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(54) Titre : VACCINS CONTRE LE CANCER POUR LE CANCER DU REIN
(54) Title: CANCER VACCINES FOR KIDNEY CANCER

(57) Abrégé/Abstract:
The invention relates to the field of cancer, in particular kidney cancer. In particular, it relates to the field of immune system directed approaches for tumor reduction and control. Some aspects of the invention relate to vaccines, vaccinations and other means of stimulating an antigen specific immune response against a tumor in individuals. Such vaccines comprise neoantigens resulting from frameshift mutations that bring out-of-frame sequences of the BAP1, PBRM1, SETD2, and VHL genes in-frame. Such vaccines are also useful for 'off the shelf' use.

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(54) Title: CANCER VACCINES FOR KIDNEY CANCER

(57) Abstract: The invention relates to the field of cancer, in particular kidney cancer. In particular, it relates to the field of immune system directed approaches for tumor reduction and control. Some aspects of the invention relate to vaccines, vaccinations and other means of stimulating an antigen specific immune response against a tumor in individuals. Such vaccines comprise neoantigens resulting from frameshift mutations that bring out-of-frame sequences of the BAP1, PBRM1, SETD2, and VHL genes in-frame. Such vaccines are also useful for 'off the shelf' use.

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Title: CANCER VACCINES FOR KIDNEY CANCER

FIELD OF THE INVENTION

The invention relates to the field of cancer, in particular kidney cancer. In particular, it relates to the field of immune system directed approaches for tumor reduction and control. Some aspects of the invention relate to vaccines, vaccinations and other means of stimulating an antigen specific immune response against a tumor in individuals. Such vaccines comprise neoantigens resulting from frameshift mutations that bring out-of-frame sequences of the BAP1, PBRM1, SETD2, and VHL genes in-frame. Such vaccines are also useful for 'off the shelf' use.

BACKGROUND OF THE INVENTION

There are a number of different existing cancer therapies, including ablation techniques (e.g., surgical procedures and radiation) and chemical techniques (e.g., pharmaceutical agents and antibodies), and various combinations of such techniques. Despite intensive research such therapies are still frequently associated with serious risk, adverse or toxic side effects, as well as varying efficacy.

There is a growing interest in cancer therapies that aim to target cancer cells with a patient's own immune system (such as cancer vaccines or checkpoint inhibitors, or T-cell based immunotherapy). Such therapies may indeed eliminate some of the known disadvantages of existing therapies, or be used in addition to the existing therapies for additional therapeutic effect. Cancer vaccines or immunogenic compositions intended to treat an existing cancer by strengthening the body's natural defenses against the cancer and based on tumor-specific neoantigens hold great promise as next-generation of personalized cancer immunotherapy. Evidence shows that such neoantigen-based vaccination can elicit T-cell responses and can cause tumor regression in patients.

Typically the immunogenic compositions/vaccines are composed of tumor antigens (antigenic peptides or nucleic acids encoding them) and may include immune stimulatory molecules like cytokines that work together to induce antigen-specific cytotoxic T-cells that target and destroy tumor cells. Vaccines containing tumor-specific and patient-specific neoantigens require the sequencing of the patients' genome and tumor genome in order to determine whether the neoantigen is tumor specific, followed by the production of personalized compositions. Sequencing, identifying the patient's specific neoantigens and preparing such personalized compositions may require a substantial amount of time, time which

may unfortunately not be available to the patient, given that for some tumors the average survival time after diagnosis is short, sometimes around a year or less.

Accordingly, there is a need for improved methods and compositions for providing subject-specific immunogenic compositions/cancer vaccines. In particular it would be desirable to have available a vaccine for use in the treatment of cancer, wherein such vaccine is suitable for treatment of a larger number of patients, and can thus be prepared in advance and provided off the shelf. There is a clear need in the art for personalized vaccines which induce an immune response to tumor specific neoantigens. One of the objects of the present disclosure is to provide personalized cancer vaccines that can be provided off the shelf. An additional object of the present disclosure is to provide cancer vaccines that can be provided prophylactically. Such vaccines are especially useful for individuals that are at risk of developing cancer.

SUMMARY OF THE INVENTION

In a preferred embodiment, the disclosure provides a vaccine for use in the treatment of kidney cancer, said vaccine comprising:

(i) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 1, an amino acid sequence having 90% identity to Sequence 1, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 1; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 2, an amino acid sequence having 90% identity to Sequence 2, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 2; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 3, an amino acid sequence having 90% identity to Sequence 3, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 3;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 4, an amino acid sequence having 90% identity to Sequence 4, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 4;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 5, an amino acid sequence having 90% identity to Sequence 5, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 5;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 6, an amino acid sequence having 90% identity to Sequence 6, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 6; and/or

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 7, an amino acid sequence having 90% identity to Sequence 7, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 7;

(ii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 19, an amino acid sequence having 90% identity to Sequence 19, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 19; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 20, an amino acid sequence having 90% identity to Sequence 20, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 20;

(iii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 189, an amino acid sequence having 90% identity to Sequence 189, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 189; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from any one of Sequences 190-192, an amino acid sequence having 90% identity to Sequences 190-192, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 190-192; and/or

(iv) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 311, an amino acid sequence having 90% identity to Sequence 311, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 311; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 312, an amino acid sequence having 90% identity to Sequence 312, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 312; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 313, an amino acid sequence having 90% identity to Sequence 313, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 313.

In a preferred embodiment, the disclosure provides a collection of frameshift-mutation peptides comprising:

(i) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 1, an amino acid sequence having 90% identity to Sequence 1, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 1; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 2, an amino acid sequence having 90% identity to Sequence 2, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 2; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 3, an amino acid sequence having 90% identity to Sequence 3, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 3;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 4, an amino acid sequence having 90% identity to Sequence 4, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 4;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 5, an amino acid sequence having 90% identity to Sequence 5, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 5;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 6, an amino acid sequence having 90% identity to Sequence 6, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 6; and/or

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 7, an amino acid sequence having 90% identity to Sequence 7, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 7;

(ii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 19, an amino acid sequence having 90% identity to Sequence 19, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 19; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 20, an amino acid sequence having 90% identity to Sequence 20, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 20;

(iii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 189, an amino acid sequence having 90% identity to Sequence 189, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 189; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from any one of Sequences 190-192, an amino acid sequence having 90% identity to Sequences 190-192, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 190-192; and/or

(iv) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 311, an amino acid sequence having 90% identity to Sequence 311, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 311; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 312, an amino acid sequence having 90% identity to Sequence 312, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 312; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 313, an amino acid sequence having 90% identity to Sequence 313, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 313.

In a preferred embodiment, the disclosure provides a peptide comprising an amino acid sequence selected from the groups:

(i) Sequences 1-18, an amino acid sequence having 90% identity to Sequences 1-18, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 1-18;

(ii) Sequences 19-188, an amino acid sequence having 90% identity to Sequences 19-188, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 19-188;

(iii) Sequences 189-310, an amino acid sequence having 90% identity to Sequences 189-310, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 189-310; and

(iv) Sequences 311-352, an amino acid sequence having 90% identity to Sequences 311-352, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 311-352.

In some embodiments of the disclosure, the peptides are linked, preferably wherein said peptides are comprised within the same polypeptide.

In a preferred embodiment, the disclosure provides one more isolated nucleic acid molecules encoding the peptides or collection of peptides as disclosed herein. In a preferred embodiment, the disclosure provides one or more vectors comprising the nucleic acid molecules disclosed herein, preferably wherein the vector is a viral vector. In a preferred embodiment, the disclosure provides a host cell comprising the isolated nucleic acid molecules or the vectors as disclosed herein.

In a preferred embodiment, the disclosure provides a binding molecule or a collection of binding molecules that bind the peptide or collection of peptides disclosed herein, where in the binding molecule is an antibody, a T-cell receptor, or an antigen binding fragment thereof.

In a preferred embodiment, the disclosure provides a chimeric antigen receptor or collection of chimeric antigen receptors each comprising i) a T cell activation molecule; ii) a transmembrane region; and iii) an antigen recognition moiety; wherein said antigen recognition moieties bind the peptide or collection of peptides disclosed herein. In a preferred embodiment, the disclosure provides a host cell or combination of host cells that express the binding molecule or collection of binding molecules, or the chimeric antigen receptor or collection of chimeric antigen receptors as disclosed herein.

In a preferred embodiment, the disclosure provides a vaccine or collection of vaccines comprising the peptide or collection of peptides, the nucleic acid molecules, the vectors, or the host cells as disclosed herein; and a pharmaceutically acceptable excipient and/or adjuvant, preferably an immune-effective amount of adjuvant.

In a preferred embodiment, the disclosure provides the vaccines as disclosed herein for use in the treatment of kidney cancer in an individual. In a preferred embodiment, the disclosure provides the vaccines as disclosed herein for prophylactic use in the prevention of kidney cancer in an individual. In a preferred embodiment, the disclosure provides the vaccines as disclosed herein for use in the preparation of a medicament for treatment of kidney cancer in an individual or for prophylactic use. In a preferred embodiment, the disclosure provides methods of treating an individual for kidney cancer or reducing the risk of developing said cancer, the method comprising administering to the individual in need thereof a therapeutically effective amount of a vaccine as disclosed herein. In some embodiments, the individual prophylactically administered a vaccine as disclosed herein has not been diagnosed with cancer.

In a preferred embodiment, the individual has kidney cancer and one or more cancer cells of the individual:

- (i) expresses a peptide having the amino acid sequence selected from Sequences 1-352, an amino acid sequence having 90% identity to any one of Sequences 1-352, or a fragment thereof comprising at least 10 consecutive amino acids of amino acid sequence selected from Sequences 1-352;
- (ii) or comprises a DNA or RNA sequence encoding an amino acid sequences of (i).

In a preferred embodiment, the disclosure provides a method of stimulating the proliferation of human T-cells, comprising contacting said T-cells with the peptide or collection of peptides, the nucleic acid molecules, the vectors, the host cell, or the vaccine as disclosed herein.

In a preferred embodiment, the disclosure provides a storage facility for storing vaccines. Preferably the facility stores at least two different cancer vaccines as disclosed herein. Preferably the storing facility stores:
a vaccine comprising:

(i) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 1, an amino acid sequence having 90% identity to Sequence 1, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 1; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 2, an amino acid sequence having 90% identity to Sequence 2, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 2; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 3, an amino acid sequence having 90% identity to Sequence 3, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 3;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 4, an amino acid sequence having 90% identity to Sequence 4, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 4;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 5, an amino acid sequence having 90% identity to Sequence 5, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 5;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 6, an amino acid sequence having 90% identity to Sequence 6, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 6; and/or

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 7, an amino acid sequence having 90% identity to Sequence 7, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 7;

and one or more vaccines selected from:
a vaccine comprising:

(ii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 19, an amino acid sequence having 90% identity to Sequence 19, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 19; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 20, an amino acid sequence having 90% identity to Sequence 20, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 20;

a vaccine comprising:

(iii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 189, an amino acid sequence having 90% identity to Sequence 189, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 189; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from any one of Sequences 190-192, an amino acid sequence having 90% identity to Sequences 190-192, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 190-192; and/or

a vaccine comprising:

(iv) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 311, an amino acid sequence having 90% identity to Sequence 311, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 311; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 312, an amino acid sequence having 90% identity to Sequence 312, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 312; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 313, an amino acid sequence having 90% identity to Sequence 313, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 313.

In a preferred embodiment, the disclosure provides a method for providing a vaccine for immunizing a patient against a cancer in said patient comprising determining the sequence of BAP1, PBRM1, SETD2, and/or VHL in cancer cells of said cancer and when the determined sequence comprises a frameshift mutation that produces a neoantigen of Sequence 1-352 or a fragment thereof, providing a vaccine comprising said neoantigen or a fragment thereof. Preferably, the vaccine is obtained from a storage facility as disclosed herein.

REFERENCE TO A SEQUENCE LISTING

The Sequence listing, which is a part of the present disclosure, includes a text file comprising amino acid and/or nucleic acid sequences. The subject matter of the Sequence listing is incorporated herein by reference in its entirety. The information recorded in computer readable form is identical to the written sequence listing. In the event of a discrepancy between the Sequence listing and the description, e.g., in regard to a sequence or sequence numbering, the description (e.g., Table 1) is leading.

DETAILED DESCRIPTION OF THE DISCLOSED EMBODIMENTS

One issue that may arise when considering personalized cancer vaccines is that once a tumor from a patient has been analysed (e.g. by whole genome or exome sequencing), neoantigens need to be selected and made in a vaccine. This may be a time consuming process, while time is something the cancer patient usually lacks as the disease progresses.

Somatic mutations in cancer can result in neoantigens against which patients can be vaccinated. Unfortunately, the quest for tumor specific neoantigens has yielded no targets that are common to all tumors, yet foreign to healthy cells. Single base pair substitutions (SNVs) at best can alter 1 amino acid which can result in a neoantigen. However, with the exception of rare site-specific oncogenic driver mutations (such as RAS or BRAF) such mutations are private and thus not generalizable.

An "off-the-shelf" solution, where vaccines are available against each potential- neoantigen would be beneficial. The present disclosure is based on the surprising finding that, despite the fact that there are infinite possibilities for frame shift mutations in the human genome, a vaccine can be developed that targets the novel amino acid sequence following a frame shift mutation in a tumor with potential use in a large population of cancer patients.

Neoantigens resulting from frame shift mutations have been previously described as potential cancer vaccines. See, for example, WO95/32731, WO2016172722 (Nantomics), WO2016/187508 (Broad), WO2017/173321 (Neon Therapeutics), US2018340944 (University of Connecticut), and WO2019/012082 (Nouscom), as well as Rahma et al. (Journal of Translational Medicine 2010 8:8) which describes peptides resulting from frame shift mutations in the von Hippel–Lindau tumor suppressor gene (VHL) and Rajasagi et al. (Blood 2014 124(3):453-462) which reports the systematic identification of personal tumor specific neoantigens.

The present disclosure provides a unique set of sequences resulting from frame shift mutations and that are shared among kidney cancer patients. The finding of shared frame shift sequences is used to define an off-the-shelf kidney cancer vaccine that can be used for both therapeutic and prophylactic use in a large number of individuals.

In the present disclosure we provide a source of common neoantigens induced by frame shift mutations, based on analysis of 10,186 TCGA tumor samples and 2774 tumor samples (see Priestley et al. 2019 at

https://doi.org/10.1101/415133). We find that these frame shift mutations can produce long neoantigens. These neoantigens are typically new to the body, and can be highly immunogenic. The heterogeneity in the mutations that are found in tumors of different organs or tumors from a single organ in different individuals has always hampered the development of specific medicaments directed towards such mutations. The number of possible different tumorigenic mutations, even in a single gene as P53 was regarded prohibitive for the development of specific treatments. In the present disclosure it was found that many of the possible different frame shift mutations in a gene converge to the same small set of 3' neo open reading frame peptides (neopeptides or NOPs). We find a fixed set of only 1,244 neopeptides in as much as 30% of all TCGA cancer patients. For some tumor classes this is higher; e.g. for colon and cervical cancer, peptides derived from only ten genes (saturated at 90 peptides) can be applied to 39% of all patients. 50% of all TCGA patients can be targeted at saturation (using all those peptides in the library found more than once). A pre-fabricated library of vaccines (peptide, RNA or DNA) based on this set can provide off the shelf, quality certified, 'personalized' vaccines within hours, saving months of vaccine preparation. This is important for critically ill cancer patients with short average survival expectancy after diagnosis.

The concept of utilizing the immune system to battle cancer is very attractive and studied extensively. Indeed, neoantigens can result from somatic mutations, against which patients can be vaccinated1-11. Recent evidence suggests that frame shift mutations, that result in peptides which are completely new to the body, can be highly immunogenic12- 15. The immune response to neoantigen vaccination, including the possible predictive value of epitope selection has been studied in great detail8, 13, 16-21 and WO2007/101227, and there is no doubt about the promise of neoantigen-directed immunotherapy. Some approaches find subject-specific neoantigens based on alternative reading frames caused by errors in translation/ transcription (WO2004/111075). Others identify subject specific neoantigens based on mutational analysis of the subjects tumor that is to be treated (WO1999/058552; WO2011/143656; US20140170178; WO2016/187508; WO2017/173321). The quest for common antigens, however, has been disappointing, since virtually all mutations are private. For SNV-derived amino acid changes, one can derive algorithms that predict likely good epitopes, but still every case is different.

A change of one amino acid in an otherwise wild-type protein may or may not be immunogenic. The antigenicity depends on a number of factors including the degree of fit of the proteasome-produced peptides in the MHC and ultimately on the repertoire of the finite T-cell system of the patient. In regards to both of these points, novel peptide sequences resulting from a frame shift mutation (referred to

herein as novel open reading frames or pNOPs) are a priori expected to score much higher. For example, a fifty amino acid long novel open reading frame sequence is as foreign to the body as a viral antigen. In addition, novel open reading frames can be processed by the proteasome in many ways, thus increasing the chance of producing peptides that bind MHC molecules, and increasing the number of epitopes will be seen by T-cell in the body repertoire.

It is has been established that novel proteins/peptides can arise from frameshift mutations[32,36]. Furthermore, tumors with a high load of frameshift mutations (micro-satellite instable tumors) have a high density of tumor infiltrating CD8+ T cells[33]. In fact, it has been shown that neo-antigens derived from frameshift mutations can elicit cytotoxic T cell responses[32,34,33]. A recent study demonstrated that a high load of frameshift indels or other mutation types correlates with response to checkpoint inhibitors[35].

Binding affinity to MHC class-I molecules was systematically predicted for frameshift indel and point mutations derived neoantigens[35].Based on this analysis, neoantigens derived from frameshifts indels result in 3 times more high-affinity MHC binders compared to point mutation derived neoantigens, consistent with earlier work[31]. Almost all frameshift derived neoantigens are so-called mutant-specific binders, which means that cells with reactive T cell receptors for those frameshift neoantigens are (likely) not cleared by immune tolerance mechanisms[35]. These data are all in favour of neo-peptides from frameshift being superior antigens.

Here we report that frame shift mutations, which are also mostly unique among patients and tumors, nevertheless converge to neo open reading frame peptides (NOPs) from their translation products that surprisingly result in common neoantigens in large groups of cancer patients. The disclosure is based, in part, on the identification of common, tumor specific novel open reading frames resulting from frame shift mutations. Accordingly, the present disclosure provides novel tumor neoantigens and vaccines for the treatment of cancer. In some embodiments, multiple neoantigens corresponding to multiple NOPs can be combined, preferably within a single peptide or a nucleic acid molecule encoding such single peptide. This has the advantage that a large percentage of the patients can be treated with a single vaccine.

While not wishing to be bound by theory, the surprisingly high number of frame shift induced novel open reading frames shared by cancer patients can be explained, at least in part, as follows. Firstly, on the molecular level, different frame shift mutations can lead to the generation of shared novel open reading

frames (or sharing at least part of a novel open reading frame). Secondly, the data presented herein suggests that frame shift mutations are strong loss-of-function mutations. This is illustrated in figure 2A, where it can be seen that the SNVs in the TCGA database are clustered within the p53 gene, presumably because mutations elsewhere in the gene do not inactive gene function. In contrast, frame shift mutations occur throughout the p53 gene (figure 2B). This suggests that frame shift mutations virtually anywhere in the p53 ORF reduce function (splice variants possibly excluded), while not all point mutations in p53 are expected to reduce function. Finally, the process of tumorigenesis naturally selects for loss of function mutations in genes that may suppress tumorigenesis. Interestingly, the present disclosure identifies frame shift mutations in genes that were not previously known as classic tumor suppressors, or that apparently do so only in some tissue tumor types (see, e.g., figure 8). These three factors are likely to contribute to the surprisingly high number of frame shift induced novel open reading frames shared by cancer patients; in particular, while frame shift mutations generally represent less than 10% of the mutations in cancer cells, their contribution to neoantigens and potential as vaccines is much higher. The high immunogenic potential of peptides resulting from frameshifts is to a large part attributable to their unique sequence, which is not part of any native protein sequence in humans, and would therefore not be recognised as 'self' by the immune system, which would lead to immune tolerance effects. The high immunogenic potential of out-of-frame peptides has been demonstrated in several recent papers.

Neoantigens are antigens that have at least one alteration that makes them distinct from the corresponding wild-type, parental antigen, e.g., via mutation in a tumor cell. A neoantigen can include a polypeptide sequence or a nucleotide sequence

As used herein the term "ORF" refers to an open reading frame. As used herein the term "neoORF" is a tumor-specific ORF (i.e., neoantigen) arising from a frame shift mutation. Peptides arising from such neo ORFs are also referred to herein as neo open reading frame peptides (NOPs) and neoantigens.

A "frame shift mutation" is a mutation causing a change in the frame of the protein, for example as the consequence of an insertion or deletion mutation (other than insertion or deletion of 3 nucleotides, or multitudes thereof). Such frameshift mutations result in new amino acid sequences in the C-terminal part of the protein. These new amino acid sequences generally do not exist in the absence of the frameshift mutation and thus only exist in cells having the mutation (e.g., in tumor cells and pre-malignant progenitor cells).

Figures 3 and 4 and the data discussed above provide the answer to the question: how many cancer patients exhibit in their tumor a frame shift in region x or gene y of the genome. The patterns result from the summation of all cancer patients. The disclosure surprisingly demonstrates that within a single cancer type (i.e. kidney cancer), the fraction of patients with a frame shift in a subset of genes is much higher than the fractions identified when looking at all cancer patients. We find that careful analysis of the data shows that frame shift mutations in only 4 genes together are found in 27% of all kidney cancers.

Novel 3' neo open reading frame peptides (i.e., NOPs) of BAP1, PBRM1, SETD2, and VHL are depicted in table 1. The NOPs, are defined as the amino acid sequences encoded by the longest neo open reading frame sequence identified. Sequences of these NOPs are represented in table 1 as follows:

VHL: Sequences 1-18.
SETD2: Sequences 19-188.
PBRM1: Sequences 189-310.
BAP1: Sequences 311-352.

The most preferred neoantigens are VHL frameshift mutation peptides, followed by PBRM1 frameshift mutation peptides, followed by BAP1 frameshift mutation peptides, followed by SETD2 frameshift mutation peptides. The preference for individual neoantigens directly correlates with the frequency of their occurrence in kidney cancer patients, with VHL frameshift mutation peptides covering up to 23% of kidney cancer patients, PBRM1 frameshift mutation peptides covering up to 9,1% of kidney cancer patients, BAP1 frameshift mutation peptides covering up to 4.4% of kidney cancer patients, SETD2 frameshift mutation peptides covering up to 4.2% of kidney cancer patients. In preferred embodiments, collections are provided comprising PBRM1 frameshift mutation peptides and SETD2 frameshift mutation peptides. In preferred embodiments, collections are provided comprising PBRM1 frameshift mutation peptides and BAP1 frameshift mutation peptides. In preferred embodiments, collections are provided comprising SETD2 frameshift mutation peptides and BAP1 frameshift mutation peptides.

Table 1 Library of NOP sequences

Sequences of NOPs including the percentage of kidney cancer patients identified in the present study with each NOP. The sequences referred to herein correspond to the sequence numbering in the table below. Different predicted alternative splice forms are indicated as "alt splice x".

Sequence	PeptideID	gene	PeptideSeq	% Kidney Cancer Patients
1	pNOP42302 alt splice a	VHL	TRASPPRSSSAIAVRASCCPYGSTSTASRSPTQRCRLARAAASTATEVTFGSSEMQGHTMGFWLTKLNYLCHLSMLTDSLFLPISHCQCIL	6,18
2	pNOP279502	VHL	SSLRITGDWTSSGRSTKIWKTTQMCRKTWSG	3,64
3	pNOP29645 alt splice b	VHL	RRRRGGVGRRGVRPGRVRPGGTGRRGGDGGRAAAARAALGELARALPGHLLQSQSARRAARMAQLRRRAAALPNAAAWHGPPHPQLPS VYSERAMPPGCPEPSQA	3,45
4	pNOP30388 alt splice b	VHL	RRRRGGVGRRGVRPGRVRPGGTGRRGGDGGRAAAARAALGELARALPGHLLQSQSARRAARMAQLRRRAAALPNAAAWHGPPHPQLPR SPLALQRCRDTRWASG	3,45
5	pNOP140916 alt splice a	VHL	TRASPPRSSSAIAVRASCCPYGSTSTASRSPTQRCRLARAAASTATECIL	2,55
6	pNOP54515	VHL	ELQETGHRQVALRRSGRPPKCAERPGAADTGAHCTSTDGRLKISVETYTVSSQLLMVLMSLDLDTGLVPSLVSKCLILRVK	2,00
7	pNOP301515	VHL	RTAYFCQYHTASVYSERAMPPGCPEPSQA	1,27
8	pNOP700435	VHL	KSDASRLSGA	0,55
9	pNOP10058 alt splice b	VHL	RRRRGGVGRRGVRPGRVRPGGTGRRGGDGGRAAAARAALGELARALPGHLLQSQSARRAARMAQLRRRAAALPNAAAWHGPPHPQLPS PHDSSGPVLRSPCPRGEHIPPGETDRCKDRNKPGSCWRRKSRPCVAWEIDLPACWEMEGLRLCGFS	<0,1
10	pNOP128393	VHL	ARRRQASKSTALKKTAGRSRAPRSPARKSPARRNWAPRRRWRPGGRGPCCAR	<0,1
11	pNOP145743 alt splice a	VHL	TRASPPRSSSAIAVRASCCPYGSTSTASRSPTQRCRLARAAASTATESS	<0,1
12	pNOP265165	VHL	LTCLLGDGGVAVVWFQLRSTSRRRKRAGWSRN	<0,1
13	pNOP352529	VHL	NAPEGGELGRGRGRRGGGRRRRVRP	<0,1
14	pNOP369816	VHL	QMQRQEQARVMLAPEEPTVCGVGN	<0,1
15	pNOP472058	VHL	GALPGEGREQDGVGTSQP	<0,1
16	pNOP519384	VHL	HRSALHINGWEIEDFC	<0,1

17	pNOP614216	VHL	SLPSWRTHSSWGD	<0,1
18	pNOP622782	VHL	CPGGRRTGTRPR	<0,1
19	pNOP42654	SETD2	ETSASCNIQATAGYYHSSSISTYSCSTITSSDSRINNCRLTALISASTASTCPSHNTLITSTSNRASGLATYTNNSSNGSTSTLTSRCSS	1,27
20	pNOP36509	SETD2	CLGFKPTVCQCTAAVLSCTVSSNHILSRTDMSNSLWCDITLFTDNSTNCTELCPAKSSVYPGATDFHSSSTRSGGTASRSSDYNSCTRAASALAA I	0,55
21	pNOP127387	SETD2	SLGSAAPLSSQTKNHCLTSQLEDSSRSRREDLLLPCDHKADSVGSSYLGKPRR	0,36
22	pNOP169956	SETD2	KPKYLLGSKKSCQFILLILKMSQISLGNRPLSKTGQIVDWEKQN	0,36
23	pNOP102433	SETD2	YTKTPALLSCQCRPPAQFRDRIIVFGIQTNSLSVYSSSTLLHSLKQPYIIKDRHVQQSMV	0,18
24	pNOP163115	SETD2	FISTYWEFRYCFISSESSTRNKGGQSNSLEMRREHISSSGCSAKE	0,18
25	pNOP175535	SETD2	HHLIHRQLHQLYRVMPSQVFSISRGNRFSQLIHKEWWYSQPQQ	0,18
26	pNOP212518	SETD2	QIISWICRPPLLPNQKPLSYLPTGRQLEIQKGRFITTM	0,18
27	pNOP238211	SETD2	RLWNTCPFLLKICWRKAKYFQLFNAGLRLRLLSLR	0,18
28	pNOP244560	SETD2	KNLLMKKHHPKMKRRVCLMWRVKGAKNSQIKQWI	0,18
29	pNOP310885	SETD2	MSKQIFPQKKKIPILGRMKKFQIVLRLV	0,18
30	pNOP516328	SETD2	EVHAEVWGCLQTQRGH	0,18
31	pNOP91847	SETD2	LDRNYPLWSKHACFHQMDFRILVGAKKKTWMIPACCIRSQKAHLEKQNLWCHHTKINSCLCQL	0,18
32	pNOP129693	SETD2	IRVTVGPGWEDGWSGKTDSRERKSCGPFCPTPVSTVLLMIHHPGEFNPADVN	<0,1
33	pNOP140653	SETD2	SRERHQCPQVQHWLGPPVSWMTSGTHSDGRNVPSKGKCHVTLILLKKMFI	<0,1
34	pNOP149559	SETD2	QITQNLHVKNISRASVALVQLLLIILMIYINLLGVQVLLHLFRVFHQE	<0,1
35	pNOP151102	SETD2	VVTFLIQERDLLPGRTGVIMGYLGNVCKRLKKKGIPYCLKEEEDQKSL	<0,1
36	pNOP164732	SETD2	LSVPEIWKRSPEMFLRISQLPGLPGRRKQSQHQSSRRENEEGTIS	<0,1
37	pNOP172893 alt splice a	SETD2	TVSEKTACRCGSHTHRKERLGLESCQRPSFEHLCPRILWRGTRS	<0,1
38	pNOP18146	SETD2	RRYIEFQRKVKLKRKTQQLNEEGMLLASEIKHLPRRLLIGQERETQTSKLKIKRKGNEEAPSHHPLLPMSGEQKGQMTDMIHQLLKRKYELKTAI NFLQRNAGSCLSKRWLNGRLRNNSNRCRTWE	<0,1
39	pNOP189678	SETD2	NFGAETYLSGTHTEHTLTVLPEVLSGTSWAVFVVDLDGRAR	<0,1
40	pNOP191028	SETD2	RLTISSRDMEKKPRNVSADQPIAGVTWEEKTESASEQQEGK	<0,1

