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Supporting Information

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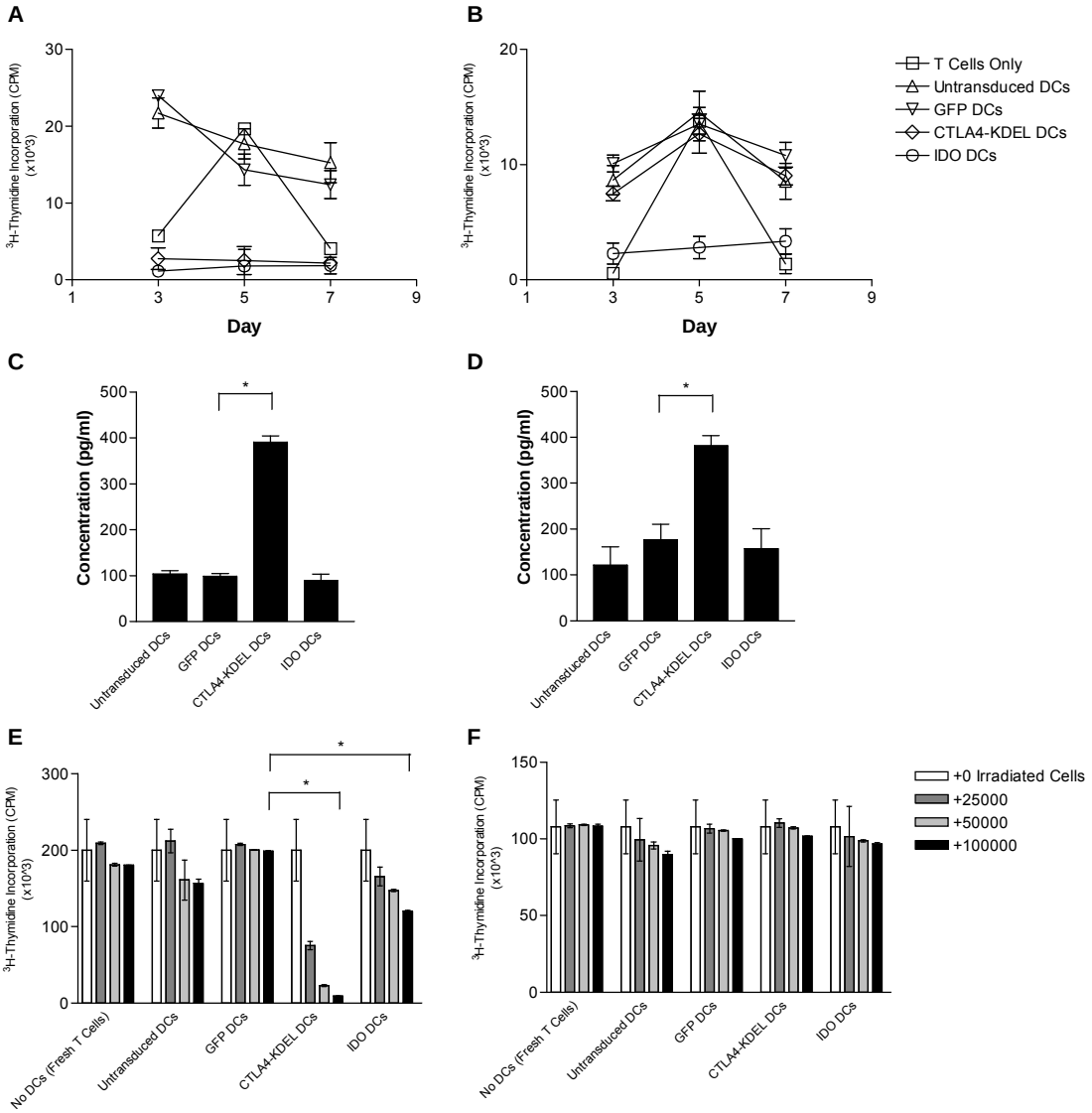
