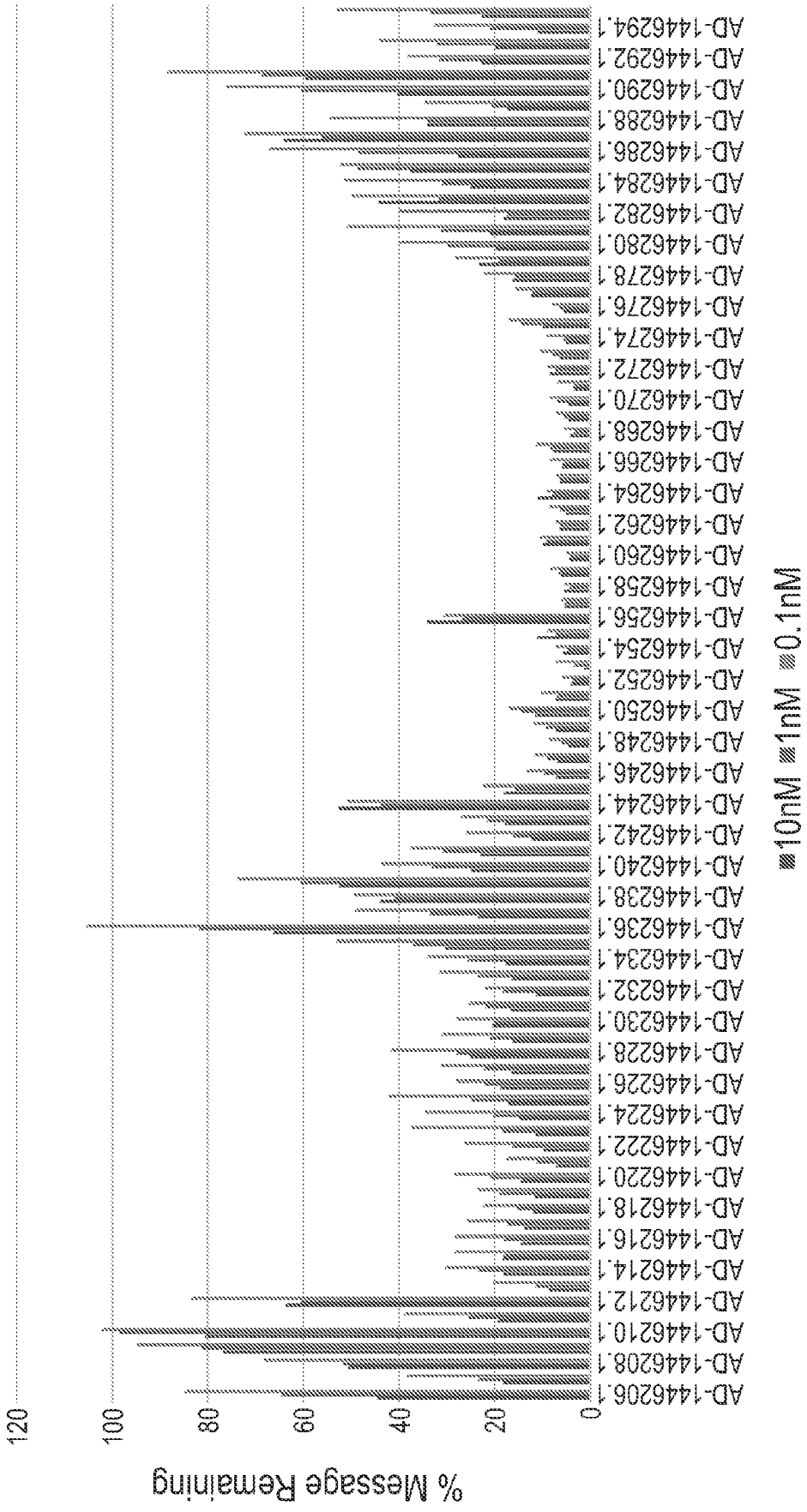

C9orf72 Antisense-targeting Duplexes In Vitro Screen
% Message Remaining
120
100
80
60
40
20
0
10nM
1nM
0.1nM
AD-1446206.1
AD-1446208.1
AD-1446210.1
AD-1446212.1
AD-1446214.1
AD-1446216.1
AD-1446218.1
AD-1446220.1
AD-1446222.1
AD-1446224.1
AD-1446226.1
AD-1446228.1
AD-1446230.1
AD-1446232.1
AD-1446234.1
AD-1446236.1
AD-1446238.1
AD-1446240.1
AD-1446242.1
AD-1446244.1
AD-1446246.1
AD-1446248.1
AD-1446250.1
AD-1446252.1
AD-1446254.1
AD-1446256.1
AD-1446258.1
AD-1446260.1
AD-1446262.1
AD-1446264.1
AD-1446266.1
AD-1446268.1
AD-1446270.1
AD-1446272.1
AD-1446274.1
AD-1446276.1
AD-1446278.1
AD-1446280.1
AD-1446282.1
AD-1446284.1
AD-1446286.1
AD-1446288.1
AD-1446290.1
AD-1446292.1
AD-1446294.1

