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SUPPLEMENTARY METHODS: Computational Model

Original compartmental model:

All computational simulations were performed using a mathematical model based on one previously described[11]. This model was designed to assess feedback mediated effects in hematopoietic culture under different conditions by inputting the microenvironmental conditions to be assessed and outputting the resulting cell population expansions. Mathematically, the model consists of a set of ordinary differential equations (ODEs) that define discrete cell populations, ranging from the long-term repopulating HSC (LTR-HSC) compartment to mature cell compartments. With each time-step, each cell has a probability of self-renewing and remaining in its current compartment of differentiating and transitioning to the subsequent compartment. The model includes 16 internal parameters that were fit using a training set of experimental data and a genetic algorithm fitting method. To simulate feedback from mature cells to the stem and progenitor populations, a set of four ODEs represent stimulatory and inhibitory feedback acting on primitive cell self-renewal and proliferation. We have previously altered this model to simulate a fed-batch feeding scheme[9] by deriving mass balance equations to identify the impact that a fed-batch system would have on the rate of soluble factor accumulation. The fed-batch version of the model was used for all subsequent steps.

Revising the model to assess lineage specific effects:

As the above described mathematical model did not explicitly consider variation in feedback from multiple specific mature cell lineage populations, we altered it to include specific mature cell lineages in this study. In the revised model, each cell that leaves the $CD34^+$ compartments via differentiation will enter one of six mature cell lineage compartments, defined to be $CD14^+$, $CD15^+$, $CD7^+$, $CD41^+$, $GlyA^+$, or other (all $CD34^-$ cells not categorized by one of the five specified phenotypes). We then assigned each

lineage a unique impact on stimulatory and inhibitory feedback signaling, by adding a weighting of 0, 0.5, or 1 to both the positive and negative feedback loops incorporated in the model. The weightings were determined from the experimental data assessing the impact of the specific mature cell populations on primitive cell expansion (**Figure 1F**). Mature cells that were classified as stimulatory (falling in the green region in **Figure 1F**) were given a weighing of 1 for the stimulatory feedback loops and a weighting of 0 for the inhibitory feedback loops. The converse was done for mature cells that were classified as inhibitory (falling in the red region). Mature cells that were classified as neutral (falling in the grey region) were given a weighing of 0.5 for both the stimulatory and inhibitory feedback loops. With this revision in place, the 16 internal parameters of the model were then refit with experimental data from the fed-batch control culture using the genetic algorithm method previously described[11].

Generating culture condition specific microenvironments in silico:

Having set-up the revived fed-batch control model, we next simulated the microenvironmental conditions observed with the DL1 and IL-6/sIL-6R culture conditions via two unique inputs for each culture condition that represented the microenvironmental changes observed experimentally. The first input was the probability of cells entering each mature cell lineage. The frequencies of cells entering each lineage were based on average experimental frequencies of mature cell lineages for each culture condition as measured by flow cytometry in **Figure 2D**. The second input was the relative proliferation rate under each culture condition. We had observed from experimental CFSE analysis and cell expansion data that the average proliferation rate changed significantly with the addition of DL1 or IL-6/sIL-6R (**Figure S1**). This in turn altered the cell density which impacted the microenvironment, as not only were the proportion of mature cells altered, but the quantity of them was also affected. In the model, the average proliferation rates of progenitors and mature cells are governed by two internal model parameters that dictate the maximum proliferation rate of Lin^- and Lin^+ cells. The relative

proliferation rate between either the DL1 condition and the control or the IL-6/sIL-6R condition and the control in **Figure S1** were used to determine a scaling factor that was applied to these maximum proliferation rate parameters. As the difference in proliferation rates for each culture condition as measured by CFSE analysis was comparable for both the primitive and mature cells, the change in proliferation did not appear to be a result of a stem cell specific mechanism. **Table S1** summarizes the model inputs that were used for each culture condition.

Simulating population outputs for each culture condition:

Once these input changes were made to mimic the microenvironment of each culture condition, the model simulations were then run to output the predicted population expansions that would result from each culture condition. If the simulated expansions closely matched the experimental expansion data, than the model would support the hypothesis that the effect of DL1 and IL-6/sIL-6R on the HSPCs was, in large part, due to the observed microenvironmental changes.

