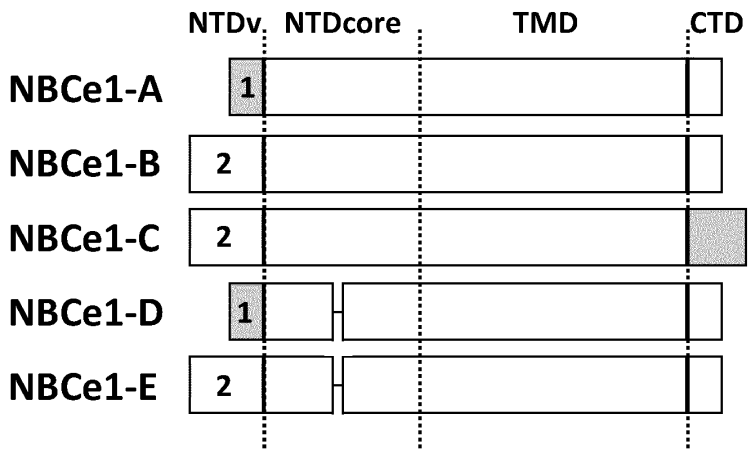
A



B

```
h-A -----------------MSTENVEGKP-------------------SNLGERG---RARS 21
h-B MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKTGHKEKKEKERI 60
m-B MEDEAVLDRGASFLKHVCDEEEVEGHHTIYIGVHVPKSYRRRRRHKRKAGHKEKKEKERI 60
                      . *:***:                    : *.:    : *

h-A S-TFL----RVVQPMFNHSIFTSAVSPAAERIRFILGEEDDSPAPPQLFTELDELLAVDG 76
h-B SENYSDKSDIENADESSSSILKPLISPAAERIRFILGEEDDSPAPPQLFTELDELLAVDG 120
m-B SENYSDKSDVENADESSSSILKPLISPAAERIRFILGEEDDSPAPPQLFTELDELLAVDG 120
    * .:           . **:.  :**********************************

h-A QEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLHSLFELRTCMEKGSIMLDREASSLP 136
h-B QEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLHSLFELRTCMEKGSIMLDREASSLP 180
m-B QEMEWKETARWIKFEEKVEQGGERWSKPHVATLSLHSLFELRTCMEKGSIMLDREASSLP 180
    ************************************************************

h-A QLVEMIVDHQIETGLLKPELKDKVTYTLLRKHRHQTKKSNLRSLADIGKTVSSASRMFTN 196
h-B QLVEMIVDHQIETGLLKPELKDKVTYTLLRKHRHQTKKSNLRSLADIGKTVSSASRMFTN 240
m-B QLVEMIADHQIETGLLKPDLKDKVTYTLLRKHRHQTKKSNLRSLADIGKTVSSASRMFSN 240
    ******.***********:*************************************:*

h-A PDNGSPAMTHRNLTSSSLNDISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFI 256
h-B PDNGSPAMTHRNLTSSSLNDISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFI 300
m-B PDNGSPAMTHRNLTSSSLNDISDKPEKDQLKNKFMKKLPRDAEASNVLVGEVDFLDTPFI 300
    ************************************************************

h-A AFVRLQQAVMLGALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKA 316
h-B AFVRLQQAVMLGALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKA 360
m-B AFVRLQQAVMLGALTEVPVPTRFLFILLGPKGKAKSYHEIGRAIATLMSDEVFHDIAYKA 360
    ************************************************************
```

FIGURE 1

```
h-A KDRHDLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMNGDTP 376
h-B KDRHDLIAGIDEFLDEVIVLPPGEWDPAIRIEPPKSLPSSDKRKNMYSGGENVQMNGDTP 420
m-B KDRHDLIAGIDEFLDEVIVLPPGEWDPTIRIEPPKSLPSSDKRKNMYSGGENVQMNGDTP 420
    ***************************:********************************

h-A HDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQALSAILFIYLAT 436
h-B HDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQALSAILFIYLAT 480
m-B HDGGHGGGGHGDCEELQRTGRFCGGLIKDIKRKAPFFASDFYDALNIQALSAILFIYLAT 480
    ************************************************************

h-A VTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAGQPLTILSSTGPVLVFERLLF 496
h-B VTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAGQPLTILSSTGPVLVFERLLF 540
m-B VTNAITFGGLLGDATDNMQGVLESFLGTAVSGAIFCLFAGQPLTILSSTGPVLVFERLLF 540
    ************************************************************
        EL2
h-A NESKDNNFDYLEFRLWIGLWSAFLCLILVATDASFLVQYFTRFTEEGFSSLISFIFIYDA 556
h-B NESKDNNFDYLEFRLWIGLWSAFLCLILVATDASFLVQYFTRFTEEGFSSLISFIFIYDA 600
m-B NESKDHNFDYLEFRLWIGLWSAFMCLVLVATDASFLVQYFTRFTEEGFSSLISFIFIYDA 600
    ******:*****************:**:********************************
                                        EL3
h-A FKKMIKLADYYPINSNFKVGYNTLFSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYH 616
h-B FKKMIKLADYYPINSNFKVGYNTLFSCTCVPPDPANISISNDTTLAPEYLPTMSSTDMYH 660
m-B FKKMIKLADYYPINSDFKVGYNTHFSCACLPPDPVNLSVSNDTTLAPEDLPTISSTDMYH 660
    *********************:******* ***:*:****.*:*:********* ***:*******
                EL3
h-A NTTFDWAFLSKKECSKYGGNLVGNNCNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFP 676
h-B NTTFDWAFLSKKECSKYGGNLVGNNCNFVPDITLMSFILFLGTYTSSMALKKFKTSPYFP 720
m-B NVTFDWAYLSKKECVKYGGKLVGNNCDFVPDITLMSFILFLGTYTSSMAMKKFKTSRYFP 720
    *.*****:****** ****:******:*********************:****** ***
                                        EL4
h-A TTARKLISDFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWV 736
h-B TTARKLISDFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGENPWWV 780
m-B TTARKLISDFAIILSILIFCVIDALVGVDTPKLIVPSEFKPTSPNRGWFVPPFGGNPWWV 780
    ************************************************** *****

h-A CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVICSLMALPW 796
h-B CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVICSLMALPW 840
m-B CLAAAIPALLVTILIFMDQQITAVIVNRKEHKLKKGAGYHLDLFWVAILMVVCSFMALPW 840
    ************************************************:**:*****

h-A YVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVFILTGLSVFMAPILKFI 856
h-B YVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVFILTGLSVFMAPILKFI 900
m-B YVAATVISIAHIDSLKMETETSAPGEQPKFLGVREQRVTGTLVFILTGLSVFMAPILKFI 900
    ************************************************************
```

FIGURE 1 - continued

```
h-A PMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQPDFIYLRHVPLRRVHLFTFLQVLC 916
h-B PMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQPDFIYLRHVPLRRVHLFTFLQVLC 960
m-B PMPVLYGVFLYMGVASLNGVQFMDRLKLLLMPLKHQPDFIYLRHVPLRRVHLFTFLQVLC 960
    ************************************************************

h-A LALLWILKSTVAAIIFPVMILALVAVRKGMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKK 976
h-B LALLWILKSTVAAIIFPVMILALVAVRKGMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKK 1020
m-B LALLWILKSTVAAIIFPVMILALVAVRKGMDYLFSQHDLSFLDDVIPEKDKKKKEDEKKK 1020
    ************************************************************

h-A KKKKGSLDSDNDDSDCPYSEKVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC 1035
h-B KKKKGSLDSDNDDSDCPYSEKVPSIKIPMDIMEQQPFLSDSKPSDRERSPTFLERHTSC 1079
m-B KKKKGSLDSDNDDSDCPYSEKVPSIKIPMDIMEQQPFLSDNKPLDRERSSTFLERHTSC 1079
    ************************************.** ***** *********

h-A (SEQ ID NO:118)
h-B (SEQ ID NO:114)
m-B (SEQ ID NO:115)
```

