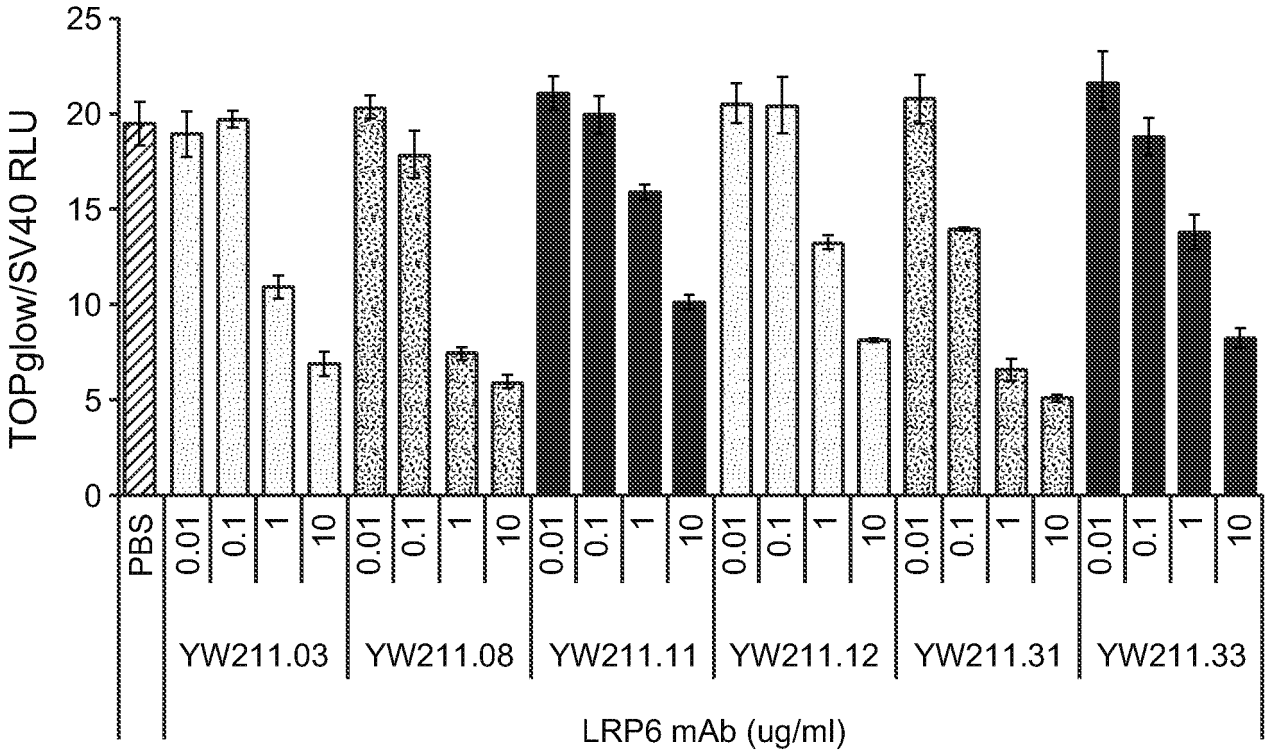

TOPglow/SV40 RLU
25
20
15
10
5
0
PBS
0.01
0.1
1
10
YW211.03
YW211.08
YW211.11
YW211.12
YW211.31
YW211.33
LRP6 mAb (ug/ml)

FIG. 1A

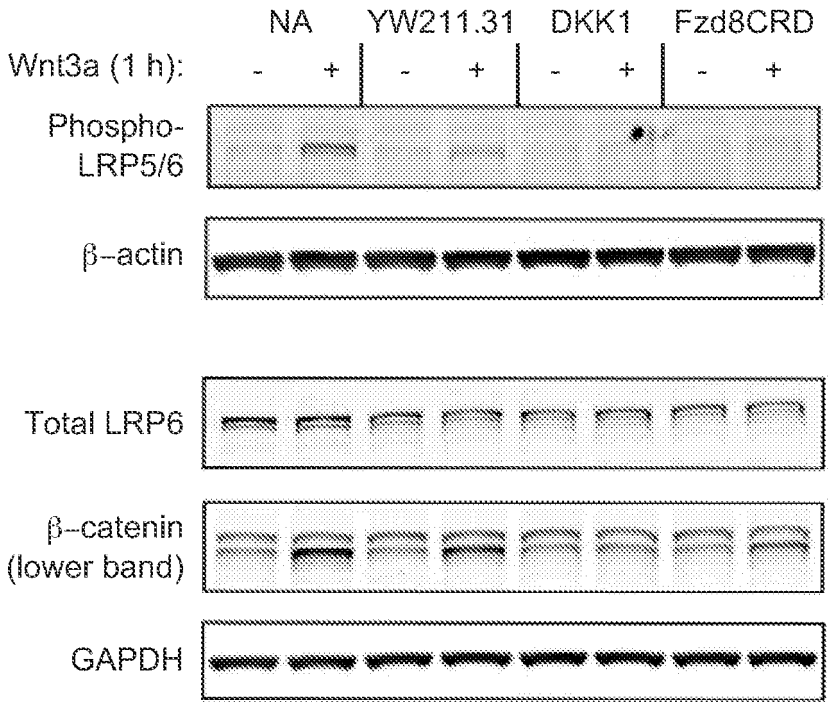

NA
YW211.31
DKK1
Fzd8CRD
Wnt3a (1 h):
- + - + - + - +
Phospho-LRP5/6
β–actin
Total LRP6
β–catenin (lower band)
GAPDH

FIG. 1B

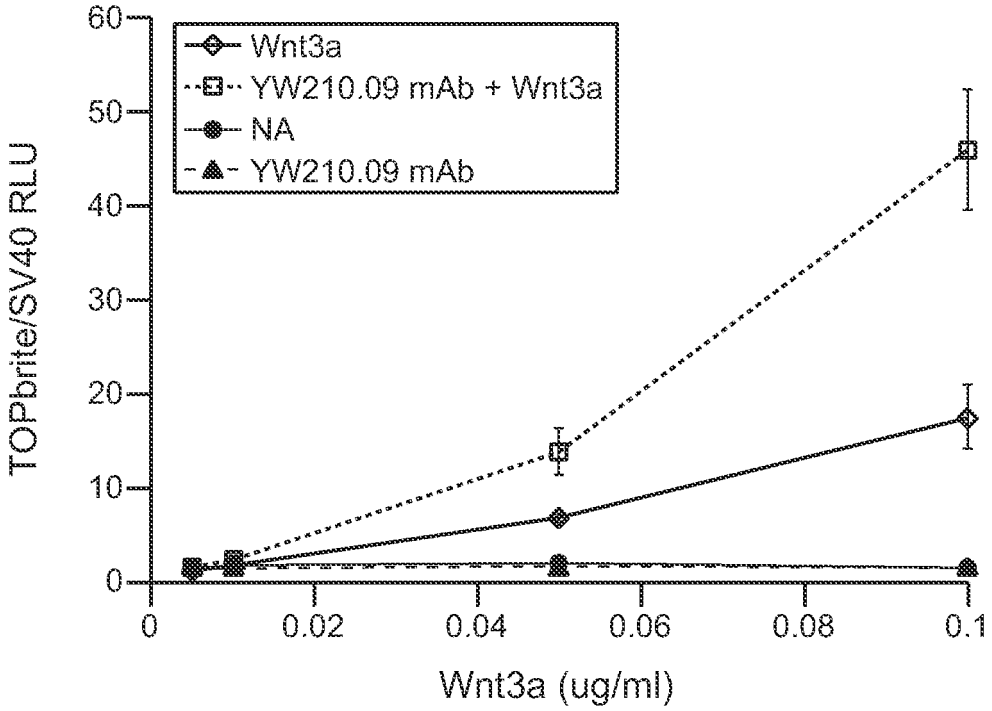

60
50
40
30
20
10
0
TOPbrite/SV40 RLU
Wnt3a
YW210.09 mAb + Wnt3a
NA
YW210.09 mAb
0
0.02
0.04
0.06
0.08
0.1
Wnt3a (ug/ml)

FIG. 1C

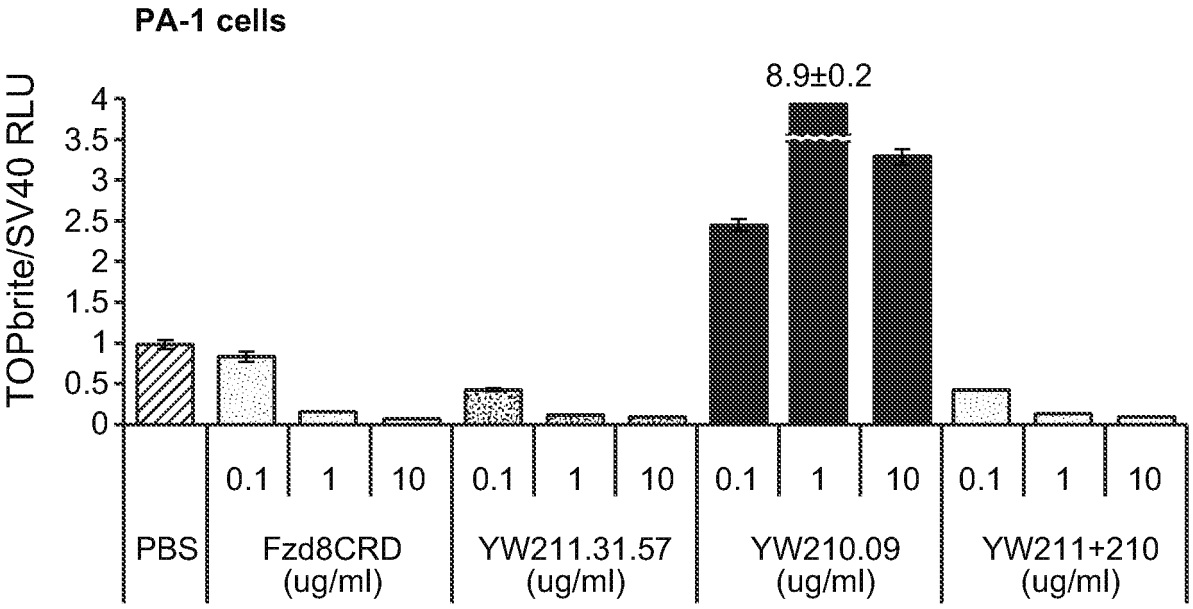

PA-1 cells
TOPbrite/SV40 RLU
8.9±0.2
0
0.5
1
1.5
2
2.5
3
3.5
4
0.1
1
10
PBS
Fzd8CRD (ug/ml)
YW211.31.57 (ug/ml)
YW210.09 (ug/ml)
YW211+210 (ug/ml)

FIG. 2A

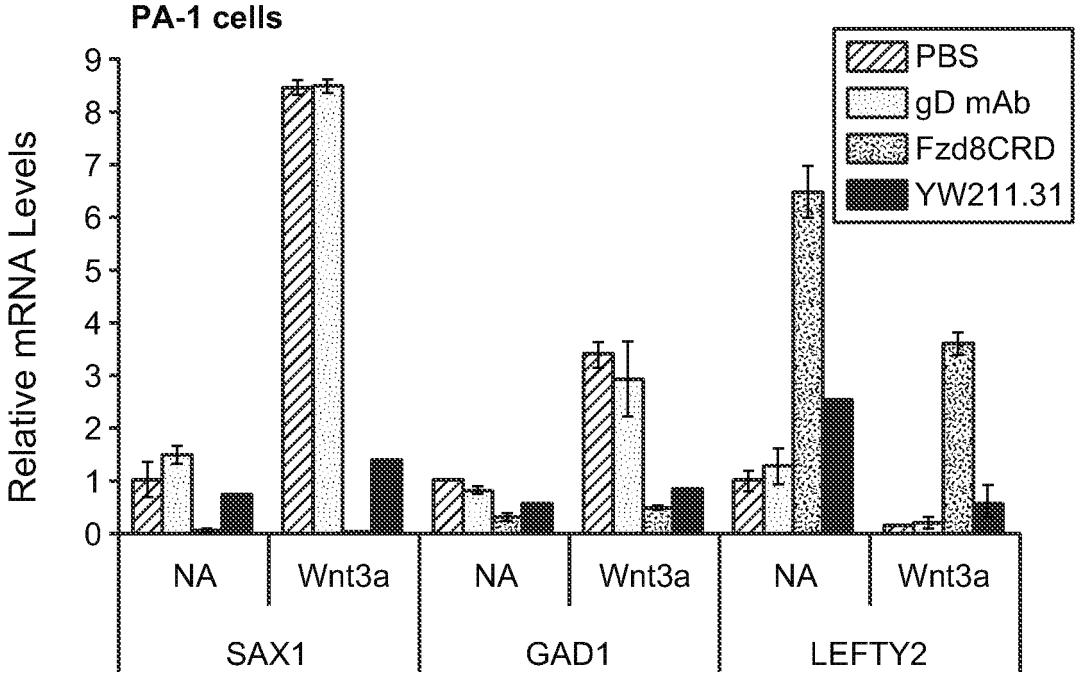

PA-1 cells
Relative mRNA Levels
PBS
gD mAb
Fzd8CRD
YW211.31
0
1
2
3
4
5
6
7
8
9
NA
Wnt3a
SAX1
GAD1
LEFTY2

