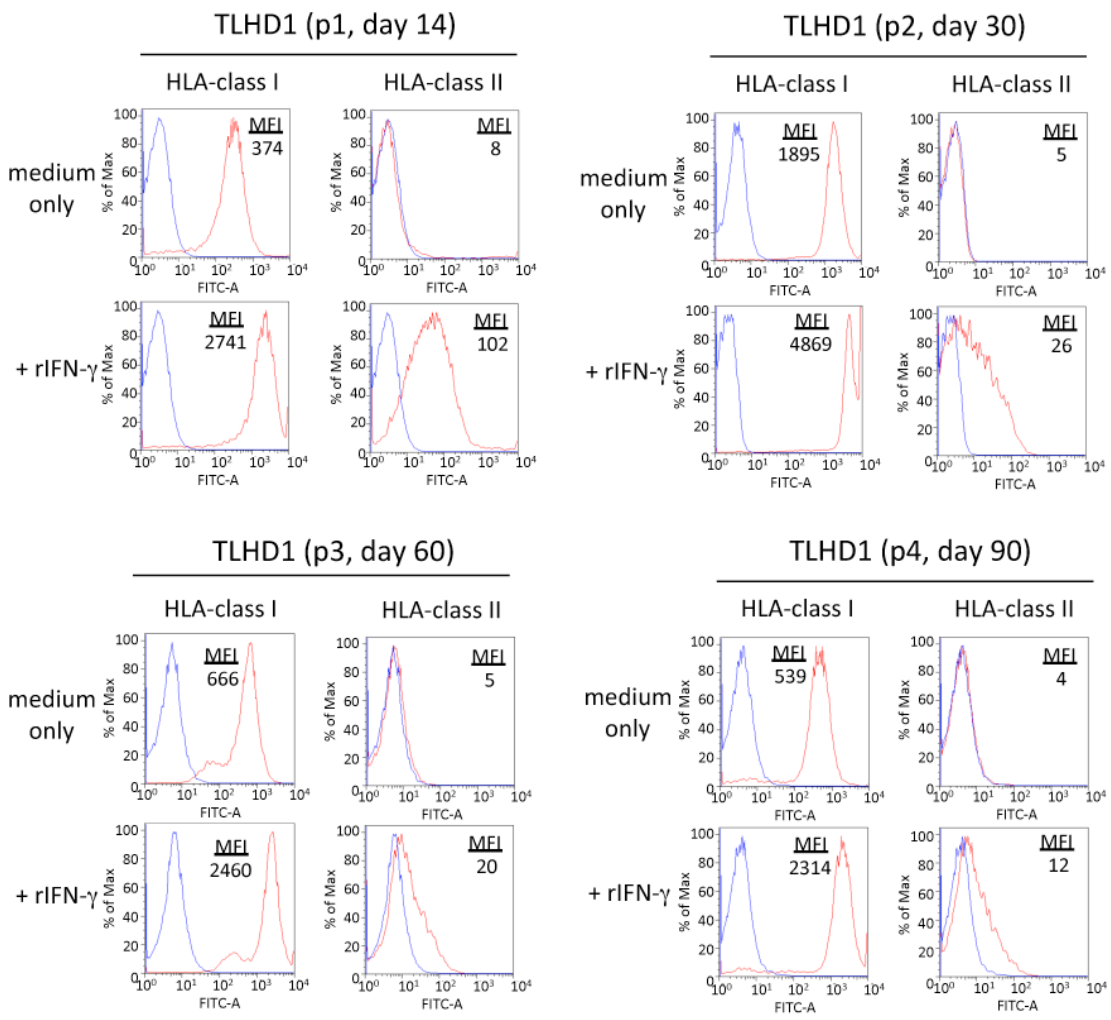
Supplementary Figure 1.



