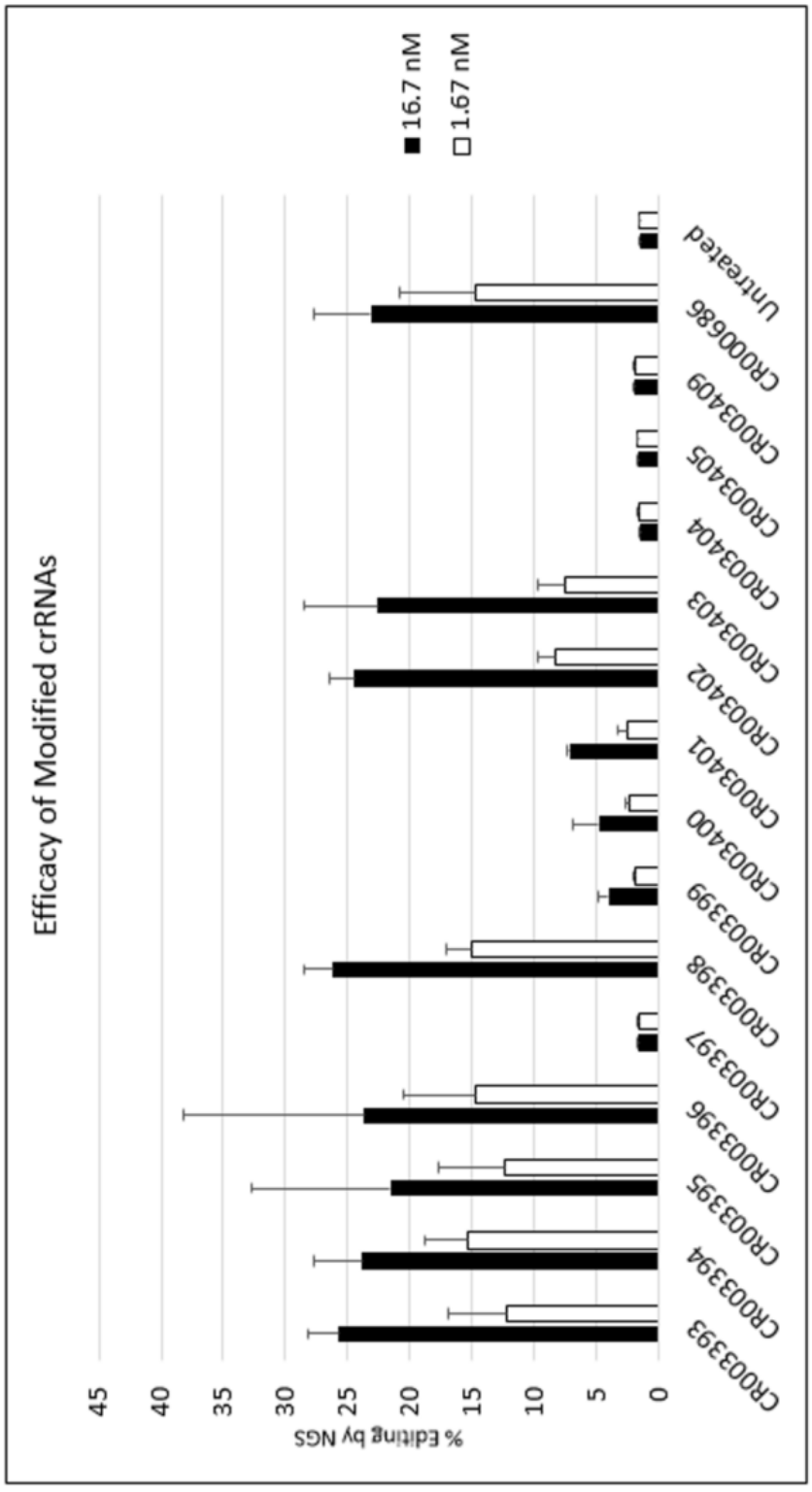

Efficacy of Modified crRNAs
% Editing by NGS
0
5
10
15
20
25
30
35
40
45
16.7 nM
1.67 nM
CR003393
CR003394
CR003395
CR003396
CR003397
CR003398
CR003399
CR003400
CR003401
CR003402
CR003403
CR003404
CR003405
CR003409
CR000686
Untreated

Figure 1

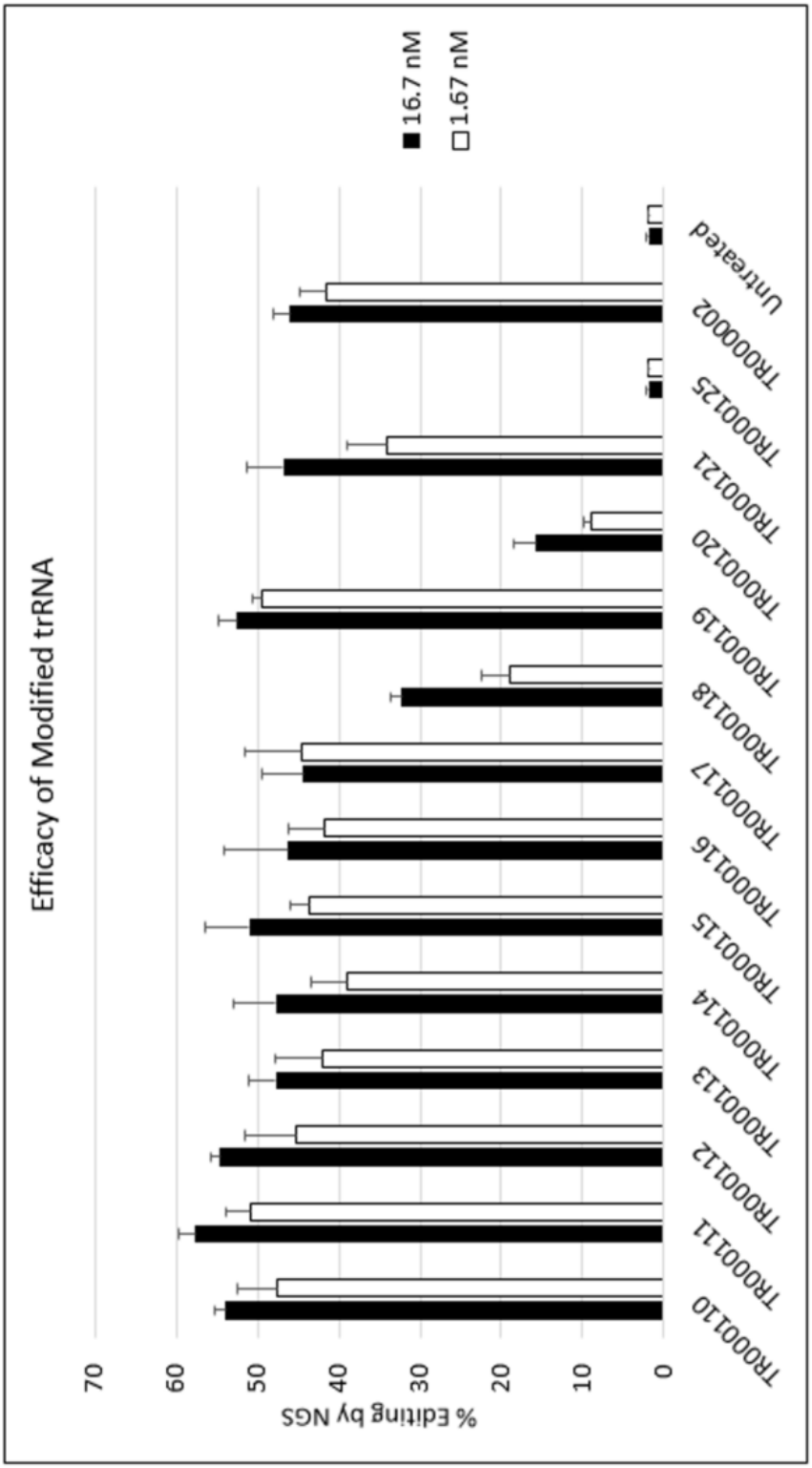

Efficacy of Modified trRNA
% Editing by NGS
0
10
20
30
40
50
60
70
16.7 nM
1.67 nM
TR000110
TR000111
TR000112
TR000113
TR000114
TR000115
TR000116
TR000117
TR000118
TR000119
TR000120
TR000121
TR000125
TR000002
Untreated

Figure 2

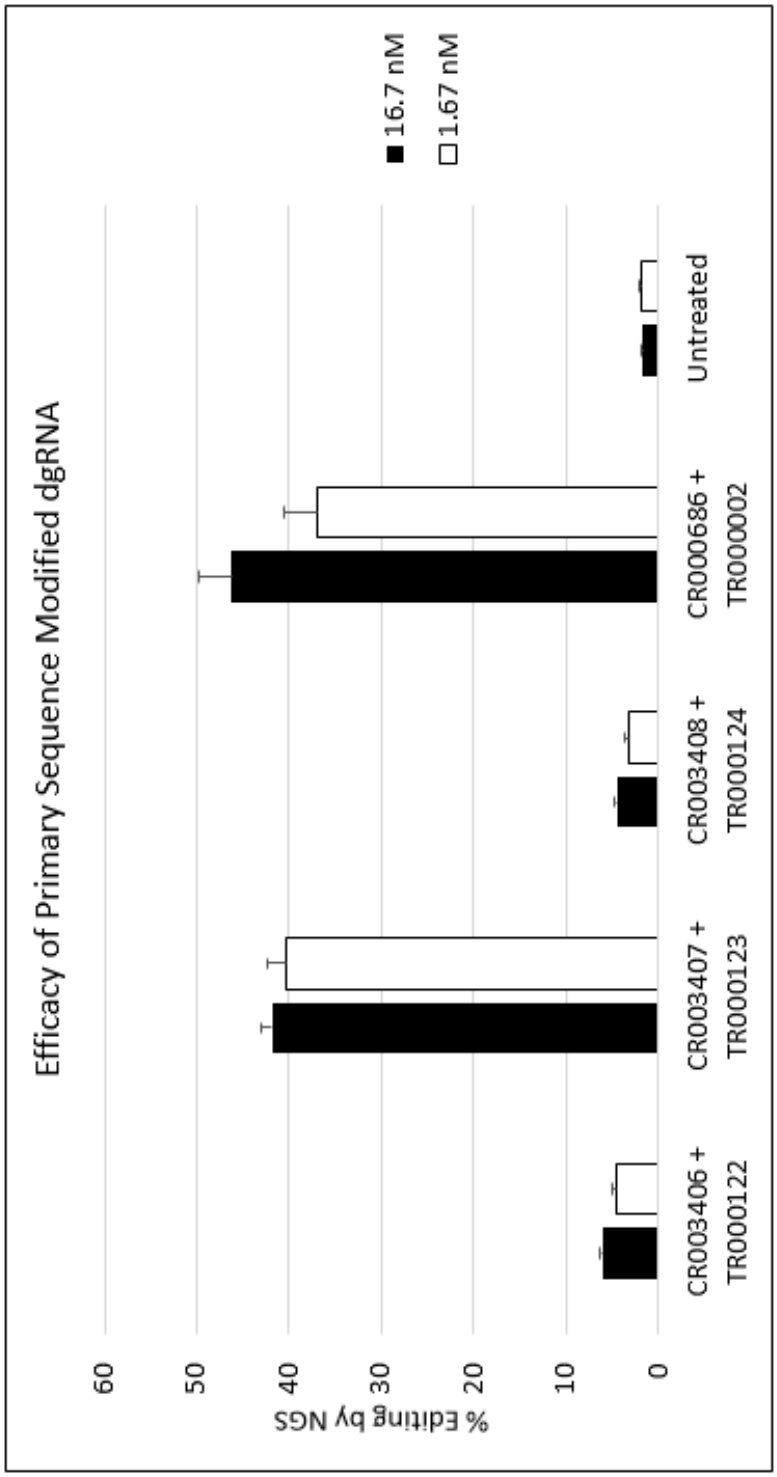

Efficacy of Primary Sequence Modified dgRNA
% Editing by NGS
60
50
40
30
20
10
0
16.7 nM
1.67 nM
CR003406 + TR000122
CR003407 + TR000123
CR003408 + TR000124
CR000686 + TR000002
Untreated

Figure 3

25 nM

	TR000110	TR000111	TR000112	TR000113	TR000114	TR000115	TR000116	TR000117	TR000118	TR000119	TR000121	TR000002
CR003393	54.1 ± 5	61.2 ± 3.1	56.7 ± 5	55.9 ± 4	53.8 ± 10.7	56.3 ± 10	52.7 ± 5.8	55.5 ± 7	40.7 ± 12.9	48.5 ± 8.1	51.3 ± 11.4	
CR003394	57.7 ± 4.7	61.5 ± 8.2	64.8 ± 6.4	65.4 ± 8.9	60.1 ± 2.5	61.6 ± 4.6	57.3 ± 7.2	57.8 ± 8.3	38.6 ± 7.9	50.9 ± 10.3	54.8 ± 8.9	
CR003395	52.4 ± 2.4	62.8 ± 6.8	61.1 ± 7.2	62.8 ± 11.1	55.9 ± 7.6	57 ± 4.9	53.4 ± 7.1	52.5 ± 8.1	38.2 ± 7.5	49.7 ± 14.4	53.1 ± 11.7	
CR003396	56 ± 3.9	56.4 ± 7.6	58.7 ± 7.3	58.1 ± 7.3	54.7 ± 4.9	58.8 ± 5	48.9 ± 2.8	52.5 ± 9.7	34.3 ± 6	48.3 ± 10.3	52.4 ± 9.5	
CR003398	50.8 ± 10.2	56.2 ± 8.2	62.5 ± 6.9	59.7 ± 8.9	56.3 ± 3.4	61.2 ± 5	53 ± 7.1	53.8 ± 9.2	29.4 ± 9.3	52.2 ± 12.2	51.9 ± 15.6	
CR003402	42.7 ± 4.5	53.3 ± 5.4	56.9 ± 11	57.4 ± 12.1	52.8 ± 9.3	55.1 ± 8.5	46.1 ± 8.4	50.5 ± 8.8	17.7 ± 6.7	45.2 ± 12	52.8 ± 11.3	
CR003403	45.8 ± 9.9	52.7 ± 8.6	59.7 ± 13.9	54.8 ± 13.7	47.2 ± 12.1	50.9 ± 8.4	43.4 ± 10.3	47 ± 13	10.5 ± 5.9	44.5 ± 15.2	46 ± 19.3	
CR000686												34.5 ± 6.4

2.5 nM

	TR000110	TR000111	TR000112	TR000113	TR000114	TR000115	TR000116	TR000117	TR000118	TR000119	TR000121	TR000002
CR003393	41.8 ± 6.2	50.4 ± 3.2	40.2 ± 4.7	43.4 ± 2	40.1 ± 4.8	43.1 ± 3.9	39.1 ± 4.2	43 ± 6	15.6 ± 3.1	34.9 ± 11.6	38.6 ± 2.2	
CR003394	45.4 ± 4.7	49.6 ± 7.9	43.9 ± 6.6	46.7 ± 5.6	40.4 ± 7.5	47.2 ± 6.9	46.8 ± 3.1	43.9 ± 2.4	13.7 ± 1.6	28.9 ± 4.3	35.9 ± 6.2	
CR003395	39 ± 10	56 ± 5.1	42.2 ± 3.5	48.2 ± 3.2	36 ± 2.8	47.4 ± 7.3	44.4 ± 5.9	41.6 ± 7	12 ± 1.4	25.8 ± 1	31.4 ± 1.8	
CR003396	34.8 ± 2.3	46.5 ± 0.4	42 ± 4.9	42.4 ± 1.8	32 ± 4	44.4 ± 5.5	41.1 ± 7.7	40.5 ± 5.1	20.7 ± 1.2	26 ± 1.2	42.4 ± 8.9	
CR003398	33.6 ± 3.3	47 ± 6.8	41.9 ± 2.6	41.9 ± 1.1	37.1 ± 4.1	43.2 ± 9.4	40.1 ± 3.9	42.9 ± 3.6	1.4 ± 0.1	34.2 ± 2	40.7 ± 7.4	
CR003402	31.2 ± 4.7	46.4 ± 5	38.5 ± 2.8	40.7 ± 3.5	29.9 ± 1.1	42.4 ± 6.6	31.7 ± 2.9	32.8 ± 4.6	7 ± 0.8	31.3 ± 2.1	46.8 ± 3.8	
CR003403	28 ± 4.3	38.3 ± 3.3	37.5 ± 4.4	36.4 ± 2.7	31.4 ± 4.6	34.6 ± 3.7	32.9 ± 4	35.8 ± 4.2	1.4 ± 0.1	34.6 ± 6.1	40.7 ± 6.6	
CR000686												21.7 ± 4.9

