

FIG. 1A

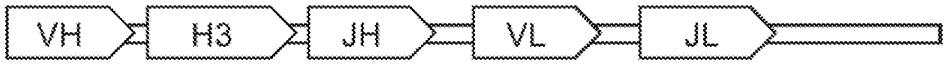
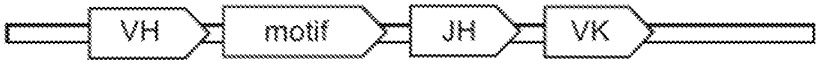


FIG. 1B



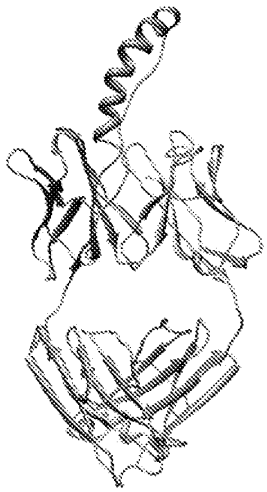


FIG. 2

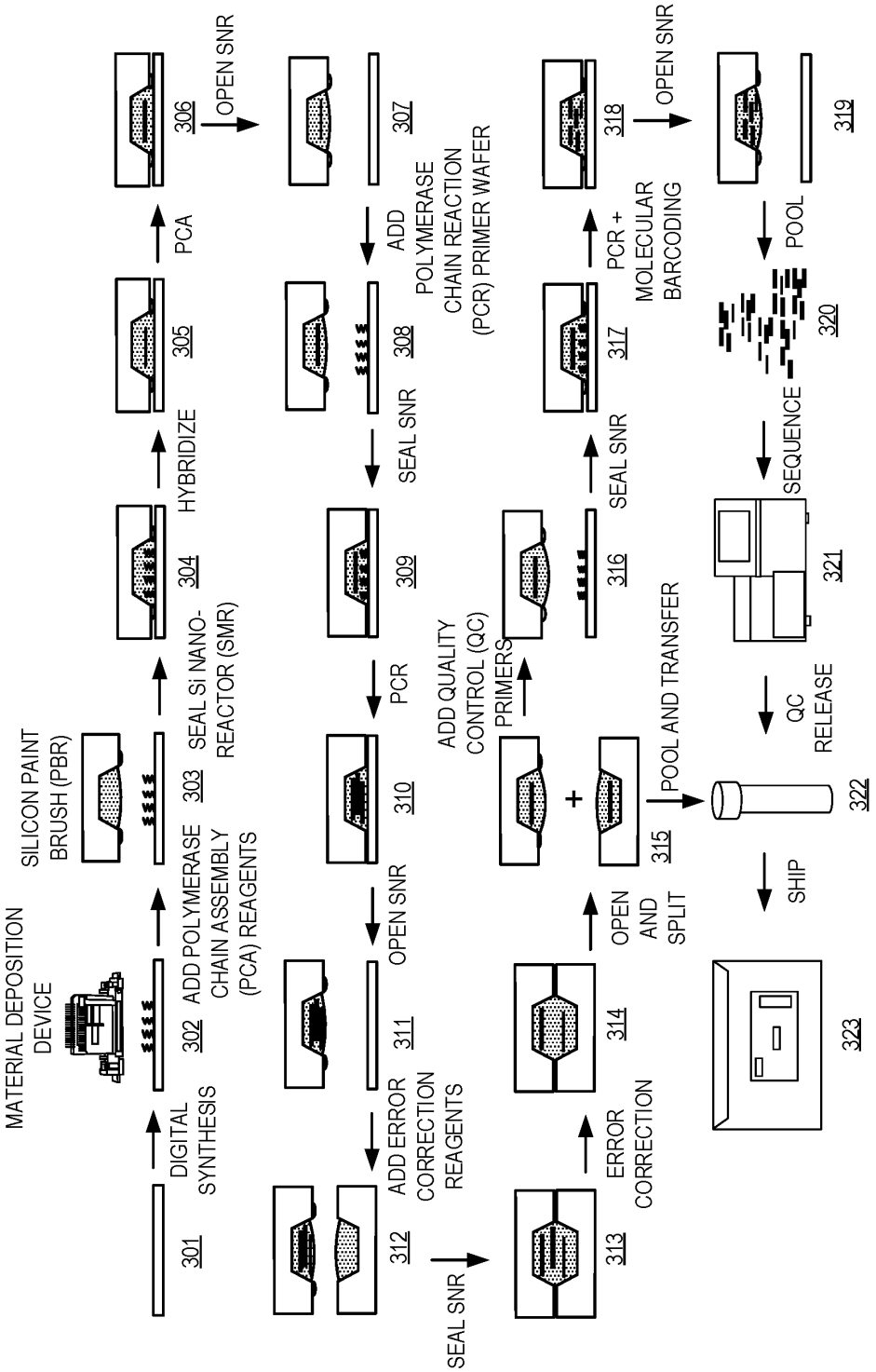

MATERIAL DEPOSITION DEVICE
SILICON PAINT BRUSH (PBR)
301
DIGITAL SYNTHESIS
302
ADD POLYMERASE CHAIN ASSEMBLY (PCA) REAGENTS
303
SEAL Si NANO-REACTOR (SMR)
304
HYBRIDIZE
305
PCA
306
OPEN SNR
307
ADD POLYMERASE CHAIN REACTION (PCR) PRIMER WAFER
308
SEAL SNR
309
PCR
310
OPEN SNR
311
ADD ERROR CORRECTION REAGENTS
312
SEAL SNR
313
ERROR CORRECTION
314
OPEN AND SPLIT
315
POOL AND TRANSFER
316
SEAL SNR
317
PCR + MOLECULAR BARCODING
318
OPEN SNR
319
POOL
320
SEQUENCE
321
QC RELEASE
322
SHIP
323
ADD QUALITY CONTROL (QC) PRIMERS

FIG. 3

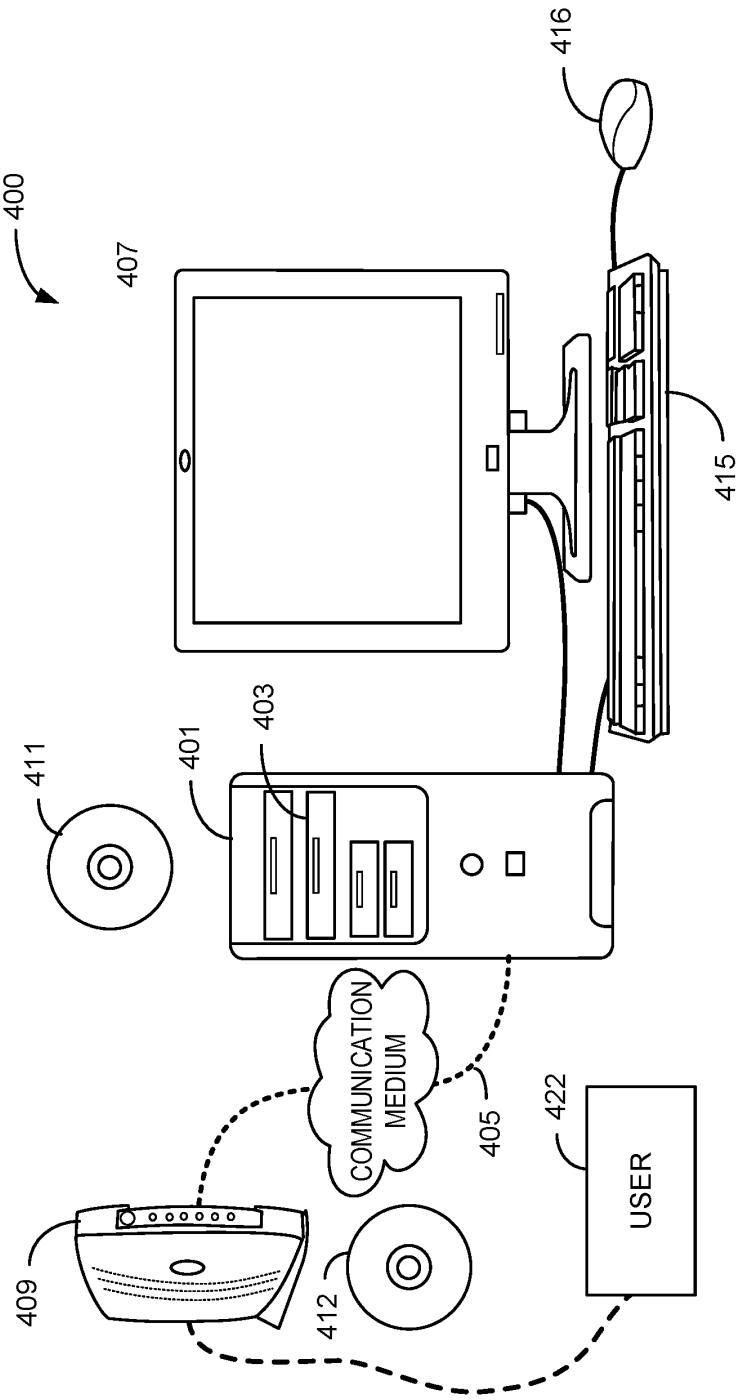

400
407
416
415
403
401
411
COMMUNICATION MEDIUM
405
422
USER
409
412

FIG. 4

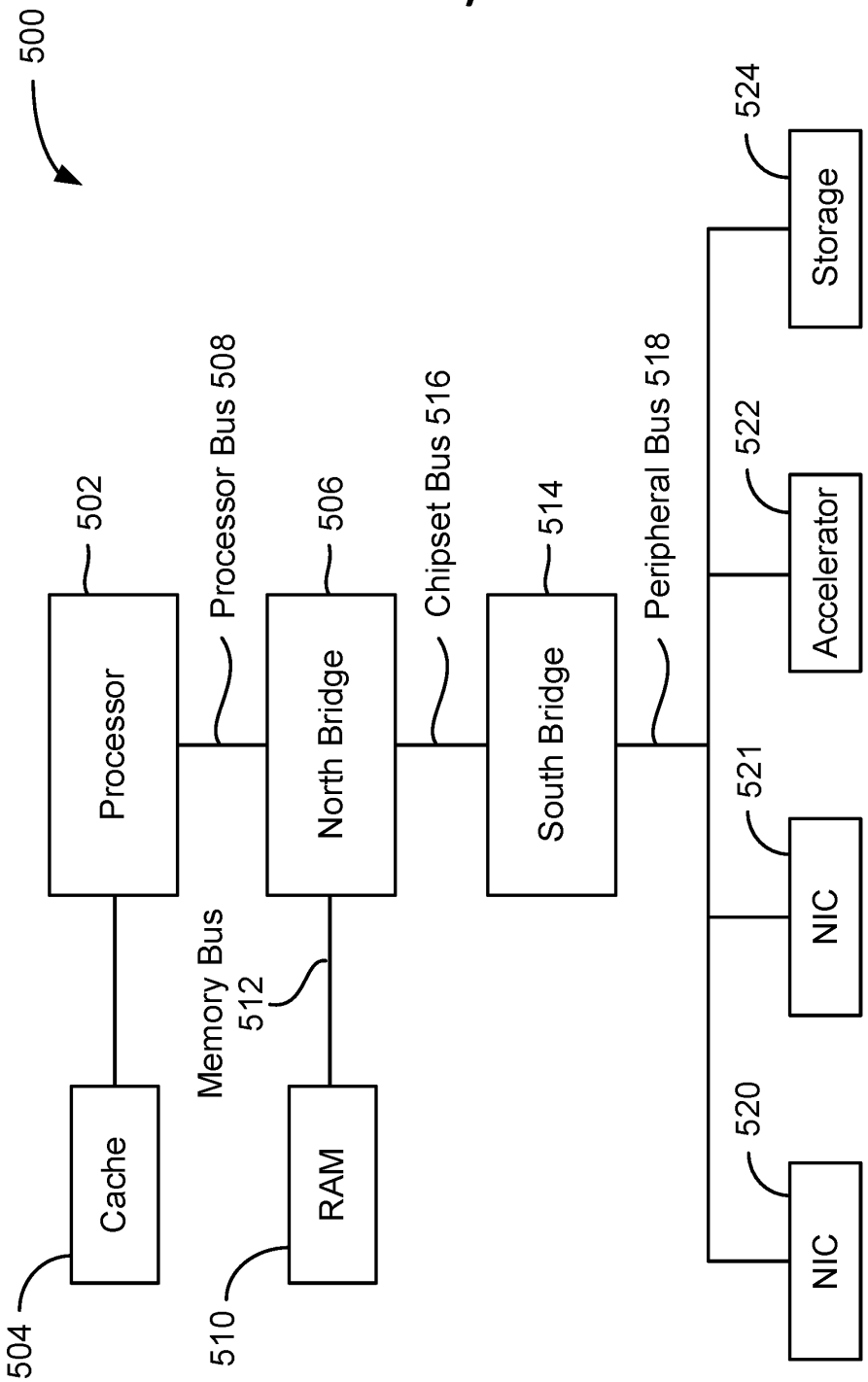

500
Processor
502
Cache
504
Processor Bus 508
North Bridge
506
Memory Bus
512
RAM
510
Chipset Bus 516
South Bridge
514
Peripheral Bus 518
Storage
524
Accelerator
522
NIC
521
NIC
520

