

(19) **DANMARK**



Patent- og
Varemærkestyrelsen

(10) **DK/EP 3513192 T3**

(12) **Oversættelse af
europæisk patentskrift**

-
- (51) Int.Cl.: **G 01 N 33/574 (2006.01)**
- (45) Oversættelsen bekendtgjort den: **2021-03-29**
- (80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2020-12-23**
- (86) Europæisk ansøgning nr.: **17771553.9**
- (86) Europæisk indleveringsdag: **2017-09-15**
- (87) Den europæiske ansøgnings publiceringsdag: **2019-07-24**
- (86) International ansøgning nr.: **GB2017052733**
- (87) Internationalt publikationsnr.: **WO2018051110**
- (30) Prioritet: **2016-09-16 GB 201615842**
- (84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**
- (73) Patenthaver: **UCL BUSINESS LTD, The Network Building , 97 Tottenham Court Road, London W1T 4TP, Storbritannien**
- (72) Opfinder: **JANES, Samuel, Canley Lodge, The Common, Harpenden, Hertfordshire AL5 3PE, Storbritannien**
KOLLURI, Krishna, Lungs for Living Research Centre, UCL Respiratory, Rayne Building, 5 University Street, London WC1E 6JF, Storbritannien
MCDERMOTT, Ultan, Room H2-09, Cancer Genome Project, Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, Storbritannien
KUMAR, Neelam, UCL Respiratory, Rayne Building, 5 University Street, London WC1E 6JF, Storbritannien
- (74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**
- (54) Benævnelse: **CELLEDØDSBIOMARKØR**
- (56) Fremdragne publikationer:
EP-A2- 2 044 434
WO-A1-2013/106002
US-A1- 2002 169 123
R POLANSKI ET AL: "Caspase-8 activation by TRAIL monotherapy predicts responses to IAPi and TRAIL combination treatment in breast cancer cell lines", CELL DEATH AND DISEASE, vol. 6, no. 10, 1 October 2015 (2015-10-01), page e1893, XP055418422, DOI: 10.1038/cddis.2015.234
PERSHANG FARSHI ET AL: "Deubiquitinases (DUBs) and DUB inhibitors: a patent review", EXPERT OPINION ON THERAPEUTIC PATENTS., vol. 25, no. 10, 16 June 2015 (2015-06-16) , pages 1191-1208, XP055406965, GB ISSN: 1354-3776, DOI: 10.1517/13543776.2015.1056737
YUAN ZHENGQIANG ET AL: "Cryopreservation of human mesenchymal stromal cells expressing TRAIL for human anti-cancer therapy", CYTOTHERAPY, vol. 18, no. 7, July 2016 (2016-07), pages 860-869, XP002774967,
M. LADANYI ET AL: "New Strategies in Pleural Mesothelioma: BAP1 and NF2 as Novel Targets for Therapeutic Development and Risk Assessment", CLINICAL CANCER RESEARCH, vol. 18, no. 17, 23 July 2012 (2012-07-23)

Fortsættes ...

, pages 4485-4490, XP055418148, US ISSN: 1078-0432, DOI: 10.1158/1078-0432.CCR-11-2375

H. WALCZAK: "Death Receptor-Ligand Systems in Cancer, Cell Death, and Inflammation", COLD SPRING HARBOR PERSPECTIVES IN BIOLOGY, vol. 5, no. 5, 1 May 2013 (2013-05-01), pages a008698-a008698, XP055418107, DOI: 10.1101/cshperspect.a008698

HAYES-JORDAN A ET AL: "474 POSTER Inhibition of PDGFR-beta increases sarcoma cell sensitivity to tumor necrosis factor related apoptosis inducing ligand, TRAIL and promotes inhibition of tumor growth in dual therapy using imantinib (Gleevac) and TRAIL", EUROPEAN JOURNAL OF CANCER. SUPPLEMENT, PERGAMON, OXFORD, GB, vol. 4, no. 12, 1 November 2006 (2006-11-01), page 145, XP027888933, ISSN: 1359-6349 [retrieved on 2006-11-01]

DESCRIPTION

[0001] The present invention relates to cell death of cancer cells, and in particular to biomarkers that may be used to identify cancer cells that are sensitive to death receptor ligand (DRL)-induced cell death. The invention also extends to prognostic methods and kits for identifying cancer cells that are sensitive to DRL-induced cell death. The invention further extends to novel compositions and therapeutic methods using such compositions for treating cancer.

[0002] Malignant pleural mesothelioma (MPM) is a rare but invariably fatal malignancy that occurs most frequently in the pleura and is almost always associated with asbestos exposure (1, 2). Although asbestos mining and usage has been the subject of legislation in many nations, there remains evidence of increased use and production of asbestos as the trend towards global industrialisation increases (3). Given a cancer latency period of anywhere from 10-40 years, it is therefore likely that in such countries asbestos-related cancers will increase over the next five decades. Current treatment options for patients diagnosed with MPM are limited. Radical surgery, for example, offers limited benefit and the cancer is refractory to most chemotherapeutic agents (4). Indeed, the current gold standard chemotherapy regimen of pemetrexed plus cisplatin offers a median overall survival of 12.1 months and a time to progression of 5.7 months (5).

[0003] Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL/Apo2L) is a member of the TNF ligand family that induces cell death in tumour cells *in vitro* and *in vivo* but not in most normal cells. Numerous chemotherapeutic drugs have also been shown to sensitize tumour cells to TRAIL-mediated cell death. While some mesothelioma cells are sensitive to TRAIL induced cell death others are resistant to the induction of cell death *in vitro* and this disruption of core-cell death machinery has been implicated in the general resistance to conventional cytotoxic agents observed clinically (11).

[0004] Certain cancers are sensitive to the pro-cell death effects of known death receptor ligands, while others are not. Without knowledge of which cancers are sensitive to DRL-induced cell death, individuals with cancer must rely on treatment with non-specific cytotoxic therapies, such as chemotherapy, with the toxicities and problems described (5). Furthermore, with no clear indication pre-treatment of whether a patient will respond or not, the benefit of such treatment is uncertain at the outset.

[0005] There is thus a compelling need for more effective therapeutic interventions for the treatment of cancers that are sensitive to cell death caused by death receptor ligand (DRL)-induced cell death. There is also a need to provide a biomarker that can be used to identify cancerous cells that are sensitive to cell death caused by death receptor ligands when bound to death receptors, such as TRAIL-receptor 1 & 2 (Death Receptors 4&5), TNF receptor and FAS receptor.

[0006] WO2013/106002 A1 discloses assays and kits for the prognosis of melanoma comprising the assessment of BAP1 protein and nucleic acid. Polanski et al, 'Caspase-8 activation by TRAIL monotherapy predicts responses to IAPi and TRAIL combination treatment in breast cancer cell lines', Cell death and disease, vol. 6, no. 10, 2015, page e1893, discloses the synergistic effect of the combination of TRAIL and Smac mimetics in the treatment of cancer. EP2044434A2 discloses

methods for identifying cancer cells that are responsive to therapeutic agents that induce apoptosis. Pershang Farshi et al, 'Deubiquitinases (DUBs) and DUB inhibitors: a patent review', Expert opinion on therapeutic patents, vol. 25, no. 10, 2015, pages 1191-1208, discusses various compounds that target deubiquitinases.

[0007] The invention is as defined in the claims.

[0008] According to a first aspect of the invention, there is provided a method of determining if an individual's cancer cell is sensitive to death receptor ligand (DRL)-induced cell death, the method comprising detecting, in a biological sample taken from the individual, for:

1. (i) the presence of a mutant *BAP1* gene or mutant BAP1 protein;
2. (ii) a reduced level of expression of a wild-type *BAP1* gene or a lower wild-type BAP1 protein concentration compared to the level of expression or protein concentration in a reference cell that is a BAP1 wild-type cell, which is resistant to DRL-induced cell death; or
3. (iii) reduced or non-binding of an ASXL protein to a wild-type BAP1 protein compared to the level of binding in a reference cell that is a BAP1 wild-type cell, which is resistant to DRL-induced cell death;

wherein the presence, in the sample, of the mutant *BAP1* gene or the mutant BAP1 protein, or of a reduced level expression of the wild-type *BAP1* gene or a lower protein concentration, or reduced or non-binding of an ASXL protein to a wild-type BAP1 protein, is indicative of the individual's cancer cell being sensitive to DRL-induced cell death.

[0009] According to a second aspect, there is provided a kit for determining if an individual's cancer cell is sensitive to DRL-induced cell death, the kit comprising detection means for detecting the expression of a mutant *BAP1* gene or mutant BAP1 protein, or for detecting a reduced level of expression of a wild-type *BAP1* gene or a lower wild-type BAP1 protein concentration compared to the level of expression or protein concentration in a reference cell that is resistant to DRL-induced cell death, or for detecting non-binding or reduced binding of an ASXL protein to a BAP1 protein compared to the level of binding in a reference cell that is a BAP1 wild-type cell that is resistant to DRL-induced cell death, wherein the presence, in the sample, of the mutant *BAP1* gene or the BAP1 protein, or of a reduced level expression of the wild-type *BAP1* gene or a lower wild-type BAP1 protein concentration, or reduced or non-binding of an ASXL protein to a wild-type BAP1 protein, is indicative of the individual's cancer cell being sensitive to DRL-induced cell death, wherein the kit comprises at least one control or reference sample, and wherein the kit comprises a negative control comprising a wild-type BAP1 protein that is resistant to DRL-induced cell death.

[0010] According to a third aspect, there is provided the in vitro use of (i) a mutant *BAP1* gene or a mutant BAP1 protein, or (ii) a cancer cell with a reduced level of expression of a wild-type *BAP1* gene or a lower wild-type BAP1 protein concentration compared to the level of expression or protein concentration in a reference cell that is a BAP1 wild-type cell, as a biomarker of sensitivity to DRL-induced cell death.

[0011] According to a fourth aspect, there is provided a composition comprising (i) a BAP1 inhibitor, and (ii) a death receptor ligand, wherein the BAP1 inhibitor is WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9, an RNAi molecule including shRNA, siRNA, miRNA, ribozymes and

antisense molecules, or a TALEN.

[0012] According to a fifth aspect, there is provided a composition comprising (i) a BAP1 inhibitor, and (ii) a death receptor ligand, for use in therapy or as a medicament, wherein the BAP1 inhibitor is WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9, an RNAi molecule including shRNA, siRNA, miRNA, ribozymes and antisense molecules, or a TALEN.

[0013] According to a sixth aspect, there is provided a composition comprising (i) a BAP1 inhibitor, and (ii) a death receptor ligand, for use in treating, preventing or ameliorating cancer, wherein the BAP1 inhibitor is WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9, an RNAi molecule including shRNA, siRNA, miRNA, ribozymes and antisense molecules, or a TALEN.

[0014] According to a seventh aspect, there is provided a pharmaceutical composition comprising the composition according to the fourth aspect, and a pharmaceutically acceptable vehicle.

[0015] According to an eighth aspect, there is provided a method for preparing the pharmaceutical composition according to the seventh aspect, the method comprising contacting a therapeutically effective amount of a BAP1 inhibitor and a death receptor ligand, and a pharmaceutically acceptable vehicle, wherein the BAP1 inhibitor is WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9, an RNAi molecule including shRNA, siRNA, miRNA, ribozymes and antisense molecules, or a TALEN.

[0016] Hence, in an example, there is provided a method of determining if an individual's cancer cell is sensitive to death receptor ligand (DRL)-induced cell death, the method comprising detecting, in a biological sample taken from the individual, for:

1. (i) the presence of a mutant *BAP1* gene or mutant BAP1 protein;
2. (ii) a reduced level of expression of a wild-type *BAP1* gene or a lower wild-type BAP1 protein concentration compared to the level of expression or protein concentration in a reference cell that is a BAP1 wildtype cell, which is resistant to DRL-induced cell death; or
3. (iii) reduced or non-binding of an ASXL protein to a wild-type BAP1 protein compared to the level of binding in a reference cell that is a BAP1 wild-type cell, which is resistant to DRL-induced cell death;

wherein the presence, in the sample, of the mutant *BAP1* gene or the mutant BAP1 protein, or of a reduced level expression of the wild-type *BAP1* gene or a lower protein concentration, or reduced or non-binding of an ASXL protein to a wild-type BAP1 protein, is indicative of the individual's cancer cell being sensitive to DRL-induced cell death.

[0017] Previous genetic analyses have identified several key recurrent alterations, including inactivation of *CDKN2A*, *NF2* and *BAP1*, as well as frequent losses and gains of a number of chromosomal arms. More recently, the first whole exome sequence analysis of 22 malignant mesothelioma patients confirmed the importance of these three genes as probable driver events in tumour development as well as identifying recurrent non-synonymous mutations in *CUL1*, an essential component of the SCF E₃ ubiquitin ligase complex (6).

[0018] *BAP1* is a tumour suppressor gene that is somatically mutated in a variety of cancer types

including mesothelioma (7), uveal melanoma (8), renal cell carcinoma (9) and cholangiocarcinomas (10). The inventors have discovered that the expression of *BAP1* mutants in cancer cells causes increased sensitivity to cell death induced by death receptor ligands, which exhibit fewer off-target effects. Tumour necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), for example, induces cell death in tumour cells *in vitro* and *in vivo* but not in most normal cells. Advantageously, the invention therefore increases the likelihood that an individual with a cancer, which is associated with expression of a mutant *BAP1* gene or synthesis of a mutant BAP1 protein, or a reduced level of expression of a wild-type *BAP1* gene or a lower wild-type BAP1 protein concentration, will exhibit a positive outcome when treated with death receptor ligand therapy. Accordingly, the method of the invention provides a prognosis. Thus, such individuals are less likely to be treated with non-specific therapies and suffer the associated undesirable side-effects.

[0019] BAP1 is a deubiquitinating enzyme encoded by the *BAP1* gene. It is an 80.4kDa protein, which comprises a nuclear-localising sequence and a ubiquitin carboxy-terminal hydrolase (UCH) domain, which provides BAP1 with deubiquitinase activity. The *BAP1* gene may be mutated in the germline or somatically in a variety of forms of cancer. The nucleotide sequence encoding one embodiment of the human wild-type *BAP1* gene (BAP1 Whole gene sequence >gi|568815595:c52410105-52401004 Homo sapiens chromosome 3) is provided herein as SEQ ID No.1, as follows:

```
GAGCGCATGCCCGCATCTGCTGTCCGACAGGCGGAAGACGAGCCCAGAGGCGGAGCAGGGCCGTCGCGC
CTTGGTGACGTCTGCCCGCGCGCGGGCGGGTGACGCGACTGGGCCCGTTGTCTGTGTGTGGGACTGAG
GGGCCCCGGGGCGGTGGGGGCTCCCGGTGGGGGCGAGCGGTGGGGAGGGAGGGCCTGGACATGGCGCTG
AGGGGCGCGCCCGCGGAAGATGAATAAGGGCTGGCTGGAGCTGGAGAGCGACCCAGGTGAGGAGGGGA
CCGGGAGGGCCAGGGGCTGGGGAGGCCGGATGGGCCCGGGACGCGCCTGCCTGACCATCACCCCCCTCCT
CTTGTCGCCCCACCCAGGCCTCTTCACCCTGCTCGTGGAAGATTTTCGGTAAGAGCCTTTTCTCCCTGCC
GGACCGGGGCTGTGGCGGGCCACCCCTGCGCCCTCACTCATCAGGGGCTGTCTTCCCTACTGCTTTCC
TTTCTCATCGCAGGTGTCAAGGGGGTGCAAGTGGAGGAGATCTACGACCTTCAGAGCAAATGTCAGGG
GTGAGTGGCTGTACACCAGGGCTGCCCCCTTACACCCAGAGTGCTGGGGAAGGTCCCAGAGAACAGGGCC
```

- 0 -

```
CCTTAGGGAAGACAGTGCCAGGAACCTACGTTGTAAATCTCACAGAAAGCAGCAGCCTTGCTCTCTG
AGTGCCCGCICCTGATCAAACCTGATACTTTCTTTTCTCCCAAACCTTTCCTTAGCGCTTCCCTTTTGTGTA
GCAGCCCCCICCCACCCCTAAGCATCCTTTGGTTTCAGCTGCTTTCTGGCCTTGCAGCGGGAAGACCC
CGGTACACACAATGTCTTTTGTGAGTGTGTAAATGTATTAATTTAGTGTGCCCATGTGTCCTTGCTT
TAATCCTGACACAAAGTCATCCTGTATTGATTGGTTGGGGTGACAAGGCCCTCCTGGGIGCCACACT
TAGAGTCTTTTCCAGTGGTCCTGCAGAAATAGATGTGTAAGAGAGTAGCAACAGTAGCAACCGTGACTG
AACCAAGAAGTCTACTTTAATTTCTGGAACAAAAGAGACTGGTGTGGGTGTTTCAATTTGCTTTCCIGAC
TGCATTGGGGCCACAAAGTGAGAAGGAGTGCCTCAGTTCTCATCAGAGTTTTTGTCTTGTCTTACTT
TGTGTTCTTACCCTGTCCCATCCTTGCCCTCAGTTCAGCTTTTCTTCTCTTACCCAGAACTATAGAC
TTCATAAGGAGACTGGGTGGACTCCTGGAGCATCACAGTCAGAGGCTTATGCTTTGCTCTGCCTGTGGC
AGGCCTTTGGTGTGTGAGGGCACAAGGCCACTTCAGACACAGTGTGGGAAGAAGCCAGGGGAGAGGGG
GGATCACAGCAAGGACACCTGAGIGATGACGCAAGGATTAATGGGAGAAAGAAGGGAATGCTG
ATTGCTTTCTCCCTTTGGCTGATCTGGCTCTGCCCTTACTTCCCCAGCCCTGTATATGGATTTATC
TTCTGTTCAAATGGATCGAAGAGCGCCGGTCCCGGCGAAAGGTCTCTACCTGGTGGATGATACGTCC
GTGATTGATGATGATATTGTGAATAACATGTTCTTTGCCACCAGGTCTGCTGGACTCTGTGCTTTGTT
TGGAGGGTGGGATGCTGCCATGTTTTGTCTGGGAAGTGGAAATGGAGGAAGACAGGAGGAGGAGATAG
GCAGATICTAGGGGTGGTAGCTACAGAAATCCTCTGGCAGAACGAACTGAACTCTTAATTCATTAAAGG
GAACAGCTTTAGAGTAGGAGGGTGTCTGAGTCCACTCTCTGTGTCTCAGATATCCAGTGGGTATITGG
TAGGTGCTTGTAAATGAATAAACATTAGGCAAAGATGAAAGGAGCTGAGAAGGGGAGTTGTCCAGATA
TGACTGACCTGCTCTGGATCCCCATTCTTGATGTATAIGGGCTTGGGGCTTGCAGTGAGGGGTGCTGTG
TATGGGIGACTATTCTTGGTTTTCACAGCTGATACCCAACTCTTGTGCAACTCATGCCTTGCTGAGCGTG
CTCCIGAACIGCAGCAGCGTGGAACCTGGGACCCACCCAGTTCGATGAAGGACTTCACCAAGGGITTC
AGCCCTGAGGTAGGCTGCAGTGCCTTCATCCTGGCTCACAGCCAACTGGGCAGATCTGACCCCTGAGGGC
CACTGGGAAIGCTACCATGATATTGGGTACTATTAGGCTGTTTCTTTTCAAATGATIGTTTAIGTT
ACATTTGACICTTAAATAAATTGTGTAAGGCCATTGTTTTTAGATGCAGTTGCGGGGAAAGGACACAGG
CCTAGGGAGGGAGGAGAGTTTCTTAAGTCAGACCATGTCAGAACCTTCTCTGTCAGGACTTTTCTCT
CAGGCCATGTTGCTTCTAGTGTCCACTAATTACCATGCAAGGCCAGCACAGTCCATCTCTTTGGGGCT
CCAGAGCTCTTTTCTGCCCCCACCAGCCTTTTAAAGAAAGTTCGTCGTGTTTCTTCCGATTCTGGAAT
GCCTCCAGGCTGCTCTCTGAAGCCTTGCCTTCCACCCATAGTCTACCTGAGGAGAAATTATTCTGATA
CGGCCCTTATTTCTTCCCCGTAGAGCAAAGGATATGCGATTGGCAATGCCCGGAGTTGGCCAAGGCC
ATAATAGGATGCGAGCTCTCTGCGAGCTCTGCGAGGTCATCTCGGCTGCGACTAGGGGAGTATCAT
```

AATAAGCCATGCCAGGTTGTGGGAGCTGTGGGAGCTGATGTGGGGTGGGAGTAGGGGGAGTATCATTT
 TTTTGGGGCCCTGACTCTGTTTTTCCCCAGGGCCGAGCCACGCCACCTCCCTGAGAAGCAGAATGGCCCTT
 AGTGCAGTGC GGACCATGGAGGCGTTCCACTTTGTGAGCTATGTGCCTATCACAGGCCGGCTCTTTGAG
 CTGGATGGGCTGAAGGTCTACCCCATTGACCATGGTAGGCACCATGAGCTGGAGGCCTGTTGGGTGTCT
 CTGCCTACCICCTAGGGAGCTGGGGCTCAGGGCCCTCTGGTATGTGGTACCCAGTGGCAGGGGTTGTCTG
 GTACCGACACCCGGCTCTGGCTGGGGTTTCACCTACACCATATTGCCCGACCAGCTCCTGATTCCCTG
 GCTCAACTGCTCTTCTGTCTTCTTCCCTTCCCACTCCTGGCCTGCCCAAACCTCAGGGTTTTCTTCTGCTG
 ATTCTTGTCTTGGTCTCCACTAGGGCCCTGGGGGGAGGACGAGGAGTGGACAGACAAGGCCCGGGCGGG
 TCATCATGGAGCGTATCGGCCCTCGCCACTGCAGGGTAAGGGCCCTGTGCCTGCCCTGTTCTACTCICIG
 GAGCTGTACCTACTTTGGGAGGGACAGAGAGTATCCAGGTGATTTGTAAATTGCAAGGCCATATGCTGA
 ATCTGGCAAGATCAGGCTTAGATCATGGGTTCTCAACTTGTTGTCTTATTTCCCTGCCTGGGCTGCCTGT
 GGCCTGCTCCTGGGTGGGCTGGGGGAGGGGCAGGCCTCAGTGGAGCCTTAGGCCAGCCCAGGTCTGCTGG
 TTCACTTCCAGATAGGGCCCTCATACAGCTTGTGGAAAGGTACCAGCTCAGGTGCCGTCATGTAAGGC
 TAGTGGCTGCCTGCCTGTTGGGGTGGGGCCCTATACCTACAGCTGCAGGTGTGACTGCAGGGAGCCCTGC
 CAGGATATCIGCCTCAACCTGATGGCGGGGCCGGGGCGGGAGCTGCTCTCACGGCTGCGGCTGTGACTG
 CAGGGAGCCCTACCACGACATCCGCTTCAACCTGATGGCAGTGGTGCCCGACCGCAGGATCAAGTATGA
 GGCCAGGCTGCATGTGCTGAAGGTGAACCGTCAGACAGTACTAGAGGCTCTGCAGCAGGTAGGTGCCCT
 TTCTTCCCTGGCCTCTGCCAGCCCAACCTCCCTGCATTCTCTCTCCCTTCCCCCACAGCATTTGTCTC
 TGATTCTGTAACATACTCTCTTGTAGATCTGGGCTTCAGCTAACCACATCTTTTCTTTGCCCCCAITGT
 GGGAAAGGTGGGACTTGGAGTGGGGAGGGAGAATAGCTTCTAAAAGGAAGTTTGGGTTTGGGTGTTTAA
 TTTCCCTGTGAGTGAATGGGTAGAGCCAAGGCCATTATTCTTTAGGTCTCAGCCCTTAGCTATTTAA
 GGTAGAAGCCCGGGTCTACCCCTTCTCCTCTGAGCCCTGGATTCTGTTGTTAGCTGATAAGAGTAACAC
 AGCCAGAGCTGATTAGACCCACAAGTCTCAAGAGTCACAGCTGCCTGAGGAGTCCAAGTCAGCCAGCA
 ACAAGTCCCCGCTGGTGTGCTGGAAGCAAAACAGGGCCCTGCAGCCTCTGAGGGCAACCACACAGGTACTG

GGGGGTTTGGGACCTCTTGTGGACCTCAGAGCCACCCGCTAATGTCTGACATGGGAGGCCATAACAGGG
 AAAGTCTTTTTTCTGGGGATGTCTTGGGCAGTGTCTTCCCCCGTCAGAAGGTAGAGGGAGAGCAGTCC
 TTCCCTAAAGAAAGGCACCTGTAAAGGGCCGCTGTATCCACAGGCCCCCTGGGCCCTTCTCTGTAAATGTA
 CACTCCCTTTCTTGTITTTCTCTAGAGGCGGTTTTTTTTTTTTTTTTTTTTTTTTTTCTTCTGCTT
 CTTTTTCCCATCTCATTCTTTGCCCTGTCTCATTGCGGGATCATGACTTAGAGCTTGCTGACTCCCAT
 TGCACCAGCTGGCTGGGCTGTTCTTCTCTGGGAAGTGTGGTTTACAGGGCCGGGGAGACTGTGAGCTT
 TTCTTGGAGATCCTACTGGAGGTCTTGCCTGTGTTCTTGGCCCTGTCTCAGATGGTGCAGAGGAGGCGGC
 TGGTTCATGCGCACAAAGCCCCATCCACAGCCCTCCCAACAAACCCAAGCTAGTGGTGAAGCCTCCAGG
 CAGCAGCCTCAATGGGGTTCACCCCAACCCCACTCCCATTTGTCCAGCGGCTGCCGGCCTTTCTAGACAA
 TCACAATTATGCCAAGTCCCCCATGCAGGTAAGCTGGGAGCACCCCTTGCAGGATTCTCTACTTGATTCT
 CTTGAGAGGCIGCAACAGGCAATTTTCCCATGTGGTTTCTTGGTGTTCATCCTTGGCATGGCTGGGTCA
 AGCTGCCTGGGCCCTGGGTGTAGGTTTCTTGCCTGATAAGAAAAGGCCCCCAACAGCAGGAGCTT
 AGGGAGGCGAGGAGAGCTCCTTTGAATTTAATCTAGTTACGTGGCTGTGGGATTAAATGTTTAGGTAC
 CCTCCTTGGTACAACITCATGGGTGGGTTTTACTGGCAAAATAAAGGCATGTGTTTCAGGGCACTCTG
 TTTCTCTTAAAACCCCTCCGTGGGGTCTATCCAGTGTAAAGTGGGTGGCAGCCTCCCCACAAGCCAAGG
 ACAGGCCATGGAACAGCTGGAGGGGTTCCGCTGACTCAGTCTGGAAAACCATGTTGGCTTTCTCTCTGG
 CTGTGAGTGTCTAGGCTCAGCCTGGGCCGAGCAGCACTTGTTTGTAACTGCCCTGGTCTTTGICCCAGG
 AGGAAGAAGACCTGGCGGCAGGTGTGGGCCGAGCGAGTTCAGTCCGCCCACCCAGCAGTACTCAG
 ATGATGAGGATGACTATGAGGATGACGAGGAGGATGACGTGCAGAACACCAACTCTGCCCTTAGGTGAG
 CCCAGCTTTCTAAGGCTACCAGGTTCTAGGTGCTTGGATCCCATCCTGAATATCTCAGTCTGTGTCTG
 AGAATGCCCTGCAGCAGATAATGTTGAGCACCTGCGGAGTTTGGGGCCCTGGGGGAGGCTGGCATGATG
 GGGCTGACCCCAAGGTCCCCAGGAAGTTTTTGGTGGGCTGGGGGGTAAGGCTGAGCACGTAAGCTTATAT
 CATGTCTATIGGAAGTGGCCTTTTAGCCAGGCCTTGAAGGATTGGTTGGGGCAGGGATGGAGGAGATG
 TGGGTGGTGGGAGGCGAGCTTTGCTGGAACACAGGGCATTGGCAAAAGGCCAGGAGTGGGATGGCTGGA
 ATAGAGGAAGTGTCTTTTGGAGACACTTGGCTGCAGCTGTGAGAACTTGATGCCAGGCTTAGCATGGCT
 AGTTCAAGTTGCTTGGACCAAGTATAAGGAGTTTTAGGGTCAGCCCTGGAGGTGCGGATGTATTIAAG
 CCATTCTGGGTACTGCTGGGTATGGTACCTTGGCCCGTTCCCTTGTCTTACATCTTCTCGGGCCCCACA
 CGTATAAGGGGAAGGGAACAGGGAAGCCAGGGGCATTGAGCGGTTCTGCTGATGGGCAACTGTGAGTGC
 TGCAGCCCAACACCATCAACGTCTTGGCTGAGAAGCTCAAAGAGTCCCAGAAGGACCTCTCAATTCTCTC
 TGTCCATCAAGACTAGCAGCGGGGCTGGGAGTCCGGCTGTGGCAGTGGCCACACACTCGCAGCCCTCAC
 CCACCCCCAGCAATGAGAGTACAGACACGGCCTCTGAGATCGGCAGTGTCTTCAACTCGCCACTGCGCT
 CGCCTATCCGCTCAGCCAACCCGACGCGGCCCTCCAGCCCTGTACCTCCCACATCTCCAAGGTGCTTT
 TTGGAGAGGATGACAGCCTGCTGCGTGTGACTGCATACGCTACAACCGTGTGTCCGTGATCTGGGTG
 CTGTCATCAGCACAGCCCTGCTGCACCTGGCTGAGGATGGGGTGTGAGTCCCTTGGCGCTGACAGGTG
 GGCTTGGACIGGCTCACTGGCCACTTGGTGCACCCAGGAGGGAGGAGGGAAGTGGCCAAGTGACCACA
 AAGTGTCTTGTGACTCTGATGATTTTTCTTGTGACCTCTCTTCCAGAGGGTGGGAAGGGTTCTTGGCCCT
 CCATCAGACCAATCCAAGGCAGCCAGGGGTCCAGCAGCCAGTGGAGAAGGAGGTGTTGGAAGCCACGG
 ACAGCAGAGAGAAGACGGGGATGGTGAGGCCTGGCGAGCCCTTGAGTGGGGAGAAAATACTCACCCAAGG
 TGAGCCTCCGTTGTGTTTTTCTCTTTAATCCTGGCAGAGGGTAAGGCCTGAGCTCCTCCIGCCCAGGT
 CCCAAGTTCTTGTGTTGGAACITTTGGTGTGAAGATTGGTGGCTGGAGCCATGTGCCAGAAGACTTTCTGG
 GTTGGGTGGTGGCAGGGGCCCTTGATAGGCATGGACTCGCTGCTCATCTTGGCTCTAGCTGCCTATTGC
 TCGTGGGGCTTTGTTGCTGGCCCCGCCCGATCAGAGGTGCAATGCTGGGTTTTGGCAGGAGCTGCTGGC
 ACTGCTGAAGTGTGTGGAGGCTGAGATTGCAAACTATGAGGCGTGCCTCAAGGAGGAGGTAGAGAAGAG
 CAAGAAGTTCAAGGTGGGTGATTTCCTCAGTGTGCTGATCTGGCCCTCTCCCGAGGTCCACTGGTGGCTG
 CTCTGGCAAGATTGGCTCCAGTGTCTCAGTCTTCTTCTCTCTTACAGATTGATGACCAGAGAAGGACC

CACAACTACGATGAGITCATCTGCACCTTTATCTCCATGCTGGCTCAGGAAGGTGAGGGGATGCGCTGC
 IGTCTTAACTGGAATGCCCTGCTGAGGGCCGTGTCTTCAGCTCCCCCTCCCCCTGGCCTCTCCTGAGGCT
 IGAGCAGACCITGGGGCACAGGGAGGGCCATGAGAGCCTCAGCTCCTGGCCTGAGGCAGCCAGCACCTG
 CTCAAGGGTCTCTACCTCTTCGCAGGCATGCTGGCCAACTAGTGGAGCAGAACATCTCCGTGCGGCGG
 CGCCAAGGGGICAGCATCGGCCGGCTCCACAAGCAGCGGAAGCCTGACCGGCGGAAACGCTCTCGCCCC
 TACAAGGCCAAGCGCCAGTGAGGACTGCTGGCCCTGACTCTGCAGCCCACTCTTGCCGTGTGGCCCTCA
 CCAGGGTCTTCCCTGCCCCACTTCCCTTTTCCAGTATTACTGAATAGTCCCAGCTGGAGAGTCCAG
 GCCCTGGGAAIGGGAGGAACCAGGCCACATTCCTTCCATCGTGCCCTGAGGCCTGACACGGCAGATCAG
 CCCCATAGTGCTCAGGAGGCAGCATCTGGAGTTGGGGCACAGCGAGGTACTGCAGCTTCCTCCACAGCC

GGCTGTGGAGCAGCAGGACCTGGCCCTTCTGCCTGGGCAGCAGAATATATATATTTTACCTATCAGAGACA
 TCTATTTTTCTGGGCTCCAACCCAACATGCCACCATGTTGACATAAGTTCCCTACCTGACTATGCTTTCT
 CTCCTAGGAGCTGTCTGGTGGGCCCAGGTCTTGTATCATGCCACGGTCCCAACTACAGGGTCCTAGC
 TGGGGGCTGGGTGGGCCCTGGGCTCTGGGCCCTGCTGCTCTAGCCCCAGCCACCAGCCTGTCCCTGTT
 GTAAGGAAGCCAGGTCTTCTCTCTTCATTCCTCTTAGGAGAGTGCCAACTCAGGGACCCAGCACTGGG
 CTGGGTTGGGAGTAGGGTGTCCCAGTGGGGTTGGGGTGAGCAGGCTGCTGGGATCCCATGGCCTGAGCA
 GAGCATGTGGGAACTGTTCAGTGGCCTGTGAACCTGTCTTCCTTGTTCCTAGCCAGGCTGTTCAAGACTGC
 TCTCCATAGCAAGGTTCTAGGGCTCTTCGCCTTCAGTGTTGTGGCCCTAGCTATGGGCCTAAATTGGGC
 TCTAGGTCTCTGTCCCTGGCGCTTGAGGCTCAGAAGAGCCTCTGTCCAGCCCCCTCAGTATTACCATGTC
 TCCCTCTCAGGGGTAGCAGAGACAGGGTTGCTTATAGGAAGCTGGCACCCTCAGCTCTTCCTGCTACT
 CCAGTTTCTCAGCCTCTGCAAGGCACTCAGGGTGGGGGACAGCAGGATCAAGACAACCCGTGGAGCC
 CCIGTGTTCCAGAGGACCTGATGCCAAGGGGTAAATGGGCCAGCAGTGCCTCTGGAGCCCAGGCCCCAA
 CACAGCCCCATGGCCTCTGCCAGATGGCTTTGAAAAAGGTGATCCAAGCAGGCCCTTTATCTGTACAT
 AGTGACTGAGTGGGGGGTGTGGCAAGTGTGGCAGCTGCCTCTGGGCTGAGCACAGCTTGACCCCTCTA
 GCCCTGTAAATACTGGATCAATGAATGAATAAACTCTCCTAAGAATCTCCTGAGAAATGAA

