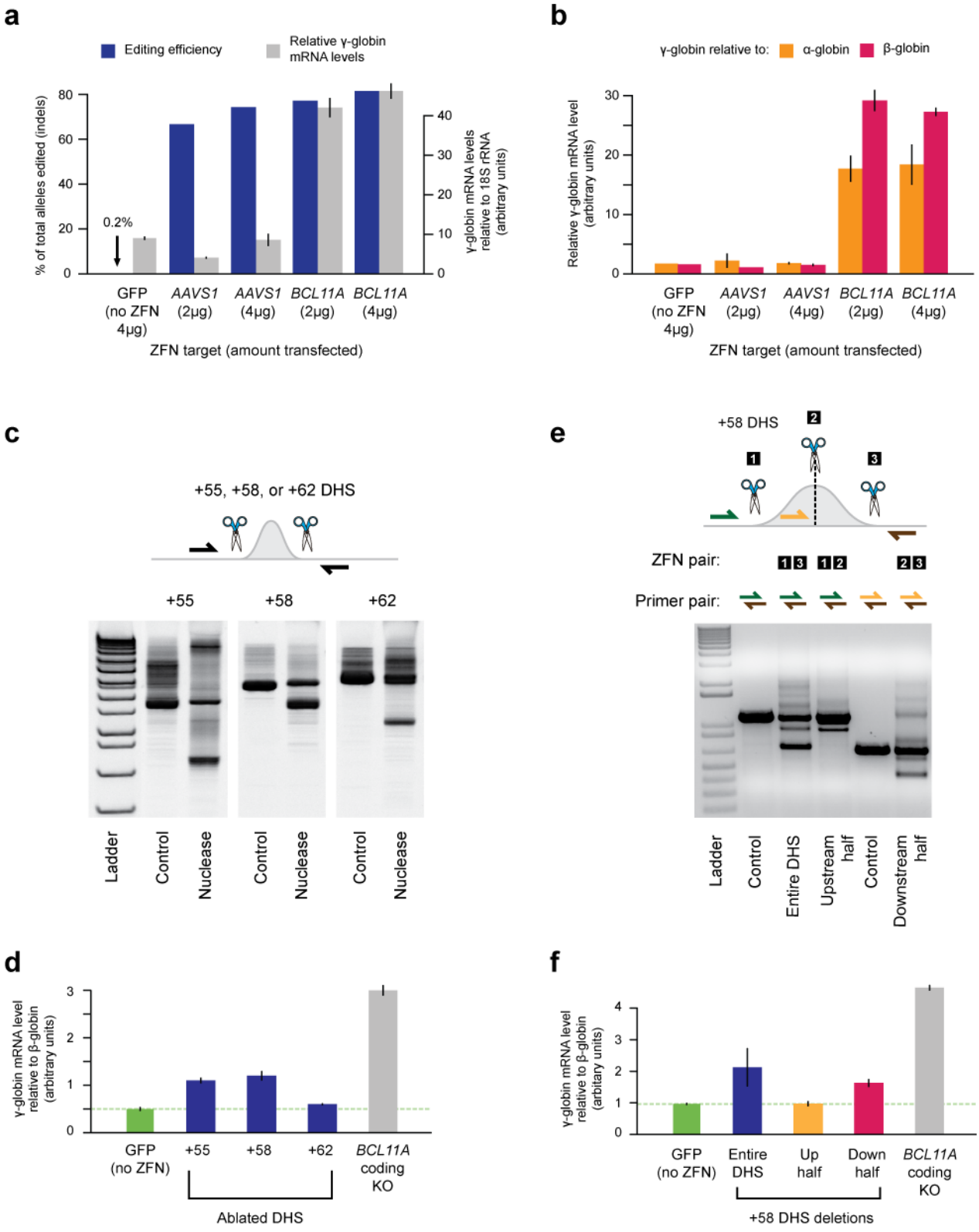
