Figure 1

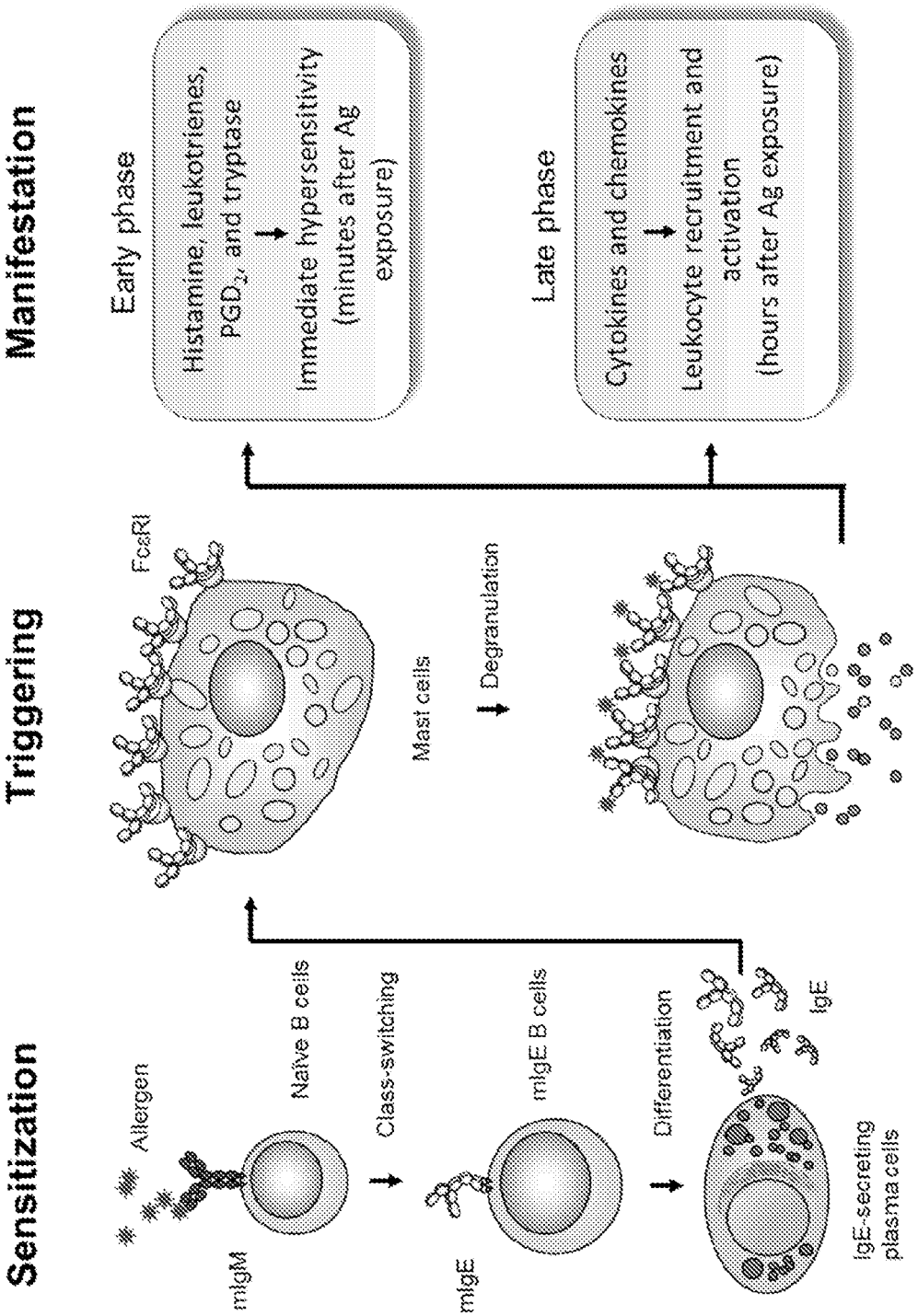

Mechanism of the IgE-mediated Allergic Disease Pathway
Sensitization
Triggering
Manifestation
Allergen
mIgM
Naïve B cells
Class-switching
mIgE
mIgE B cells
Differentiation
IgE
IgE-secreting plasma cells
FcεRI
Mast cells
Degranulation
Early phase
Histamine, leukotrienes, PGD2, and tryptase
Immediate hypersensitivity (minutes after Ag exposure)
Late phase
Cytokines and chemokines
Leukocyte recruitment and activation
(hours after Ag exposure)

Figure 2A

Figure 2B

Figure 3

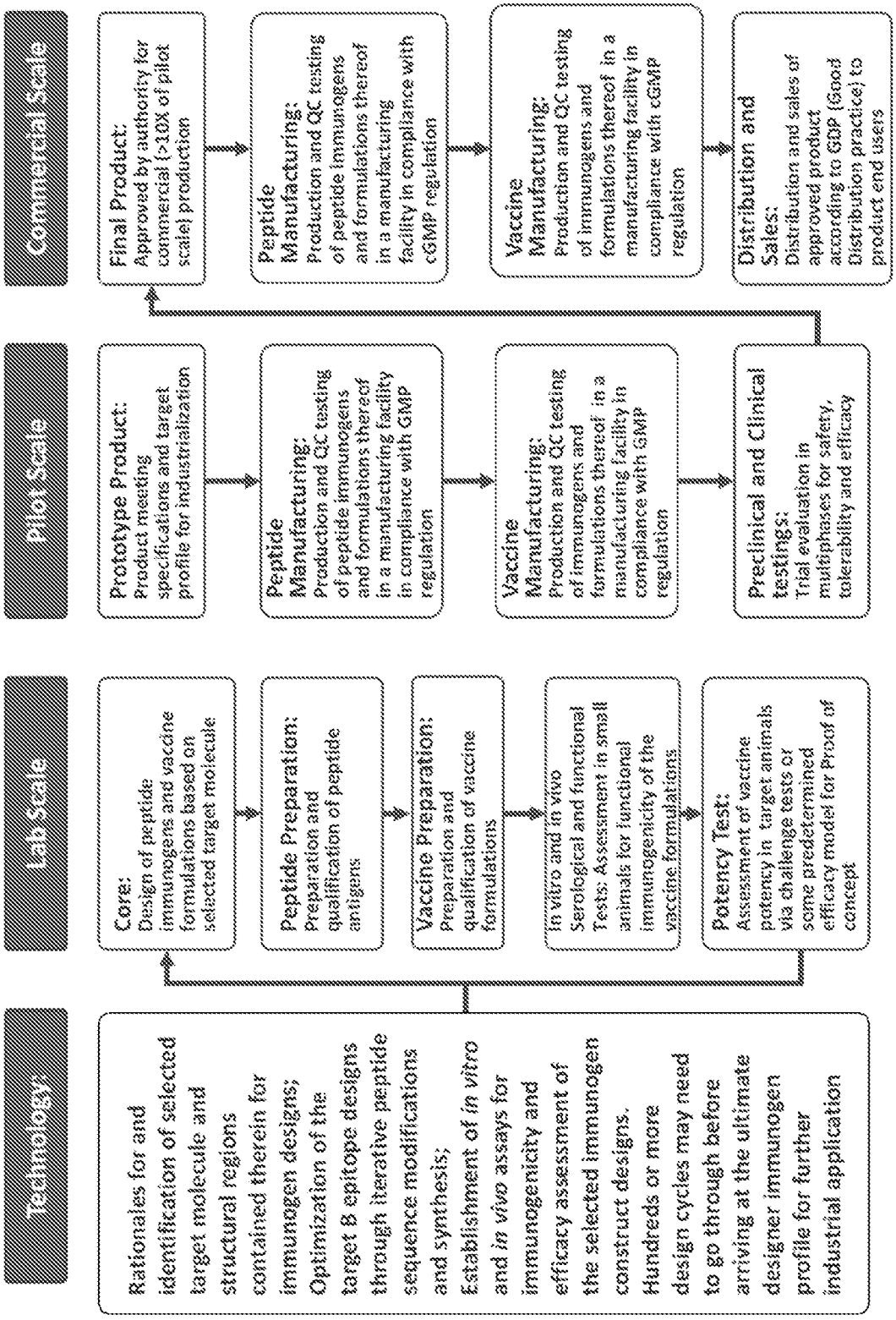

Technology
Lab Scale
Pilot Scale
Commercial Scale
Rationales for and identification of selected target molecule and structural regions contained therein for immunogen designs; Optimization of the target B epitope designs through iterative peptide sequence modifications and synthesis; Establishment of in vitro and in vivo assays for immunogenicity and efficacy assessment of the selected immunogen construct designs. Hundreds or more design cycles may need to go through before arriving at the ultimate designer immunogen profile for further industrial application
Core: Design of peptide immunogens and vaccine formulations based on selected target molecule
Peptide Preparation: Preparation and qualification of peptide antigens
Vaccine Preparation: Preparation and qualification of vaccine formulations
In vitro and in vivo Serological and functional Tests: Assessment in small animals for functional immunogenicity of the vaccine formulations
Potency Test: Assessment of vaccine potency in target animals via challenge tests or some predetermined efficacy model for Proof of concept
Prototype Product: Product meeting specifications and target profile for industrialization
Peptide Manufacturing: Production and QC testing of peptide immunogens and formulations thereof in a manufacturing facility in compliance with GMP regulation
Vaccine Manufacturing: Production and QC testing of immunogens and formulations thereof in a manufacturing facility in compliance with GMP regulation
Preclinical and Clinical testings: Trial evaluation in multiphases for safety, tolerability and efficacy
Final Product: Approved by authority for commercial (>10X of pilot scale) production
Peptide Manufacturing: Production and QC testing of peptide immunogens and formulations thereof in a manufacturing facility in compliance with cGMP regulation
Vaccine Manufacturing: Production and QC testing of immunogens and formulations thereof in a manufacturing facility in compliance with cGMP regulation
Distribution and Sales: Distribution and sales of approved product according to GDP (Good Distribution practice) to product end users

