IMM450 Written Report — Salma Ragheb — Supervisor: Dr. Norman Iscove

Phenotypically Distinguishing Leukemia-Initiating Cells from Inert Leukemia Blasts in a HOXB4-Transgene Dependent Leukemia Model

0. Abstract

At the top of the hematopoietic hierarchy are the hematopoietic stem cells (HSCs), whose distinguishing feature is their ability to sustain self-renewal indefinitely. As stem cell progeny differentiate towards mature blood cells, they lose the ability to self-renew and the expression of homeobox genes. The Iscove lab has been studying if loss of self-renewal is related to downregulation of homeobox genes. To do so, expression of a homeobox gene implicated in self-renewal, HOXB4, is enforced in HSCs via retroviral infection. The cells these HOXB4-infected HSCs differentiate into will continue to express HOXB4, enforcing HOXB4 in lineage-committed progenitors (LCPs) — where expression usually stops. Immortalized by the HOXB4 transgene, these progenitors accumulate gene mutations. In the Iscove lab, a myeloid leukemia arose 18 months after HOXB4-transduction in mice. The cells reconstituting this leukemia: the leukemia-initiating cells (LICs) exist as 1/63 cells of the leukemia cell population in BM. My aim is to find the LIC markers that might distinguish the LIC from the rest of the inert blasts in the leukemia cell population. My key accomplishment was finding that the majority of the LICs based on the results of injecting sorted HOXB4 leukemic BM fractions in vivo fall into the cKit+Sca1-CD150-Flt3-CD34+ FcyR$_{med}$CD49b-CD93-CD56+ fractions.

1. Background

1a. Hematopoietic Hierarchy

The hematopoietic system has a hierarchy with cell stages that vary in self-renewing capacity and degree of differentiation. At the beginning of the hierarchy are the most primitive cells: the long-term hematopoietic stem cells (LT-HSCs) *(Fig 1)*. These HSCs have two key characteristics that are relevant to this paper: sustained self-renewing capacity and endogenous expression of the homeobox genes. Self-renewal refers to the capacity of HSCs to replicate into

copies of themselves that retain identical self-renewing capacity (Lim et al. 2017). Differentiation of HSCs into specific lineages is accompanied by a diminished self-renewing capacity and loss of homeobox expression (Lim et al. 2017). Multipotent HSCs eventually differentiate into oligopotent lineage-committed progenitors (LCPs), which later differentiate into unipotent terminally differentiated cells that belong in one lineage and are functionally particular *(Fig 1).* From HSCs to LCPs, the expression of the homeobox genes is increasingly downregulated. The LCP stage in which the homeobox genes are silenced is also the stage during which self-renewing capacity is lost. The Iscove lab studies if there is a correlation between the downregulation of homeobox genes and diminished self-renewal in LCPs.

1b. HOXB4-Dependent Leukemia Model

HOXB4 – a transcription factor in the homeobox family – has been implicated in enhancing self-renewal (Antonchuk et al. 2001, 2002). In the Iscove Lab, expression of HOXB4 had been enforced in LCPs where HOXB4 isn't naturally expressed. This was done by means of infecting normal donor HSCs with a HOXB4-VENUS retrovirus. VENUS is a fluorescent protein that serves as a visual proxy for the expression of the HOXB4 transgene.

Long before I joined the lab, recipient mice had been irradiated as to deplete their own bone marrow (BM), and the donor HOXB4-infected HSCs had been injected to generate HOXB4-reconstituted marrow in the recipients *(Fig 2)*. The differentiated progeny of these HOXB4-infected HSCs will also intergenerationally express the HOXB4 transgene. Since LCPs don't express endogenous HOXB4, the LCPs of HOXB4-infected HSCs acquired a self-renewing capacity characteristic of HSCs (Lim et al. 2017). LCPs replicated indefinitely with sustained self-renewal, creating immortalized clones of themselves, which accumulated gene mutations that eventually become leukemogenic (Argiropoulos and Humphries 2007). Leukemic

transformation has been observed in the Iscove lab model after in vivo passage of HOXB4-immortalized marrow cells into recipient mice for periods beyond 18 months *(Fig 2)*. The mAML1 line of murine acute myeloid leukemia was derived in this way. One key property of this leukemia is its continuing dependence on the HOXB4 transgene. After excision of HOXB4 using a cre-loxP strategy, the leukemia regresses and disappears in host mice. This HOXB4-dependent leukemia had been thawed or maintained in transplantation from one mouse to another before I joined the lab for this project.

Typically, only a minority of cells can regenerate a leukemia population after transplant. The mAML1 line was analyzed by limiting dilution analysis in which varying numbers of cells were transplanted into irradiated recipient mice. The analysis estimated that only 1 in every 63 mAML cells engrafted and regenerated the leukemia, suggesting that this line might share the hierarchical structure typical of human AML samples. The analysis also showed that a single mAML1 leukemia initiating cell regenerates a lethal leukemia burden within 30-35 days in a mouse. The leukemia blasts, which also make up the leukemia population, do not contribute to the fate of the leukemia and are inert in this respect.

1c. Rationale for Marker Selection

My project is to characterize the surface marker phenotype of the rare LICs in the HOXB4-transgene dependent mAML1 model in a way that might allow these cells to be distinguished from most of the inert leukemia blasts. If the 1 in 63 cells that can regenerate the leukemia are distinct from the majority of the leukemia cells, they might be expected to differ in the gene expression of cell-surface markers that would be selected for by staining with antibodies that target the markers and subsequent cell-sorting. The markers sorted into binary or ternary fractions which were later injected in vivo are: cKit, Sca1, CD150, Flt3, CD34, FcyR, CD49b,

CD93, CD56. Since AML has been reported to preserve remnants of normal precursor hierarchy, and this HOXB4-dependent leukemia is an AML model, the first 7 markers are of interest because their expression is well-studied in the hierarchy *(Fig 3)*. Studying the windows of expression of these milestone stem-cell and differentiation hematopoietic markers is aligned with the objective of this project because it contextualizes the phenotype of the LIC with what has been covered in the literature about the markers that make up this distinguishing phenotype *(Fig 3; Table 1)*. Outside of the proteins whose surface expression is well studied in the hierarchy, I also turned to markers associated with AML. CD93 is a transmembrane receptor that was found to be highly expressed in leukemia stem cells, AML progenitor cells, and even more differentiated AML cells in humans (Jia et al. 2022). Compared to normal HSCs, CD56 expression was 53-fold higher in LSC population in humans diagnosed with AML (Alegretti et al. 2011). The additional markers only titrated, without being later sorted and injected, are CD115, CD38, CD366, CD131, CD96, B220, CD116, CD123, and CD33.

2. Methodology

2a. Harvesting Leukemic HOXB4-reconstituted Marrow for Sort or Titration

The HOXB4-dependent leukemia described in part *(1b)* had been thawed or maintained in transplantation from one mouse to another before I joined the lab. For my project, I harvested leukemic cells from one of the leukemic HOXB4-reconstituted donor mice by flushing bone marrow (BM) from femurs and tibias using a syringe and sort media, then lysed RBCs with ACK buffer so that only the WBCs remained. This leukemic whole BM is then stained with Fc block for 15 minutes to prevent non-specific binding. This stage is skipped when the marker to be titrated or sorted for is FcyR.

2b. Titrating Antibodies

To prepare for a titration, HOXB4 leukemic BM is harvested as described in part *(2a).* The cells are then stained in the marker of interest in 5 different concentrations — 1:30, 1:100, 1:300, 1:900, 1:1500. The tubes are then run on the flow cytometer, and the dilution that yields the best separation between the +ve and the -ve fractions of the marker is selected. The StainIndex plugin is used on FlowJo to best determine the optimal dilution to be used if the marker is deemed interesting to sort for and subsequently inject into mice. All titration gates on optimal dilutions of markers not sorted for are shown in *(Fig 14)*.

2c. Sorting and Gating Strategy

If the expression of the marker is found to be interesting in a titration experiment, a sort is scheduled. Whether or not the marker is interesting depends on whether there is bimodality or heterogeneity of marker expression. For example, a marker might be interesting to sort for if there is even a vaguely distinct smaller subpopulation because it might contain the rare LICs. To prepare for a sort, HOXB4 leukemic BM is harvested as described in *(2a).* LBM cells are stained with the marker of interest for about 30 minutes and then sorted into binary or ternary fractions by cell sorters *(Fig 4).* Then, the two binary or three ternary fractions were injected into two or three mice irradiated by 7 Gys, along with a radioprotective dose (per mouse) of 2 $x10^6$ normal BM. For example, CD34+ cells were injected into a cohort of mice and CD34- cells were injected into another cohort. For FcyR, instead of a binary sort, a ternary sort was performed because the Iscove lab found that FcyR expression falls over three populations ($FcyR_{hi}$, $FcyR_{med}$, $FcyR_{lo}$) rather than two (Mazzanti 2019). All gating strategy used during each marker sort is shown in *(Fig 5-12)*.

