Group	n	Graft loss days	MST
Saline	5	28, 28, 31, 31, 31	31
PEG	3	28, 35, 35	35
FR104 (1 mg/kg)	5	31, 42, 42, 56, 56	42
FR104 (5 mg/kg)	5	42, 42, 56, 63, 63	56
CTLA-4 Ig (2 mg/kg)	5	31, 31, 31, 31, 35	31
CTLA-4 Ig (10 mg/kg)	5	28, 31, 31, 31, 35	31

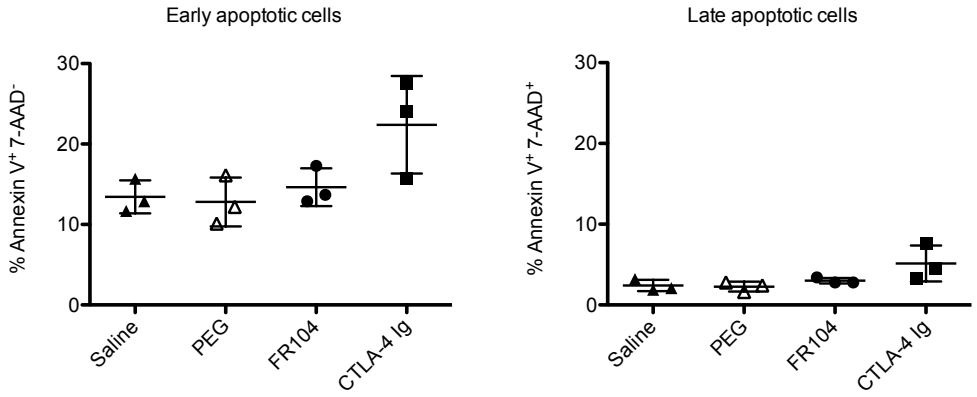
Table 1. *Skin graft survival times for each treatment group in Figure 2.*

Group	n	Graft loss days	MST
Saline	3	32, 34, 36	34
Saline + Tregs	5	42, 44, 72, >100, >100	72
FR104	5	34, 42, 56, 62, 64	56
FR104 + Tregs	5	36, >100, >100, >100, >100	>100
CTLA-4 Ig	5	32, 32, 40, 40, 40	40
CTLA-4 Ig + Tregs	5	34, 36, 36, 38, 38	36

Table 2. *Skin graft survival times of each treatment group in Figure 6.*

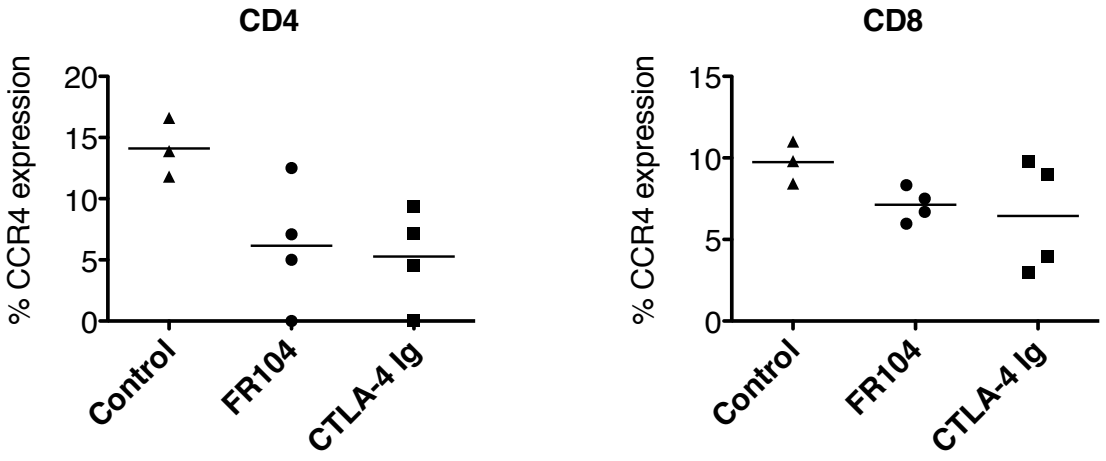
Group	n	Graft loss days	MST
Saline + Tregs	5	30, 34, 44, 44, 47	44
FR104 + Tregs	5	54, 61, 89, >100, >100	89
CTLA-4 Ig + Tregs	5	27, 30, 30, 34, 44	30

