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Supplemental Information

Flow-Cytometry Platform for Intracellular Detection of FVIII in Blood Cells: A New Tool to Assess Gene Therapy Efficiency for Hemophilia A

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Figure S1

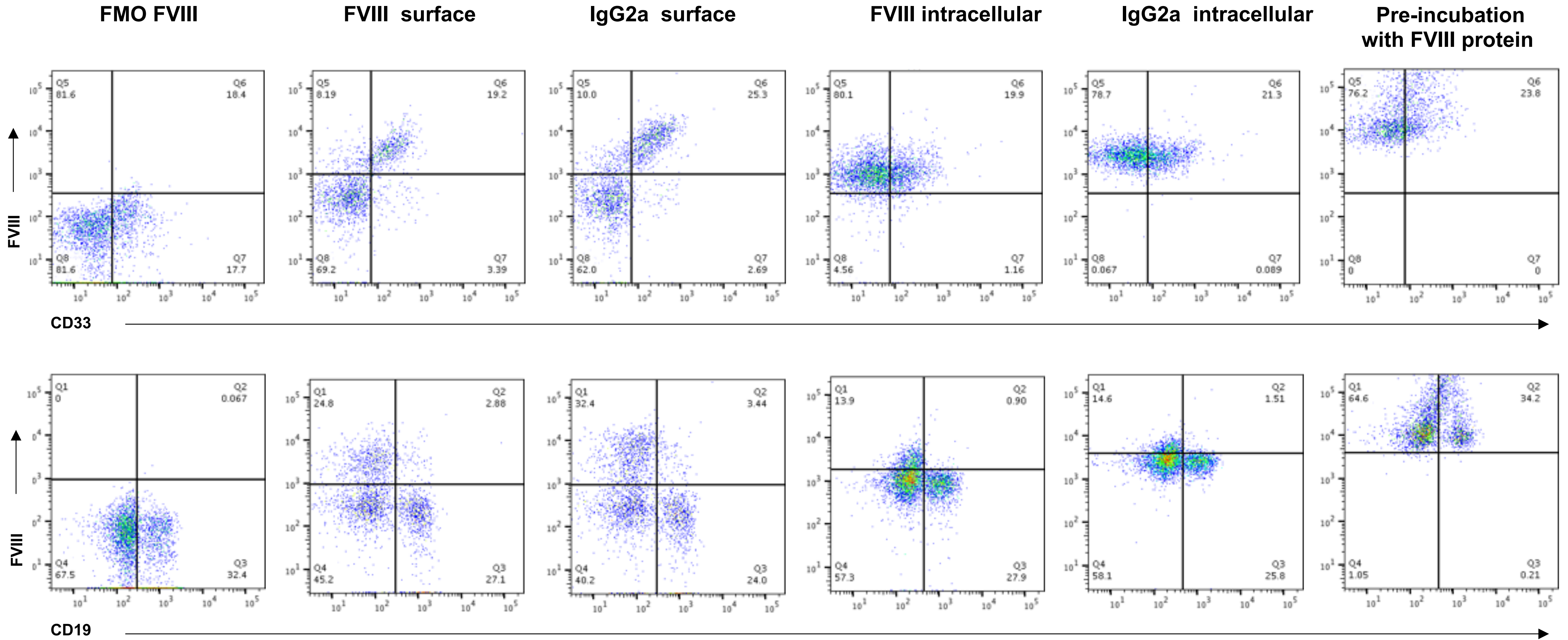


Figure S1. FVIII PBMC staining and evaluation of protein unspecific binding

Fresh PBMCs were stained with IgG2a Dylight custom-labelled anti FVIII GMA®Ab 8002 anti A1 domain, or with an unspecific Dylight custom–labelled IgG2a on the surface and intracellularly. Sub-phenotypes CD19 and CD33 are displayed. Intracellular staining was repeated after co-incubation with FVIII recombinant protein, 15’ at RT (last column right).

