

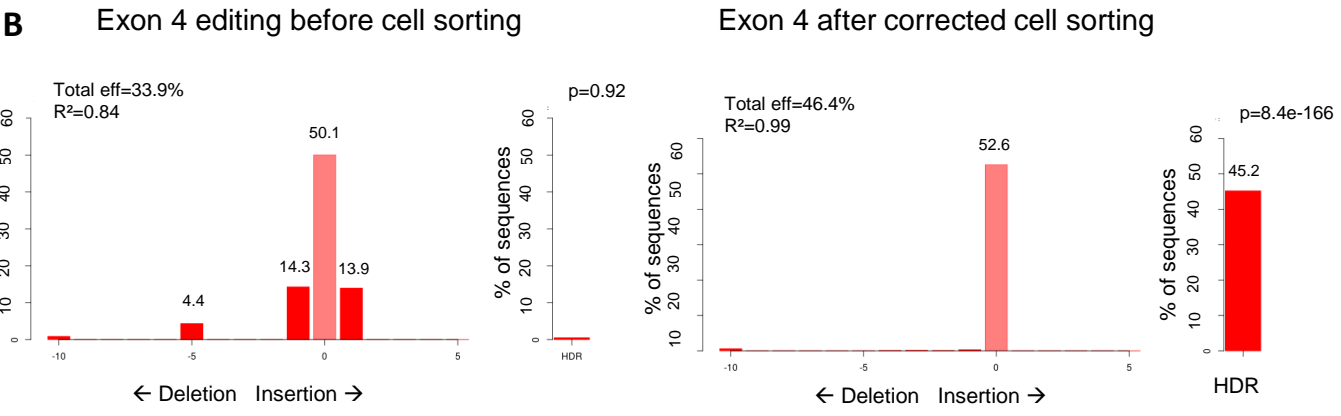
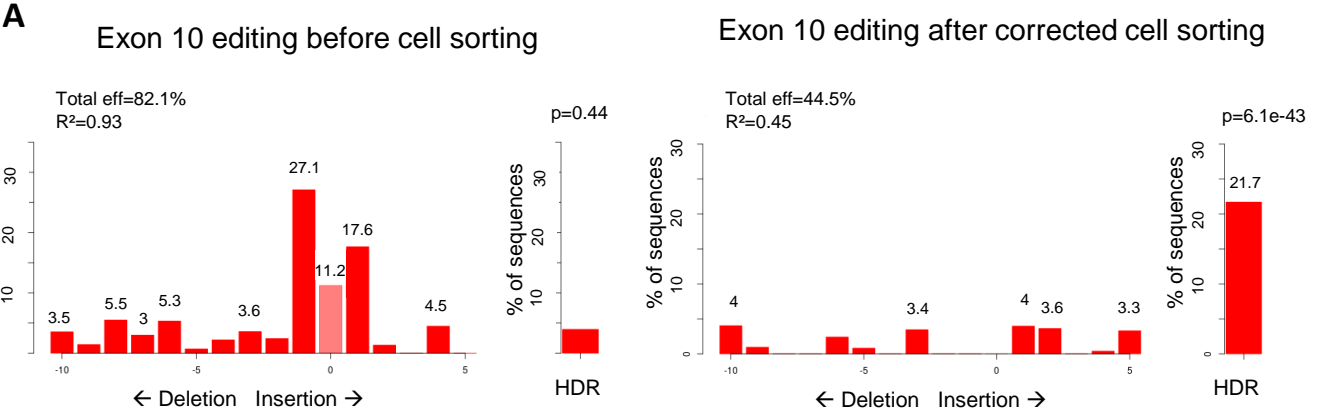
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Supplemental Information

**Mutation-Specific Guide RNA for Compound Heterozygous Porphyria
On-target Scarless Correction by CRISPR/Cas9 in Stem Cells**

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Supplemental fig 1: TIDER analyses related to Figure 2B and 3A



Supplemental fig 2: off-targets analysis of UROS exon 4 targeting (related to figure 7)