2d. Blood Analysis after Injection of Sorted Fractions

The mice were bled first after 5-14 days since injections of sorted fractions and every 3-6 days since then to collect data points of how the VENUS+ percentage increases over time. Peripheral blood was collected from saphenous veins of mice; the RBCs were lysed with ACK buffer for 5-10 minutes to keep WBCs only. An example of the gating strategy used in blood analysis for a mouse which had been injected with CD34+ fraction is shown in *(Fig 15)*. The group that becomes leukemic will show a VENUS+ leukemic population like in the right panel of *(Fig 15)*. This VENUS+ population indicates that the HOXB4-transduced leukemia donor cells had expanded to detectable numbers in the recipient since injection. This detectability of a VENUS+ population reflects the transfer of leukemia-initiating cells. Since the recipient mouse itself doesn't inherently circulate VENUS+ cells, any increasing VENUS+% in the recipient indicates leukemic reconstitution from the injected HOXB4-transduced sorted cells. Only LICs can divide into clones like this and sustain them. Therefore, increasing VENUS+% indicate the presence of LICs. This process of harvesting BM, sorting, injecting, and analyzing blood had been repeated for each of the sorted markers.

3. Results

The mice developed leukemia at different speeds. That is, the rate at which this leukemia developed differed among the separate fractions. Using these different rates, an estimate of how many LICs were sorted into each fraction was calculated to determine which fractions contain the majority of the LICs. Injecting only one LIC into a recipient mouse makes it 50% VENUS+ by day 30-35 since injection; this is an Iscove lab finding that helps serve as a baseline for this calculation. For simplicity, the method of calculation is shown in *(Fig 13)* using only four mice which had been injected with four different fractions. After calculating the raw LIC numbers per fraction, they were converted into percentages of the total LICs in one marker. For example, out

of the total LICs in the cKit marker fractions, 88.4% were in the cKit+ fraction and 11.6% were in the cKit- fraction. Continuing with the rest of the binary markers, most of the LICs were sorted into the Sca1-, CD150-, Flt3-, CD34+, $FcyR_{med}$, CD49b-, CD93-, and CD56+ fractions *(Fig 16)*. For FcyR, with ternary expression, the majority of the LICs were sorted into the $FcyR_{med}$ fraction *(Fig 16)*.

The fractions that enriched for the majority of the LICs did not identify a minority phenotype that distinguishes LIC from inert blasts. While the majority of the LICs ended up in one of the two or three fractions, some LICs still made it into the other fraction(s), which means LICs are not exclusive to the fraction with the majority of the LICs. Only CD150 contained no LICs in the minor, positive fraction; mice injected with the CD150+ fraction did not develop leukemia even past 55 days. As per *(Fig 16)*, since the compound phenotype with the majority of LICs is cKit+Sca1-CD150-Flt3-CD34+ $FcyR_{med}$CD49b-CD93-CD56+, the marker expression of the LICs always tracks the fraction that contains the majority leukemia cell population; since the LICs are rare, it is expected that they should be enriched for by the fraction with the minority leukemia cell population. From *(Fig 5-12)*, the minority populations are cKit, Sca1+, CD150+, Flt3+, Fcy_{hi}, CD34-, CD49b+, CD93+, CD56- respectively; no marker enriched for the LICs in the minority population. For the markers only titrated, based on the plots of the optimal dilutions of each titrated marker in *(Fig 14)*, only CD115 and CD33 are worth sorting for because there is a minority population that might contain the LICs.

4. Discussion

4a. Interpretation of Results

The purpose of this project is to distinguish the LICs from the inert leukemia blasts. Because the LICs are the minority in the leukemia population, markers that show two

populations one of which a minority population — separate based on significantly different level of expression than the majority — are good candidates for a sort. In FlowJo, this corresponds to a marker where the VENUS+ leukemia cell population shows heterogeneity. Ideally, heterogeneity in marker expression would manifest two distinct populations when the expression of the marker is plotted, one of which often shows a minor population. An example of a parameter that shows heterogeneity is the VENUS parameter in *(Fig 15)* because it shows two distinct populations. If the marker of interest is one that significantly distinguishes LICs from blasts, the marker must ideally fulfill two criteria: first, it would yield two distinct populations and second, the LICs would end up in the minor population. Given that the LICs have the majority phenotype for all sorted markers despite their rarity (1/63) in the leukemia cell population, the markers sorted for did not significantly distinguish the LICs. As such, no model of a distinguishing phenotype for the LICs in this HOXB4-dependent mAML1 could be built based on these experiments.

4b. Impact of Project in Area of Interest & Future Directions

Isolating the LICs further from the inert blasts would allow us to study what differentiates the cells that reconstitute the leukemia from those that are inert even though they both make up the leukemia population. How might the phenotypic profiles of each of them contribute to their different roles in maintaining leukemic clones? Since the markers sorted for didn't help in distinguishing the LICs from the blasts, it is worth reconsidering the baseline of 1 LIC in 63 inert blasts and performing another limiting dilution experiment to get a better proportion of LICs.

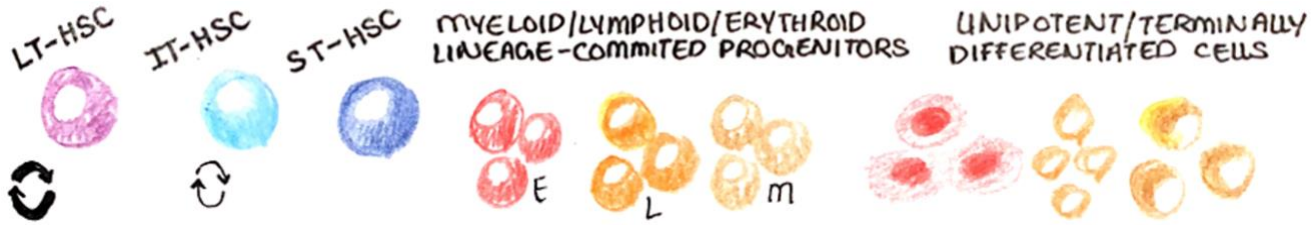


Figure 1. ***Cell stages in the hematopoietic hierarchy.*** *Cells on the left are most primitive and cells on the right are more mature. Self-renewal diminishes and HOXB4-expression is downregulated as cells advance from left to right.*

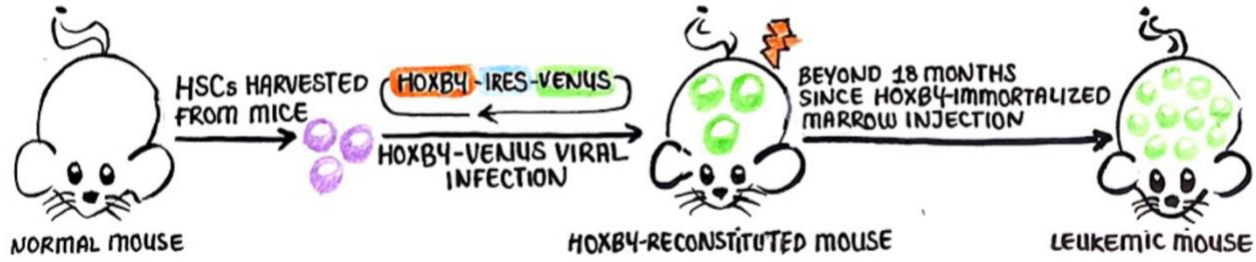


Figure 2. ***Making a HOXB4-leukemic mouse.*** *Normal HSCs were harvested from a donor mouse, infected with a HOXB4-VENUS retrovirus, and injected into an irradiated recipient mouse. The HOXB4-transgene also exerts its expression in lineage-differentiated progeny of HSCs, which are immortalized by means of self-renewal conferred to them by the transduction. Beyond 18 months, this HOXB4-reconstituted BM accumulates enough mutations to become leukemic BM. This is a HOXB4-transgene dependent leukemia model (mAML1).*

