Supplemental Materials

Type 1-polarized DC immunotherapeutic contains heterogeneous populations with IL-12p70 production restricted to a rare subset

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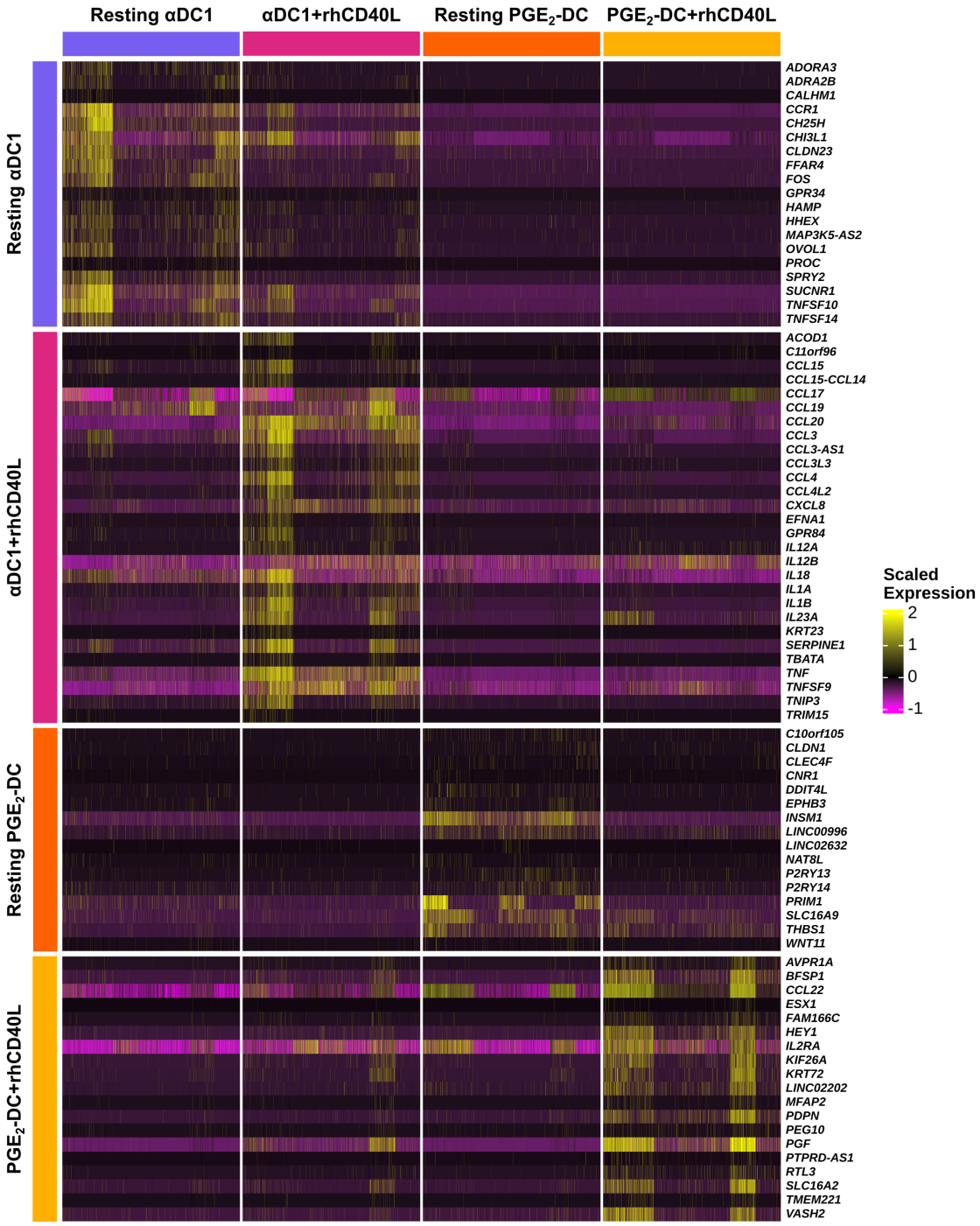
Supplemental Methods

