Supplementary Figures
for
Single-cell heterogeneity in Sézary syndrome

Terkild B. Buus, Andreas Willerslev-Olsen, Simon Fredholm, Edda Blümel, Claudia Nastasi, Maria Gluud, Tengpeng Hu, Lise M. Lindahl, Lars Iversen, Hanne Fogh, Robert Gniadecki, Ivan V. Litvinov, Jenny L. Persson, Charlotte M. Bonefeld, Carsten Geisler, Jan P. Christensen, Thorbjørn Krejsgaard, Thomas Litman, Anders Woetmann and Niels Ødum

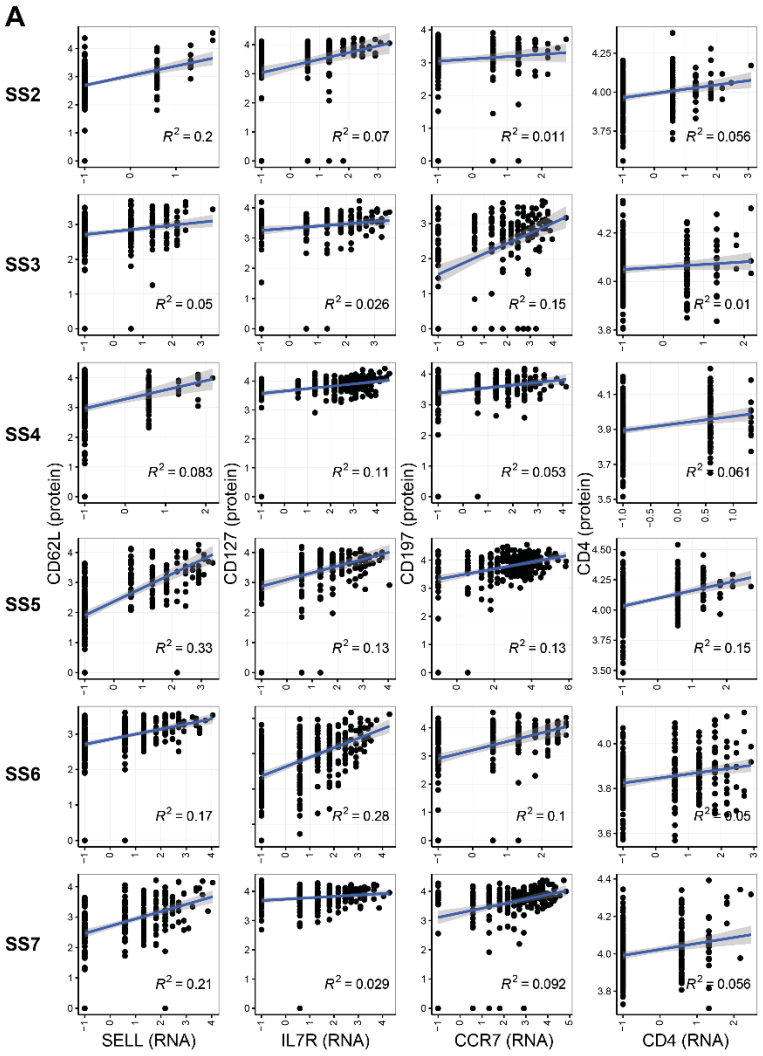
Figure S1: Validation of single-cell RNA sequencing

