

Supplemental Data

[methyl-3H]-thymidine survival

Fibroblasts were plated in 6-well culture dishes (7500 cells per well) in quadruplicate (0 J/m²) or triplicate (others) in 3 ml medium. Two day after seeding, cells were washed with PBS and UV irradiated (0-8 J/m²; 254 nm Philips TUV lamp). Five days after irradiation cells were pulse-labeled with [methyl-3H]-thymidine (40-60 Ci/mmol; 5 μCi/ml; Amersham Biosciences), chased for 30 minutes in unlabeled medium, washed with PBS, lysed in 0.25 M NaOH and harvested. Cell lysates were transferred into scintillation flasks and supplemented with 7.5 ml Hionic Fluor scintillation fluid (Packard). Each sample was counted in the scintillation counter for 10 minutes and results were expressed as the percentage of counts obtained from the non-treated dishes (set as 100%).

Unscheduled DNA synthesis (UDS) assay

For UDS 1 x 10⁵ cells were seeded onto 24 mm cover slips and UV irradiated with 16 J/m² after 1 day. The cells were washed once with PBS and incubated for 3 hours in culture medium containing 10 μM 5-ethynyl-2'-deoxyuridine (EdU; Thermo Fisher Scientific). After EdU incorporation, cells were fixed in 4% formaldehyde/PBS, washed twice with 3% BSA/PBS, permeabilized for 20 minutes in 0.5% Triton/PBS and washed once with PBS. Cells were incubated for 30 minutes with fluorescent dye coupling buffer containing 10 mM CuSO₄ and Alexa Fluor 594 azide (Click-iT, Thermo Fisher Scientific). After washing with PBS, cells were mounted in vectashield, containing 1.5 μg/ml DAPI. UDS levels were expressed as a percentage of the average fluorescence intensity in the nucleus of wild-type cells, which was set at 100%. The mean fluorescence is determined with a confocal microscope (Zeiss LSM 700) from at least 50 cells. Images were processed using ImageJ.

Recovery of RNA synthesis (RRS) assay

For RRS 1×10^5 cells were seeded onto 24 mm cover slips and UV irradiated with 8 J/m^2 after 1 day. The cells were washed once with PBS and incubated for 2 h in culture medium containing $100 \text{ }\mu\text{M}$ 5-ethynyl-uridine (EU) at different time-points after UV (2 hours or 24 hours). After EU incorporation, cells were fixed in 4% formaldehyde/PBS, washed twice with 3% BSA/PBS, permeabilized for 20 minutes in 0.5% Triton/PBS and washed once with PBS. Cells were incubated for 30 minutes with fluorescent dye coupling buffer containing 10mM CuSO_4 and Alexa Fluor 594 azide (Click-iT, Thermo Fisher Scientific). After washing with PBS, cells were mounted in vectashield, containing $1.5 \text{ }\mu\text{g/ml}$ DAPI. RRS levels were expressed as a percentage of the average fluorescence intensity in the nucleus of non-irradiated wild-type cells, which was set at 100%. The mean fluorescence is determined with a confocal microscope (Zeiss LSM 700) from at least 50 cells. Images were processed using ImageJ.

Legends to Supplemental Figures

Figure S1. Characterization of DNA Repair capacity

(A) UV-survival assay measuring UV sensitivities in triplicate culture dishes of patient fibroblasts (TTD218UT, TTD251HE), NER-defective XP-A cells (XP25RO) and NER-proficient (wild-type) controls (C4RO, C5RO). (B) Global NER activities measured as UV-induced unscheduled DNA synthesis (UDS) using EdU incorporation, visualized by fluorescence-conjugated azide (Click-iT assay). Shown are representative pictures from the UDS experiment performed on primary fibroblasts of TTD218UT (left panel) or TTD241HE (right panel) and compared to C5RO primary fibroblasts. UDS-derived fluorescence is shown in red and nuclear staining in blue (DAPI). (C) Quantification of the UDS experiments. Mean intensities of at least 50 nuclei are expressed as percentages of those in normal cells assayed in parallel. The error bars indicate SEM.

