| Ab name | Clone | Fluorochrome | Reference | Brand |
|---|---|---|---|---|
| CD90 | 5E10 | APC | 559869 | BD Bioscience |
| CD34 | 581 | PE-Cy7 | 343516 | Biolegend |
| CD34 | 581 | PerCPCy5.5 | 343522 | Biolegend |
| CD38 | HB-7 | FITC | 356610 | Biolegend |
| CD45RA | HI100 | PE | 304108 | Biolegend |
| CD45RA | REA1047 | APCVio770 | 130-117-747 | MiltenyiBiotec |
| CD45RA | HI100 | BV421 | 562885 | BD Biosciences |
| CD45 | REA747 | VioGreen | 130-110-638 | MiltenyiBiotec |
| CD45 | HI30 | PE | 304008 | Biolegend |
| CD14 | M5E2 | PE | 301850 | Biolegend |
| CD15 | W6D3 | PE | 323006 | Biolegend |
| CD3 | SK7 | APCCy7 | 344818 | Biolegend |
| CD19 | SJ25C1 | PerCPCy5.5 | 45-0198-42 | Life Technologies |
| CD45 (mouse) | 30-F11 | APC | 17-0451-83 | eBiosciences |
| Ki67 | B56 | FITC | 556026 | BD Biosciences |
| Phospho-p38MAPK (Thr130/Tyr182) | 3D7 | PE | 6908S | Cell Signaling |
| 8-Hydroxy-2’-deoxyguanosine (8oxodG) | N45.1 | purified | ab48508 | abcam |
| NRF2 | polyclonal | Purified (rabbit) | ab31163 | abcam |
| Goat-anti-rabbit | polyclonal | AF647 | A-21244 | Life Technologies |
| Goat-anti-mouse | polyclonal | AF647 | A-21235 | Life Technologies |

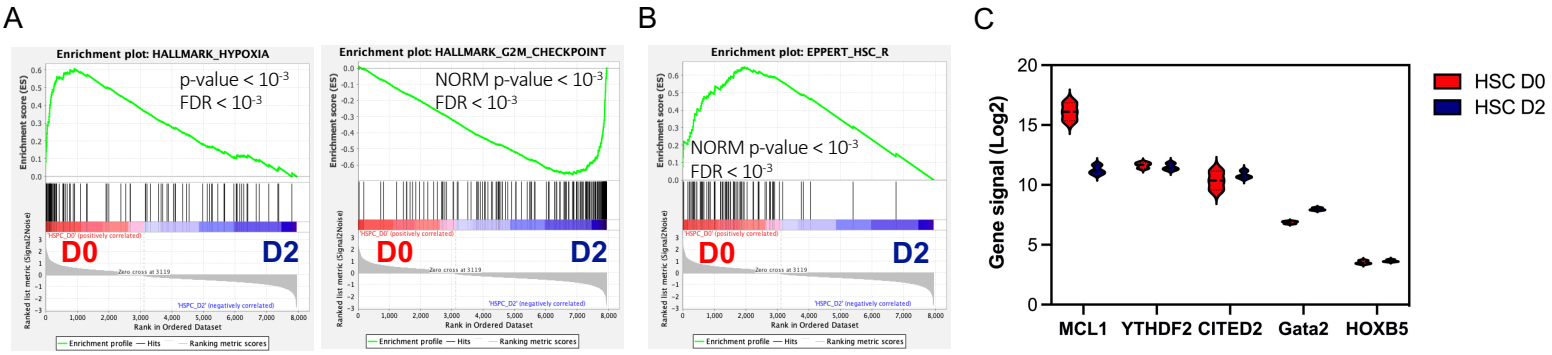
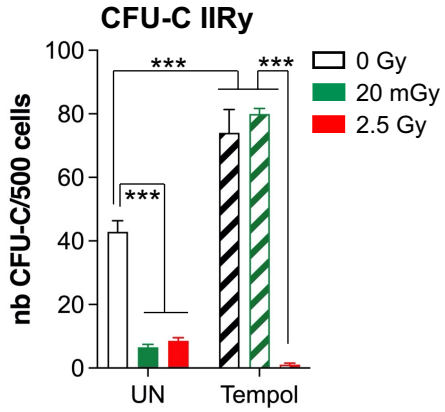
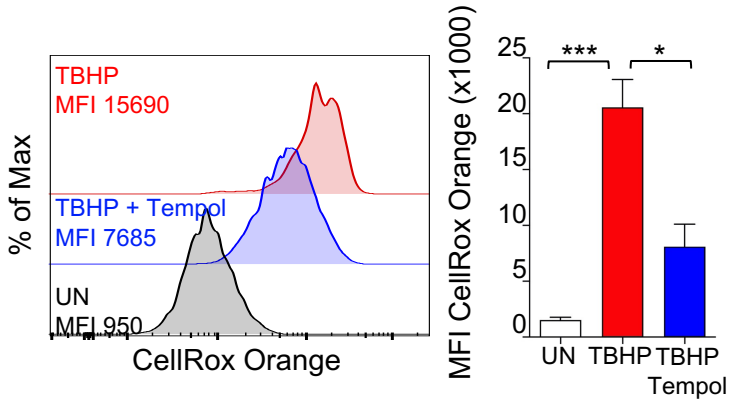



