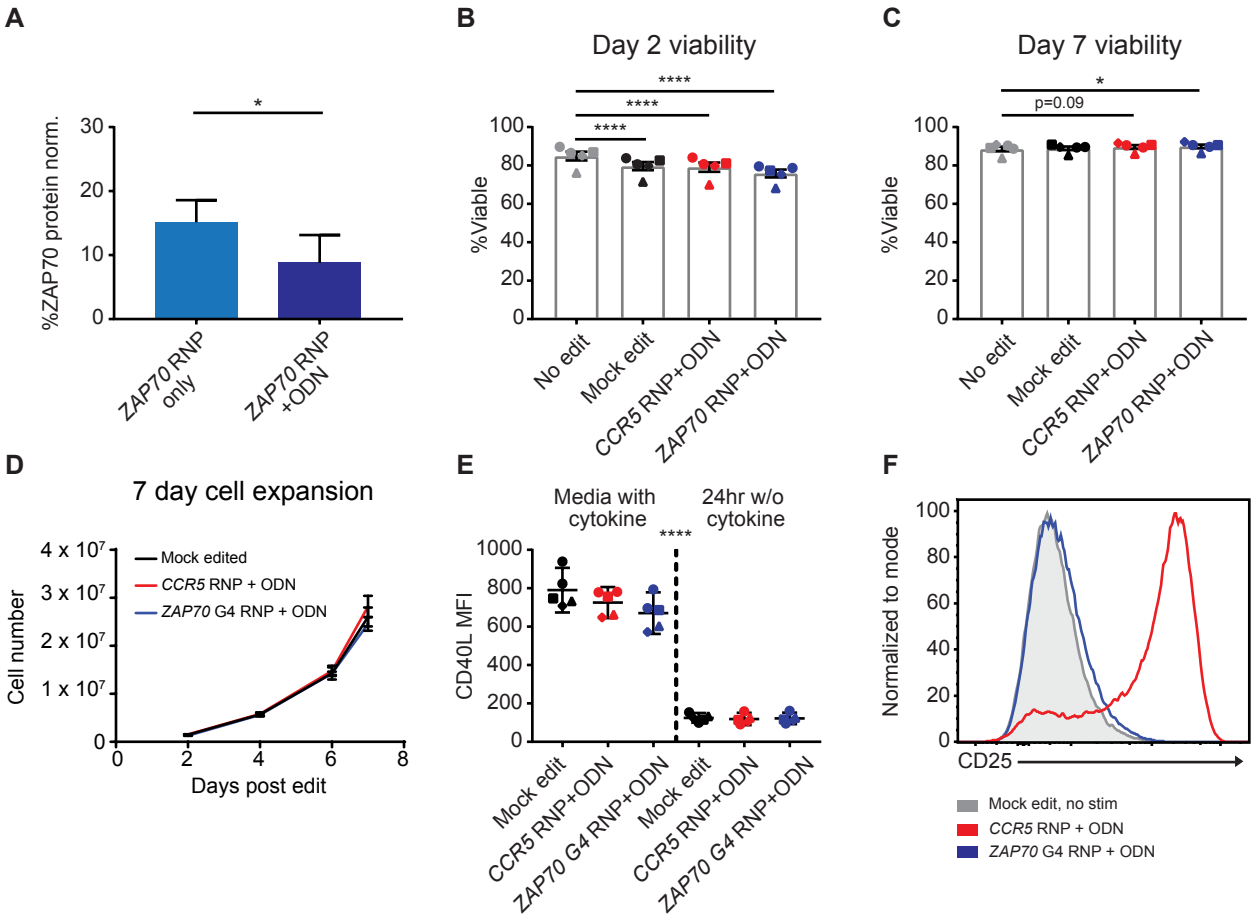
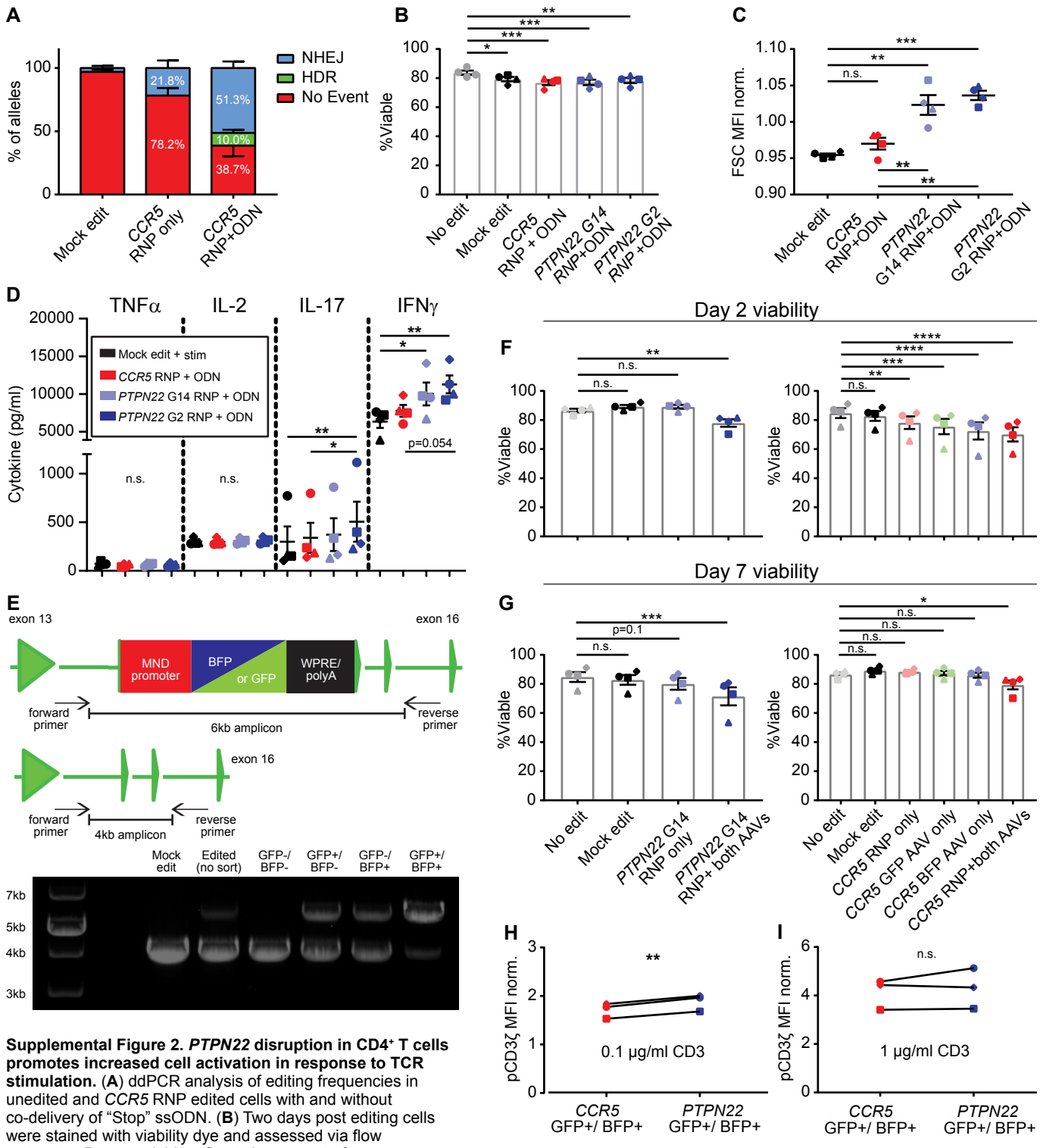
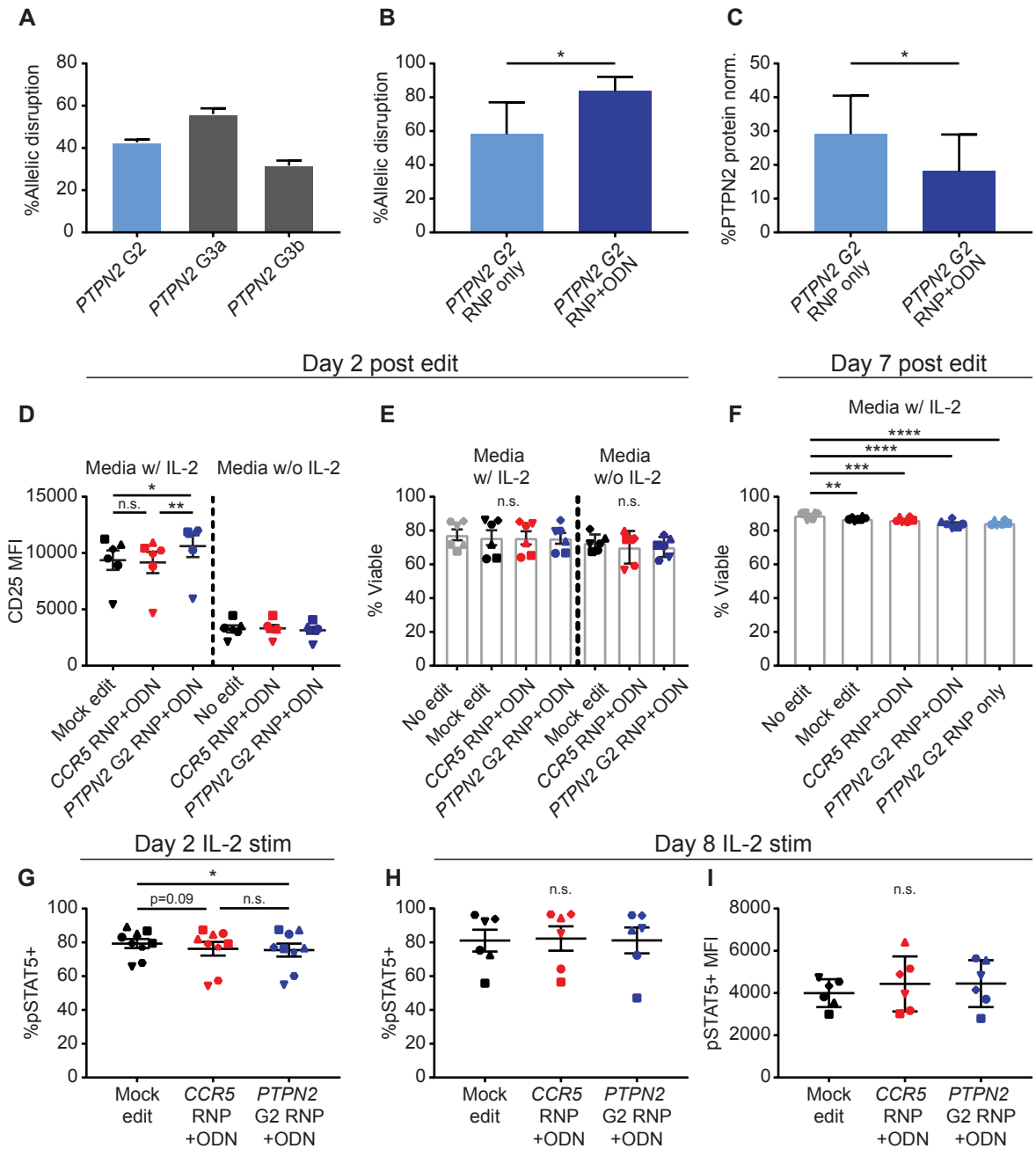


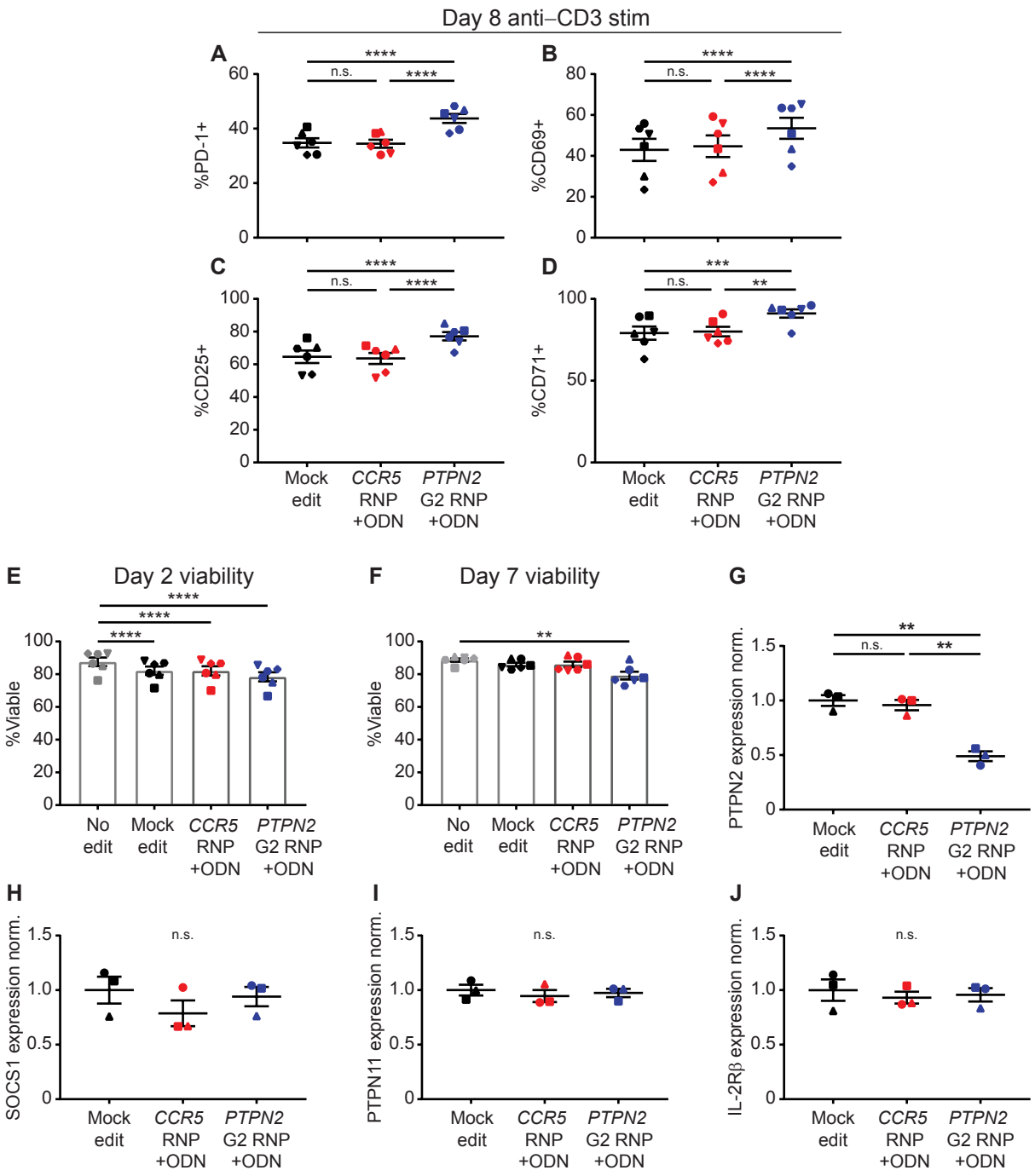
Supplemental Figure 1. *ZAP70* disruption in CD4⁺ T cells is highly efficient and results in minimal impact on cell viability and expansion post-editing. (**A**) Quantified ZAP70 protein expression comparing western blots of CD4⁺ T cells from the same donors that had been transfected with *ZAP70* G4 RNP alone vs. *ZAP70* G4 RNP plus an ssODN containing a stop codon. Protein expression values are relative to actin and normalized to mock edited values from the same T cell donor (n=5 human samples (4 independent donors plus 1 repeat donor, repeat donors were run in separate experiments), paired t test). (**B-C**) After editing, cells were expanded in high cytokine culture for 7 days. At 2 days (**B**) and 7 days (**C**) post-editing, cells were stained with