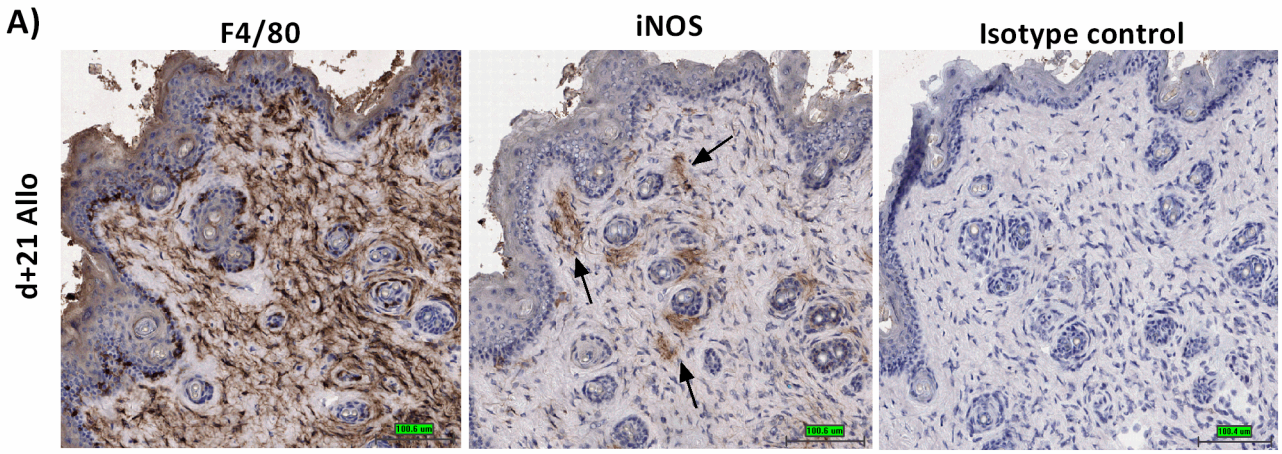
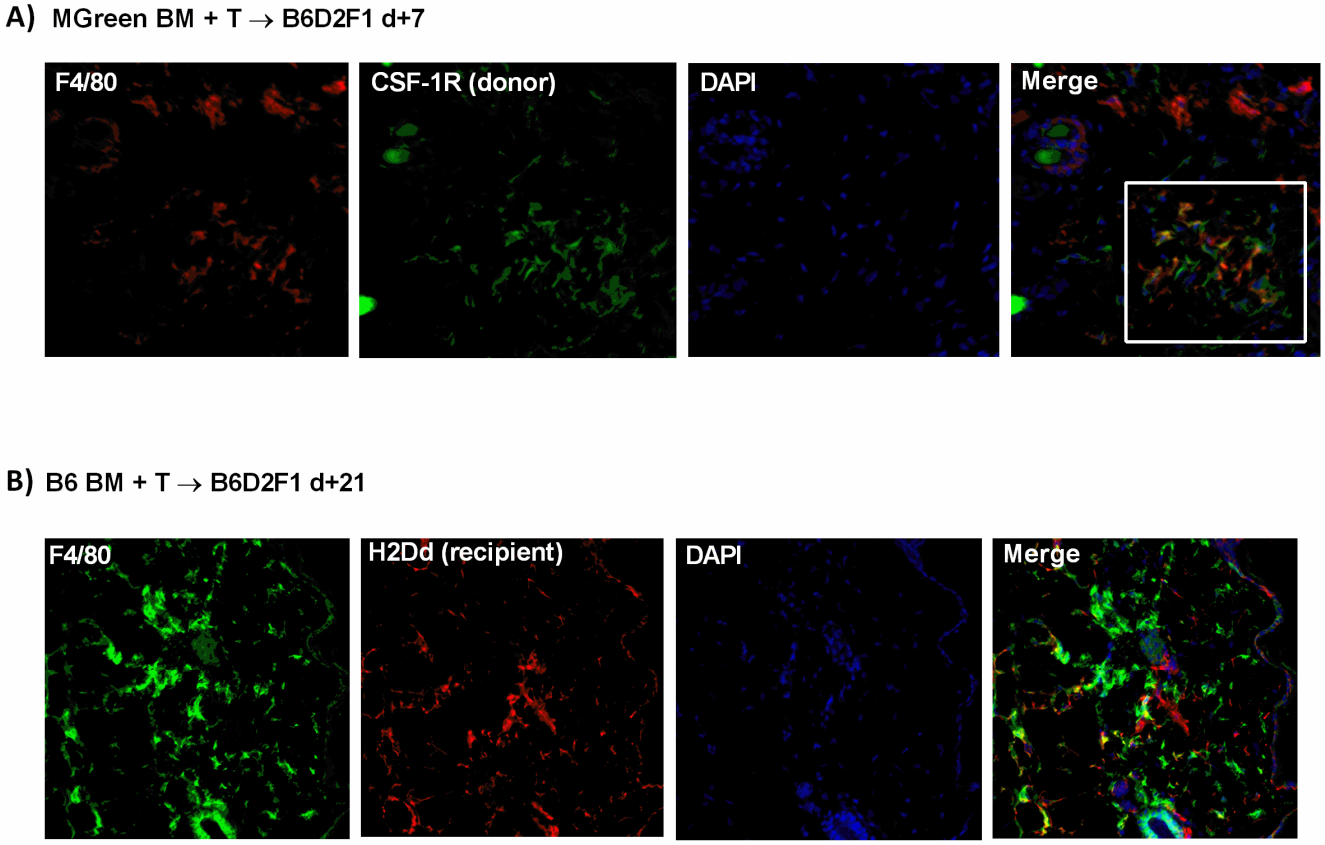
Supplemental figure 1.