FIG. 2B

Tumor type	Cell line	Fold change in autocrine Wnt signaling with treatment		
		YW211.31.57	YW210.09	Fzd8CRD
Teratocarcinoma	Ntera-2	0.33	1.60	0.07
	PA-1	0.11	3.34	0.08
Breast	Hs578T	1.98	1.27	0.60
NSCLC	EKVX	3.16	0.95	0.34
	NCI-H23	2.15	0.37	0.35
	NCI-H358	1.43	0.66	0.47
	NCI-H2030	6.10	2.02	0.29
Melanoma	M14	1.69	0.44	0.36
Hepatocellular	JHH-1	6.25	2.01	0.63
Soft tissue sarcoma	SW872	3.42	1.81	0.67
	HT-1080	2.90	1.44	0.43

FIG. 3

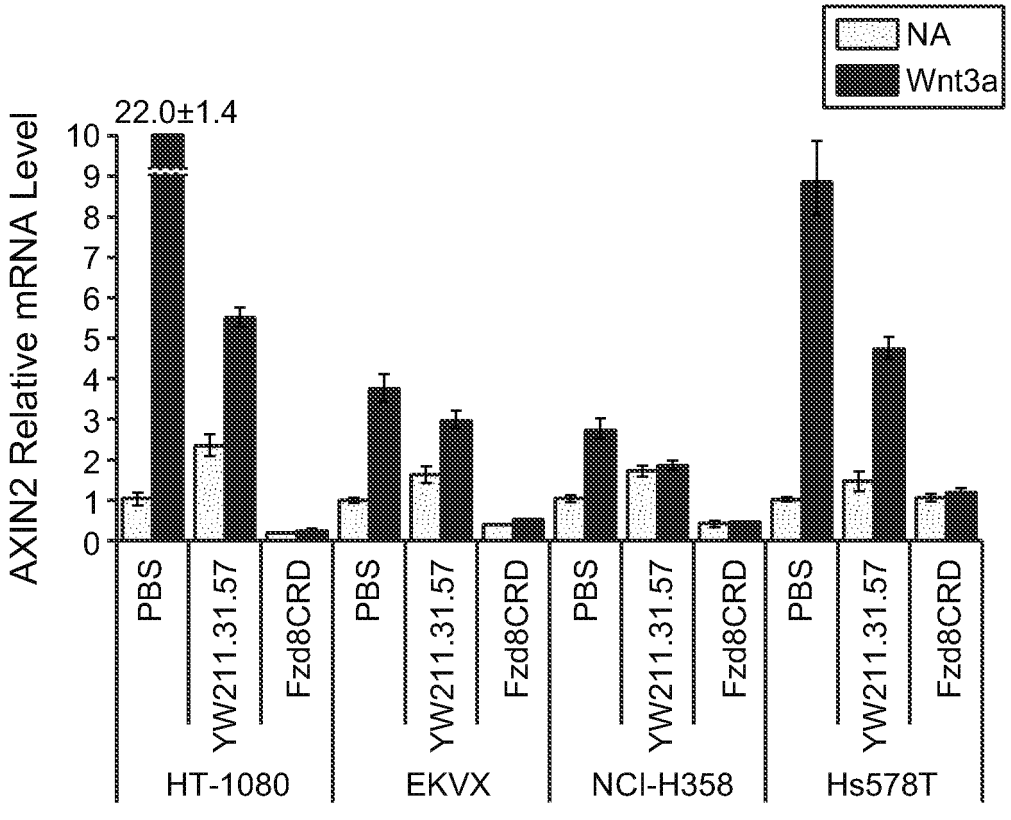


FIG. 4A

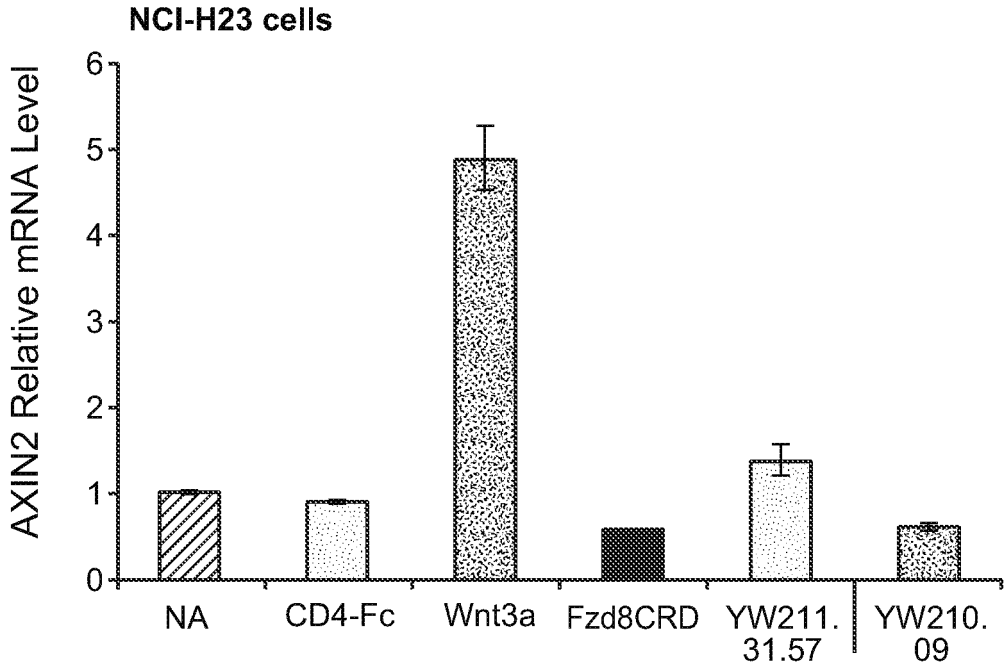

NCI-H23 cells
AXIN2 Relative mRNA Level
0
1
2
3
4
5
6
NA
CD4-Fc
Wnt3a
Fzd8CRD
YW211.31.57
YW210.09

FIG. 4B

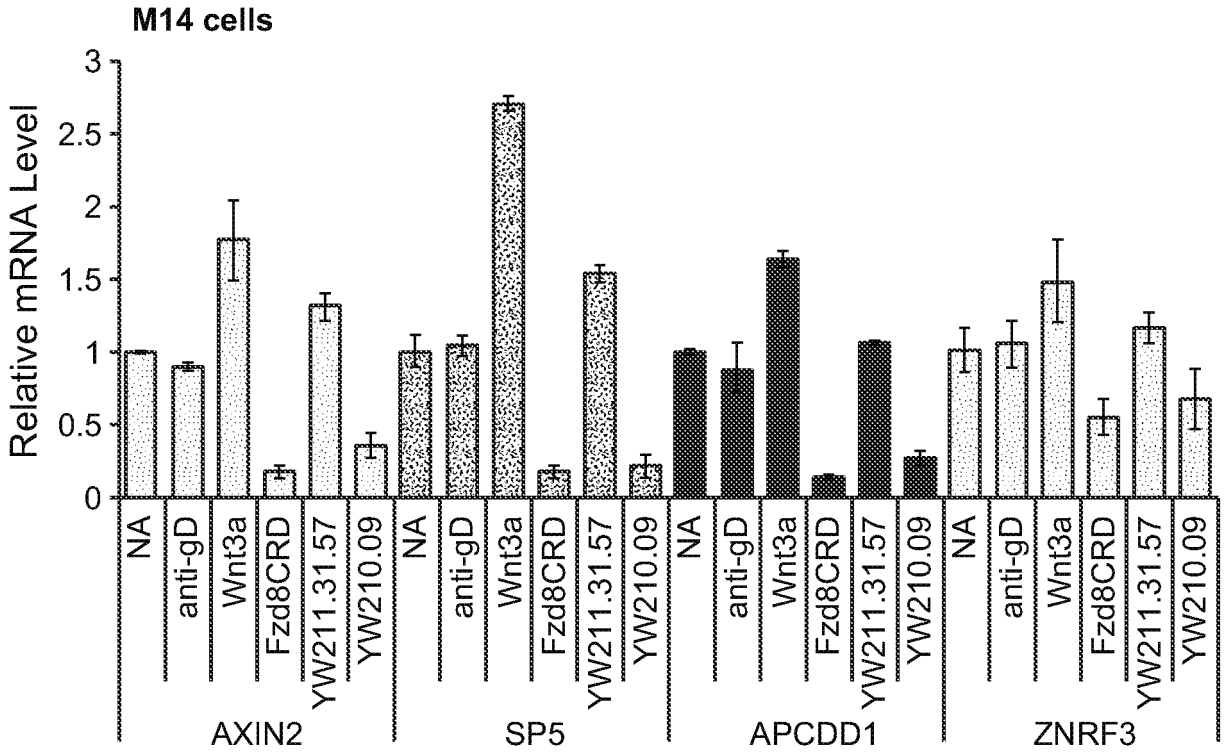

M14 cells
Relative mRNA Level
0
0.5
1
1.5
2
2.5
3
NA
anti-gD
Wnt3a
Fzd8CRD
YW211.31.57
YW210.09
AXIN2
SP5
APCDD1
ZNRF3

FIG. 4C

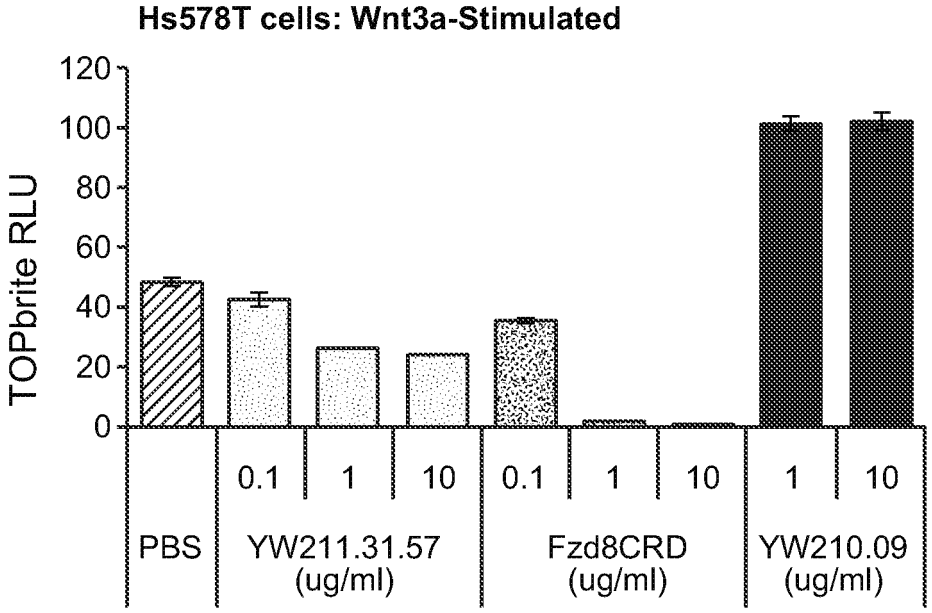

Hs578T cells: Wnt3a-Stimulated
TOPbrite RLU
120
100
80
60
40
20
0
0.1
1
10
0.1
1
10
1
10
PBS
YW211.31.57 (ug/ml)
Fzd8CRD (ug/ml)
YW210.09 (ug/ml)

