

Supplementary Information

Engineering T Cell-Selective Chemokine Inhibitors Through Network-Level Directed Evolution

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Supplementary Fig. 1 | Propidium iodide staining for peptide cytotoxicity assays.

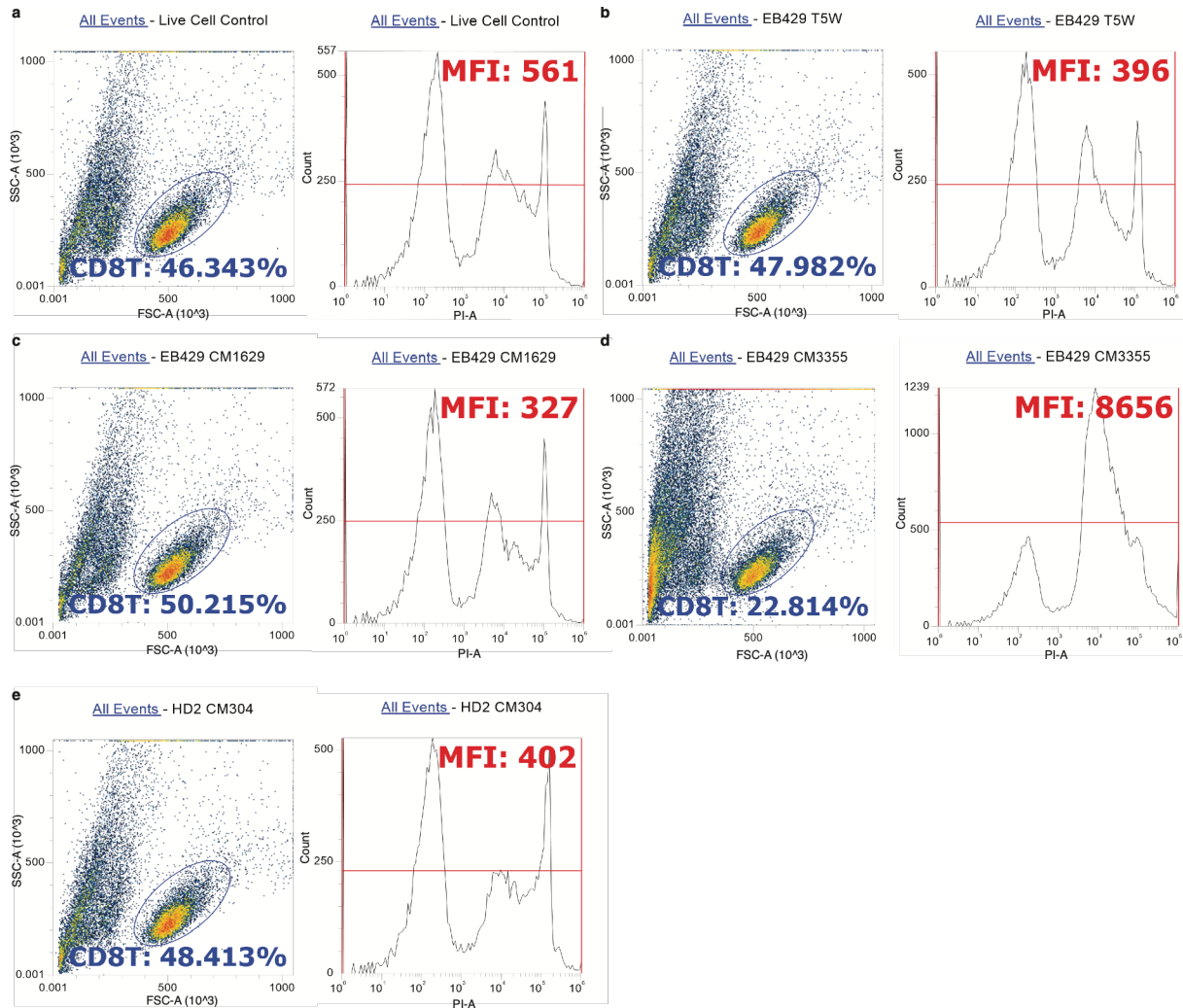
Supplementary Fig. 2 | IMiD-chemokine pools elicit dose-dependent migration responses.

Supplementary Fig. 3 | Effect of scrambling peptide sequences

Supplementary Fig. 4 | Dose-dependent inhibition of cell migration by peptides.