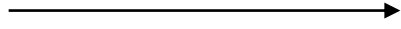
Position	Locus	Off-target sequence	Mismatche(s) number	MIT score	% InDels
1	intron:VSIG10L	GGAAGGGGCTGAGTTACGTTTGG	3	0,9	7
2	intron:KCTD5	GGCAGCAGCAGGGTTATGTTGGG	3	0,6	<loq
3	intron:AC141586.5	GGCAGCAGCAGGGTTATGTTGGG	3	0,6	<loq
4	intergenic:RP11-157N3.1-RP11-411H5.1	GGAATCATCAGAGTTACTTTGGG	3	0,6	<loq
5	intergenic:MRPS11P1-RP5-1177M21.1	GGACCCAGCTGAGTTAAGTTTGG	3	0,5	<loq
6	intergenic:PLCB1-AL121898.1	GGAACAAGGGGAGTTACGTTAGG	4	0,4	5
7	intergenic:KLF4-RP11-438P9.1	GAAAGCAGCAGAGTCATGTTGGG	3	0,3	<loq
8	intron:DSCR8	GGAAGCAGCAGAGTTACAGTTGG	2	0,3	<loq
9	intergenic:ATP6V0E1P4-AL591415.1	GGAGTCAGCACAGTTAGGTTAGG	4	0,3	<loq
10	intergenic:TACR3-RNU6-635P	AGAAGCATCAGGGTTATGTTAGG	4	0,3	<loq

CRISPOR-predicted off-targets analysis. Each predicted off-target was sequenced and analysed by ICE to evaluate Indels.

Supplemental fig 3: hiPSC-CEP karyotype after three rounds of Cas9 gRNA-4 nucleofections (related to figure 7)



CEP-hiPSC



Exon 4 edited CEP-hiPSC

Supplementary table 1: Primers sequence (5' to 3'), related to Figure 1D

PCR Primers sequence (5' to 3')

<i>UROS</i> exon 4 Forward	TAGTTCCAGGCACATAGTAAGCAC
<i>UROS</i> exon 4 Reverse	AGGAGGTGAACAACGAATAGACAG
<i>UROS</i> exon 10 Forward	GAGAGCGTGGATGCAGA
<i>UROS</i> exon 10 Reverse	ACAGCAACCATACACTCAG

sgRNA sequence (5' to 3')

Exon 4 gRNA-4	GGAAGCAGCAGAGTTACGTT
Exon 10 gRNA-10	TGCAGCCATCGGCCCCACTA

HDR template sequence (5' to 3')

Exon 4 80nt-ssODN	CATTTTTACCAGCCCCAGAGCAGTGGAAAGCAGCAGAGCTCTGT TTGGAGCAAACAATAAACTGAAGGTGAGGGTGGGT
Exon 10 75nt-ssODN	GTTTTCTCTTCTGTCTTTATAGTTTGCAGCCATCGGGCCCACTAC GGCTCGCGCGCTGGCCGCCAGGGCCTTCC

PCR Primers for off-target analysis (5' to 3')

Off-target 1 Forward	TTCCTCGCTAAGTGGGTAGC
Off-target 1 Reverse	GGGGCCAGAAAGAGGATAAG
Off-target 2 Forward	AGGTGAAGGTTTTGTGTGCGA
Off-target 2 Reverse	CAGTGAGGAATGAGGGTGGAG
Off-target 3 Forward	AGGTGAAGGTTTTGTGTGCGA
Off-target 3 Reverse	CAGTGAGGAATGAGGGTGGAG
Off-target 4 Forward	GCGTTCTTTTCCCTTACATCCTC
Off-target 4 Reverse	GCATACAAAATGTCCCAAA
Off-target 5 Forward	TCCGCTCTTTGGTGTTTAGC
Off-target 5 Reverse	CAGGCTAAGTCTGGGTCTGG
Off-target 6 Forward	CTTCCCTCCTCAACACAGGT
Off-target 6 Reverse	CAGGCAATGAGAAGGTGACA
Off-target 7 Forward	ACCCTCTGTCTTGTGTAGACT
Off-target 7 Reverse	AGCCTCAGCCTCATCATGTTT
Off-target 8 Forward	TTGATAGGAAGAAGGAATGCTG
Off-target 8 Reverse	TTCCTCCTCTTCCCTCCATCTC
Off-target 9 Forward	GACTTGCCTGTTTCGGACAT
Off-target 9 Reverse	CAGAGCCACCAAATGCAAG
Off-target 10 Forward	ACTGTCCACCATTTCTCACCA
Off-target 10 Reverse	ATCCTTCTCTTTGCCAGACC