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Supplemental Information

Cut and Paste: Efficient Homology-Directed Repair of a Dominant Negative *KRT14* Mutation via CRISPR/Cas9 Nickases

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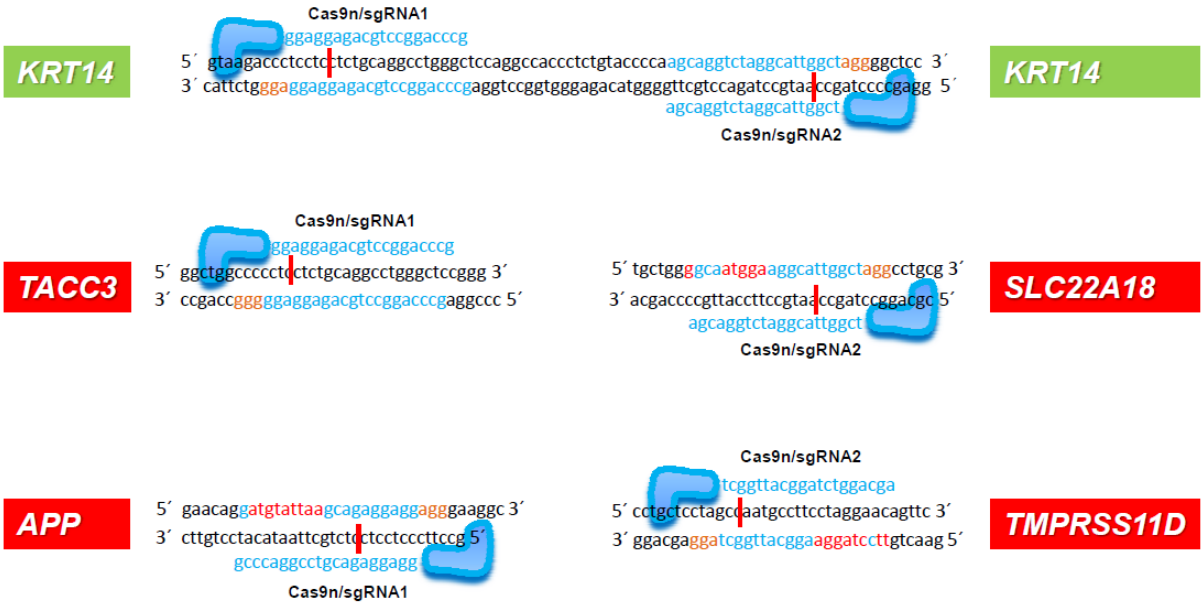
Supplementary Data