Figure 4

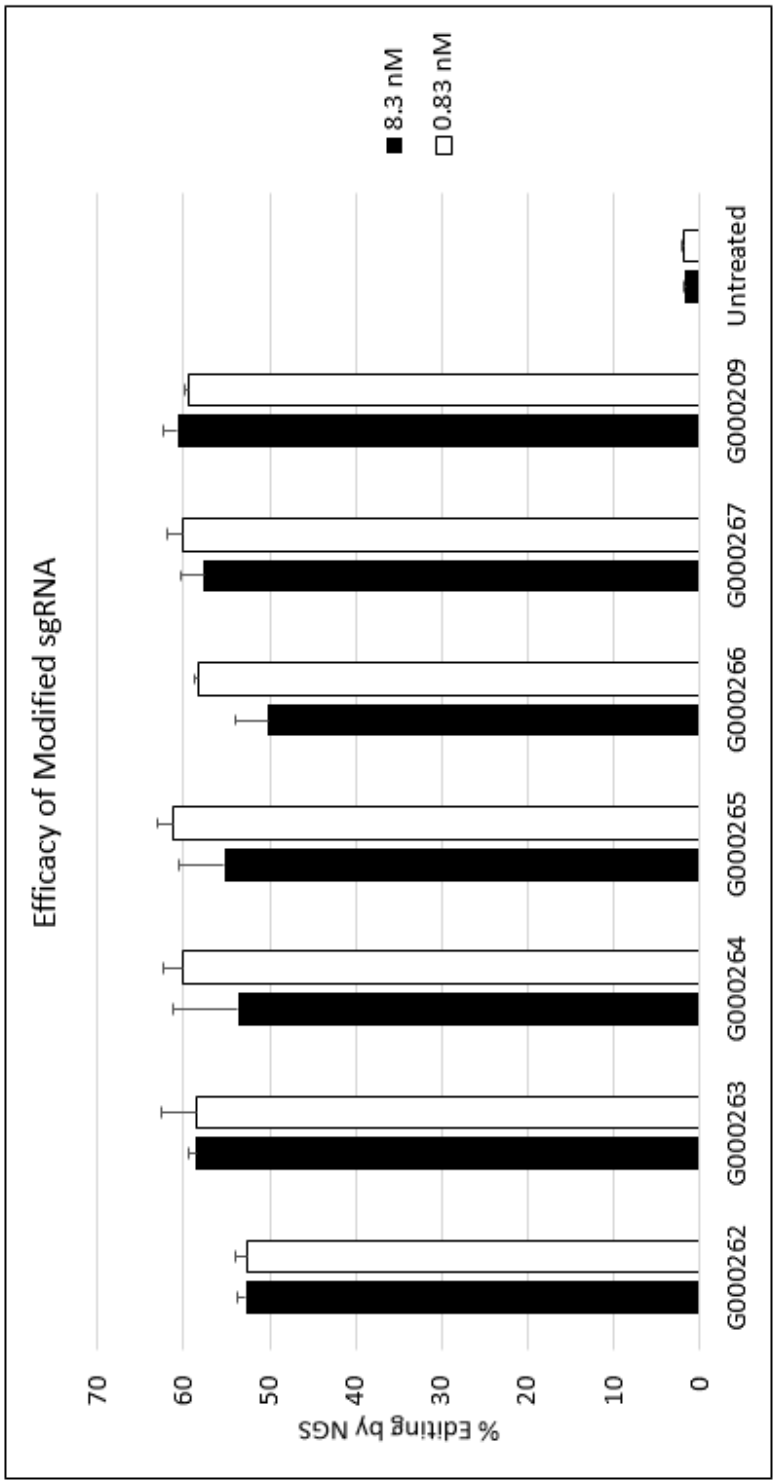

Efficacy of Modified sgRNA
% Editing by NGS
0
10
20
30
40
50
60
70
8.3 nM
0.83 nM
G000262
G000263
G000264
G000265
G000266
G000267
G000209
Untreated

Figure 5

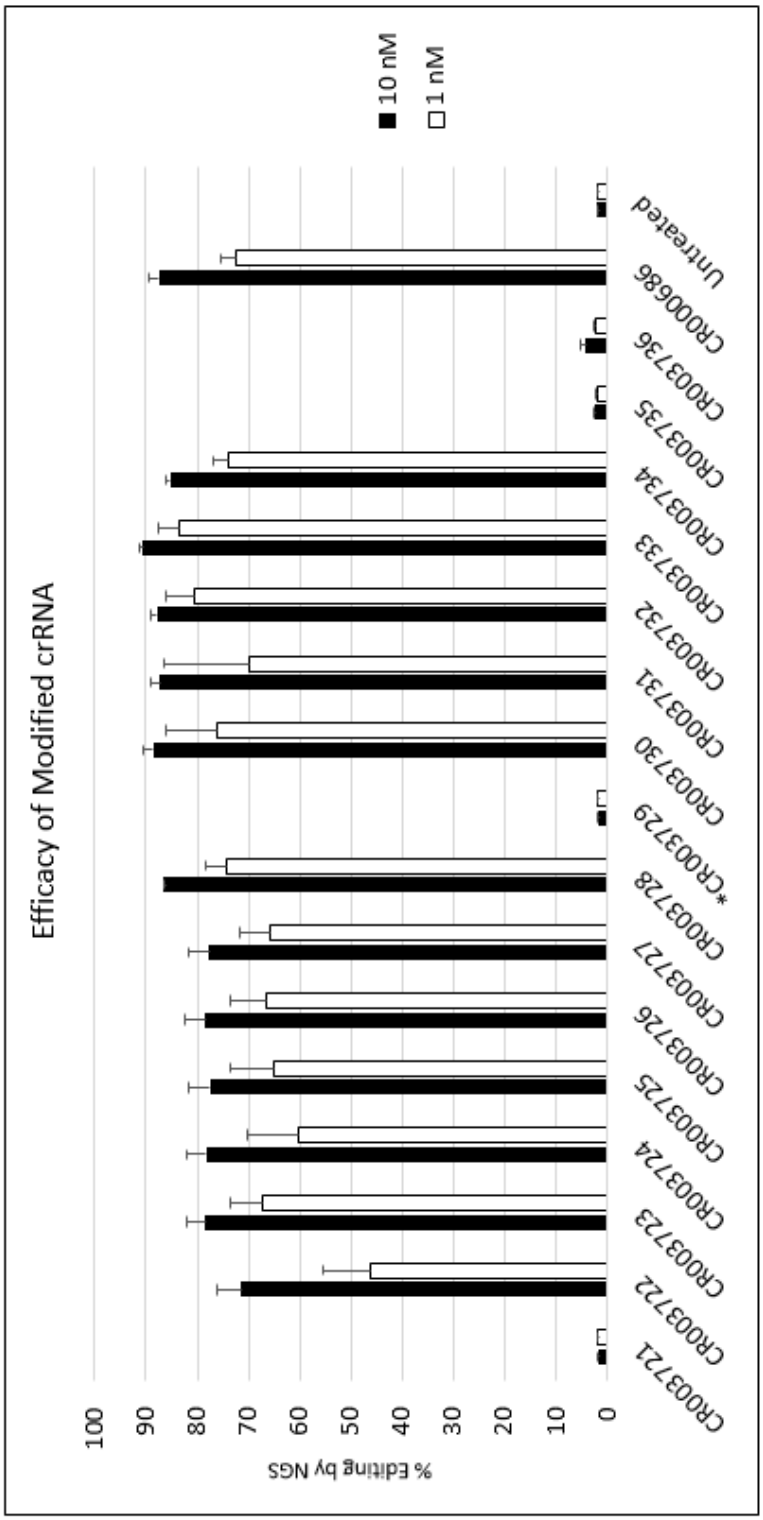

Efficacy of Modified crRNA
% Editing by NGS
100
90
80
70
60
50
40
30
20
10
0
10 nM
1 nM
CR003721
CR003722
CR003723
CR003724
CR003725
CR003726
CR003727
*CR003728
CR003729
CR003730
CR003731
CR003732
CR003733
CR003734
CR003735
CR003736
CR000686
Untreated

Figure 6

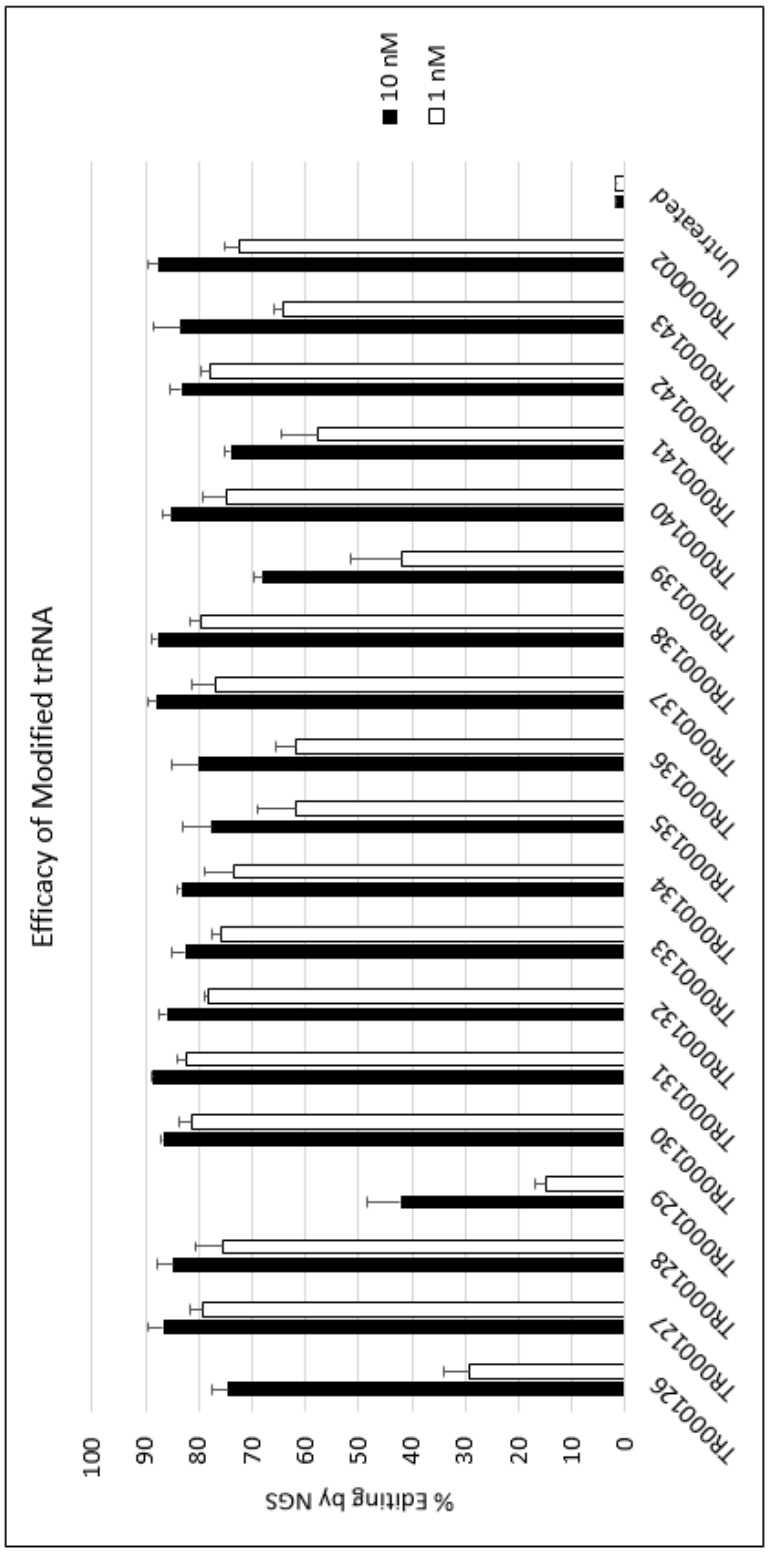

Efficacy of Modified trRNA
% Editing by NGS
100
90
80
70
60
50
40
30
20
10
0
10 nM
1 nM
TR000126
TR000127
TR000128
TR000129
TR000130
TR000131
TR000132
TR000133
TR000134
TR000135
TR000136
TR000137
TR000138
TR000139
TR000140
TR000141
TR000142
TR000143
TR000002
Untreated

Figure 7

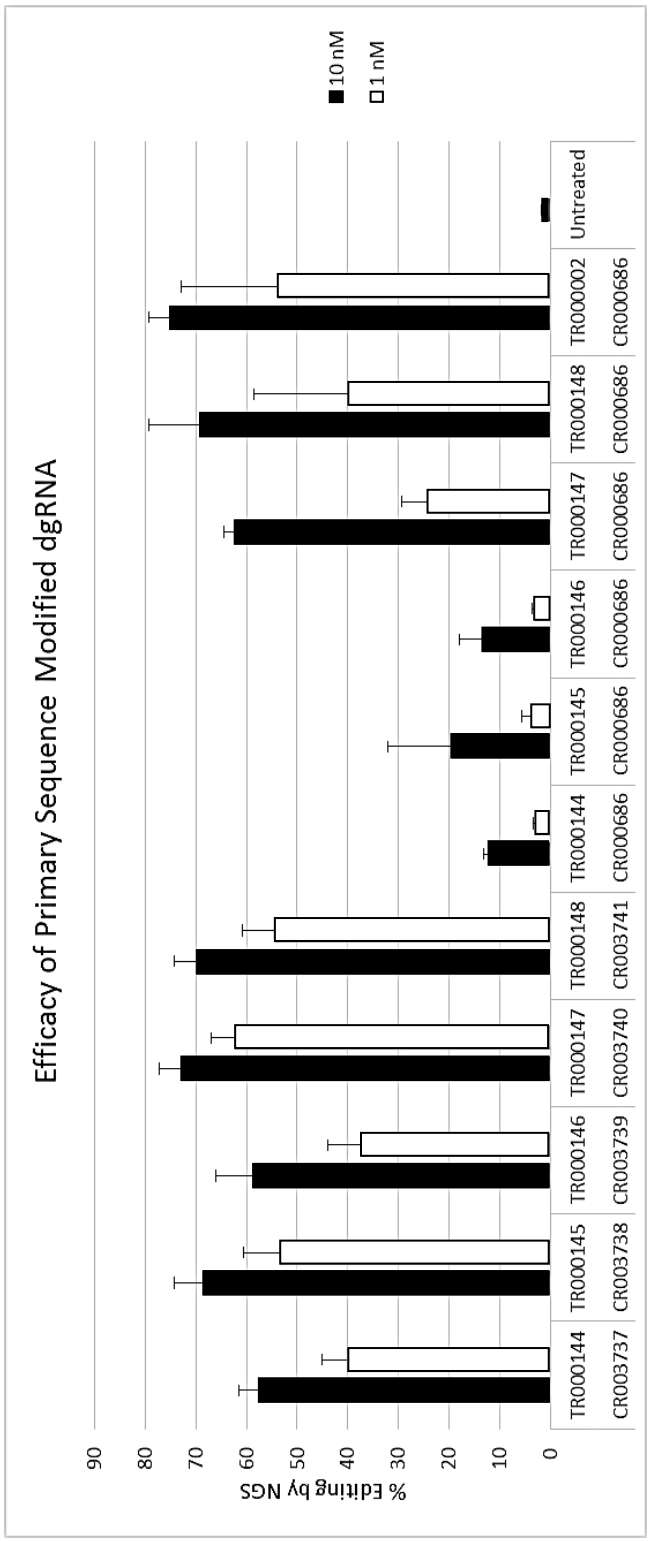

Efficacy of Primary Sequence Modified dgRNA
% Editing by NGS
10 nM
1 nM
0
10
20
30
40
50
60
70
80
90
TR000144
CR003737
TR000145
CR003738
TR000146
CR003739
TR000147
CR003740
TR000148
CR003741
TR000144
CR000686
TR000145
CR000686
TR000146
CR000686
TR000147
CR000686
TR000148
CR000686
TR000002
CR000686
Untreated