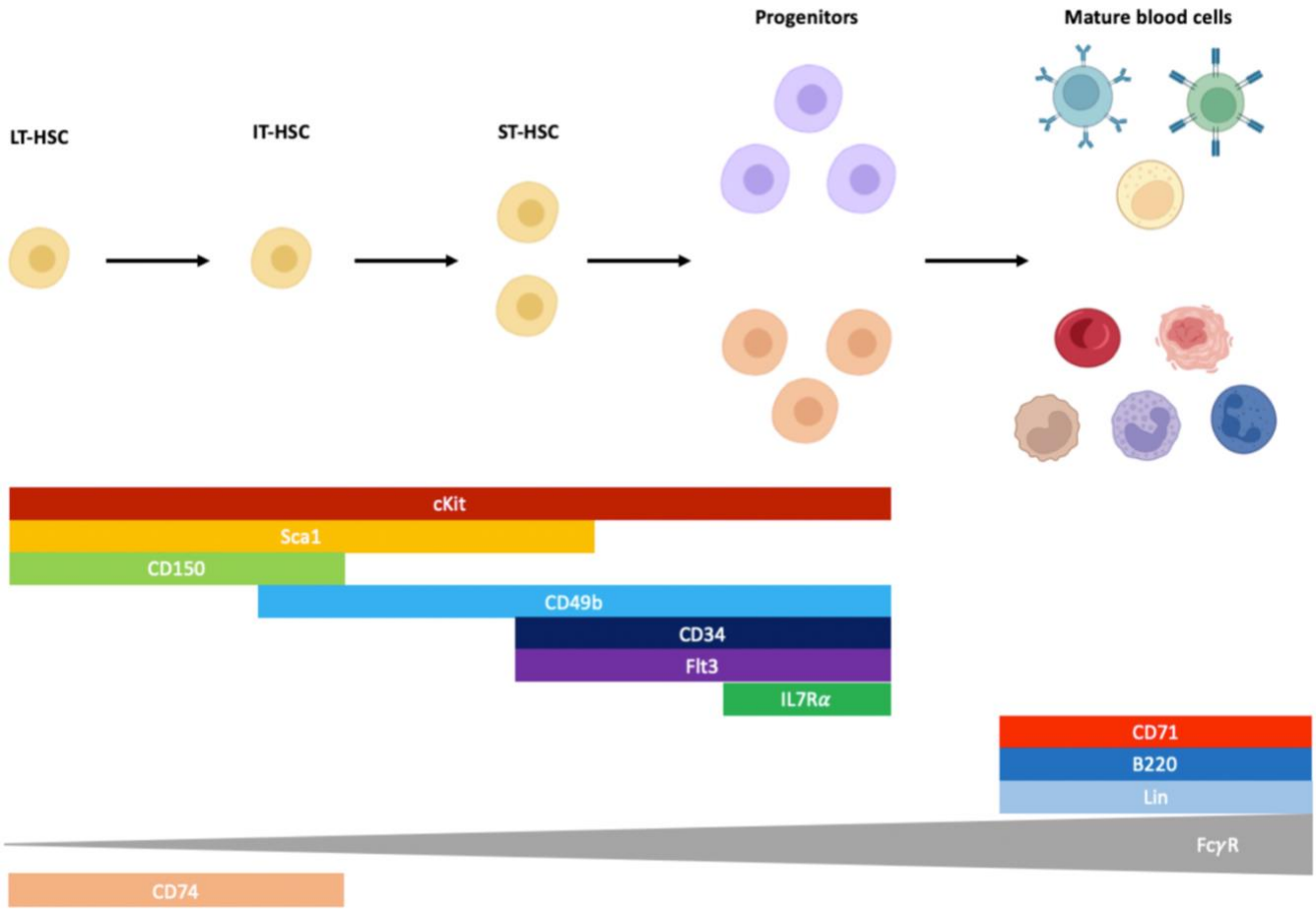


Figure 3. ***Expression of markers in primitive to mature stages of the hematopoietic hierarch****y (Won 2022). LT-HSC: long-term hematopoietic stem cell (which reconstitute differentiated clones for 4 -11 months posttransplant); IT-HSCs (whose clones persist for 6–8 months and then die thereafter); and ST-HSCs (which reconstitute differentiated hematopoietic clones for 4-6 weeks) (Benveniste et al. 2010). Progenitors refer to lineage-committed progenitors (LCPs).*

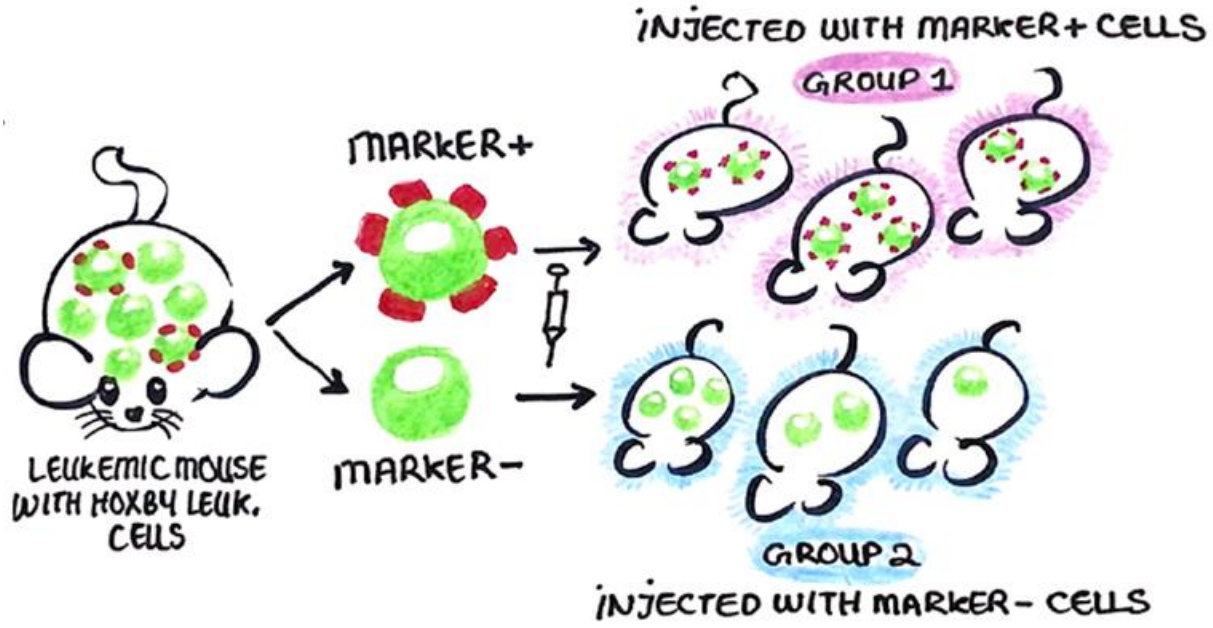


Figure 4. Leukemic HOXB4-reconstituted BM was harvested and sorted into marker fractions which were then transplanted into separate cohorts.

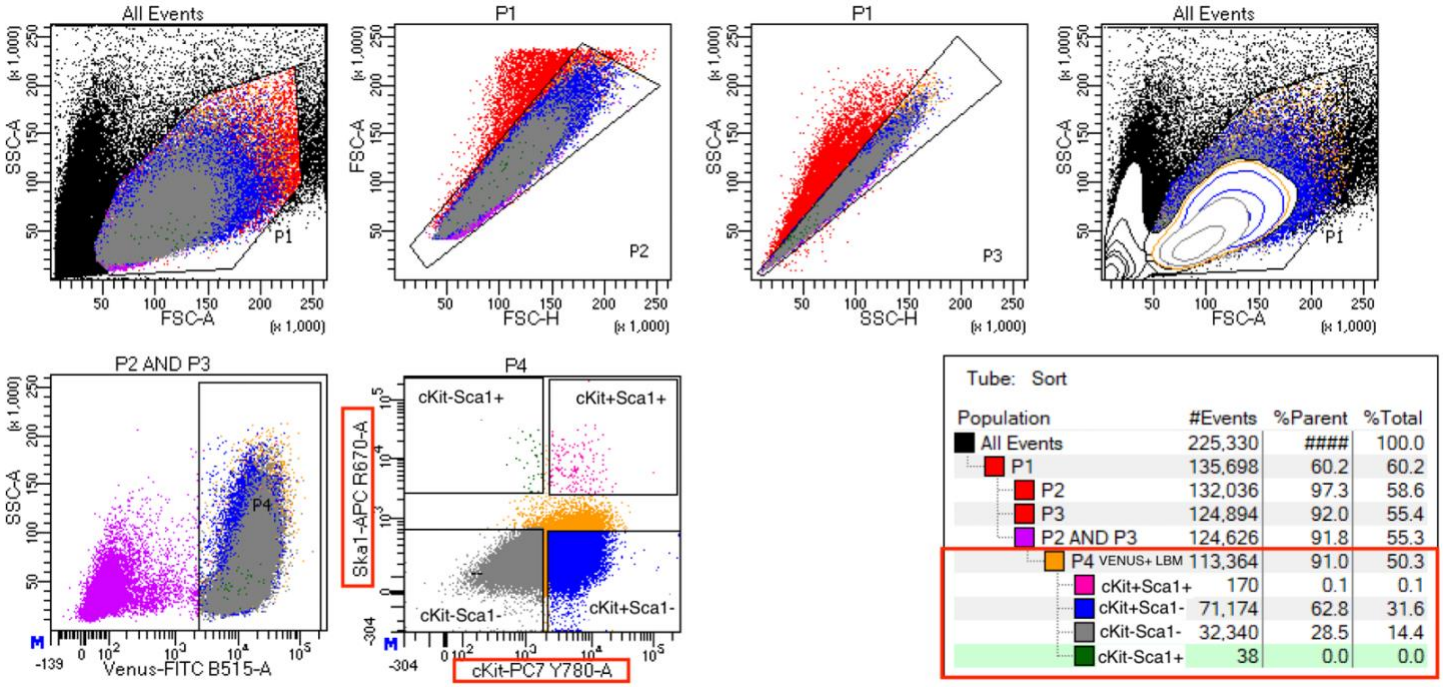


Figure 5. ***Gating Strategy for cKit and Sca1 Surface Markers.*** *P1 is to only gate the lymphocytes. P2 and P3 remove doublets and debris. P4 is to select only the VENUS+ cells (the leukemia cell population). P4 is then taken onto a cKit vs Sca1 plot and VENUS+ cells are divided into quadrant gates of varying cKitSca1 expression. In the population hierarchy on the bottom right, the important column is (% Parent) because it shows the % of each quadrant fraction out of the VENUS+ cells.*