[SEQ ID No.1]

[0020] The cDNA sequence encoding one embodiment of the human wild-type *BAP1* gene is provided herein as SEQ ID No. 2, as follows:

ATGAATAAGGGCTGGCTGGAGCTGGAGAGCGACCCAGGCCTCTTACCCCTGCTCGTGGAAGATTTCGGT
 GTCAAGGGGGTGCAAGTGGAGGAGATCTACGACCTTCAGAGCAAATGTCAGGGCCCTGTATATGGATTT
 ATCTTCTGTTCAAATGGATCGAAGAGCGCCGGTCCCGGCGAAAGGTCTCTACCTTGGTGGATGATACG
 TCCGTGATTGATGATGATATTGTGAATAACATGTTCTTTGCCACCAGCTGATACCCAACCTCTTGTCGA
 ACTCATGCCTTGCTGAGCGTGCTCCTGAACCTGCAGCAGCGTGACCTGGGACCCACCCTGAGTCGCATG
 AAGGACTTCACCAAGGGTTTCAGCCCTGAGAGCAAAGGATATGCGATTGGCAATGCCCCGGAGTTGGCC
 AAGGCCCATAAATAGCCATGCCAGGCCCGAGCCACGCCACCTCCCTGAGAAGCAGAATGGCCTTAGTGCA
 GTGCGGACCATGGAGGCGTTCACCTTTGTGAGCTATGTGCCTATCACAGGCCGGCTCTTTGAGCTGGAT
 GGGCTGAAGGTCTACCCCATTGACCATGGGCCCTGGGGGGAGGACGAGGAGTGGACAGACAAGGCCCGG
 CGGGTCATCATGGAGCGTATCGGCCTCGCCACTGCAGGGGAGCCCTACCACGACATCCGCTTCAACCTG
 ATGGCAGTGGTGGCCGACCGCAGGATCAAGTATGAGGCCAGGCTGCATGTGCTGAAGGTGAACCGTCAG
 ACAGTACTAGAGGCTCTGCAGCAGCTGATAAGAGTAACACAGCCAGAGCTGATTACAGACCCACAAGTCT
 CAAGAGTCAAGCTGCCTGAGGAGTCCAAGTCAGCCAGCAACAAGTCCCCGCTGGTGCTGGAAGCAAAC
 AGGGCCCCCTGCAGCCTCTGAGGGCAACCACACAGATGGTGCAGAGGAGGCGGCTGGTTTCATGCGCACA
 GCCCCATCCACAGCCCTCCCAACAAACCCAAGCTAGTGGTGAAGCCTCCAGGCAGCAGCCTCAATGGG
 GTTACCCCCAACCCCACTCCCATGTGTCAGCGGCTGCCGGCCTTTCTAGACAATCACAATTATGCCAAG
 TCCCCCATGCAGGAGGAAGAAGACCTGGCGGCAGGTGTGGGCCGAGCCGAGTTCAGTCCGCCCACCC
 CAGCAGTACTCAGATGATGAGGATGACTATGAGGATGACGAGGAGGATGACGTGCAGAACACCAACTCT
 GCCCTTAGGTATAAGGGGAAGGGAACAGGGAAGCCAGGGGCATGAGCGGTCTGCTGATGGGCAACTG
 TCAGTGCTGCAGCCCAACACCATCAACGTCTTGGCTGAGAAGCTCAAAGAGTCCCAGAAGGACCTCTCA
 ATTCTCTGTCCATCAAGACTAGCAGCGGGGCTGGGAGTCCGGCTGTGGCAGTGGCCACACACTCGCAG
 CCCTCACCCACCCCAAGCAATGAGAGTACAGACACGGCCTCTGAGATCGGCAGTGCTTTCAACTCGCCA
 CTGCGCTCGCCTATCCGCTCAGCCAACCCGACGCGGCCCTCCAGCCCTGTCACCTCCCACATCTCCAAG
 GTGCTTTTTGGAGAGGATGACAGCCTGCTGCGTGTGACTGCATACGCTACAACCGTGCTGTCCGTGAT
 CTGGGTCTGTCAICAGCACAGGCCTGCTGCACCTGGCTGAGGATGGGGTGCTGAGTCCCCTGGCGCTG
 ACAGAGGGTGGGAAGGGTTCTCGCCCTCCATCAGACCAATCCAAGGCAGCCAGGGGTCCAGCAGCCCA
 GTGGAGAAGGAGGTCGTGGAAGCCACGGACAGCAGAGAGAAGACGGGGATGGTGAGGCCTGGCGAGCCC
 TTGAGTGGGGAGAAATACTCACCCAAGGAGCTGCTGGCACTGCTGAAGTGTGTGGAGGCTGAGATTGCA
 AACTATGAGGCGTGCTCAAGGAGGAGGTAGAGAAGAGGAAGAAGTTCAAGATTGATGACCAGAGAAGG
 ACCCACAACCTACGATGAGTTCATCTGCACCTTTATCTCCATGCTGGCTCAGGAAGGCATGCTGGCCAAC
 CTAGTGGAGCAGAACATCTCCGTGCGGCGGCGCCAAGGGGTGAGCATCGGCCGGCTCCACAAGCAGCGG
 AAGCTGACCGGCGGAAACGCTCTCGCCCCCTACAAGGCCAAGCGCCAGTGA

[SEQ ID No.2]

[0021] The amino acid sequence of one embodiment of human wild-type BAP1 is referred to herein as SEQ ID No. 3, as follows:

MNKGWLELESDPGLFTLLVEDFGVKG VQVEEIYDLQSKCQGPVYGFIFL FKWIEERRSRRKVSTLVDDT
 SVIDDDIVNNMFFAHQLIPNSCATHALLSVLLNCSSVDLGPTLSRMKDFTKGFSPE SKGYAIGNAPELA
 KAHNSHARPEPRHLPEKQNGLSAVRTMEAFHFVSYVPITGRLELDGLKVYPIDHGPWGEDEEWTDKAR
 RVIMERIGLATAGEPYHDIRFNLMAVVPDRRIKYEARLHVLKVN RQTVLEALQQLIRVTQPELIQTHKS
 QESQLPEESKSASNKSPVLLEANRAPAAASEGNHTDGAEAAAGSCAQAPSHSPPNKP KLVVKPPGSSLNG
 VHPNFTPIVQRLPAFLDNHNYAKSPMQEEEDLAAGVGRSRVPVRPPQQYSDD EDDYEDDEEDDVQNTNS
 ALRYKKGKGTGKPGALSGSADGQLSVLQPN TINVLAEKLKESQKDLSIPLSIKTSSGAGSPAVAVPTH SQ
 PSPTPSNESTDTASEIGSAFNSPLRSPIRSANPTRPSSPV TSHISKVLFGEDDSLLRVDCIRYNRAVRD
 LGPVI STGLLHLAEDGVLSP LALTEGGKGSSPSIRPIQGSQGS SSPVEKEVVEATDSREKTGMVRPGE P
 LSGEKYSPKELLALLKCVEAEIANYEACLKEEVEKRKKFKIDDQRTHNYDEFICTFISMLAQEGMLAN
 LVEQNISVRRRQGVSIGRLHKQRKPDRKR SRPYKAKRQ

[SEQ ID No.3]

[0022] Tumours arise due to mutations in proto-oncogenes or tumour suppressor genes. A gain-of-function mutation in a proto-oncogene converts it into an oncogene, which causes tumourigenesis. Such gain-of-function mutations are usually missense mutations in the DNA bases, which confer a change in the amino acid sequence, or a gain of copy number of the gene. A loss-of-function mutation in a tumour suppressor gene will also lead to tumourigenesis. A loss-of-function mutation in a tumour suppressor however is not restricted to the change of a few amino acids. Any change in an amino acid which impairs the function of the protein, results in a loss-of-function mutation. Hence, unlike oncogenes, it is not always possible to identify a specific mutation which results in the loss of function of a tumour suppressor gene. Loss-of-function truncating mutations of tumour suppressors are therefore typically identified throughout the coding exons of a gene.

[0023] BAP1 is a tumour suppressor gene. According to the invention, a mutant *BAP1* gene is one that comprises a mutation. The mutant *BAP1* gene is a gene that encodes a non-functional or enzymatically inactive BAP1 protein, or a BAP1 protein that exhibits reduced binding to an ASXL protein compared to the level of binding in a reference cell, which is resistant to DRL-induced cell death. Thus, a mutant BAP1 protein is one that is non-functional or enzymatically inactive or incapable of binding to an ASXL protein or exhibits reduced binding to an ASXL protein compared to the level of binding in a reference cell, which is resistant to DRL-induced cell death. As described in the Examples (see Figure 7d), BAP1 has surprisingly been shown to form a complex with ASXL1, ASXL2 or ASXL3. These proteins will be collectively referred to herein as an ASXL protein. BAP1-ASXL complexes have been shown to deubiquitinate Histone 2A, and other substrates. Inhibiting the formation of this complex renders BAP1 non-functional or enzymatically inactive.

[0024] The mutant BAP1 protein may be a full-length protein with mutations at specific loci. The mutant BAP1 protein may be a partial or complete deletion or mutation of the wild-type BAP1 protein. Partial deletion or mutation may occur in the nuclear localisation sequence (NLS), the active site of wild-type BAP1, the binding site of ASXL, or at any place in the gene that would result in the loss of function of BAP1. Mutation may include one or more point mutations. Point mutations may be a substitution, an insertion, a deletion or a frameshift mutation.

[0025] A reduced level of expression of a wild-type *BAP1* gene compared to the level of expression in a reference cell may result in a lower wild-type BAP1 protein concentration compared to the protein concentration in the same reference cell. The reduced level of expression of the wild-type

BAP1 gene may be at least a 10%, 15%, 25%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or a 100% reduction compared to the reference cell. Similarly, the lower concentration of wild-type BAP1 protein may be lower by at least 10%, 15%, 25%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% compared to the reference cell. The skilled person would know how to detect the extent of the reduction in *BAP1* gene expression or lower BAP1 protein expression.

[0026] Lower expression of wild-type BAP1 protein may be caused by epigenetic silencing, methylation or low levels of *BAP1* gene expression. The term "non-functional BAP1 protein" can refer to but is not limited to a BAP1 protein that does not exhibit deubiquitinase (enzyme) activity. The skilled person would appreciate that standard assays for measuring deubiquitinase activity include, but are not limited to, fluorescence assays using a fluorogenic substrate, such as ubiquitin-amidomethylcoumarin; and HPLC assays using ubiquitin ethyl ester or ubiquitin fusion peptides as model substrates to monitor deubiquitinating activity.

[0027] In one embodiment, the amino acid sequence of a mutant BAP1 gene (p.R60Q) is referred to herein as SEQ ID NO. 4, as follows:

```
1  mnkgwleles dpglftllve dfgvkgvqve eiyclqskcq gpvygfiflf kwieerrsrq
61  kvstlvddts vidddivnm ffahqlipns cathallsvl lncssvdlgp tlsrmkdftk
```

- 11 -

```
121  gfspekgya ignapelaka hnsharpepr hlpekngls avrtmeafhf vsyvpitgrl
181  feldglkvyp idhgpwgede ewtdkarrvi meriglatag epyhdirfnl mavvpdrrik
241  yearlhvlkv nrqtvlealq qlirvtqpel iqthksqesq lpeesksasn ksplvleanr
301  apaasegnht dgaeaaagsc aqapshsppn kpklvvkppg sslngvhpnp tpivqrlpaf
361  ldnhnyaksp mgeeedlaag vgrsrvpvrp pqgysddedd yeddeeddvq ntosalrykg
421  kgtgkpgals gsadgqlsvl qpntinvlae klkesqkdl iplsiktssg agspavavpt
481  hsqpsptpsn estdtaseig safnsplrps irsanptrps spvtshiskv lfgeddslr
541  vdcirynrav rdlgpvistg llhlaedgvl splalteggk gsspsirpiq gsqgssspve
601  kevveatdsr ektgmvrpge plsgekyspk ellallkcve aeianyeacl keevekrkkf
661  kiddqrrthn ydefictfis mlaqegmlan lveqnisvrr rggvsigrh kqrkpdrrkr
721  srpykakrq
```

[SEQ ID No.4]

[0028] Therefore, the amino acid sequence of the mutant BAP1 protein may be encoded by SEQ ID NO. 4 or a fragment or variant thereof.

[0029] In one embodiment, the nucleotide sequence of a mutant *BAP1* gene is referred to herein as SEQ ID No. 5, as follows:

```
ATGAATAAGGGCTGGCTGGAGCTGGAGAGCGACCCAGGCCTCTTCACCCTGCTCGTGGAAGATTTGGT
GTCAAGGGGGGTGCAAGTGGAGGAGATCTACGACCTTCAGAGCAAATGTCAGGGCCCTGTATATGGATTT
ATCTTCCTGTTCAAATGGATCGAAGAGCGCCGGTCCCGGCAAAAGGTCTCTACCTTGGTGGATGATACG
TCCGTGATTGATGATGATATTGTGAATAACATGTTCTTTGCCACACAGCTGATACCCAACCTCTTGTCGA
ACTCATGCCTTGCTGAGCGTGCTCCTGAACTGCAGCAGCGTGGACCTGGGACCCACCCTGAGTCGCATG
AAGGACTTCACCAAGGGTTTCAGCCCTGAGAGCAAAGGATATGCGATTGGCAATGCCCCGGAGTTGGCC
AAGGCCCATAGCCATGCCAGGCCCGAGCCACGCCACCTCCCTGAGAAGCAGAAATGGCCTTAGTGCA
GTGCGGACCATGGAGGCGTTCCACTTTGTCAGCTAIGTGCCTATCACAGGCCGGCTCTTTGAGCTGGAT
GGGCTGAAGGTCTACCCCATGACCATGGGCCCTGGGGGGAGGACGAGGAGTGGACAGACAAGGCCCGG
CGGGTCATCATGGAGCGTATCGGCCTCGCCACTGCAGGGGAGCCCTACACGACATCCGCTTCAACCTG
ATGGCAGTGGTGGCCGACCGCAGGATCAAGTATGAGGCCAGGCTGCATGTGCTGAAGGTGAACCGTCAG
ACAGTACTAGAGGCTCTGCAGCAGCTGATAAGAGTAACACAGCCAGAGCTGATTCAGACCCACAAGTCT
CAAGAGTCACAGCTGCCTGAGGAGTCCAAGTCAGCCAGCAACAAGTCCCCGCTGGTGCTGGAAGCAAAC
AGCCCGCCTCCAGCCTCTCAGCCCAAGCAGCAGATCCTCCAGCAGCAGCCCGCTCCTTCATCCCGCAG
```

AGGGCCCCTGAGCCCTGAGGGCAACACACAGATGGTGCAGAGGAGGCGGCTGGTTCATGCGCAAA
 GCCCCATCCACAGCCCTCCCAACAAACCCAAGCTAGTGGTGAAGCCTCCAGGCAGCAGCCTCAATGGG
 GTTCACCCCAACCCCACTCCCATTTGTCCAGCGGCTGCCGGCCTTTCTAGACAATCACAATTATGCCAAG
 TCCCCCATGCAGGAGGAAGAAGACCTGGCGGCAGGTGTGGGCCGAGCCGAGTTCCAGTCCGCCCACCC
 CAGCAGTACTCAGATGATGAGGATGACTATGAGGATGACGAGGAGGATGACGTGCAGAACACCAACTCT
 GCCCTTAGGTATAAGGGGAAGGGAACAGGGAAGCCAGGGGCATTGAGCGGTTCTGCTGATGGGCAACTG
 TCAGTGCTGCAGCCCAACACCATCAACGTCCTTGGCTGAGAAGCTCAAAGAGTCCCAGAAGGACCTCTCA
 ATTCTCTGTCCATCAAGACTAGCAGCGGGGCTGGGAGTCCGGCTGTGGCAGTGGCCACACACTCGCAG
 CCCTCACCCACCCCAAGCAATGAGAGTACAGACACGGCCTCTGAGATCGGCAGTGCTTTCAACTCGCCA
 CTGCGCTCGCCTATCCGCTCAGCCAACCCGACGCGGCCCTCCAGCCCTGTCACCTCCCACATCTCCAAG
 GTGCTTTTTTGGAGAGGATGACAGCCTGCTGCGTGTGACTGCATACGCTACAACCGTGCTGTCCGTGAT
 CTGGGTCCTGTCATCAGCACAGGCCTGCTGCACCTGGCTGAGGATGGGGTGCTGAGTCCCCTGGCGCTG

 ACAGAGGGTGGAAGGGTTCCTCGCCCTCCATCAGACCAATCCAAGGCAGCCAGGGGTCCAGCAGCCCA
 GTGGAGAAGGAGGTCTGTGAAGCCACGGACAGCAGAGAGAAGACGGGGATGGTGAGGCCTGGCGAGCCC
 TTGAGTGGGGAGAAATACTCACCCAAGGAGCTGCTGGCACTGCTGAAGTGTGTGGAGGCTGAGATTGCA
 AACTATGAGGCGTGCTCAAGGAGGAGGTAGAGAAGAGGAAGAAGTTCAAGATTGATGACCAGAGAAGG
 ACCCACAACCTACGATGAGTTCATCTGCACCTTTATCTCCATGCTGGCTCAGGAAGGCATGCTGGCCAAC
 CTAGTGGAGCAGAACATCTCCGTGCGGCGGCCAAGGGGTCAGCATCGGCCGGCTCCACAAGCAGCGG
 AAGCTGACCGGCGGAACGCTCTCGCCCTACAAGGCCAAGCGCCAGTGA

[SEQ ID No.5]

[0030] Therefore, the nucleotide sequence of the mutant *BAP1* gene may be encoded by SEQ ID NO. 5 or a fragment or variant thereof.

[0031] Reduced or non-binding between an ASXL protein (ASXL1, ASXL2 or ASXL3) and a wild-type BAP1 protein may be at least a 10%, 15%, 25%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or a 100% reduction compared to a reference cell in which binding between a wild-type BAP1 protein and an ASXL protein occurs. The skilled person would know how to detect the extent of the reduction in wild-type BAP1 protein binding to an ASXL protein. Standard assays for measuring binding between a wild-type BAP1 protein and an ASXL protein include but are not limited to protein complex immunoprecipitation or fluorescence resonance energy transfer (FRET).

[0032] The term "DRL-induced cell death" can refer to, but is not limited to, apoptosis and other types of cell death caused by a death receptor ligand (DRL), such as necroptosis and necrosis. Thus, the term "death receptor ligand" refers to any agent that binds to a cellular receptor and induces death of the cell on which the receptor is located. The term "cell death" can refer to cellular apoptosis, necrosis and necroptosis. Preferably, it refers to cellular apoptosis. Apoptosis refers to programmed cell death caused by activation of an apoptotic signal transduction pathway. This may be achieved through the binding of a DRL to a death receptor. Death receptor ligands may be TRAIL, TNFalpha, FAS ligand (FASL), recombinant TRAIL (dulanermin), antibodies to death receptors, especially antibodies to death receptors of the ligand TRAIL (such as mapatumumab, drozitumumab, conatumumab, lexatumumab, tigatuzumab, LBY-135), or a combination thereof.

[0033] The DRL may be an agonist molecule such as Medi-3039 or any agent that activates an apoptotic signal transduction pathway. An extrinsic apoptotic signal transduction pathway may be the FAS ligand pathway, the TNFalpha pathway or the TRAIL pathway.

[0034] The term "express(ed) or expression" can refer to a transcribed gene (i.e. DNA), or corresponding RNA that has been translated into a polypeptide or protein. Expression of a *BAP1* mutant gene or BAP1 polypeptide may be detected in any compartment of the cell (e.g. in the nucleus, cytosol, the Endoplasmic Reticulum, the Golgi apparatus or the intracellular surface of the plasma membrane).

[0035] Detecting according to (i), (ii) or (iii) of this disclosure may comprise the use of any one of the following assays for detecting the presence of a gene or its corresponding protein in a sample: polymerase chain reaction (PCR); northern blotting; hybridisation-based detection techniques; flow cytometry; immunoassays, such as enzyme-linked immunosorbent assays (ELISAs), an enzyme immunoassay (EIAs), radioimmunoassay (RIAs), Western Blots, immuno-precipitation or immunohistochemistry; immunofluorescence; chromogenic (enzyme activity) assays; fluorometric imaging plate reader (FLIPR) assay; high performance liquid chromatography (HPLC) tandem mass spectrometry (MS/MS).

[0036] The biological sample is preferably a cancerous bodily sample taken from a test individual. Detection for the presence of a *BAP1* mutant gene or mutant BAP1 protein in the sample is therefore preferably carried out *in vitro*. The sample may comprise tissue, blood, plasma, serum, spinal fluid, urine, sweat, saliva, sputum, tears, breast aspirate, prostate fluid, seminal fluid, vaginal fluid, stool, cervical scraping, amniotic fluid, intraocular fluid, mucous, moisture in breath, animal tissue, cell lysates, tumour tissue, hair, skin, buccal scrapings, nails, bone marrow, cartilage, prions, bone powder, ear wax, or combinations thereof. The sample may be a biopsy.

[0037] In another example, the sample may be contained within the test subject, which may be an experimental animal (e.g. a mouse or rat) or a human, wherein the method is an *in vivo* based test. Alternatively, the sample may be an *ex vivo* sample or an *in vitro* sample. Therefore, the cells being tested may be in a tissue sample (for *ex vivo* based tests) or the cells may be grown in culture (an *in vitro* sample). Preferably, the biological sample is an *ex vivo* sample.

[0038] The inventors have developed a prognostic kit for determining a subject's sensitivity or otherwise to DRL-induced cell death.

[0039] According to another example, there is provided a kit for determining if an individual's cancer cell is sensitive to DRL-induced cell death, the kit comprising detection means for detecting the expression of a mutant *BAP1* gene or mutant BAP1 protein, or for detecting a reduced level of expression of a wild-type *BAP1* gene or a lower wild-type BAP1 protein concentration compared to the level of expression or protein concentration in a reference cell that is resistant to DRL-induced cell death, or for detecting non-binding or reduced binding of an ASXL protein to a BAP1 protein compared to the level of binding in a reference cell that is a BAP1 wild-type cell that is resistant to DRL-induced cell death, wherein the presence, in the sample, of the mutant *BAP1* gene or the BAP1 protein, or of a reduced level expression of the wild-type *BAP1* gene or a lower wild-type BAP1 protein concentration, or reduced or non-binding of an ASXL protein to a wild-type BAP1 protein, is indicative of the individual's cancer cell being sensitive to DRL-induced cell death.

[0040] Preferably, the kit is used to provide a prognosis for an individual being treated with a death receptor ligand (DRL) or any agents activating the apoptotic pathways. A death receptor ligand may

be TRAIL, TNFalpha, FAS ligand (FASL), recombinant TRAIL (dulanermin), death receptor antibodies (such as mapatumumab, drozitumumab, conatumumab, lexatumumab, tigatuzumab), death receptor agonists, such as Medi-3038 or Medi-3039, or a combination thereof.

[0041] Preferably, the kit comprises at least one control or reference sample. The kit may comprise a negative control and/or a positive control. A negative control may comprise a wild-type BAP1 protein that is resistant to DRL-induced cell death. A positive control may comprise a mutant BAP1 mRNA, or mutant BAP1 protein, or a blank sample. The skilled person will appreciate that the level of mRNA in a sample is indicative of the level of gene expression in a cell.

[0042] The detection means is preferably configured to detect the expression or the concentration of a mutant BAP1 protein or mRNA, or wild-type BAP1 mRNA or BAP1 protein in the biological sample taken from the test individual. The presence of the mutant BAP1 mRNA or mutant BAP1 protein, or reduced level of expression of wild-type BAP1 mRNA or lower protein expression to the control, in the sample, is indicative that the test sample is sensitive to DRL-induced cell death. The level of expression of the wild-type BAP1 mRNA or concentration of BAP1 protein in the biological sample may be reduced or lower compared to the concentration or level of expression of the wild-type BAP1 mRNA or protein in a negative control. The reduction in expression may be at least a 10%, 15%, 25%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% reduction compared to the negative control. Similarly, the concentration of the wild-type BAP1 protein in the biological sample may be lower by at least a 10%, 15%, 25%, 35%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%. Conversely, the absence of a mutant BAP1 gene or protein, or normal to high expression of the wild-type BAP1 gene or protein, in the sample, is indicative that the sample is insensitive to DRL-induced cell death.

[0043] Detection of a mutation in a BAP1 gene or protein can be achieved using a number of sequencing approaches. In one approach, whole exome or targeted gene sequencing is undertaken with massively parallel sequencing of tumour DNA with target genes enriched using commercially available RNA baits.

[0044] In another example, a capillary sequencing approach is utilised with PCR primers designed to each of the exons in the BAP1 gene footprint. Thus, the detection means may be a primer.

[0045] In one example, the nucleic acid sequence of a forward primer used to detect exon 12 (chr3:52438255-52438817F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 6 as follows:

acctagaacctggtagccttag [SEQ ID No. 6]

[0046] In one example, the nucleic acid sequence of a forward primer used to detect exon 8 (chr3:5244063i-5244H38F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 7 as follows:

gtacagctccagagagtagaac [SEQ ID No. 7]

[0047] In one example, the nucleic acid sequence of a forward primer used to detect exon 1 (chr3:52443644-52444094F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 8 as follows:

tcttaccgaaatcttccacgag [SEQ ID No. 8]

[0048] In one example, the nucleic acid sequence of a forward primer used to detect exon 3 (chr3:52443356-52443839F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 9 as follows:

ctgctgcttctgtgagatttt [SEQ ID No. 9]

[0049] In one example, the nucleic acid sequence of a forward primer used to detect exon 6 (chr3:52436404-52436905F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 10 as follows:

agggcattccagttaagacag [SEQ ID No. 10]

[0050] In one example, the nucleic acid sequence of a forward primer used to detect exon 17 (chr3:52436105-52436652F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 11 as follows:

caagagtgggctgcagag [SEQ ID No. 11]

[0051] In one example, the nucleic acid sequence of a forward primer used to detect exon 6 (chr3:52441201-52441691F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 12 as follows:

actaaggccattctgcttctc [SEQ ID No. 12]

[0052] In one example, the nucleic acid sequence of a forward primer used to detect exon 4 (chr3:52442276-52442837F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 13 as follows:

atcccaccctccaaacaaag [SEQ ID No. 13]

[0053] In one example, the nucleic acid sequence of a forward primer used to detect exon 13a (chr3:52437218-52437786F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 14 as follows:

caccaagtggccagtgag [SEQ ID No. 14]

[0054] In one example, the nucleic acid sequence of a forward primer used to detect exon 13b (chr3:52437388-52437956F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 15 as follows:

ggctgtcatcctctccaaaa [SEQ ID No. 15]

[0055] In one example, the nucleic acid sequence of a forward primer used to detect exon 13c (chr3:52437558-52438125F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 16 as follows:

gagggctgcgagtggtg [SEQ ID No. 16]

[0056] In one example, the nucleic acid sequence of a forward primer used to detect exon 14 (chr3:52436940-52437529F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 17 as follows:

ctctgccaggattaaaggagaa [SEQ ID No. 17]

[0057] In one example, the nucleic acid sequence of a forward primer used to detect exon 9 (chr3:52440055-52440607F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 18 as follows:

gaatgcagggagggttg [SEQ ID No. 18]

[0058] In one example, the nucleic acid sequence of a forward primer used to detect exon 5 (chr3:52441760-52442308F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 19 as follows:

acccaatatcatgtggtagcat [SEQ ID No. 19]

[0059] In one example, the nucleic acid sequence of a forward primer used to detect exon 2 (chr3:52443516-52443974F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 20 as follows:

aaggacagcccctgatga [SEQ ID No. 20]

[0060] In one example, the nucleic acid sequence of a forward primer used to detect exon 7 (chr3:52440976-52441547F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 21 as follows:

gtaggcagagacaccaac [SEQ ID No. 21]

[0061] In one example, the nucleic acid sequence of a forward primer used to detect exon 15 (chr3:52436581-52437102F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 22 as follows:

ccttctctggtcatcaatctgt [SEQ ID No. 22]

[0062] In one example, the nucleic acid sequence of a forward primer used to detect exon 10 (chr3:52439567-52440143F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 23 as follows:

ctctgaggtccacaagaggt [SEQ ID No. 23]

[0063] In one example, the nucleic acid sequence of a forward primer used to detect exon 11 (chr3:52438912-52439525F) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 24 as follows:

tcaagtagagaatcctgcaagg [SEQ ID No. 24]

[0064] In one example, the nucleic acid sequence of a reverse primer used to detect exon 12 (chr3:52438255-52438817R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 25 as follows:

gagcagcactgtttgtaactg [SEQ ID No. 25]

[0065] In one example, the nucleic acid sequence of a reverse primer used to detect exon 8 (chr3:52440631-52441138R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 26 as follows:

ctcaactgctcttctgtctt [SEQ ID No. 26]

[0066] In one example, the nucleic acid sequence of a reverse primer used to detect exon 1

(chr3:52443644-52444094R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 27 as follows:

gagggagggcctggacat [SEQ ID No. 27]

[0067] In one example, the nucleic acid sequence of a reverse primer used to detect exon 3 (chr3:52443356-52443839R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 28 as follows:

ctgtccttcctactgctttc [SEQ ID No. 28]

[0068] In one example, the nucleic acid sequence of a reverse primer used to detect exon 16 (chr3:52436404-52436905R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 29 as follows:

gaagttcaaggtgggtgatttc [SEQ ID No. 29]

[0069] In one example, the nucleic acid sequence of a reverse primer used to detect exon 17 (chr3:52436105-52436652R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 30 as follows:

ctcagctcctggcctgag [SEQ ID No. 30]

[0070] In one example, the nucleic acid sequence of a reverse primer used to detect exon 6 (chr3:52441201-52441691R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 31 as follows:

ggagaaattattctgatacgcc [SEQ ID No. 31]

[0071] In one example, the nucleic acid sequence of a reverse primer used to detect exon 4 (chr3:52442276-52442837R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 32 as follows:

gaagggaatgctgattgtcttc [SEQ ID No. 32]

[0072] In one example, the nucleic acid sequence of a reverse primer used to detect exon 13a (chr3:52437218-52437786R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 33 as follows:

ctatccgctcagccaacc [SEQ ID No. 33]

[0073] In one example, the nucleic acid sequence of a reverse primer used to detect exon 13b (chr3:52437388-52437956R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 34 as follows:

ctctcaattcctctgtccatca [SEQ ID No. 34]

[0074] In one example, the nucleic acid sequence of a reverse primer used to detect exon 13c (chr3:52437558-52438125R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 35 as follows:

cgttccttgcttcacatct [SEQ ID No. 35]

[0075] In one example, the nucleic acid sequence of a reverse primer used to detect exon 14 (chr3:52436940-52437529R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 36 as follows:

follows:

tcctgcactctgatgatttct [SEQ ID No. 36]

[0076] In one example, the nucleic acid sequence of a reverse primer used to detect exon 9 (chr3:52440055-52440607R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 37 as follows:

gatatctgcctcaacctgatgg [SEQ ID No. 37]

[0077] In one example, the nucleic acid sequence of a reverse primer used to detect exon 5 (chr3:52441760-52442308R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 38 as follows:

gtgctgtgtatgggtgacta [SEQ ID No. 38]

[0078] In one example, the nucleic acid sequence of a reverse primer used to detect exon 2 (chr3:52443516-52443974R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 39 as follows:

gaagatgaataagggctggct [SEQ ID No. 39]

[0079] In one example, the nucleic acid sequence of a reverse primer used to detect exon 7 (chr3:52440976-52441547R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 40 as follows:

tgatgtgggtgggagtag [SEQ ID No. 40]

[0080] In one example, the nucleic acid sequence of a reverse primer used to detect exon 15 (chr3:52436581-52437102R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 41 as follows:

cccgatcagaggtgcaat [SEQ ID No. 41]

[0081] In one example, the nucleic acid sequence of a reverse primer used to detect exon 10 (chr3:52439567-52440143R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 42 as follows:

agctatttaaggtagaagcccg [SEQ ID No. 42]

[0082] In one example, the nucleic acid sequence of a reverse primer used to detect exon 11 (chr3:52438912-52439525R) of wild-type BAP1 mRNA is referred to herein as SEQ ID No. 43 as follows:

actgtgagcttttcttgagat [SEQ ID No. 43]

[0083] The skilled person would appreciate that the binding of an ASXL protein to a wild-type BAP1 protein may be achieved using a variety of techniques known in the art, which include protein complex immunoprecipitation, Bimolecular Fluorescence complementation, Affinity electrophoresis, Immunoelectrophoresis, chemical cross linking, Proximity ligation assay and FRET.

[0084] The inventors believe that their findings (i.e. that cells containing a mutant *BAP1* gene or a mutant BAP1 protein, or cells expressing reduced levels of a wild-type *BAP1* gene or with a lower concentration of BAP1 protein, are sensitive to DRL-induced cell death) means that they have

identified a robust biomarker for sensitivity to DRL-induced cell death.

[0085] Therefore, in another example, there is provided a use of (i) a mutant *BAP1* gene or a mutant BAP1 protein, or (ii) a cancer cell with a reduced level of expression of a wild-type *BAP1* gene or a lower wild-type BAP1 protein concentration compared to the level of expression or protein concentration in a reference cell that is a BAP1 wild-type cell, as a biomarker of sensitivity to DRL-induced cell death.