Figure 8

10 nM

	TR000127	TR000128	TR000130	TR000134	TR000135	TR000136	TR000137	TR000138	TR000139	TR000142	TR000143	TR000002
CR003723	18 ± 13.8	22.8 ± 4.1	24 ± 2.6	26.9 ± 2.8	20.8 ± 5	22.2 ± 5.7	26.7 ± 5	23.6 ± 4.1	13.7 ± 2.4	22.3 ± 4.4	10.8 ± 2.2	18.5 ± 1.5
CR003725	28.4 ± 1.2	29.2 ± 4.2	27.9 ± 1.3	30.6 ± 3.4	26.1 ± 3.8	25.9 ± 4.4	30 ± 3	26.9 ± 2	18.5 ± 2.2	25.1 ± 0.9	23.1 ± 1.7	
CR003726	30.4 ± 1.1	31.3 ± 3.2	30.6 ± 2.9	32 ± 2.2	29.8 ± 2.5	29.9 ± 2.8	32.3 ± 1	28.7 ± 2.2	20.9 ± 2.4	27 ± 1.4	23.6 ± 2.3	24.9 ± 1.1
CR003727	31.6 ± 2.1	27 ± 2.7	29.6 ± 0.8	32.1 ± 1.3	26.7 ± 1.2	27.2 ± 5.2	31 ± 2	28.1 ± 0.7	19.2 ± 0.7	26.7 ± 2.1	22.5 ± 1.2	25.6 ± 3
CR003728	34.6 ± 2.4	2.4 ± 0.1	34.9 ± 2.6	37.7 ± 3.1	33.1 ± 2.1	33.1 ± 4	35.4 ± 0.9	32.4 ± 2.9	25.8 ± 4.8	30.7 ± 2.7	28.2 ± 2.2	28.6 ± 0.7
CR003729	34.8 ± 0.9	32.3 ± 1.5	33.8 ± 1.1	34.8 ± 2.5	29.8 ± 1.8	31.5 ± 3.6	32.6 ± 1.7	31 ± 1.9	25.4 ± 3.7	28.6 ± 0.5	25.6 ± 1.1	27.8 ± 0.6
CR003734	27.5 ± 0.3	21.5 ± 1.5	29.8 ± 1.6	29.5 ± 1.6	13.7 ± 2.1	14.6 ± 1.2	31 ± 2.4	28 ± 2.5	7 ± 1.1	25.5 ± 1.3	3.4 ± 0.1	22.7 ± 2.8
CR000686	23 ± 2.8	22.4 ± 3.9	23.6 ± 6.2	26.4 ± 1	22.2 ± 2	19.5 ± 1.1	24.6 ± 3	27.4 ± 2.6	12.2 ± 1.7	22.9 ± 1.8	20.2 ± 1.1	22.6 ± 3

1 nM

	TR000127	TR000128	TR000130	TR000134	TR000135	TR000136	TR000137	TR000138	TR000139	TR000142	TR000143	TR000002
CR003723	18.3 ± 14.1	16 ± 12.1	25.3 ± 0.8	22.5 ± 3.4	14.3 ± 10.6	17.5 ± 2	23.1 ± 3.8	21 ± 1.4	8.8 ± 1.4	18.3 ± 0.7	8.3 ± 1.5	14.6 ± 0.4
CR003725	26.9 ± 1.8	25.1 ± 4.1	25 ± 2.7	24.8 ± 2.3	23.1 ± 2.8	16.2 ± 12.1	17.3 ± 13.1	22.5 ± 1	15.2 ± 1.7	22.7 ± 1.5	19.7 ± 3.1	
CR003726	16.8 ± 12.9	26.4 ± 2	23.6 ± 2.6	25.2 ± 1.5	22.7 ± 3.4	20.7 ± 1.5	26.6 ± 1.4	20.4 ± 0.9	13.3 ± 0.3	21.6 ± 1.5	18.6 ± 0.5	17 ± 1
CR003727	24.1 ± 3.1	20.3 ± 1.4	21 ± 1.3	23.6 ± 2.6	19.7 ± 1.7	20.3 ± 1.4	15.3 ± 11.3	20.2 ± 1.5	11.7 ± 1.7	20.7 ± 0.7	15.1 ± 0.8	19.7 ± 4.2
CR003728	26.9 ± 0.8	2.4 ± 0.1	28.2 ± 2.4	27.9 ± 0.6	26.4 ± 3.2	22.9 ± 1.2	26.4 ± 1.9	24.8 ± 0.5	18.8 ± 1.8	23.3 ± 1.4	20.3 ± 1.1	23.4 ± 3.7
CR003729	26.3 ± 2.5	30.5 ± 4.6	28.3 ± 2.7	28.6 ± 2	25.3 ± 3.1	24.6 ± 1.6	25 ± 0.3	23.7 ± 0.6	17.1 ± 1.7	21.9 ± 0.5	21 ± 2	22 ± 2
CR003734	18.9 ± 5.3	15.2 ± 3	21.4 ± 1.7	20.1 ± 0.1	8.5 ± 1.7	7.4 ± 0.9	20.4 ± 0.8	20.4 ± 1.4	3 ± 0.1	19.3 ± 0.8	2.5 ± 0.3	17.3 ± 2.1
CR000686	16.2 ± 1	17.3 ± 3.4	18.3 ± 3.3	20.5 ± 5	15.4 ± 1.2	14.8 ± 3	18.9 ± 3.6	20.1 ± 1.7	7.9 ± 2.3	17.5 ± 3.3	15.4 ± 1	14.1 ± 1.6

Figure 9

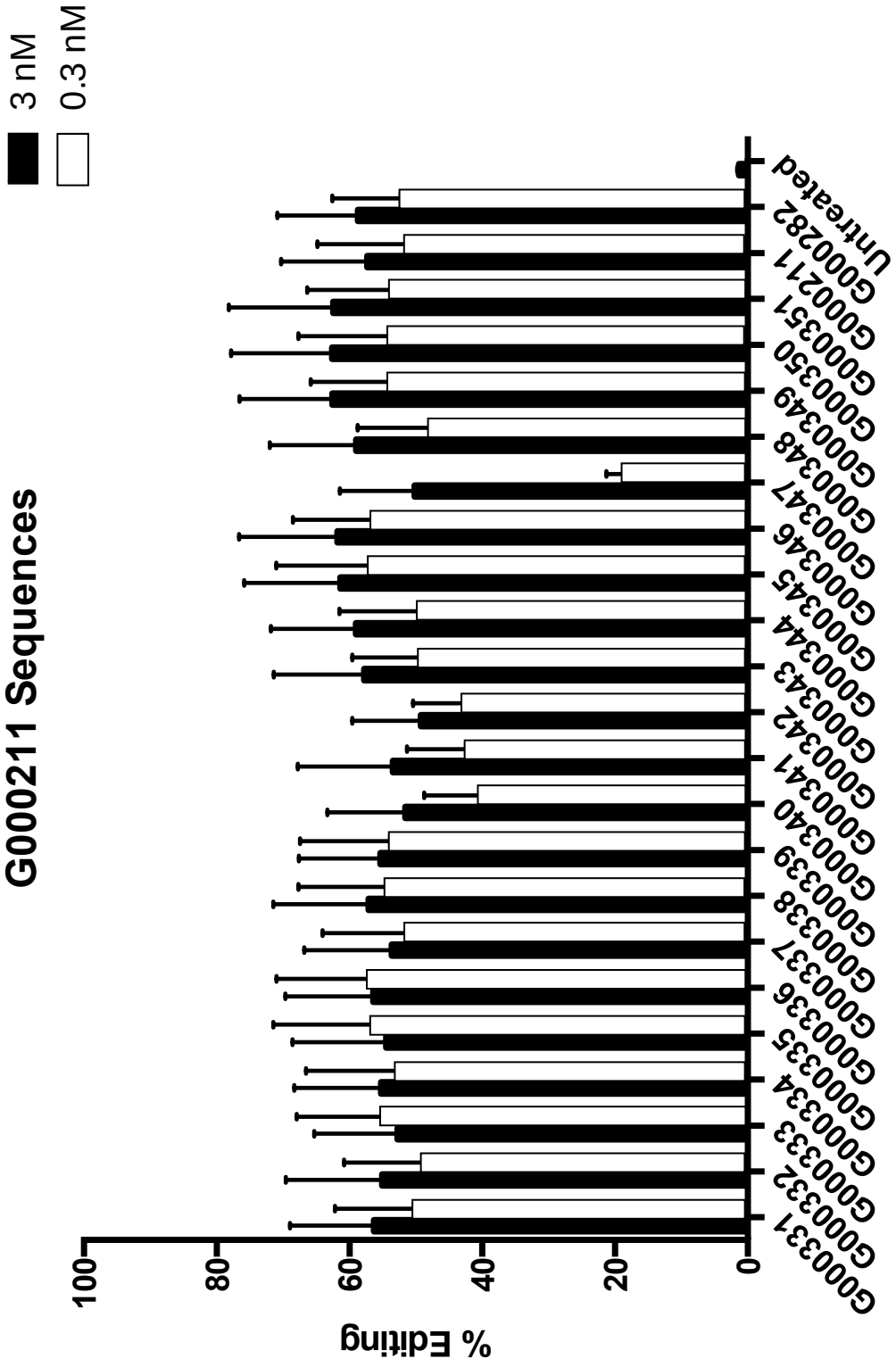

3 nM
0.3 nM
G000211 Sequences
% Editing
100
80
60
40
20
0
G000331
G000332
G000333
G000334
G000335
G000336
G000337
G000338
G000339
G000340
G000341
G000342
G000343
G000344
G000345
G000346
G000347
G000348
G000349
G000350
G000351
G000211
G000282
Untreated

Figure 10

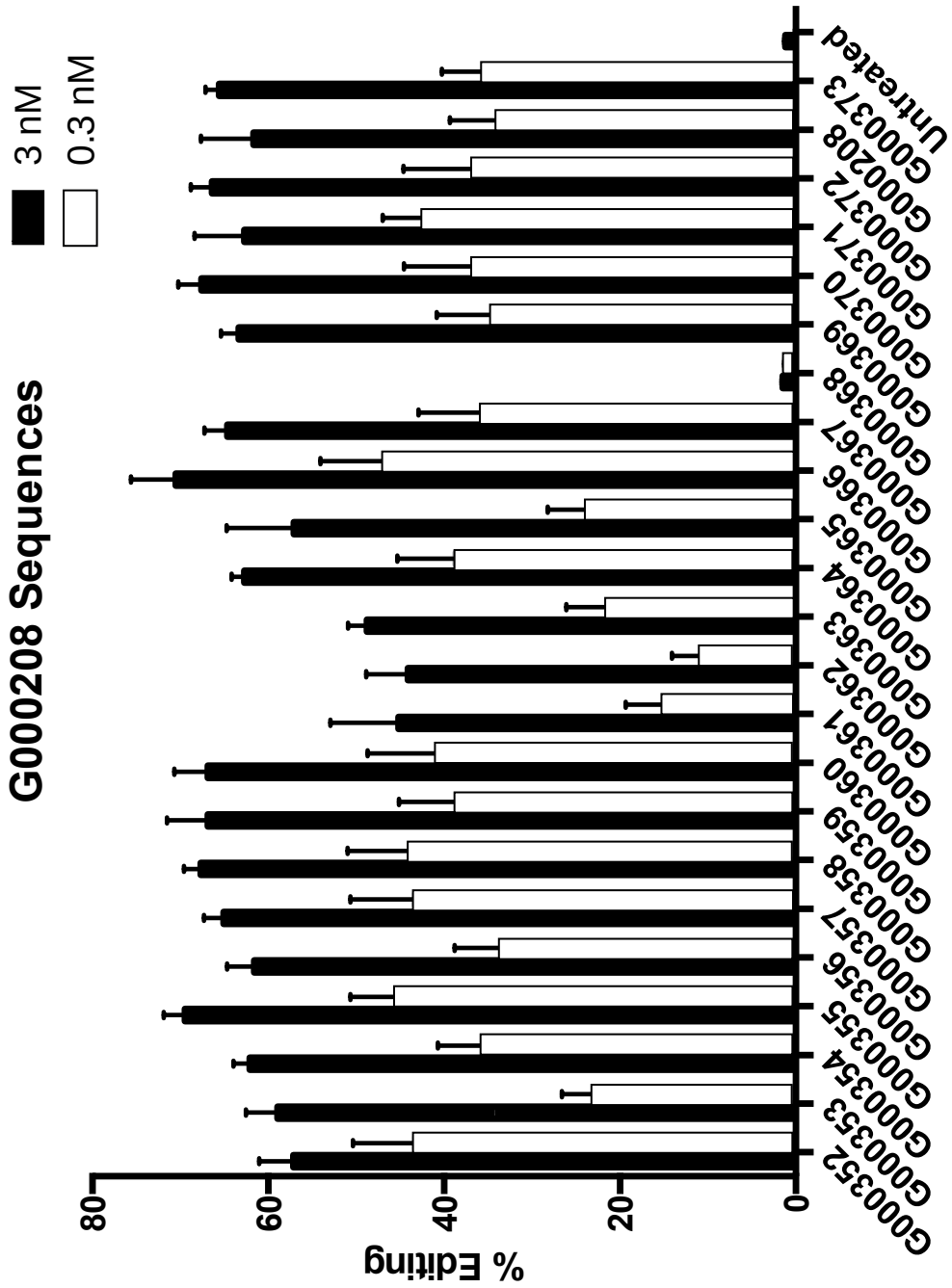

3 nM
0.3 nM
G000208 Sequences
% Editing
0
20
40
60
80
G000352
G000353
G000354
G000355
G000356
G000357
G000358
G000359
G000360
G000361
G000362
G000363
G000364
G000365
G000366
G000367
G000368
G000369
G000370
G000371
G000372
G000208
G000373
Untreated

Figure 11

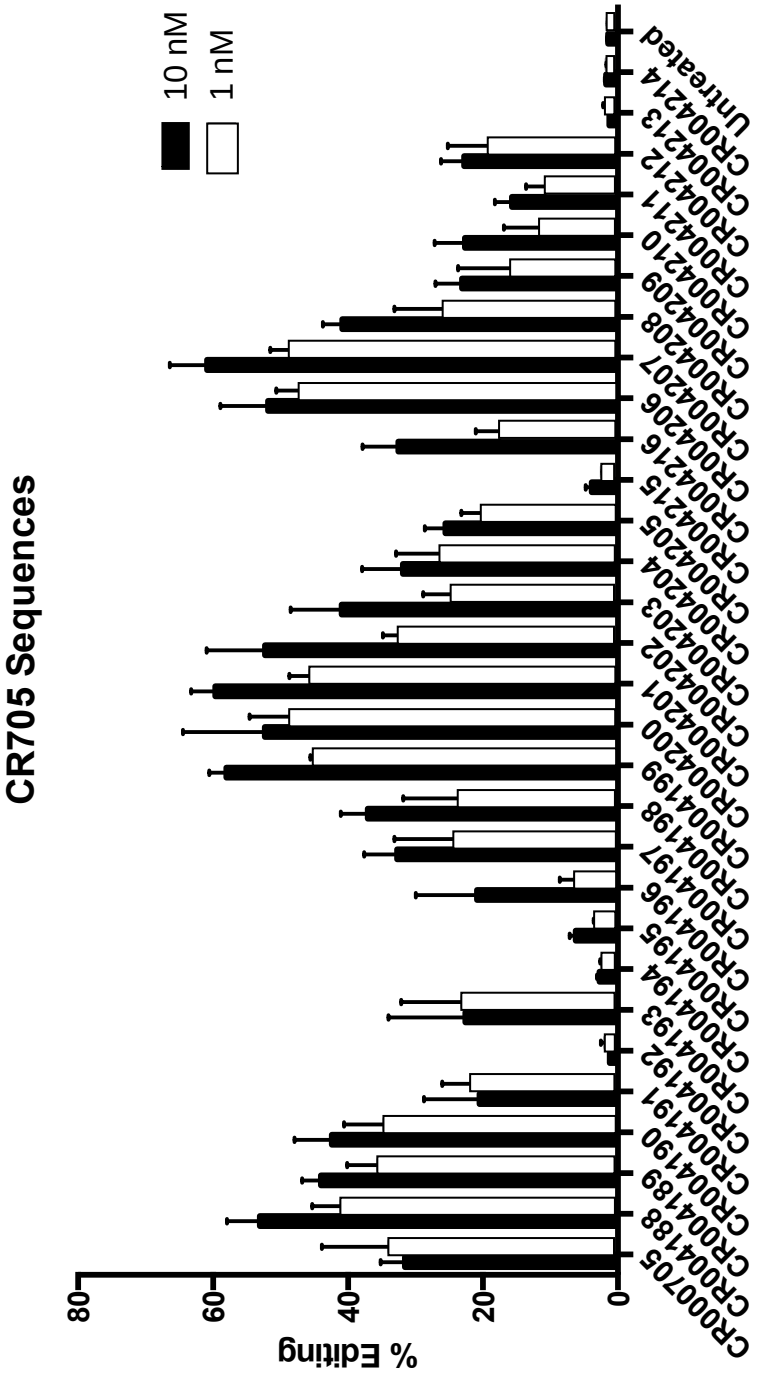

CR705 Sequences
% Editing
0
20
40
60
80
10 nM
1 nM
CR000705
CR004188
CR004189
CR004190
CR004191
CR004192
CR004193
CR004194
CR004195
CR004196
CR004197
CR004198
CR004199
CR004200
CR004201
CR004202
CR004203
CR004204
CR004205
CR004215
CR004216
CR004206
CR004207
CR004208
CR004209
CR004210
CR004211
CR004212
CR004213
Untreated