Figure 4

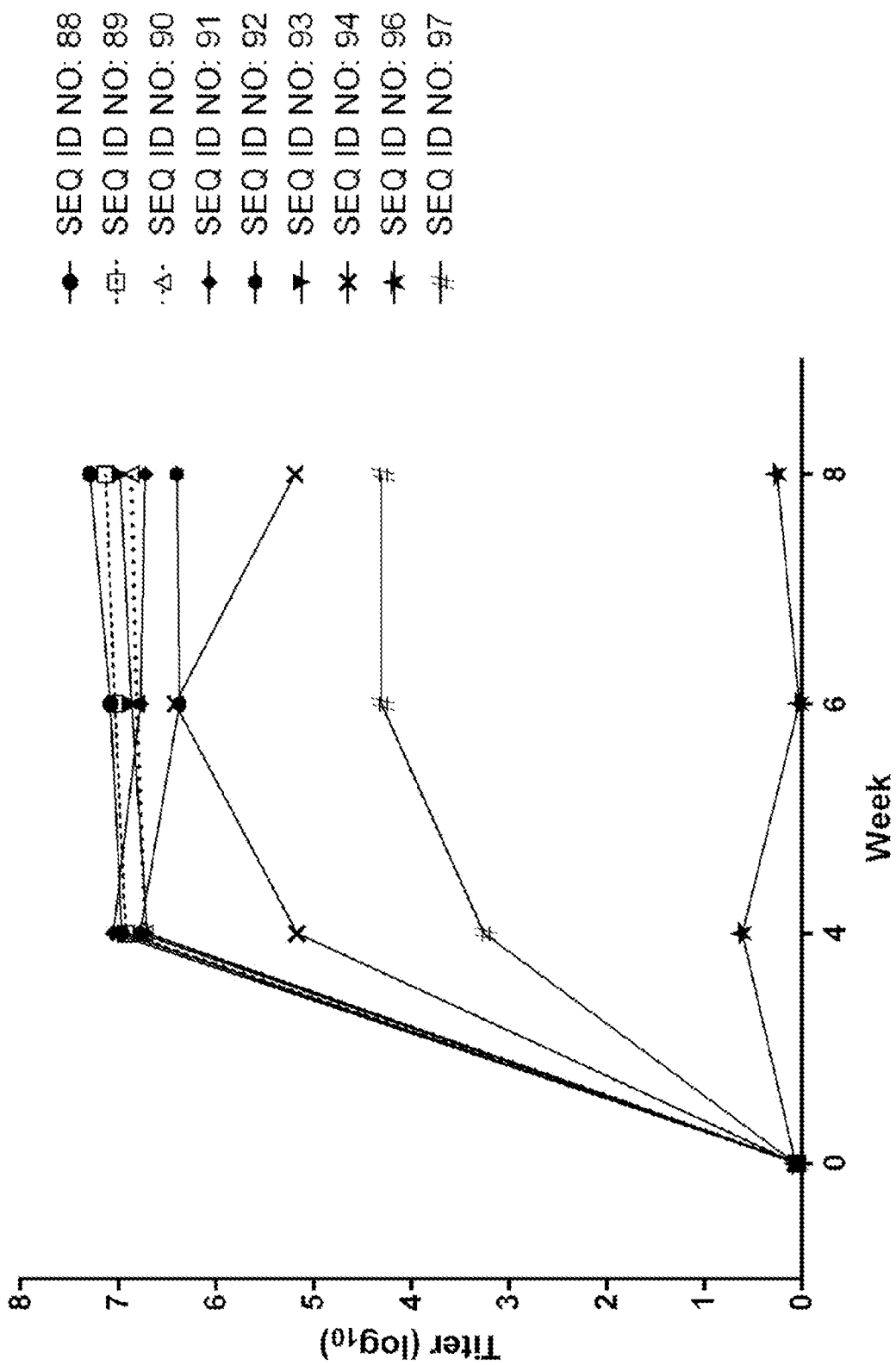
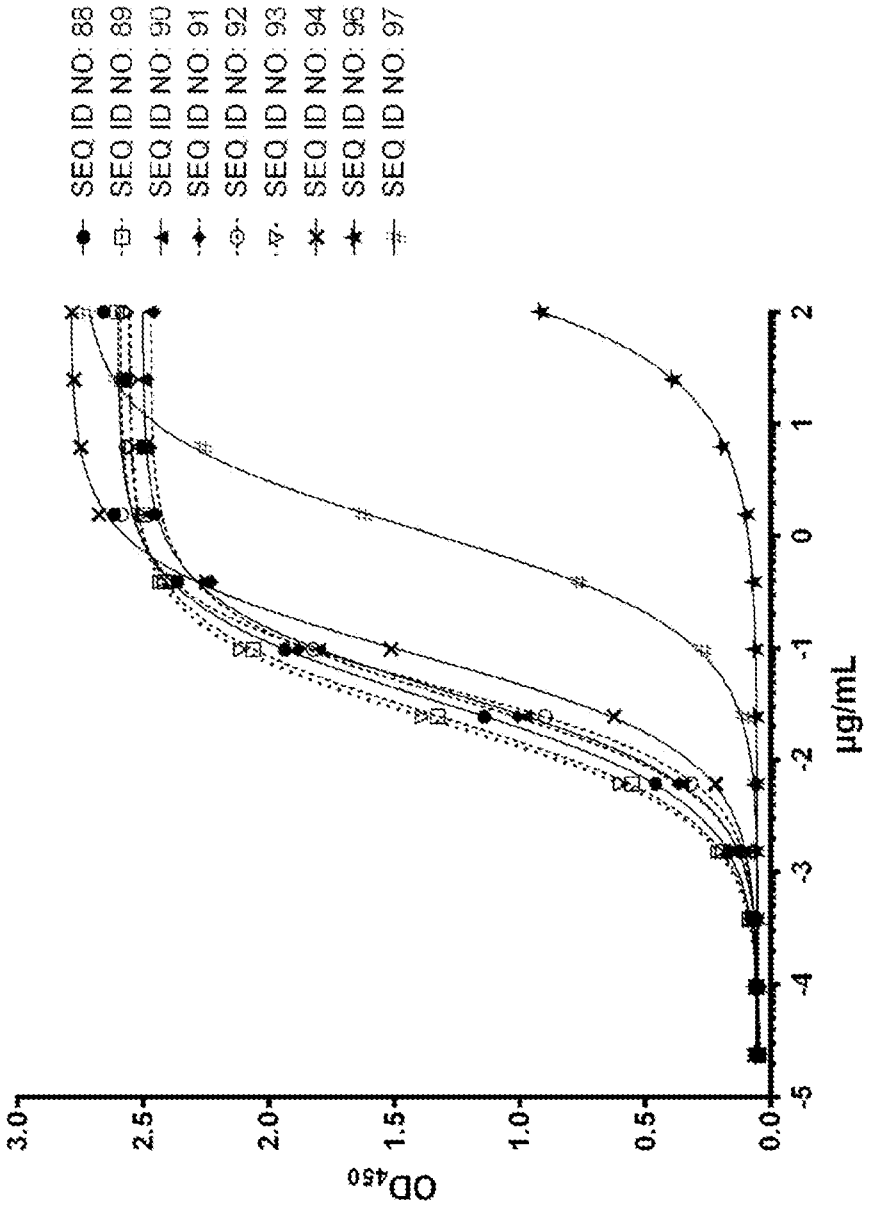

SEQ ID NO: 88
SEQ ID NO: 89
SEQ ID NO: 90
SEQ ID NO: 91
SEQ ID NO: 92
SEQ ID NO: 93
SEQ ID NO: 94
SEQ ID NO: 96
SEQ ID NO: 97
Titer (log10)
Week
0
1
2
3
4
5
6
7
8
0
4
6
8

Figure 5



SEQ ID NO	EC_{50} (µg/mL)
88	0.03320
89	0.02368
90	0.03980
91	0.03561
92	0.04522
93	0.02111
94	0.08892
96	>5
97	1.127

Figure 6A

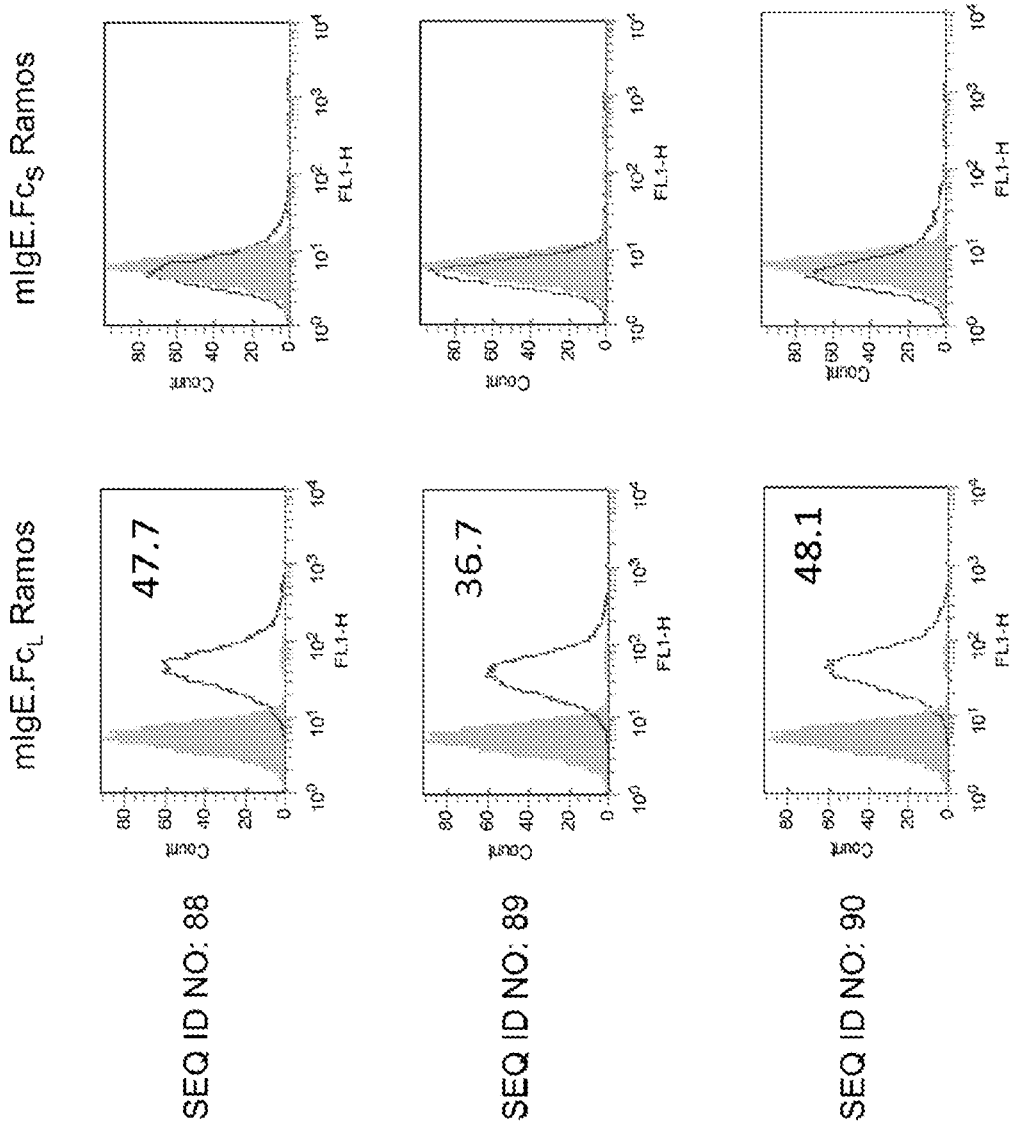

mIgE.Fc$_L$ Ramos
mIgE.Fc$_S$ Ramos
47.7
36.7
48.1
SEQ ID NO: 88
SEQ ID NO: 89
SEQ ID NO: 90
Count
FL1-H

