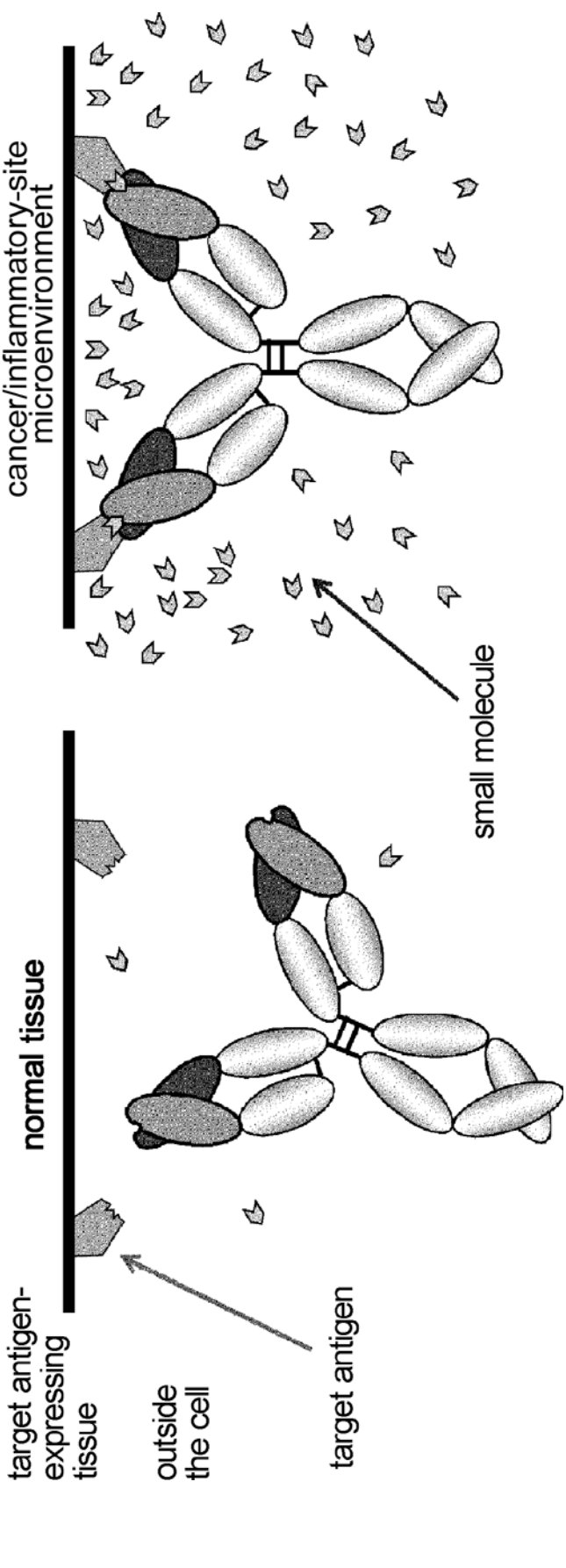

cancer/inflammatory-site microenvironment
small molecule
normal tissue
target antigen-expressing tissue
outside the cell
target antigen

FIG. 1

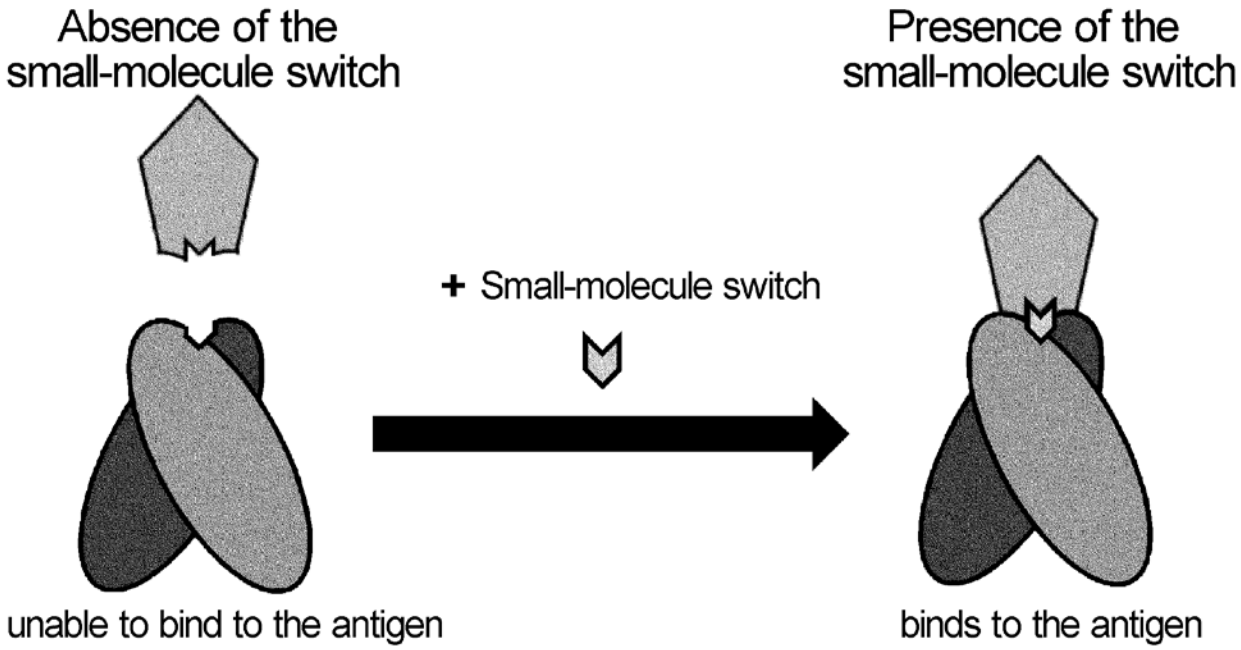

Absence of the
small-molecule switch
Presence of the
small-molecule switch
+ Small-molecule switch
unable to bind to the antigen
binds to the antigen

FIG. 2

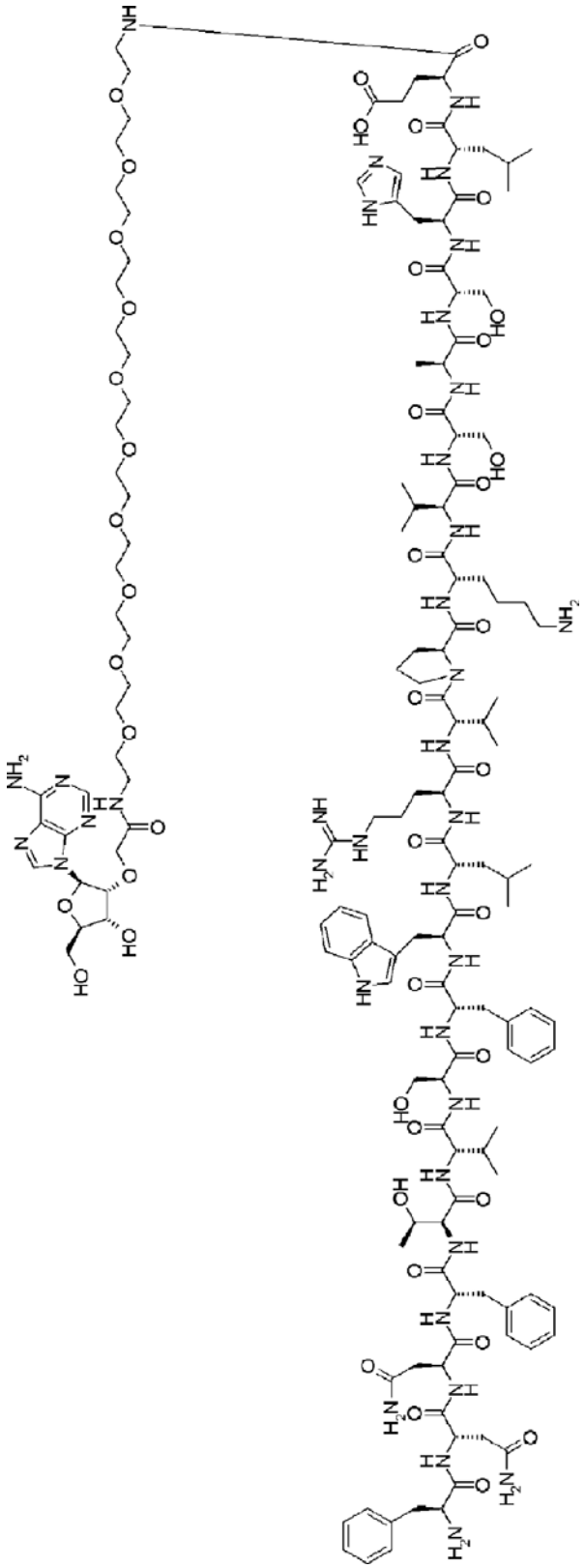


FIG. 3

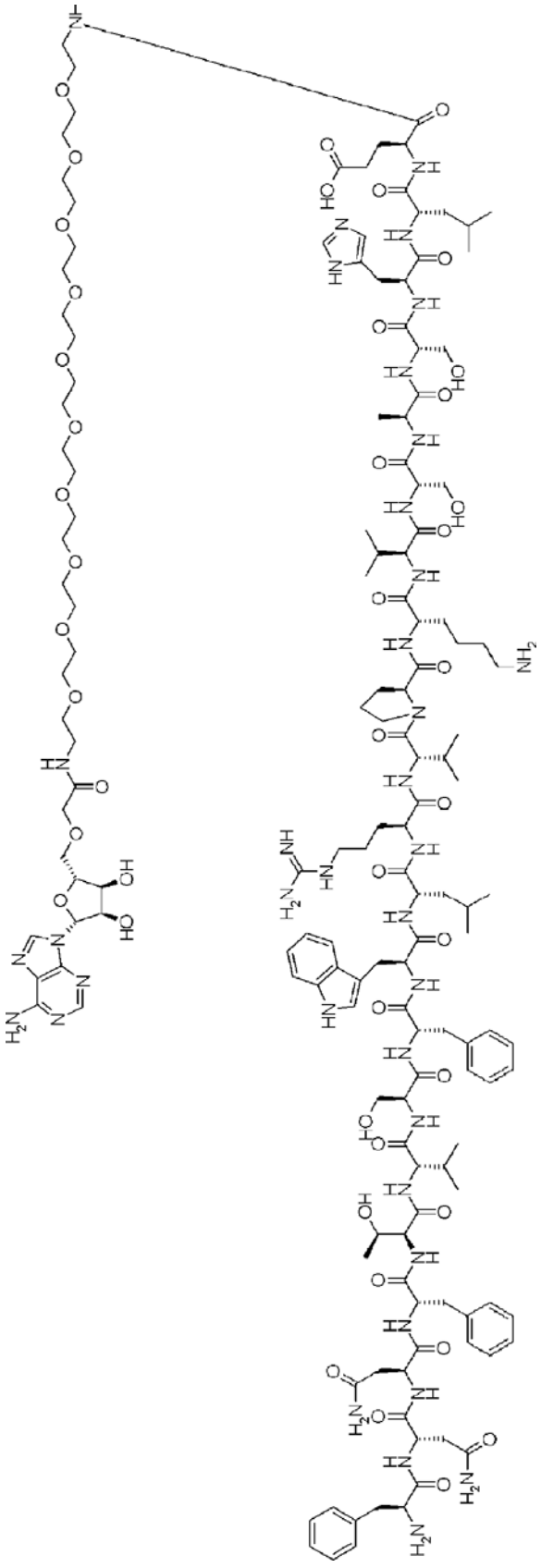


FIG. 4

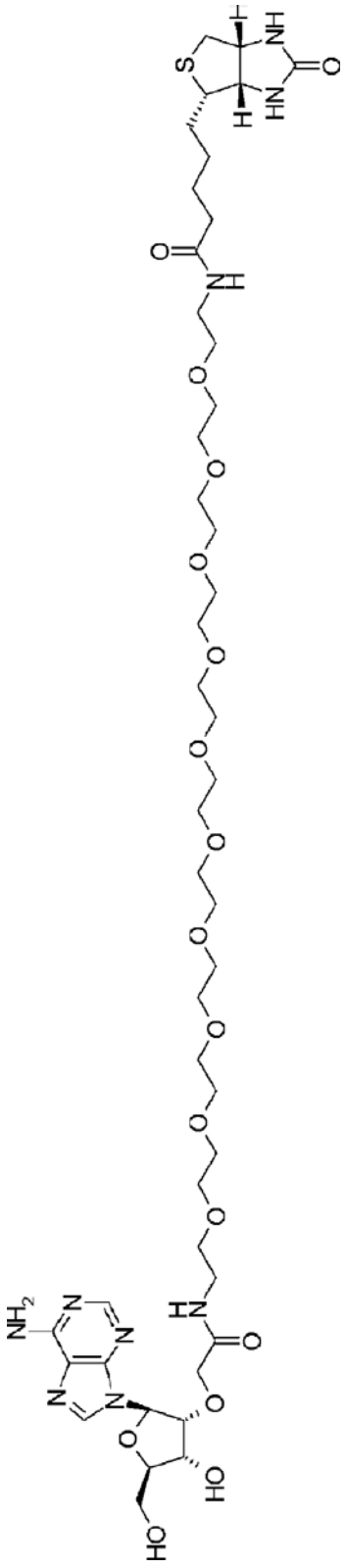


FIG. 5

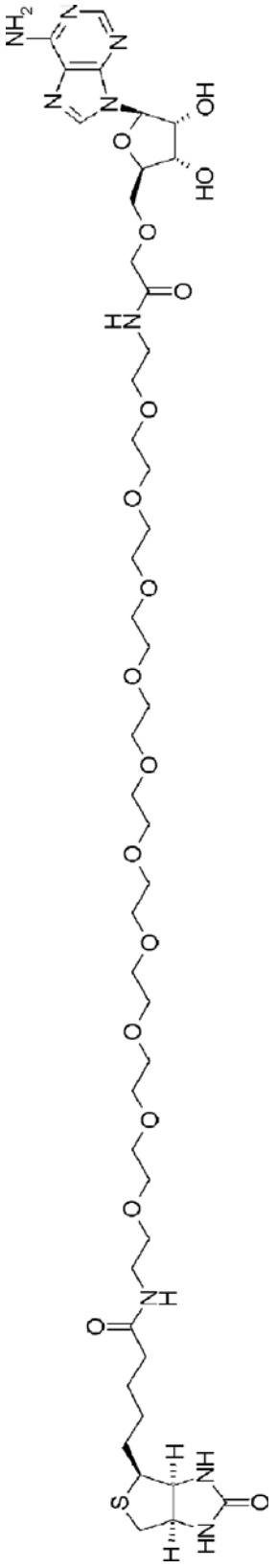


FIG. 6

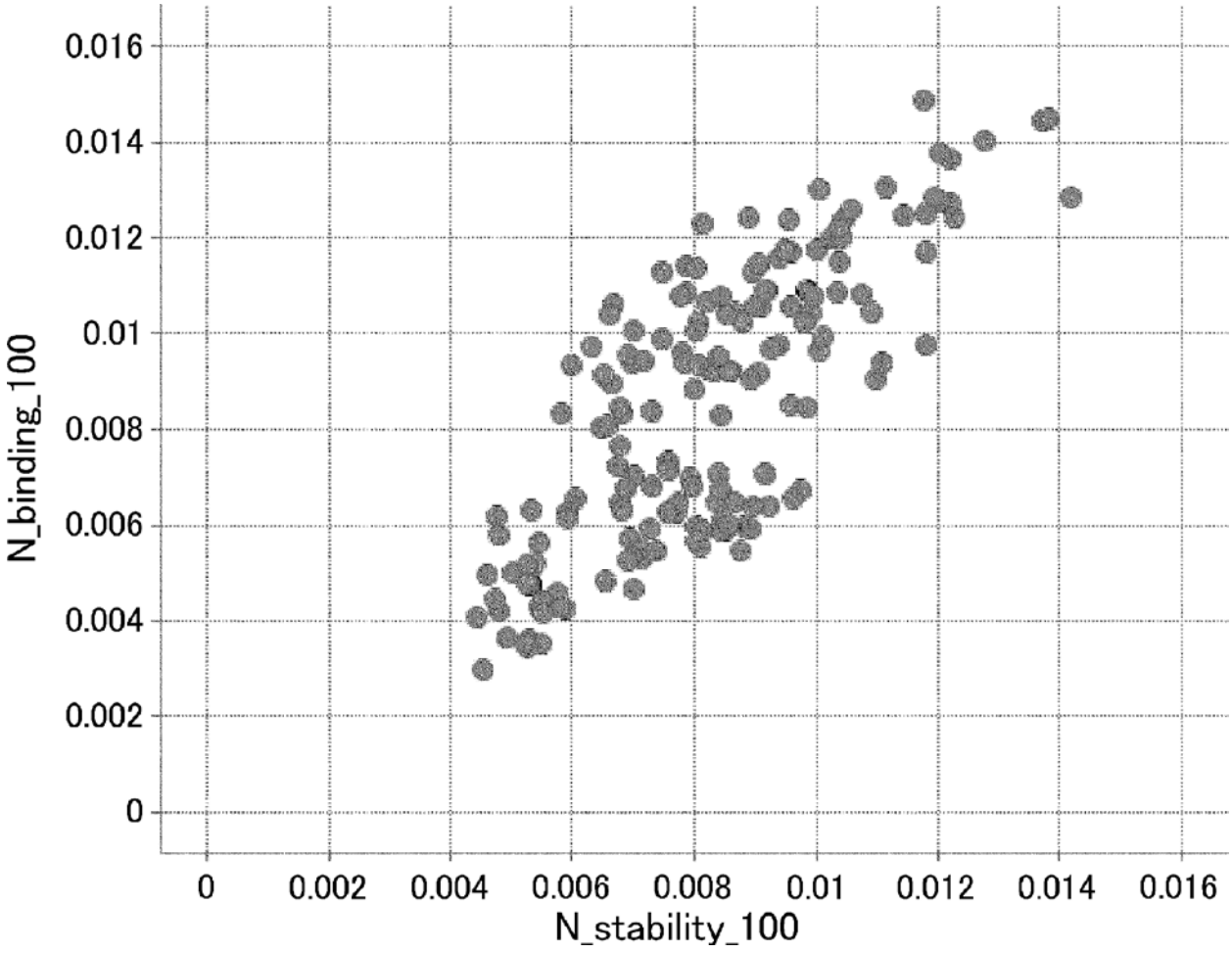

0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
N_binding_100
0
0.002
0.004
0.006
0.008
0.01
0.012
0.014
0.016
N_stability_100