Single cell multiomics analysis

αDC1 and PGE_2-DC from n = 7 independent study participants were sampled for single cell multiomics analysis using the BD Rhapsody Single-Cell Analysis System (RRID:SCR_027096) based on their combined representation of the spectrum of CD40L-induced IL-12p70 production. The mature DC were harvested on culture day 6, washed to remove maturation cytokines, and replated in a fresh ultra-low attachment 24 well plate before stimulation with 1 μg/mL rhCD40L for 4 hours. The cells were then collected and labeled with condition-specific oligomer-conjugated multiplexing antibodies (BD Biosciences Cat# 633781, RRID:AB_2870299). For n = 3 participants, the multiplexed cells were stained with the following antibodies to analyze surface protein expression: CD19 (clone HIB19; BD Biosciences Cat# 940247, RRID:AB_2876128), CD274 (clone MIH1; BD Biosciences Cat# 940035, RRID:AB_2875926), CD3 (clone SK7; BD Biosciences Cat# 940000, RRID:AB_2875891), CD86 (clone 2331 (FUN-1); BD Biosciences Cat# 940025, RRID:AB_2875916), HLA-DR (clone G46-6; BD Biosciences Cat# 940010, RRID:AB_2875901), and CD169 (clone 7-239; BD Biosciences Cat# 940223, RRID:AB_2876104). For n = 4 participants, the multiplexed cells were then stained with the OMICS-One APC panel of oligomer-conjugated AbSeq antibodies (BD Biosciences Cat# 572435) and 12 additional AbSeq antibodies (see Table S1) to analyze surface protein expression. Barcoded cells were then mixed in equal parts before loading onto a BD Rhapsody high-throughput cartridge per manufacturer's instructions for single cell capture using the BD Rhapsody Express instrument. Quality control metrics were

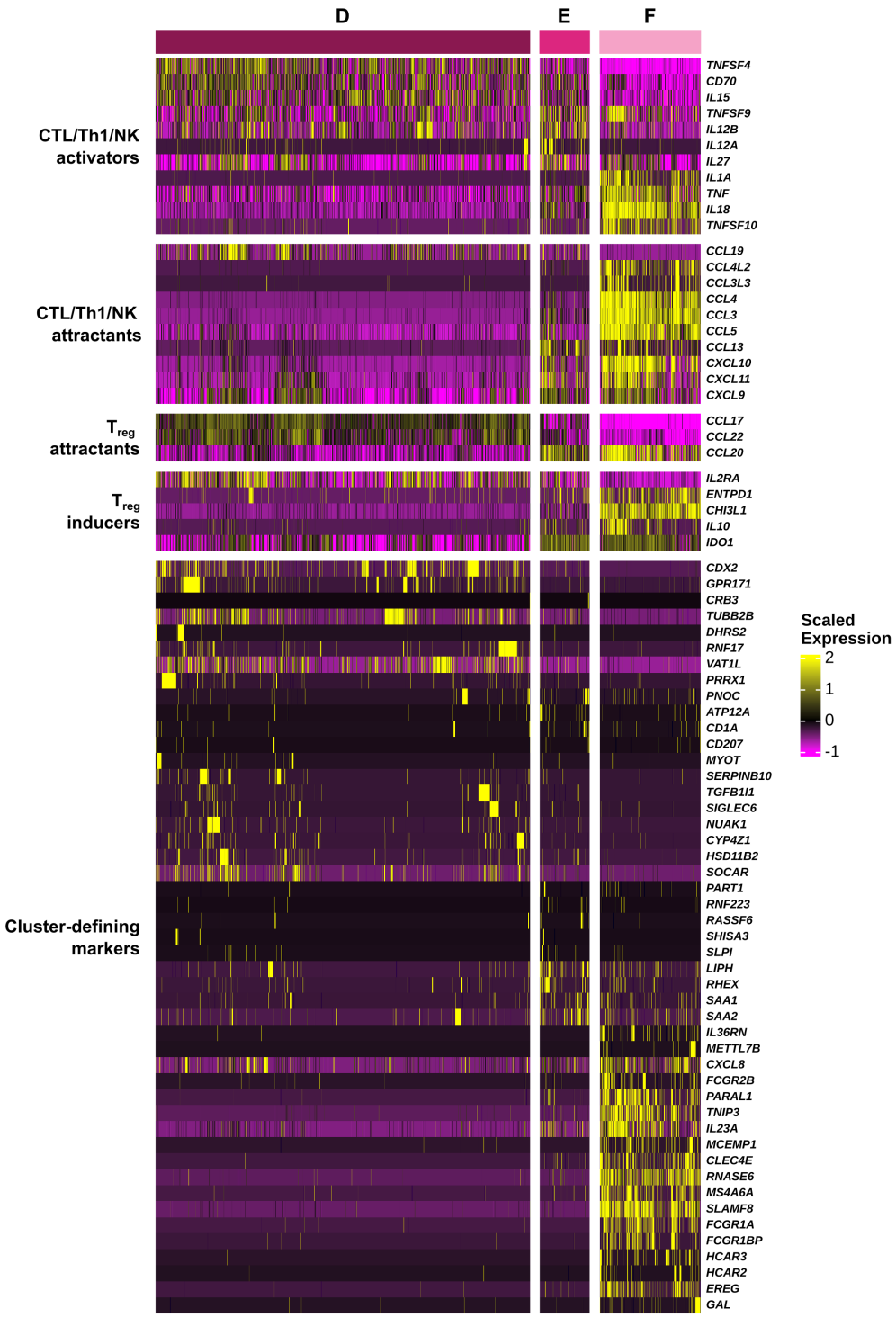
verified using the BD Rhapsody Scanner. cDNA libraries of the whole transcriptome, multiplexing barcodes, and AbSeq barcodes were then prepared according to manufacturer's specifications. Pooled libraries were submitted to the Emory Yerkes National Primate Research Center Genomics Core (RRID:SCR_026418) for sequencing on an Illumina NovaSeq 6000 (RRID:SCR_016387) or the UCLA Technology Center for Genomics and Bioinformatics (RRID:SCR_012204) for sequencing on an Illumina NovaSeq X (RRID:SCR_024569). Demultiplexing, initial cell calling, quality filtering, alignment, and annotation were performed using the BD Rhapsody Whole Transcriptome Analysis pipeline on the Seven Bridges Genomics platform (multiple versions, RRID:SCR_008308). The resulting Seurat objects were then imported into RStudio (R version 4.5.0, RRID:SCR_000432), and Seurat (version 5.0.3, RRID:SCR_016341) was used for further quality control (87). After removing multiplets and cells with mitochondrial reads >25%, n = 149,535 cells were included in the analysis. Samples were log normalized using Seurat::NormalizeData and the first 3 participants were batch-corrected using the RPCA method implemented in Seurat::IntegrateData. Because the average sequencing depth (83,368.42 reads/cell) was greater for these 3 participants compared to the others (48,083.84 reads/cell), the remaining 4 participants were reference-mapped using that integrated dataset as the reference before downstream analysis. After re-scaling the mapped data, differential gene expression analysis was performed using MAST (version 1.33.0) with regression of percent mitochondrial transcripts and participant as implemented in Seurat::FindMarkers. Cells were randomly downsampled for Supplemental Figure S1 for DEG analysis for computational efficiency. The data were plotted using the SCpubr