Figure 6B

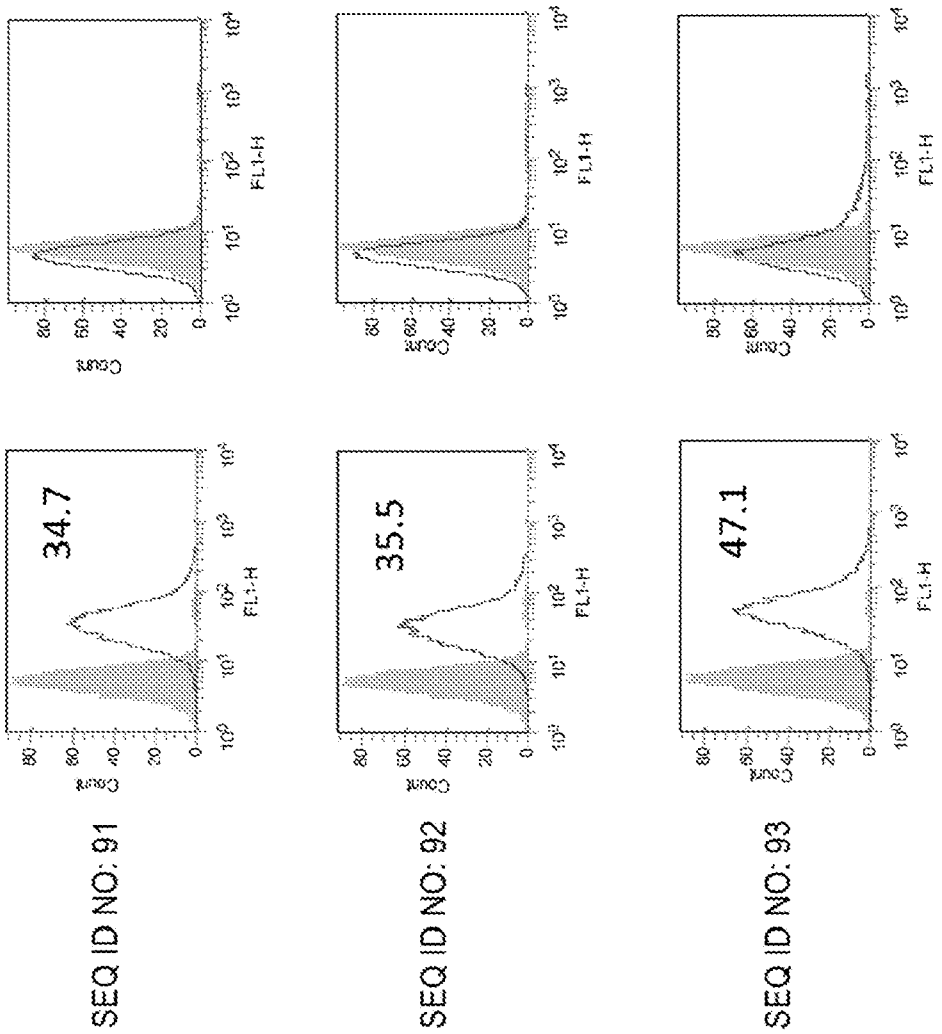

mIgE.Fc$_L$ Ramos
mIgE.Fc$_S$ Ramos
34.7
35.5
47.1
SEQ ID NO: 91
SEQ ID NO: 92
SEQ ID NO: 93
Count
FL1-H

Figure 6C

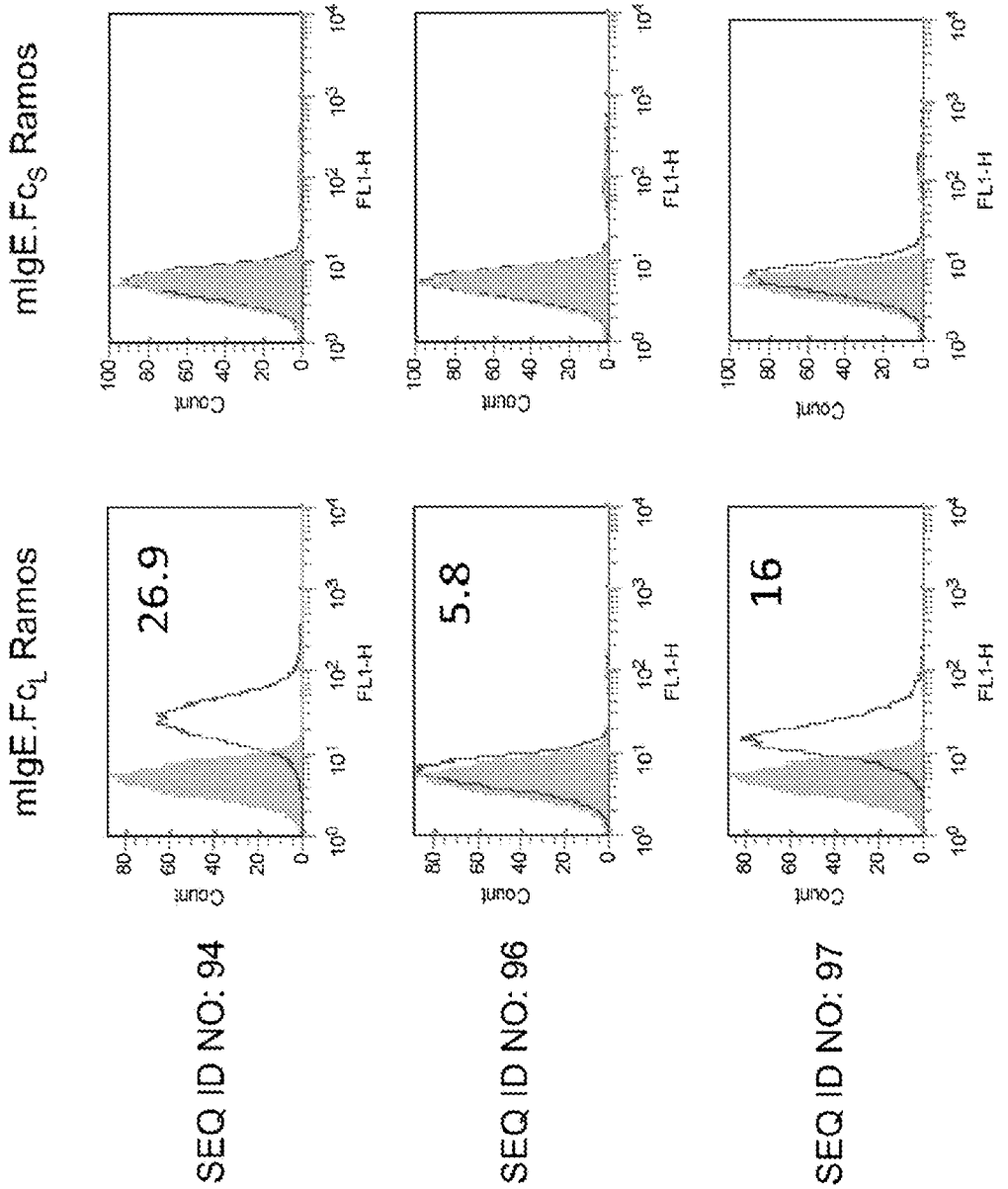
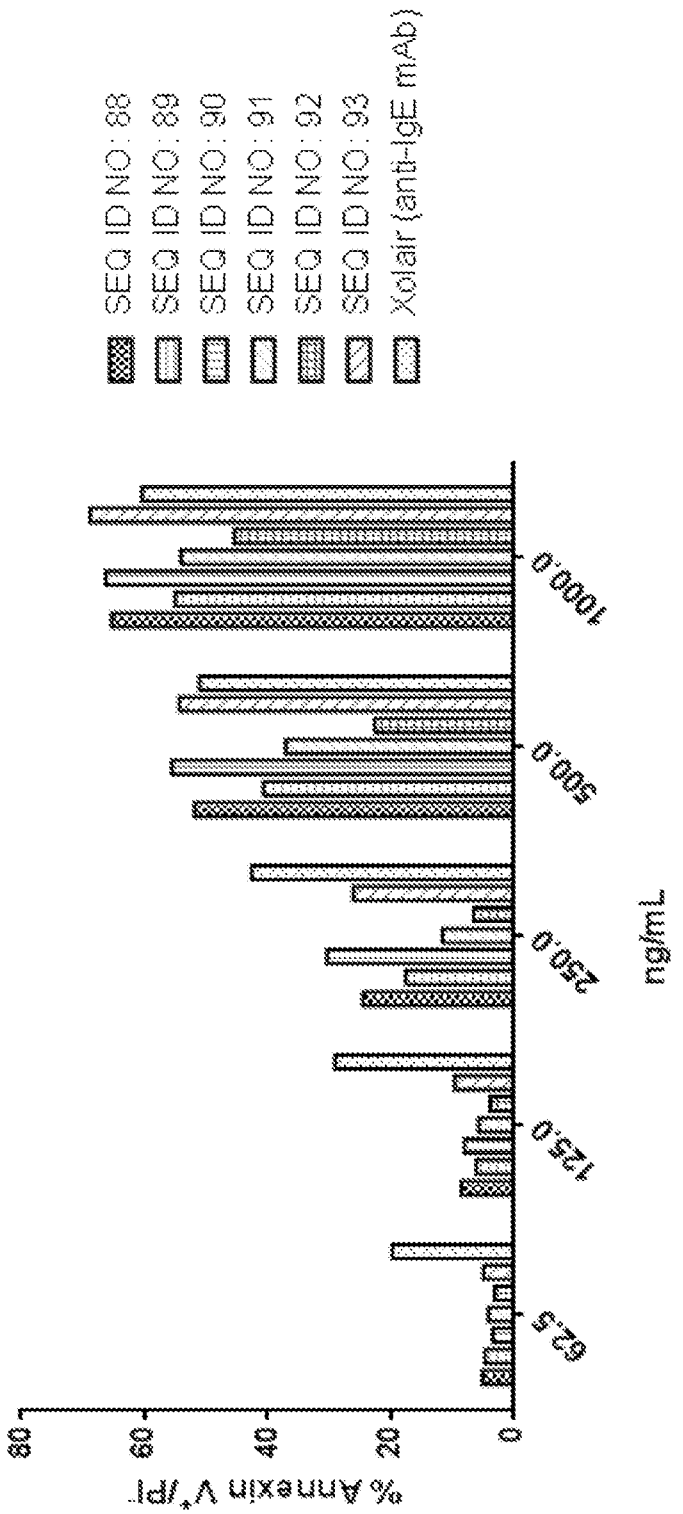

mIgE.Fc$_L$ Ramos
mIgE.Fc$_S$ Ramos
26.9
5.8
16
SEQ ID NO: 94
SEQ ID NO: 96
SEQ ID NO: 97
Count
FL1-H

