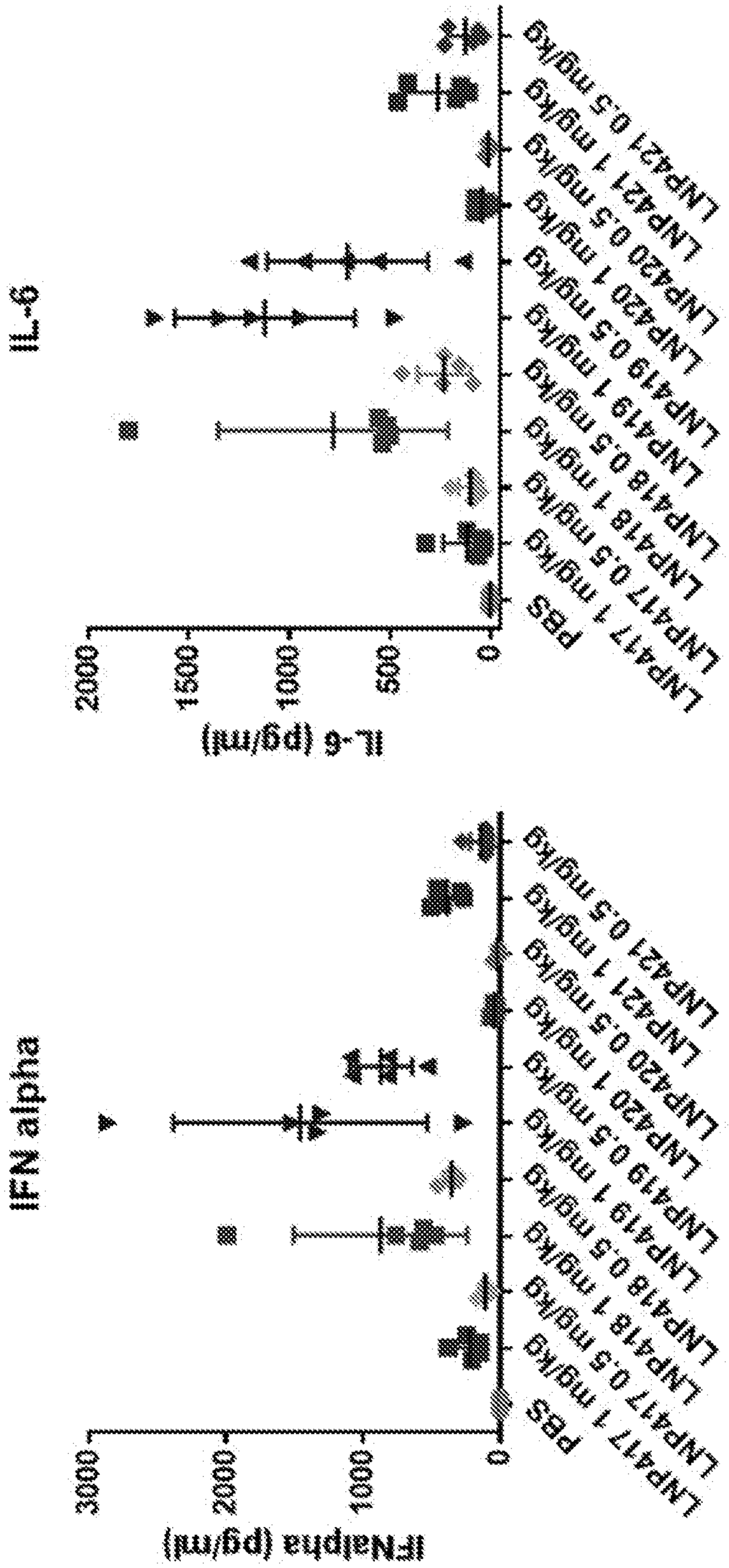
IL-6
IL-6 (pg/ml)
2000
1500
1000
500
0
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg
IFN alpha
IFNalpha (pg/ml)
3000
2000
1000
0
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

Fig. 1B

Fig. 1A

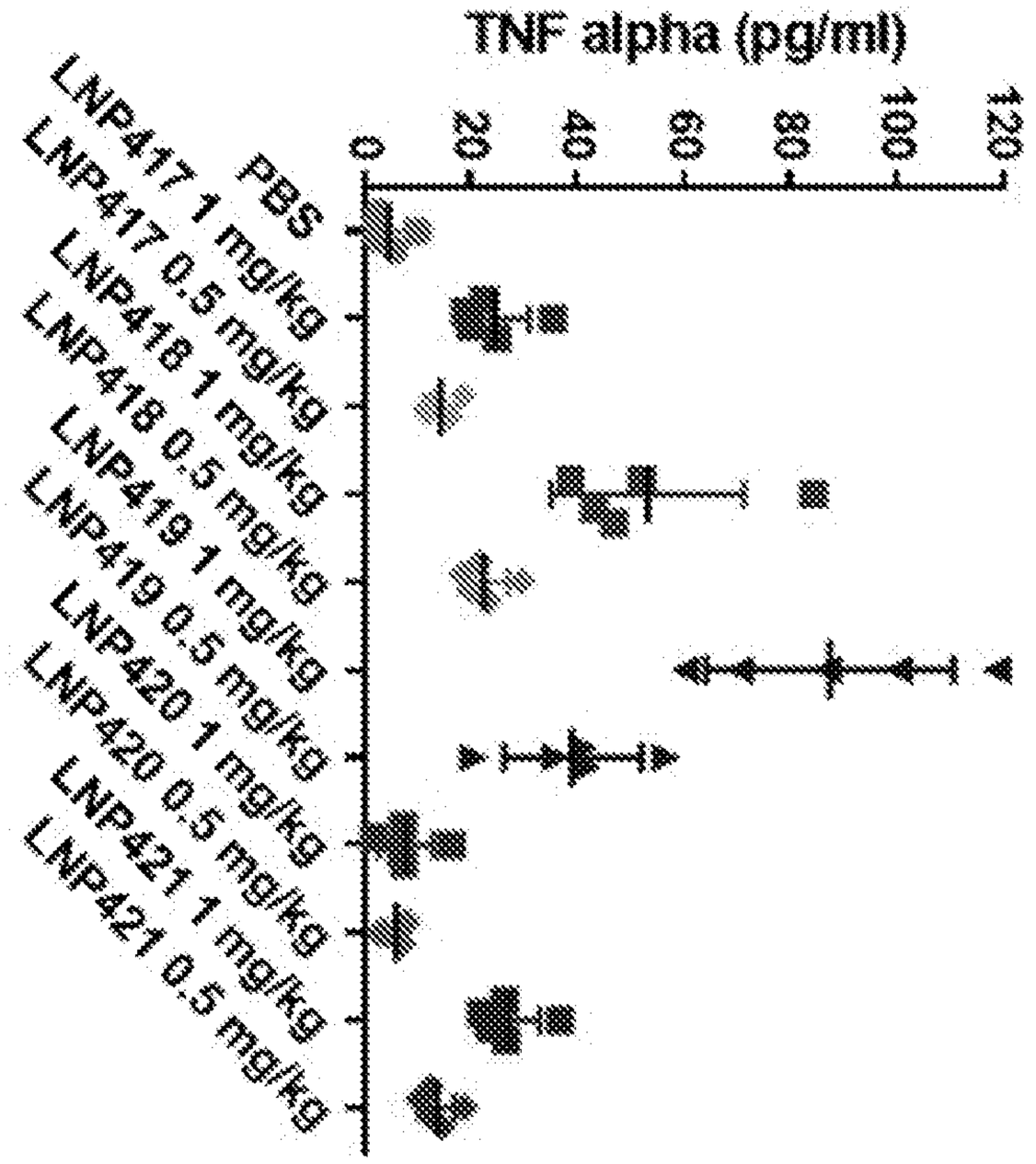
TNF alpha (pg/ml)
0
20
40
60
80
100
120
TNF alpha
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

Fig. 1C

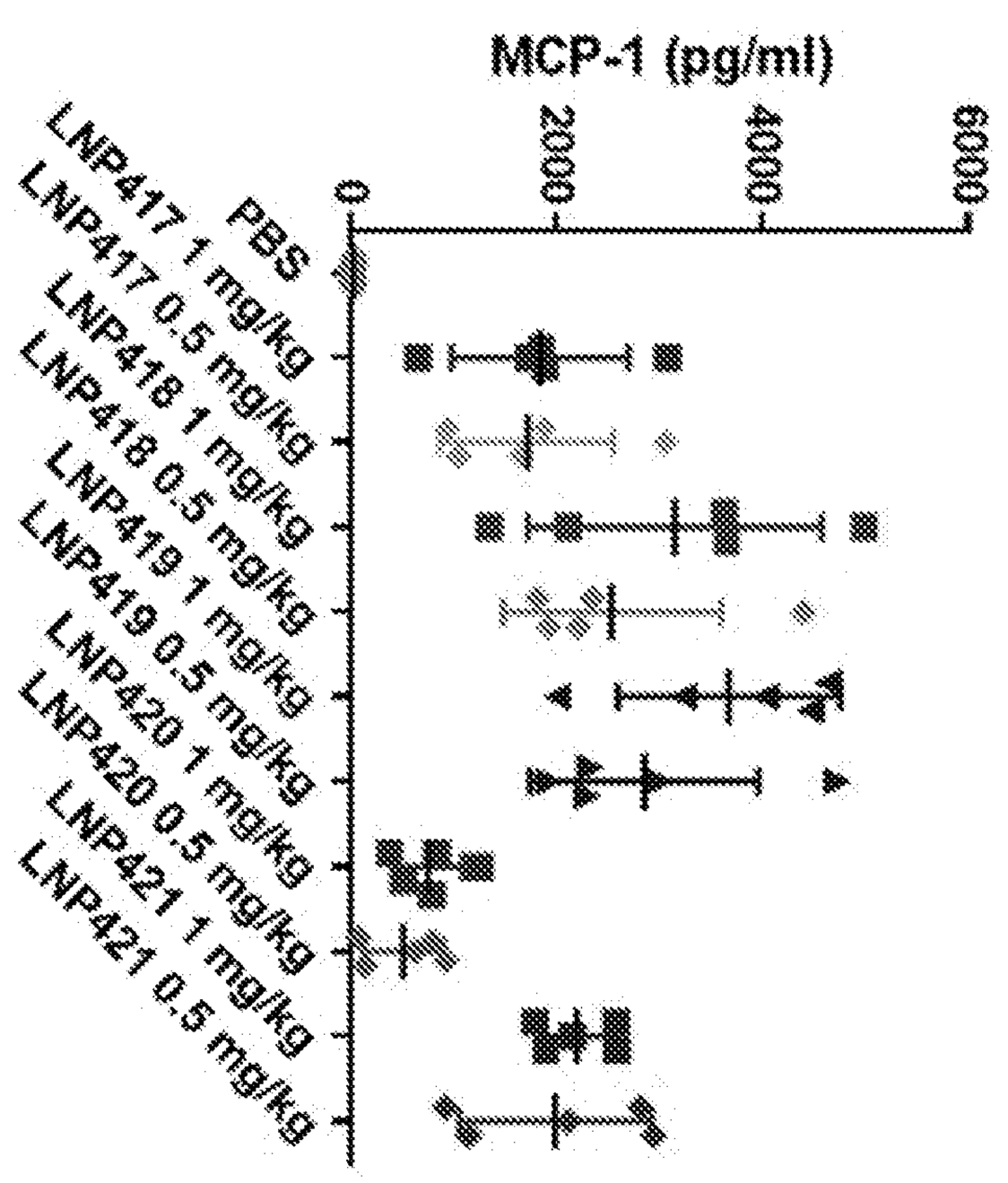
MCP-1 (pg/ml)
0
2000
4000
6000
MCP-1
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

Fig. 1D

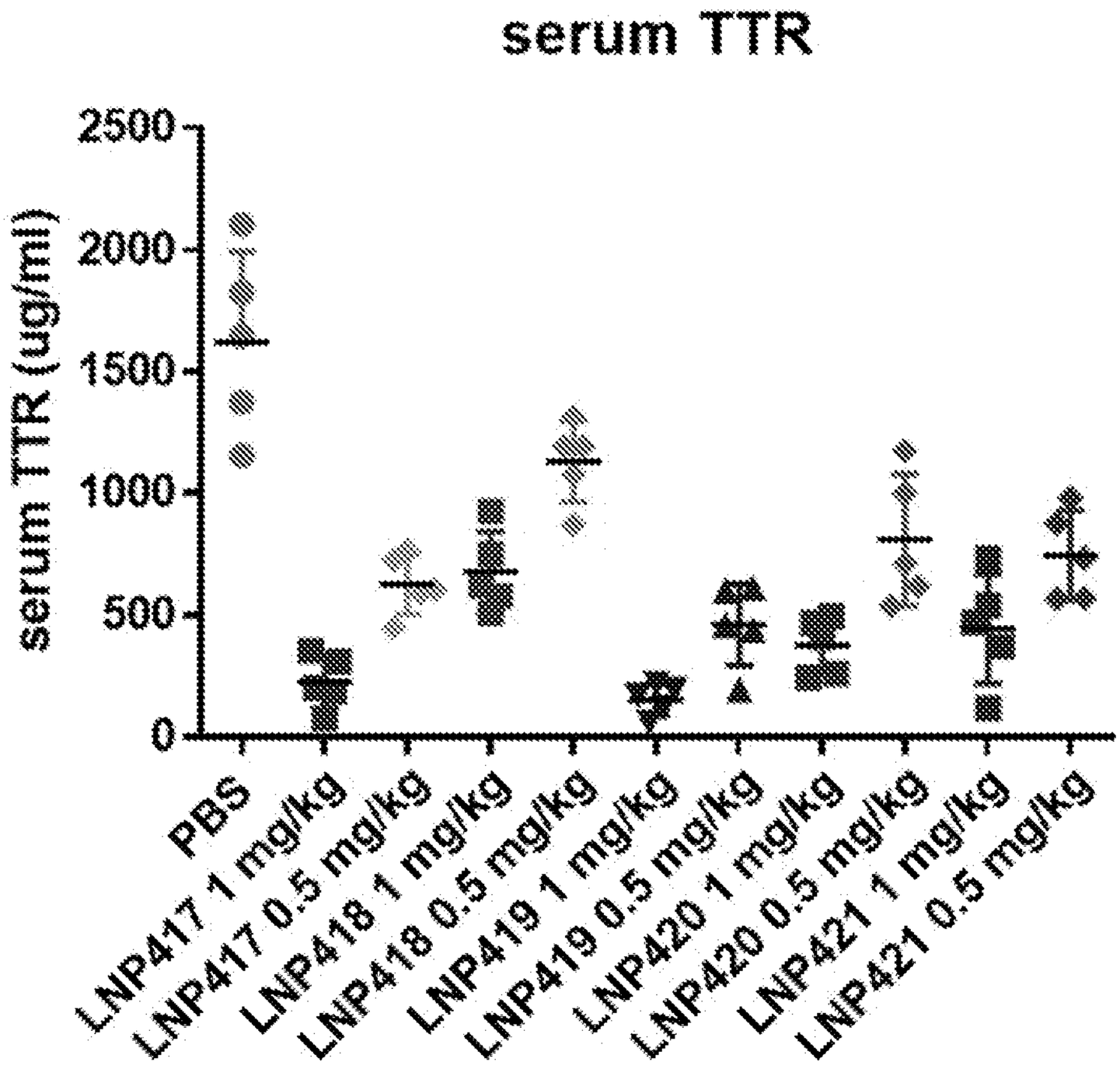
serum TTR
serum TTR (ug/ml)
2500
2000
1500
1000
500
0
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

Fig. 2A

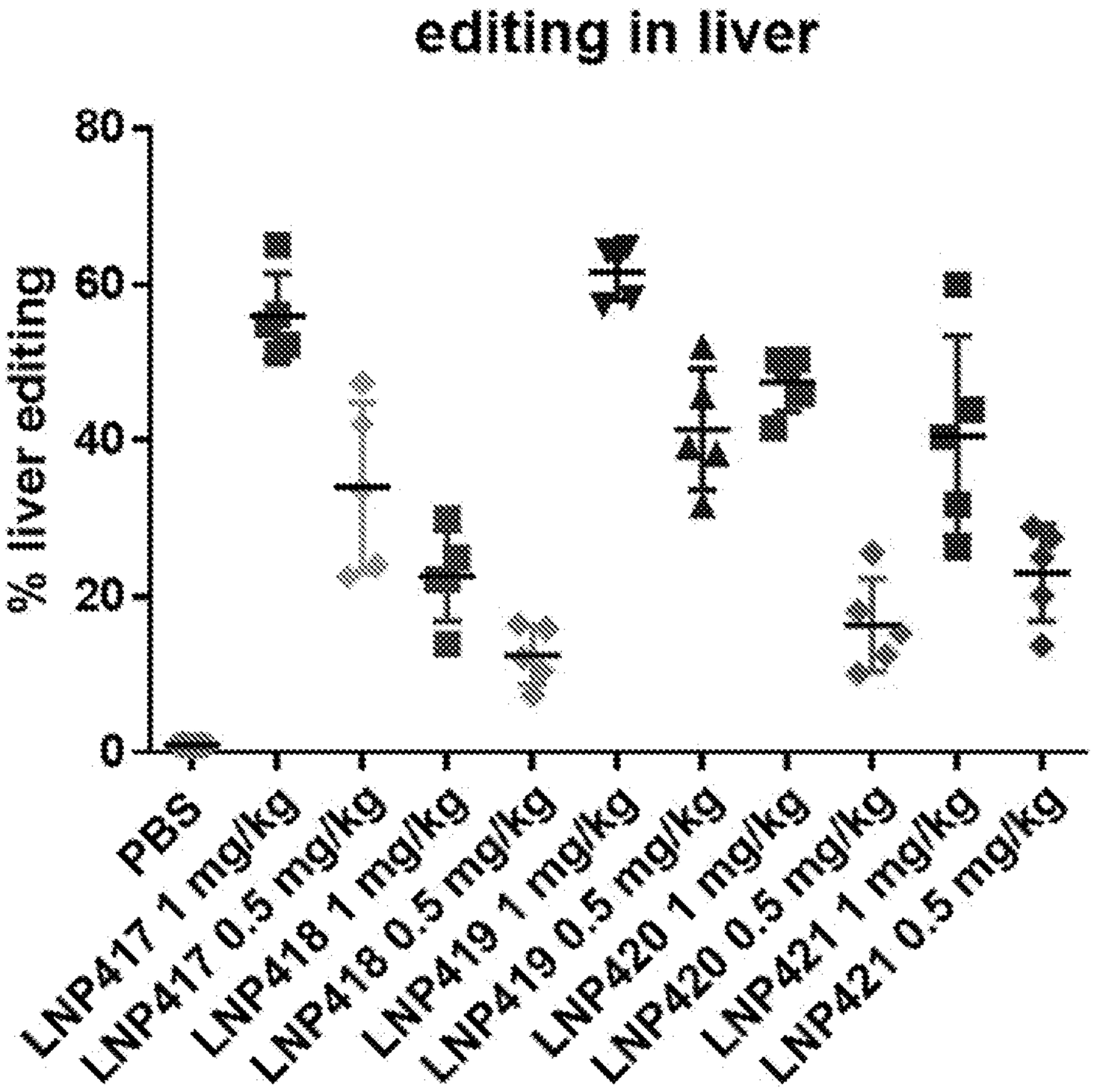
editing in liver
% liver editing
0
20
40
60
80
PBS
LNP417 1 mg/kg
LNP417 0.5 mg/kg
LNP418 1 mg/kg
LNP418 0.5 mg/kg
LNP419 1 mg/kg
LNP419 0.5 mg/kg
LNP420 1 mg/kg
LNP420 0.5 mg/kg
LNP421 1 mg/kg
LNP421 0.5 mg/kg

Fig. 2B

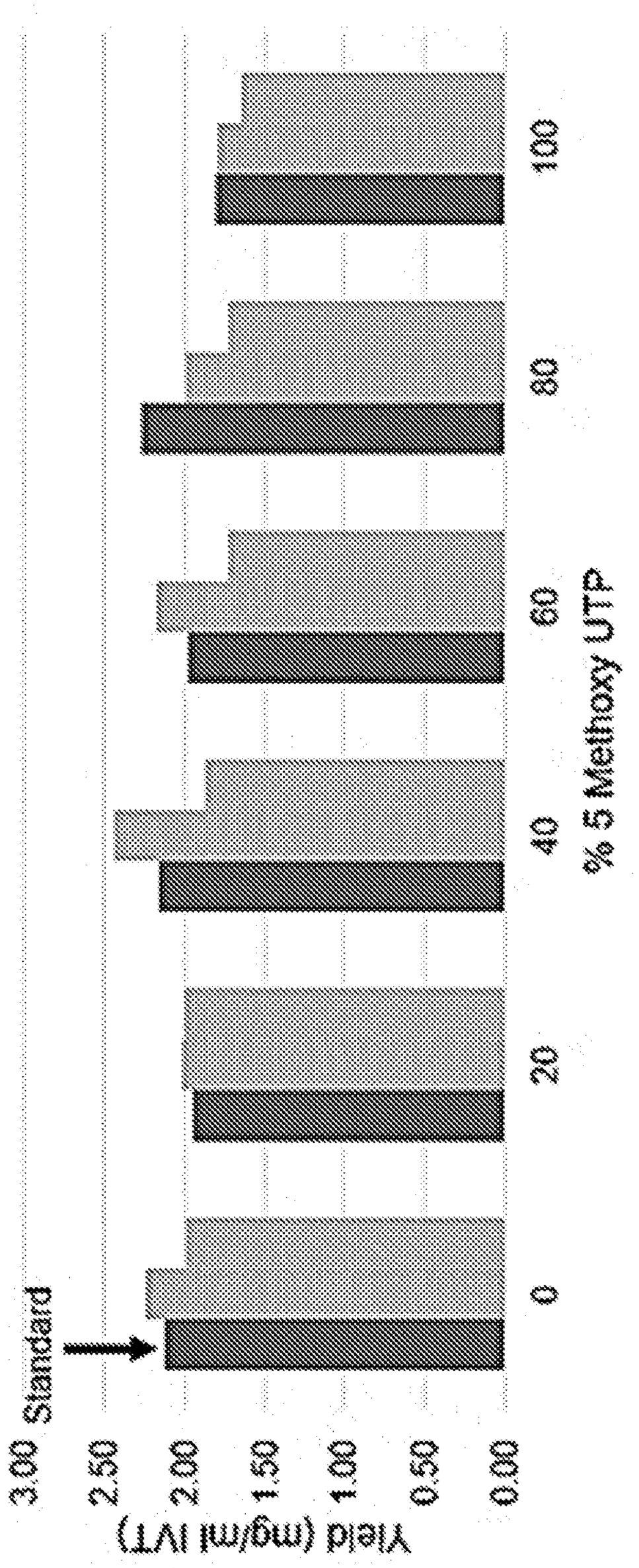
Yield (mg/ml IVT)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Standard
0
20
40
% 5 Methoxy UTP
60
80
100

Fig. 3

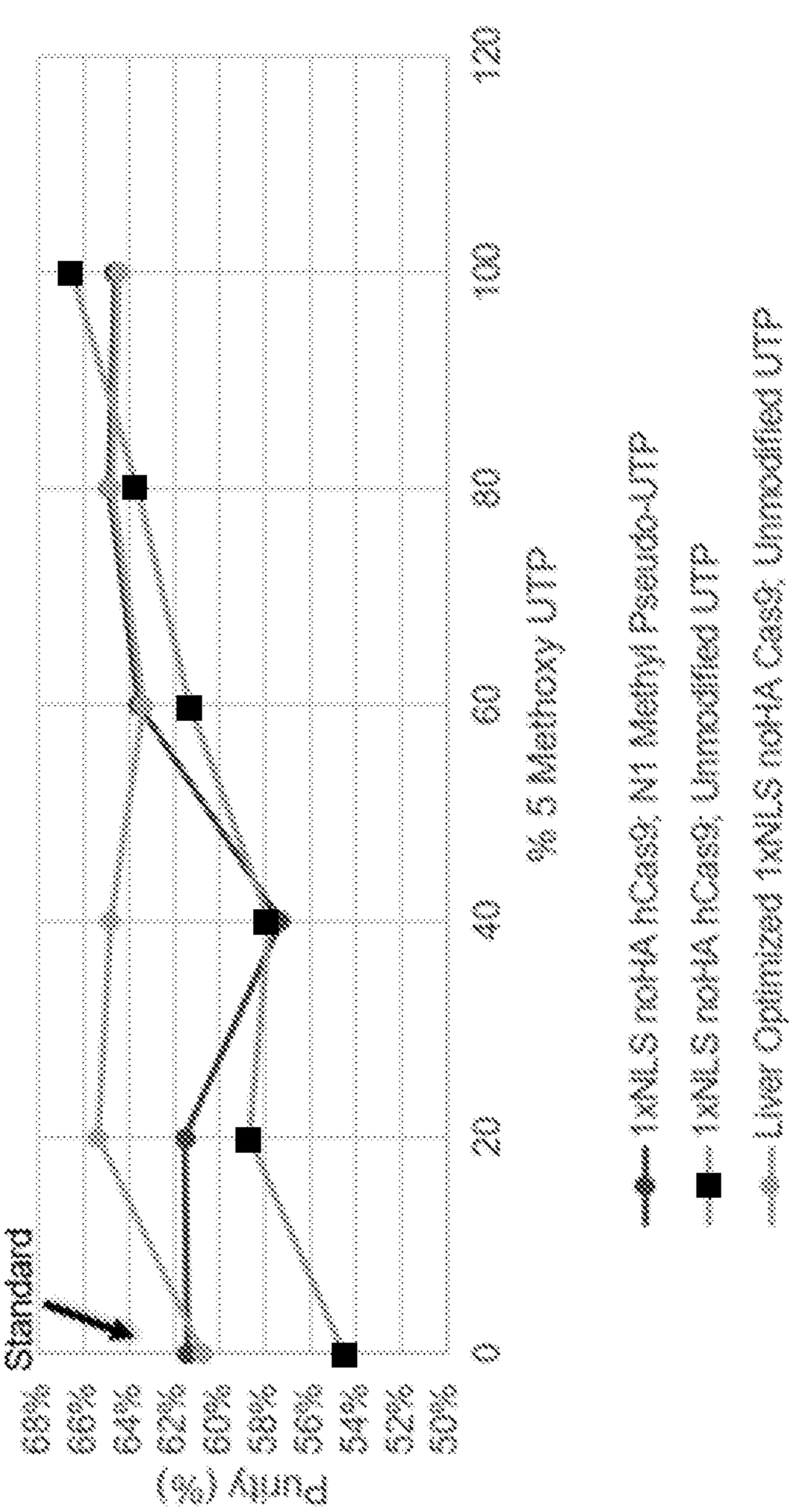
Standard
0
20
40
60
80
100
120
% Standard Purity
1xhIL6 moAb hCasp, 5 Methoxy UTP
1xhIL6 moAb hCasp, N1 Methyl Pseudo-UTP
1xhIL6 moAb hCasp, Unmodified UTP
1.lvey Optimized 1xhIL6 moAb Casp, Unmodified UTP
Purity (%)
60%
65%
70%
75%
80%
85%
90%
95%