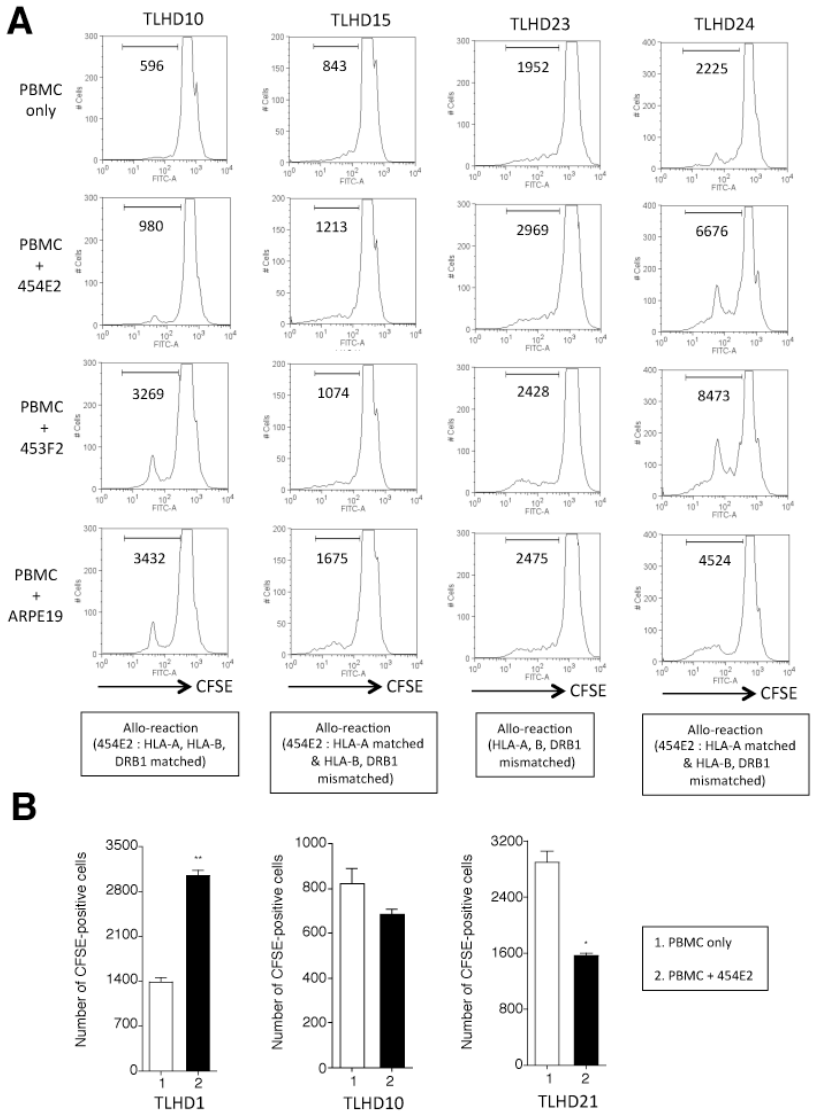
Expression of HLA-class II on IFN-γ–treated iPS-RPE cells. iPS-RPE cells (n=6) were cultured for 48 hr in the presence or absence (medium only) of recombinant IFN-γ and stained with anti-HLA-class II antibody. As controls, other human RPE cells (ES cell–derived RPE cells and fetal RPE cells) and control human cells (cornea endothelial cells, fibroblasts, and iPS cells) were also prepared. MFI - mean fluorescence intensity.

Supplementary Figure 2.