FIGURE 1 - continued

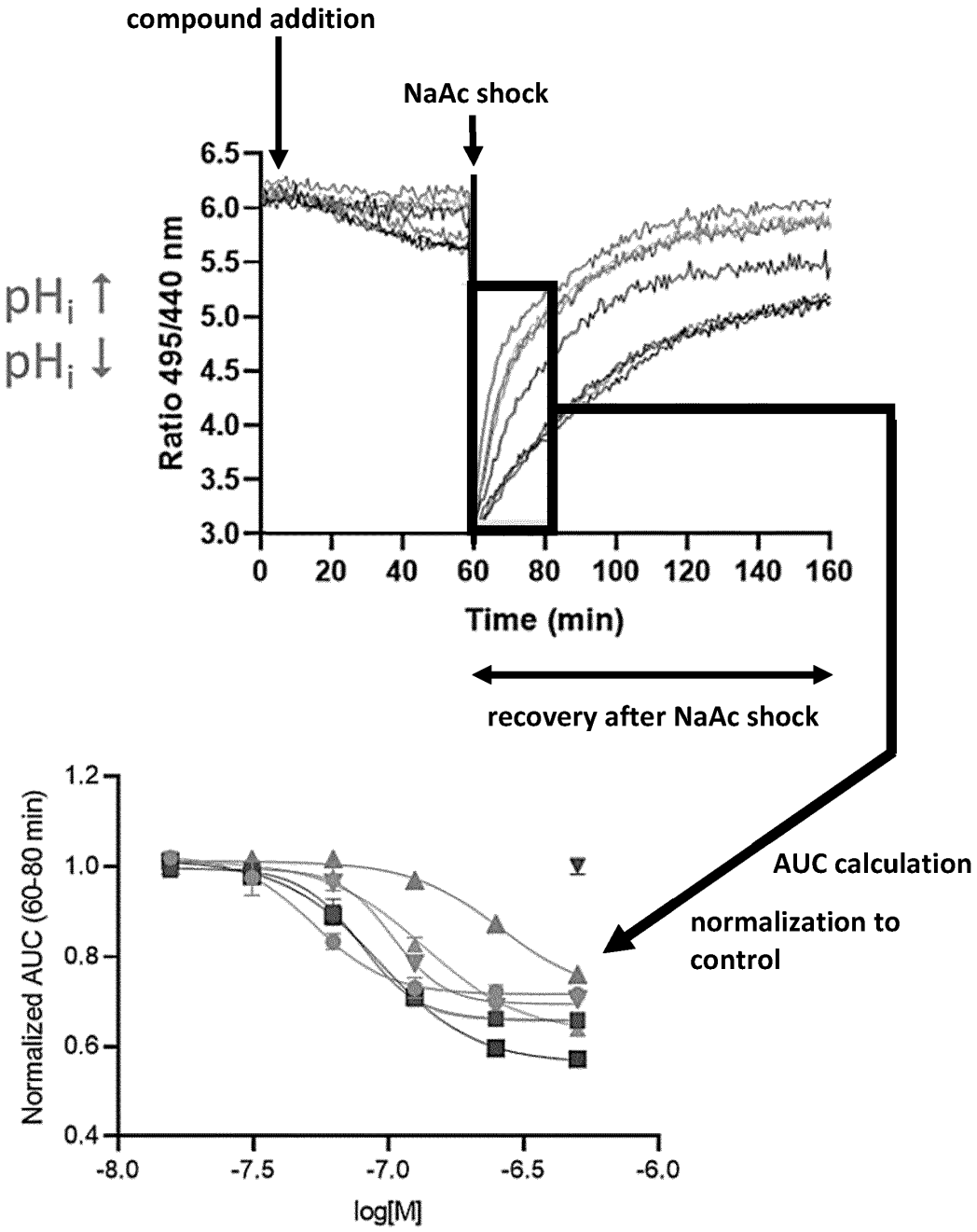

compound addition
NaAc shock
pH$_i$ ↑
pH$_i$ ↓
Ratio 495/440 nm
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
0
20
40
60
80
100
120
140
160
Time (min)
recovery after NaAc shock
AUC calculation
normalization to control
Normalized AUC (60-80 min)
1.2
1.0
0.8
0.6
0.4
-8.0
-7.5
-7.0
-6.5
-6.0
log[M]