Figure S2. Characterization of recovery of RNA Synthesis

Transcription-coupled NER activities measured as recovery of RNA Synthesis (RRS) in UV-exposed primary fibroblasts using EU incorporation and subsequent Click-iT Assay. (A-B) Shown are representative pictures at the indicated time points (time of EU pulse-labeling after UV irradiation) from the RRS experiment performed on primary fibroblasts of TTD218UT (A) or TTD241HE (B) and compared to C5RO primary fibroblasts. RRS-derived fluorescence is shown in red and nuclear staining in blue (DAPI). (C) Quantification of the EU-incorporation (measure for RNA synthesis) at the indicated time points after UV. Mean intensities of at least 50 nuclei are expressed as percentages of those in normal cells. The error bars indicate SEM.

Figure S3. TFIIIE protein level is reduced in TFIIIE β -mutated TTD cells.

(A-C) Immuno-fluorescence analysis of TTD218UT_{sv} and wild-type control (C5RO_{sv} and MRC5_{sv}) cells, stained for (A) TFIIIE β , (B) TFIIIE α or (C) XPB (TFIIH) and DNA was stained with DAPI (blue). Quantification of the mean intensities (n=50 nuclei), expressed as percentage of the mean intensity in normal cells, is shown beneath the representative images. Error bars indicate SEM. (D) Immuno-blot analysis to determine TFIIIE protein levels of Sv40-immortalized patient cells (TTD218UT_{sv}) compared to two wild-type controls (MRC5_{sv}, C5RO_{sv}). (E) Quantification of the immune-blot. The band intensities of TFIIIE β or TFIIIE α were normalized to Tubulin and expressed as percentage of control cells.

Figure S4. TFIIIE β complementation in TTD218UT_{sv} cells

Shown are representative pictures of an immuno-fluorescence experiment performed with MRC5_{sv}, C5RO_{sv}, TTD218UT_{sv} and TTD218UT_{sv} cells complemented with TFIIIE β ^{WT}-GFP. Cells were fixed and stained for (A) TFIIIE β (B) TFIIIE α , and DNA was stained with DAPI. Quantification of the immuno-fluorescence experiments is shown below the images. Mean intensities of at least 50 nuclei are expressed as percentages of those in normal cells, is shown beneath the representative images. The error bars indicate SEM. TFIIIE β ^{WT}-GFP expressing cells are indicated under the images marked with GFP.

Figure S5. Transcription levels in primary fibroblasts at 37°C and 40°C

(A) Overall transcription in patient fibroblasts TTD218UT (middle panel) and TTD241HE (bottom panel) and wild-type control cells, C5RO (top panel). Cells were cultured for 72 h at 37°C or 40°C and transcription levels were measured by incorporated ethynyl-uridine using a Click-iT assay. (B) Quantification of the transcription experiment. Mean intensities of at least

50 nuclei are expressed as percentages of the mean intensity in normal cells. The error bars indicate the SEM.

Figure S6. iPSC erythroid differentiation

In vitro erythroblast differentiation as performed and shown in Figure 3A were performed in triplicate. Shown are representative pictures of the second (left panel) and third experiment (right panel) of cytopins and Giemsa-Grünwald staining. Arrows indicate multinucleated cells. Quantification of multinucleated cells are shown in Figure 3B.

Figure S7. iPSC erythroid specification and differentiation

iPS cells, derived from TTD218UT (TTD) fibroblasts, control fibroblasts (control F) and peripheral blood mononuclear cell (control E) were differentiated to erythroblasts, which were further expanded and subsequently differentiated to poly/orthochromatic normoblasts as indicated in material and methods. (A) **Cells** were harvested as suspension cells from the iPSC colony differentiation after 12 days. Panels represent flow cytometry dot plots from 2 independent experiments depicting CD71 versus CD235 (or GPA) to assess the purity, production and differentiation status of erythroid cells as described before (1) at the day of harvest (day 0, upper panels) and after 7 days (**lower panels**) of erythroblast expansion on Epo, SCF and Dex using a culture system as described before (2). CD71+CD235+ cells are defined as erythroid cells. Note that at the day of harvest (day 0) erythroid cells are identified but purity is still low. However, after 7 days of expansion the cells are primarily CD71+/CD235+ erythroblasts (>93%) and contaminating non-erythroid cells are limited. (B) **Cytopins** of the 7 days expanded cultures in A were stained with Giemsa/Max Grünwald and benzidine (haemoglobin stains brown). A low seeding cytopin and high seeding cytopins are depicted for control (upper panels) and TTD (lower panels), which look similar in all

