**Protocol Descriptions for Publication**

**Luminex -EMD Millipore Human 80 Plex kits:** This assay was performed by the Human Immune Monitoring Center at Stanford University- Immunoassay Team.  Kits were purchased from EMD Millipore Corporation, Burlington, MA., and run according to the manufacturer’s recommendations with modifications described as follows: H80 kits include 3 panels: Panel 1 is Milliplex HCYTA-60K-PX48. Panel 2 is Milliplex HCP2MAG-62K-PX23. Panel 3 includes the Milliplex HSP1MAG-63K-06 and HADCYMAG-61K-03 (Resistin, Leptin and HGF) to generate a 9 plex. The assay setup followed recommended protocol: Briefly: samples were diluted 3-fold (Panel 1&2) and 10-fold for Panel 3. 25ul of the diluted sample was mixed with antibody-linked magnetic beads in a 96-well plate and incubated overnight at 4°C with shaking. Cold and Room temperature incubation steps were performed on an orbital shaker at 500-600 rpm. Plates were washed twice with wash buffer in a BioTek ELx405 washer (BioTek Instruments, Winooski, VT). Following one-hour incubation at room temperature with biotinylated detection antibody, streptavidin-PE was added for 30 minutes with shaking. Plates were washed as described above and PBS added to wells for reading in the Luminex FlexMap3D Instrument with a lower bound of 50 beads per sample per cytokine. **Each sample was measured with single or duplicate replicates [specify per your study]**.  Custom Assay Chex control beads were purchased and added to all wells (Radix BioSolutions, Georgetown, Texas). Wells with a bead count <50 were flagged, and data with a bead count <20 were excluded.

**Luminex – Thermo-Fisher/Life technologies Mouse 48 plex Kits:**This assay was performed by the Human Immune Monitoring Center at Stanford University.  Mouse 48 plex Procarta kits (EPX480-20834-901) were purchased from Thermo-Fisher/Life Technologies, Santa Clara, California, USA, and used according to the manufacturer’s recommendations with modifications as described. Briefly: Beads were added to a 96 well plate and washed in a BioTek ELx405 washer. Samples were added to the plate containing the mixed antibody-linked beads and incubated overnight at 4°C with shaking. Cold (4oC) and Room temperature incubation steps were performed on an orbital shaker at 500-600 rpm. Following the overnight incubation plates were washed in a BioTek ELx405 washer and biotinylated detection antibody added for 60 minutes at room temperature with shaking. Plate was washed as described and streptavidin-PE was added for 30 minutes at room temperature. Plate was washed as above and reading buffer was added to the wells. Each sample was measured in duplicate or single wells [specify per your study]. Plates were read using a **Luminex 200 or a FM3D FlexMap instrument** with a lower bound of 50 beads per sample per cytokine. Custom Assay Chex control beads were purchased from Radix BioSolutions, Georgetown, Texas; and added to all wells.

**Olink Immunoassay:** This assay was performed by the Human Immune Monitoring Center at Stanford University.  The samples were subjected to Olink multiplex assay with Inflammatory panel (Olink Bioscience, Uppsala, Sweden), according to the manufacturer’s instructions. The inflammatory panel includes 92 proteins associated with human inflammatory conditions. Briefly, an incubation master mix containing pairs of oligonucleotide-labeled antibodies to each protein was added to the samples and incubated for 16 hours at 4 ºC. Each protein was targeted with two different epitope-specific antibodies increasing the specificity of the assay. Presence of the target protein in the sample brought the partner probes in close proximity, allowing the formation of a double strand oligonucleotide polymerase chain reaction (PCR) target. On the following day, the extension master mix in the sample initiated the specific target sequences to be detected and generated amplicons using PCR in 96 well plate. For the detection of the specific protein, Dynamic array integrated fluidic Circuit (IFC) 96x96 chip was primed, loaded with 92 protein specific primers and mixed with sample amplicons including three inter-plate controls (IPS) and three negative controls (NC). Real time microfluidic qPCR was performed in Biomark (Fluidigm, San Francisco, CA) for the target protein quantification.

Data were analyzed using Real time PCR analysis software via ΔΔCt method and Normalized Protein Expression (NPX) manager. One NPX difference equals to the doubling of the protein concentration.

