R175H

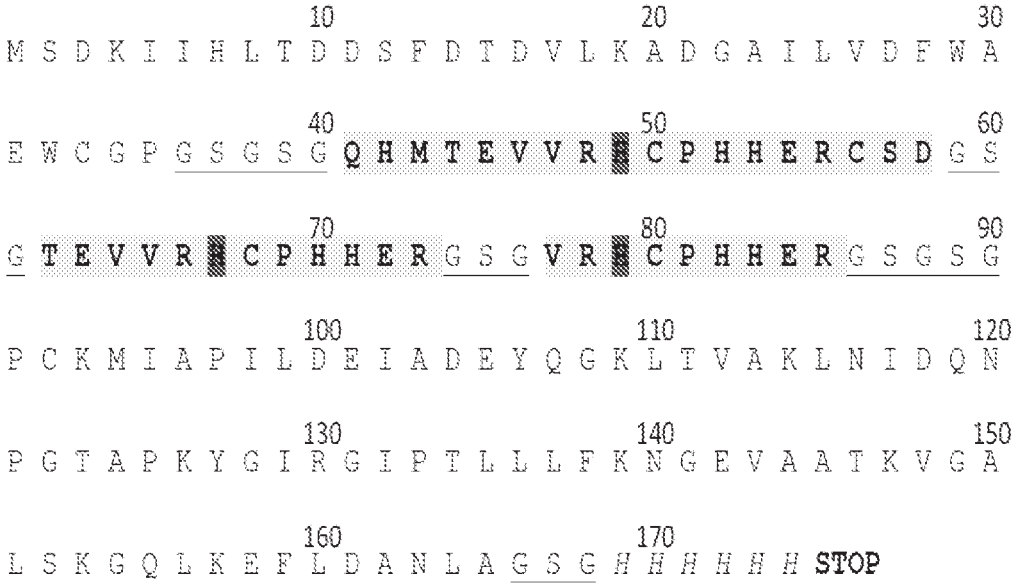


Figure 1A

R248Q

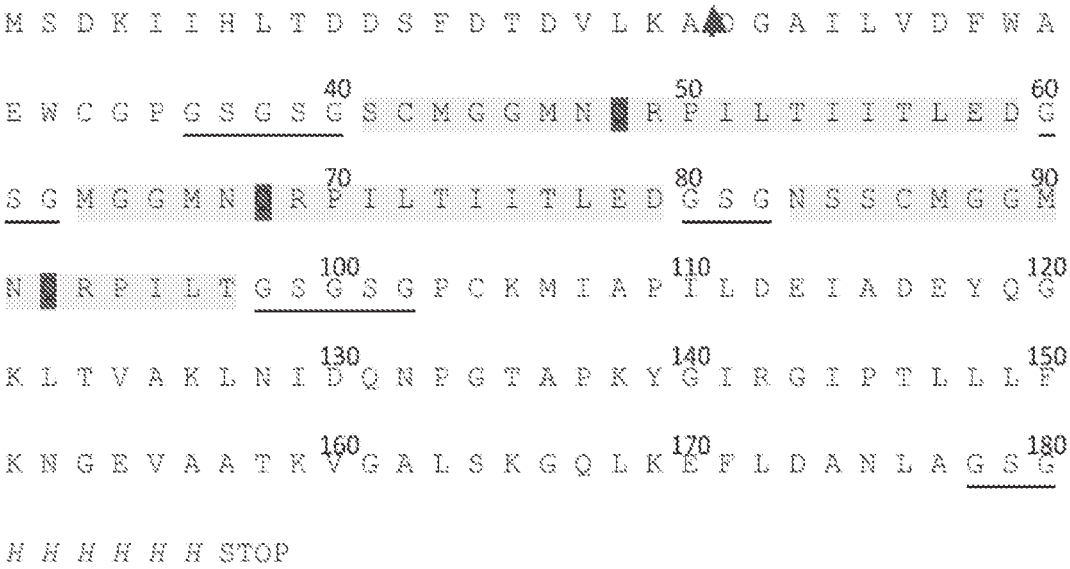


Figure 1B

R273H

```
                    10                  20                  30
M S D K I I H L T D D S F D T D V L K A D G A I L V D F W A
                    40                  50                  60
E W C G P G S G S G R N S F E V H V C A G S G N L L G R N S
                    70                  80                  90
F E V H V C A C G S G G R N S F E V H V C A C P G S G S G P
                   100                 110                 120
C K M I A P I L D E I A D E Y Q G K L T V A K L N I D Q N P
                   130                 140                 150
G T A P K Y G I R G I P T L L L F K N G E V A A T K V G A L
                   160                 170
S K G Q L K E F L D A N L A G S G H H H H H H STOP
```

Figure 1C

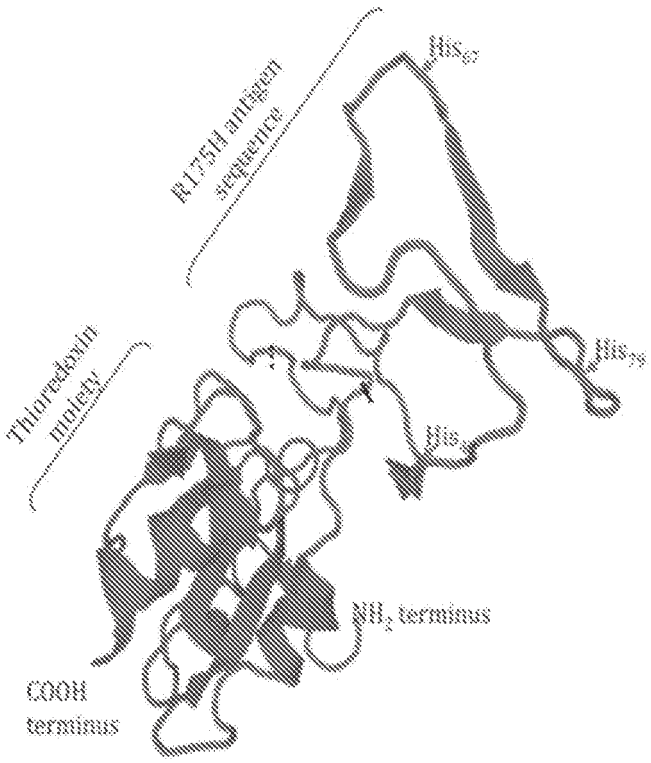


Figure 2A

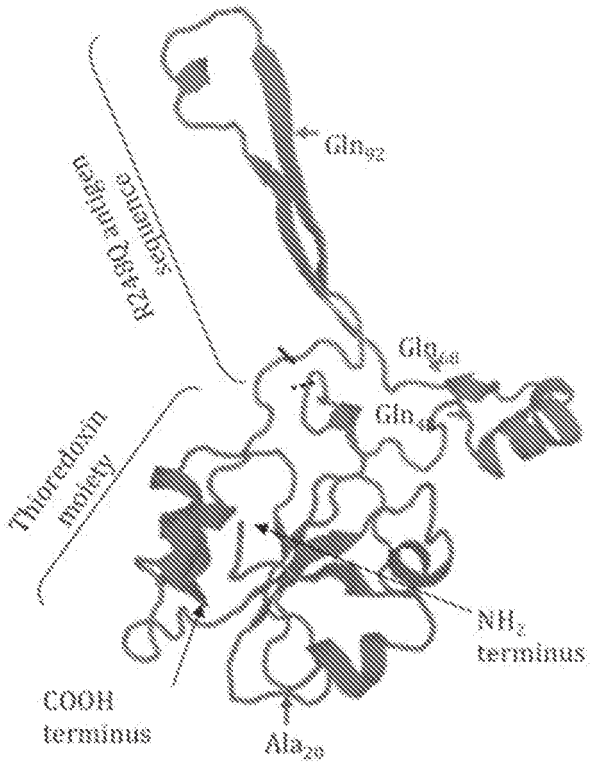


Figure 2B

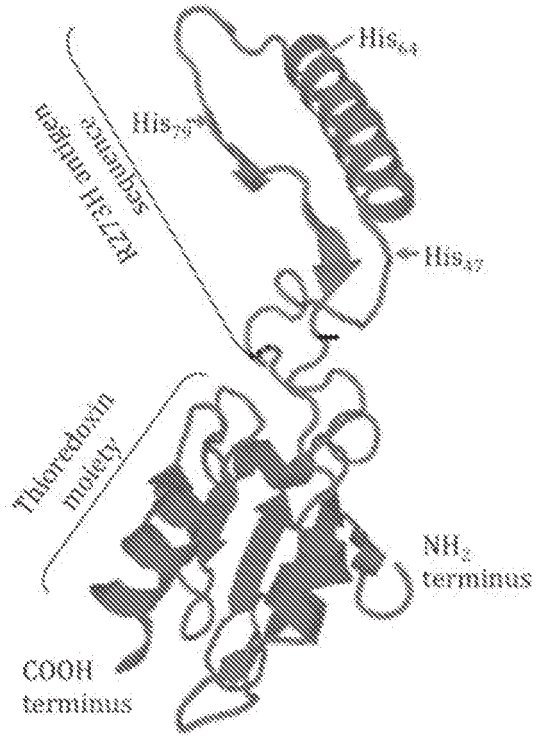


Figure 2C

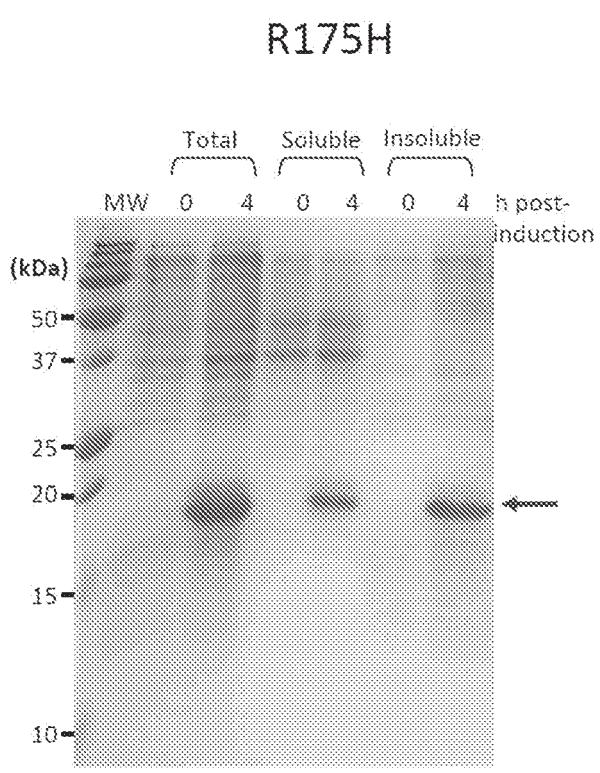


Figure 3A

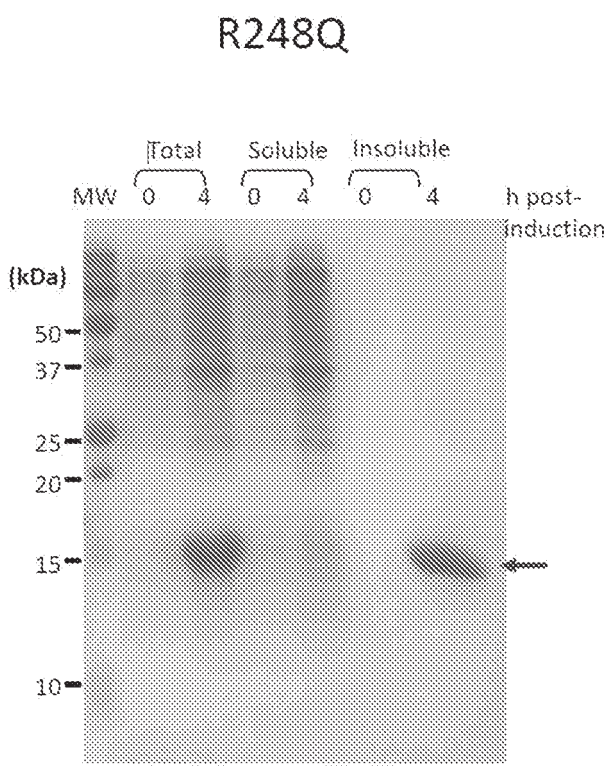


Figure 3B

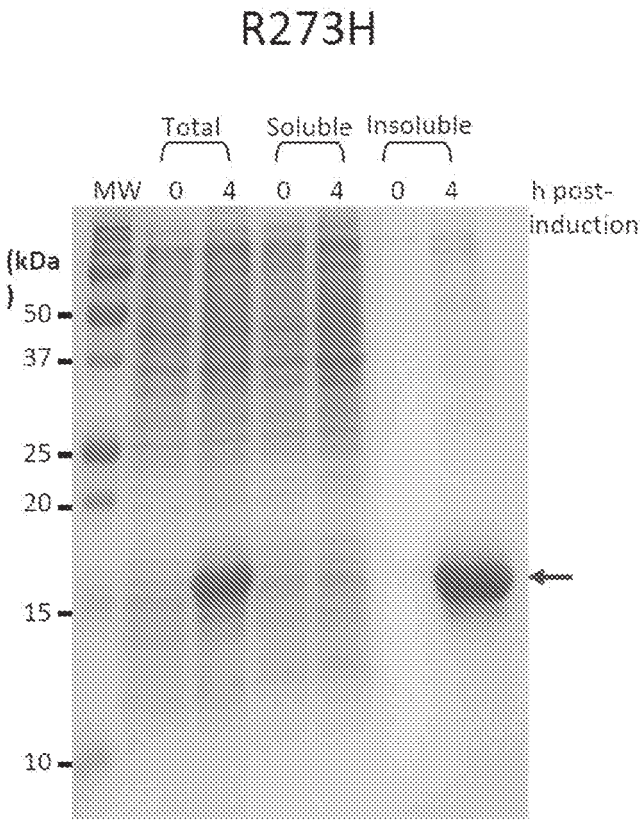

R273H
Total
Soluble
Insoluble
MW
0
4
0
4
0
4
h post-induction
(kDa)
50
37
25
20
15
10

Figure 3C

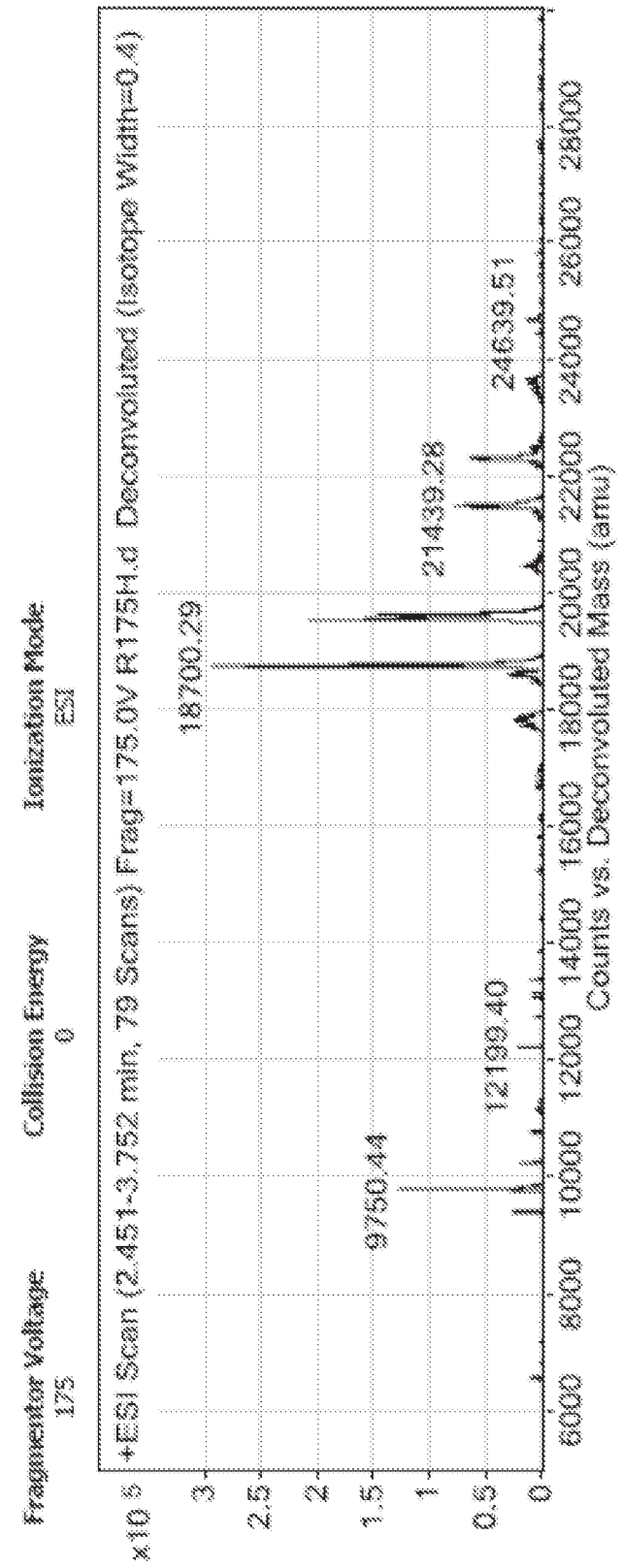

R175H'
Fragmentor Voltage
175
Collision Energy
0
Ionization Mode
ESI
+ESI Scan (2.451-3.752 min, 79 Scans) Frag=175.0V R175H.d Deconvoluted (Isotope Width=0.4)
x10 5
18700.29
21439.28
24639.51
9750.44
12199.40
Counts vs. Deconvoluted Mass (amu)

Figure 4A

R248Q

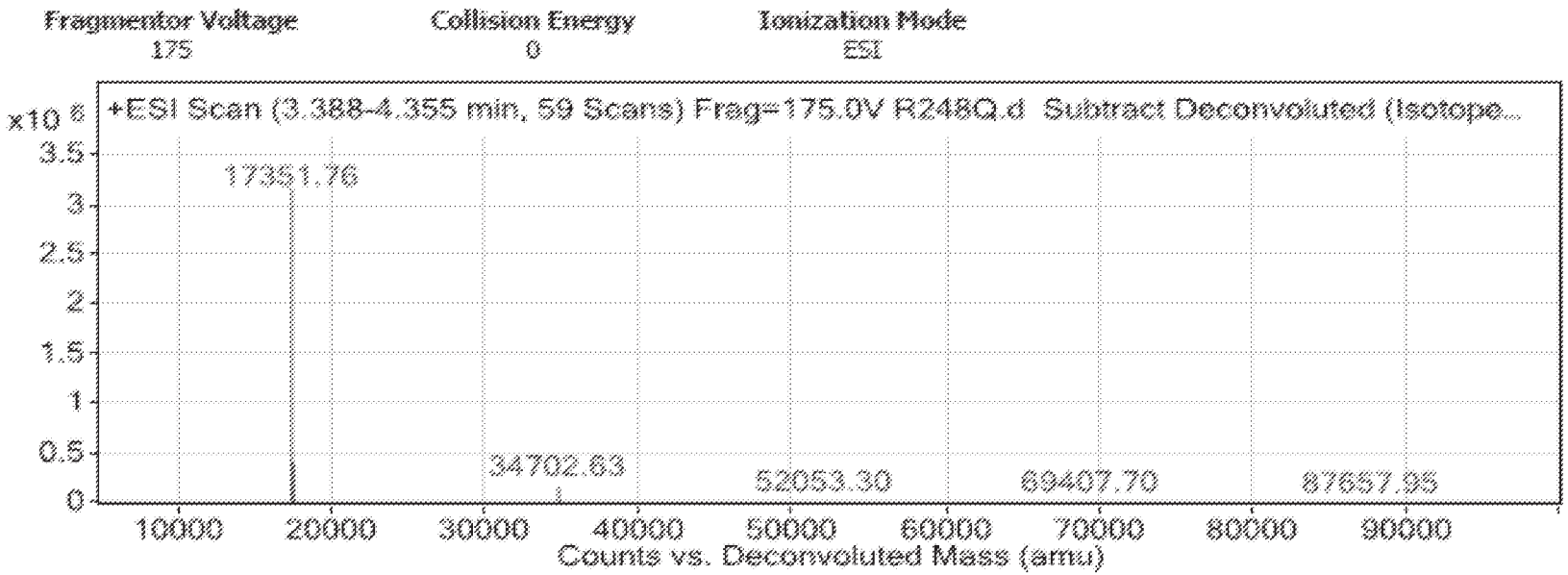


Figure 4B

α-R175H

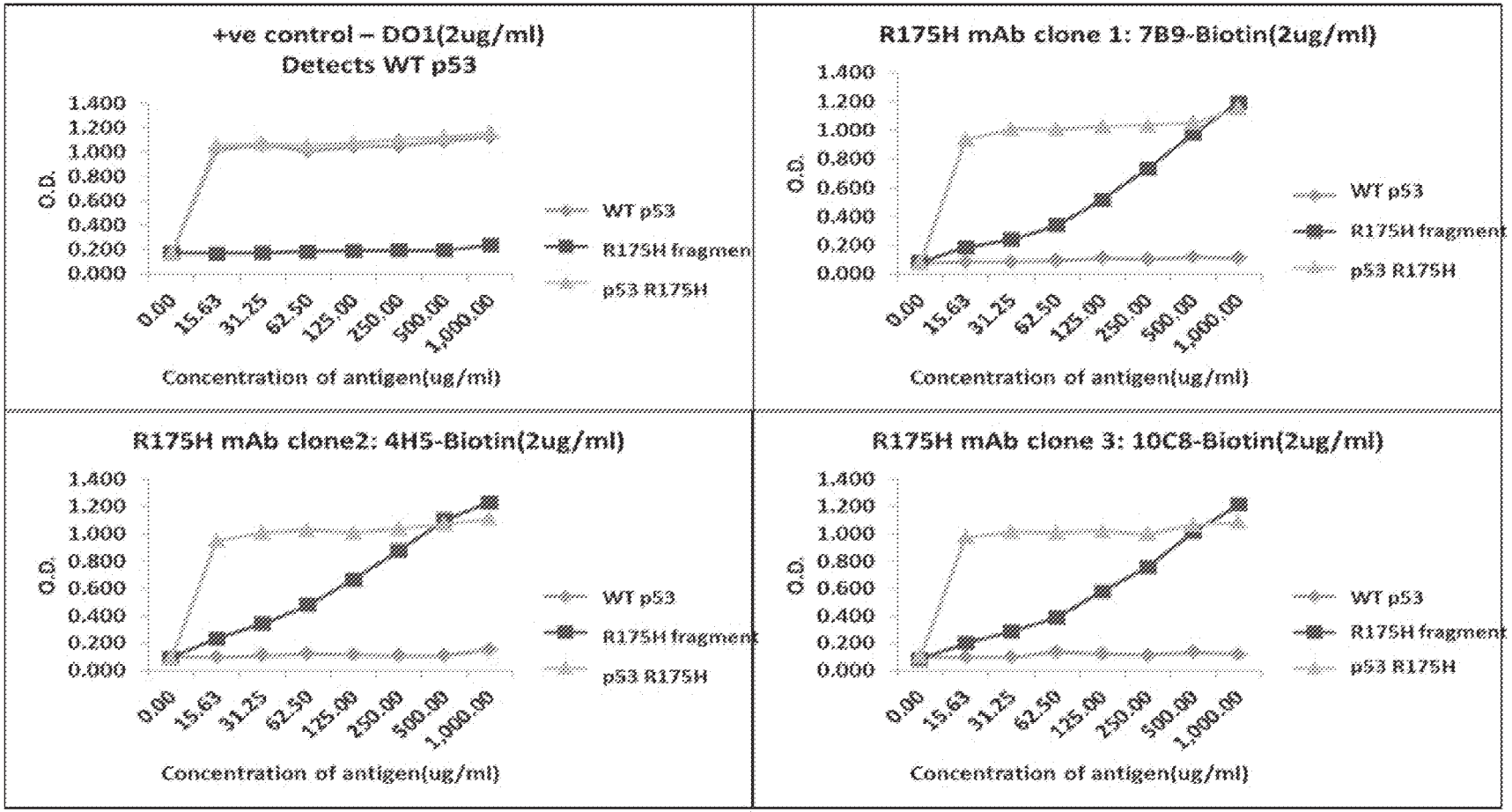


Figure 5A

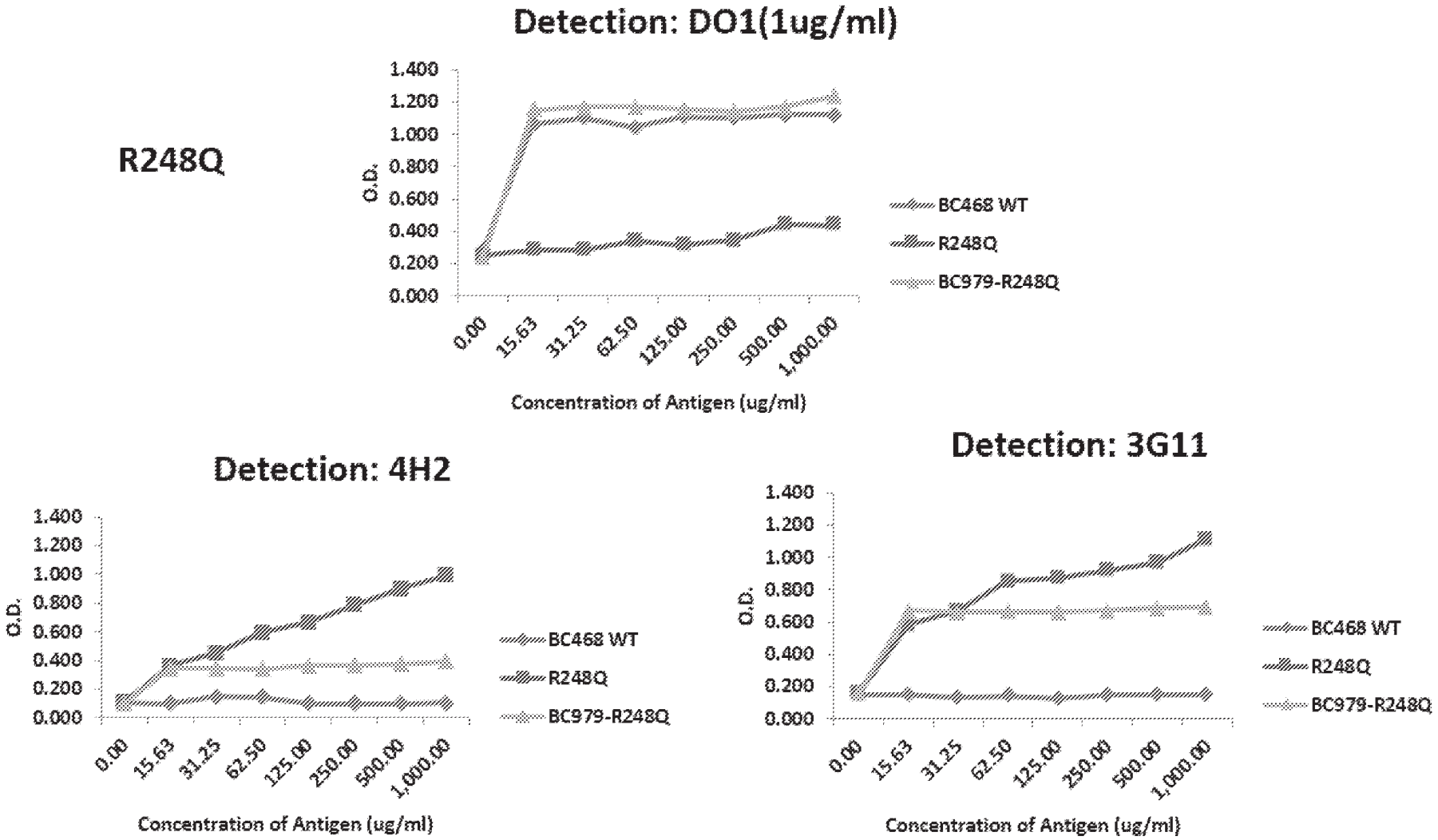
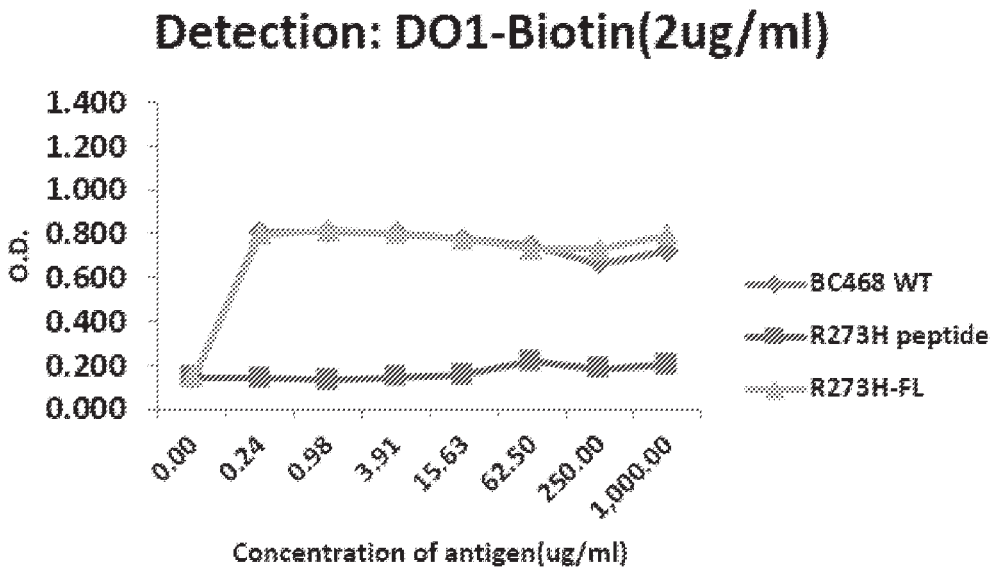

Detection: DO1(1ug/ml)
R248Q
O.D.
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0.00
15.63
31.25
62.50
125.00
250.00
500.00
1,000.00
Concentration of Antigen (ug/ml)
BC468 WT
R248Q
BC979-R248Q
Detection: 4H2
Detection: 3G11

Figure 5B

R273H



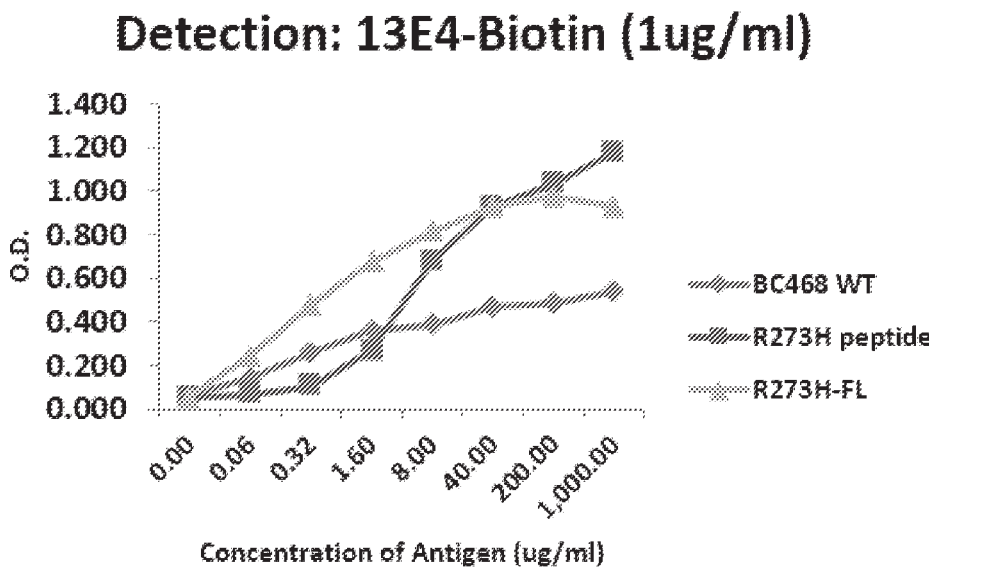


Figure 5C

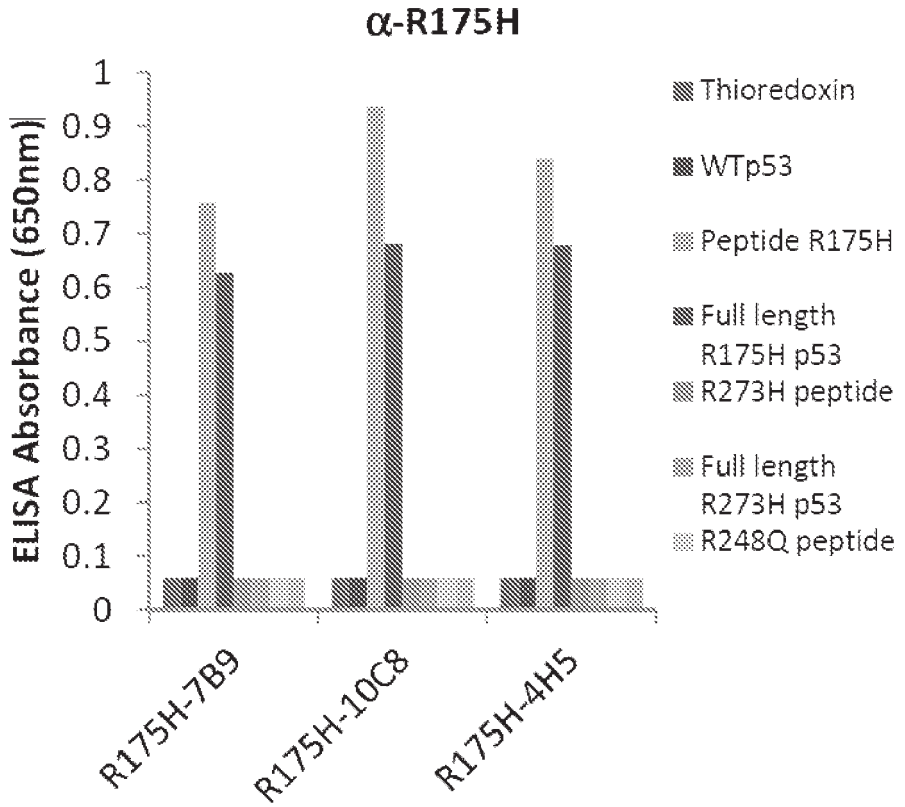


Figure 6A

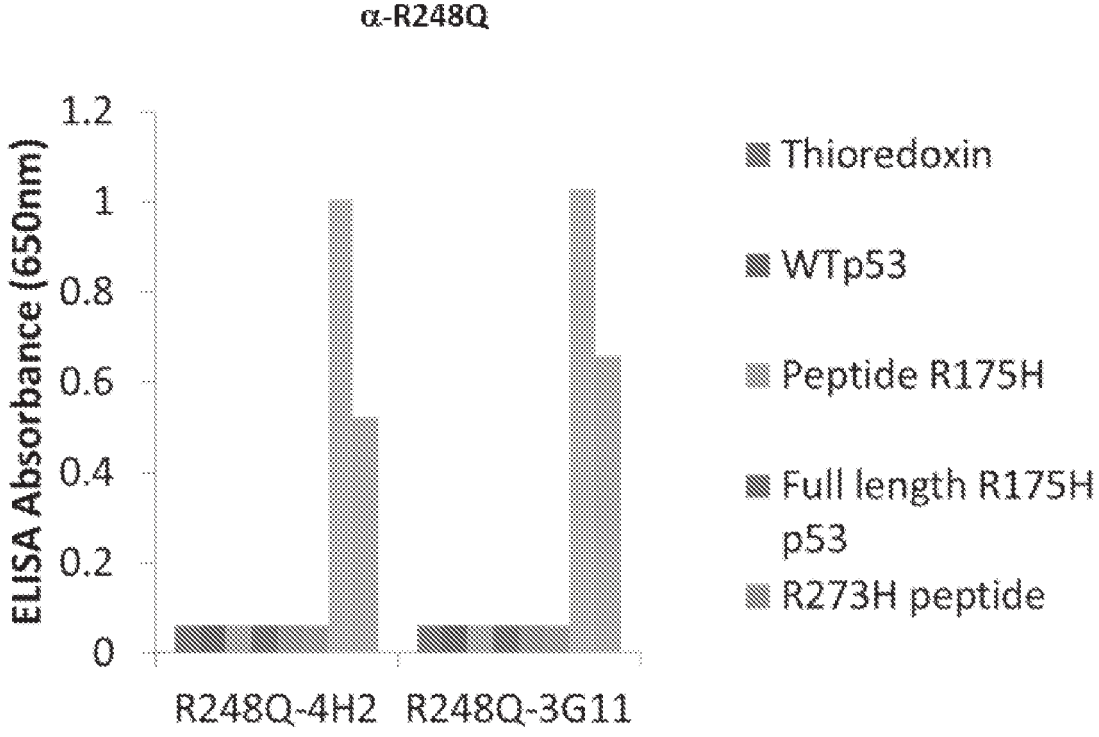


Figure 6B

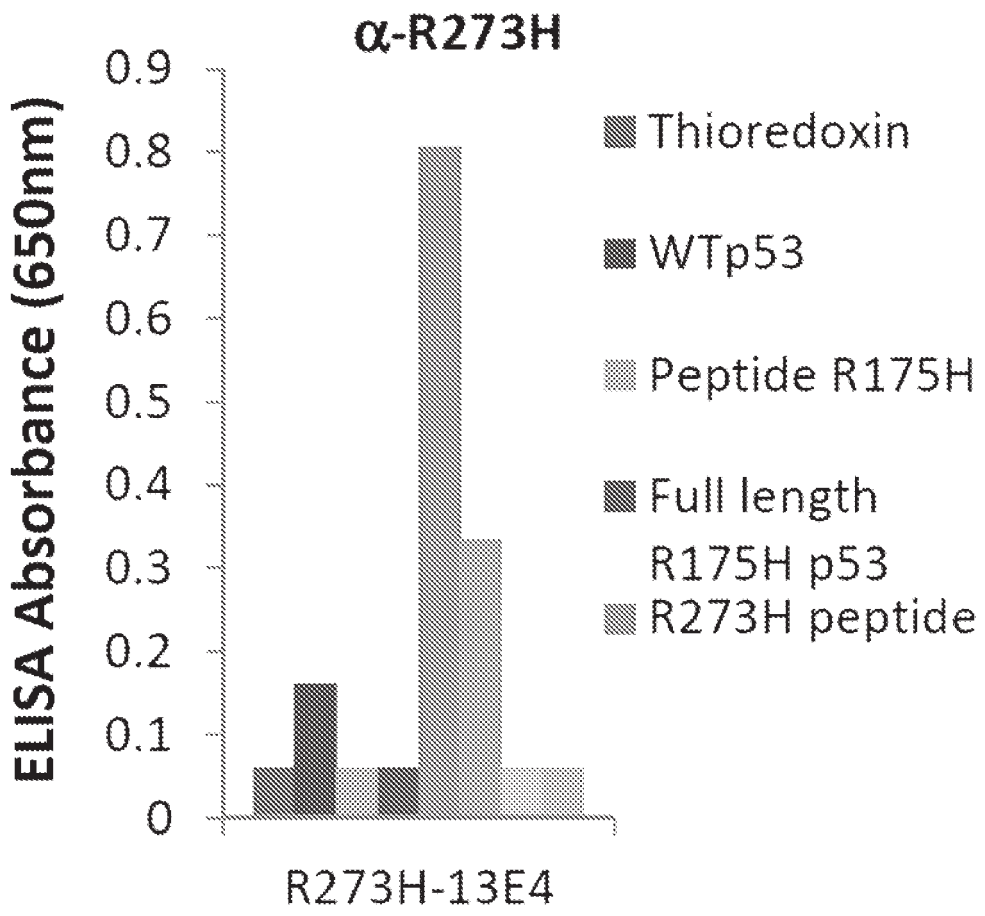


Figure 6C

Anti-R175H mAbs

10C8

WTp53	QHMTEVVRRCPHHERCSD
R175H	QHMTEVVRHCPHHERCSD
6	----GGVLHLPHHTRN--
3	-----ADNHEPHPLACD-
4	-------T-RHQPHHSLPPR
8	--------T-PHQPHHSFTPR
7	---VSTGVHYPHHSA-----
2	---VDMHHYPHHYKD---
5	-------ILASHRPHHYGY-
1	---LCTPLLHPIHHL---