Table 3. *Skin graft survival times of each treatment group in Figure 7.*



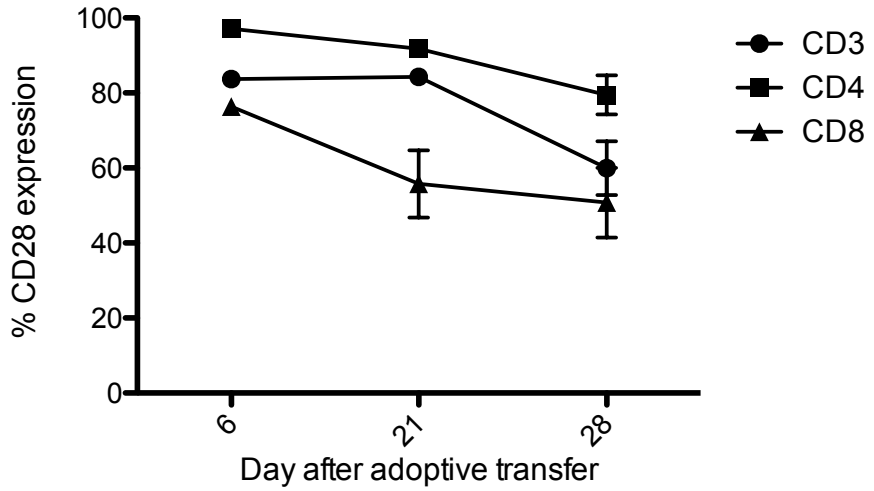
Supplementary Figure S1. FR104 and CTLA-4 Ig do not promote apoptosis *in vivo*

$5x10^6$ human PBMCs were adoptively transferred by intraperitoneal injection on day 0. Mice were treated intravenously on day 1 and day 3 with saline (n=3), PEG (n=3), FR104 (n=3) or CTLA-4 Ig (n=3). Peritoneal cells were harvested on day 6 after adoptive transfer. Annexin V levels were similar between groups indicating that FR104 and CTLA-4 Ig do not promote apoptosis. Data are shown as mean ± SD.



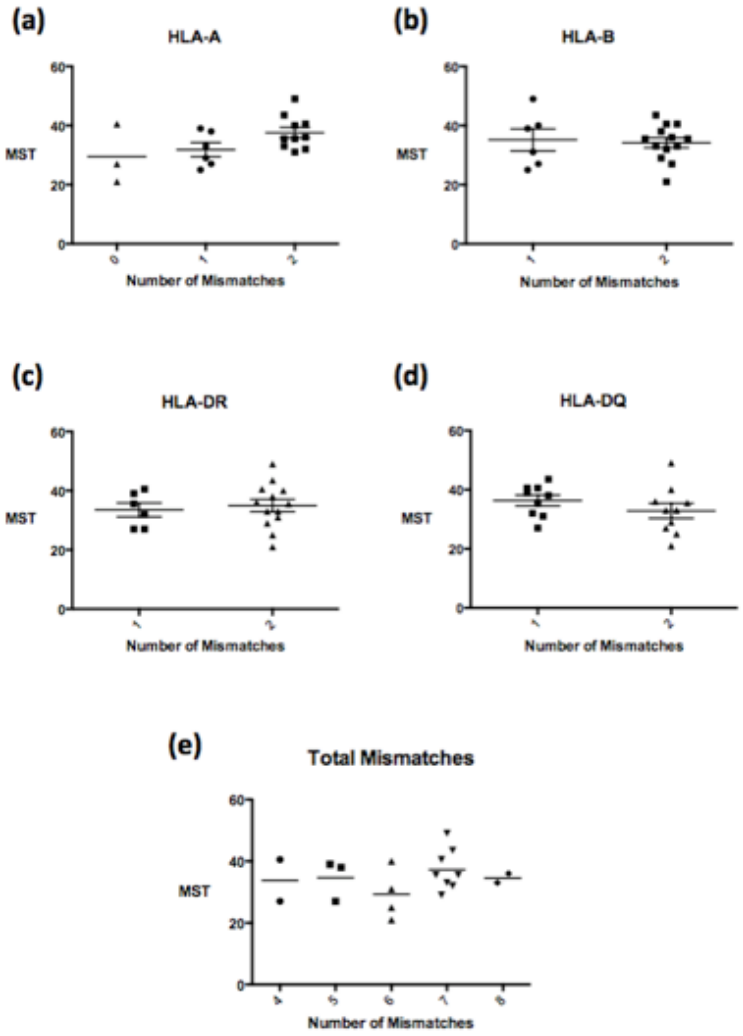
Supplementary Figure S2. FR104 and CTLA-4 Ig do not suppress CCR4 expression on T lymphocytes *in vivo*

CCR4 expression on $CD4^+$ and $CD8^+$ T lymphocyte in peripheral blood was analysed by flow cytometry from mice treated with saline, PEG, FR104 or CTLA-4 Ig at day 28 after adoptive transfer of PBMCs.