FIG. 1

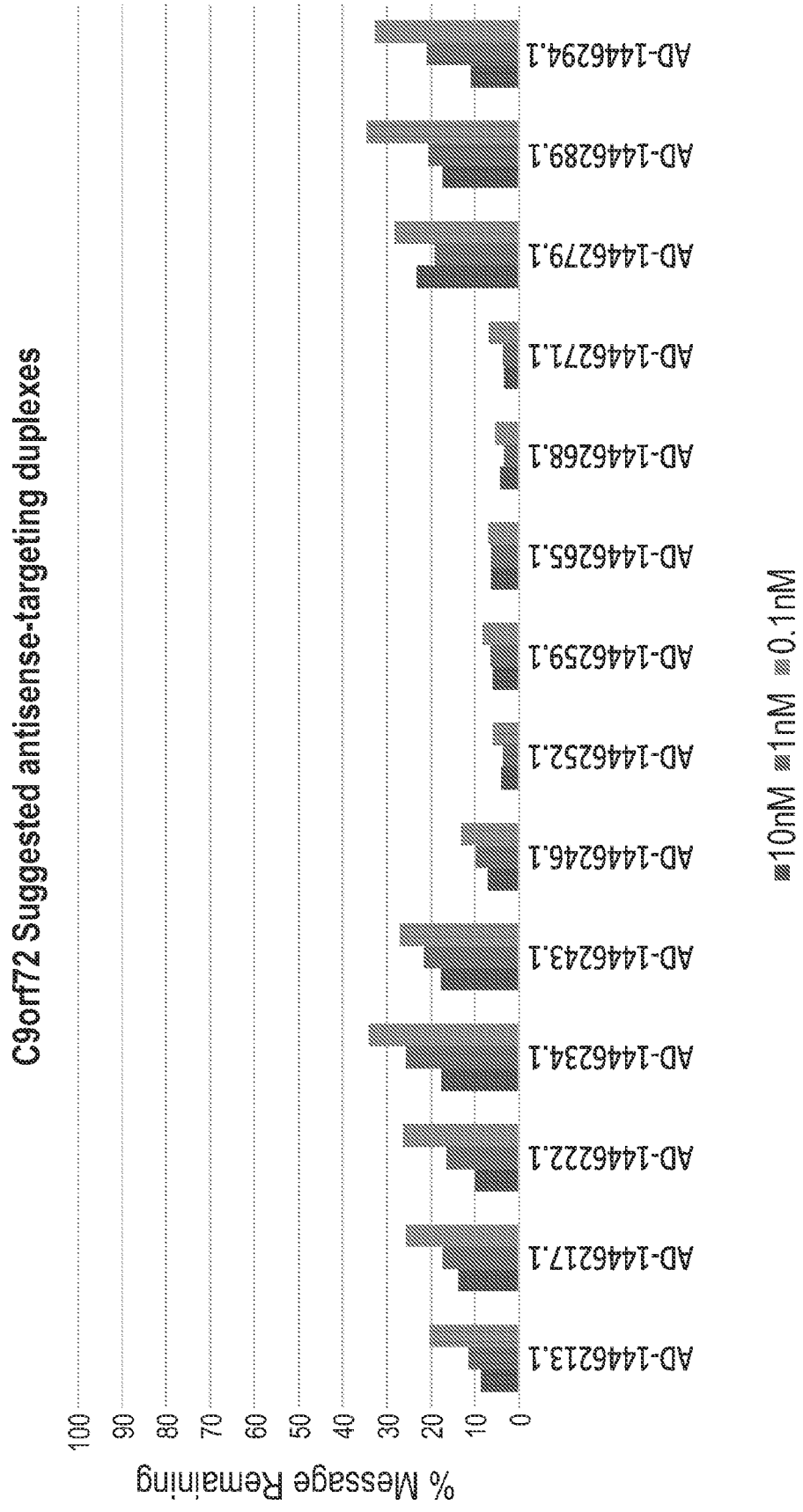

C9orf72 Suggested antisense-targeting duplexes
% Message Remaining
100
90
80
70
60
50
40
30
20
10
0
AD-1446213.1
AD-1446217.1
AD-1446222.1
AD-1446234.1
AD-1446243.1
AD-1446246.1
AD-1446252.1
AD-1446259.1
AD-1446265.1
AD-1446268.1
AD-1446271.1
AD-1446279.1
AD-1446289.1
AD-1446294.1
10nM 1nM 0.1nM