Figure S2

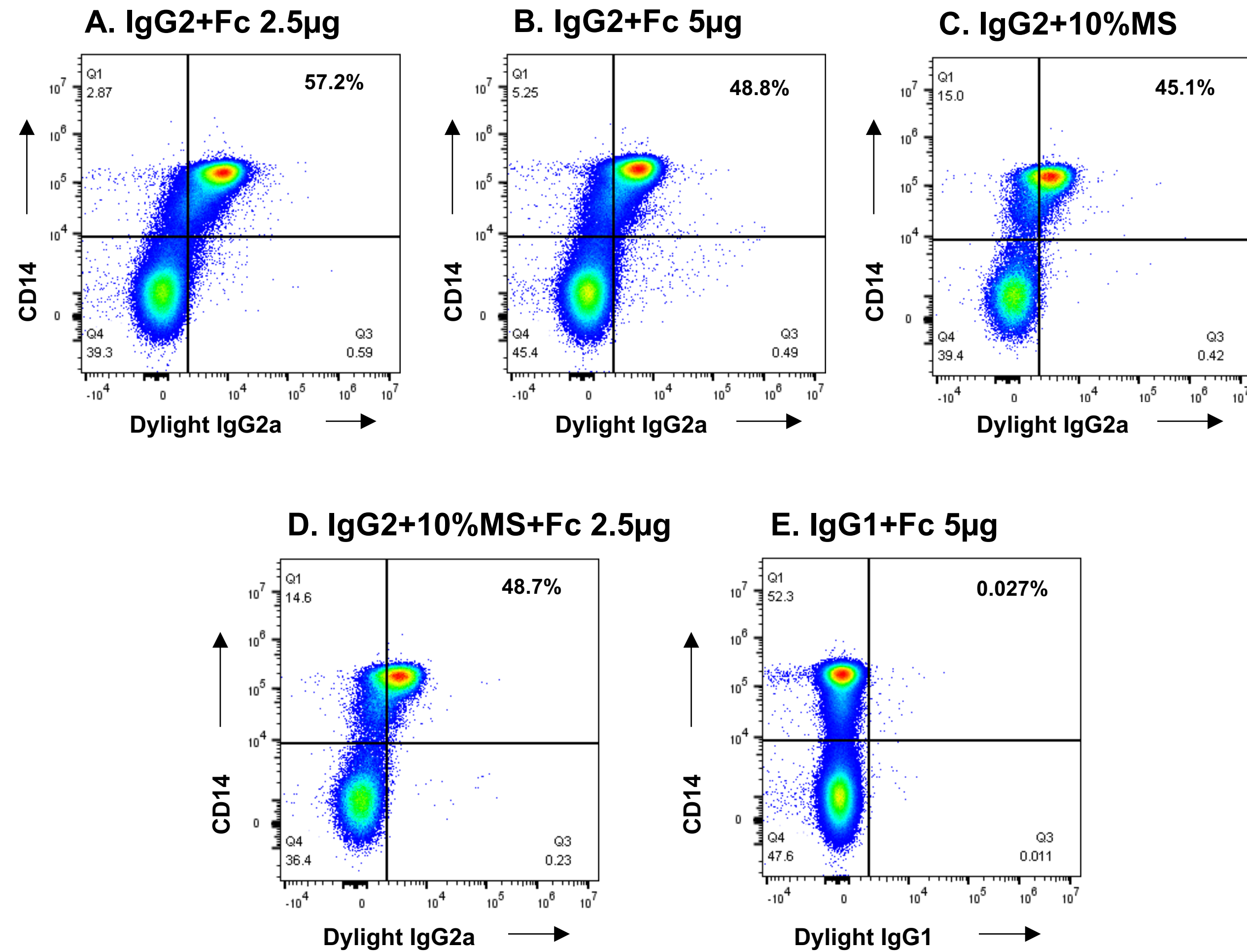


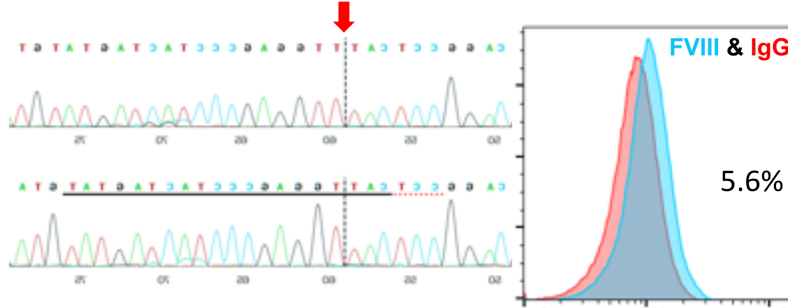
Figure S2. Fc Block Versus Mouse serum block and IgG2a vs IgG1 staining on human peripheral CD14+ cells

FC experiment using Dylight 650 NHS Ester custom-labelled antibodies for comparison of different blocking types: comparing the efficacy of different concentrations of Fc blocking (**A-E**). 