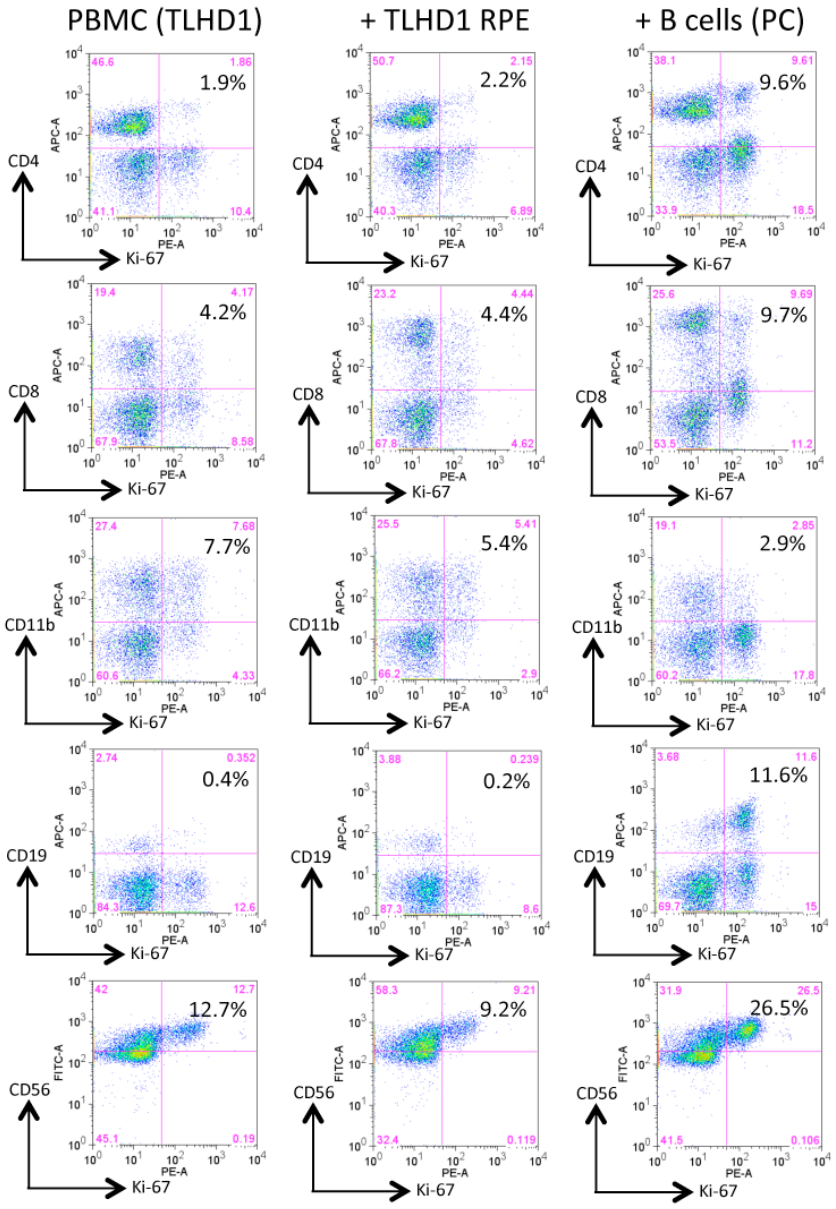
Expression of HLA-class I and class II on human iPS cell–derived RPE cells at different culture stages. TLHD1 iPS-RPE cells were prepared from passage 1 (p1), p2, p3, or p4 and stained with anti-HLA-class I or -class II antibody. Cells were cultured with recombinant IFN-γ for 48 hr. Other human iPS-RPE cells, 454E2 RPE cells, yielded similar results (data not shown). MFI - mean fluorescence intensity.

Supplementary Figure 3.