aspects. Note the absence of the multinucleated and cell size phenotype in the TTD sample as observed later in poly/orthochromatic cells during terminal differentiation (see main text). (C) Expanded erythroblasts of A and B were terminally differentiated for 3 days as indicated in material and methods at 37°C and 39°C as depicted. Erythroblast differentiation is accompanied with decreased expression of CD71 but maintained expression of CD235 and absence of CD34 and CD36. Flow cytometry dot plots depict CD71/CD235 in upper panels and CD34/CD36 in the lower panels. **Please note** the decreased expression of CD71 compared to figure A, indicating differentiation which is similar between control and TTD (patient). CD34 and CD36 are absent at this stage (lower panels) and no differences were observed between control and TTD samples. (D) Erythroid cells after 3 days of differentiation were pelleted and photographed to show hemoglobinization, which was comparable between control and TTD at both 37°C and 39°C. These data indicate that erythroid specification of iPSC lines is not blocked and that differentiation of erythroblasts can progress normally as defined by marker expression.

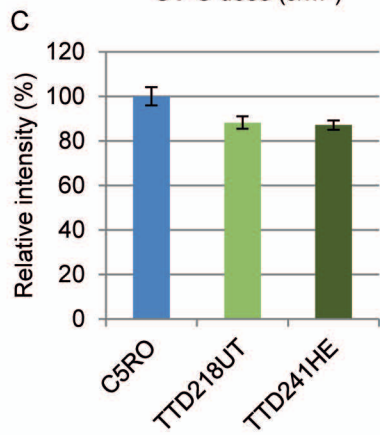
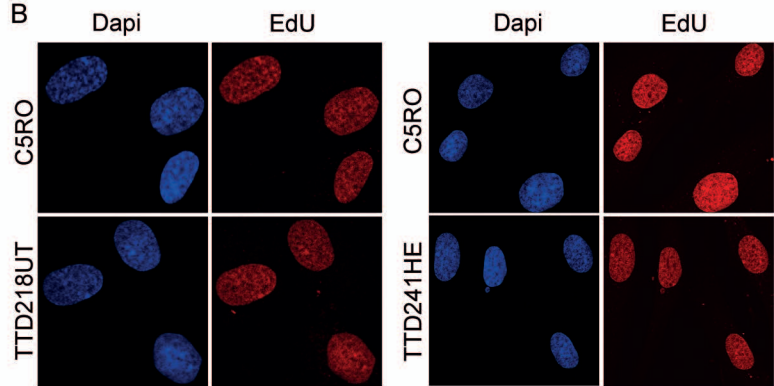
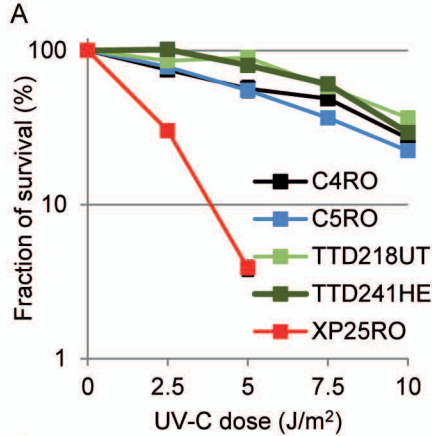
Figure S8. Hemoglobinization in erythroid cells

