Figure 1

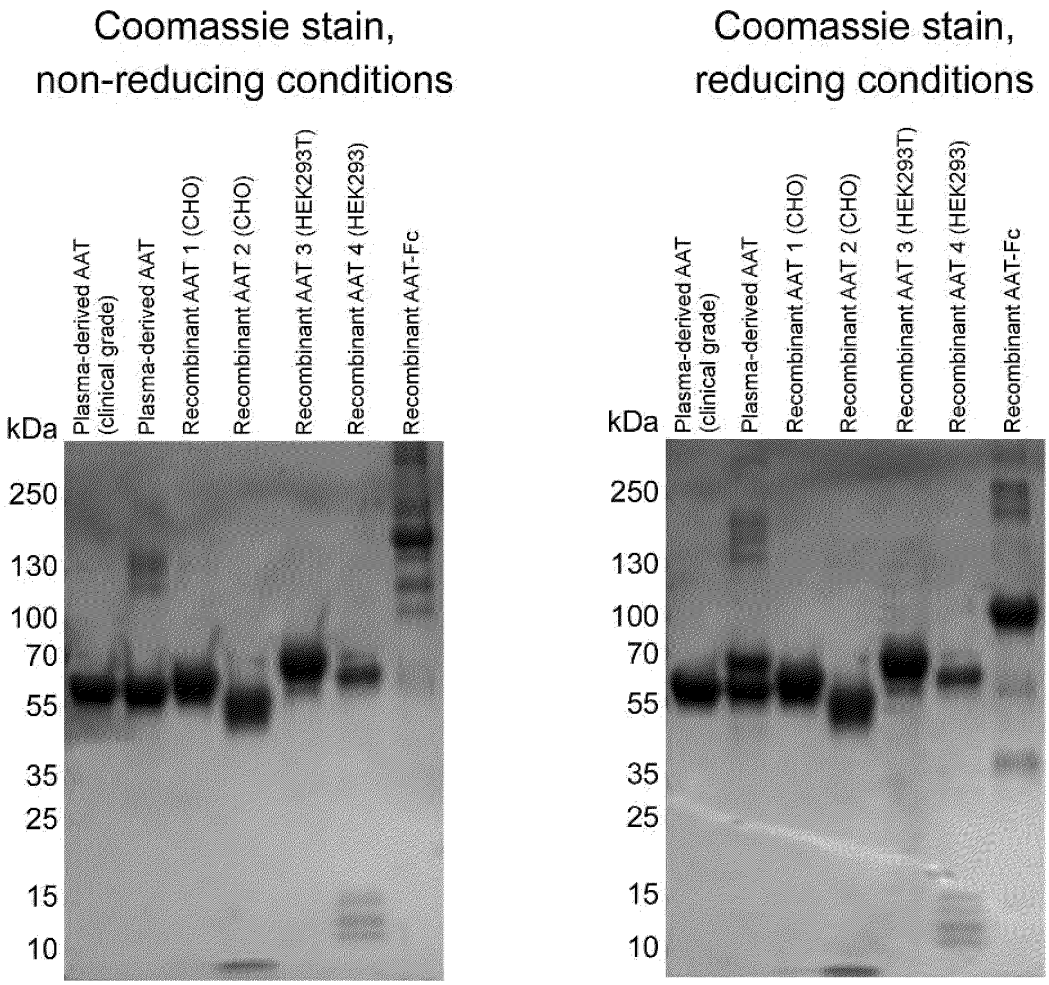

Coomassie stain,
non-reducing conditions
Coomassie stain,
reducing conditions
kDa
250
130
100
70
55
35
25
15
10
Plasma-derived AAT (clinical grade)
Plasma-derived AAT
Recombinant AAT 1 (CHO)
Recombinant AAT 2 (CHO)
Recombinant AAT 3 (HEK293T)
Recombinant AAT 4 (HEK293)
Recombinant AAT-Fc

Figure 2

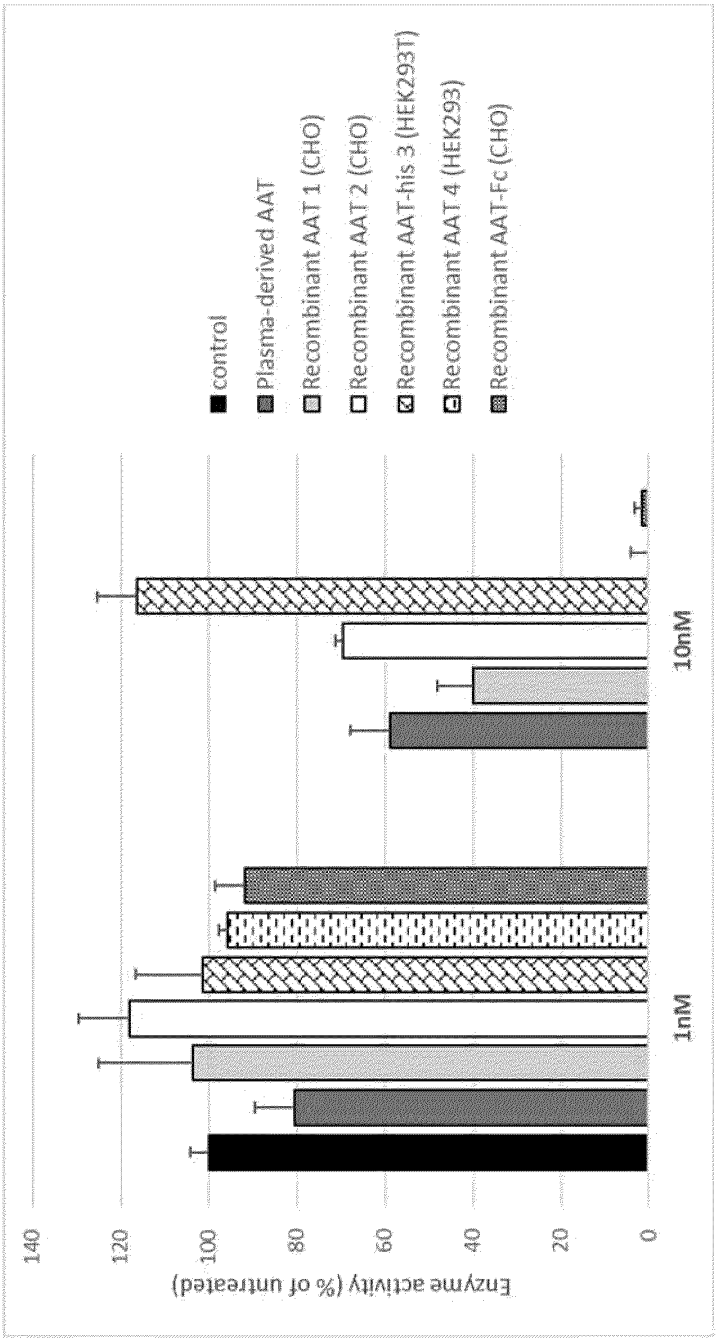

control
Plasma-derived AAT
Recombinant AAT 1 (CHO)
Recombinant AAT 2 (CHO)
Recombinant AAT-his 3 (HEK293T)
Recombinant AAT 4 (HEK293)
Recombinant AAT-Fc (CHO)
Enzyme activity (% of untreated)
0
20
40
60
80
100
120
140
1nM
10nM