FIG. 2

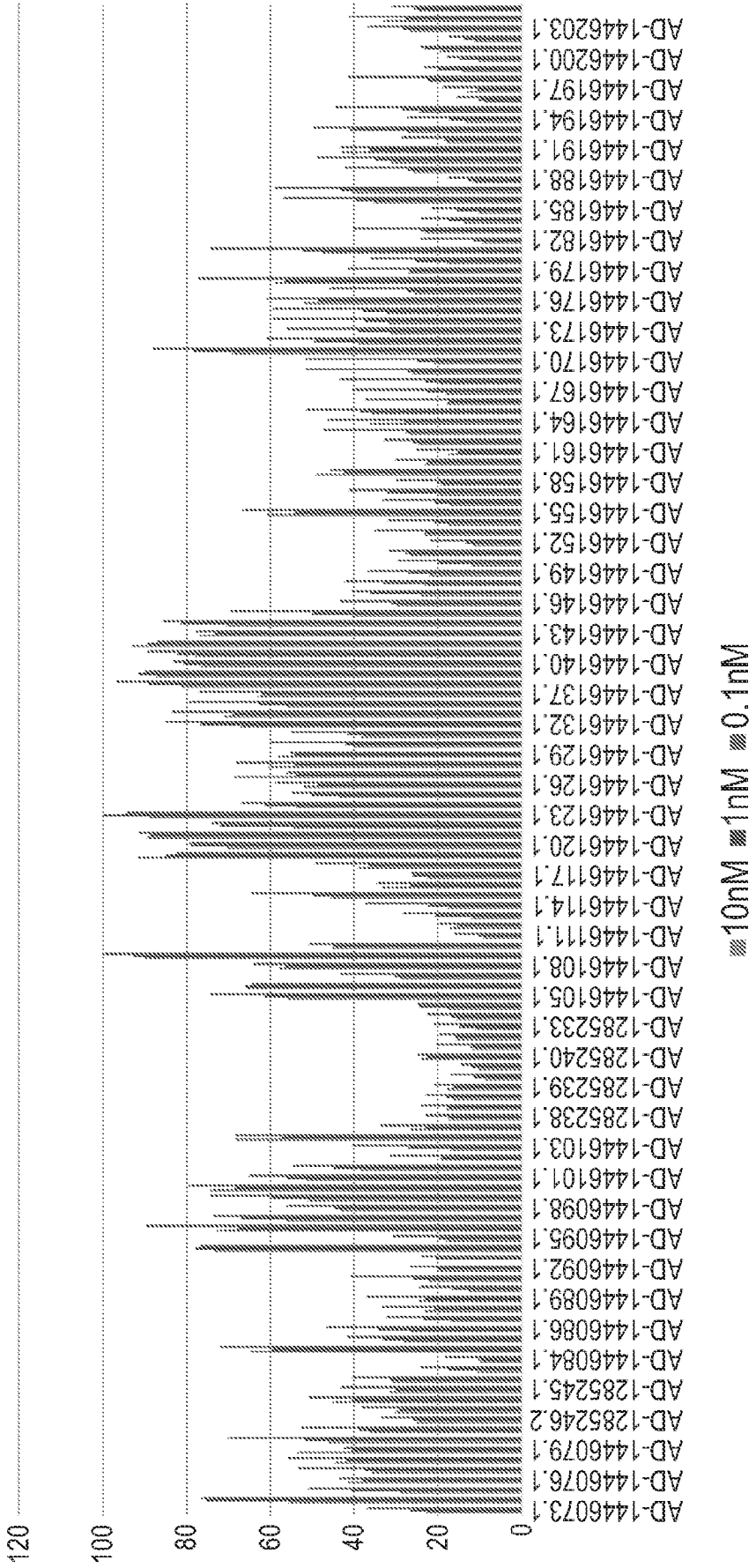

C9orf72 Expanded Sense In Vitro Screen
120
100
80
60
40
20
0
10nM 1nM 0.1nM
AD-1446073.1
AD-1446076.1
AD-1446079.1
AD-1285246.2
AD-1285245.1
AD-1446084.1
AD-1446086.1
AD-1446089.1
AD-1446092.1
AD-1446095.1
AD-1446098.1
AD-1446101.1
AD-1446103.1
AD-1285238.1
AD-1285239.1
AD-1285240.1
AD-1285233.1
AD-1446105.1
AD-1446108.1
AD-1446111.1
AD-1446114.1
AD-1446117.1
AD-1446120.1
AD-1446123.1
AD-1446126.1
AD-1446129.1
AD-1446132.1
AD-1446137.1
AD-1446140.1
AD-1446143.1
AD-1446146.1
AD-1446149.1
AD-1446152.1
AD-1446155.1
AD-1446158.1
AD-1446161.1
AD-1446164.1
AD-1446167.1
AD-1446170.1
AD-1446173.1
AD-1446176.1
AD-1446179.1
AD-1446182.1
AD-1446185.1
AD-1446188.1
AD-1446191.1
AD-1446194.1
AD-1446197.1
AD-1446200.1
AD-1446203.1