[0086] The inventors have developed a method of treating subjects suffering from cancer, and especially those suffering from a cancer that is completely or partially insensitive to death receptor ligand-induced cell death.

[0087] The death receptor ligand may be administered as a monotherapy or in combination with other agents that are capable of killing cancer cells.

[0088] The "subject" or "individual" may be a vertebrate, mammal, or domestic animal. Hence, medicaments according to this disclosure may be used to treat any mammal, for example livestock (e.g. a horse), pets, or may be used in other veterinary applications. Most preferably, the subject is a human being.

[0089] As mentioned above, mesothelioma cells are resistant to the induction of cell death. This disruption of core-cell death machinery has been implicated in the resistance to conventional cytotoxic agents generally observed clinically. It is believed that disruption of cell death machinery is mediated downstream through elevated expression of anti-cell death proteins such as members of the IAP (inhibitors of apoptosis) family or the BCL-2 family. The IAP family consist of 8 members (*BIRC2*, *BIRC3*, *BIRC5*, *BIRC6*, *BIRC7*, *BIRC8*, *NAIP*, *XIAP*). The defining feature of an IAP protein is the presence of a ~70-amino acid baculovirus IAP repeat (BIR) domain that mediates protein-protein interactions (12). Through these domains, IAP members act as endogenous inhibitors of caspases, the main executioners of cell death, and act through either direct caspase inhibition or ubiquitin-mediated regulation of caspase degradation (13).

[0090] The inventors have demonstrated that *BAP1* gene expression can be reduced in mesothelioma cell lines using a BAP1 shRNA-expressing lentivirus and that this reduction of *BAP1* gene expression results in increased sensitivity to DLR-induced cell death in these cell lines. Accordingly, the inventors have determined that it is possible to sensitise to DRL-induced cell death, an individual suffering from a cancer that is normally insensitive to DRL-induced cell death, by administering a BAP1 inhibitor.

[0091] Thus, a BAP1 inhibitor is any agent that targets the BAP1 gene or protein directly, whereas an agent that mimics the effect of BAP1 inhibition is a molecule that targets a signalling molecule downstream of the BAP1 gene/protein signalling pathway.

[0092] A BAP1 inhibitor may be any molecule that inactivates BAP1 protein function, such as a deubiquitinase inhibitor or antagonist, or which silences or reduces BAP1 gene or protein expression or function (such as a small molecule inhibitor of BAP1), or which prevents or reduces binding of a wild-type BAP1 protein to an ASXL protein. The BAP1 inhibitor or agent may also be

any molecule that mutates a wild-type *BAP1* gene to create a mutant *BAP1* gene. Thus, the BAP1 inhibitor or agent may be an RNAi molecule, including shRNA, siRNA, miRNA, ribozymes and antisense molecules; a TALEN (Transcriptional Activator Like-Effector Nuclease); or a CRISPR/CAS9 nuclease. The inventors have demonstrated in the Examples below that *BAP1* gene expression can be reduced in mesothelioma cell lines using shRNA expressing lentivirus and that this reduction of *BAP1* gene expression results in increased sensitivity to DLR-induced cell death in these cell lines.

[0093] In addition to a BAP1 inhibitor, an agent that mimics the effect of BAP1 inhibition is an agent that targets signalling or effector molecules downstream of BAP1, such agents may be used to induce DLR resistance in cancer cell. In one example, the BAP1 inhibitor may be an IAP inhibitor (a SMAC mimetic). An IAP may be BIRC2, BIRC3, BIRC5, BIRC6, BIRC7, BIRC8, NAIP, XIAP or a fragment thereof. The inventors have found that BAP1 expression increases the expression of BIRC3 protein and that inhibition of BIRC3 protein with an IAP inhibitor, such as LCL161, results in sensitization to DRL induced cell death. An IAP inhibitor is an agent that reduces expression or translation of an IAP, or renders an IAP functionally inactive.

[0094] The IAP inhibitor or agent may also be any molecule that mutates an IAP gene or protein to create a mutant IAP gene or protein. Thus, the IAP1 inhibitor or agent may be an RNAi molecule, including shRNA, siRNA, miRNA, ribozymes and antisense molecules; a TALEN (Transcriptional Activator Like-Effector Nuclease); or a CRISPR/CAS9 nuclease. An agent that mimics the effect of BAP1 inhibition may be an RNA helicase inhibitor, such as, YK-4279 or a tyrosine kinase inhibitor, such as sorafenib.

[0095] The inventors believe that their surprising observation (i.e. that BAP1 plays a role in regulating DRL-induced cell death of cancer cells) can be used to develop a novel targeted approach for treatment of any cancer comprising mutants of BAP1.

[0096] Thus, in another example, there is provided a composition comprising (i) a BAP1 inhibitor or an agent that mimics the effect of BAP1 inhibition and (ii) a death receptor ligand.

[0097] In another example, there is provided a composition comprising (i) a BAP1 inhibitor or an agent that mimics the effect of BAP1 inhibition and (ii) a death receptor ligand, for use in therapy or as a medicament.

[0098] In another example, there is provided a composition comprising (i) a BAP1 inhibitor or an agent that mimics the effect of BAP1 inhibition and (ii) a death receptor ligand, for use in treating, preventing or ameliorating cancer.

[0099] The BAP1 inhibitor or agent that mimics the effect of BAP1 inhibition and the death receptor ligand may be administered simultaneously, or the BAP1 inhibitor may be administered prior to the death receptor ligand.

[0100] It will be appreciated that administration of the BAP1 inhibitor first sensitizes the subject to DRL-induced cell death. Then, administration of the death receptor ligand can be effectively used to induce cell death in cancerous cells.

[0101] Preferably, the BAP1 inhibitor is any molecule or approach which inactivates BAP1 protein function, such as a deubiquitinase inhibitor or antagonist, or which silences or reduces *BAP1* gene transcription or BAP1 protein expression, or which mutates a wild-type *BAP1* gene to create a BAP1 mutant gene and protein. The BAP1 inhibitor may be WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9 or a small molecule inhibitor of BAP1.

[0102] Preferred death receptor ligands may be, but are not limited to TRAIL, TNFalpha, FAS ligand (FASL), recombinant TRAIL (dulanermin), death receptor antibodies (such as mapatumumab, drozitumumab, conatumumab, lexatumumab, tigatuzumab, LBY-135) or death receptor agonists such as Medi 3038 or Medi 3039, or a combination thereof.

[0103] Mesothelioma (or Malignant Pleural Mesothelioma) is a rare form of cancer that develops from cells of the mesothelium, the protective lining that covers many of the internal organs of the body. The most common anatomical site for mesothelioma is the pleura (the outer lining of the lungs and internal chest wall), but it can also arise in the peritoneum (the lining of the abdominal cavity), the pericardium (the sac that surrounds the heart) or the tunica vaginalis (a sac that surrounds the testis). Thus, the cancer which may be treated according to any this disclosure may be selected from mesothelioma, Malignant Pleural Mesothelioma, uveal melanoma, melanoma, non-melanoma skin cancer, renal cancers, cholangiocarcinomas, lung cancers, cancer of the pleura, abdominal cancer, peritoneal cancer, cancer of the pericardium, head and neck cancers, brain cancers, breast cancers, liver and biliary tract cancers, gastrointestinal cancers including upper and lower tracts, urothelial cancers, prostate cancers, testicular cancer, cancer of the tunica vaginalis, ovarian cancers, cervical cancers, sarcomas, lymphomas and leukaemia.

[0104] More preferably, the cancer which is treated is mesothelioma. Most preferably, the cancer which is treated is an asbestos-induced cancer.

[0105] The compositions according to this disclosure may have a number of different forms depending, in particular, on the manner in which the composition is to be used. Thus, for example, the composition may be in the form of a powder, tablet, capsule, liquid, ointment, cream, gel, hydrogel, aerosol, spray, micellar solution, transdermal patch, liposome suspension or any other suitable form that may be administered to a person or animal in need of treatment. It will be appreciated that the vehicle of medicaments according to this disclosure should be one which is well tolerated by the subject to whom it is given.

[0106] Compositions according to this disclosure may be used in a number of ways. For instance, oral administration may be required, in which case the agents may be contained within a composition that may, for example, be ingested orally in the form of a tablet, capsule or liquid. Compositions comprising agents of this disclosure may be administered by inhalation (e.g. intranasally). Compositions may also be formulated for topical use. For instance, creams or ointments may be applied to the skin.

[0107] Agents or compositions according to this disclosure may also be incorporated within a slow- or delayed-release device. Such devices may, for example, be inserted on or under the skin, and the medicament may be released over weeks or even months. The device may be located at least adjacent to the treatment site. Such devices may be particularly advantageous when long-term

treatment with agents used according to this disclosure is required and which would normally require frequent administration (e.g. at least daily injection).

[0108] In an example, compositions and agents according to this disclosure may be administered to a subject by injection into the blood stream or directly into a site requiring treatment. For example, the medicament may be injected at least adjacent to a sensitive cell, or within a tumour. Injections may be intravenous (bolus or infusion) or subcutaneous (bolus or infusion), or intradermal (bolus or infusion), or intrapleural.

[0109] It will be appreciated that the amount of the composition and agent that is required is determined by its biological activity and bioavailability, which in turn depends on the mode of administration, the physicochemical properties of the modulator and whether it is being used as a monotherapy or in a combined therapy. The frequency of administration will also be influenced by the half-life of the BAP1 inhibitors and DRLs within the subject being treated. Optimal dosages to be administered may be determined by those skilled in the art, and will vary with the particular agent in use, the strength of the pharmaceutical composition, the mode of administration, and the advancement of the cancer. Additional factors depending on the particular subject being treated will result in a need to adjust dosages, including subject age, weight, gender, diet, and time of administration.

[0110] Generally, a daily dose of between 0.01µg/kg of body weight and 500mg/kg of body weight of the agents (e.g. the inhibitor or the DRL) according to this disclosure may be used for treating, ameliorating, or preventing cancer, depending upon which agent is used. More preferably, the daily dose is between 0.01mg/kg of body weight and 400mg/kg of body weight, more preferably between 0.1mg/kg and 200mg/kg body weight, and most preferably between approximately 1mg/kg and 100mg/kg body weight.

[0111] The composition or agent(s) may be administered before, during or after onset of the cancer. Daily doses may be given as a single administration (e.g. a single daily injection). Alternatively, the agent may require administration twice or more times during a day. As an example, agents may be administered as two (or more depending upon the severity of the disease being treated) daily doses of between 25mg and 7000 mg (i.e. assuming a body weight of 70 kg). A subject receiving treatment may take a first dose upon waking and then a second dose in the evening (if on a two dose regime) or at 3- or 4-hourly intervals thereafter. Alternatively, a slow release device may be used to provide optimal doses of agents according to this disclosure to a patient without the need to administer repeated doses.

[0112] Known procedures, such as those conventionally employed by the pharmaceutical industry (e.g. in *in vivo* experimentation, clinical trials, etc.), may be used to form specific formulations comprising the agents according to this disclosure and precise therapeutic regimes (such as daily doses of the agents and the frequency of administration). The inventors believe that they are the first to describe a pharmaceutical composition for treating cancer, based on the use of a BAP1 inhibitor to sensitise the subject to death receptor-induced cell death, and a death receptor ligand, which induces cell death of the cells that express death receptors and previously insensitive to death receptor-induced cell death.

[0113] According to another example, there is provided a pharmaceutical composition comprising the composition according to this disclosure, and a pharmaceutically acceptable vehicle.

[0114] According to another example, there is provided a method for preparing the pharmaceutical composition according to this disclosure, the method comprising contacting a therapeutically effective amount of a BAP1 inhibitor or agent that mimics the effect of BAP1 inhibition and a death receptor ligand, and a pharmaceutically acceptable vehicle.

[0115] A "therapeutically effective amount" of BAP1 inhibitor is any amount which, when administered to a subject, is the amount needed to sensitise an individual's cells to death receptor-induced cell death. A "therapeutically effective amount" of death receptor ligand is any amount which, when administered to a subject, is the amount needed to treat the cancer, or produce the desired effect.

[0116] For example, the therapeutically effective amount of active agent (i.e. BAP1 inhibitor and a death receptor ligand) used may be from about 0.01 mg/kg body weight to about 800 mg/kg body weight, and preferably from about 0.01 mg to about 500 mg. It is preferred that the amount of agent is an amount from about 0.1 mg to about 250 mg, and most preferably from about 0.1 mg/ kg body weight to about 20 mg/ kg body weight.

[0117] A "pharmaceutically acceptable vehicle" as referred to herein, is any known compound or combination of known compounds that are known to those skilled in the art to be useful in formulating pharmaceutical compositions.

[0118] In one example, the pharmaceutically acceptable vehicle may be a solid, and the composition may be in the form of a powder or tablet. A solid pharmaceutically acceptable vehicle may include one or more substances which may also act as flavouring agents, lubricants, solubilisers, suspending agents, dyes, fillers, glidants, compression aids, inert binders, sweeteners, preservatives, dyes, coatings, or tablet-disintegrating agents. The vehicle may also be an encapsulating material. In powders, the vehicle is a finely divided solid that is in admixture with the finely divided active agents according to this disclosure. In tablets, the active agent (e.g. the peptide, antibody, DRL or BAP1 inhibitor) may be mixed with a vehicle having the necessary compression properties in suitable proportions and compacted in the shape and size desired. The powders and tablets preferably contain up to 99% of the active agents. Suitable solid vehicles include, for example calcium phosphate, magnesium stearate, talc, sugars, lactose, dextrin, starch, gelatin, cellulose, polyvinylpyrrolidone, low melting waxes and ion exchange resins. In another example, the pharmaceutical vehicle may be a gel and the composition may be in the form of a cream or the like. However, the pharmaceutical vehicle may be a liquid, and the pharmaceutical composition is in the form of a solution. Liquid vehicles are used in preparing solutions, suspensions, emulsions, syrups, elixirs and pressurized compositions. The active agent according to this disclosure may be dissolved or suspended in a pharmaceutically acceptable liquid vehicle such as water, an organic solvent, a mixture of both or pharmaceutically acceptable oils or fats. The liquid vehicle can contain other suitable pharmaceutical additives such as solubilisers, emulsifiers, buffers, preservatives, sweeteners, flavouring agents, suspending agents, thickening agents, colours, viscosity regulators, stabilizers or osmo-regulators. Suitable examples of liquid vehicles for oral and parenteral administration include water (partially containing additives as above, e.g.

cellulose derivatives, preferably sodium carboxymethyl cellulose solution), alcohols (including monohydric alcohols and polyhydric alcohols, e.g. glycols) and their derivatives, and oils (e.g. fractionated coconut oil and arachis oil). For parenteral administration, the vehicle can also be an oily ester such as ethyl oleate and isopropyl myristate. Sterile liquid vehicles are useful in sterile liquid form compositions for parenteral administration. The liquid vehicle for pressurized compositions can be a halogenated hydrocarbon or other pharmaceutically acceptable propellant.

[0119] Liquid pharmaceutical compositions, which are sterile solutions or suspensions, can be utilised by, for example, intramuscular, intrathecal, epidural, intraperitoneal, intravenous and particularly subcutaneous injection. The composition or antibody may be prepared as a sterile solid composition that may be dissolved or suspended at the time of administration using sterile water, saline, or other appropriate sterile injectable medium.

[0120] The agents and compositions of this disclosure may be administered orally in the form of a sterile solution or suspension containing other solutes or suspending agents (for example, enough saline or glucose to make the solution isotonic), bile salts, acacia, gelatin, sorbitan monoleate, polysorbate 80 (oleate esters of sorbitol and its anhydrides copolymerized with ethylene oxide) and the like. The agent or composition according to this disclosure can also be administered orally either in liquid or solid composition form. Compositions and agents suitable for oral administration include solid forms, such as pills, capsules, granules, tablets, and powders, and liquid forms, such as solutions, syrups, elixirs, and suspensions.

[0121] Forms useful for parenteral administration include sterile solutions, emulsions, and suspensions.

[0122] It will be appreciated that this disclosure extends to any nucleic acid or peptide or variant, derivative or analogue thereof, which comprises substantially the amino acid or nucleic acid sequences of any of the sequences referred to herein, including variants or fragments thereof. The terms "substantially the amino acid/nucleotide/peptide sequence", "variant" and "fragment", can be a sequence that has at least 40% sequence identity with the amino acid/nucleotide/peptide sequences of any one of the sequences referred to herein, for example 40% identity with the polypeptide identified as SEQ ID Nos. 3 or 4, and so on.

[0123] Amino acid/polynucleotide/polypeptide sequences with a sequence identity which is greater than 50%, more preferably greater than 65%, 70%, 75%, and still more preferably greater than 80% sequence identity to any of the sequences referred to are also envisaged. Preferably, the amino acid/polynucleotide/polypeptide sequence has at least 85% identity with any of the sequences referred to, more preferably at least 90%, 92%, 95%, 97%, 98%, and most preferably at least 99% identity with any of the sequences referred to herein.

[0124] The skilled technician will appreciate how to calculate the percentage identity between two amino acid/polynucleotide/polypeptide sequences. In order to calculate the percentage identity between two amino acid/polynucleotide/polypeptide sequences, an alignment of the two sequences must first be prepared, followed by calculation of the sequence identity value. The percentage identity for two sequences may take different values depending on:- (i) the method used to align the sequences, for example, ClustalW, BLAST, FASTA, Smith-Waterman (implemented in different

programs), or structural alignment from 3D comparison; and (ii) the parameters used by the alignment method, for example, local vs global alignment, the pair-score matrix used (e.g. BLOSUM62, PAM250, Gonnet etc.), and gap-penalty, e.g. functional form and constants.

[0125] Having made the alignment, there are many different ways of calculating percentage identity between the two sequences. For example, one may divide the number of identities by: (i) the length of shortest sequence; (ii) the length of alignment; (iii) the mean length of sequence; (iv) the number of non-gap positions; or (v) the number of equivalenced positions excluding overhangs. Furthermore, it will be appreciated that percentage identity is also strongly length dependent. Therefore, the shorter a pair of sequences is, the higher the sequence identity one may expect to occur by chance. Hence, it will be appreciated that the accurate alignment of protein or DNA sequences is a complex process. The popular multiple alignment program ClustalW (Thompson et al., 1994, Nucleic Acids Research, 22, 4673-4680; Thompson et al., 1997, Nucleic Acids Research, 24, 4876-4882) is a preferred way for generating multiple alignments of proteins or DNA in accordance with this disclosure. Suitable parameters for ClustalW may be as follows: For DNA alignments: Gap Open Penalty = 15.0, Gap Extension Penalty = 6.66, and Matrix = Identity. For protein alignments: Gap Open Penalty = 10.0, Gap Extension Penalty = 0.2, and Matrix = Gonnet. For DNA and Protein alignments: ENDGAP = -1, and GAPDIST = 4. Those skilled in the art will be aware that it may be necessary to vary these and other parameters for optimal sequence alignment. Preferably, calculation of percentage identities between two amino acid/polynucleotide/polypeptide sequences may then be calculated from such an alignment as $(N/T) \times 100$, where N is the number of positions at which the sequences share an identical residue, and T is the total number of positions compared including gaps but excluding overhangs. Hence, a most preferred method for calculating percentage identity between two sequences comprises (i) preparing a sequence alignment using the ClustalW program using a suitable set of parameters, for example, as set out above; and (ii) inserting the values of N and T into the following formula:- Sequence Identity = $(N/T) \times 100$.

[0126] Alternative methods for identifying similar sequences will be known to those skilled in the art. For example, a substantially similar nucleotide sequence will be encoded by a sequence which hybridizes to any sequences referred to herein or their complements under stringent conditions. By stringent conditions, we mean the nucleotide hybridises to filter-bound DNA or RNA in 3x sodium chloride/sodium citrate (SSC) at approximately 45°C followed by at least one wash in 0.2x SSC/0.1% SDS at approximately 20-65°C. Alternatively, a substantially similar polypeptide may differ by at least 1, but less than 5, 10, 20, 50 or 100 amino acids from the sequences shown in SEQ ID Nos. 3 or 4.

[0127] Due to the degeneracy of the genetic code, it is clear that any nucleic acid sequence described herein could be varied or changed without substantially affecting the sequence of the protein encoded thereby, to provide a variant thereof. Suitable nucleotide variants are those having a sequence altered by the substitution of different codons that encode the same amino acid within the sequence, thus producing a silent change. Other suitable variants are those having homologous nucleotide sequences but comprising all, or portions of, sequence, which are altered by the substitution of different codons that encode an amino acid with a side chain of similar biophysical properties to the amino acid it substitutes, to produce a conservative change. For example small non-polar, hydrophobic amino acids include glycine, alanine, leucine, isoleucine, valine, proline, and

methionine. Large non-polar, hydrophobic amino acids include phenylalanine, tryptophan and tyrosine. The polar neutral amino acids include serine, threonine, cysteine, asparagine and glutamine. The positively charged (basic) amino acids include lysine, arginine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. It will therefore be appreciated which amino acids may be replaced with an amino acid having similar biophysical properties, and the skilled technician will know the nucleotide sequences encoding these amino acids.

[0128] All of the features described herein (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined with any of the above aspects in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

[0129] For a better understanding of the invention, and to show how embodiments of the same may be carried into effect, reference will now be made, by way of example, to the accompanying diagrammatic drawings, in which:-

Figure 1A shows various types of mutations in different malignant pleural mesothelioma (MPM) cell lines. **Figure 1B** is an analysis of TCGA exomes for enrichment of BAP1 loss of function mutations. **Figure 1c** is a schematic of *BAP1* gene exons with Mutations annotated from 5180 TCGA exomes.

Figure 2A is a volcano plot showing drug-genome interactions in MPM. The volcano plot displays the mean delta AUC by genotype for 92 library compounds. The Y-axis denotes adjusted p-Value, X-axis denotes effect size. Size of circle indicates number of mutant lines in cohort tested. **Figure 2B** is a histogram which shows the results of a 6-day viability assay for multiple mesothelioma cell lines (n=19). rTRAIL (40ng/ml). Met 5a is a mesothelial normal control line. **Figure 2C** is a scatter plot showing AUC following 6 day viability assay in response to 40ng/ml rTRAIL (normalised to DMSO treated control) in cell lines discretized by BAP1 mutation status. BAP1 mutation status significantly correlates with response to rTRAIL. Two-tailed t-test, p=0.015.

Figure 3A shows the results of a viability assay in which various MPM cells lines are treated with rTRAIL. MPM cell lines were treated with a dose range from 0.5ng/ml to 100ng/ml and cell viability was measured using Syto-60 assay. Based on their cell viability, the cell lines were classified into resistant (red), partially sensitive (orange) and sensitive (green). Cell lines were western blotted to probe the expression of BAP1 protein expression. **Figure 3B** shows the results of a viability assay in which various MPM cells lines are treated with rTRAIL. Three BAP1 wild-type cell lines (MPP-89, H2869 & H2818) and four BAP1 mutant cell lines (H2722, H2461, H28 and H2731) were treated with TRAIL (0-1000 ng/ml) for 24 hours and cell death quantified using an Annexin V/DAPI cell death assay.

Figure 4 is a graph (and Western blot), which shows that knocking down BAP1 in a BAP1 wild-type mesothelioma cell line H2818 confers increased cell death response to rTRAIL.

Figure 5A is an immunoblot for BAP1 protein in BAP1 null mesothelioma lines following transfection with empty vector and BAP1 expression vector. **Figure 5B** is a dose-response curve for an Annexin V/DAPI cell death assay performed with rTRAIL on the BAP1 null H226 parental line, a BAP1 wt overexpressing stable line, and BAP1 C91 hydrolase inactive stable cell line. **Figure 5C** is a graph showing the effect of rTRAIL on cell viability of an untransduced H226 cell line, a BAP1 (transduced)

cell line, and a H226 cell line with the NLS deleted.

Figure 6A shows that cell death is significantly dysregulated with the loss of the BAP1 catalytic ubiquitin hydrolase domain. Comparing GEX profile of C91 variant (catalytically inactive) BAP1 transduced H226 with H226 BAP1 wild-type transduced H226 Kegg pathway analysis on significantly dysregulated genes analysis with adj p <0.05 and FDR <20%. **Figure 6B** is a graph showing RMA normalised gene centered mRNA expression of IAP genes BIRC2 and BIRC3 in C91 mutant BAP1 vs wild-type BAP1 expressing H226 cell line. The Western blot shows dysregulation of IAP family proteins in H226 cell line expressing catalytically inactivated C91 mutant BAP1.

Figure 7A is a volcano plot showing drug-genome interactions when rTRAIL was used as an anchor drug in combination with the library of 94 single agent compounds. Synergy was described using delta AUC metric. **Figure 7B & 7C** are graphs showing the effect of rTRAIL on cell viability of MPM cells in the presence of LCL161. TRAIL resistant MPM cells were treated with either 0-1000 ng/ml of TRAIL alone or a combination of 5 μ M LCL161 and 0-1000 ng/ml of TRAIL for 24 hours and cell death was quantified by Annexin V/DAPI assay. **Figure 7D** is a graph showing the effect of rTRAIL on cell viability of cells in the presence of the IAP inhibitor, LCL161. BAP1-transduced or BAP1 C91A-transduced H226 cells were treated either with 0-1000 ng/ml of TRAIL alone or combination of 5 μ M LCL161 and 0-1000 ng/ml of TRAIL for 24 hours and cell death was quantified by Annexin V/DAPI assay.

Figure 8A is a graph showing the effect of rTRAIL on the viability of various cancer cell lines. Bladder (RT4) and Breast (HCC1187) cancer cell lines with nonsense mutations in BAP1 show sensitivity to rTRAIL while renal cell cancer cell lines (769P & RCC10RGB) with missense mutation and wild-type renal (BB65RCC) and bladder cancer (SW1710) cell lines are resistant to TRAIL. **Figure 8B** shows that knockdown of BAP1 in Breast cancer cell line MDA MB-231 increases sensitivity to rTRAIL.

Figure 9A is a protocol of an *in vivo* experiment. **Figure 9B** is a box plot showing the weight of tumours extracted from mice injected with mutant or wild-type BAP1-expressing cells after treatment with rTRAIL. Tumour weights of mutated BAP1 xenografts are significantly smaller than wild-type BAP1 xenografts after TRAIL treatment. **Figure 9C** is a graph showing that TRAIL treatment reduces the tumour burden (measured by bioluminescence) of mutated BAP1 xenografts when compared to TRAIL treated wild-type BAP1 xenografts or untreated BAP1 mutated and BAP1 wild-type xenografts.

Figure 10 is a graph, which shows that BAP1 the that catalytic domain of BAP1 also regulates the sensitivity of H226 cells to the cell death-inducing ligands, TRAIL, FASL and TNF α . Untransduced BAP1-negative H226 cells, BAP1-expressing and catalytically dead BAP1-expressing H226 cells were treated with 100 ng/ml of FASL, TRAIL and TNF-alpha for 24 hours and cell death was quantified by Annexin V/DAPI assay. * p<0.05 indicating significant difference between untransduced H226 cells H226 BAP1 expressing cells. NS no significant difference between untransduced H226 cells and BAP1 C91A transduced cells. # p<0.05 indicating significant difference between untransduced H226 cells and BAP1 C91A transduced cells.

Figure 11 is a graph, which shows that BAP1 that catalytic domain of BAP1 also regulates the sensitivity of H226 cells to the cell death inducing ligands, TRAIL, FASL and TNF α . Untransduced BAP1-negative H226 cells, BAP1-expressing, ASXL binding site deleted BAP1-expressing H226 cells

and catalytically dead BAPi-expressing H226 cells were treated with 100 ng/ml of FASL, TRAIL and TNF-alpha for 24 hours and cell death was quantified by Annexin V/DAPI assay.

Examples

[0130] The inventors have discovered that mutation of the *BAP1* tumour suppressor gene confers sensitivity to therapeutic modulation of the apoptotic pathway in human cancers. They have explored and validated this association in malignant pleural mesothelioma, bladder carcinoma and breast carcinoma and have evidence that it can be extended to between 1-36% human cancers, including renal cell carcinoma, and cervical cancer and uveal melanoma. Although the data described herein focuses on rTRAIL, a recombinant protein that activates the TRAIL pathway by binding to TRAIL receptor 1 (TRAIL-R1, also known as death receptor 4; DR4) and TRAIL-R2 (also known as DR5), BAP1 is also found to modulate other pro-apoptotic pathways, such as the FAS ligand pathway or the TNF pathway or intrinsic apoptotic pathway (see Example 6).

MATERIALS AND METHODS

Whole exome sequencing

[0131] DNA was extracted using the column extraction technique as per manufacturer's instructions (QIAGEN). Genomic libraries were prepared using the Illumina paired end sample prep kit following the manufacturer's instructions. Exome enrichment was performed using the Agilent SureSelect Human All Exon 50Mb kit following the manufacturer's recommended protocol. Each exome was sequenced using the 75-bp paired end protocol on an Illumina HiSeq 2000 DNA Analyser to produce approximately 5-10 Gb of sequence per exome. Sequencing reads were aligned to the human genome (NCBI build GrCh 37) using the Burrows- Wheeler aligner (BWA) algorithm with default settings (17). Unmapped reads and PCR duplicates were excluded from the analysis. Average coverage of the cell line exomes at 20x or higher was 80%.

Copy Number Annotation

[0132] DNA was extracted as above. DNA was outsourced to AROS for SNP 6.0 array (<http://arosab/services/microarrays/genotyping/>). Copy number annotation was derived from the PICNIC algorithm [18].

Variant detection

[0133] The CaVEMan algorithm was used to call single nucleotide substitutions [19]. The algorithm

uses a naive Bayesian classifier to estimate the posterior probability of each possible genotype (wild-type, germline or somatic mutation) at each base. To call insertions and deletions, split read mapping was implemented as a modification of the Pindel Algorithm [19]. Pindel searches for one read anchored on the genome with the other read mapped in two portions, spanning a putative insertion/ deletion. For both algorithms, an identical putative normal from the CGP panel of tumours was nominated that has been used in all cell lines studied without available matched normal tissue. Significant post processing filtering against various panels of normal was subsequently undertaken to eliminate as many germline single nucleotide polymorphisms as possible. These include the 1000 genomes database, DB SNP, and an internal panel of CGP normal. Following these steps missense variants were annotated using the FATHM algorithm (Cancer Genome Project) as to potential functional consequence of the variant.

Combination (genome-drug) therapeutic screen approaches

[0134] Manual "single dose" combination screening was undertaken using 96 well formats. Cells were plated on day 1 in previously optimized seeding densities in 180µl of media. On day 2 20 µl of a 10X concentration of media from a stock of drugs was added. Cells were then allowed to grow for 72hrs or 6 days and fixed at the end of the assay. Drug wells were compared to DMSO treated control wells.

[0135] Single agent high throughput 5 point viability screening was undertaken in 384 well formats [18] using robotic liquid handling with fixing with 4% paraformaldehyde and staining for viability with Syto60 nucleic acid dye (Invitrogen) (see below). Single agent dose response curves were derived for each library of 85-95 drugs according to the experiment used, and log IC50 or area under the curve (AUC) metrics were derived for each library compound in each cell line according to a previously derived formula [18]. Using this data various 2-drug synergy was measured with a Delta AUC metric.

[0136] A binary event matrix was compiled for the cell lines in the mesothelioma screen by aggregating copy number and exome data and this was used as input classifiers for genomic correlation. Data from this therapeutic screen was then analysed using a Multivariate Analysis of Variance (MANOVA) [18] to annotate the sensitizing effect of genotype on dose response. The results are presented as a volcano plot demonstrating significance of the interaction (above a Benjamin Hochberg false discovery threshold) and magnitude of effect size.

Analyses of TCGA data

[0137] Frequency of BAP1 truncating mutations in various cancer types is based upon data generated by The Cancer Genome Atlas (TCGA) Research Network: <http://cancergenome.nih.gov/>.

Cell culture

[0138] 293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), nonessential amino acids, 50 U/mL penicillin, 50 µg/mL streptomycin, and 1% sodium pyruvate. Human mesothelioma cell lines were cultured in RPMI-1640 medium supplemented with 10% FBS, 1% penicillin-streptomycin and 1% sodium pyruvate (H2369, H2373, H2461, H2591, H2595, H2722, H2731, H2795, H2803, H2804, H2869, H290, H513, IST-MES1, MPP-89, MSTO-211H, NCI-H2052, NCI-H2452, NCI-H226, NCI-H28) or Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM/F-12) supplemented with 10% FBS, nonessential amino acids, 50 U/mL penicillin, 50 µg/mL streptomycin, and 1% sodium pyruvate (H2818, H2810). Cells were maintained at 37°C at 5% CO₂.

Western blotting

[0139] Cell monolayers were washed in phosphate buffered saline (PBS) and lysed in radioimmunoprecipitation assay (RIPA) buffer (Sigma-Aldrich) and protease inhibitors (Complete-mini; Roche) on ice. Lysates were centrifuged at 14000 rpm for ten minutes and the supernatant aspirated. Protein concentration was calculated from a standard curve of bovine serum albumin using the BCA assay (Calbiotech) according to the manufacturer's instructions. Lysates were prepared to the appropriate concentration and 4x Laemelli buffer and 10x reducing agent added prior to the sample being heated at 70°C for ten minutes. Lysates were subjected to SDS-PAGE on pre-cast 4-12% Bis-Tris gels (Invitrogen) at 200V for 1 hr. Protein was transferred onto a nitrocellulose membrane using an iBlot gel transfer device (Invitrogen) as per manufacturer's instructions. Membranes were blocked in 5% milk in tris-buffered saline with Tween 20 (TBS-T) before the addition of primary antibody (at 1:1000 in TBS-T unless otherwise stated) overnight at 4°C. The following day the membrane was washed three times in TBS-T and secondary antibody added (at 1:2500 in TBS-T). Antibodies used include BAP1 (C-4; Santa Cruz sc-28383), alpha tubulin (Cell Signalling #2125), c-IAP1 (Cell signaling #7065), cIAP2 (Cell signaling, #3130), Livin (Cell Signalling #5471), Survivin (Cell signaling, #2803), Alexa Fluor® 488 (Invitrogen A-21202). Immunoblots were imaged using an ImageQuant™ LAS 4000 biomolecular imager.

