Figures

Figure 1:

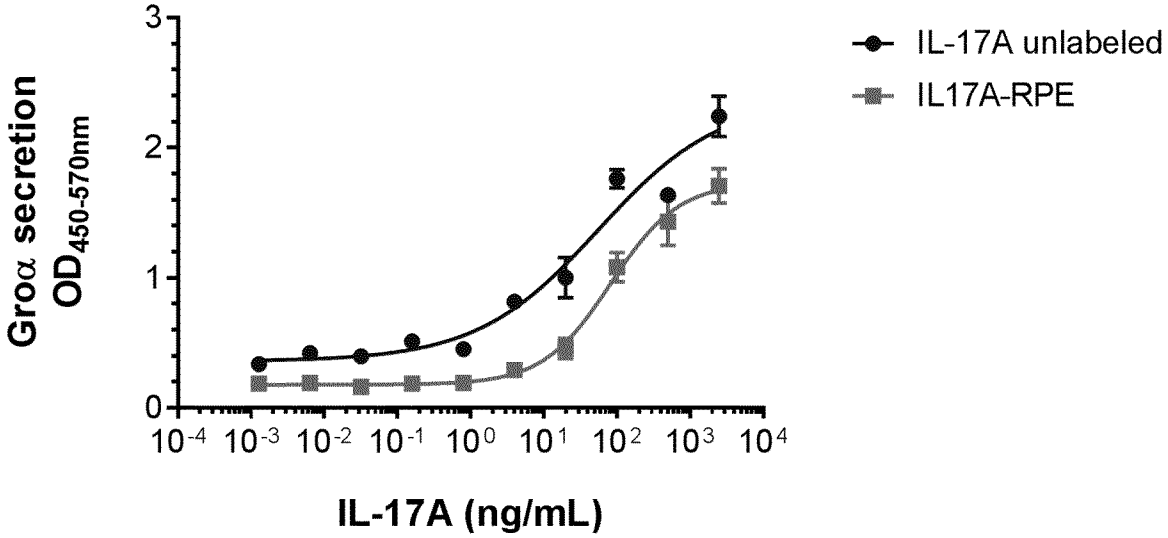
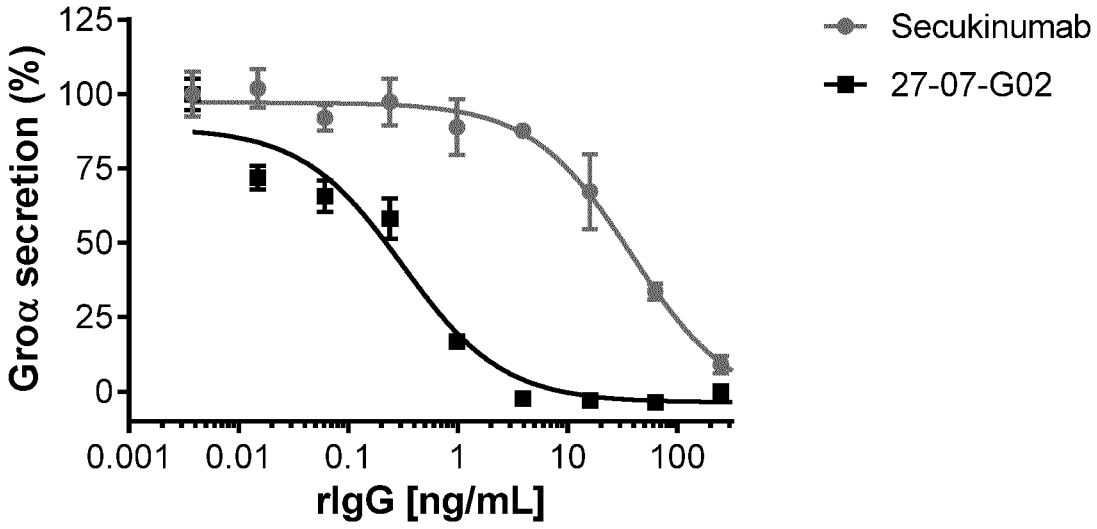


Figure 2:

(A)


Secukinumab
27-07-G02
Groα secretion (%)
125
100
75
50
25
0
0.001
0.01
0.1
1
10
100
rIgG [ng/mL]

(B)

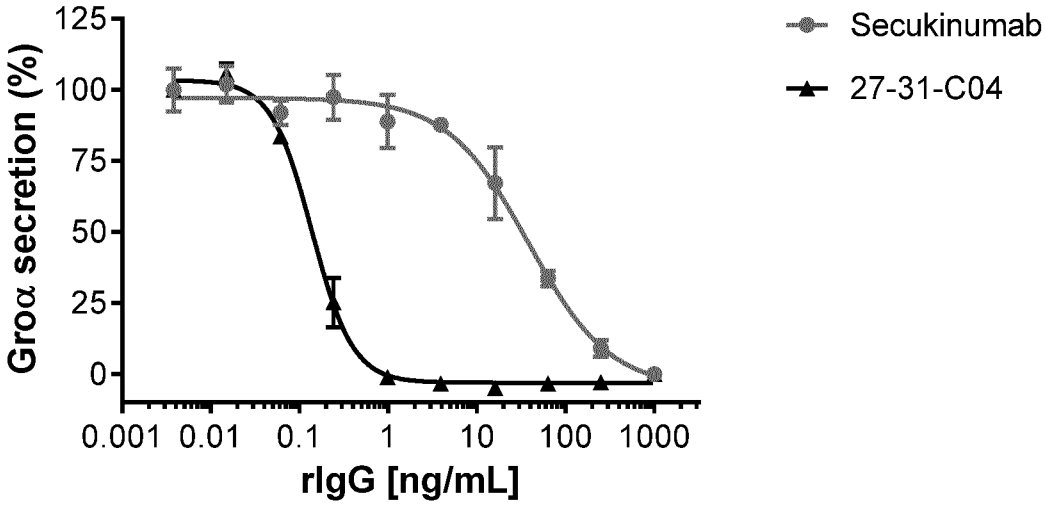
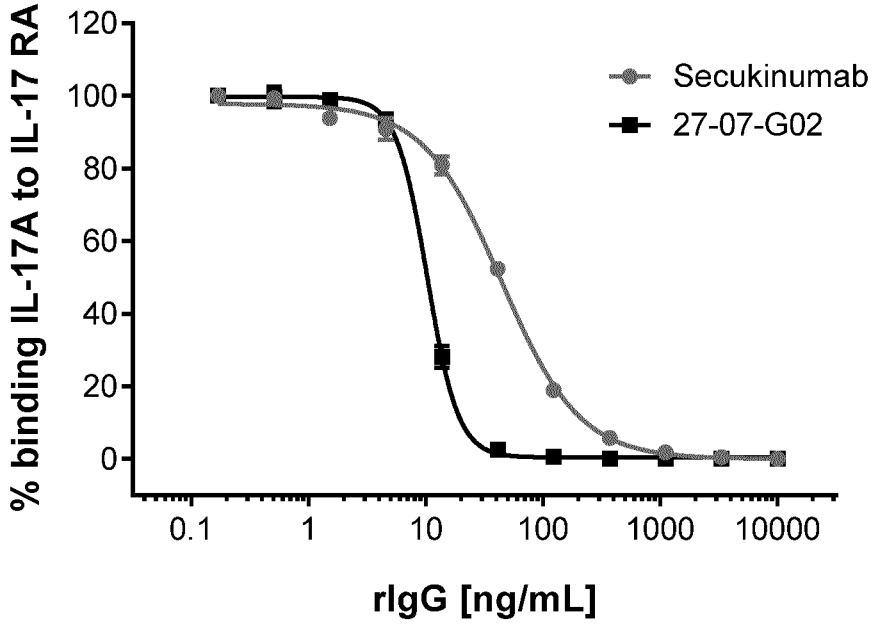

Secukinumab
27-31-C04
Groα secretion (%)
125
100
75
50
25
0
0.001
0.01
0.1
1
10
100
1000
rIgG [ng/mL]

Figure 3:

(A)


% binding IL-17A to IL-17 RA
120
100
80
60
40
20
0
0.1
1
10
100
1000
10000
rIgG [ng/mL]
Secukinumab
27-07-G02

(B)

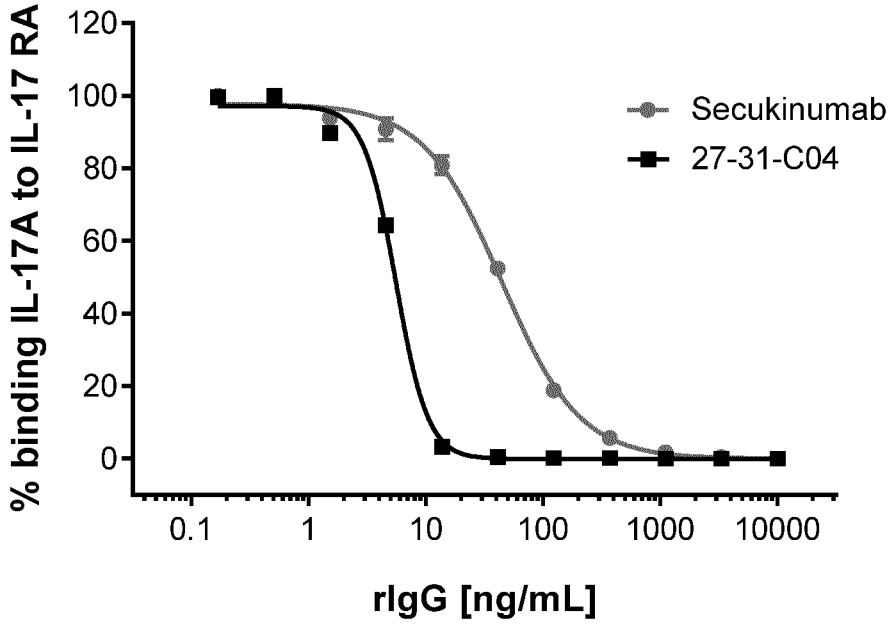
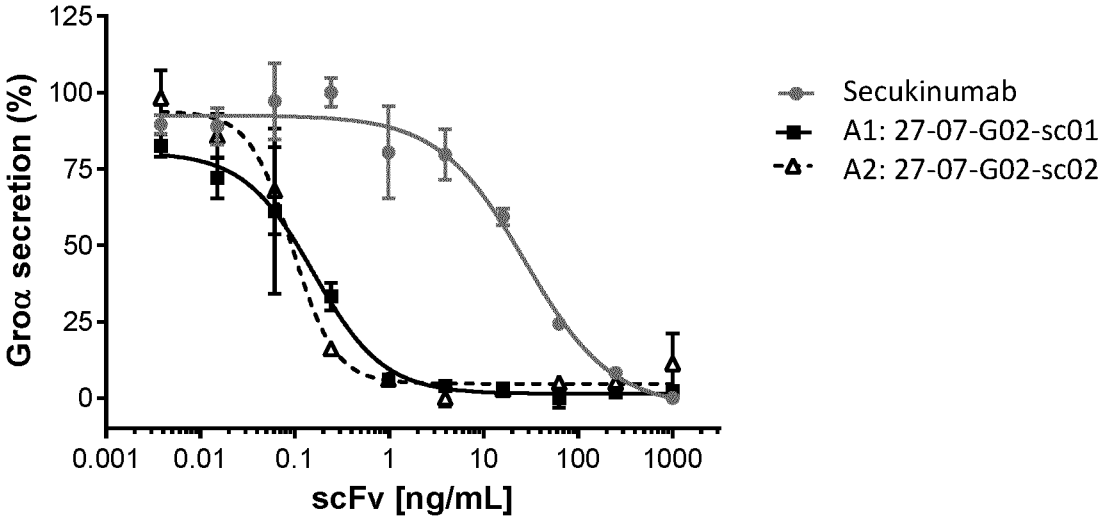

% binding IL-17A to IL-17 RA
120
100
80
60
40
20
0
0.1
1
10
100
1000
10000
rIgG [ng/mL]
Secukinumab
27-31-C04

Figure 4:

(A)



(B)

