SUPPORTING MATERIAL

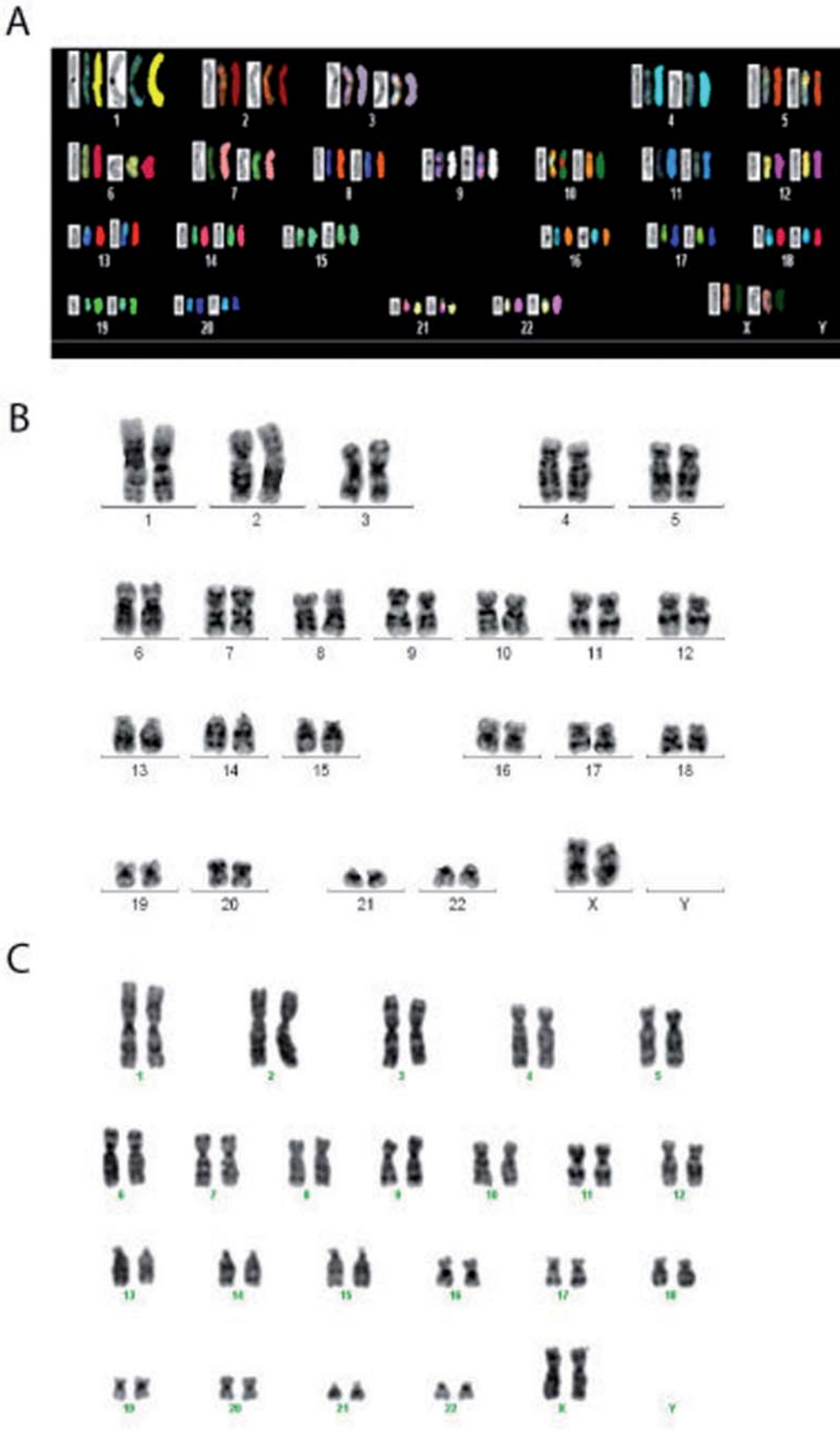
Figure S1: Karyotype stability.
Proliferating cells were incubated with Colcemid (2 hours at 37ºC) in order to arrest mitotic cells in metaphase. Cells were then shocked in a hypotonic solution (KCl 7,5 mM) and subsequently fixed with acetic acid 1: 3 methanol (at least 5 times) and dropped onto coverslips for chromosome staining. (A) SKY analysis of hVM1 cells, showing a normal karyotype, without any chromosomal translocation, duplication or abnormal chromosome number. (B-C) G-Banding karyotype of hVM1-ø and hVM1-high Bcl-X_L cell lines (the transplanted cell lines), showing a normal chromosomal distribution. Pictures are representative of 20 metaphases analyzed for each cell line, consistently giving the same results.