Supplemental Figure 1. F4/80^{+} macrophages express low levels of iNOS 21 days post-transplant

A) Representative IHC images of from lethally irradiated B6 recipients that received G-CSF mobilised Balb/c (CD45.1) grafts. IHC for F4/80 and iNOS expression illustrates that at 21 days post-transplant minimal F4/80^{+} cells are iNOS positive. Specificity of staining was confirmed using a matched isotype control. Slides viewed using Aperio Image Scope software at x5 magnification.

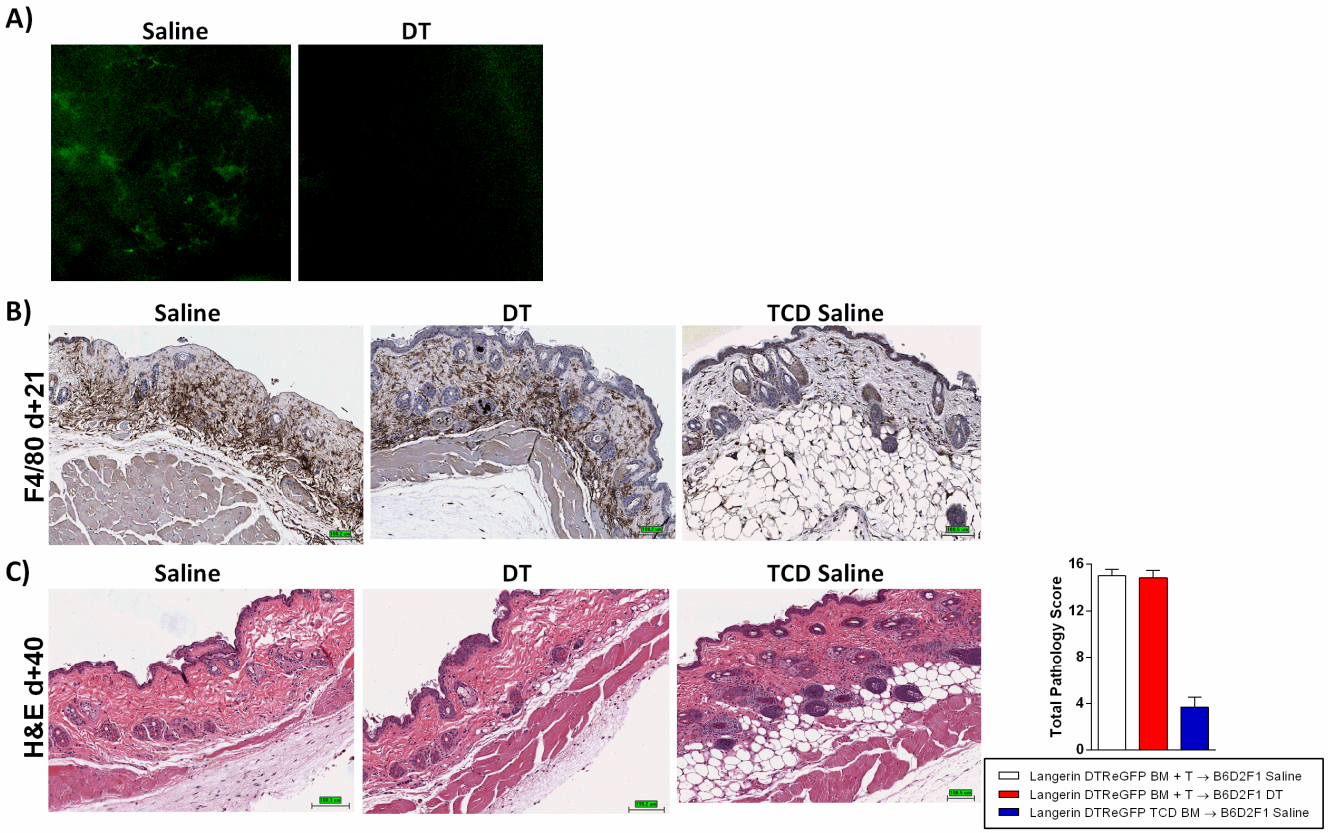
Supplemental Figure 2.