Figure 12A

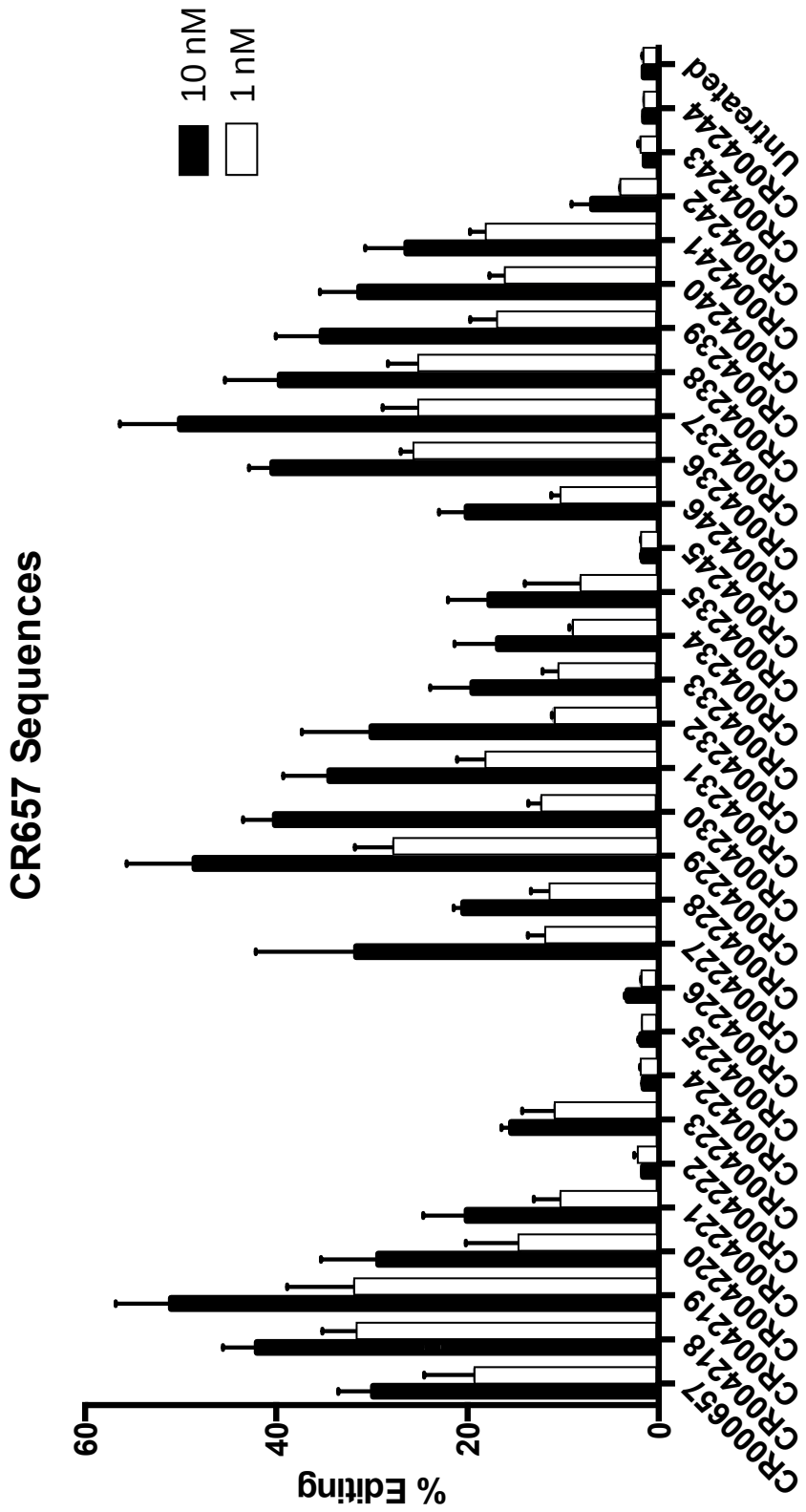

10 nM
1 nM
CR657 Sequences
% Editing
0
20
40
60
CR000657
CR004218
CR004219
CR004220
CR004221
CR004222
CR004223
CR004224
CR004225
CR004226
CR004227
CR004228
CR004229
CR004230
CR004231
CR004232
CR004233
CR004234
CR004235
CR004245
CR004246
CR004236
CR004237
CR004238
CR004239
CR004240
CR004241
CR004242
CR004243
Untreated

Figure 12B

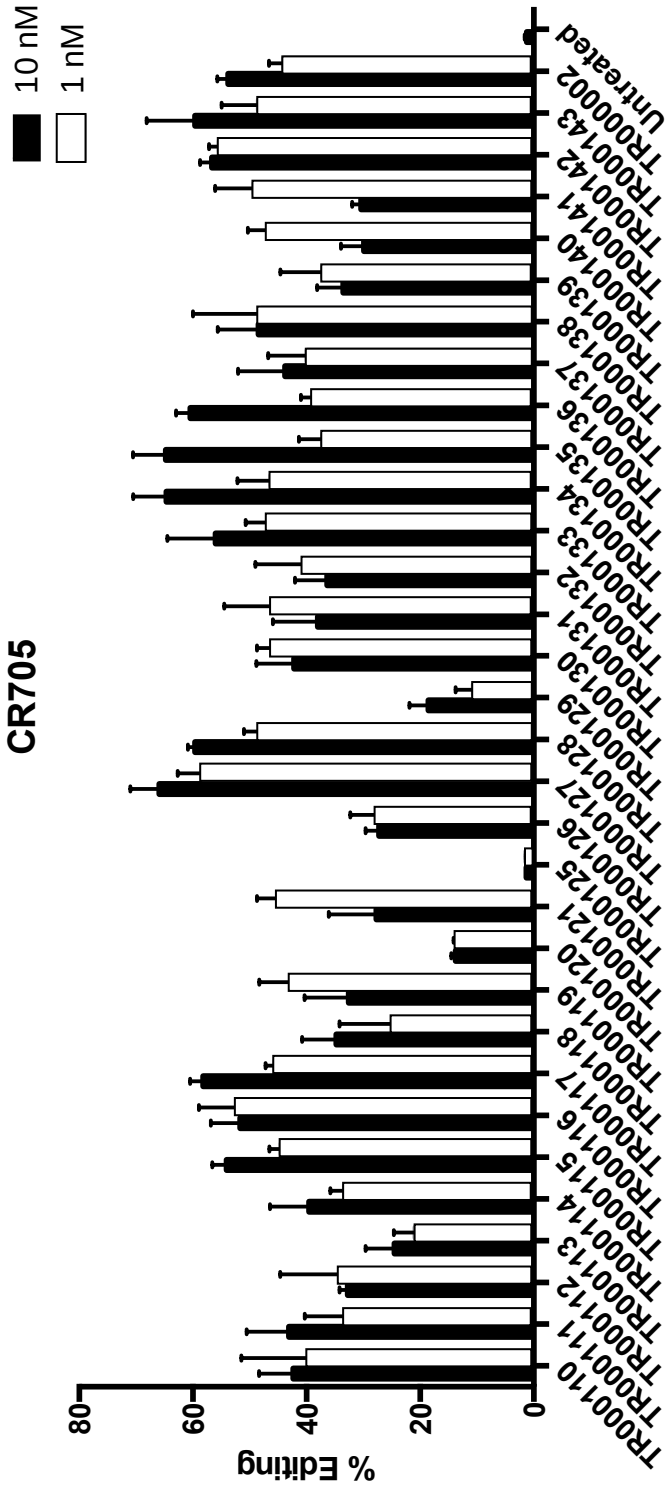

10 nM
1 nM
CR705
% Editing
0
20
40
60
80
TR0001 10
TR0001 11
TR0001 12
TR0001 13
TR0001 14
TR0001 15
TR0001 16
TR0001 17
TR0001 18
TR0001 19
TR0001 20
TR0001 21
TR0001 25
TR0001 26
TR0001 27
TR0001 28
TR0001 29
TR0001 30
TR0001 31
TR0001 32
TR0001 33
TR0001 34
TR0001 35
TR0001 36
TR0001 37
TR0001 38
TR0001 39
TR0001 40
TR0001 41
TR0001 42
TR0001 43
Untreated 02

Figure 13A

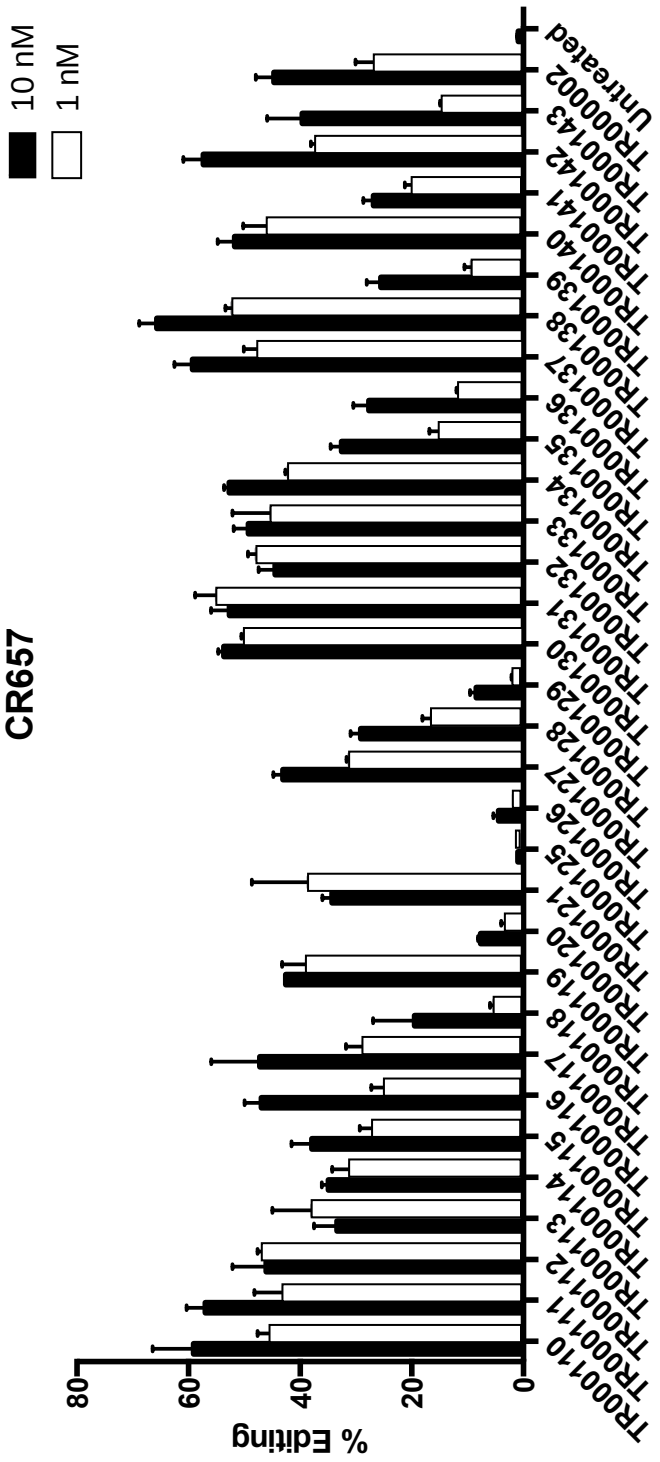

10 nM
1 nM
CR657
% Editing
0
20
40
60
80
Untreated
TR0000002
TR0000143
TR0000142
TR0000141
TR0000140
TR0000139
TR0000138
TR0000137
TR0000136
TR0000135
TR0000134
TR0000133
TR0000132
TR0000131
TR0000130
TR0000129
TR0000128
TR0000127
TR0000126
TR0000125
TR0000121
TR0000120
TR0000119
TR0000118
TR0000117
TR0000116
TR0000115
TR0000114
TR0000113
TR0000112
TR0000111
TR0000110

