To test our strategy in therapeutic conditions, we assessed correction of globin imbalance in patients' HSPCs. As positive control, HSPCs were transduced with a LV encoding for a bAS3 transgene under the control of the b-globin gene promoter and its mini-locus control region, currently in clinical trial for thalassemia (LV bAS3). 13, RNP transfection was very efficient both in erythroid liquid culture and CFC in generating InDels (90.5% 6 7.1 for RNP and 90.4% 6 9.0 for RNP1AAV, mean 6 SD; Figure 5A), HBA2 deletion (0.95 6 0.06 HBA2 copies/cell for RNP and 0.77 6 0.15 for RNP1AAV, mean 6 SD; Figure 5B) and bAS3 KI (0.80 6 0.21 copies/cell for RNP1AAV, mean 6 SD; Figure 5C). Globin mRNA analysis of single BFU-E colonies derived from b0- and b1-edited HSPCs showed that a/b imbalance was improved in all conditions, in accordance with the reduced number of HBA2 genes (supplemental Figure 4G) and with the stronger effect resulting from the concomitant a downregulation and bAS3 expression (Figure 5F). mRNA globin imbalance (measured as a/b-like globins ratio) was ameliorated in all conditions (Figure 5D), with bAS3 KI cells performing better than HBA2-deleted erythroblasts because of bAS3 expression (Figure 5E; supplemental Figure 4C). Of note, modified HSPCs retained proper erythroid differentiation (supplemental Figure 4D) and multilineage potential (CFC assay; supplemental Figure 4F), although we observed some toxicity associated with the editing procedure (supplemental Figure 4E). In particular, we tested the HBA2 deletion approach in b1 cells and the combination of the a-deletion and bAS3 KI strategy in b1- and b0 -thalassemic HSPCs (supplemental Figure 4A-B). HSPCs were transfected with RNP and transduced with AAV as described previously (Figure 3A). Overall, these data show that we can modify b-thalassemia HSPCs and reduce their a/b globin balance, without affecting HSPCs potential.