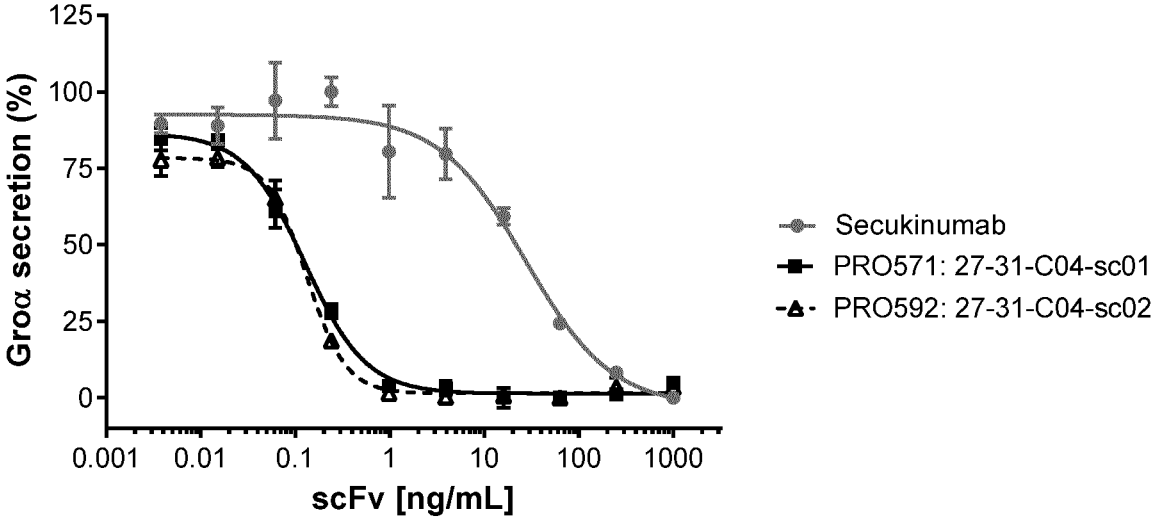
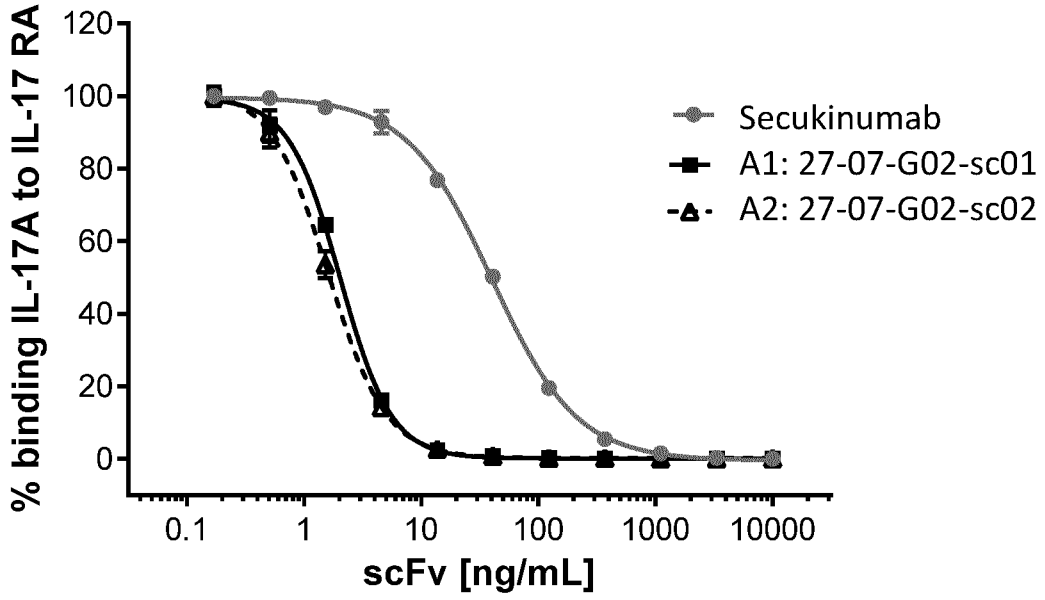


Figure 4

Figure 5:

(A)


% binding IL-17A to IL-17 RA
120
100
80
60
40
20
0
0.1
1
10
100
1000
10000
scFv [ng/mL]
Secukinumab
A1: 27-07-G02-sc01
A2: 27-07-G02-sc02

(B)

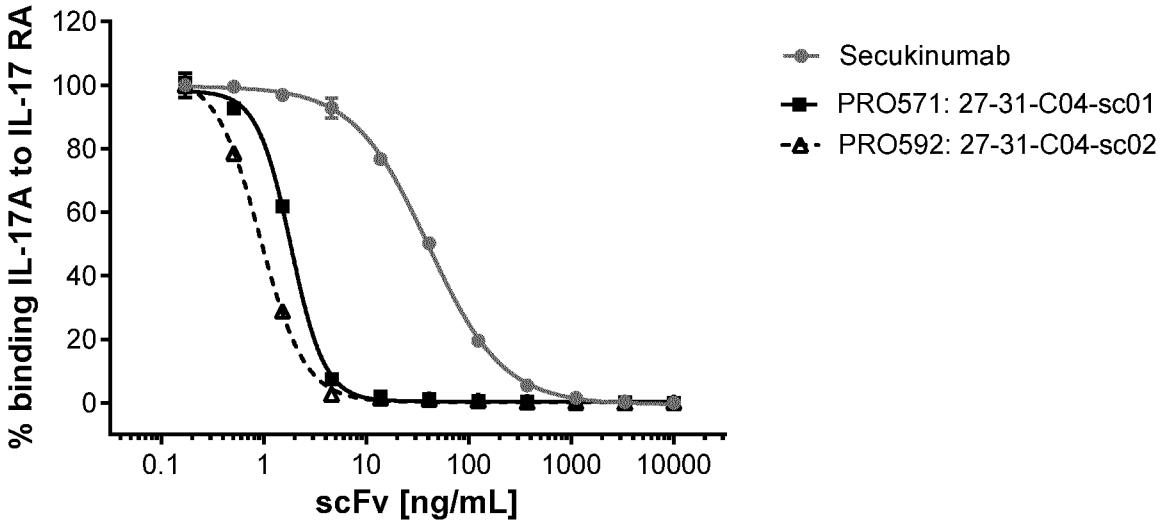
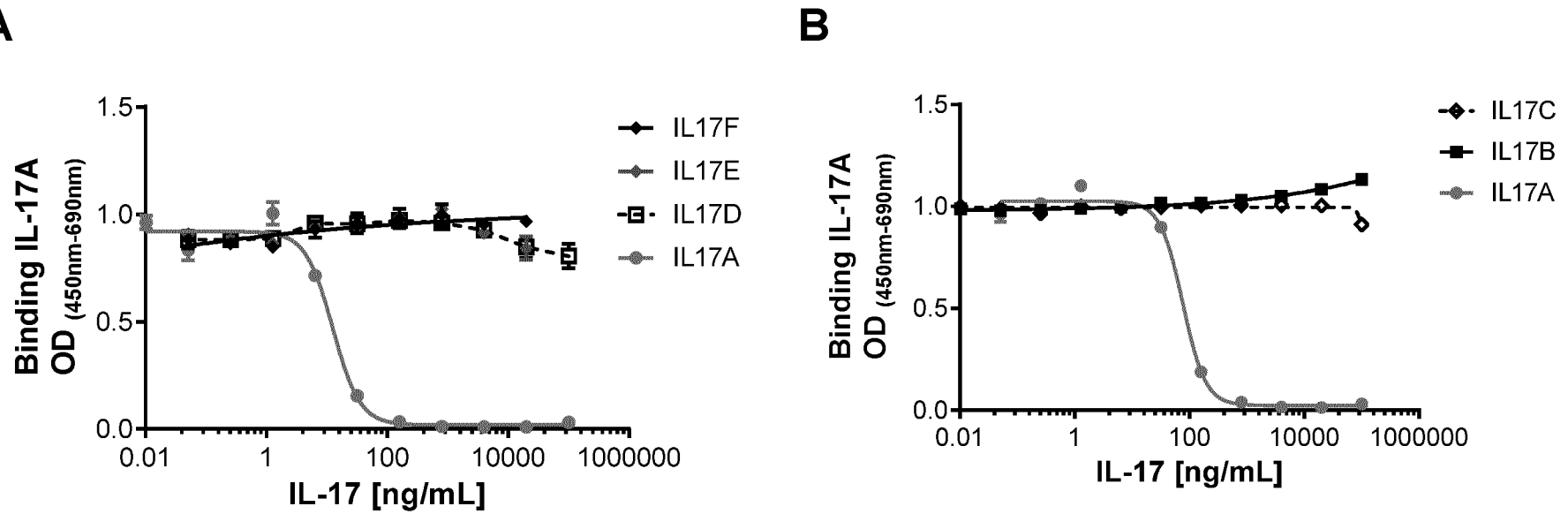

% binding IL-17A to IL-17 RA
120
100
80
60
40
20
0
0.1
1
10
100
1000
10000
scFv [ng/mL]
Secukinumab
PRO571: 27-31-C04-sc01
PRO592: 27-31-C04-sc02

Figure 6:

(I)


A
Binding IL-17A
OD (450nm-690nm)
1.5
1.0
0.5
0.0
0.01
1
100
10000
1000000
IL-17 [ng/mL]
IL17F
IL17E
IL17D
IL17A
B
Binding IL-17A
OD (450nm-690nm)
1.5
1.0
0.5
0.0
0.01
1
100
10000
1000000
IL-17 [ng/mL]
IL17C
IL17B
IL17A

(II)

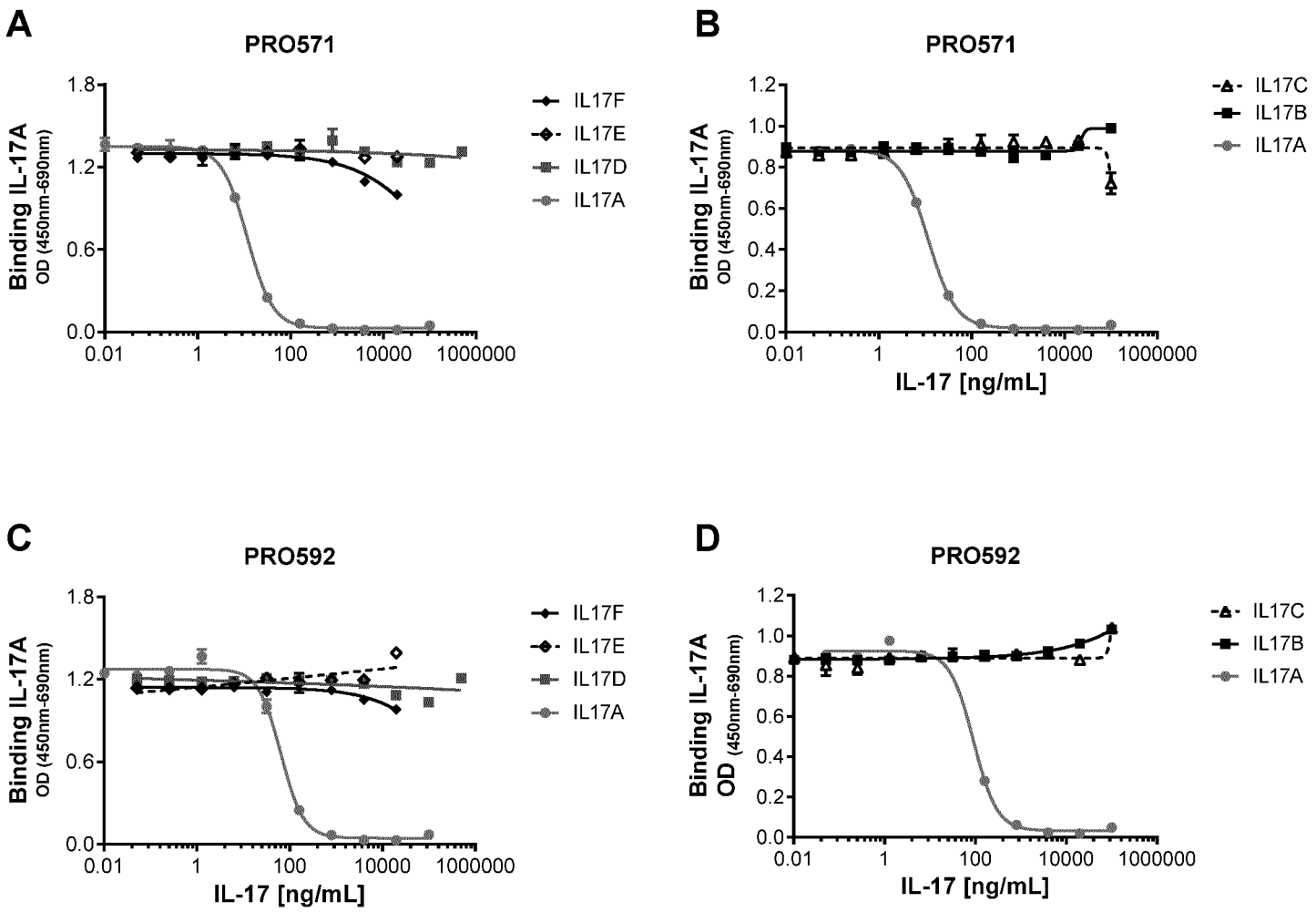
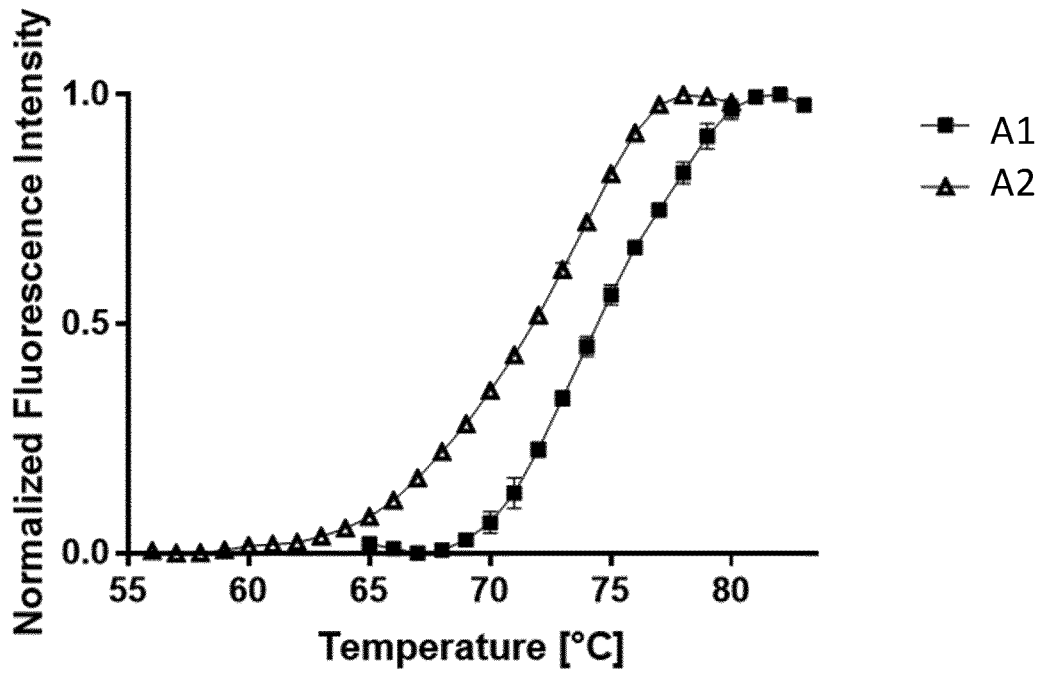

