Cell Stem Cell, Volume *1*

Supplemental Data

Imaging Hematopoietic Precursor Division in Real Time

Mingfu Wu, Hyog Young Kwon, Frederique Rattis, Jordan Blum, Chen Zhao, Rina Ashkenazi, Trachette L. Jackson, Nicholas Gaiano, Tim Oliver, and Tannishtha Reya

Supplemental Experimental Procedures

Criteria for Scoring Division Patterns

Target cells were reviewed in movie replay. CFP intensity was used as a control and the intensity levels did not change significantly or consistently in one direction during imaging. Both CFP and GFP intensity were scored by Pixel intensity unit (PIU). PIU of individual cells was counted by subtracting the average PIU of the surrounding background from the average PIU of the area occupied by the cell body. Cells with a PIU < 25 were not tracked. Scoring of symmetric or asymmetric division was determined by an objective criterion of the ratio of GFP fluorescence between the mother and daughter cells. Cells were considered to have similar levels of fluorescence if their PIU ratio was within 1.5 fold of each other. A division was scored as symmetric commitment if both daughters had downregulated GFP expression to a level at least 3 fold less than the mother. A division was scored as an asymmetric cell division if the PIU of one daughter was similar to that of the mother (within 1.5 fold) while the PIU of the other daughter was at least 3 fold less than that of the mother. A division was scored as a symmetric renewal division if the PIU of the mother was within 1.5 fold of the PIU of the daughters. The final PIU score was determined 10-12 time points (each time point=10 minutes) before the daughters entered the second division. These criteria were applied to scoring of all images.

Sequences of primers for Notch ligand PCR

Jagged 1 (5'-AGA AGT CAG AGT TCA GAG GCG TCC –3' and 5'-AGT AGA AGG CTG TCA CCA AGC AAC-3'), Jagged 2 (5'- AGC CAC GGA GCA GTC ATT TG-3' and 5'- TCG GAT TCC AGA GCA GAT AGC G-3'), Delta like 1 (5'- AAC CAT GAA CAA CCT AGC

CAA TT-3’ and 5’- CAT GGT CCC CGT GAA AGT C-3’), Delta like 4 (5’- AGG TGC CAC TTC GGT TAC ACA G-3’ and 5’-CAA TCA CAC ACT CGT TCC TCT CTT C-3’), and GAPDH (5’-CCT GGA GAA ACC TGC CAA GTA TG-3’ and 5’-AGA GTG GGA GTT GCT GTT GAA GTC-3’) .

List of Viral Constructs

MSCV-Numb-CFP, MSCV-Numb::GFP, MSCV-NUP98-HOXA9-IRES-CFP, MSCV-IRES-CFP, MSCV-IRES-GFP and MSCV-BCR-ABL IRES-CFP.