**Luminex -EMD Millipore Magnetic kits:** This assay was performed by the Human Immune Monitoring Center at Stanford University.  Kits were purchased from EMD Millipore Corporation, Burlington, MA., and used according to the manufacturer’s recommendations with modifications described as follows. Briefly: samples were mixed with antibody-linked magnetic beads on a 96-well plate and incubated overnight at 4°C with shaking. Cold and Room temperature incubation steps were performed on an orbital shaker at 500-600 rpm. Plates were washed twice with wash buffer in a Biotek ELx405 washer. Following one hour incubation at room temperature with biotinylated detection antibody, streptavidin-PE was added for 30 minutes with shaking. Plates were washed as described and PBS added to wells for reading in the Luminex FlexMap3D Instrument with a lower bound of 50 beads per sample per cytokine. **Each sample was measured in duplicate or singlet**.  Custom Assay Chex control beads were purchased from Radix BioSolutions, Georgetown, Texas, and added to all wells.

**Luminex -Polystyrene bead kits:**  This assay was performed in the Human Immune Monitoring Center at Stanford University.  Human 51-plex or Mouse 26 plex kits were purchased from Affymetrix and used according to the manufacturer’s recommendations with modifications as described below. Briefly, samples were mixed with antibody-linked polystyrene beads on 96-well filter-bottom plates and incubated at room temperature for 2 h followed by overnight incubation at 4°C. Room temperature incubation steps were performed on an orbital shaker at 500-600 rpm. Plates were vacuum filtered and washed twice with wash buffer, then incubated with biotinylated detection antibody for 2 h at room temperature. Samples were then filtered and washed twice as above and re-suspended in streptavidin-PE. After incubation for 40 minutes at room temperature, two additional vacuum washes were performed, and the samples re-suspended in Reading Buffer. Each sample was measured in duplicate. Plates were read using a Luminex 200 instrument with a lower bound of 100 beads per sample per cytokine. Custom assay Control beads by Radix BioSolutions are added to all wells.

**Flow Cytometry Immunophenotyping:**This assay was performed by the Human Immune Monitoring Center at Stanford University.  PBMC were thawed in warm media, washed twice and resuspended at 1x10^7 viable cells/mL. 50 uL cells per well were stained for 45 min at room temperature with the antibodies shown in the table [above] (all reagents from BD Biosciences, San Jose, CA). Cells were washed three times with FACS buffer (PBS supplemented with 2% FBS and 0.1% sodium azide), and resuspended in 200 uL FACS buffer. 100,000 lymphocytes per sample were collected using DIVA 6.0 software on an LSRII flow cytometer (BD Biosciences). Data analysis was performed using FlowJo v9.3 by gating on live cells based on forward versus side scatter profiles, then on singlets using forward scatter area versus height, followed by cell subset-specific gating.

**Phosphoflow PBMC CyTOF:**This assay was performed by the Human Immune Monitoring Center at Stanford University.  PBMC were thawed in warm media, washed twice, counted by Vi-Cell (Beckman Coulter) and resuspended at 5x10^6 viable cells/mL. 200 uL of cells were plated per well in 96-well deep-well plates. After resting for 1 hour at 37C, cells were stimulated by adding 50 ul of stim (IFNa, IL-6, IL-7, IL-10, IL-21, LPS or PMA/ionomycin) or left unstimulated and incubated at 37°C for 15 minutes. Cells were then fixed with paraformaldehyde, washed twice with CyFACS buffer (PBS supplemented with 2% BSA, 2 mM EDTA, and 0.1% sodium azide) and stained for 30 min at room temperature with 20 mL of surface antibody cocktail. Cells were washed twice with CyFACS, permeabilized with 100% methanol and kept at -80C overnight. The next day, cells were washed with CyFACS buffer and resuspended in 20 mL intracellular antibody cocktail in CyFACS for 30 min at room temperature before washing twice in CyFACS. Cells were resuspended in 100 mL iridium-containing DNA intercalator (1:2000 dilution in 2% PFA in PBS) and incubated at room temperature for 20 min. Cells were washed once with CyFACS buffer and twice with MilliQ water. Cells were diluted to 750x10^5 cells/mL in MilliQ water and acquired on CyTOF. Data analysis was performed using FlowJo v10 by gating on intact cells based on the iridium isotopes from the intercalator, then on singlets by Ir191 vs cell length followed by cell subset-specific gating.  If Cell-ID™ 20-Plex Pd Barcoding, Fluidigm was used, fixed samples were barcoded, stained and acquired as single multiplex sample or samples.