Cell viability assays

[0140] Adherent cell lines were seeded 24 hours before drugging. Cells were trypsinised and counted before seeding at the optimal density for the size of well (96 or 384) and duration of assay. 72hr after drug treatment cells were fixed with 4% paraformaldehyde for 30 minutes. Following two washes of dH₂O 100µl Syto60 nucleic acid stain (Invitrogen) was added at a final concentration of 1µM and plates fixed for 1hr at room temperature. Quantification of fluorescent signal was achieved using excitation/emission wavelength of 630/695 nM.

Cell death assays

[0141] Adherent mesothelioma cell lines were plated in a 96 well plate at approximately 10000 cells per well. Cells were plated and given 1 day to adhere at which time drug was added. After 48 hrs

media, including floating cells, was collected from each well. The remaining adherent cells were washed with PBS and mobilised with 0.05% trypsin in EDTA. All cells were collected into tubes containing the previously removed media and pelleted by centrifugation (300g, 5 minutes). Cells were then re-suspended in 1X Annexin V binding buffer with 10µl/1ml concentration of Annexin V-647 antibody (Invitrogen) and incubated for 15 minutes at room temperature. DAPI (2µg/ml) was then added to each sample before flow cytometry analysis as below. Annexin V-/DAPI- cells were judged to be viable, AnnexinV+/DAPI- cells were considered to be undergoing apoptosis (early apoptotic phase), and Annexin V+/DAPI+ cells were considered late apoptotic or necrotic, and recorded as dead.

Flow cytometry analyses

[0142] Cells were washed with phosphate buffered saline and fixed by incubation in 4% paraformaldehyde for 20 minutes at room temperature. For intracellular BAP1 staining, fixed cells were permeabilised in 0.1% triton X-100 in PBS for 20 minutes on ice, washed twice with PBS, incubated with primary antibody (C-4; Santa Cruz sc-28383,1:100) for 20 minutes on ice, washed twice again and incubated with a fluorescent secondary antibody (Alexa Fluor® 488, 1:200 (Invitrogen A-21202)). Cells were washed twice with PBS and suspended in PBS for flow cytometry analysis. Cells analysed as part of the cell death assays were prepared as above.

[0143] Flow cytometry analysis was conducted on an LSRFortessa cell analyser (BD Biosciences) and data analysed with FlowJo software.

mRNA microarray

[0144] The mRNA from catalytically inactive BAP1 expressing H226 cells (H226 C91A) and WT BAP1 expressing H226 cells (H226 BAP1) was extracted and run on an Illumina HT12 array.

Pathway analysis

[0145] The significantly differentially expressed genes identified from the mRNA microarray were analysed using KEGG pathway analysis.

Plasmids

[0146] The cDNA full-length clone of human BAP1 was obtained in a pCMV6-AC backbone (Origene, SC117256), which was cloned into a PCCL.CMV lentiviral backbone for all further experiments. BAP1 mutant constructs were generated using site directed mutagenesis kits (NEB) and confirmed by full length DNA sequencing. Short hairpin RNAs were obtained through UCL RNAi library in a GIPZ shRNAmir lentiviral vector ([Dharmacon V2LHS41473](#)). The sequence (SEQ ID NO. 44) for the short hairpin is as follows:

TAAAGGTGCAGATGAACTC [SEQ ID NO. 44]

Lentivirus production and concentration

[0147] Lentiviruses were generated by transfection of 293T cells with the lentivirus vector plasmids together with the packaging plasmid pCMVdR8.2 and envelope plasmid pMDG.2 using jetPEI (Polyplus Transfection) as the transfection reagent. The 293T cells were incubated at 37°C and the medium containing the lentiviruses harvested at 24 and 48 hrs. The lentivirus was concentrated by ultracentrifugation at 18000rpm for 2hours at 4°C (SW28 rotor, Optima LE80K Ultracentrifuge, Beckman) and stored at -80°C before use.

[0148] Lentivirus titration was performed by transducing 293T cells with serial dilutions of virus in the presence of 4µg/ml polybrene. After 4 days cells were analyzed for the percentage of BAP1 positive cells using flow cytometry. Viral was calculated as follows:

Titre (transduction units (TU)/ml) = Proportion of BAP1 positive cells x number of seeded cells / volume of virus (ml)

[0149] MPM cells were then transduced with a range of multiplicity of infections (MOIs) in the presence of 4µg/ml polybrene and transduction efficacy assessed by flow cytometry analysis. The optimal population (lowest MOI at which >90% transduction achieved) was selected for further experiments.

shRNA experiments

[0150] Lentivirus encoding shRNA targeting BAP1 was generated as per the lentivirus production protocol above. MPM cells (H2818) were transduced and treated with puromycin 200µg/mL until a pure population was achieved. Immunoblotting was performed to assess efficacy of the shRNA knockdown.

Animals

[0151] All animal studies were approved by the University College London Biological Services Ethical Review Committee and licensed under the UK Home Office regulations and the Evidence for the Operation of Animals (Scientific Procedures) Act 1986 (Home Office, London, UK). Mice were purchased from Charles River, kept in individually ventilated cages under specific pathogen-free conditions and had access to sterile-irradiated food and autoclaved water *ad libitum*.

Xenograft mouse models

[0152] Groups of 8 week old NOD.CB17-Prkdcscid/NcrCrl (NOD SCID) mice (Charles River) were

injected on each flank with 1 million cells of luciferase transduced mesothelioma cell lines (H226 BAP and H226 C91A) in a 1:1 mixture of matrigel and media. When tumours were established, as assessed by bioluminescent imaging (IVIS), at 14 days following injection of tumour cells, treatment was began with either vehicle or isoleucine zipper TRAIL (izTRAIL) [20]. Either vehicle or izTRAIL were given intraperitoneally once daily at a dose of 600mcg for the duration of the experiment. Tumour size was assessed at days 0, 13, 19, 26 and 41 using bioluminescent imaging (IVIS). Mice were culled at 42 days and tumours removed and weighed. Six mice per group were treated. Researchers were not blinded in these experiments.

Statistical analyses

[0153] Statistical analysis was performed using GraphPad Prism V.4 (GraphPad Software). *In vivo* experiments with multiple groups were analysed using repeated measures ANOVA, and single-group data were assessed using Student t test. All *in vitro* experiments were performed in triplicate unless specified otherwise.

Example 1 - TRAIL targets BAP1 mutant mesothelioma cells

[0154] The inventors carried out a combinatorial chemical screen in 15 mesothelioma cell lines (together with the Met5a mesothelial normal control line) using 94 small molecule inhibitors and chemotherapy agents (see Table 1) either alone or in combination with the ligand tumour necrosis factor (TNF)-related cell death inducing ligand (TRAIL). To detect examples of extreme drug sensitivity, the inventors analysed for statistical associations between response and the mutational status of these cell lines based on a set of 8 genes recently identified as being candidate cancer genes in mesothelioma (see Figure 1A). Of note *BAP1* mutations are well recognised across cancer types (see Figure 1B and C). The largest effect of a mutation on drug response was that of mesothelioma cells harbouring a mutation in the deubiquitinase BAP1 and treated with TRAIL (see Figure 2A). There was no significant effect on cell viability observed in the control normal mesothelial cell line MET-5A included in the screen (see Figure 2B). *BAP1* mutant cells were significantly more sensitive to TRAIL than their wild-type counterparts (see Figures 2B & 2C). Furthermore, the *BAP1* mutations detected in these cell lines would be predicted to be truncating (see Table 2). The inventors confirmed by immunoblot that *BAP1* mutations were usually associated with loss of protein expression and the mutant cell lines are generally sensitive to TRAIL (see Figure 3A).

Table 1 - Compounds used in combinatorial chemical screen with 15 mesothelioma cell lines together with the Met5a mesothelial normal control line.

compound	target	Targeted process/pathway	min conc (uM)	max conc (uM)
AICAR	AMPK agonist	metabolism	7.81	2000
Camptothecin	DNA topoisomerase I	DNA replication	0.0004	0.1
Vinblastine	Microtubules	cytoskeleton	0.0004	0.1
Cisplatin	DNA crosslinking	DNA replication	0.0234	6

compound	target	Targeted process/pathway	min conc (uM)	max conc (uM)
Docetaxel	Microtubules	cytoskeleton	0.0000	0.0125
Gefitinib	EGFR	EGFR signalling	0.0020	0.5
ABT-263	Bcl-2, Bcl-xL, and Bcl-w	apoptosis regulation	0.0078	2
Vorinostat	HDAC inhibitor Class I, IIa, IIb, IV	chromatin histone acetylation	0.0391	10
Nilotinib	Bcr-Abl	ABL signalling	0.0078	2
AZD-2281	PARP1/2	Genome integrity	0.0195	5
Bosutinib	SRC, ABL, TEC	ABL signalling	0.0078	2
Lenalidomide	TNF alpha	other	0.0195	5
Axitinib	PDGFR, KIT, VEGFR	RTK signalling	0.0078	2
AZD7762	Chk 1/2	Genome integrity	0.0078	2
GW 441756	Trk A	RTK signalling	0.0078	2
CEP-701	FLT3, JAK2, NTRK1, RET	RTK signalling	0.0078	2
SB 216763	GSKa/b	WNT signalling	0.0391	10
17-AAG	Hsp90	other	0.0039	1
AMG-706	VEGFR, RET, c-KIT, PDGFR	RTK signalling	0.0078	2
KU-55933	ATM	Genome integrity	0.0391	10
BIBW2992	EGFR, HER2	EGFR signalling	0.0020	0.5
GDC-0449	SMO	other	0.0391	10
PLX4720	RAF	ERK MAPK signalling	0.0391	10
BX-795	TBK1, PDK1, IKK, AURKB/C	other	0.0195	5
NU-7441	DNAPK	Genome integrity	0.0078	2
SL 0101-1	RSK, AURKB, PIM3	ERK MAPK signalling	0.0391	10
BI-D1870	RSK1/2/3/5, PLK1, AURKB	cell cycle	0.0195	5
BIRB 0796	p38, JNK2	JNK and p38 signalling	0.0391	10
JNK Inhibitor VIII	JNK	JNK and p38 signalling	0.0391	10
681640	Wee1, Chk1	cell cycle	0.0078	2
Nutlin-3a	p53-MDM2 interaction	p53 pathway	0.0313	8

compound	target	Targeted process/pathway	min conc (uM)	max conc (uM)
mirin	MRE11-Rad50-Nbs1 complex	cell cycle	0.3906	100
PD-173074	FGFR1, FGFR3	RTK signalling	0.0078	2
ZM-447439	Aurora B	mitosis	0.0156	4
RO-3306	Cdk1	cell cycle	0.0195	5
MK-2206	AKT1/2	PI3K signalling	0.0156	4
PD-0332991	Cdk 4/6	cell cycle	0.0156	4
PF477736	Chk 1 (Chk2)	cell cycle	0.0039	1
GW843682X (AN-13)	Plk1	mitosis	0.0195	5
NVP-BEZ235	PI3K Class 1 and mTORC1/2	PI3K signalling	0.0010	0.25
GDC0941	PI3K (class 1)	PI3K signalling	0.0156	4
AZD8055	mTORC1/2	TOR signalling	0.0078	2
PD-0325901	MEK 1/2	ERK MAPK signalling	0.0010	0.25
AZD6482	PI3K beta	PI3K signalling	0.0195	5
Obatoclax Mesylate	Bcl-2, Bcl-x1, Mcl-1	apoptosis regulation	0.0391	10
EHT 1864	Rac GTPases	cytoskeleton	0.0391	10
BMS-708163	gamma-secretase complex	other	0.0195	5
5-Fluorouracil	antimetabolite	mitosis	0.0781	20
Paclitaxel	Beta subunit of tubulin	cytoskeleton	0.0000	0.01
PF-02341066	MET, ALK	RTK signalling	0.0039	1
Sorafenib	PDGFR, KIT, VEGFR	RTK signalling	0.0156	4
BI-2536	Plk1,2,3	mitosis	0.0020	0.5
BMS-536924	IGF-1R	IGFR signalling	0.0156	4
GSK1904529A	IGF-1R and IR	IGFR signalling	0.0195	5
AKT inhibitor VIII	AKT1/2/3	PI3K signalling	0.0195	5
PF-4708671	p70 S6KA	TOR signalling	0.0391	10
JNJ-26854165	MDM2	p53 pathway	0.0391	10
LY317615	PKC beta	other	0.0391	10
BMS-754807	IGF-1R/IR	IGFR signalling	0.0391	10
TW 37	BCL-2, BCL-XL	apoptosis regulation	0.0195	5
		apoptosis		

compound	target	Targeted process/pathway	min conc (uM)	max conc (uM)
Embelin	XIAP	regulation	0.0391	10
Erlotinib	EGFR	EGFR signalling	0.0078	2
AZ628	BRAF	ERK MAPK signalling	0.0078	2
AG-014699	PARP1/2	Genome integrity	0.0195	5
Gemcitabine	nucleoside analog	DNA replication	0.0391	10
GSK269962A	ROCK1/2	cytoskeleton	0.0195	5
SB-505124	TGFbetaR-I (ALK5)	other	0.0391	10
Tamoxifen	ER	other	0.0195	5
Fulvestrant	ER	other	0.0039	1
Anastrozole	ER	other	0.0391	10
JQ1	BRD2, BRD3, BRD4	chromatin other	0.0039	1
YK 4-279	RNA helicase A	other	0.0391	10
CHIR-99021	GSK3B	WNT signalling	0.0391	10
(5Z)-7 - Oxozeaenol	TAK1	other	0.0391	10
FK866	NAMPT inhibitor	metabolism	0.0039	1
BMS-345541	IKK-beta	other	0.0391	10
AZ960	JAK2	other	0.0391	10
BMN-673	PARP	Genome integrity	0.0391	10
XAV 939	Tankyrase (PARP5a)	WNT signalling	0.0195	5
GSK1120212	MEK1, MEK2	ERK MAPK signalling	0.0039	1
GSK2118436	BRAF	ERK MAPK signalling	0.0391	10
Temozolomide	DNA alkylating agent	DNA replication	0.1172	30
Olaparib + Temozolomide	DNA damage response	Genome integrity	0.0391	10
AZD2281	PARP	Genome integrity	0.0391	10
Bicalutamide	Androgen receptor	other	0.0391	10
PF-562271	FAK	cytoskeleton	0.0391	10
PAC-1	Caspase 3 activator	apoptosis regulation	0.0391	10
INCB-18424	JAK1, JAK2, TYK2	other	0.0391	10

compound	target	Targeted process/pathway	min conc (uM)	max conc (uM)
OSI-906	IGFR-1	IGFR signalling	0.0098	2.5
Epirubicin	DNA damage	DNA replication	0.0391	10
Cyclophosphamide	DNA alkylating agent	DNA replication	0.0391	10
Carboplatin	DNA damage	DNA replication	0.0391	10
Everolimus	mTOR	TOR signalling	0.0195	5
LCL161	SMAC mimetic	apoptosis regulation	0.0391	10
rTRAIL	Death receptor ligand	apoptosis regulation	0.39ng/ml	100ng/ml
DMSO	CONTROL	NA		

Table 2 - BAP1 mutation status in selected cell lines.

SAMPLE_NAME	COSMIC_ID	DESCRIPTION	ZYGOSITY	rTRAIL response	BAP1 mRNA expression
H226		Deletion	Homozygous	Sensitive	
H2461	1290810	Deletion - Frameshift	heterozygous	Sensitive	0.32
H2722	1290812	HomDel	homozygous	Resistant	-2.52
H2731	1240134	Essential Splice	heterozygous	Sensitive	-0.41
H2795	1290813	Essential Splice	heterozygous	Sensitive*	-0.45
H2804	1240136	Essential Splice	heterozygous	Sensitive	0.51
IST-MES 1	907173	Essential Splice	homozygous	Unknown	-0.33
NCI-H2452	908462	Substitution - Missense	homozygous	Resistant	-0.04
NCI-H28	908470	Essential Splice	heterozygous	Sensitive	1.27
NCI-H226	905941	HomDel	homozygous	Sensitive**	ND
H2595	1240132	Wild-type	heterozygous	Unknown	-2.05
H2369	1290808	Wild-type	heterozygous	Resistant	-0.64
H2373	1290809	Wild-type	heterozygous	Resistant	1.65
H2591	1240131	Wild-type	heterozygous	Resistant	0.93
H2803	1240135	Wild-type	heterozygous	Resistant	0.62
H2810	1240137	Wild-type	heterozygous	Resistant	1.06
H2818	1290814	Wild-type	heterozygous	Resistant	0.41
H2869	1240138	Wild-type	heterozygous	Resistant	-0.67

SAMPLE_NAME	COSMIC_ID	DESCRIPTION	ZYGOSITY	rTRAIL response	BAP1 mRNA expression
H290	1240139	Wild-type	heterozygous	Unknown	-0.15
H513	1240141	Wild-type	heterozygous	Resistant	0.11
MPP-89	908150	Wild-type	heterozygous	Resistant	0.54
MSTO-211H	908152	Wild-type	heterozygous	Sensitive**	-0.28
NCI-H2052	688058	Wild-type	heterozygous	Resistant	0.92

Example 2 - Modulation of BAP1 expression determines TRAIL sensitivity through activation of cell death

[0155] TRAIL binds via two active transmembrane death receptors, DR4 and DR5, triggering a caspase cascade and subsequently cell death. The viability effect of TRAIL observed in *BAP1* mutant cells was indeed associated with an increased fraction of cells stained with the apoptotic marker Annexin V (see Figure 3B).

[0156] The inventors therefore next examined whether modulation of BAP1 expression in mesothelioma cells resulted in changes in TRAIL sensitivity. The ablation of BAP1 protein with the use of a lentiviral shRNA in the *BAP1* wild-type cell line H2818 promoted a shift towards increased sensitivity in the BAP1 null compared to the BAP1 competent parental line (see Figure 4). The *BAP1* null cell line NCI-H226, which possesses a homozygous deletion of BAP1, was transduced with a BAP1 expression vector to restore expression of wild-type full length *BAP1* (see Figure 5A) or the catalytically dead C91A mutant. Treatment of the null NCI-H226 cell line with a dose range of TRAIL resulted in increased cell death which was significantly reduced in the BAP1 expressing H226 cell line (see Figure 5b). The C91A variant however phenocopied the response of the BAP1 null parental cell line indicating a functional Ubiquitin hydrolase catalytic domain is critical for sensitivity to TRAIL. The nuclear localization signal (NLS) also plays a key role in imparting TRAIL resistance as deletion of NLS results in significant reduction in BAP1 induced TRAIL resistance (see Figure 5c).

Example 3- Loss of BAP1 expression and function modulates components of the apoptotic machinery

[0157] The H226 mesothelioma cell line harbours a homozygous deletion of *BAP1*, resulting in complete loss of BAP1 expression. The inventors further examined the effect of this catalytically inactive BAP1 on differential mRNA gene expression as well as carrying out a signalling pathway impact analysis (SPIA), as previously described (PMID 18990722). Among those pathways significantly altered when comparing wild-type versus c91a mt BAP1, was that of cell death pathways (see Figure 6A) (http://www.genome.jp/dbget-bin/www_bget?path:map04210). In particular, there was decreased expression of members of the IAP family in H226 cells stably

transduced with the catalytically dead C91A mutant (see Figure 6B). The largest effects were seen in CIAP2, and this was confirmed by western blot (see Figure 6C).

Example 4 - Combination drug screen demonstrates synergy between SMAC mimetic LCL161 and rTRAIL in BAP1 competent cell lines.

[0158] rTRAIL was used as an anchor drug in combination with the library of 94 single agent compounds described above. Synergy was described using delta AUC metric (ref Wessles *et al*) and this was correlated with the previously described genomic subgroups. The inventors have shown that drugs such as SMAC mimetic LCL161, DNA helicase inhibitor YK-4279 and the tyrosine kinase inhibitor sorafenib to increase the efficacy of DRL - induced apoptosis in otherwise resistant cells. One of the most synergistic findings of this screen was the association of sensitivity to the SMAC mimetic LCL161 and rTRAIL in *BAP1* wild-type MPM (see Figure 7 A). This was validated by treating TRAIL resistant wild-type *BAP1* expressing cells with combination of LCL161 and TRAIL. Further validation was also performed in the H226 cell lines stably expressing wild-type *BAP1* that was previously demonstrated to be resistant to TRAIL alone (see Figure 5 and 7B-D). The combination of the IAP inhibitor, LCL161, and TRAIL showed a synergistic increase in cell death in the both mutant and wild-type line indicating that DRL induced cell death in *BAP1* mutant and wild-type cells can be enhanced by combining with other agents (see Figure 7B-D). Figure 7D in particular shows that both *BAP1* mutant and wild-type cells undergo cell death in response to treatment with the combination of TRAIL and LCL161. Endogenous SMAC/Diablo is a specific natural inhibitor of IAP's (14). This data suggests that in the *BAP1* competent state, *BAP1* loss can be phenocopied by specifically mimicking this inhibitory effect on IAPs resulting in a net inactivation of IAP's and sensitivity to rTRAIL. This would lend further support to the idea that the *BAP1*/ extrinsic apoptotic pathway perturbation seen is related to a specific dysregulation of net activity of IAP's.

Example 5 - Extension of BAP1/TRAIL effect to other tissues harboring BAP1 loss of function mutations

[0159] The deubiquitinase *BAP1* is frequently mutated in pleural mesothelioma (36%), uveal melanoma (47%) and intrahepatic cholangiocarcinomas (25%) as previously noted. To determine whether additional *BAP1* mutant tumours occur that may also be amenable to this therapeutic approach, the inventors extended this analysis to a cohort of 5180 tumour samples in 20 cancer types using variant data from The Cancer Genome Atlas (TCGA) (<http://cancergenome.nih.gov/>). Truncating *BAP1* mutations were also observed in a diverse range of cancer types, with frequencies of up to 6% (see Figure 1b and c) Carbone, M. *et al*, Nature Reviews Cancer 13, 153-159 (March 2013). When the inventors extended their analysis to a panel of 1001 cancer cell lines that had previously been submitted for whole exome and copy number analysis, they identified 17 cell lines harbouring truncating mutations in *BAP1* (http://cancer.sanger.ac.uk/cancergenome/projects/cell_lines/). These included clear cell kidney cancer, bladder cancer and breast cancer cell lines. Treatment of these cell lines with TRAIL resulted in a marked viability effect compared to *BAP1* wild-type cell lines from the same cancer type (see Figure 8A). The inventors also inactivated *BAP1* using a lentiviral shRNA in the breast cancer cell line, MDA-MB231, and observed an exaggerated apoptotic response to rTRAIL (see Figure 8B). This suggests that TRAIL therapy may be efficacious

in other forms of cancer (in addition to mesothelioma), and that inhibition of BAP1 or by targeting the pathway which BAP1 induces TRAIL resistance it is possible to sensitise cancer cells to TRAIL.

[0160] The inventors demonstrated the efficacy of targeting BAP1 mutant cells with TRAIL *in vivo* by mice xenograft models. Mutant and wild-type BAP1 cells were injected subcutaneously into left and right flanks of mice. The mice were treated with either rTRAIL or vehicle (see figure 9A). The tumours were weighed at the end of the experiment and the weights of mutated BAP1 tumours in mice that received TRAIL was significantly less than wild-type BAP1 tumours with TRAIL treatment or mutant and wild-type tumours of mice with vehicle treatment (see figure 9B). The tumour burden was tracked in real time over a period of 4 weeks. The tumour burden of mutated BAP1 xenografts in mice that received TRAIL was significantly less than wild-type BAP1 xenografts with TRAIL treatment or mutant and wild-type xenografts of mice with vehicle treatment (see figure 9C).

Example 6 - Role of ASXL binding site on of BAP1 function

[0161] The inventors have also demonstrated that a mutation in the ASXL protein binding site of the *BAP1* gene impairs BAP1-induced TRAIL resistance (see Figure 11). BAP1 has been shown to form a complex with proteins ASXL1, ASXL2 or ASXL3. Mutation of the binding site for ASXL protein inhibits formation of BAP1-ASXL complexes. The BAP1-ASXL complex has been shown to deubiquitinate Histone 2A, and other substrates, and both BAP1 and ASXL1, ASXL2, or ASXL3 are required for this deubiquitination function. This complex is an important regulator of the Polycomb Repressor Complex and gene transcription. The inventors have shown that the *BAP1* wild-type and ASXL3 mutant (truncating mutation) cell line H513 is TRAIL sensitive. Hence loss of function of ASXL1, ASXL2 or ASXL3 increases the sensitivity of cells to DRL induced cell death. Mutations of ASXL1, ASXL2 or ASXL3 also predict sensitivity to DLR and hence can be used as a biomarker for cell death independent of BAP1 mutational status.

Example 7- Extension of BAP1/TRAIL effect to other extrinsic death pathways

[0162] Although the data in this application focus on rTRAIL, a recombinant protein that activates the TRAIL pathway by binding to DR4 and DR5 receptors, the observed BAP1 mutation-sensitisation extends to other extrinsic apoptotic pathways including the FAS ligand pathway and the TNFalpha pathway (see figure 10).

Summary

[0163] The inventors have found that BAP1 is an important regulator of whether a cell will undergo cell death in response to the activation of a death receptor by a death receptor ligand, such as TRAIL, TNF alpha (TNFα) and FAS ligand (FASL). Specifically, non-functional or low expression of wild-type BAP1 causes cells to become sensitive to death receptor ligand-induced cell death. Consequently, it has been discovered that a mutant BAP1 gene or a mutant BAP1 protein, or a cancer cell with low expression of a wild-type BAP1 protein may be used as a biomarker of

sensitivity to DRL-induced cell death.

References

[0164]

1. 1. Lanphear BP, Buncher CR. Latent period for malignant mesothelioma of occupational origin. *Journal of occupational medicine : official publication of the Industrial Medical Association*. 1992;34(7):718-21. Epub 1992/07/01.
2. 2. Lemen RA, Dement JM, Wagoner JK. Epidemiology of asbestos-related diseases. *Environmental health perspectives*. 1980;34:1-11. Epub 1980/02/01.
3. 3. Algranti E, Saito CA, Carneiro AP, Moreira B, Mendonca EM, Bussacos MA. The next mesothelioma wave: Mortality trends and forecast to 2030 in Brazil. *Cancer epidemiology*. 2015. Epub 2015/09/01.
4. 4. Treasure T, Waller D, Tan C, Entwisle J, O'Brien M, O'Byrne K, et al. The Mesothelioma and Radical surgery randomized controlled trial: the Mars feasibility study. *Journal of thoracic oncology: official publication of the International Association for the Study of Lung Cancer*. 2009;4(10):1254-8. Epub 2009/08/08.
5. 5. Vogelzang NJ, Rusthoven JJ, Symanowski J, Denham C, Kaukel E, Ruffie P, et al. Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesothelioma. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*. 2003;21(14):2636-44. Epub 2003/07/16.
6. 6. Guo G, Chmielecki J, Goparaju C, Heguy A, Dolgalev I, Carbone M, et al. Whole-exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A, and CUL1 in malignant pleural mesothelioma. *Cancer research*. 2015;75(2):264-9. Epub 2014/12/10.
7. 7. Bott M, Brevet M, Taylor BS, Shimizu S, Ito T, Wang L, et al. The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesothelioma. *Nature genetics*. 2011;43(7):668-72. Epub 2011/06/07.
8. 8. Martin M, Masshofer L, Temming P, Rahmann S, Metz C, Bornfeld N, et al. Exome sequencing identifies recurrent somatic mutations in EIFAX and SF3B1 in uveal melanoma with disomy 3. *Nature genetics*. 2013;45(8):933-6. Epub 2013/06/26.
9. 9. Gerlinger M, Horswell S, Larkin J, Rowan AJ, Salm MP, Varela I, et al. Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencing. *Nature genetics*. 2014;46(3):225-33. Epub 2014/02/04.
10. 10. Chan-On W, Nairismagi ML, Ong CK, Lim WK, Dima S, Pairojkul C, et al. Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancers. *Nature genetics*. 2013;45(12):1474-8. Epub 2013/11/05.
11. 11. Narasimhan SR, Yang L, Gerwin BI, Broaddus VC. Resistance of pleural mesothelioma cell lines to apoptosis: relation to expression of Bcl-2 and Bax. *The American journal of physiology*. 1998;275(1 Pt 1):L165-71. Epub 1998/08/05.
12. 12. Hinds MG, Norton RS, Vaux DL, Day CL. Solution structure of a baculoviral inhibitor of apoptosis (IAP) repeat. *Nature structural biology*. 1999;6(7):648-51. Epub 1999/07/15.
13. 13. de Almagro MC, Vucic D. The inhibitor of apoptosis (IAP) proteins are critical regulators of signaling pathways and targets for anti-cancer therapy. *Experimental oncology*. 2012;34(3):200-11. Epub 2012/10/17.

14. 14. Yu J, Wang P, Ming L, Wood MA, Zhang L. SMAC/Diablo mediates the proapoptotic function of PUMA by regulating PUMA-induced mitochondrial events. *Oncogene*. 2007;26(29):4189-98. Epub 2007/01/24.
15. 15. Behjati S et al. Recurrent PTPRB and PLCG1 mutations in angiosarcoma. *Nat Genet*. 2014 Apr;46(4):376-9. doi: 10.1038/ng.2921.
16. 16. Tarpey PS et al Frequent mutation of the major cartilage collagen gene COL2A1 in Chondrosarcoma. *Nat Genet*. 2013 Aug; 45(8): 923-926.
17. 17. Li, H and Durbin, R Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics*, 2009. 25(14): p1754-60.
18. 18. Garnett, M.J., et al., Systemic identification of genomic markers of drug sensitivity in cancer cells. *Nature*, 2012. 483 (7391): p.473-81.
19. 19. Behjati, S. et al., Mobile DNA in cancer. Extensive transduction of nonrepetitive DNA mediated by Li retrotransposition in cancer genome. *Science*, 2014. 345(6196): p.1251343.
20. 20. Ganten T, Koschny R, Sykora J, Schulze-Bergkamen H, Buchler P, Haas T, et al. Preclinical differentiation between apparently safe and potentially hepatotoxic applications of TRAIL either alone or in combination with chemotherapeutic drugs. *Clin Cancer Res*. 2006;12:2640-6

SEQUENCE LISTING

[0165]