FIG. 3

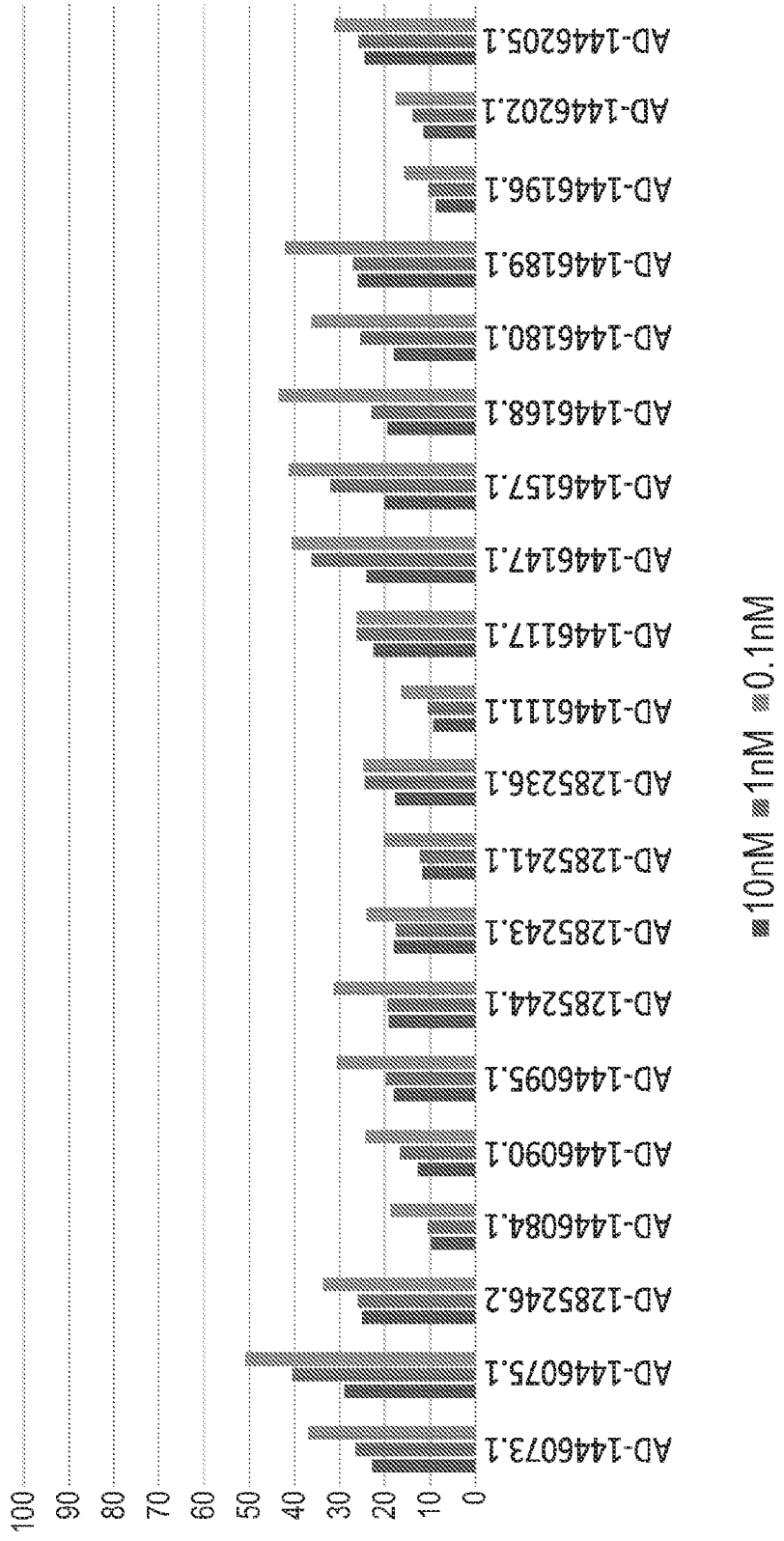

C9orf72 Expanded Sense Suggested Duplexes
100
90
80
70
60
50
40
30
20
10
0
AD-1446073.1
AD-1446075.1
AD-1285246.2
AD-1446084.1
AD-1446090.1
AD-1446095.1
AD-1285244.1
AD-1285243.1
AD-1285241.1
AD-1285236.1
AD-1446111.1
AD-1446117.1
AD-1446147.1
AD-1446157.1
AD-1446168.1
AD-1446180.1
AD-1446189.1
AD-1446196.1
AD-1446202.1
AD-1446205.1
10nM 1nM 0.1nM

