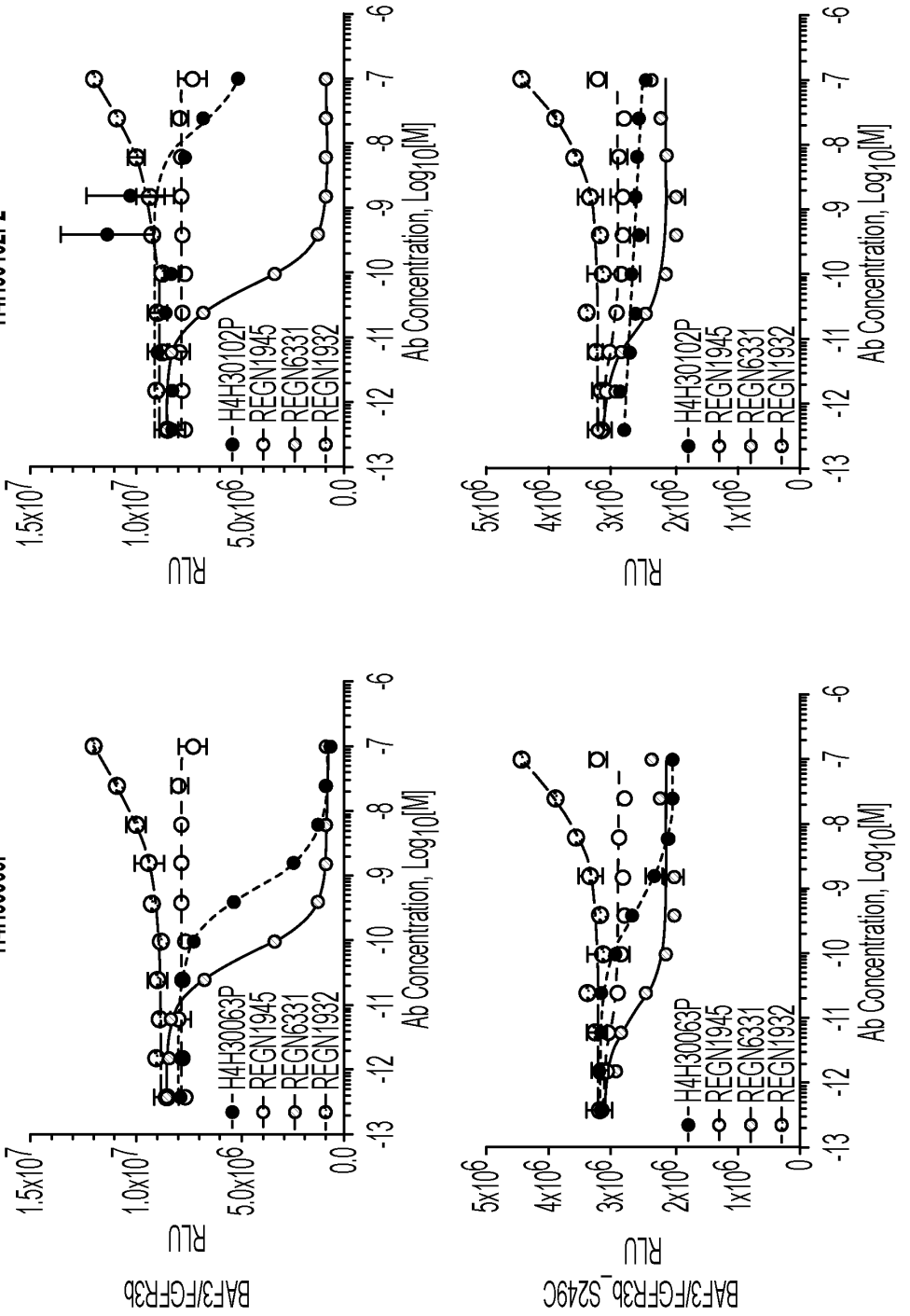

H4H30102P2
H4H30063P
BAF3/FGFR3b
BAF3/FGFR3b_S249C
RLU
Ab Concentration, Log10[M]
H4H30102P
H4H30063P
REGN1945
REGN6331
REGN1932

FIG. 1

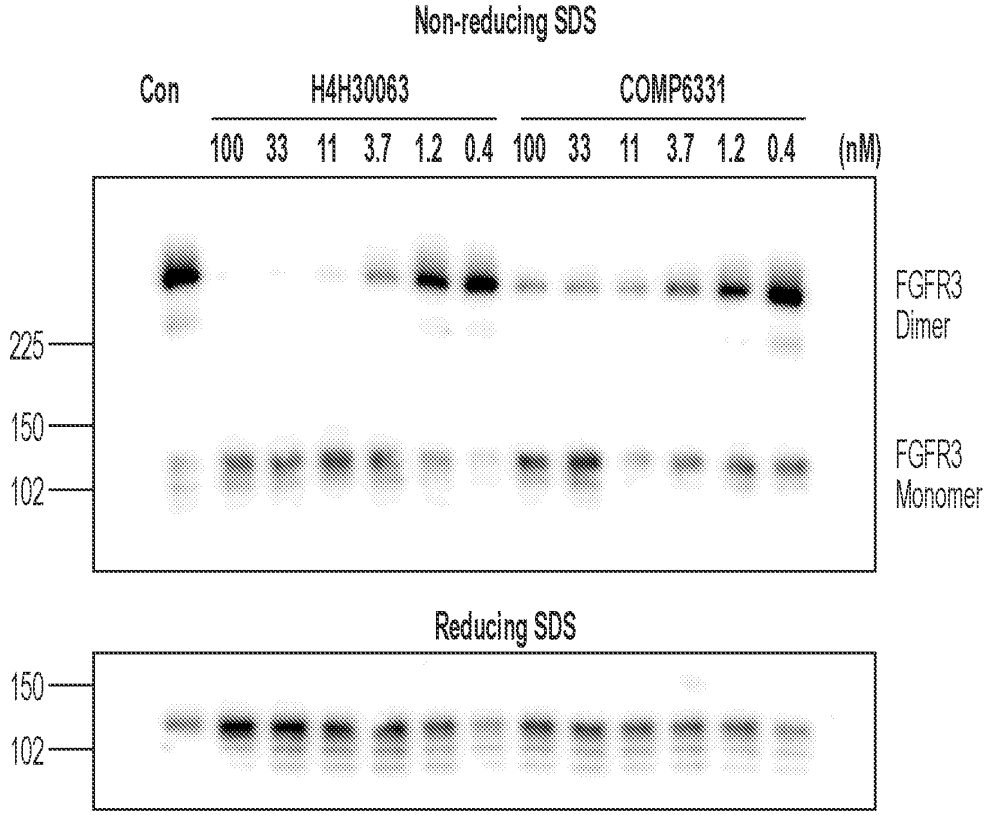

Non-reducing SDS
Con
H4H30063
COMP6331
100 33 11 3.7 1.2 0.4 100 33 11 3.7 1.2 0.4 (nM)
225
150
102
FGFR3 Dimer
FGFR3 Monomer
Reducing SDS
150
102