Figure 3

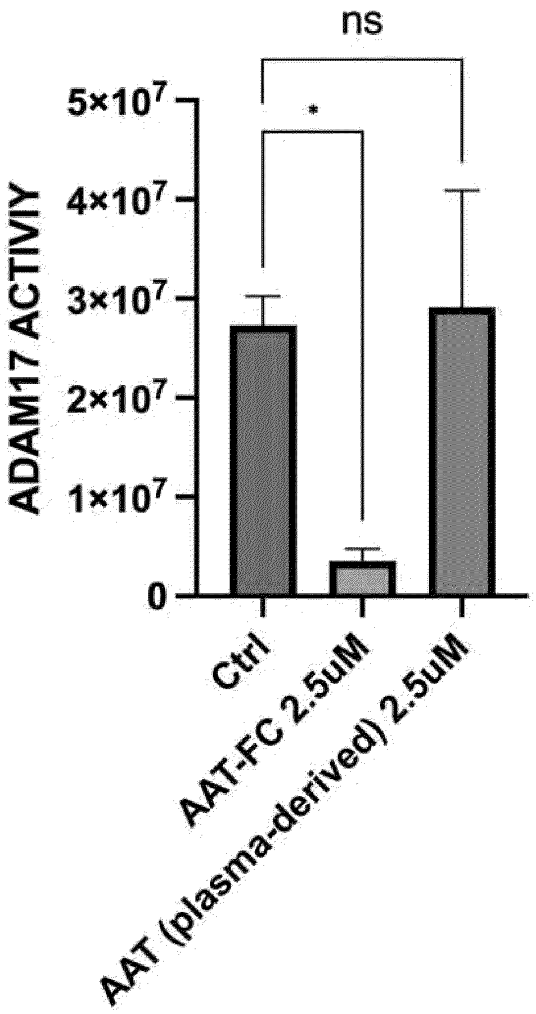

ns
*
5×10^7
4×10^7
3×10^7
2×10^7
1×10^7
0
ADAM17 ACTIVIY
Ctrl
AAT-FC 2.5uM
AAT (plasma-derived) 2.5uM

Figure 4

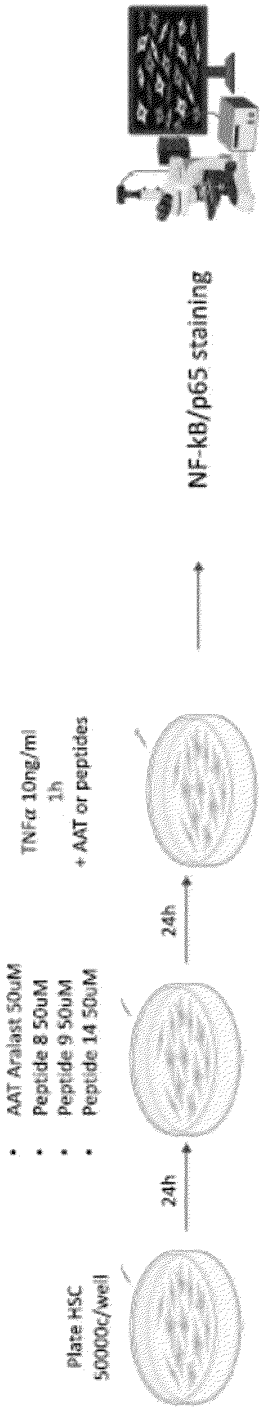


Plate HSC
50000c/well
24h
AAT Aralast 50uM
Peptide 8 50uM
Peptide 9 50uM
Peptide 14 50uM
24h
TNFα 10ng/ml
1h
+ AAT or peptides
NF-kB/p65 staining

Figure 5

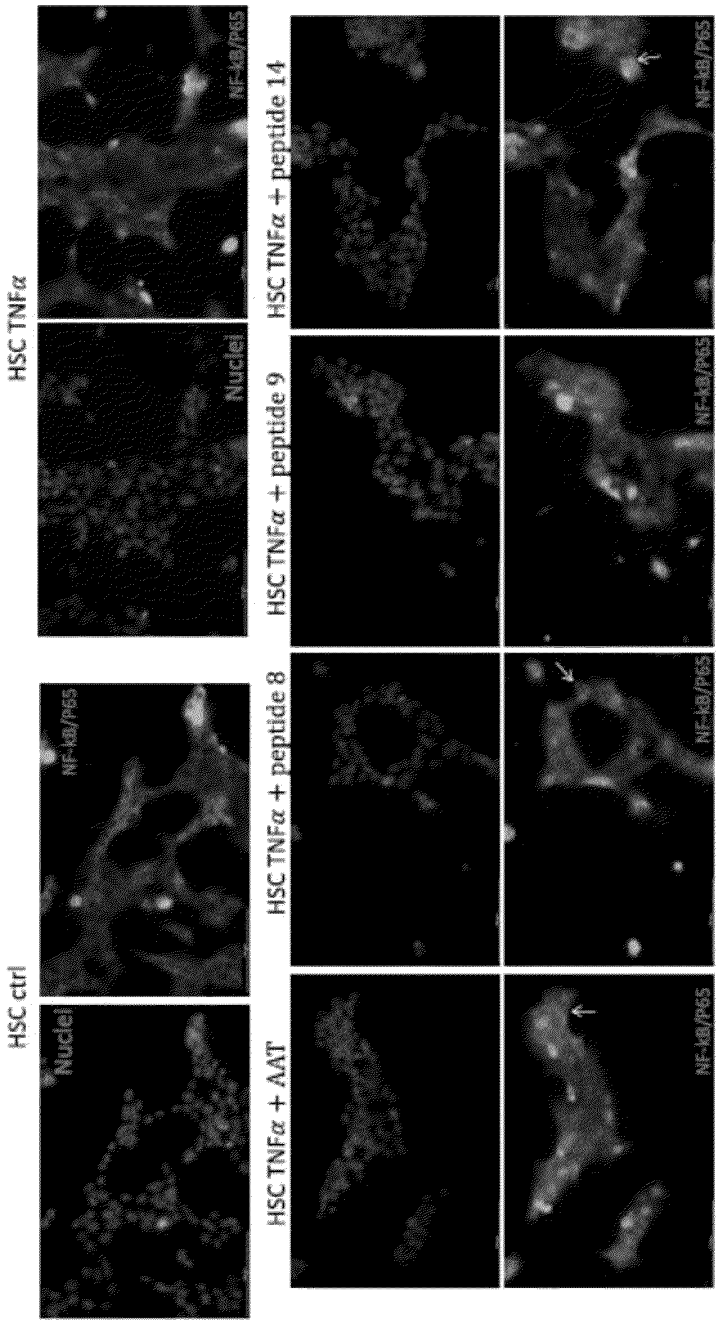
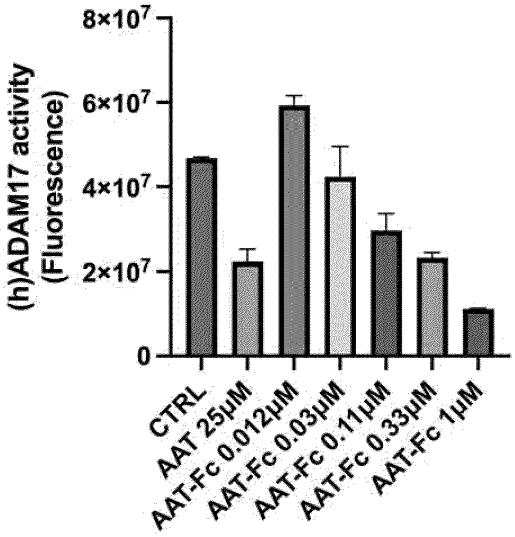

