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Supplemental Information

Disruption of the BCL11A Erythroid Enhancer Reactivates Fetal Hemoglobin in Erythroid Cells of Patients with β-Thalassemia Major

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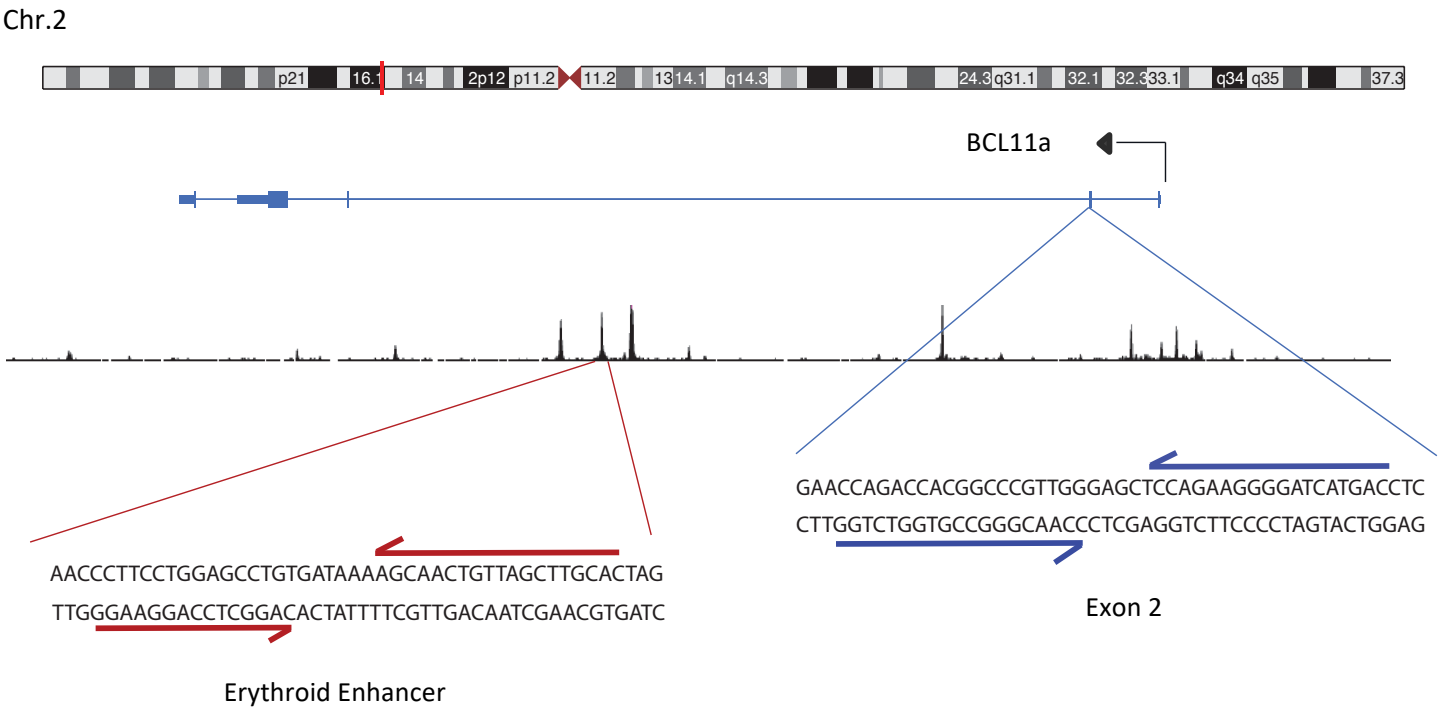
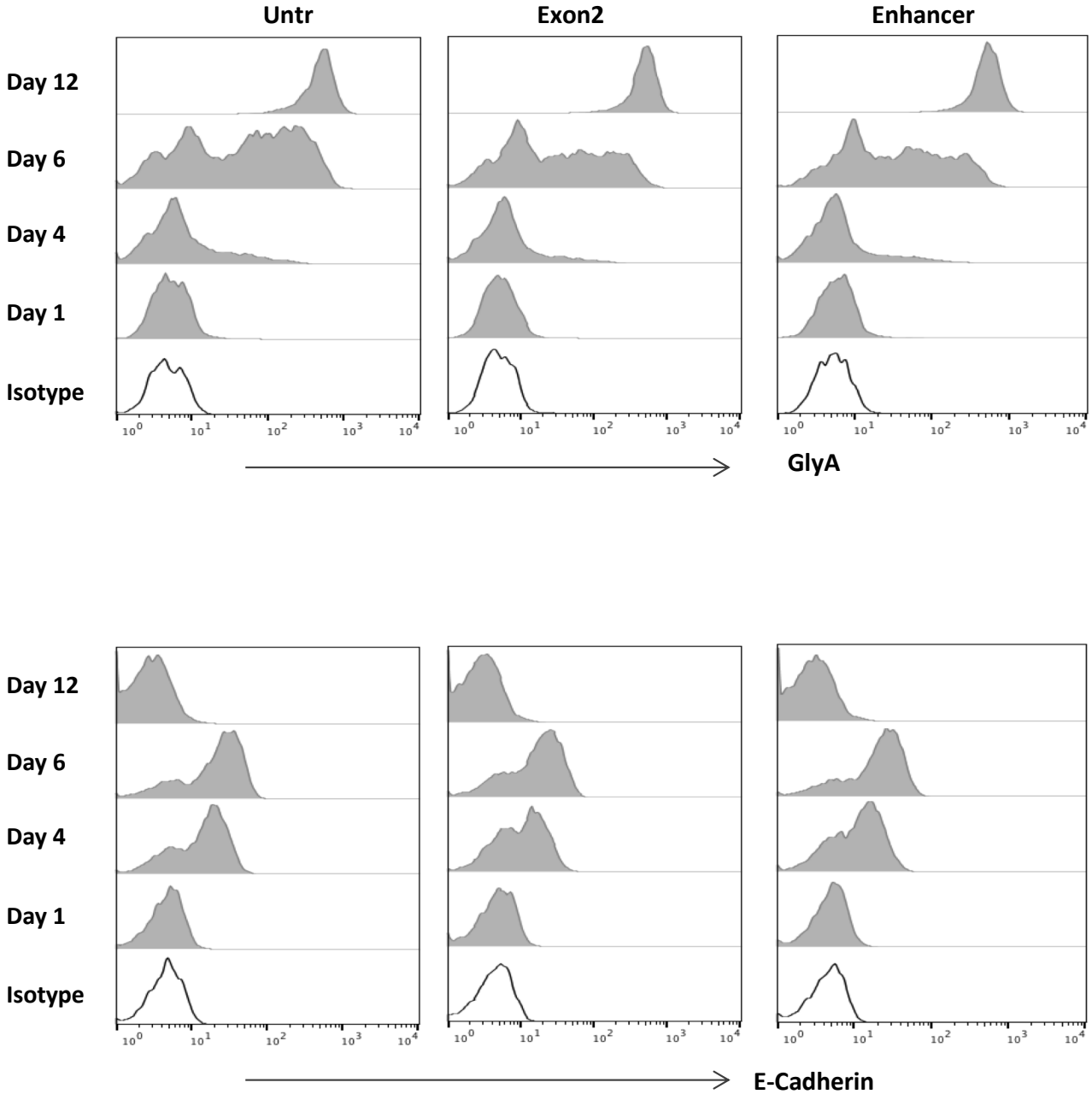
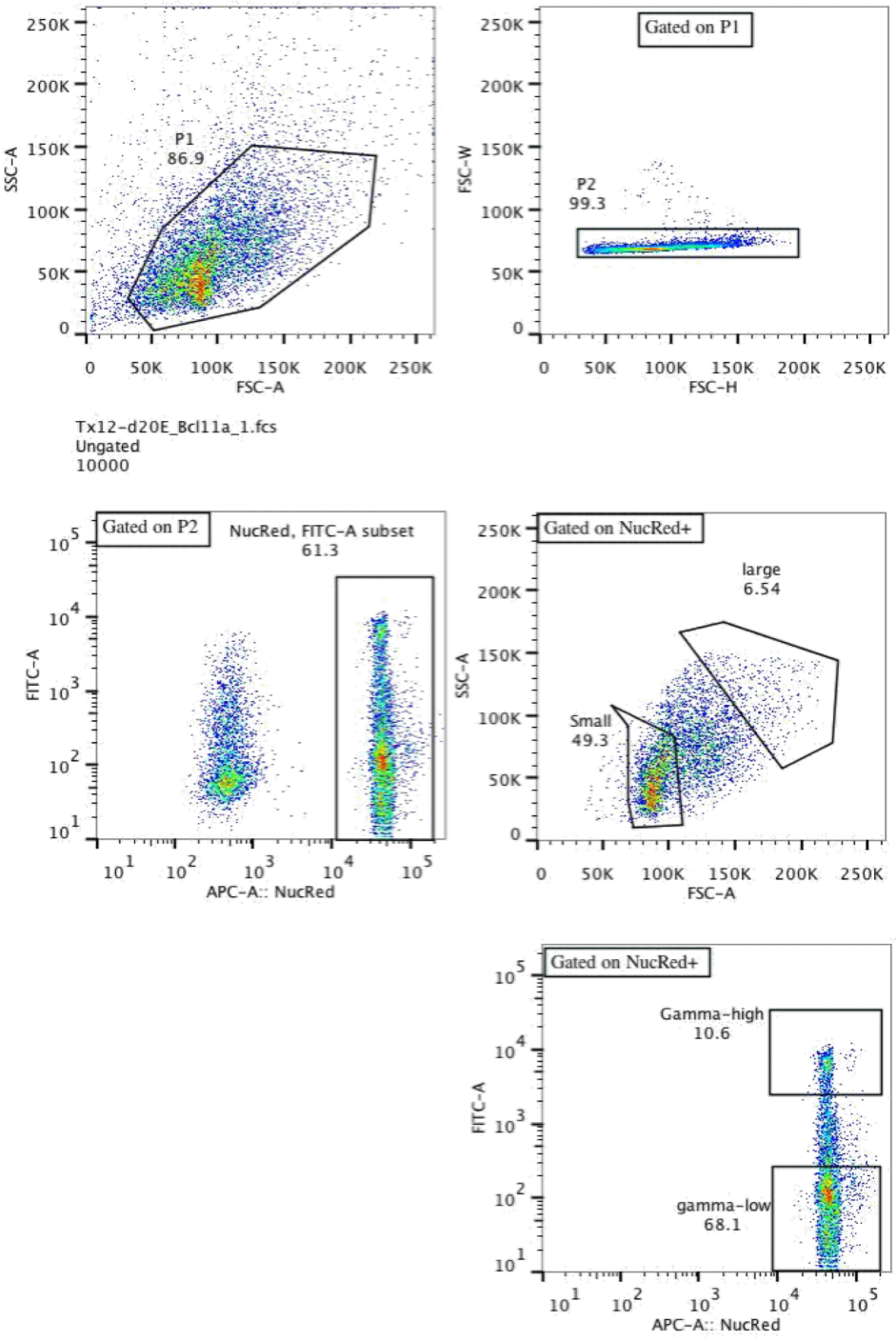


