Figure 1

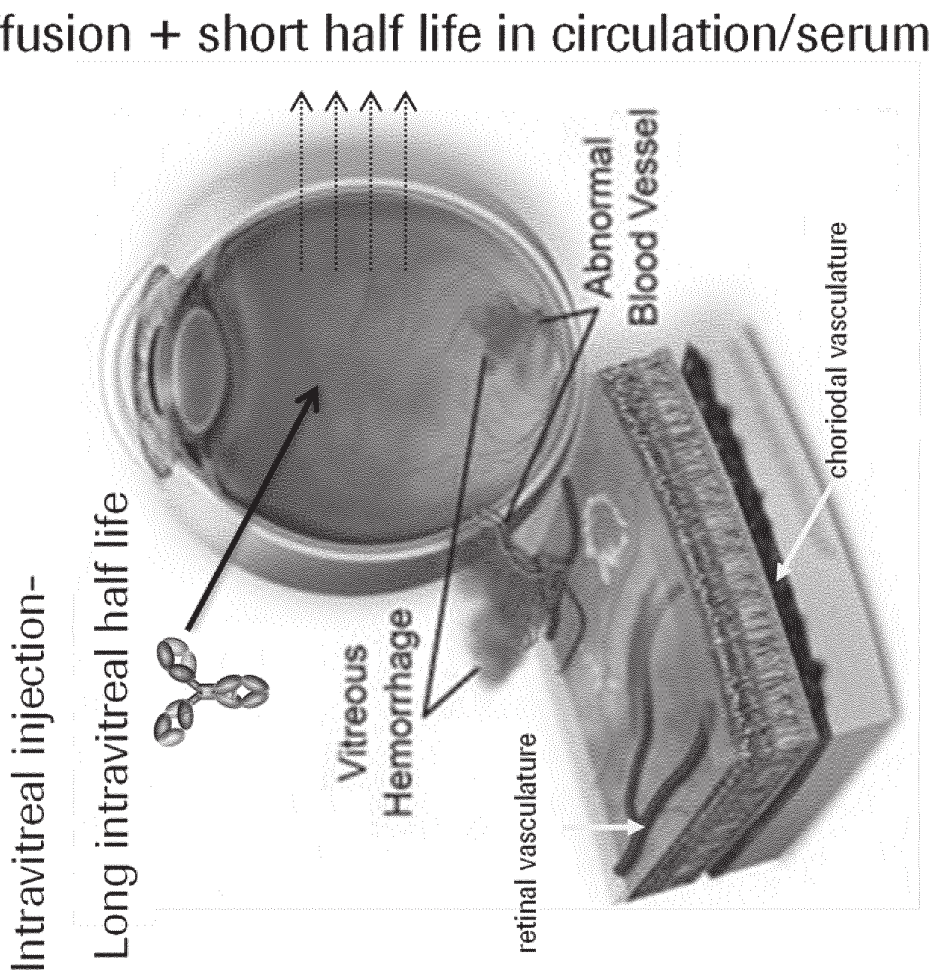

Diffusion + short half life in circulation/serum
Intravitreal injection-
Long intravitreal half life
Vitreous Hemorrhage
Abnormal Blood Vessel
retinal vasculature
choriodal vasculature

Figure 2

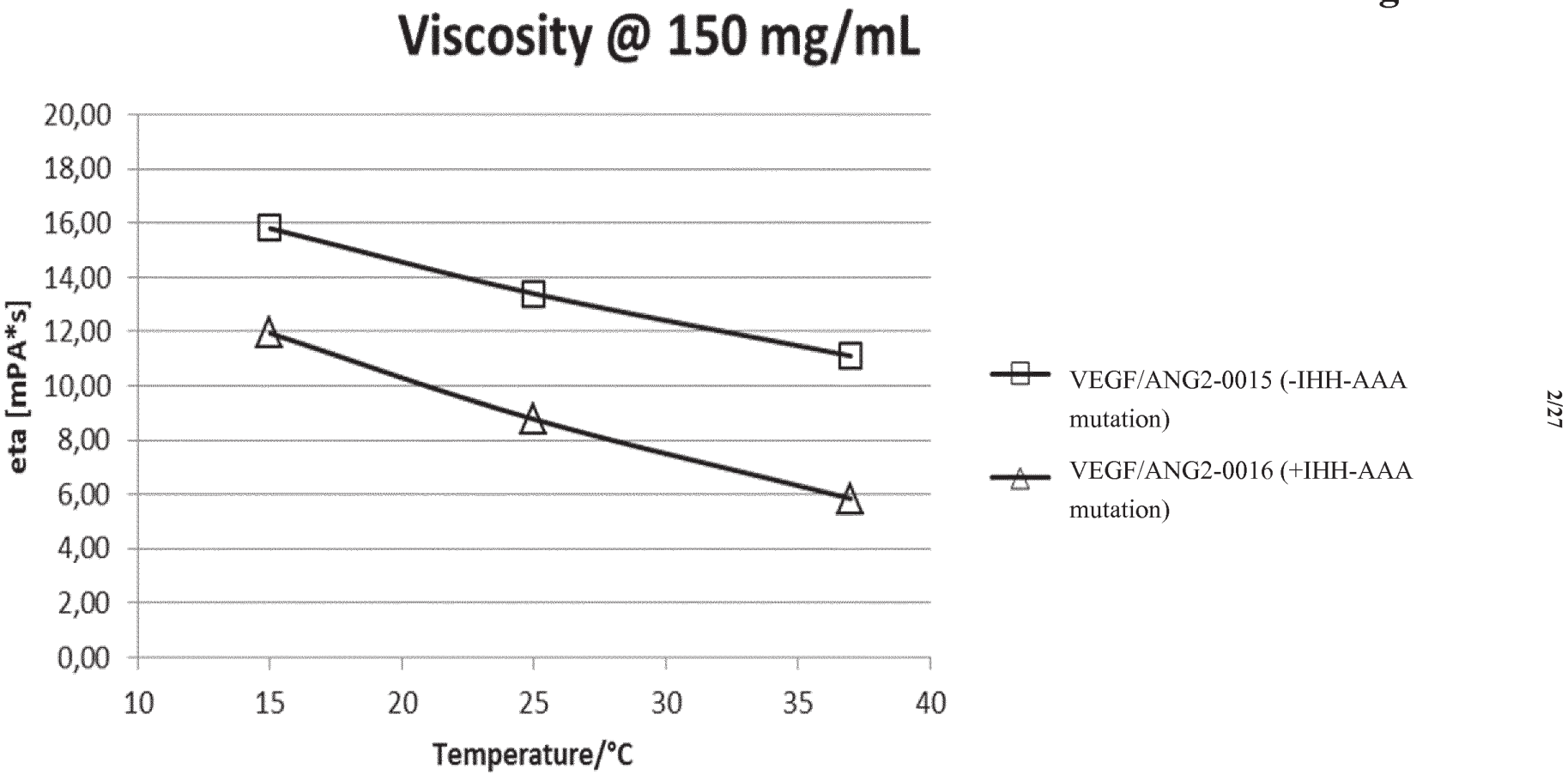

Viscosity @ 150 mg/mL
eta [mPA*s]
20,00
18,00
16,00
14,00
12,00
10,00
8,00
6,00
4,00
2,00
0,00
10
15
20
25
30
35
40
Temperature/°C
VEGF/ANG2-0015 (-IHH-AAA mutation)
VEGF/ANG2-0016 (+IHH-AAA mutation)

Figure 3

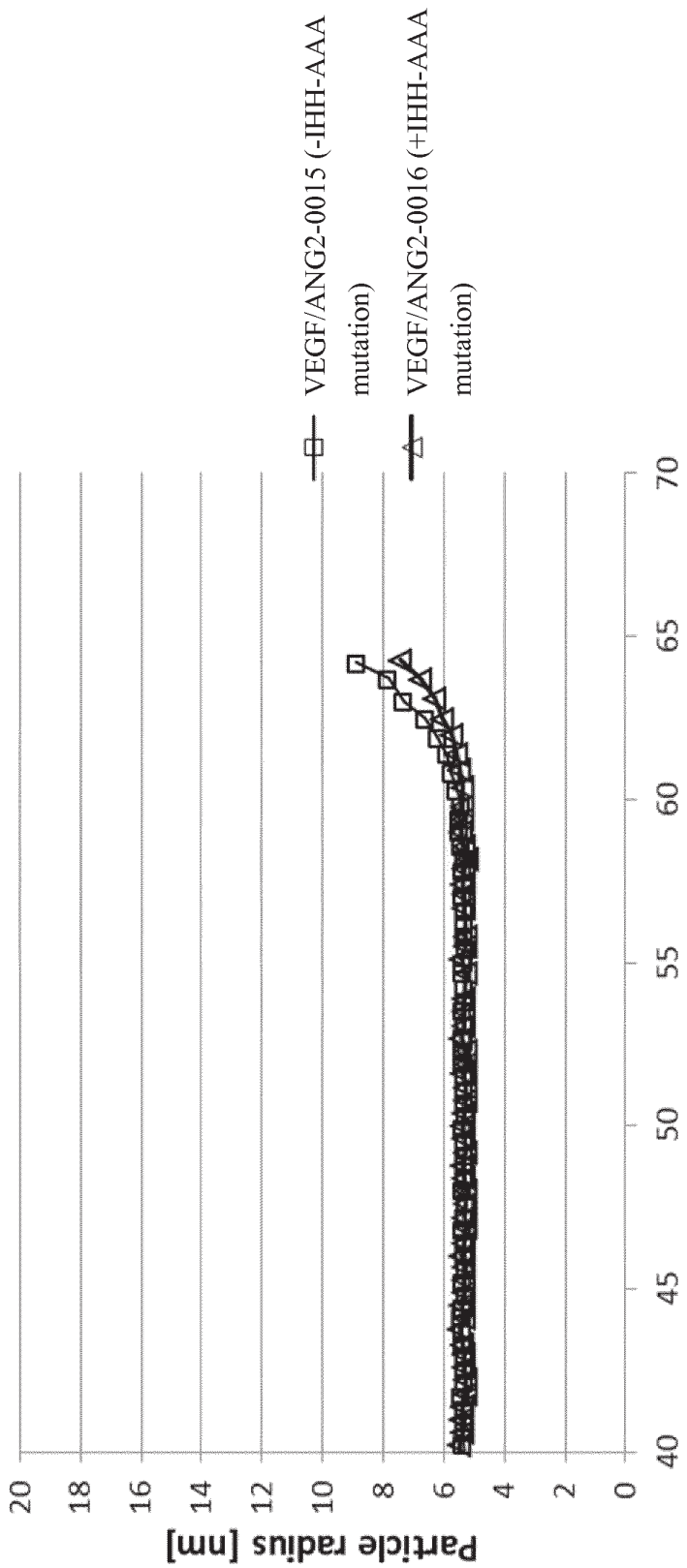

DLS Tagg
Particle radius [nm]
Temperature [°C]
VEGF/ANG2-0015 (-IHH-AAA mutation)
VEGF/ANG2-0016 (+IHH-AAA mutation)