A
PRO571
Binding IL-17A OD (450nm-690nm)
1.8
1.2
0.6
0.0
0.01
1
100
10000
1000000
IL17F
IL17E
IL17D
IL17A
B
PRO571
Binding IL-17A OD (450nm-690nm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.01
1
100
10000
1000000
IL-17 [ng/mL]
IL17C
IL17B
IL17A
C
PRO592
Binding IL-17A OD (450nm-690nm)
1.8
1.2
0.6
0.0
0.01
1
100
10000
1000000
IL-17 [ng/mL]
IL17F
IL17E
IL17D
IL17A
D
PRO592
Binding IL-17A OD (450nm-690nm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.01
1
100
10000
1000000
IL-17 [ng/mL]
IL17C
IL17B
IL17A

Figure 7:

(A)


Normalized Fluorescence Intensity
1.0
0.5
0.0
55
60
65
70
75
80
Temperature [°C]
A1
A2

(B)

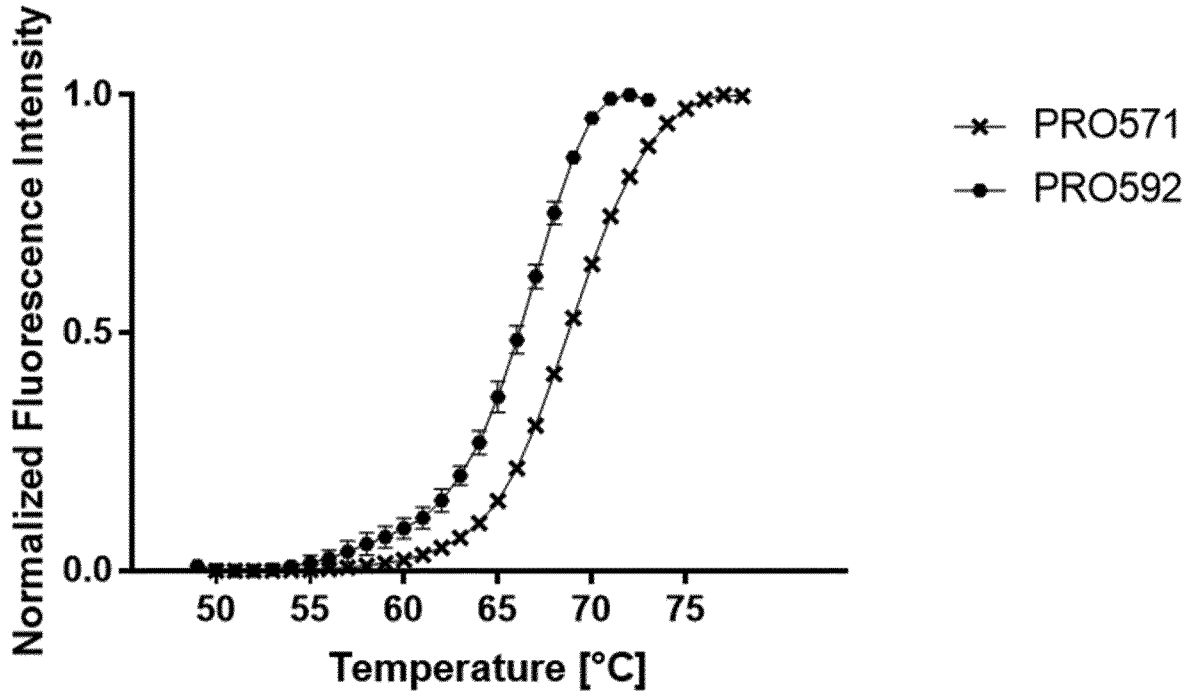
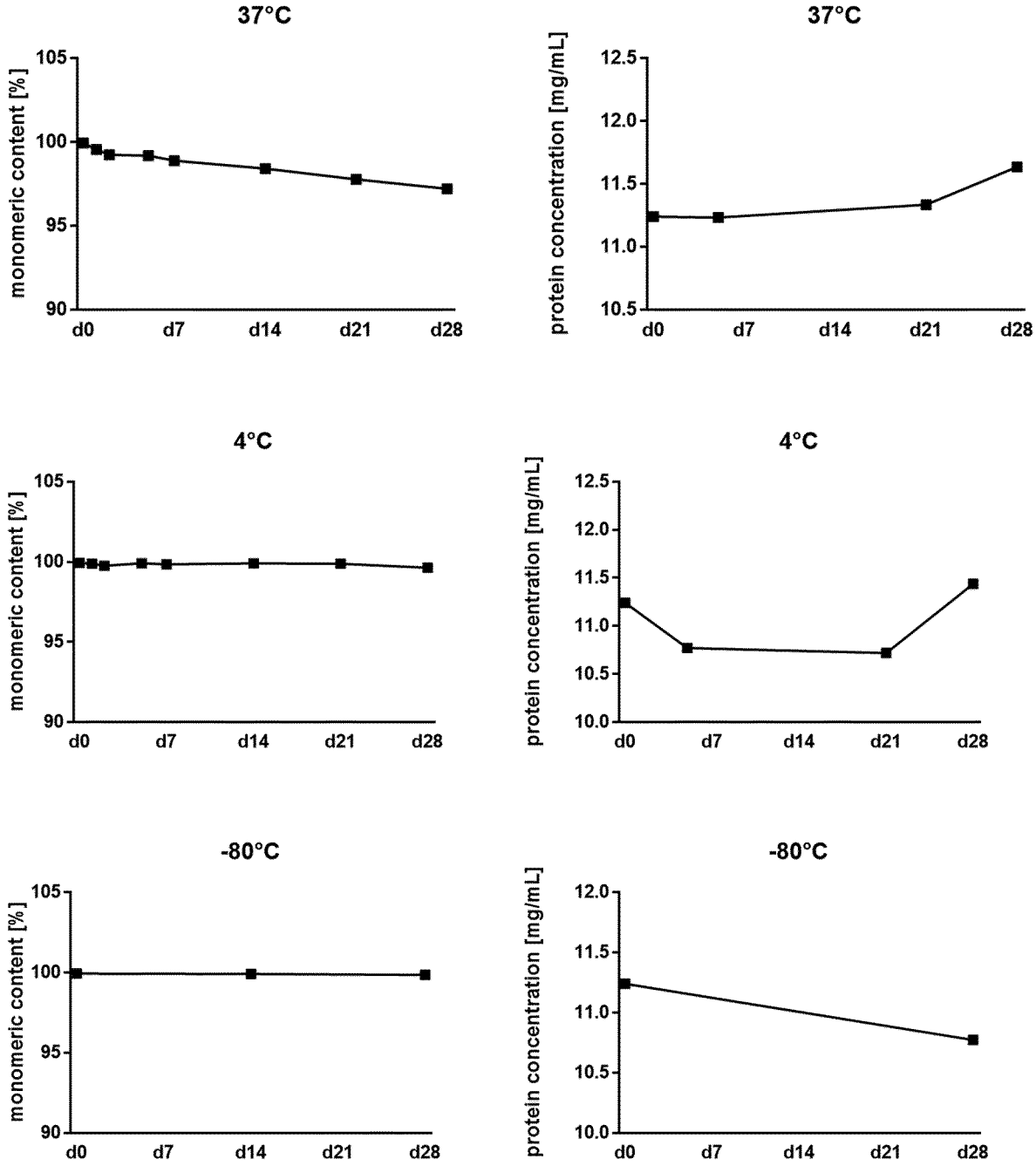

Normalized Fluorescence Intensity
1.0
0.5
0.0
50
55
60
65
70
75
Temperature [°C]
PRO571
PRO592

Figure 8:

(A)


37°C
monomeric content [%]
105
100
95
90
d0
d7
d14
d21
d28
37°C
protein concentration [mg/mL]
12.5
12.0
11.5
11.0
10.5
d0
d7
d14
d21
d28
4°C
monomeric content [%]
105
100
95
90
d0
d7
d14
d21
d28
4°C
protein concentration [mg/mL]
12.5
12.0
11.5
11.0
10.5
10.0
d0
d7
d14
d21
d28
-80°C
monomeric content [%]
105
100
95
90
d0
d7
d14
d21
d28
-80°C
protein concentration [mg/mL]
12.0
11.5
11.0
10.5
10.0
d0
d7
d14
d21
d28

(B)

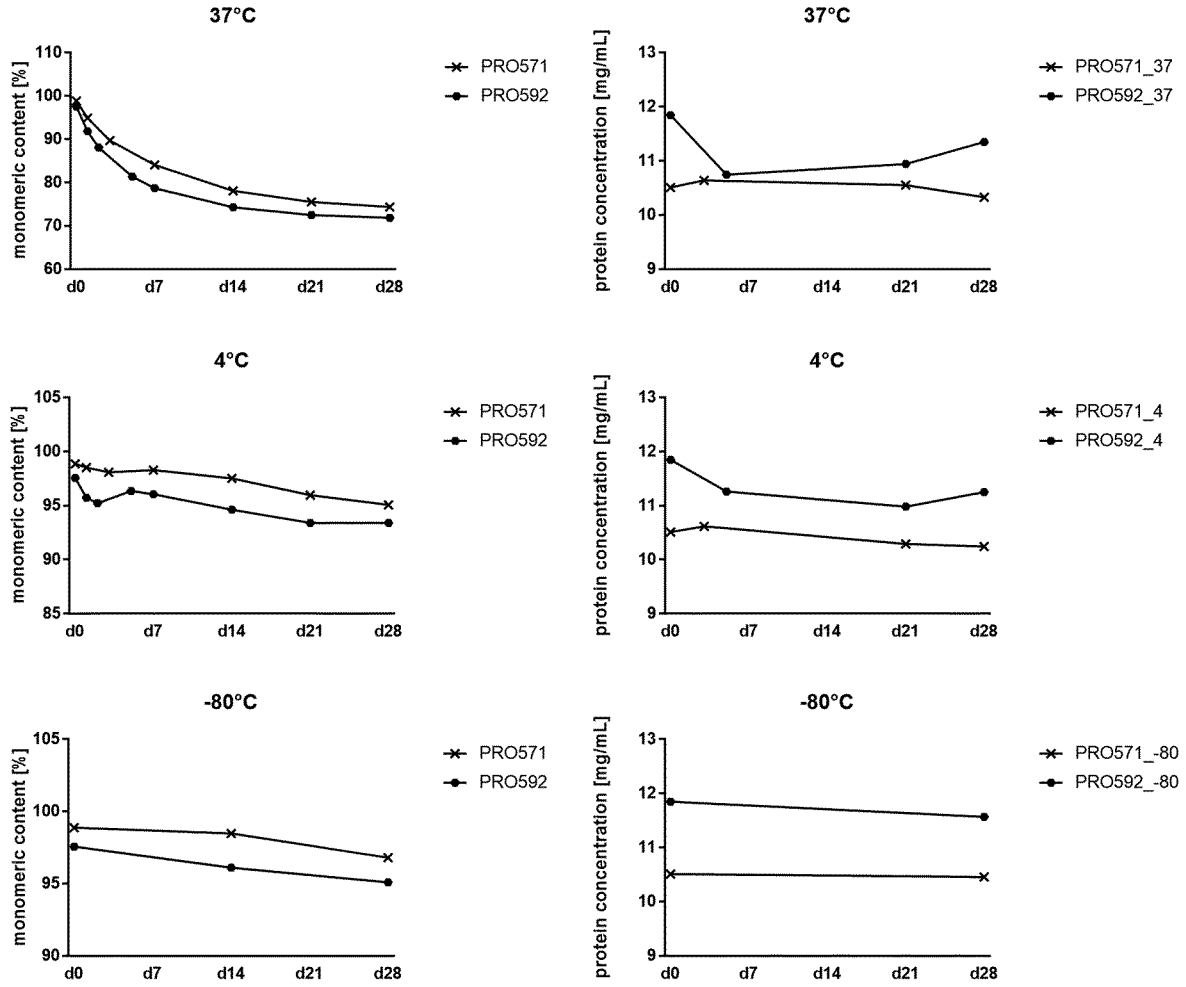
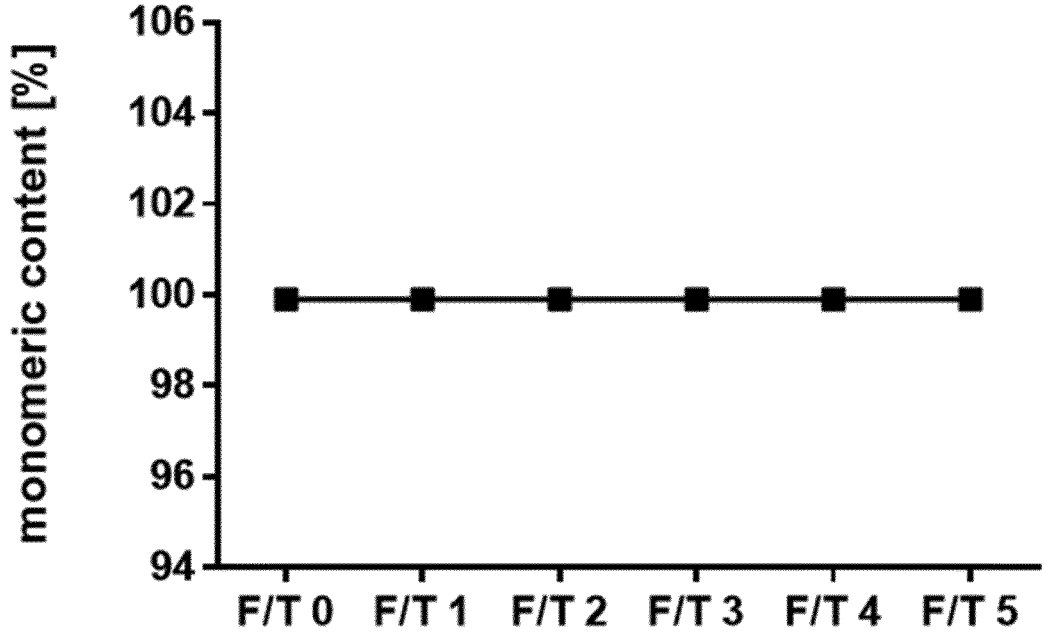