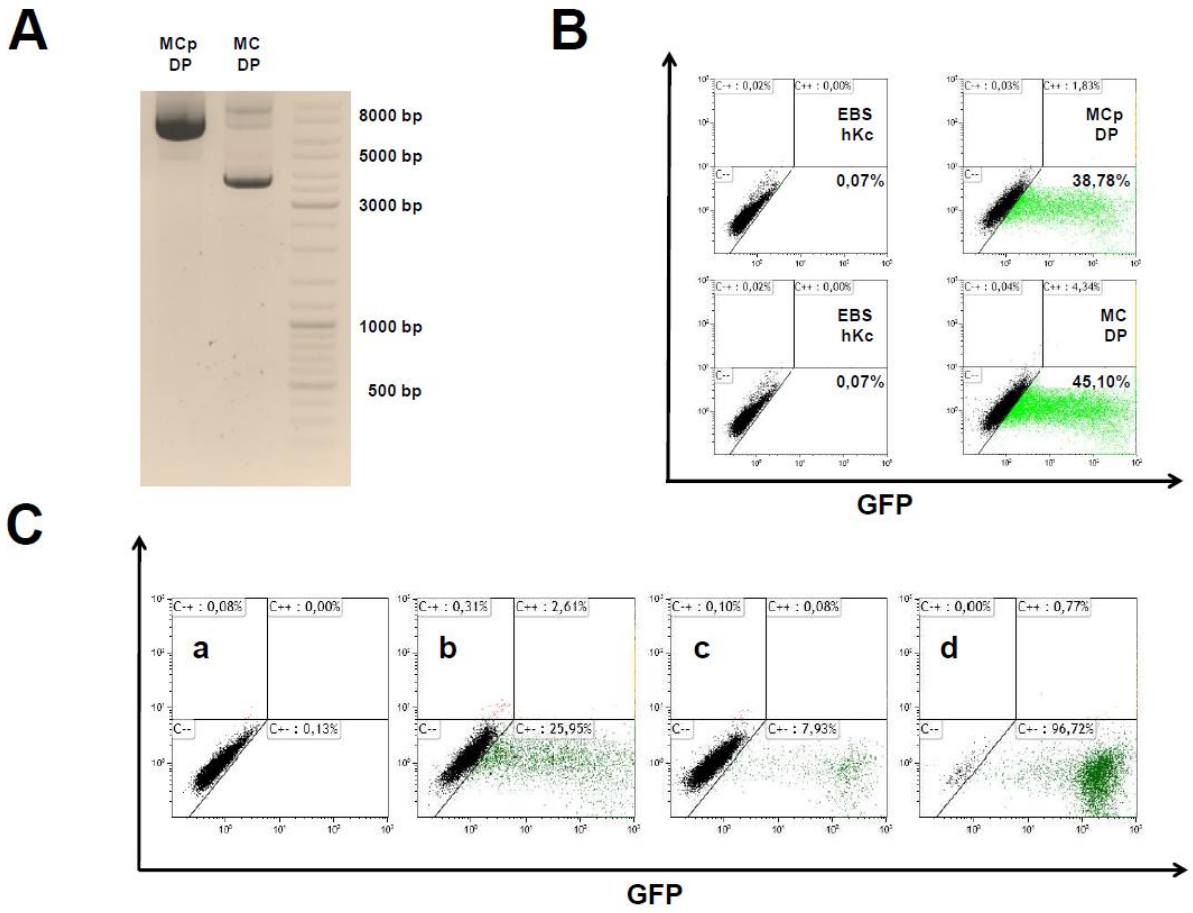
HEK 293

sgRNA 1	3´ggaggagacgtccggacccg 5´						
localization	target sequence	Cas9n/sgRNA1+2			spCas9/sgRNA1		
on-target sgRNA1	on-target sgRNA1	PAM+2	PAM+3	PAM+4	PAM+2	PAM+3	PAM+4
Chr. 17 (41583086 to 41583064) *KRT14* Intron 7 (sense)	5´cctcctcctctgcaggcctgggc 3´	0,31	0,31	0,24	3,38	6,56	16,10
off-targets sgRNA1	off-targets sgRNA1	PAM+/- 2	PAM+/- 3	PAM+/- 4	PAM+/- 2	PAM+/- 3	PAM+/- 4
Chr. 4 (1738178 to 1738200) *TACC3* Intron 10 (sense)	5´ccccctcctctgcaggcctgggc 3´	0,00	0,00	0,00	0,00	0,00	4,37
Chr. 21 (26917895 to 26917917) *APP* Intron 7 (antisense)	3´ctacataattcgtctcctcctcc 5´	0,35	0,17	0,00	0,23	0,05	0,00
Chr. 21 (44907189 to 44907211) *ITGB2* Intron 4 (antisense)	3´ttaaggtccgcgtctcctcctcc 5´	0,15	0,37	0,37	0,00	0,00	0,00
Chr. 17 (2787805 to 2787827) *RAP1GAP2* Intron 2 (sense)	5´cctcctcctctgctggccttccac 3´	0,00	0,00	0,16	0,00	0,00	2,50
Chr. 21 (42294475 to 42294497) *ABCG1* Intron 14 (sense)	5´gagcctcctctgcaggaggccag 3´	0,00	0,00	0,00	0,00	0,00	0,00
Chr. 21 (44724272 to 44724294) *PDXK* Intron 4 (sense)	5´cttcctcctctgcacgcctccgt 3´	0,08	0,00	0,00	0,87	0,00	0,00