FIG. 5

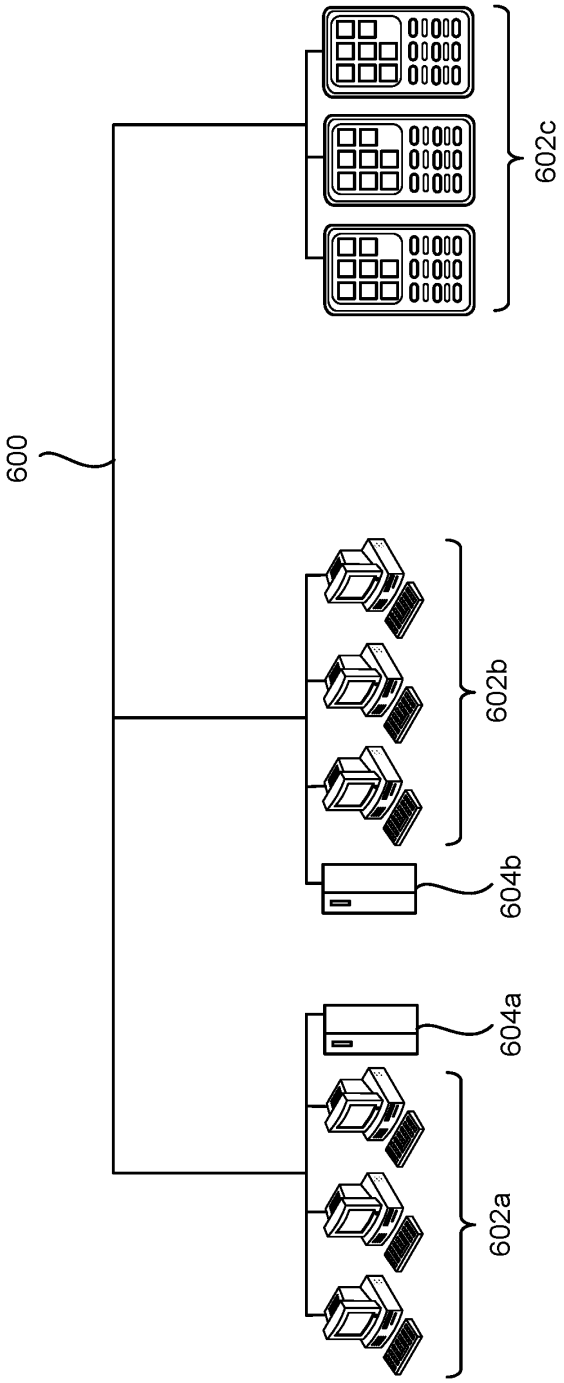


FIG. 6

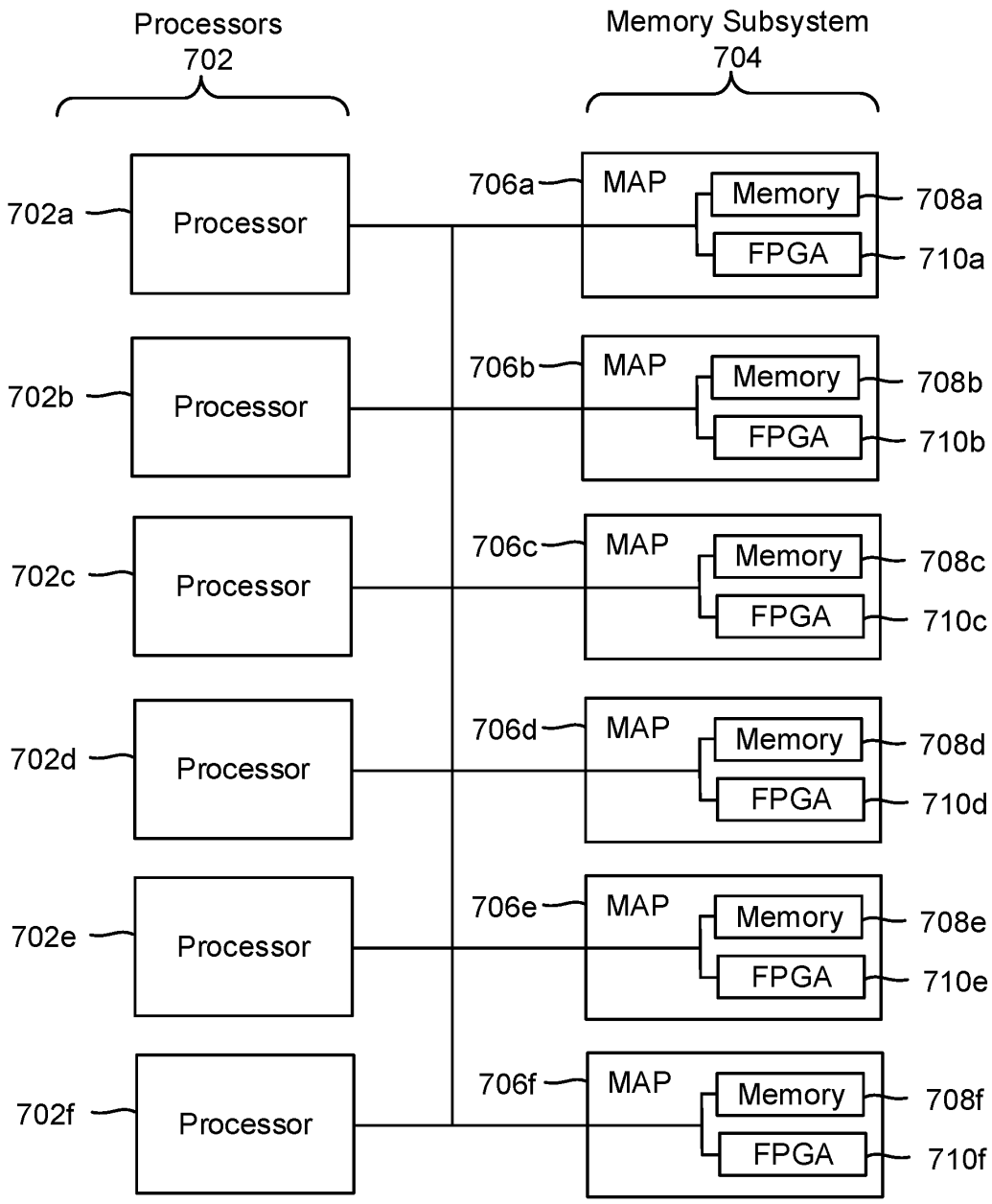

Processors
702
Memory Subsystem
704
702a
Processor
706a
MAP
Memory
FPGA
708a
710a
702b
Processor
706b
MAP
Memory
FPGA
708b
710b
702c
Processor
706c
MAP
Memory
FPGA
708c
710c
702d
Processor
706d
MAP
Memory
FPGA
708d
710d
702e
Processor
706e
MAP
Memory
FPGA
708e
710e
702f
Processor
706f
MAP
Memory
FPGA
708f
710f

FIG. 7

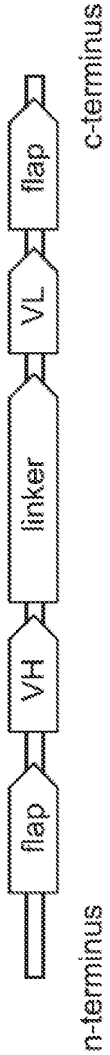


FIG. 8A



FIG. 8B

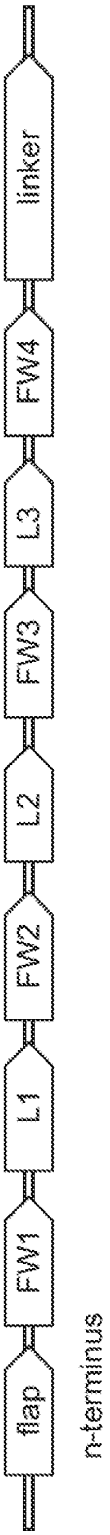


FIG. 8C

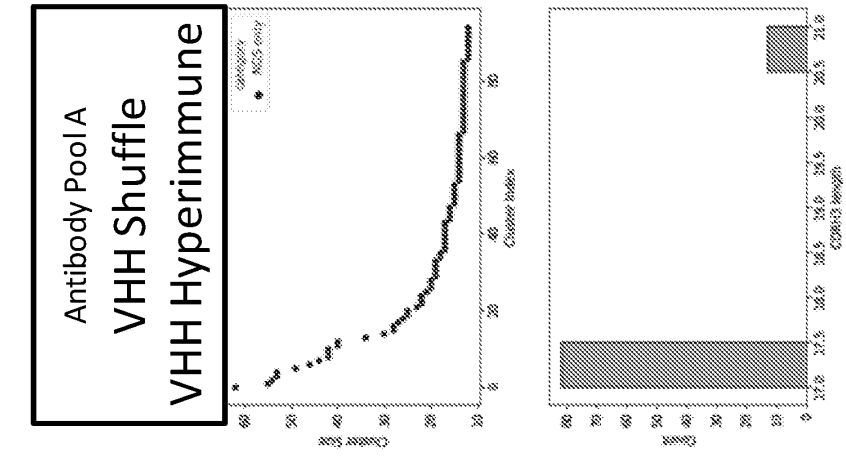


FIG. 9A

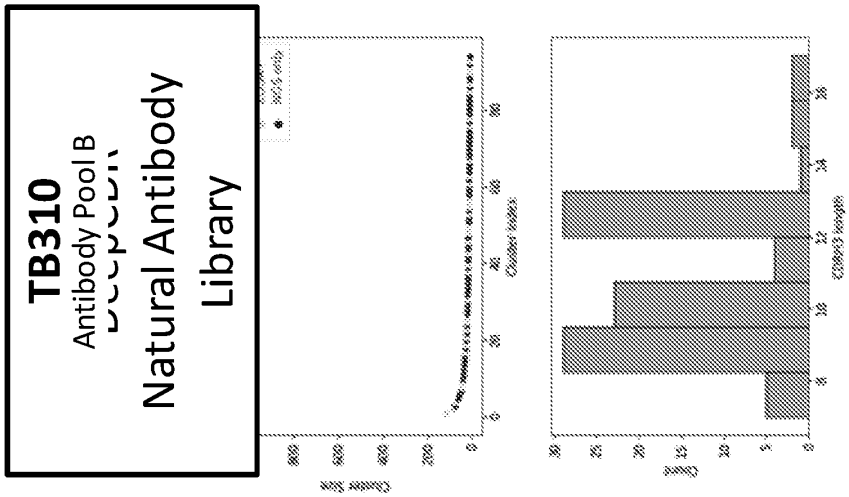


FIG. 9B

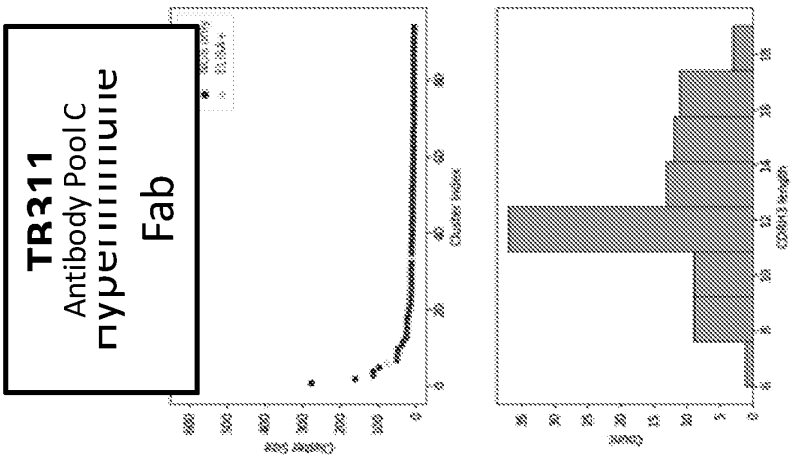


FIG. 9C

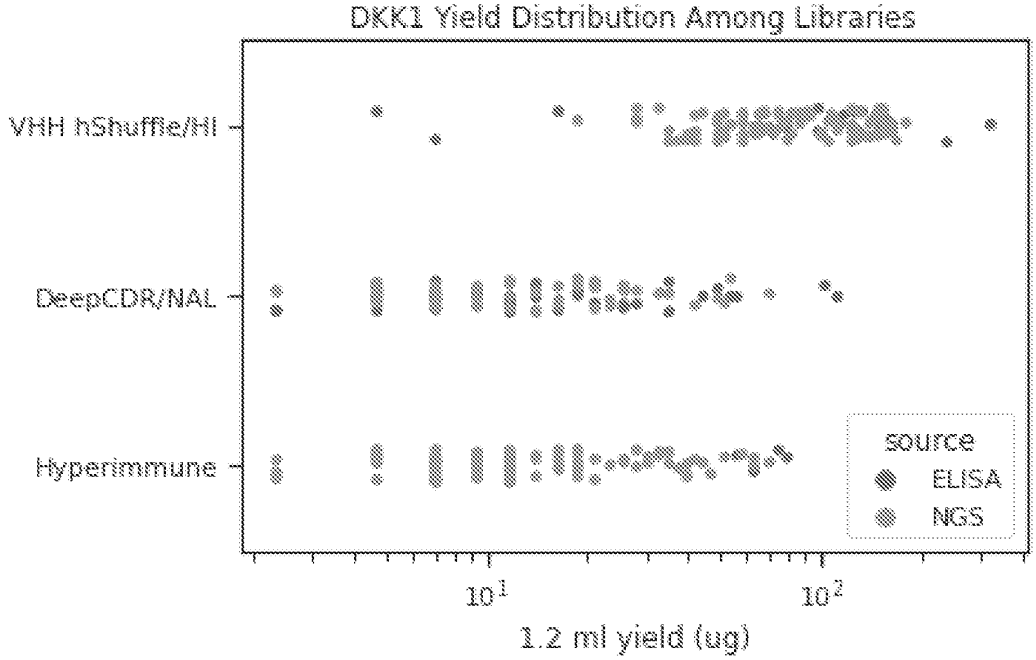


FIG. 10A

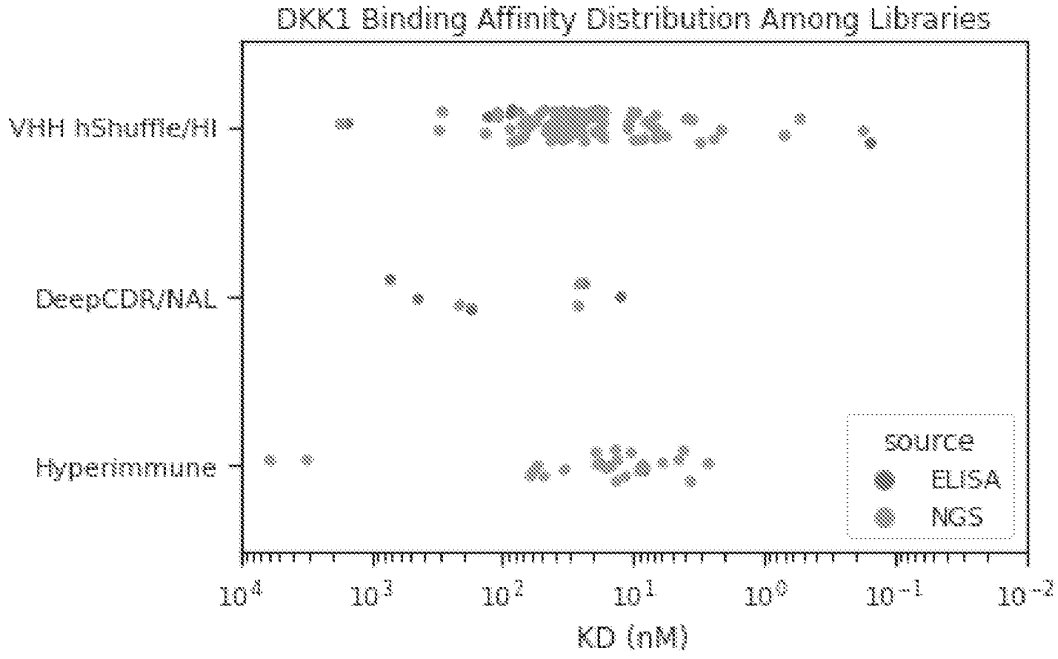


FIG. 10B

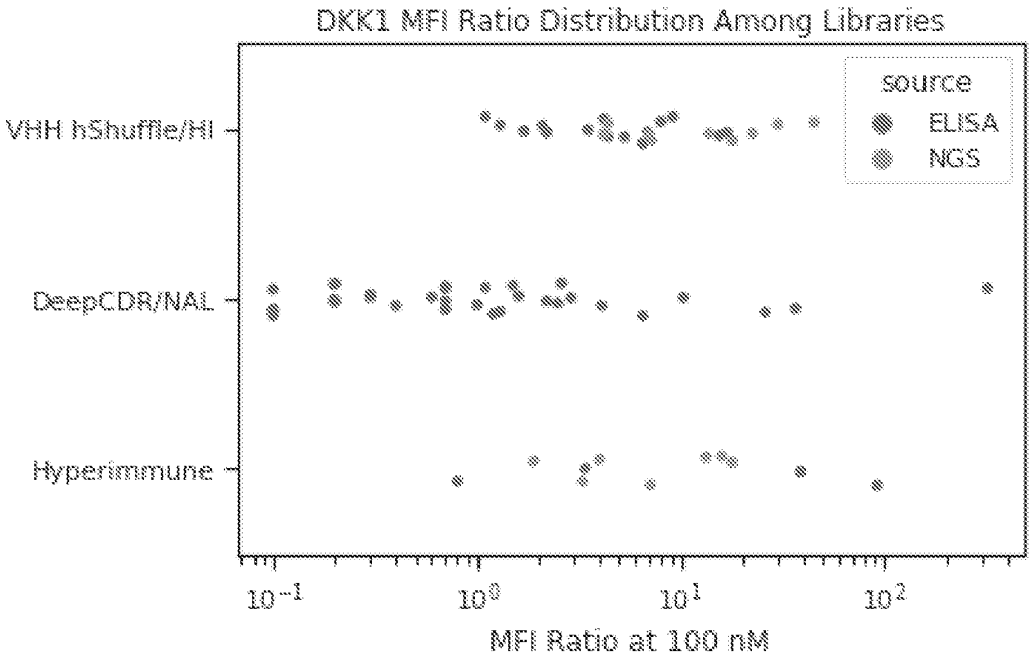

DKK1 MFI Ratio Distribution Among Libraries
source
ELISA
NGS
VHH hShuffle/HI
DeepCDR/NAL
Hyperimmune
10^{-1}
10^{0}
10^{1}
10^{2}
MFI Ratio at 100 nM