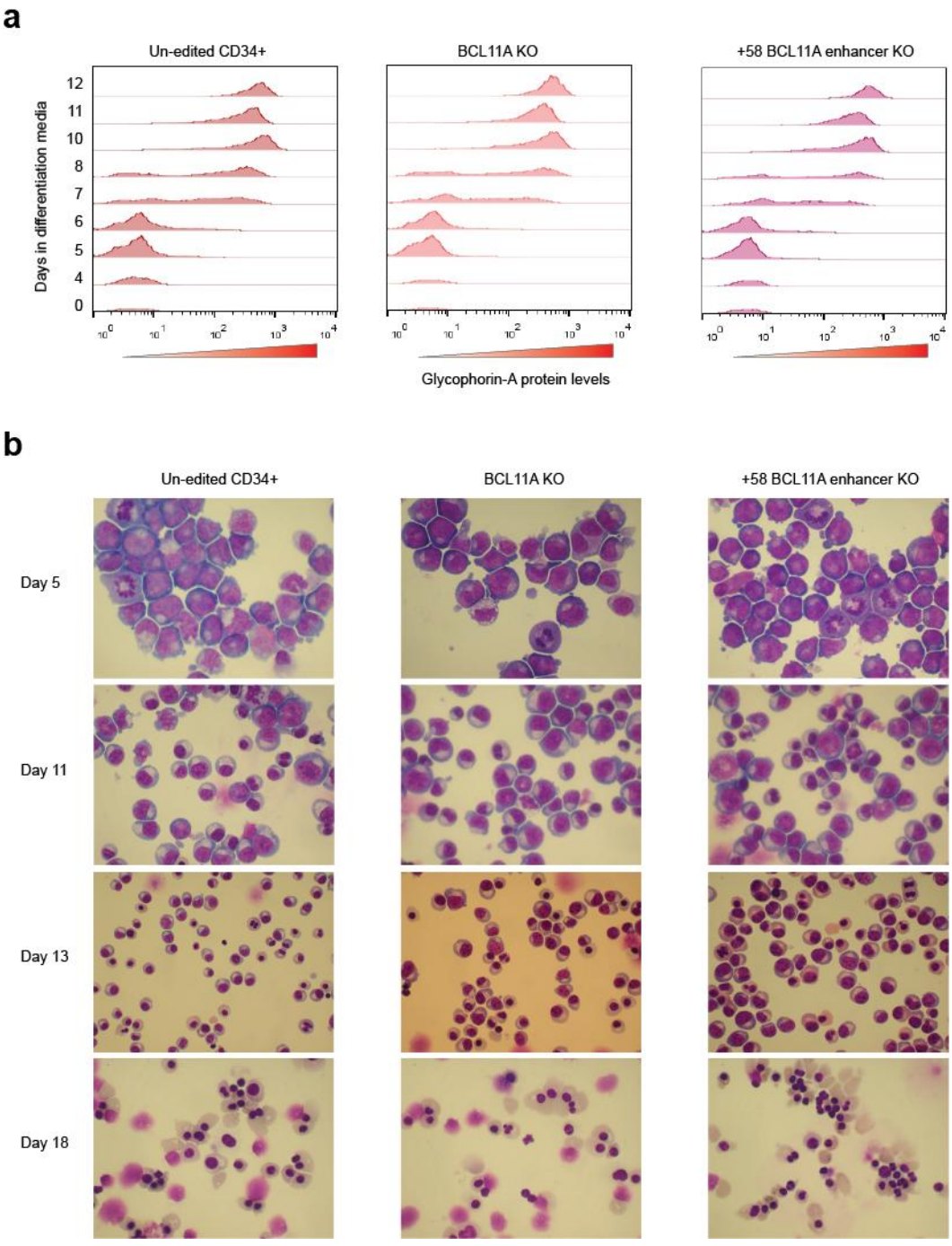


