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Supplemental Information

CRISPR/Cas9-Mediated Treatment Ameliorates the Phenotype of the Epidermolytic Palmoplantar Keratoderma-like Mouse

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SUPPLEMENTAL INFORMATION

Supplemental Figures

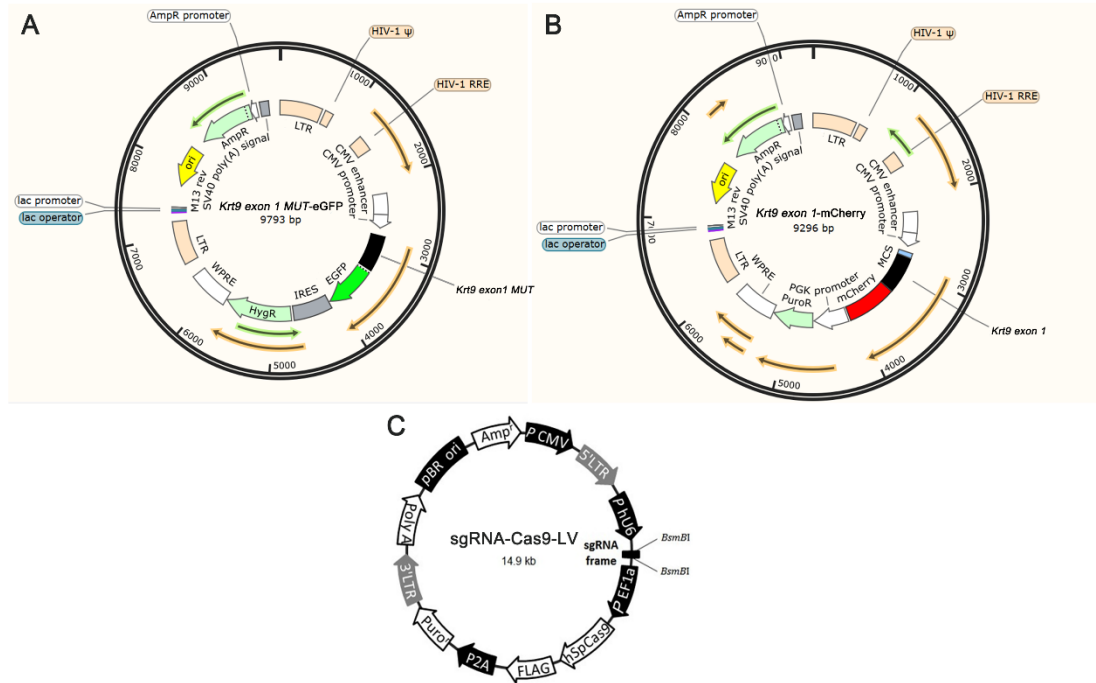


Figure S1. Constructed Vectors Used. (A, B) Diagrams of two plasmids used to establish the HeLa Hez cell line which stably expressed both *Krt9* exon1-eGFP and *Krt9* exon1 MUT-mCherry. (C) Diagram of LV delivery system for CRISPR-Cas9. Cas9-LV, sg1-Cas9-LV, and sg2-Cas9-LV all had the FLAG-expressing frame. While Cas9-LV had no sgRNA frame, sg1-Cas9-LV and sg2-Cas9-LV each had a different sgRNA frame.