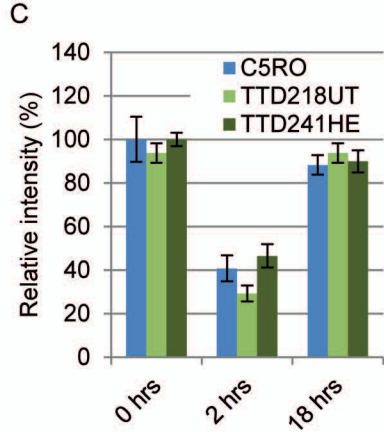
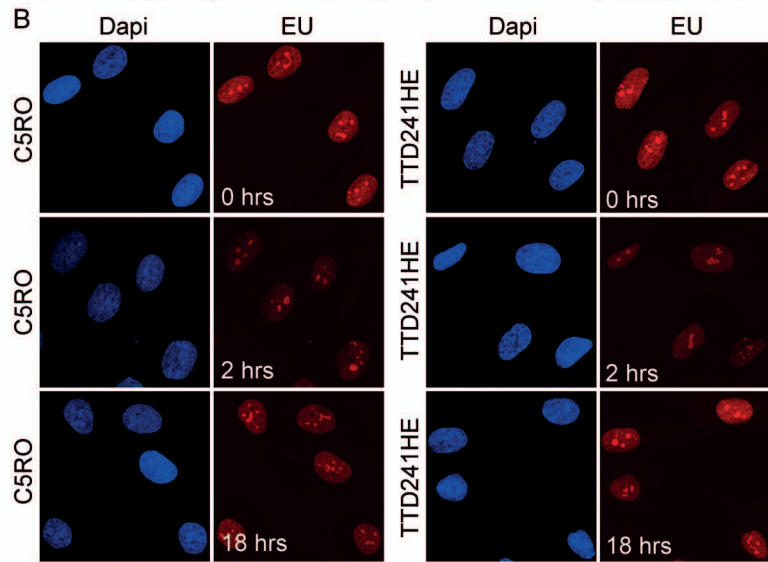
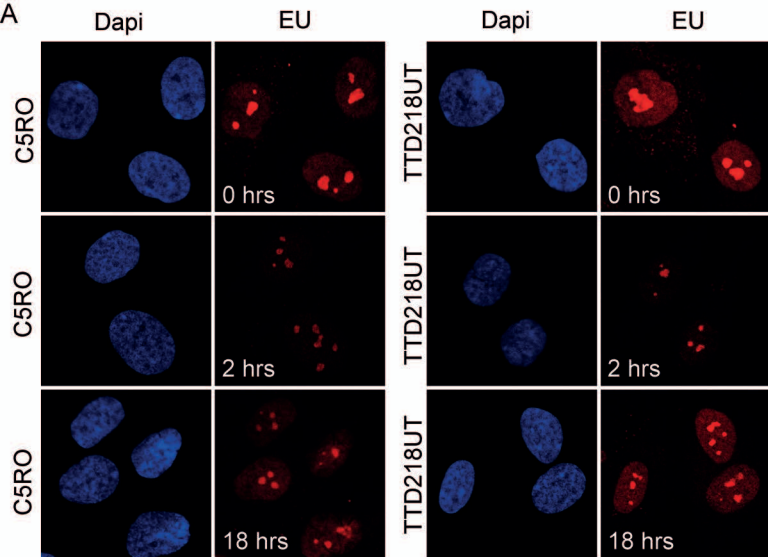
HPLC analysis from 3 independent experiments of TTD-derived erythroid cells and control cells (Control E; iPS-derived from blood). HPLC graphs and corresponding hemoglobin peakcalling were obtained as indicated in material and methods. The called peaks depicted as bar graphs shown in the main Figure 3D as percentages of hemoglobin variants, are indicated in the HPLC.