FIG. 4

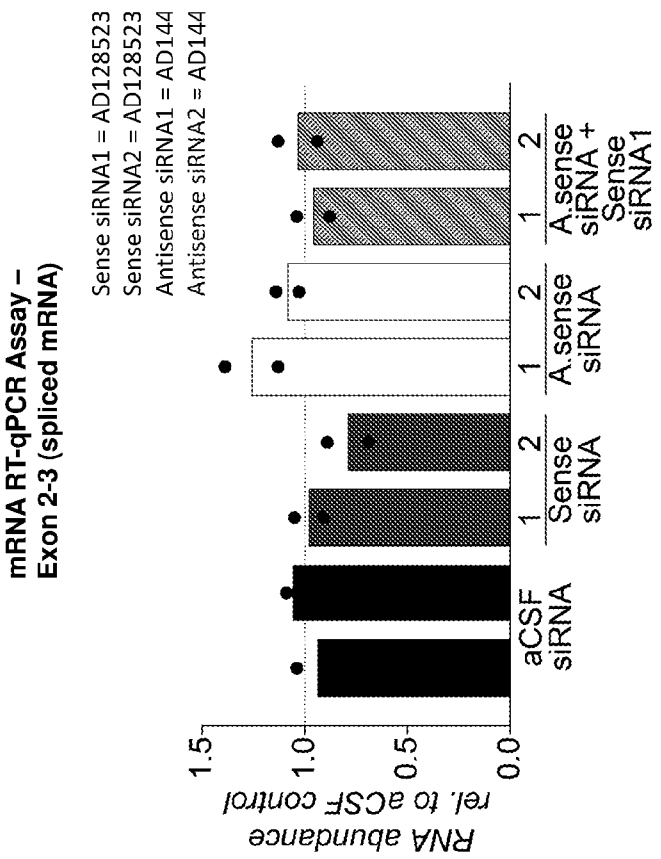

mRNA RT-qPCR Assay –
Exon 2-3 (spliced mRNA)
Sense siRNA1 = AD128523
Sense siRNA2 = AD128523
Antisense siRNA1 = AD144
Antisense siRNA2 = AD144
RNA abundance
rel. to aCSF control
1.5
1.0
0.5
0.0
aCSF siRNA
1
2
Sense siRNA
1
2
A.sense siRNA
1
2
A.sense siRNA + Sense siRNA1

FIG. 5B

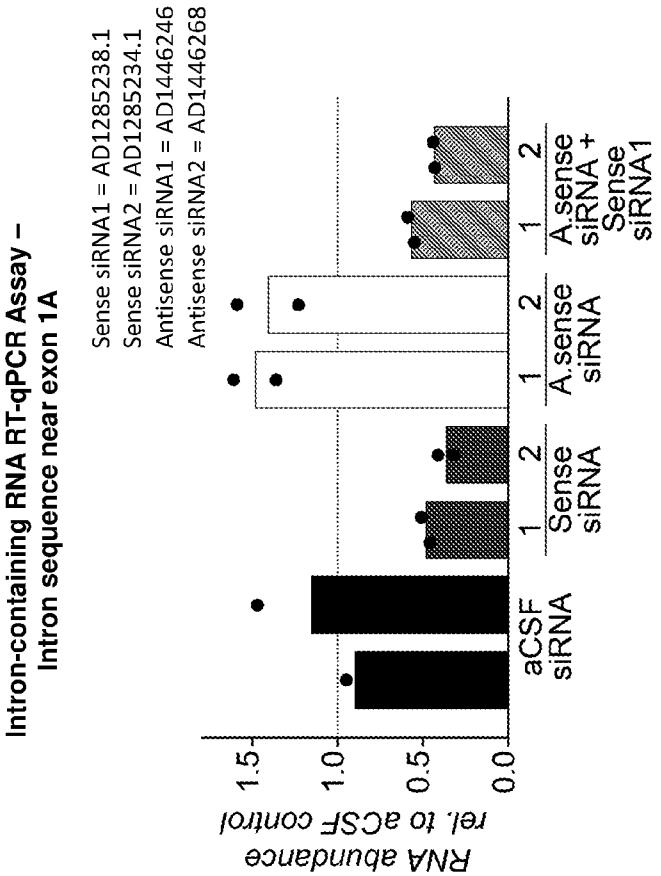

Intron-containing RNA RT-qPCR Assay –
Intron sequence near exon 1A
Sense siRNA1 = AD1285238.1
Sense siRNA2 = AD1285234.1
Antisense siRNA1 = AD1446246
Antisense siRNA2 = AD1446268
RNA abundance
rel. to aCSF control
1.5
1.0
0.5
0.0
aCSF siRNA
1
2
Sense siRNA
1
2
A.sense siRNA
1
2
A.sense siRNA + Sense siRNA1