FIG. 7

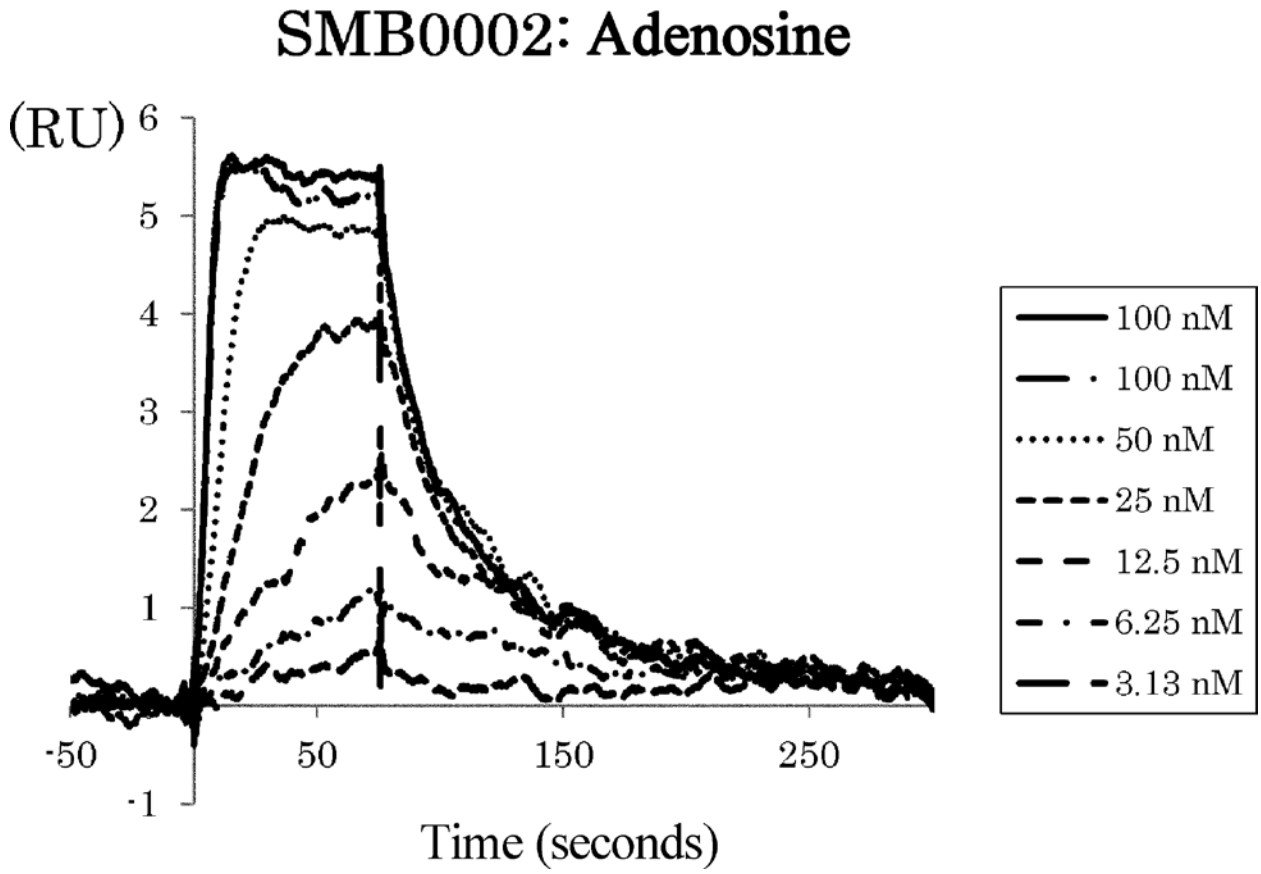

SMB0002: Adenosine
(RU)
6
5
4
3
2
1
-1
-50
50
150
250
Time (seconds)
100 nM
100 nM
50 nM
25 nM
12.5 nM
6.25 nM
3.13 nM

FIG. 8A

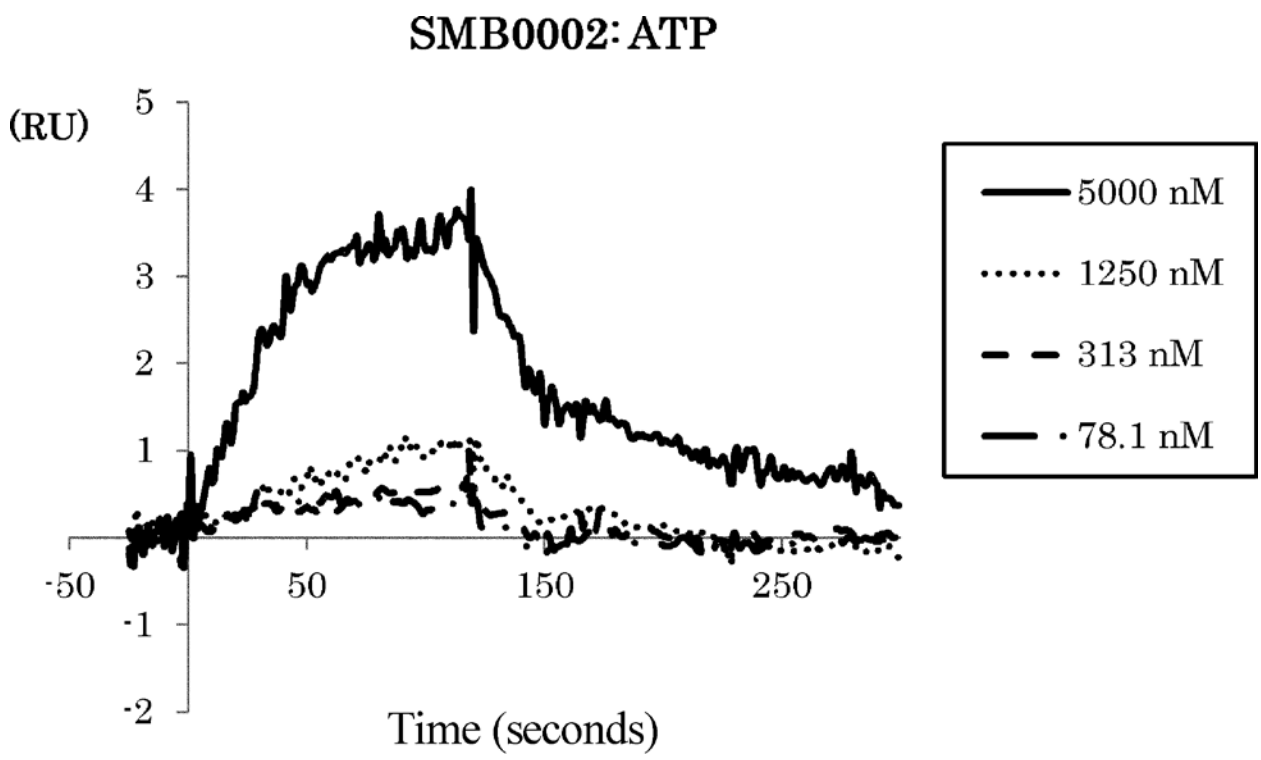

SMB0002: ATP
(RU)
5
4
3
2
1
-1
-2
-50
50
150
250
Time (seconds)
5000 nM
1250 nM
313 nM
78.1 nM

FIG. 8B

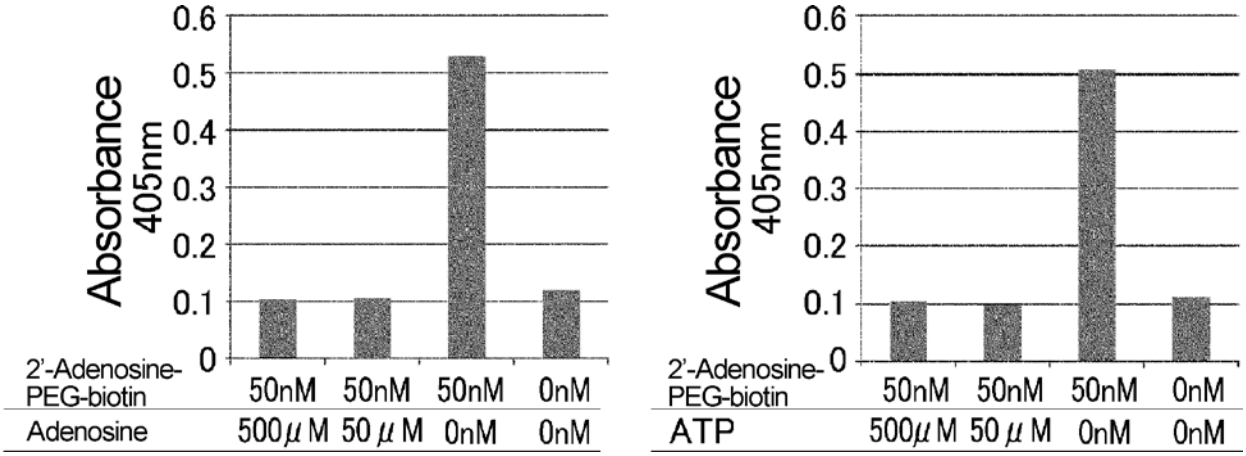

Absorbance 405nm
0.6
0.5
0.4
0.3
0.2
0.1
0
2'-Adenosine-PEG-biotin
50nM 50nM 50nM 0nM
Adenosine
500μM 50μM 0nM 0nM
Absorbance 405nm
0.6
0.5
0.4
0.3
0.2
0.1
0
2'-Adenosine-PEG-biotin
50nM 50nM 50nM 0nM
ATP
500μM 50μM 0nM 0nM

FIG. 9

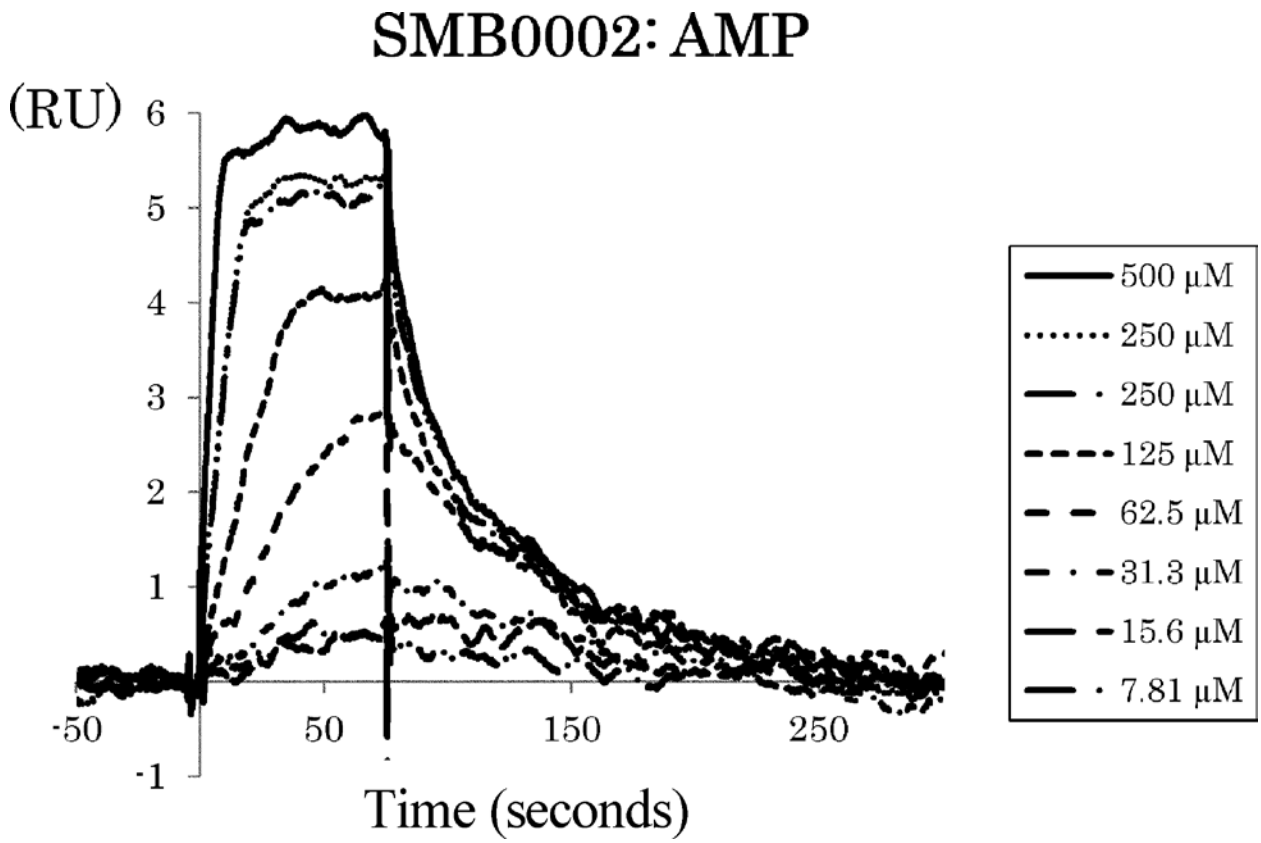

SMB0002: AMP
(RU)
6
5
4
3
2
1
-1
-50
50
150
250
Time (seconds)
500 µM
250 µM
250 µM
125 µM
62.5 µM
31.3 µM
15.6 µM
7.81 µM

FIG. 10A

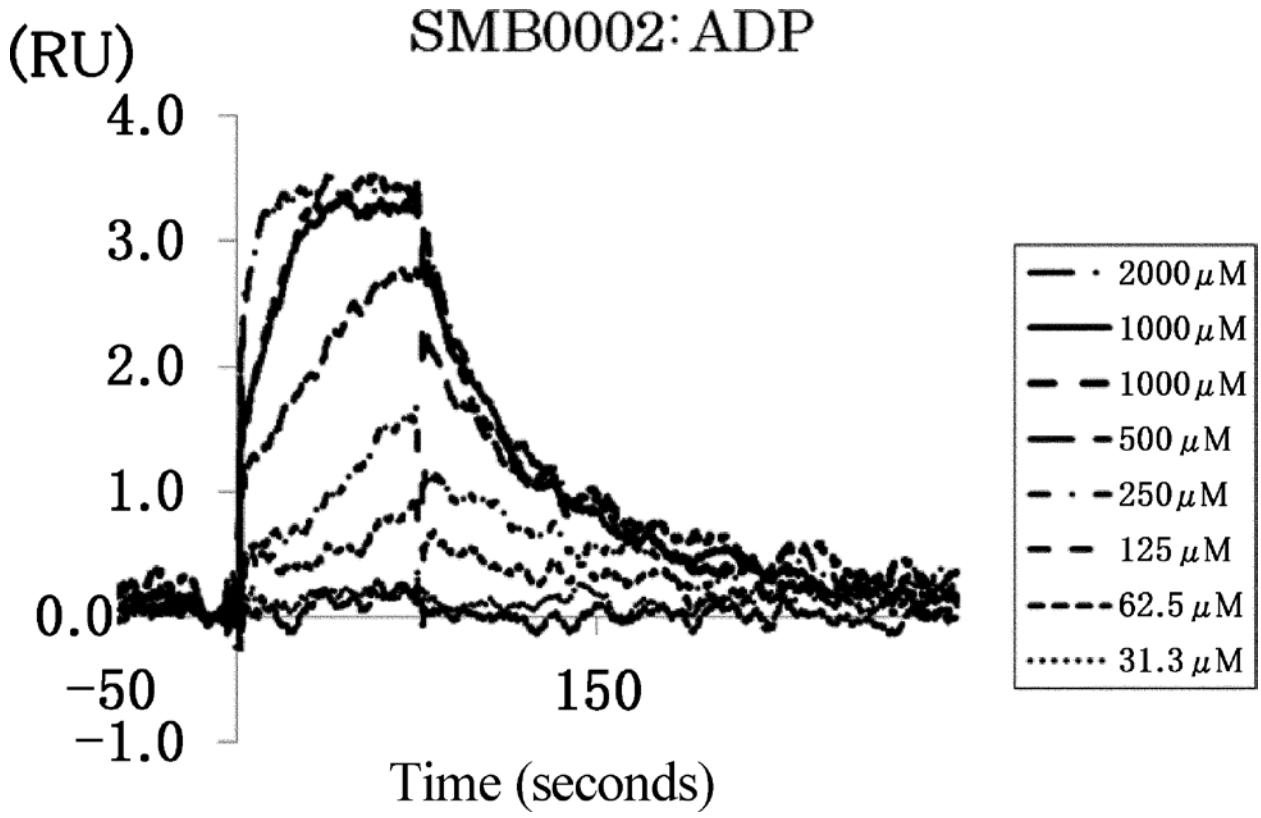

SMB0002: ADP
(RU)
4.0
3.0
2.0
1.0
0.0
−1.0
−50
150
Time (seconds)
2000 μM
1000 μM
1000 μM
500 μM
250 μM
125 μM
62.5 μM
31.3 μM

