Figure 1

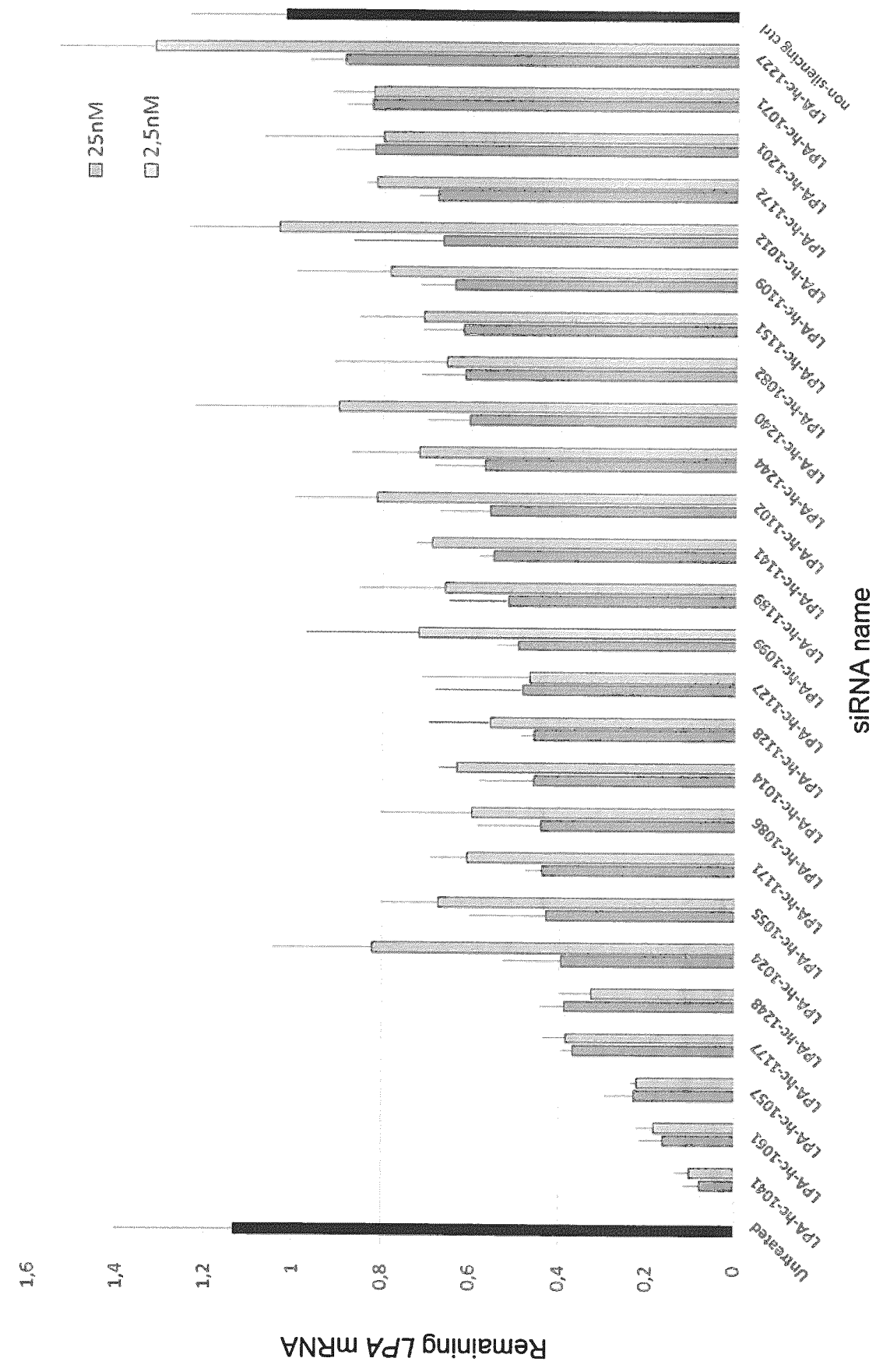

25nM
2,5nM
Remaining LPA mRNA
0
0,2
0,4
0,6
0,8
1
1,2
1,4
1,6
siRNA name
Untreated
LPA-hc-1041
LPA-hc-1061
LPA-hc-1057
LPA-hc-1177
LPA-hc-1248
LPA-hc-1024
LPA-hc-1055
LPA-hc-1171
LPA-hc-1086
LPA-hc-1014
LPA-hc-1128
LPA-hc-1127
LPA-hc-1099
LPA-hc-1189
LPA-hc-1141
LPA-hc-1102
LPA-hc-1244
LPA-hc-1240
LPA-hc-1082
LPA-hc-1151
LPA-hc-1109
LPA-hc-1012
LPA-hc-1172
LPA-hc-1201
LPA-hc-1071
LPA-hc-1227
non-silencing ctrl

Figure 2A

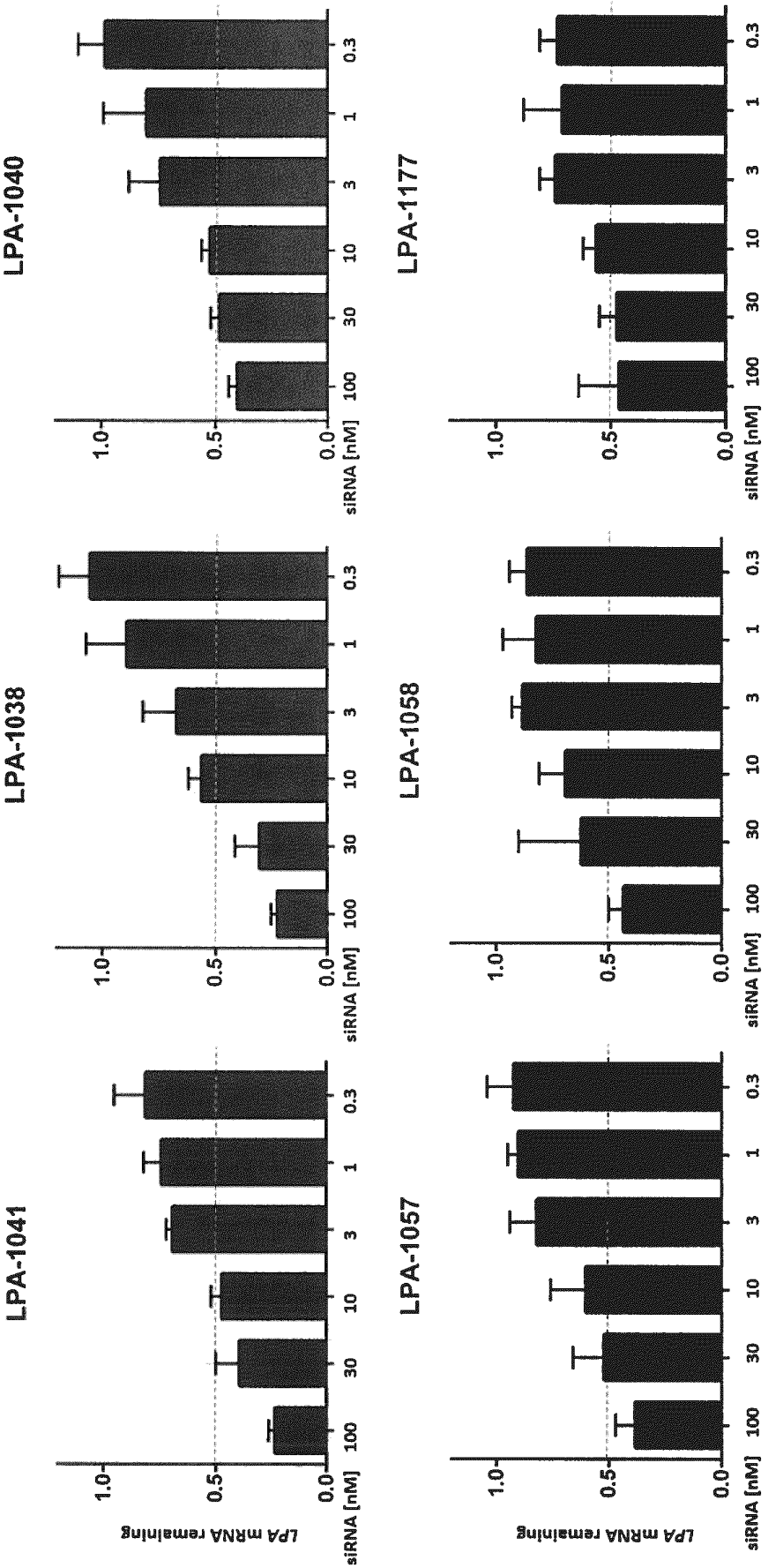

LPA-1040
LPA-1038
LPA-1041
LPA-1177
LPA-1058
LPA-1057
LPA mRNA remaining
siRNA [nM]
1.0
0.5
0.0
100
30
10
3
1
0.3