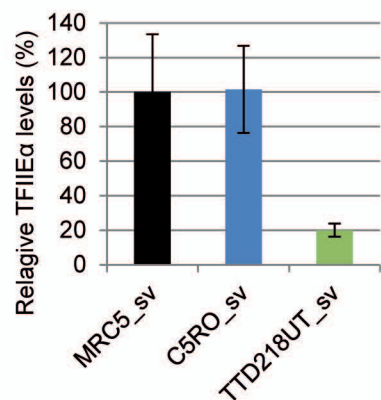
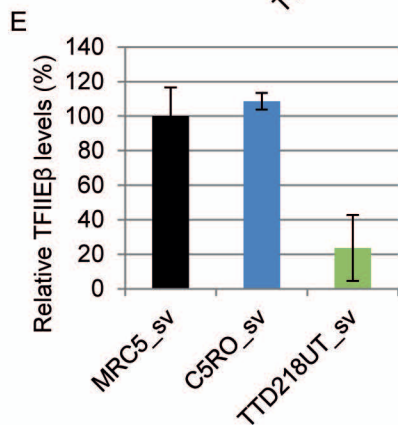
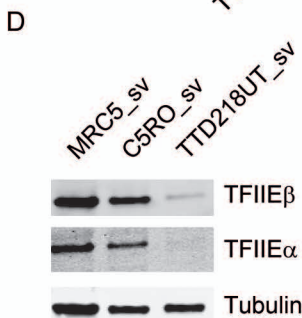
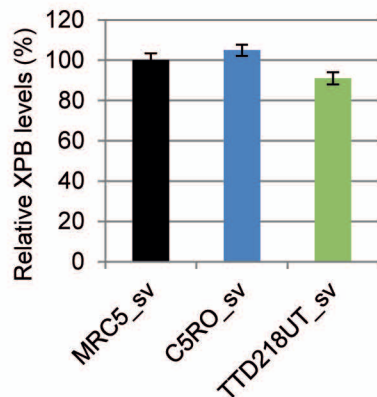
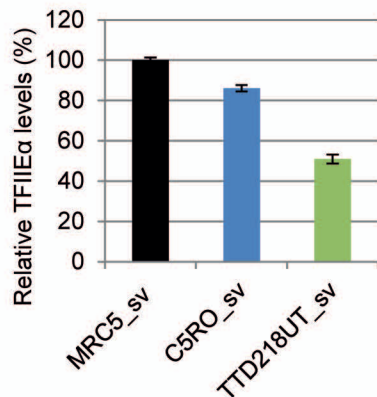
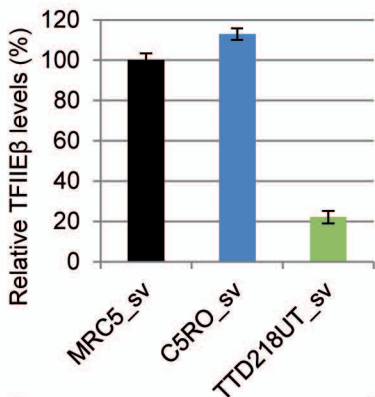
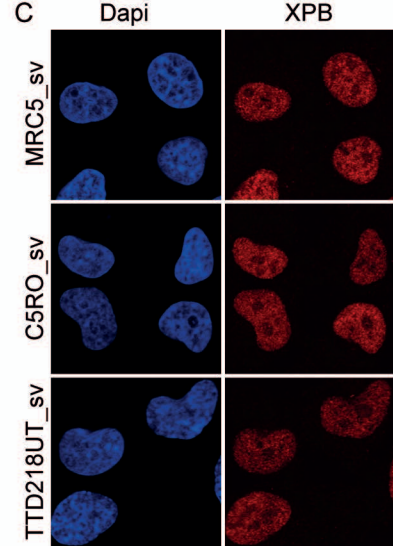
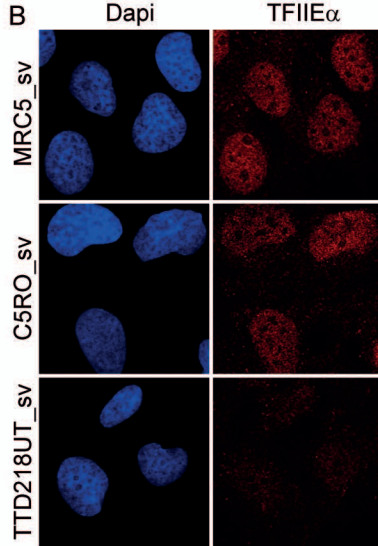
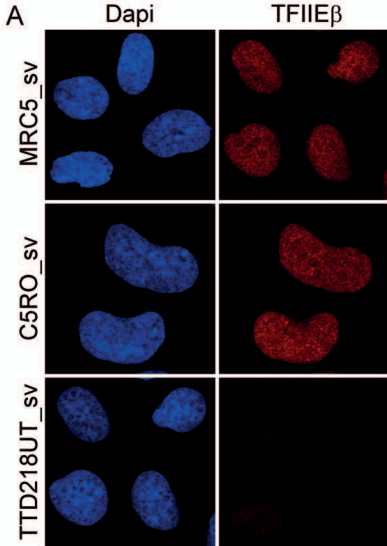
References