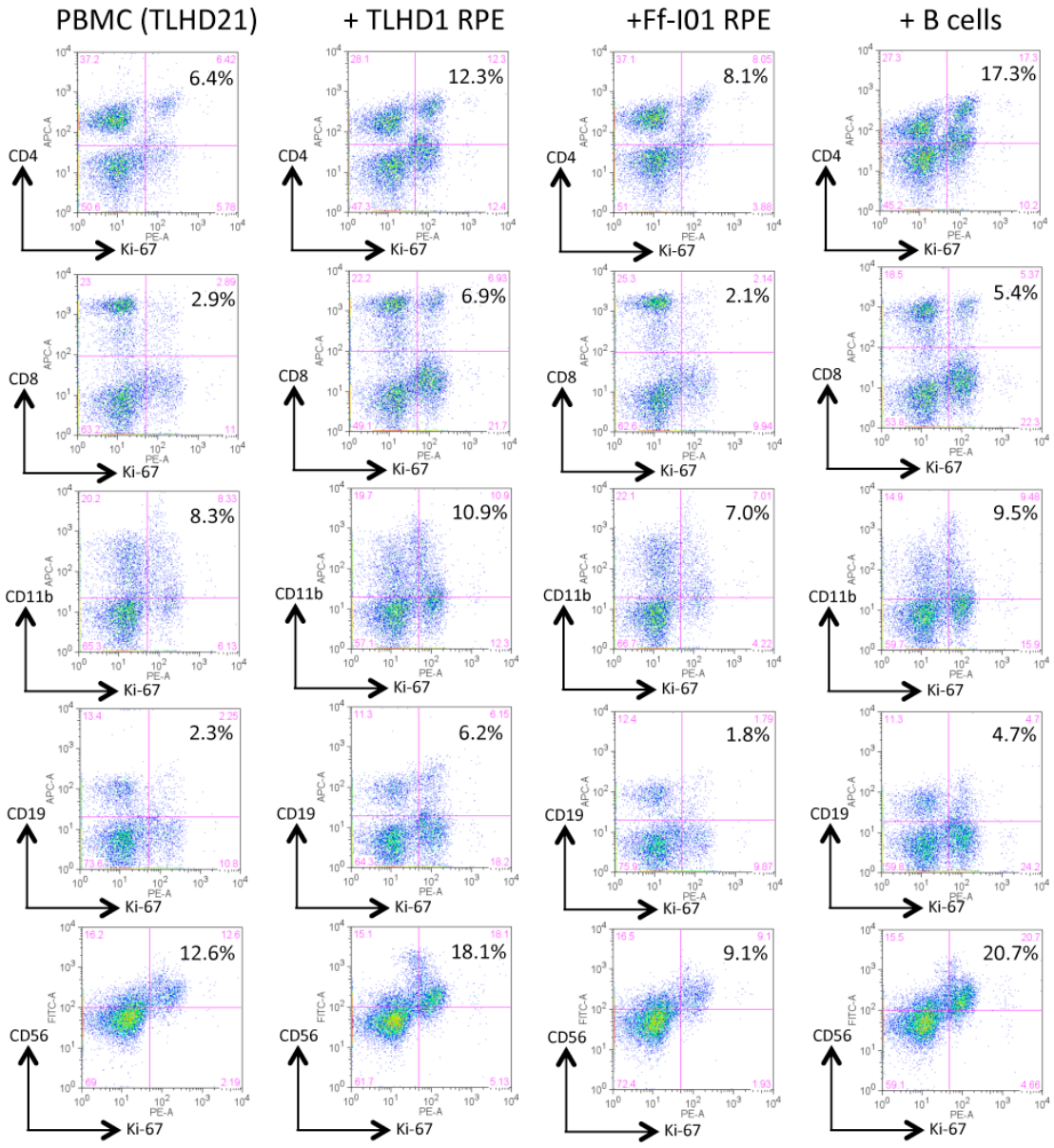
Results of PBMC-RPE MLR assay. (**A**) MHC homozygote iPS-RPE cells (target cells; 454E2, 453F2), ARPE-19 RPE cell lines, and B cells (data not shown) were cultured with CFSE-labeled PBMC (effector cells) from TLHD10, 15, 23, or 24 donors for 96-120 hr (effector: target ratio = 100:1). The only HLA-matched combination was TLHD10 PBMC and 454E2 iPS-RPE cells; no other combinations were HLA matched. Numbers in the histogram indicate CFSE-positive cells. (**B**) We statistically analyzed these MLR data between TLHD1, TLHD10, and TLHD21 donors and 454E2 iPS-RPE cells. The mean and standard deviation of the number of CFSE-positive cells are shown. Data represent the means ± S.E.M of three independent experiments. * P <0.05, ** P <0.005, as compared to PBMC only (open bar).

