

Supplementary Information

Standardized 11-color flow cytometry panel for the functional phenotyping of human T regulatory cells.

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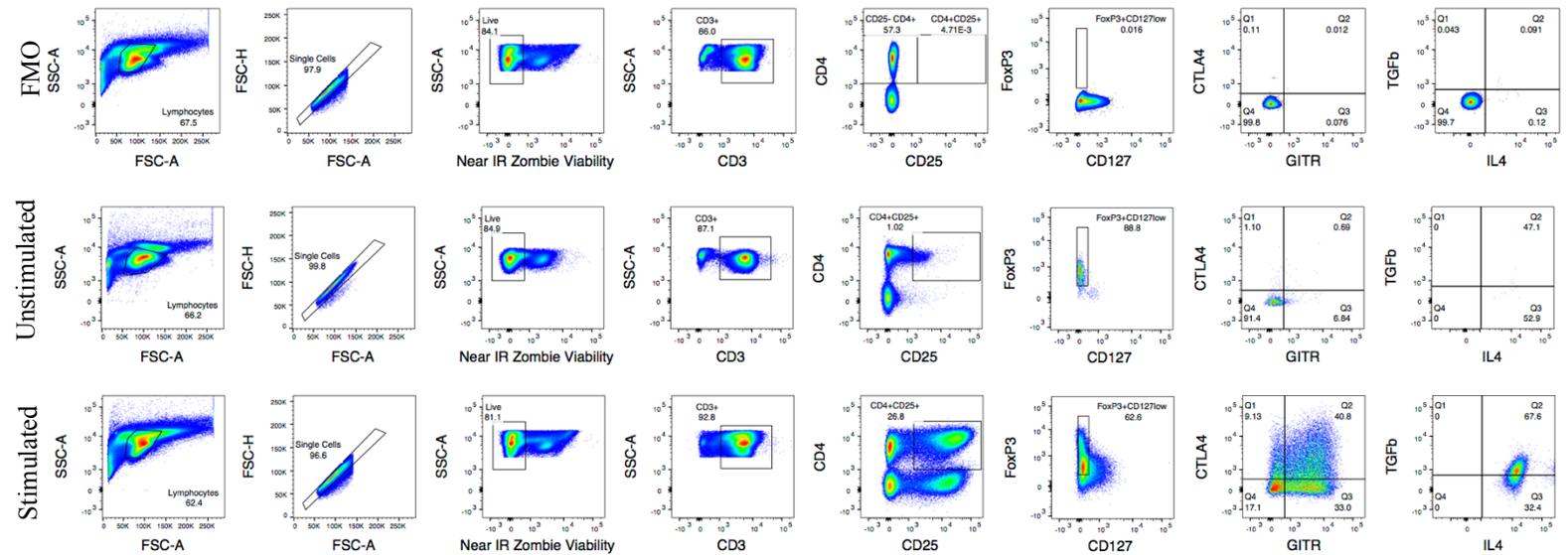
Table S1