Supplementary Figure 1

Location-specific effects on fetal globin following genome editing of the *BCL11A* locus in CD34^{+} cells.

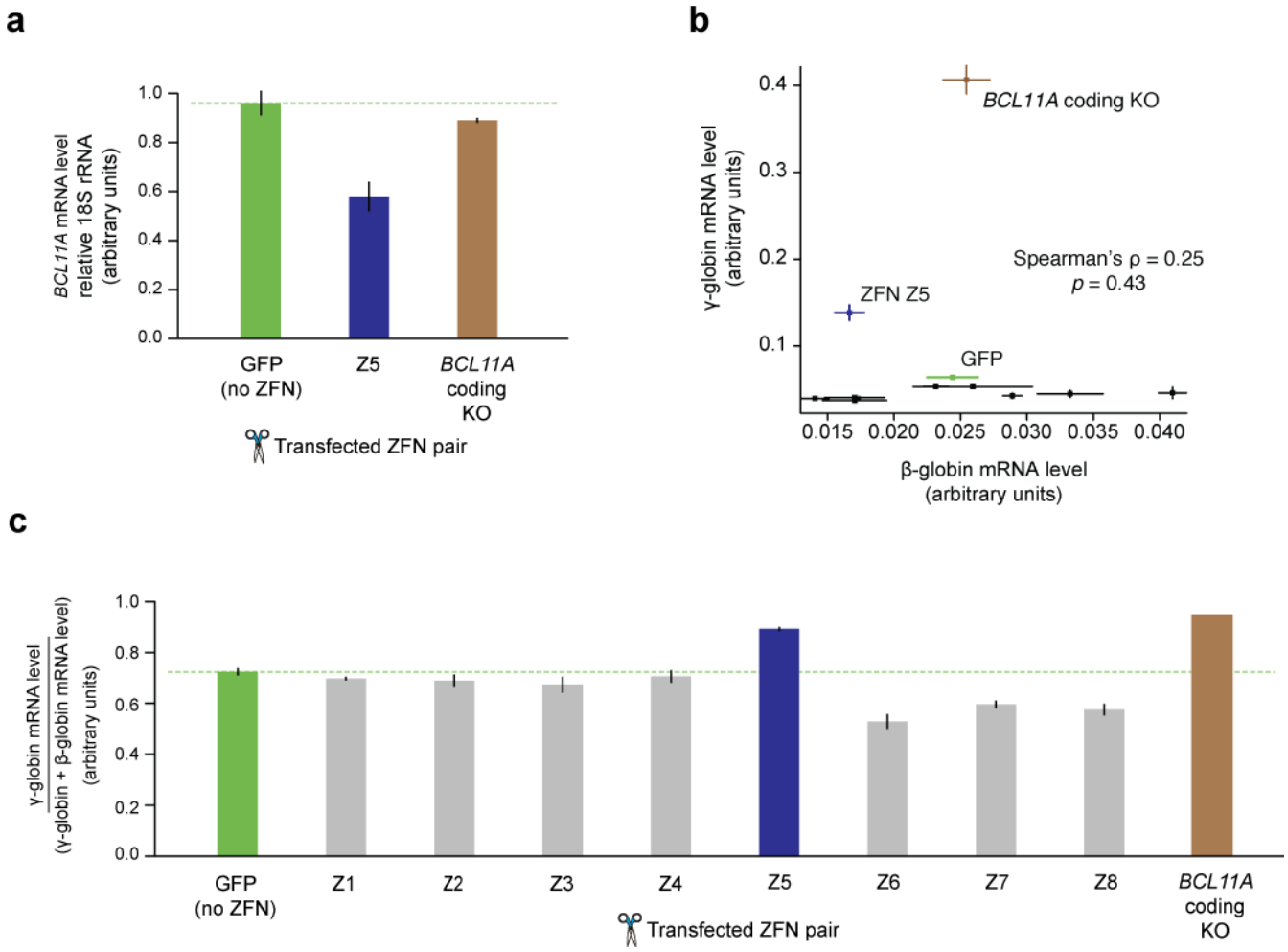
(**a**) Bar chart showing the editing efficacy (blue bars) of ZFNs in primary human $CD34^+$ cells targeting open reading frames and their effect on relative γ-globin mRNA expression (grey bars). mRNAs encoding ZFNs targeting coding sequence or control (GFP) were transfected into $CD34^+$ cells and the relative level of γ-globin was measured following *in vitro* erythropoiesis. Editing efficiency is computed as the percentage of genotyped alleles bearing an indel. Both γ-globin and 18S rRNA were measured independently in replicate (each $n = 2$) and then combinatorially normalized to each other (total $n = 4$). Bars indicate the mean and error bars indicate the standard deviation of the normalized data. (**b**) Same as **a** except γ-globin expression relative to α-globin (orange bars) or β-globin (pink bars). (**c**) Deletion of individual DNase I hypersensitive sites (DHSs) from the *BCL11A* erythroid enhancer. Human $CD34^+$ cells were electroporated with mRNA encoding control (GFP) or TALENs designed to delete the indicated DHS were genotyped by PCR following *in vitro* erythropoiesis. (**d**) Effect of TALEN-driven deletion of individual DHSs comprising the *BCL11A* enhancer on relative γ-globin mRNA levels following *in vitro* erythropoiesis. Both γ-globin and β-globin were measured independently in replicate (each $n = 2$) and then combinatorially normalized to each other (total $n = 4$). Bars indicate the mean and error bars indicate the standard deviation of the normalized data. (**e**) Same as **c** deleting specific stretches of the +58 DHS as indicated. (**f**) Same as **d** for regions indicated in **e**.