viability dye and assessed via flow cytometry. Percent viable reflects the percent of events collected from each culture that were identified as single, live, lymphocytes (n=5, matched one-way ANOVA with a Dunnet post hoc test referenced to unedited cells). (**D**) Cell expansion post-editing stratified by editing condition (n=5 human samples (4 independent donors plus 1 repeat donor, repeat donors were run in separate experiments), lines represent mean, brackets represent SEM). (**E**) Summary flow data for CD40L expression in cells from the same edited cultures +/- 24-hour rest in cytokine free media (lines and error bars represent mean +/- SEM, n=5 human samples (4 independent donors plus 1 repeat sample, repeat samples were run in separate experiments), matched one-way ANOVA). (**F**) Representative overlay of flow cytometry histograms for CD25 expression in indicated editing conditions from the same donor at 24 hours post anti-CD3 stimulation. (**A-C**) Bars represent mean +/- SEM. RNP - ribonucleoprotein, ODN or ssODN - single stranded oligo-deoxynucleotide. Shapes in summary plots correspond to individual donors. All data is from 2 independent experiments. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.



Supplemental Figure 2. *PTPN22* disruption in CD4⁺ T cells promotes increased cell activation in response to TCR stimulation. (**A**) ddPCR analysis of editing frequencies in unedited and *CCR5* RNP edited cells with and without co-delivery of "Stop" ssODN. (**B**) Two days post editing cells were stained with viability dye and assessed via flow cytometry. Percent viable reflects the percent of events collected from each culture that were identified as single, live, lymphocytes. (**C-D)** Cells that had been edited and cultured as in Fig.2D-F were stimulated with plate bound anti-CD3 for 48 hours. (**C**) Summary data of median