41	pNOP197618	SETD2	QMIVKKRRIGINRMDPIFQTSPINFFYPFRKTRGQCKHLK	<0,1
42	pNOP198489	SETD2	SCSALGGTFYRTPFCPSTSTSGATCGSSCGSFQFPVCGPE	<0,1
43	pNOP201541 alt splice b	SETD2	ELPKTFLQKKPRNVSADQPIAGVTWEEKTESASEQQEGK	<0,1
44	pNOP204603	SETD2	PLPPTPSYCWLLSALHLIFRKLSFLRAGMLLYSNLVSSP	<0,1
45	pNOP208142	SETD2	AVKKQVLRRNPHNLKASFLVQNLMKILYGLLQVKDHMI	<0,1
46	pNOP21038	SETD2	GNLHDLKQTEMINILAIQNLKEILGMYLPDVDQKESDGGADLTLGLREALELIYPIPGQNDLIIMTLIVATIGAPLIERGRAILGHTQITEHERVLT QKKSIRRHTQGVPHLIPLLTET	<0,1
47	pNOP213911	SETD2	SQGRLSGILLLGKAQEMMPALSMKLRWTWELQHMMKTP	<0,1
48	pNOP219165	SETD2	LNVLLGVQMGIIVPIDGFRENSMQMWKSYSQKRKAGA	<0,1
49	pNOP237811	SETD2	QYSKKPSPVCCGCAKEFYFAHGRNKSLFFSEQSKL	<0,1
50	pNOP244795	SETD2	KWKLVIPYLLQKNLPHQSQGWNWAKFILRNICFM	<0,1
51	pNOP261712	SETD2	DTTCGWLALIRRAQKLRLGLLSRKAFYHVSAT	<0,1
52	pNOP267031	SETD2	QVKPVLKLISFSFIKEQRRIRKFLLHSPVENK	<0,1
53	pNOP272114	SETD2	EGFKHTLTVLPEVLSGTSWAVFVVDLDGRAR	<0,1
54	pNOP27510 alt splice c	SETD2	SSCSRSRLRRWGISTTRSTRPLKKKKMRQRLKMCRKQVSSKDQCSKVLLLVDFCPKAPKQKLIWKNRDDRRCHSASALQRKLCRIGFSLHLAM KSKVILQTLQLYLFR	<0,1
55	pNOP277421	SETD2	QKERRINLIEILSECSVSVHLFLKMKELKVK	<0,1
56	pNOP292377	SETD2	YDSSHEKPDHCLLLHLIVGFFLPYTLYSGS	<0,1
57	pNOP311275	SETD2	NPKMDCERTTEGWVFYHQTGSFRLRVNV	<0,1
58	pNOP329486	SETD2	TSKEKQRSIQKRDVPVHRPVPEPLPET	<0,1
59	pNOP331645	SETD2	ARGGSTGGSETTATDAEPGNDITTAL	<0,1
60	pNOP33233	SETD2	HHHCPMTLLVIMPRIIPLLVTHQVIPCRPMWIPATLMLERCSCPHPAWTQCVLLLLMIMLSPWWDILQNPFLPLHQYQWCHMWQLLWKF PVPSMWPRVMV	<0,1
61	pNOP33646	SETD2	SVTVVVMPQKLCLQFMKIILALLKVQMMKVIQKIQIRMIAVFQETVSSLLWLCQRILLCPWKKQVLVLLGAVKVIDTILTIGKMRDWSQGDIC MRKNLKV	<0,1
62	pNOP338557	SETD2	NTKPRSTLRSTCRSLGLFTNPKRTLN	<0,1
63	pNOP340580	SETD2	RKRKNCSHNSYSHNSCLNAKLIVKPT	<0,1

64	pNOP342316 alt splice c	SETD2	SSCSRSRLRRWGISTTRSTRPLSFLG	<0,1
65	pNOP343672	SETD2	VKEMGILVRIHRVLIHHSTHLILPPS	<0,1
66	pNOP357840	SETD2	TLTGNLTAKWEELPQLKTLNIWLAS	<0,1
67	pNOP358911	SETD2	WCSTPRLQRCCLASAGPRPSSGTEL	<0,1
68	pNOP359043	SETD2	WLELKLWSRNLPVWNSYRTHTHSPA	<0,1
69	pNOP360928	SETD2	CPASSLCWLPTRLSHAGLCGSQQP	<0,1
70	pNOP362588	SETD2	FGHQTPGQLERPKGGISNSKEKSN	<0,1
71	pNOP371897	SETD2	SGGRAGRDISAPRFSTGWALLCHG	<0,1
72	pNOP372037	SETD2	SIRKCPCRGRGRIAVTTATPTTAA	<0,1
73	pNOP373154	SETD2	THRPHLRLHRLHLPKPQHSHHQHQ	<0,1
74	pNOP376360	SETD2	CDWIGIIPFGQSMHAFIKWISEY	<0,1
75	pNOP377069	SETD2	DEAAAAAAASEDGGFLRPGAPDP	<0,1
76	pNOP377612	SETD2	EDILKAYLISFLFLQRPKDIILF	<0,1
77	pNOP383516	SETD2	MKEEKEDMCILLMTQKLYFLLVI	<0,1
78	pNOP394982	SETD2	FGRTGTTEGVIQLQPYKENFAE	<0,1
79	pNOP397465	SETD2	IIACLNFLILRVMGLIVQVIQM	<0,1
80	pNOP399481	SETD2	LKNQLIRGFLAAKPKIQTYTVL	<0,1
81	pNOP407617	SETD2	VFLPLVRYHMWMACTHQKSSET	<0,1
82	pNOP409009	SETD2	YLIYCRRIFPTKVKGGIGQNSF	<0,1
83	pNOP418203	SETD2	MDLLGQNLIWQHFALLKLMLF	<0,1
84	pNOP418296	SETD2	MKKSNSLILLVYRHLVQKVVN	<0,1
85	pNOP424480	SETD2	SSSEGVCTKQKHPLLFHGPEE	<0,1
86	pNOP429517	SETD2	CWKGAPAHTQHGPSVFSCSL	<0,1
87	pNOP429922	SETD2	DLLLRDGKKRQVFFKTRKRI	<0,1
88	pNOP432391	SETD2	GQGCCFIQIWSPALSTRRRK	<0,1

89	pNOP436619	SETD2	LQGPTAMEGMCQARENAMLL	<0,1
90	pNOP437076	SETD2	LWSLWWTQVSAKCRTVWWDT	<0,1
91	pNOP44117	SETD2	MEIEKKKMRQRLKMCRKQVSSKDQCSKVLLLVDFCPKAPKQKLIWKNRDDRRCHSASALQRKLCRIGFSLHLAMKSKVILQTLQLYLFR	<0,1
92	pNOP443088	SETD2	SIKKFLSHRIKYKWISWGRI	<0,1
93	pNOP460543	SETD2	QRKAKKYSEKRCPSSSSSA	<0,1
94	pNOP462663	SETD2	RVILKVMVSFRTERKLEWR	<0,1
95	pNOP46371	SETD2	HKKKKMRQRLKMCRKQVSSKDQCSKVLLLVDFCPKAPKQKLIWKNRDDRRCHSASALQRKLCRIGFSLHLAMKSKVILQTLQLYLFR	<0,1
96	pNOP464819	SETD2	TIATLLCVTLKLKILSLQL	<0,1
97	pNOP467110	SETD2	WVIWEMFARGSRRREFHIA	<0,1
98	pNOP467219	SETD2	YEGFLLQNQRFRHILYFER	<0,1
99	pNOP470113	SETD2	DNRCHSKRKLLSFHESQL	<0,1
100	pNOP472466	SETD2	GHHPILNLIGTVKLRPLT	<0,1
101	pNOP474911	SETD2	ISERTTNYNCTRIFRSRY	<0,1
102	pNOP475195	SETD2	KCAENRFHQRTNVQRCCF	<0,1
103	pNOP475627	SETD2	KKFRVFKACRERNNSRSR	<0,1
104	pNOP475977	SETD2	KPHEGLEKAQDSRSRHLQ	<0,1
105	pNOP476615	SETD2	KYVGGKQSTSNYSTLVSD	<0,1
106	pNOP477103	SETD2	LIKILKEKRLGLQLHQID	<0,1
107	pNOP478859	SETD2	NFQQALKRKEILKRAQHL	<0,1
108	pNOP479862	SETD2	PEASGRDYKDFGTLAHSY	<0,1
109	pNOP483413	SETD2	RRGELGSTGWIPFFRPVR	<0,1
110	pNOP485089	SETD2	SPFWNVMGCLCCGSGWQS	<0,1
111	pNOP486790	SETD2	TRKGCCWLQRSNTCPEDS	<0,1
112	pNOP488264	SETD2	WKMVRVSLIKTRCSAYPG	<0,1
113	pNOP497968	SETD2	KKMISKIEGLLKKGGRK	<0,1

114	pNOP503210	SETD2	PSGSPKFRGNRESQNTF	<0,1
115	pNOP506077 alt splice d	SETD2	RRNDLVRRIQTHTHSPA	<0,1
116	pNOP513180	SETD2	ASRYFLKKRRFPYWEG	<0,1
117	pNOP515847	SETD2	ELPLSREDALFSAIHR	<0,1
118	pNOP519358	SETD2	HRHSQETNVSQTENYK	<0,1
119	pNOP528494	SETD2	REKETKKLPLTTLFCL	<0,1
120	pNOP529595	SETD2	RPRKSPRQQKQTPPVN	<0,1
121	pNOP533091	SETD2	THVYASYDCGLFQNSS	<0,1
122	pNOP540029	SETD2	EGFSGWRARSSDGKW	<0,1
123	pNOP542259	SETD2	GLGFLPPNWFLQAQS	<0,1
124	pNOP545135	SETD2	ISSIPSERQGVSAST	<0,1
125	pNOP546281	SETD2	KLVSYLHLNQKLTLK	<0,1
126	pNOP547748	SETD2	LHQGSLSPCSHLKWL	<0,1
127	pNOP549104	SETD2	MCIWNQNQLYVIVEI	<0,1
128	pNOP550126	SETD2	NIVERYSIIKSLKLE	<0,1
129	pNOP550494	SETD2	NRAQREQRHKTRRTY	<0,1
130	pNOP555604	SETD2	RSMHETKTSITISWP	<0,1
131	pNOP560211	SETD2	VKIAWTVQSLMQPVS	<0,1
132	pNOP562314	SETD2	YSKPSSCTSSGRLDS	<0,1
133	pNOP571897	SETD2	ITAVNQIVKPKNGL	<0,1
134	pNOP573430	SETD2	KRKGWVSSSIKSIK	<0,1
135	pNOP582365	SETD2	RNREESGNFFYTVQ	<0,1
136	pNOP582849 alt splice d	SETD2	RRNDLVRRIQWMES	<0,1
137	pNOP585794	SETD2	STVRILRTWSAMRM	<0,1

138	pNOP588522	SETD2	VTAGKVTRSFRKRL		<0,1
139	pNOP603675	SETD2	LLARQWLLGSKIR		<0,1
140	pNOP604883	SETD2	LYECAFGIKTSYM		<0,1
141	pNOP605041	SETD2	MFFSVSKWGLLFQ		<0,1
142	pNOP605550	SETD2	NAERTHLQFWMQC		<0,1
143	pNOP606242	SETD2	NLTLWVTQIQRKP		<0,1
144	pNOP613396	SETD2	SDCWARVGGWVVR		<0,1
145	pNOP617878	SETD2	VIWPPNSWTVGKT		<0,1
146	pNOP625134 alt splice b	SETD2	ELPKTFLRTPLS		<0,1
147	pNOP625852	SETD2	EYIACSYTTQHT		<0,1
148	pNOP626121	SETD2	FILFQVRTISLL		<0,1
149	pNOP626457	SETD2	FRKGICFQGEQA		<0,1
150	pNOP629408	SETD2	HLHLLMQHHYQQ		<0,1
151	pNOP630920	SETD2	IRKRATAEQISL		<0,1
152	pNOP632708	SETD2	KQILSRTLYLIV		<0,1
153	pNOP641668	SETD2	RFCTDFFKSKIT		<0,1
154	pNOP645655	SETD2	SNSRTSTRFPNR		<0,1
155	pNOP647445	SETD2	TGKNRIEFFFLL		<0,1
156	pNOP648378	SETD2	TSGSQADSRCYE		<0,1
157	pNOP649199	SETD2	VKRERPRQANSK		<0,1
158	pNOP649226	SETD2	VLATDHFPKQAR		<0,1
159	pNOP652844	SETD2	AQKLTQTLPRN		<0,1
160	pNOP661751	SETD2	HPGHCWLLPQQ		<0,1
161	pNOP662108	SETD2	HVGKIVLIVFS		<0,1
162	pNOP663661	SETD2	KARMAKRILIN		<0,1

163	pNOP663940	SETD2	KERFFKKLRKK	<0,1
164	pNOP665029	SETD2	KPGAQLIPANG	<0,1
165	pNOP668907	SETD2	MPLKKEIALVS	<0,1
166	pNOP675389	SETD2	RKCLFNRKKEE	<0,1
167	pNOP676055	SETD2	RNRTSGVTTPR	<0,1
168	pNOP677462	SETD2	RWKEEASILQN	<0,1
169	pNOP678458	SETD2	SIFAQRHQNKS	<0,1
170	pNOP684868	SETD2	WSCPRNCVYSS	<0,1
171	pNOP686009	SETD2	AARRAANLFF	<0,1
172	pNOP687273	SETD2	ARRQGWQRGS	<0,1
173	pNOP690677	SETD2	ECETQNQGVH	<0,1
174	pNOP692910	SETD2	FRRTRNSNIE	<0,1
175	pNOP696399	SETD2	HMIAMIVLRN	<0,1
176	pNOP698375	SETD2	IYPFKTYTQV	<0,1
177	pNOP698819	SETD2	KFDRSLKICM	<0,1
178	pNOP699449	SETD2	KKRKTRNLFR	<0,1
179	pNOP700367	SETD2	KRRRRTCAYF	<0,1
180	pNOP700416	SETD2	KRYSVCIFPM	<0,1
181	pNOP704029	SETD2	MNCLFLSPNL	<0,1
182	pNOP709667	SETD2	QSQWPCHIHQ	<0,1
183	pNOP71440	SETD2	VGTSLKKSLLPRISNLTVAMELVVDTSISKMQNSMVGHVITGKAMVTGIQDQVDLLELGLCMIELKDKYQIP	<0,1
184	pNOP717155	SETD2	TFYRGTPEVV	<0,1
185	pNOP717203	SETD2	TGIPTRVIIF	<0,1
186	pNOP80237 alt splice a	SETD2	TVSEKTACRCGSHTHRKERLGLESCQRPSFKRSPEMFLRISQLPGLPGRRKQSQHQSSRRENEEGTIS	<0,1
187	pNOP85768	SETD2	CSKVLLLVDFCPKAPKQKLIWKNRDDRRCHSASALQRKLCRIGFSLHLAMKSKVILQTLQLYLFR	<0,1

188	pNOP91732	SETD2	KENLKGLQKMKQLKDVVLPLMNWDSDEGHHILSMTVVLPVINLPFQNLYPSLINLKILSVVQN	<0,1
189	pNOP100723	PBRM1	LLTSSFFLTMQSPIISQILLNIKPLANSGICTFEQEMSLFRKEKQMTKMMMKMGKTIRAQ	1,82
190	pNOP136779	PBRM1	WVAIRQAFHLCRAQLMALLAWAACSHFTLGGLHPTIFRQVCLASRASHHRV	0,55
191	pNOP152717	PBRM1	GLQHQVEVHMDNRWEFWGLQGSRHHLHIPAHIQLDPLSYSSQQHPCL	0,55
192	pNOP18284	PBRM1	ASVWSCLSRNTLSYAQKTSEMRMFLSVNHGILPKPNLLRKLNCGPCPSAQSGLSLGMCLCLWFAWPLYLQMQIKVMMRRIQTTQRTVELKT ILTWKRKKKMSLWKCPMVNQVATTLSSSITMTCG	0,55
193	pNOP108150	PBRM1	RLATVSSSSPMAWCVLVWAELKKYGFEMELHIFMAPSSFTQKKQSMSPQKCSTKKKYF	0,36
194	pNOP143190	PBRM1	ILLPCAMNSIIPSETIRMNRADFSVSSSLGHQSEEINQTIMKWFLSPLT	0,36
195	pNOP146830	PBRM1	CSGMPGTIMRRAPRFIMMHISWRSYSRRKGKSWAHCLMMMTWLLPNSS	0,36
196	pNOP245326 alt splice a	PBRM1	LRPNTQTTLSGSSAAWWGQNGEILRQPRKQNMKA	0,36
197	pNOP297770	PBRM1	LGRMLKIIATVGLIYFKSICLKYWNEQEG	0,36
198	pNOP302582	PBRM1	STKMLLFYTKSCLKHAETWREMRTLMSQM	0,36
199	pNOP323570	PBRM1	LQIHQDVSLANFFRNCLLKCNIQIIMQ	0,36
200	pNOP46601 alt splice b	PBRM1	NEKKKKEKLKRVKIPLVLQASQAYIAHTARTVALKTACTMLEITSMWNLQRPTYNHISSVLKDCGRIQLKKKFLRVTITTKFQLVKF	0,36
201	pNOP139958	PBRM1	QVLHTVKAALVKREIPLASITVIKEQYKEVVYQQLQWHFNMAQKVKKMLL	0,18
202	pNOP154131	PBRM1	NFSSFLLKFVMNSAKMERFFFHRHSAIPQNICIMMWRKRERKNCQKK	0,18
203	pNOP188417	PBRM1	HVFSESVLCCHSRTSSPAGQLKYQKMTFCFVRAATMRATSR	0,18
204	pNOP264907	PBRM1	LNLFTICLQRKNTLIITSKLKCPYHYNRSEQN	0,18
205	pNOP303604	PBRM1	VIKEQYKEVVYQQLQWHFNMAQKVKKMLL	0,18
206	pNOP324121	PBRM1	MMKFTTSENQLFLRRSHHLCWKRRSSC	0,18
207	pNOP367819	PBRM1	NCSKLCRQRRKSLPGETISRTETA	0,18
208	pNOP396093	PBRM1	GPTPRLLFRGAQPPGGDRMEKS	0,18
209	pNOP504975	PBRM1	RGNDRRHEADVPECQAL	0,18
210	pNOP520755	PBRM1	KEHKKAANENLIQCCS	0,18
211	pNOP547617	PBRM1	LGKGKRRCPCGNVQW	0,18

212	pNOP548688	PBRM1	LSITSAMTNMLVKRE	0,18
213	pNOP592499	PBRM1	CLIMPVHTMSRSL	0,18
214	pNOP613848	PBRM1	SHSCSYKSIRTSH	0,18
215	pNOP83912	PBRM1	KMFNHIITFLLYRSIKSCGSVAILVTGGGGKEQRRIIFIAFYCTSQGHFYIRTLLISYFNLFVILS	0,18
216	pNOP150133	PBRM1	RSWIPWVPREEELPPLPAVSAGTLMMGTILCQHQAQAGKGGDFPIFQL	<0,1
217	pNOP155811	PBRM1	SWIPWVPREEELPPLPAVSAGTLMMGTILCQHQAQAGKGGDFPIFQL	<0,1
218	pNOP170046	PBRM1	KSYSMLFLKLESQVQAEDFVTYLWLNHPKRTILIIIKSSWSQWT	<0,1
219	pNOP179578	PBRM1	WVPREEELPPLPAVSAGTLMMGTILCQHQAQAGKGGDFPIFQL	<0,1
220	pNOP180157	PBRM1	CAQFSHLQASSKIAASYAGKEERACQERRYRGRRQHDLFSHL	<0,1
221	pNOP18247 alt splice c	PBRM1	AAGERASSTATAAECFSPSRHPCGGSHGGGATTNTNGDAQSAVDTCCRCDEPRSGPYGRDSSTRWKSIWTTGGSFGASRAAGTTSISRPTSS WTPCHTAANNTHVCSSPTKDPAASSLRGLPEIH	<0,1
222	pNOP189150	PBRM1	LLHAMKRESQKQKASLPLWMFQILFISFMTQLGVVGITKGS	<0,1
223	pNOP189879	PBRM1	NTLKDSVRSPTALASGIRHWQLEDATSICRKNRRAAYPLTG	<0,1
224	pNOP196761 alt splice d	PBRM1	NEETGFSRRWLCSGKAEEVGFHGFQEKKSYLPFQQCQRGL	<0,1
225	pNOP200354	PBRM1	AGPHALHTPTVYPKVCQRQCKEGRLQTENQHEWLHPVQQ	<0,1
226	pNOP217261	PBRM1	GAYRSQDHCPEDTEWKLQKYSCNGQRYRSPRKKCQNL	<0,1
227	pNOP219611 alt splice d	PBRM1	NEETGFSRRWLCSEEVGFHGFQEKKSYLPFQQCQRGL	<0,1
228	pNOP227023 alt splice e	PBRM1	KVEMMILKRWEKKIVSLPQSLPKAVQRRKAPNGKST	<0,1
229	pNOP227832 alt splice d	PBRM1	NEETGFSRRWLCSEVGFHGFQEKKSYLPFQQCQRGL	<0,1
230	pNOP245327 alt splice a	PBRM1	LRPNTQTTLSGSSAAWWGQNGEILRQPRKQNMKV	<0,1
231	pNOP258302 alt splice f	PBRM1	SLQPPLILVVPKEKGTLMTVRCWVSGGYPVKRT	<0,1
232	pNOP27190	PBRM1	HGQHAATSPWGASTPPSSARCAWPPGHPTTGCDEPRSGPYGRDSSTRWKSIWTTGGSFGASRAAGTTSISRPTSSWTPCHTAANNTHVCS SPTKDPAASSLRGLPEIH	<0,1
233	pNOP280248	PBRM1	TVDHAHQLSQVCPSGCASACGSRGLCICKCR	<0,1

234	pNOP291935	PBRM1	WAPFCVNTRPKQEKEETFQSSNCRSYCRVP	<0,1
235	pNOP296775	PBRM1	ISSQKMPKLIMSLALKYSRMQIQLKKYFI	<0,1
236	pNOP303912	PBRM1	VYPKVCQRQCKEGRLQTENQHEWLHPVQQ	<0,1
237	pNOP314359	PBRM1	SGRNLPHDMYSRKVCCVVIQGLPLLQAN	<0,1
238	pNOP320508	PBRM1	GLSCPKCDFADSRAYPQSFCVSHESSG	<0,1
239	pNOP324586	PBRM1	NLLLQKTNCSSEGAITFAGKEDPVARS	<0,1
240	pNOP336473	PBRM1	KQHVPCWRLRLCGTCRGQPTTTYRLY	<0,1
241	pNOP364497	PBRM1	HVAEGWRLCLHQVPWPGASSCGQN	<0,1
242	pNOP365921	PBRM1	KSMGSRWSCIFLWPHLHSPRRNRA	<0,1
243	pNOP370708	PBRM1	RKSTFFSTLPVFSSLLEGDPGAAS	<0,1
244	pNOP380983	PBRM1	ISRPLPRGYRMEATKVFMQWPKI	<0,1
245	pNOP386577	PBRM1	RGRVRSRKHHFLYGCFKSFLSAL	<0,1
246	pNOP39757 alt splice b	PBRM1	NEKKKKEKLKRVKIPLVLQASQAYIAHTARTVALKTACTMLEITSMWNLQRPTYNHISSVLKDCGRIQLVKNGCMAVGFTDQMKHSTWLHE NF	<0,1
247	pNOP407746	PBRM1	VKSSNEDSIQLGCSQTDRSFTQ	<0,1
248	pNOP40838 alt splice b	PBRM1	NEKKKKELKRVKIPLVLQASQAYIAHTARTVALKTACTMLEITSMWNLQRPTYNHISSVLKDCGRIQLVKNGCMAVGFTDQMKHSTWLHENF	<0,1
249	pNOP416668	PBRM1	KSSTRRSFISNYNGTSIWLRK	<0,1
250	pNOP425265	PBRM1	TLQKWRDSSFTGTQLYHKTFA	<0,1
251	pNOP434493	PBRM1	KAHGHGKNSKSHDGQQVPRY	<0,1
252	pNOP448870	PBRM1	CLKMPNAIMCPIQPSTSEF	<0,1
253	pNOP449322	PBRM1	CTYPGEVTQGEKERAGPTA	<0,1
254	pNOP452067	PBRM1	GKMLQRFFSRNSCCGSQLS	<0,1
255	pNOP453139	PBRM1	HGFQEKKSYLPFQQCQRGL	<0,1
256	pNOP460219	PBRM1	QKFLLWIPTFLTNHPLHLT	<0,1
257	pNOP461097	PBRM1	RFLWCCRPLRLTSHIQPGL	<0,1

258	pNOP471164	PBRM1	ERQADEEIQRIEEVFTLC		<0,1
259	pNOP475150	PBRM1	KAKGPTPPWQMPSGAFEI		<0,1
260	pNOP475643	PBRM1	KKKFLRVTIITKFQLVKF		<0,1
261	pNOP484932 alt splice f	PBRM1	SLQPPLILVVPKEKVKRT		<0,1
262	pNOP489695	PBRM1	AGVFDLQRCSCSTQSPA		<0,1
263	pNOP499515	PBRM1	LKEKYFFLHITSLLQLT		<0,1
264	pNOP501126	PBRM1	NAHITTTDPNKTEESRI		<0,1
265	pNOP505051	PBRM1	RHDGWLSARPSTFAGPS		<0,1
266	pNOP513619	PBRM1	CGEREKGKIAKRNRGR		<0,1
267	pNOP51984 alt splice a	PBRM1	LRPNTQTTLSGSSAAWWGQNGEILRQPRKQNMKSGQLKLLSSRRESEQHSNSSRVLLPEQAPLWGLSWGWCHHQHQWGCSIS		<0,1
268	pNOP521733	PBRM1	KRLKLNIMKWLSQVFE		<0,1
269	pNOP531143	PBRM1	SIGTSKKDESDRFRNI		<0,1
270	pNOP535461	PBRM1	WPTSTKILTLWLRTLS		<0,1
271	pNOP548322	PBRM1	LPHQRPSGFFTQRPT		<0,1
272	pNOP550107	PBRM1	NIPPGYTKISRKRSF		<0,1
273	pNOP554243	PBRM1	RIFSSLLEGDPGAAS		<0,1
274	pNOP555796	PBRM1	RTILIRGVAASVPYF		<0,1
275	pNOP566375	PBRM1	EGSPPQCHISEASL		<0,1
276	pNOP574911	PBRM1	LLKSPWTWKKFEVT		<0,1
277	pNOP577711	PBRM1	NSRQVCGHVCQGIL		<0,1
278	pNOP585233	PBRM1	SRLQTLGFVPSNKK		<0,1
279	pNOP590879	PBRM1	AHKNVLQKRSISE		<0,1
280	pNOP592931	PBRM1	CSGTPSTFAKHTT		<0,1
281	pNOP60025	PBRM1	RCDEPRSGPYGRDSSTRWKSIWTTGGSFGASRAAGTTSISRPTSSWTPCHTAANNTHVCSSPTKDPAASSLRGLPEIH		<0,1

282	pNOP601294	PBRM1	KIKIHDSNAAETK		<0,1
283	pNOP603087	PBRM1	LDENPTETKNGRV		<0,1
284	pNOP617063	PBRM1	TSFSTSASDPPGQ		<0,1
285	pNOP617777	PBRM1	VGRVAFLLKNQNT		<0,1
286	pNOP617867	PBRM1	VIRMMREDATAIL		<0,1
287	pNOP622052	PBRM1	AWLSSIQGCKFN		<0,1
288	pNOP626183	PBRM1	FKSLSTIFLCQS		<0,1
289	pNOP626974 alt splice g	PBRM1	GDWLFEALVVLW		<0,1
290	pNOP627099	PBRM1	GFPLDLSCLTTI		<0,1
291	pNOP632437	PBRM1	KMVVWLLVLPTK		<0,1
292	pNOP63453	PBRM1	QVGSDTGSSKTRRPFVERTGEPPTLSLAEKQRGPHHHGRCPLAPSRFDAPGHPQHSPSIQPRKCLITSLRFFYIEA		<0,1
293	pNOP647364	PBRM1	TFLPFAFKEKIP		<0,1
294	pNOP656432	PBRM1	EEYRQLRGQSS		<0,1
295	pNOP659059 alt splice g	PBRM1	GDWLFEALVVL		<0,1
296	pNOP660715	PBRM1	GTKAKKSTRLL		<0,1
297	pNOP662459	PBRM1	IGQIQKYMKMQ		<0,1
298	pNOP669764	PBRM1	NHLGANGLENN		<0,1
299	pNOP679970	PBRM1	SSRARFRQKTL		<0,1
300	pNOP694253	PBRM1	GLHPTWLQPD		<0,1
301	pNOP698245	PBRM1	ITVFCQNQIF		<0,1
302	pNOP700951	PBRM1	KWKSMMMLIC		<0,1
303	pNOP710206	PBRM1	RCSRTSAVFY		<0,1
304	pNOP712256	PBRM1	RRCCFSCCTL		<0,1
305	pNOP712598	PBRM1	RRSWSSFLKP		<0,1