Figure S2: Bcl-X_L prevents AIF translocation from mitochondria to the nucleus during cell differentiation. Absence of effect of JNK inhibition on cell death.
A) ICC for AIF detection in dividing (left panels) or 3 days differentiated cells (right panels). hVM1-ø cells were also treated for the whole differentiation process with 50µM z-VAD-fmk. In dividing cells there is no AIF translocation to the nucleus. After differentiation, untreated or z-VD-fmk treated hVM1-ø displayed a nuclear localization of AIF whereas in Bcl-X_L cell lines AIF was localized in mitochondria. Nuclei were counterstained with TO-PRO. Scale bar=10µm.
B) Effect of SP600125 mediated JNK inhibition on cell death was analyzed by FACS quantification of DNA fragmentation (Sub G0-G1 area) in 3 days differentiated cells. Data represent mean ± S.E.M. of 3 independent experiments. No significant differences were found between 15µM SP600125 treated and untreated cells (-), neither in hVM1-ø nor in hVM1-high-Bcl-X_L cells.

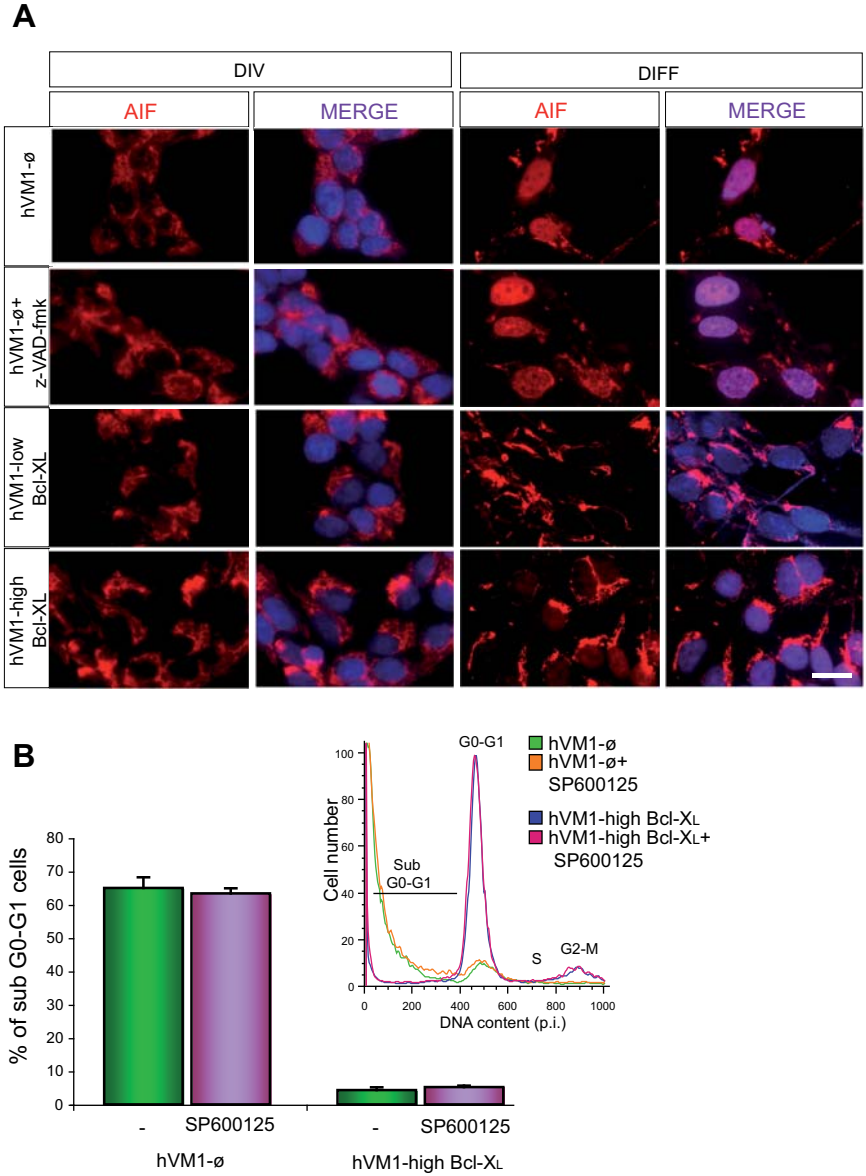
Figure S3: Mitochondrial membrane potential in differentiation hVM1 cells is preserved by Bcl-X_L.
$\Delta\Psi_{mit}$ was determined in dividing cells and in cells differentiated for 3 and 5 days (n=3 for each sample) using MitoprobeTM DilC1(5) and propidium iodide. All the cells were selected for the analyses (region R1 in panel A). The protonophore CCCP was used as a positive control for depolarized mitochondria, and Camptothecin (CA) for apoptosis, in dividing hVM-Ø-GFP cells (B). The decrese in $\Delta\Psi_{mit}$ in the viable cells (negative for PI, lower region of the plots) is denoted by arrows. Note the decrease in $\Delta\Psi_{mit}$ in hVM-Ø-GFP cells differentiated for 5 days (panel C, bottom, in 33% of the total number of cells).