FIG. 4D

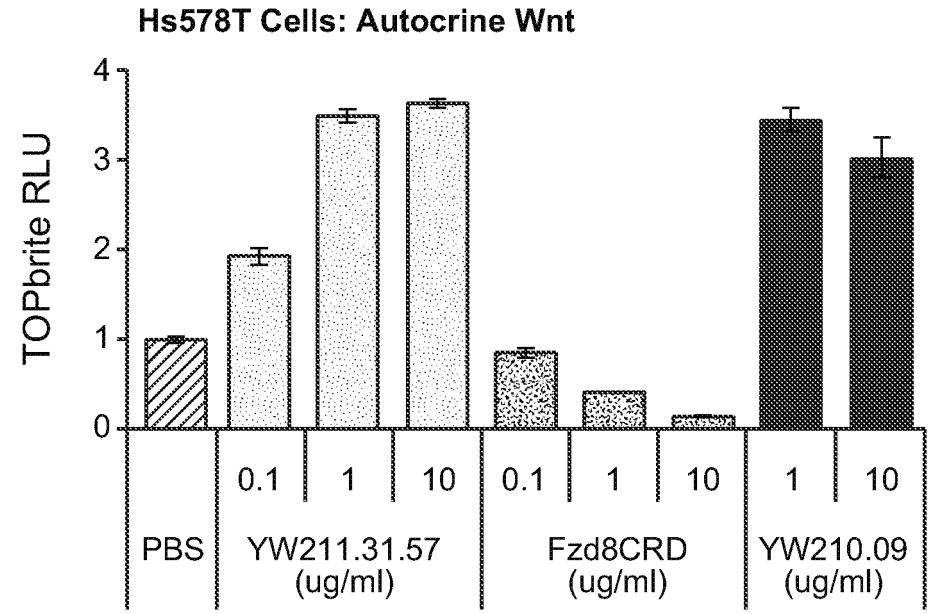

Hs578T Cells: Autocrine Wnt
TOPbrite RLU
4
3
2
1
0
0.1
1
10
0.1
1
10
1
10
PBS
YW211.31.57 (ug/ml)
Fzd8CRD (ug/ml)
YW210.09 (ug/ml)

FIG. 4E

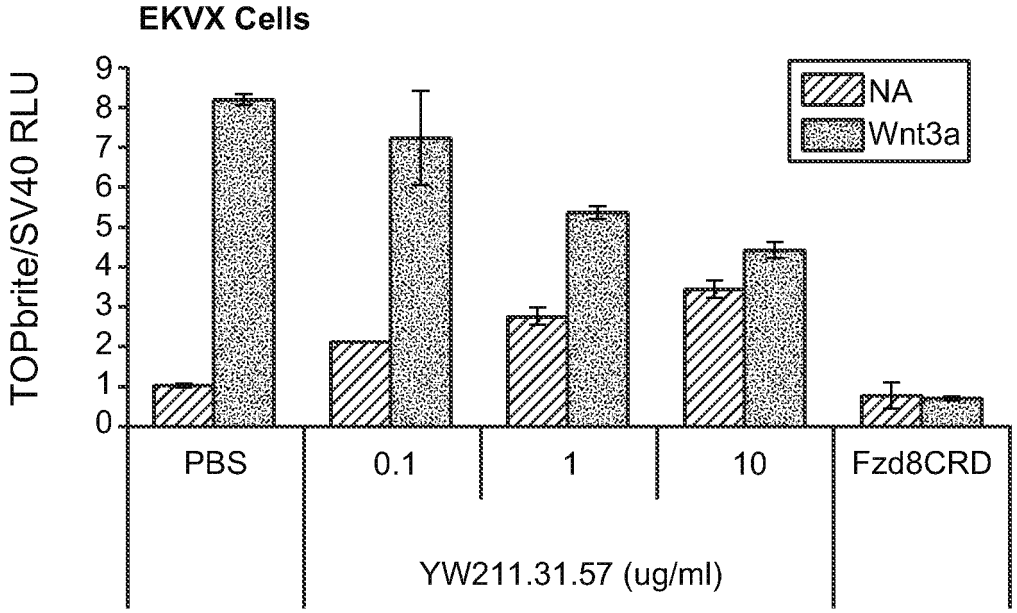

EKVX Cells
TOPbrite/SV40 RLU
0
1
2
3
4
5
6
7
8
9
NA
Wnt3a
PBS
0.1
1
10
Fzd8CRD
YW211.31.57 (ug/ml)

FIG. 4F

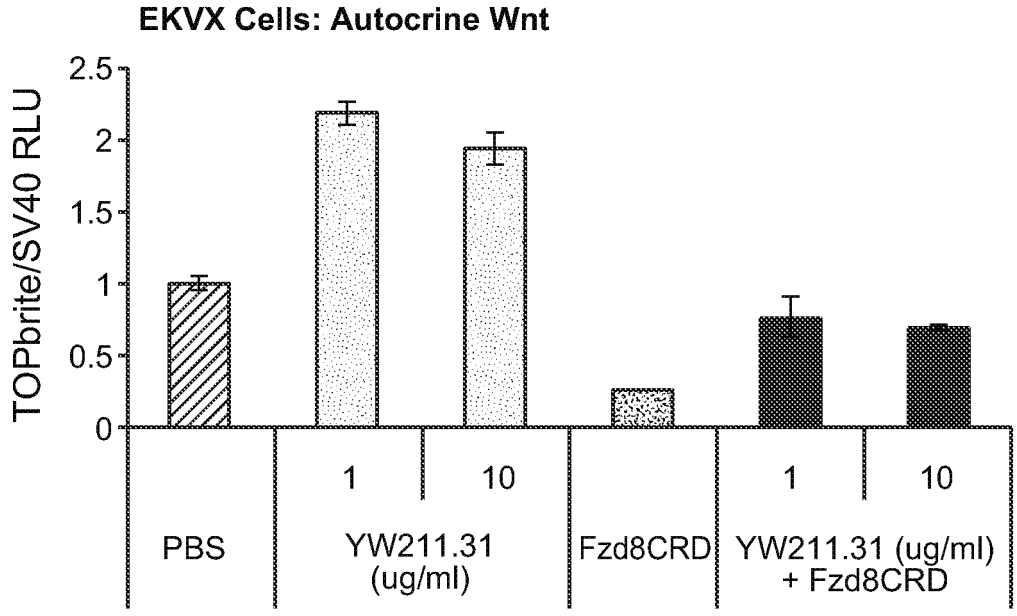

EKVX Cells: Autocrine Wnt
TOPbrite/SV40 RLU
0
0.5
1
1.5
2
2.5
PBS
1
10
YW211.31 (ug/ml)
Fzd8CRD
1
10
YW211.31 (ug/ml) + Fzd8CRD

FIG. 4G

Expression	HEK293 Cells			Hs578T Cells		
	Fold change in Wnt signaling with treatment			Fold change in Wnt signaling with treatment		
	YW211.31.57	YW210.09	Fzd8CRD	YW211.31.57	YW210.09	Fzd8CRD
Wnt3	0.07	1.86	0.14	0.39	2.02	0.18
Wnt3a	0.07	3.79	0.09	0.52	3.23	0.12
Wnt1	2.16	0.20	0.06	1.83	0.48	0.15
Wnt2	6.18	0.10	0.14	3.36	0.22	0.06
Wnt2b	3.24	0.24	0.33	3.11	0.51	0.13
Wnt6	4.41	0.34	0.71	2.67	0.62	0.15
Wnt8a	3.37	0.37	0.38	3.09	0.95	0.37
Wnt9a	1.47	0.51	0.35			
Wnt9b	1.38	0.67	0.92	4.21	0.95	0.41
Wnt10b	3.03	0.16	0.16	2.53	0.58	0.27
Wnt4				2.29	2.60	0.26
Wnt7a	3.54	1.42	0.22	5.83	4.72	0.14
Wnt7b	3.90	0.76	0.16	4.63	2.95	0.12
Wnt10a	2.53	1.07	0.30	2.88	0.66	0.11
autocrine Wnt				2.56	1.87	0.59

FIG. 5

FIG. 6

Expression	Fold change in Wnt signaling with treatment		
	YW211.31.57	YW210.09	Fzd8CRD
Wnt3a-Fzd4	0.25	0.95	1.06
Wnt3a-Fzd5	0.09	0.93	0.99
Wnt1-Fzd4	4.41	0.38	1.31
Wnt1-Fzd5	4.63	0.13	1.23
Wnt3a-LRP6	0.85	1.20	0.97
Wnt1-LRP6	1.23	1.12	0.28
LRP6	0.40	1.28	1.13

FIG. 7

Expression	Fold change in Wnt signaling with treatment		
	YW211.31.57	YW210.09	YW211.31.57 + YW210.09
Wnt3a	0.07	3.79	0.03
Wnt1	1.57	0.30	0.42
Wnt8A	3.37	0.37	0.22
Wnt9B	1.38	0.67	0.46
Wnt10B	3.03	0.16	0.08
Wnt7A	3.54	1.42	1.98
Wnt7B	3.90	0.76	1.80
Wnt10A	2.53	1.07	0.83

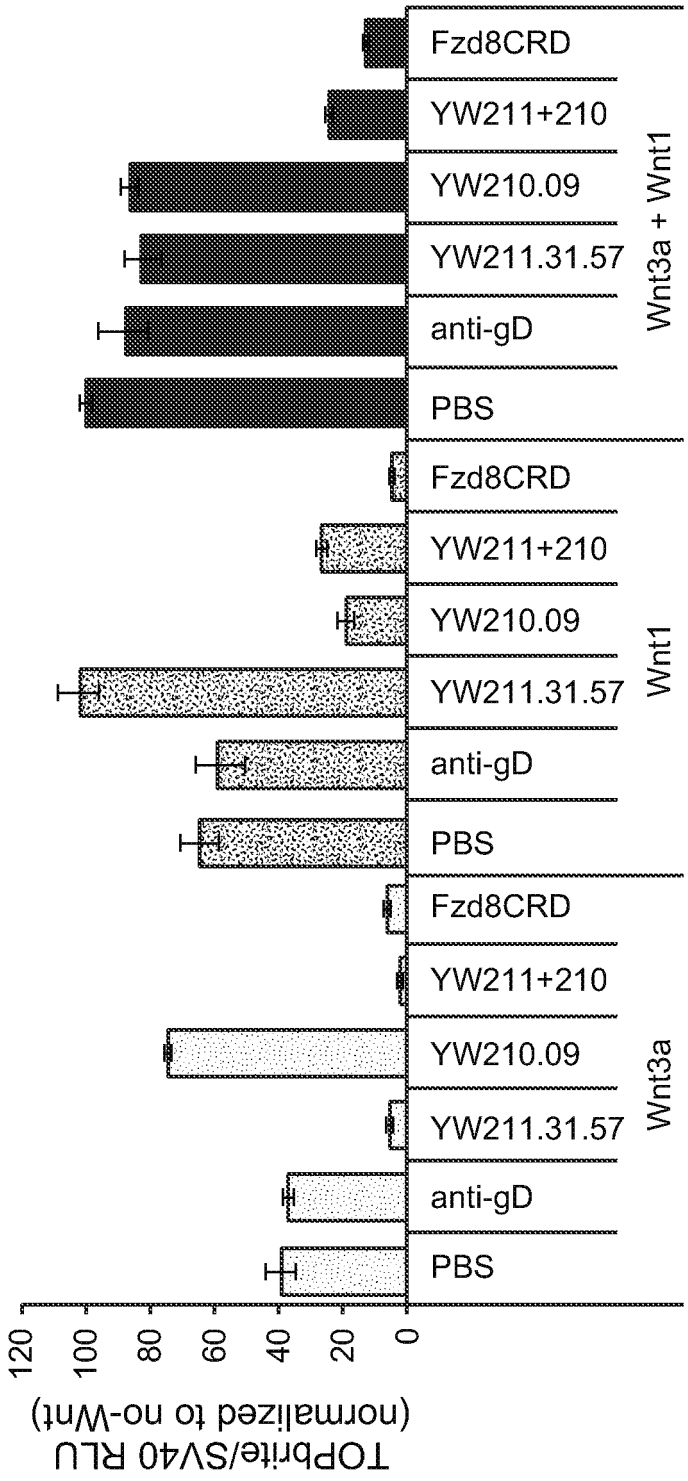

TOPbrite/SV40 RLU
(normalized to no-Wnt)
120
100
80
60
40
20
0
Wnt3a
Wnt1
Wnt3a + Wnt1
PBS
anti-gD
YW211.31.57
YW210.09
YW211+210
Fzd8CRD

