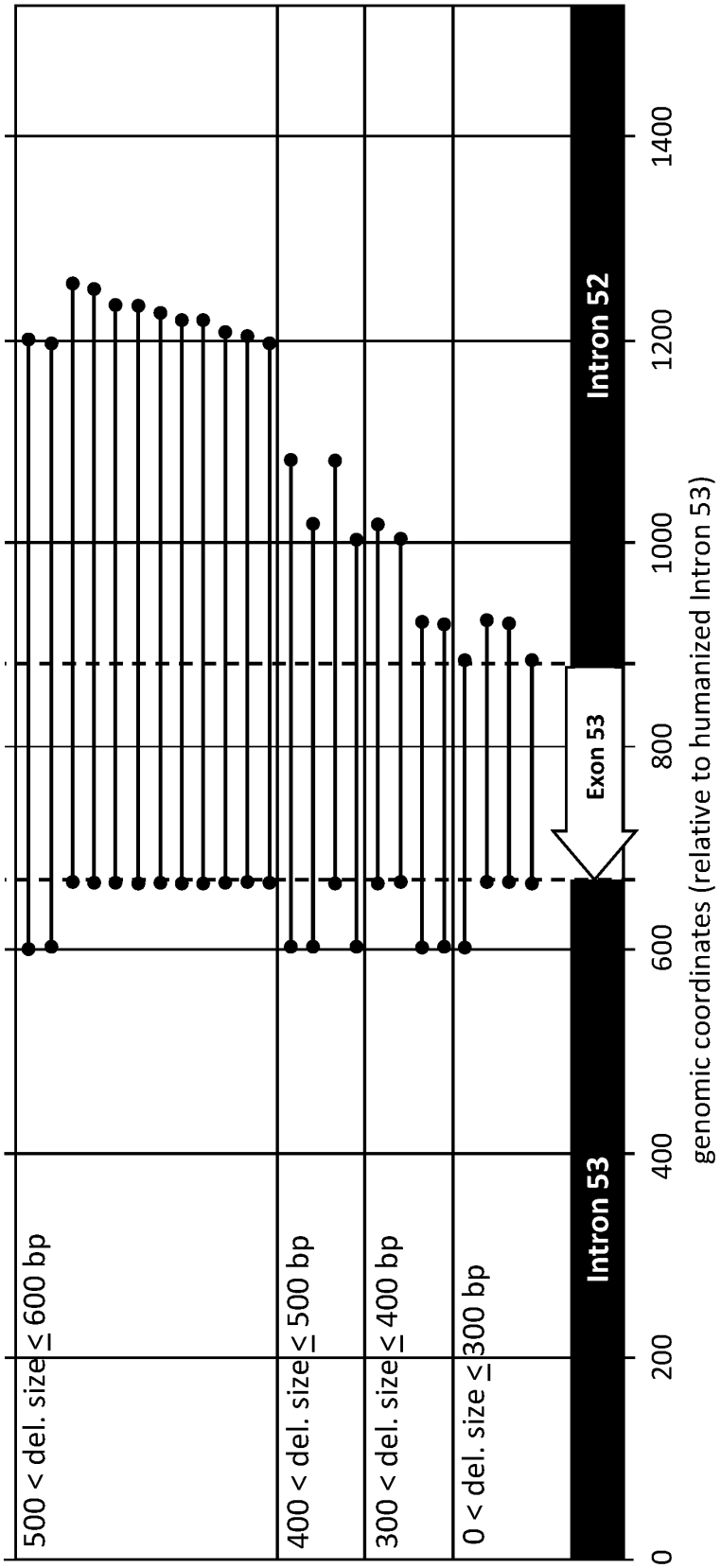

500 < del. size ≤ 600 bp
400 < del. size ≤ 500 bp
300 < del. size ≤ 400 bp
0 < del. size ≤ 300 bp
Intron 53
Exon 53
Intron 52
0
200
400
600
800
1000
1200
1400
genomic coordinates (relative to humanized Intron 53)

