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Supporting Information

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V_4C_3 MXene Immune Profiling and Modulation of T Cell-Dendritic Cell Function and Interaction

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The supporting information includes Supporting Figures S1-S5 and Supporting Table 2:

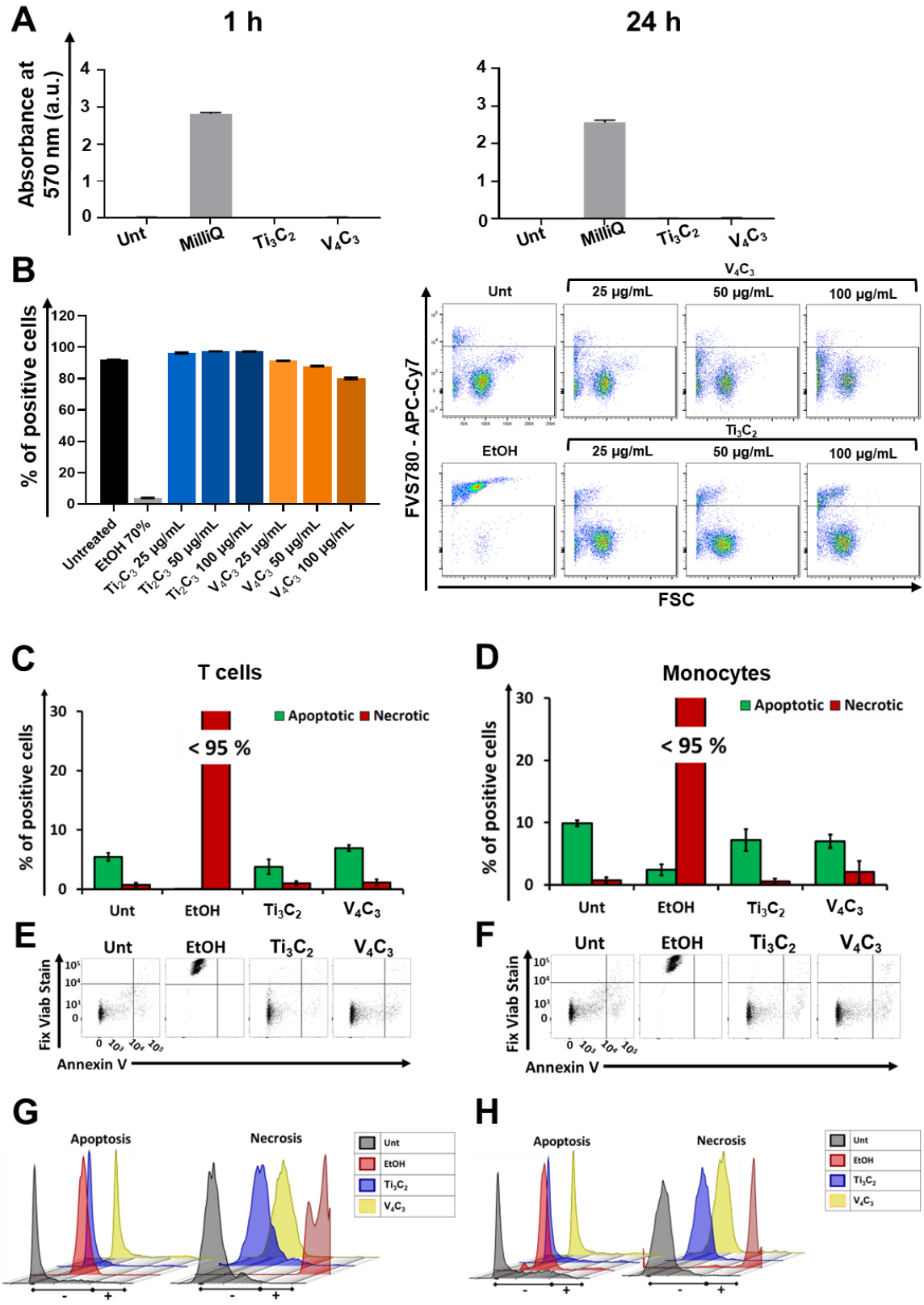

Figure S1. **Effects of MXenes on RBC lysis, PBMC viability, and primary T cell and monocyte apoptosis and necrosis. A)** Histograms of RBCs treated with 50 µg/mL of Ti_3C_2 or V_4C_3 for 1 and 24 h. Sample Absorbance was measured with Sunrise TECAN Infinite M200PRO microplate reader

at the wavelength of 570 - 620 nm. MilliQ water was used as a positive control to induce cell lysis. **B)** PBMCs were treated with Ti_3C_2 or V_4C_3 (25, 50, and 100 µg/mL) for 24 h or left untreated (Unt). EtOH 70% was used as a positive control to induce cell death. PBMCs were stained with Fixable Viability Staining 780. Histograms