Supplementary Figure 2

Effect of coding and regulatory DNA editing on erythroid development and maturation.

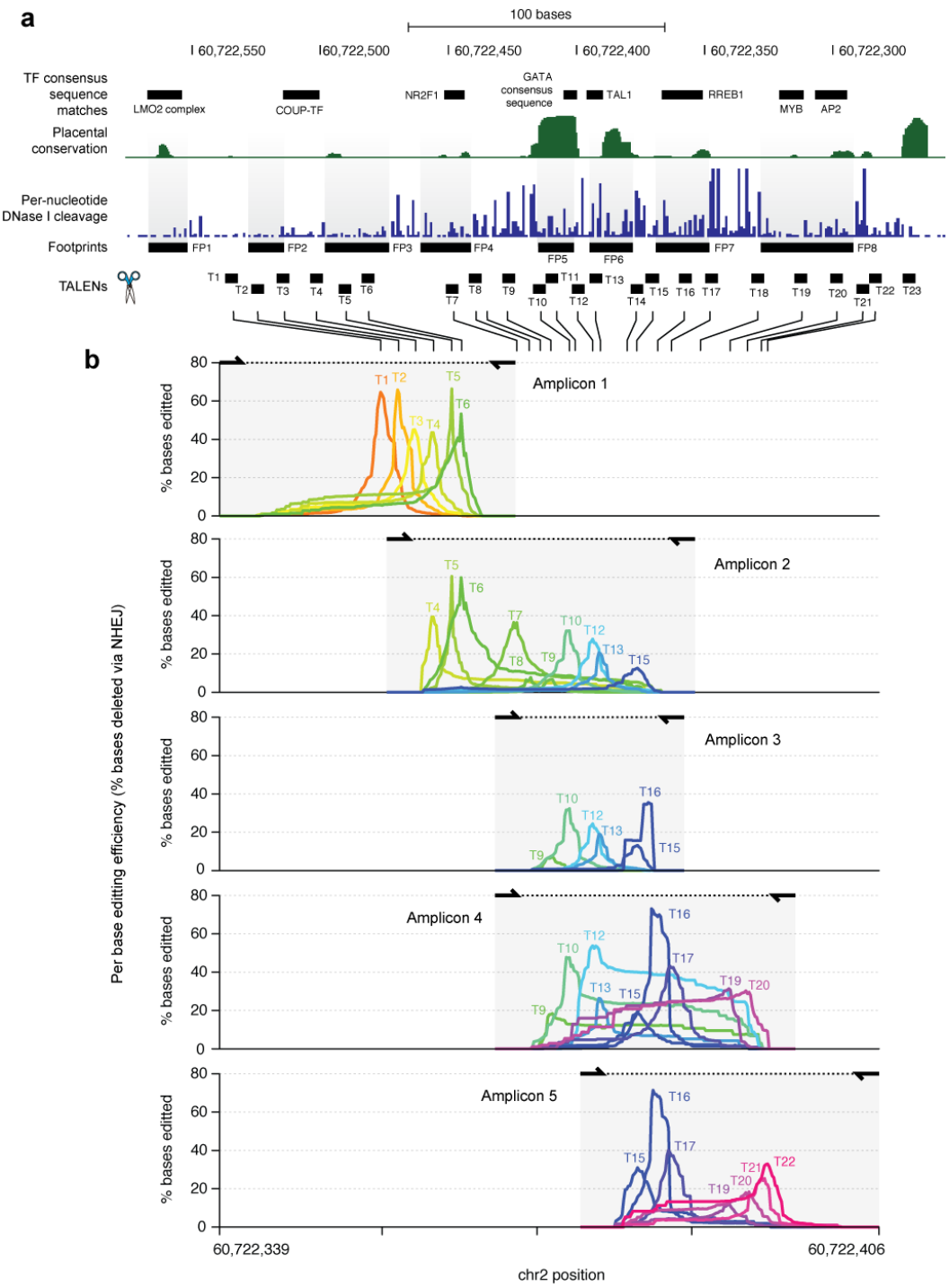
(**a**) FACS analysis of differentiating $CD34^+$ cells for the erythroid cell-surface protein glycophorin-A with type of edit (none, *BCL11A* coding KO or enhancer ablation) vs. days in differentiation media. (**b**) Exemplary morphology of differentiating $CD34^+$ cells as in **a**. Cells were centrifuged onto slides and stained using the Wright-Giemsa technique.



Supplementary Figure 3

Effect of coding and regulatory DNA editing on *BCL11A* and globin mRNA expression levels.