FIG. 8A

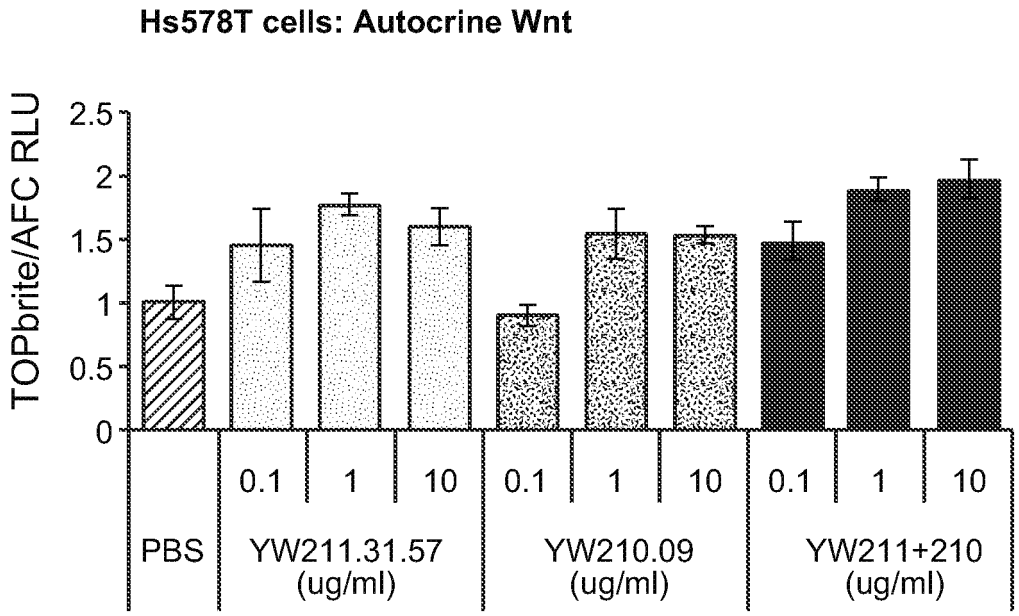

Hs578T cells: Autocrine Wnt
TOPbrite/AFC RLU
2.5
2
1.5
1
0.5
0
0.1
1
10
0.1
1
10
0.1
1
10
PBS
YW211.31.57 (ug/ml)
YW210.09 (ug/ml)
YW211+210 (ug/ml)

FIG. 8B

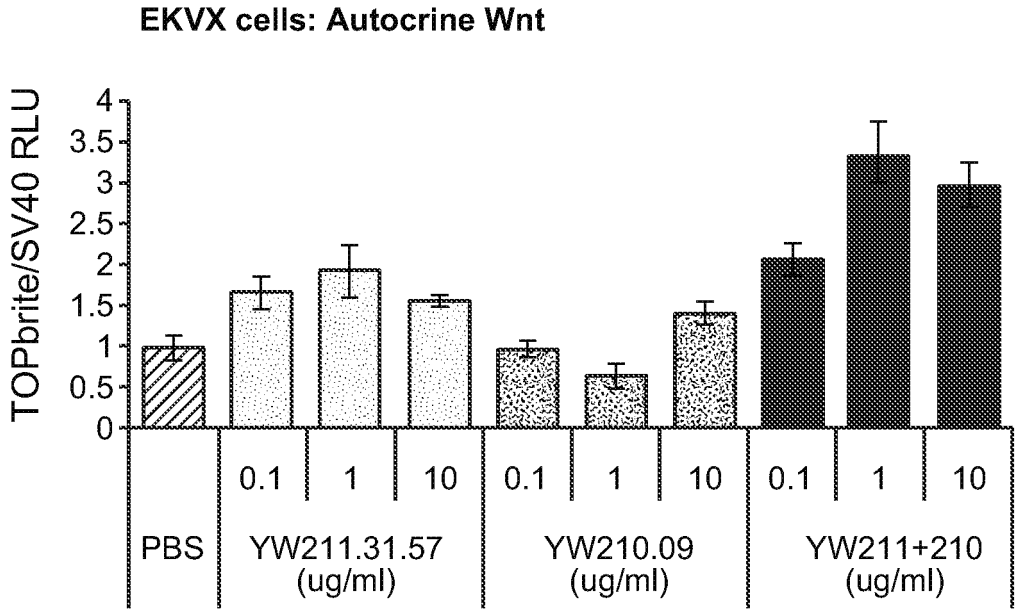

EKVX cells: Autocrine Wnt
TOPbrite/SV40 RLU
4
3.5
3
2.5
2
1.5
1
0.5
0
0.1
1
10
0.1
1
10
0.1
1
10
PBS
YW211.31.57 (ug/ml)
YW210.09 (ug/ml)
YW211+210 (ug/ml)

FIG. 8C

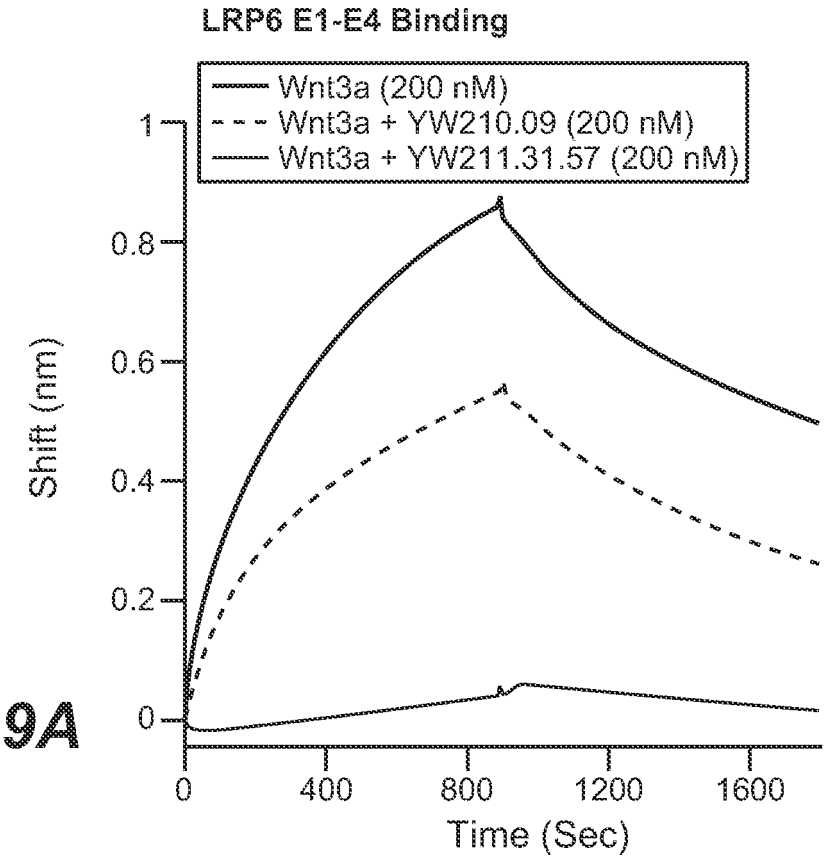

LRP6 E1-E4 Binding
Wnt3a (200 nM)
Wnt3a + YW210.09 (200 nM)
Wnt3a + YW211.31.57 (200 nM)
Shift (nm)
0
0.2
0.4
0.6
0.8
1
0
400
800
1200
1600
Time (Sec)

FIG. 9A

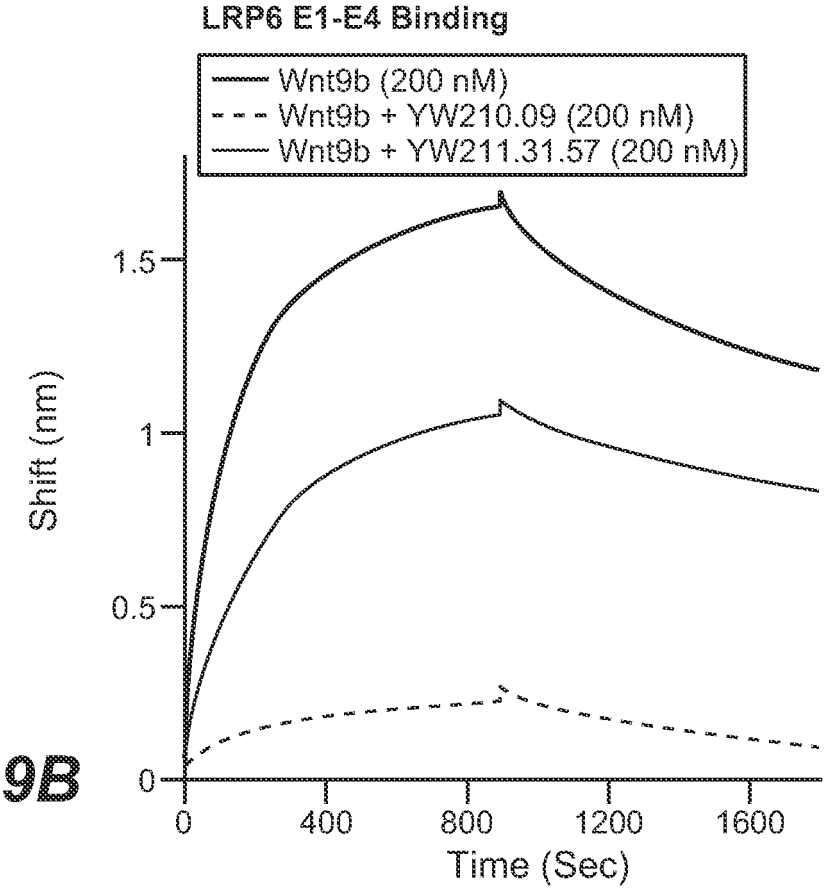

LRP6 E1-E4 Binding
Wnt9b (200 nM)
Wnt9b + YW210.09 (200 nM)
Wnt9b + YW211.31.57 (200 nM)
Shift (nm)
0
0.5
1
1.5
0
400
800
1200
1600
Time (Sec)

FIG. 9B

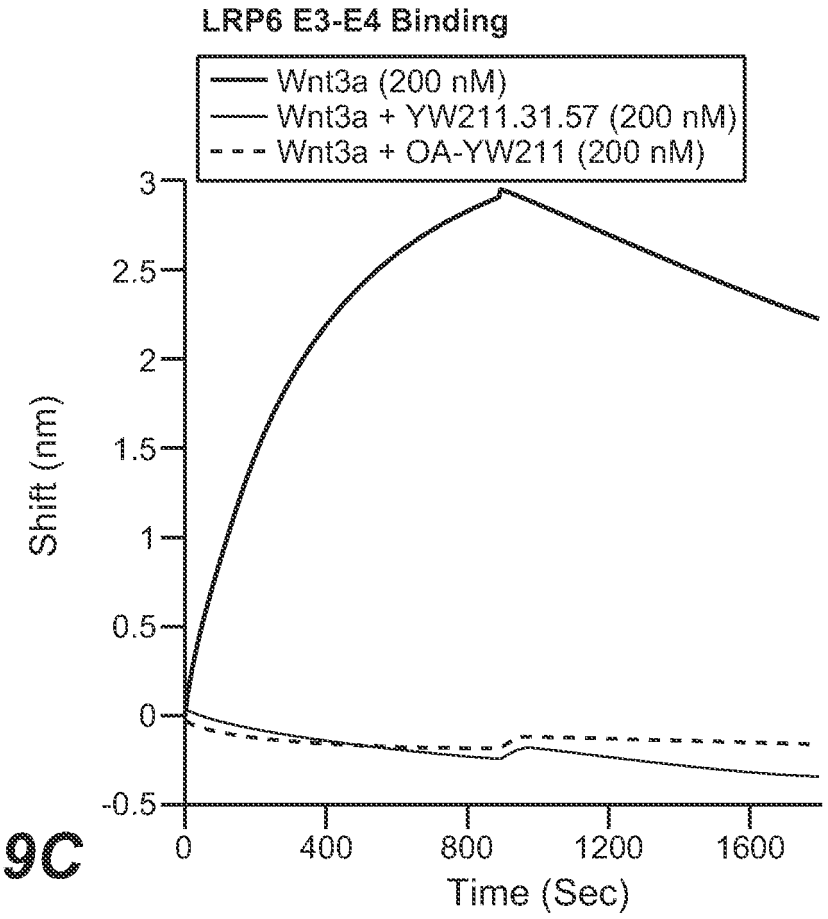

LRP6 E3-E4 Binding
Wnt3a (200 nM)
Wnt3a + YW211.31.57 (200 nM)
Wnt3a + OA-YW211 (200 nM)
Shift (nm)
3
2.5
2
1.5
1
0.5
0
-0.5
0
400
800
1200
1600
Time (Sec)