Fig. 4

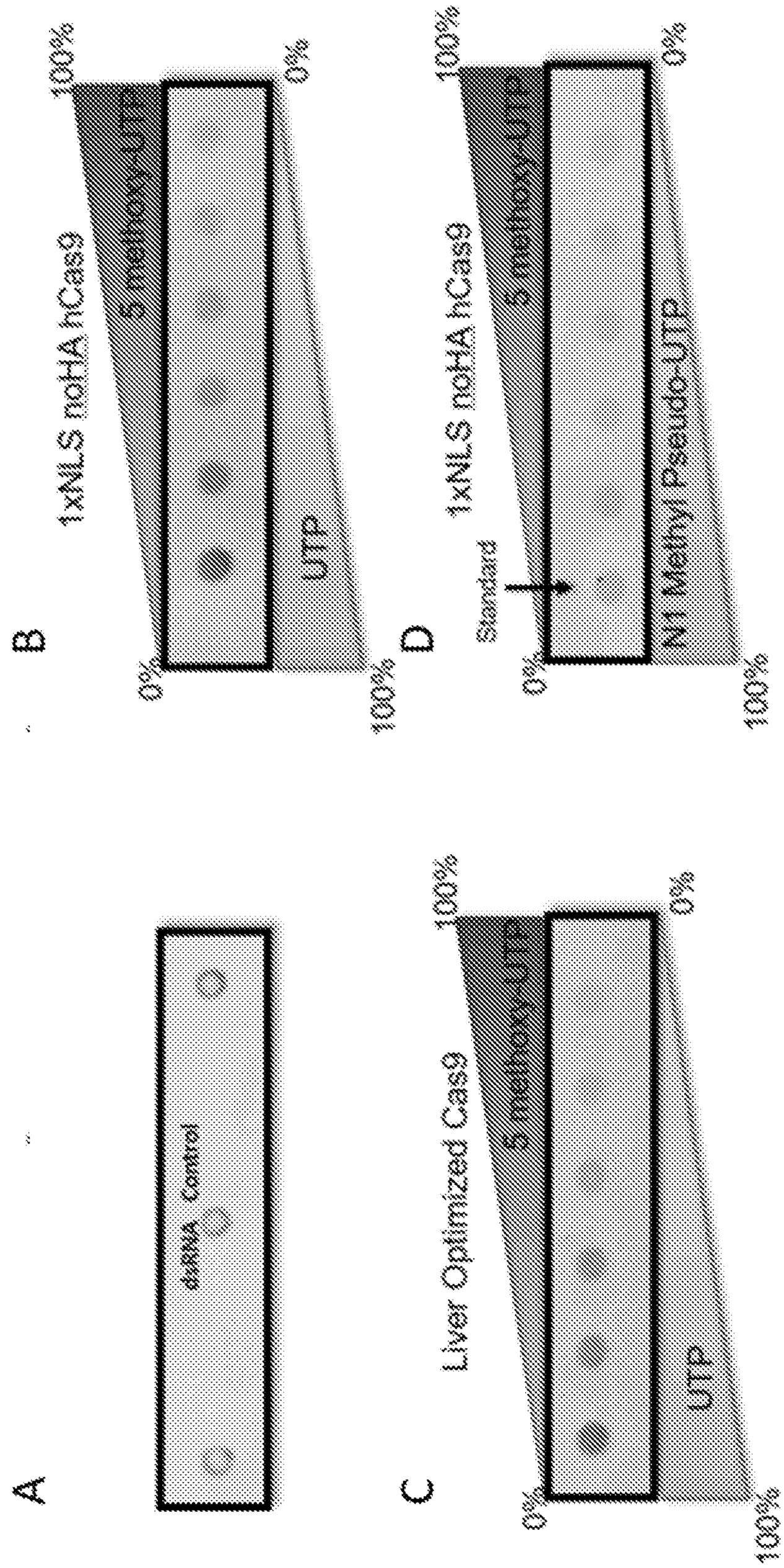
A
Control
dsRNA
B
1xNLS noHA hCas9
5 methoxyUTP
UTP
100%
0%
C
Liver Optimized Cas9
5 methoxyUTP
UTP
100%
0%
D
1xNLS noHA hCas9
Standard
N1 Methyl Pseudo-UTP
100%
0%

Fig. 5

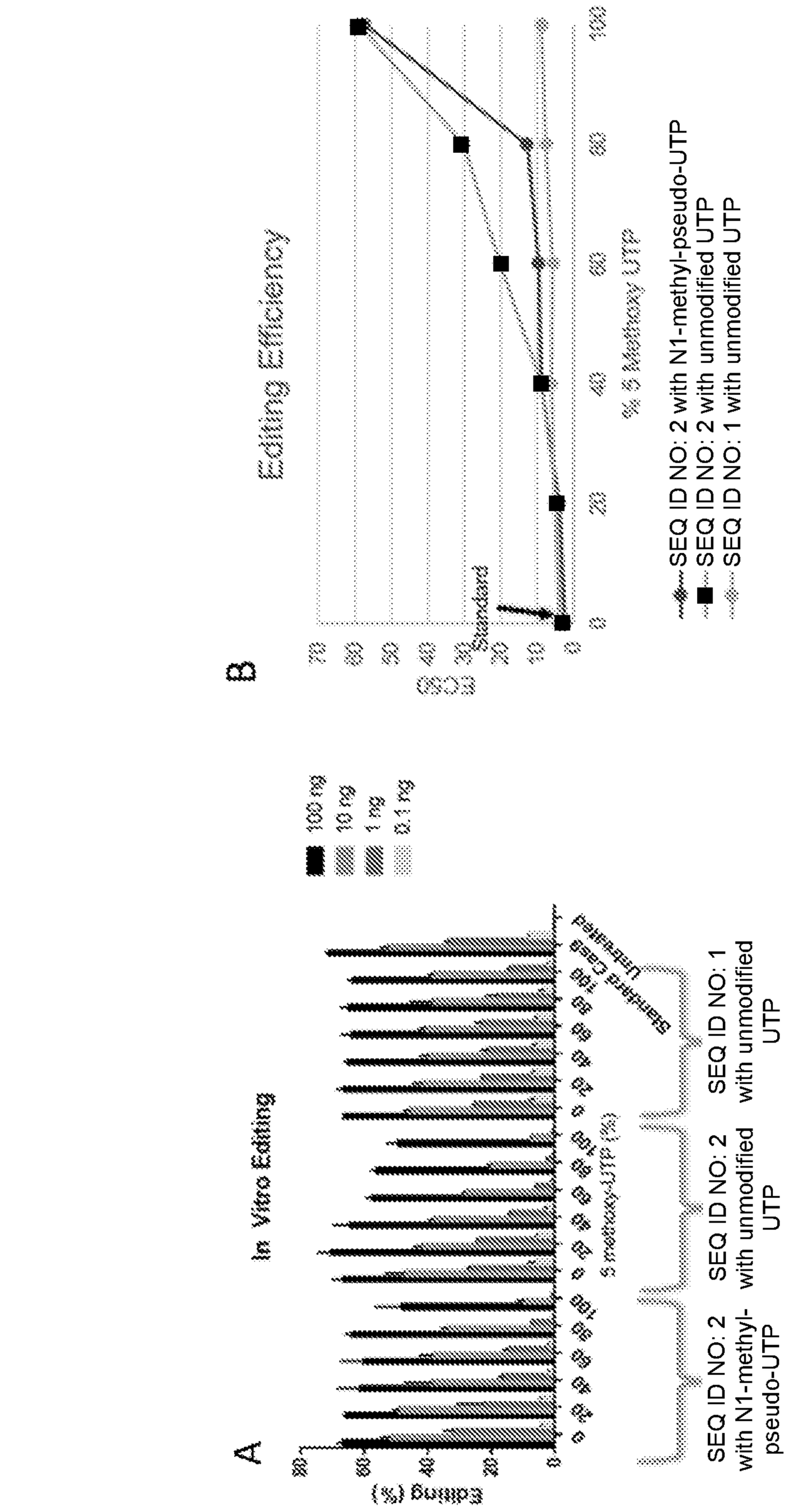
A
In Vitro Editing
Editing (%)
Standard
Standard Cas9
Unmodified
5 methoxy-UTP (%)
100 mg
10 mg
1 mg
0.1 mg
SEQ ID NO: 2 with N1-methyl-pseudo-UTP
SEQ ID NO: 2 with unmodified UTP
SEQ ID NO: 1 with unmodified UTP
B
Editing Efficiency
ED50
Standard
% 5 Methoxy UTP
SEQ ID NO: 2 with N1-methyl-pseudo-UTP
SEQ ID NO: 2 with unmodified UTP
SEQ ID NO: 1 with unmodified UTP

Fig. 6

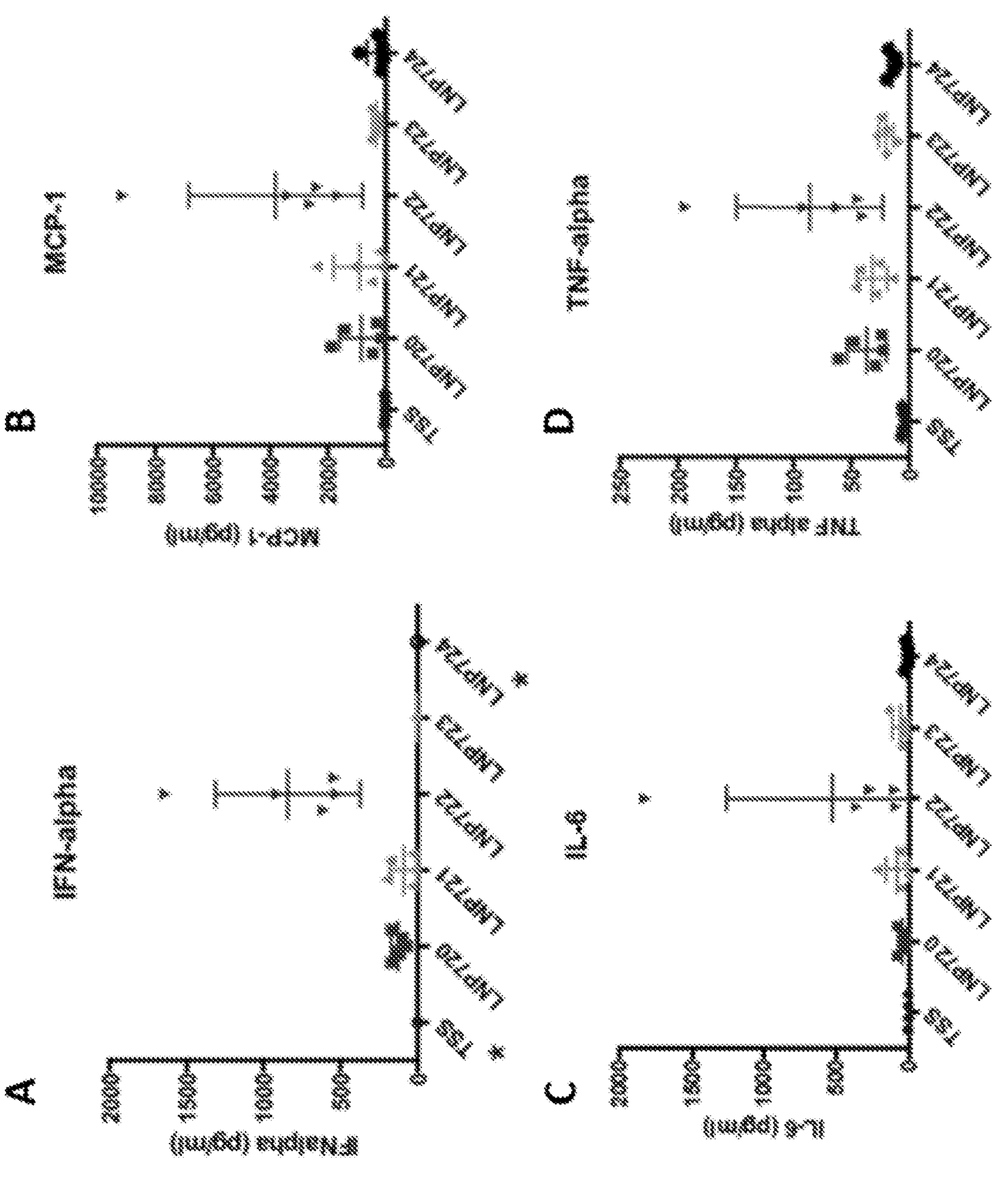
A
IFN-alpha
IFNalpha (pg/ml)
2000
1500
1000
500
0
TSS
LNP72b
LNP72h
LNP72i
LNP72j
LNP72k
B
MCP-1
MCP-1 (pg/ml)
3000
2000
C
IL-6
IL-6 (pg/ml)
D
TNF-alpha
TNF alpha (pg/ml)

Fig. 7

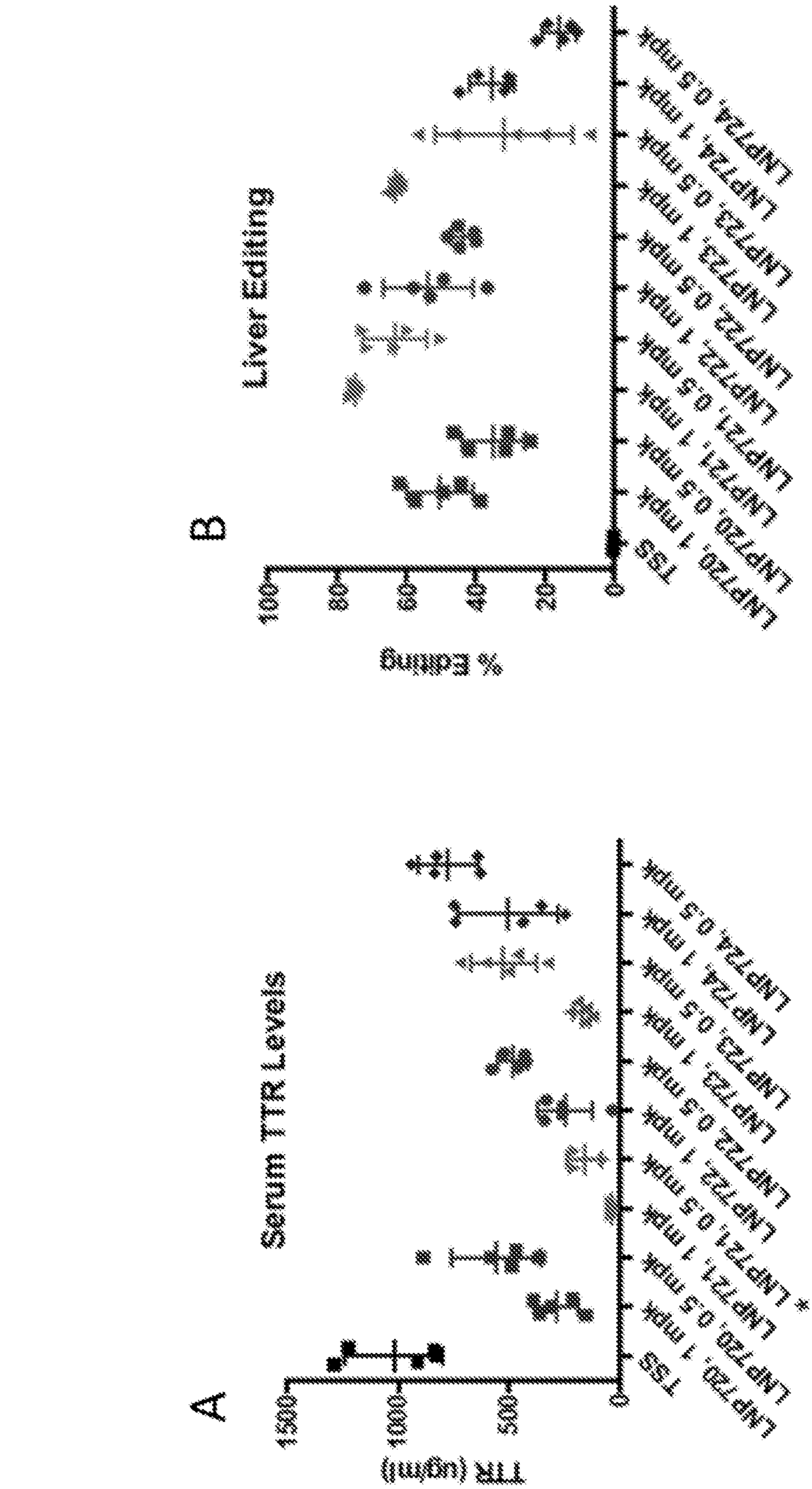
A
Serum TTR Levels
TTR (ug/ml)
1500
1000
500
0
TSS
LNP720, 1 mpk
LNP720, 0.5 mpk
LNP721, 1 mpk
LNP721, 0.5 mpk
LNP722, 1 mpk
LNP722, 0.5 mpk
LNP723, 1 mpk
LNP723, 0.5 mpk
LNP724, 1 mpk
LNP724, 0.5 mpk
* LNP724, 1 mpk
LNP724, 0.5 mpk
B
Liver Editing
% editing
100
80
60
40
20
0
TSS
LNP720, 1 mpk
LNP720, 0.5 mpk
LNP721, 1 mpk
LNP721, 0.5 mpk
LNP722, 1 mpk
LNP722, 0.5 mpk
LNP723, 1 mpk
LNP723, 0.5 mpk
LNP724, 1 mpk
LNP724, 0.5 mpk

Fig. 8

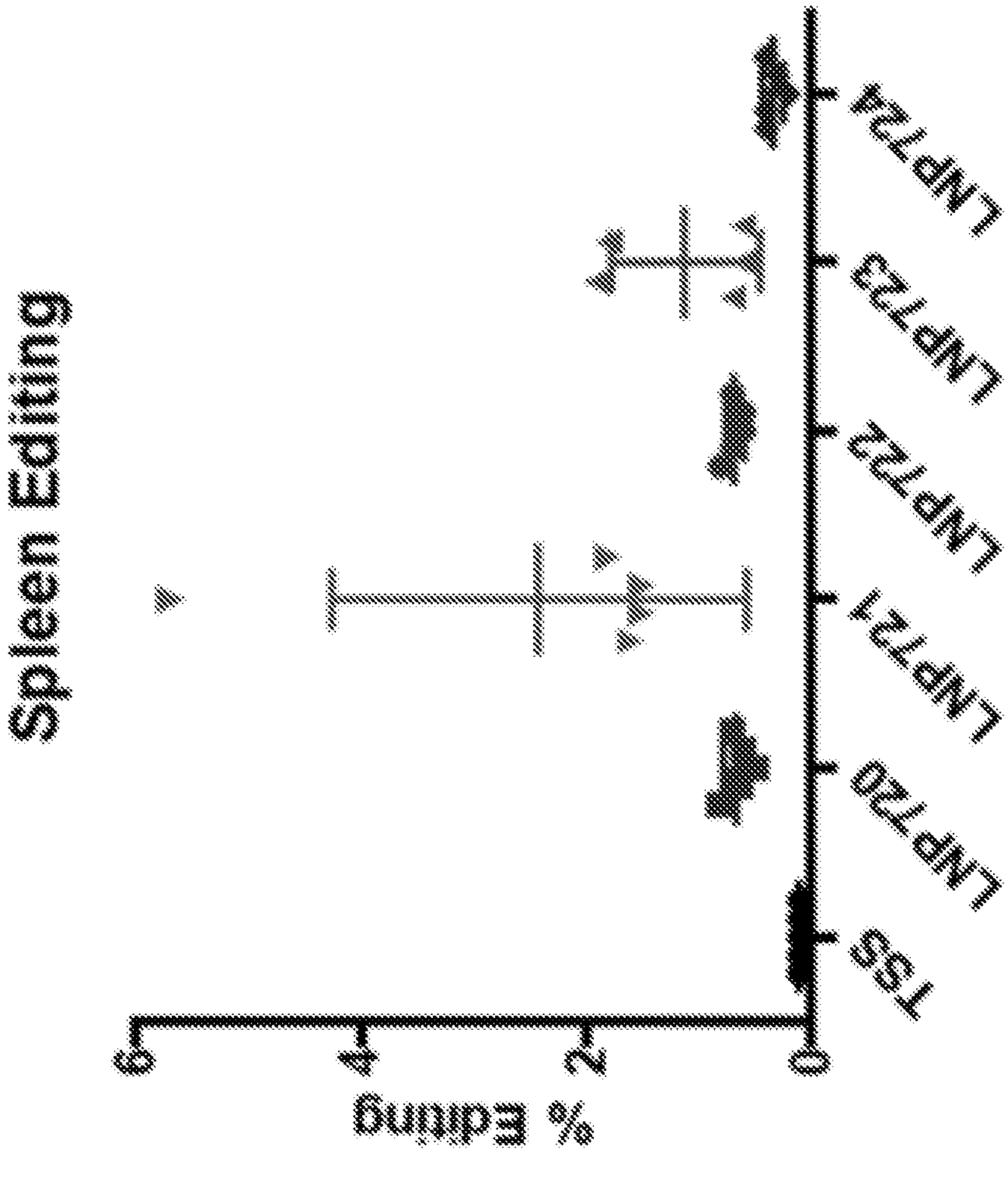
Spleen Editing
% editing
0
2
4
6
TSS
LNP72b
LNP72a
LNP72c
LNP72d

Fig. 9

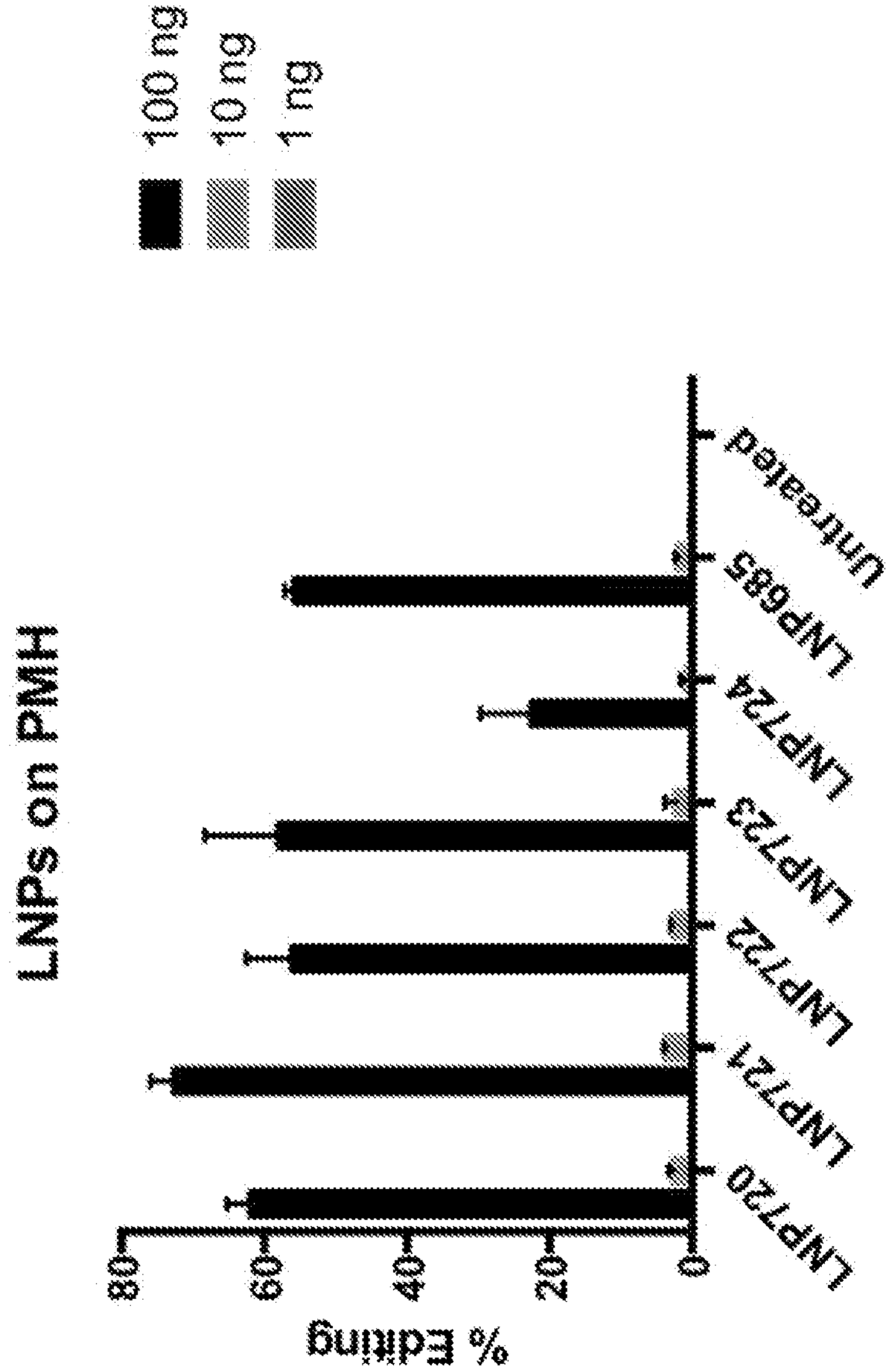
100 ng
10 ng
1 ng
LNPs on PMH
% Editing
0
20
40
60
80
LNP720
LNP721
LNP722
LNP723
LNP724
LNP685
Untreated