37°C
monomeric content [%]
110
100
90
80
70
60
d0
d7
d14
d21
d28
PRO571
PRO592
37°C
protein concentration [mg/mL]
13
12
11
10
9
PRO571_37
PRO592_37
4°C
monomeric content [%]
105
100
95
90
85
PRO571
PRO592
4°C
protein concentration [mg/mL]
PRO571_4
PRO592_4
-80°C
monomeric content [%]
PRO571
PRO592
-80°C
protein concentration [mg/mL]
PRO571_-80
PRO592_-80

Figure 9:

(A)


monomeric content [%]
106
104
102
100
98
96
94
F/T 0
F/T 1
F/T 2
F/T 3
F/T 4
F/T 5

(B)

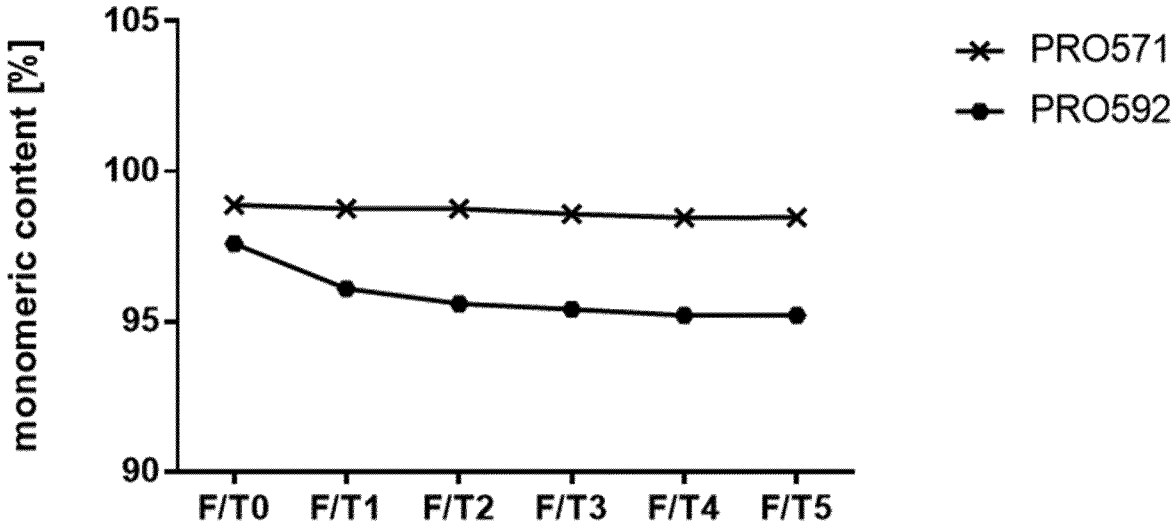

monomeric content [%]
105
100
95
90
F/T0
F/T1
F/T2
F/T3
F/T4
F/T5
PRO571
PRO592

Figure 10:

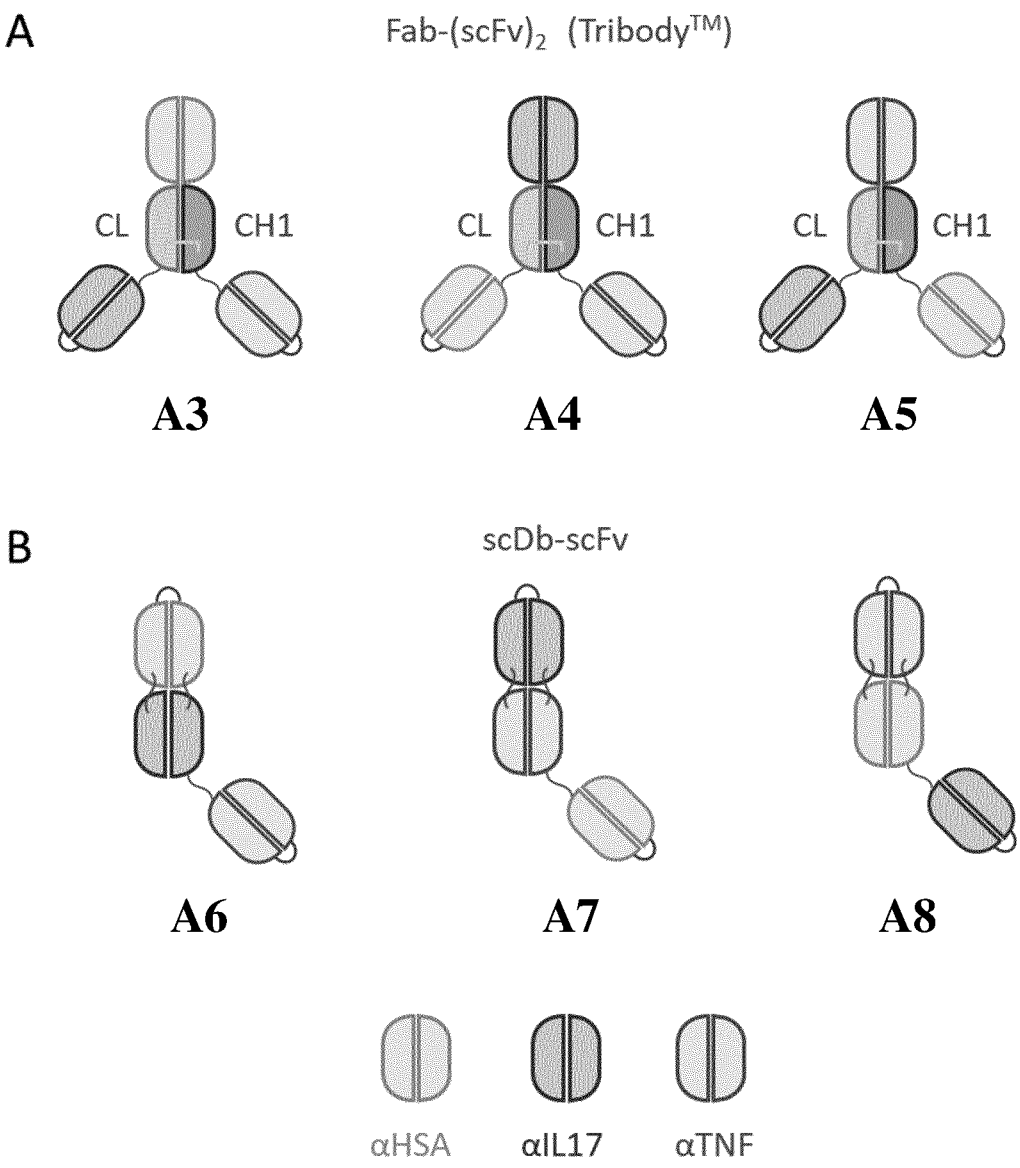
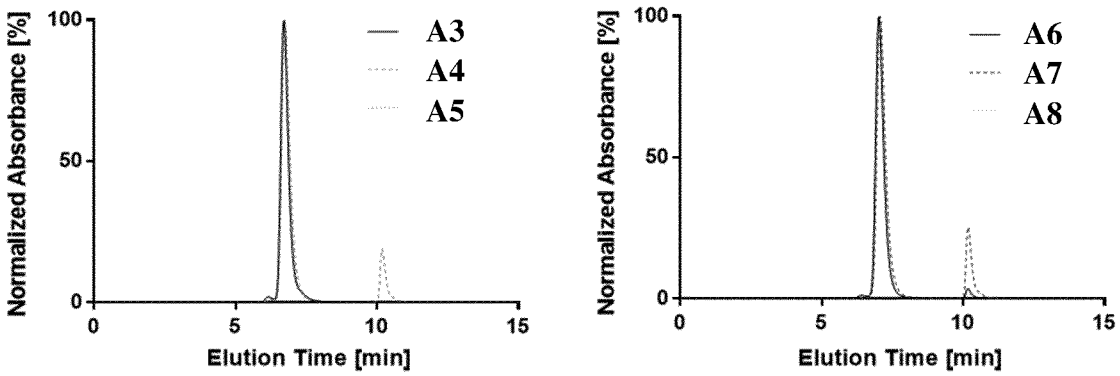

A
Fab-(scFv)2 (Tribody™)
CL
CH1
CL
CH1
CL
CH1
A3
A4
A5
B
scDb-scFv
A6
A7
A8
αHSA
αIL17
αTNF

Figure 11:

A


Normalized Absorbance [%]
100
50
0
0
5
10
15
Elution Time [min]
A3
A4
A5
Normalized Absorbance [%]
100
50
0
0
5
10
15
Elution Time [min]
A6
A7
A8

B

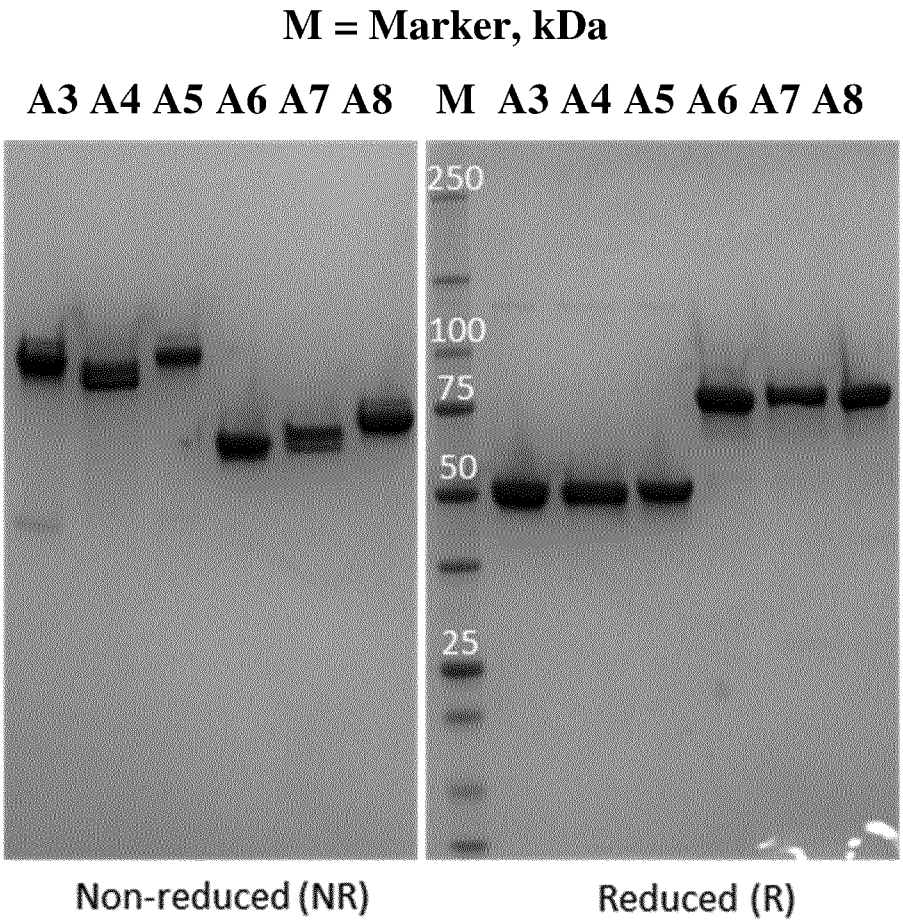

M = Marker, kDa
A3 A4 A5 A6 A7 A8
M
A3 A4 A5 A6 A7 A8
250
100
75
50
25
Non-reduced (NR)
Reduced (R)

Figure 12:

