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SUPPLEMENTARY INFORMATION

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A clinically meaningful metric of immune age derived from high-dimensional longitudinal monitoring

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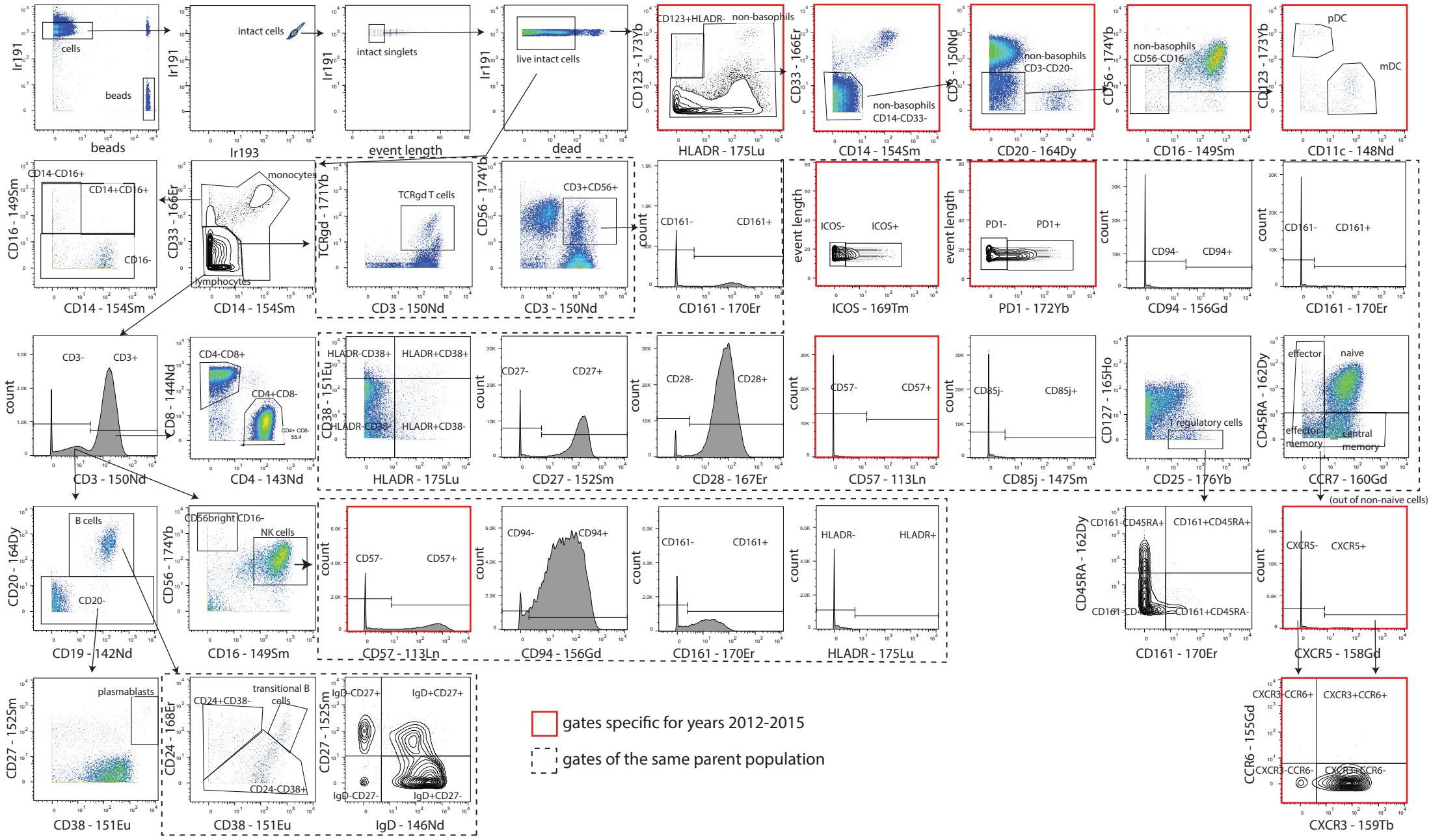
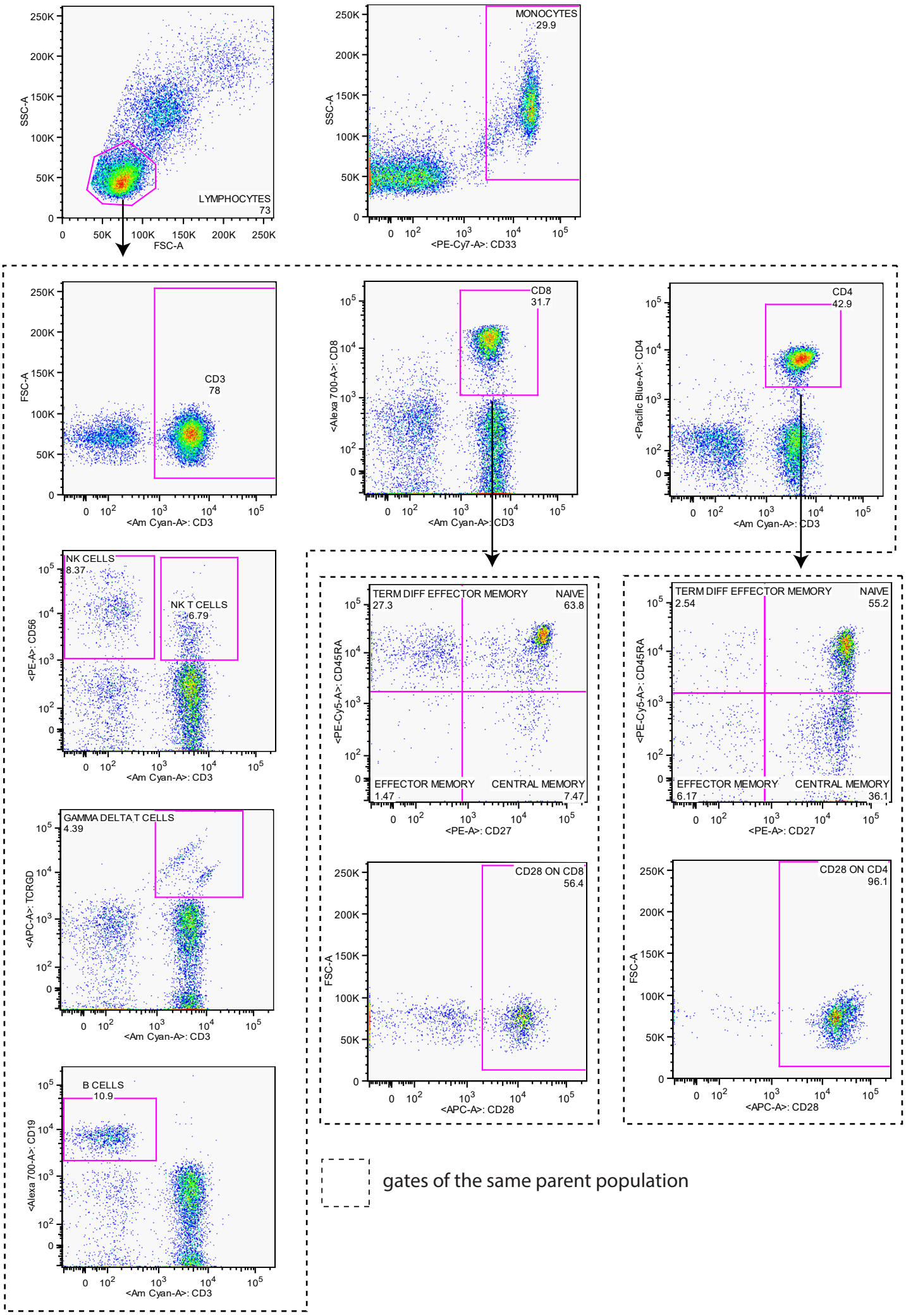
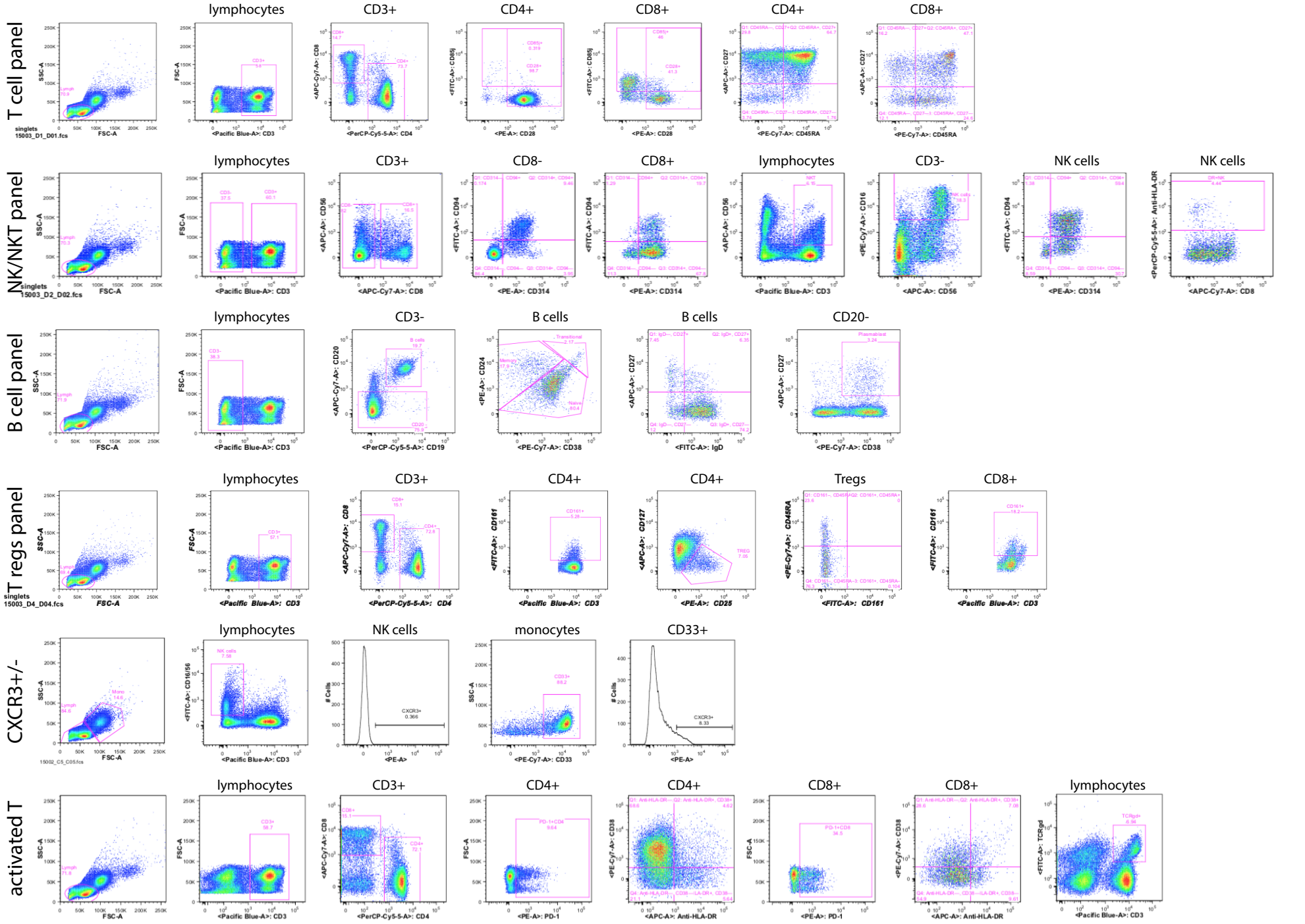