and dot plots show the percentage (%) of positive live cells. **C-D)** Cells were treated with 50 µg/mL of Ti_3C_2 or V_4C_3 for 24 h or left untreated (Unt). EtOH 70% was used as a positive control to induce cell death. PBMCs were stained with Annexin V for apoptosis and Fixable Viability Staining 780 for necrosis. T cells were gated for CD3 marker while monocytes were gated for CD14 marker. Both apoptotic and necrotic cells were evaluated and expressed as percentage of total cell number. **E-F)** Representative dot-plot analysis for T cells and monocytes. **G-H)** Histograms showing apoptotic and necrotic cells.

A

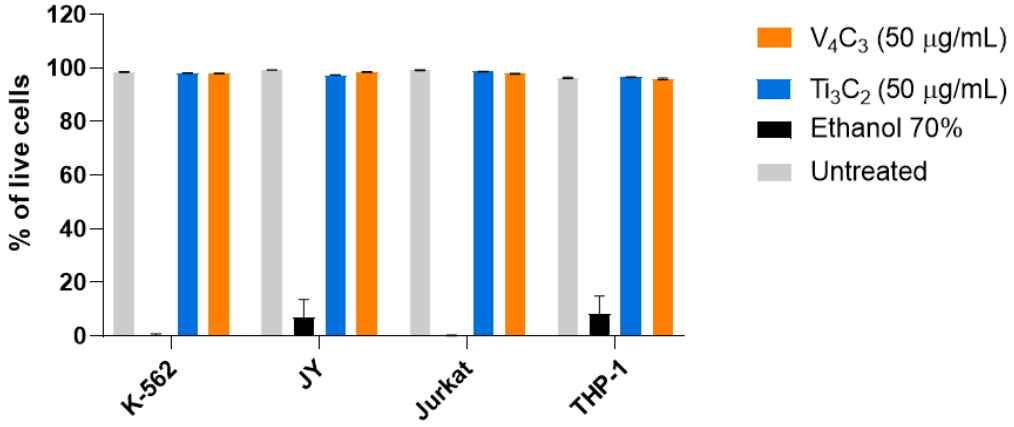

Figure S2. Impact on viability of K-562, JY, Jurkat, and THP-1 cell lines. Cell viability after treatment with 50 µg/mL of V_4C_3 or Ti_3C_2 for 24 h. After material exposure, cells were stained with Fixable Viability Staining Zombie NIR and then analyzed by flow cytometry. The histogram indicates the percentage of live cells on total single cells.