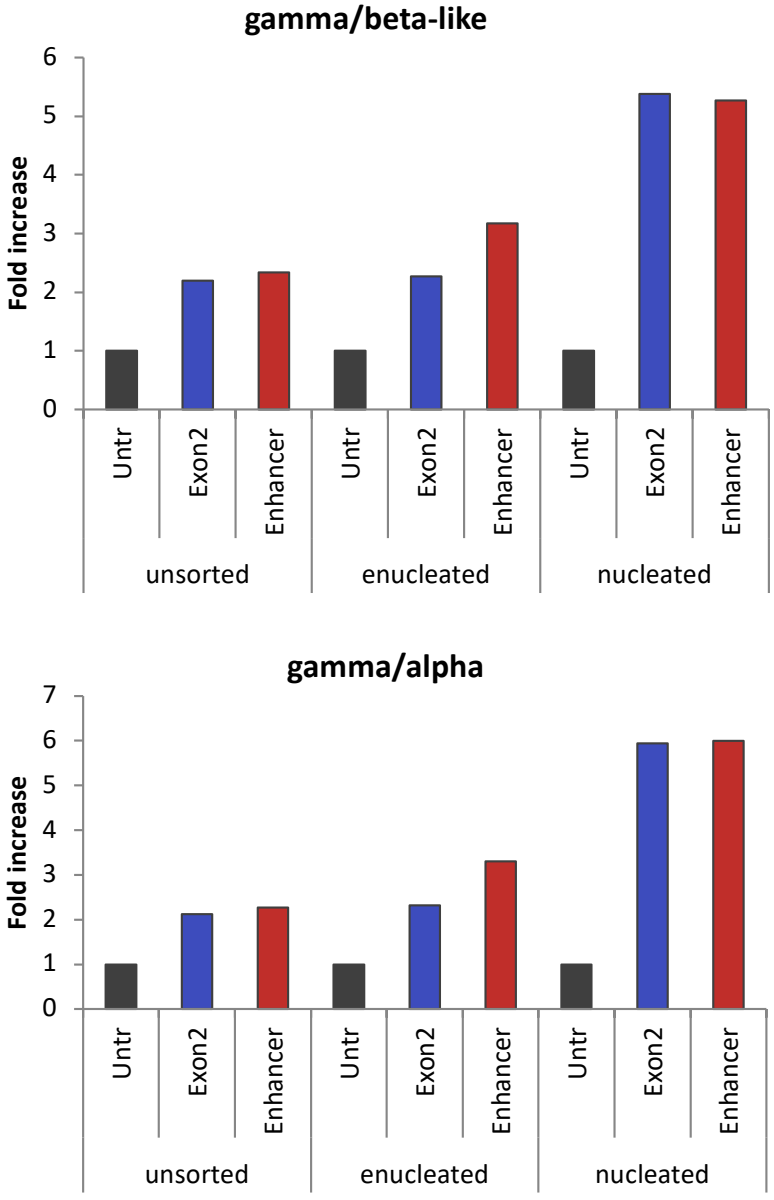
Supp.Figure 1. Binding sequences of the ZFNs targeting the BCL11a Exon 2 and the erythroid enhancer (DHS +58).