FIG. 9C

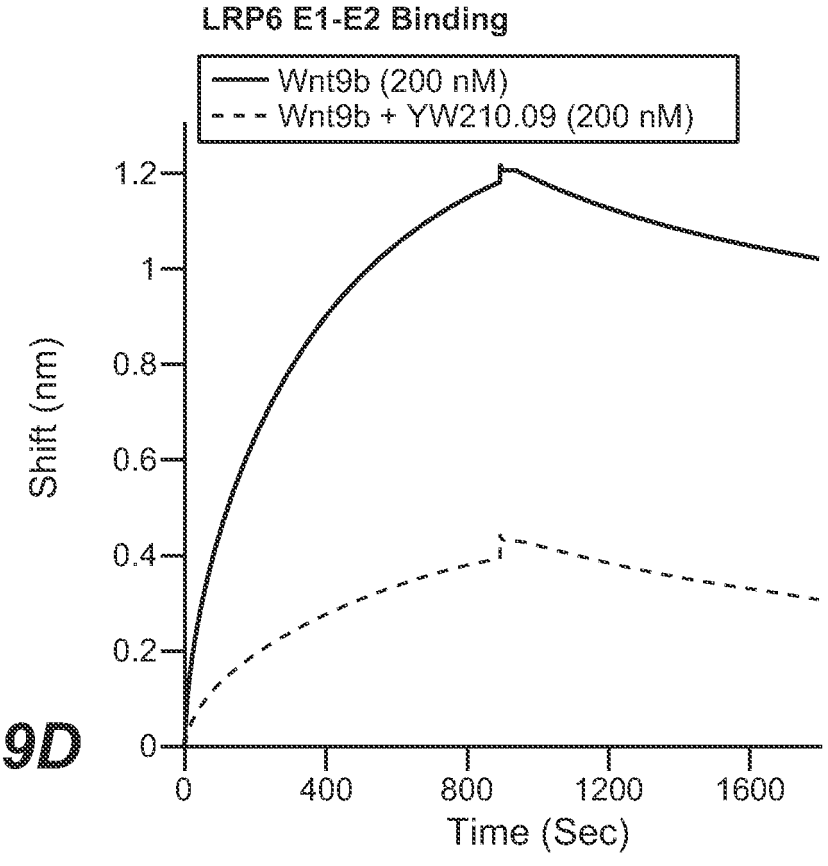

LRP6 E1-E2 Binding
Wnt9b (200 nM)
Wnt9b + YW210.09 (200 nM)
Shift (nm)
1.2
1
0.8
0.6
0.4
0.2
0
0
400
800
1200
1600
Time (Sec)

