Supplemental Figures and Tables

Structural Perturbations of Exon Skipping Edits within the Dystrophin D20:24 Region

Xin Niu and Nick Menhart

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Figure S1

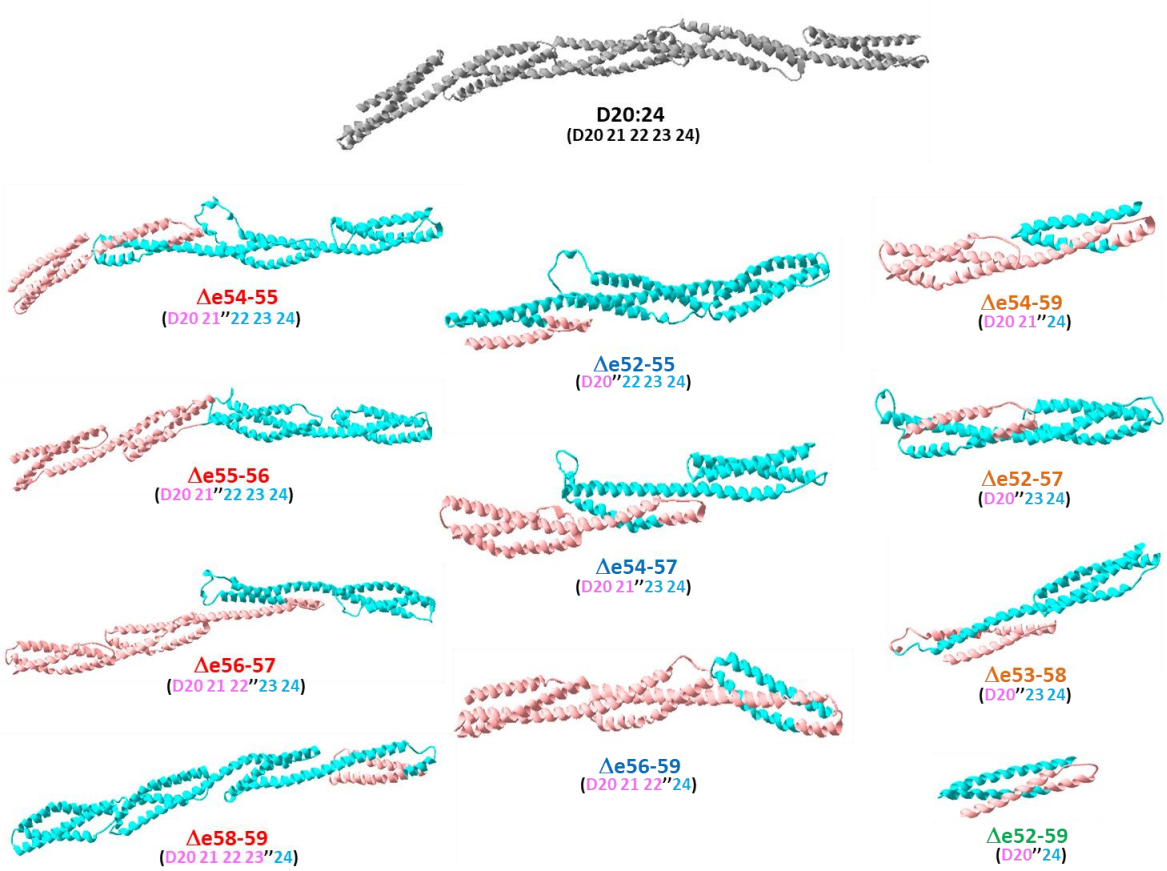

Figure S1. Target rod models showing edit site in relation to STR structure. Target proteins were modelled using the Robetta protein modelling server. At the top, the 5-STR D20:24 unedited rod is shown in grey. Below, the eleven targeted exon edited proteins are shown with the N-terminal region before the edit site in pink, and the C-terminal region after the site in blue. Also shown below the name of each edit is the STR structure in the same coloring scheme. Partial STRs are denoted as 'X' (N-terminal region) or 'Y (C-terminal fraction). Thus, hybrid STRs become X"Y. We can see that only Δe55-56 has the exon edit junction at an STR junction, between the 2nd and 3rt STR there. All others have edit junctions internal to STRs, so creating a hybrid STR at the edit site.