Fig. 10

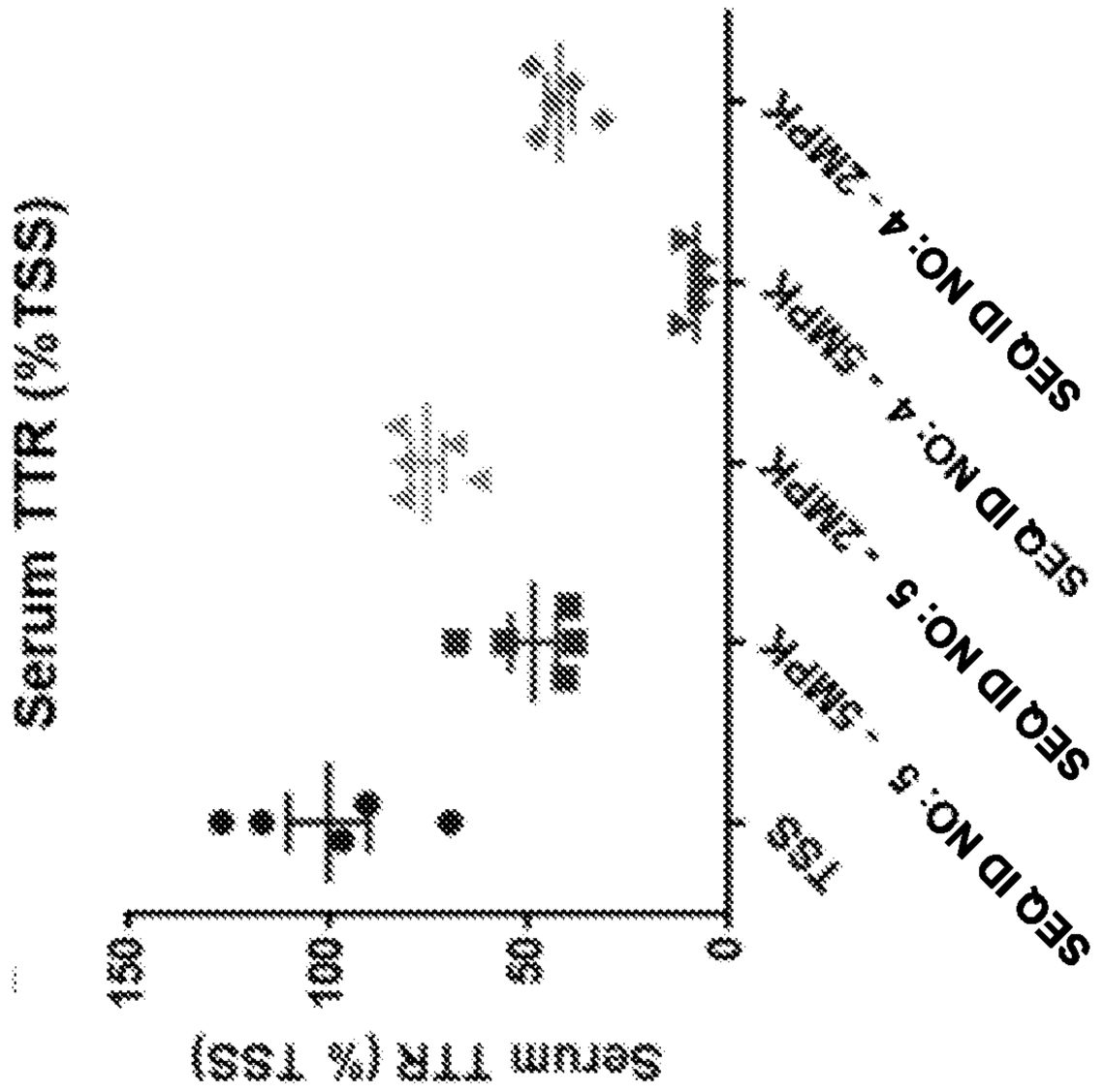

Fig. 11B

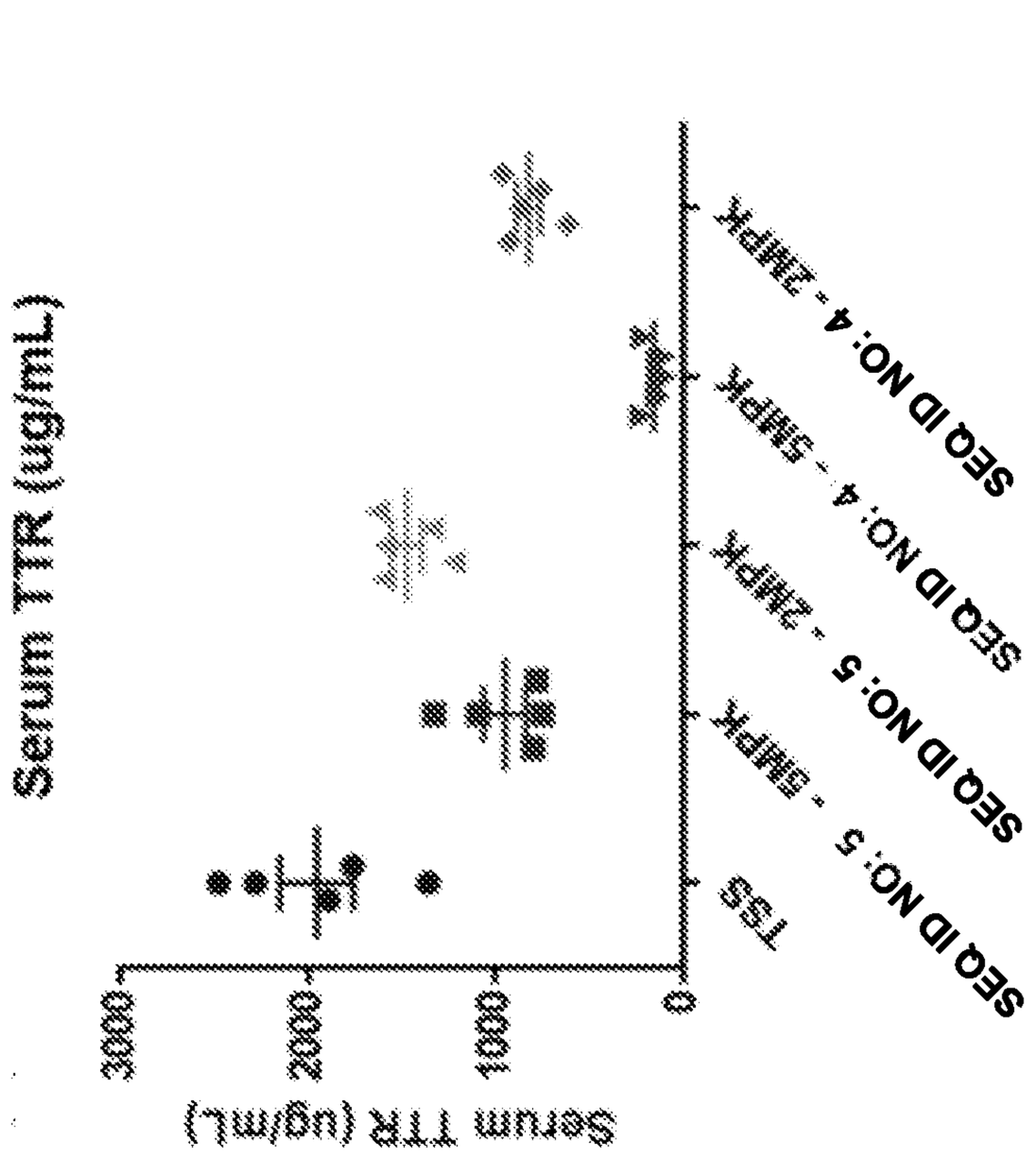

Fig. 11A

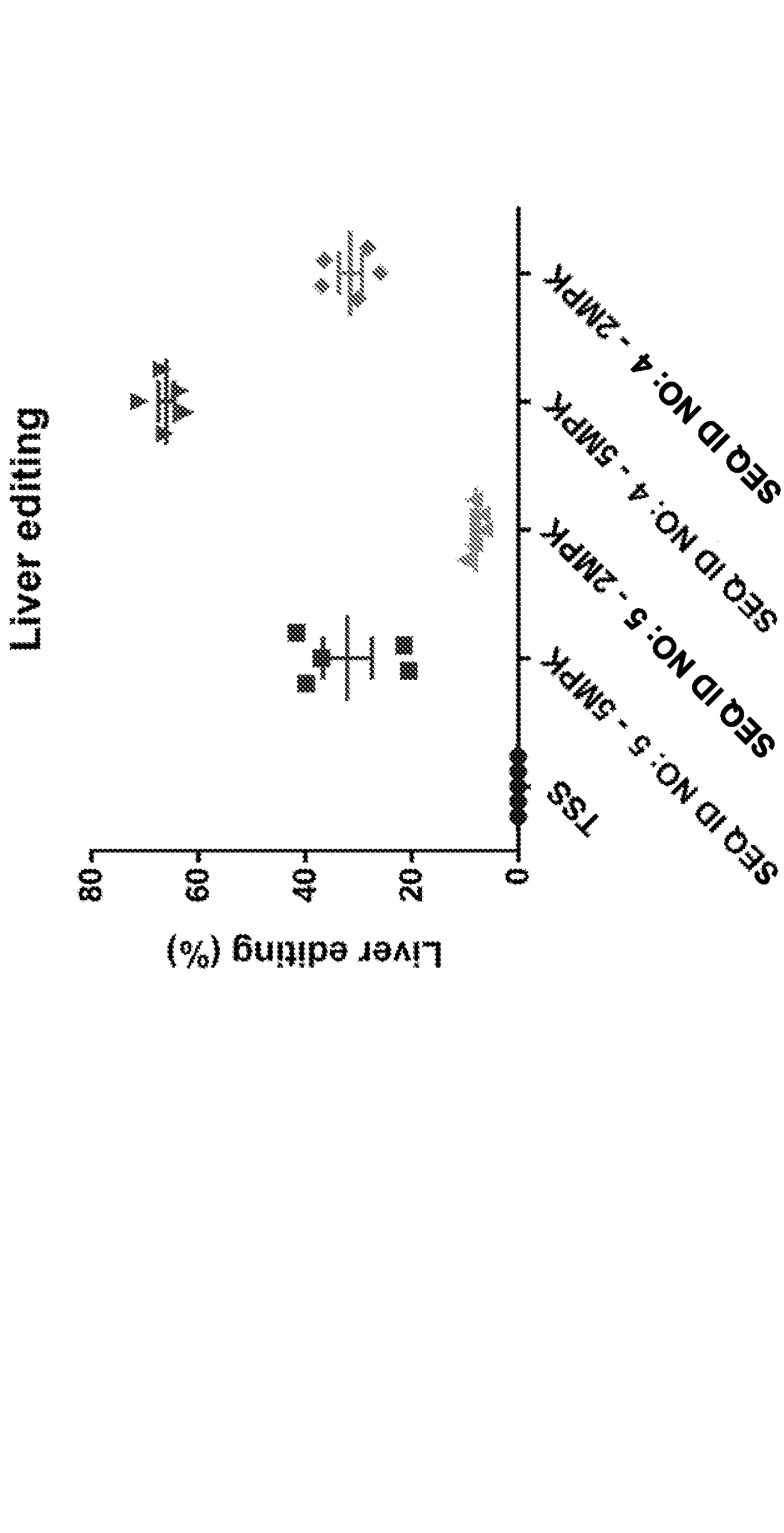
Liver editing (%)
0
20
40
60
80
TSS
SEQ ID NO: 5 - 5MPK
SEQ ID NO: 5 - 2MPK
SEQ ID NO: 4 - 5MPK
SEQ ID NO: 4 - 2MPK
Liver editing

Fig. 12

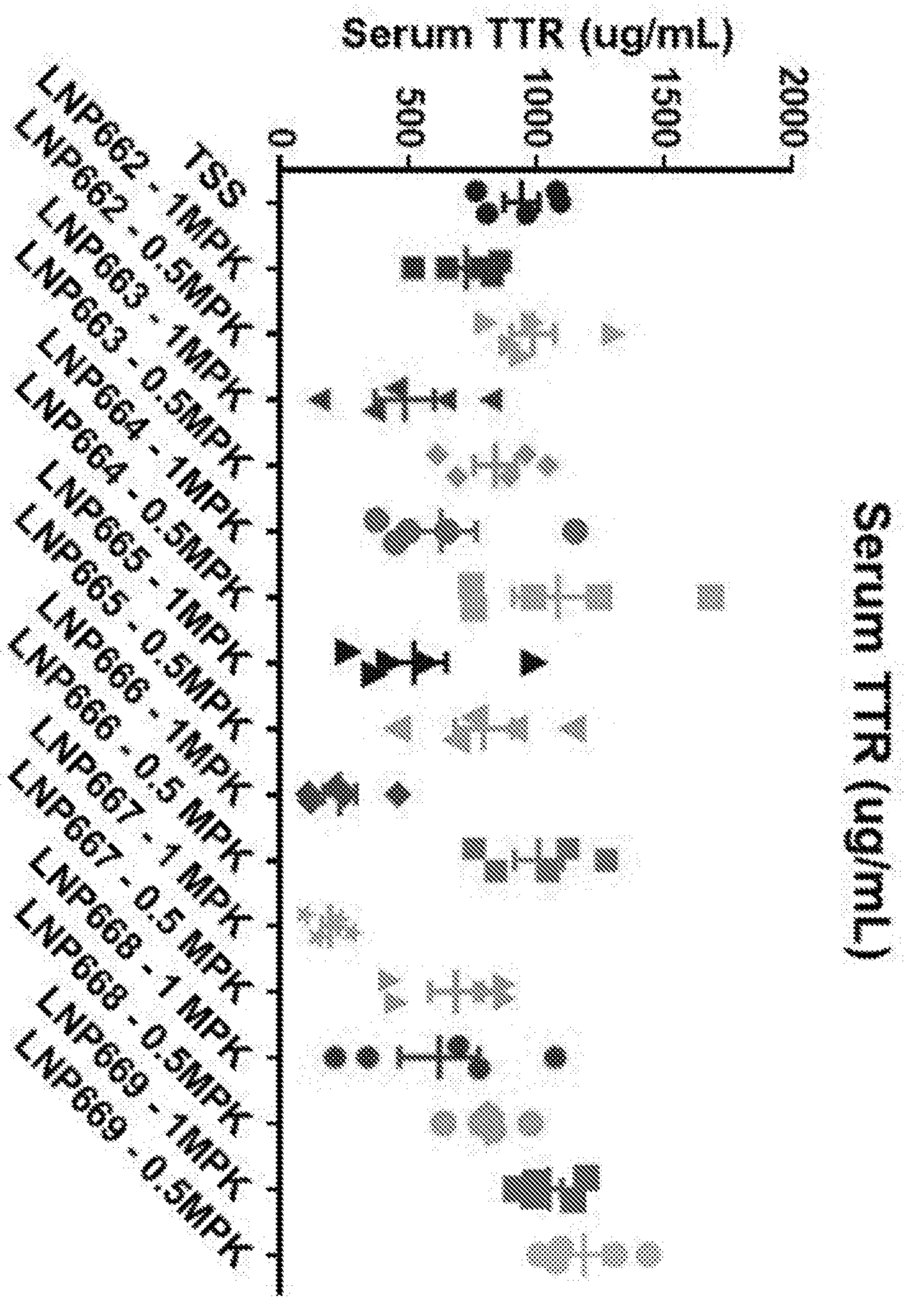
Serum TTR (ug/mL)
2000
1500
1000
500
0
TSS
LNP662 - 1MPK
LNP662 - 0.5MPK
LNP663 - 1MPK
LNP663 - 0.5MPK
LNP664 - 1MPK
LNP664 - 0.5MPK
LNP665 - 1MPK
LNP665 - 0.5MPK
LNP666 - 1MPK
LNP666 - 0.5 MPK
LNP667 - 1 MPK
LNP667 - 0.5 MPK
LNP668 - 1 MPK
LNP668 - 0.5MPK
LNP669 - 1MPK
LNP669 - 0.5MPK

Fig. 13A

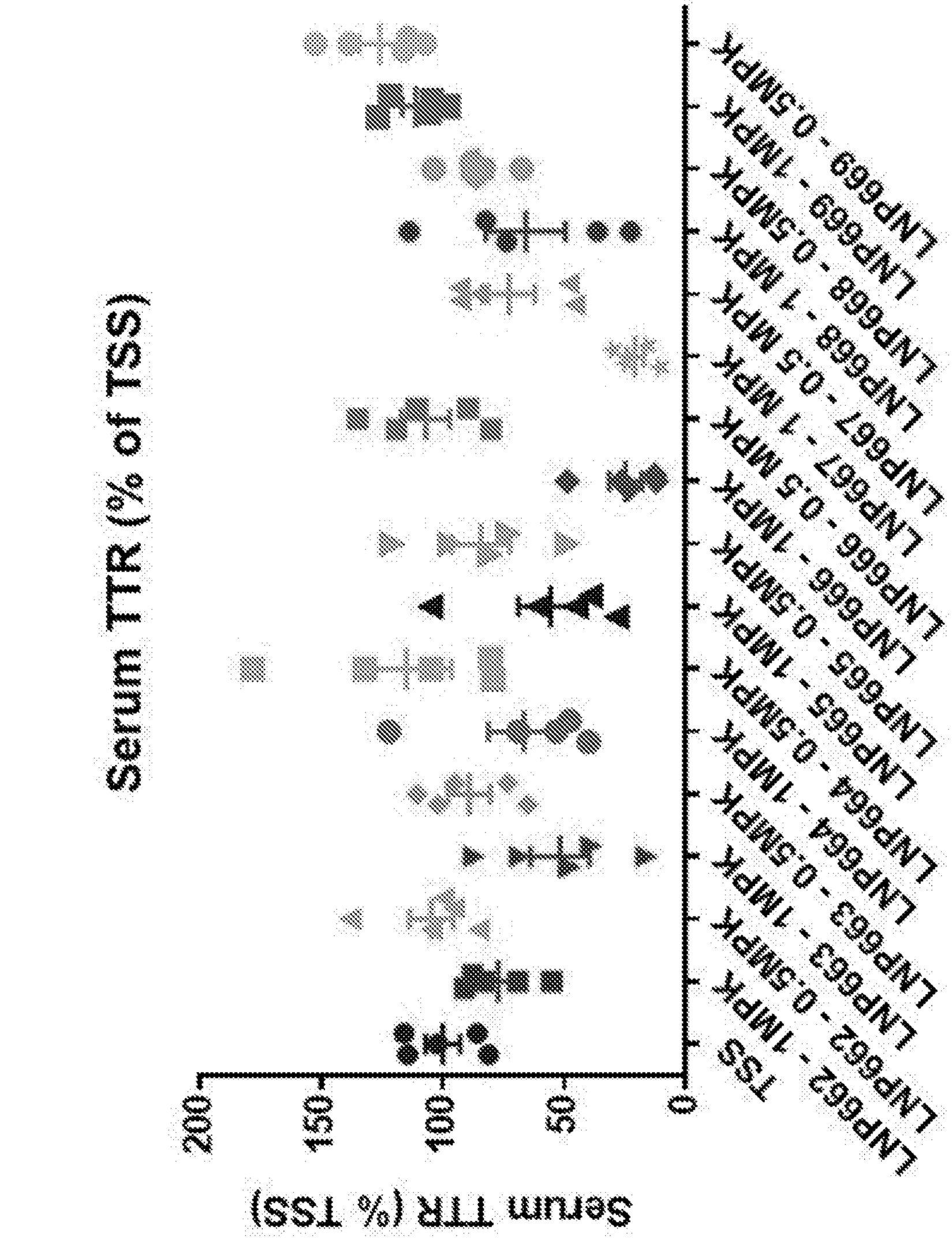
Serum TTR (% of TSS)
200
150
100
50
0
TSS
TSS - 1MPK
LNP662 - 0.5MPK
LNP662 - 1MPK
LNP663 - 0.5MPK
LNP663 - 1MPK
LNP664 - 0.5MPK
LNP664 - 1MPK
LNP665 - 0.5MPK
LNP665 - 1MPK
LNP666 - 0.5MPK
LNP666 - 1MPK
LNP667 - 0.5MPK
LNP667 - 1MPK
LNP668 - 0.5MPK
LNP668 - 1MPK
LNP669 - 0.5MPK
LNP669 - 1MPK

Fig. 13B

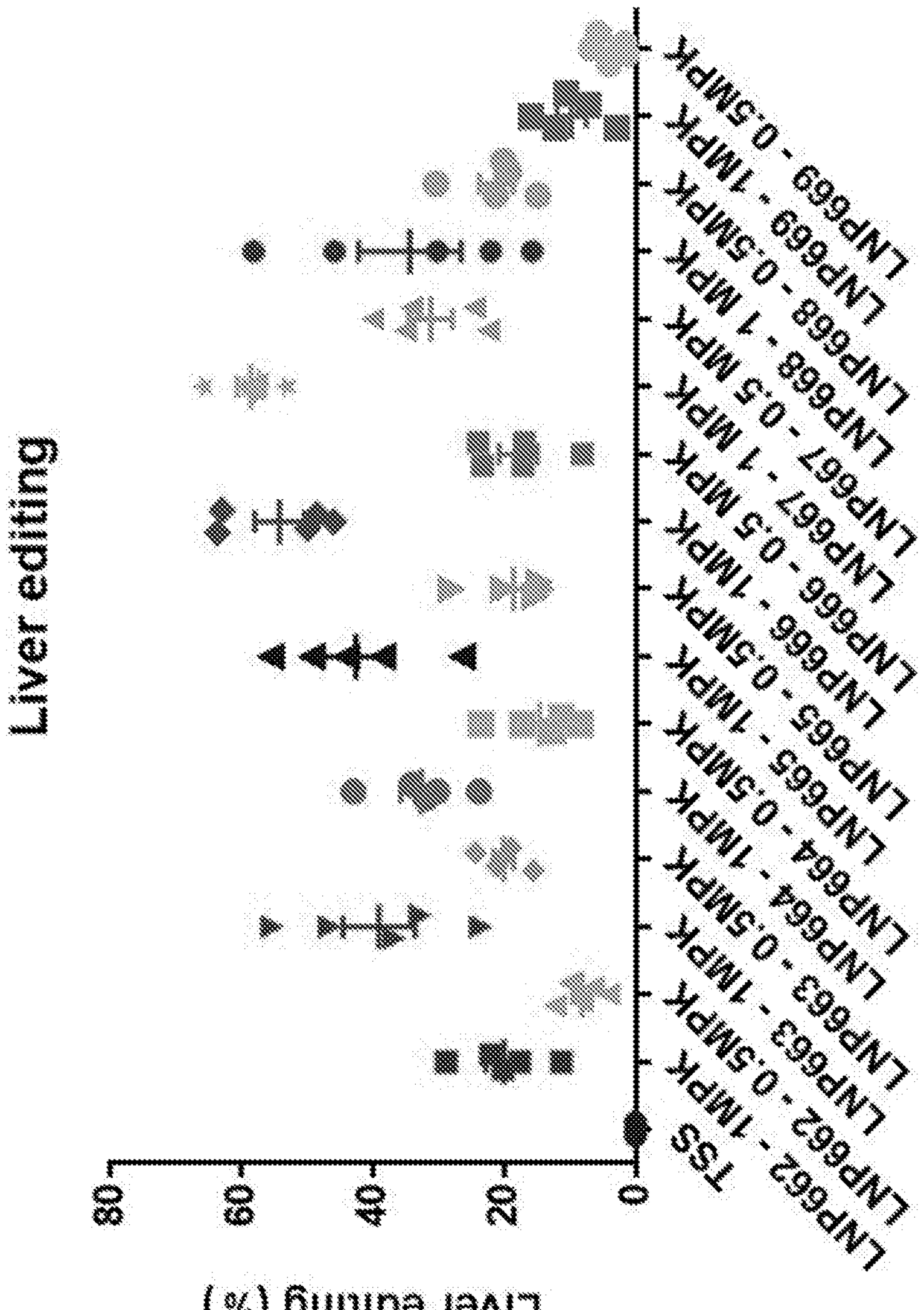
Liver editing (%)
0
20
40
60
80
TSS - 1MPK
LNP662 - 0.5MPK
LNP662 - 1MPK
LNP663 - 0.5MPK
LNP663 - 1MPK
LNP664 - 0.5MPK
LNP664 - 1MPK
LNP665 - 0.5MPK
LNP665 - 1MPK
LNP666 - 0.5MPK
LNP666 - 1MPK
LNP667 - 0.5 MPK
LNP667 - 1 MPK
LNP668 - 0.5 MPK
LNP668 - 1 MPK
LNP669 - 0.5MPK
LNP669 - 1MPK

Fig. 13C

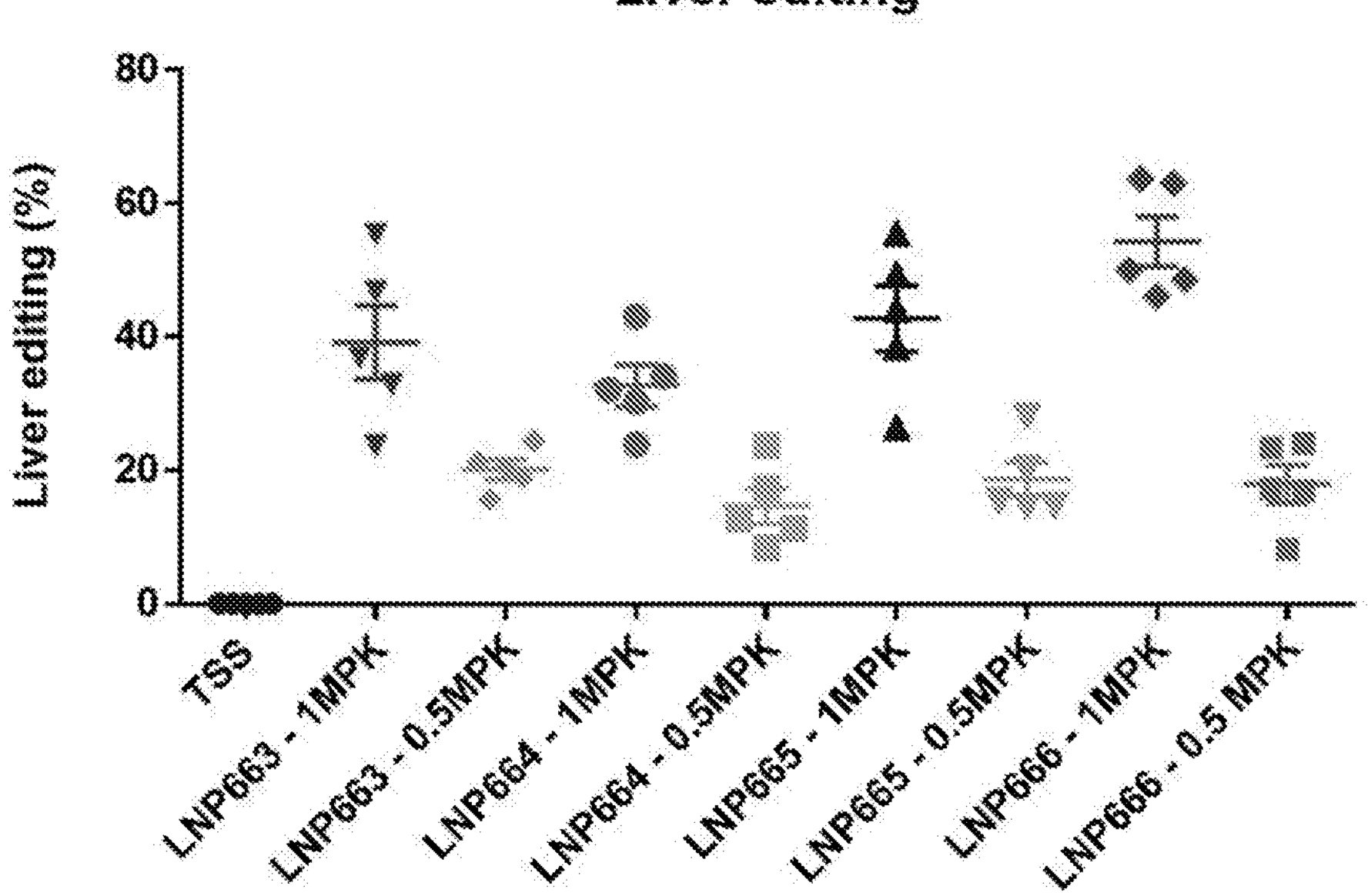
Liver editing
Liver editing (%)
0
20
40
60
80
TSS
LNP663 - 1MPK
LNP663 - 0.5MPK
LNP664 - 1MPK
LNP664 - 0.5MPK
LNP665 - 1MPK
LNP665 - 0.5MPK
LNP666 - 1MPK
LNP666 - 0.5 MPK

Fig. 13D

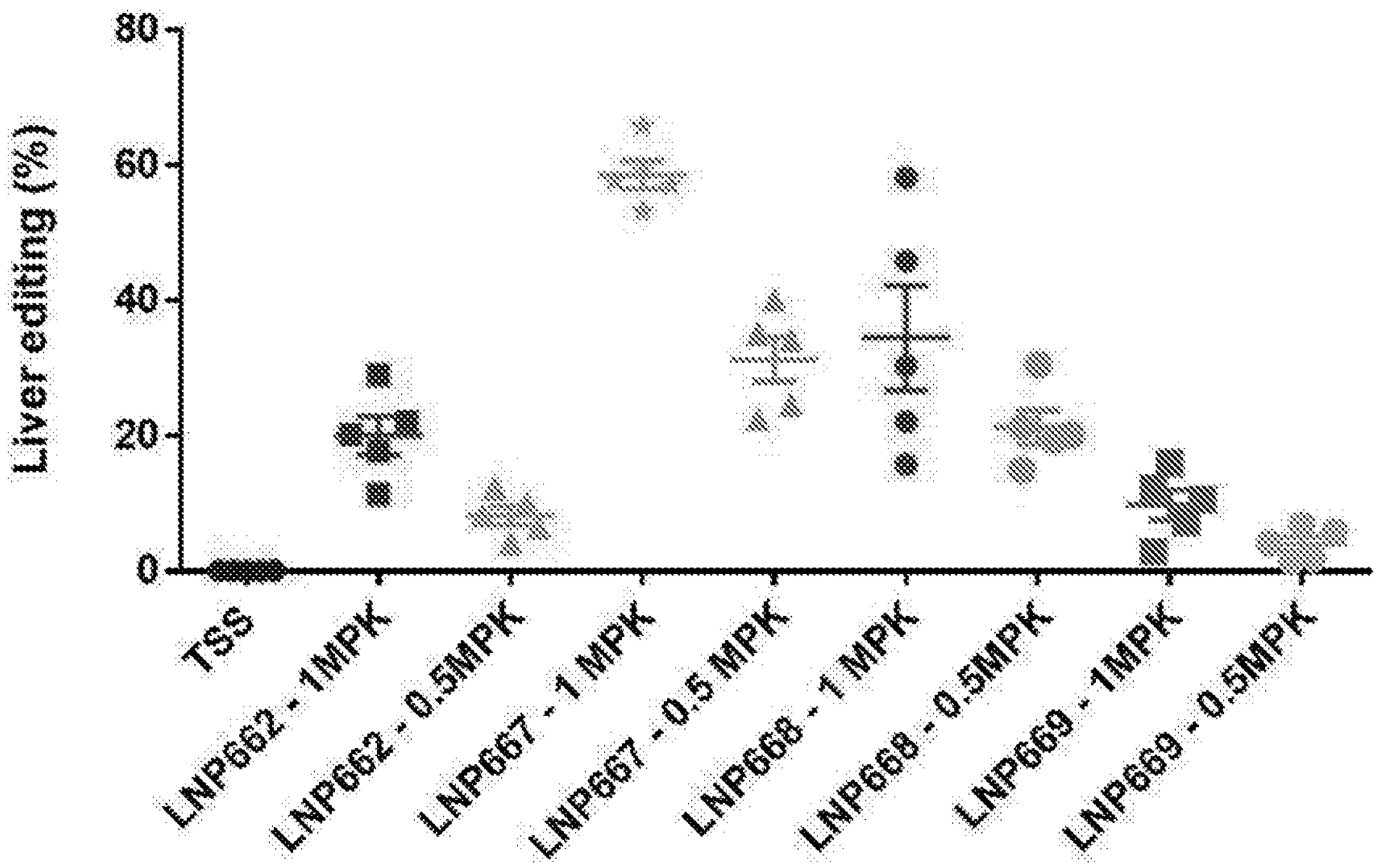
Liver editing
Liver editing (%)
0
20
40
60
80
TSS
LNP662 - 1MPK
LNP662 - 0.5MPK
LNP667 - 1 MPK
LNP667 - 0.5 MPK
LNP668 - 1 MPK
LNP668 - 0.5MPK
LNP669 - 1MPK
LNP669 - 0.5MPK