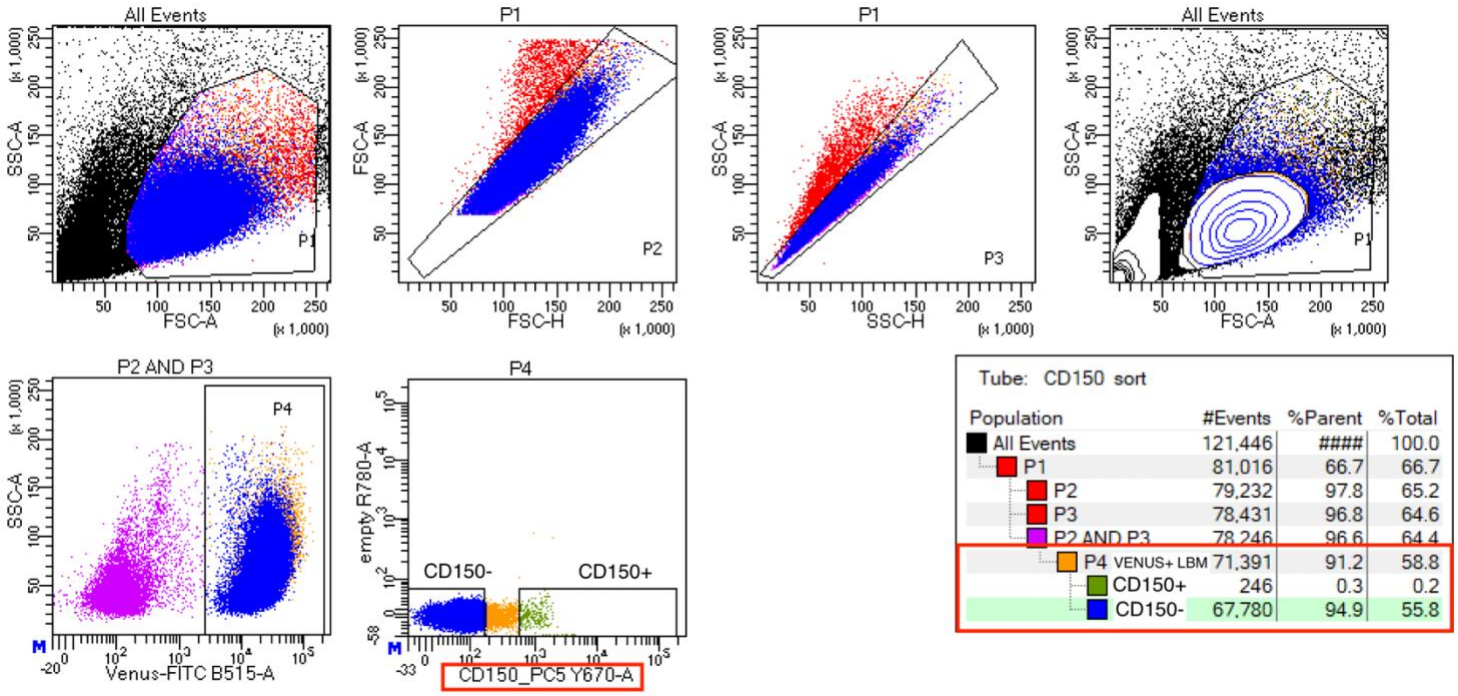


Figure 6. ***Gating Strategy for CD150 Surface Marker****. P1 is to only gate the lymphocytes. P2 and P3 remove doublets and debris. P4 is to select only the VENUS+ cells (the leukemia cell population). P4 is then taken onto another plot of CD150 on x-axis and VENUS+ cells are divided into binary fractions of CD150- and CD150+. In the population hierarchy on the bottom right, the important column is (% Parent) because it shows the % of each fraction out of the VENUS+ cells.*

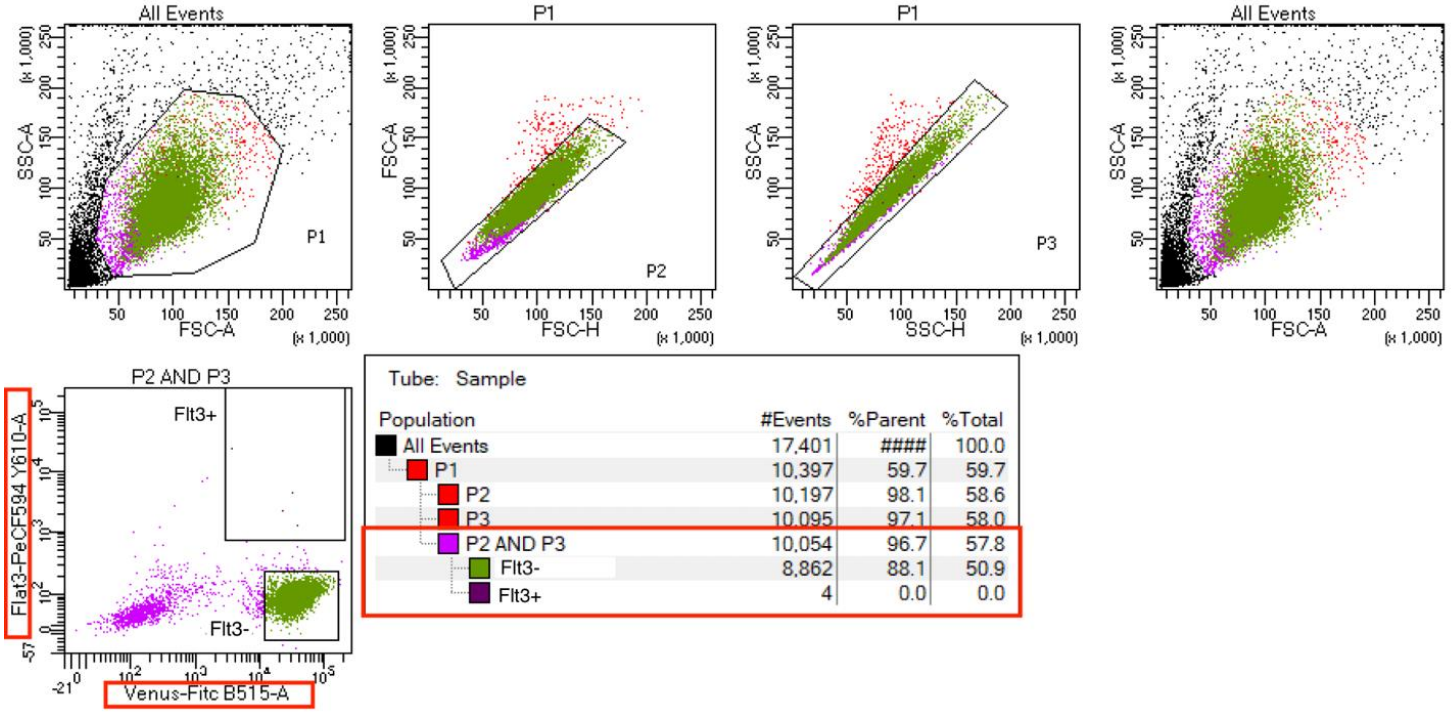


Figure 7. ***Gating Strategy for Flt3 Surface Marker.*** *P1 is to only gate the lymphocytes. P2 and P3 remove doublets and debris. P2 and P3 are then taken onto a VENUS vs Flt3 plot. The VENUS+ cells on the right half of the plot are divided into binary fractions of Flt3+ and Flt3-. In the population hierarchy, the important column is (% Parent) because it shows the % of each VENUS+ fraction out of the lymphocytes (from which debris and doublets are removed).*