HSC ctrl
Nuclei
NF-kB/P65
HSC TNFα
Nuclei
NF-kB/P65
HSC TNFα + AAT
NF-kB/P65
HSC TNFα + peptide 8
NF-kB/P65
HSC TNFα + peptide 9
NF-kB/P65
HSC TNFα + peptide 14
NF-kB/P65

Figure 6

A


(h)ADAM17 activity
(Fluorescence)
8×10^7
6×10^7
4×10^7
2×10^7
0
CTRL
AAT 25µM
AAT-Fc 0.012µM
AAT-Fc 0.03µM
AAT-Fc 0.11µM
AAT-Fc 0.33µM
AAT-Fc 1µM

B

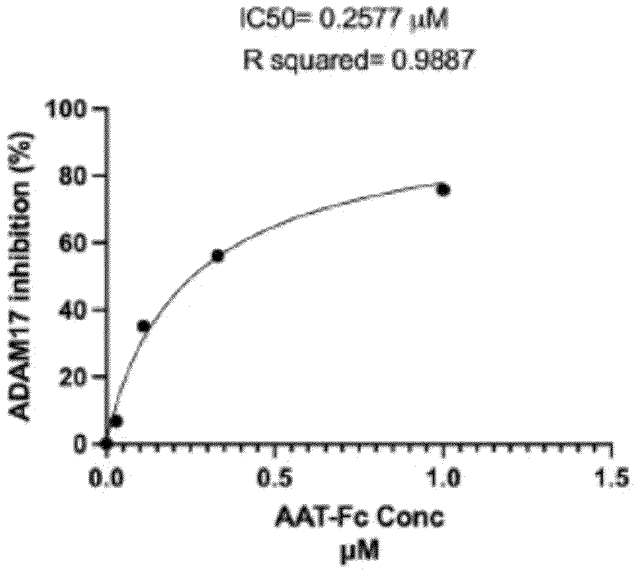

IC50= 0.2577 µM
R squared= 0.9887
ADAM17 inhibition (%)
100
80
60
40
20
0
0.0
0.5
1.0
1.5
AAT-Fc Conc
µM

Figure 7

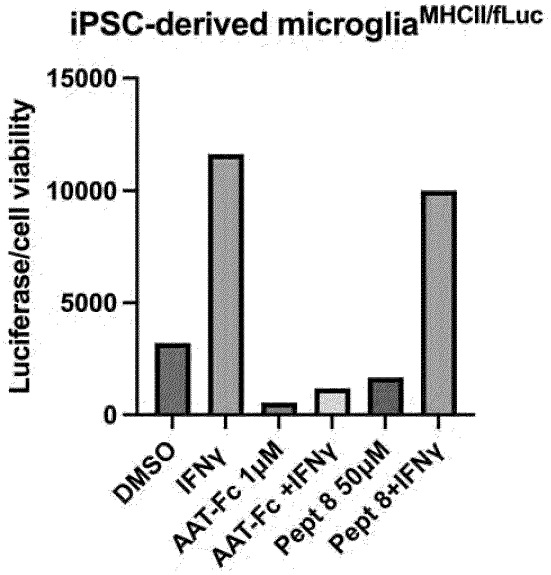
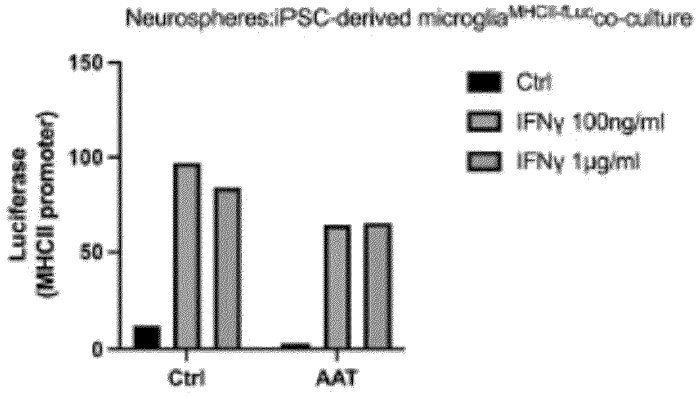


Figure 8



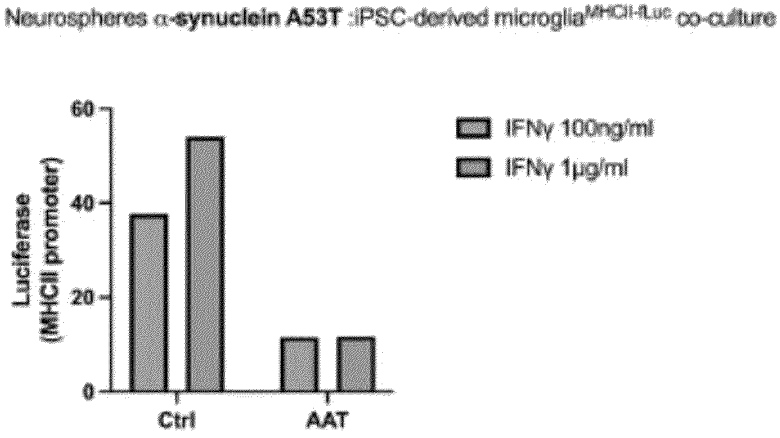
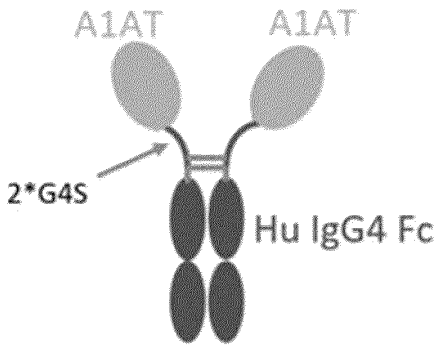
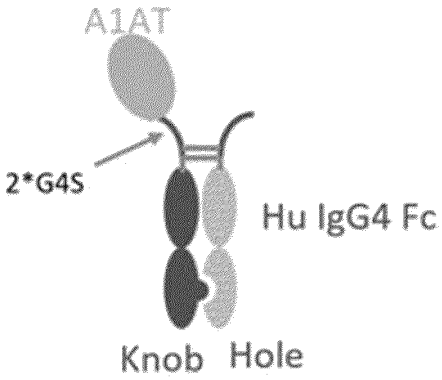