Fig. 13E

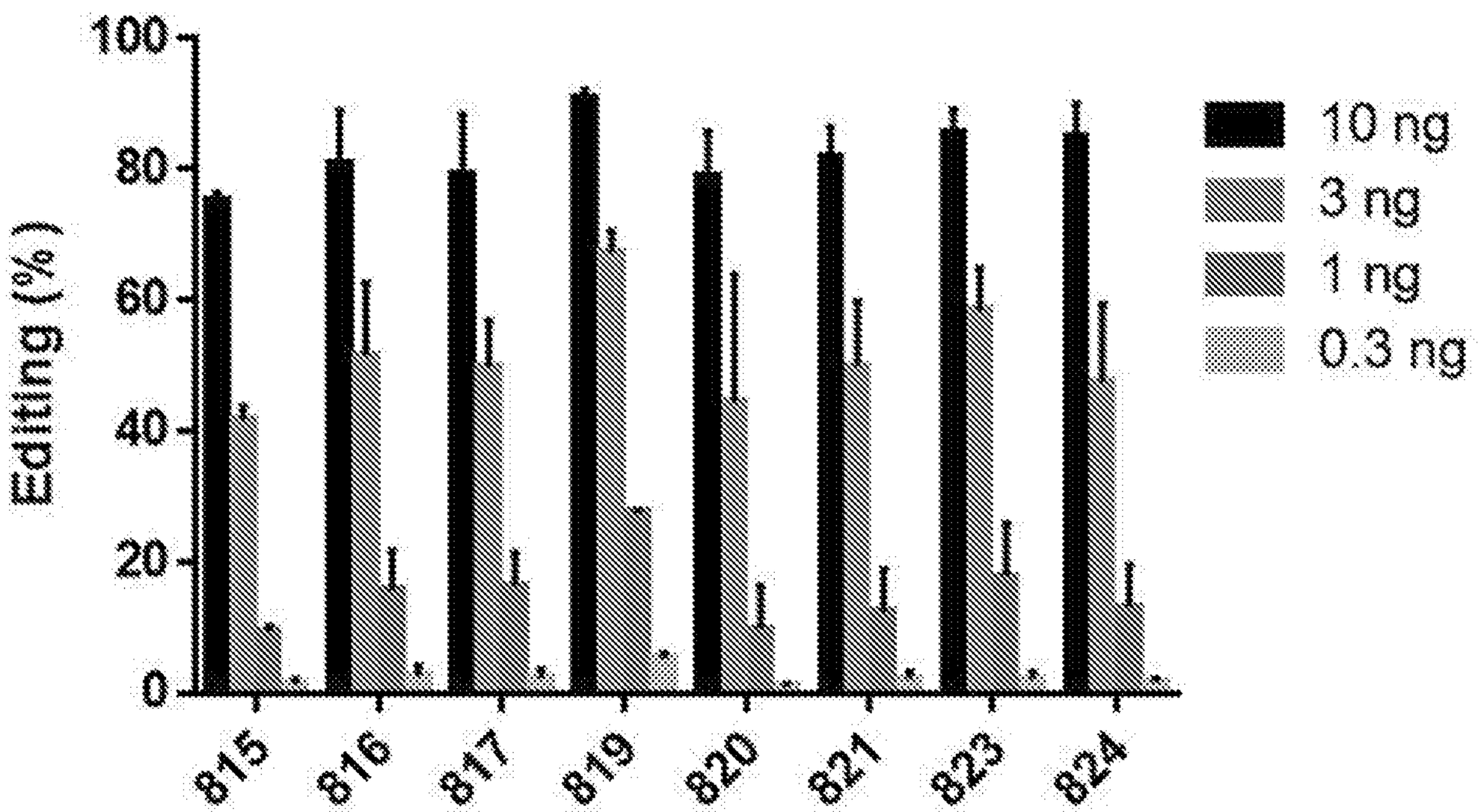
Editing (%)
100
80
60
40
20
0
815
816
817
819
820
821
823
824
10 ng
3 ng
1 ng
0.3 ng

Fig. 14

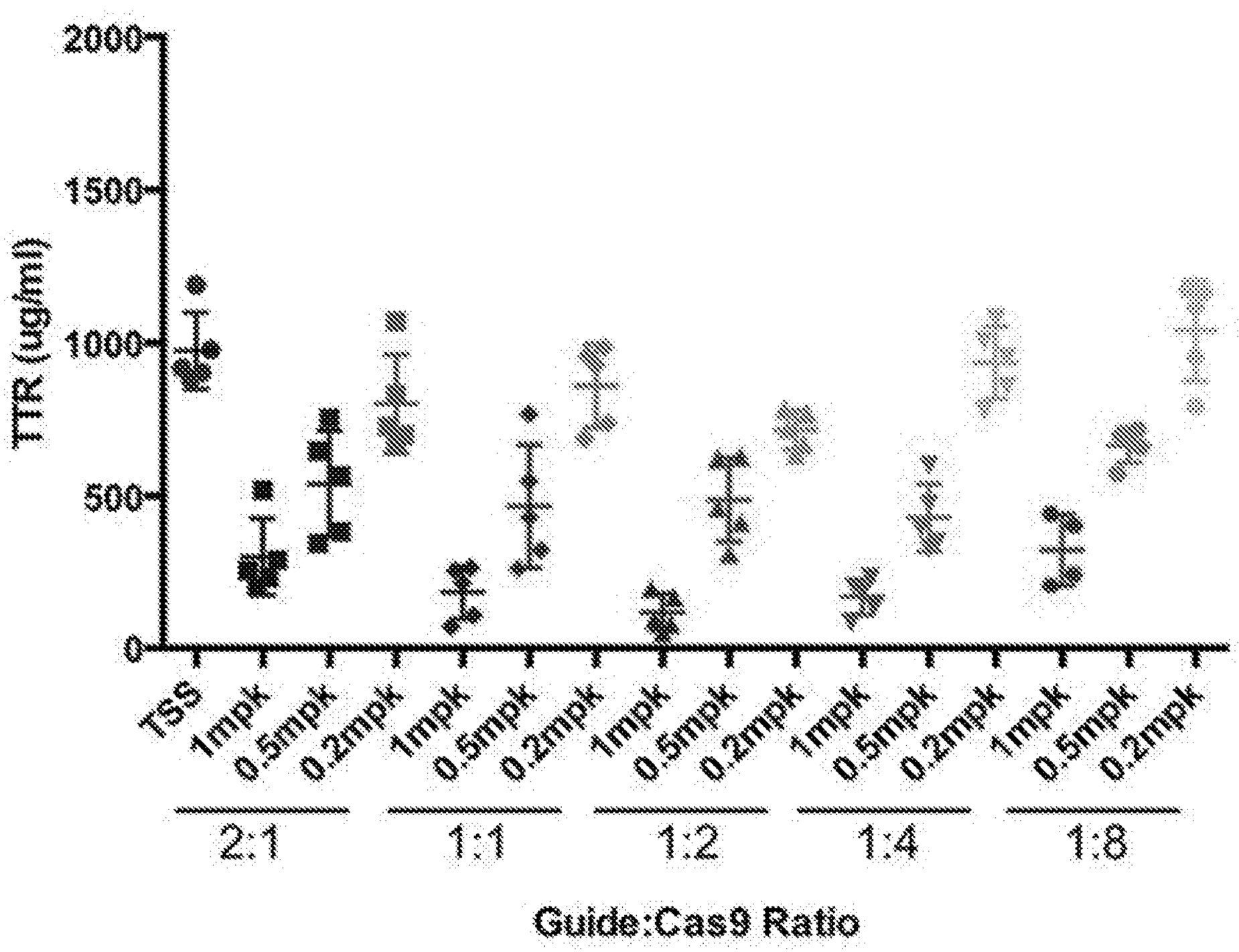
Serum TTR levels - SEQ ID NO: 5
TTR (ug/ml)
2000
1500
1000
500
0
TSS
1mpk
0.5mpk
0.2mpk
2:1
1:1
1:2
1:4
1:8
Guide:Cas9 Ratio

Fig. 15A

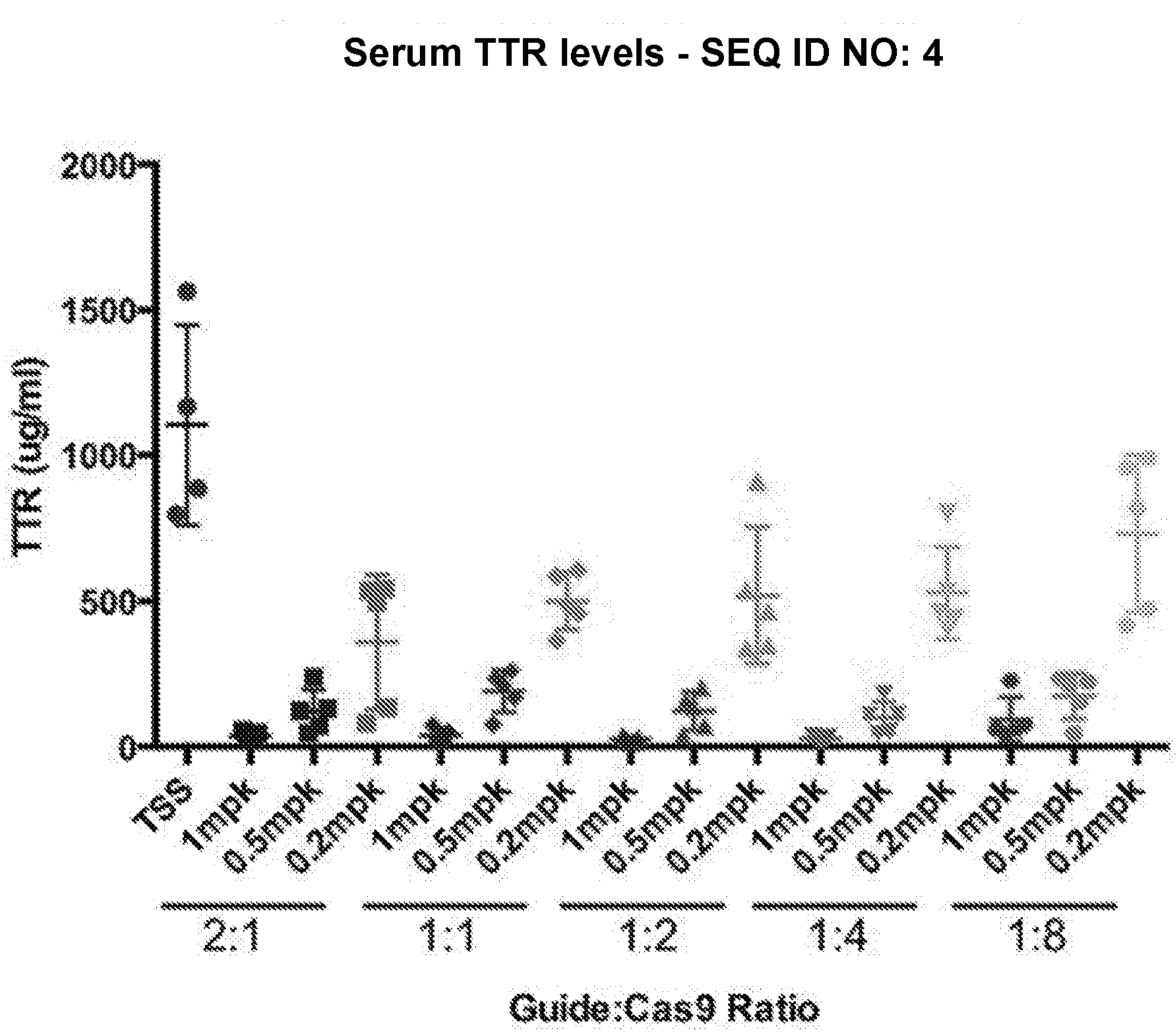
Serum TTR levels - SEQ ID NO: 4
TTR (ug/ml)
2000
1500
1000
500
0
TSS
1mpk
0.5mpk
0.2mpk
2:1
1:1
1:2
1:4
1:8
Guide:Cas9 Ratio

Fig. 15B

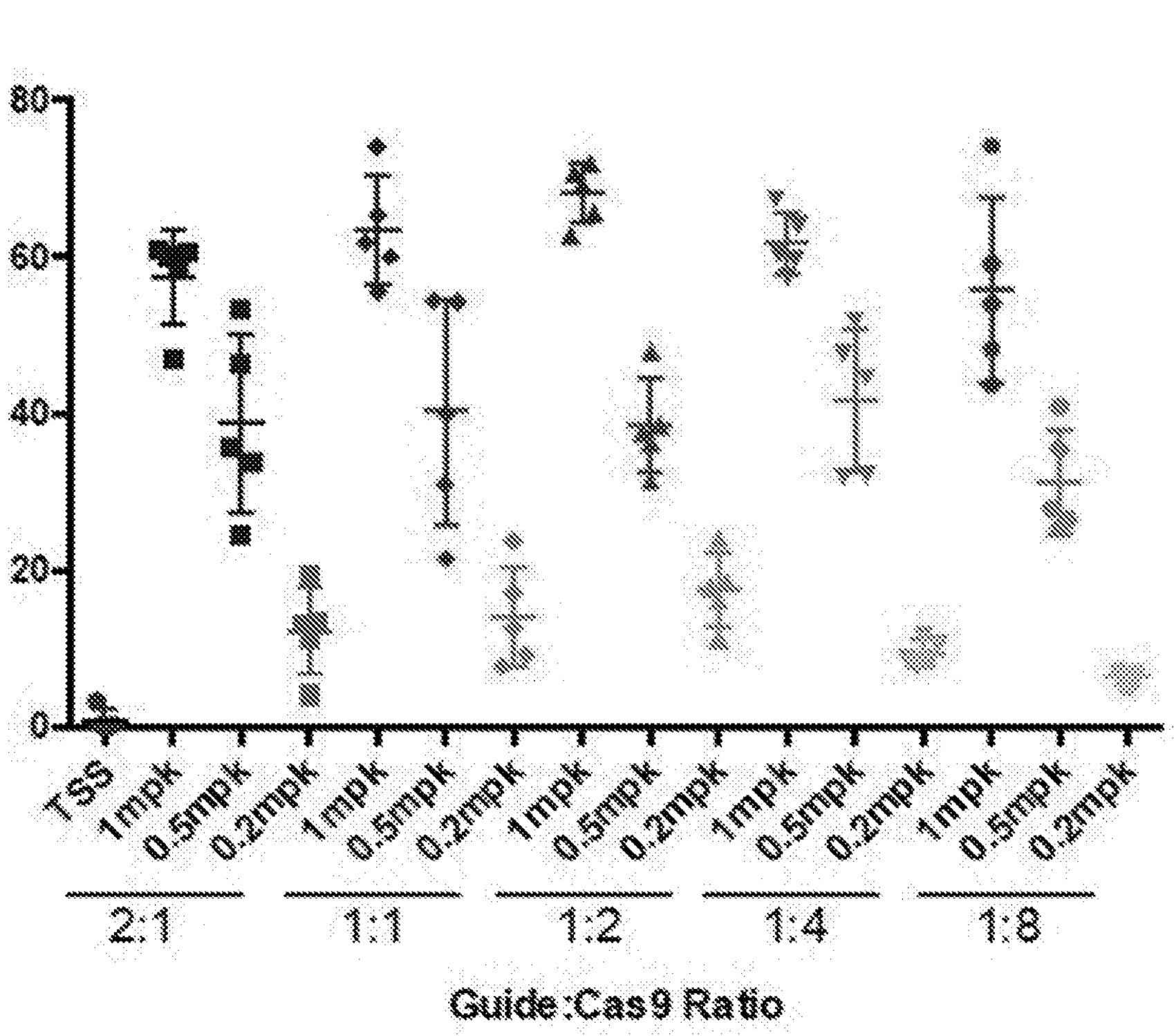
Liver Editing - SEQ ID NO: 5
80
60
40
20
0
TSS
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
2:1
1:1
1:2
1:4
1:8
Guide:Cas9 Ratio

Fig. 16A

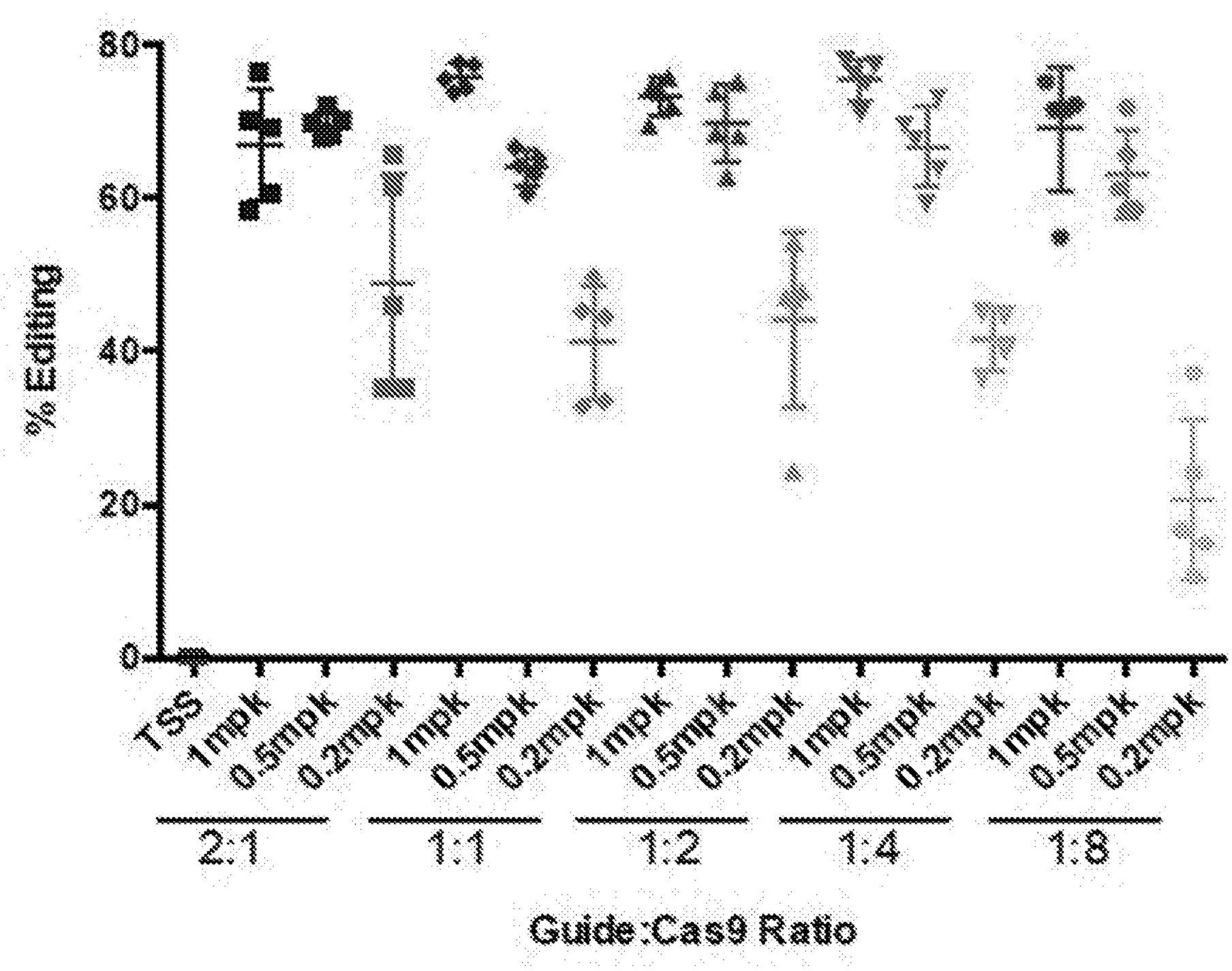
Liver Editing - SEQ ID NO: 4
% Editing
80
60
40
20
0
TSS
1mpk
0.5mpk
0.2mpk
2:1
1:1
1:2
1:4
1:8
Guide:Cas9 Ratio

Fig. 16B

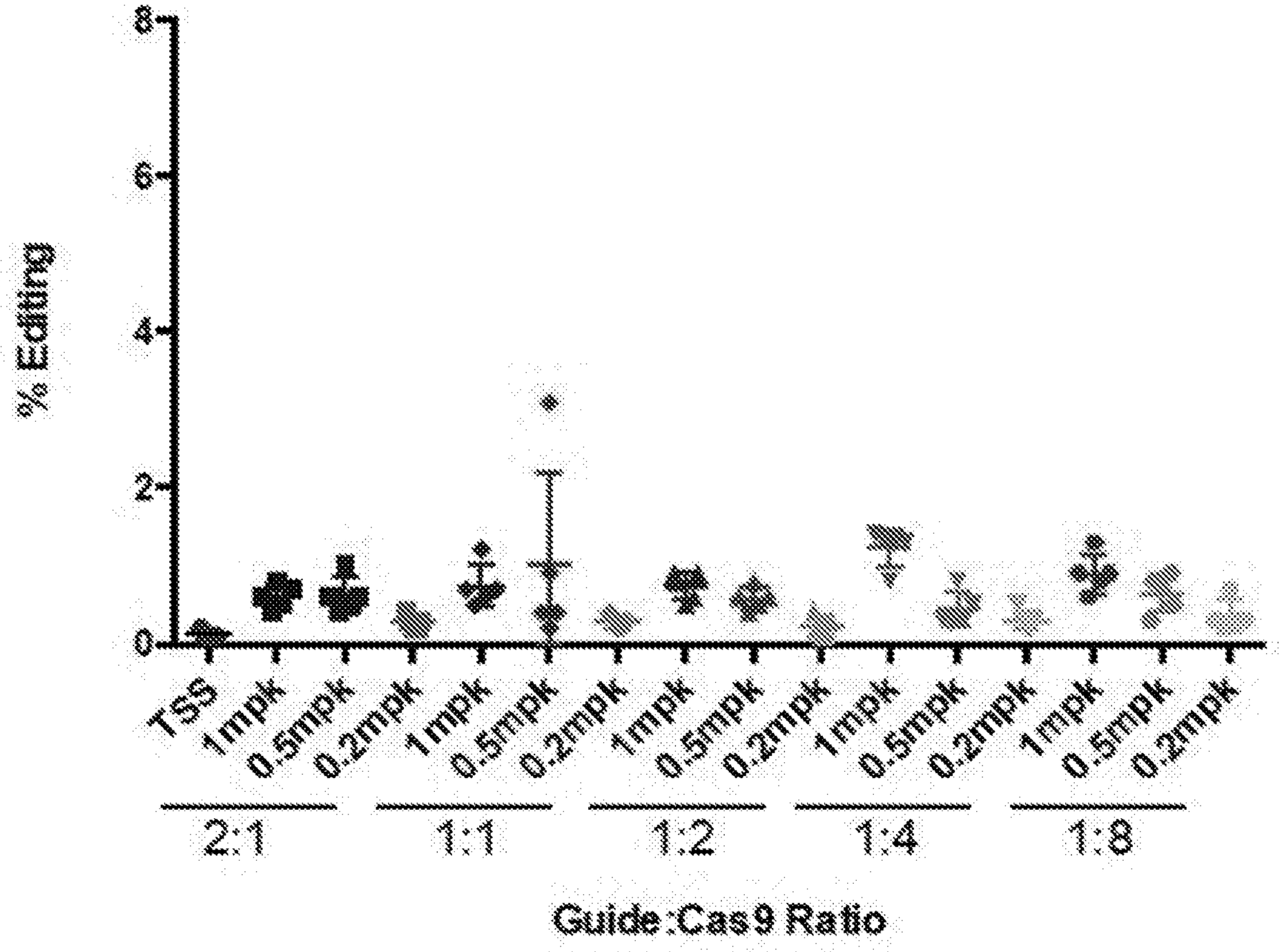
Spleen Editing - SEQ ID NO: 5
% Editing
0
2
4
6
8
TSS
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
2:1
1:1
1:2
1:4
1:8
Guide:Cas9 Ratio

Fig. 17A

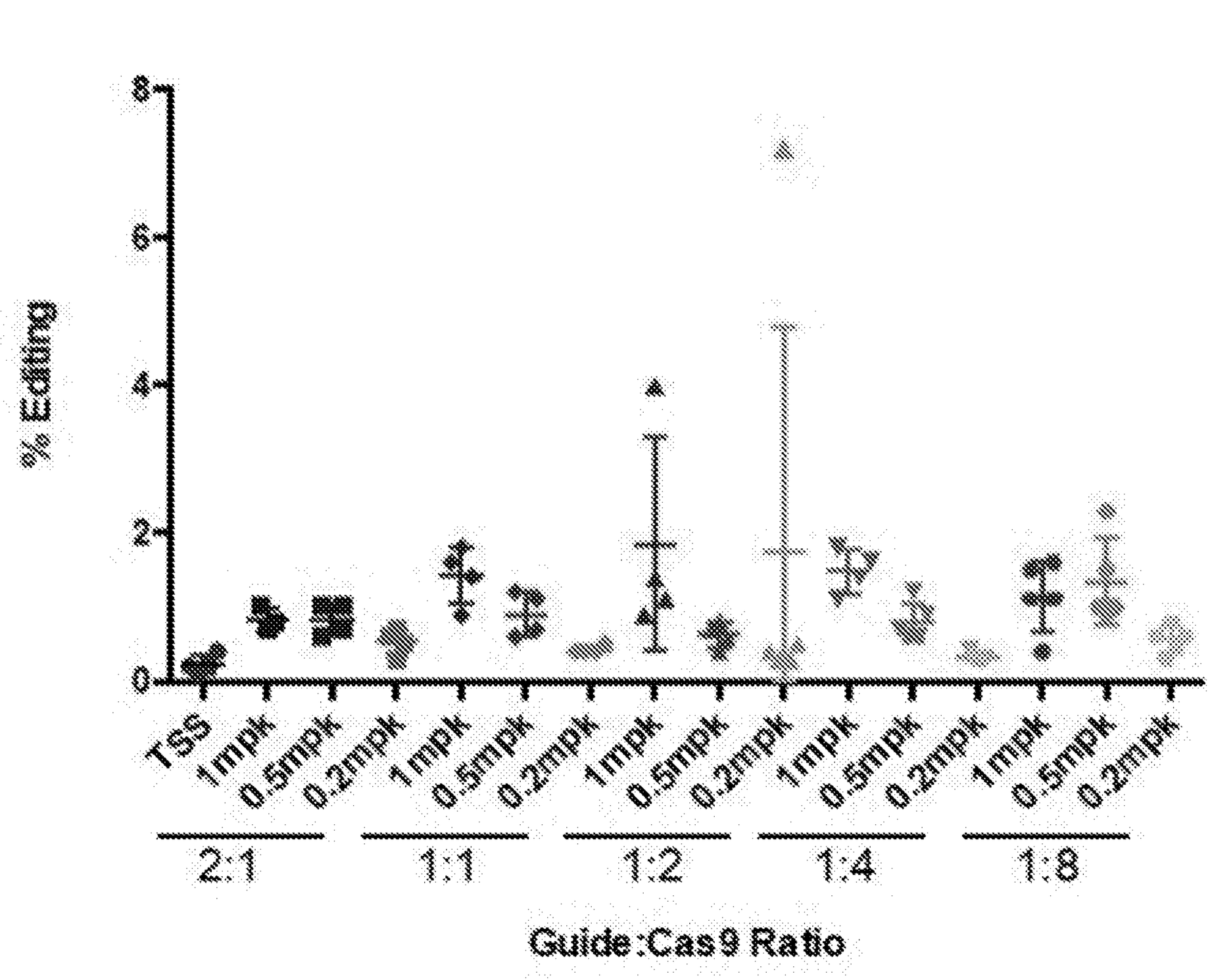
Spleen Editing - SEQ ID NO: 4
% Editing
0
2
4
6
8
TSS
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
1mpk
0.5mpk
0.2mpk
2:1
1:1
1:2
1:4
1:8
Guide:Cas9 Ratio