FIG. 10C

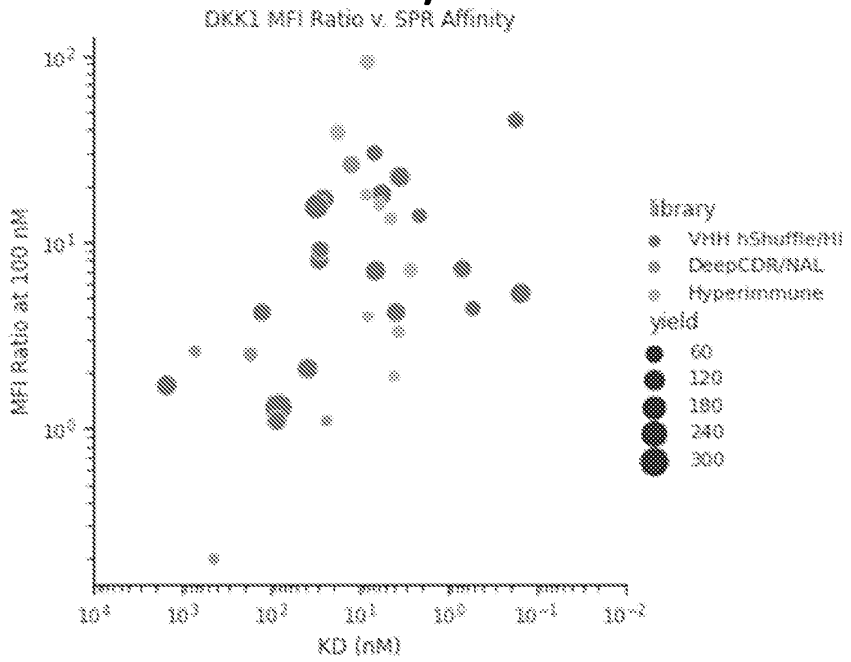


FIG. 11A

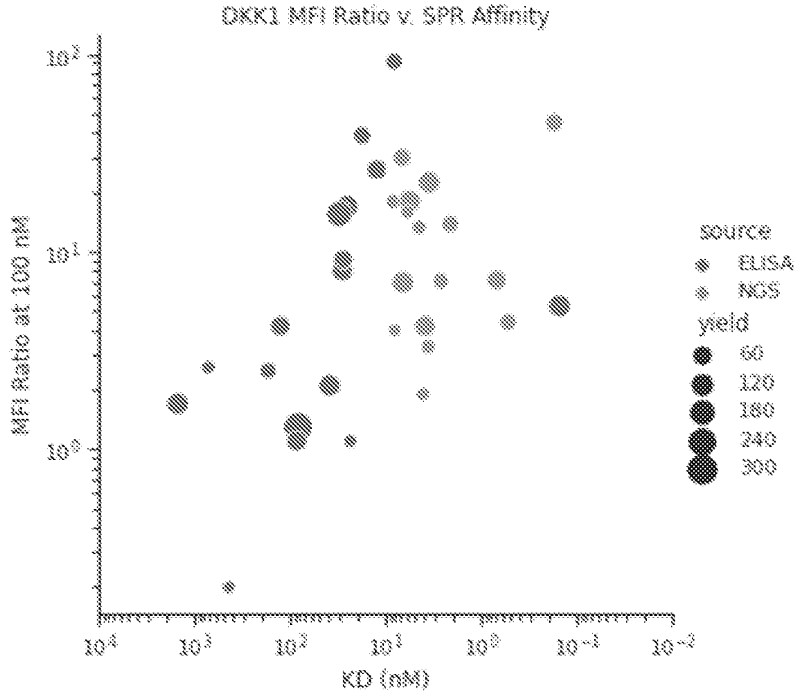


FIG. 11B

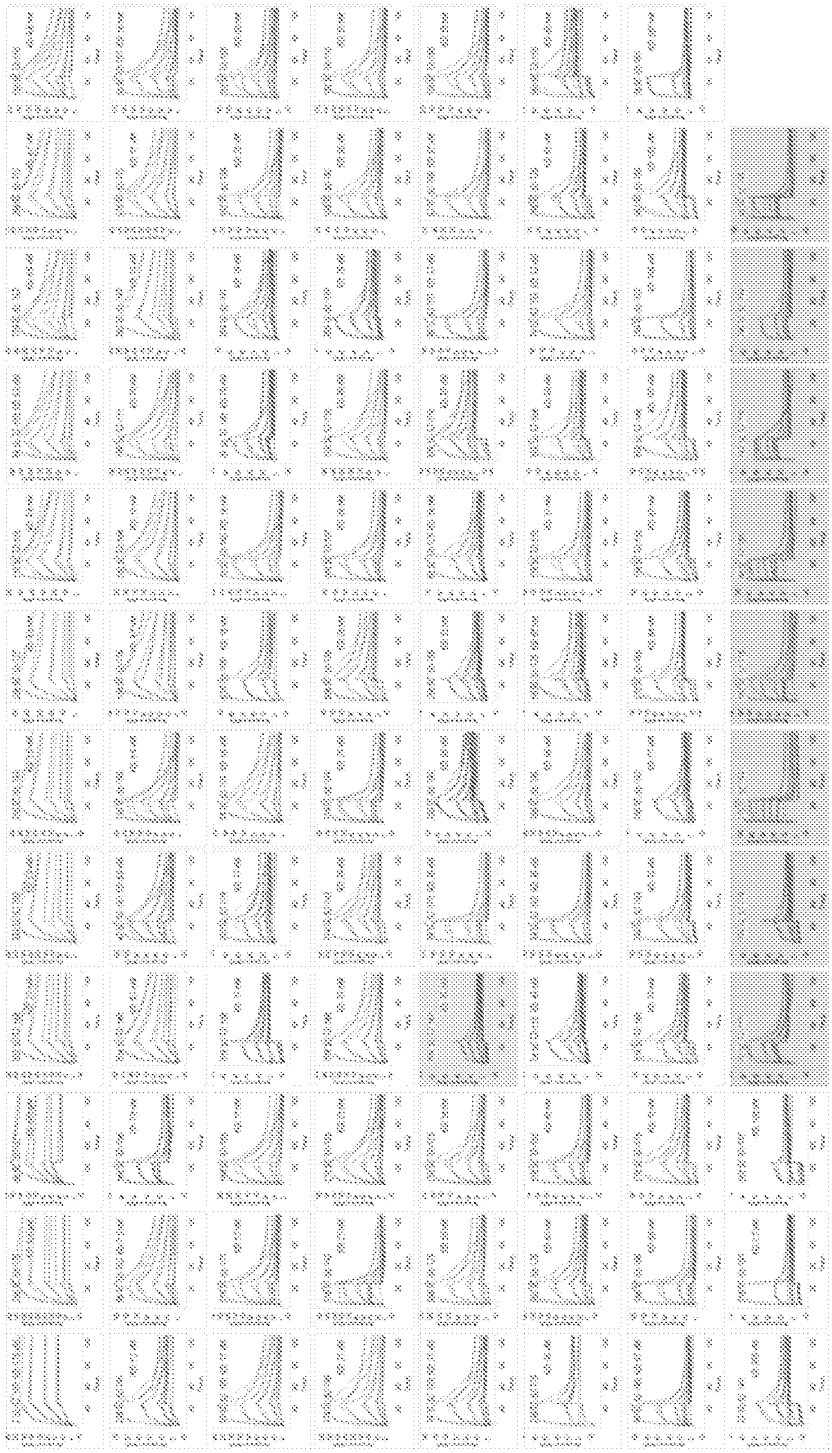


FIG. 12A

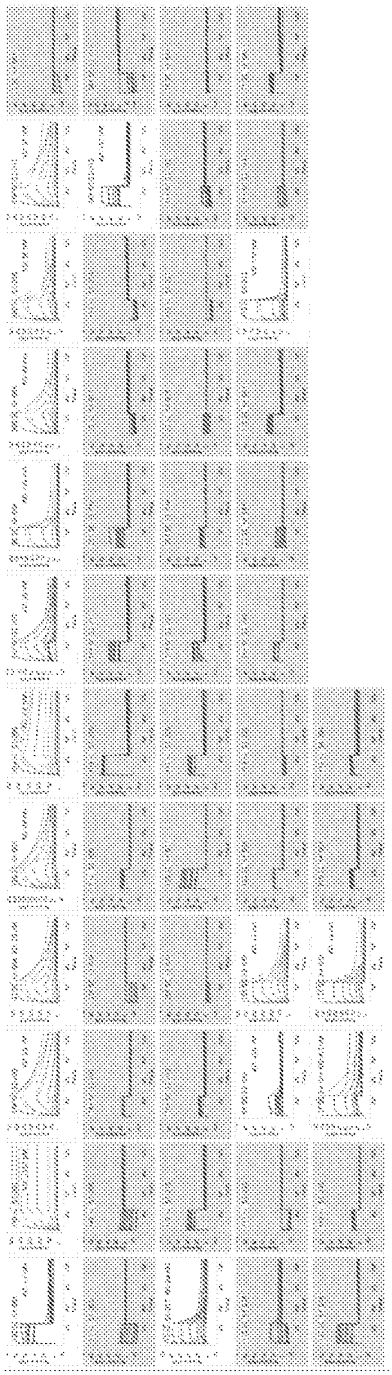


FIG. 12B

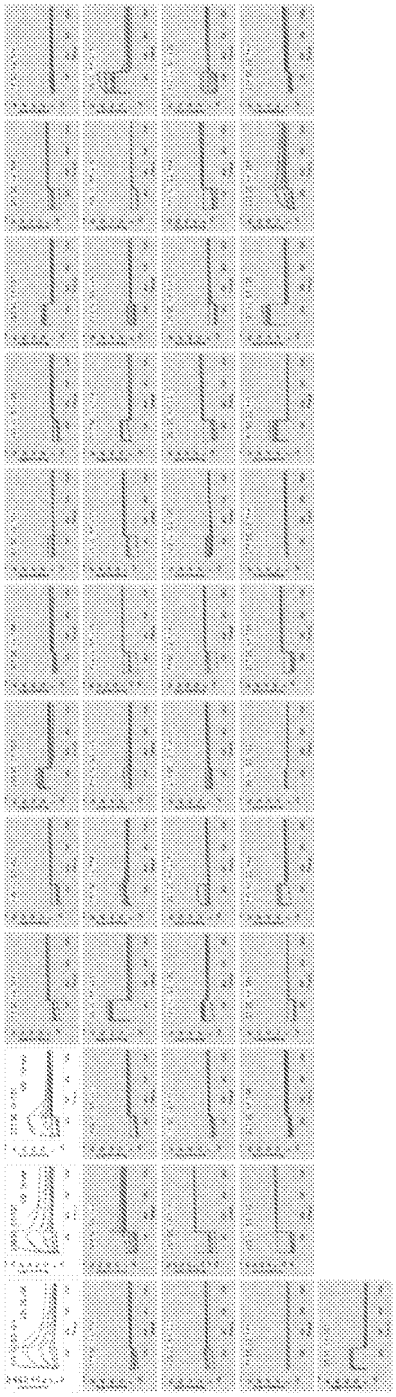


FIG. 12C

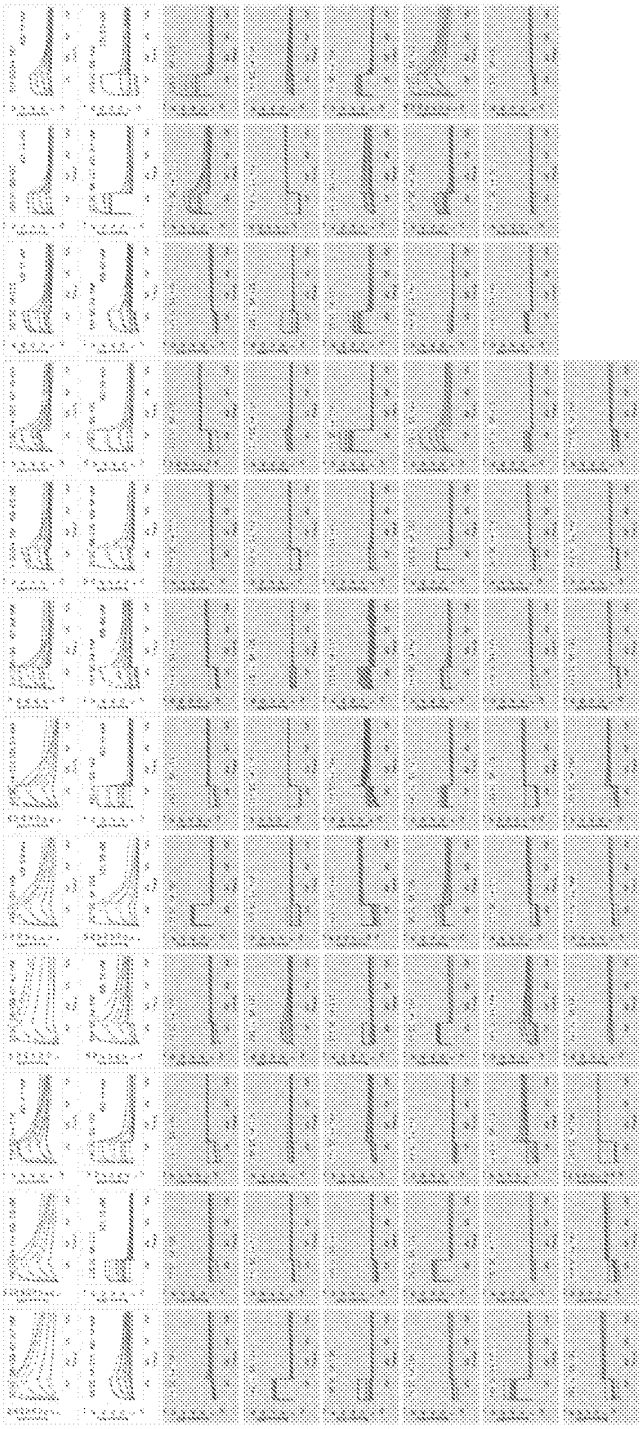


FIG. 12D

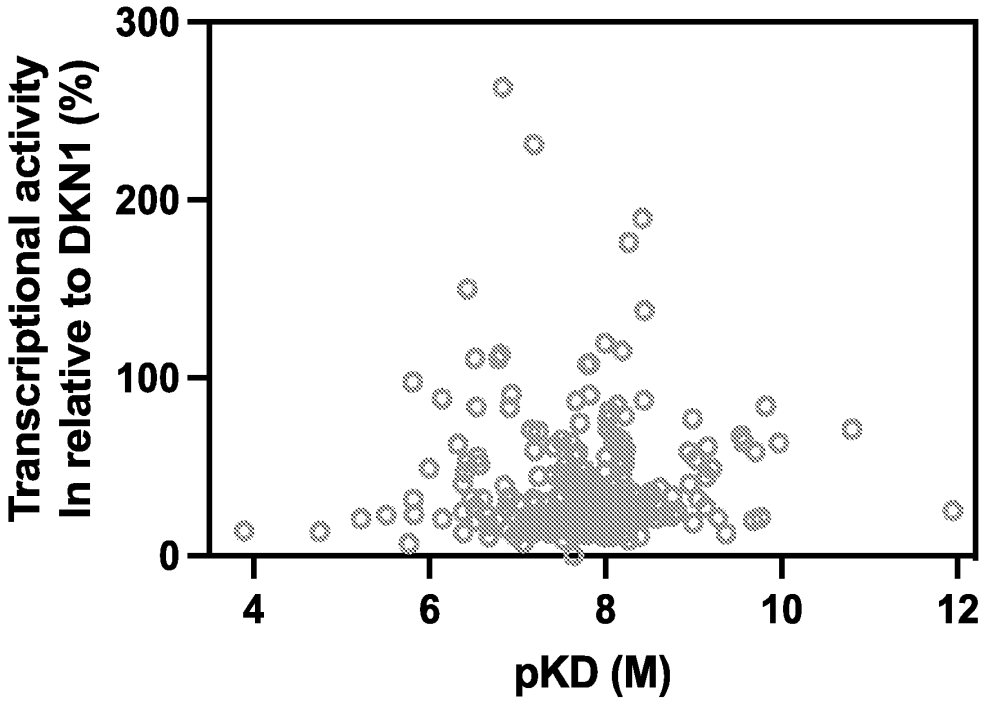

Transcriptional activity
In relative to DKN1 (%)
300
200
100
0
4
6
8
10
12
pKD (M)