Supplementary Figure 4.



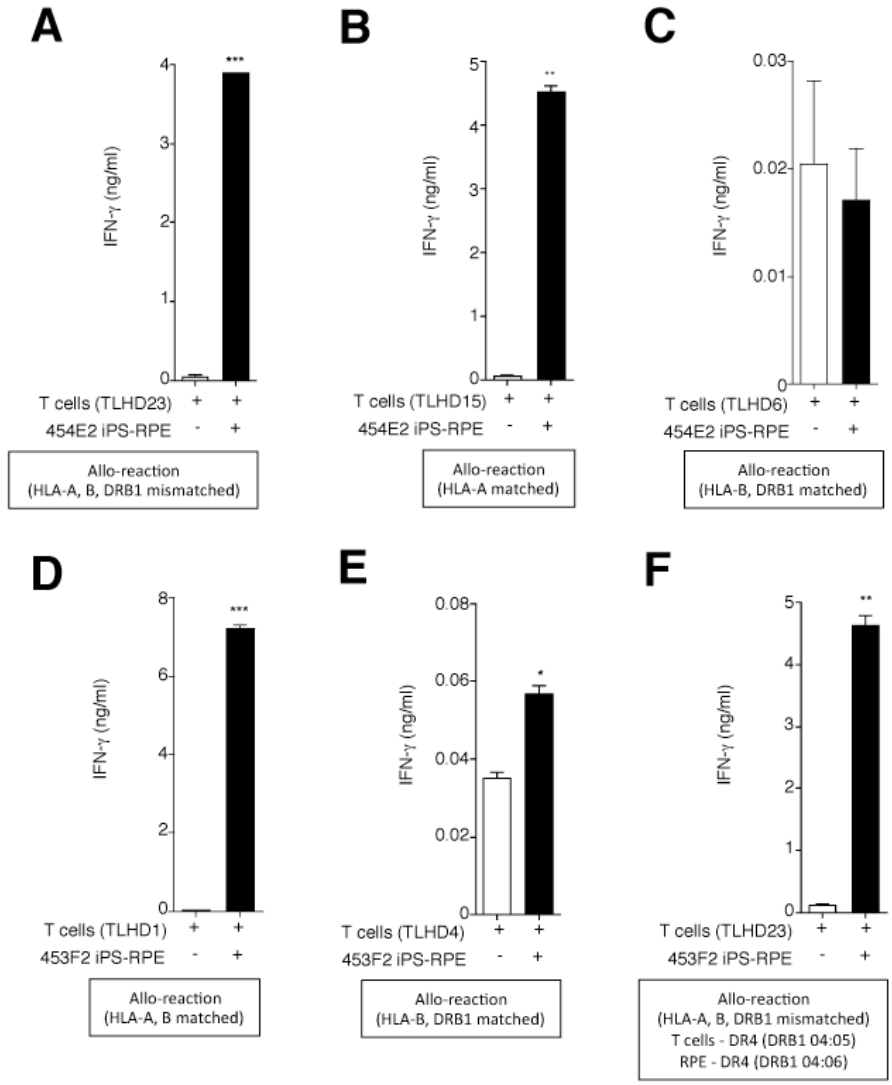
PBMC-RPE MLR assay (Ki-67 proliferation) with autogenic iPS cell–RPE cells. To evaluate another PBMC-RPE MLR assay with allogeneic HLA homozygote iPS-RPE cells (454E2, 453F2, and Ff-I01) and B cells as positive control cells, we first used autogenic iPS cell–RPE cells (TLHD1 PBMC and TLHD1 iPS-RPE cells), and we stained harvested PBMC after co-cultures: $CD4^+$ cells (helper T cells), $CD8^+$ cells (cytotoxic T cells), $CD11b^+$ cells (macrophages/monocytes), $CD19^+$ cells (B cells), and $CD56^+$ (NK cells). As expected, TLHD1 PBMC did not respond to auto-RPE cells in vitro, whereas all types of immune cells in PBMC (except CD11b) greatly responded to allogeneic B cells (PC).