Fig. 17B

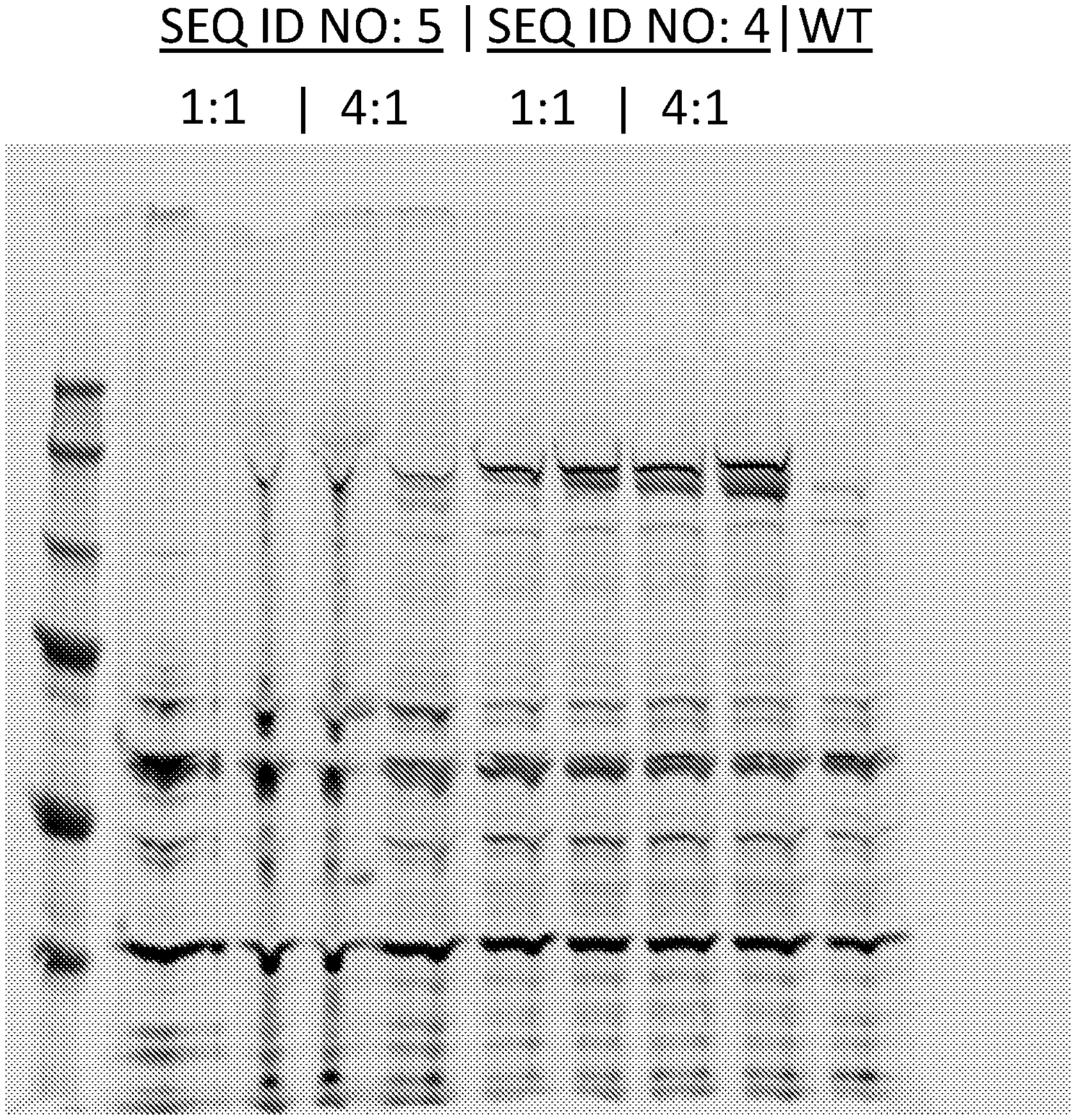

Fig. 18

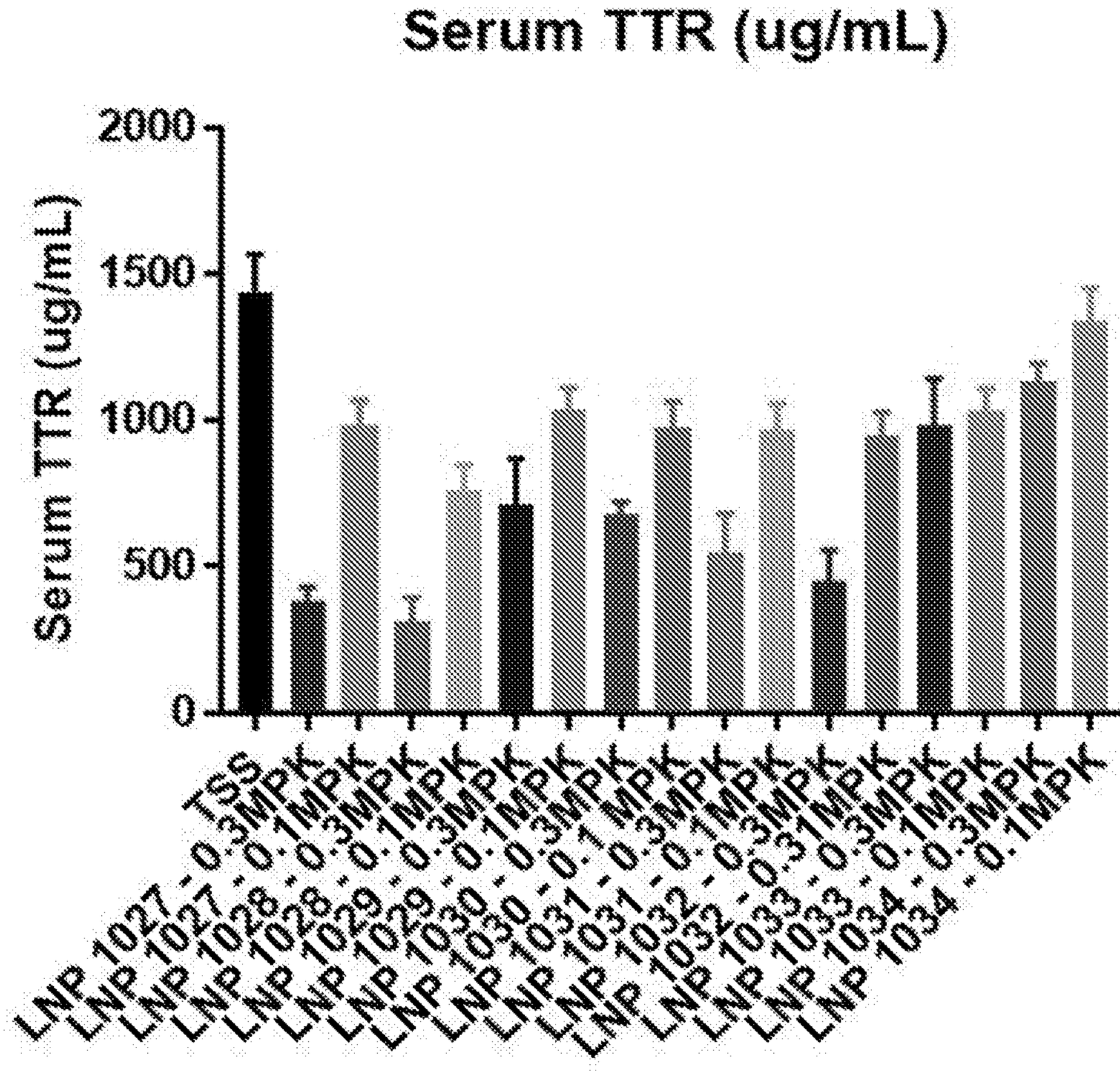
Serum TTR (ug/mL)
2000
1500
1000
500
0
Serum TTR (ug/mL)
TSS
LNP 1027 - 0.3MPK
LNP 1027 - 0.1MPK
LNP 1028 - 0.3MPK
LNP 1028 - 0.1MPK
LNP 1029 - 0.3MPK
LNP 1029 - 0.1MPK
LNP 1030 - 0.3MPK
LNP 1030 - 0.1MPK
LNP 1031 - 0.3MPK
LNP 1031 - 0.1MPK
LNP 1032 - 0.3MPK
LNP 1032 - 0.1MPK
LNP 1033 - 0.3MPK
LNP 1033 - 0.1MPK
LNP 1034 - 0.3MPK
LNP 1034 - 0.1MPK

Fig. 19A

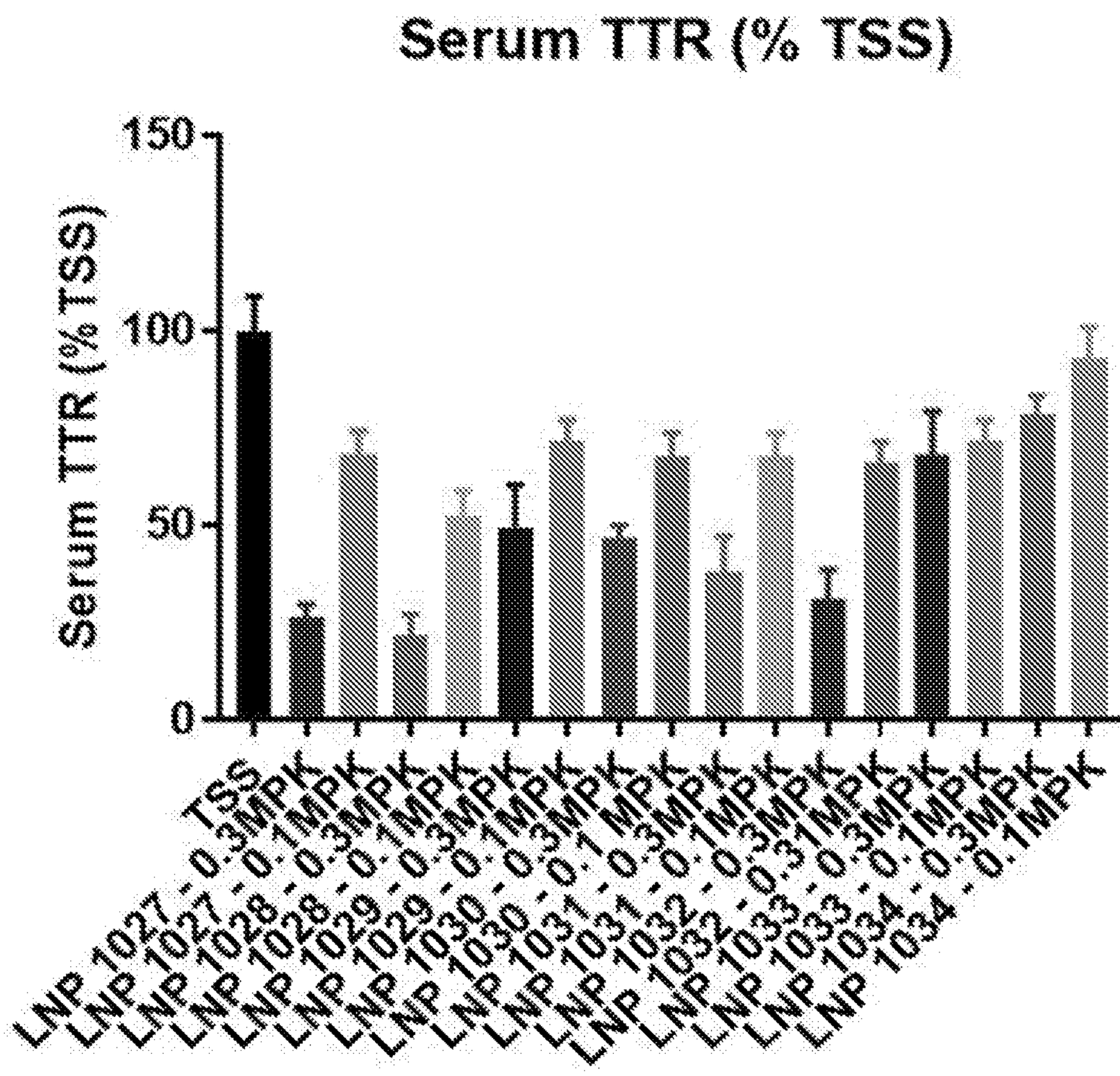
Serum TTR (% TSS)
Serum TTR (% TSS)
150
100
50
0
TSS
LNP 1027 - 0.3MPK
LNP 1027 - 0.1MPK
LNP 1028 - 0.3MPK
LNP 1028 - 0.1MPK
LNP 1029 - 0.3MPK
LNP 1029 - 0.1MPK
LNP 1030 - 0.3MPK
LNP 1030 - 0.1MPK
LNP 1031 - 0.3MPK
LNP 1031 - 0.1MPK
LNP 1032 - 0.3MPK
LNP 1032 - 0.31MPK
LNP 1033 - 0.3MPK
LNP 1033 - 0.1MPK
LNP 1034 - 0.3MPK
LNP 1034 - 0.1MPK

Fig. 19B

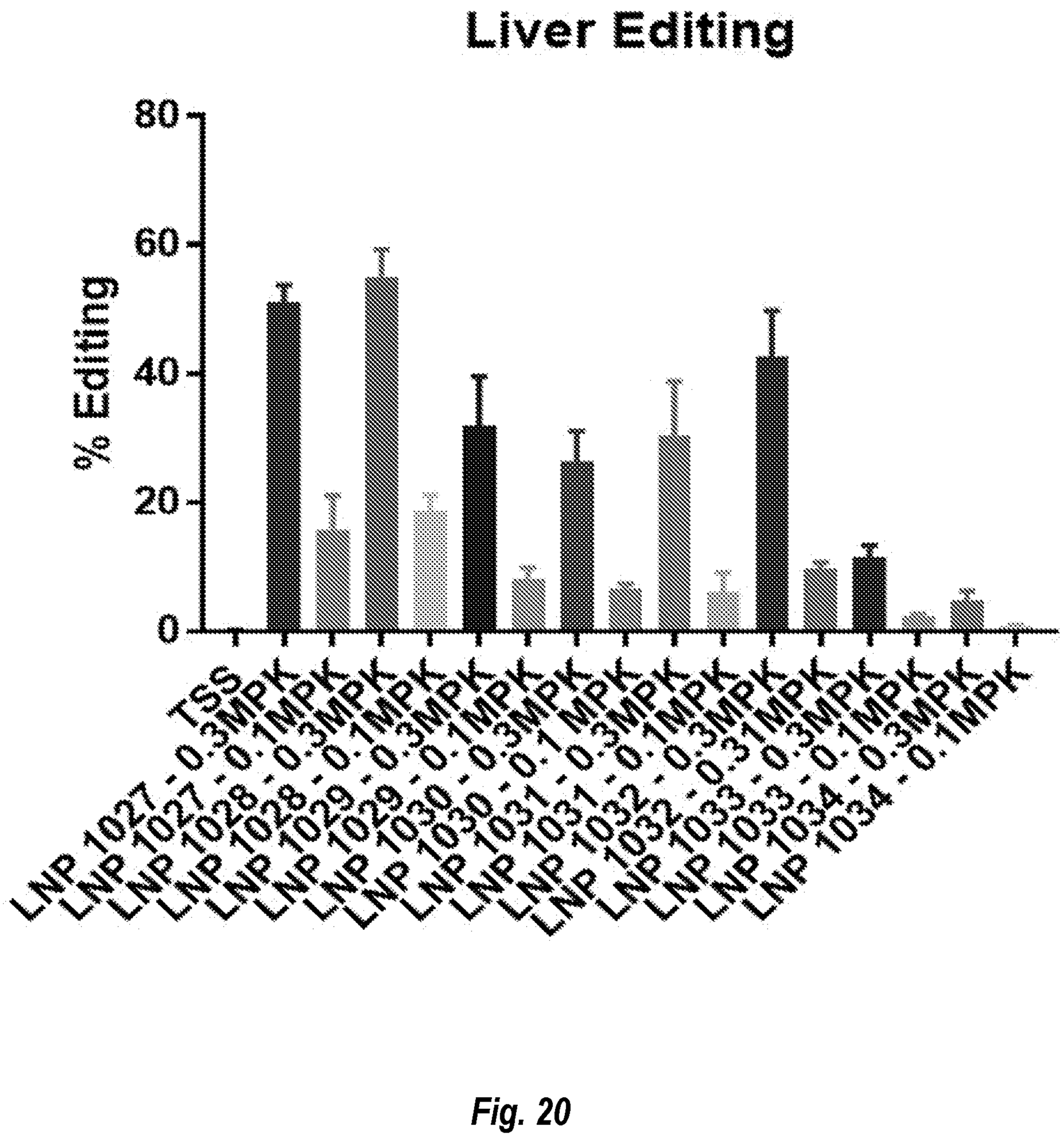
Liver Editing
% Editing
0
20
40
60
80
TSS
LNP 1027 - 0.3MPK
LNP 1027 - 0.1MPK
LNP 1028 - 0.3MPK
LNP 1028 - 0.1MPK
LNP 1029 - 0.3MPK
LNP 1029 - 0.1MPK
LNP 1030 - 0.3MPK
LNP 1030 - 0.1MPK
LNP 1031 - 0.3MPK
LNP 1031 - 0.1MPK
LNP 1032 - 0.3MPK
LNP 1032 - 0.1MPK
LNP 1033 - 0.3MPK
LNP 1033 - 0.1MPK
LNP 1034 - 0.3MPK
LNP 1034 - 0.1MPK

Fig. 20

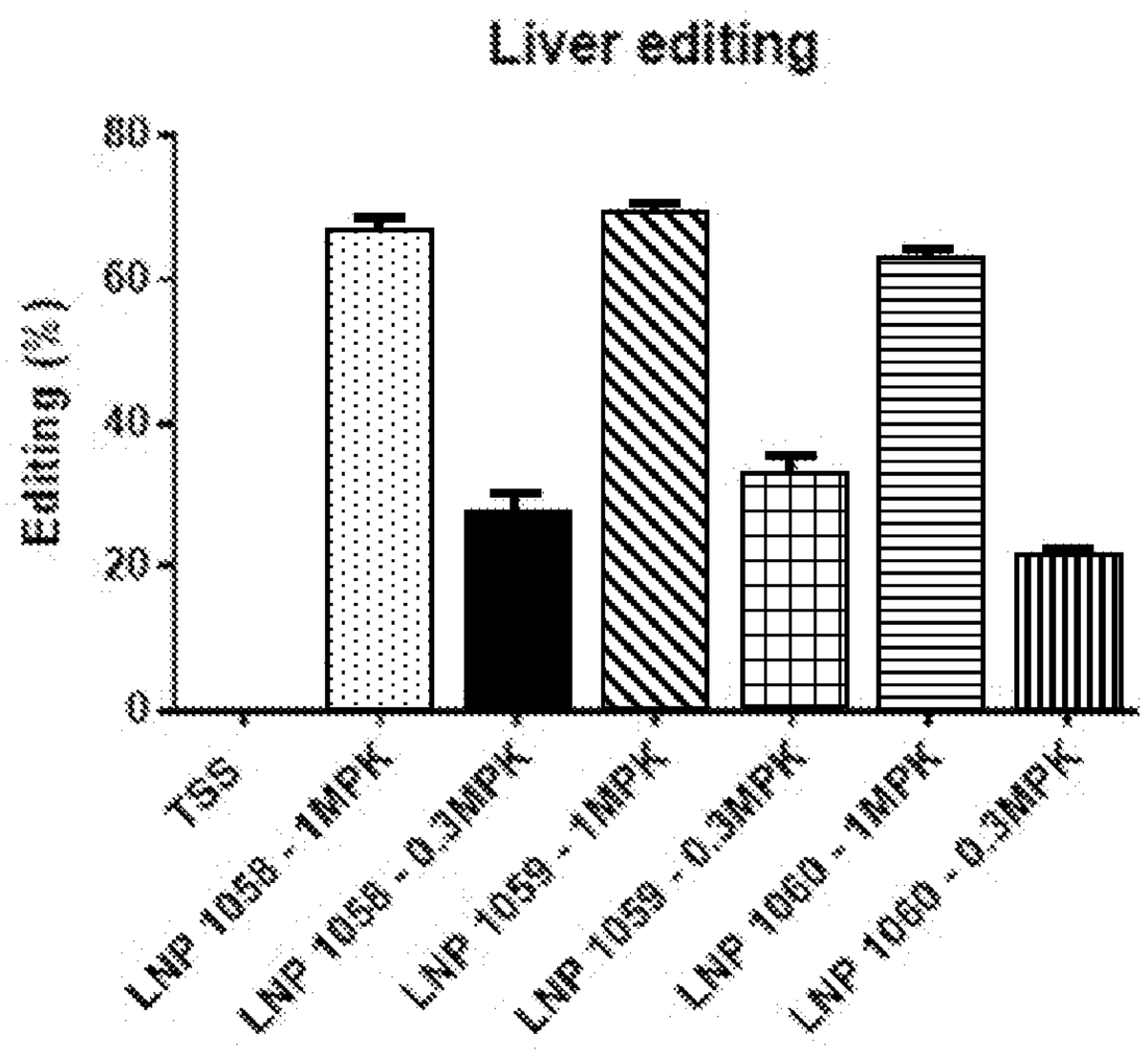

Fig. 21A

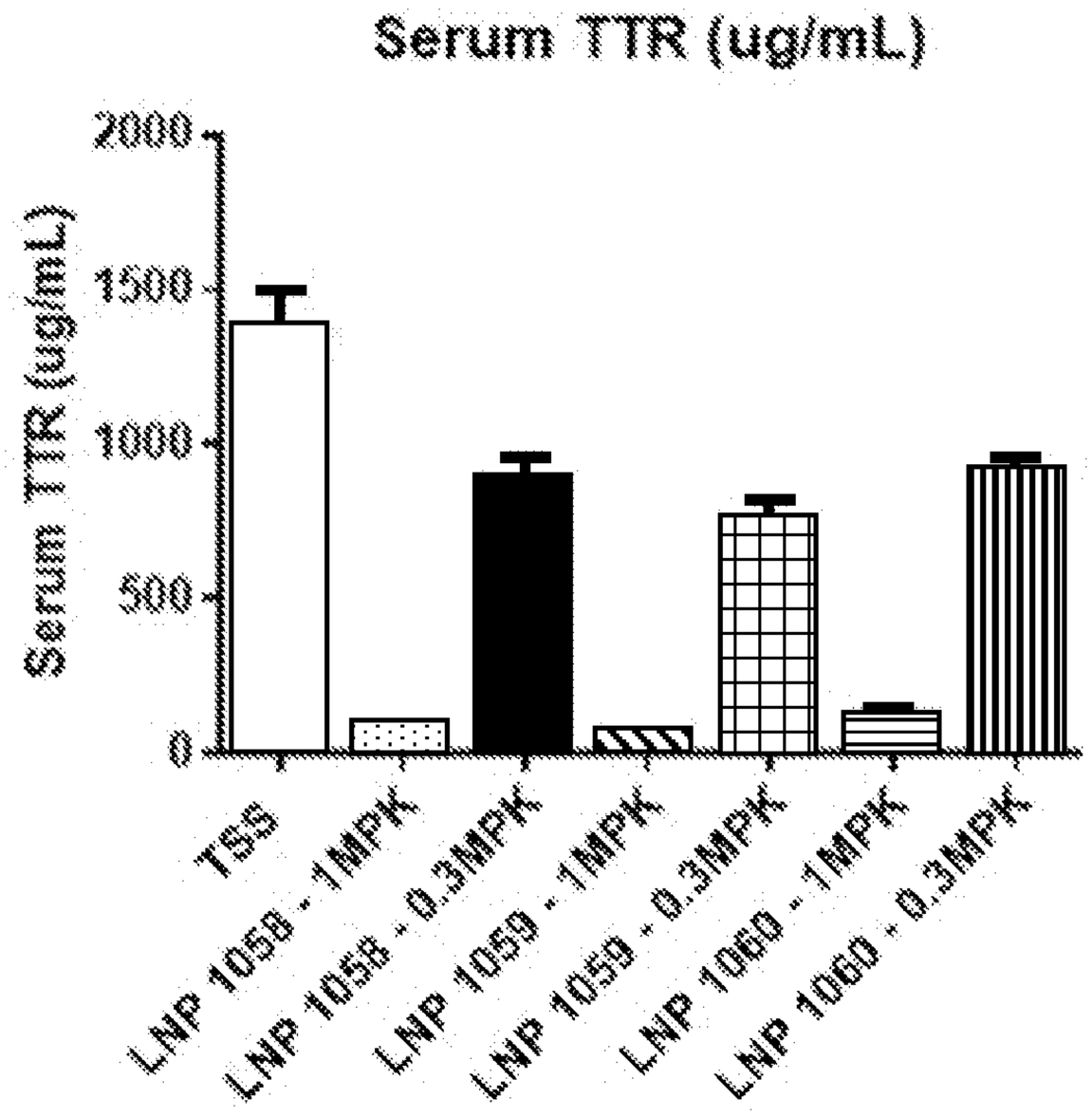

Fig. 21B

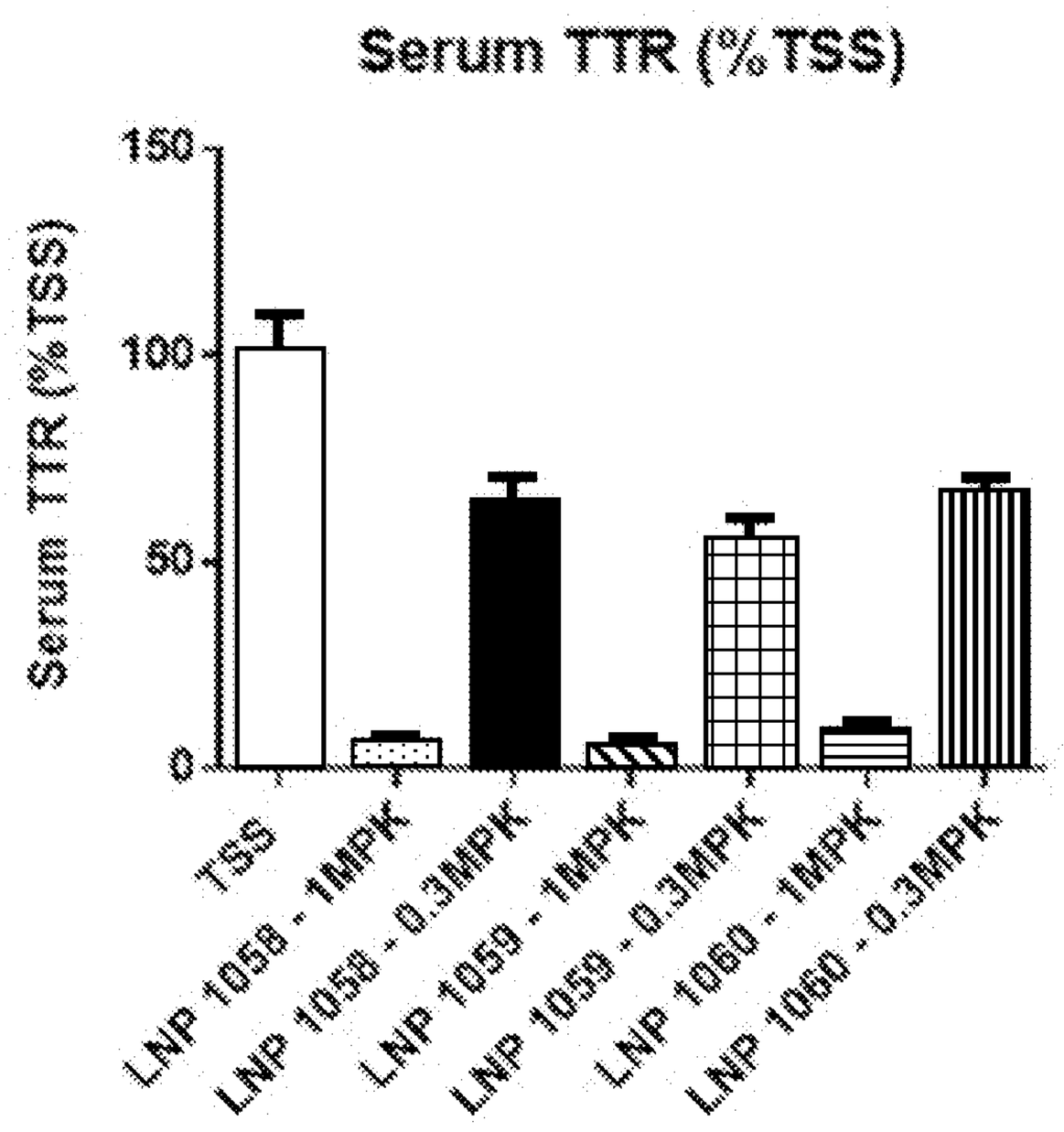
Serum TTR (%TSS)
Serum TTR (%TSS)
150
100
50
0
TSS
LNP 1058 - 1MPK
LNP 1058 - 0.3MPK
LNP 1059 - 1MPK
LNP 1059 - 0.3MPK
LNP 1060 - 1MPK
LNP 1060 - 0.3MPK