FIG. 13

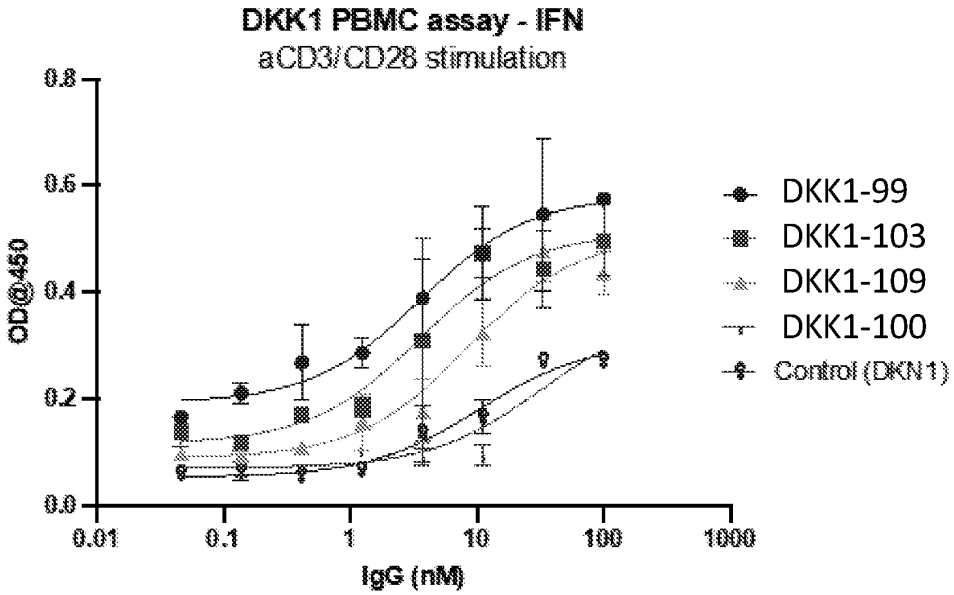


FIG. 14A

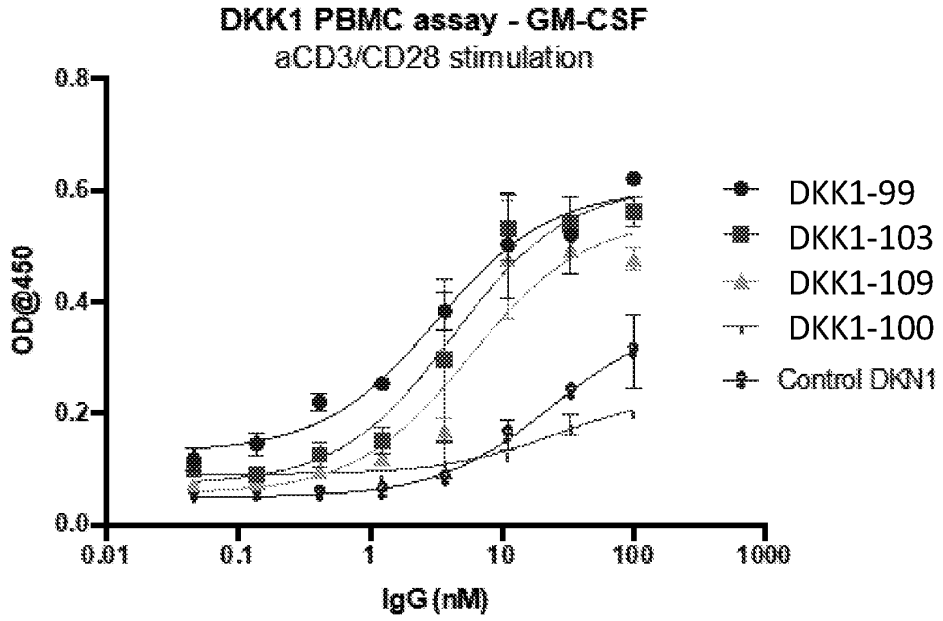


FIG. 14B

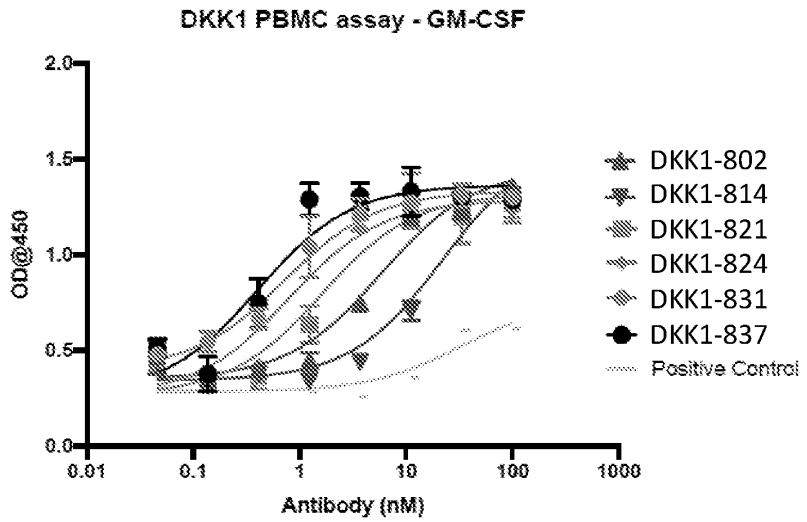


FIG. 14C

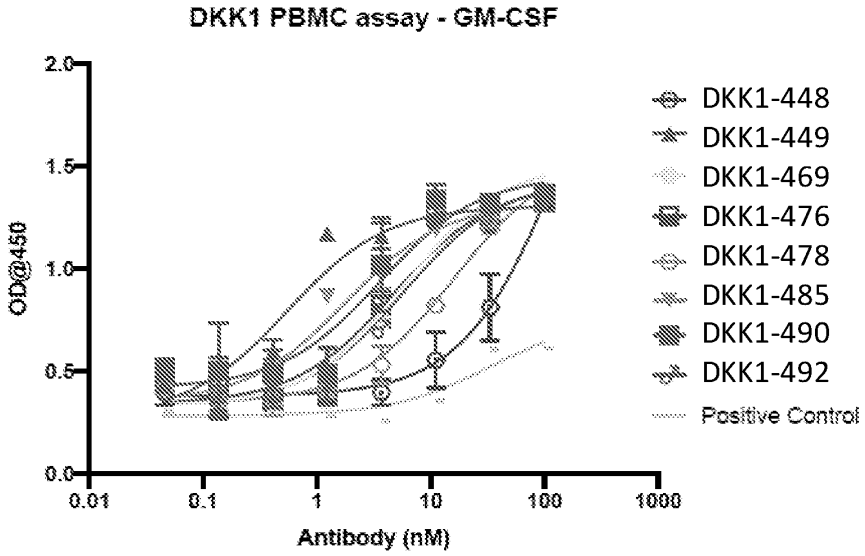


FIG. 14D

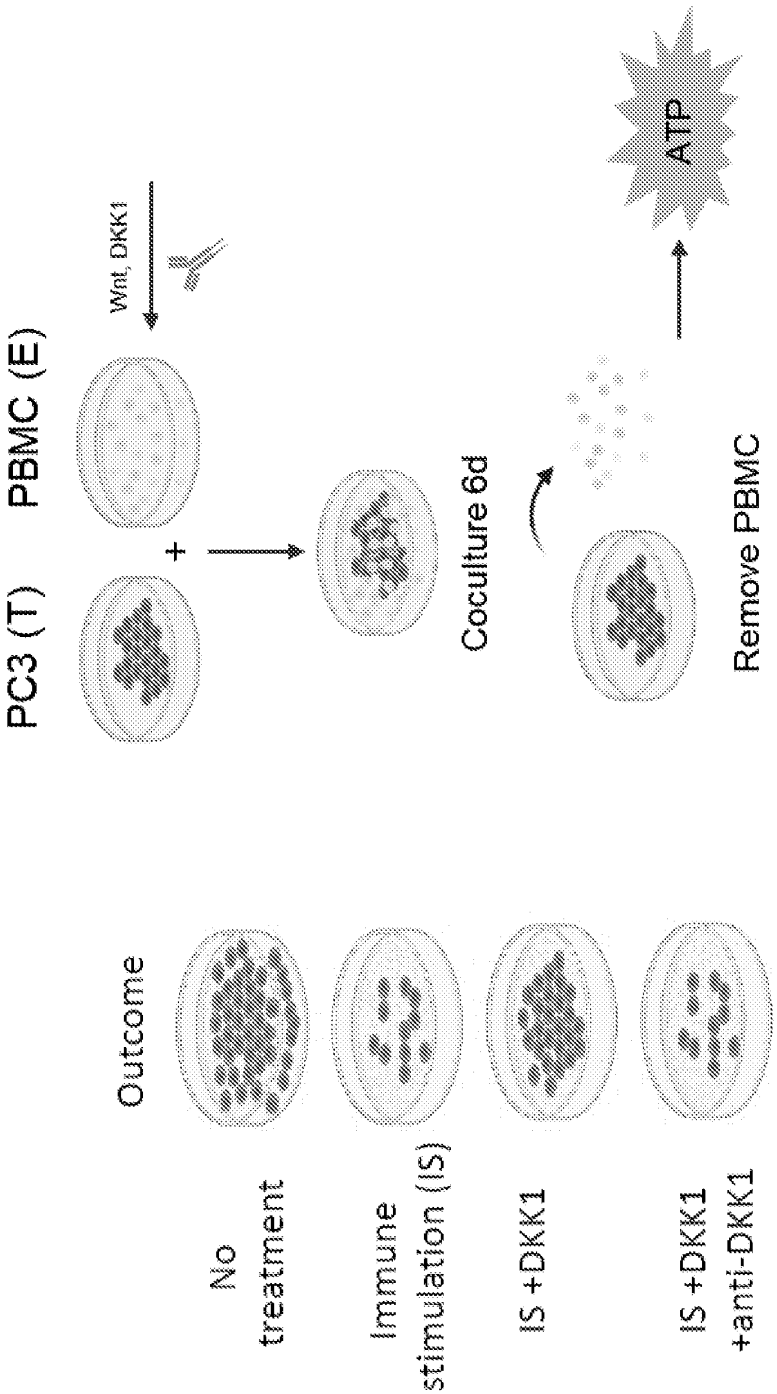

PC3 (T)
PBMC (E)
Wnt, DKK1
+
Coculture 6d
ATP
Remove PBMC
Outcome
No treatment
Immune stimulation (IS)
IS +DKK1
IS +DKK1 +anti-DKK1
Readout: PC3 ATP

