FIG. 1A

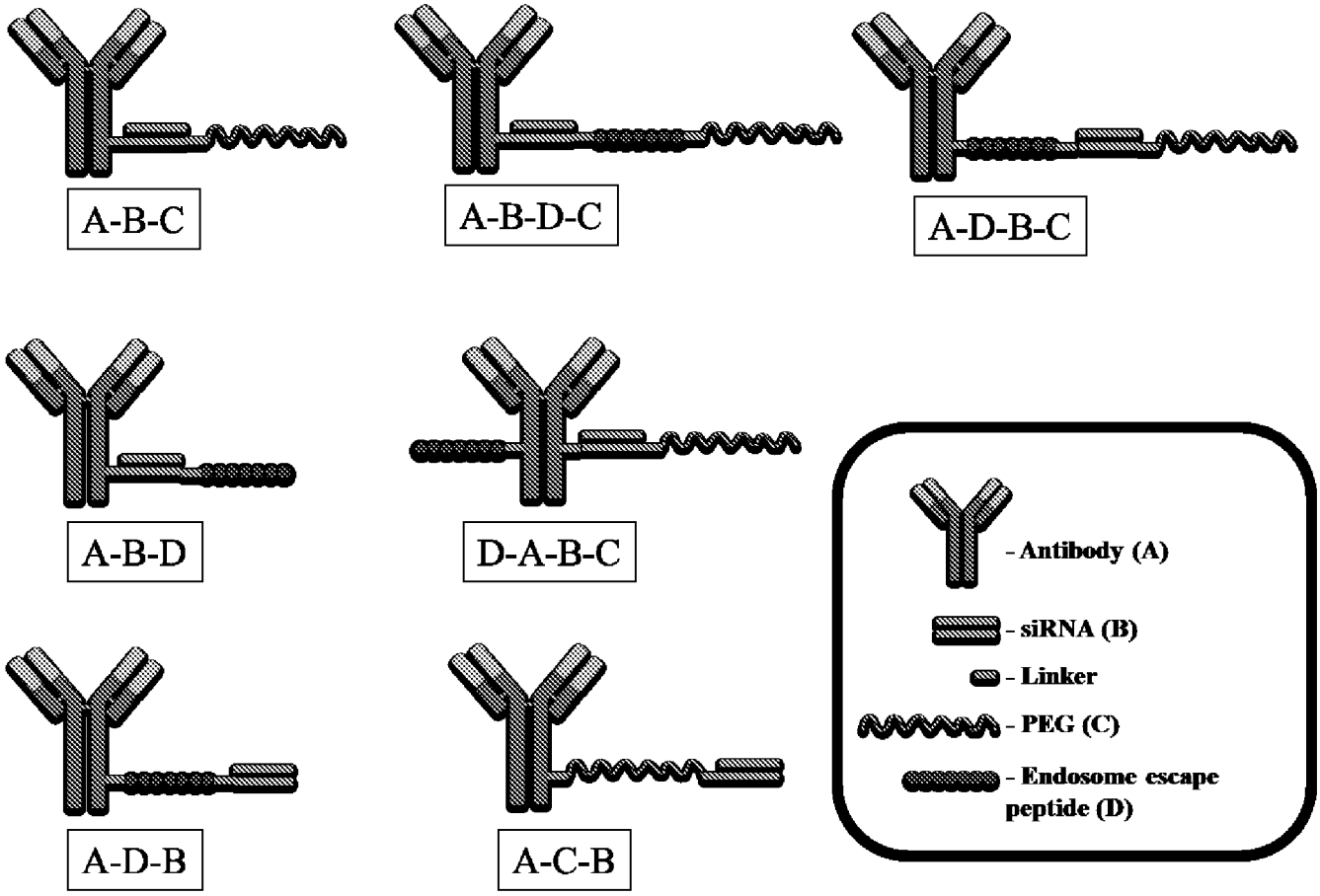

A-B-C
A-B-D-C
A-D-B-C
A-B-D
D-A-B-C
A-D-B
A-C-B
- Antibody (A)
- siRNA (B)
- Linker
- PEG (C)
- Endosome escape peptide (D)

FIG. 1B

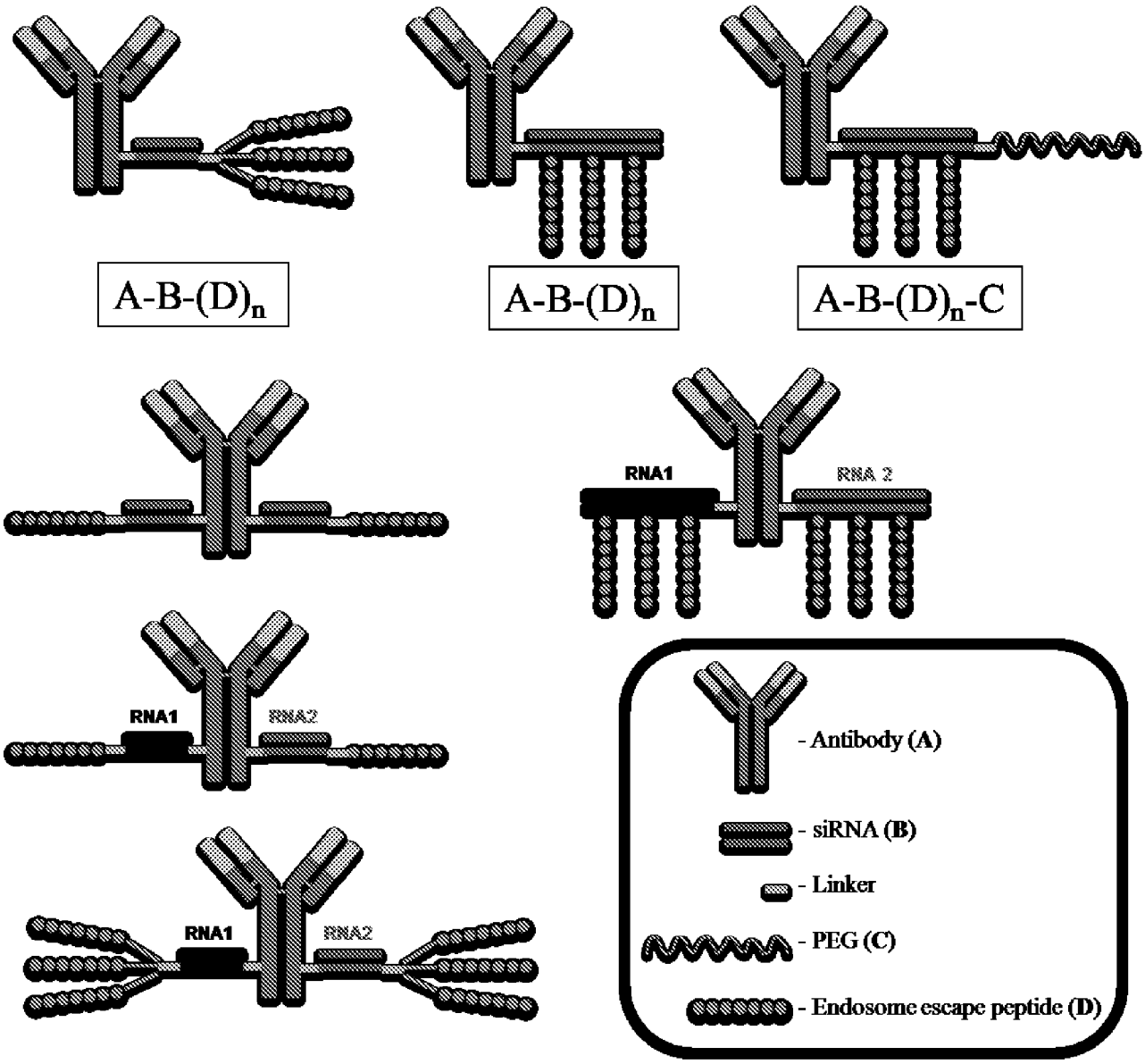

A-B-(D)n
A-B-(D)n
A-B-(D)n-C
RNA1
RNA 2
RNA1
RNA2
RNA1
RNA2
- Antibody (A)
- siRNA (B)
- Linker
- PEG (C)
- Endosome escape peptide (D)

FIG. 1C

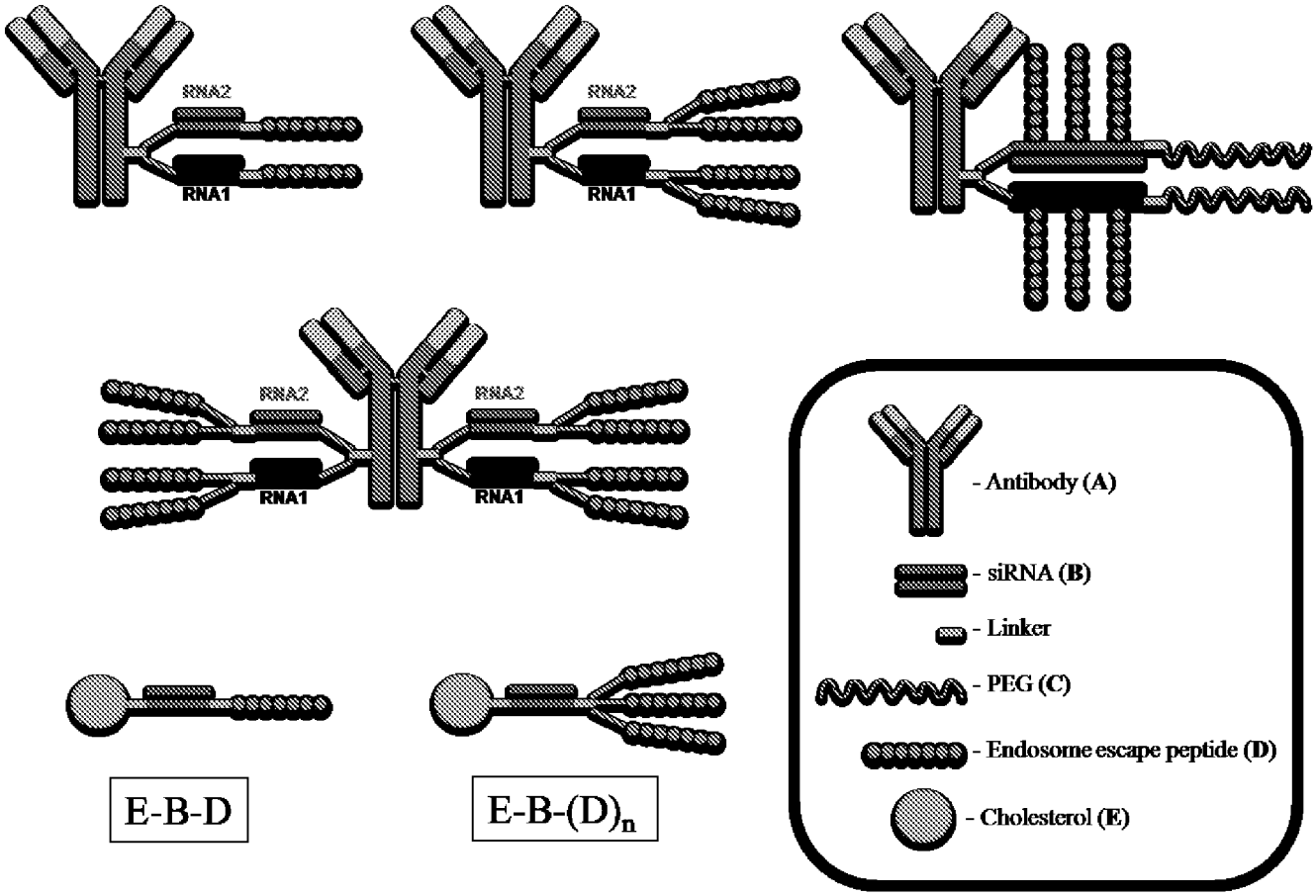

RNA2
RNA1
RNA2
RNA1
RNA2
RNA2
RNA1
RNA1
- Antibody (A)
- siRNA (B)
- Linker
- PEG (C)
- Endosome escape peptide (D)
- Cholesterol (E)
E-B-D
E-B-(D)n

FIG. 2

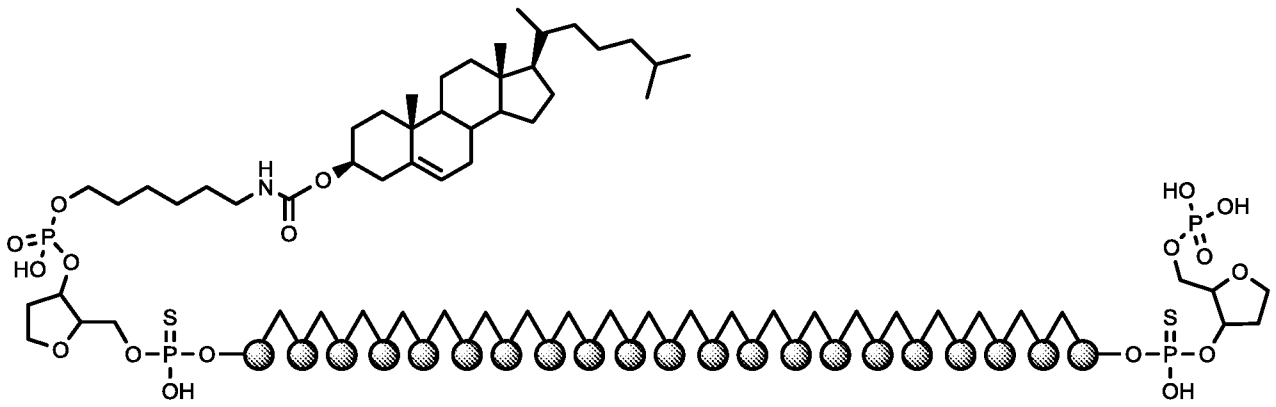
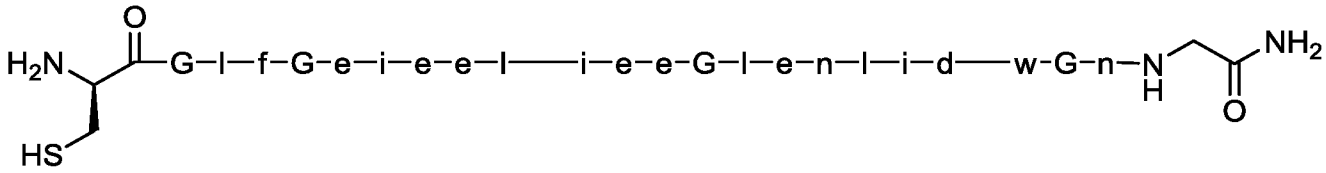
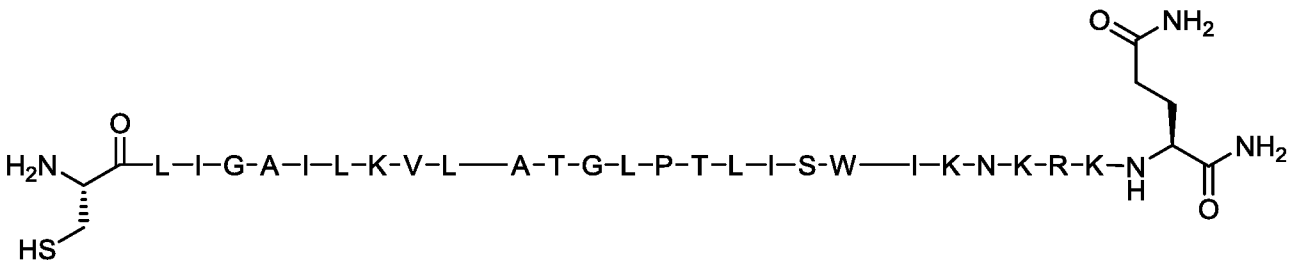


FIG. 3



(SEQ ID NO: 2055)

FIG. 4



(SEQ ID NO: 2060)

FIG. 5

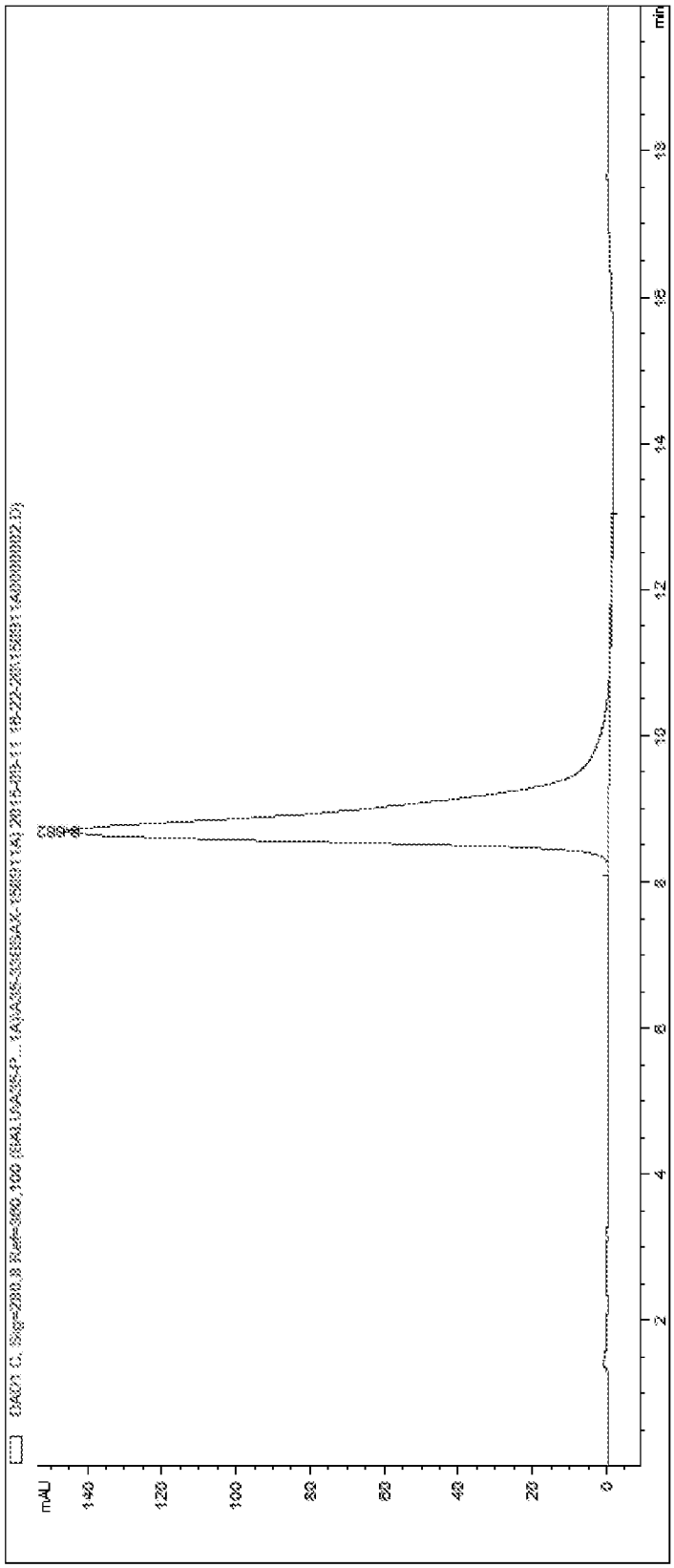
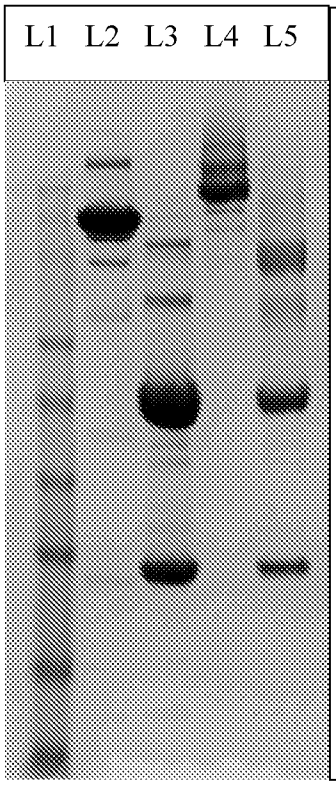


FIG. 6



L1. HMW Standard
L2. EGFR-Ab – Non-reduced
L3. EGFR-Ab – Reduced
L4. EGFR-Ab-PEG20kDa-siRNA – Non-reduced
L5. EGFR-Ab-PEG20kDa-siRNA - Reduced

FIG. 7

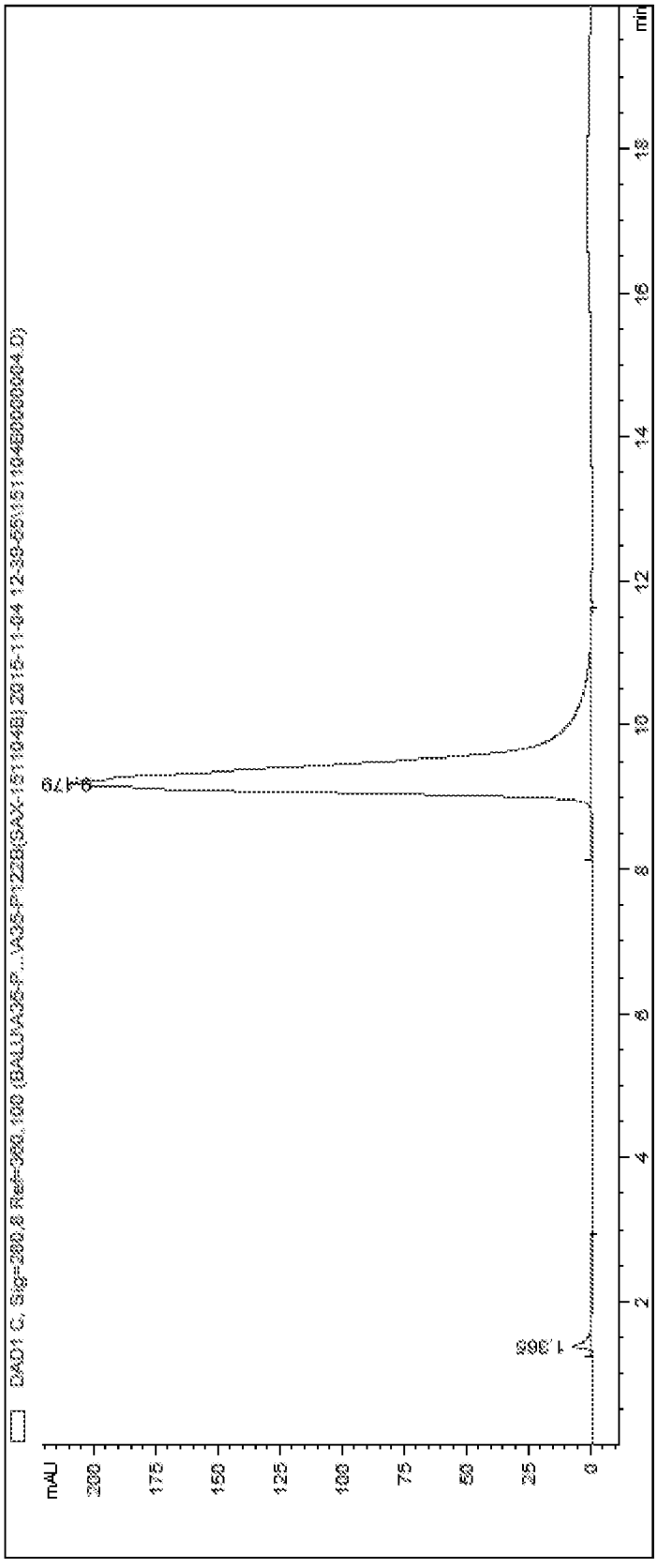

mAU
200
175
150
125
100
75
50
25
0
2
4
6
8
10
12
14
16
18
min
1.365
9.179

FIG. 8

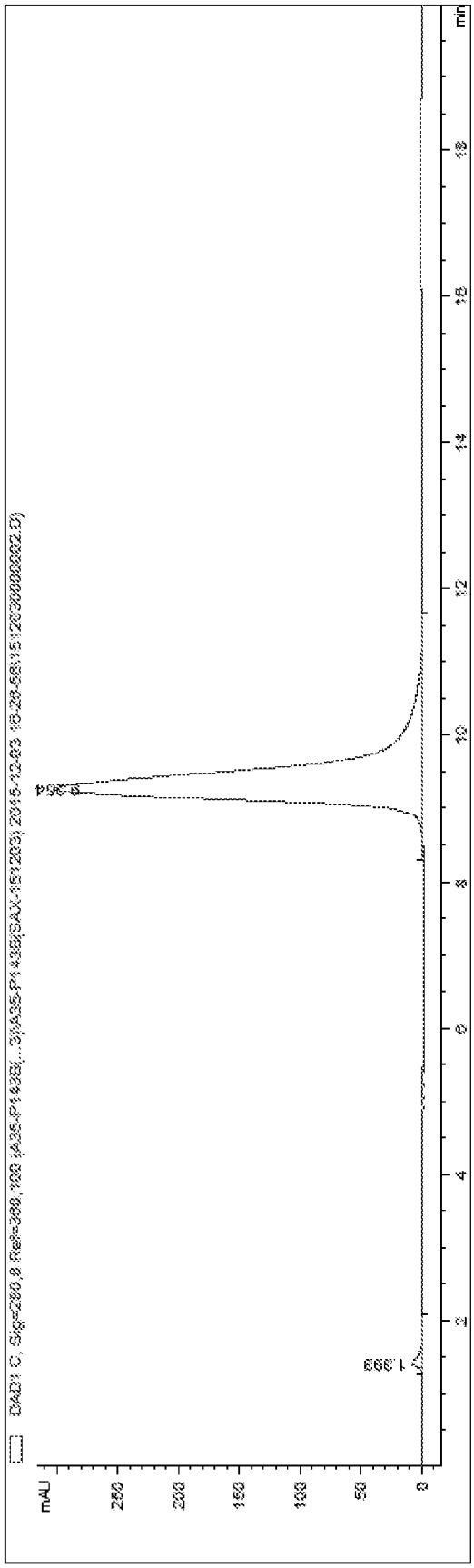


FIG. 9

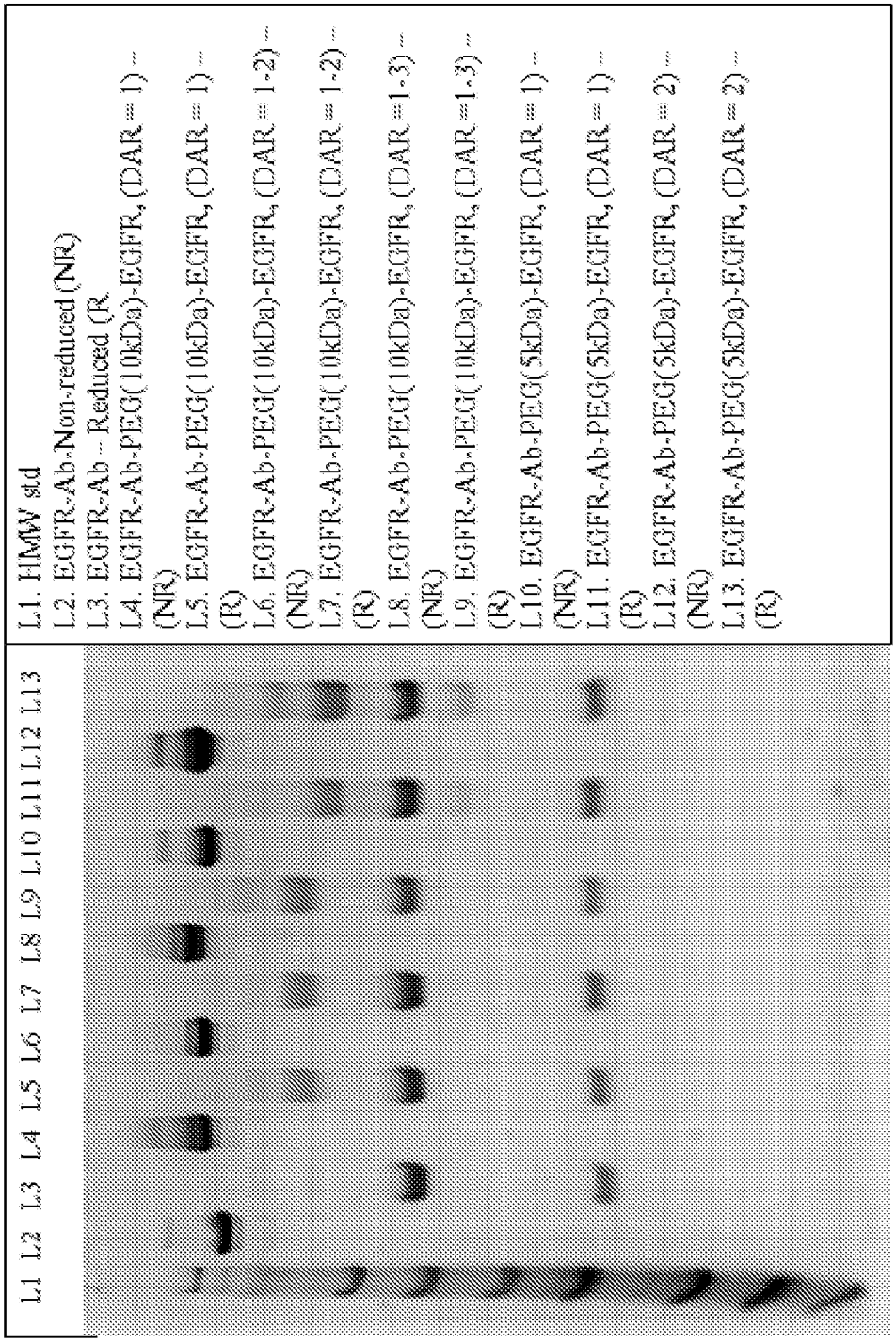

L1 L2 L3 L4 L5 L6 L7 L8 L9 L10 L11 L12 L13
L1. HMW std
L2. EGFR-Ab-Non-reduced (NR)
L3. EGFR-Ab – Reduced (R
L4. EGFR-Ab-PEG(10kDa)-EGFR, (DAR = 1) – (NR)
L5. EGFR-Ab-PEG(10kDa)-EGFR, (DAR = 1) – (R)
L6. EGFR-Ab-PEG(10kDa)-EGFR, (DAR = 1-2) – (NR)
L7. EGFR-Ab-PEG(10kDa)-EGFR, (DAR = 1-2) – (R)
L8. EGFR-Ab-PEG(10kDa)-EGFR, (DAR =1-3) – (NR)
L9. EGFR-Ab-PEG(10kDa)-EGFR, (DAR =1-3) – (R)
L10. EGFR-Ab-PEG(5kDa)-EGFR, (DAR = 1) – (NR)
L11. EGFR-Ab-PEG(5kDa)-EGFR, (DAR = 1) – (R)
L12. EGFR-Ab-PEG(5kDa)-EGFR, (DAR = 2) – (NR)
L13. EGFR-Ab-PEG(5kDa)-EGFR, (DAR = 2) – (R)

FIG.10

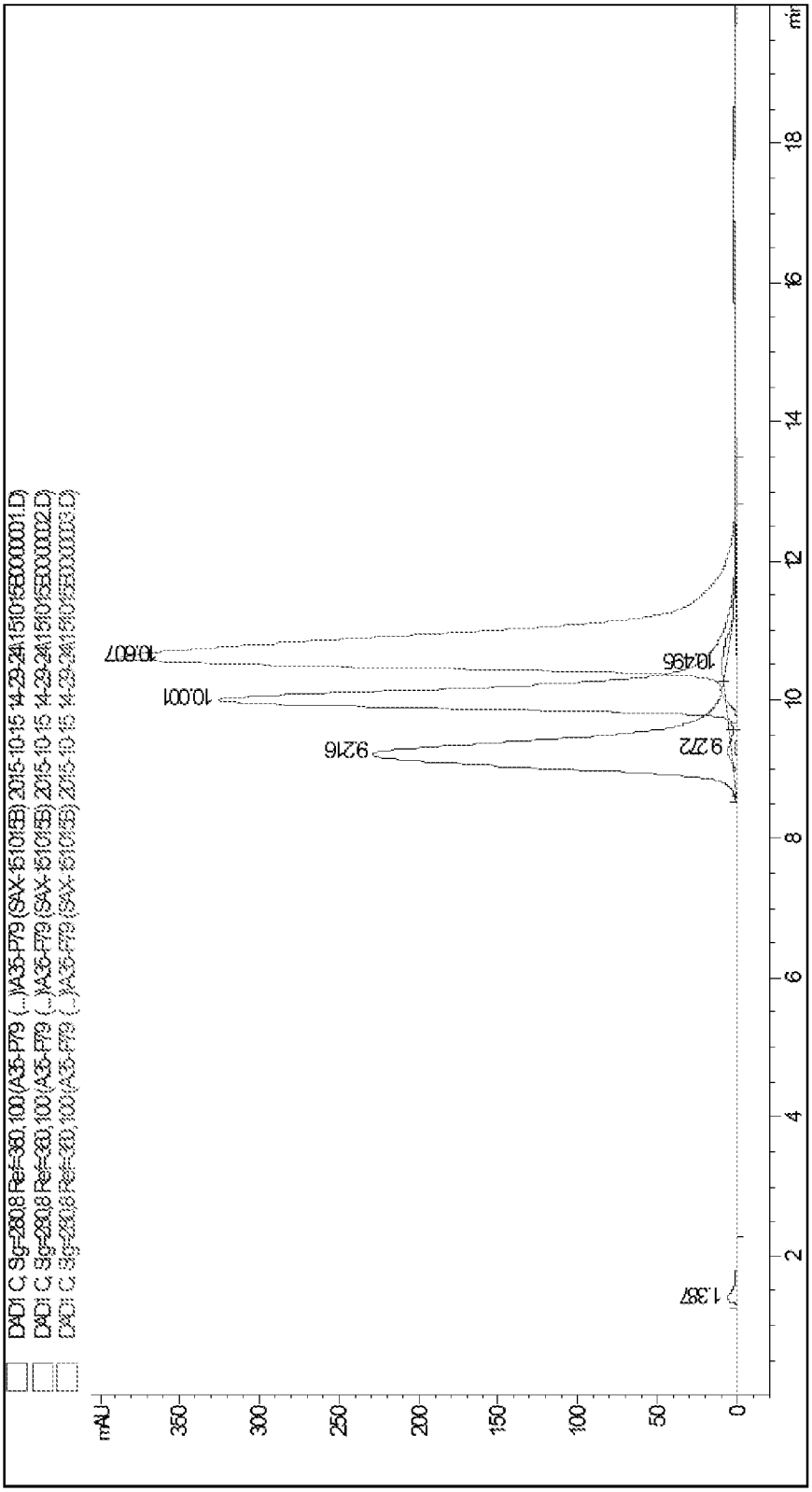

DAD1 C, Sig=280,8 Ref=360,100 (A35-P79 (...)A35-P79 (SAX-151015B) 2015-10-15 14-29-24\151015B0000001.D)
DAD1 C, Sig=280,8 Ref=360,100 (A35-P79 (...)A35-P79 (SAX-151015) 2015-10-15 14-29-24\151015B0000002.D)
DAD1 C, Sig=280,8 Ref=360,100 (A35-P79 (...)A35-P79 (SAX-151015) 2015-10-15 14-29-24\151015B0000003.D)
mAU
350
300
250
200
150
100
50
0
1.387
9.216
9.272
10.001
10.496
10.807
2
4
6
8
10
12
14
16
18
min

FIG. 11

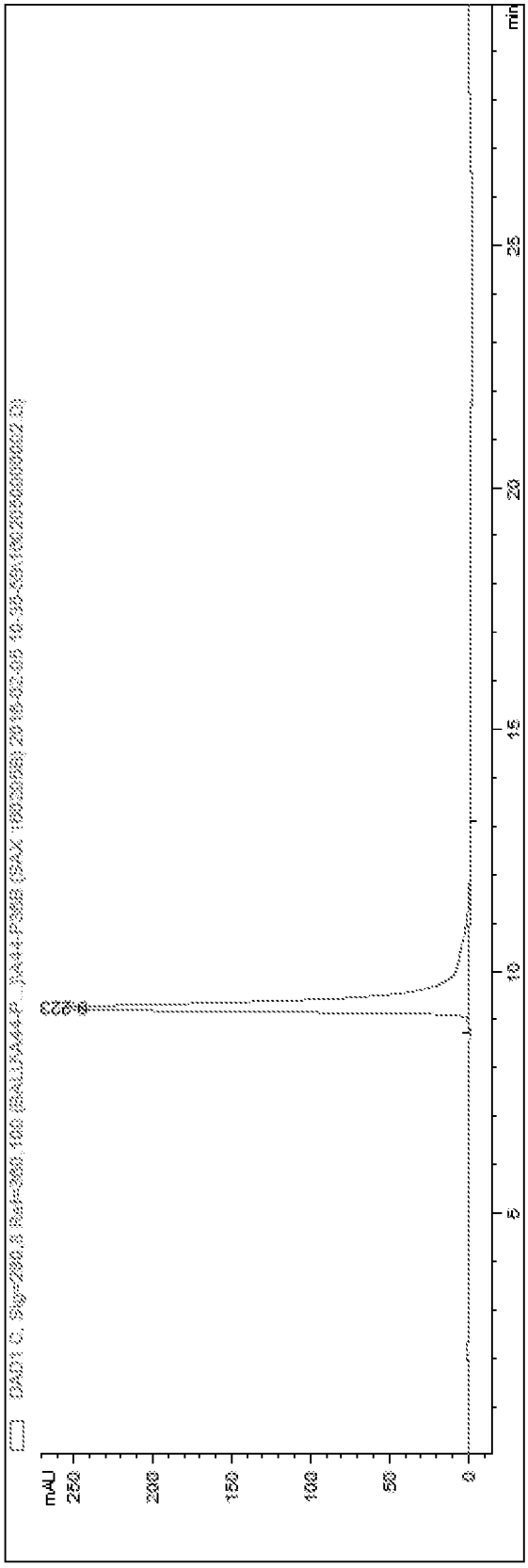


FIG. 12

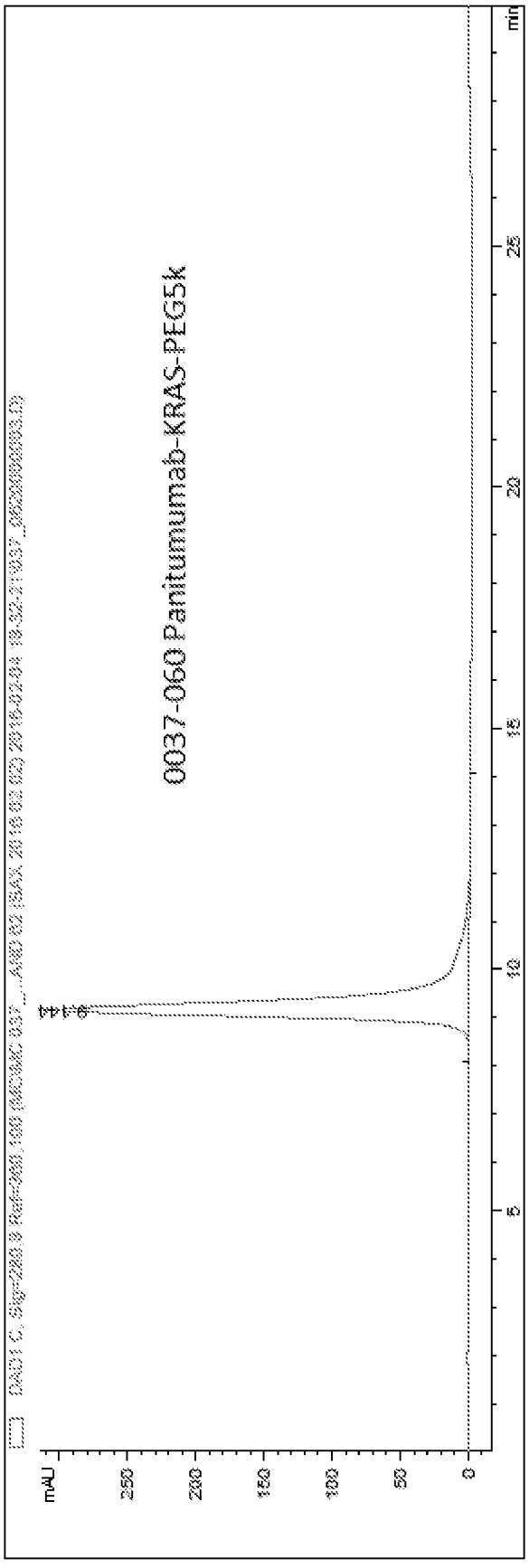

0037-060 Panitumumab-KRAS-PEG5k
mAU
250
200
150
100
50
0
5
10
15
20
25
min

FIG. 13

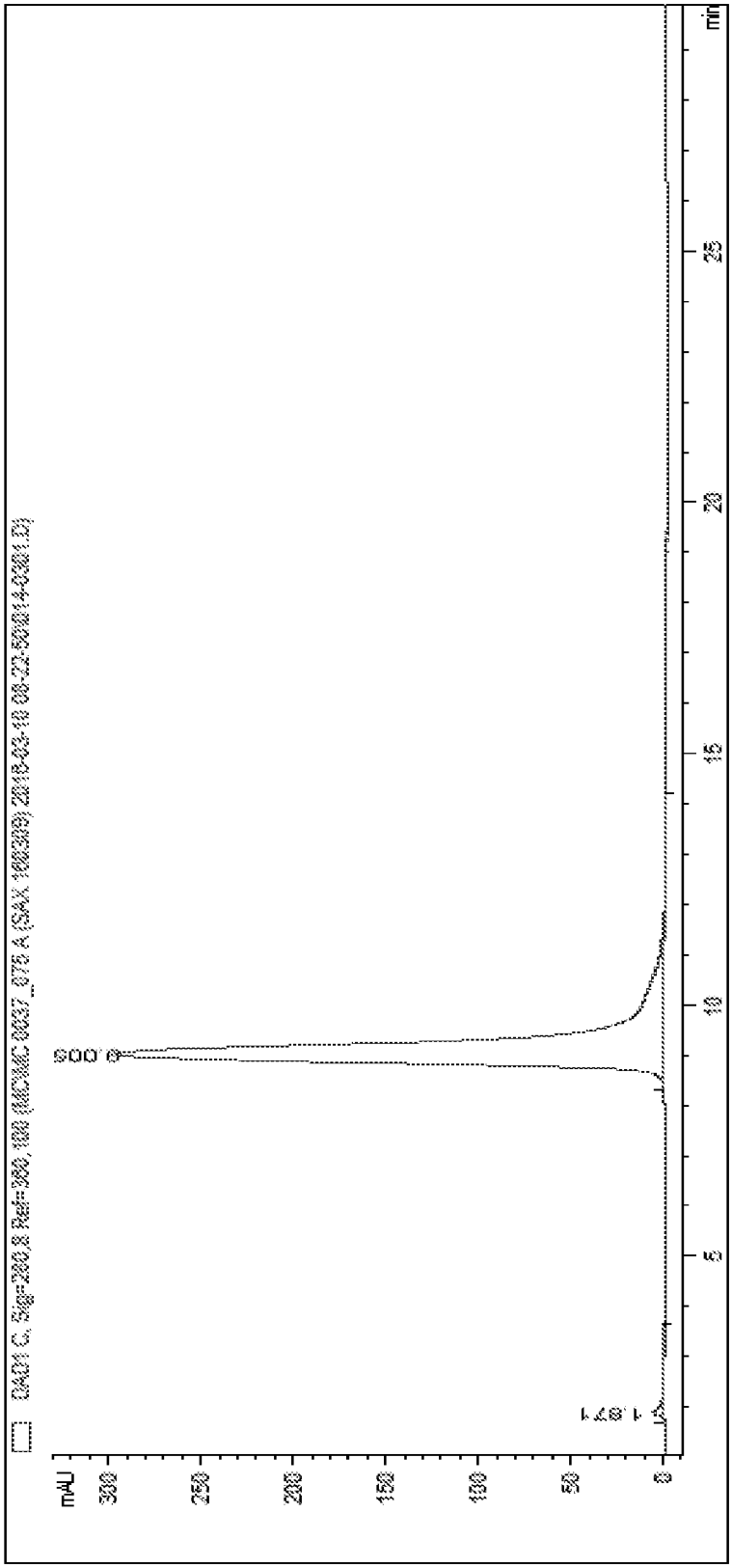


FIG. 14

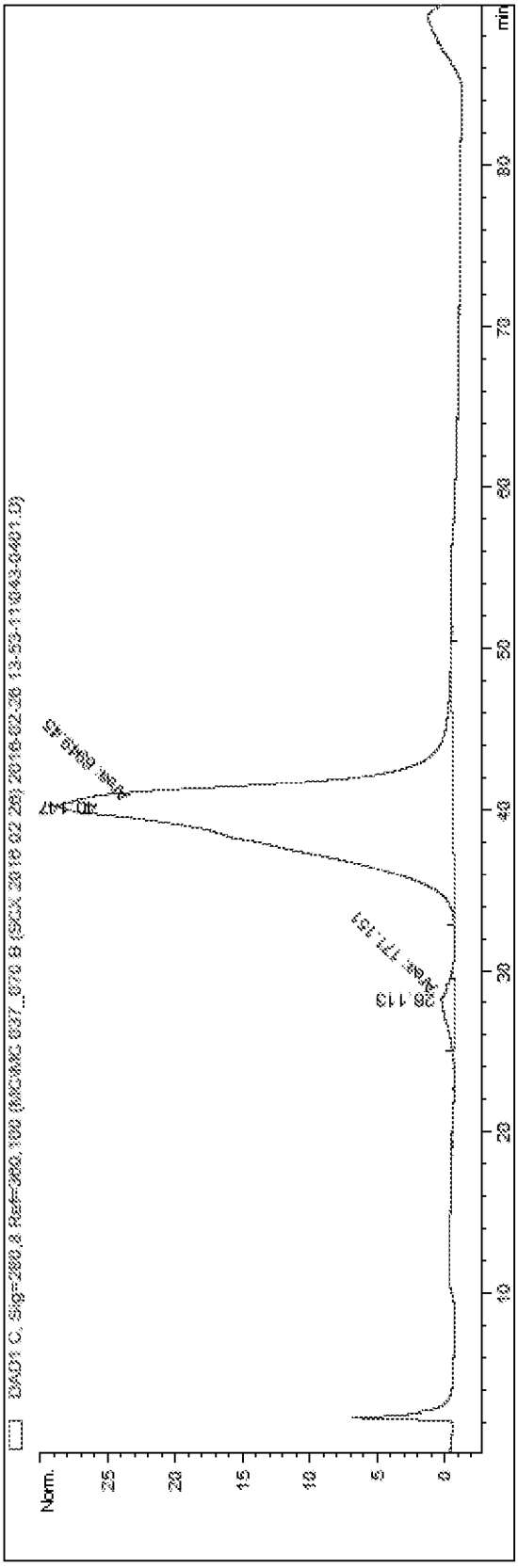


FIG. 15

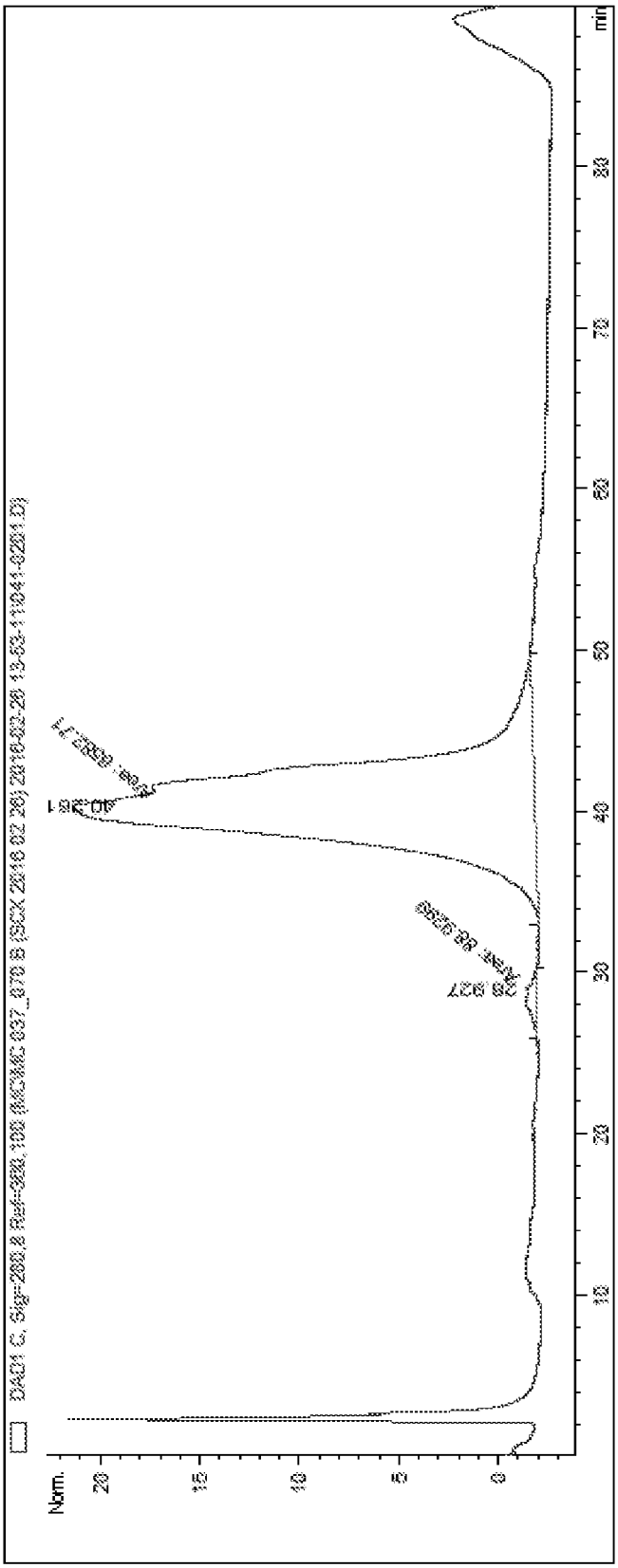


FIG. 16

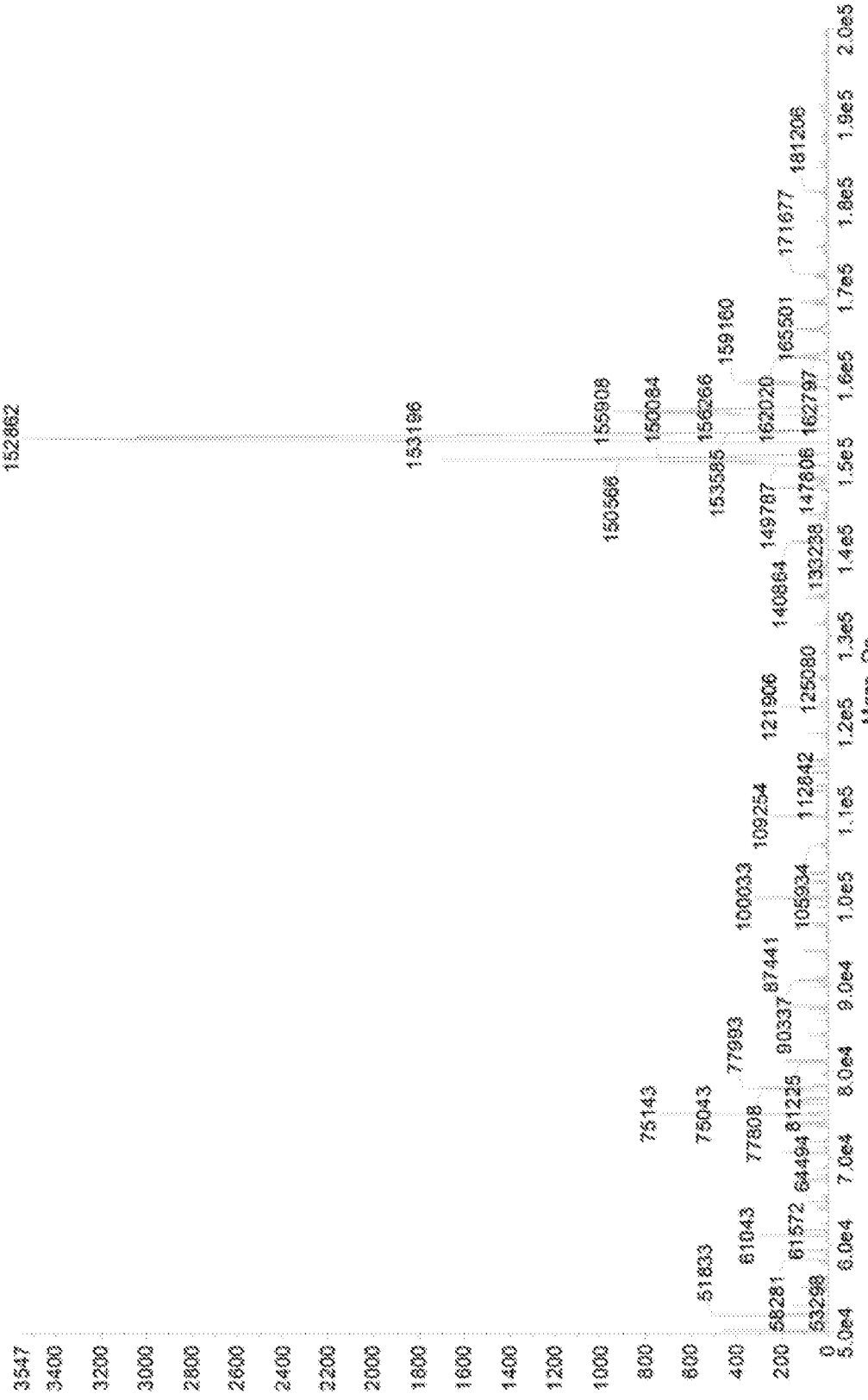

3547
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
5.0e4
6.0e4
7.0e4
8.0e4
9.0e4
1.0e5
1.1e5
1.2e5
1.3e5
1.4e5
1.5e5
1.6e5
1.7e5
1.8e5
1.9e5
2.0e5
Mass, Da
152862
153196
155908
150084
156266
159160
165501
171677
181206
150566
153585
162020
162797
149787
147606
140864
133238
121906
125080
112842
109254
100033
105934
87441
90337
77993
75143
75043
77808
81225
64494
61572
61043
51833
58281
53298

FIG. 17

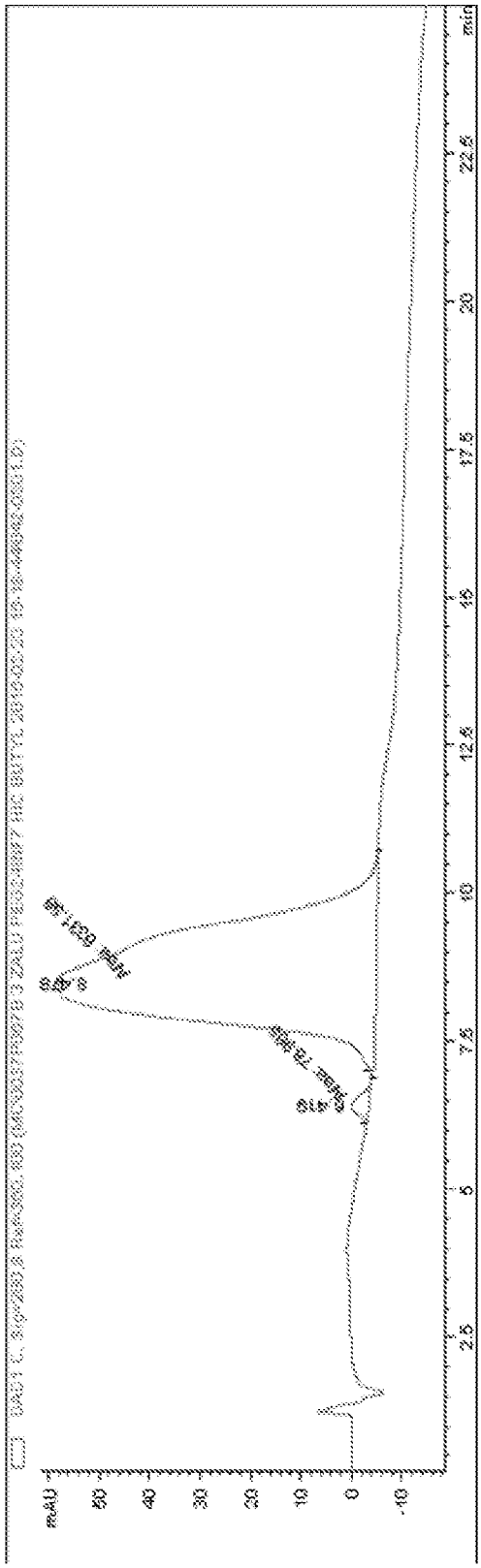


FIG. 18

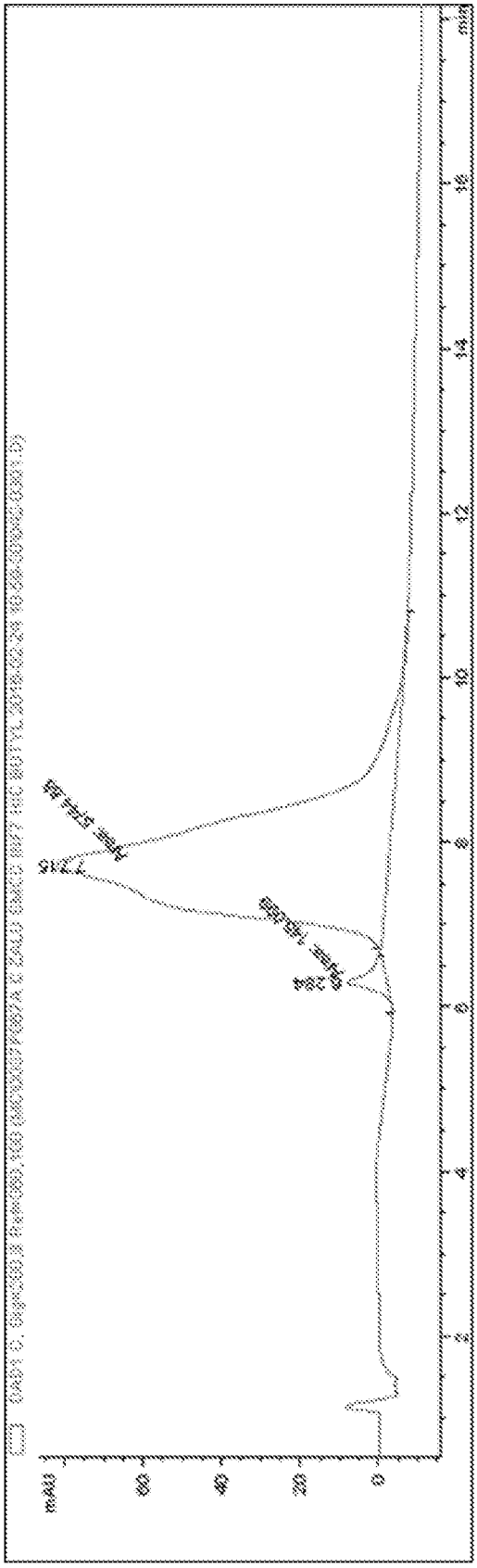


FIG. 19

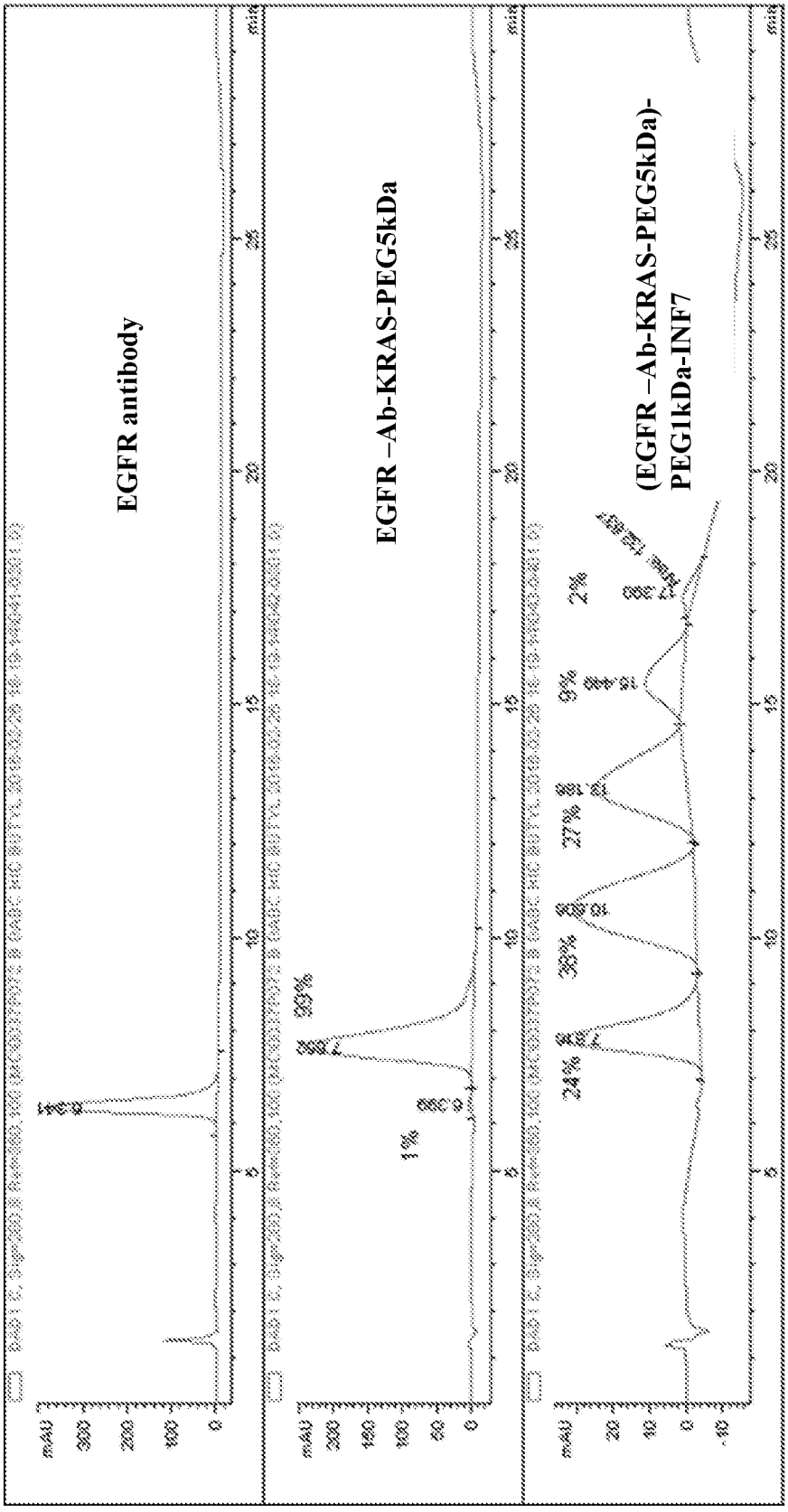

EGFR antibody
EGFR –Ab-KRAS-PEG5kDa
(EGFR –Ab-KRAS-PEG5kDa)-PEG1kDa-INF7
mAU
min
1%
89%
24%
36%
27%
9%
2%