<110> UCL Business PLC

<120> CELL DEATH BIOMARKER

<130> AXH/75476PCT1

<150> GB1615842.0

<151> 2016-09-16

<160> 44

<170> PatentIn version 3.5

<210> 1

<211> 9102

<212> DNA

<213> Homo sapiens

<400> 1

gagcgcacatgc ccgcacatctgc tgtccgacag gcggaagacg agcccagagg cggagcaggg	60
ccgtcgcgcgc ttggtgacgt ctgccgcgcg cgcgggcgcg tgacgcgcact gggcccgttg	120
tctgtgtgtg ggactgaggg gccccggggg cggtgggggc tcccgggtgg ggcagcgggtg	180
gggagggagg gcctggacat ggcgcctgagg ggccgcgcccg cgggaagatg aataagggct	240
ggctggagct ggagagcgac ccaggtgagg aggggaccgg gagggccagg ggctggggag	300

gccgggatggg	cccgggacgc	gcctgcctga	ccatcacccc	ctcctcttgt	cgccccaccc	360
aggcctcttc	acccctgctcg	tggaagattt	cggtaagagc	cttttctccc	tgccggaccg	420
gggctgtggc	ggcccacccc	tgcgccctca	ctcatcaggg	gctgtccttc	cctactgctt	480
tcctttcctc	atcgcaggtg	tcaagggggg	gcaagtggag	gagatctacg	accttcagag	540
caaatgtcag	gggtgagtgg	ctgtacacca	gggctgcccc	ttacaccag	agtgtgggg	600
aaggtcccag	agaacagggc	cccttaggga	agacagtgcc	aggaacccta	cgttgtaaaa	660
tctcacagaa	agcagcagcc	ttgctctctg	agtgcccgct	cctgatcaaa	ctgatacttt	720
cttttctccc	aaactttcct	tagcgcttcc	ctttttgtag	cagccccctc	cccacccta	780
agcatccttt	ggttcagctg	ctttcctggc	cttgcacggg	gaagaccccg	gtcacacaat	840
gtcttttgtg	cagtttgtga	atgtattaat	tttagtgtgc	ccatgtgtcc	ttggctttaa	900
tcctgacaca	aagtcatcct	gtattgattg	gttgggggtga	caaggccct	cctgggtgcc	960
cacacttaga	gtcttttccc	agtggtcctg	cagaatagat	gtgtaagaga	gtagcaacag	1020
tagcaaccgt	gactgaacca	agaagtctac	tttaatttcc	tggaaacaaa	gagactggtg	1080
tgggtgttca	tttgctttcc	tgactgcatt	ggggcccaca	agtgagaagg	agtgcctcag	1140
ttcctcatca	gagtttttgt	tcttgtctta	ctttgtgttc	ctaccctgtc	ccatccttgg	1200
ccctcagttc	cagcttttct	tctcttacct	agaactatag	acttcataag	gagactgggt	1260
ggactcctgg	agcatcacag	tcagaggctt	atgctttgct	ctgcctgtgg	caggcctttg	1320
gtgtgtgagg	gcacaaggcc	acttcagaca	cagtgttggg	aagaagccag	gggagagggg	1380
ggatcacagc	aaggacacct	gagtgatgac	gcagtgcaaa	ggattaatgg	gagaaagaag	1440
ggaatgctga	ttgtcttctc	ccctttggct	gatctggctc	tgccccttac	ttccccagc	1500
cctgtatatg	gatttatctt	cctgttcaaa	tggatcgaag	agcgccggtc	ccggcgaaag	1560
gtctctacct	tggtggatga	tacgtccgtg	attgatgatg	atattgtgaa	taacatgttc	1620
tttgcccacc	aggtctgctg	gactctgtgc	tttgtttgga	gggtgggatg	ctgccatggt	1680
tttgcttggg	aagtggaaat	ggaggaagac	aggaggagga	gataggcaga	ttotaggggt	1740
ggtagctaca	gaaatcctct	ggcagaacga	actgaactct	taattcatta	aagggaacag	1800
ctttagagta	ggaggggtgc	tgagtcact	ctctgtgtcc	tcagatatcc	agtgggtatt	1860
tggtaggtgc	ttgttaaattg	aataaacatt	aggcaaagat	gaaaggagct	gagaagggga	1920
gttgtccaga	tatgactgac	ctgctctgga	tcccattct	tgatgtatat	gggcttgggg	1980
cttgacgtga	ggggtgctgt	gtatgggtga	ctattcttgg	tttcacagct	gatacccaac	2040
tcttgtgcaa	ctcatgcctt	gctgagcgtg	ctcctgaact	gcagcagcgt	ggacctggga	2100
cccacctga	gtcgcatgaa	ggacttcacc	aagggtttca	gccctgaggt	aggctgcagt	2160
gccttcaccc	tggtcacag	ccaactgggc	agatctgacc	ctgagggcca	ctgggaatgc	2220
taccacatga	tattgggtac	tattaggctg	tttctttttc	aaatgattgt	ttatgttaca	2280
tttgactctt	aaataaattg	tgtaaggcca	ttgttttttag	atgcagttgc	ggggaaagga	2340
cacaggccta	gggagggagg	agagtttcct	taagtcagac	catgtcagaa	ccttctctgt	2400
caggactttt	cctctcaggc	catgttgctt	cctagtgtcc	actaattacc	atgcaaggcc	2460
agcacagtcc	atctcttttg	ggctccagag	ctcttttctg	ccccaccag	ccttttaaga	2520
aagtctgtct	gtgttccttc	cgattcctgg	aatgcctcca	ggctgctctc	tgaagctttg	2580
ccttccaccc	atagtcctac	ctgaggagaa	attattctga	tacggcctta	ttttcttccc	2640

cgtagagcaa aggatatgcg attggcaatg ccccgaggtt ggccaaggcc cataatagcc	2700
atgccagggtg tgtgggagct gtgggagctg atgtggggtg ggagtagggg gagtatcatt	2760
ttttgggccc tgactctgtt tttccccagg cccgagccac gccacctccc tgagaagcag	2820
aatggcctta gtgcagtgcg gaccatggag gcgttccact ttgtcagcta tgtgcctatc	2880
acaggccggc tctttgagct ggatgggctg aaggtctacc ccattgacca tggtaggcac	2940
catgagctgg aggcctgttg ggtgtctctg cctacctcct agggagctgg ggctcagggc	3000
cctctggtat gtggtaccca gtggcagggg ttgtcggtag cgacacccgg ctctggctgg	3060
ggtttcaccc tacaccatat tgcccagcca gctcctgatt ccctggctca actgctcttc	3120
tctgtcttcc ttccactcc tggcctgcc aaactcaggg ttctcttctc gctgattcct	3180
tgtcttggtc tccactaggg ccctgggggg aggacgagga gtggacagac aaggcccggc	3240
gggtcatcat ggagcgtatc ggctcgcca ctgcagggtg agggccctgt gcctgcctg	3300
ttctactctc tggagctgta cctactttgg gagggacaga gagtatccag gtgatttgta	3360
aattgcaagg ccatatggtg aatctggcaa gatcaggctt agatcatggg ttctcaactt	3420
gttgtcttat ttctgcctg ggctgcctgt ggctgctcc tgggtgggct gggggagggg	3480
caggcctcag tggagcctta ggcagcccag gtctgctggt tcacttccag ataggccct	3540
catacagctt gttggaaggt accagctcag gtgcctggca tgtatggcta gtcgctgcct	3600
gcctgttggg gtggggccta tacctacagc tgcaggtgtg actgcaggga gccctgccag	3660
gatatctgcc tcaacctgat ggcggggccg gggcgggagc tgctctcacg gctgcggctg	3720
tgactgcagg gagccctacc acgacatccg cttcaacctg atggcagtgg tgcccagccg	3780
caggatcaag tatgaggcca ggctgcatgt gctgaagggtg aaccgtcaga cagtactaga	3840
ggctctgcag caggtaggtg ccctttcttc ctggcctctg ccagacccaa ccctccctgc	3900
attcctcttc ccttccccca cagcatttgt ctctgattcg tgaacatact ctcttgtaga	3960
tctgggcttc agctaaccac atcttttctt tgccccatt gtgggaaagg tgggacttgg	4020
agtggggagg gagaatagct tctaaaagga agtttgggtt tgggtgtttt atttccctgt	4080
gagtgaatgg gtagagccaa ggccattatt cctttaggtc ctcagccctt agctatttaa	4140
ggtagaagcc cgggtctacc ctttctcttc tgagccctgg attctgttgt tagctgataa	4200
gagtaacaca gccagagctg attcagacct acaagtctca agagtcacag ctgcctgagg	4260
agtccaagtc agccagcaac aagtccccgc tgggtgctgga agcaaacagg gccctgcag	4320
cctctgaggg caaccacaca ggtactgggg ggtttgggac ctcttggtga cctcagagcc	4380
acccgctaatt gtctgacatg ggaggcctaa acagggaaag tctttttctg gggatgtcct	4440
tgggcagtgt tcttcccccg tcagaaggta gagggagagc agtccttccc taaagaaagg	4500
cacctgtaaa gggccgctgt taccacaggc ccctgggccc ttctctgtaa tgtacactcc	4560
ctttcttgtt ttctctagag ggggtttttt tttttttttt tttttttttt tttcttctg	4620
cttctttttt cccatctcat tctttgccct gtctcattgc gggatcatga cttagagctt	4680
gctgactccc attgcaccag ctggctgggc tgttcttctc tgggaagtgc tggttcacag	4740
ggccggggag actgtgagct tttcttgag atcctactgg aggtcctgcc tgtgttcttg	4800
ccctgtctca gatggtgcag aggaggcggc tggttcatgc gcacaagccc catcccacag	4860
ccctcccaac aaadccaagc tagtggtgaa gcctccaggc agcagcctca atggggttca	4920
ccccaacccc actcccattg tccagcggct gccggccttt ctagacaatc acaattatgc	4980
-----	5040

caagtcacccc atgcaggtaa gctgggagca cccttcagcagg attctctact tgattctctt 5040
 gagaggctgc aacaggcaat tttcccatgt ggttccttgg tgttcacccct tggcatggct 5100
 gggctcaagct gcctgggcct gggttgctag gttcctctgc ctgatatgaa aaggccccc 5160
 caacagcagg agcttaggga ggcagggaga gctcctttga atttaactca gttacgtggc 5220
 tgtgggatta aatgttttagg tcacgctcct tggtagaact tcatgggttg ggttttactg 5280
 gcaaaataaa ggcattgtgt tcagggcact ctgtttctct taaaaccctt ccgtgggggt 5340
 ctatccagtg taagtgggtg gcagcctccc cacaagccaa ggacaggcca tggacagct 5400
 ggaggggttc cgctgactca gtctggaaaa ccatgttggc tttctctctg gctgtgagtg 5460
 tctaggctca gcctgggccc agcagcactt gtttgtaact gccctggtct ttgtcccagg 5520
 aggaagaaga cctggcggca ggtgtgggccc gcagccgagt tccagtccgc ccacccagc 5580
 agtactcaga tgatgaggat gactatgagg atgacgagga ggatgacgtg cagaacacca 5640
 actctgccct taggtcagcc cagctttcta aggctaccag gttctaggtg ctccggatcc 5700
 catcctgaat atctcagctt gtgtctgaga atgccctgca gcagataatg ttgagcacct 5760
 gcggagtttg gggccctggg ggaggctggc atgatggggc tgacccagcag tcccaggaa 5820
 gtttttggtg ggctgggggg taaggctgag cacgtaagct tatatcatgt cctattggaa 5880
 gtggcctttt agccaggcct tgaaggattg gttggggcag ggatggagga gatgtgggtg 5940
 gtggggaggc agctttgctg gaacacaggg cattggcaaa aggccaggag tgggatggct 6000
 ggaatagagg aagtgtcttt tgaggacact tggctgcagc tgtcagaact tgatgccagg 6060
 cttagcatgg ctagttcaag ttgcttggac caagtataag gagtttttagg gtcagcccct 6120
 ggaggtcggg atgtatttaa gccattctgg gtactgctgg gtatgggtcac ctggcccgtt 6180
 cccttgcttc acatcttctc gggcccccaca ggtataaggg gaagggaaca gggaagccag 6240
 gggcattgag cggttctgct gatgggcaac tgtcagtgct gcagcccaac accatcaacg 6300
 tcttggtgta gaagctcaaa gagtcccaga aggacctctc aattcctctg tccatcaaga 6360
 ctagcagcgg ggcctgggagt ccggctgtgg cagtgcaccac aactcgcag ccctcaccca 6420
 ccccagcaa tgagagtaca gacacggcct ctgagatcgg cagtgccttc aactcgccac 6480
 tgcgctcgcc tatccgctca gccaaaccga cggggccctc cagccctgtc acctcccaca 6540
 tctccaaggt gctttttgga gaggatgaca gcctgctgag tgttgactgc atacgctaca 6600
 accgtgctgt ccgtgatctg ggtcctgtca tcagcacagg cctgctgcac ctggctgagg 6660
 atgggggtgt gagtccctg gcgctgacag gtgggccttg gactggctca ctggccactt 6720
 ggtgcaccca ggaggagga ggggaagtggc caagtgacca caaagtgtcc tgcactctga 6780
 tgattttctt gtgacctctc tcccagagg gtgggaaggg ttctcgcgcc tccatcagac 6840
 caatccaagg cagccagggg tccagcagcc cagtggagaa ggaggtcgtg gaagccacgg 6900
 acagcagaga gaagacgggg atggtgaggc ctggcgagcc cttgagtggg gagaaatact 6960
 caccacaggt gagcctccgt tgtggttttc tcctttaatc ctggcagagg gtaaggcctg 7020
 agctcctcct gccaggtgc caagttcttg attggaactt tgggtgtgaag attggtggct 7080
 ggagccatgt gccagaagac tttctgggtt ggggtggtggc aggggccttg ataggcatgg 7140
 actcgtgct catccttgcc tctagctgcc tattgctcgt ggggctttgt tgctggccc 7200
 ccccgatcag aggtgcaatg ctgggttttg gcaggagctg ctggcactgc tgaagtgtgt 7260
 ggaggctgag attgcaaact atgaggcgtg cctcaaggag gaggtagaga agaggaagaa 7320
 gttcaagggt ggtgatttct ccagttgcct gatctggcct ctcccaggt ccactggtgg 7380

ctgctctggc aagattggct ccagtgtctt cagtcttctt ctctcctaca gattgatgac	7440
cagagaagga cccacaacta cgatgagttc atctgcacct ttatctccat gctggctcag	7500
gaaggtgagg ggatgcgctg ctgtcttaac tggaatgccc tgctgagggc cgtgtccttc	7560
agctcccctc ccctggcctc tcctgaggct tgagcagacc ttggggcaca gggagggcca	7620
tgagagcctc agctcctggc ctgaggcagc cagcacctgc tcaagggctt ctacctcttc	7680
gcaggcatgc tggccaacct agtggagcag aacatctccg tgcgggcgcg ccaaggggtc	7740
agcatcggcc ggctccacaa gcagcggag cctgaccggc ggaaacgctc tcgcccctac	7800
aaggccaagc gccagtgagg actgctggcc ctgactctgc agcccactct tgccgtgtgg	7860
ccctcaccag ggtccttccc tgccccactt ccccttttcc cagtattact gaatagtccc	7920
agctggagag tccaggccct gggaatggga ggaaccaggc cacattcctt ccatcgtgcc	7980
ctgaggcctg acacggcaga tcagcccccatt agtgcctcagg aggcagcattc tggagttggg	8040
gcacagcgag gtactgcagc ttccctccaca gccggctgtg gagcagcagg acctggccct	8100
tctgcctggg cagcagaata tatatcttac ctatcagaga catctatttt tctgggctcc	8160
aaccaacat gccaccatgt tgacataagt tcctacctga ctatgctttc tctcctagga	8220
gctgtcctgg tgggcccagg tccttgatc atgccacggt cccaactaca gggtcctagc	8280
tgggggcctg ggtgggccct gggctctggg ccctgctgct ctagccccag ccaccagcct	8340
gtccctgttg taaggaagcc aggtcttctc tcttcattcc tcttaggaga gtgccaaact	8400
cagggacca gcactgggct ggggtgggag taggggtgtc cagtgggggt ggggtgagca	8460
ggctgctggg atcccatggc ctgagcagag catgtgggaa ctgttcagtg gcctgtgaac	8520
tgtcttcctt gttctagcca ggctgttcaa gactgctctc catagcaagg ttctagggct	8580
cttcgccttc agtgttgagg ccctagctat gggcctaaat tgggctctag gtctctgtcc	8640
ctggcgcttg aggtcagaa gagcctctgt ccagcccctc agtattacca tgtctccctc	8700
tcaggggtag cagagacagg gttgcttata ggaagctggc accactcagc tcttcctgct	8760
actccagttt cctcagcctc tgcaaggcac tcaggggtggg ggacagcagg atcaagacaa	8820
cccgttgagg ccctgtgtt ccagaggacc tgatgccaaag gggtaatggg ccagcagtg	8880
cctctggagc ccaggcccca acacagcccc atggcctctg ccagatggct ttgaaaaagg	8940
tgatccaagc agggcccttt atctgtacat agtgactgag tgggggggtg tggcaagtgt	9000
ggcagctgcc tctgggctga gcacagcttg acccctctag ccctgtaaa tactggatca	9060
atgaatgaat aaaactctcc taagaatctc ctgagaaatg aa	9102

<210> 2

<211> 2190

<212> DNA

<213> Homo sapiens

<400> 2

atgaataagg gctggctgga gctggagagc gaccagggcc tcttcaccct gctcgtggaa	60
gatttcgggtg tcaaggggggt gcaagtggag gagatctacg accttcagag caaatgtcag	120
ggccctgtat atggatttat ctccctgttc aaatggatcg aagagcgccg gtcccggcga	180
aaggtctcta ccttgggtgga tgatacgtcc gtgattgatg atgatattgt gaataacatg	240
ttctttgccc accagctgat acccaactct tgtgcaactc atgccttgct gagcgtgctc	300

```

ctgaactgca gcagcgtgga cctgggaccc accctgagtc gcatgaagga cttcaccaag      360
ggtttcagcc ctgagagcaa aggatatgcg attggcaatg ccccgaggtt ggccaaggcc      420
cataatagcc atgccaggcc cgagccacgc cacctccctg agaagcagaa tggccttagt      480
gcagtgcgga ccatggaggc gttccacttt gtcagctatg tgcctatcac aggccggctc      540
tttgagctgg atgggctgaa ggtctacccc attgaccatg ggccctgggg ggaggacgag      600
gagtggacag acaaggcccg gcgggtcatc atggagcgtg tcggcctcgc cactgcaggg      660
gagccctacc acgacatccg cttcaacctg atggcagtggt tgcccgaccg caggatcaag      720
tatgaggcca ggctgcatgt gctgaagggtg aaccgtcaga cagtactaga ggctctgcag      780
cagctgataa gagtaacaca gccagagctg attcagaccc acaagtctca agagtcacag      840
ctgcctgagg agtccaagtc agccagcaac aagtccccgc tgggtgctgga agcaaacagg      900
gcccctgcag cctctgaggg caaccacaca gatggtgcag aggaggcggc tggttcatgc      960
gcacaagccc catcccacag ccctcccaac aaacccaagc tagtggtgaa gcctccaggc     1020
agcagcctca atgggggttca cccaacccc actccattg tccagcggct gccggccttt     1080
ctagacaatc acaattatgc caagtcccc atgcaggagg aagaagacct ggcggcaggc     1140
gtgggccgca gccgagttcc agtccgcca cccagcagt actcagatga tgaggatgac     1200
tatgaggatg acgaggagga tgacgtgcag aacaccaact ctgcccttag gtataagggg     1260
aagggaacag ggaagccagg ggcattgagc ggttctgctg atgggcaact gtcagtgctg     1320
cagcccaaca ccatcaacgt cttggctgag aagctcaaag agtcccagaa ggacctctca     1380

attcctctgt ccatcaagac tagcagcggg gctgggagtc cggctgtggc agtgcccaca     1440
cactcgcagc cctcacccac cccagcaat gagagtacag acacggcctc tgagatcggc     1500
agtgttttca actcgccact gcgctcgctt atccgctcag ccaacccgac gcggccctcc     1560
agccctgtca cctcccacat ctccaagggtg ctttttggag aggatgacag cctgctgcgt     1620
gttgactgca tacgctacaa ccgtgctgtc cgtgatctgg gtcctgtcat cagcacaggc     1680
ctgctgcacc tggctgagga tggggtgctg agtcccctgg cgctgacaga gggtaggaag     1740
ggttcctcgc cctccatcag accaatccaa ggcagccagg ggtccagcag cccagtggag     1800
aaggaggtcg tggaagccac ggacagcaga gagaagacgg ggatggtgag gcctggcgag     1860
cccttgagtg gggagaaata ctacccaag gagctgctgg cactgctgaa gtgtgtggag     1920
gctgagattg caaactatga ggcgtgcctc aaggaggagg tagagaagag gaagaagttc     1980
aagattgatg accagagaag gaccacaac tacgatgagt tcatctgcac ctttatctcc     2040
atgctggctc aggaaggcat gctggccaac ctagtgagc agaacatctc cgtgcggcgg     2100
cgccaagggg tcagcatcgg ccggctccac aagcagcggg agcctgaccg gcggaaacgc     2160
tctcgccctt acaaggccaa gcgccagtga                                     2190

```

<210> 3

<211> 729

<212> PRT

<213> Homo sapiens

<400> 3

```

Met Asn Lys Gly Trp Leu Glu Leu Glu Ser Asp Pro Gly Leu Phe Thr
1           5           10           15

```

Leu Leu Val Glu Asp Phe Gly Val Lys Gly Val Gln Val Glu Glu Ile
 20 25 30
 Tyr Asp Leu Gln Ser Lys Cys Gln Gly Pro Val Tyr Gly Phe Ile Phe
 35 40 45
 Leu Phe Lys Trp Ile Glu Glu Arg Arg Ser Arg Arg Lys Val Ser Thr
 50 55 60
 Leu Val Asp Asp Thr Ser Val Ile Asp Asp Asp Ile Val Asn Asn Met
 65 70 75 80
 Phe Phe Ala His Gln Leu Ile Pro Asn Ser Cys Ala Thr His Ala Leu
 85 90 95
 Leu Ser Val Leu Leu Asn Cys Ser Ser Val Asp Leu Gly Pro Thr Leu
 100 105 110
 Ser Arg Met Lys Asp Phe Thr Lys Gly Phe Ser Pro Glu Ser Lys Gly
 115 120 125
 Tyr Ala Ile Gly Asn Ala Pro Glu Leu Ala Lys Ala His Asn Ser His
 130 135 140
 Ala Arg Pro Glu Pro Arg His Leu Pro Glu Lys Gln Asn Gly Leu Ser
 145 150 155 160
 Ala Val Arg Thr Met Glu Ala Phe His Phe Val Ser Tyr Val Pro Ile
 165 170 175
 Thr Gly Arg Leu Phe Glu Leu Asp Gly Leu Lys Val Tyr Pro Ile Asp
 180 185 190
 His Gly Pro Trp Gly Glu Asp Glu Glu Trp Thr Asp Lys Ala Arg Arg
 195 200 205
 Val Ile Met Glu Arg Ile Gly Leu Ala Thr Ala Gly Glu Pro Tyr His
 210 215 220
 Asp Ile Arg Phe Asn Leu Met Ala Val Val Pro Asp Arg Arg Ile Lys
 225 230 235 240
 Tyr Glu Ala Arg Leu His Val Leu Lys Val Asn Arg Gln Thr Val Leu
 245 250 255
 Glu Ala Leu Gln Gln Leu Ile Arg Val Thr Gln Pro Glu Leu Ile Gln
 260 265 270
 Thr His Lys Ser Gln Glu Ser Gln Leu Pro Glu Glu Ser Lys Ser Ala
 275 280 285
 Ser Asn Lys Ser Pro Leu Val Leu Glu Ala Asn Arg Ala Pro Ala Ala
 290 295 300
 Ser Glu Gly Asn His Thr Asp Gly Ala Glu Glu Ala Ala Gly Ser Cys
 305 310 315 320
 Ala Gln Ala Pro Ser His Ser Pro Pro Asn Lys Pro Lys Leu Val Val
 325 330 335

Lys Pro Pro Gly Ser Ser Leu Asn Gly Val His Pro Asn Pro Thr Pro
 340 345 350

Ile Val Gln Arg Leu Pro Ala Phe Leu Asp Asn His Asn Tyr Ala Lys
 355 360 365

Ser Pro Met Gln Glu Glu Glu Asp Leu Ala Ala Gly Val Gly Arg Ser
 370 375 380

Arg Val Pro Val Arg Pro Pro Gln Gln Tyr Ser Asp Asp Glu Asp Asp
 385 390 395 400

Tyr Glu Asp Asp Glu Glu Asp Asp Val Gln Asn Thr Asn Ser Ala Leu
 405 410 415

Arg Tyr Lys Gly Lys Gly Thr Gly Lys Pro Gly Ala Leu Ser Gly Ser
 420 425 430

Ala Asp Gly Gln Leu Ser Val Leu Gln Pro Asn Thr Ile Asn Val Leu
 435 440 445

Ala Glu Lys Leu Lys Glu Ser Gln Lys Asp Leu Ser Ile Pro Leu Ser
 450 455 460

Ile Lys Thr Ser Ser Gly Ala Gly Ser Pro Ala Val Ala Val Pro Thr
 465 470 475 480

His Ser Gln Pro Ser Pro Thr Pro Ser Asn Glu Ser Thr Asp Thr Ala
 485 490 495

Ser Glu Ile Gly Ser Ala Phe Asn Ser Pro Leu Arg Ser Pro Ile Arg
 500 505 510

Ser Ala Asn Pro Thr Arg Pro Ser Ser Pro Val Thr Ser His Ile Ser
 515 520 525

Lys Val Leu Phe Gly Glu Asp Asp Ser Leu Leu Arg Val Asp Cys Ile
 530 535 540

Arg Tyr Asn Arg Ala Val Arg Asp Leu Gly Pro Val Ile Ser Thr Gly
 545 550 555 560

Leu Leu His Leu Ala Glu Asp Gly Val Leu Ser Pro Leu Ala Leu Thr
 565 570 575

Glu Gly Gly Lys Gly Ser Ser Pro Ser Ile Arg Pro Ile Gln Gly Ser
 580 585 590

Gln Gly Ser Ser Ser Pro Val Glu Lys Glu Val Val Glu Ala Thr Asp
 595 600 605

Ser Arg Glu Lys Thr Gly Met Val Arg Pro Gly Glu Pro Leu Ser Gly

610

615

620

Glu Lys Tyr Ser Pro Lys Glu Leu Leu Ala Leu Leu Lys Cys Val Glu
 625 630 635 640

Ala Glu Ile Ala Asn Tyr Glu Ala Cys Leu Lys Glu Glu Val Glu Lys
645 650 655

Arg Lys Lys Phe Lys Ile Asp Asp Gln Arg Arg Thr His Asn Tyr Asp
660 665 670

Glu Phe Ile Cys Thr Phe Ile Ser Met Leu Ala Gln Glu Gly Met Leu
675 680 685

Ala Asn Leu Val Glu Gln Asn Ile Ser Val Arg Arg Arg Gln Gly Val
690 695 700

Ser Ile Gly Arg Leu His Lys Gln Arg Lys Pro Asp Arg Arg Lys Arg
705 710 715 720

Ser Arg Pro Tyr Lys Ala Lys Arg Gln
725

<210> 4

<211> 729

<212> PRT

<213> Homo sapiens

<400> 4

Met Asn Lys Gly Trp Leu Glu Leu Glu Ser Asp Pro Gly Leu Phe Thr
1 5 10 15

Leu Leu Val Glu Asp Phe Gly Val Lys Gly Val Gln Val Glu Glu Ile
20 25 30

Tyr Asp Leu Gln Ser Lys Cys Gln Gly Pro Val Tyr Gly Phe Ile Phe
35 40 45

Leu Phe Lys Trp Ile Glu Glu Arg Arg Ser Arg Gln Lys Val Ser Thr
50 55 60

Leu Val Asp Asp Thr Ser Val Ile Asp Asp Asp Ile Val Asn Asn Met
65 70 75 80

Phe Phe Ala His Gln Leu Ile Pro Asn Ser Cys Ala Thr His Ala Leu
85 90 95

Leu Ser Val Leu Leu Asn Cys Ser Ser Val Asp Leu Gly Pro Thr Leu

100

105

110

Ser Arg Met Lys Asp Phe Thr Lys Gly Phe Ser Pro Glu Ser Lys Gly
115 120 125

Tyr Ala Ile Gly Asn Ala Pro Glu Leu Ala Lys Ala His Asn Ser His
130 135 140

Ala Arg Pro Glu Pro Arg His Leu Pro Glu Lys Gln Asn Gly Leu Ser
145 150 155 160

Ala Val Arg Thr Met Glu Ala Phe His Phe Val Ser Tyr Val Pro Ile
165 170 175

Thr Gly Arg Leu Phe Glu Leu Asp Gly Leu Lys Val Tyr Pro Ile Asp
 180 185 190
 His Gly Pro Trp Gly Glu Asp Glu Glu Trp Thr Asp Lys Ala Arg Arg
 195 200 205
 Val Ile Met Glu Arg Ile Gly Leu Ala Thr Ala Gly Glu Pro Tyr His
 210 215 220
 Asp Ile Arg Phe Asn Leu Met Ala Val Val Pro Asp Arg Arg Ile Lys
 225 230 235 240
 Tyr Glu Ala Arg Leu His Val Leu Lys Val Asn Arg Gln Thr Val Leu
 245 250 255
 Glu Ala Leu Gln Gln Leu Ile Arg Val Thr Gln Pro Glu Leu Ile Gln
 260 265 270
 Thr His Lys Ser Gln Glu Ser Gln Leu Pro Glu Glu Ser Lys Ser Ala
 275 280 285
 Ser Asn Lys Ser Pro Leu Val Leu Glu Ala Asn Arg Ala Pro Ala Ala
 290 295 300
 Ser Glu Gly Asn His Thr Asp Gly Ala Glu Glu Ala Ala Gly Ser Cys
 305 310 315 320
 Ala Gln Ala Pro Ser His Ser Pro Pro Asn Lys Pro Lys Leu Val Val
 325 330 335
 Lys Pro Pro Gly Ser Ser Leu Asn Gly Val His Pro Asn Pro Thr Pro
 340 345 350
 Ile Val Gln Arg Leu Pro Ala Phe Leu Asp Asn His Asn Tyr Ala Lys
 355 360 365
 Ser Pro Met Gln Glu Glu Glu Asp Leu Ala Ala Gly Val Gly Arg Ser
 370 375 380
 Arg Val Pro Val Arg Pro Pro Gln Gln Tyr Ser Asp Asp Glu Asp Asp
 385 390 395 400
 Tyr Glu Asp Asp Glu Glu Asp Asp Val Gln Asn Thr Asn Ser Ala Leu
 405 410 415
 Arg Tyr Lys Gly Lys Gly Thr Gly Lys Pro Gly Ala Leu Ser Gly Ser
 420 425 430
 Ala Asp Gly Gln Leu Ser Val Leu Gln Pro Asn Thr Ile Asn Val Leu
 435 440 445
 Ala Glu Lys Leu Lys Glu Ser Gln Lys Asp Leu Ser Ile Pro Leu Ser
 450 455 460
 Ile Lys Thr Ser Ser Gly Ala Gly Ser Pro Ala Val Ala Val Pro Thr
 465 470 475 480
 His Ser Gln Pro Ser Pro Thr Pro Ser Asn Glu Ser Thr Asp Thr Ala
 485 490 495

Ser Glu Ile Gly Ser Ala Phe Asn Ser Pro Leu Arg Ser Pro Ile Arg
500 505 510

Ser Ala Asn Pro Thr Arg Pro Ser Ser Pro Val Thr Ser His Ile Ser
515 520 525

Lys Val Leu Phe Gly Glu Asp Asp Ser Leu Leu Arg Val Asp Cys Ile
530 535 540

Arg Tyr Asn Arg Ala Val Arg Asp Leu Gly Pro Val Ile Ser Thr Gly
545 550 555 560

Leu Leu His Leu Ala Glu Asp Gly Val Leu Ser Pro Leu Ala Leu Thr
565 570 575

Glu Gly Gly Lys Gly Ser Ser Pro Ser Ile Arg Pro Ile Gln Gly Ser
580 585 590

Gln Gly Ser Ser Ser Pro Val Glu Lys Glu Val Val Glu Ala Thr Asp
595 600 605

Ser Arg Glu Lys Thr Gly Met Val Arg Pro Gly Glu Pro Leu Ser Gly
610 615 620

Glu Lys Tyr Ser Pro Lys Glu Leu Leu Ala Leu Leu Lys Cys Val Glu
625 630 635 640

Ala Glu Ile Ala Asn Tyr Glu Ala Cys Leu Lys Glu Glu Val Glu Lys
645 650 655

Arg Lys Lys Phe Lys Ile Asp Asp Gln Arg Arg Thr His Asn Tyr Asp
660 665 670

Glu Phe Ile Cys Thr Phe Ile Ser Met Leu Ala Gln Glu Gly Met Leu
675 680 685

Ala Asn Leu Val Glu Gln Asn Ile Ser Val Arg Arg Arg Gln Gly Val
690 695 700

Ser Ile Gly Arg Leu His Lys Gln Arg Lys Pro Asp Arg Arg Lys Arg
705 710 715 720

Ser Arg Pro Tyr Lys Ala Lys Arg Gln
725

<210> 5

<211> 2190

<212> DNA

<213> Homo sapiens

<400> 5

atgaataagg gctggctgga gctggagagc gaccaggcc tcttcaccct gctcgtggaa	60
gatttcggtg tcaagggggt gcaagtggag gagatctacg accttcagag caaatgtcag	120
ggccctgtat atggatttat cttcctgttc aaatggatcg aagagcgccg gtcccgcaa	180
aaggtctcta ccttggtgga tgatacgtcc gtgattgatg atgatattgt gaataacatg	240
ttctttgccc accagctgat acccaactct tgtgcaactc atgccttgct gagcgtgctc	300

ctgaactgca gcagcgtgga cctgggaccc accctgagtc gcatgaagga cttcaccaag	360
ggtttcagcc ctgagagcaa aggatatgcg attggcaatg ccccgaggtt ggccaaggcc	420
cataatagcc atgccaggcc cgagccacgc cacctccctg agaagcagaa tggccttagt	480
gcagtgcgga ccatggaggc gttccacttt gtcagctatg tgcctatcac aggcgggctc	540
tttgagctgg atgggctgaa ggtctacccc attgaccatg ggccctgggg ggaggacgag	600
gagtggacag acaaggcccc gcgggtcatc atggagcgta tcggcctcgc cactgcaggg	660
gagccctacc acgacatccg cttcaacctg atggcagtgg tgcccgaccg caggatcaag	720
tatgaggcca ggctgcatgt gctgaagggtg aaccgtcaga cagtactaga ggctctgcag	780
cagctgataa gagtaacaca gccagagctg attcagaccc acaagtctca agagtcacag	840
ctgcctgagg agtccaagtc agccagcaac aagtccccgc tgggtgctgga agcaaacagg	900
gccctgcag cctctgaggg caaccacaca gatgggtgcag aggaggcggc tggttcatgc	960
gcacaagccc catcccacag ccctcccaac aaaccaagc tagtggtgaa gcctccaggc	1020
agcagcctca atggggttca cccaacccc actcccattg tccagcggct gccggccttt	1080
ctagacaatc acaattatgc caagtcccc atgcaggagg aagaagacct ggccggcagg	1140
gtgggcccga gccgagttcc agtccgccc cccagcagt actcagatga tgaggatgac	1200
tatgaggatg acgaggagga tgacgtgcag aacaccaact ctgcccttag gtataagggg	1260
aagggaacag ggaagccagg ggcattgagc ggttctgctg atgggcaact gtcagtgctg	1320
cagcccaaca ccatcaacgt cttggctgag aagctcaaag agtcccagaa ggacctctca	1380
attcctctgt ccatcaagac tagcagcggg gctgggagtc cggctgtggc agtgcccaca	1440
cactcgcagc cctcacccac cccagcaat gagagtacag acacggcctc tgagatcggc	1500
agtgctttca actcgccact gcgctcgct atccgctcag ccaacccgac gcggccctcc	1560
agccctgtca cctcccacat ctccaagggtg ctttttgag aggatgacag cctgctgcgt	1620
gttgactgca tacgctacaa ccgtgctgtc cgtgatctgg gtctgtcat cagcacaggc	1680
ctgctgcacc tggctgagga tggggtgctg agtcccctgg cgctgacaga ggggtgggaag	1740
ggttcctcgc cctccatcag accaatccaa ggcagccagg ggtccagcag cccagtggag	1800
aaggaggctc tggaagccac ggacagcaga gagaagacgg ggatgggtgag gcctggcgag	1860
cccttgagtg gggagaaata ctcacccaag gagctgctgg cactgctgaa gtgtgtggag	1920
gctgagattg caaactatga ggcgtgcctc aaggaggagg tagagaagag gaagaagttc	1980
aagattgatg accagagaag gaccacaaac tacgatgagt tcatctgcac ctttatctcc	2040
atgctggctc aggaaggcat gctggccaac ctagtggagc agaacatctc cgtgcggcgg	2100
cgccaagggg tcagcatcgg ccggctccac aagcagcggg agcctgaccg gcggaaacgc	2160
tctcgccctt acaaggccaa gcgccagtga	2190

<210> 6

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 6
acctagaacc tggtagcctt ag 22

