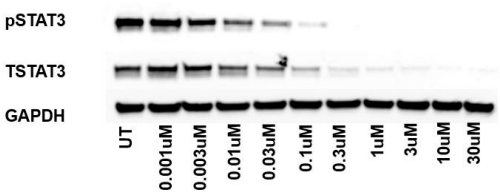
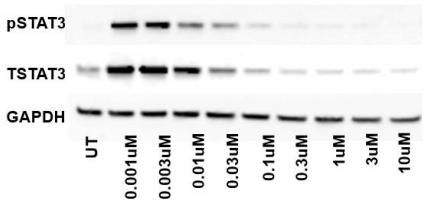
Supplemental Figure 8. Knockdown/potency of mouse STAT3 ASO and danvatirsen in cultured mouse and human macrophages

A.

Mouse M2 macrophages IC50 50nM
Treated with mouse STAT3 ASO

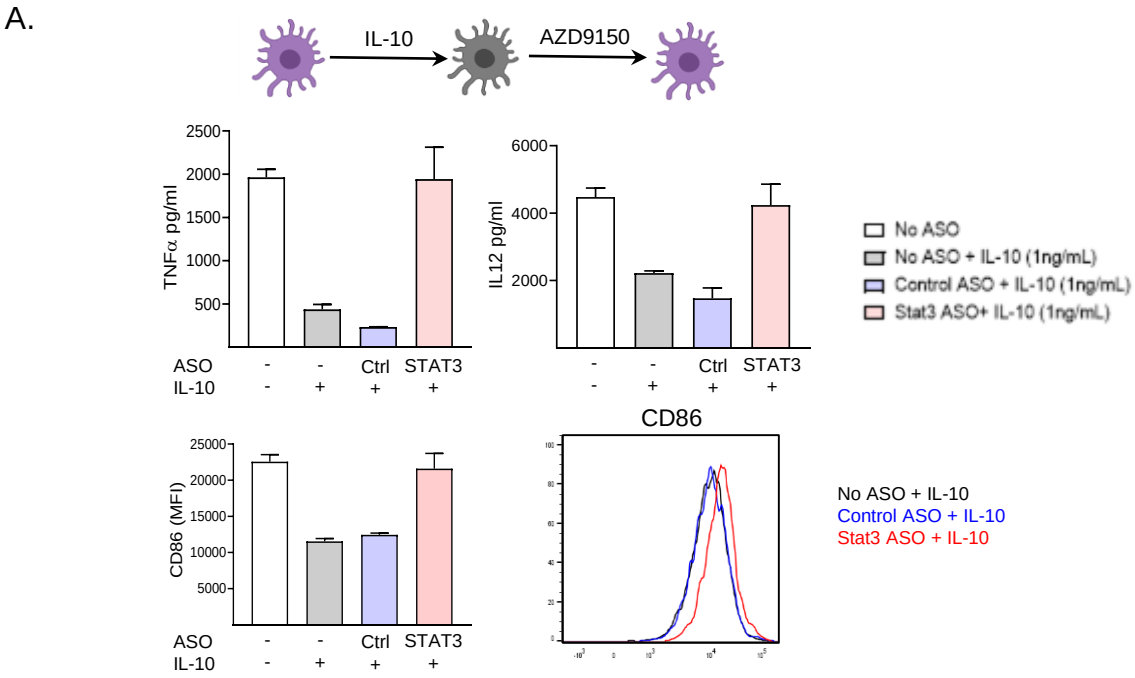


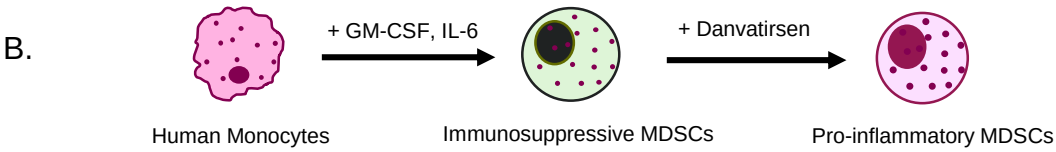
Human Macrophages: IC50 60nM
Treated with danvatirsen

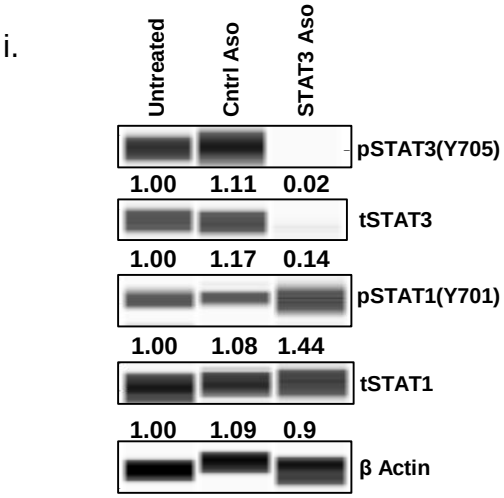


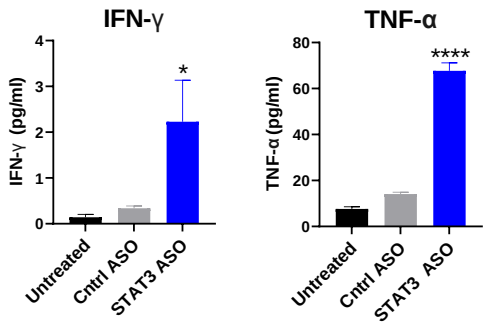
Supplemental Figure 8. A. Mouse or human 'suppressive' macrophages were treated with the mSTAT3 ASO or danvatirsen, respectively, at the concentrations indicated, for 3 days. Cell lysates were harvested for western blot analysis of phospho-stat3 or total stat3.

