Clinical data suggest that a reduction of a–globin to 75% to 25% of its physiological levels is safe and beneficial to patients with b–thalassemia.3,35 The most common natural mutations affecting a–globin synthesis are gene deletions that remove a single HBA gene from 1 or both chromosomes generating –a/aa or –a/–a genotypes (–a3.7 and –a4.2deletions36) (Figure 1A).Effects of genetic modifiers can be either mediated by HbF or independent of HbF: Genetic modifiers affecting sickle cell disease (SCD) may be linked to increased fetal hemoglobin (HbF) expression.To establish a correlation between a–globin expression and number of HBA genes, we generated multiple cell clones with mono– or biallelic HBA2 deletions (–a/aa and –a/–a, respectively, n 5 3 per genotype) and we showed a significant amelioration of the a/b–like globin imbalance upon deletion of HBA2, with the –a/–a clones being indistinguishable from wild–type HUDEP–2 cells (Figure 1D; supplemental Figure 1D).To minimize the possibility of generating a a–globin KO, the sgRNA was designed to target the 59UTR (HBA15) of HBA1 and HBA2 (Figure 1A), where the presence of InDels resulting from doublestrand breaks (DSBs) does not affect a–globin production.37 As a b0–thalassemia cell model, we used immortalized HUDEP–2, which can differentiate and express adult hemoglobin (supplemental Figure 1A–B), and we knocked out b–globin genes (HUDEP–2 b0) (supplemental Figure 1C).We transfected both wild–type HUDEP–2 and HUDEP–2 b0 with RNP targeting HBA and we achieved efficient editing (83.1% 6 12.1 and 77.3% 6 18.2, respectively, n 5 3; Figure 1B) and genomic deletion of HBA2 gene (0.9 6 0.2 and 0.9 6 0.1 HBA2 copy per cell, n 5 3; Figure 1C), which resulted in a decrease of a–globin messenger RNA (mRNA) expression upon erythroid differentiation (Figure 1D).