SUPPLEMENTARY FIGURES

FIGURE S1

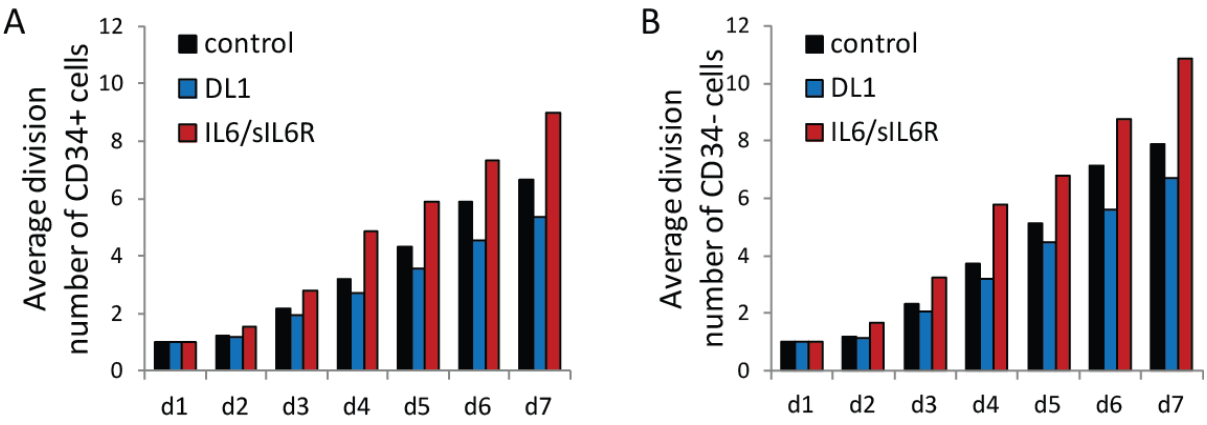

Figure S1: Comparison of cell division rate among culture conditions. CFSE staining was used to assess average cell division number of **A)** $CD34^+$ and **B)** $CD34^-$ cells when Lin^- cells were cultured in the fed-batch system under control, DL1, or IL-6/sIL-6R conditions. The addition of DL1 decreased cell division rates while the addition of IL-6 and sIL-6R increased cell division rates as compared to controls.

