Supplemental Information for:

Unsupervised Capture and Profiling of Rare Immune Cells using Multi-Directional Magnetic Ratcheting

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S1. Description and Citations on Sample Sparing Assays and the Challenges Associated with Antigen-Specific Immune Cell Monitoring

The NIH has previously outlined the outstanding need for sample sparing assays in the analysis of the immune system: https://grants.nih.gov/grants/guide/rfa-files/RFA-AI-14-027.html

In general, the need for sample sparing assays of immune cell state or function is driven by 1) the need to obtain simultaneous multi-parameter analysis of immune function (i.e. functional, protein, and gene expression data from the same samples), and 2) the need to monitor continuously or during conditions causing a weakened immune system, in which a limited amount of sample (e.g. blood) can be obtained by the patient.

Several research groups have also summarized the challenge and need for providing assays for antigen specific immune cell response that are compatible with low volume or rare cell concentrations, including:

1. Phetsouphanh, C et al; Detecting Antigen-Specific T Cell Responses: From Bulk Populations to Single Cells. Int J Mol Sci, 2015, 16(8), 18878-18893
2. Shen, C. et al; Frequency and reactivity of antigen-specific T cells were concurrently measured through the combination of artificial antigen-present cell, MACS, and Elispot., Scientific Reports, 7, 16400, 2017
3. Smith, SG et al; Assay Optimization and Technology Transfer for multi-site immune-monitoring in vaccine trials. PLoS One, 2017, 12(10), e0184391

To summarize the findings in these and other government panels and publications, they describe FACS and MACS based separation and analysis technology as capable of rare cell detection and enumeration in controlled laboratory conditions, but not compatible for reproducible use and affordable/continuous monitoring in immune therapy and vaccine trials. There exists a great need to provide new tools that change the way researchers/clinicians interface with and analyse rare samples from clinical trials and clinical monitoring programs.

S2. Comparison of Magnetic Ratcheting Separation to Standard Flow Cytometry

In conjunction with the methods and data outlined in figure 4B, the team took the same sample loaded with a predefined number of magnetic bead labelled cells (specific for CD4/8, as described in the main text methods section) mixed in a background of at least 1 million non-labelled PBMCs, and analysed the sample using a cytoflex volumetric flow cytometer (Beckman Coulter). However, in this experiment the sample was diluted so that the number of expected "pre-labelled" cells varied from 3000 cells/microliter (a 1:20 target cell to PBMC ratio or 150,000 total) to 3 cells/microliter (a 1:20000 target cell to PBMC ratio or 150 total) within an analysis time and input volume that matched those performed on the magnetic ratchet (i.e. 20-50 microliter input (diluted to achieve minimum input for the cytometer interface), and less than 30-minute total analysis time). A cytometer event was quantified as a detected target cell when the fluorescence intensity of the event was greater than the baseline level (as measured using a separate sample of PBMCs mixed with a non-specific/non-target fluorescently labelled antibody) and the forward/side scatter position fell in a pre-defined scatter intensity level consistent with T cells (as measured using a separate sample of PBMCs). The results in figure S1 show that when challenged with the same separation parameters (i.e. the same sample volume and separation times), the magnetic ratcheting approach out-performed a standard cytometer, as an expected number of cells greater than 1,500 was necessary to provide a statistically detected target cell population. The magnetic ratchet was tested with as few as 50 target cells (in the MOG specific tetramer capture tests), and will be further characterized for additional rare cells applications (less than 50 cells) in subsequent publications.