306	pNOP713384	PBRM1	RWARQSGHSD		<0,1
307	pNOP713614	PBRM1	SAISRLLCNN		<0,1
308	pNOP717507	PBRM1	TKTRRRKKRS		<0,1
309	pNOP88057 alt splice c	PBRM1	AAGERASSTATAAECFSPSRHPCGGSHGGGATTNTNGDAQSAVDTCCRHDGWLSARPSTFAGPS		<0,1
310	pNOP91822 alt splice e	PBRM1	KVEMMILKRWEKKIVRSLNLLLYLSFRPPWPVSWTSCPTHPHSLPQSLPKAVQRRKAPNGKST		<0,1
311	pNOP13160	BAP1	AVLLMGNCQCCSPTPSTSWLRSSKSPRRTSQFLCPSRLAAGLGVRLWQCPHTRSPHPPPAMRVQTRPLRSAVLSTRHCARLSAQPTRRGPPA LSPPTSPRCFLERMTACCVLTAYATTVLSVIWVLSSAQACCTWLRMGC		1,09
312	pNOP179558	BAP1	WSRTSPCGGAKGSASAGSTSSGSLTGGNALAPTRPSASEDCWP		0,73
313	pNOP403156	BAP1	QRGWESGCGSAHTLAALTHPQQ		0,55
314	pNOP185542	BAP1	VHLHLYLHAGSGRHAGQPSGAEHLRAAAPRGQHRPAPQAAEA		0,36
315	pNOP82605	BAP1	TITIMPSPPCRRKKTWRQVWAAAEFQSAHPSSTQMMRMTMRMTRRMTCRTPTLPLGIRGREQGSQGH		0,36
316	pNOP144488 alt splice a	BAP1	PWALGGGRGVDRQGPAGHHGAYRPRHCRGALPRHPLQPDGSGARPQDQV		0,18
317	pNOP372160	BAP1	SLQAAASMGFTPTPLPLSSGCRPF		0,18
318	pNOP396500	BAP1	GVQVSQQQVPAGAGSKQGPCSL		0,18
319	pNOP42739	BAP1	GQPHRWCRGGGWFMRTSPIPQPSQQTQASGEASRQQPQWGSPQPHSHCPAAAGLSRQSQLCQVPHAGGRRPGGRCGPQPSSSPPTPA VLR		0,18
320	pNOP492413	BAP1	EAQRVPEGPLNSSVHQD		0,18
321	pNOP64587 alt splice b	BAP1	GWGAESPGADRGWEGFLALHQTNPRQPGVQQPSGEGGRGSHGQQREDGDGEAWRALEWGEILTQGAAGTAEVCGG		0,18
322	pNOP91275	BAP1	FRPTSLKSHSCLRSPSQPATSPRWCWKQTGPLQPLRATTQMVQRRRLVHAHKPHPTALPTNPS		0,18
323	pNOP100199 alt splice b	BAP1	GWGAESPGADRGWEGFLALHQTNPRQPGVQQPSGEGGRGSHGQQREDGDGAAGTAEVCGG		<0,1
324	pNOP159625	BAP1	PAETLSPLQGQAPVRTAGPDSAAHSCRVALTRVLPCPTSPFPSITE		<0,1
325	pNOP163874	BAP1	HVLCPPADTQLLCNSCLAERAPELQQRGPGTHPESHEGLHQGFQP		<0,1
326	pNOP165665 alt splice c	BAP1	QRVGRVPRPPSDQSKAARGPAAQWRRRSWKPRTAERRRGWSCWHC		<0,1
327	pNOP178195 alt splice d	BAP1	RSTPLTMGPGGRTRSGQTRPGGSSWSVSASPLQGSPTTTSAST		<0,1

328	pNOP178196 alt splice d	BAP1	RSTPLTMGPGGRTRSGQTRPGGSSWSVSASPLQGSSMRPGCMC	<0,1
329	pNOP194903	BAP1	GQAACAEGEPSDSTRGSAAADKSNTARADSDPQVSRVTAA	<0,1
330	pNOP197712 alt splice c	BAP1	QRVGRVPRPPSDQSKAARGPAAQWRRRSWKPRTAERRRGW	<0,1
331	pNOP216613	BAP1	DRQCFQLATALAYPLSQPDAALQPCHLPHLQGAFWRG	<0,1
332	pNOP229607	BAP1	RRGRSSRLMTREGPTTTMSSSAPLSPCWLRKACWPT	<0,1
333	pNOP231133 alt splice e	BAP1	VGRNTHPSCLLLVGLCCWPAPIRGAMLGFGRSCWHC	<0,1
334	pNOP277235 alt splice a	BAP1	PWALGGGRGVDRQGPAGHHGAYRPRHCRDQV	<0,1
335	pNOP34900 alt splice b	BAP1	GWGAESPGADRGWEGFLALHQTNPRQPGVQQPSGEGGRGSHGQQREDGDGEAWRALEWGEILTQAAYCSWGFVAGPPRSEVQCWVL AGAAGTAEVCGG	<0,1
336	pNOP410821	BAP1	CSADHGGVPLCQLCAYHRPAL	<0,1
337	pNOP508542	BAP1	SVWRLRLQTMRRASRRR	<0,1
338	pNOP511363	BAP1	WQWCPTAGSSMRPGCMC	<0,1
339	pNOP516062	BAP1	EQRICDWQCPGVGQGP	<0,1
340	pNOP51715 alt splice b	BAP1	GWGAESPGADRGWEGFLALHQTNPRQPGVQQPSGEGGRGSHGQQREDGDAAYCSWGFVAGPPRSEVQCWVLAGAAGTAEVCGG	<0,1
341	pNOP543182	BAP1	GVPQGGGREEEEVQD	<0,1
342	pNOP560984	BAP1	WATVSAAAQHHQRLG	<0,1
343	pNOP568350	BAP1	GEGNREARGIERFC	<0,1
344	pNOP613742	BAP1	SGSCHQHRPAAPG	<0,1
345	pNOP617773 alt splice e	BAP1	VGRNTHPRSCWHC	<0,1
346	pNOP706421	BAP1	PCQARATPPP	<0,1
347	pNOP716714	BAP1	TAAAWTWDPP	<0,1
348	pNOP722094	BAP1	YPTLVQLMPC	<0,1
349	pNOP75322	BAP1	RTSPRVSALRAKDMRLAMPRSWPRPIIAMPGPSHATSLRSRMALVQCGPWRRSTLSAMCLSQAGSLSWMG	<0,1

350	pNOP76739	BAP1	IRAGWSWRATQASSPCSWKISVSRGCKWRRSTTFRANVRALYMDLSSCSNGSKSAGPGERSLPWWMIRP	<0,1
351	pNOP79648 alt splice c	BAP1	QRVGRVPRPPSDQSKAARGPAAQWRRRSWKPRTAERRRGCCLLLVGLCCWPAPIRGAMLGFGRSCWHC	<0,1
352	pNOP88716	BAP1	GLAGAGERPRPLHPARGRFRCQGGASGGDLRPSEQMSGPCIWIYLPVQMDRRAPVPAKGLYLGG	<0,1

In a preferred embodiment the disclosure provides one or more frameshift-mutation peptides (also referred to herein as 'neoantigens') comprising an amino acid sequence selected from the groups:

(i) Sequences 1-18, an amino acid sequence having 90% identity to Sequences 1-18, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 1-18;

(ii) Sequences 19-188, an amino acid sequence having 90% identity to Sequences 19-188, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 19-188;

(iii) Sequences 189-310, an amino acid sequence having 90% identity to Sequences 189-310, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 189-310; and

(iv) Sequences 311-352, an amino acid sequence having 90% identity to Sequences 311-352, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 311-352.

As will be clear to a skilled person, the preferred amino acid sequences may also be provided as a collection of tiled sequences, wherein such a collection comprises two or more peptides that have an overlapping sequence. Such 'tiled' peptides have the advantage that several peptides can be easily synthetically produced, while still covering a large portion of the NOP. In an exemplary embodiment, a collection comprising at least 3, 4, 5, 6, 10, or more tiled peptides each having between 10-50, preferably 12-45, more preferably 15-35 amino acids, is provided. As described further herein, such tiled peptides are preferably directed to the C-terminus of a pNOP. As will be clear to a skilled person, a collection of tiled peptides comprising an amino acid sequence of Sequence X, indicates that when aligning the tiled peptides and removing the overlapping sequences, the resulting tiled peptides provide the amino acid sequence of Sequence X, albeit present on separate peptides. As is also clear to a skilled person, a collection of tiled peptides comprising a fragment of 10 consecutive amino acids of Sequence X, indicates that when aligning the tiled peptides and removing the overlapping sequences, the resulting tiled peptides provide the amino acid sequence of the fragment, albeit present on separate peptides. When providing tiled peptides, the fragment preferably comprises at least 20 consecutive amino acids of a sequence as disclosed herein.

Specific NOP sequences cover a large percentage of kidney cancer patients. Preferred NOP sequences, or subsequences of NOP sequence, are those that target the largest percentage of kidney cancer patients. Preferred sequences are, preferably in this order of preference, Sequence 1 (6.2% of kidney cancer patients) and Sequence 2 (3.6% of kidney cancer patients), Sequence 3, 4 (each covering 3.5%

of kidney cancer patients), Sequence 5 (2.6% of kidney cancer patients), Sequence 6 (2% of kidney cancer patients), Sequence 189 (1.8% of kidney cancer patients), Sequence 7, 19 (each covering 1.3% of kidney cancer patients), Sequence 311 (1.1% of kidney cancer patients), Sequence 312 (0.7% of kidney cancer patients), Sequence 8, 20, 190-192, 313 (each covering 0.6% of kidney cancer patients), Sequence 21-22, 193-200, 314-315 (each covering 0.4% of kidney cancer patients), Sequence 23-31, 201-215, 316-322 (each covering 0.2% of kidney cancer patients), and all other Sequences listed in Table 1 and not mentioned in this paragraph (each covering <0.1% of kidney cancer patients).

As discussed further herein, neoantigens also include the nucleic acid molecules (such as DNA and RNA) encoding said amino acid sequences. The preferred sequences listed above are also the preferred sequences for the embodiments described further herein.

Preferably, the neoantigens and vaccines disclosed herein induce an immune response, or rather the neoantigens are immunogenic. Preferably, the neoantigens bind to an antibody or a T-cell receptor. In preferred embodiments, the neoantigens comprise an MHCI or MHCII ligand.

The major histocompatibility complex (MHC) is a set of cell surface molecules encoded by a large gene family in vertebrates. In humans, MHC is also referred to as human leukocyte antigen (HLA). An MHC molecule displays an antigen and presents it to the immune system of the vertebrate. Antigens (also referred to herein as 'MHC ligands') bind MHC molecules via a binding motif specific for the MHC molecule. Such binding motifs have been characterized and can be identified in proteins. See for a review Meydan et al. 2013 BMC Bioinformatics 14:S13.

MHC-class I molecules typically present the antigen to CD8 positive T-cells whereas MHC-class II molecules present the antigen to CD4 positive T-cells. The terms "cellular immune response" and "cellular response" or similar terms refer to an immune response directed to cells characterized by presentation of an antigen with class I or class II MHC involving T cells or T-lymphocytes which act as either "helpers" or "killers". The helper T cells (also termed CD4+ T cells) play a central role by regulating the immune response and the killer cells (also termed cytotoxic T cells, cytolytic T cells, CD8+ T cells or CTLs) kill diseased cells such as cancer cells, preventing the production of more diseased cells.

In preferred embodiments, the present disclosure involves the stimulation of an anti-tumor CTL response against tumor cells expressing one or more tumor-

expressed antigens (i.e., NOPs) and preferably presenting such tumor-expressed antigens with class I MHC.

In some embodiments, an entire NOP (e.g., Sequence 1) may be provided as the neoantigen (i.e., peptide). The length of the NOPs identified herein vary from around 10 to around 140 amino acids. Preferred NOPs are at least 20 amino acids in length, more preferably at least 30 amino acids, and most preferably at least 50 amino acids in length. While not wishing to be bound by theory, it is believed that neoantigens longer than 10 amino acids can be processed into shorter peptides, e.g., by antigen presenting cells, which then bind to MHC molecules.

In some embodiments, fragments of a NOP can also be presented as the neoantigen. The fragments comprise at least 8 consecutive amino acids of the NOP, preferably at least 10 consecutive amino acids, and more preferably at least 20 consecutive amino acids, and most preferably at least 30 amino acids. In some embodiments, the fragments can be about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, about 25, about 26, about 27, about 28, about 29, about 30, about 31, about 32, about 33, about 34, about 35, about 36, about 37, about 38, about 39, about 40, about 41, about 42, about 43, about 44, about 45, about 46, about 47, about 48, about 49, about 50, about 60, about 70, about 80, about 90, about 100, about 110, or about 120 amino acids or greater. Preferably, the fragment is between 8-50, between 8-30, or between 10-20 amino acids. As will be understood by the skilled person, fragments greater than about 10 amino acids can be processed to shorter peptides, e.g., by antigen presenting cells.

The specific mutations resulting in the generation of a neo open reading frame may differ between individuals resulting in differing NOP lengths. However, as depicted in, e.g., Figure 2, such individuals share common NOP sequences, in particular at the C-terminus of an NOP. While suitable fragments for use as neoantigens may be located at any position along the length of an NOP, fragments located near the C-terminus are preferred as they are expected to benefit a larger number of patients. Preferably, fragments of a NOP correspond to the C-terminal (3') portion of the NOP, preferably the C-terminal 10 consecutive amino acids, more preferably the C-terminal 20 consecutive amino acids, more preferably the C-terminal 30 consecutive amino acids, more preferably the C-terminal 40 consecutive amino acids, more preferably the C-terminal 50 consecutive amino acids, more preferably the C-terminal 60 consecutive amino acids, more preferably the C-terminal 70 consecutive amino acids, more preferably the C-terminal 80 consecutive amino acids, more preferably the C-terminal 90 consecutive amino acids, and most preferably the C-terminal 100 or more consecutive amino acids. As

is clear to a skilled person, the C-terminal amino acids need not include the, e.g., 1-5 most C-terminal amino acids. In some embodiments a subsequence of the preferred C-terminal portion of the NOP may be highly preferred for reasons of manufacturability, solubility and MHC binding strength.

Suitable fragments for use as neoantigens can be readily determined. The NOPs disclosed herein may be analysed by known means in the art in order to identify potential MHC binding peptides (i.e., MHC ligands). Suitable methods are described herein in the examples and include in silico prediction methods (e.g., ANNPRED, BIMAS, EPIMHC, HLABIND, IEDB, KISS, MULTIPRED, NetMHC, PEPVAC, POPI, PREDEP, RANKPEP, SVMHC, SVRMHC, and SYFFPEITHI, see Lundegaard 2010 130:309-318 for a review). MHC binding predictions depend on HLA genotypes, furthermore it is well known in the art that different MHC binding prediction programs predict different MHC affinities for a given epitope. While not wishing to be limited by such predictions, at least 60% of NOP sequences as defined herein, contain one or more predicted high affinity MHC class I binding epitope of 10 amino acids, based on allele HLA-A0201 and using NetMHC4.0.

A skilled person will appreciate that natural variations may occur in the genome resulting in variations in the sequence of an NOP. Accordingly, a neoantigen of the disclosure may comprise minor sequence variations, including, e.g., conservative amino acid substitutions. Conservative substitutions are well known in the art and refer to the substitution of one or more amino acids by similar amino acids. For example, a conservative substitution can be the substitution of an amino acid for another amino acid within the same general class (e.g., an acidic amino acid, a basic amino acid, or a neutral amino acid). A skilled person can readily determine whether such variants retain their immunogenicity, e.g., by determining their ability to bind MHC molecules.

Preferably, a neoantigen has at least 90% sequence identity to the NOPs disclosed herein. Preferably, the neoantigen has at least 95% or 98% sequence identity. The term "% sequence identity" is defined herein as the percentage of nucleotides in a nucleic acid sequence, or amino acids in an amino acid sequence, that are identical with the nucleotides, resp. amino acids, in a nucleic acid or amino acid sequence of interest, after aligning the sequences and optionally introducing gaps, if necessary, to achieve the maximum percent sequence identity. The skilled person understands that consecutive amino acid residues in one amino acid sequence are compared to consecutive amino acid residues in another amino acid sequence. Methods and computer programs for alignments are well known in the art. Sequence identity is calculated over substantially the whole length, preferably the whole (full) length, of a sequence of interest.

The disclosure also provides at least two frameshift-mutation derived peptides (i.e., neoantigens), also referred to herein as a 'collection' of peptides. Preferably the collection comprises at least 3, at least 4, at least 5, at least 10, at least 15, or at least 20, or at least 50 neoantigens. In some embodiments, the collections comprise less than 20, preferably less than 15 neoantigens. Preferably, the collections comprise the top 20, more preferably the top 15 most frequently occurring neoantigens in cancer patients. The neoantigens are selected from

(i) Sequences 1-18, an amino acid sequence having 90% identity to Sequences 1-18, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 1-18;

(ii) Sequences 19-188, an amino acid sequence having 90% identity to Sequences 19-188, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 19-188;

(iii) Sequences 189-310, an amino acid sequence having 90% identity to Sequences 189-310, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 189-310; and

(iv) Sequences 311-352, an amino acid sequence having 90% identity to Sequences 311-352, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 311-352.

Preferably, the collection comprises at least two frameshift-mutation derived peptides corresponding to the same gene. Preferably, a collection is provided comprising:

(i) at least two frameshift-mutation derived peptides, wherein each peptide, or collection of tiled peptides, comprises a different amino acid sequence selected from Sequences 1-18, an amino acid sequence having 90% identity to Sequences 1-18, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 1-18;

(ii) at least two frameshift-mutation derived peptides, wherein each peptide, or collection of tiled peptides, comprises a different amino acid sequence selected from Sequences 19-188, an amino acid sequence having 90% identity to Sequences 19-188, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 19-188;

(iii) at least two frameshift-mutation derived peptides, wherein each peptide, or collection of tiled peptides, comprises a different amino acid sequence selected from Sequences 189-310, an amino acid sequence having 90% identity to Sequences 189-310, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 189-310; or

(iv) at least two frameshift-mutation derived peptides, wherein each peptide, or collection of tiled peptides, comprises a different amino acid sequence selected from Sequences 311-352, an amino acid sequence having 90% identity to

Sequences 311-352, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 311-352.

In some embodiments, the collection comprises two or more neoantigens corresponding to the same NOP. For example, the collection may comprise two (or more) fragments of Sequence 1 or the collection may comprise a peptide having Sequence 1 and a peptide having 95% identity to Sequence 1.

Preferably, the collection comprises two or more neoantigens corresponding to different NOPs. In some embodiments, the collection comprises two or more neoantigens corresponding to different NOPs of the same gene. For example the peptide may comprise the amino acid sequence of Sequence 1 (or a fragment or collection of tiled fragments thereof) and the amino acid sequence of Sequence 2 (or a fragment or collection of tiled fragments thereof).

Preferably, the collection comprises Sequences 1-4, preferably 1-7, more preferably 1-18 (or a fragment or collection of tiled fragments thereof).

Preferably, the collection comprises Sequences 19-20, preferably 19-31, more preferably 19-188 (or a fragment or collection of tiled fragments thereof).

Preferably, the collection comprises Sequences 189-192, preferably 189-215, more preferably 189-310 (or a fragment or collection of tiled fragments thereof).

Preferably, the collection comprises Sequences 311-313, preferably 311-322, more preferably 311-352 (or a fragment or collection of tiled fragments thereof).

In some embodiments, the collection comprises two or more neoantigens corresponding to different NOPs of different genes. For example the collection may comprise a peptide having the amino acid sequence of Sequence 1 (or a fragment or collection of tiled fragments thereof) and a peptide having the amino acid sequence of Sequence 19 (or a fragment or collection of tiled fragments thereof). Preferably, the collection comprises at least one neoantigen from group (i) and at least one neoantigen from group (ii); at least one neoantigen from group (i) and at least one neoantigen from group (iii); at least one neoantigen from group (i) and at least one neoantigen from group (iv); at least one neoantigen from group (i) and at least one neoantigen from group (v); at least one neoantigen from group (ii) and at least one neoantigen from group (iii); at least one neoantigen from group (ii) and at least one neoantigen from group (iv); or at least one neoantigen from group (iii) and at least one neoantigen from group (iv). Preferably, the collection comprises at least one neoantigen from group (i), at least one neoantigen from group (ii), and at least one neoantigen from group (iii). Preferably, the collection comprises at least one neoantigen from each of groups (i) to (iv).

In a preferred embodiment, the collections disclosed herein include Sequence 1 and Sequence 2 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection further includes one or both of Sequence 3 and 4 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection further includes, Sequence 5 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection even further includes Sequence 6 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection even further includes Sequence 189 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection even further includes one or both of Sequence 7 and 19 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection even further includes Sequence 311 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection even further includes Sequence 312 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection even further includes Sequence 8, 20, 190-192, 313 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection even further includes Sequence 21-22, 193-200, 314-315 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection even further includes Sequence 23-31, 201-215, 316-322 (or a variant or fragment or collection of tiled fragments thereof as disclosed herein). In preferred embodiments, the collection even further includes all other Sequences listed in Table 1 and not mentioned in this paragraph (or a variant or fragment or collection of tiled fragments thereof as disclosed herein).

Such collections comprising multiple neoantigens have the advantage that a single collection (e.g, when used as a vaccine) can benefit a larger group of patients having different frameshift mutations. This makes it feasible to construct and/or test the vaccine in advance and have the vaccine available for off-the-shelf use. This also greatly reduces the time from screening a tumor from a patient to administering a potential vaccine for said tumor to the patient, as it eliminates the time of production, testing and approval. In addition, a single collection consisting of multiple neoantigens corresponding to different genes will limit possible resistance mechanisms of the tumor, e.g. by losing one or more of the targeted neoantigens.

In preferred embodiments, the neoantigens (i.e., peptides) are directly linked. Preferably, the neoantigens are linked by peptide bonds, or rather, the

neoantigens are present in a single polypeptide. Accordingly, the disclosure provides polypeptides comprising at least two peptides (i.e., neoantigens) as disclosed herein. In some embodiments, the polypeptide comprises 3, 4, 5, 6, 7, 8, 9, 10 or more peptides as disclosed herein (i.e., neoantigens). Such polypeptides are also referred to herein as 'polyNOPs'. A collection of peptides can have one or more peptides and one or more polypeptides comprising the respective neoantigens.

In an exemplary embodiment, a polypeptide of the disclosure may comprise 10 different neoantigens, each neoantigen having between 10-400 amino acids. Thus, the polypeptide of the disclosure may comprise between 100-4000 amino acids, or more. As is clear to a skilled person, the final length of the polypeptide is determined by the number of neoantigens selected and their respective lengths. A collection may comprise two or more polypeptides comprising the neoantigens which can be used to reduce the size of each of the polypeptides.

In some embodiments, the amino acid sequences of the neoantigens are located directly adjacent to each other in the polypeptide. For example, a nucleic acid molecule may be provided that encodes multiple neoantigens in the same reading frame. In some embodiments, a linker amino acid sequence may be present. Preferably a linker has a length of 1, 2, 3, 4 or 5, or more amino acids. The use of linker may be beneficial, for example for introducing, among others, signal peptides or cleavage sites. In some embodiments at least one, preferably all of the linker amino acid sequences have the amino acid sequence VDD.

As will be appreciated by the skilled person, the peptides and polypeptides disclosed herein may contain additional amino acids, for example at the N- or C-terminus. Such additional amino acids include, e.g., purification or affinity tags or hydrophilic amino acids in order to decrease the hydrophobicity of the peptide. In some embodiments, the neoantigens may comprise amino acids corresponding to the adjacent, wild-type amino acid sequences of the relevant gene, i.e., amino acid sequences located 5' to the frame shift mutation that results in the neo open reading frame. Preferably, each neoantigen comprises no more than 20, more preferably no more than 10, and most preferably no more than 5 of such wild-type amino acid sequences.

In preferred embodiments, the peptides and polypeptides disclosed herein have a sequence depicted as follows:

A-B-C-(D-E)$_n$, wherein

- A, C, and E are independently 0-100 amino acids
- B and D are amino acid sequences as disclosed herein and selected from sequences 1-352, or an amino acid sequence having 90% identity to Sequences 1-

352, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 1-352,

- n is an integer from 0 to 500.

Preferably, B and D are different amino acid sequences. Preferably, n is an integer from 0-200. Preferably A, C, and E are independently 0-50 amino acids, more preferably independently 0-20 amino acids.

The peptides and polypeptides disclosed herein can be produced by any method known to a skilled person. In some embodiments, the peptides and polypeptide are chemically synthesized. The peptides and polypeptide can also be produced using molecular genetic techniques, such as by inserting a nucleic acid into an expression vector, introducing the expression vector into a host cell, and expressing the peptide. Preferably, such peptides and polypeptide are isolated, or rather, substantially isolated from other polypeptides, cellular components, or impurities. The peptide and polypeptide can be isolated from other (poly)peptides as a result of solid phase protein synthesis, for example. Alternatively, the peptides and polypeptide can be substantially isolated from other proteins after cell lysis from recombinant production (e.g., using HPLC).

The disclosure further provides nucleic acid molecules encoding the peptides and polypeptide disclosed herein. Based on the genetic code, a skilled person can determine the nucleic acid sequences which encode the (poly)peptides disclosed herein. Based on the degeneracy of the genetic code, sixty-four codons may be used to encode twenty amino acids and translation termination signal.

In a preferred embodiment, the nucleic acid molecules are codon optimized. As is known to a skilled person, codon usage bias in different organisms can effect gene expression level. Various computational tools are available to the skilled person in order to optimize codon usage depending on which organism the desired nucleic acid will be expressed. Preferably, the nucleic acid molecules are optimized for expression in mammalian cells, preferably in human cells. Table 2 lists for each acid amino acid (and the stop codon) the most frequently used codon as encountered in the human exome.

Table 2 – most frequently used codon for each amino acid and most frequently used stop codon.

A	GCC
C	TGC
D	GAC
E	GAG
F	TTC

G	GGC
H	CAC
I	ATC
K	AAG
L	CTG
M	ATG
N	AAC
P	CCC
Q	CAG
R	CGG
S	AGC
T	ACC
V	GTG
W	TGG
Y	TAC
Stop	TGA

In preferred embodiments, at least 50%, 60%, 70%, 80%, 90%, or 100% of the amino acids are encoded by a codon corresponding to a codon presented in Table 2.

In preferred embodiments, the nucleic acid molecule encodes for a linker amino acid sequence in the peptide. Preferably, the nucleic acid sequence encoding the linker comprises at least one codon triplet that codes for a stop codon when a frameshift occurs. Preferably, said codon triplet is chosen from the group consisting of: ATA, CTA, GTA, TTA, ATG, CTG, GTG, TTG, AAA, AAC, AAG, AAT, AGA, AGC, AGG, AGT, GAA, GAC, GAG, and GAT. These codons do not code for a stop codon, but could create a stop codon in case of a frame shift, such as when read in the +1, +2, +4, +, 5, etc. reading frame. For example, two amino acid encoding sequences are linked by a linker amino acid encoding sequence as follows (linker amino acid encoding sequence in bold):

CTATACAGGCGA**ATG**AGATTATG

Resulting in the following amino acid sequence (amino acid linker sequence in bold): LYRR**M**RL

In case of a +1 frame shift, the following sequence is encoded:

YTGE**[stop]**DY

This embodiment has the advantage that if a frame shift occurs in the nucleotide sequence encoding the peptide, the nucleic acid sequence encoding the linker will terminate translation, thereby preventing expression of (part of) the

native protein sequence for the gene related to peptide sequence encoded by the nucleotide sequence.

In some preferred embodiments, the linker amino acid sequences are encoded by the nucleotide sequence GTAGATGAC. This linker has the advantage that it contains two out of frame stop codons (TAG and TGA), one in the +1 and one in the -1 reading frame. The amino acid sequence encoded by this nucleotide sequence is VDD. The added advantage of using a nucleotide sequence encoding for this linker amino acid sequence is that any frame shift will result in a stop codon.

The disclosure also provides binding molecules and a collection of binding molecules that bind the neoantigens disclosed herein and or a neoantigen/MHC complex. In some embodiments the binding molecule is an antibody, a T-cell receptor, or an antigen binding fragment thereof. In some embodiments the binding molecule is a chimeric antigen receptor comprising i) a T cell activation molecule; ii) a transmembrane region; and iii) an antigen recognition moiety; wherein said antigen recognition moieties bind the neoantigens disclosed herein and or a neoantigen/MHC complex.

The term “antibody” as used herein refers to an immunoglobulin molecule that is typically composed of two identical pairs of polypeptide chains, each pair of chains consisting of one “heavy” chain with one “light” chain. The human light chains are classified as kappa and lambda. The heavy chains comprise different classes namely: mu, delta, gamma, alpha or epsilon. These classes define the isotype of the antibody, such as IgM, IgD, IgG IgA and IgE, respectively. These classes are important for the function of the antibody and help to regulate the immune response. Both the heavy chain and the light chain comprise a variable domain and a constant region. Each heavy chain variable region (VH) and light chain variable region (VL) comprises complementary determining regions (CDR) interspersed by framework regions (FR). The variable region has in total four FRs and three CDRs. These are arranged from the amino- to the carboxyl-terminus as follows: FR1. CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the light and heavy chain together form the antibody binding site and define the specificity for the epitope.

The term "antibody" encompasses murine, humanized, deimmunized, human, and chimeric antibodies, and an antibody that is a multimeric form of antibodies, such as dimers, trimers, or higher-order multimers of monomeric antibodies. The term antibody also encompasses monospecific, bispecific or multi-specific antibodies, and any other modified configuration of the immunoglobulin molecule that comprises an antigen recognition site of the required specificity.