Figure 13B

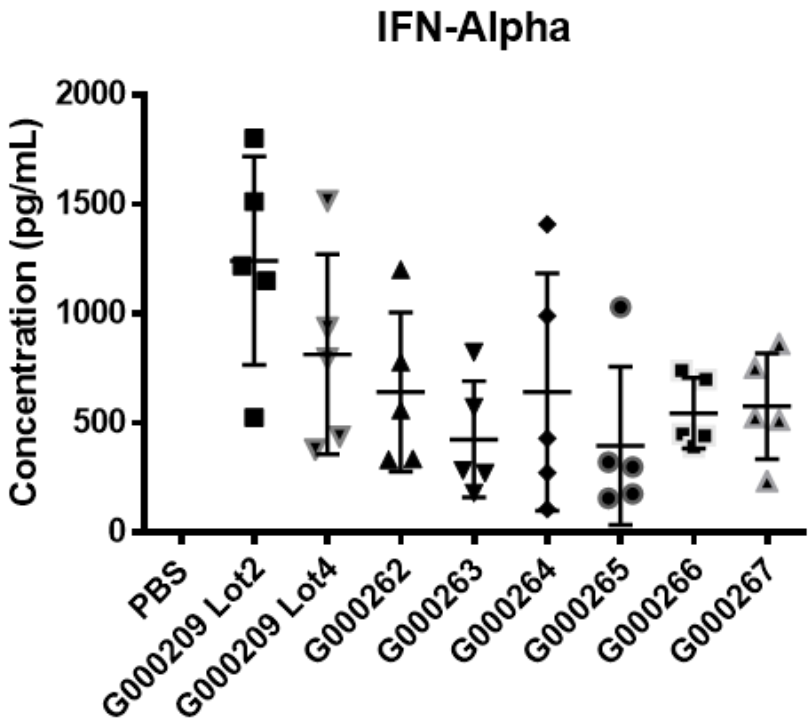


Figure 14A

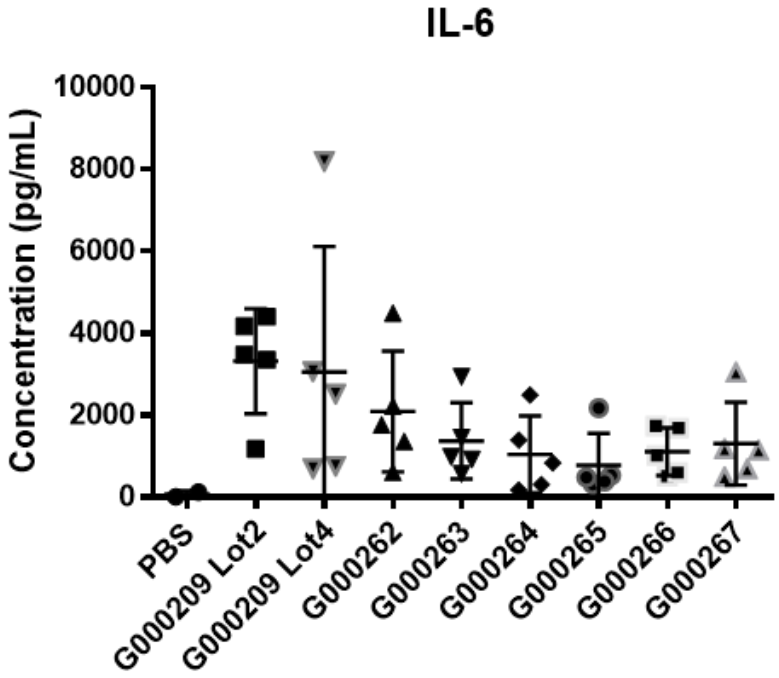


Figure 14B

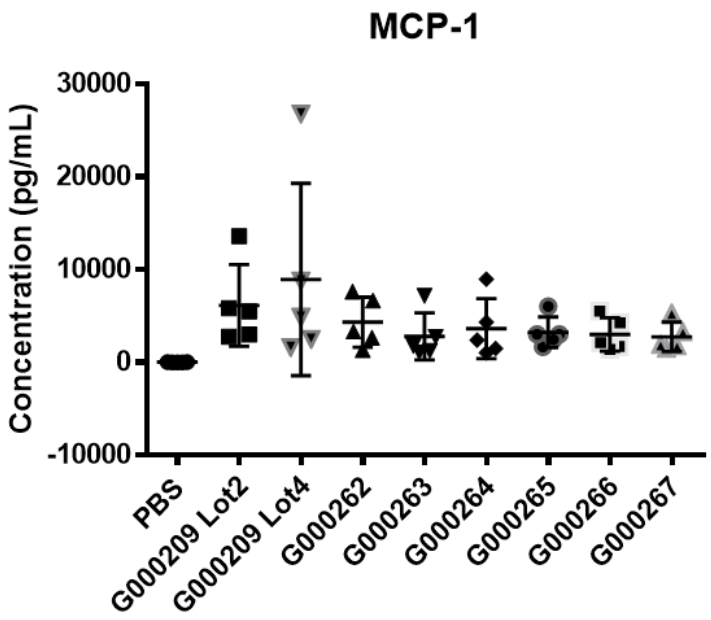


Figure 14C

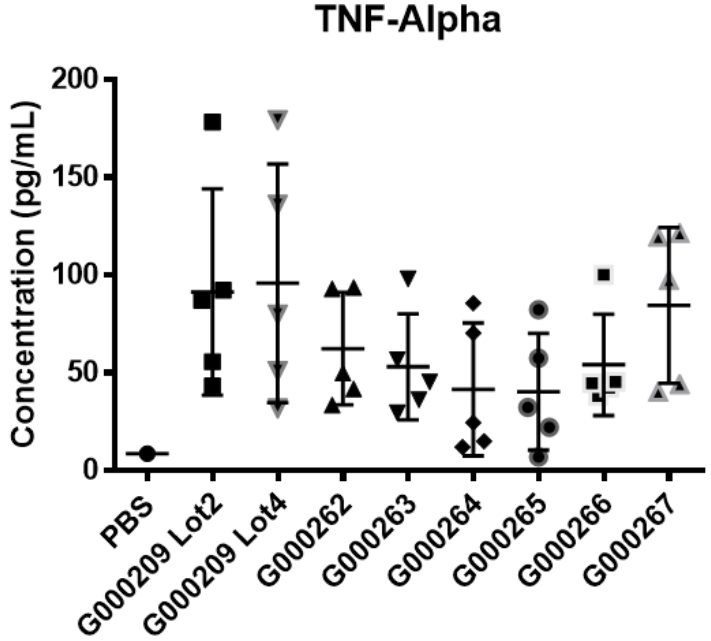


Figure 14D

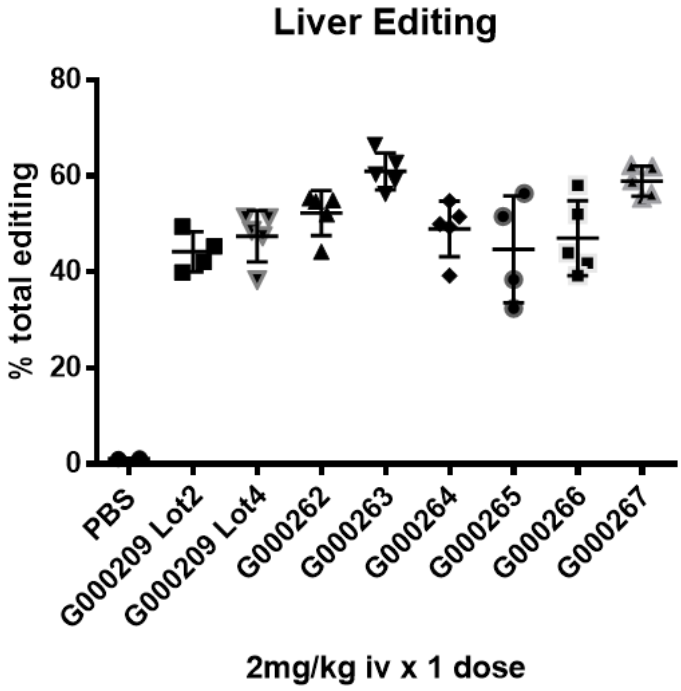


Figure 15A

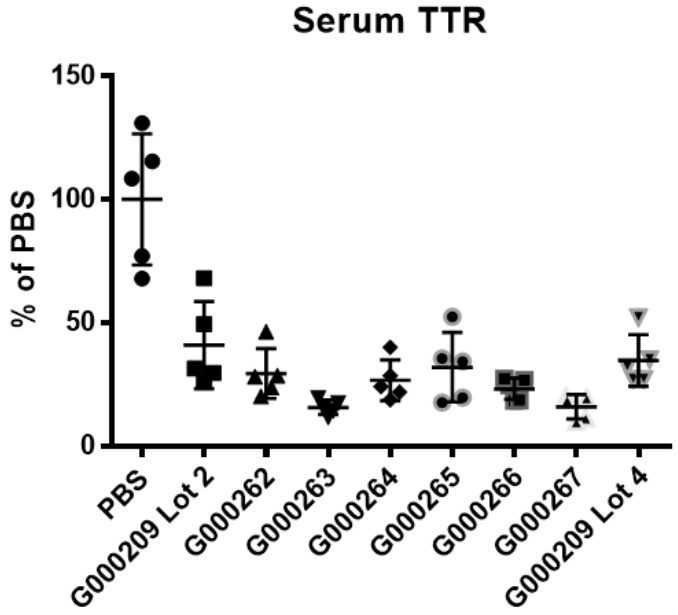
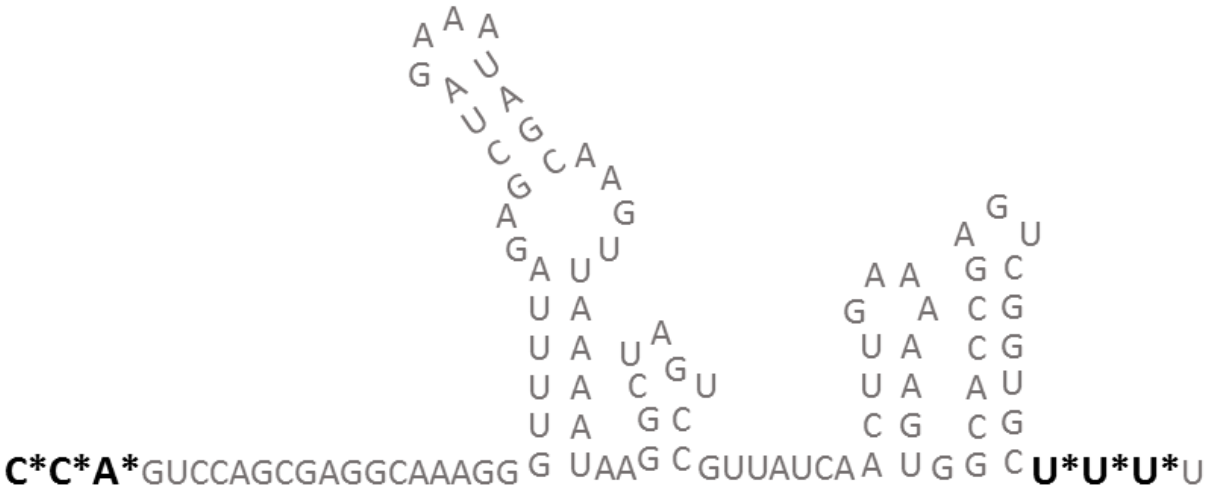


Figure 15B

Guide	Average % Editing	Std. Dev.
G209 Lot#2	*44.2*	*4.1*
G209 Lot#4	*47.5*	*5.3*
G262	*52.2*	*4.7*
G263	*60.9*	*3.8*
G264	*48.9*	*5.8*
G265	*44.7*	*11.1*
G266	*47.0*	*7.8*
G267	*58.9*	*3.1*

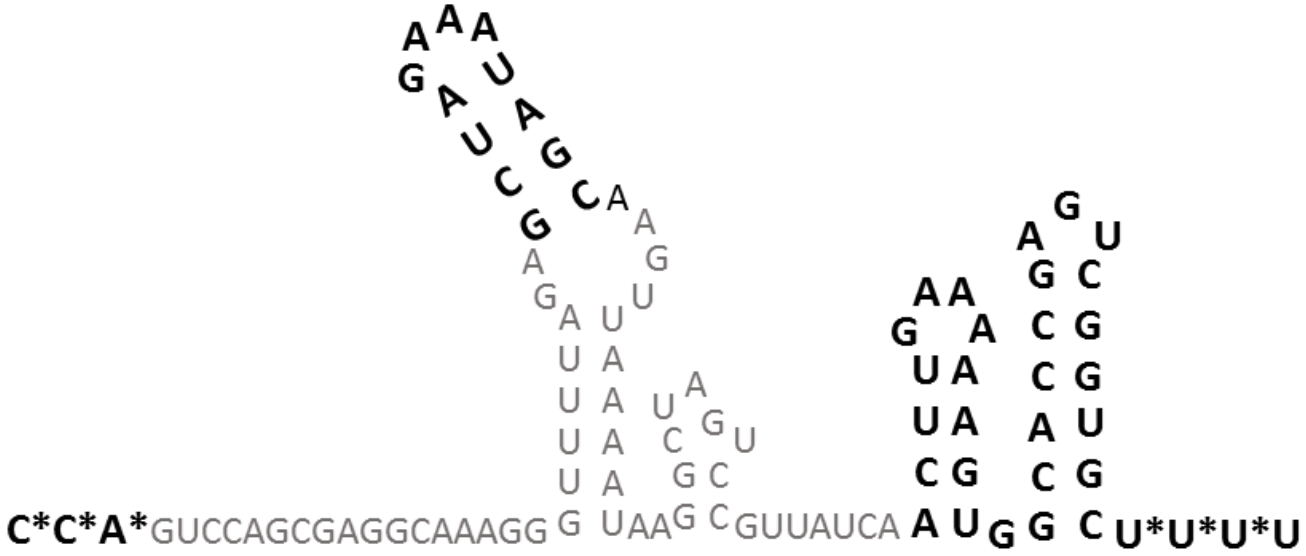
Figure 15C



* = Phosphorothioate

End-modified sgRNA
G000209

Figure 15D



* = Phosphorothioate

Highly modified sgRNA
G000267

Figure 15E

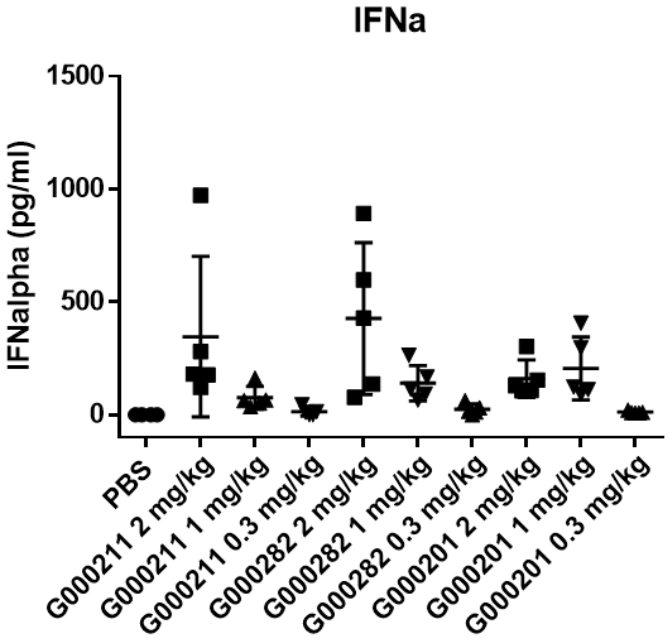


Figure 16A

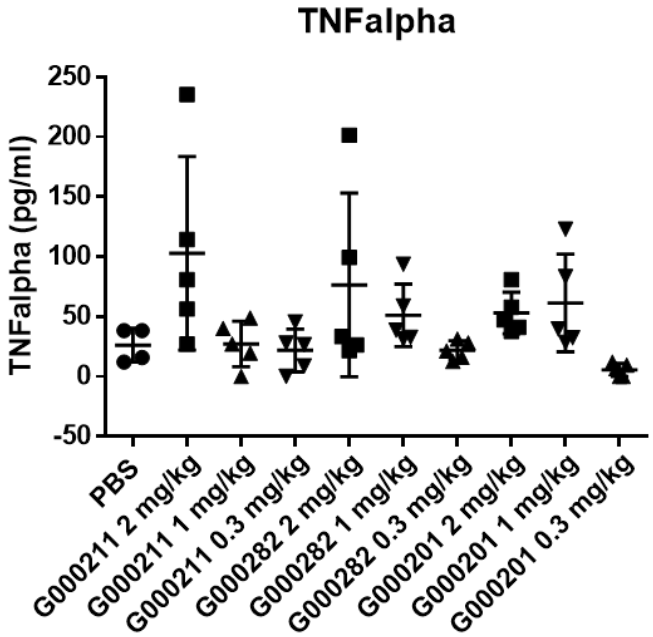


Figure 16B

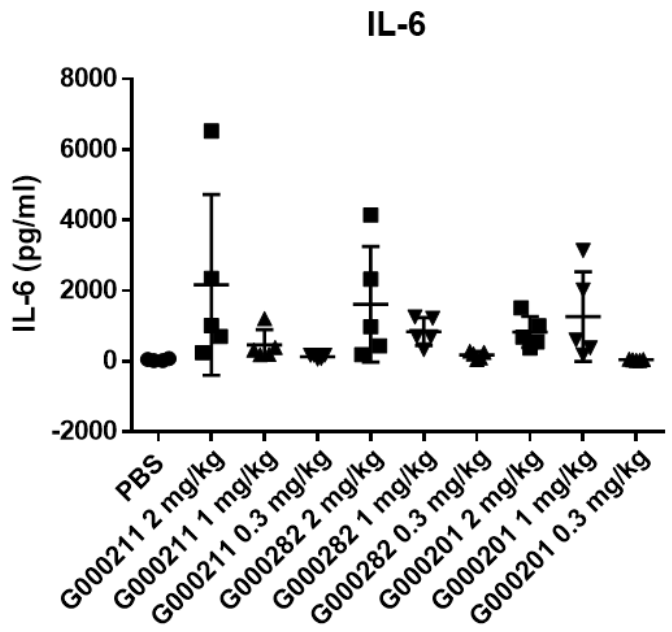


Figure 16C

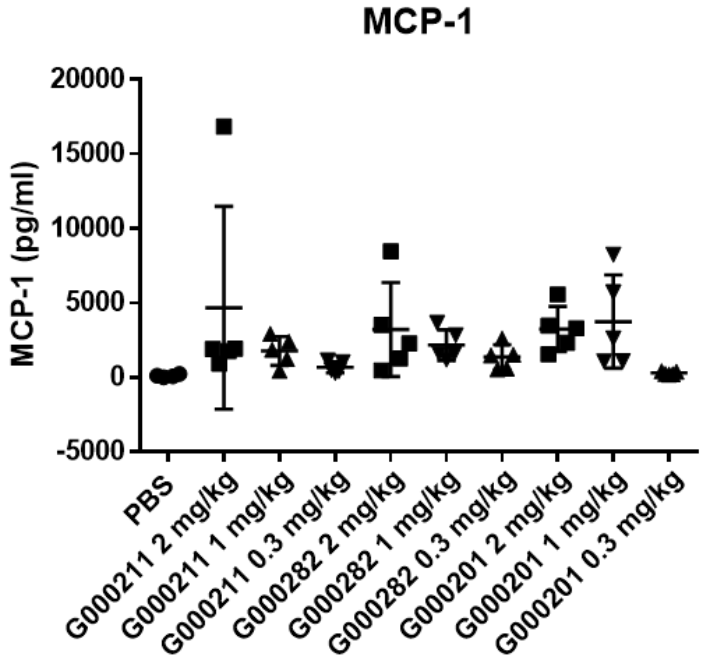


Figure 16D

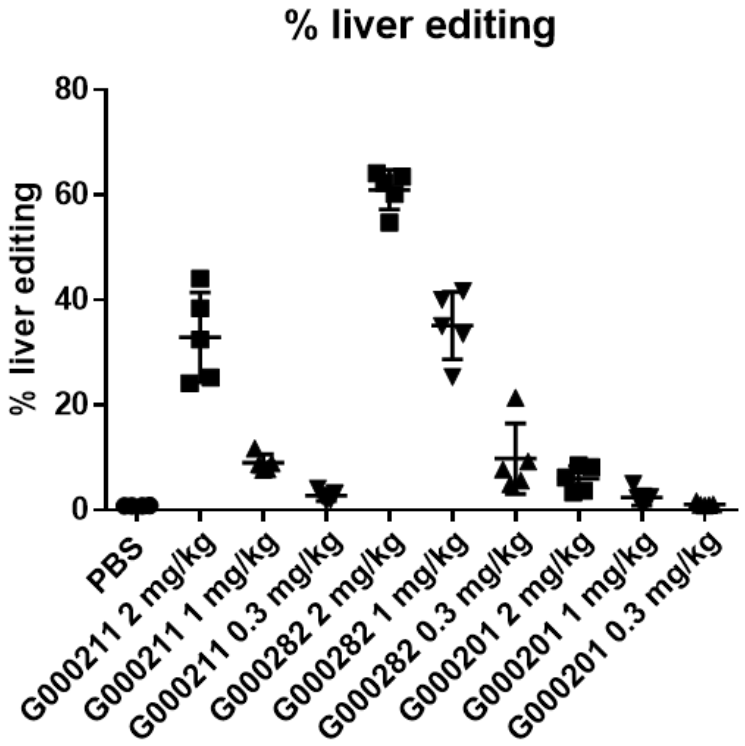


Figure 17A

	Average % Editing	Std. Dev.
PBS	0.8370155	0.03184162
G211 2 mg/kg	32.89082	8.520595
G211 1 mg/kg	9.024511	1.640143
G211 0.3 mg/kg	2.762495	0.9668095
G282 2 mg/kg	60.99886	3.792423
G282 1 mg/kg	35.13641	6.434229
G282 0.3 mg/kg	9.812781	6.713302
G284 2 mg/kg	6.007987	2.434861
G284 1 mg/kg	2.413099	1.540902
G284 0.3 mg/kg	1.130903	0.3189707