FIG. 2

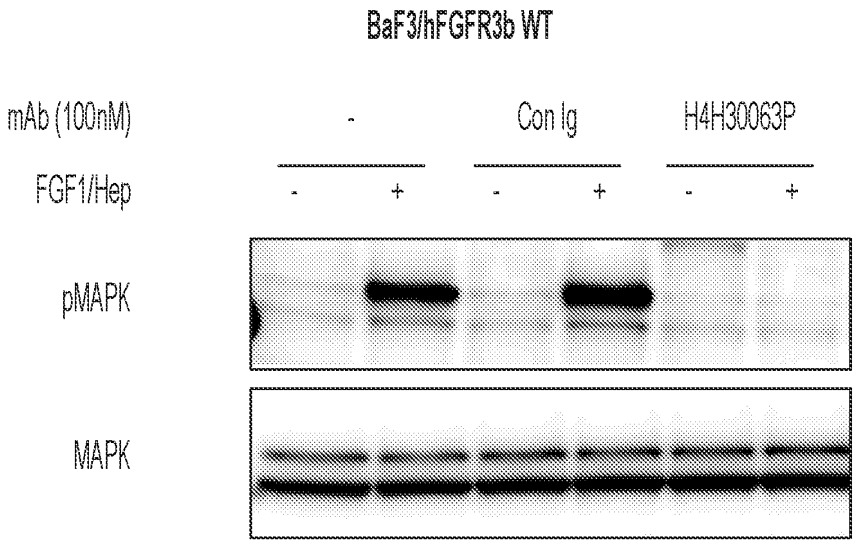

BaF3/hFGFR3b WT
mAb (100nM)
-
Con Ig
H4H30063P
FGF1/Hep
- + - + - +
pMAPK
MAPK

FIG. 3

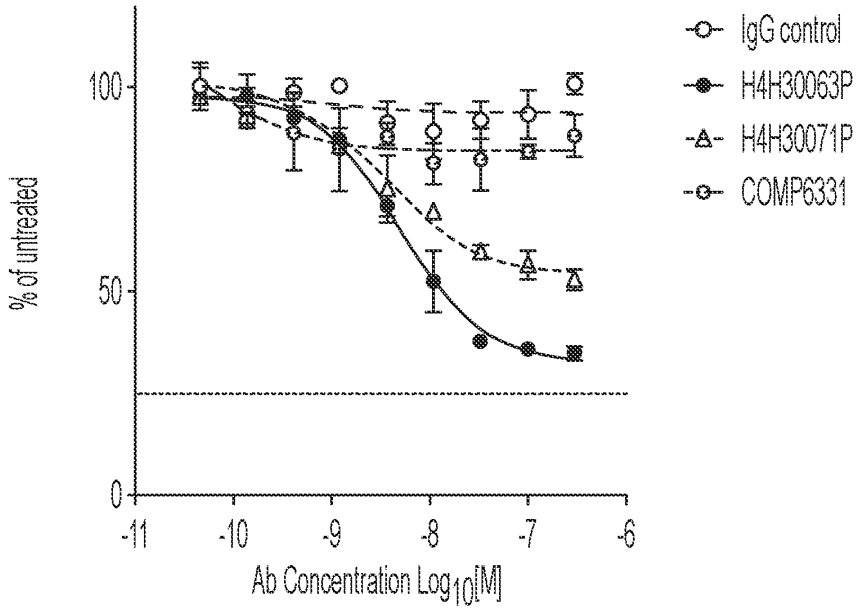

IgG control
H4H30063P
H4H30071P
COMP6331
% of untreated
100
50
0
-11
-10
-9
-8
-7
-6
Ab Concentration Log10[M]

FIG. 4

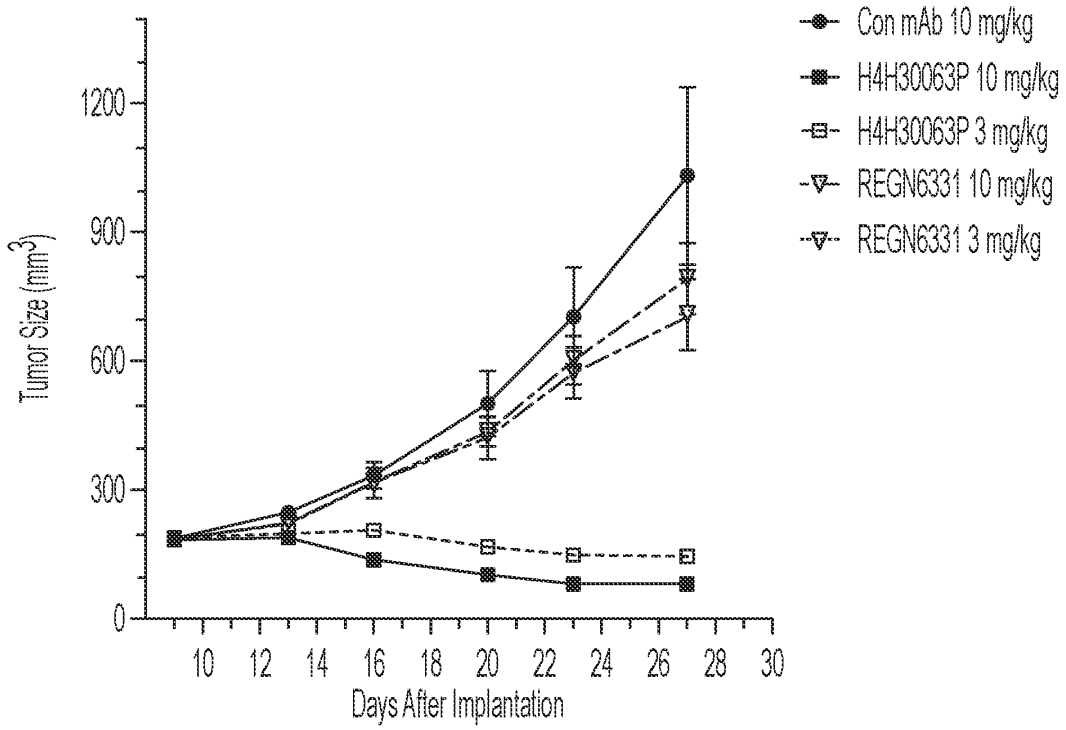

Con mAb 10 mg/kg
H4H30063P 10 mg/kg
H4H30063P 3 mg/kg
REGN6331 10 mg/kg
REGN6331 3 mg/kg
Tumor Size (mm3)
0
300
600
900
1200
10
12
14
16
18
20
22
24
26
28
30
Days After Implantation

FIG. 5

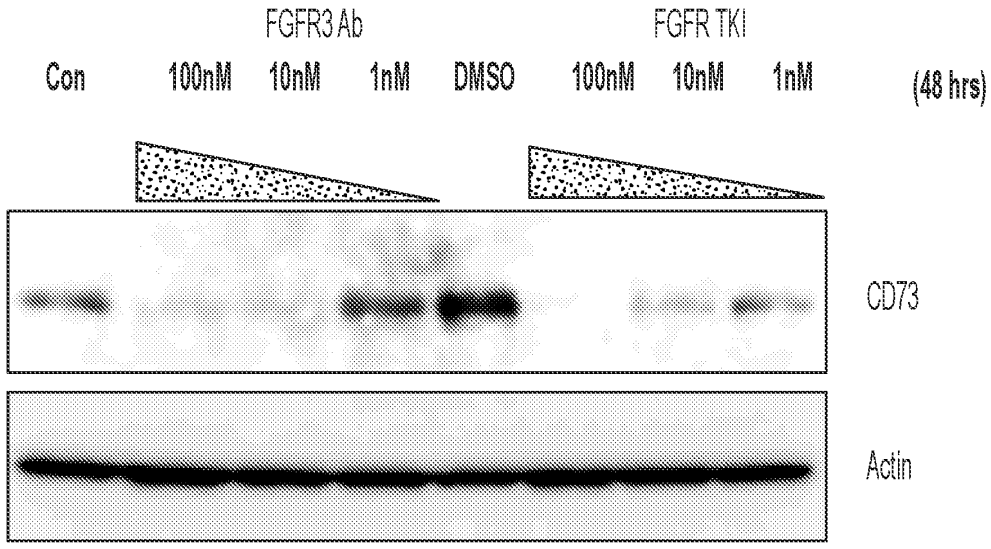

FGFR3 Ab
FGFR TKI
Con
100nM
10nM
1nM
DMSO
100nM
10nM
1nM
(48 hrs)
CD73
Actin

FIG. 6

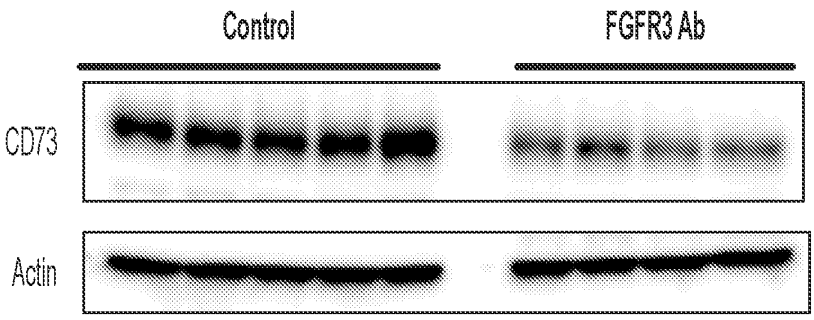


FIG. 7

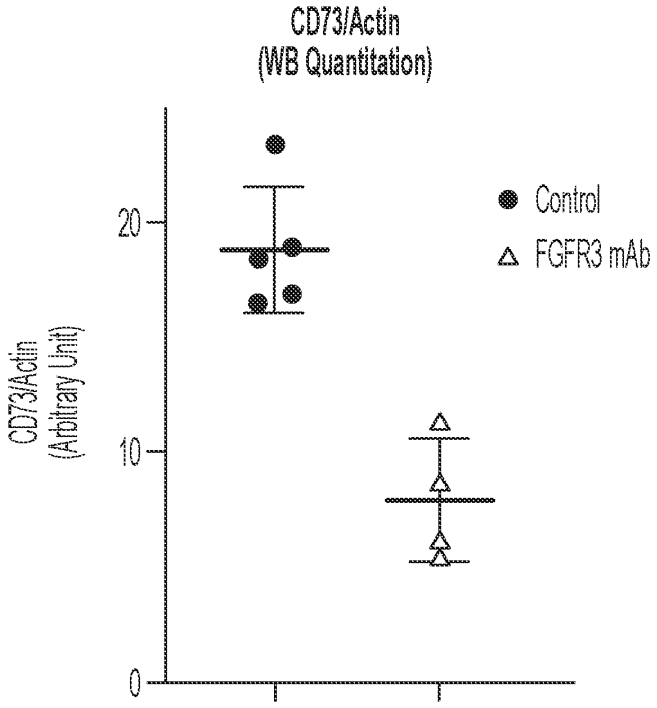

CD73/Actin
(WB Quantitation)
CD73/Actin
(Arbitrary Unit)
20
10
0
Control
FGFR3 mAb