Figure 4

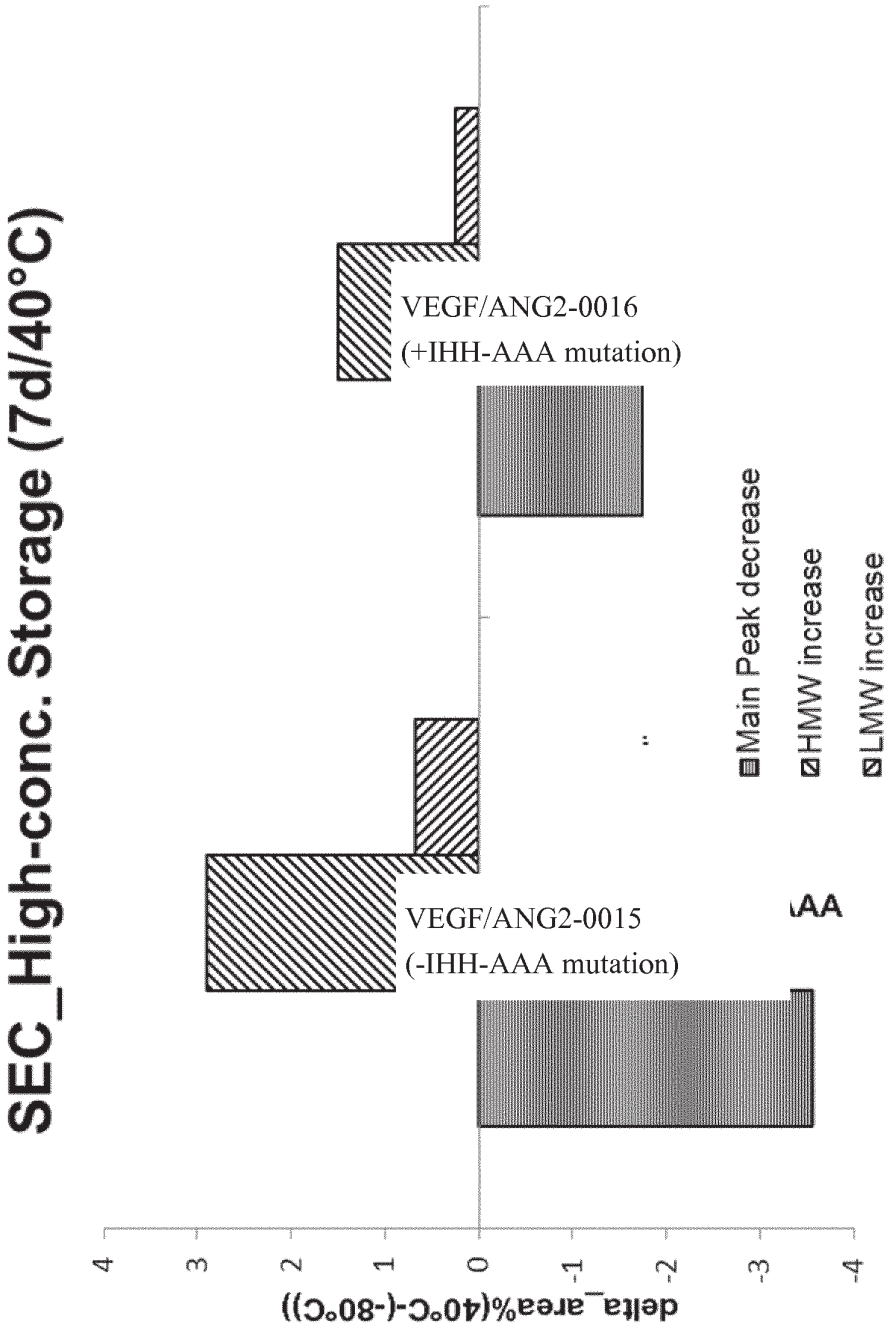

SEC_High-conc. Storage (7d/40°C)
VEGF/ANG2-0016
(+IHH-AAA mutation)
VEGF/ANG2-0015
(-IHH-AAA mutation)
Main Peak decrease
HMW increase
LMW increase
delta_area%(40°C-(-80°C))
4
3
2
1
0
-1
-2
-3
-4

Figure 5A

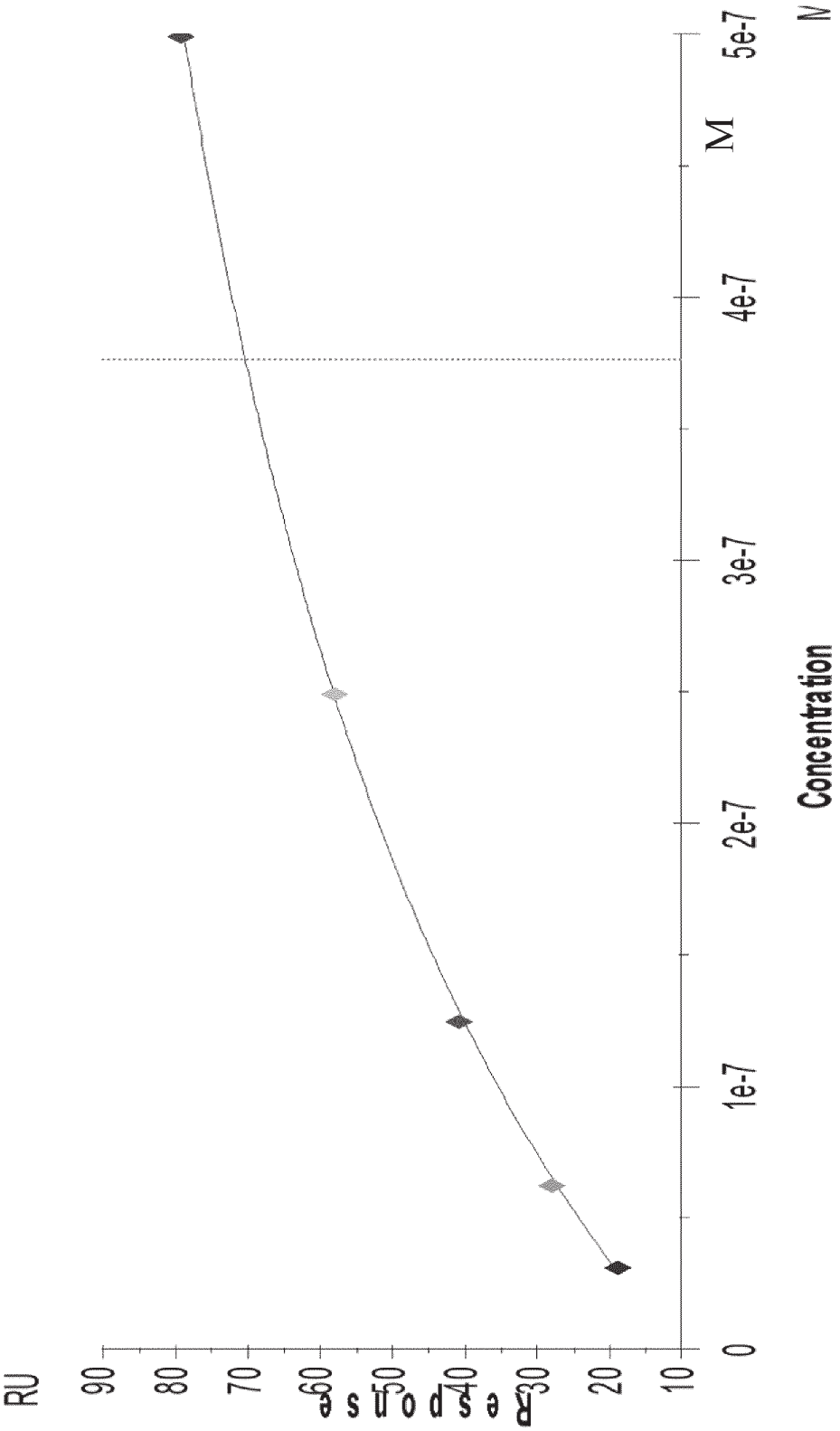

RU
90
80
70
60
50
40
30
20
10
Response
0
1e-7
2e-7
3e-7
4e-7
5e-7
M
Concentration
M

Figure 5B

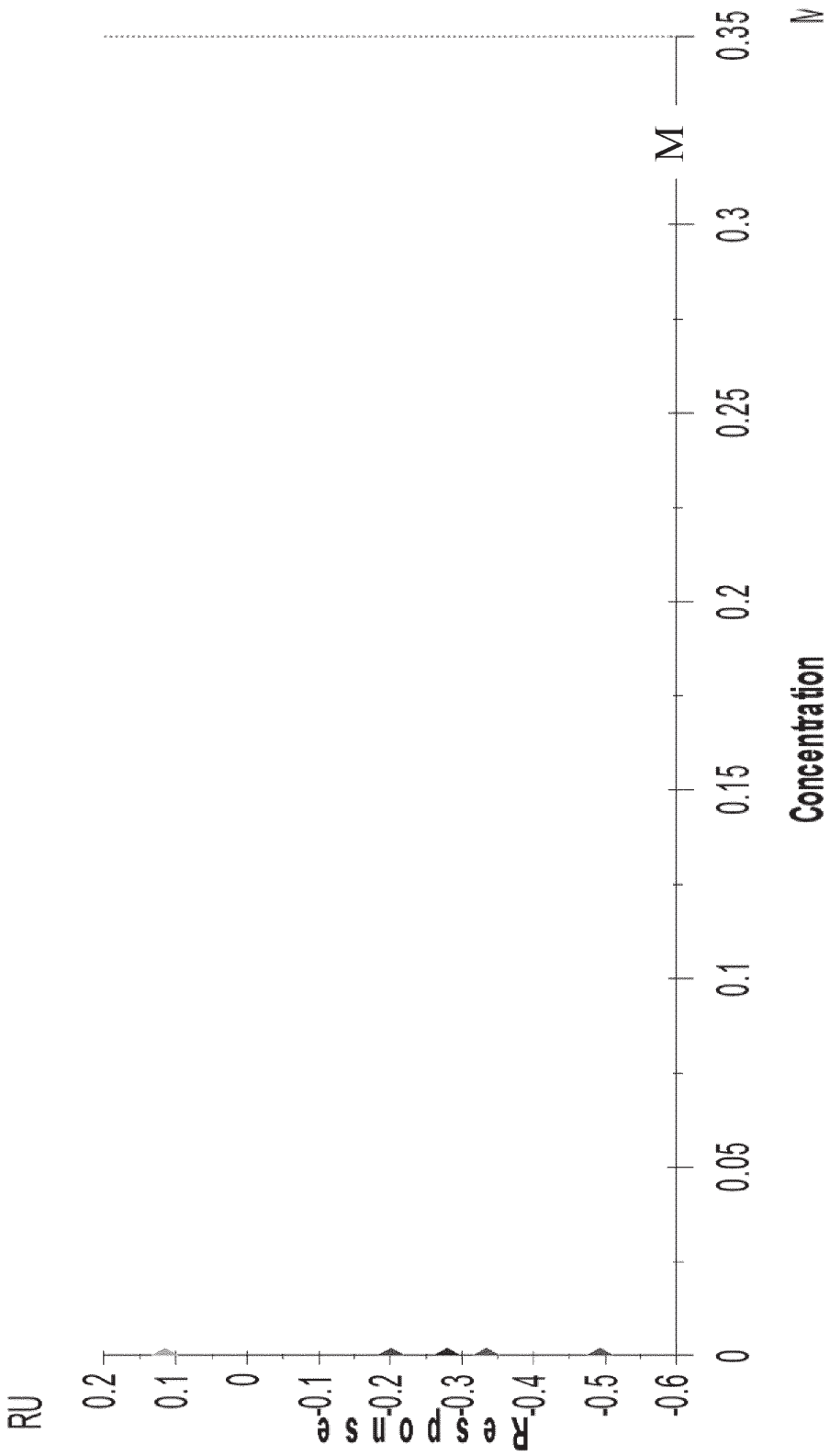

RU
0.2
0.1
0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
Response
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
M
Concentration

Figure 6

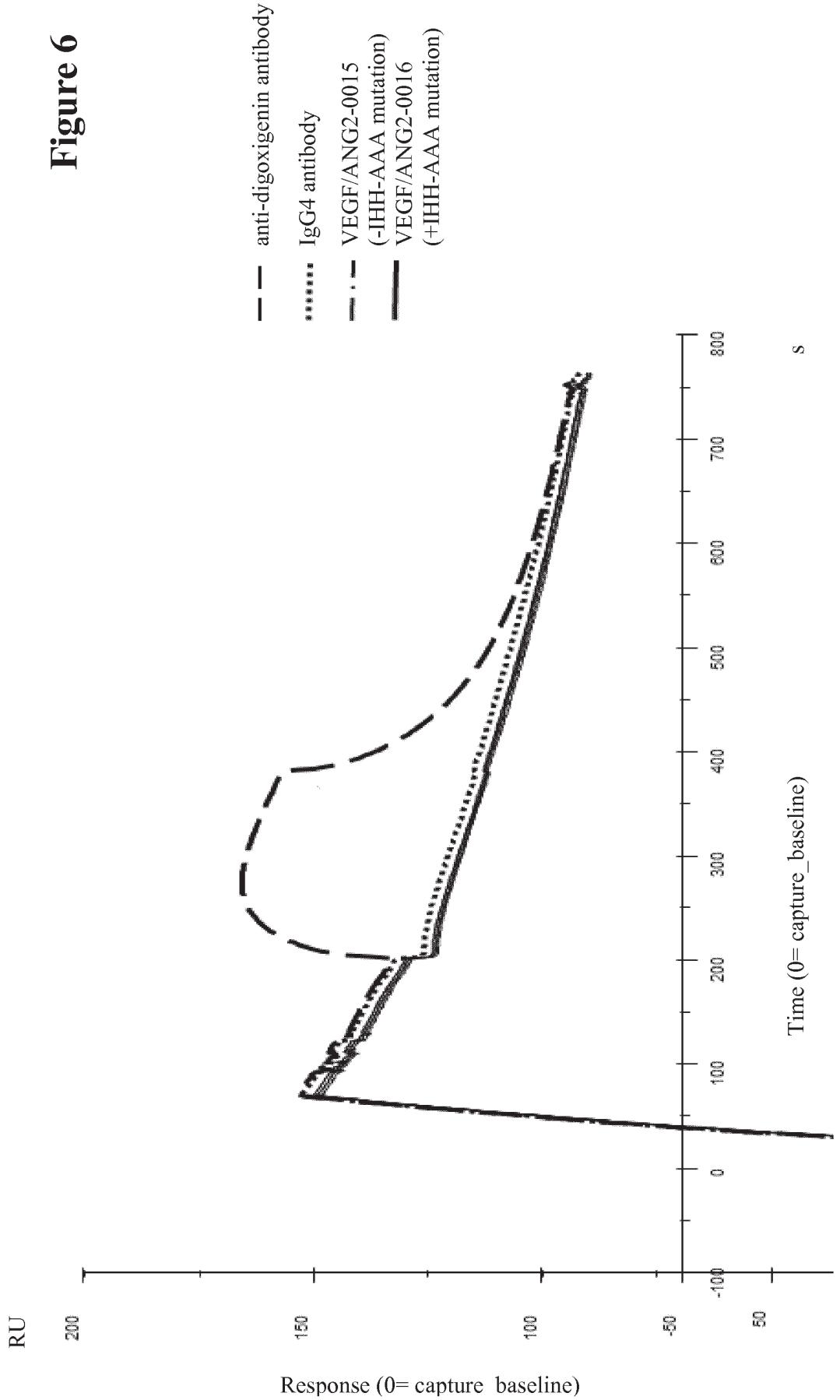

anti-digoxigenin antibody
IgG4 antibody
VEGF/ANG2-0015 (-IHH-AAA mutation)
VEGF/ANG2-0016 (+IHH-AAA mutation)
RU
200
150
100
-50
50
-100
0
100
200
300
400
500
600
700
800
s
Time (0= capture_baseline)
Response (0= capture baseline)