FIGURE 2

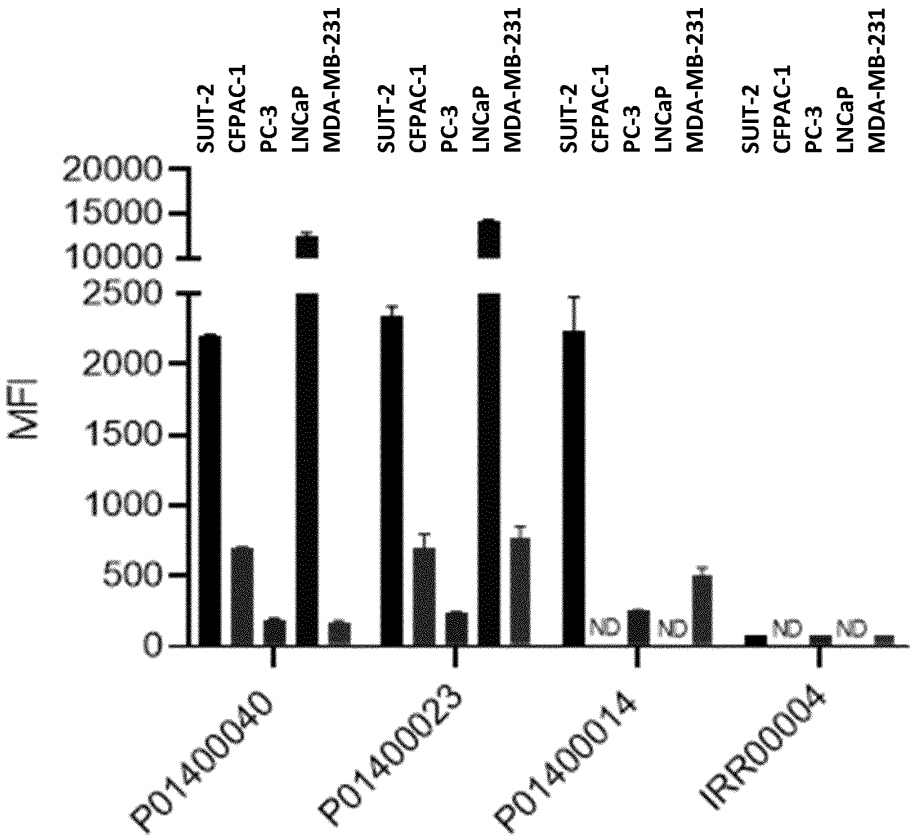


FIGURE 3

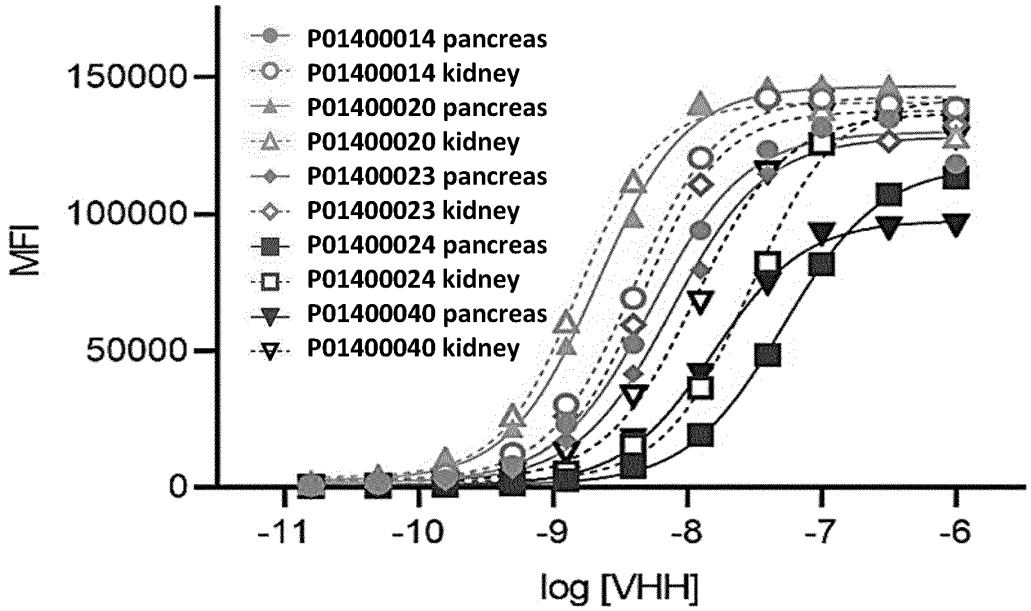


FIGURE 4

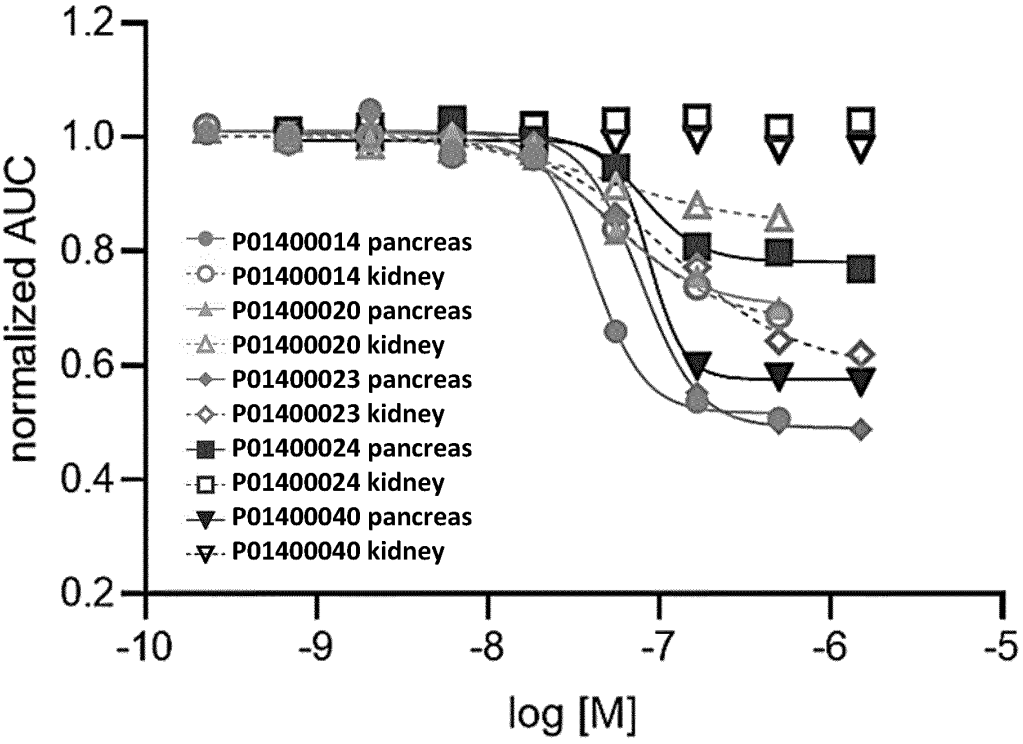


FIGURE 5

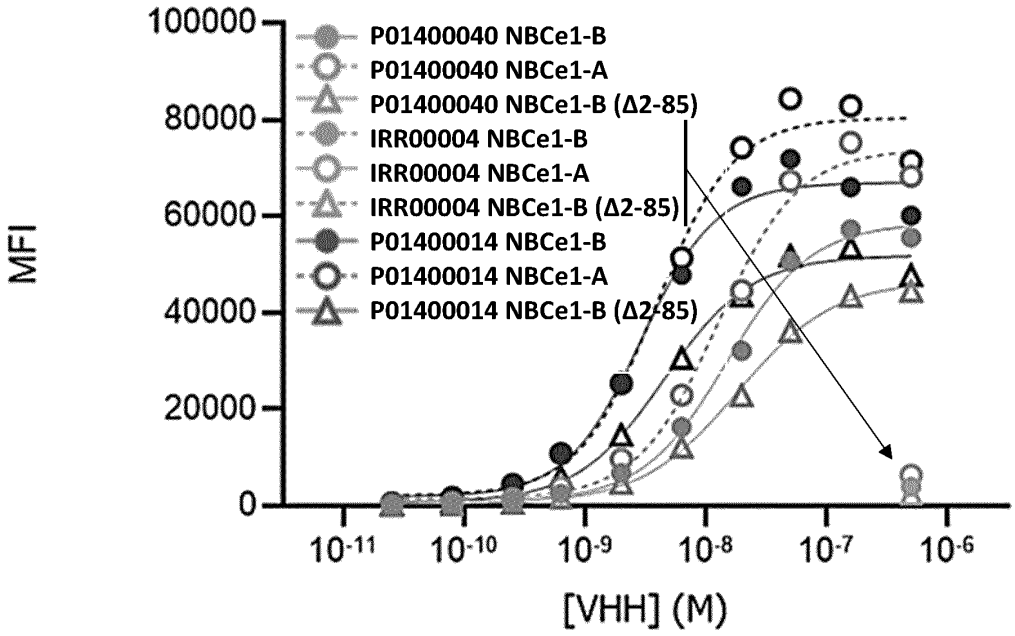


FIGURE 6

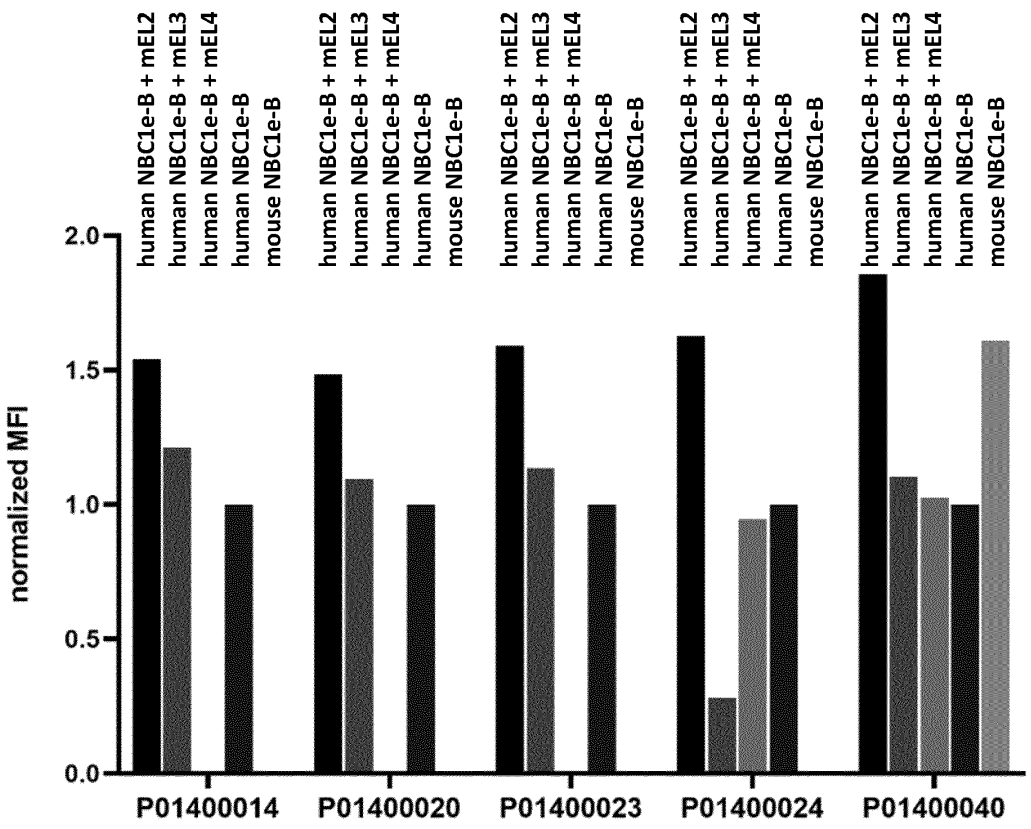

normalized MFI
2.0
1.5
1.0
0.5
0.0
human NBC1e-B + mEL2
human NBC1e-B + mEL3
human NBC1e-B + mEL4
human NBC1e-B
mouse NBC1e-B
P01400014
P01400020
P01400023
P01400024
P01400040

FIGURE 7

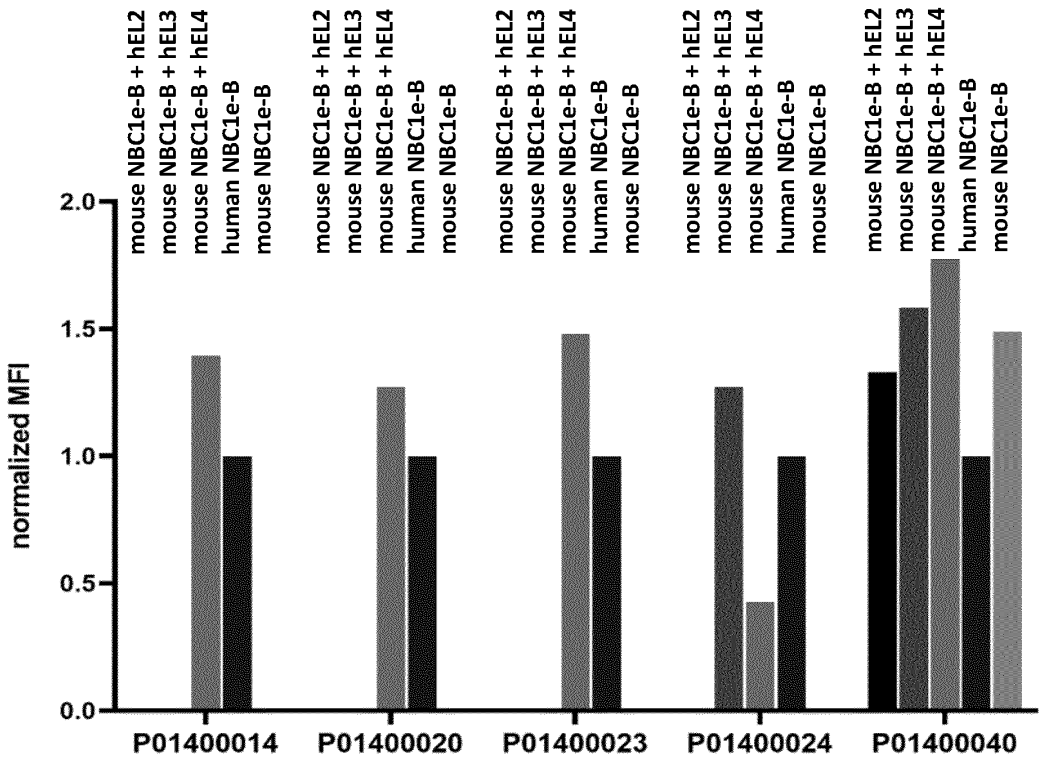
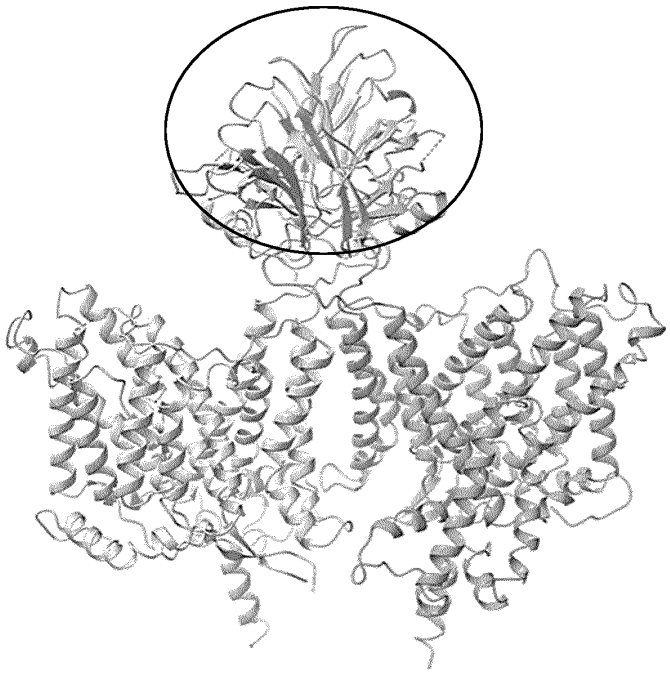