FIG. 10B

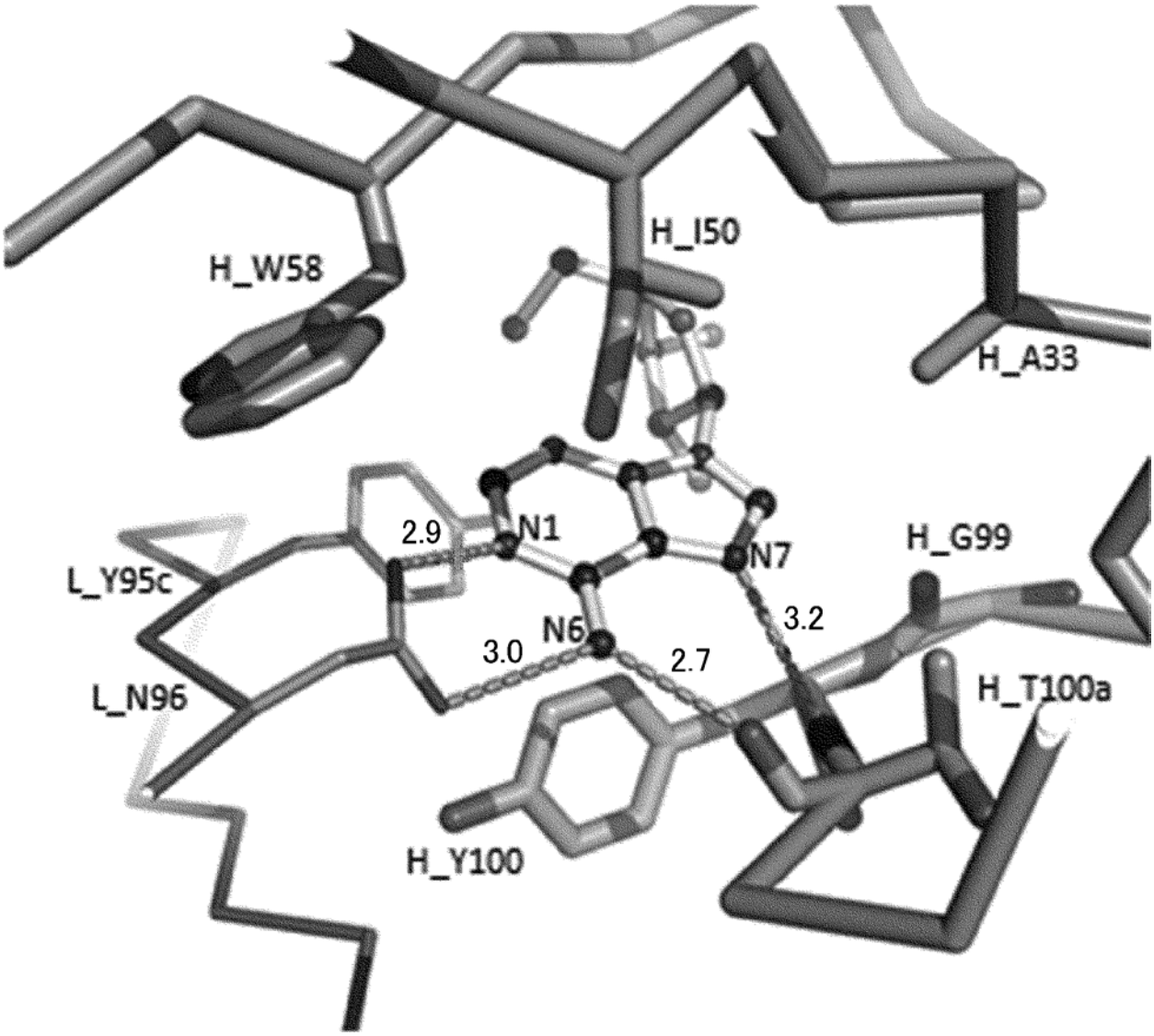

H_I50
H_W58
H_A33
2.9
N1
N7
H_G99
L_Y95c
3.2
N6
3.0
2.7
L_N96
H_T100a
H_Y100

FIG. 11A

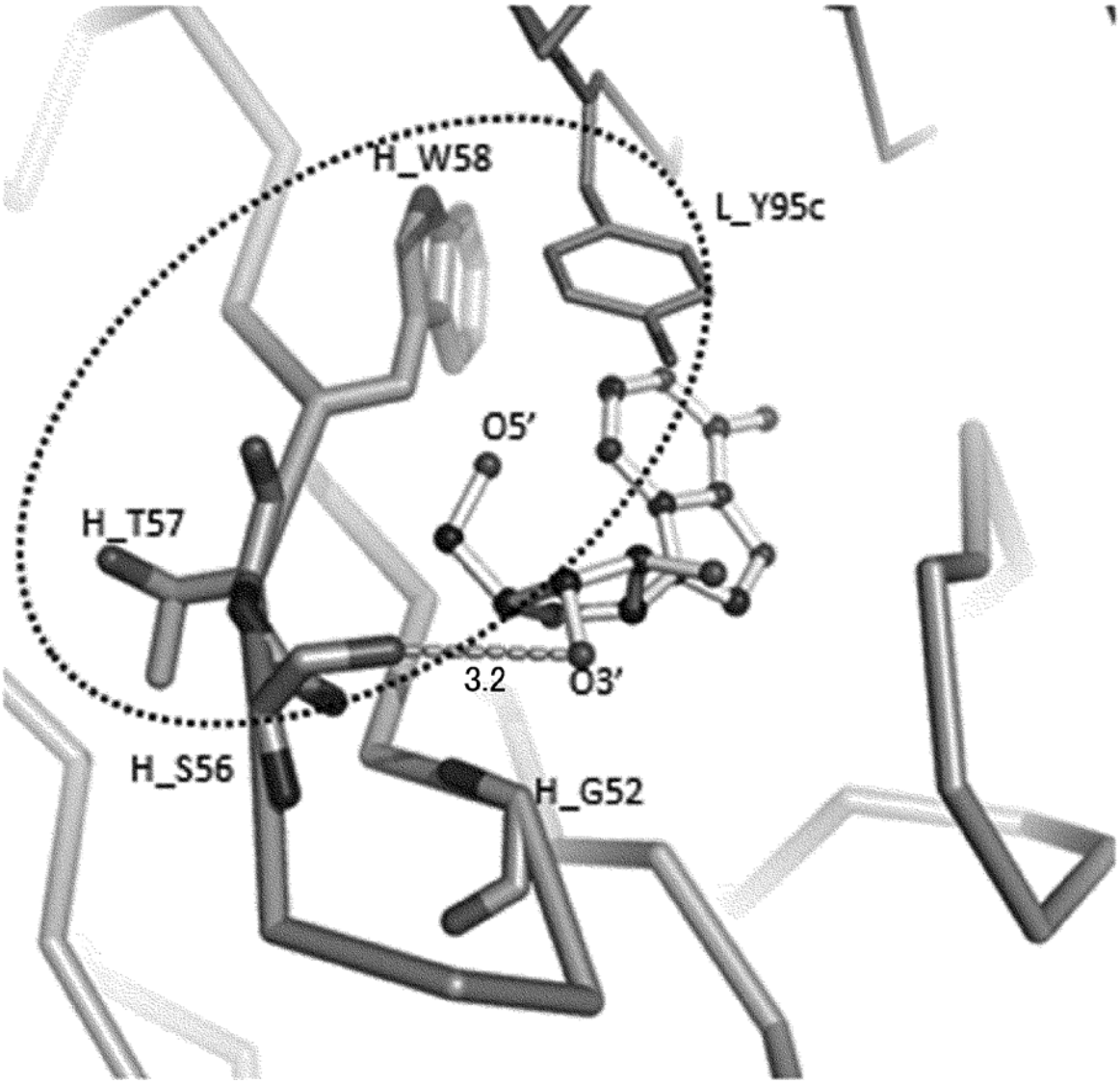


FIG. 11B

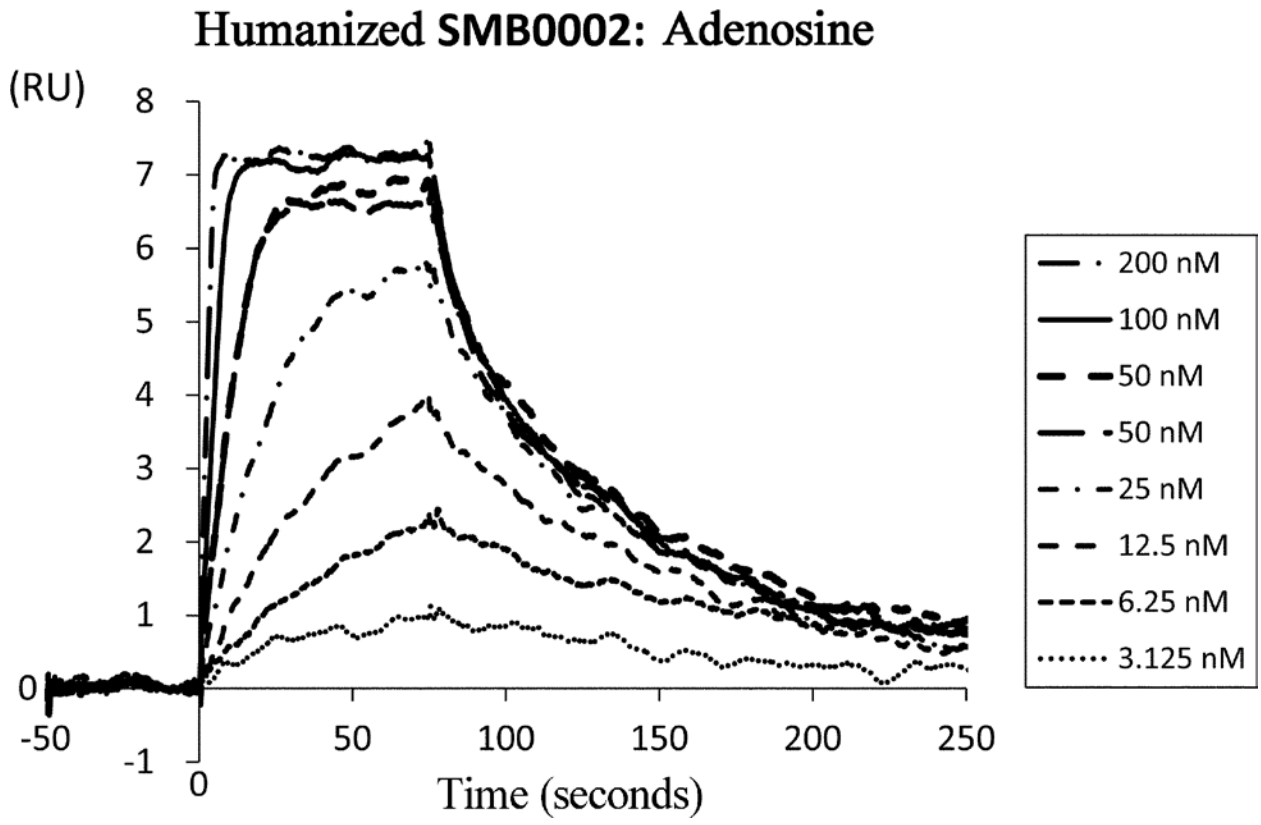

Humanized SMB0002: Adenosine
(RU)
8
7
6
5
4
3
2
1
0
-1
-50
0
50
100
150
200
250
Time (seconds)
200 nM
100 nM
50 nM
50 nM
25 nM
12.5 nM
6.25 nM
3.125 nM

FIG. 12

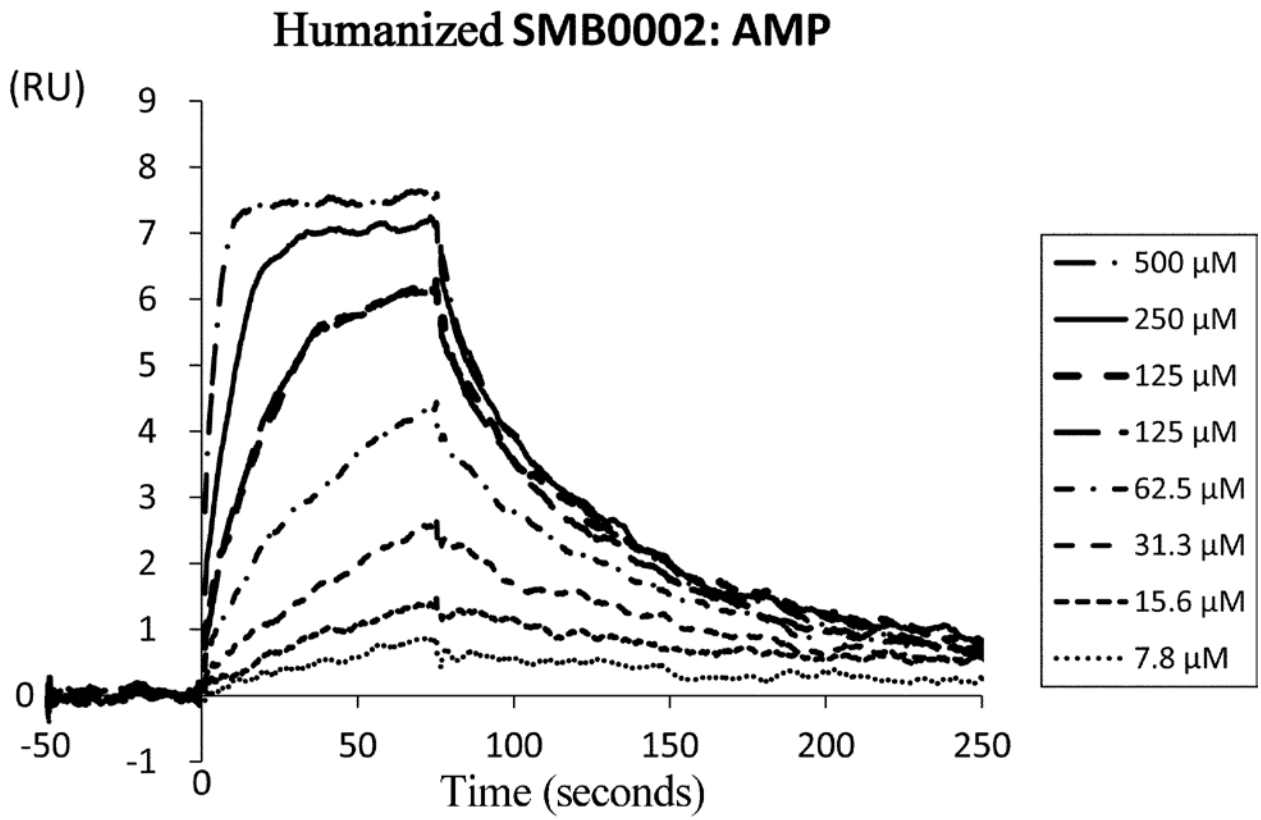

Humanized SMB0002: AMP
(RU)
9
8
7
6
5
4
3
2
1
0
-1
-50
0
50
100
150
200
250
Time (seconds)
500 µM
250 µM
125 µM
125 µM
62.5 µM
31.3 µM
15.6 µM
7.8 µM

FIG. 13

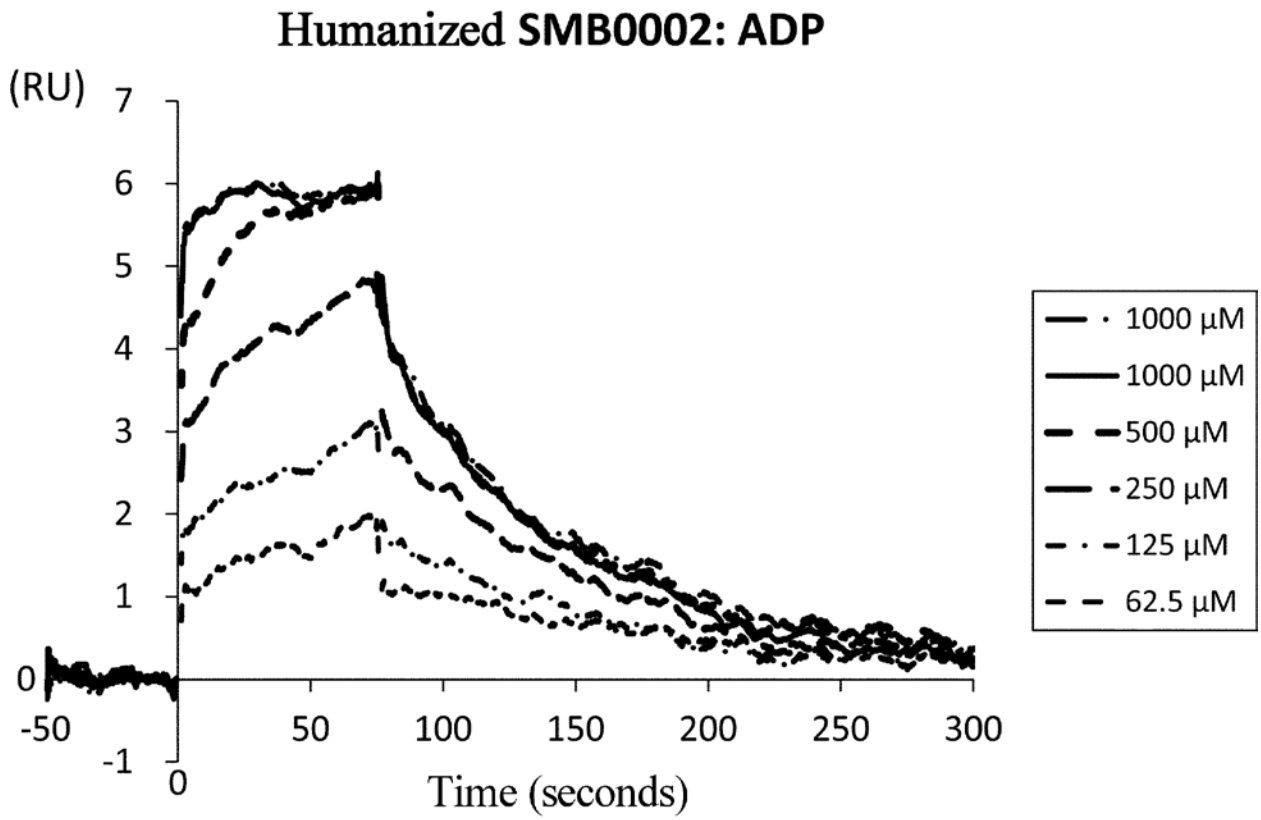

Humanized SMB0002: ADP
(RU)
7
6
5
4
3
2
1
0
-1
-50
0
50
100
150
200
250
300
Time (seconds)
1000 µM
1000 µM
500 µM
250 µM
125 µM
62.5 µM

FIG. 14

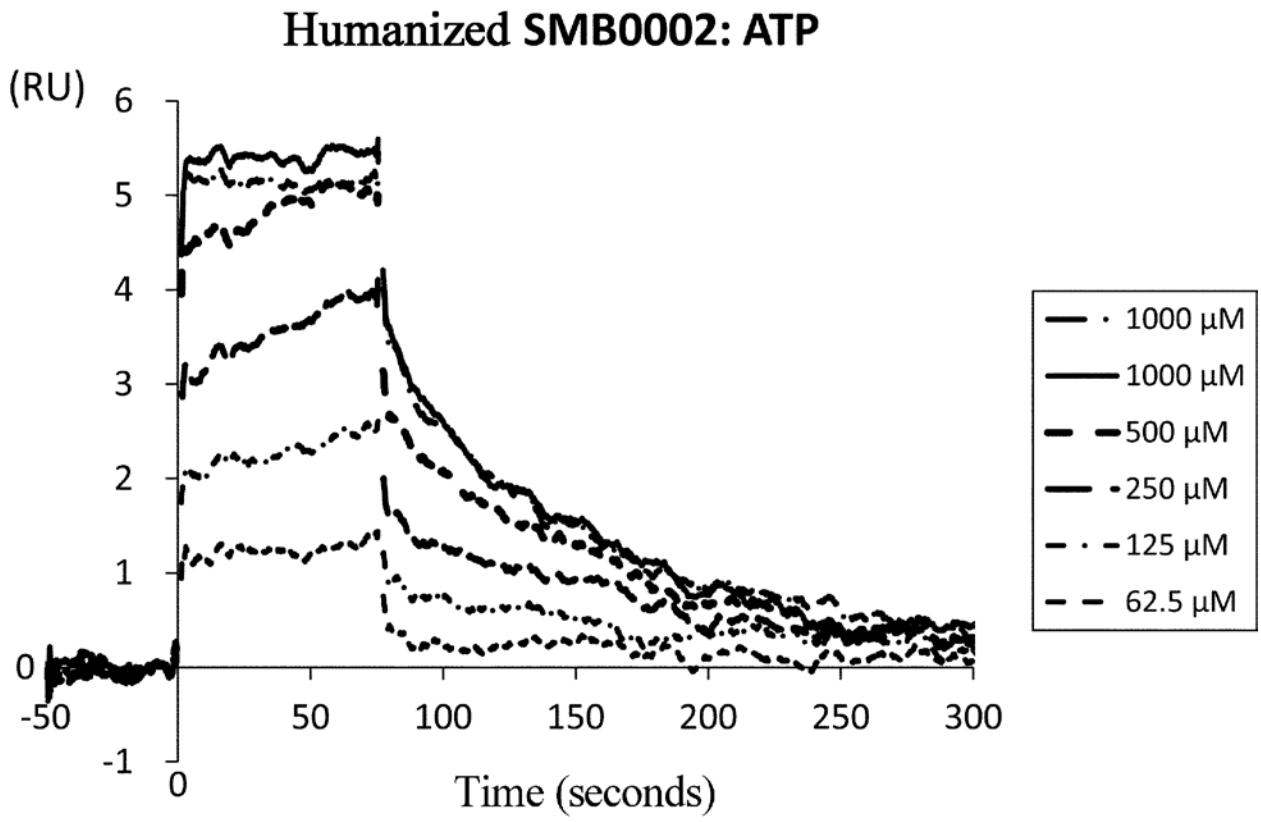

Humanized SMB0002: ATP
(RU)
6
5
4
3
2
1
0
-1
-50
0
50
100
150
200
250
300
Time (seconds)
1000 µM
1000 µM
500 µM
250 µM
125 µM
62.5 µM