Figure 7A

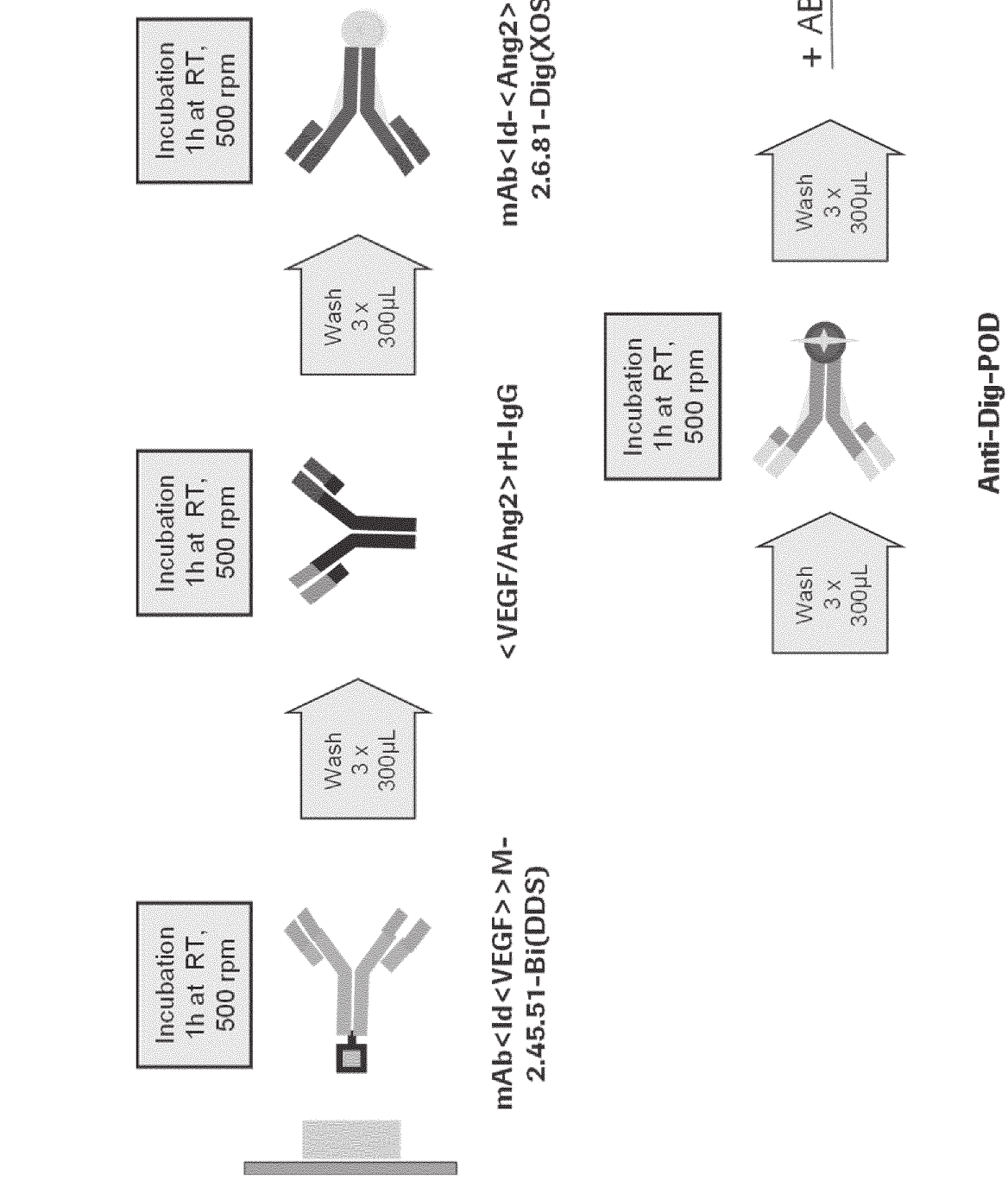

Incubation 1h at RT, 500 rpm
Wash 3 x 300µL
mAb<Id<VEGF>>M-2.45.51-Bi(DDS)
Incubation 1h at RT, 500 rpm
Wash 3 x 300µL
<VEGF/Ang2>rH-IgG
Incubation 1h at RT, 500 rpm
Wash 3 x 300µL
mAb<Id-<Ang2>>M-2.6.81-Dig(XOSu)
Incubation 1h at RT, 500 rpm
Wash 3 x 300µL
Anti-Dig-POD
+ ABTS
S

Figure 7B

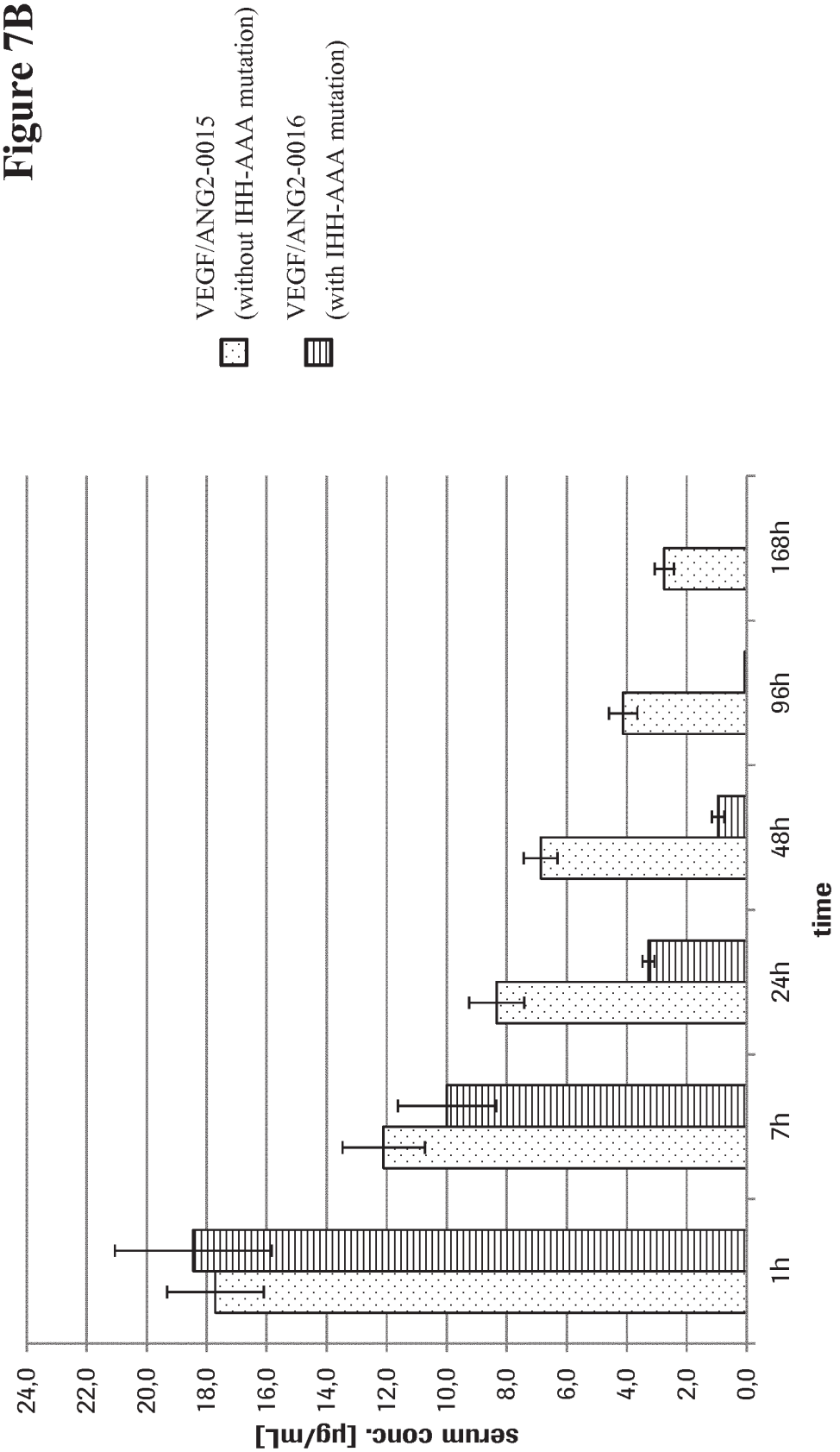

VEGF/ANG2-0015
(without IHH-AAA mutation)
VEGF/ANG2-0016
(with IHH-AAA mutation)
serum conc. [µg/mL]
24,0
22,0
20,0
18,0
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
1h
7h
24h
48h
96h
168h
time

Figure 7C

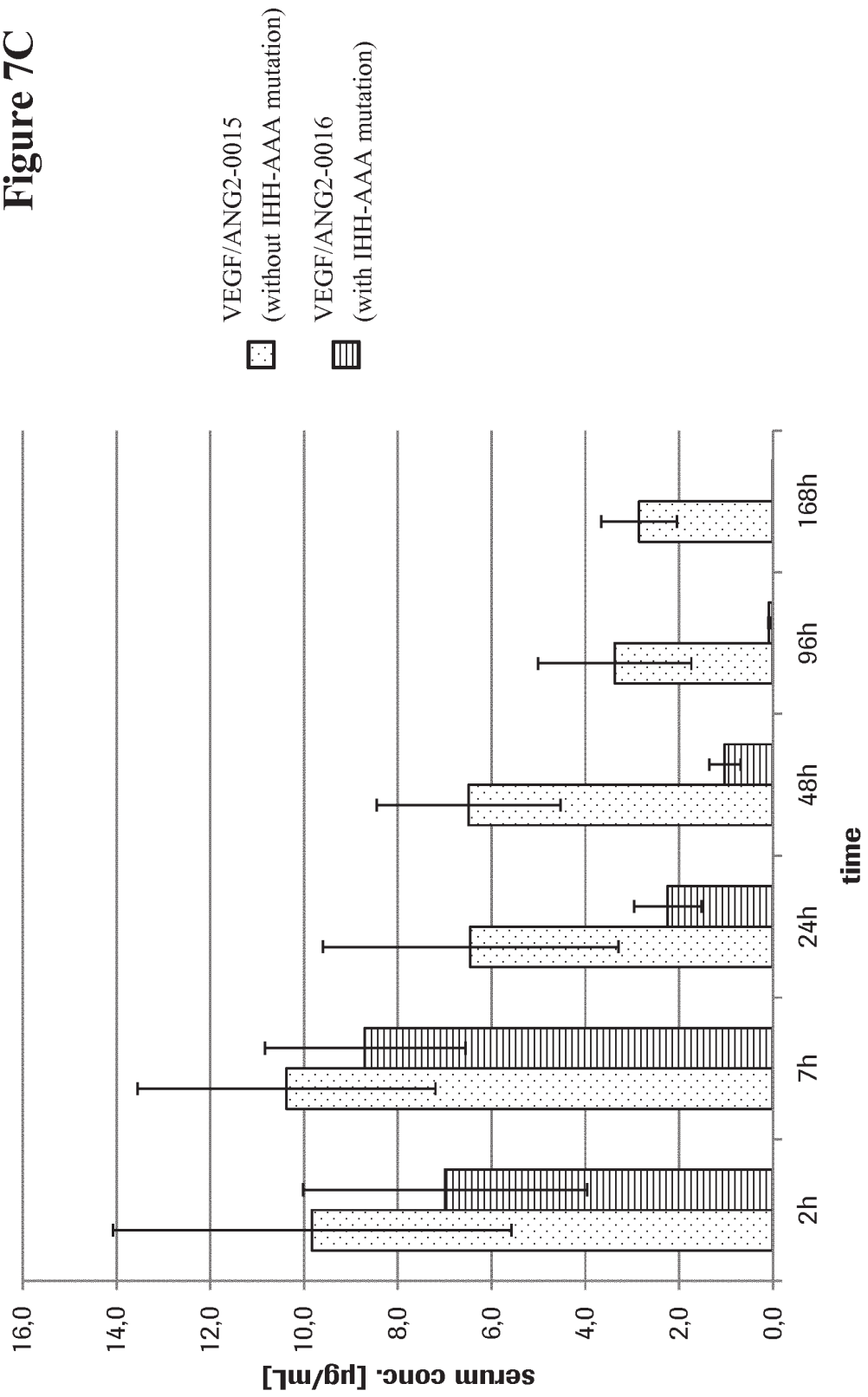

VEGF/ANG2-0015
(without IHH-AAA mutation)
VEGF/ANG2-0016
(with IHH-AAA mutation)
serum conc. [pg/mL]
16,0
14,0
12,0
10,0
8,0
6,0
4,0
2,0
0,0
2h
7h
24h
48h
96h
168h
time