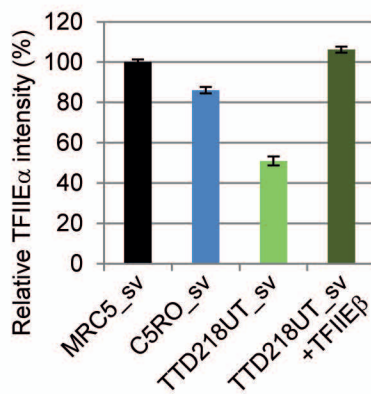
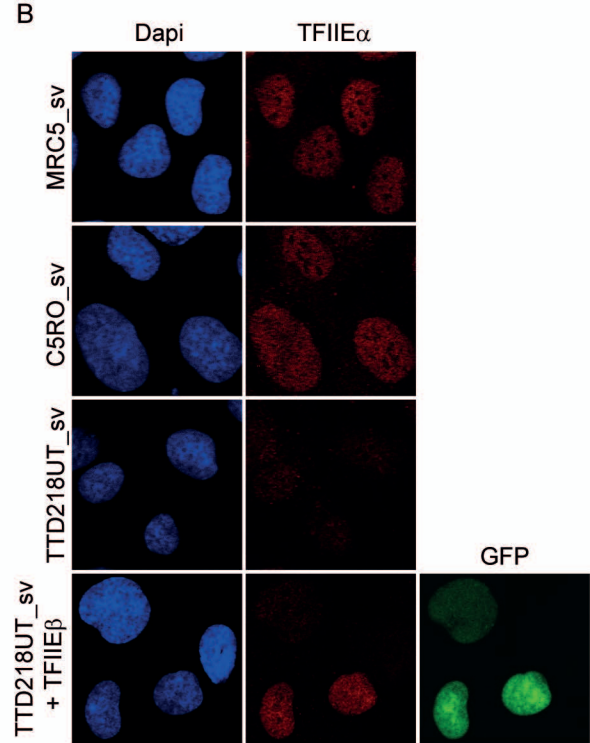
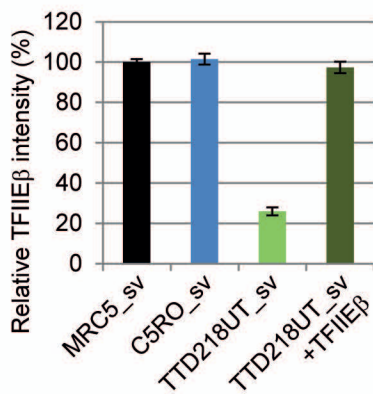
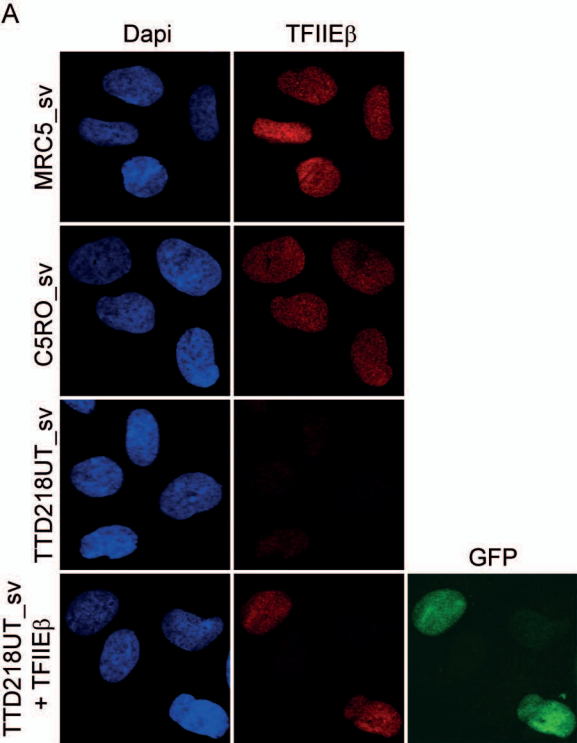
1. Socolovsky M, *et al.* (2001) Ineffective erythropoiesis in *Stat5a(-/-)5b(-/-)* mice due to decreased survival of early erythroblasts. *Blood* 98(12):3261-3273.
2. van den Akker E, Satchwell TJ, Pellegrin S, Daniels G, & Toye AM (2010) The majority of the in vitro erythroid expansion potential resides in CD34(-) cells,

outweighing the contribution of CD34(+) cells and significantly increasing the erythroblast yield from peripheral blood samples. *Haematologica* 95(9):1594-1598.

