Systemic T-Cell Activation Landscapes Elicited Following Intramuscular and Intranasal Immunization with the Active NDV-Vectored Anti-COVID-19 Vaccine "Patria" in Humans

Claudia Carranza [1,†], Alejandro Torres-Flores [2,†], Dámaris P. Romero-Rodríguez [3], Constantino López-Macías [4], Martha Torres [1] and and Esmeralda Juárez [5,*]

Supplementary material

Table S1. List of antibodies used for flow cytometry staining.

Antibody against	*Fluorochrome*	*Supplier*	*Cat. number*	*Dilution (ml)*	*RRID*
CD3	AF700	Biolegend	344821	1:33	AB_2563419
CD4	PerCPCy5.5	Biolegend	300530	1:100	AB_893322
CD8	BV605	Biolegend	300936	1:33	AB_2832575
CD8	PE/Cy7	Biolegend	980910	1:100	AB_2876774
CD16	BV510	Biolegend	302048	1:100	AB_2562085
CD19	APC-Cy7	Biolegend	302218	1:100	AB_314248
CD19	APC	BD	555415	1:40	AB_398597
CD14	APC-Cy7	Biolegend	325620	1:100	AB_830693
CD14	PE CF594	BD	562335	1:100	AB_11153663
CD56	PE	Biolegend	362508	1:100	AB_2563925
CD69	BV421	Biolegend	310930	1:25	AB_2561909
CD137	APC	Biolegend	309810	1:33	AB_830672
OX40	PE-Cy7	Biolegend	350012	1:100	AB_10901161
IFN-γ	FITC	BD	554551	1:200	AB_395473
TNF-α	APC	Biolegend	502912	1:33	AB_315264
IL-2	BV605	Biolegend	500332	1:40	AB_2563877