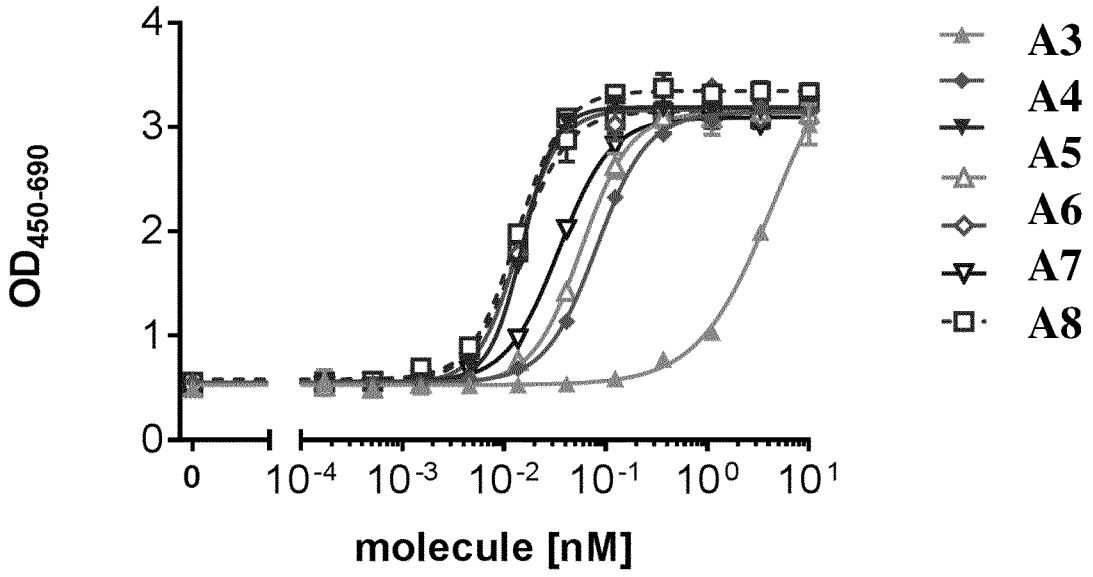

OD450-690
4
3
2
1
0
0
10-4
10-3
10-2
10-1
100
101
molecule [nM]
A3
A4
A5
A6
A7
A8

Figure 13:

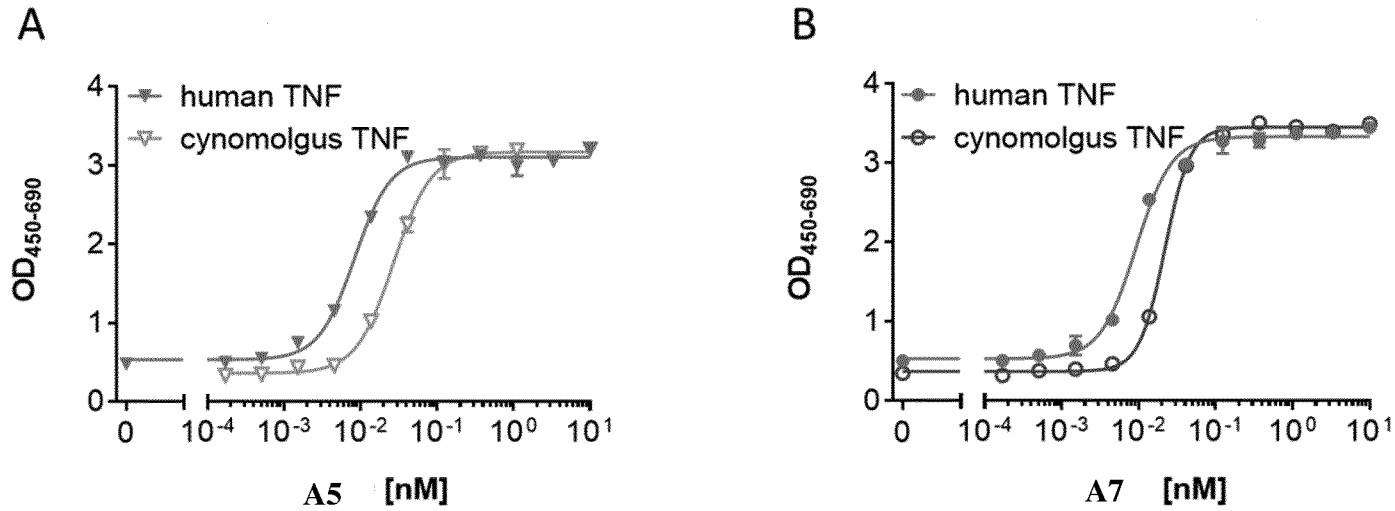
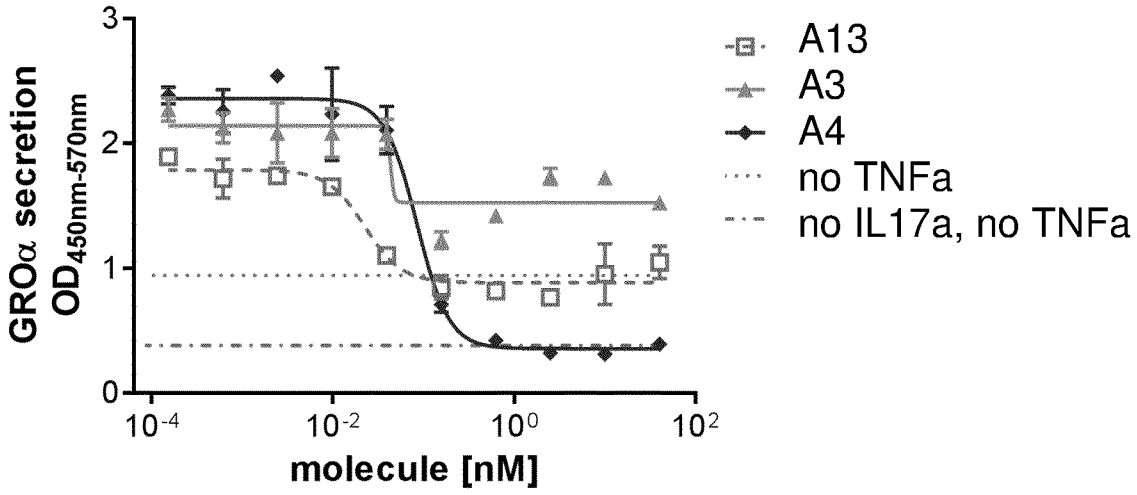

A
human TNF
cynomolgus TNF
$OD_{450-690}$
0
1
2
3
4
0
10^{-4}
10^{-3}
10^{-2}
10^{-1}
10^{0}
10^{1}
A5 [nM]
B
human TNF
cynomolgus TNF
$OD_{450-690}$
A7 [nM]

Figure 14:

A


GROα secretion
OD450nm-570nm
molecule [nM]
A13
A3
A4
no TNFa
no IL17a, no TNFa

B

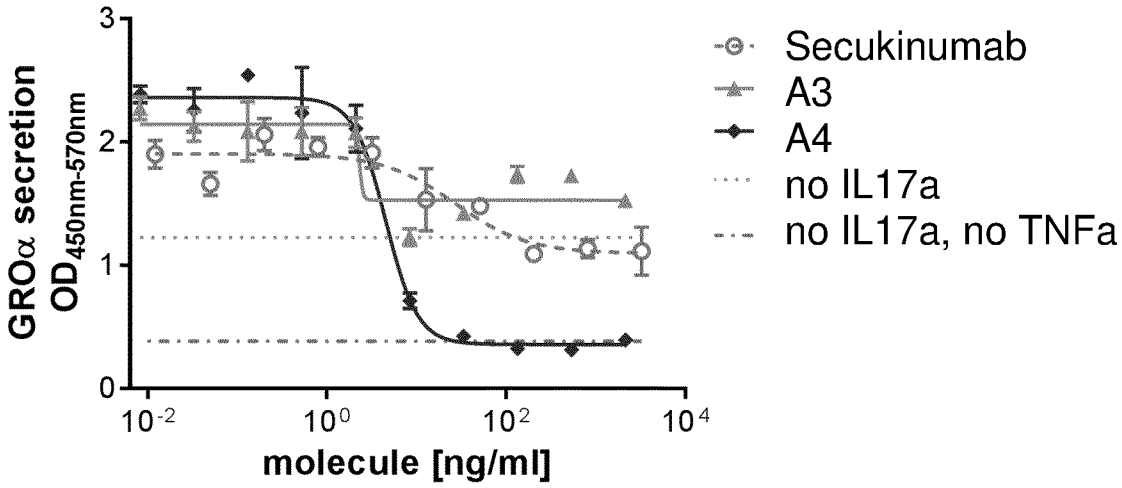

GROα secretion
OD450nm-570nm
molecule [ng/ml]
Secukinumab
A3
A4
no IL17a
no IL17a, no TNFa

Figure 14 (contd.):

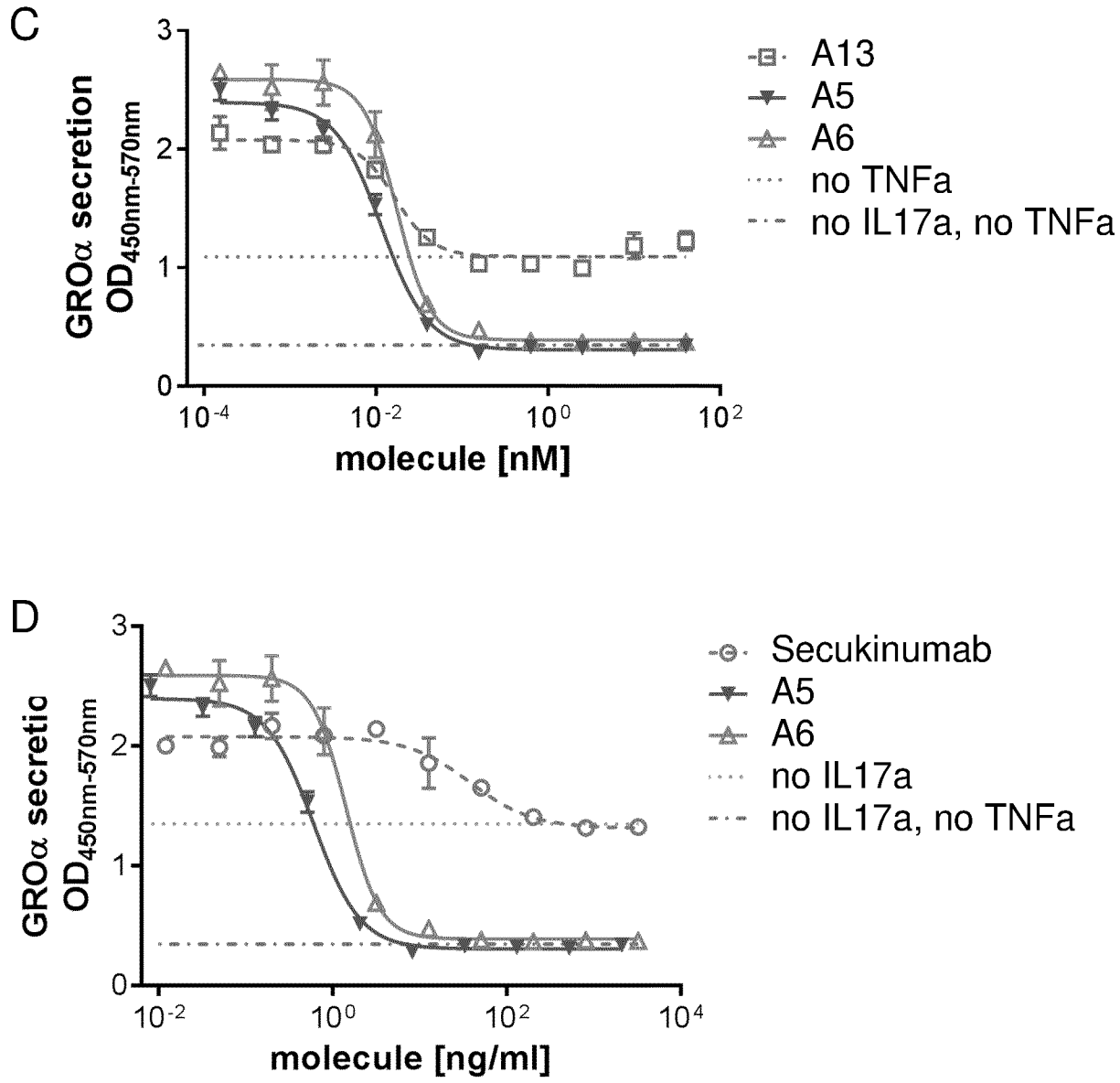
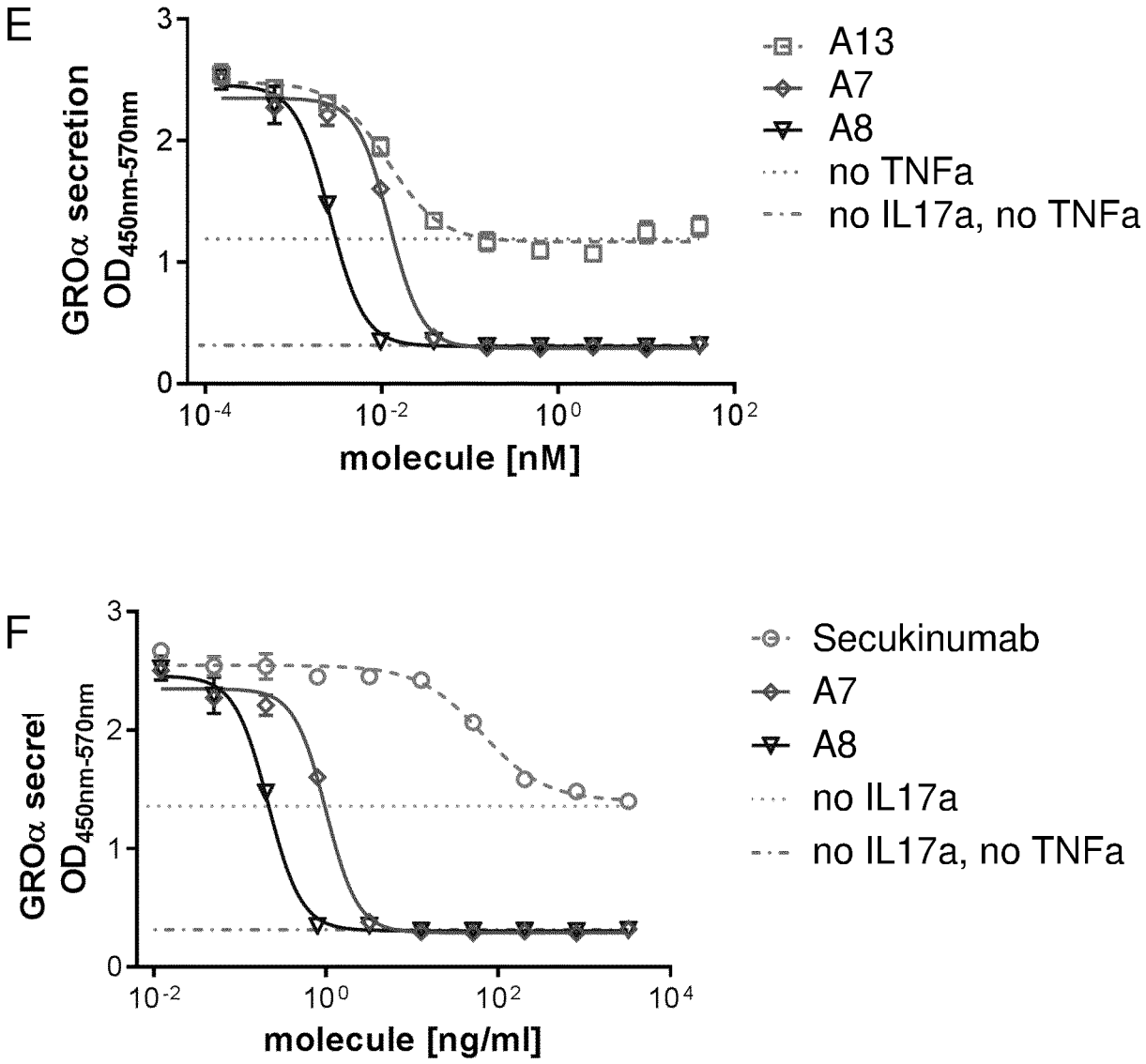