Figure 7



Cell line: mIgE.Fc$_L$ Ramos
Antibodies: purified guinea pig anti-IgE-EMPD IgG

SEQ ID NO:	88	89	90	91	92	93	Xolair
EC_{50} (ng/mL)	303.8	345.3	277.5	403.9	536	302	121.4

Figure 8

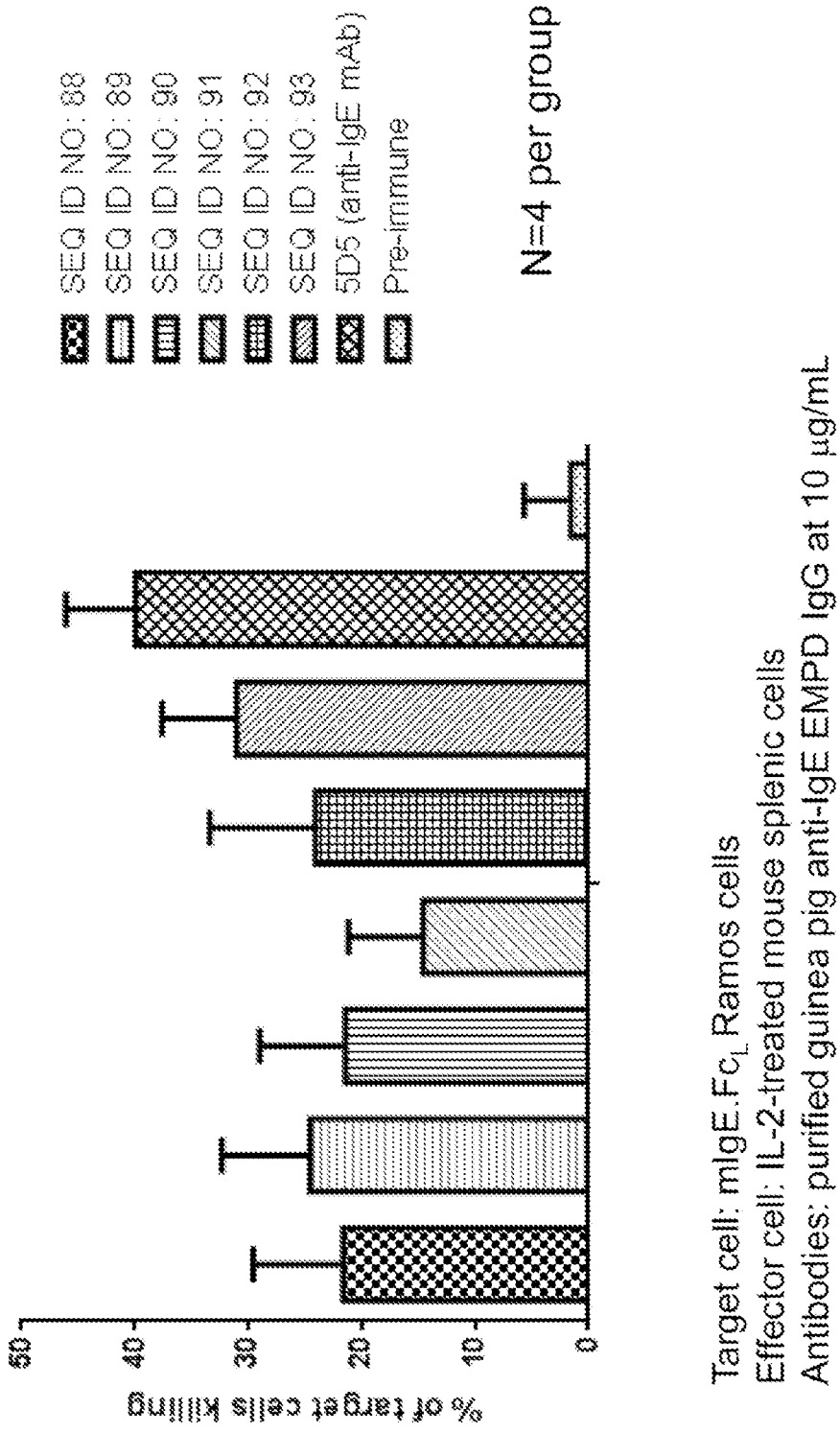

E/T=1:30
% of target cells killing
0
10
20
30
40
50
SEQ ID NO: 88
SEQ ID NO: 89
SEQ ID NO: 90
SEQ ID NO: 91
SEQ ID NO: 92
SEQ ID NO: 93
5D5 (anti-IgE mAb)
Pre-immune
N=4 per group
Target cell: mIgE.Fc$_L$ Ramos cells
Effector cell: IL-2-treated mouse splenic cells
Antibodies: purified guinea pig anti-IgE EMPD IgG at 10 μg/mL

Figure 9

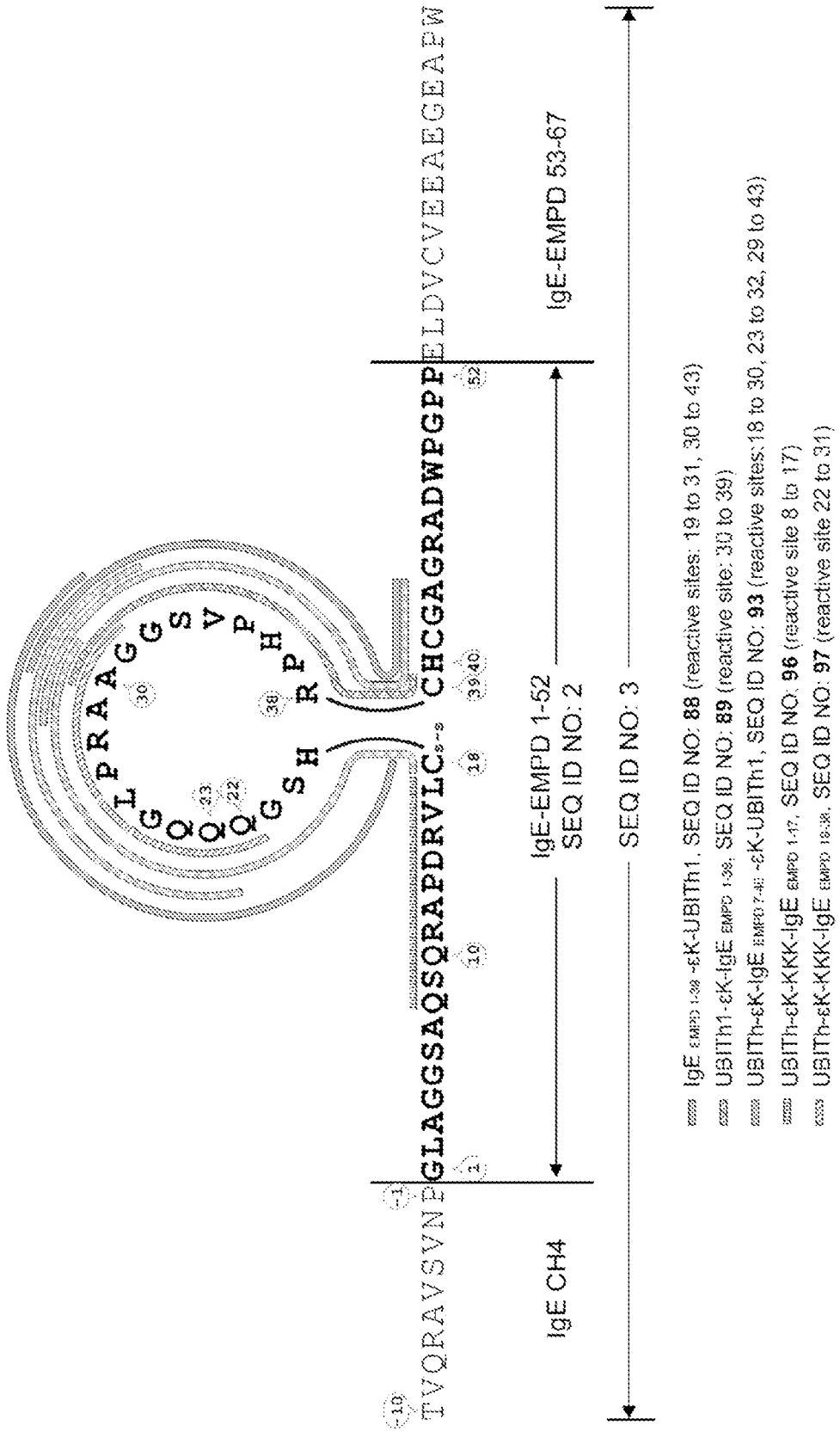

TVQRAVSVNP
GLAGGSAQSQRAPDRVLC
CHCGAGRADWPGPP
ELDVCVEEAEGEAPW
IgE CH4
IgE-EMPD 1-52
SEQ ID NO: 2
IgE-EMPD 53-67
SEQ ID NO: 3
IgE EMPD 1-38 -εK-UBITh1, SEQ ID NO: 88 (reactive sites: 19 to 31, 30 to 43)
UBITh1-εK-IgE EMPD 1-38, SEQ ID NO: 89 (reactive site: 30 to 39)
UBITh-εK-IgE EMPD 17-46 -εK-UBITh1, SEQ ID NO: 93 (reactive sites:18 to 30, 23 to 32, 29 to 43)
UBITh-εK-KKK-IgE EMPD 1-17, SEQ ID NO: 96 (reactive site 8 to 17)
UBITh-εK-KKK-IgE EMPD 18-38, SEQ ID NO: 97 (reactive site 22 to 31)

Figure 10

- *Animals:* F1 offspring (hIGHE x Balb/c).
- *Groups:* Placebo and 3 vaccination groups.
- *Vaccine dose:* Immunize on weeks 0, 3, and 5 with doses at 9 (low), 18 (medium) or 40 (high) µg per dose or adjuvant placebo by *i.m.* route.
- *Allergen:* Immunize on week 10 and 16 with 50 µg papain/TiterMax Gold adjuvant by subcutaneous route.