Figure 2B

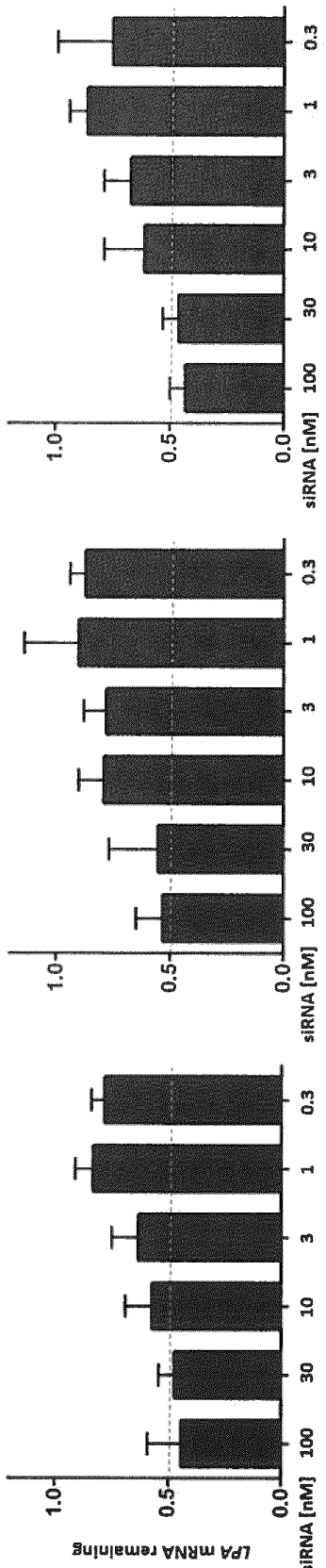
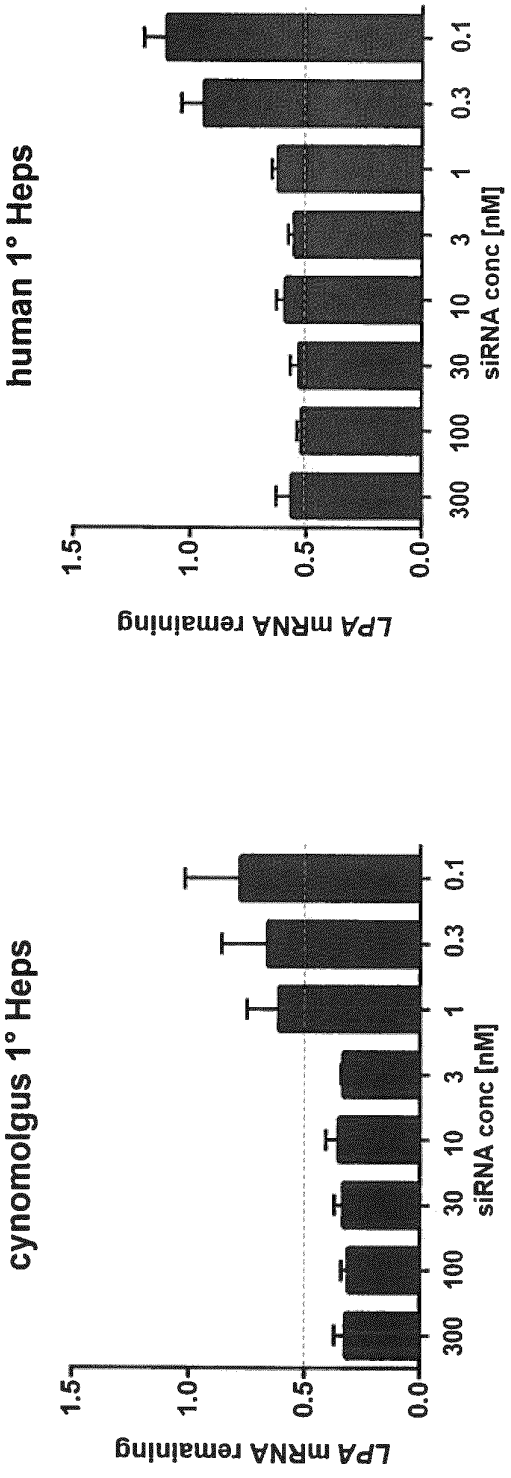


Figure 3



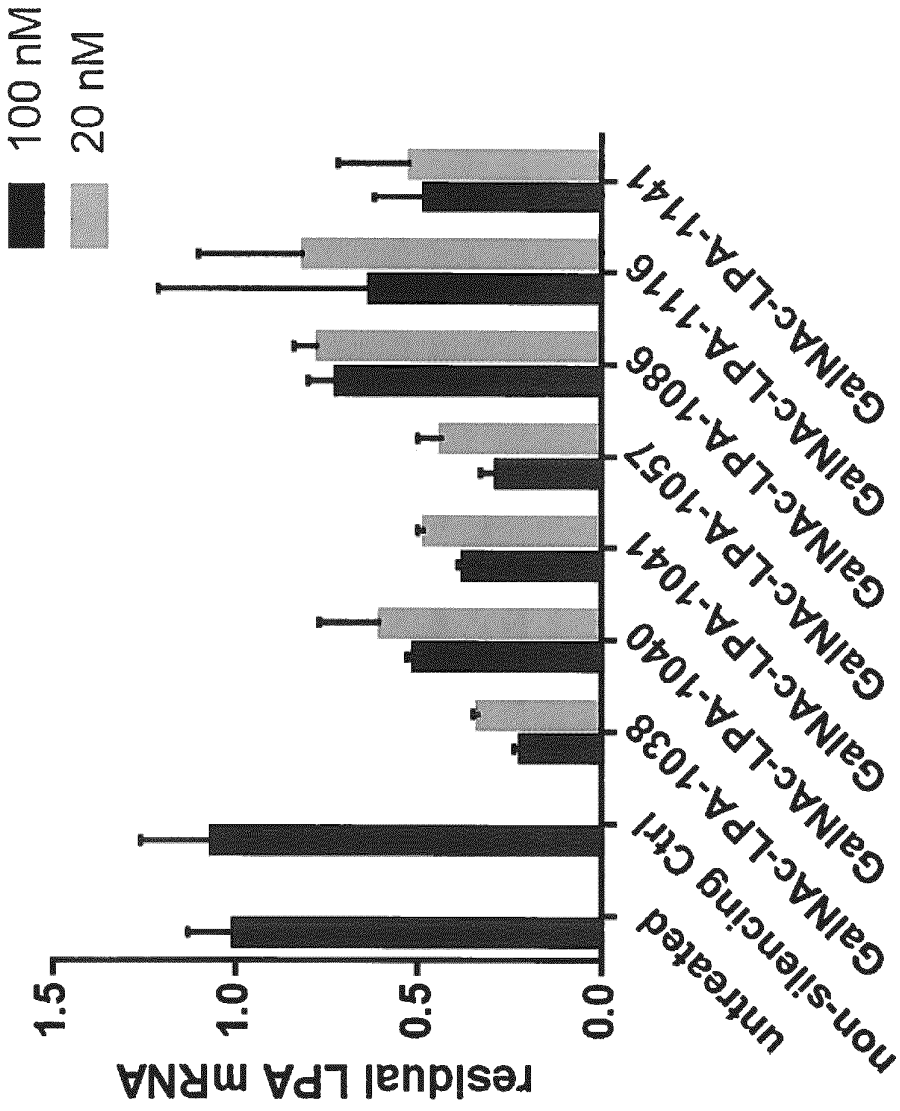

100 nM
20 nM
residual LPA mRNA
1.5
1.0
0.5
0.0
untreated
non-silencing Ctrl
GalNAc-LPA-1038
GalNAc-LPA-1040
GalNAc-LPA-1041
GalNAc-LPA-1057
GalNAc-LPA-1086
GalNAc-LPA-1116
GalNAc-LPA-1141