(version 3.0.0; preprint citation), EnhancedVolcano (version 1.26.0, RRID:SCR_018931), ComplexHeatmap (version 2.24.1, RRID:SCR_017270), scCustomize (version 3.0.1, RRID:SCR_024675), and UpSetR (version 1.4.0, RRID:SCR_026112) packages (88-92). After filtering for DEGs with p adj < 0.01 and $|\log_2 FC| > 1$, pathway enrichment analysis was conducted on those gene sets using the pathfindR package with the BioGRID PIN (version 2.6.0) (93). Proteomic data were normalized using centered log ratio as implemented in Seurat::NormalizeData.



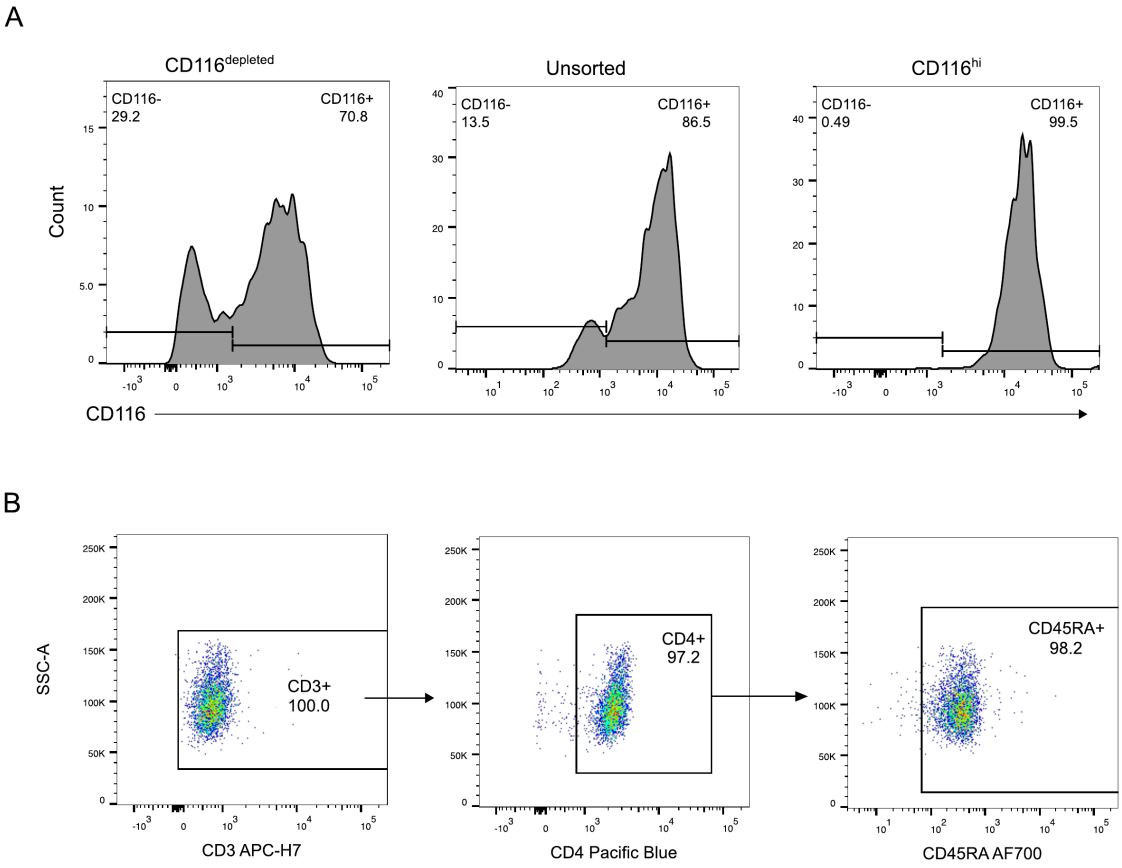
Supplemental Figure S1: Differentially expressed genes across αDC1 and PGE_2-DC ± rhCD40L stimulation.