Supplementary Figure S3. Time course of CD28 expression on human T cells in humanised mice.

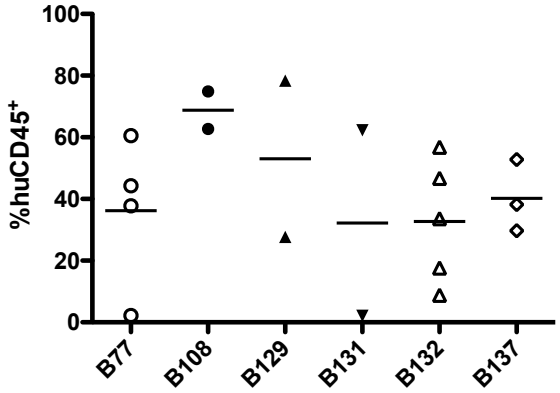
CD28 expression on human T cells was measured from day 6 to day 28 *in vivo* (n=3). Expression levels altered but followed a similar trend among groups.



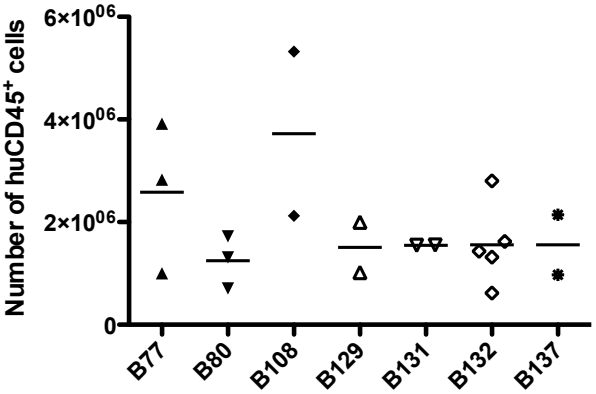
Supplementary Figure S4. The degree of HLA mismatch between skin and PBMC donor dose not significantly alter the kinetics of rejection.

Pooled data are presented from multiple assays performed in humanized skin transplant model. BALB/c- Rag2$^{-/-}$cγ$^{-/-}$ mice received a human skin transplant and 5 weeks later an adoptive transfer of 5x10^6 PBMC from a donor allogeneic to the skin graft donor. 19 separate groups of mice (minimum n=3 mice per group) were transplanted using a distinct skin and PBMC donor combination for each group of mice. The MST for each group of mice was calculated. PBMC and skin graft donors were tissue typed and the number of HLA mismatches between PBMC donor and skin graft donor calculated for each group across the HLA loci of HLA-A, HLA-B, HLA-DR and HLA-DQ. The number of HLA mismatches is shown plotted against the MST for that particular group for: **(a)** mismatches in the HLA-A locus; **(b)** mismatches in the HLA-B locus; **(c)** mismatches in the HLA-DR locus; **(d)** mismatches in the HLA-DQ locus; and **(e)** total mismatches across HLA-A, HLA-B, HLA-DR and HLA-DQ. There was no significant reduction in MST with increased total HLA mismatches (p=0.5703, with total mismatches ranging from 4 to 8 and a median of 7 HLA mismatches) or with increased mismatches in any one particular locus (HLA-A: p=0.1043, HLA-B: p=0.7853, HLA-DR: p=0.6823, HLA-DQ: p=0.2877, with 1 or 2 mismatches in each locus apart from HLA-A where mismatches ranged from 0 to 2).

A.



B.



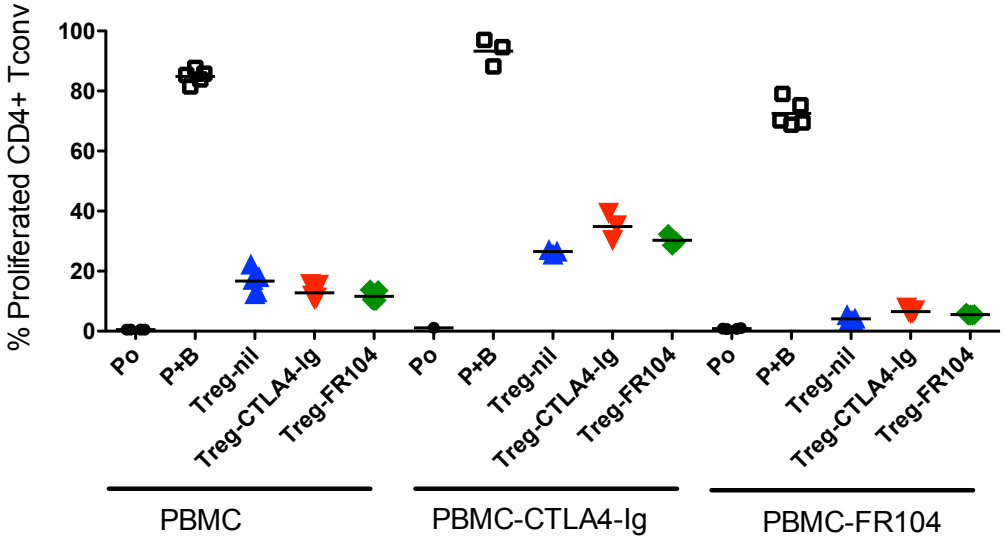
C.

