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Supplemental Information

Genome Editing-Mediated Utrophin Upregulation in Duchenne Muscular Dystrophy Stem Cells

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Supplementary Methods

Off-targets prediction and validation

To determine the potential off target activity of guide RNAs we have used the COSMID off-target prediction analysis tool (https://crispr.bme.gatech.edu/)[43] which rank order the off-target sites based on sequence similarity with the guide RNA provided. Top ranked homologous sites for each guide RNA are PCR amplified and sequenced to screen for any mutation in the genomic DNA of edited clonal cell lines.

Dual sgRNA mediated *UTRN*IMTR deletion efficiency quantification

To determine the cutting efficiency of the sgRNA pairs tested in this study, we transfected HEK 293T cells with plasmids expressing the SaCas9 and dual sgRNAs (sgRNA 1&3, sgRNA 1&4, sgRNA 2&4 and sgRNA 2&3). Three days after the transfected cells were lysed and PCR screened with *UTRN* forward and reverse primer mentioned before. Equal amount of PCR product and 5 µl of TrackIT 100 bp DNA ladder were run on 2% agarose gel. The gel image was captured using a G:Box imaging system (SYNGENE). Both unedited and UTRNΔIMTR edited PCR products in each lane were quantified using ImageJ software v2.0 in reference with the appropriate size DNA ladder band in the same gel. Deletion efficiency of each pair of sgRNA were shown as percentage of number of DNA copies in edited PCR product normalized with DNA copies in the total PCR product.

Utrophin protein expression in hiPSC differentiated myotubes

The wild type, unedited DMD and the edited *UTRN*ΔIMTR hiPSC cell lines were differentiated by MyoD overexpression and total 10 μg cell protein extracts were loaded for utrophin western blotting. α-Tubulin was used as loading control.

Supplementary Figure legends

Supplementary Figure 1. *UTRN*IMTR Deletion efficiency of sgRNA pairs

The DNA gel shows PCR product from unedited and *UTRN*ΔIMTR edited genome in HEK 293T cells transfected with SaCas9 and different sgRNA pairs. The sgRNA pair 1&4 shows maximum deletion efficiency (56%).

Supplementary Figure 2. Expression of β-dystroglycan (β-DG) in differentiated myotubes

Wild type, DMD and *UTRN*ΔIMTR myotubes were stained with mouse anti β-DG (green) and DAPI (blue). Scale bar = 200 μm. The wild type myotubes show higher expression of cytoplasmic β-DG. In absence of dystrophin, the DMD myotubes show lack of β-DG staining. Whereas, *UTRN*ΔIMTR myotubes show restoration of β-DG expression compared with the DMD myotubes.

Supplementary Figure 3. SaCas9-GFP-SgRNA1&4 plasmid map

The plasmid map shows expression cassette SaCas9 with C-terminal EGFP transgene under EF1-α promoter. The guide RNA pairs are cloned under U6 promoter.

Supplementary Figure 4. Utrophin protein expression in hiPSC differentiated myotubes

Utrophin expression in wild type, DMD and *UTRN*ΔIMTR hiPSC cell line derived myotubes were quantified by western blotting. A. Representative western blot shows expression of utrophin in wild type, DMD and edited *UTRN*ΔIMTR hiPSC differentiated myotubes. α-Tubulin was used as loading control. B. Densitometric analysis of the utrophin western blot to quantify utrophin expression in the myotubes. Bars represent mean ± SEM (Mean from three different

experiments with three different wells each, n=9). Difference in utrophin expression between DMD and *UTRN*ΔIMTR myotubes were statistically analyzed by Kruskal-Wallis test (*P=0.04).

Supplementary Tables

Table S1. Oligonucleotide sequences for guide RNAs cloning

Name	Guide RNA Sequence	Sense Oligo (5'-3')	Antisense oligo (5'-3')
sg1	TCTATGTCACTGCTTCTACAG	CACCGTCTATGTCACTGCTTCTACAG	AAACCTGTAGAAGCAGTGACATAGAC
sg2	GGTACCTCCACCTACATCTTT	CACCGGGTACCTCCACCTACATCTTT	AAACAAAGATGTAGGTGGAGGTACCC
sg3	CATAAAGCAGTTTCCAATGCA	CACCGCATAAAGCAGTTTCCAATGCA	AAACTGCATTGGAAACTGCTTTATGC
sg4	GAAGACACCAAATCTACAACT	CACCGGAAGACACCAAATCTACAACT	AAACAGTTGTAGATTTGGTGTCTTCC

Table S2. Off-target sites of guide RNA1.

	Chromosome position	Query type	Mismatch	Cut site	COSMID Score
1	Chr12:111687717-111687742	Del 9	2	111687733	1.68
2	Chr6:145581830-145581855	Del 9	2	145581846	1.85
3	Chr4:97683403-97683428	Del 14	2	97683412	2.99
4	Chr4:97683403-97683428	Del 12	2	97683412	2.99
5	Chr7:118654093-118654118	Del 19	2	118654109	6.81

Table S3. Off-target sites of guide RNA4.