Consensus: **167**QHMTEVVRHCPHHERCSD**184**

10C8, 7B9, 4H5 epitope

Figure 7A

Anti-R248Q mAbs

4H2

```
WTp53          GMNRRPILTIITLEDS
R248Q          GMNQRPILTIITLEDS
1        ------ELWQNVRPFLWV----------
2        ----------SRPADHWPRHNE----
3        ----------SSRPMAHWPIKV------
4        ----------VSSRPSSHWPI----------
5        ---SNTPHWPGRPGI----------
6        ----------INTPRDWAYGGL---------
7        ----------FNQPPRSGRVI----------
```

Consensus: **245** GMNQ RP ILTIITLEDS **260**
4H2 epitope

Figure 7B

Anti-R273H mAb

13E4

```
WTp53    ----GRNSFEVRVCAC---------
R273H    ----GRNSFEVHVCAC---------
4        -----GK-TYSYHMQLYT-------
3        ---------- YHVGGILGRLFM
5        ------YNTFPYHLGYTL---------
2        ---DNASSSKVHHGH----------
1        ---------- TFVHGNTWTIVN-
6        ---TNTMHAVHHPL----------
```

Consensus: **267**RNSFEVHVCACPGR**280**
13E4 epitope

Figure 7C

Anti-R175H mAbs

7B9

WTp53	QHMTEVVRRCPHHERCSD
R175H	QHMTEVVRHCPHHERCSD
4	-----YNQTHHPHHQEY–
9	---GAPEVHHPAHQA----
7	-----KTLS-HAPHHSKE---
8	-----HSPV-HTPHGSGK--
5	---------TPHQPHHSFTPR
6	----DSHFLHYPHHSN----
2	------ESTGHSPHQHN---
3	------STPVHTPHHAHY--
1	----SGSLQHRPHPRN----

4H5

WTp53	QHMTEVVRRCPHHERCSD
R175H	QHMTEVVRHCPHHERCSD
8	---VGSPQHWPHHSN---
9	---VGSPQHWPHHCN---
2	---VGSPQHWPHHSN---
7	---VGSPQHWPHHSN---
1	---VGSPQHWPHHSK---
3	---LEVN-HWGHHYRT-
5	---GGSG-HQSGHRP----
6	----SLG-HKSHHFIAA---
10	---FDLRDYMHMPHH------

Consensus: **167**QHMTEVVRHCPHHERCSD**184**

10C8, 7B9, 4H5 epitope

Figure 8A

Anti-R248Q mAbs

3G11

WTp53	GMNRRPILTIITLEDS
R248Q	GMNQRPILTIITLEDS
1	---------- SSTHYSWSLMDA
2	--------- GPSCHYCFTGLP----
3	---------- GPSCHYCFTGLP----
4	WSVPPGRHIHYS----------
5	WSVPPGRHIHYS----------
10	------ FSVPSGHYIRAN--------
8	------ FSVRPFHYAQDN-------
7	------ FSVPFDRIQETP---------
9	------ FSVRPYDPQIEN--------
6	----- HSVWYANGSVKT ------

Consensus: **215**SVVVPYEPPEVGSDCTTIHYN **235**

3G11 epitope

Figure 8B

4H5 Alanine Scan on R175H

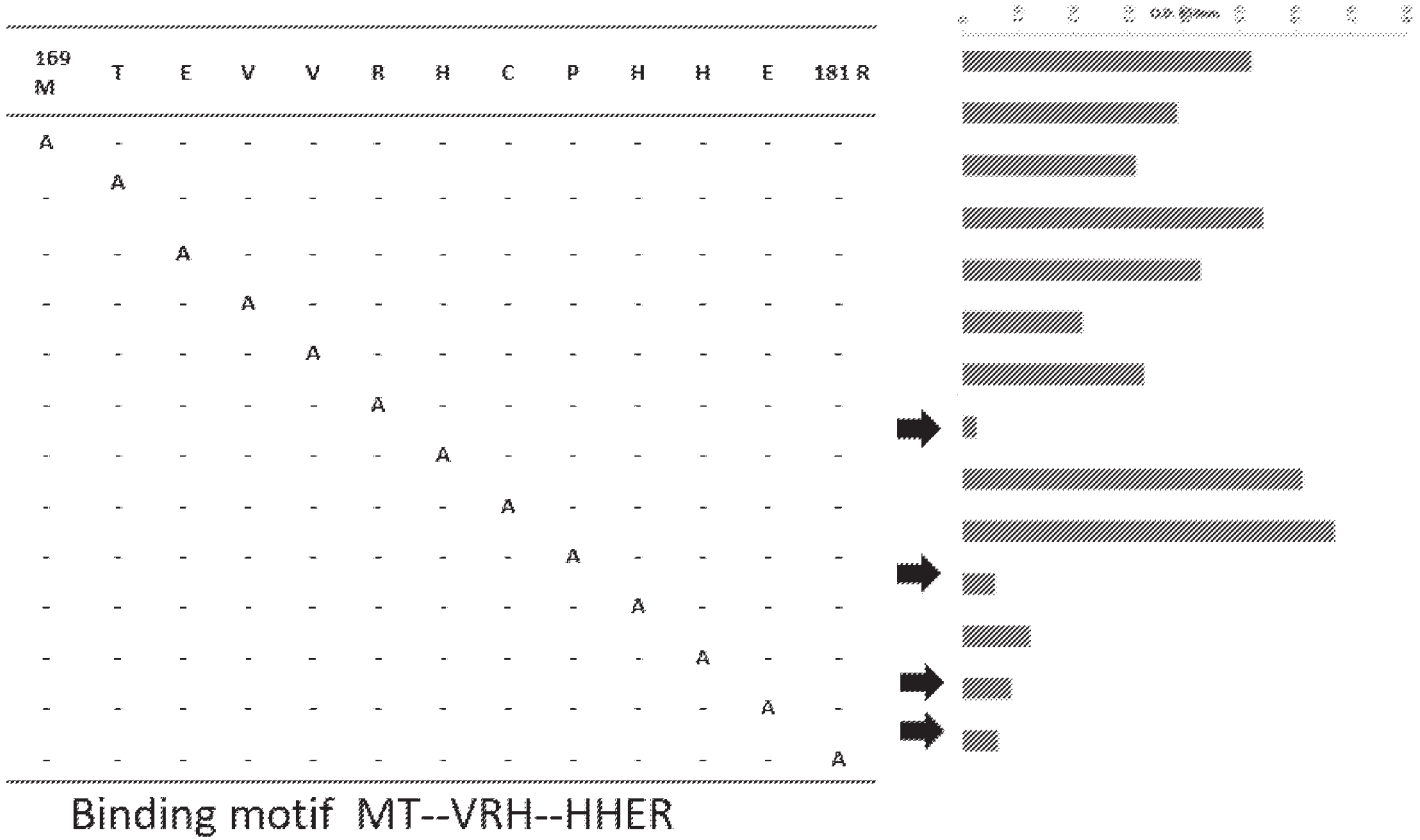


Figure 9A

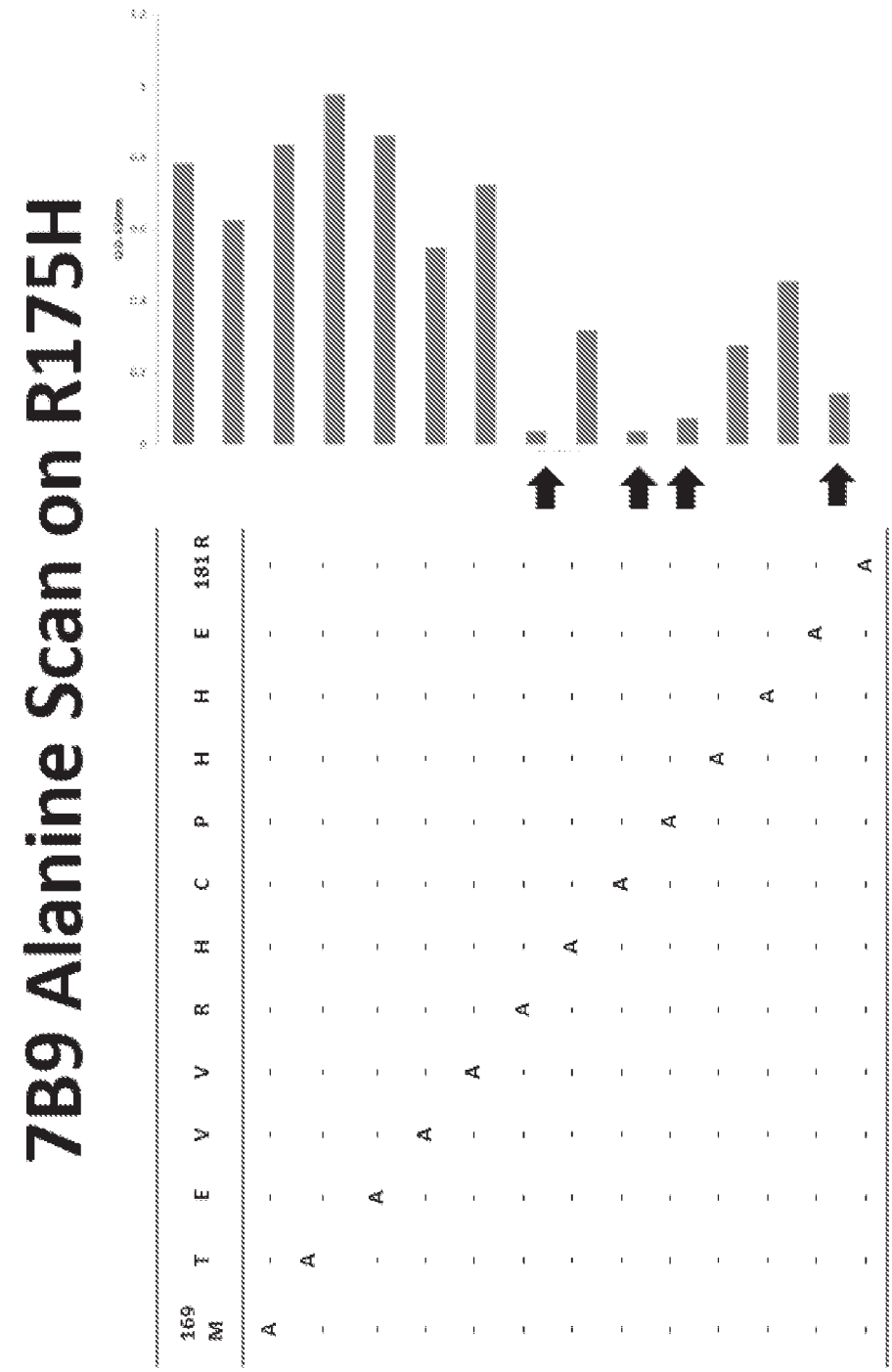

7B9 Alanine Scan on R175H
169 M T E V V R H C P H H E 181 R
Binding motif -T--VRHCPHHER

Figure 9B

10C8 Alanine Scan on R175H

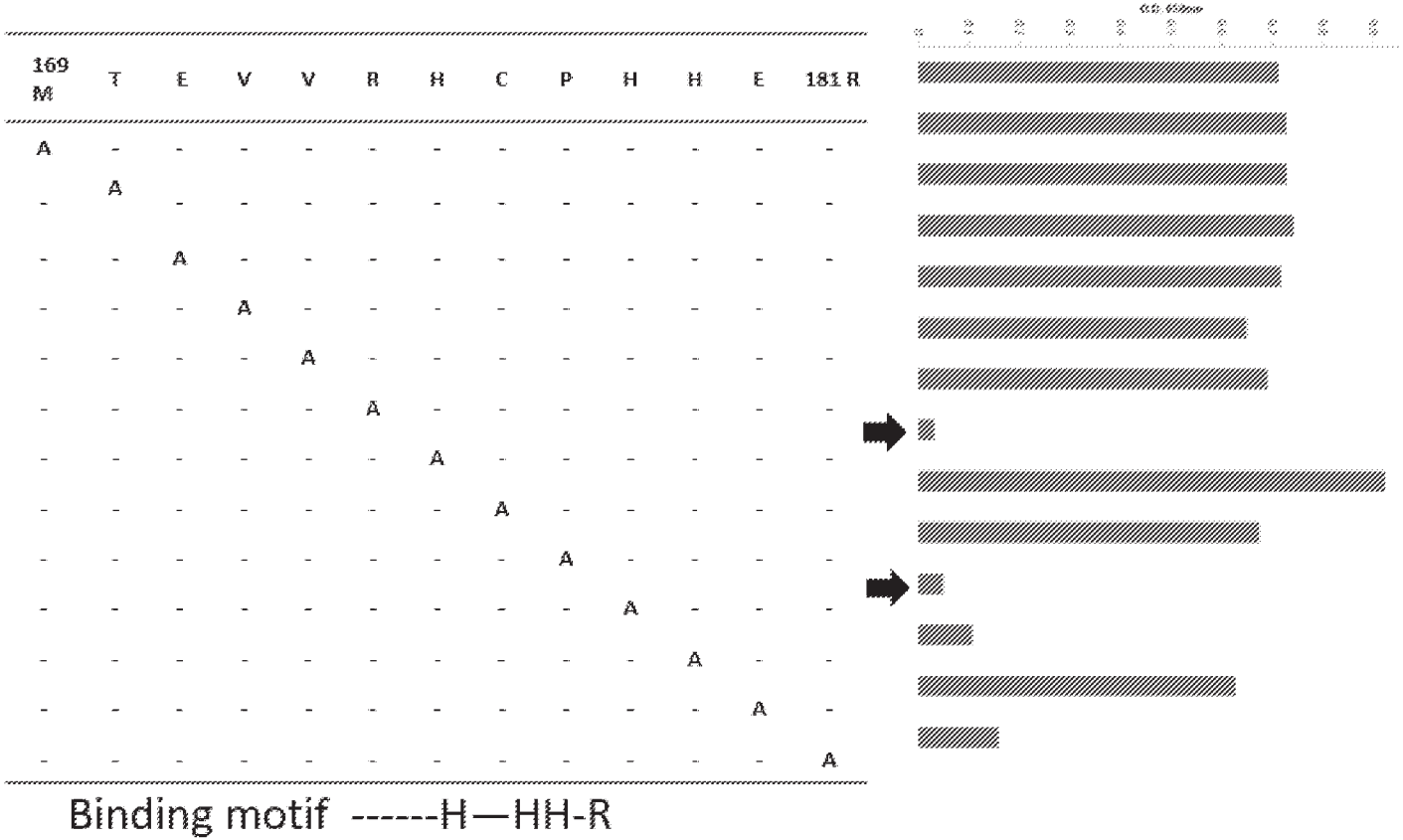


Figure 9C

13E4 Alanine Scan on R273H

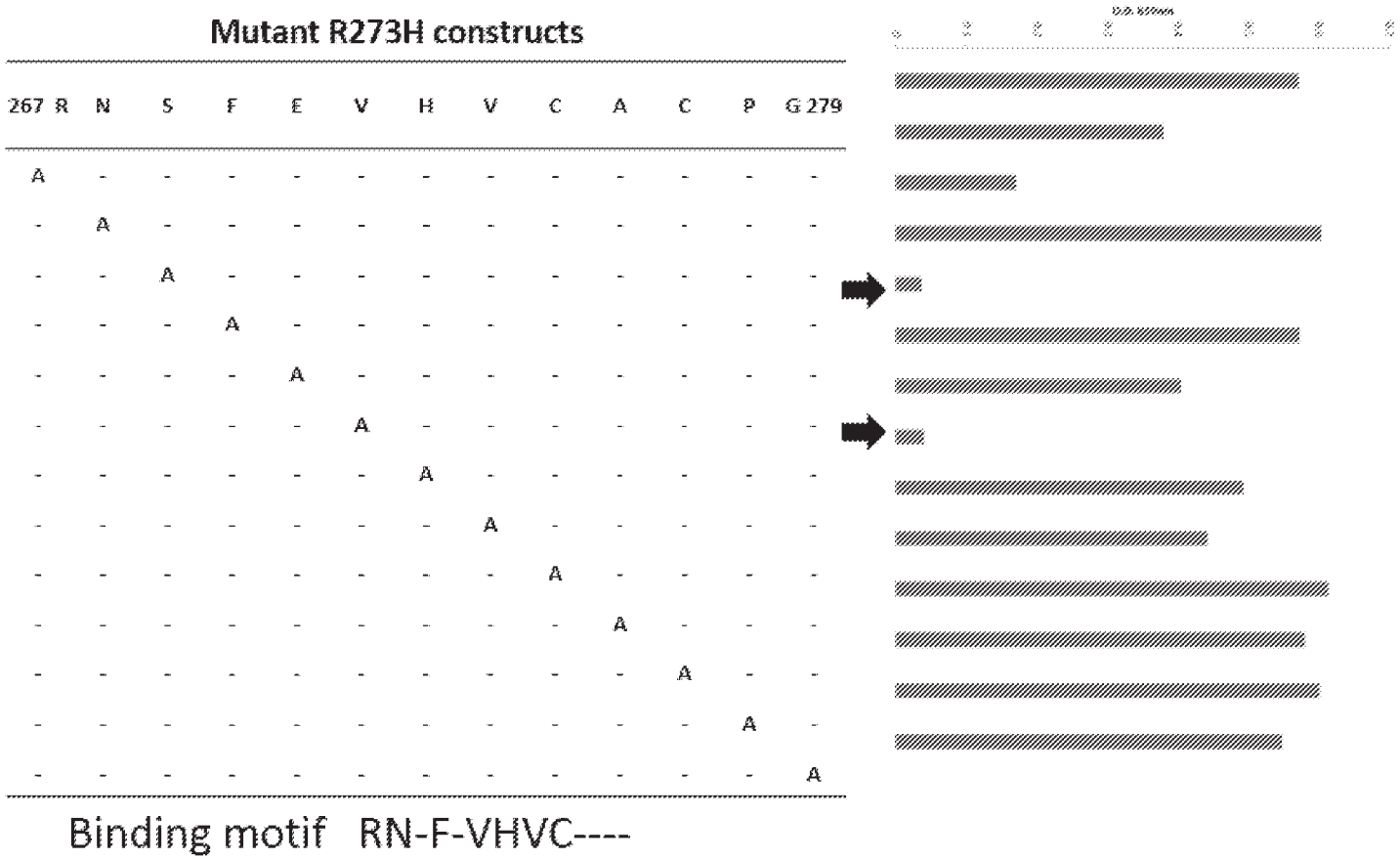


Figure 9D

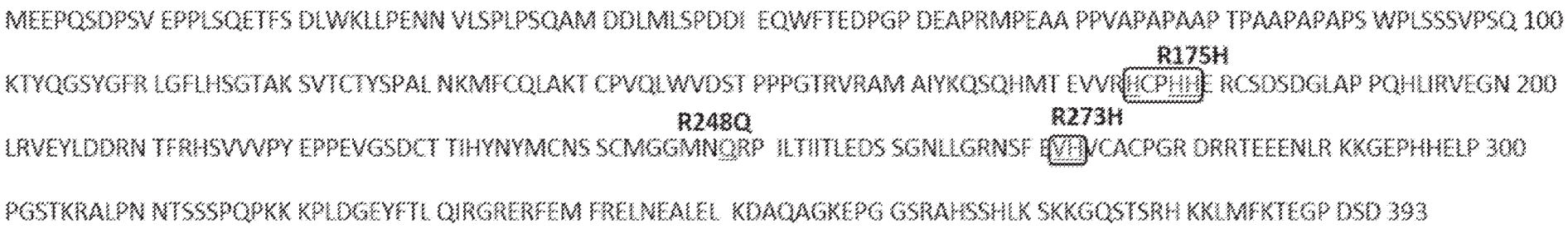

MEEPQSDPSV EPPLSQETFS DLWKLLPENN VLSPLPSQAM DDLMLSPDDI EQWFTEDPGP DEAPRMPEAA PPVAPAPAAP TPAAPAPAPS WPLSSSVPSQ 100
R175H
KTYQGSYGFR LGFLHSGTAK SVTCTYSPAL NKMFCQLAKT CPVQLWVDST PPPGTRVRAM AIYKQSQHMT EVVRHCPHHE RCSDSDGLAP PQHLIRVEGN 200
R248Q
R273H
LRVEYLDDRN TFRHSVVVPY EPPEVGSDCT TIHYNYMCNS SCMGGMNQRP ILTIITLEDS SGNLLGRNSF EVHVCACPGR DRRTEEENLR KKGEPHHELP 300
PGSTKRALPN NTSSSPQPKK KPLDGEYFTL QIRGRERFEM FRELNEALEL KDAQAGKEPG GSRAHSSHLK SKKGQSTSRH KKLMFKTEGP DSD 393

Figure 10

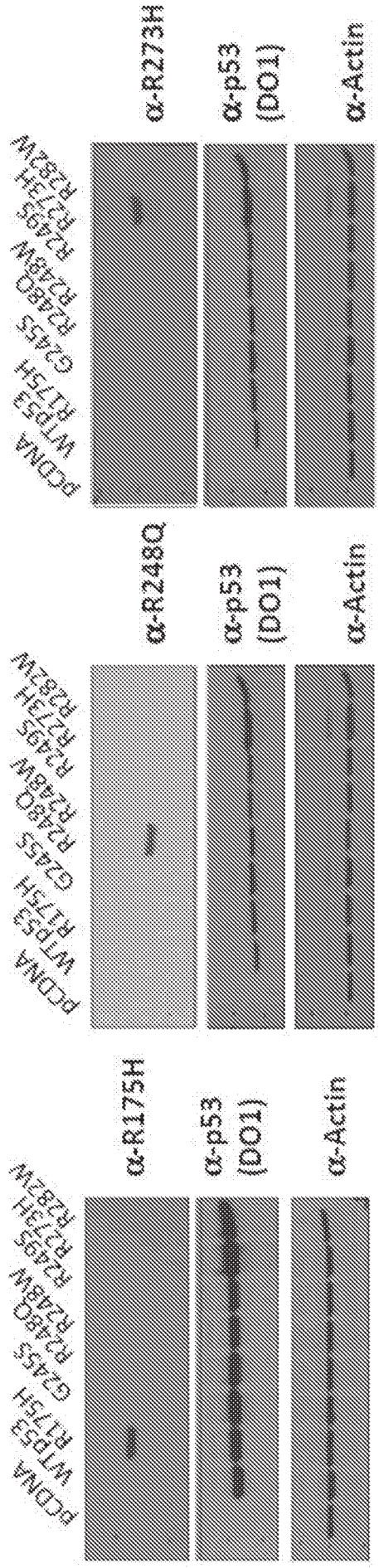

pCDNA
WTp53
R175H
G245S
R248Q
R248W
R249S
R273H
R282W
α-R175H
α-p53 (DO1)
α-Actin
α-R248Q
α-R273H

Figure 11

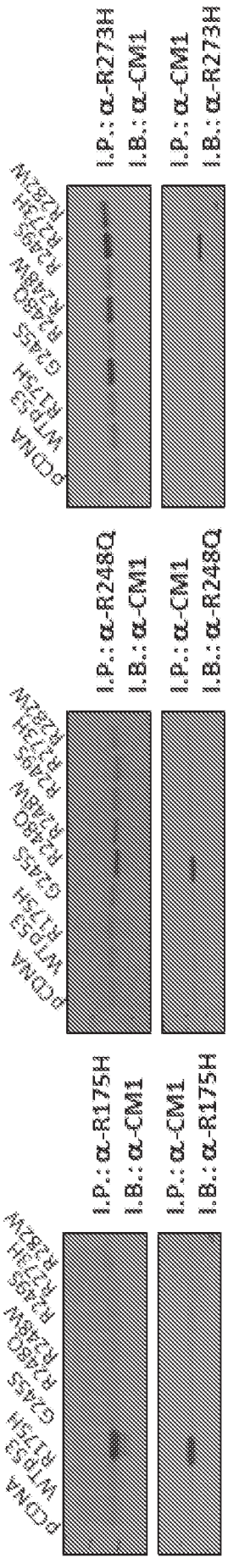

pCDNA WTp53 R175H G245S R248Q R273H R282W
I.P.: α-R175H
I.B.: α-CM1
I.P.: α-CM1
I.B.: α-R175H
pCDNA WTp53 R175H G245S R248Q R273H R282W
I.P.: α-R248Q
I.B.: α-CM1
I.P.: α-CM1
I.B.: α-R248Q
pCDNA WTp53 R175H G245S R248Q R273H R282W
I.P.: α-R273H
I.B.: α-CM1
I.P.: α-CM1
I.B.: α-R273H

Figure 12

Cell lines used in study	p53 status
A549, MCF-7, HCT116, MRC5, RKO, U2OS	Wild-type
AU565	R175H
SKBR3	R175H
HEC-1A	R248Q
RD	R248W
786-O	R248W
PLC-PRF/15	R249S
KNS-62	R249S
SW480	R273H
A431	R273H

Figure 13

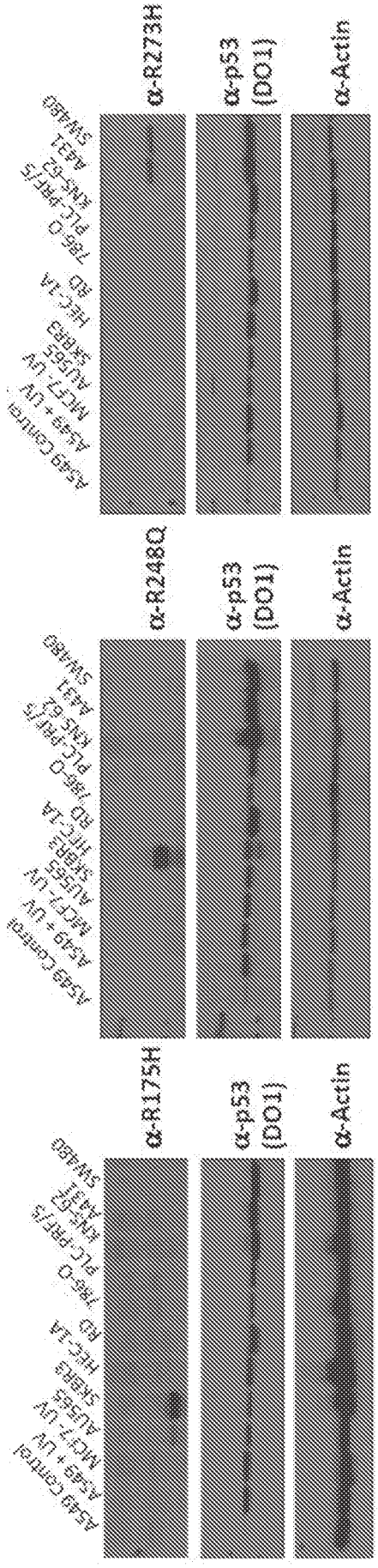

α-R175H
α-p53 (DO1)
α-Actin
α-R248Q
α-p53 (DO1)
α-Actin
α-R273H
α-p53 (DO1)
α-Actin

Figure 14

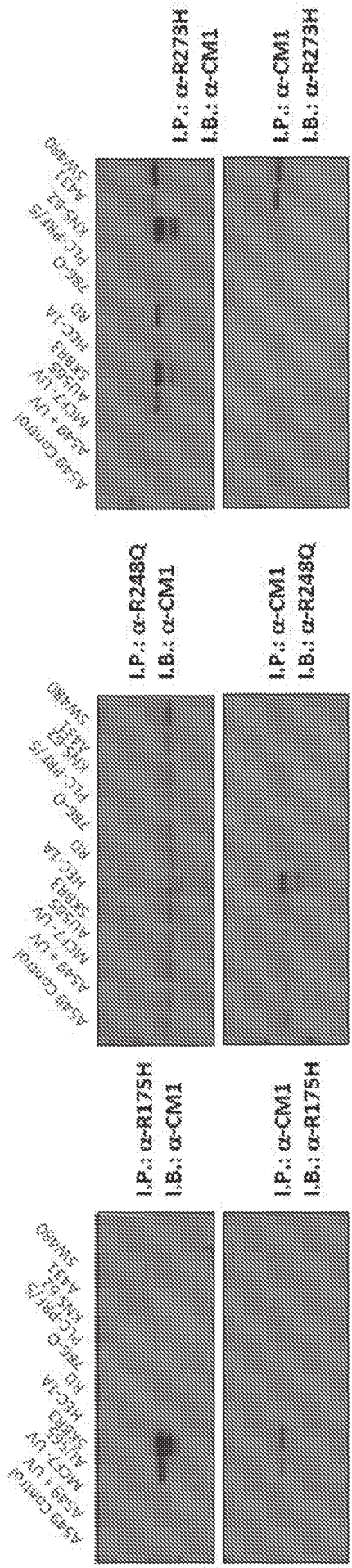

I.P.: α-R175H
I.B.: α-CM1
I.P.: α-CM1
I.B.: α-R175H
I.P.: α-R248Q
I.B.: α-CM1
I.P.: α-CM1
I.B.: α-R248Q
I.P.: α-R273H
I.B.: α-CM1
I.P.: α-CM1
I.B.: α-R273H