FIG. 8

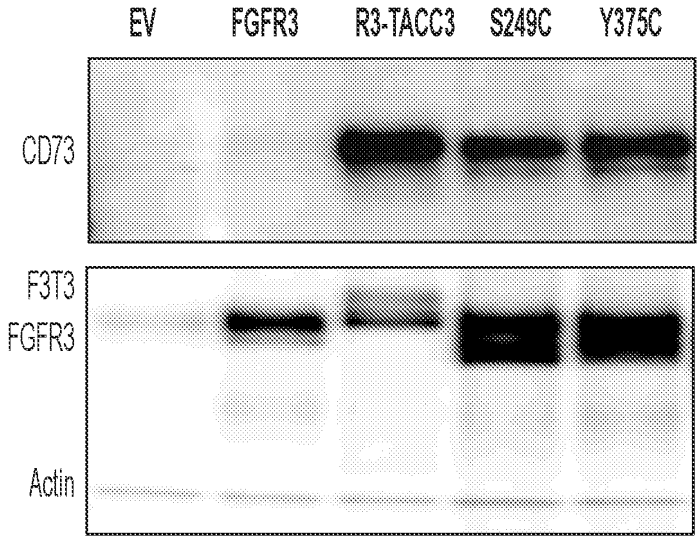


FIG. 9

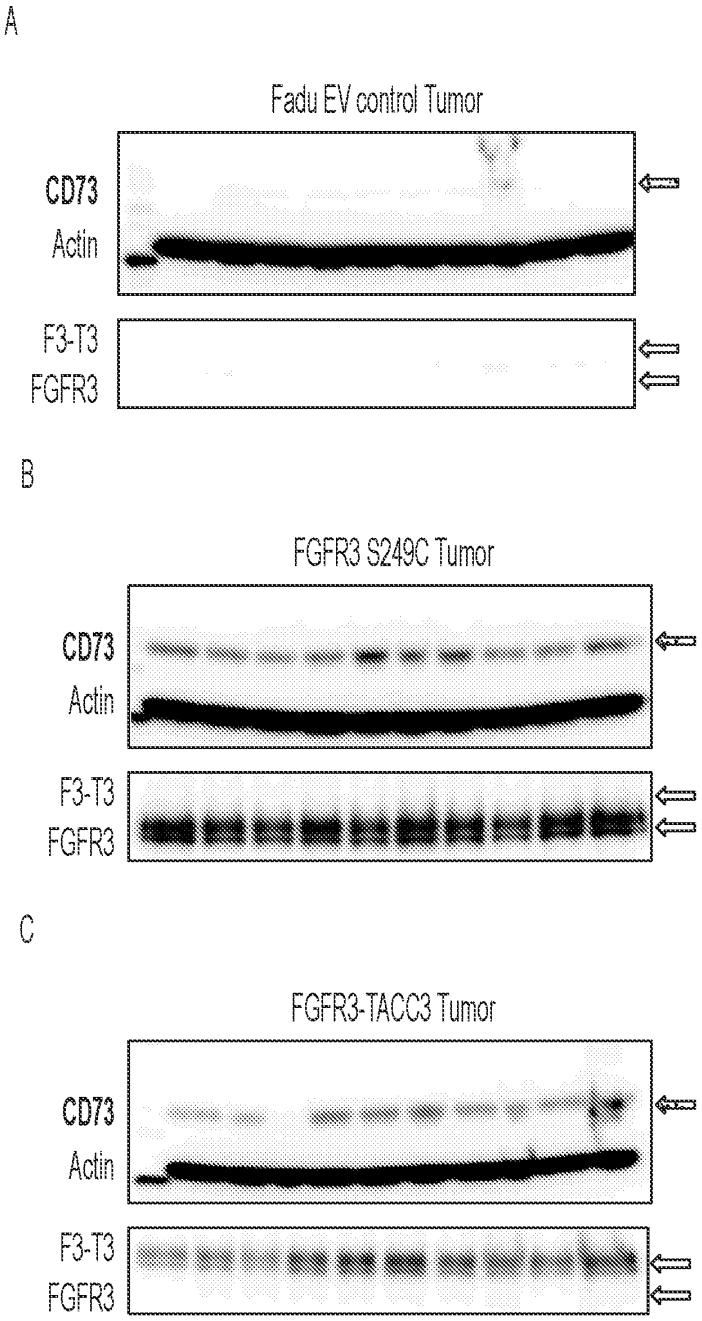

A
Fadu EV control Tumor
CD73
Actin
F3-T3
FGFR3
B
FGFR3 S249C Tumor
CD73
Actin
F3-T3
FGFR3
C
FGFR3-TACC3 Tumor
CD73
Actin
F3-T3
FGFR3

FIG. 10

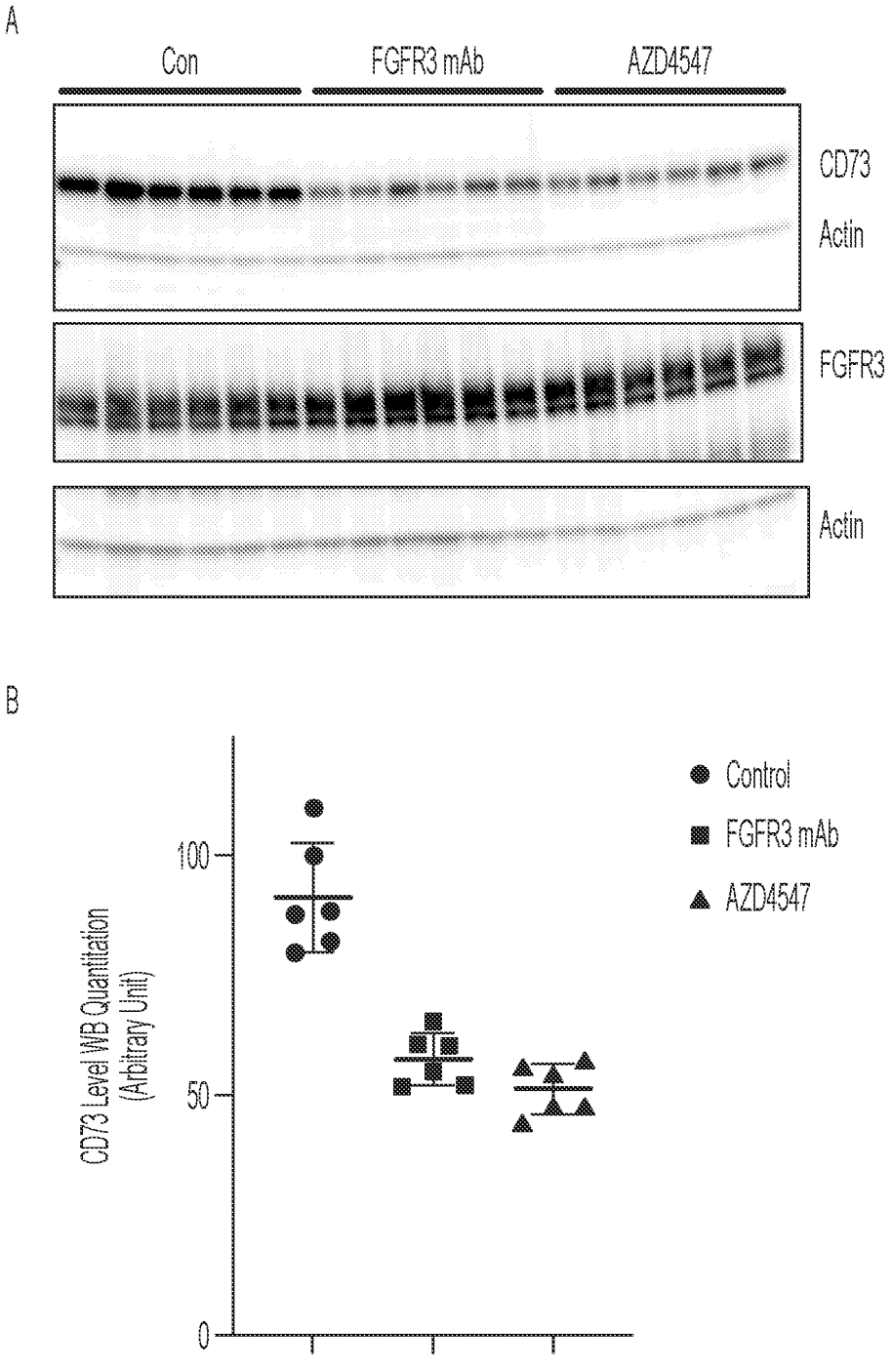

A
Con
FGFR3 mAb
AZD4547
CD73
Actin
FGFR3
Actin
B
CD73 Level WB Quantitation (Arbitrary Unit)
100
50
0
Control
FGFR3 mAb
AZD4547