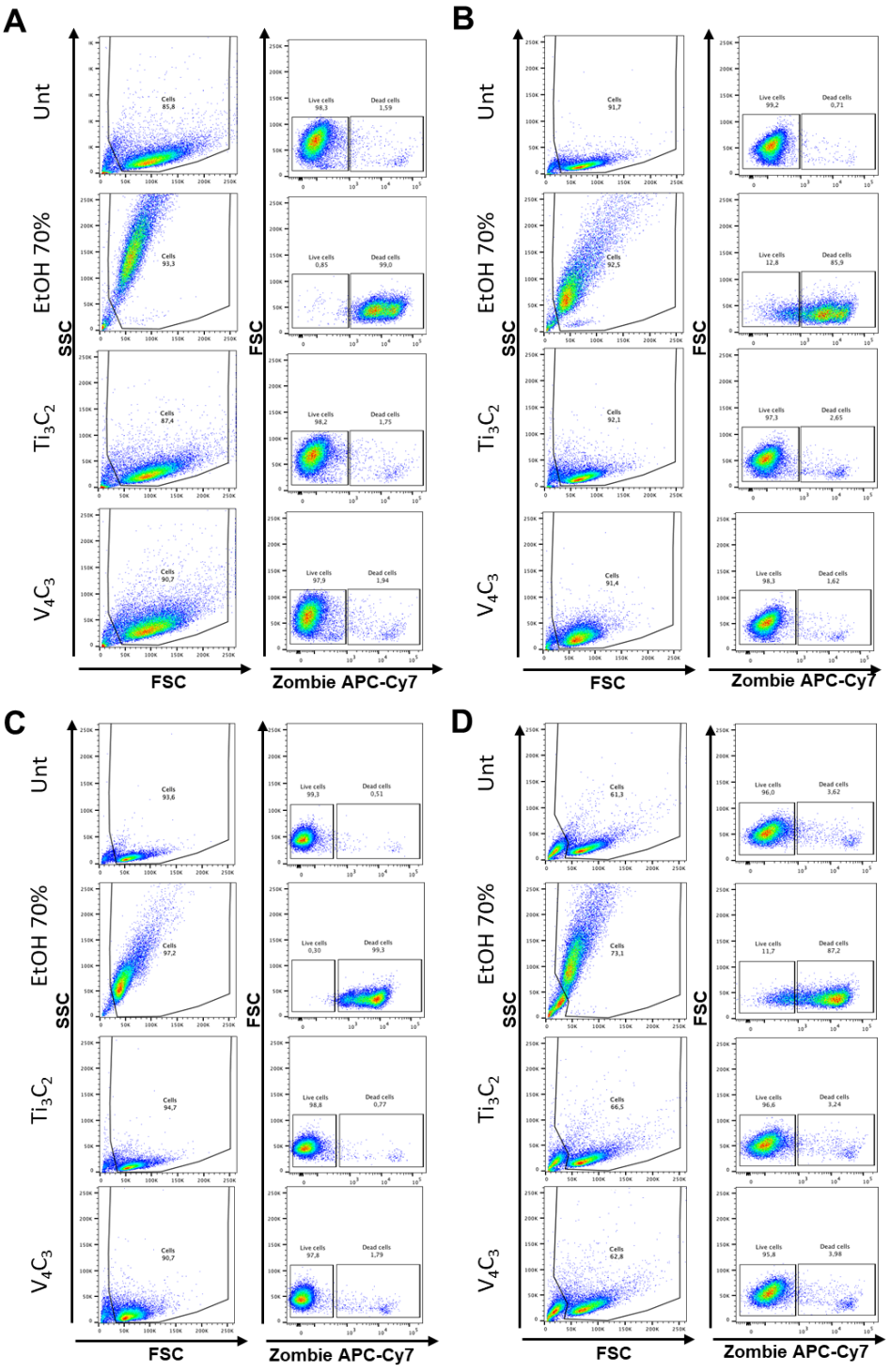

Figure S3. A) Viability of K562, **B)** JY, **C)** Jurkat, and **D)** THP-1 cell lines after treatment with 50 µg/mL of V_4C_3 or Ti_3C_2 for 24 h. After material exposure, cells were stained with Fixable Viability Staining Zombie NIR and then analyzed by flow cytometry. Dot plots indicate the percentage of live cells on total single cells.