Figure 15

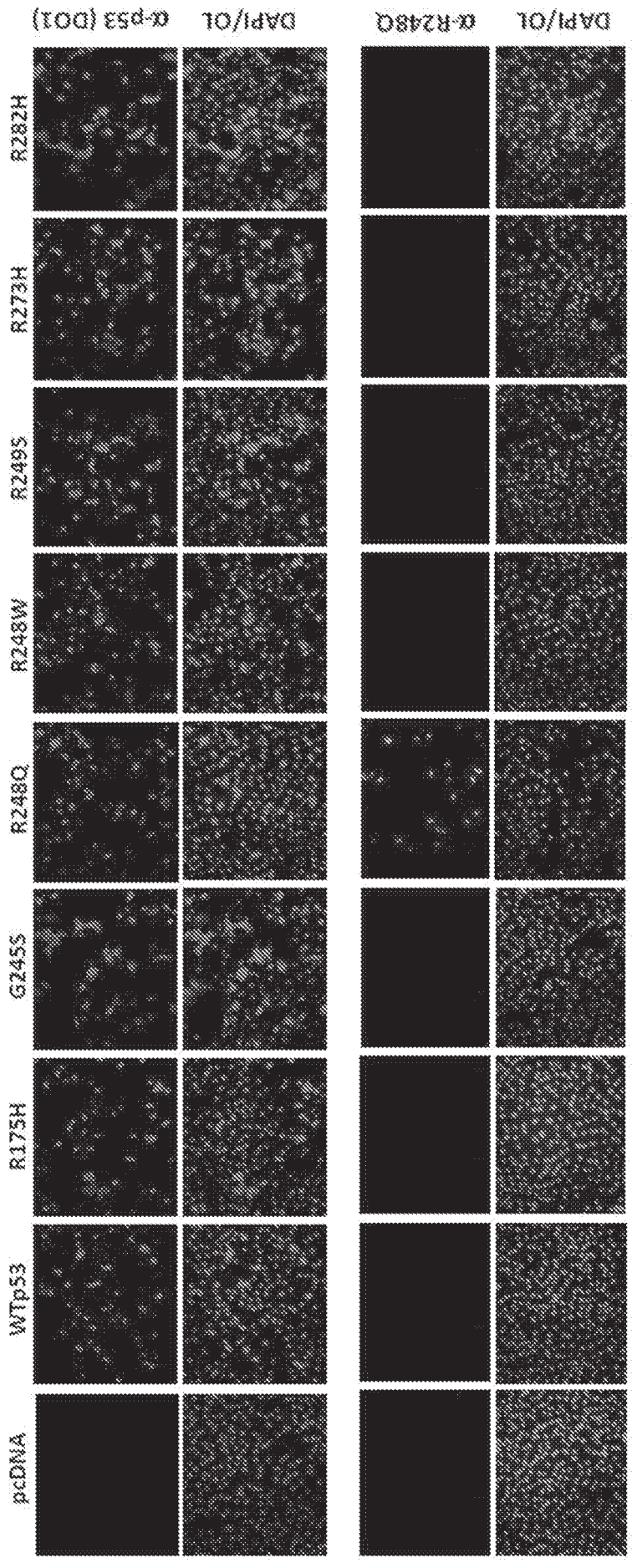

pcDNA
WTp53
R175H
G245S
R248Q
R248W
R249S
R273H
R282H
DAPI/OL
α-R248Q
DAPI/OL
α-p53 (DO1)

Figure 16

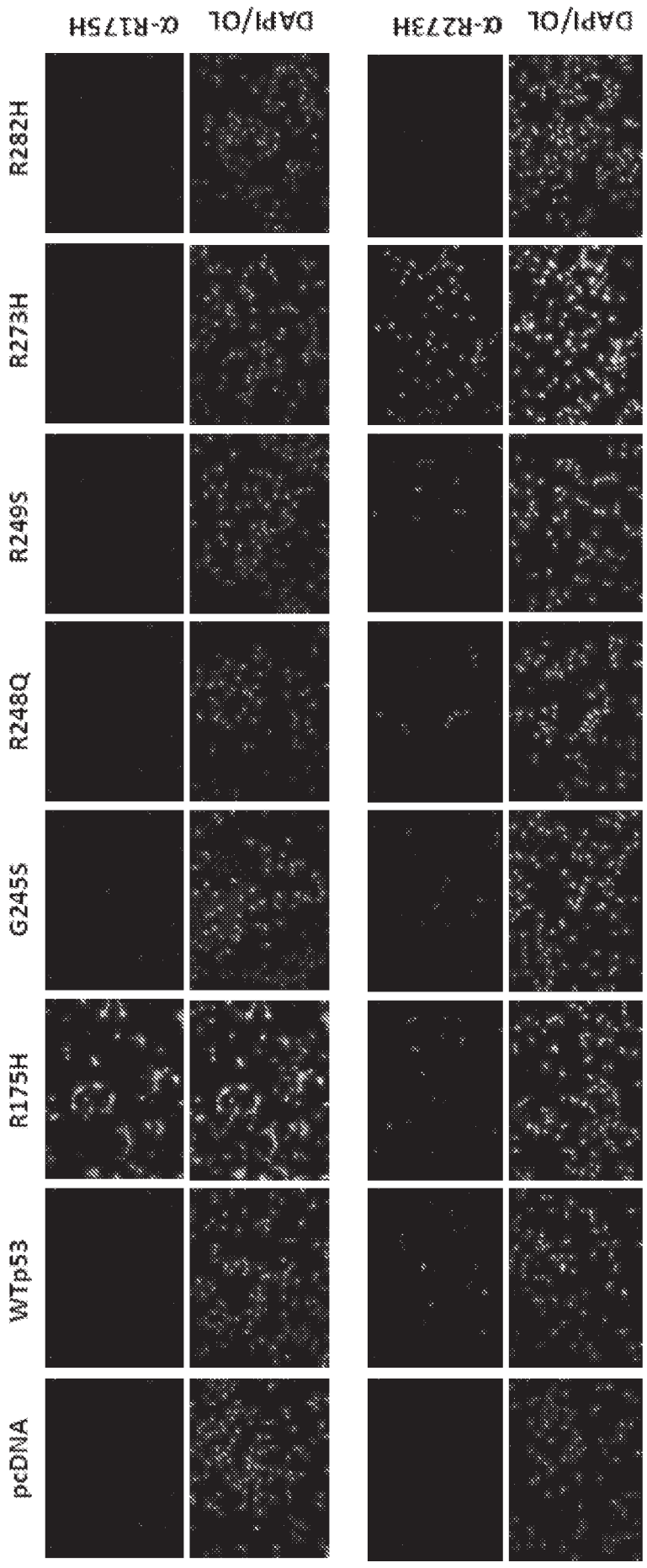


Figure 16 (cont.)

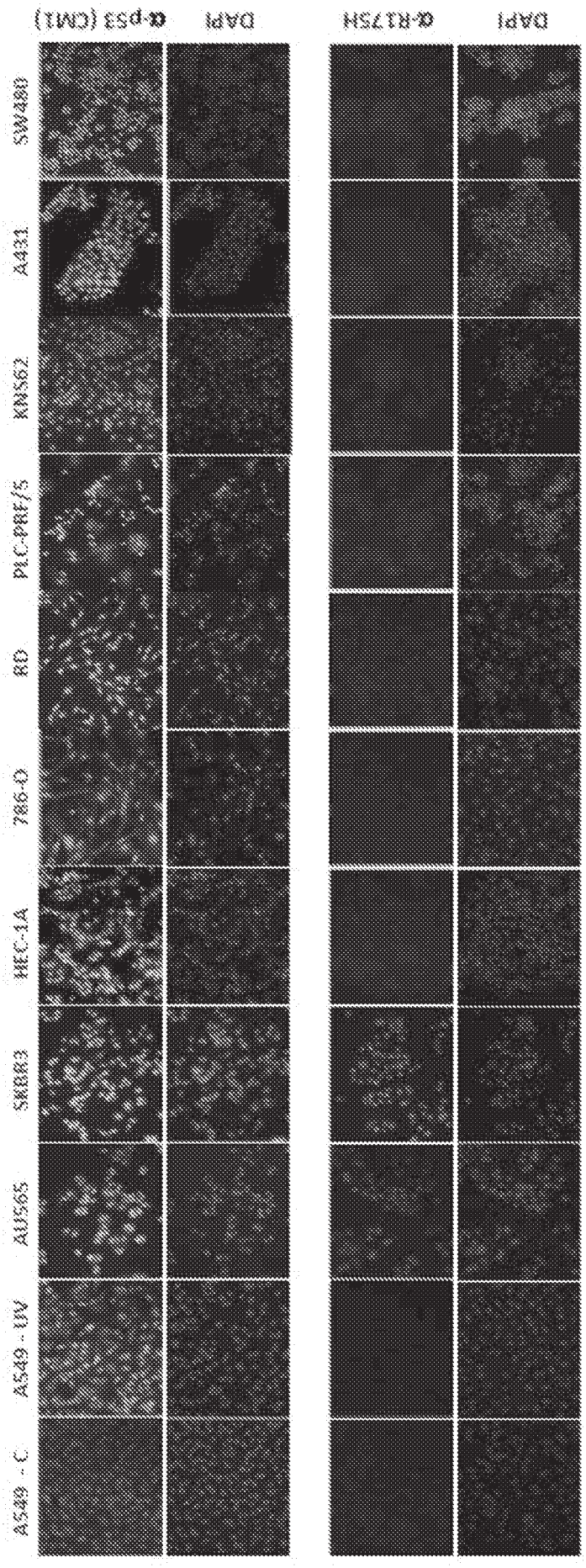

α-p53 (CM1)
DAPI
α-R175H
DAPI
A549 - C
A549 - UV
AU565
SKBR3
MEC-1A
786-O
BD
PLC-PRF/5
K562
A431
SW480

Figure 17A

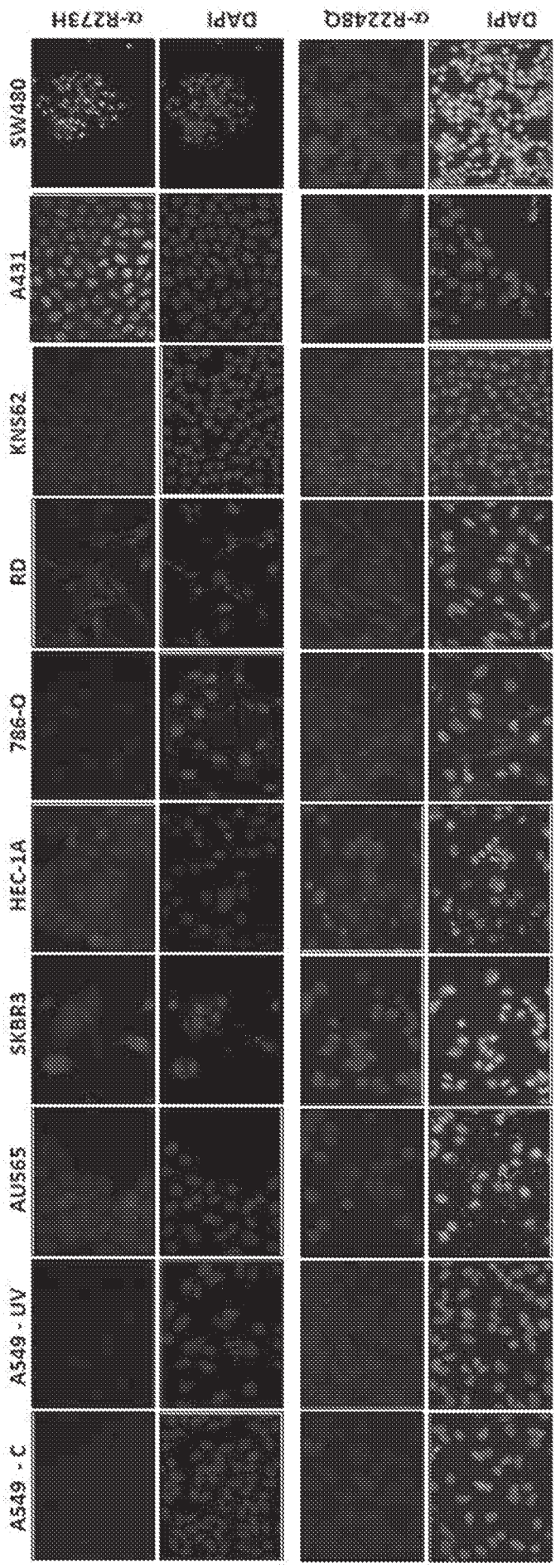


Figure 17A (cont.)

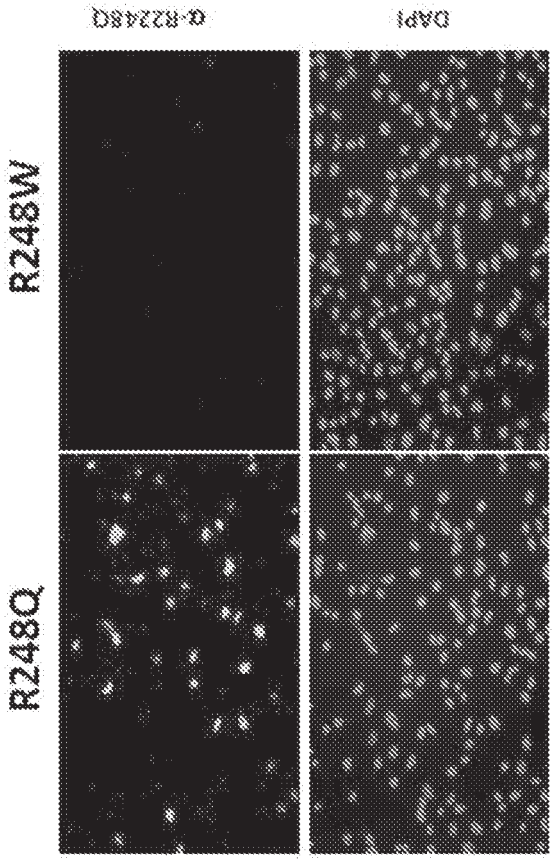


Figure 17B

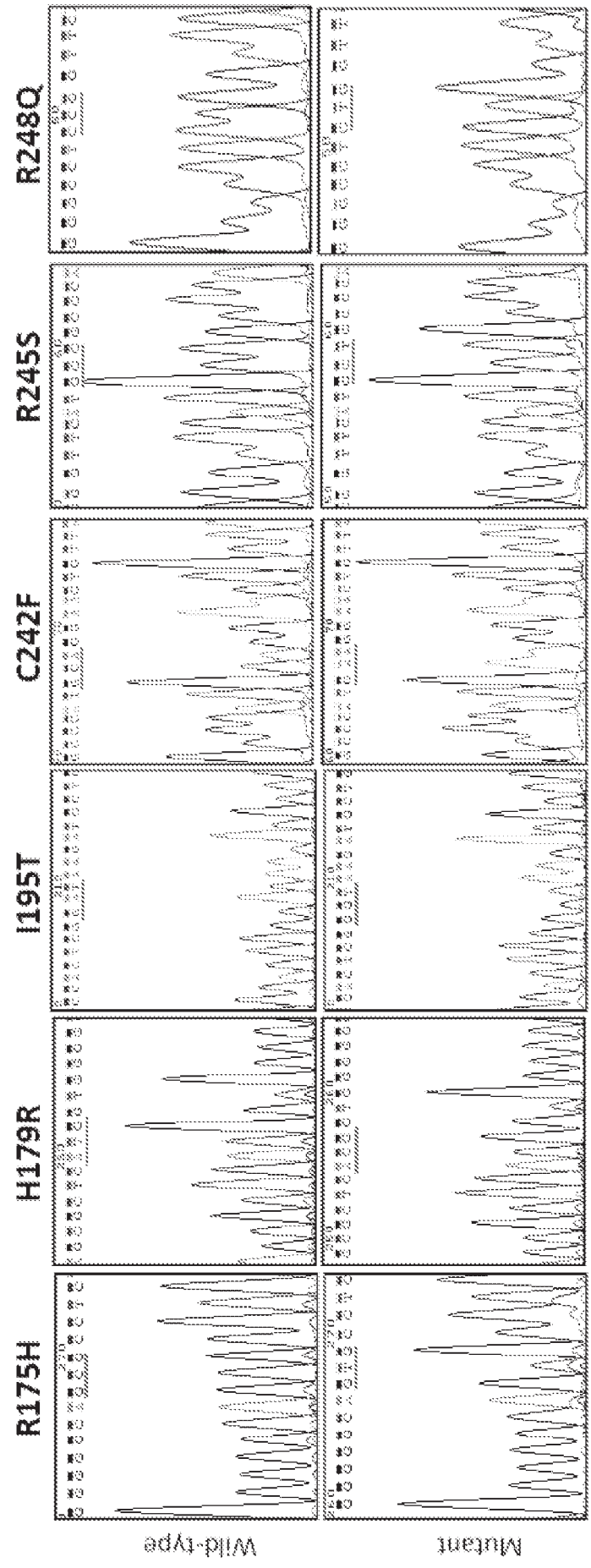


Figure 18A

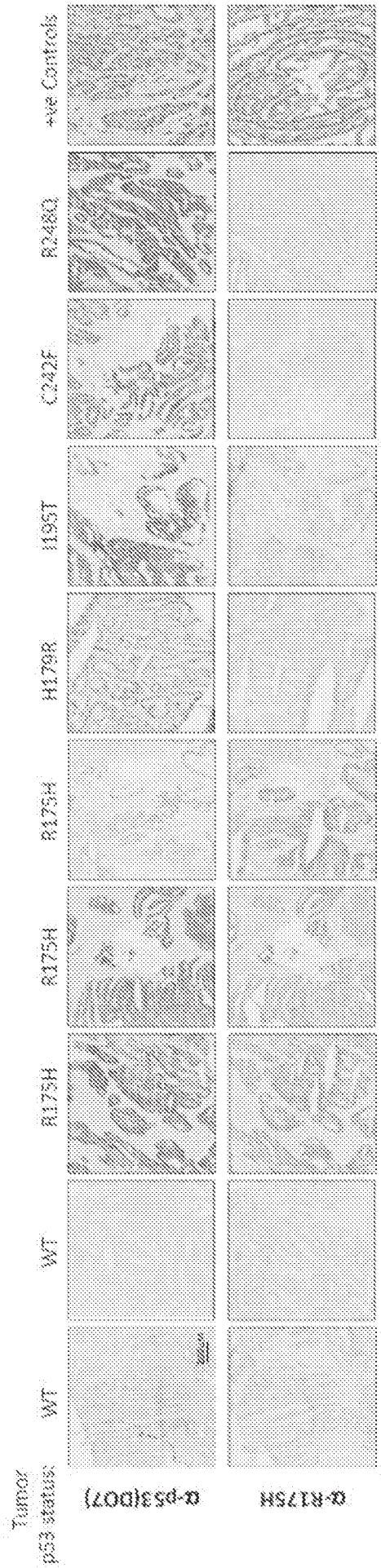

Tumor p53 status
WT
WT
R175H
R175H
R175H
H179R
I195T
C242F
R248Q
+ve Controls
α-p53(DO7)
α-R175H

Figure 18B

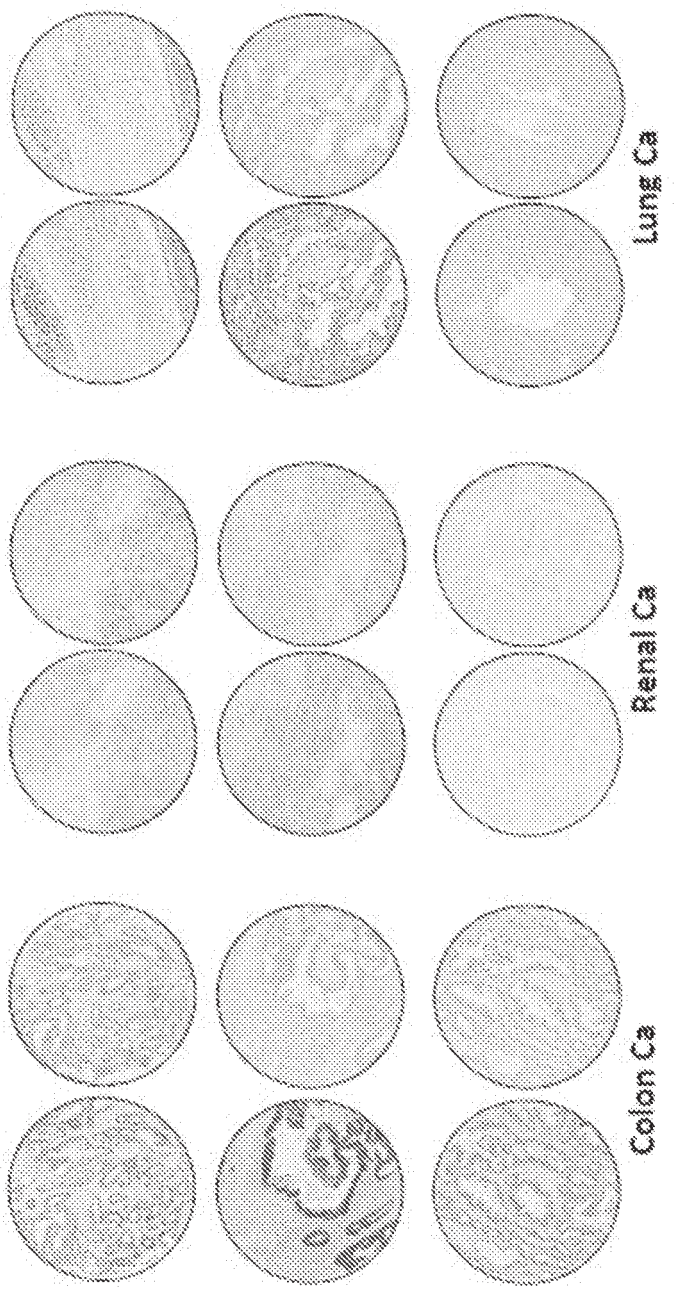

α-p53(DO7)
α-R175H
α-p53(DO7)
α-R175H
α-p53(DO7)
α-R175H
Double positive
Single positive
Double negative
Breast Ca
Colon Ca
Renal Ca
Lung Ca

Figure 19

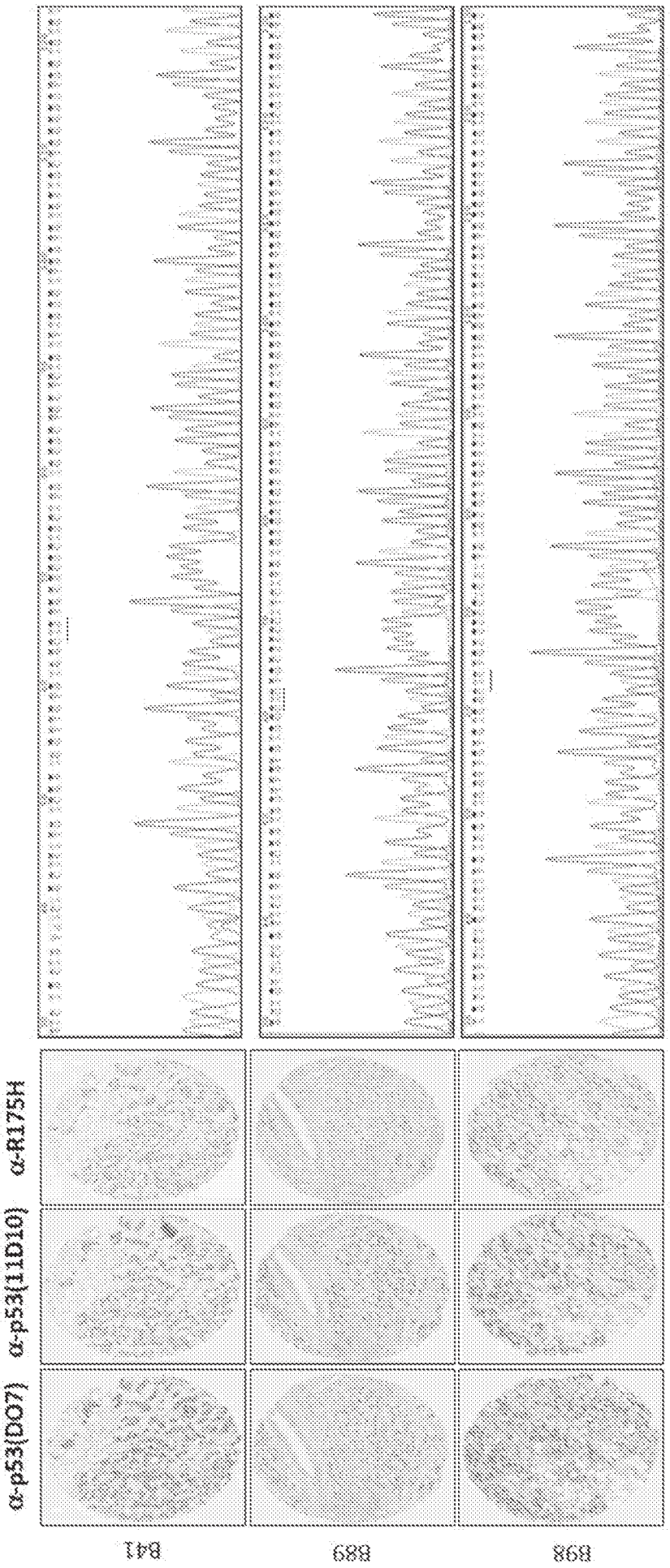


Figure 20A

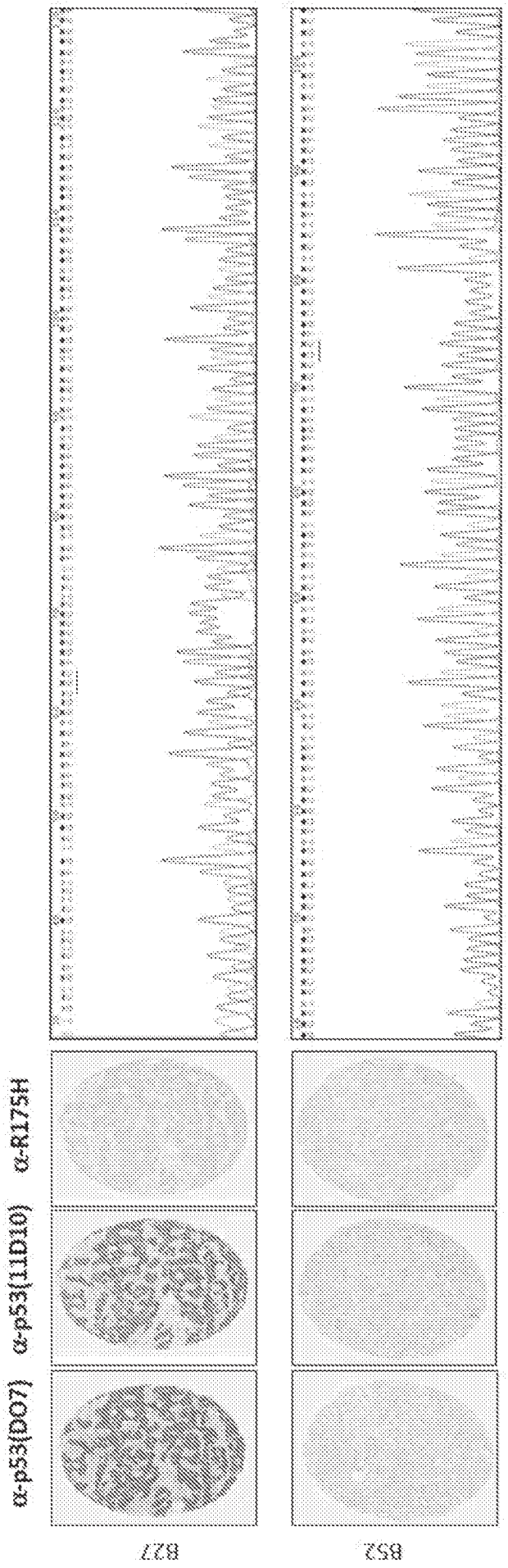


Figure 20B

Combined data from analysis of human tumor microarrays by immunohistochemistry

TMAs * used in study	Total samples = n	α-pan-p53† (DO7) n (%)	α-R175H† n (%)
Colon	99	60 (60)	5
Breast	102	72 (70)	5
Lung	169	114 (67)	8
Prostate	231	27 (11)	0
Renal	196	68 (34)	3

*: TMA: tumor microarray

Figure 21


R175
|P53_HUMAN MTEVVRRCPHHERCSD-SDG-LAPPQHLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVG 226
|P53_CERAE MTEVVRRCPHHERCSD-SDG-LAPPQHLIRVEGNLRVEYSDDRNTFRHSVVVPYEPPEVG 226
|P53_MACMU MTEVVRRCPHHERCSD-SDG-LAPPQHLIRVEGNLRVEYSDDRNTFRHSVVVPYEPPEVG 226
|P53_MACFU MTEVVRRCPHHERCSD-SDG-LAPPQHLIRVEGNLRVEYSDDRNTFRHSVVVPYEPPEVG 226
|P53_MACFA MTEVVRRCPHHERCSD-SDG-LAPPQHLIRVEGNLRVEYSDDRNTFRHSVVVPYEPPEVG 226
|P53_TUPGB VTEVVRRCPHHERCSD-SDG-LAPPQHLIRVEGNLHAEYSDDRNTFRHSVVVPYEPPEVG 226
|P53_MARMO MTEVVRRCPHHERCSD-SDG-LAPPQHLIRVEGNLRAEYLDDRNTFRHSVVVPYEPPEVG 224
|P53_RABIT MTEVVRRCPHHERCSD-SDG-LAPPQHLIRVEGNLRAEYLDDRNTFRHSVVVPYEPPEVG 223
|P53_DELLE MTEVVRRCPHHERCSDYSDG-LAPPQHLIRVEGNLRAEYLDDRNTFRHSVVVPYEPPEVG 220
|P53_PIG MTEVVRRCPHHERSSDYSDG-LAPPQHLIRVEGNLRAEYLDDRNTFRHSVVVPYEPPEVG 219
|P53_CANFA VTEVVRRCPHHERCSDSSDG-LAPPQHLIRVEGNLRAKYLDDRNTFRHSVVVPYEPPEVG 214
|P53_FELCA MTEVVRRCPHHERCPDSSDG-LAPPQHLIRVEGNLHAKYLDDRNTFRHSVVVPYEPPEVG 219
|P53_CAVPO MTEVVRRCPHHERCSD-SDG-LAPPQHLIRVEGNLHAEYVDDRTTFRHSVVVPYEPPEVG 224
|P53_SHEEP MTEVVRRCPHHERSSDYSDG-LAPPQHLIRVEGNLRAEYFDDRNTFRHSVVVPYESPEIE 215
|P53_MESAU MTEVVRRCPHHERSSE-GDG-LAPPQHLIRVEGNMHAEYLDDKQTFRHSVVVPYEPPEVG 229
|P53_CRIGR MTEVVRRCPHHERSSE-GDS-LAPPQHLIRVEGNLHAEYLDDKQTFRHSVVVPYEPPEVG 226
|P53_BOVIN MTEVVRRCPHHERSSDYSDG-LAPPQHLIRVEGNLRAEYLDDRNTFRHSVVVPYESPEID 219
|P53_BOSIN MTEVVRRCPHHERSSDYSDG-LAPPQHLIRVEGNLRAEYLDDRNTFRHSVVVPYESPEID 219
|P53_RAT MTEVVRRCPHHERCSD-GDG-LAPPQHLIRVEGNPYAEYLDDRQTFRHSVVVPYEPPEVG 224
|P53_MOUSE MTEVVRRCPHHERCSD-GDG-LAPPQHLIRVEGNLYPEYLEDRQTFRHSVVVPYEPPEAG 220
|P53_HORSE MTEVVRRCPHHERCSDSSDG-LAPPQHLIRVEGNLRAEYLDDRNTFRHSVVVPYEPPEVG 177
|P53_EQUAS MTEVVRRCPHHERCSDSSDG-LAPPQHLIRVEGNLRAEYLDDRNTLRHSVVVPYEPPEVG 102
|P53_CHICK VAEVVRRCPHHERCGGGTDG-LAPAQHLIRVEGNPQARYHDDETTKRHSVVVPYEPPEVG 212
|P53_XENLA VAEVVKRCPHHERSVEPGED-AAPPSHLMRVEGNLQAYYMEDVNSGRHSVCVPYEGPQVG 201
|P53_ONCMY VADVVRRCPHHQSTSENNEG-PAPRGHLVRVEGNQRSEYMEDGNTLRHSVLVPYEPPQVG 215
|P53_DANRE VAEVVRRCPHHERTPDG-DN-LAPAGHLIRVEGNQRANYREDNITLRHSVFVPYEAPQLG 194
|P53_ICTPU VAEVVRRCPHHERSNDSSDG-PAPPGHLLRVEGNSRAVYQEDGNTQAHSVVVPYEPPQVG 202
|P53_XIPMA VGEVVKRCPHHQ-SEDLSDN-KS---HLIRVEGSQLAQYFEDPNTRRHSVTVPYERPQLG 189
|P53_XIPHE VGEVVKRCPHHQ-SEDLSDN-KS---HLIRVEGSQLAQYFEDPNTRRHSVTVPYERPQLG 189

Figure 22

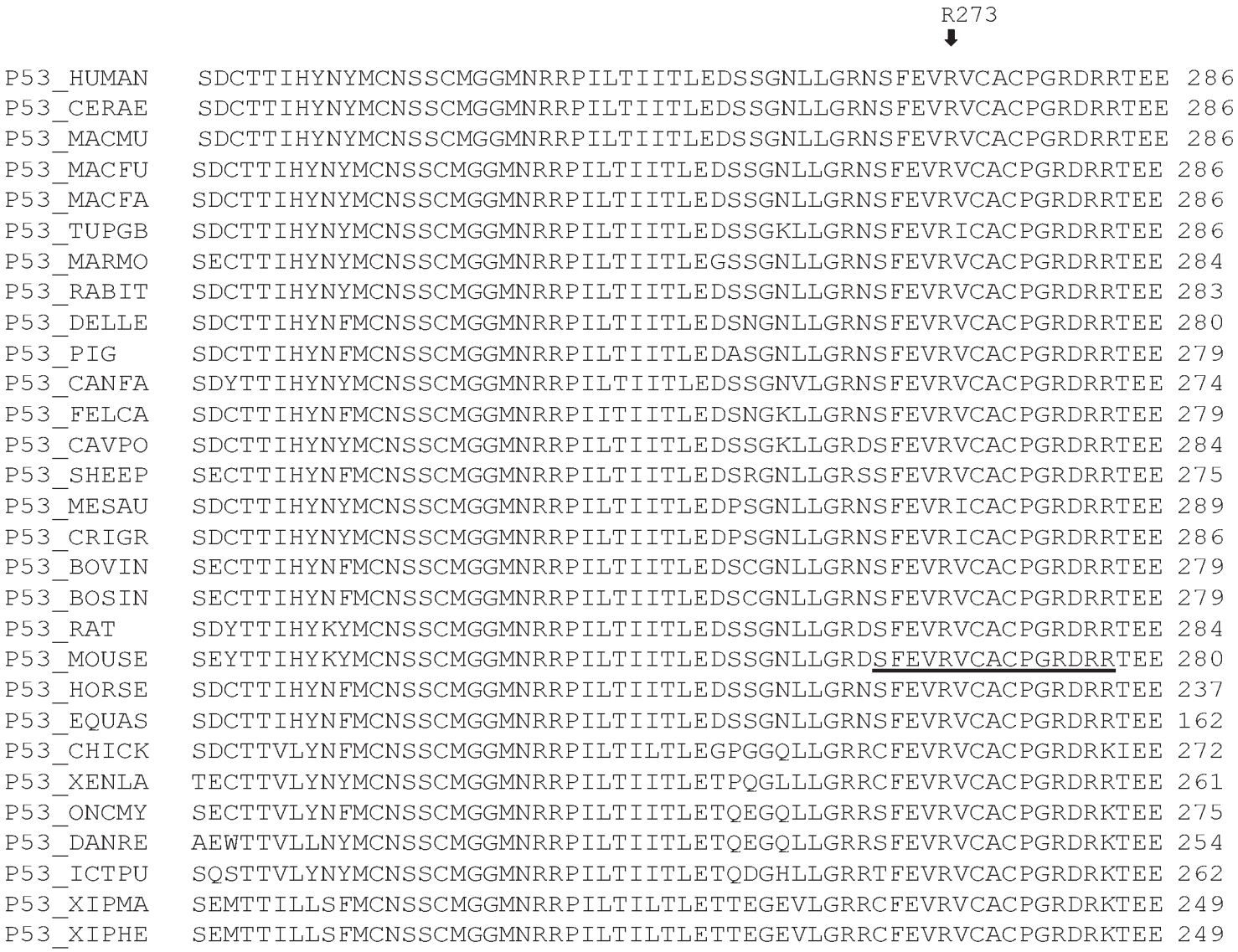


Figure 22 (cont.)