FIG. 11

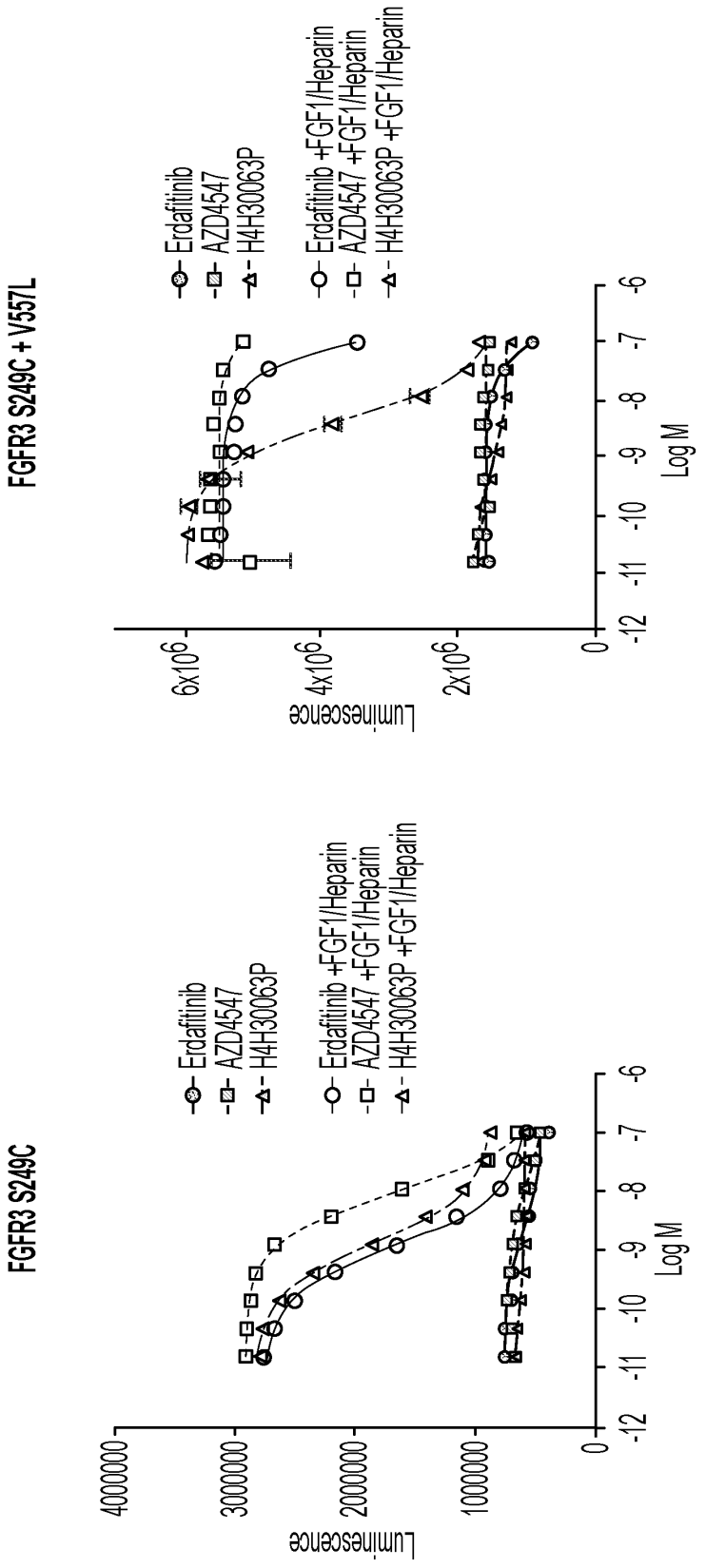

FGFR3 S249C + V557L
FGFR3 S249C
Erdafitinib
AZD4547
H4H30063P
Erdafitinib +FGF1/Heparin
AZD4547 +FGF1/Heparin
H4H30063P +FGF1/Heparin
Luminescence
Log M
6x10^6
4x10^6
2x10^6
4000000
3000000
2000000
1000000
0
-6
-7
-8
-9
-10
-11
-12

FIG. 12A

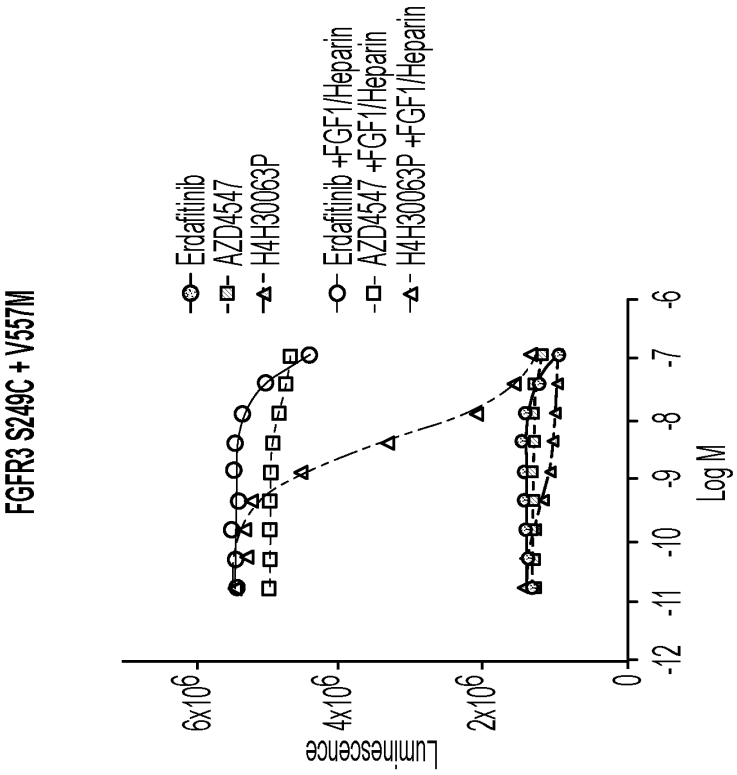
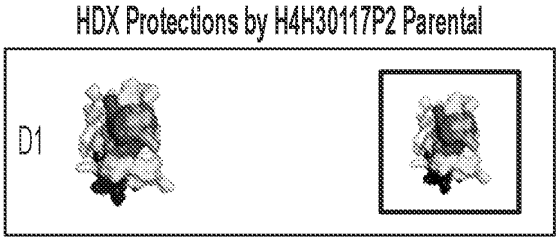

FGFR3 S249C + V557M
Erdafitinib
AZD4547
H4H30063P
Erdafitinib +FGF1/Heparin
AZD4547 +FGF1/Heparin
H4H30063P +FGF1/Heparin
Luminescence
6x10^6
4x10^6
2x10^6
0
-12
-11
-10
-9
-8
-7
-6
Log M

FIG. 12B

Antigen: REGN3152 [hFGFR3b_ecto(E23-G377).mmH]

Antibody	FGFR3b residues with significant changes in deuterium%	Sequence coverage
H4H30117P2	60-86 SCPPPGGGPMGPTVWVKDGTGLVPSER (SEQ ID: 245) 107-118 YSCRQRLTQRVL (SEQ ID NO: 246) *D1*	96.1%
H4H30063P	163-170 LLAVPAAN (SEQ ID NO: 247) 245-258 VLERSPHRPILQAG (SEQ ID NO: 248) *D2* 305-315 YVTVLKSWISE (SEQ ID NO: 249) *D3* 319-324 ADVRLR (SEQ ID NO: 250) 337-352 LCRATNFIGVAEKAFW (SEQ ID NO: 251)	90.1%