FIG. 20

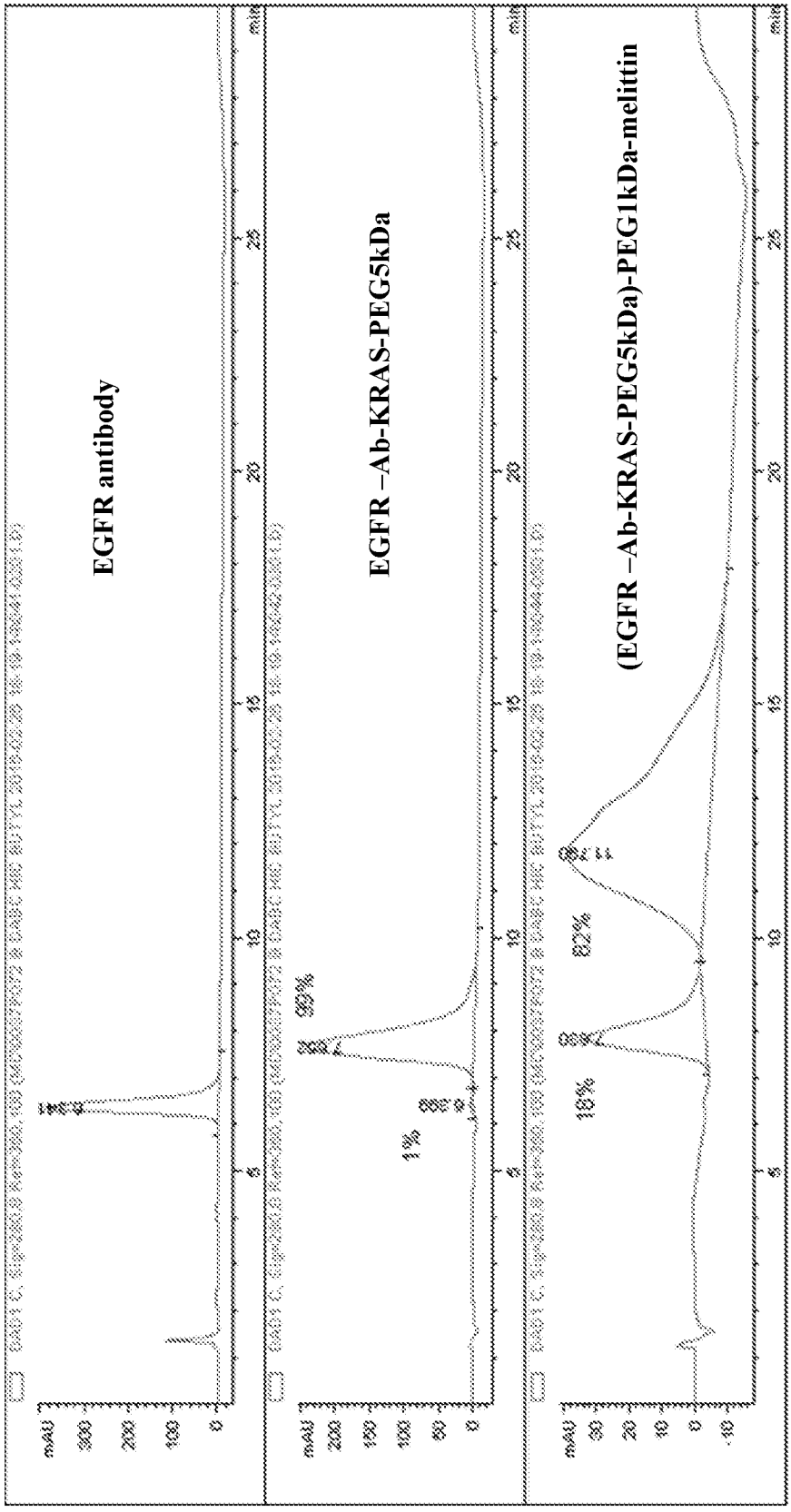

EGFR antibody
EGFR –Ab-KRAS-PEG5kDa
(EGFR –Ab-KRAS-PEG5kDa)-PEG1kDa-melittin
99%
1%
82%
18%
mAU
min

FIG. 21

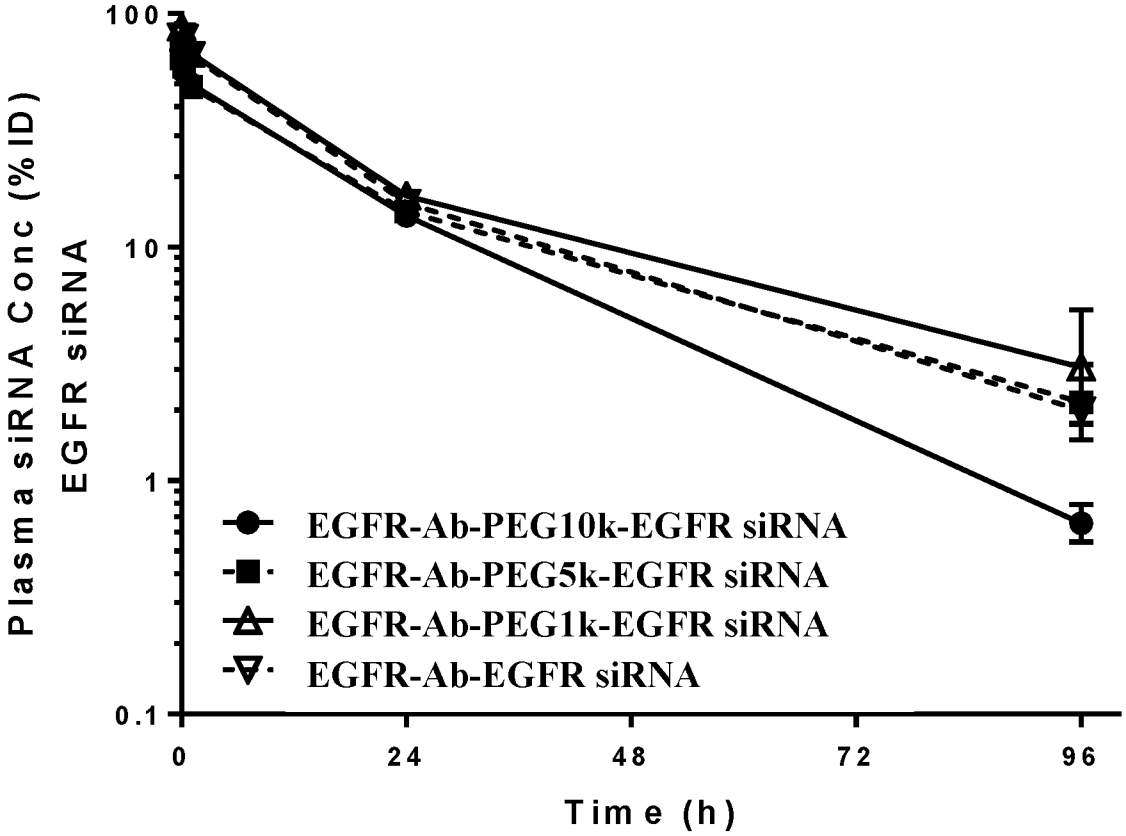

100
10
1
0.1
Plasma siRNA Conc (%ID)
EGFR siRNA
0
24
48
72
96
Time (h)
EGFR-Ab-PEG10k-EGFR siRNA
EGFR-Ab-PEG5k-EGFR siRNA
EGFR-Ab-PEG1k-EGFR siRNA
EGFR-Ab-EGFR siRNA

FIG. 22

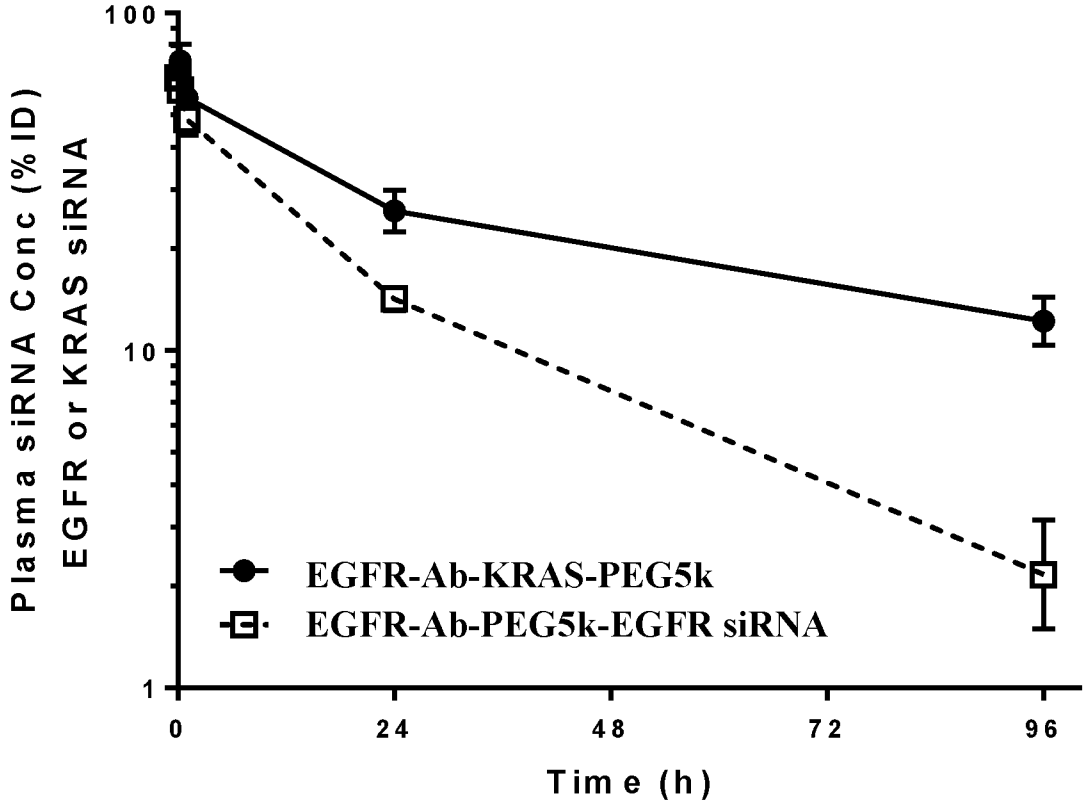

100
10
1
Plasma siRNA Conc (% ID)
EGFR or KRAS siRNA
EGFR-Ab-KRAS-PEG5k
EGFR-Ab-PEG5k-EGFR siRNA
0
24
48
72
96
Time (h)

FIG. 23

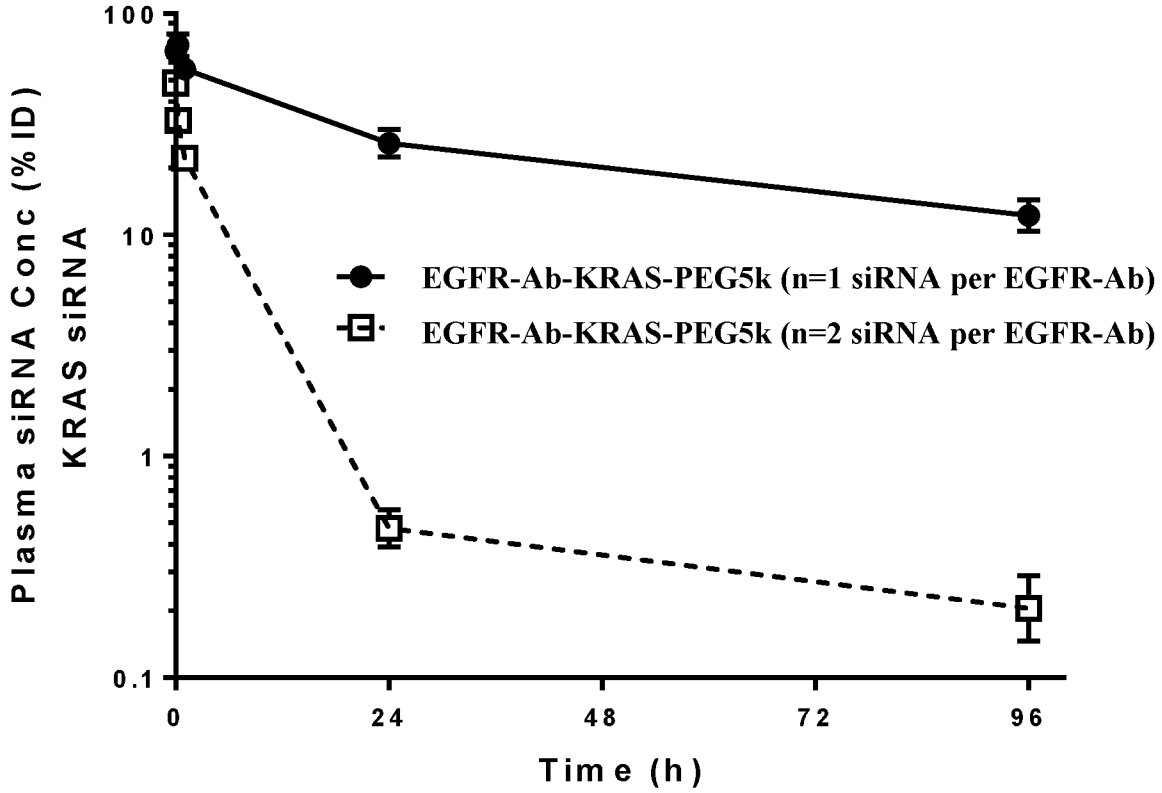

100
10
1
0.1
Plasma siRNA Conc (% ID)
KRAS siRNA
EGFR-Ab-KRAS-PEG5k (n=1 siRNA per EGFR-Ab)
EGFR-Ab-KRAS-PEG5k (n=2 siRNA per EGFR-Ab)
0
24
48
72
96
Time (h)

FIG. 24

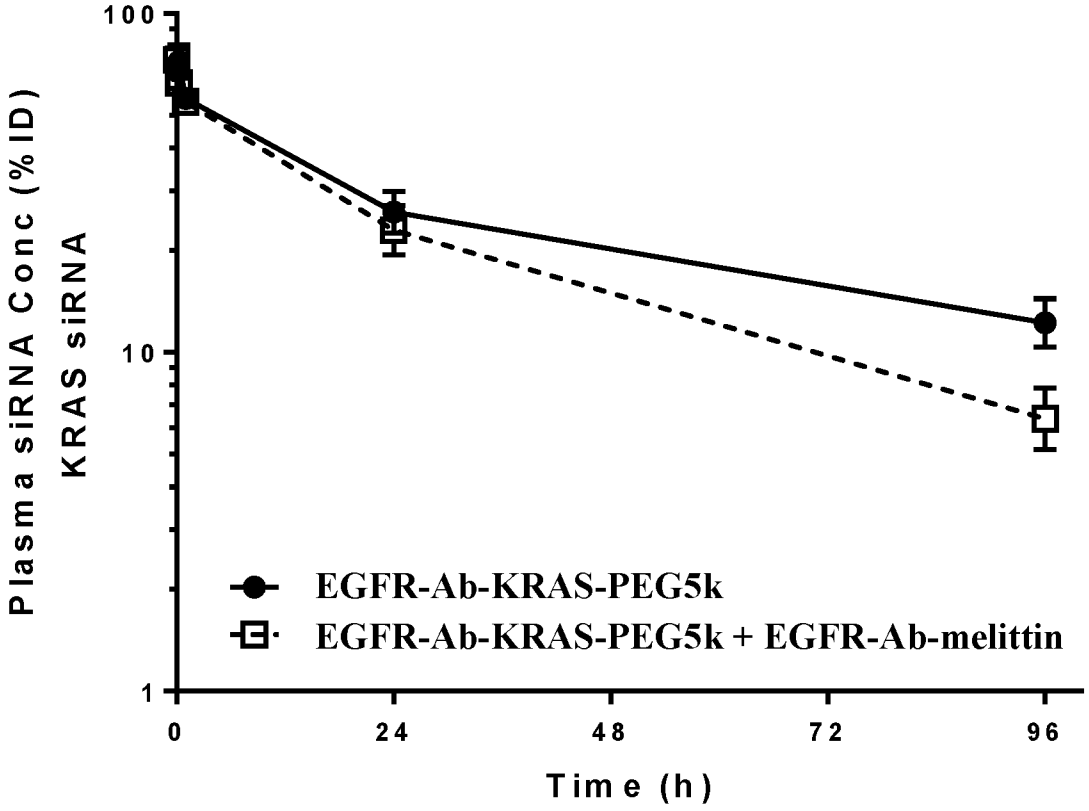

100
10
1
Plasma siRNA Conc (%ID)
KRAS siRNA
0
24
48
72
96
Time (h)
EGFR-Ab-KRAS-PEG5k
EGFR-Ab-KRAS-PEG5k + EGFR-Ab-melittin

FIG. 25

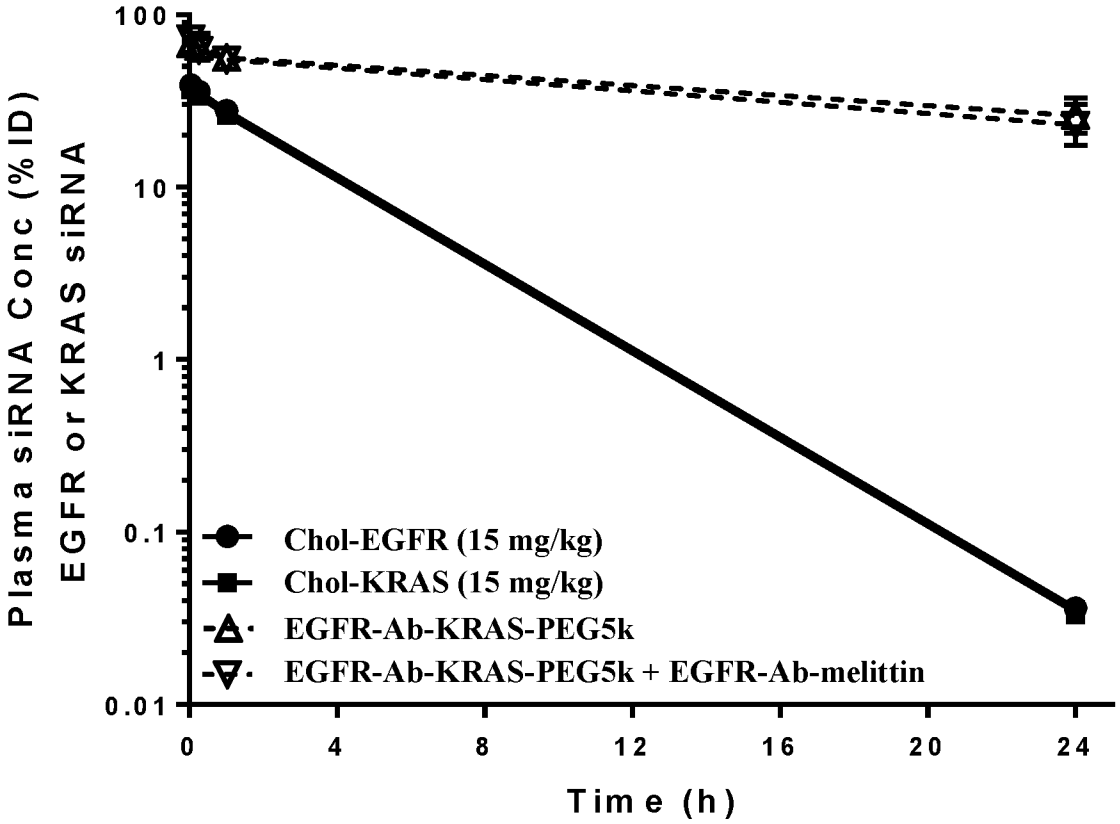

Plasma siRNA Conc (% ID)
EGFR or KRAS siRNA
100
10
1
0.1
0.01
0
4
8
12
16
20
24
Time (h)
Chol-EGFR (15 mg/kg)
Chol-KRAS (15 mg/kg)
EGFR-Ab-KRAS-PEG5k
EGFR-Ab-KRAS-PEG5k + EGFR-Ab-melittin

FIG. 26A

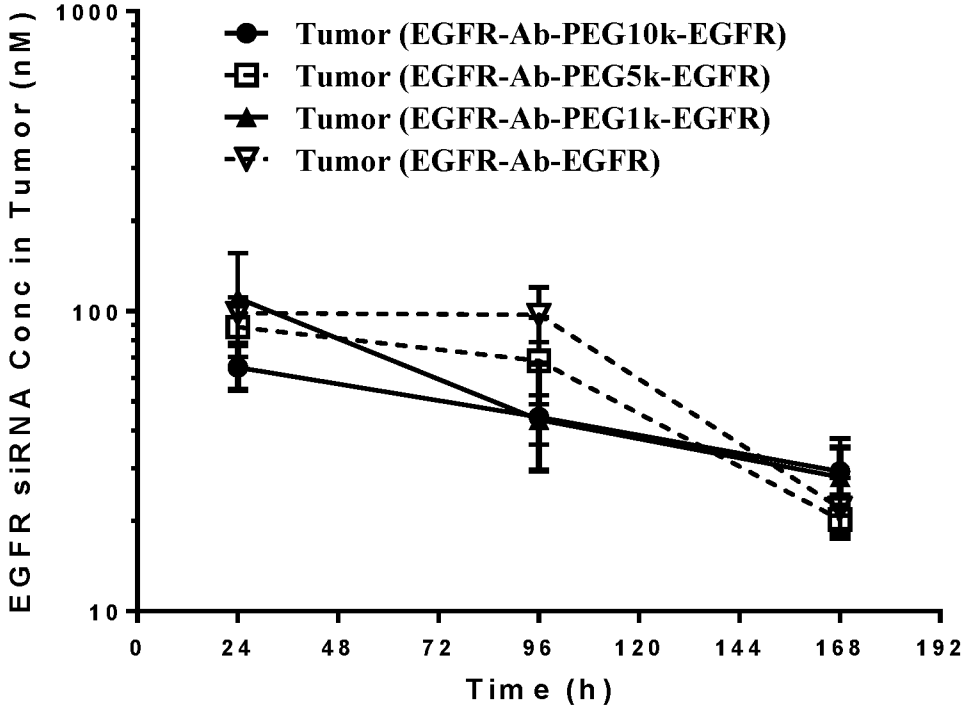


FIG. 26B

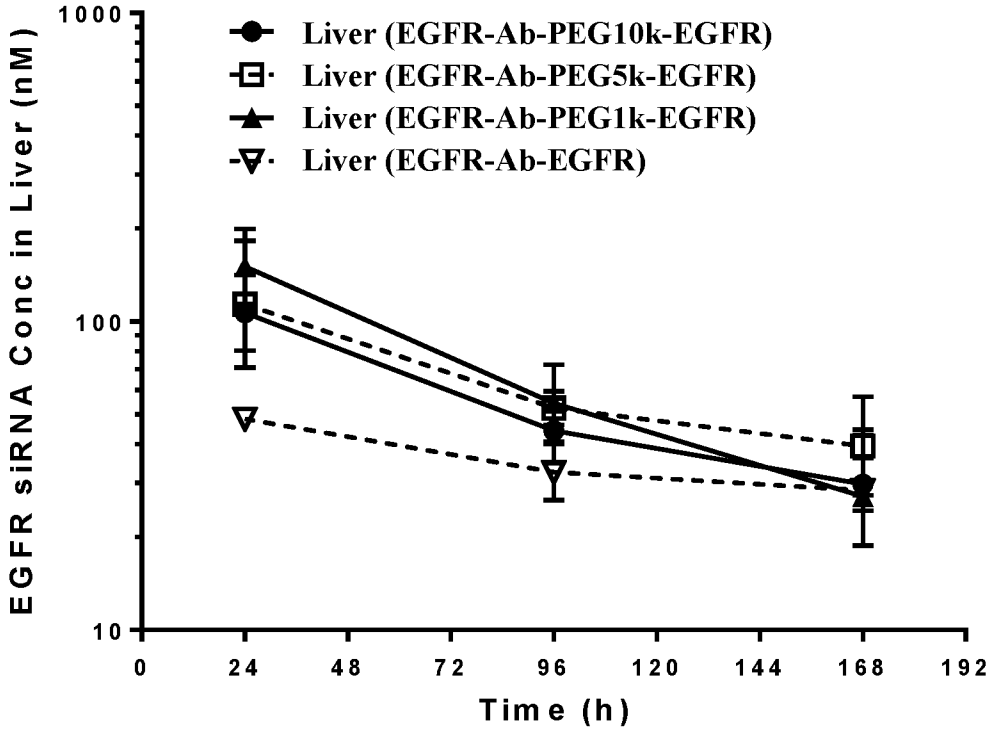


FIG. 27

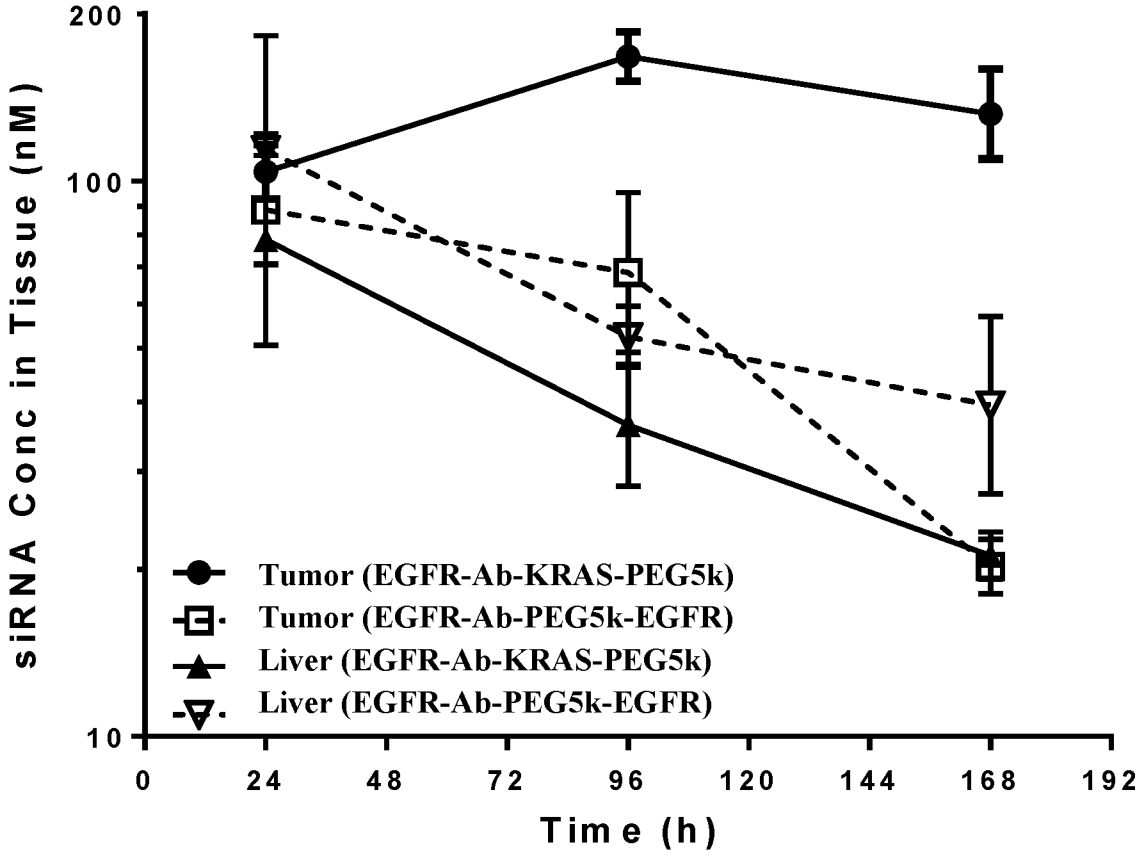

200
100
10
siRNA Conc in Tissue (nM)
0
24
48
72
96
120
144
168
192
Time (h)
Tumor (EGFR-Ab-KRAS-PEG5k)
Tumor (EGFR-Ab-PEG5k-EGFR)
Liver (EGFR-Ab-KRAS-PEG5k)
Liver (EGFR-Ab-PEG5k-EGFR)

FIG. 28

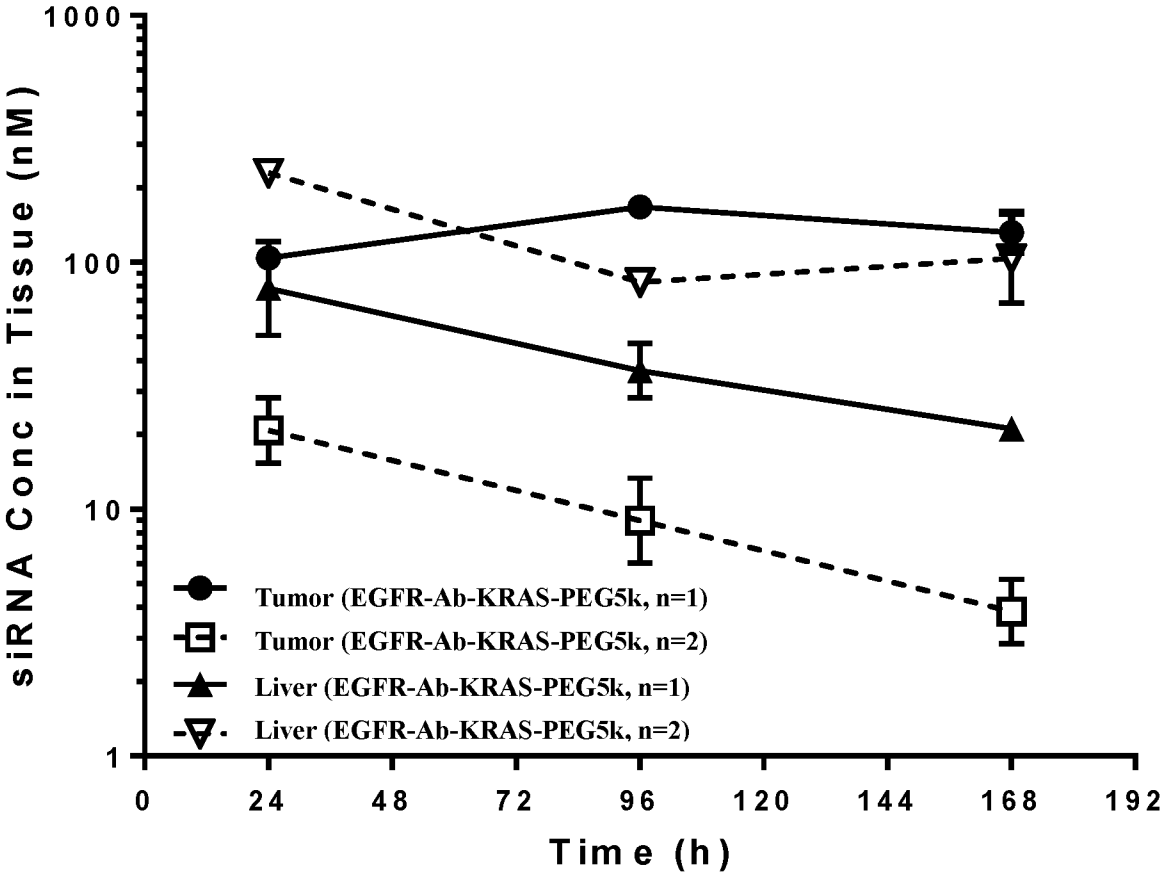

1000
100
10
1
siRNA Conc in Tissue (nM)
0
24
48
72
96
120
144
168
192
Time (h)
Tumor (EGFR-Ab-KRAS-PEG5k, n=1)
Tumor (EGFR-Ab-KRAS-PEG5k, n=2)
Liver (EGFR-Ab-KRAS-PEG5k, n=1)
Liver (EGFR-Ab-KRAS-PEG5k, n=2)

FIG. 29

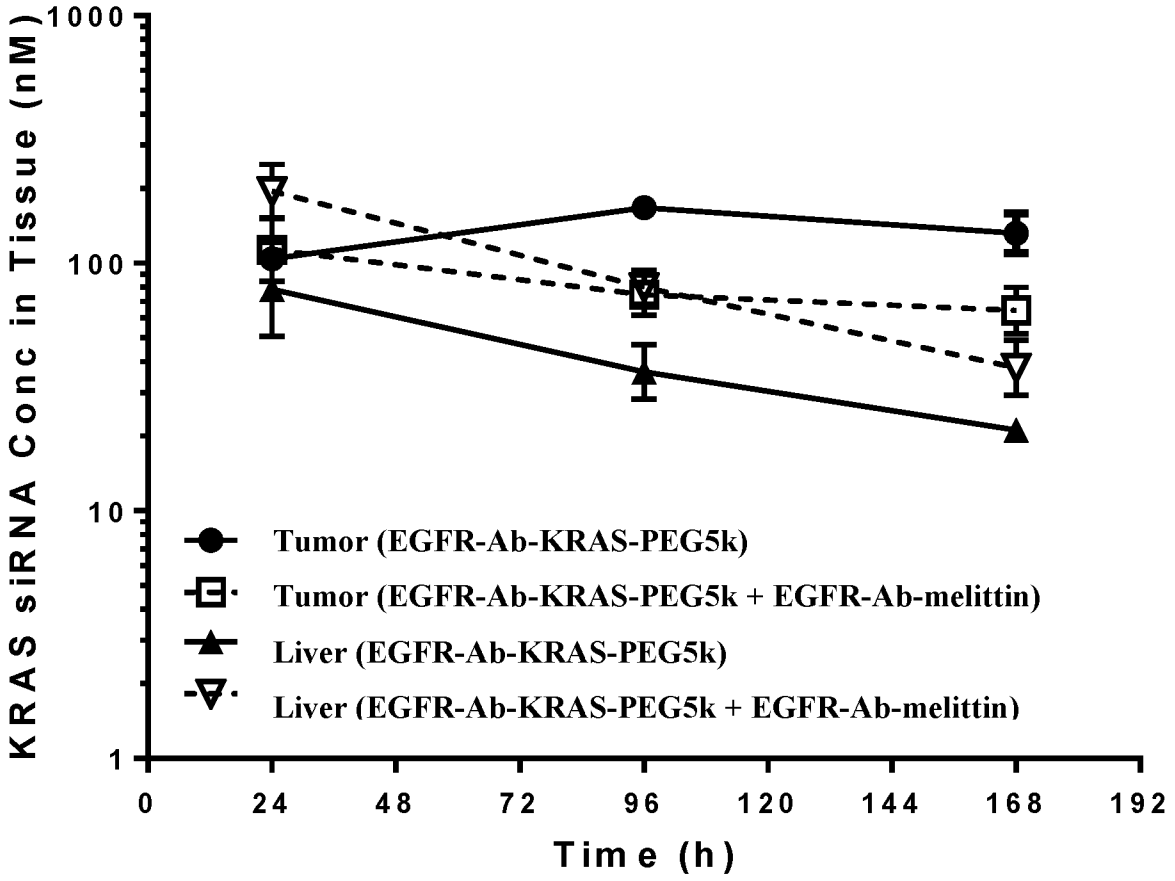

KRAS siRNA Conc in Tissue (nM)
1000
100
10
1
0
24
48
72
96
120
144
168
192
Time (h)
Tumor (EGFR-Ab-KRAS-PEG5k)
Tumor (EGFR-Ab-KRAS-PEG5k + EGFR-Ab-melittin)
Liver (EGFR-Ab-KRAS-PEG5k)
Liver (EGFR-Ab-KRAS-PEG5k + EGFR-Ab-melittin)

FIG. 30

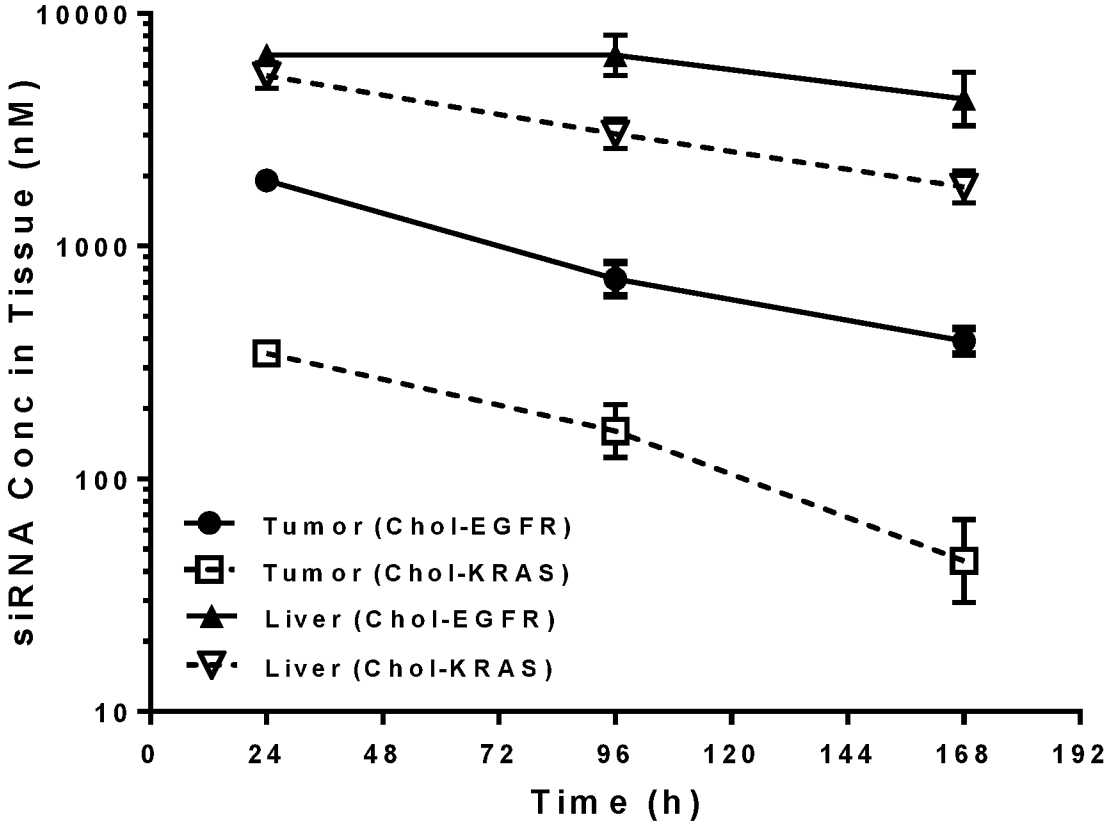

10000
1000
100
10
siRNA Conc in Tissue (nM)
0
24
48
72
96
120
144
168
192
Time (h)
Tumor (Chol-EGFR)
Tumor (Chol-KRAS)
Liver (Chol-EGFR)
Liver (Chol-KRAS)

FIG. 31A

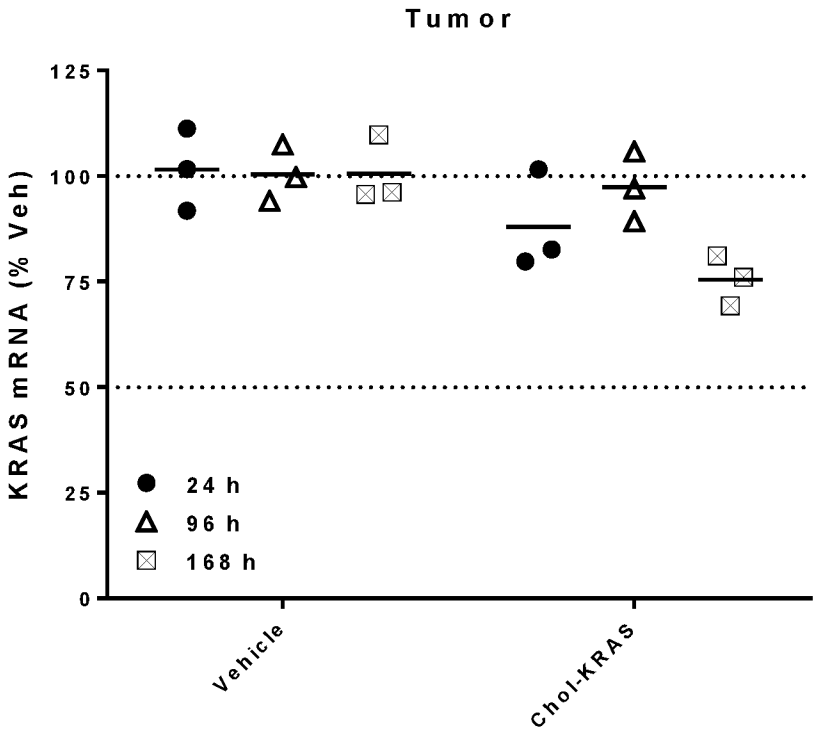


FIG. 31B

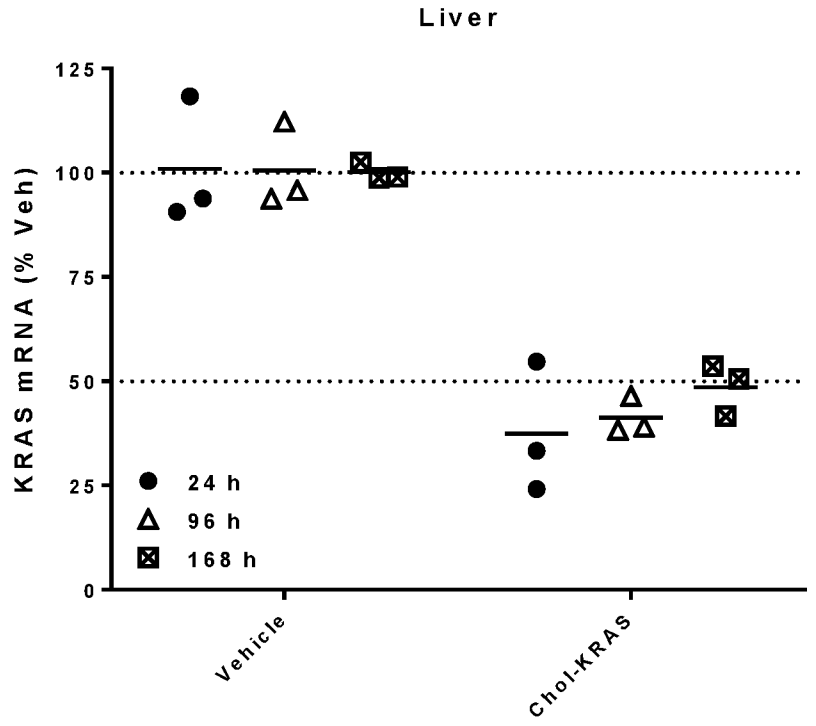


FIG. 32

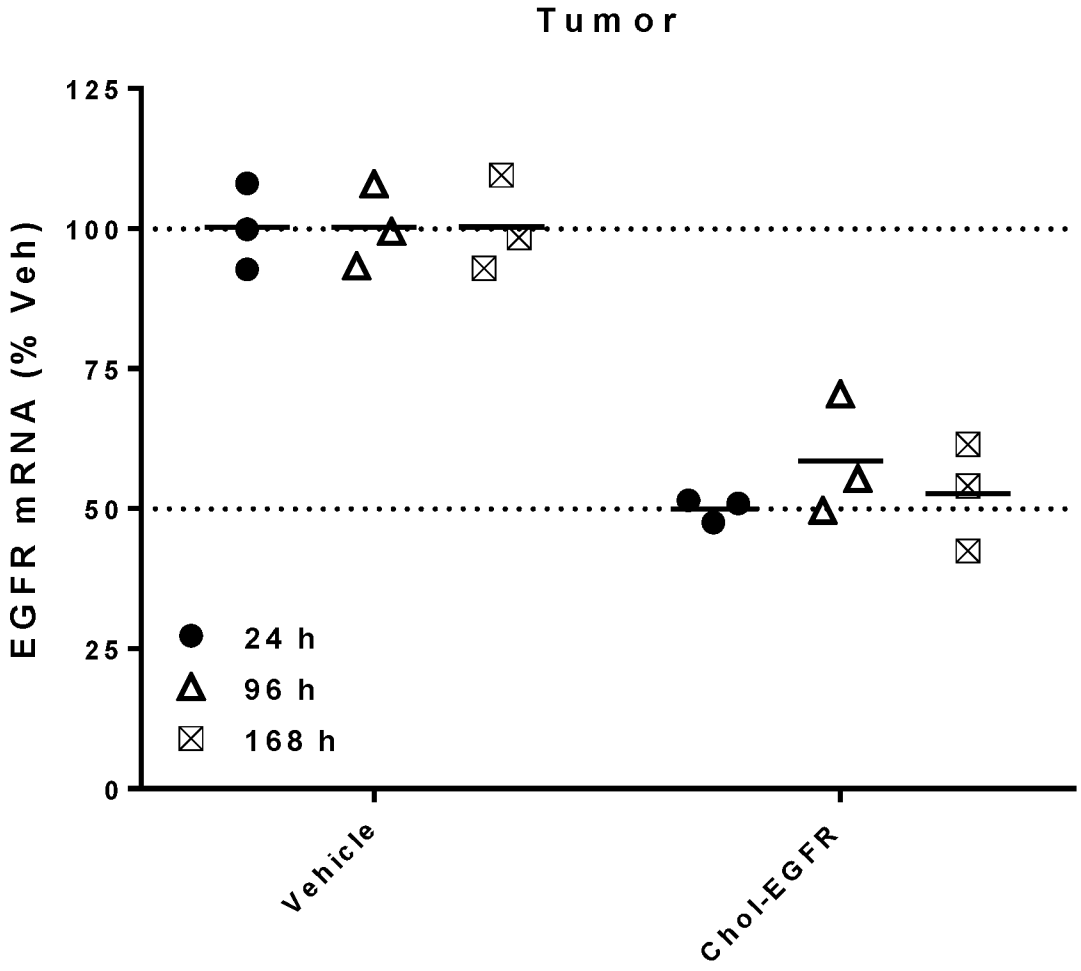

Tumor
EGFR mRNA (% Veh)
125
100
75
50
25
0
24 h
96 h
168 h
Vehicle
Chol-EGFR

FIG. 33

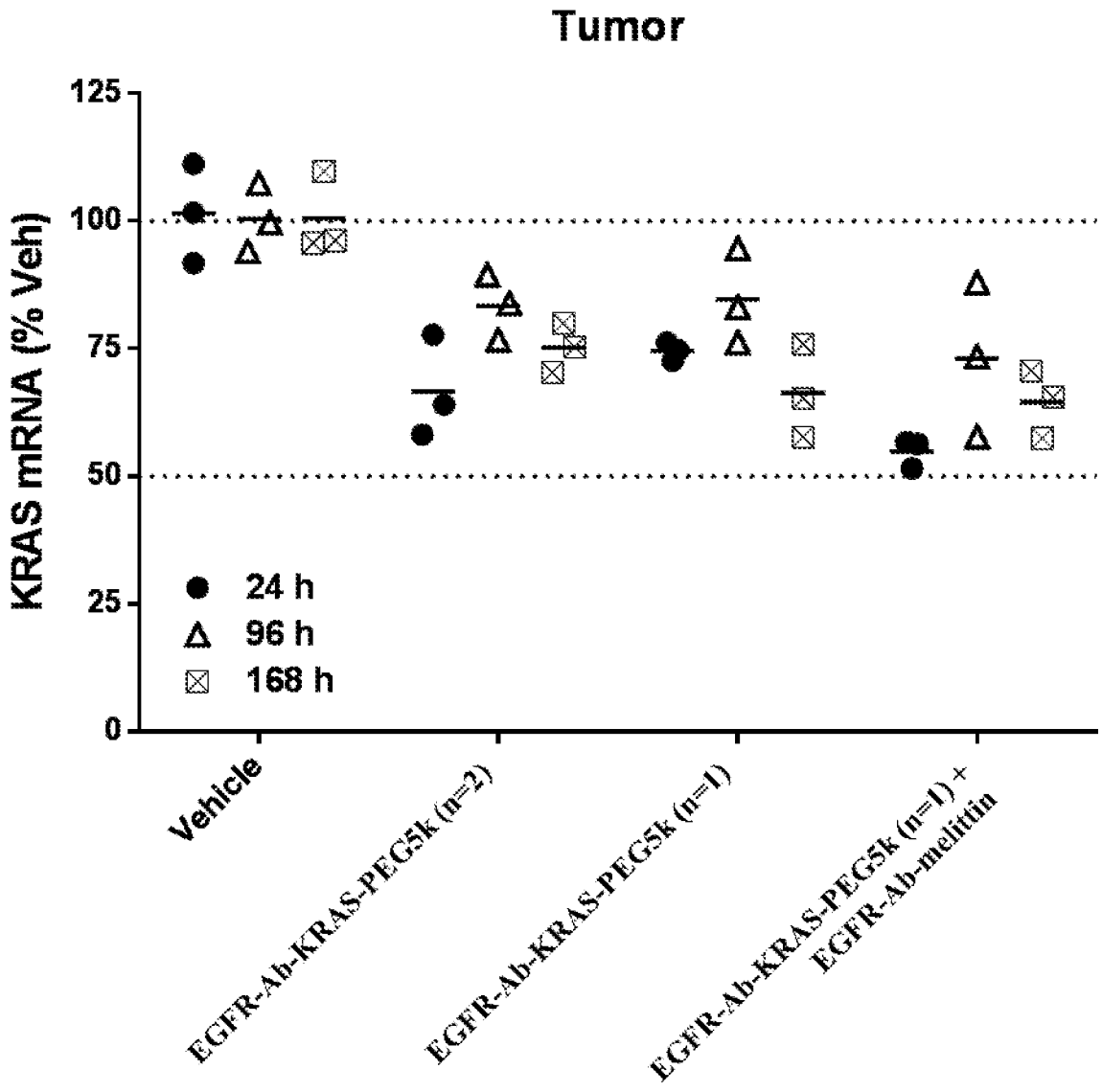

Tumor
KRAS mRNA (% Veh)
125
100
75
50
25
0
24 h
96 h
168 h
Vehicle
EGFR-Ab-KRAS-PEG5k (n=2)
EGFR-Ab-KRAS-PEG5k (n=1)
EGFR-Ab-KRAS-PEG5k (n=1) + EGFR-Ab-melittin

FIG. 34

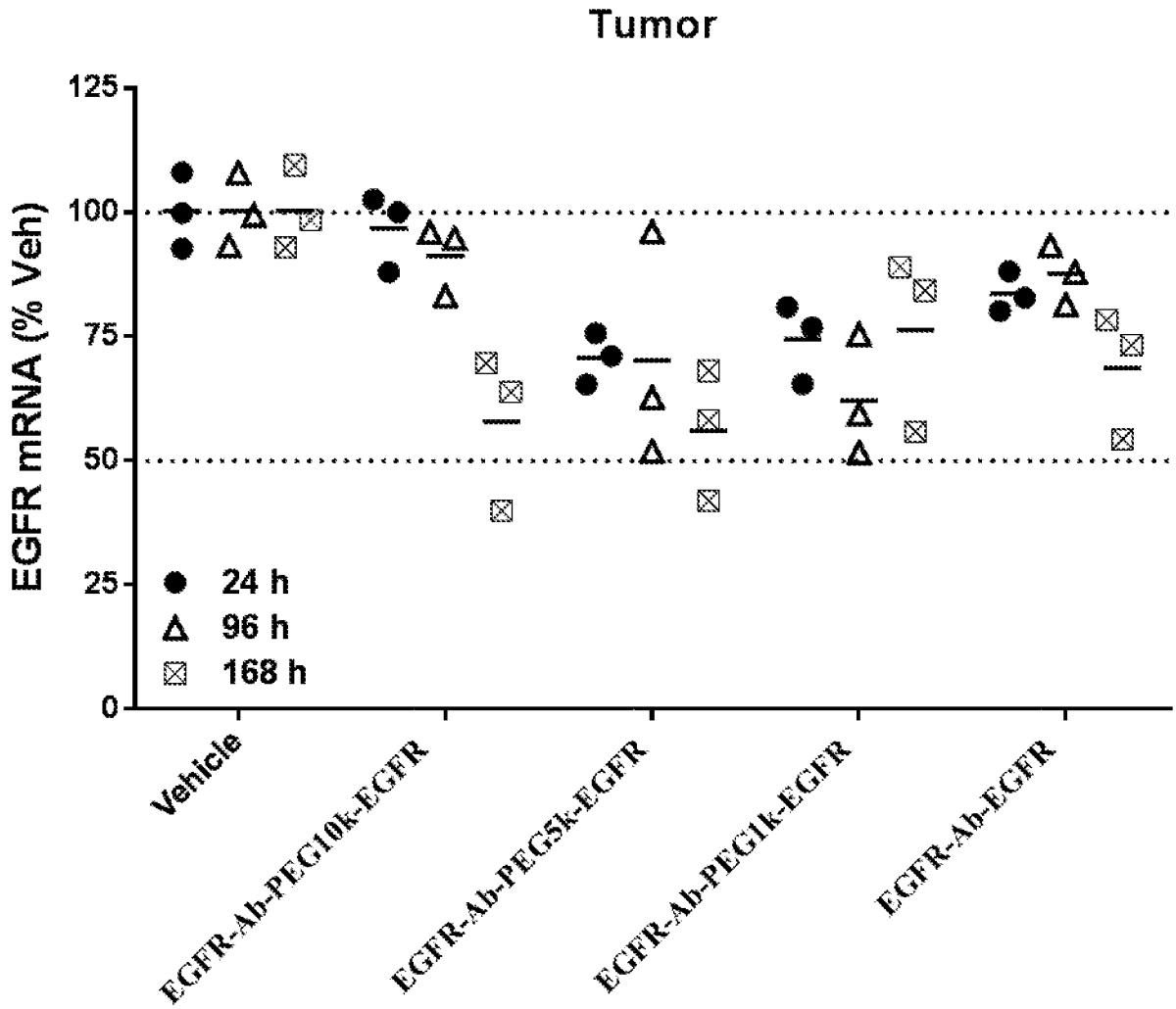

Tumor
EGFR mRNA (% Veh)
125
100
75
50
25
0
24 h
96 h
168 h
Vehicle
EGFR-Ab-PEG10k-EGFR
EGFR-Ab-PEG5k-EGFR
EGFR-Ab-PEG1k-EGFR
EGFR-Ab-EGFR

FIG. 35

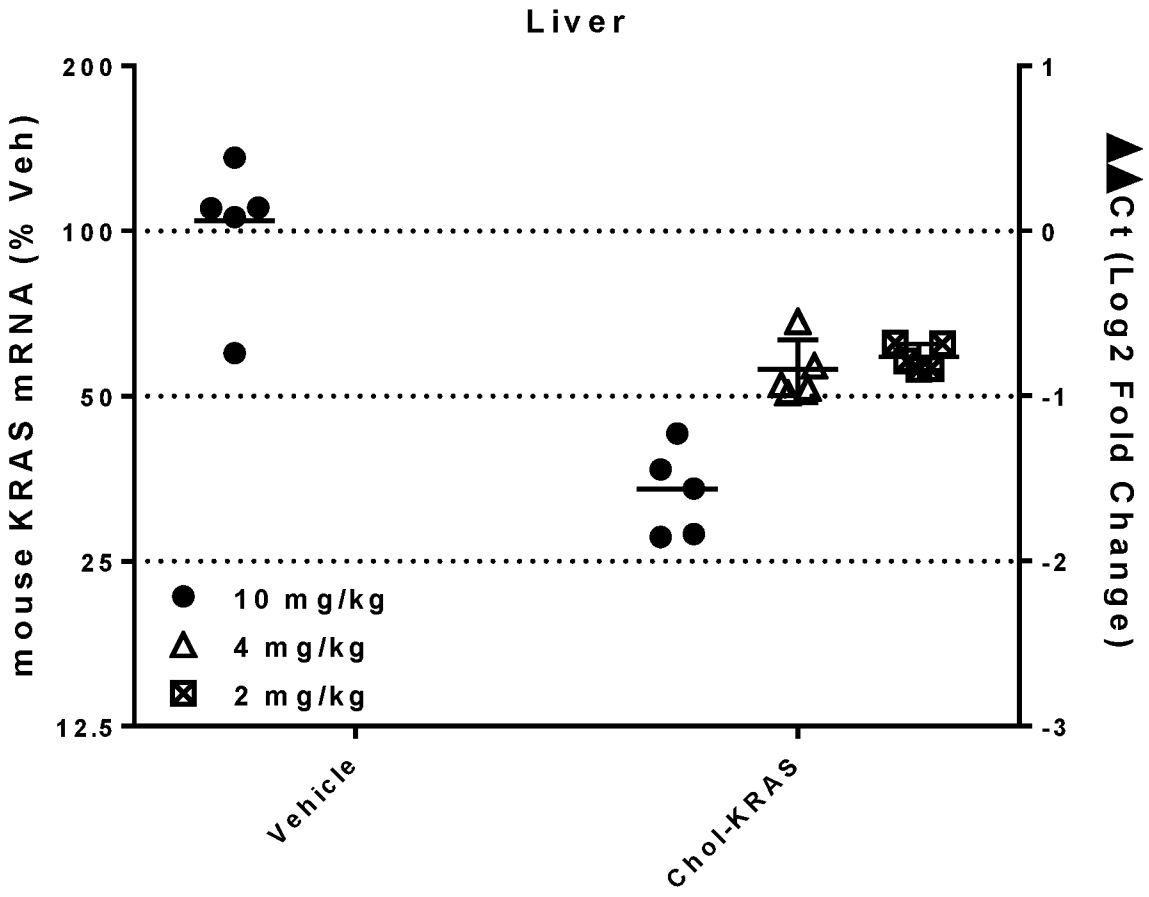

Liver
mouse KRAS mRNA (% Veh)
200
100
50
25
12.5
ΔΔCt (Log2 Fold Change)
1
0
-1
-2
-3
10 mg/kg
4 mg/kg
2 mg/kg
Vehicle
Chol-KRAS