**Phosphoflow whole blood CYTOF**: This assay was performed by the Human Immune Monitoring Center at Stanford University. 200 ul aliquots of fresh whole blood sample were plated per well in 96-well deep-well plates or cluster tubes. After resting for 1 hour at 37C, cells were stimulated by adding 50 ul of stim (e.g., IFNa, IL-6, IL-7, IL-10, IL-21, LPS or PMA/ionomycin) or left unstimulated and incubated at 37°C for 15 minutes. Cells were then fixed with Proteomic Stabilizer (Smart Tube, Inc., San Carlos, CA) and kept at -80C. Upon thawing, samples were washed with Smart Tube Thaw-Lyse buffer twice, and with CyFACS buffer (PBS supplemented with 2% BSA, 2 mM EDTA, and 0.1% sodium azide) once.  They were then stained for 30 min at room temperature with 20 mL of surface antibody cocktail. Cells were washed twice with CyFACS, permeabilized with 100% methanol and kept at -80C overnight. The next day, cells were washed with CyFACS buffer and resuspended in 20 mL intracellular antibody cocktail in CyFACS for 30 min at room temperature before washing twice in CyFACS. Cells were resuspended in 100 mL iridium-containing DNA intercalator (1:2000 dilution in 2% PFA in PBS) and incubated at room temperature for 20 min. Cells were washed once with cyFACS buffer and twice with MilliQ water. Cells were diluted to 750x10^5 cells/mL in MilliQ water and acquired on CyTOF. Data analysis was performed using FlowJo v10 by gating on intact cells based on the iridium isotopes from the intercalator, then on singlets by Ir191 vs cell length followed by cell subset-specific gating.  If Cell-ID™ 20-Plex Pd Barcoding, Fluidigm was used, fixed samples were barcoded, stained and acquired as single multiplex sample or samples.

**Phosphoepitope Flow Cytometry (Cytokine stimulation, pSTAT readouts):**This assay was performed by the Human Immune Monitoring Center at Stanford University.  PBMC were thawed in warm media, washed twice and resuspended at 0.5x10^6 viable cells/mL. 200 uL of cells were plated per well in 96-well deep-well plates. After resting for 1 hour at 37C, cells were stimulated by adding 50 ul of cytokine (IFNa, IFNg, IL-6, IL-7, IL-10, IL-2, or IL-21) and incubated at 37°C for 15 minutes. The PBMCs were then fixed with paraformaldeyde, permeableized with methanol, and kept at -80C overnight. Each well was bar-coded using a combination of Pacific Orange and Alexa-750 dyes (Invitrogen, Carlsbad, CA) and pooled in tubes. The cells were washed with FACS buffer (PBS supplemented with 2% FBS and 0.1% soium azide), and stained with the following antibodies (all from BD Biosciences, San Jose, CA): CD3 Pacific Blue, CD4 PerCP-Cy5.5, CD20 PerCp-Cy5.5, CD33 PE-Cy7, CD45RA Qdot 605, pSTAT-1 AlexaFluor488, pSTAT-3 AlexaFluor647, pSTAT-5 PE. The samples were then washed and resuspended in FACS buffer. 100,000 cells per stimulation condition were collected using DIVA 6.0 software on an LSRII flow cytometer (BD Biosciences). Data analysis was performed using FlowJo v9.3 by gating on live cells based on forward versus side scatter profiles, then on singlets using forward scatter area versus height, followed by cell subset-specific gating.

**Intracellular cytokine staining:**This assay was performed by the Human Immune Monitoring Center at Stanford University.  PBMC were thawed in warm media, washed twice and resuspended at 1x10^7 viable cells/mL. 200 uL of cells were plated per well in 96-well V-bottom plates. After overnight rest at 37C, activation reagents [specify here] + 10 ug/mL brefeldin A (Sigma, St. Louis, MO) were added and the cells incubated for 6 hours at 37°C. EDTA was then added to 15 mM final concentration, and the cells washed with PBS. They were then stained with Red LIVE/DEAD cell viability dye (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. After washing with FACS buffer (PBS supplemented with 2% FBS and 0.1% sodium azide), cells were resuspended in 200 uL of 1x BD FACS Lysing Solution (BD Biosciences), and the cells incubated for 10 min at room temperature. They were then centrifuged at 500 x G for 5 min, and resuspended in 200 uL of 1x BD FACS Permeabilizing Solution 2 (BD Biosciences). After 10 min at room temperature, the cells were washed twice with FACS buffer, and resuspended in 200 uL FACS buffer. Staining with cytokine-specific antibodies [insert list here] was then performed for 1 h at room temperature, followed by two additional washes with FACS buffer. 100,000 CD3+ cells per sample were collected using DIVA 6.0 software on an LSRII flow cytometer (BD Biosciences). Data analysis was performed using FlowJo v9.3 by gating on live cells based on forward versus side scatter profiles, then on singlets using forward scatter area versus height, followed by dead cell exclusion using Red LIVE/DEAD viability dye, and then cell subset-specific gating.

