Supplemental Materials

CRISPR-Cas9 KO Cell Line Generation and Development of a Cell-Based Potency Assay for rAAV-FKRP Gene Therapy

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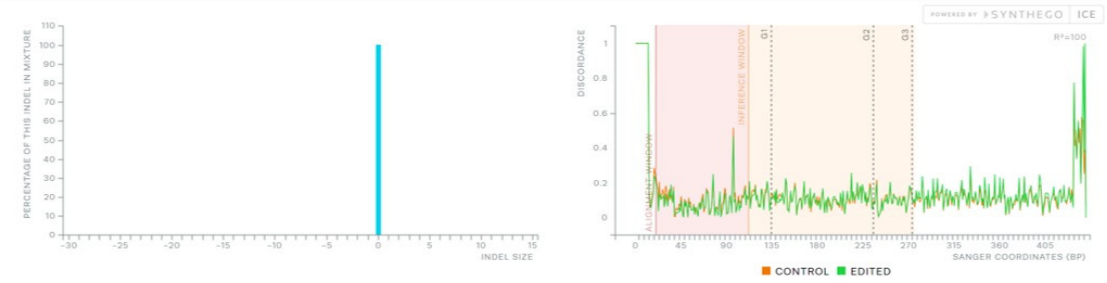
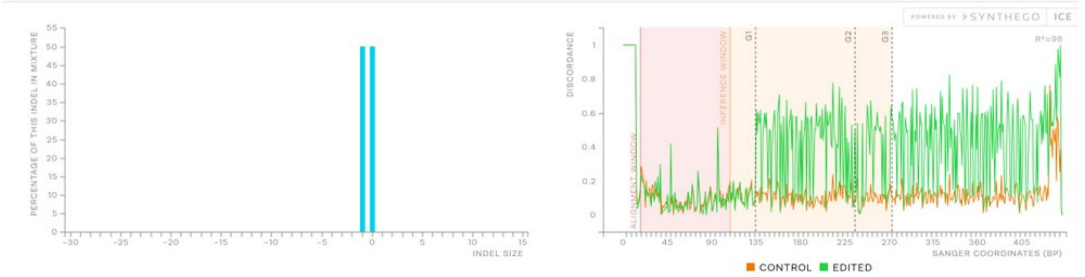
A

Guide Targets	PAM Sequences	Indel %	Model Fit (R^2)
g1 CTGGCAGCGGGTGAGCCGCA	g1 TGG	0	1
g2 AGCCTAGGAATTCCCGGGCC	g2 CGG		
g3 GCACCAGGACGGTGACACGG	g3 GGG		



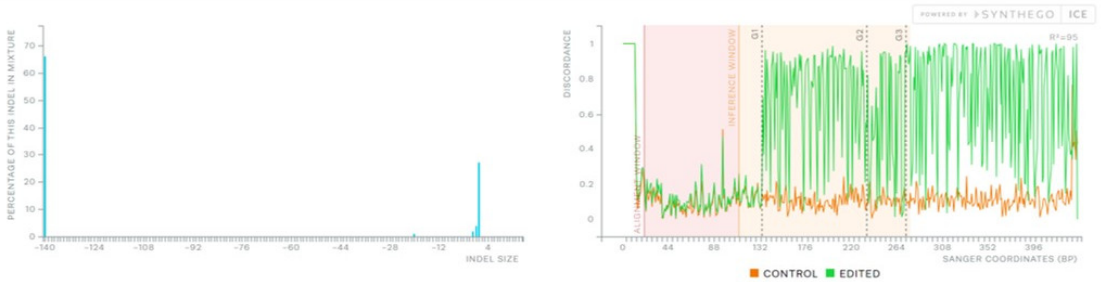
B

Guide Targets	PAM Sequences	Indel %	Model Fit (R^2)	Knockout-Score
g1 CTGGCAGCGGGTGAGCCGCA	g1 TGG	50	0.98	50
g2 AGCCTAGGAATTCCCGGGCC	g2 CGG			
g3 GCACCAGGACGGTGACACGG	g3 GGG			



C

Guide Targets	PAM Sequences	Indel %	Model Fit (R^2)	Knockout-Score
g1 CTGGCAGCGGGTGAGCCGCA	g1 TGG	96	0.95	96
g2 AGCCTAGGAATTCCCGGGCC	g2 CGG			
g3 GCACCAGGACGGTGACACGG	g3 GGG			