Chr. 21 (40725550 to 40725572) *IGSF5* Intron 7 (sense)	5´actcctcctctgcaggatgaaca 3´	0,00	0,00	0,07	0,13	0,06	0,00
Chr. 21 (36962293 to 36962315) *HLCS* Intron 1 (antisense)	3´gatgctcctctgcaggccgcagg 5´	0,30	0,00	0,00	0,47	0,00	0,00
sgRNA 2	5´agcaggtctaggcattggct 3´						
localization	target sequence	Cas9n/sgRNA1+2			spCas9/sgRNA2		
on-target sgRNA2	on-target sgRNA2	PAM-2	PAM-3	PAM-4	PAM-2	PAM-3	PAM-4
Chr. 17 (41583040 to 41583018) *KRT14* Intron 7 (antisense)	3´tcgtccagatccgtaaccgatcc 5´	0,00	0,00	0,00	0,00	1,84	28,65
off-targets sgRNA2	off-targets sgRNA2	PAM+/- 2	PAM+/- 3	PAM+/- 4	PAM+/- 2	PAM+/- 3	PAM+/- 4
Chr. 11 (2936955 to 2936977) *SLC22A18* Intron 6 (antisense)	3´ccgttaccttccgtaaccgatcc 5´	0,00	0,00	0,00	0,00	0,00	0,00
Chr. 4 (67871082 to 67871104) *TMPRSS11D* Intron 1 (sense)	5´cctagccaatgccttcctaggaa 3´	0,00	0,00	0,00	0,20	0,09	0,43
Chr. 4 (113999328 to 113999350) *ANK2* Intron 1 (antisense)	3´cttaataaacccgtaaccgatcc 5´	0,00	0,00	0,00	0,28	0,23	0,00
Chr. 11 (52028437 to 52028459) *AAMDC* Intron 3 (antisense)	3´acccaatatccgtaaccgattt 5´	0,00	0,00	0,00	0,03	0,00	0,00
Chr. 5 (36657983 to 36658005) *SLC1A3* Intron 3 (antisense)	3´acttagatatccgtaaccgatta 5´	0,12	0,04	0,00	0,00	0,03	0,00