FIG. 5A

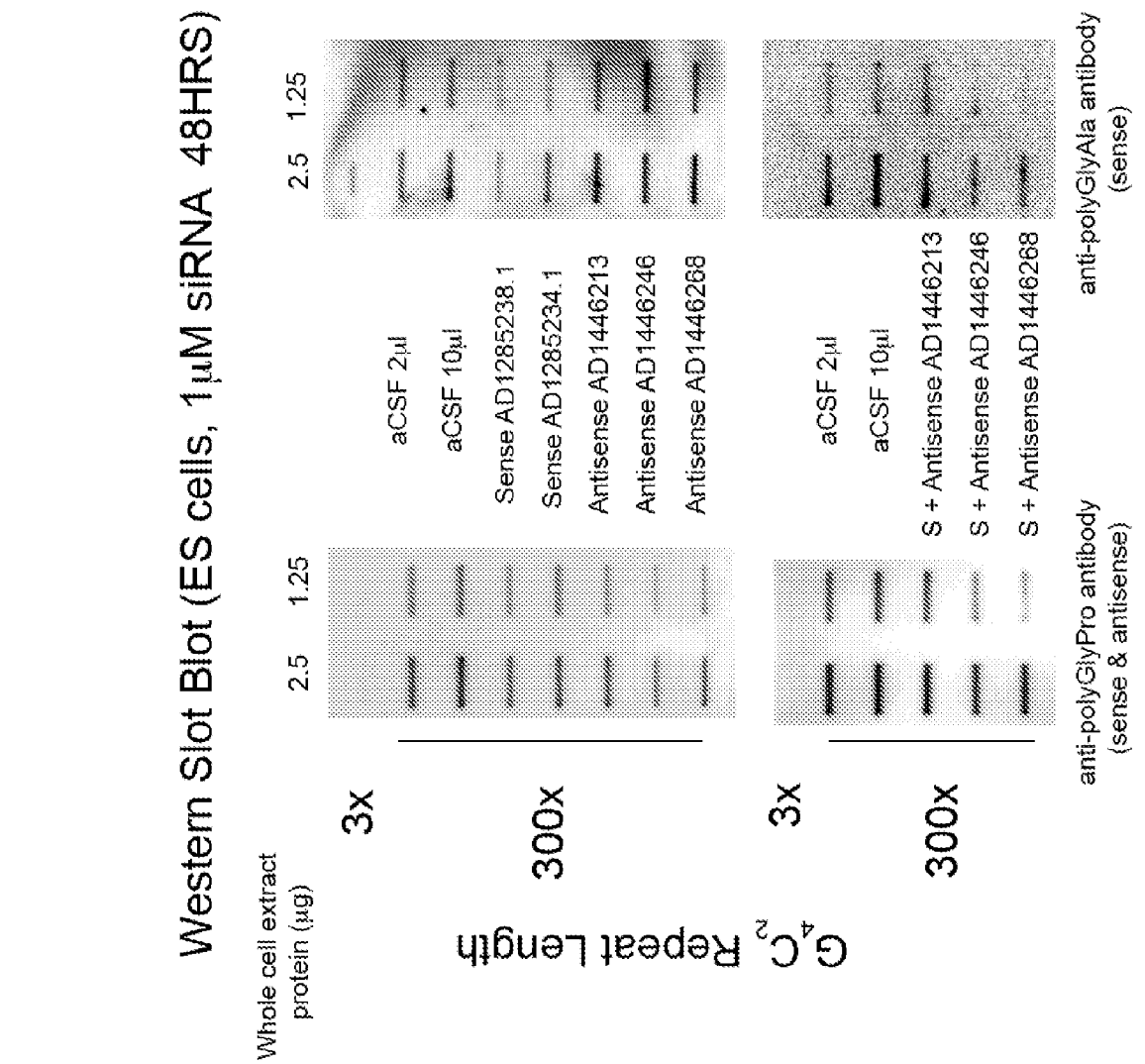

Western Slot Blot (ES cells, 1μM siRNA 48HRS)
Whole cell extract protein (μg)
2.5 1.25
2.5 1.25
3x
300x
3x
300x
G4C2 Repeat Length
aCSF 2μl
aCSF 10μl
Sense AD1285238.1
Sense AD1285234.1
Antisense AD1446213
Antisense AD1446246
Antisense AD1446268
aCSF 2μl
aCSF 10μl
S + Antisense AD1446213
S + Antisense AD1446246
S + Antisense AD1446268
S = AD1285238.1
anti-polyGlyPro antibody (sense & antisense)
anti-polyGlyAla antibody (sense)