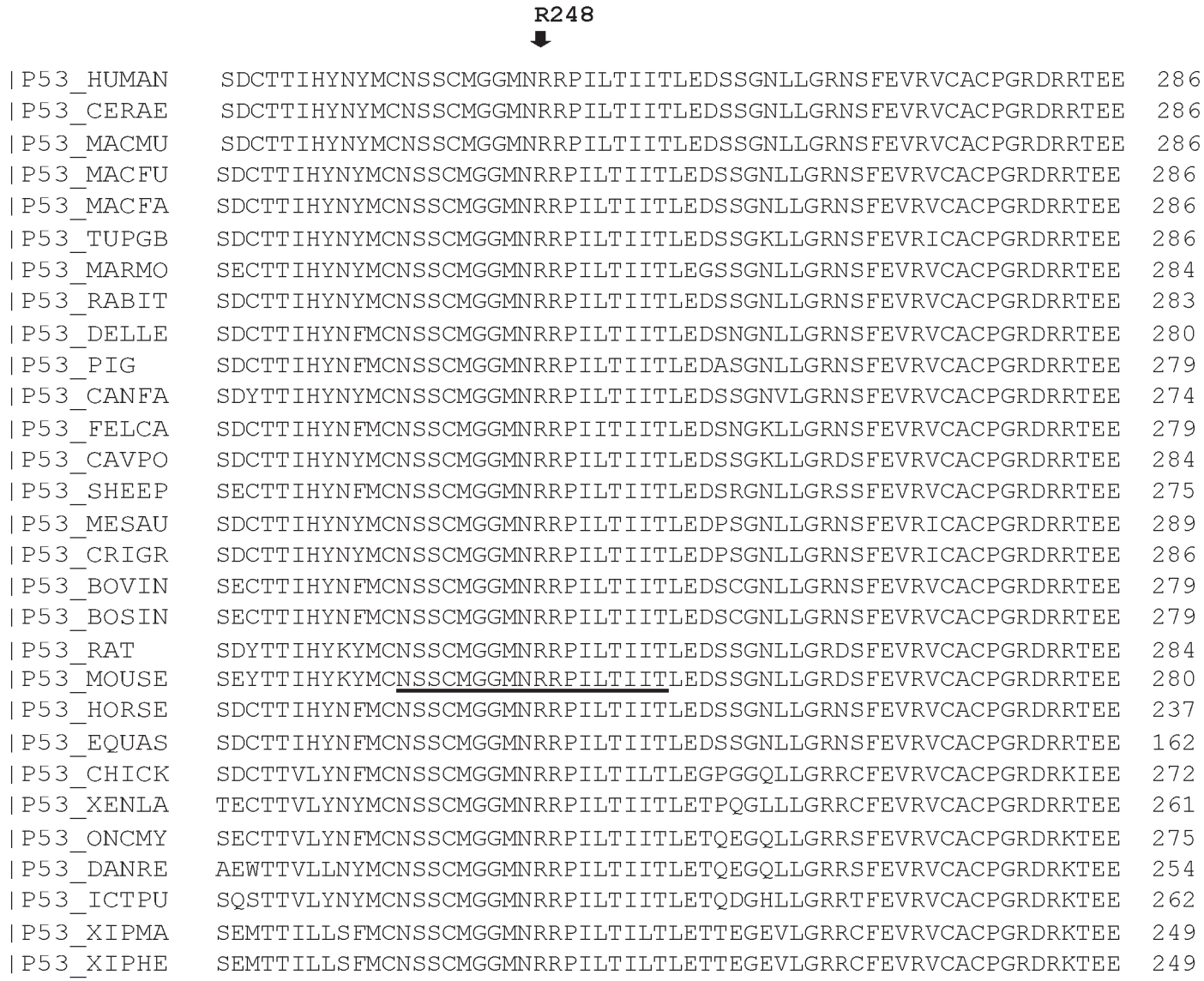


Figure 22 (cont.)

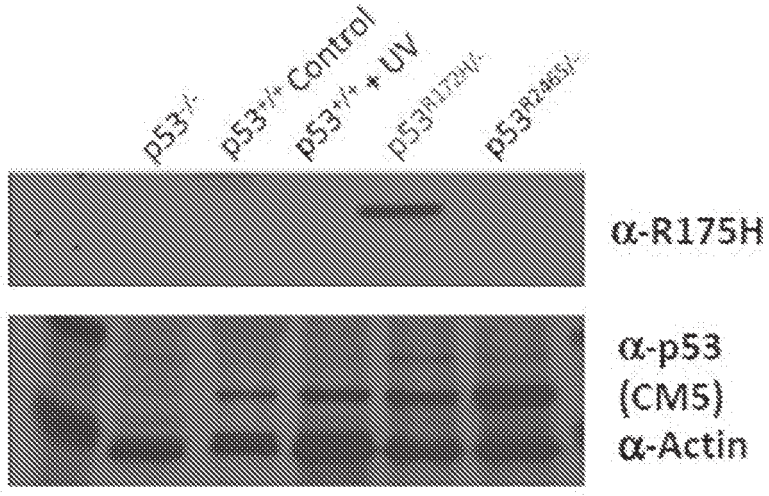


Figure 23A

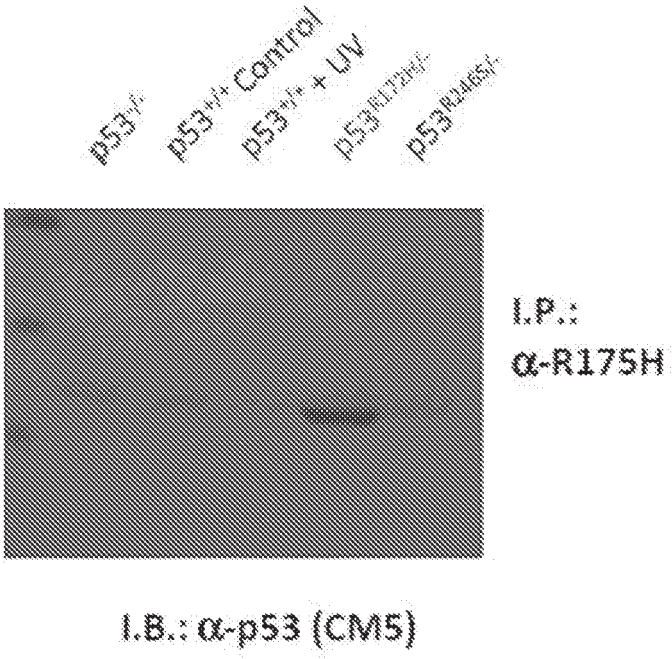


Figure 23B

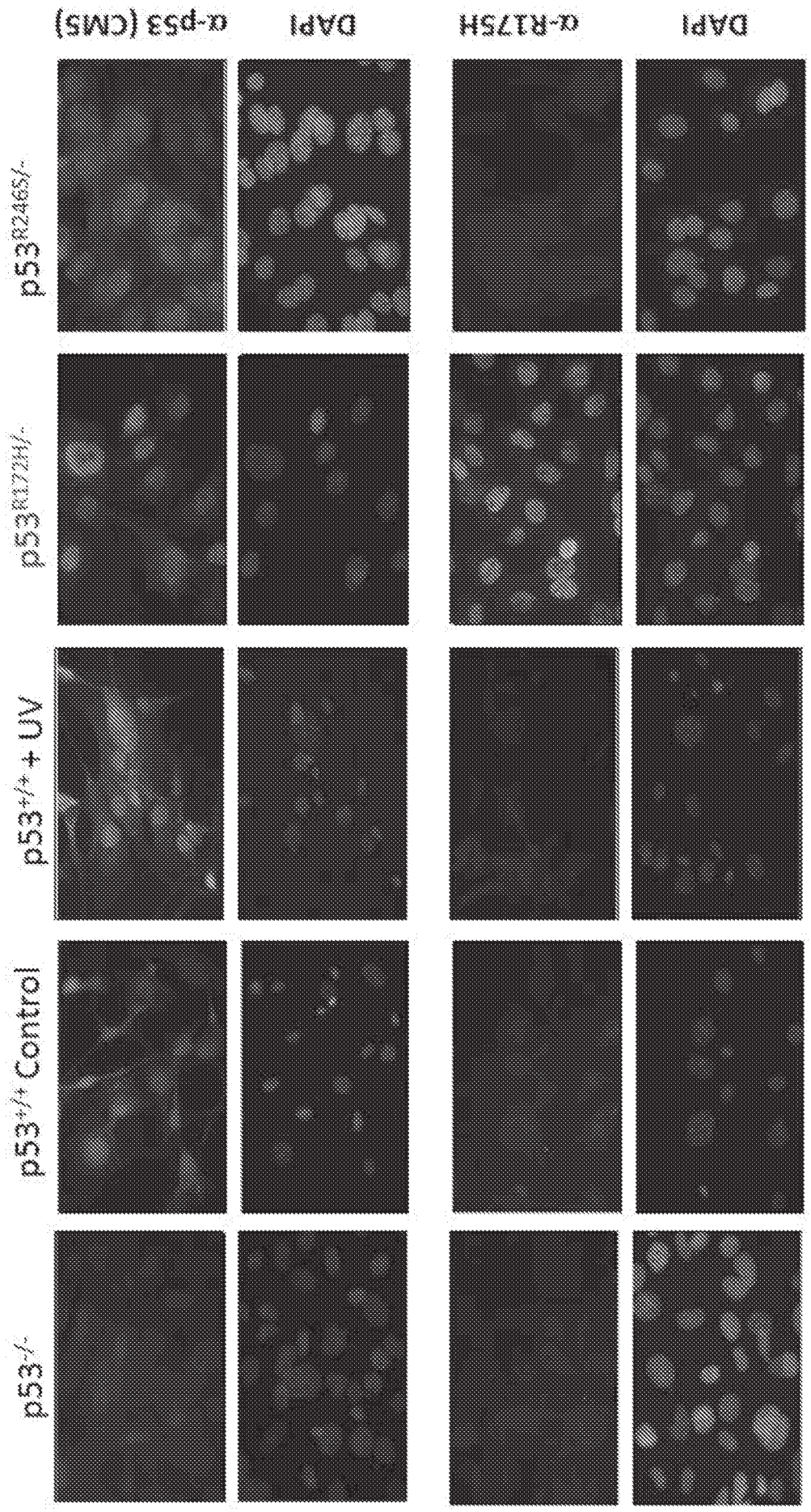

p53-/-
p53+/+ Control
p53+/+ + UV
p53R172H/-
p53R246S/-
α-p53 (CM5)
DAPI
α-R175H
DAPI

Figure 23C

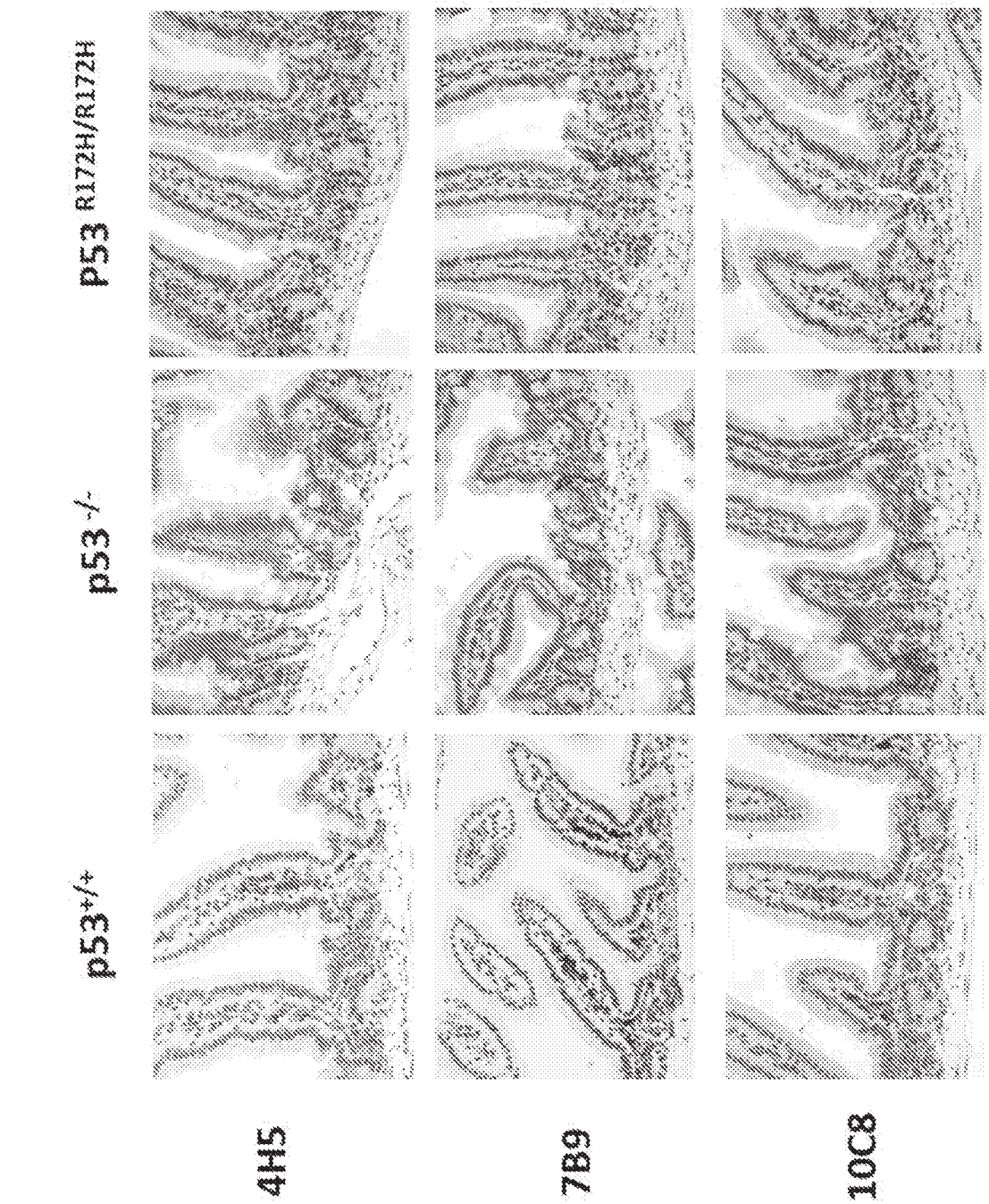


Figure 23D

Anti-R175H clone 4H5

DIVLTQSTSSLSVSAGERVTLSCKSSQSLLNSGNQKSYLAWYQQKPGQPPKLLIYGASTRE SGVPDRFTGSGSETDFTLTISSVQPEDLAVYYCQNDHSYPLTFGAGTKLELK (SEQ ID NO:18)

LC-CDR1:	QSLLNSGNQKSY	(SEQ ID NO:19)
LC-CDR2:	GAS	(SEQ ID NO:20)
LC-CDR3:	QNDHSYPLT	(SEQ ID NO:21)

Anti-R175H clone 7B9

DIVMTQSPSSLSVSGGEKVTMSCKSSQSLLNSGNQKSNLAWYQQKPGQPPKLLIYGASTR ESGVPDRFAGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGGGTKLELK (SEQ ID NO:22)

LC-CDR1:	QSLLNSGNQKSN	(SEQ ID NO:23)
LC-CDR2:	GAS	(SEQ ID NO:20)
LC-CDR3:	QNDHSYPLT	(SEQ ID NO:21)

Anti-R175H clone 10C8

DIVMTQSPSSLSVSGGEKVTMSCKSSQSLLNSGNQKSNLAWYQQKPGQPPKLLIYGASTR ESGVPDRFAGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGGGTKLELK (SEQ ID NO:22)

LC-CDR1:	QSLLNSGNQKSN	(SEQ ID NO:23)
LC-CDR2:	GAS	(SEQ ID NO:20)
LC-CDR3:	QNDHSYPLT	(SEQ ID NO:21)

Figure 24

Anti-R175H clone 4H5

EVQLQQSGPELVKPGASVKISCKTSGFTFTEYTMHWMKQSHGRSLEWIGRIDPNNGVTVY NQKFKVKATLTVDRSSSTAYLELRSLTSEDSAVYYCARWGGDYVTGGGTTLTVSS (SEQ ID NO:24)

HC-CDR1:	GFTFTEYT	(SEQ ID NO:25)
HC-CDR2:	IDPNNGVT	(SEQ ID NO:26)
HC-CDR3:	ARWGGDYV	(SEQ ID NO:27)

Anti-R175H clone 7B9

EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWMKQSHGKSLEWIGRINPYSGGTVY NQKFKGKATLTVDKSSSTAYMELRSLTSDDSAVYYCARWGGDYVTGGGTTLTVSS (SEQ ID NO:28)

HC-CDR1:	GYTFTEYT	(SEQ ID NO:29)
HC-CDR2:	INPYSGGT	(SEQ ID NO:30)
HC-CDR3:	ARWGGDYV	(SEQ ID NO:27)

Anti-R175H clone 10C8

EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWMKQSHGKSLEWIGRINPYSGGTVY NQKFKGKATLTVDKSSSTAYMELRSLTSDDSAVYYCARWGGDYVTGGGTTLTVSS (SEQ ID NO:28)

HC-CDR1:	GYTFTEYT	(SEQ ID NO:29)
HC-CDR2:	INPYSGGT	(SEQ ID NO:30)
HC-CDR3:	ARWGGDYV	(SEQ ID NO:27)

Figure 25

Anti R175H Clone	CDR 1	CDR 2	CDR 3
Light Chain			
4H5	QSLLNSGNQKSY (SEQ ID NO:19)	GAS (SEQ ID NO:20)	QNDHSYPLT (SEQ ID NO:21)
7B9	QSLLNSGNQKSN (SEQ ID NO:23)	GAS (SEQ ID NO:20)	QNDHSYPLT (SEQ ID NO:21)
10C8	QSLLNSGNQKSN (SEQ ID NO:23)	GAS (SEQ ID NO:20)	QNDHSYPLT (SEQ ID NO:21)
Consensus	QSLLNSGNQKSX_1 (SEQ ID NO:31) where X_1 = Y or N	GAS (SEQ ID NO:20)	QNDHSYPLT (SEQ ID NO:21)
Heavy Chain			
4H5	GFTFTEYT (SEQ ID NO:25)	IDPNNGVT (SEQ ID NO:26)	ARWGGDYV (SEQ ID NO:27)
7B9	GYTFTEYT (SEQ ID NO:29)	INPYSGGT (SEQ ID NO:30)	ARWGGDYV (SEQ ID NO:27)
10C8	GYTFTEYT (SEQ ID NO:29)	INPYSGGT (SEQ ID NO:30)	ARWGGDYV (SEQ ID NO:27)
Consensus	GX_2TFTEYT (SEQ ID NO:32) where X_2 = F or Y	IX_3P$X_4$$X_5GX_6$T (SEQ ID NO:33) where X_3 = D or N; X_4 = N or Y; X_5 = N or S; and X_6 = V or G	ARWGGDYV (SEQ ID NO:27)

Figure 26

Light chain variable domains

Anti-R175H clone 4H5

>4H5_aa_L
DIVLTQSTSSLSVSAGERVTLSCKSSQSLLNSGNQKSYLAWYQQKPGQPPKLLIYGASTRE
SGVPDRFTGSGSETDFTLTISSVQPEDLAVYYCQNDHSYPLTFGAGTKLELK [SEQ ID
NO:18]

>4H5_ntd_L
GATATTGTGCTCACCCAATCTACATCCTCCCTGAGTGTGTCAGCTGGAGAGAGGGTCA
CTTTGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAAAAGAGTTACTTG
GCCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAACTGTTGATCTACGGGGCATCCA
CTAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGAAACCGATTTCAC
TCTTACCATCAGCAGTGTGCAGCCTGAAGACCTGGCAGTTTATTATTGTCAGAATGATC
ATAGTTATCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO: 34]

Anti-R175H clone 7B9

>7B9_aa_L
DIVMTQSPSSLSVSGGEKVTMSCKSSQSLLNSGNQKSNLAWYQQKPGQPPKLLIYGASTR
ESGVPDRFAGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGGGTKLELK [SEQ ID
NO:22]

>7B9_ntd_L
GATATTGTGATGACCCAGTCTCCATCCTCCCTGAGTGTGTCAGGAGGAGAGAAGGTCA
CTATGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAAAAGAGCAACTTG
GCCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAATTGTTGATCTATGGGGCATCCA
CTAGGGAATCTGGGGTCCCTGATCGCTTCGCAGGCAGTGGATCTGGAACCGATTTCAC
TCTTACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAAAATGATC
ATAGTTATCCGCTCACGTTCGGTGGTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO: 35]

Anti-R175H clone 10C8

>10C8_aa_L
DIVMTQSPSSLSVSGGEKVTMSCKSSQSLLNSGNQKSNLAWYQQKPGQPPKLLIYGASTR
ESGVPDRFAGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGGGTKLELK [SEQ ID
NO: 22]

>10C8_ntd_L
GATATTGTGATGACCCAGTCTCCATCCTCCCTGAGTGTGTCAGGAGGAGAGAAGGTCA
CTATGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAAAAGAGCAACTTG
GCCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAATTGTTGATCTATGGGGCATCCA
CTAGGGAATCTGGGGTCCCTGATCGCTTCGCAGGCAGTGGATCTGGAACCGATTTCAC
TCTTACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAAAATGATC
ATAGTTATCCGCTCACGTTCGGTGGTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO: 36]

Figure 27

Heavy chain variable domains

Anti-R175H clone 4H5

>4H5_aa_H
EVQLQQSGPELVKPGASVKISCKTSGFTFTEYTMHWMKQSHGRSLEWIGRIDPNNGVTVY
NQKFKVKATLTVDRSSSTAYLELRSLTSEDSAVYYCARWGGDYVTGGGTTLTVSS [SEQ ID
NO: 24]

>4H5_ntd_H
GAGGTTCAGCTGCAGCAGTCTGGACCTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGA
TATCTTGCAAGACTTCCGGATTCACATTCACTGAATACACCATGCACTGGATGAAACAG
AGCCATGGAAGGAGCCTTGAGTGGATCGGACGTATTGATCCTAACAATGGTGTTACTGT
TTATAACCAGAAGTTCAAGGTCAAGGCCACATTGACTGTGGACAGGTCCTCCAGCACA
GCCTATCTGGAGCTCCGCAGTCTGACGTCTGAGGACTCTGCAGTCTATTACTGTGCAA
GATGGGGTGGTGACTACGTCACGGGGGGAGGCACCACTCTCACAGTCTCCTCA [SEQ
ID NO: 37]

Anti-R175H clone 7B9

>7B9_aa_H
EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWMKQSHGKSLEWIGRINPYSGGTVY
NQKFKGKATLTVDKSSSTAYMELRSLTSDDSAVYYCARWGGDYVTGGGTTLTVSS [SEQ
ID NO: 28]

>7B9_ntd_H
GAGGTCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAG
ATATCCTGCAAGACTTCTGGCTACACTTTCACTGAATACACCATGCACTGGATGAAGCA
GAGCCATGGAAAGAGCCTTGAGTGGATTGGACGTATTAATCCTTATAGTGGTGGTACTG
TCTACAACCAGAAGTTCAAGGGCAAGGCCACATTGACTGTGGACAAGTCCTCCAGCAC
AGCCTATATGGAGCTCCGCAGCCTGACATCTGATGATTCTGCAGTCTATTACTGTGCAA
GATGGGGTGGTGACTACGTCACGGGGGGAGGCACCACTCTCACAGTCTCCTCA [SEQ
ID NO: 38]

Anti-R175H clone 10C8

>10C8_aa_H
EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWMKQSHGKSLEWIGRINPYSGGTVY
NQKFKGKATLTVDKSSSTAYMELRSLTSDDSAVYYCARWGGDYVTGGGTTLTVSS [SEQ
ID NO: 28]

>10C8_ntd_H
GAGGTGCAGCTTCAGCAGTCGGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAG
ATATCCTGCAAGACTTCTGGCTACACTTTCACTGAATACACCATGCACTGGATGAAGCA
GAGCCATGGAAAGAGCCTTGAGTGGATTGGACGTATTAATCCTTATAGTGGTGGTACTG
TCTACAACCAGAAGTTCAAGGGCAAGGCCACATTGACTGTGGACAAGTCCTCCAGCAC
AGCCTATATGGAGCTCCGCAGCCTGACATCTGATGATTCTGCAGTCTATTACTGTGCAA
GATGGGGTGGTGACTACGTCACGGGGGGAGGCACCACTCTCACAGTCTCCTCA [SEQ
ID NO: 39]

Figure 27 (cont.)

Anti-R248Q clone 3G11

DIVLTQTPLTLSVTIGQPASISCKSNQSLLYSDGKTYLNWLLQRPGQSPKRLIYLVSKLDSGV
PDRFTGSGSGTDFTLKISRVEAEDLGLYYCWQGTHFPLTFGAGTKLELK (SEQ ID NO:40)

LC-CDR1:	QSLLYSDGKTY	(SEQ ID NO:41)
LC-CDR2:	LVS	(SEQ ID NO:42)
LC-CDR3:	WQGTHFPLT	(SEQ ID NO:43)

Anti-R248Q clone 4H2

DIVITQSPLTLSVTIGQPASISCKSDQSLLYSDGKTYLNWLLQRPGQSPKRLIYLVSELDSGV
PDRFTGSGSGTDFTLKISRVEAEDLGLYYCWQGTHFPLTFGAGTKLELK (SEQ ID NO:44)

LC-CDR1:	QSLLYSDGKTY	(SEQ ID NO:41)
LC-CDR2:	LVS	(SEQ ID NO:42)
LC-CDR3:	WQGTHFPLT	(SEQ ID NO:43)

Figure 28

Anti-R248Q clone 3G11

DVQLQQSGPELVKPGASVKMSCKASGYTFTDYYVKWVKQSPGQSLEWIGDIHPKNGGTN YNQKFKGKAALTVDKSSSTAYMQLNSLTSEDSAVYFCAKMGGYDDYWGQGTTLTVSS (SEQ ID NO:45)

HC-CDR1:	GYTFTDYY	(SEQ ID NO:46)
HC-CDR2:	IHPKNGGT	(SEQ ID NO:47)
HC-CDR3:	AKMGGYDDY	(SEQ ID NO:48)

Anti-R248Q clone 4H2

QVQLKQSGPELVKPGASVKMSCKASGYTFTDYYLKWVRQSHGKSLEWIGDIDPKNGGTNY NQKFKGKATLTVDKSSSTAYMQLNSLTSEDSAVYYCAKQGGFDDYWGQGTTLTVSS (SEQ ID NO:49)

HC-CDR1:	GYTFTDYY	(SEQ ID NO:46)
HC-CDR2:	IDPKNGGT	(SEQ ID NO:50)
HC-CDR3:	AKQGGFDDY	(SEQ ID NO:51)

Figure 29

Anti-R248Q Clone	CDR 1	CDR 2	CDR 3
Light Chain			
3G11	QSLLYSDGKTY (SEQ ID NO:41)	LVS (SEQ ID NO:42)	WQGTHFPLT (SEQ ID NO:43)
4H2	QSLLYSDGKTY (SEQ ID NO:41)	LVS (SEQ ID NO:42)	WQGTHFPLT (SEQ ID NO:43)
Consensus	QSLLYSDGKTY (SEQ ID NO:41)	LVS (SEQ ID NO:42)	WQGTHFPLT (SEQ ID NO:43)
Heavy Chain			
3G11	GYTFTDYY (SEQ ID NO:46)	IHPKNGGT (SEQ ID NO:47)	AKMGGYDDY(SEQ ID NO:48)
4H2	GYTFTDYY (SEQ ID NO:46)	IDPKNGGT (SEQ ID NO:50)	AKQGGFDDY (SEQ ID NO:51)
Consensus	GYTFTDYY (SEQ ID NO:46)	IX_7PKNGGT (SEQ ID NO:52) where X_7 = H or D	AKX_8GGX_9DDY (SEQ ID NO:53) where X_8 = M or Q; and X_9 = Y or F

Figure 30

Light chain variable domains

Anti-R248Q clone 3G11

>3G11_aa_L
DIVLTQTPLTLSVTIGQPASISCKSNQSLLYSDGKTYLNWLLQRPGQSPKRLIYLVSKLDSGV
PDRFTGSGSGTDFTLKISRVEAEDLGLYYCWQGTHFPLTFGAGTKLELK [SEQ ID NO:40]

>3G11_ntd_L
GATATTGTGCTGACACAGACTCCACTCACTTTGTCGGTTACCATTGGACAACCAGCCTC
CATCTCTTGCAAGTCAAATCAGAGCCTCTTATATAGTGATGGAAAGACATATTTGAATTG
GTTATTACAGAGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTAAACTGG
ACTCTGGAGTCCCTGACAGGTTCACTGGCAGTGGATCAGGGACAGATTTCACACTGAA
AATCAGCAGAGTGGAGGCTGAGGATTTGGGACTTTATTATTGCTGGCAAGGTACACATT
TTCCTCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO: 54]

Anti-R248Q clone 4H2

>4H2_aa_L
DIVITQSPLTLSVTIGQPASISCKSDQSLLYSDGKTYLNWLLQRPGQSPKRLIYLVSELDSGV
PDRFTGSGSGTDFTLKISRVEAEDLGLYYCWQGTHFPLTFGAGTKLELK [SEQ ID NO:44]

>4H2_ntd_L
GACATTGTGATCACACAGTCTCCACTCACTTTGTCGGTTACCATTGGACAACCAGCCTC
CATCTCTTGCAAGTCAGATCAGAGCCTCTTATATAGTGATGGAAAGACATATTTGAATTG
GCTATTACAGAGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTGAACTG
GACTCTGGAGTCCCTGACAGGTTCACTGGCAGTGGATCAGGGACAGATTTCACACTGA
AAATCAGCAGAGTGGAGGCTGAGGATTTGGGACTTTATTATTGCTGGCAAGGTACACAT
TTTCCTCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO: 55]

Figure 31

Heavy chain variable domains

Anti-R248Q clone 3G11

>3G11_aa_H
DVQLQQSGPELVKPGASVKMSCKASGYTFTDYYVKWVKQSPGQSLEWIGDIHPKNGGTN
YNQKFKGKAALTVDKSSSTAYMQLNSLTSEDSAVYFCAKMGGYDDYWGQGTTLTVSS
[SEQ ID NO:45]

>3G11_ntd_H
GATGTGCAGCTTCAGCAGTCAGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGA
TGTCCTGTAAGGCTTCTGGATACACATTCACTGACTACTATGTGAAGTGGGTGAAGCAG
AGTCCTGGACAGAGCCTTGAGTGGATTGGAGATATTCATCCTAAGAACGGTGGTACTAA
CTACAACCAGAAGTTCAAGGGCAAGGCCGCATTGACTGTGGACAAATCCTCCAGCACA
GCCTACATGCAGCTCAACAGCCTGACATCTGAGGACTCTGCAGTCTATTTCTGTGCAAA
AATGGGAGGCTACGACGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
[SEQ ID NO: 56]

Anti-R248Q clone 4H2

>4H2_aa_H
QVQLKQSGPELVKPGASVKMSCKASGYTFTDYYLKWVRQSHGKSLEWIGDIDPKNGGTNY
NQKFKGKATLTVDKSSSTAYMQLNSLTSEDSAVYYCAKQGGFDDYWGQGTTLTVSS [SEQ
ID NO:49]

>4H2_ntd_H
CAGGTGCAGCTGAAGCAGTCAGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAG
ATGTCCTGTAAGGCTTCTGGATACACATTCACTGACTACTATCTGAAGTGGGTGAGGCA
GAGTCATGGAAAGAGCCTTGAGTGGATTGGAGATATAGATCCCAAGAATGGTGGTACT
AATTACAACCAGAAGTTTAAGGGCAAGGCCACATTGACTGTGGACAAATCCTCCAGCAC
AGCCTACATGCAGCTCAACAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAA
AACAGGGGGGGTTCGACGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
[SEQ ID NO: 57]

Figure 31 (cont.)