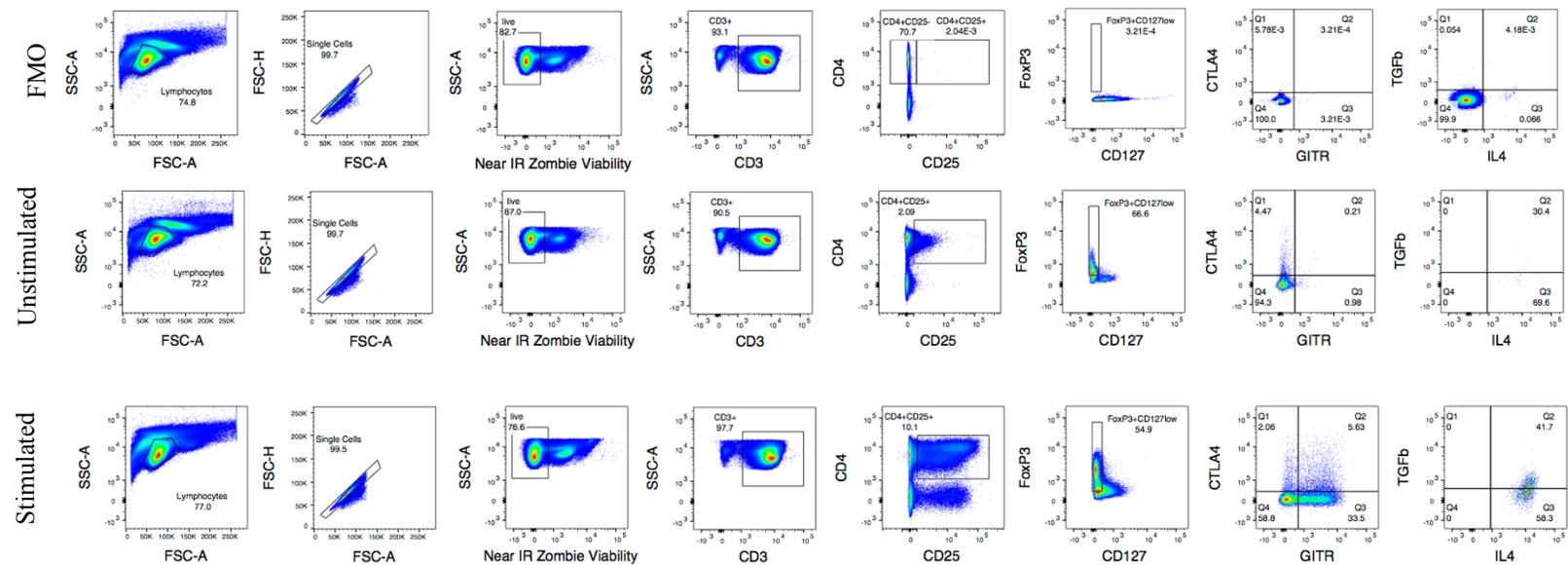
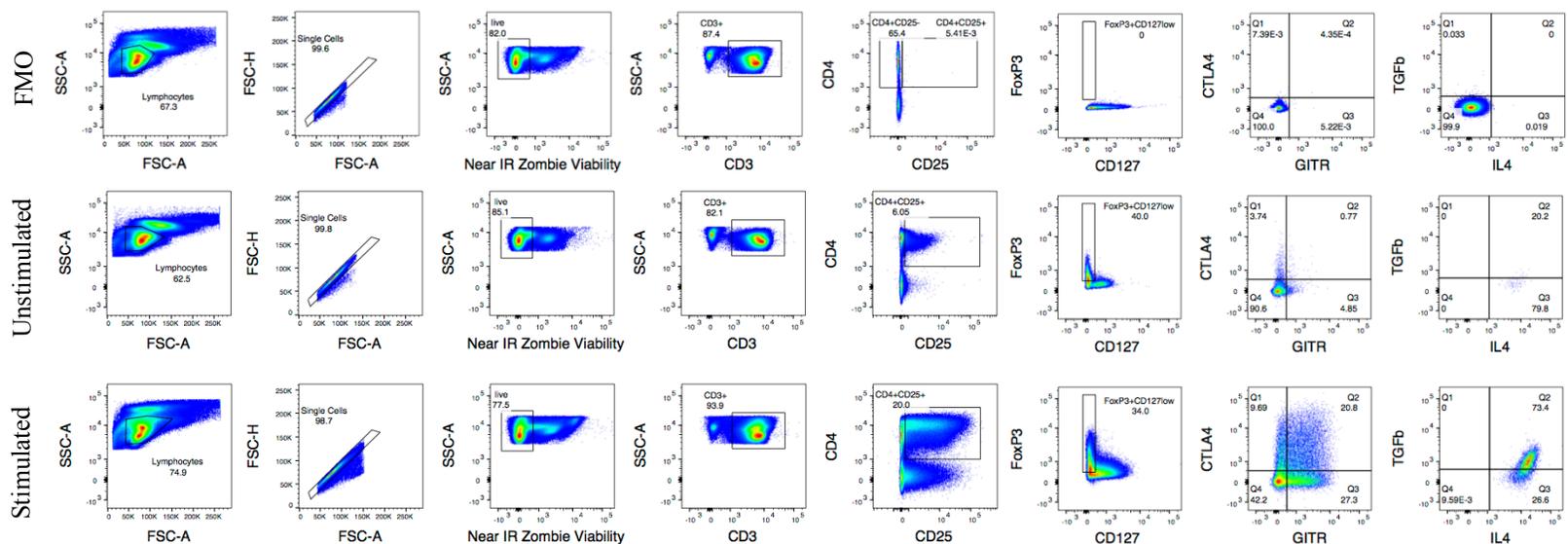
Figure S1

Fluorophore	Run 1	Run 2	Run 3	Average
FCS-A	380	450	430	420
SSC-A	180	170	180	176.67
Alexa Fluor 700-A	600	600	600	600
BV 786-A	565	565	580	570
BV421-A	410	410	410	410
BV510-A	415	415	415	415
BV650-A	500	540	510	516.67
PE-A	505	505	505	505
FITC-A	400	400	400	400
PE-Cy 5-5-A	555	555	555	555
APC-A	580	580	580	580
PerCP-Cy 5-5-A	565	565	565	565
Zombie NIR-A	525	525	525	525