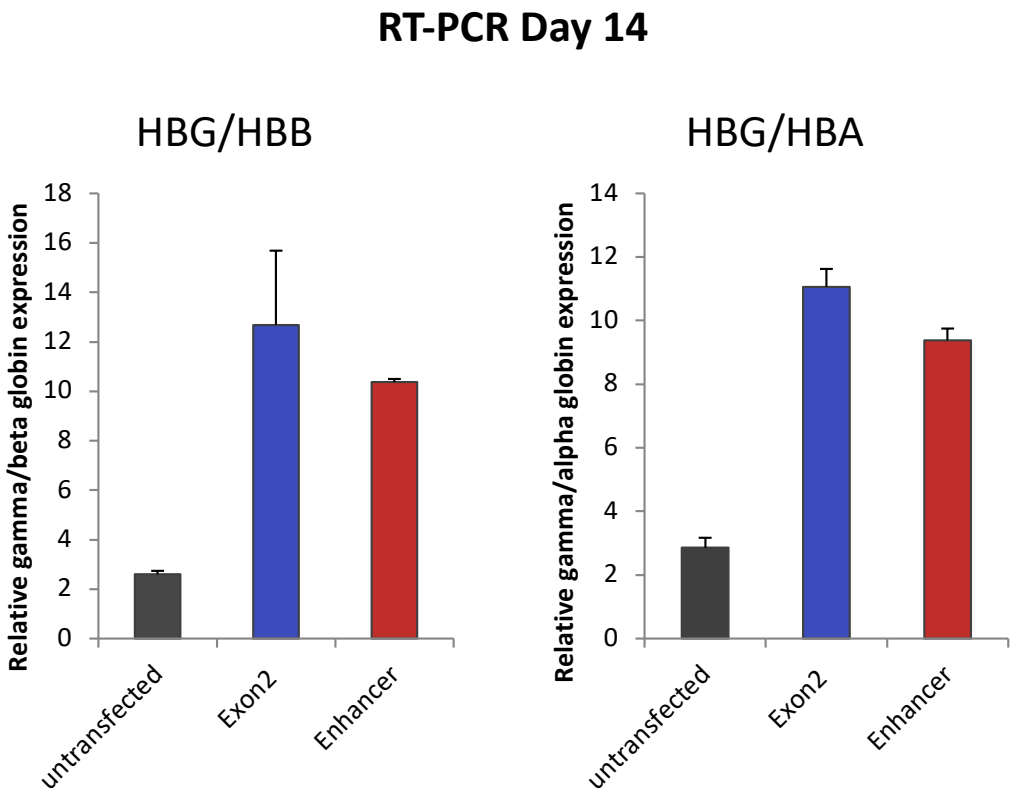
Supp.Figure 2. Concurrent erythroid differentiation of untransfected, exon2 and enhancer-ZFN transfected wild type cells based on early (E-cadherin) and late (GlyA) erythroid surface markers.



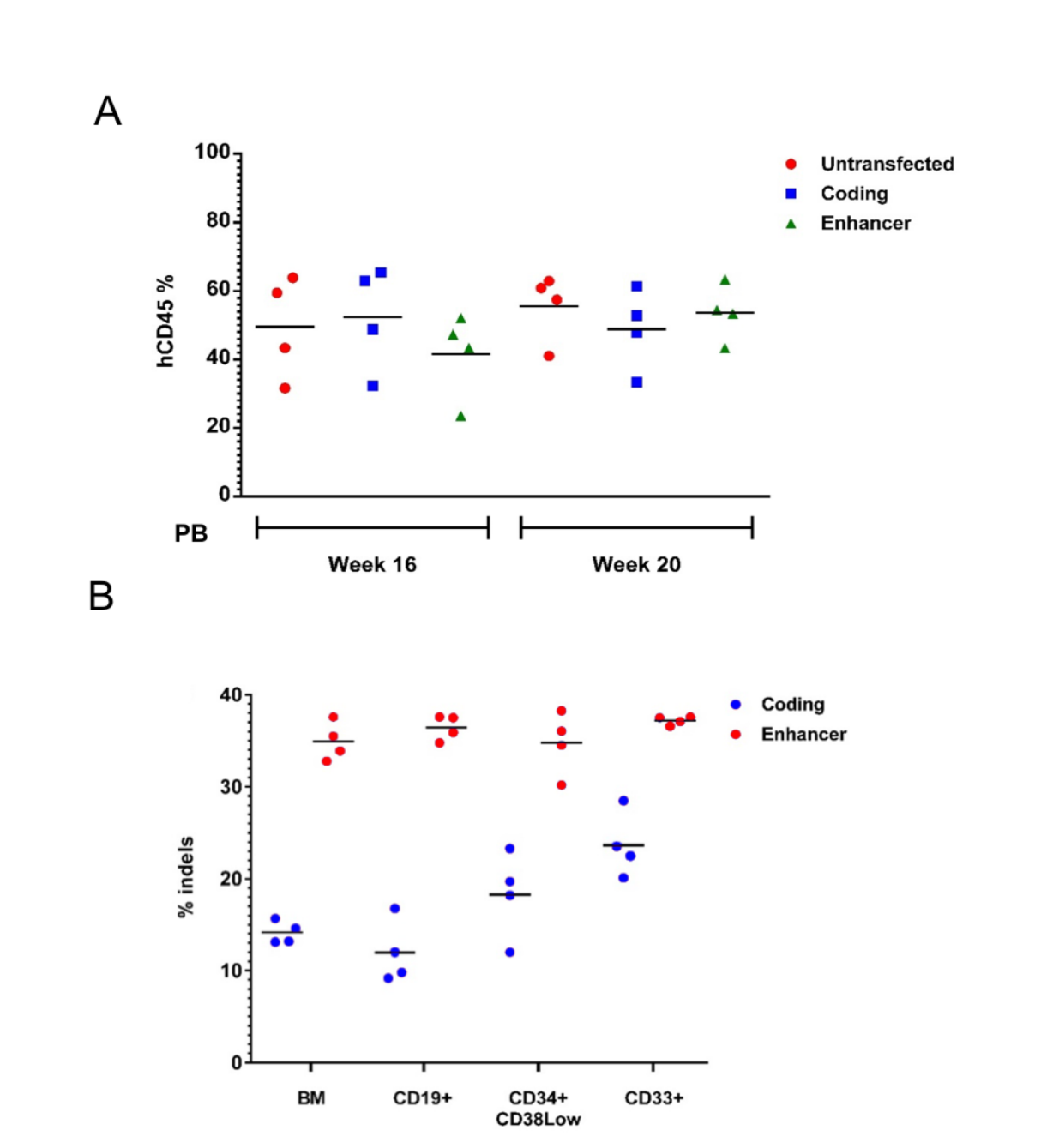
Supp.Figure 3. Gating strategy for sorting erythroid cells based on their size (small/large) and/or based on HbF epression (high/low). Representative dot plots from the exon2-edited sample.



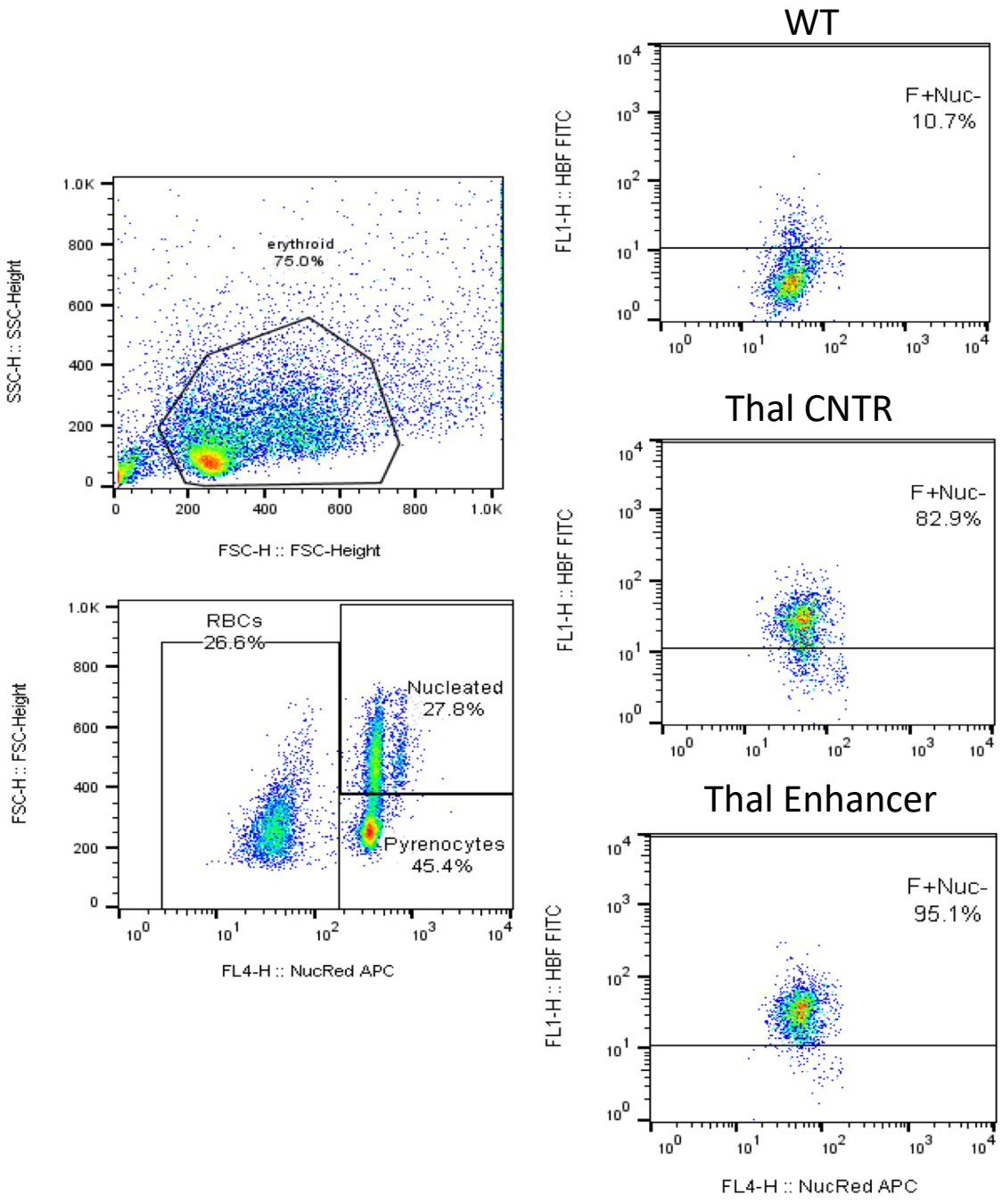