Figure 7D

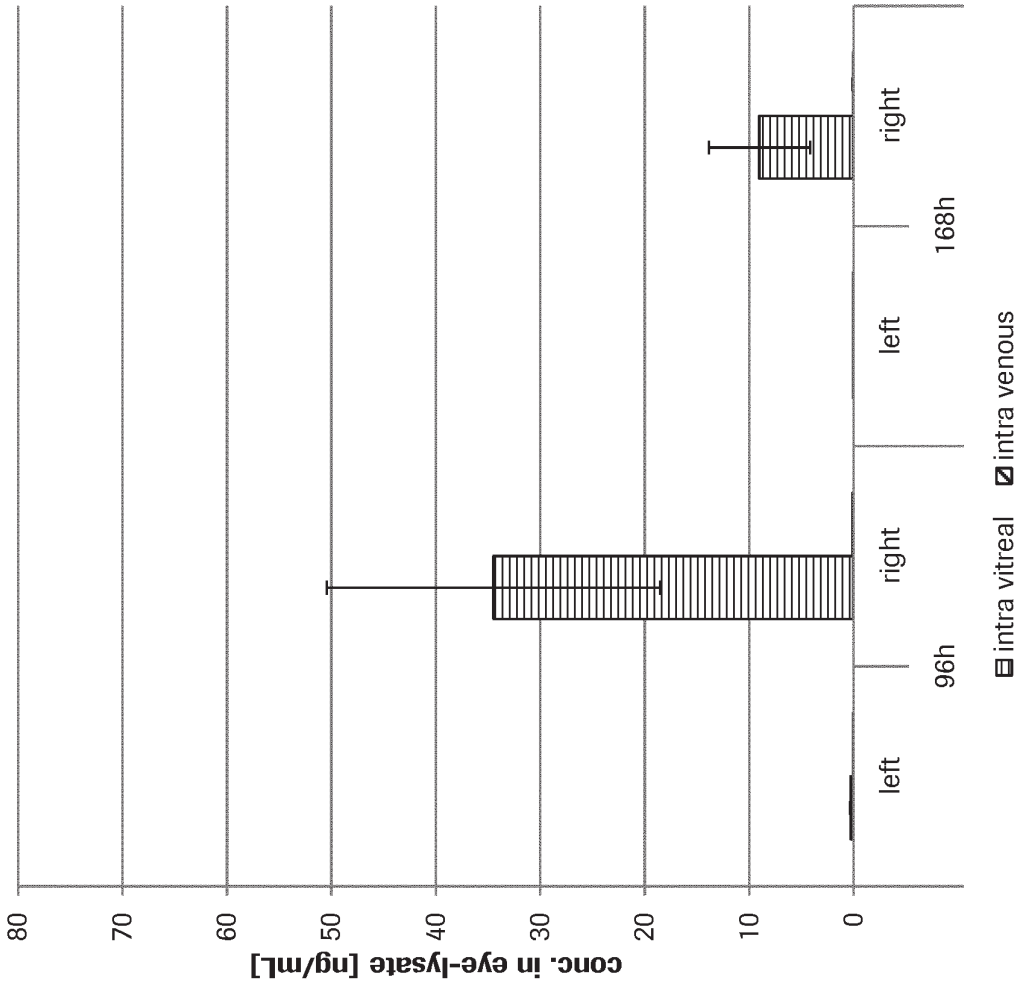

conc. in eye-lysate [ng/mL]
80
70
60
50
40
30
20
10
0
left
right
left
right
96h
168h
intra vitreal
intra venous

Figure 7E

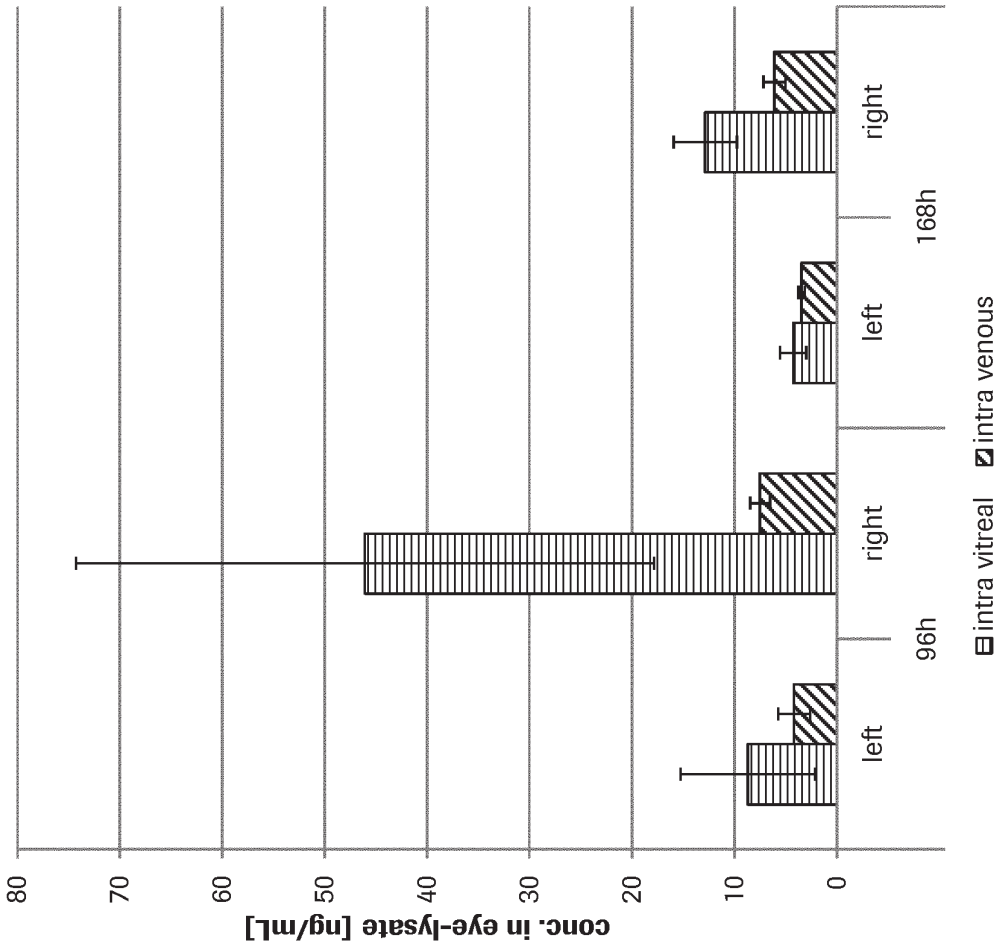

conc. in eye-lysate [ng/mL]
0
10
20
30
40
50
60
70
80
left
right
left
right
96h
168h
intra vitreal
intra venous

Figure 8A

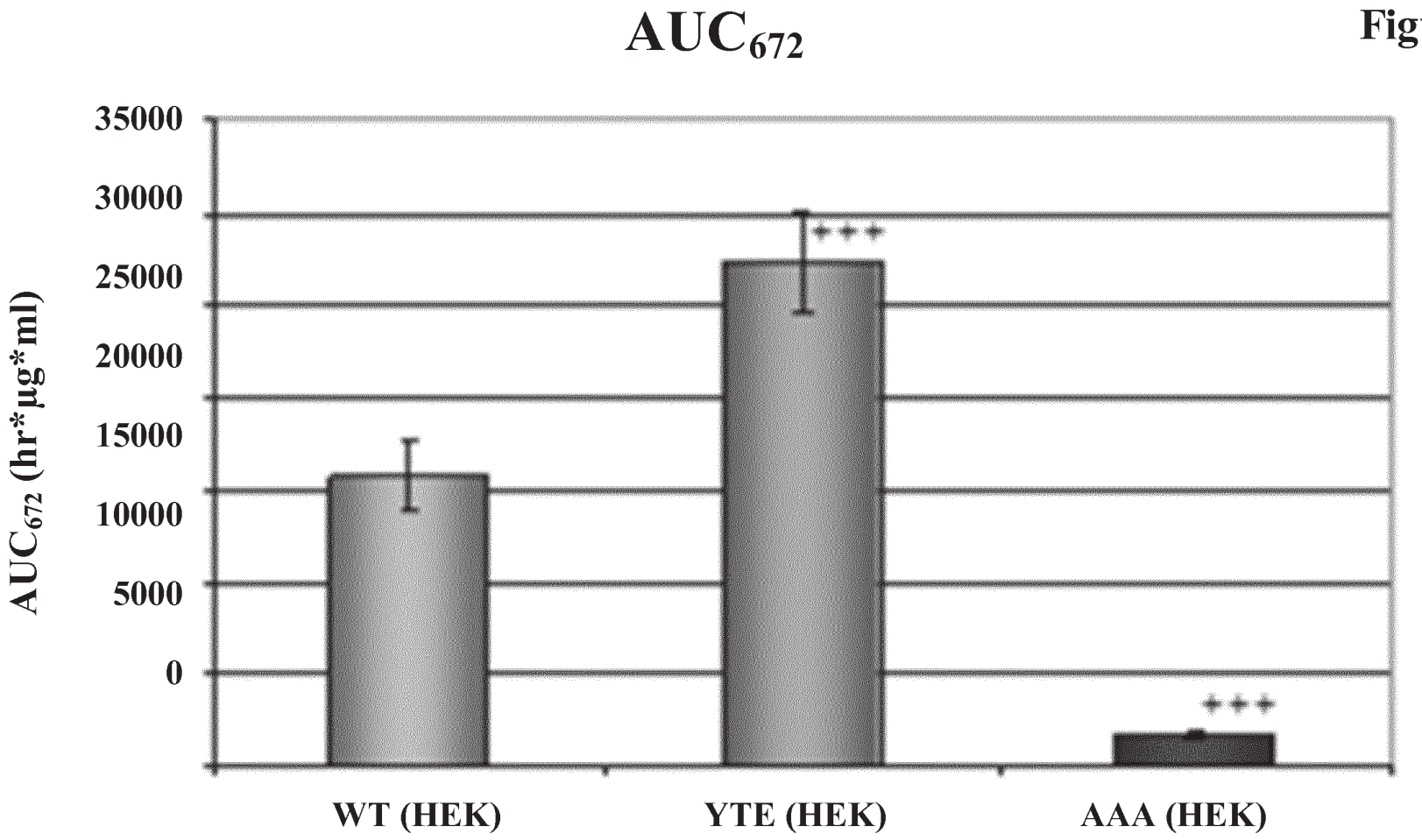

AUC672
AUC672 (hr*µg*ml)
35000
30000
25000
20000
15000
10000
5000
0
+++
+++
WT (HEK)
YTE (HEK)
AAA (HEK)