FIG. 15

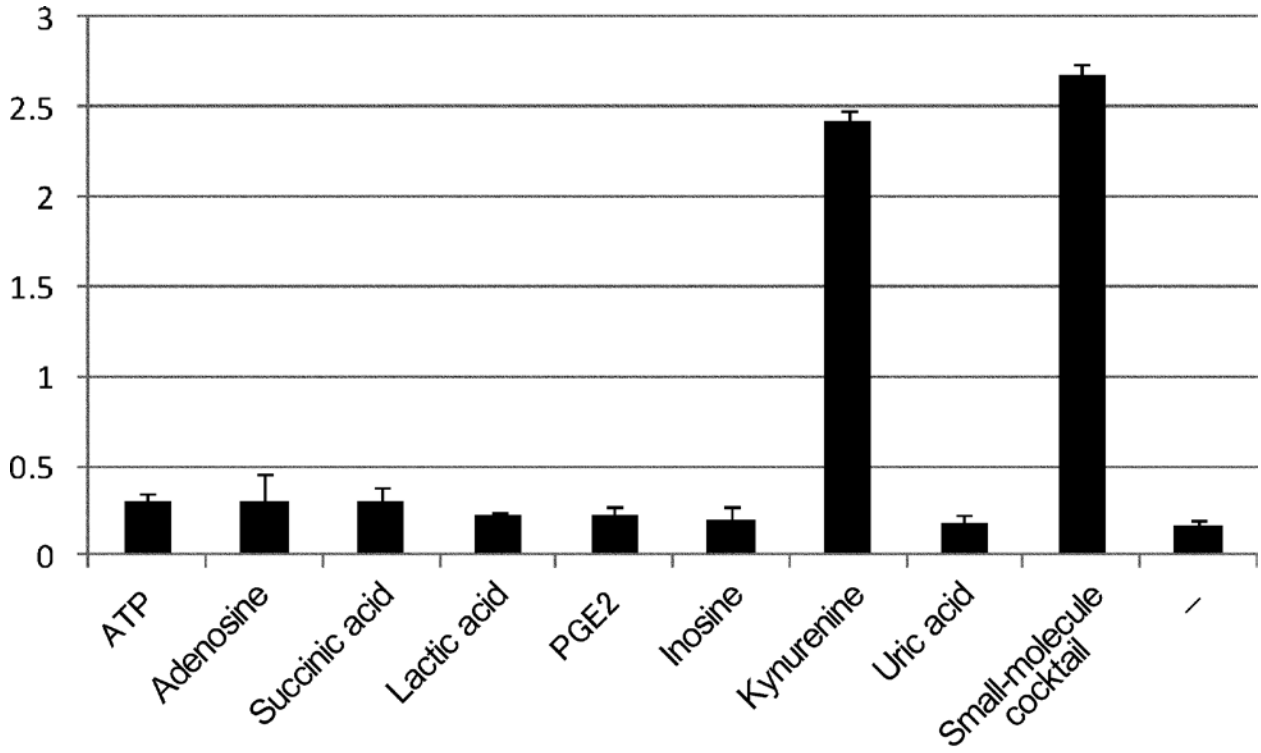

3
2.5
2
1.5
1
0.5
0
ATP
Adenosine
Succinic acid
Lactic acid
PGE2
Inosine
Kynurenine
Uric acid
Small-molecule cocktail
–

FIG. 16

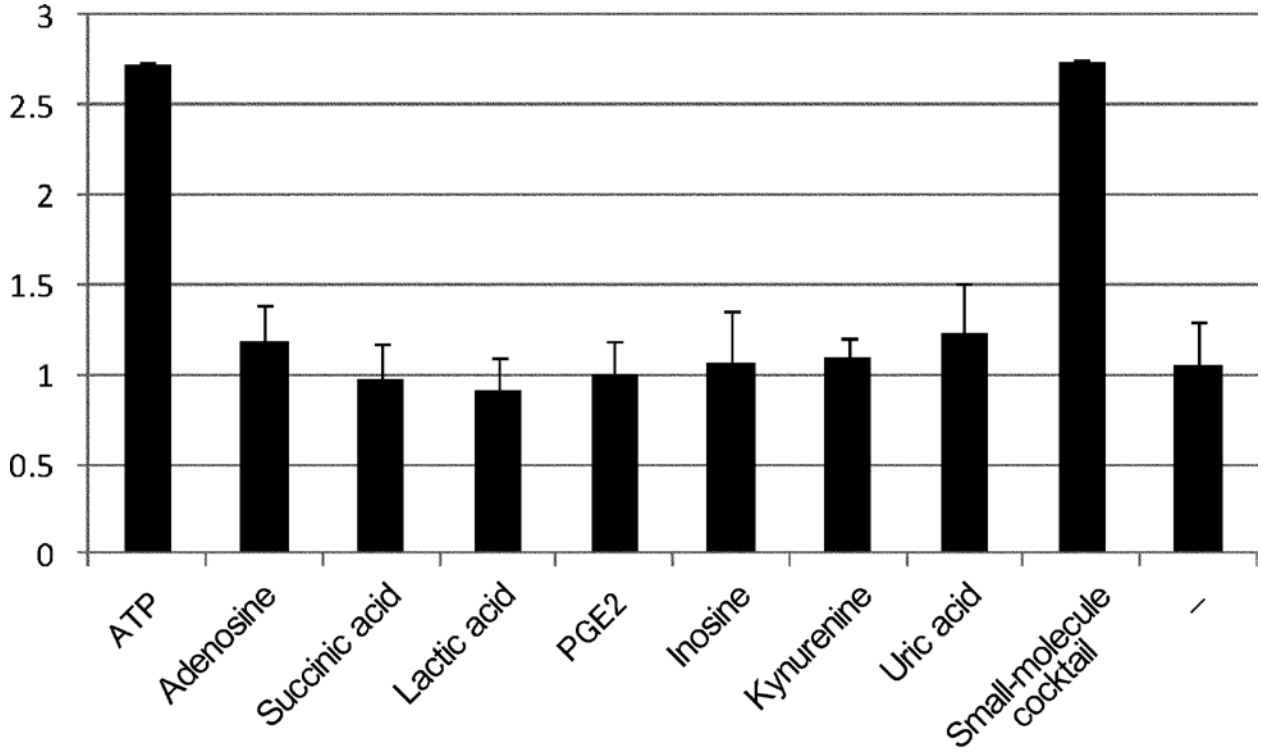

3
2.5
2
1.5
1
0.5
0
ATP
Adenosine
Succinic acid
Lactic acid
PGE2
Inosine
Kynurenine
Uric acid
Small-molecule cocktail
–

FIG. 17

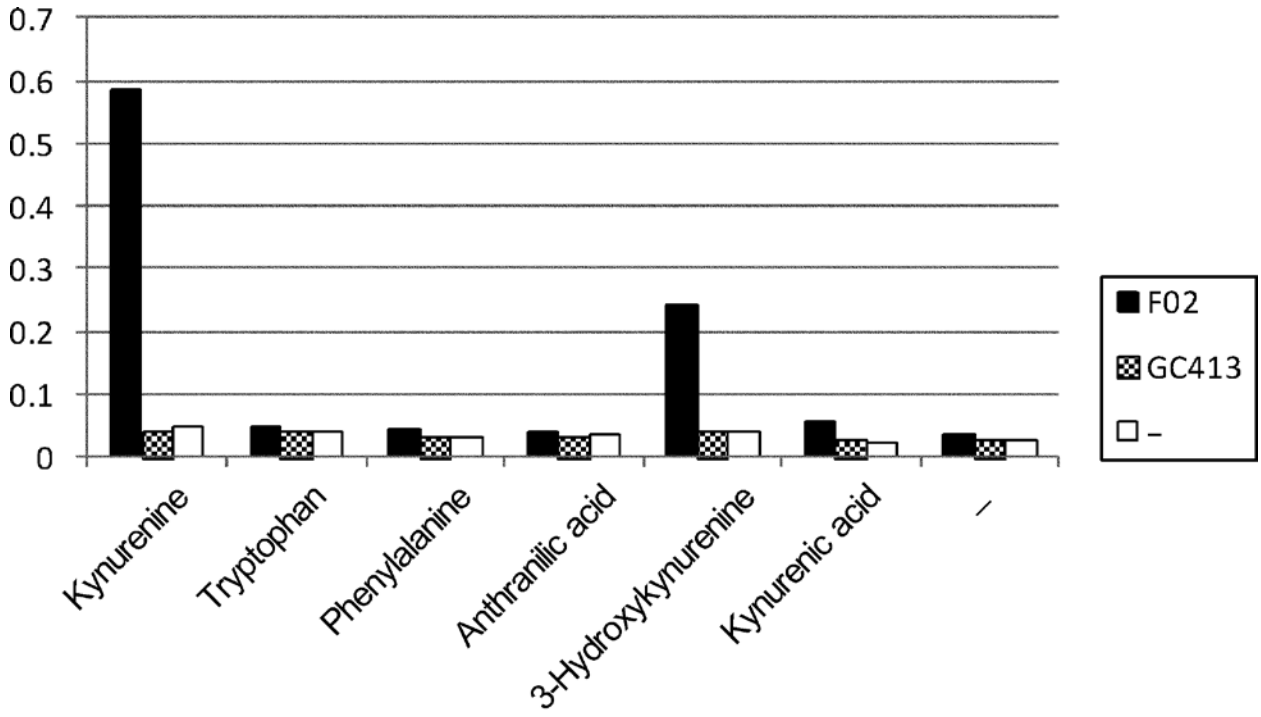

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Kynurenine
Tryptophan
Phenylalanine
Anthranilic acid
3-Hydroxykynurenine
Kynurenic acid
–
F02
GC413
–

FIG. 18

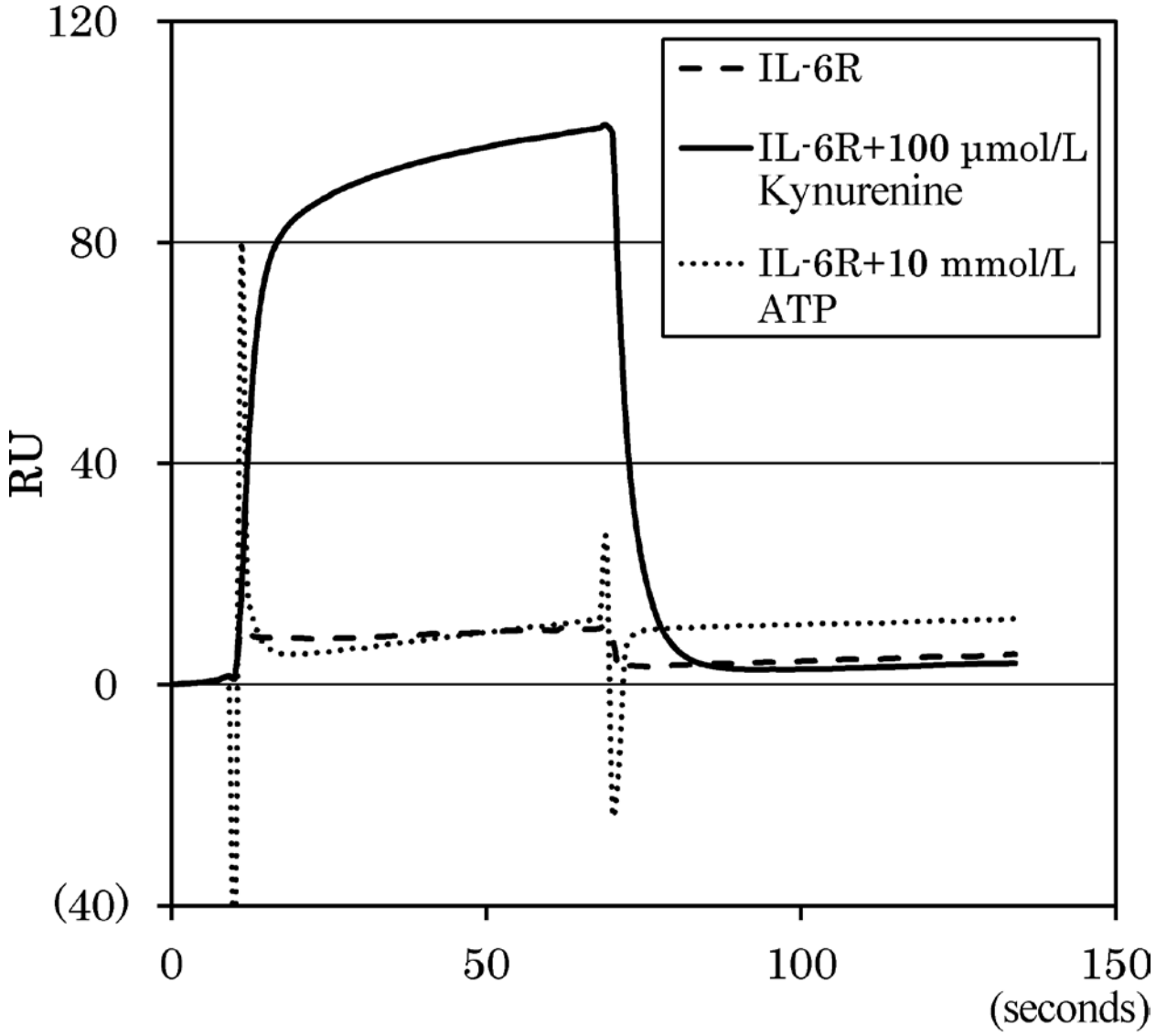

120
80
40
0
(40)
RU
0
50
100
150
(seconds)
IL-6R
IL-6R+100 µmol/L Kynurenine
IL-6R+10 mmol/L ATP

FIG. 19

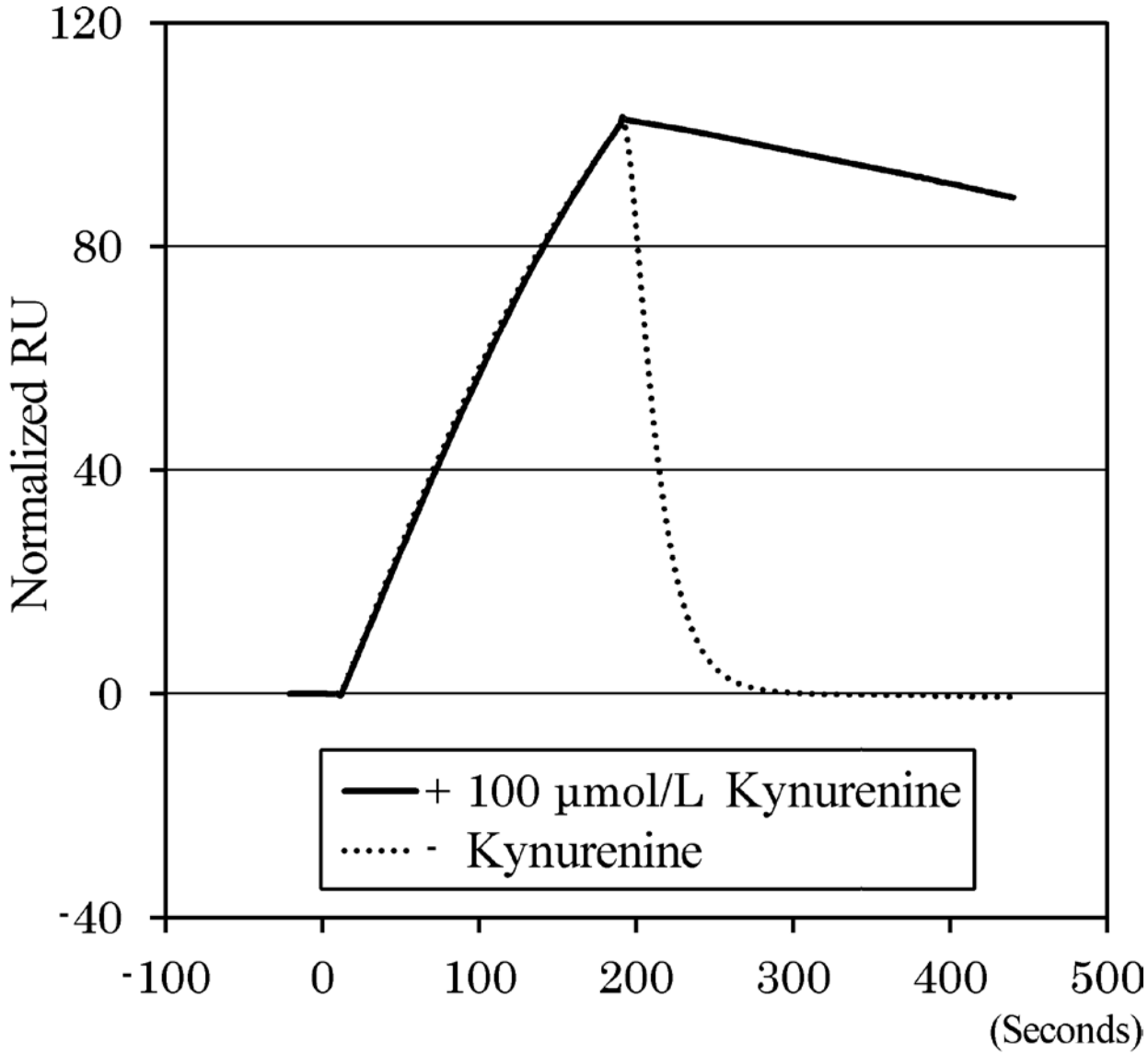

120
80
40
0
-40
Normalized RU
-100
0
100
200
300
400
500
(Seconds)
+ 100 µmol/L Kynurenine
- Kynurenine

FIG. 20

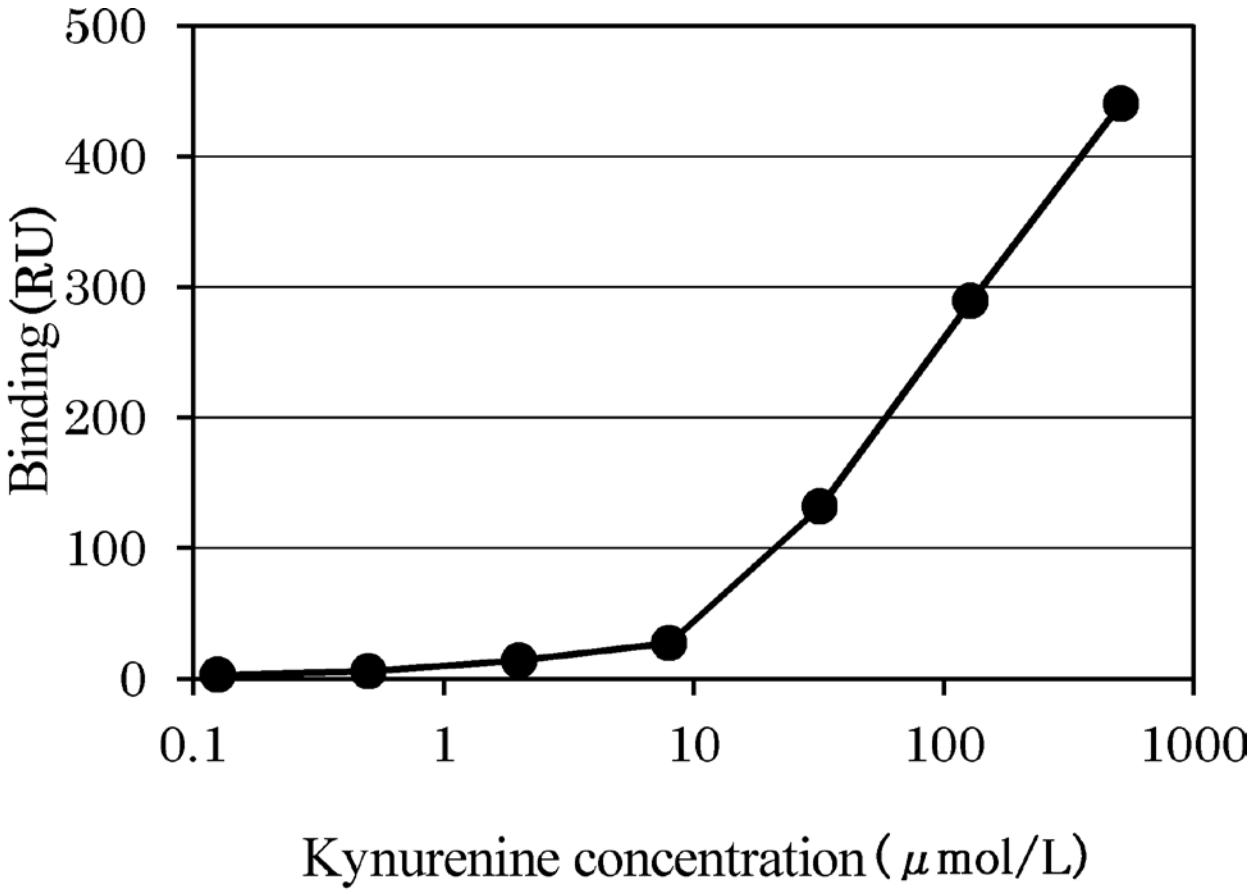

500
400
300
200
100
0
Binding (RU)
0.1
1
10
100
1000
Kynurenine concentration (μmol/L)

