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Supplementary information for Optimized Method for Studying Inflammasome Activation in Human Monocytes/Macrophages Derived From CRISPR-Engineered Cord Blood CD34$^+$ Progenitors

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1. Nucleofection programs EO-100, DZ-100, and ER-100 were evaluated using the pmaxGFP control plasmid supplied with the Lonza 4D-Nucleofector kit. Cells nucleofected without pmaxGFP showed no detectable green fluorescent protein (GFP)-positive cells and served as the negative control. Increasing GFP expression was accompanied by reduced live-cell recovery. After 24 h, EO-100 produced the highest overall live cell recovery (36.1%) but the lowest nucleofection efficiency (49.4%). DZ-100 showed intermediate live-cell recovery (20.6%) and nucleofection efficiency (72.5%), whereas ER-100 produced the highest nucleofection efficiency (95.8%) but the lowest live-cell recovery (6.7%) (Figure S1). These results demonstrate the trade-off between cell recovery and nucleofection efficiency across the three programs. Based on its balance between live cell recovery and nucleofection efficiency, DZ-100 was selected for subsequent CRISPR/Cas9 editing experiments.

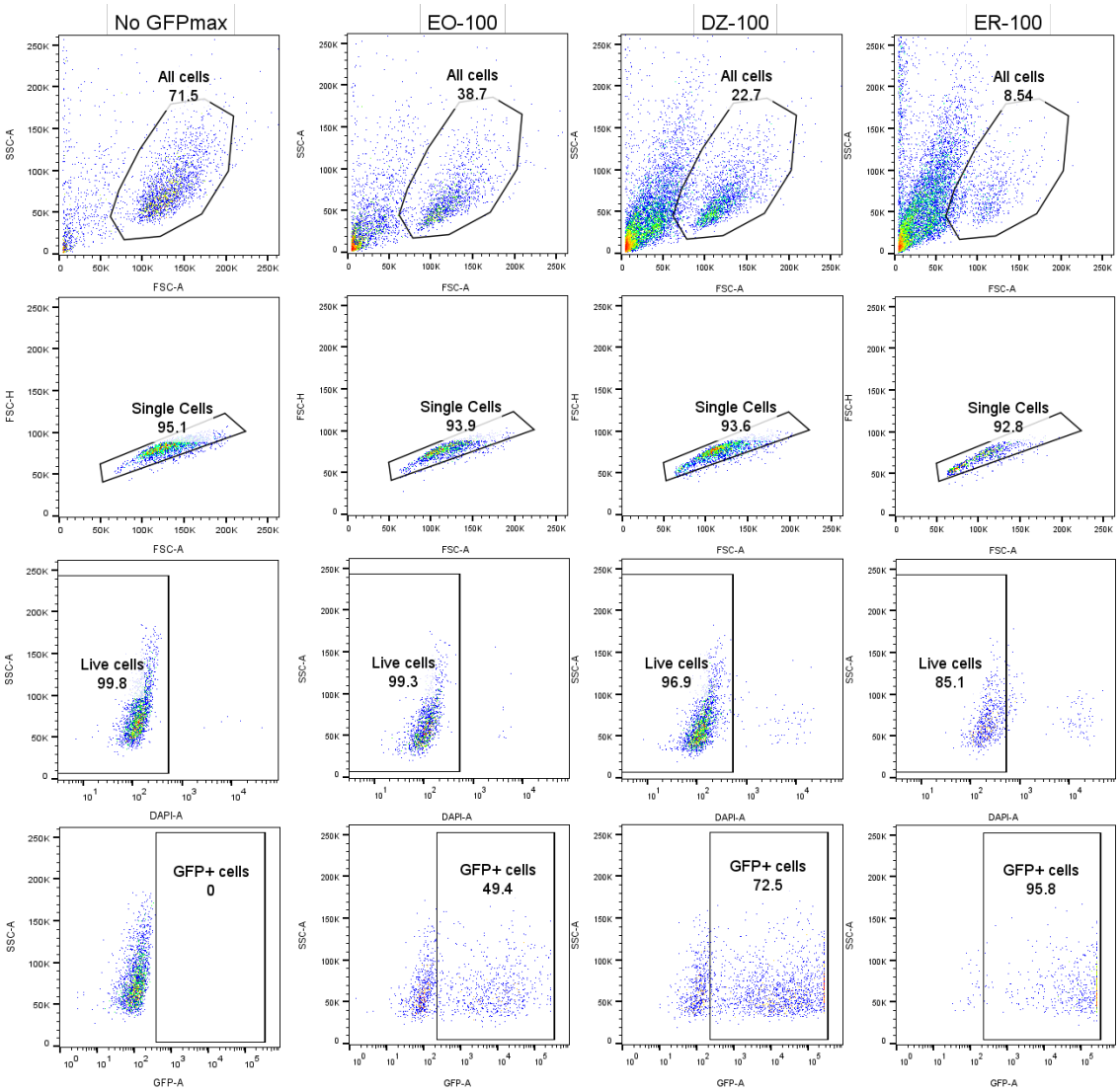


Figure S1. Optimization of nucleofection programs for human CB CD34$^+$ HSPCs using pmaxGFP. Representative flow cytometry plots comparing the EO-100, DZ-100, and ER-100 nucleofection programs using the pmaxGFP control plasmid supplied with the Lonza 4D-Nucleofector kit. Cells nucleofected without pmaxGFP were included as a negative control. Events were sequentially gated to identify intact cells based on forward-scatter area (FSC-A) and side-scatter area (SSC-A), singlets based on FSC-A and forward-scatter height (FSC-H), DAPI-negative live cells, and GFP-positive cells. Values within each gate represent