FIGURE S2

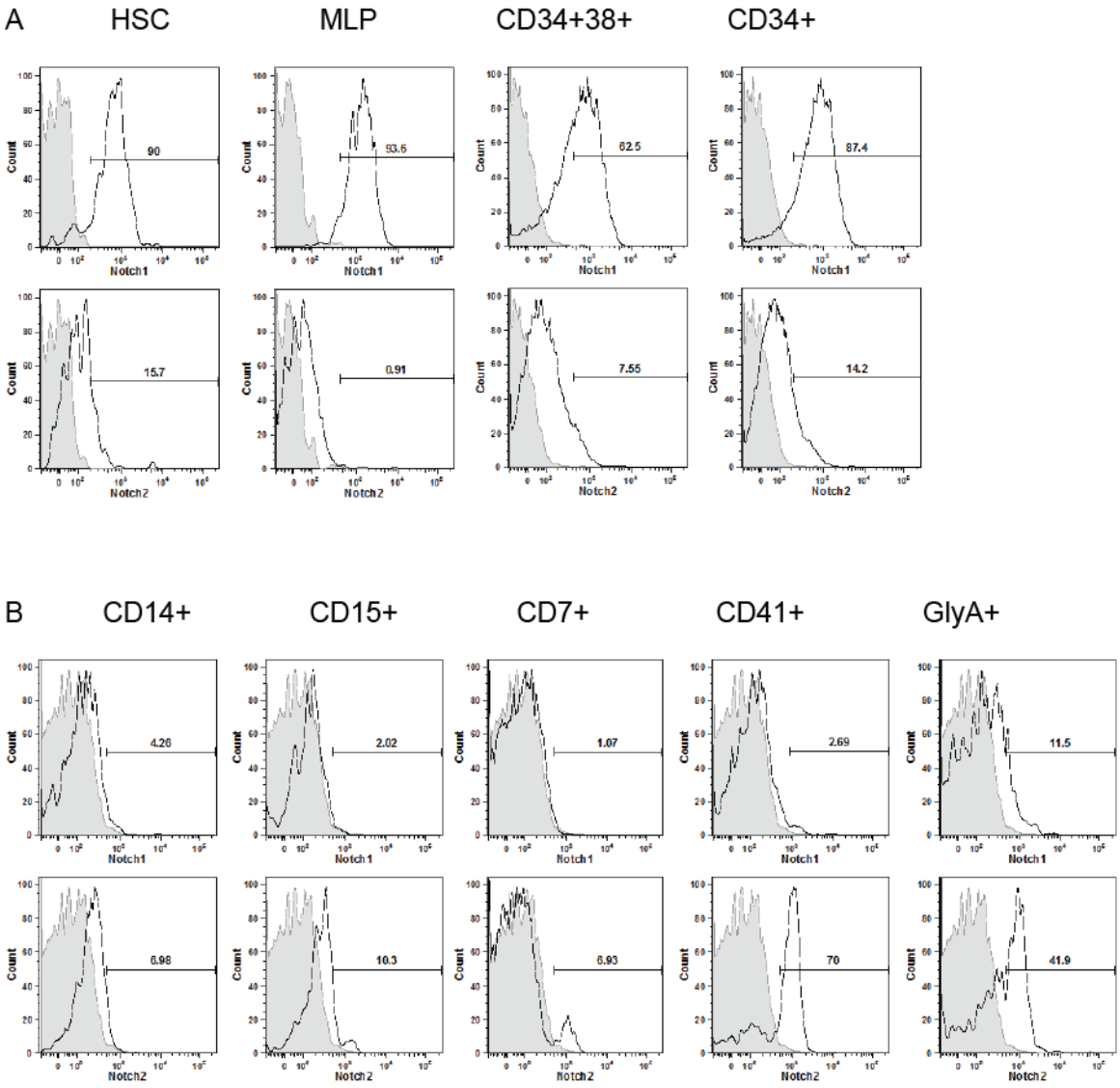

Figure S2: Protein expression of Notch1 and Notch2 receptors on hematopoietic populations. Protein expression of Notch1 and Notch2 was measured by flow cytometry on **A)** freshly isolated Lin- cells and **B)** red-blood cell depleted UCB cells. The Notch receptors were co-stained with surface markers for specific cell sub-populations, as indicated. (HSC = $Lin^{-}CD34^{+}CD38^{-}CD90^{+/-}$ $CD49f^{+}CD45RA^{-}$, MLP = $Lin^{-}CD34^{+}CD38^{-}CD90^{-}CD45RA^{+}$.)

FIGURE S3

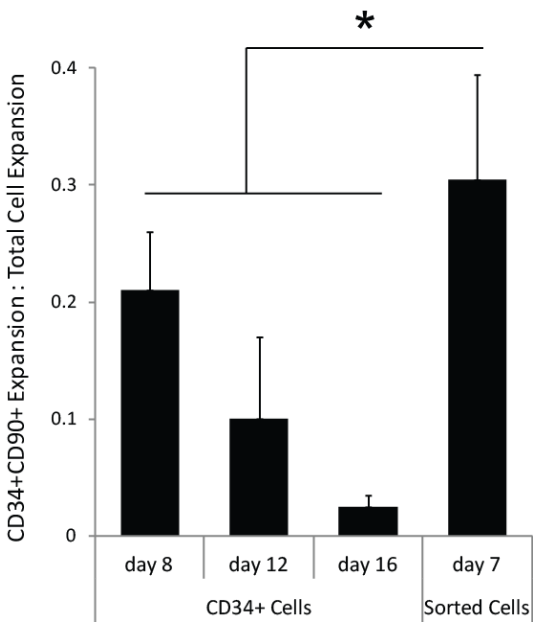

Figure S3: Comparison of relative cell expansion between heterogeneous and purified seeding populations. Ratio of expansion of $CD34^{+}CD90^{+}$ cells to expansion of total cells between cultures seeded with heterogenous $CD34^{+}$ cells and cultured for 8, 12, or 16 days, and cultures seeded with $Lin^{-}Rho^{lo}CD34^{+}CD38^{-}CD45RA^{-}CD49f^{+}$ cells (sorted cells) and cultured for 7 days.