mouse NBC1e-B + hEL2
mouse NBC1e-B + hEL3
mouse NBC1e-B + hEL4
human NBC1e-B
mouse NBC1e-B
normalized MFI
2.0
1.5
1.0
0.5
0.0
P01400014
P01400020
P01400023
P01400024
P01400040

FIGURE 8

A



B

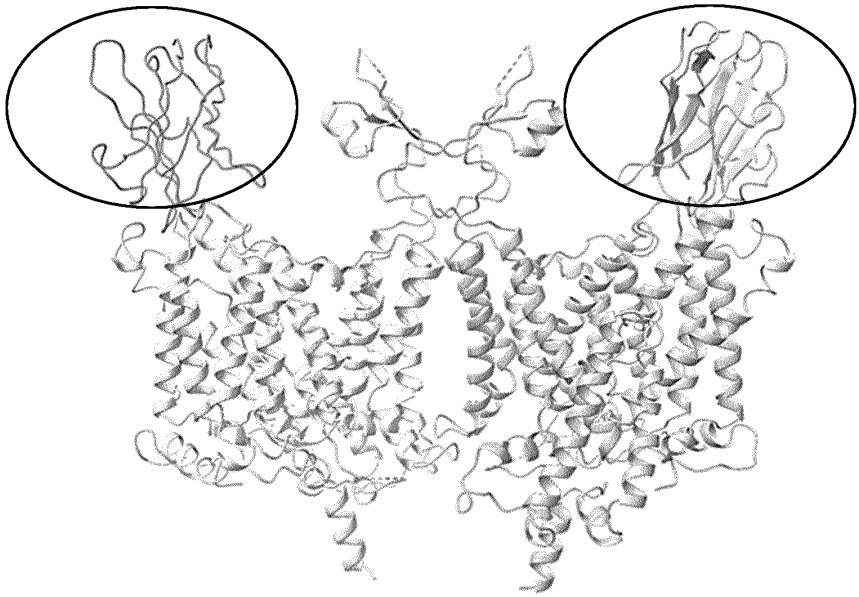
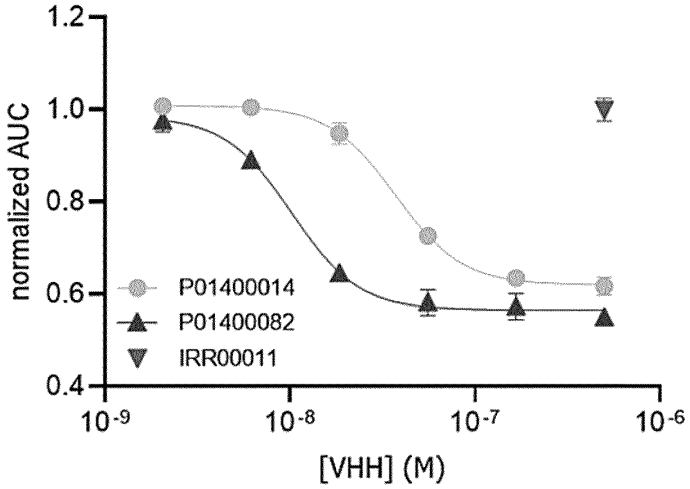
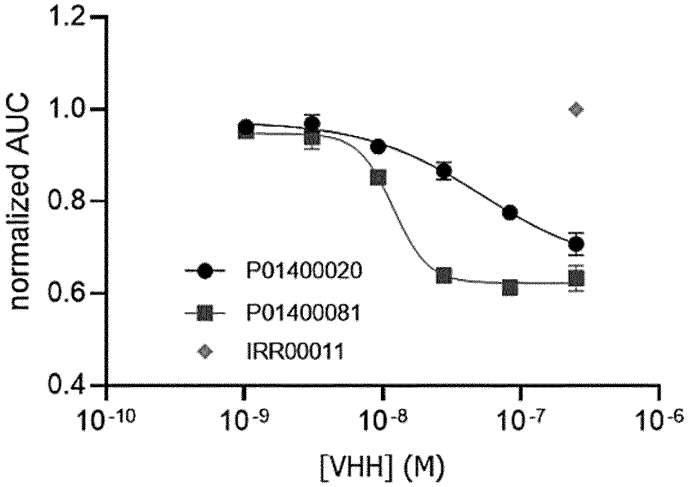


FIGURE 9

A



B



C

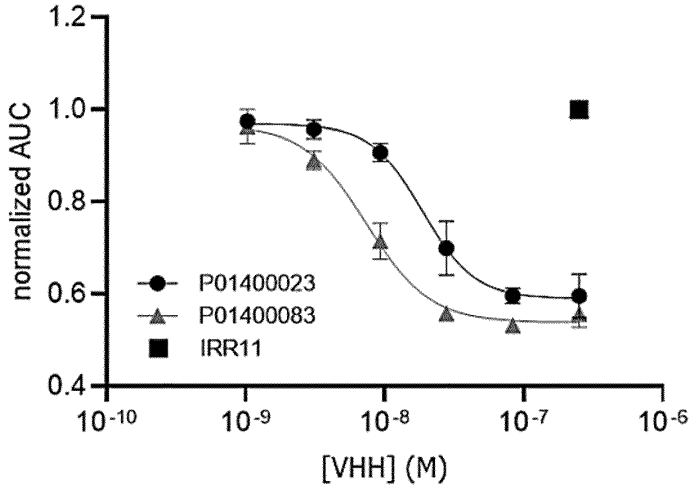
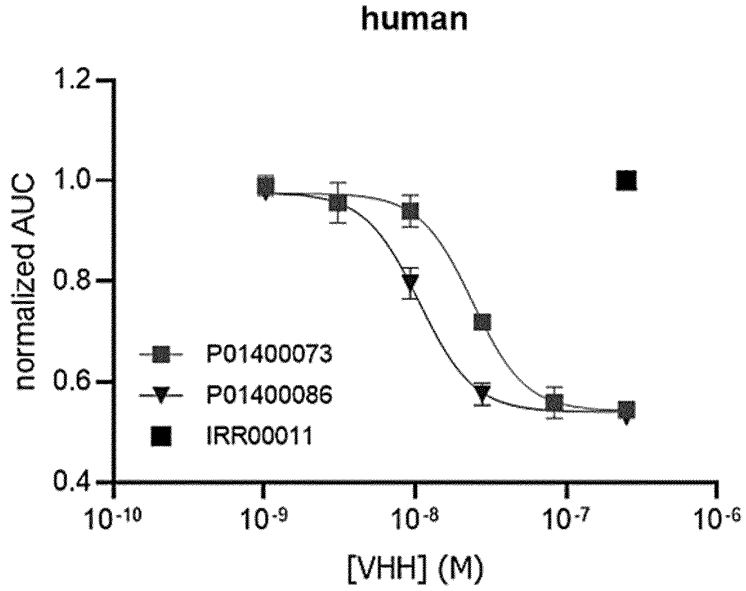


FIGURE 10

A


human
normalized AUC
1.2
1.0
0.8
0.6
0.4
P01400073
P01400086
IRR00011
10-10
10-9
10-8
10-7
10-6
[VHH] (M)