D

Guide Targets	PAM Sequences	Indel %	Model Fit (R^2)	Knockout-Score
g1 CTGGCAGCGGGTGAGCCGCA	g1 TGG	100	0.86	90
g2 AGCCTAGGAATTCCCGGGCC	g2 CGG			
g3 GCACCAGGACGGTGACACGG	g3 GGG			

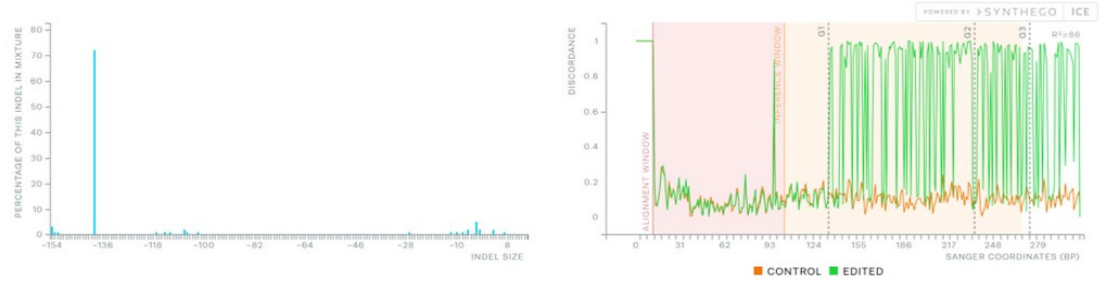


Figure S1. Gene editing efficiency and frequency of InDels determined using the ICE Software. **(A)** Representation of InDels in the non-edited wt clones (X/X); Top: sgRNAs sequences used and percentage of InDels for the mixture of sgRNAs. Bottom left: ICE InDels profiles generated by the sgRNAs. Bottom right: Comparison of the reference non-edited sequence (control in orange) and the non-edited wt clone (edited in green). **(B)** Representation of InDels in the edited heterozygous clones (X/A); Top: sgRNAs sequences used and percentage of InDels for the mixture of sgRNAs. Bottom left: ICE InDels profiles generated by the sgRNAs. Bottom right: Comparison of the reference non-edited sequence (control in orange) and the edited heterozygous clone (edited in green). **(C)** Representation of InDels in the edited compound heterozygous clones (A'/A); Top: sgRNAs sequences used and percentage of InDels for the mixture of sgRNAs. Bottom left: ICE InDels profiles generated by the sgRNAs. Bottom right: Comparison of the reference sequence non-edited (control in orange) and the edited compound heterozygous clone (edited in green). **(D)** Representation of InDels in the edited homozygous clones (A/A); Top: sgRNAs sequences used and percentage of InDels for the mixture of sgRNAs. Bottom left: ICE InDels profiles generated by the sgRNAs. Bottom right: Comparison of the reference non-edited sequence (control in orange) and the edited homozygous clone (edited in green).