Figure 9



Construct 1

Figure 10



Construct 2

Figure 11

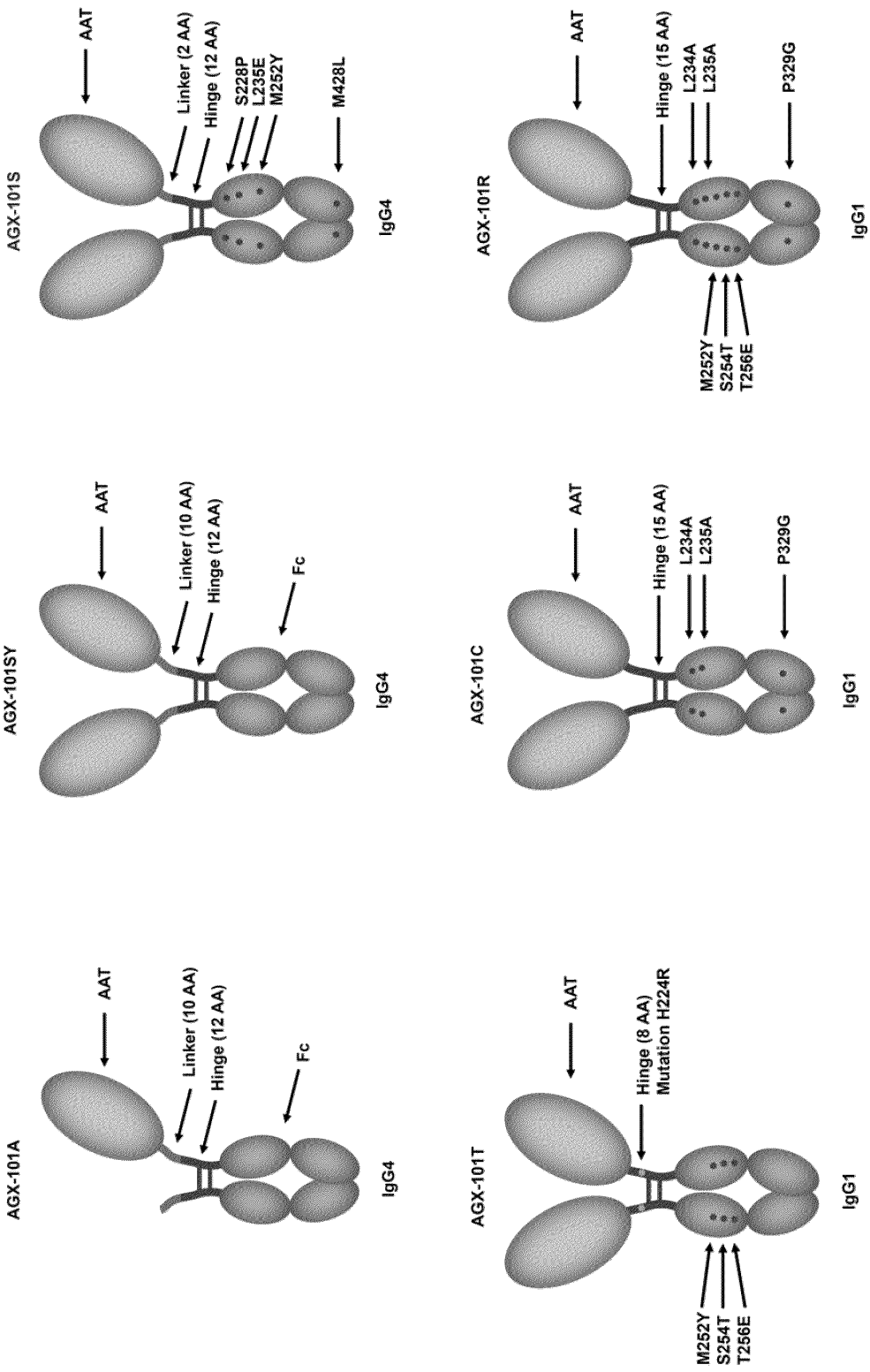
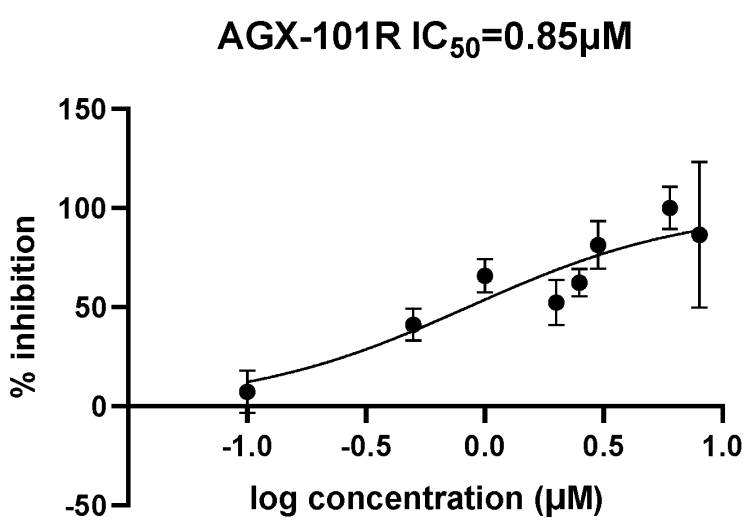

AGX-101S
AAT
Linker (2 AA)
Hinge (12 AA)
S228P
L235E
M252Y
M428L
IgG4
AGX-101R
AAT
Hinge (15 AA)
L234A
L235A
P329G
M252Y
S254T
T256E
IgG1
AGX-101SY
AAT
Linker (10 AA)
Hinge (12 AA)
Fc
IgG4
AGX-101C
AAT
Hinge (15 AA)
L234A
L235A
P329G
IgG1
AGX-101A
AAT
Linker (10 AA)
Hinge (12 AA)
Fc
IgG4
AGX-101T
AAT
Hinge (8 AA)
Mutation H224R
M252Y
S254T
T256E
IgG1

Figure 12

A


AGX-101R IC50=0.85µM
150
100
50
0
-50
% inhibition
-1.0
-0.5
0.0
0.5
1.0
log concentration (µM)

B

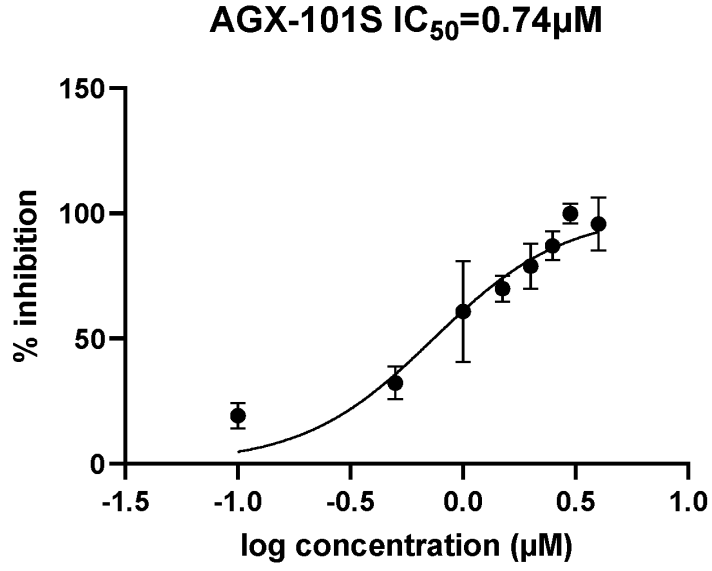
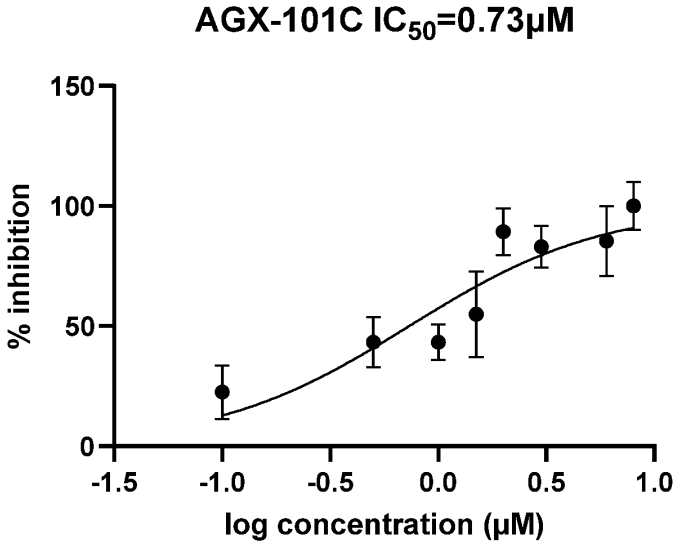