FIGURE S4

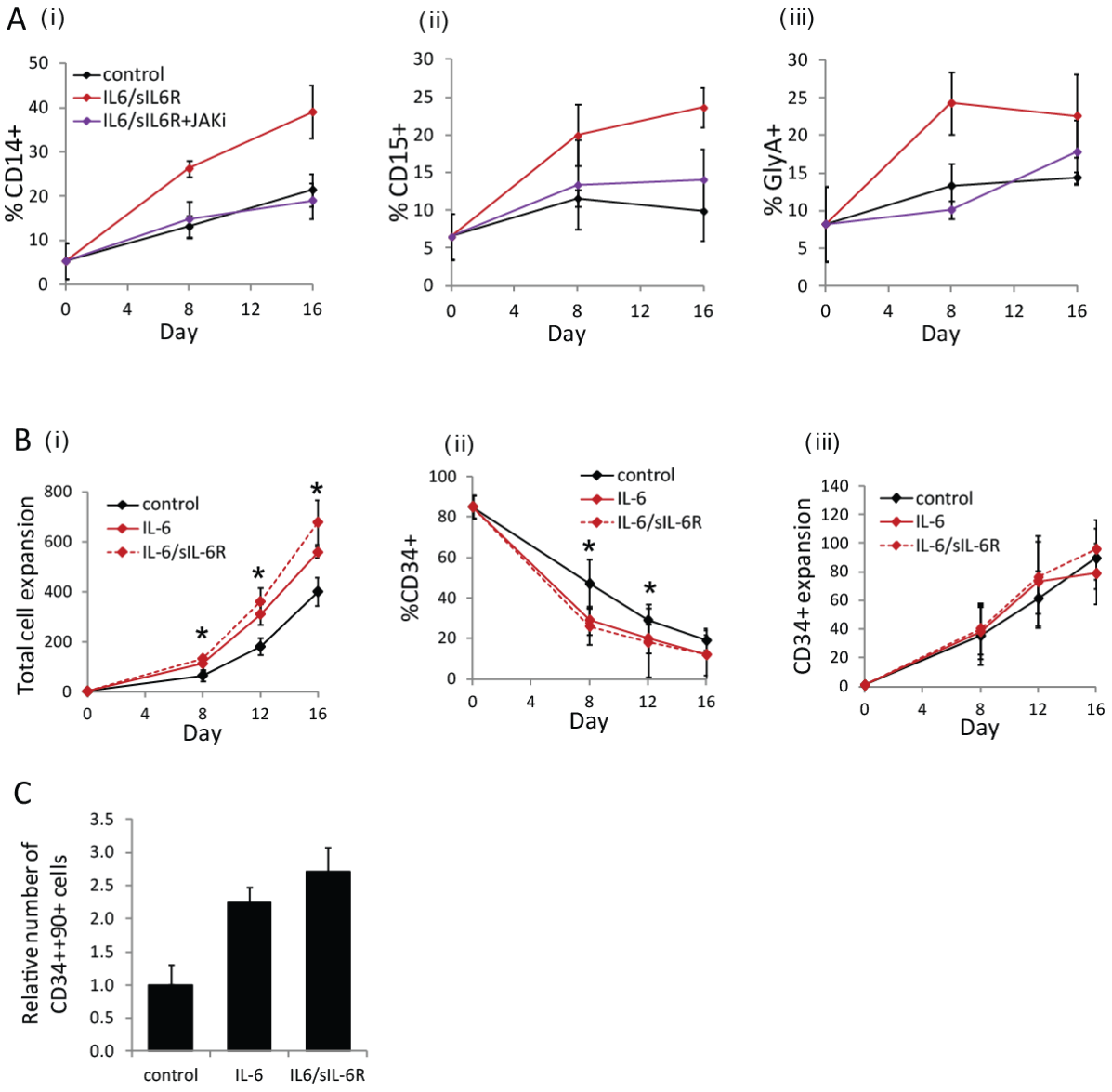

Figure S4: The effect of IL-6/sIL-6R on mature cell production and primitive cell expansion. A) The addition of IL-6/sIL-6R increased production of **(i)** $CD14^+$ cells; **(ii)** $CD15^+$ cells; and **(iii)** $GlyA^+$ cells. This effect could be reversed by the addition of JAKi, confirming that the phenotype changes were JAK-STAT dependent. **B)** The addition of IL-6/sIL-6R to culture caused: **(i)** a significant increase in total cell expansion but; **(ii)** a decrease in the frequency of $CD34^+$ cells. This resulted in **(iii)** no significant difference in the $CD34^+$ expansion with IL-6 or IL-6/sIL-6R as compared to the fed-batch control. **C)** When $Lin^-Rho^{lo}CD34^+CD38^-CD45RA^-CD49f^+$ cells were cultured with IL-6 or IL-6/sIL-6R, primitive $CD34^+CD90^+$ cells were expanded above what is seen with the control. This impact was not seen on the bulk $CD34^+$ cell cultures.