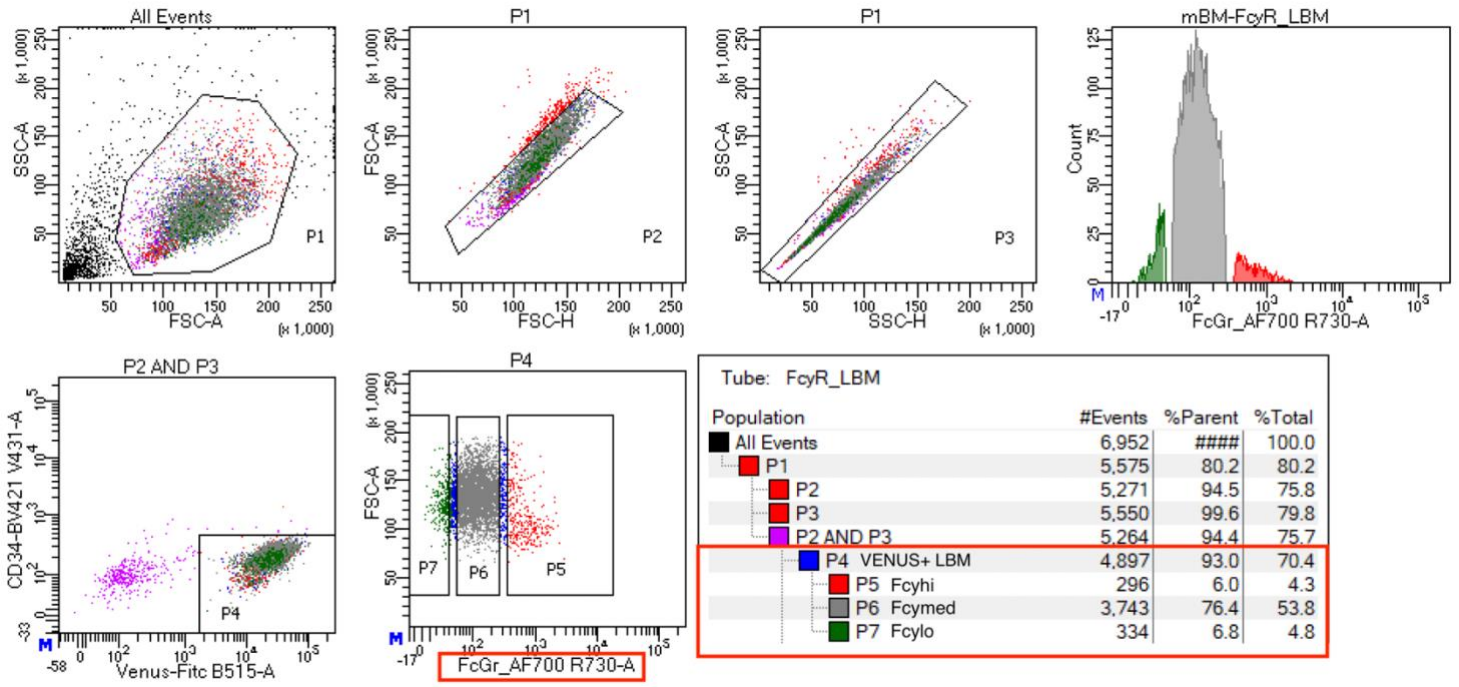


Figure 8. ***Gating Strategy for FcyR Surface Marker.*** P1 is to only gate the lymphocytes. P2 and P3 remove doublets and debris. P4 is to select only the VENUS+ cells. In the P4 gate, the three FcyR fractions are gated in a FcyR vs SSC plot. In the population hierarchy on the bottom right, the important column is (% Parent) because it shows the % of each fraction out of the VENUS+ population.

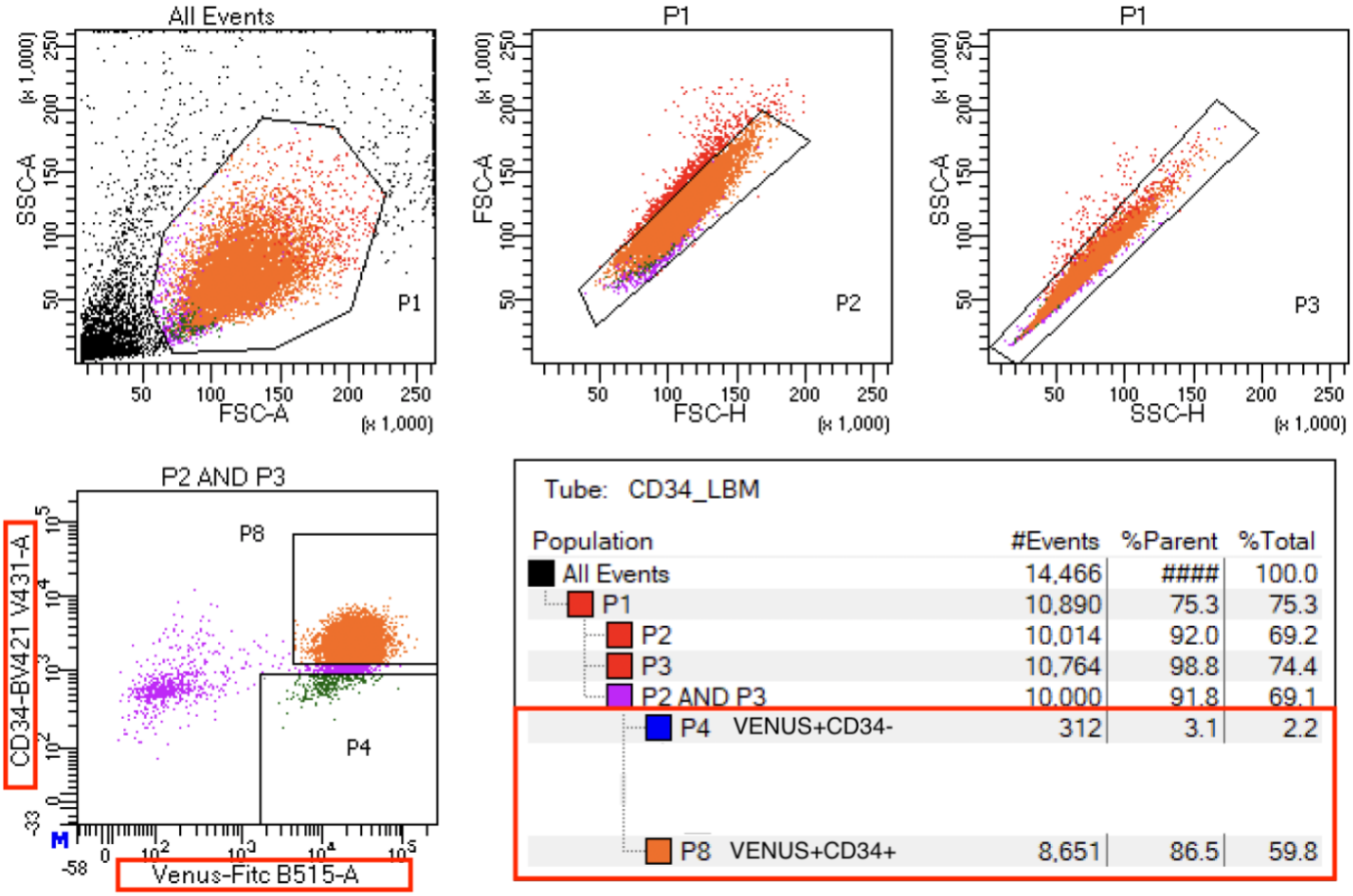


Figure 9. ***Gating Strategy for CD34 Surface Marker.*** *P1 is to only gate the lymphocytes. P2 and P3 remove doublets and debris. P2 and P3 are then taken onto a VENUS vs CD34 plot. The VENUS+ cells on the right half of the plot are divided into binary fractions of CD34+ and CD34-. In the population hierarchy, the important column is (% Parent) because it shows the % of each VENUS+ fraction out of the lymphocytes (from which debris and doublets are removed).*