<210> 7
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Primer

<400> 7
gtacagctcc agagagtaga ac 22

<210> 8
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Primer

<400> 8
tcttaccgaa atctccacg ag 22

<210> 9
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Primer

<400> 9
ctgctgcttt ctgtgagatt tt 22

<210> 10
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Primer

<400> 10
agggcattcc agttaagaca g 21

<210> 11
<211> 18
<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 11

caagagtggg ctgcagag 18

<210> 12

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 12

actaaggcca ttctgcttct c 21

<210> 13

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 13

atcccaccct ccaaacaag 20

<210> 14

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 14

caccaagtgg ccagtgag 18

<210> 15

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 15

ggctgtcatc ctctcaaaa 20

<210> 16
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic Primer

<400> 16
 gagggctgcg agtgtgtg 18

<210> 17
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic Primer

<400> 17
 ctctgccagg attaaaggag aa 22

<210> 18
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic Primer

<400> 18
 gaatgcaggg aggggtgg 18

<210> 19
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic Primer

<400> 19
 acccaatatc atgtggtagc at 22

<210> 20
 <211> 18
 <212> DNA
 <213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 20

aaggacagcc cctgatga 18

<210> 21

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 21

gtaggcagag acacccaac 19

<210> 22

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 22

ccttctctgg tcataaatct gt 22

<210> 23

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 23

ctctgaggtc cacaagaggt 20

<210> 24

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 24

tcaagtagag aatcctgcaa gg 22

<210> 25

<211> 22

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Primer

<400> 25
gagcagcact tgtttgtaac tg 22

<210> 26
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Primer

<400> 26
ctcaactgct cttctctgtc tt 22

<210> 27
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Primer

<400> 27
gagggagggc ctggacat 18

<210> 28
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Primer

<400> 28
ctgtccttcc ctactgctt c 21

<210> 29
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic Primer

<400> 29

gaagttcaag gtgggtgatt tc 22

<210> 30

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 30

ctcagctcct ggccctgag 18

<210> 31

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 31

ggagaaatta ttctgatacg gcc 23

<210> 32

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 32

gaagggaatg ctgattgtct tc 22

<210> 33

<211> 18

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 33

ctatccgctc agccaacc 18

<210> 34

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 34

ctctcaattc ctctgtccat ca 22

<210> 35

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 35

cgttcccttg cttcacatct 20

<210> 36

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 36

tcctgcactc tgatgatttt ct 22

<210> 37

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 37

gatattgcc tcaacctgat gg 22

<210> 38

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic Primer

<400> 38

gtgctgtgta tgggtgacta 20

<210> 39

<211> 21
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Primer

 <400> 39
 gaagatgaat aagggctggc t 21

 <210> 40
 <211> 19
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Primer

 <400> 40
 tgatgtgggg tgggagtag 19

 <210> 41
 <211> 18
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Primer

 <400> 41
 cccgatcaga ggtgcaat 18

 <210> 42
 <211> 22
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Primer

 <400> 42
 agctatttaa ggtagaagcc cg 22

 <210> 43
 <211> 22
 <212> DNA
 <213> Artificial Sequence

 <220>
 <223> Synthetic Primer

<400> 43

actgtgagct ttcttgag at 22

<210> 44

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic sequence for short hairpin

<400> 44

taaaggtgca gatgaactc 19

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- [WO2013106002A1](#) [0006]
- [EP2044434A2](#) [0006]
- [GB1615842A](#) [0165]

Non-patent literature cited in the description

- **POLANSKI et al.** Caspase-8 activation by TRAIL monotherapy predicts responses to IAPi and TRAIL combination treatment in breast cancer cell lines *Cell death and disease*, 2015, vol. 6, 10e1893- [0006]
- **PERSHANG FARSHI et al.** Deubiquitinases (DUBs) and DUB inhibitors: a patent review *Expert opinion on therapeutic patents*, 2015, vol. 25, 101191-1208 [0006]
- **THOMPSON et al.** *Nucleic Acids Research*, 1994, vol. 22, 4673-4680 [0125]
- **THOMPSON et al.** *Nucleic Acids Research*, 1997, vol. 24, 4876-4882 [0125]
- **CARBONE, M. et al.** *Nature Reviews Cancer*, 2013, vol. 13, 153-159 [0159]
- **LANPHEAR BPBUNCHEER CRL** Latent period for malignant mesothelioma of occupational origin *Journal of occupational medicine : official publication of the Industrial Medical*

Association, 1992, vol. 34, 7718-21 [0164]

- **LEMEN RADEMENT JMWAGONER JKE**Epidemiology of asbestos-related diseasesEnvironmental health perspectives., 1980, vol. 34, 1-11 [0164]
- **ALGRANTI ESAITO CACARNEIRO APMOREIRA BMENDONCA EMBUSSACOS MA**The next mesothelioma wave: Mortality trends and forecast to 2030 in BrazilCancer epidemiology, 2015, [0164]
- **TREASURE TWALLER DTAN CENTWISLE JO'BRIEN MO'BYRNE K et al.**The Mesothelioma and Radical surgery randomized controlled trial: the Mars feasibility studyJournal of thoracic oncology: official publication of the International Association for the Study of Lung Cancer, 2009, vol. 4, 101254-8 [0164]
- **VOGELZANG NJRUSTHOVEN JJSYMANOWSKI JDENHAM CKAUKEL ERUFFIE P et al.**Phase III study of pemetrexed in combination with cisplatin versus cisplatin alone in patients with malignant pleural mesotheliomaJournal of clinical oncology: official journal of the American Society of Clinical Oncology, 2003, vol. 21, 142636-44 [0164]
- **GUO GCHMIELECKI JGOPARAJU CHEGUY ADOLGALEV ICARBONE M et al.**Whole-exome sequencing reveals frequent genetic alterations in BAP1, NF2, CDKN2A, and CUL1 in malignant pleural mesotheliomaCancer research, 2015, vol. 75, 2264-9 [0164]
- **BOTT MBRENET MTAYLOR BSSHIMIZU SITO TWANG L et al.**The nuclear deubiquitinase BAP1 is commonly inactivated by somatic mutations and 3p21.1 losses in malignant pleural mesotheliomaNature genetics, 2011, vol. 43, 7668-72 [0164]
- **MARTIN MMASSHOFER LTEMING PRAHMANN SMETZ CBORNFELD N et al.**Exome sequencing identifies recurrent somatic mutations in EIFAX and SF3B1 in uveal melanoma with disomy 3Nature genetics, 2013, vol. 45, 8933-6 [0164]
- **GERLINGER MHORSWELL SLARKIN JROWAN AJSALM MPVARELA I et al.**Genomic architecture and evolution of clear cell renal cell carcinomas defined by multiregion sequencingNature genetics, 2014, vol. 46, 3225-33 [0164]
- **CHAN-ON WNAIRISMAGI MLONG CKLIM WKDIMA SPAIROJKUL C et al.**Exome sequencing identifies distinct mutational patterns in liver fluke-related and non-infection-related bile duct cancersNature genetics, 2013, vol. 45, 121474-8 [0164]
- **NARASIMHAN SRYANG LGERWIN BIBROADDUS VC**Resistance of pleural mesothelioma cell lines to apoptosis: relation to expression of Bcl-2 and BaxThe American journal of physiology, 1998, vol. 275, L165-71 [0164]
- **HINDS MGNORTON RSVAUX DLDAY CL**Solution structure of a baculoviral inhibitor of apoptosis (IAP) repeatNature structural biology, 1999, vol. 6, 7648-51 [0164]
- **DE ALMAGRO MCVUCIC D.**The inhibitor of apoptosis (IAP) proteins are critical regulators of signaling pathways and targets for anti-cancer therapyExperimental oncology., 2012, vol. 34, 3200-11 [0164]
- **YU JWANG PMING LWOOD MAZHANG L.**SMAC/Diablo mediates the proapoptotic function of PUMA by regulating PUMA-induced mitochondrial eventsOncogene, 2007, vol. 26, 294189-98 [0164]
- **BEHJATI S et al.**Recurrent PTPRB and PLCG1 mutations in angiosarcomaNat Genet., 2014, vol. 46, 4376-9 [0164]
- **TARPEY PS et al.**Frequent mutation of the major cartilage collagen gene COL2A1 in ChondrosarcomaNat Genet., 2013, vol. 45, 8923-926 [0164]
- **LI, HDURBIN, R**Fast and accurate short read alignment with Burrows-Wheeler transformBioinformatics, 2009, vol. 25, 141754-60 [0164]

- **GARNETT, M.J. et al.** Systemic identification of genomic markers of drug sensitivity in cancer cells *Nature*, 2012, vol. 483, 7391473-81 [\[0164\]](#)
- **BEHJATI, S. et al.** Mobile DNA in cancer. Extensive transduction of nonrepetitive DNA mediated by Li retrotransposition in cancer genome *Science*, 2014, vol. 345, 61961251343- [\[0164\]](#)
- **GANTEN TKOSCHNY RSYKORA JSCHULZE-BERGKAMEN HBUCHLER PHAAS T et al.** Preclinical differentiation between apparently safe and potentially hepatotoxic applications of TRAIL either alone or in combination with chemotherapeutic drugs *Clin Cancer Res.*, 2006, vol. 12, 2640-6 [\[0164\]](#)

PATENTKRAV

1. Fremgangsmåde til bestemmelse af, om en persons cancercelle er sensitiv over for dødsreceptorligand (DRL)-induceret celledød, hvilken fremgangsmåde omfatter påvisning, i en biologisk prøve taget fra personen, af:

(i) tilstedeværelsen af et mutant *BAP1*-gen eller mutant BAP1-protein;

(ii) et reduceret ekspressionsniveau af et vildtype-*BAP1*-gen eller en lavere vildtype-BAP1-proteinkoncentration sammenlignet med ekspressionsniveauet eller proteinkoncentrationen i en referencecelle, der er en BAP1-vildtype-celle, der er resistent over for DRL-induceret celledød; eller

(iii) reduceret eller ikke-binding af et ASXL-protein til et vildtype-BAP1-protein sammenlignet med bindingsniveauet i en referencecelle, der er en BAP1-vildtype-celle, der er resistent over for DRL-induceret celledød;

hvor tilstedeværelsen, i prøven, af det mutante *BAP1*-gen eller det mutante BAP1-protein, eller af et reduceret ekspressionsniveau af vildtype-*BAP1*-genet eller en lavere proteinkoncentration, eller reduceret eller ikke-binding af et ASXL-protein til et vildtype-BAP1-protein, indikerer, at personens cancercelle er sensitiv over for DRL-induceret celledød.

2. Fremgangsmåde ifølge krav 1, hvor det mutante *BAP1*-gen er et gen, der koder for et ikke-funktionelt eller enzymatisk inaktivt BAP1-protein, eller et BAP1-protein, der udviser reduceret binding til et ASXL-protein sammenlignet med bindingsniveauet i en referencecelle, der er resistent over for DRL-induceret celledød, eventuelt hvor ASXL-proteinet er ASXL1, ASXL2 eller ASXL3.

3. Fremgangsmåde ifølge krav 1, hvor et mutant BAP1-protein er ét, der er ikke-funktionelt eller enzymatisk inaktivt eller ude af stand til at binde til et ASXL-protein eller udviser reduceret binding til et ASXL-protein sammenlignet med bindingsniveauet i en referencecelle, der er resistent over for DRL-induceret celledød.

4. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det reducerede ekspressionsniveau er mindst en 10 %, 15 %, 25 %, 35 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 % eller en 100 % reduktion sammenlignet med referencecellen, og hvor den lavere koncentration af vildtype-BAP1-protein kan være mindst 10 %, 15 %, 25 %, 35 %, 45 %, 50 %, 55 %, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 % eller 100 % lavere

sammenlignet med referencecellen.

5. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det mutante BAP1-proteins aminosyresekvens er kodet for af SEQ ID NO: 4.

5

6. Fremgangsmåde ifølge et hvilket som helst af de foregående krav, hvor det mutante *BAP1*-gens nukleotidsekvens er kodet for af SEQ ID NO: 5.

7. Kit til bestemmelse af, om en persons cancercelle er sensitiv over for DRL-induceret celledød, hvilket kit omfatter påvisningsmidler til påvisning af ekspresionen af et mutant *BAP1*-gen eller mutant BAP1-protein, eller til påvisning af et reduceret ekspressionsniveau af et vildtype-*BAP1*-gen eller en lavere vildtype-BAP1-proteinkoncentration sammenlignet med ekspressionsniveauet eller proteinkoncentration i en referencecelle, der er resistent over for DRL-induceret celledød, eller til påvisning af ikke-binding eller reduceret binding af et ASXL-protein til et BAP1-protein sammenlignet med bindingsniveauet i en referencecelle, der er en BAP1-vildtype-celle, der er resistent over for DRL-induceret celledød, hvor tilstedeværelsen, i prøven, af det mutante *BAP1*-gen eller BAP1-proteinet, eller af et reduceret ekspressionsniveau af vildtype-*BAP1*-genet eller en lavere vildtype-BAP1-proteinkoncentration, eller reduceret eller ikke-binding af et ASXL-protein til et vildtype-BAP1-protein, indikerer, at personens
10
15
20
cancercelle er sensitiv over for DRL-induceret celledød, hvor kittet omfatter mindst én kontrol- eller referenceprøve, og hvor kittet omfatter en negativ kontrol omfattende et vildtype-BAP1-protein, der er resistent over for DRL-induceret celledød.

8. Kit ifølge krav 7, hvor kittet omfatter en positiv kontrol omfattende et mutant BAP1-mRNA, eller mutant BAP1-protein eller en blindprøve.
25

9. In vitro-anvendelse af (i) et mutant *BAP1*-gen eller et mutant BAP1-protein, eller (ii) en cancercelle med et reduceret ekspressionsniveau af et vildtype-*BAP1*-gen eller en lavere vildtype-BAP1-proteinkoncentration sammenlignet med ekspressionsniveauet eller proteinkoncentration i en referencecelle, der er en BAP1-vildtype-celle, som en biomarkør af sensitivitet for DRL-induceret celledød.
30

10. Sammensætning, der omfatter (i) en BAP1-hæmmer og (ii) en dødsreceptorligand, hvor BAP1-hæmmeren er WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9,

et RNAi-molekyle, der indbefatter shRNA, siRNA, miRNA, ribozymmer og antisense-molekyler eller en TALEN.

11. Sammensætning, der omfatter (i) en BAP1-hæmmer og (ii) en dødsreceptorligand, til
5 anvendelse i terapi eller som et lægemiddel, hvor BAP1-hæmmeren er WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9, et RNAi-molekyle, der indbefatter shRNA, siRNA, miRNA, ribozymmer og antisense-molekyler eller en TALEN.

12. Sammensætning, der omfatter (i) en BAP1-hæmmer og (ii) en dødsreceptorligand, til
10 anvendelse i behandling, forebyggelse eller lindring af cancer, hvor BAP1-hæmmeren er WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9, et RNAi-molekyle, der indbefatter shRNA, siRNA, miRNA, ribozymmer og antisense-molekyler eller en TALEN.

13. Sammensætning, der omfatter (i) en BAP1-hæmmer og (ii) en dødsreceptorligand, til
15 anvendelse ifølge krav 12, hvor BAP1-hæmmeren er WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9, et RNAi-molekyle, der indbefatter shRNA, siRNA, miRNA, ribozymmer og antisense-molekyler eller en TALEN, hvor canceren er mesotheliom, malignt pleuralt mesotheliom, uvealt melanom, melanom, ikke-melanom-hudcancer, renalcancer, lungecancer, lungehindecancer, abdominal cancer, peritoneal cancer, cancer i pericardiet, en
20 hoved- eller halscancer, hjerne-cancer, brystcancer, lever- eller galdevejscancer, gastrointestinal cancer indbefattende øvre og nedre veje, urothelcancer, prostatacancer, testiscancer, cancer i tunica vaginalis, ovariecancer, cervixcancer, sarkom, lymfom eller leukæmi.

14. Farmaceutisk sammensætning, der omfatter sammensætningen ifølge krav 10 og et
25 farmaceutisk acceptabelt vehikel.

15. Fremgangsmåde til fremstilling af den farmaceutiske sammensætning ifølge krav 14, hvilken fremgangsmåde omfatter at etablere kontakt mellem en terapeutisk virksom mængde af en BAP1-hæmmer og en dødsreceptorligand, og et farmaceutisk acceptabelt vehikel, hvori
30 BAP1-hæmmeren er WP1130, Usp9x, Usp5, Usp14, Usp24, UCH37, b-AP15, CRISPR/CAS9, et RNAi-molekyle, der indbefatter shRNA, siRNA, miRNA, ribozymmer og antisense-molekyler eller en TALEN.