*FIG. 1*

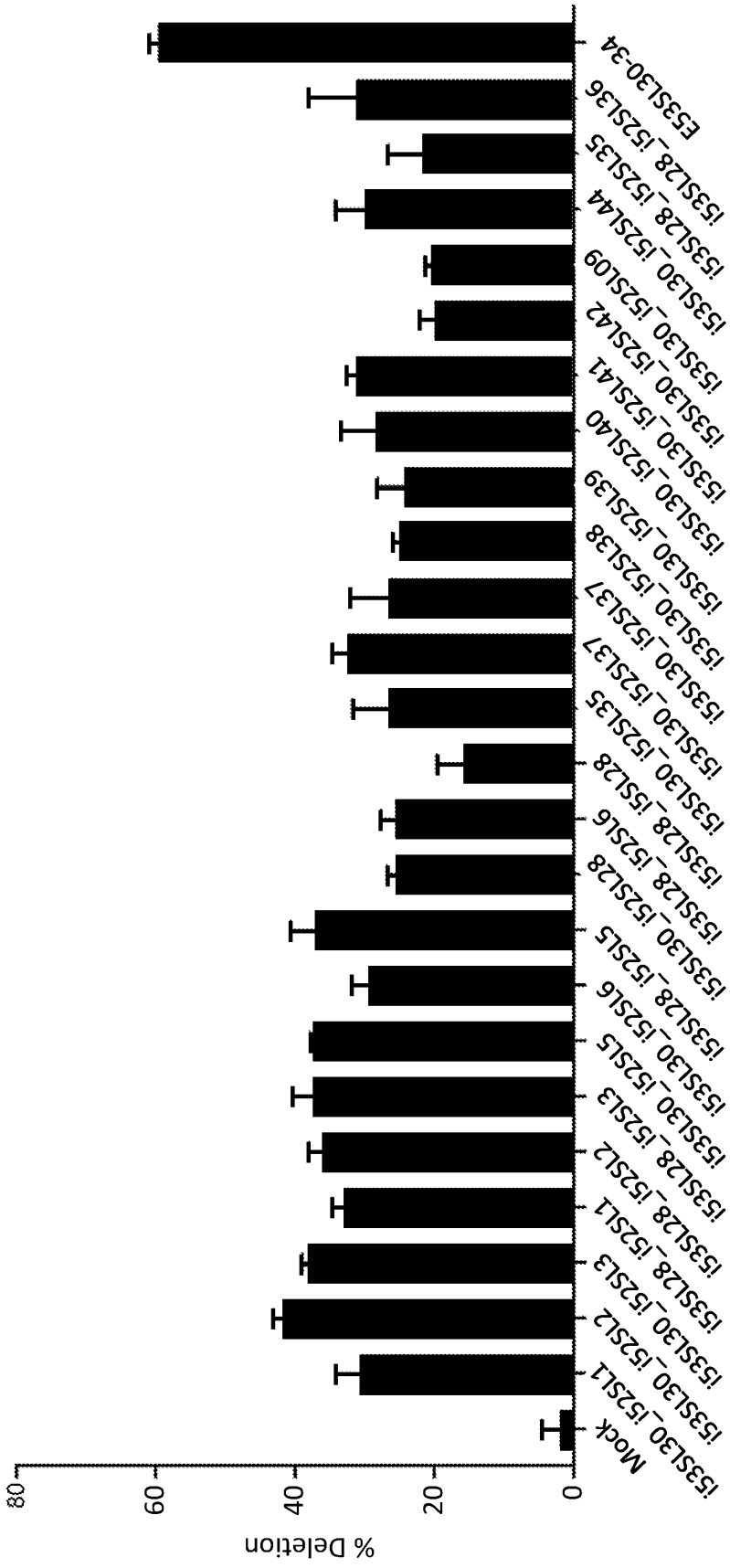

% Deletion
0
20
40
60
80
Mock
i53sL30_i52sL1
i53sL30_i52sL2
i53sL30_i52sL3
i53sL28_i52sL1
i53sL28_i52sL2
i53sL28_i52sL3
i53sL30_i52sL5
i53sL28_i52sL6
i53sL30_i52sL5
i53sL28_i52sL6
i53sL30_i52sL8
i53sL28_i52sL8
i53sL28_i52sL6
i53sL30_i52sL8
i53sL30_i52sL35
i53sL30_i52sL37
i53sL30_i52sL37
i53sL30_i52sL38
i53sL30_i52sL39
i53sL30_i52sL40
i53sL30_i52sL41
i53sL30_i52sL42
i53sL30_i52sL09
i53sL28_i52sL44
i53sL28_i52sL35
E53sL30-36
E53sL30-34