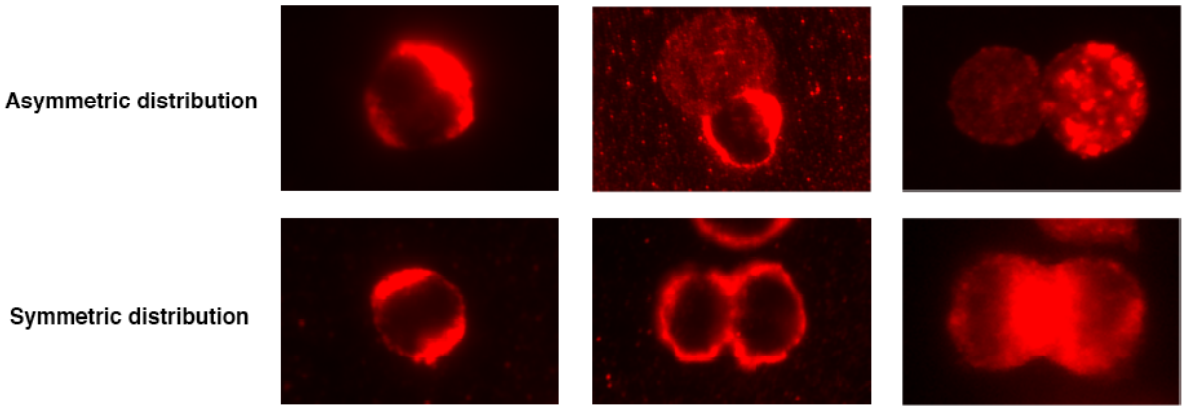
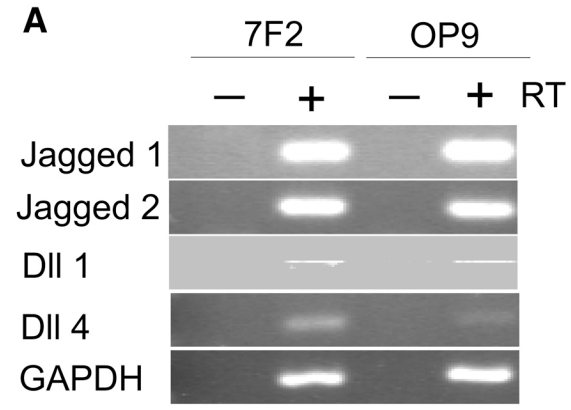
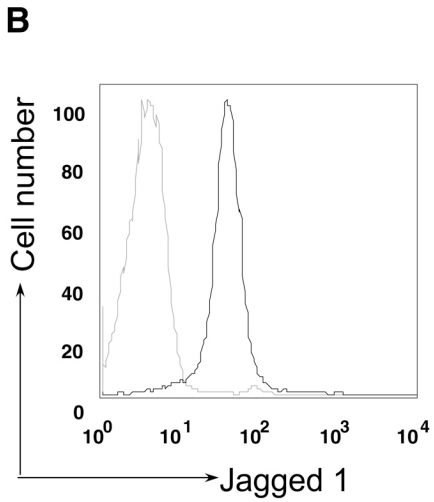
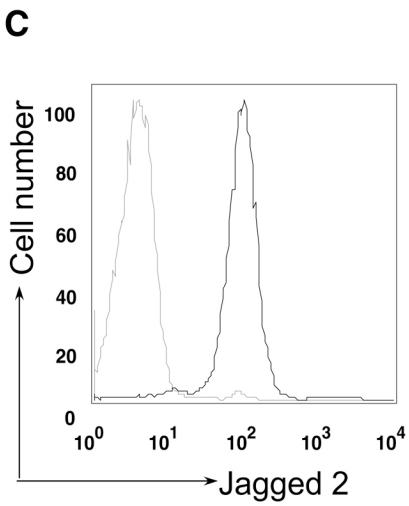


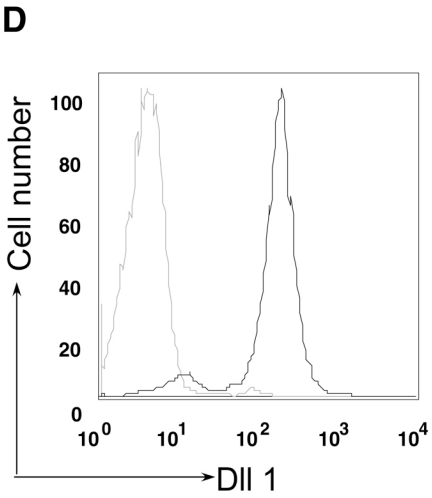
Figure S1. Patterns of numb distribution and localization in mitotic cells

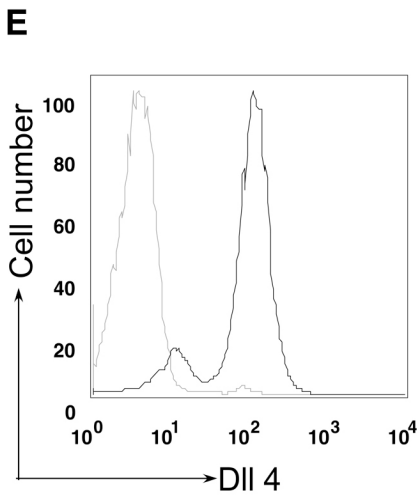
Panels show examples of cells with different patterns of asymmetric and symmetric distribution of numb. Mitotic cells in metaphase (PH3 positive, data not shown) typically display Numb localization at the cortex and/or membrane (n>100) (Left column). On average, approximately 85% of cells at telophase appear to display Numb localization at the cortex and/or membrane (middle column), while 5% and 10% of telophase cells display Numb localization in the cytoplasm and cleavage furrow respectively (upper and lower panels, right column) (n>50).











