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Supplemental information

Multiplex CRISPR/Cas9 genome editing in hematopoietic stem cells for fetal hemoglobin reinduction generates chromosomal translocations

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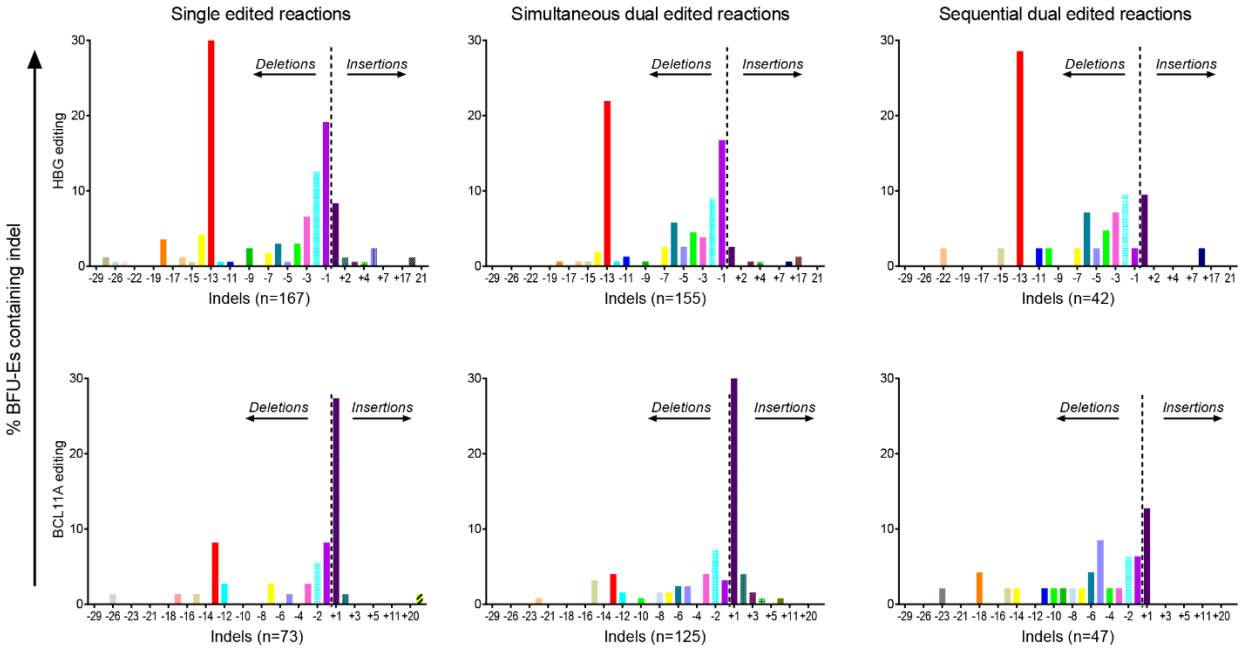
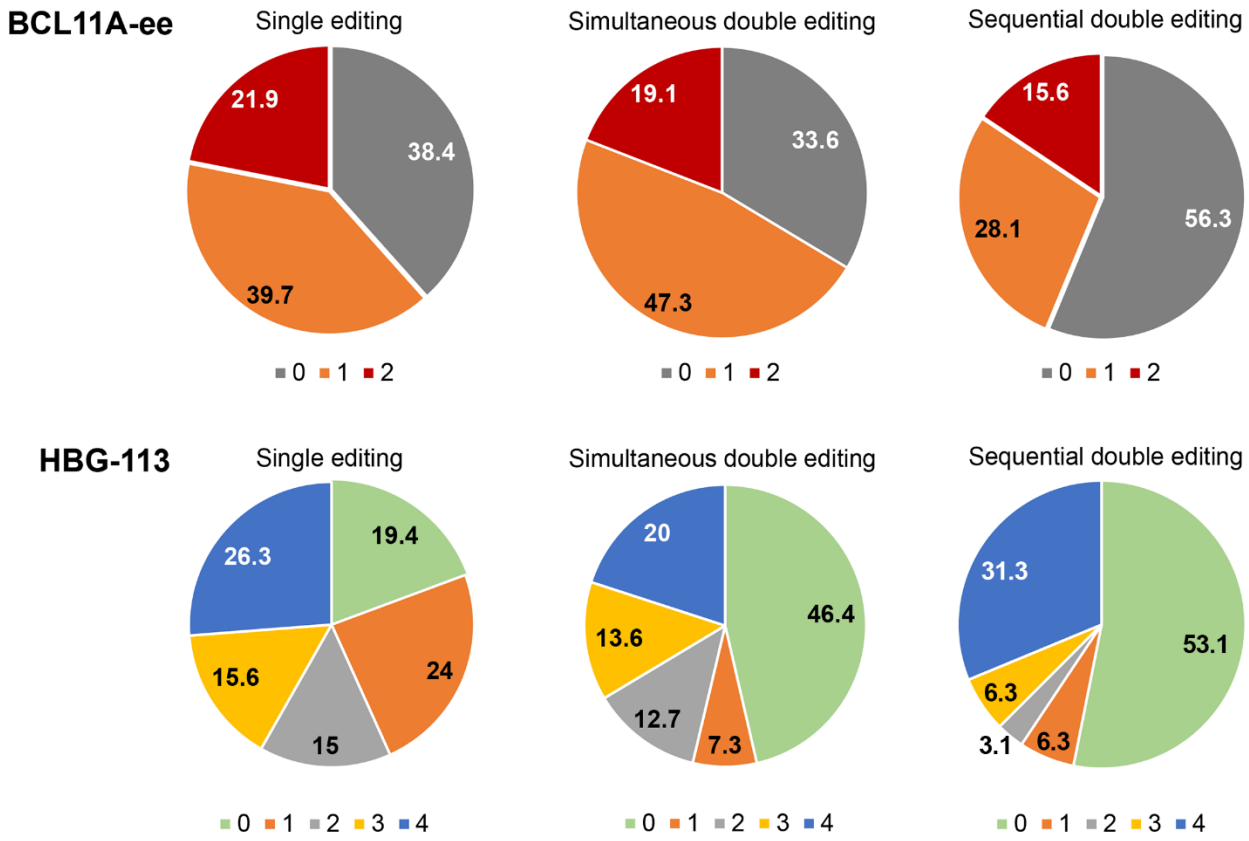
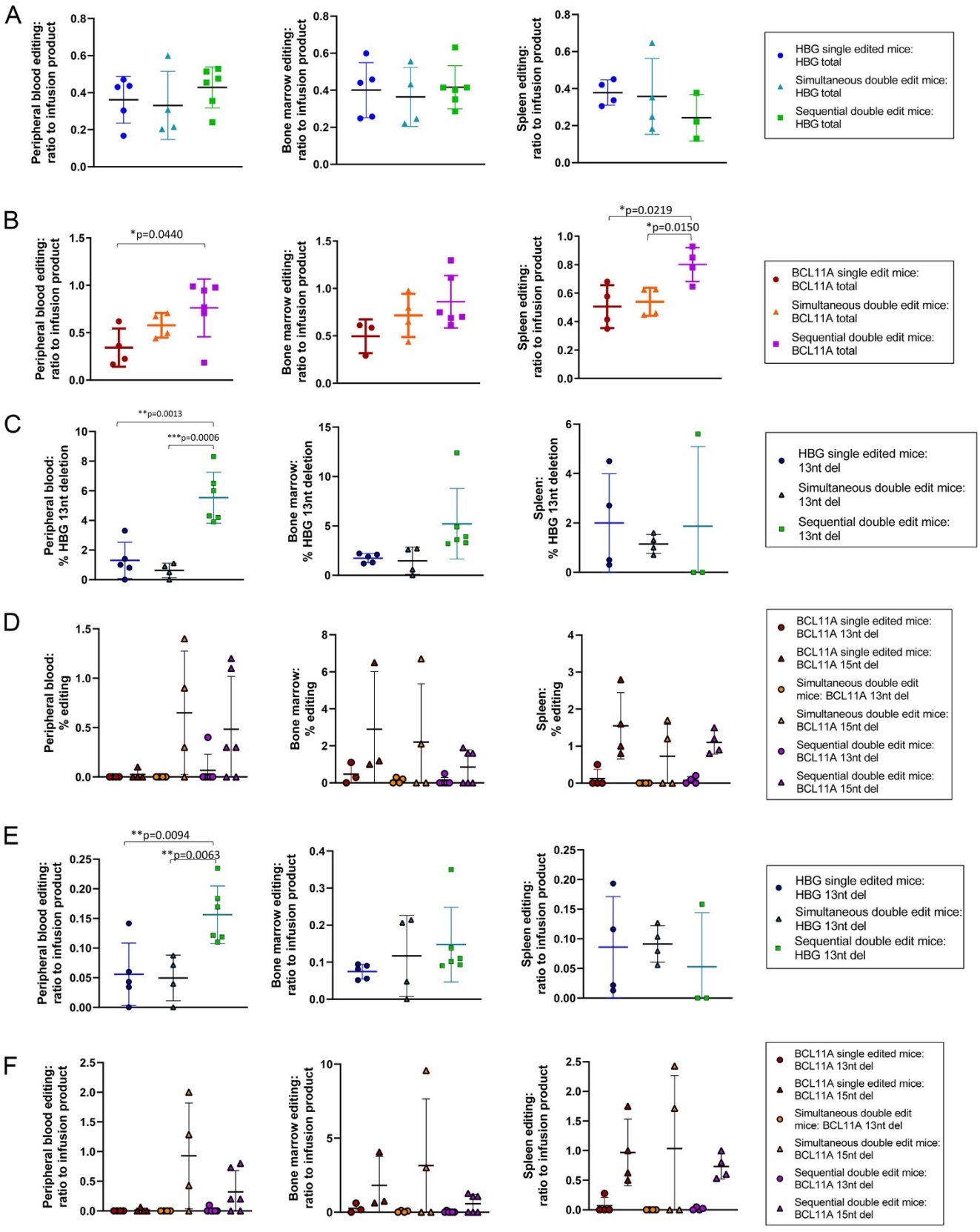