Supplementary Figure 5.



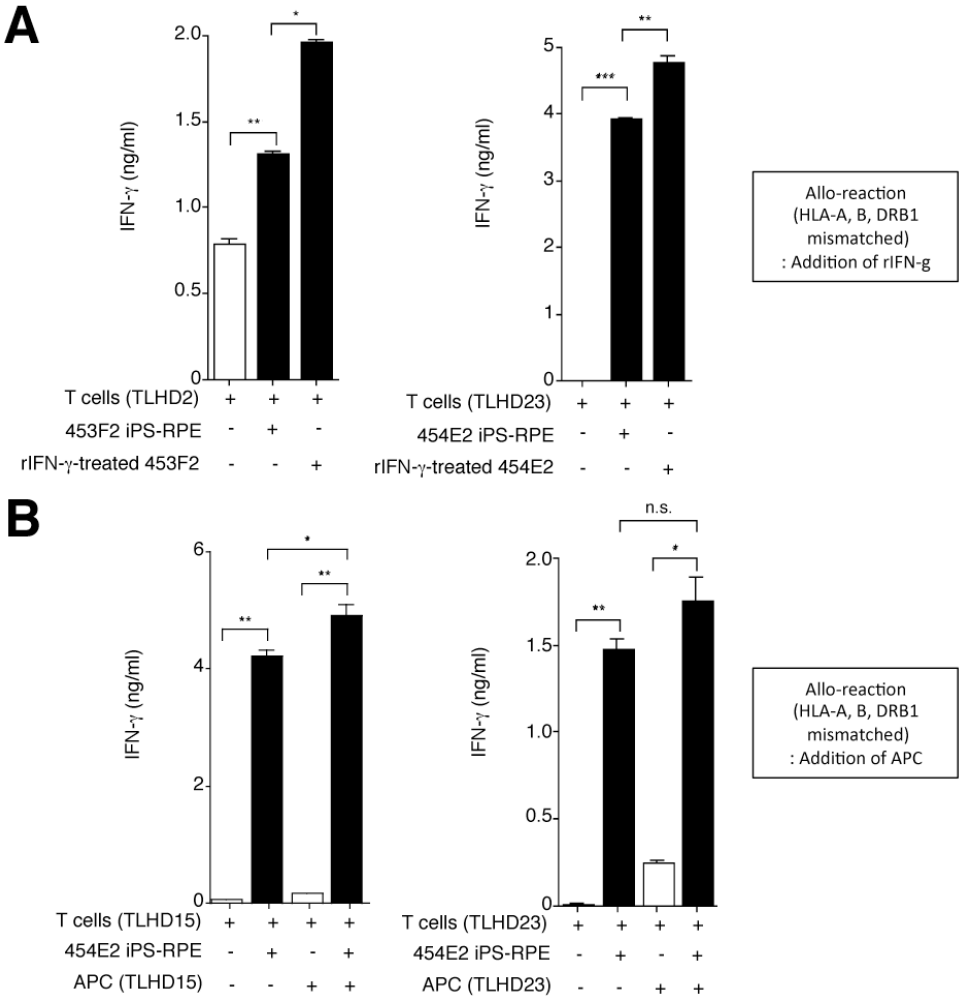
PBMC-RPE MLR assay by Ki-67 proliferation. TLHD21 PBMC versus TLHD1 iPS-RPE cells = HLA-A, B, DRB1 all mismatched, and TLHD21 PBMC versus Ff-I01 iPS-RPE cells = HLA-A, B, DRB1 all matched.

Supplementary Figure 6.