Figure 4

Figure 5

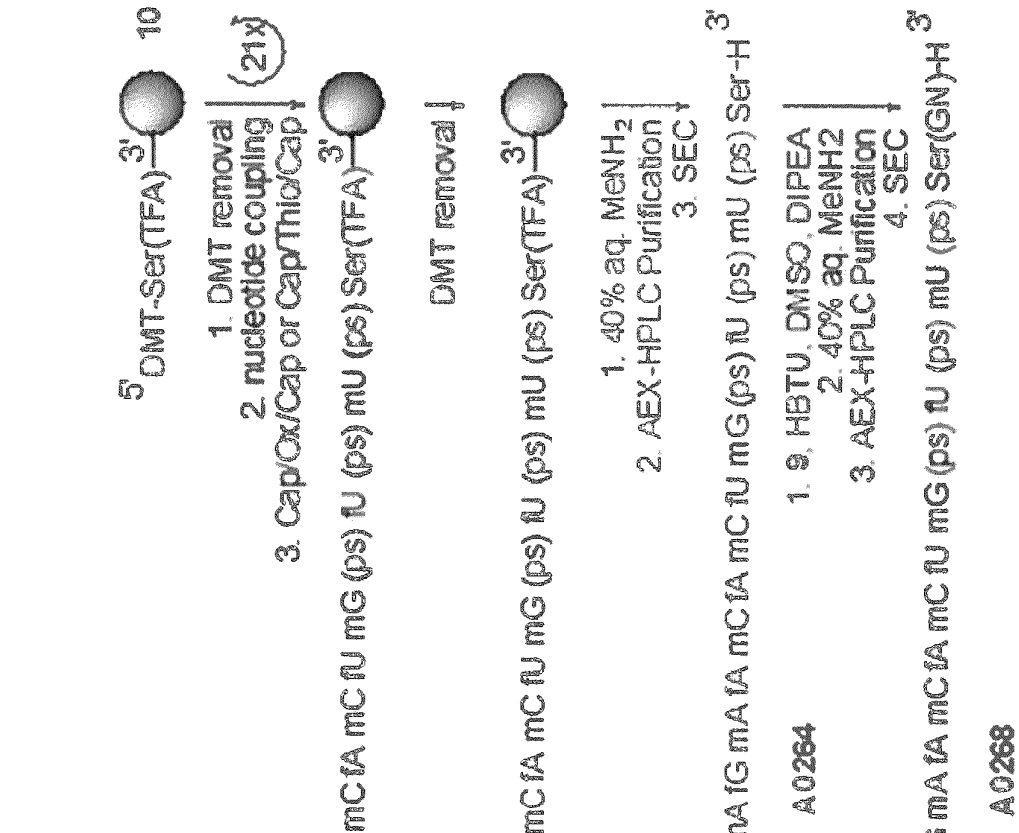

5' DMT-Ser(TFA) 3' 10
1. DMT removal
2. nucleotide coupling
3. Cap/Cap or Cap/Thio/Cap
21x
5' DMT-mU (ps) fU (ps) mA fU mA fG mA fG mC fA mA fG mA fA mC fA mC fU mG (ps) fU (ps) mU (ps) Ser(TFA) 3'
DMT removal
5' mU (ps) fU (ps) mA fU mA fG mA fG mC fA mA fG mA fA mC fA mC fU mG (ps) fU (ps) mU (ps) Ser(TFA) 3'
1. 40% aq. MeNH2
2. AEX-HPLC Purification
3. SEC
5' mU (ps) fU (ps) mA fU mA fG mA fG mC fA mA fG mA fA mC fA mC fU mG (ps) fU (ps) mU (ps) Ser-H 3'
A 0264
1. 9, HBTU, DMSO, DIPEA
2. 40% aq. MeNH2
3. AEX-HPLC Purification
4. SEC
5' mU (ps) fU (ps) mA fU mA fG mA fG mC fA mA fG mA fA mC fA mC fU mG (ps) fU (ps) mU (ps) Ser(GN)-H 3'
A 0268