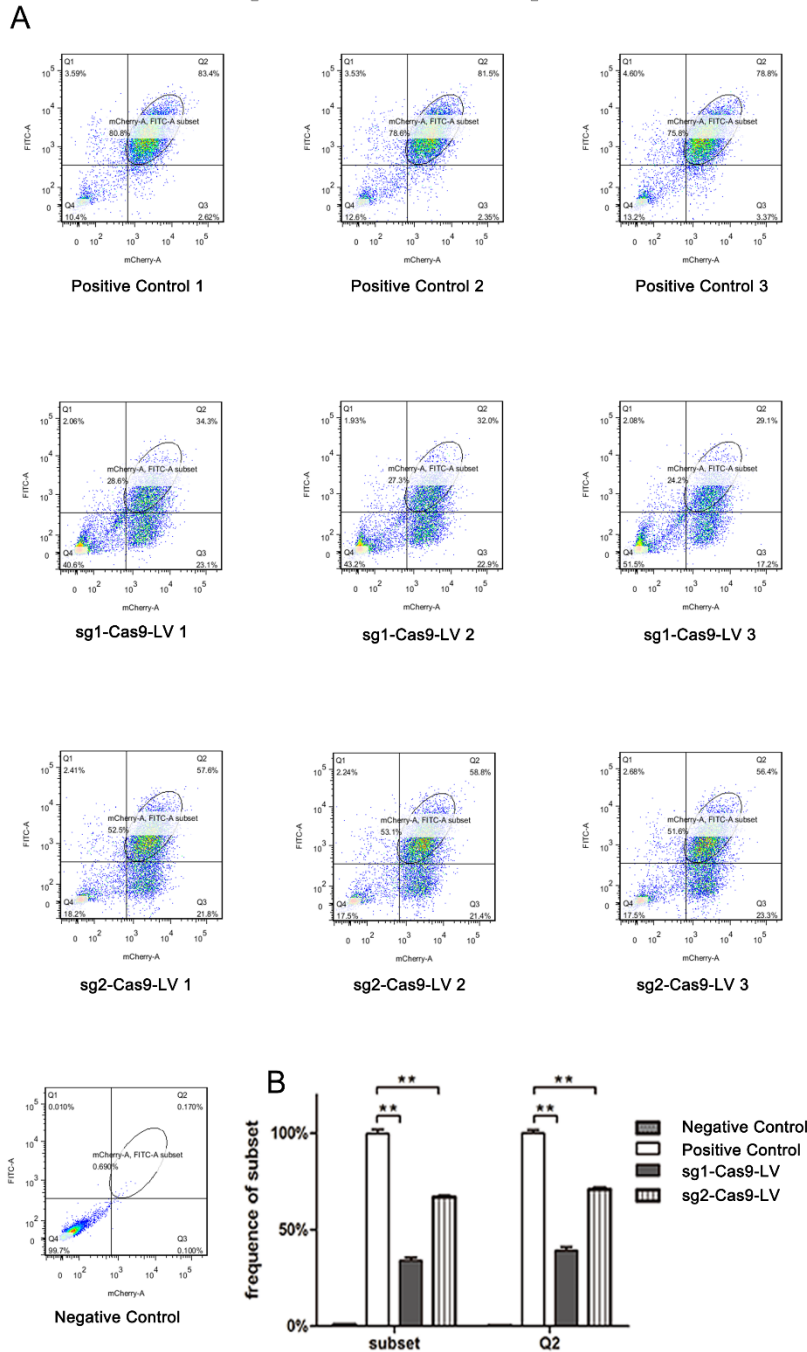


Figure S2. Flow Cytometric Analysis of Efficacy of CRISPR/Cas9 System. (A) FACs of HeLa Hezcells; circle and Q2 are subsets of cells with mCherry and eGFP fluorescence. The HeLa Hez cells were treated with different LVs. (B) Analyses of subset (circled) and Q2. 10,000 cells were collected from each sample (mean \pm SEM; $n=3$; $**P<0.01$). Each experiment was repeated at least three times.



Figure S3. Sanger Sequencing Analysis of T-A Clones. (A, B) *Krt9/c.434delAinsGGCT* was the mutation site. As the KI-*Krt9* mice had other bases (GGCT) inserted within exon 1 of *Krt9*, these bases could be identified by Sanger sequencing. Sequencing analyses of a total of 72 clones after sg1-Cas9-LV treatment revealed 31 mutated clones and 41 wild-type clones. Three clones with the mutated allele were targeted by sg1-Cas9-LV.