2,5 µg/100 µl buffer (**A.**), 5 µg/100 µl buffer (**B.**), Mouse serum 10% final concentration (**C.**), 2,5 µg/100 µl buffer (**D.**) and IgG1 isotype control (**E.**).

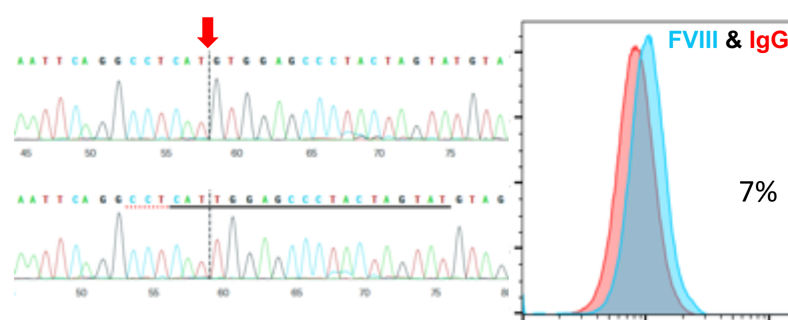
Figure S3

A. U937

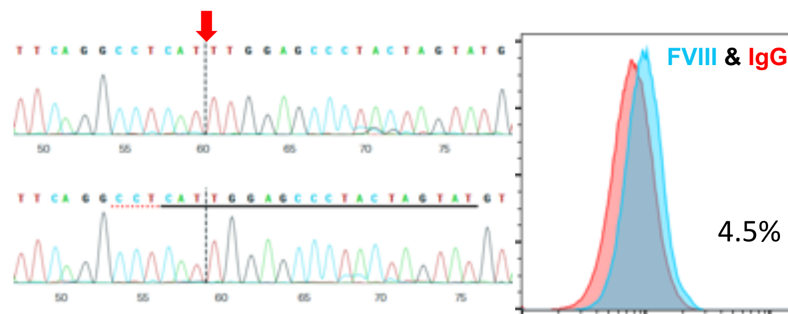
Clone C7 (+1bp)

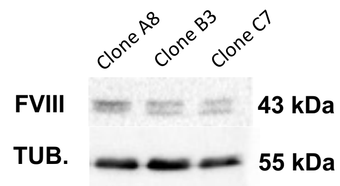


Clone B3 (+1bp)