Supp.Figure 4. Gamma-globin/beta-like globin and gamma-globin/alpha-globin fold increase in normal cells after editing as evaluated by HPLC (day 20 of the erythroid differentiation).



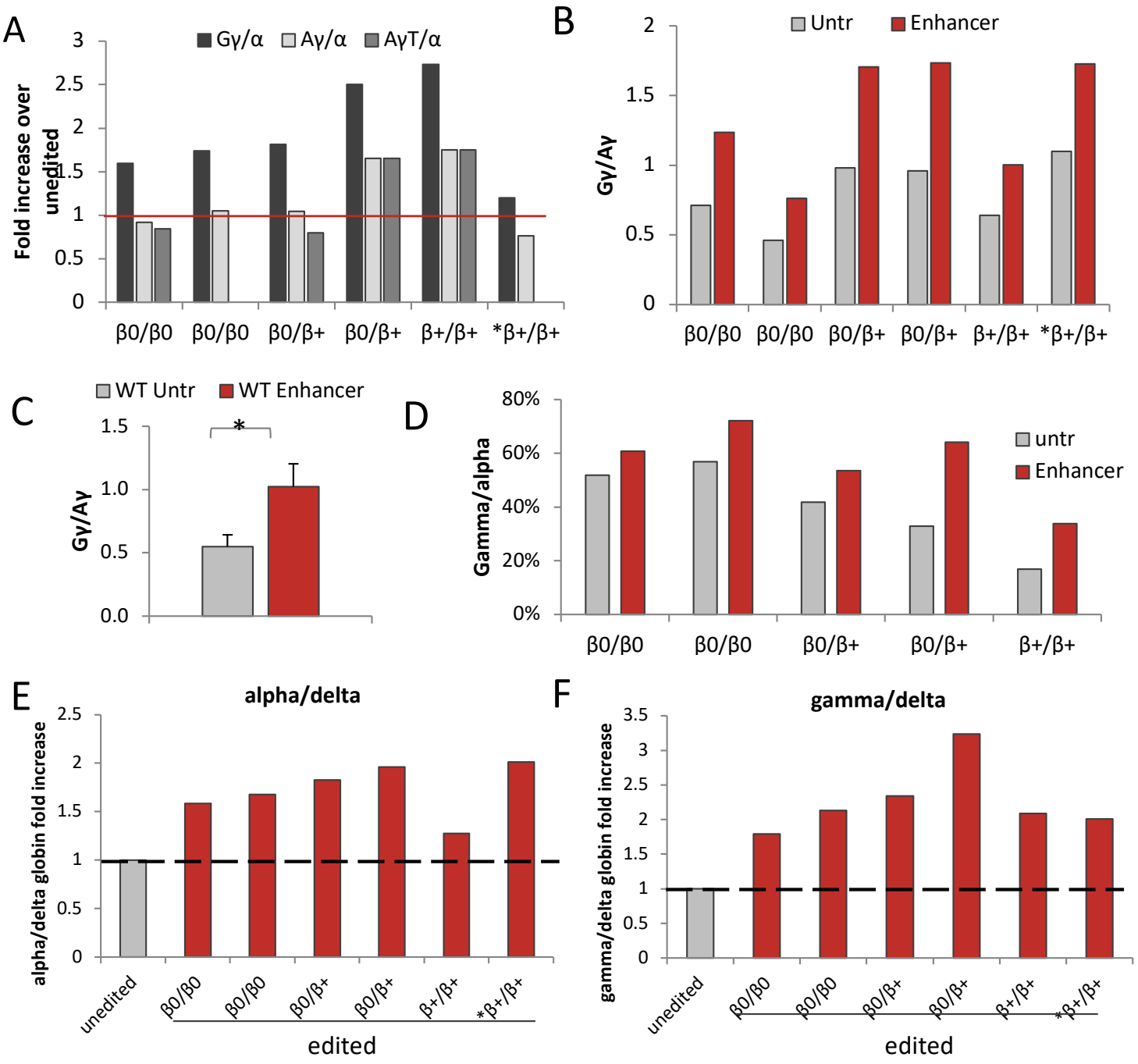
Supp.Figure 5. Relative gamma-globin/beta-globin and gamma-globin/alpha-globin ratios as evaluated by RT-PCR. Both γ-globin and β-globin were measured independently in replicate (each n = 2) and then combinatorically normalized to each other (total n = 4). Bars and error represent mean ± s.d. of the normalized data.



Supp.Figure 6. A. human cell chimerism in the BM of primary recipients 16 and 20 weeks post transplantation. B. Indel % in different lineages in the bone marrow of primary recipients 20 weeks post transplantation.



Supp.Figure 7. HbF+ cell frequency within the red blood cells of wild type and thalassemic (β+/β+: IVSII-745/IVSII-745) edited and unedited samples.



Supp.Figure 8. A. G-gamma, A-gamma and (in some donors) AT-gamma chains increase in thalassemic edited samples. B. Gγ/Aγ ratio in unedited and edited cells. C. Gγ/Aγ ratio in WT samples from three donors unedited and edited with the enhancer ZFNs. D. Gamma-globin/alpha-globin percentage. The *β+/β+ sample (IVSII-745/IVSII-745) is excluded. E. The alpha-globin/delta-globin fold increase in all thalassemic edited samples. F. The gamma-globin/delta-globin fold increase in all thalassemic edited samples. All data were collected on day 20 of the erythroid culture. Genotype of samples from left to right in all panels: IVSI-1/IVSI-1, CD39/CD39, CD39/ IVSI-110, CD39/ IVSI-110, IVSI-110/ IVSI-110, IVSII-745/IVSII-745.