	Chromosome position	Query type	Mismatch	Cut site	COSMID Score
1	Chr6:47204675-47204701	No indel	3	47204692	1.1
2	Chr5:32834524-32834549	Del 18	2	32834540	1.57
3	Chr1:180279087-180279114	Ins 18	2	180279105	2.14
4	Chr5:26710353-26710380	Ins 11	2	26710371	5.32
5	Chr15:82779792-82779818	No indel	3	82779801	5.85

Table S4. Primer sequences used for off-target sites PCR amplification.

Target	Forward primer (5'-3')	Reverse primer (5'-3')
Off-target1/sgRNA1	AGTAGCACCTCTCCCCAGG	CTGAGGCAGGAAGCTTGAA

	T	C
Off-target2/sgRNA1	TTGCAATTGTTTTTGGCATC	CTATGCCCAAATAGCCAAGG
Off-target3/4/sgRNA1	ACAATGAGCCCTTACCCAGA	GCATCTCGTGTCTCAACATCA
Off-target5/sgRNA 1	GCCAGGAAGTCCAAGATCAG	GCAAACATCGTTTTGTGAAGG
Off-target1/sgRNA 4	TGCACACAAGGTAAGCCAAA	GAACCAGGGGAGTGATCTGA
Off-target2/sgRNA 4	CCCTCATCACAGGCAGTTTT	TTCACTCGGTGTTTCTGACG
Off-target3/sgRNA 4	AAAAAGACCCACCCATCCTT	CAACAGCGCAAGACTCTGTC
Off-target4/sgRNA 4	CTGATGCCCACCTGCTAAGT	GGCTGTGGTGAGCCATTATT
Off-target5/sgRNA 4	TGCAGTGAGCTGAGACCTTG	AGGGCTAGTAGGGAGCGTGT

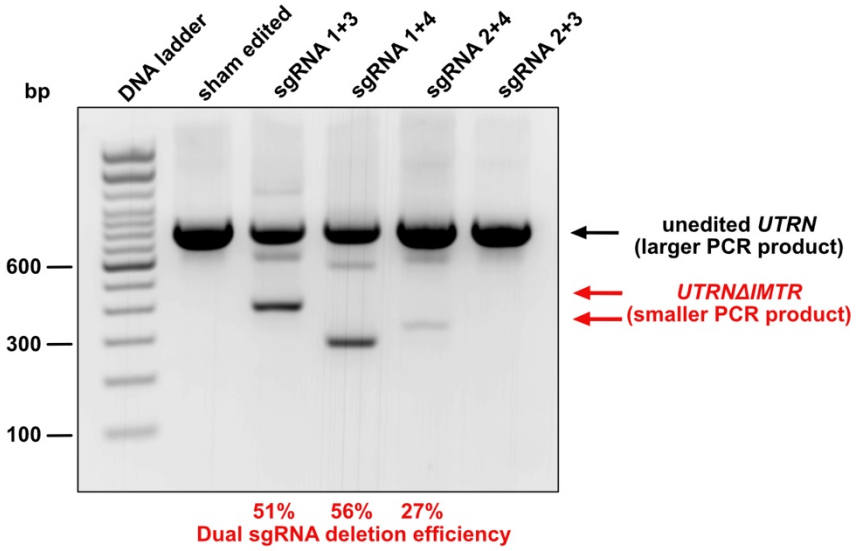
Table S5. Primer sequences used for qPCR.

Target	Forward primer (5'-3')	Reverse primer (5'-3')
MyoD1	TACCCAAGGTGGAGATCCTG	ATAGATCATGGGCGGTTCAG
NANOG	CAAAGGCAAACAACCCACTT	TCTGCTGGAGGCTGAGGTAT
MyoG	CAGTGCCATCCAGTACATCG	AGGTTGTGGGCATCTGTAGG
MyoD1endo	CCCAAGGTGGAGATCCTG	CCGCTGTAGTCCATCATGC
GAPDH	GAGTCAACGGATTTGGTCGT	GACAAGCTTCCCGTTCTCAG

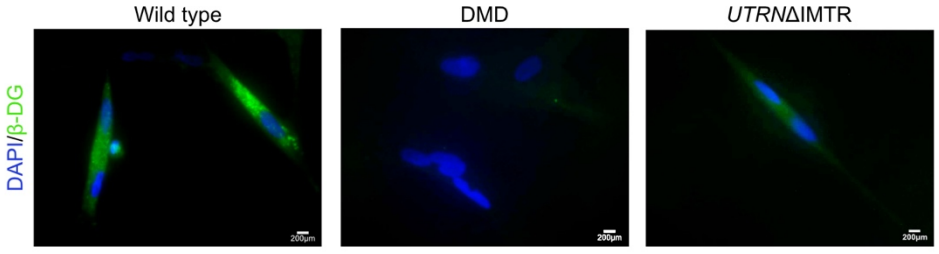
Supplemental References

1. Cradick, TJ, Qiu, P, Lee, CM, Fine, EJ, and Bao, G (2014). COSMID: A Web-based Tool for Identifying and Validating CRISPR/Cas Off-target Sites. *Mol Ther Nucleic Acids***3**: e214.

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3