FIG. 21

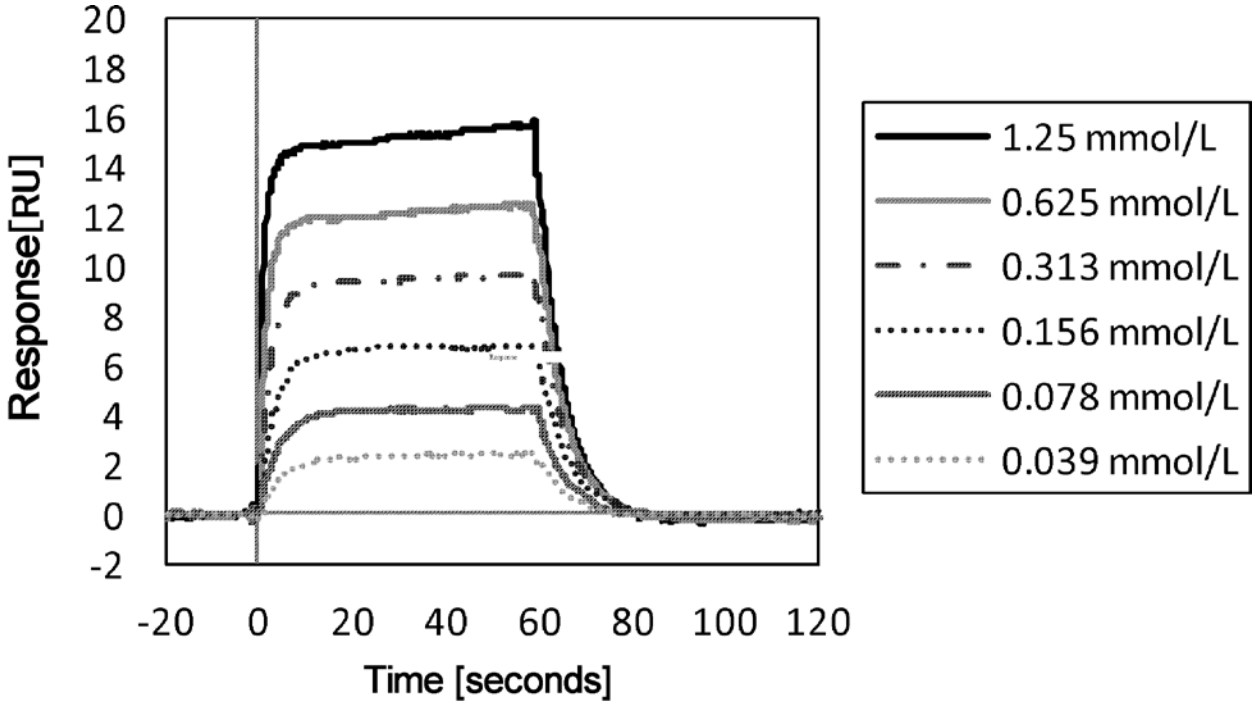

Response[RU]
20
18
16
14
12
10
8
6
4
2
0
-2
-20
0
20
40
60
80
100
120
Time [seconds]
1.25 mmol/L
0.625 mmol/L
0.313 mmol/L
0.156 mmol/L
0.078 mmol/L
0.039 mmol/L

FIG. 22

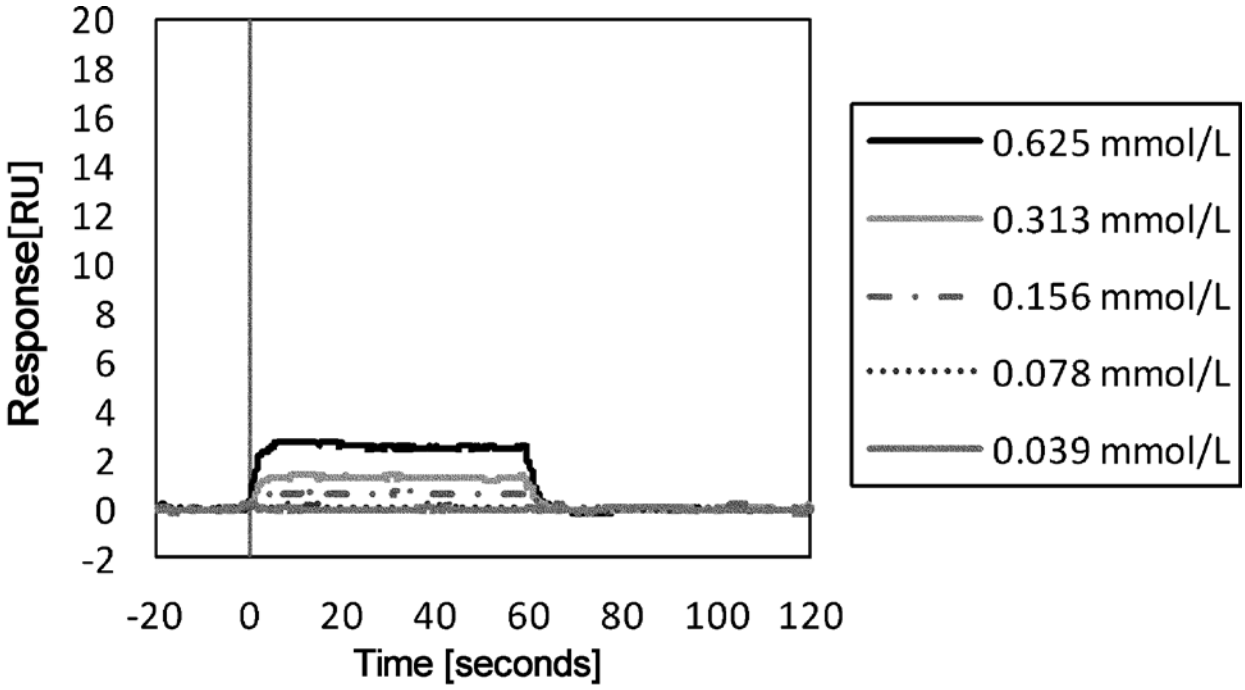

Response[RU]
20
18
16
14
12
10
8
6
4
2
0
-2
-20
0
20
40
60
80
100
120
Time [seconds]
0.625 mmol/L
0.313 mmol/L
0.156 mmol/L
0.078 mmol/L
0.039 mmol/L

FIG. 23

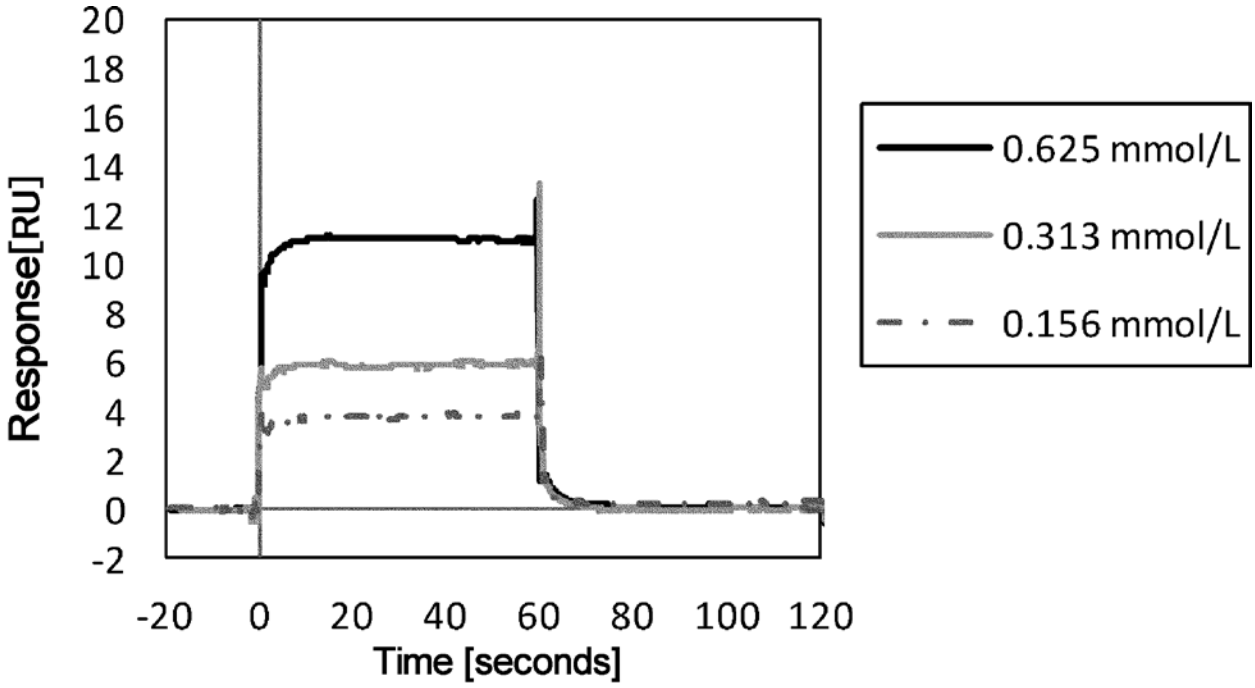

Response[RU]
20
18
16
14
12
10
8
6
4
2
0
-2
-20
0
20
40
60
80
100
120
Time [seconds]
0.625 mmol/L
0.313 mmol/L
0.156 mmol/L

FIG. 24

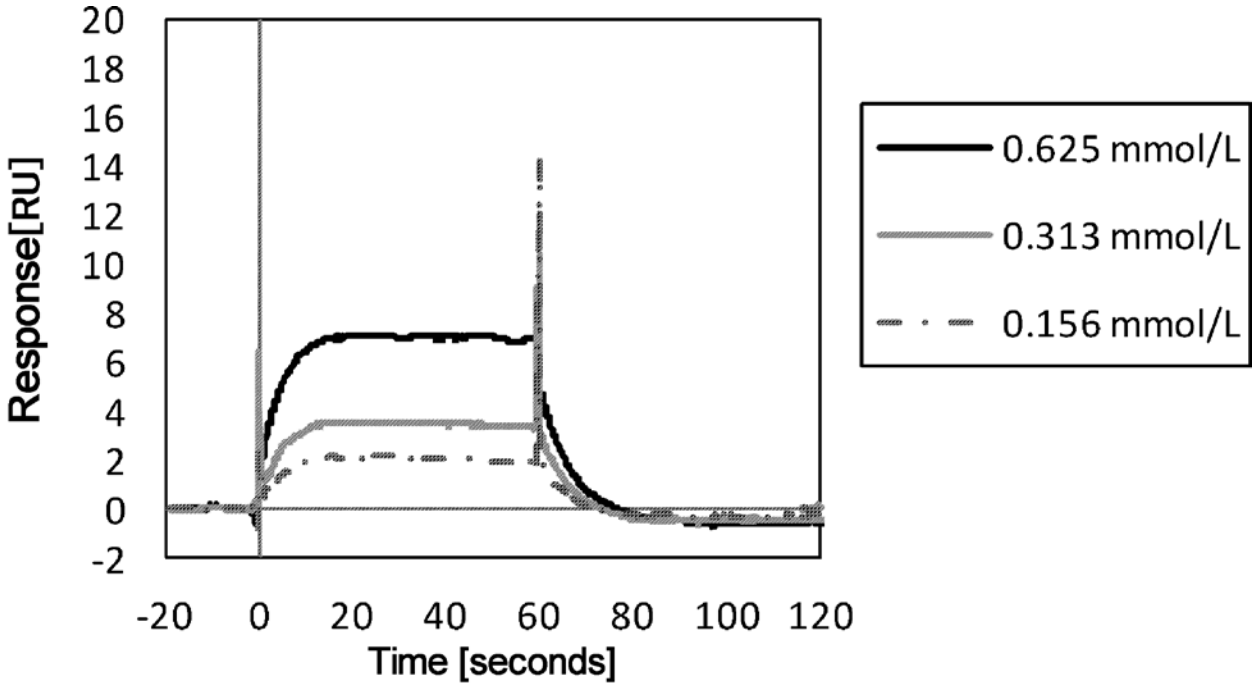

Response[RU]
20
18
16
14
12
10
8
6
4
2
0
-2
-20
0
20
40
60
80
100
120
Time [seconds]
0.625 mmol/L
0.313 mmol/L
0.156 mmol/L

FIG. 25

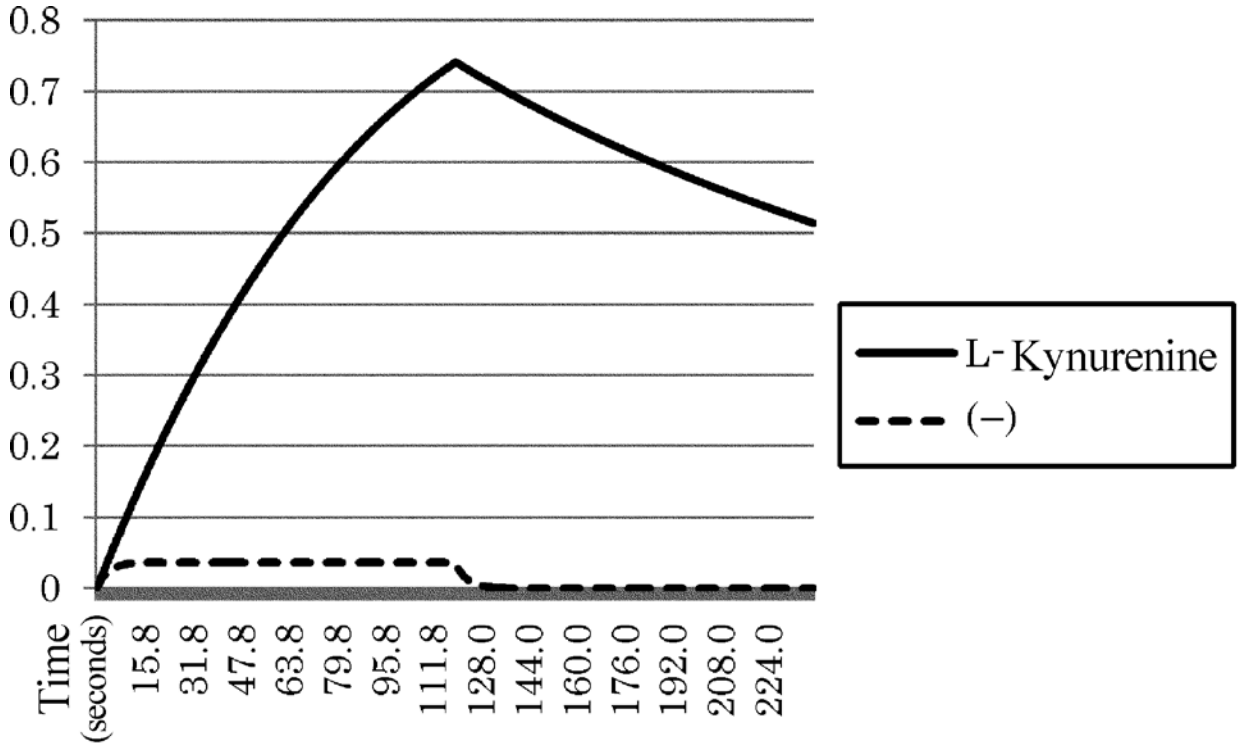

0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Time (seconds)
15.8
31.8
47.8
63.8
79.8
95.8
111.8
128.0
144.0
160.0
176.0
192.0
208.0
224.0
L-Kynurenine
(–)

FIG. 26

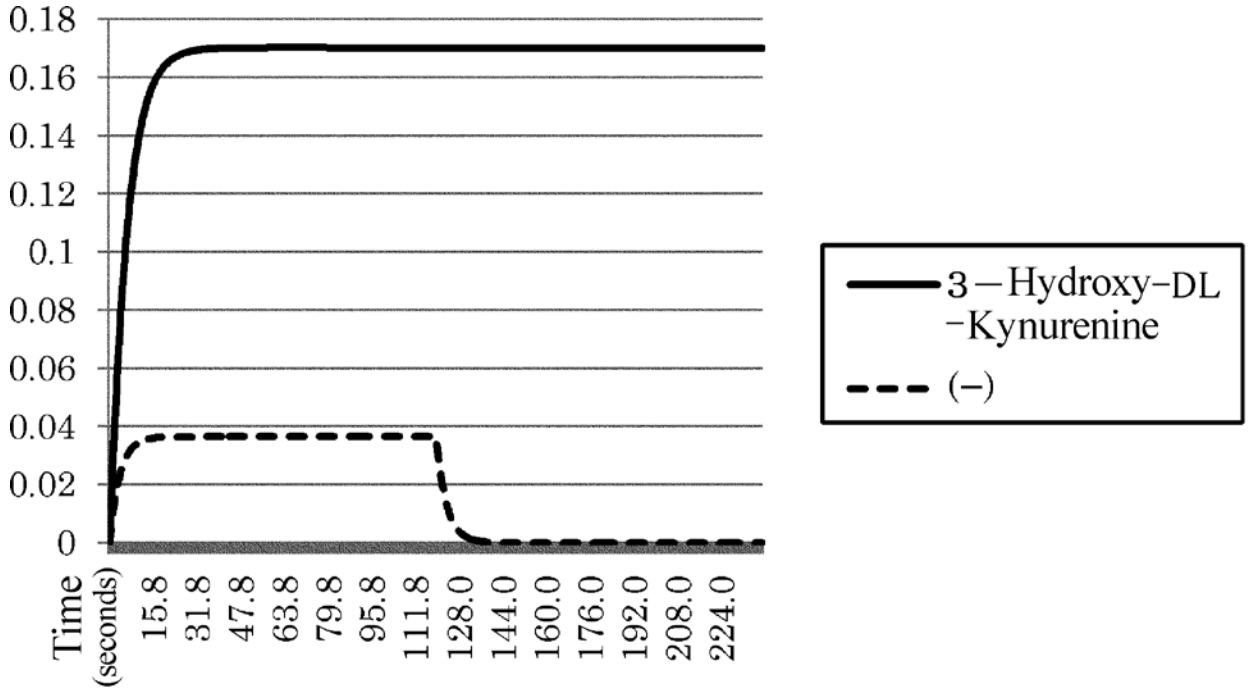

0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Time (seconds)
15.8
31.8
47.8
63.8
79.8
95.8
111.8
128.0
144.0
160.0
176.0
192.0
208.0
224.0
3－Hydroxy-DL-Kynurenine
(－)

FIG. 27

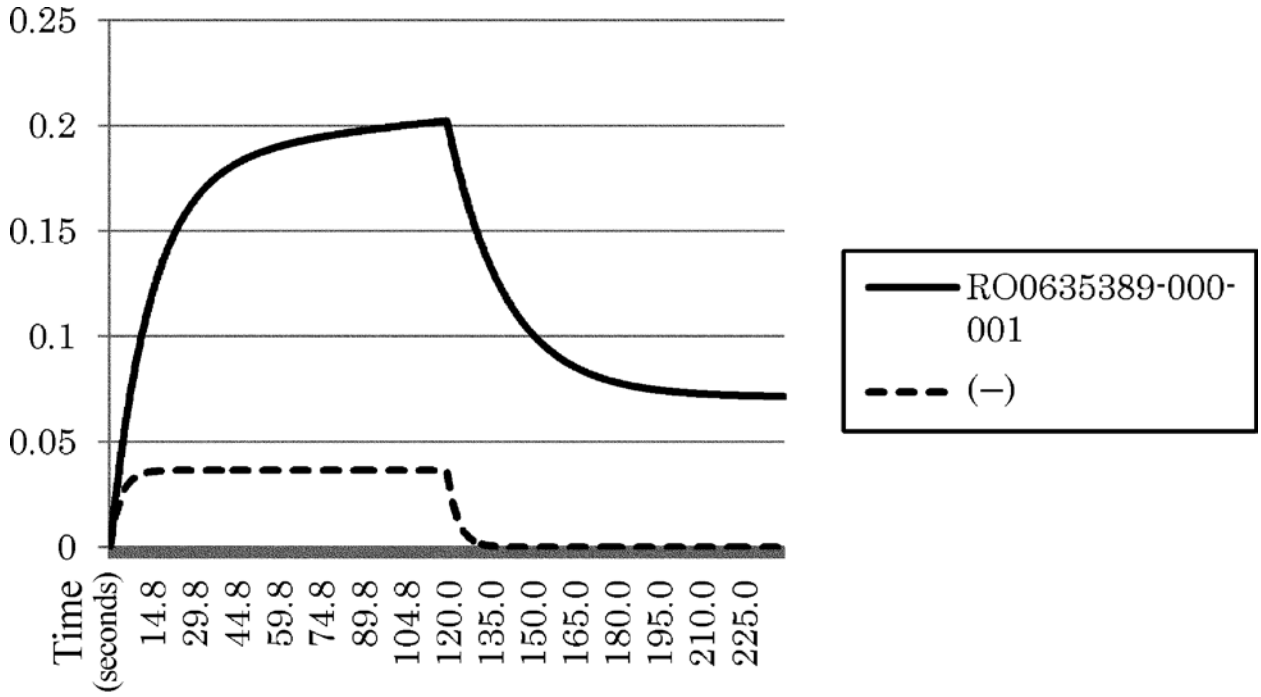

0.25
0.2
0.15
0.1
0.05
0
Time (seconds)
14.8
29.8
44.8
59.8
74.8
89.8
104.8
120.0
135.0
150.0
165.0
180.0
195.0
210.0
225.0
RO0635389-000-001
(–)