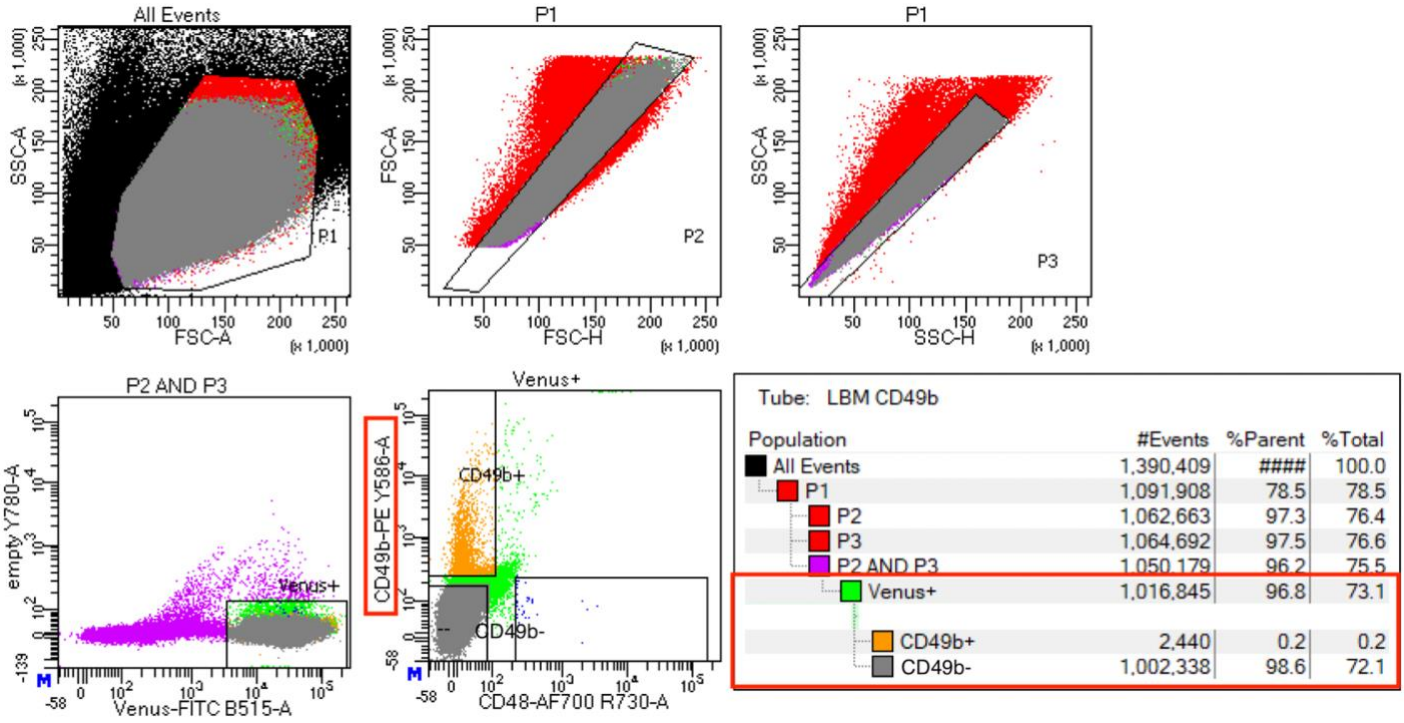


Figure 10. ***Gating Strategy for CD49b Surface Marker****. P1 is to only gate the lymphocytes. P2 and P3 remove doublets and debris. The Venus+ gate is then taken onto a CD49b vs CD48 plot and VENUS+ cells are divided into binary fractions of CD49b- and CD49b+. The cells are not stained with CD48 antibody; the CD48 parameter is an empty channel just to plot CD49b against another parameter. In the population hierarchy on the bottom right, the important column is (% Parent) because it shows the % of each fraction out of the Venus+ gate cells.*

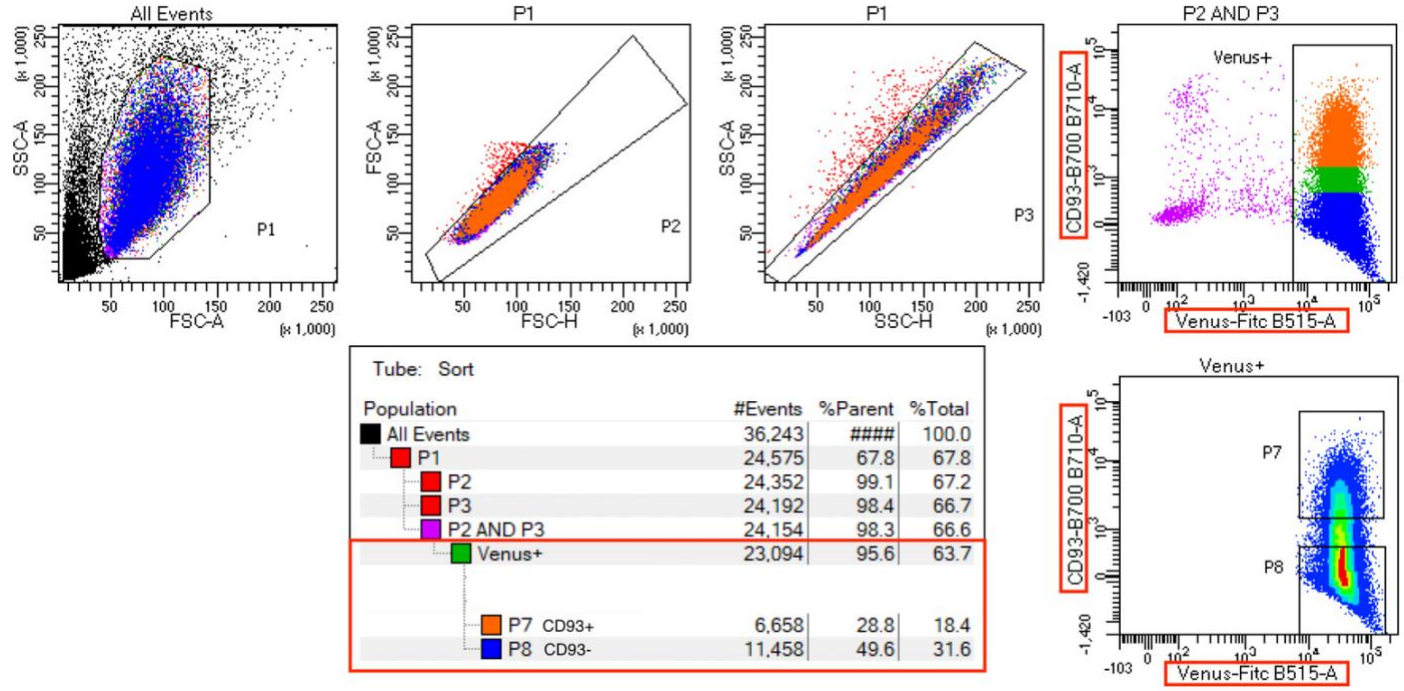


Figure 11. ***Gating Strategy for CD93 Surface Marker****. P1 is to only gate the lymphocytes, excluding RBCs in the bottom left corner. P2 and P3 remove doublets and debris. The Venus+ gate in the 4th plot on the top right is then taken onto a CD93 vs Venus plot and VENUS+ cells are divided into binary fractions of CD93+ and CD93-. In the population hierarchy on the bottom, the important column is (% Parent) because it shows the % of each fraction out of the Venus+ gate. The plot on the bottom left corner show the same thing as the plot above it, just in pseudocolor mode.*

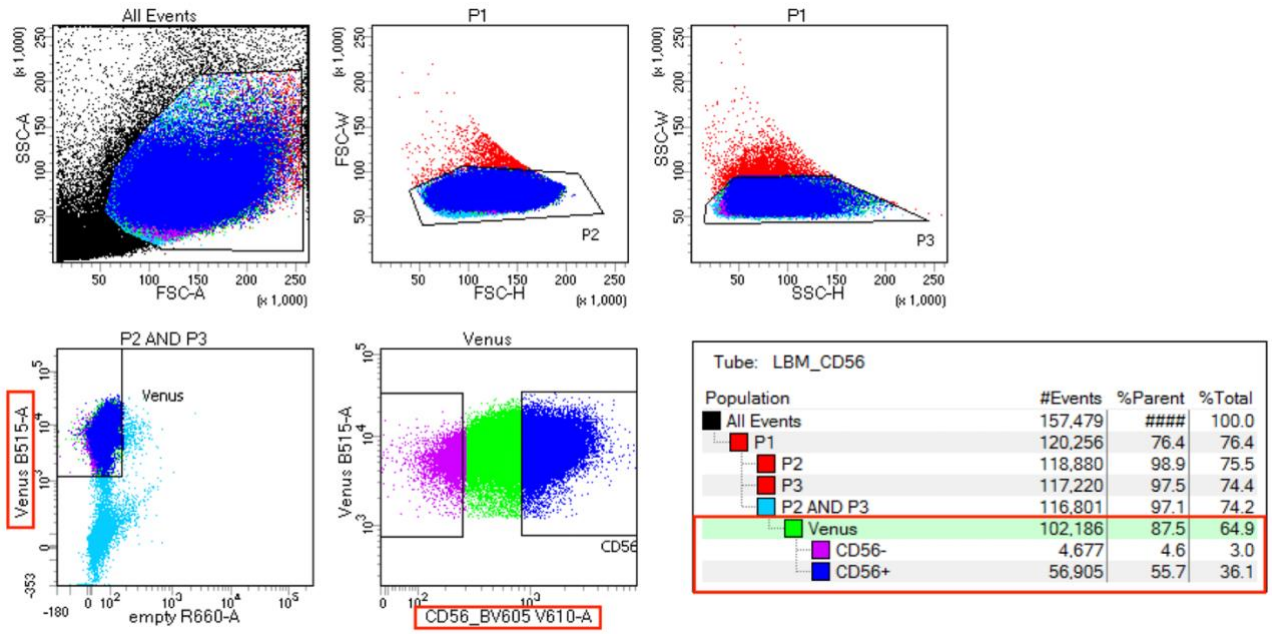


Figure 12. ***Gating Strategy for CD56 Surface Marker****. P1 is to only gate the lymphocytes, excluding RBCs in the bottom left corner. P2 and P3 remove doublets and debris. The Venus+ gate in the 1st plot on the bottom row is then taken onto a CD56 vs Venus plot and VENUS+ cells are divided into binary fractions of CD56+ and CD56-. In the population hierarchy on the bottom right, the important column is (% Parent) because it shows the % of each fraction out of the Venus+ gate.*