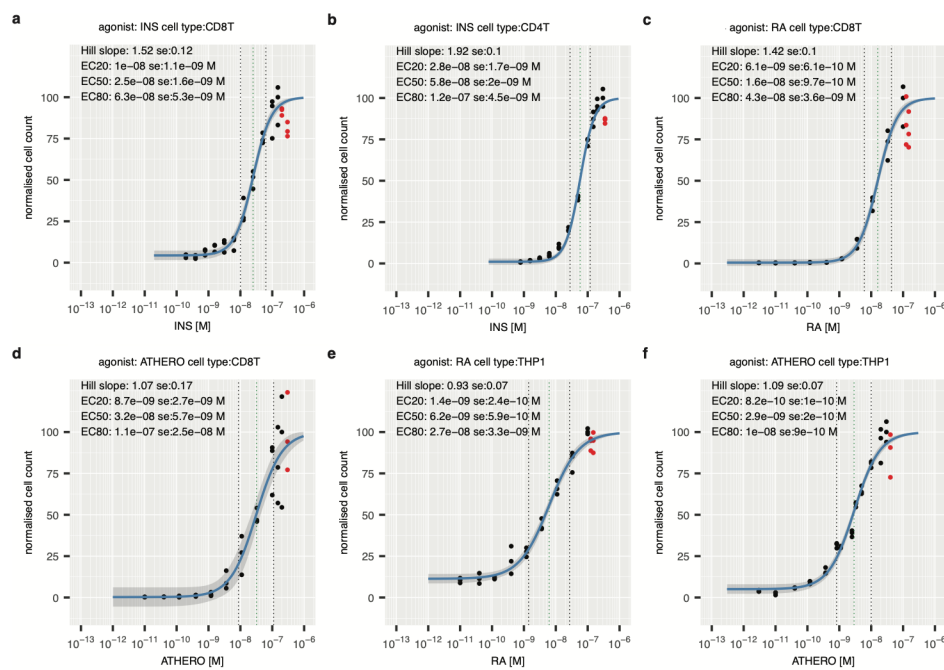
Supplementary Fig. 5 | Residue level interaction tile-plot

Supplementary Fig. 6 | Cell receptor labelling of CD8⁺ and CD4⁺ T cells.



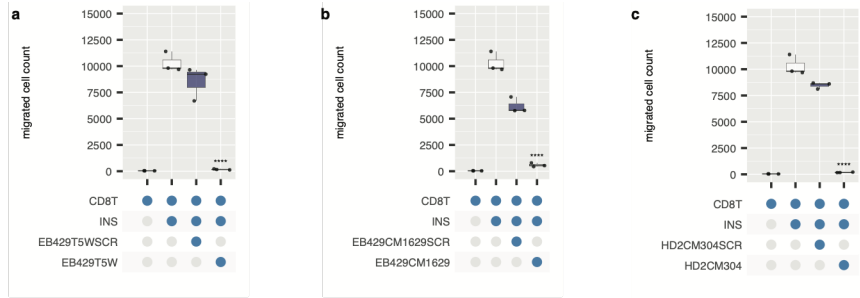
Supplementary Fig. 1 | Propidium iodide staining for peptide cytotoxicity assays.

a-e, Representative flow cytometry dot plots (left) and corresponding propidium iodide median fluorescence intensity (MFI) histograms (right) for CD8⁺ T cells incubated with or without peptides. Dot plots show forward scatter height (FSC-H; x-axis) versus side scatter height (SSC-H; y-axis). CD8⁺ T cells are identified by the gate labelled “CD8T”. Histograms show cell count (y-axis) versus fluorescence intensity (x-axis), with the median fluorescence intensity indicated in each panel. Median fluorescent intensity was calculated from the entire histogram as indicated by the red gate. **a**, untreated control; **b**, EB429T5W; **c**, EB429CM1629; **d**, EB429CM3355; **e**, HD2CM304.



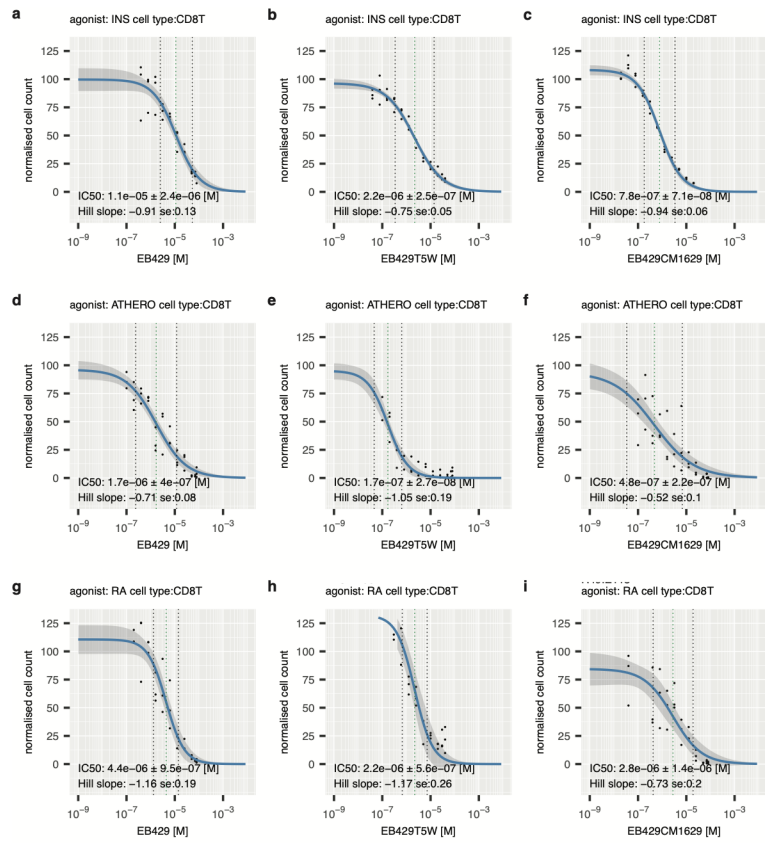
Supplementary Fig. 2 | IMID-chemokine pools elicit dose-dependent migration responses.