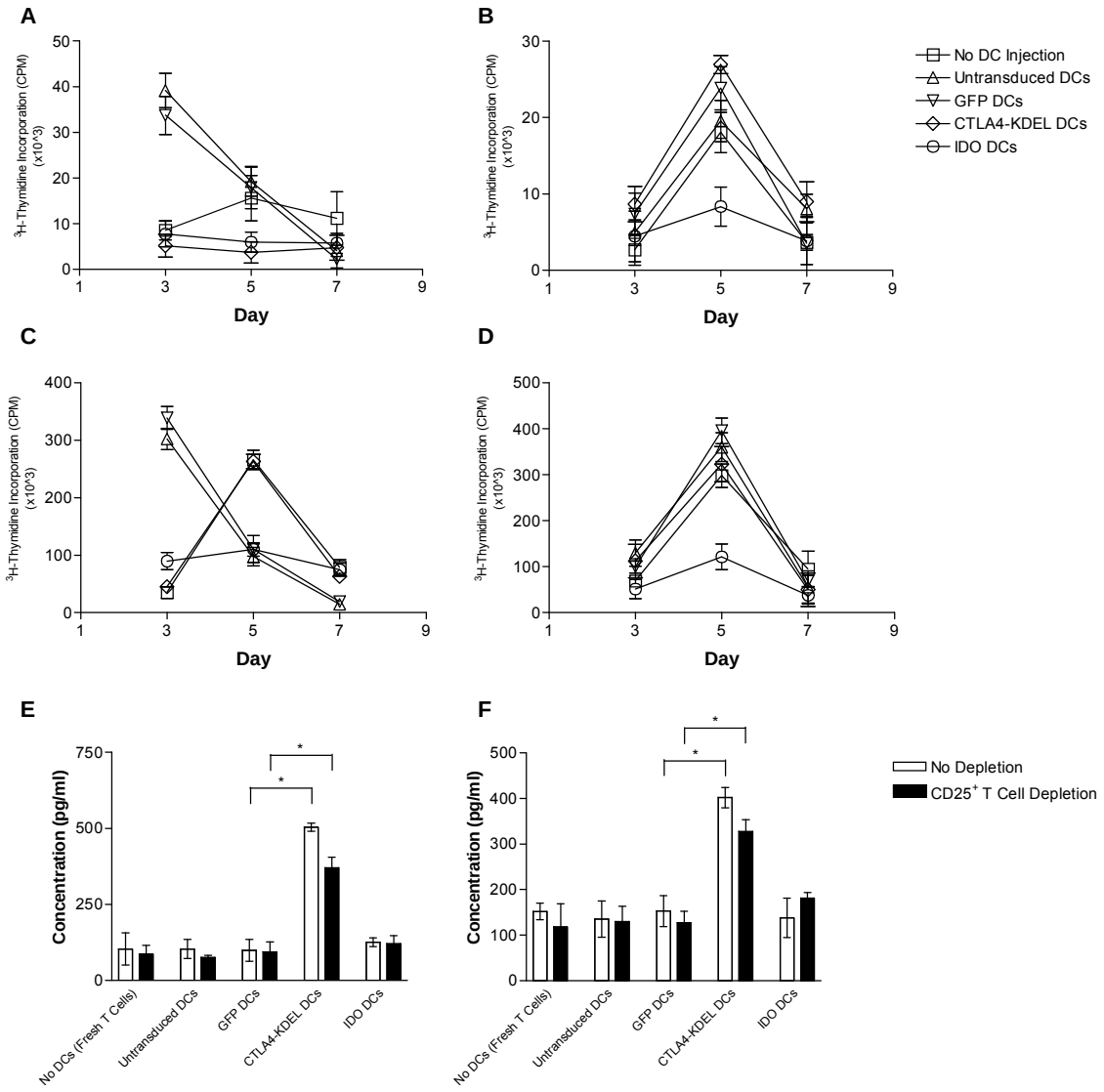
Dendritic cell modification as a route to inhibiting corneal graft rejection by the indirect pathway of allorecognition