Activation reagents: PMA, PHA, Ionomycin (Sigma, St. Louis, MO); anti-CD3/CD28 antibodies (Invitrogen, Carlsbad, CA); CMV pp65 + IE-1 peptide mix (JPT, Berlin, Germany).

Standard Ab cocktail: CD3 V500, CD8 V450, CD4 PerCP Cy5.5, IFNgamma FITC, IL17 PE, IL2 PE Cy7, TNF AlexaFluor 700, IL-22 APC.

**CyTOF Immunophenotyping:**This assay was performed in the Human Immune Monitoring Center at Stanford University. PBMCs were thawed in warm RPMI+FBS media containing benzonase, washed twice, resuspended in CyFACS buffer (PBS supplemented with 2% BSA, 2 mM EDTA, and 0.1% soium azide), and viable cells were counted by Vicell. Cells were added to a U-bottom microtiter plate at 1.5 million viable cells/well and washed once by pelleting and resuspension in fresh CyFACS buffer. The cells were stained for 60 min at room temperature with 50 uL of the following antibody-polymer conjugate cocktail: [insert Ab list here]. All antibodies were from purified unconjugated, carrier-protein-free stocks from BD Biosciences, Biolegend, or R&D Systems. The polymer and metal isotopes were from Fluidigm Corporation. The cells were washed twice by pelleting and resuspension with 500 uL FACS buffer. The cells were resuspended in 100 uL PBS buffer containing 2 ug/mL Live-Dead (DOTA-maleimide (Macrocyclics) containing natural-abundance indium). The cells were washed twice by pelleting and resuspension with 500 uL PBS. The cells were resuspended in 100 uL 2% PFA in PBS and placed at 4C overnight. The next day, 500 uL CyFACS was added and the cells were pelleted. The cells were resuspended in 100 uL eBiosciences permeabilization buffer (1x in PBS) and placed on ice for 45 min before washing once with 500 uL CyFACS.The cells were resuspended in 100 uL iridium-containing DNA intercalator (1:2000 dilution in 1x PBS; Fluidigm) and incubated at room temperature for 20 min. The cells were washed once with 500 uL CyFACS and twice in 500 uLMilliQ water. The cells were diluted to 0.8M/mL in MilliQ water containing a 10x dilution of EQ Normalization beads (Fluidigm) before injection into the CyTOF (Fluidigm). Data analysis was performed using FlowJo v10 by gating on cells based on iridium signal, then intact cells based on both the iridium isotopes from the intercalator, then on singlets by Ir191 vs cell length, then on live cells (Indium-LiveDead minus population), followed by cell subset-specific gating.

**CyTOF Intracellular Cytokine Staining (ICS) Assay:** CyTOF ICS was performed at the Human Immune Monitoring Center as previously described (Subrahmanyam & Maecker). Briefly, frozen PBMC were thawed and washed twice in complete medium (RPMI supplemented with Pen-Strep and L-glutamine). Cell counts were obtained using a Vi-Cell XR cell viability analyzer (Beckman Coulter, Brea, California). 2 × 106 cells per sample were rested in 96-well U-bottom plates overnight at 37 °C, 5% CO2. After resting, secretion inhibitors brefeldin A (5μg/ml) and monensin (5μg/ml) (Sigma-Aldrich, St. Louis, MO) were added along with 10 ng/ml PMA and 1μg/ml Ionomycin (Sigma). All samples were incubated for 4 h at 37 °C. Thereafter, 2 mM EDTA was added for 15 min at room temperature. Cells were washed twice in CyFACS buffer (1X diluted from 10X PBS, (Rockland Immunochemicals, Pottstown, PA) supplemented with 0.1% BSA and 0.05% sodium azide). A cell-surface antibody cocktail consisting of pre conjugated antibodies (Fluidigm, South San Francisco, CA) as well as in-house conjugated antibodies (Table) was prepared in CyFACS and added to the cells for 45 min on ice. All antibody cocktails were filtered using 0.1μm spin filters (Millipore, Darmstadt, Germany) to remove possible antibody aggregates before staining. Cells were washed twice in CyFACS after surface staining, and 1:3000115In -DOTA Maleimide (5 mg/ml) live/dead stain was added for 30 min on ice. The cells were then fixed in 2% paraformaldehyde (PFA) in PBS at 4 °C overnight. These fixed cells were washed twice in 1X permeabilization buffer (eBioscience, San Diego, CA). Intracellular staining cocktail, also consisting of Fluidigm pre-conjugated as well as in house conjugated antibodies was prepared in 1X permeabilization buffer and added to the cells for 45 min on ice (Table). After washing three times in CyFACS, cells were stained with Intercalator-Ir (Fluidigm) diluted as per the manufacturer’s recommendations in 2% PFA in PBS, and incubated for 20 min at room temperature. Finally, cells were washed twice in CyFACS and three times in MilliQ water. EQ Four Element Calibration Beads from Fluidigm were added as per the manufacturer’s directions prior to running. Data were acquired on a Helios mass cytometer (Fluidigm).