Representative dose-response curves showing effects of the indicated IMID-chemokine pools on CD8⁺ T cells, CD4⁺ T cells, and THP1 migration. Inhibitor concentration (M) is shown on the x-axis and percent migration normalised to maximum response (100%) on the y-axis. Technical replicates (n = 3) are shown as individual points. Curves (solid lines) and 95% confidence intervals (shaded) were fitted using a three-parameter log-logistic model with maximum constrained to 100%. EC20, EC50 and EC80 (M) ± standard error are indicated. Data points showing reduction from the peak response (red) were excluded from curve fitting.



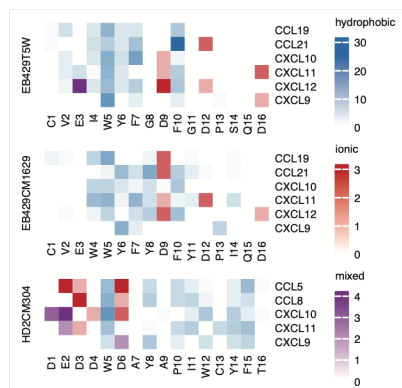
Supplementary Fig. 3 | Effect of scrambling peptide sequences

Effect of scrambling of peptide sequences on cell migration induced by the "INS" pool. Scrambled controls in each figure are indicated by the suffix "SCR". Y-axis shows migrated cell count. Migration is normalised to the median response to chemoattractant alone (set to 10,000). Box-and-whisker plots show centre line, median; box, 25th to 75th percentile; whiskers, 1.5x interquartile range. Points indicate biologically independent replicates. Individual experimental conditions are indicated as blue-filled circles. Control conditions are shown in blue. Statistics: two-sided Dunnett's test with multiple-comparisons correction; **** $p \leq 0.0001$, *** $p \leq 0.001$, ** $p \leq 0.01$, * $p \leq 0.05$. Exact p -values and replicate numbers are provided in Supplementary Data.