Figure11

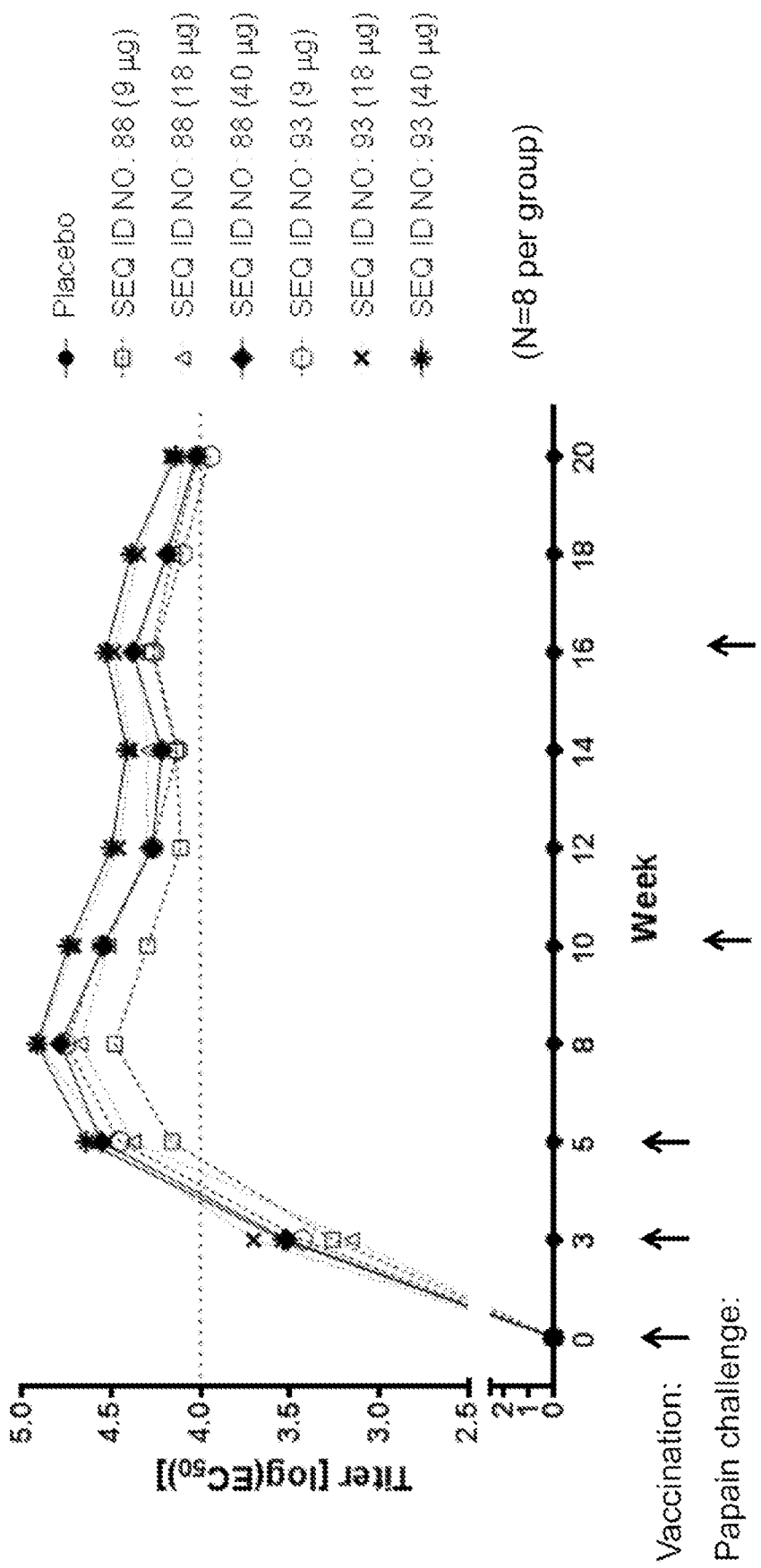

Antibody titers [Log(EC50 of dilution factor] to recombinant γ1-em67 protein
Placebo
SEQ ID NO: 86 (9 µg)
SEQ ID NO: 86 (18 µg)
SEQ ID NO: 86 (40 µg)
SEQ ID NO: 93 (9 µg)
SEQ ID NO: 93 (18 µg)
SEQ ID NO: 93 (40 µg)
(N=8 per group)
Titer [log(EC50)]
5.0
4.5
4.0
3.5
3.0
2.5
2
1
0
0
3
5
8
10
12
14
16
18
20
Week
Vaccination:
Papain challenge:

Figure 12

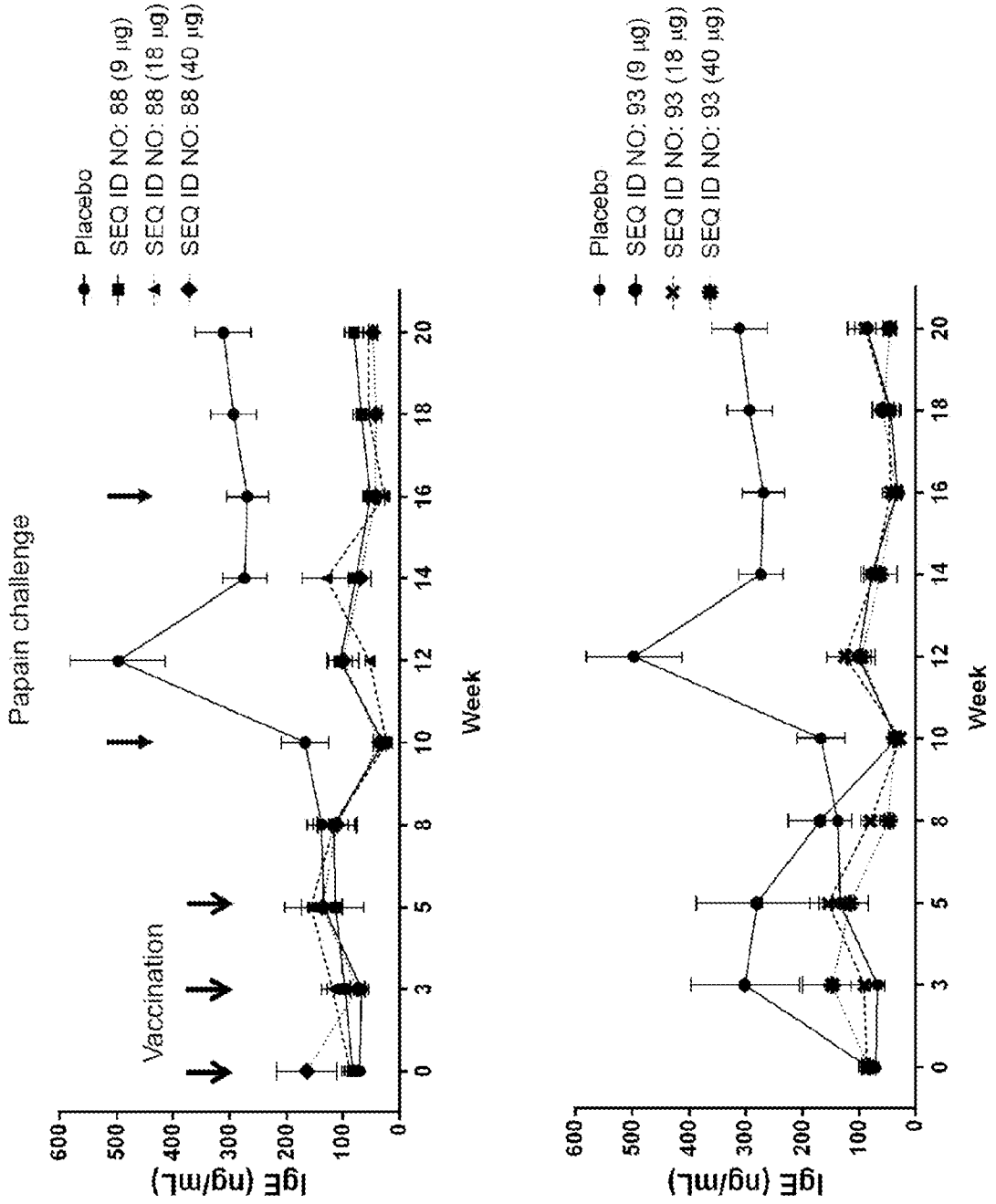

Papain challenge
Vaccination
Placebo
SEQ ID NO: 88 (9 µg)
SEQ ID NO: 88 (18 µg)
SEQ ID NO: 88 (40 µg)
IgE (ng/mL)
Week
Placebo
SEQ ID NO: 93 (9 µg)
SEQ ID NO: 93 (18 µg)
SEQ ID NO: 93 (40 µg)
IgE (ng/mL)
Week