FIG. 9D

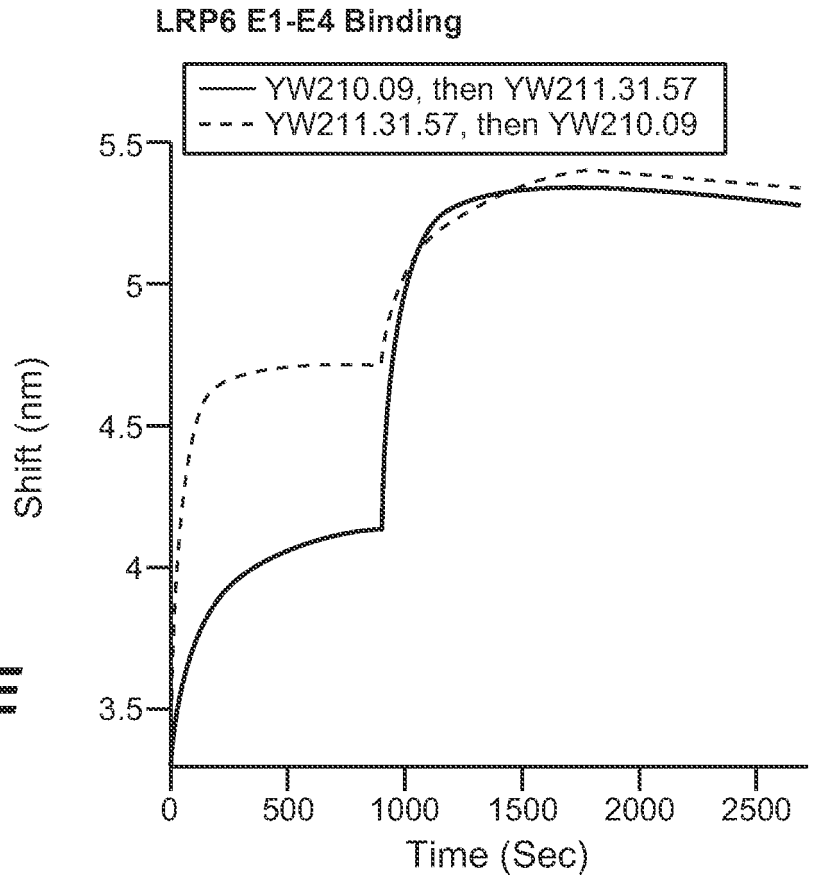


FIG. 9E

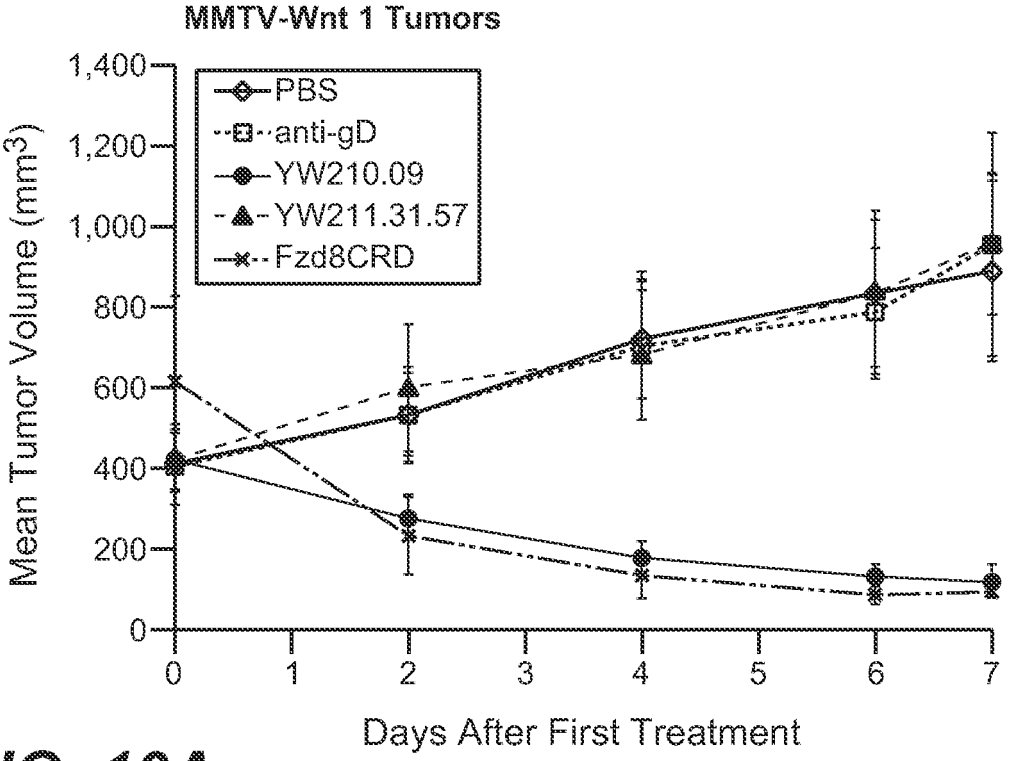


FIG. 10A

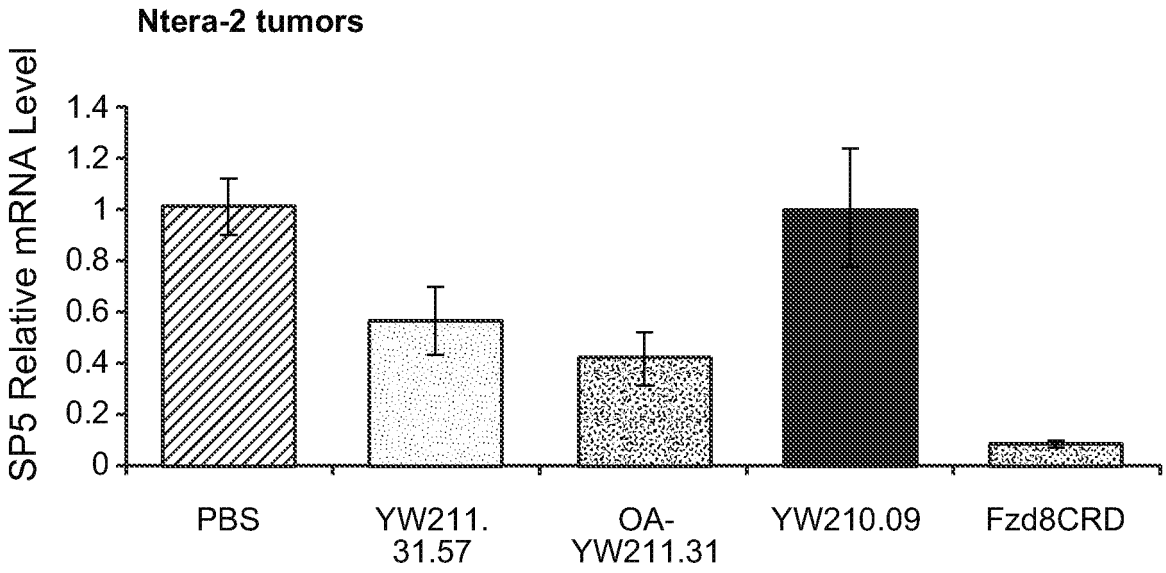


FIG. 10B

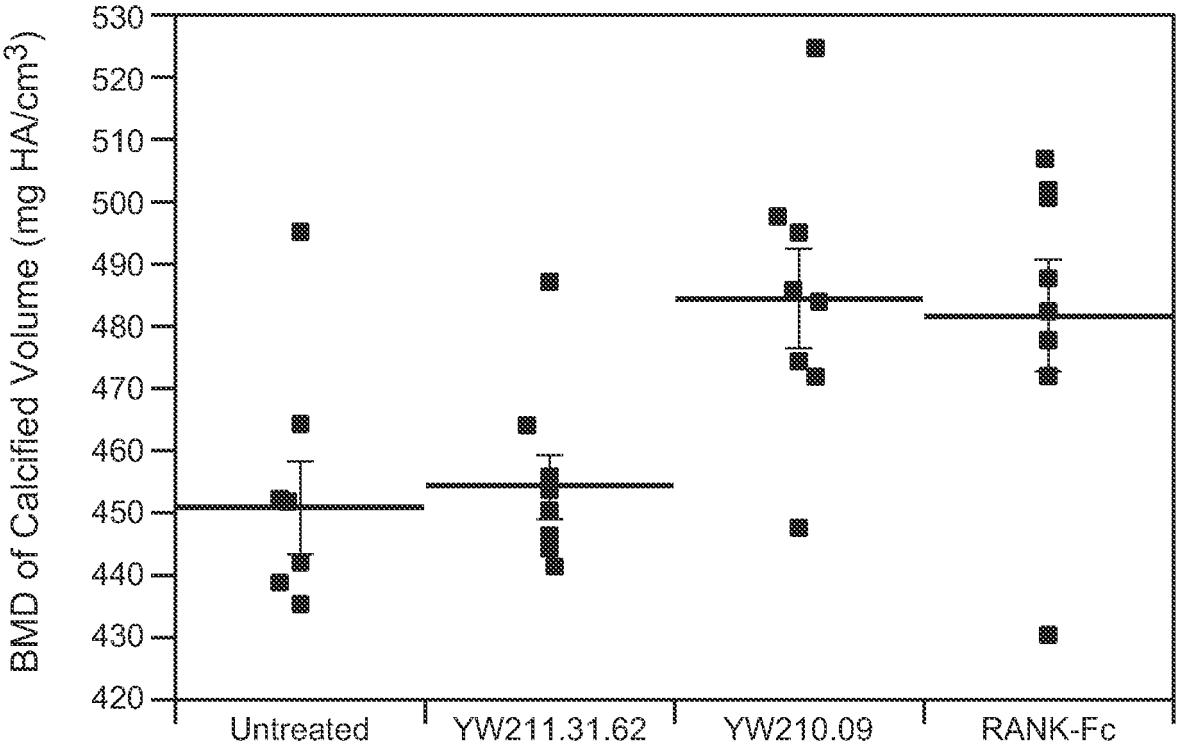


FIG. 10C

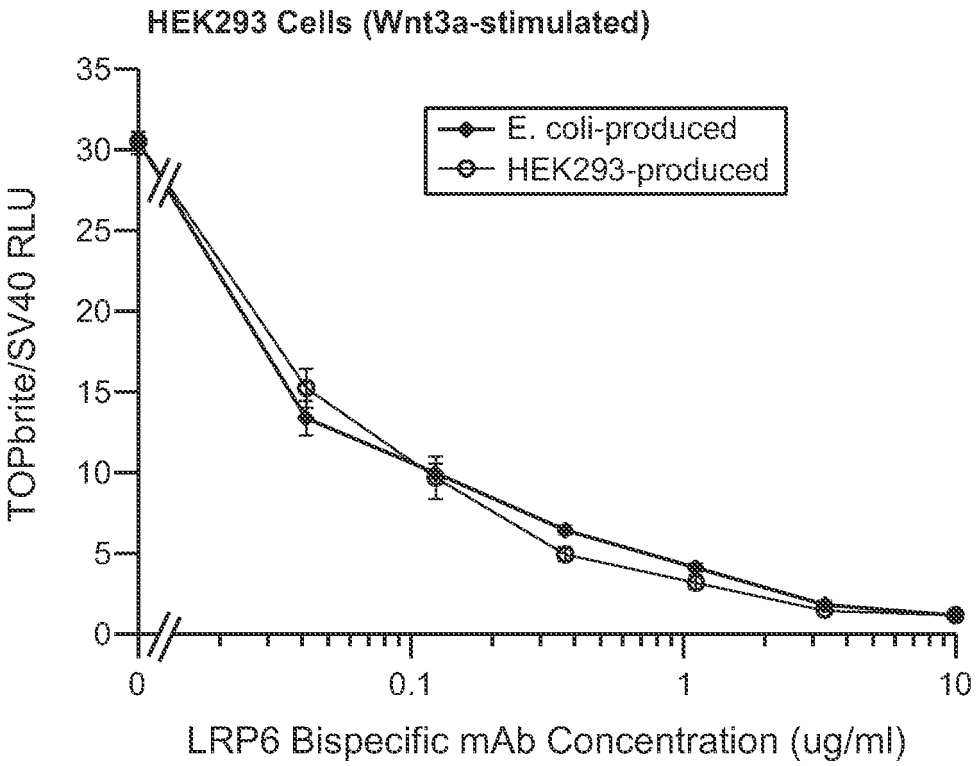

HEK293 Cells (Wnt3a-stimulated)
E. coli-produced
HEK293-produced
TOPbrite/SV40 RLU
35
30
25
20
15
10
5
0
0
0.1
1
10
LRP6 Bispecific mAb Concentration (ug/ml)

FIG. 11A

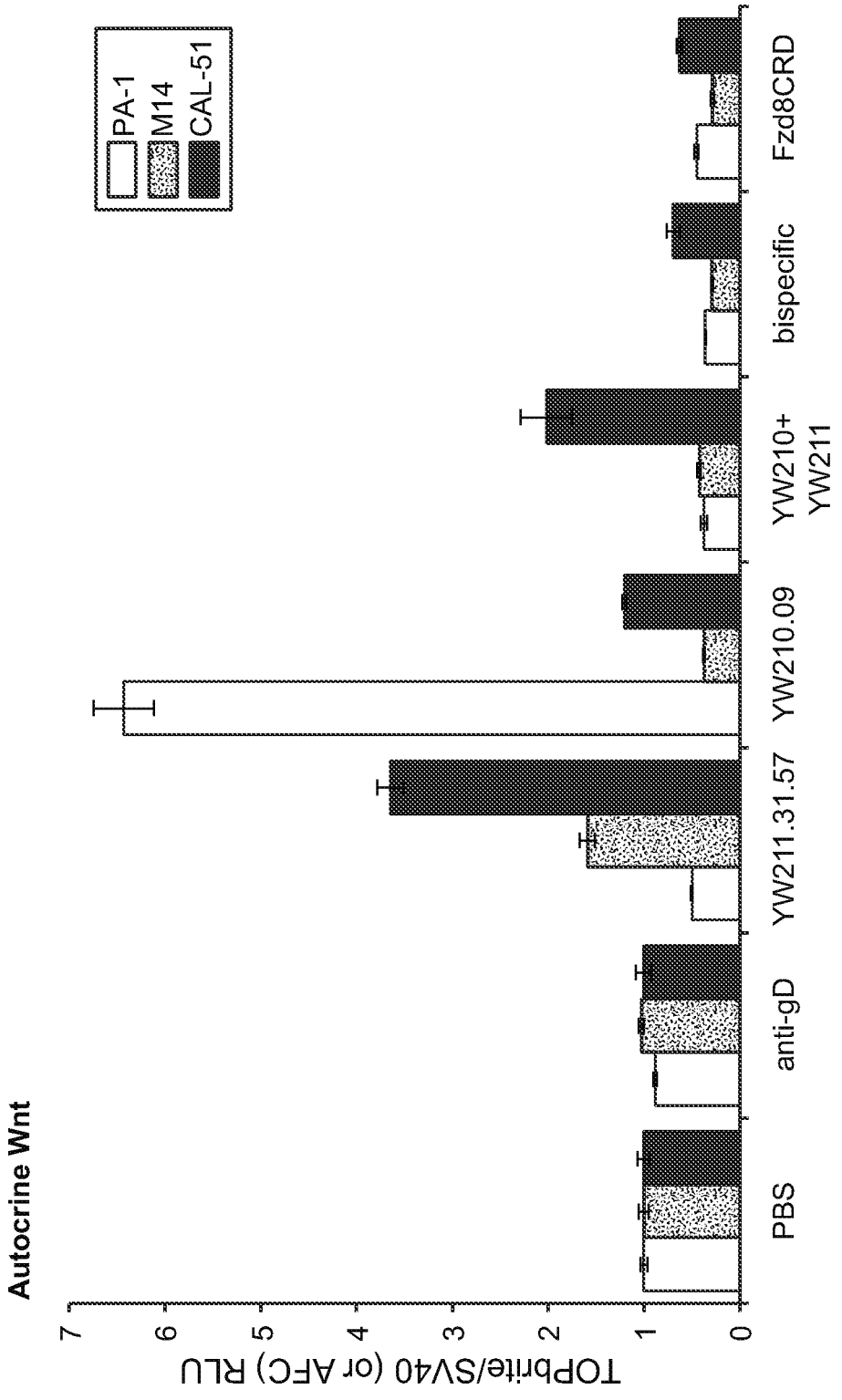

Autocrine Wnt
TOPbrite/SV40 (or AFC) RLU
0
1
2
3
4
5
6
7
PA-1
M14
CAL-51
PBS
anti-gD
YW211.31.57
YW210.09
YW210+ YW211
bispecific
Fzd8CRD

FIG. 11B

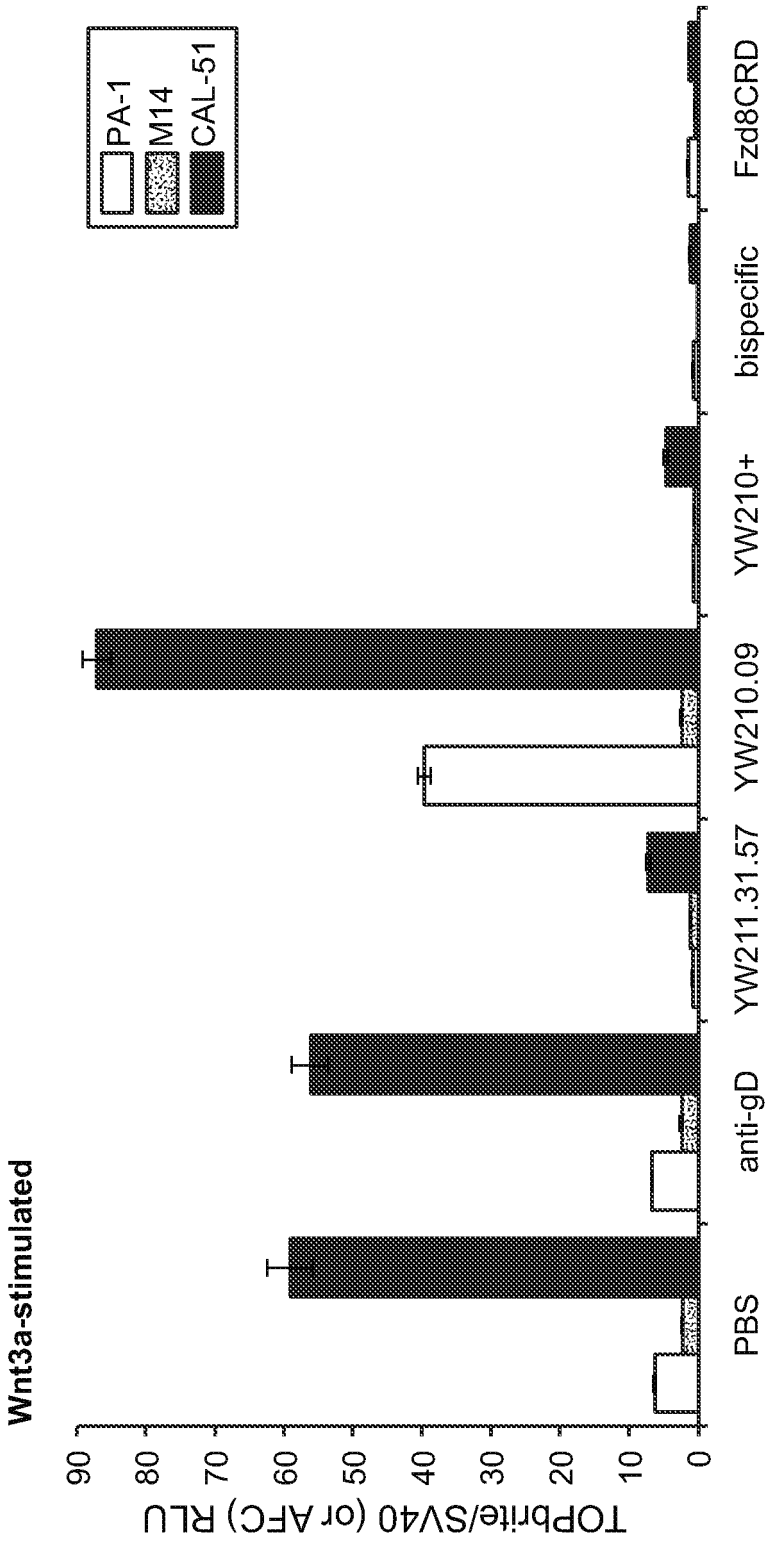

Wnt3a-stimulated
TOPbrite/SV40 (or AFC) RLU
0
10
20
30
40
50
60
70
80
90
PA-1
M14
CAL-51
PBS
anti-gD
YW211.31.57
YW210.09
YW210+ YW211
bispecific
Fzd8CRD