Figure 17B

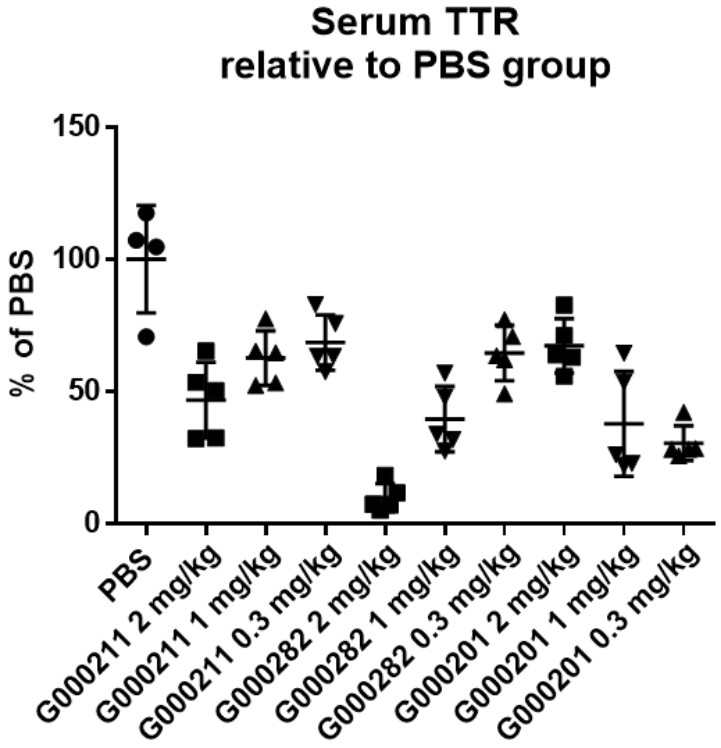


Figure 17C

	Average % TTR Reduction	Std. Dev.
PBS	100.000	20.31276
G211 2 mg/kg	46.73565	14.33137
G211 1 mg/kg	62.66805	10.30656
G211 0.3 mg/kg	68.51203	10.40399
G282 2 mg/kg	9.890765	5.288372
G282 1 mg/kg	39.58118	12.35095
G282 0.3 mg/kg	64.59702	10.51861
G284 2 mg/kg	67.28742	10.217
G284 1 mg/kg	37.76873	19.77835
G284 0.3 mg/kg	30.4822	6.612638

Figure 17D

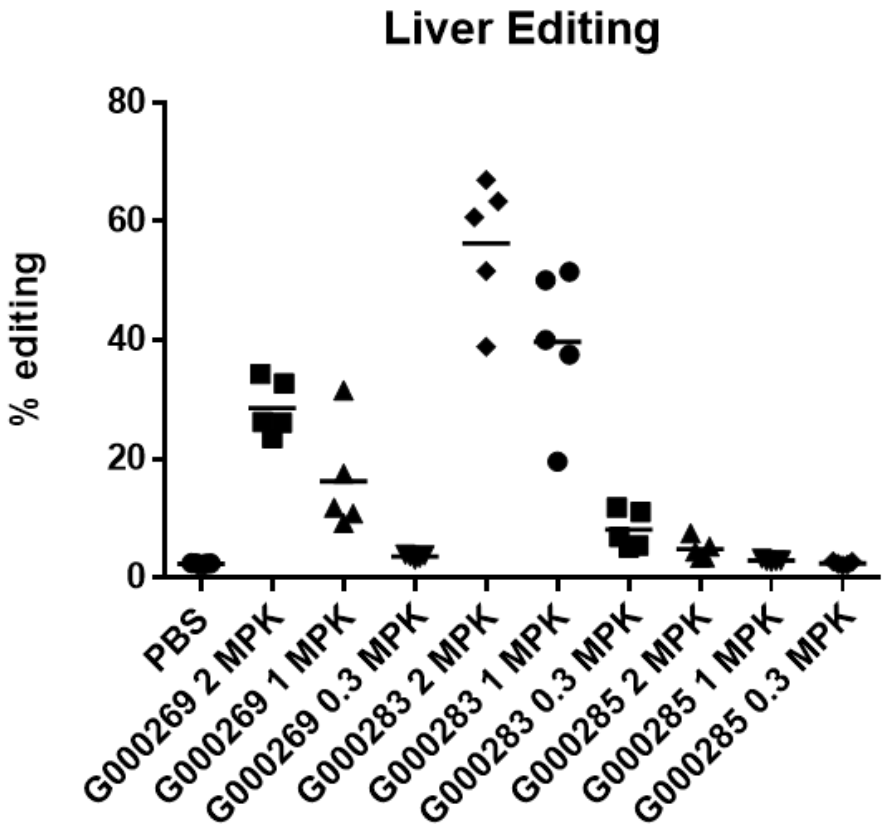

Liver Editing
% editing
80
60
40
20
0
PBS
G000269 2 MPK
G000269 1 MPK
G000269 0.3 MPK
G000283 2 MPK
G000283 1 MPK
G000283 0.3 MPK
G000285 2 MPK
G000285 1 MPK
G000285 0.3 MPK

Figure 18A

Guide	Dose	Average % Editing
PBS		*2.38*
G269	*2 MPK*	*28.57*
	1 MPK	*16.21*
	0.3 MPK	*3.54*
G283	*2 MPK*	*56.32*
	1 MPK	*39.73*
	0.3 MPK	*8.08*
G285	*2 MPK*	*4.80*
	1 MPK	*2.90*
	0.3 MPK	*2.44*