Figure 13

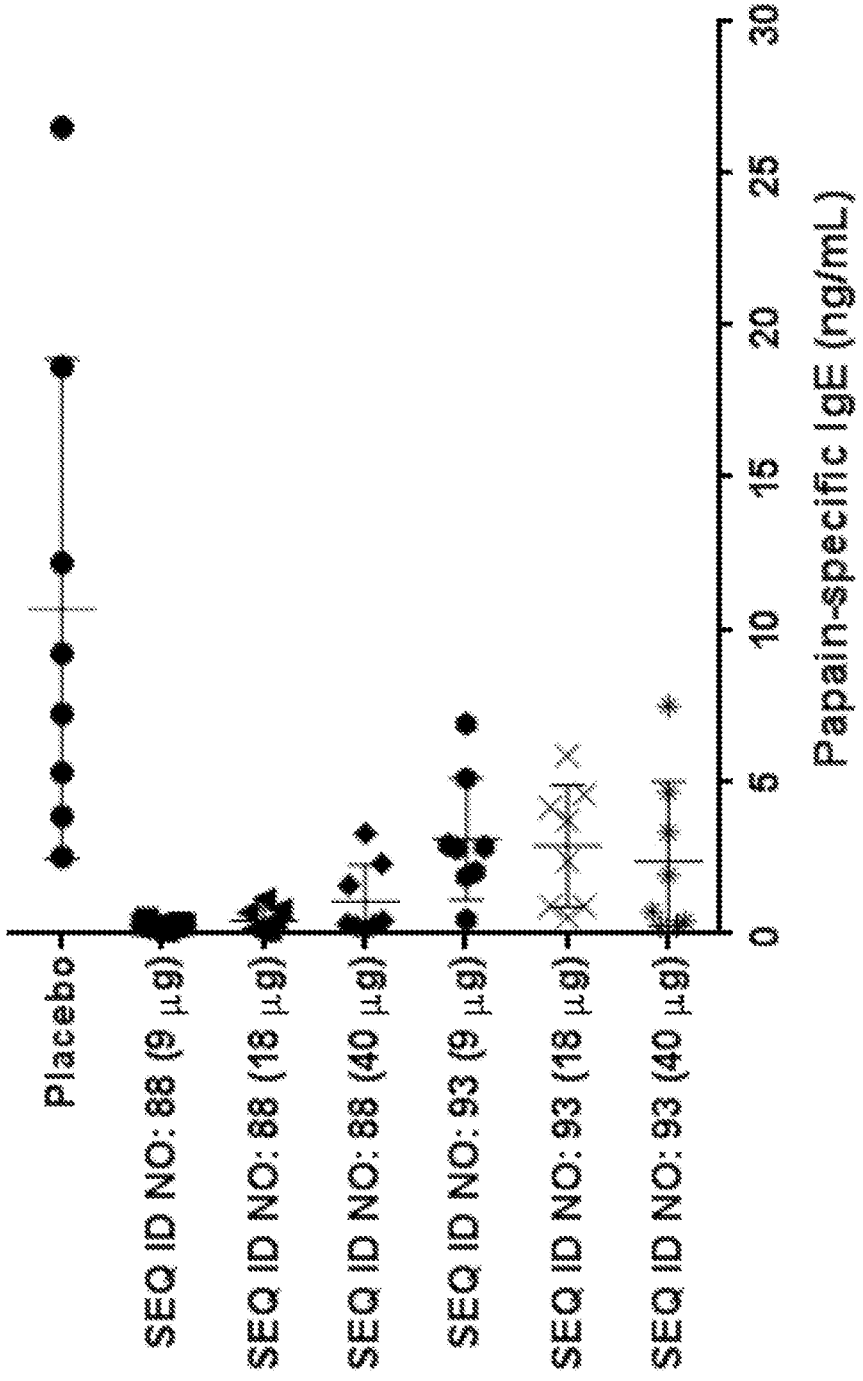

Placebo
SEQ ID NO: 88 (9 µg)
SEQ ID NO: 88 (18 µg)
SEQ ID NO: 88 (40 µg)
SEQ ID NO: 93 (9 µg)
SEQ ID NO: 93 (18 µg)
SEQ ID NO: 93 (40 µg)
0
5
10
15
20
25
30
Papain-specific IgE (ng/mL)

Figure 14

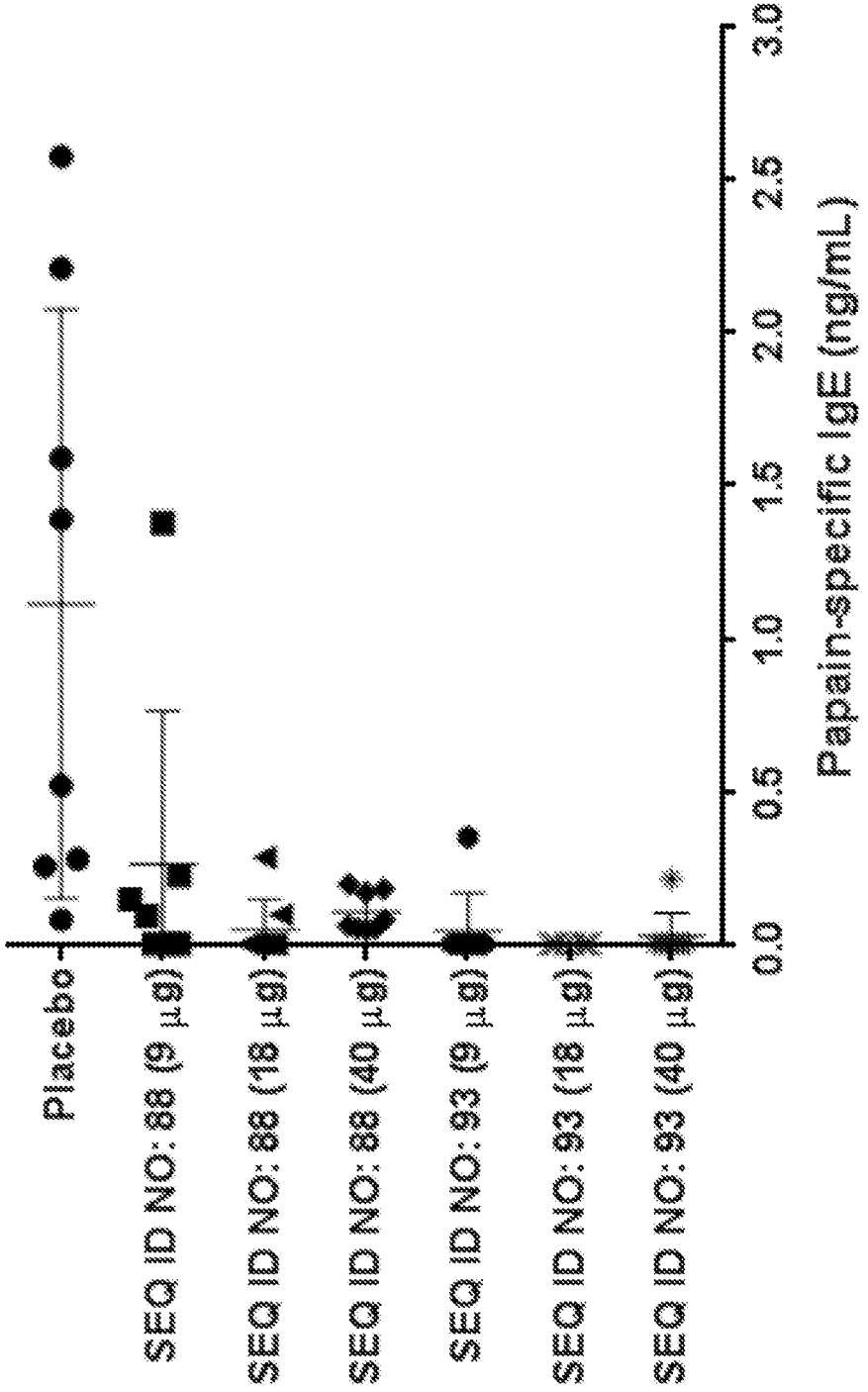
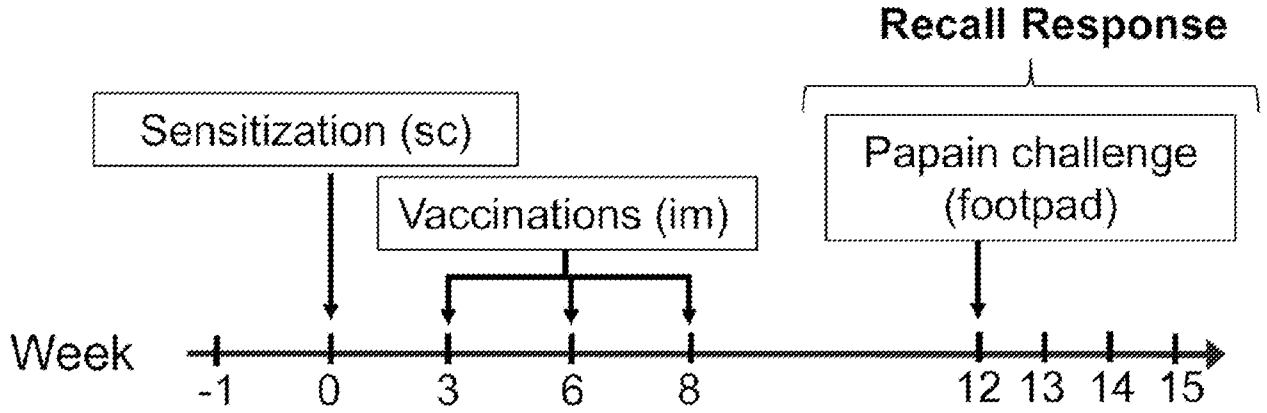

Placebo
SEQ ID NO: 88 (9 µg)
SEQ ID NO: 88 (18 µg)
SEQ ID NO: 88 (40 µg)
SEQ ID NO: 93 (9 µg)
SEQ ID NO: 93 (18 µg)
SEQ ID NO: 93 (40 µg)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
Papain-specific IgE (ng/mL)

Figure 15

Experimental Protocol for Efficacy Evaluation of IgE-EMPD Vaccine Formulations On Sensitization/Recall Responses in Human IGHE Knockin Hybrid Mice



- *Animals:* F1 offspring (HulGHE x Balb/c).
- *Groups:* Placebo and vaccination groups.
- *Vaccine dose:* Immunize on weeks 3, 6, and 8 with a dose of 40 µg or adjuvant placebo by *i.m.* route.
- *Allergen:* Sensitize on week 0 with 50 µg papain/TiterMax Gold adjuvant by subcutaneous route and recall on week 12 with 50 µg papain/PBS solution via footpad.