Supplementary Figure S1. Indel patterns in individual BFU-Es following single or dual CRISPR/Cas9 editing. Indel patterns observed at HBG-113 and BCL11A-ee within individual BFU-Es were analyzed, following single, simultaneous dual or sequential dual editing reactions. A reduction in larger indels was observed with simultaneous dual editing, particularly at the HBG-113 locus, consistent with saturation of the MMEJ repair pathway. *Abbreviations*: BCL11A: BCL11A-ee; HBG: HBG-113.



Supplementary Figure S2. Distribution of allele numbers edited in each reaction at each locus, on analysis of single BFU-Es (%)


A
Peripheral blood editing: ratio to infusion product
Bone marrow editing: ratio to infusion product
Spleen editing: ratio to infusion product
HBG single edited mice: HBG total
Simultaneous double edit mice: HBG total
Sequential double edit mice: HBG total
B
*p=0.0440
*p=0.0219
*p=0.0150
BCL11A single edit mice: BCL11A total
Simultaneous double edit mice: BCL11A total
Sequential double edit mice: BCL11A total
C
**p=0.0013
***p=0.0006
Peripheral blood: % HBG 13nt deletion
Bone marrow: % HBG 13nt deletion
Spleen: % HBG 13nt deletion
HBG single edited mice: 13nt del
Simultaneous double edit mice: 13nt del
Sequential double edit mice: 13nt del
D
Peripheral blood: % editing
Bone marrow: % editing
Spleen: % editing
BCL11A single edited mice: BCL11A 13nt del
BCL11A single edited mice: BCL11A 15nt del
Simultaneous double edit mice: BCL11A 13nt del
Simultaneous double edit mice: BCL11A 15nt del
Sequential double edit mice: BCL11A 13nt del
Sequential double edit mice: BCL11A 15nt del
E
**p=0.0094
**p=0.0063
HBG single edited mice: HBG 13nt del
Simultaneous double edit mice: HBG 13nt del
Sequential double edit mice: HBG 13nt del
F
BCL11A single edited mice: BCL11A 13nt del
BCL11A single edited mice: BCL11A 15nt del
Simultaneous double edit mice: BCL11A 13nt del
Simultaneous double edit mice: BCL11A 15nt del
Sequential double edit mice: BCL11A 13nt del
Sequential double edit mice: BCL11A 15nt del

Supplementary Figure S3. Editing in humanized mouse tissues following transplantation with single or dual-edited CD34$^+$ cells.

(A) HBG-113 total editing within human cells in the PB, BM and spleen at necropsy: ratio to editing frequency within infusion product for each arm (mean ±SD). **(B)** BCL11A-ee total editing within human cells in the PB, BM and spleen at necropsy: ratio to editing frequency within infusion product for each arm (mean ±SD). **(C)** HBG-113 13-nt deletion frequencies within human cells in the PB, BM and spleen at necropsy (mean ±SD). **(D)** BCL11A-ee 13- and 15-nt deletion frequencies within human cells in the PB, BM and spleen at necropsy (mean ±SD). **(E)** HBG-113 13-nucleotide deletion frequencies within human cells in the PB, BM and spleen at necropsy: ratio to editing frequency within infusion product for each arm (mean ±SD). **(F)** BCL11A-ee 13- and 15-nt deletion frequencies within human cells in the PB, BM and spleen at necropsy: ratio to editing frequency within infusion product for each arm (mean ±SD).

Abbreviations: BCL11A: BCL11A-ee; HBG: HBG-113; nt: nucleotide.