Figure 18B

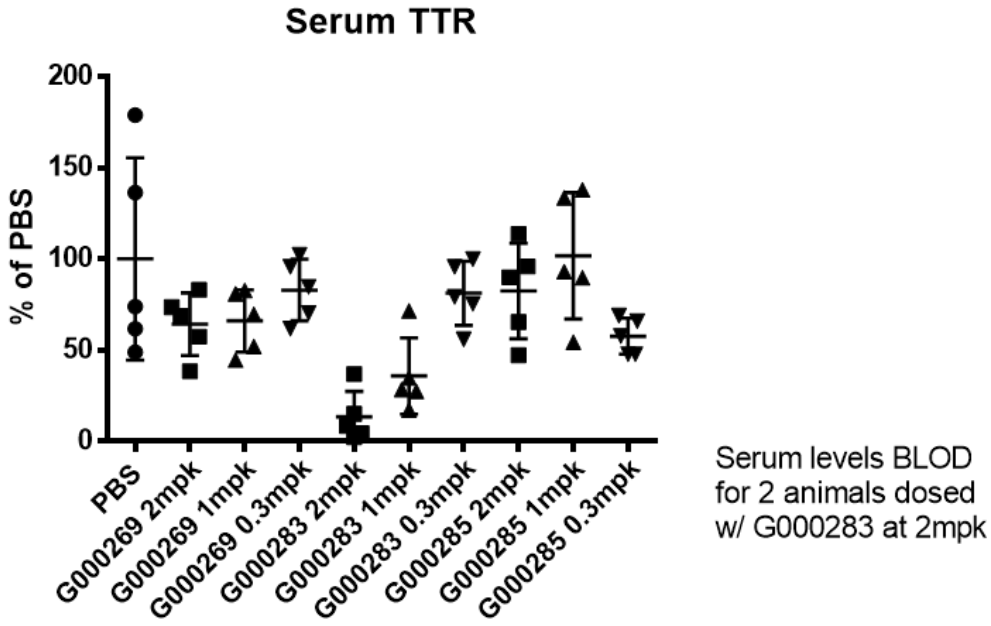


Figure 18C

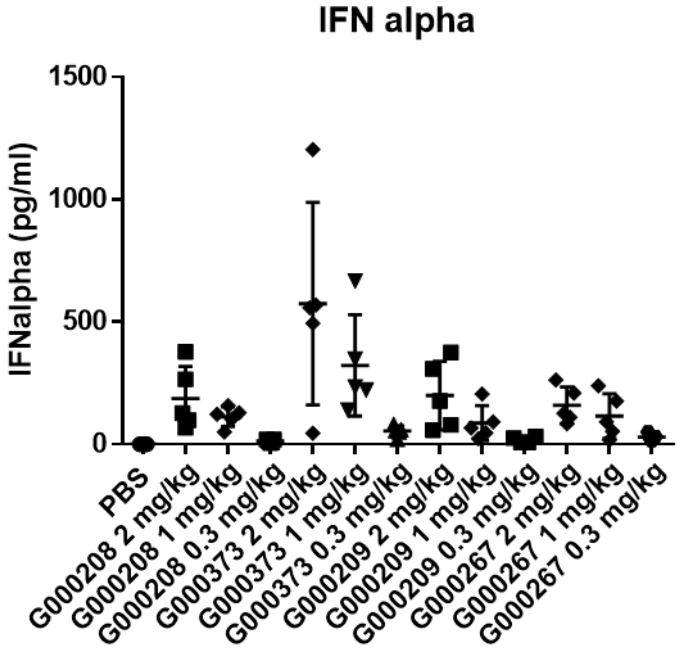


Figure 19A

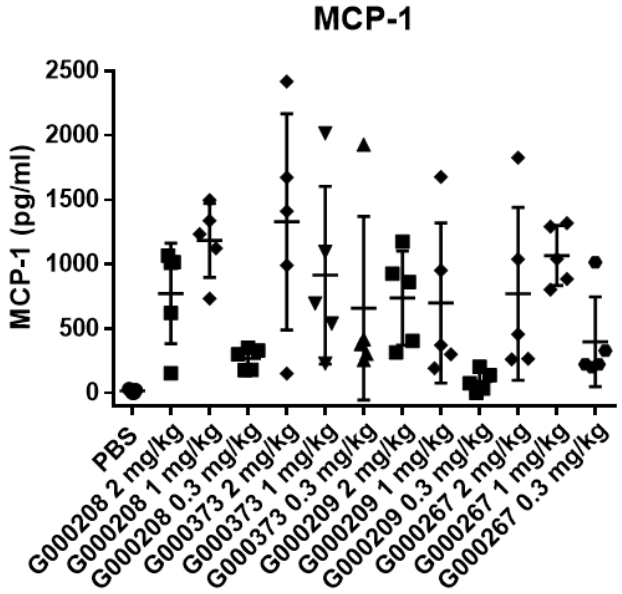


Figure 19B

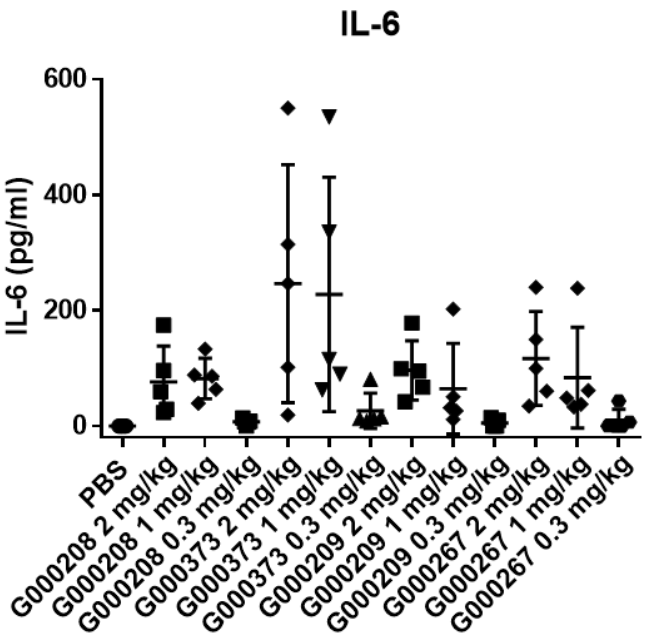


Figure 19C

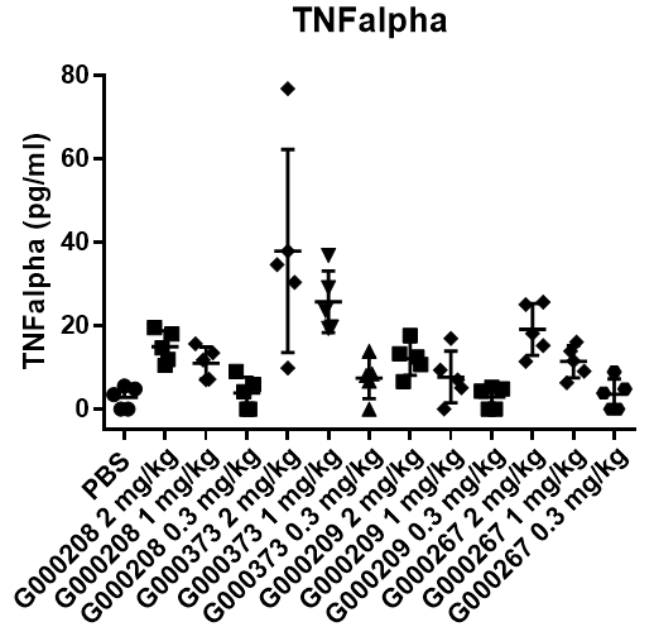


Figure 19D

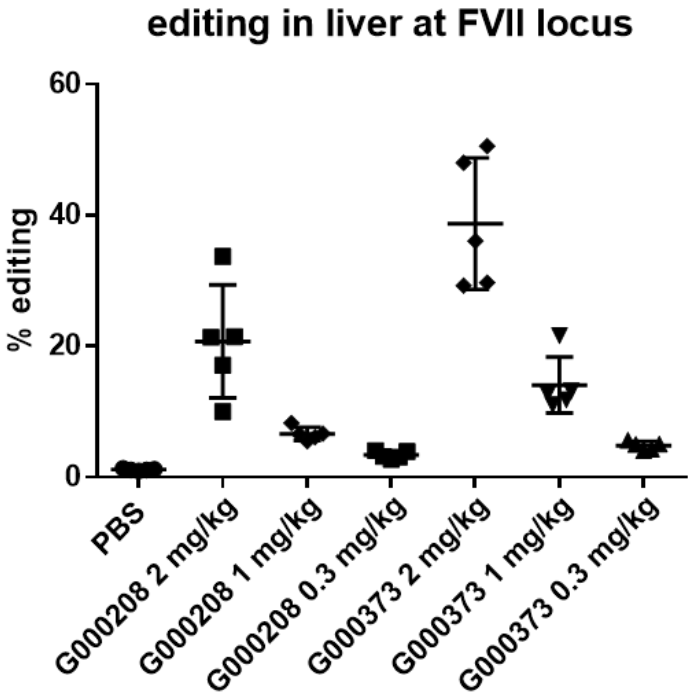


Figure 20A

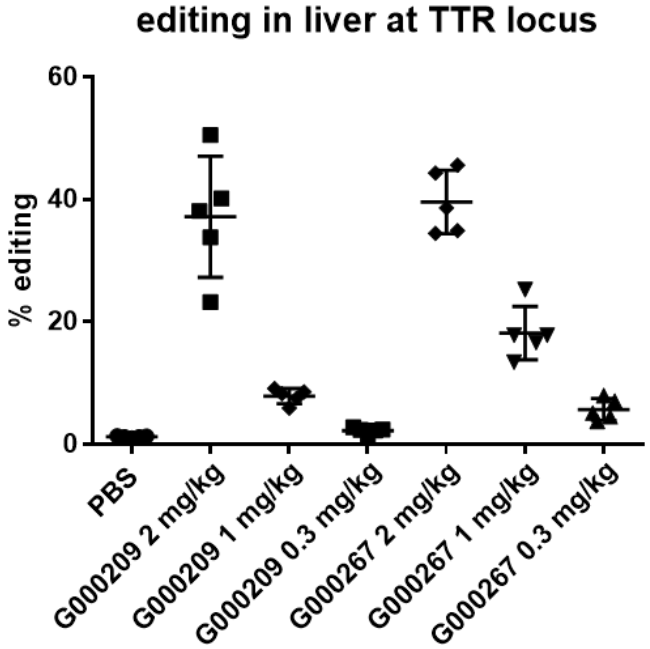
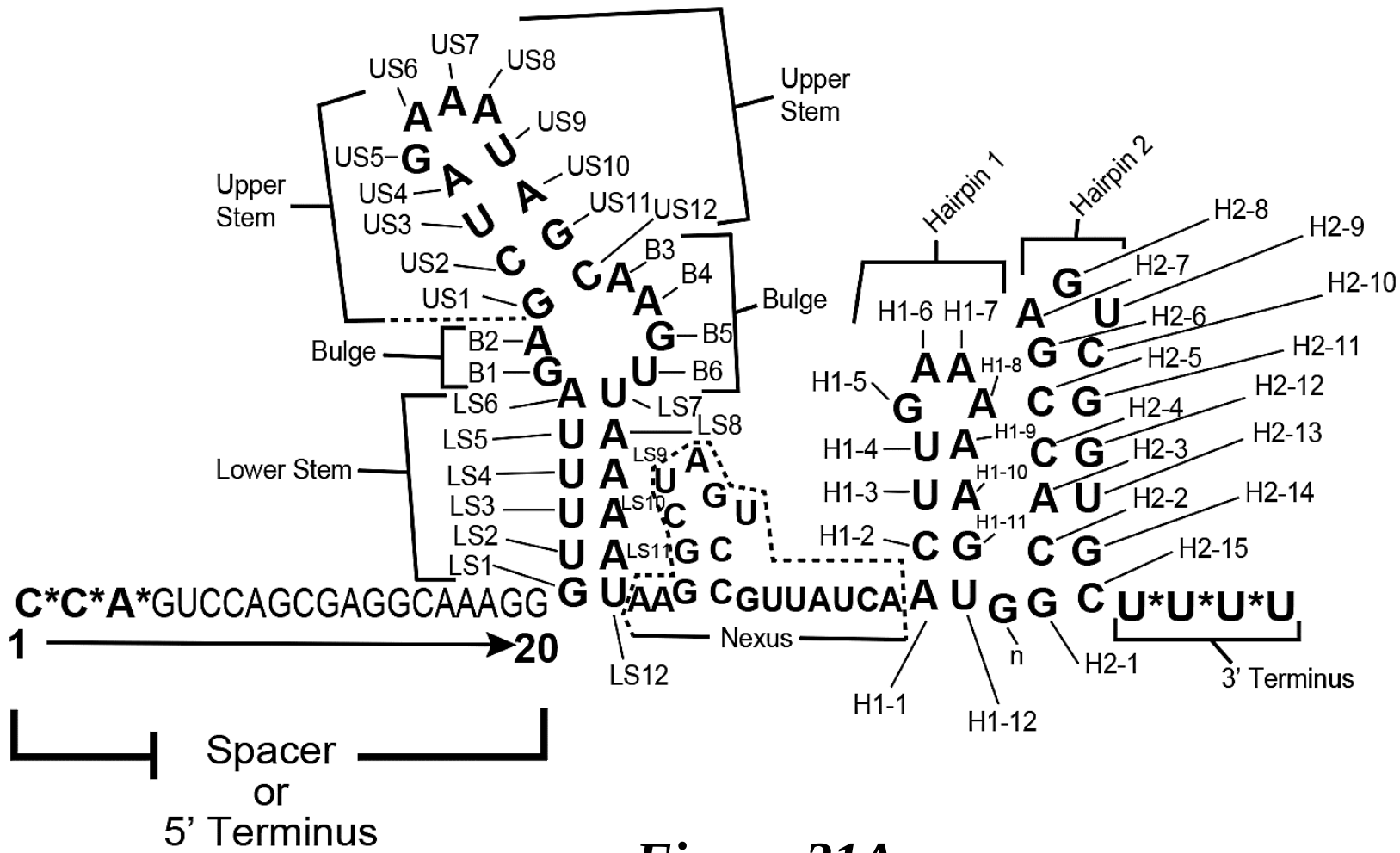
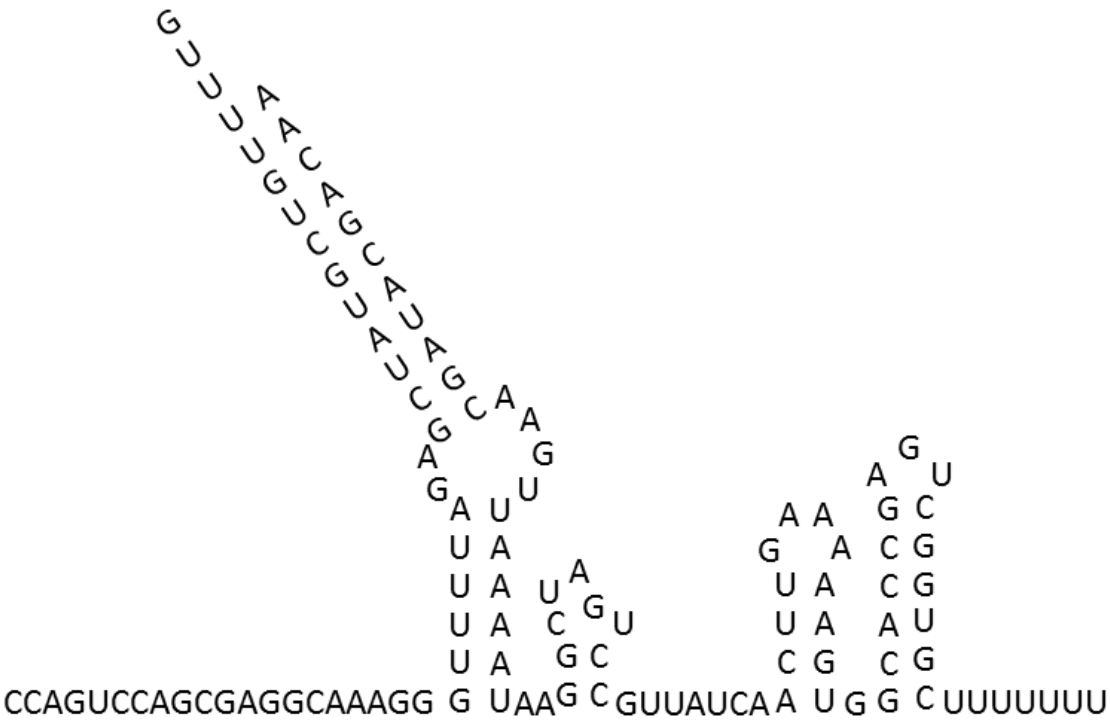


Figure 20B



Upper Stem
Upper Stem
Bulge
Bulge
Lower Stem
Hairpin 1
Hairpin 2
Nexus
3' Terminus
C*C*A*GUCCAGCGAGGCAAAGG
1
20
Spacer or 5' Terminus
US1
US2
US3
US4
US5
US6
US7
US8
US9
US10
US11
US12
B1
B2
B3
B4
B5
B6
LS1
LS2
LS3
LS4
LS5
LS6
LS7
LS8
LS9
LS10
LS11
LS12
H1-1
H1-2
H1-3
H1-4
H1-5
H1-6
H1-7
H1-8
H1-9
H1-10
H1-11
H1-12
H2-1
H2-2
H2-3
H2-4
H2-5
H2-6
H2-7
H2-8
H2-9
H2-10
H2-11
H2-12
H2-13
H2-14
H2-15
n

Figure 21A



dgRNA
CR000686 + TR000002

Figure 21B

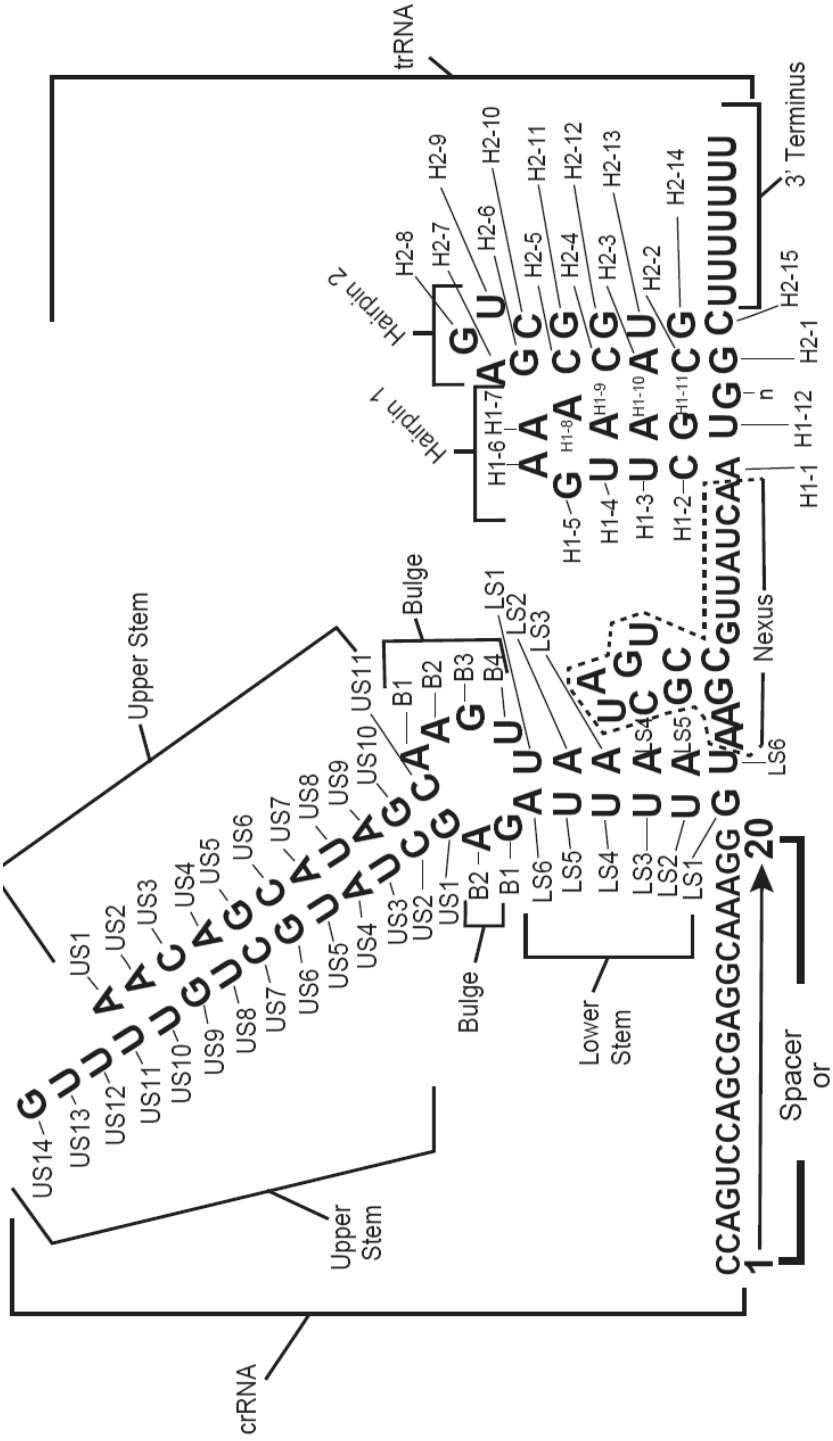

dgRNA
CR000686 + TR000002
crRNA
trRNA
Upper Stem
Lower Stem
Bulge
Hairpin 1
Hairpin 2
Nexus
Spacer or 5' Terminus
3' Terminus

Figure 21C

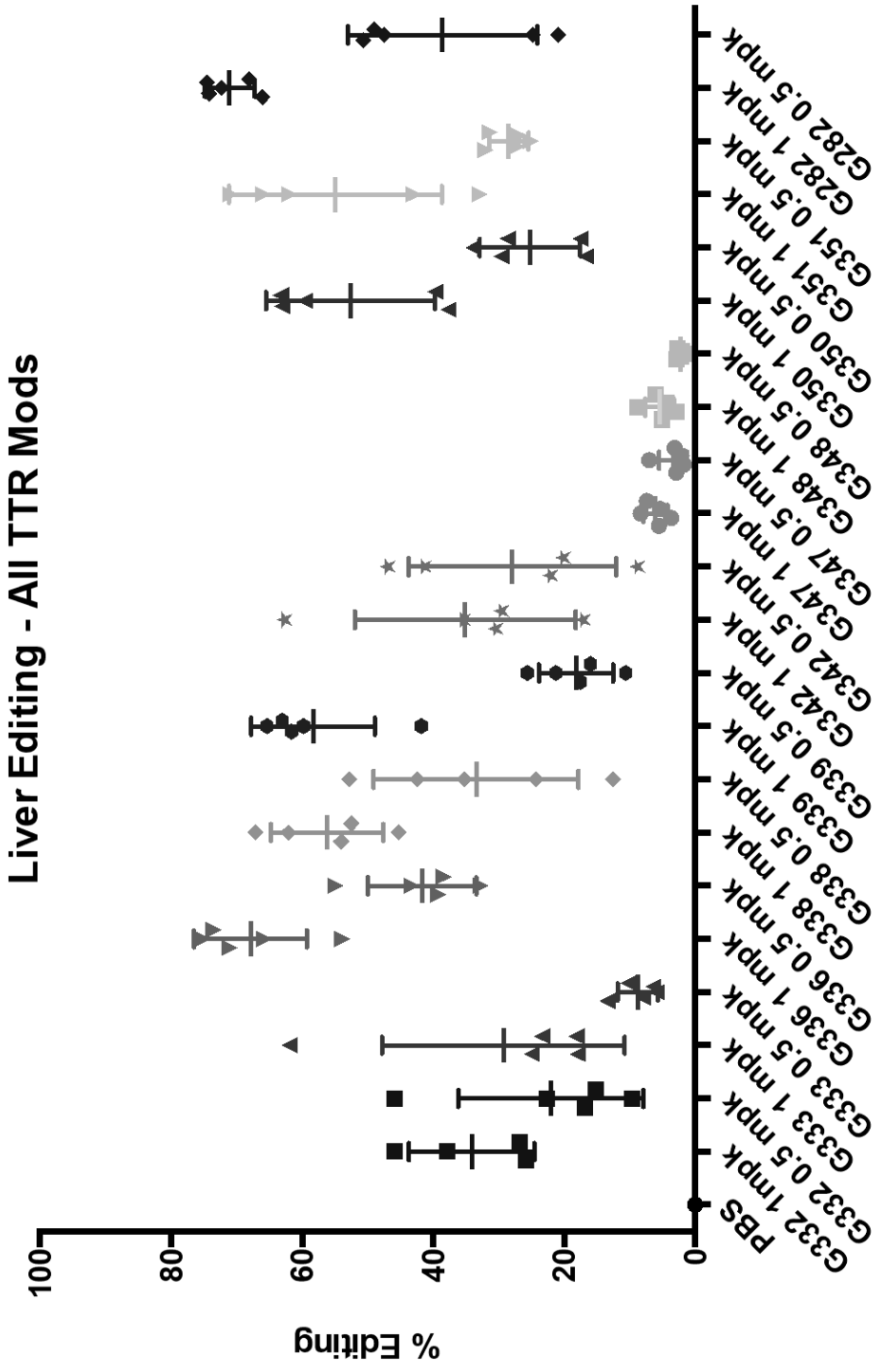

Liver Editing - All TTR Mods
% Editing
100
80
60
40
20
0
PBS
G332 1mpk
G332 0.5 mpk
G333 1 mpk
G333 0.5 mpk
G336 1 mpk
G336 0.5 mpk
G338 1 mpk
G338 0.5 mpk
G339 1 mpk
G339 0.5 mpk
G342 1 mpk
G342 0.5 mpk
G347 1 mpk
G347 0.5 mpk
G348 1 mpk
G348 0.5 mpk
G350 1 mpk
G350 0.5 mpk
G351 1 mpk
G351 0.5 mpk
G282 1 mpk
G282 0.5 mpk

Figure 22A

Group	Average % Editing	Std. Dev.
PBS	0	0
G332 1mpk	34.14	9.575
G332 0.5 mpk	22.08	14.13
G333 1 mpk	29.28	18.53
G333 0.5 mpk	8.801	3.082
G336 1 mpk	67.92	8.609
G336 0.5 mpk	41.72	8.277
G338 1 mpk	56.25	8.561
G338 0.5 mpk	33.48	15.63
G339 1 mpk	58.36	9.444
G339 0.5 mpk	18.23	5.654
G342 1 mpk	35.12	16.84
G342 0.5 mpk	27.94	15.83
G347 1 mpk	6.073	1.825
G347 0.5 mpk	3.399	2.13
G348 1 mpk	5.464	2.171
G348 0.5 mpk	2.333	0.448
G350 1 mpk	52.66	12.88
G350 0.5 mpk	25.27	7.695
G351 1 mpk	54.94	16.24
G351 0.5 mpk	28.52	3.014
G282 1 mpk	71.08	3.789
G282 0.5 mpk	38.6	14.45