FIG. 11C

Expression	HEK293 Cells						Hs578T Cells					
	Fold change in Wnt signaling with treatment						Fold change in Wnt signaling with treatment					
	anti-gD	YW211.31.57	YW210.09	YW210+YW211	bispecific	Fzd8CRD	anti-gD	YW211.31.57	YW210.09	YW210+YW211	bispecific	Fzd8CRD
vector	0.80	1.06	0.97	0.87	0.83	0.77	1.06	1.22	0.99	1.21	0.84	0.70
Wnt3	1.05	0.09	4.26	0.06	0.04	0.25	0.79	0.12	2.29	0.21	0.03	0.19
Wnt3a	1.07	0.14	4.08	0.08	0.03	0.26	0.86	0.26	2.97	0.31	0.07	0.22
Wnt1	1.21	2.53	0.22	0.56	0.06	0.08	0.86	1.32	0.30	0.31	0.10	0.21
Wnt2	0.83	3.70	0.13	0.23	0.09	0.15	0.67	2.91	0.30	0.42	0.27	0.28
Wnt2b	1.08	2.56	0.32	0.30	0.25	0.43						
Wnt6	0.94	5.00	0.37	0.45	0.30	0.61	0.86	3.19	0.48	0.72	0.33	0.22
Wnt8a	1.08	5.39	0.28	0.30	0.21	0.45						
Wnt9a	1.03	2.27	0.91	0.74	0.31	0.79						
Wnt9b	1.27	1.81	0.87	0.57	0.24	1.22	0.89	2.22	0.50	0.34	0.24	0.74
Wnt10b	1.28	3.19	0.22	0.32	0.04	0.40	0.90	2.30	0.42	0.51	0.26	0.61
Wnt4												
Wnt7a	1.22	5.76	1.65	3.67	0.50	0.48	0.95	4.63	2.92	3.66	0.89	0.29
Wnt7b	1.16	5.54	0.90	3.69	0.59	0.38	1.03	4.06	2.29	6.46	0.59	0.20
Wnt10a	1.30	2.48	1.01	1.56	0.38	0.42	0.92	2.94	0.55	1.04	0.20	0.19
Wnt1+Wnt3a	1.16	1.16	1.13	0.30	0.08	0.13	0.86	0.70	0.86	0.46	0.16	0.22

FIG. 12

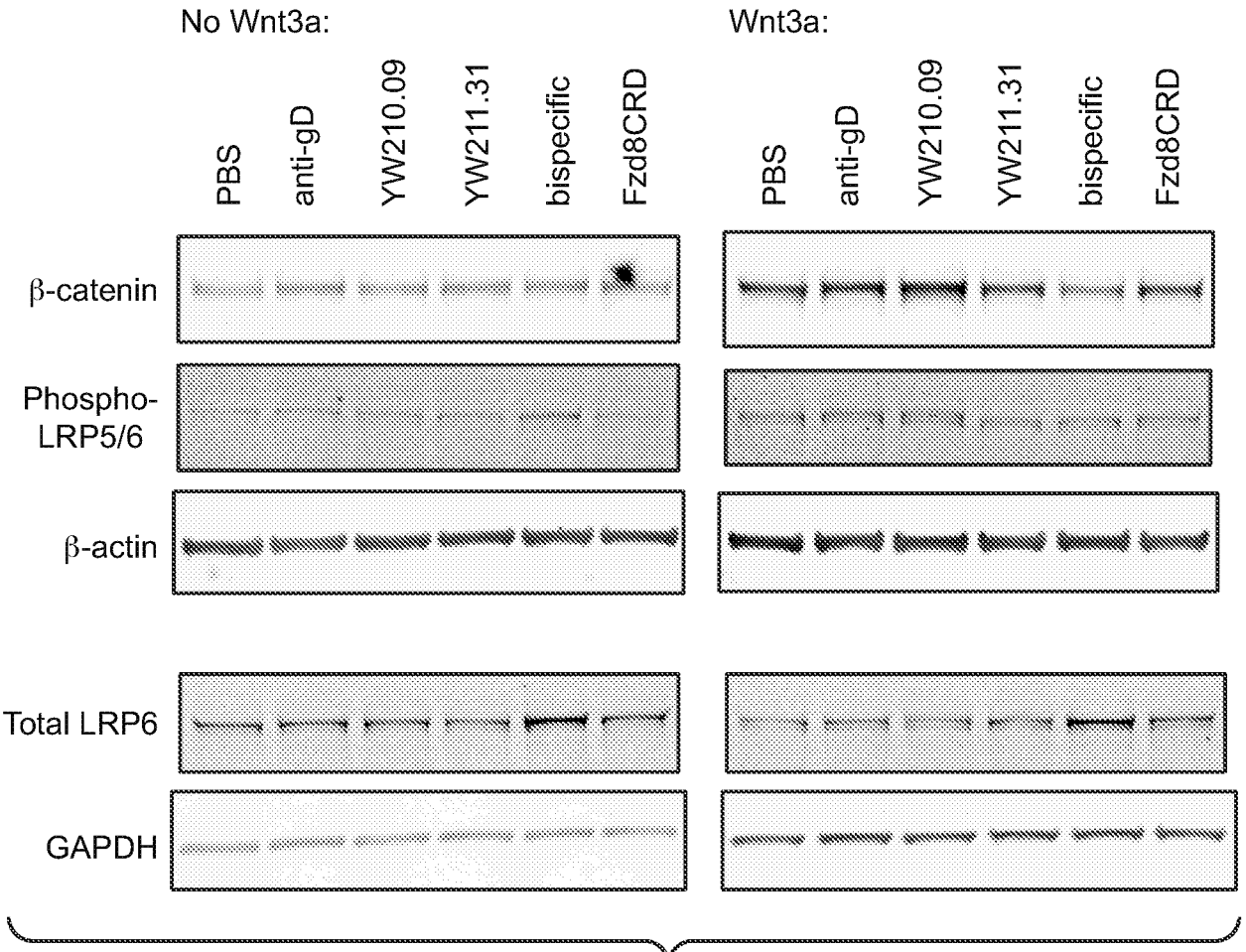


FIG. 13A

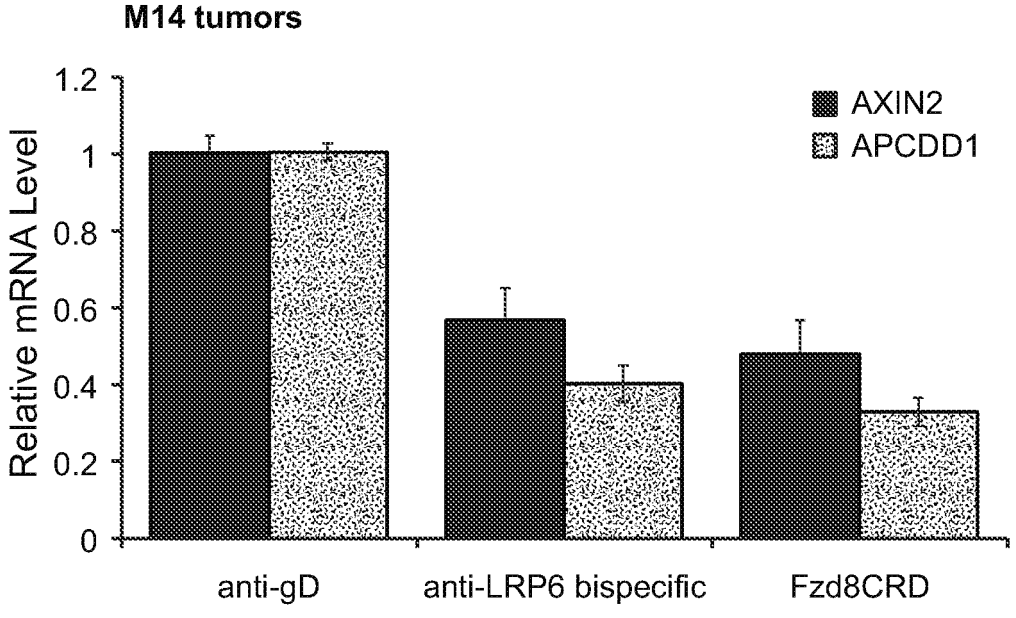


FIG. 13B

FIG. 14

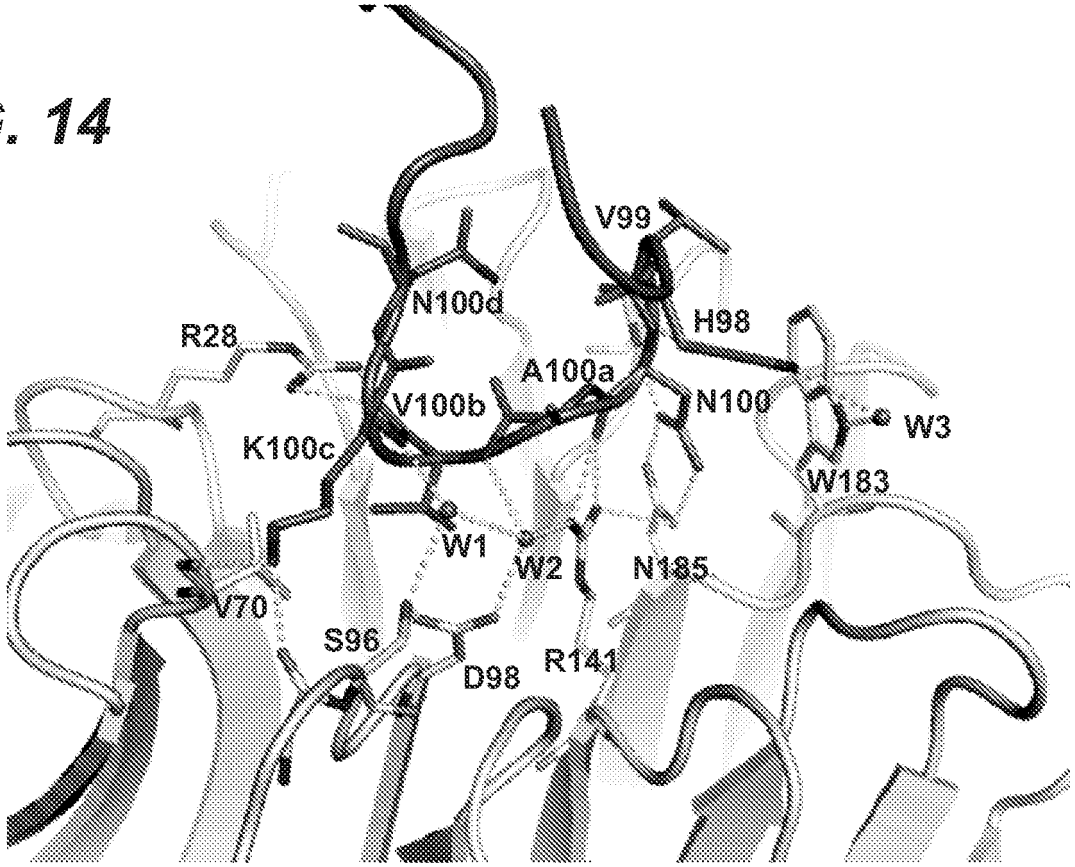
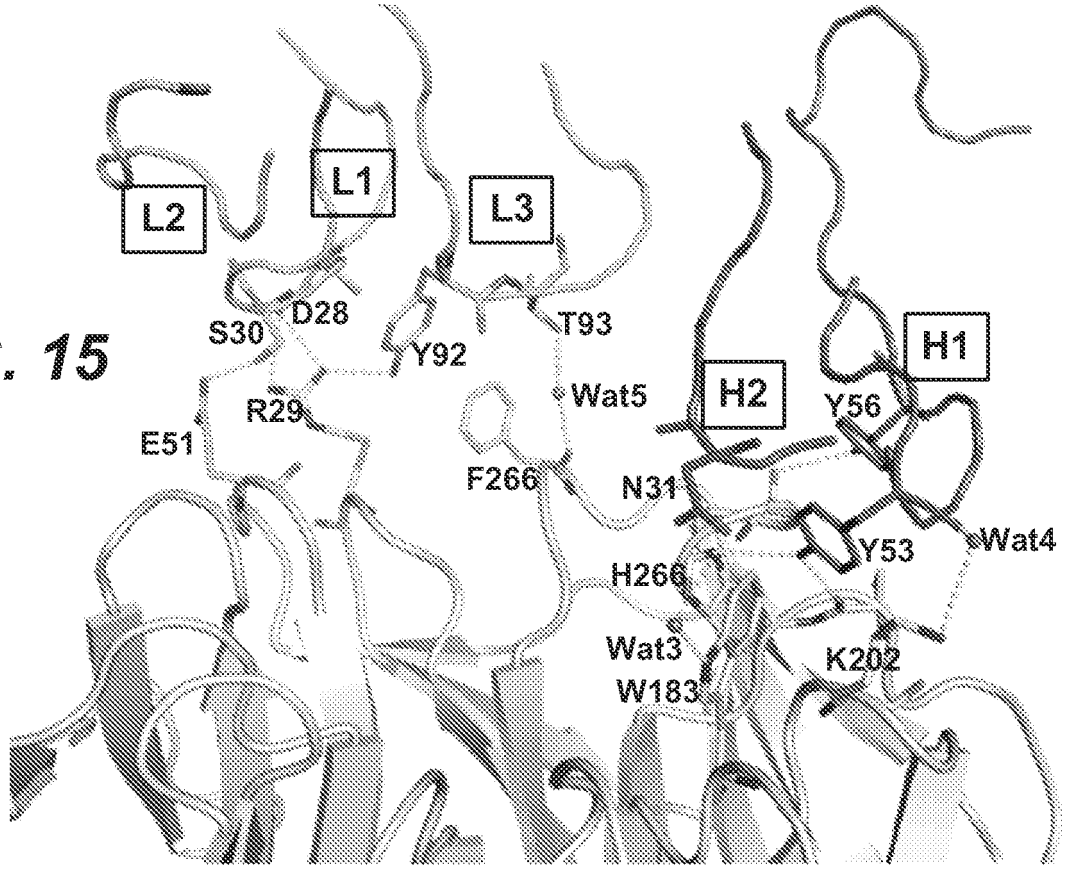