Anti-R273H clone 13E4

DIVMTQSPLSLPVNLGDQVSLSCRSSQSIVHNNGDTYLEWYLQKPGQSPKLLIYKVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHLPLTFGSGTKLELK (SEQ ID NO:58)

LC-CDR1:	QSIVHNNGDTY	(SEQ ID NO:59)
LC-CDR2:	KVS	(SEQ ID NO:60)
LC-CDR3:	FQGSHLPLT	(SEQ ID NO:61)

Figure 32

Anti-R273H clone 13E4

EVQLLETGGDLVTPGGSLKLSCAASGFSFSDYYMYWVRQTPEKRLEWVATISVGGTYTFY SDNVKGRFVISRDNARKNLHLEMNSLKSEDAAMYYCVRDGNDGKFLGYWGQGTFVTVTV S (SEQ ID NO:62)

HC-CDR1:	GFSFSDYY	(SEQ ID NO:63)
HC-CDR2:	ISVGGTYT	(SEQ ID NO:64)
HC-CDR3:	VRDGNDGKFLG	(SEQ ID NO:65)

Figure 33

Anti-R273H clone 13E4

Light chain variable domain

>13E4_aa_L
DIVMTQSPLSLPVNLGDQVSLSCRSSQSIVHNNGDTYLEWYLQKPGQSPKLLIYKVSNRFS
GVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHLPLTFGSGTKLELK [SEQ ID NO:58]

>13E4_ntd_L
GATATTGTGATGACACAATCTCCACTCTCCCTGCCTGTCAATCTTGGAGATCAAGTCTC
CCTCTCTTGCAGATCTAGTCAGAGCATTGTACATAATAATGGAGACACCTATTTAGAATG
GTACCTACAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGAT
TTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAA
GATCAGCAGAGTGGAGGCTGAGGATCTGGGAATTTATTACTGCTTTCAAGGTTCACATC
TTCCGCTCACGTTCGGTTCTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO: 66]

Heavy chain variable domain

>13E4_aa_H
EVQLLETGGDLVTPGGSLKLSCAASGFSFSDYYMYWVRQTPEKRLEWVATISVGGTYTFY
SDNVKGRFVISRDNARKNLHLEMNSLKSEDAAMYYCVRDGNDGKFLGYWGQGTFVTVTV
S [SEQ ID NO:62]

>13E4_ntd_H
GAAGTGCAGCTGTTGGAGACTGGGGGAGACTTAGTGACGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAGCCTCTGGATTCAGCTTCAGTGACTATTATATGTATTGGGTTCGCCA
GACTCCGGAAAAGAGGCTGGAGTGGGTCGCAACTATTAGTGTTGGTGGTACATACACC
TTCTATTCAGACAATGTGAAGGGGAGATTCGTCATCTCCAGAGACAATGCCAGGAAAAA
TCTGCATCTGGAAATGAACAGTCTGAAGTCTGAGGACGCAGCCATGTATTACTGTGTAA
GAGATGGCAACGATGGAAAATTTCTTGGGTACTGGGGCCAGGGGACTTTCGTCACTGT
CACT [SEQ ID NO: 67]

Figure 34

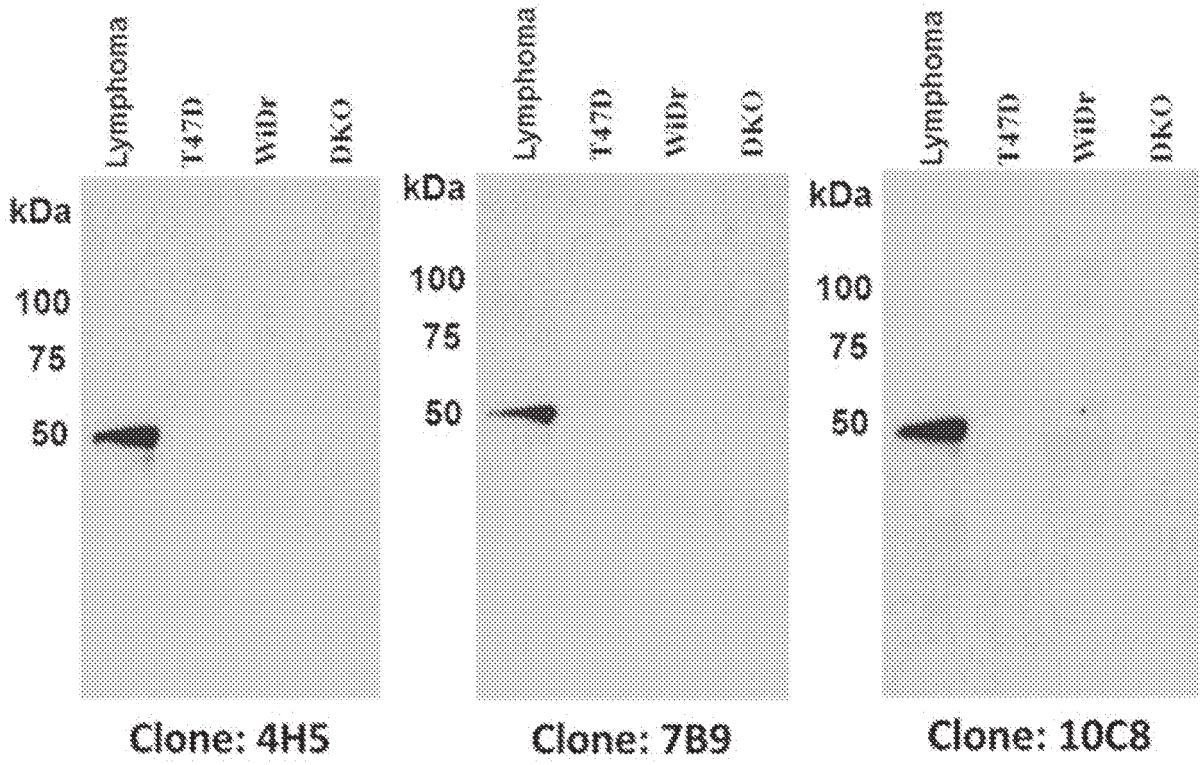


Figure 35

Clone: 4H5

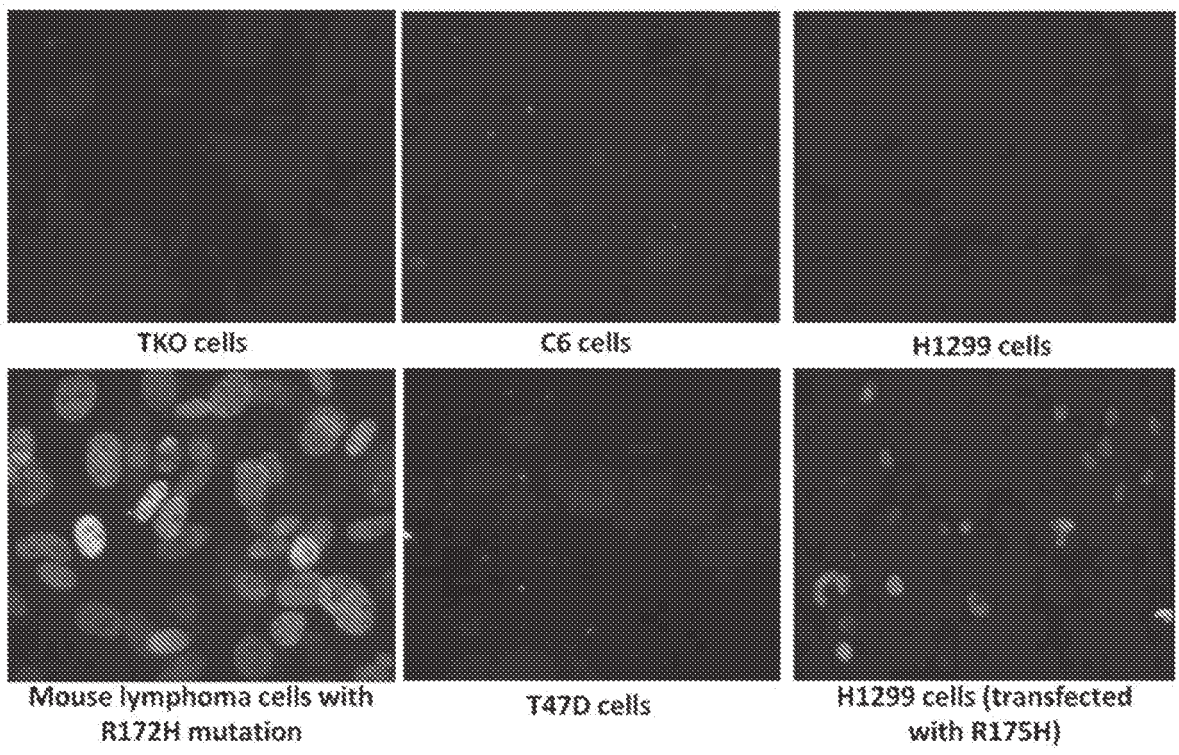


Figure 36A

Clone: 7B9

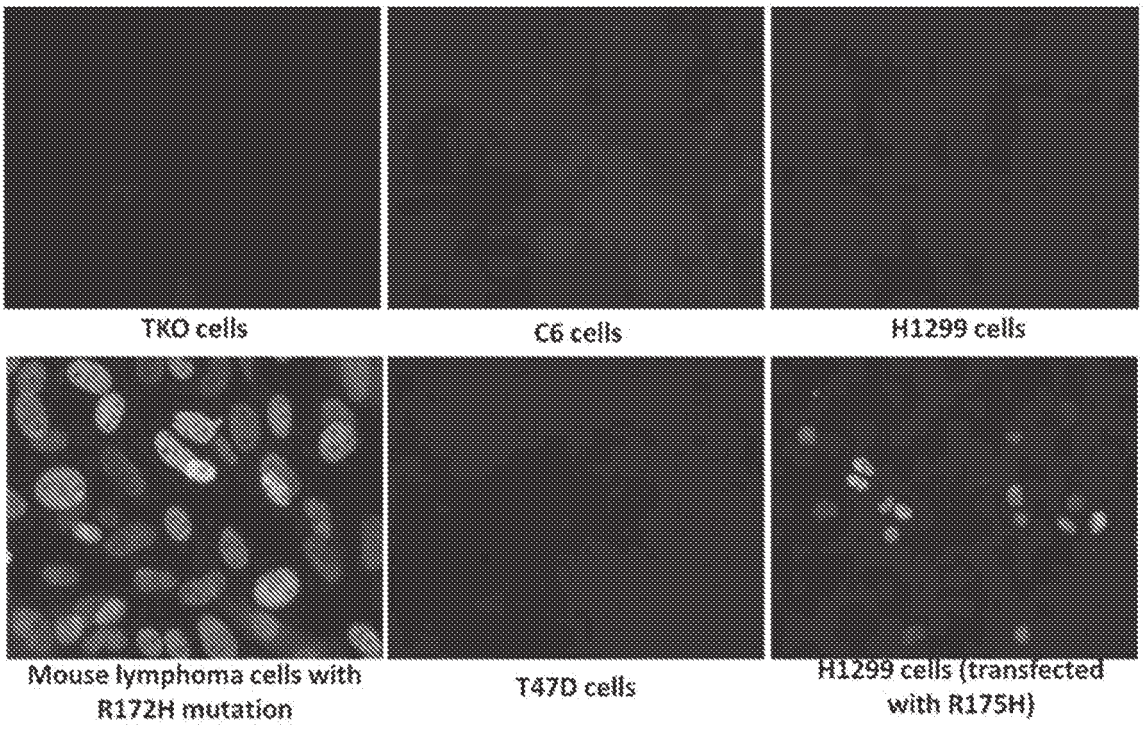


Figure 36B

Clone: 10C8

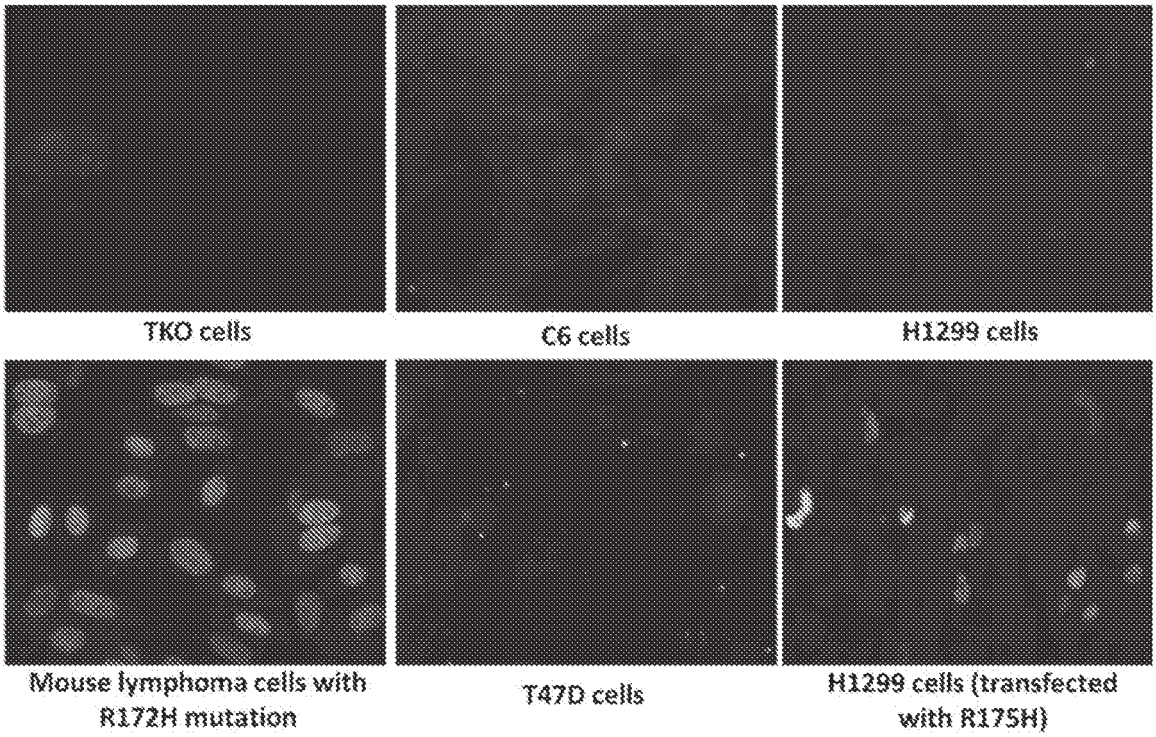


Figure 36C

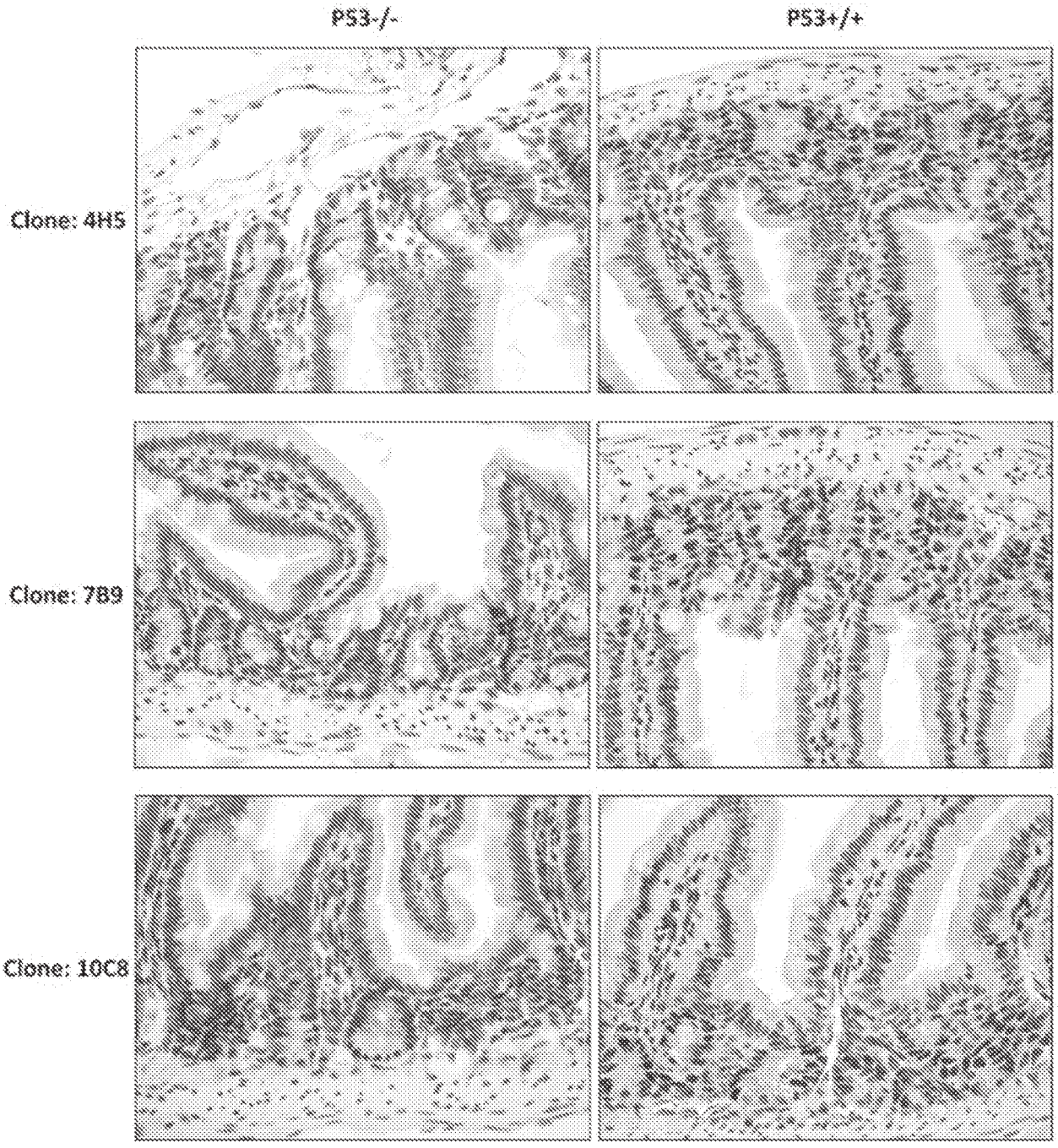


Figure 37

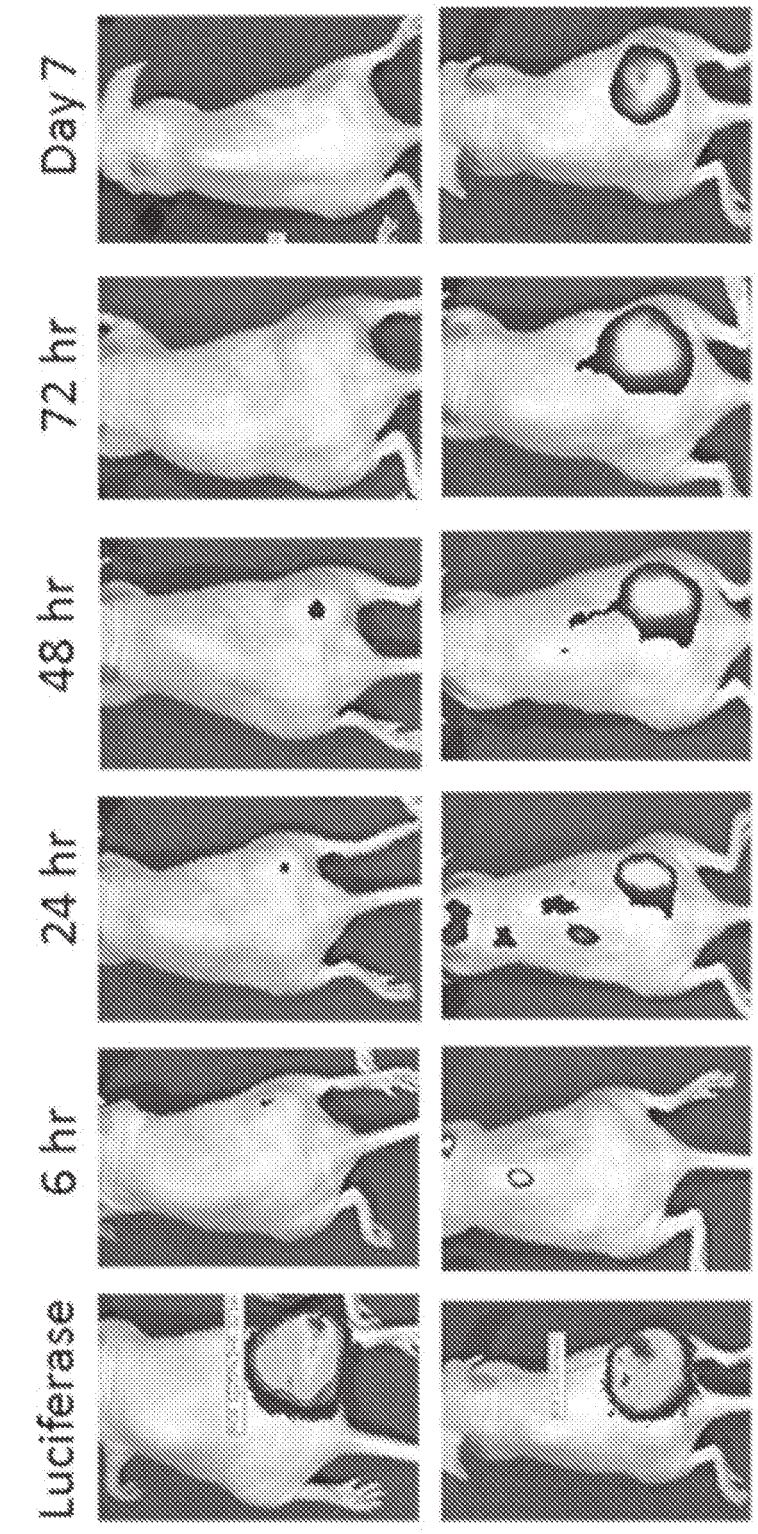


Figure 38

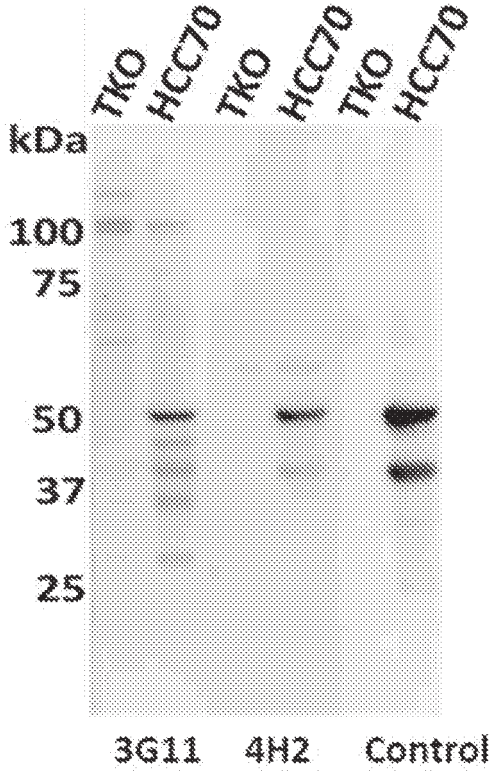


Figure 39

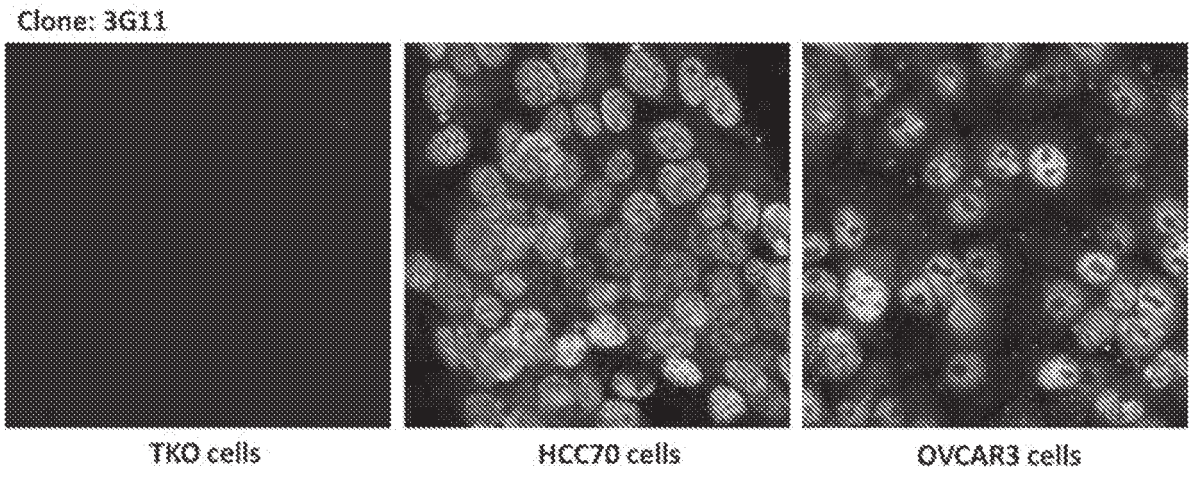


Figure 40A

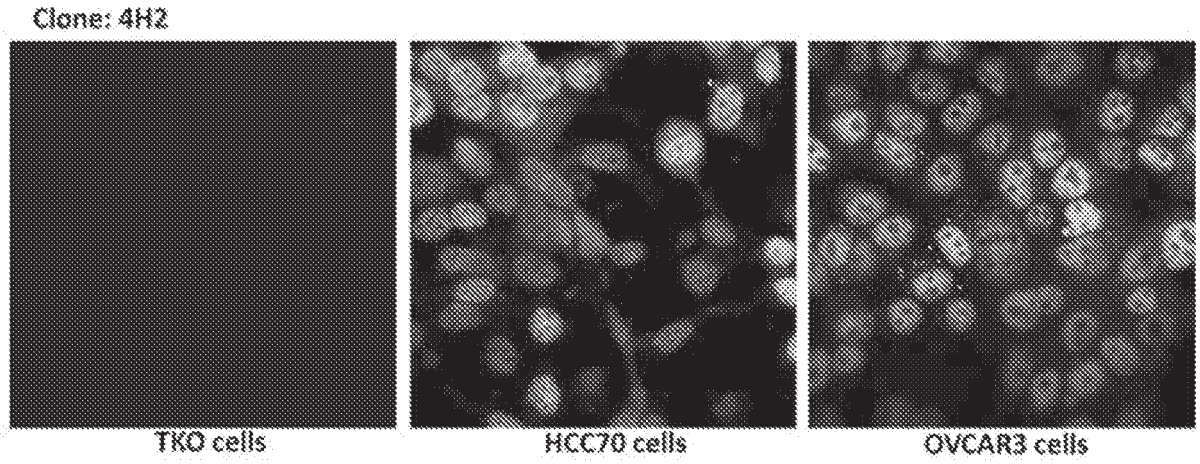


Figure 40B

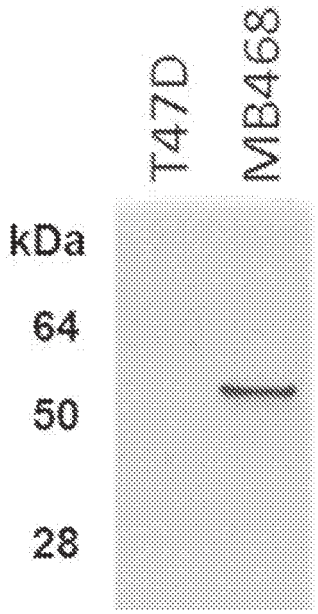


Figure 41

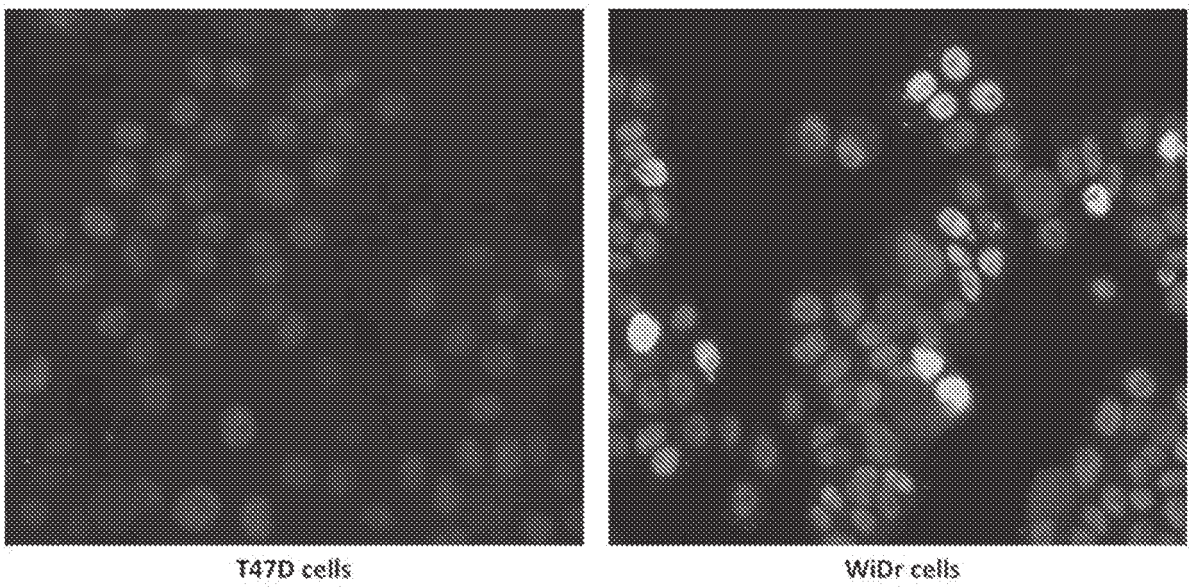


Figure 42

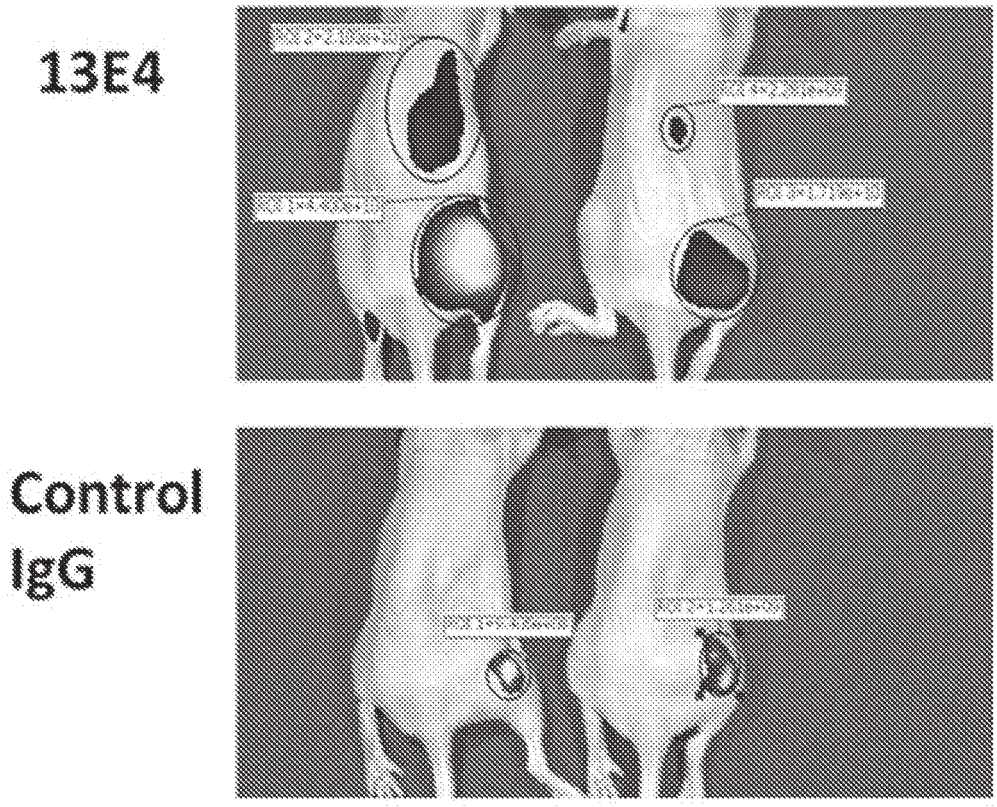


Figure 43

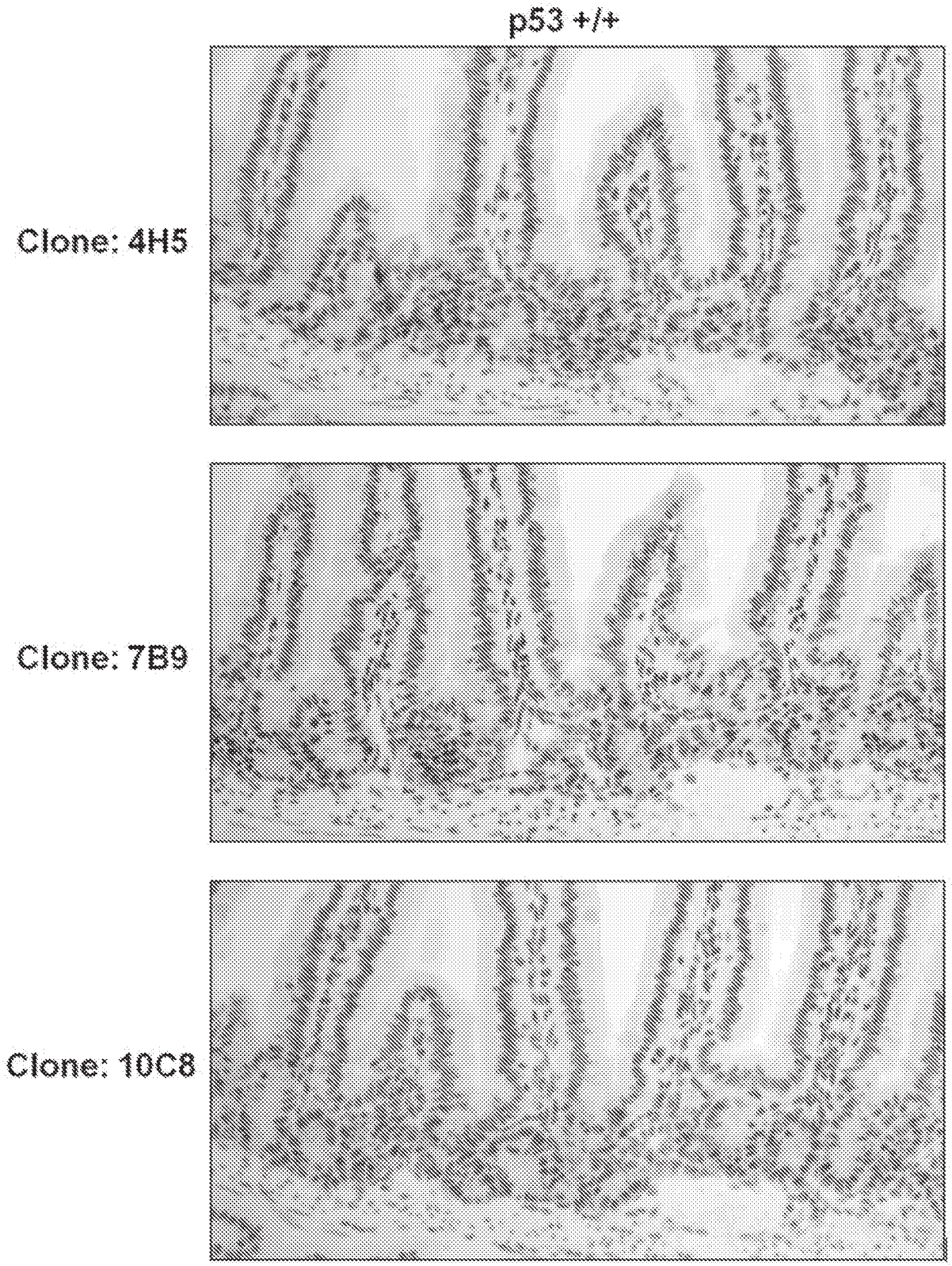


Figure 44

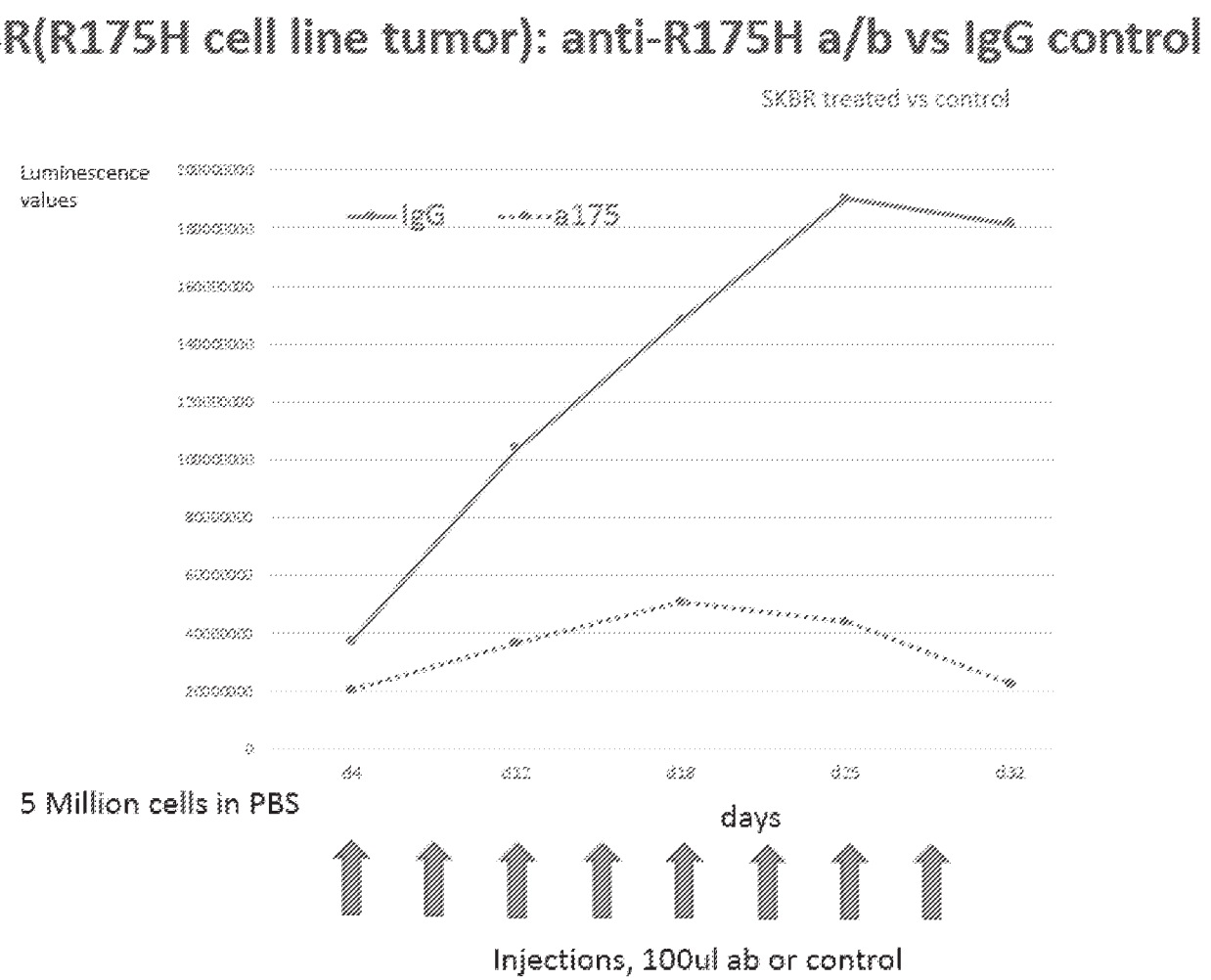

SKBR(R175H cell line tumor): anti-R175H a/b vs IgG control
SKBR treated vs control
Luminescence values
IgG
a175
d4
d11
d18
d25
d32
5 Million cells in PBS
days
Injections, 100ul ab or control