PBMC donor	MST (days)	HLA mismatches
B77	41	7
B108	31	6
B129	35.5	8
B131	35.5	7
B132	33	7
B137	27	5

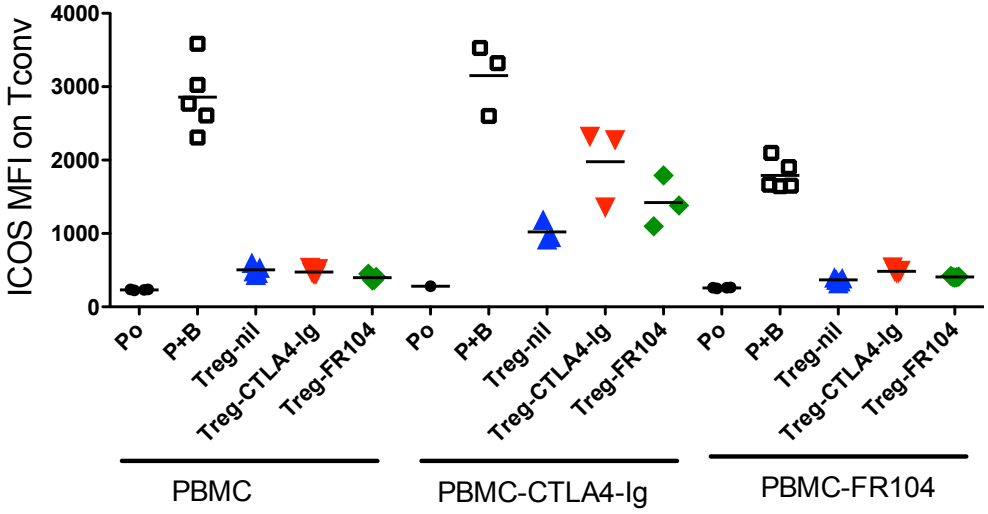
Supplementary Figure S5. Reconstitution analysis of BALB/c-Rag2-/-cγ$^{-/-}$ mice receiving a human skin graft and 5x10^6 allogeneic PBMC.

