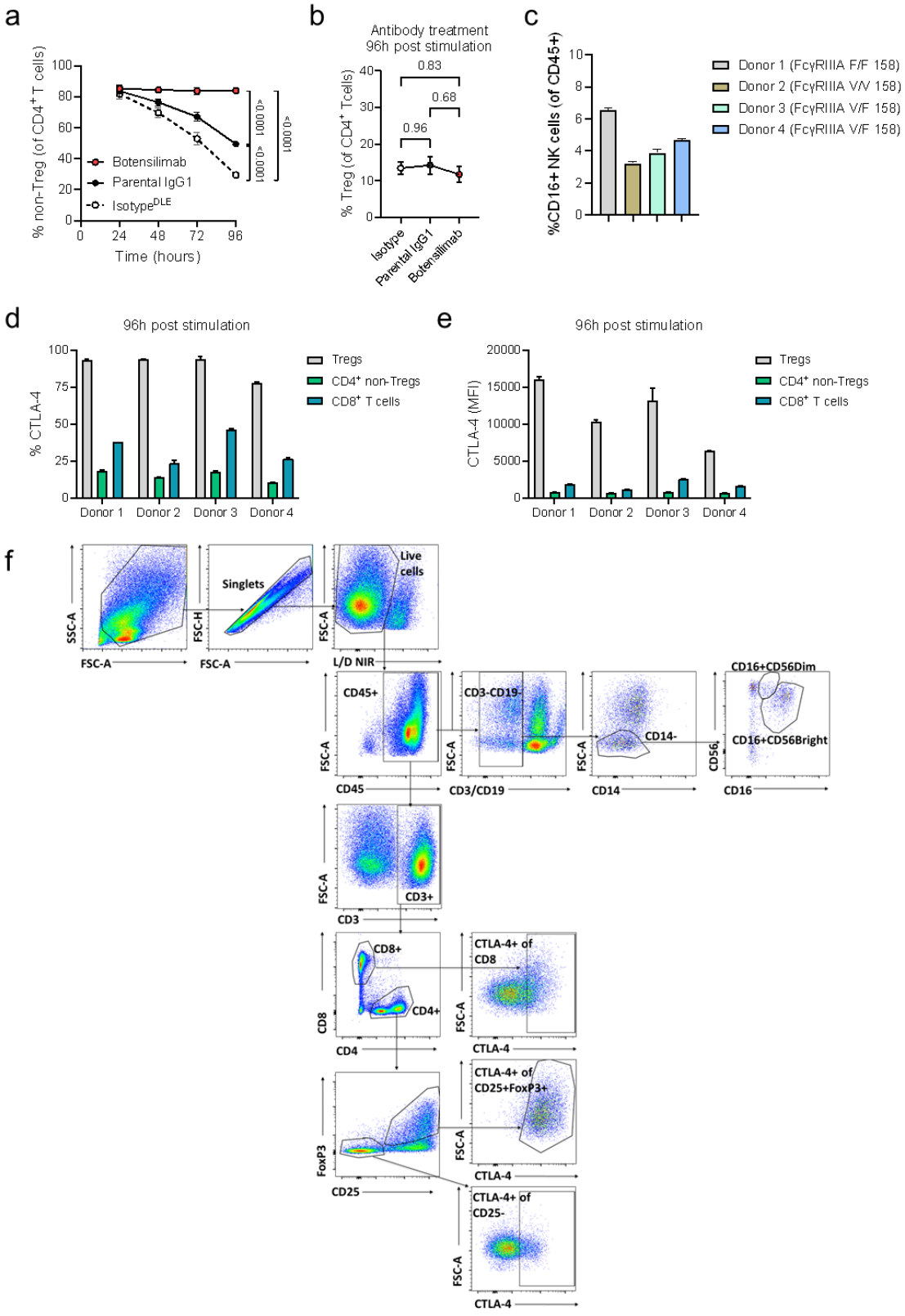
Supplementary Figure S9



Supplementary Figure S9. Botensilimab does not decrease CD4+ non-regulatory T cells (non-Tregs) from stimulated human PBMC cultures. (a) $CD4^+$ non-Treg frequencies from staphylococcal enterotoxin A (SEA)-stimulated healthy donor PBMCs treated with 5 μg/ml of botensilimab, parental IgG1, or $IgG1^{DLE}$ by flow cytometry (n=4 donors). **(b)** Percentage of Treg cells among CD4+ T cells from SEA-stimulated PBMCs. Cultures were treated with botensilimab, parental IgG1, or isotype antibodies 96h post-SEA peptide stimulation of PBMCs (n=4 donors). **(c)** Percentage of FcγRIIIA ($CD16^+$)-expressing NK cells, **(d)** percentage of CTLA-4+, and **(e)** mean fluorescent intensity (MFI) of CTLA-4 on Tregs, CD4+ non-Tregs and CD8+ T cells at 96h post-SEA peptide stimulation. **(f)** Representative flow cytometry gating strategy for $CD4^+$ and $CD8^+$ T cell subsets, and $CD16^+$ NK cells profiled from staphylococcal enterotoxin A (SEA) superantigen-stimulated PBMC cultures are shown. CD8+ T cells ($CD3^+$, $CD4^-$ $CD8^+$), $CD4^+$ non-Tregs ($CD3^+$, $CD4^+$ $CD8^-$, $CD25^-$, $FoXP3^-$), Tregs ($CD3^+$, $CD4^+$ $CD8^-$, $CD25^+$, $FoXP3^+$) were defined in each T cell subpopulation. NK cells were defined as $CD3^-$, $CD19^-$, $CD14^-$, $CD56^+$. Data are represented as mean ± s.e.m **(a-e)**. Data were analyzed using a **(a)** two-way ANOVA or **(b)** one-way ANOVA followed by a Tukey's multiple comparisons test.