FIG. 36

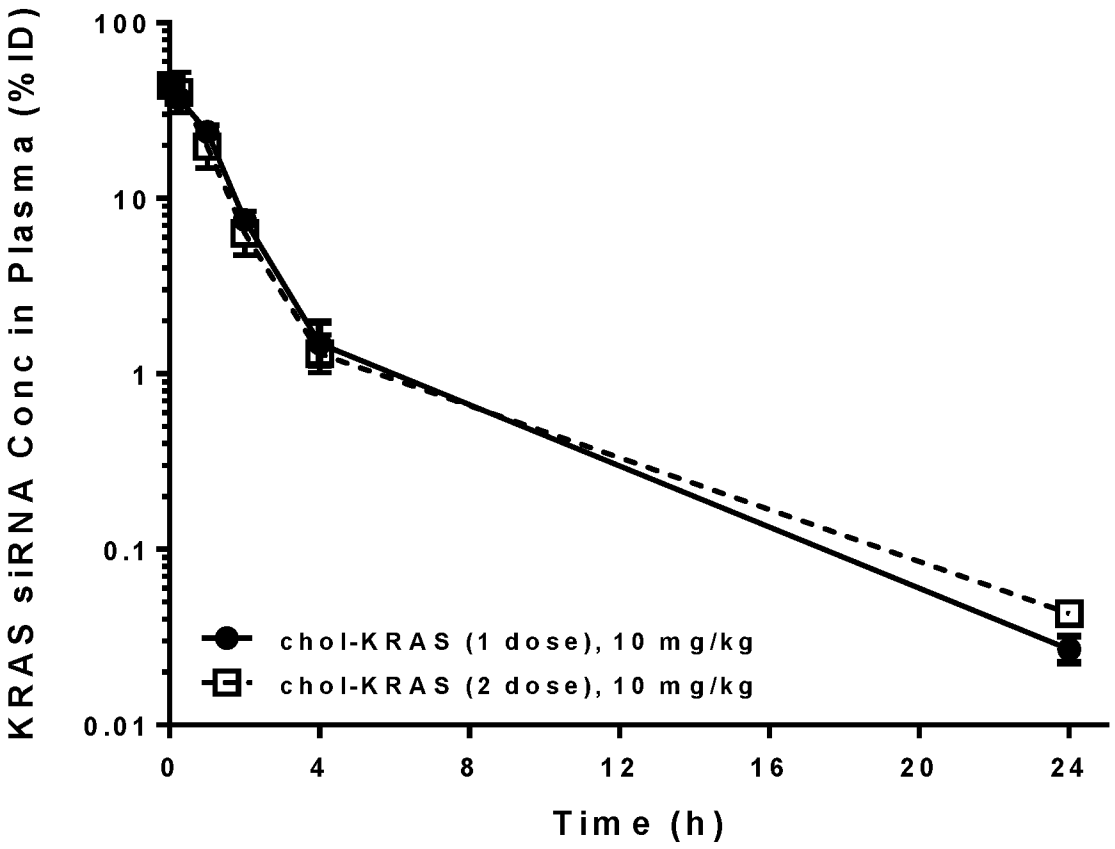

KRAS siRNA Conc in Plasma (%ID)
100
10
1
0.1
0.01
0
4
8
12
16
20
24
Time (h)
chol-KRAS (1 dose), 10 mg/kg
chol-KRAS (2 dose), 10 mg/kg

FIG. 37

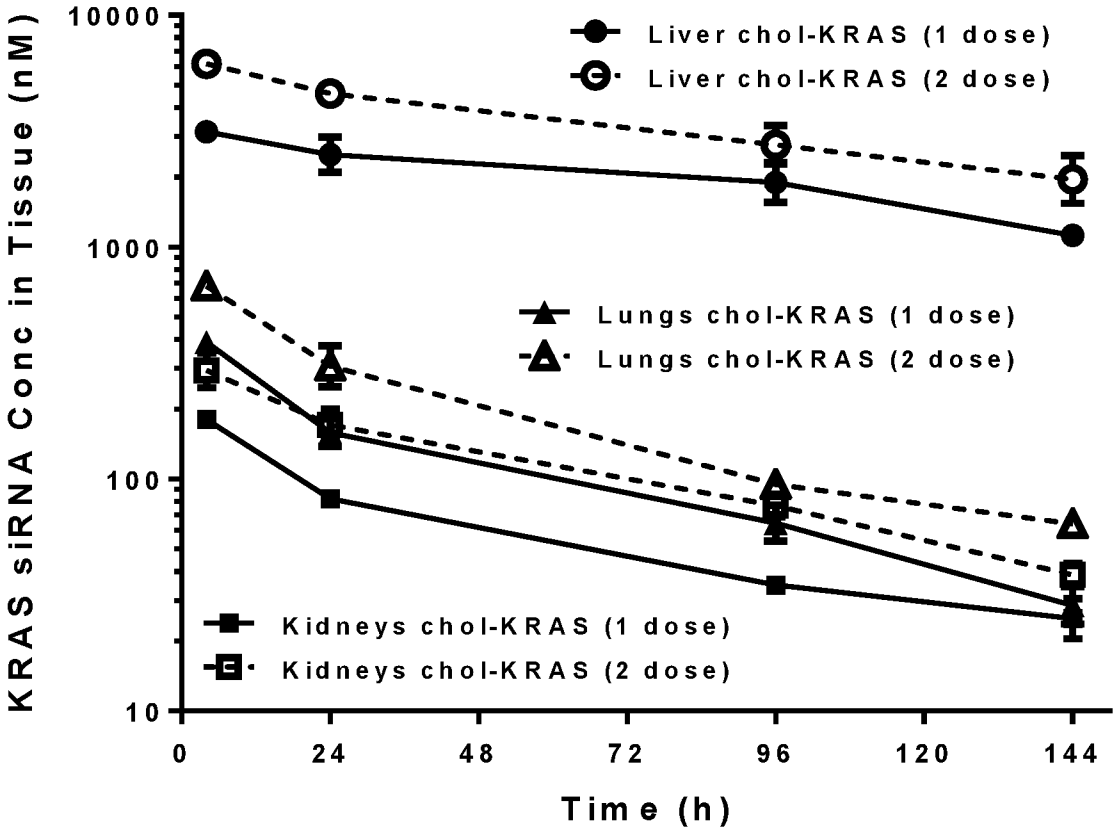

KRAS siRNA Conc in Tissue (nM)
10000
1000
100
10
0
24
48
72
96
120
144
Time (h)
Liver chol-KRAS (1 dose)
Liver chol-KRAS (2 dose)
Lungs chol-KRAS (1 dose)
Lungs chol-KRAS (2 dose)
Kidneys chol-KRAS (1 dose)
Kidneys chol-KRAS (2 dose)

FIG. 38A

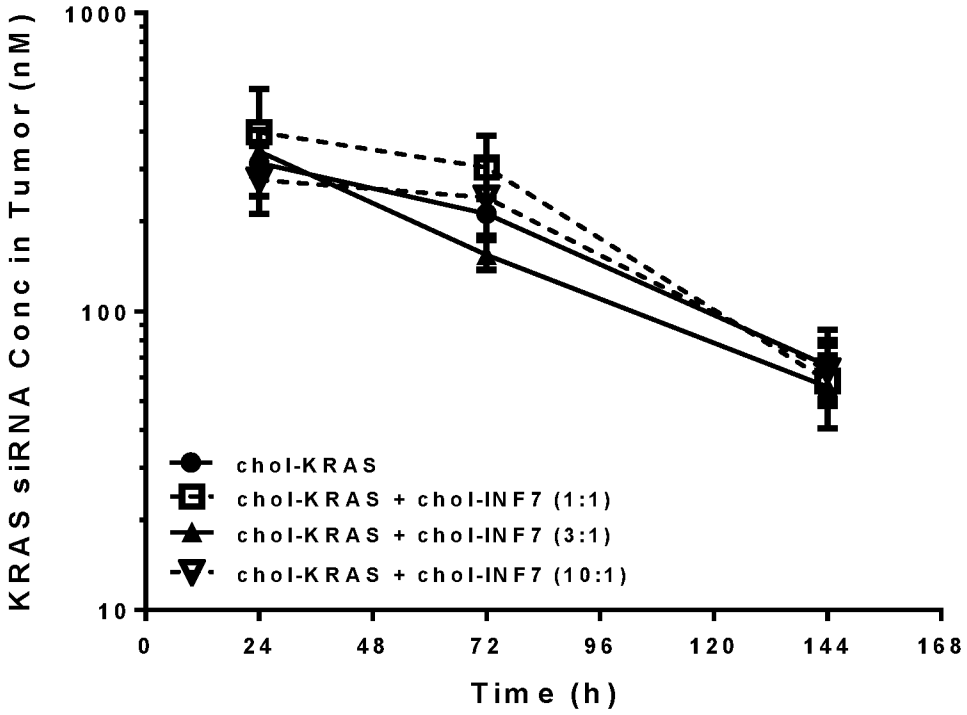


FIG. 38B

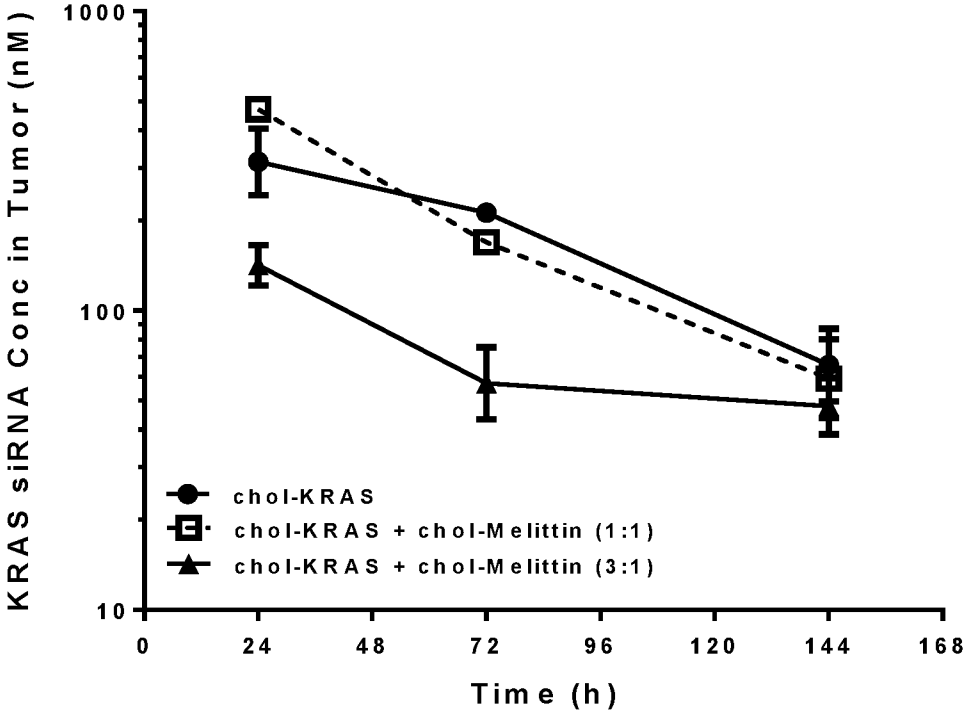


FIG. 39A

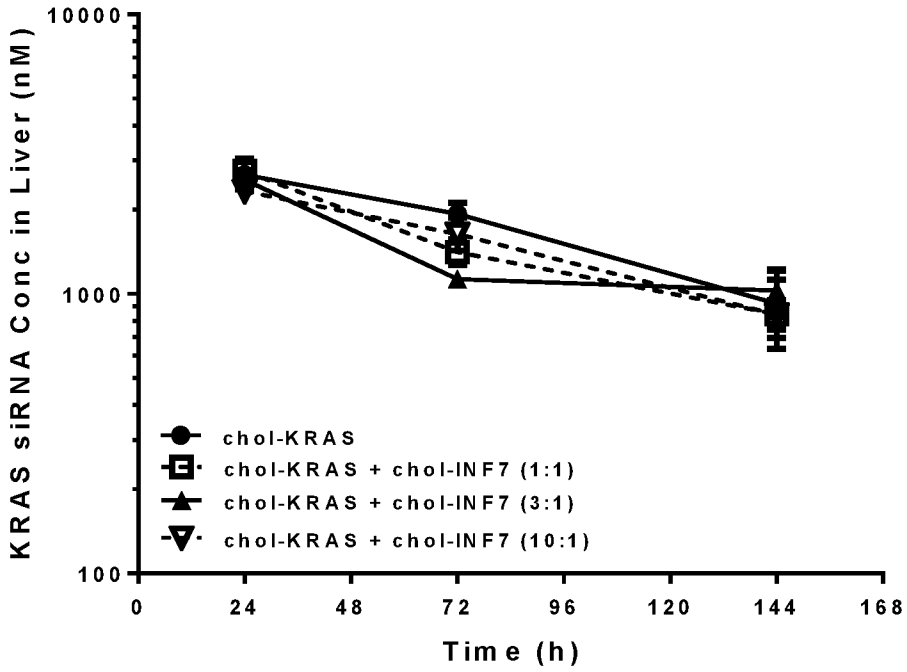


FIG. 39B

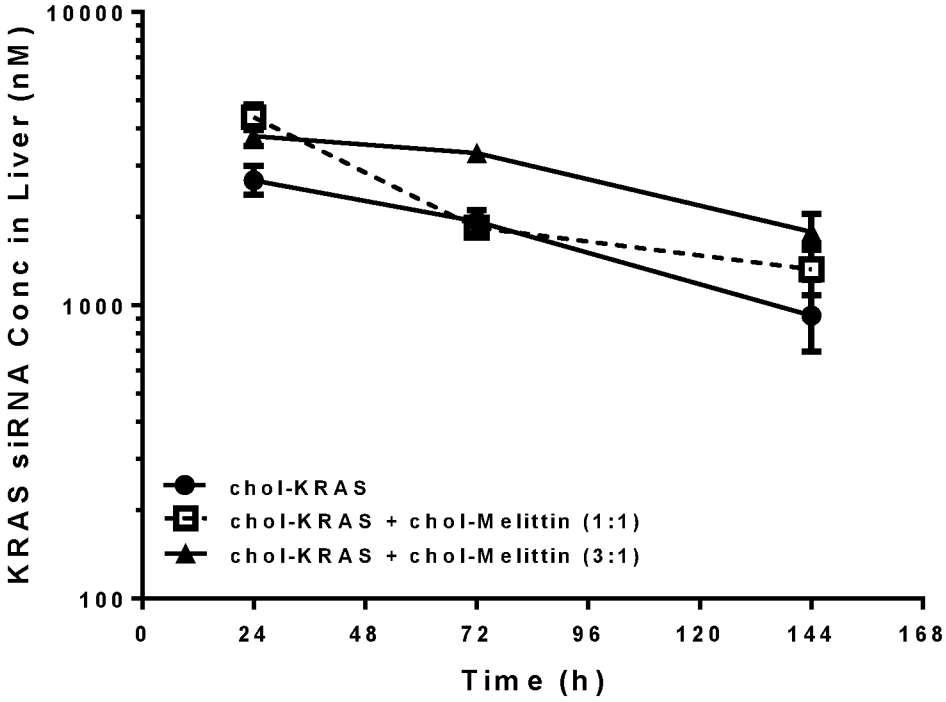


FIG. 40A

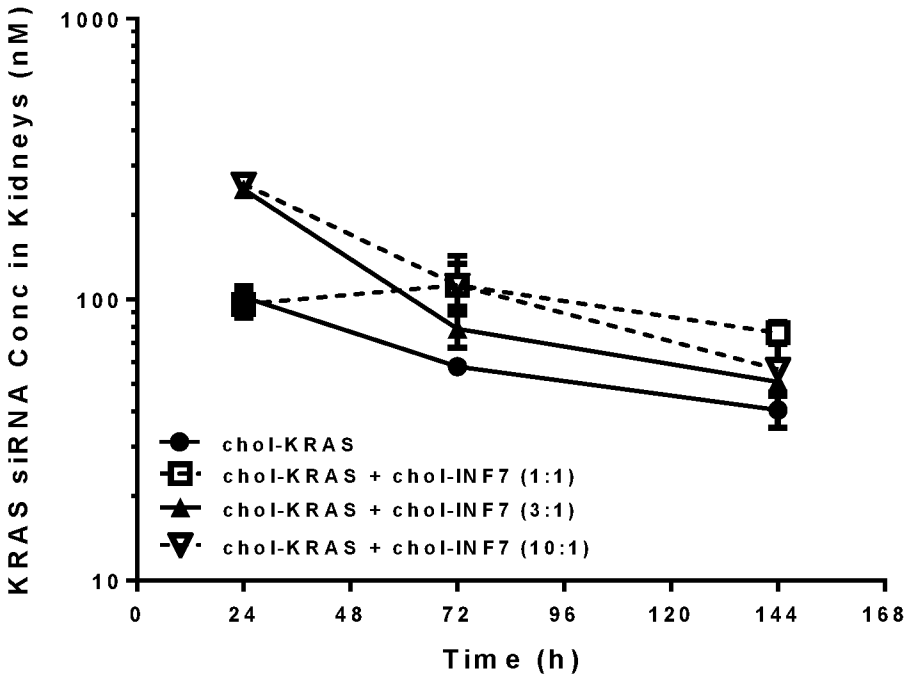


FIG. 40B

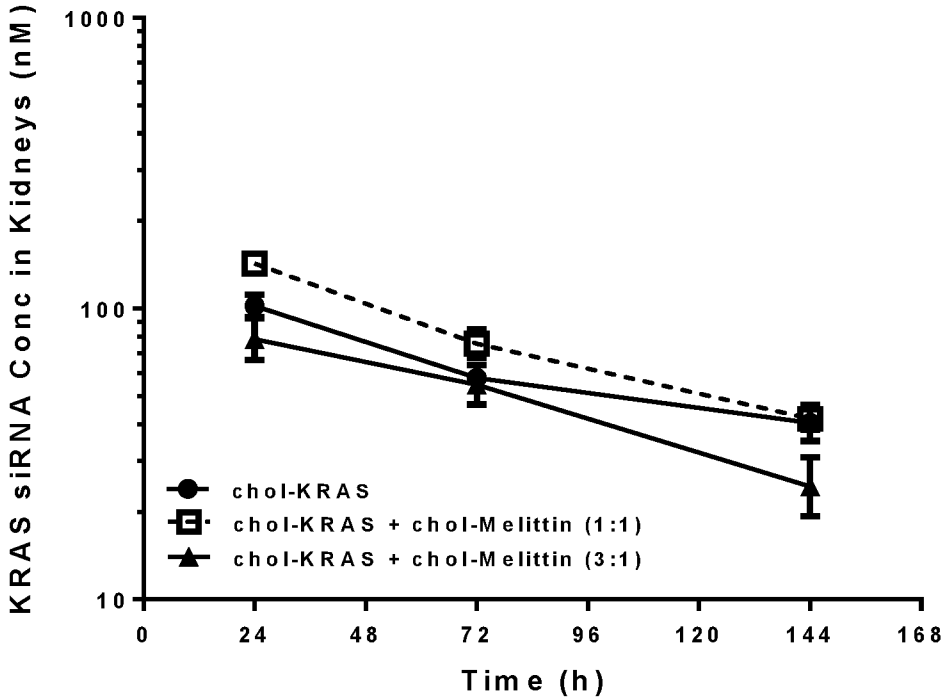


FIG. 41A

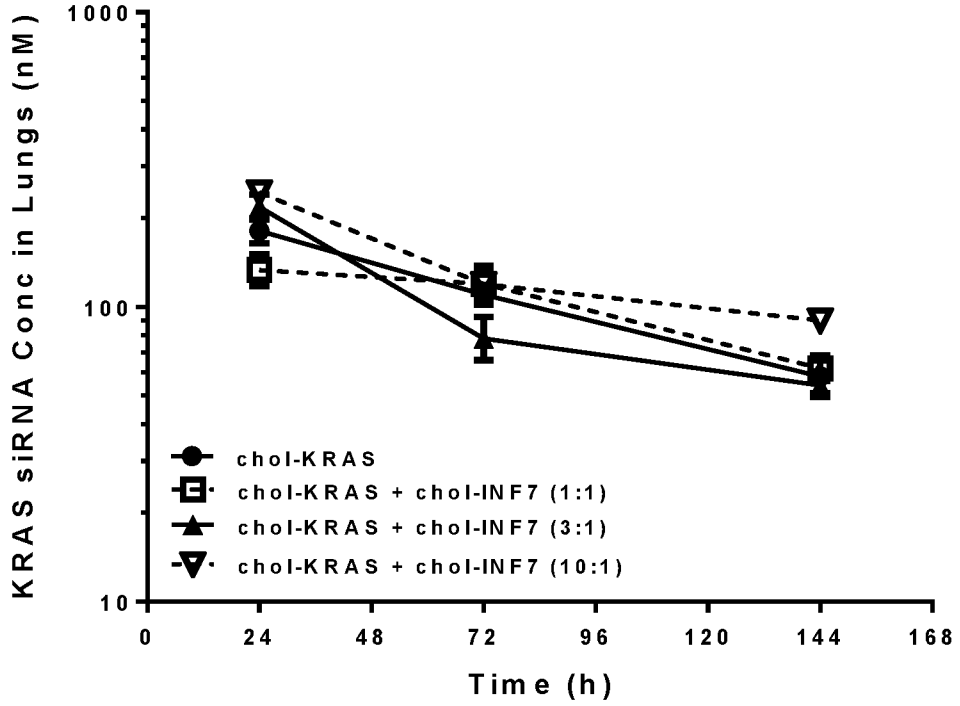


FIG. 41B

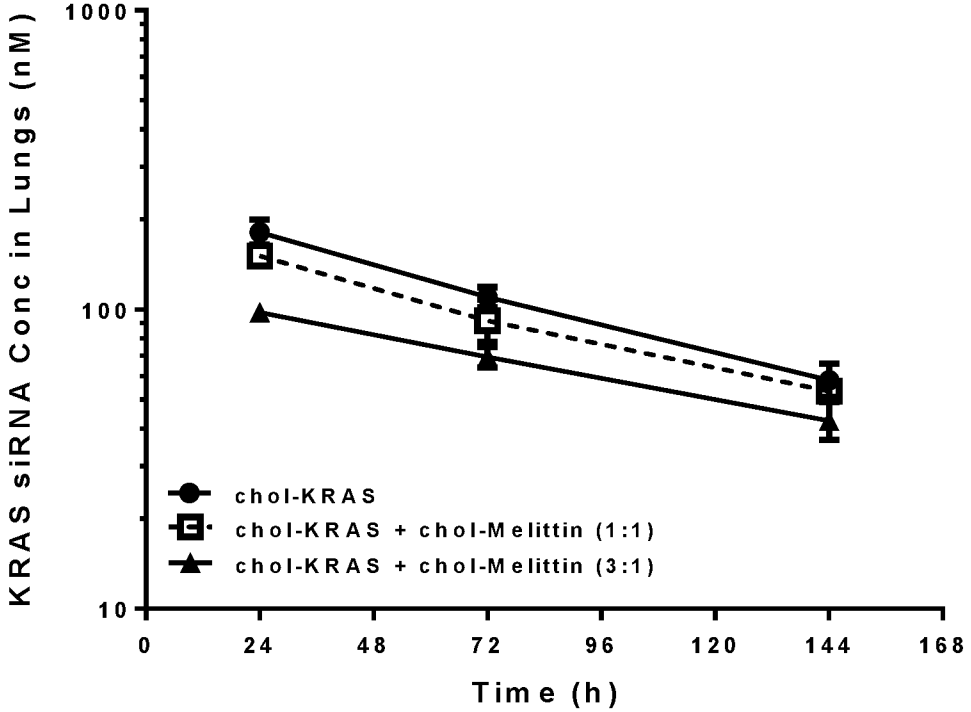


FIG. 42

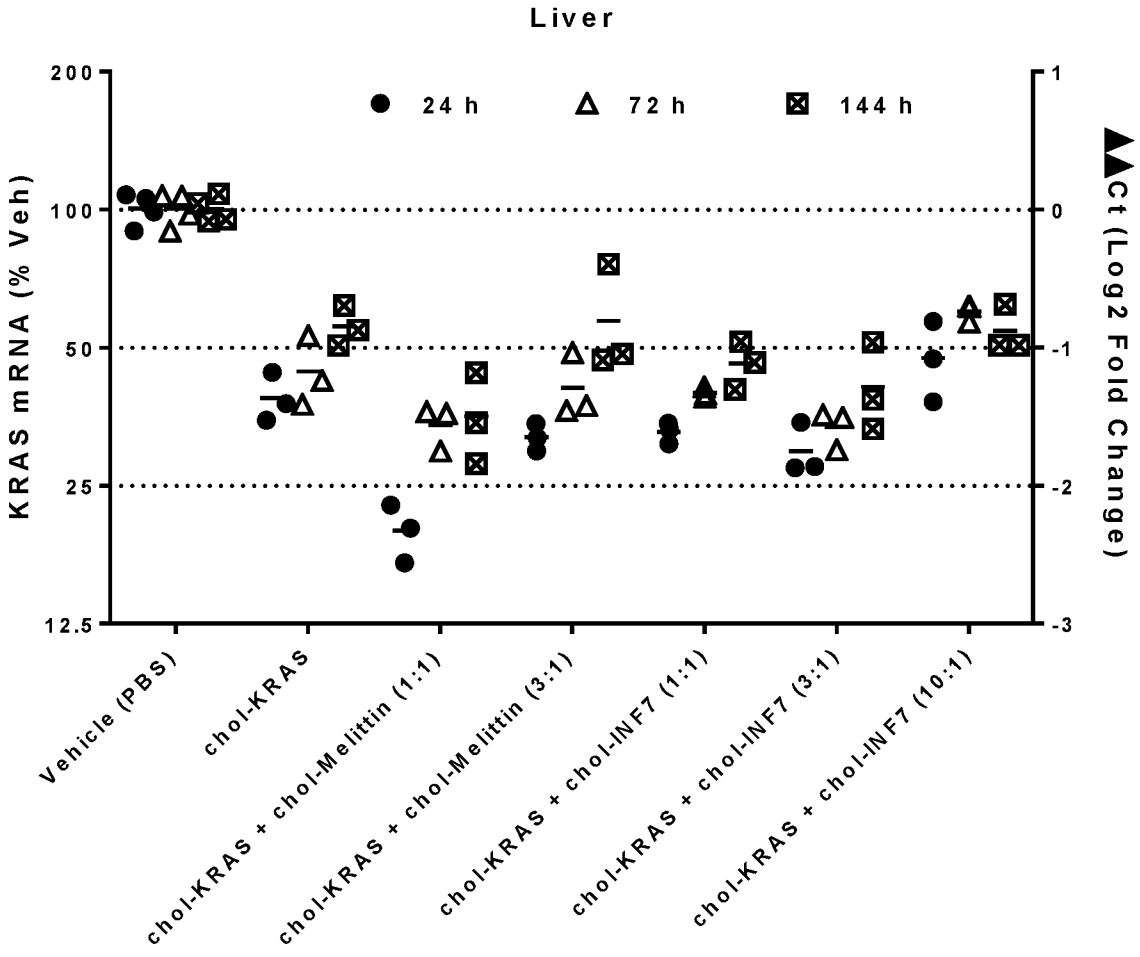

Liver
24 h
72 h
144 h
KRAS mRNA (% Veh)
ΔΔCt (Log2 Fold Change)
200
100
50
25
12.5
1
0
-1
-2
-3
Vehicle (PBS)
chol-KRAS
chol-KRAS + chol-Melittin (1:1)
chol-KRAS + chol-Melittin (3:1)
chol-KRAS + chol-INF7 (1:1)
chol-KRAS + chol-INF7 (3:1)
chol-KRAS + chol-INF7 (10:1)

FIG. 43A

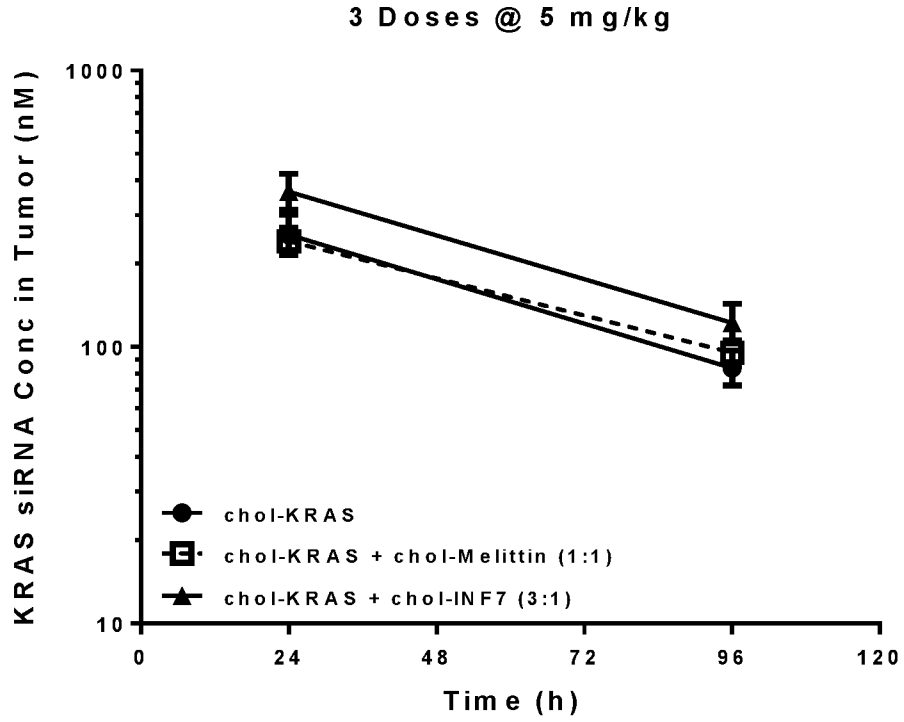


FIG. 43B

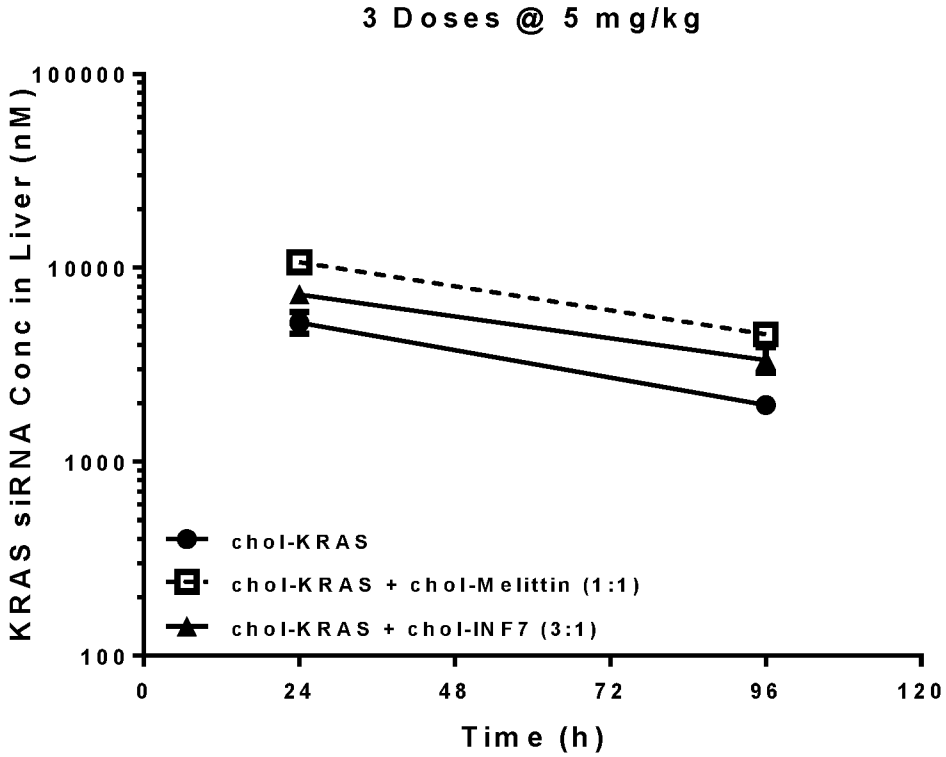


FIG. 44A

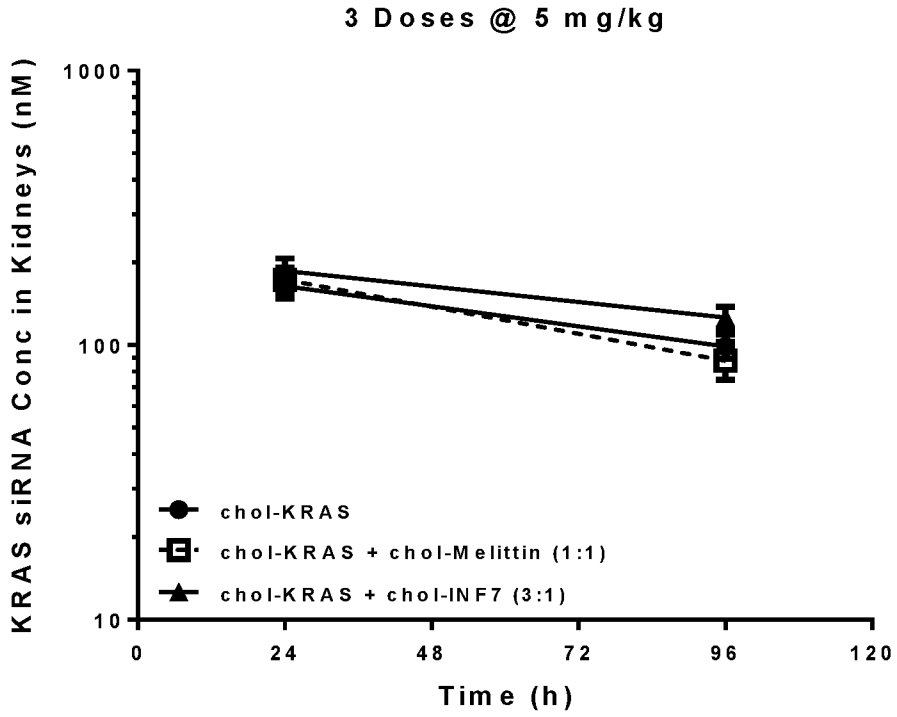


FIG. 44B

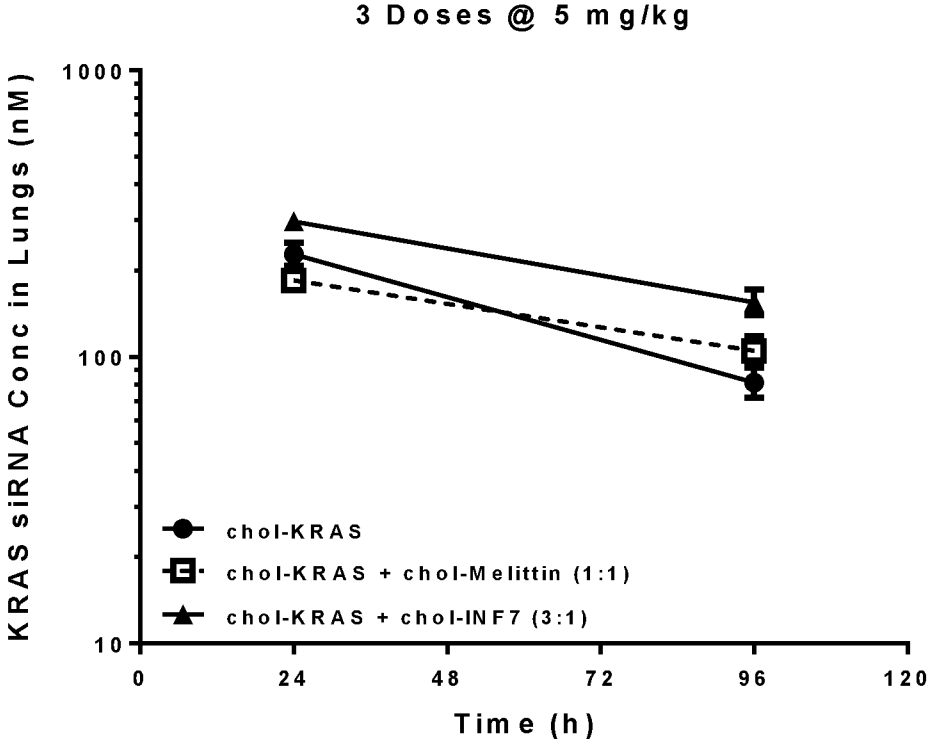


FIG. 45

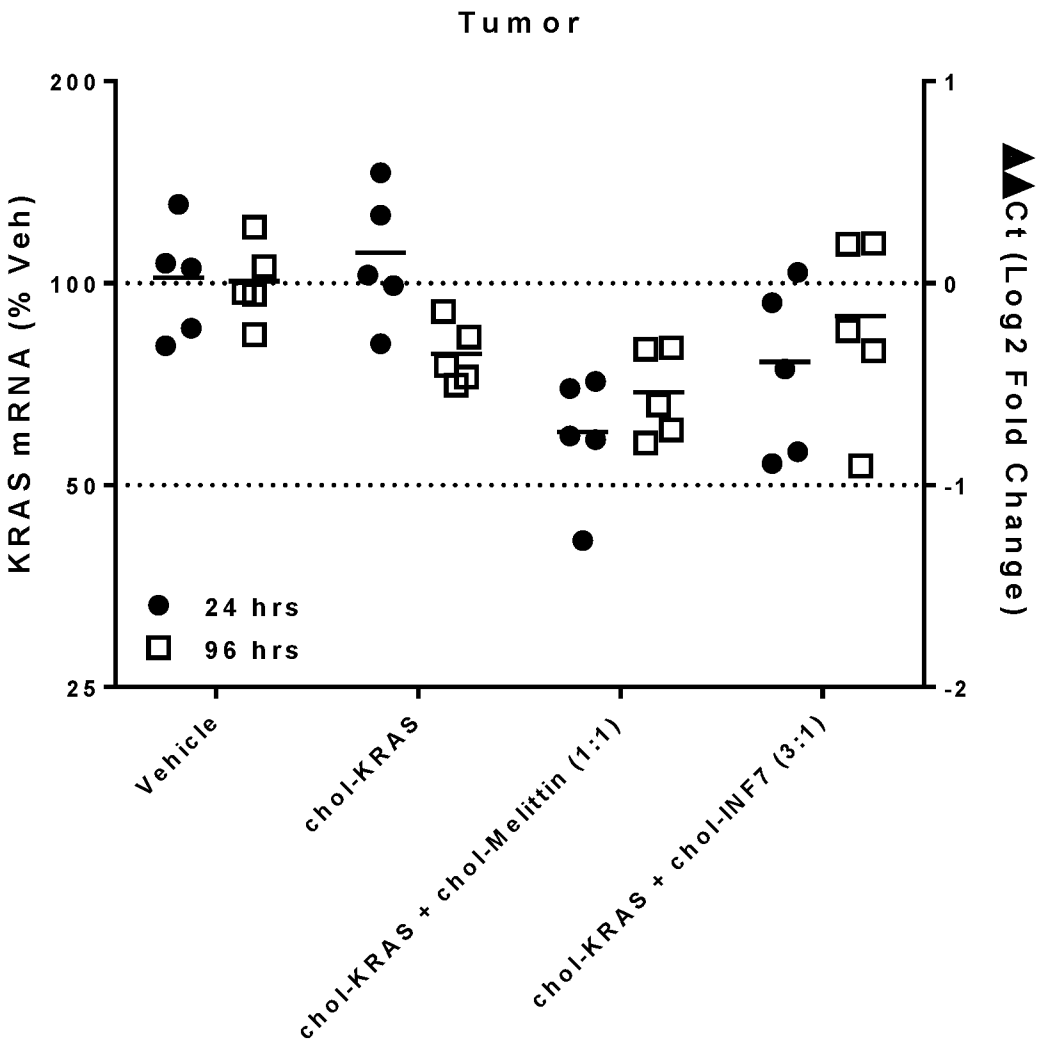

Tumor
KRAS mRNA (% Veh)
ΔΔCt (Log2 Fold Change)
200
100
50
25
1
0
-1
-2
24 hrs
96 hrs
Vehicle
chol-KRAS
chol-KRAS + chol-Melittin (1:1)
chol-KRAS + chol-INF7 (3:1)

FIG. 46

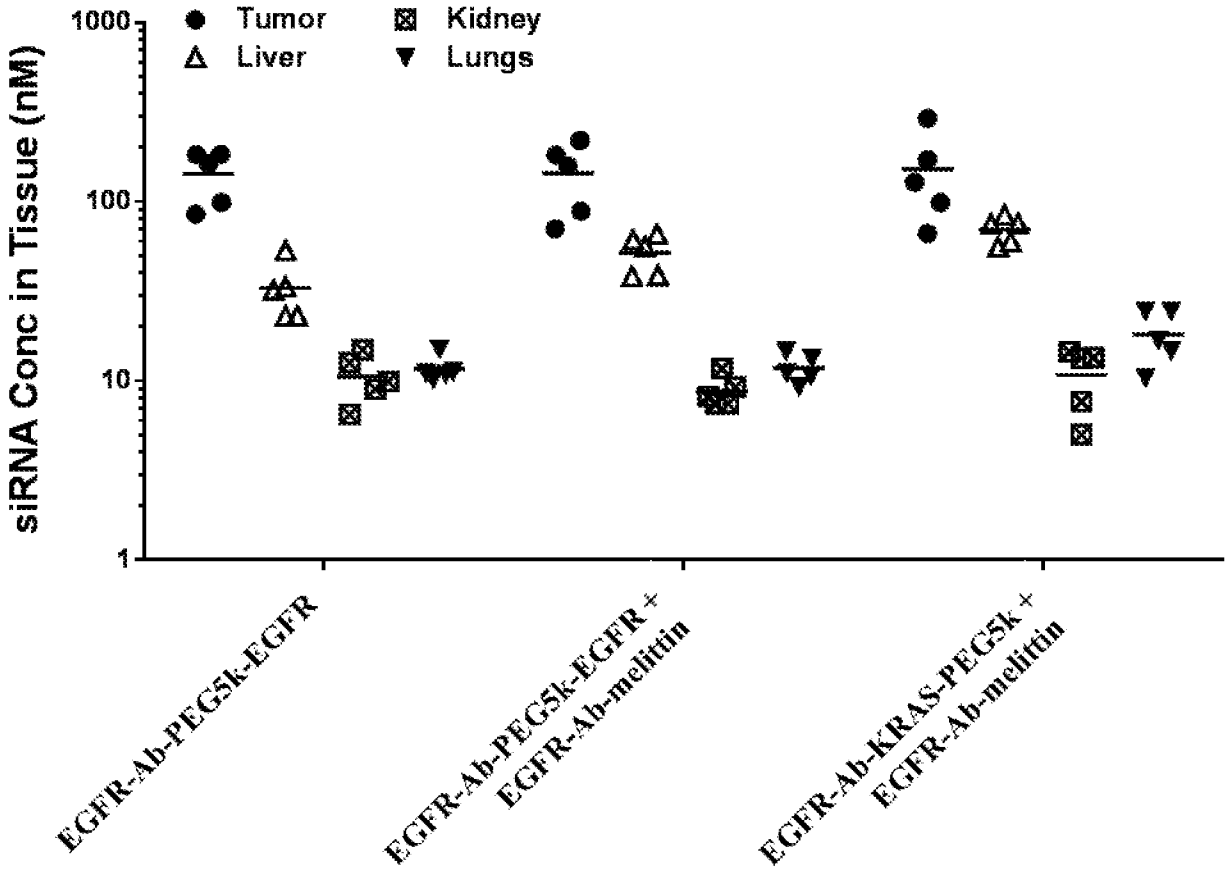

siRNA Conc in Tissue (nM)
1000
100
10
1
Tumor
Liver
Kidney
Lungs
EGFR-Ab-PEG5k-EGFR
EGFR-Ab-PEG5k-EGFR + EGFR-Ab-melittin
EGFR-Ab-KRAS-PEG5k + EGFR-Ab-melittin

FIG. 47A

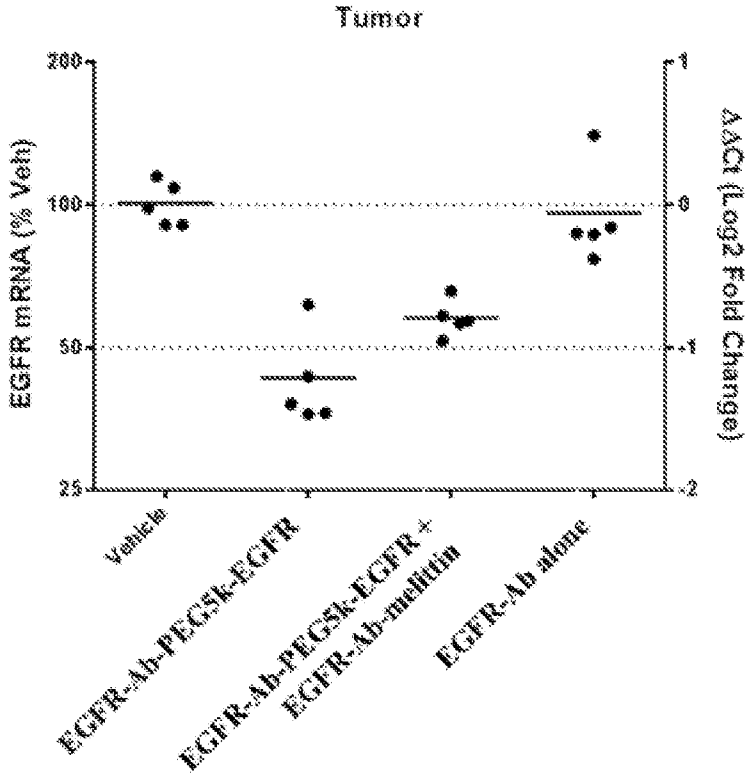


FIG. 47B

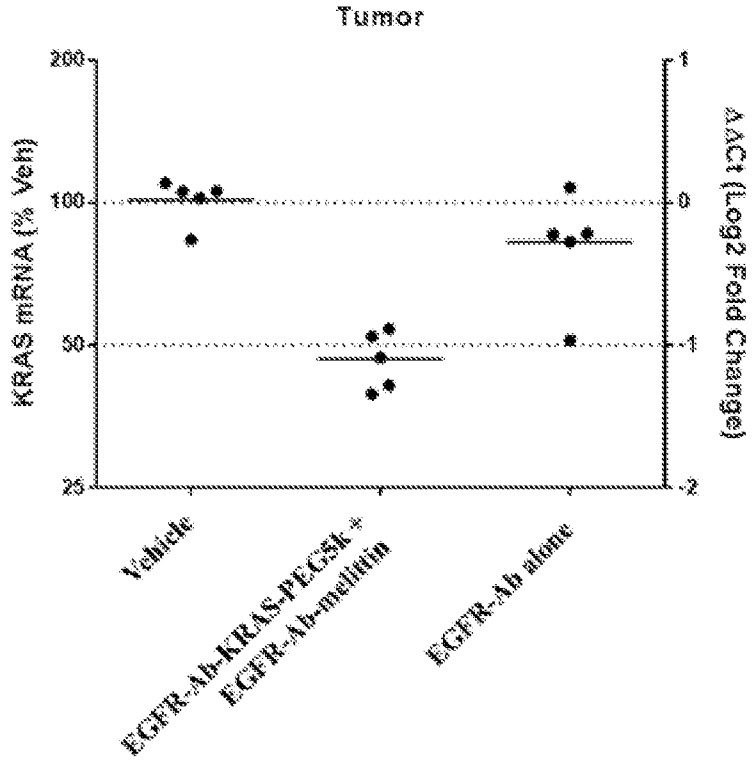


FIG. 48

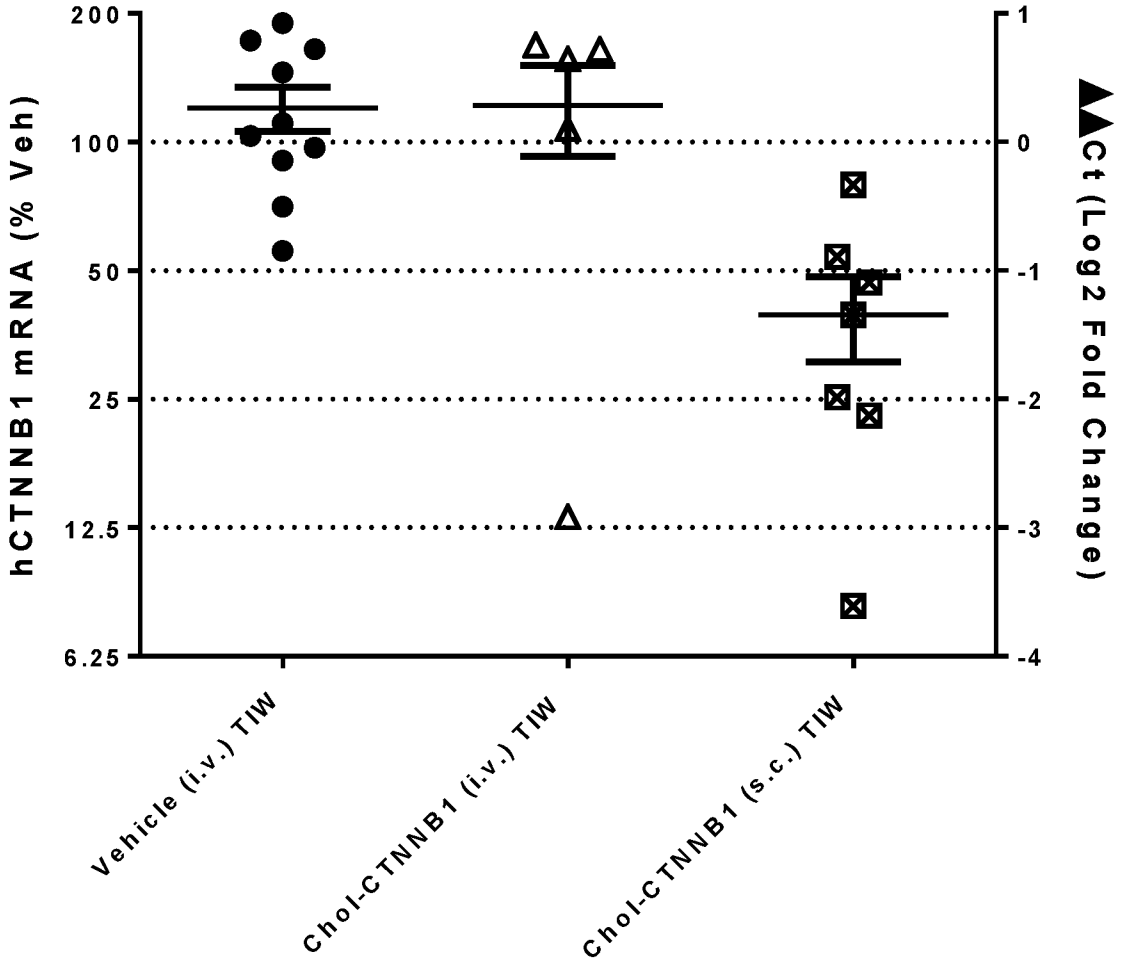

hCTNNB1 mRNA (% Veh)
200
100
50
25
12.5
6.25
ΔΔCt (Log2 Fold Change)
1
0
-1
-2
-3
-4
Vehicle (i.v.) TIW
ChoI-CTNNB1 (i.v.) TIW
ChoI-CTNNB1 (s.c.) TIW

FIG. 49

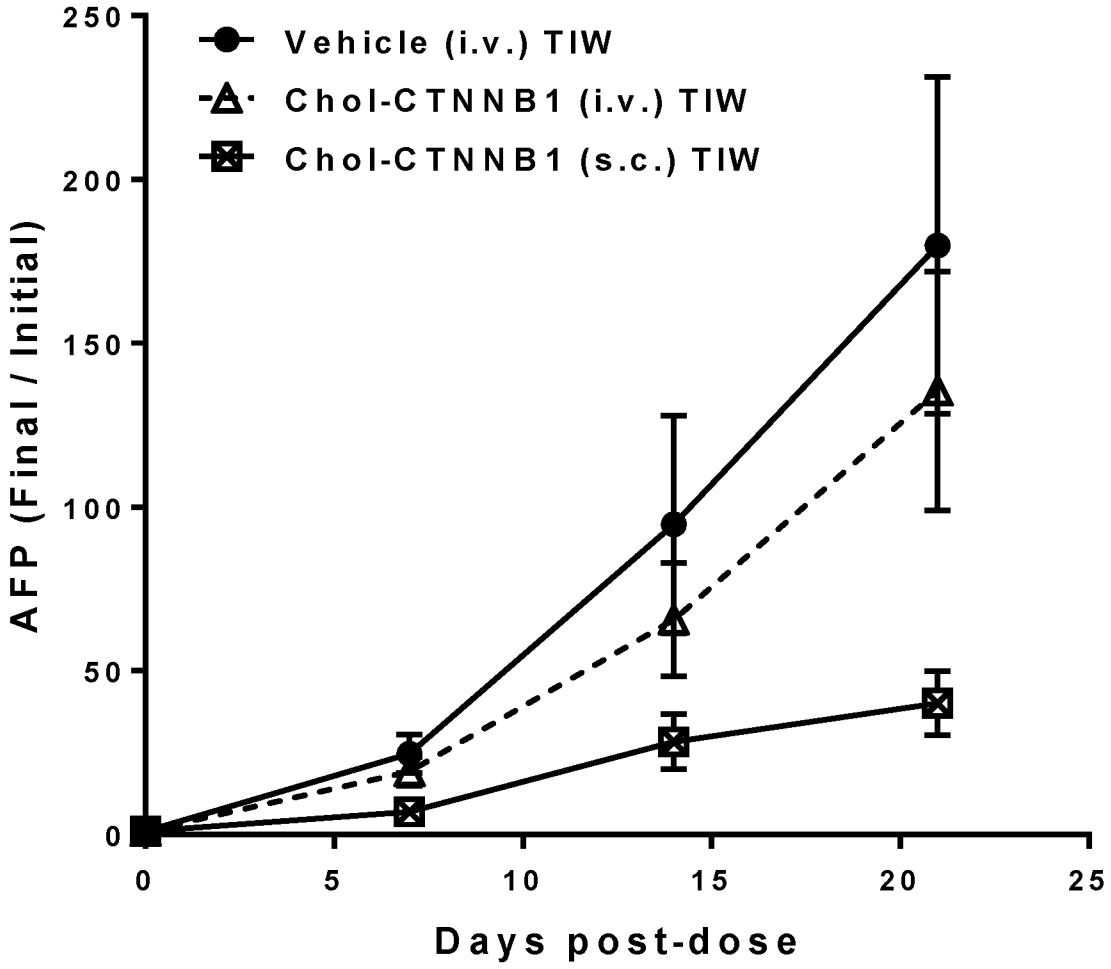

Vehicle (i.v.) TIW
Chol-CTNNB1 (i.v.) TIW
Chol-CTNNB1 (s.c.) TIW
AFP (Final / Initial)
250
200
150
100
50
0
0
5
10
15
20
25
Days post-dose

FIG. 50A

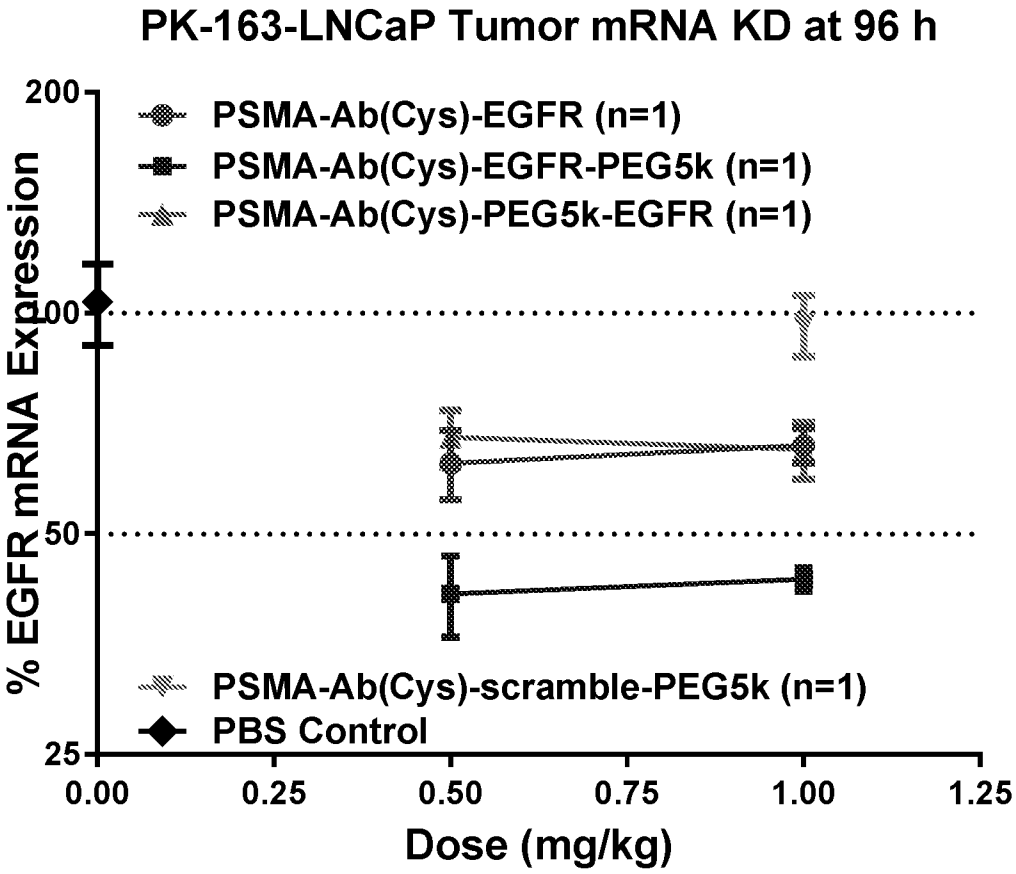

PK-163-LNCaP Tumor mRNA KD at 96 h
PSMA-Ab(Cys)-EGFR (n=1)
PSMA-Ab(Cys)-EGFR-PEG5k (n=1)
PSMA-Ab(Cys)-PEG5k-EGFR (n=1)
PSMA-Ab(Cys)-scramble-PEG5k (n=1)
PBS Control
% EGFR mRNA Expression
200
100
50
25
0.00
0.25
0.50
0.75
1.00
1.25
Dose (mg/kg)