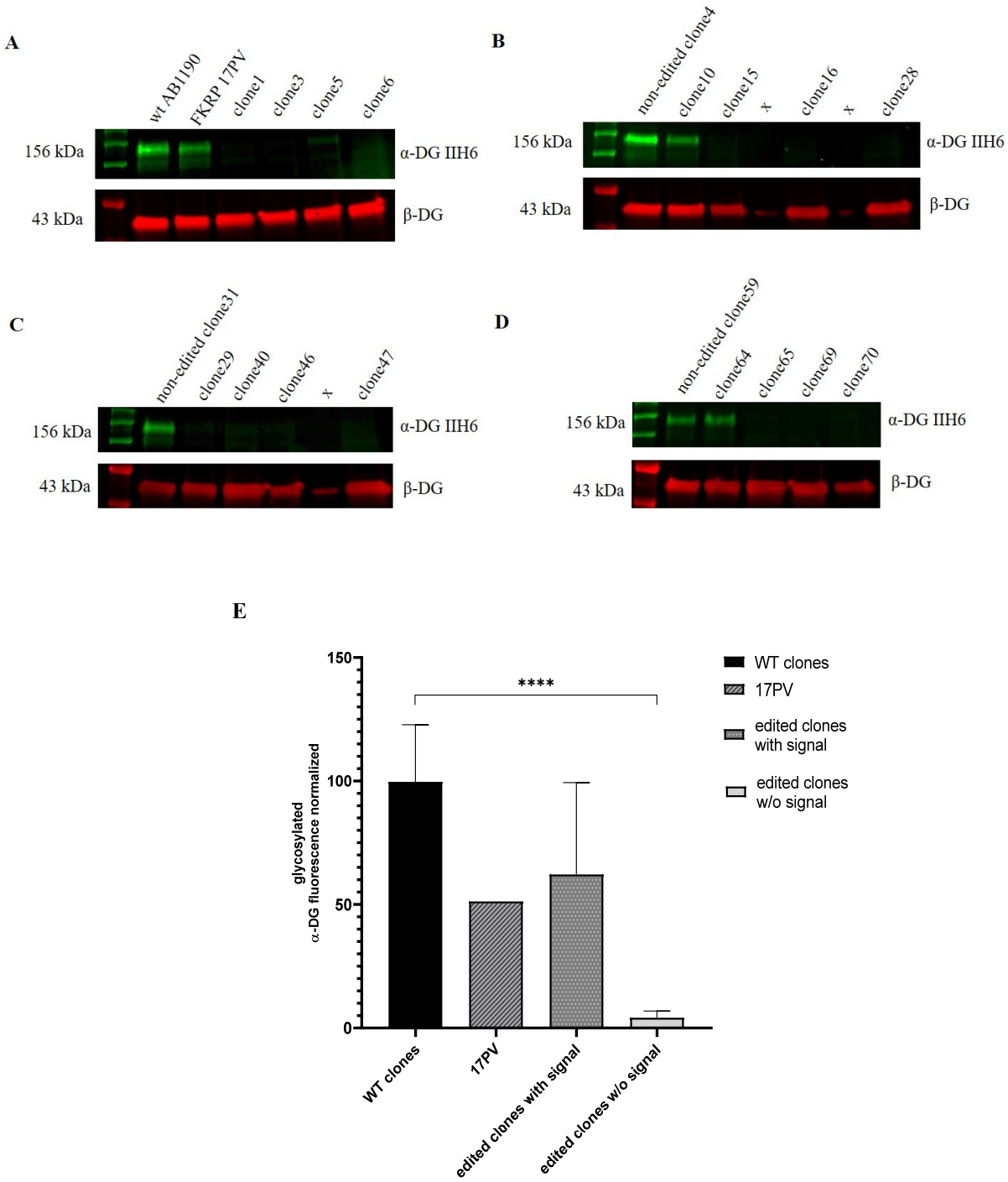


Figure S2. Expression of glycosylated α-DG in the selection of 16 clones. **(A)** Expression of the glycosylated α-DG using IIH6 antibody by western blot from the original wt myotubes (wt AB1190), the immortalized LGMDR9 patients myotubes (FKRP 17PV), and the edited clones (clone 1, clone 3, clone 5 and clone 6). β-DG was used as housekeeping protein. **(B)** Expression of the glycosylated α-DG using IIH6 antibody by western blot from the non-edited clone 4 and the edited clones (clone 10, clone 15, clone 16 and clone 28). β-DG was used as housekeeping protein. The "x" corresponds to an empty well. **(C)** Expression of the glycosylated α-DG using IIH6 antibody by western blot from the non-edited clone 31 and the edited clones (clone 29, clone 40, clone 46 and clone 47). β-DG was used as housekeeping protein. The "x" corresponds to an empty well. **(D)** Expression of the glycosylated α-DG using IIH6 antibody by western blot from the non-edited clone 59 and the edited clones (clone 64, clone 65, clone 69 and clone 70). β-DG was used as housekeeping protein. **(E)**

Quantification of glycosylated α-DG normalized in 4 groups: WT/non edited clone (AB1190/clone 4, 31 and 59), FKRP 17PV cell line, edited clones with glycosylated α-DG signal (clones 5, 10 and 64), edited clones with no glycosylated α-DG signal (clones 1, 3, 6, 15, 16, 28, 29, 40, 47, 65, 69 and 70). **** p-value < 0.0001 following an unpaired t-test.