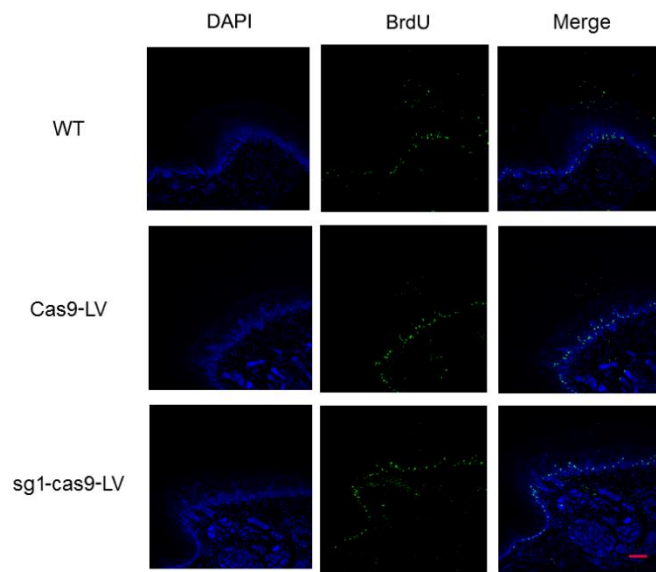


Figure S4. Immunofluorescence Against BrdU. IF staining against BrdU indicated amelioration in the epidermis of pads after sg1-Cas9-LV treatment. Scale bar, 50 μ m.

Supplemental Tables

Table S1. List of Predicted Top 49 Potential Off-target Sites

	Sequence	Locus	Gene
1	CAGTCTGGCCTCTTGGCTCCCAG	chr16: +90882305	<i>Evalc</i>
2	CCATCTGCCCTCTTGGCTCTGGG	chr14: +25613172	<i>Zmiz1</i>
3	GCGTCTGGCTTCTTGGCTCTAGG	chr9: +111451036	<i>Dclk3</i>
4	CAGTTTGCCTTCTTGGCTCAAAG	chr17: +8690449	<i>Pde10a</i>
5	TCCTCTGGCCTCATGGCTCACAG	chr5: +35367777	
6	CCTTCTGGCATCTTGGATCATAG	chr1: -135242443	
7	TCCTCTACCCTCTTGGCTCATGG	chr5: -35726673	<i>Sh3tc1</i>
8	CTGTCTTCCATCTTGGCTCAAGG	chr5: -142554011	
9	CCCTTTTGCTTCTTGGCTCAGAG	chr13: -40573456	
10	CATTCTGTTCTCTTGGCTCAGAG	chr17: +81953655	
11	CCTTACGACCTCTTGGCTCACAG	chr6: -56055534	
12	CCTACAGTCCTCTTGGCTCATGG	chr18: -5507756	<i>Gm10125</i>
13	CTGTTTGGCTACTTGGCTCATAG	chr1: -141190474	
14	CCCTCTGGCATCTTAGCTCAGAG	chr2: -91136498	<i>Mybpc3</i> (exonic)
15	CCCTCTGCCTCTTGGCTTAAGG	chr5: +45434088	<i>Qdpr</i> (exonic)
16	CCTCCTGGCTGCTTGGCTCACAG	chr12: -77928545	
17	CCATCTGCCTACTTGGCTCATAG	chr18: +10503415	<i>Greb11</i>
18	CATTCTGTCCTTTTGGCTCACAG	chr6: -148197489	<i>Ergic2</i>
19	GTCTCTGGCCTCTTGGCTCTGGG	chr4: -124255444	
20	CAGACTGCCCTCTTGGCTCCAAG	chr11: -95402645	
21	CTGGCTGTCCTCTTGGCTCTAAG	chr11: +44788874	<i>Ebfl</i>
22	CCTCTTGGCCTCTTGGCTCCCAG	chr2: -181430900	<i>Zbtb46</i>
23	CAGACTGGCTTTTTGGCTCATGG	chr2: -169325990	
24	TCGTCTGCCTTCTTGGCTCTGAG	chr9: +67972906	<i>Vps13c</i> (exonic)
25	CTCTCTGGCTTCTTGGCTCCAGG	chr17: -48899189	
26	GTGGCTGGCCTCTTGGTTCACAG	chr2: -173417917	
27	GCAGCTGGCCTCTTGGGTCAGAG	chr11: +35865560	<i>Wwc1</i>
28	GGGCCTGGCCTCCTGGCTCATGG	chr17: +90027127	
29	CAGTTTTGACTCTTGGCTCAGGG	chr2: +172958569	
30	GCCCCTGGCCTCATGGCTCATAG	chr10: -68004046	<i>Rtkn2</i>
31	CGGGCTGTCCTCCTGGCTCATAG	chr9: +47096948	
32	CAGTACCCCTCTTGGCTCAGGG	chr16: -3854315	<i>Zfp174</i>