**Figure S1 related to Figure 1:** GSEA analysis of the data using (A) hallmark gene-sets and (B)EPPERT_HSC signature: several pathways are significantly different: NORM p value $<10^{-3}$, FDR$<10^{-3}$. (C) MCL1 gene expression is also downregulated in D2 HSC compared to D0 HSC, however some other genes known to be involved in HSC self-renewal as well are not differentiation regulated by culture conditions.

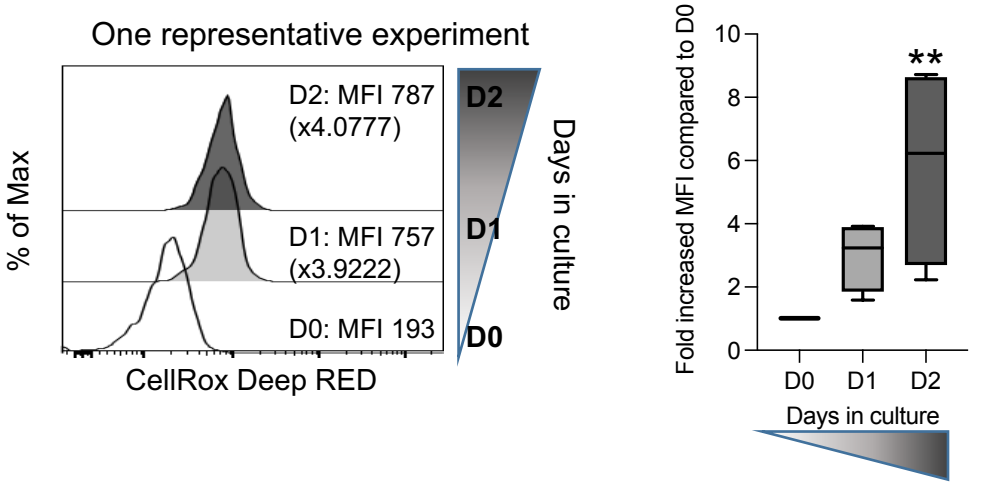
A




B




C




**<u>Figure S2 related to Figure 2:</u>** (A) human HSC were treated or not with tempol then exposed or not to 20 mGy or 2.5 Gy irradiation then serial CFU-C plating assays were performed. Here is represented the number of colonies generate in the secondary plating (CFU-C IIRy) n=3 independent experiments ***p<0.001. Two-ways anova (B) CellRox Orange staining of $CD34^{+}$ cells after treatment with pro and antioxidants: demonstration of the antioxidant activity of Tempol. n>3. *p<0.05, ***p<0.001. Two-ways anova. (C) Kinetic of ROS production over time in culture. Untreated CD34+ cells were cultured and ROX levels measured in HSC fraction at D0, D1 and D2 of culture. 4 independent experiments with 4 independent CB samples, Two-ways anova with Kruskal-Wallis test **p<0.01.