flow values for forward scatter. (**D**) Concentration of cytokines present in supernatant as determined by ELISA. (**E**) Schematic of PCR based detection of AAV mediated HDR gene editing in gDNA and representative agarose gel of amplified gDNA from *PTPN22* bi-allelically edited CD4⁺ T cells, FACS-sorted based on editing outcome. Large band reflects alleles with AAV homology cassette within the amplicon site. (**F-G**) Following editing using RNP and rAAV6, CD4⁺ T cells were expanded in culture for 7 days. At 2 days (**F**) and 7 days (**G**) post-editing cells were stained with viability dye and assessed via flow cytometry. Cells were either unedited, mock edited, RNP edited only (*PTPN22* or *CCR5*) or edited with a combination of RNP and AAVs containing homology donor cassettes. Percent viable reflects the percent of events collected from each culture that were identified as a single, live, lymphocytes. (**H-I**) CD4⁺ T cells bi-allelically edited (GFP+/BFP+) at either the *CCR5* or *PTPN22* loci from the same donor were stimulated using mouse anti-human CD3 (at final concentrations listed) and goat anti-mouse crosslinker (at half the concentration of anti-CD3 used). Cells were fixed at several timepoints post stimulation and analyzed for CD3ζ phosphorylation by flow cytometry. Median pCD3ζ values show 2' stimulation relative to the same sample's unstimulated value. (**B-D, F-G**) Significance determined by matched one-way ANOVA with Dunnet post hoc test (**B, F-G**) or Tukey's correction (**C-D**), n=4. (**H-I**) Significance determined by paired t test, n=3. **(A-D)** Data is from 2 independent experiments. RNP – ribonucleoprotein, ODN or ssODN - single stranded oligo-deoxynucleotide. All bars represent mean +/- SEM. Shapes in summary plots correspond to individual donors. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.