Fig. 21C

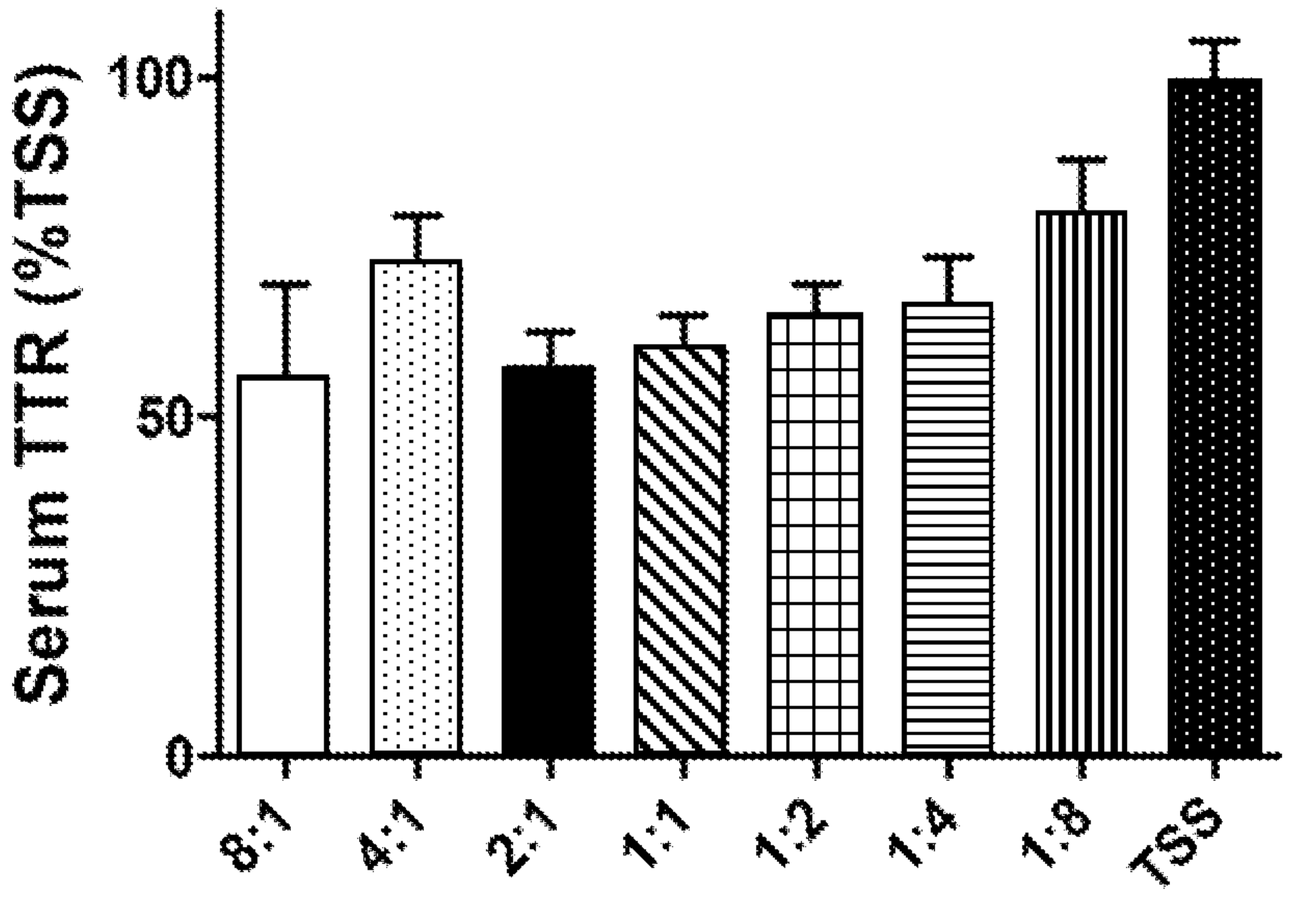

Fig. 22A

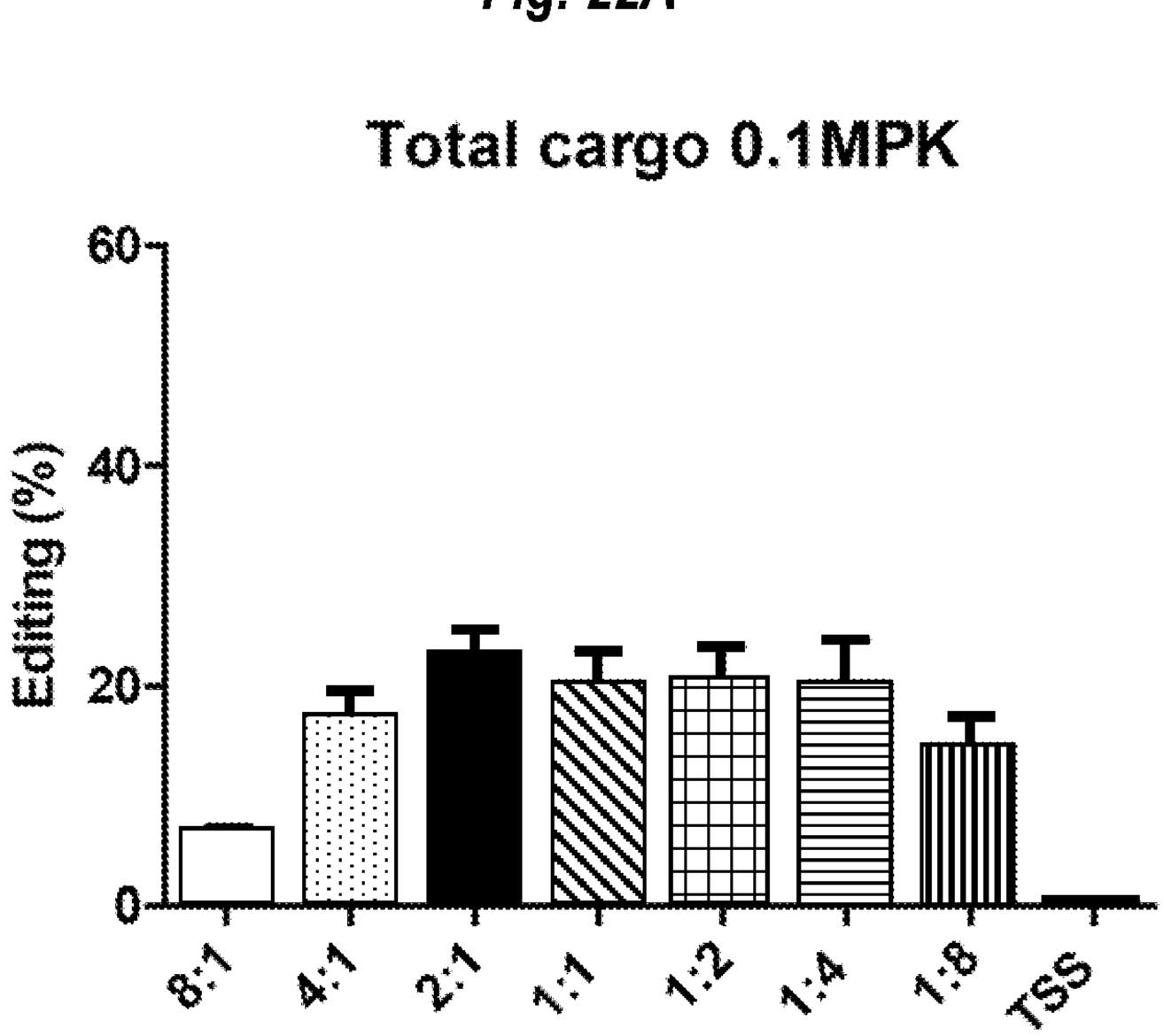

Fig. 22B

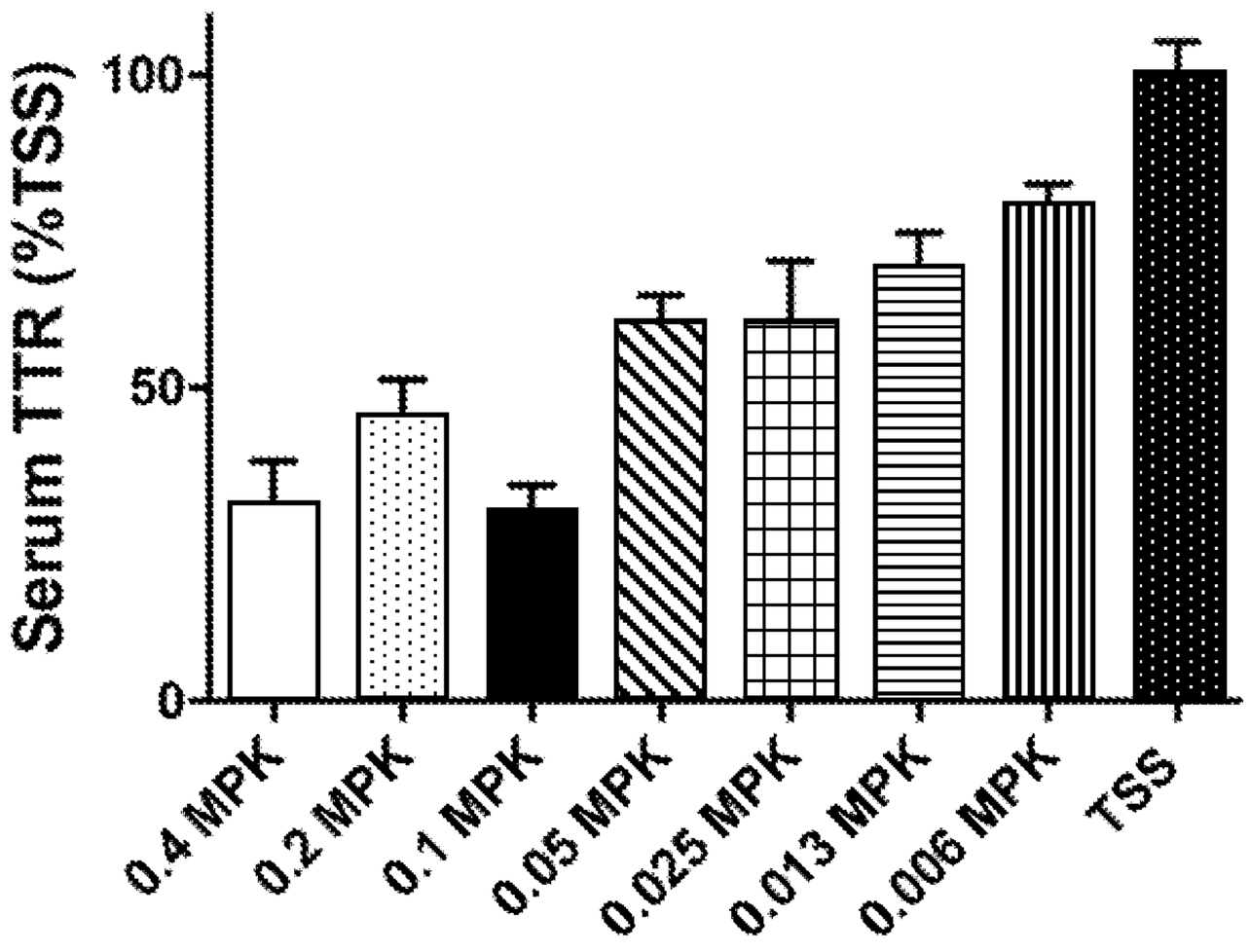

Fig. 22C

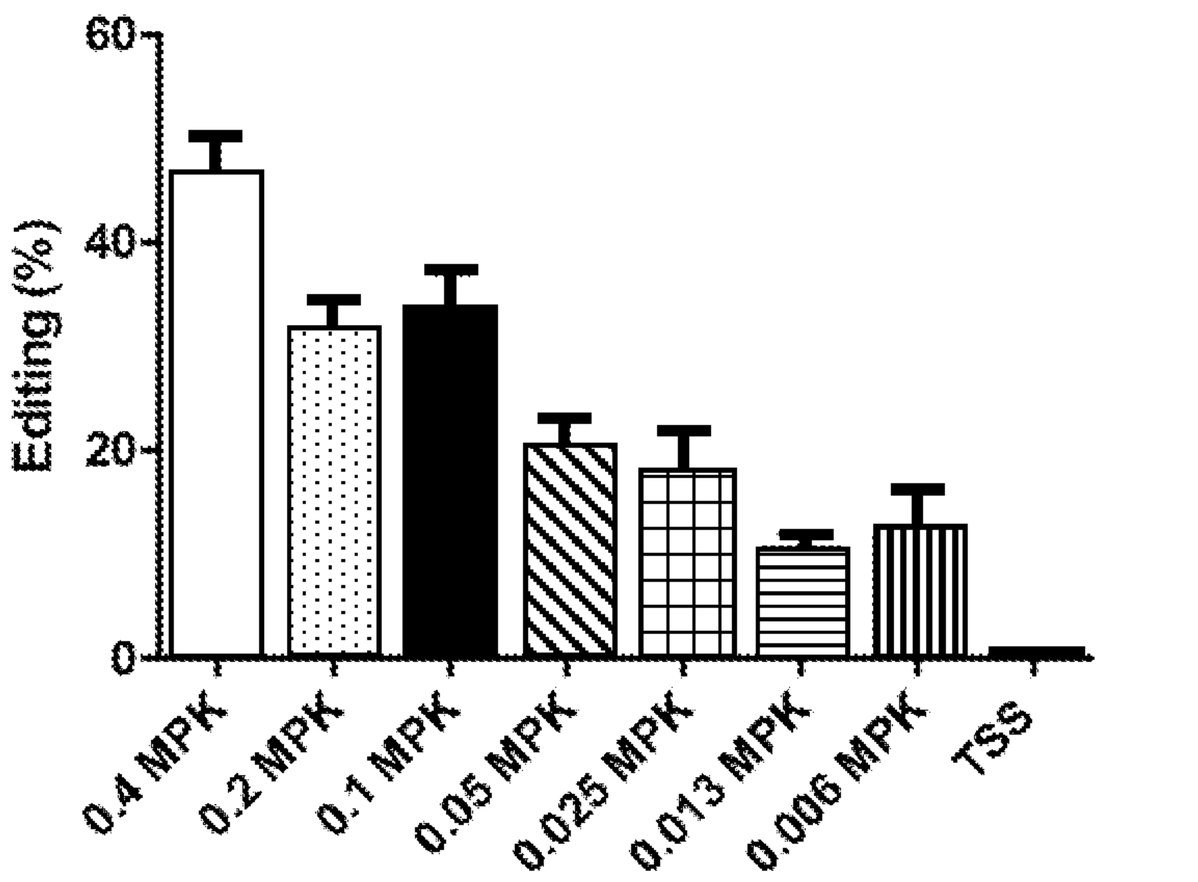

Fig. 22D

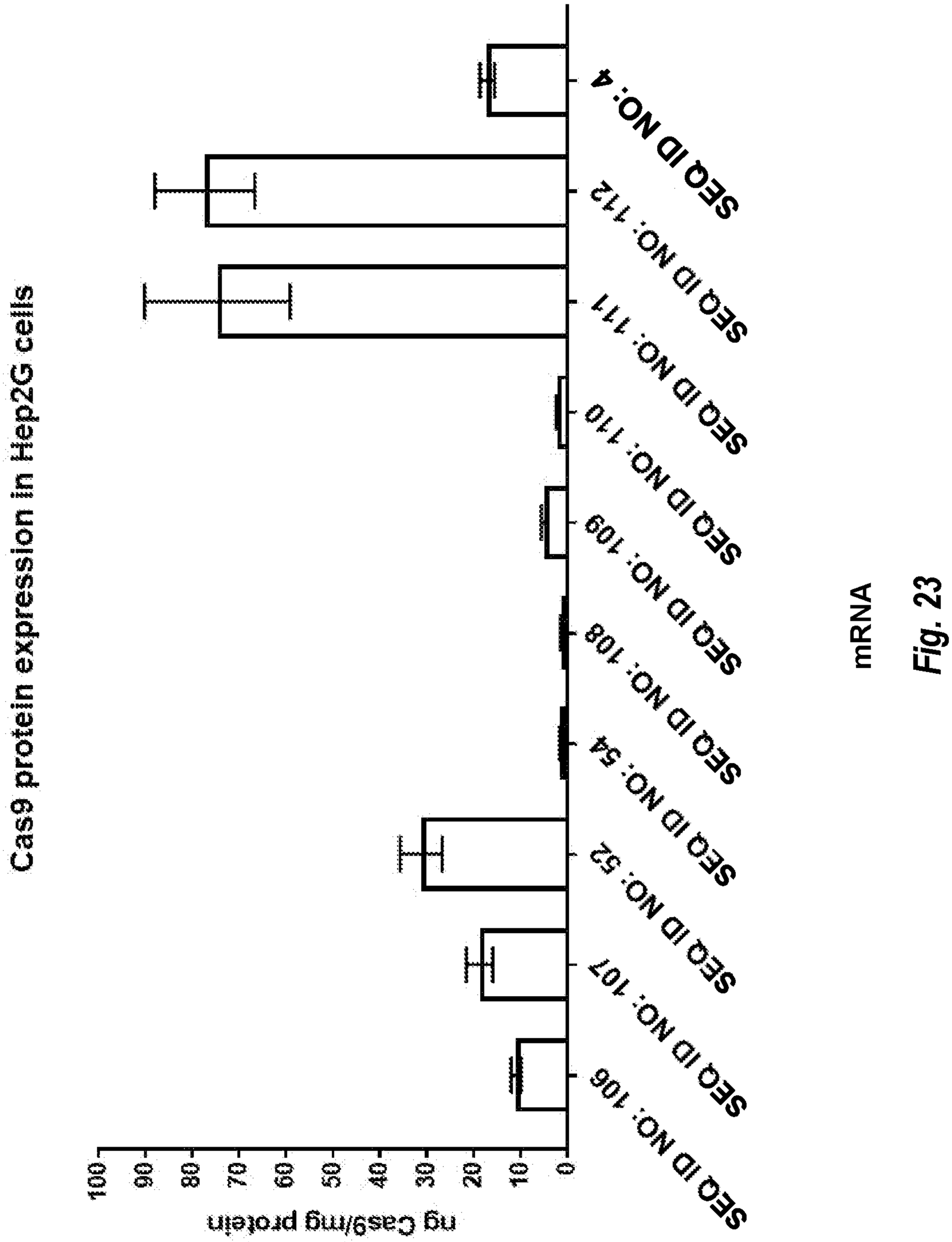
Cas9 protein expression in Hep2G cells
ng Cas9/mg protein
0
10
20
30
40
50
60
70
80
90
100
SEQ ID NO: 106
SEQ ID NO: 107
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 108
SEQ ID NO: 109
SEQ ID NO: 110
SEQ ID NO: 111
SEQ ID NO: 112
SEQ ID NO: 4
mRNA

Fig. 23

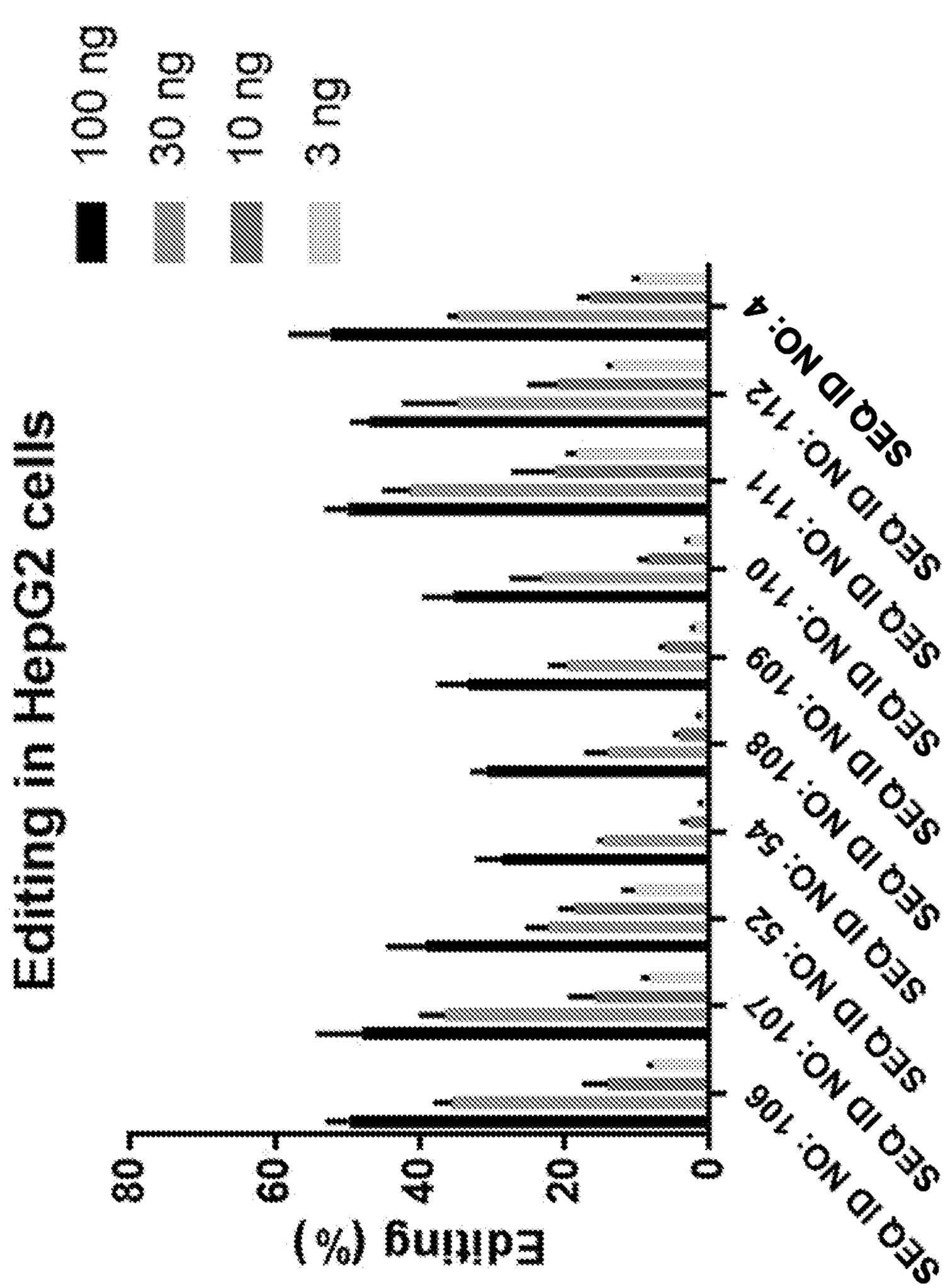
100 ng
30 ng
10 ng
3 ng
Editing in HepG2 cells
Editing (%)
0
20
40
60
80
SEQ ID NO: 106
SEQ ID NO: 107
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 108
SEQ ID NO: 109
SEQ ID NO: 110
SEQ ID NO: 111
SEQ ID NO: 112
SEQ ID NO: 4

Fig. 24

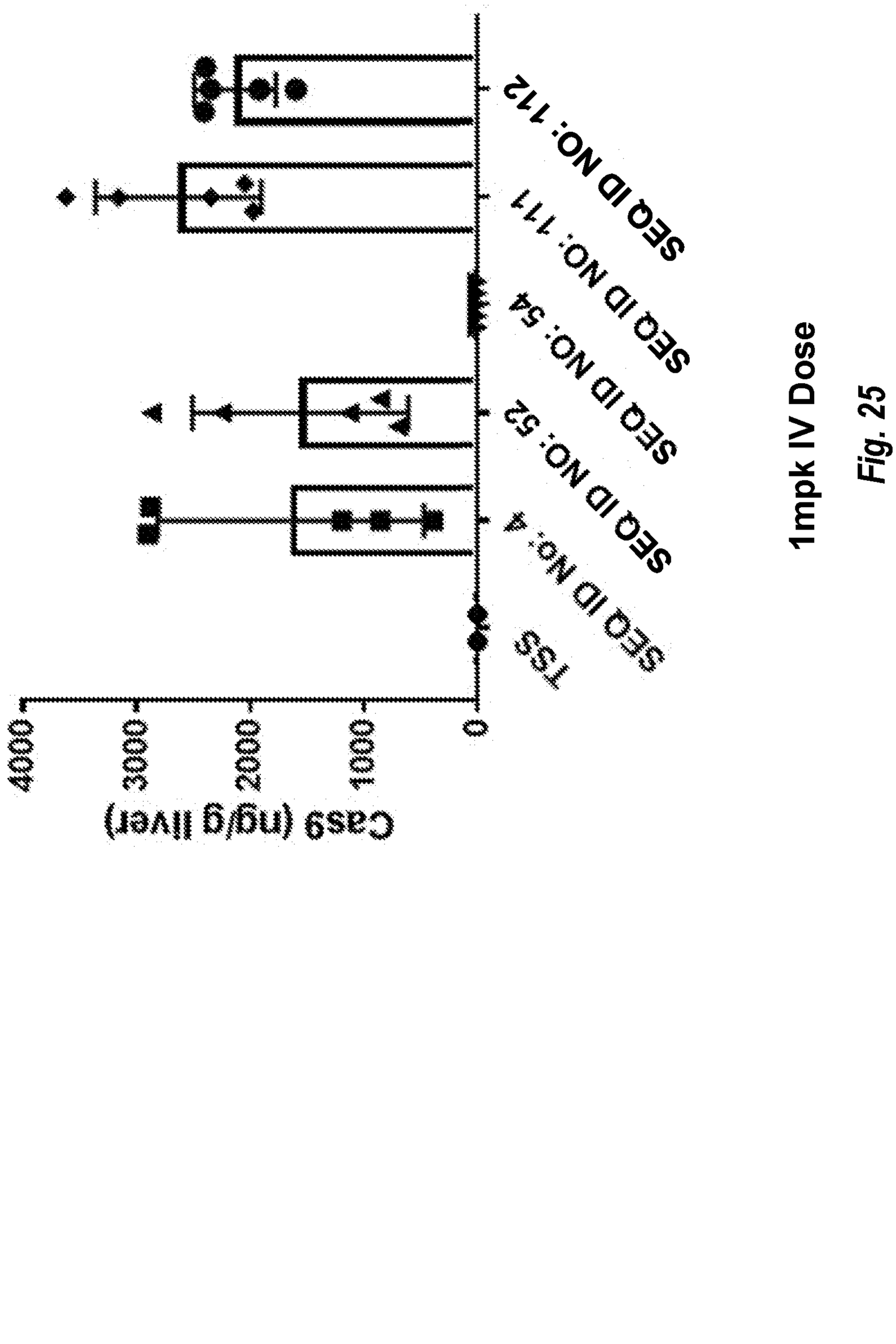
Cas9 (ng/g liver)
4000
3000
2000
1000
0
TSS
SEQ ID NO: 4
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 111
SEQ ID NO: 112
1mpk IV Dose

Fig. 25

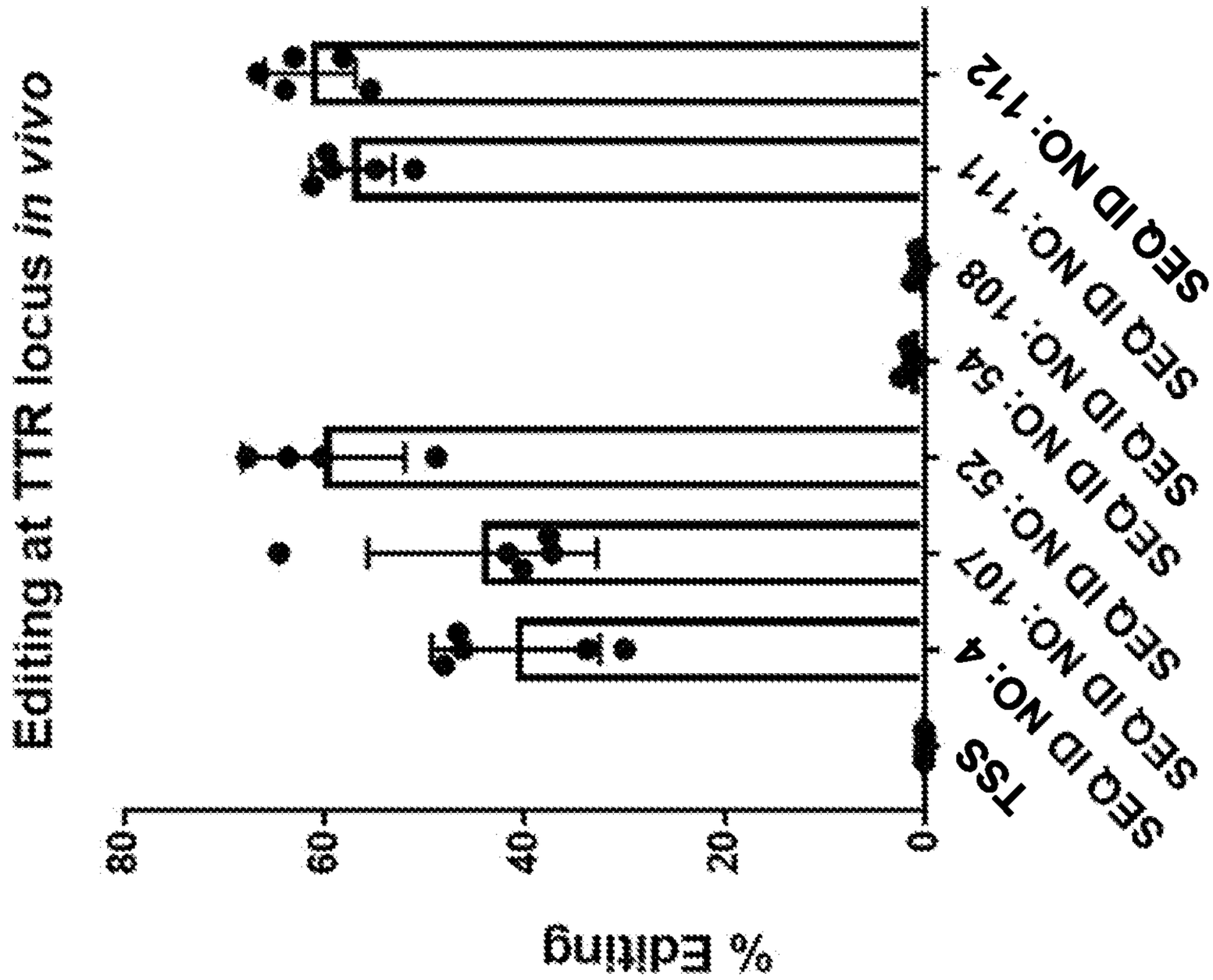
Editing at TTR locus in vivo
% Editing
80
60
40
20
0
TSS
SEQ ID NO: 4
SEQ ID NO: 107
SEQ ID NO: 52
SEQ ID NO: 54
SEQ ID NO: 108
SEQ ID NO: 111
SEQ ID NO: 112
0.1mpk IV Dose