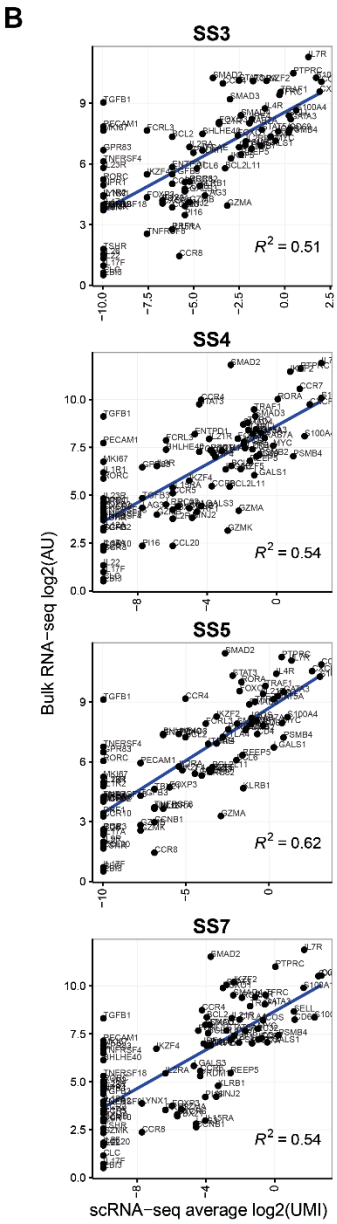
A) Correlation between index sorted surface marker intensity (y-axis; log10 of fluorescence intensity) and single-cell mRNA expression of the corresponding gene (x-axis; log2 of UMI count) from six Sézary Syndrome (SS) patients (SS2-SS7).

B) Correlation of the 110 genes included in the targeted single-cell RNA-sequencing experiment between the average single-cell mRNA expression and bulk mRNA expression from sorted malignant T cells form four SS patients.

C) Single-cell co-expression of index sorted surface markers located in the t-distributed stochastic neighbor embedding (t-SNE) maps included in Figure 3B and colored by their fluorescence intensity.

Figure S1


A
SS2
SS3
SS4
SS5
SS6
SS7
CD62L (protein)
CD127 (protein)
CD197 (protein)
CD4 (protein)
SELL (RNA)
IL7R (RNA)
CCR7 (RNA)
CD4 (RNA)


B
SS3
SS4
SS5
SS7
Bulk RNA−seq log2(AU)
scRNA−seq average log2(UMI)

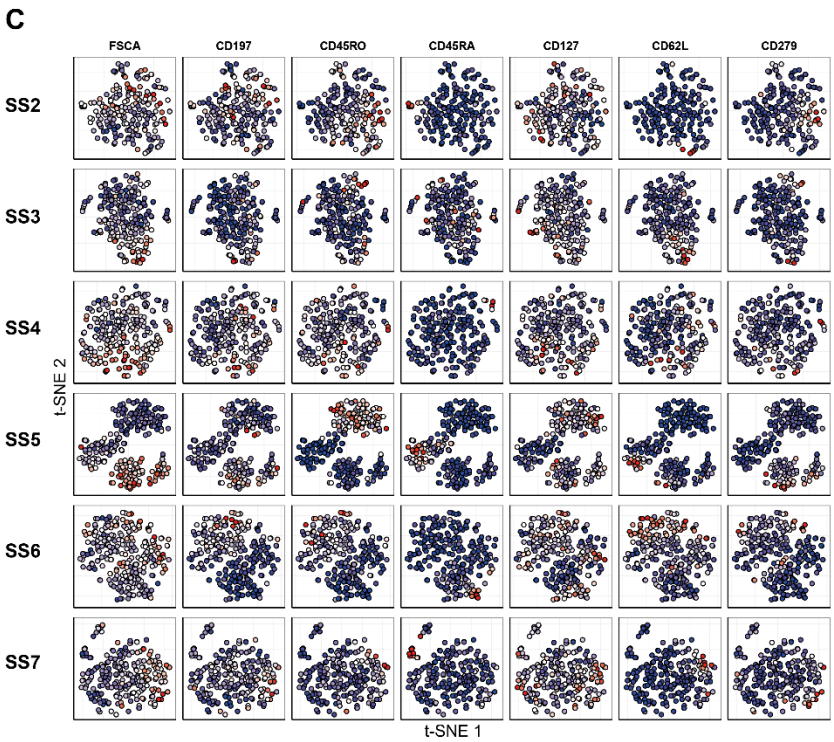

C
FSCA
CD197
CD45RO
CD45RA
CD127
CD62L
CD279
SS2
SS3
SS4
SS5
SS6
SS7
t-SNE 2
t-SNE 1

Figure S2-S6: HDAC inhibitor treatment affect some malignant subpopulations, but not all.
Similar to the results shown in Figure 4 from five Sézary Syndrome patient are presented in Figures S2-S6 (SS4: Figure S2, SS5: Figure S3, SS6: Figure S4, SS7: Figure S5, SS9: Figure S6)

A) Co-expression of surface marker expression within malignant cells from SS patients visualized by t-distributed stochastic neighbor embedding (t-SNE) plots colored by fluorescence intensity of the indicated markers or by automated clustering using the PhenoGraph algorithm showing all (left) or reduced number of clusters (right).