FIG. 28

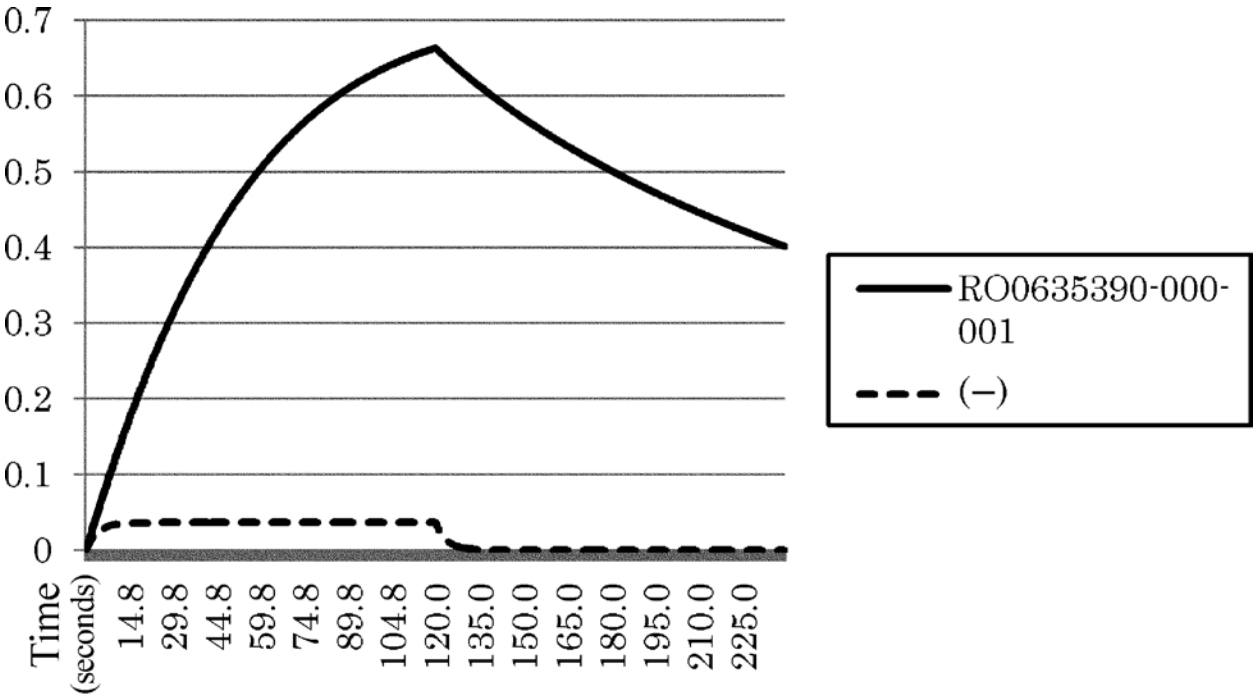

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Time (seconds)
14.8
29.8
44.8
59.8
74.8
89.8
104.8
120.0
135.0
150.0
165.0
180.0
195.0
210.0
225.0
RO0635390-000-001
(–)

FIG. 29

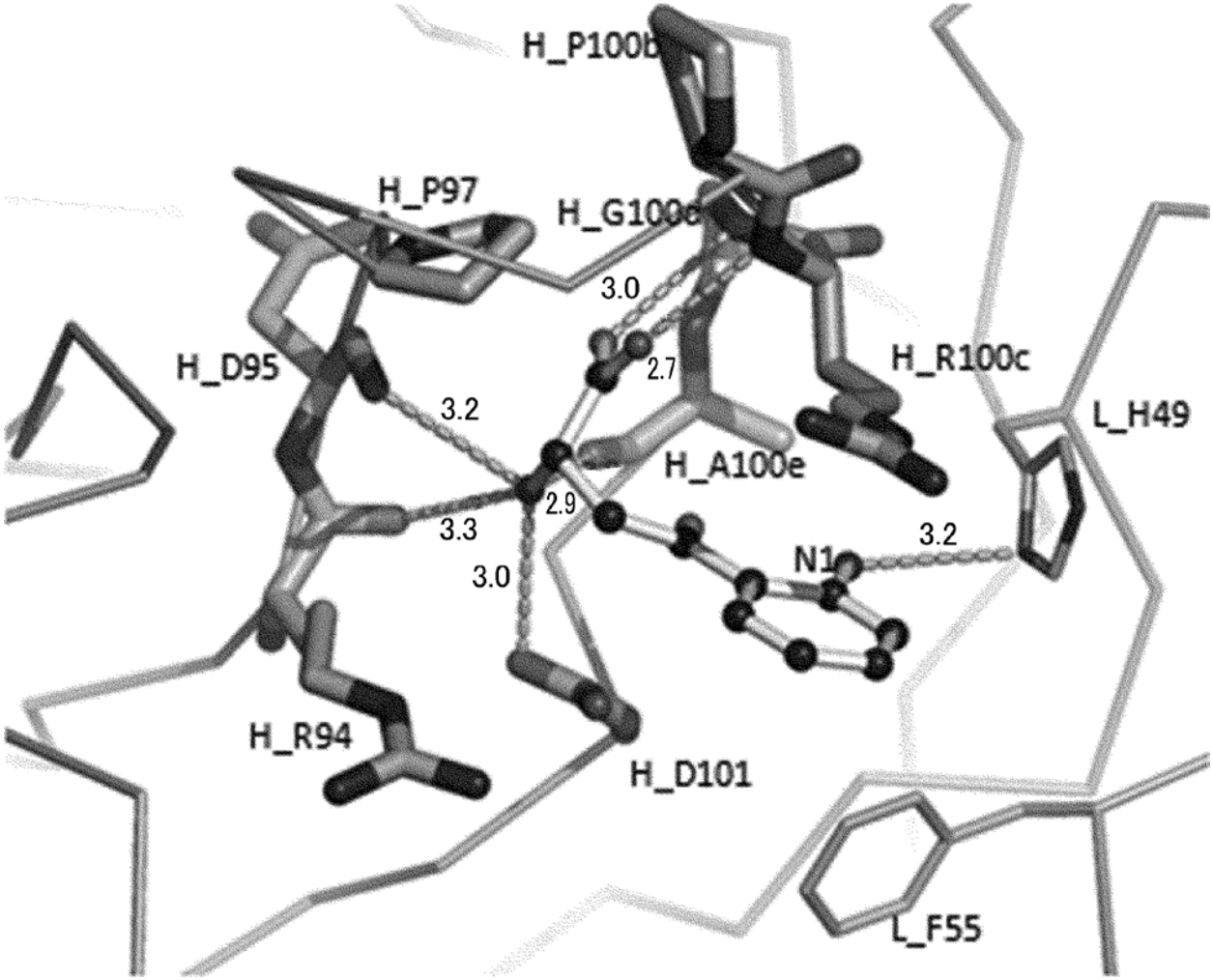

H_P100b
H_P97
H_G100d
3.0
H_D95
2.7
H_R100c
3.2
L_H49
H_A100e
2.9
3.3
3.2
N1
3.0
H_R94
H_D101
L_F55

FIG. 30

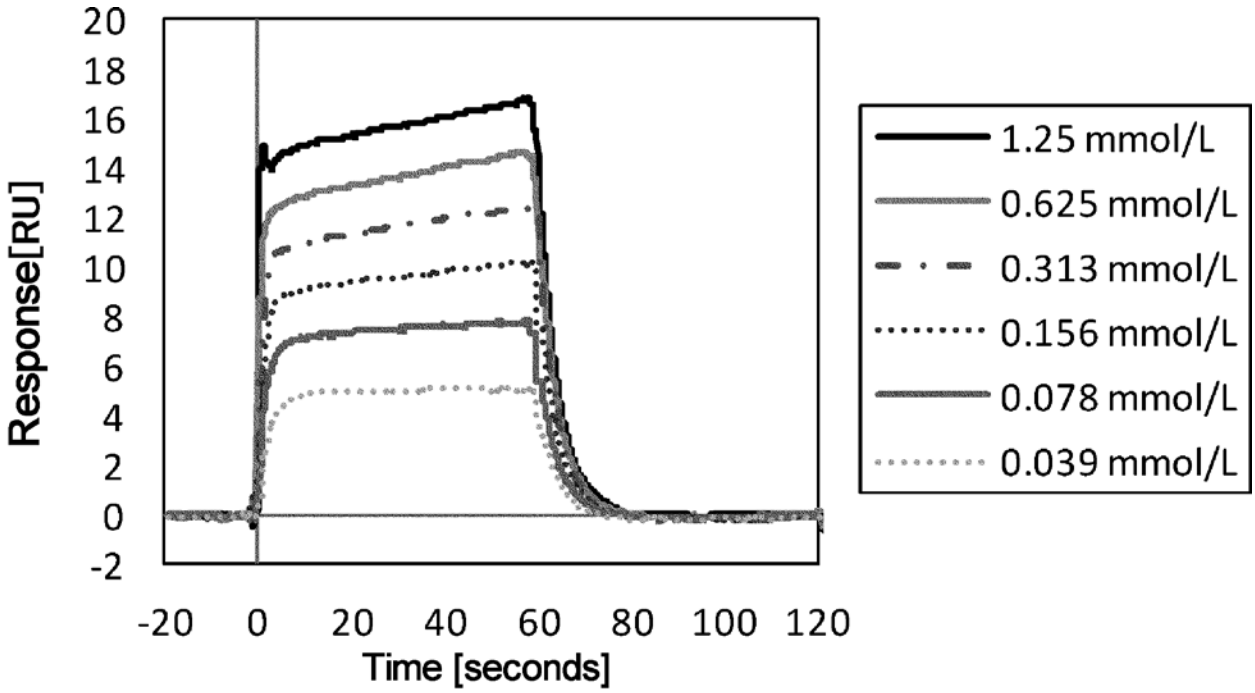

Response[RU]
20
18
16
14
12
10
8
6
4
2
0
-2
-20
0
20
40
60
80
100
120
Time [seconds]
1.25 mmol/L
0.625 mmol/L
0.313 mmol/L
0.156 mmol/L
0.078 mmol/L
0.039 mmol/L

FIG. 31

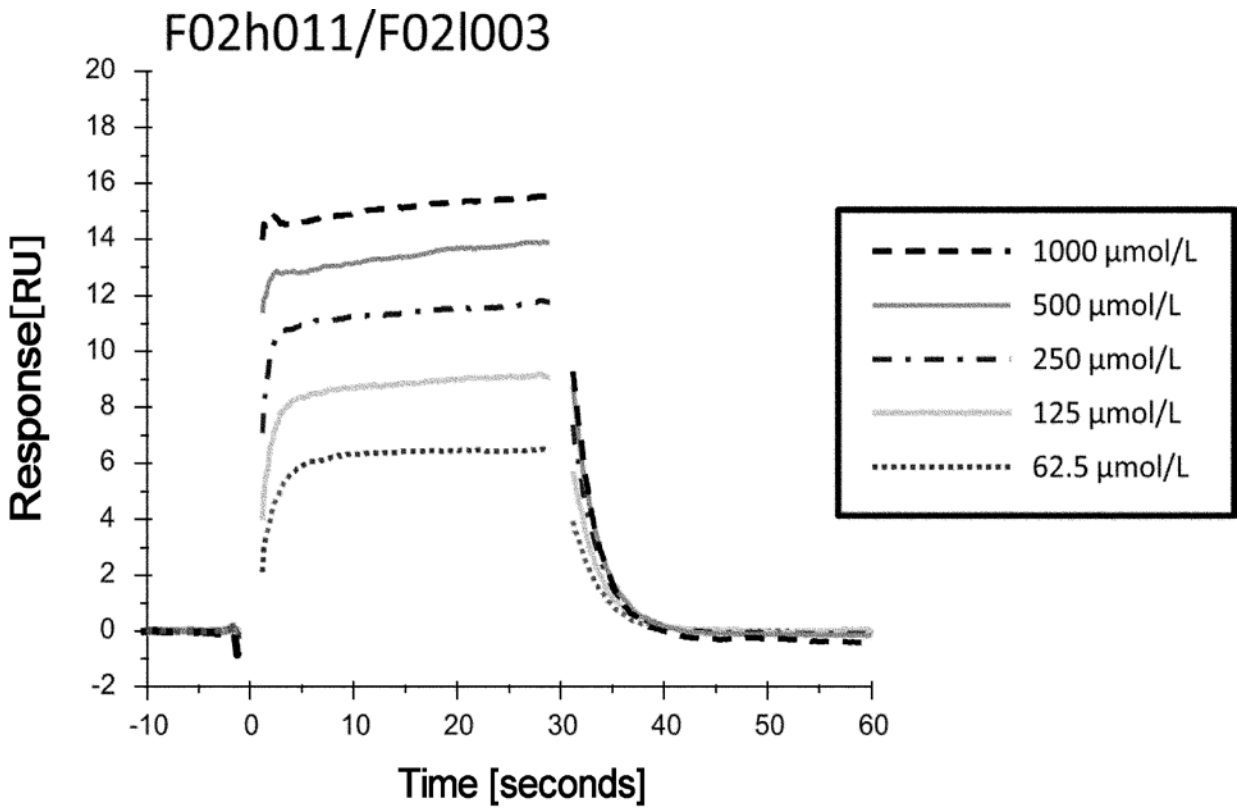

F02h011/F02l003
Response[RU]
Time [seconds]
1000 µmol/L
500 µmol/L
250 µmol/L
125 µmol/L
62.5 µmol/L

FIG. 32

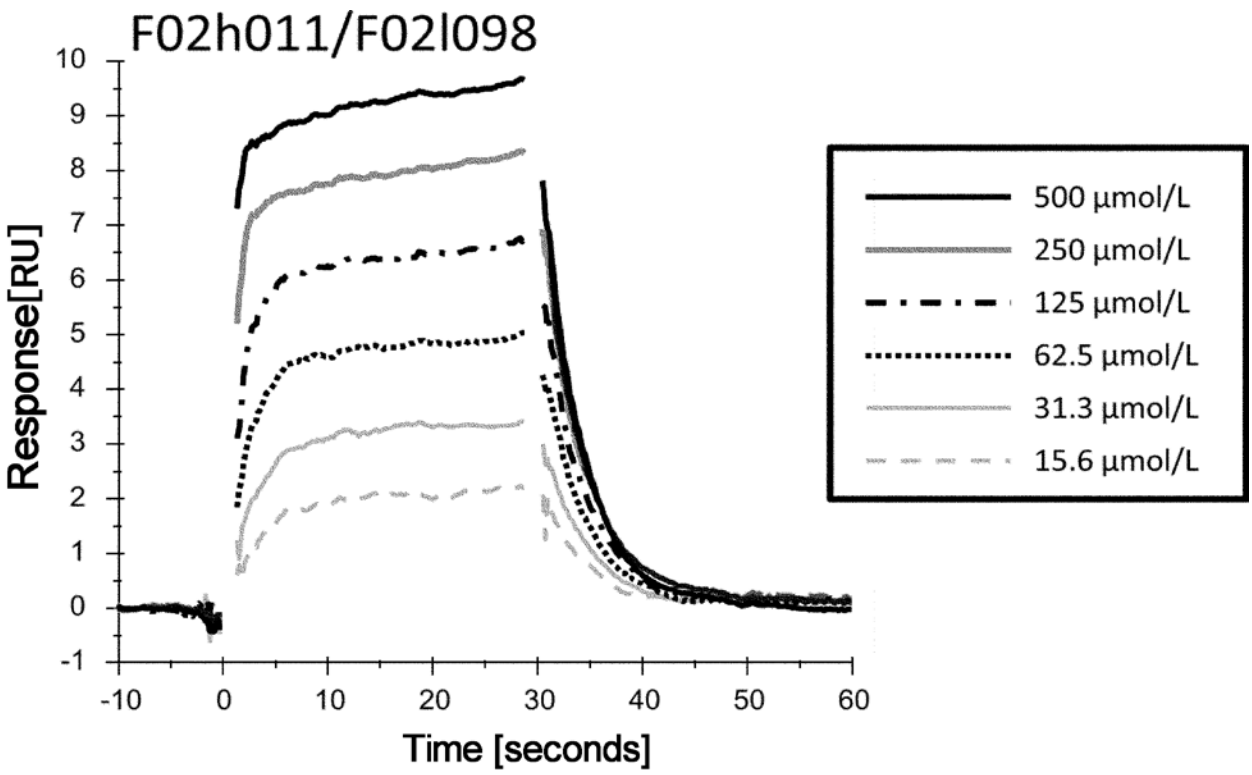

F02h011/F02l098
Response[RU]
Time [seconds]
500 µmol/L
250 µmol/L
125 µmol/L
62.5 µmol/L
31.3 µmol/L
15.6 µmol/L