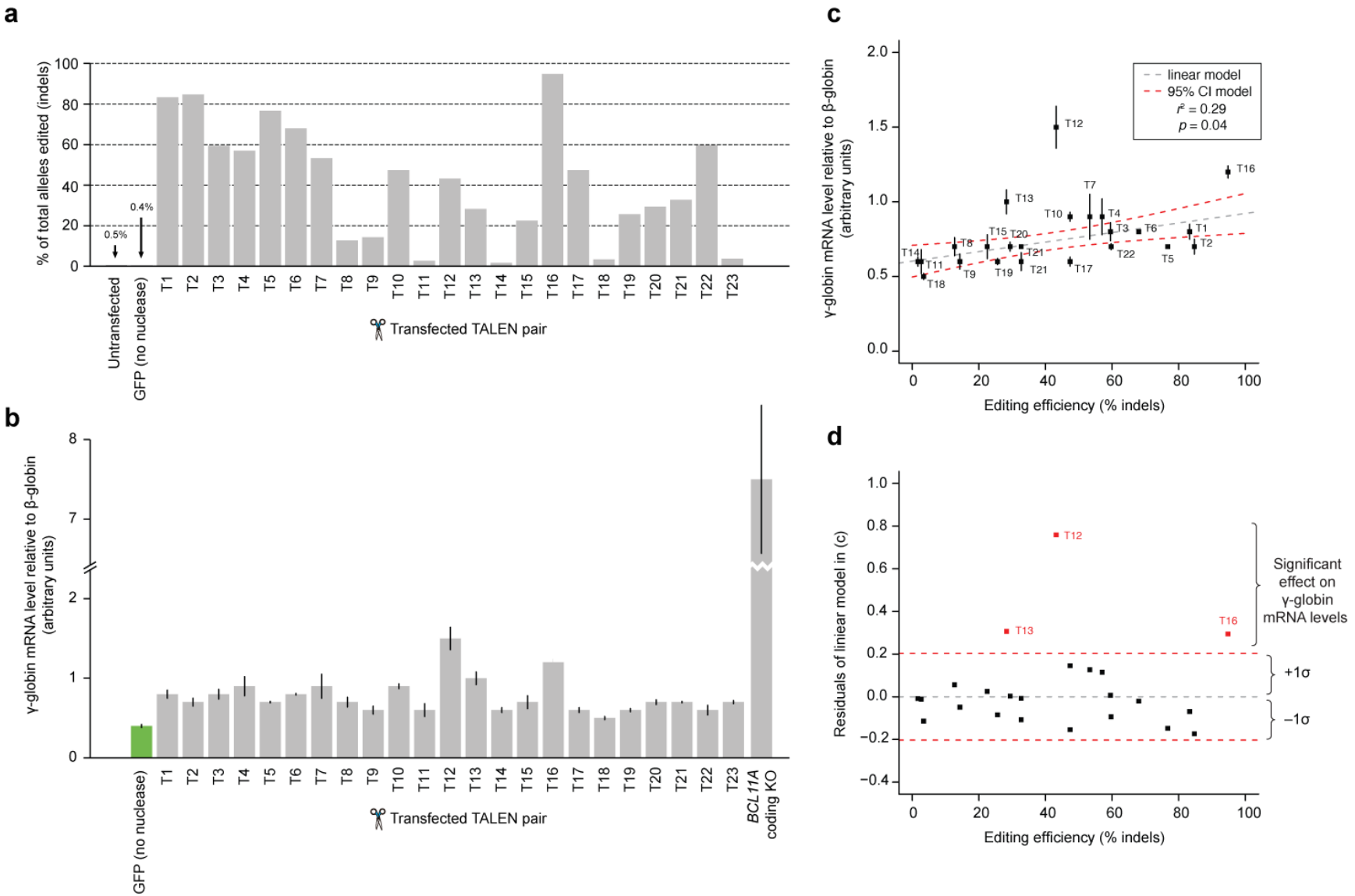
(**a**) Levels of *BCL11A* mRNA relative to 18S rRNA following electroporation of human $CD34^+$ cells with mRNA encoding ZFNs or control (GFP) and *in vitro* erythropoiesis. Both *BCL11A* and 18S rRNA were measured independently in replicate (each $n = 2$) and then combinatorially normalized to each other (total $n = 4$). Bars indicate the mean and error bars indicate the standard deviation of the normalized data. (**b**) Scatterplot of γ-globin vs. β-globin mRNA levels in $CD34^+$-derived erythroid cells after exposure to ZFNs targeting DNase I footprints within the *BCL11A* erythroid enhancer (black) or the coding region of *BCL11A* (brown). Both γ-globin and β-globin were measured independently in replicate (each $n = 2$) and then combinatorially normalized to each other (total $n = 4$). Bars indicate the mean and error bars indicate the standard deviation of the normalized data. (**c**) Levels of γ-globin mRNA normalized to that of total β-like globin (γ-globin + β-globin) following electroporation of human $CD34^+$ cells with mRNA encoding ZFNs or control (GFP) and *in vitro* erythropoiesis. Both γ-globin and β-globin were measured independently in replicate (each $n = 2$) and then combinatorially normalized to each other (total $n = 4$). Bars indicate the mean and error bars indicate the standard deviation of the normalized data.



Supplementary Figure 4

Fine-resolution unbiased reverse genetic tiling analysis of the +58 DHS.