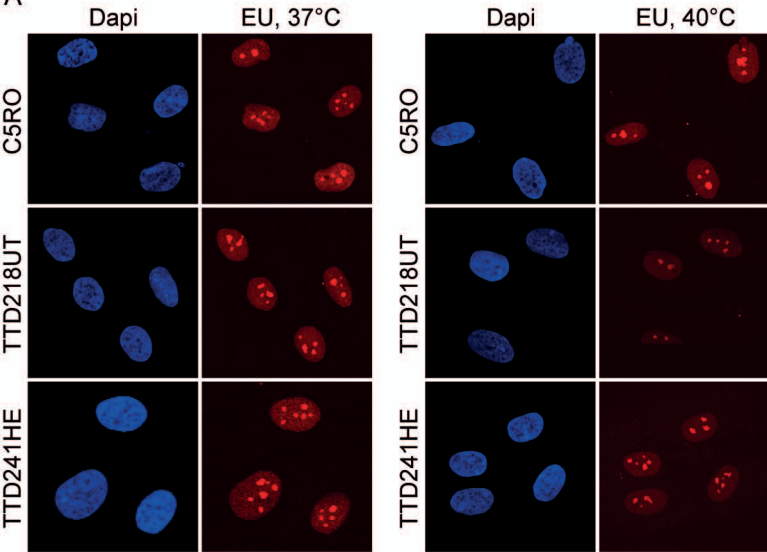




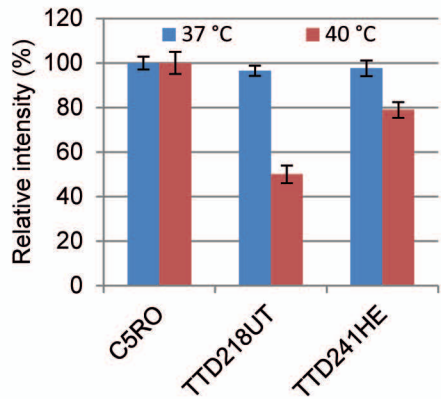




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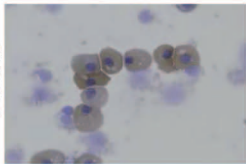


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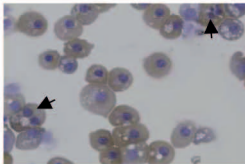


37°C

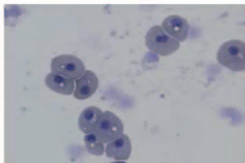
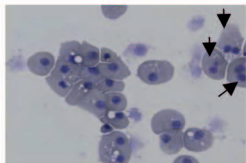
Control



37°C

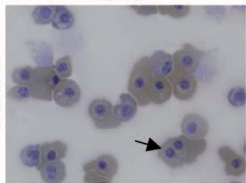


TTD

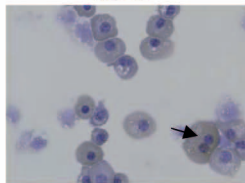


39°C

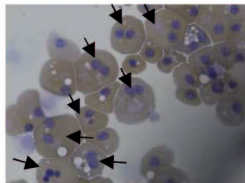
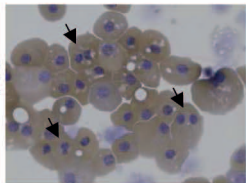
Control