Wu et al Supplementary Figure S2

Figure S2. Notch ligands are expressed in stromal cells and hematopoietic precursors

(A) RNA generated from 7F2 or OP9 cells was reverse transcribed, and RT-PCR performed to determine expression of Notch ligands. RNA without reverse transcriptase was used to control for genomic contamination. (B-E) KLSC cells were sorted, stained with either isotype control antibody (gray) or antibodies to Notch ligands (black) and then analyzed by FACS.

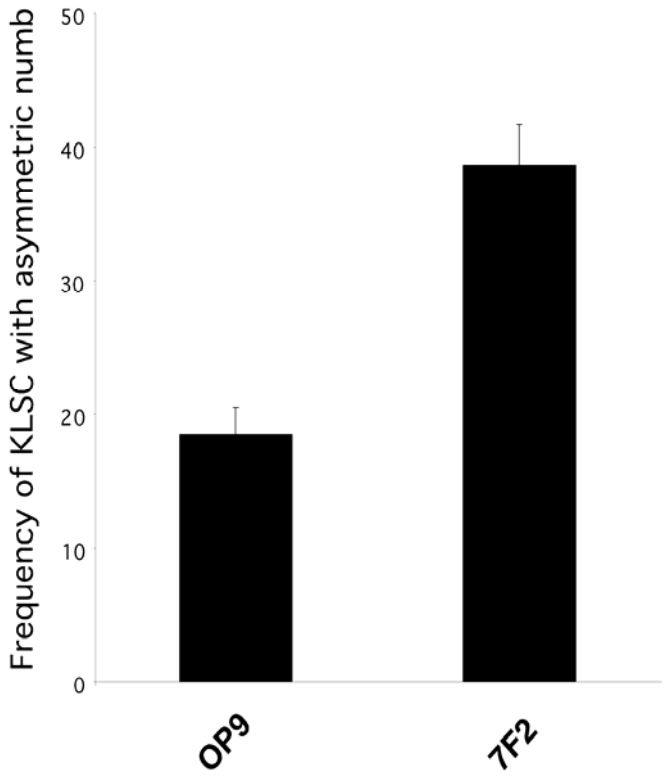
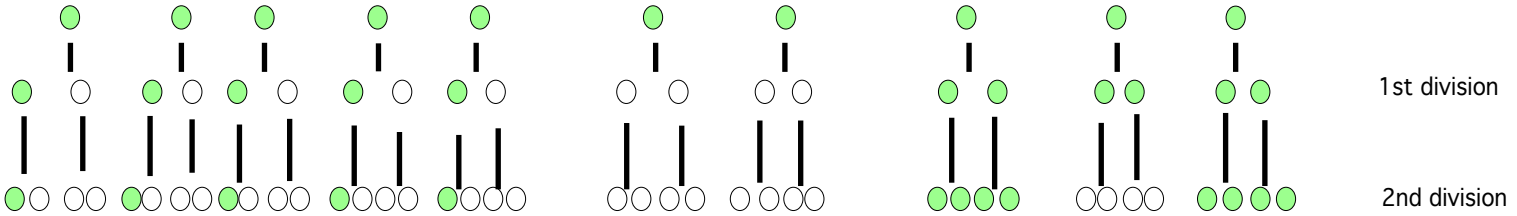


Figure S3. Differential frequency of asymmetric numb distribution in KLSC GFP+ cells co-cultured with 7F2 or OP9 stroma

KLSC GFP+ cells were loaded on cover slips plated with OP9 or 7F2 cells, cultured overnight and Nocodozale added (10nm). After 24 hours, cells were fixed and subsequently stained to visualize phosphorylated Histone 3 (to identify mitotic cells), and numb. Graph shows frequency of cells with asymmetric distribution of numb when cultured on OP9 or 7F2 stroma.