Figure 6

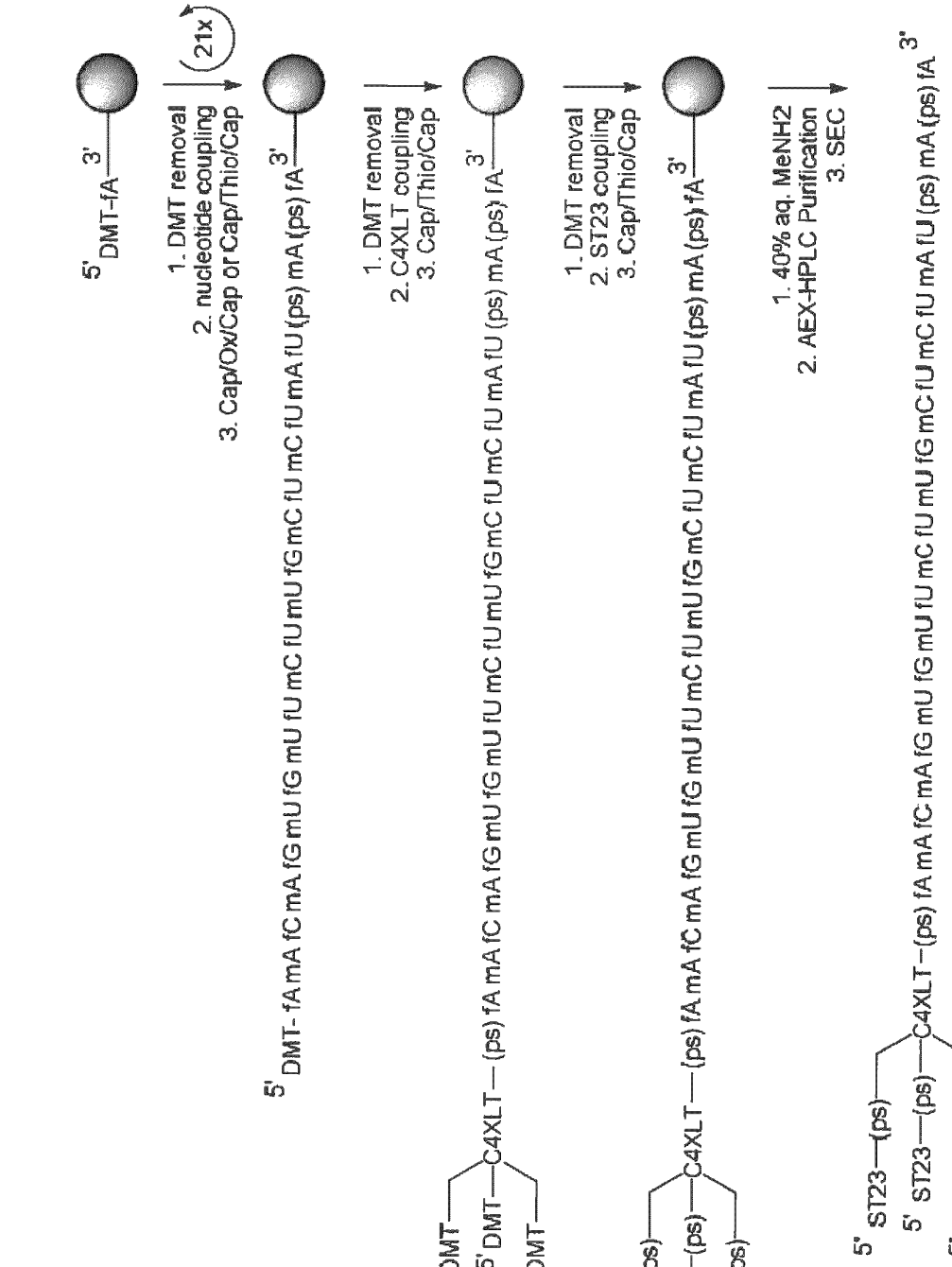

5' DMT-fA 3'
1. DMT removal
2. nucleotide coupling
3. Cap/Ox/Cap or Cap/Thio/Cap
21x
5' DMT- fA mA fC mA fG mU fG mU fU mC fU mU fG mC fU mC fU mA fU (ps) mA (ps) fA 3'
1. DMT removal
2. C4XLT coupling
3. Cap/Thio/Cap
5' DMT
5' DMT
C4XLT
(ps) fA mA fC mA fG mU fG mU fU mC fU mU fG mC fU mC fU mA fU (ps) mA (ps) fA 3'
5' DMT
1. DMT removal
2. ST23 coupling
3. Cap/Thio/Cap
5' ST23(Ac)3
5' ST23(Ac)3
5' ST23(Ac)3
(ps)
(ps)
(ps)
C4XLT
(ps) fA mA fC mA fG mU fG mU fU mC fU mU fG mC fU mC fU mA fU (ps) mA (ps) fA 3'
1. 40% aq. MeNH2
2. AEX-HPLC Purification
3. SEC
5' ST23
5' ST23
5' ST23
(ps)
(ps)
(ps)
C4XLT
(ps) fA mA fC mA fG mU fG mU fU mC fU mU fG mC fU mC fU mA fU (ps) mA (ps) fA 3'
A0006

Figure 7a

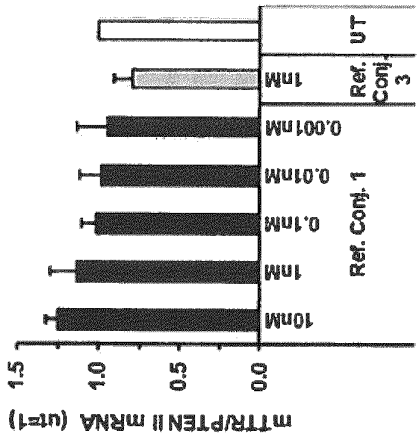


Figure 7b

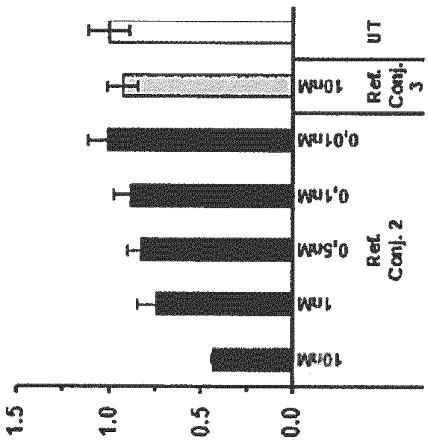


Figure 7c



Figure 8

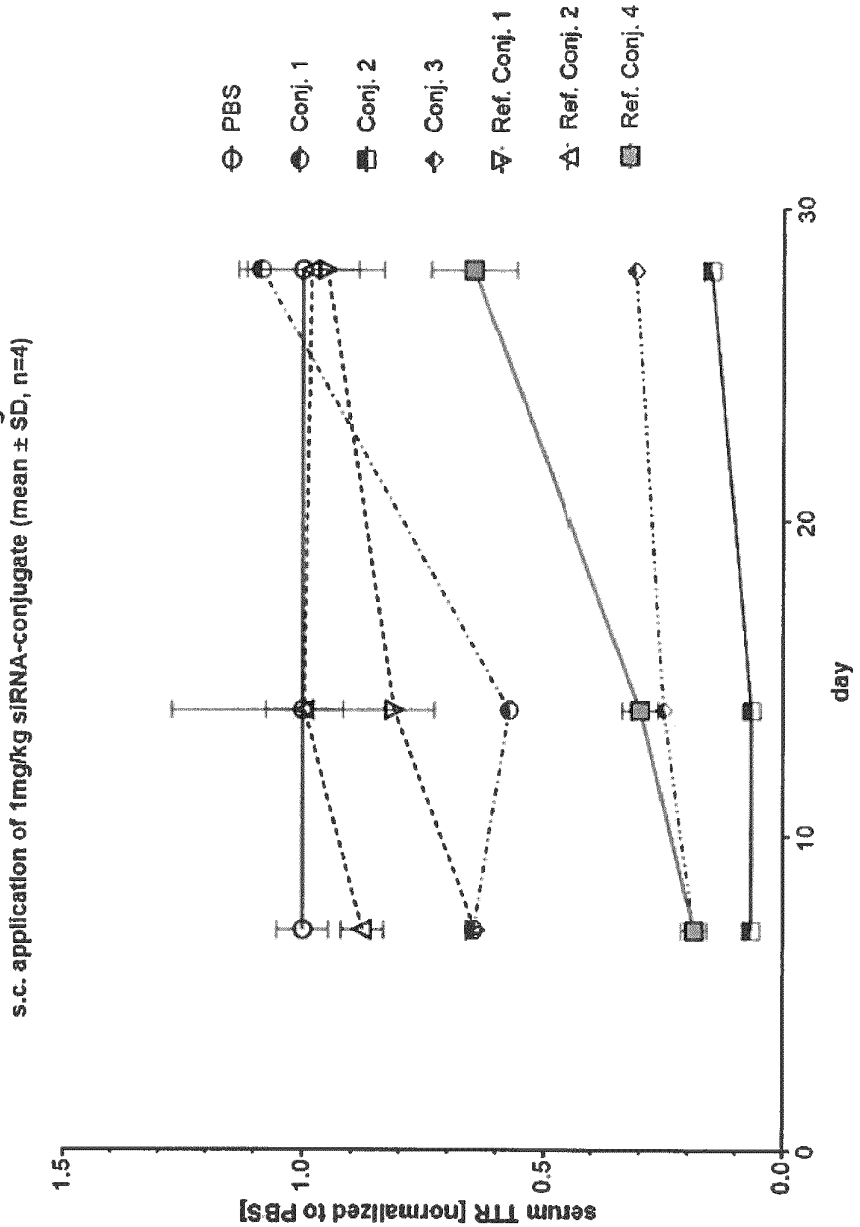

time course of serum TTR in C57BL/6 following
s.c. application of 1mg/kg siRNA-conjugate (mean ± SD, n=4)
serum TTR [normalized to PBS]
day
PBS
Conj. 1
Conj. 2
Conj. 3
Ref. Conj. 1
Ref. Conj. 2
Ref. Conj. 4
0.0
0.5
1.0
1.5
0
10
20
30