B

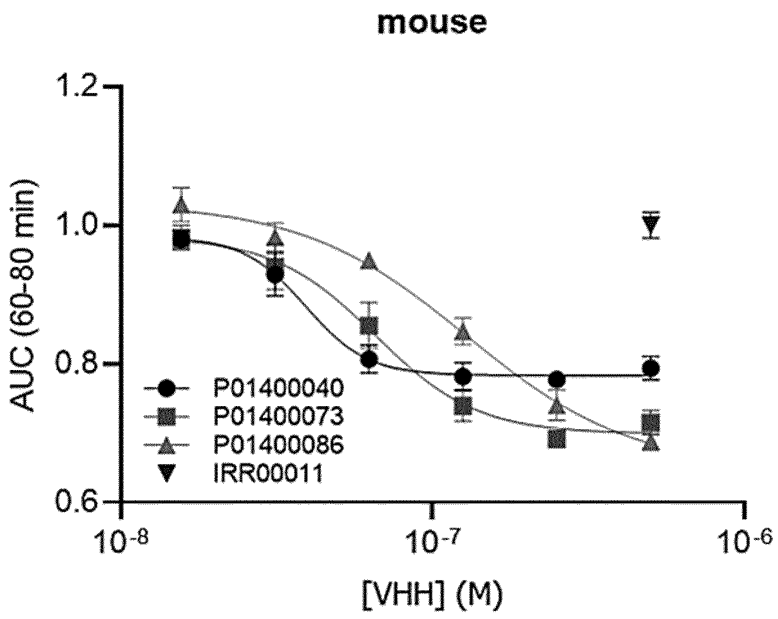

mouse
AUC (60-80 min)
1.2
1.0
0.8
0.6
P01400040
P01400073
P01400086
IRR00011
10-8
10-7
10-6
[VHH] (M)

FIGURE 11

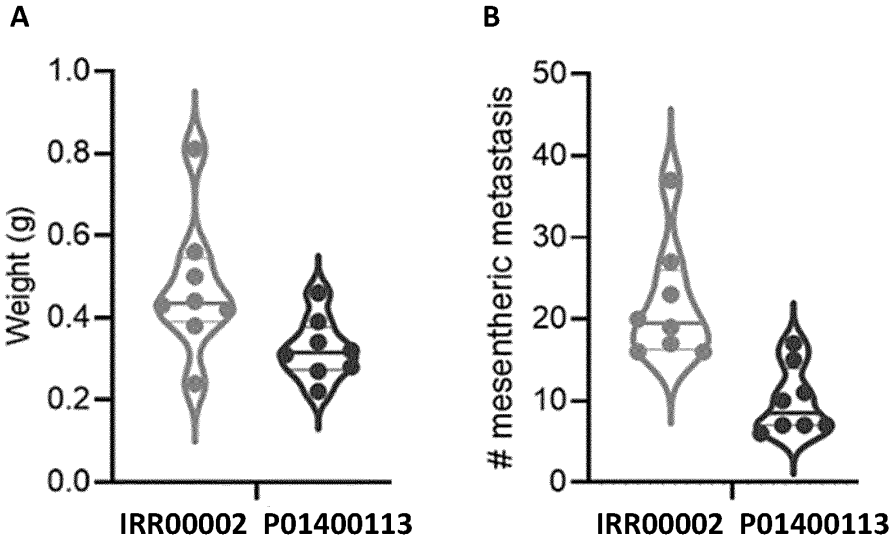

A
1.0
0.8
0.6
0.4
0.2
0.0
Weight (g)
IRR00002
P01400113
B
50
40
30
20
10
0
mesentheric metastasis
IRR00002
P01400113

FIGURE 12

```
                FR1                 CDR1      FR2          CDR2

41 EVQLVESGGGLVQSGGSLRLSCVPSGRTSS IQTMA WFRQAPGKTREFVA GIRWSVGSTAY 60
40 EVQLVESGGGLVQAGGSLRLSCTTSGRTSR IQTMA WFRQAPGKLREFVA GIRWSVGSTAY 60
24 EVQLVESGGGLVQPGGSLRLSCATSGRTST IQTMA WFRQAPGKLREFVA AIRWSVGSTAY 60
46 EVQLVESGGGLVQAGGSLRLSCVASGRTSS IQTMA WFRQAPGKSREFVA AIRWSVGSAAY 60
21 EVQLVESGGGLVQPGGSLRLSCAASTATVS TYTMG WFRQAPGKEREFVA GIMRNADTTLY 60
20 EVQLVESGGGLVQPGGSLRLSCAASTATFS DYTVG WFRQAPGKEREFVA GIMRRAETTIY 60
12 EVQLVESGGGLVQPGGSLRLSCAASIATFS DYTVA WFRQAPGKEREFVA GIMRRAETTIY 60
14 EVQLVESGGGLVQPGGSLRLSCATSGRTFS GYAIG WFRQAPGKEREFVA AISRSGTRTVY 60
23 EVQLVESGGGLVQPGGSLRLSCVASGRTFT GYAMG WFRQAPGKEREFVA AVSRSGTRVIY 60
   ************* ********. *  *    ::. ******** ***** .:      . *

   CDR2              FR3                        CDR3        FR4

41 HNSVK GRFTISRDSYQTTLYLQMNNLKPEDTAVYYCAA DRDLSSSDSTTSQTTYDY WGRG 120
40 LNSVK GRFTISRDPLATTLYLQMNNLKSEDTAVYYCAA TTKLTSSDSTTLQDTYDY WGRG 120
24 LNSVK GRFTISRDPLATTLYLQMNSLRPEDTAVYYCAA DRTFSDSDSVTLQATYDY WGQG 120
46 LNSVK GRFTISRDYLKSTLYLQMNNLQPEDTAVYYCAA DRRVSSSDSTTLQATYDY WGQG 120
21 GDSVK GRFTISRDNAKNTVYLQMNSLRPEDTAVYYCAA RPS--M-RIQTTVRQYDS WGQG 117
20 GDSVK GRFTISRDNAKNTVYLQMNSLRPEDTAVYYCAA RPS--M-RIQTTARQYDS WGQG 117
12 GDSVK GRFTVSRGNARNTVYLQMNSLRPEDTAVYYCAA RPS--M-RIQTTARQYDS WGQG 117
14 ADSVK GRFTIARDSAKSTTYLQMNSLRPEDTAVYYCAA TTGSTN----SPITDYAY WGQG 116
23 ADSVK GRFTISKDNAQGTVYLQMNSLRPEDTAVYYCAA DHKPST-AWVSARGDMDF WGQG 119
    :*** ****:::.    * *****.*: **********           :        **:*

   FR4

41 TLVTVSS     127 (SEQ ID NO:21)
40 TLVTVSS     127 (SEQ ID NO:20)
24 TLVTVSS     127 (SEQ ID NO:19)
46 TLVTVSS     127 (SEQ ID NO:22)
21 TLVTVSS     124 (SEQ ID NO:18)
20 TLVTVSS     124 (SEQ ID NO:17)
12 TLVTVSS     124 (SEQ ID NO:16)
14 TLVTVSS     123 (SEQ ID NO:15)
23 TLVTVSS     126 (SEQ ID NO:14)
   *******
```

FIGURE 13

VHH	CDR1 Kabat	CDR2 Kabat	CDR3 Kabat
P01400014	GYAIG (SEQ ID NO:30)	AISRSGTRTVYADSVK (SEQ ID NO:43)	TTGSTNSPITDYAY (SEQ ID NO:54)
P01400020	DYTVG (SEQ ID NO:27)	GIMRRAETTIYGDSVK (SEQ ID NO:41)	RPSMRIQTTARQYDS (SEQ ID NO:53)
P01400023	GYAMG (SEQ ID NO:31)	AVSRSGTRVIYADSVK (SEQ ID NO:44)	DHKPSTAWVSARGDMDF (SEQ ID NO:55)
P01400040	IQTMA (SEQ ID NO:23)	GIRWSVGSTAYLNSVK (SEQ ID NO:36)	TTKLTSSDSTTLQDTYDY (SEQ ID NO:48)

VHH	CDR1 IMGT	CDR2 IMGT	CDR3 IMGT
P01400014	GRTFSGYA (SEQ ID NO:90)	ISRSGTRT (SEQ ID NO:92)	AATTGSTNSPITDYAY (SEQ ID NO:94)
P01400020	TATFSDYT (SEQ ID NO:96)	IMRRAETT (SEQ ID NO:98)	AARPSMRIQTTARQYDS (SEQ ID NO:100)
P01400023	GRTFTGYA (SEQ ID NO:102)	VSRSGTRV (SEQ ID NO:104)	AADHKPSTAWVSARGDMDF (SEQ ID NO:106)
P01400040	GRTSRIQT (SEQ ID NO:108)	IRWSVGST (SEQ ID NO:110)	AATTKLTSSDSTTLQDTYDY (SEQ ID NO:112)

VHH	CDR1 AbM	CDR2 AbM	CDR3 AbM
P01400014	GRTFSGYAIG (SEQ ID NO:91)	AISRSGTRTV (SEQ ID NO:93)	TTGSTNSPITDYAY (SEQ ID NO:95)
P01400020	TATFSDYTVG (SEQ ID NO:97)	GIMRRAETTI (SEQ ID NO:99)	RPSMRIQTTARQYDS (SEQ ID NO:101)
P01400023	GRTFTGYAMG (SEQ ID NO:103)	AVSRSGTRVI (SEQ ID NO:105)	DHKPSTAWVSARGDMDF (SEQ ID NO:107)
P01400040	GRTSRIQTMA (SEQ ID NO:109)	GIRWSVGSTA (SEQ ID NO:111)	TTKLTSSDSTTLQDTYDY (SEQ ID NO:113)