AGX-101S IC50=0.74µM
150
100
50
0
% inhibition
-1.5
-1.0
-0.5
0.0
0.5
1.0
log concentration (µM)

Figure 12 Continued

C



D

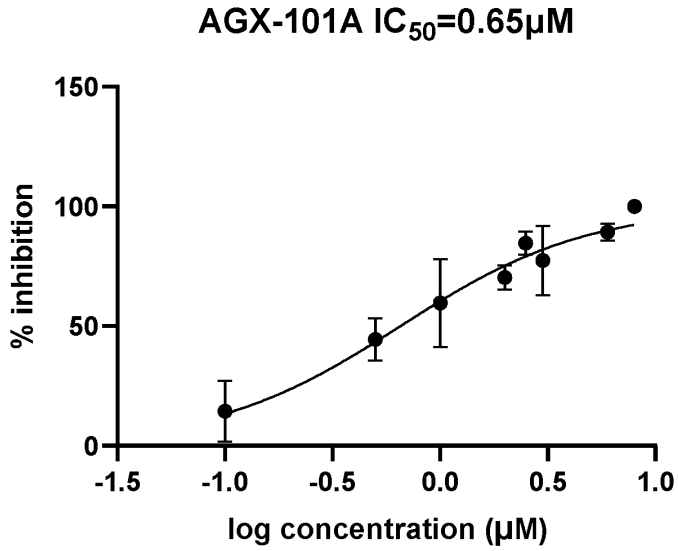


Figure 12 Continued

E

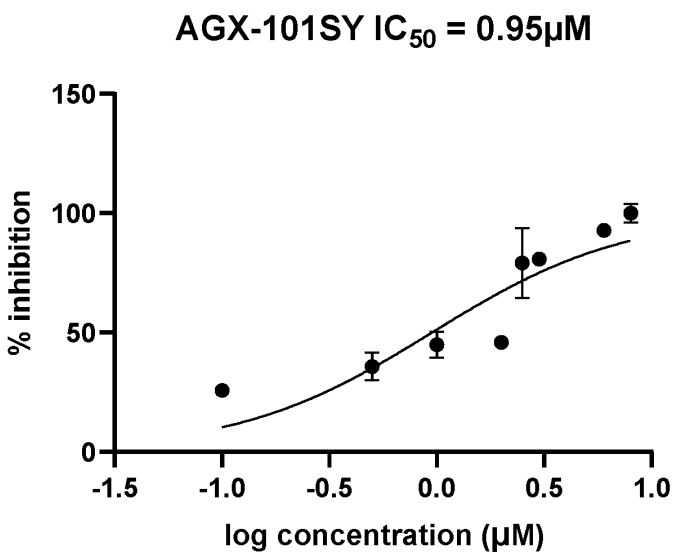
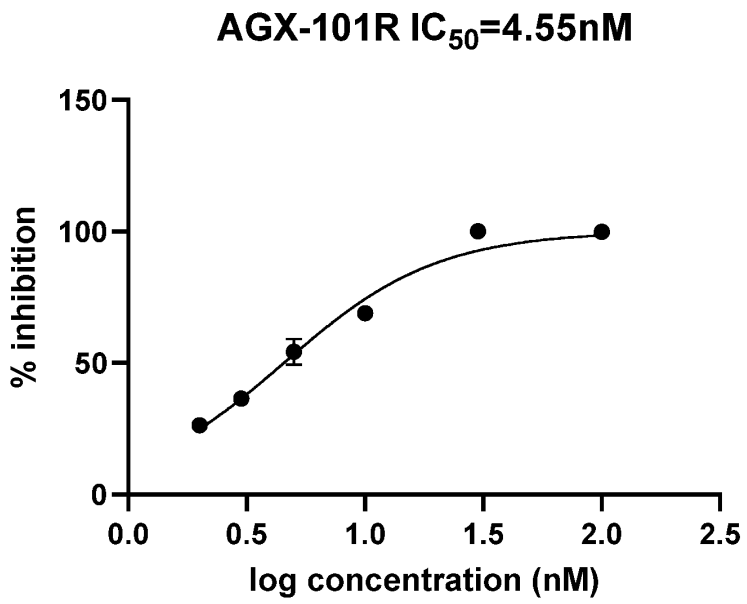


Figure 13

A


AGX-101R IC50=4.55nM
150
100
50
0
% inhibition
0.0
0.5
1.0
1.5
2.0
2.5
log concentration (nM)

B

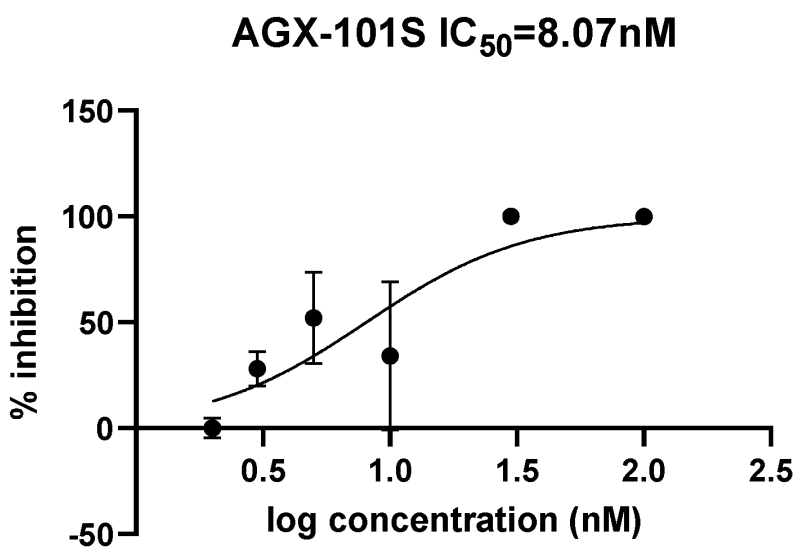
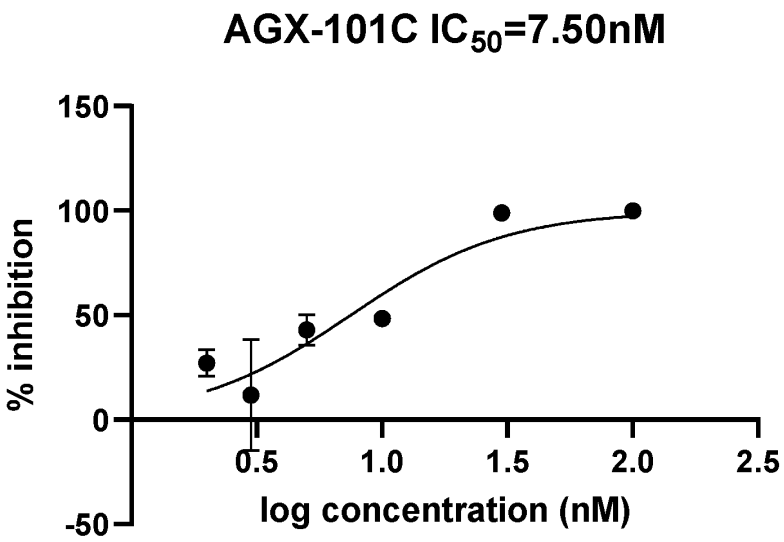