Figure 22B

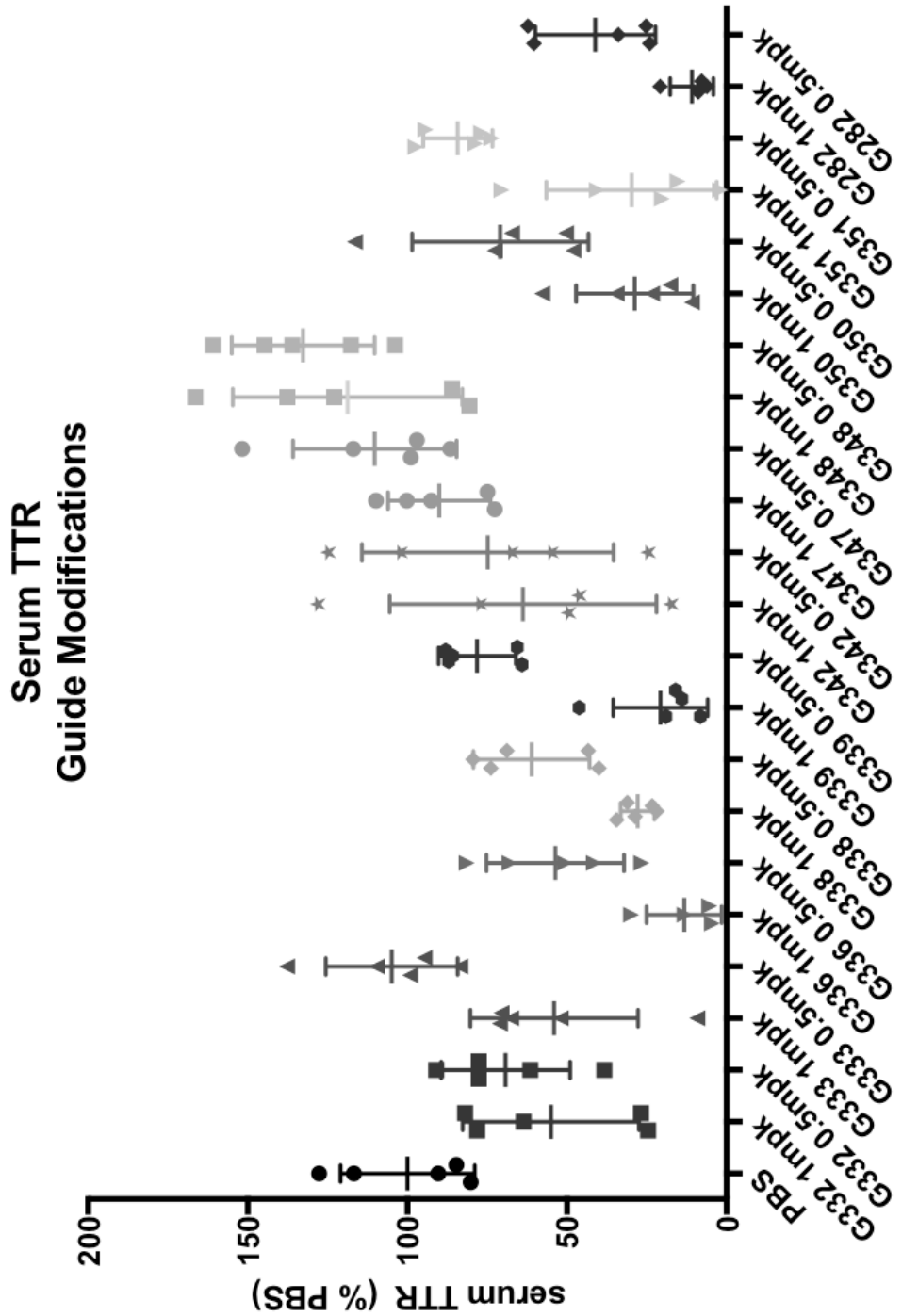

Serum TTR
Guide Modifications
serum TTR (% PBS)
200
150
100
50
0
PBS
G332 1mpk
G332 0.5mpk
G333 1mpk
G333 0.5mpk
G336 1mpk
G336 0.5mpk
G338 1mpk
G338 0.5mpk
G339 1mpk
G339 0.5mpk
G342 1mpk
G342 0.5mpk
G347 1mpk
G347 0.5mpk
G348 1mpk
G348 0.5mpk
G350 1mpk
G350 0.5mpk
G351 1mpk
G351 0.5mpk
G282 1mpk
G282 0.5mpk

Figure 22C

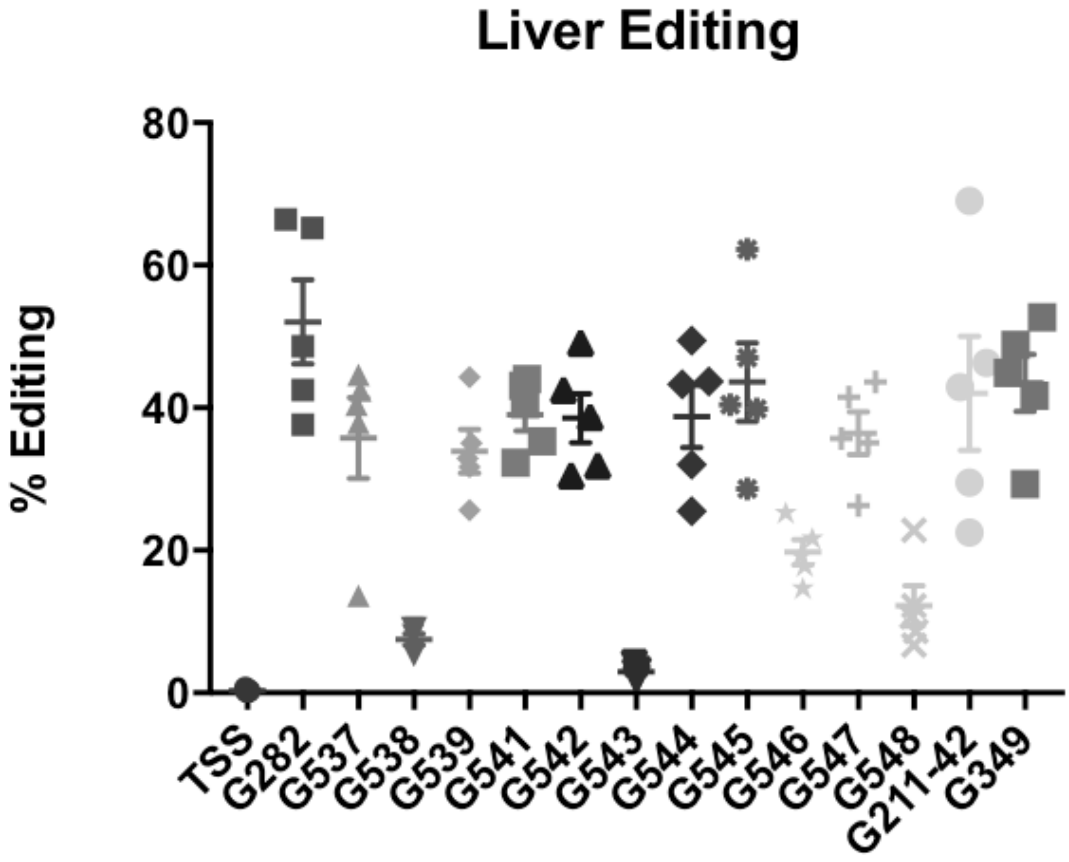

Liver Editing
% Editing
80
60
40
20
0
TSS
G282
G537
G538
G539
G541
G542
G543
G544
G545
G546
G547
G548
G211-42
G349

Figure 23A

Guide	Average % Editing
TSS	0.32
G282	52.06
G537	35.78
G538	7.5
G539	33.9
G541	39.04
G542	38.54
G543	2.96
G544	38.78
G545	43.6
G546	19.74
G547	36.44
G548	12.2
G211-42	42.04
G349	43.48

Figure 23B

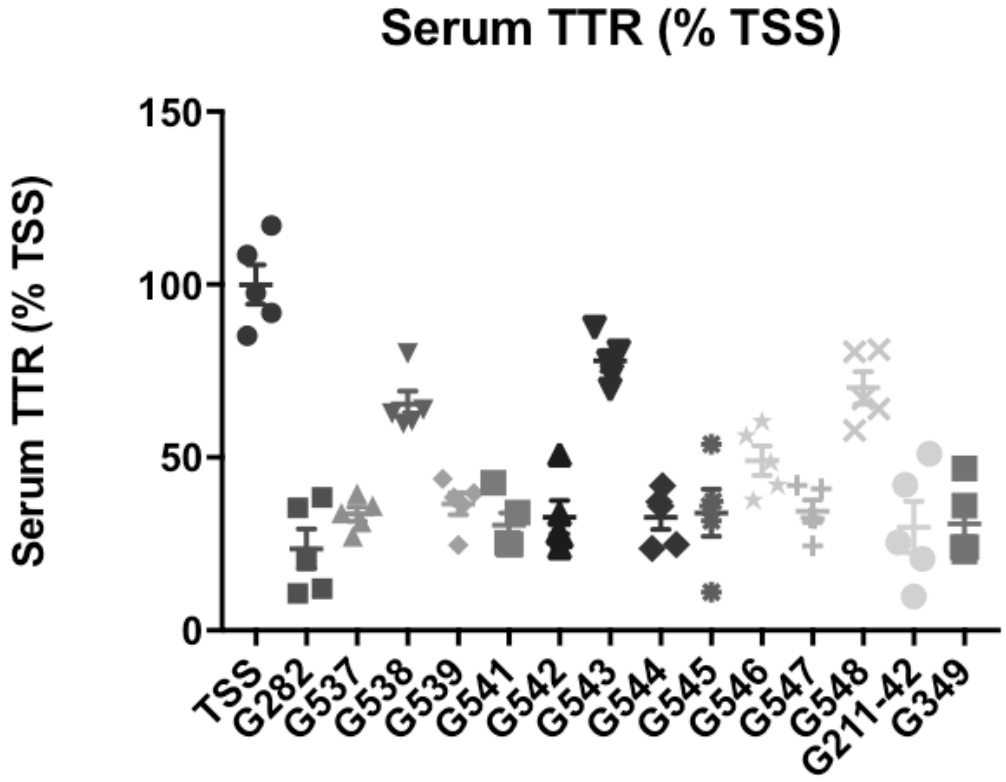

Serum TTR (% TSS)
Serum TTR (% TSS)
150
100
50
0
TSS
G282
G537
G538
G539
G541
G542
G543
G544
G545
G546
G547
G548
G211-42
G349

Figure 23C

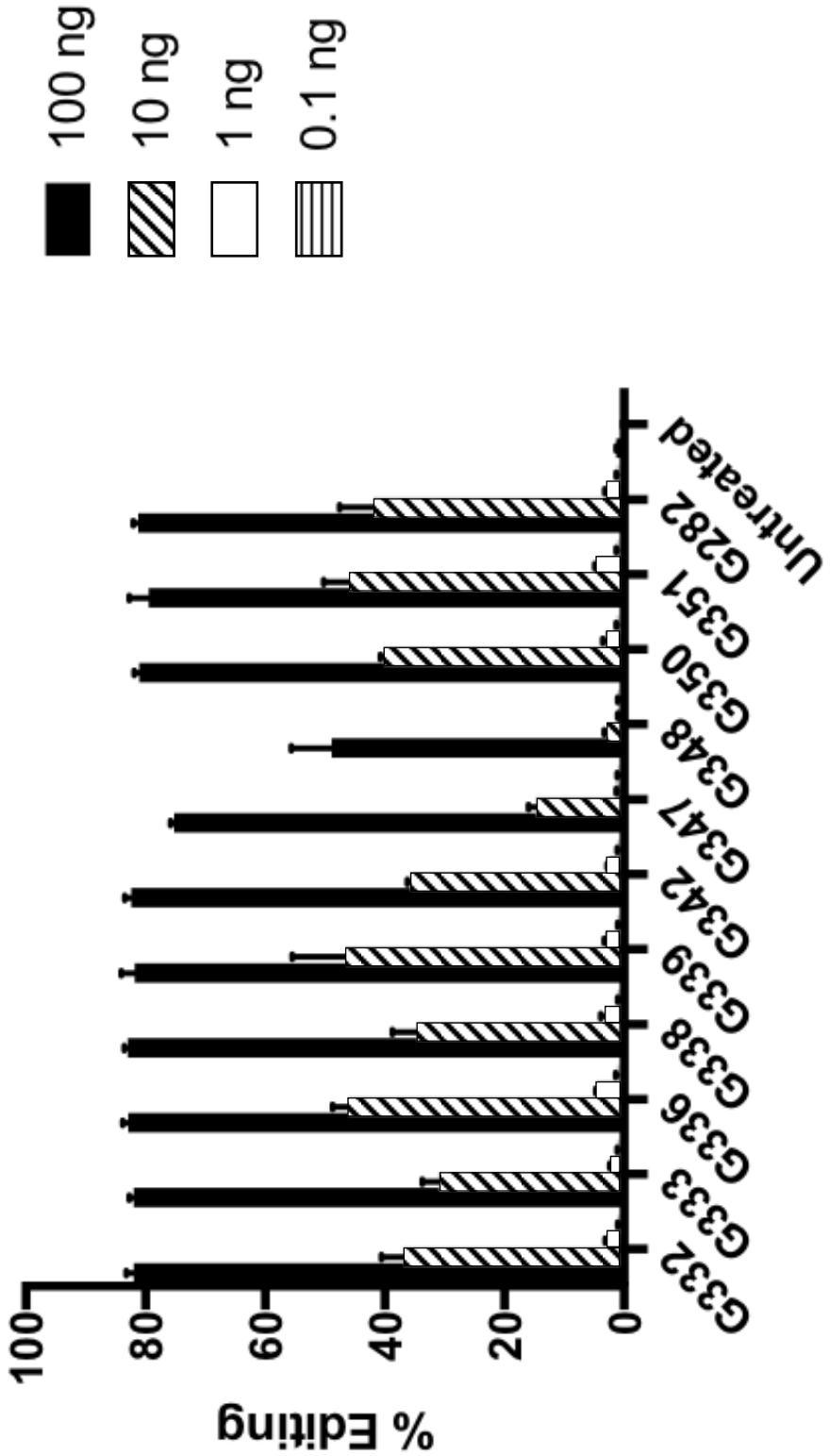

100 ng
10 ng
1 ng
0.1 ng
% Editing
100
80
60
40
20
0
G332
G333
G336
G338
G339
G342
G347
G348
G350
G351
G282
Untreated

Figure 24A

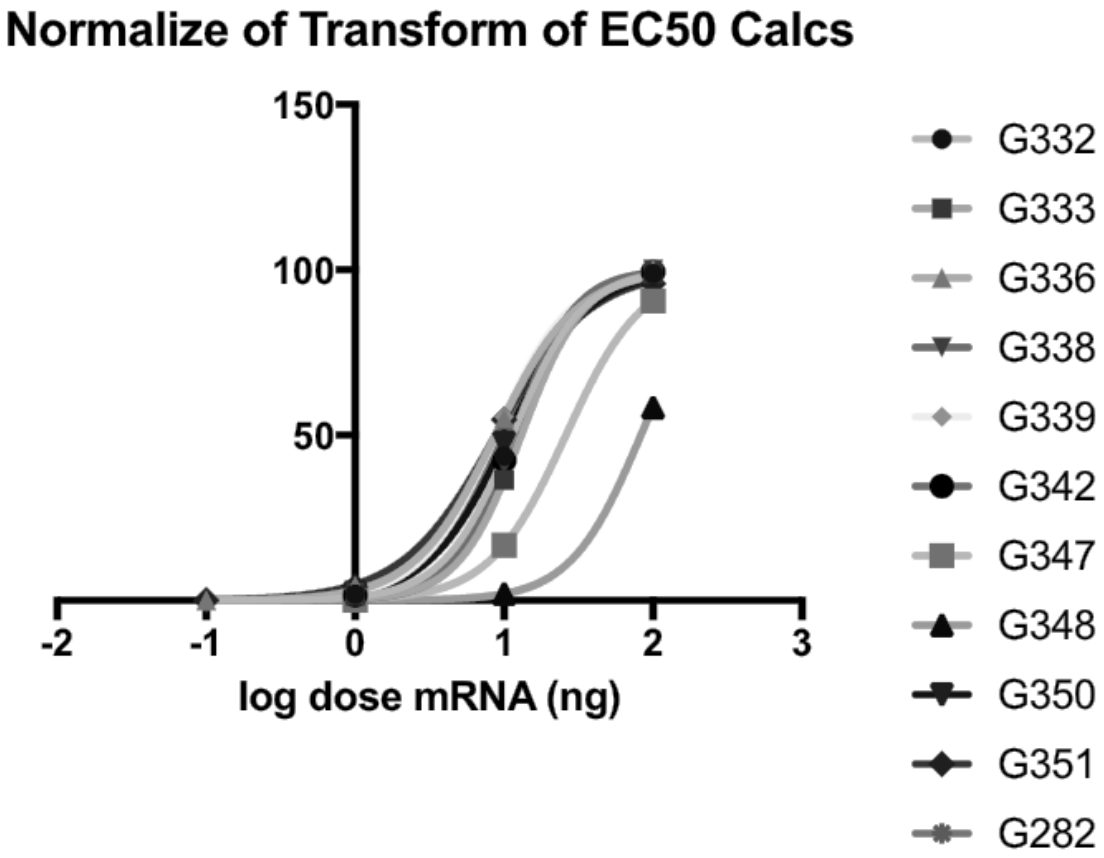

Normalize of Transform of EC50 Calcs
150
100
50
-2
-1
0
1
2
3
log dose mRNA (ng)
G332
G333
G336
G338
G339
G342
G347
G348
G350
G351
G282

Figure 24B

GUIDE	EC50
G332	11.42
G333	13.07
G336	8.735
G338	11.80
G339	8.778
G342	11.60
G347	26.07
G348	83.09
G350	10.57
G351	8.797
G282	10.04

Figure 24C