Reference: Subrahmanyam PB, Maecker HT. Cytof measurement of immunocompetence across major immune cell types. Curr Protoc Cytom. 2017;82:9.54.1–9.54.12. <https://doi.org/10.1002/cpcy.27>

**RNA extraction and microarray--whole blood (PAXgene), Illumina microarray:**Work was performed by the Human Immune Monitoring Center at Stanford University.  RNA sampling and extraction: Blood was collected directly into PAXgene Blood RNA Tube (BD PreAnalytix) using a 21-gauge butterfly needle and catheter so that tubes could be held low and vertical to maintain the vacuum on top of the stabilization solution. Tubes were inverted 10 times. Each tube drew a maximum of 2.5 ml of blood. Samples were incubated in collection tubes at room temperature then stored at -80C within 4h. Total RNA was isolated according to the manufacturer’s Instructions by using a PAXgene RNA blood kit (Qiagen).The entire procedure was carried out at room temperature with the QIAcube automated robot (Qiagen) . Total RNA yield was assessed using the Thermo Scientific NanoDrop 1000 micro-volume spectrophotometer (absorbance at 260 nm and the ratio of 260/280 and 260/230). RNA integrity was assessed using the Agilent’s Bioanalyzer NANO Lab-on-Chip instrument (Agilent).

Microarray processing and analysis: Biotinylated, amplified antisense complementary RNA (cRNA) targets were prepared from 200 to 250 ng of the total RNA using the Illumina RNA amplification kit (Applied Biosystems/Ambion). Seven hundred and fifty nanograms of labeled cRNA was hybridized overnight to Illumina Human HT-12 V3 BeadChip arrays (Illumina), which contained >48,000 probes. The arrays were then washed, blocked, stained and scanned on an Illumina BeadStation 500 following the manufacturer’s protocols. BeadStudio/GenomeStudio software (Illumina) was used to generate signal intensity values from the scans. For normalization, the software was used to subtract background and scale average signal intensity for each sample to the global average signal intensity for all samples. A gene expression analysis software program, GeneSpring GX version 7.3.1 (Agilent Technologies), was used to perform further normalization. Transcripts meeting the filtering criteria were subjected to hierarchical clustering using GeneSpring.

**RNA extraction and microarray--PBMC or sorted cells, Agilent microarray:**Work was performed by the Human Immune Monitoring Center at Stanford University.  RNA sampling and extraction: PBMC or sorted cell populations (< 1x10^7 viable cells) were collected in 1ml TRIzol (Invitrogen) and stored at -80c until use). Total RNA was isolated according to the TRIzol protocol (Invitrogen) or RNeasy Mini Kit (Qiagen). For using the RNeasy Mini Kit, the entire procedure was carried out at room temperature with the QIAcube automated robot (Qiagen) . Total RNA yield was assessed using the Thermo Scientific NanoDrop 1000 micro-volume spectrophotometer (absorbance at 260 nm and the ratio of 260/280 and 260/230). RNA integrity was assessed using the Agilent’s Bioanalyzer NANO Lab-on-Chip instrument (Agilent).