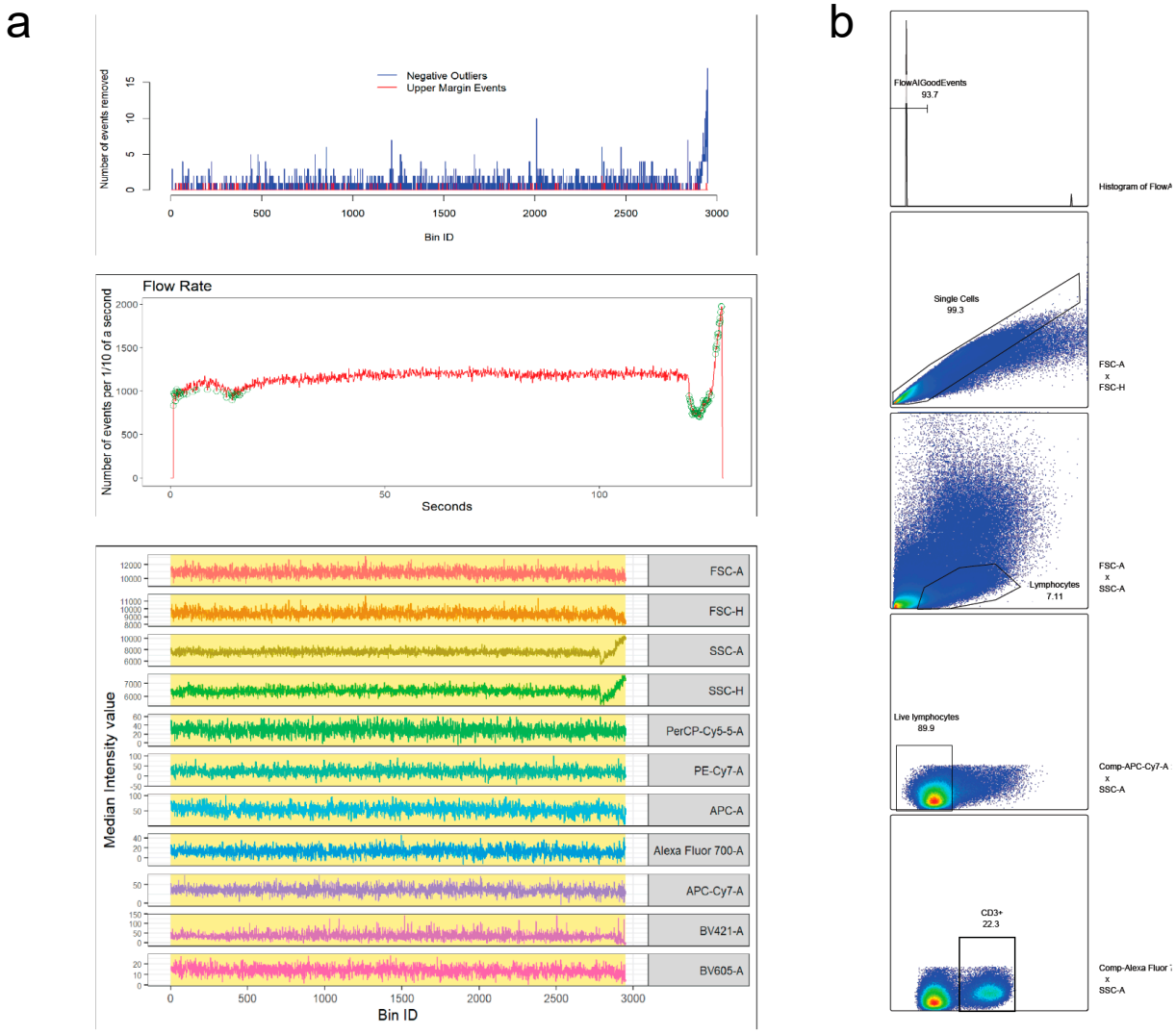


Figure S1. Flow cytometry quality control and gating strategy. Raw flow cytometry data were initially processed using the FlowAI algorithm to identify and remove anomalous events based on acquisition stability, flow rate, and fluorescence signal consistency, thereby retaining high-quality events for downstream analysis (a). Subsequently, sequential gating was applied to select singlets and lymphocytes based on light-scatter properties, and live cells by excluding Live/Dead^{+} events. The remaining lymphocytes were then separated into CD3^{+} and CD3^{-} compartments for subsequent immunophenotypic and computational analyses (b).