Figure 8B

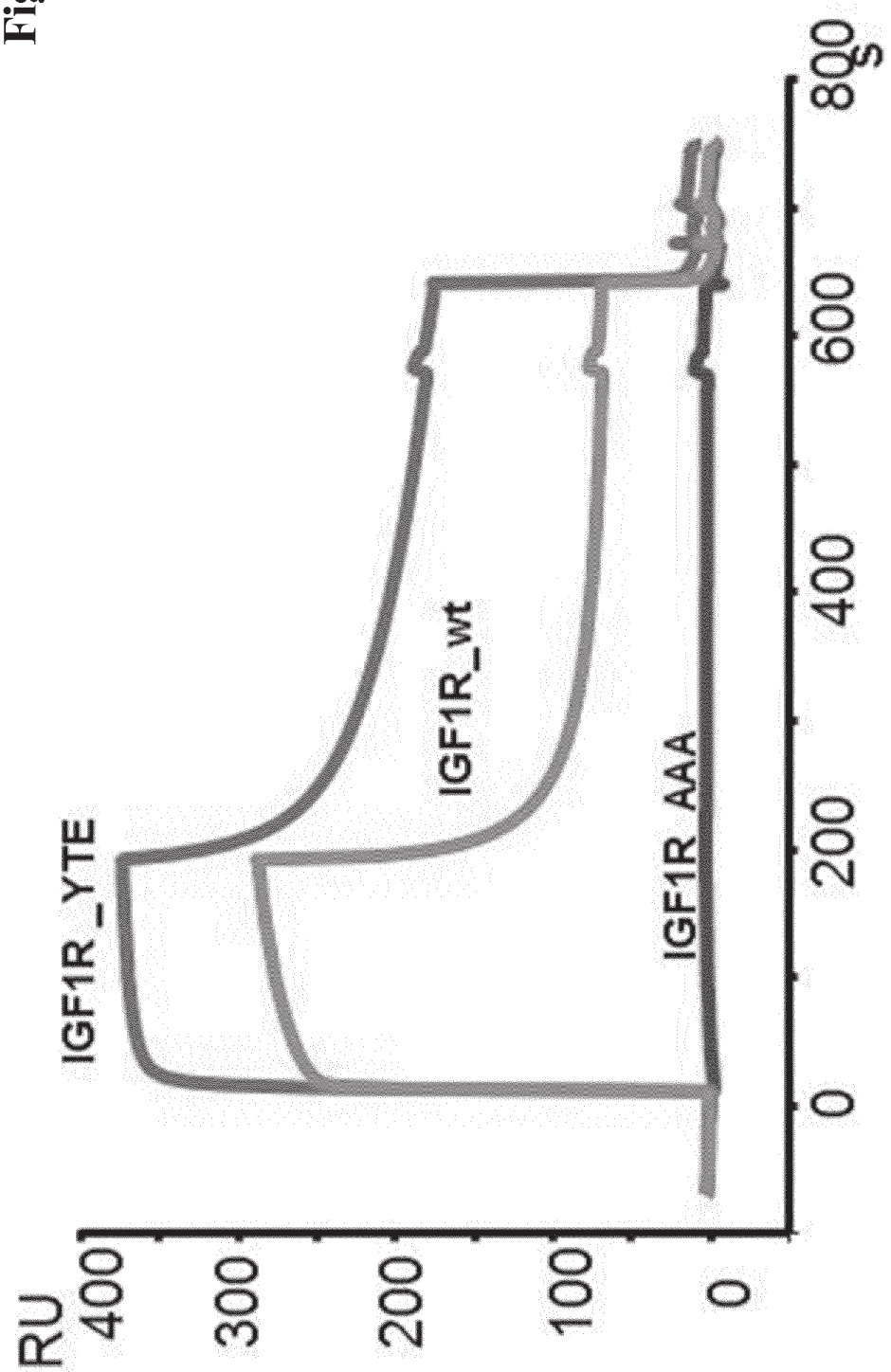

RU
400
300
200
100
0
0
200
400
600
800
s
IGF1R_YTE
IGF1R_wt
IGF1R_AAA

Figure 8C

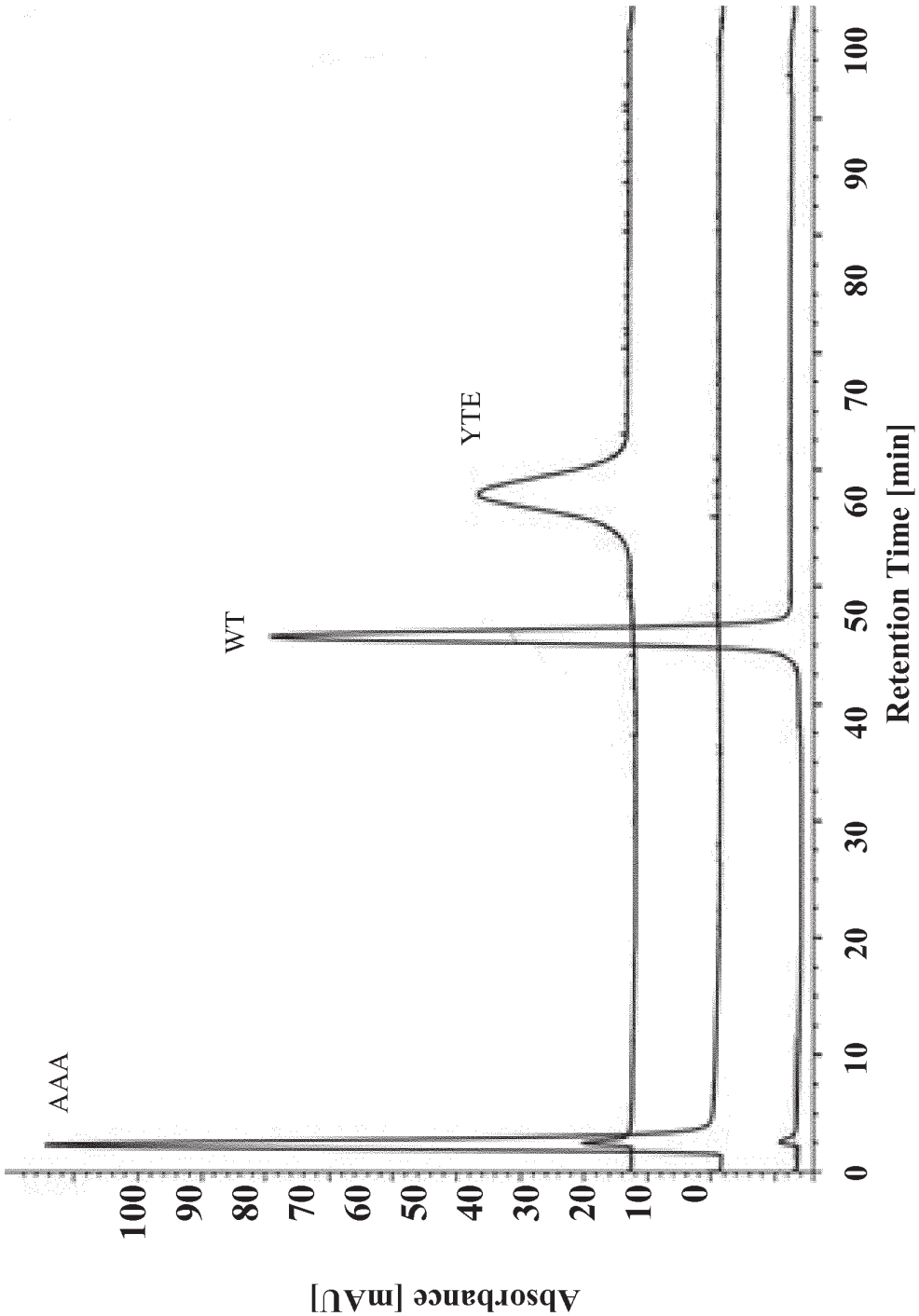
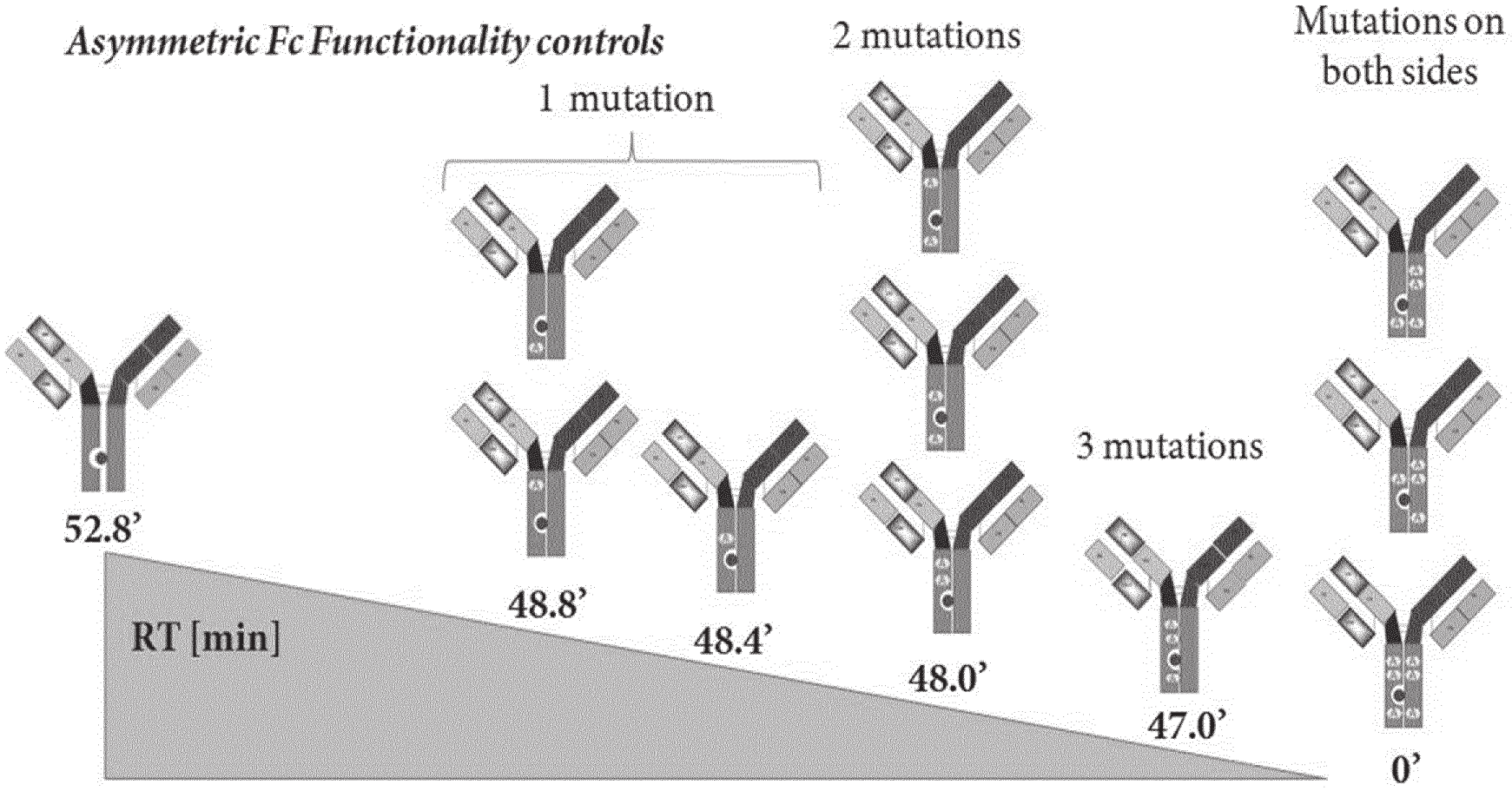

AAA
WT
YTE
Absorbance [mAU]
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
Retention Time [min]

Figure 9


Asymmetric Fc Functionality controls
1 mutation
2 mutations
Mutations on both sides
3 mutations
52.8'
48.8'
48.4'
48.0'
47.0'
0'
RT [min]

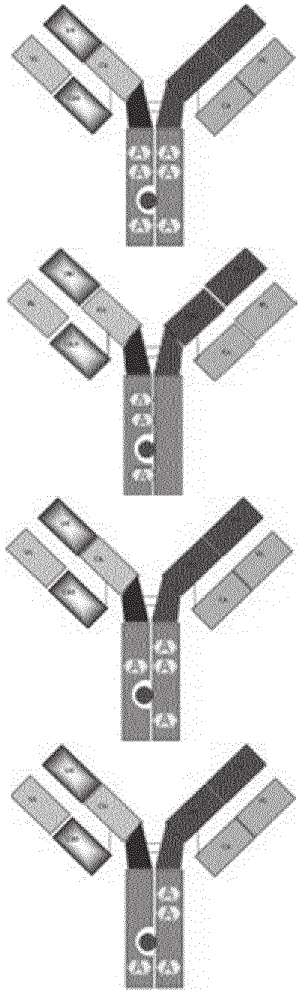


Figure 10

Knob			Hole			
Pos 1	Pos 2	Pos 3	Pos 1	Pos 2	Pos 3	
I253A	H310A	H435A	I253A	H310A	H435A	No binding
---	---	---	I253A	H310A	H435A	Binding
I253A	H310A	H435A	---	H310A	---	No binding
I253A	H310A	H435A	---	---	H435A	No binding