FIG. 15A

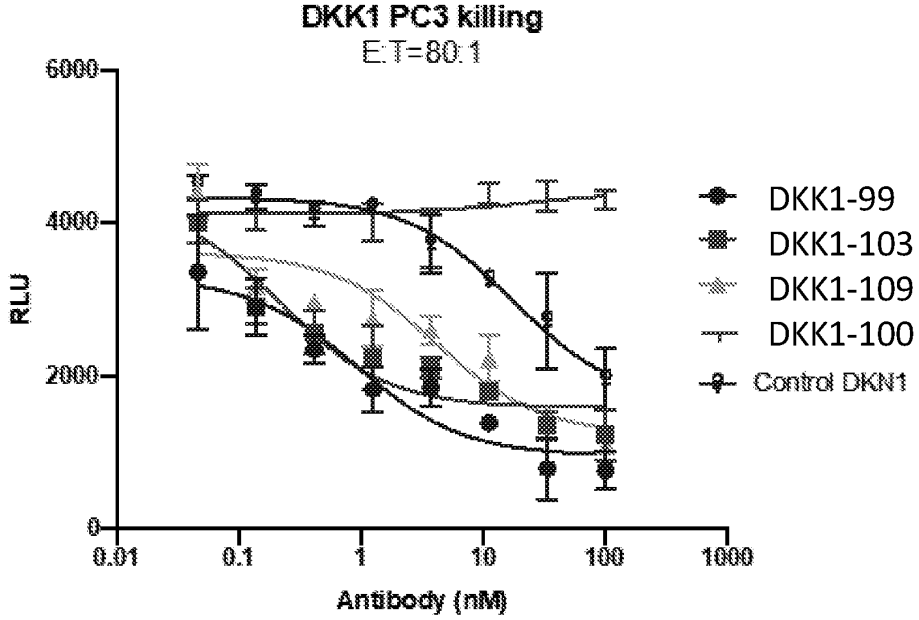


FIG. 15B

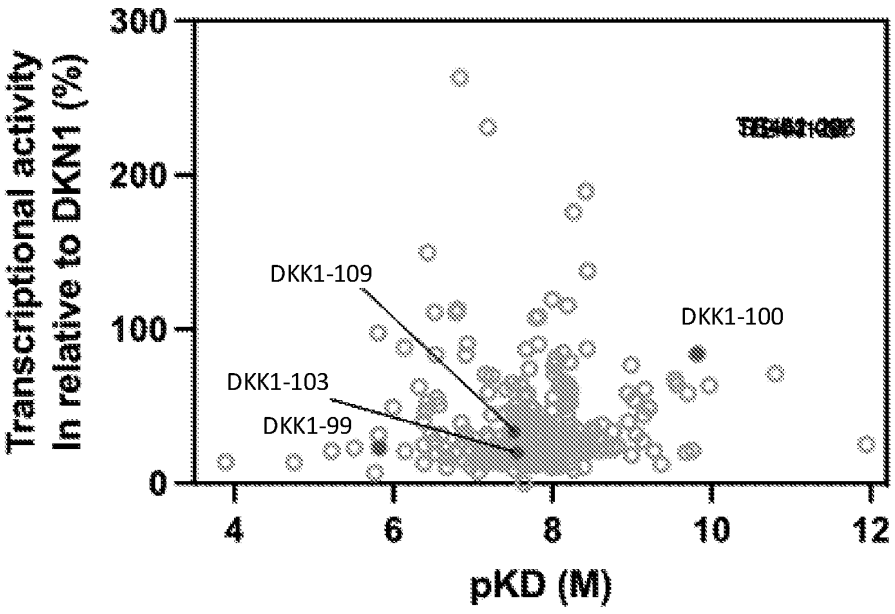


FIG. 15C

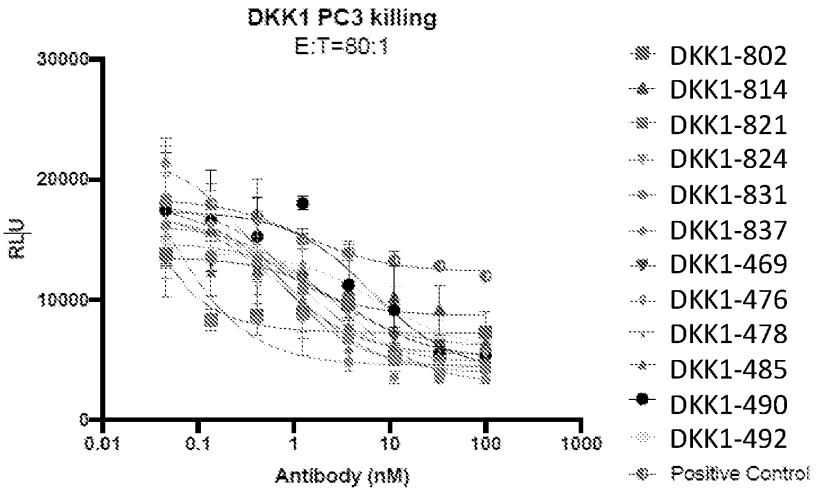


FIG. 15D

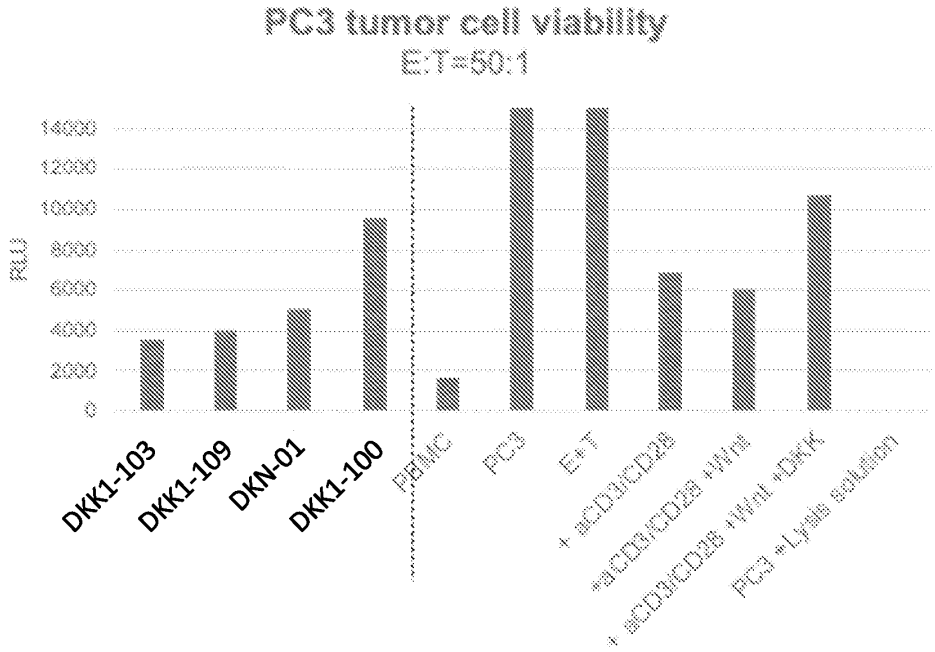


FIG. 15E

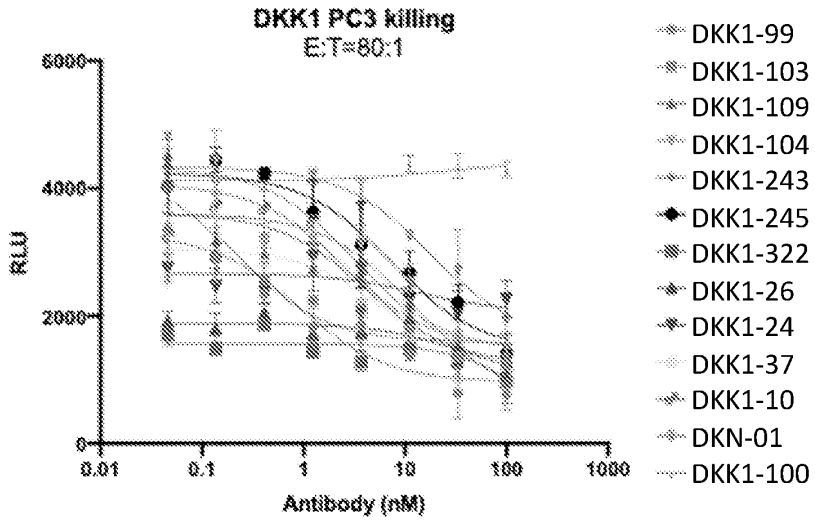


FIG. 15F

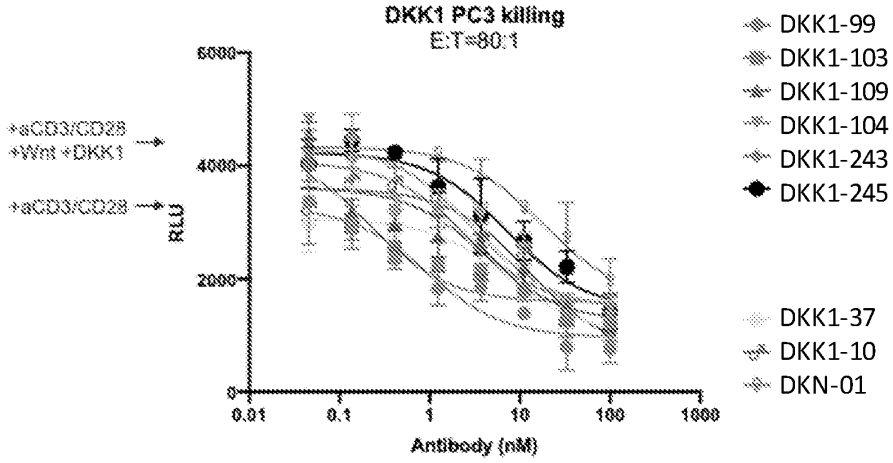


FIG. 15G

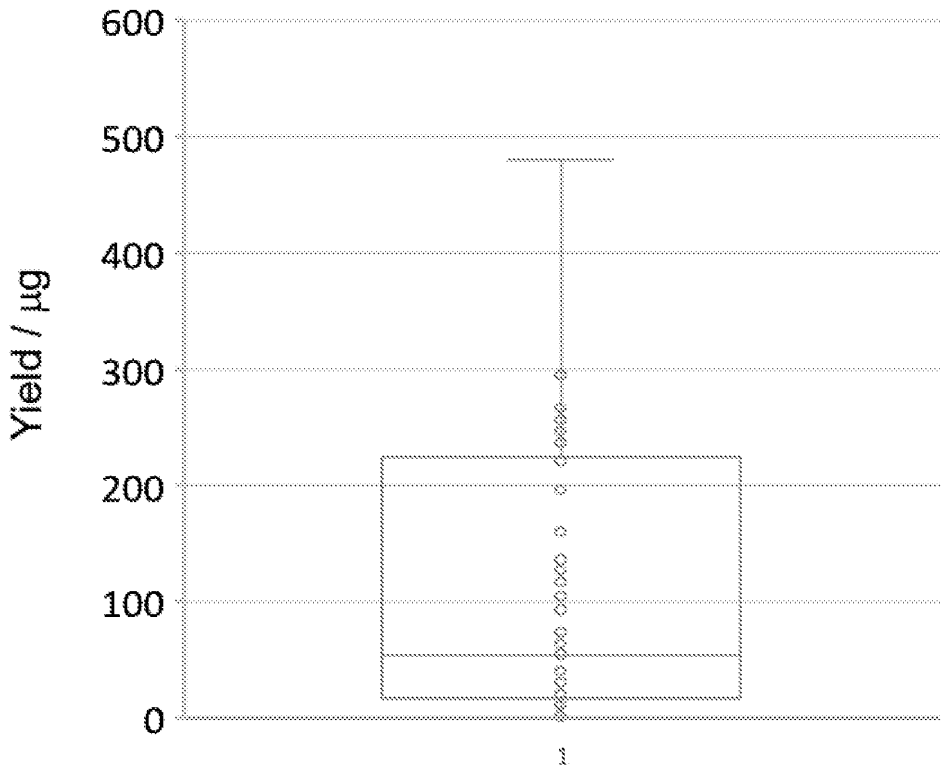


FIG. 16

Bins	ID	1 DKK1-24	1 DKK1-825	1 DKK1-28	1 DKN-01	2 DKK1-37	2 DKK1-109	2 DKK1-93	2 DKK1-100	2 DKK1-245	2 DKK1-99	2 DKK1-103	2 DKK1-10	2 DKK1-243
Bins														
1	DKK1-24													
1	DKK1-825													
1	DKK1-28													
1	DKN-01													
2	DKK1-37													
2	DKK1-109													
2	DKK1-93													
2	DKK1-100													
2	DKK1-245													
2	DKK1-99													
2	DKK1-103													
2	DKK1-10													
2	DKK1-243													

FIG. 17A

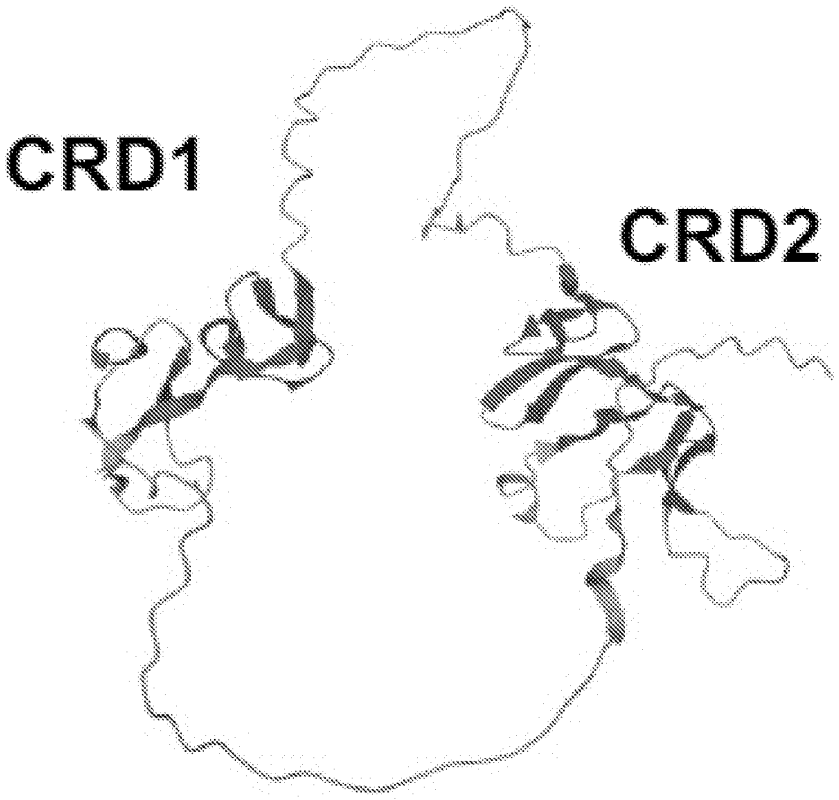


FIG. 17B

Name	DKK1 CRD1	DKK1 CRD2	R&D DKK1
	K_D (M)	K_D (M)	K_D (M)
DKK1-99 IgG2	5.22E-06	N/A	2.49E-07
DKK1-103 IgG2	3.71E-09	N/A	7.35E-08
DKK1-109 IgG2	9.29E-09	N/A	4.46E-08
DKK1-100 IgG2	N/A	2.08E-10	7.73E-10
Positive Control	N/A	7.91E-10	9.77E-10
DKK1-805	N/A	3.25E-09	5.22E-08
DKK1-820	N/A	7.54E-09	8.74E-09
DKK1-821	N/A	N/A	3.65E-08
DKK1-824	5.59E-09	1.45E-05	5.58E-08
DKK1-825	N/A	6.90E-10	6.36E-11
DKK1-830	N/A	1.69E-10	3.22E-11
DKK1-831	2.49E-08	N/A	1.09E-07
DKK1-837	2.26E-08	N/A	4.21E-08
DKK1-839	N/A	1.68E-11	4.94E-12
DKK1-739	N/A	4.43E-11	1.57E-09
DKK1-744	N/A	1.49E-09	3.14E-11
DKK1-448	N/A	2.57E-09	8.23E-08
DKK1-449	9.22E-08	N/A	1.41E-07
DKK1-473	N/A	1.09E-09	1.61E-09
DKK1-477	N/A	2.77E-10	1.73E-09
DKK1-478	N/A	7.08E-09	2.75E-09
DKK1-481	N/A	N/A	1.12E-08
DKK1-485	2.79E-09	N/A	1.56E-08