FIGURE 14

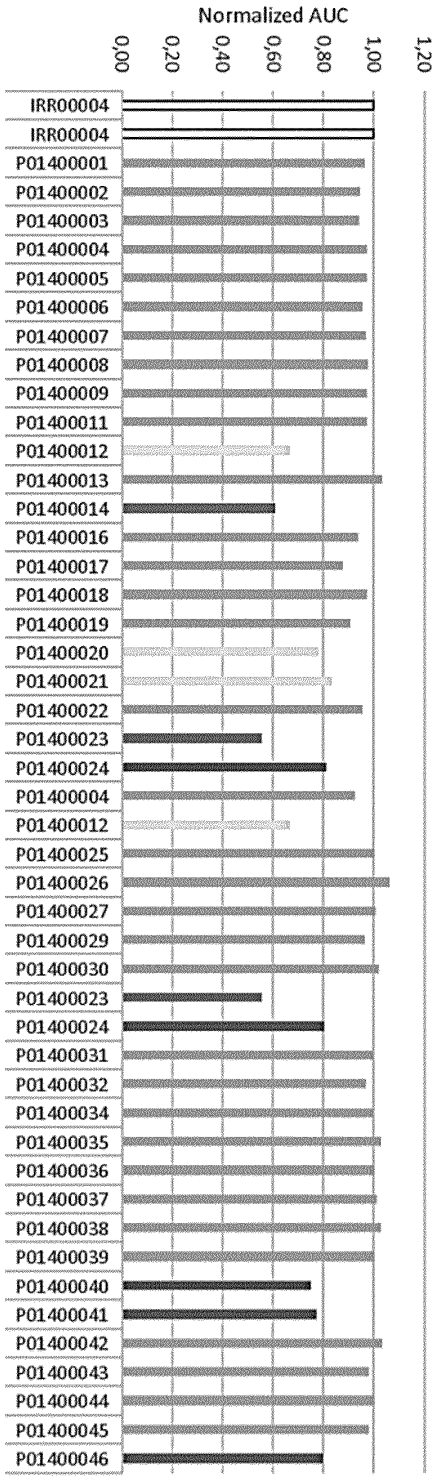

Normalized AUC
0,00
0,20
0,40
0,60
0,80
1,00
1,20
IRR00004
IRR00004
P01400001
P01400002
P01400003
P01400004
P01400005
P01400006
P01400007
P01400008
P01400009
P01400011
P01400012
P01400013
P01400014
P01400016
P01400017
P01400018
P01400019
P01400020
P01400021
P01400022
P01400023
P01400024
P01400004
P01400012
P01400025
P01400026
P01400027
P01400029
P01400030
P01400023
P01400024
P01400031
P01400032
P01400034
P01400035
P01400036
P01400037
P01400038
P01400039
P01400040
P01400041
P01400042
P01400043
P01400044
P01400045
P01400046

FIGURE 15

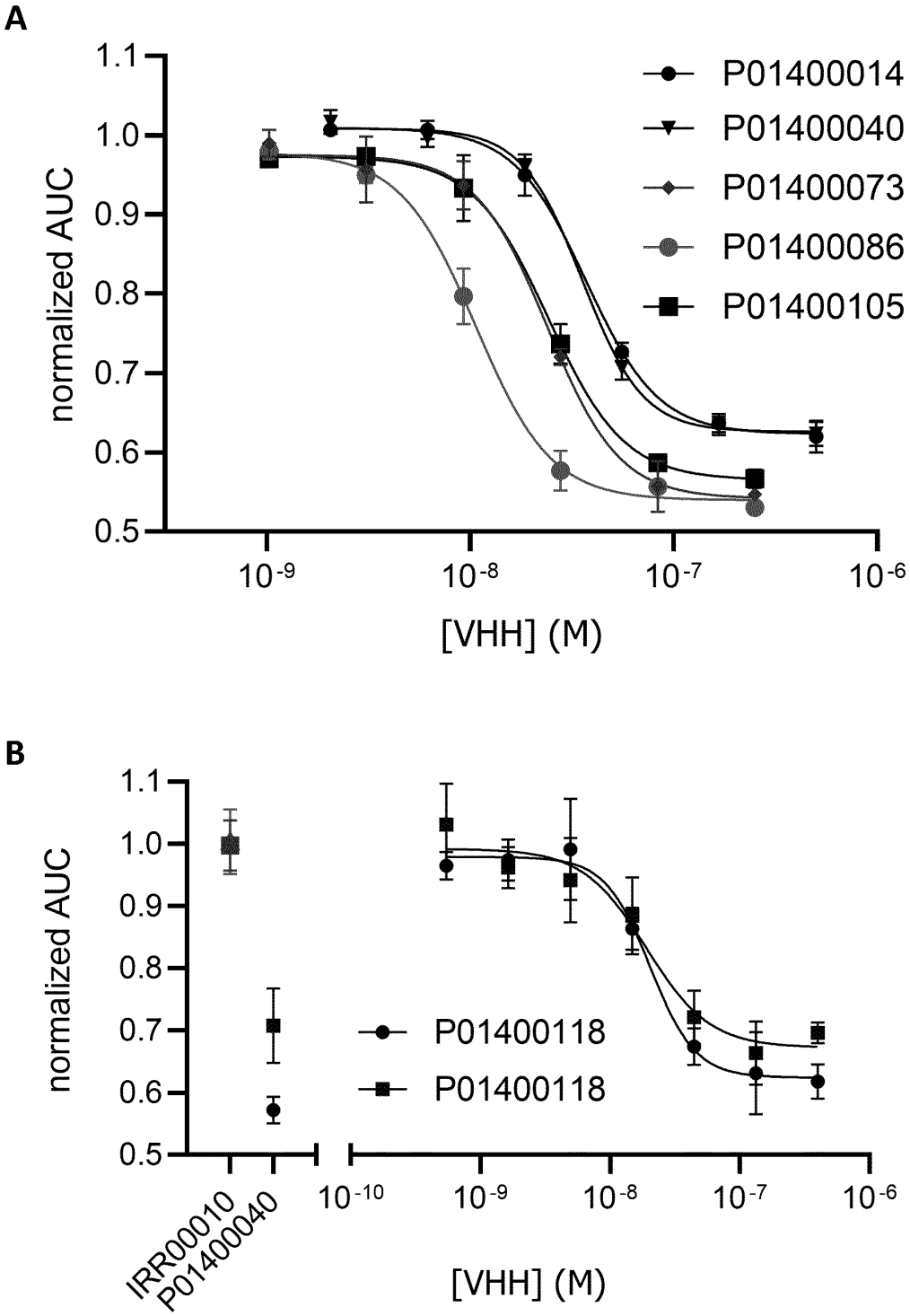

A
1.1
1.0
0.9
0.8
0.7
0.6
0.5
normalized AUC
P01400014
P01400040
P01400073
P01400086
P01400105
10-9
10-8
10-7
10-6
[VHH] (M)
B
1.1
1.0
0.9
0.8
0.7
0.6
0.5
normalized AUC
P01400118
P01400118
IRR00010
P01400040
10-10
10-9
10-8
10-7
10-6
[VHH] (M)