Heatmap displaying scaled expression of the top differentially expressed genes (DEGs) identified in each DC type both with and without rhCD40L stimulation (n = 7 participants). Equal numbers of cells were sampled from each participant within each condition for visualization. Columns represent individual cells grouped by condition, and rows represent individual genes. Genes are grouped by the condition in which they are most highly expressed, as indicated by the row annotations at left. DEGs were determined by running MAST with regression of percent mitochondrial transcripts and participant on a randomly downsampled set of 10,000 cells per condition.



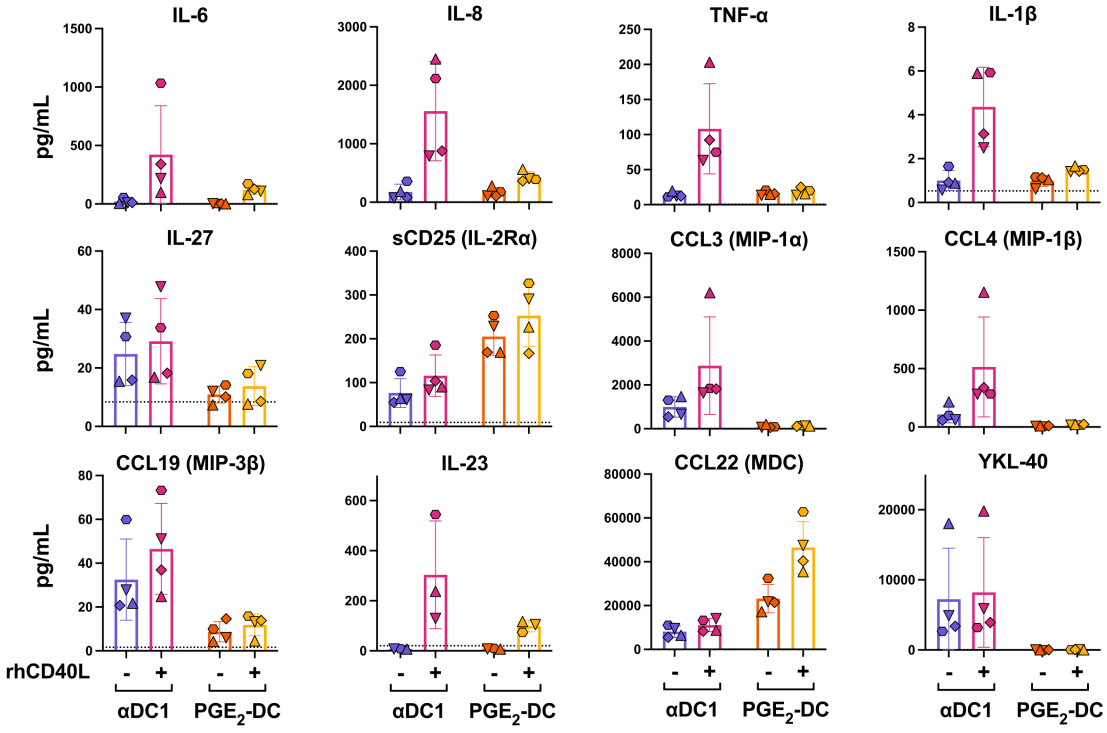
Supplemental Figure S2: Differentially expressed genes across rhCD40L-stimulated αDC1 subclusters.