Fig. 26

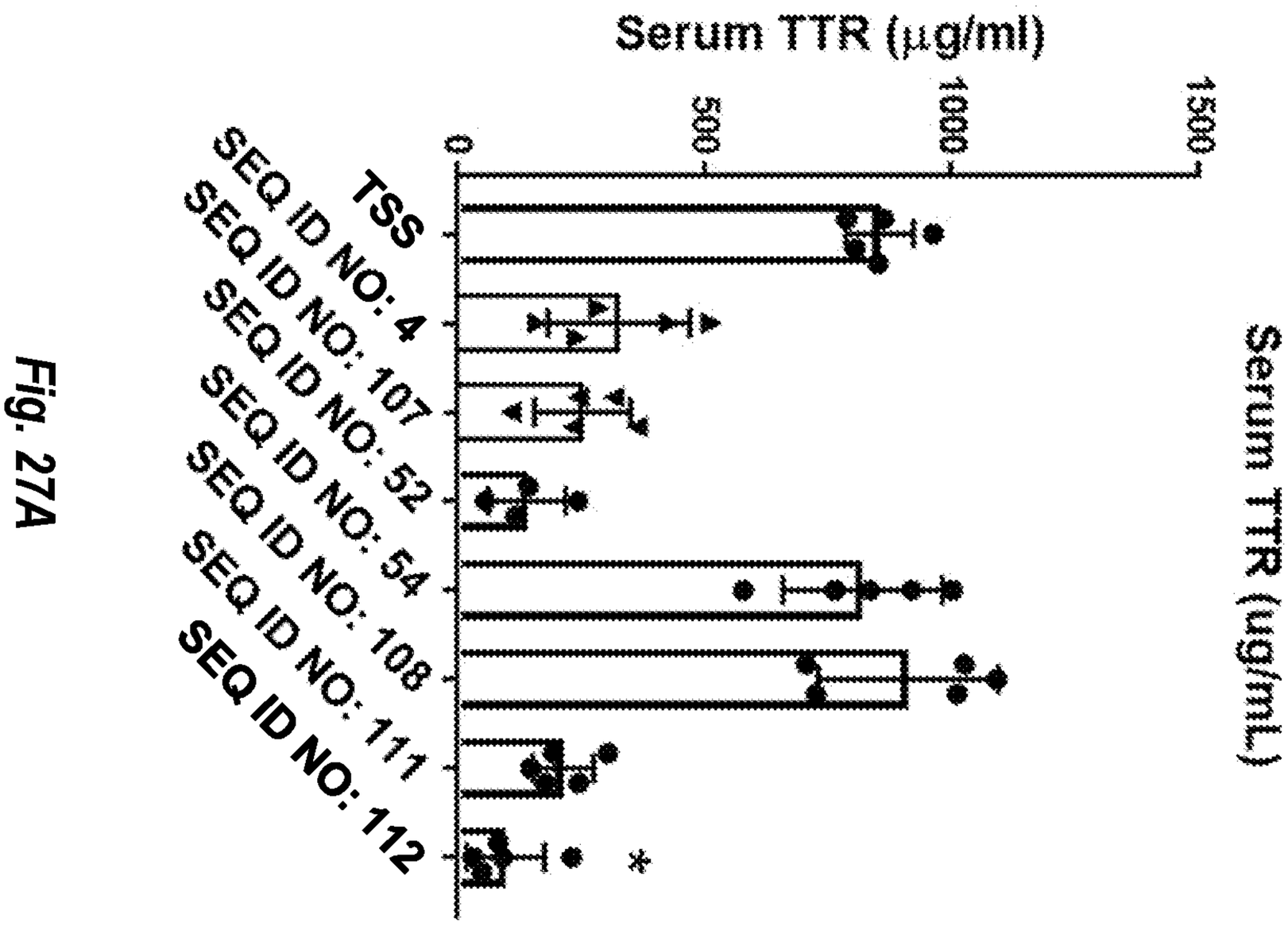

Fig. 27A

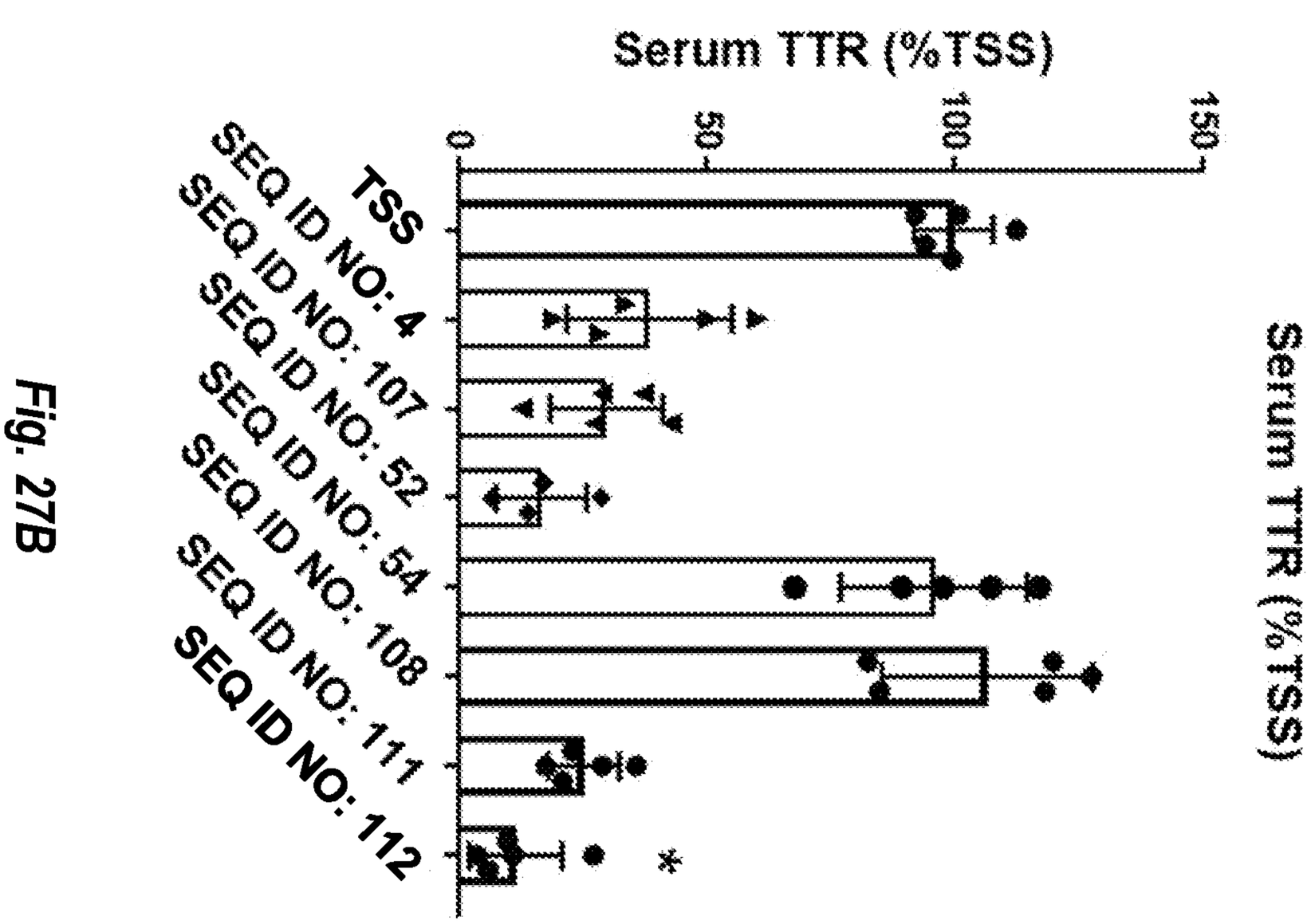

Fig. 27B

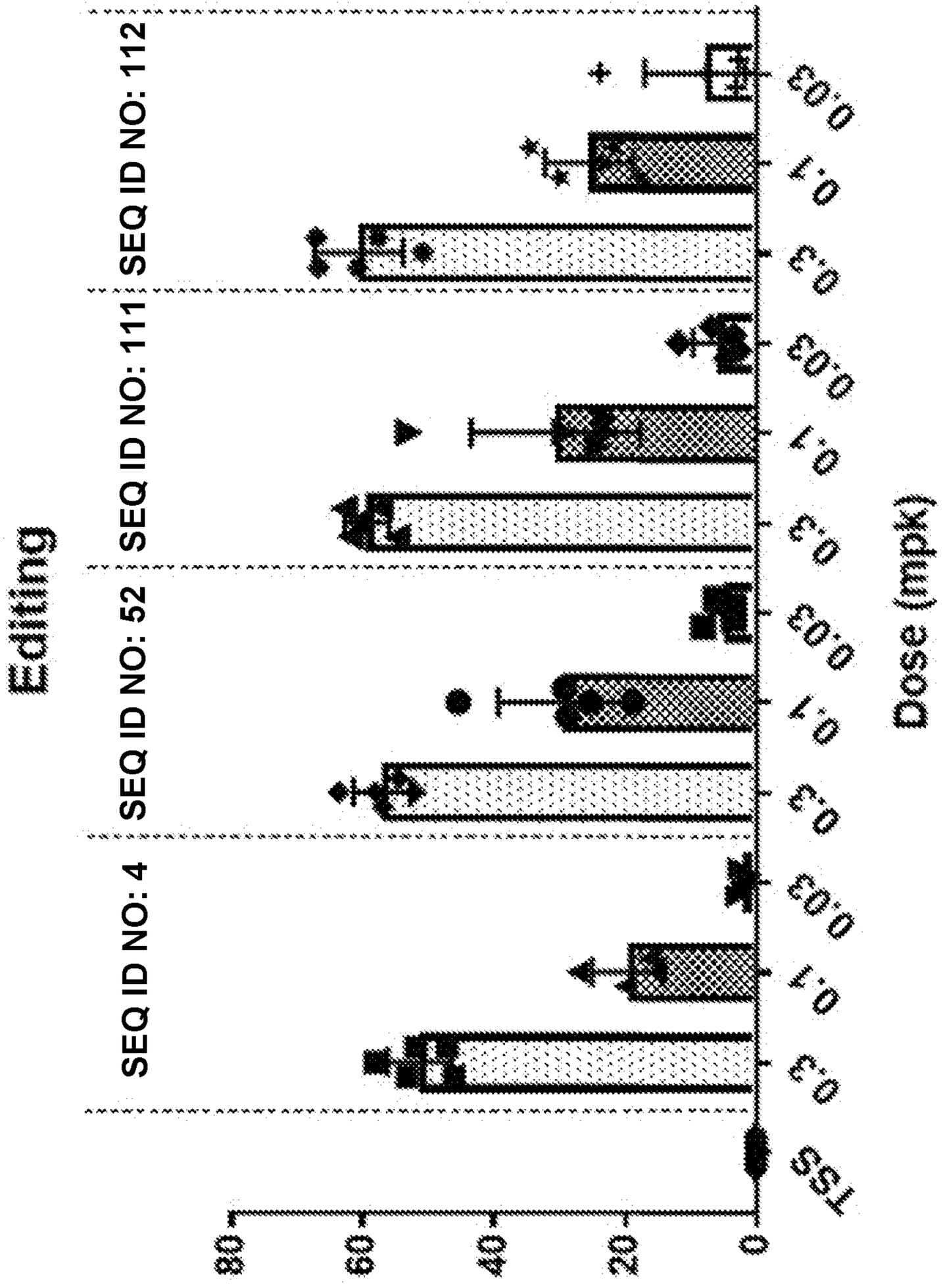
Editing (TTR, liver)
SEQ ID NO: 4
SEQ ID NO: 52
SEQ ID NO: 111
SEQ ID NO: 112
TSS
0.3
0.1
0.03
0.3
0.1
0.03
Dose (mpk)

Fig. 28

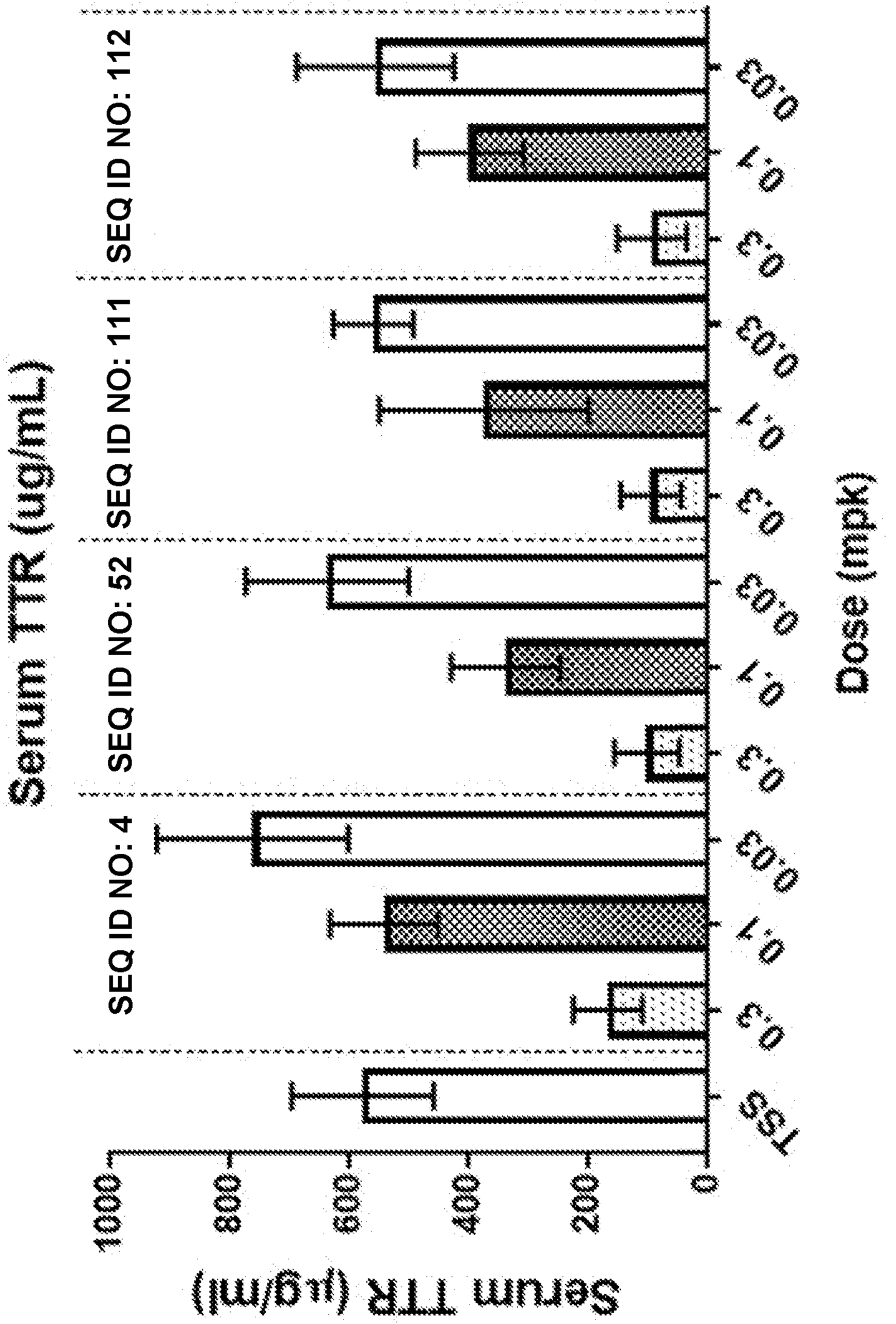
Serum TTR (ug/mL)
SEQ ID NO: 4
SEQ ID NO: 52
SEQ ID NO: 111
SEQ ID NO: 112
TBS
0.3
1.0
3.0
0.3
1.0
3.0
0.3
1.0
3.0
0.3
1.0
3.0
Dose (mpk)
0
200
400
600
800
1000
Serum TTR (μg/ml)

Fig. 29A

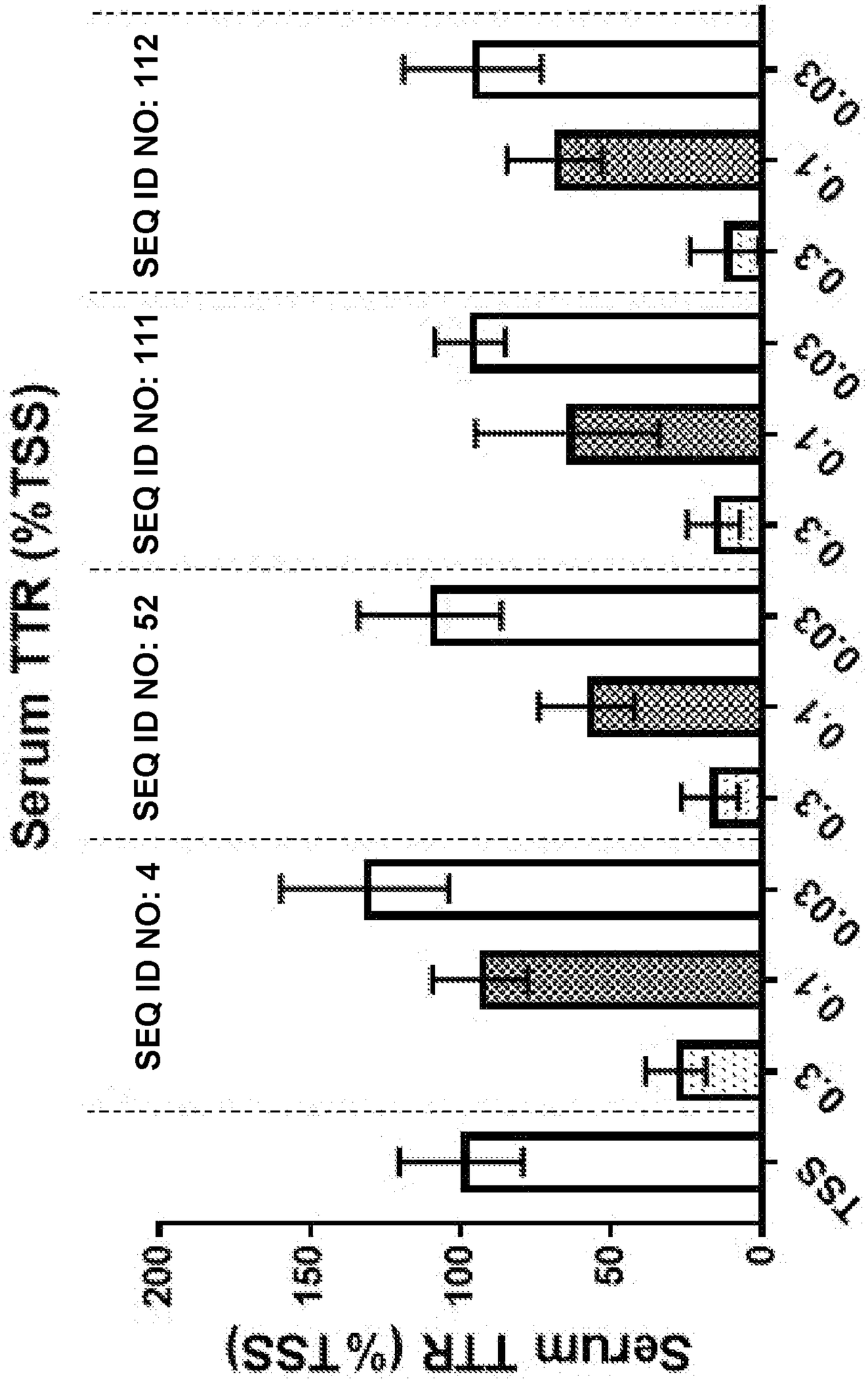
Serum TTR (%TSS)
200
150
100
50
0
TSS
0.3
0.1
0.03
SEQ ID NO: 4
SEQ ID NO: 52
SEQ ID NO: 111
SEQ ID NO: 112
Dose (mpk)

Fig. 29B

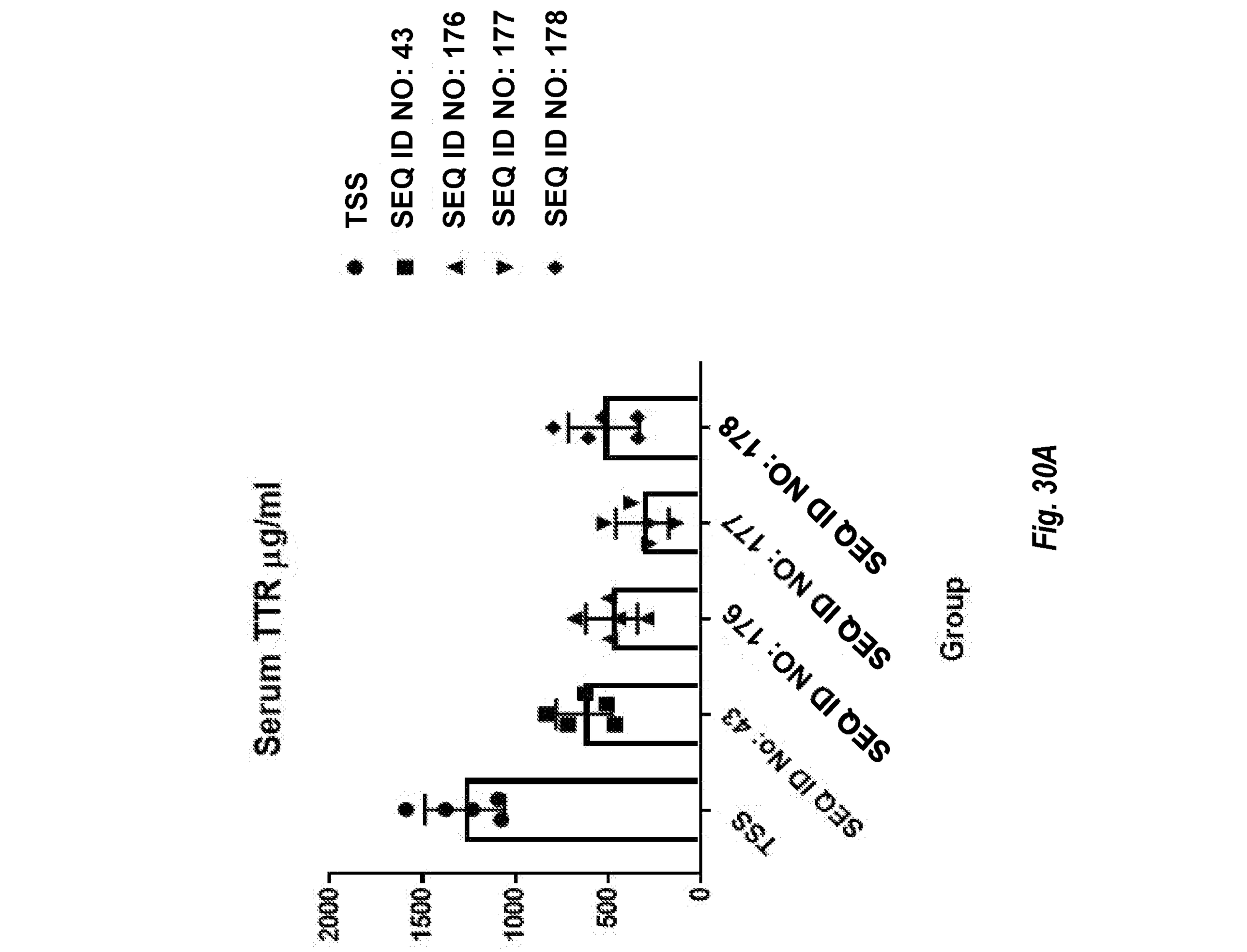
TSS
SEQ ID NO: 43
SEQ ID NO: 176
SEQ ID NO: 177
SEQ ID NO: 178
Serum TTR µg/ml
2000
1500
1000
500
0
TSS
SEQ ID No: 43
SEQ ID NO: 176
SEQ ID NO: 177
SEQ ID NO: 178
Group
Serum TTR ug/ml

Fig. 30A

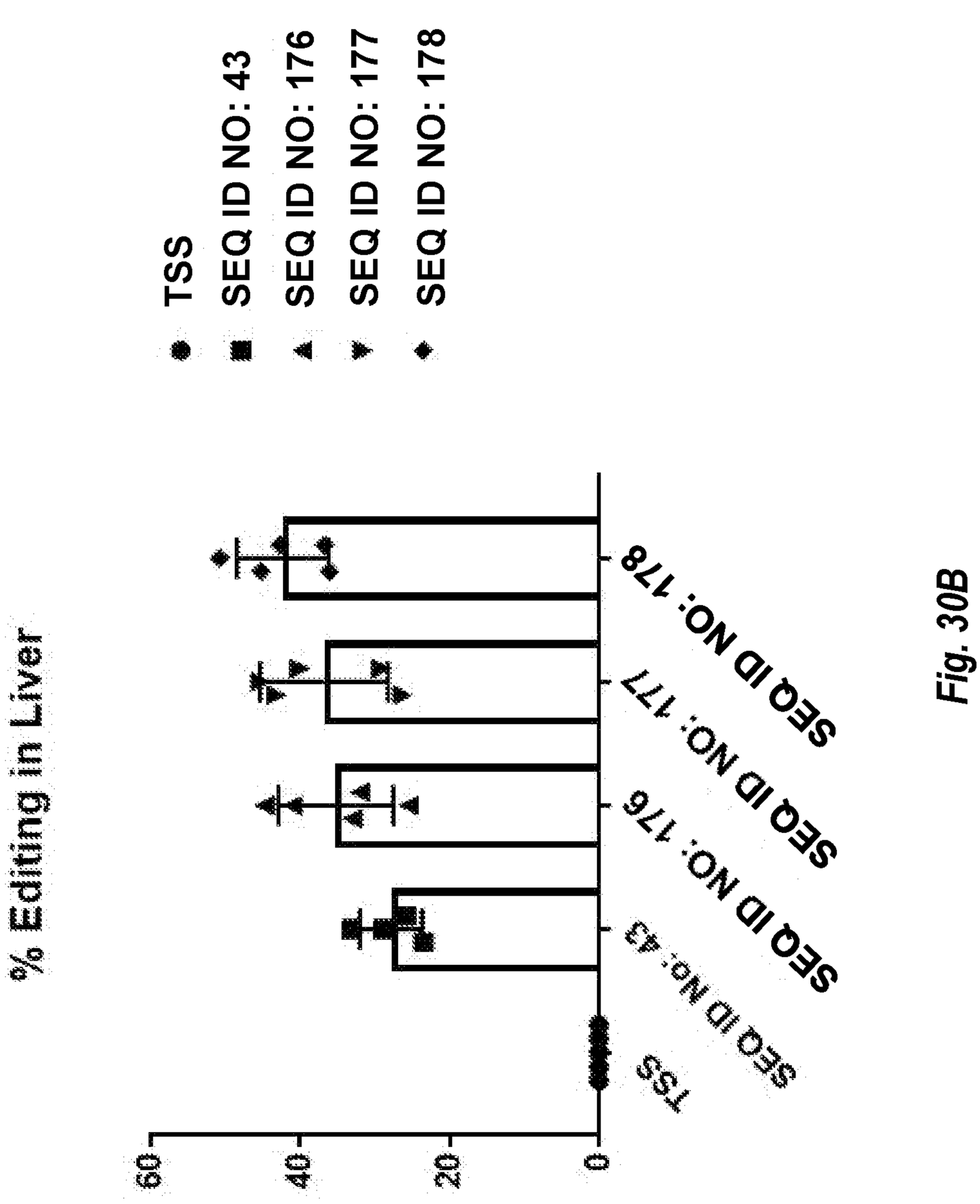
TSS
SEQ ID NO: 43
SEQ ID NO: 176
SEQ ID NO: 177
SEQ ID NO: 178
% Editing in Liver
60
40
20
0
%Editing
TSS
SEQ ID No: 43
SEQ ID NO: 176
SEQ ID NO: 177
SEQ ID NO: 178