Figure S2

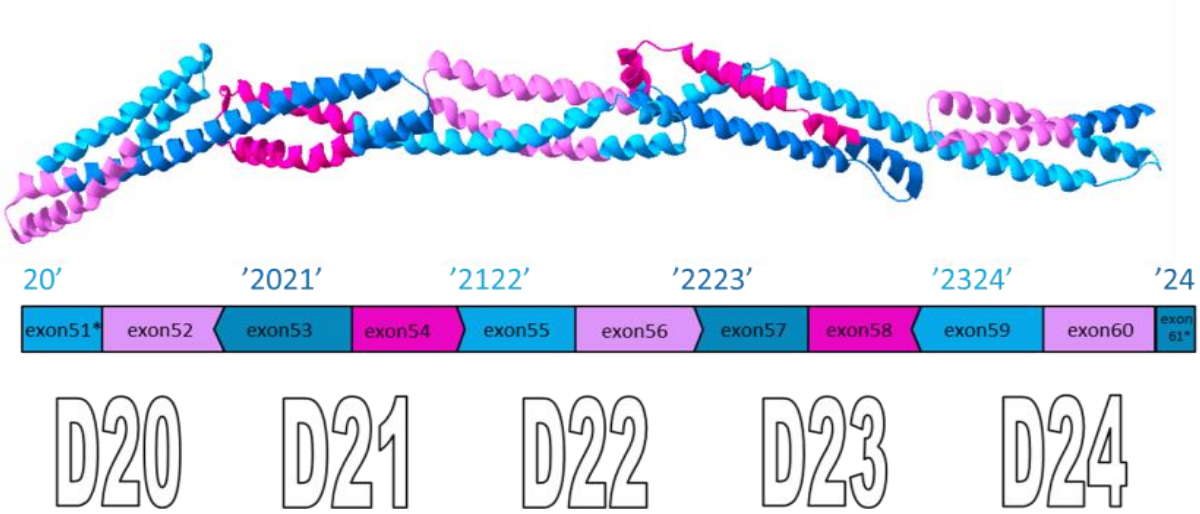

Figure S2. STR structure in relation to exon structure. The modelled D20:24 native rod consists of five STRs, and is presented with the exons colored alternately magenta and blue (the intensity of each is modulated for clarity, where they overlap in the 3-dimnesional modelled structure). We see that the model is consistent with a unified rod, where the last helix of one STR propagates directly into the first STR of the next. Exon reading frame is shown by the shape the vertical edges for the exons: **|** is frame 0 (i.e. at codon boundary); **>** is frame 1 (i.e. between 1st and 2nd base of a codon). **<** is -1 frame (i.e. between 2nd and 3rd base of a codon). Viable edits need to match up compatible edges to ensure proper reading frame.

Figure S3

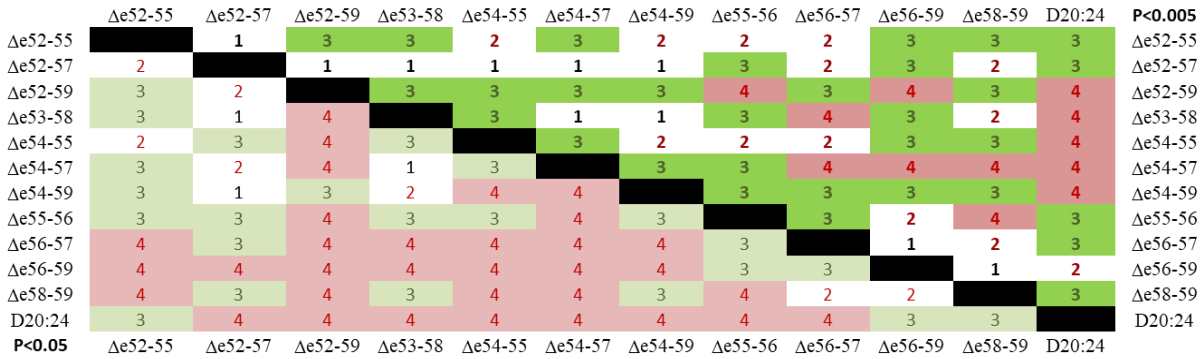

Figure S3. Significance of Differences in Edited Rod Properties The number of comparisons of four protein property parameters (f_a, Tm, ΔH, PK_{50}) among all 12 rods (D20:24 wild-type plus 11 exon-edit targets) that are statistically significant for all pair-wise comparisons is presented. In the upper right triangle the standard was *highly significan*t, P<0.005; and in the lower left the standard was *significant* P<0.05. Of the 66 pairwise comparisons, 50% of them show significance at P<0.05 in all four measured properties, and 85% in at least three. For highly statistically significant differences. P<0.005, 18% achieved this in four measurements, and 65% in at least three methods. This emphasizes that the nature of the edit site has a large impact on protein properties.

Table S1

edit in this study	relevant DMD defects and potential AON therapies							
	DMD defect	Leiden database identifier	Leiden database # Dec 2020	3' repair exon skip	DMD defect	Leiden database identifier	Leiden database # Dec 2020	5' repair exon skip
Δe54-55	Δe54	53i_54i	30	exon55**	Δe55	54i_55i	51	exon54***
Δe55-56	Δe56	54i_55i	51	exon56	Δe56	55i_56i	22	exon55**
Δe56-57	Δe57	55i_56i	22	exon57	Δe57	56i_57i	3	exon56
Δe58-59	Δe58	57i_58i	2	exon59	Δe59	58i_59i	2	exon58
Δe52-55	Δe52-54	51i_54i	49	exon55**	Δe53-55	52i_55i	69	exon52**
Δe54-57	Δe54-55	53i_56i	2	exon57	Δe55-57	54i_57i	0	exon54**
Δe56-59	Δe56-58	55i_58i	0	exon59	Δe56-59	56i_59i	1	exon56
Δe52-57	Δe52-56	51i_56i	2	exon57	Δe53-57	52i_57i	5	exon52**
Δe53-58	Δe53-57	52i_57i	5	exon58	Δe54-58	53i_58i	0	exon53*
Δe54-59	Δe54-58	53i_58i	0	exon59	Δe55-59	54i_59i	1	exon54***
Δe52-59	Δe52-58	51i_58i	1	exon59	Δe53-59	52i_59i	3	exon52**

* AONs targeting exon 53 are approved: golodirsen or viltolarsen

** in preclincal development, Sarepta

*** was in preclinical development at Wave Lifesciences

Table S1 Known DMD defects relevant to targets studies. The edits in this study are the endpoints of exon skipping repair of DMD defects as shown, by skipping exons on the 5' or 3' side of the specific defects show. The number of known patients in the public Leiden DMD database is shown as of December 2020 (as well as the edit notation used in that database). One target, Δe53-57, is amenable to exon skipping by currently available therapeutics, golodirsen or viltolarsen. Seven other targets in this study are relevant to AONs that are, or recently were, in development pipelines. The Wave Lifesciences program was recently discontinued due to failure of their stereo-pure platform to meet clinical targets; but this platform issue does not detract from the observation that exon 54 is a relevant target.