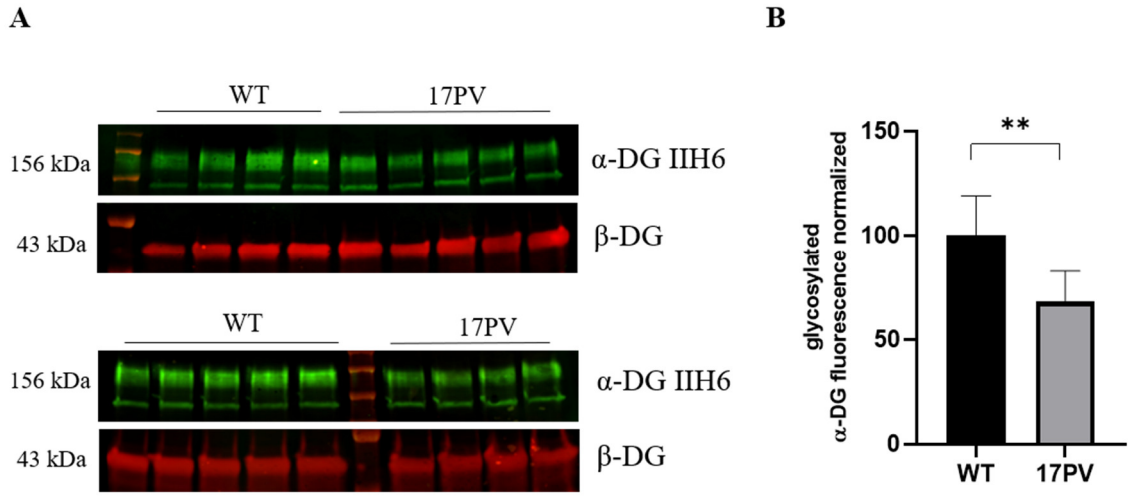


Figure S3. Expression of glycosylated α-DG in WT and FKRP immortalized cell lines. **(A)** Expression of the glycosylated α-DG using IIH6 antibody by western blot from the original wt myotubes (wt AB1190) and the immortalized LGMDR9 patients myotubes (FKRP 17PV). Two different experiments were performed. **(B)** Quantification and normalization of glycosylated α-DG normalized (n=9 per cell lines). ** p-value = 0.0011 following an unpaired t-test.