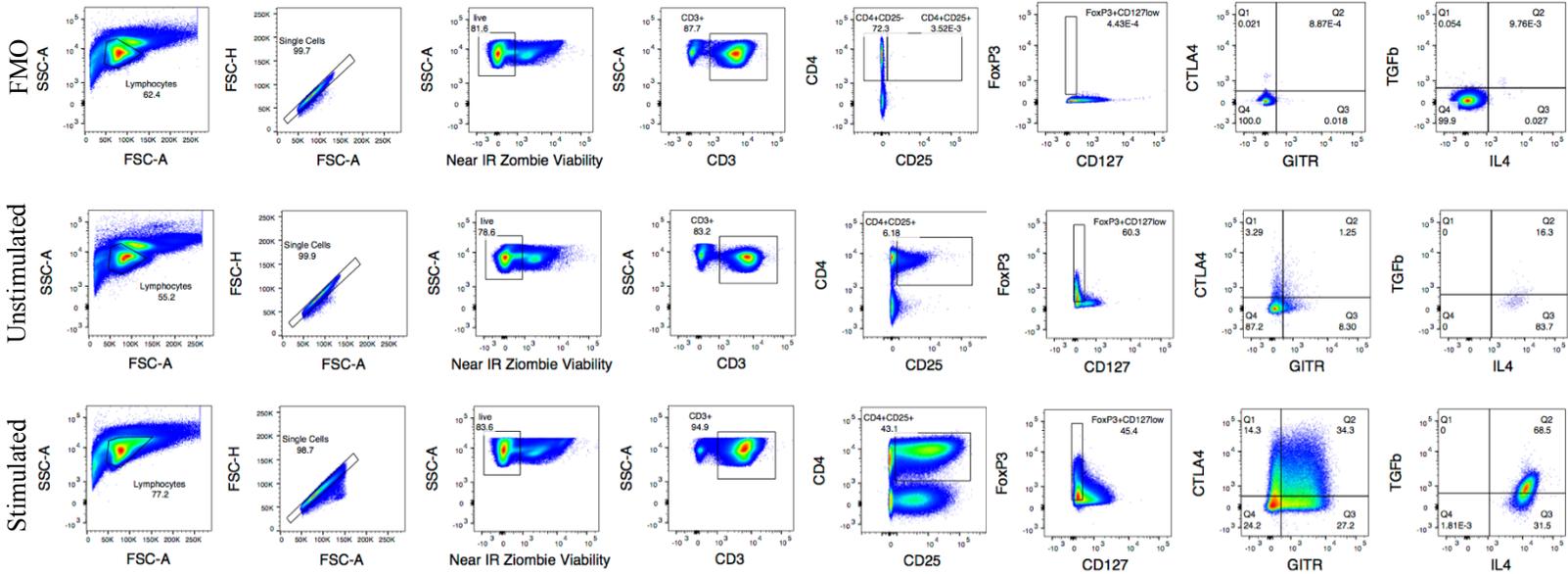
Supplemental Table 1. Voltages set on BD LSR Fortessa X-20 using single color controls.