*FIG. 2*

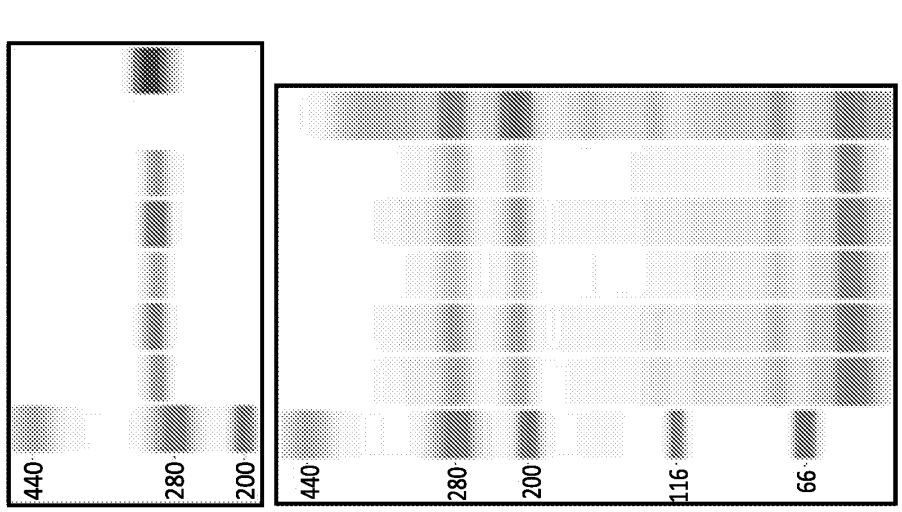
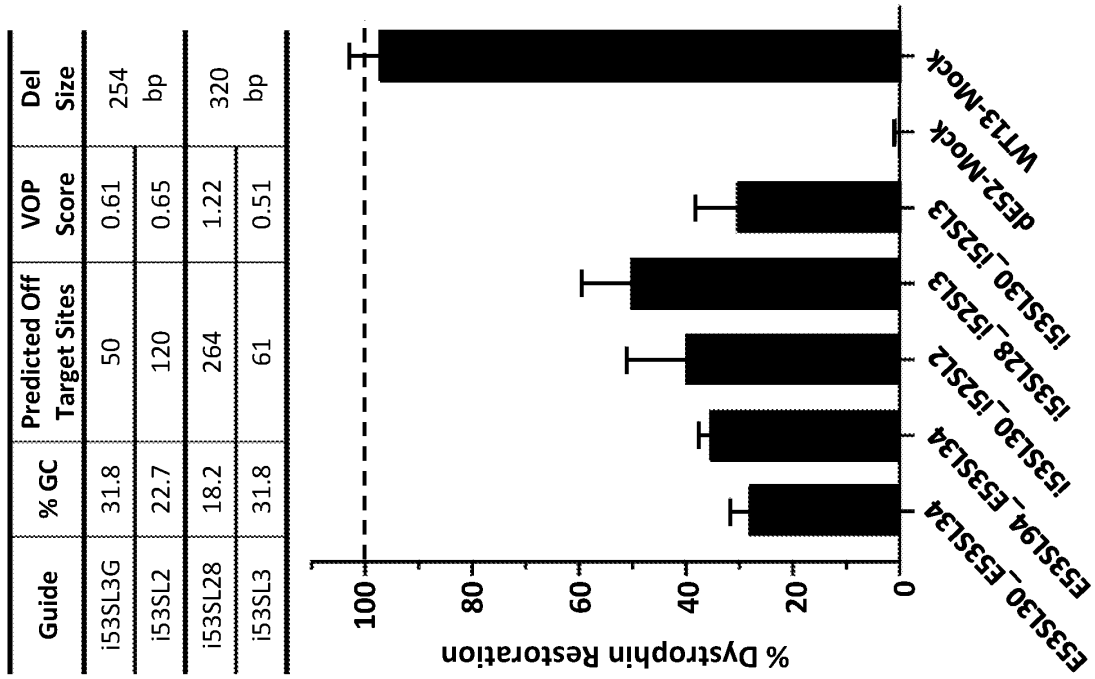



FIG. 3A




| Guide | % GC | Predicted Off Target Sites | VOP Score | Del Size |
|---|---|---|---|---|
| i53SL3G | 31.8 | 50 | 0.61 | 254 bp |
| i53SL2 | 22.7 | 120 | 0.65 | |
| i53SL28 | 18.2 | 264 | 1.22 | 320 bp |
| i53SL3 | 31.8 | 61 | 0.51 | |