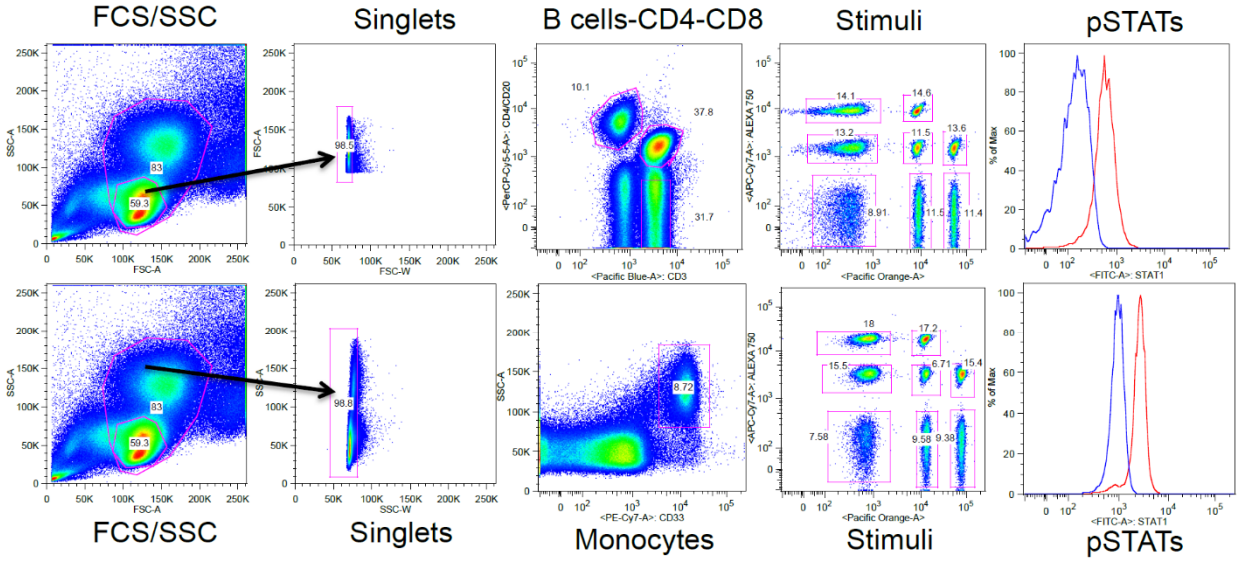
Supplementary Figure 1: CyTOF gating strategy. Gating from parental populations is assigned with arrows. Red frames denote gates that are specific to the years 2012-15, whereas dashed frames denote gates from the same parental population.