Figure 11

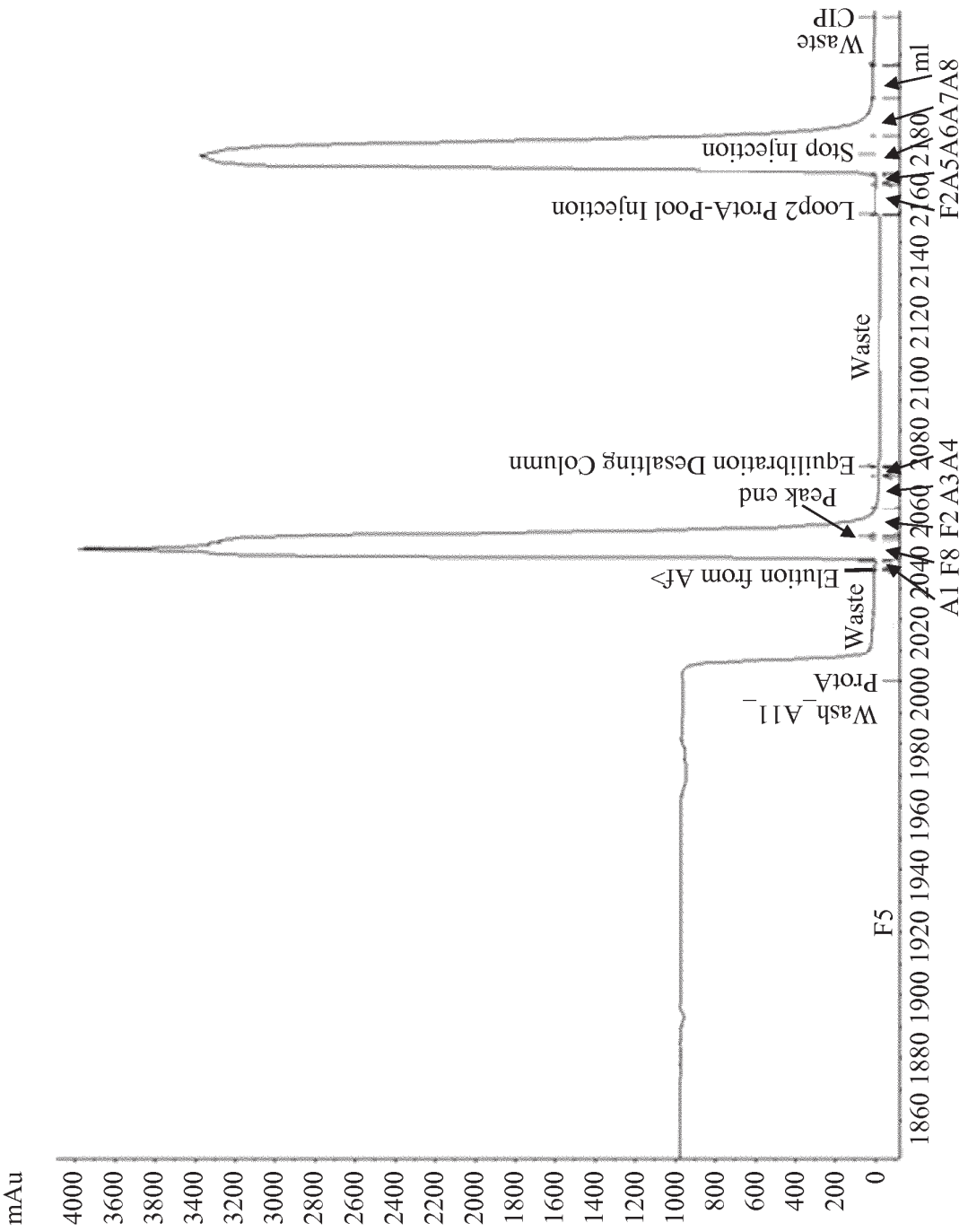

mAu
4000
3600
3800
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1860 1880 1900 1920 1940 1960 1980 2000 2020 2040 2060 2080 2100 2120 2140 2160 2180
ml
F5
Wash_A11_
ProtA
Waste
Elution from AF>
Peak end
Equilibration Desalting Column
Waste
Loop2 ProtA-Pool Injection
Stop Injection
Waste
CIP
A1 F8 F2 A3A4
F2A5A6A7A8

Figure 12

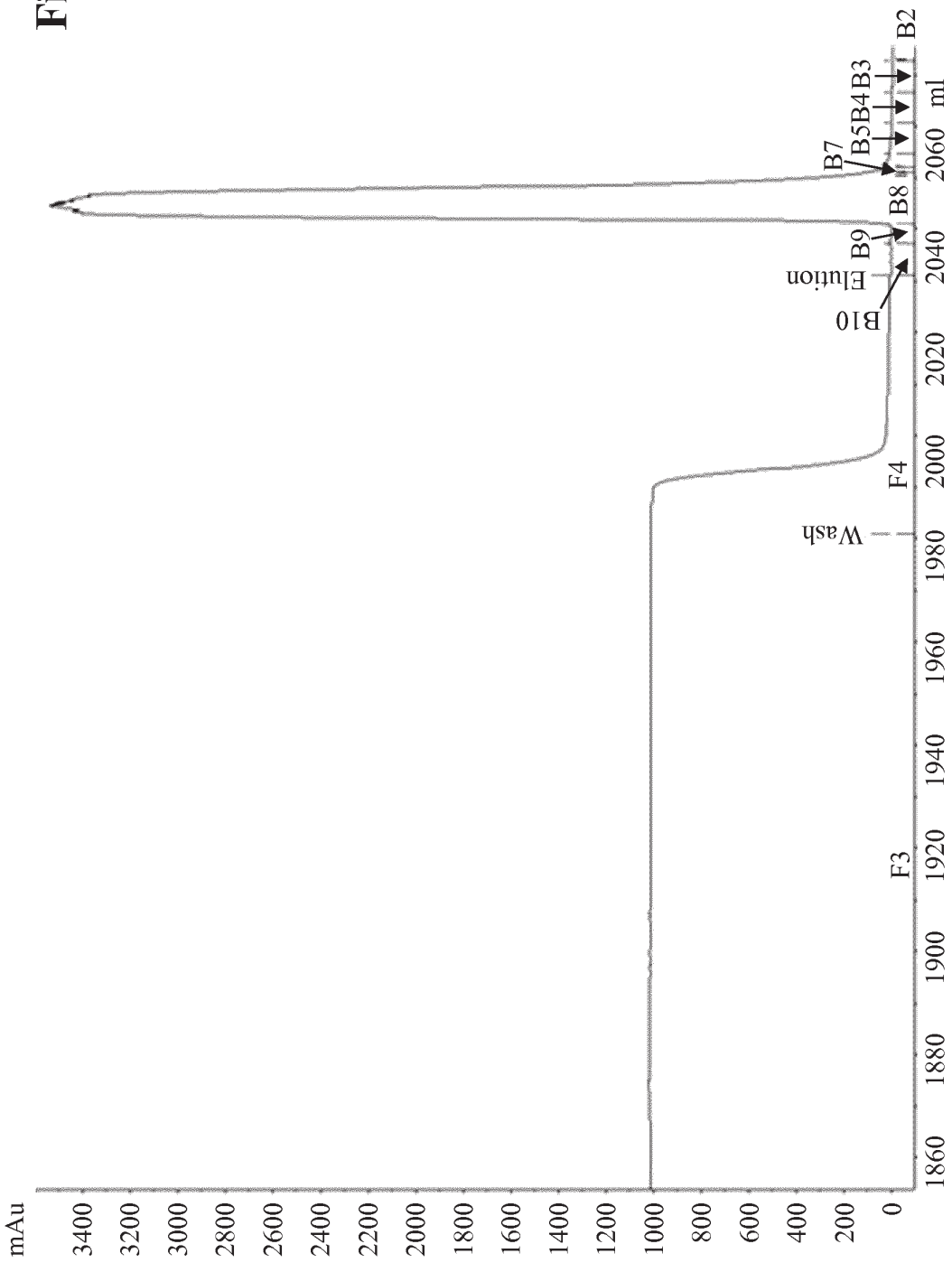

mAu
3400
3200
3000
2800
2600
2400
2200
2000
1800
1600
1400
1200
1000
800
600
400
200
0
1860
1880
1900
1920
1940
1960
1980
2000
2020
2040
2060
ml
F3
F4
Wash
B10
Elution
B9
B8
B7
B5
B4
B3
B2

Figure 13

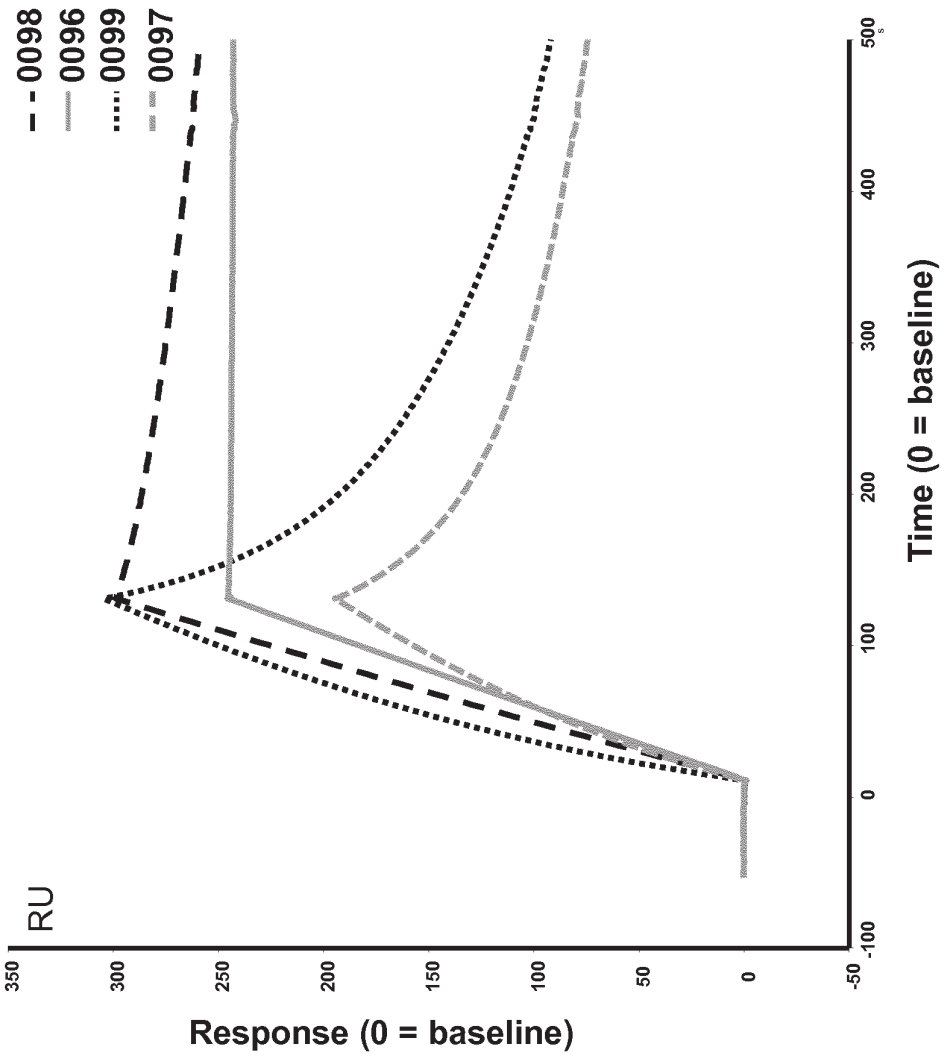

0098
0096
0099
0097
RU
350
300
250
200
150
100
50
0
-50
-100
Response (0 = baseline)
-100
0
100
200
300
400
500
s
Time (0 = baseline)

Figure 14

sample	mutation (hole)	RT [min]
anti-VEGF/ANG2 antibody (0096)	-	51.88
anti-VEGF/ANG2 antibody (0097)	I253A/H310A/H435A	46.61
anti-VEGF/ANG2 antibody (0098)	H310A/H433A/Y436A	46.67
anti-VEGF/ANG2 antibody (0099)	L251D/L314D/L432D	46.25
anti-VEGF/ANG2 antibody (0100)	M252Y/S254T/T256E	56.17