Figure 16

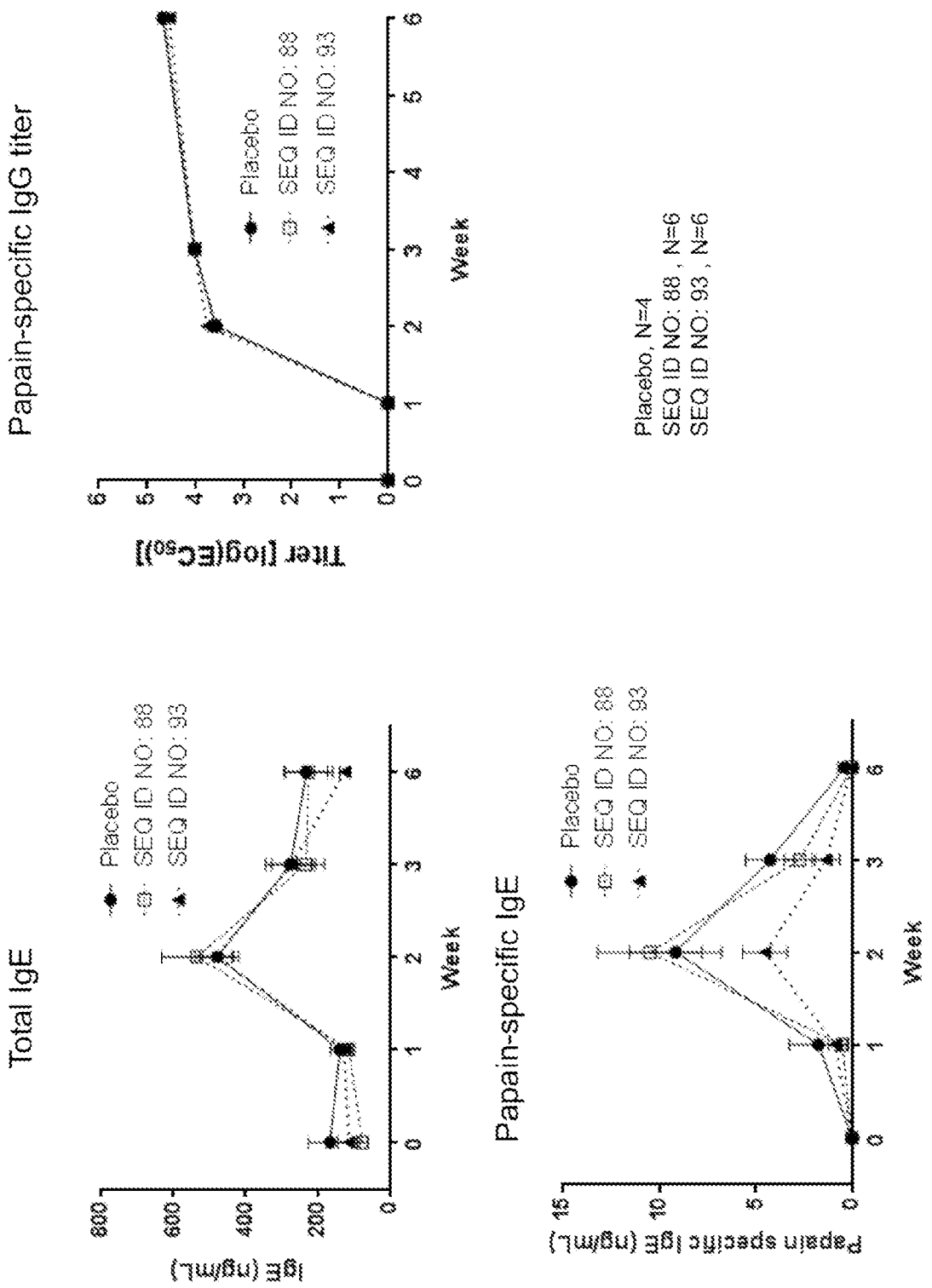

Papain-specific IgG titer
Titer [log(EC50)]
Week
Placebo
SEQ ID NO: 88
SEQ ID NO: 93
Placebo, N=4
SEQ ID NO: 88 , N=6
SEQ ID NO: 93 , N=6
Total IgE
IgE (ng/mL)
Papain-specific IgE
Papain specific IgE (ng/mL)

Figure 17

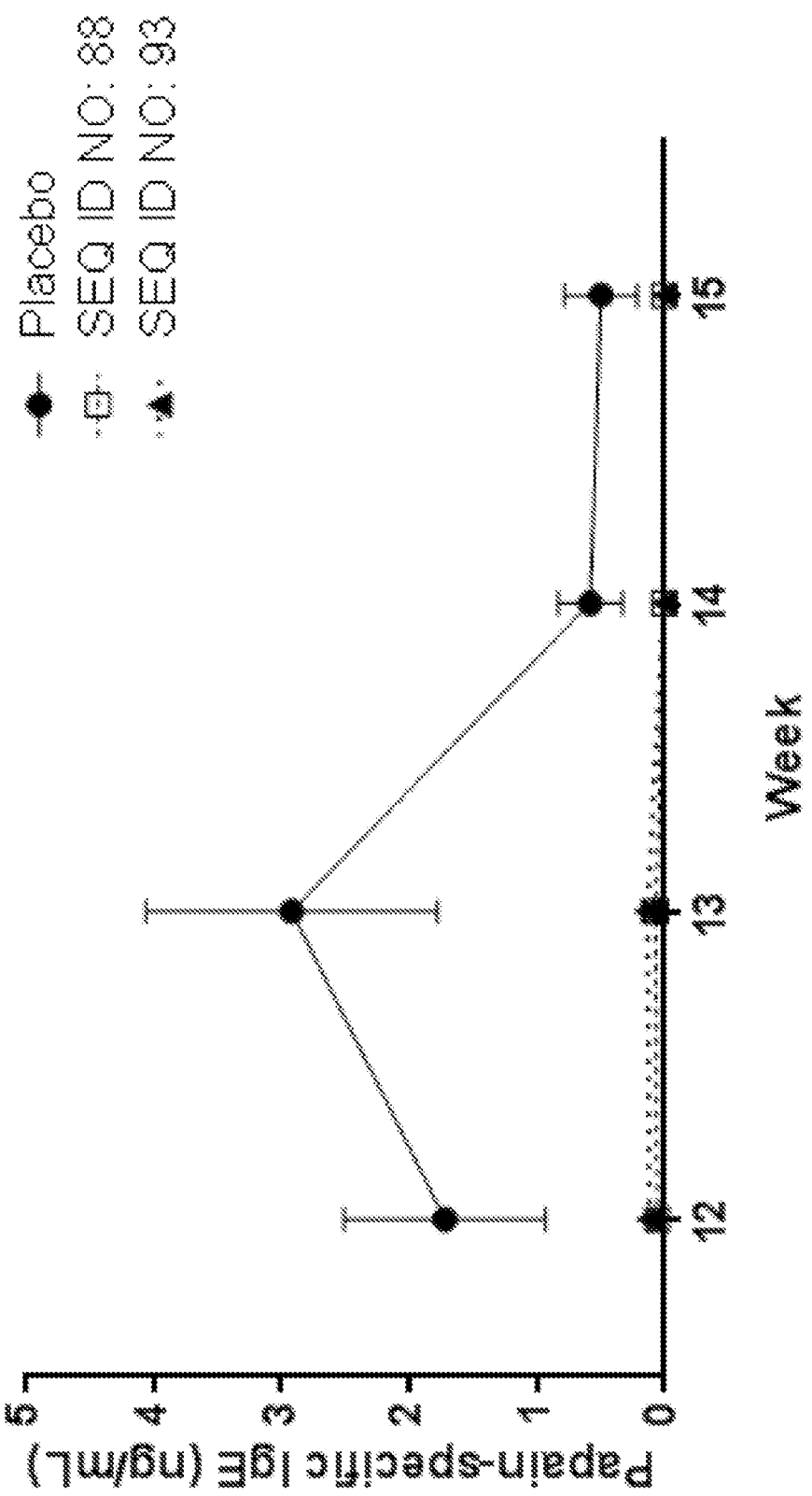

Placebo
SEQ ID NO: 88
SEQ ID NO: 93
Papain-specific IgE (ng/mL)
0
1
2
3
4
5
12
13
14
15
Week