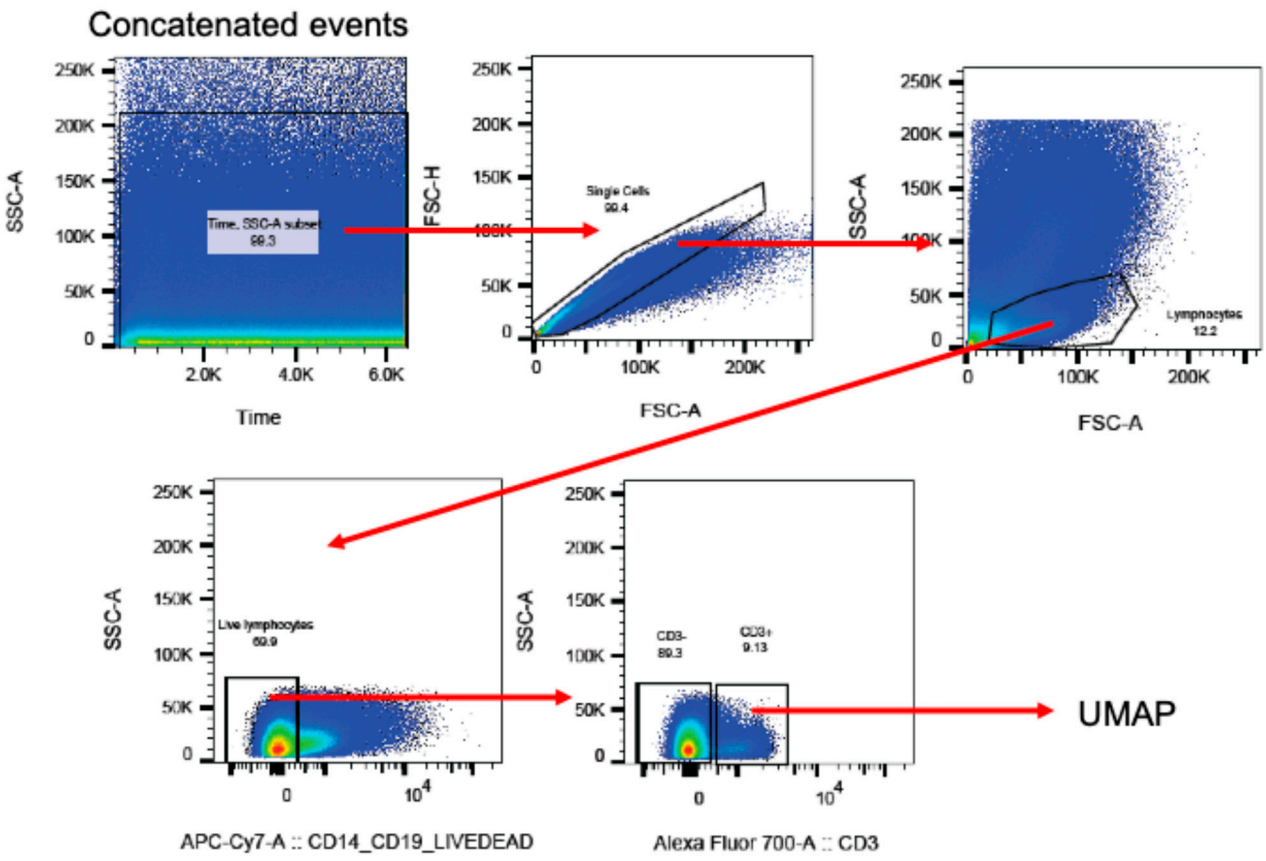


Figure S2. Gating strategy and preprocessing workflow for high-dimensional analysis. Flow cytometry data were initially subjected to quality control using the FlowAI algorithm to remove anomalous events. High-quality events from all samples were subsequently processed through sequential gating to select time-stable events, singlets, and lymphocytes based on light scatter properties. Dead cells, along with $CD14^+$ and $CD19^+$ cells, were excluded using a dump gate, and the remaining lymphocytes were separated into $CD3^+$ and $CD3^-$ compartments for downstream computational analysis. The resulting populations were exported for UMAP visualization and FlowSOM clustering.