Figure S4: TH stain does not co-localize with GABA, Glutamate or Serotonin.
ICC for TH, GABA, Glutamate (Glut) and 5-HT in seven days differentiated cells. Nuclei were counterstained with Hoechst. Scale bar=10µm.

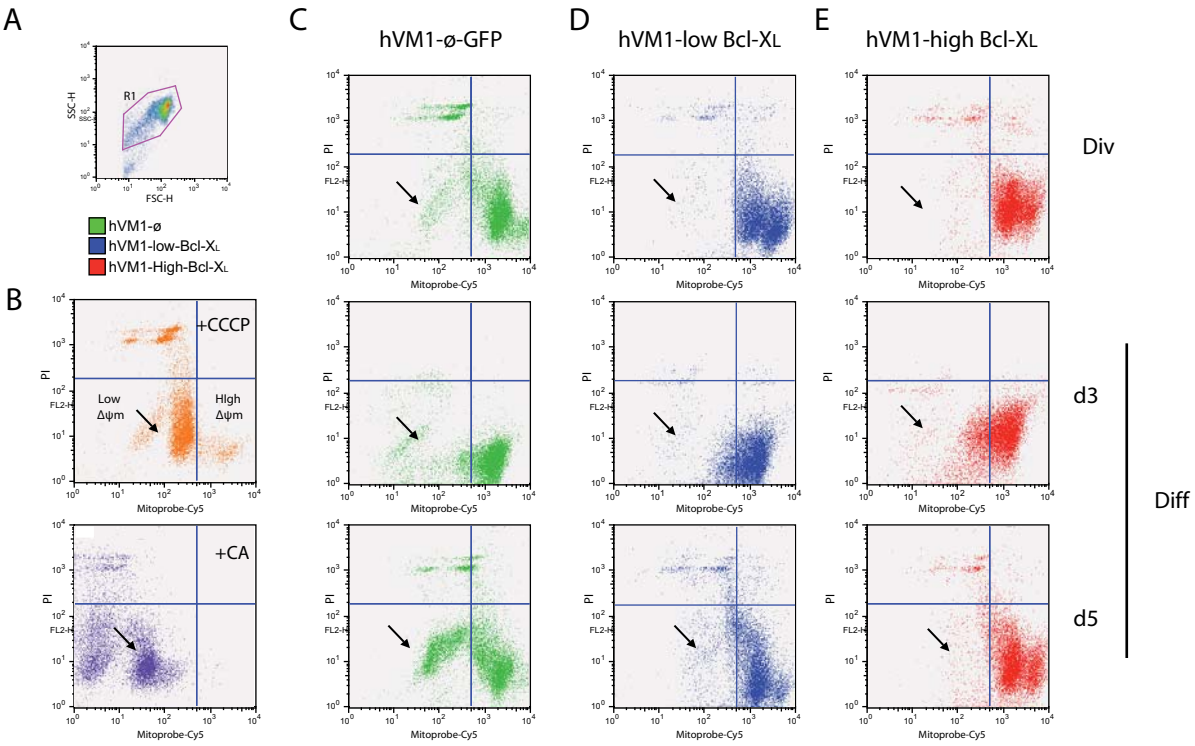
Figure S5: Cell cycle analysis of hVM1 derived stem cell lines under proliferation and differentiation conditions
Cellular phases of the cell cycle were studied by PI staining and FACS analysis in proliferating cells (Div) or after 3 and 7 days of differentiation (d3 and d7). **A)** Cells were FACS selected accorded to their size and complexity, gating only living cells for cell cycle analysis. Plots depict experimental data reported in the histograms in panels **B)** and **C)**. Cells in active mitosis were represented by those in S-G2-M phases of the cell cycle **(B)**. Quiescent cells were quantified as percentage of cells in G0-G1 phases of the cell cycle **(C)**. Note that after 3 days of differentiation, the number of proliferative cells (in S-G2-M phase) significantly decreased, increasing at the same time the number of cells in G0-G1 phases. Data represent mean ± S.E.M. of 3 independent experiments. ($p<0.05$, * *vs.* Div, ANOVA, post-hoc Fisher test).