Figure 9

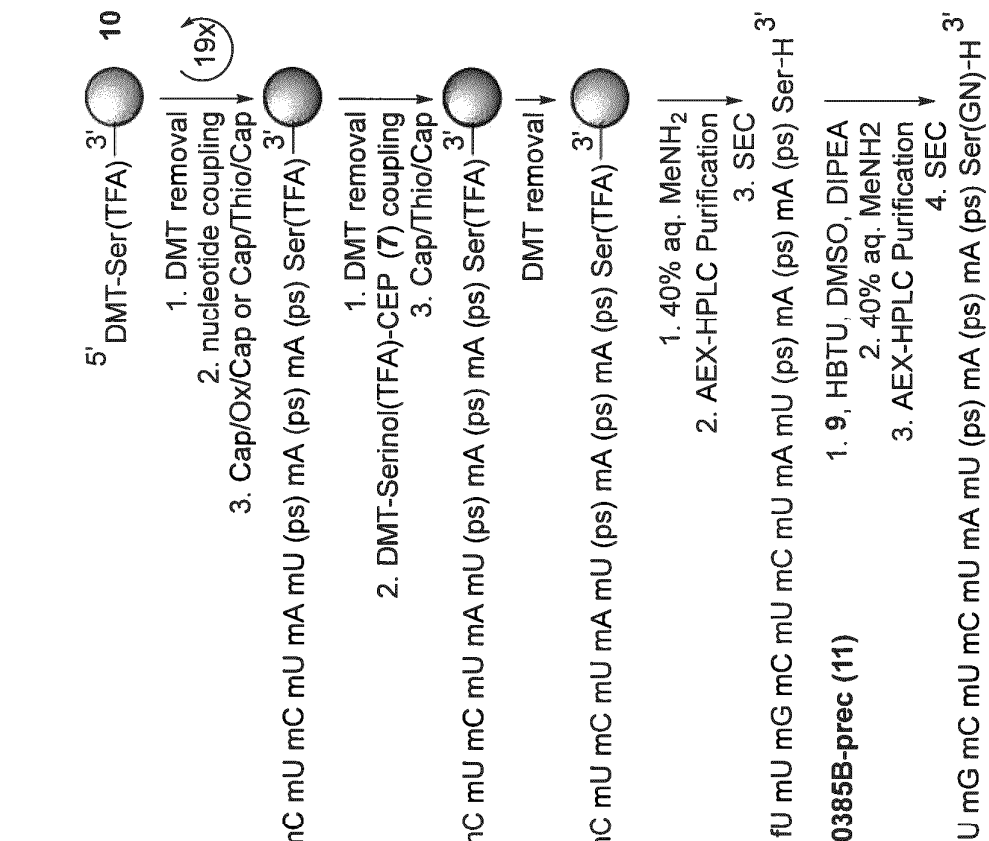
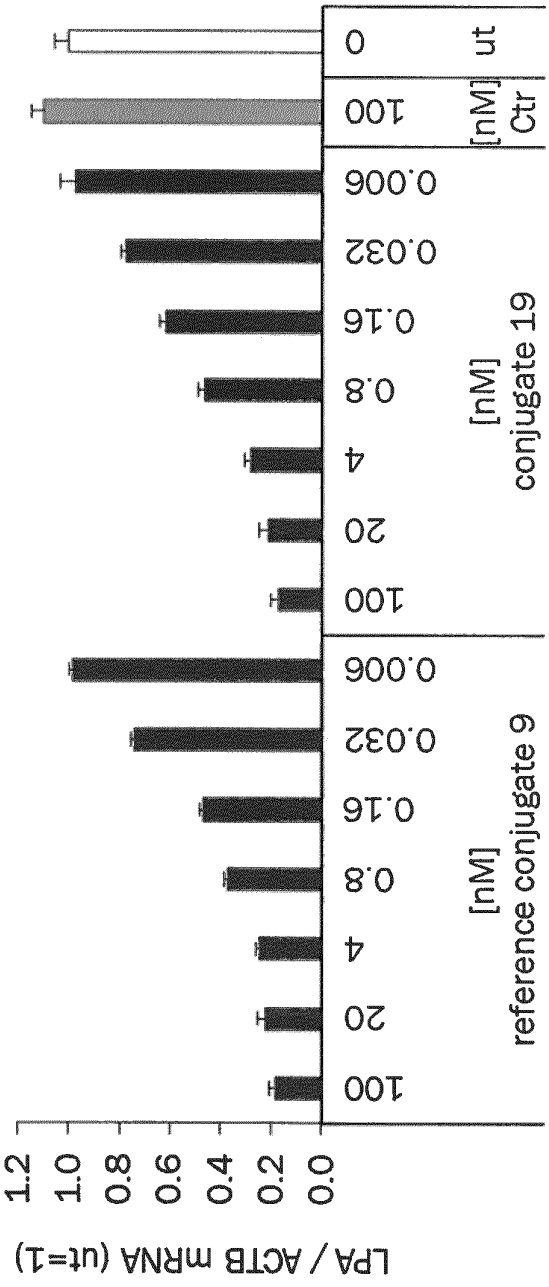

5' DMT-Ser(TFA) 3' 10
1. DMT removal
2. nucleotide coupling
3. Cap/Ox/Cap or Cap/Thio/Cap
19x
5' DMT-mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) Ser(TFA) 3'
1. DMT removal
2. DMT-Serinol(TFA)-CEP (7) coupling
3. Cap/Thio/Cap
5' DMT-Ser(TFA) (ps) mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) Ser(TFA) 3'
DMT removal
5' H-Ser(TFA) (ps) mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) Ser(TFA) 3'
1. 40% aq. MeNH2
2. AEX-HPLC Purification
3. SEC
5' H-Ser (ps) mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) Ser-H 3'
X0385B-prec (11)
1. 9, HBTU, DMSO, DIPEA
2. 40% aq. MeNH2
3. AEX-HPLC Purification
4. SEC
5' H-Ser(GN) (ps) mC (ps) mA (ps) mG mU mG mU fU fC fU mU mG mC mU mC mU mA mU (ps) mA (ps) Ser(GN)-H 3'
X0385B (12)

Figure 10


LPA / ACTB mRNA (ut=1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
100
20
4
0.8
0.16
0.032
0.006
[nM]
reference conjugate 9
100
20
4
0.8
0.16
0.032
0.006
[nM]
conjugate 19
100
[nM]
Ctr
0
ut