DRAWINGS

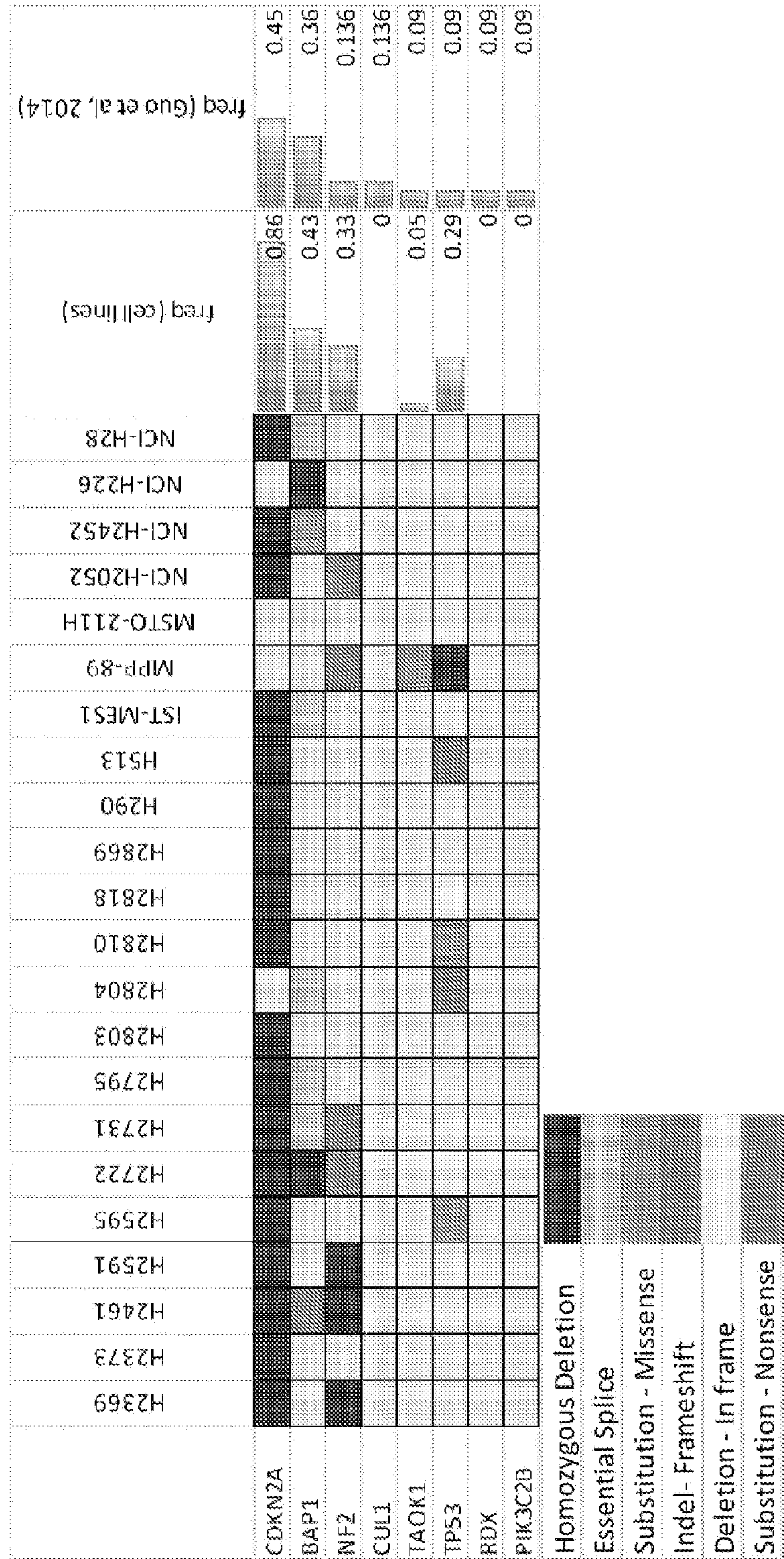


Fig. 1A

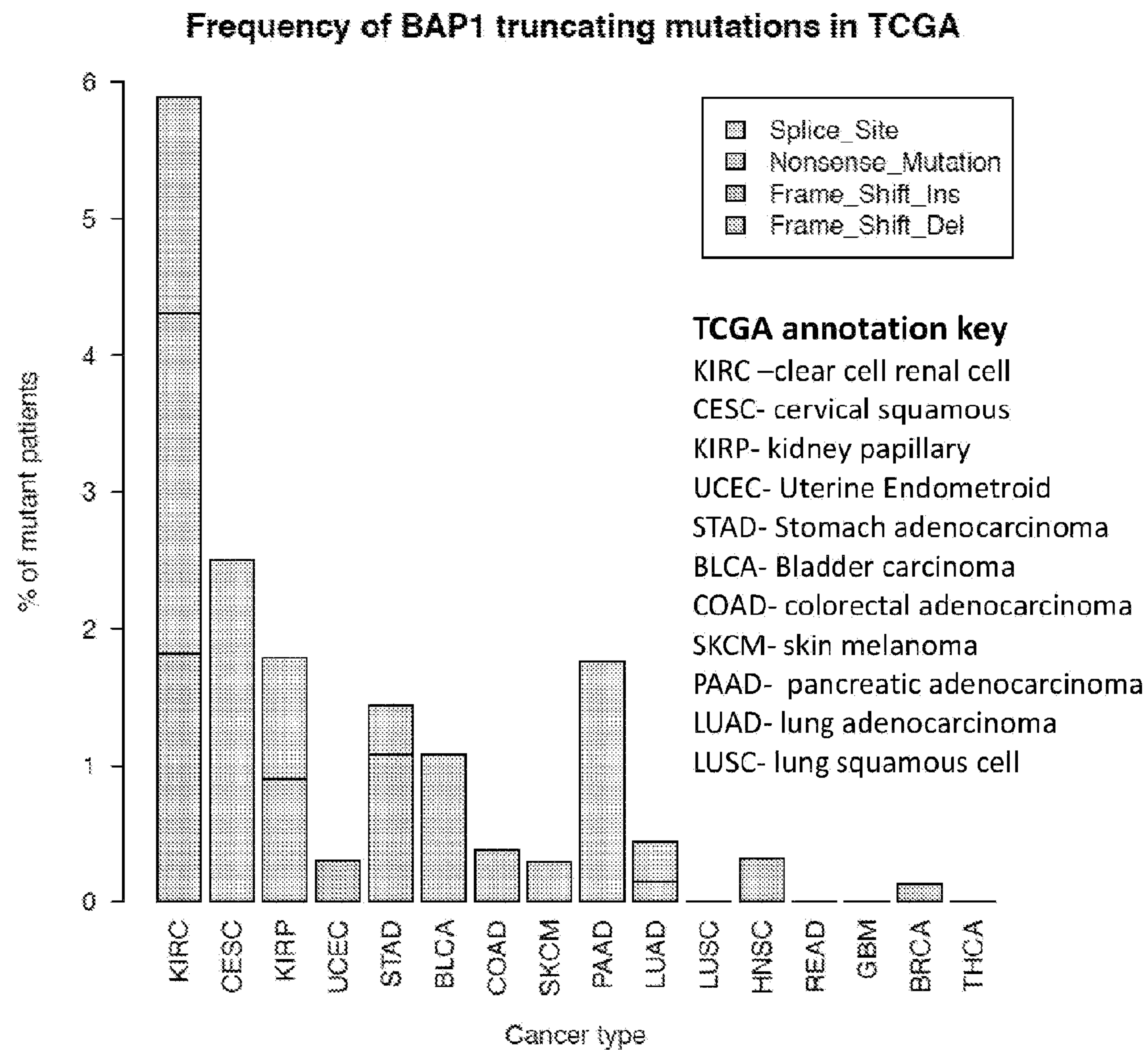


Fig. 1B

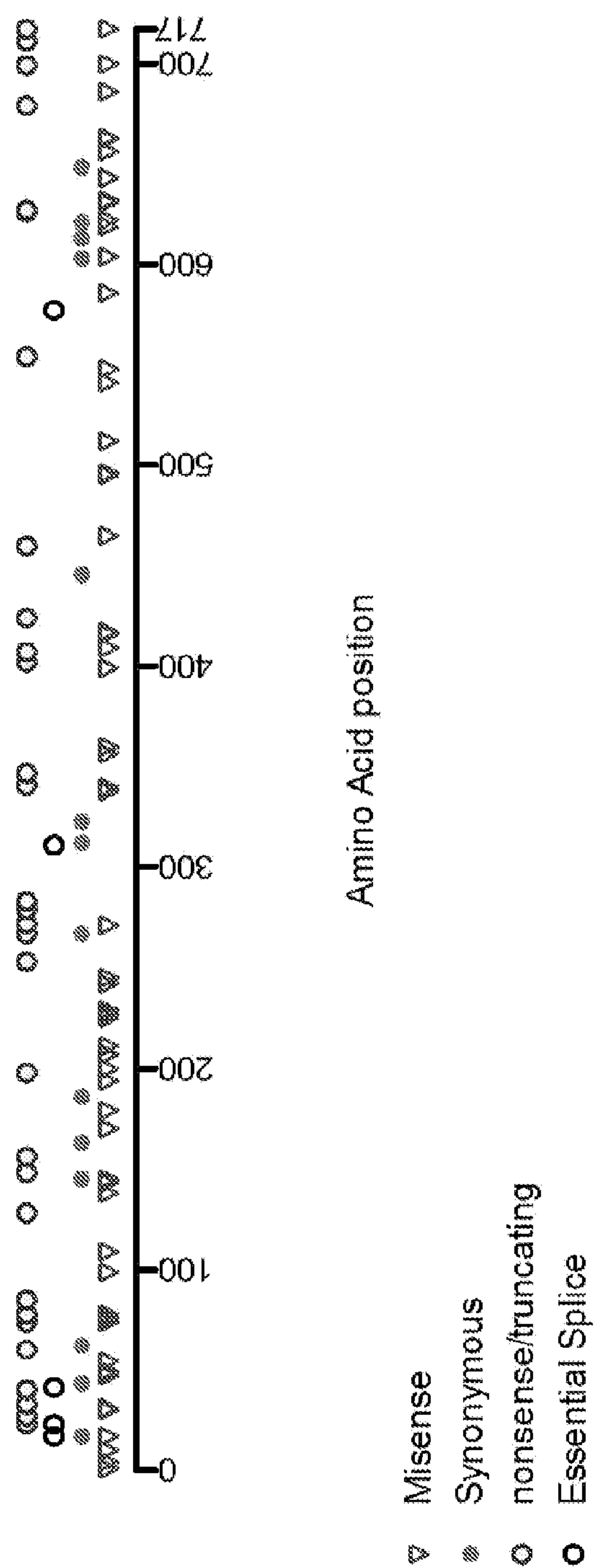


Fig. 1C

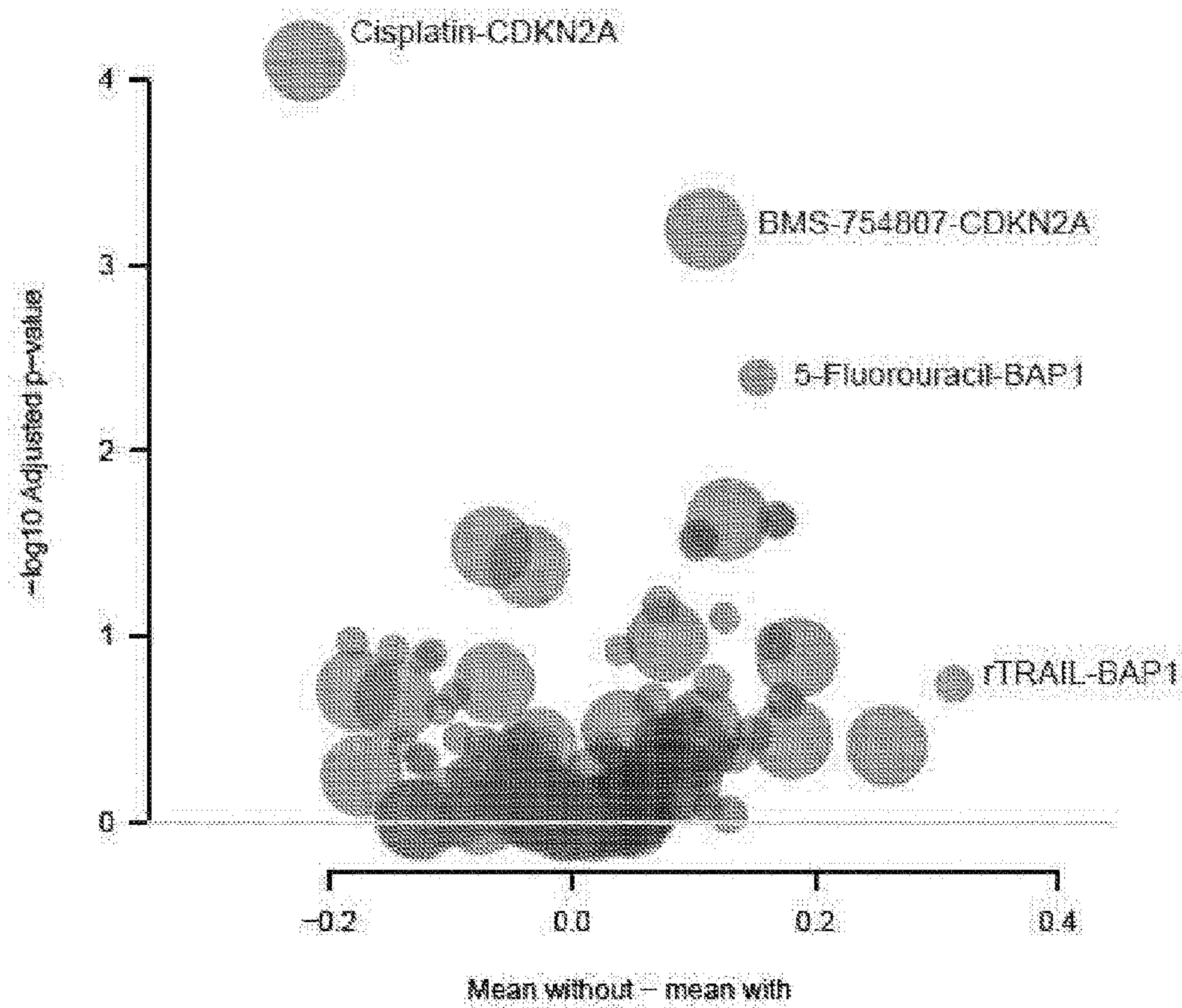


Fig. 2A

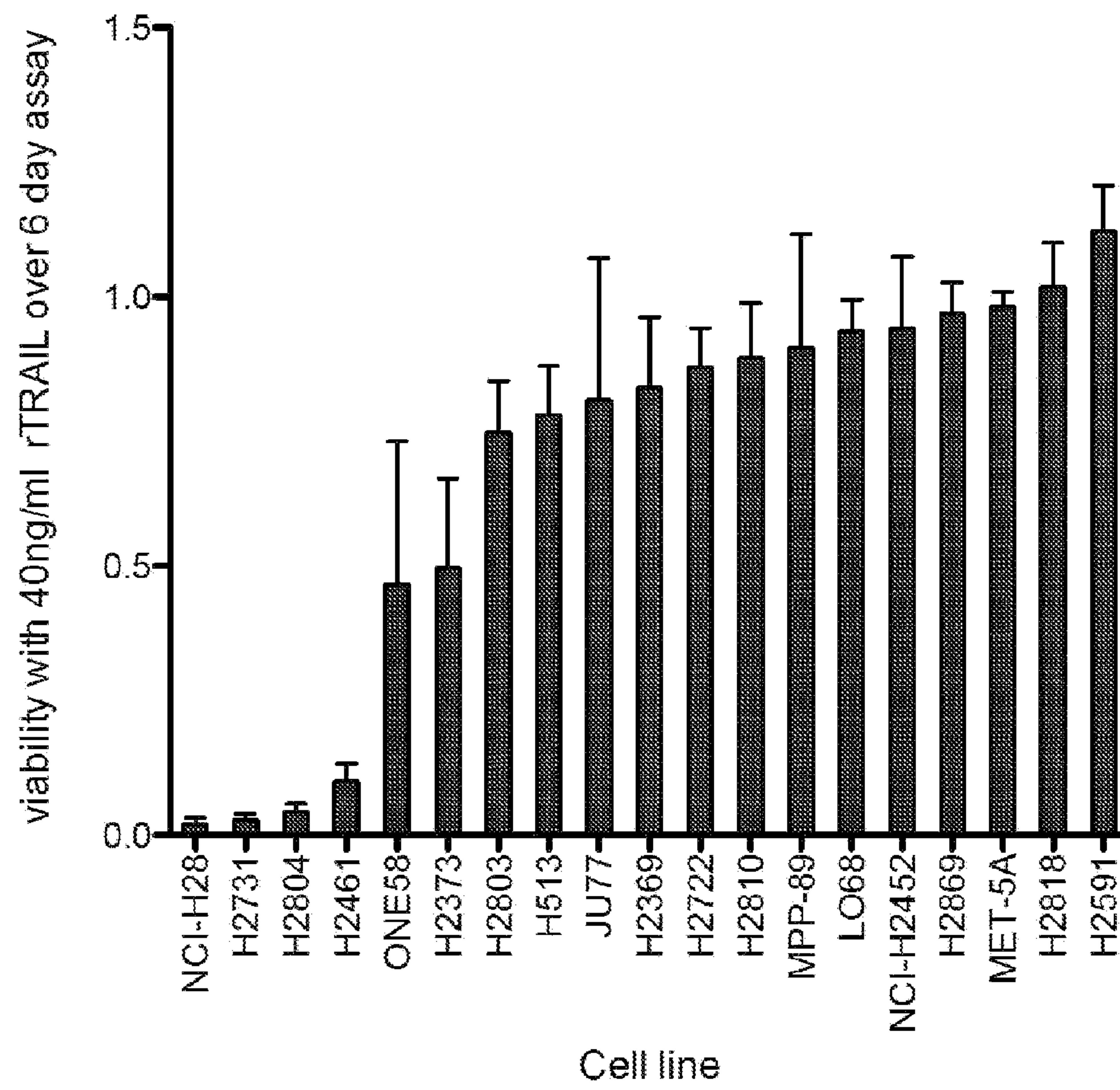


Fig. 2B

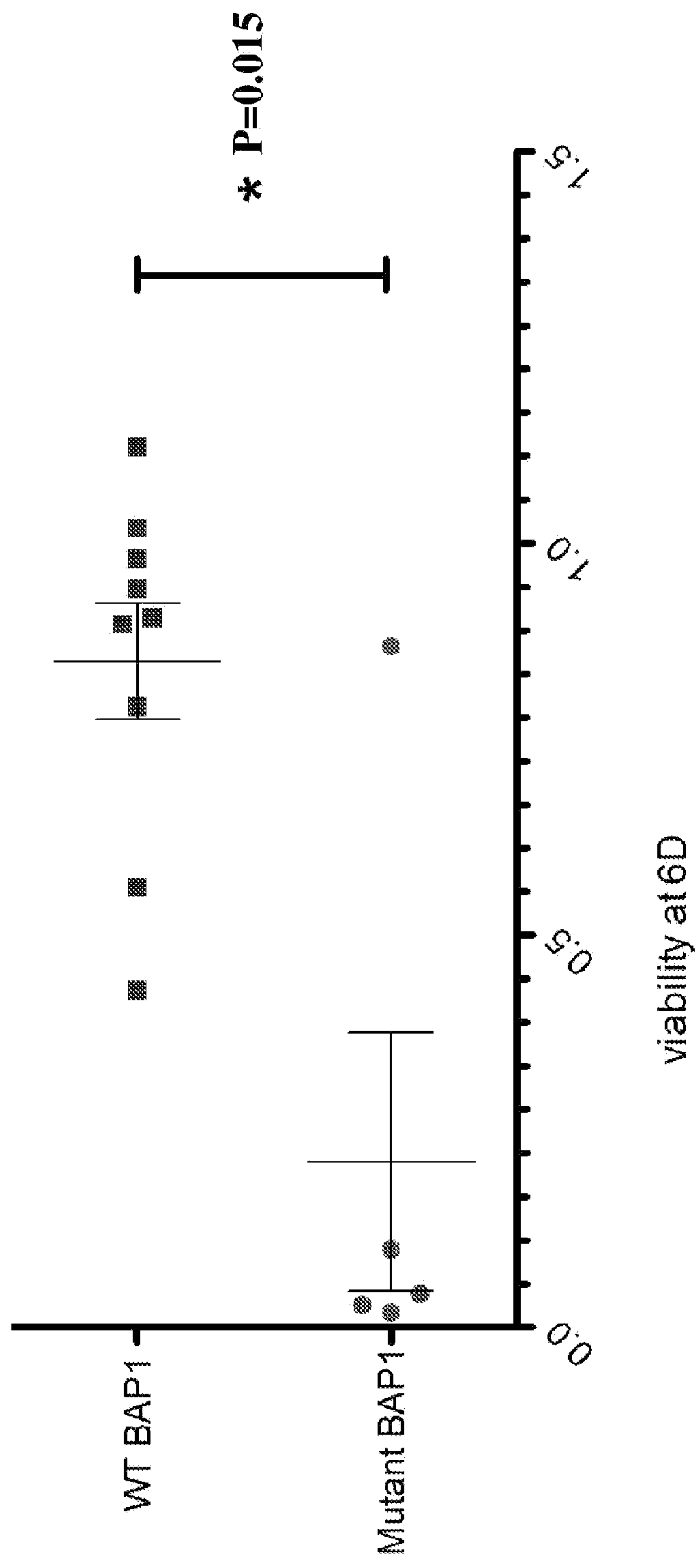


Fig. 2C

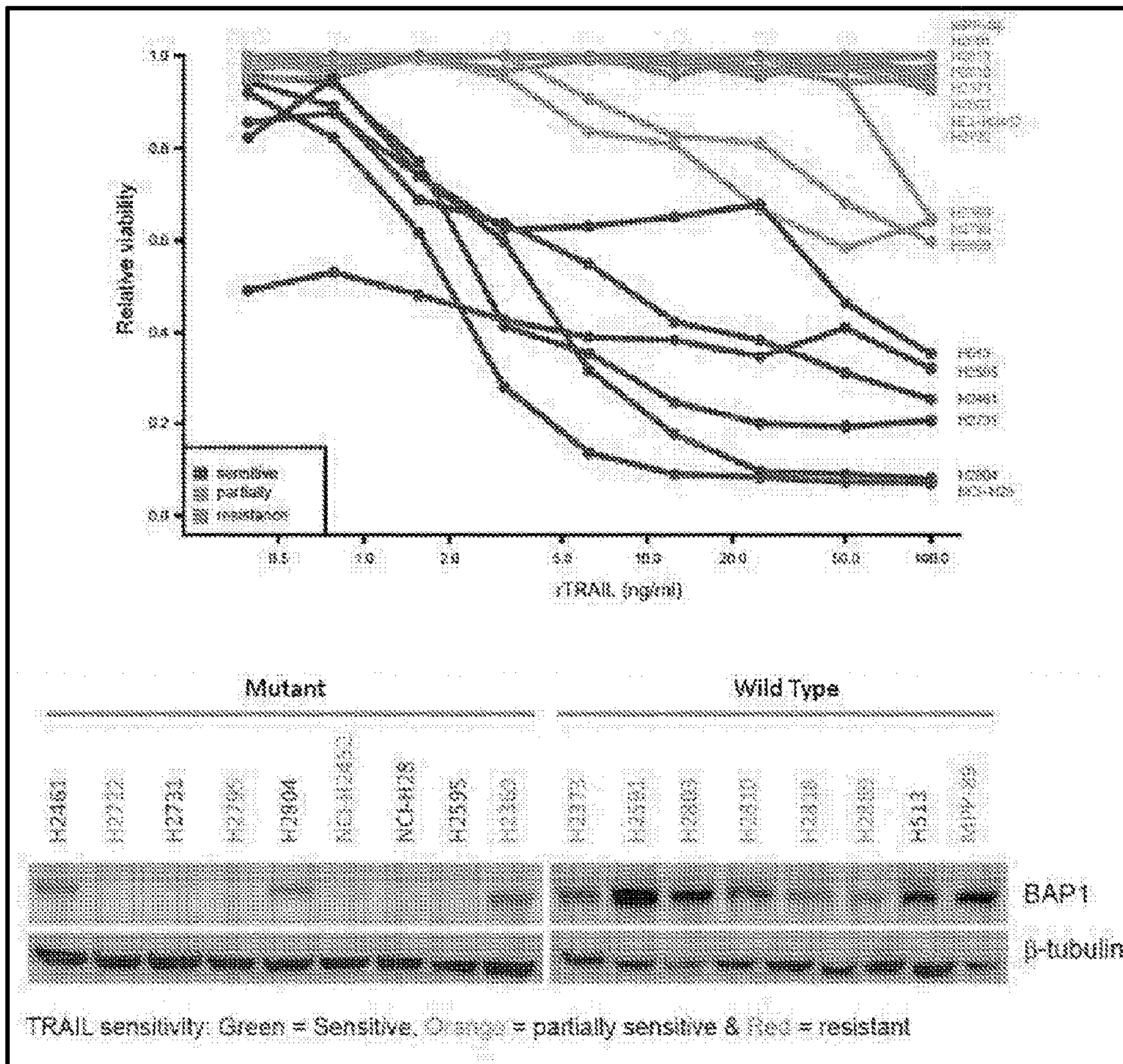


Fig. 3A

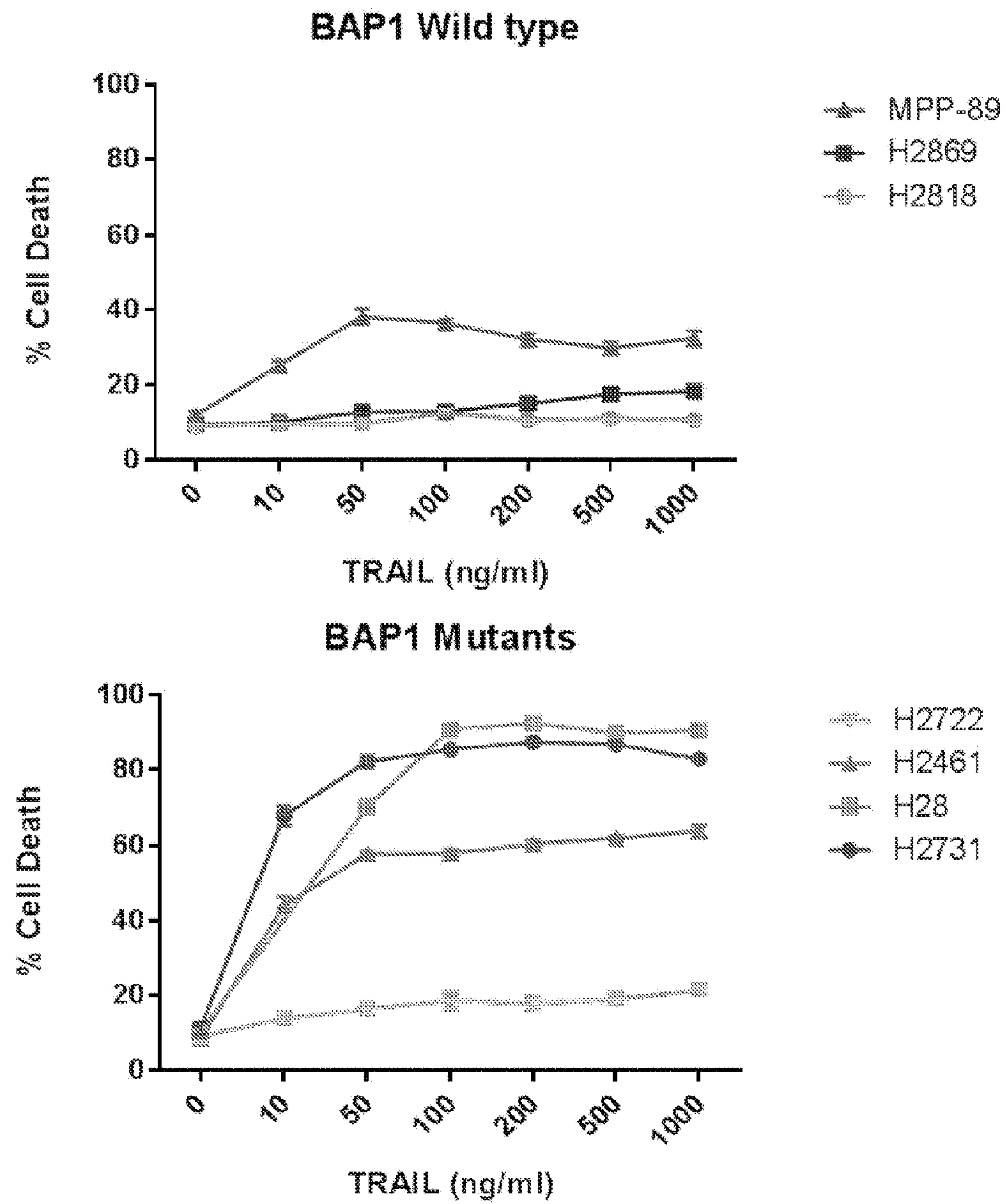


Fig. 3B

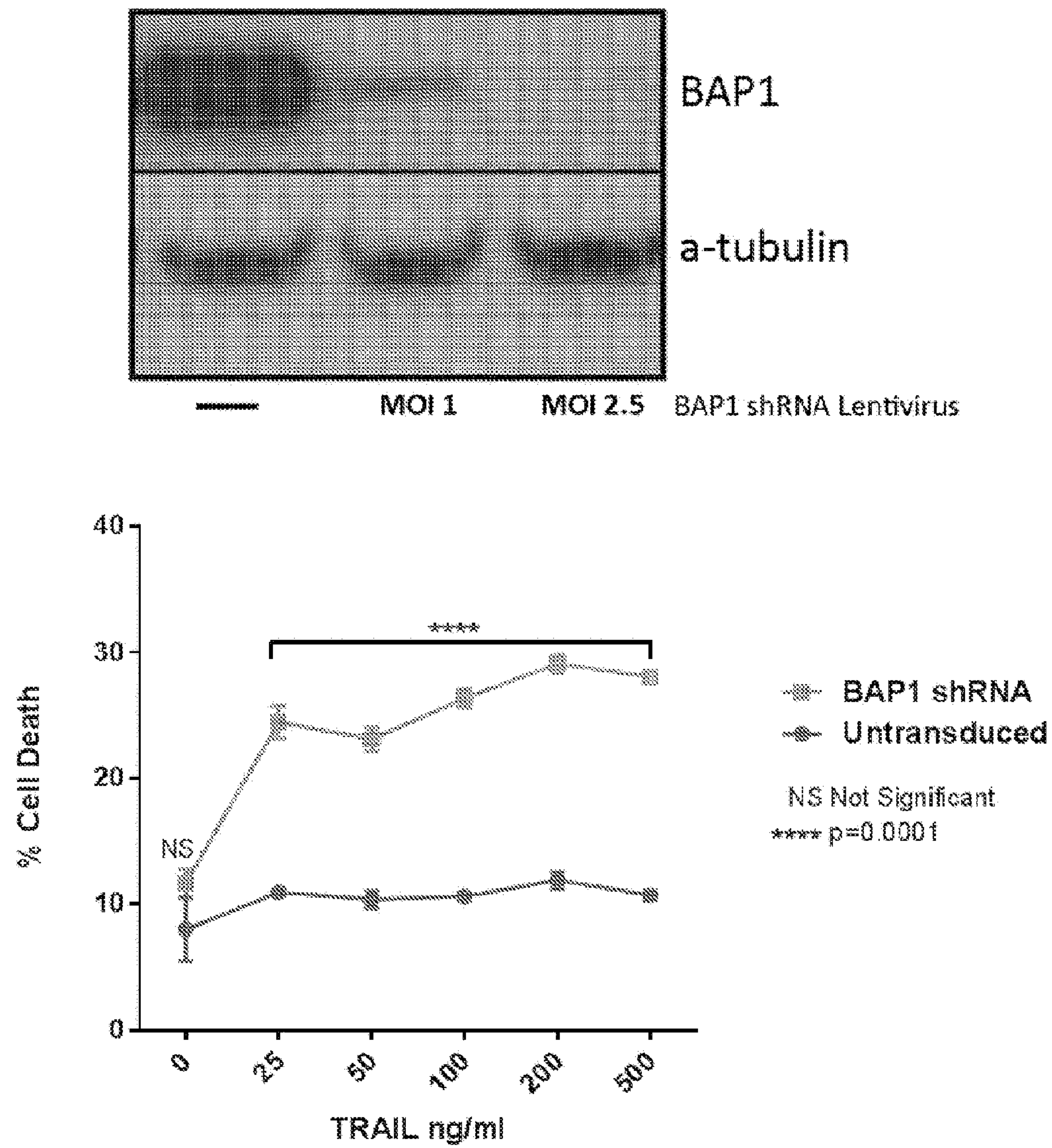


Fig. 4

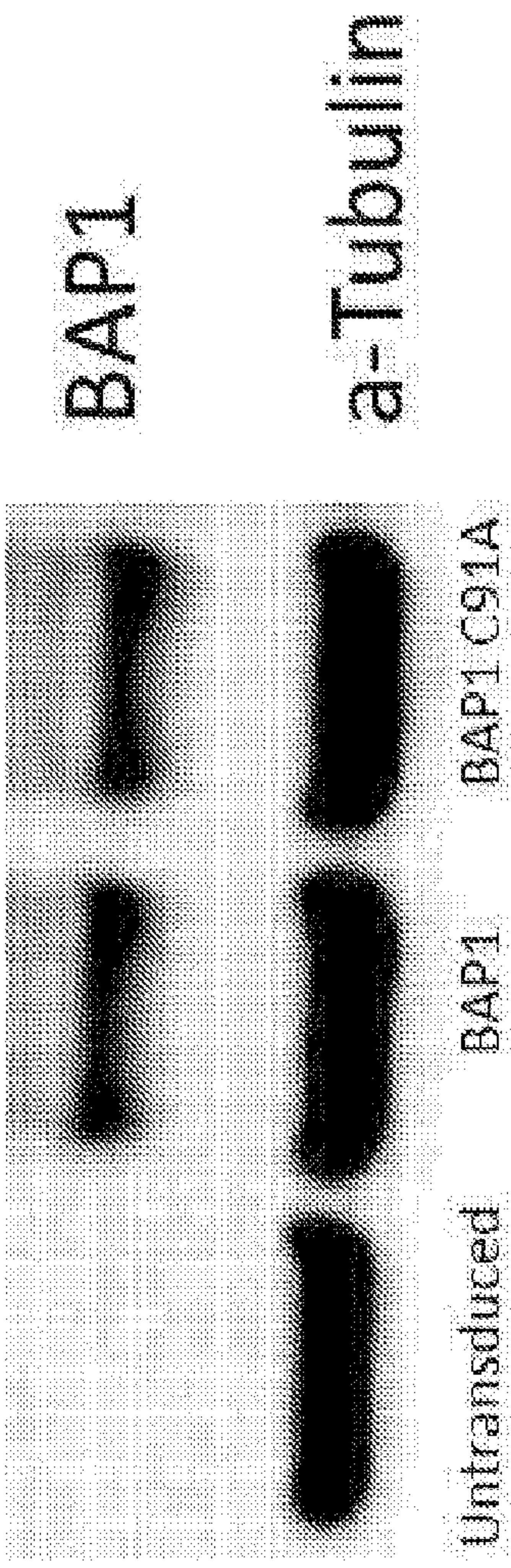


Fig. 5A

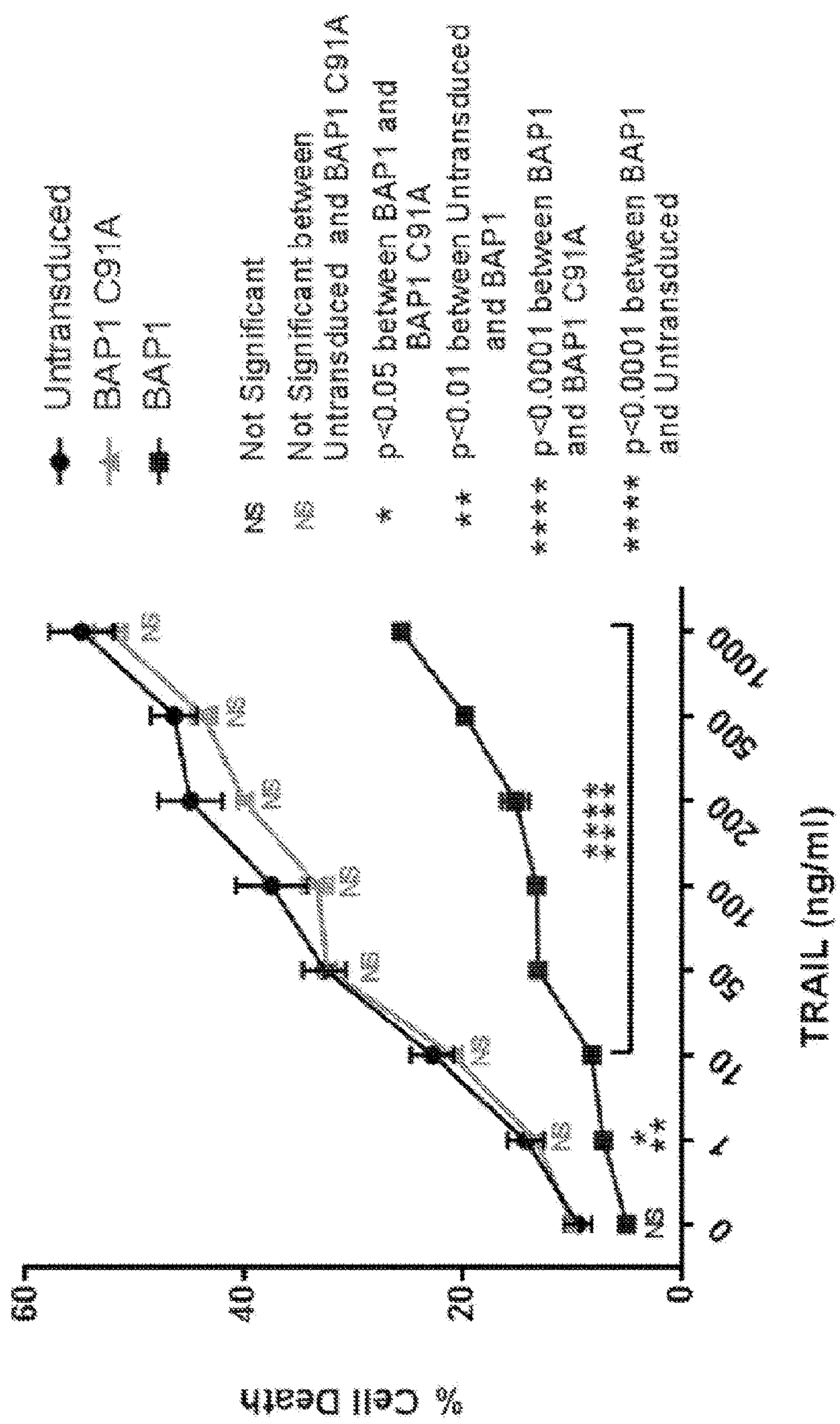


Fig. 5B

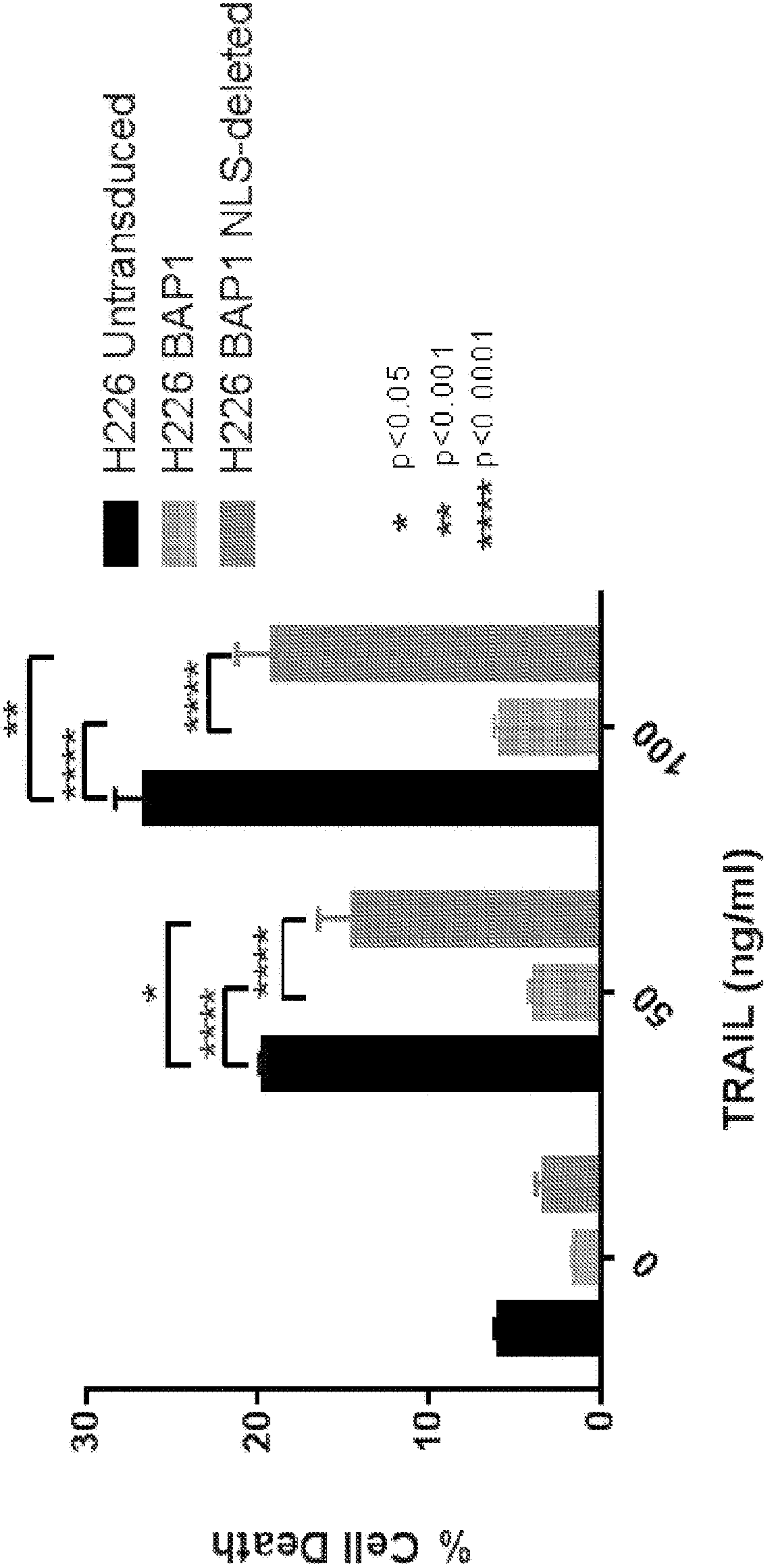