Figure 14 (contd.):



SUBSTITUTE SHEET (RULE 26)

Figure 15:

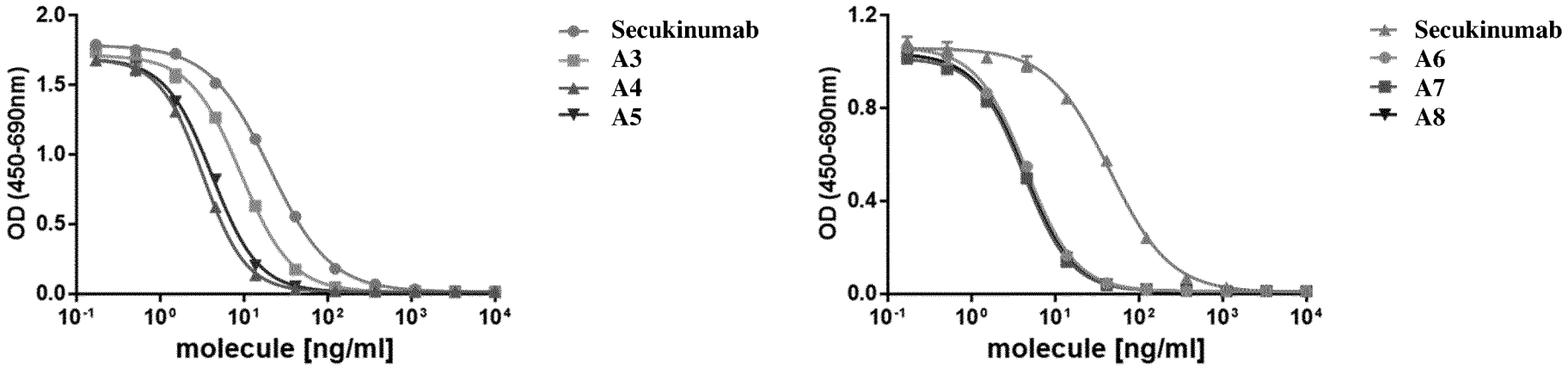

OD (450-690nm)
molecule [ng/ml]
Secukinumab
A3
A4
A5
Secukinumab
A6
A7
A8

Figure 16:

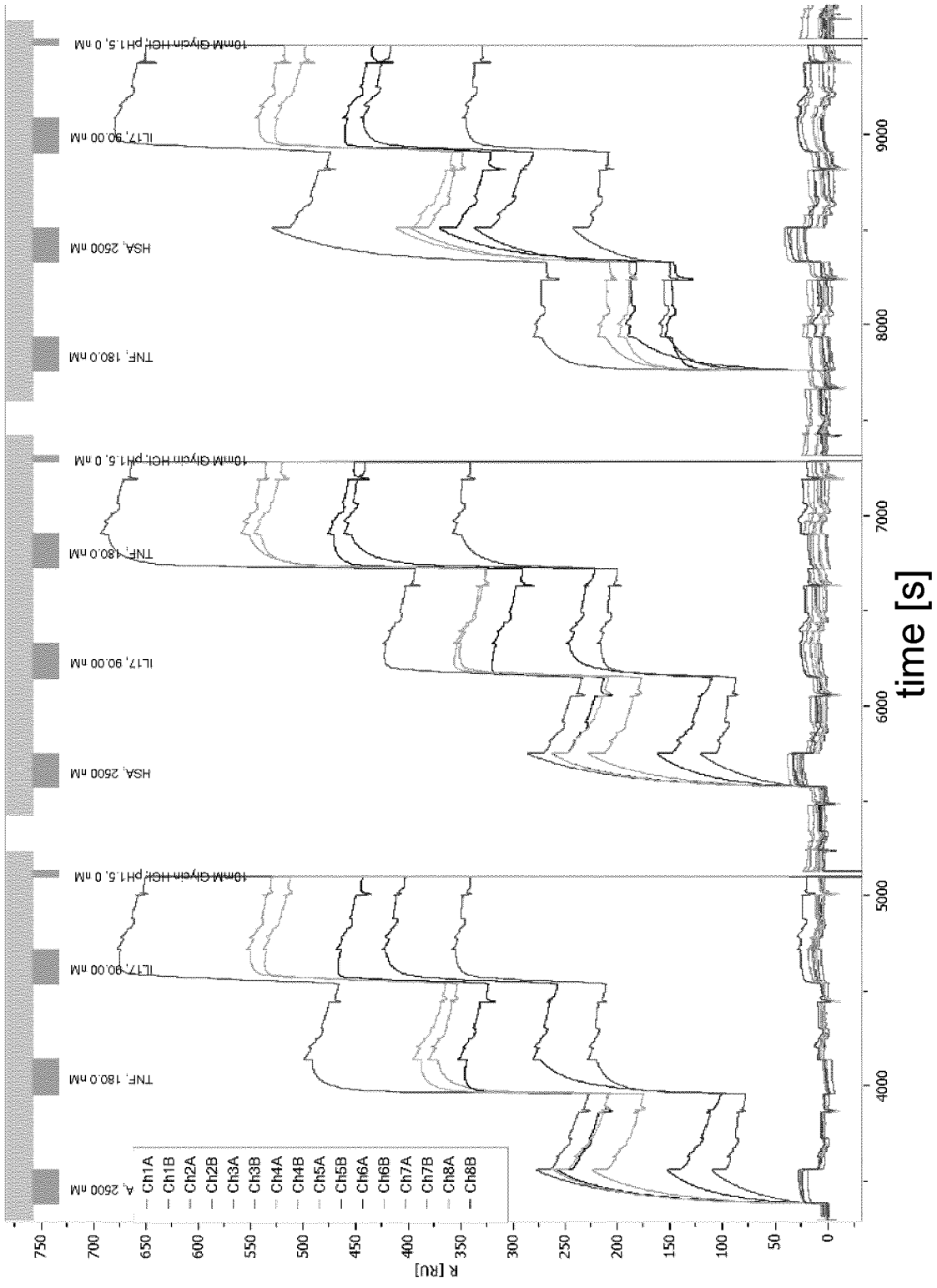

R [RU]
time [s]
Ch1A
Ch1B
Ch2A
Ch2B
Ch3A
Ch3B
Ch4A
Ch4B
Ch5A
Ch5B
Ch6A
Ch6B
Ch7A
Ch7B
Ch8A
Ch8B
A, 2500 nM
TNF, 180.0 nM
IL17, 90.00 nM
10mM Glycin-HCl; pH1.5, 0 nM
HSA, 2500 nM
TNF, 180.0 nM
IL17, 90.00 nM
10mM Glycin-HCl; pH1.5, 0 nM
HSA, 2500 nM
TNF, 180.0 nM
IL17, 90.00 nM
10mM Glycin-HCl; pH1.5, 0 nM

Figure 16 (contd.):

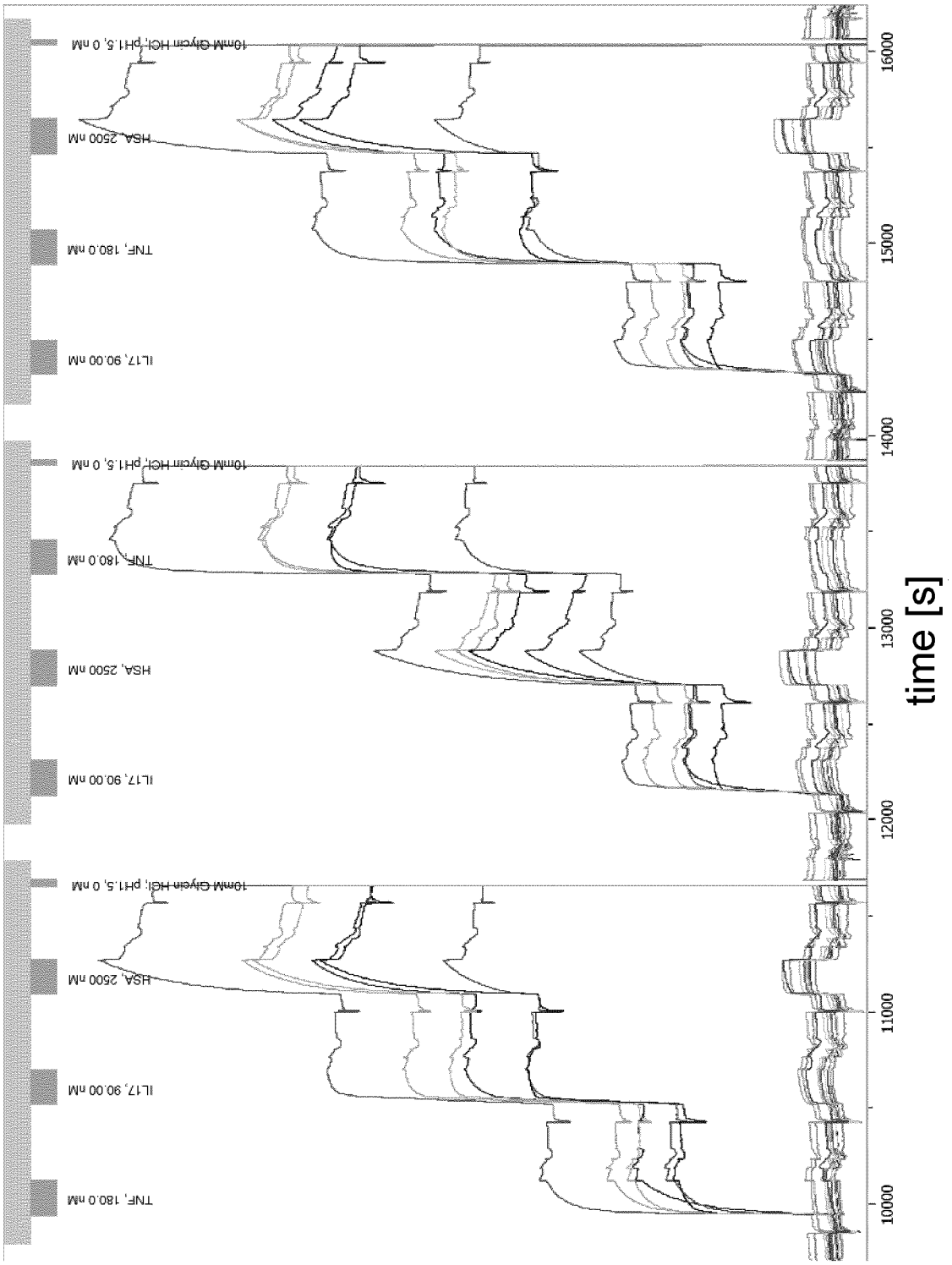


Figure 17:

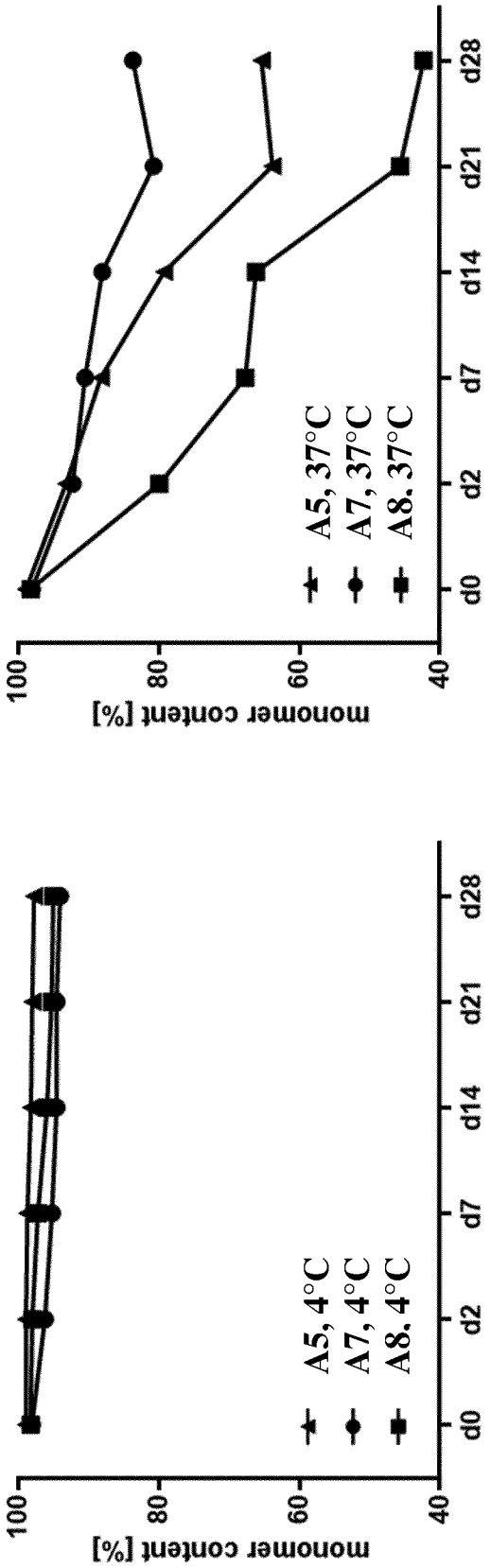

monomer content [%]
100
80
60
40
d0
d2
d7
d14
d21
d28
A5, 4°C
A7, 4°C
A8. 4°C
monomer content [%]
100
80
60
40
d0
d2
d7
d14
d21
d28
A5, 37°C
A7, 37°C
A8. 37°C

Figure 18:

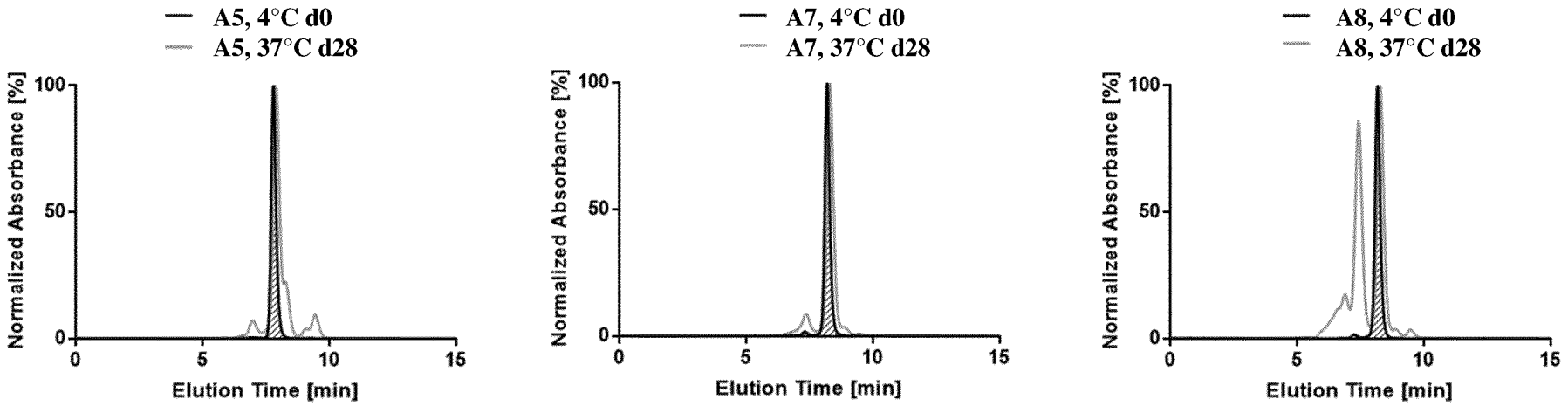

A5, 4°C d0
A5, 37°C d28
Normalized Absorbance [%]
100
50
0
0
5
10
15
Elution Time [min]
A7, 4°C d0
A7, 37°C d28
Normalized Absorbance [%]
100
50
0
0
5
10
15
Elution Time [min]
A8, 4°C d0
A8, 37°C d28
Normalized Absorbance [%]
100
50
0
0
5
10
15
Elution Time [min]

Figure 19:

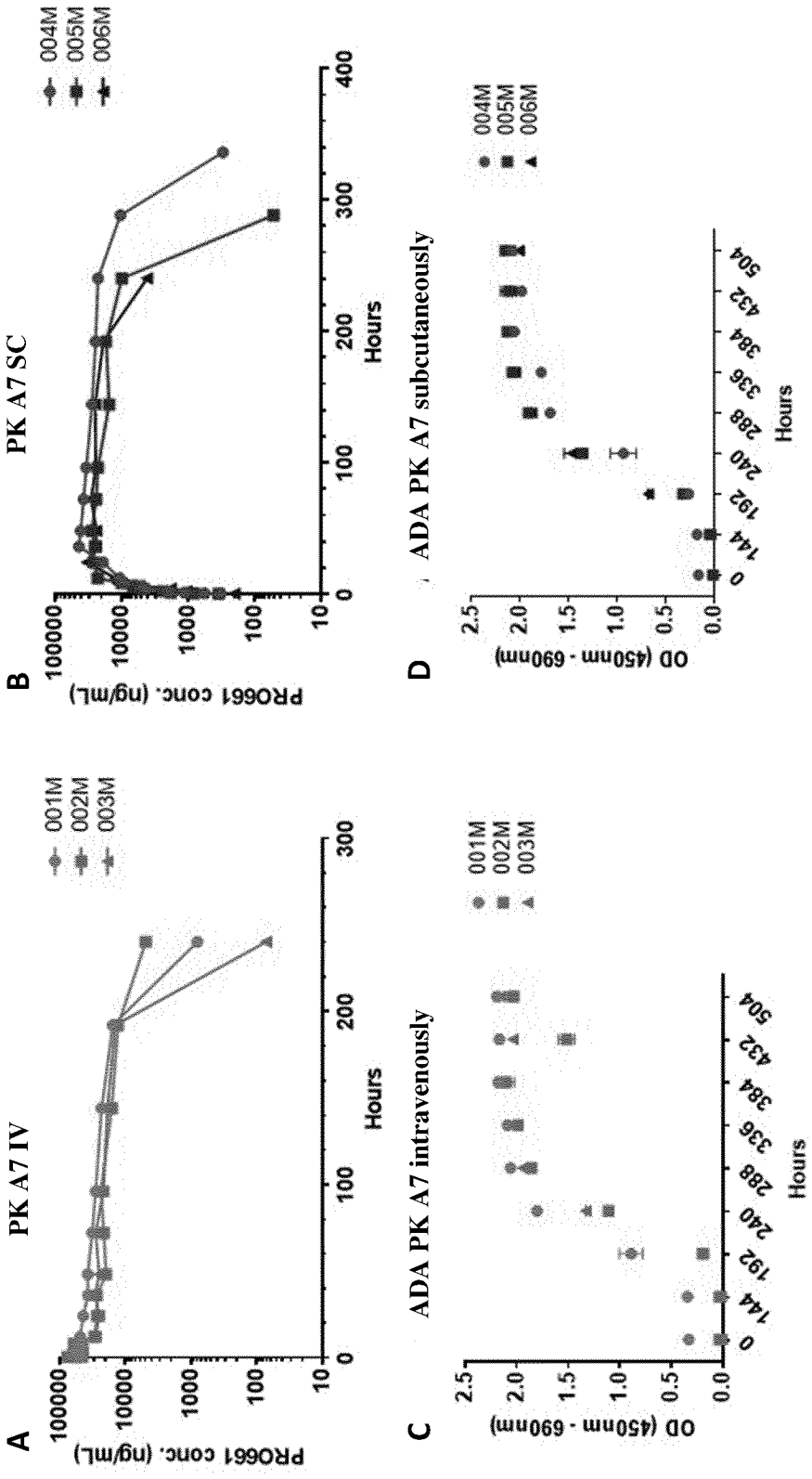

A
PK A7 IV
001M
002M
003M
PRO661 conc. (ng/mL)
Hours
B
PK A7 SC
004M
005M
006M
PRO661 conc. (ng/mL)
Hours
C
ADA PK A7 intravenously
001M
002M
003M
OD (450nm - 690nm)
Hours
D
ADA PK A7 subcutaneously
004M
005M
006M
OD (450nm - 690nm)
Hours

Figure 20:

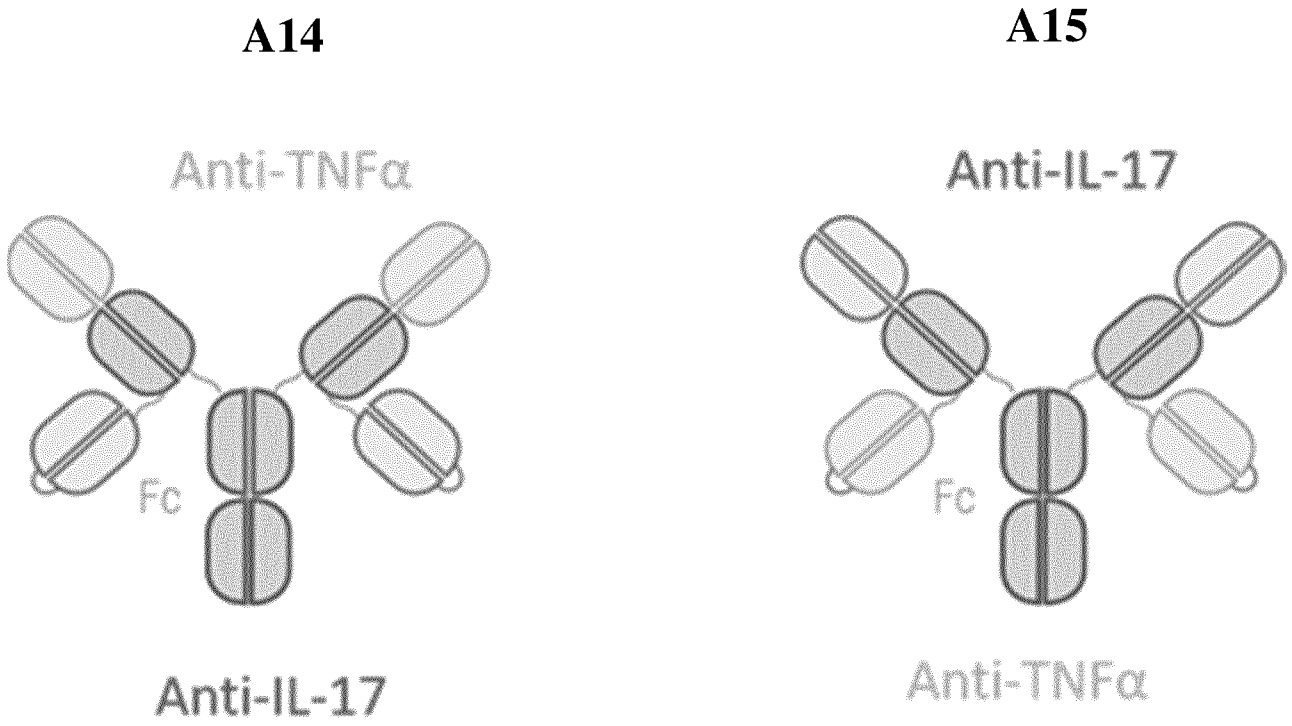


Figure 21:

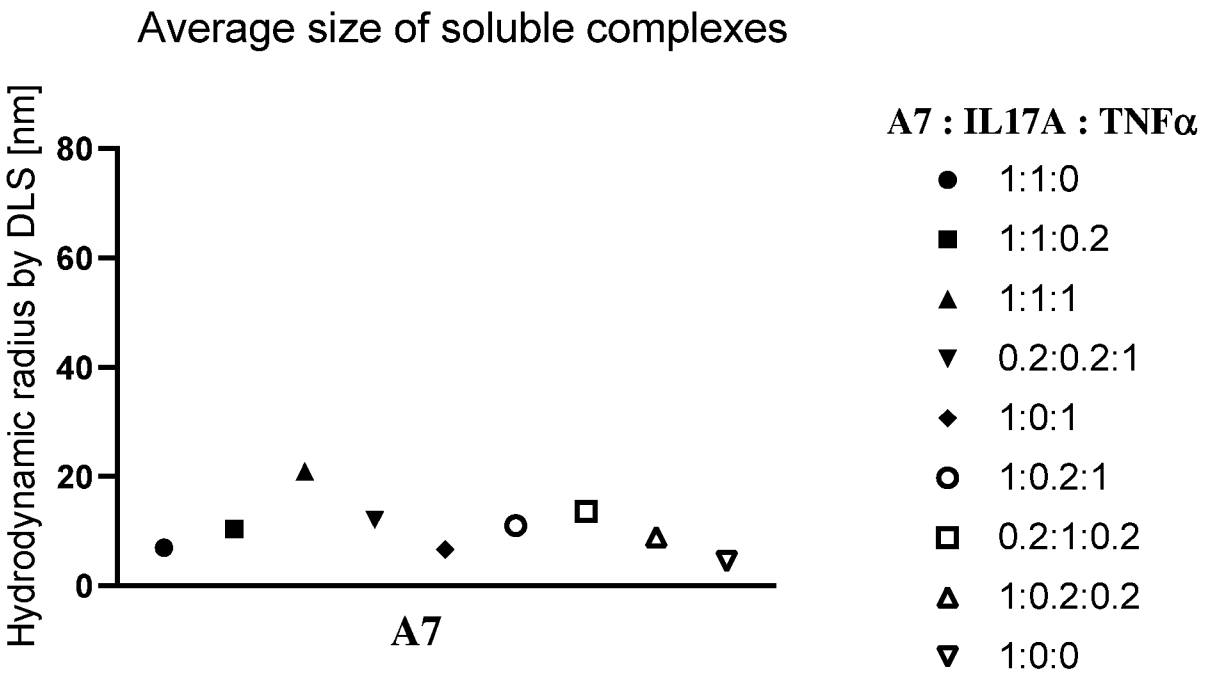

Average size of soluble complexes
Hydrodynamic radius by DLS [nm]
80
60
40
20
0
A7
A7 : IL17A : TNFα
1:1:0
1:1:0.2
1:1:1
0.2:0.2:1
1:0:1
1:0.2:1
0.2:1:0.2
1:0.2:0.2
1:0:0