Recognition of HLA-A, B, DRB1 on allogeneic iPS-RPE cells from HLA homozygous donors by CD4$^+$ T cells obtained from MHC-matched donors. The graph indicates ELISA data for IFN-γ production by CD4$^+$ T cells exposed to 454E2 or 453F2 iPS-RPE cells. (**A**) TLHD23 T cells vs 454E2 RPE cells (HLA-A, B, and DRB1 mismatched), (**B**) TLHD15 T cells vs 454E2 RPE cells (HLA-A matched, HLA-B and DRB1 mismatched), (**C**) TLHD6 T cells vs 454E2 RPE cells (HLA-B and DRB1 matched, HLA-A mismatched), (**D**) TLHD1 T cells vs 453F2 RPE cells (HLA-A and B matched, HLA-DRB1 mismatched), (**E**) TLHD4 T cells vs 453F2 RPE cells (HLA- B and DRB1 matched, HLA-A mismatched), (**F**) TLHD23 T cells vs 453F2 RPE cells (HLA-DR serotype matched, but HLA-DRB1 genotype mismatched). Data represent the means ± S.E.M of three independent experiments. * P <0.05, ** P <0.005, *** P <0.0005, as compared to the control (open bar).

Supplementary Figure 7.



Response to allogeneic HLA homozygous iPS-RPE cells by $CD4^+$ T cells in the presence of recombinant IFN-γ or antigen-presenting cells (APC) (**A**) Purified $CD4^+$ T cells were cultured with iPS-RPE cells or IFN-γ–pretreated iPS-RPE cells for 48 hr, and the levels of IFN-γ in the supernatants were measured. The graph indicates data for IFN-γ production by $CD4^+$ T cells exposed to target iPS-RPE cells. Left panel – TLHD2 T cells vs 453F2 RPE cells (untreated) or IFN-γ–pretreated cells. Right panel – TLHD23 T cells vs 454E2 RPE cells (untreated) or IFN-γ–pretreated cells. (**B**) $CD4^+$ T cells were cultured with 454E2 iPS-RPE cells plus autogenic APC (left panel – TLHD15 T cells plus APC, right panel – TLHD23 T cells plus APC). Data represent the means ± S.E.M of three independent experiments. * P <0.05, ** P <0.005, *** P <0.0005, as compared to the $CD4^+$ T cells alone (open bar). n.s. – not significant.

Supplementary Table 1. Summary for HLA-allele type in T cells from healthy donors