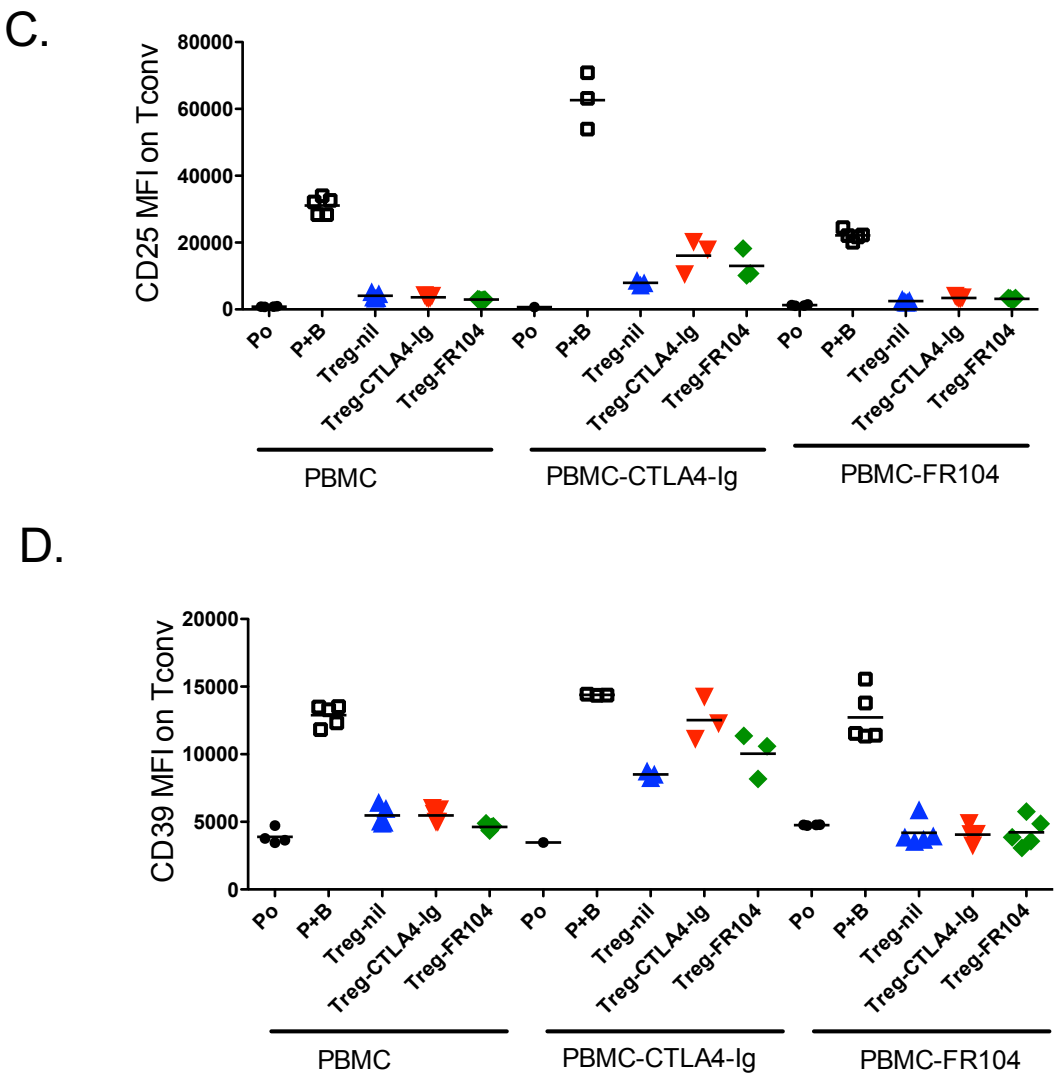
BALB/c-Rag2$^{-/-}$cγ$^{-/-}$ mice received a human skin transplant and 5 weeks later were reconstituted with 5x10^6 allogeneic PBMC. (a) % human CD45$^+$ cells and (b) total human CD45$^+$ cell numbers from cellular preparation of spleens taken at the point of skin graft rejection from BALB/c-Rag2$^{-/-}$cγ$^{-/-}$ mice which had received a human skin graft and 5x10^6 allogeneic PBMC 5 weeks later. Each B- number represents a separate skin rejection assay where a particular skin and PBMC donor was used.. Data are represented as individual data points + mean. (c) The number of HLA mismatches (across HLA-A, B, DR and DQ) between skin and PBMC donors and the respective MST is shown for each group of mice. At

A.


% Proliferated CD4+ Tconv
100
80
60
40
20
0
Po
P+B
Treg-nil
Treg-CTLA4-Ig
Treg-FR104
PBMC
PBMC-CTLA4-Ig
PBMC-FR104

B.


ICOS MFI on Tconv
4000
3000
2000
1000
0
Po
P+B
Treg-nil
Treg-CTLA4-Ig
Treg-FR104
PBMC
PBMC-CTLA4-Ig
PBMC-FR104



Supplementary Figure S6. CTLA-4Ig protects conventional T cells from Treg-mediated suppression

PBMCs were VPD-stained and pre-treated in vitro with CTLA-4Ig, FR104, or no pre-treatment (indicated on lower section of figures). PBMC were then assessed in an in vitro suppression assays with Tregs which were also pre-treated with CTLA-4If, FR104, or no pre-treatment. (A). In all assays, PBMC proliferated in response to stimulation with anti-CD3/anti-CD28 beads (P+B), but not in the absence of beads (Po). After addition of Tregs, PBMC proliferation was suppressed regardless of whether Tregs were pre-incubated with an immunosuppressive agent. However, PBMCs that were pre-treated with CTLA-4Ig were significantly resistant to Treg-mediated suppression (middle panel, one-way ANOVA $p<0.0001$), whereas PBMCs pre-treated with FR104 were suppressed to a similar degree as non-pre-treated PBMCs (right panel). Similarly, CTLA-4Ig pre-treated PBMCs exhibited higher levels of activation marker expression (mean fluorescence intensity, MFI) than those pre-treated with FR104 ($p<0.0001$ for each): (B) ICOS, (C) CD25 and (D) CD39. Data are presented as individual data points + mean.