Fig. 5C

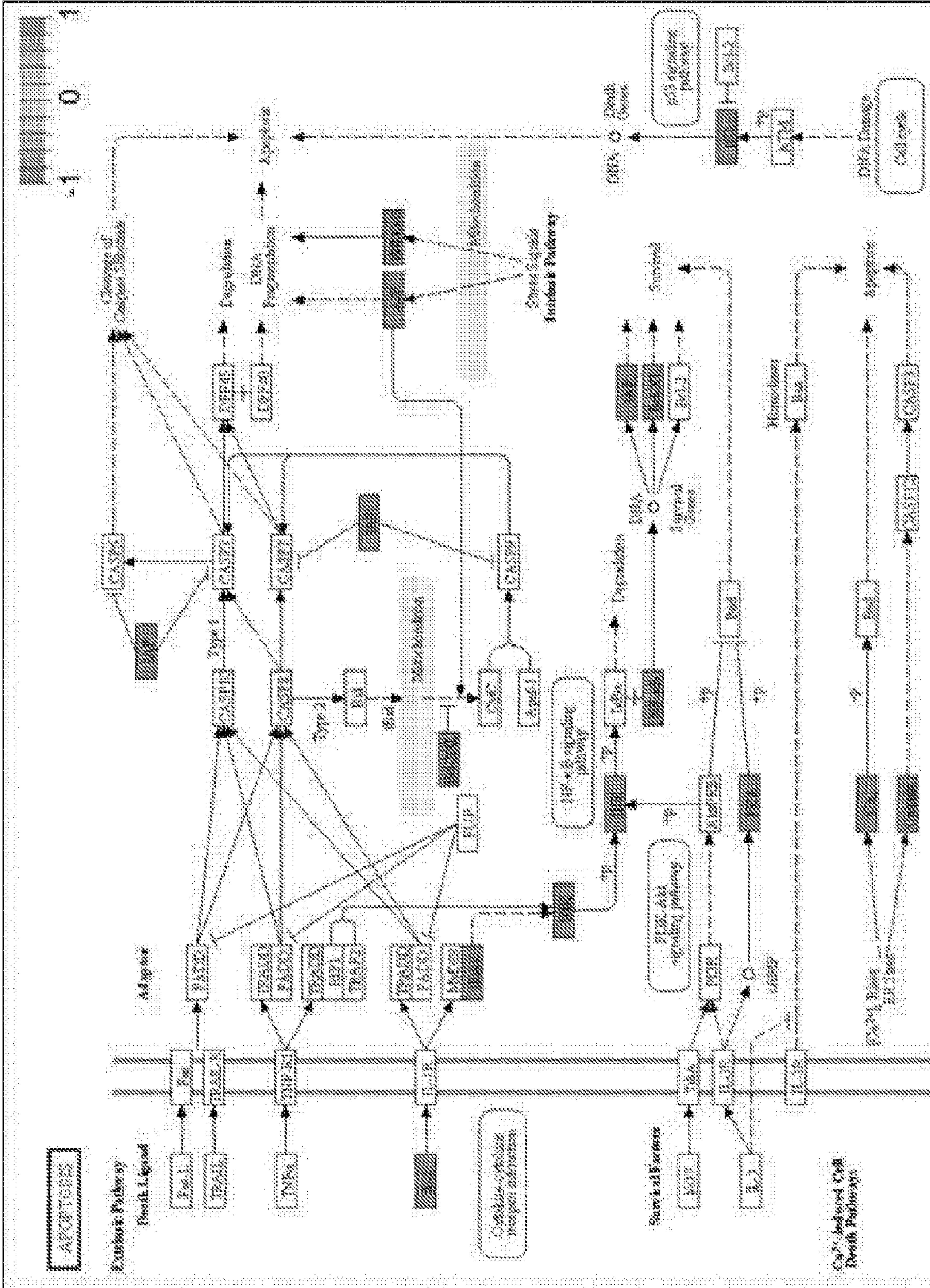


Fig. 6A

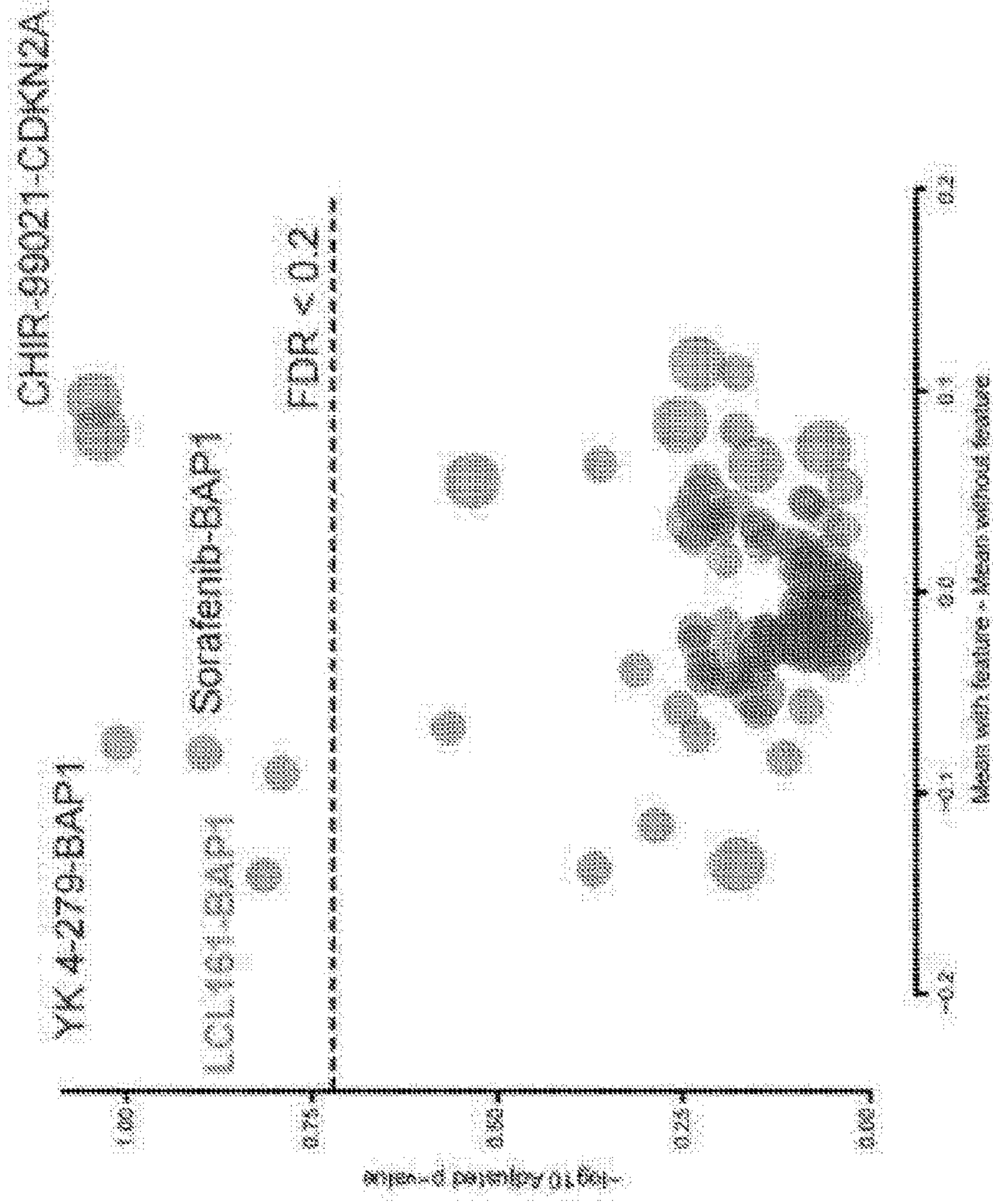


Fig. 7A

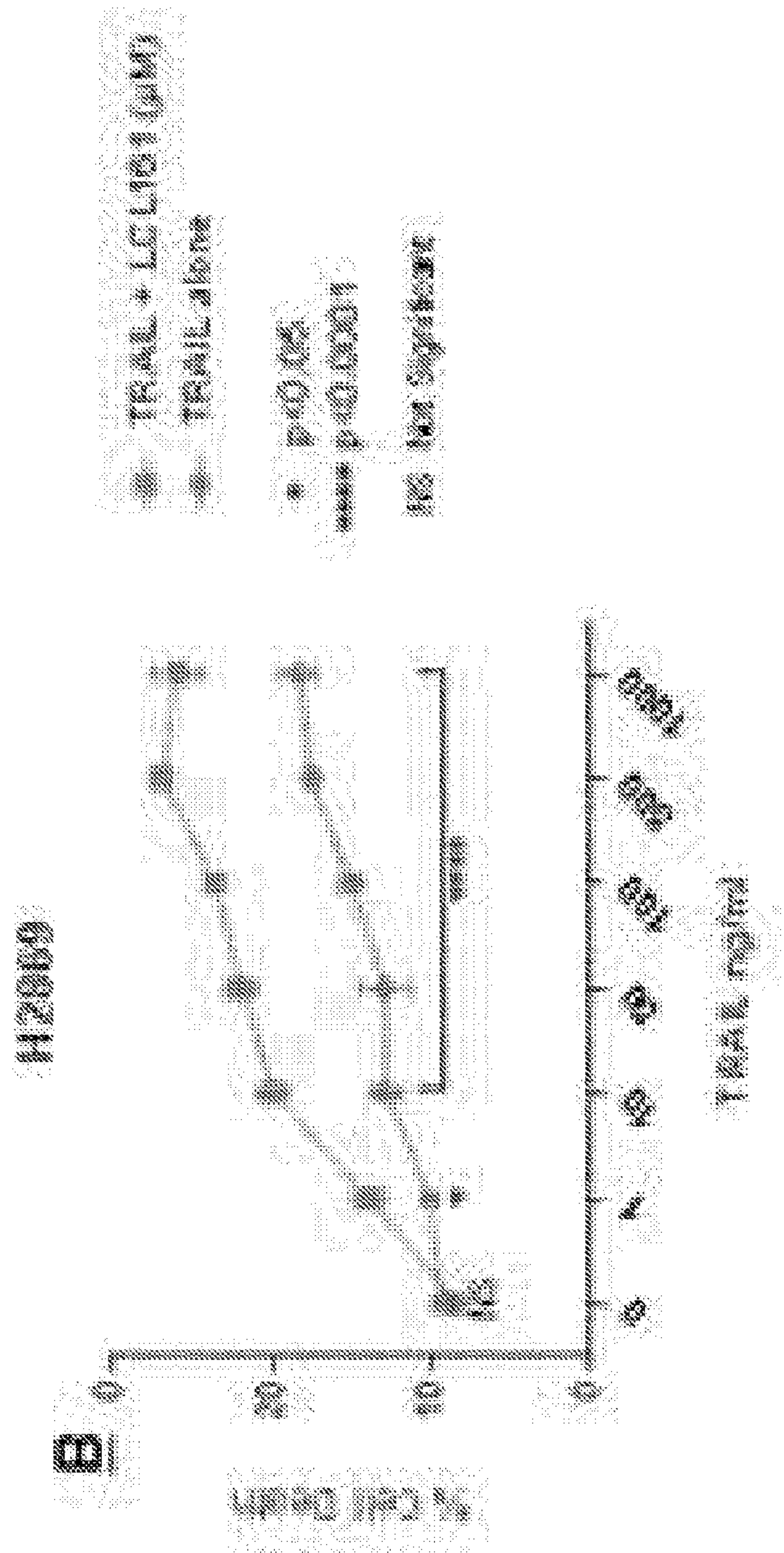


Fig. 7B

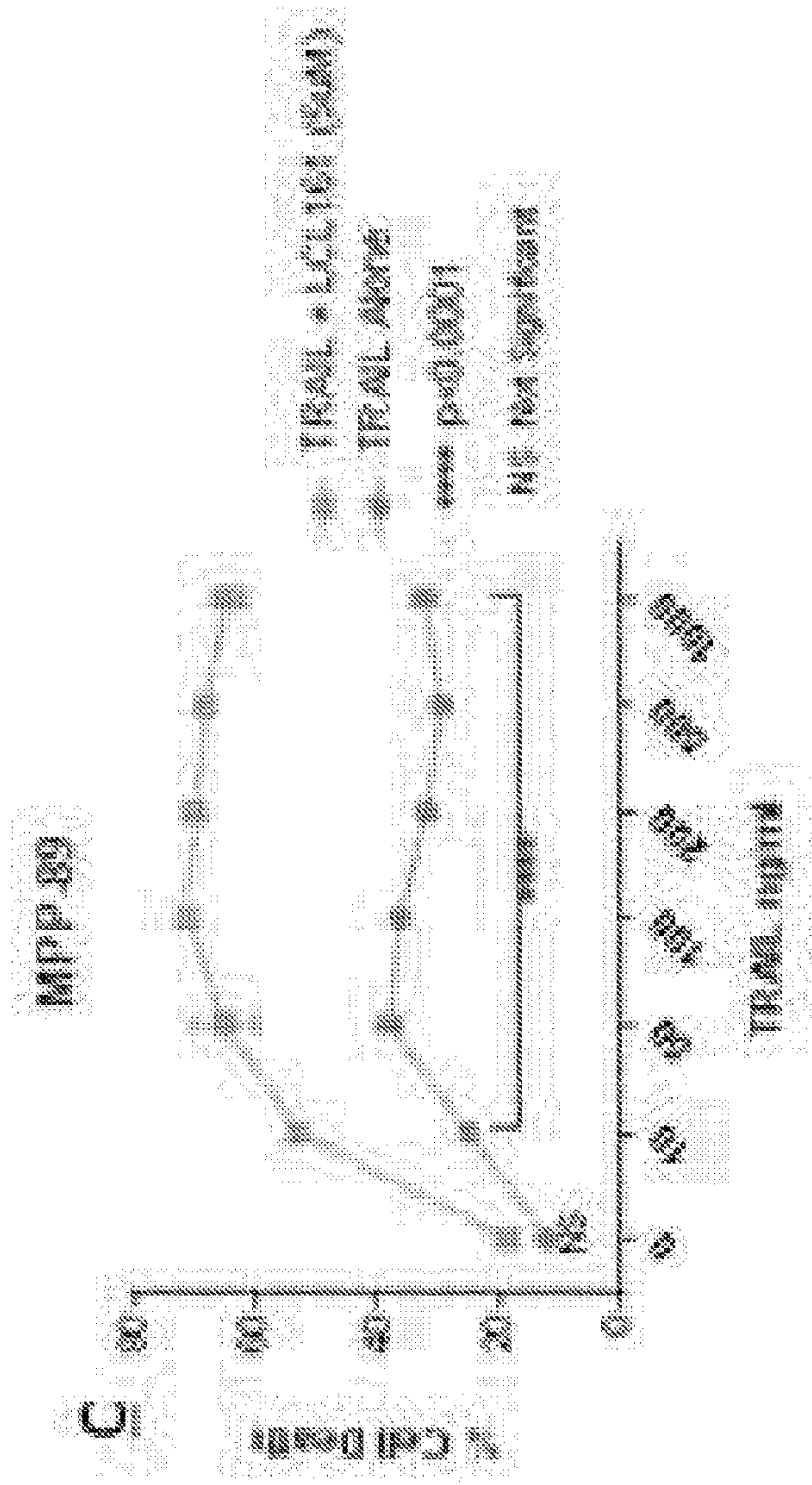


Fig. 7C

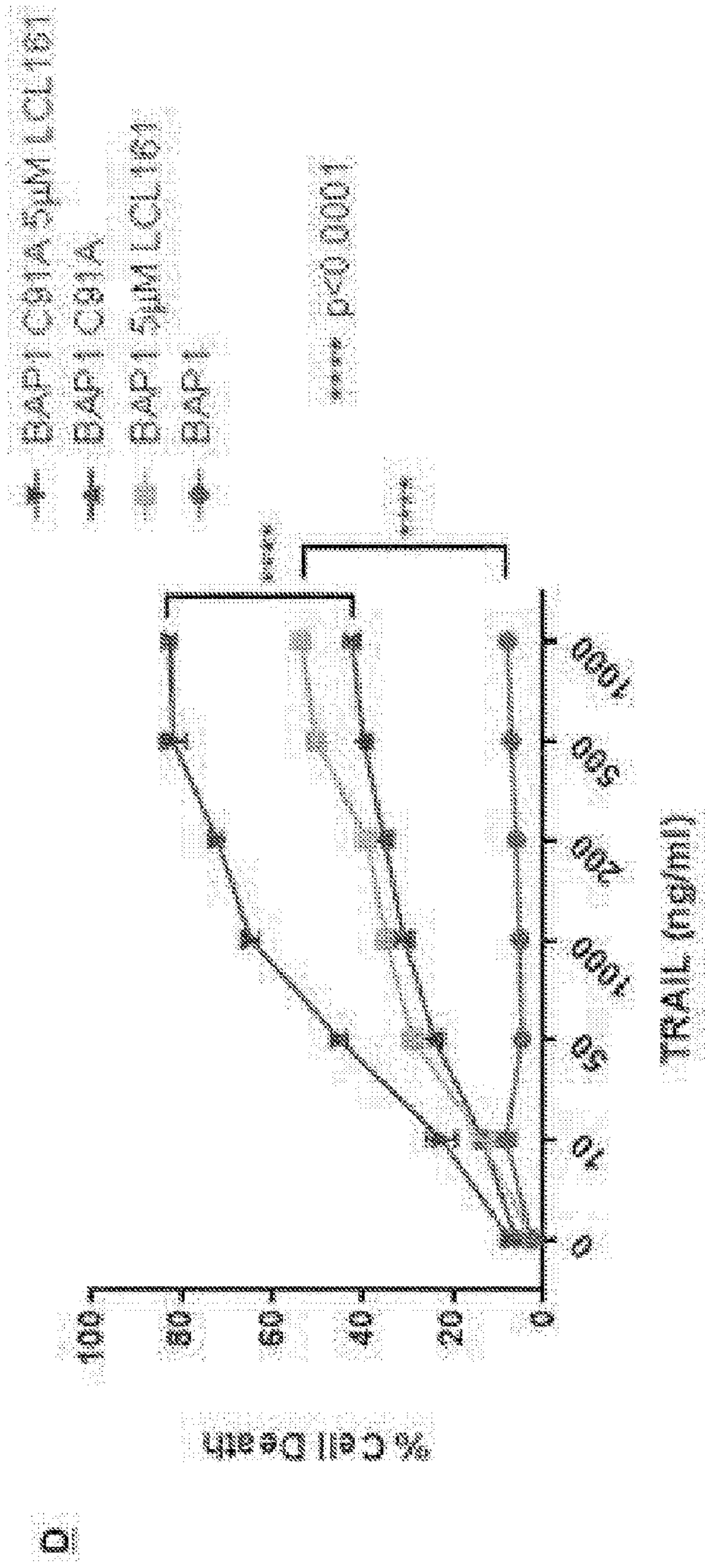


Fig. 7D

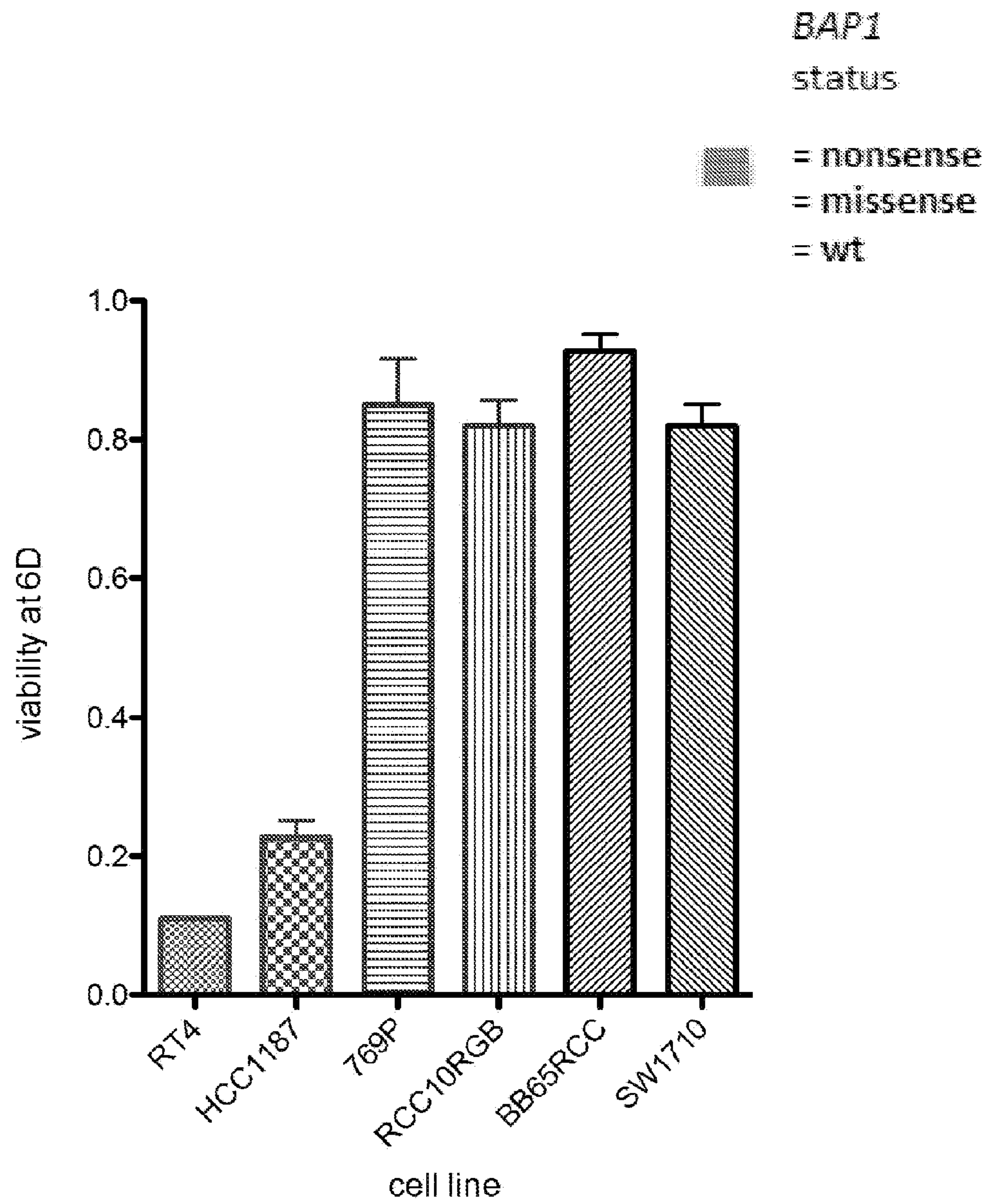


Fig. 8A

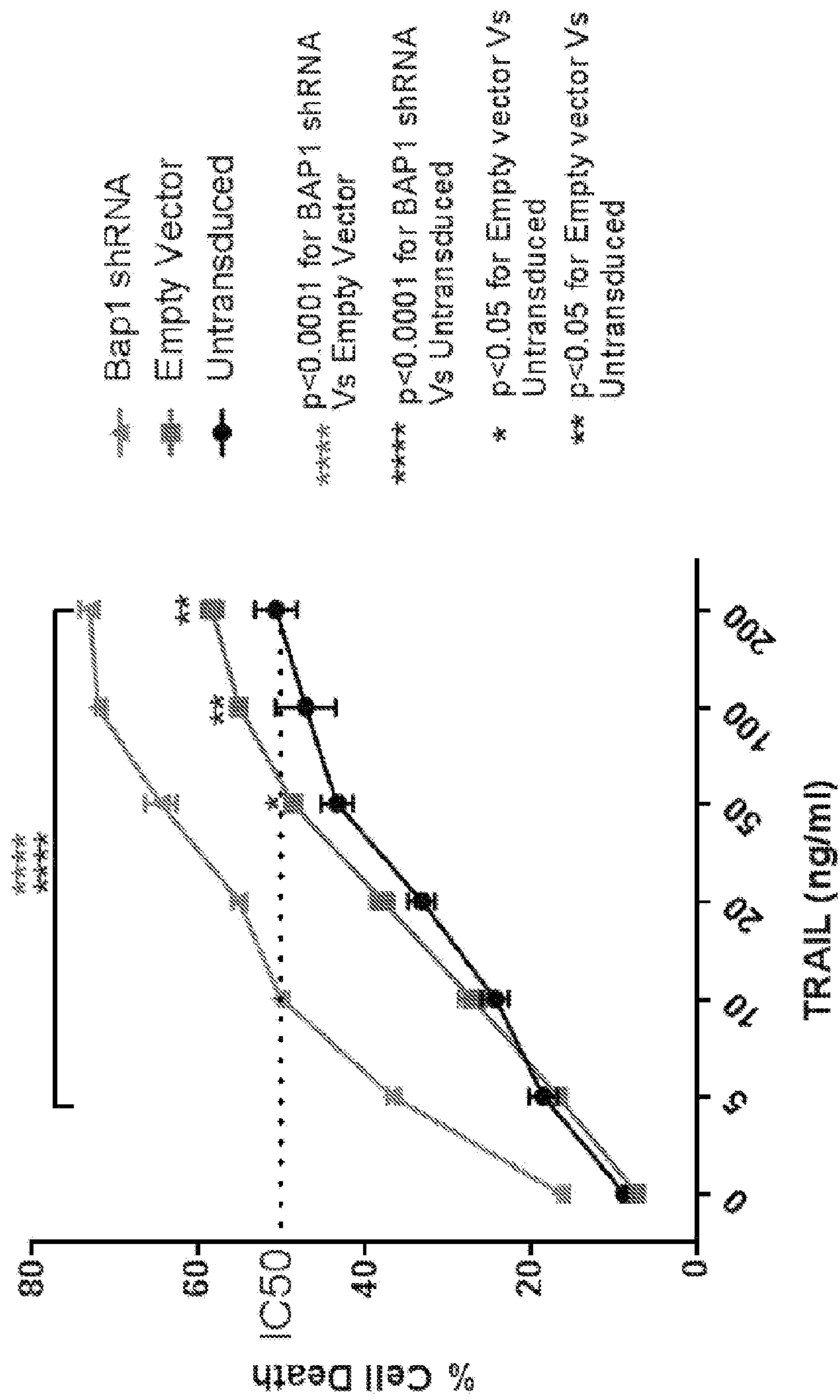


Fig. 8B

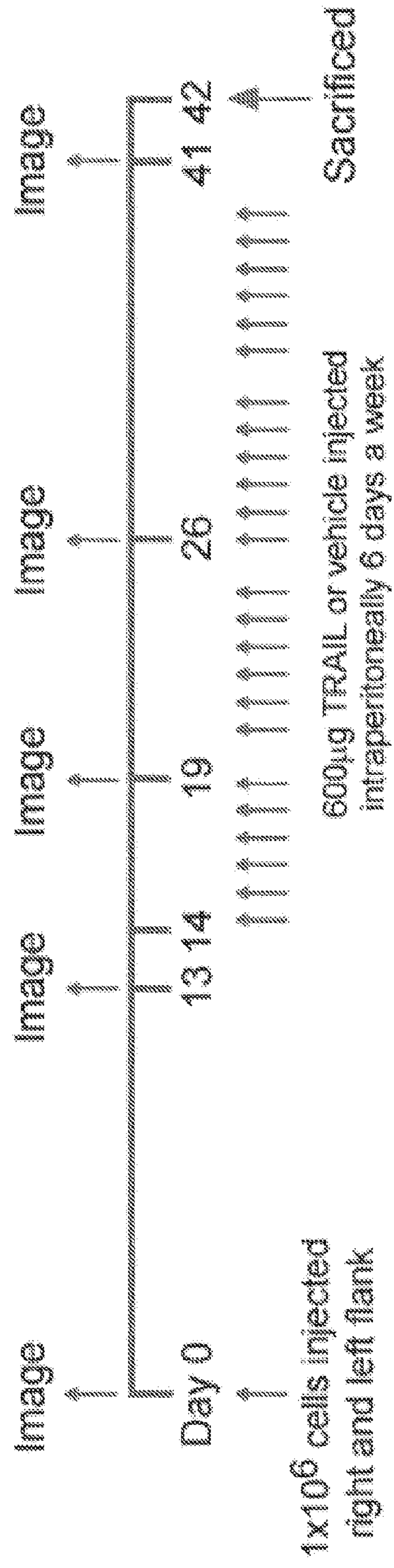


Fig. 9A

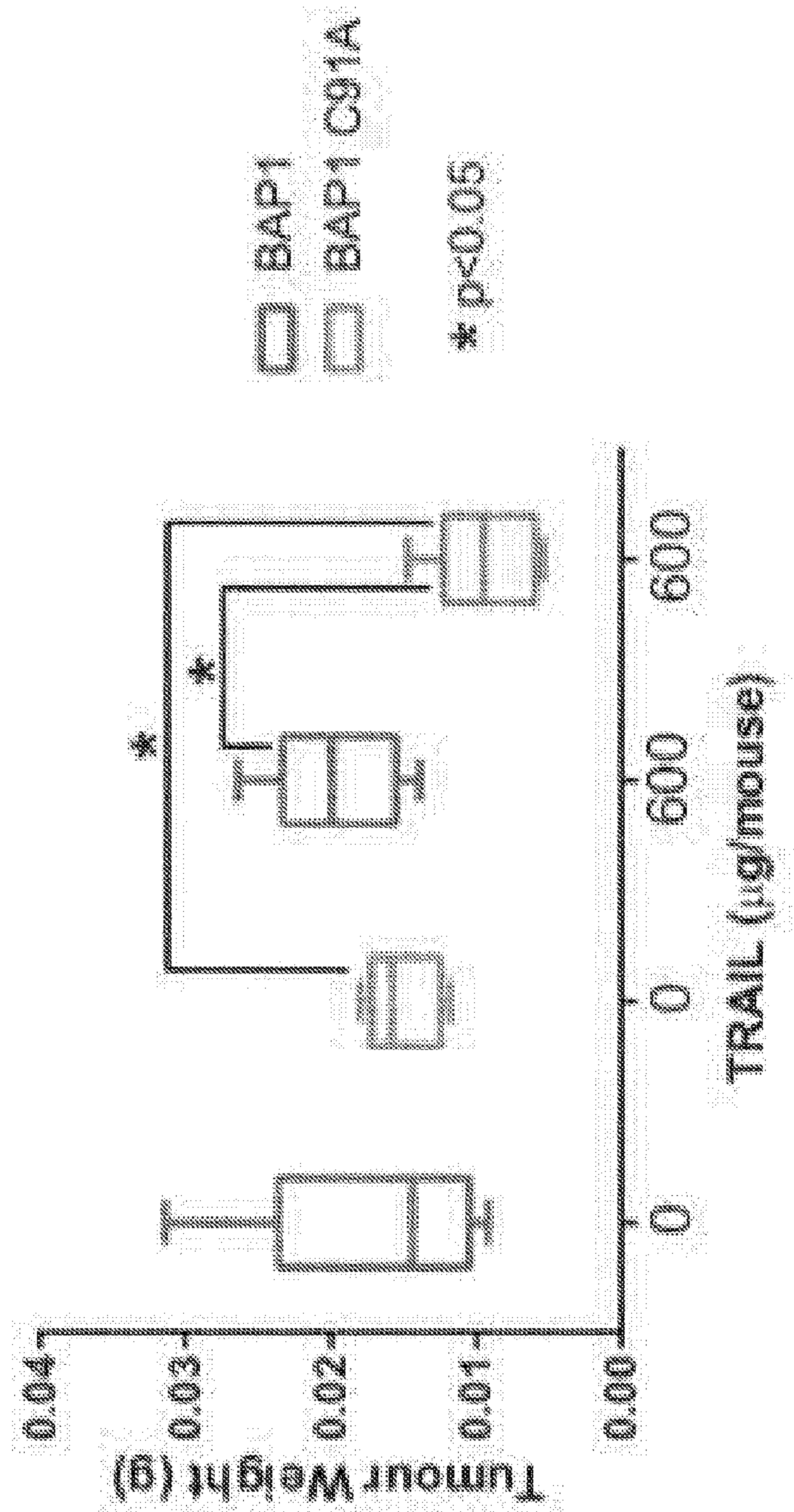


Fig. 9B

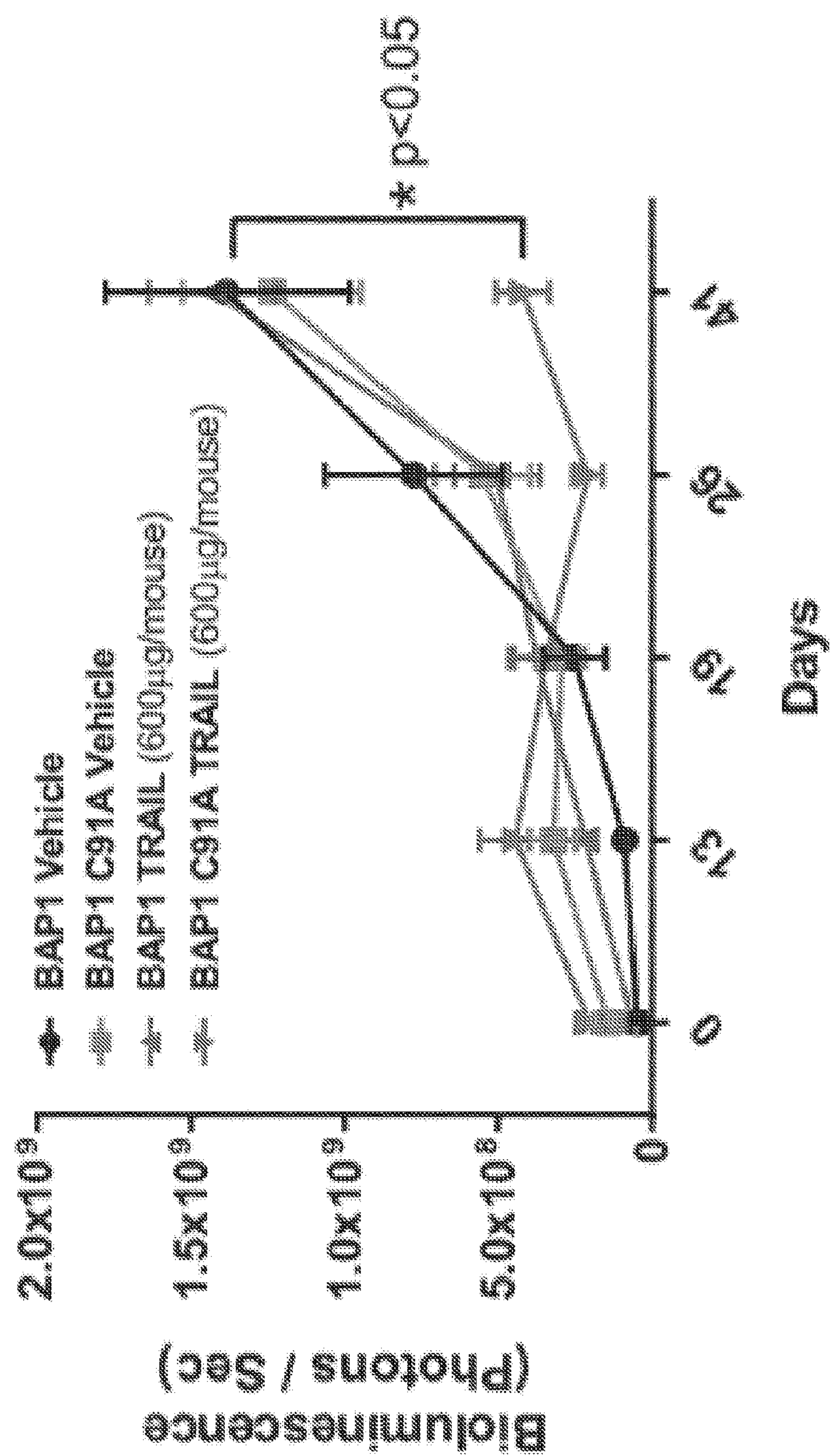


Fig. 9C

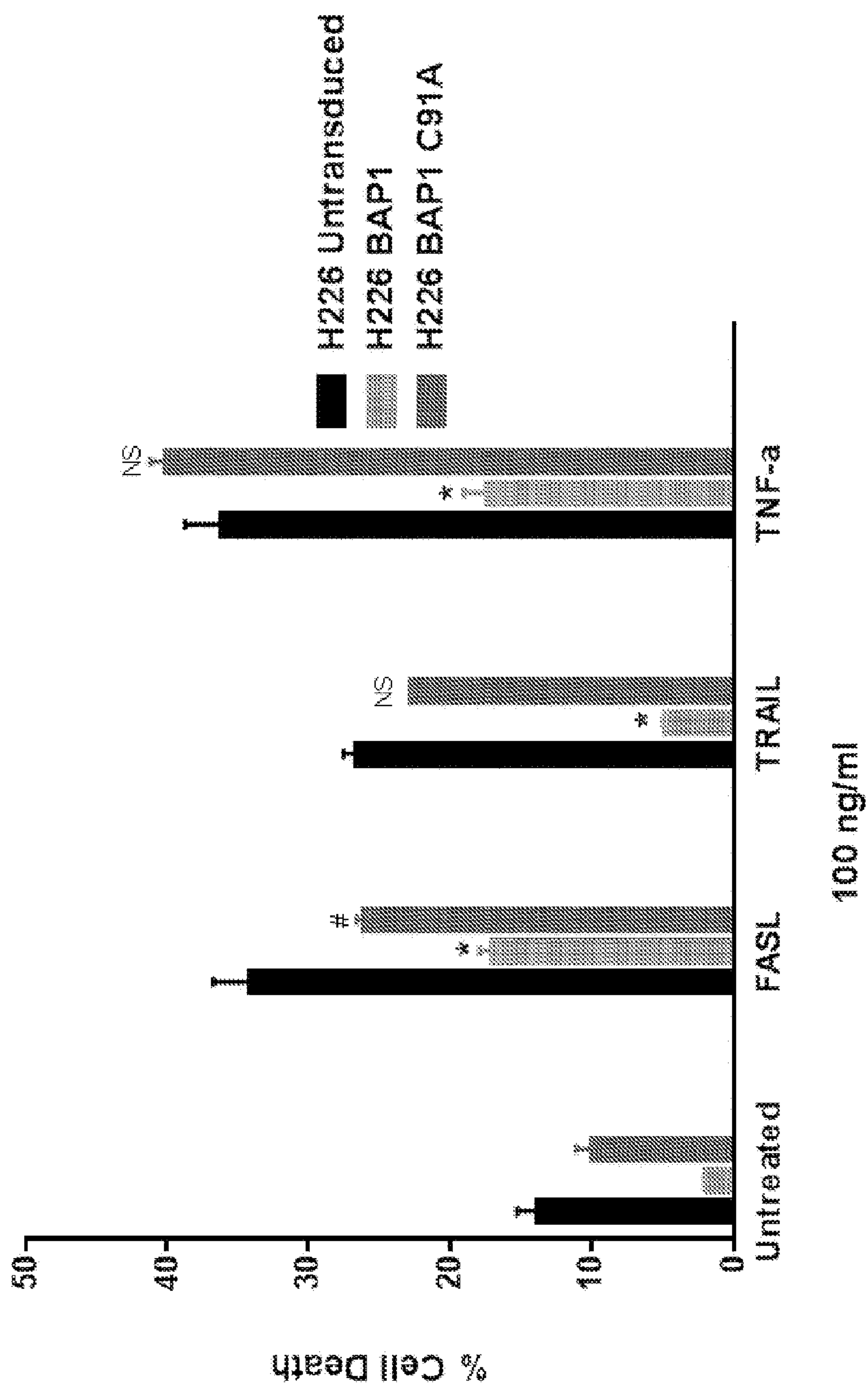


Fig. 10

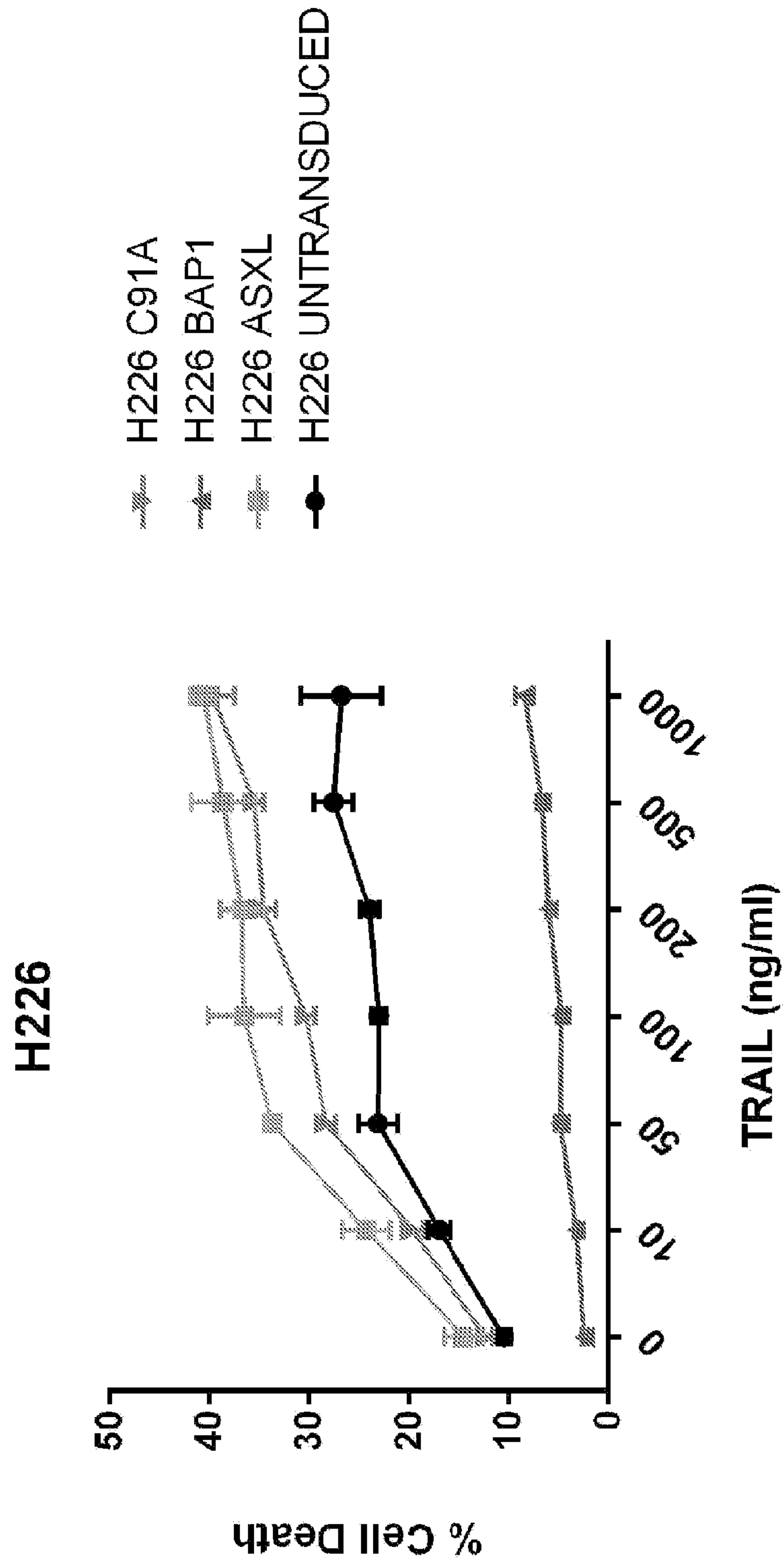


Fig. 11