39°C

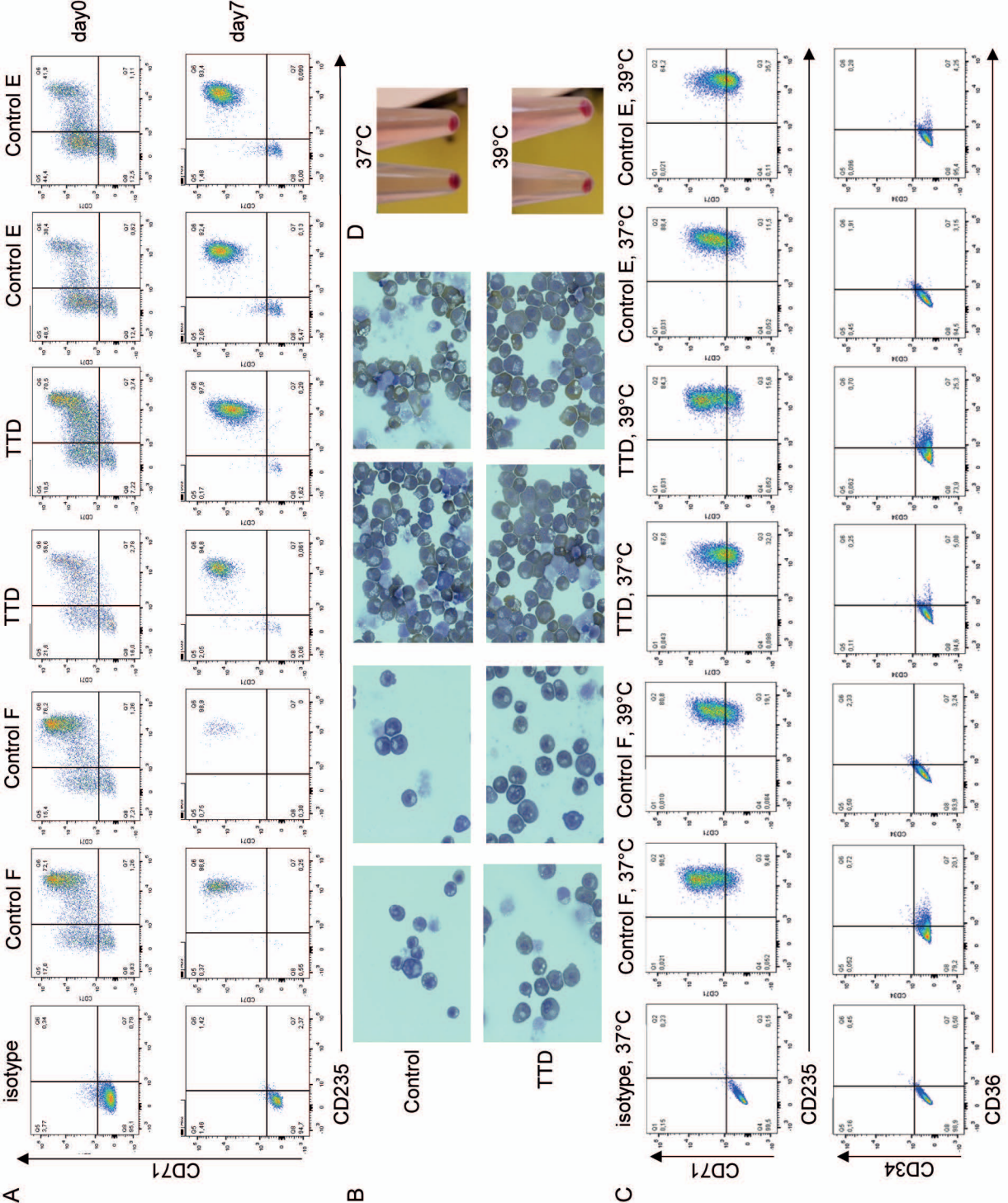


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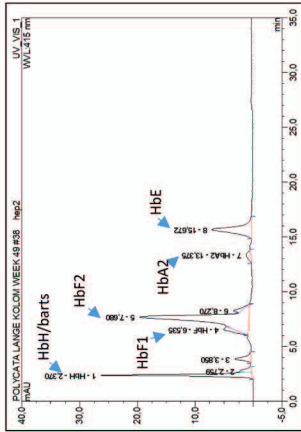


Experient 2

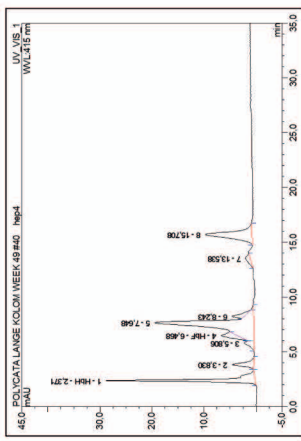
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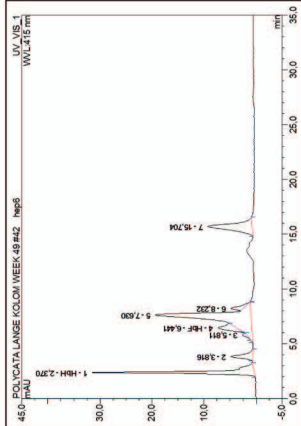
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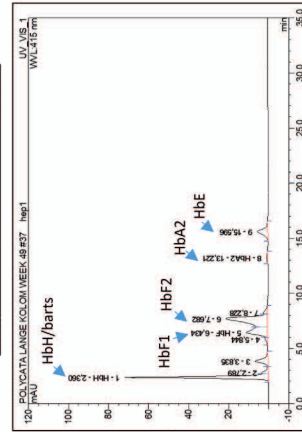
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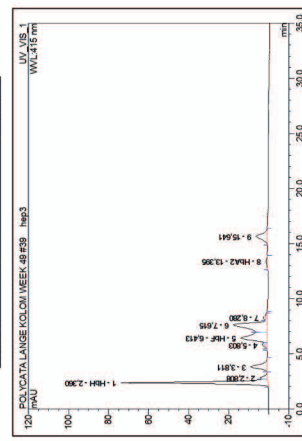
Control E, Experiment 3



TTD, Experiment 1



TTD, Experiment 2



TTD, Experiment 3