Preferably, an antibody or antigen binding fragment thereof as disclosed herein is a humanized antibody or antigen binding fragment thereof. The term "humanized antibody" refers to an antibody that contains some or all of the CDRs from a non-human animal antibody while the framework and constant regions of the antibody contain amino acid residues derived from human antibody sequences. Humanized antibodies are typically produced by grafting CDRs from a mouse antibody into human framework sequences followed by back substitution of certain human framework residues for the corresponding mouse residues from the source antibody. The term “deimmunized antibody” also refers to an antibody of non-human origin in which, typically in one or more variable regions, one or more epitopes have been removed, that have a high propensity of constituting a human T-cell and/or B-cell epitope, for purposes of reducing immunogenicity. The amino acid sequence of the epitope can be removed in full or in part. However, typically the amino acid sequence is altered by substituting one or more of the amino acids constituting the epitope for one or more other amino acids, thereby changing the amino acid sequence into a sequence that does not constitute a human T-cell and/or B-cell epitope. The amino acids are substituted by amino acids that are present at the corresponding position(s) in a corresponding human variable heavy or variable light chain as the case may be.

In some embodiments, an antibody or antigen binding fragment thereof as disclosed herein is a human antibody or antigen binding fragment thereof. The term "human antibody" refers to an antibody consisting of amino acid sequences of human immunoglobulin sequences only. Human antibodies may be prepared in a variety of ways known in the art.

As used herein, antigen-binding fragments include Fab, F(ab'), F(ab')2, complementarity determining region (CDR) fragments, single-chain antibodies (scFv), bivalent single-chain antibodies, and other antigen recognizing immunoglobulin fragments.

In some embodiments, the antibody or antigen binding fragment thereof is an isolated antibody or antigen binding fragment thereof. The term "isolated" as used herein refer to material which is substantially or essentially free from components which normally accompany it in nature.

In some embodiments, the antibody or antigen binding fragment thereof is linked or attached to a non-antibody moiety. In preferred embodiments, the non-antibody moiety is a cytotoxic moiety such as auristatins, maytanasines, calicheasmicins, duocarymycins, a-amanitin, doxorubicin, and centanamycin.

Other suitable cytotoxins and methods for preparing such antibody drug conjugates are known in the art; see, e.g., WO2013085925A1 and WO2016133927A1.

Antibodies which bind a particular epitope can be generated by methods known in the art. For example, polyclonal antibodies can be made by the conventional method of immunizing a mammal (e.g., rabbits, mice, rats, sheep, goats). Polyclonal antibodies are then contained in the sera of the immunized animals and can be isolated using standard procedures (e.g., affinity chromatography, immunoprecipitation, size exclusion chromatography, and ion exchange chromatography). Monoclonal antibodies can be made by the conventional method of immunization of a mammal, followed by isolation of plasma B cells producing the monoclonal antibodies of interest and fusion with a myeloma cell (see, e.g., Mishell, B. B., et al., Selected Methods In Cellular Immunology, (W.H. Freeman, ed.) San Francisco (1980)). Peptides corresponding to the neoantiens disclosed herein may be used for immunization in order to produce antibodies which recognize a particular epitope. Screening for recognition of the epitope can be performed using standard immunoassay methods including ELISA techniques, radioimmunoassays, immunofluorescence, immunohistochemistry, and Western blotting. See, Short Protocols in Molecular Biology, Chapter 11, Green Publishing Associates and John Wiley & Sons, Edited by Ausubel, F. M et al., 1992. In vitro methods of antibody selection, such as antibody phage display, may also be used to generate antibodies recognizing the neoantigens disclosed herein (see, e.g., Schirrmann et al. Molecules 2011 16:412-426).

T-cell receptors (TCRs) are expressed on the surface of T-cells and consist of an α chain and a β chain. TCRs recognize antigens bound to MHC molecules expressed on the surface of antigen-presenting cells. The T-cell receptor (TCR) is a heterodimeric protein, in the majority of cases (95%) consisting of a variable alpha (α) and beta (β) chain, and is expressed on the plasma membrane of T-cells. The TCR is subdivided in three domains: an extracellular domain, a transmembrane domain and a short intracellular domain. The extracellular domain of both α and β chains have an immunoglobulin-like structure, containing a variable and a constant region. The variable region recognizes processed peptides, among which neoantigens, presented by major histocompatibility complex (MHC) molecules, and is highly variable. The intracellular domain of the TCR is very short, and needs to interact with CD3ζ to allow for signal propagation upon ligation of the extracellular domain.

With the focus of cancer treatment shifted towards more targeted therapies, among which immunotherapy, the potential of therapeutic application of tumor-directed T-cells is increasingly explored. One such application is adoptive T-cell

therapy (ATCT) using genetically modified T-cells that carry chimeric antigen receptors (CARs) recognizing a particular epitope (Ref Gomes-Silva 2018). The extracellular domain of the CAR is commonly formed by the antigen-specific subunit of (scFv) of a monoclonal antibody that recognizes a tumor-antigen (Ref Abate-Daga 2016). This enables the CAR T-cell to recognize epitopes independent of MHC-molecules, thus widely applicable, as their functionality is not restricted to individuals expressing the specific MHC-molecule recognized by the TCR. Methods for engineering TCRs that bind a particular epitope are known to a skilled person. See, for example, US20100009863A1, which describes methods of modifying one or more structural loop regions. The intracellular domain of the CAR can be a TCR intracellular domain or a modified peptide to enable induction of a signaling cascade without the need for interaction with accessory proteins. This is accomplished by inclusion of the CD3ζ-signalling domain, often in combination with one or more co-stimulatory domains, such as CD28 and 4-1BB, which further enhance CAR T-cell functioning and persistence (Ref Abate-Daga 2016).

The engineering of the extracellular domain towards an scFv limits CAR T-cell to the recognition of molecules that are expressed on the cell-surface. Peptides derived from proteins that are expressed intracellularly can be recognized upon their presentation on the plasma membrane by MHC molecules, of which human form is called human leukocyte antigen (HLA). The HLA-haplotype generally differs among individuals, but some HLA types, like HLA-A*02:01, are globally common. Engineering of CAR T-cell extracellular domains recognizing tumor-derived peptides or neoantigens presented by a commonly shared HLA molecule enables recognition of tumor antigens that remain intracellular. Indeed CAR T-cells expressing a CAR with a TCR-like extracellular domain have been shown to be able to recognize tumor-derived antigens in the context of HLA-A*02:01 (Refs Zhang 2014, Ma 2016, Liu 2017).

In some embodiments, the binding molecules are monospecific, or rather they bind one of the neoantigens disclosed herein. In some embodiments, the binding molecules are bispecific, e.g., bispecific antibodies and bispecific chimeric antigen receptors.

In some embodiments, the disclosure provides a first antigen binding domain that binds a first neoantigen described herein and a second antigen binding domain that binds a second neoantigen described herein. The first and second antigen binding domains may be part of a single molecule, e.g., as a bispecific antibody or bispecific chimeric antigen receptor or they may be provided on separate molecules, e.g., as a collection of antibodies, T-cell receptors, or chimeric antigen receptors. In some embodiments, 3, 4, 5 or more antigen binding domains

are provided each binding a different neoantigen disclosed herein. As used herein, an antigen binding domain includes the variable (antigen binding) domain of a T-cell receptor and the variable domain of an antibody (e.g., comprising a light chain variable region and a heavy chain variable region).

The disclosure further provides nucleic acid molecules encoding the antibodies, TCRs, and CARs disclosed herein. In a preferred embodiment, the nucleic acid molecules are codon optimized as disclosed herein.

The disclosure further provides vectors comprising the nucleic acids molecules disclosed herein. A "vector" is a recombinant nucleic acid construct, such as plasmid, phase genome, virus genome, cosmid, or artificial chromosome, to which another nucleic acid segment may be attached. The term "vector" includes both viral and non-viral means for introducing the nucleic acid into a cell in vitro, ex vivo or in vivo. The disclosure contemplates both DNA and RNA vectors. The disclosure further includes self-replicating RNA with (virus-derived) replicons, including but not limited to mRNA molecules derived from mRNA molecules from alphavirus genomes, such as the Sindbis, Semliki Forest and Venezuelan equine encephalitis viruses.

Vectors, including plasmid vectors, eukaryotic viral vectors and expression vectors are known to the skilled person. Vectors may be used to express a recombinant gene construct in eukaryotic cells depending on the preference and judgment of the skilled practitioner (see, for example, Sambrook et al., Chapter 16). For example, many viral vectors are known in the art including, for example, retroviruses, adeno-associated viruses, and adenoviruses. Other viruses useful for introduction of a gene into a cell include, but a not limited to, arenavirus, herpes virus, mumps virus, poliovirus, Sindbis virus, and vaccinia virus, such as, canary pox virus. The methods for producing replication-deficient viral particles and for manipulating the viral genomes are well known. In preferred embodiments, the vaccine comprises an attenuated or inactivated viral vector comprising a nucleic acid disclosed herein.

Preferred vectors are expression vectors. It is within the purview of a skilled person to prepare suitable expression vectors for expressing the inhibitors disclosed hereon. An “expression vector” is generally a DNA element, often of circular structure, having the ability to replicate autonomously in a desired host cell, or to integrate into a host cell genome and also possessing certain well-known features which, for example, permit expression of a coding DNA inserted into the vector sequence at the proper site and in proper orientation. Such features can include, but are not limited to, one or more promoter sequences to direct transcription initiation of the coding DNA and other DNA elements such as enhancers,

polyadenylation sites and the like, all as well known in the art. Suitable regulatory sequences including enhancers, promoters, translation initiation signals, and polyadenylation signals may be included. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector. The expression vectors may also contain a selectable marker gene which facilitates the selection of host cells transformed or transfected. Examples of selectable marker genes are genes encoding a protein such as G418 and hygromycin which confer resistance to certain drugs, ß- galactosidase, chloramphenicol acetyltransferase, and firefly luciferase.

The expression vector can also be an RNA element that contains the sequences required to initiate translation in the desired reading frame, and possibly additional elements that are known to stabilize or contribute to replicate the RNA molecules after administration. Therefore when used herein the term DNA when referring to an isolated nucleic acid encoding the peptide according to the invention should be interpreted as referring to DNA from which the peptide can be transcribed or RNA molecules from which the peptide can be translated.

Also provided for is a host cell comprising an nucleic acid molecule or a vector as disclosed herein. The nucleic acid molecule may be introduced into a cell (prokaryotic or eukaryotic) by standard methods. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art recognized techniques to introduce a DNA into a host cell. Such methods include, for example, transfection, including, but not limited to, liposome-polybrene, DEAE dextran-mediated transfection, electroporation, calcium phosphate precipitation, microinjection, or velocity driven microprojectiles ("biolistics"). Such techniques are well known by one skilled in the art. See, Sambrook et al. (1989) Molecular Cloning: A Laboratory Manaual (2 ed. Cold Spring Harbor Lab Press, Plainview, N.Y.). Alternatively, one could use a system that delivers the DNA construct in a gene delivery vehicle. The gene delivery vehicle may be viral or chemical. Various viral gene delivery vehicles can be used with the present invention. In general, viral vectors are composed of viral particles derived from naturally occurring viruses. The naturally occurring virus has been genetically modified to be replication defective and does not generate additional infectious viruses, or it may be a virus that is known to be attenuated and does not have unacceptable side effects.

Preferably, the host cell is a mammalian cell, such as MRC5 cells (human cell line derived from lung tissue), HuH7 cells (human liver cell line), CHO-cells

(Chinese Hamster Ovary), COS-cells (derived from monkey kidney (African green monkey), Vero-cells (kidney epithelial cells extracted from African green monkey), Hela-cells (human cell line), BHK-cells (baby hamster kidney cells, HEK-cells (Human Embryonic Kidney), NSO-cells (Murine myeloma cell line), C127-cells (nontumorigenic mouse cell line), PerC6®-cells (human cell line, Crucell), and Madin-Darby Canine Kidney(MDCK) cells. In some embodiments, the disclosure comprises an in vitro cell culture of mammalian cells expressing the neoantigens disclosed herein. Such cultures are useful, for example, in the production of cell-based vaccines, such as viral vectors expressing the neoantigens disclosed herein.

In some embodiments the host cells express the antibodies, TCRs, or CARs as disclosed herein. As will be clear to a skilled person, individual polypeptide chains (e.g., immunoglobulin heavy and light chains) may be provided on the same or different nucleic acid molecules and expressed by the same or different vectors. For example, in some embodiments, a host cell is transfected with a nucleic acid encoding an α-TCR polypeptide chain and a nucleic acid encoding a β-polypeptide chain.

In preferred embodiments, the disclosure provides T-cells expressing a TCR or CAR as disclosed herein. T cells may be obtained from, e.g., peripheral blood mononuclear cells, bone marrow, lymph node tissue, cord blood, thymus tissue, spleen tissue, and tumors. Preferably, the T-cells are obtained from the individual to be treated (autologous T-cells). T-cells may also be obtained from healthy donors (allogenic T-cells). Isolated T-cells are expanded in vitro using established methods, such as stimulation with cytokines (IL-2). Methods for obtaining and expanding T-cells for adoptive therapy are well known in the art and are also described, e.g., in EP2872533A1.

The disclosure also provides vaccines comprising one or more neoantigens as disclosed herein. In particular, the vaccine comprises one or more (poly)peptides, antibodies or antigen binding fragments thereof, TCRs, CARS, nucleic acid molecules, vectors, or cells (or cell cultures) as disclosed herein.

The vaccine may be prepared so that the selection, number and/or amount of neoantigens (e.g., peptides or nucleic acids encoding said peptides) present in the composition is patient-specific. Selection of one or more neoantigens may be based on sequencing information from the tumor of the patient. For any frame shift mutation found, a corresponding NOP is selected. Preferably, the vaccine comprises more than one neoantigen corresponding to the NOP selected. In case multiple frame shift mutations (multiple NOPs) are found, multiple neoantigens corresponding to each NOP may be selected for the vaccine.

The selection may also be dependent on the specific type of cancer, the status of the disease, earlier treatment regimens, the immune status of the patient, and, HLA-haplotype of the patient. Furthermore, the vaccine can contain individualized components, according to personal needs of the particular patient.

As is clear to a skilled person, if multiple neoantigens are used, they may be provided in a single vaccine composition or in several different vaccines to make up a vaccine collection. The disclosure thus provides vaccine collections comprising a collection of tiled peptides, collection of peptides as disclosed herein, as well as nucleic acid molecules, vectors, or host cells as disclosed herein. As is clear to a skilled person, such vaccine collections may be administered to an individual simultaneously or consecutively (e.g., on the same day) or they may be administered several days or weeks apart.

Various known methods may be used to administer the vaccines to an individual in need thereof. For instance, one or more neoantigens can be provided as a nucleic acid molecule directly, as "naked DNA". Neoantigens can also be expressed by attenuated viral hosts, such as vaccinia or fowlpox. This approach involves the use of a virus as a vector to express nucleotide sequences that encode the neoantigen. Upon introduction into the individual, the recombinant virus expresses the neoantigen peptide, and thereby elicits a host CTL response. Vaccination using viral vectors is well-known to a skilled person and vaccinia vectors and methods useful in immunization protocols are described in, e.g., U.S. Patent No. 4722848. Another vector is BCG (Bacille Calmette Guerin) as described in Stover et al. (Nature 351:456-460 (1991)).

Preferably, the vaccine comprises a pharmaceutically acceptable excipient and/or an adjuvant. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like. Suitable adjuvants are well-known in the art and include, aluminum (or a salt thereof, e.g., aluminium phosphate and aluminium hydroxide), monophosphoryl lipid A, squalene (e.g., MF59), and cytosine phosphoguanine (CpG), montanide, liposomes (e.g. CAF adjuvants, cationic adjuvant formulations and variations thereof), lipoprotein conjugates (e.g. Amplivant), Resiquimod, Iscomatrix, hiltonol, poly-ICLC (polyriboinosinic-polyribocytidylic acid-polylysine carboxymethylcellulose). A skilled person is able to determine the appropriate adjuvant, if necessary, and an immune-effective amount thereof. As used herein, an immune-effective amount of adjuvant refers to the amount needed to increase the vaccine's immunogenicity in order to achieve the desired effect.

The disclosure also provides the use of the neoantigens disclosed herein for the treatment of disease, in particular for the treatment of kidney cancer (also referred to as renal cancer) in an individual. In preferred embodiments, the cancer is renal clear cell carcinoma (KIRC). Approximately 70% of all kidney cancer is renal clear cell carcinoma. It is within the purview of a skilled person to diagnose an individual with as having kidney cancer.

As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, or inhibiting the progress of a disease, or reversing, alleviating, delaying the onset of, or inhibiting one or more symptoms thereof. Treatment includes, e.g., slowing the growth of a tumor, reducing the size of a tumor, and/or slowing or preventing tumor metastasis.

The term 'individual' includes mammals, both humans and non-humans and includes but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines. Preferably, the human is a mammal.

As used herein, administration or administering in the context of treatment or therapy of a subject is preferably in a "therapeutically effective amount", this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of the disease being treated. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners.

The optimum amount of each neoantigen to be included in the vaccine composition and the optimum dosing regimen can be determined by one skilled in the art without undue experimentation. The composition may be prepared for injection of the peptide, nucleic acid molecule encoding the peptide, or any other carrier comprising such (such as a virus or liposomes). For example, doses of between 1 and 500 mg 50 µg and 1.5 mg, preferably 125 µg to 500 µg, of peptide or DNA may be given and will depend from the respective peptide or DNA. Other methods of administration are known to the skilled person. Preferably, the vaccines may be administered parenterally, e.g., intravenously, subcutaneously, intradermally, intramuscularly, or otherwise.

For therapeutic use, administration may begin at or shortly after the surgical removal of tumors. This can be followed by boosting doses until at least symptoms are substantially abated and for a period thereafter.

In some embodiments, the vaccines may be provided as a neoadjuvant therapy, e.g., prior to the removal of tumors or prior to treatment with radiation or chemotherapy. Neoadjuvant therapy is intended to reduce the size of the tumor before more radical treatment is used. For that reason being able to provide the vaccine off-the-shelf or in a short period of time is very important.

Also disclosed herein, the vaccine is capable of initiating a specific T-cell response. It is within the purview of a skilled person to measure such T-cell responses either in vivo or in vitro, e.g. by analyzing IFN-γ production or tumor killing by T-cells. In therapeutic applications, vaccines are administered to a patient in an amount sufficient to elicit an effective CTL response to the tumor antigen and to cure or at least partially arrest symptoms and/or complications.

The vaccine disclosed herein can be administered alone or in combination with other therapeutic agents. The therapeutic agent is for example, a chemotherapeutic agent, radiation, or immunotherapy, including but not limited to checkpoint inhibitors, such as nivolumab, ipilimumab, pembrolizumab, or the like. Any suitable therapeutic treatment for a particular, cancer may be administered.

The term "chemotherapeutic agent" refers to a compound that inhibits or prevents the viability and/or function of cells, and/or causes destruction of cells (cell death), and/or exerts anti-tumor/anti-proliferative effects. The term also includes agents that cause a cytostatic effect only and not a mere cytotoxic effect. Examples of chemotherapeutic agents include, but are not limited to bleomycin, capecitabine, carboplatin, cisplatin, cyclophosphamide, docetaxel, doxorubicin, etoposide, interferon alpha, irinotecan, lansoprazole, levamisole, methotrexate, metoclopramide, mitomycin, omeprazole, ondansetron, paclitaxel, pilocarpine, rituxitnab, tamoxifen, taxol, trastuzumab, vinblastine, and vinorelbine tartrate.

Preferably, the other therapeutic agent is an anti-immunosuppressive/immunostimulatory agent, such as anti-CTLA antibody or anti-PD-1 or anti-PD-L1. Blockade of CTLA-4 or PD-L1 by antibodies can enhance the immune response to cancerous cells. In particular, CTLA-4 blockade has been shown effective when following a vaccination protocol.

As is understood by a skilled person the vaccine and other therapeutic agents may be provided simultaneously, separately, or sequentially. In some

embodiments, the vaccine may be provided several days or several weeks prior to or following treatment with one or more other therapeutic agents. The combination therapy may result in an additive or synergistic therapeutic effect.

As disclosed herein, the present disclosure provides vaccines which can be prepared as off-the-shelf vaccines. As used herein "off-the-shelf" means a vaccine as disclosed herein that is available and ready for administration to a patient. For example, when a certain frame shift mutation is identified in a patient, the term "off-the-shelf" would refer to a vaccine according to the disclosure that is ready for use in the treatment of the patient, meaning that, if the vaccine is peptide based, the corresponding polyNOP peptide may, for example already be expressed and for example stored with the required excipients and stored appropriately, for example at -20 ^{0}C or -80 ^{0}C. Preferably the term "off-the-shelf" also means that the vaccine has been tested, for example for safety or toxicity. More preferably the term also means that the vaccine has also been approved for use in the treatment or prevention in a patient. Accordingly, the disclosure also provides a storage facility for storing the vaccines disclosed herein. Depending on the final formulation, the vaccines may be stored frozen or at room temperature, e.g., as dried preparations. Preferably, the storage facility stores at least 20 or at least 50 different vaccines, each recognizing a neoantigen disclosed herein.

The present disclosure also contemplates methods which include determining the presence of NOPs in a tumor sample. In a preferred embodiment, a tumor of a patient can be screened for the presence of frame shift mutations and an NOP can be identified that results from such a frame shift mutation. Based on the NOP(s) identified in the tumor, a vaccine comprising the relevant NOP(s) can be provided to immunize the patient, so the immune system of the patient will target the tumor cells expressing the neoantigen. An exemplary workflow for providing a neoantigen as disclosed herein is as follows. When a patient is diagnosed with a cancer, a biopsy may be taken from the tumor or a sample set is taken of the tumor after resection. The genome, exome and/or transcriptome is sequenced by any method known to a skilled person. The outcome is compared, for example using a web interface or software, to the library of NOPs disclosed herein. A patient whose tumor expresses one of the NOPs disclosed herein is thus a candidate for a vaccine comprising the NOP (or a fragment thereof).

Accordingly, the disclosure provides a method for determining a therapeutic treatment for an individual afflicted with cancer, said method comprising determining the presence of a frame shift mutation which results in the expression of an NOP selected from sequences 1-352. Identification of the expression of an NOP indicates that said individual should be treated with a vaccine corresponding

to the identified NOP. For example, if it is determined that tumor cells from an individual express Sequence 1, then a vaccine comprising Sequence 1 or a fragment thereof is indicated as a treatment for said individual.

Accordingly, the disclosure provides a method for determining a therapeutic treatment for an individual afflicted with cancer, said method comprising
a. performing complete, targeted or partial genome, exome, ORFeome, or transcriptome sequencing of at least one tumor sample obtained from the individual to obtain a set of sequences of the subject-specific tumor genome, exome, ORFeome, or transcriptome;
b. comparing at least one sequence or portion thereof from the set of sequences with one or more sequences selected from:
(i) Sequences 1-18;
(ii) Sequences 19-188;
(iii) Sequences 189-310; and
(iv) Sequences 311-352;
c. identifying a match between the at least one sequence or portion thereof from the set of sequences and a sequence from groups (i) to (v) when the sequences have a string in common representative of at least 8 amino acids to identify a neoantigen encoded by a frameshift mutation;
wherein a match indicates that said individual is to be treated with the vaccine as disclosed herein.

As used herein the term "sequence" can refer to a peptide sequence, DNA sequence or RNA sequence. The term "sequence" will be understood by the skilled person to mean either or any of these, and will be clear in the context provided. For example, when comparing sequences to identify a match, the comparison may be between DNA sequences, RNA sequences or peptide sequences, but also between DNA sequences and peptide sequences. In the latter case the skilled person is capable of first converting such DNA sequence or such peptide sequence into, respectively, a peptide sequence and a DNA sequence in order to make the comparison and to identify the match. As is clear to a skilled person, when sequences are obtained from the genome or exome, the DNA sequences are preferably converted to the predicted peptide sequences. In this way, neo open reading frame peptides are identified.

As used herein the term "exome" is a subset of the genome that codes for proteins. An exome can be the collective exons of a genome, or also refer to a subset of the exons in a genome, for example all exons of known cancer genes.

As used herein the term "transcriptome" is the set of all RNA molecules is a cell or population of cells. In a preferred embodiment the transcriptome refers to all mRNA.

In some preferred embodiments the genome is sequenced. In some preferred embodiments the exome is sequenced. In some preferred embodiments the transcriptome is sequenced. In some preferred embodiments a panel of genes is sequenced, for example BAP1, PBRM1, SETD2, and VHL. In some preferred embodiments a single gene is sequenced. Preferably the transcriptome is sequenced, in particular the mRNA present in a sample from a tumor of the patient. The transcriptome is representative of genes and neo open reading frame peptides as defined herein being expressed in the tumor in the patient.

As used herein the term "sample" can include a single cell or multiple cells or fragments of cells or an aliquot of body fluid, taken from an individual, by means including venipuncture, excretion, ejaculation, massage, biopsy, needle aspirate, lavage sample, scraping, surgical incision, or intervention or other means known in the art. The DNA and/or RNA for sequencing is preferably obtained by taking a sample from a tumor of the patient. The skilled person knowns how to obtain samples from a tumor of a patient and depending on the nature, for example location or size, of the tumor. Preferably the tumor is a kidney tumor. Preferably the sample is obtained from the patient by biopsy or resection. The sample is obtained in such manner that is allows for sequencing of the genetic material obtained therein. In order to prevent a less accurate identification of at least one antigen, preferably the sequence of the tumor sample obtained from the patient is compared to the sequence of other non-tumor tissue of the patient, usually blood, obtained by known techniques (e.g. venipuncture).

Identification of frame shift mutations can be done by sequencing of RNA or DNA using methods known to the skilled person. Sequencing of the genome, exome, ORFeome, or transcriptome may be complete, targeted or partial. In some embodiments the sequencing is complete (whole sequencing). In some embodiments the sequencing is targeted. With targeted sequencing is meant that purposively certain region or portion of the genome, exome, ORFeome or transcriptome are sequenced. For example targeted sequencing may be directed to only sequencing for sequences in the set of sequences obtained from the cancer patient that would provide for a match with one or more of the sequences in the sequence listing, for example by using specific primers. In some embodiment only portion of the genome, exome, ORFeome or transcriptome is sequenced. The skilled person is well-aware of methods that allow for whole, targeted or partial sequencing of the genome, exome, ORFeome or transcriptome of a tumor sample of a patient. For example any suitable sequencing-by-synthesis platform can be used including the Genome

Sequencers from Illumina/Solexa, the Ion Torrent system from Applied BioSystems, and the RSII or Sequel systems from Pacific Biosciences. Alternatively Nanopore sequencing may be used, such as the MinION, GridION or PromethION platform offered by Oxford Nanopore Technologies. The method of sequencing the genome, exome, ORFeome or transcriptome is not in particular limited within the context of the present invention.

Sequence comparison can be performed by any suitable means available to the skilled person. Indeed the skilled person is well equipped with methods to perform such comparison, for example using software tools like BLAST and the like, or specific software to align short or long sequence reads, accurate or noisy sequence reads to a reference genome, e.g. the human reference genome GRCh37 or GRCh38. A match is identified when a sequence identified in the patients material and a sequence as disclosed herein have a string, i.e. a peptide sequence (or RNA or DNA sequence encoding such peptide (sequence) in case the comparison is on the level of RNA or DNA) in common representative of at least 8, preferably at least 10 adjacent amino acids. Furthermore, sequence reads derived from a patients cancer genome (or transcriptome) can partially match the genomic DNA sequences encoding the amino acid sequences as disclosed herein, for example if such sequence reads are derived from exon/intron boundaries or exon/exon junctions, or if part of the sequence aligns upstream (to the 5' end of the gene) of the position of a frameshift mutation. Analysis of sequence reads and identification of frameshift mutations will occur through standard methods in the field. For sequence alignment, aligners specific for short or long reads can be used, e.g. BWA (Li and Durbin, Bioinformatics. 2009 Jul 15;25(14):1754-60) or Minimap2 (Li, Bioinformatics. 2018 Sep 15;34(18):3094-3100). Subsequently, frameshift mutations can be derived from the read alignments and their comparison to a reference genome sequence (e.g. the human reference genome GRCh37) using variant calling tools, for example Genome Analysis ToolKit (GATK), and the like (McKenna et al. Genome Res. 2010 Sep;20(9):1297-303).

A match between an individual patient's tumor sample genome or transcriptome sequence and one or more NOPs disclosed herein indicates that said tumor expresses said NOP and that said patient would likely benefit from treatment with a vaccine comprising said NOP (or a fragment thereof). More specifically, a match occurs if a frameshift mutation is identified in said patient's tumor genome sequence and said frameshift leads to a novel reading frame (+1 or -1 with respect to the native reading from of a gene). In such instance, the predicted out-of-frame peptide derived from the frameshift mutation matches any of the sequences 1- 352 as disclosed herein. In some embodiments, said patient is

administered said NOP (e.g., by administering the peptides, nucleic acid molecules, vectors, host cells or vaccines as disclosed herein).

In some embodiments, the methods further comprise sequencing the genome, exome, ORFeome, or transcriptome (or a part thereof) from a normal, non-tumor sample from said individual and determining whether there is a match with one or more NOPs identified in the tumor sample. Although the neoantigens disclosed herein appear to be specific to tumors, such methods may be employed to confirm that the neoantigen is tumor specific and not, e.g., a germline mutation.

The disclosure further provides the use of the neoantigens and vaccines disclosed herein in prophylactic methods from preventing or delaying the onset of kidney cancer. Approximately 1.5-2% of individuals will develop kidney cancer and the neo open reading frames disclosed herein occur in up to 27% of kidney cancer patients. Prophylactic vaccination based on frameshift resulting peptides disclosed herein would thus provide protection to approximately 0.5% of the general population. The vaccine may be specifically used in a prophylactic setting for individuals that have an increased risk of developing kidney cancer. For example, prophylactic vaccination is expected to provide possible protection to around 22% of individuals having a germline predisposition mutation as referred to in Table 3 and who would have developed kidney cancer as a result of their predisposing mutation. In some embodiments, the prophylactic methods are useful for individuals who are genetically related to individuals afflicted with kidney cancer. In some embodiments, the prophylactic methods are useful for the general population.