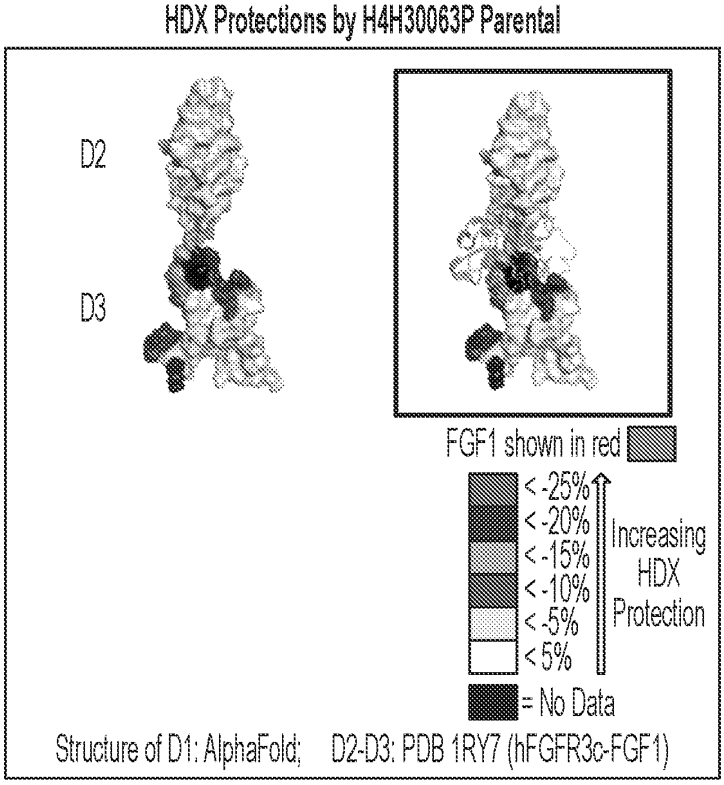


FIG. 13

Antigen: REGN3152 [hFGFR3b_ecto(E23-G377).mmH]

Antibody	FGFR3b residues with significant changes in deuterium%		Sequence coverage
H4H30045P	44-58 GQQEQLVFGSGDAVE (SEQ ID NO: 252) 87-94 VLVGPQRL (SEQ ID NO: 253)	*D1*	96.1%
H4H30108P2	245-258 VLERSPHRPILQAG (SEQ ID NO: 254) 274-283 HCKVYSDAQP (SEQ ID NO: 255) 305-324 YVTVLKSWISESVEADVRLR (SEQ ID NO: 256) 337-351 LCRATNFIGVAEKAF (SEQ ID NO: 257)	*D2* *D3*	96.1%

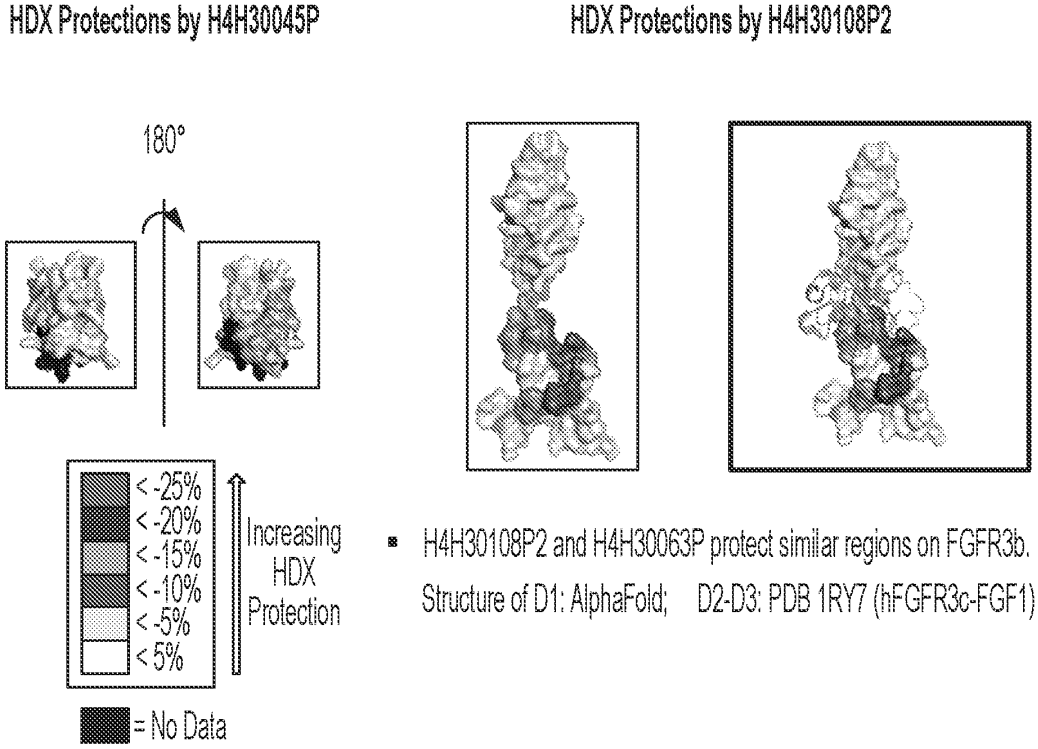
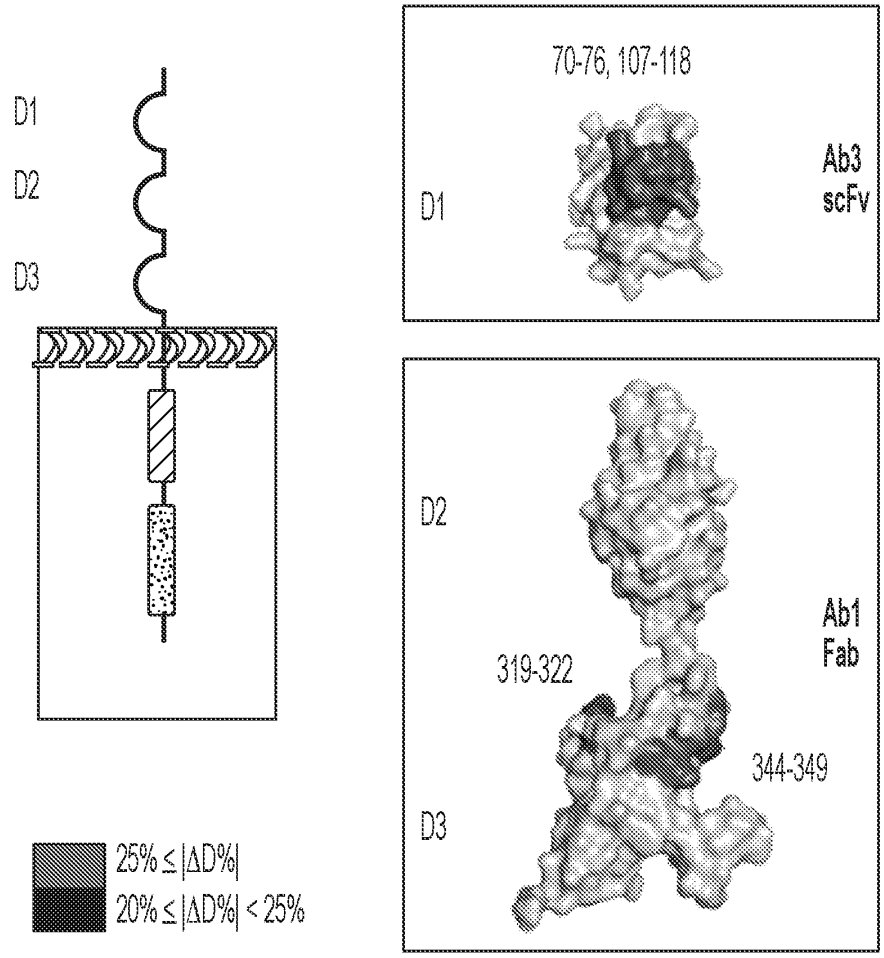