7F2

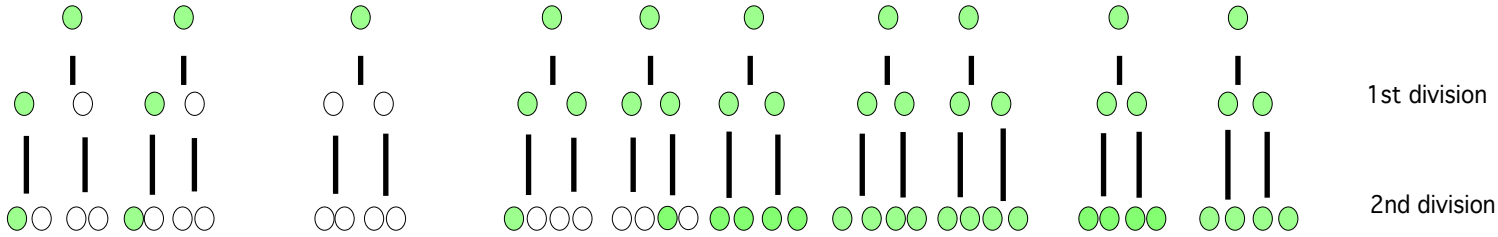
Number of cells for each type of division based on observed frequency 1st division: 10 GFP+ cells : 3.3=renewal, 1.7 (2.0)= commitment, 5.0=asymmetric
Number of cells for each type of division based on observed frequency 2nd division 11 GFP+ cells: 3.6 (4.0)=renewal, 1.8 (2.0) commitment, 5.5 (5)=asymmetric



Total GFP+=13
Total GFP-= 27

OP9

Number of cells for each type of division based on observed frequency 1st division 10 GFP+ cells : 6.5 (7)=renewal, 1.3= commitment, 2.2=asymmetric
Number of cells for each type of division based on observed frequency 2nd division 16 GFP+ cells: 10.4 (10)=renewal, 2.08 commitment, 3.5 (4)=asymmetric

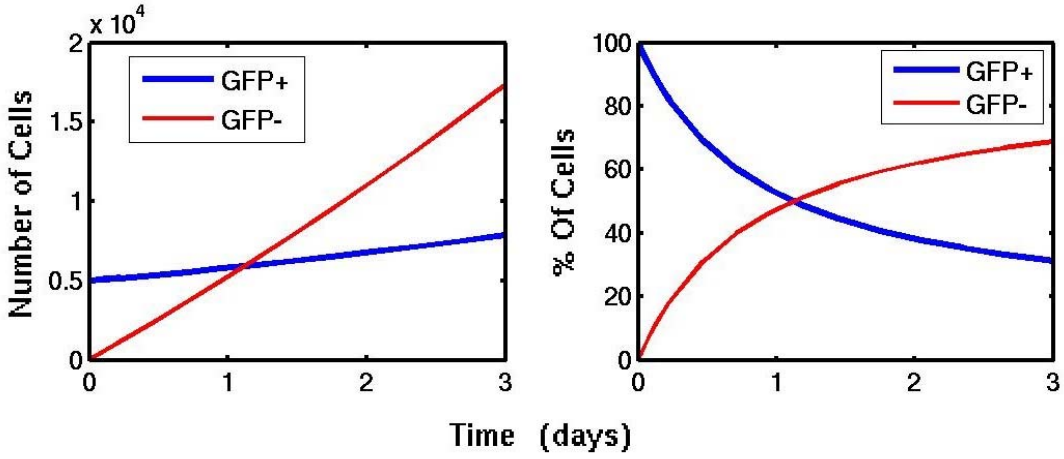


Total GFP+=24
Total GFP-= 16

Wu et al Supplementary Figure S4A

B.