In some embodiments, the individual is at risk of developing cancer. It is understood to a skilled person that being at risk of developing cancer indicates that the individual has a higher risk of developing cancer than the general population; or rather the individual has an increased risk over the average of developing cancer. Such risk factors are known to a skilled person and include being a male, increased age, in particular being 40 years or older; smoking, having advanced kidney disease, having von Hippel-Lindau (VHL) disease or inherited papillary renal cell carcinoma, having a family history of kidney cancer, asbestos exposure, and having a mutation in a gene that predisposes an individual to kidney cancer.

In some embodiments, said individual has a germline mutation in a gene that increases the chance that the individual will develop kidney cancer, preferably the mutation is in the ATM, ATR, BRCA1, BRIP1, CBL, CHEK2, DROSHA, FANCL, FH, FLCN, GJB2, MUTYH, PRDM9, RECQL, RECQL3, SDHA, and/or SPC gene. Predisposing mutations in the ATM, ATR, BRCA1, BRIP1, CBL, CHEK2, DROSHA, FANCL, FH, FLCN, GJB2, MUTYH, PRDM9, RECQL,

RECQL3, SDHA, and SPC genes are known to a skilled person and such mutations can be identified in individuals. Preferably, the prophylactic methods disclosed herein comprise determining the presence of a predisposing mutation in one or more of the ATM, ATR, BRCA1, BRIP1, CBL, CHEK2, DROSHA, FANCL, FH, FLCN, GJB2, MUTYH, PRDM9, RECQL, RECQL3, SDHA, and SPC genes and prophylactically administering the vaccine disclosed herein to an individual having said predisposing mutation in one or more of the ATM, ATR, BRCA1, BRIP1, CBL, CHEK2, DROSHA, FANCL, FH, FLCN, GJB2, MUTYH, PRDM9, RECQL, RECQL3, SDHA, and SPC genes.

As used herein, "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, the verb “to consist” may be replaced by “to consist essentially of” meaning that a compound or adjunct compound as defined herein may comprise additional component(s) than the ones specifically identified, said additional component(s) not altering the unique characteristic of the invention.

The articles “a” and “an” are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, “an element” means one element or more than one element.

The word “approximately” or “about” when used in association with a numerical value (approximately 10, about 10) preferably means that the value may be the given value of 10 more or less 1% of the value.

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

For the purpose of clarity and a concise description features are described herein as part of the same or separate embodiments, however, it will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Frame shift initiated translation in the TCGA (n=10,186) cohort is of sufficient size for immune presentation. A. Peptide length distribution of frame shift mutation initiated translation up to the first encountered stop codon. Dark shades are unique peptide sequences derived from frameshift mutations, light shade indicates the total sum (unique peptides derived from frameshifts multiplied by number of patients containing that frameshift). B. Gene distribution of peptides with length 10 or longer and encountered in up to 10 patients.

Figure 2 *Neo open reading frame peptides (TCGA cohort) converge on common peptide sequences.* Graphical representation in an isoform of TP53, where amino acids are colored distinctly. A. somatic single nucleotide variants, B. positions of frame shift mutations on the -1 and the +1 frame. C. amino acid sequence of TP53. D. Peptide (10aa) library (n=1,000) selection. Peptides belonging to -1 or +1 frame are separated vertically E,F pNOPs for the different frames followed by all encountered frame shift mutations (rows), translated to a stop codon (lines) colored by amino acid.

Figure 3 *A recurrent peptide selection procedure can generate a 'fixed' library to cover up to 50% of the TCGA cohort.* Graph depicts the number of unique patients from the TCGA cohort (10,186 patients) accommodated by a growing library of 10-mer peptides, picked in descending order of the number patients with that sequence in their NOPs. A peptide is only added if it adds a new patient from the TCGA cohort. The dark blue line shows that an increasing number of 10-mer peptides covers an increasing number of patients from the TCGA cohort (up to 50% if using 3000 unique 10-mer peptides). Light shaded blue line depicts the number of patients containing the peptide that was included (right Y-axis). The best peptide covers 89 additional patients from the TCGA cohort (left side of the blue line), the worst peptide includes only 1 additional patient (right side of the blue line).

Figure 4 For some cancers up to 70% of patients contain a recurrent NOP. TCGA cohort ratio of patients separated by tumor type that could be 'helped' using optimally selected peptides for genes encountered most often within a cancer. Coloring represents the ratio, using 1, 2 .. 10 genes, or using all encountered genes (lightest shade)

Figure 5 *Examples of NOPs.* Selection of genes containing NOPs of 10 or more amino acids.

Figure 6 *Frame shift presence in mRNA from 58 CCLE colorectal cancer cell lines.* a. Cumulative counting of RNAseq allele frequency (Samtools mpileup (XO:1/all)) at the genomic position of DNA detected frame shift mutations.
b. IGV examples of frame shift mutations in the BAM files of CCLE cell lines.

Figure 7 *Example of normal isoforms, using shifted frame.*
Genome model of CDKN2A with the different isoforms are shown on the minus strand of the genome. Zoom of the middle exon depicts the 2 reading frames that are encountered in the different isoforms.

Figure 8 *Gene prevalence vs Cancer type.*
Percentage of frameshift mutations (resulting in peptides of 10 aa or longer), assessed by the type of cancer in the TCGA cohort. Genes where 50% or more of the frameshifts occur within a single tumor type are indicated in bold. . Cancer type abbreviations are as follows:

LAML Acute Myeloid Leukemia
ACC Adrenocortical carcinoma
BLCA Bladder Urothelial Carcinoma
LGG Brain Lower Grade Glioma
BRCA Breast invasive carcinoma
CESC Cervical squamous cell carcinoma and endocervical adenocarcinoma
CHOL Cholangiocarcinoma
LCML Chronic Myelogenous Leukemia
COAD Colon adenocarcinoma
CNTL Controls
ESCA Esophageal carcinoma
GBM Glioblastoma multiforme
HNSC Head and Neck squamous cell carcinoma
KICH Kidney Chromophobe
KIRC Kidney renal clear cell carcinoma
KIRP Kidney renal papillary cell carcinoma
LIHC Liver hepatocellular carcinoma
LUAD Lung adenocarcinoma
LUSC Lung squamous cell carcinoma
DLBC Lymphoid Neoplasm Diffuse Large B-cell Lymphoma
MESO Mesothelioma
MISC Miscellaneous
OV Ovarian serous cystadenocarcinoma
PAAD Pancreatic adenocarcinoma
PCPG Pheochromocytoma and Paraganglioma
PRAD Prostate adenocarcinoma
READ Rectum adenocarcinoma
SARC Sarcoma
SKCM Skin Cutaneous Melanoma
STAD Stomach adenocarcinoma
TGCT Testicular Germ Cell Tumors
THYM Thymoma
THCA Thyroid carcinoma
UCS Uterine Carcinosarcoma
UCEC Uterine Corpus Endometrial Carcinoma
UVM Uveal Melanoma

Figure 9 *NOPs in the MSK-IMPACT study*

Frame shift analysis in the targeted sequencing panel of the MSK-IMPACT study, covering up to 410 genes in more 10,129 patients (with at least 1 somatic mutation). a. FS peptide length distribution, b. Gene count of patients containing NOPs of 10 or more amino acids. c. Ratio of patients separated by tumor type that

possess a neo epitope using optimally selected peptides for genes encountered most often within a cancer. Coloring represents the ratio, using 1, 2 .. 10 genes, or using all encountered genes (lightest shade) d. Examples of NOPs for 4 genes.

Figure 10-13 Out-of-frame peptide sequences based on frameshift mutations in kidney cancer patients, for Fig 10 (VHL), Fig 11 (PBRM1), Fig 12 (BAP1), and Fig 13 (SET2D).

EXAMPLES

We have analyzed 10,186 cancer genomes from 33 tumor types of the 40 TCGA (The Cancer Genome Atlas[22]) and focused on the 143,444 frame shift mutations represented in this cohort. Translation of these mutations after re-annotation to a RefSeq annotation, starting in the protein reading frame, can lead to 70,439 unique peptides that are 10 or more amino acids in length (a cut off we have set at a size sufficient to shape a distinct epitope in the context of MHC (figure 1a). The list of genes most commonly represented in the cohort and containing such frame shift mutations is headed nearly exclusively by tumor driver genes, such as NF1, RB, BRCA2 (figure 1b) whose whole or partial loss of function apparently contributes to tumorigenesis. Note that a priori frame shift mutations are expected to result in loss of gene function more than a random SNV, and more independent of the precise position. NOPs initiated from a frameshift mutation and of a significant size are prevalent in tumors, and are enriched in cancer driver genes. Alignment of the translated NOP products onto the protein sequence reveals that a wide array of different frame shift mutations translate in a common downstream stretch of neo open reading frame peptides ('NOPs'), as dictated by the -1 and +1 alternative reading frames. While we initially screened for NOPs of ten or more amino acids, their open reading frame in the out-of-frame genome often extends far beyond that search window. As a result we see (figure 2) that hundreds of different frame shift mutations all at different sites in the gene nevertheless converge on only a handful of NOPs. Similar patterns are found in other common driver genes (figure 5). Figure 2 illustrates that the precise location of a frame shift does not seem to matter much; the more or less straight slope of the series of mutations found in these 10,186 tumors indicates that it is not relevant for the biological effect (presumably reduction/loss of gene function) where the precise frame shift is, as long as translation stalls in the gene before the downstream remainder of the protein is expressed. As can also be seen in figure 2, all frame shift mutations alter the reading frame to one of the two alternative frames. Therefore, for potential immunogenicity the relevant information is the sequence of the alternative ORFs and more precisely, the encoded peptide sequence between 2 stop codons. We term these peptides 'proto Neo Open Reading Frame peptides' or pNOPs, and generated a full list of all thus defined out of frame protein encoding regions in the human genome, of 10 amino acids or longer. We refer to the total sum of all Neo-ORFs as

the Neo-ORFeome. The Neo-ORFeome contains all the peptide potential that the human genome can generate after simple frame-shift induced mutations. The size of the Neo-ORFeome is 46.6 Mb. To investigate whether or not Nonsense Mediated Decay would wipe out frame shift mRNAs, we turned to a public repository containing read coverage for a large collection of cell lines (CCLE). We processed the data in a similar fashion as for the TCGA, identified the locations of frame shifts and subsequently found that, in line with the previous literature[23-25], at least a large proportion of expressed genes also contained the frame shift mutation within the expressed mRNAs (figure 6). On the mRNA level, NOPs can be detected in RNAseq data. We next investigated how the number of patients relates to the number of NOPs. We sorted 10-mer peptides from NOPs by the number of new patients that contain the queried peptide. Assessed per tumor type, frame shift mutations in genes with very low to absent mRNA expression were removed to avoid overestimation. Of note NOP sequences are sometimes also encountered in the normal ORFeome, presumably as result of naturally occuring isoforms (e,g, figure 7). Also these peptides were excluded. We can create a library of possible 'vaccines' that is optimally geared towards covering the TCGA cohort, a cohort large enough that, also looking at the data presented here, it is representative of future patients (figure 10). Using this strategy 30% of all patients can be covered with a fixed collection of only 1,244 peptides of length 10 (figure 3). Since tumors will regularly have more than 1 frame shift mutation, one can use a 'cocktail' of different NOPs to optimally attack a tumor. Indeed, given a library of 1,244 peptides, 27% of the covered TCGA patients contain 2 or more 'vaccine' candidates. In conclusion, using a limited pool with optimal patient inclusion of vaccines, a large proportion of patients is covered. Strikingly, using only 6 genes (TP53, ARID1A, KMT2D, GATA3, APC, PTEN), already 10% of the complete TCGA cohort is covered. Separating this by the various tumor types, we find that for some cancers (like Pheochromocytoma and Paraganglioma (PCPG) or Thyroid carcinoma (THCA)) the hit rate is low, while for others up to 39% can be covered even with only 10 genes (Colon adenocarcinoma (COAD) using 60 peptides, Uterine Corpus Endometrial Carcinoma (UCEC) using 90 peptides), figure 4. At saturation (using all peptides encountered more than once) 50% of TCGA is covered and more than 70% can be achieved for specific cancer types (COAD, UCEC, Lung squamous cell carcinoma (LUSC) 72%, 73%, 73% respectively). As could be expected, these roughly follow the mutational load in the respective cancer types. In addition some frame shifted genes are highly enriched in specific tumor types (e.g. VHL, GATA3. figure 8). We conclude that at saturating peptide coverage, using only very limited set of genes, a large cohort of patients can be provided with off the shelf vaccines. To validate the presence of NOPs, we used the targeted sequencing data on 10,129 patients from the MSK-IMPACT cohort 26. For the 341-410 genes assessed in this cohort, we obtained strikingly similar results in terms of genes frequently affected

by frame shifts and the NOPs that they create (figure 9). Even within this limited set of genes, 86% of the library peptides (in genes targeted by MSK-IMPACT) were encountered in the patient set. Since some cancers, like glioblastoma or pancreatic cancer, show survival expectancies after diagnosis measured in months rather than years (e.g. see 27), it is of importance to move as much of the work load and time line to the moment before diagnosis. Since the time of whole exome sequencing after biopsy is currently technically days, and since the scan of a resulting sequence against a public database describing these NOPs takes seconds, and the shipment of a peptide of choice days, a vaccination can be done theoretically within days and practically within a few weeks after biopsy. This makes it attractive to generate a stored and quality controlled peptide vaccine library based on the data presented here, possibly with replicates stored on several locations in the world. The synthesis in advance will - by economics of scale - reduce costs, allow for proper regulatory oversight, and can be quality certified, in addition to saving the patient time and thus provide chances. The present invention will likely not replace other therapies, but be an additional option in the treatment repertoire. The advantages of scale also apply to other means of vaccination against these common neoantigens, by RNA- or DNA--based approaches (e.g. 28), or recombinant bacteria (e.g. 29). The present invention also provides neoantigen directed application of the CAR-T therapy (For recent review see 30, and references therein), where the T-cells are directed not against a cell-type specific antigens (such as CD19 or CD20), but against a tumor specific neoantigen as provided herein. E.g. once one functional T-cell against any of the common p53 NOPs (figure 2) is identified, the recognition domains can be engineered into T-cells for any future patient with such a NOP, and the constructs could similarly be deposited in an off-the-shelf library. In the present invention, we have identified that various frame shift mutations can result in a source for common neo open reading frame peptides, suitable as pre-synthesized vaccines. This may be combined with immune response stimulating measures such as but not limited checkpoint inhibition to help instruct our own immune system to defeat cancer.

Up to 5% of kidney cancers are a result of a heritable germline mutation. Von Hipple Lindau (VHL)-disease is a well known renal cancer disorder with a genetic basis (Schmidt and Linehan Semin Oncol. 2016 Oct; 43(5): 566–574). Germline mutations in the VHL gene, or other genes, such as BAP1, can predispose an individual to kidney cancer.

Thus, there is an opportunity for prophylactic vaccination to reduce the risk of kidney cancer in individuals with predisposition mutations in these genes. In addition, other non-genetic risk factors may play a role in development of kidney cancer, such as high blood pressure and smoking.A prophylactic vaccine would be

of highest efficacy if it vaccinates against (i) strongly immunogenic antigens, and (ii) antigens that are expected to be present in a large proportion of kidney tumors (observed in patients with predisposition mutations).

We exploited a recent data source from the Hartwig Medical Foundation (see Priestley et al. 2019 at https://doi.org/10.1101/415133) for the presence of targetable neoantigens in kidney tumors in patients with germline mutations in a range of cancer predisposition genes. Amongst 106 kidney tumors, we found that 18 (17%) of the patients carrying those tumors have a mutation in one or more possible predisposition genes (Table 3).

Table3: germline mutations in cancer predisposition genes observed in kidney cancer patients.

Gene	Count
ATM	1
ATR	1
BRCA1	1
BRIP1	1
CBL	1
CHEK2	2
DROSHA	1
FANCL	2
FH	1
FLCN	1
GJB2	2
MUTYH	1
PRDM9	1
RECQL	1
RECQL4	1
SDHA	1
XPC	1

Next, we explored the idea of using neo-open reading frame peptides, resulting from somatic frameshift mutations, as an attractive source of neoantigens in human cancers. Therefore, we calculated the number of kidney cancer patients in the HMF data resource with frameshift mutations leading to possible out of frame neo-peptides. Neo-peptides larger than or equal to 10 amino acids are most frequently found in VHL (12.3%) and PBRM1 (7.5%).

To explore the possibility for prophylactic vaccination of individuals at risk for kidney cancer based on germline predisposing mutations, we determined the genes

for which out-of-frame peptide sequences are found among patients with germline predisposition mutations.
Out of all 18 kidney cancer patients with presumed germline predisposition mutations (in any possible predisposition gene), we found 4 (22%) patients that have a frameshift leading to a neo-peptide in VHL or PBRM1.
We conclude that a considerable fraction of kidney cancer patients with predisposing germline mutations, may benefit from vaccination against frameshift-induced neopeptides. With a peptide vaccine covering only 2 genes (VHL, PBRM1), for 22% of patients with germline mutations, kidney cancer development can possibly be prevented.

Methods:
TCGA frameshift mutations – Frame shift mutations were retrieved from Varscan and mutect files per tumor type via https://portal.gdc.cancer.gov/. Frame shift mutations contained within these files were extracted using custom perl scripts and used for the further processing steps using HG38 as reference genome build.

CCLE frameshift mutations - For the CCLE cell line cohort, somatic mutations were retrieved from http://www.broadinstitute.org/ccle/data/browseData?conversationPropagation=begin (CCLE_hybrid_capture1650_hg19_NoCommonSNPs_NoNeutralVariants_CDS_2012.02.20.maf). Frame shift mutations were extracted using custom perl scripts using hg19 as reference genome.

Refseq annotation - To have full control over the sequences used within our analyses, we downloaded the reference sequences from the NCBI website (2018-02-27) and extracted mRNA and coding sequences from the gbff files using custom perl scripts. Subsequently, mRNA and every exon defined within the mRNA sequences were aligned to the genome (hg19 and hg38) using the BLAT suite. The best mapping locations from the psl files were subsequently used to place every mRNA on the genome, using the separate exons to perform fine placement of the exonic borders. Using this procedure we also keep track of the offsets to enable placement of the amino acid sequences onto the genome.

Mapping genome coordinate onto Refseq - To assess the effect of every mentioned frame shift mutation within the cohorts (CCLE or TCGA), we used the genome coordinates of the frameshifts to obtain the exact protein position on our reference sequence database, which were aligned to the genome builds. This step was performed using custom perl scripts taking into account the codon offsets and strand orientation, necessary for the translation step described below.

Translation of FS peptides - Using the reference sequence annotation and the positions on the genome where a frame shift mutation was identified, the frame shift mutations were used to translate peptides until a stop codon was encountered. The NOP sequences were recorded and used in downstream analyses as described in the text.

Verification of FS mRNA expression in the CCLE colorectal cancer cell lines - For a set of 59 colorectal cancer cell lines, the HG19 mapped bam files were downloaded from https://portal.gdc.cancer.gov/ . Furthermore, the locations of FS mutations were retrieved from CCLE_hybrid_capture1650_hg19_NoCommonSNPs_NoNeutralVariants_CDS_2012.02.20.maf (http://www.broadinstitute.org/ccle/data/browseData?conversationPropagation=begin), by selection only frameshift entries. Entries were processed similarly to to the TCGA data, but this time based on a HG19 reference genome. To get a rough indication that a particular location in the genome indeed contains an indel in the RNAseq data, we first extracted the count at the location of a frameshift by making use of the pileup function in samtools. Next we used the special tag XO:1 to isolate reads that contain an indel in it. On those bam files we again used the pileup function to count the number of reads containing an indel (assuming that the indel would primarily be found at the frameshift instructed location). Comparison of those 2 values can then be interpreted as a percentage of indel at that particular location. To reduce spurious results, at least 10 reads needed to be detected at the FS location in the original bam file.

Defining peptide library - To define peptide libraries that are maximized on performance (covering as many patients with the least amount of peptides) we followed the following procedure. From the complete TCGA cohort, FS translated peptides of size 10 or more (up to the encountering of a stop codon) were cut to produce any possible 10-mer. Then in descending order of patients containing a 10-mer, a library was constructed. A new peptide was added only if an additional patient in the cohort was included. peptides were only considered if they were seen 2 or more times in the TCGA cohort, if they were not filtered for low expression (see Filtering for low expression section), and if the peptide was not encountered in the orfeome (see Filtering for peptide presence orfeome). In addition, since we expect frame shift mutations to occur randomly and be composed of a large array of events (insertions and deletions of any non triplet combination), frame shift mutations being encountered in more than 10 patients were omitted to avoid focusing on potential artefacts. Manual inspection indicated that these were cases with e.g. long stretches of Cs, where sequencing errors are common.

Filtering for low expression - Frameshift mutations within genes that are not expressed are not likely to result in the expression of a peptide. To take this into account we calculated the average expression of all genes per TCGA entity and arbitrarily defined a cutoff of 2 log2 units as a minimal expression. Any frameshift mutation where the average expression within that particular entity was below the cutoff was excluded from the library. This strategy was followed, since mRNA gene expression data was not available for every TCGA sample that was represented in the sequencing data set. Expression data (RNASEQ v2) was pooled and downloaded from the R2 platform (http://r2.amc.nl). In current sequencing of new tumors with the goal of neoantigen identification such mRNA expression studies are routine and allow routine verification of presence of mutant alleles in the mRNA pool.

Filtering for peptide presence orfeome - Since for a small percentage of genes, different isoforms can actually make use of the shifted reading frame, or by chance a 10-mer could be present in any other gene, we verified the absence of any picked peptide from peptides that can be defined in any entry of the reference sequence collection, once converted to a collection of tiled 10-mers.

Generation of cohort coverage by all peptides per gene To generate overviews of the proportion of patients harboring exhaustive FS peptides starting from the most mentioned gene, we first pooled all peptides of size 10 by gene and recorded the largest group of patients per tumor entity. Subsequently we picked peptides identified in the largest set of patients and kept on adding a new peptide in descending order, but only when at least 1 new patient was added. Once all patients containing a peptide in the first gene was covered, we progressed to the next gene and repeated the procedure until no patient with FS mutations leading to a peptide of size 10 was left.

proto-NOP (pNOP) and Neo-ORFeome proto - NOPs are those peptide products that result from the translation of the gene products when the reading frame is shifted by -1 or +1 base (so out of frame). Collectively, these pNOPs form the Neo-Orfeome.As such we generated a pNOP reference base of any peptide with length of 10 or more amino acids, from the RefSeq collection of sequences. Two notes: the minimal length of 10 amino acids is a choice; if one were to set the minimal window at 8 amino acids the total numbers go up a bit, e.g. the 30% patient covery of the library goes up. On a second note: we limited our definition to ORFs that can become in frame after a single insertion deletion on that location; this includes obviously also longer insertion or deletion stretches than +1 or -1. The definition has not taken account more complex events that get an out-of-frame ORF in frame,

such as mutations creating or deleting splice sites, or a combination of two frame shifts at different sites that result in bypass of a natural stop codon; these events may and will occur, but counting those in will make the definition of the Neo-ORFeome less well defined. For the magnitude of the numbers these rare events do not matter much.

Visualizing nops - Visualization of the nops was performed using custom perl scripts, which were assembled such that they can accept all the necessary input data structures such as protein sequence, frameshifted protein sequences, somatic mutation data, library definitions, and the peptide products from frameshift translations.

Detection of frameshift resulting neopeptides in breast cancer patients with cancer predisposition mutations – Somatic and germline mutation data were downloaded from the supplementary files attached to the manuscript posted here: https://www.biorxiv.org/content/biorxiv/early/2019/01/16/415133.full.pdf. Frameshift mutations were selected from the somatic mutation files and out-of-frame peptides were predicted using custom Perl and Python scripts, based on the human reference genome GRCh37. Out-of-frame peptides were selected based on their length (>= 10 amino acids) and mapped against out of frame peptide sequences for each possible alternative transcript for genes present in the human genome, based on Ensembl annotation (ensembl.org).

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Claims

1. A vaccine for use in the treatment of kidney cancer, said vaccine comprising:

(i) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 1, an amino acid sequence having 90% identity to Sequence 1, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 1; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 2, an amino acid sequence having 90% identity to Sequence 2, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 2; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 3, an amino acid sequence having 90% identity to Sequence 3, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 3;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 4, an amino acid sequence having 90% identity to Sequence 4, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 4;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 5, an amino acid sequence having 90% identity to Sequence 5, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 5;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 6, an amino acid sequence having 90% identity to Sequence 6, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 6; and/or

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 7, an amino acid sequence having 90% identity to Sequence 7, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 7;

(ii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 19, an amino acid sequence having 90% identity to Sequence 19, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 19; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 20, an amino acid sequence having 90% identity to Sequence 20, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 20;

(iii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 189, an amino acid sequence having 90% identity to Sequence 189, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 189; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from any one of Sequences 190-192, an amino acid sequence having 90% identity to Sequences 190-192, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 190-192; and/or

(iv) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 311, an amino acid sequence having 90% identity to Sequence 311, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 311; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 312, an amino acid sequence having 90% identity to Sequence 312, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 312; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 313, an amino acid sequence having 90% identity to Sequence 313, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 313.

2. A collection of frameshift-mutation peptides comprising:

(i) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 1, an amino acid sequence having 90% identity to Sequence 1, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 1; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 2, an amino acid sequence having 90% identity to Sequence 2, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 2; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 3, an amino acid sequence having 90% identity to Sequence 3, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 3;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 4, an amino acid sequence having 90% identity to Sequence 4, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 4;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 5, an amino acid sequence having 90% identity to Sequence

5, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 5;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 6, an amino acid sequence having 90% identity to Sequence 6, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 6; and/or

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 7, an amino acid sequence having 90% identity to Sequence 7, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 7;

(ii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 19, an amino acid sequence having 90% identity to Sequence 19, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 19; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 20, an amino acid sequence having 90% identity to Sequence 20, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 20;

(iii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 189, an amino acid sequence having 90% identity to Sequence 189, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 189; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from any one of Sequences 190-192, an amino acid sequence having 90% identity to Sequences 190-192, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 190-192; and/or

(iv) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 311, an amino acid sequence having 90% identity to Sequence 311, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 311; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 312, an amino acid sequence having 90% identity to Sequence 312, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 312; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 313, an amino acid sequence having 90% identity to Sequence 313, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 313.

3. A peptide, or collection of tiled peptides, comprising an amino acid sequence selected from the groups:

(i) Sequences 1-18, an amino acid sequence having 90% identity to Sequences 1-18, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 1-18;

(ii) Sequences 19-188, an amino acid sequence having 90% identity to Sequences 19-188, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 19-188;

(iii) Sequences 189-310, an amino acid sequence having 90% identity to Sequences 189-310, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 189-310; and

(iv) Sequences 311-352, an amino acid sequence having 90% identity to Sequences 311-352, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 311-352.

4. The vaccine of claim 1, the collection of claim 2, or the peptide of claim 3, wherein said peptides are linked, preferably wherein said peptides are comprised within the same polypeptide.

5. One or more isolated nucleic acid molecules encoding the collection of peptides according to claim 2 or 4 or the peptide of claim 3 or 4, preferably wherein the nucleic acid is codon optimized.

6. One or more vectors comprising the nucleic acid molecules of claim 5, preferably wherein the vector is a viral vector.

7. A host cell comprising the isolated nucleic acid molecules according to claim 5 or the vectors according to claim 6.

8. A binding molecule or a collection of binding molecules that bind the peptide or collection of peptides according to any one of claims 2-4, where in the binding molecule is an antibody, a T-cell receptor, or an antigen binding fragment thereof.

9. A chimeric antigen receptor or collection of chimeric antigen receptors each comprising i) a T cell activation molecule; ii) a transmembrane region; and iii) an antigen recognition moiety;
wherein said antigen recognition moieties bind the peptide or collection of peptides according to any one of claims 2-4.

10. A host cell or combination of host cells that express the binding molecule or collection of binding molecules according to claim 8 or the chimeric antigen receptor or collection of chimeric antigen receptors according to claim 9.

11. A vaccine or collection of vaccines comprising the peptide or collection of peptides according to any one of claims 2-4, the nucleic acid molecules of claim 5, the vectors of claim 6, or the host cell of claim 7 or 10; and a pharmaceutically acceptable excipient and/or adjuvant, preferably an immune-effective amount of adjuvant.

12. The vaccine or collection of vaccines of claim 11 for use in the treatment of kidney cancer in an individual, preferably wherein the vaccine or collection of vaccines is used in a neo-adjuvant setting.

13. The vaccine or collection of vaccines for use according to claim 12, wherein said individual has kidney cancer and one or more cancer cells of the individual:

- (i) expresses a peptide having the amino acid sequence selected from Sequences 1-352, an amino acid sequence having 90% identity to any one of Sequences 1-352, or a fragment thereof comprising at least 10 consecutive amino acids of amino acid sequence selected from Sequences 1-352;
- (ii) or comprises a DNA or RNA sequence encoding an amino acid sequences of (i).

14. The vaccine or collection of vaccines of claim 11 for prophylactic use in the prevention of cancer in an individual, preferably wherein the cancer is kidney cancer.

15. The vaccine or collection of vaccines for use according to of any one of claims 12-14, wherein said individual is at risk for developing cancer, preferably wherein said individual has a germline mutation in the ATM, ATR, BRCA1, BRIP1, CBL, CHEK2, DROSHA, FANCL, FH, FLCN, GJB2, MUTYH, PRDM9, RECQL, RECQL3, SDHA, and/or SPC gene.