Supplementary Figure 2: Flow cytometry 2007-2008 gating strategy. Gating from parental populations is assigned with arrows. Dashed frames denote gates from the same parental population.



Supplementary Figure 3: Flow cytometry 2009 gating strategy. Different panels are ordered by row. Parent populations are shown on top of the corresponding gate.



Barcode key

	Unstim	IFN-a	IFN-g	IL-6	IL-7	IL-10	IL-21	IL-2
Pacific Orange	-	Low	High	-	Low	-	Low	High
Alexa Fluor 750	-	-	-	Low	Low	High	High	Low

Supplementary Figure 4: phospho-flow gating strategy. Stimuli were debarcoded to derive cellular abundance of phosphorylated antigens in each cell population.

Supplementary Note 1 – Relation of Immune Features at Baseline with Future Clinical Events

Relation of immune-features with a certain disease is an open question that has been previously addressed by hypothesis-driven studies where the disease was intentionally well-represented in the study cohort. As a longitudinal study, our data may be utilized to relate the levels of specific immune-features at baseline with future clinical outcome, allowing for identification of causal relationships between a disease and age-related immune-features. To identify such correlations, we examined the clinical records of the older-adults for events that occurred during the study and stratified their clinical diagnoses into high-resolution classes (**Supplementary Table 13)**. This classification yielded more than 300 unique diagnoses, where each was diagnosed on average in 2.9% of the older adult population. We reasoned that because of this sparseness, demonstration of a causal effect between immune-features and these diagnoses will be underpowered. Thus, we classified the diagnoses into larger and less-specific groups to yield 24 clinical categories, each was diagnosed on average in 21.9% of the older adult population (**Supplementary Table 13**).
Next, to identify causal relationships between immune-cell levels whose abundance change with age and these clinical categories, we applied a survival analysis in which for each combination of a cell subset and a clinical category, the future occurrence of events in the category was regressed against age, gender and the cell-subset frequency, as measured at some baseline year (specifically: 2007 or 2008, in order to maximize the number of future events). We identified some significant relationships between baseline cellular frequencies and future occurrence of a clinical category ($P < 0.05$, by multivariate Cox regression model incorporating baseline frequency in addition to age and gender). However, the large number of hypotheses, which equals to the number of age-related immune-features times the number of clinical categories, resulted with no significant correlation following multiple hypotheses correction (applied using Benjamini-Hochberg methodology). We concluded that the heterogeneity of clinical phenotypes existing in our cohort stemming from the absence of a specific driving clinical hypothesis prevents identification of disease-causing immune-features.
Similar to individual immune-features, applying a similar clinical association analysis on baseline IMM-AGE score as measured in 2010 with future occurrence of evets related to clinical categories resulted with no significant association due to multiple testing.