V99
N100d
H98
R28
A100a
V100b
N100
W3
K100c
W183
W1
W2
N185
V70
S96
D98
R141

FIG. 15


L1
L2
L3
H1
H2
S30
D28
T93
Y92
Wat5
R29
E51
Y56
F266
N31
Y53
Wat4
H266
Wat3
K202
W183

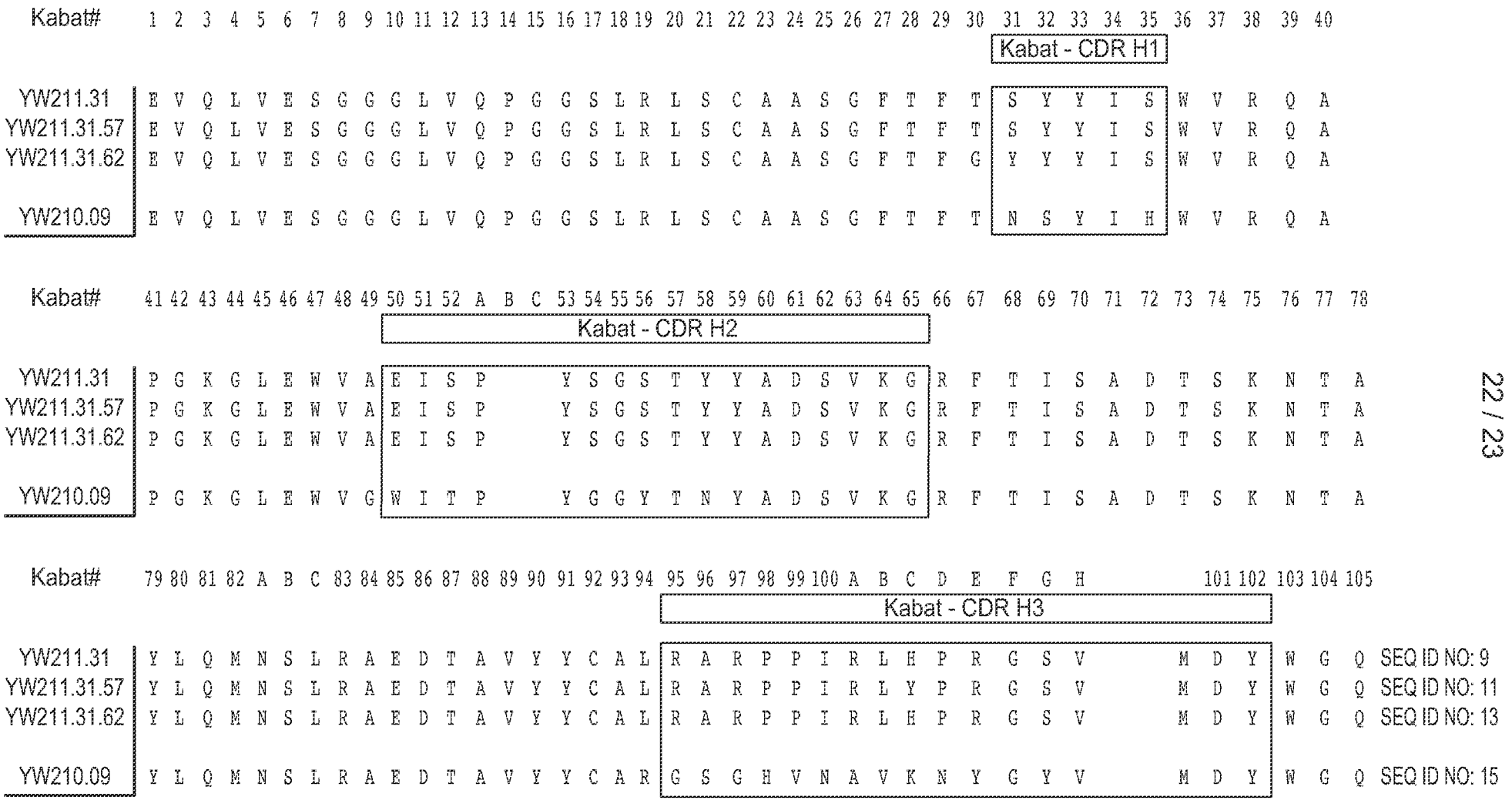

Kabat# 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40
Kabat - CDR H1
YW211.31 E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F T S Y Y I S W V R Q A
YW211.31.57 E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F T S Y Y I S W V R Q A
YW211.31.62 E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F G Y Y Y I S W V R Q A
YW210.09 E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F T N S Y I H W V R Q A
Kabat# 41 42 43 44 45 46 47 48 49 50 51 52 A B C 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78
Kabat - CDR H2
YW211.31 P G K G L E W V A E I S P Y S G S T Y Y A D S V K G R F T I S A D T S K N T A
YW211.31.57 P G K G L E W V A E I S P Y S G S T Y Y A D S V K G R F T I S A D T S K N T A
YW211.31.62 P G K G L E W V A E I S P Y S G S T Y Y A D S V K G R F T I S A D T S K N T A
YW210.09 P G K G L E W V G W I T P Y G G Y T N Y A D S V K G R F T I S A D T S K N T A
Kabat# 79 80 81 82 A B C 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 A B C D E F G H 101 102 103 104 105
Kabat - CDR H3
YW211.31 Y L Q M N S L R A E D T A V Y Y C A L R A R P P I R L H P R G S V M D Y W G Q SEQ ID NO: 9
YW211.31.57 Y L Q M N S L R A E D T A V Y Y C A L R A R P P I R L Y P R G S V M D Y W G Q SEQ ID NO: 11
YW211.31.62 Y L Q M N S L R A E D T A V Y Y C A L R A R P P I R L H P R G S V M D Y W G Q SEQ ID NO: 13
YW210.09 Y L Q M N S L R A E D T A V Y Y C A R G S G H V N A V K N Y G Y V M D Y W G Q SEQ ID NO: 15

FIG. 16

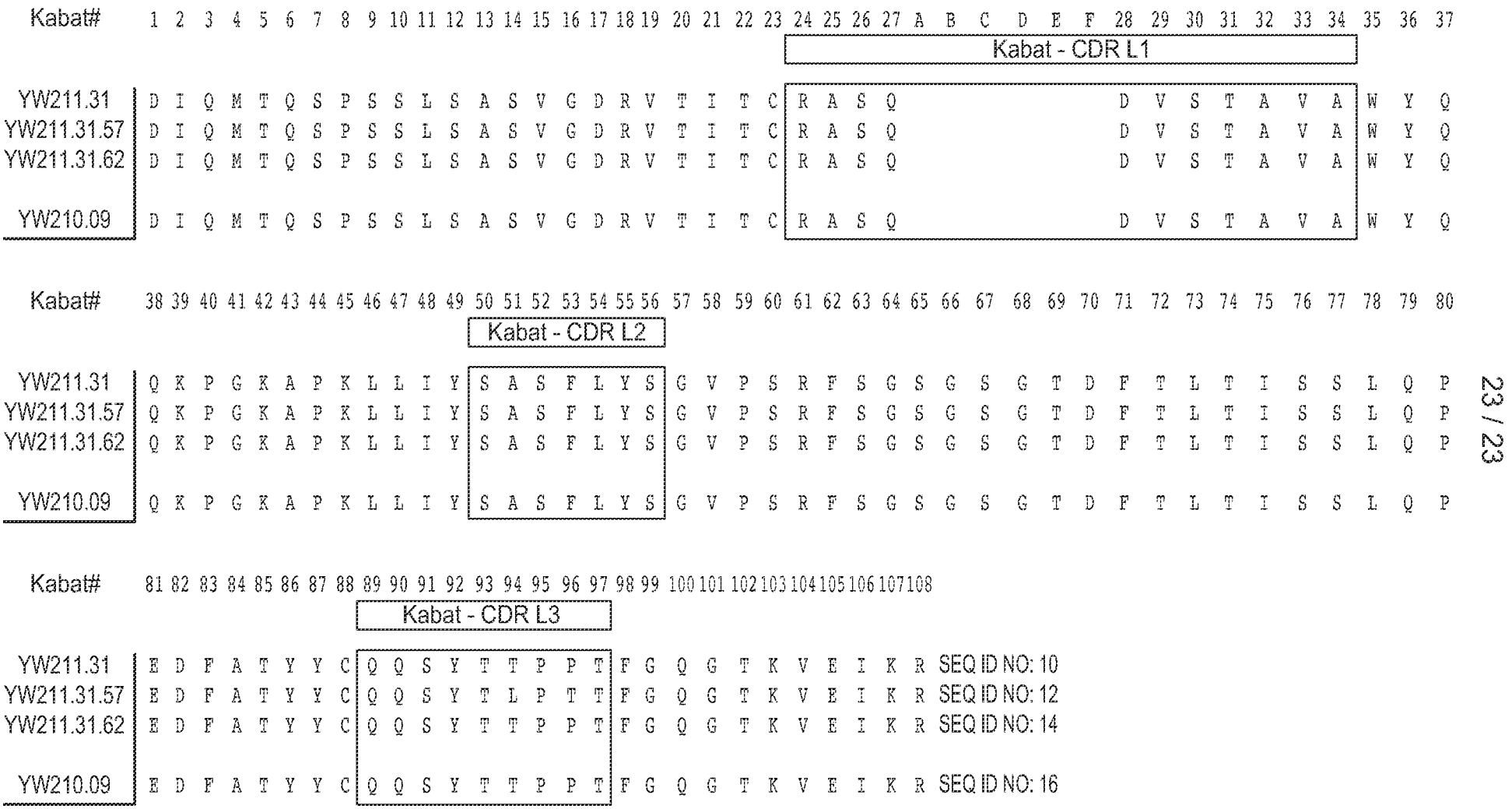

Kabat#
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 A B C D E F 28 29 30 31 32 33 34 35 36 37
Kabat - CDR L1
YW211.31 D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V S T A V A W Y Q
YW211.31.57 D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V S T A V A W Y Q
YW211.31.62 D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V S T A V A W Y Q
YW210.09 D I Q M T Q S P S S L S A S V G D R V T I T C R A S Q D V S T A V A W Y Q
Kabat#
38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
Kabat - CDR L2
YW211.31 Q K P G K A P K L L I Y S A S F L Y S G V P S R F S G S G S G T D F T L T I S S L Q P
YW211.31.57 Q K P G K A P K L L I Y S A S F L Y S G V P S R F S G S G S G T D F T L T I S S L Q P
YW211.31.62 Q K P G K A P K L L I Y S A S F L Y S G V P S R F S G S G S G T D F T L T I S S L Q P
YW210.09 Q K P G K A P K L L I Y S A S F L Y S G V P S R F S G S G S G T D F T L T I S S L Q P
Kabat#
81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108
Kabat - CDR L3
YW211.31 E D F A T Y Y C Q Q S Y T T P P T F G Q G T K V E I K R SEQ ID NO: 10
YW211.31.57 E D F A T Y Y C Q Q S Y T L P T T F G Q G T K V E I K R SEQ ID NO: 12
YW211.31.62 E D F A T Y Y C Q Q S Y T T P P T F G Q G T K V E I K R SEQ ID NO: 14
YW210.09 E D F A T Y Y C Q Q S Y T T P P T F G Q G T K V E I K R SEQ ID NO: 16

FIG. 17