FIGURE 16

C

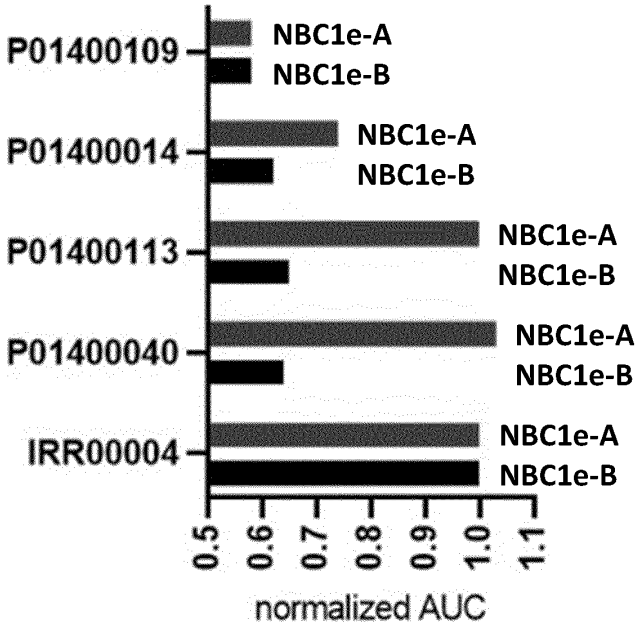


FIGURE 16 – continued

A

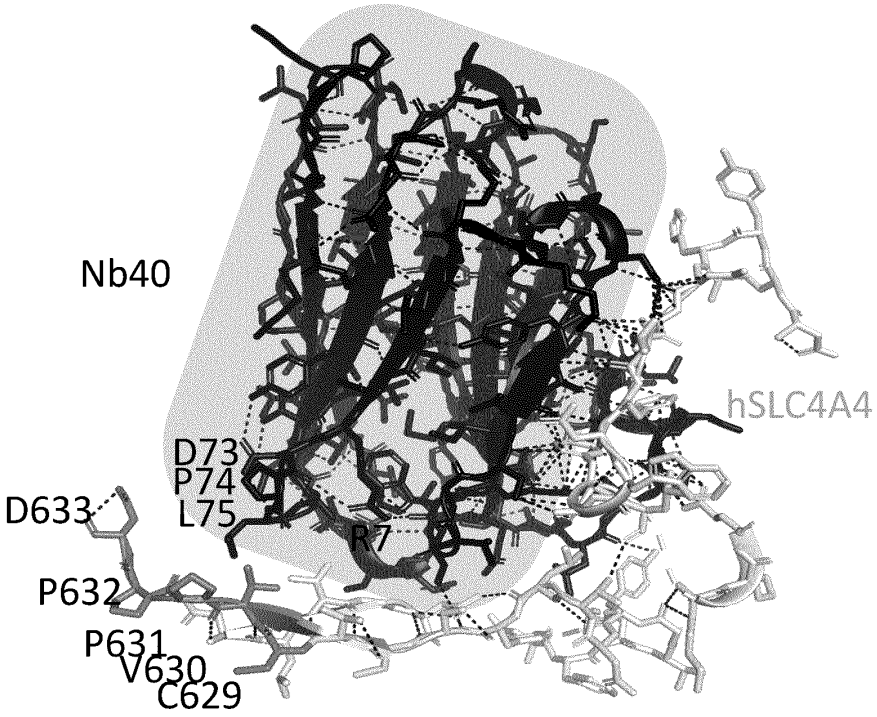


FIGURE 17

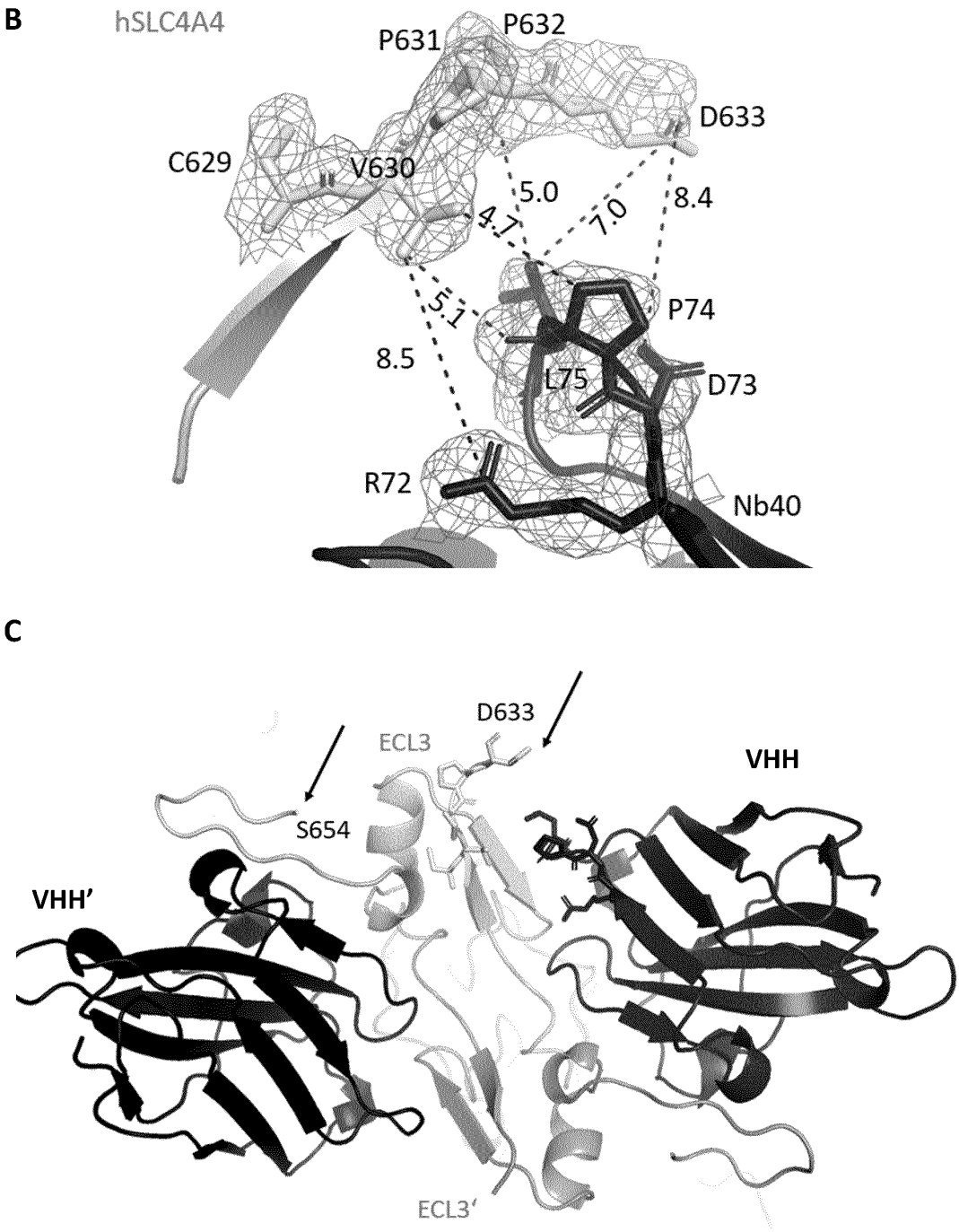
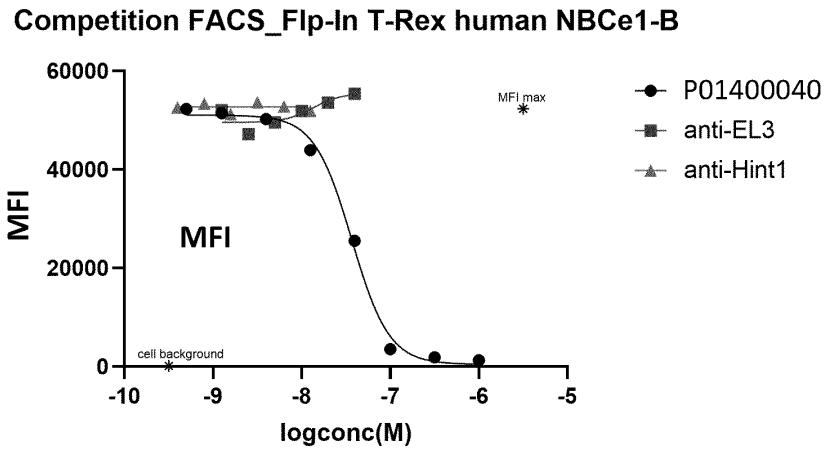
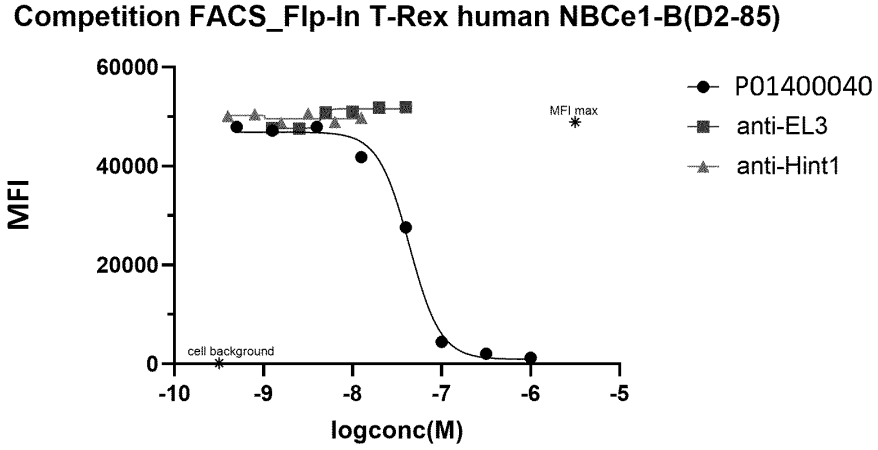


FIGURE 17 – continued


Competition FACS_Flp-In T-Rex human NBCe1-B
60000
40000
20000
0
MFI
MFI max
MFI
cell background
-10
-9
-8
-7
-6
-5
logconc(M)
P01400040
anti-EL3
anti-Hint1


Competition FACS_Flp-In T-Rex human NBCe1-B(D2-85)
60000
40000
20000
0
MFI
MFI max
cell background
-10
-9
-8
-7
-6
-5
logconc(M)
P01400040
anti-EL3
anti-Hint1