Figure 45

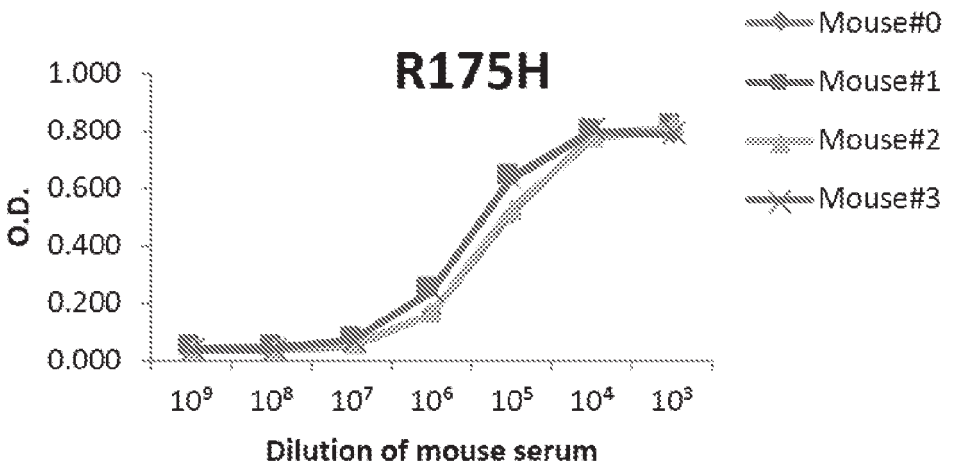


Figure 46A

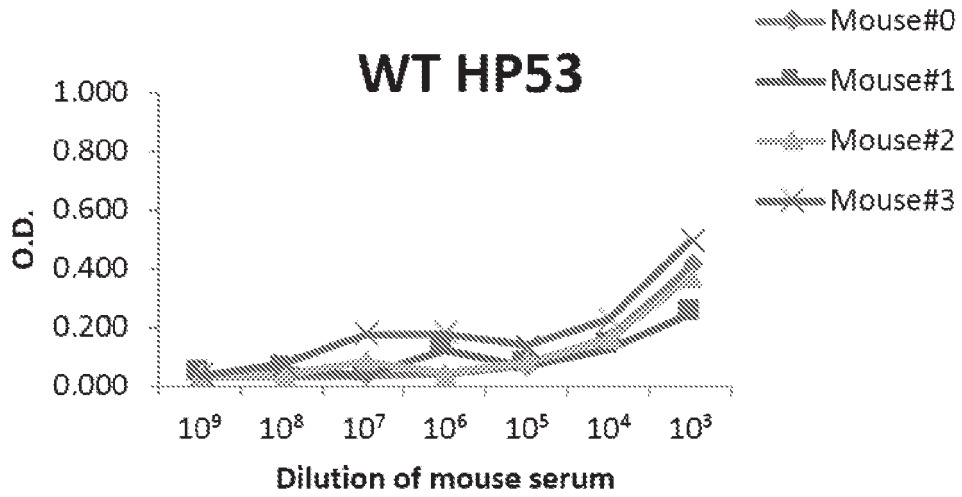


Figure 46B

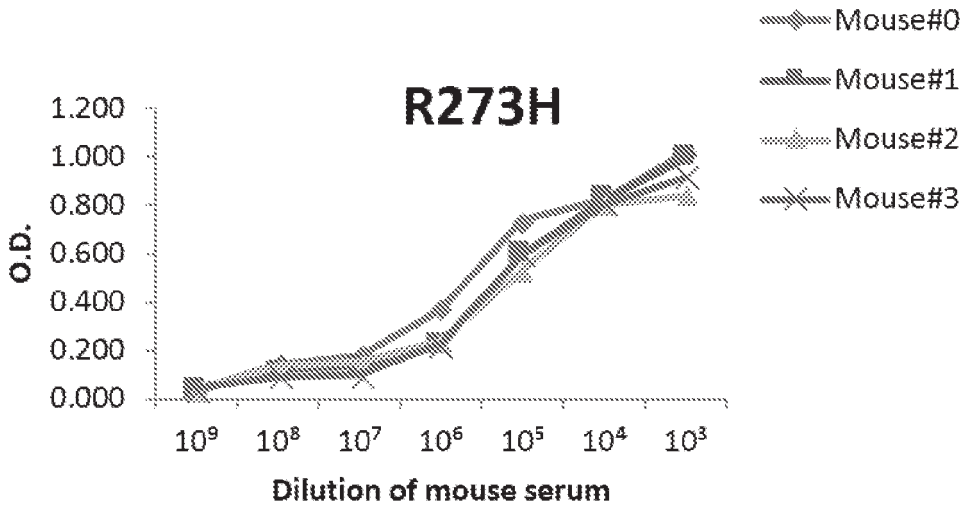


Figure 46C

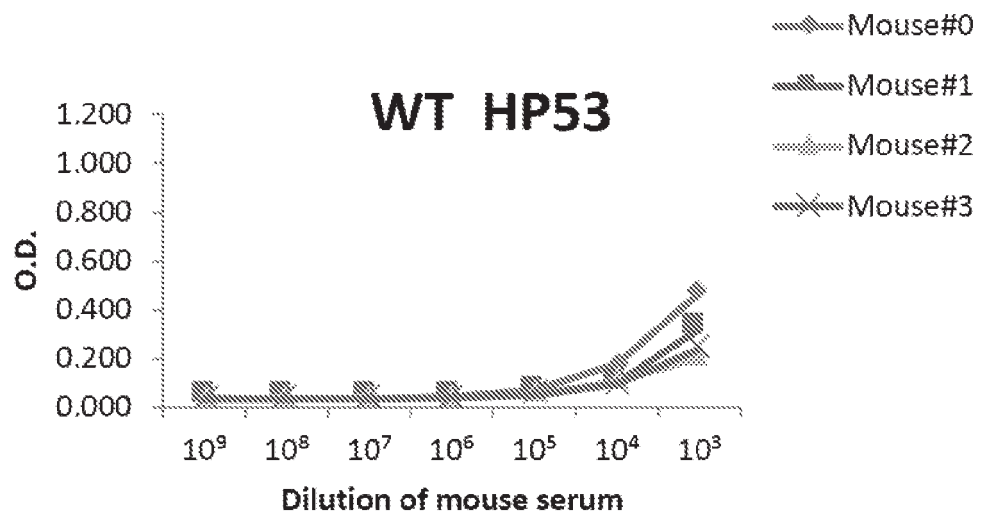


Figure 46D

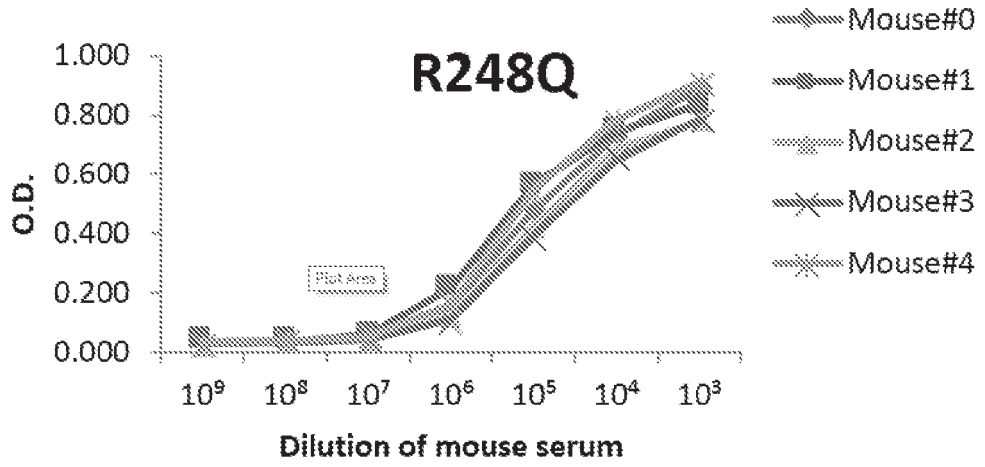


Figure 46E

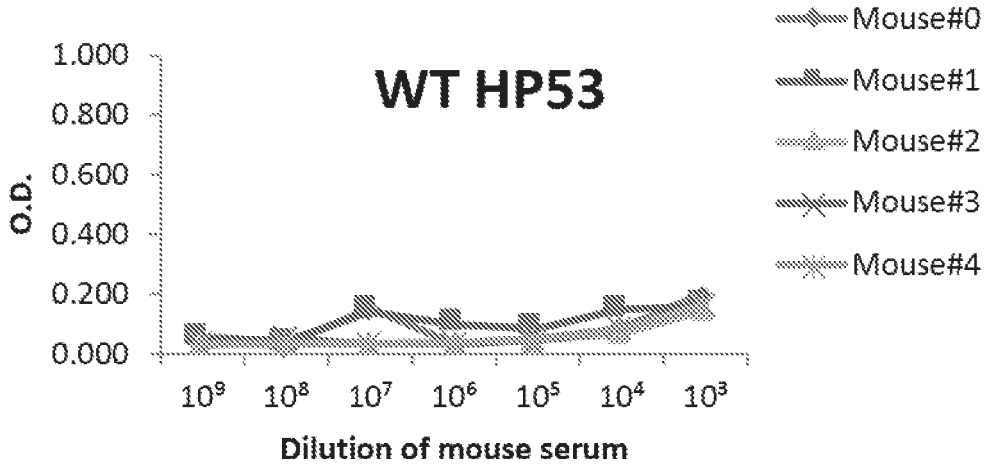
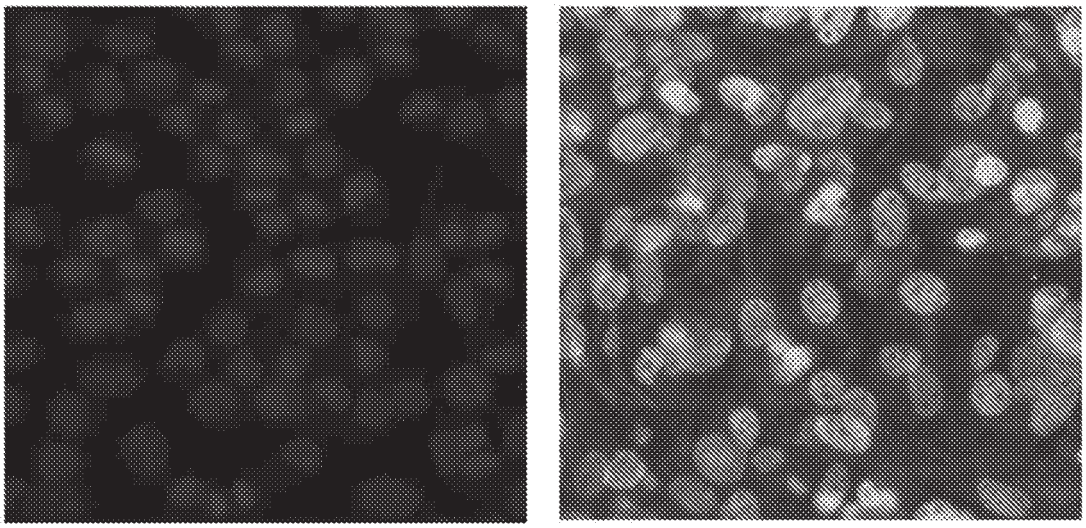


Figure 46F

R175H

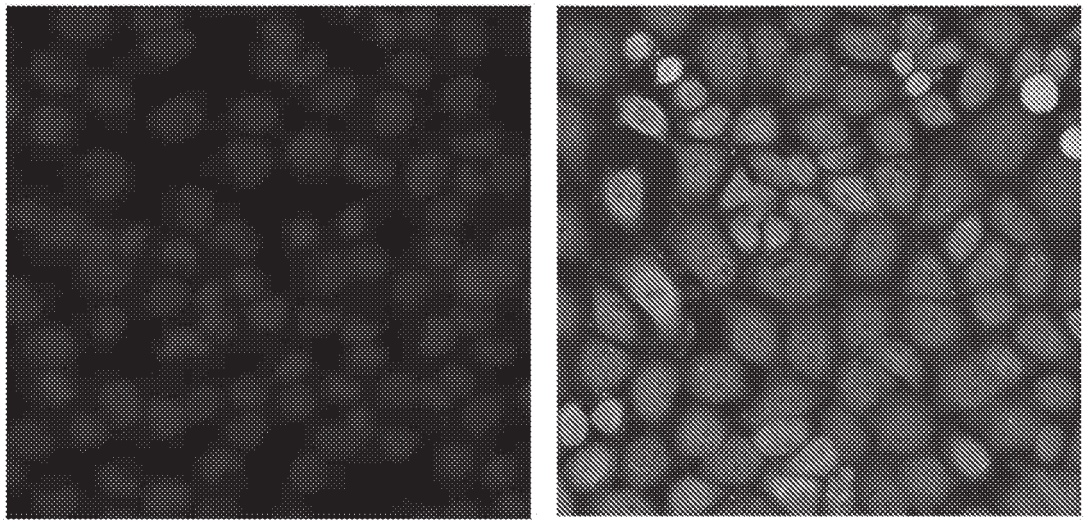


Normal mouse serum on R175H positive cells

R175H Mouse serum on R175H positive cells

Figure 47A

R248Q

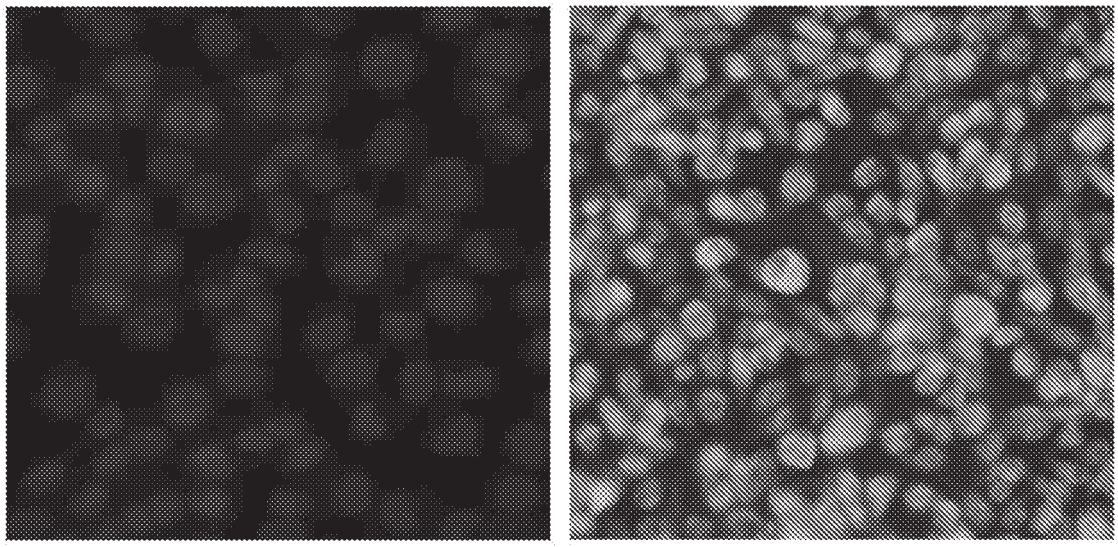


Normal mouse serum on R248Q positive cells

R248Q Mouse serum on R248Q positive cells

Figure 47B

R273H



Normal mouse serum on R273H positive cells

R273H Mouse serum on R273H positive cells

Figure 47C

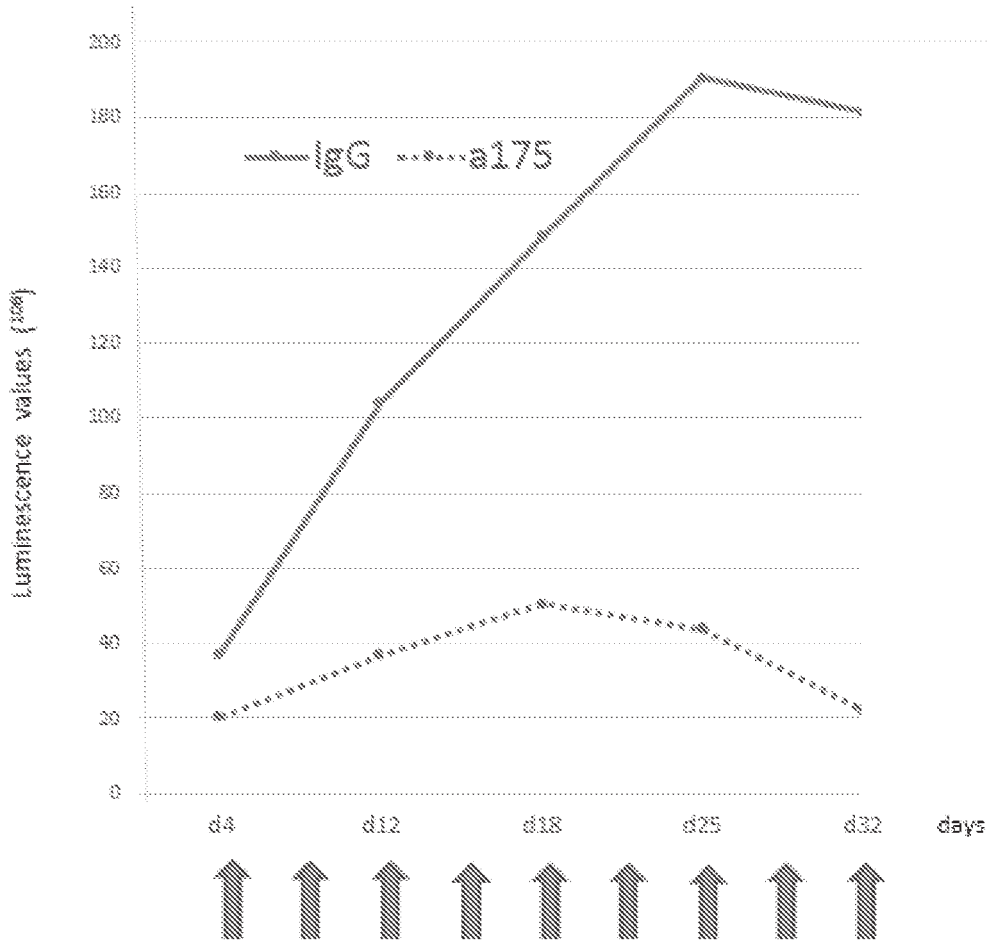

IgG
a175
Luminescence values (RLU)
0
20
40
60
80
100
120
140
160
180
200
d4
d12
d18
d25
d32
days

Figure 48

Mouse-human chimeric anti-R175H clone 4H5

DIVLTQSTSSLSVSAGERVTLSCKSSQSLLNSGNQKSYLAWYQQKPGQPPKLLIYGASTRE SGVPDRFTGSGSETDFTLTISSVQPEDLAVYYCQNDHSYPLTFGAGTKLELK (SEQ ID NO:237)

LC-CDR1:	QSLLNSGNQKSY	(SEQ ID NO:19)
LC-CDR2:	GAS	(SEQ ID NO:20)
LC-CDR3:	QNDHSYPLT	(SEQ ID NO:21)

Mouse-human chimeric anti-R175H clone 7B9

DIVMTQSPSSLSVSGGEKVTMSCKSSQSLLNSGNQKSNLAWYQQKPGQPPKLLIYGASTR ESGVPDRFAGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGGGTKLELK (SEQ ID NO:238)

LC-CDR1:	QSLLNSGNQKSN	(SEQ ID NO:23)
LC-CDR2:	GAS	(SEQ ID NO:20)
LC-CDR3:	QNDHSYPLT	(SEQ ID NO:21)

Mouse-human chimeric anti-R175H clone 10C8

DIVMTQSPSSLSVSGGEKVTMSCKSSQSLLNSGNQKSNLAWYQQKPGQPPKLLIYGASTR ESGVPDRFAGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGGGTKLELK (SEQ ID NO:238)

LC-CDR1:	QSLLNSGNQKSN	(SEQ ID NO:23)
LC-CDR2:	GAS	(SEQ ID NO:20)
LC-CDR3:	QNDHSYPLT	(SEQ ID NO:21)

Figure 49

Mouse-human chimeric anti-R175H clone 4H5

EVQLQQSGPELVKPGASVKISCKTSGFTFTEYTMHWMKQSHGRSLEWIGRIDPNNGVTVY NQKFKVKATLTVDRSSSTAYLELRSLTSEDSAVYYCARWGGDYVTGGGTTLTVSS (SEQ ID NO:239)

HC-CDR1:	GFTFTEYT	(SEQ ID NO:25)
HC-CDR2:	IDPNNGVT	(SEQ ID NO:26)
HC-CDR3:	ARWGGDYV	(SEQ ID NO:27)

Mouse-human chimeric anti-R175H clone 7B9

EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWMKQSHGKSLEWIGRINPYSGGTVY NQKFKGKATLTVDKSSSTAYMELRSLTSDDSAVYYCARWGGDYVTGGGTTLTVSS (SEQ ID NO:240)

HC-CDR1:	GYTFTEYT	(SEQ ID NO:29)
HC-CDR2:	INPYSGGT	(SEQ ID NO:30)
HC-CDR3:	ARWGGDYV	(SEQ ID NO:27)

Mouse-human chimeric anti-R175H clone 10C8

EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWMKQSHGKSLEWIGRINPYSGGTVY NQKFKGKATLTVDKSSSTAYMELRSLTSDDSAVYYCARWGGDYVTGGGTTLTVSS (SEQ ID NO:241)

HC-CDR1:	GYTFTEYT	(SEQ ID NO:29)
HC-CDR2:	INPYSGGT	(SEQ ID NO:30)
HC-CDR3:	ARWGGDYV	(SEQ ID NO:27)

Figure 50

Light chain variable domains

Mouse-human chimeric anti-R175H clone 4H5

>MH 4H5_aa_L
DIVLTQSTSSLSVSAGERVTLSCKSSQSLLNSGNQKSYLAWYQQKPGQPPKLLIYGASTRE
SGVPDRFTGSGSETDFTLTISSVQPEDLAVYYCQNDHSYPLTFGAGTKLELK [SEQ ID
NO:237]

>MH 4H5_ntd_L
GATATTGTGcTCaCCCaATCTACATCCTCCCTGAGTGTGTCAGCTGGAGAGAGGGTCAC
TTTGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAAAAGAGTTACTTGG
CCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAACTGTTGATCTACGGGGCATCCAC
TAGGGAATCTGGGGTCCCTGATCGCTTCACAGGCAGTGGATCTGAAACCGATTTCACT
CTTACCATCAGCAGTGTGCAGCCTGAAGACCTGGCAGTTTATTATTGTCAGAATGATCA
TAGTTATCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO:242]

Mouse-human chimeric anti-R175H clone 7B9

>MH 7B9_aa_L
DIVMTQSPSSLSVSGGEKVTMSCKSSQSLLNSGNQKSNLAWYQQKPGQPPKLLIYGASTR
ESGVPDRFAGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGGGTKLELK [SEQ ID
NO:238]

>MH 7B9_ntd_L
GATATTGTGATGACCCAGTCTCCATCCTCCCTGAGTGTGTCAGGAGGAGAGAAGGTCA
CTATGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAAAAGAGCAACTTG
GCCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAATTGTTGATCTATGGGGCATCCA
CTAGGGAATCTGGGGTCCCTGATCGCTTCGCAGGCAGTGGATCTGGAACCGATTTCAC
TCTTACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAAAATGATC
ATAGTTATCCGCTCACGTTCGGTGGTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO:243]

Mouse-human chimeric anti-R175H clone 10C8

>MH 10C8_aa_L
DIVMTQSPSSLSVSGGEKVTMSCKSSQSLLNSGNQKSNLAWYQQKPGQPPKLLIYGASTR
ESGVPDRFAGSGSGTDFTLTISSVQAEDLAVYYCQNDHSYPLTFGGGTKLELK [SEQ ID
NO:238]

>MH 10C8_ntd_L
GATATTGTGATGACCCAGTCTCCATCCTCCCTGAGTGTGTCAGGAGGAGAGAAGGTCA
CTATGAGCTGCAAGTCCAGTCAGAGTCTGTTAAACAGTGGAAATCAAAAGAGCAACTTG
GCCTGGTACCAGCAGAAACCAGGGCAGCCTCCTAAATTGTTGATCTATGGGGCATCCA
CTAGGGAATCTGGGGTCCCTGATCGCTTCGCAGGCAGTGGATCTGGAACCGATTTCAC
TCTTACCATCAGCAGTGTGCAGGCTGAAGACCTGGCAGTTTATTACTGTCAAAATGATC
ATAGTTATCCGCTCACGTTCGGTGGTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO:243]

Figure 51

Heavy chain variable domains

Mouse-human chimeric anti-R175H clone 4H5

>MH 4H5_aa_H
EVQLQQSGPELVKPGASVKISCKTSGFTFTEYTMHWMKQSHGRSLEWIGRIDPNNGVTVY
NQKFKVKATLTVDRSSSTAYLELRSLTSEDSAVYYCARWGGDYVTGGGTTLTVSS [SEQ ID
NO:239]

>MH 4H5_ntd_H
GAGGTTCAGCTGCAGCAGTCTGGACCTGAACTGGTGAAGCCTGGGGCTTCAGTGAAGA
TATCTTGCAAGACTTCCGGATTCACATTCACTGAATACACCATGCACTGGATGAAACAG
AGCCATGGAAGGAGCCTTGAGTGGATCGGACGTATTGATCCTAACAATGGTGTTACTGT
TTATAACCAGAAGTTCAAGGTCAAGGCCACATTGACTGTGGACAGGTCCTCCAGCACA
GCCTATCTGGAGCTCCGCAGTCTGACGTCTGAGGACTCTGCAGTCTATTACTGTGCAA
GATGGGGTGGTGACTACGTCACGGGGGGAGGCACCACTCTCACAGTCTCCTCA [SEQ
ID NO:244]

Mouse-human chimeric anti-R175H clone 7B9

>MH 7B9_aa_H
EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWMKQSHGKSLEWIGRINPYSGGTVY
NQKFKGKATLTVDKSSSTAYMELRSLTSDDSAVYYCARWGGDYVTGGGTTLTVSS [SEQ
ID NO:240]

>MH 7B9_ntd_H
GAGGTCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAG
ATATCCTGCAAGACTTCTGGCTACACTTTCACTGAATACACCATGCACTGGATGAAGCA
GAGCCATGGAAAGAGCCTTGAGTGGATTGGACGTATTAATCCTTATAGTGGTGGTACTG
TCTACAACCAGAAGTTCAAGGGCAAGGCCACATTGACTGTGGACAAGTCCTCCAGCAC
AGCCTATATGGAGCTCCGCAGCCTGACATCTGATGATTCTGCAGTCTATTACTGTGCAA
GATGGGGTGGTGACTACGTCACGGGGGGAGGCACCACTCTCACAGTCTCCTCA [SEQ
ID NO:245]

Mouse-human chimeric anti-R175H clone 10C8

>MH 10C8_aa_H
EVQLQQSGPELVKPGASVKISCKTSGYTFTEYTMHWMKQSHGKSLEWIGRINPYSGGTVY
NQKFKGKATLTVDKSSSTAYMELRSLTSDDSAVYYCARWGGDYVTGGGTTLTVSS [SEQ
ID NO:241]

>MH 10C8_ntd_H
GAGGTGCAGCTTCaGCAGTCGGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAG
ATATCCTGCAAGACTTCTGGCTACACTTTCACTGAATACACCATGCACTGGATGAAGCA
GAGCCATGGAAAGAGCCTTGAGTGGATTGGACGTATTAATCCTTATAGTGGTGGTACTG
TCTACAACCAGAAGTTCAAGGGCAAGGCCACATTGACTGTGGACAAGTCCTCCAGCAC
AGCCTATATGGAGCTCCGCAGCCTGACATCTGATGATTCTGCAGTCTATTACTGTGCAA
GATGGGGTGGTGACTACGTCACGGGGGGAGGCACCACTCTCACAGTCTCCTCA [SEQ
ID NO:246]

Figure 52

Mouse-human chimeric anti-R273H clone 13E4

DIVMTQSPLSLPVNLGDQVSLSCRSSQSIVHNNGDTYLEWYLQKPGQSPKLLIYKVSNRFS GVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHLPLTFGSGTKLELK (SEQ ID NO:247)

LC-CDR1:	QSIVHNNGDTY	(SEQ ID NO:59)
LC-CDR2:	KVS	(SEQ ID NO:60)
LC-CDR3:	FQGSHLPLT	(SEQ ID NO:61)

Figure 53

Mouse-human chimeric anti-R273H clone 13E4

EVQLLETGGDLVTPGGSLKLSCAASGFSFSDYYMYWVRQTPEKRLEWVATISVGGTYTFY SDNVKGRFVISRDNARKNLHLEMNSLKSEDAAMYYCVRDGNDGKFLGYWGQGTFVTVTV S (SEQ ID NO:248)

HC-CDR1:	GFSFSDYY	(SEQ ID NO:63)
HC-CDR2:	ISVGGTYT	(SEQ ID NO:64)
HC-CDR3:	VRDGNDGKFLG	(SEQ ID NO:65)

Figure 54

Mouse-human chimeric anti-R273H clone 13E4 light chain variable domain

>MH 13E4_aa_L
DIVMTQSPLSLPVNLGDQVSLSCRSSQSIVHNNGDTYLEWYLQKPGQSPKLLIYKVSNRFS
GVPDRFSGSGSGTDFTLKISRVEAEDLGIYYCFQGSHLPLTFGSGTKLELK [SEQ ID
NO:247]

>MH 13E4_ntd_L
GATATTGTGATGACACAATCTCCACTCTCCCTGCCTGTCAATCTTGGAGATCAAGTCTC
CCTCTCTTGCAGATCTAGTCAGAGCATTGTACATAATAATGGAGACACCTATTTAGAATG
GTACCTACAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGAT
TTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAA
GATCAGCAGAGTGGAGGCTGAGGATCTGGGAATTTATTACTGCTTTCAAGGTTCACATC
TTCCGCTCACGTTCGGTTCTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO:249]

Figure 55

Mouse-human chimeric anti-R273H clone 13E4 heavy chain variable domain

>MH 13E4_aa_H
EVQLLETGGDLVTPGGSLKLSCAASGFSFSDYYMYWVRQTPEKRLEWVATISVGGTYTFY
SDNVKGRFVISRDNARKNLHLEMNSLKSEDAAMYYCVRDGNDGKFLGYWGQGTFVTVTV
S [SEQ ID NO:248]

>MH 13E4_ntd_H
GAAGTGCAGCTGTTGGAGACTGGGGGAGACTTAGTGACGCCTGGAGGGTCCCTGAAA
CTCTCCTGTGCAGCCTCTGGATTCAGCTTCAGTGACTATTATATGTATTGGGTTCGCCA
GACTCCGGAAAAGAGGCTGGAGTGGGTCGCAACTATTAGTGTTGGTGGTACATACACC
TTCTATTCAGACAATGTGAAGGGGAGATTCGTCATCTCCAGAGACAATGCCAGGAAAAA
TCTGCATCTGGAAATGAACAGTCTGAAGTCTGAGGACGCAGCCATGTATTACTGTGTAA
GAGATGGCAACGATGGAAAATTTCTTGGGTACTGGGGCCAGGGGACTTTCGTCACTGT
CACTGTCTCT [SEQ ID NO:250]

Figure 56

Mouse-human chimeric anti-R248Q clone 3G11

DIVLTQTPLTLSVTIGQPASISCKSNQSLLYSDGKTYLNWLLQRPGQSPKRLIYLVSKLDSGV
PDRFTGSGSGTDFTLKISRVEAEDLGLYYCWQGTHFPLTFGAGTKLELK (SEQ ID NO:251)

LC-CDR1:	QSLLYSDGKTY	(SEQ ID NO:41)
LC-CDR2:	LVS	(SEQ ID NO:42)
LC-CDR3:	WQGTHFPLT	(SEQ ID NO:43)

Mouse-human chimeric anti-R248Q clone 4H2

DIVITQSPLTLSVTIGQPASISCKSDQSLLYSDGKTYLNWLLQRPGQSPKRLIYLVSELDSGV
PDRFTGSGSGTDFTLKISRVEAEDLGLYYCWQGTHFPLTFGAGTKLELK (SEQ ID NO:252)

LC-CDR1:	QSLLYSDGKTY	(SEQ ID NO:41)
LC-CDR2:	LVS	(SEQ ID NO:42)
LC-CDR3:	WQGTHFPLT	(SEQ ID NO:43)

Figure 57

Mouse-human chimeric anti-R248Q clone 3G11

DVQLQQSGPELVKPGASVKMSCKASGYTFTDYYVKWVKQSPGQSLEWIGDIHPKNGGTN YNQKFKGKAALTVDKSSSTAYMQLNSLTSEDSAVYFCAKMGGYDDYWGQGTTLTVSS (SEQ ID NO:253)

HC-CDR1:	GYTFTDYY	(SEQ ID NO:46)
HC-CDR2:	IHPKNGGT	(SEQ ID NO:47)
HC-CDR3:	AKMGGYDDY	(SEQ ID NO:48)

Mouse-human chimeric anti-R248Q clone 4H2

QVQLKQSGPELVKPGASVKMSCKASGYTFTDYYLKWVRQSHGKSLEWIGDIDPKNGGTNY NQKFKGKATLTVDKSSSTAYMQLNSLTSEDSAVYYCAKQGGFDDYWGQGTTLTVSS (SEQ ID NO:254)