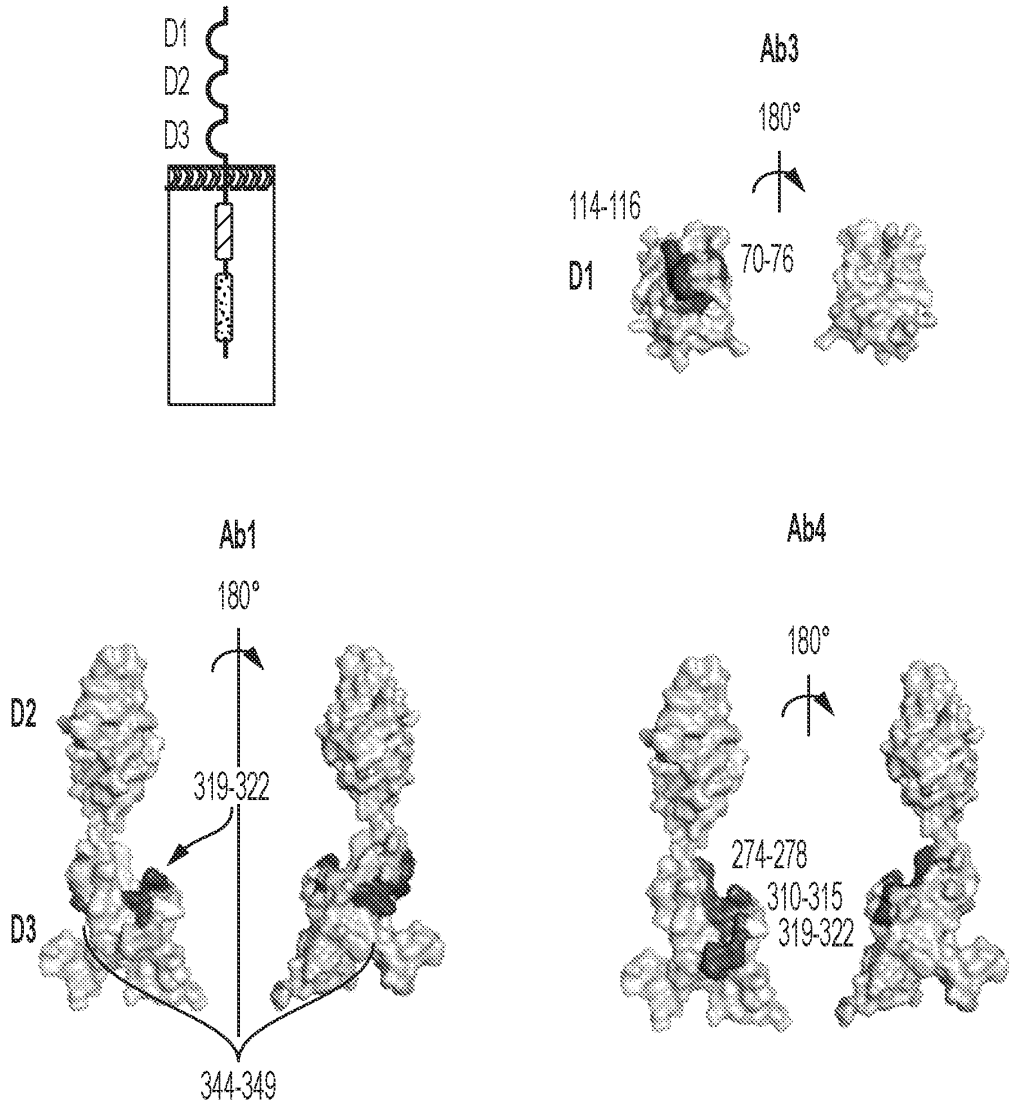
FIG. 14



Ab code used in figures:

Ab1 H4H30063P, Ab3 H4H30117P2, Ab4 H4H30108P2, Ab5 H4H30045P2

FIG. 15



Ab code used in figures:

Ab1 H4H30063P, Ab3 H4H30117P2, Ab4 H4H30108P2, Ab5 H4H30045P2

FIG. 16A

Antibody	FGFR3b residues with significant changes in deuterium%
FGFR3 Ab1	319-322 ADVR (SEQ ID NO: 258) 344-349 IGVAEK (SEQ ID NO: 259)

Antibody	FGFR3b residues with significant changes in deuterium%
FGFR3 Ab3	70-76 GPTVWVK (SEQ ID NO: 260) 114-116 TQR

Antibody	FGFR3b residues with significant changes in deuterium%
FGFR3 Ab4	274-278 HCKVY (SEQ ID NO: 261) 310-315 KSWISE (SEQ ID NO: 262) 319-322 ADVR (SEQ ID NO: 258)

Ab code used in figures:
Ab1 H4H30063P, Ab3 H4H30117P2, Ab4 H4H30108P2, Ab5 H4H30045P2

FIG. 16B

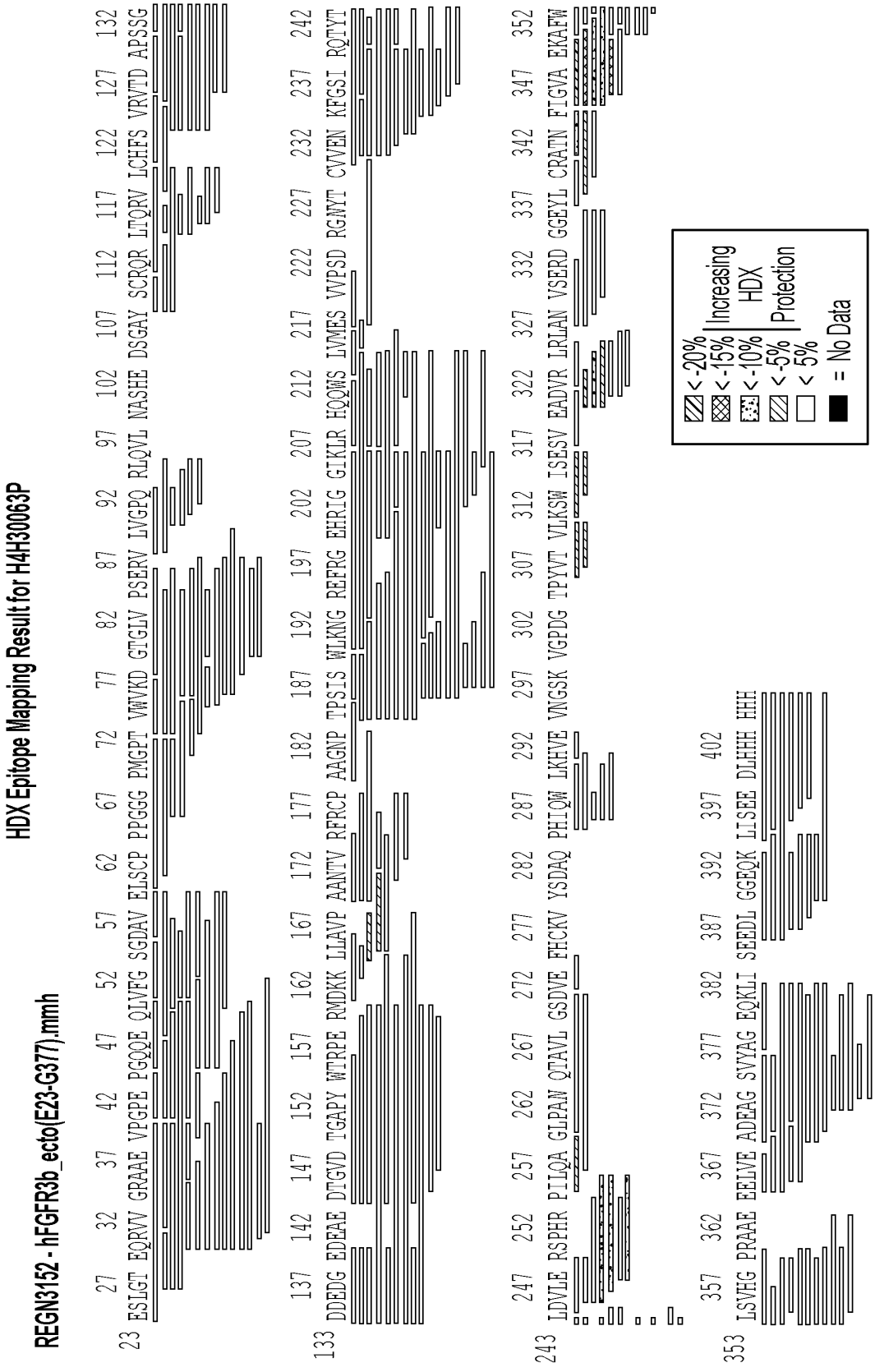

HDX Epitope Mapping Result for H4H30063P
REGN3152 - hFGFR3b_ecto(E23-G377).mmh
< -20%
< -15%
< -10%
< -5%
< 5%
= No Data
Increasing HDX Protection

