LETTER TO THE EDITOR

Optimizing CRISPR methodology for precise gene editing in the erythroid cell line BEL-A with high efficiency generation of a sickle cell anemia model

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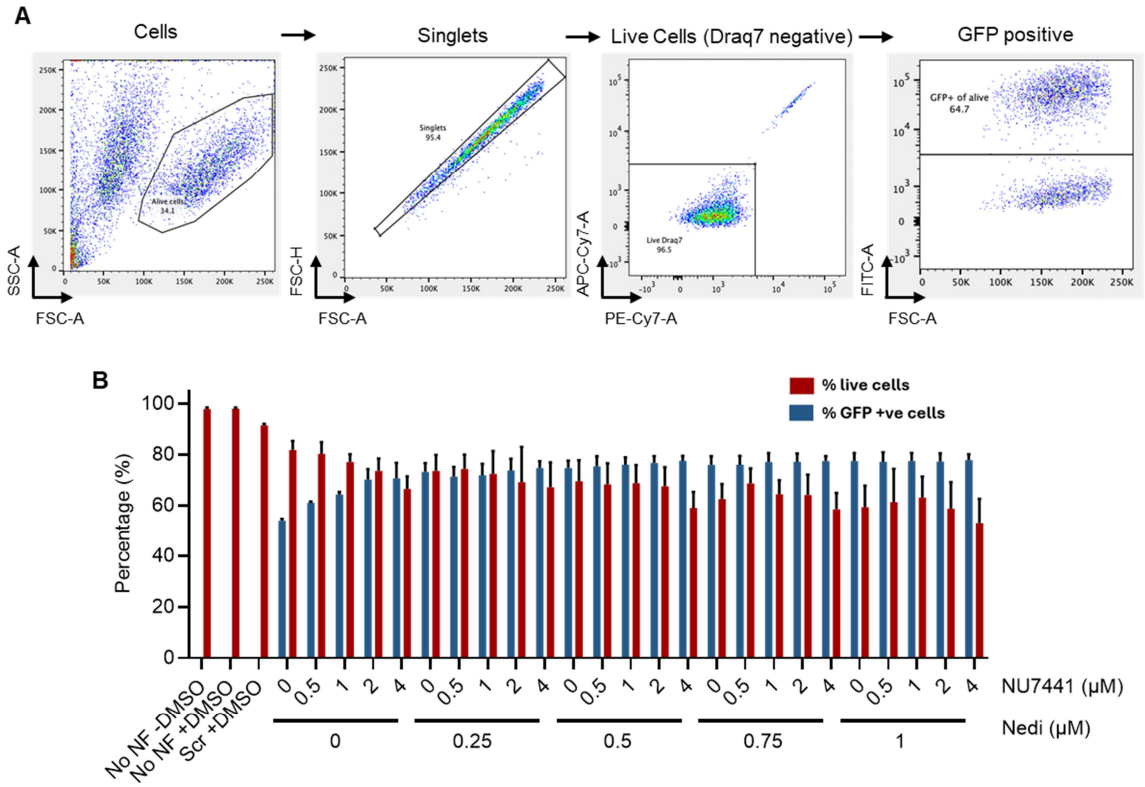
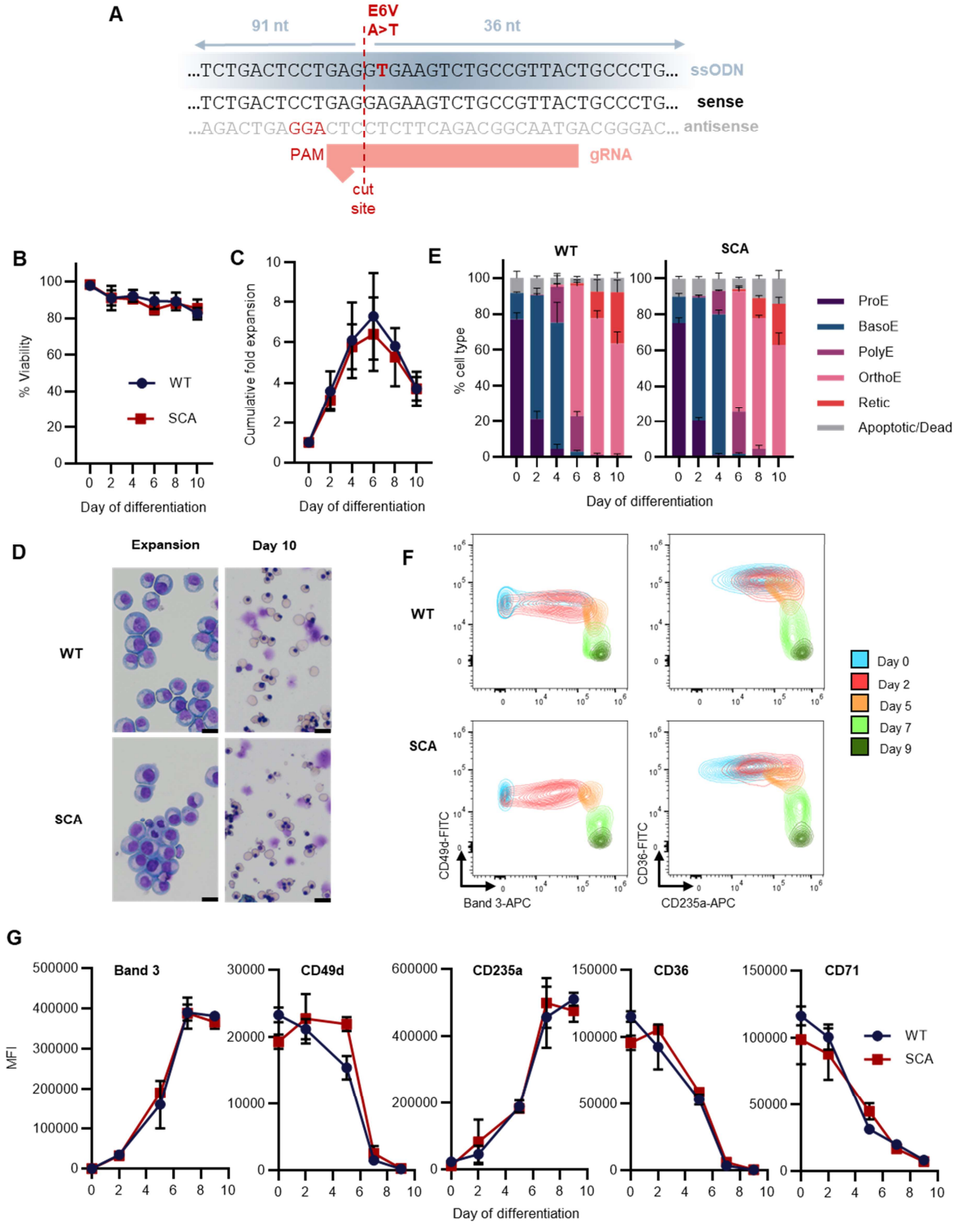
Supplementary Figure 1