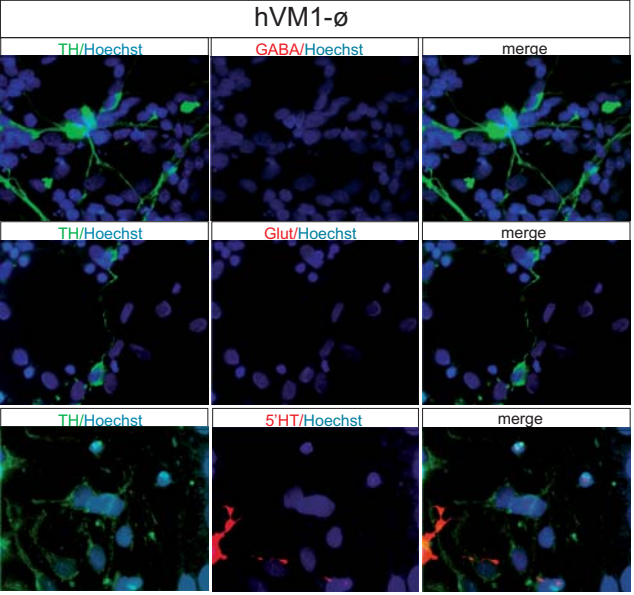
Courtois et al. Fig. S1

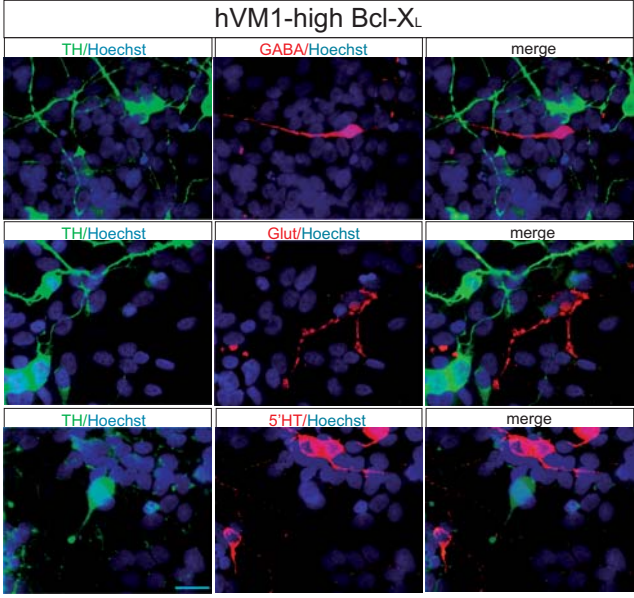


Courtois et al. Fig. S2