FIG. 50B

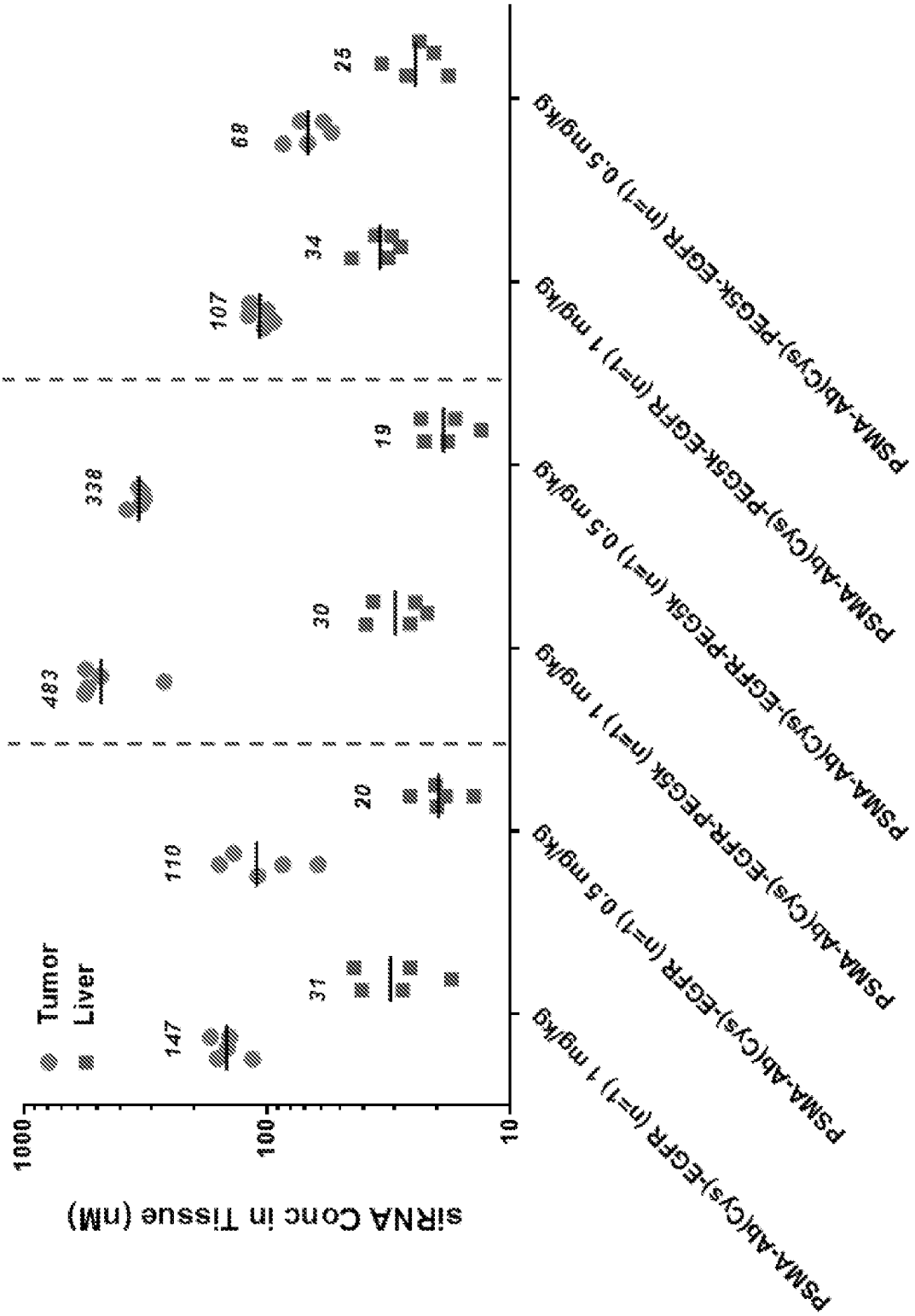

PK-163-LNCaP at 96 h
Tumor
Liver
siRNA Conc in Tissue (nM)
1000
100
10
483
338
30
19
107
34
68
25
147
110
31
20
PSMA-Ab(Cys)-EGFR (n=1) 1 mg/kg
PSMA-Ab(Cys)-EGFR (n=1) 0.5 mg/kg
PSMA-Ab(Cys)-EGFR-PEG5k (n=1) 1 mg/kg
PSMA-Ab(Cys)-EGFR-PEG5k (n=1) 0.5 mg/kg
PSMA-Ab(Cys)-PEG5k-EGFR (n=1) 1 mg/kg
PSMA-Ab(Cys)-PEG5k-EGFR (n=1) 0.5 mg/kg

FIG. 51A

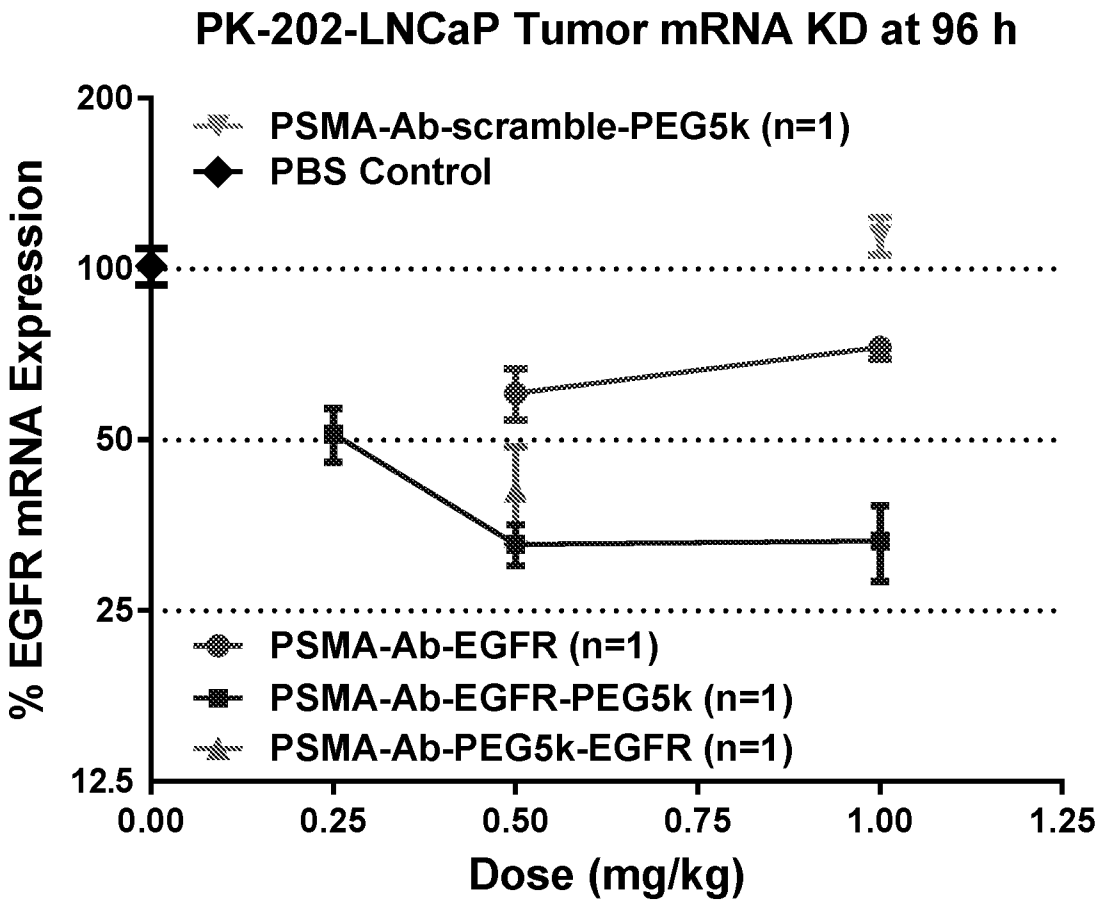

PK-202-LNCaP Tumor mRNA KD at 96 h
% EGFR mRNA Expression
200
100
50
25
12.5
PSMA-Ab-scramble-PEG5k (n=1)
PBS Control
PSMA-Ab-EGFR (n=1)
PSMA-Ab-EGFR-PEG5k (n=1)
PSMA-Ab-PEG5k-EGFR (n=1)
0.00
0.25
0.50
0.75
1.00
1.25
Dose (mg/kg)

FIG. 51B

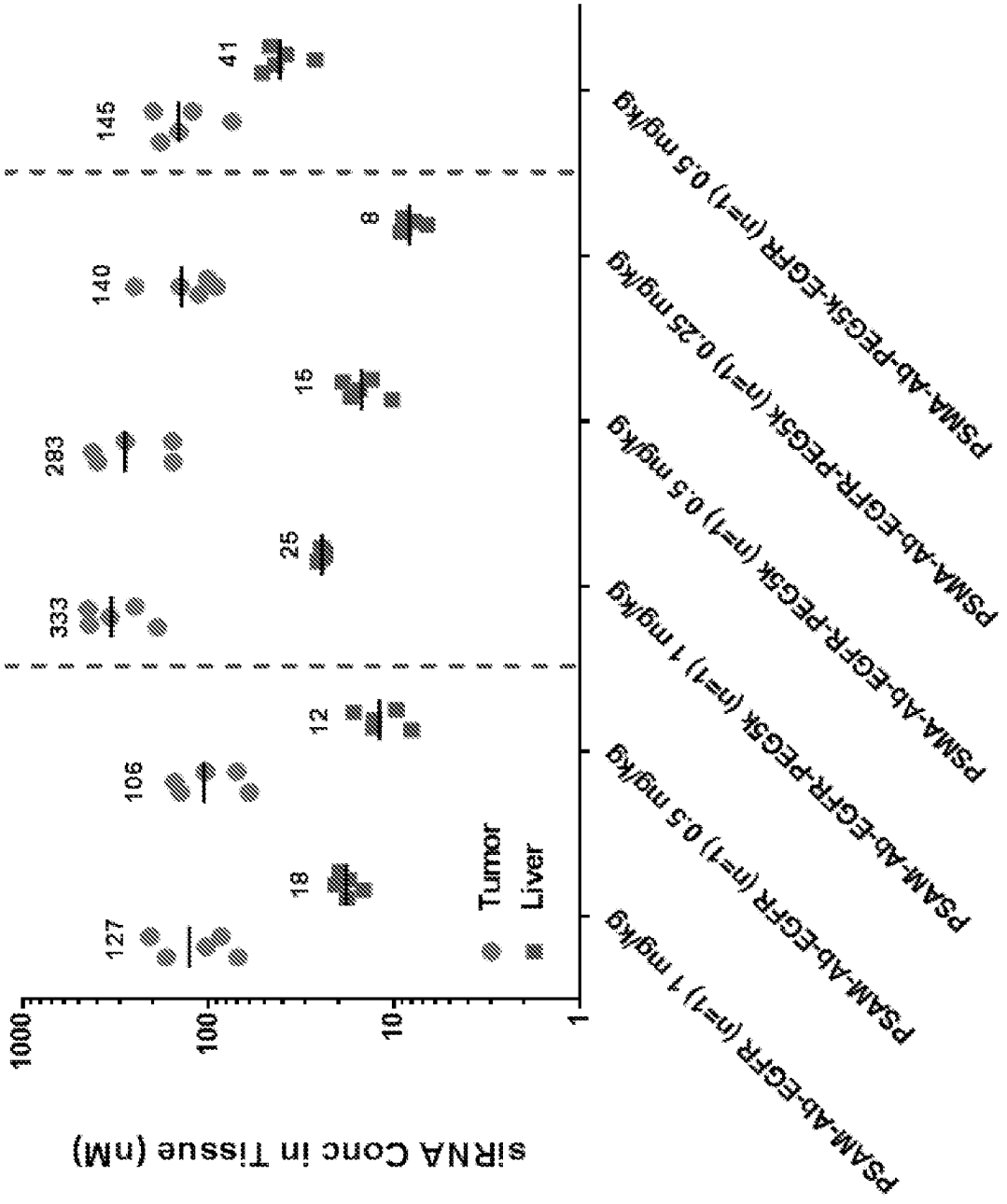

PK-202-LNCaP at 96 h
siRNA Conc in Tissue (nM)
1000
100
10
1
Tumor
Liver
127
106
18
12
333
283
25
140
15
145
8
41
PSMA-Ab-EGFR (n=1) 1 mg/kg
PSMA-Ab-EGFR (n=1) 0.5 mg/kg
PSMA-Ab-EGFR-PEG5k (n=1) 1 mg/kg
PSMA-Ab-EGFR-PEG5k (n=1) 0.5 mg/kg
PSMA-Ab-EGFR-PEG5k (n=1) 0.25 mg/kg
PSMA-Ab-PEG5k-EGFR (n=1) 0.5 mg/kg

FIG. 52

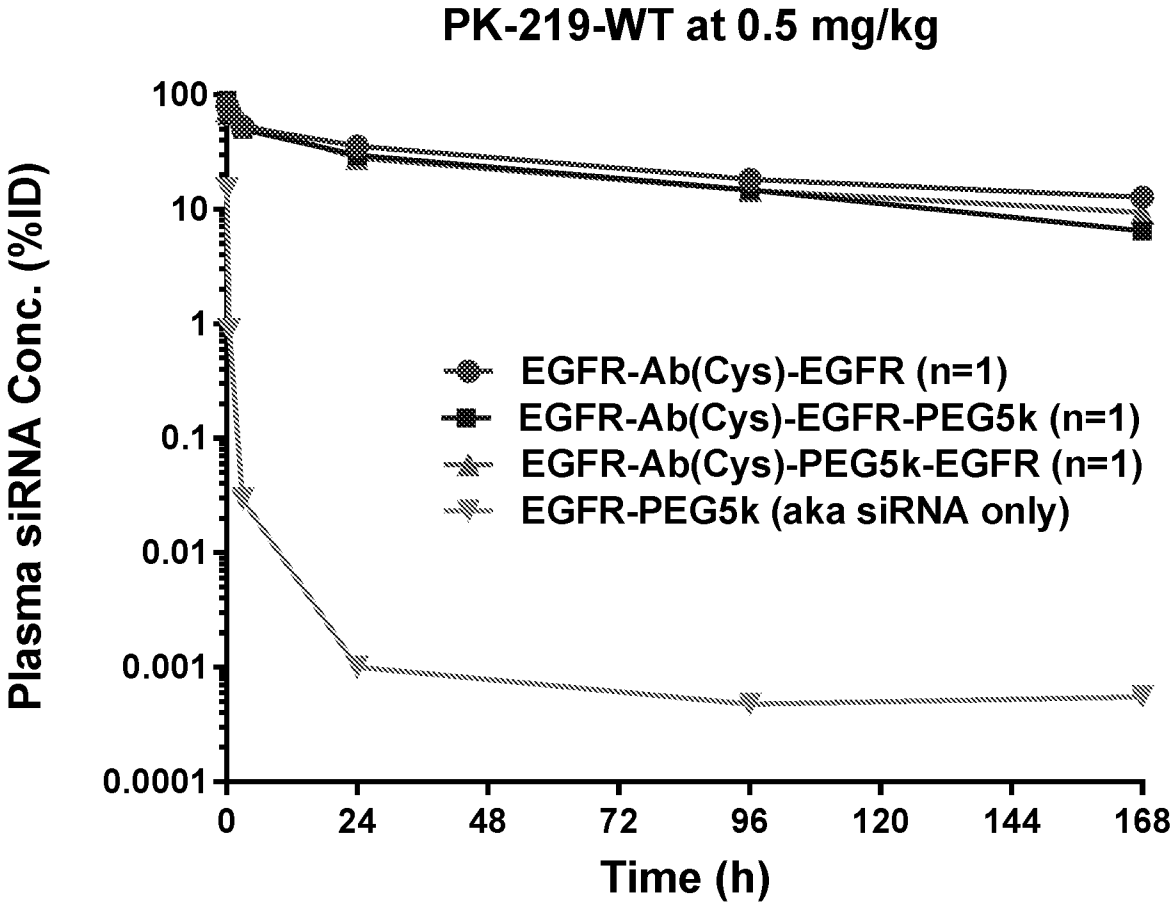

PK-219-WT at 0.5 mg/kg
Plasma siRNA Conc. (%ID)
100
10
1
0.1
0.01
0.001
0.0001
0
24
48
72
96
120
144
168
Time (h)
EGFR-Ab(Cys)-EGFR (n=1)
EGFR-Ab(Cys)-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-PEG5k-EGFR (n=1)
EGFR-PEG5k (aka siRNA only)

FIG. 53A

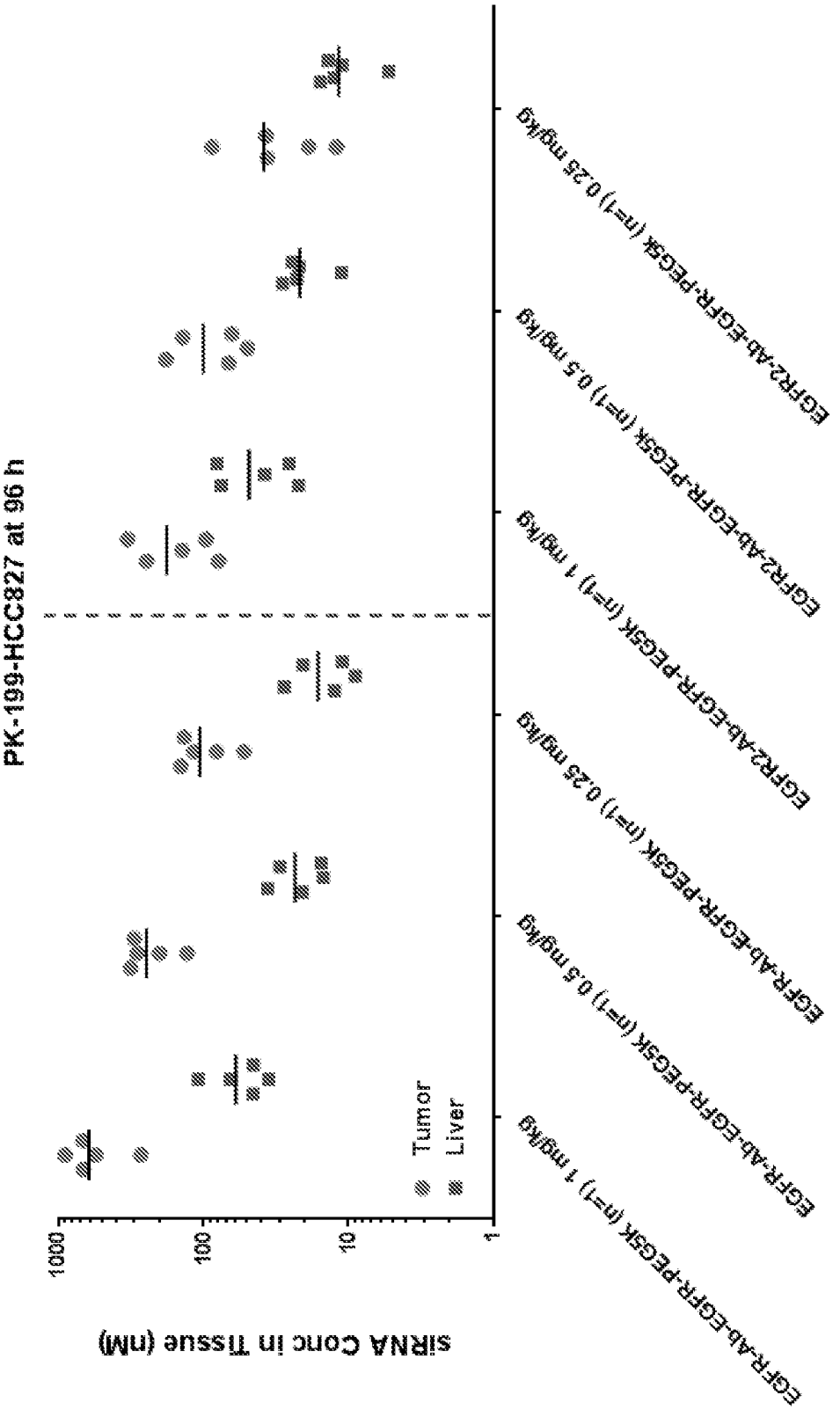

PK-199-HCC827 at 96 h
siRNA Conc in Tissue (nM)
1000
100
10
1
Tumor
Liver
EGFR-Ab-EGFR-PEG5K (n=1) 1 mg/kg
EGFR-Ab-EGFR-PEG5K (n=1) 0.5 mg/kg
EGFR-Ab-EGFR-PEG5K (n=1) 0.25 mg/kg
EGFR2-Ab-EGFR-PEG5K (n=1) 1 mg/kg
EGFR2-Ab-EGFR-PEG5K (n=1) 0.5 mg/kg
EGFR2-Ab-EGFR-PEG5K (n=1) 0.25 mg/kg

FIG. 53B

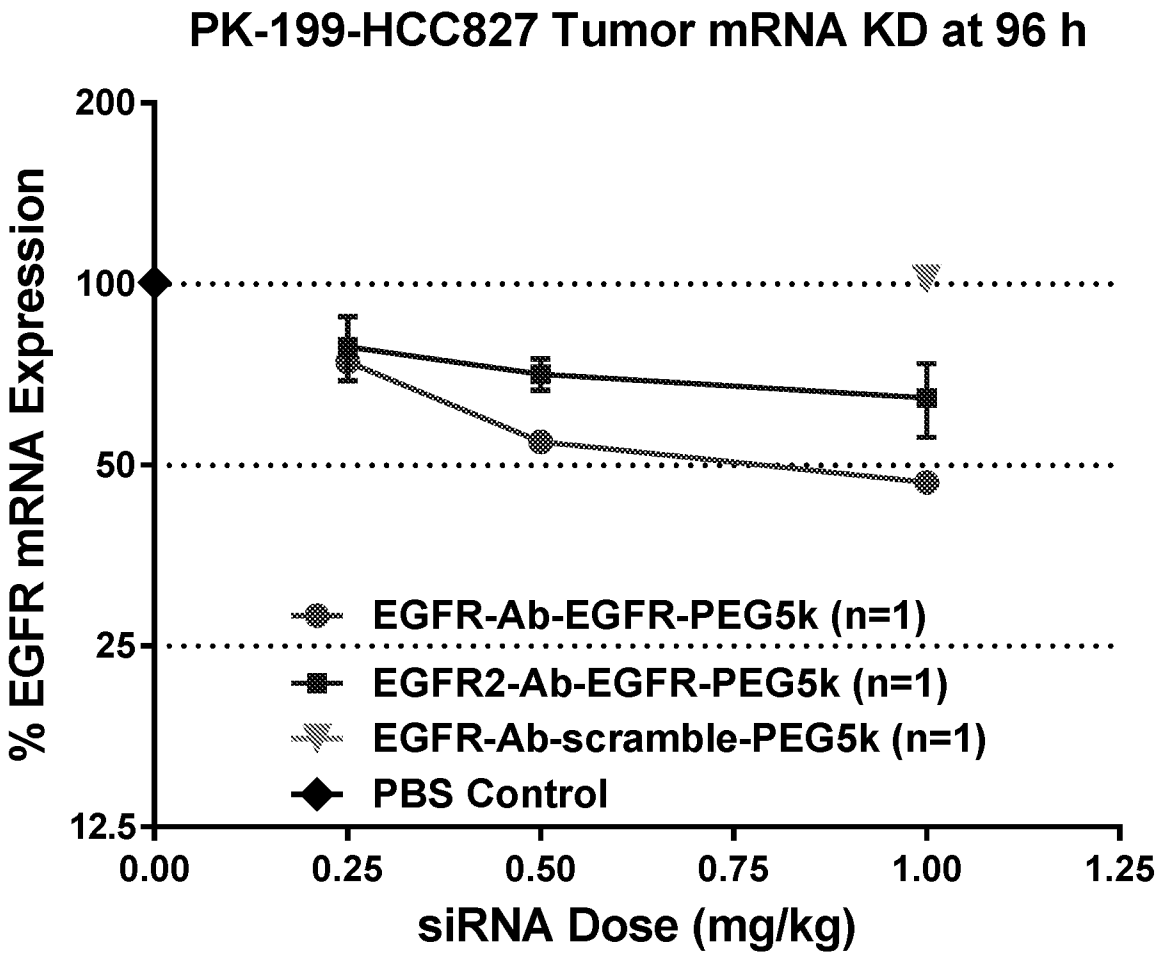
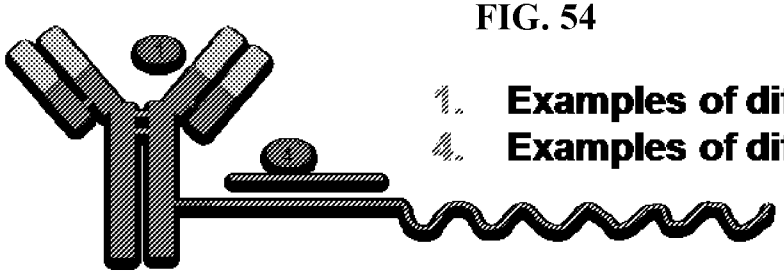

PK-199-HCC827 Tumor mRNA KD at 96 h
% EGFR mRNA Expression
200
100
50
25
12.5
0.00
0.25
0.50
0.75
1.00
1.25
siRNA Dose (mg/kg)
EGFR-Ab-EGFR-PEG5k (n=1)
EGFR2-Ab-EGFR-PEG5k (n=1)
EGFR-Ab-scramble-PEG5k (n=1)
PBS Control

FIG. 54



1. **Examples of different A's (mAbs and Fabs)**
4. **Examples of different B's (siRNA targets)**

mAb	mAb target	In vitro KD	siRNA target				
			KRAS	EGFR	CTNNB1	AR	HPRT
Zalutumumab*	Human EGFR		√ PK-237-HCC827	√ PK/PD, PK-199-HCC827 and PK239-HCC827			
Panitumumab*	Human EGFR		√ PK-129-WT	√ PK/PD, PK-199-HCC827			
Herceptin	Human EGFR			√ PK/PD, PK-234-HCC827 and PK-236			
ANT4044*	Human PSMA			√ PK-129-WT, PK-123-LNCaP		√ PK-160-LNCaP	
mASGR*	Mouse ASGR		√ PK-253-WT		√ PK-257-WT		
CD71* (Fab)	Mouse transferrin	√ B cells			√ PK-245-WT		√ PK-258-WT, PK-254-WT

√ - Tested *in vitro*

√ - Tested *in vivo*

FIG. 55

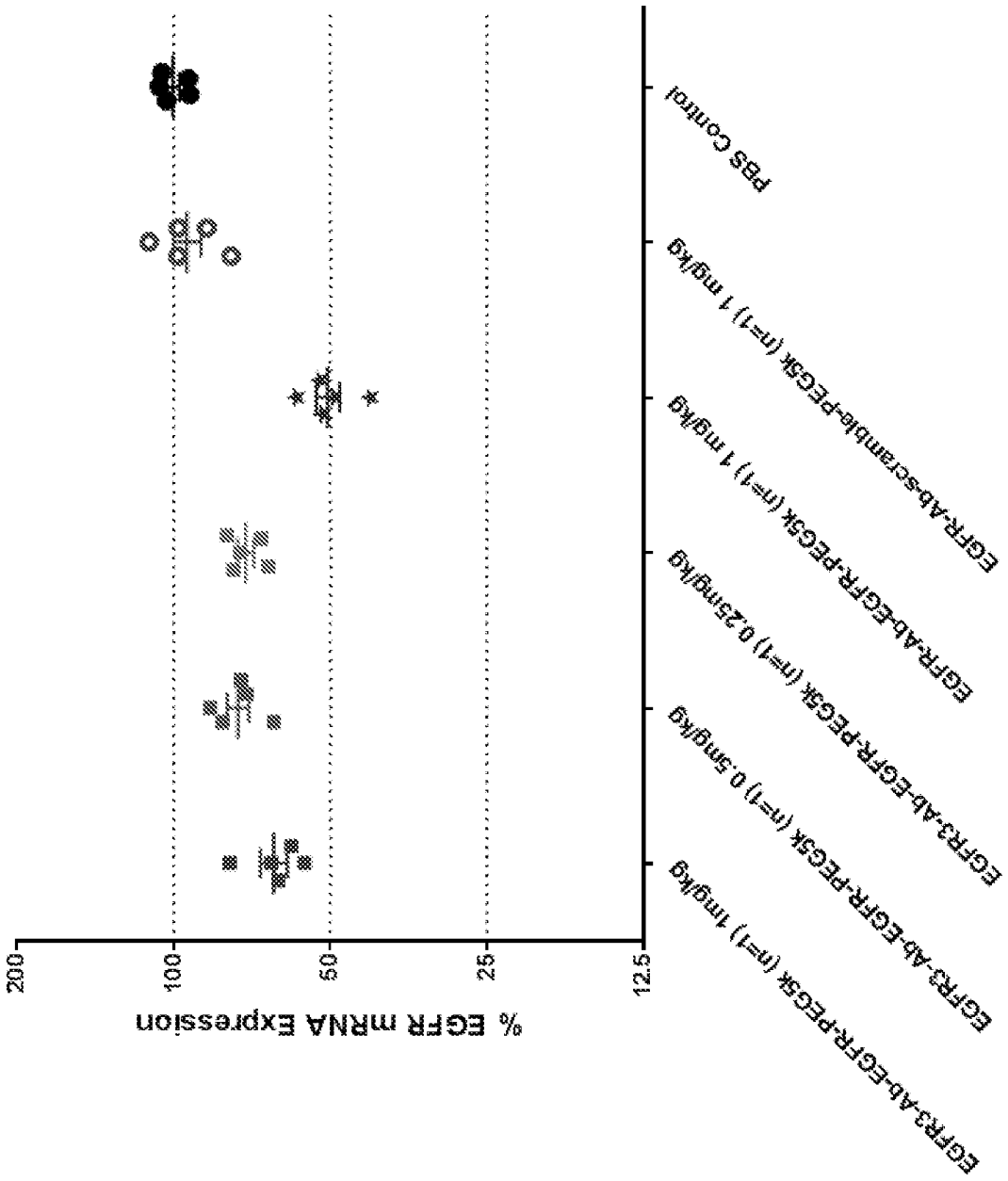

PK-236-HCC827 Tumor EGFR mRNA at 96 h
% EGFR mRNA Expression
200
100
50
25
12.5
PBS Control
EGFR-Ab-scrambled-PEG5k (n=1) 1 mg/kg
EGFR-Ab-EGFR-PEG5k (n=1) 1 mg/kg
EGFR3-Ab-EGFR-PEG5k (n=1) 0.25mg/kg
EGFR3-Ab-EGFR-PEG5k (n=1) 0.5mg/kg
EGFR3-Ab-EGFR-PEG5k (n=1) 1mg/kg

FIG. 56A

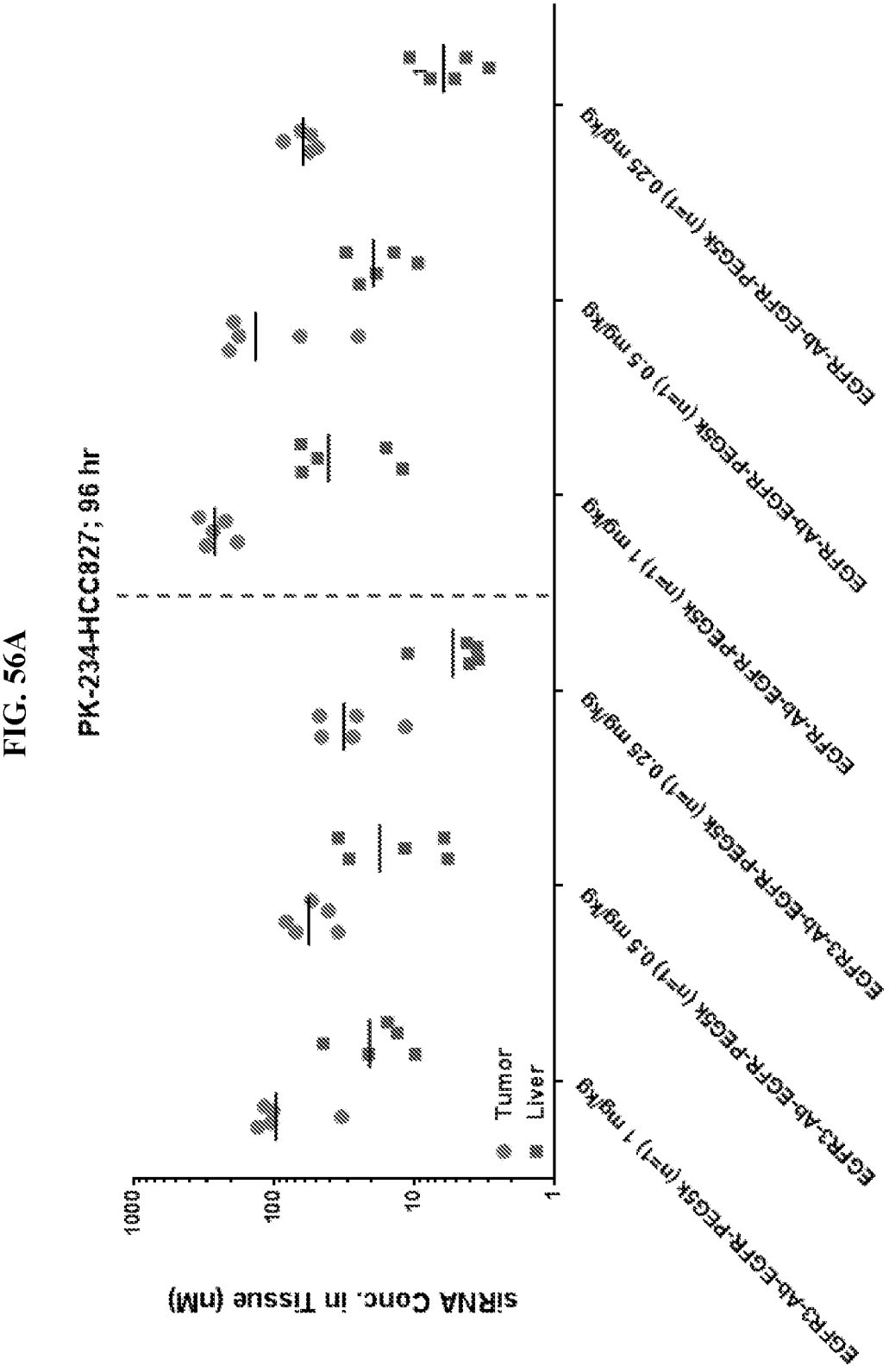

PK-234-HCC827; 96 hr
siRNA Conc. in Tissue (nM)
1000
100
10
1
Tumor
Liver
EGFR3-Ab-EGFR-PEG5k (n=1) 1 mg/kg
EGFR3-Ab-EGFR-PEG5k (n=1) 0.5 mg/kg
EGFR3-Ab-EGFR-PEG5k (n=1) 0.25 mg/kg
EGFR-Ab-EGFR-PEG5k (n=1) 1 mg/kg
EGFR-Ab-EGFR-PEG5k (n=1) 0.5 mg/kg
EGFR-Ab-EGFR-PEG5k (n=1) 0.25 mg/kg

FIG. 56B

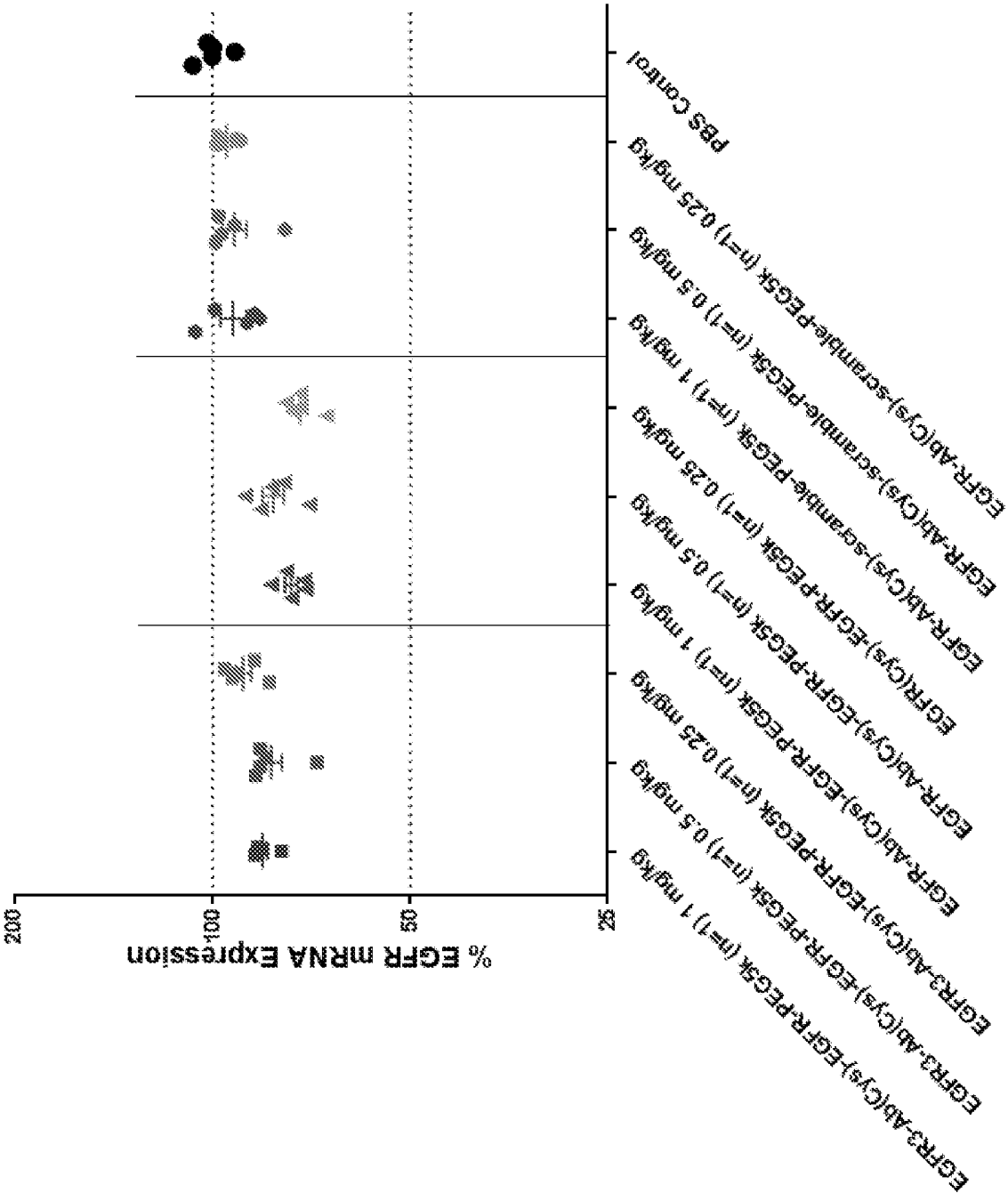

PK-234-HCC827 Tumor EGFR mRNA at 96 h
% EGFR mRNA Expression
200
100
50
25
PBS Control
EGFR-Ab(Cys)-scramble-PEG5k (m=1) 0.25 mg/kg
EGFR-Ab(Cys)-scramble-PEG5k (m=1) 0.5 mg/kg
EGFR-Ab(Cys)-scramble-PEG5k (m=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (m=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (m=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (m=1) 1 mg/kg
EGFR3-Ab(Cys)-EGFR-PEG5k (m=1) 0.25 mg/kg
EGFR3-Ab(Cys)-EGFR-PEG5k (m=1) 0.5 mg/kg
EGFR3-Ab(Cys)-EGFR-PEG5k (m=1) 1 mg/kg

FIG. 57A

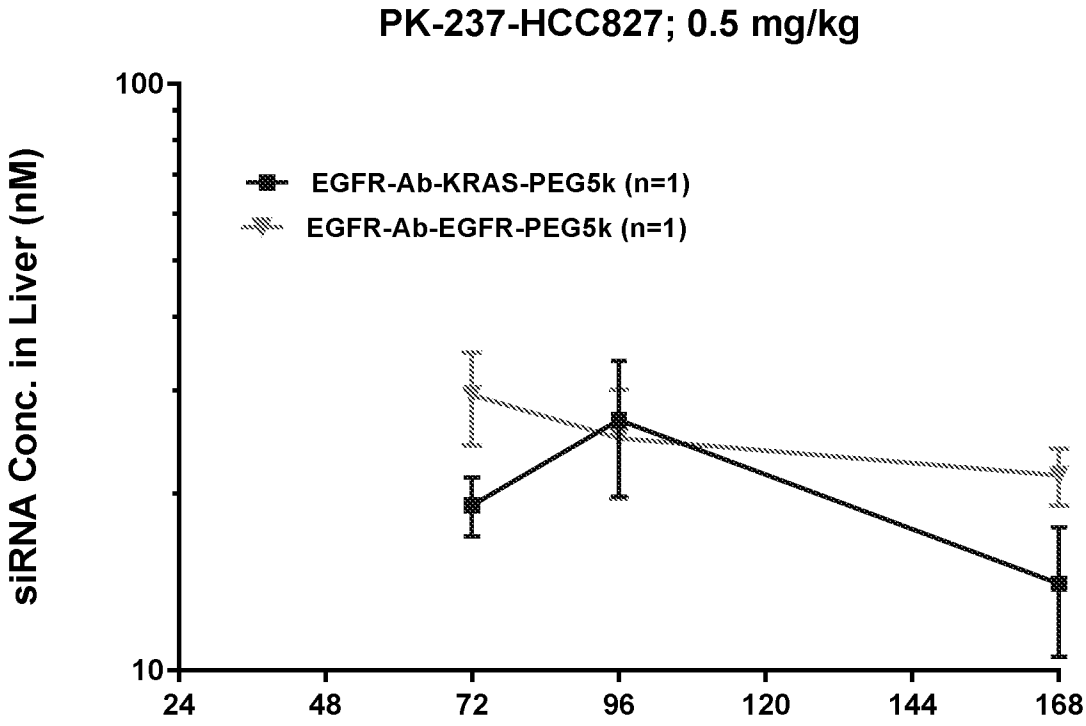


FIG. 57B

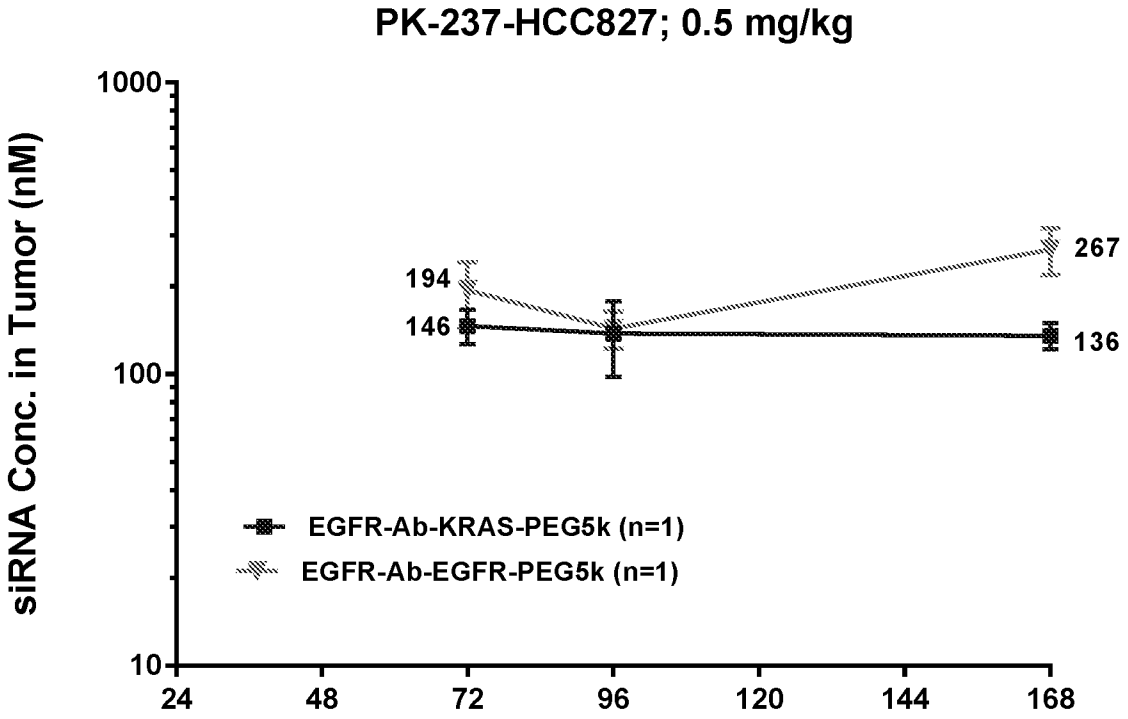


FIG. 57C

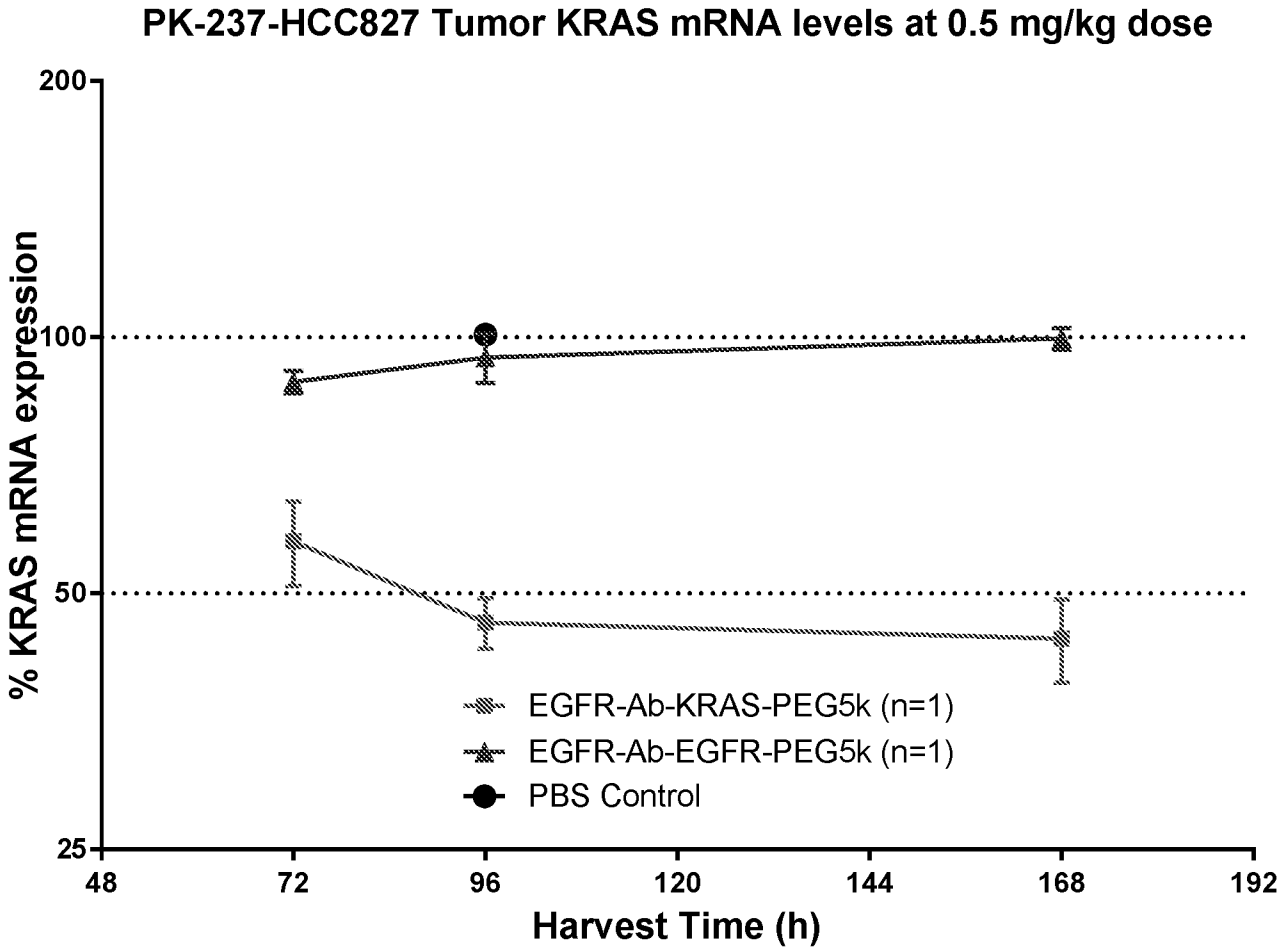

PK-237-HCC827 Tumor KRAS mRNA levels at 0.5 mg/kg dose
% KRAS mRNA expression
200
100
50
25
48
72
96
120
144
168
192
Harvest Time (h)
EGFR-Ab-KRAS-PEG5k (n=1)
EGFR-Ab-EGFR-PEG5k (n=1)
PBS Control

FIG. 58A

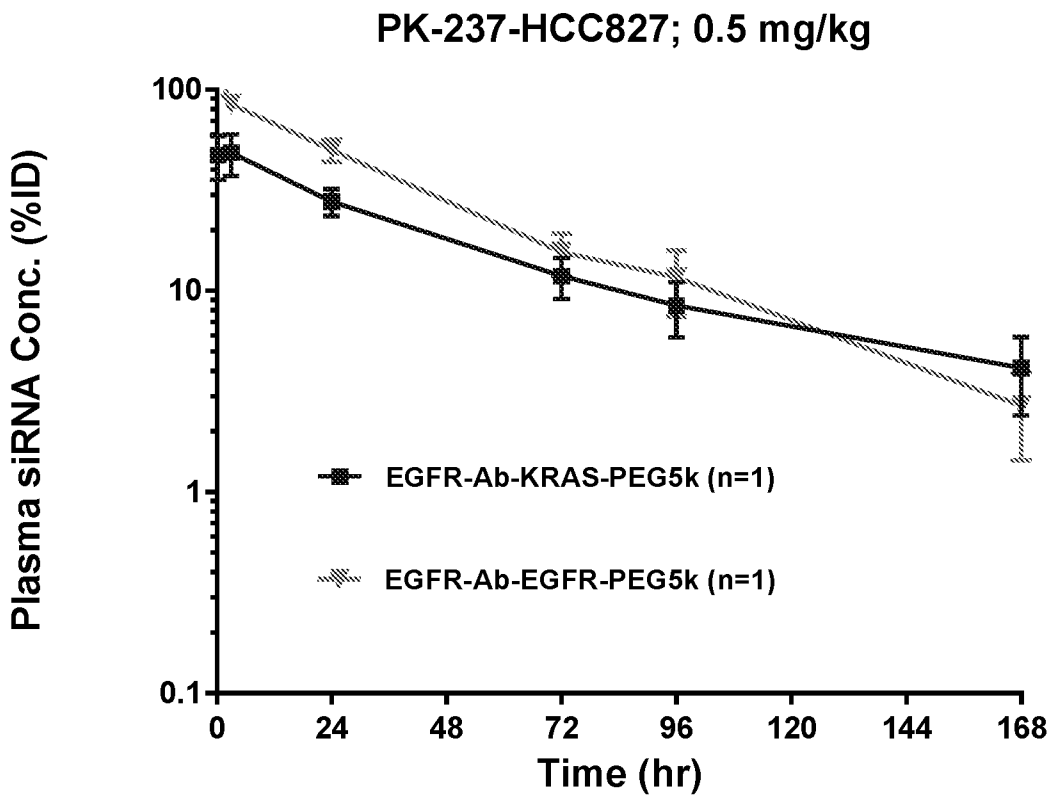


FIG. 58B

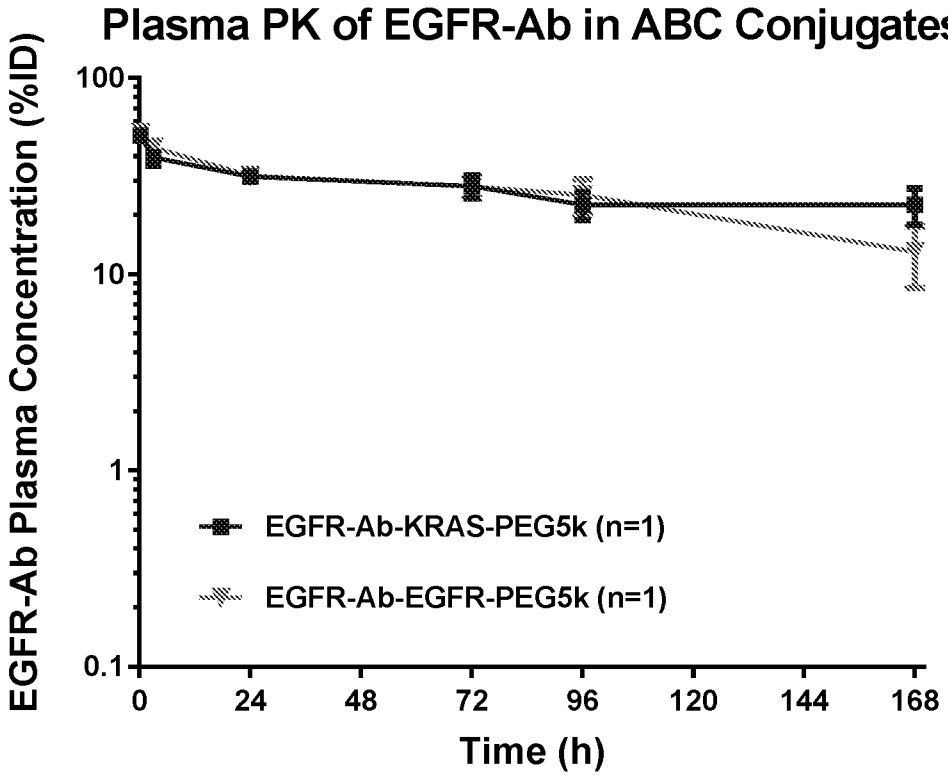


FIG. 59A

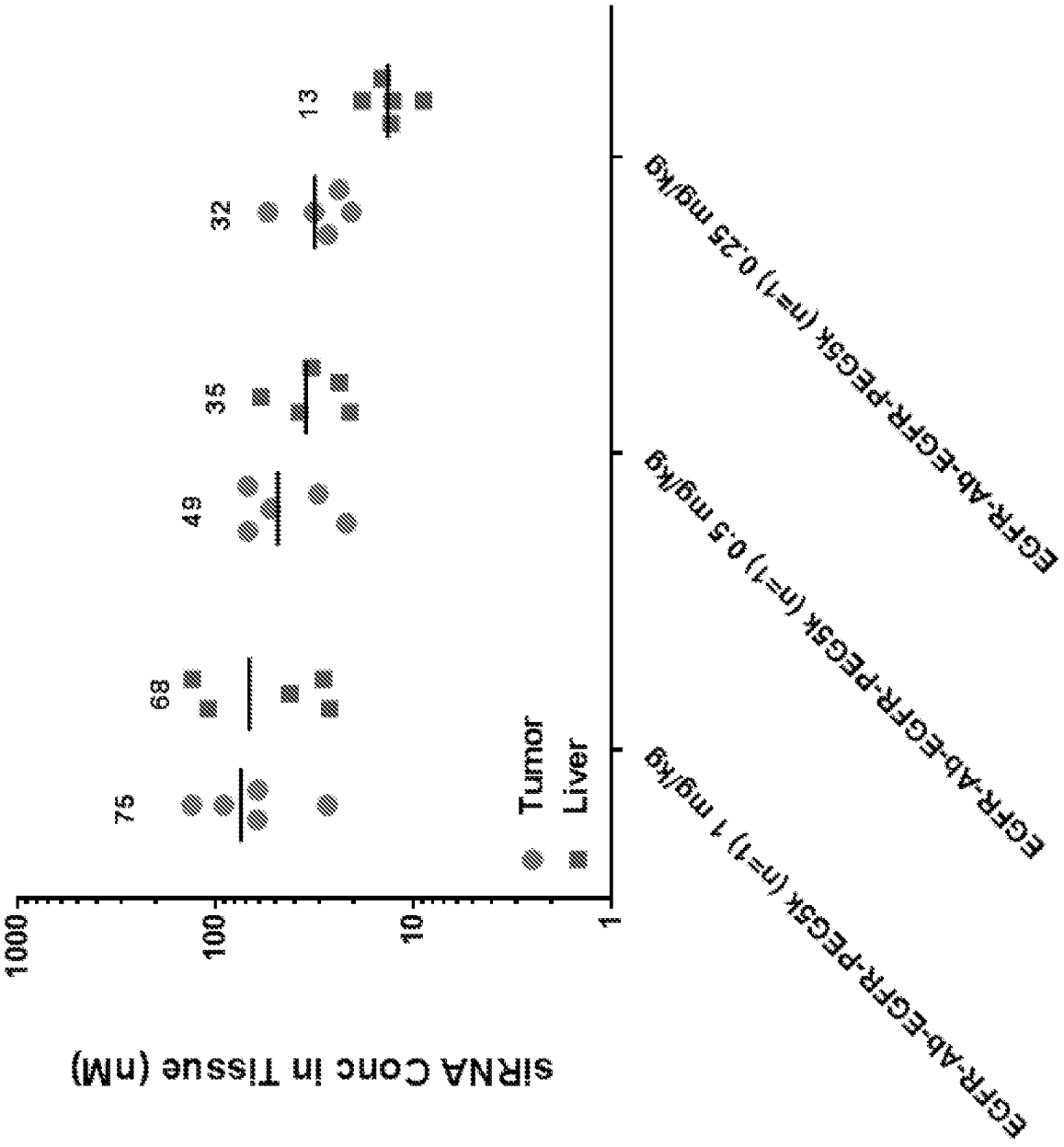

PK-187-Hep3B; 96 h
siRNA Conc in Tissue (nM)
1000
100
10
1
Tumor
Liver
75
68
49
35
32
13
EGFR-Ab-EGFR-PEG5k (m=1) 1 mg/kg
EGFR-Ab-EGFR-PEG5k (m=1) 0.5 mg/kg
EGFR-Ab-EGFR-PEG5k (m=1) 0.25 mg/kg

FIG. 59B

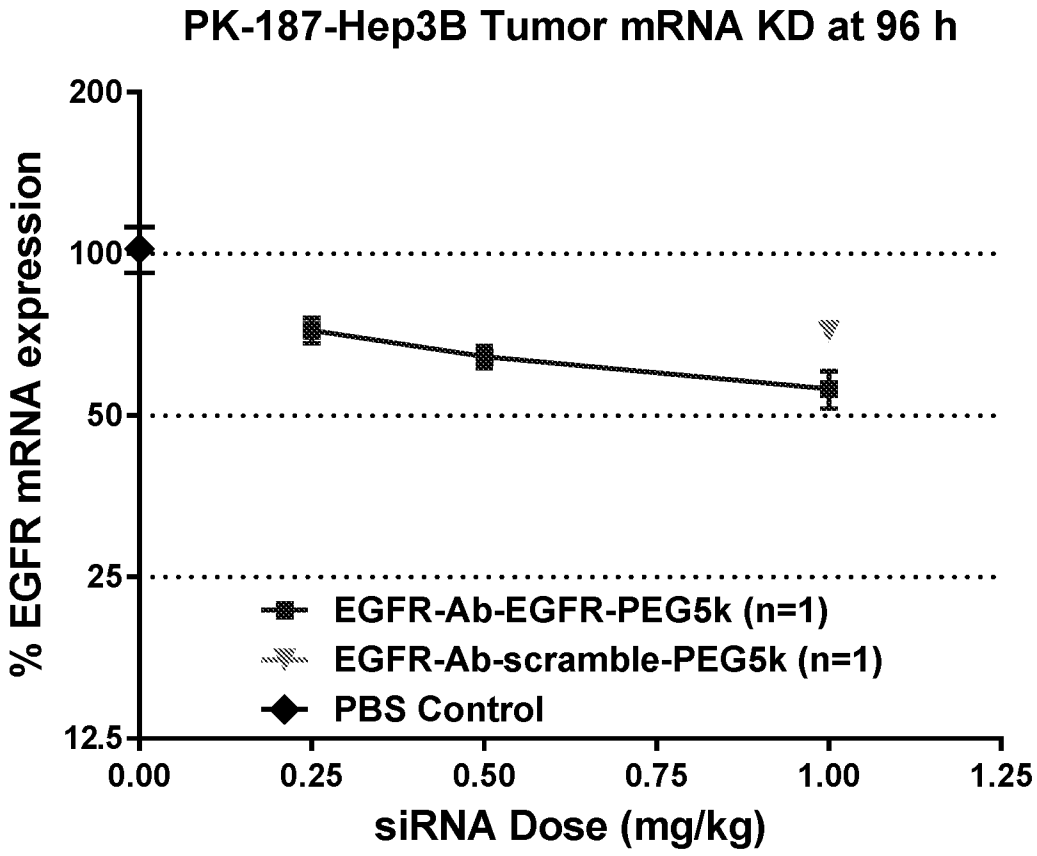

PK-187-Hep3B Tumor mRNA KD at 96 h
% EGFR mRNA expression
200
100
50
25
12.5
0.00
0.25
0.50
0.75
1.00
1.25
siRNA Dose (mg/kg)
EGFR-Ab-EGFR-PEG5k (n=1)
EGFR-Ab-scramble-PEG5k (n=1)
PBS Control