B-G) Changes in the malignant subpopulations following treatment with increasing concentrations of two HDAC inhibitors: (B-D) Romidepsin or (E-G) SAHA colored by reduced PhenoGraoh clusters. (B & E) Visualized by changes in t-SNE plots of clustered cells. (C, D, F & G) Visualized by stacked barplots of (C & F) cluster frequency or (D & G) total cell counts.

H) Single-cell heatmaps of malignant cells treated with increasing concentrations of Romidepsin. Rows are distributed by reduced PhenoGraph clusters (left bar) and colored by fluorescence intensity of the indicated markers. Violin plots (top) display the overall expression range of the indicated markers within the total malignant population.

Figure S2

SS4

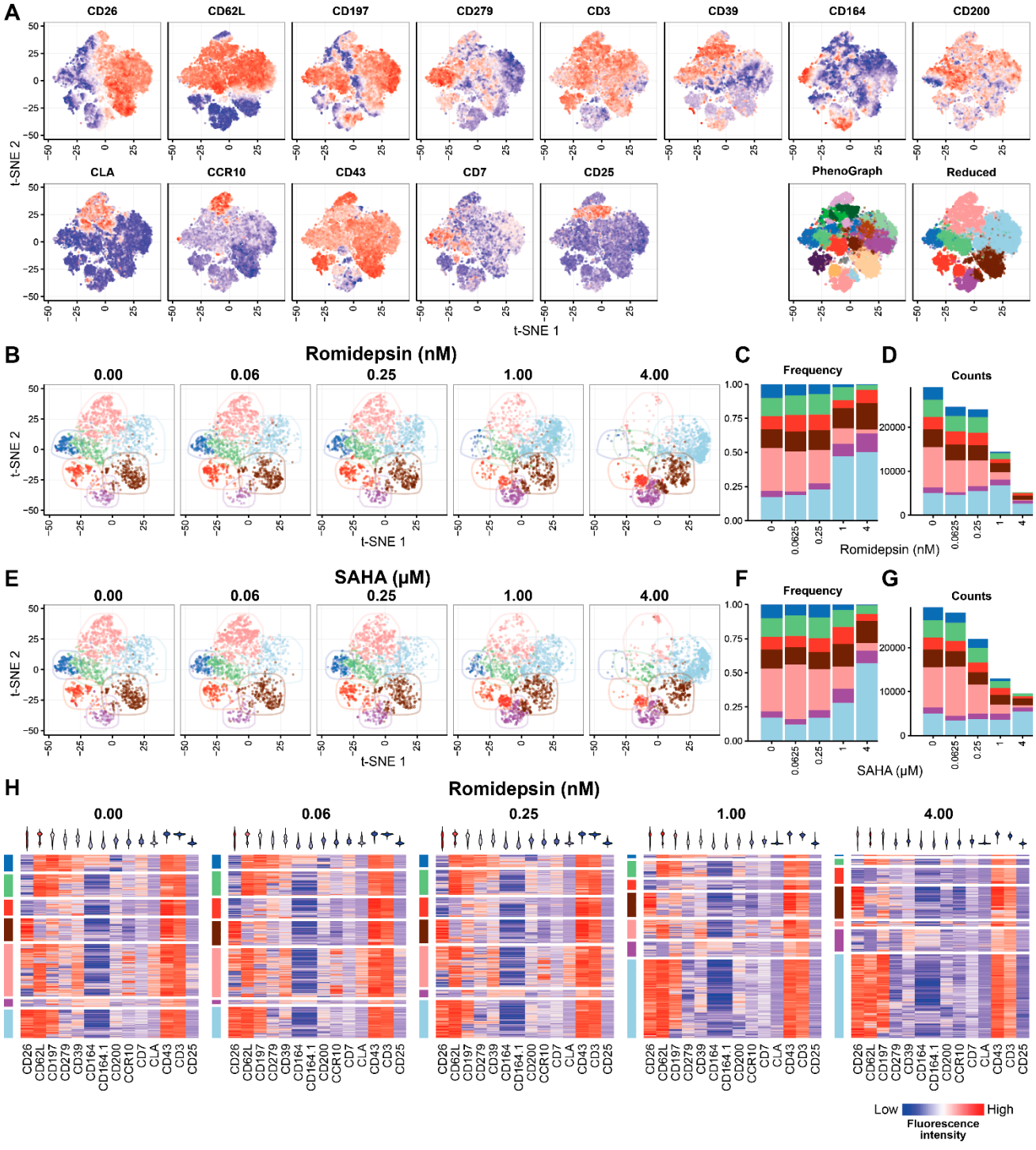


Figure S3

SS5

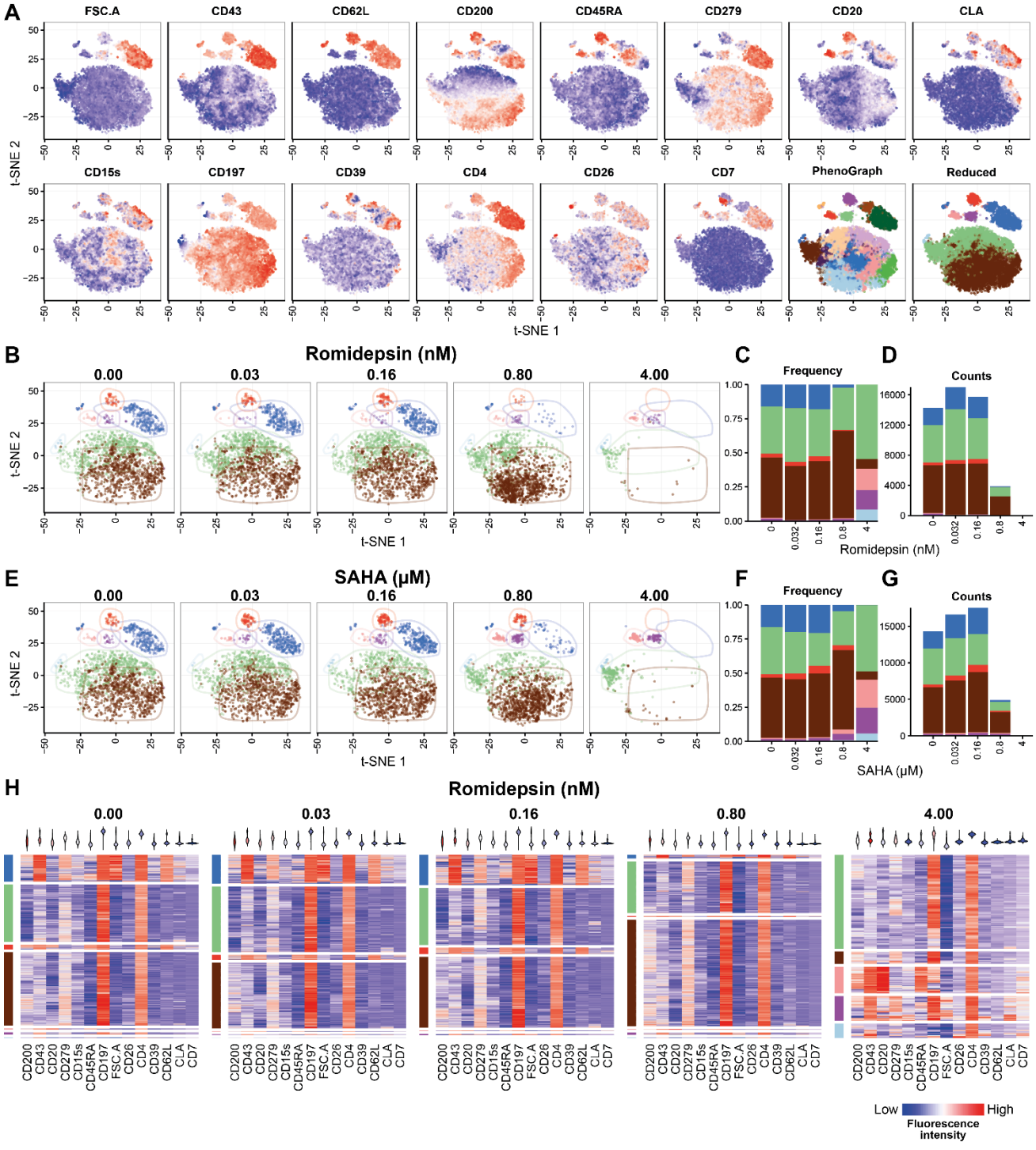


Figure S4
SS6

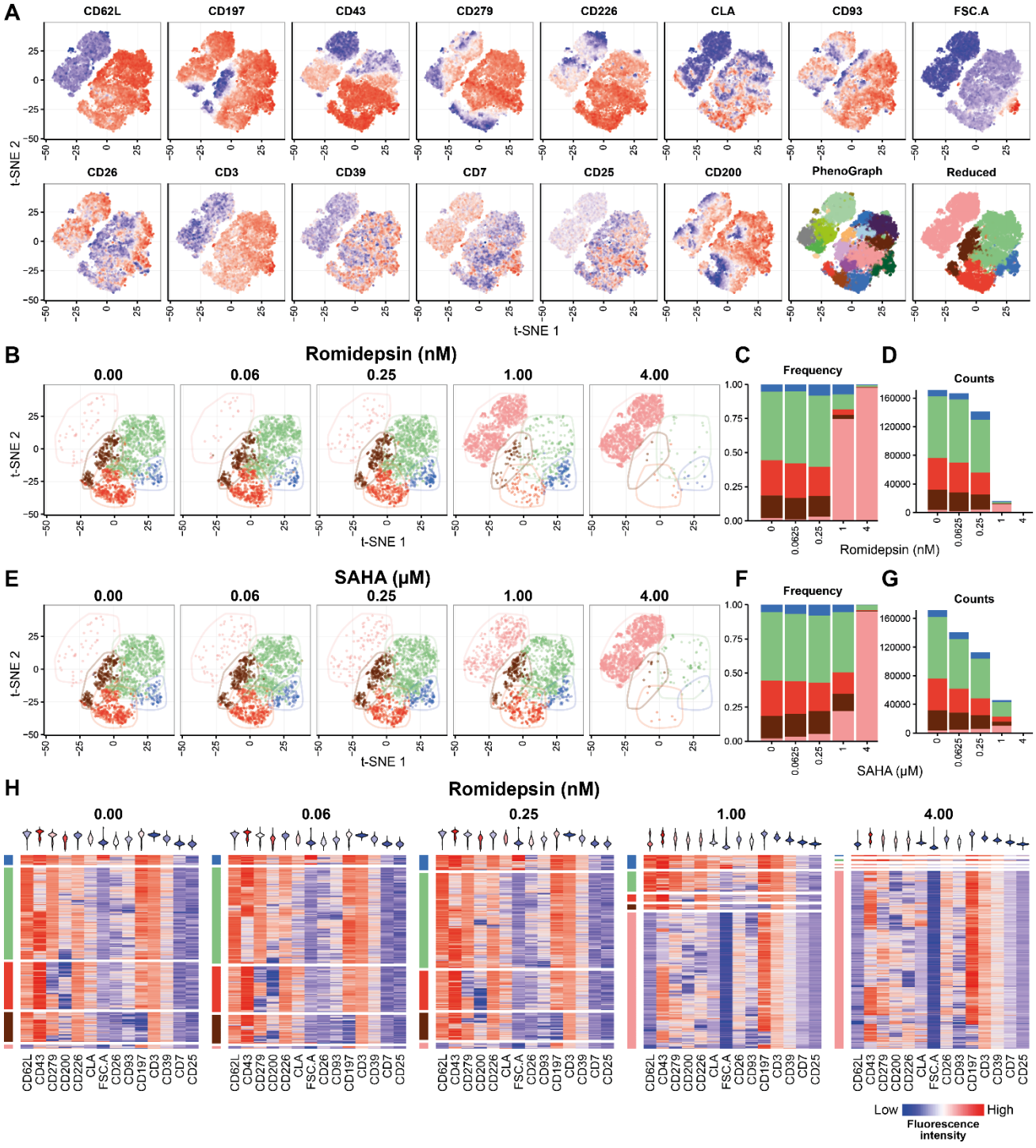


Figure S5

SS7

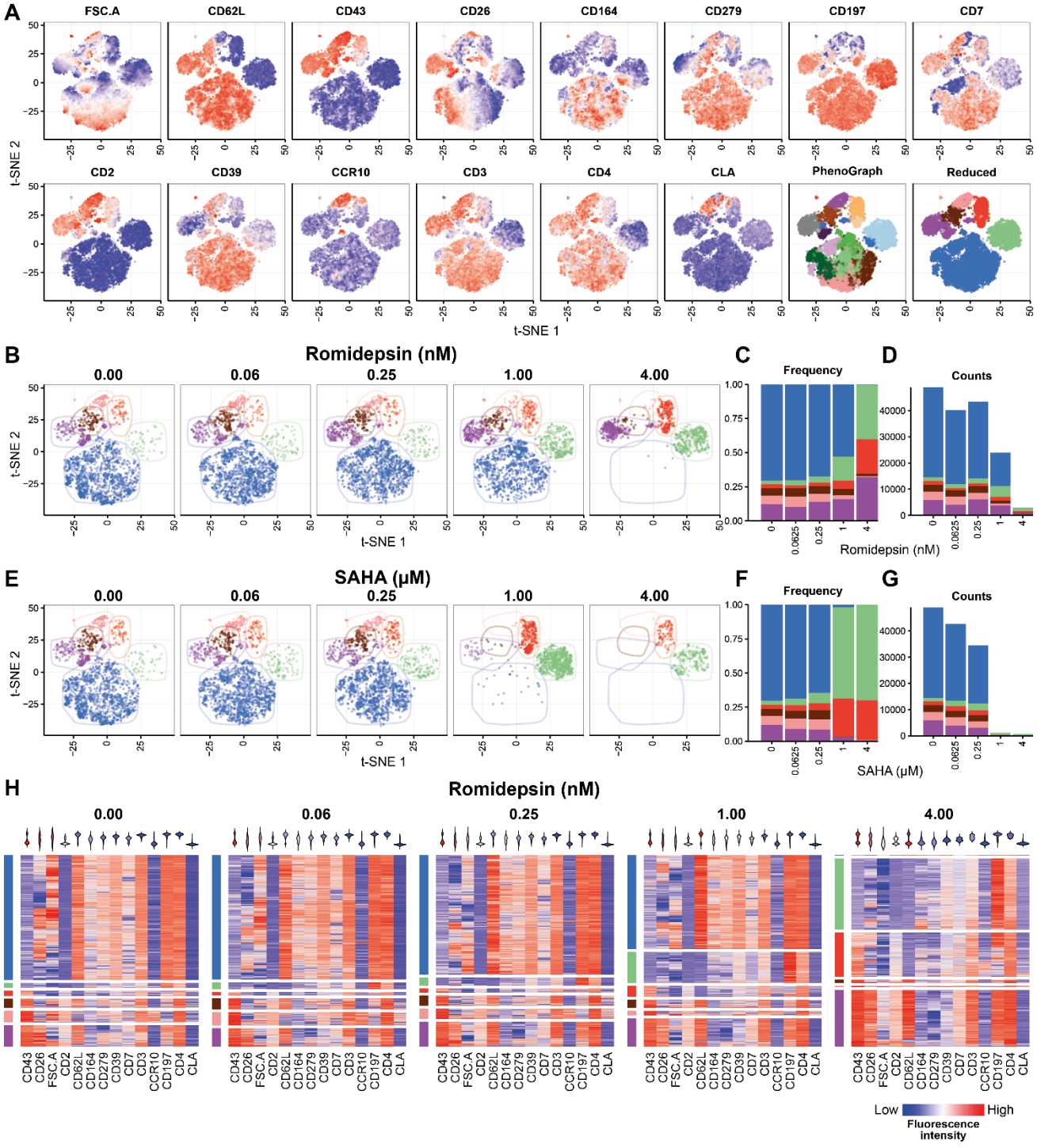


Figure S6

SS9