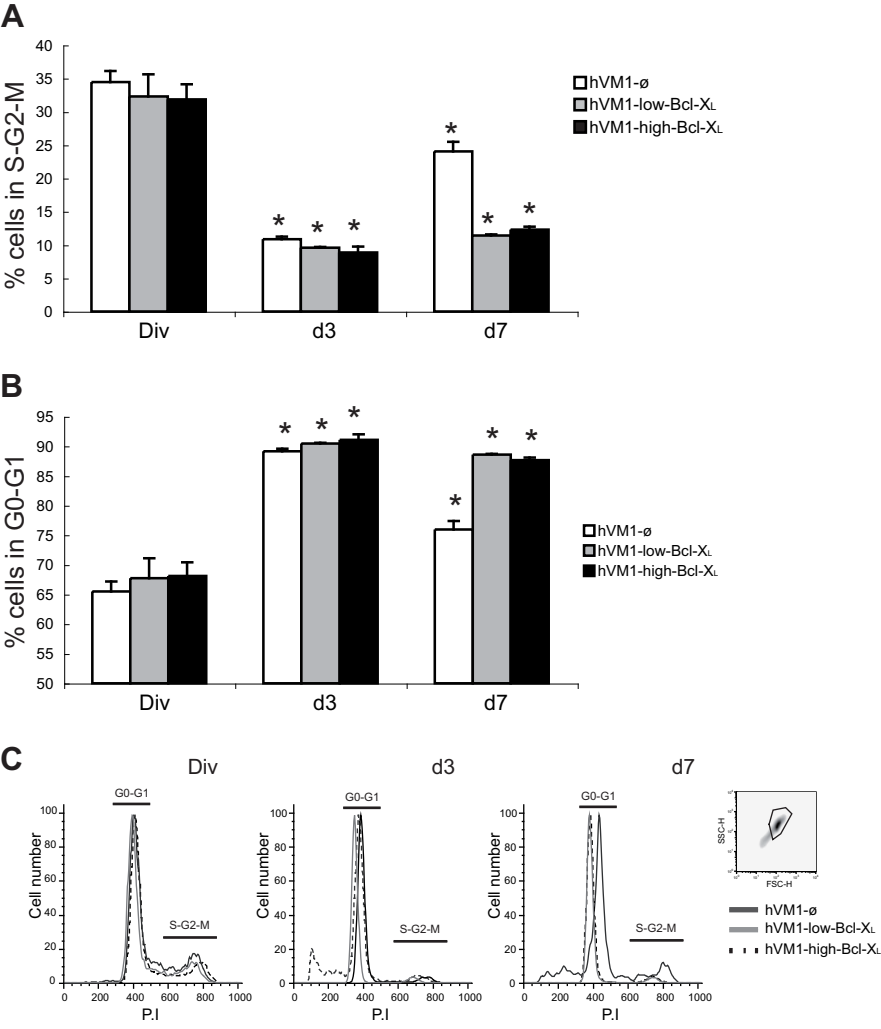


Courtois et al. Fig. S3





Courtois et al. Fig. S4



Courtois et al. Fig. S5