Supplemental Figure 2. Distribution of donor and host macrophages post-transplant.

A) Representative IF images at 7 days post-transplant from skin of lethally irradiated B6D2F1 recipients which received BM + T cell grafts from MacGreen mice (CSF-1R promoter driving eGFP). IF confirms the presence of donor macrophages ($F4/80^{+}CSF\text{-}1R^{+}$) in the skin by day 7 post-transplant (Boxed area in merged image).B) Representative IF images at 21 days post-transplant from skin of lethally irradiated B6D2F1($H2D^{d}$) recipients which received BM + T cell grafts from C57Bl/6 mice. IF illustrates that by 21 days post-transplant there are few $H2D^{d}$+ (Red) cells that are $F4/80^{+}$ (Green), with the majority of $F4/80^{+}$ cells (Green) being $H2D^{dneg}$ (i.e. very minimal numbers of merged yellow cells). Images were captured using an Applied precision delta vision deconvolution microscope (x20 magnification), viewed using ImageJ 1.44p.

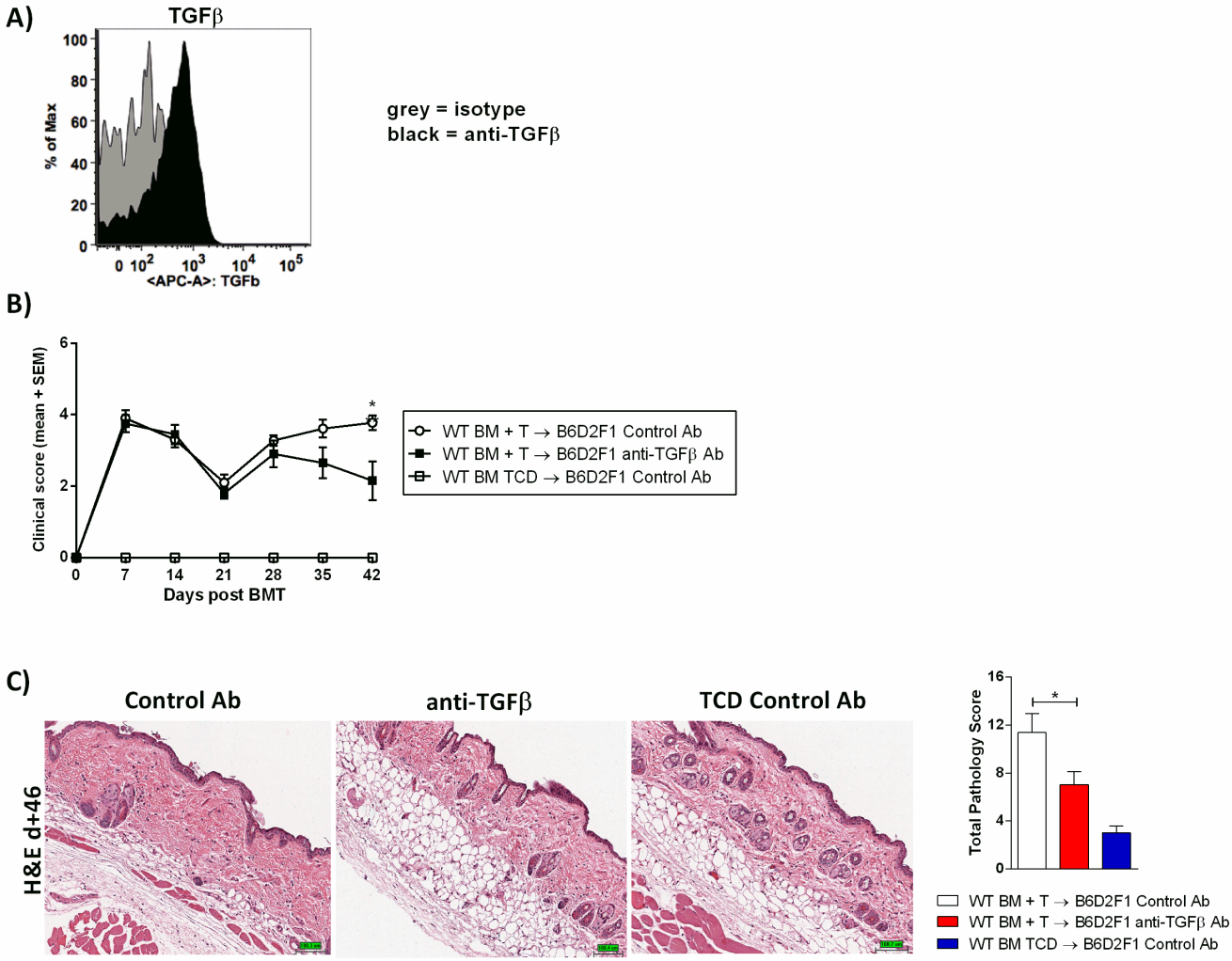
Supplemental Figure 3.



Supplemental Figure 3. Langerhans cells do not contribute to chronic GVHD in skin

A) Representative IF images from lethally irradiated B6D2F1 recipients that received BM + T cell grafts from langerin-DTReGFP mice and treated with saline or DT from *d*+7 to *d*+40 . Image illustrates the depletion of $eGFP^+$ cells in the epidermis after DT treatment. B) IHC for F4/80 expression at *d*+21 confirms that administration of DT did not affect $F4/80^+$ macrophage infiltration. C) H&E images and semi-quantitative