AGX-101S IC50=8.07nM
150
100
50
0
-50
% inhibition
0.5
1.0
1.5
2.0
2.5
log concentration (nM)

Figure 13 Continued

C



D

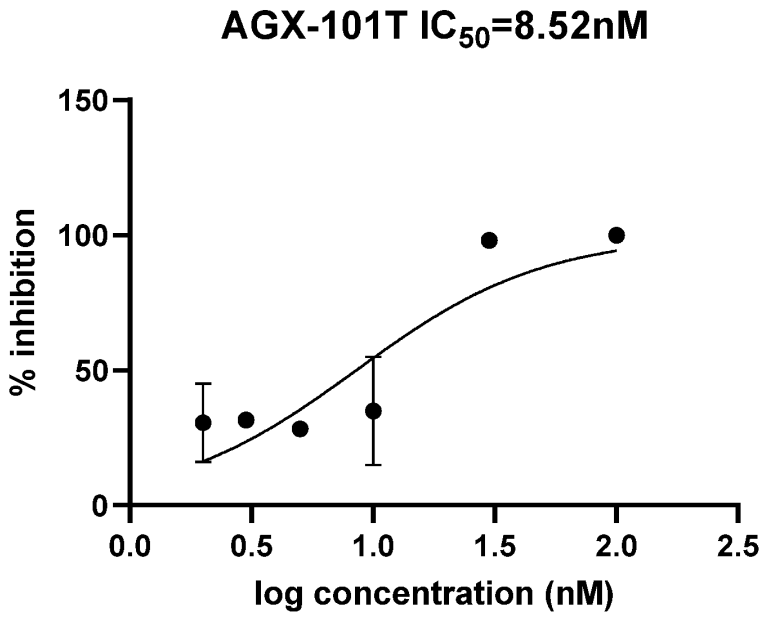
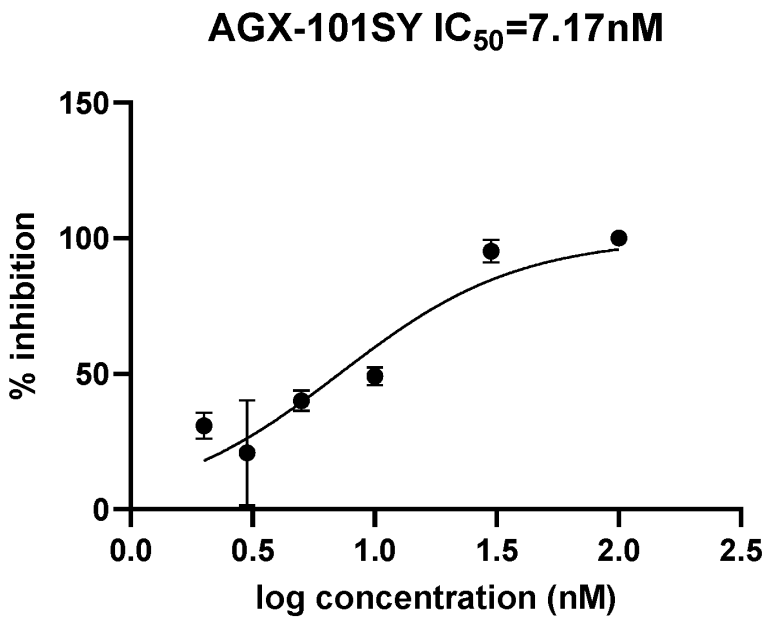


Figure 13 Continued

E



F

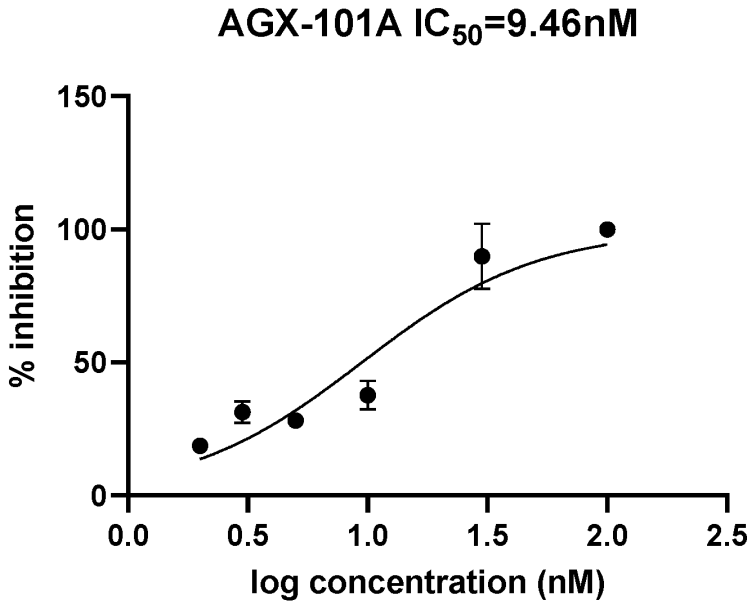
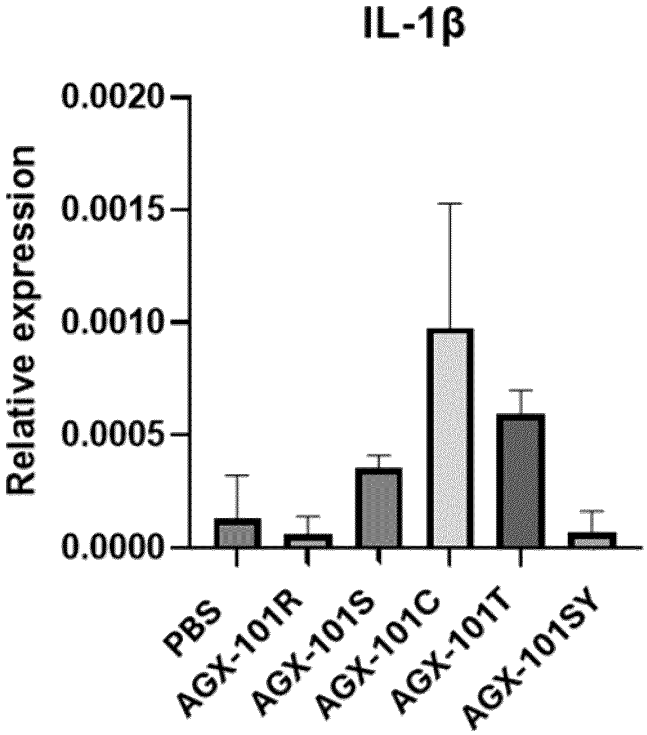