FIG. 17C

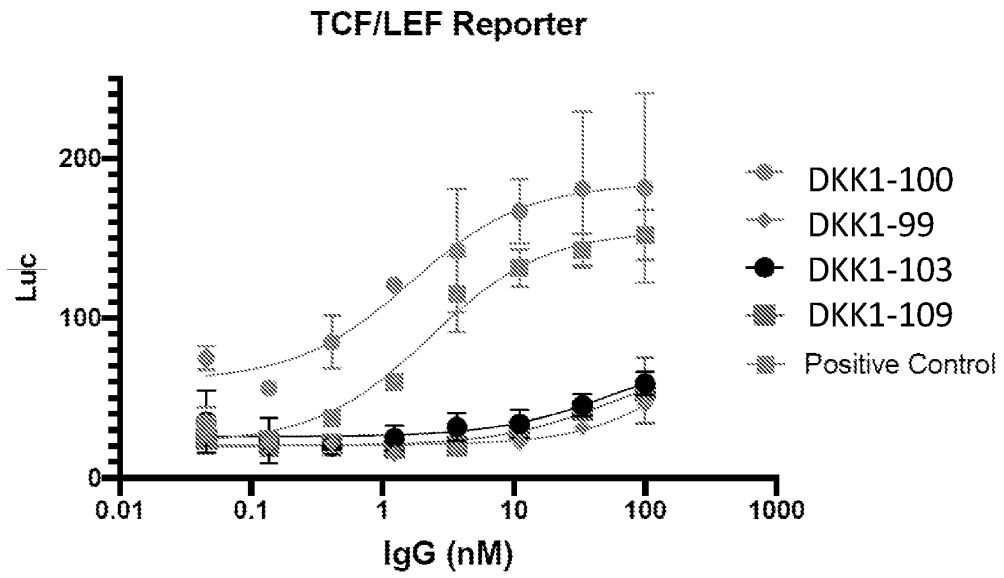


FIG. 18A

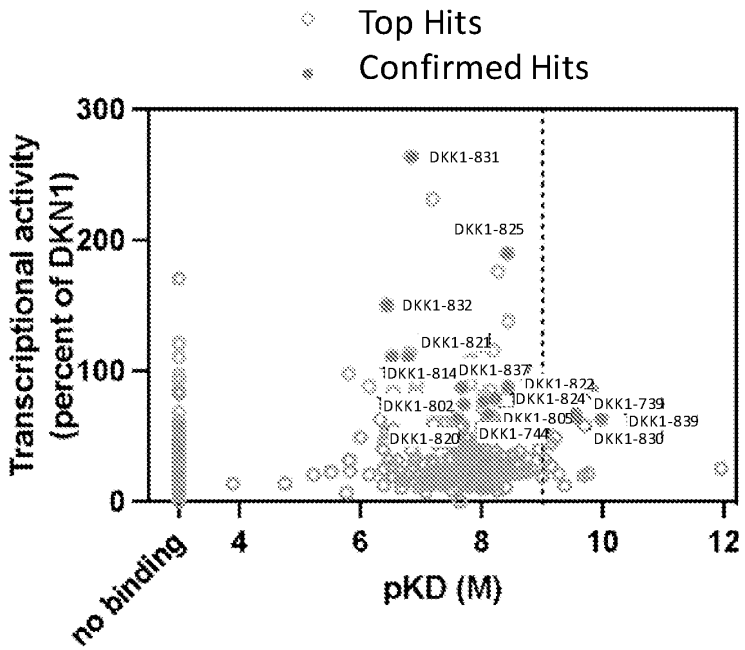


FIG. 18B

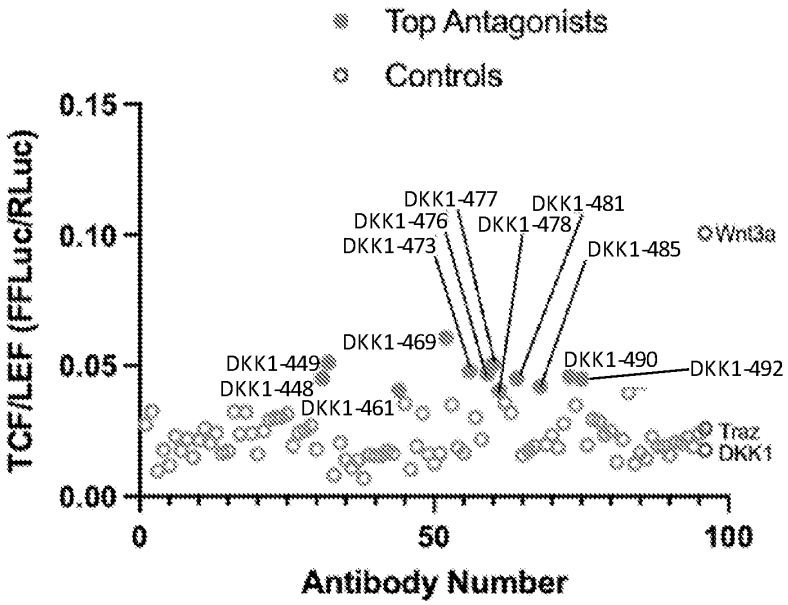


FIG. 18C

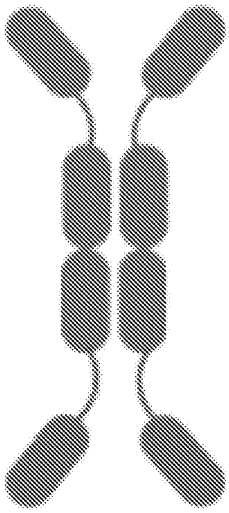


FIG. 19A

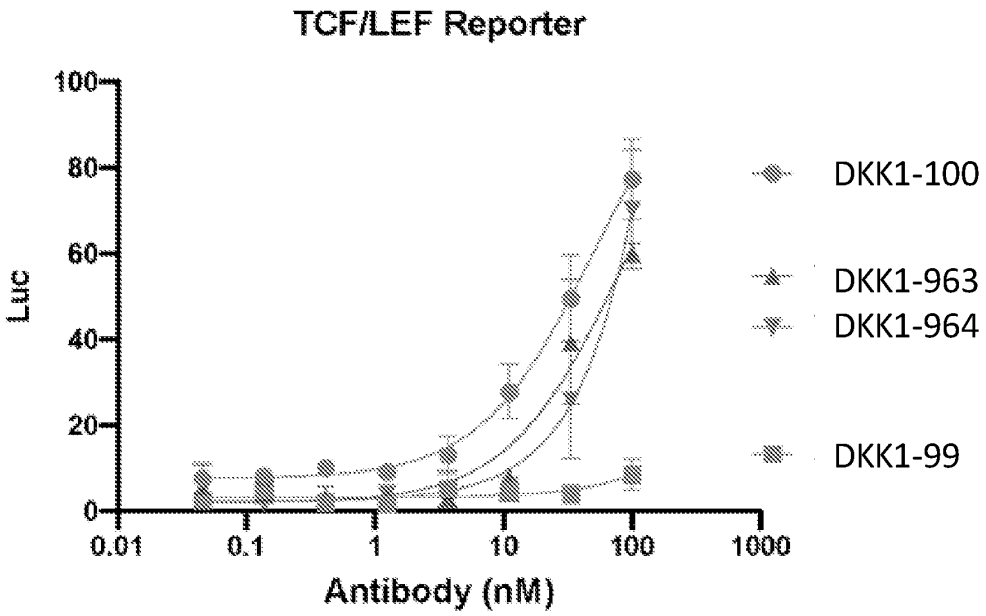


FIG. 19B

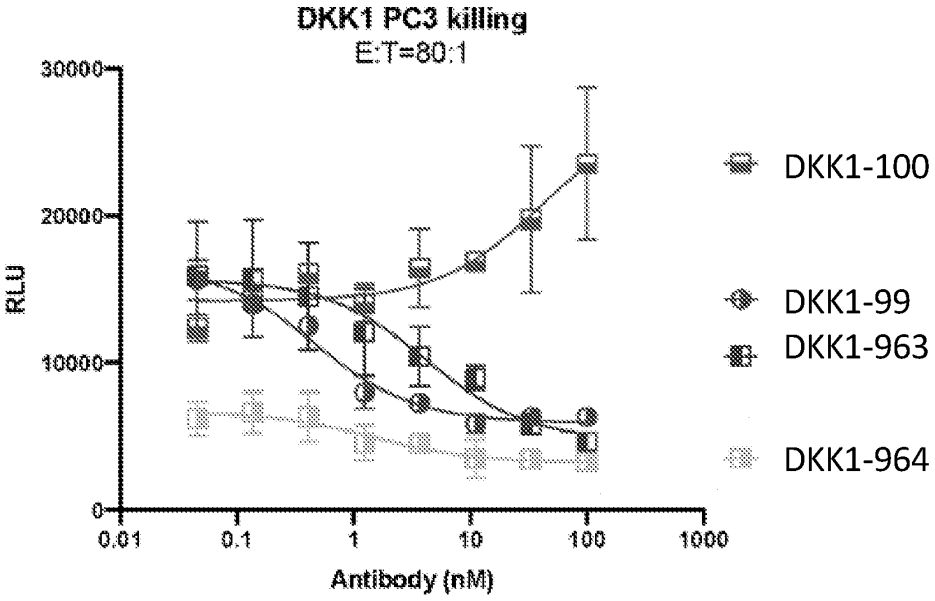


FIG. 19C

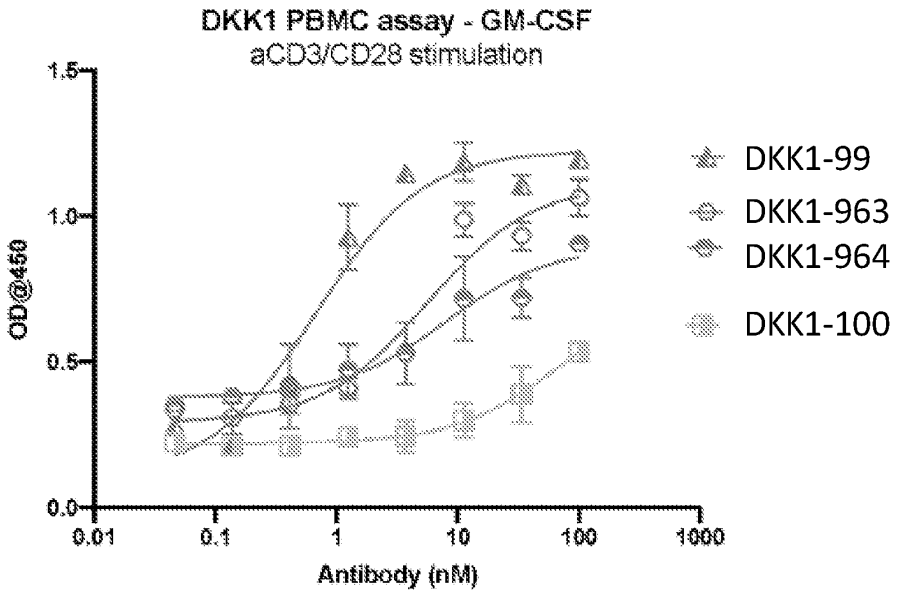

DKK1 PBMC assay - GM-CSF
aCD3/CD28 stimulation
OD@450
1.5
1.0
0.5
0.0
0.01
0.1
1
10
100
1000
Antibody (nM)
DKK1-99
DKK1-963
DKK1-964
DKK1-100

FIG. 19D

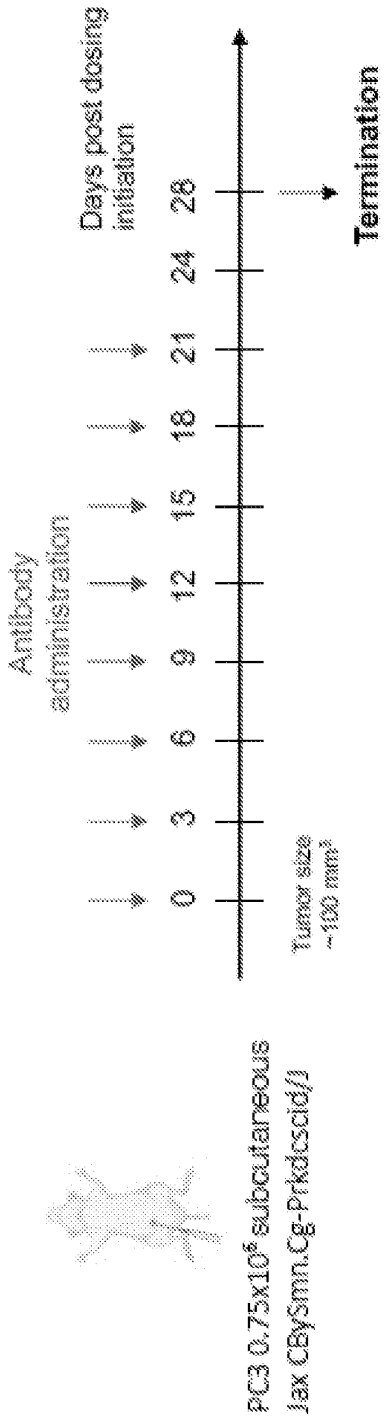

Days post dosing initiation
0
3
6
9
12
15
18
21
24
28
Termination
Antibody administration
Tumor size ~100 mm³
PC3 0.75x10⁶ subcutaneous
Jax CBySmn.Cg-Prkdcscid/J

FIG. 20A

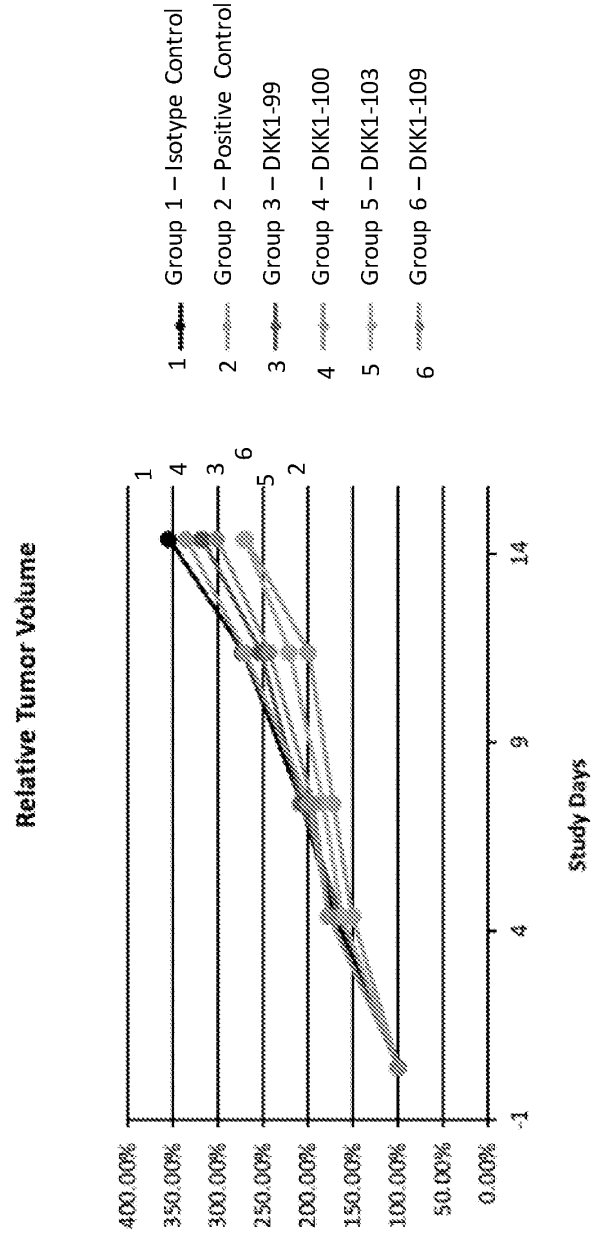

Relative Tumor Volume
400.00%
350.00%
300.00%
250.00%
200.00%
150.00%
100.00%
50.00%
0.00%
-1
4
9
14
Study Days
1
2
3
4
5
6
1 Group 1 – Isotype Control
2 Group 2 – Positive Control
3 Group 3 – DKK1-99
4 Group 4 – DKK1-100
5 Group 5 – DKK1-103
6 Group 6 – DKK1-109

FIG. 20B

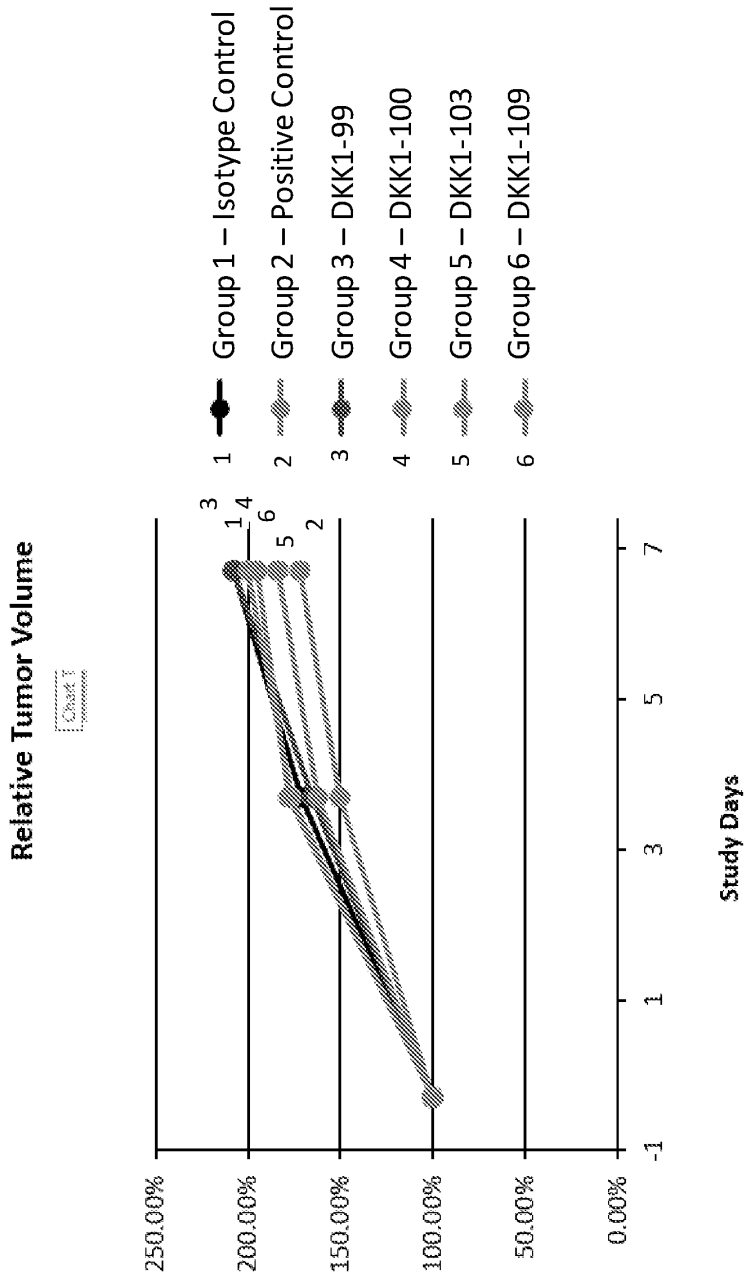

Relative Tumor Volume
Chart 1
Group 1 – Isotype Control
Group 2 – Positive Control
Group 3 – DKK1-99
Group 4 – DKK1-100
Group 5 – DKK1-103
Group 6 – DKK1-109
250.00%
200.00%
150.00%
100.00%
50.00%
0.00%
-1
1
3
5
7
Study Days

FIG. 20C

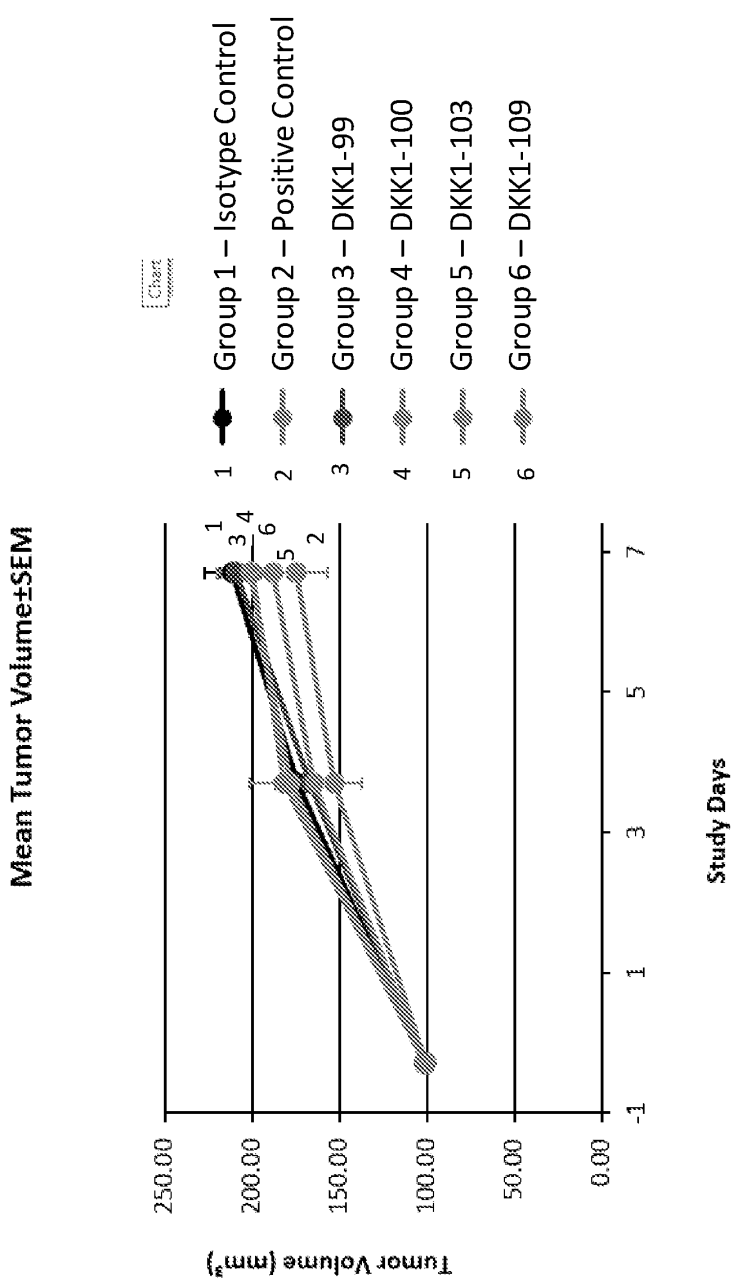

Mean Tumor Volume±SEM
Chart
Group 1 – Isotype Control
Group 2 – Positive Control
Group 3 – DKK1-99
Group 4 – DKK1-100
Group 5 – DKK1-103
Group 6 – DKK1-109
Tumor Volume (mm³)
250.00
200.00
150.00
100.00
50.00
0.00
-1
1
3
5
7
Study Days

FIG. 20D

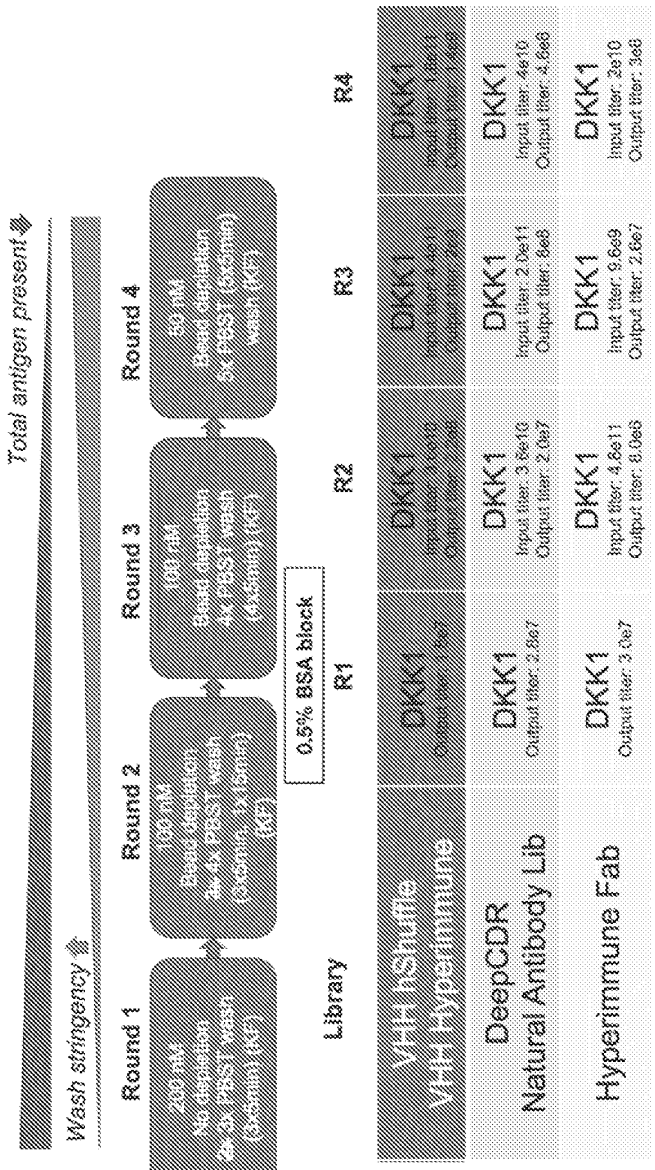

Total antigen present
Wash stringency
Round 1
200 nM
No depletion
2x-3x PBST wash
(3x5min) (KF)
Round 2
100 nM
Bead depletion
2x-4x PBST wash
(3x5min, 1x15min)
(KF)
Round 3
100 nM
Bead depletion
4x PBST wash
(4x5min) (KF)
Round 4
50 nM
Bead depletion
5x PBST (5x5min)
wash (KF)
0.5% BSA block
Library
R1
R2
R3
R4
VHH hShuffle
VHH Hyperimmune
DKK1
DKK1
DKK1
DKK1
DeepCDR
Natural Antibody Lib
DKK1
Output titer: 2.8e7
DKK1
Input titer: 3.6e10
Output titer: 2.0e7
DKK1
Input titer: 2.0e11
Output titer: 8e8
DKK1
Input titer: 4e10
Output titer: 4.6e6
Hyperimmune Fab
DKK1
Output titer: 3.0e7
DKK1
Input titer: 4.8e11
Output titer: 8.0e6
DKK1
Input titer: 9.6e9
Output titer: 2.6e7
DKK1
Input titer: 2e10
Output titer: 3e6