FIG. 60

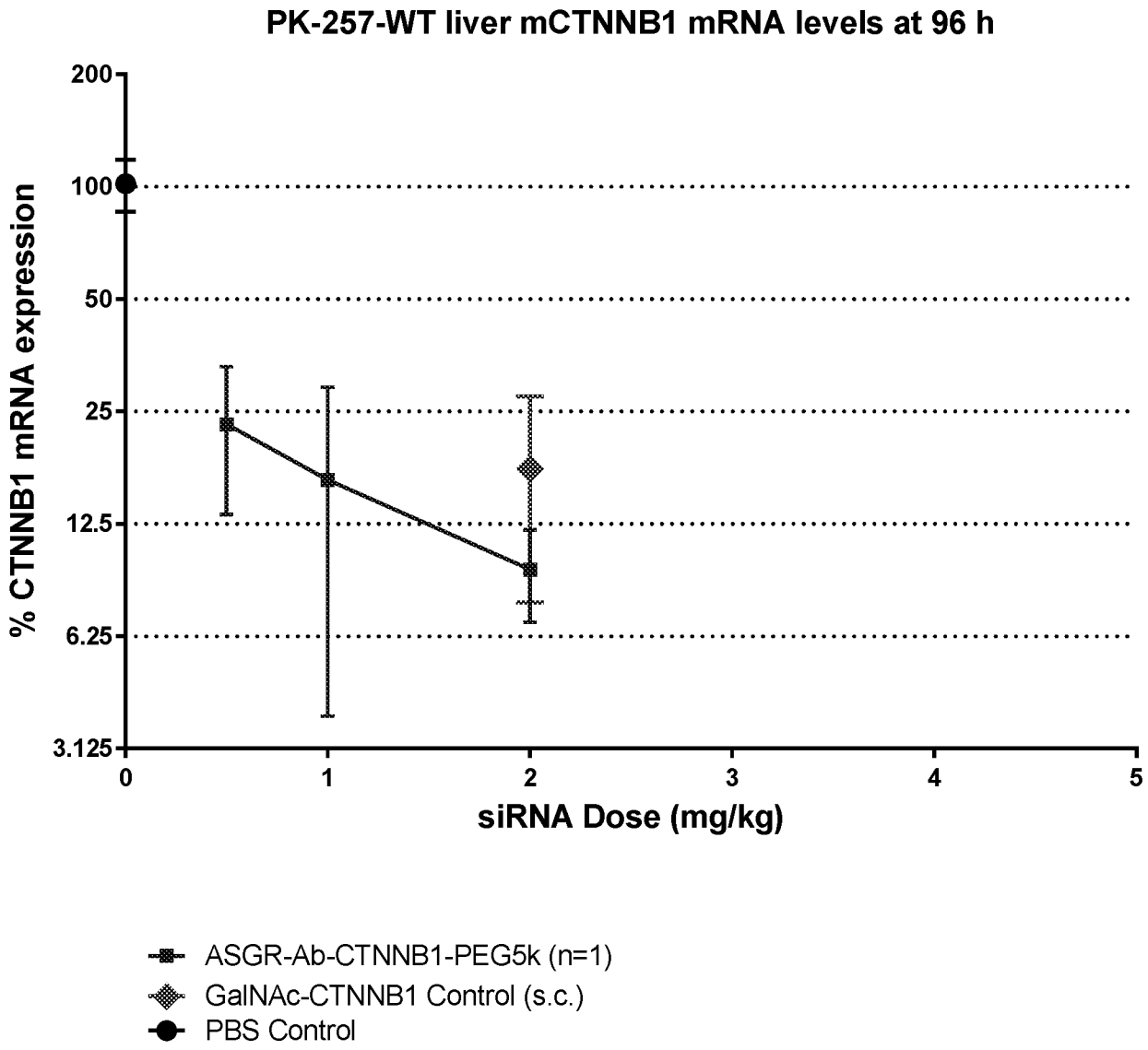

PK-257-WT liver mCTNNB1 mRNA levels at 96 h
% CTNNB1 mRNA expression
200
100
50
25
12.5
6.25
3.125
0
1
2
3
4
5
siRNA Dose (mg/kg)
ASGR-Ab-CTNNB1-PEG5k (n=1)
GalNAc-CTNNB1 Control (s.c.)
PBS Control

FIG. 61

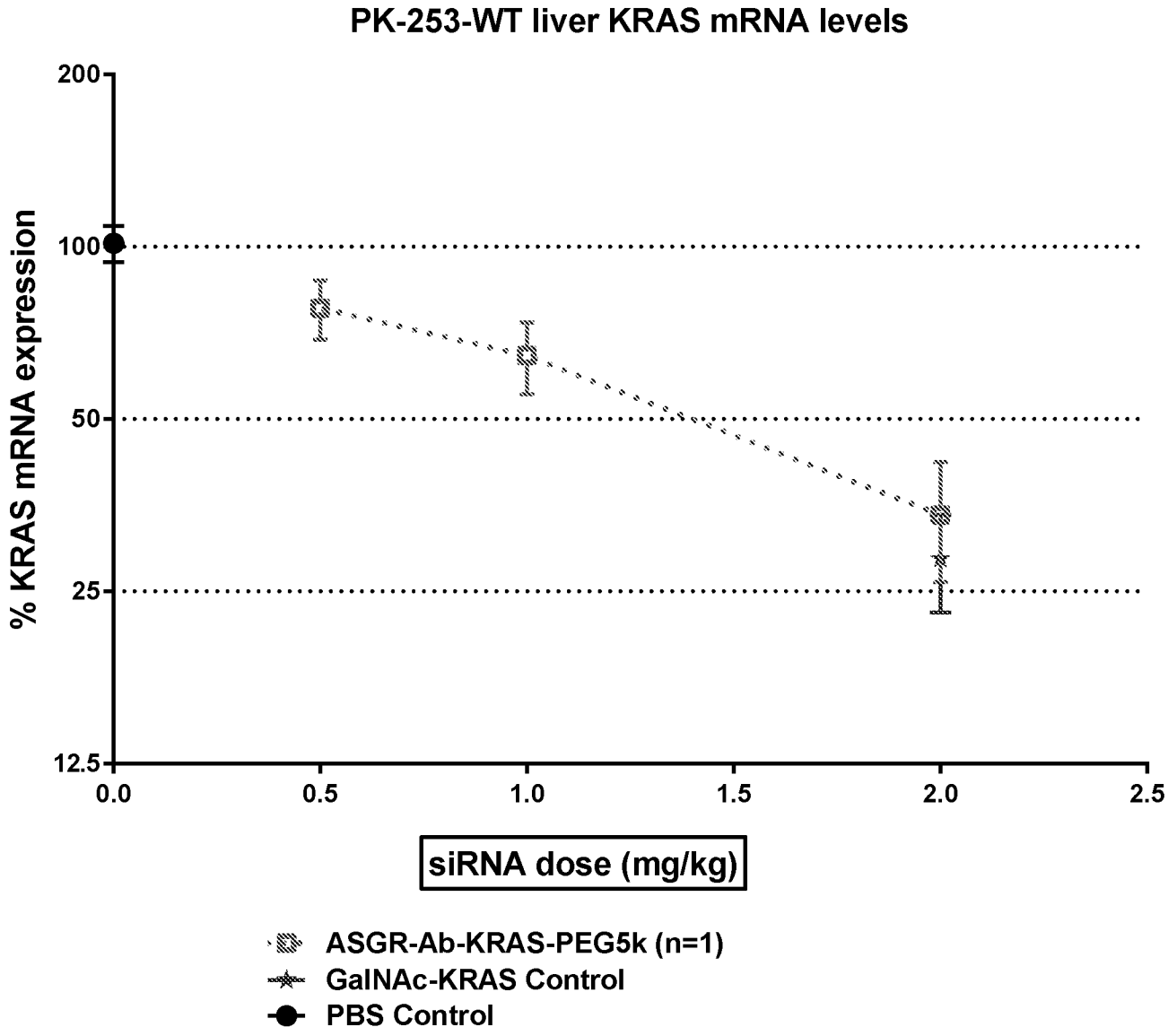

PK-253-WT liver KRAS mRNA levels
% KRAS mRNA expression
200
100
50
25
12.5
0.0
0.5
1.0
1.5
2.0
2.5
siRNA dose (mg/kg)
ASGR-Ab-KRAS-PEG5k (n=1)
GalNAc-KRAS Control
PBS Control

FIG. 62

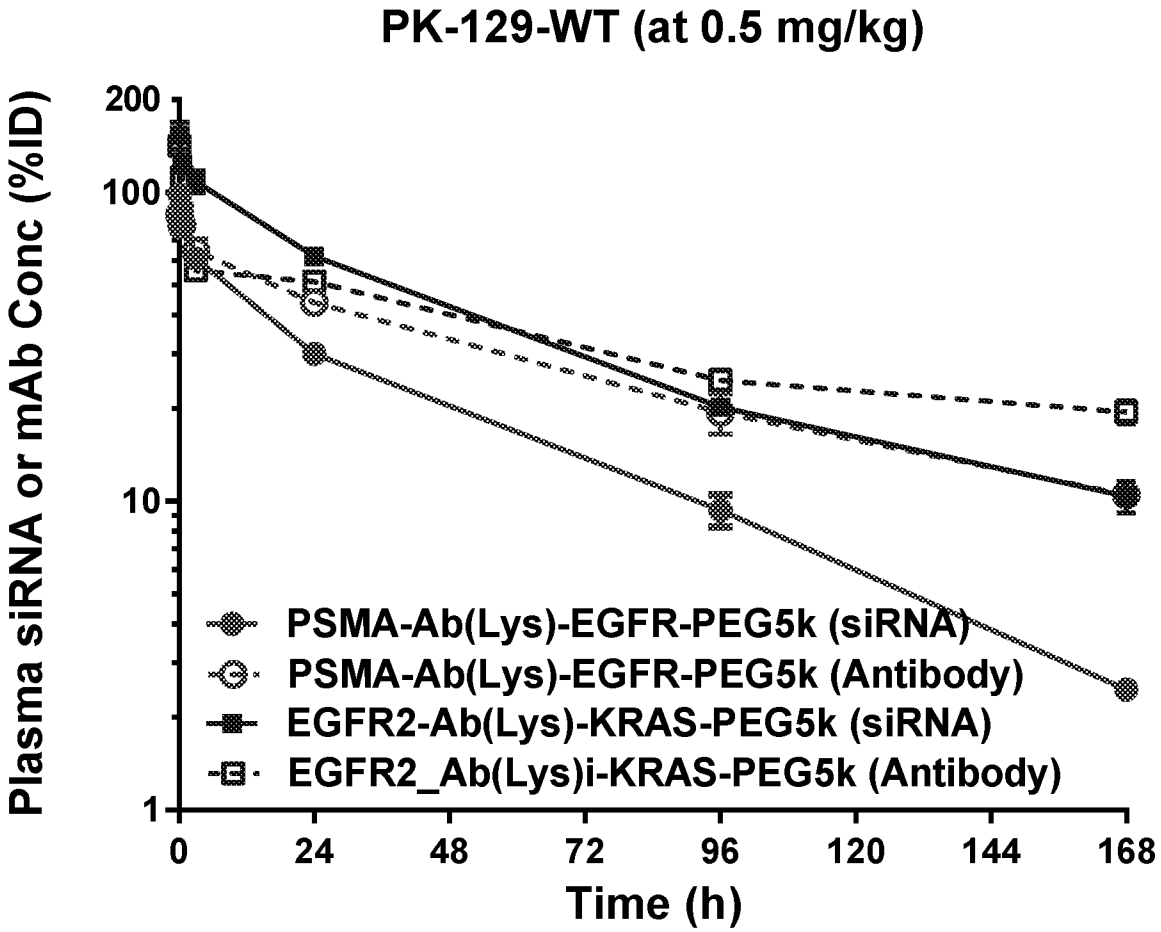

PK-129-WT (at 0.5 mg/kg)
Plasma siRNA or mAb Conc (%ID)
200
100
10
1
0
24
48
72
96
120
144
168
Time (h)
PSMA-Ab(Lys)-EGFR-PEG5k (siRNA)
PSMA-Ab(Lys)-EGFR-PEG5k (Antibody)
EGFR2-Ab(Lys)-KRAS-PEG5k (siRNA)
EGFR2_Ab(Lys)i-KRAS-PEG5k (Antibody)

FIG. 63A

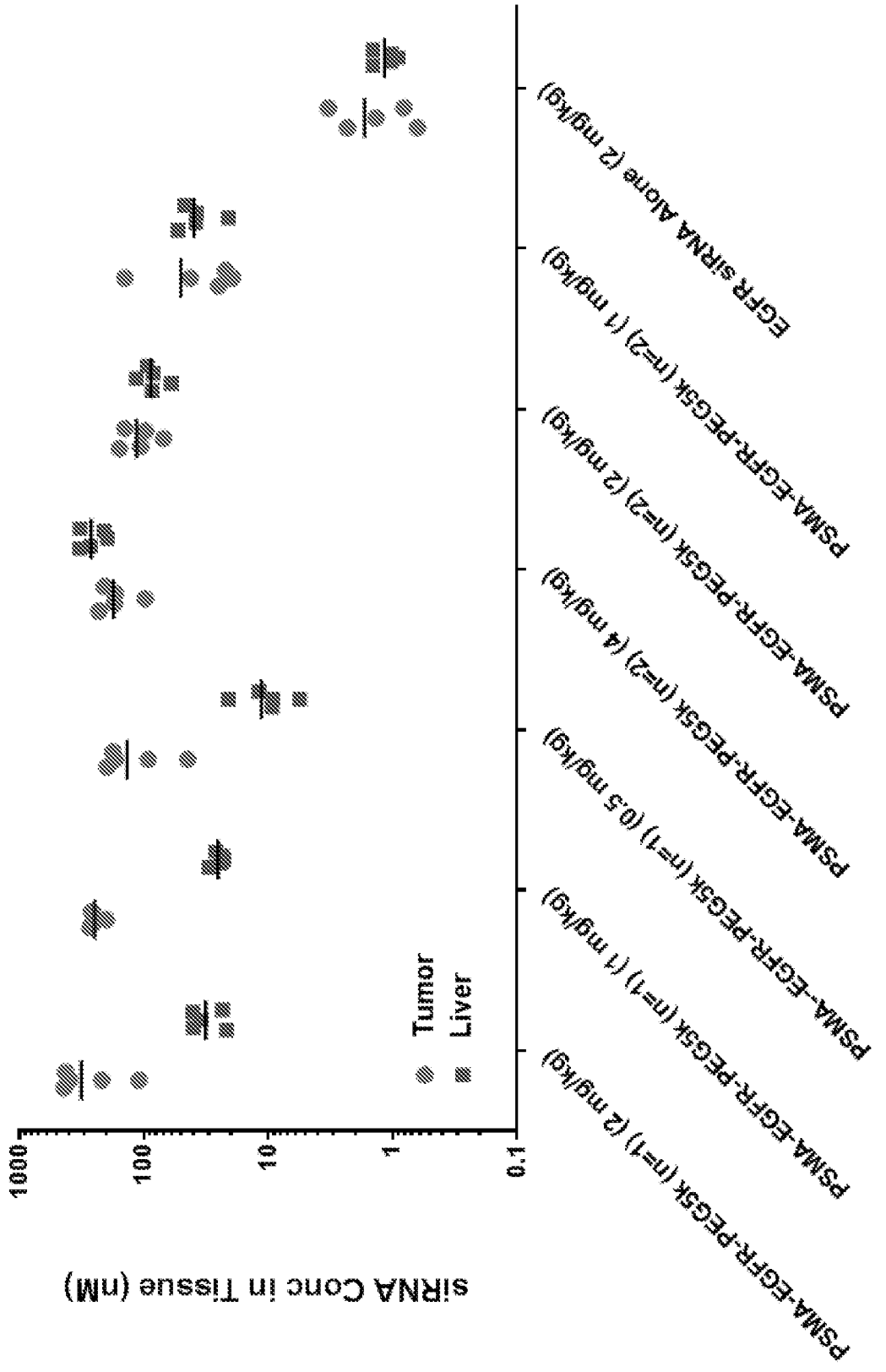

PK-123-LNCaP, 72 h
siRNA Conc in Tissue (nM)
1000
100
10
1
0.1
Tumor
Liver
PSMA-EGFR-PEG5k (n=1) (2 mg/kg)
PSMA-EGFR-PEG5k (n=1) (1 mg/kg)
PSMA-EGFR-PEG5k (n=1) (0.5 mg/kg)
PSMA-EGFR-PEG5k (n=2) (4 mg/kg)
PSMA-EGFR-PEG5k (n=2) (2 mg/kg)
PSMA-EGFR-PEG5k (n=2) (1 mg/kg)
EGFR siRNA Alone (2 mg/kg)

FIG. 63B

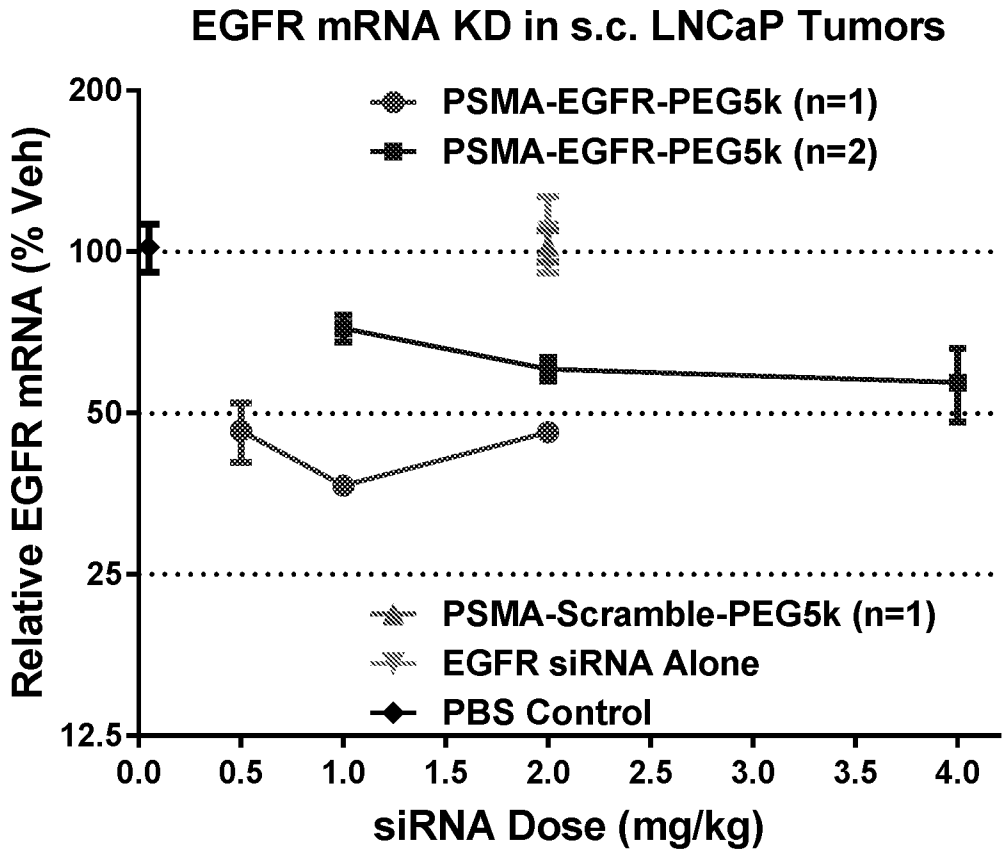

EGFR mRNA KD in s.c. LNCaP Tumors
PSMA-EGFR-PEG5k (n=1)
PSMA-EGFR-PEG5k (n=2)
PSMA-Scramble-PEG5k (n=1)
EGFR siRNA Alone
PBS Control
Relative EGFR mRNA (% Veh)
200
100
50
25
12.5
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
siRNA Dose (mg/kg)

FIG. 64A

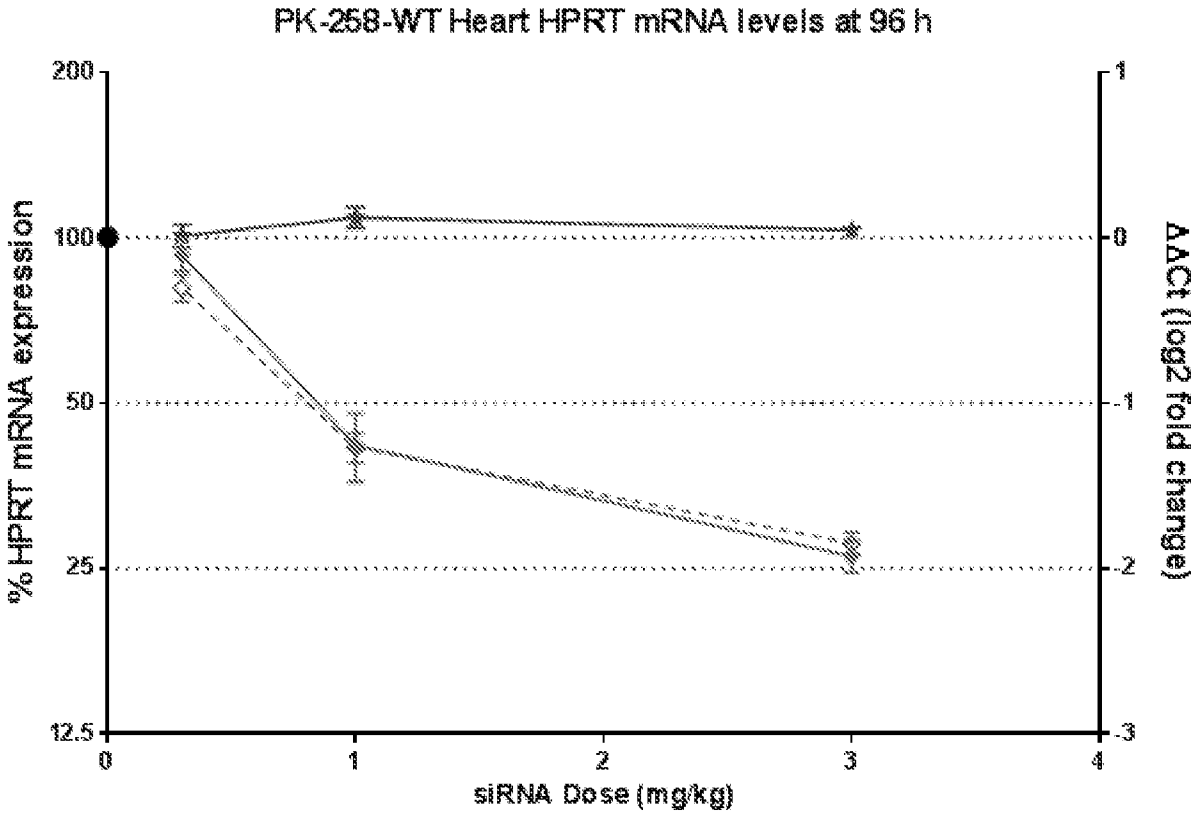

PK-258-WT Heart HPRT mRNA levels at 96 h
% HPRT mRNA expression
200
100
50
25
12.5
ΔΔCt (log2 fold change)
1
0
-1
-2
-3
0
1
2
3
4
siRNA Dose (mg/kg)
Anti-B cell Ab(Cys)-HPRT-PEG5k (n=1)
Anti-B cell Ab(Lys)-HPRT-PEG5k (n=1)
Anti-B cell Ab(Cys)-scramble-PEG5k (n=1)
PBS Control

FIG. 64B

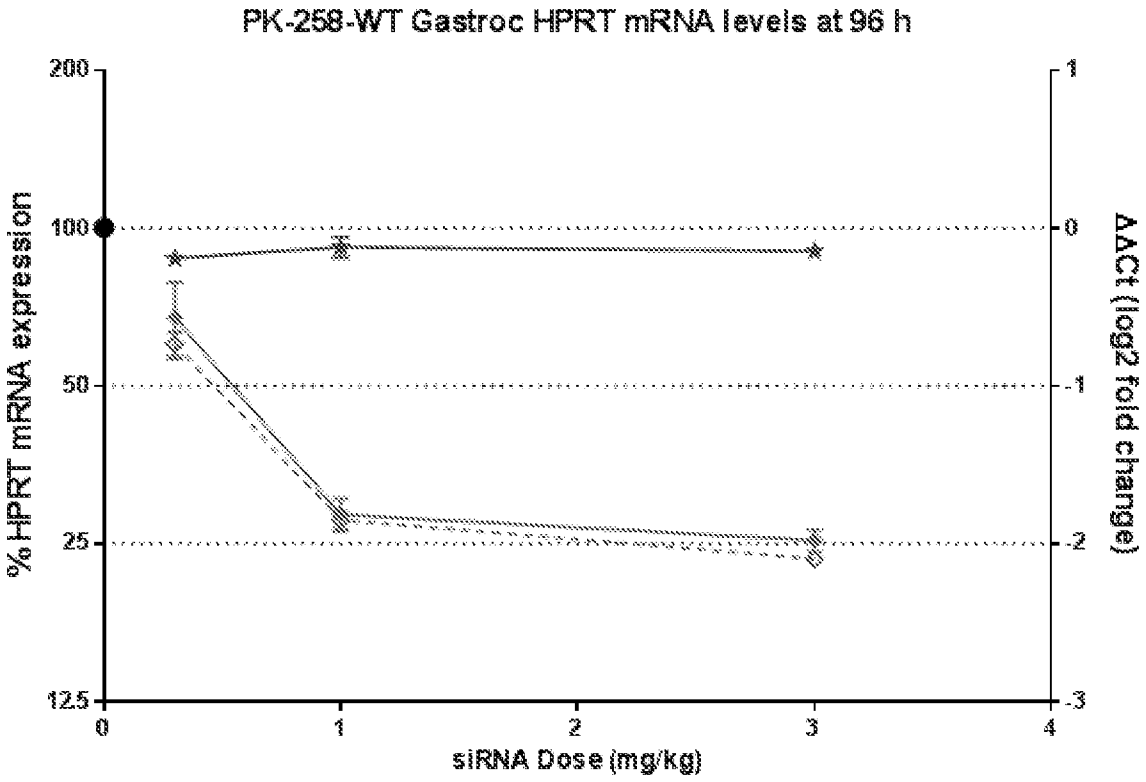

PK-258-WT Gastroc HPRT mRNA levels at 96 h
% HPRT mRNA expression
200
100
50
25
12.5
ΔΔCt (log2 fold change)
1
0
-1
-2
-3
0
1
2
3
4
siRNA Dose (mg/kg)
Anti-B cell Ab(Cys)-HPRT-PEG5k (n=1)
Anti-B cell Ab(Lys)-HPRT-PEG5k (n=1)
Anti-B cell Ab(Cys)-scramble-PEG5k (n=1)
PBS Control

FIG. 64C

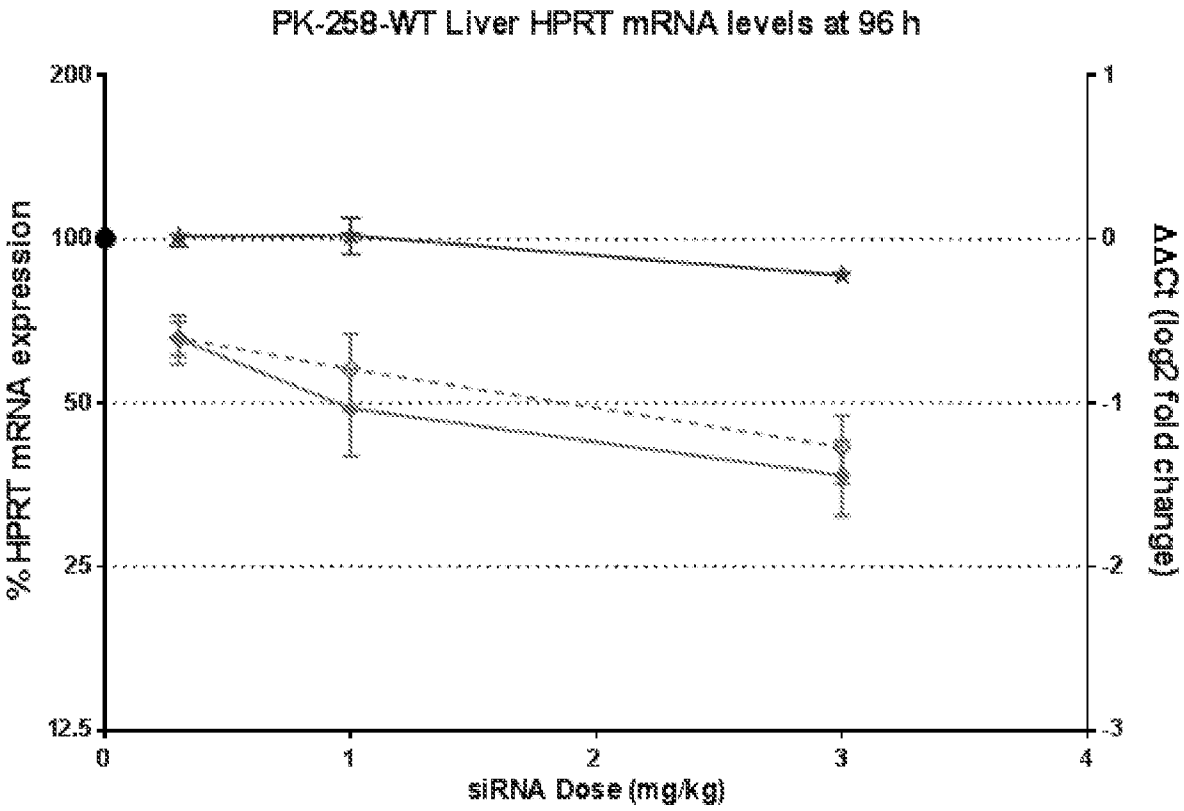

PK-258-WT Liver HPRT mRNA levels at 96 h
% HPRT mRNA expression
ΔΔCt (log2 fold change)
siRNA Dose (mg/kg)
200
100
50
25
12.5
1
0
-1
-2
-3
0
1
2
3
4
Anti-B cell Ab(Cys)-HPRT-PEG5k (n=1)
Anti-B cell Ab(Lys)-HPRT-PEG5k (n=1)
Anti-B cell Ab(Cys)-scramble-PEG5k (n=1)
PBS Control

FIG. 64D

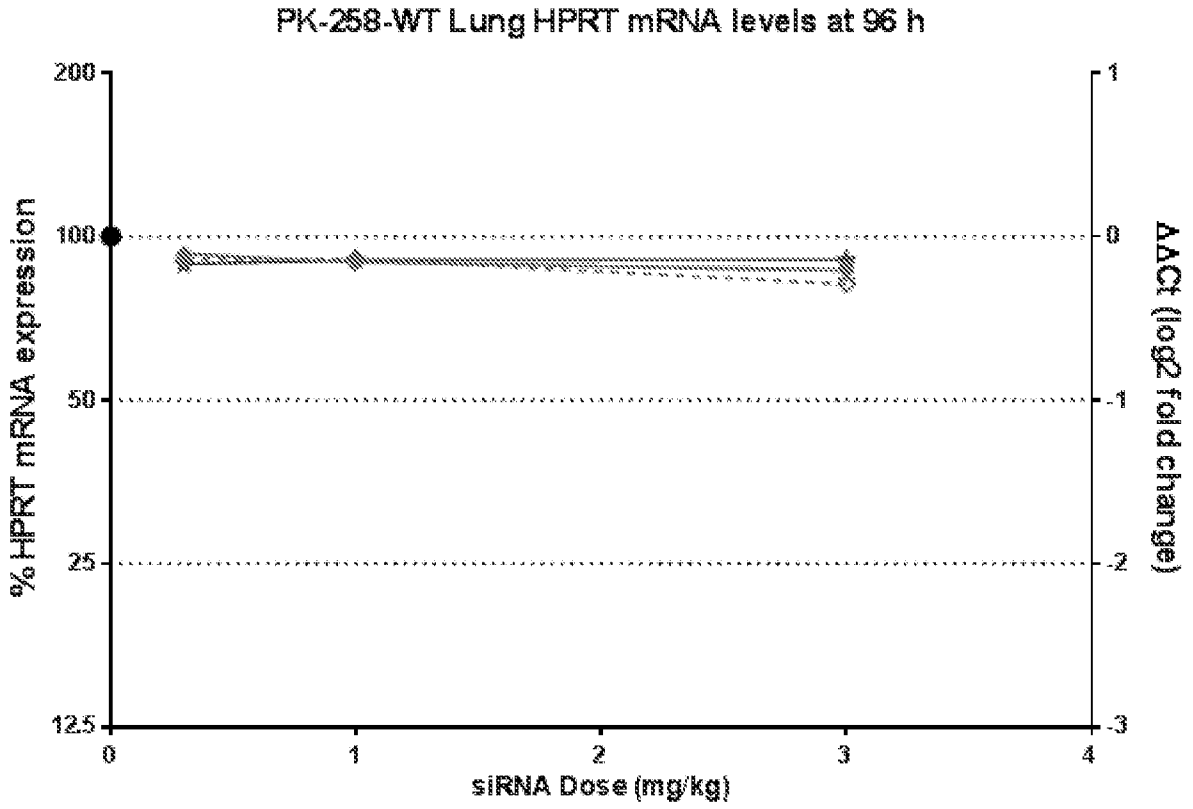

PK-258-WT Lung HPRT mRNA levels at 96 h
% HPRT mRNA expression
200
100
50
25
12.5
ΔΔCt (log2 fold change)
1
0
-1
-2
-3
0
1
2
3
4
siRNA Dose (mg/kg)
Anti-B cell Ab(Cys)-HPRT-PEG5k (n=1)
Anti-B cell Ab(Lys)-HPRT-PEG5k (n=1)
Anti-B cell Ab(Cys)-scramble-PEG5k (n=1)
PBS Control

FIG. 64E

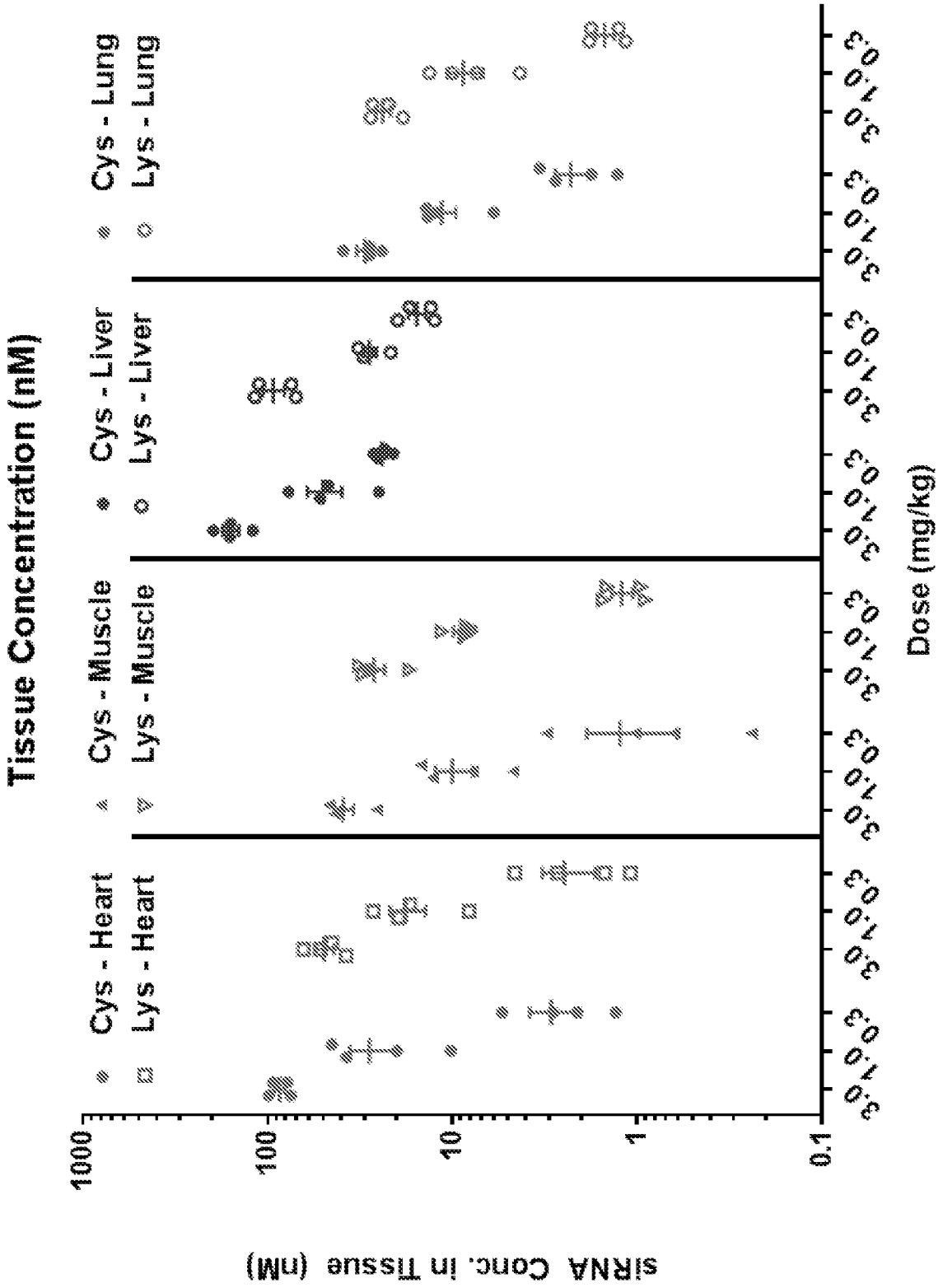

Tissue Concentration (nM)
Cys - Heart
Lys - Heart
Cys - Muscle
Lys - Muscle
Cys - Liver
Lys - Liver
Cys - Lung
Lys - Lung
siRNA Conc. in Tissue (nM)
1000
100
10
1
0.1
3.0
1.0
0.3
Dose (mg/kg)

FIG. 65A

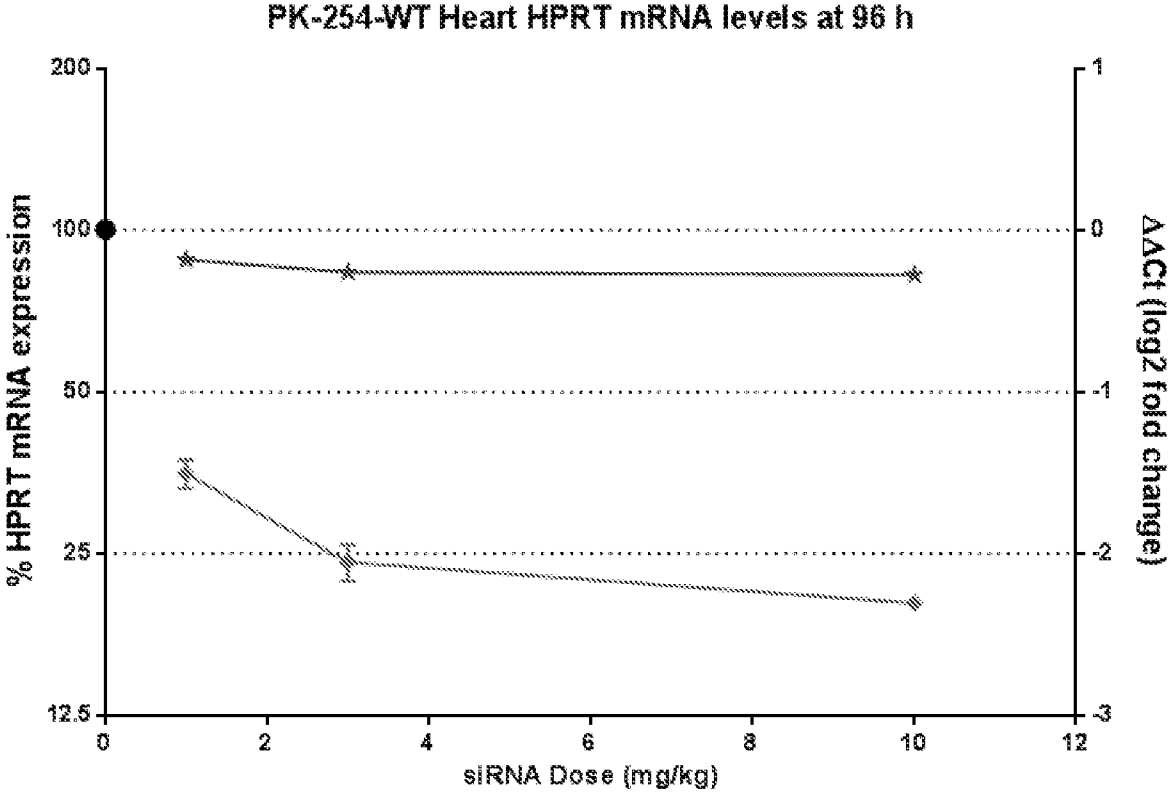

PK-254-WT Heart HPRT mRNA levels at 96 h
% HPRT mRNA expression
ΔΔCt (log2 fold change)
200
100
50
25
12.5
1
0
-1
-2
-3
0
2
4
6
8
10
12
siRNA Dose (mg/kg)
Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)
Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)
PBS Control

FIG. 65B

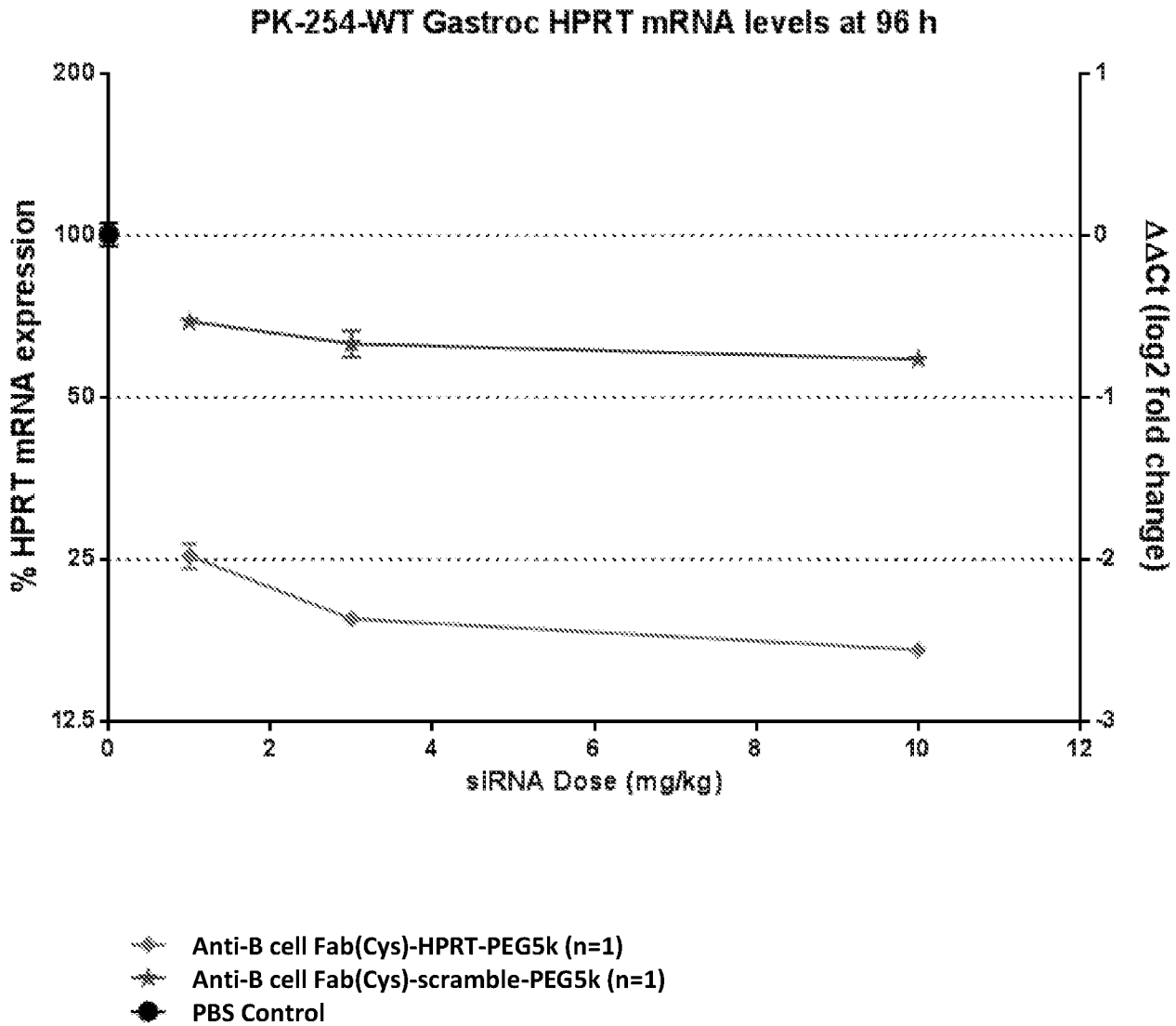

PK-254-WT Gastroc HPRT mRNA levels at 96 h
% HPRT mRNA expression
200
100
50
25
12.5
ΔΔCt (log2 fold change)
1
0
-1
-2
-3
0
2
4
6
8
10
12
siRNA Dose (mg/kg)
Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)
Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)
PBS Control

FIG. 65C

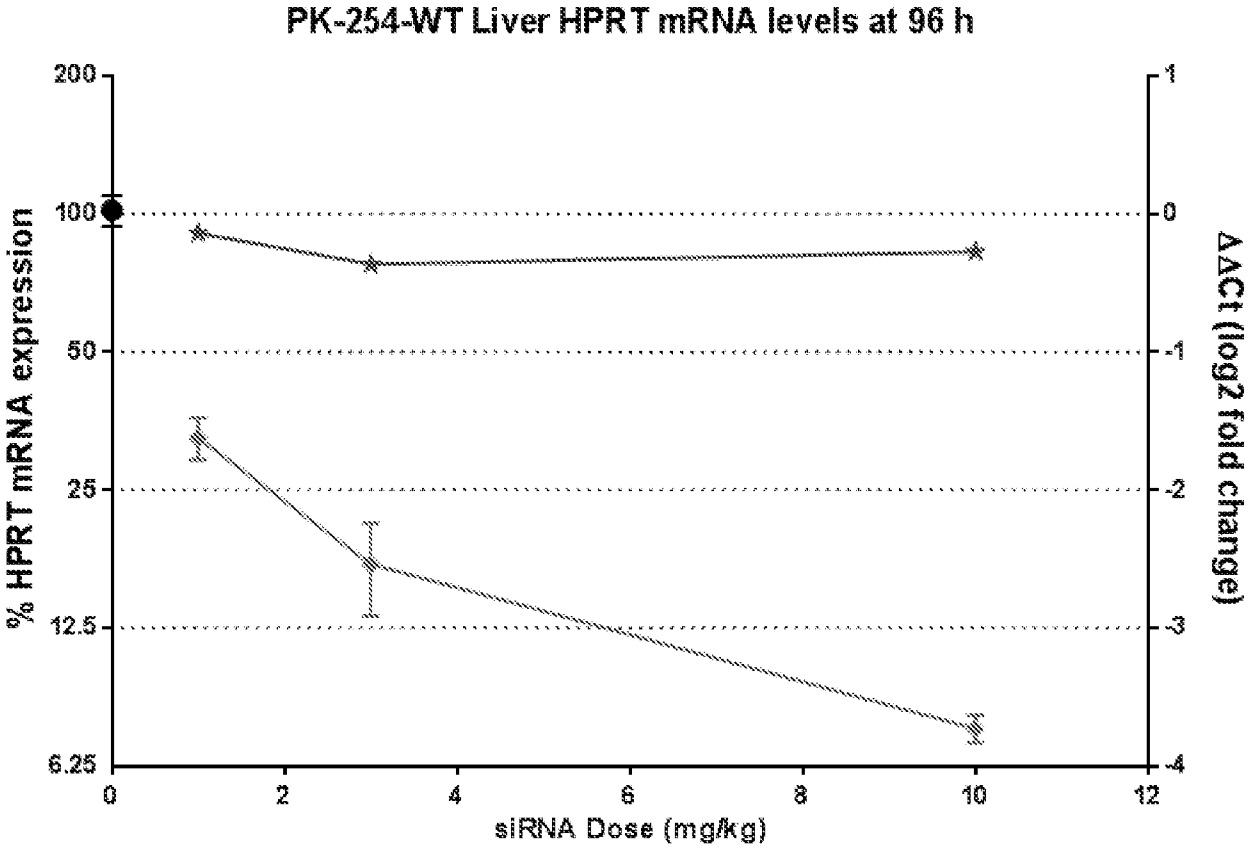

PK-254-WT Liver HPRT mRNA levels at 96 h
% HPRT mRNA expression
ΔΔCt (log2 fold change)
200
100
50
25
12.5
6.25
1
0
-1
-2
-3
-4
0
2
4
6
8
10
12
siRNA Dose (mg/kg)
Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)
Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)
PBS Control

FIG. 65D

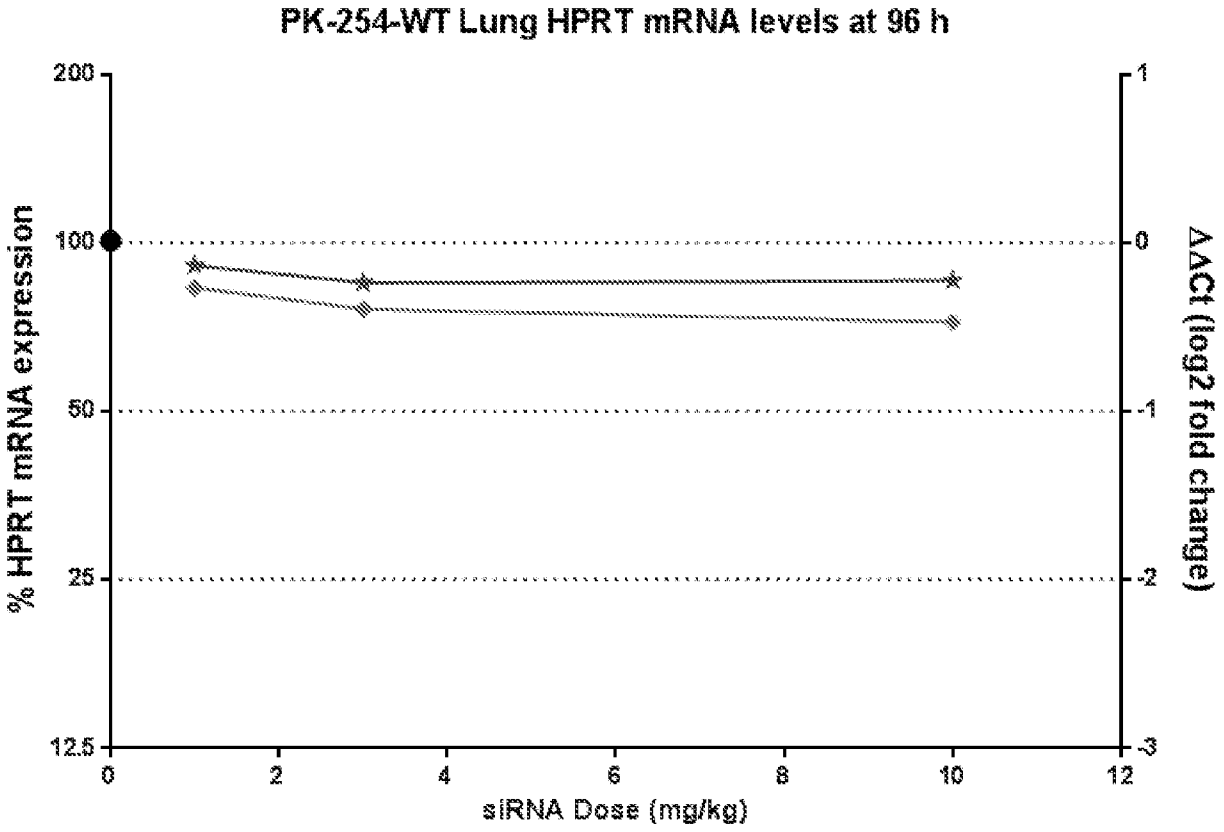

PK-254-WT Lung HPRT mRNA levels at 96 h
% HPRT mRNA expression
ΔΔCt (log2 fold change)
200
100
50
25
12.5
1
0
-1
-2
-3
0
2
4
6
8
10
12
siRNA Dose (mg/kg)
Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)
Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)
PBS Control

FIG. 65E

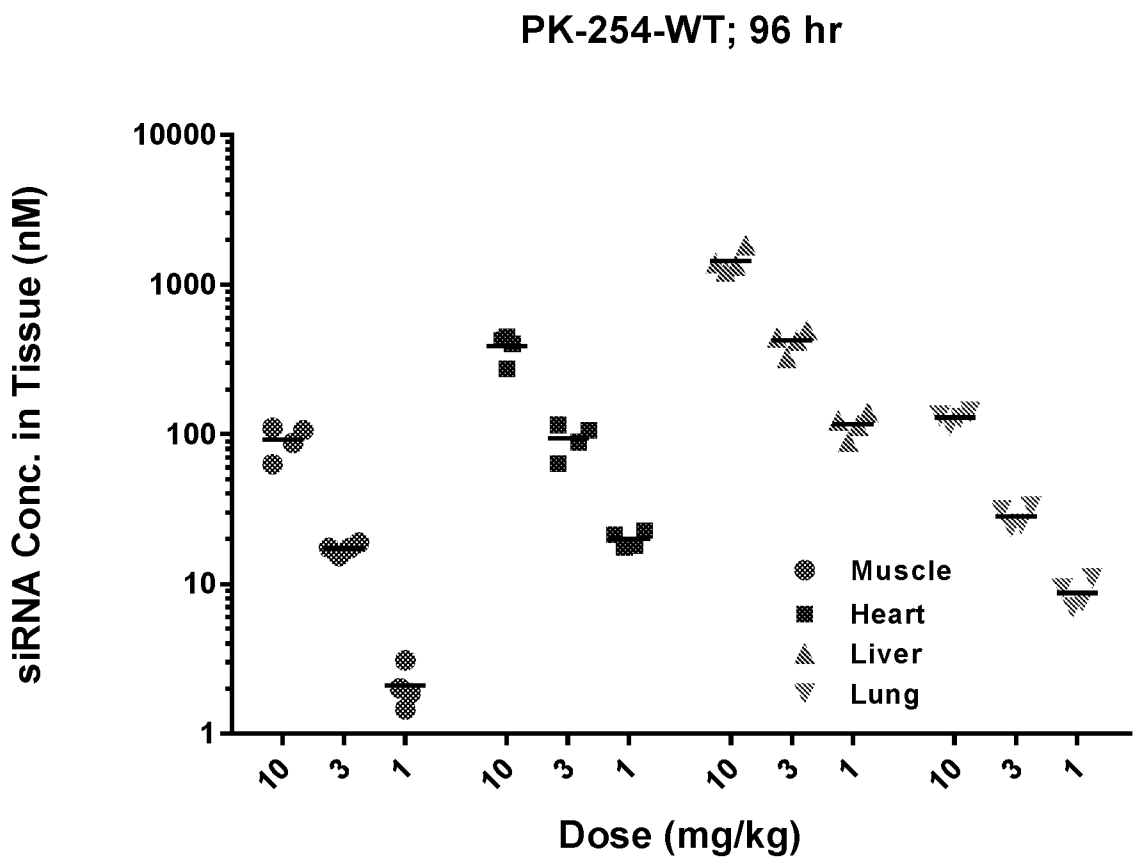

PK-254-WT; 96 hr
siRNA Conc. in Tissue (nM)
10000
1000
100
10
1
Muscle
Heart
Liver
Lung
10
3
1
10
3
1
10
3
1
10
3
1
Dose (mg/kg)

FIG. 66A

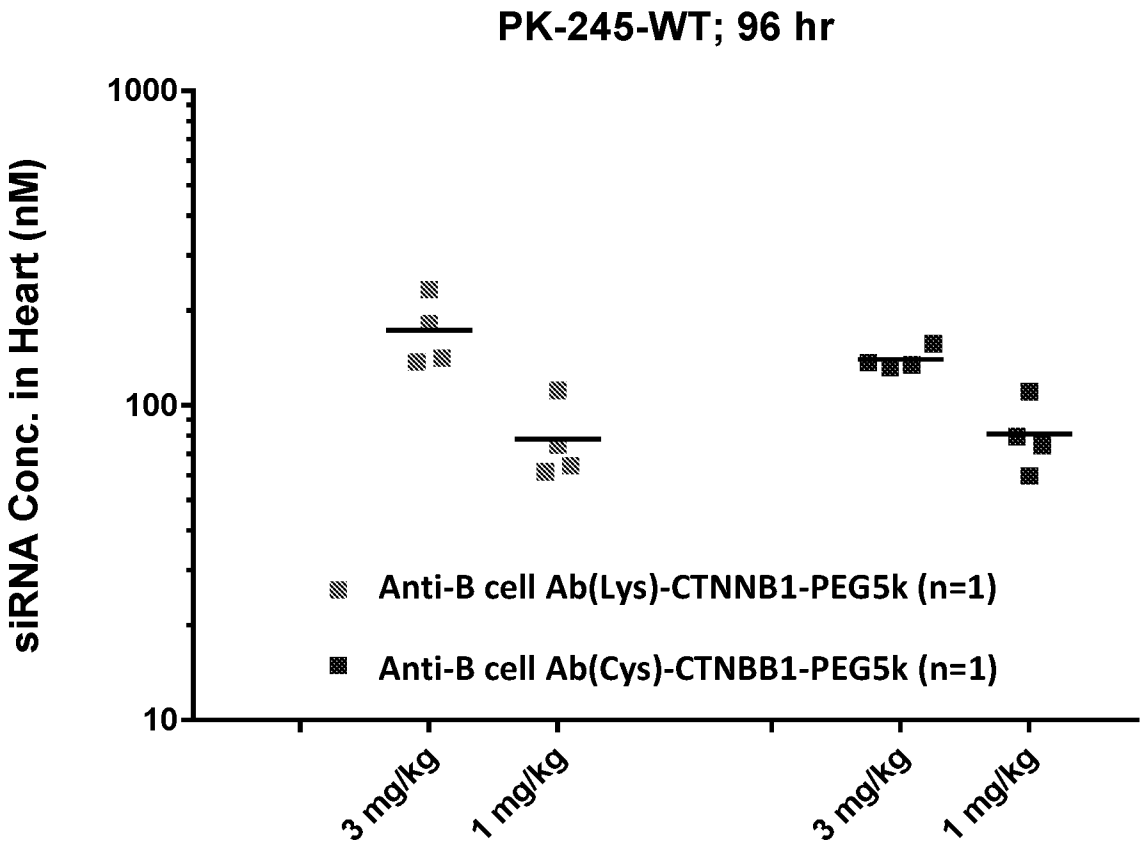

PK-245-WT; 96 hr
siRNA Conc. in Heart (nM)
1000
100
10
Anti-B cell Ab(Lys)-CTNNB1-PEG5k (n=1)
Anti-B cell Ab(Cys)-CTNBB1-PEG5k (n=1)
3 mg/kg
1 mg/kg
3 mg/kg
1 mg/kg