FIG. 3B

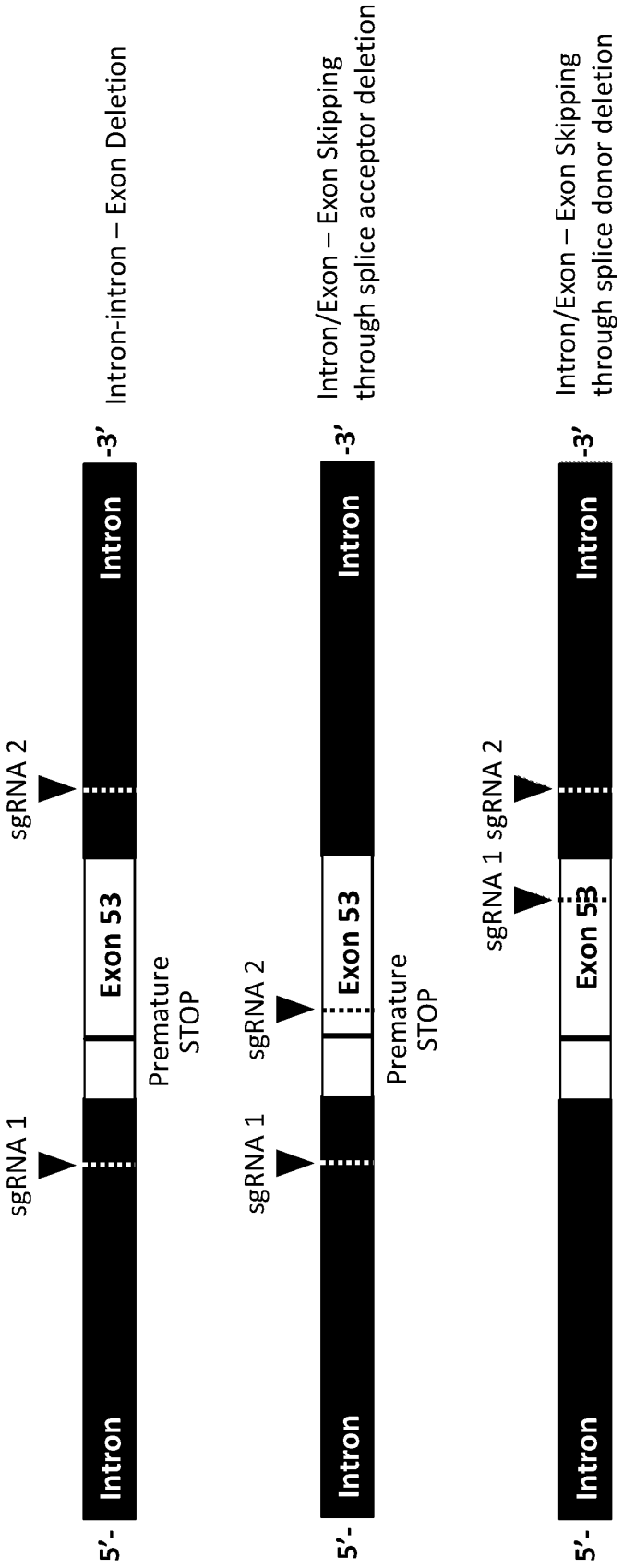

Intron-intron – Exon Deletion
Intron/Exon – Exon Skipping through splice acceptor deletion
Intron/Exon – Exon Skipping through splice donor deletion
5'-
Intron
Exon 53
Premature STOP
sgRNA 1
sgRNA 2
-3'