FIG. 33

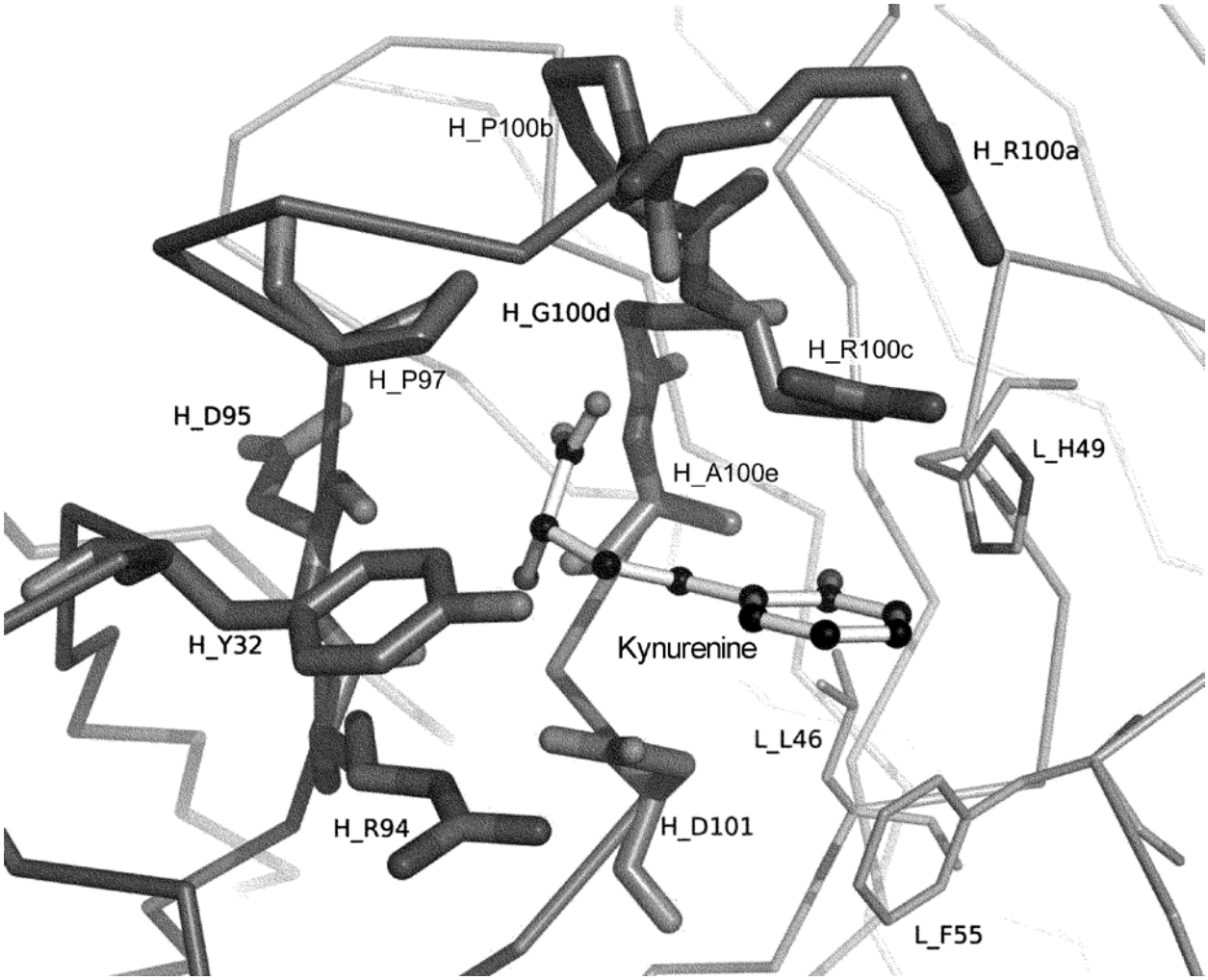

H_P100b
H_R100a
H_G100d
H_R100c
H_P97
H_D95
L_H49
H_A100e
H_Y32
Kynurenine
L_L46
H_R94
H_D101
L_F55

FIG. 34



FIG. 35

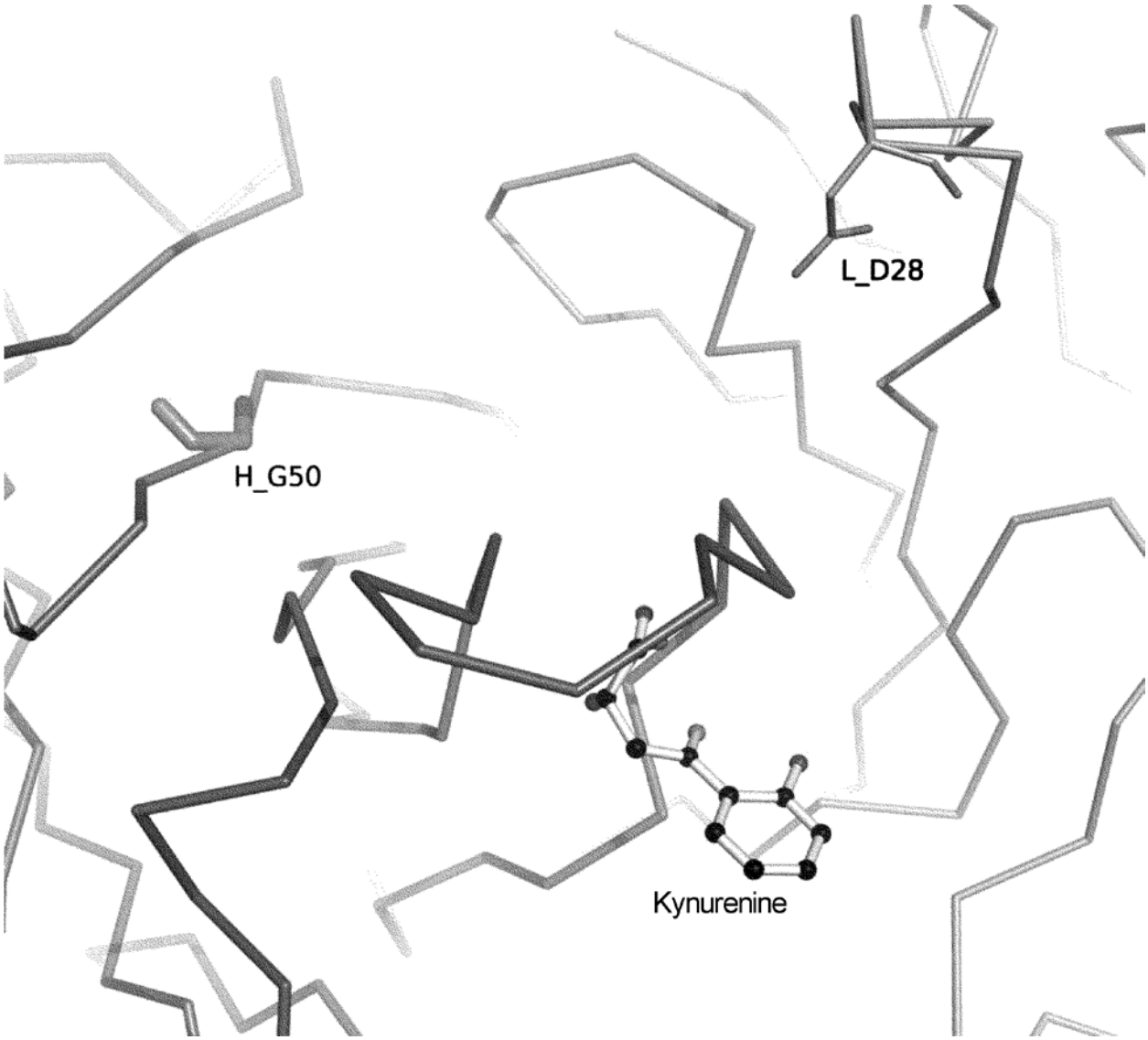


FIG. 36

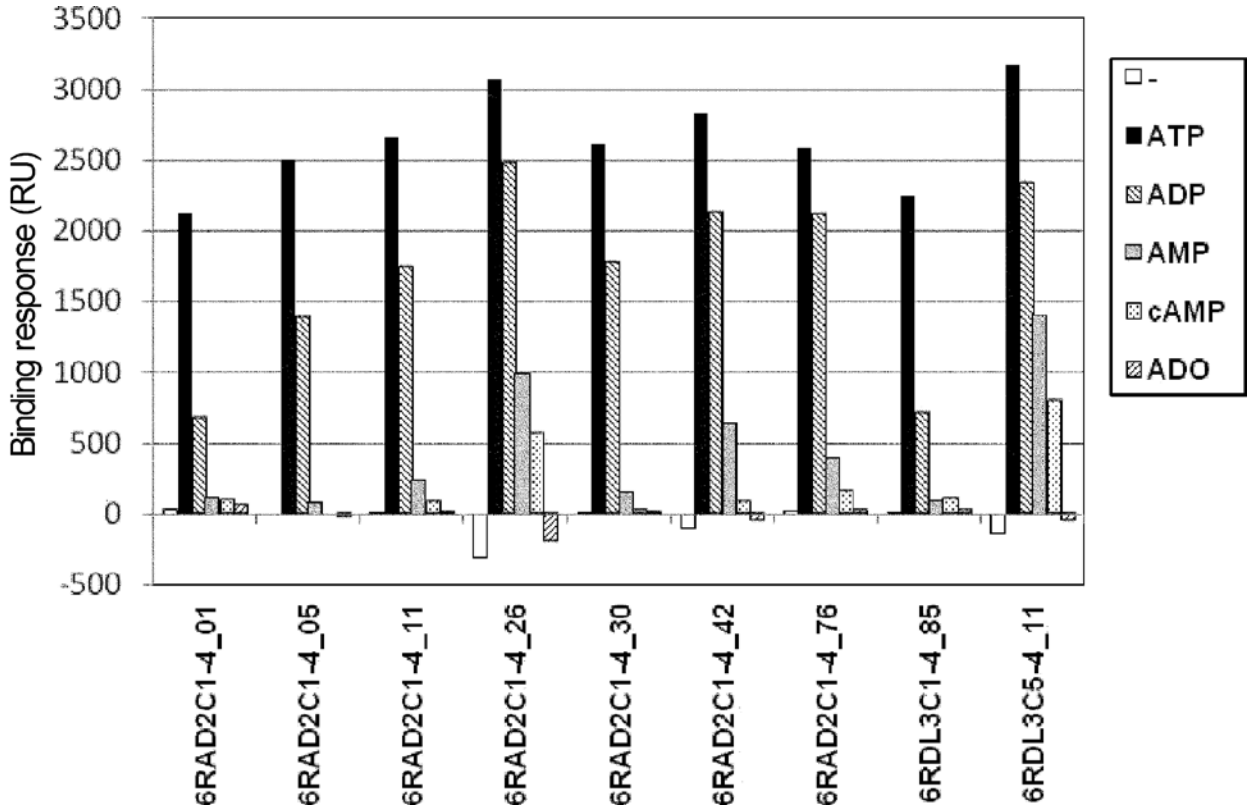

3500
3000
2500
2000
1500
1000
500
0
-500
Binding response (RU)
-
ATP
ADP
AMP
cAMP
ADO
6RAD2C1-4_01
6RAD2C1-4_05
6RAD2C1-4_11
6RAD2C1-4_26
6RAD2C1-4_30
6RAD2C1-4_42
6RAD2C1-4_76
6RDL3C1-4_85
6RDL3C5-4_11

FIG. 37

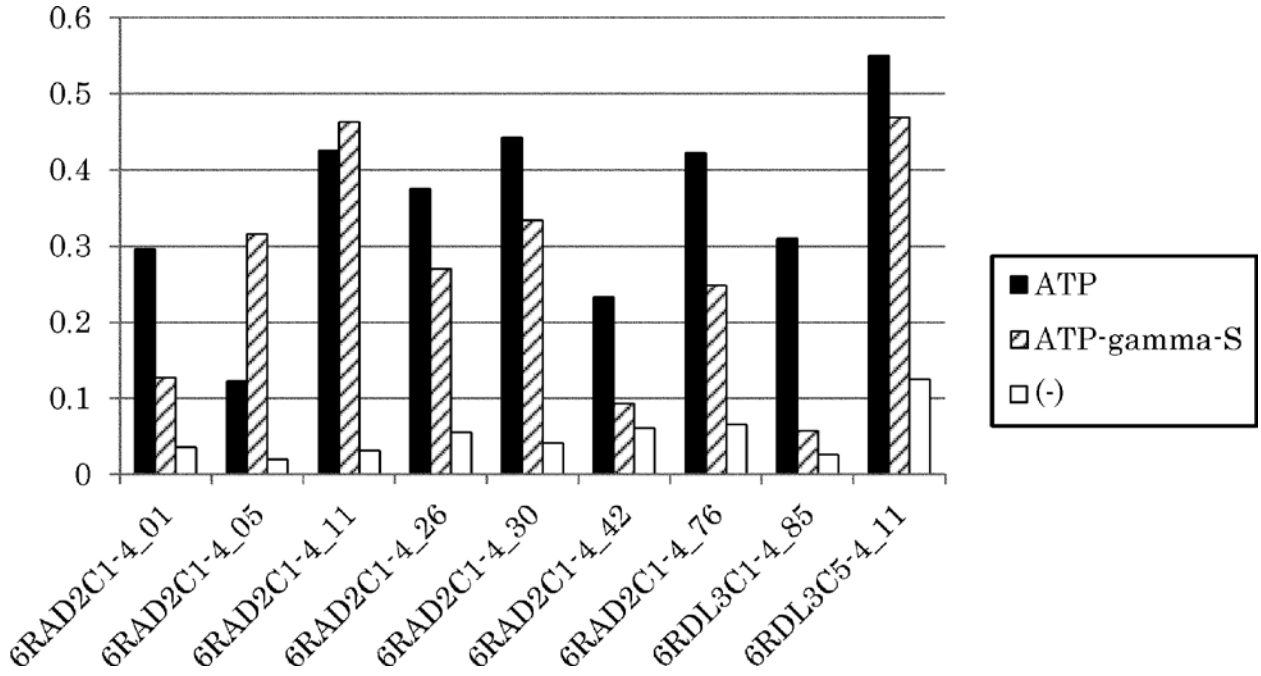

0.6
0.5
0.4
0.3
0.2
0.1
0
6RAD2C1-4_01
6RAD2C1-4_05
6RAD2C1-4_11
6RAD2C1-4_26
6RAD2C1-4_30
6RAD2C1-4_42
6RAD2C1-4_76
6RDL3C1-4_85
6RDL3C5-4_11
ATP
ATP-gamma-S
(-)

FIG. 38

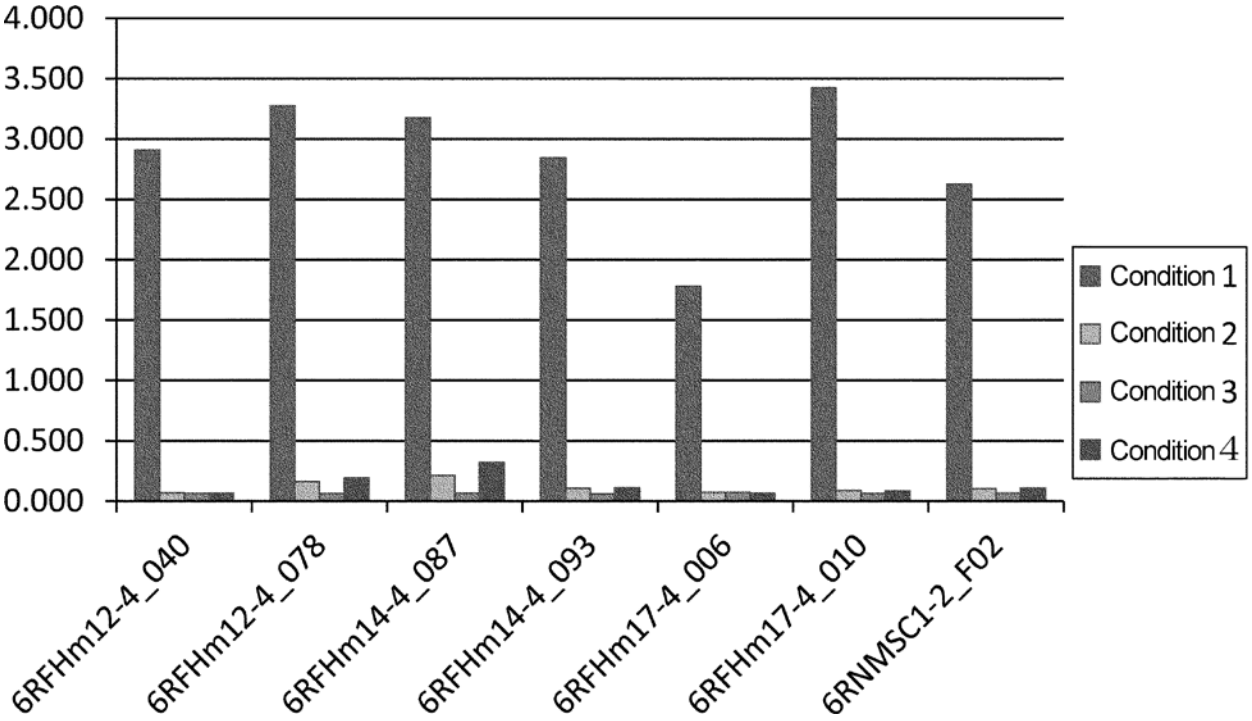

4.000
3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Condition 1
Condition 2
Condition 3
Condition 4
6RFHm12-4_040
6RFHm12-4_078
6RFHm14-4_087
6RFHm14-4_093
6RFHm17-4_006
6RFHm17-4_010
6RNMSC1-2_F02

FIG. 39

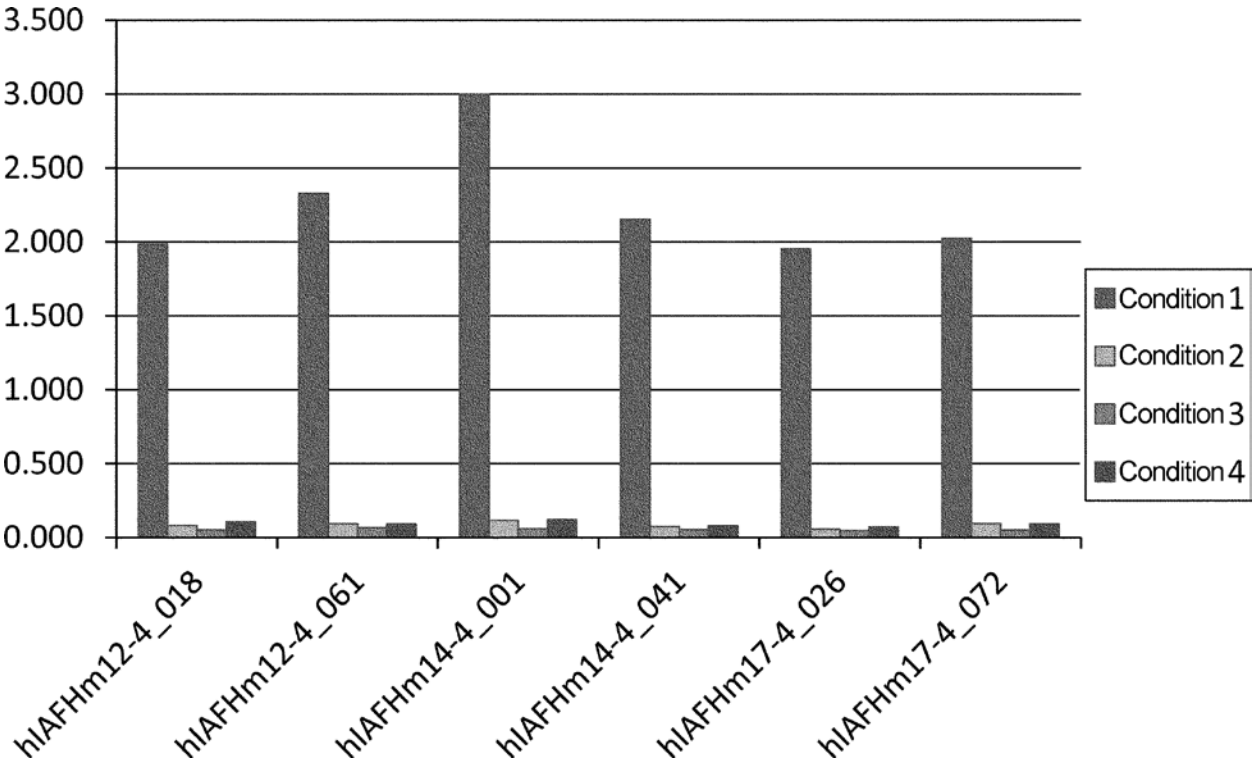

3.500
3.000
2.500
2.000
1.500
1.000
0.500
0.000
Condition 1
Condition 2
Condition 3
Condition 4
hIAFHm12-4_018
hIAFHm12-4_061
hIAFHm14-4_001
hIAFHm14-4_041
hIAFHm17-4_026
hIAFHm17-4_072