A

days since injection	11			15			20			25			26			29			34		
fraction	%	%/(100-%)	ln[%/(100-%)]	%	%/(100-%)	ln[%/(100-%)]	%	%/(100-%)	ln[%/(100-%)]	%	%/(100-%)	ln[%/(100-%)]	%	%/(100-%)	ln[%/(100-%)]	%	%/(100-%)	ln[%/(100-%)]	%	%/(100-%)	ln[%/(100-%)]
Kit-Sca1+	-	-	-	-	-	-	-	-	-	1.26	0.0	-4.4	-	-	-	13.4	0.2	-1.9	48.2	0.9	-0.1
Kit+Sca1-	24.7	0.3	-1.1	57.0	1.3	0.3	85.5	5.9	1.8	-	-	-	-	-	-	-	-	-	-	-	-
Kit-Sca1-	0.4	0.0	-5.5	2.2	0.0	-3.8	18.3	0.2	-1.5	73.1	2.7	1.0	-	-	-	-	-	-	-	-	-
Flt3-	0.2	0.0	-6.2	1.2	0.0	-4.4	4.2	0.0	-3.1	-	-	-	37.5	0.6	-0.5	-	-	-	-	-	-

B

	from plot A slope	td
baseline	0.47	1.47
Kit+Sca1-	0.32	2.17
Kit-Sca1-	0.47	1.49
Flt3-	0.37	1.88
mean	0.41	1.75

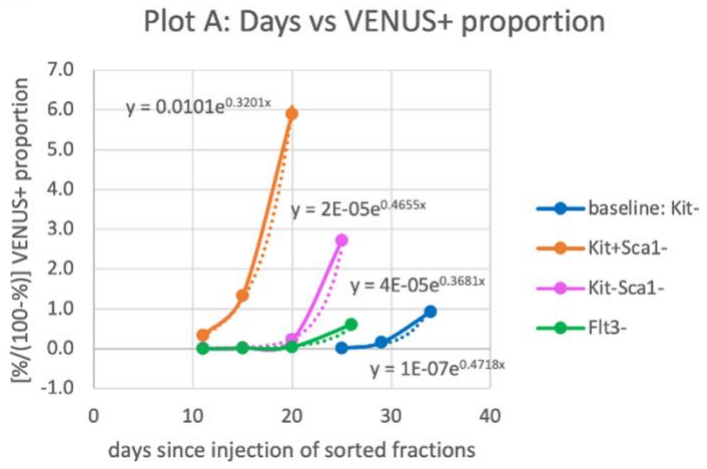
C

td= 0.6931/slope
ln(b)=y(mean ln) - (slopemean*xmean)
x(1)=(ln(1)-lnb)/meanslope
doublings=offset from baseline/meanTd
offset from baseline: baseline x(1) - fraction x(1)
[x(mean), y(exp ln mean)]: 1st point in imposed slopes
[x(1), 1]: 2nd point in imposed slopes

D

x(mean)	y(mean ln)	y(exp ln mean)	ln(b)	x(1)	y=1	offset from baseline	doublings	LSCs injected: 2^doublings	
29.33	-2.1	0.12	-14.02	34.50	1	0.00	0	1	baseline
15.33	0.3	1.37	-5.92	14.56	1	19.94	11.38	2672.504472	Kit+Sca1-
17.75	-2.5	0.09	-9.67	23.78	1	10.72	6.12	69.46936653	Kit-Sca1-
18.00	-3.6	0.03	-10.88	26.77	1	7.73	4.41	21.28163703	Flt3-

E



F

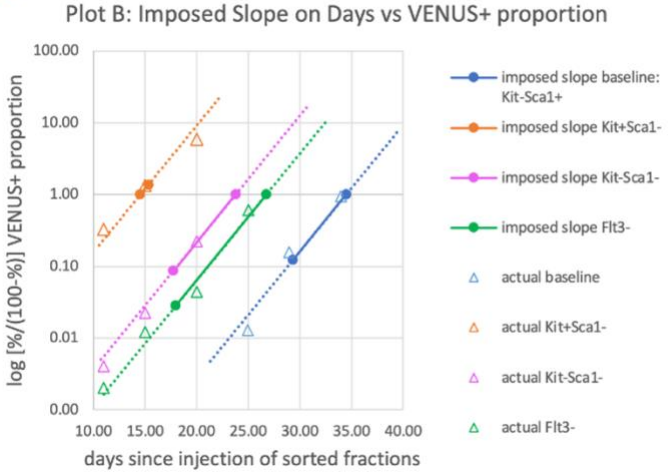


Figure 13. ***Steps to calculate the LICs per mouse*** *using the baseline that 1 LIC injected into a mouse makes it 50% VENUS-HOXB4+ by day 30-35. Examples of four mice injected with three different fractions are shown. In* **A** *the first row is of the days since injection of the sorted fractions indicated on the left column. In* **A&E** *%/(100-%) is plotted instead of % because %/(100-%) tracks the VENUS+% in a direct fashion. The last column in* **D** *represents the number of LICs injected in each of the mice in the labelled fractions.* **C** *is a list corresponding to the formulas used in* ***A, B and D****.* **E** *is the plot of days vs units of VENUS+. The slopes in the second column of B are derived from plot E.* **F** *is a plot in which the average slope was imposed for all fractions to easily compare other mice to the baseline mouse. The distance difference between a fraction and the baseline at the x(1) intercept is the "offset from baseline" column in* ***D*** *that allows estimation of the LICs (labelled LSCs here) injected per fraction.* ***(not shown)*** *This calculation of "LSCs injected" is done with all the mice injected with the +ve fraction of a particular marker and all the mice injected with the -ve fraction of the same marker. These raw numbers (of the total LSCs in the +ve fraction or of the total LSCs in the negative fraction of a marker) are divided by the total number of LICs in both fractions of the marker to yield a percentage which is then used to make bar graphs for each marker as shown in (fig 16).*