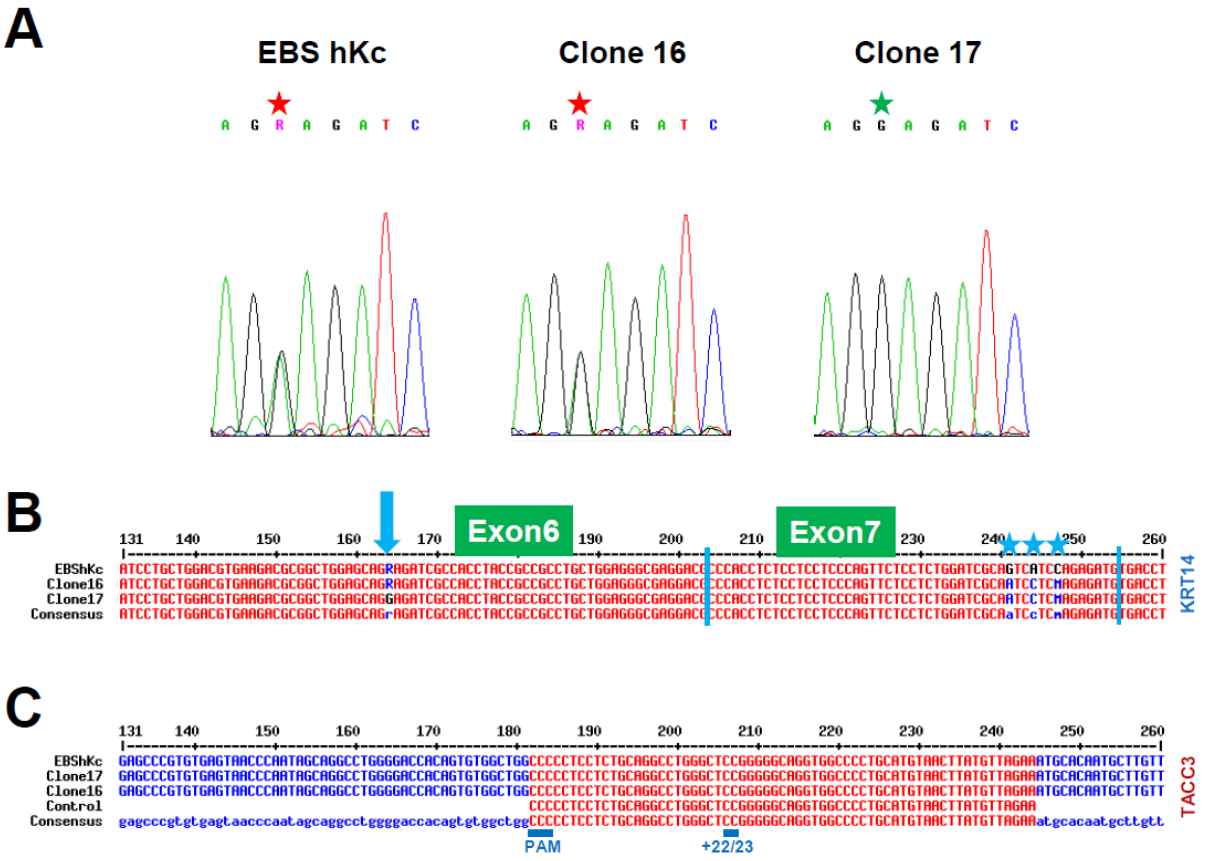
Supplementary Table 1: DNA cleavage activity of the respective nucleases at potential off-target regions for sgRNA1 and sgRNA2. DNA cleavage activities of sgRNAs at on-target and off-target regions are represented as % indels relative to untreated control. The cut-off for cleavage activity was set to 0.5%. Colored boxes indicate cleavage activity of the respective Cas9/sgRNA at on-target and off-target regions. On-target and off-target region sequences are listed, with red letters indicating the PAM position, blue letters indicating mismatches between the sgRNA on-target binding sites and the potential off-target regions and black letters indicating matches between on-target and off-target regions.



Supplementary Figure 1: Schematic depiction of on-target and off-target cutting sites. DNA cleavage sites of the two sgRNAs at on-target and predicted off-target regions are shown, with orange letters indicating the PAM position, red letters indicating mismatches between the sgRNA on-target binding sites and the potential off-target regions and blue letters indicating matches between on-target and off-target regions. Two predicted off-targets are depicted for each sgRNA, showing either antisense strand or sense strand targeting.



Supplementary Figure 2: Transfection efficiency and selection efficiency using a MC donor plasmid. (A) Size comparison of the parental MC donor plasmid (MCp-DP) and the MC donor plasmid. **(B)** Flow cytometric analysis of EBS-gen sev keratinocytes 48 hours post transfection revealed differences in transfection efficiencies. **(C)** Selection and sorting strategy for the different CRISPR/Cas9 gene editing approaches. Representative FACS blots show (a) untreated patient cells, treated cells (b) after transfection with the MC DP, (c) after blasticidin selection start and (d) after blasticidin selection stop.



Supplementary Figure 3: Correction of the dominantly inherited missense mutation (c.1231G>A) within exon 6 of *KRT14* gene in single cell clones. (A) Amplification of endogenous *KRT14* using a forward primer binding to exon 3 and a reverse primer binding exon 7 and the integrated restriction sites (EcoRI and NheI sequence). Correction of the mutation within exon 6 of *KRT14* was confirmed by sequence analysis of the PCR product. Red star: heterozygous mutation (A>G) within exon 6; green star: repaired mutation (A>G) within exon 6 of targeted allele. **(B)** Amplification of expressed *KRT14* (cDNA) using a forward primer binding to the exon 5/exon 6 junction and a reverse primer binding to exon 8. Correction of the mutation and accurate integration of the MC sequence was confirmed by sequence analysis of the PCR product. Blue arrow: position of the mutation (position 164); R indicates the heterozygous mutation. G indicates correction of the mutation. blue stars: silent mutations. **(C)** Sequence analysis of the predicted off-target region intron 10 of *TACC3*. The predicted off-target region for sgRNA1 was PCR-amplified using a primer pair binding specifically within *TACC3* intron 10. Genomic DNA isolated from single cell clones treated with Cas9n-sgRNA1+2/ MC–DP and untreated EBS keratinocytes was used as PCR template. PAM +22/23 represents positions of typical on-target indel formations within intron 7 of *KRT14* via double nicking. Sequence alignment was performed with the software "Multiple sequence alignment with hierarchical clustering" (21).