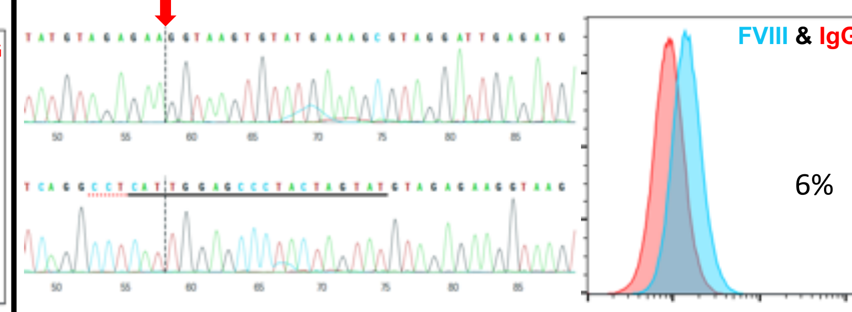
Clone A8 (+1bp)



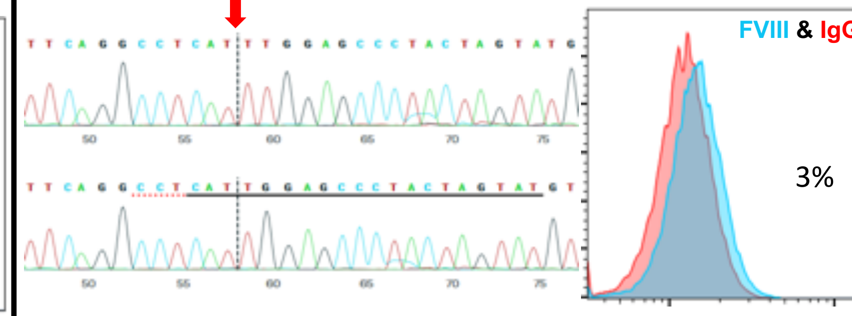


B. HECV

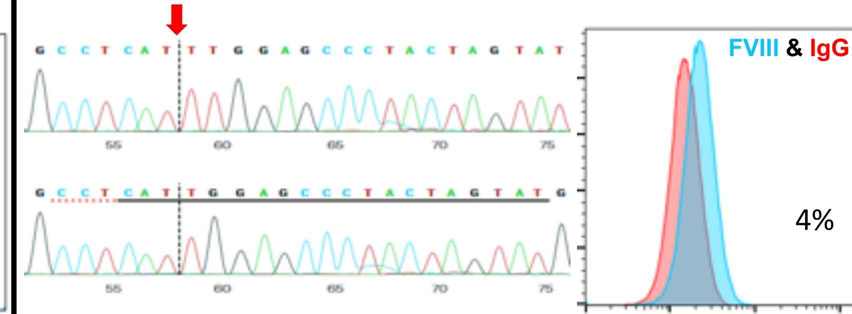
Clone A2 (+1bp)

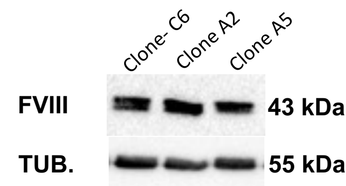


Clone A5 (-25bp)



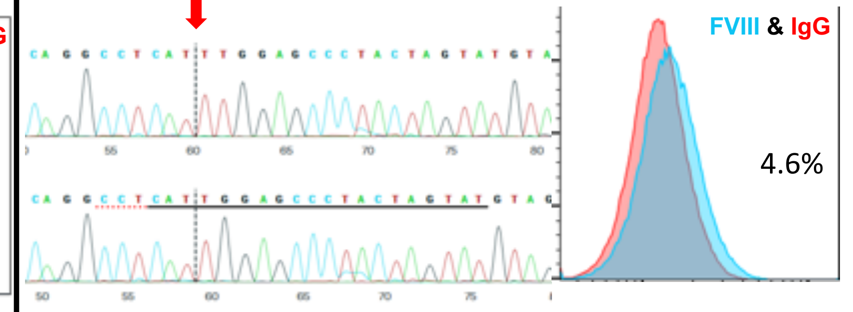
Clone C6 (+1bp)