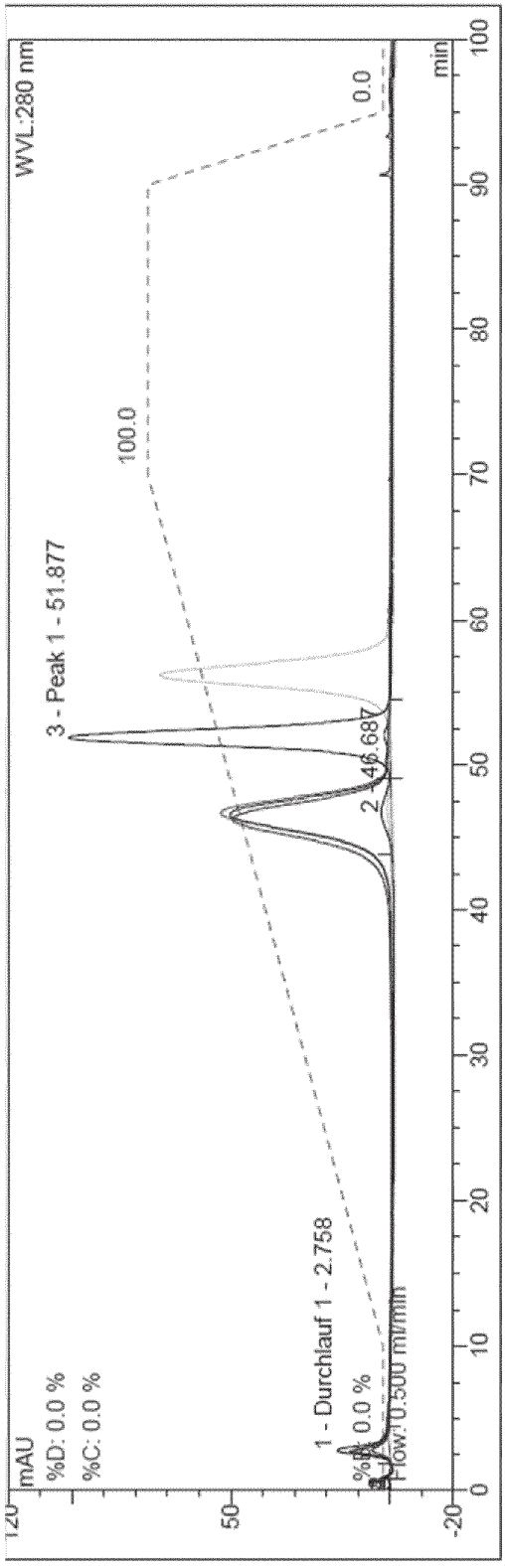


Figure 15

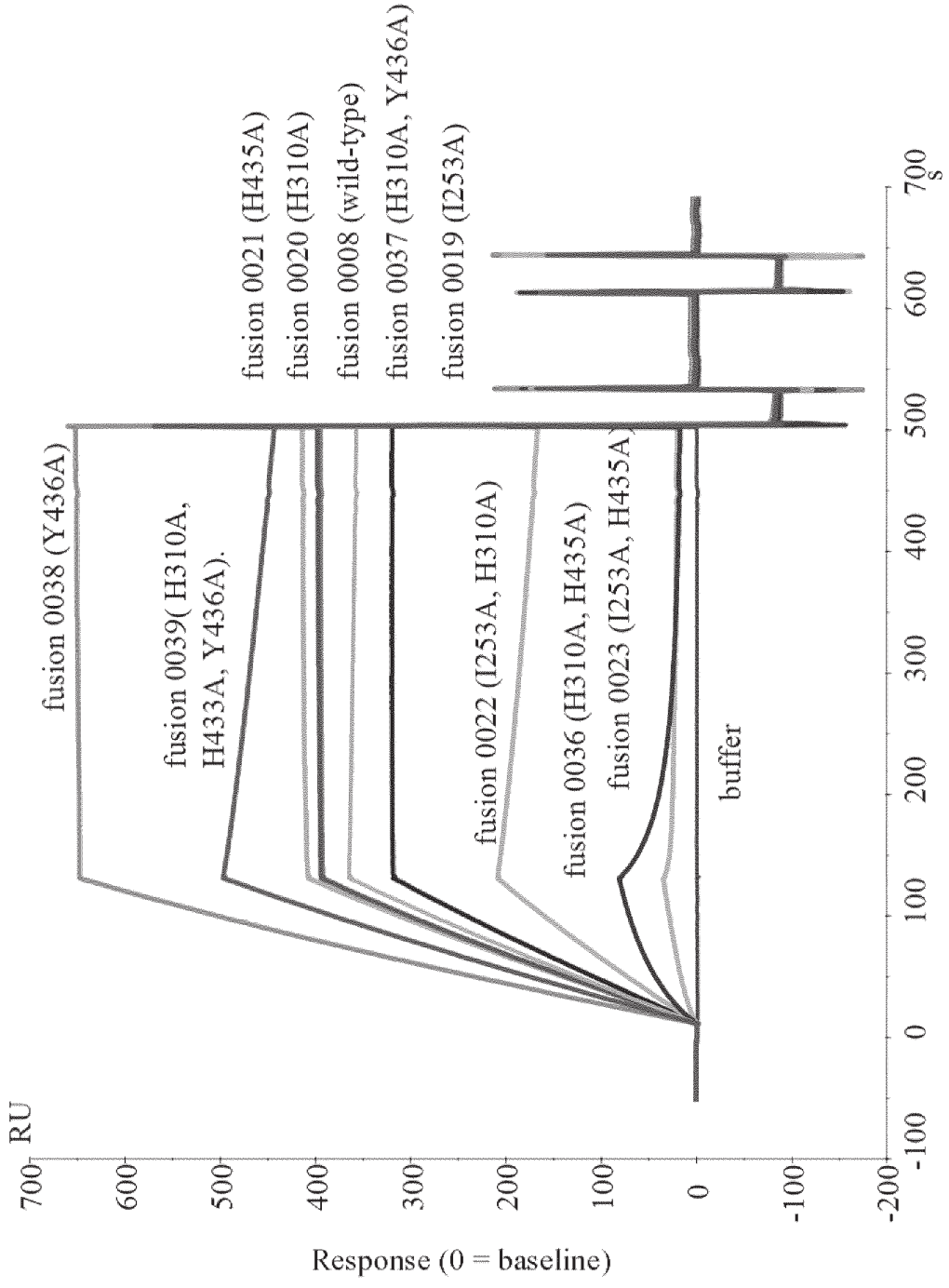

RU
700
600
500
400
300
200
100
0
-100
-200
Response (0 = baseline)
-100
0
100
200
300
400
500
600
700
s
Time (0 = baseline)
fusion 0038 (Y436A)
fusion 0039(H310A, H433A, Y436A).
fusion 0021 (H435A)
fusion 0020 (H310A)
fusion 0008 (wild-type)
fusion 0037 (H310A, Y436A)
fusion 0019 (I253A)
fusion 0022 (I253A, H310A)
fusion 0036 (H310A, H435A)
fusion 0023 (I253A, H435A)
buffer

Figure 16

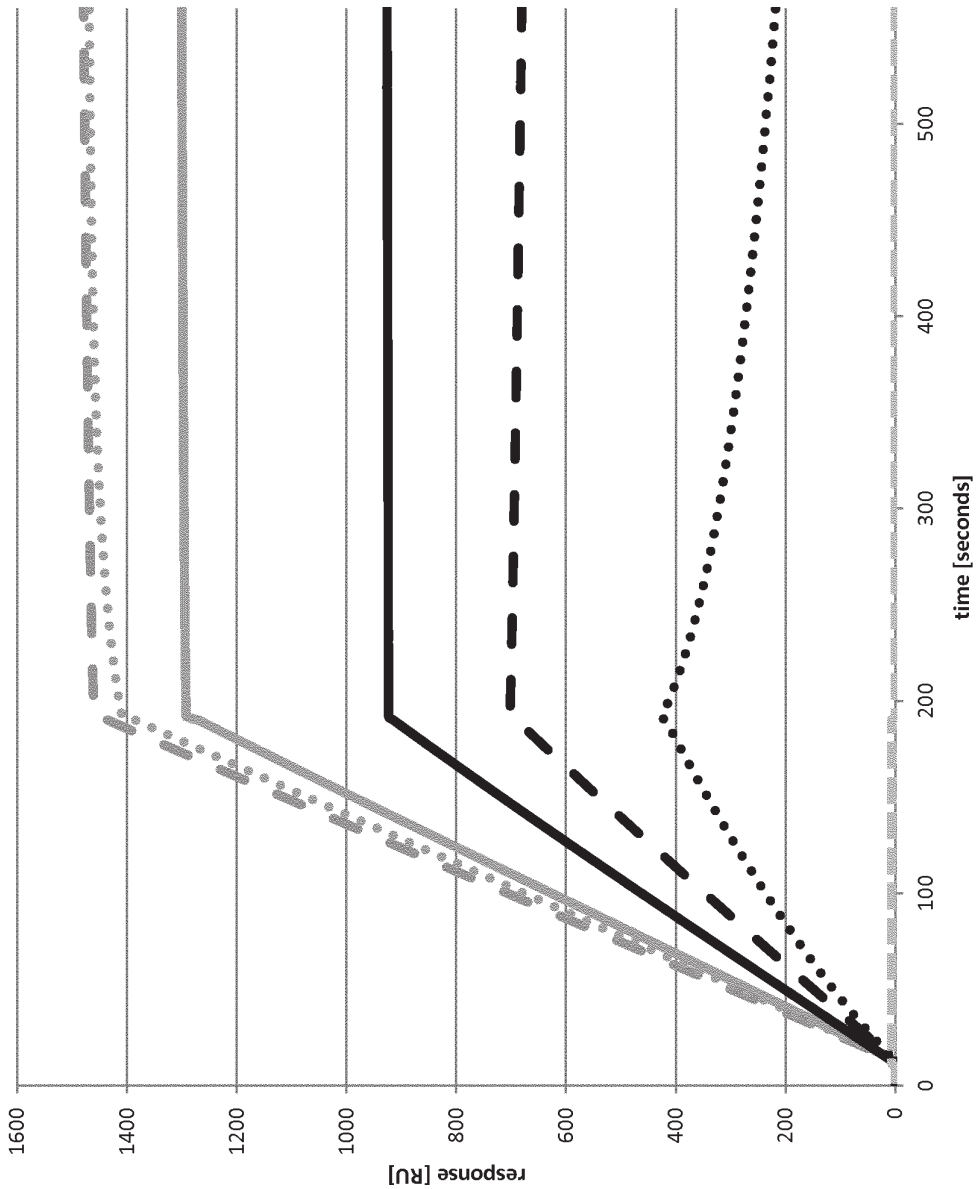

IGF-1R 0033 (wild-type)
IGF-1R 0035
IGF-1R 0045
VEGF/ANG2 0098
VEGF/ANG2 0121
VEGF/ANG2 wild-type
Fab as control
response [RU]
time [seconds]
0
200
400
600
800
1000
1200
1400
1600
0
100
200
300
400
500

Figure 17

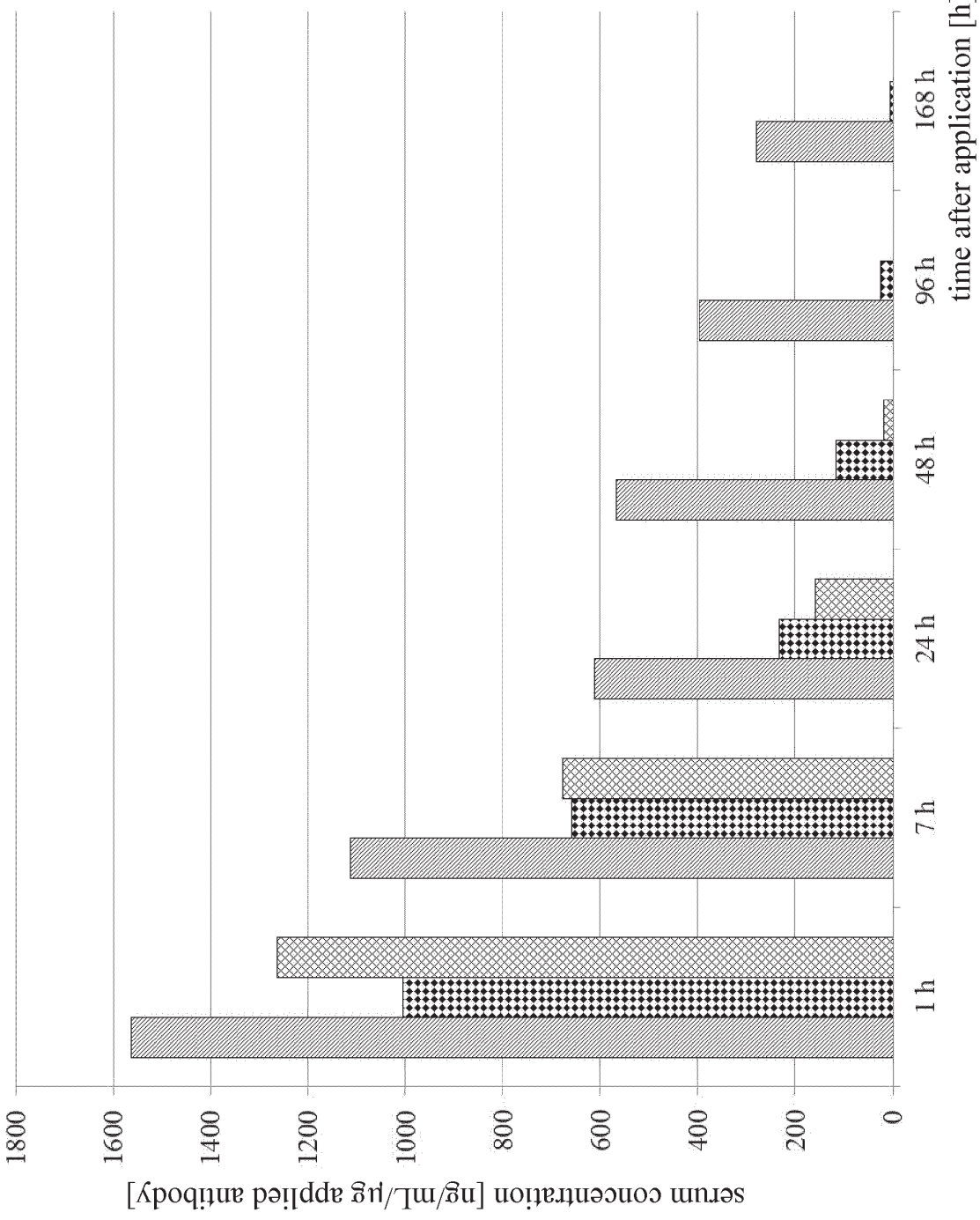

IGF-1R 0033
IGF-1R 0035
IGF-1R 0045
serum concentration [ng/mL/µg applied antibody]
0
200
400
600
800
1000
1200
1400
1600
1800
1 h
7 h
24 h
48 h
96 h
168 h
time after application [h]