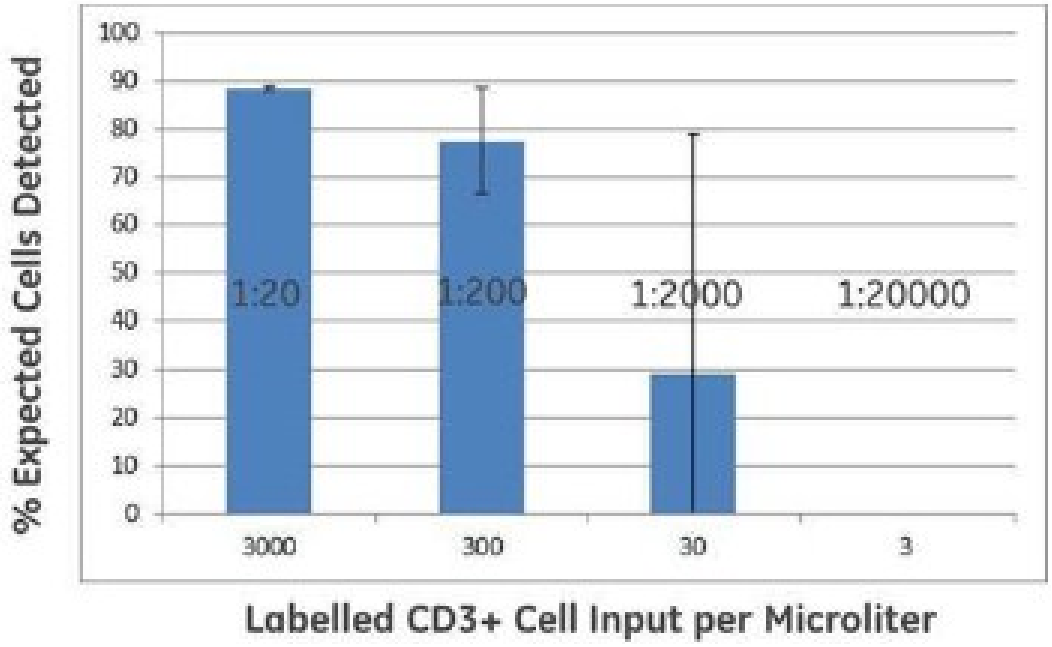

Figure S1. A plot showing the number of expected cells versus detected cells identified using a volumetric flow cytometer using the same sample as analysed by the magnetic ratchet in figure 4B. The total number of cells input was 3000 per microliter (or 150,000), 300 per microliter (or 15,000), 30 per microliter (or 1,500), and 3 per microliter (or 150).

In an additional experiment to compare the ratcheting technique to standard separation technologies, the team expanded the data outlined in figure 4C. In this case the same number (i.e. ~150) of CD154+ pre-labelled cells were spiked into a background of 1 million PBMCs. The cells were then run through the magnetic ratchet or through a MACS column per manufacturer's instruction (https://www.miltenyibiotec.com/US-en/products/macs-cell-separation/cell-separation-reagents/microbeads-and-isolation-kits/t-cells/cd154-microbead-kit-human.html/), and the experiment was run in triplicate. Ratcheted cells in the platform were imaged in place on the ratchet as described in the methods section, while the eluate from the MACS separation was pipetted onto a slide for imaging and quantification. While the ratchet and MACS column separation achieved similar separation efficiencies (ratchet capture efficiency shown in figure 4C in the text, MACS capture efficiency 57 +/- 14%), the purity of the CD154+ cells in the ratcheted sample was 95 +/- 1.4% compared to 2.8 +/1 0.27% from the MACS column (shown in figure S2). In this experiment, the sample volume was diluted for the MACS separation (following manufacturer's instruction), allowing for sufficient capture. However, the "binary" separation nature of the MACS column (i.e. capture everything/every cell with magnetic beads attached as it passes the column, with no method of separating

target vs. non-specific binding events/cells), did not provide a high purity sample, while the quantitative separation capability of the magnetic ratchet enabled additional separation and purification of the CD154+ cell population.

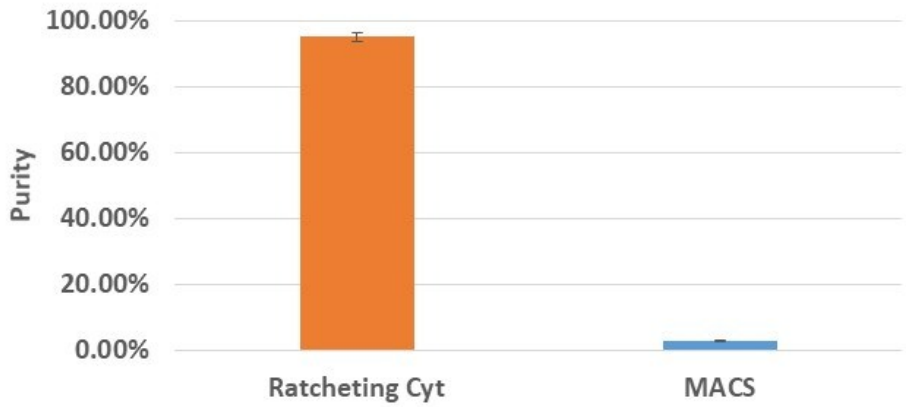

Figure S2. Additional data from a CD154+ spiked cell experiment, in which 150 CD154+ cells were spiked into 1 million background PBMCs and then separated on either the magnetic ratchet or a MACS column. The ratchet enabled further separation of the target CD154+ cells away from contaminated (non-labelled) cells, and a higher separation purity compared to the MACS column.