HC-CDR1:	GYTFTDYY	(SEQ ID NO:46)
HC-CDR2:	IDPKNGGT	(SEQ ID NO:50)
HC-CDR3:	AKQGGFDDY	(SEQ ID NO:51)

Figure 58

Mouse-human chimeric anti-R248Q clone 3G11 light chain variable domain

>MH 3G11_aa_L
DIVLTQTPLTLSVTIGQPASISCKSNQSLLYSDGKTYLNWLLQRPGQSPKRLIYLVSKLDSGV
PDRFTGSGSGTDFTLKISRVEAEDLGLYYCWQGTHFPLTFGAGTKLELK [SEQ ID NO:251]

>MH 3G11_ntd_L
GATATTGTGCTGACACAGACTCCACTCACTTTGTCGGTTACCATTGGACAACCAGCCTC
CATCTCTTGCAAGTCAAATCAGAGCCTCTTATATAGTGATGGAAAGACATATTTGAATTG
GTTATTACAGAGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTAAACTGG
ACTCTGGAGTCCCTGACAGGTTCACTGGCAGTGGATCAGGGACAGATTTCACACTGAA
AATCAGCAGAGTGGAGGCTGAGGATTTGGGACTTTATTATTGCTGGCAAGGTACACATT
TTCCTCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO:255]

Mouse-human chimeric anti-R248Q clone 4H2 light chain variable domain

>MH 4H2_aa_L
DIVITQSPLTLSVTIGQPASISCKSDQSLLYSDGKTYLNWLLQRPGQSPKRLIYLVSELDSGV
PDRFTGSGSGTDFTLKISRVEAEDLGLYYCWQGTHFPLTFGAGTKLELK [SEQ ID NO:252]

>MH 4H2_ntd_L
GACATTGTGATCACACAGTCTCCACTCACTTTGTCGGTTACCATTGGACAACCAGCCTC
CATCTCTTGCAAGTCAGATCAGAGCCTCTTATATAGTGATGGAAAGACATATTTGAATTG
GCTATTACAGAGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTGAACTG
GACTCTGGAGTCCCTGACAGGTTCACTGGCAGTGGATCAGGGACAGATTTCACACTGA
AAATCAGCAGAGTGGAGGCTGAGGATTTGGGACTTTATTATTGCTGGCAAGGTACACAT
TTTCCTCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAA [SEQ ID NO:256]

Figure 59

Mouse-human chimeric anti-R248Q clone 3G11 heavy chain variable domain

>MH 3G11_aa_H
DVQLQQSGPELVKPGASVKMSCKASGYTFTDYYVKWVKQSPGQSLEWIGDIHPKNGGTN
YNQKFKGKAALTVDKSSSTAYMQLNSLTSEDSAVYFCAKMGGYDDYWGQGTTLTVSS
[SEQ ID NO:253]

>MH 3G11_ntd_H
GATGTGCAGCTTCAGCAGTCAGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGA
TGTCCTGTAAGGCTTCTGGATACACATTCACTGACTACTATGTGAAGTGGGTGAAGCAG
AGTCCTGGACAGAGCCTTGAGTGGATTGGAGATATTCATCCTAAGAACGGTGGTACTAA
CTACAACCAGAAGTTCAAGGGCAAGGCCGCATTGACTGTGGACAAATCCTCCAGCACA
GCCTACATGCAGCTCAACAGCCTGACATCTGAGGACTCTGCAGTCTATTTCTGTGCAAA
AATGGGAGGCTACGACGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
[SEQ ID NO:257]

Mouse-human chimeric anti-R248Q clone 4H2 heavy chain variable domain

>MH 4H2_aa_H
QVQLKQSGPELVKPGASVKMSCKASGYTFTDYYLKWVRQSHGKSLEWIGDIDPKNGGTNY
NQKFKGKATLTVDKSSSTAYMQLNSLTSEDSAVYYCAKQGGFDDYWGQGTTLTVSS [SEQ
ID NO:254]

>MH 4H2_ntd_H
CAGGTGCAGCTGAAGCAGTCAGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAG
ATGTCCTGTAAGGCTTCTGGATACACATTCACTGACTACTATCTGAAGTGGGTGAGGCA
GAGTCATGGAAAGAGCCTTGAGTGGATTGGAGATATAGATCCCAAGAATGGTGGTACT
AATTACAACCAGAAGTTTAAGGGCAAGGCCACATTGACTGTGGACAAATCCTCCAGCAC
AGCCTACATGCAGCTCAACAGCCTGACATCTGAGGACTCTGCAGTCTATTACTGTGCAA
AACAGGGGGGGTTCGACGACTACTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA
[SEQ ID NO:258]

Figure 60

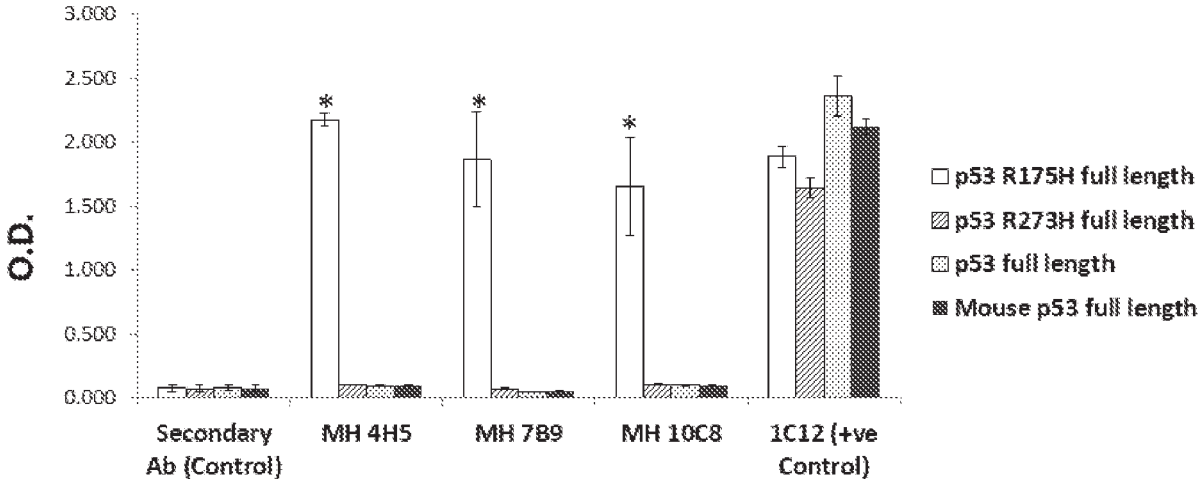


Figure 61

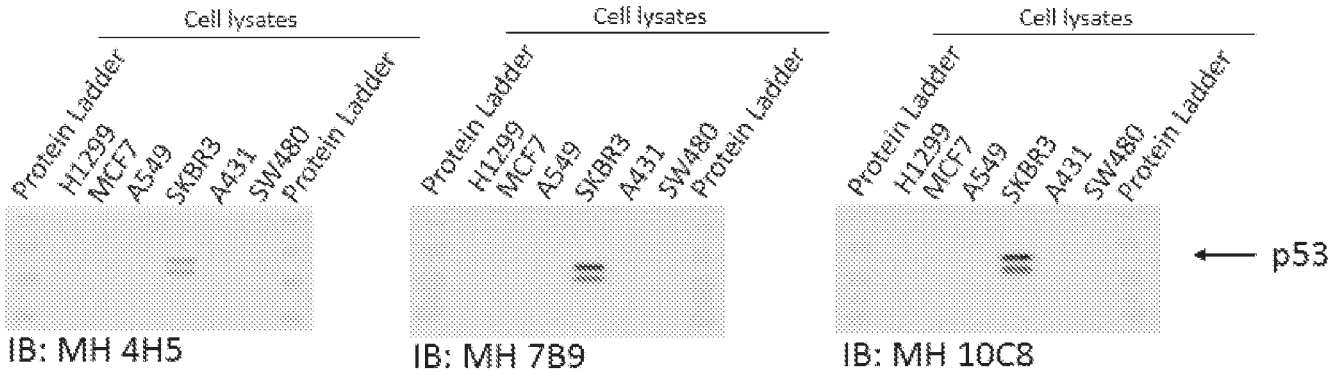


Figure 62

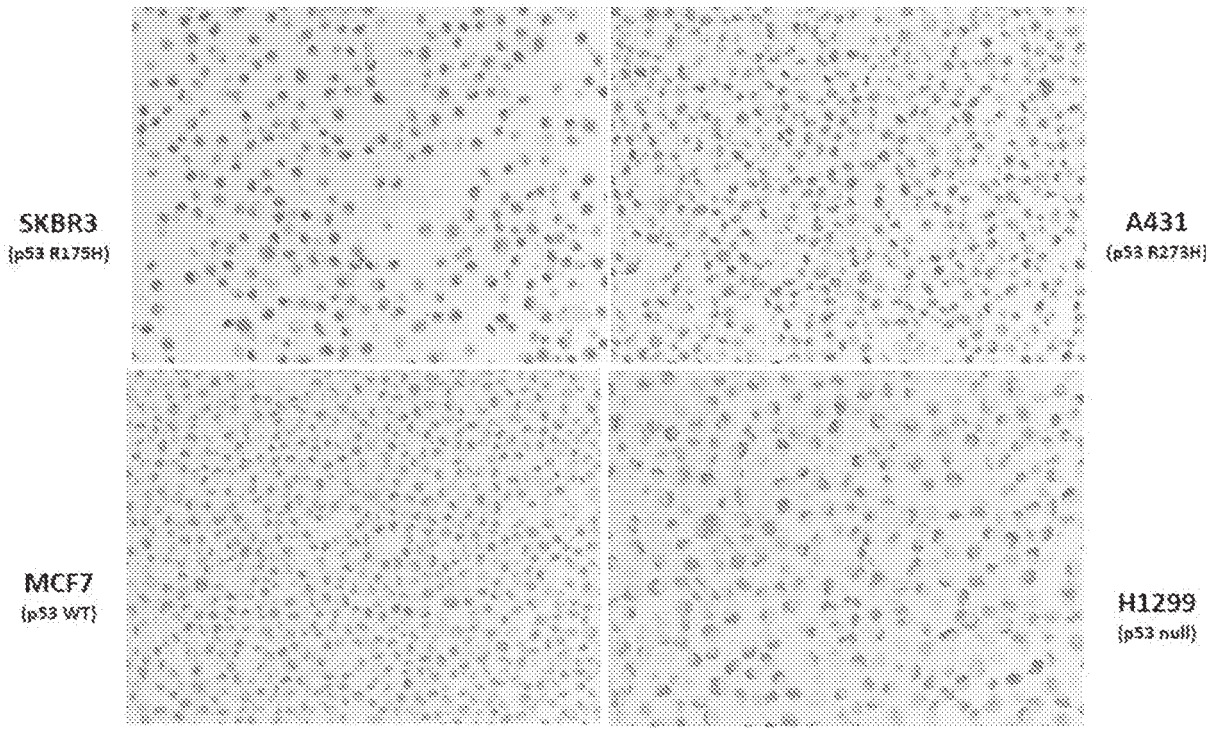


Figure 63

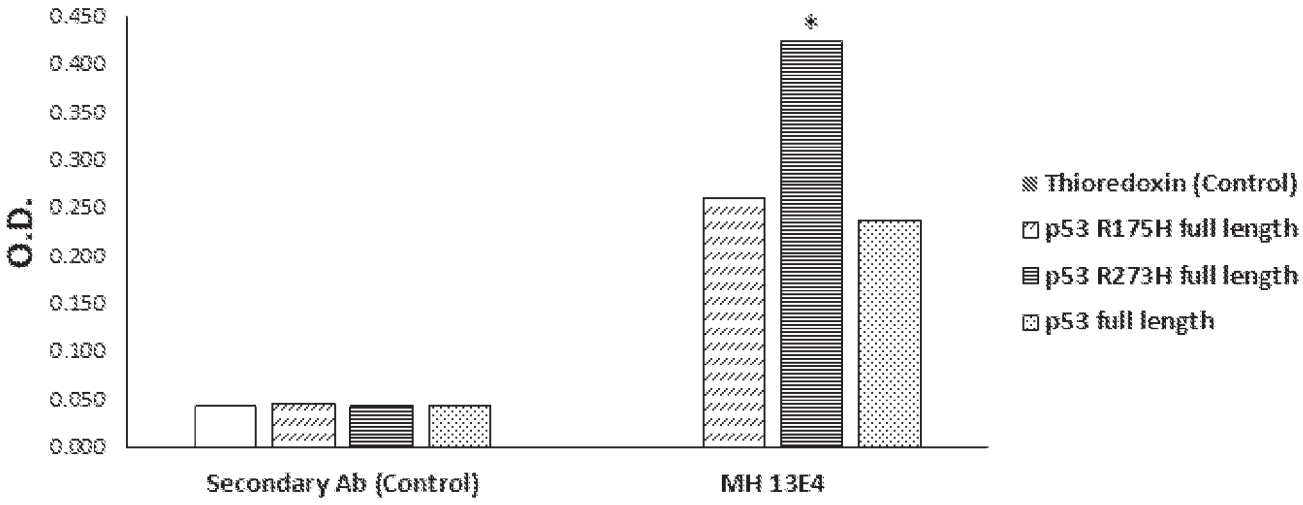


Figure 64

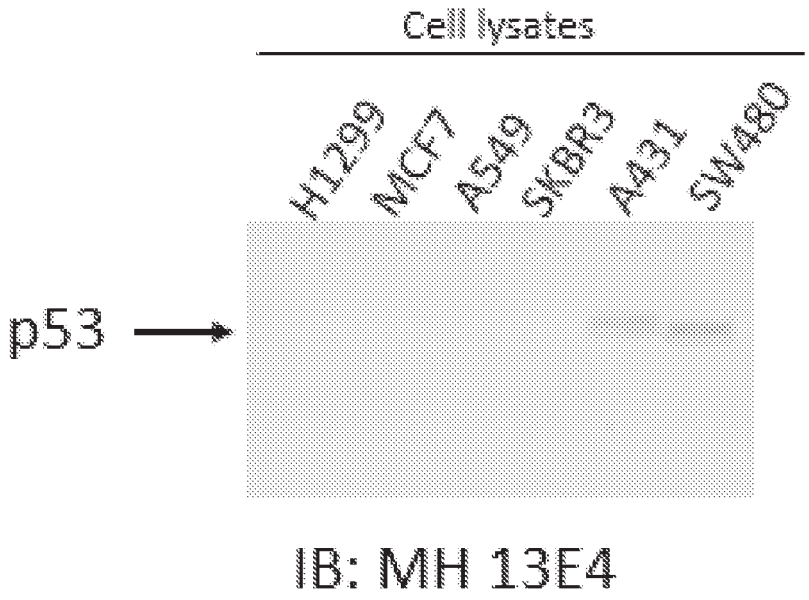


Figure 65

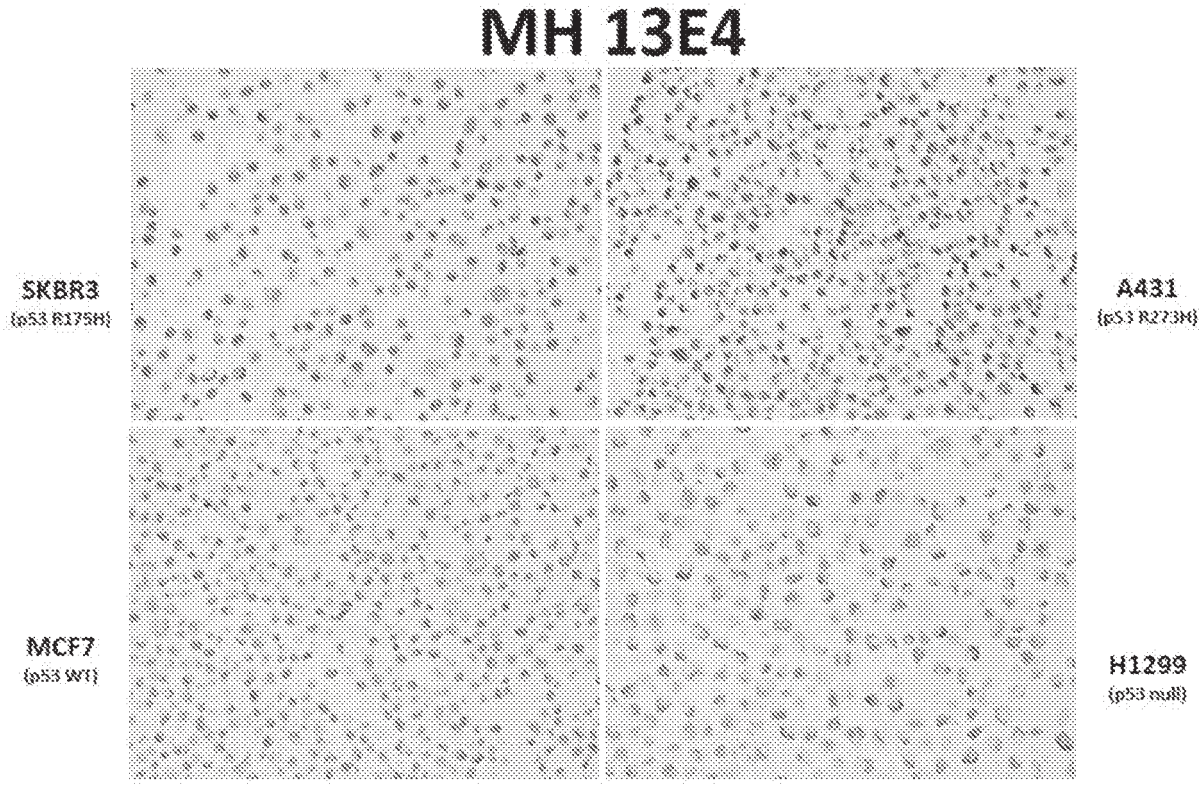
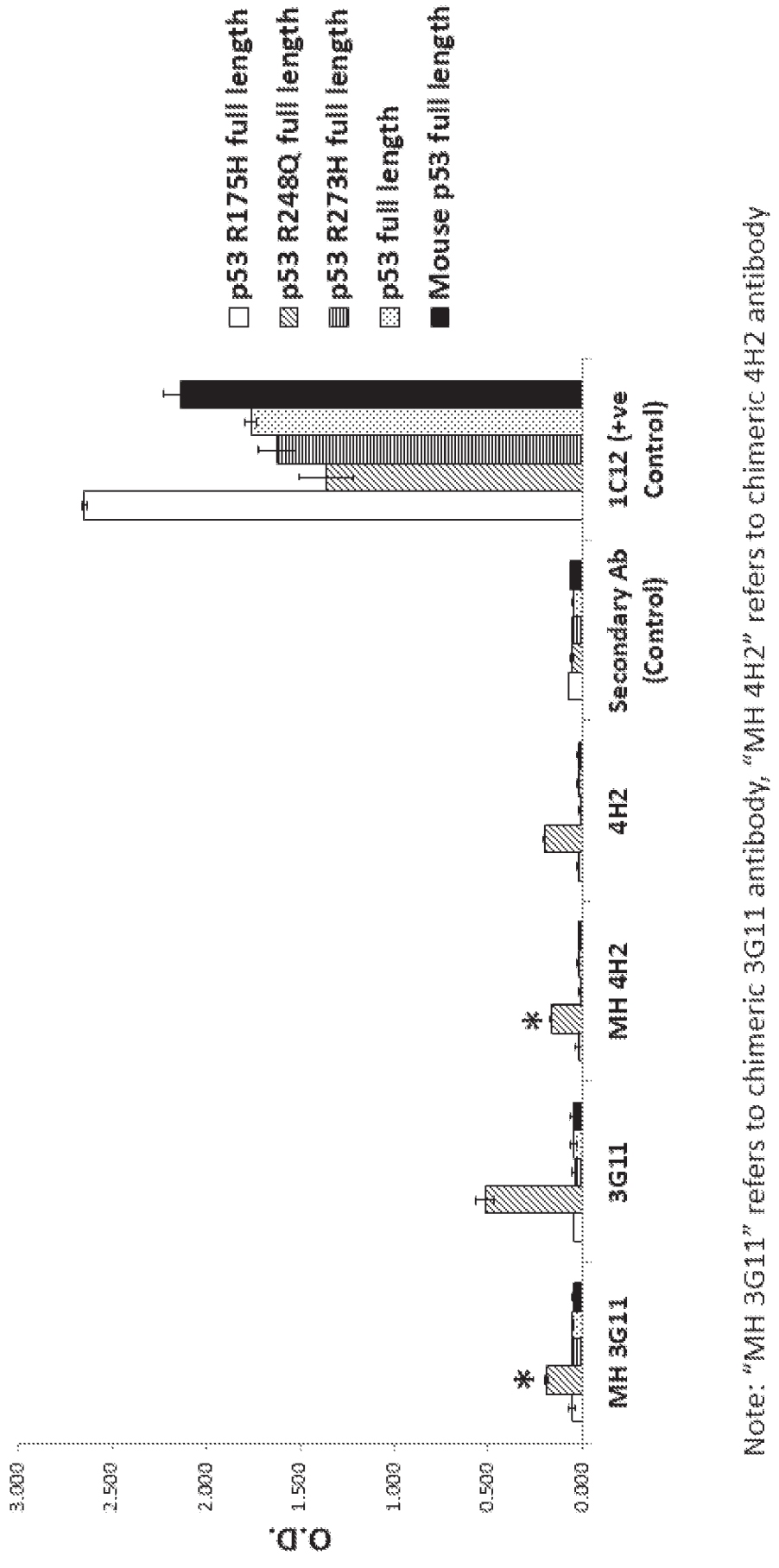


Figure 66



Note: "MH 3G11" refers to chimeric 3G11 antibody, "MH 4H2" refers to chimeric 4H2 antibody

Figure 67

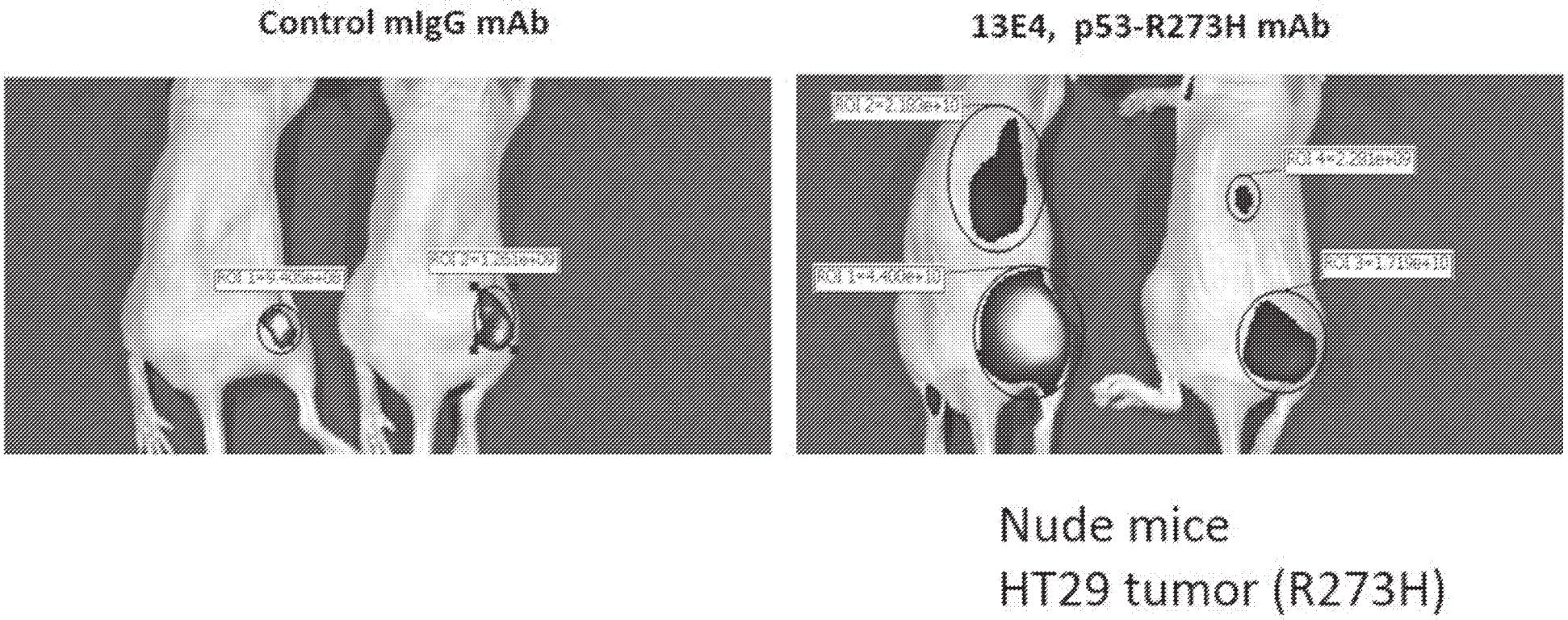

Control mIgG mAb
13E4, p53-R273H mAb
Nude mice
HT29 tumor (R273H)

Figure 68

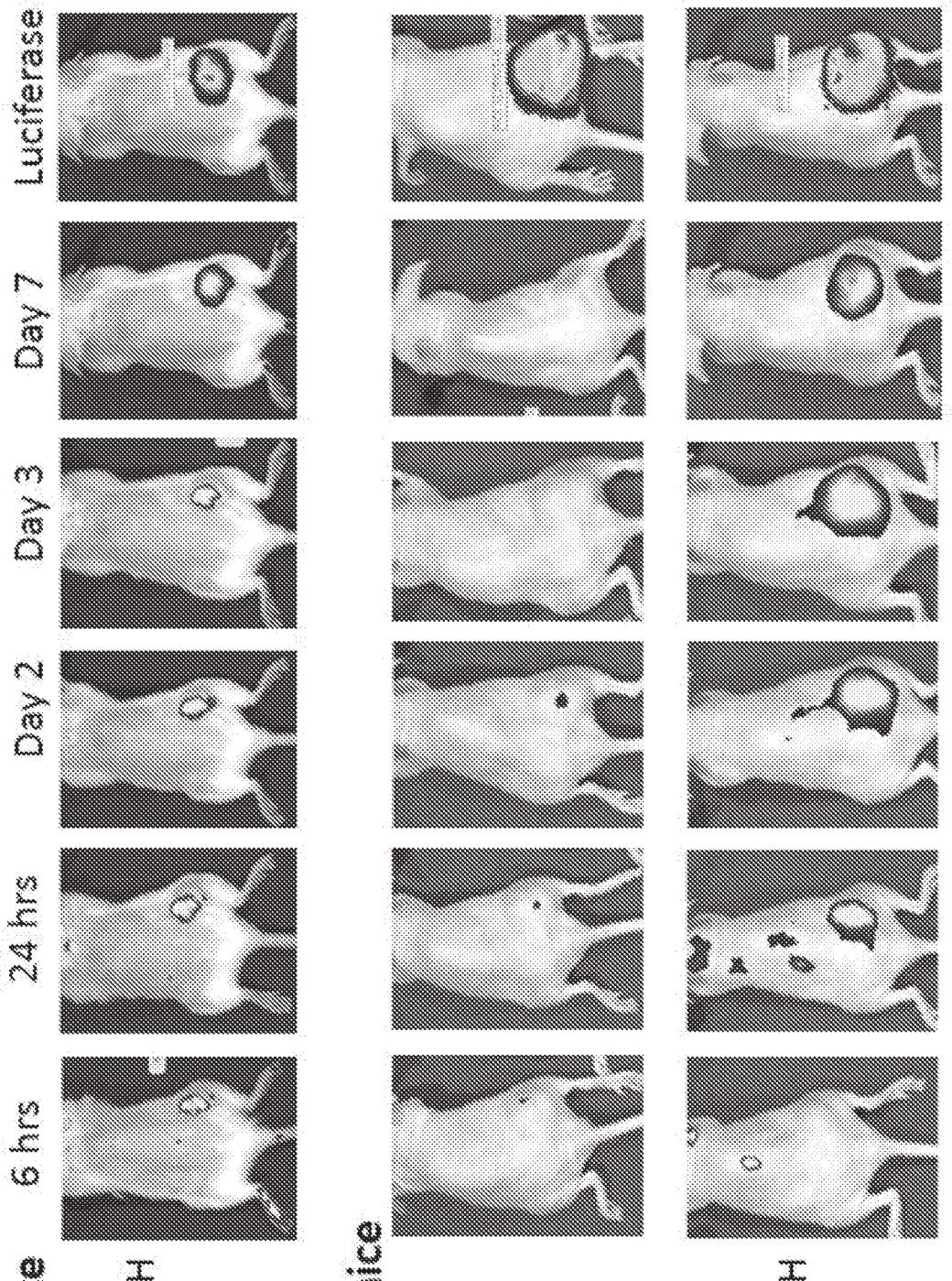

B6 mice
7B9
Anti-R175H
6 hrs
24 hrs
Day 2
Day 3
Day 7
Luciferase
Nude mice
IgG
control
4H5
Anti-R175H

