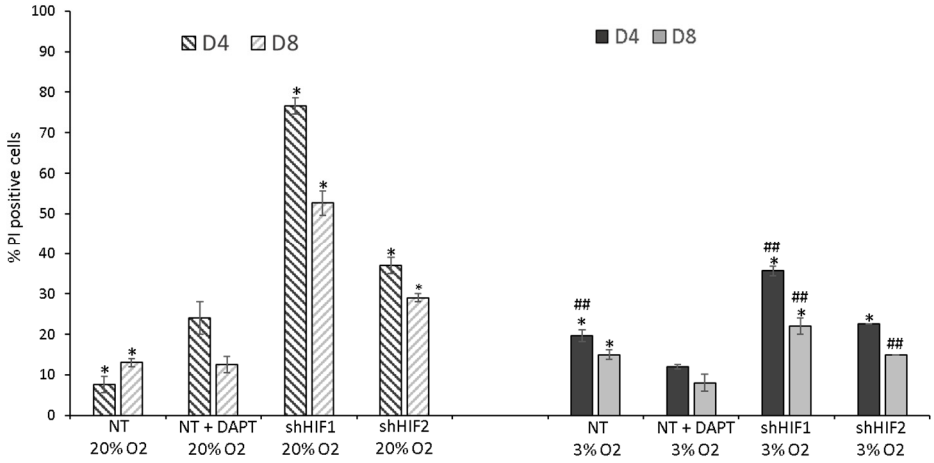
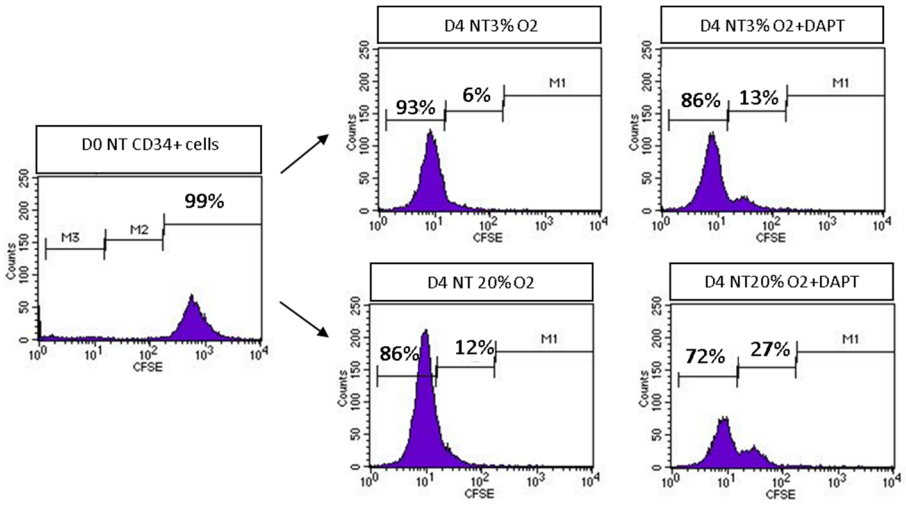
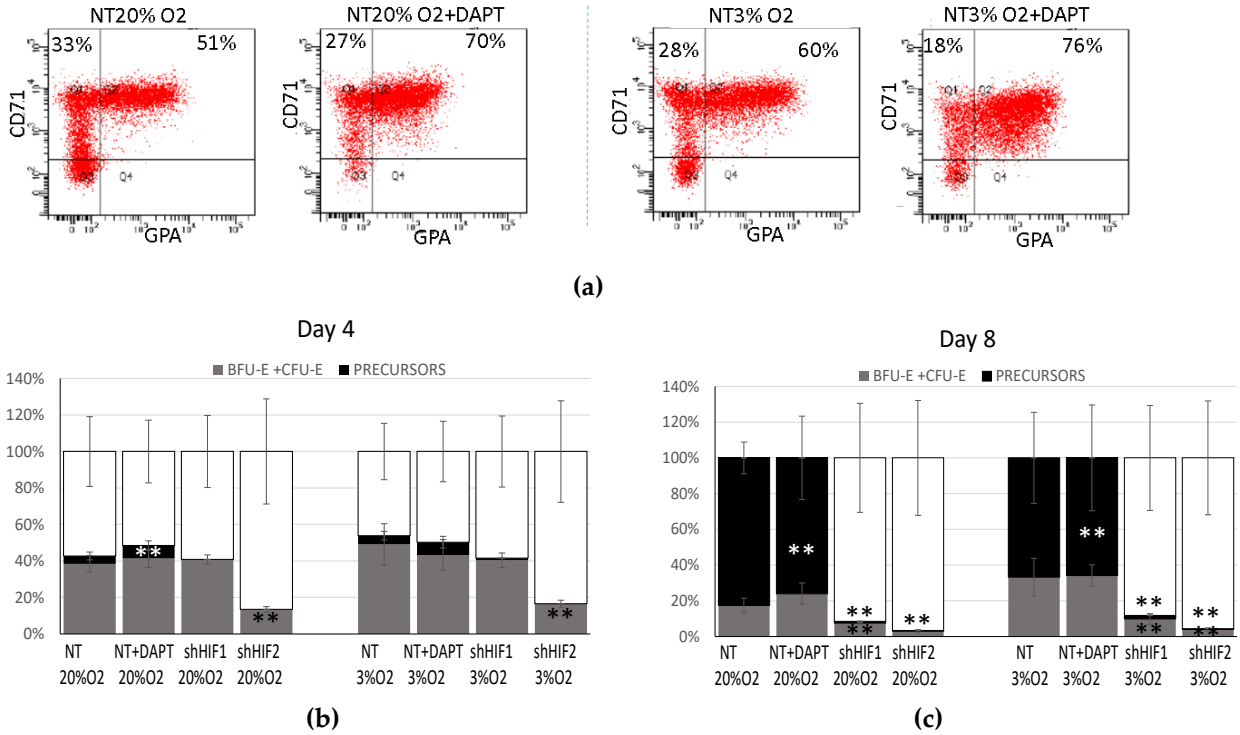
Supplementary Material



