

Legend to Supplementary Figures.

Supplementary Figure 1

Early HES-1 mRNA expression is derived from FL hematopoietic cells.

(A) Quantitative real-time gene expression analysis of HES-1 and EBF expression in OP9-DL1 cells, in c-Kit⁺ FL cells (0), and in cocultures after 1 and 3 days. All expression levels were calculated relative to β -actin. These samples are not re-sorted after coculture, but the contribution of OP9-DL1 cells to the signal can be given an upper limit by the dilution of a gene expressed in the stromal cells, EBF. Note that EBF signals are diluted 30x between pure OP9-DL1 cells and the hematopoietic cells in the OP9-DL1 cocultures, while HES-1 levels in the cocultures are much higher. (B) Quantitative real-time gene expression analysis of HES-1, Deltex1 and Nrarp in c-Kit⁺ FL cells re-sorted after 0 or 2 days of OP9-control  or OP9-DL1  stromal coculture. All expression levels were calculated relative to β -actin.

Supplementary Figure 2

Expression of transcription factors in different sorted sub-populations of cells when co-cultured on OP9-control and OP9-DL1 stromal cells for 4 days.

Lineage negative c-kit⁺ cells were cultured on OP9-control stromal cells  or on OP9-DL1 stromal cells  for 4 days. Diversification of cells during culture was reflected by the appearance of distinct subpopulations with different patterns of expression of c-kit, CD27, Mac-1 (CD11b), and Gr-1 (Ly-6G). The cells were then sorted into sub-populations as indicated in the figure. Leftmost bars: the initial FL c-kit⁺ cells on day 0; input to both culture conditions. Total progeny: FL-derived cells sorted by light scatter only to deplete OP9 cells after 4 days of culture.

Lineage specific transcription factor expression was detected by quantitative PCR in the sorted sub-populations of FL cells. All expression levels were calculated relative to β -actin.

Supplementary Figure 3

Induction of PAX-5 expression in 4 day OP9-DL1 precultured c-Kit⁺CD27⁺ FL precursors after transfer onto OP9-control stromal cells.

Quantitative real-time PAX-5 gene expression analysis of sorted 4 day OP9-control and OP9-DL1 precultured c-Kit⁺CD27⁺ cells. OP9-DL1 derived cells were also replated onto fresh OP9-control stromal cells with samples taken after 2 and 4 days of additional culture (data derived from Figure 6 of the manuscript). Units of expression are exactly as in Fig. 6B, with the level of PAX-5 induced in the CD27⁺ c-kit⁺ cells after 4 days of primary culture on OP9-control set to 1. Note the close match to PAX-5 induced after 4 days in switch culture.