Figure 69

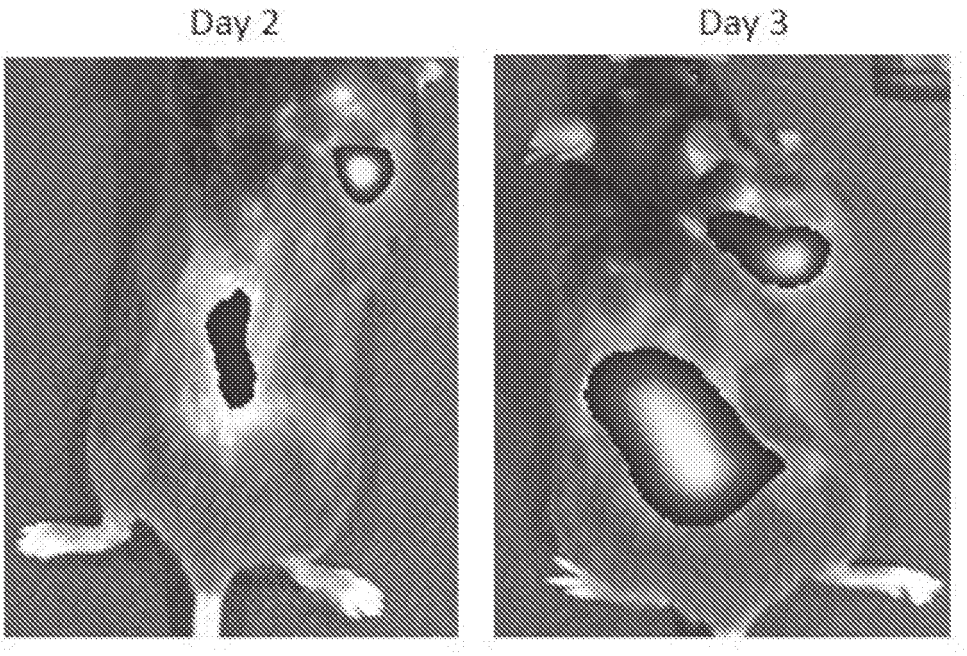


Figure 70

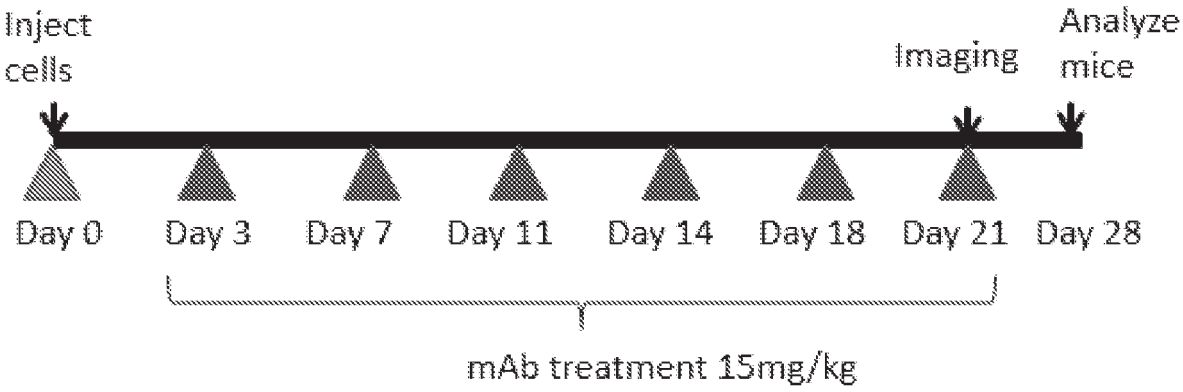


Figure 71A

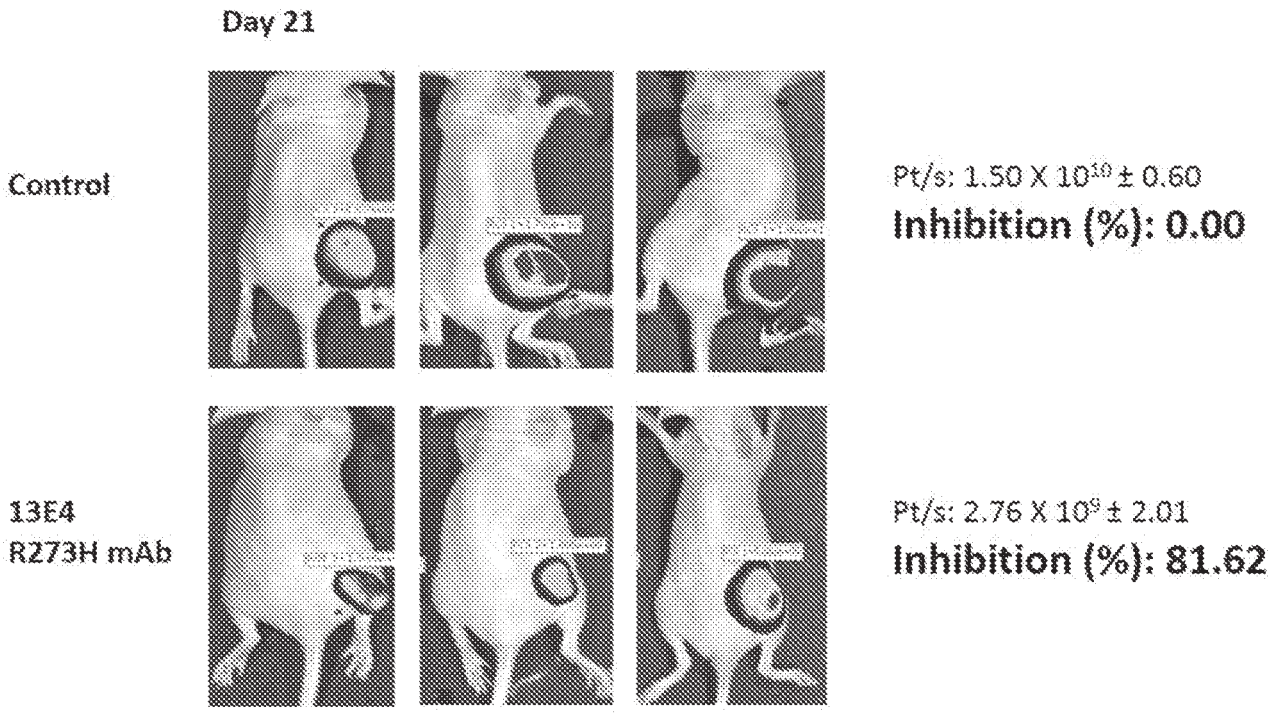


Figure 71B

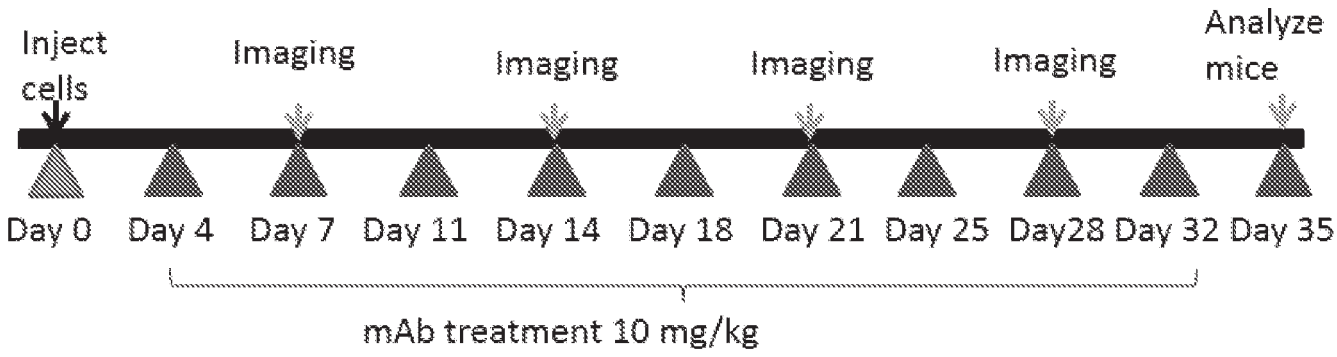


Figure 72A

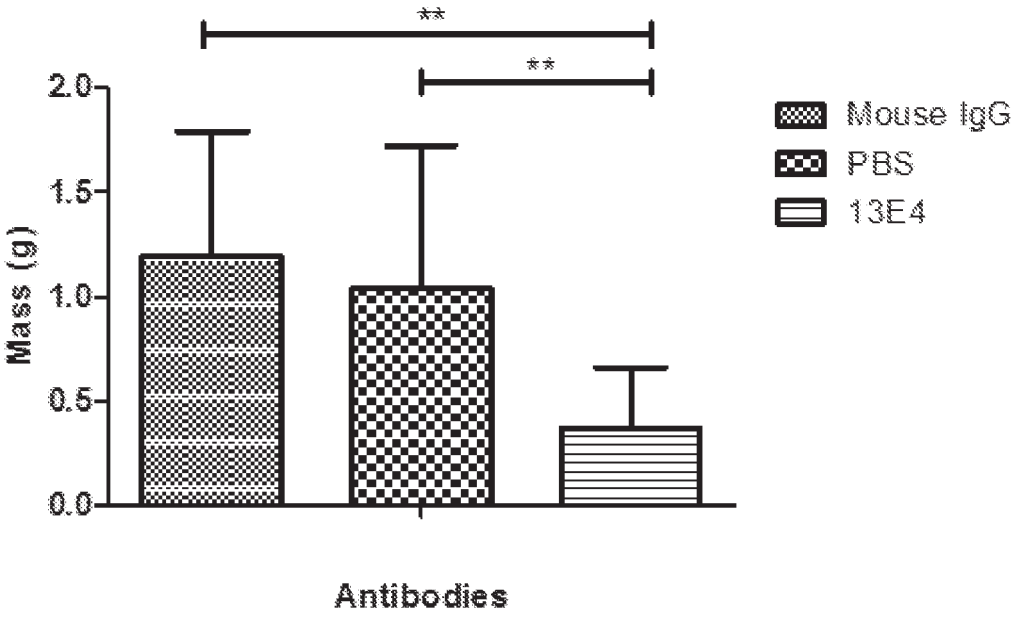


Figure 72B

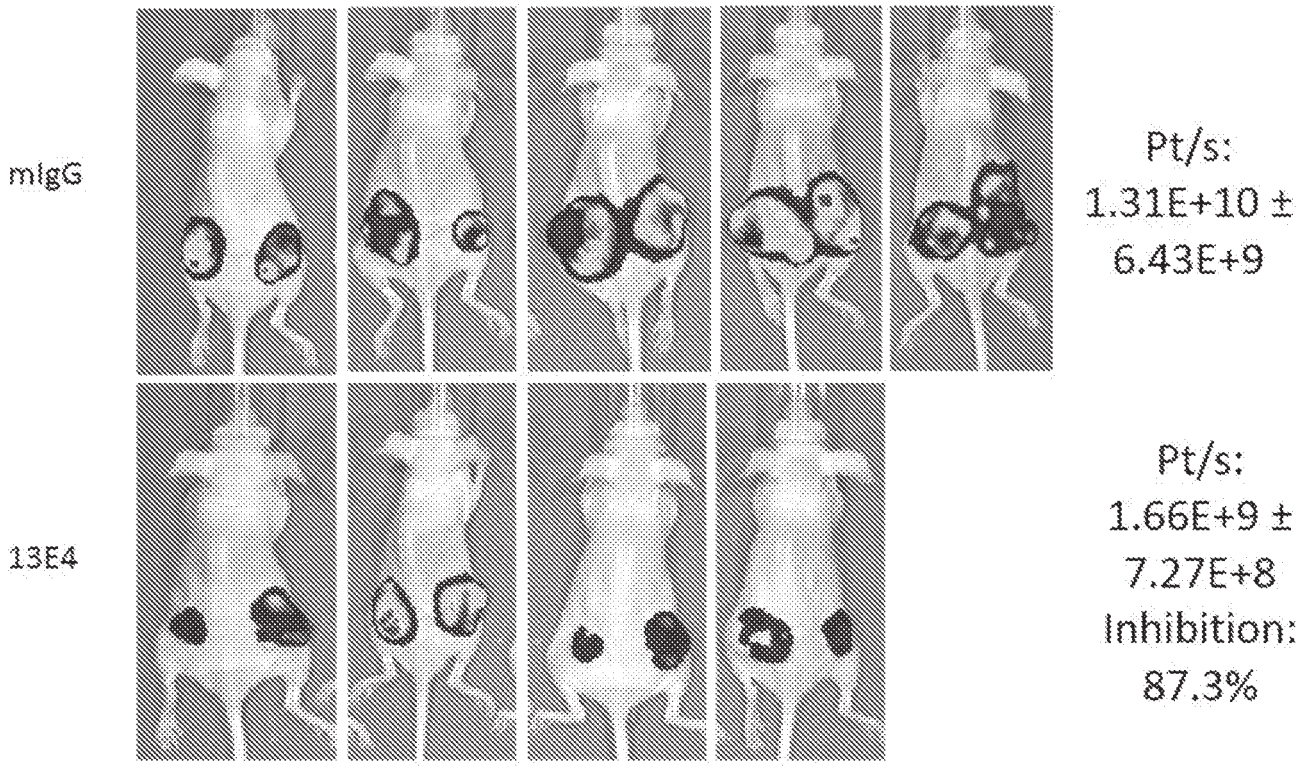


Figure 72C

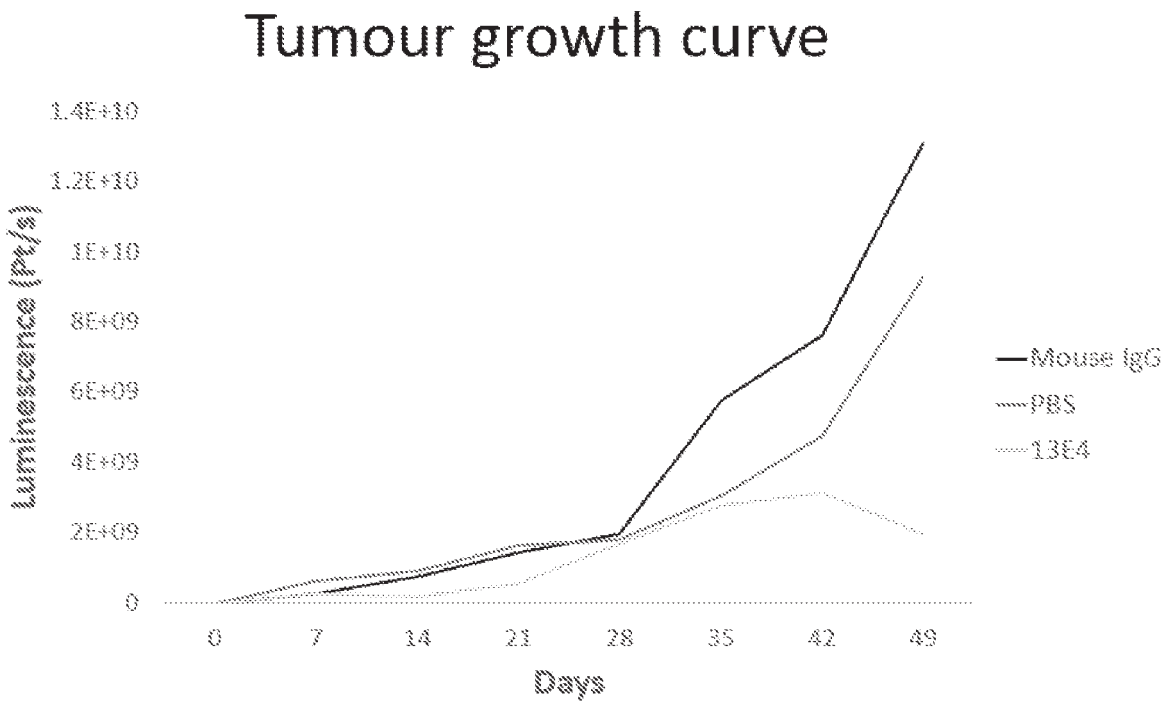


Figure 72D

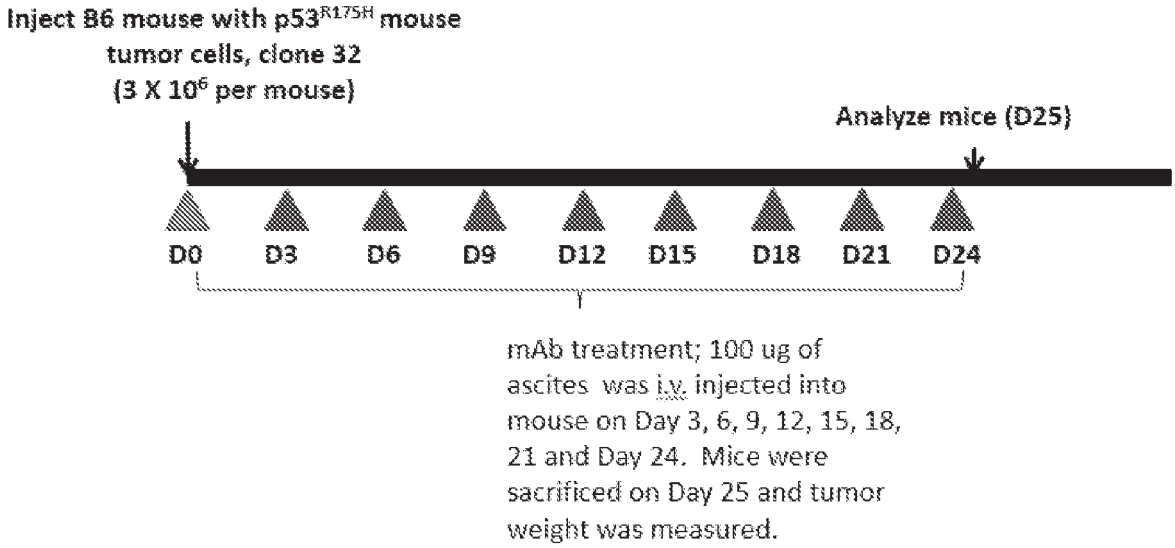


Figure 73A

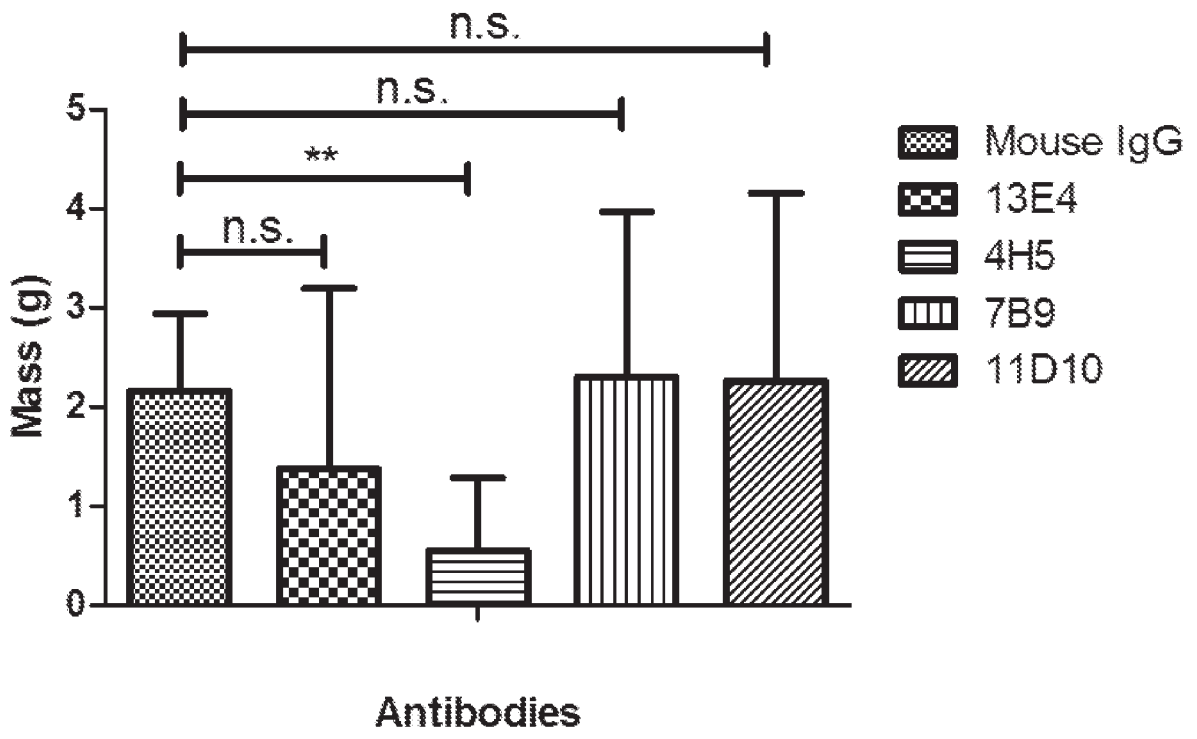


Figure 73B

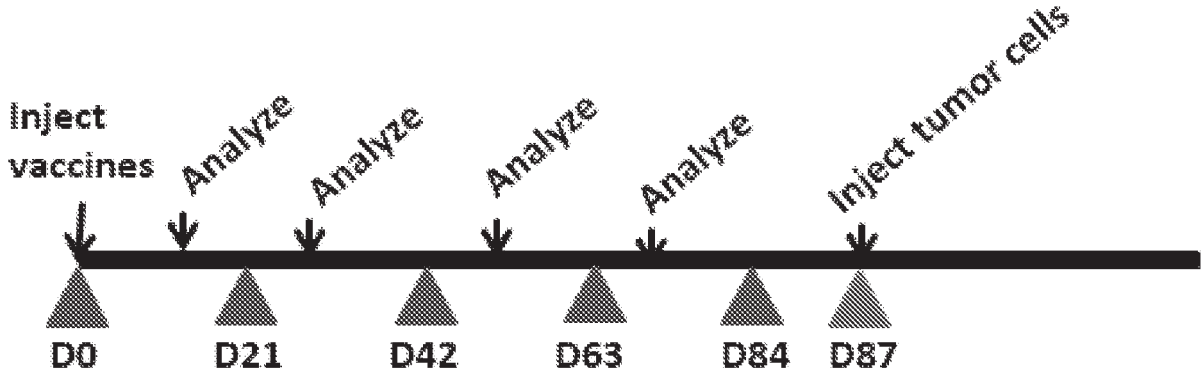


Figure 74A

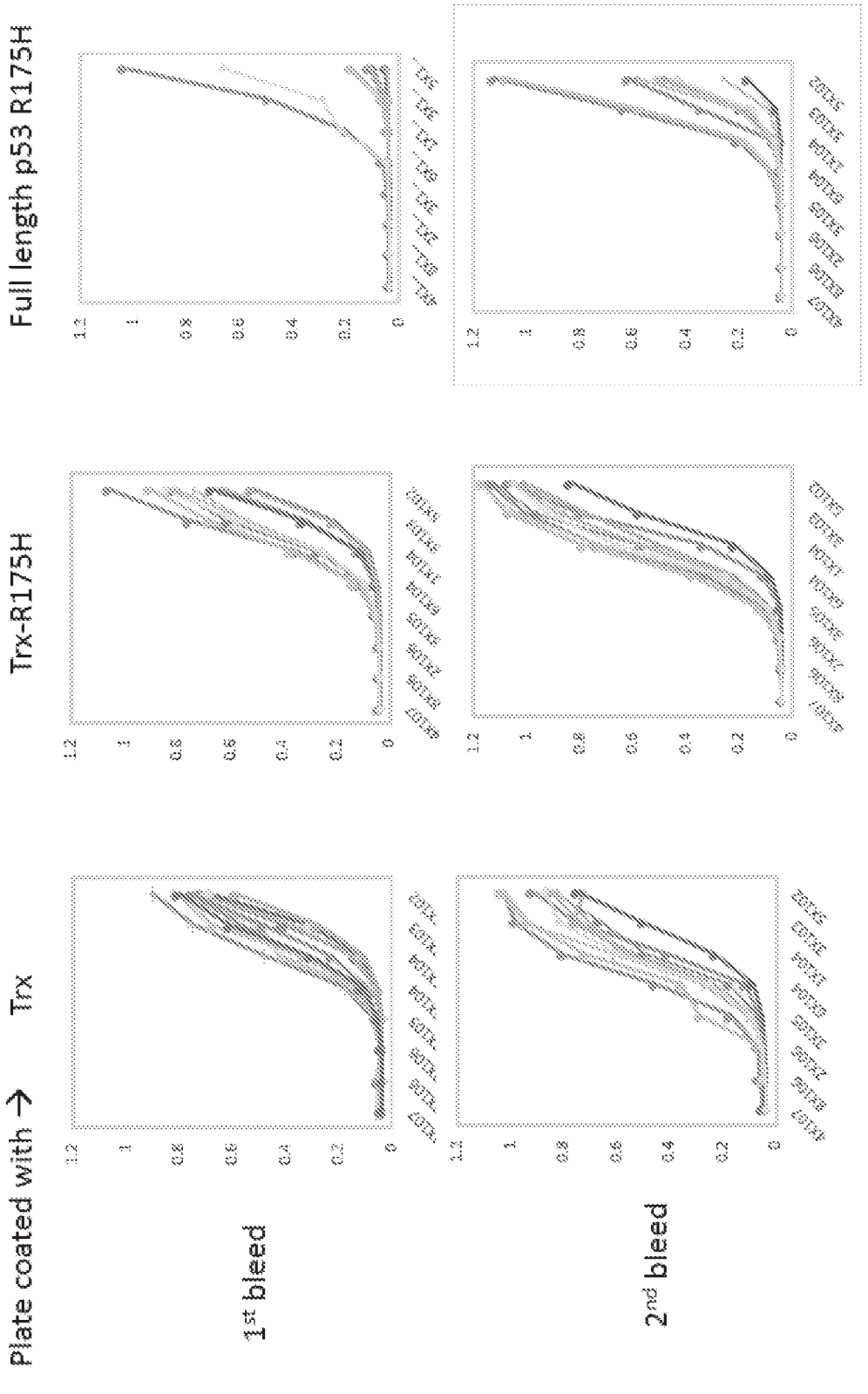


Plate coated with →
Trx
Trx-R175H
Full length p53 R175H
1st bleed
2nd bleed
Fold of serum dilution

Figure 74B

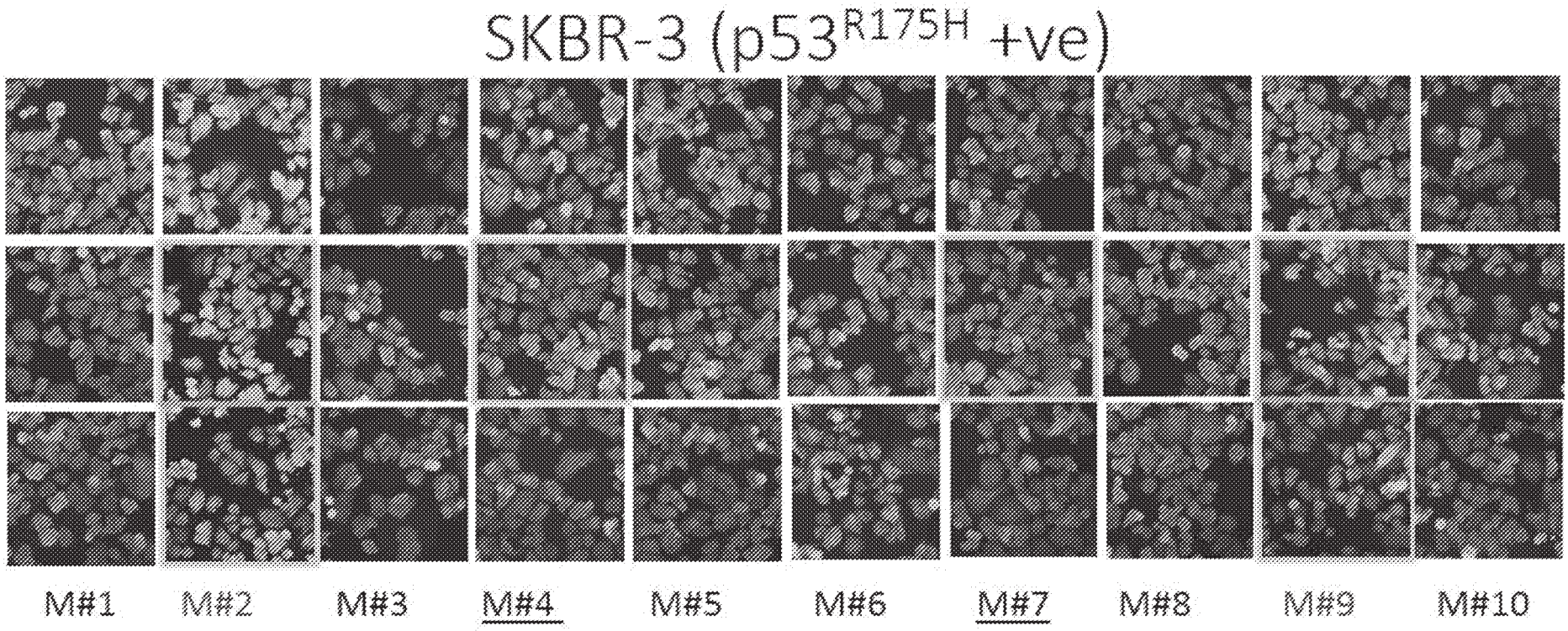

SKBR-3 (p53R175H +ve)
M#1
M#2
M#3
M#4
M#5
M#6
M#7
M#8
M#9
M#10
Row 1: 1st bleed
Row 2: 2nd bleed
Row 3: 3rd bleed
4H5 (+ve ctrl)

Figure 74C

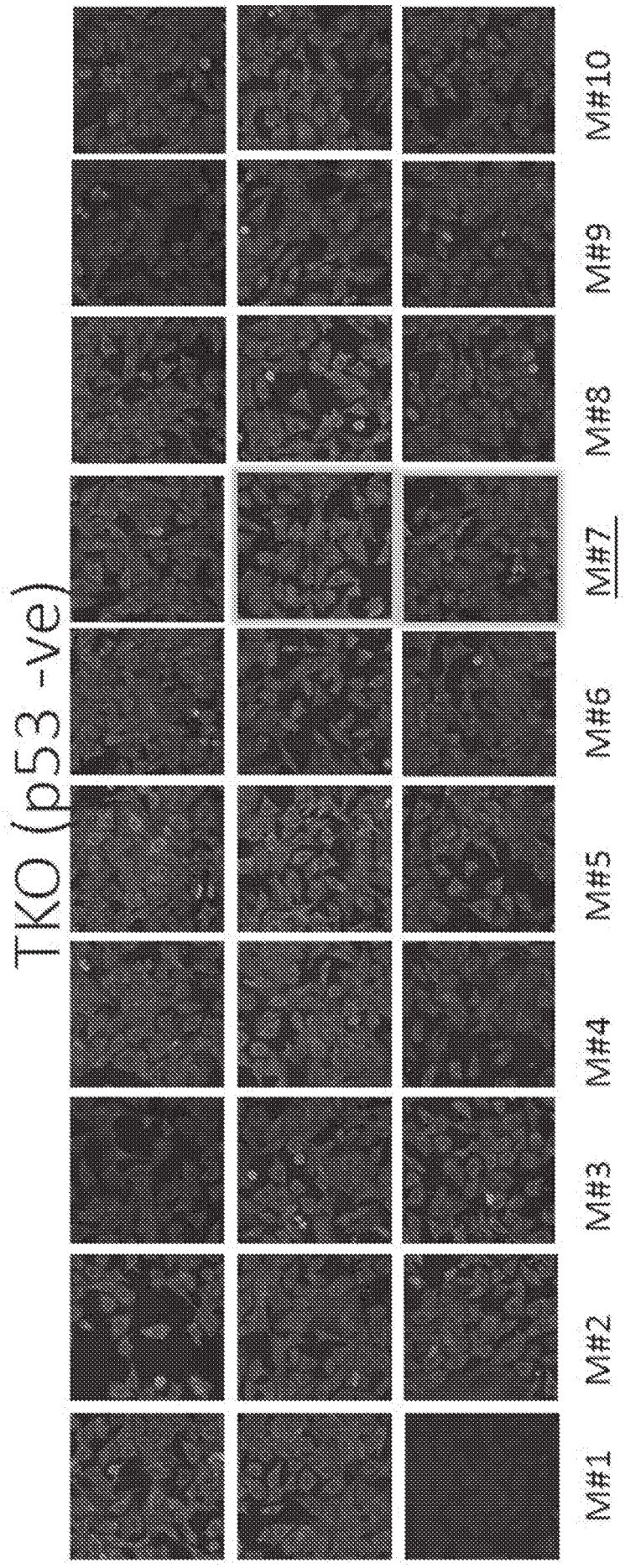

TKO (p53 -ve)
M#1
M#2
M#3
M#4
M#5
M#6
M#7
M#8
M#9
M#10
Row 1: 1st bleed
Row 2: 2nd bleed
Row 3: 3rd bleed
4H5 (+ve ctrl)

Figure 74D

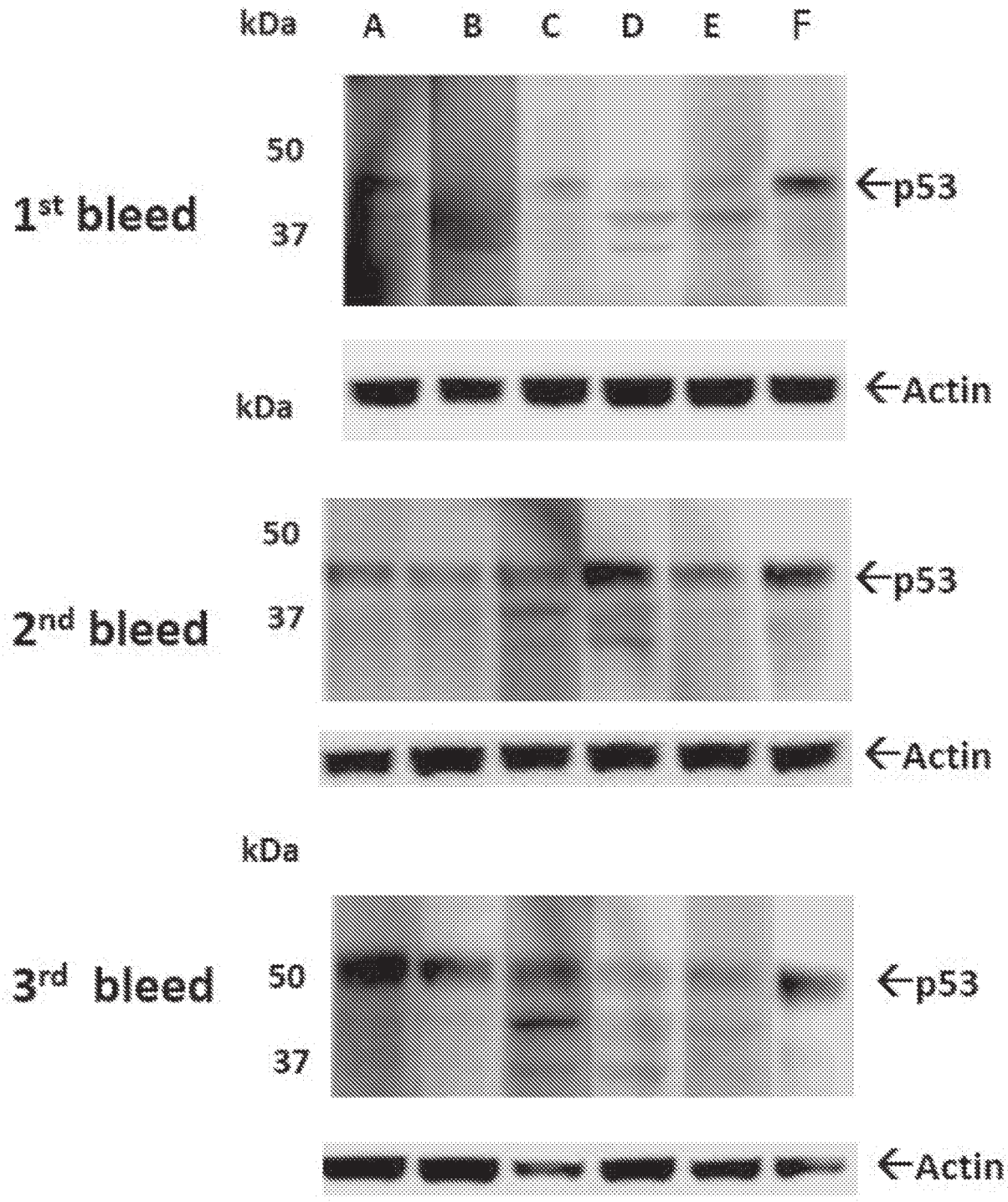

kDa
A
B
C
D
E
F
50
37
1st bleed
←p53
kDa
←Actin
50
37
2nd bleed
←p53
←Actin
kDa
50
37
3rd bleed
←p53
←Actin

Figure 74E

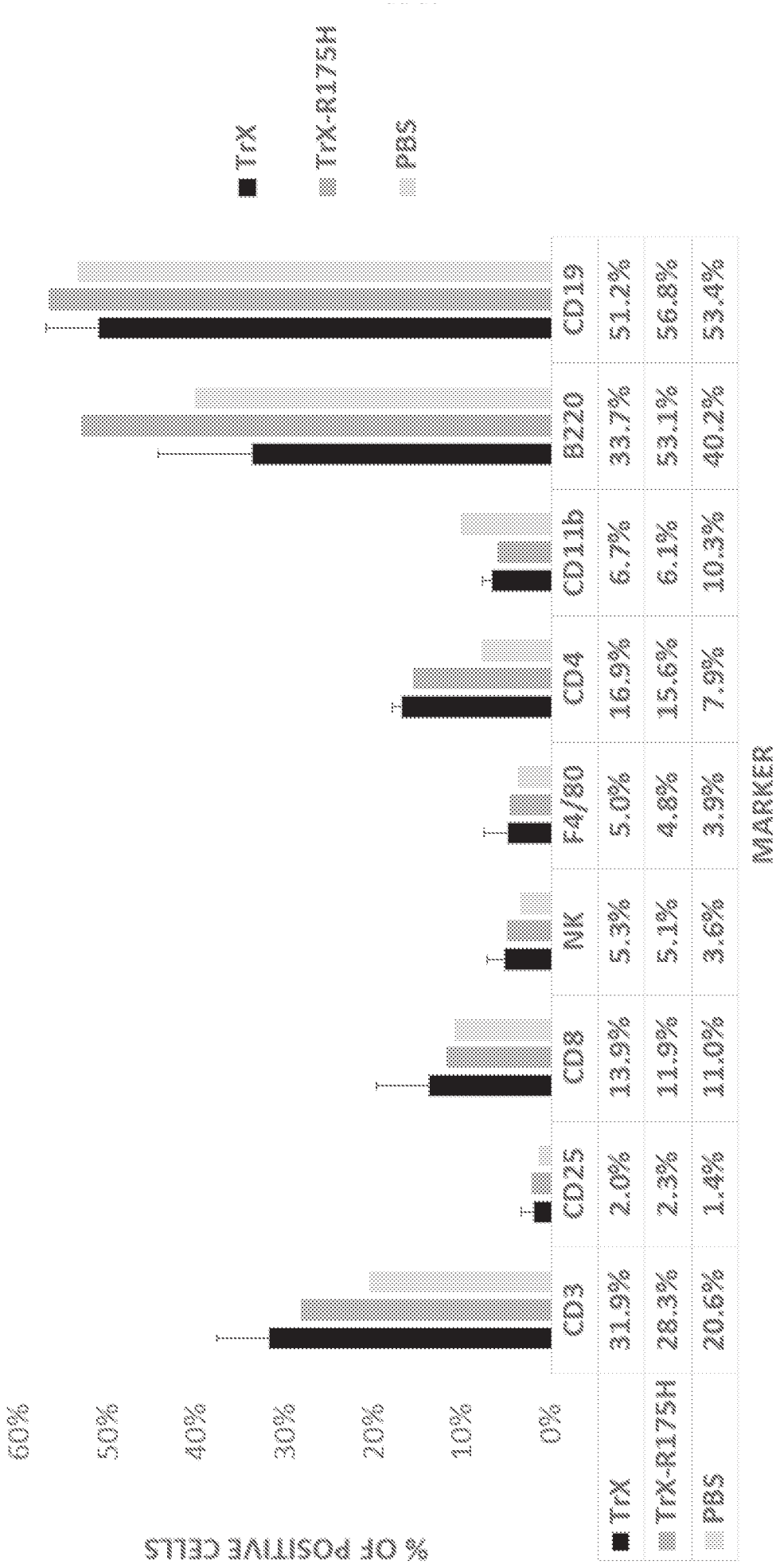

TrX
TrX-R175H
PBS
% OF POSITIVE CELLS
60%
50%
40%
30%
20%
10%
0%
MARKER
CD3
CD25
CD8
NK
F4/80
CD4
CD11b
B220
CD19
TrX 31.9% 2.0% 13.9% 5.3% 5.0% 16.9% 6.7% 33.7% 51.2%
TrX-R175H 28.3% 2.3% 11.9% 5.1% 4.8% 15.6% 6.1% 53.1% 56.8%
PBS 20.6% 1.4% 11.0% 3.6% 3.9% 7.9% 10.3% 40.2% 53.4%

Figure 74F