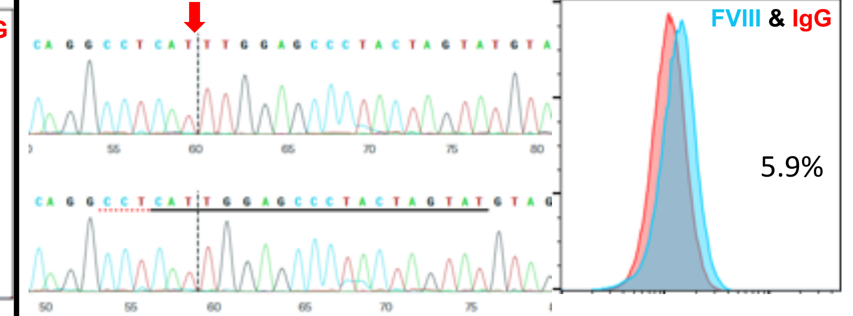


C. HELA

Clone A7 (+1bp)



Clone D3 (+1bp)



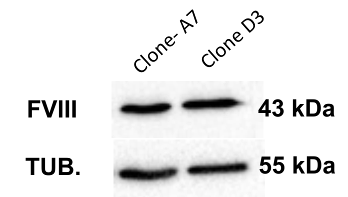
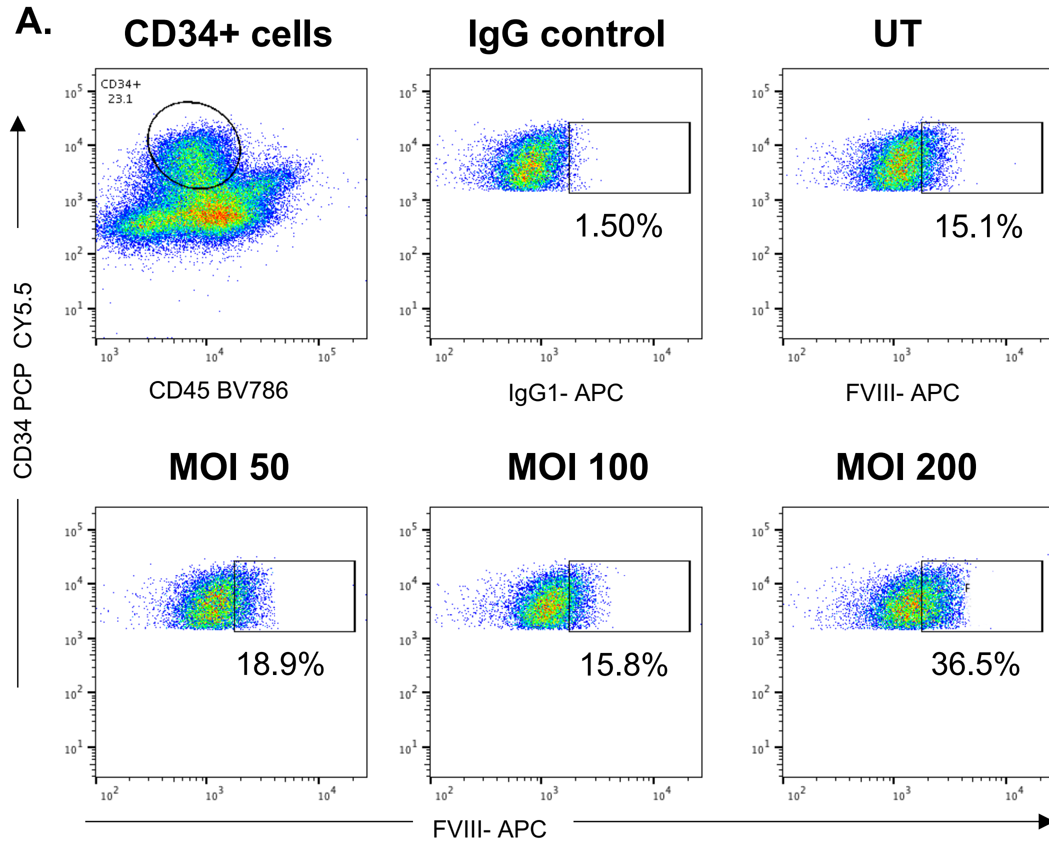