FIG. 6A

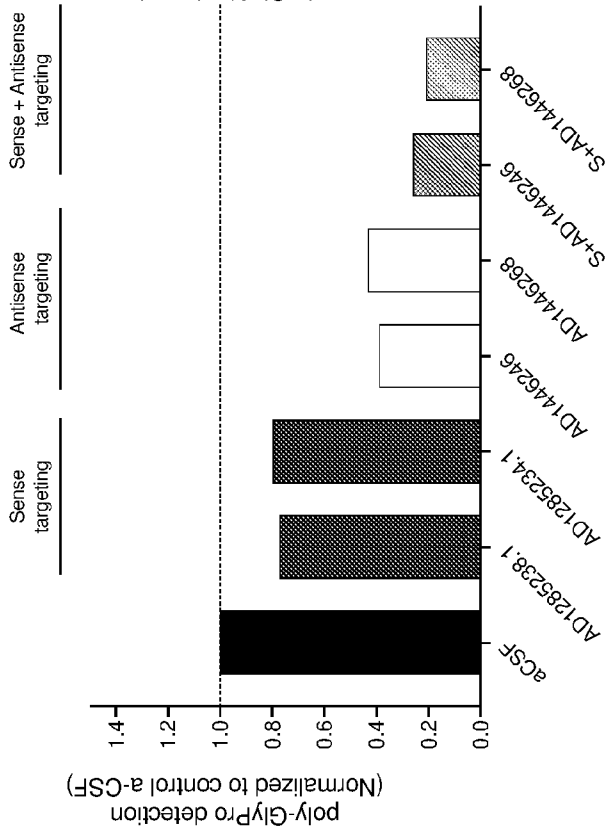

poly-GlyPro detection
(Normalized to control a-CSF)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aCSF
AD12852381
AD12852341
AD14462461
AD14462681
S+AD14462461
S+AD14462681
Sense targeting
Antisense targeting
Sense + Antisense targeting

FIG. 6B

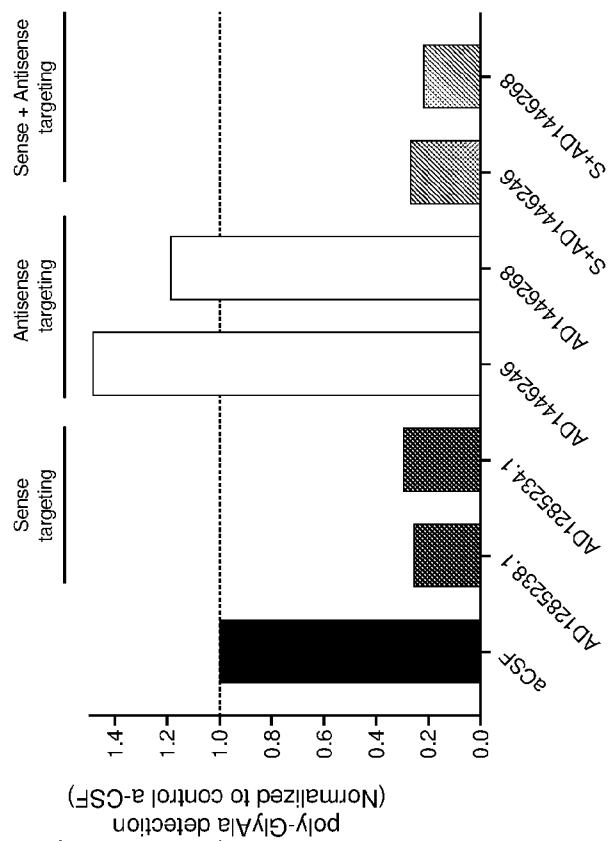

poly-GlyAla detection
(Normalized to control a-CSF)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
aCSF
AD12852381
AD12852341
AD14462461
AD14462681
S+AD14462461
S+AD14462681
Sense targeting
Antisense targeting
Sense + Antisense targeting

FIG. 6C

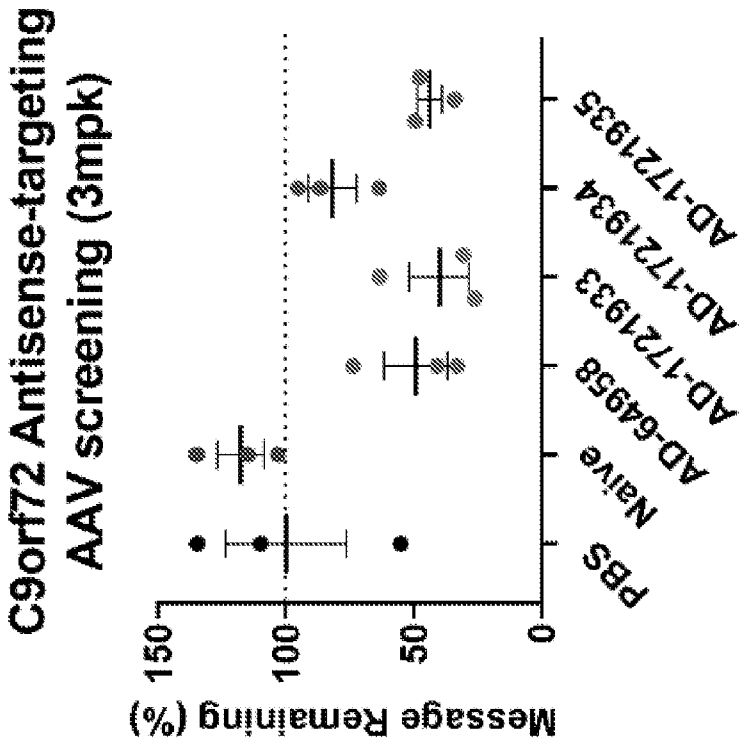

C9orf72 Antisense-targeting
AAV screening (3mpk)
Message Remaining (%)
150
100
50
0
PBS
Naïve
AD-649558
AD-1721933
AD-1721934
AD-1721935