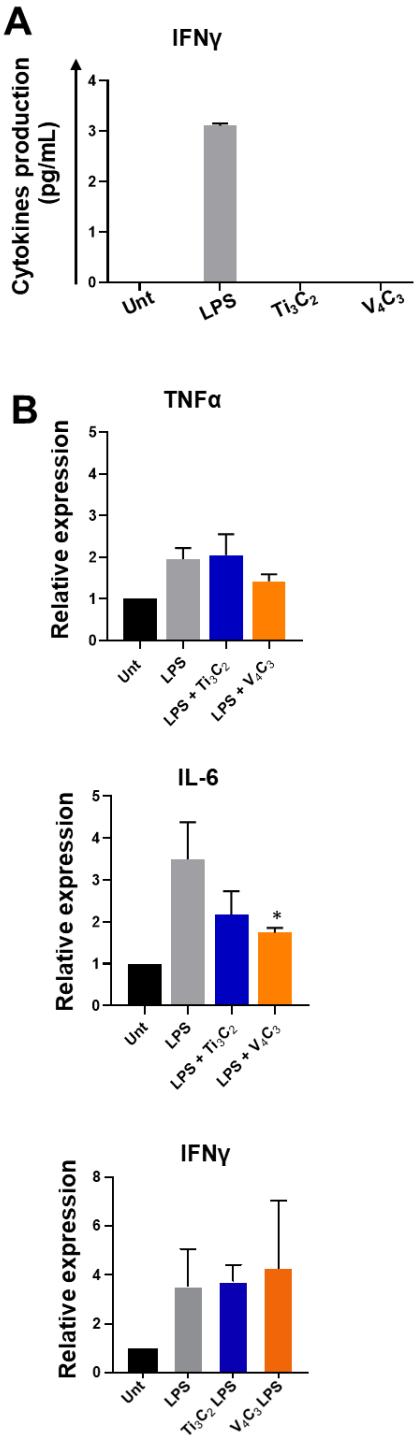

Figure S4. Cytokine release and gene expression with LPS priming. PBMCs were treated with 50 µg/mL of Ti_3C_2 or V_4C_3 for 24 h with LPS priming or left untreated. LPS (2 µg/mL) was used as a positive control. **A)** Cytokine release (IFNγ) was assessed by cytokine bead array in flow cytometry and expressed as pg/mL. **B)** Gene expression (TNFα, IL-6, and IFNγ) versus untreated (unt) was evaluated by RT-qPCR and normalized to Gapdh by $2^{-\Delta\Delta CT}$ method (represented as Log2 fold change). All the experiments were performed at least as three independent triplicate (*$p < 0.05$, **$p < 0.01$ Statistical analysis performed by two-tales student t-test).