FIG. 40

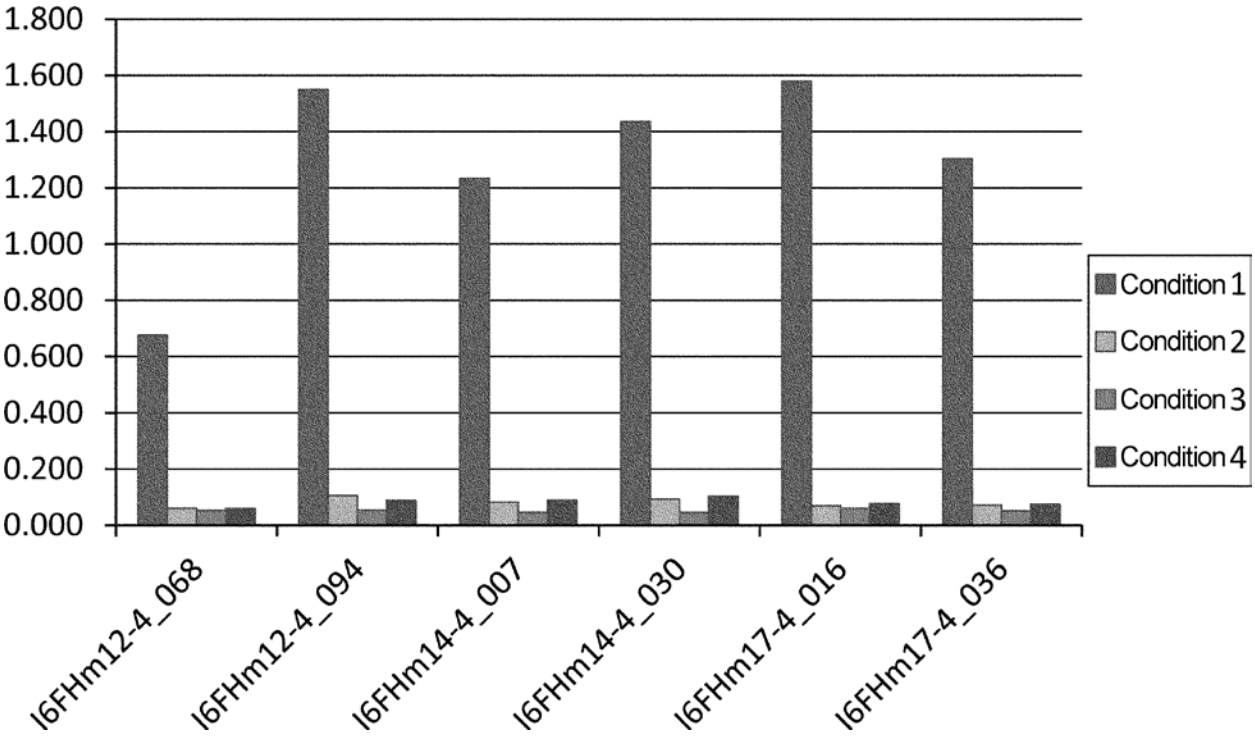

1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
Condition 1
Condition 2
Condition 3
Condition 4
I6FHm12-4_068
I6FHm12-4_094
I6FHm14-4_007
I6FHm14-4_030
I6FHm17-4_016
I6FHm17-4_036

FIG. 41

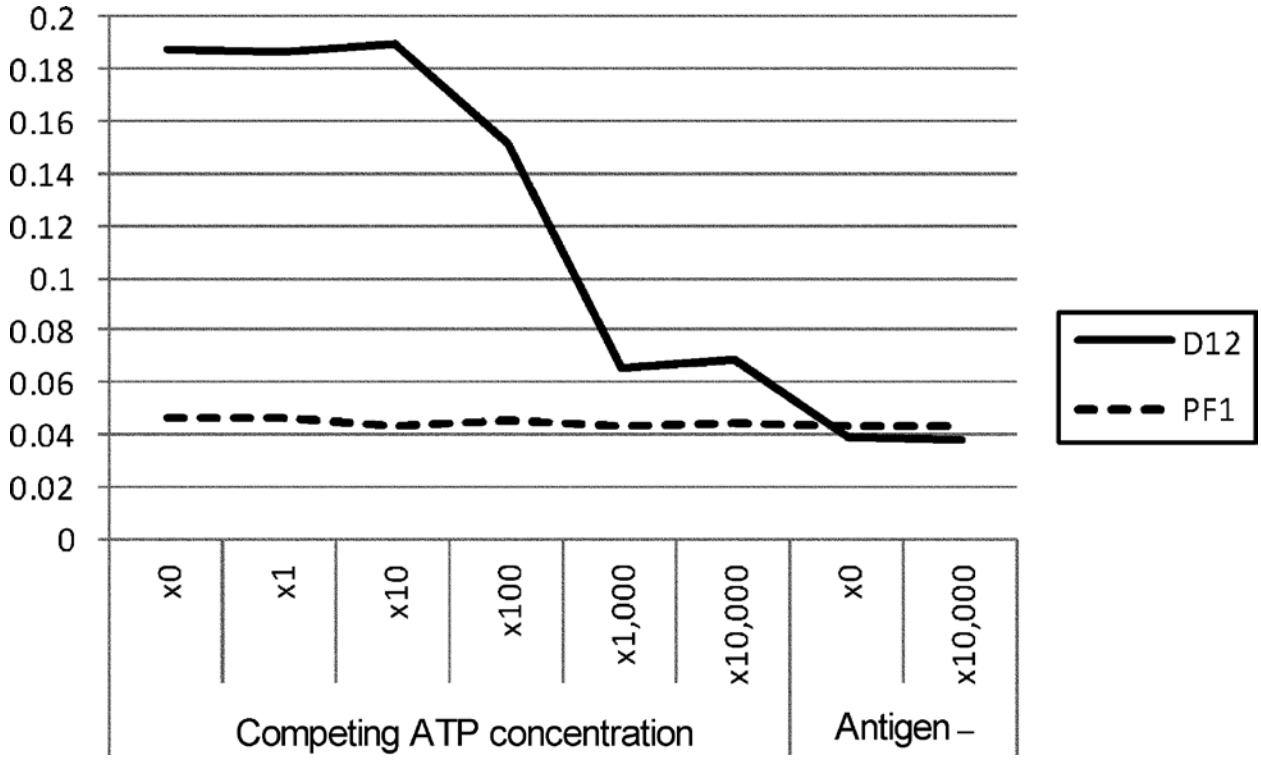

0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
x0
x1
x10
x100
x1,000
x10,000
x0
x10,000
Competing ATP concentration
Antigen –
D12
PF1

FIG. 42

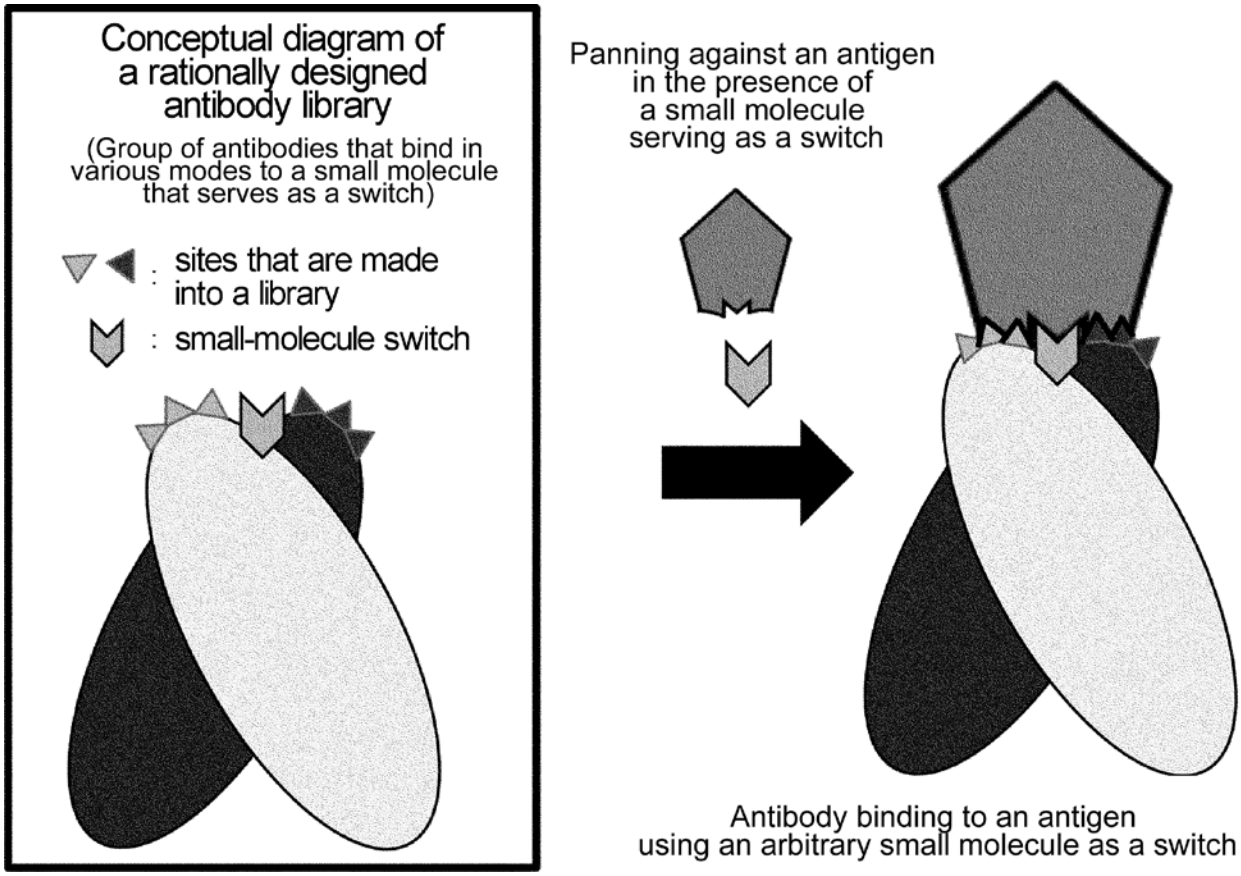

Conceptual diagram of a rationally designed antibody library
(Group of antibodies that bind in various modes to a small molecule that serves as a switch)
: sites that are made into a library
: small-molecule switch
Panning against an antigen in the presence of a small molecule serving as a switch
Antibody binding to an antigen using an arbitrary small molecule as a switch

FIG. 43

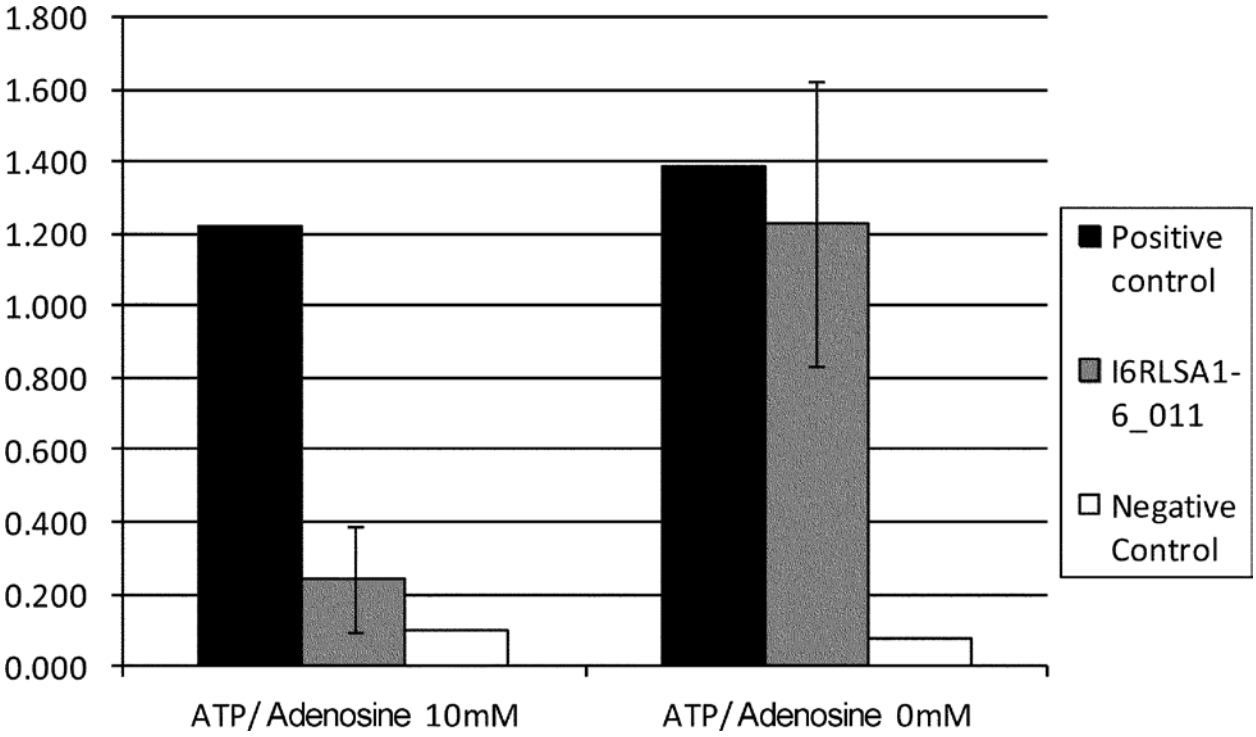

1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
ATP/Adenosine 10mM
ATP/Adenosine 0mM
Positive control
I6RLSA1-6_011
Negative Control

FIG. 44

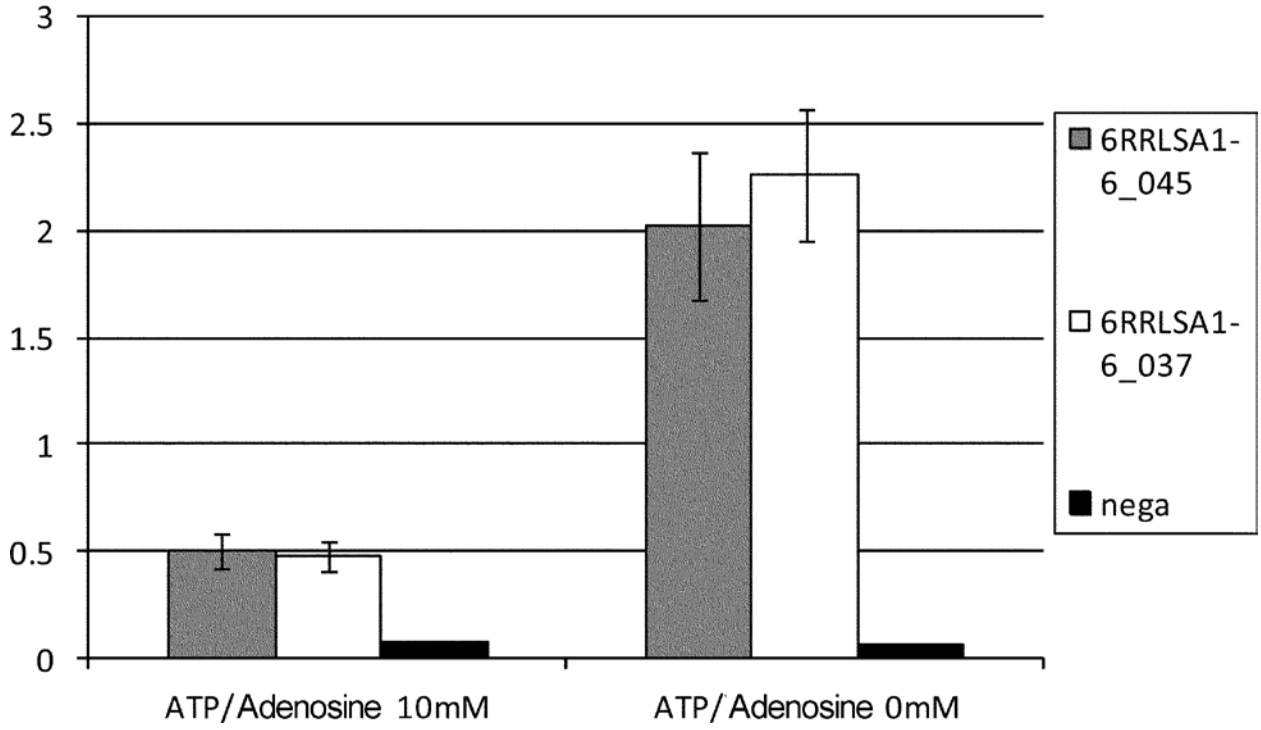

3
2.5
2
1.5
1
0.5
0
ATP/Adenosine 10mM
ATP/Adenosine 0mM
6RRLSA1-6_045
6RRLSA1-6_037
nega

FIG. 45

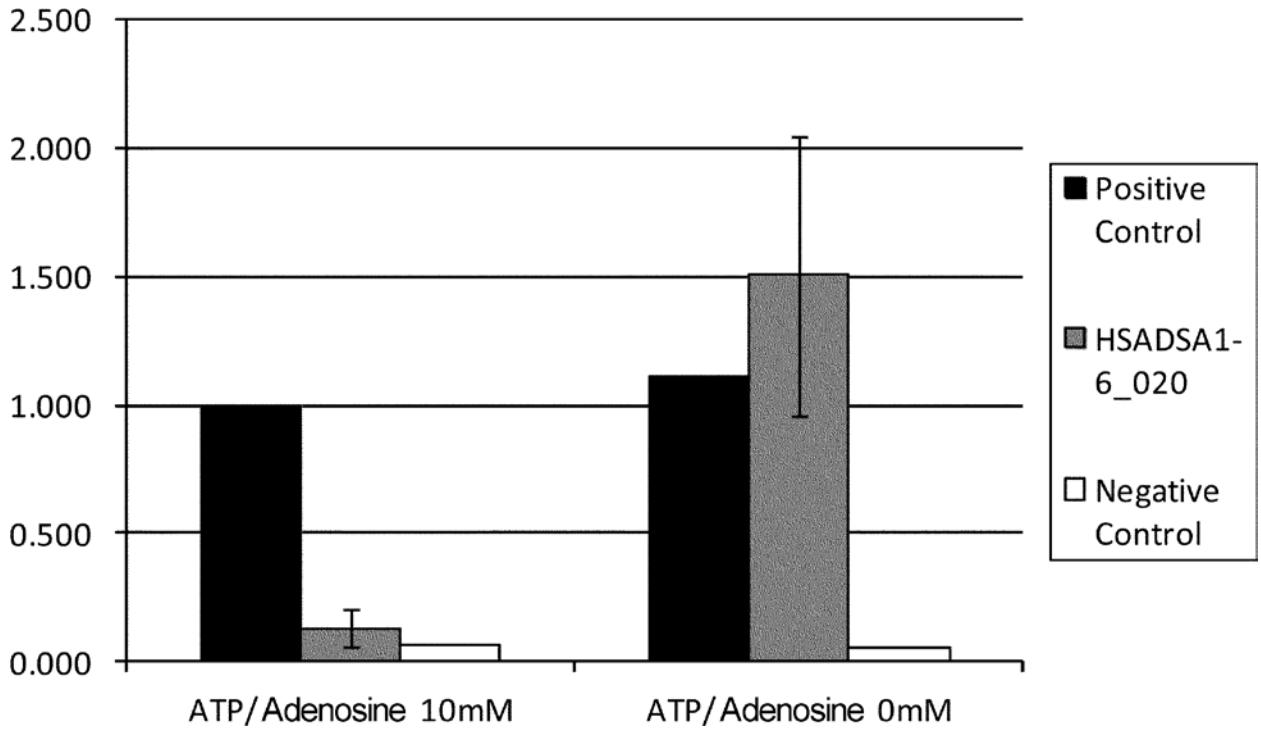

2.500
2.000
1.500
1.000
0.500
0.000
ATP/Adenosine 10mM
ATP/Adenosine 0mM
Positive Control
HSADSA1-6_020
Negative Control

FIG. 46