			(exonic)
33	CCCACCTGGCTCTTGGCTCAGAG	chr2: +116705891	
34	CAGTCTTGCCTCTTAGCTCACAG	chr12: +110681716	<i>1700001k1</i> <i>9Rik</i>
35	CGGCCCCGCCTCTTGGCTCATGG	chr13: +17786816	<i>Cdk13</i>
36	CTGTAATGCCTCTTGGCTCACAG	chr5: -112962525	<i>Adrbk2</i>
37	CCTTTTGCCTCCTGGCTCACGG	chr5: +148511528	<i>Ubl3</i>
38	CATTCTGGCTTCTTGGTTCACAG	chr11: +95134670	
39	CAGTCTGACATCCTGGCTCAAGG	chr7: -87242684	
40	GCGTGGGGTCTCTTGGCTCATGG	chr5: +147914988	
41	CCGTGTTCCCACTTGGCTCAGAG	chr13: +35849660	<i>Cdyl</i>
42	CAGCCTGTCCTCTTGGCTTAGAG	chr12: +92172149	<i>Gm40598</i>
43	CTTCCTGGCCTCTTGGCTAAGAG	chr9: +50277419	
44	CTCTCTGTCCTCTTGGCTGAGGG	chr17: -27930058	<i>Anks1</i>
45	CGGTAAGGCCTGTTGGCTCAGAG	chr9: +62634220	
46	TCTTCTGGTCTCTTGGCTCTCGG	chr5: +114851500	
47	CCGTCTGATCTCTTAGCTCAAAG	chr15: +73789447	<i>Morh5</i>
48	TAGTCTGTCCTCTTAGCTCAGGG	chr3: -45836873	<i>Gm32340</i>
49	ACCTCTAGCCTCATGGCTCAAAG	chr6: +55004645	

Table S2. Summary of DNA Sequencing Results within the Top 10 Off-target Sites

	Sequence	Locus	Treated/Untreated
1	CAGTCTGGCCTCTTGGCTCCCAG	chr16: +90882305	0/20
2	CCATCTGCCCTCTTGGCTCTGGG	chr14: +25613172	0/20
3	GCGTCTGGCTTCTTGGCTCTAGG	chr9: +111451036	0/20
4	CAGTTTGCCTTCTTGGCTCAAAG	chr17: +8690449	0/20
5	TCCTCTGGCCTCATGGCTCACAG	chr5: +35367777	1/19
6	CCTTCTGGCATCTTGGATCATAG	chr1: -135242443	1/19
7	TCCTCTACCCTCTTGGCTCATGG	chr5: -35726673	0/20
8	CTGTCTTCCATCTTGGCTCAAGG	chr5: -142554011	0/20
9	CCCTTTGCTTCTTGGCTCAGAG	chr13: -40573456	0/20
10	CATTCTGTTCTCTTGGCTCAGAG	chr17: +81953655	1/19