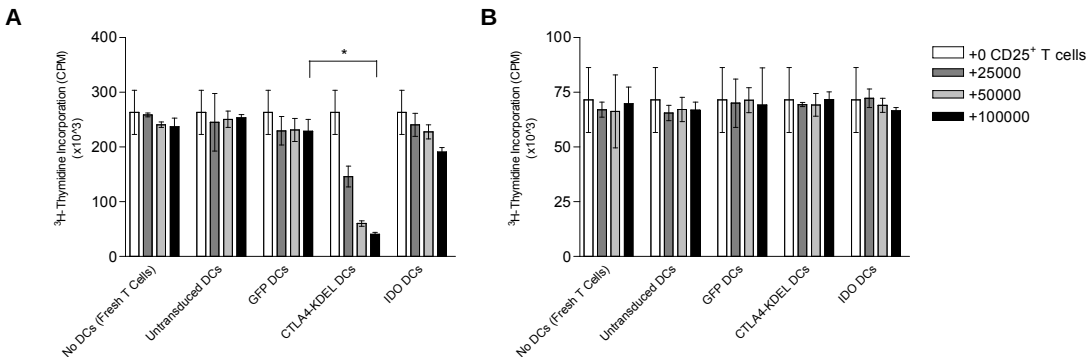


SUPPORTING FIGURE 1. Induction of donor alloantigen-specific T-cell anergy and regulation in vitro.

BALB/c DCs were transduced either with EIAV GFP (control), EIAV CTLA4-KDEL or EIAV IDO, followed by stimulation with LPS. The transduced (and untransduced) DCs were subsequently incubated in vitro with C3H/He-derived $CD4^+$ T cells. After 10 days, the T cells were harvested and rechallenged in vitro with **(A)** donor BALB/c DCs or **(B)** third-party C57BL/6 DCs, and $CD4^+$ T-cell proliferation assessed by thymidine incorporation on days 3, 5 and 7. **(C-D)** Culture supernatant from the donor DC rechallenge assay was harvested for detection of the immunoregulatory cytokines **(C)** TGF-β and **(D)** IL-10 by ELISA. **(E-F)** In addition, after 10 days of the primary DC - $CD4^+$ T-cell coculture, the T cells were harvested, irradiated and added (0-10^5 $CD4^+$ T cells) to a primary MLR between freshly-isolated C3H-derived $CD4^+$ T cells and **(E)** donor BALB/c DCs or **(F)** third-party C57BL/6 DCs. T-cell proliferation was assessed by thymidine incorporation after 5 days. All results are shown as the mean ± SD of triplicate wells and are representative of three independent experiments performed. * $p<0.05$, two-tailed *t*-test..