FIG. 7

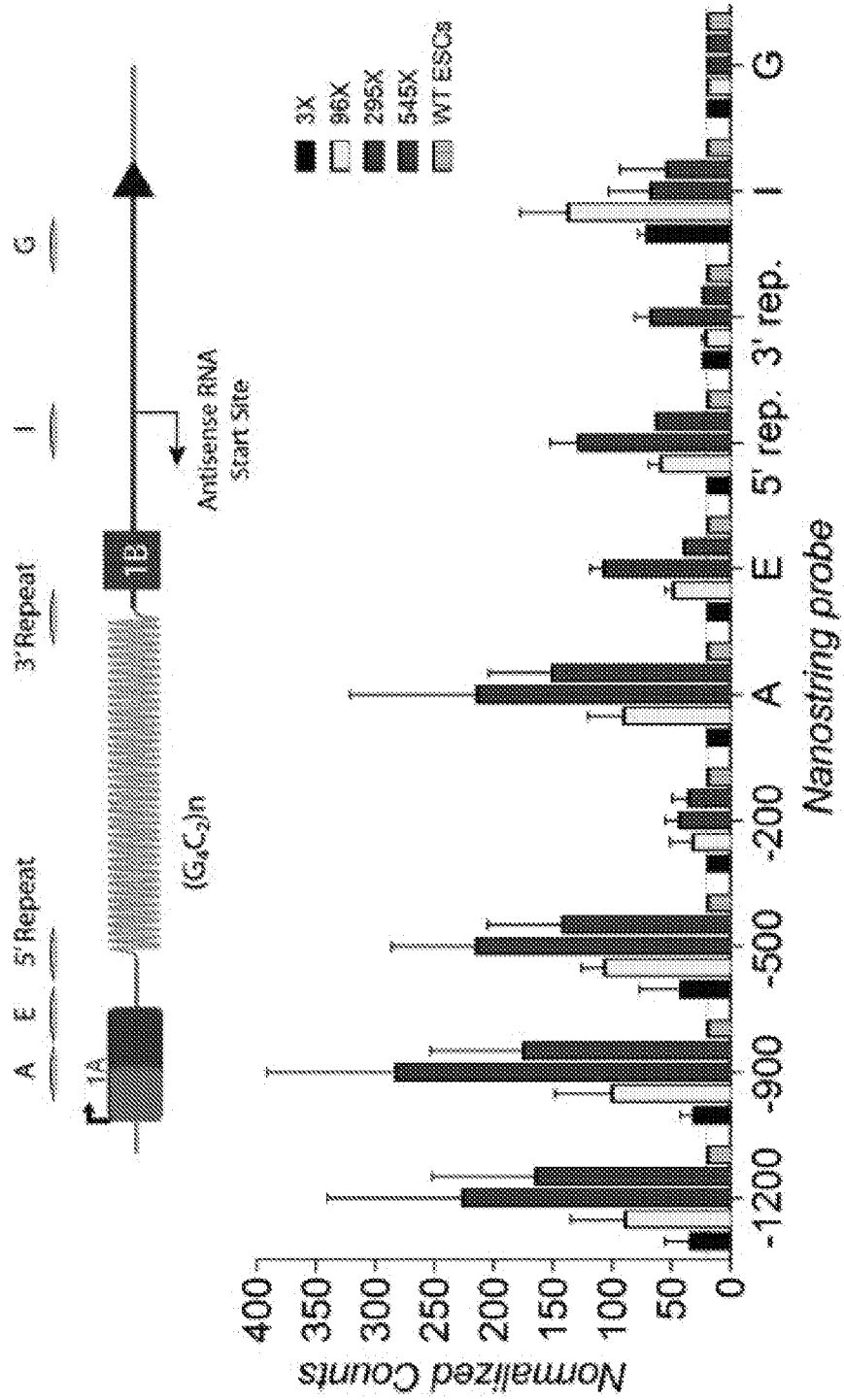

A
E
5' Repeat
1A
1B
3' Repeat
I
G
(G4C2)n
Antisense RNA
Start Site
3X
96X
295X
545X
WT ESCs
Normalized Counts
400
350
300
250
200
150
100
50
0
-1200
-900
-500
-200
A
E
5' rep.
3' rep.
I
G
Nanostring probe

FIG. 8