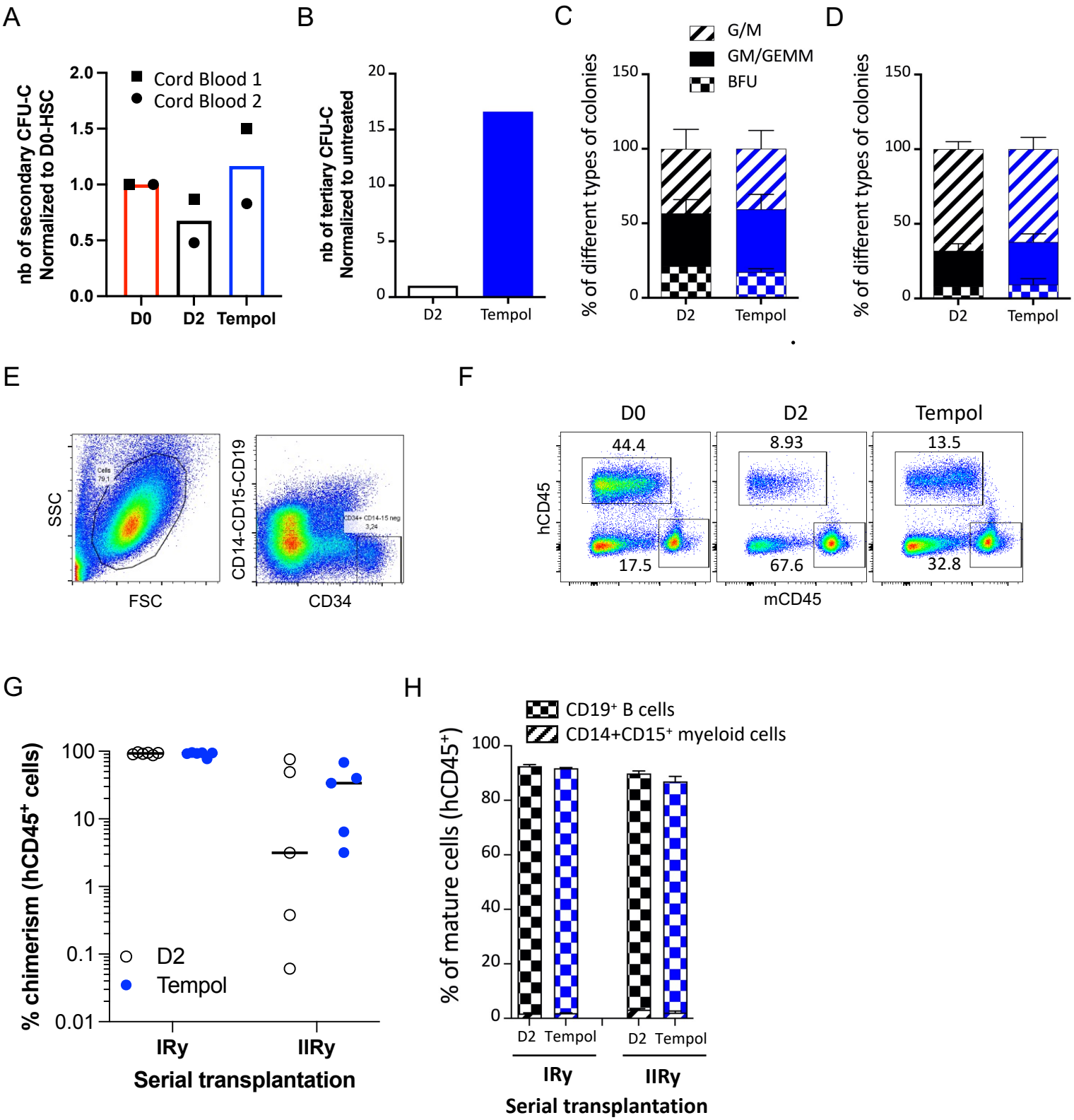



**Figure S3 related to Figure 3**. (D) HSC from 2 independent cord blood samples were either seeded directly in CFU-C medium (D0, red) or first treated or not with Tempol, cultured for 48h and then seeded in CFU-C medium (D2, black and Tempol, blue). Colonies were quantified 10 to 12 days after. (B) Tertiary CFU-C: 10% of total cells of equivalent secondary plates were seeded in triplicate. n=1. (C-D). Repartition of the different types of colonies in primary and secondary CFU-C respectively n=5. (E) Sorting strategy for extended LTC-IC. (F) FACS dot plot of bone marrow staining with antibodies against human and murine CD45. Representative example of each condition at the median of reconstitution (G) $CD34^+$ cells were treated or not with Tempol then cultivated 2 days in transduction medium then transplanted into NSG mice (250000 cells/mice). Serial transplantation assay was performed. Left % of chimerism at the first and the second transplantation. Median of % of chimerism for the secondary transplantation, 3.16% and 33.67% for D2 and Tempol-D2 conditions respectively (H) among $hCD45^+$ cells, the % of mature cells ($CD19^+$ B cells and CD14+15 myeloid cells) in the first and the second transplantation. One experiment, n>5 per condition.