Supplemental Figure 3. *PTPN2* disruption in CD4⁺ T cells is most efficient with RNP and ssODN co-delivery and impacts the cell surface phenotype in media containing IL-2. (**A**) ICE analysis of indel frequency in primary human CD4+ T cells using 3 alternative gRNAs delivered as RNPs. Percent allelic disruption represents estimated indel frequency in edited cell compared to unedited controls (n=3). (**B**) ICE analysis of indel frequency in primary human CD4⁺ T cells using *PTPN2* G2 RNP +/- stop codon containing ssODNs. Percent allelic disruption represents estimated indel frequency in edited cell compared to unedited controls (n=5, paired t test). (**C**) Quantified PTPN2 protein expression comparing western blots of CD4⁺ T cells from the same donors edited with *PTPN2* G2 RNP +/- stop codon containing ssODN. Protein expression values are relative to actin and normalized to unedited values from the same T cell donor (n=6, paired t test). (**D**) Median CD25 expression in edited cells cultured for 2 days with or without IL-2 as in Fig. 5B (n=6, matched two-way ANOVA with Tukey's correction stratified by presence of IL-2 in media). (**E-F**) Two and seven days, post editing cells were stained with viability dye and assessed via flow cytometry. Percent viable reflects the percent of events collected from each culture that were identified as single, live, lymphocytes (n=6, (**E**) matched two-way ANOVA with Tukey's correction, stratified by presence of IL-2 in media, (**F**) matched one-way ANOVA with a Dunnet post-hoc test referenced to unedited cells). (**G**) Response to IL-2 stimulation for experiments described in Fig.5E&F as measured by %pSTAT5 positive over background levels (n=9). (**H-I**) Percent response (**H**) and median pSTAT5 fluorescence (**I**) following a 20-minute IL-2 stimulation. Prior to simulation, edited cells were expanded for 7 days in IL-2 supplemented media and rested for 24-hours in cytokine free media as described in Fig.5B (n=6). All bars and lines represent mean +/- SEM. RNP - ribonucleoprotein, ODN or ssODN - single stranded oligo-deoxynucleotide. Shapes correspond to individual donors. All data is from 2-3 independent experiments. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.



Supplemental Figure 4. *PTPN2* disruption in CD4⁺ T cells alters the response to TCR stimulation and reduces long-term viability in high cytokine media. (**A-D**) Summary data for all donors showing percentage of cells staining positive for PD-1 (**A**), CD69 (**B**), CD25 (**C**), or CD71 (**D**) for control and edited T cell populations in response to 24-hour anti-CD3 stimulation (n=6). (**E-F**) Viability assessment of edited and control populations at Day 2 (**E**) and Day 7 (**F**) post-editing using cytokine media (IL-2, IL-7, IL-15) as in Fig 7A. Cells were stained with viability dye and assessed via flow cytometry. Percent viable reflects the percent of events collected from each culture that were identified as single, live, lymphocytes (n=6). (**G-J**) CD4⁺ T cells that were edited, expanded and rested as in Fig.7A, were assessed for baseline mRNA expression levels for PTPN2 (**G**), SOCS1 (**H**), PTPN11 (**I**), and IL-2Rβ (**J**) by RT-qPCR (Cq values normalized to housekeeping gene *B2M*, then normalized to the average adjusted Cq value of mock edited cells, n=3). All data analyzed with matched one-way ANOVA with Tukey's correction or Dunnet post-hoc test. Lines and error bars represent mean +/- SEM. RNP - ribonucleoprotein, ODN or ssODN - single stranded oligo-deoxynucleotide. Shapes in summary plots correspond to individual donors. All data is from 2 independent experiments. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001.