(**a**) Map of engineered TALENs tiling the +58 DHS with respect to per-nucleotide conservation, DNase I cleavage and computationally predicted protein-DNA interactions (motifs and footprints). (**b**) Per-nucleotide editing efficiencies of individual TALEN pairs determined by amplicon sequencing (Online Methods). Each panel shows different amplicons tiling the +58 DHS.



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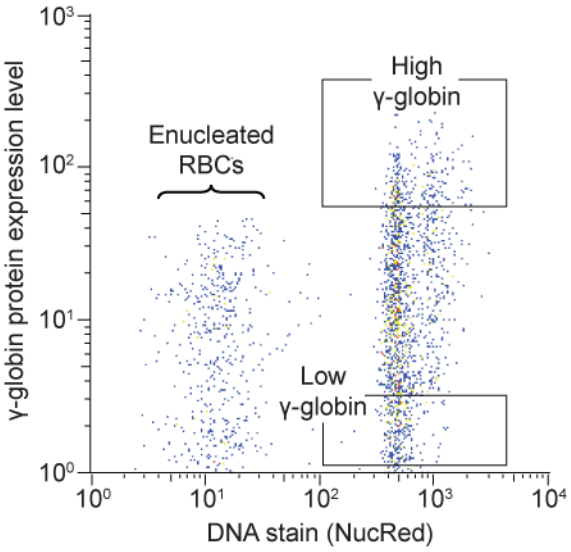
Supplementary Figure 5

Effect of TALEN-mediated editing on γ-globin mRNA expression.