Figure 22:


Average size of soluble complexes
Hydrodynamic radius by DLS [nm]
80
60
40
20
0
A14
A14 : IL17A : TNFα
1:1:0
1:1:0.2
1:1:1
0.2:0.2:1
1:0:1
1:0.2:1
0.2:1:0.2
1:0.2:0.2
1:0:0

Figure 23:

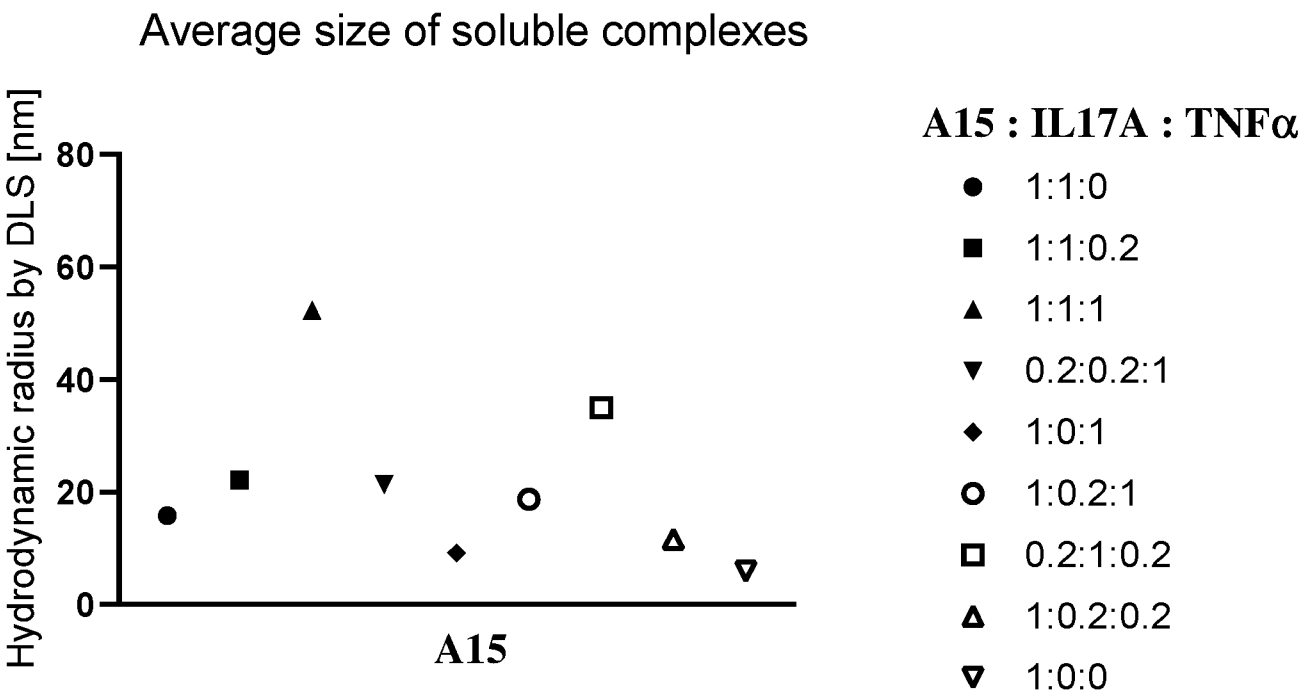

Average size of soluble complexes
Hydrodynamic radius by DLS [nm]
80
60
40
20
0
A15
A15 : IL17A : TNFα
1:1:0
1:1:0.2
1:1:1
0.2:0.2:1
1:0:1
1:0.2:1
0.2:1:0.2
1:0.2:0.2
1:0:0

Figure 24:

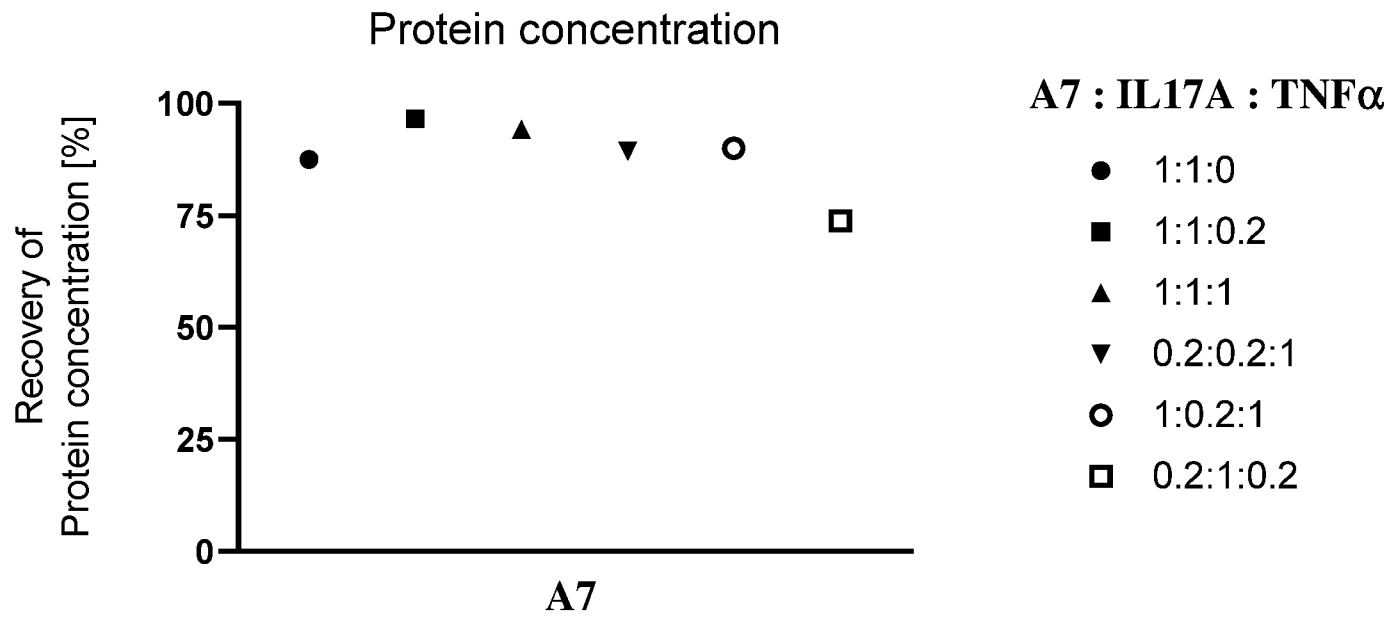

Protein concentration
Recovery of Protein concentration [%]
100
75
50
25
0
A7
A7 : IL17A : TNFα
1:1:0
1:1:0.2
1:1:1
0.2:0.2:1
1:0.2:1
0.2:1:0.2

Figure 25:

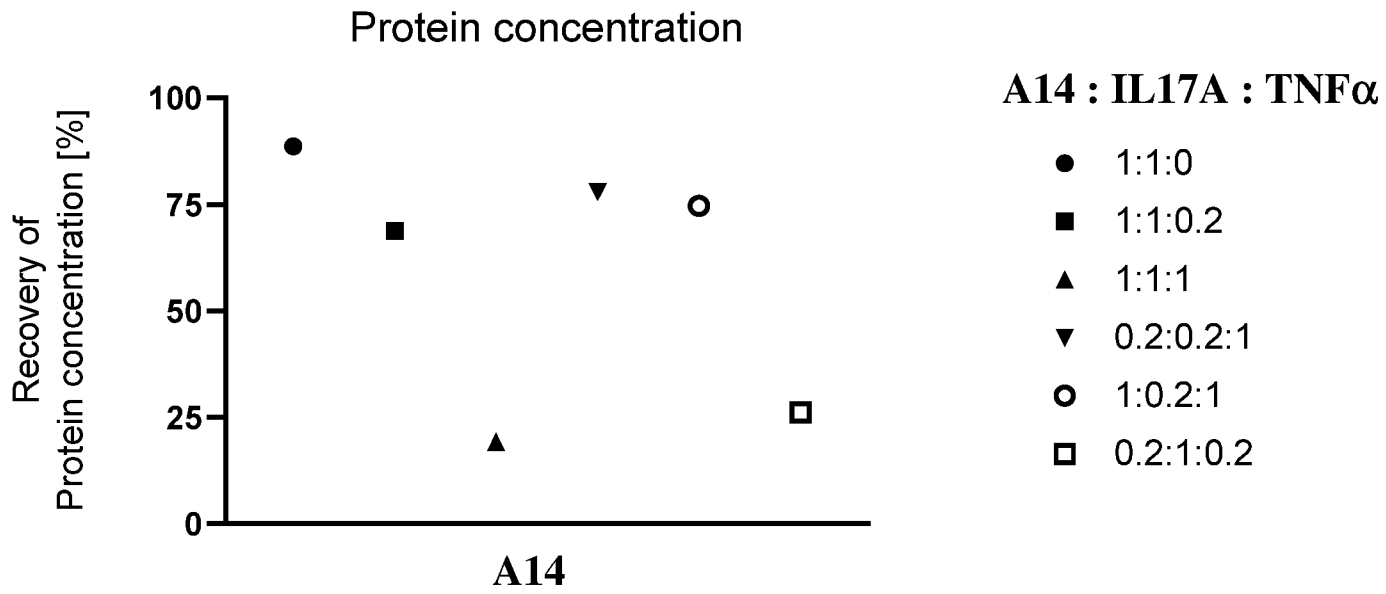
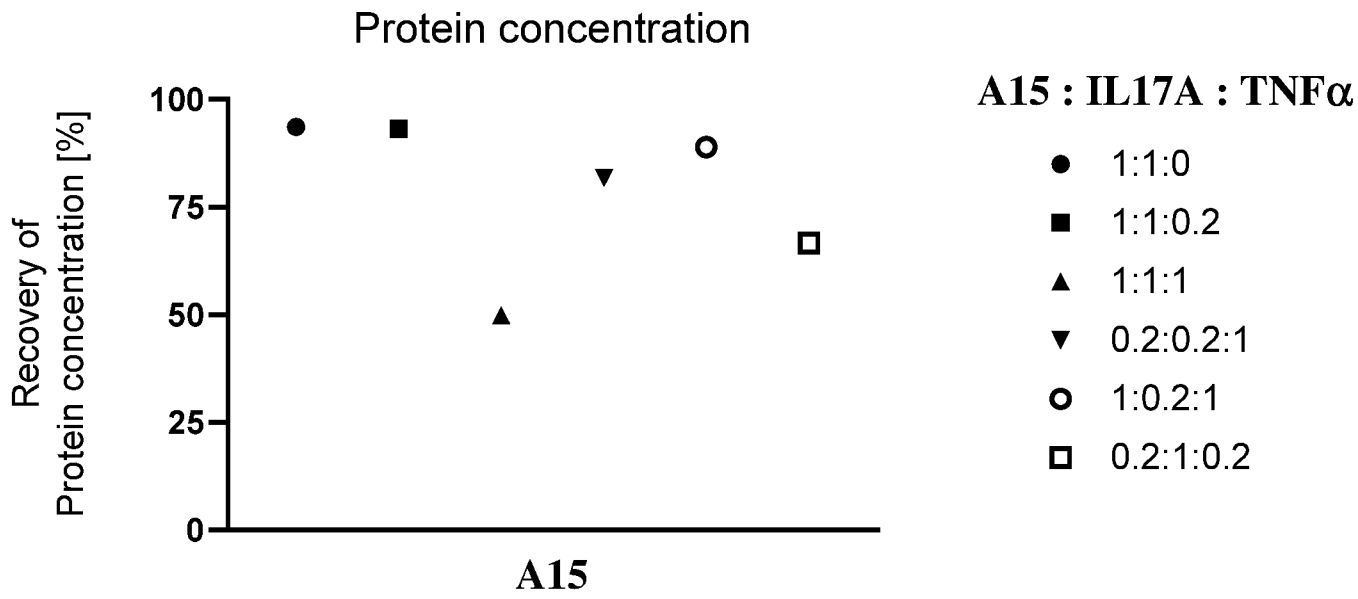

Protein concentration
Recovery of Protein concentration [%]
100
75
50
25
0
A14
A14 : IL17A : TNFα
1:1:0
1:1:0.2
1:1:1
0.2:0.2:1
1:0.2:1
0.2:1:0.2

Figure 26:


Protein concentration
Recovery of
Protein concentration [%]
100
75
50
25
0
A15
A15 : IL17A : TNFα
1:1:0
1:1:0.2
1:1:1
0.2:0.2:1
1:0.2:1
0.2:1:0.2