No.	T cells	HLA-A	HLA-B	HLA-C	HLA-DRB1	HLA-DQB1	HLA-DPB1
1	TLHD1	11:01/-	15:01/67:01	04:01/07:02	09:01/16:02	03:03/05:02	02:02/05:01
2	TLHD2	26:01/A31:01	39:01/51:01	07:02/14:02	09:01/15:01	03:03/06:02	02:01/05:01
3	TLHD3	02:06/11:01	35:01/54:01	01:02/03:04	04:05/08:02	03:02/04:01	05:01/-
4	TLHD4	02:01/03:01	15:01/51:01	04:01/15:02	04:02/04:06	03:02/-	02:01/05:01
5	TLHD5	24:02/33:03	07:02/44:03	07:02/14:03	01:01/13:02	05:01/06:04	04:01/05:01
6	TLHD6	11:01/26:02	39:01/52:01	07:02/12:02	08:03/15:02	06:01/-	04:02/09:01
7	TLHD7	24:02/26:01	35:01/54:01	01:02/07:02	14:05/15:01	05:03/06:02	02:01/05:01
8	TLHD8	26:01/26:03	13:01/35:01	03:03/03:04	12:02/15:01	03:01/06:02	02:01/05:01
9	TLHD9	24:02/26:03	51:01/54:01	01:02/14:02	04:05/12:01	03:01/04:01	05:01/09:01
10	TLHD10	24:02/31:01	40:02/52:01	03:04/12:02	15:01/15:02	06:01/06:02	02:01/09:01
11	TLHD11	24:02/-	07:02/59:01	01:02/07:02	04:05/09:01	03:03/04:01	04:02/-
12	TLHD12	24:02/26:01	51:01/54:01	01:02/14:02	14:03/14:05	03:01/05:03	05:01/-
13	TLHD13	24:02/26:01	40:02/56:01	01:02/03:04	04:05/14:54	04:01/05:03	03:01/05:01
14	TLHD14	01:01/24:02	37:01/51:01	06:02/14:02	09:01/10:01	03:03/05:01	04:01/09:01
15	TLHD15	02:01/24:02	48:01/54:01	01:02/18:01	04:05/-	04:01/-	02:01/05:01
16	TLHD16	02:01/31:01	15:01/40:02	03:03/15:02	09:01/-	03:03/-	05:01/-
17	TLHD17	24:02/31:01	54:01/55:02	01:02/-	04:05/09:01	03:03/04:01	02:01/05:01
18	TLHD18	02:01/11:01	15:01/52:01	01:02/12:02	14:54/15:02	05:02/06:01	02:02/09:01
19	TLHD19	02:01/33:03	35:01/44:03	03:03/14:03	13:02/15:02	06:01/06:04	04:01/09:01
20	TLHD20	02:01/24:02	44:03/51:01	14:02/14:03	09:01/13:02	03:03/06:04	02:01/05:01
21	TLHD21	24:02/24:20	39:01/52:01	07:02/12:02	09:01/15:02	06:01/03:03	05:01/09:01
22	TLHD22	24:02/26:02	39:01/40:06	07:02/08:01	08:03/14:06	06:01/03:01	02:01/03:01
23	TLHD23	02:01/33:03	46:01/54:01	01:02/-	04:05/08:03	04:01/06:01	05:01/09:01
24	TLHD24	24:02/33:03	40:02/44:03	03:04/14:03	08:03/14:54	05:03/06:01	02:01/-
25	TLHD25	24:02/33:03	44:03/55:02	12:03/14:03	04:05/13:02	04:01/06:04	02:02/05:01
26	TLHD26	02:01/26:03	40:06/44:03	08:01/14:03	04:05/08:03	04:01/06:01	04:02/05:01

The sample of HLA typing was all PBMC (n=26).

Supplementary Table 2. Results for T-cell response to HLA homozygous iPS-RPE cells

No.	T cells	T-cell response to 454E2*	HLA-matched to 454E2 iPS-RPE
1	TLHD1	(+)	
2	TLHD2	(+)	
3	TLHD3	(+)	
4	TLHD4	(+)	
5	TLHD5	(+)	A*24:02
6	TLHD6	(-)	B*52:01/C*12:02/DRB1*15:02 /DQB1*06:01
7	TLHD7	(+)	A*24:02
8	TLHD8	(+)	
9	TLHD9	(+)	A*24:02
10	TLHD10	(-)	A*24:02 /B*52:01/C*12:02/DRB1*15:02 /DQB1*06:01
11	TLHD11	(-)	A*24:02
12	TLHD12	(+)	A*24:02
13	TLHD13	(+)	A*24:02
14	TLHD14	(+)	A*24:02
15	TLHD15	(+)	A*24:02
16	TLHD16	(+)	
17	TLHD17	(+)	A*24:02
18	TLHD18	(-)	B*52:01/C*12:02/DRB1*15:02 /DQB1*06:01
19	TLHD19	(-)	DRB1*15:02 /DQB1*06:01
20	TLHD20	(+)	A*24:02
21	TLHD21	(-)	A*24:02/B*52:01/C*12:02/DRB1*15:02/DQB1*06:01
22	TLHD22	(+)	A*24:02 /DQB1*06:01
23	TLHD23	(+)	
24	TLHD24	(+)	A*24:02
25	TLHD25	(+)	A*24:02
26	TLHD26	(+)	DQB1*06:01

* T-cell response to 454E2 - Compared with IFN-γ ELISA data of T cells without RPE (controls), (+) indicates high, and (-) indicates low or similar levels in the results of T cells exposed to 454E2 iPS-RPE cells.