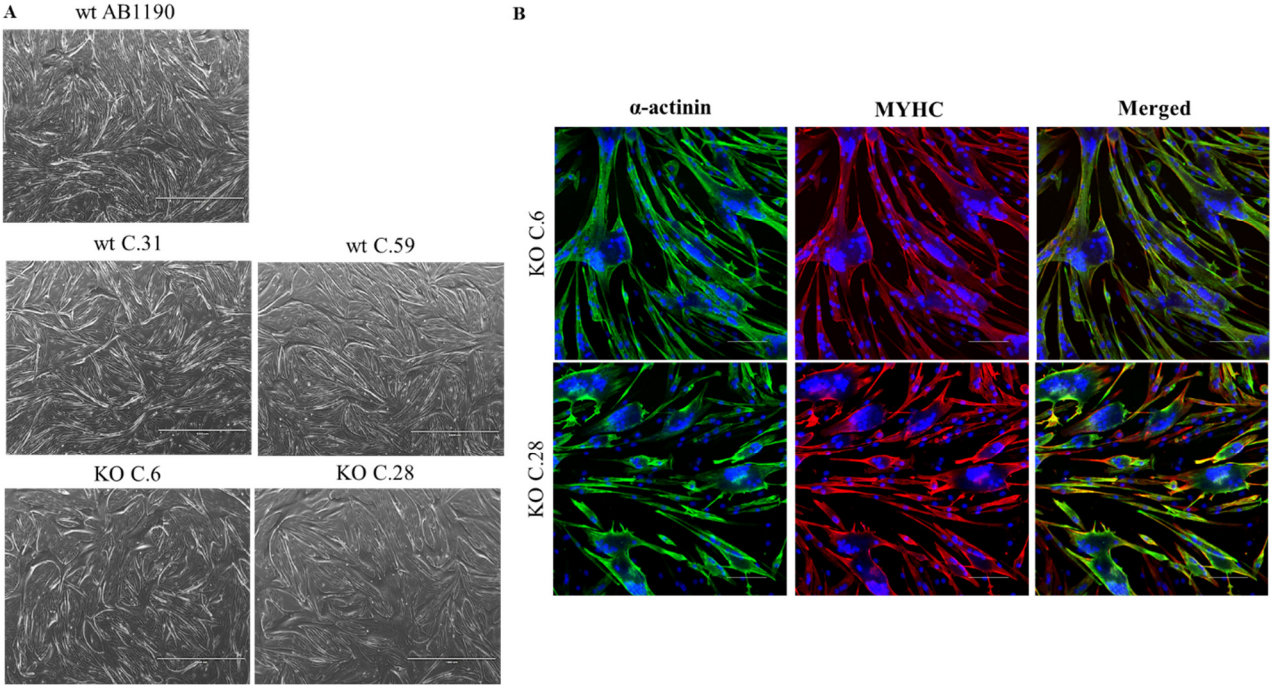


Figure S4. Myotube differentiation observed by microscopic analysis and immunofluorescence staining in edited clones. (A) Brightfield pictures of the original wt cells (AB1190), the non-edited wt clones (wt C.31 and wt C.59) and the edited wt clones (KO C.6 and KO C.28) at myotube stage. Scale bars = 1,000 μm. (B) Immunofluorescence staining (α-actinin and MYHC) of the edited clones (KO C.6 and KO C.28). Cell nuclei were labelled with DAPI dye. Scale bars = 100 μm.

Table S1. *In silico* analysis of off-targets with CRISPOR TEFOR **(A)** Top 10 locus of off-targets for sgRNA1. **(B)** Top 10 locus of off-targets for sgRNA2. **(C)** Top 10 locus of off-targets for sgRNA3.

A

Locus	mismatchCount	cfdOfftargetScore	chrom	start	end	strand
FKRP	**0**	**100**	**chr19**	**46755455**	**46755477**	**-**
intergenic:RP11-1151B14.2-RP11-1151B14.3	4	0.51	chr18	56112872	56112894	-
intergenic:AL136967.1-RP11-328M4.2	3	0.46	chr6	41384109	41384131	+
intron:ZNF862	4	0.40	chr7	149551791	149551813	-
intergenic:RP11-128M1.1-TGM3	3	0.34	chr20	2268800	2268822	+
exon:DBNL/PGAM2/AC017116.11	4	0.34	chr7	44101156	44101178	-
intergenic:LMBR1-RNF32	3	0.31	chr7	156462214	156462236	+
intron:MYO18B	4	0.30	chr22	26145944	26145966	+
intergenic:RP11-386G21.2-XKR4	4	0.30	chr8	56083410	56083432	+
intergenic:RP11-761N21.1-RP11-520A21.1	4	0.29	chr3	40950300	40950322	-
intergenic:RXRA-RP11-473E2.2	4	0.28	chr9	137393918	137393940	-

B

Locus	mismatchCount	cfdOfftargetScore	chrom	start	end	strand
FKRP	**0**	**100**	**chr19**	**46755556**	**46755578**	**+**
intergenic:CTC-461H2.2-RHPN2	4	0.71	chr19	33554035	33554057	+
intergenic:KCNN3-PMVK	3	0.67	chr1	154845237	154845259	-
lnc:AC006116.17/ZSCAN5A/Y_RNA	3	0.62	chr19	56826681	56826703	+
intergenic:RNU7-145P-RP11-142I20.1	4	0.53	chr18	38950440	38950462	-
intergenic:RPS23P3-RNU6-699P	4	0.50	chr4	67430059	67430081	+
lnc:RP11-50E11.2/RP11-50E11.3	4	0.41	chr10	52397448	52397470	-
intron:DFNB31	4	0.40	chr9	117170569	117170591	-
intergenic:COMP-UPF1	4	0.39	chr19	18924078	18924100	-
exon:C6orf226	4	0.33	chr6	42858415	42858437	-
exon:CD22	3	0.30	chr19	35820497	35820519	+

C

Locus	mismatchCount	cfdOfftargetScore	chrom	start	end	strand
FKRP	**0**	**100**	**chr19**	**46755594**	**46755616**	**-**
exon:PYGM	4	0.57	chr11	64518824	64518846	+
intron:RP11-890B15.2	4	0.54	chr11	130723063	130723085	-
intron:GPR56	4	0.39	chr16	57692790	57692812	+
intergenic:SNAP25-AS1-SDAD1P2	4	0.37	chr20	10360573	10360595	+
intron:AGAP3	3	0.33	chr7	150805390	150805412	-
intergenic:MDH2-AC005077.14	4	0.32	chr7	75698884	75698906	+
intron:CUX1	4	0.31	chr7	101818983	101819005	-
intron:AC008271.1	4	0.31	chr2	15831614	15831636	-
intergenic:RP11-324D17.2-RP11-324D17.1	4	0.30	chr16	54276113	54276135	-
intergenic:VPS37D-DNAJC30/WBSCR22	4	0.26	chr7	73090050	73090072	+