Figure 14

A


IL-1β
Relative expression
0.0020
0.0015
0.0010
0.0005
0.0000
PBS
AGX-101R
AGX-101S
AGX-101C
AGX-101T
AGX-101SY

B

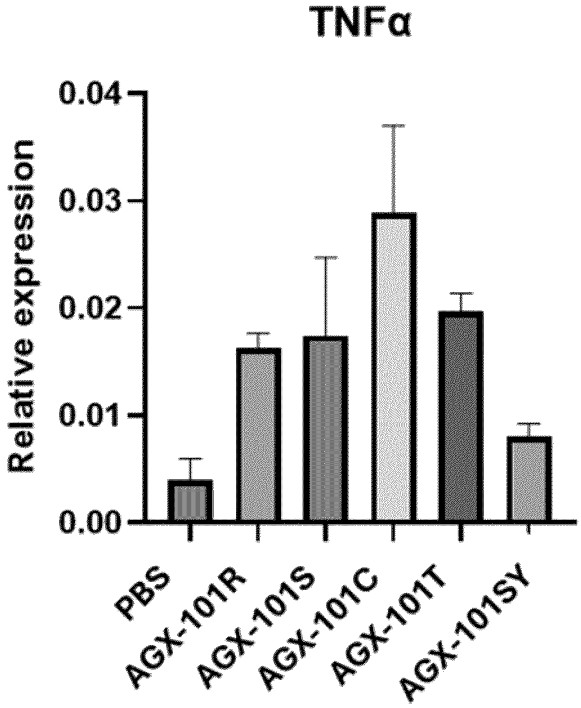
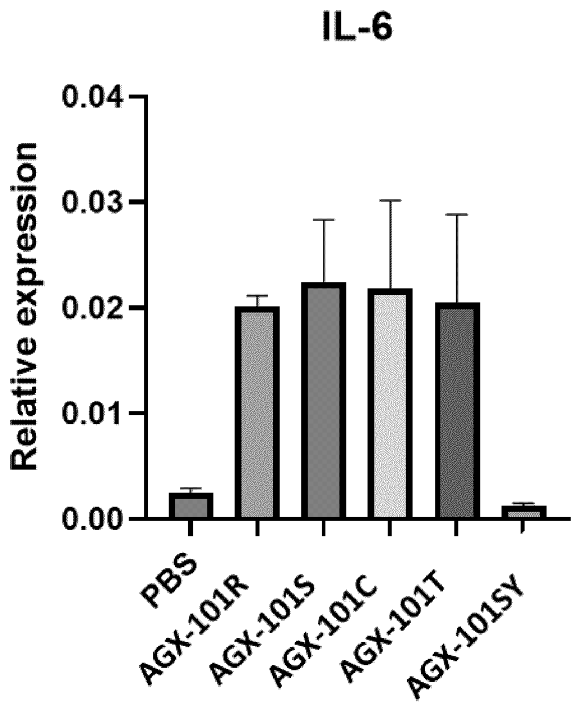

TNFα
Relative expression
0.04
0.03
0.02
0.01
0.00
PBS
AGX-101R
AGX-101S
AGX-101C
AGX-101T
AGX-101SY

Figure 14 Continued

C



D

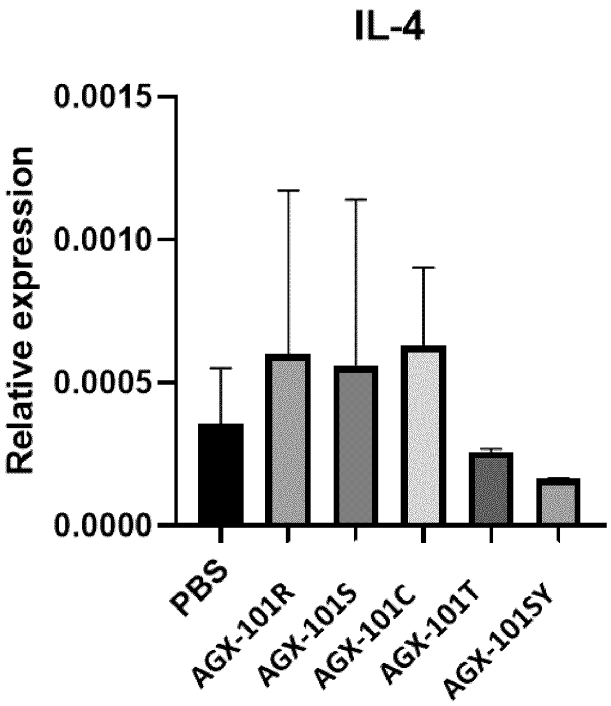


Figure 15

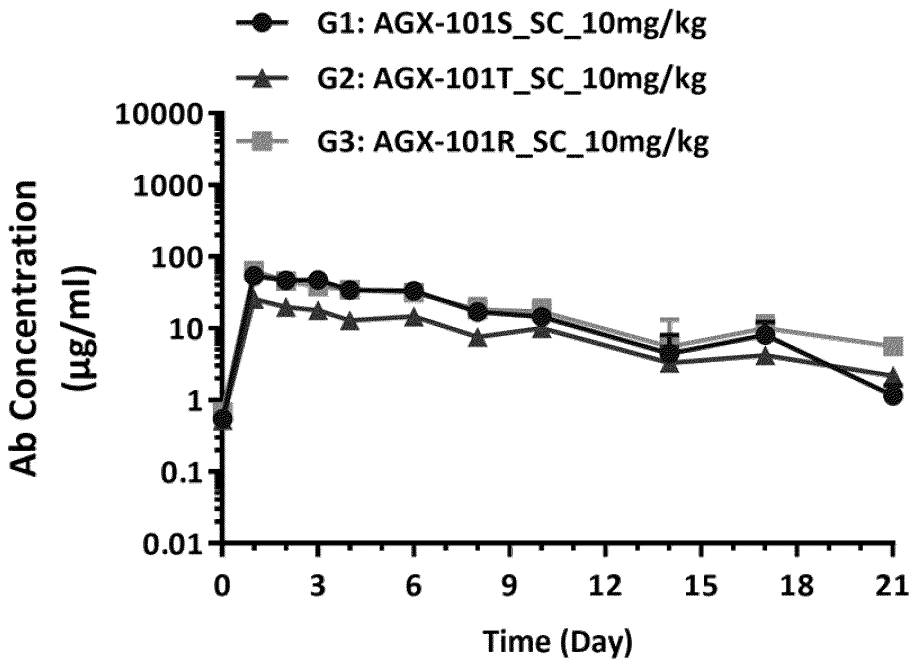
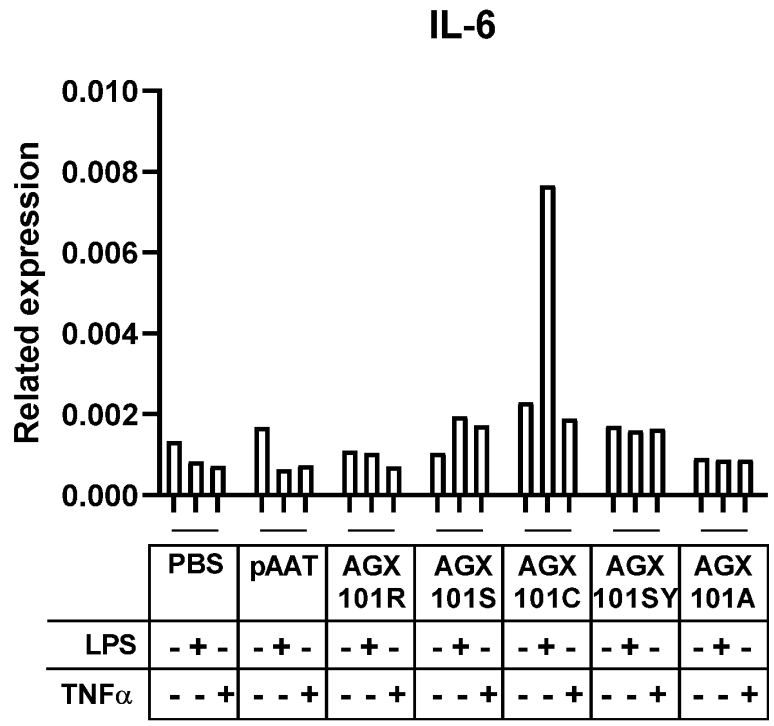