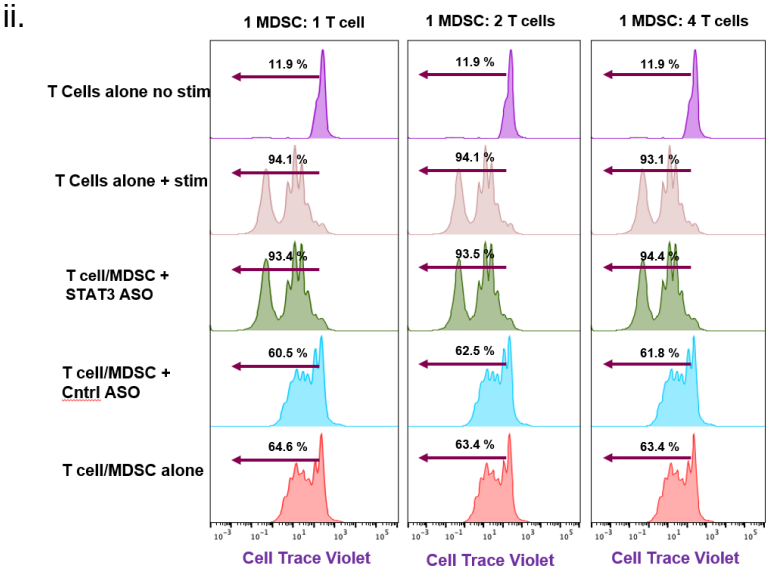
Supplemental Figure 9. Effect of Danvatirsen treatment in human DCs, MDSCs and human T cells





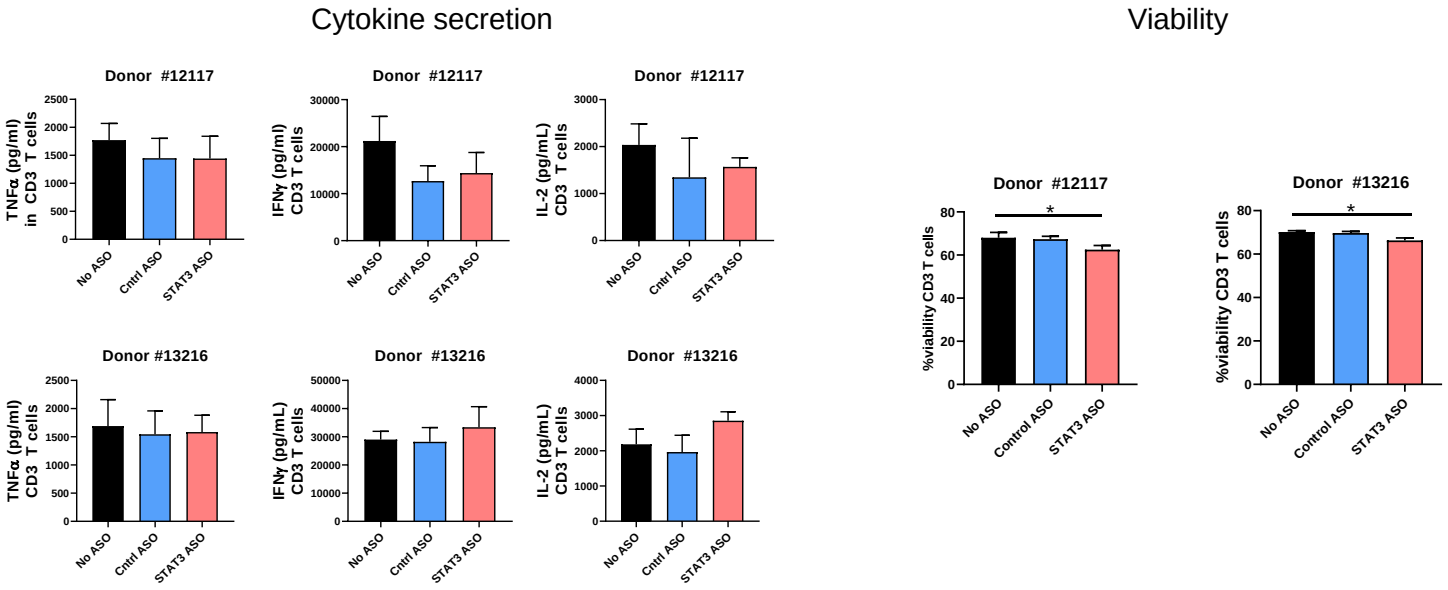






Supplemental Figure 9. Effect of Danvatirsen treatment in human DCs, MDSCs and human T cells

C.



Supplemental Figure 9. A. Human monocytes differentiated into dendritic cells using GM-CSF and IL-4, followed by IL-10 treatment to mimic a suppressed TME. Human DC cultures were subsequently treated with 5uM Control ASO or 5uM Danvatirsen, and analyzed for cytokine secretion and cell surface expression of CD80/CD86. **B.** Human monocytes differentiated into MDSCs using GM-CSF and IL-6 followed by treatment with 10uM danvatirsen. Supernatants were harvested for analysis of cytokines IFN γ and TNF **i**. Lysates were analyzed for pSTAT3(Y785), tSTAT3, pSTAT1(Y701), tSTAT1, **β**-Actin by western blot. ii. Human T cells isolated from autologous PBMCS were co-cultured with above treated MDSC and proliferation was measured using CellTrace Violet cell proliferation assay. Experiment is representative of n=3. **C.** Human T cells isolated from PBMCs were cultured ex vivo and treated with control ASO or danvatirsen (5uM) for 3 days, to evaluate cytokine secretion and viability (CD3 T cells only). Data was generated with two human donors, in triplicate, and One-way ANOVA to calculate statistics (*p<0.05 **p<0.01 ***p<0.001).