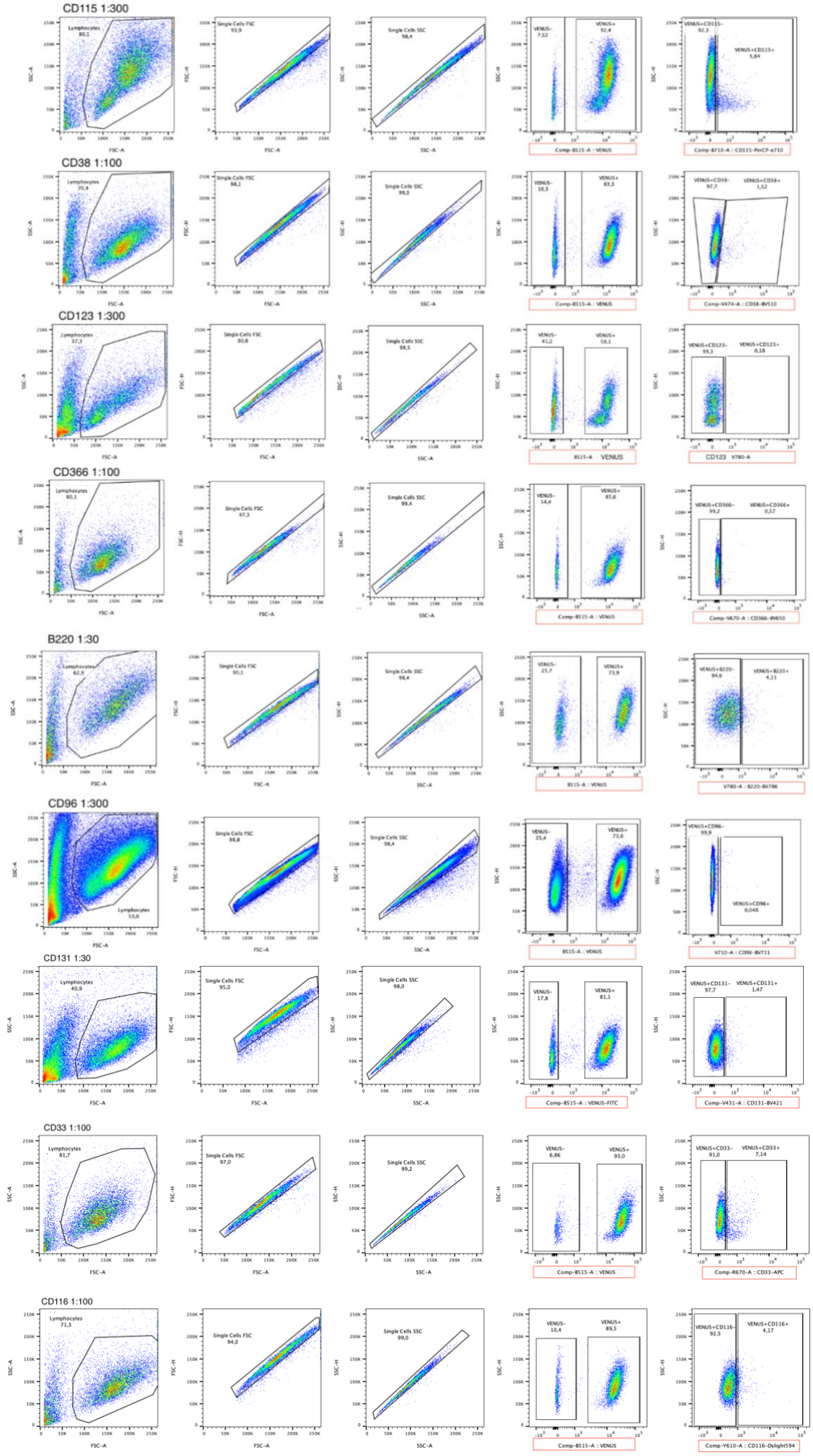


Figure 14. ***Optimal dilutions*** *of CD115, CD38, CD123, CD366, B220, CD96, CD131, CD33, CD116 are shown respectively. These markers were not sorted for.*

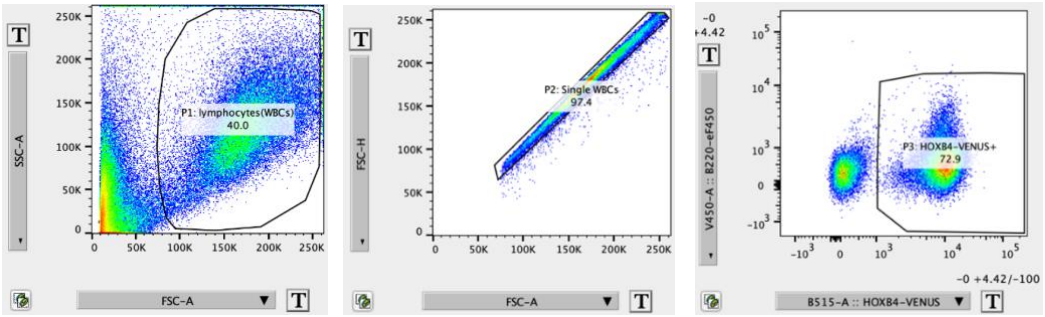
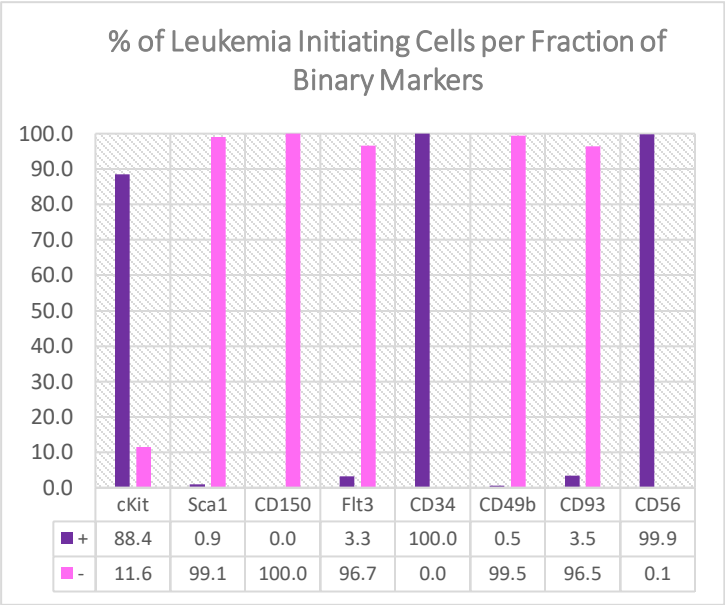
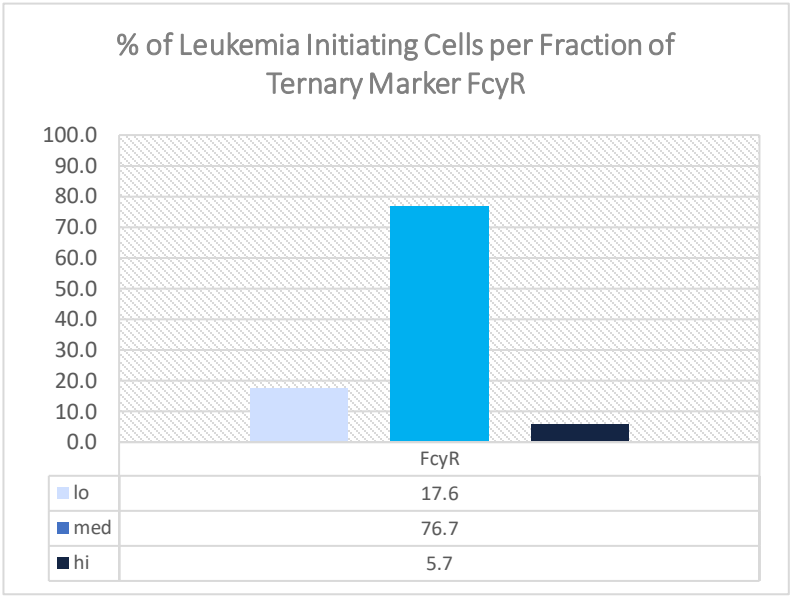


Figure 15. **Example of blood analysis plots after injection of sorted fraction that contains LICs.** P1 selects only the WBCs, leaving the RBCs that weren't lysed by ACK in the lower left corner. P2 gates only the single WBCs, excluding out doublets or debris. P3 gates the VENUS+ population out of the single WBCs only. In this case, it doesn't matter what is plotted as the second parameter against the B515-A: HOXB4-VENUS because in blood analysis of the WBCs, we are only looking at one parameter: the presence or absence of the HOXB4-transgene in each cell (visually represented as the presence or absence of VENUS).



	cKit	Sca1	CD150	Flt3	CD34	CD49b	CD93	CD56
+	88.4	0.9	0.0	3.3	100.0	0.5	3.5	99.9
-	11.6	99.1	100.0	96.7	0.0	99.5	96.5	0.1



	FcyR
lo	17.6
med	76.7
hi	5.7

Figure 16. Percentages of how many LICs per fraction of one marker using calculations in *(Fig 13).*

Marker	What it is	Window of expression in hematopoietic hierarchy
Sca1	stem cell antigen-1	- enriches for HSCs (Spangrude et al. 1988, Mazzanti 2019) - marks LT-HSCs, IT-HSCs, ST-HSCs (Holmes and Stanford 2007, Mazzanti 2019)
cKit	stem cell factor receptor	- marks LT-HSCs, IT-HSCs, ST-HSCs, LCPs *(Fig 3)* - Sca1-cKit+ corresponds differentiation shift from ST-HSC to LCPs
CD150	signaling lymphocytic activation molecule 1 (SLAMF1) (Won 2022)	- marks LT-HSC & IT-HSC - ST-HSC, LCPs, & terminally-differentiated cells are CD150-
Flt3	transmembrane receptor tyrosine kinase	- marks the portion of LT-HSC that are quiescent - expression ceases in IT-HSCs & ST-HSCs - expression persists in LCPs
CD34	transmembrane phosphoglycoprotein (Civin et al. 1984)	- marks ST-HSC & LCPs because indicate exit from quiescence
FcyR	CD16 (FcγRIII) and CD32 (FcγRII)	- LT-HSC don't express FcγR - LCPs (which are cKit+Sca1-) express FcyR
CD49b	integrin alpha subunit of a cell adhesion glycoprotein	- expressed in IT-HSCs, ST-HSCs, and progenitors, but not in LT-HSCs

Table 1. Marker Windows of Expression

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