FIG. 17

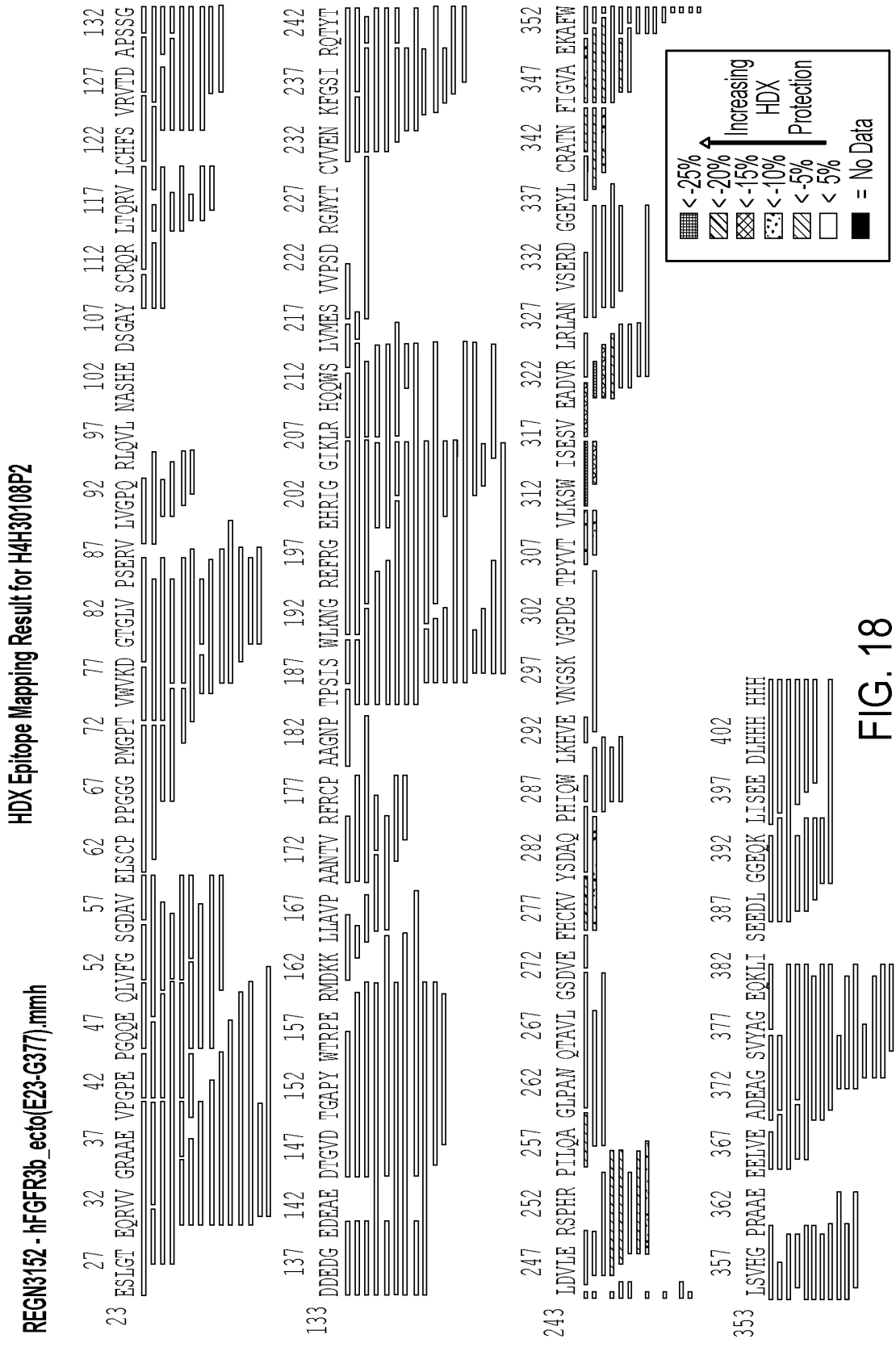

REGN3152 - hFGFR3b_ecto(E23-G377).mmh
HDX Epitope Mapping Result for H4H30108P2
< -25%
< -20%
< -15%
< -10%
< -5%
< 5%
= No Data
Increasing HDX Protection

FIG. 18

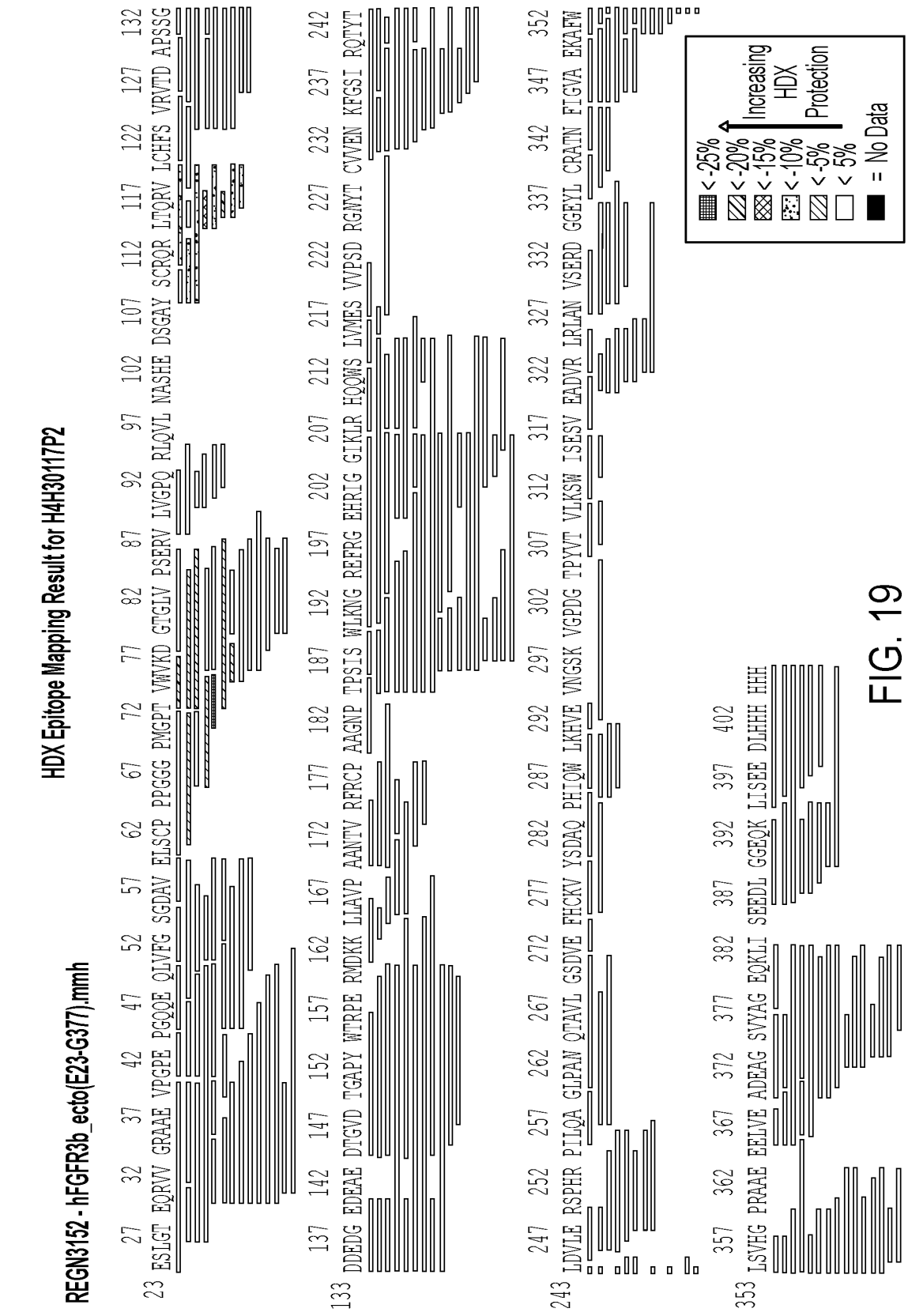

HDX Epitope Mapping Result for H4H30117P2
REGN3152 - hFGFR3b_ecto(E23-G377).mmh
< -25%
< -20%
< -15%
< -10%
< -5%
< 5%
= No Data
Increasing HDX Protection