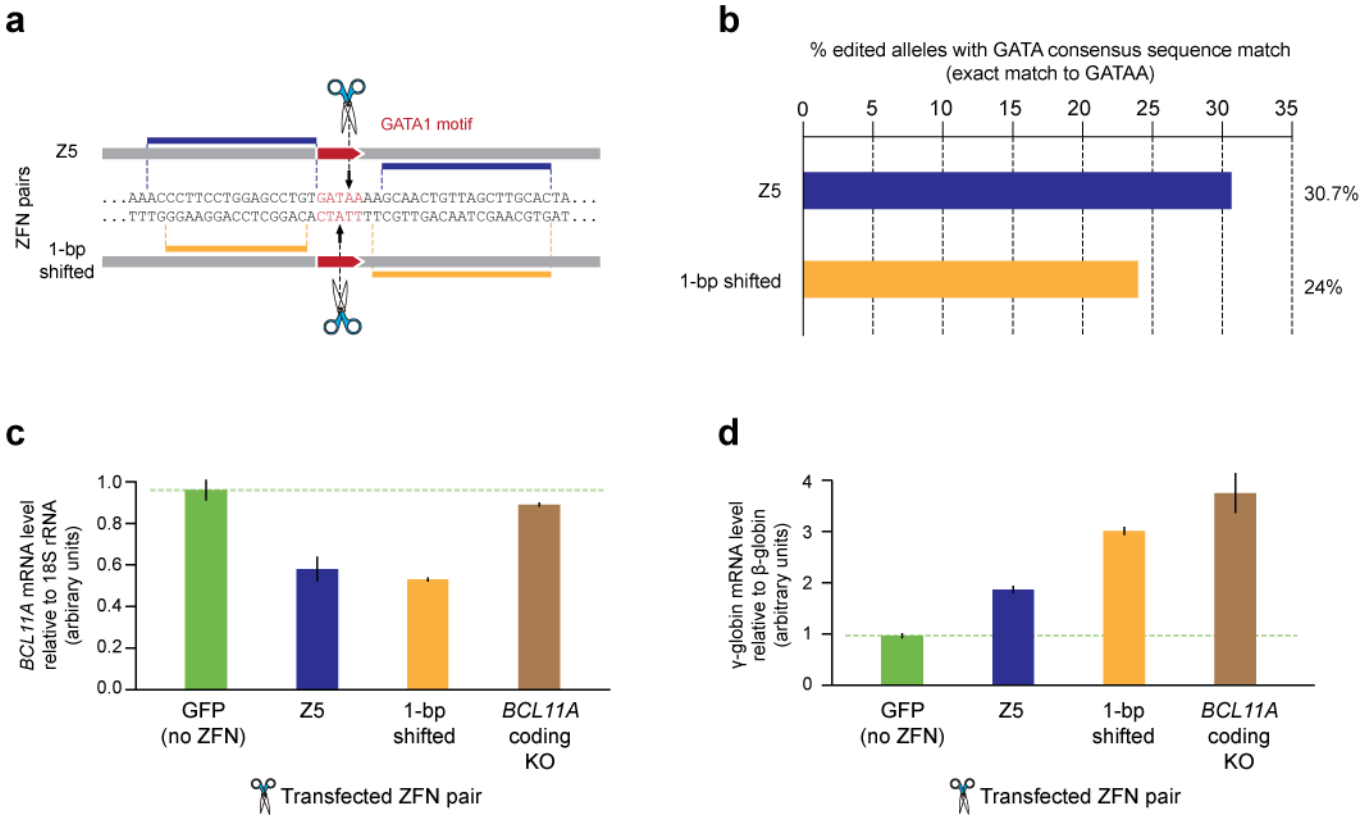
(**a**) Bar chart showing the editing efficacy of TALENs shown in **Supplementary Figure 2**. Editing efficiency is computed as the percentage of genotyped alleles bearing an indel in the overall cell population determined via amplicon sequencing (Online Methods). (**b**) Levels of γ-globin mRNA relative to that of β-globin following electroporation of human $CD34^+$ cells with mRNA encoding TALENs (shown in **Supplementary Fig. 4**) and *in vitro* erythropoiesis. Both γ-globin and β-globin were measured independently in replicate (each $n = 2$) and then combinatorially normalized to each other (total $n = 4$). Bars indicate the mean and error bars indicate the standard deviation of the normalized data. (**c**) Relative γ-globin mRNA levels (to β-globin) (mean ± s.d.; from **b**) vs. TALEN editing efficiency (from **a**). Dotted grey line is the best-fit robust linear model (Online Methods). Dotted red line indicates the 95% CI of the linear model mean. (**d**) Residual plot of linear model in **c** used to determine TALEN edits significantly associated with increased γ-globin expression. The red dotted lines show the 1 standard deviation of the residuals; samples that exceed this threshold are associated with significant changes in their γ-globin expression.



Supplementary Figure 6

Cell sorting on γ-globin protein expression levels.