G1: AGX-101S_SC_10mg/kg
G2: AGX-101T_SC_10mg/kg
G3: AGX-101R_SC_10mg/kg
Ab Concentration (µg/ml)
10000
1000
100
10
1
0.1
0.01
0
3
6
9
12
15
18
21
Time (Day)

Figure 16

A


IL-6
Related expression
0.010
0.008
0.006
0.004
0.002
0.000
PBS
pAAT
AGX 101R
AGX 101S
AGX 101C
AGX 101SY
AGX 101A
LPS - + - - + - - + - - + - - + - - + - - + -
TNFα - - + - - + - - + - - + - - + - - + - - +

B

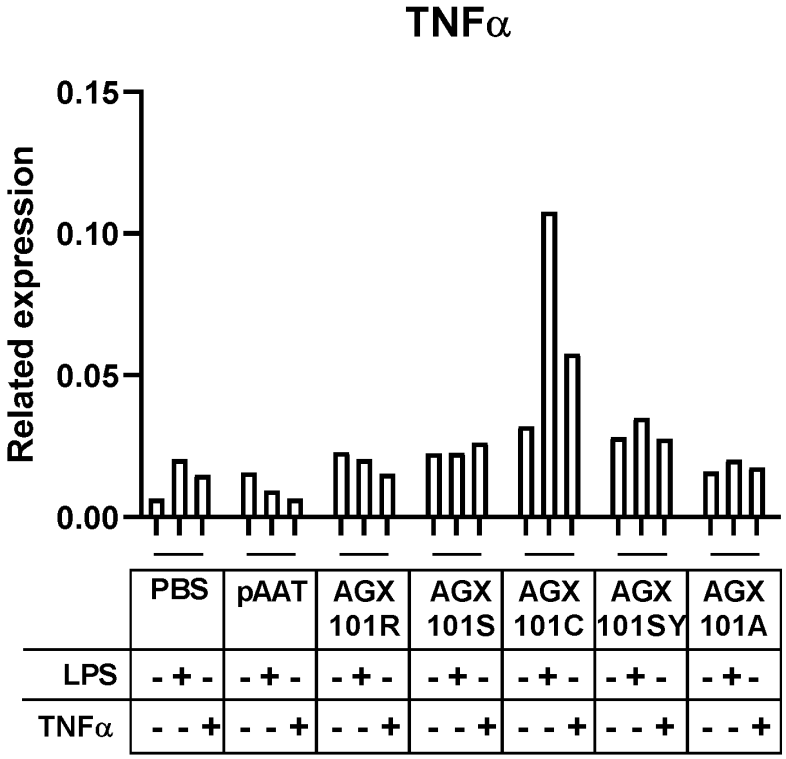

TNFα
Related expression
0.15
0.10
0.05
0.00
PBS
pAAT
AGX 101R
AGX 101S
AGX 101C
AGX 101SY
AGX 101A
LPS - + - - + - - + - - + - - + - - + - - + -
TNFα - - + - - + - - + - - + - - + - - + - - +

Figure 16 Continued

C

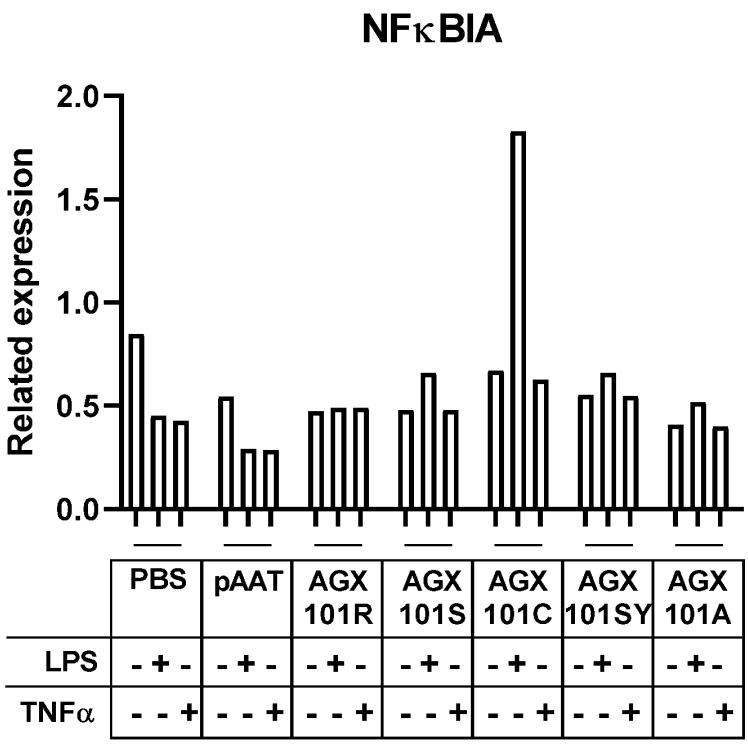


Figure 16 Continued

D

	pdAAT		AGX-101R		AGX-101S		AGX-101C		AGX-101SY		AGX-101A	
	+LPS	+TNFα	+LPS	+TNFα	+LPS	+TNFα	+LPS	+TNFα	+LPS	+TNFα	+LPS	+TNFα
IL-6	0.00083	0.00072	0.00104	**0.00070**	0.00194	**0.00171**	0.00766	0.00766	0.00159	0.00164	0.00087	0.00087
TNFα	0.00929	0.00643	0.02031	**0.01513**	0.02252	**0.02618**	0.10766	0.05759	0.03489	0.02757	0.02016	0.01741
NFKBIA	0.29163	0.28440	**0.48879**	0.49017	**0.65849**	0.47840	1.82915	0.62580	0.65972	0.54573	0.51678	0.39961