histopathology, confirmed that depletion of $langerin^+$ cells post-transplant did not alter cutaneous pathology (p=0.861) (n=5 saline, n=6 DT, n=3 TCD Saline). Statistical comparisons were calculated using 2-tailed Mann Whitney tests and data is represented as mean ± SEM. IF images were viewed on an Olympus BX61 Nuance microscope (x63 magnification), images viewed using ImageJ 1.44p. H&E slides viewed using Aperio Image Scope software at x5 magnification.

Supplemental Figure 4.



Supplemental Figure 4. TGF-β blockade post-transplant attenuates chronic GVHD.

A) Representative FACS histogram illustrating TGF-β expression by $CD11b^{+}F4/80^{+}Ly6C^{lo}$ PB monocytes from B6D2F1 recipients of B6 BM + T grafts on *d*+46 post-transplant after 2hr culture with 100ng LPS. Gating strategy is the same as shown in figure 3. B) Lethally irradiated B6D2F1 recipients of B6 BM + T cells grafts were treated with either control or anti-TGFβ antibody from *d*+7 to *d*+46 post-transplant (20ug/3x weekly). Mice which received anti-TGFβ treatment had a significantly lower GVHD clinical scores by *d*+42 post-transplant (*p=0.035). C) H&E images and semi-quantitative histopathology, confirmed that anti-TGFβ treatment post-transplant resulted in a significant reduction in cutaneous pathology (*p=0.018; n=8 control Ab, n=11 anti-TGFβ, n=3 TCD control Ab). Statistically significant differences were calculated using 2-tailed Mann Whitney test and data is represented as mean ± SEM. Slides viewed using Aperio Image Scope software at x5 magnification.