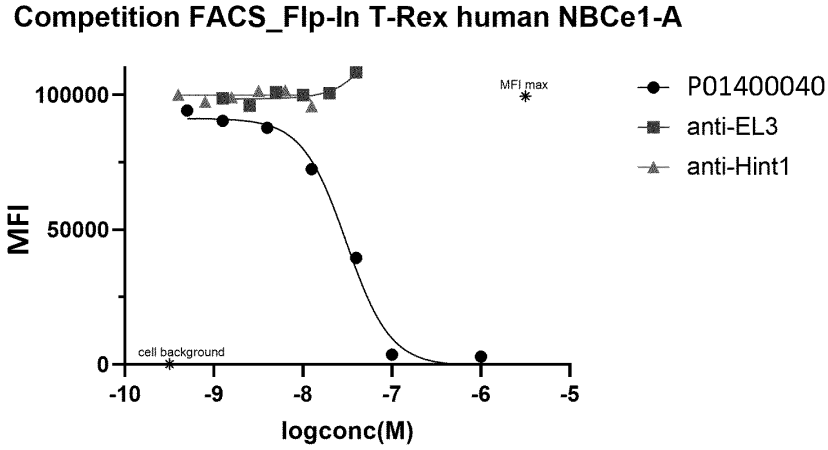

Competition FACS_Flp-In T-Rex human NBCe1-A
100000
50000
0
MFI
MFI max
cell background
-10
-9
-8
-7
-6
-5
logconc(M)
P01400040
anti-EL3
anti-Hint1

FIGURE 18

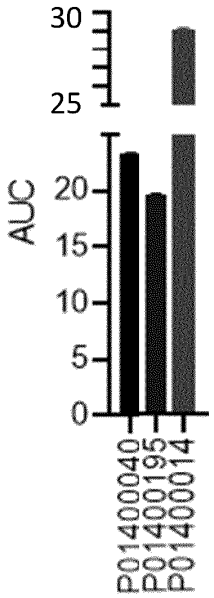


FIGURE 19

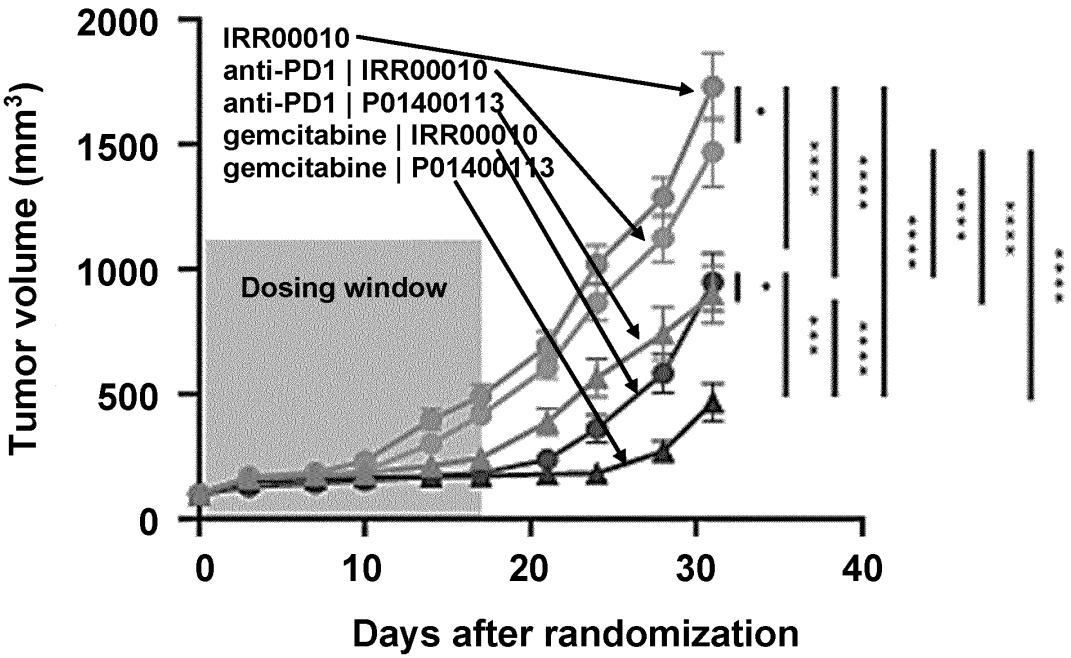


FIGURE 20

A

```
IMGT#              911       20        3035   40         50       5962       70
                   ||        |         ||     |          |        ||         |
huIGHV3/JH EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVSVISSDGSSTYYADS
P01400040  ............A........TT..R.SRIQT.A.F......LR.F.AG.RWSVG..A.LN.
P01400119  ......................TT..R.SRIQT.A.F......LR.F.AG.RWSVG..A.LN.

P01400024  ......................T..R.STIQT.A.F......LR.F.AA.RWSVG..A.LN.
P01400046  ............A........V...R.S.IQT.A.F......SR.F.AA.RWSVG.AA.LN.
P01400041  ............S........VP..R.S.IQT.A.F......TR.F.AG.RWSVG..A.HN.

IMGT#              80        90        100  105  111.3112.4    117120
                   |         |         |    |        ||        |   |
huIGHV3/JH VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR-----------------WGQGTLVTVSS
P01400040  ..........PLAT.......N.KS.........ATTKLTSSDSTTLQDTYDY..R........
P01400119  ..........PLAT.........P.........ATTKLTSSDSTTLQDTYDY..R........

P01400024  ..........PLAT.........P.........ADRTFSDSDSVTLQATYDY...........
P01400046  ..........YL.S.......N.QP.........ADRRVSSSDSTTLQATYDY...........
P01400041  ..........SYQT.......N.KP.........ADRDLSSSDSTTSQTTYDY..R........

huIGHV3/JH (SEQ ID NO:148)
P01400040  (SEQ ID NO:20)
P01400119  (SEQ ID NO:138)
P01400024  (SEQ ID NO:19)
P01400046  (SEQ ID NO:22)
P01400041  (SEQ ID NO:21)
```

B

```
IMGT#              911       20        3035   40         50       5962       70
                   ||        |         ||     |          |        ||         |
huIGHV3/JH EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVSVISSDGSSTYYADS
P01400014  ......................T..R...G..IG.F......ER.F.AA..RS.TR.V....
P01400131  ......................T..R...G..IG.F......ER.F.AA..RS.TR.V....

IMGT#              80        90        100  105111.1112.2  117120
                   |         |         |    |      ||      |   |
huIGHV3/JH VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAR-------------WGQGTLVTVSS
P01400014  .......A..SA.S.T........P.........ATTGSTNSPITDYAY...........
P01400131  ..........SA.S.T........P.........ATTGSTNSPITDYAY...........

huIGHV3/JH (SEQ ID NO:148)
P01400014  (SEQ ID NO:15)
P01400131  (SEQ ID NO:139)
```

FIGURE 21

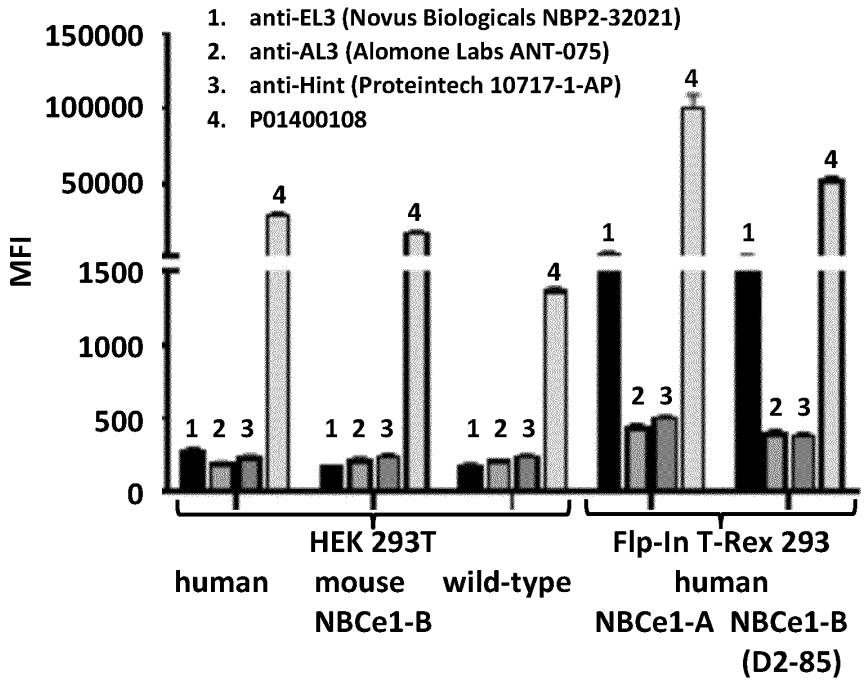

1. anti-EL3 (Novus Biologicals NBP2-32021)
2. anti-AL3 (Alomone Labs ANT-075)
3. anti-Hint (Proteintech 10717-1-AP)
4. P01400108
MFI
150000
100000
50000
1500
1000
500
0
HEK 293T
human
mouse
NBCe1-B
wild-type
Flp-In T-Rex 293
human
NBCe1-A
NBCe1-B
(D2-85)

FIGURE 22