FIG. 66B

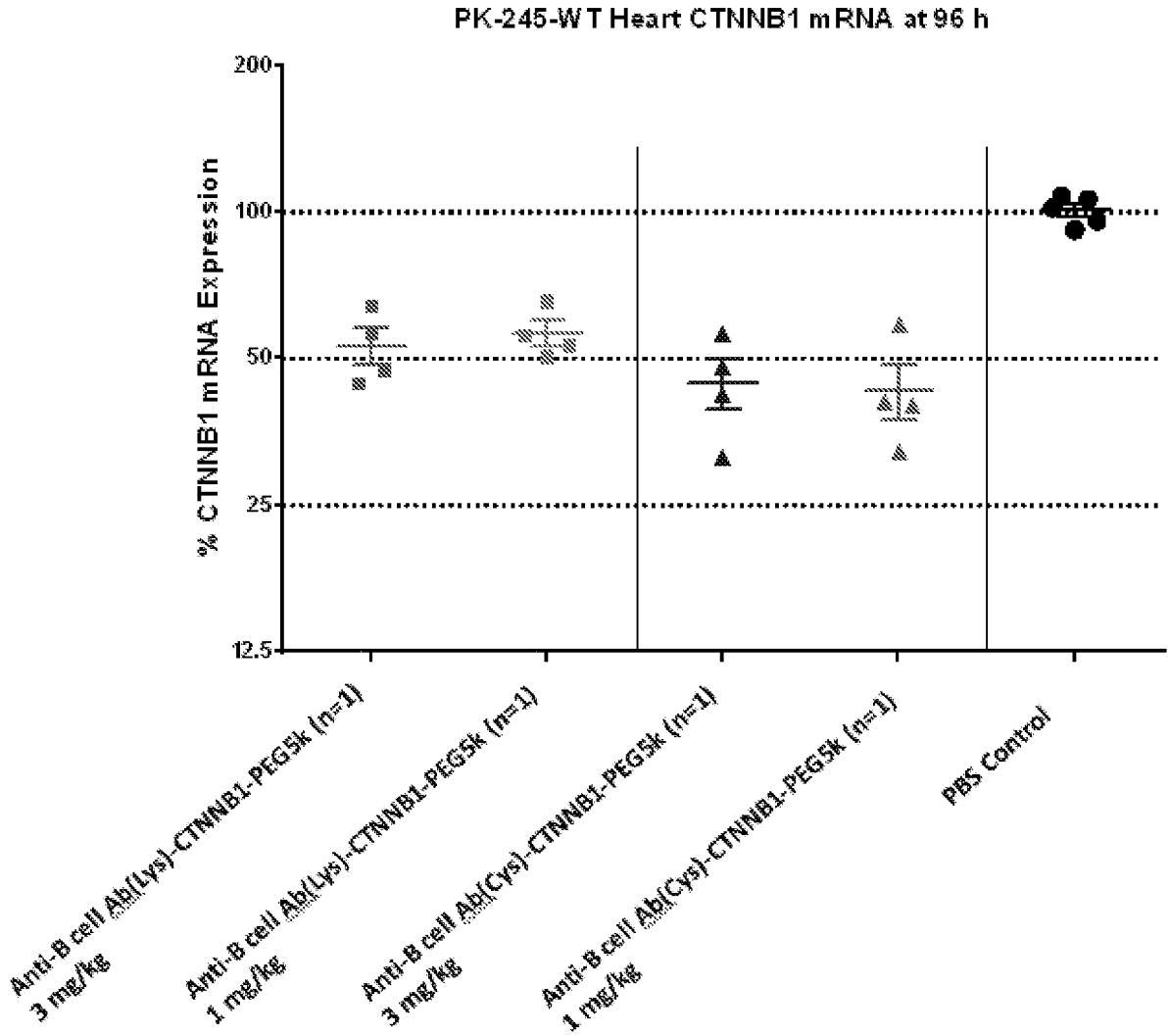

PK-245-WT Heart CTNNB1 mRNA at 96 h
% CTNNB1 mRNA Expression
200
100
50
25
12.5
Anti-B cell Ab(Lys)-CTNNB1-PEG5k (n=1) 3 mg/kg
Anti-B cell Ab(Lys)-CTNNB1-PEG5k (n=1) 1 mg/kg
Anti-B cell Ab(Cys)-CTNNB1-PEG5k (n=1) 3 mg/kg
Anti-B cell Ab(Cys)-CTNNB1-PEG5k (n=1) 1 mg/kg
PBS Control

FIG. 66C

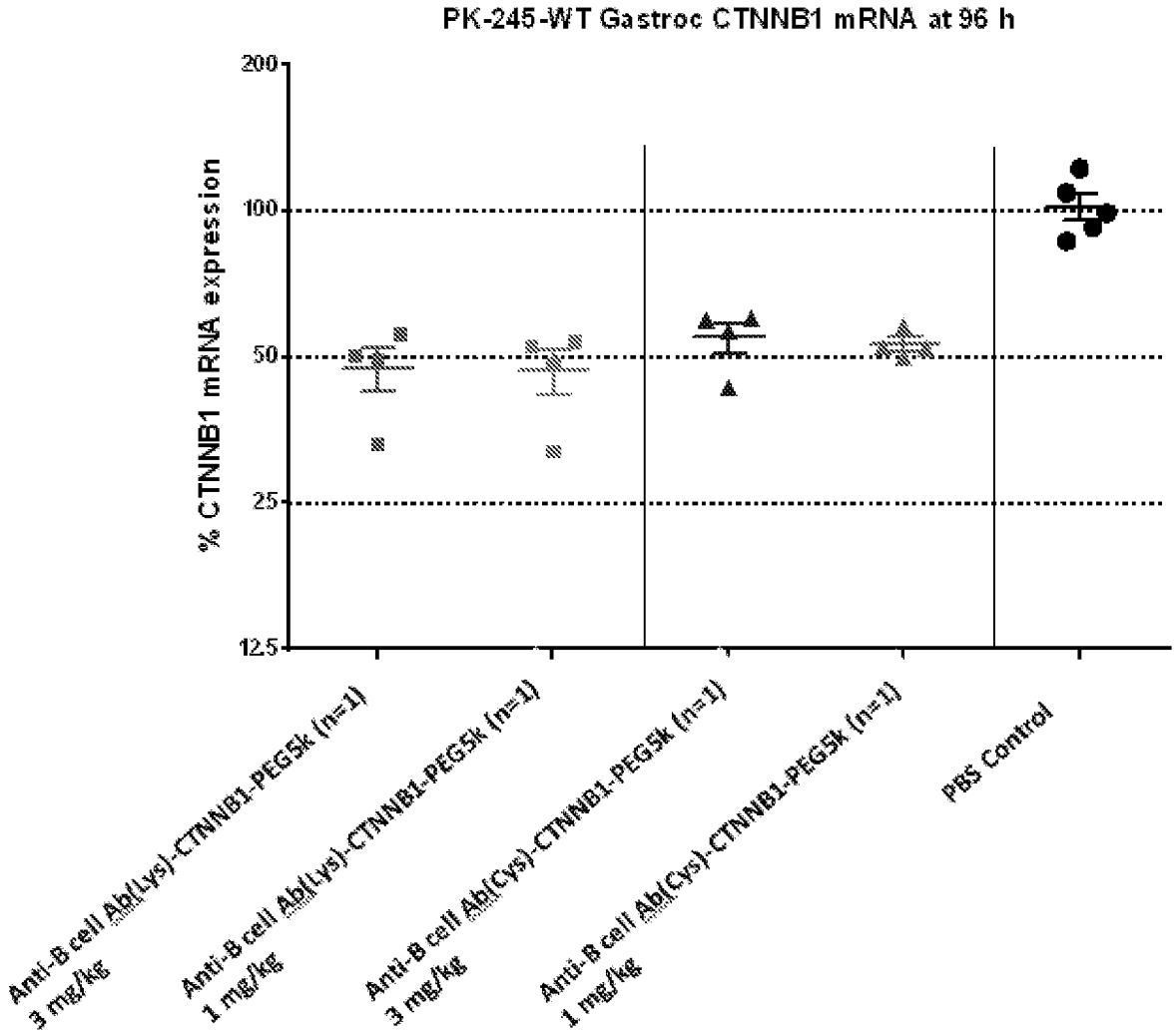

PK-245-WT Gastroc CTNNB1 mRNA at 96 h
% CTNNB1 mRNA expression
200
100
50
25
12.5
Anti-B cell Ab(Lys)-CTNNB1-PEG5k (n=1) 3 mg/kg
Anti-B cell Ab(Lys)-CTNNB1-PEG5k (n=1) 1 mg/kg
Anti-B cell Ab(Cys)-CTNNB1-PEG5k (n=1) 3 mg/kg
Anti-B cell Ab(Cys)-CTNNB1-PEG5k (n=1) 1 mg/kg
PBS Control

FIG. 66D

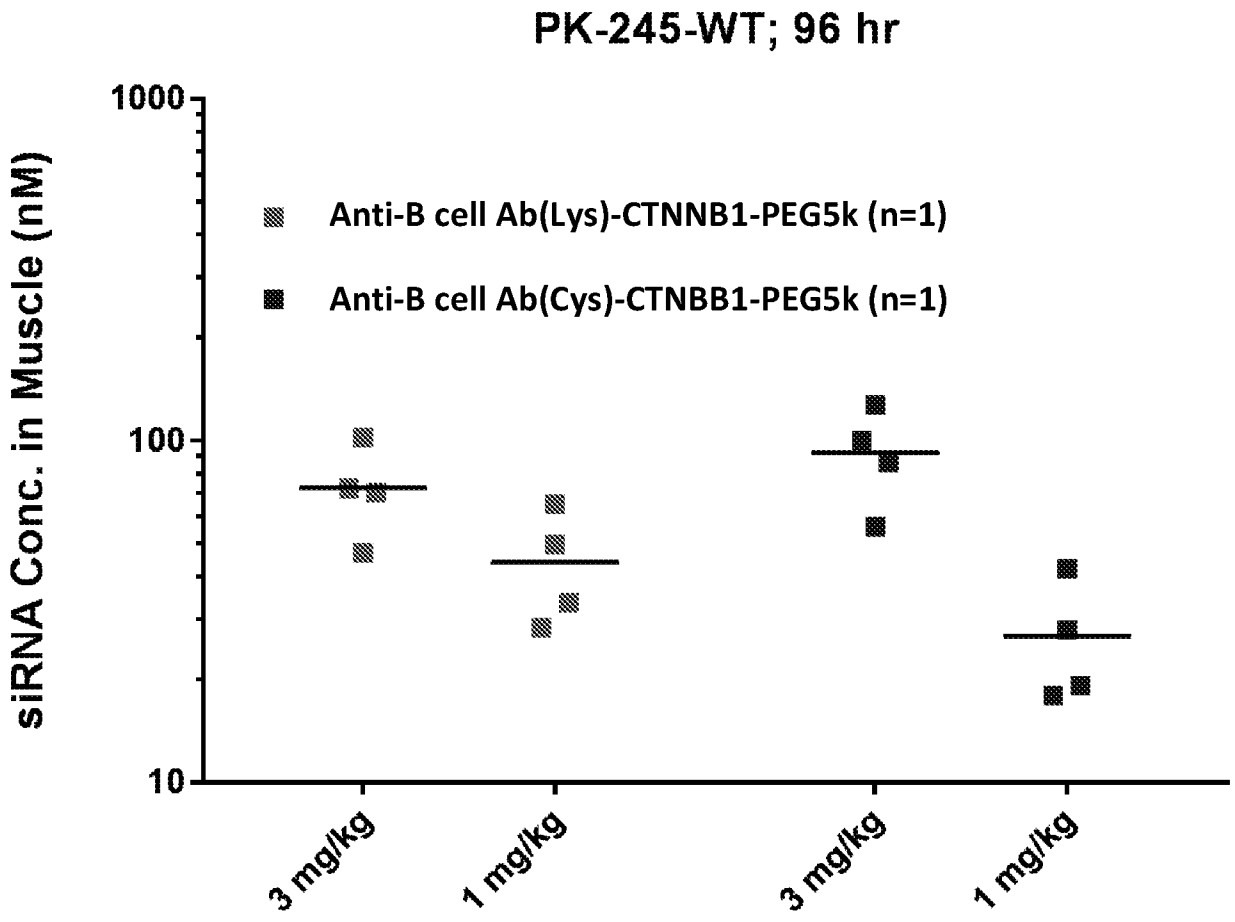

PK-245-WT; 96 hr
siRNA Conc. in Muscle (nM)
1000
100
10
Anti-B cell Ab(Lys)-CTNNB1-PEG5k (n=1)
Anti-B cell Ab(Cys)-CTNBB1-PEG5k (n=1)
3 mg/kg
1 mg/kg
3 mg/kg
1 mg/kg

FIG. 67A

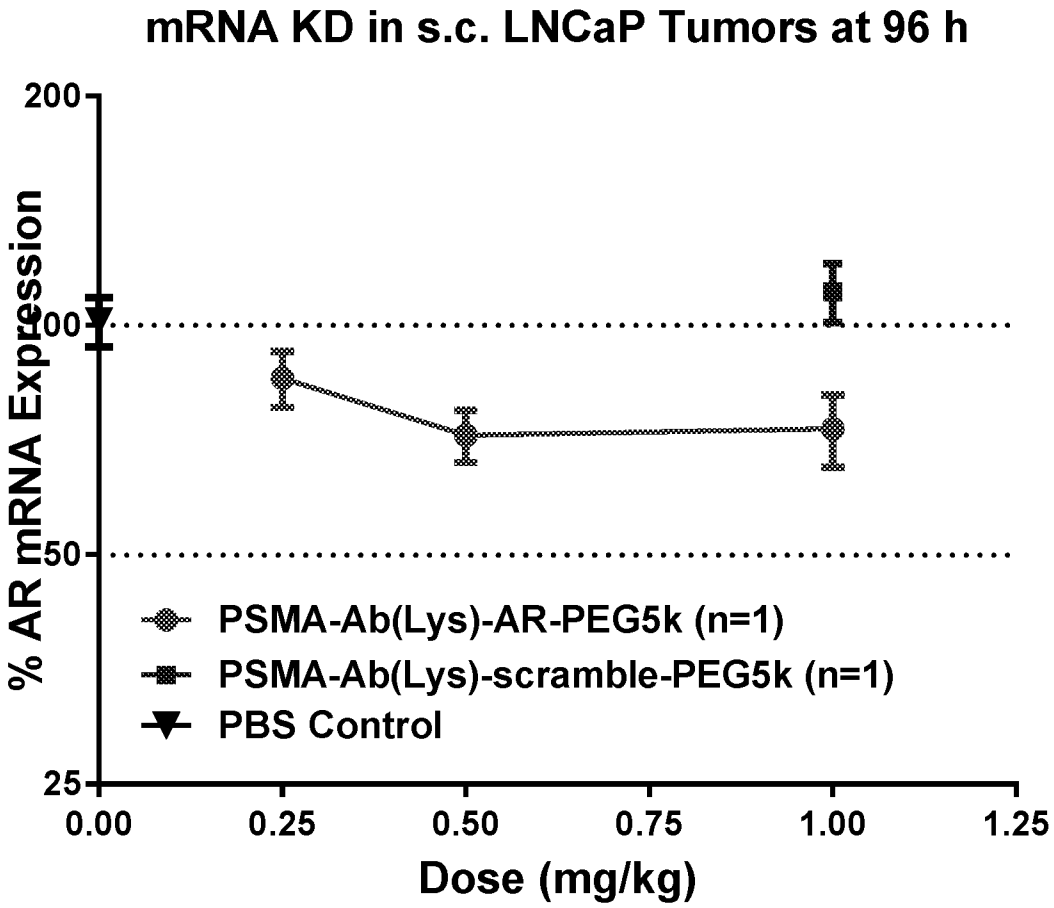

mRNA KD in s.c. LNCaP Tumors at 96 h
% AR mRNA Expression
200
100
50
25
0.00
0.25
0.50
0.75
1.00
1.25
Dose (mg/kg)
PSMA-Ab(Lys)-AR-PEG5k (n=1)
PSMA-Ab(Lys)-scramble-PEG5k (n=1)
PBS Control

FIG. 67B

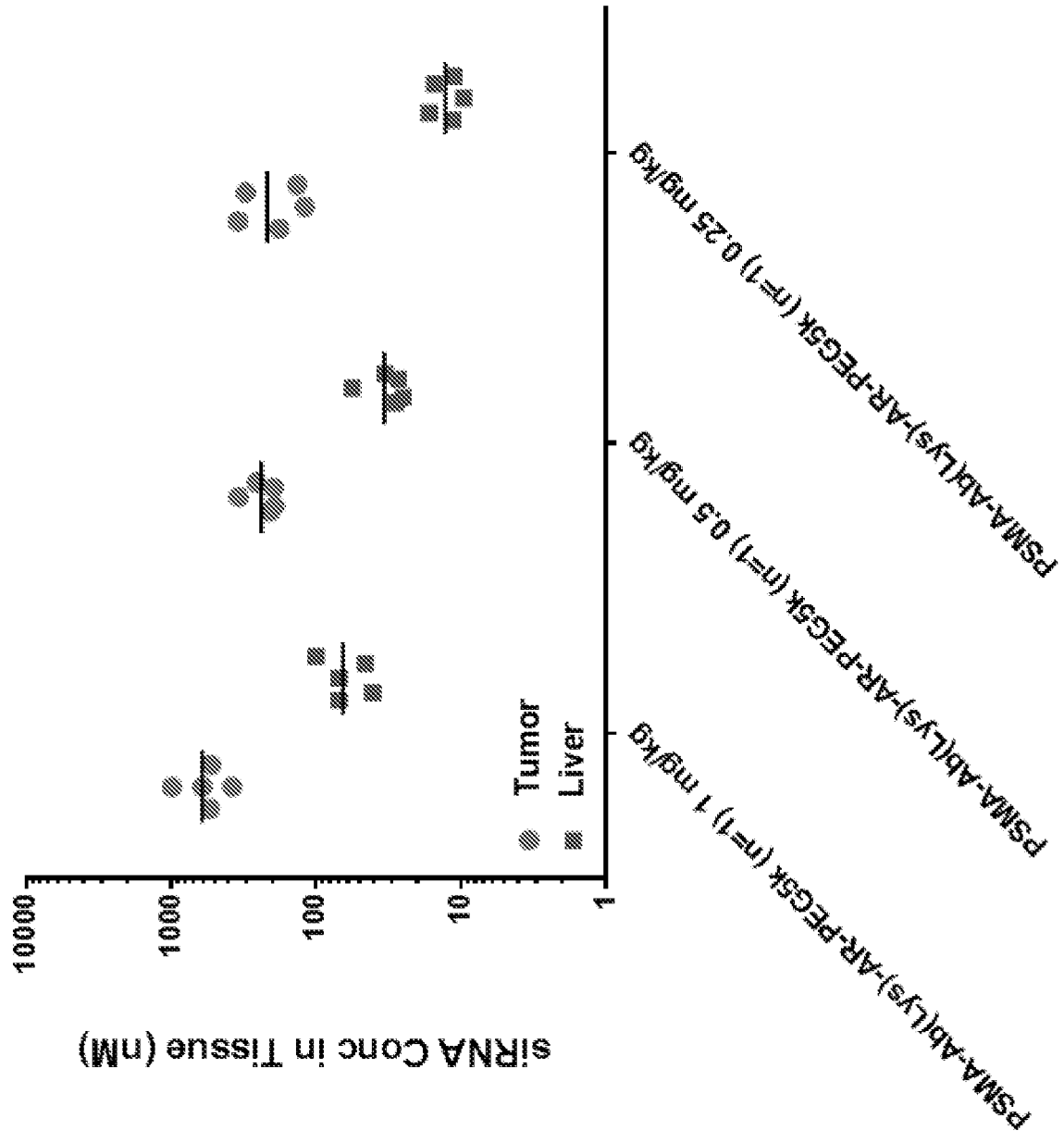
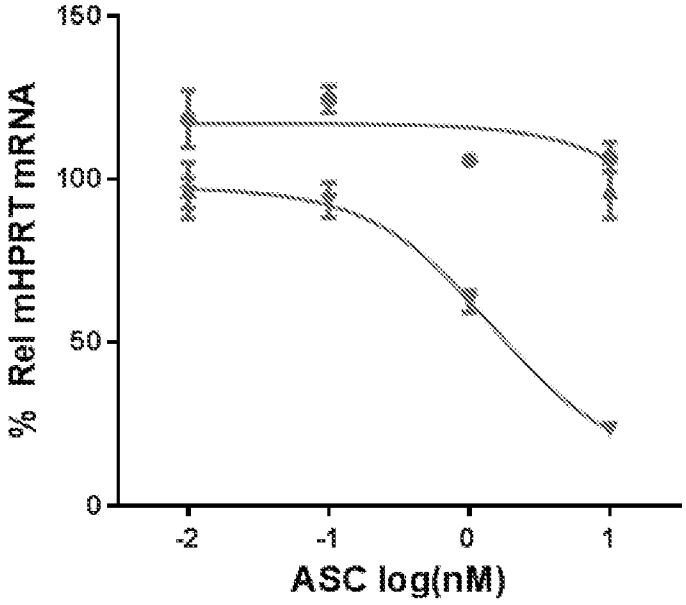
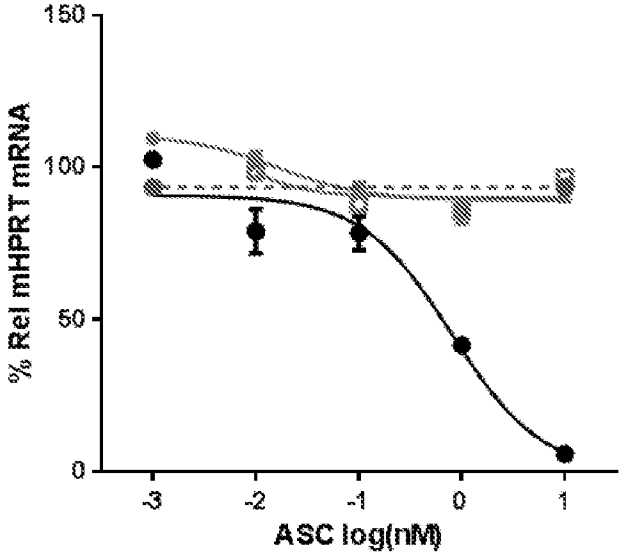

PK-160-LNCaP; 96 h
siRNA Conc in Tissue (nM)
10000
1000
100
10
1
Tumor
Liver
PSMA-Abl(Lys)-AR-PEG5k (n=1) 1 mg/kg
PSMA-Abl(Lys)-AR-PEG5k (n=1) 0.5 mg/kg
PSMA-Abl(Lys)-AR-PEG5k (n=1) 0.25 mg/kg

FIG. 68A



Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)
Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)
Vehicle

FIG. 68B



Vehicle
Anti-B cell Fab(Cys)-scramble-PEG5k (n=1)
Anti-B cell Fab(Cys)-HPRT-PEG5k (n=1)
Anti-B cell mAb

FIG. 69A

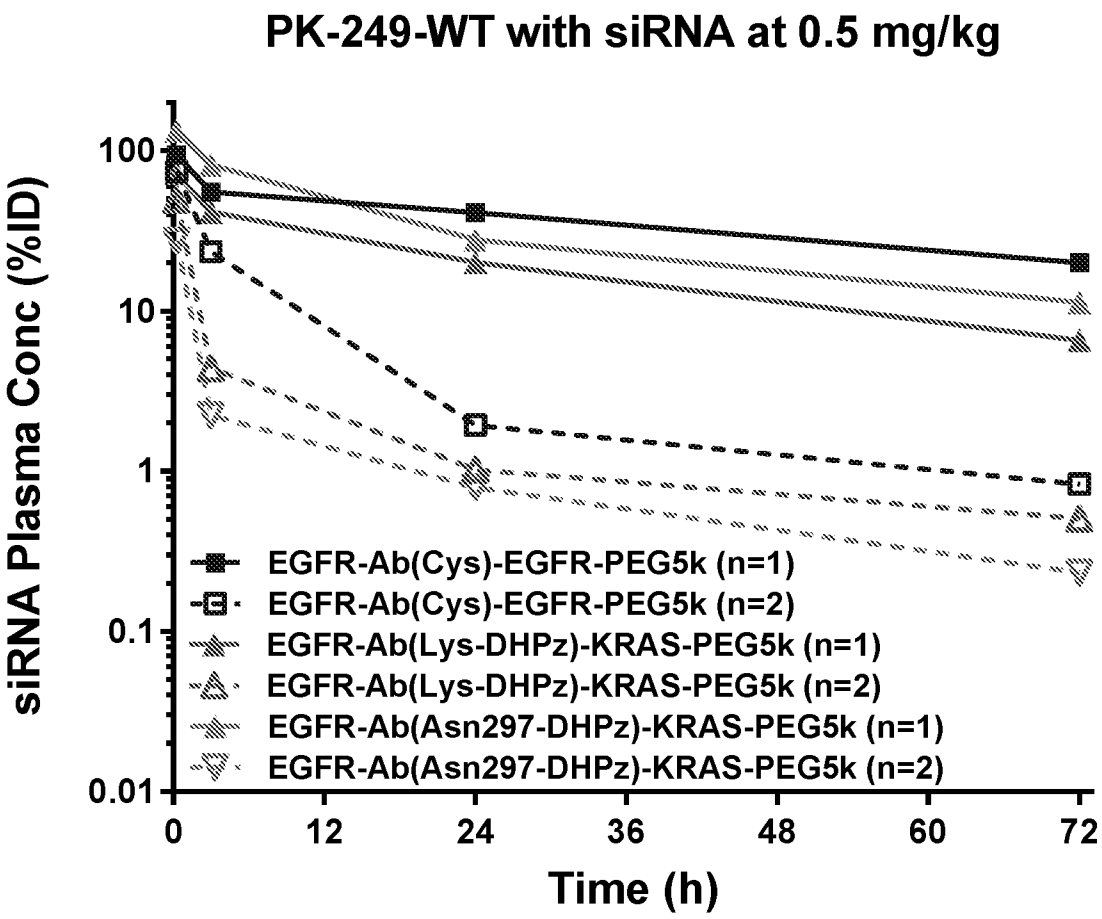

PK-249-WT with siRNA at 0.5 mg/kg
siRNA Plasma Conc (%ID)
100
10
1
0.1
0.01
0
12
24
36
48
60
72
Time (h)
EGFR-Ab(Cys)-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-EGFR-PEG5k (n=2)
EGFR-Ab(Lys-DHPz)-KRAS-PEG5k (n=1)
EGFR-Ab(Lys-DHPz)-KRAS-PEG5k (n=2)
EGFR-Ab(Asn297-DHPz)-KRAS-PEG5k (n=1)
EGFR-Ab(Asn297-DHPz)-KRAS-PEG5k (n=2)

FIG. 69B

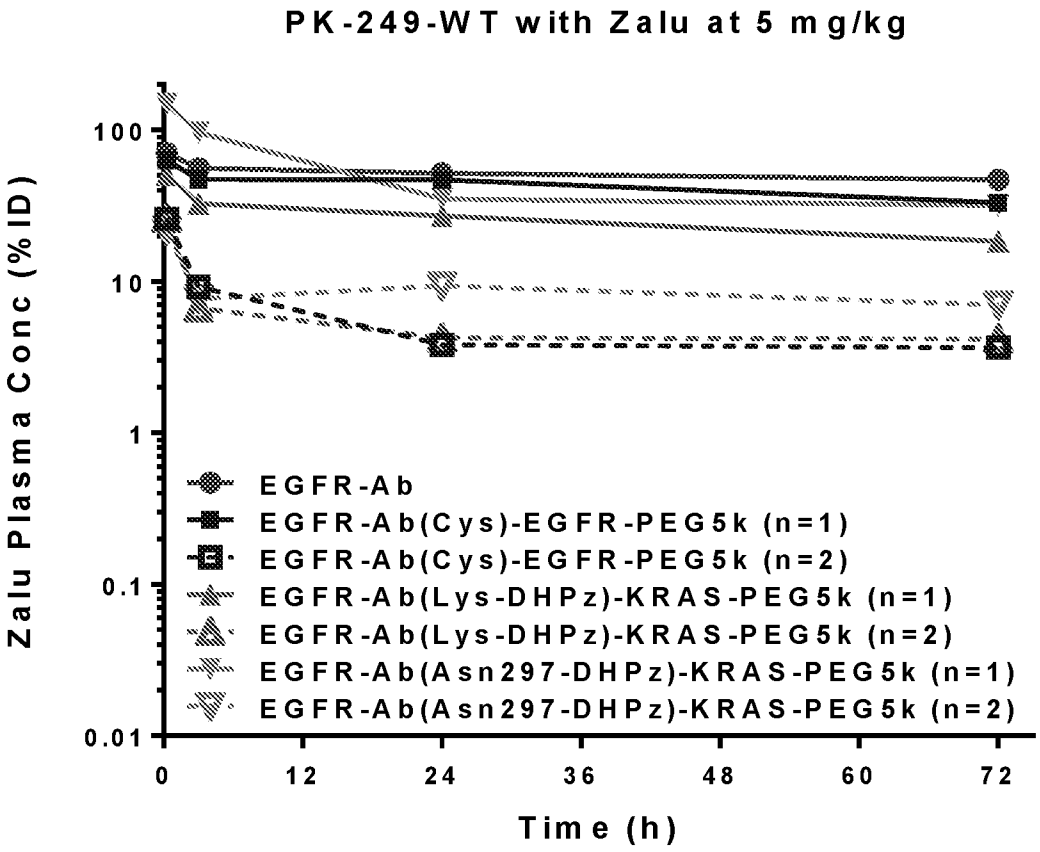

PK-249-WT with Zalu at 5 mg/kg
Zalu Plasma Conc (%ID)
100
10
1
0.1
0.01
0
12
24
36
48
60
72
Time (h)
EGFR-Ab
EGFR-Ab(Cys)-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-EGFR-PEG5k (n=2)
EGFR-Ab(Lys-DHPz)-KRAS-PEG5k (n=1)
EGFR-Ab(Lys-DHPz)-KRAS-PEG5k (n=2)
EGFR-Ab(Asn297-DHPz)-KRAS-PEG5k (n=1)
EGFR-Ab(Asn297-DHPz)-KRAS-PEG5k (n=2)

FIG. 70A

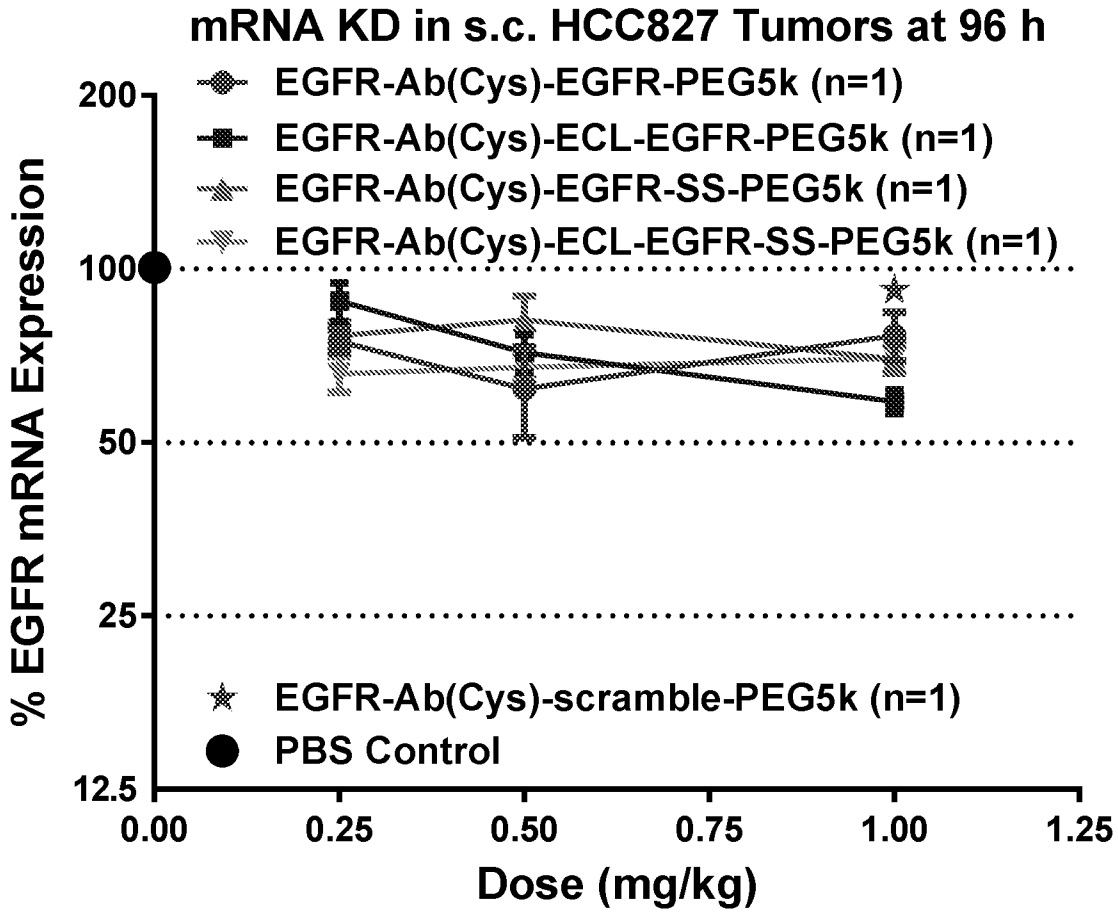

mRNA KD in s.c. HCC827 Tumors at 96 h
EGFR-Ab(Cys)-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1)
EGFR-Ab(Cys)-ECL-EGFR-SS-PEG5k (n=1)
EGFR-Ab(Cys)-scramble-PEG5k (n=1)
PBS Control
% EGFR mRNA Expression
200
100
50
25
12.5
0.00
0.25
0.50
0.75
1.00
1.25
Dose (mg/kg)

FIG. 70B

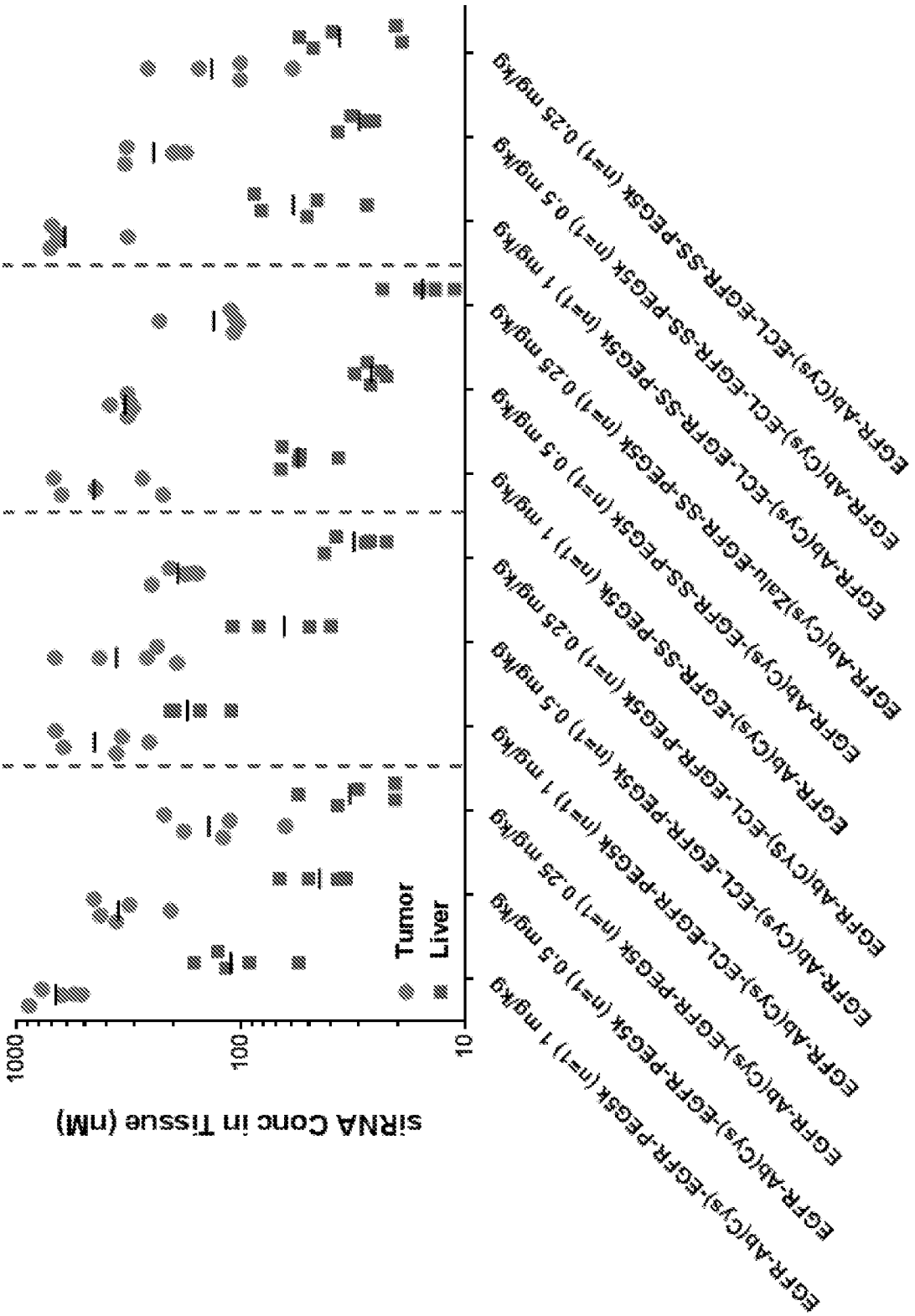

PK-180-HCC827; 96 h
siRNA Conc in Tissue (nM)
1000
100
10
Tumor
Liver
EGFR-Ab(Cys)-EGFR-PEG5k (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (n=1) 0.25 mg/kg
EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1) 1 mg/kg
EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1) 0.25 mg/kg
EGFR-Ab(Cys)2alu-EGFR-SS-PEG5k (n=1) 1 mg/kg
EGFR-Ab(Cys)-ECL-EGFR-SS-PEG5k (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-ECL-EGFR-SS-PEG5k (n=1) 0.25 mg/kg

FIG. 70C

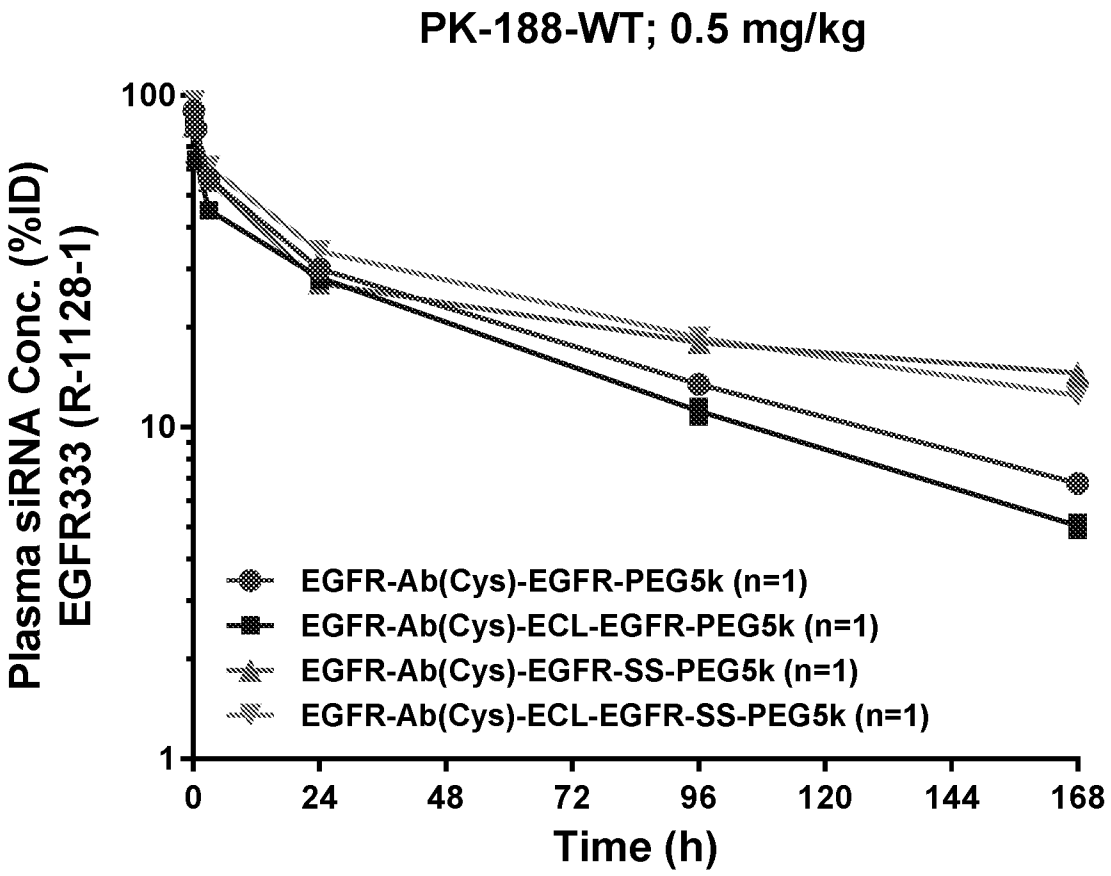

PK-188-WT; 0.5 mg/kg
Plasma siRNA Conc. (%ID)
EGFR333 (R-1128-1)
100
10
1
0
24
48
72
96
120
144
168
Time (h)
EGFR-Ab(Cys)-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1)
EGFR-Ab(Cys)-ECL-EGFR-SS-PEG5k (n=1)

FIG. 71A

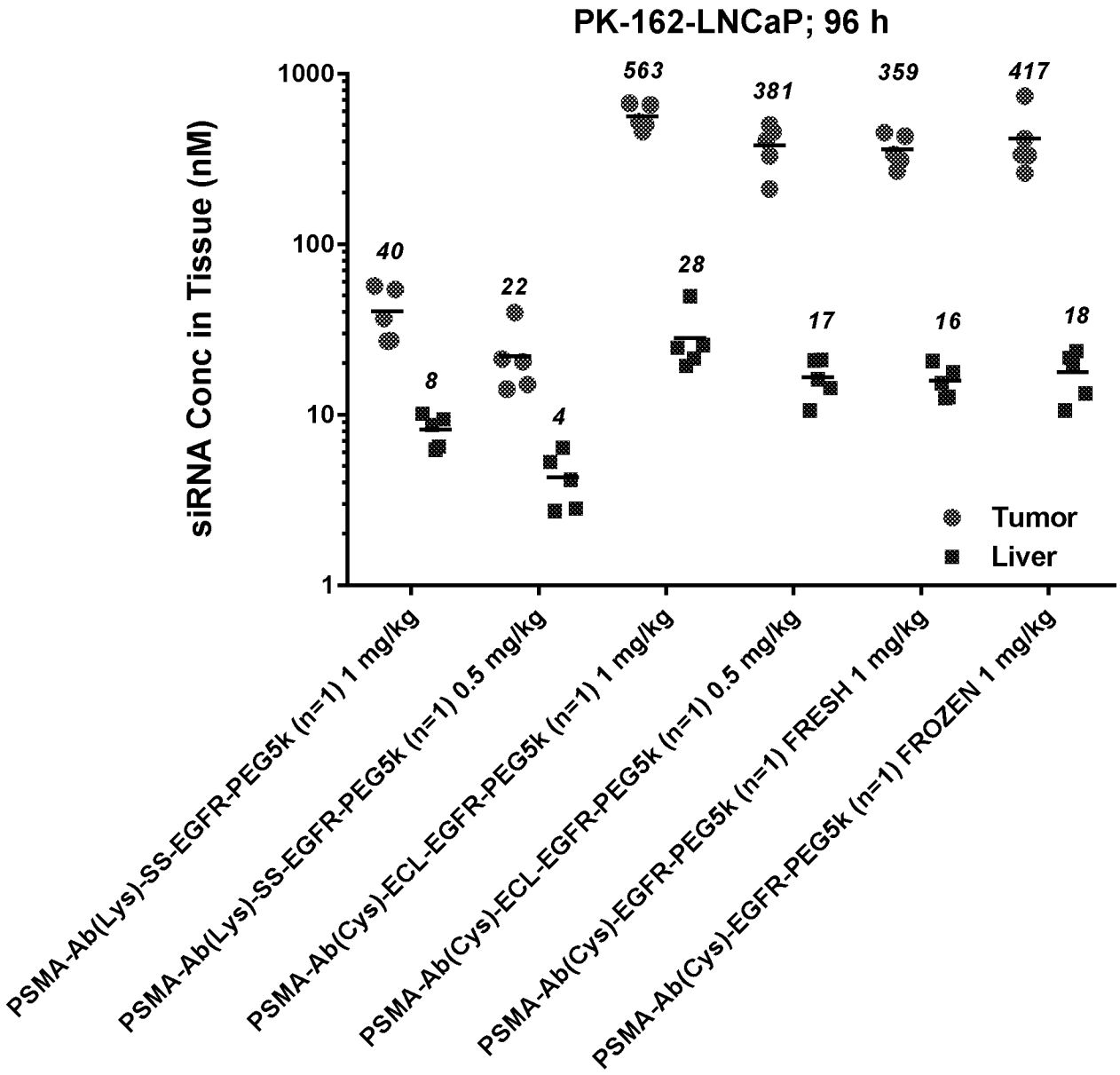

PK-162-LNCaP; 96 h
siRNA Conc in Tissue (nM)
1000
100
10
1
40
8
22
4
563
28
381
17
359
16
417
18
Tumor
Liver
PSMA-Ab(Lys)-SS-EGFR-PEG5k (n=1) 1 mg/kg
PSMA-Ab(Lys)-SS-EGFR-PEG5k (n=1) 0.5 mg/kg
PSMA-Ab(Cys)-ECL-EGFR-PEG5k (n=1) 1 mg/kg
PSMA-Ab(Cys)-ECL-EGFR-PEG5k (n=1) 0.5 mg/kg
PSMA-Ab(Cys)-EGFR-PEG5k (n=1) FRESH 1 mg/kg
PSMA-Ab(Cys)-EGFR-PEG5k (n=1) FROZEN 1 mg/kg

FIG. 71B

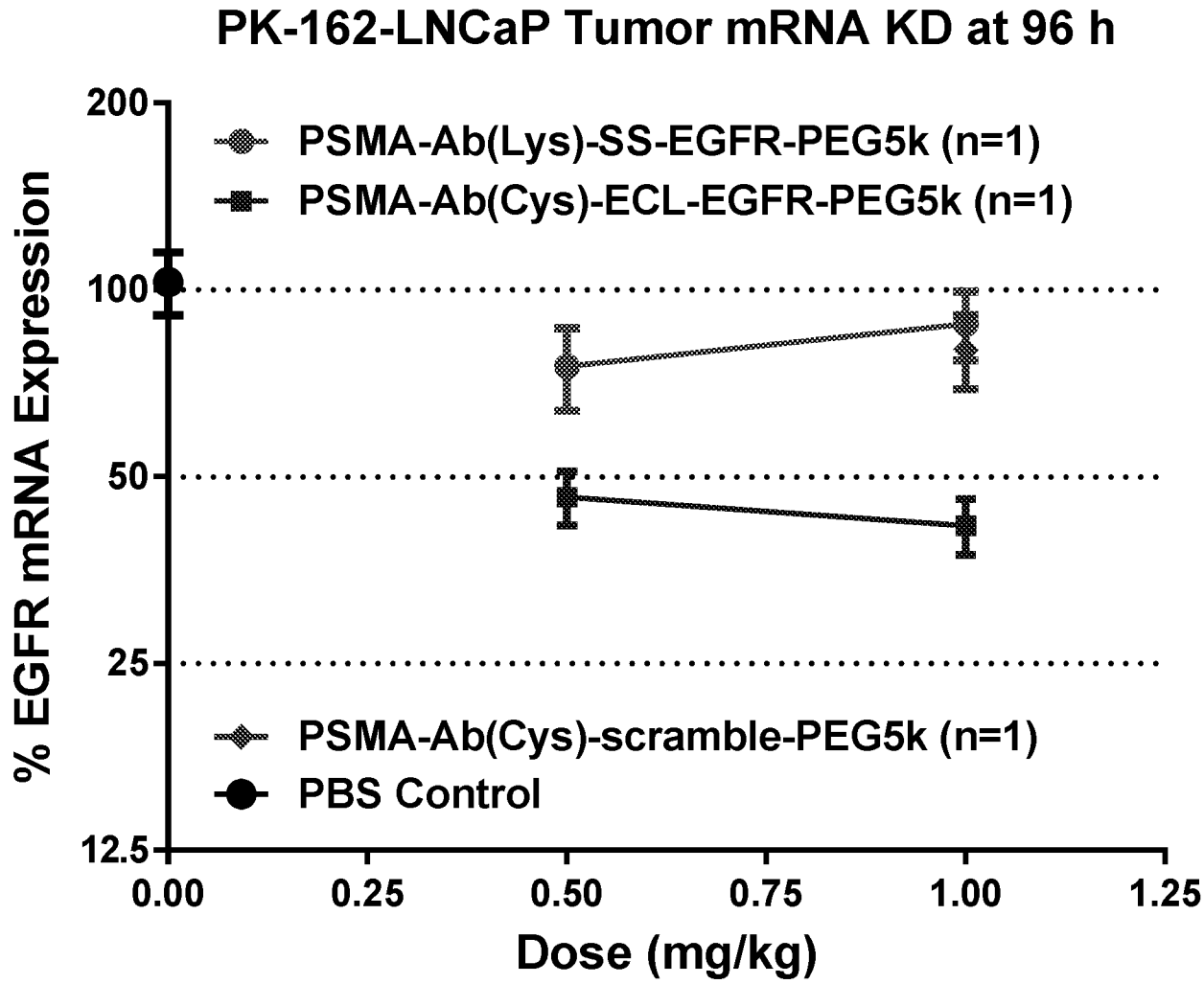

PK-162-LNCaP Tumor mRNA KD at 96 h
% EGFR mRNA Expression
200
100
50
25
12.5
PSMA-Ab(Lys)-SS-EGFR-PEG5k (n=1)
PSMA-Ab(Cys)-ECL-EGFR-PEG5k (n=1)
PSMA-Ab(Cys)-scramble-PEG5k (n=1)
PBS Control
0.00
0.25
0.50
0.75
1.00
1.25
Dose (mg/kg)

FIG. 71C

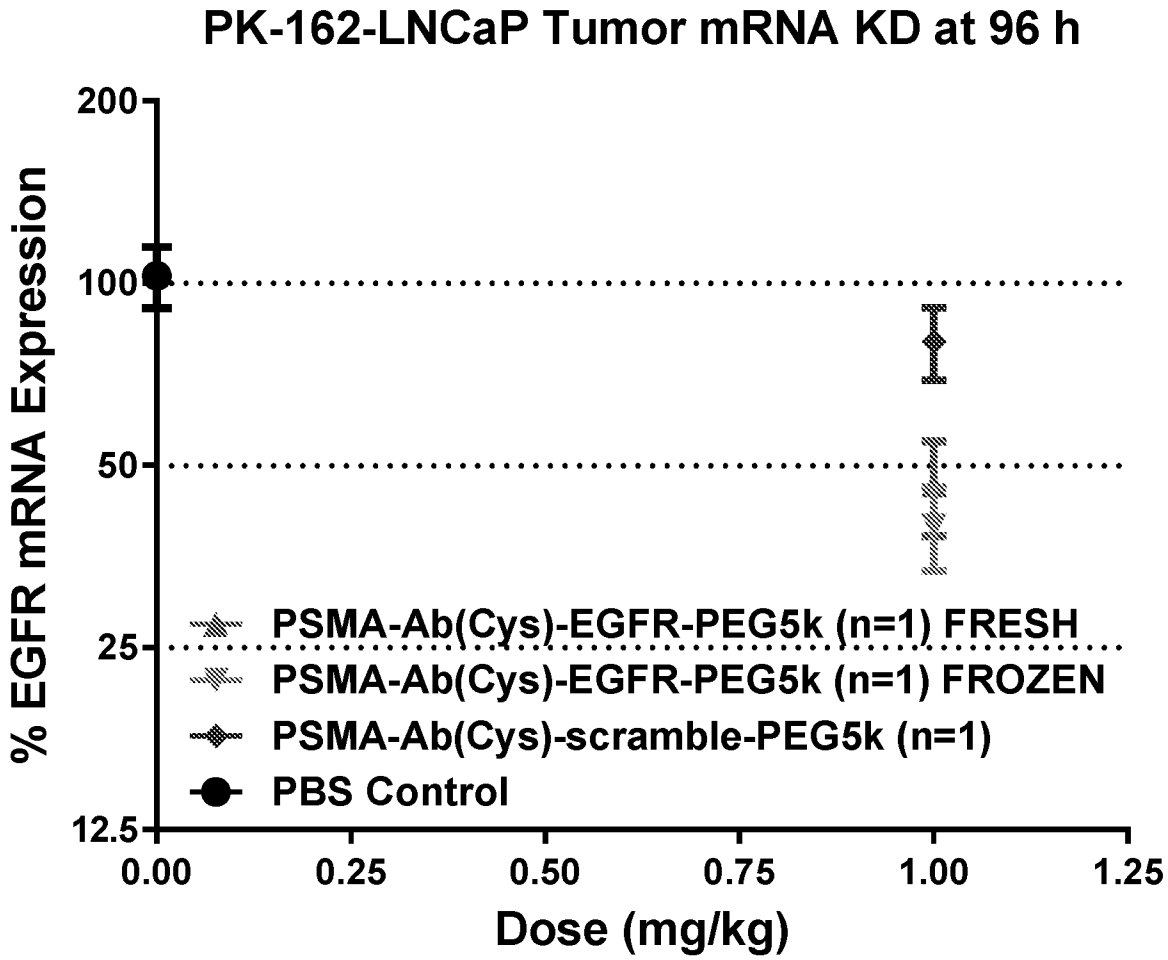

PK-162-LNCaP Tumor mRNA KD at 96 h
% EGFR mRNA Expression
200
100
50
25
12.5
0.00
0.25
0.50
0.75
1.00
1.25
Dose (mg/kg)
PSMA-Ab(Cys)-EGFR-PEG5k (n=1) FRESH
PSMA-Ab(Cys)-EGFR-PEG5k (n=1) FROZEN
PSMA-Ab(Cys)-scramble-PEG5k (n=1)
PBS Control

FIG. 72A

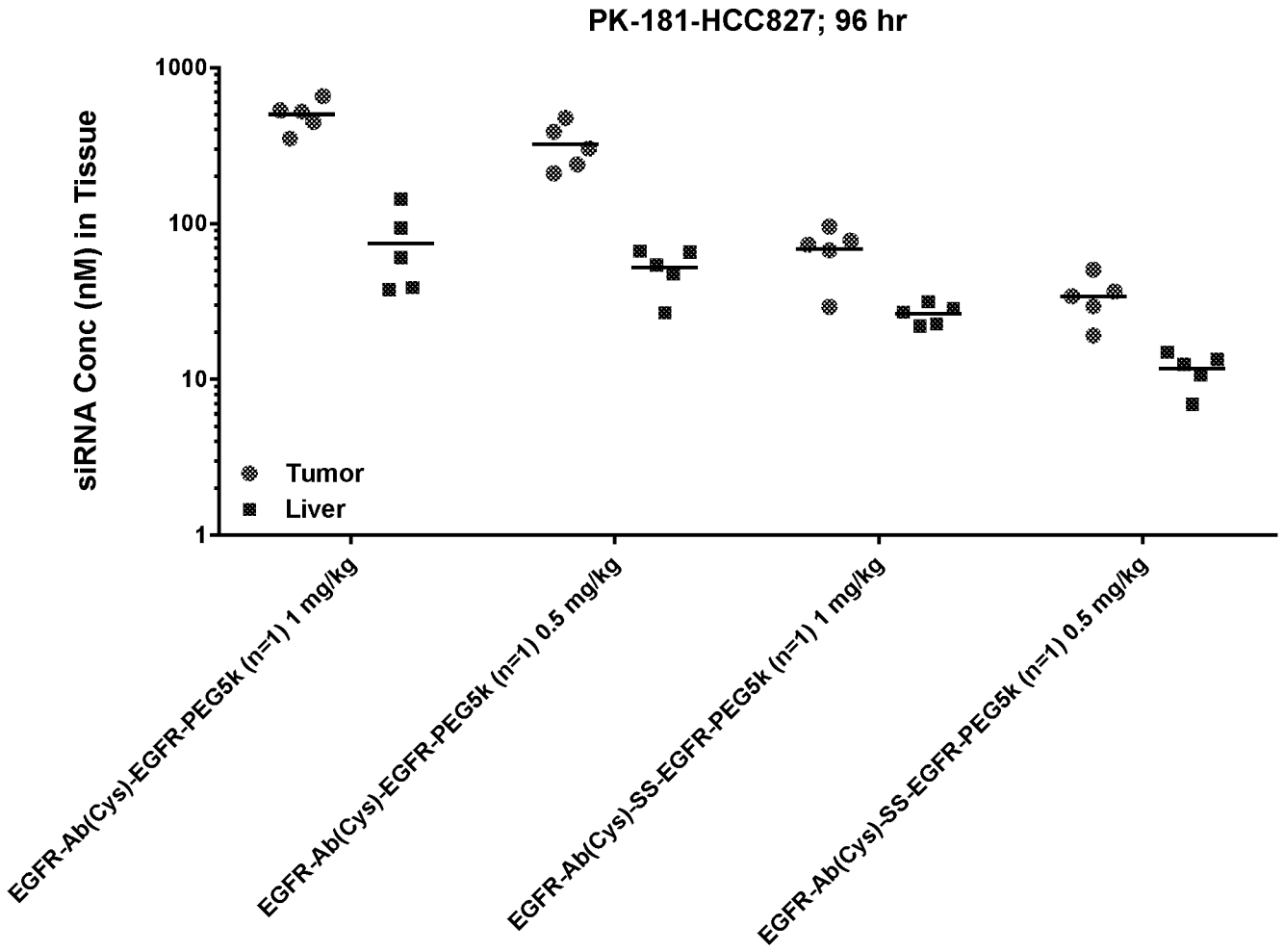

PK-181-HCC827; 96 hr
siRNA Conc (nM) in Tissue
1000
100
10
1
Tumor
Liver
EGFR-Ab(Cys)-EGFR-PEG5k (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-SS-EGFR-PEG5k (n=1) 1 mg/kg
EGFR-Ab(Cys)-SS-EGFR-PEG5k (n=1) 0.5 mg/kg