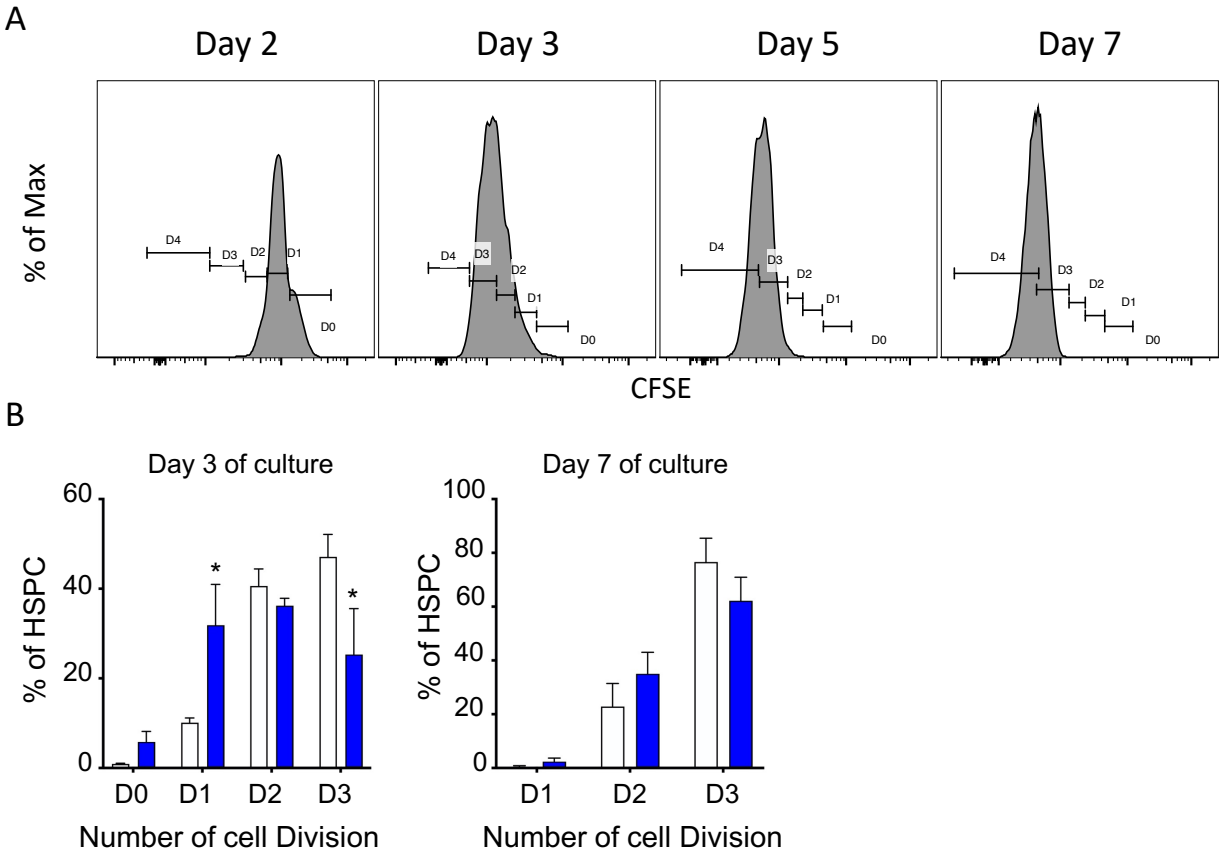



**Figure S4 related to Figure 4:** (A) Example of gate to distinguish cell divisions from day 2 to day 7 (B) Quantification of the % of HSC depending on cell division after 3 and 7 days of culture. 3 independent experiments. One-way ANOVA test, ** $p<0.01$