FIG. 21

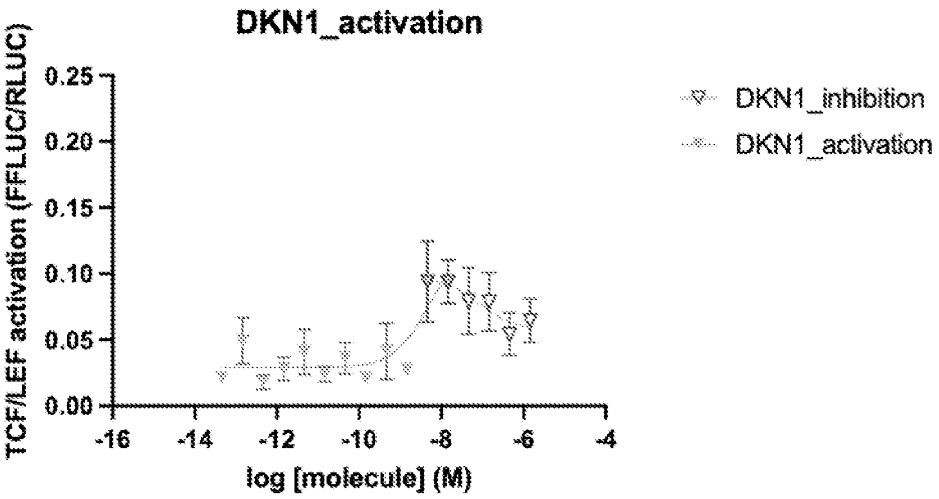


FIG. 22A

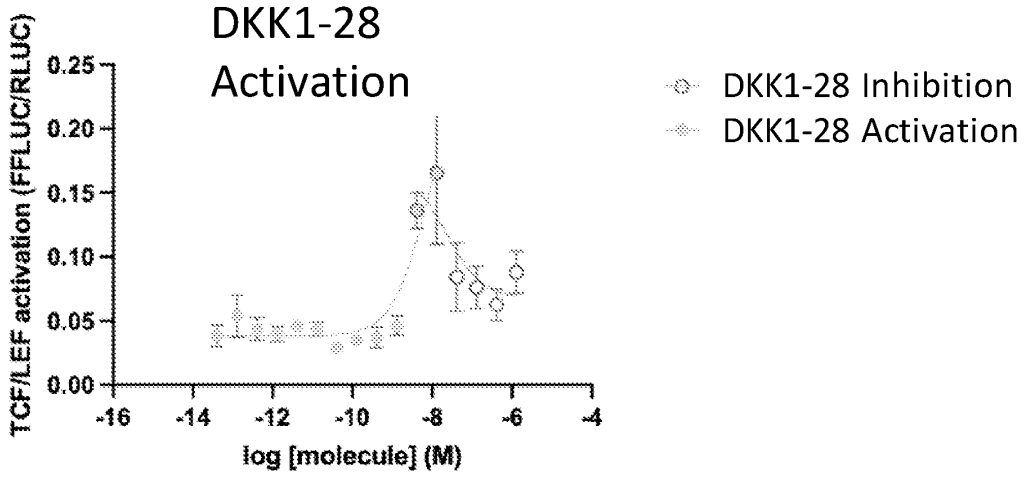


FIG. 22B

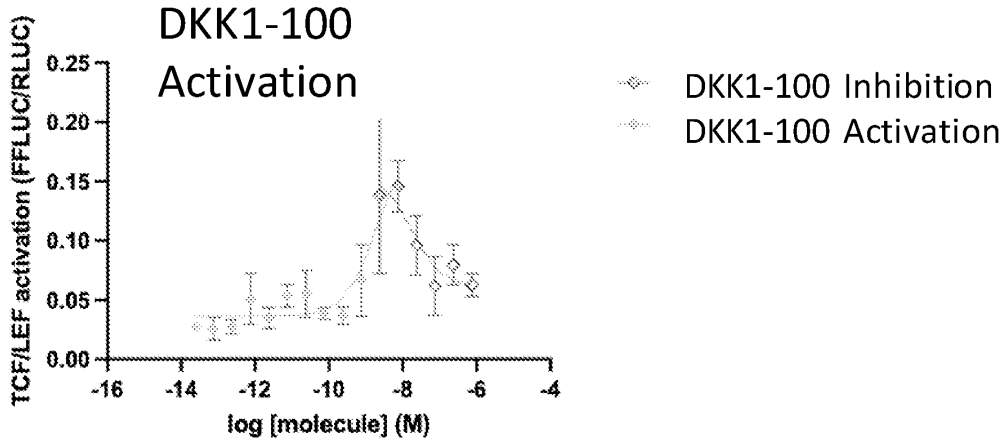


FIG. 22C

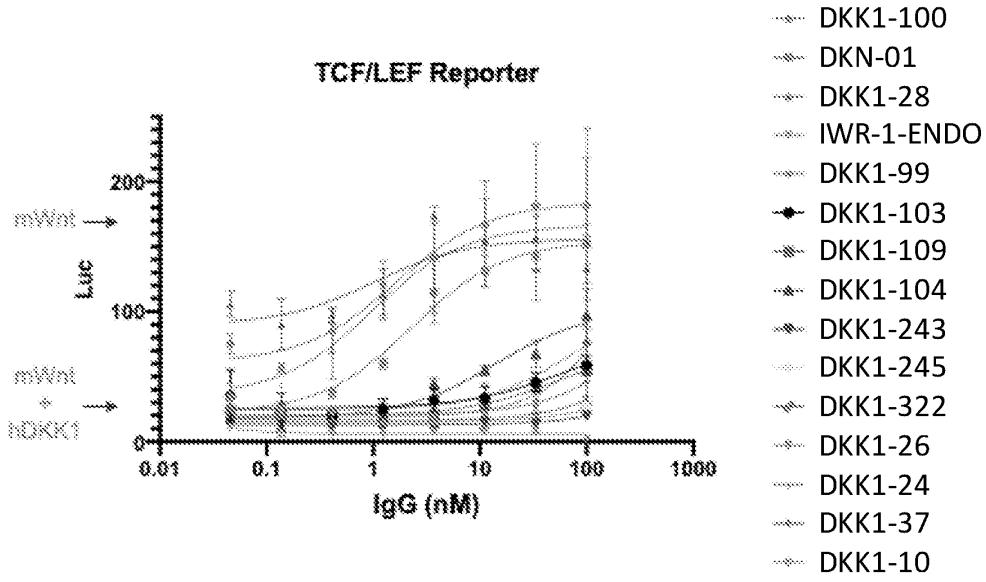


FIG. 23A

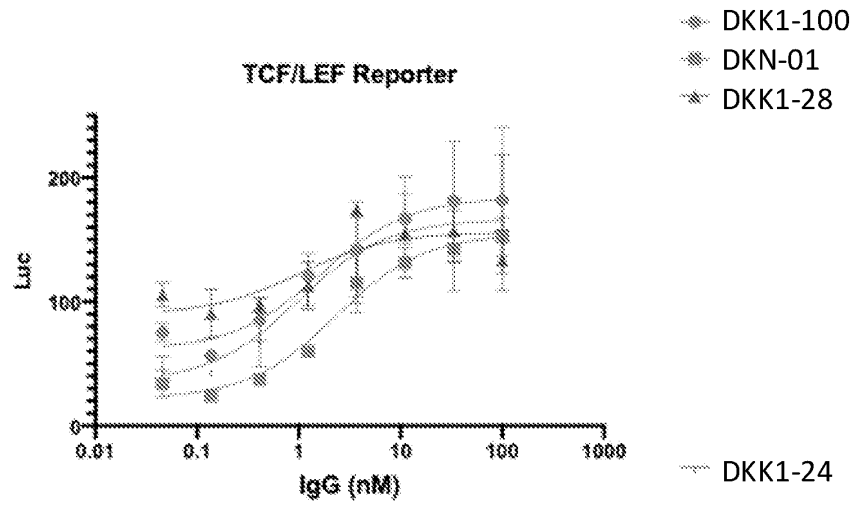


FIG. 23B

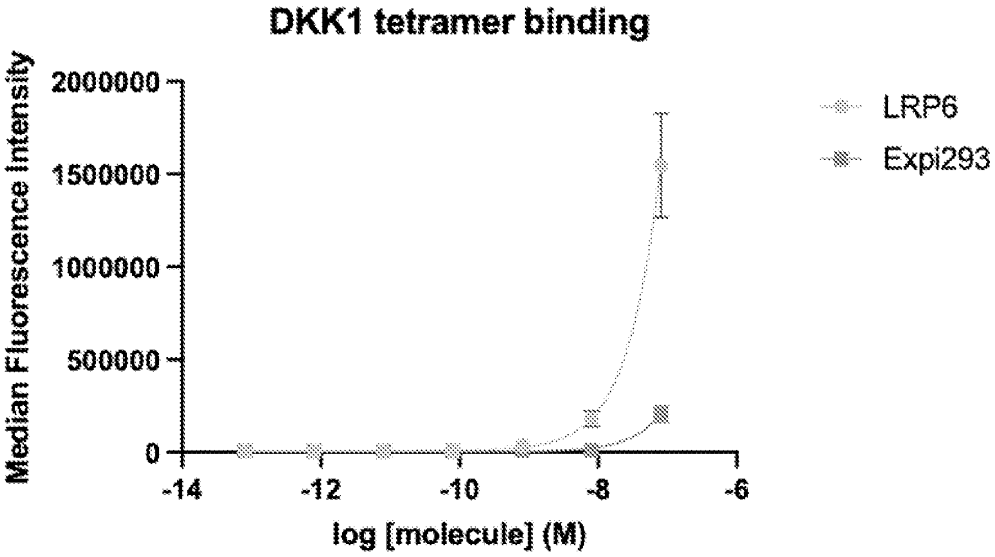


FIG. 24

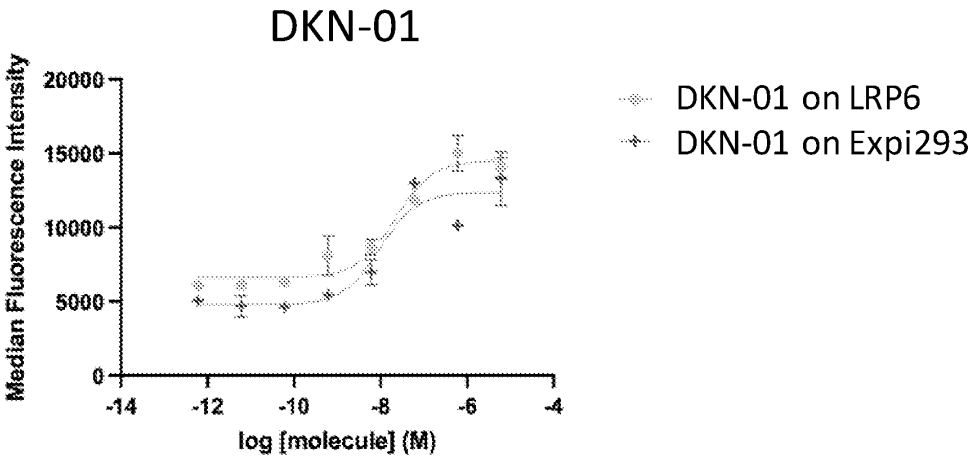


FIG. 25A

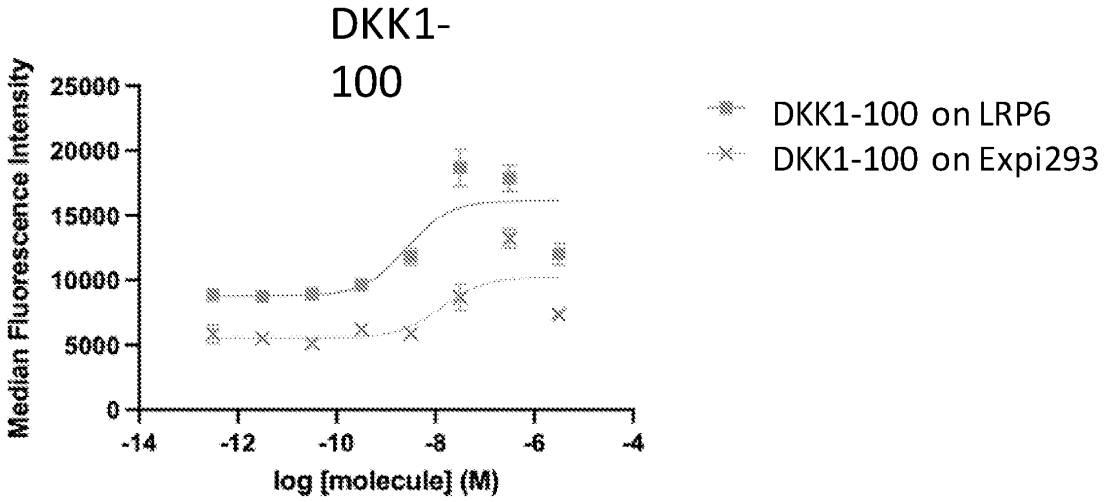


FIG. 25B

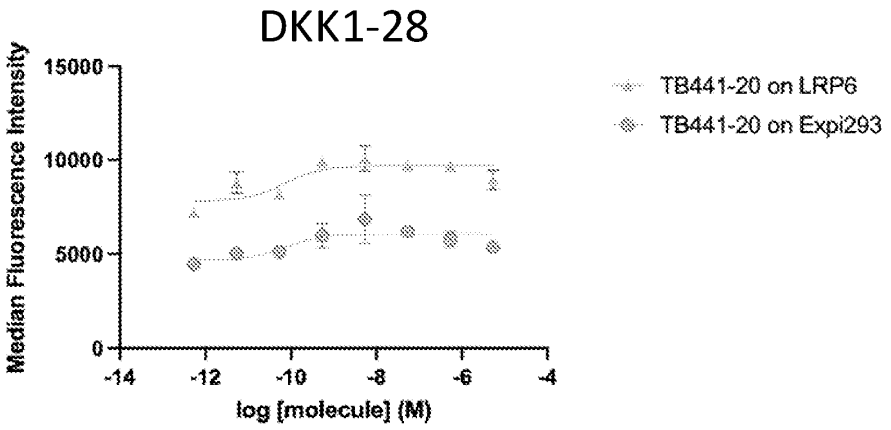


FIG. 25C

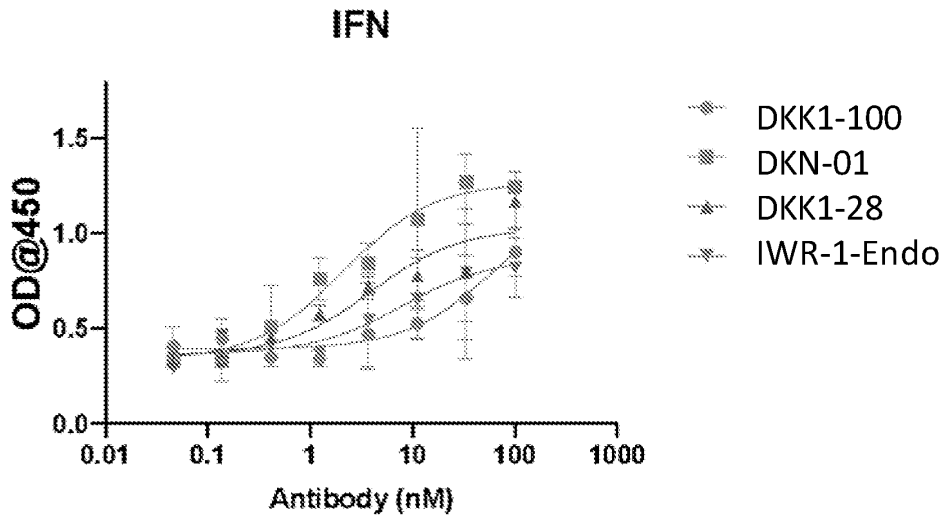


FIG. 26A