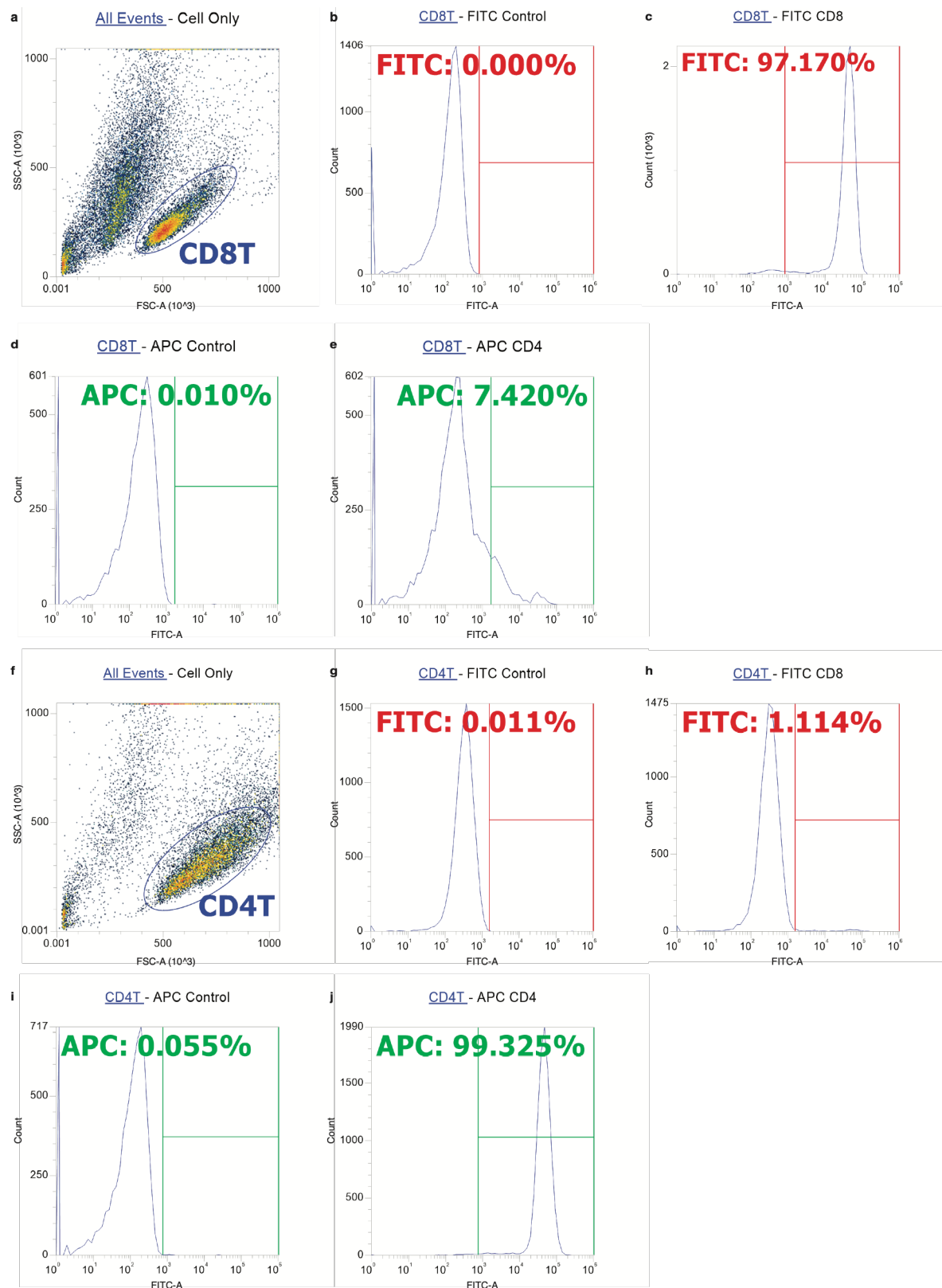
Supplementary Fig. 4 | Dose-dependent inhibition of cell migration by peptides.

Representative dose-response curves illustrating the effects of the indicated human chemokine pools on cell migration. The y-axis shows the percentage of migrated cells, normalized to maximal migration (defined as 100%). The x-axis indicates inhibitor concentration (M). Technical replicates at each inhibitor concentration ($n = 3$) are shown as individual data points. Dose-response curves (solid blue lines) and 95% confidence intervals (grey ribbons) were fitted using a three-parameter log-logistic model with the maximum response constrained to zero. Dotted green lines denote the IC₅₀, while dotted black lines indicate the IC₂₀ and IC₈₀ values. The Hill slope and IC₅₀ (M, estimated from the x-intercept) $\pm$ standard error (SE) are reported in each plot. E numbers denote individual experiments.



Supplementary Fig. 5 | Residue level interaction tile-plot

Heatmaps show Arpeggio-defined atomic contacts between each peptide residue (columns) and individual chemokines (rows). For each peptide–chemokine pair, tiles represent the number of unique atom–atom contacts involving that peptide residue, grouped by interaction type. Hydrophobic (blue) and ionic (red) interactions were identified from Arpeggio output and counted as distinct contact pairs based on unique atom identifiers. Interaction counts are aggregated at the peptide residue level. White tiles indicate no detected contacts. Mixed interactions (purple) denote residues that formed both hydrophobic and ionic contacts with the same chemokine. In these cases, the colour intensity reflects the summed number of hydrophobic and ionic contacts for that residue–chemokine pair. Colour scales are normalised to the global maximum interaction count observed across all peptides to allow direct comparison between heatmaps.



Supplementary Fig. 6 | Cell receptor labelling of CD8+ and CD4+ T cells.

a, f, Representative flow cytometry dot plots of CD8+ T cells and CD4+ T cells isolated as described in methods. Dot plots show forward scatter height (FSC-A; x-axis) versus side scatter height (SSC-A; y-axis). CD8+ T and CD4+ T cells are identified by the gates labelled "CD8T" and "CD4T" respectively. **b-e, g-j,** Histograms showing cell count (y-axis) versus fluorescence intensity (x-axis). The cell type and antibody used for labelling is indicated above the histogram plot. % labelled cells are indicated in each plot. Gating thresholds were determined using FITC control (red), or APC control (green) conjugated antibodies. **b-e,** Histograms of isolated CD8+T cells. **g-j,** Histograms of isolated CD4+T cells.