Supplementary Figure 1: (A) Gating strategy for GFP to BFP fluorescent conversion assay**.** The proportion of cells exhibiting GFP fluorescence (GFP positive; GFP +ve) corresponds to the editing efficiency via HDR, reflecting successful incorporation of the desired edits. Events (<10,000 per samples) were gated to identify live single cells, with the GFP +ve events calculated as a proportion of this population. **(B)** Trialling a combination of NU7441 and Nedisertib (Nedi) small molecule HDR enhancers. Cells were cultured with small molecules for 24 hours following transfection. The percentage of live cells was quantified using DRAQ7 staining, and the percentage of GFP-positive (GFP +ve) cells was calculated as a proportion of live cells. Results show mean ± SD, *n* = 2 independent experiments. All conditions used 3 µg Cas9, a gRNA to Cas9 ratio of 1:2.5, 100 pmol of template and 5 × 10^4 cells in buffer P3 (Lonza). NF; Nucleofection. Scr; Scrambled gRNA control.

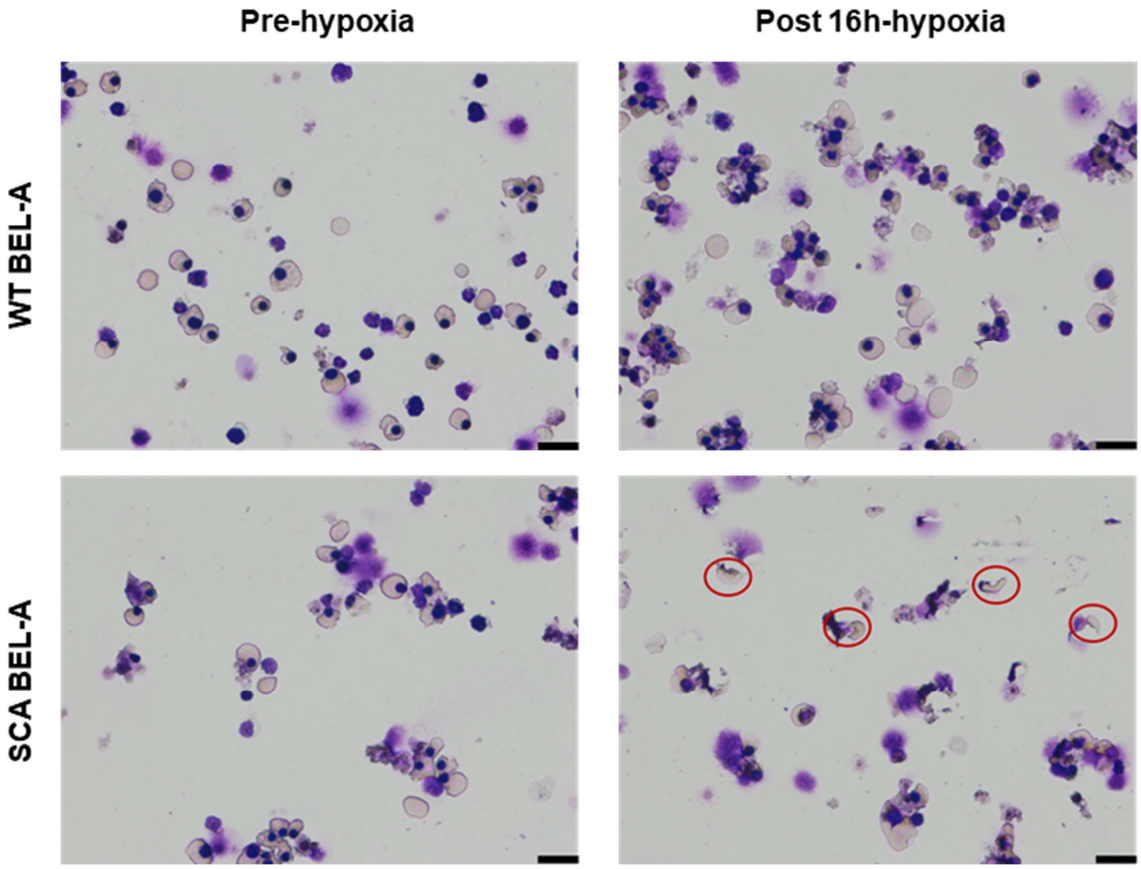
Supplementary Figure 2



Supplementary Figure 2: Generation and characterisation of SCA BEL-A. (A) Schematic of PGE strategy to introduce the E6V A>T SCA (sickle cell anemia) mutation in BEL-A cells. **(B)** Percentage viability and **(C)** Cumulative fold expansion curves of WT (wildtype) and SCA BEL-A during erythroid differentiation. **(D)** Representative Leishman's stained cytospin images of expanding and day 10 differentiated WT and SCA

BEL-A cells, scale bars 20 μm. **(E)** Erythroid differentiation morphology quantification (ProE, proerythroblast; BasoE, basophilic erythroblast; PolyE, polychromatic erythroblast; OrthoE, orthochromatic erythroblast; Retic, reticulocyte). ≥200 cells counted per cytospin. **(F)** Representative flow cytometry plots tracking cell surface marker levels during WT and SCA BEL-A differentiation. Cells were dual stained with anti-CD36 or anti-α4-integrin FITC conjugates (both Miltenyi Biotec) and anti-band 3 (BRIC71) or anti-glycophorin A (BRIC256) primary antibodies (both IBGRL, Filton) used in conjunction with an IgG1 APC secondary (Biolegend). **(G)** Median fluorescence intensity (MFI) of Band 3, alpha 4 integrin (CD49d), glycophorin A (CD235a), CD36 and CD71 during WT and SCA BEL-A differentiation. Results show mean ± SD, n=3. PAM; protospacer adjacent motif.

Supplementary Figure 3



Supplementary Figure 3: Hypoxia treatment of WT and SCA BEL-A. Representative Leishman's stained cytospin images of WT (wildtype) and SCA (sickle cell anemia) BEL-A cells pre- and post-16-hour hypoxia exposure to identify irreversibly sickled reticulocytes (red circles). Scale bars 20 µm.