FIG. 72B

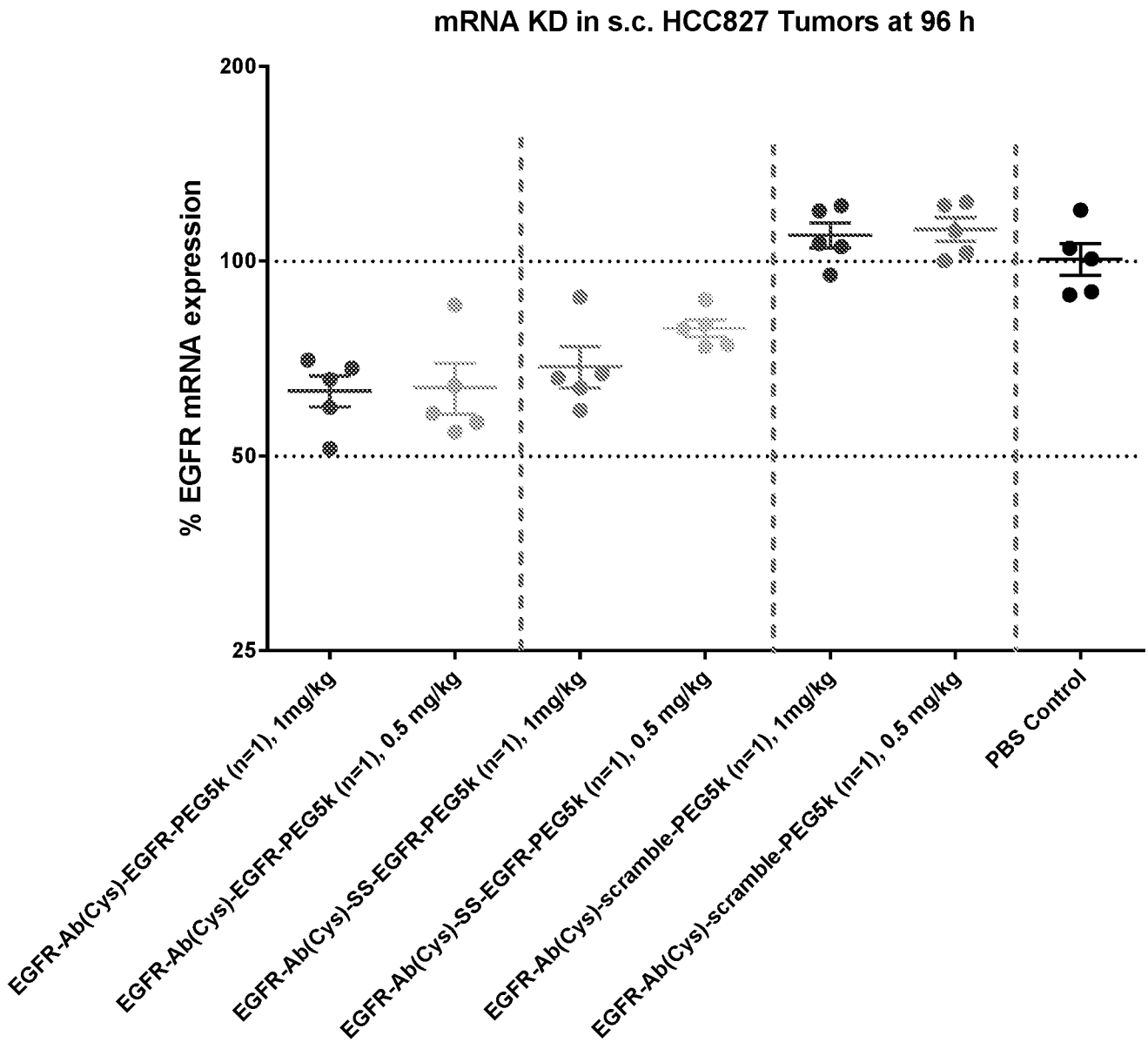

mRNA KD in s.c. HCC827 Tumors at 96 h
% EGFR mRNA expression
200
100
50
25
EGFR-Ab(Cys)-EGFR-PEG5k (n=1), 1mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (n=1), 0.5 mg/kg
EGFR-Ab(Cys)-SS-EGFR-PEG5k (n=1), 1mg/kg
EGFR-Ab(Cys)-SS-EGFR-PEG5k (n=1), 0.5 mg/kg
EGFR-Ab(Cys)-scramble-PEG5k (n=1), 1mg/kg
EGFR-Ab(Cys)-scramble-PEG5k (n=1), 0.5 mg/kg
PBS Control

FIG. 73A

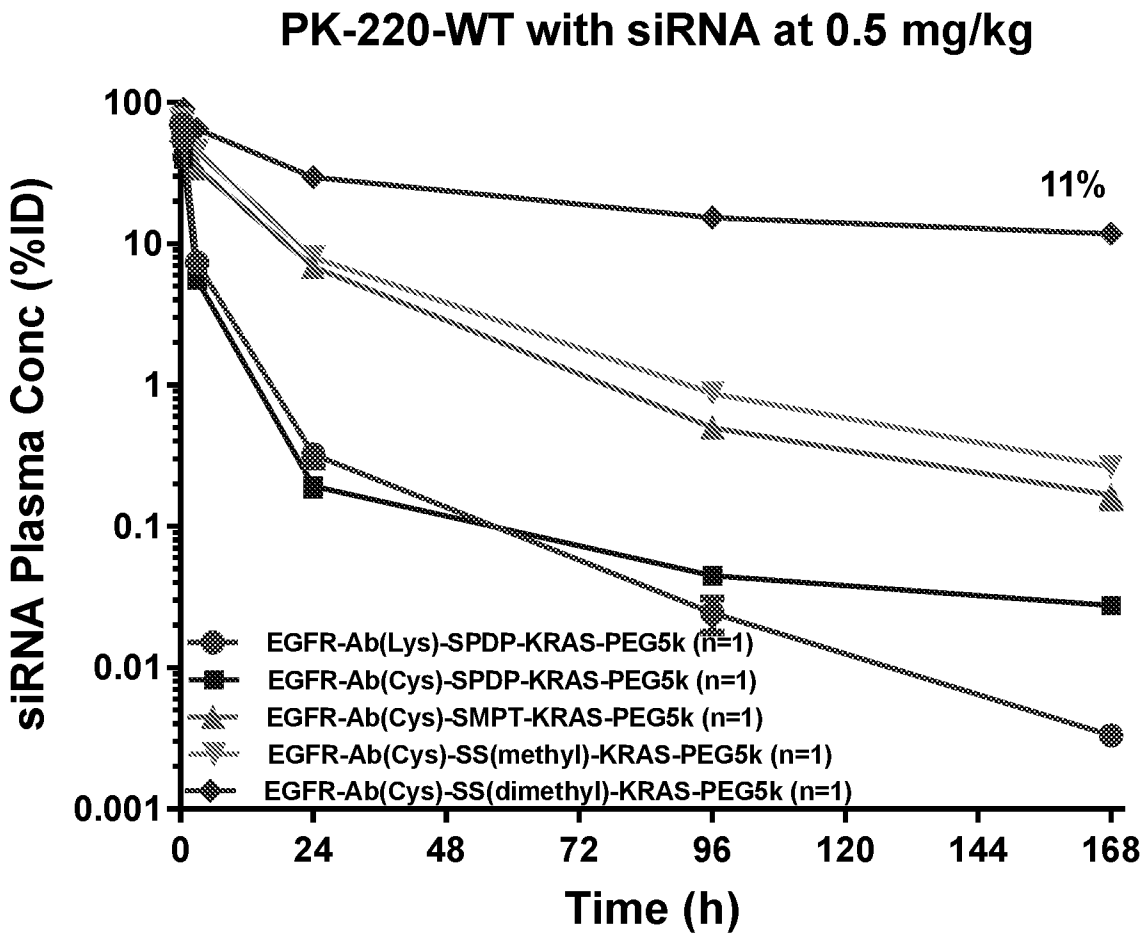

PK-220-WT with siRNA at 0.5 mg/kg
siRNA Plasma Conc (%ID)
100
10
1
0.1
0.01
0.001
0
24
48
72
96
120
144
168
Time (h)
11%
EGFR-Ab(Lys)-SPDP-KRAS-PEG5k (n=1)
EGFR-Ab(Cys)-SPDP-KRAS-PEG5k (n=1)
EGFR-Ab(Cys)-SMPT-KRAS-PEG5k (n=1)
EGFR-Ab(Cys)-SS(methyl)-KRAS-PEG5k (n=1)
EGFR-Ab(Cys)-SS(dimethyl)-KRAS-PEG5k (n=1)

FIG. 73B

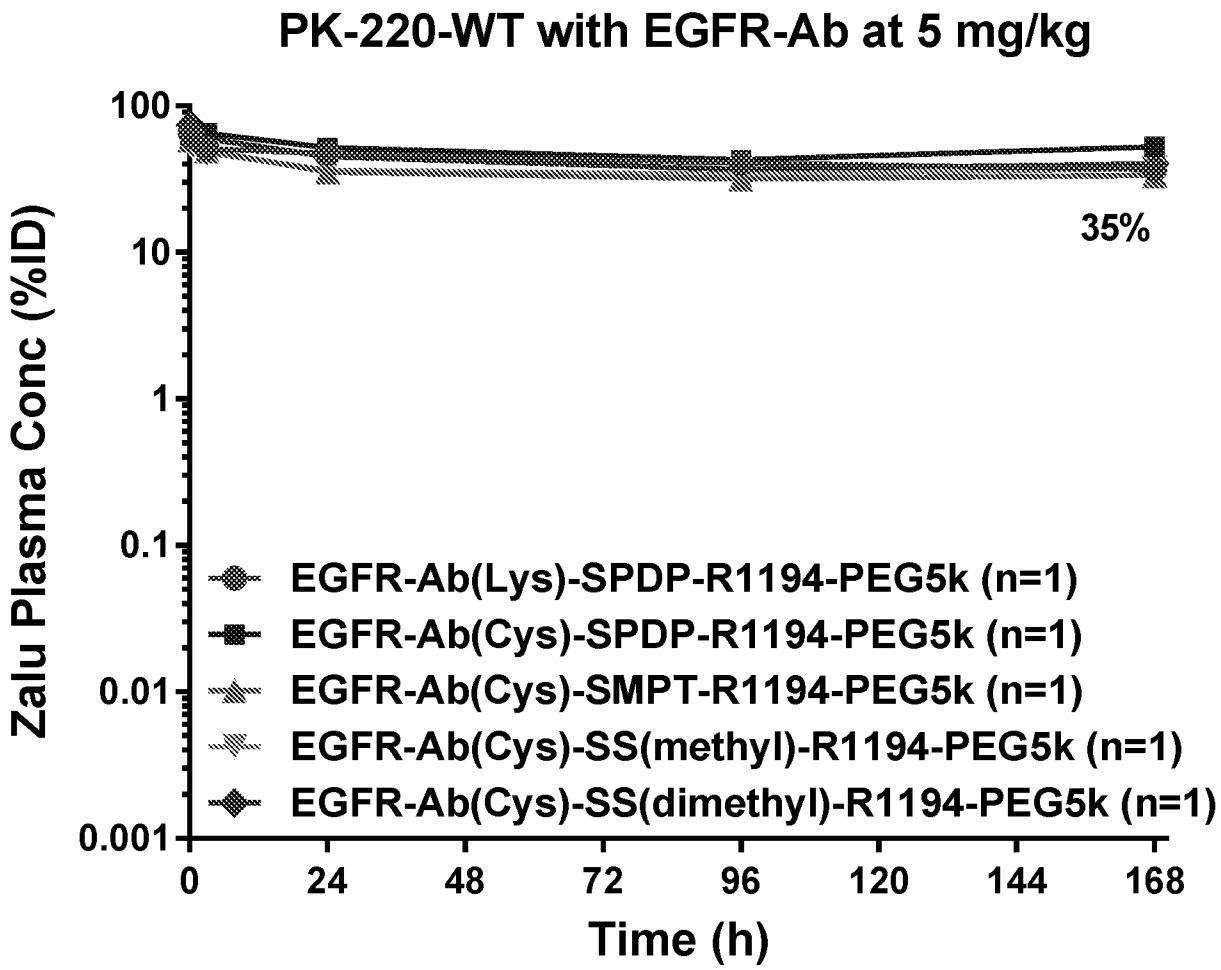

PK-220-WT with EGFR-Ab at 5 mg/kg
Zalu Plasma Conc (%ID)
100
10
1
0.1
0.01
0.001
35%
EGFR-Ab(Lys)-SPDP-R1194-PEG5k (n=1)
EGFR-Ab(Cys)-SPDP-R1194-PEG5k (n=1)
EGFR-Ab(Cys)-SMPT-R1194-PEG5k (n=1)
EGFR-Ab(Cys)-SS(methyl)-R1194-PEG5k (n=1)
EGFR-Ab(Cys)-SS(dimethyl)-R1194-PEG5k (n=1)
0
24
48
72
96
120
144
168
Time (h)

FIG. 74

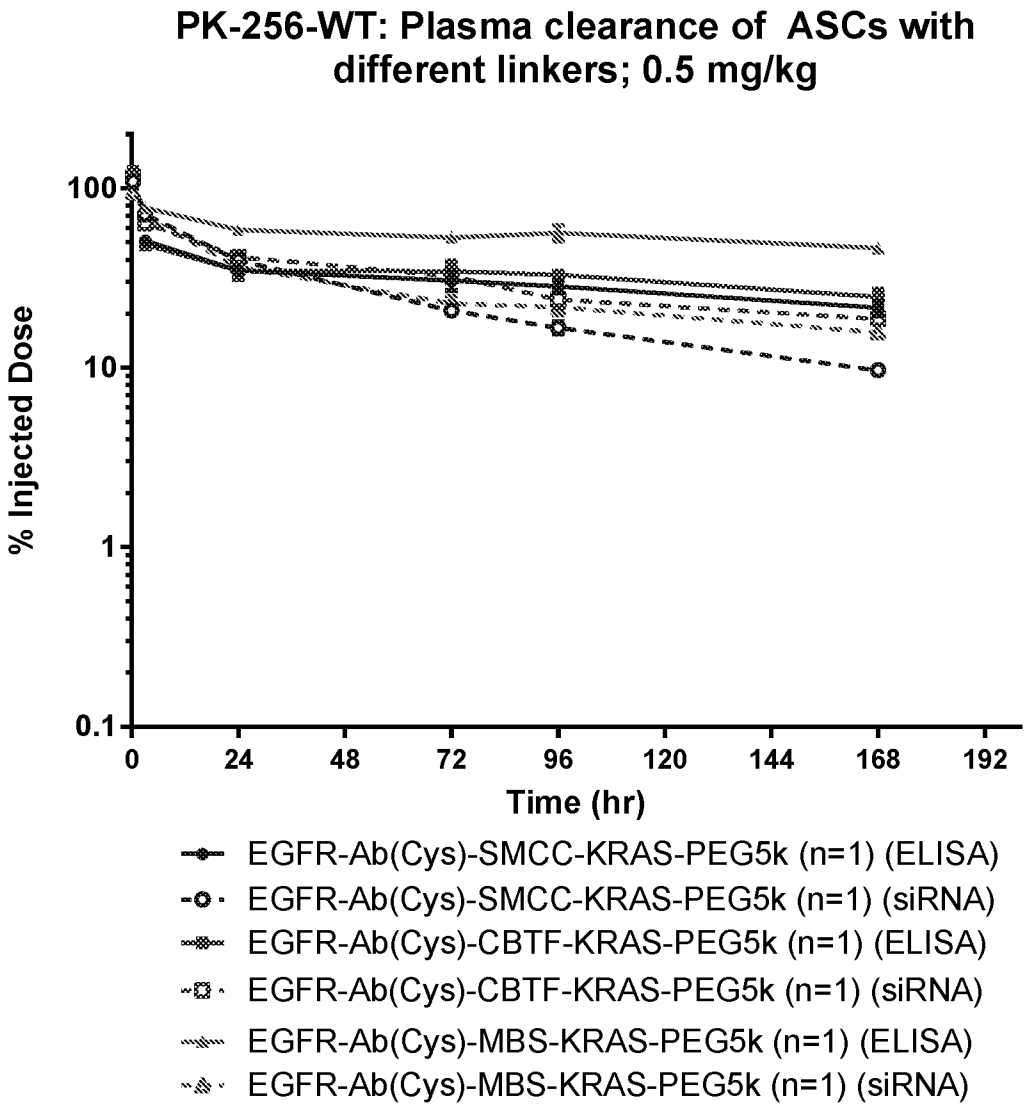

PK-256-WT: Plasma clearance of ASCs with different linkers; 0.5 mg/kg
% Injected Dose
100
10
1
0.1
0
24
48
72
96
120
144
168
192
Time (hr)
EGFR-Ab(Cys)-SMCC-KRAS-PEG5k (n=1) (ELISA)
EGFR-Ab(Cys)-SMCC-KRAS-PEG5k (n=1) (siRNA)
EGFR-Ab(Cys)-CBTF-KRAS-PEG5k (n=1) (ELISA)
EGFR-Ab(Cys)-CBTF-KRAS-PEG5k (n=1) (siRNA)
EGFR-Ab(Cys)-MBS-KRAS-PEG5k (n=1) (ELISA)
EGFR-Ab(Cys)-MBS-KRAS-PEG5k (n=1) (siRNA)

FIG. 75A

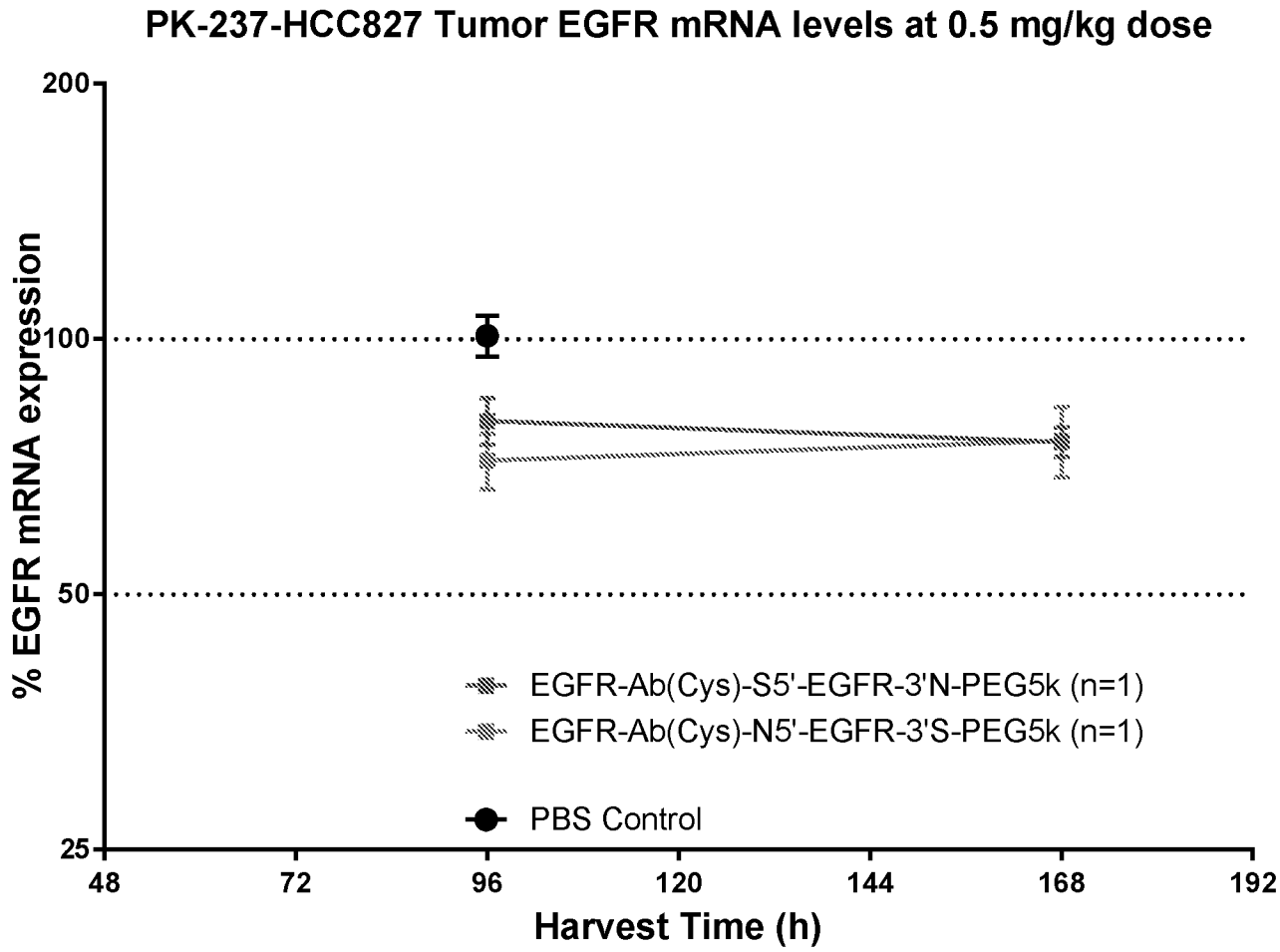

PK-237-HCC827 Tumor EGFR mRNA levels at 0.5 mg/kg dose
% EGFR mRNA expression
200
100
50
25
48
72
96
120
144
168
192
Harvest Time (h)
EGFR-Ab(Cys)-S5'-EGFR-3'N-PEG5k (n=1)
EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)
PBS Control

FIG. 75B

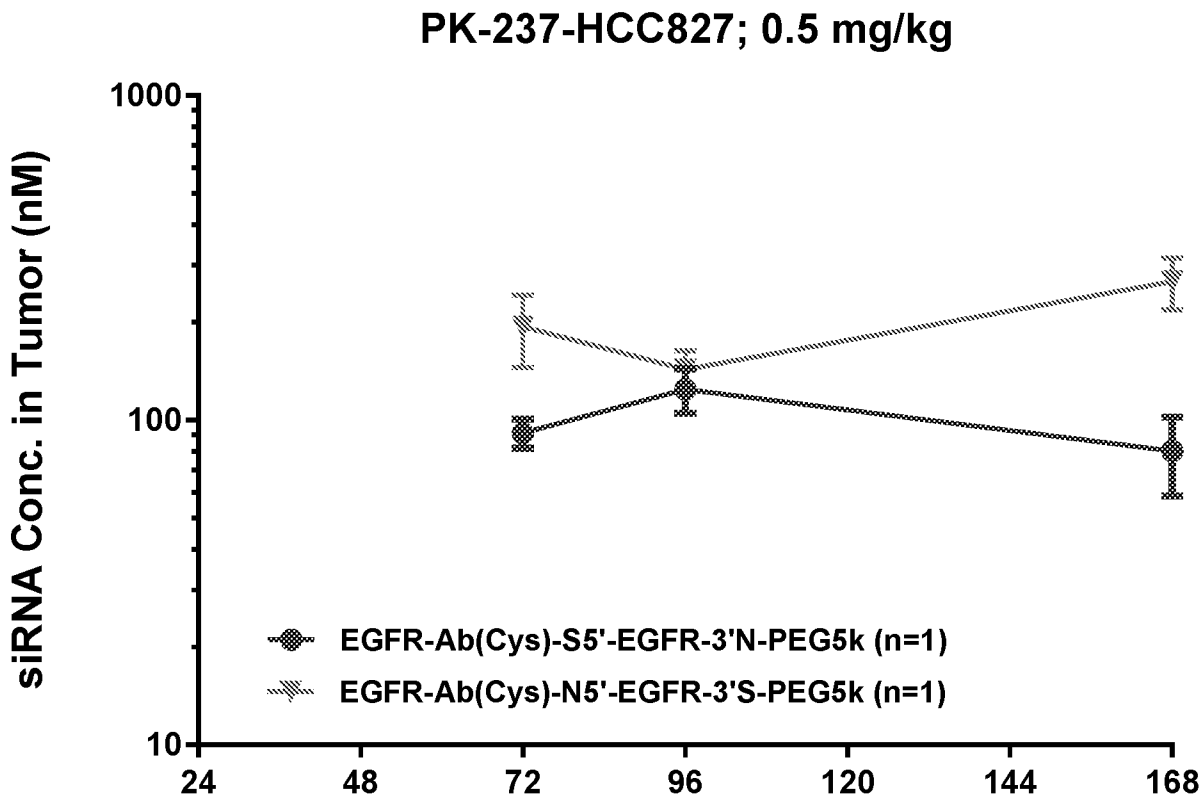

PK-237-HCC827; 0.5 mg/kg
siRNA Conc. in Tumor (nM)
1000
100
10
24
48
72
96
120
144
168
EGFR-Ab(Cys)-S5'-EGFR-3'N-PEG5k (n=1)
EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)

FIG. 75C

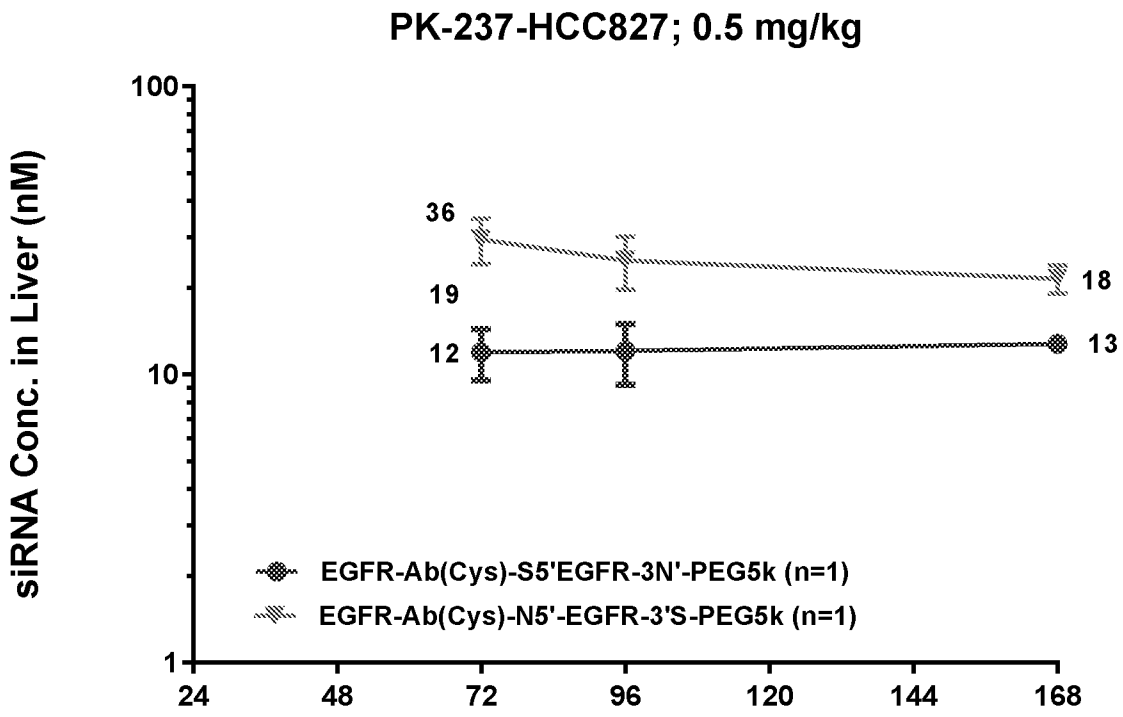

PK-237-HCC827; 0.5 mg/kg
siRNA Conc. in Liver (nM)
100
10
1
24
48
72
96
120
144
168
36
19
12
18
13
EGFR-Ab(Cys)-S5'EGFR-3N'-PEG5k (n=1)
EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)

FIG. 75D

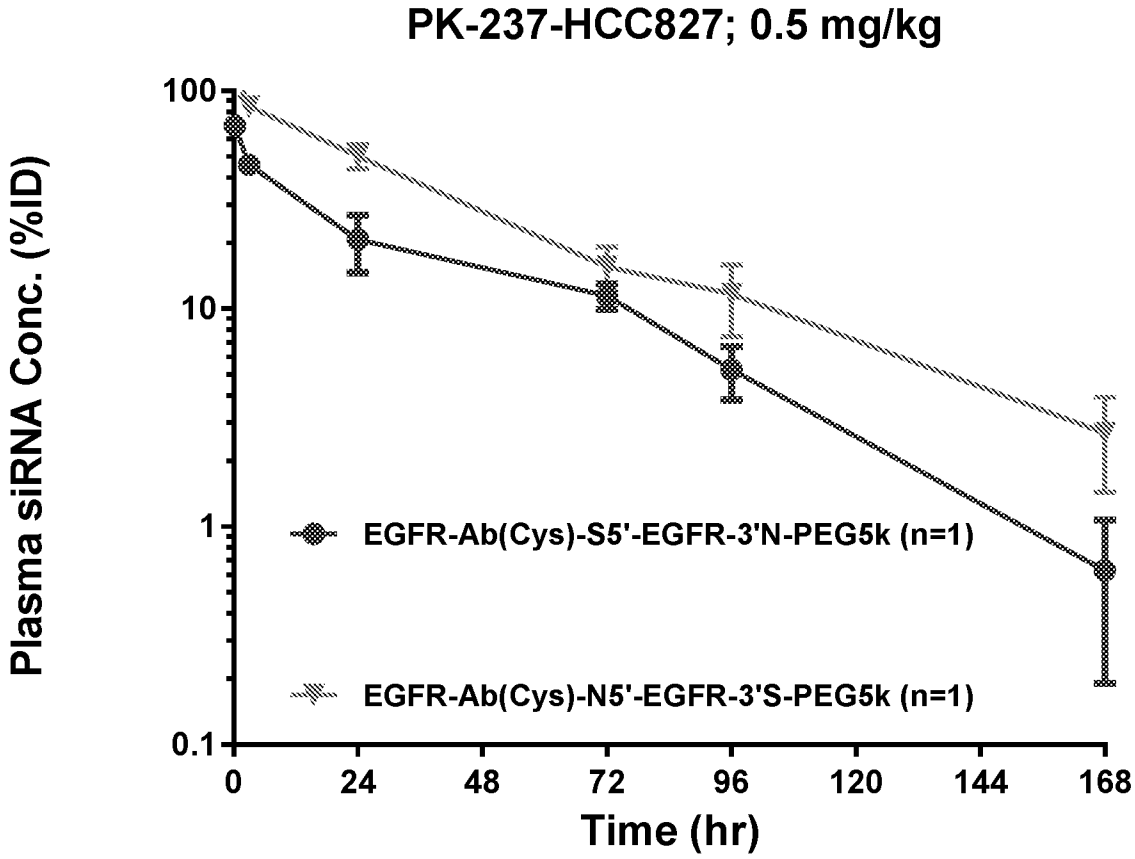

PK-237-HCC827; 0.5 mg/kg
Plasma siRNA Conc. (%ID)
100
10
1
0.1
0
24
48
72
96
120
144
168
Time (hr)
EGFR-Ab(Cys)-S5'-EGFR-3'N-PEG5k (n=1)
EGFR-Ab(Cys)-N5'-EGFR-3'S-PEG5k (n=1)

FIG. 76A

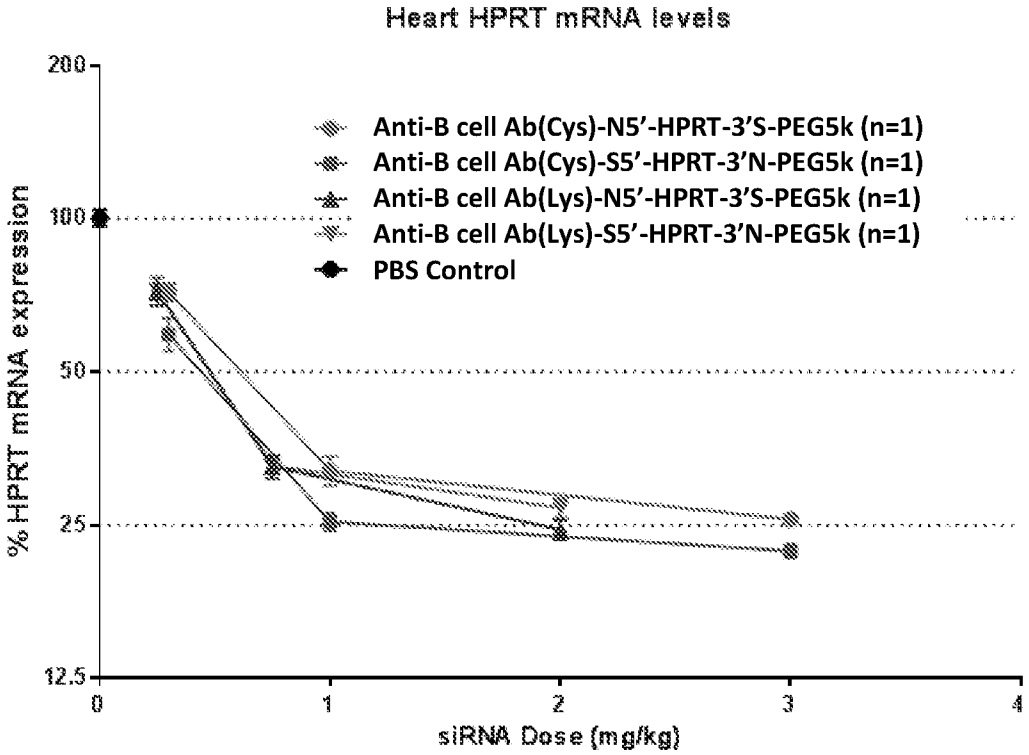


FIG. 76B

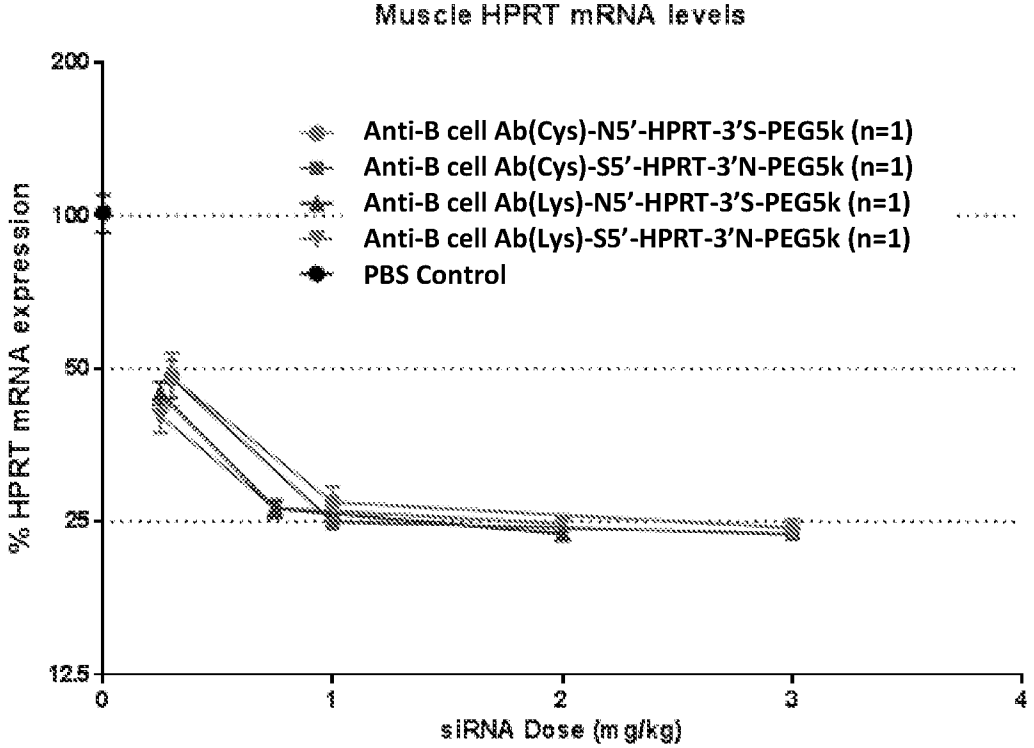


FIG. 76C

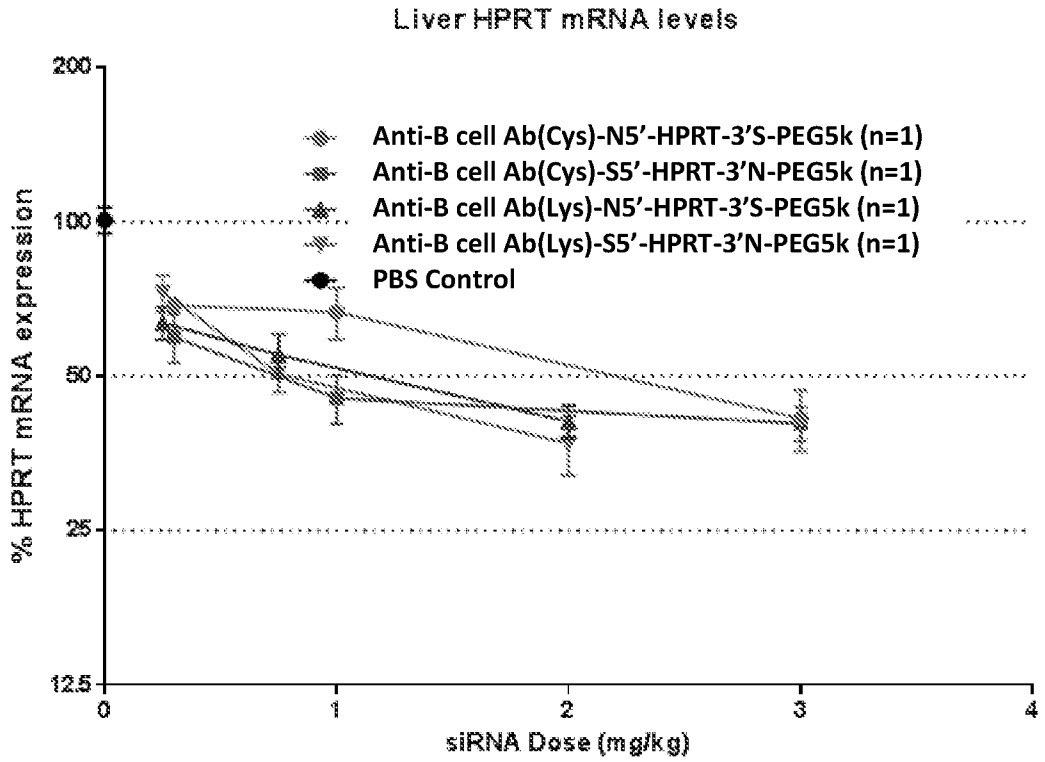


FIG. 76D

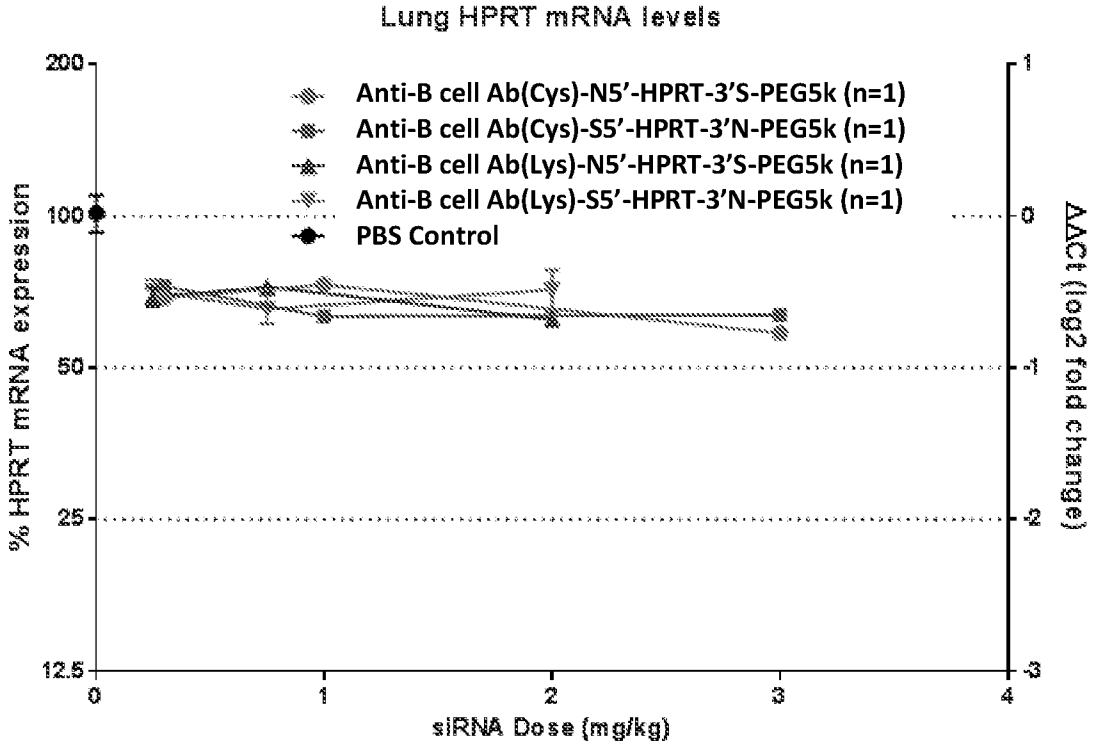


FIG. 77A

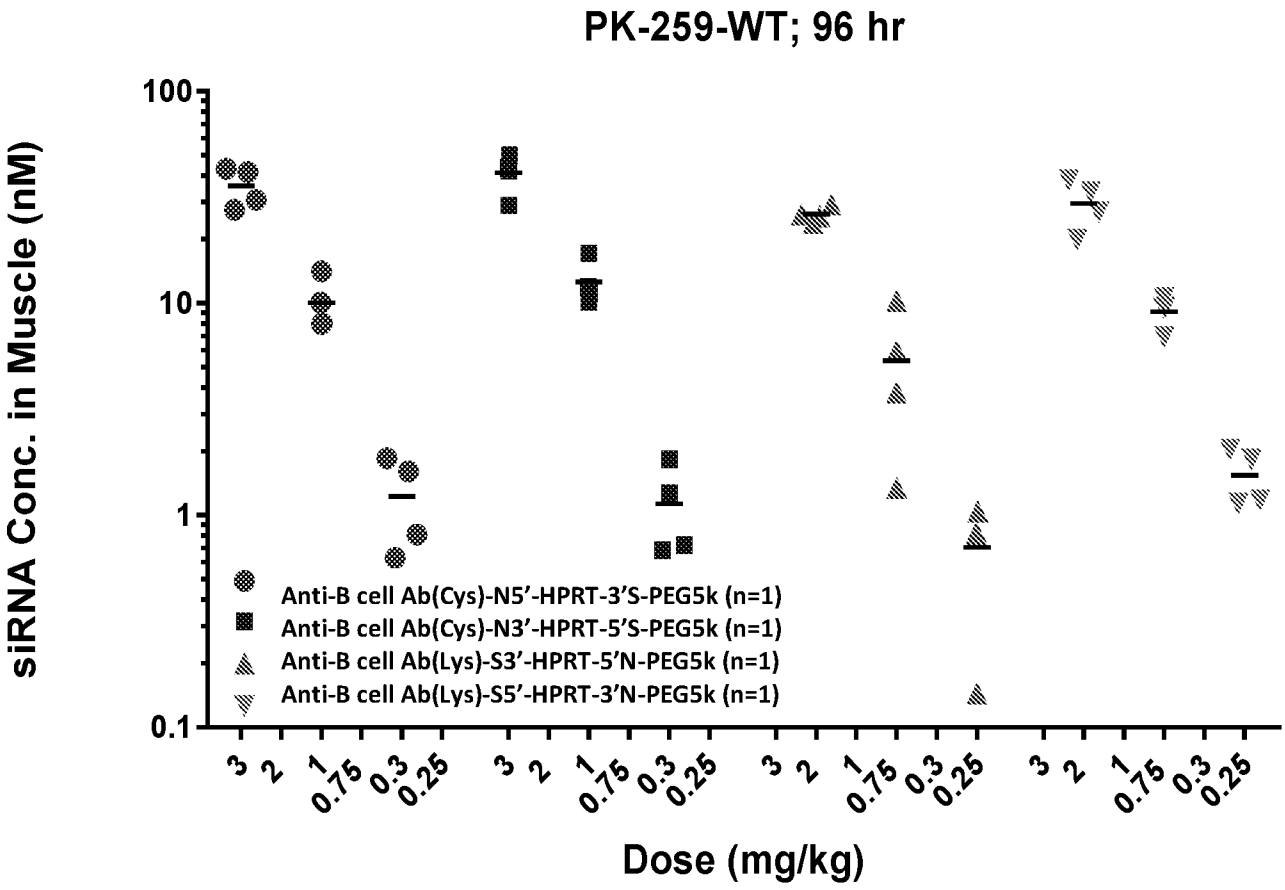

PK-259-WT; 96 hr
siRNA Conc. in Muscle (nM)
100
10
1
0.1
Anti-B cell Ab(Cys)-N5'-HPRT-3'S-PEG5k (n=1)
Anti-B cell Ab(Cys)-N3'-HPRT-5'S-PEG5k (n=1)
Anti-B cell Ab(Lys)-S3'-HPRT-5'N-PEG5k (n=1)
Anti-B cell Ab(Lys)-S5'-HPRT-3'N-PEG5k (n=1)
3 2 1 0.75 0.3 0.25 3 2 1 0.75 0.3 0.25 3 2 1 0.75 0.3 0.25 3 2 1 0.75 0.3 0.25
Dose (mg/kg)

FIG. 77B

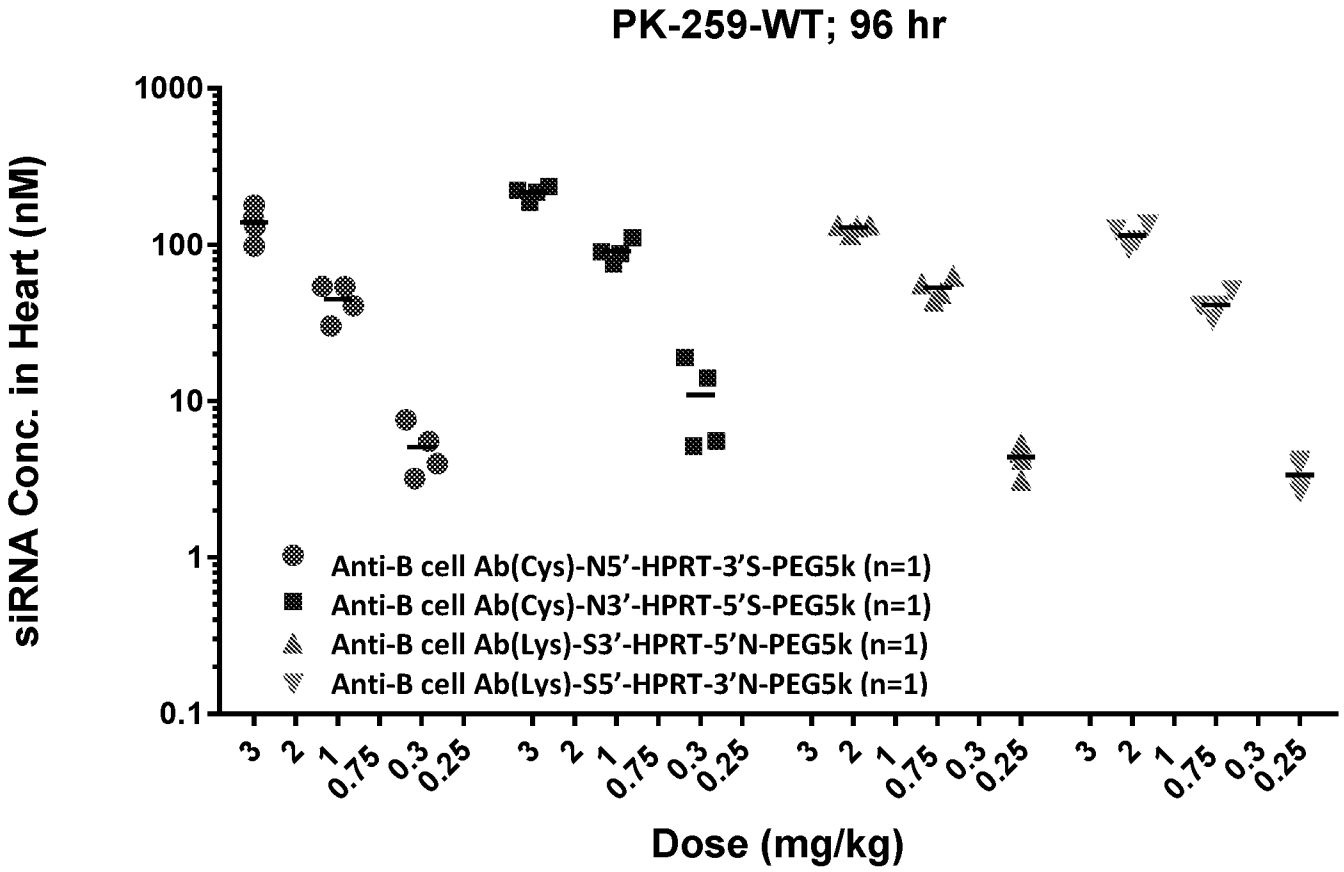

PK-259-WT; 96 hr
siRNA Conc. in Heart (nM)
1000
100
10
1
0.1
Anti-B cell Ab(Cys)-N5'-HPRT-3'S-PEG5k (n=1)
Anti-B cell Ab(Cys)-N3'-HPRT-5'S-PEG5k (n=1)
Anti-B cell Ab(Lys)-S3'-HPRT-5'N-PEG5k (n=1)
Anti-B cell Ab(Lys)-S5'-HPRT-3'N-PEG5k (n=1)
3 2 1 0.75 0.3 0.25 3 2 1 0.75 0.3 0.25 3 2 1 0.75 0.3 0.25 3 2 1 0.75 0.3 0.25
Dose (mg/kg)

FIG. 77C

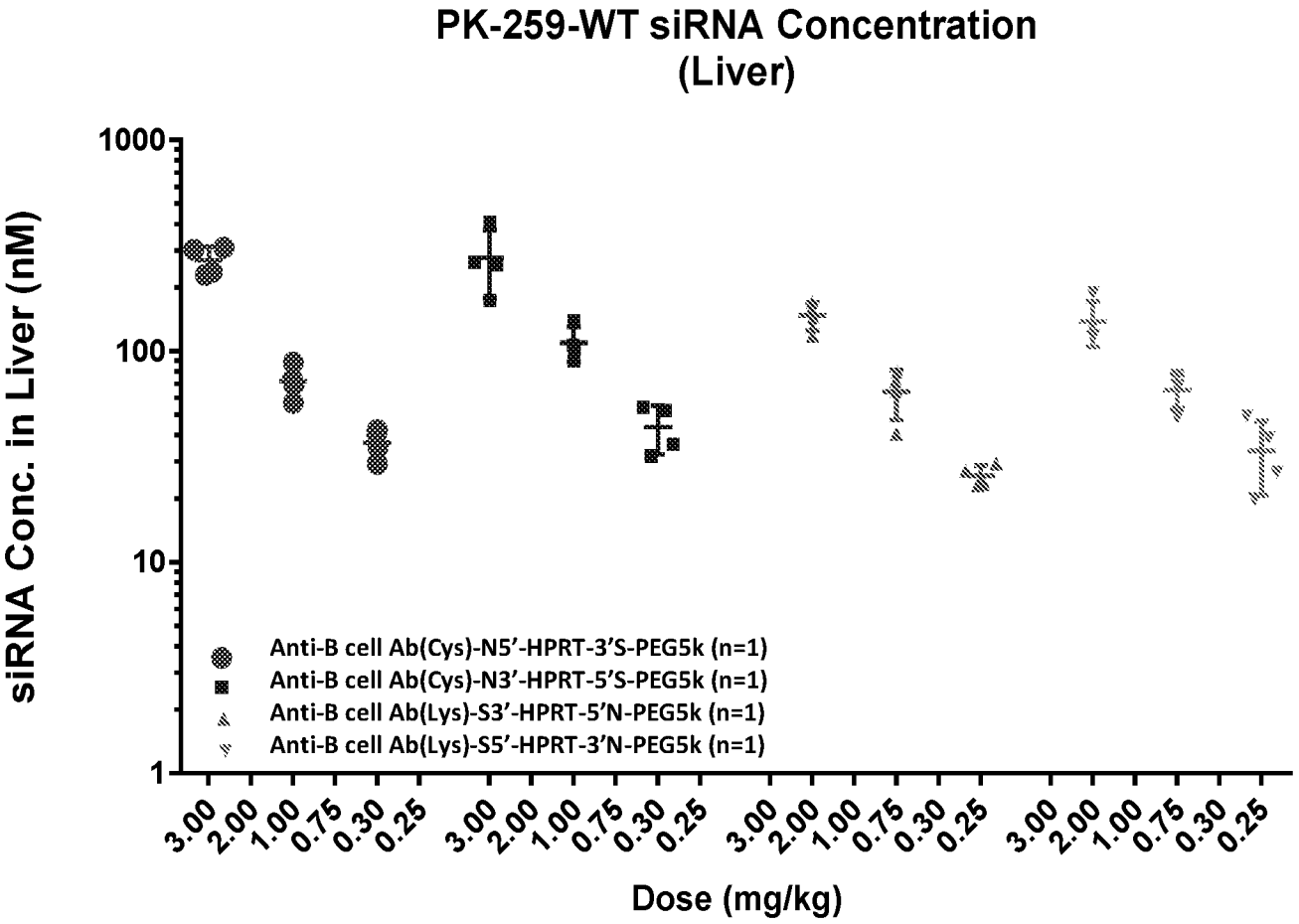

PK-259-WT siRNA Concentration
(Liver)
siRNA Conc. in Liver (nM)
1000
100
10
1
Anti-B cell Ab(Cys)-N5'-HPRT-3'S-PEG5k (n=1)
Anti-B cell Ab(Cys)-N3'-HPRT-5'S-PEG5k (n=1)
Anti-B cell Ab(Lys)-S3'-HPRT-5'N-PEG5k (n=1)
Anti-B cell Ab(Lys)-S5'-HPRT-3'N-PEG5k (n=1)
3.00 2.00 1.00 0.75 0.30 0.25 3.00 2.00 1.00 0.75 0.30 0.25 3.00 2.00 1.00 0.75 0.30 0.25 3.00 2.00 1.00 0.75 0.30 0.25
Dose (mg/kg)

FIG. 77D

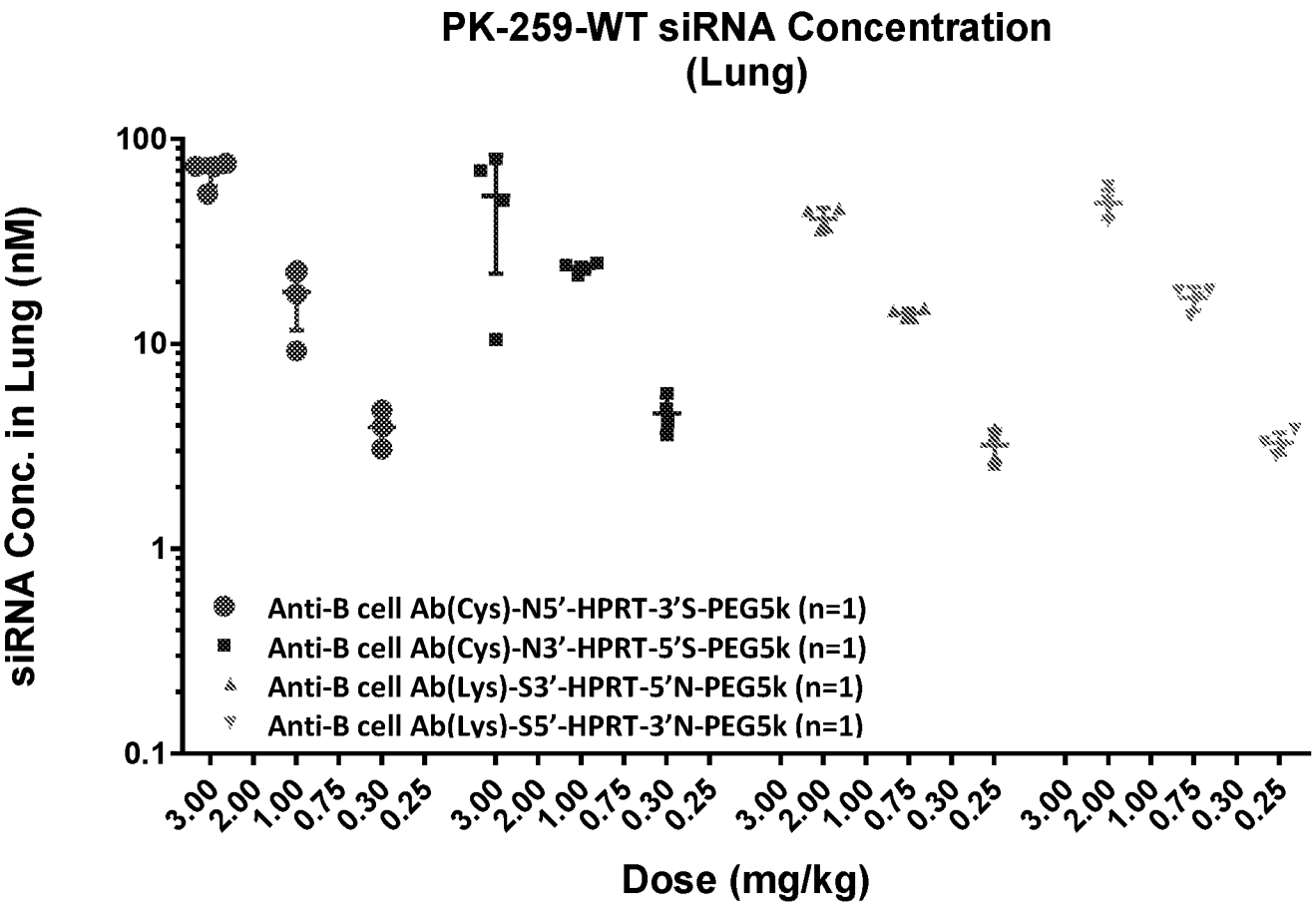

PK-259-WT siRNA Concentration
(Lung)
siRNA Conc. in Lung (nM)
100
10
1
0.1
Anti-B cell Ab(Cys)-N5'-HPRT-3'S-PEG5k (n=1)
Anti-B cell Ab(Cys)-N3'-HPRT-5'S-PEG5k (n=1)
Anti-B cell Ab(Lys)-S3'-HPRT-5'N-PEG5k (n=1)
Anti-B cell Ab(Lys)-S5'-HPRT-3'N-PEG5k (n=1)
3.00 2.00 1.00 0.75 0.30 0.25 3.00 2.00 1.00 0.75 0.30 0.25 3.00 2.00 1.00 0.75 0.30 0.25 3.00 2.00 1.00 0.75 0.30 0.25
Dose (mg/kg)