Figure 18B

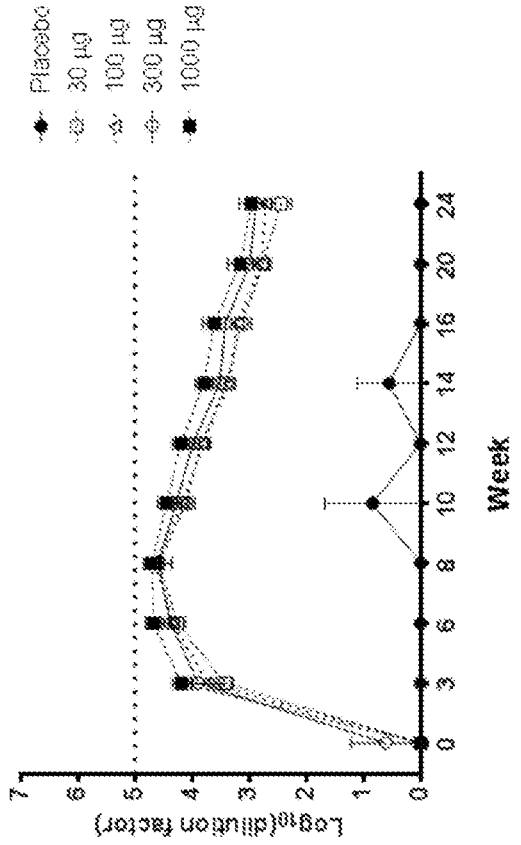


Figure 18A

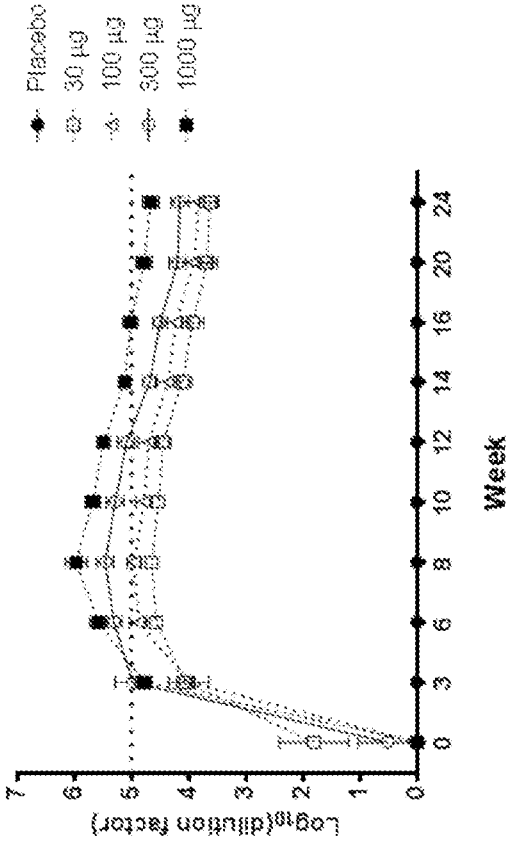


Figure 19

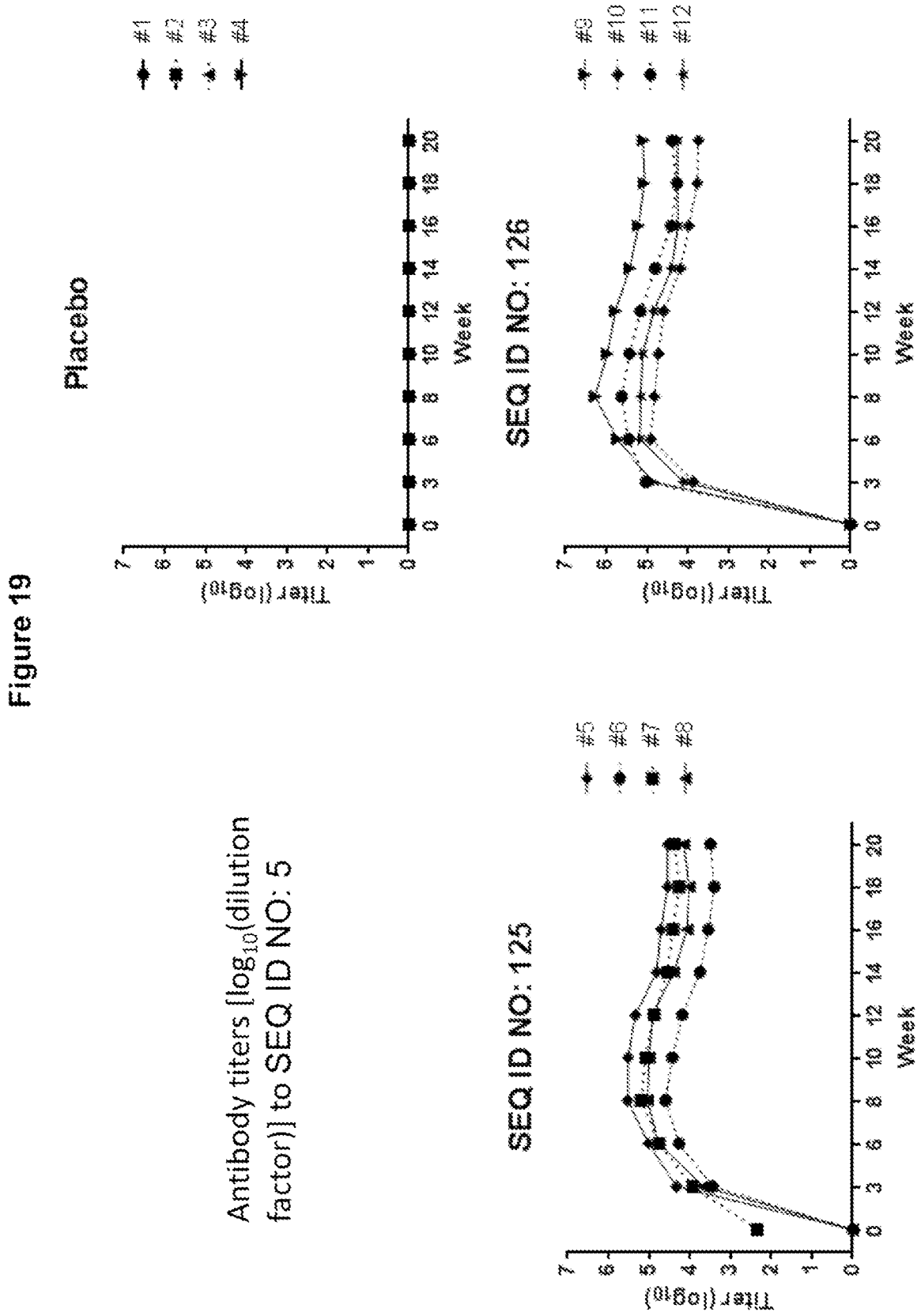

Placebo
#1
#2
#3
#4
SEQ ID NO: 126
#9
#10
#11
#12
SEQ ID NO: 125
#5
#6
#7
#8
Antibody titers [log10(dilution factor)] to SEQ ID NO: 5
Titer (log10)
Week
0
1
2
3
4
5
6
7
0
3
6
8
10
12
14
16
18
20

Figure 20

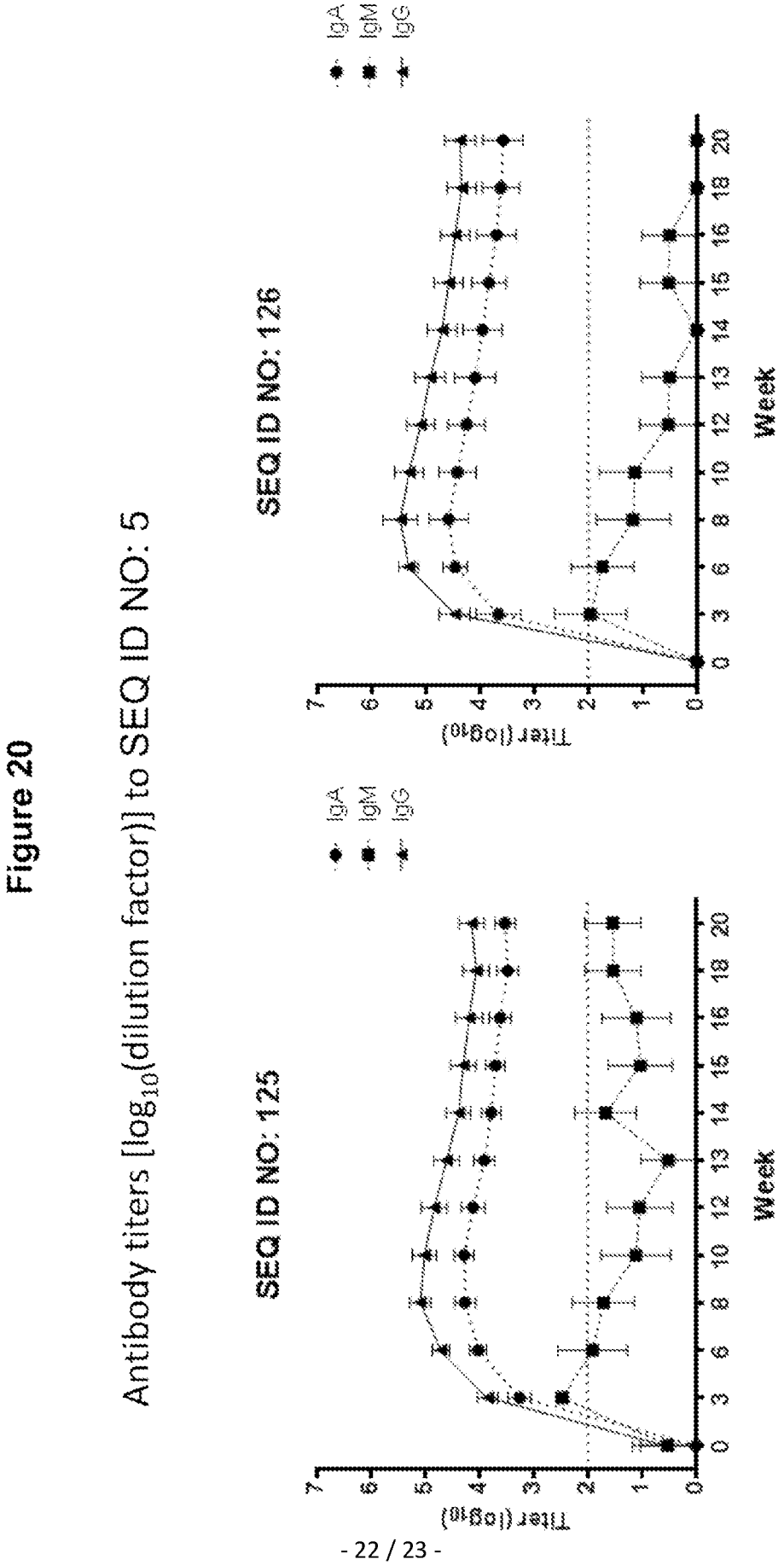
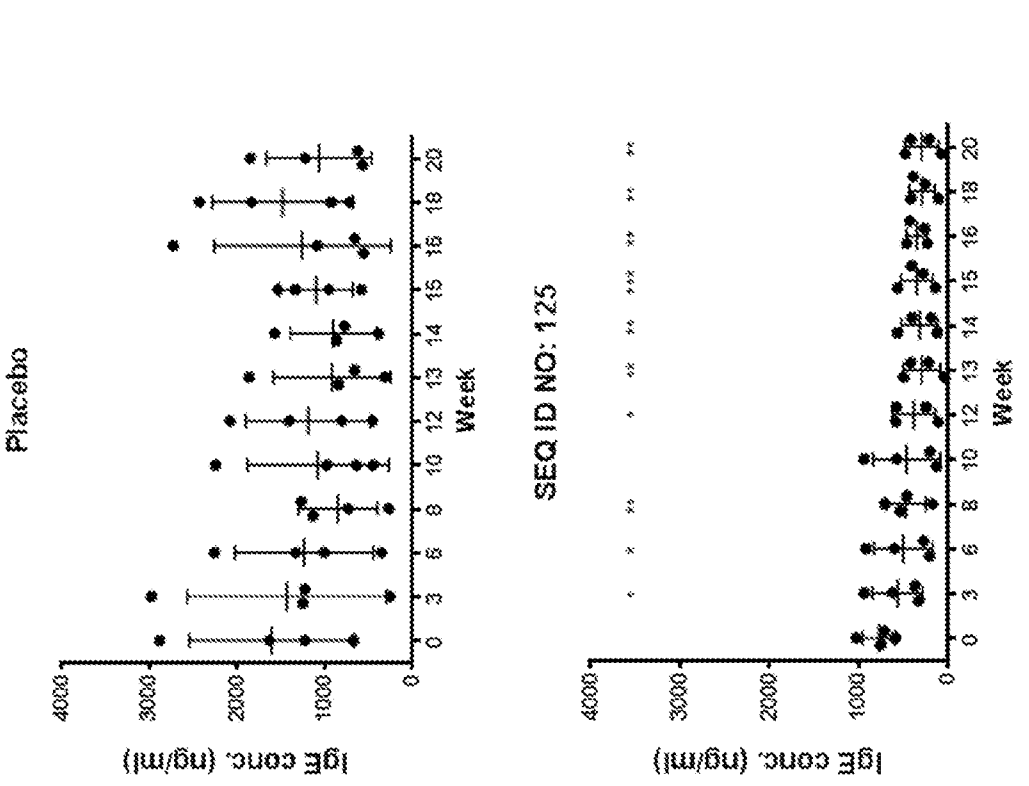

Antibody titers [log10(dilution factor)] to SEQ ID NO: 5
SEQ ID NO: 125
SEQ ID NO: 126
IgA
IgM
IgG
Titer (log10)
Week
0
1
2
3
4
5
6
7
0
3
6
8
10
12
13
14
15
16
18
20

Figure 21


Placebo
IgE conc. (ng/ml)
Week
SEQ ID NO: 125
IgE conc. (ng/ml)
Week
SEQ ID NO: 126
IgE conc. (ng/ml)
Week