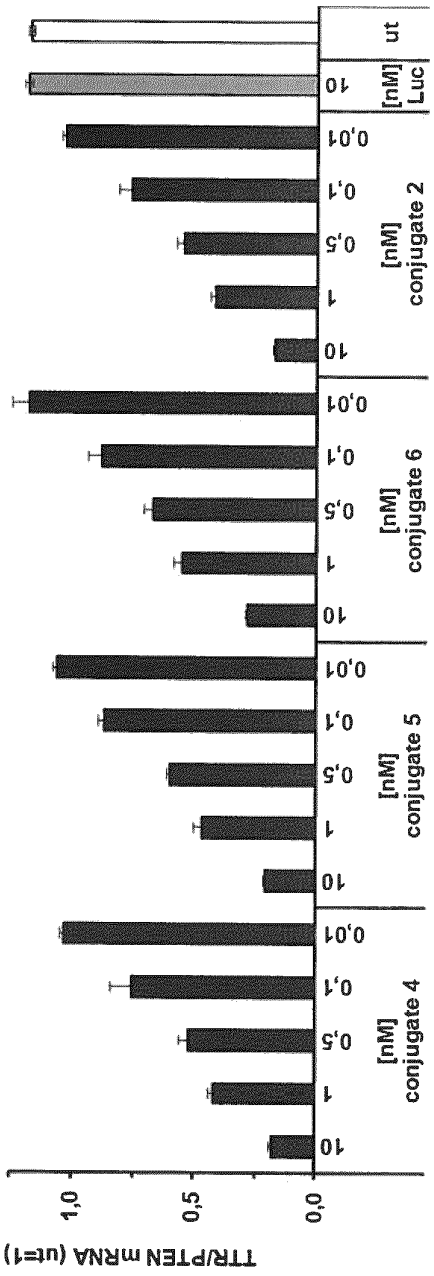


Figure 11A

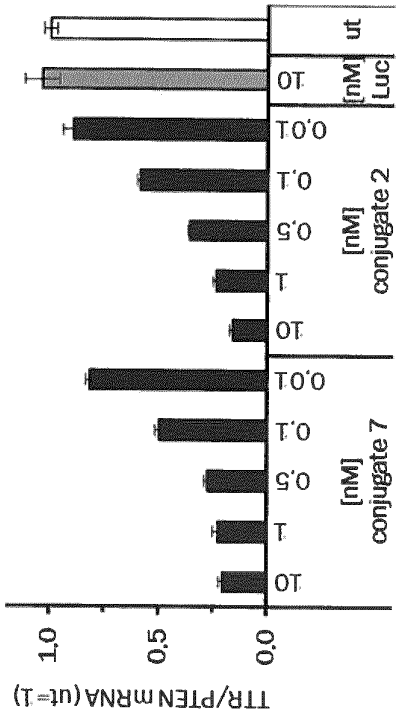


Figure 11B

Figure 12A

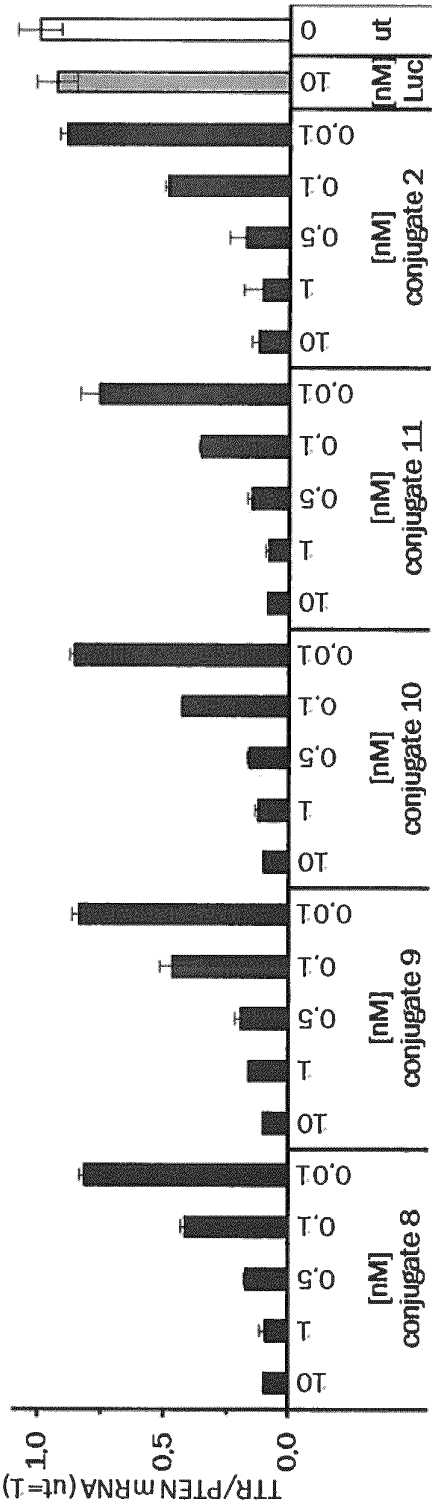
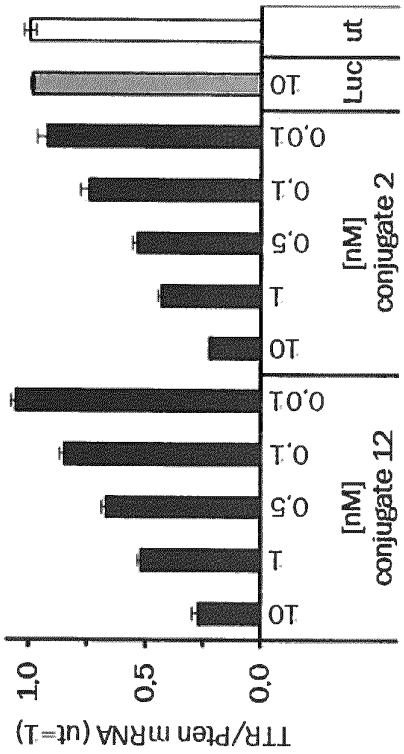


Figure 12B



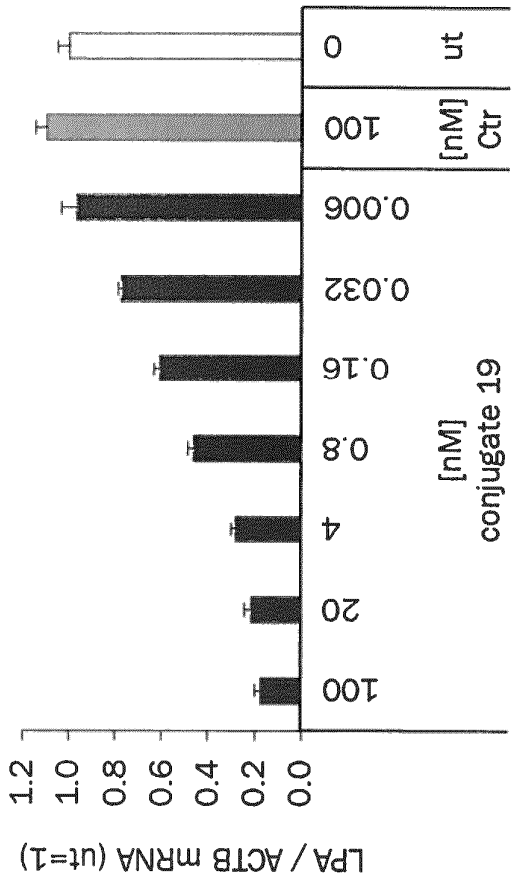

LPA / ACTB mRNA (ut=1)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
100
20
4
0.8
0.16
0.032
0.006
100
0
[nM]
conjugate 19
[nM]
Ctr
ut

Figure 13

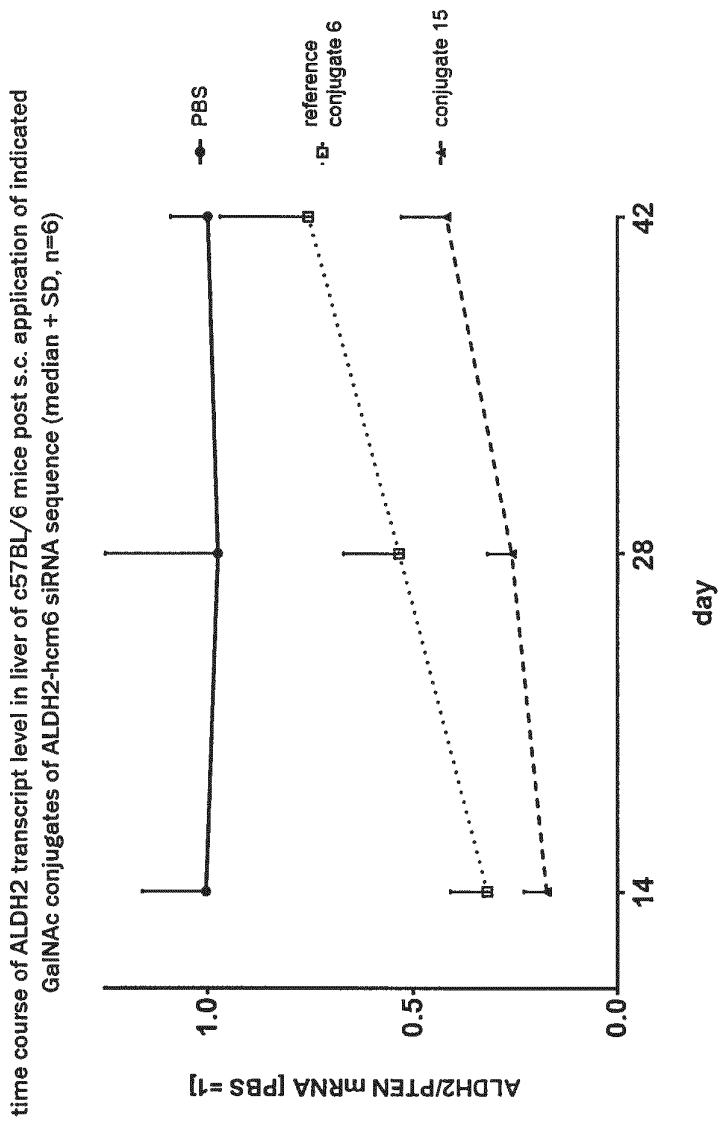

time course of ALDH2 transcript level in liver of c57BL/6 mice post s.c. application of indicated GalNAc conjugates of ALDH2-hcm6 siRNA sequence (median + SD, n=6)
PBS
reference conjugate 6
conjugate 15
ALDH2/PTEN mRNA [PBS =1]
1.0
0.5
0.0
14
28
42
day