7F2



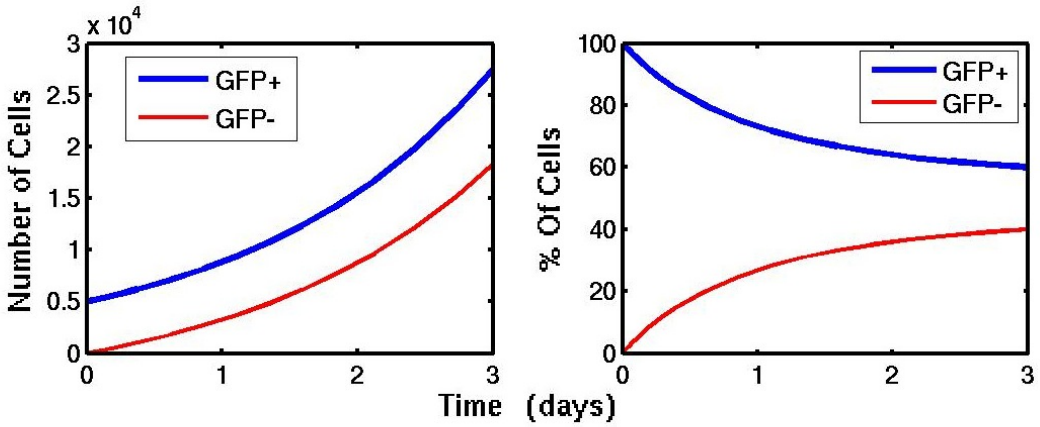
Input Parameters based on experimental observations

ρ (division rate per day)	1.2
ϕ_s symmetric renewal division frequency	.33
ϕ_a asymmetric division frequency	.5
ϕ_d symmetric commitment division frequency	.17
δ_s death rate of GFP+ cells	.034
δ_n death rate of GFP- cells	.06
Input Cell Number (GFP+KLSC)	5000

Predicted Results based on mathematical modeling

Number (%) of GFP+ cells after 3 days	7913 (32%)
Number (%) of GFP- cells after 3 days	17175 (68%)

C. **OP9**



Input Parameters based on experimental observations

ρ (division rate per day)	1.1
ϕ_s symmetric renewal division frequency	.65
ϕ_a asymmetric division frequency	.25
ϕ_d symmetric commitment division frequency	.10
δ_s death rate of GFP+ cells	.034
δ_n death rate of GFP- cells	.06
Input Cell Number (GFP+KLSC)	5000

Predicted Results based on mathematical modeling

Number (%) of GFP+ cells after 3 days	27000 (60%)
Number (%) of GFP- cells after 3 days	18000 (40%)

Figure S4. Modeling differentiation of HSCs on 7F2 and OP9 stroma

(A) 10 GFP+ cells are modeed to undergo divisions according to the frequency observed on either 7F2 (top) or OP9 (bottom) cells to visualize how an observed division pattern can result in greater or lesser differentiation in the final pool of daughter cells. GFP+ cells are shown in green, and GFP- daughters in white. Cells are modeled to undergo two rounds of division. Number of cells undergoing each type of division are calculated by multiplying the frequency with the number of GFP+ cells at each division (numbers are rounded if needed, and shown in parenthesis). GFP- cells were only observed to undergo division into two GFP- daughters experimentally, and thus are modeled to follow that pattern.

(B, C) Mathematical modeling of growth and differentiation of GFP+KLSCs cultured on 7F2 or OP9 stroma for 3 days. Input parameters include frequencies of each type of division, total division rate and rate of death of GFP+ and GFP- cells based on experimental observations. Left panel shows absolute numbers of GFP+ and GFP- cells generated with a starting population of 5000 GFP+ cells on either 7F2 (B) or OP9 cells (C). Right panel shows the relative frequency of GFP+ and GFP- cells on 7F2 (B) or OP9 cells (C).