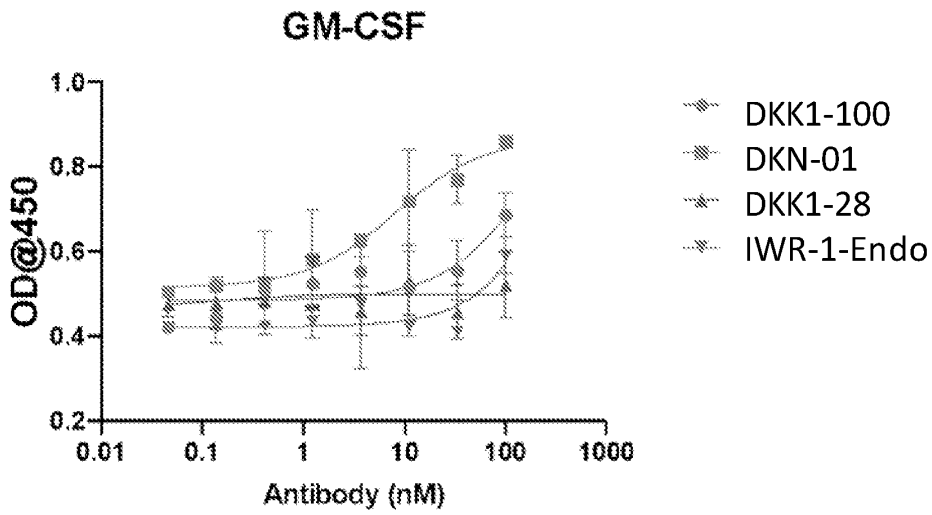


FIG. 26B

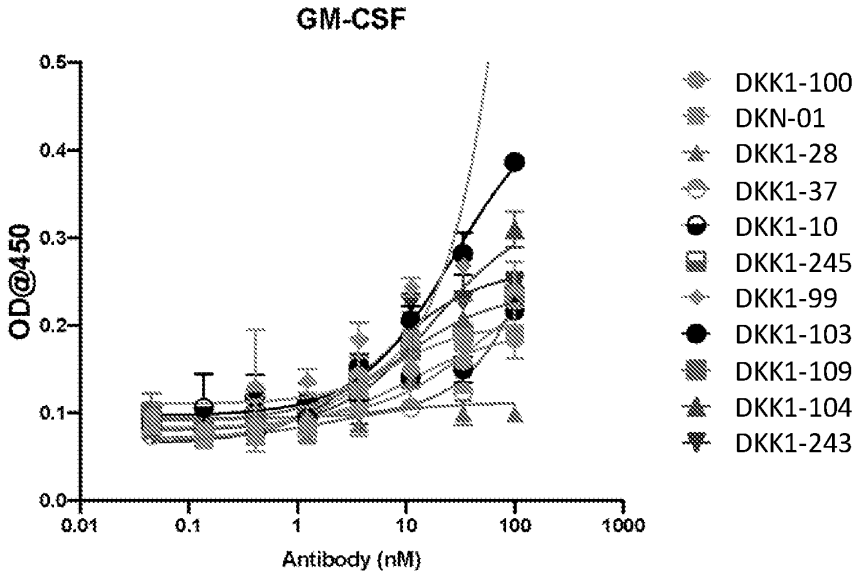


FIG. 27A

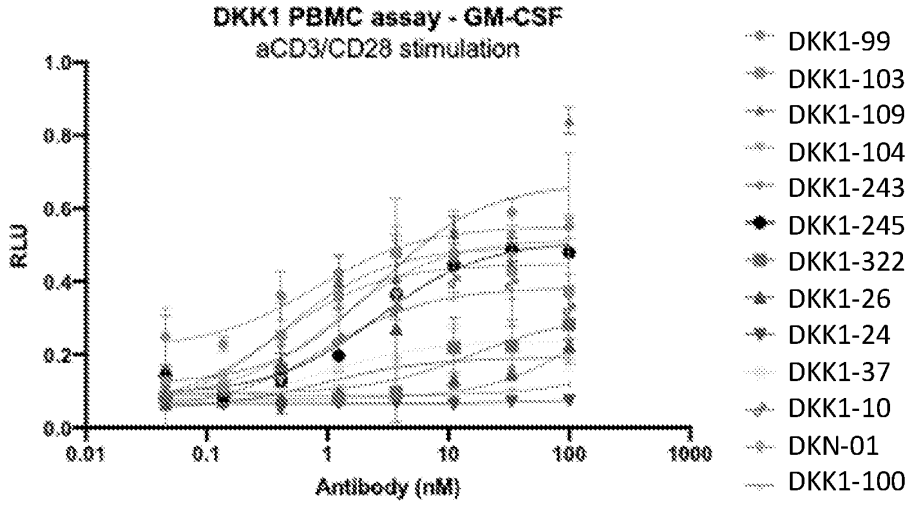


FIG. 27B

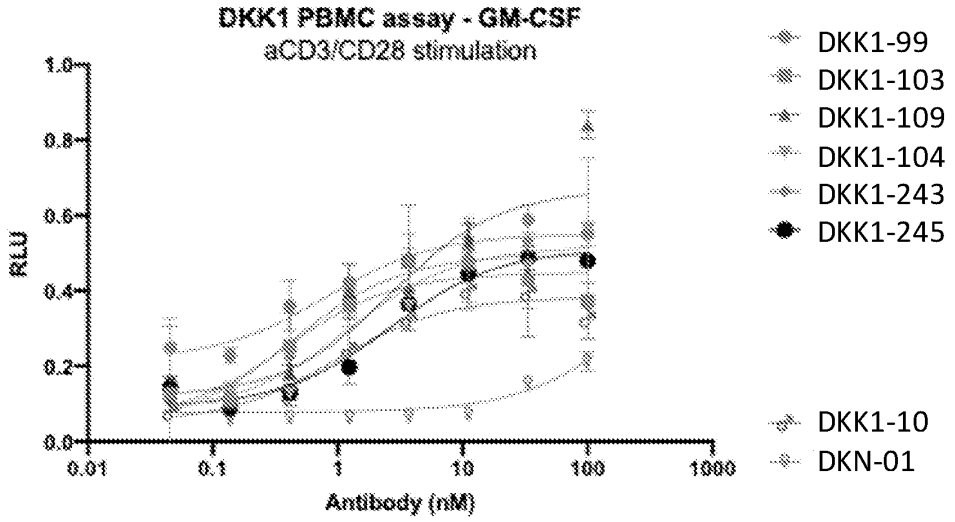


FIG. 27C

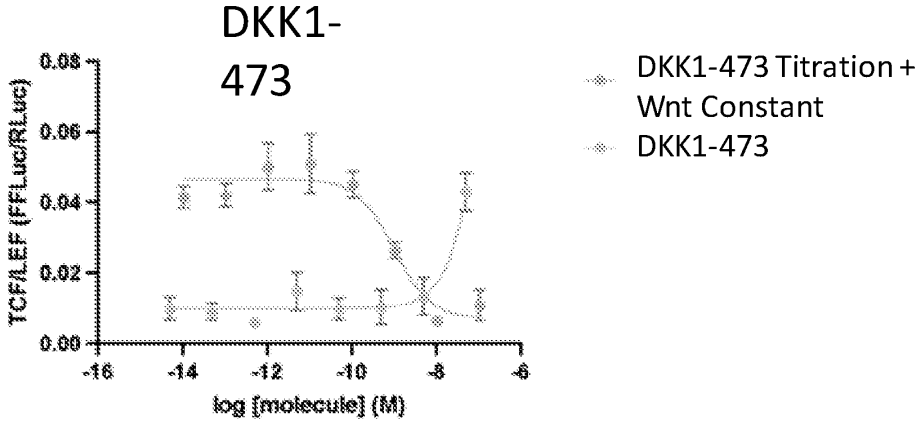


FIG. 28A

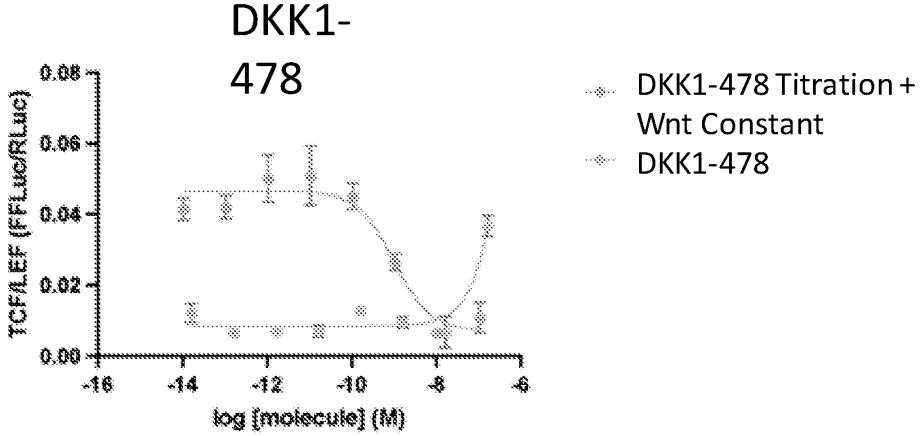


FIG. 28B

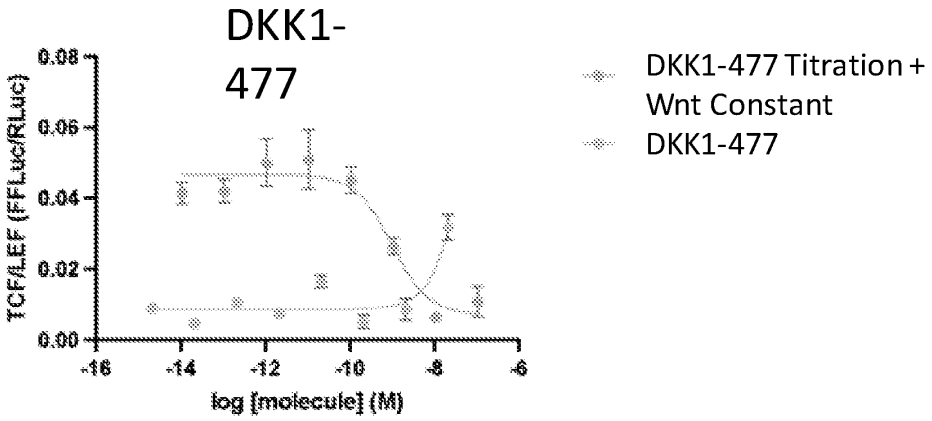


FIG. 28C

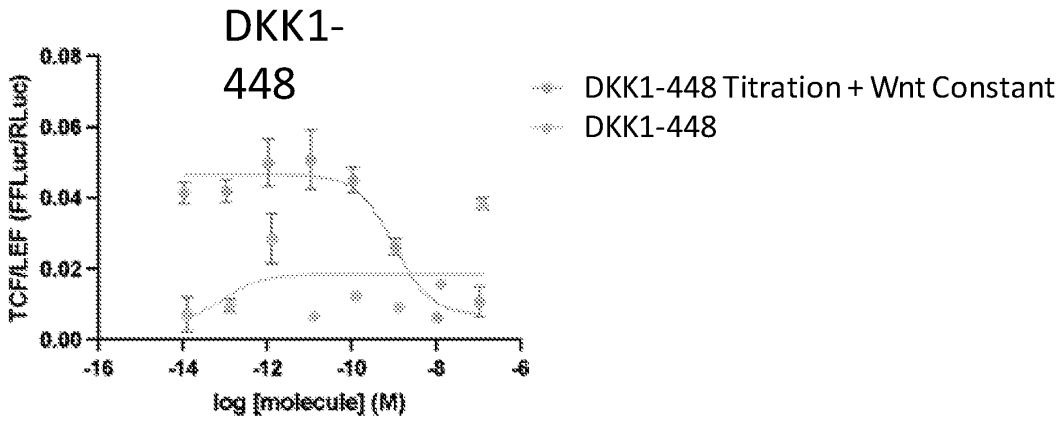


FIG. 28D

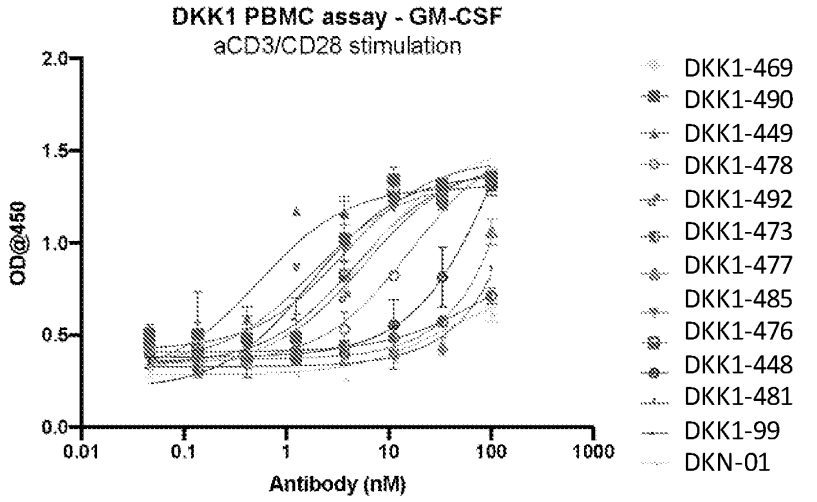


FIG. 29A

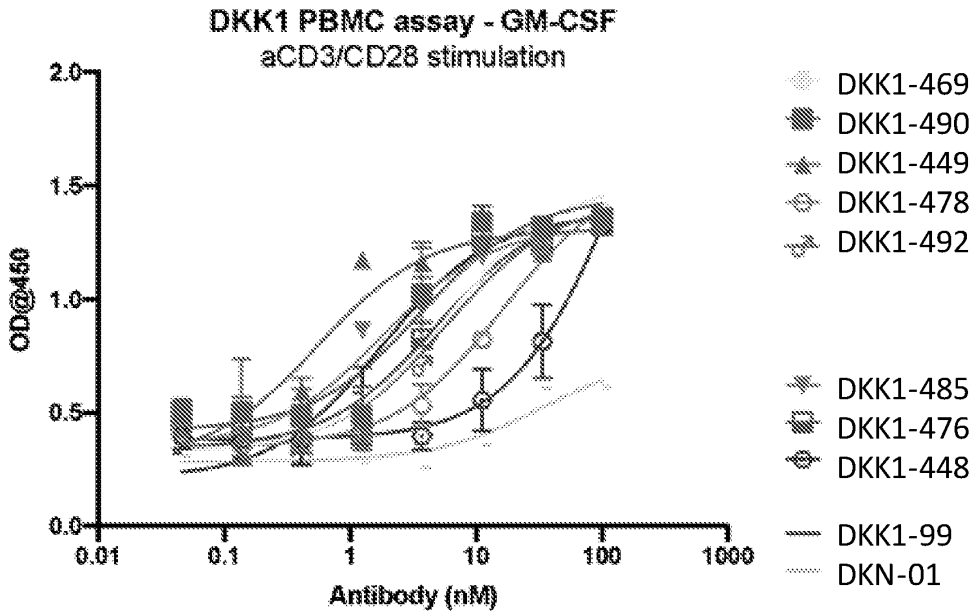


FIG. 29B

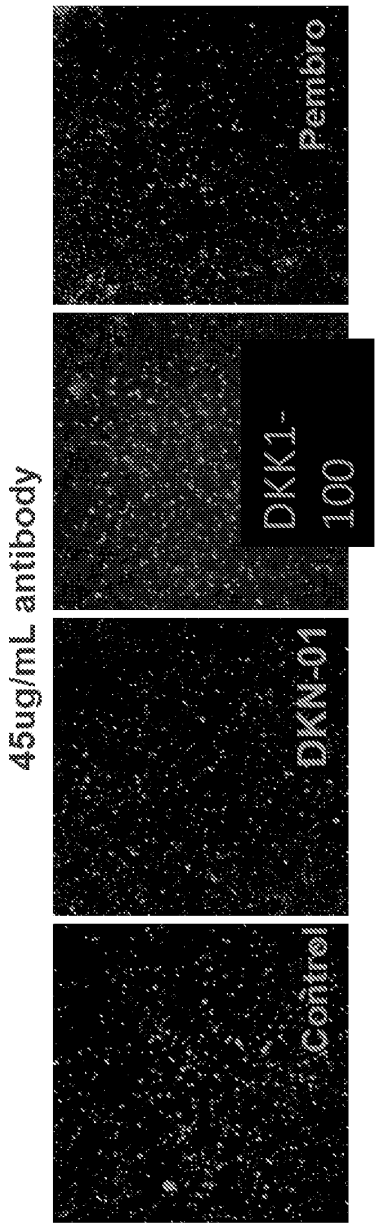


FIG. 30