Figure 14

Figure 15

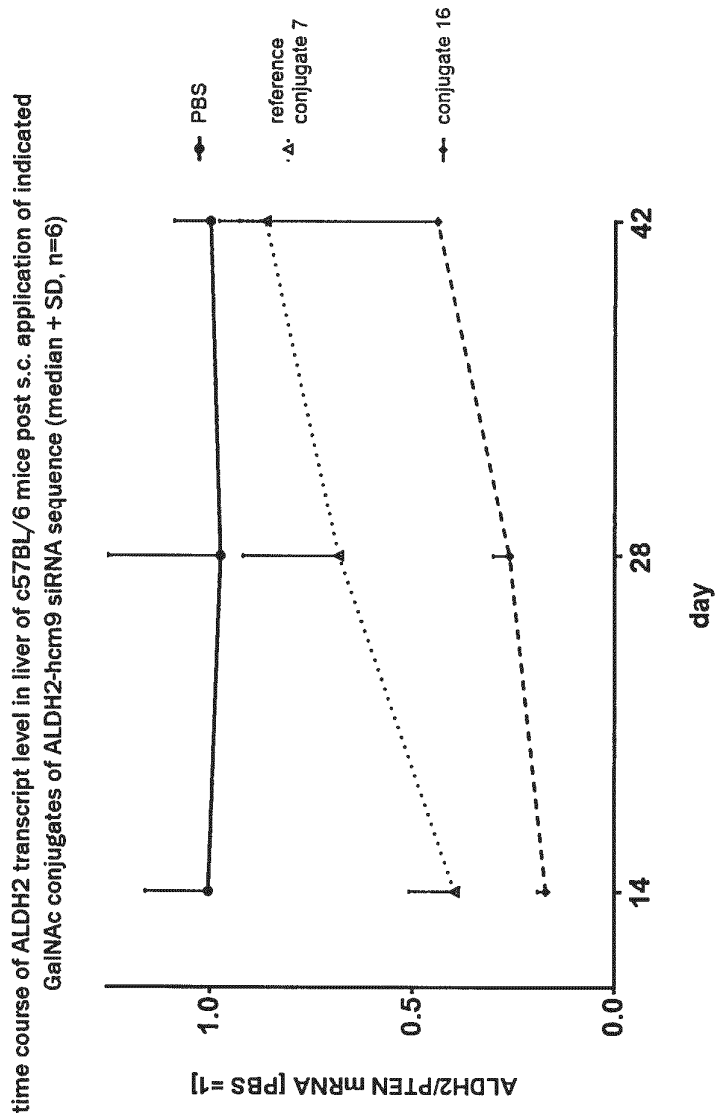

time course of ALDH2 transcript level in liver of c57BL/6 mice post s.c. application of indicated GalNAc conjugates of ALDH2-hcm9 siRNA sequence (median + SD, n=6)
PBS
reference conjugate 7
conjugate 16
ALDH2/PTEN mRNA [PBS =1]
1.0
0.5
0.0
14
28
42
day

Figure 16

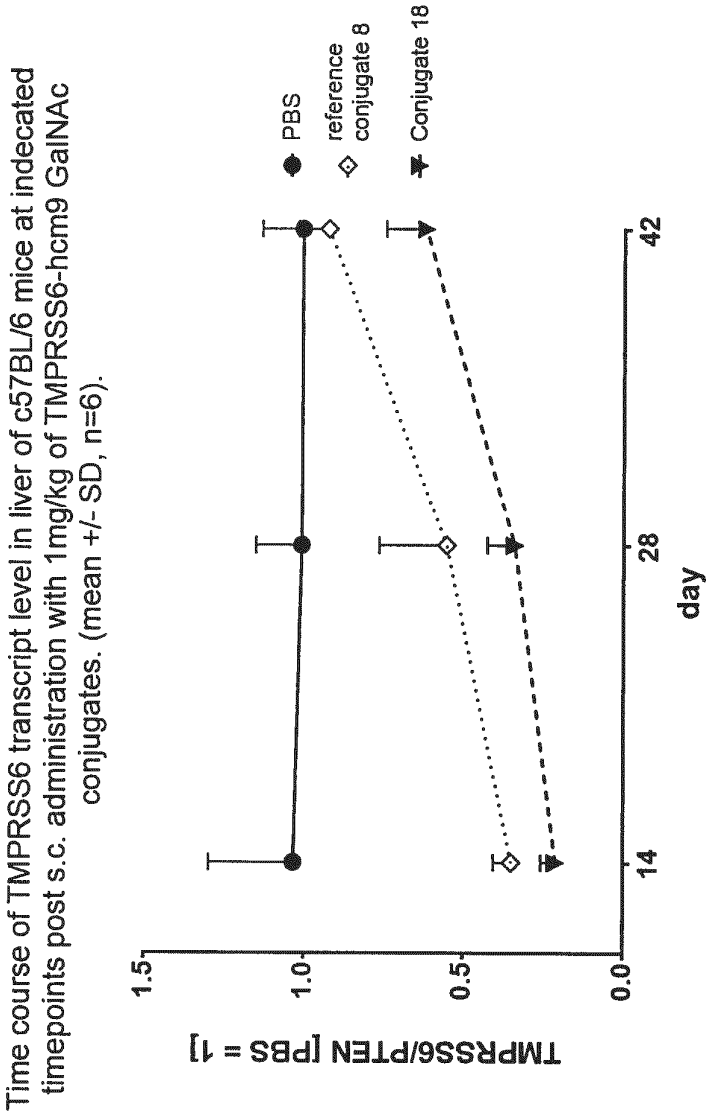

Time course of TMPRSS6 transcript level in liver of c57BL/6 mice at indecated timepoints post s.c. administration with 1mg/kg of TMPRSS6-hcm9 GalNAc conjugates. (mean +/- SD, n=6).
TMPRSS6/PTEN [PBS = 1]
1.5
1.0
0.5
0.0
14
28
42
day
PBS
reference conjugate 8
Conjugate 18