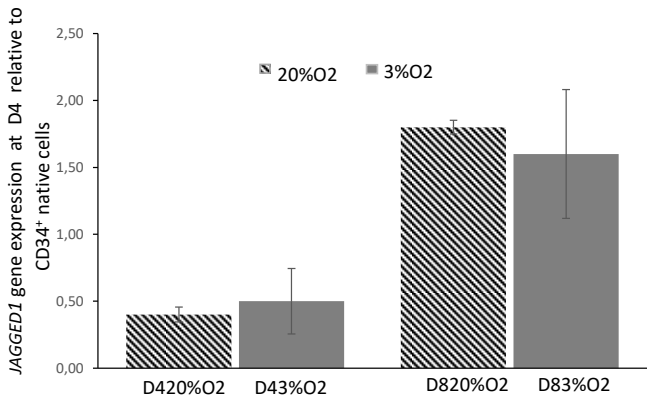

**Supplementary Figure S1. Cell survival**. Cell harvested at day 4 and day 8 culture propagated from non-transduced CD34⁺ cells in presence or absence of Notch γ-secretase inhibitor DAPT as well as transduced CD34⁺ cells with shHIF1α or with shHIF2α at 20 and 3% O2 were stained with propidium iodide (PI). The bars show percentage of PI positive cells representing dead cells. The values are given as a mean ± SD of three independent experiments. . Asterisks indicate a significant difference in the respect to NT condition, at $p < 0.05$ (*); Mann-Whitney test. NT, non-transduced CD34⁺ cells. $p < 0.01$ (##).




**Supplementary Figure S2. Tracking cell division history by CFSE staining**. Non-transduced CD34⁺cells were labeled with CFSE dye and culture for four days at 3%, and 20% O2 in the presence or absence the Notch signaling inhibitor DAPT. Histograms present CFSE intensity obtained by analyzing the cells at Day 0 (D0) and after 4 days (D4) of cultures. One representative out of three independent experiments is showed.




**Supplementary Figure S3. Distribution of cellular types in time-course of erythroid culture**. Eight-day erythroid cultures were propagated from non-transduced CD34⁺ cells in presence or absence of Notch γ-secretase inhibitor DAPT as well as transduced CD34⁺ cells with shHIF1α or with shHIF2α at 20 and 3% O2. At the figure **a)** scatter plot represent expression of transferrin receptor CD71 versus glycophorine A (GPA). One representative of five independent experiments is shown.The total number erythroid of progenitors was calculated by addition of the total number of BFU-E and CFU-E obtained at Day 4 **(b)** and Day 8 of culture **(c).** The total number of precursors cells was calculated based on the % of CD71⁺/GPA⁺ cells of total cells obtained at the same time-points. The number of non-erythroid cells (not exclusively committed to erythroid lineage) was calculated using a substraction : total number of cell – (BFU-E+ CFU-E + erythroid precursors). The bars represent the mean values ± SD of seven independent experiments. **.** Asterisks indicate a significant difference in the respect to NT condition, at $p < 0.05$ (*), $p < 0.01$ (**),Mann-Whitney test. NT, non-transduced CD34⁺ cells.




**Supplementary Figure S4. Expression of Notch ligand *JAGGED1*.** Gene expression was evaluated in erythroid cells obtained in day 4 and of culture of non–transduced CD34+ cells at 20 and 3% O2 by real-time polymerase chain reaction analysis. Bars represent relative gene expression in respect to native CD34+ CB cells. Data are showed as mean ± SD of three independent experiments

| Gene | Primer | Sequence |
|---|---|---|
| hu β-aktin | forward | 5'-ATTGGCAATGAGCGGTTC-3' |
| hu β-aktin | reverse | 5'-GGATGCCACAGGACTCCAT-3' |
| hu HES-1 | forward | 5'-GATGTGAAGGCTTTCAAGACAGA-3' |
| hu HES-1 | reverse | 5'-GGAAAATGGCTTCCTTAGGC-3' |
| hu HEY-2 | forward | 5'-CCAGCAGTGCATCAGTATGTC-3' |
| hu HEY-2 | reverse | 5-'CAGGCACTTACGAAACACGA-3' |
| hu Jagged-1 | forward | 5'-TGCCAAGTGCCAGGAAGT-3' |
| hu Jagged-2 | reverse | 5'-GCCCCATCTGGTATCACACT-3’ |

**Supplementary Table S1. Real-time polymerase chain reaction primers**