16. A method of stimulating the proliferation of human T-cells, comprising contacting said T-cells with the peptide or collection of peptides according to any one of claims 2-4, the nucleic acid molecules of claim 5, the vectors of claim 6, the host cell of claim 7 or 10, or the vaccine of claim 11.

17. A method of treating an individual for kidney cancer or reducing the risk of developing said cancer, the method comprising administering to the individual in need thereof the vaccine of claim 11, preferably wherein the individual has a germline mutation in the ATM, ATR, BRCA1, BRIP1, CBL, CHEK2, DROSHA, FANCL, FH, FLCN, GJB2, MUTYH, PRDM9, RECQL, RECQL3, SDHA, and/or SPC gene.

18. A storage facility for storing vaccines, said facility storing at least two different cancer vaccines of claim 11.

19. The storage facility for storing vaccines according to claim 18, wherein said facility stores a vaccine comprising:

(i) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 1, an amino acid sequence having 90% identity to Sequence 1, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 1; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 2, an amino acid sequence having 90% identity to Sequence 2, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 2; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 3, an amino acid sequence having 90% identity to Sequence 3, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 3;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 4, an amino acid sequence having 90% identity to Sequence 4, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 4;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 5, an amino acid sequence having 90% identity to Sequence 5, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 5;

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 6, an amino acid sequence having 90% identity to Sequence 6, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 6; and/or

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 7, an amino acid sequence having 90% identity to Sequence 7, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 7;

and one or more vaccines selected from:

a vaccine comprising:

(ii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 19, an amino acid sequence having 90% identity to Sequence 19, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 19; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 20, an amino acid sequence having 90% identity to Sequence 20, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 20;

a vaccine comprising:

(iii) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 189, an amino acid sequence having 90% identity to Sequence 189, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 189; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from any one of Sequences 190-192, an amino acid sequence having 90% identity to Sequences 190-192, or a fragment thereof comprising at least 10 consecutive amino acids of Sequences 190-192; and/or

a vaccine comprising:

(iv) a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 311, an amino acid sequence having 90% identity to Sequence 311, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 311; and

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 312, an amino acid sequence having 90% identity to Sequence 312, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 312; preferably also comprising

a peptide, or a collection of tiled peptides, having the amino acid sequence selected from Sequence 313, an amino acid sequence having 90% identity to Sequence 313, or a fragment thereof comprising at least 10 consecutive amino acids of Sequence 313.

20. A method for providing a vaccine for immunizing a patient against a cancer in said patient comprising determining the sequence of BAP1, PBRM1, SETD2, and/or VHL in cancer cells of said cancer and when the determined sequence comprises a frameshift mutation that produces a neoantigen of Sequence 1-352 or a fragment thereof, providing a vaccine of claim 11 comprising said neoantigen or a fragment thereof.

21. The method of claim 20, wherein the vaccine is obtained from a storage facility of claim 18 or claim 19.

Fig. 1a

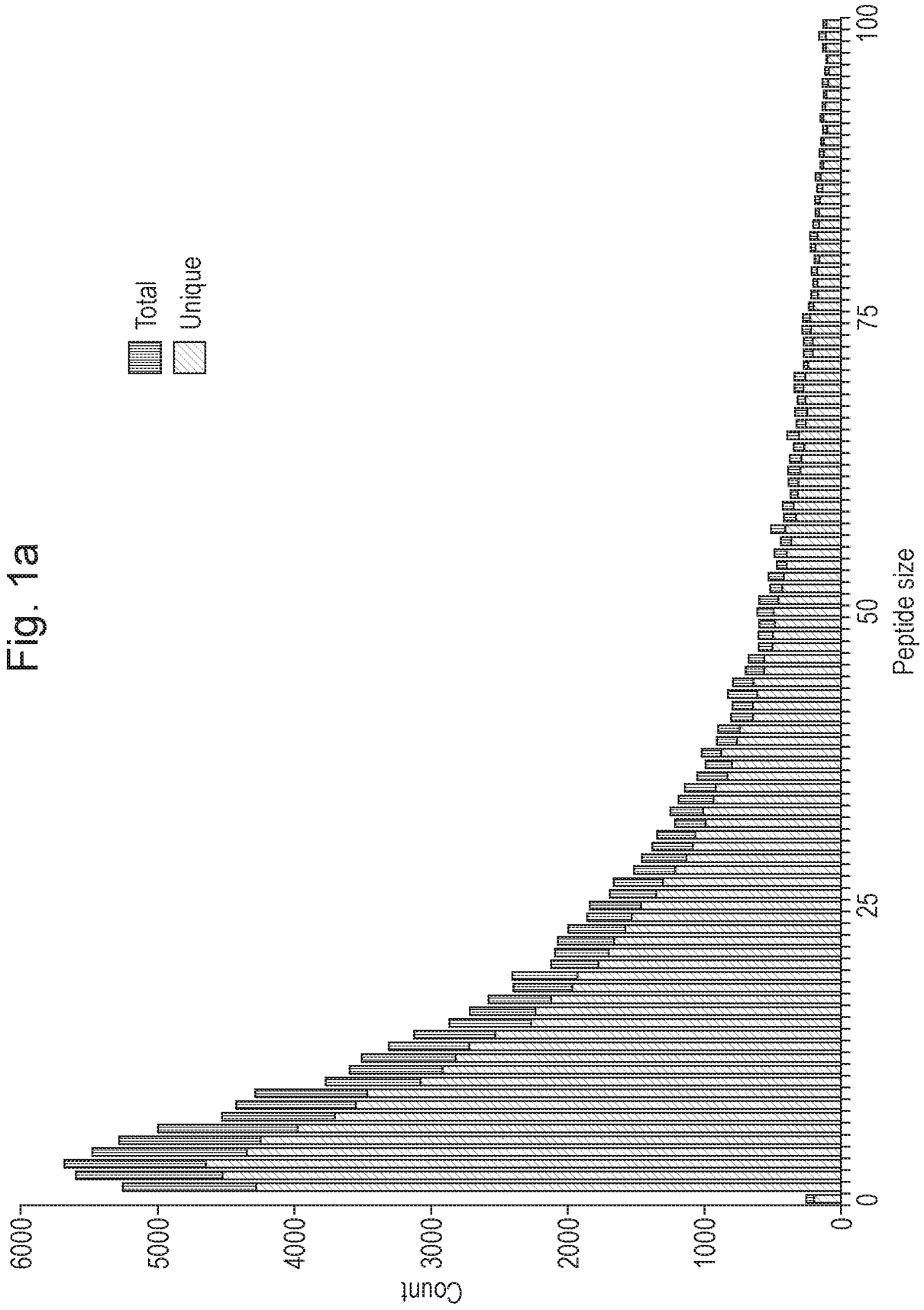

Total
Unique
Count
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5000
4000
3000
2000
1000
0
0
25
50
75
100
Peptide size

Fig. 1b

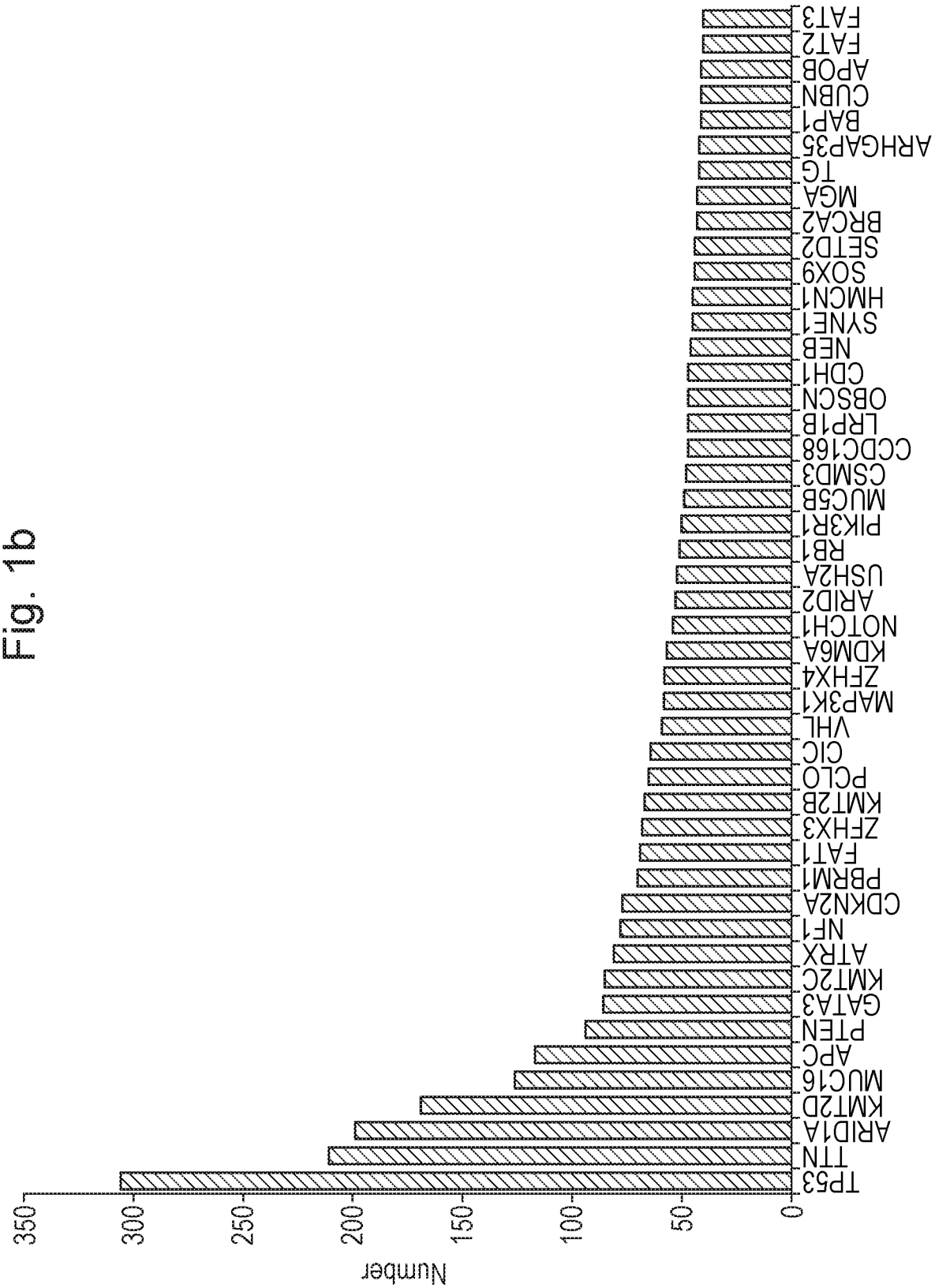

Number
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100
150
200
250
300
350
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KMT2D
MUC16
APC
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KMT2C
ATRX
NF1
CDKN2A
PBRM1
FAT1
ZFHX3
KMT2B
PCLO
CIC
VHL
MAP3K1
ZFHX4
KDM6A
NOTCH1
ARID2
USH2A
RB1
PIK3R1
MUC5B
CSMD3
CCDC168
LRP1B
OBSCN
CDH1
NEB
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TG
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BAP1
CUBN
APOB
FAT2
FAT3

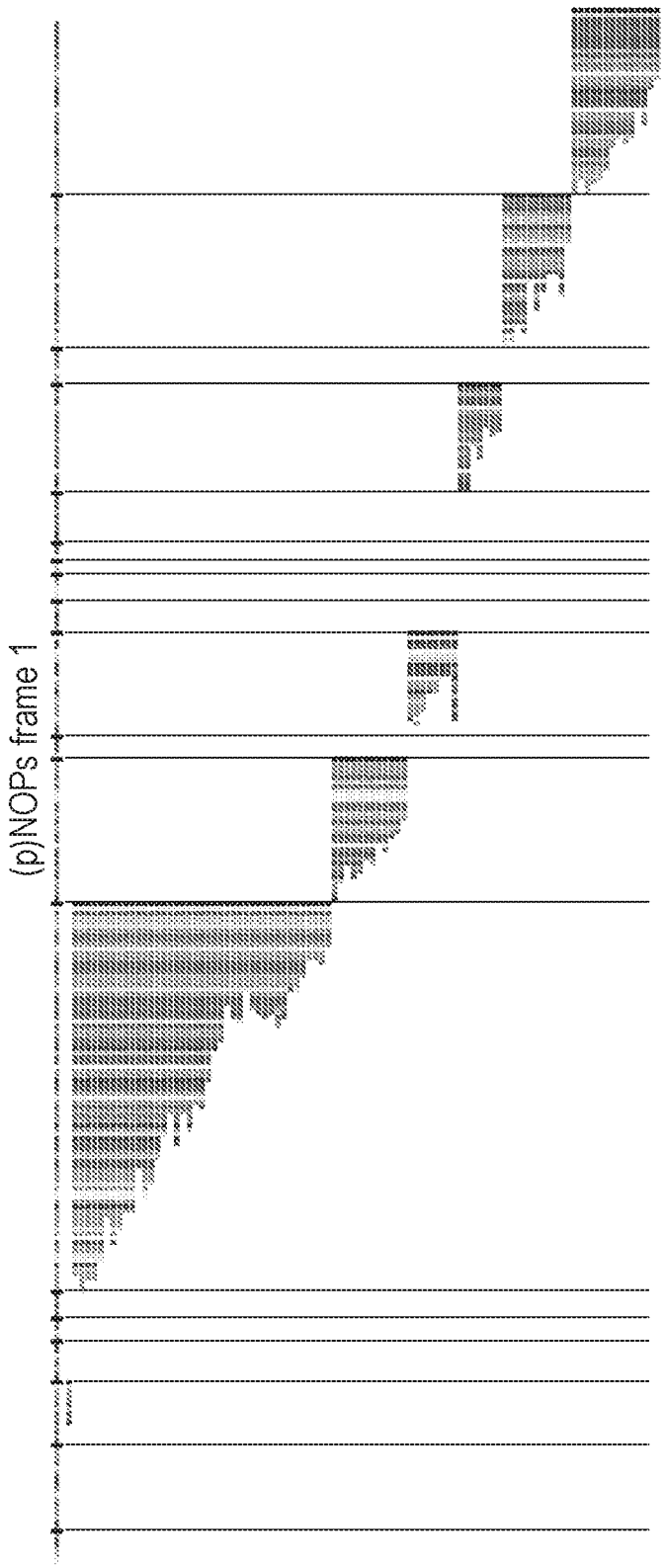
Fig. 2A

Fig. 2B

Fig. 2C

Fig. 2D

Fig. 2E



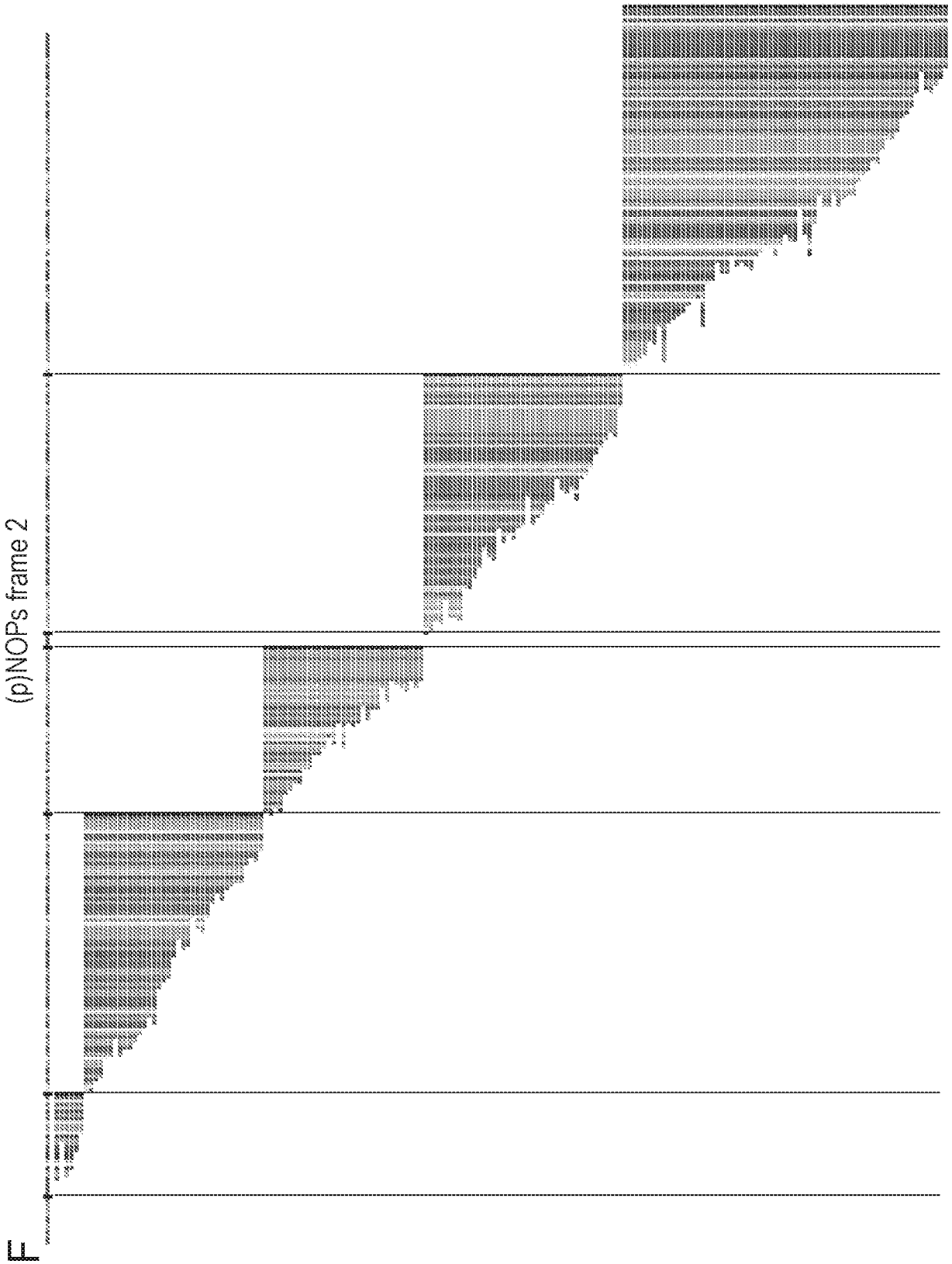


Fig. 2F

Fig. 3

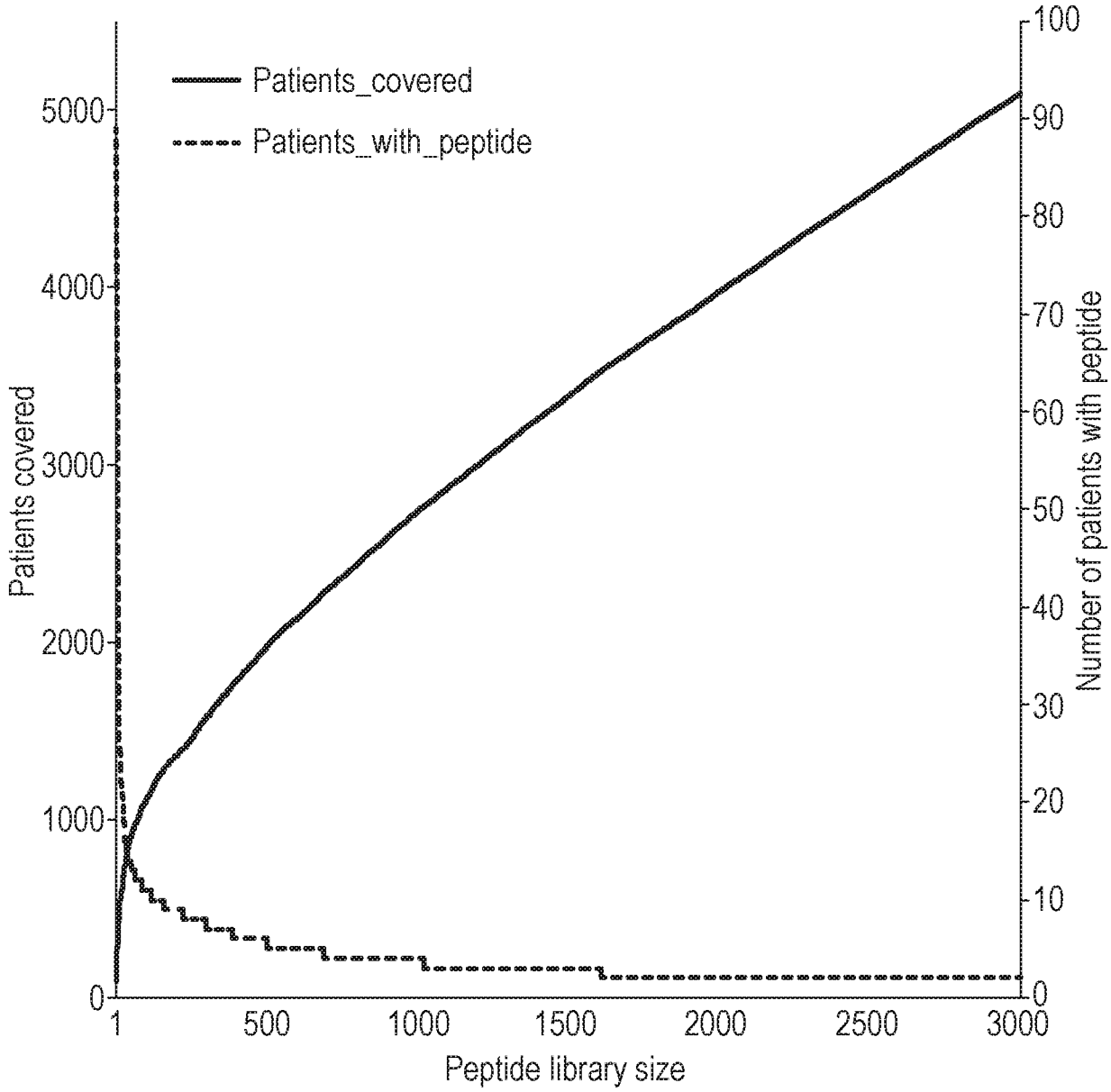

Patients_covered
Patients_with_peptide
Patients covered
Number of patients with peptide
Peptide library size
0
1000
2000
3000
4000
5000
0
10
20
30
40
50
60
70
80
90
100
1
500
1000
1500
2000
2500
3000

Fig. 4

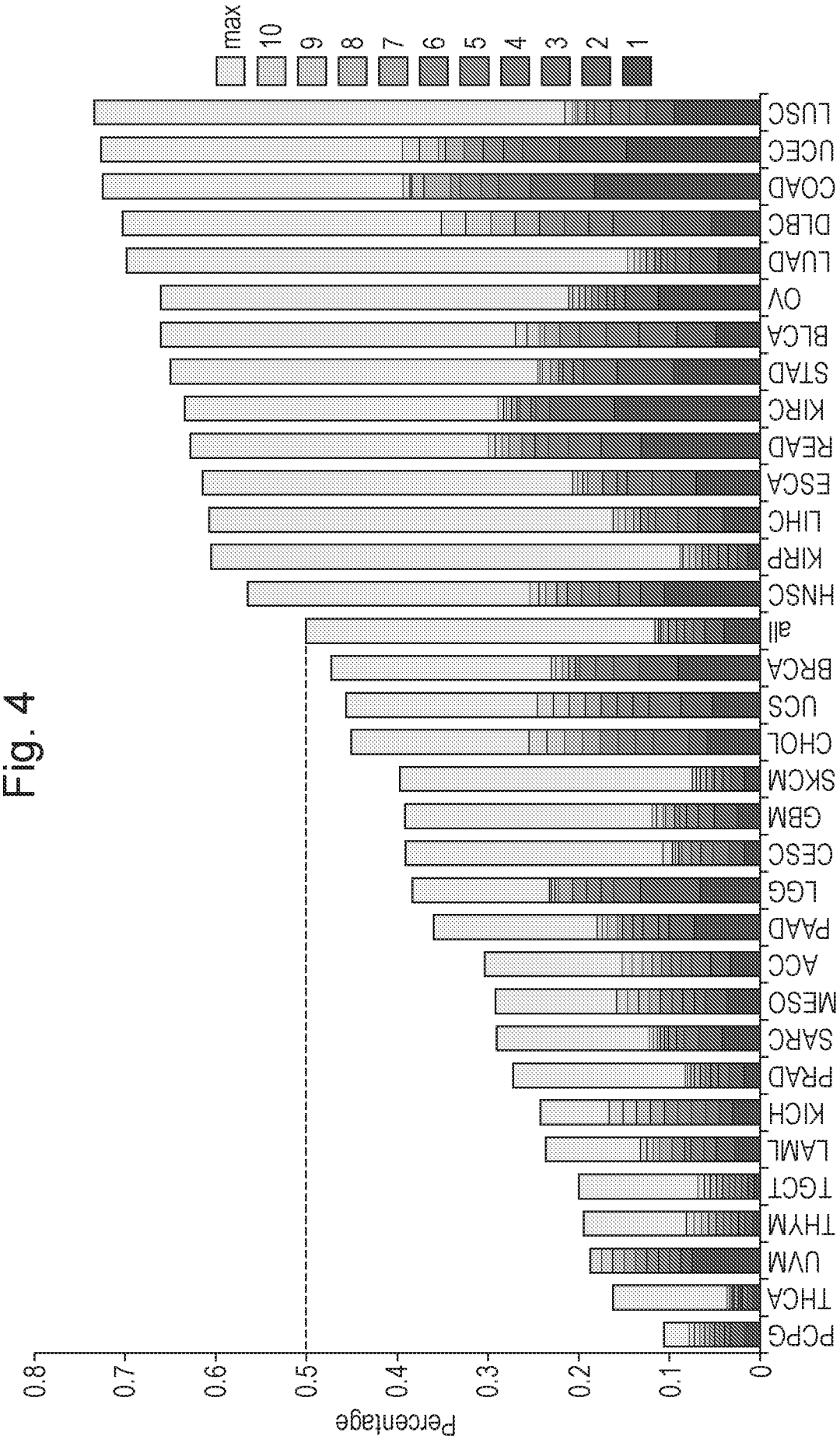

max
10
9
8
7
6
5
4
3
2
1
LUSC
UCEC
COAD
DLBC
LUAD
OV
BLCA
STAD
KIRC
READ
ESCA
LIHC
KIRP
HNSC
all
BRCA
UCS
CHOL
SKCM
GBM
CESC
LGG
PAAD
ACC
MESO
SARC
PRAD
KICH
LAML
TGCT
THYM
UVM
THCA
PCPG
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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Percentage

Fig. 5A

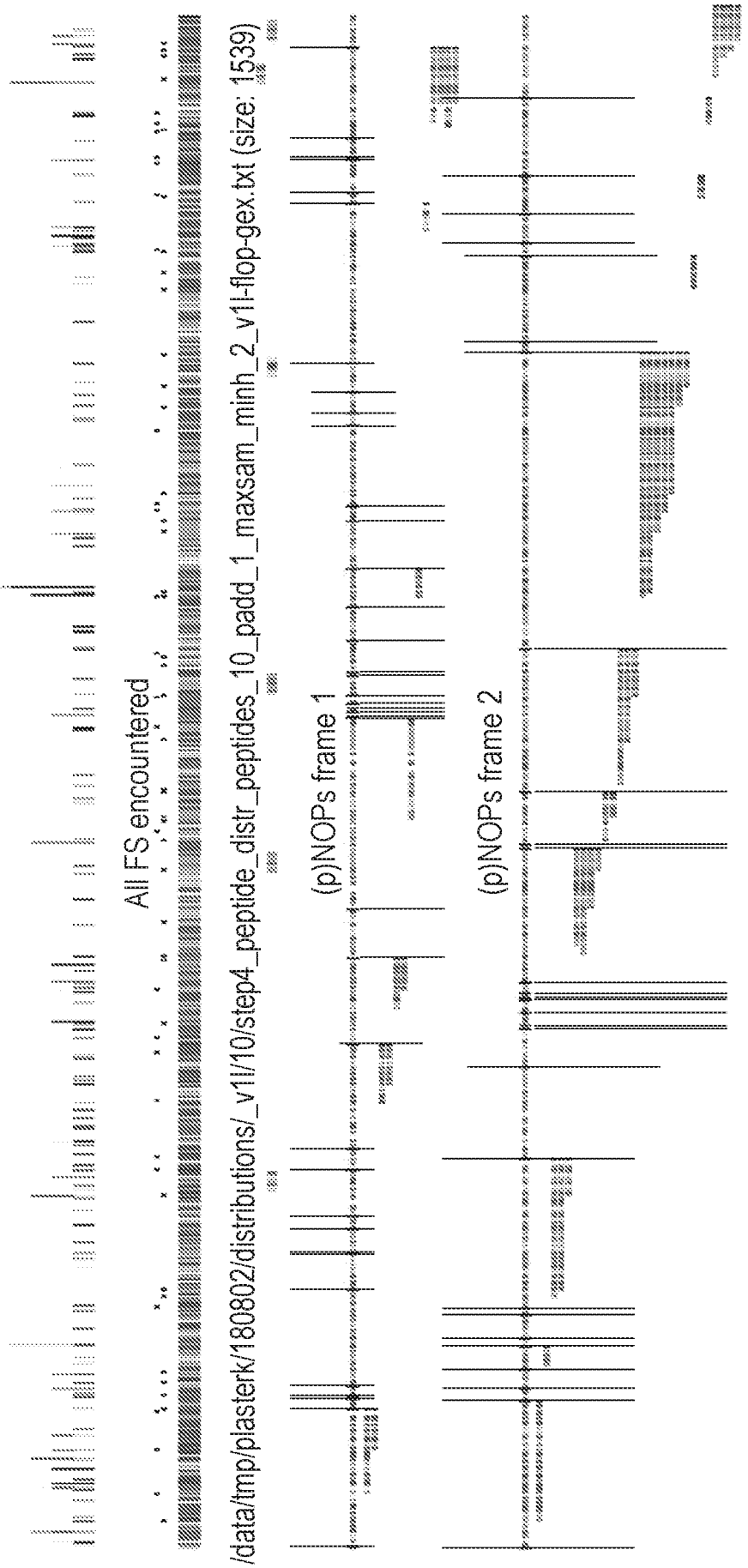

BAP1-NM_004656
Somatic SNVs
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5B