FIG. 78A

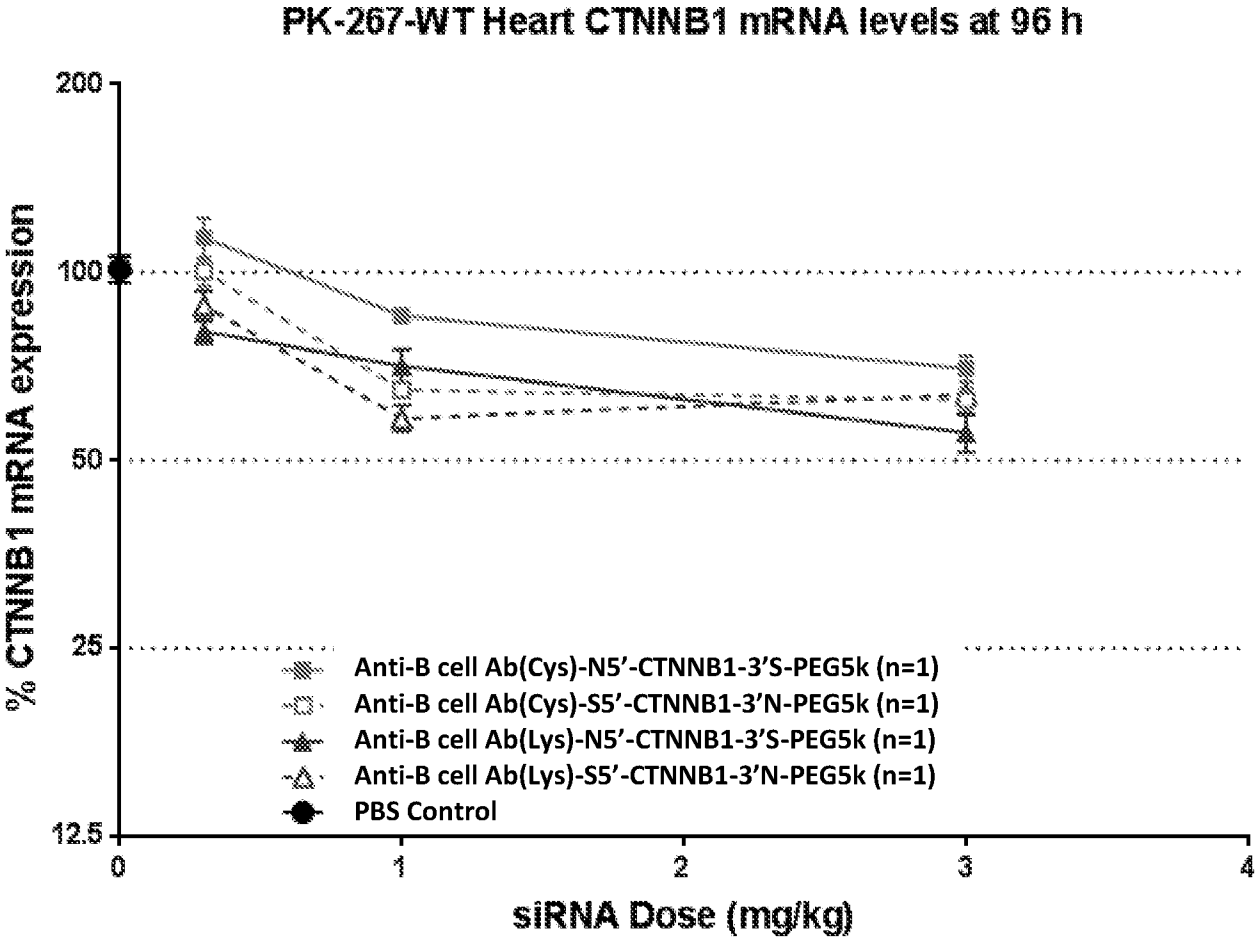

PK-267-WT Heart CTNNB1 mRNA levels at 96 h
% CTNNB1 mRNA expression
200
100
50
25
12.5
0
1
2
3
4
siRNA Dose (mg/kg)
Anti-B cell Ab(Cys)-N5'-CTNNB1-3'S-PEG5k (n=1)
Anti-B cell Ab(Cys)-S5'-CTNNB1-3'N-PEG5k (n=1)
Anti-B cell Ab(Lys)-N5'-CTNNB1-3'S-PEG5k (n=1)
Anti-B cell Ab(Lys)-S5'-CTNNB1-3'N-PEG5k (n=1)
PBS Control

FIG. 78B

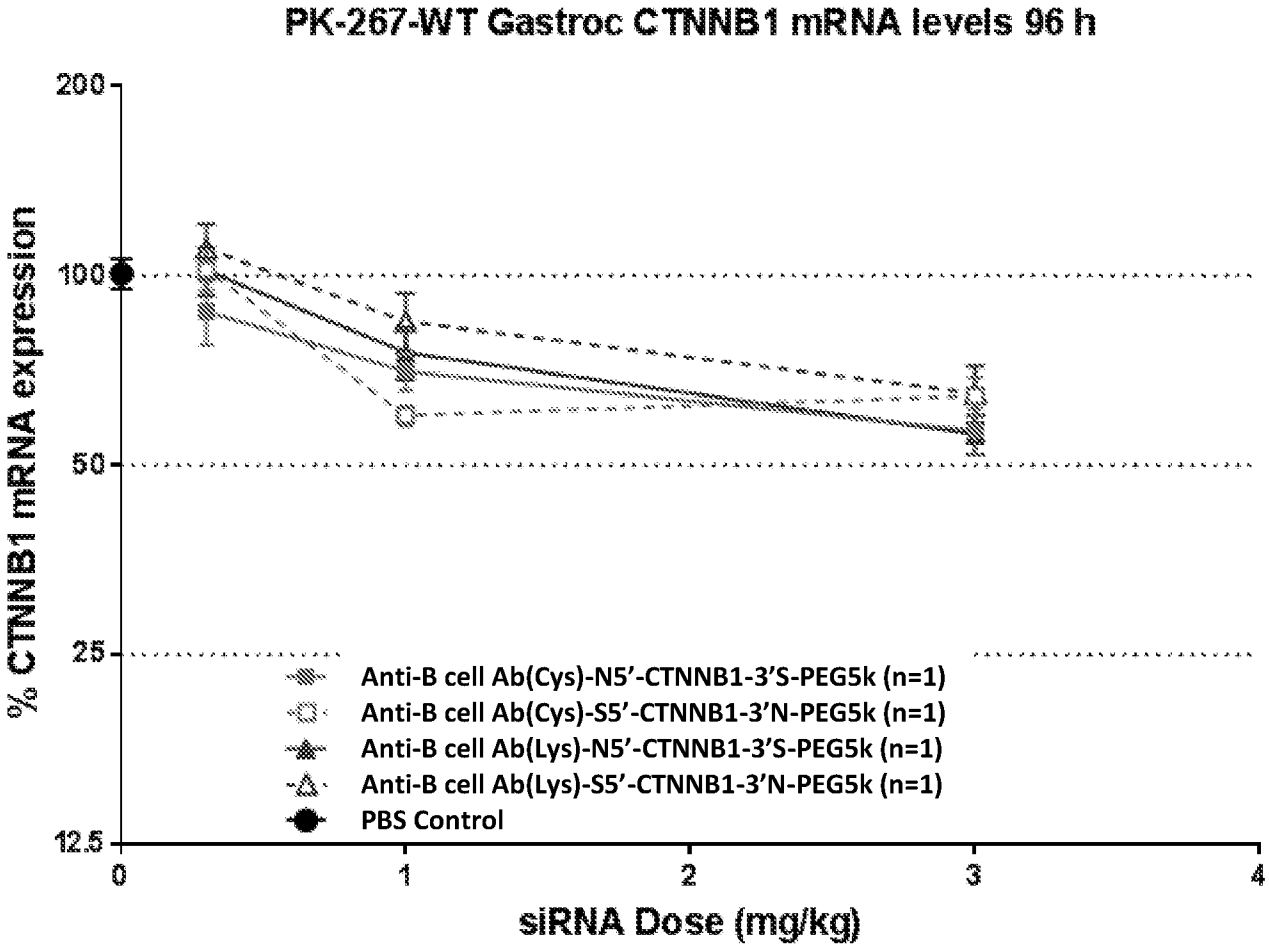

PK-267-WT Gastroc CTNNB1 mRNA levels 96 h
% CTNNB1 mRNA expression
200
100
50
25
12.5
0
1
2
3
4
siRNA Dose (mg/kg)
Anti-B cell Ab(Cys)-N5'-CTNNB1-3'S-PEG5k (n=1)
Anti-B cell Ab(Cys)-S5'-CTNNB1-3'N-PEG5k (n=1)
Anti-B cell Ab(Lys)-N5'-CTNNB1-3'S-PEG5k (n=1)
Anti-B cell Ab(Lys)-S5'-CTNNB1-3'N-PEG5k (n=1)
PBS Control

FIG. 78C

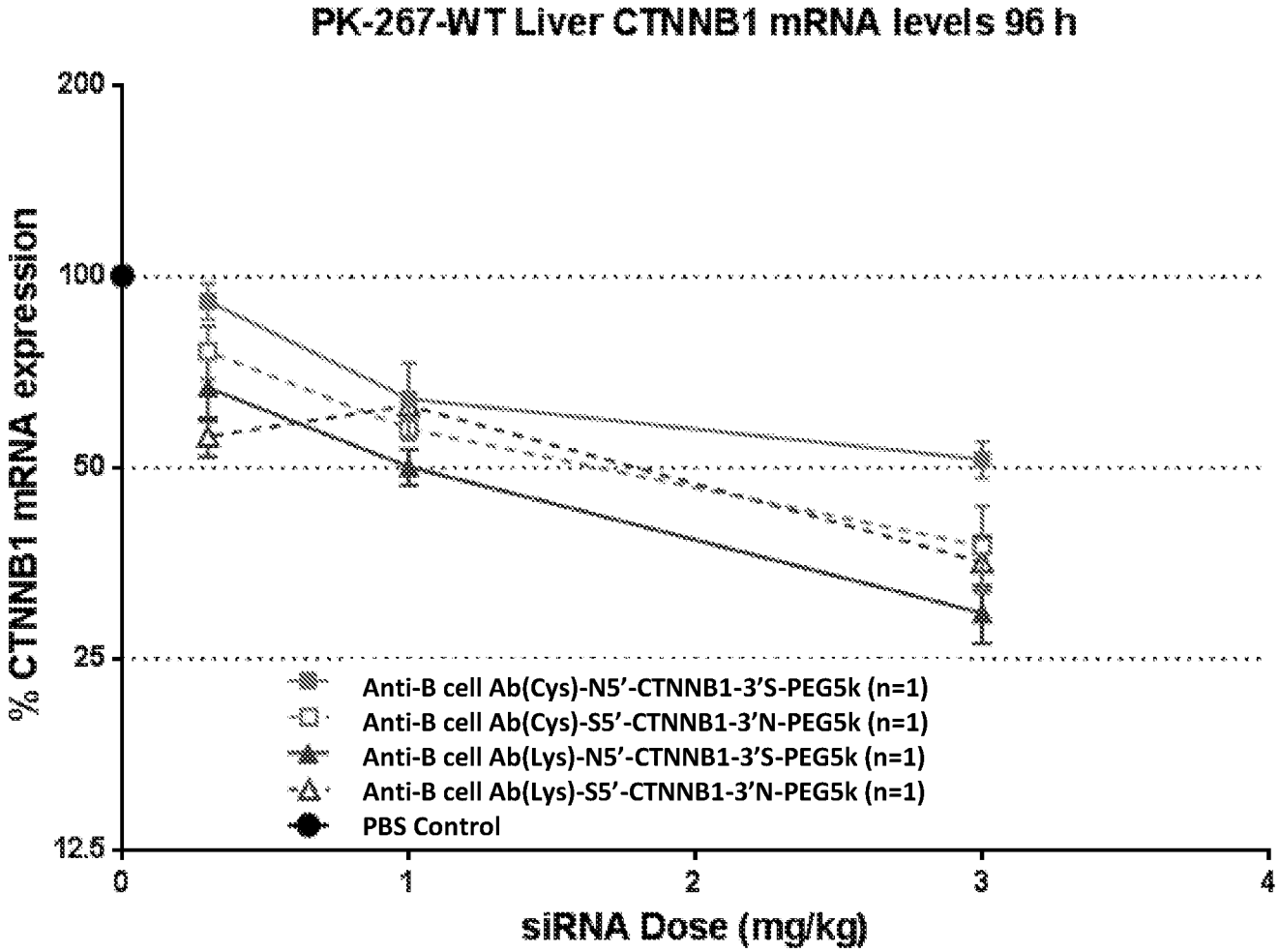

PK-267-WT Liver CTNNB1 mRNA levels 96 h
% CTNNB1 mRNA expression
200
100
50
25
12.5
0
1
2
3
4
siRNA Dose (mg/kg)
Anti-B cell Ab(Cys)-N5'-CTNNB1-3'S-PEG5k (n=1)
Anti-B cell Ab(Cys)-S5'-CTNNB1-3'N-PEG5k (n=1)
Anti-B cell Ab(Lys)-N5'-CTNNB1-3'S-PEG5k (n=1)
Anti-B cell Ab(Lys)-S5'-CTNNB1-3'N-PEG5k (n=1)
PBS Control

FIG. 78D

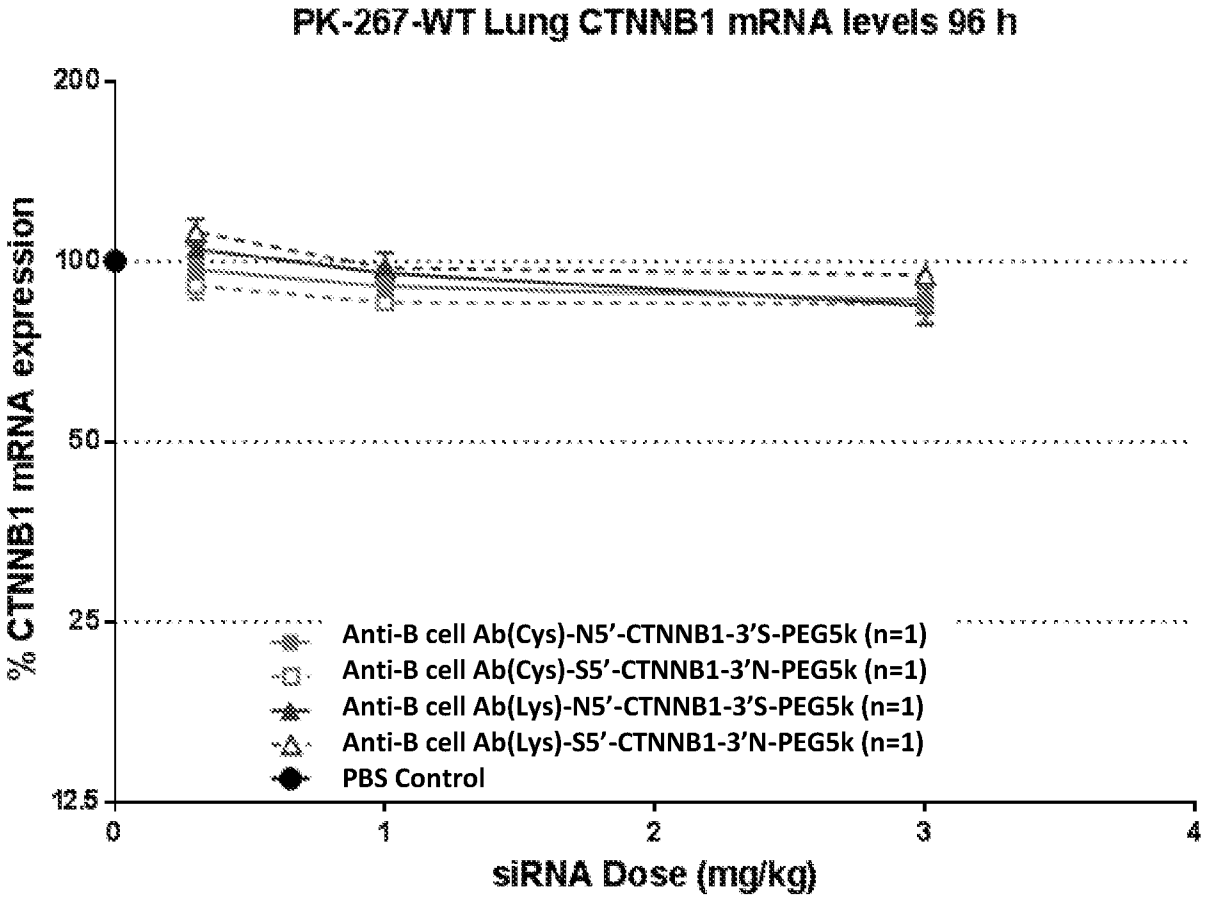

PK-267-WT Lung CTNNB1 mRNA levels 96 h
% CTNNB1 mRNA expression
200
100
50
25
12.5
0
1
2
3
4
siRNA Dose (mg/kg)
Anti-B cell Ab(Cys)-N5'-CTNNB1-3'S-PEG5k (n=1)
Anti-B cell Ab(Cys)-S5'-CTNNB1-3'N-PEG5k (n=1)
Anti-B cell Ab(Lys)-N5'-CTNNB1-3'S-PEG5k (n=1)
Anti-B cell Ab(Lys)-S5'-CTNNB1-3'N-PEG5k (n=1)
PBS Control

FIG. 79

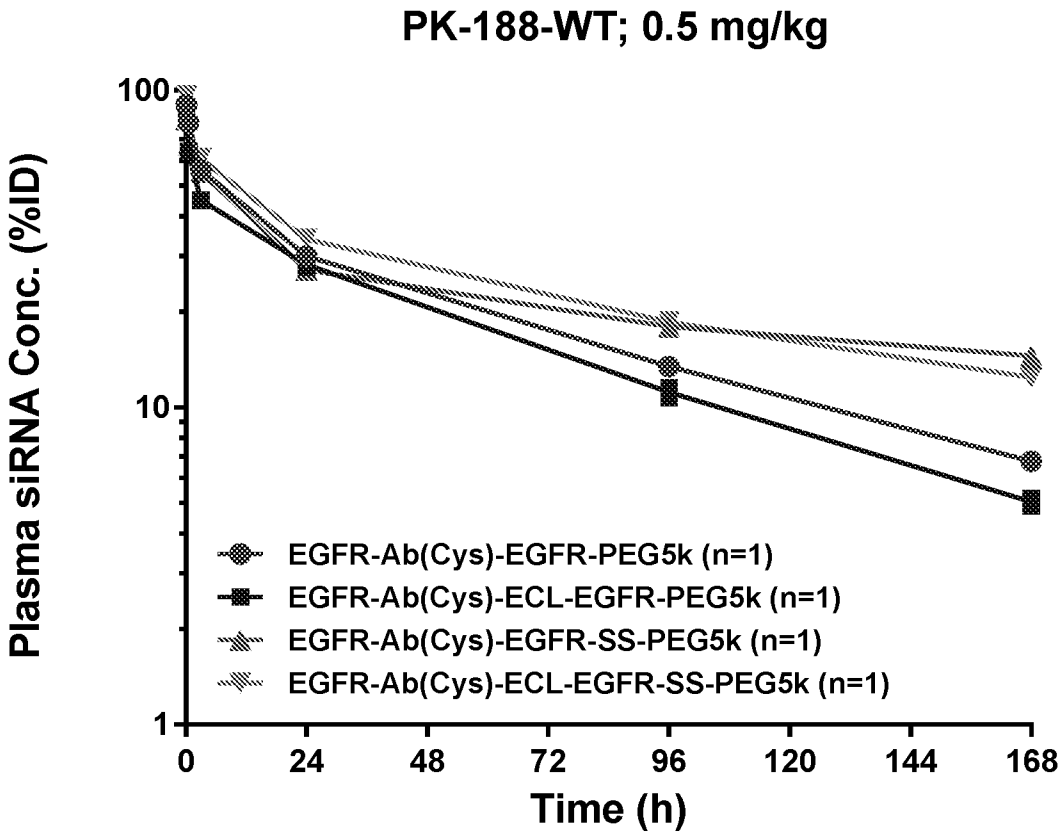

PK-188-WT; 0.5 mg/kg
Plasma siRNA Conc. (%ID)
100
10
1
0
24
48
72
96
120
144
168
Time (h)
EGFR-Ab(Cys)-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-ECL-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-EGFR-SS-PEG5k (n=1)
EGFR-Ab(Cys)-ECL-EGFR-SS-PEG5k (n=1)

FIG. 80A

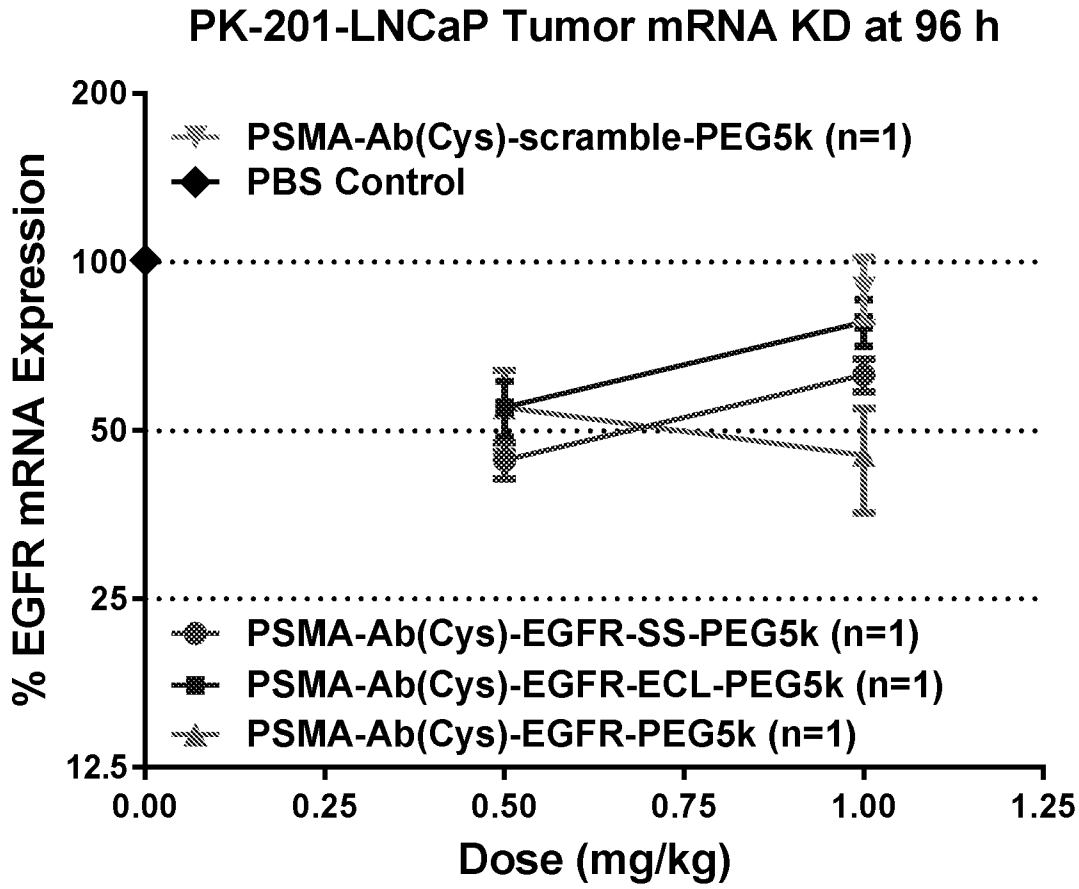

PK-201-LNCaP Tumor mRNA KD at 96 h
% EGFR mRNA Expression
200
100
50
25
12.5
PSMA-Ab(Cys)-scramble-PEG5k (n=1)
PBS Control
PSMA-Ab(Cys)-EGFR-SS-PEG5k (n=1)
PSMA-Ab(Cys)-EGFR-ECL-PEG5k (n=1)
PSMA-Ab(Cys)-EGFR-PEG5k (n=1)
0.00
0.25
0.50
0.75
1.00
1.25
Dose (mg/kg)

FIG. 80B

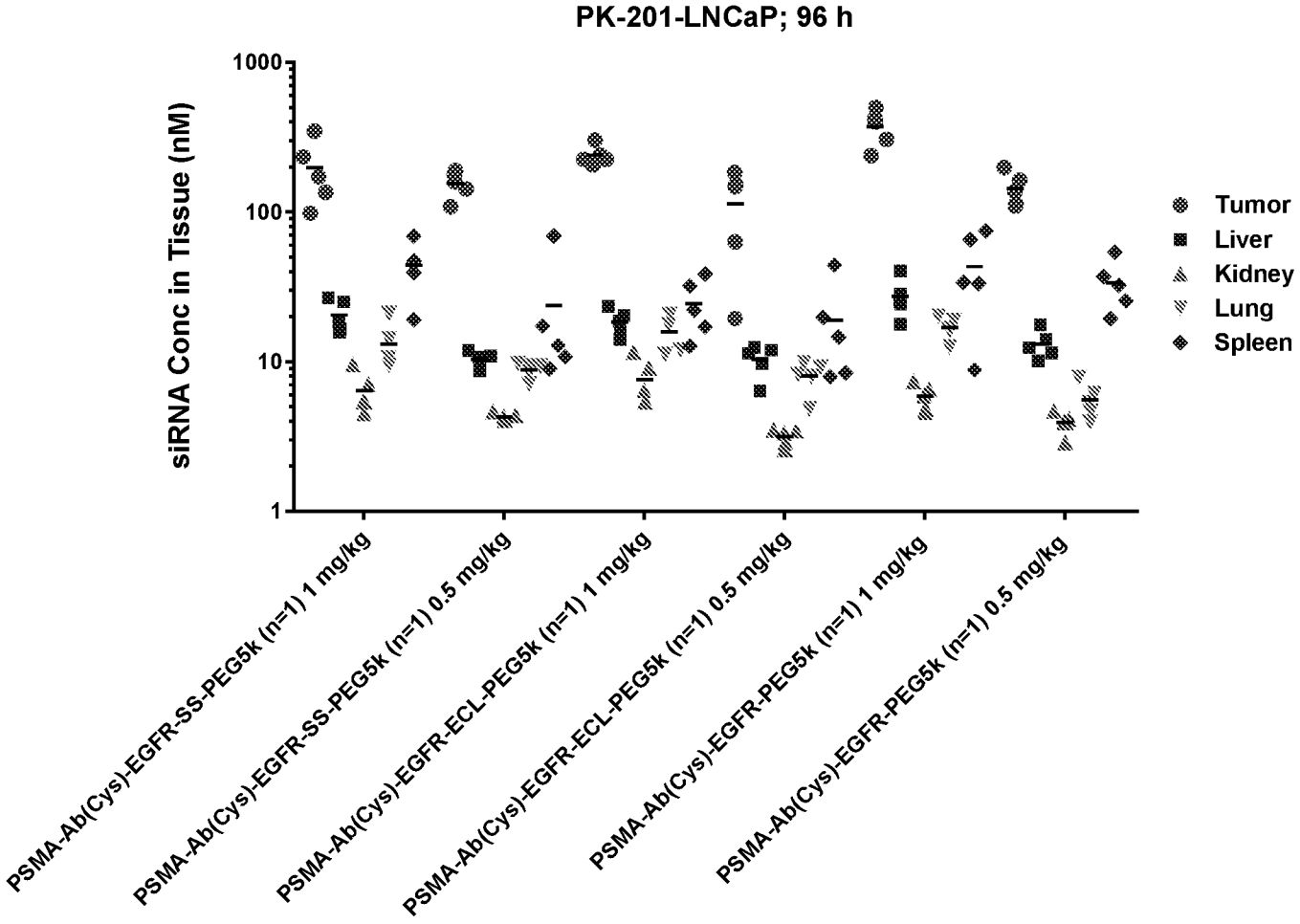

PK-201-LNCaP; 96 h
siRNA Conc in Tissue (nM)
1000
100
10
1
Tumor
Liver
Kidney
Lung
Spleen
PSMA-Ab(Cys)-EGFR-SS-PEG5k (n=1) 1 mg/kg
PSMA-Ab(Cys)-EGFR-SS-PEG5k (n=1) 0.5 mg/kg
PSMA-Ab(Cys)-EGFR-ECL-PEG5k (n=1) 1 mg/kg
PSMA-Ab(Cys)-EGFR-ECL-PEG5k (n=1) 0.5 mg/kg
PSMA-Ab(Cys)-EGFR-PEG5k (n=1) 1 mg/kg
PSMA-Ab(Cys)-EGFR-PEG5k (n=1) 0.5 mg/kg

FIG. 81A

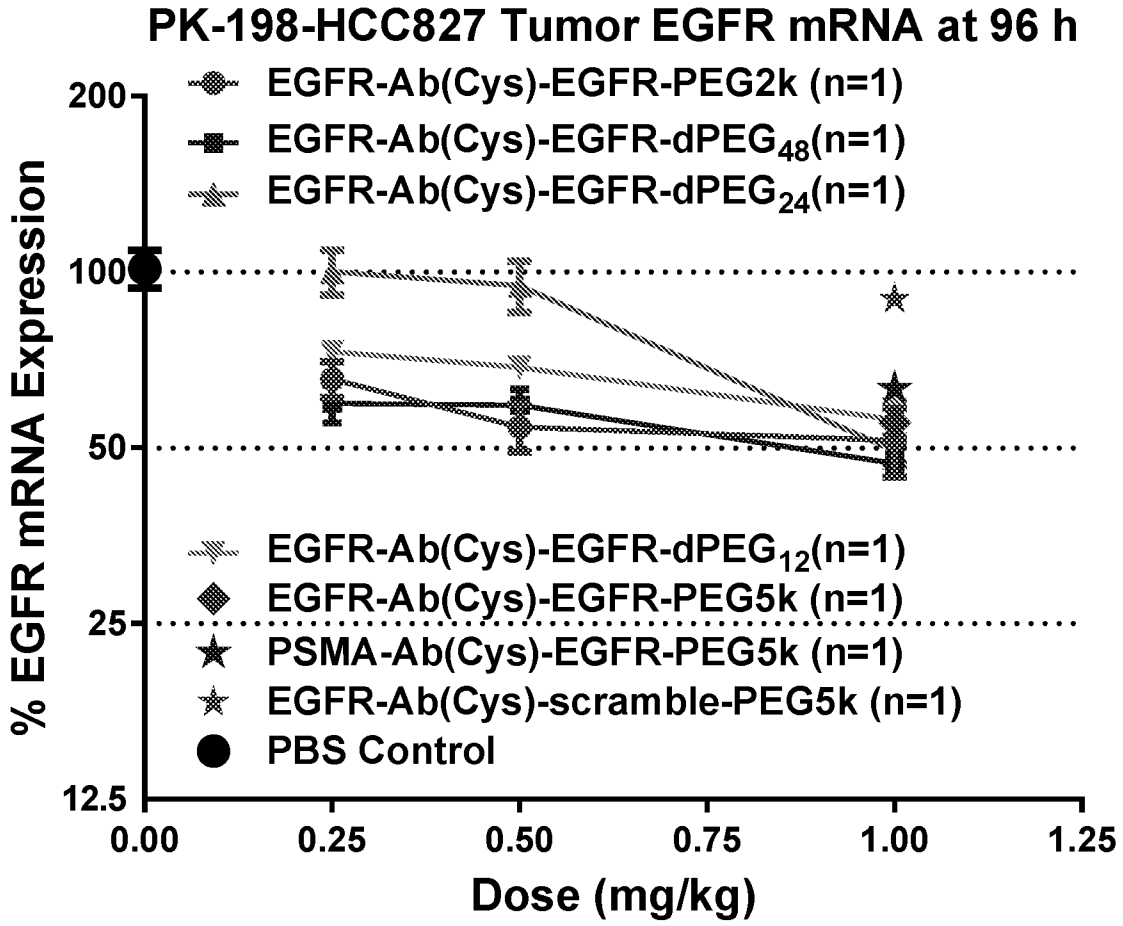

PK-198-HCC827 Tumor EGFR mRNA at 96 h
EGFR-Ab(Cys)-EGFR-PEG2k (n=1)
EGFR-Ab(Cys)-EGFR-dPEG48(n=1)
EGFR-Ab(Cys)-EGFR-dPEG24(n=1)
EGFR-Ab(Cys)-EGFR-dPEG12(n=1)
EGFR-Ab(Cys)-EGFR-PEG5k (n=1)
PSMA-Ab(Cys)-EGFR-PEG5k (n=1)
EGFR-Ab(Cys)-scramble-PEG5k (n=1)
PBS Control
% EGFR mRNA Expression
200
100
50
25
12.5
0.00
0.25
0.50
0.75
1.00
1.25
Dose (mg/kg)

FIG. 81B

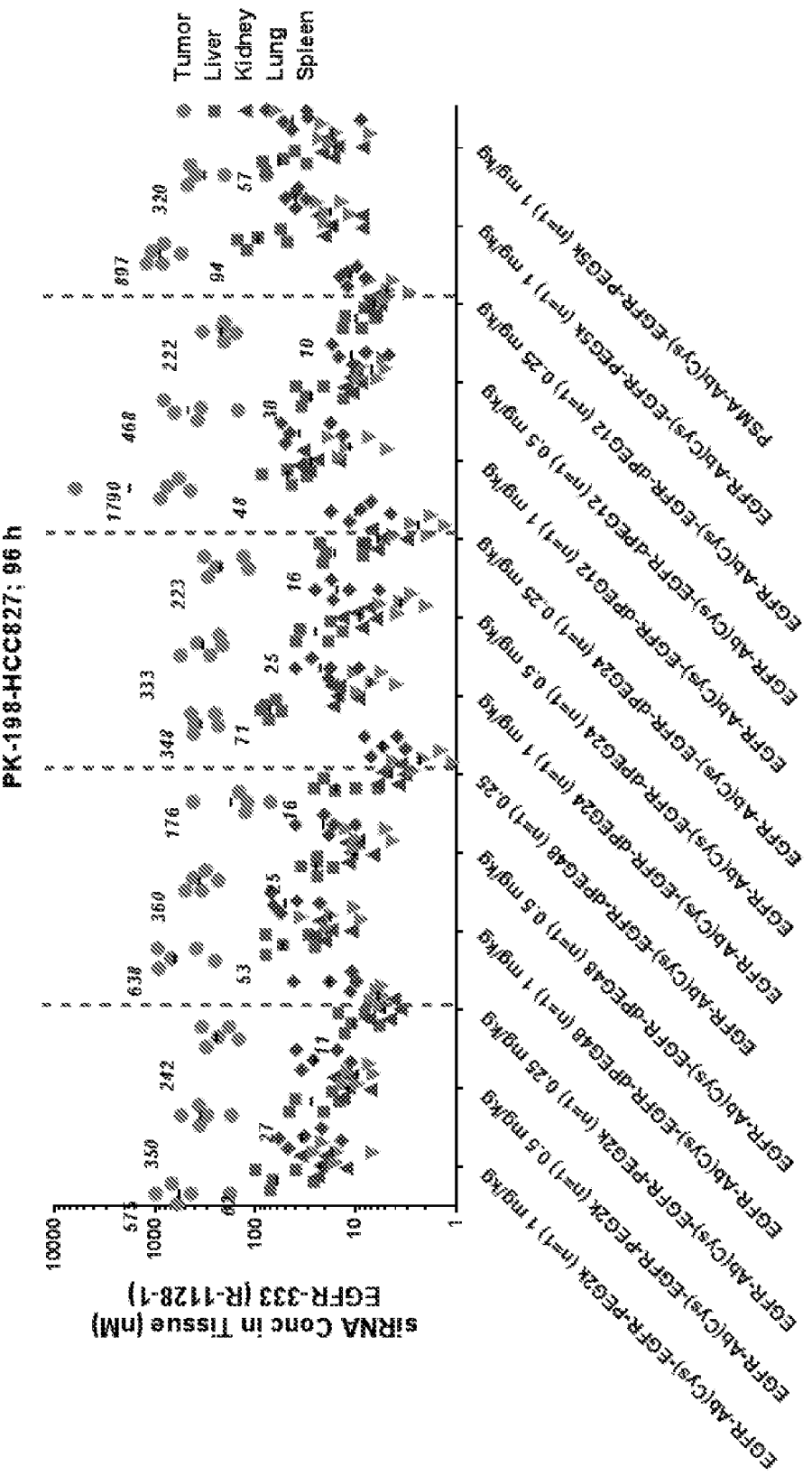

PK-198-HCC827; 96 h
siRNA Conc in Tissue (nM)
EGFR-333 (R-1128-1)
Tumor
Liver
Kidney
Lung
Spleen
EGFR-Ab(Cys)-EGFR-PEG2k (m=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-PEG2k (m=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-PEG2k (m=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-dPEG48 (m=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-dPEG48 (m=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-dPEG48 (m=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-dPEG24 (m=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-dPEG24 (m=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-dPEG24 (m=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-dPEG12 (m=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-dPEG12 (m=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-dPEG12 (m=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (m=1) 1 mg/kg
PSMA-Ab(Cys)-EGFR-PEG5k (m=1) 1 mg/kg

FIG. 82

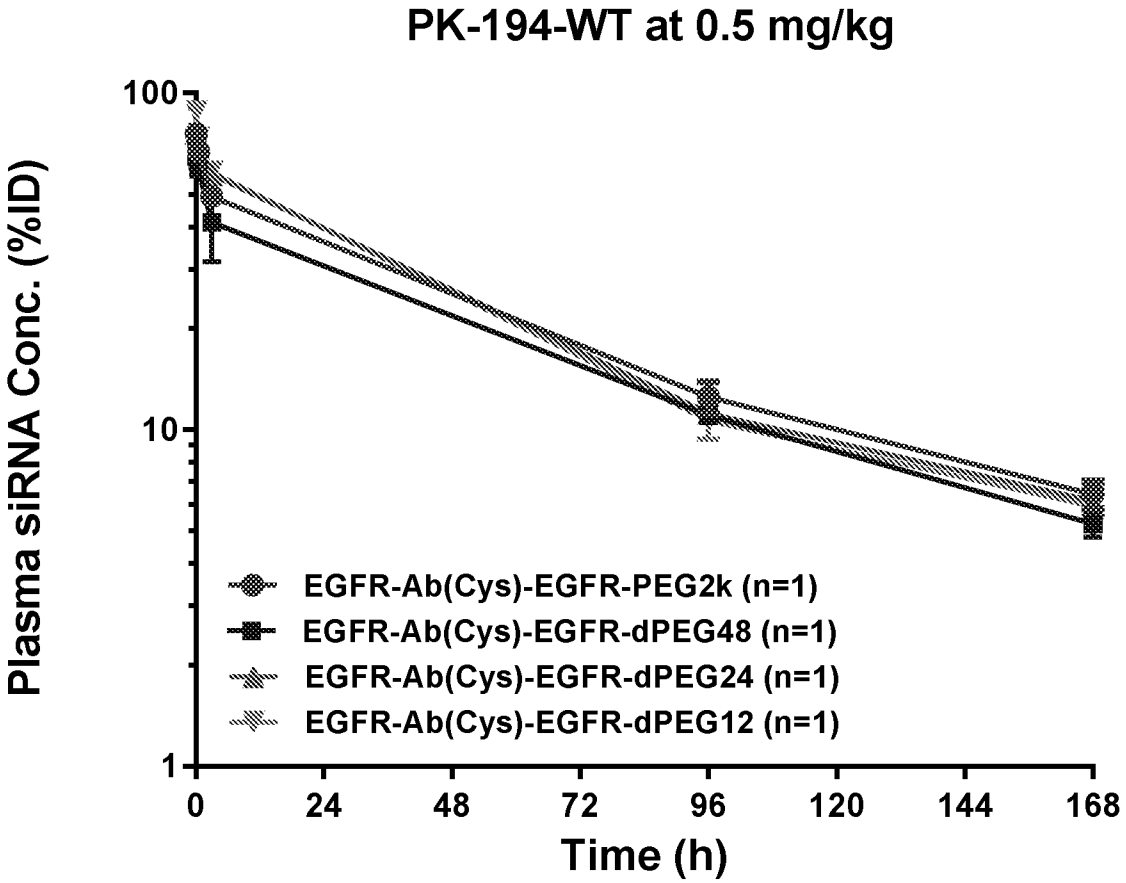

PK-194-WT at 0.5 mg/kg
Plasma siRNA Conc. (%ID)
100
10
1
0
24
48
72
96
120
144
168
Time (h)
EGFR-Ab(Cys)-EGFR-PEG2k (n=1)
EGFR-Ab(Cys)-EGFR-dPEG48 (n=1)
EGFR-Ab(Cys)-EGFR-dPEG24 (n=1)
EGFR-Ab(Cys)-EGFR-dPEG12 (n=1)

FIG. 83

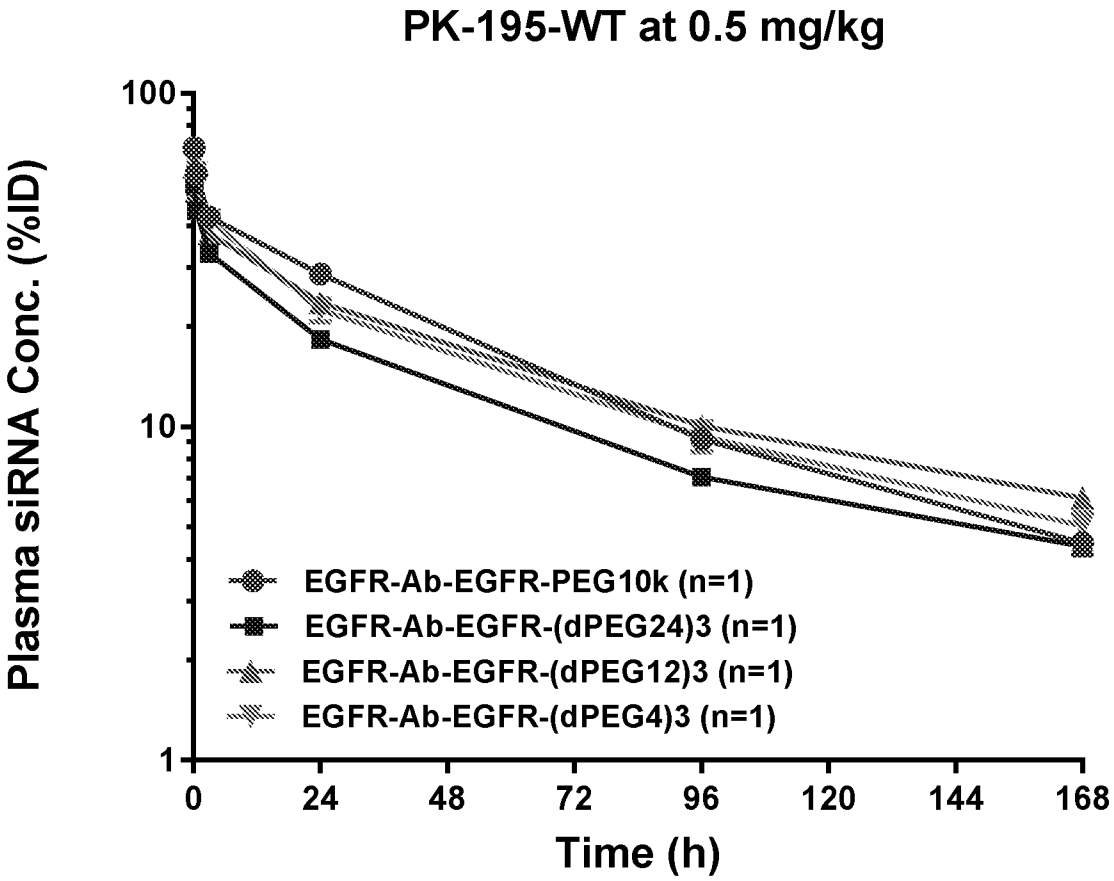

PK-195-WT at 0.5 mg/kg
Plasma siRNA Conc. (%ID)
100
10
1
0
24
48
72
96
120
144
168
Time (h)
EGFR-Ab-EGFR-PEG10k (n=1)
EGFR-Ab-EGFR-(dPEG24)3 (n=1)
EGFR-Ab-EGFR-(dPEG12)3 (n=1)
EGFR-Ab-EGFR-(dPEG4)3 (n=1)

FIG. 84

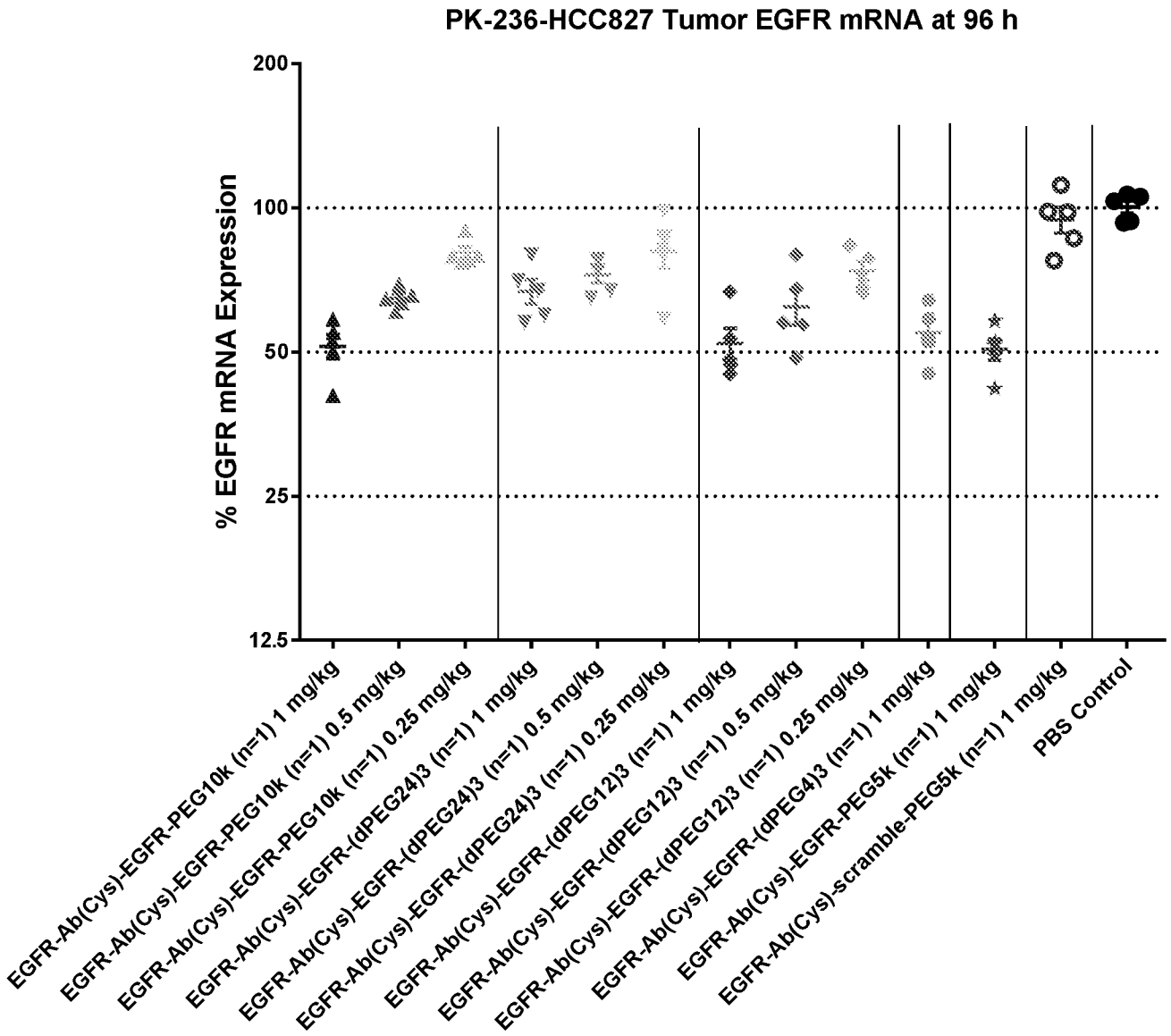

PK-236-HCC827 Tumor EGFR mRNA at 96 h
% EGFR mRNA Expression
200
100
50
25
12.5
EGFR-Ab(Cys)-EGFR-PEG10k (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-PEG10k (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-PEG10k (n=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG24)3 (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG24)3 (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG24)3 (n=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG12)3 (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG12)3 (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG12)3 (n=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG4)3 (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (n=1) 1 mg/kg
EGFR-Ab(Cys)-scramble-PEG5k (n=1) 1 mg/kg
PBS Control

FIG. 85

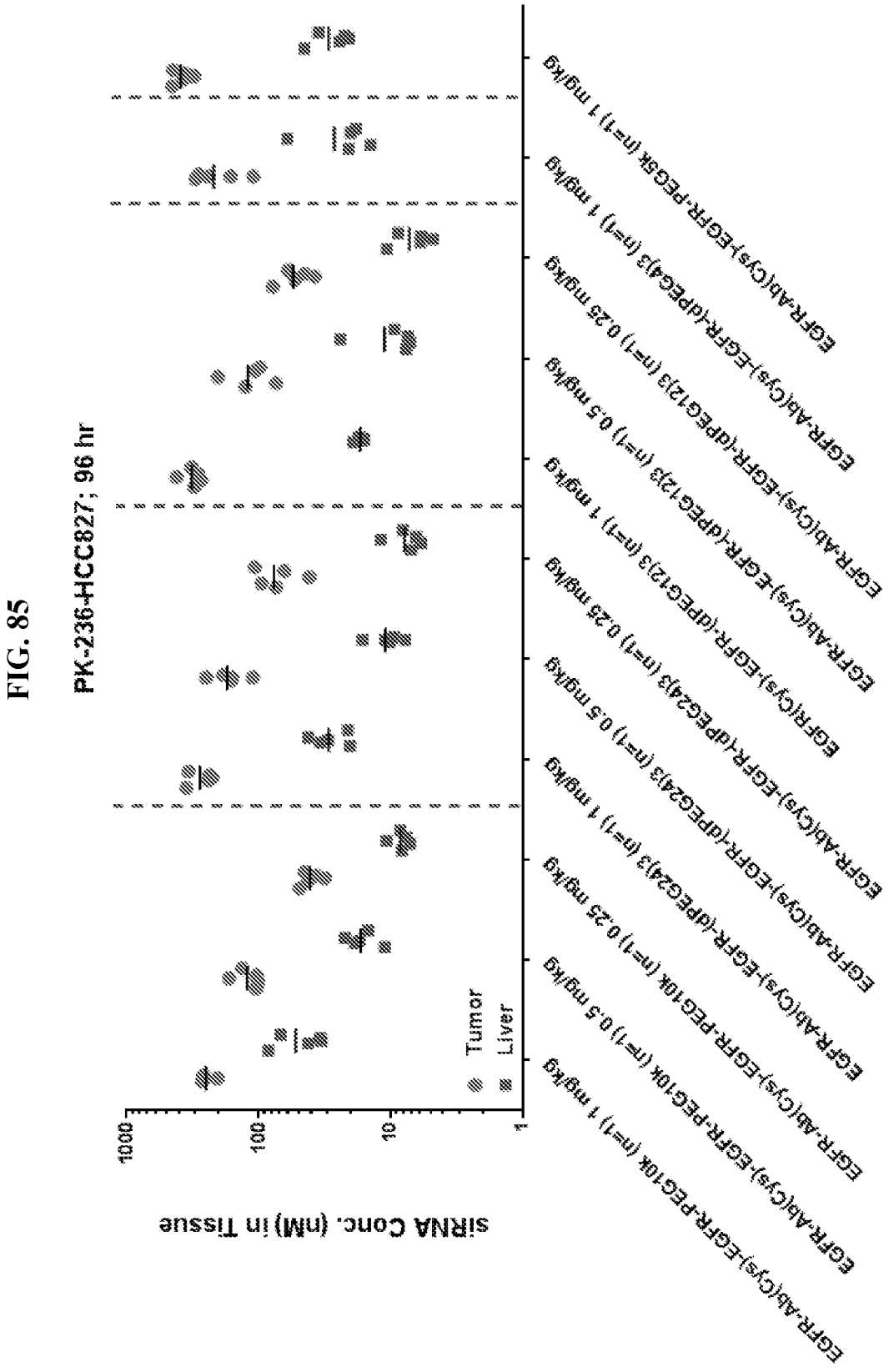

PK-236-HCC827; 96 hr
siRNA Conc. (nM) in Tissue
1000
100
10
1
Tumor
Liver
EGFR-Ab(Cys)-EGFR-PEG-10k (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-PEG-10k (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-PEG-10k (n=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG24)3 (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG24)3 (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG24)3 (n=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG12)3 (n=1) 1 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG12)3 (n=1) 0.5 mg/kg
EGFR-Ab(Cys)-EGFR-(dPEG12)3 (n=1) 0.25 mg/kg
EGFR-Ab(Cys)-EGFR-PEG5k (n=1) 1 mg/kg

FIG. 86

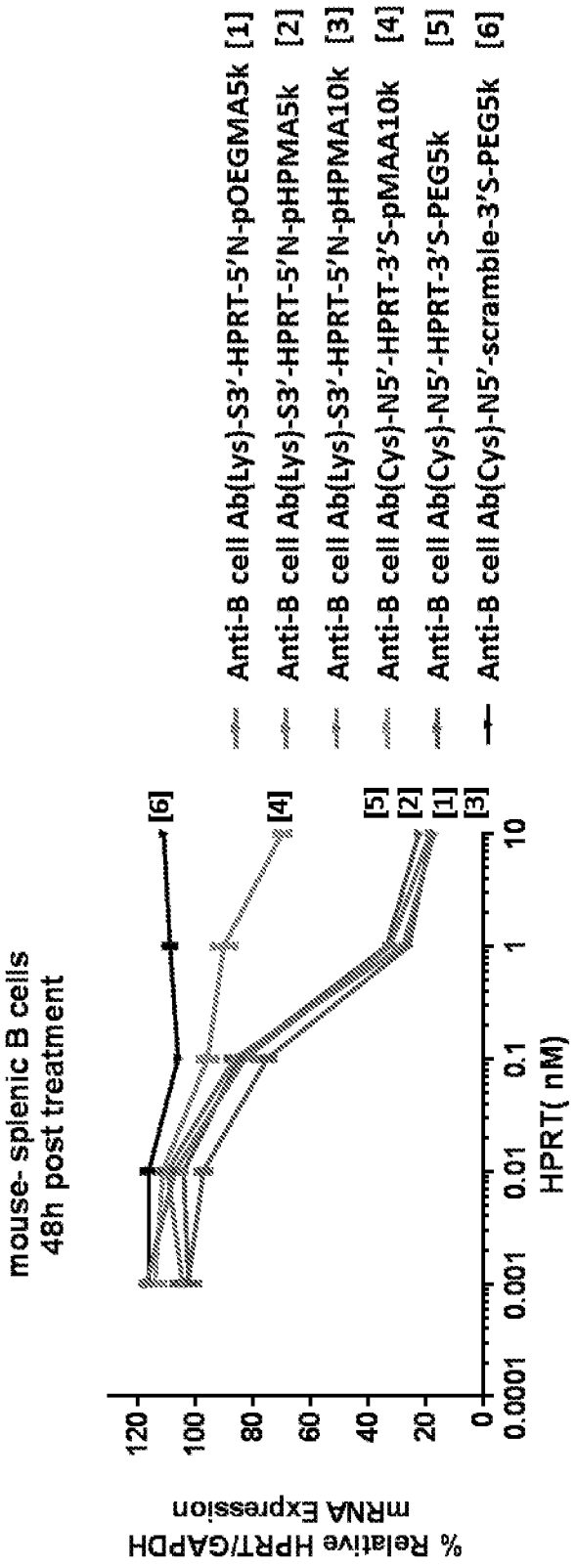
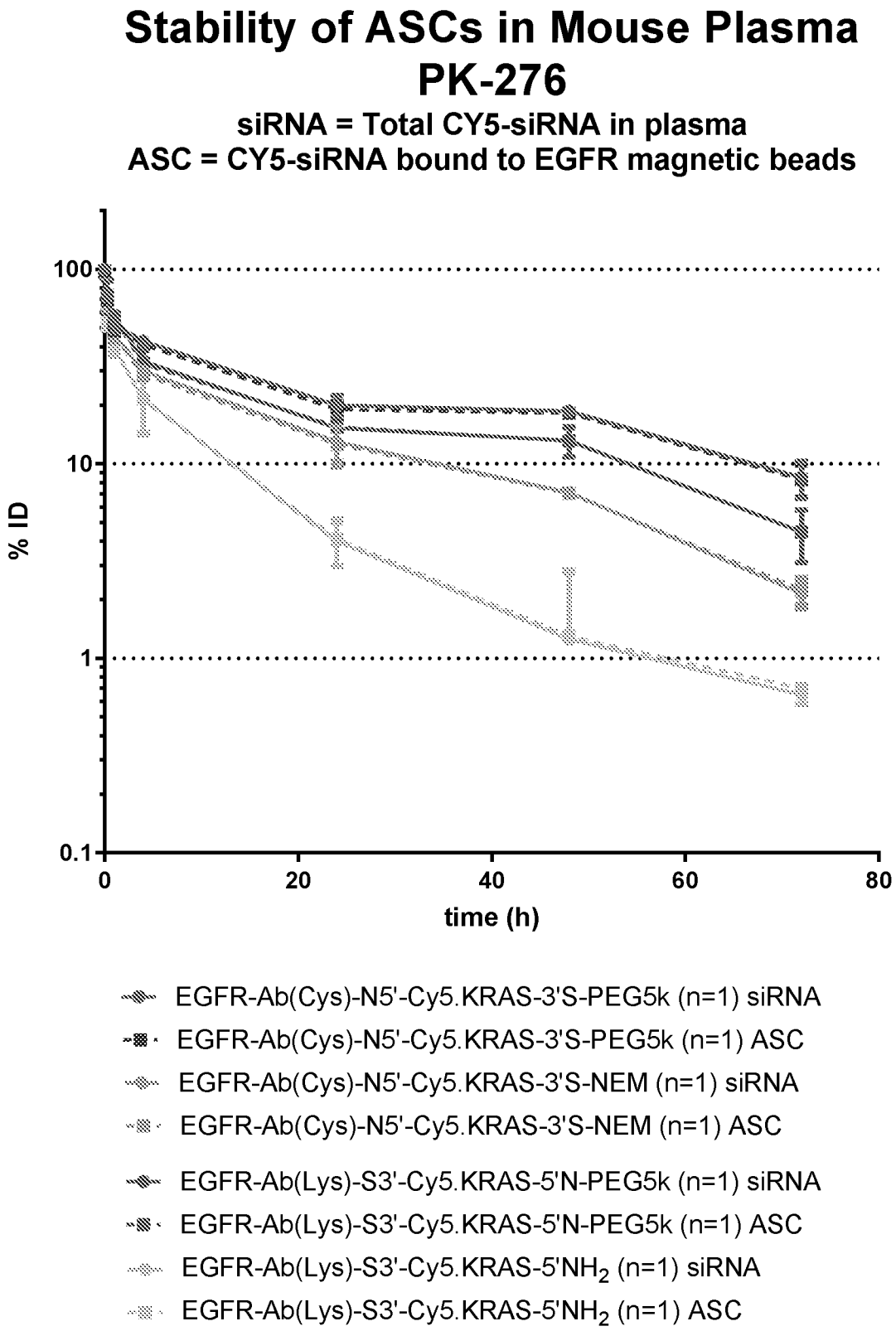

mouse- splenic B cells
48h post treatment
% Relative HPRT/GAPDH mRNA Expression
HPRT(nM)
0.0001
0.001
0.01
0.1
1
10
0
20
40
60
80
100
120
[6]
[4]
[5]
[2]
[1]
[3]
Anti-B cell Ab(Lys)-S3'-HPRT-5'N-pOEGMA5k [1]
Anti-B cell Ab(Lys)-S3'-HPRT-5'N-pHPMA5k [2]
Anti-B cell Ab(Lys)-S3'-HPRT-5'N-pHPMA10k [3]
Anti-B cell Ab(Cys)-N5'-HPRT-3'S-pMAA10k [4]
Anti-B cell Ab(Cys)-N5'-HPRT-3'S-PEG5k [5]
Anti-B cell Ab(Cys)-N5'-scramble-3'S-PEG5k [6]

FIG. 87


Stability of ASCs in Mouse Plasma
PK-276
siRNA = Total CY5-siRNA in plasma
ASC = CY5-siRNA bound to EGFR magnetic beads
100
10
1
0.1
% ID
0
20
40
60
80
time (h)
EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S-PEG5k (n=1) siRNA
EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S-PEG5k (n=1) ASC
EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S-NEM (n=1) siRNA
EGFR-Ab(Cys)-N5'-Cy5.KRAS-3'S-NEM (n=1) ASC
EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'N-PEG5k (n=1) siRNA
EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'N-PEG5k (n=1) ASC
EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'NH2 (n=1) siRNA
EGFR-Ab(Lys)-S3'-Cy5.KRAS-5'NH2 (n=1) ASC