Scatterplot of fluorescence-activated cell sorting (FACS) of *in vitro* generated erythrocytes following targeted disruption of footprint 5 within the +58 DHS using ZFNs. Boxes indicate the FACS gates used to isolate populations of erythroblasts bearing high and low levels of γ-globin protein.



Supplementary Figure 7

Fine-resolution analysis of the GATA1 motif within the +58 DHS using ZFN-driven genome editing.

(**a**) Diagram of the engineered target specificity of ZFN pairs Z5 (top) and 1-bp shift (bottom). Blue and yellow indicate the predicted binding sites for the individual ZFN monomers. (**b**) Proportion of edited (containing an indel; e.g., non-reference) alleles genotyped that retain a match to the GATA1 consensus sequence following electroporation of either Z5 or 1-bp-shifted mRNA encoding ZFNs and *in vitro* erythropoiesis. (**c**) Levels of *BCL11A* mRNA relative to 18S rRNA following electroporation of human $CD34^+$ cells with mRNA encoding ZFNs or control (GFP) and *in vitro* erythropoiesis. Both *BCL11A* and 18S rRNA were measured independently in replicate (each $n = 2$) and then combinatorially normalized to each other (total $n = 4$). Bars indicate the mean and error bars indicate the standard deviation of the normalized data. (**d**) Same as **c** for of levels of γ-globin mRNA relative to that of β-globin.