FIG. 19

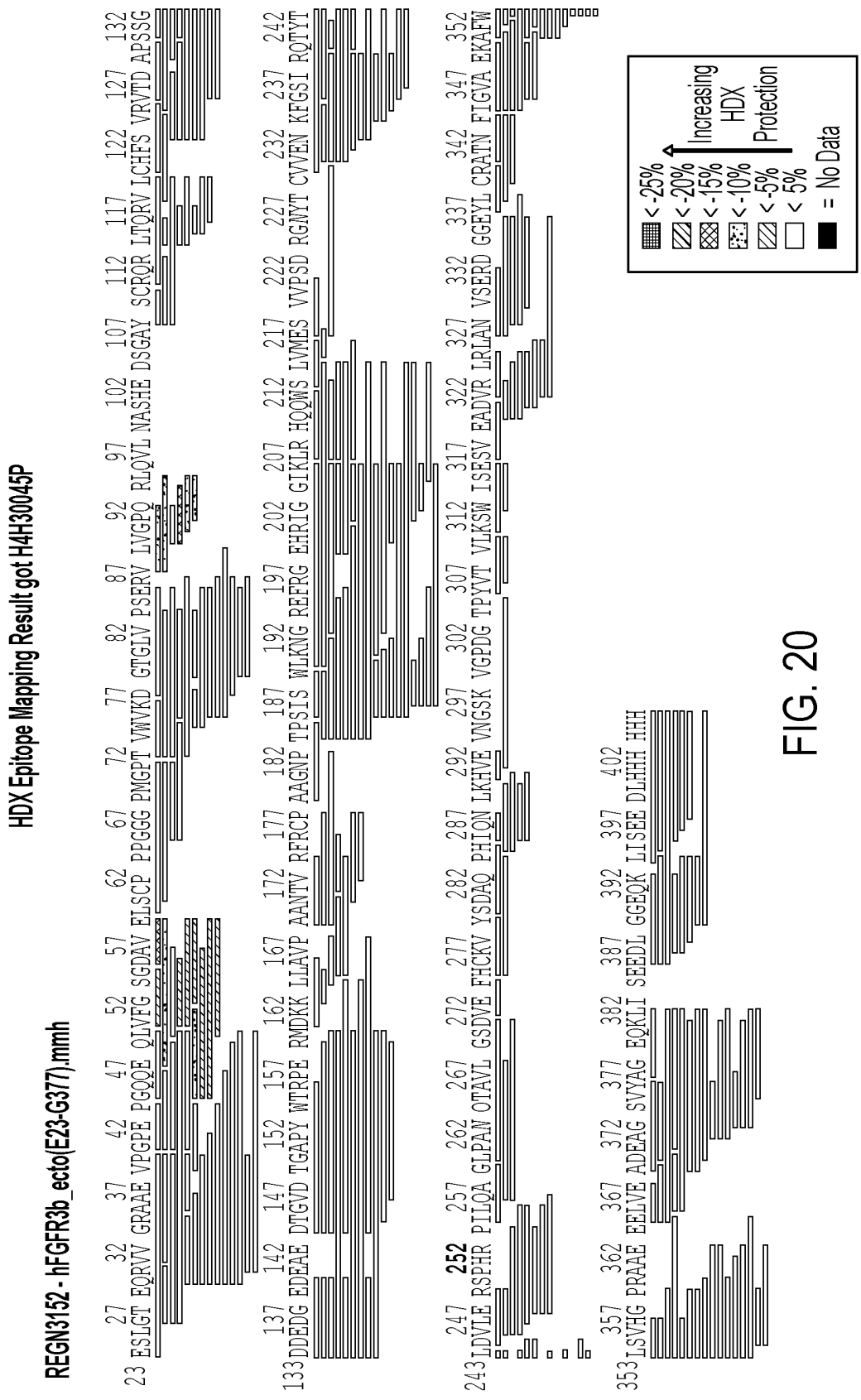

HDX Epitope Mapping Result got H4H30045P
REGN3152 - hFGFR3b_ecto(E23-G377).mmh
< -25%
< -20%
< -15%
< -10%
< -5%
< 5%
= No Data
Increasing HDX Protection

FIG. 20

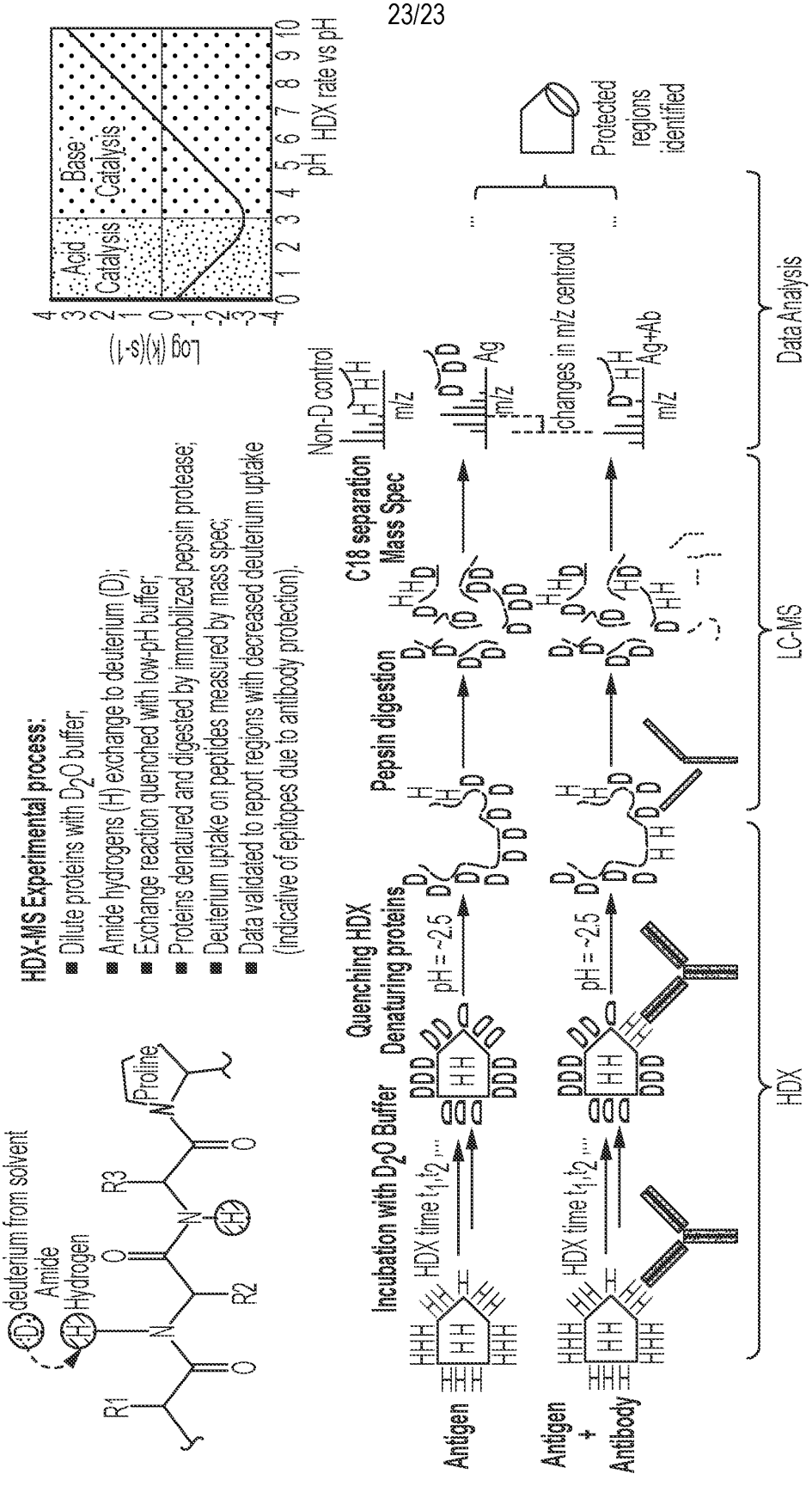

HDX-MS Experimental process:
Dilute proteins with D2O buffer;
Amide hydrogens (H) exchange to deuterium (D);
Exchange reaction quenched with low-pH buffer;
Proteins denatured and digested by immobilized pepsin protease;
Deuterium uptake on peptides measured by mass spec;
Data validated to report regions with decreased deuterium uptake (indicative of epitopes due to antibody protection).
deuterium from solvent
Amide Hydrogen
Proline
Acid Catalysis
Base Catalysis
pH HDX rate vs pH
Incubation with D2O Buffer
HDX time t1,t2,...
Quenching HDX
Denaturing proteins
pH = ~2.5
Pepsin digestion
C18 separation
Mass Spec
Non-D control
m/z
Ag
Ag+Ab
changes in m/z centroid
Protected regions identified
Antigen
Antigen + Antibody
HDX
LC-MS
Data Analysis

FIG. 21