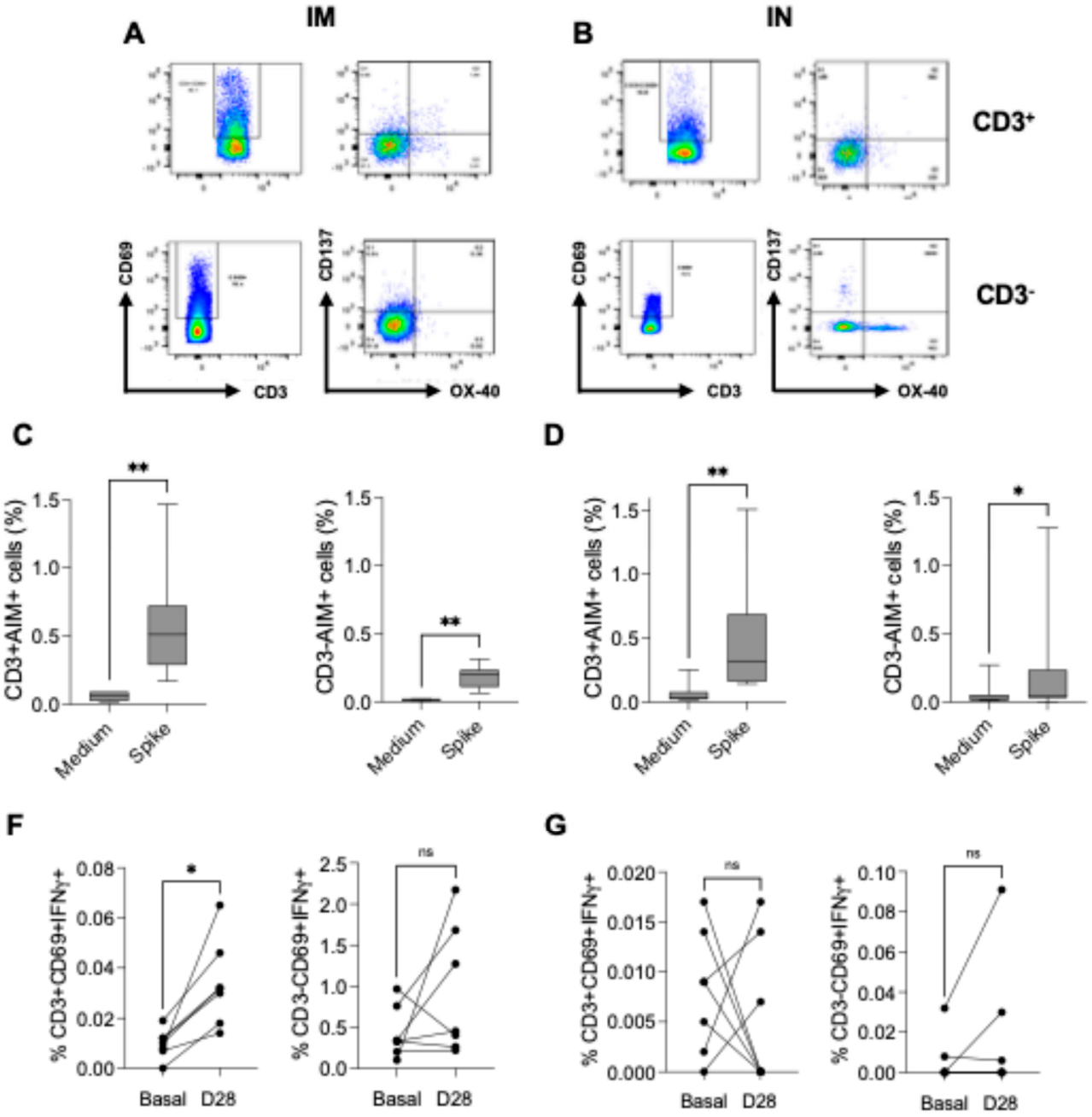


Figure S3. Representative gating strategy and conventional AIM analysis of Spike-responsive CD3⁺ and CD3⁻ lymphocytes following AVX/COVID-12 vaccination. Whole blood cells were stimulated in vitro with recombinant SARS-CoV-2 Wuhan Spike protein or culture medium alone for 18 h, and activation-induced marker (AIM) expression was evaluated by flow cytometry. (A,B) Representative gating strategy used to identify AIM⁺ CD4⁺ T cells (CD69⁺OX40⁺), AIM⁺ CD8⁺ T cells (CD69⁺CD137⁺), and activation-associated CD3⁻ cells following intramuscular (IM) (A) or intranasal (IN) (B) vaccination. Frequencies of Spike-responsive AIM⁺ CD4⁺, AIM⁺ CD8⁺, and activation-associated CD3⁻ cells (C, D) and IFNγ-producing cells (F, G) were measured 28 days after IM (C, F, n = 8) or IN (D, G, n = 9) vaccination. Data are presented as box plots showing the median and interquartile range, or as before-after comparisons at 28 days post-vaccination. Statistical comparisons were performed using the Mann–Whitney U test. *p < 0.05; **p < 0.01.