Fig. 30B

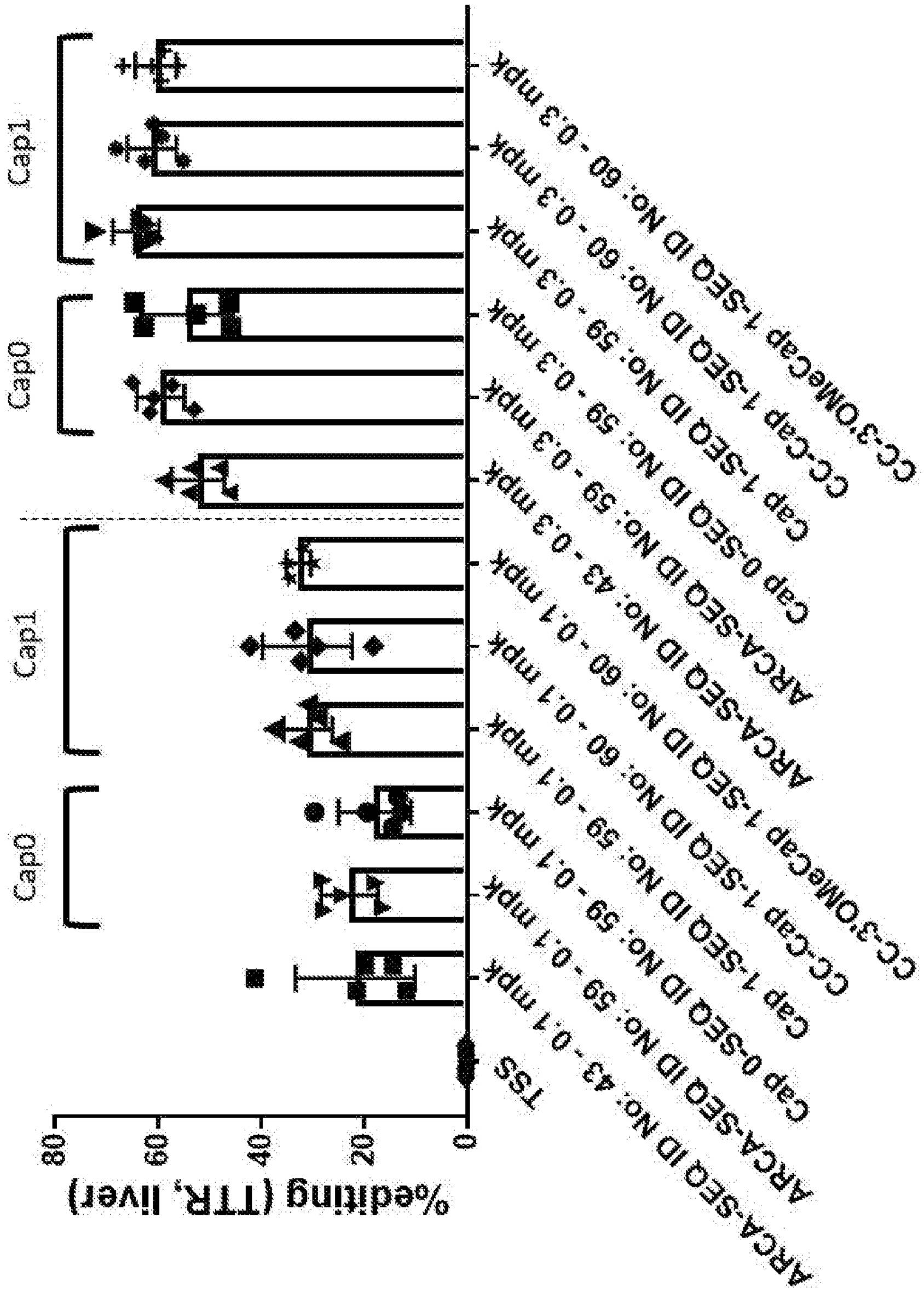
%editing (TTR, liver)
0
20
40
60
80
TSS
ARCA-SEQ ID No: 43 - 0.1 mpk
CC-3'OMeCap 1-SEQ ID No: 43 - 0.1 mpk
Cap 0-SEQ ID No: 43 - 0.1 mpk
CC-Cap 1-SEQ ID No: 43 - 0.1 mpk
ARCA-SEQ ID No: 59 - 0.1 mpk
CC-3'OMeCap 1-SEQ ID No: 59 - 0.1 mpk
Cap 0-SEQ ID No: 59 - 0.1 mpk
CC-Cap 1-SEQ ID No: 59 - 0.1 mpk
ARCA-SEQ ID No: 60 - 0.1 mpk
CC-3'OMeCap 1-SEQ ID No: 60 - 0.1 mpk
Cap 0-SEQ ID No: 60 - 0.1 mpk
CC-Cap 1-SEQ ID No: 60 - 0.1 mpk
ARCA-SEQ ID No: 43 - 0.3 mpk
CC-3'OMeCap 1-SEQ ID No: 43 - 0.3 mpk
Cap 0-SEQ ID No: 43 - 0.3 mpk
CC-Cap 1-SEQ ID No: 43 - 0.3 mpk
ARCA-SEQ ID No: 59 - 0.3 mpk
CC-3'OMeCap 1-SEQ ID No: 59 - 0.3 mpk
Cap 0-SEQ ID No: 59 - 0.3 mpk
CC-Cap 1-SEQ ID No: 59 - 0.3 mpk
ARCA-SEQ ID No: 60 - 0.3 mpk
CC-3'OMeCap 1-SEQ ID No: 60 - 0.3 mpk
Cap0
Cap1
Cap0
Cap1

Fig. 31

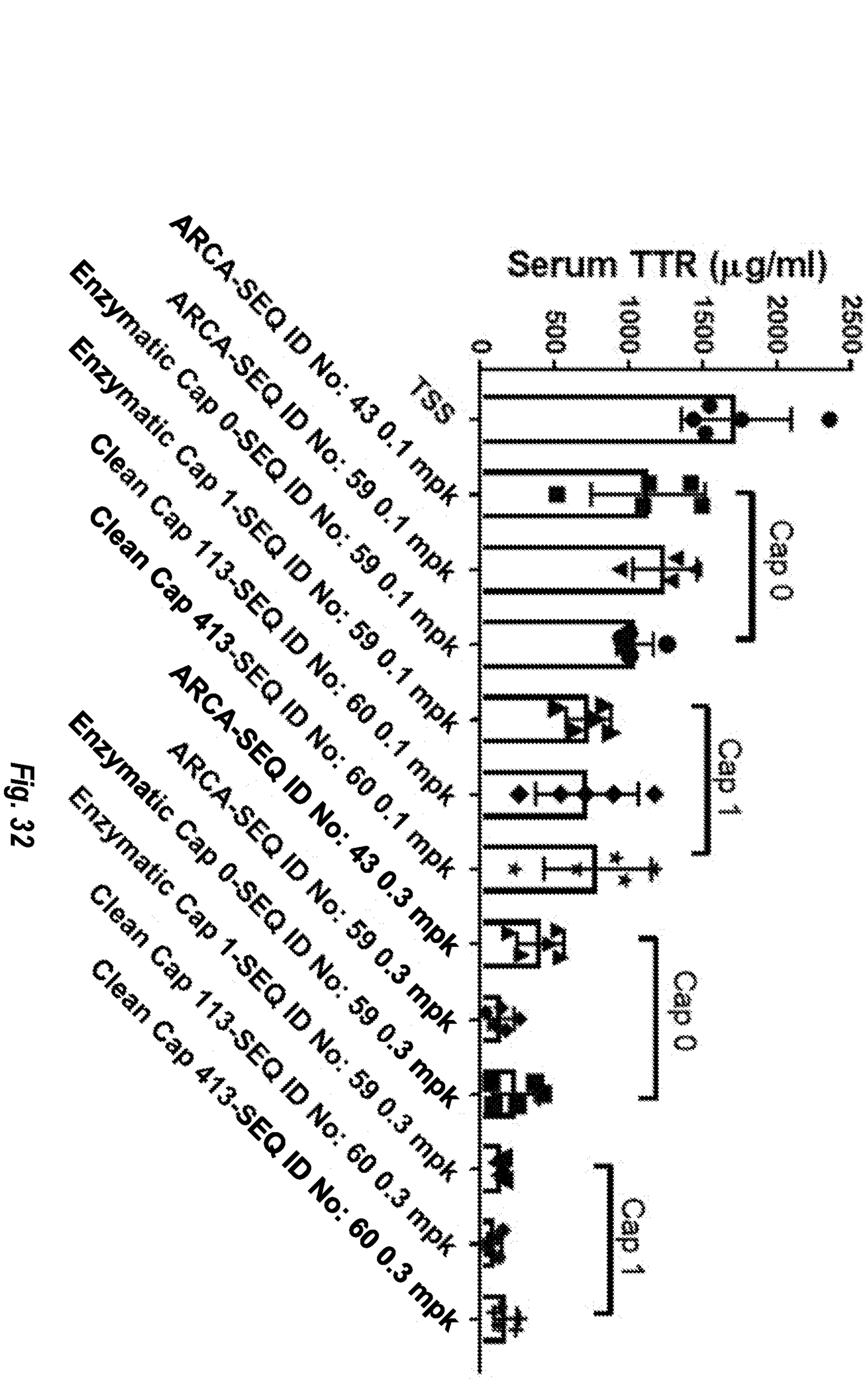
Serum TTR (µg/ml)
0
500
1000
1500
2000
2500
TSS
Cap 0
Cap 1
ARCA-SEQ ID No: 43 0.1 mpk
ARCA-SEQ ID No: 59 0.1 mpk
Enzymatic Cap 0-SEQ ID No: 59 0.1 mpk
Enzymatic Cap 1-SEQ ID No: 59 0.1 mpk
Clean Cap 113-SEQ ID No: 60 0.1 mpk
Clean Cap 413-SEQ ID No: 60 0.1 mpk
ARCA-SEQ ID No: 43 0.3 mpk
ARCA-SEQ ID No: 59 0.3 mpk
Enzymatic Cap 0-SEQ ID No: 59 0.3 mpk
Enzymatic Cap 1-SEQ ID No: 59 0.3 mpk
Clean Cap 113-SEQ ID No: 60 0.3 mpk
Clean Cap 413-SEQ ID No: 60 0.3 mpk

Fig. 32

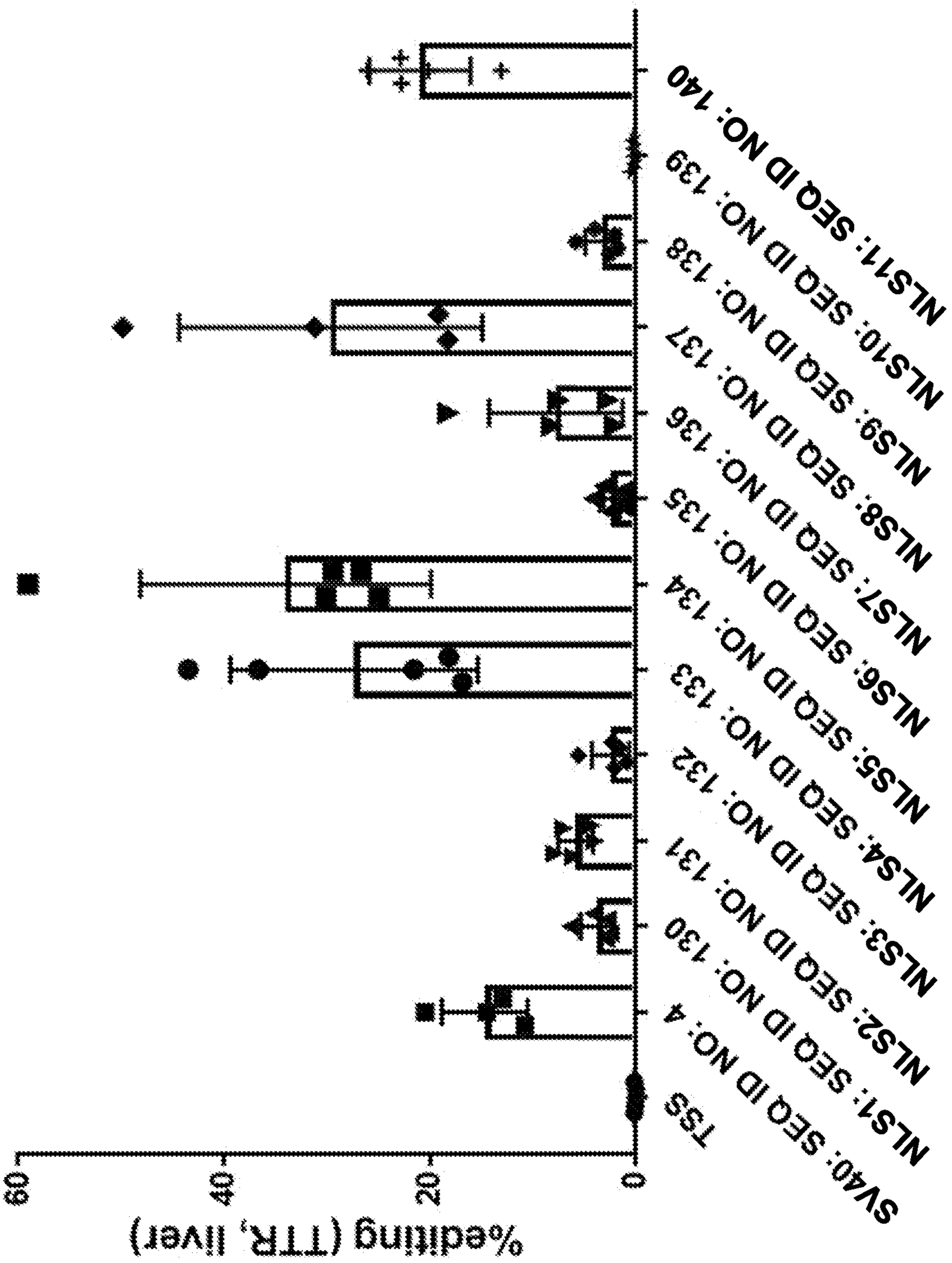
%editing (TTR, liver)
0
20
40
60
TSS
SV40: SD
NLS1: SEQ ID NO: 4
NLS2: SEQ ID NO: 130
NLS3: SEQ ID NO: 131
NLS4: SEQ ID NO: 132
NLS5: SEQ ID NO: 133
NLS6: SEQ ID NO: 134
NLS7: SEQ ID NO: 135
NLS8: SEQ ID NO: 136
NLS9: SEQ ID NO: 137
NLS10: SEQ ID NO: 138
NLS11: SEQ ID NO: 139
NLS11: SEQ ID NO: 140

Fig. 33

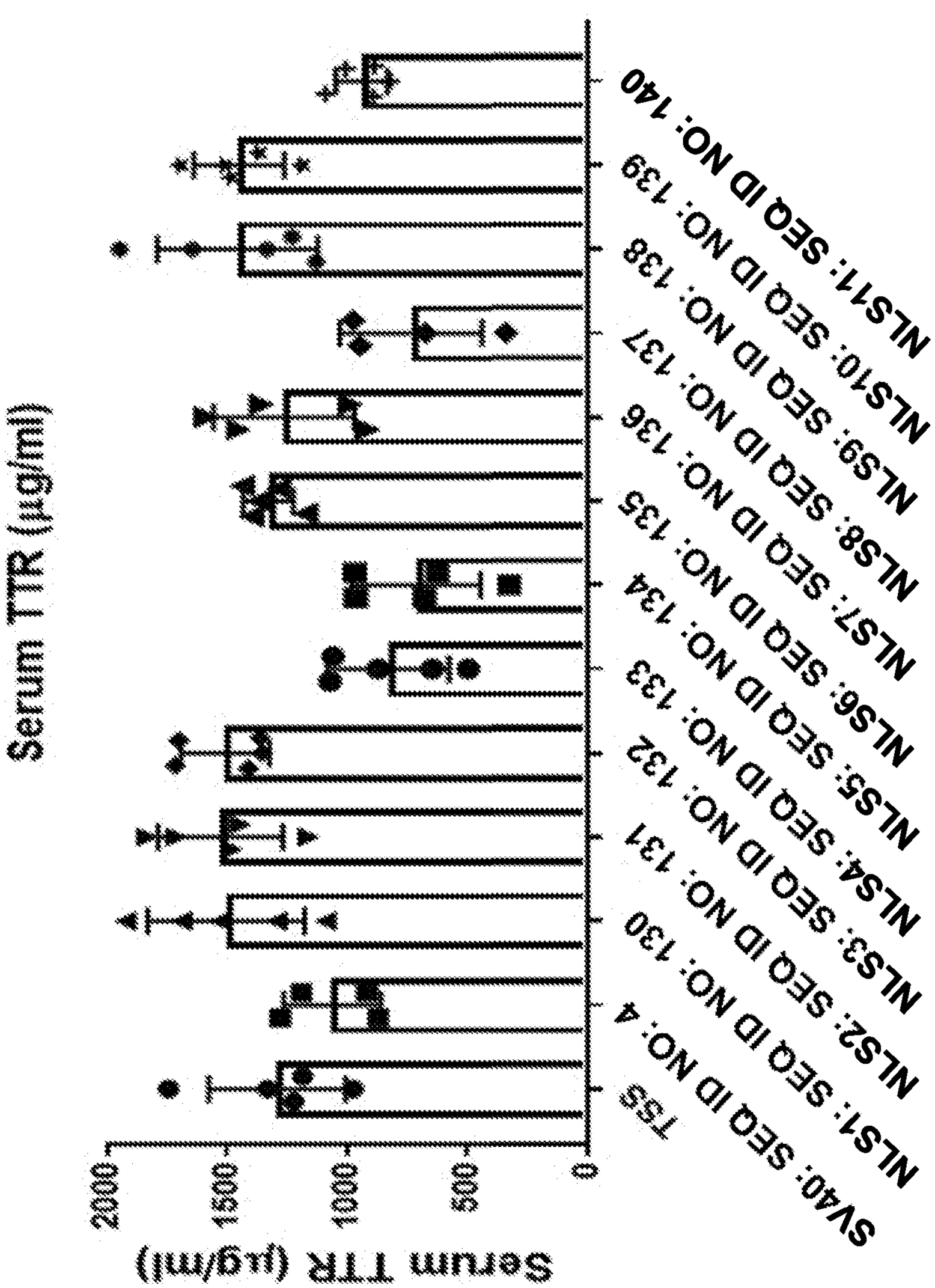
Serum TTR (μg/ml)
0
500
1000
1500
2000
TSS
SVA40: SEQ ID NO: 4
NLS1: SEQ ID NO: 130
NLS2: SEQ ID NO: 131
NLS3: SEQ ID NO: 132
NLS4: SEQ ID NO: 133
NLS5: SEQ ID NO: 134
NLS6: SEQ ID NO: 135
NLS7: SEQ ID NO: 136
NLS8: SEQ ID NO: 137
NLS9: SEQ ID NO: 138
NLS10: SEQ ID NO: 139
NLS11: SEQ ID NO: 140

Fig. 34A

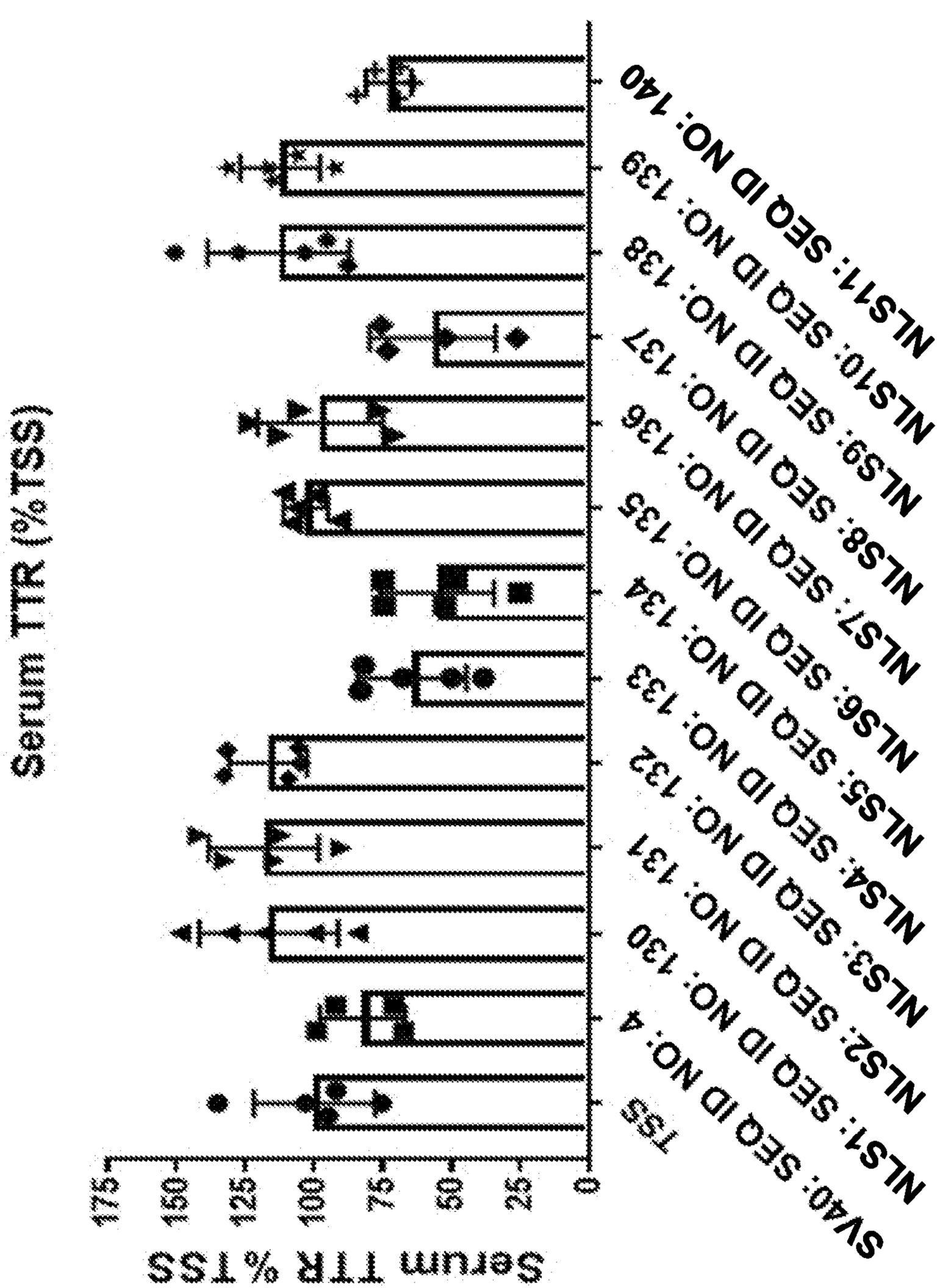
Serum TTR (%TSS)
175
150
125
100
75
50
25
0
TSS
SVA40: SEQ ID NO: 4
NLS1: SEQ ID NO: 130
NLS2: SEQ ID NO: 131
NLS3: SEQ ID NO: 132
NLS4: SEQ ID NO: 133
NLS5: SEQ ID NO: 134
NLS6: SEQ ID NO: 135
NLS7: SEQ ID NO: 136
NLS8: SEQ ID NO: 137
NLS9: SEQ ID NO: 138
NLS10: SEQ ID NO: 139
NLS11: SEQ ID NO: 140

Fig. 34B

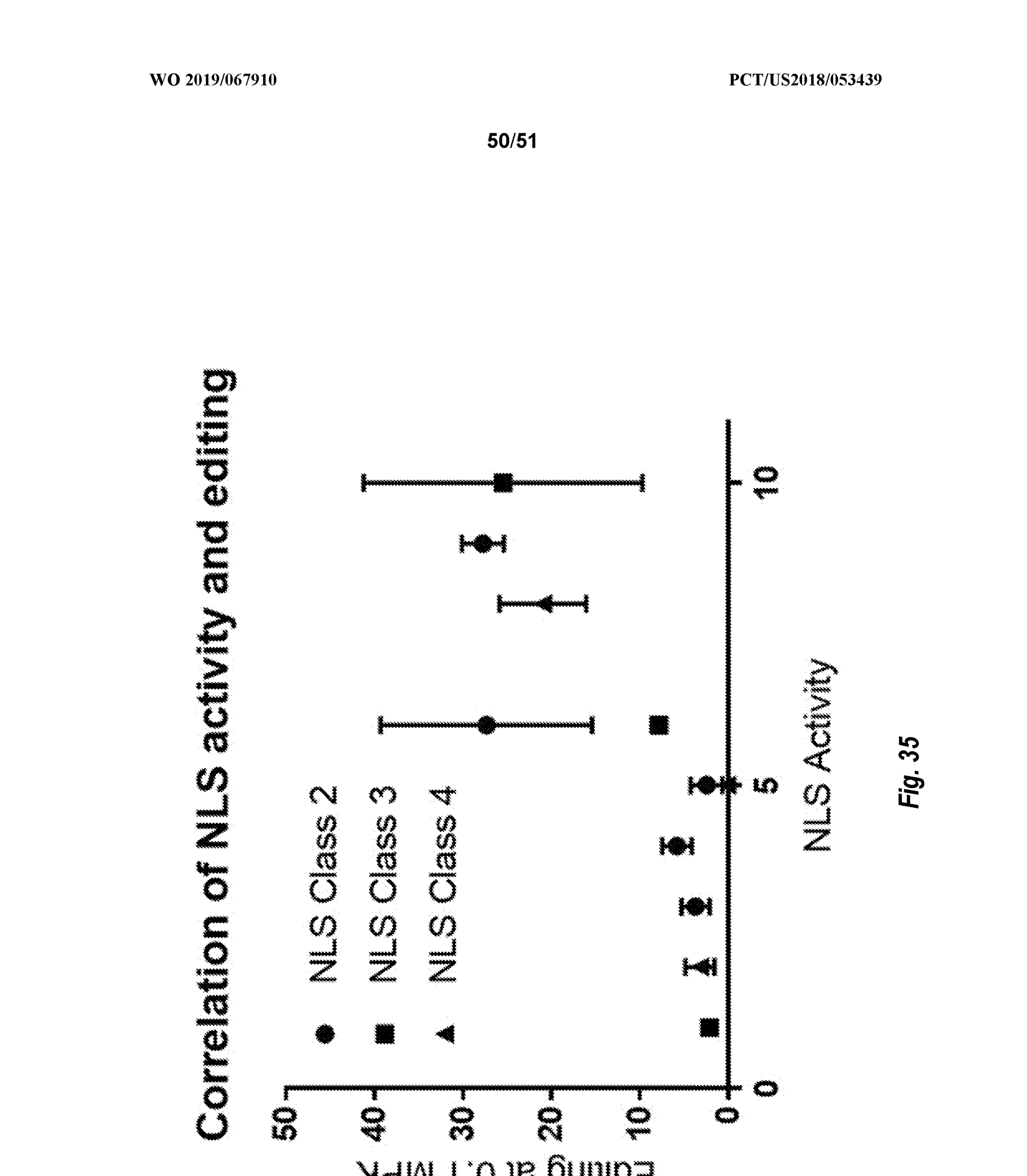
Correlation of NLS activity and editing
NLS Class 2
NLS Class 3
NLS Class 4
Editing at 0.1 MPK
NLS Activity
0
5
10
0
10
20
30
40
50

Fig. 35

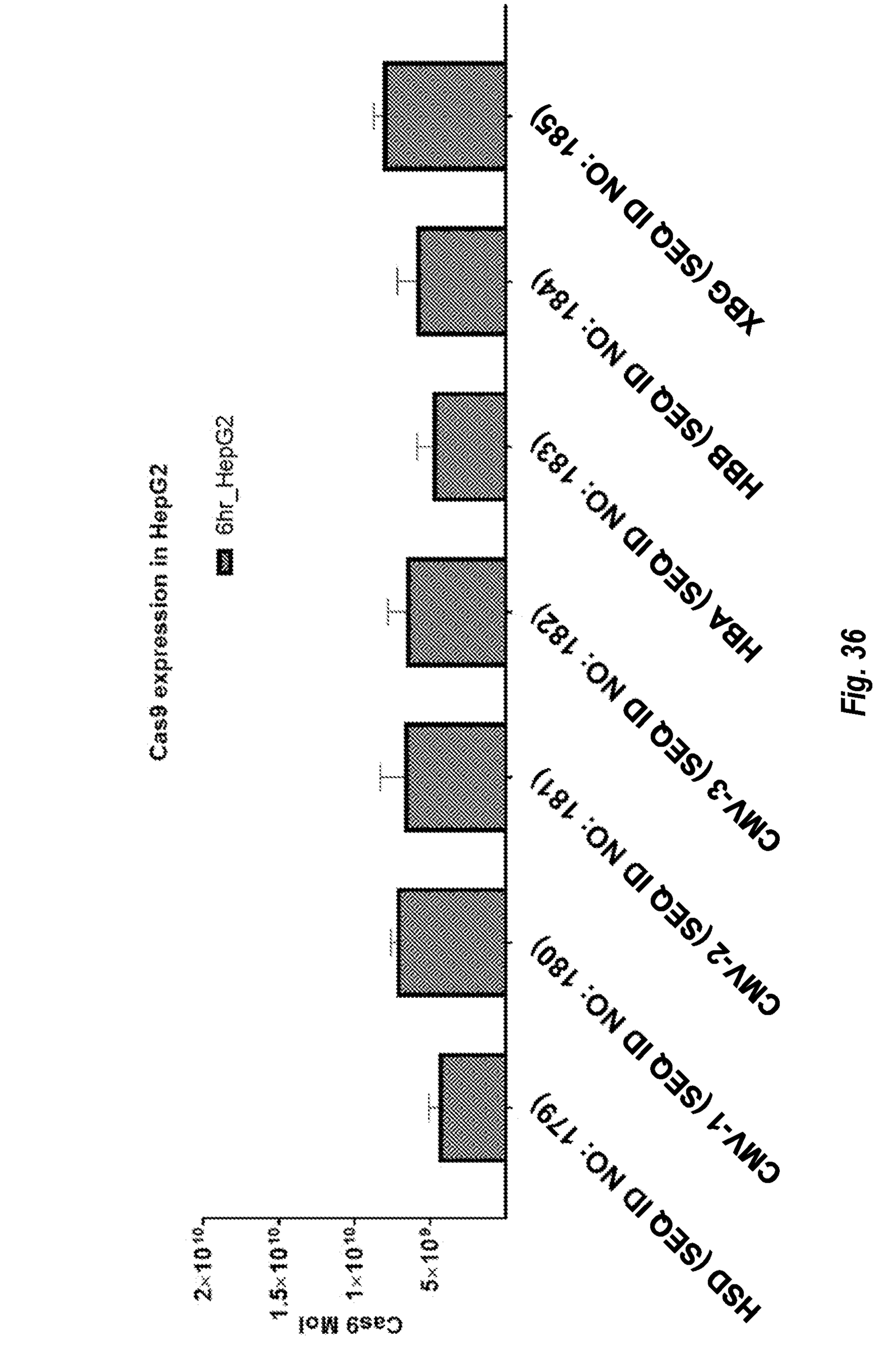
Cas9 expression in HepG2
6hr_HepG2
Cas9 MoI
2×10^10
1.5×10^10
1×10^10
5×10^9
HSD (SEQ ID NO: 179)
CMV-1 (SEQ ID NO: 180)
CMV-2 (SEQ ID NO: 181)
CMV-3 (SEQ ID NO: 183)
HBA (SEQ ID NO: 182)
HBB (SEQ ID NO: 184)
XBG (SEQ ID NO: 185)

Fig. 36