A

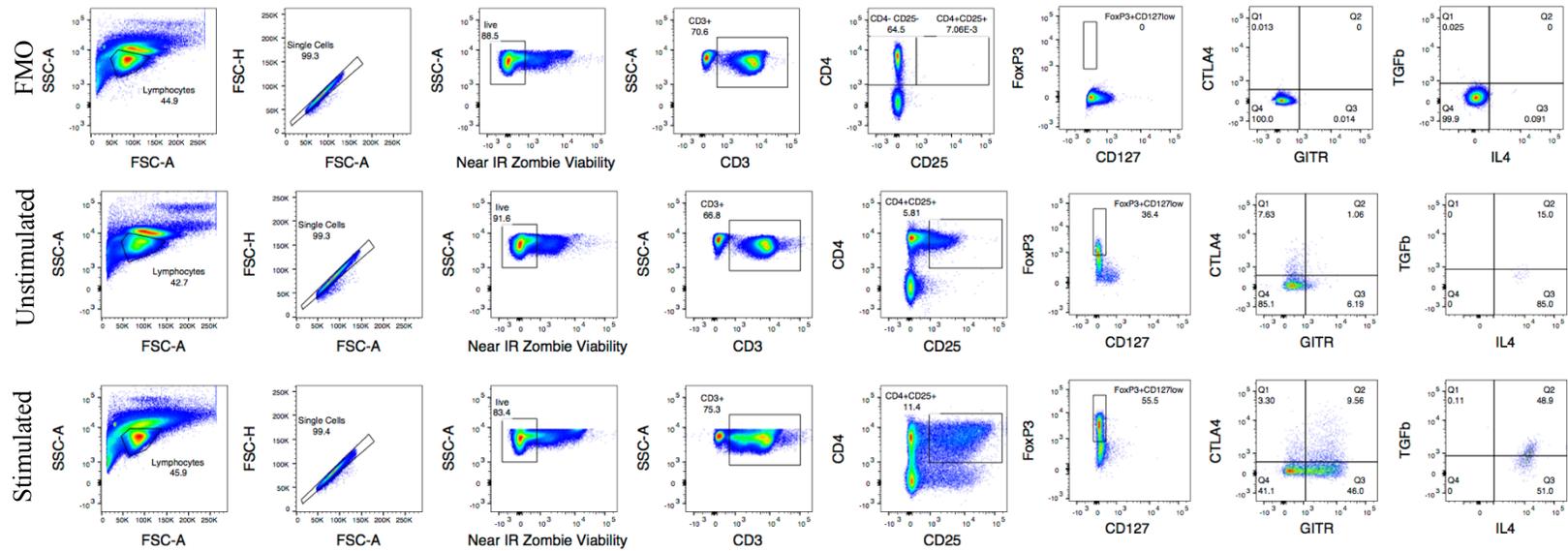


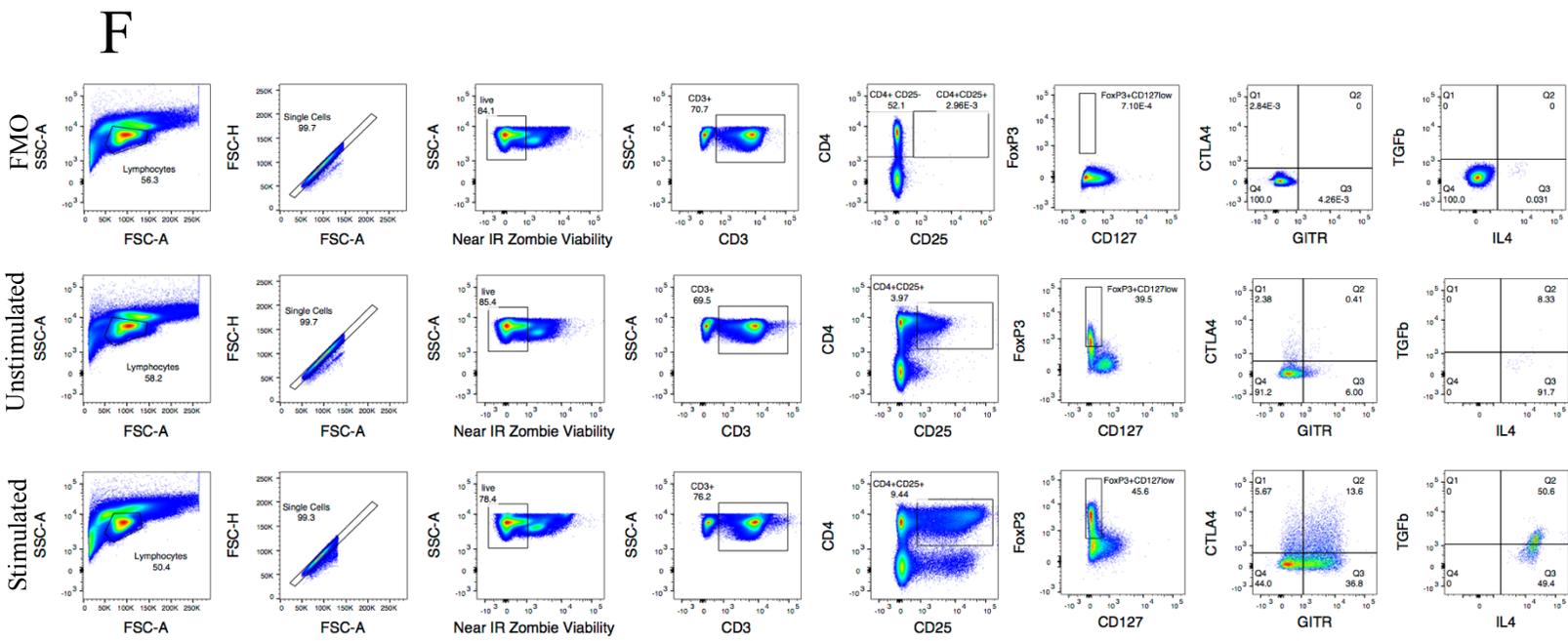
B**C**

D



E





Supplemental Figure 1. Gating strategy each of the 6 donors, Donors A – F, each donor is shown in the figure with the letter caption corresponding to the donor. FMO controls for each individual donor were used to establish gates. Figures are labeled with the donor. The first row is the FMO control for individual donor. The FMO was stained for CD3, CD4, CD8, CD25, and Zombie NIR. The second row is the no stimulation sample for each donor. These samples were stained with the full panel. The third row is the 96hr CD3/CD28+ stimulated sample. These samples were stained with the full panel. Within the FMO, all checkpoint and cytokine gates are shown on a CD4+ CD25- parent population; for stimulated and unstimulated cells, the checkpoint and cytokine gates are shown on a CD4+ CD25+ parent population.