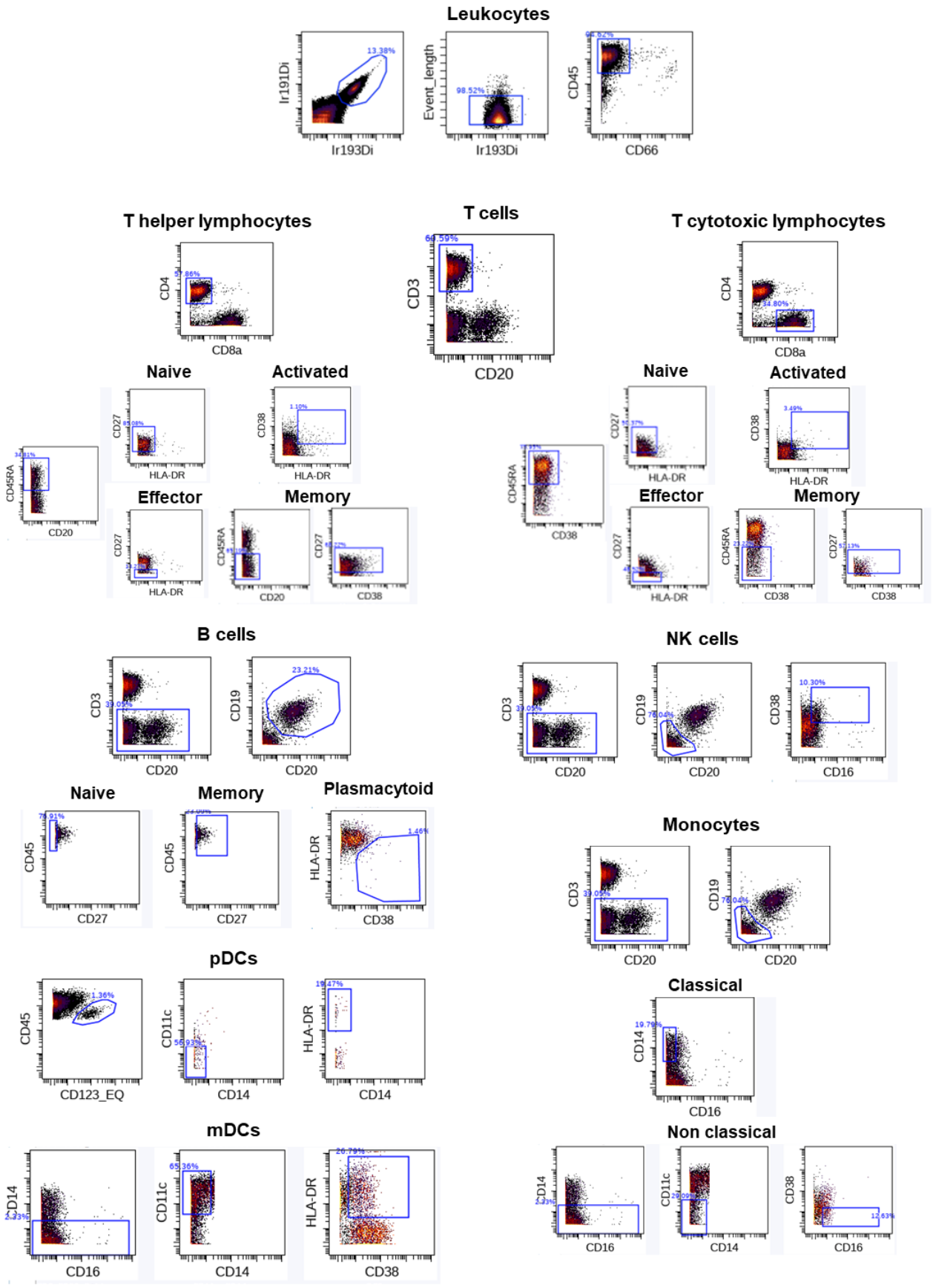

Figure S5. Human primary immune cell subpopulations gating strategy. Dot plots showing the gating strategy used for the identification of the different immune cell subpopulations by CyTOF.

Antibody	**Fluorochrome**	**Clone**	**Manufacturer**	**Catalogue number**
Fixable Viability Stain 780 (FVS780)	APC Cy7		BD Horizon	565388
Annexin V	PE		BD Horizon	560930
Anti-CD25	PE	M-A251	BD Bioscience	557138
Anti-CD69	FITC	FN50	BD Horizon	561928
Anti-CD4	BV421	RM4-5	Biolegend	100543
Anti-*I-A/I-E*	BV421	M5/114.15.2	Biolegend	107631
Zombie NIR fixable viability dye	APC Cy7		Biolegend	423105
Anti-CD11c	BV605	N418	Biolegend	117333
Anti-CD154	PE	MR1	Miltenyi	130102467
Anti-CD40	APC	FGK45.5	Miltenyi	130102547

Table S2. Table reporting all the antibodies used for the flow cytometry experiments. Each antibody was used in the concentration suggested by the manufacturer.