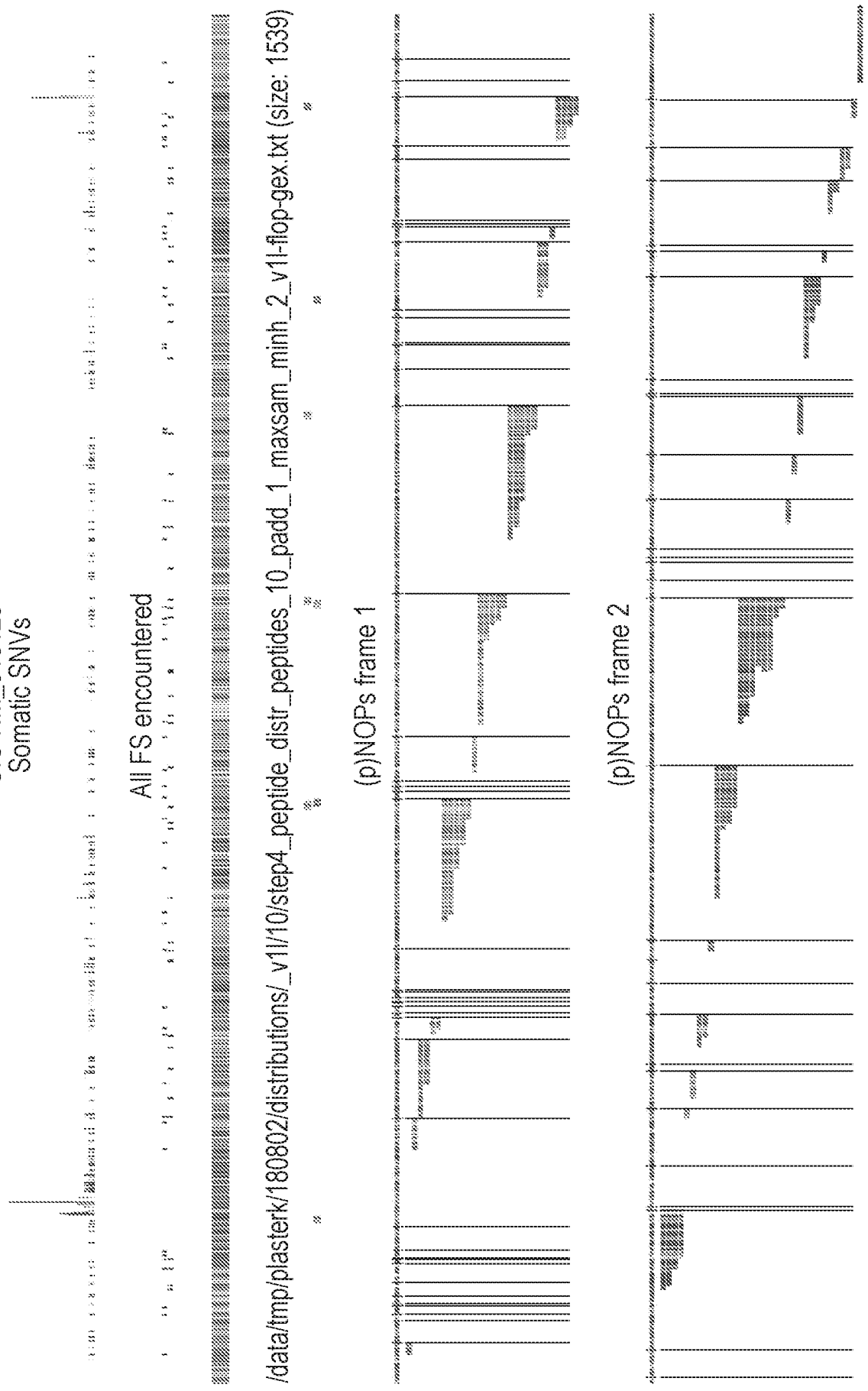

CIC-NM_015125
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5C

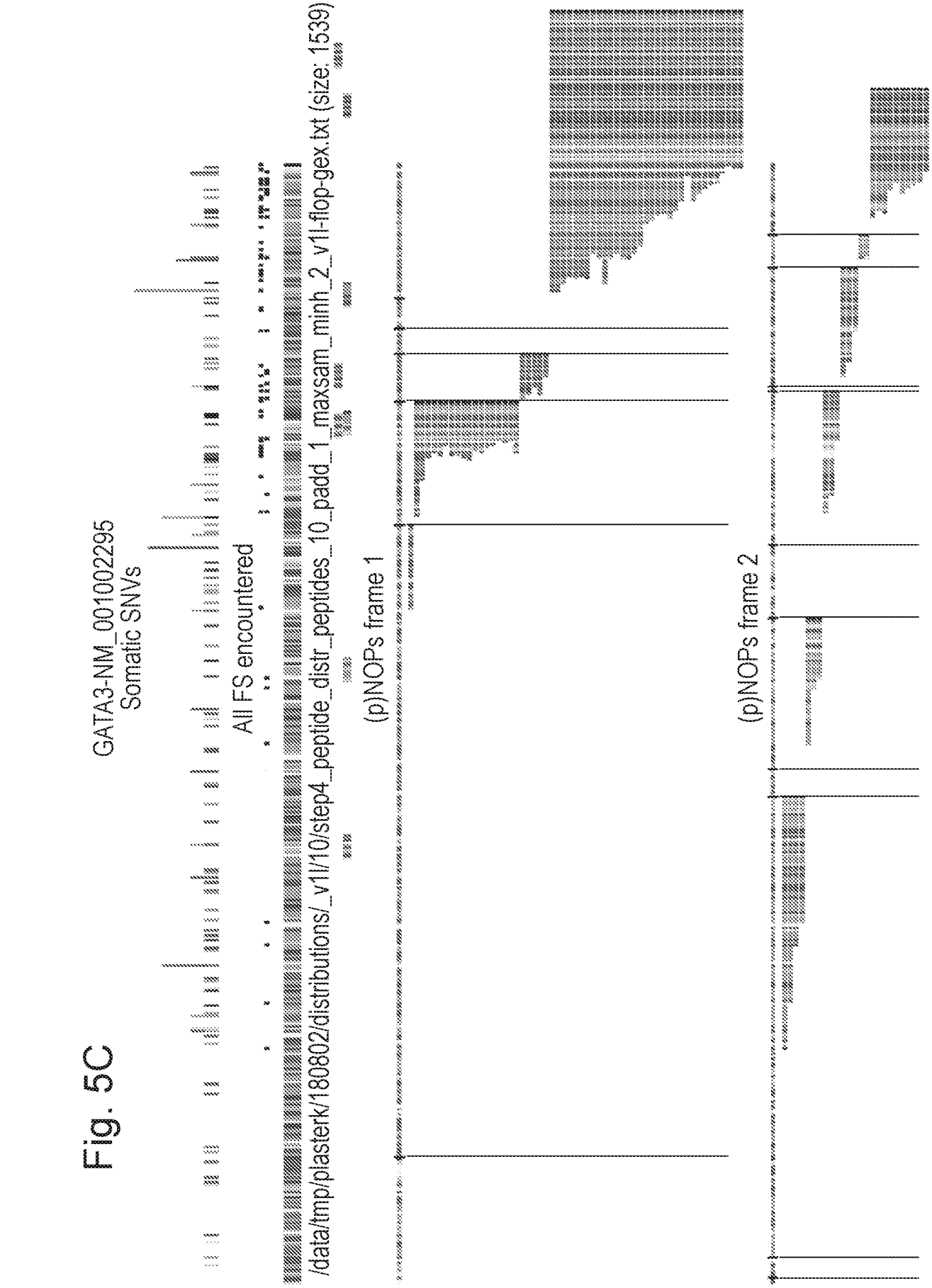

GATA3-NM_001002295
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5D

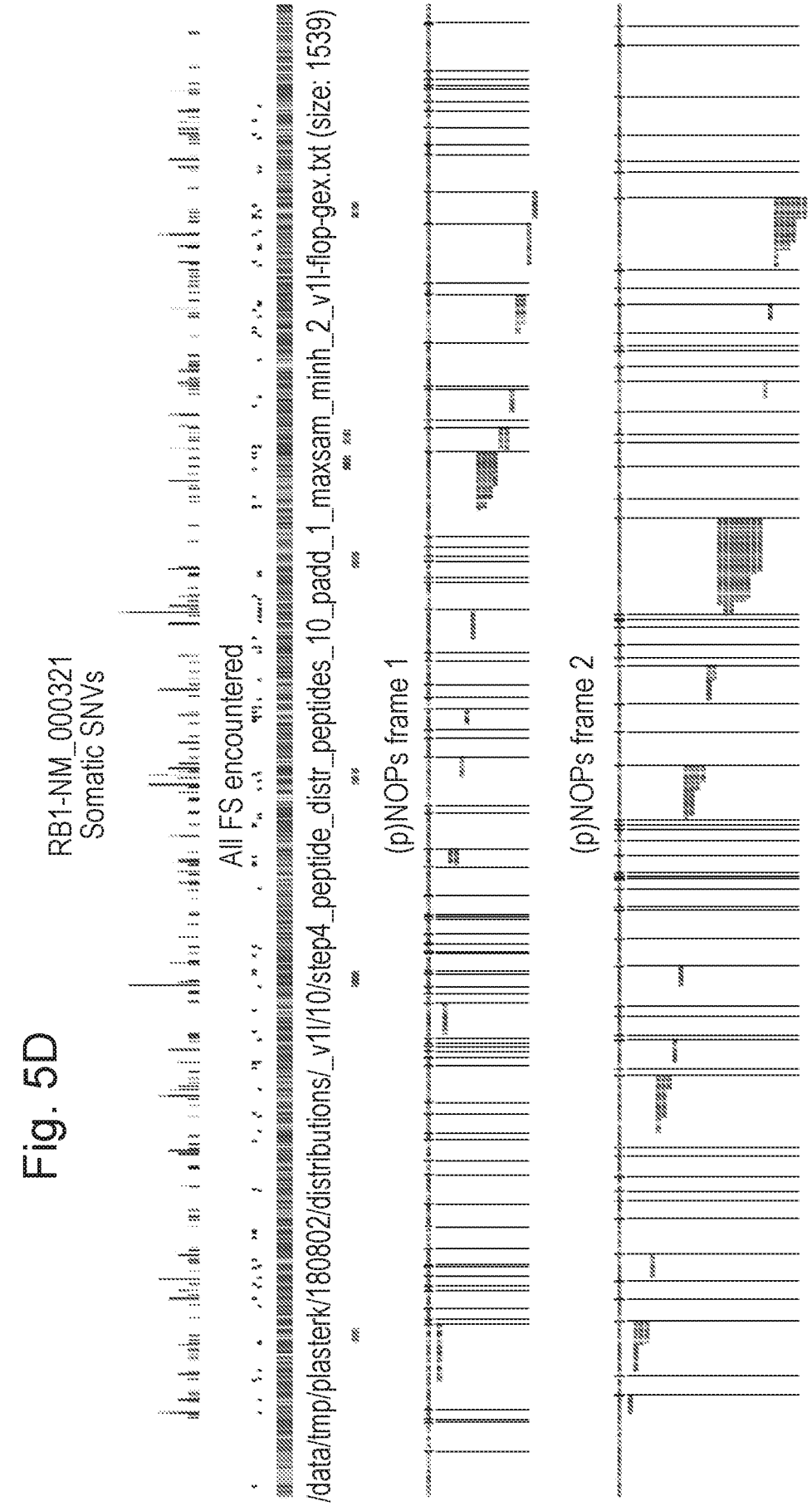

RB1-NM_000321
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5E

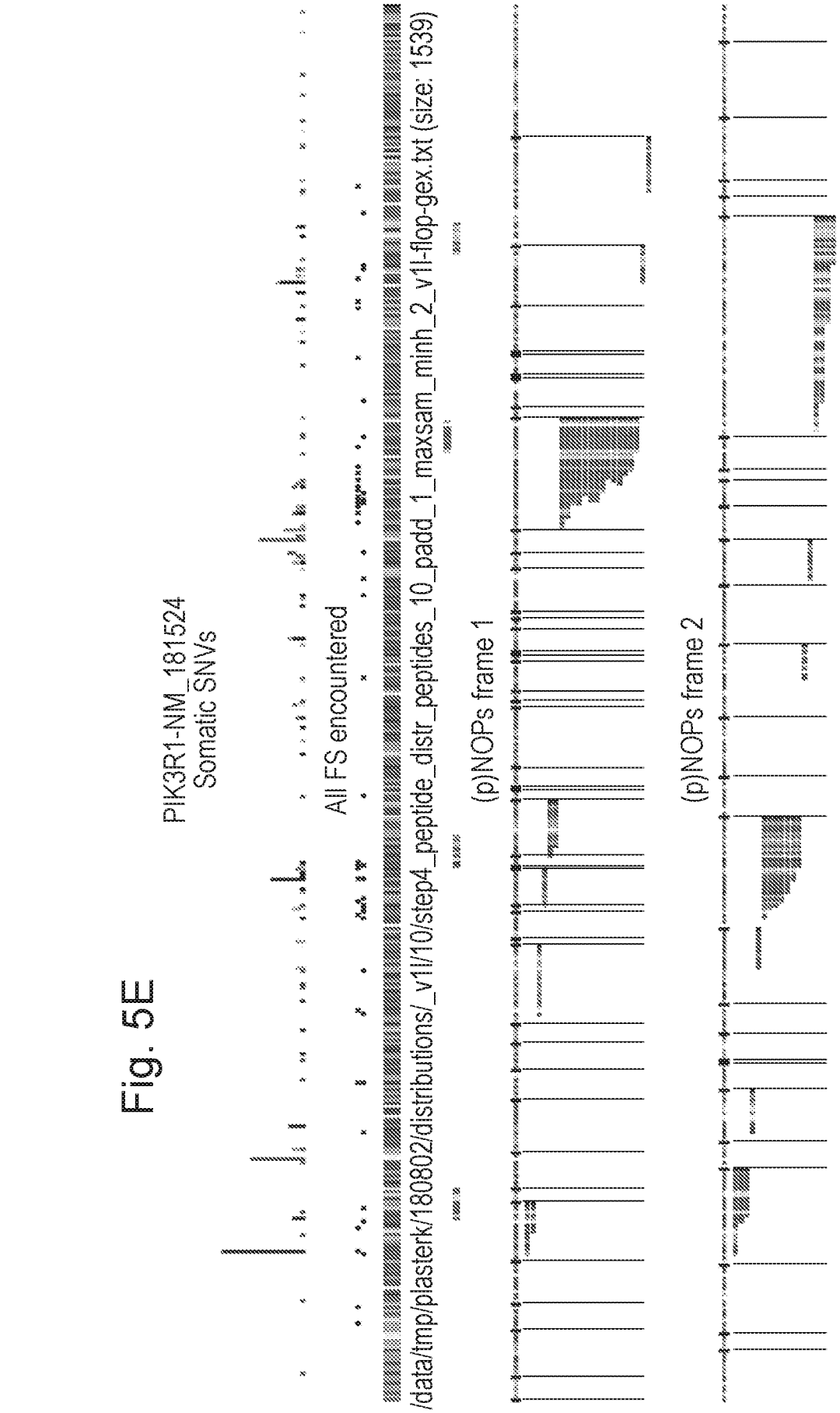

PIK3R1-NM_181524
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5F

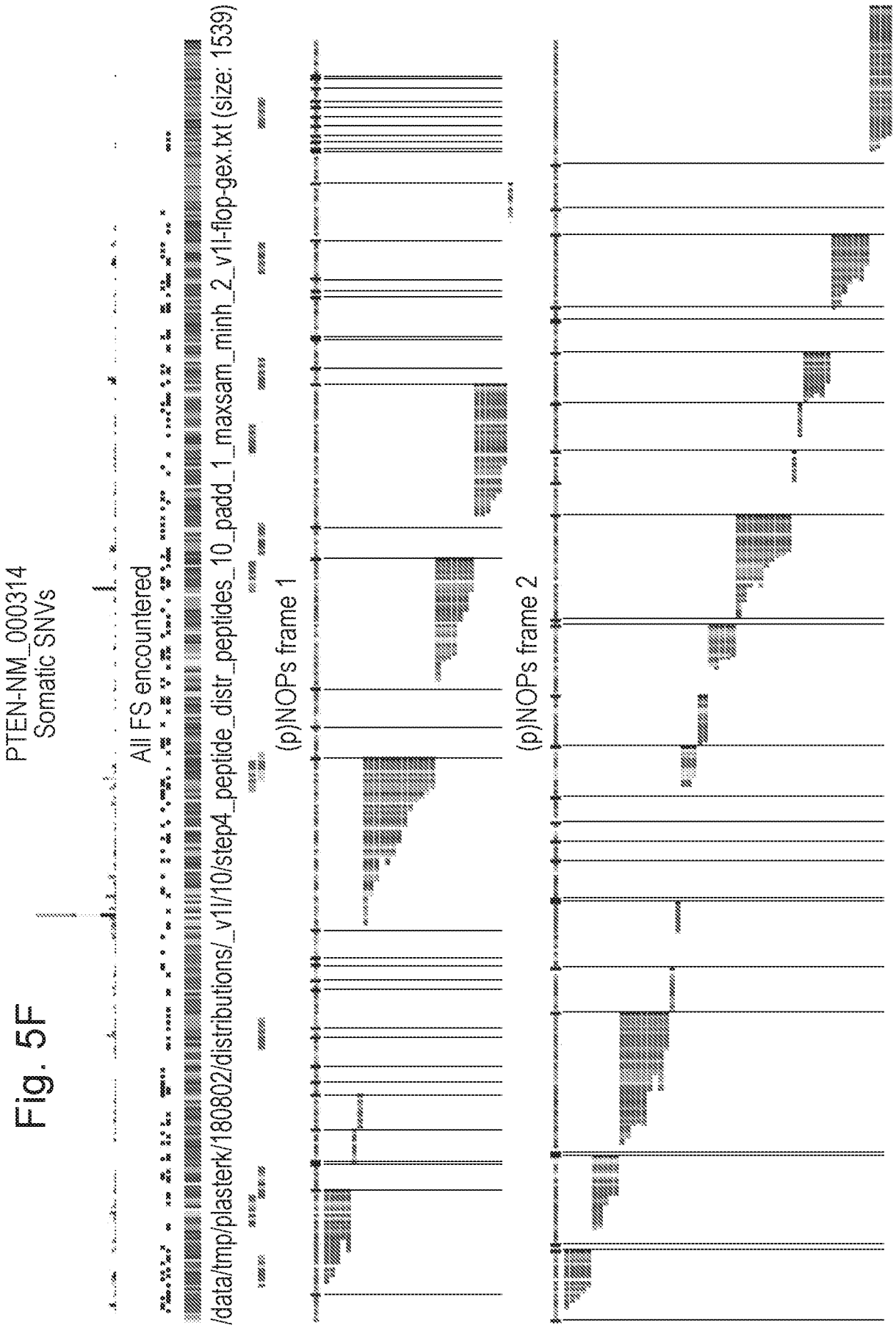

PTEN-NM_000314
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5G

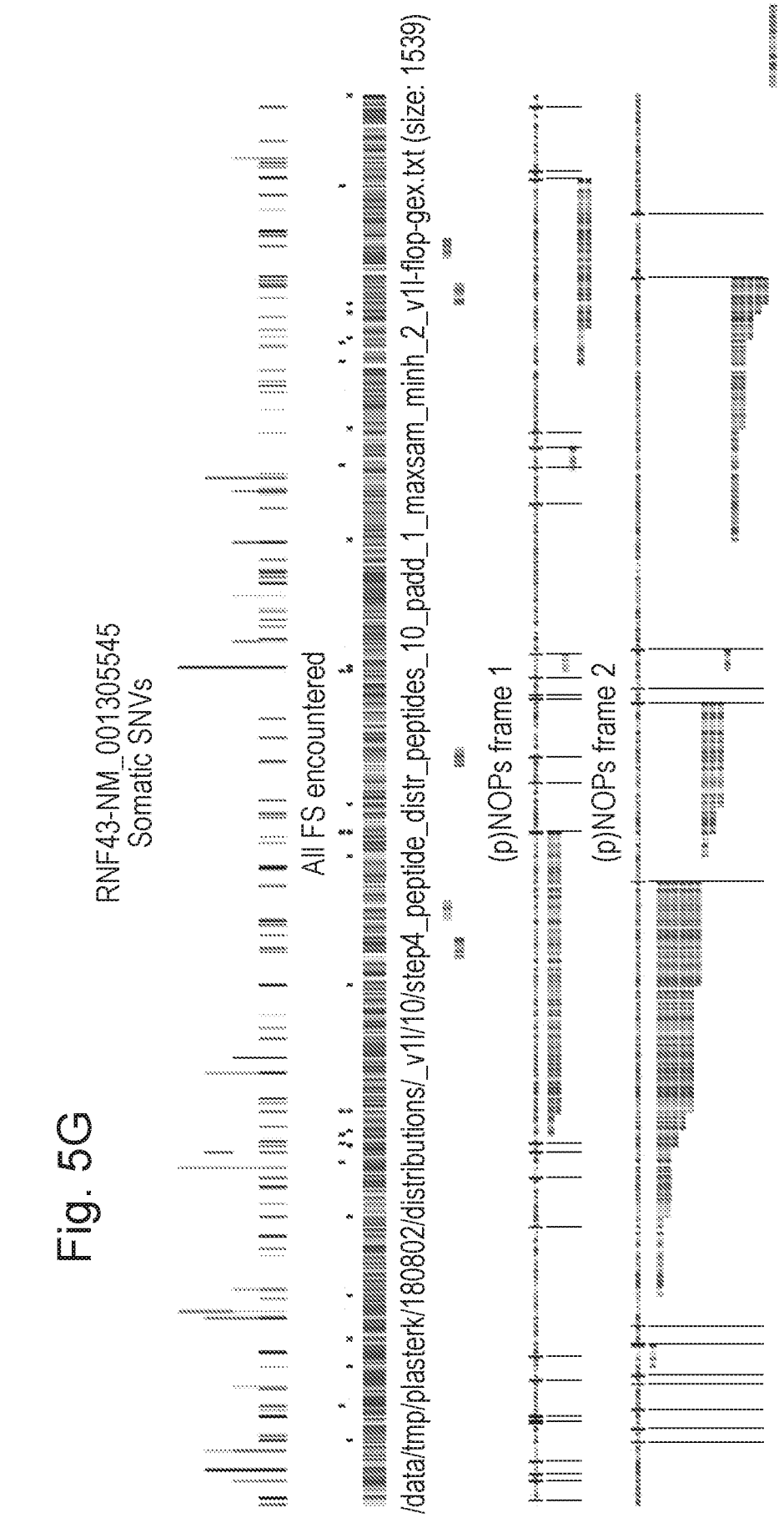

RNF43-NM_001305545
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5H

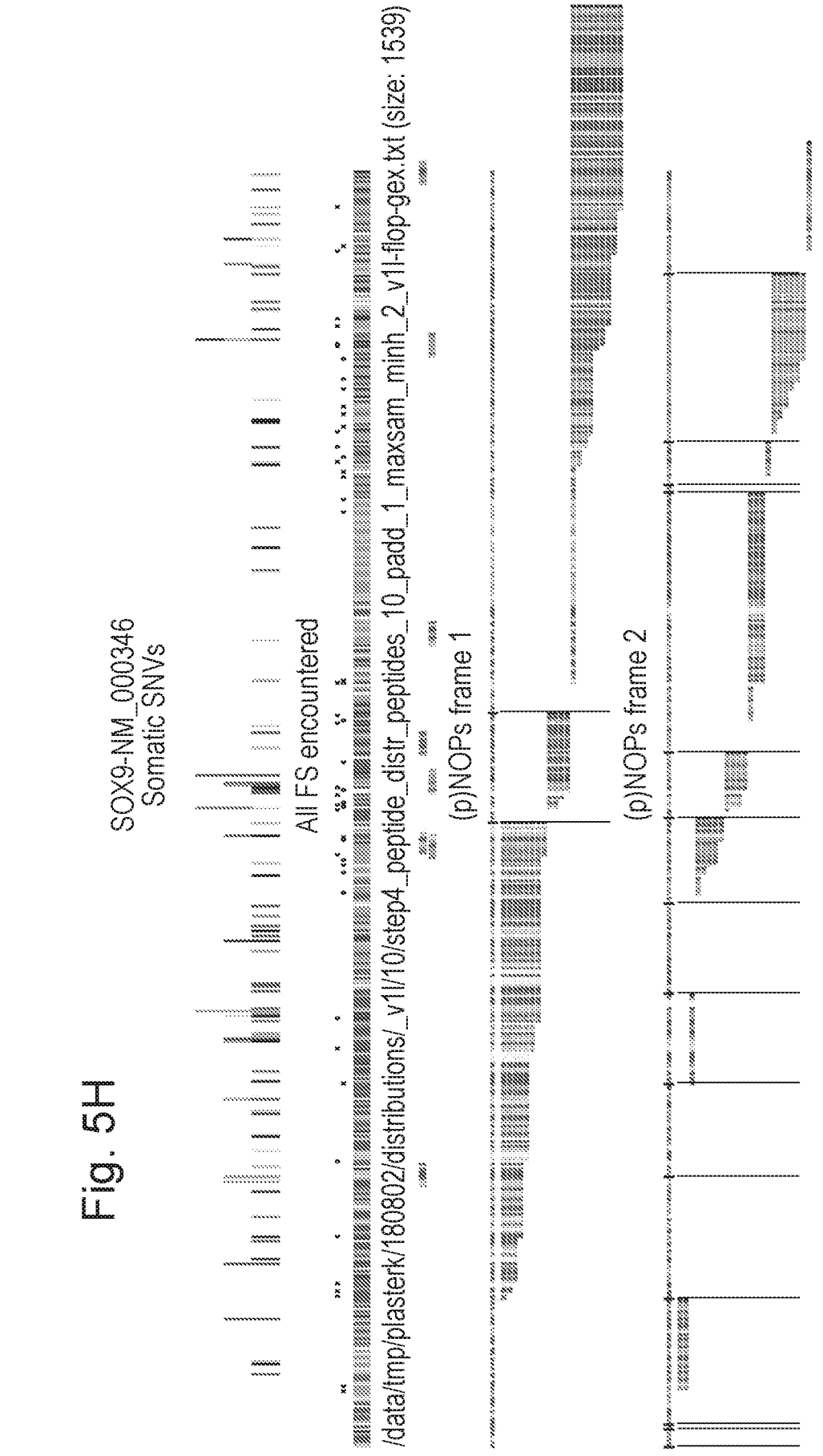

SOX9-NM_000346
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5I

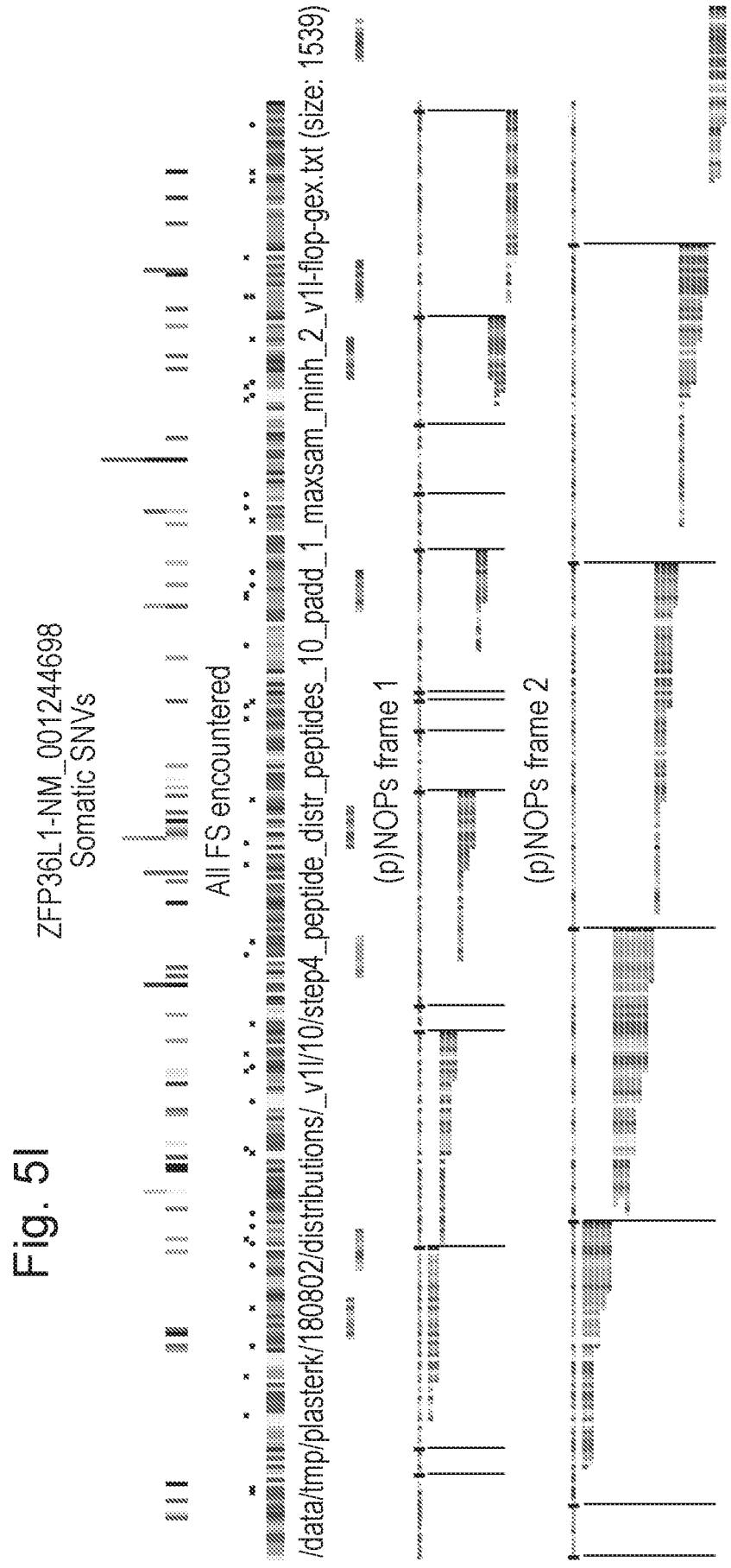

ZFP36L1-NM_001244698
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5J

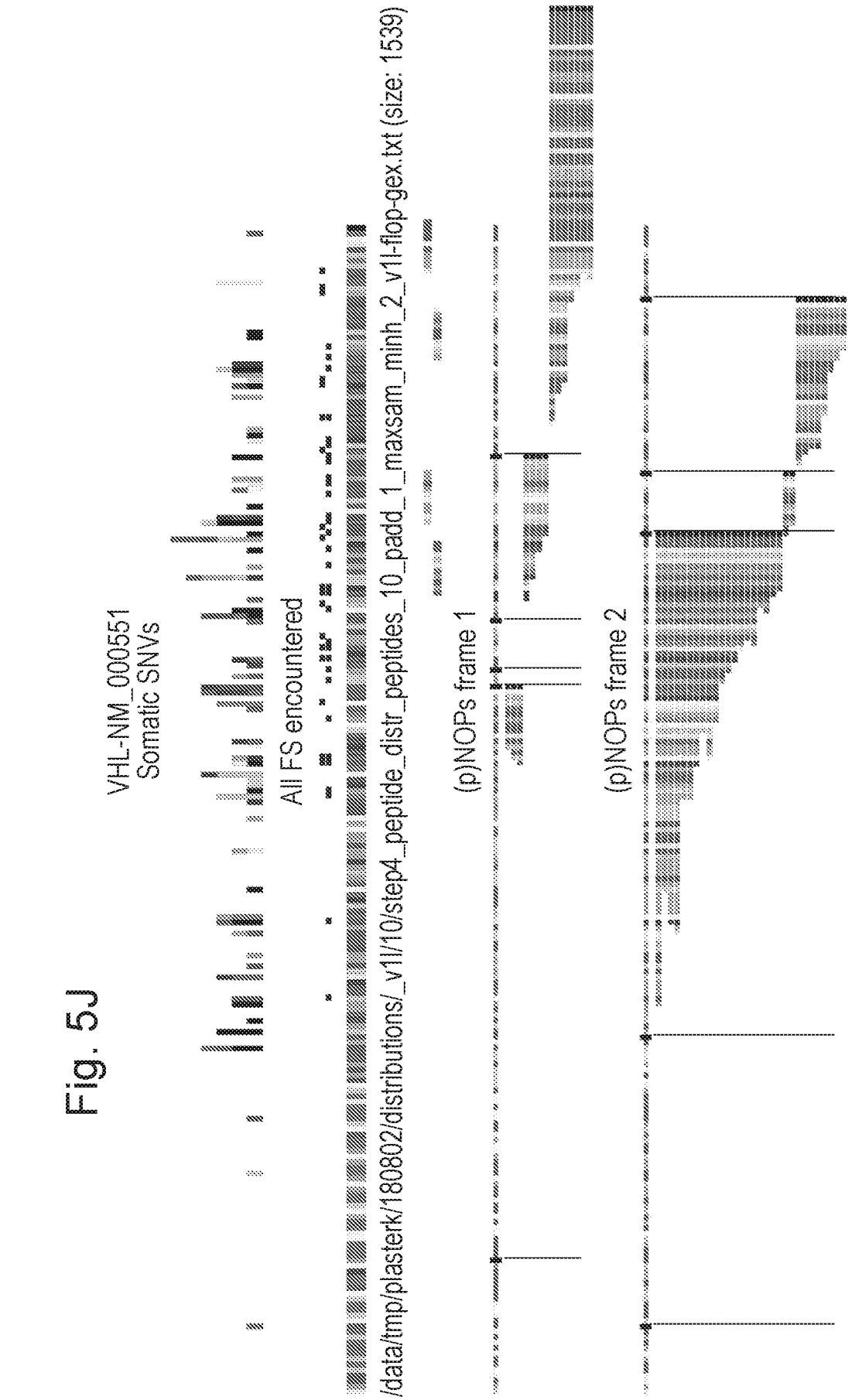

VHL-NM_000551
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 5K

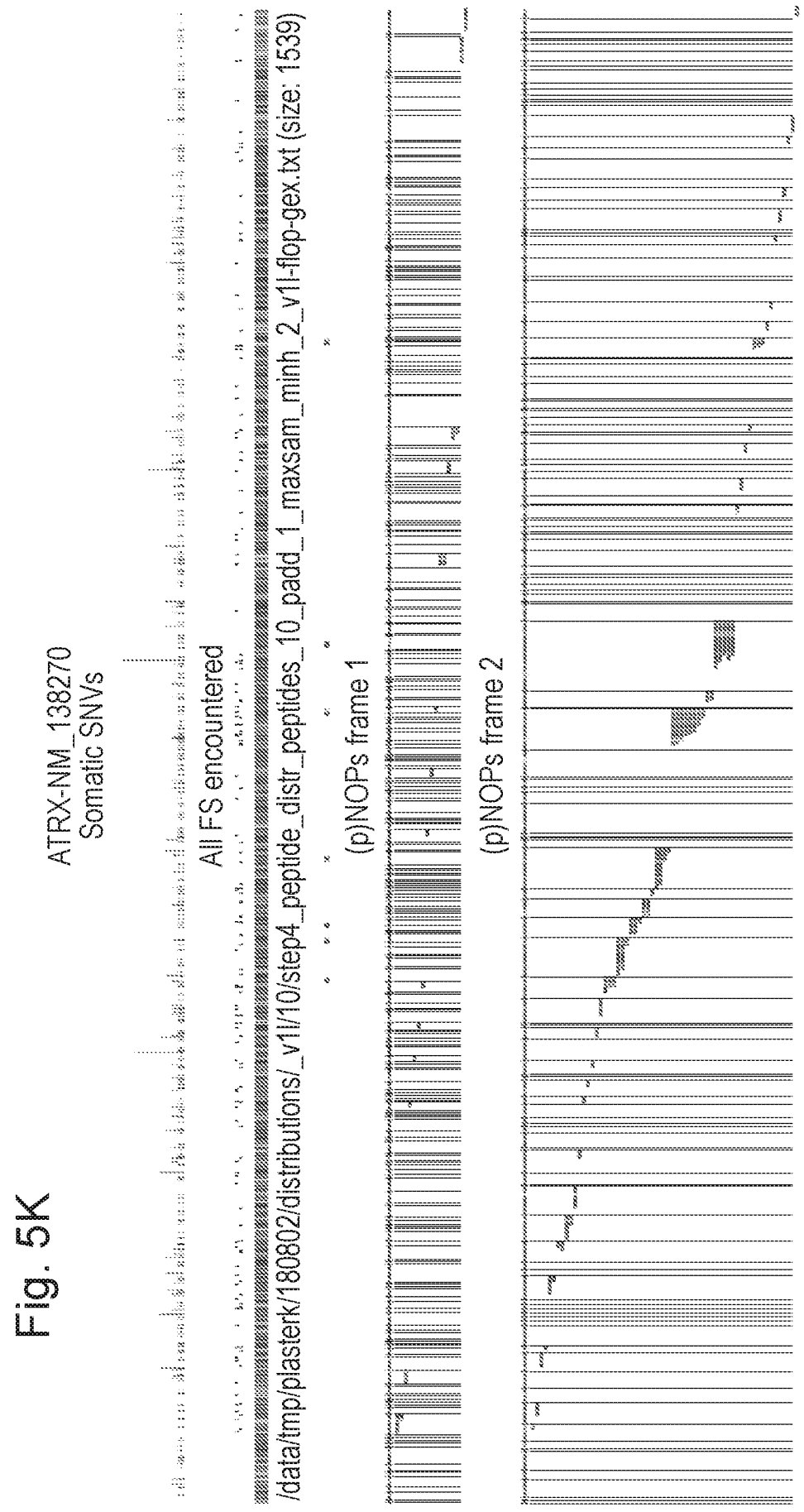

ATRX-NM_138270
Somatic SNVs
All FS encountered
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(p)NOPs frame 1
(p)NOPs frame 2

Fig. 6A

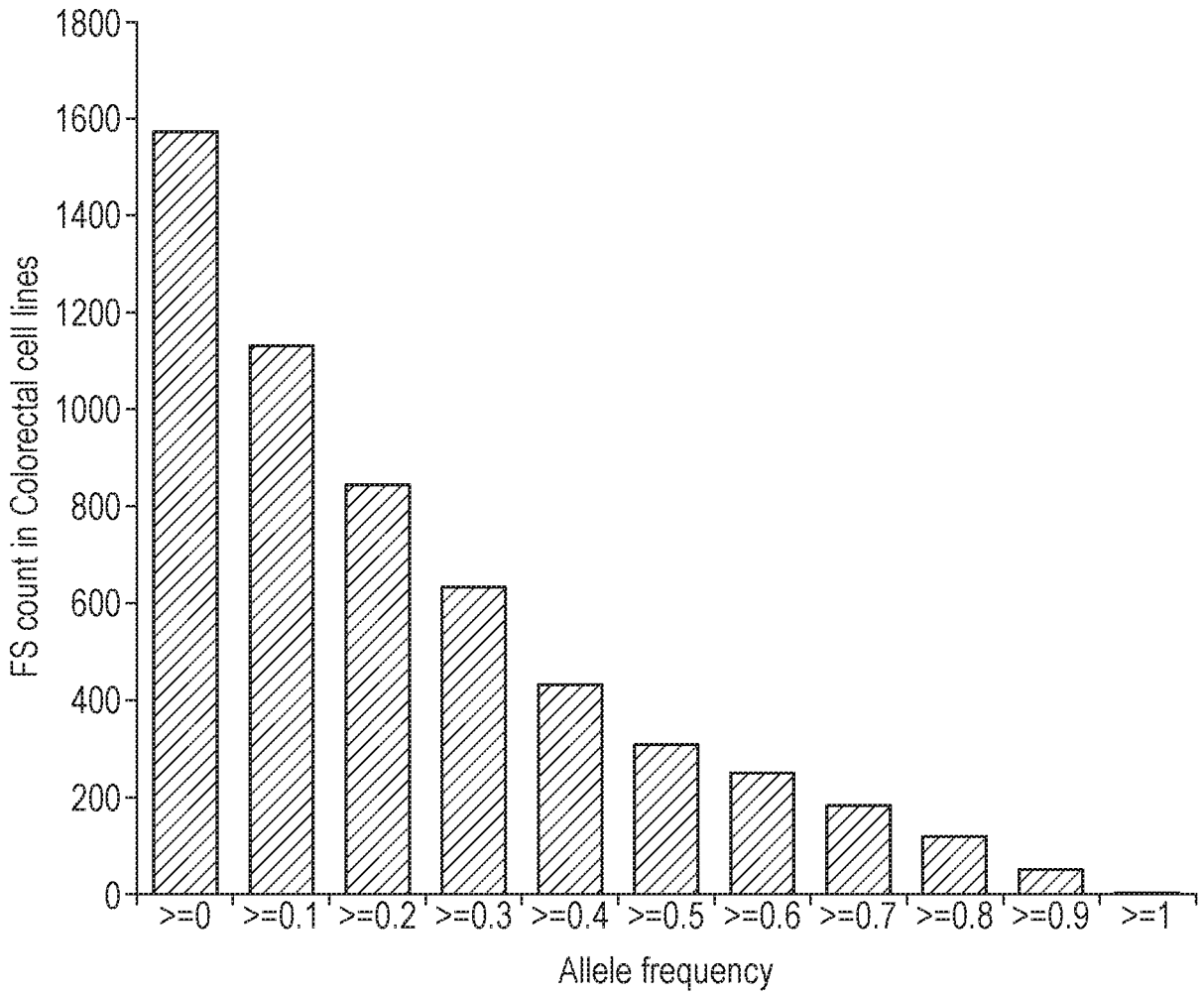

1800
1600
1400
1200
1000
800
600
400
200
0
FS count in Colorectal cell lines
>=0
>=0.1
>=0.2
>=0.3
>=0.4
>=0.5
>=0.6
>=0.7
>=0.8
>=0.9
>=1
Allele frequency

Fig. 6B

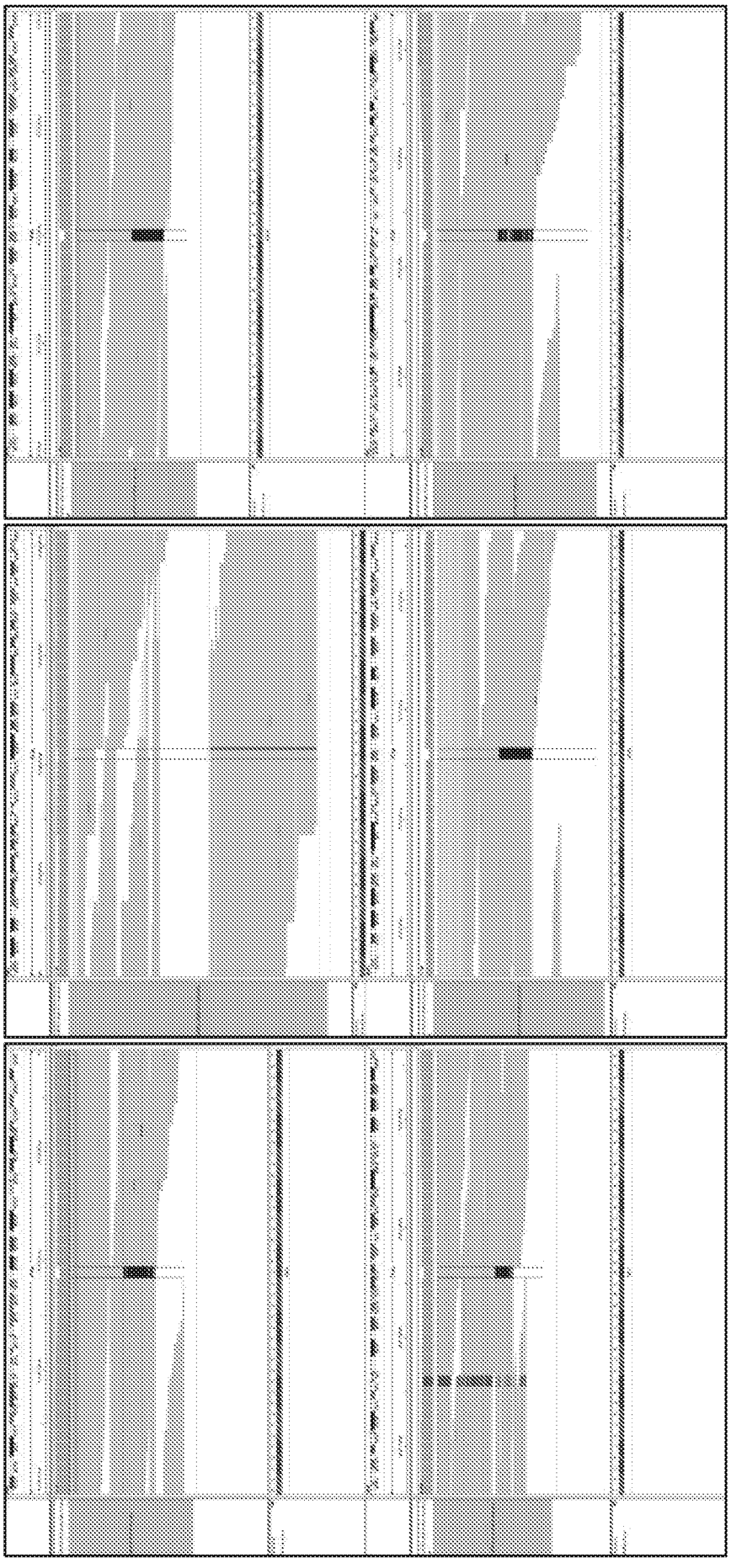