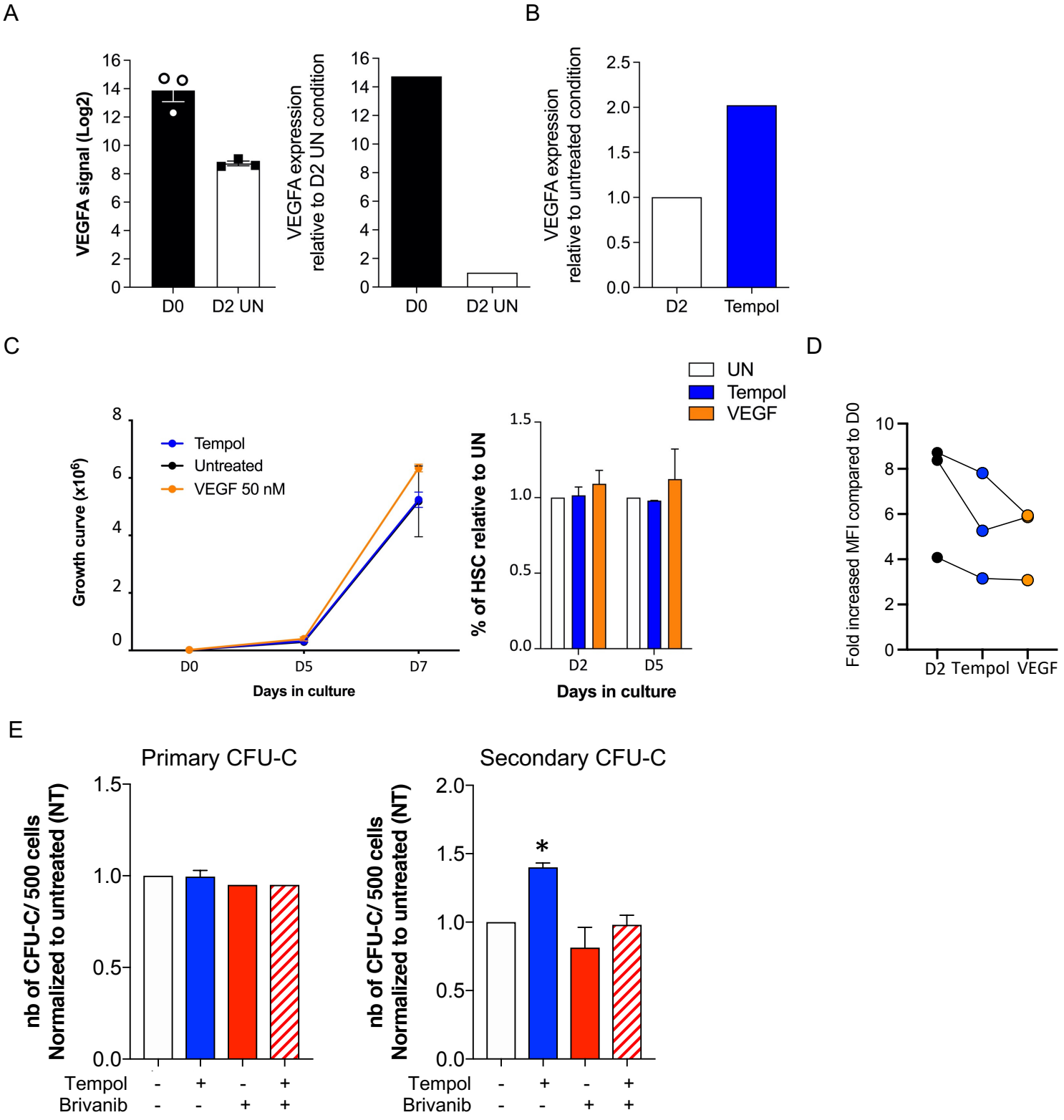



**Figure S5 related to Figure 5:** (A) VEGA expression in uncultured D0 HSC vs culture untreated D2 HSC, microarray signal (Left, n=3) and qPCR experiment (Right, n=1). (B) Validation of the microarray data by qPCR. (C) $CD34^+$ cells were first pre-treated with Tempol (blue), VEGFa (orange) or remained untreated (white) and then cultured several day as indicated. Curves representing the number of total cells (Left) and histograms indicating % of HSC normalized to % of HSC in untreated condition after 2 and 5 days of culture (Right) (D) ROS levels were measured at D0 and D2 of culture for each condition, here is represented the fold increased MFI of CellRox Deep RED probe at D2 compared to D0. 3 independents experiments using $CD34^+$ cells from 3 independent CB samples (E) sorted-HSC were first pre-treated with Tempol (blue), Brivanib (red), Brivanib and Tempol (hatched red bar) or remained untreated (white) then cultured for 2 days before being seeded in CFU-C medium Primary CFU-C (Left) and Secondary CFU-C (Right) were quantified 10-12 days later. 2 independent experiments.