Microarray processing and analysis: Cy3 and/or Cy5 labeled, amplified antisense complementary RNA (cRNA) targets were prepared from 20 to 500 ng of the total RNA using the QuickAmp Labeling kit or the Low Input Quick Amp Labeling Kit (Agilent). 850 ug of labeled cRNA was hybridized overnight to Agilent Whole Human Genome 4 x 44 K slides, which contain 44,000 probes, including 19,596 Entrez Gene RNAs; or to SurePrint G3 Human Gene Expression 8x60k slides, which contain 60,000 probes, including 27,958 Entrez Gene RNAs and 7,419 lincRNAs. The arrays were then washed, blocked, stained and scanned on the Agilent microarray scanner following the manufacturer’s protocols. Data were extracted using Agilent Feature Extraction Software. Microarray normalization was performed by GeneSpring GX 11.0 software. Further statistical and bioinformatic analyses were done with Ingenuity Pathway Analysis software (Ingenuity Systems).Transcripts meeting the filtering criteria were subjected to hierarchical clustering using GeneSpring.

**Biomark Microfluidic qPCR Array:**This assay was performed by the Human Immune Monitoring Center at Stanford University.  For 10-50 ng total RNA, reverse transcription of the RNA to cDNA was performed at 50°C for 15 minutes using the High Capacity Reverse Transcription kit (ABI). For cell samples, RT was performed directly on a 96-well PCR plate (ABI) containing lysis buffer (Invitrogen) by using SuperScript III One-Step RT-PCR System with PlatinumTaq (CellDirect kit, Invitrogen). PreAmp was performed on a thermocycler using the TaqMan PreAmp Master Mix Kit (Invitrogen) added to cDNA and pooled Taqman assays. RT enzyme was inactivated and the Taq polymerase reaction was started by bringing the sample to 95 °C for 2 minutes. The cDNA was preamplified by denaturing for 10 (total RNA) to 18 (single cell) cycles at 95°C for 15 seconds, annealing at 60°C for 4 minutes. The resulting cDNA product was diluted 1:2 with 1x TE buffer (Invitrogen). 2X Applied Biosystems Taqman Master Mix , Fluidigm Sample Loading Reagent, and preamplified cDNA were mixed and loaded into the 48.48 Dynamic Array (Fluidigm) sample inlets, followed by loading 10X assays into the assay inlets. Manufacturer’s instructions for chip priming, pipetting, mixing, and loading onto the BioMark system were followed. Real-time PCR was carried out with the following conditions: 10 min at 95°C, followed by 50 cycles of 15 sec at 95°C and 1 min at 60°C. Data was analyzed using Fluidigm software. All reactions were performed in duplicate or triplicate, and Ct values were normalized to the GAPDH or 18S positive control.

**Single cell TCRseq+phenotyping:**This assay was performed by the Human Immune Monitoring Center at Stanford University. This method was based on Han et al., Nature Biotechnology 32, 684-692 (2014). Using single FACS-sorted T cells in 96-well plates, TCR sequence and gene expression analysis were obtained by a series of three nested PCR reactions. Cells were sorted directly into RT-PCR buffer. For the first reaction, reverse transcription and preamplification were performed with a One-Step RT-PCR kit (Qiagen) using multiplex PCR with multiple Vα and Vβ region primers, Cα and Cβ region primers, and primers for selected T cell phenotyping genes, in a 20-μl reaction. A 25-cycle first RT-PCR reaction was done per manufacturer's instructions using the following cycling conditions: 50 °C 30 min; 95 °C 15 min; 94 °C 30 s, 62 °C 1 min, 72 °C 1 min × 25 cycles; 72 °C 5 min; 4 °C. Next, a 1-μl aliquot of the first reaction was used as a template for a second 20-μl PCR using HotStarTaq DNA polymerase (Qiagen) for either TCR sequencing or phenotyping. The cycling conditions were: 95 °C 15 min; 94 °C 30 s, 64 °C 1 min, 72 °C 1 min × 25 cycles (TCR) or 35 cycles (phenotyping); 72 °C 5 min; 4 °C. A 1-μl aliquot of the second PCR product was used as a template for the third 20-μl PCR reaction, which incorporated barcodes and enabled sequencing on the Illumina MiSeq platform. The PCR products were combined at equal proportion by volume, run on a 1.2% agarose gel, and a band around 350 to 380 bp was excised and gel purified using a Qiaquick gel extraction kit (Qiagen). This purified product was then sequenced on Miseq for a pair-ended 2x250 run. The fastq files were used to run a customized TCRseq informatics pipeline, which assigned reads to wells, totaled phenotyping gene reads per well, assigned TCR Va, Ja, Vb, and Jb usage, along with alternate Va/Ja where applicable, and reported CDR3a and CDR3b sequences.