localization	HEK293 (mean coverage; reads)	EBS hKc (mean coverage; reads)	forward primer	reverse primer
on-target	**on-target**	**on-target**	**on-target**	**on-target**
KRT14 **Intron 7**	**1847**	**10075**	5´- ACCATGTATCTAATGATCCTGTCCTTTTCTA -3´	5´- CTCAGCCCCTCACGGAG -3´
off-targets sgRNA1	**off-targets sgRNA1**	**off-targets sgRNA1**	**off-targets sgRNA1**	**off-targets sgRNA1**
TACC3 **Intron 10**	**2043**	**6629**	5´- CCAACATCCACACCAGTGTTGT -3´	5´- GGCACCCTTTCTGCAGAAAGTA -3´
APP **Intron 7**	**1041**	**4549**	5´- GGAAGGAAGGAAGGAAAGAGTCATG -3´	5´- GCACTCTTCTACCAAAACATTTTCAGT -3´
ITGB2 **Intron 4**	**462**	**2172**	5´- CCATTGTGGTCTTCCTGGGTTT -3´	5´- CTAGAGGCAGAGGAGGAGGCGATA -3´
RAP1GAP2 **Intron 2**	**1312**	**4335**	5´- GCTCATGTAGCAAGTGGCTTCA -3´	5´- GGGAAGGCTACAGGAAGGATGA -3´
ABCG1 **Intron 14**	**450**	**1638**	5´- GCAGACGGCAGTCTGTTGAG -3´	5´- GAAAGTGGCCACCTGGAGTT -3´
PDXK **Intron 4**	**954**	**2827**	5´- CCCTAAAGGTTATACGAGGGACAAGT -3´	5´- CCACTATACGAGCACACTGACA -3´
IGSF5 **Intron 7**	**1367**	**4819**	5´- GGAATGCTGGAAAACTAGACCTACT -3´	5´- ATGCAATCTGGAATACACATGGGA -3´
HLCS **Intron 1**	**599**	**2273**	5´- GTTCTGCTTCAAGTATAAAAGGAGACACA -3´	5´- CCGGTTAGCTACTGTGTGCTAA -3´
off-targets sgRNA2	**off-targets sgRNA2**	**off-targets sgRNA2**	**off-targets sgRNA2**	**off-targets sgRNA2**
SLC22A18 **Intron 6**	**2711**	**2053**	5´- GTCCTCAGCTTCACCTGCAT -3´	5´- CACTTGGCTTCTATGATGTCAGCA -3´
TMPRSS11D **Intron 1**	**2084**	**4277**	5´- TTATCTGTCTCCCTCCACCAGTT -3´	5´- TGAAAGGGAAACACTTTGAGTTTCTTTAAA -3´
ANK2 **Intron 1**	**1111**	**2550**	5´- AAGTACACATGCAAGCCCTGAA -3´	5´- TCCCTGAGTCTTATAAATTTTTCCACCAAA -3´
AAMDC **Intron 3**	**2477**	**2962**	5´- CCGGCTATCCTCCAAATTCTTAACA -3´	5´- TGTTACCTGGATGTGAGACATGGA -3´
SLC1A3 **Intron 3**	**3972**	**5858**	5´- GCTACCAGTTGCAGCATTGACT -3´	5´- GTGAGAATGCCATCATTTGACCAAG -3´

Supplementary Table 2: NGS primer combinations and mean coverage of the designed panel.