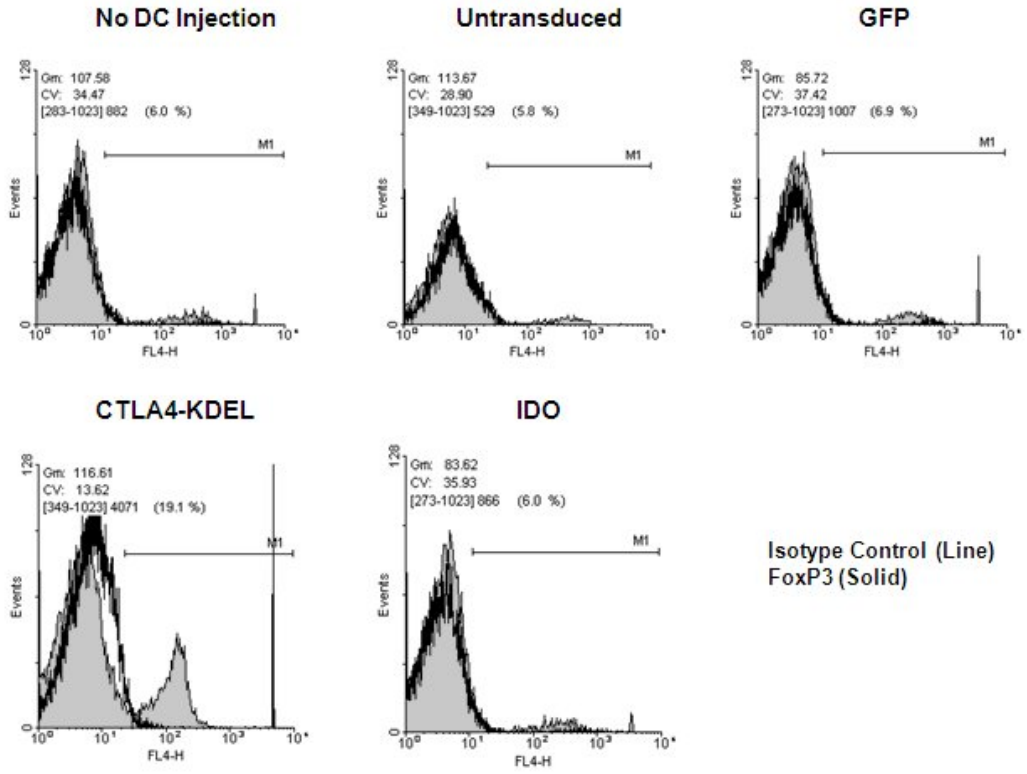


SUPPORTING FIGURE 2. Induction of donor alloantigen-specific T-cell anergy in vivo and production of immunoregulatory cytokines.
2.5x10^6 BALB/c DCs (either untransduced, or transduced with EIAV GFP (control), EIAV CTLA4-KDEL or EIAV IDO) were injected intravenously into C3H/He mice. **(A-D)** After 10 days, CD4$^+$ T cells purified from the spleens of injected mice were rechallenged in vitro with **(A)** donor BALB/c DCs or **(B)** third-party C57BL/6 DCs, and CD4$^+$ T-cell proliferation assessed by thymidine incorporation on days 3, 5 and 7. The purified CD4$^+$ T cells were also rechallenged with **(C)** donor BALB/c DCs or **(D)** third-party C57BL/6 DCs in the presence of 100 U/ml exogenous IL-2. **(E-F)** Culture supernatants from donor DC rechallenge assays (including those from CD4$^+$CD25$^+$ T-cell depleted-rechallenge assays) were harvested on day 7 for detection of the immunoregulatory cytokines **(E)** TGF-β and **(F)** IL-10 by ELISA. All results are shown as the mean ± SD of triplicate wells and are representative of three independent experiments performed. * p<0.05, two-tailed t-test.



SUPPORTING FIGURE 3. Generation of donor alloantigen-specific Treg cells in vivo with capacity for linked suppression.

$2.5x10^6$ BALB/c DCs (either untransduced, or transduced with EIAV GFP (control), EIAV CTLA4-KDEL or EIAV IDO) were injected intravenously into C3H/He mice. **(A-B)** After 10 days, $CD4^+CD25^+$ T cells purified from the spleens of injected mice were irradiated and added in increasing numbers (0-10^5 $CD4^+CD25^+$ T cells) to a primary MLR between freshly-isolated C3H-derived $CD4^+$ T cells and **(A)** donor BALB/c DCs or **(B)** third-party C57BL/6 DCs. T-cell proliferation was assessed by thymidine incorporation after 5 days. All results are shown as the mean ± SD of triplicate wells and are representative of three independent experiments performed. * $p<0.05$, two-tailed *t*-test.



SUPPORTING FIGURE 4. Generation of FoxP3$^+$CD4$^+$CD25$^+$ T cells in vivo
2.5x10^6 BALB/c DCs (either untransduced, or transduced with EIAV-GFP (control), EIAV-CTLA4-KDEL or EIAV-IDO) were injected intravenously into C3H/He mice. After 10 days, CD4$^+$CD25$^+$ T cells purified from the spleens of injected mice were stained and analysed by flow cytometry for expression of FoxP3. The results shown in are representative of three independent experiments.