FIG. 4

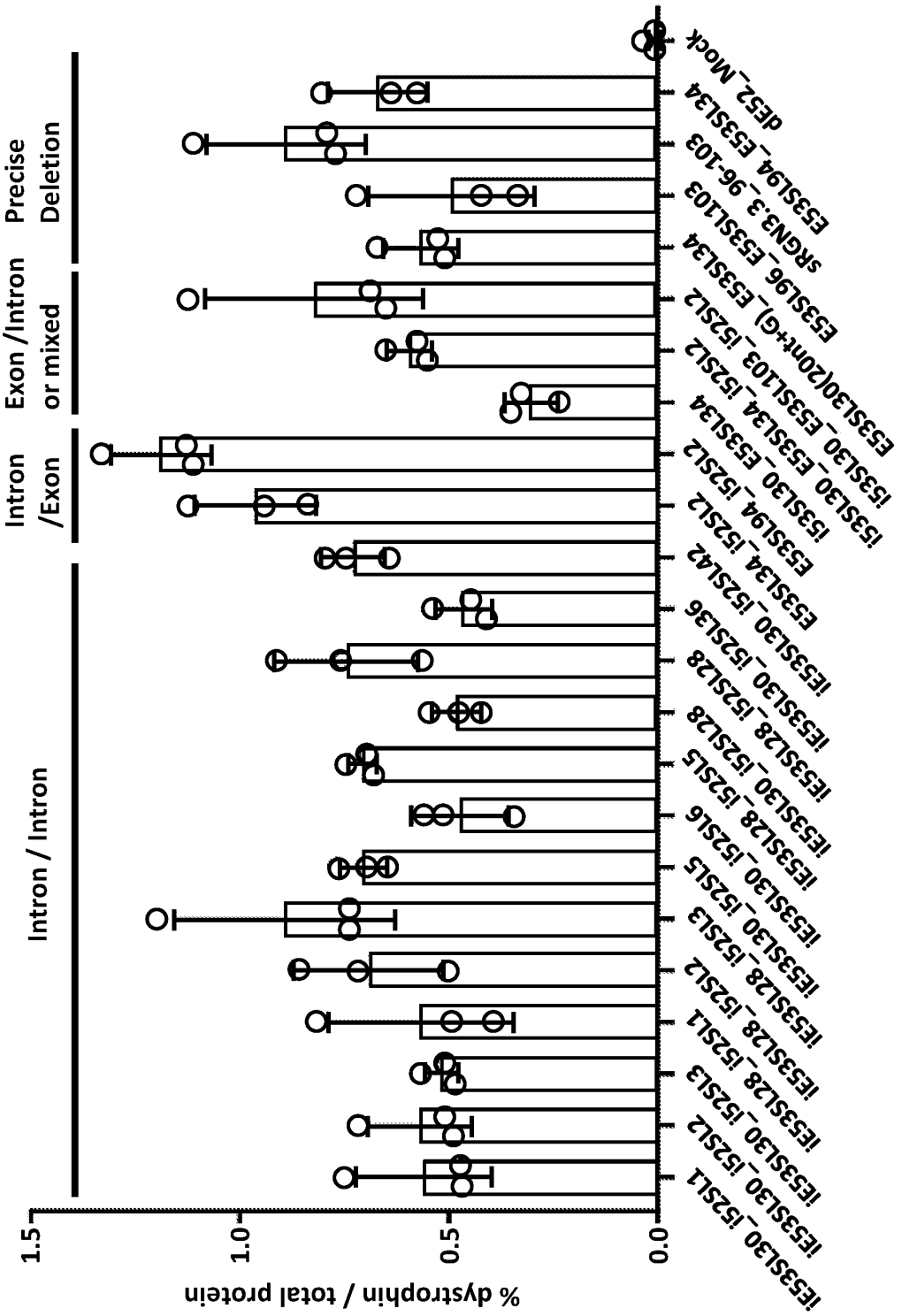

% dystrophin / total protein
0.0
0.5
1.0
1.5
Intron / Intron
Intron /Exon
Exon /Intron or mixed
Precise Deletion
i53SL30_i52SL1
i53SL30_i52SL2
i53SL28_i52SL3
i53SL28_i52SL1
i53SL28_i52SL2
i53SL30_i52SL3
i53SL30_i52SL5
i53SL28_i52SL6
i53SL30_i52SL5
i53SL28_i52SL8
i53SL30_i52SL8
i53SL30_i52SL6
i53SL28_i52SL2
i53SL4_i52SL2
i53SL9_i52SL2
i53SL30_i52SL2
i53SL30_E53SL34_i52SL2
i53SL30_E53SL103_i52SL2
E53SL30(20nt+G)_i52SL2
E53SL96_E53SL34
sRGN3.3_96-103
E53SL94_E53SL103
dE52_Mock