Heatmap displaying scaled expression of differentially expressed genes (DEGs) identified in each rhCD40L-stimulated αDC1 subcluster (D, E, F) (n = 7 participants). Equal numbers of cells were sampled from each cluster for visualization. Columns represent individual cells grouped by subcluster; rows represent individual genes. Genes are grouped into CTL/Th1/NK cell activators or attractants, Treg attractants or inducers, and remaining cluster-specific markers as indicated by the row annotations at left. DEGs were determined by running MAST with regression of percent mitochondrial transcripts and participant.



Supplemental Figure S3: CD116-based magnetic separation of αDC1 and purity of naïve CD4+ T cells used for priming.

(A) CD116 expression by flow cytometry in the CD116^{depleted}, unsorted, and CD116^{hi} fractions following magnetic separation of mature αDC1, with the percentage of CD116^{+} and CD116^{-} cells indicated in each gate; the CD116^{depleted} fraction remained 70.8% CD116^{+}, reflecting enrichment rather than absolute purification. (B) Representative sequential gating of enriched naïve CD4^{+} T cells used in mixed lymphocyte reactions, showing CD3^{+} (100.0%), CD4^{+} (97.2%), and CD45RA^{+} (98.2%) populations.



Supplemental Figure S4: Multiplex analysis of cytokine and chemokine secretion by αDC1 and PGE_2-DC ± rhCD40L.

Concentrations of IL-6, IL-8, TNF-α, IL-1β, IL-27, sCD25 (IL-2Rα), CCL3 (MIP-1α), CCL4 (MIP-1β), CCL19 (MIP-3β), CCL22 (MDC), and YKL-40 in supernatants collected from αDC1 and PGE_2-DC following 24-hour stimulation with or without rhCD40L (1 µg/mL) were measured by multiplex electrochemiluminescence assay (Meso Scale Discovery) (n = 4 participants). IL-23 concentration was determined after the same stimulation by ELISA (n = 3 overlapping participants). Values were normalized to cellular ATP content. Dashed lines indicate assay lower limits of detection, and values below that limit are represented as half of the lower limit of detection for analysis purposes. Bars represent mean ± SD; individual participant values are shown with unique symbols. Low n precluded statistical analysis.

Supplemental Table S1. AbSeq antibodies used for CITE-seq proteomic analysis

Target	Clone	BD cat #	RRID
CD1c	F10/21A3	OMICS-One APC/Myeloid-Cell Protein Panel (572435)	AB_2875974
CD11c	B-LY6		AB_2875915
CD11b	M1/70		AB_2875899
CD14	MPHIP9		AB_2875896
CD15	W6D3		AB_2876152
CD16	3G8		AB_2875897
CD32	FLI8.26		AB_2875960
CD33	WM53		AB_2875922
CD36	CLB-IVC7		AB_2876105
CD40	5C3		AB_2875940
CD45	HI30		AB_2875893
CD64	10.1		AB_2875914
CD85k	ZM3.8		AB_2876140
CD80	L307.4		AB_2875927
CD116	hGMCSFR-M1		AB_2876186
CD103	BER-ACT8		AB_2875958
CD115 (CSF1R)	9-4D2-1E4		AB_2876106
CD123	7G3		AB_2875911
CD141	1A4		AB_2875970
CD162	KPL-1		AB_2876108
CD163	GHI/61		AB_2875949
CD169	7-239		AB_2876104
CD192 (CCR2)	LS132.1D9		AB_2876163
CD195 (CCR5)	2D7/CCR5		AB_2875941
CD206	19.2		AB_2875959
CD273	MIH18		AB_2875962
CD274	MIH1		AB_2875926
HLA-DR	G46-6		AB_2875901
FCeR1a	AER-37		AB_2876102
VISTA	MIH65.rMAb		AB_2876339
CD137	4B4-1	940055	AB_2875946
CD49d	9F10	940059	AB_2875950
CD63	H5C6	940243	AB_2876124
CD81	JS-81	940052	AB_2875943
CD18	6.7	940086	AB_2875977
CD85j	GHI/75	940249	AB_2876130
HLA-DQ	TU169	940233	AB_2876114
CD89	A59	940277	AB_2876155
CCR7	L1-A	940394	AB_2876258
CD25	2A3	940009	AB_2875900
CD83	HB15e	940054	AB_2875945
CD86	2331 (FUN-1)	940025	AB_2875916