FIGURE S5

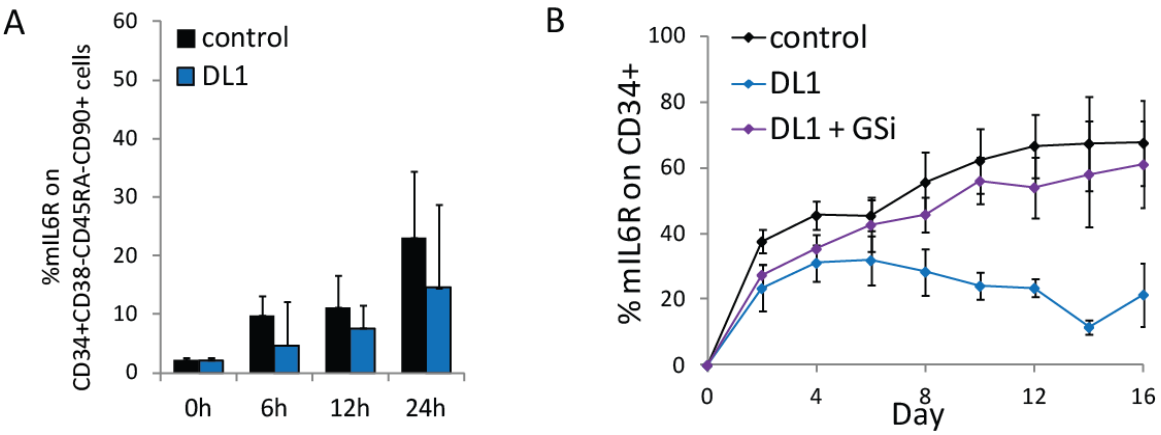

Figure S5: The effect of DL1 and GSi on mIL-6R. A) mIL-6R measured on $CD34^{+}CD38^{-}45RA^{-}CD90^{+}$ cells. **B)** The effect of DL1 on mIL6R was inhibited by the addition of a γ-secretase inhibitor (GSi), confirming that it is a Notch-mediated effect.

SUPPLEMENTARY TABLES

Supplementary Table 1: Inputs to mathematical model for each culture condition, to simulate microenvironmental conditions.

	Control	**DL1**	**IL6/sIL6R**
Mature Cell Frequencies (weightings for stimulatory/inhibitory feedback)			
CD14+ (0/1)	20%	5%	30%
CD15+ (0/1)	15%	5%	25%
CD7+ (0.5/0.5)	2%	10%	2%
CD41+ (1/0)	30%	30%	20%
GlyA+ (0/1)	5%	5%	12%
Other (0.5/0.5)	28%	45%	11%
Max Proliferation Rate Scaling Factor			
Lin- cells (u_{max})	1	0.8	1.8
Lin+ cells (u_{+})	1	0.8	1.8

Supplementary Table 2: Summary of week 16 in vivo data used for limiting dilution analysis.

	Cell Dose	Day 0 equivalent	Engrafted mice/ Total mice	HSC frequency
Fresh	1000	1000	1/4	1/6115
	5000	5000	3/4	
	15000	15000	3/4	
	25000	25000	4/4	
Day 12 FB	50000	320.5	1/4	1/754
	125000	801.3	3/4	
	250000	1602.6	3/4	
	500000	3205.1	4/4	
Day 12 FB+DL1	50000	362.3	3/5	1/550
	125000	905.8	5/5	
	250000	1811.6	4/5	
	500000	3623.2	3/3	
Day 16 FB	50000	121.1	0/5	1/1540
	125000	302.7	0/5	
	250000	605.3	2/4	
	500000	1210.7	2/3	
Day 16 FB+DL1	50000	114.4	1/4	1/969
	125000	286.0	0/5	
	250000	572.1	3/5	
	500000	1144.2	2/3	