Figure 18

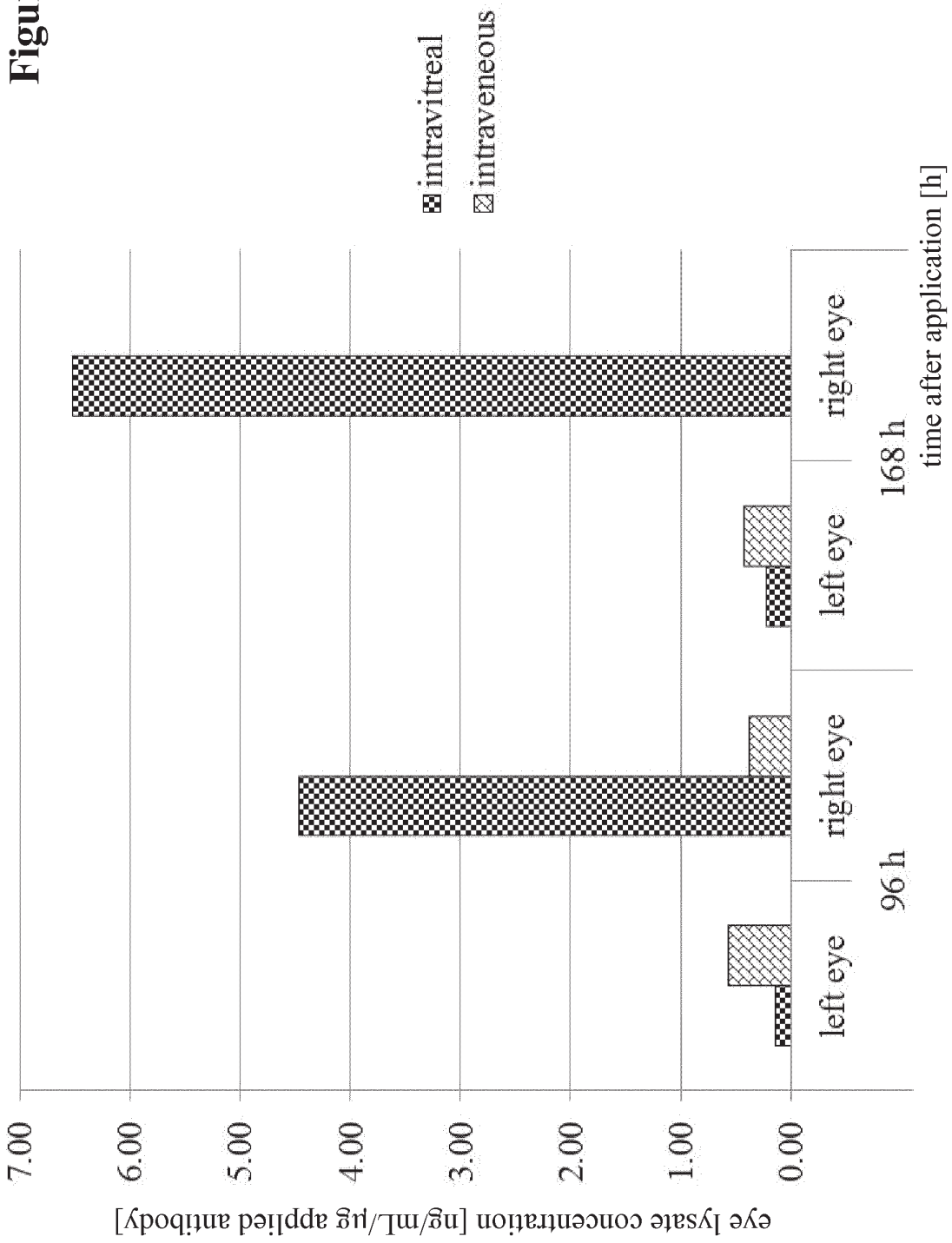

intravitreal
intraveneous
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
eye lysate concentration [ng/mL/µg applied antibody]
left eye
right eye
96 h
left eye
right eye
168 h
time after application [h]

Figure 19

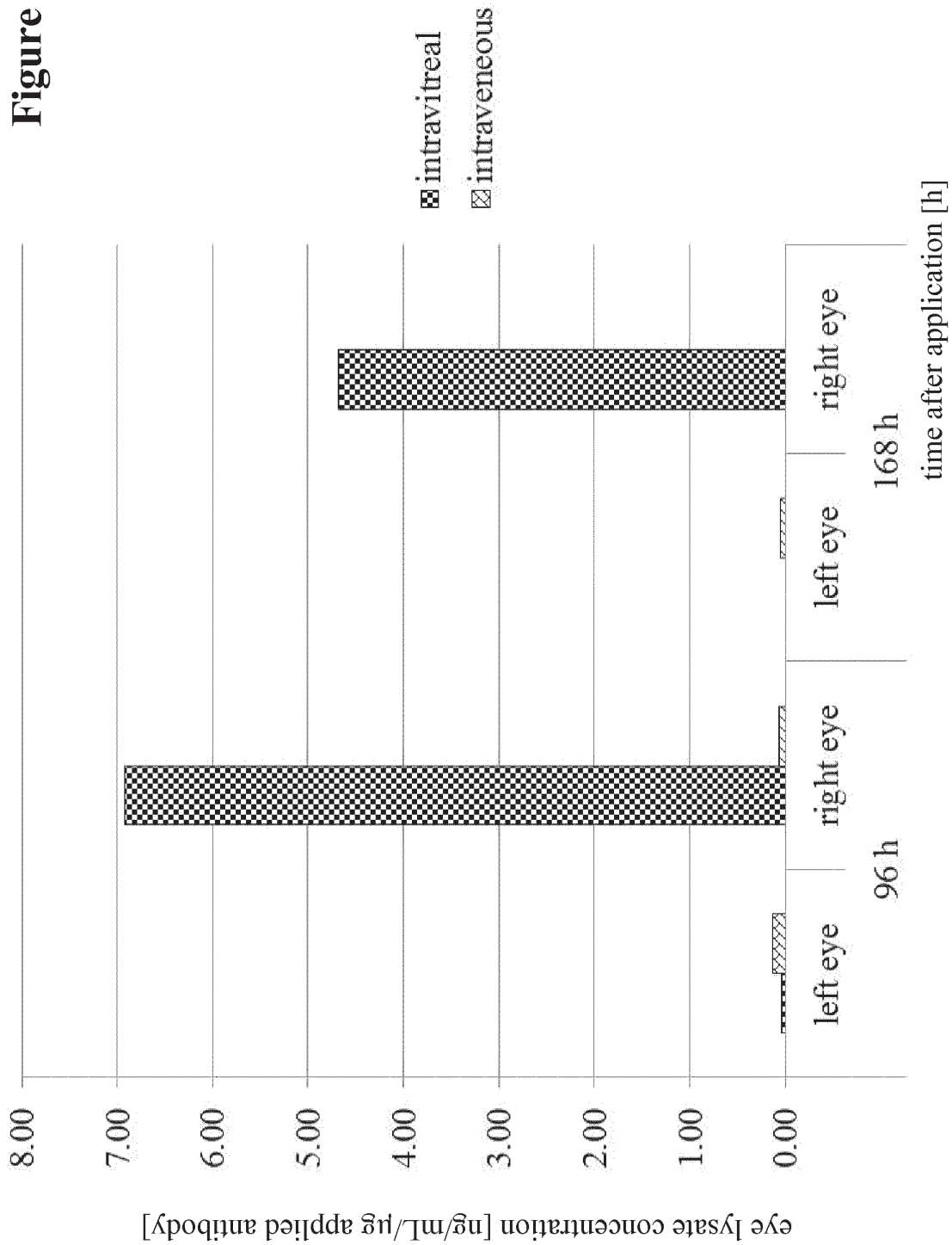
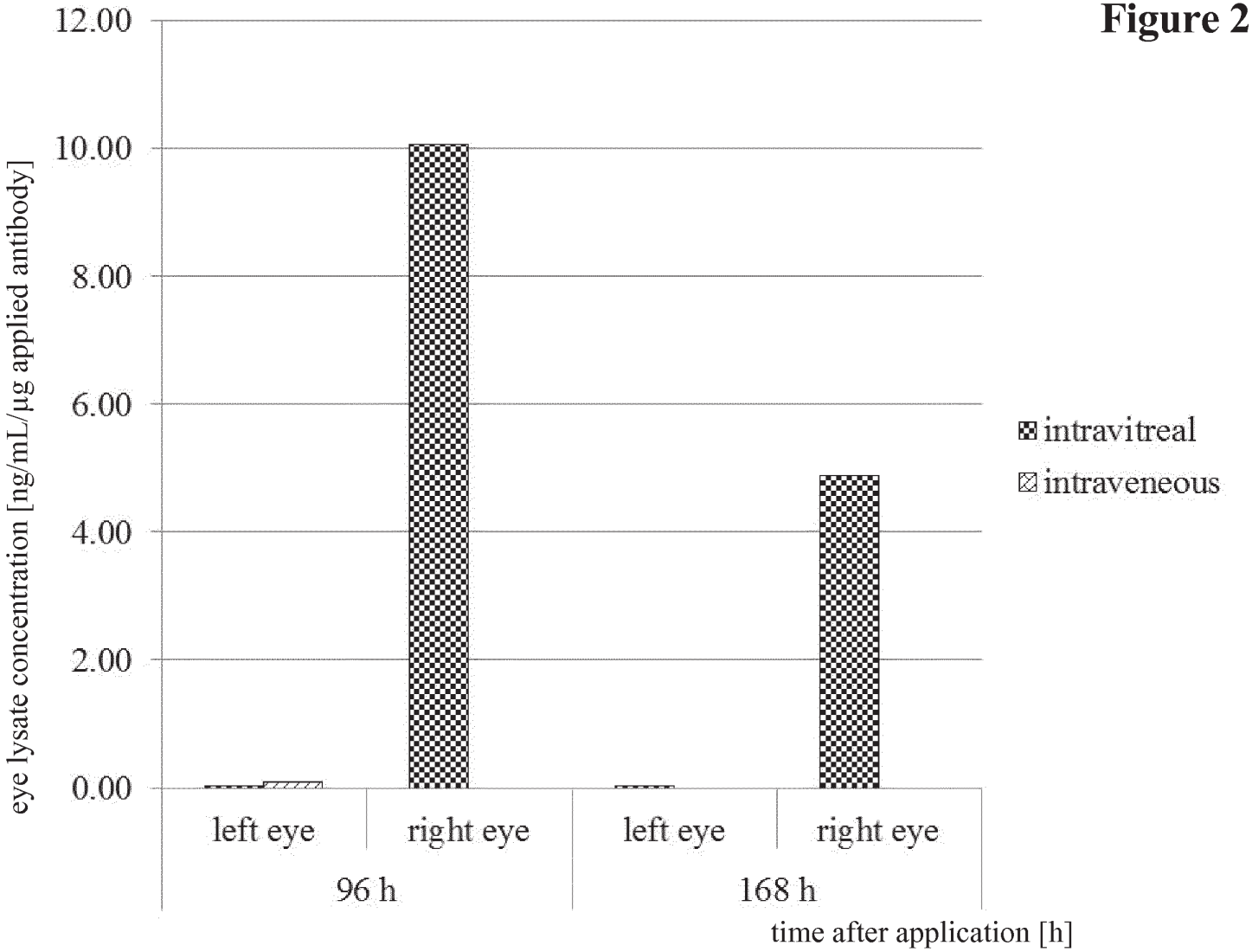

intravitreal
intraveneous
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
eye lysate concentration [ng/mL/µg applied antibody]
left eye
right eye
left eye
right eye
96 h
168 h
time after application [h]

Figure 20


12.00
10.00
8.00
6.00
4.00
2.00
0.00
eye lysate concentration [ng/mL/µg applied antibody]
left eye
right eye
left eye
right eye
96 h
168 h
time after application [h]
intravitreal
intraveneous