the percentage of the corresponding parent population. Overall live cell recovery was calculated as the percentage of DAPI-negative singlets among all acquired events. Nucleofection efficiency was determined as the percentage of GFP-positive cells among DAPI-negative live singlets.

2. Editing of the *AAVS1* control population was assessed by PCR, Sanger sequencing, and TIDE analysis. PCR amplification generated products of approximately 557 bp in both *AAVS1*-edited and *TET2*-edited samples (Figure S2A). Alignment of forward and reverse Sanger sequencing reads identified a single-base deletion in the *AAVS1*-edited population relative to the *TET2*-edited population, which served as the unedited reference at the *AAVS1* locus (Figure S2B). TIDE analysis estimated an overall editing efficiency of 87.4%, predominantly comprising a 1-bp deletion with an estimated frequency of 85.6% ($R^2 = 0.94$; Figure S2C).

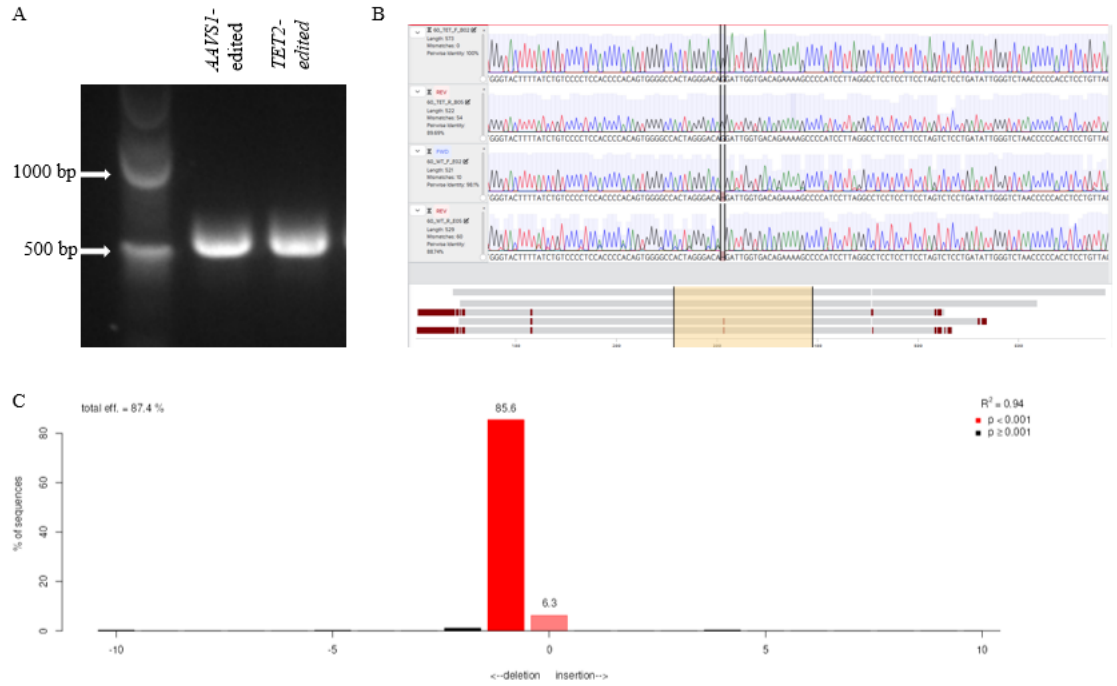


Figure S2. Validation of CRISPR/Cas9-mediated editing at the *AAVS1* locus. (A) Representative agarose gel showing PCR products spanning the CRISPR/Cas9 target site at the *AAVS1* locus. Both *AAVS1*-edited and *TET2*-edited cells produced amplicons of approximately 557 bp. (B) Representative Sanger sequencing chromatograms and sequence alignment of the *AAVS1* target region using Benchling. The upper two chromatograms show the *TET2*-edited control sequence, obtained using the forward and reverse sequencing primers, respectively. The lower two chromatograms show the bulk *AAVS1*-edited population, obtained using the forward and reverse sequencing primers, respectively. Alignment of these sequences identifies a single-base deletion, indicated between the two black vertical lines. (C) TIDE analysis estimated an overall editing efficiency of 87.4%, with a 1-bp deletion accounting for 85.6% of sequences ($R^2 = 0.94$).