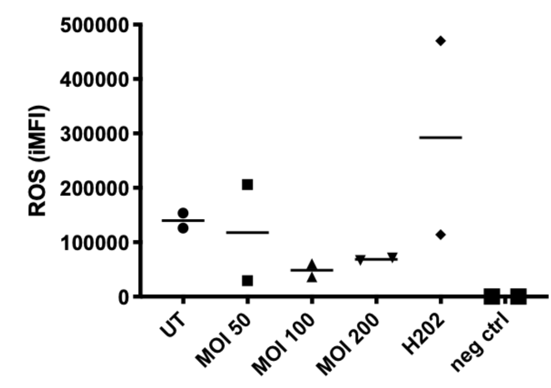
Figure S3. Sequence analysis of cell lines before and after gene editing. For each clone the gene edited sequence (top lane) and its unmodified counterpart (bottom lane) are shown, after analysis with Synthego knock out Sanger sequencing software. In the bottom lanes (unmodified sequences) the gRNA target sequence is underlined with dashed lines.

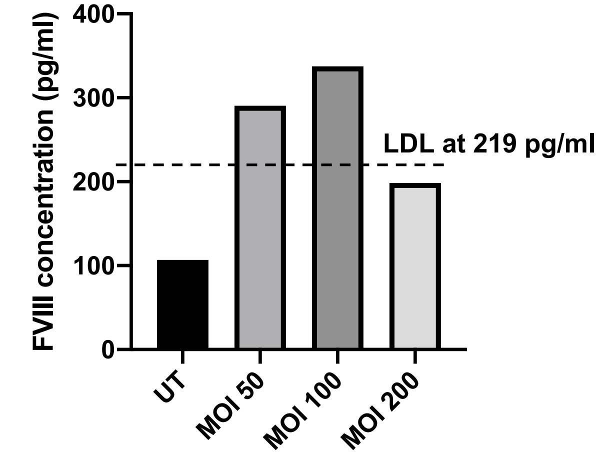
In the right side, for each panel, the rate of FC FVIII positivity - over respective IgG staining - is shown.

Corresponding western blot images of the clones after gene editing are also shown. The same WB membranes were stripped and used for Tubulin after FVIII staining and development.

Figure S4







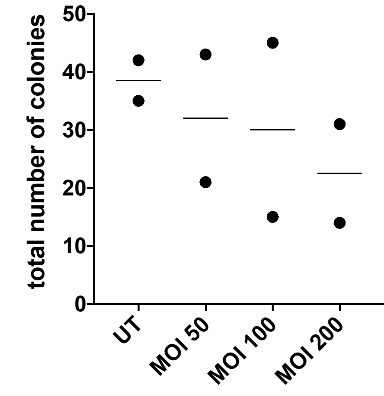


Figure S4. Transduction of Hematopoietic (CD34+) stem cells and FVIII expression
FC experiment on transduced CD34^{+} cells at different MOIs using monoclonal anti-A2 domain FVIII antibody labelled with Zenon AF647 with IgG1 as isotype control (**A**). FC evaluation of ROS for the same experiment. IMFI (% of ROS positive cells x MFI) is shown for each condition. The iMFI was preferred, to normalize for technical variations. Two technical replicates of the same experiment are shown (dots). Cells treated with H2O2 served as positive control, and cells not exposed to CellRox as negative control (**B**). FVIII secretion measurement by ELISA. Average of triplicates measurements of 1 experiment is shown. Lower Detection Limit (LDL) of the assay was 219 pg/ml (**C**). Methylcellulose CFU colonies assay after transduction. Total number of colonies after 14 days is shown (**D**).