FIG. 5

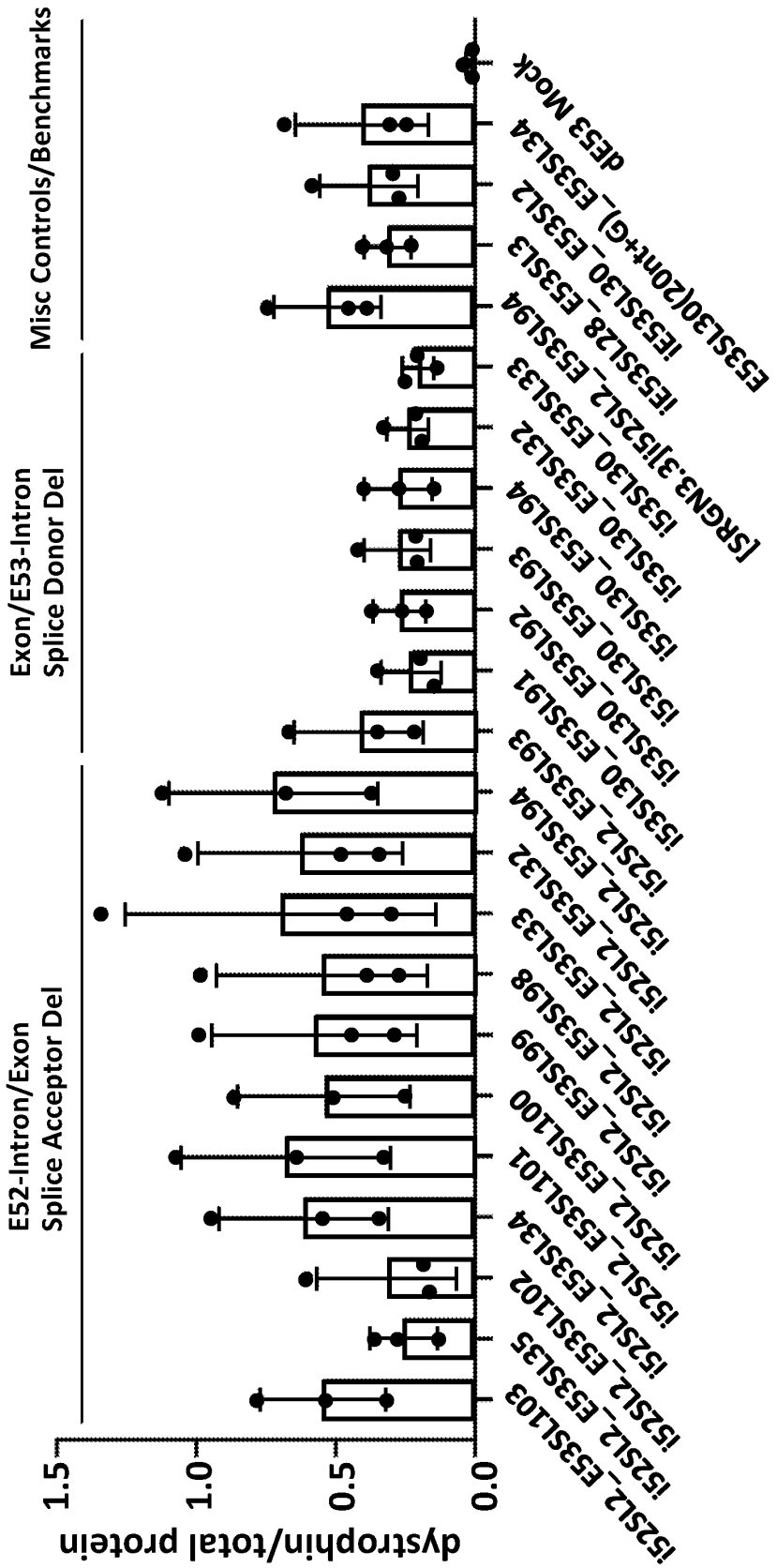

dystrophin/total protein
1.5
1.0
0.5
0.0
E52-Intron/Exon
Splice Acceptor Del
Exon/E53-Intron
Splice Donor Del
Misc Controls/Benchmarks
i52i2_E53iL103
i52i2_E53iL35
i52i2_E53iL102
i52i2_E53iL34
i52i2_E53iL101
i52i2_E53iL100
i52i2_E53iL99
i52i2_E53iL98
i52i2_E53iL33
i52i2_E53iL32
i52i2_E53iL94
i53iL30_E53iL93
i53iL30_E53iL91
i53iL30_E53iL92
i53iL30_E53iL93
i53iL30_E53iL94
i53iL30_E53iL32
[5RGN3.3]i52i2_E53iL33
i53iL28_E53iL94
i53iL30_E53iL3
E53iL30(20nt+G)_E53iL2
dE53 Mock

*FIG. 6*