Figure 17

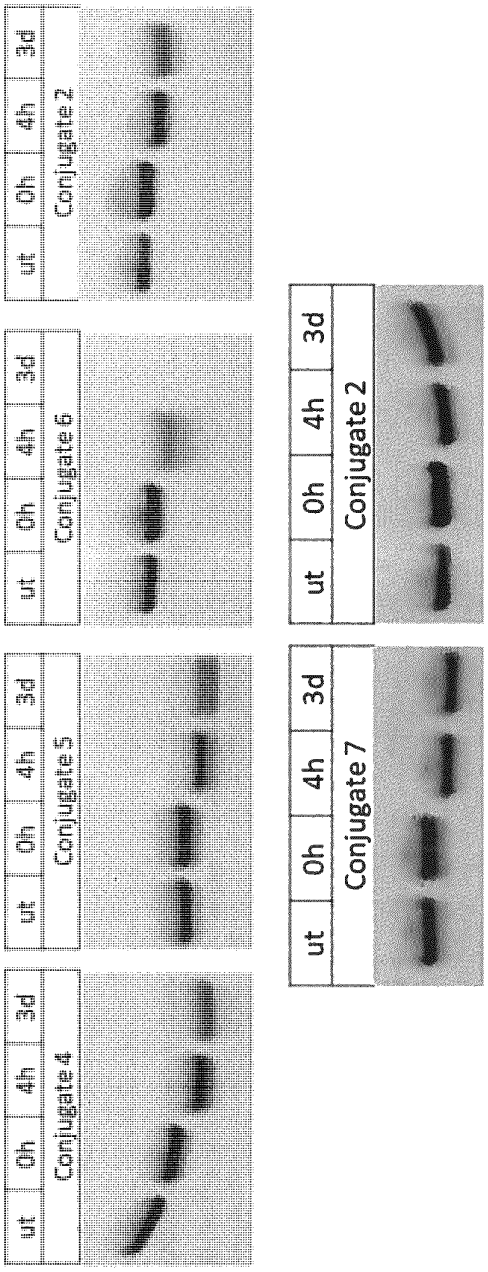


Figure 18

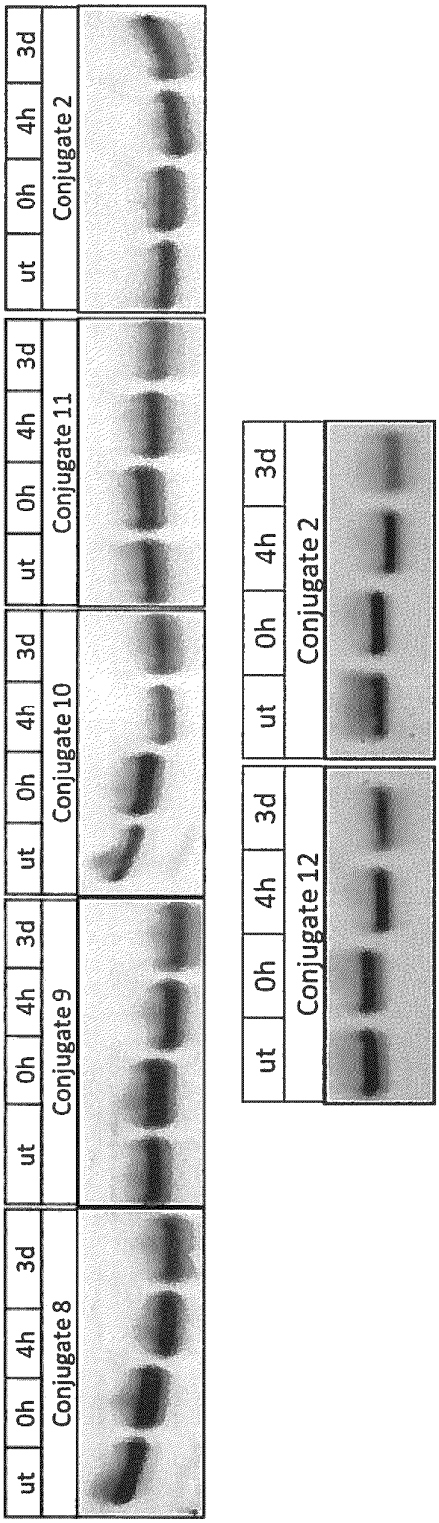


Figure 19

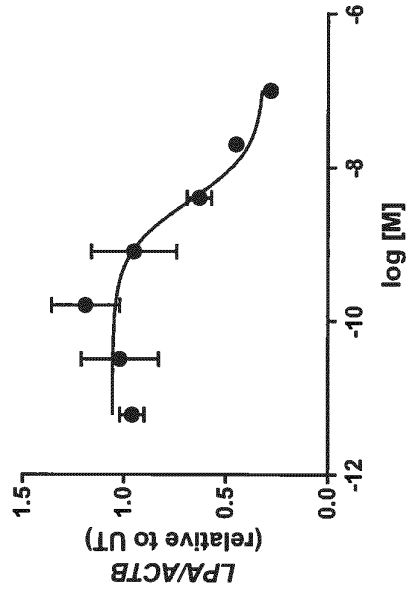


Figure 20

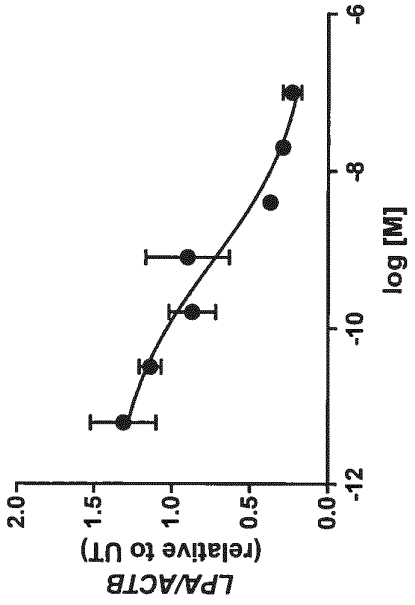


Figure 21

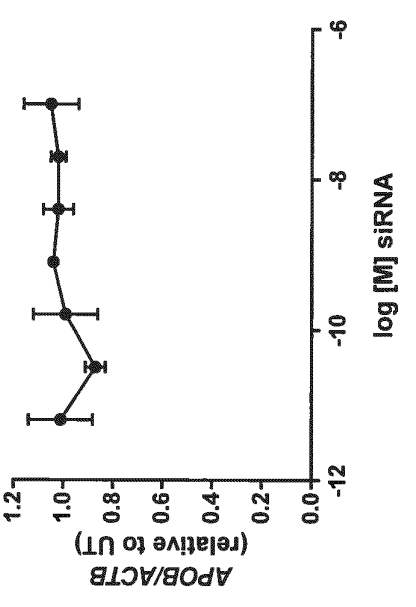


Figure 22

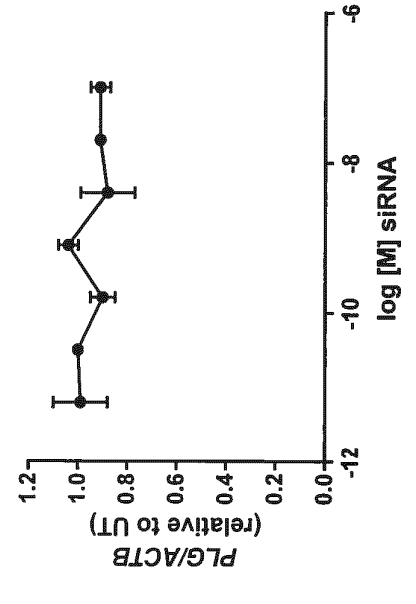


Figure 23

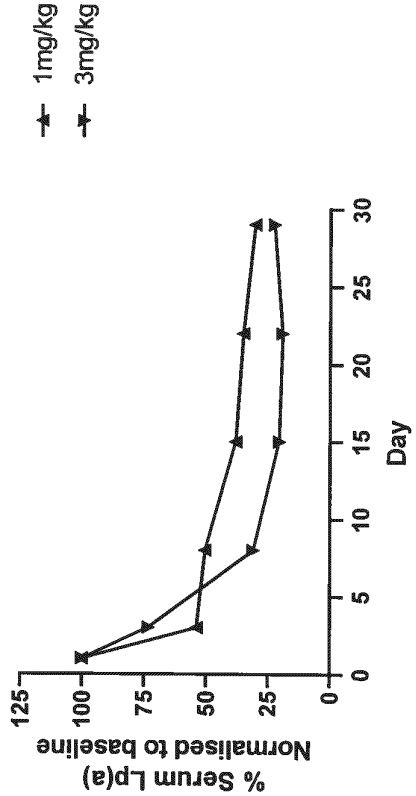


Figure 24