Fig. 7

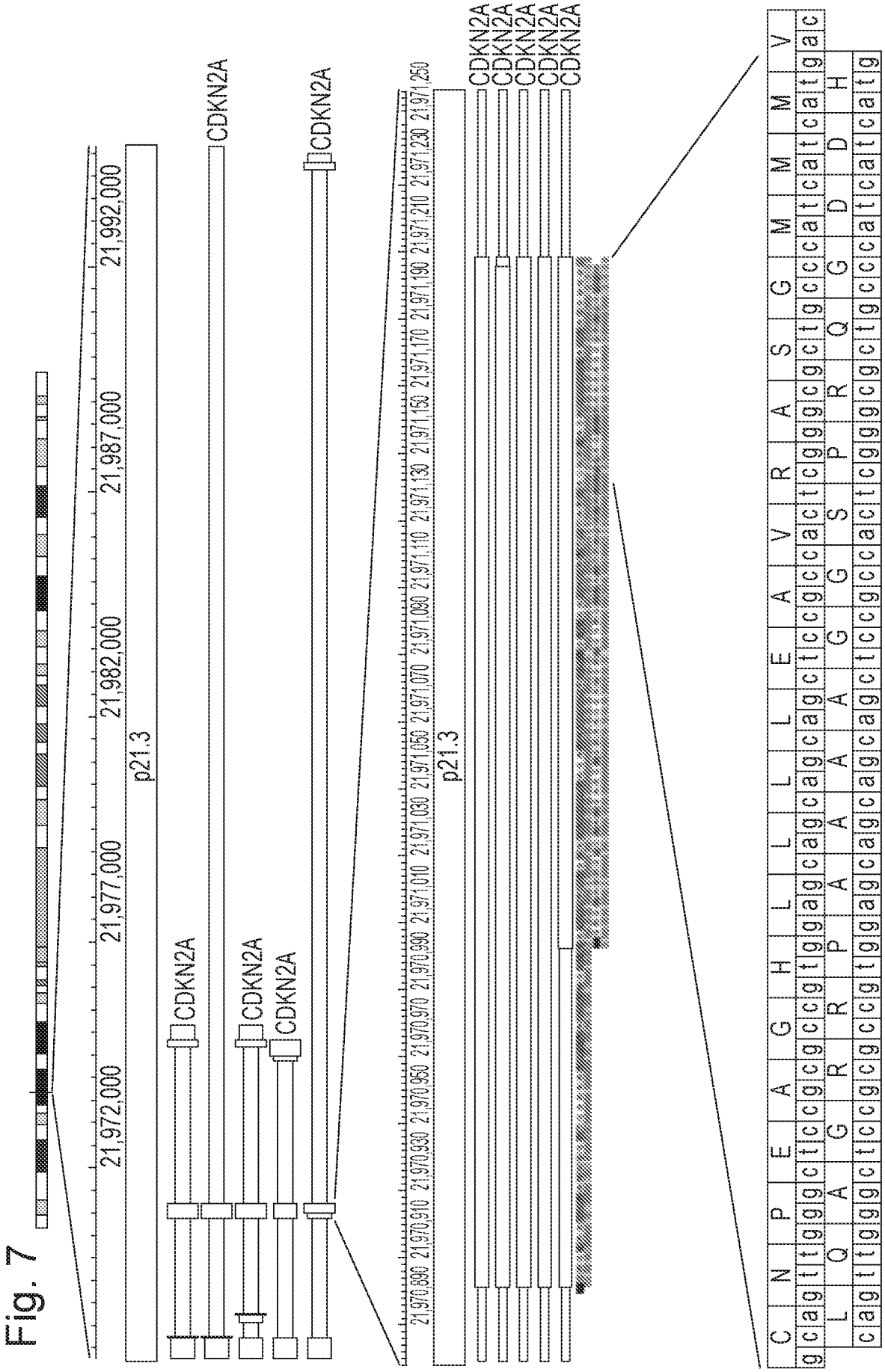

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21,982,000
21,987,000
21,992,000
p21.3
CDKN2A
CDKN2A
CDKN2A
CDKN2A
21,970,590 21,970,610 21,970,630 21,970,650 21,970,670 21,970,690 21,971,010 21,971,030 21,971,050 21,971,070 21,971,090 21,971,110 21,971,130 21,971,150 21,971,170 21,971,190 21,971,210 21,971,230 21,971,250
p21.3
CDKN2A
CDKN2A
CDKN2A
CDKN2A
CDKN2A
C N P E A G H L L L L L E A V R A S G M M M V
g c a g t t g g g c t c c g g c c g t g g a g c a g c a g c a g c a g c t c c g c c a c t c g g g c g c t g c c c a t c a t c a t g a c
L Q A G R R P A A A A A A G S P R Q G D D H
c a g t t g g g c t c c g g c c g t g g a g c a g c a g c a g c t c c g c c a c t c g g g c g c t g c c c a t c a t c a t g

Fig. 8

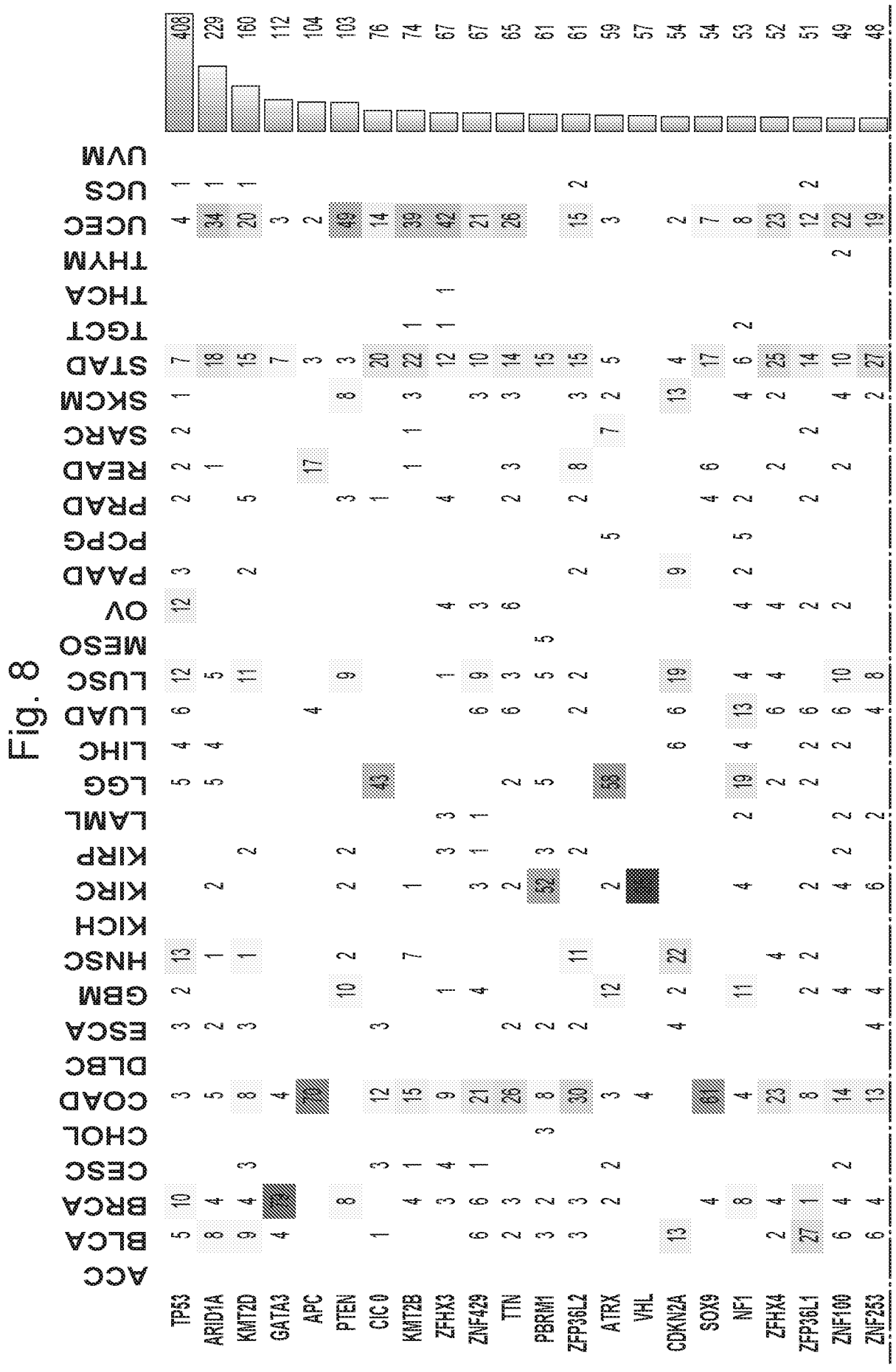

ACC
BLCA
BRCA
CESC
CHOL
COAD
DLBC
ESCA
GBM
HNSC
KICH
KIRC
KIRP
LAML
LGG
LIHC
LUAD
LUSC
MESO
OV
PAAD
PCPG
PRAD
READ
SARC
SKCM
STAD
TGCT
THCA
THYM
UCEC
UCS
UVM
TP53
ARID1A
KMT2D
GATA3
APC
PTEN
CIC 0
KMT2B
ZFHX3
ZNF429
TTN
PBRM1
ZFP36L2
ATRX
VHL
CDKN2A
SOX9
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ZFP36L1
ZNF100
ZNF253

Fig. 8 (Cont. I)

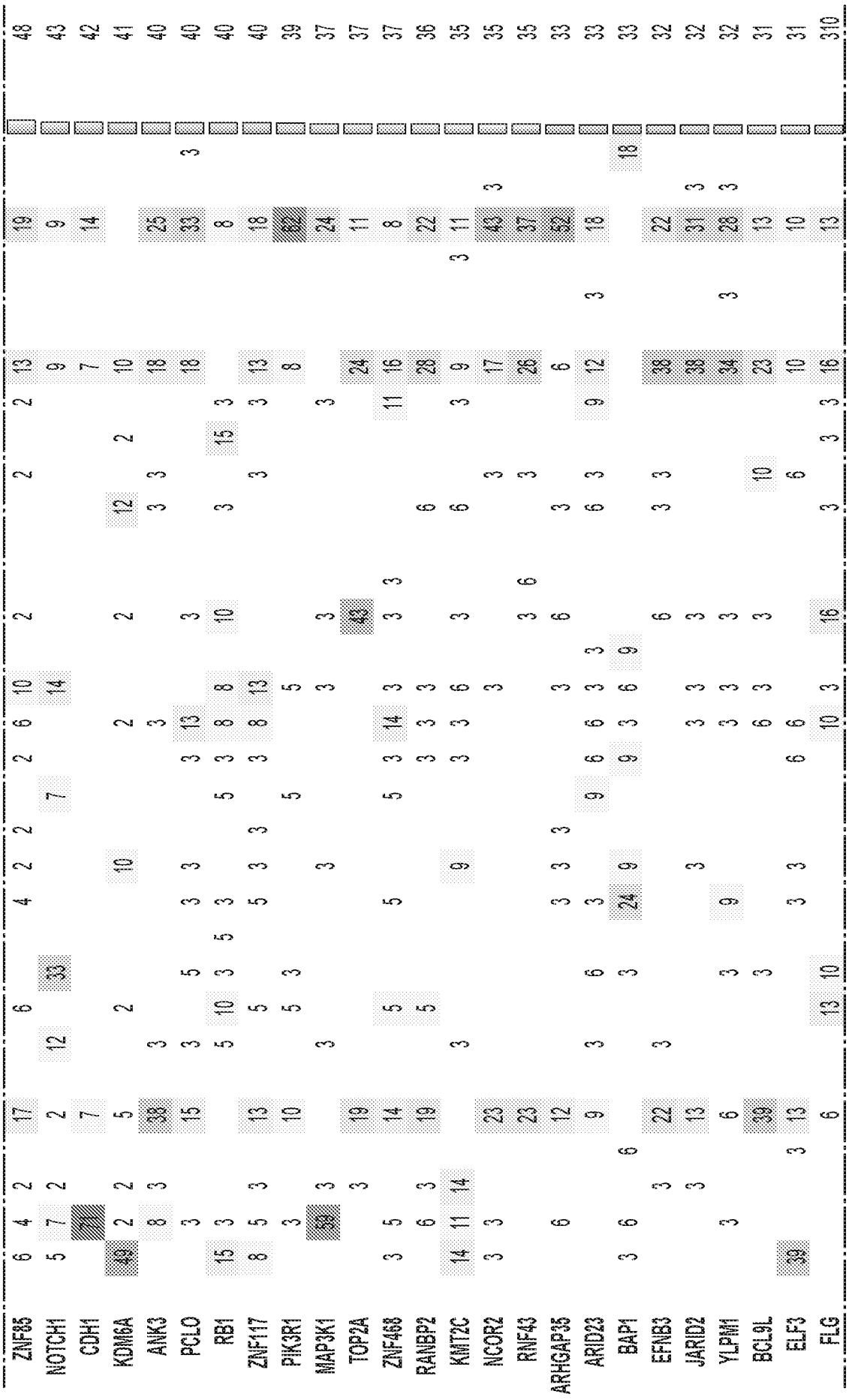

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ANK3
PCLO
RB1
ZNF117
PIK3R1
MAP3K1
TOP2A
ZNF468
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KMT2C
NCOR2
RNF43
ARHGAP35
ARID23
BAP1
EFNB3
JARID2
YLPM1
BCL9L
ELF3
FLG

Fig. 8 (Cont. III)

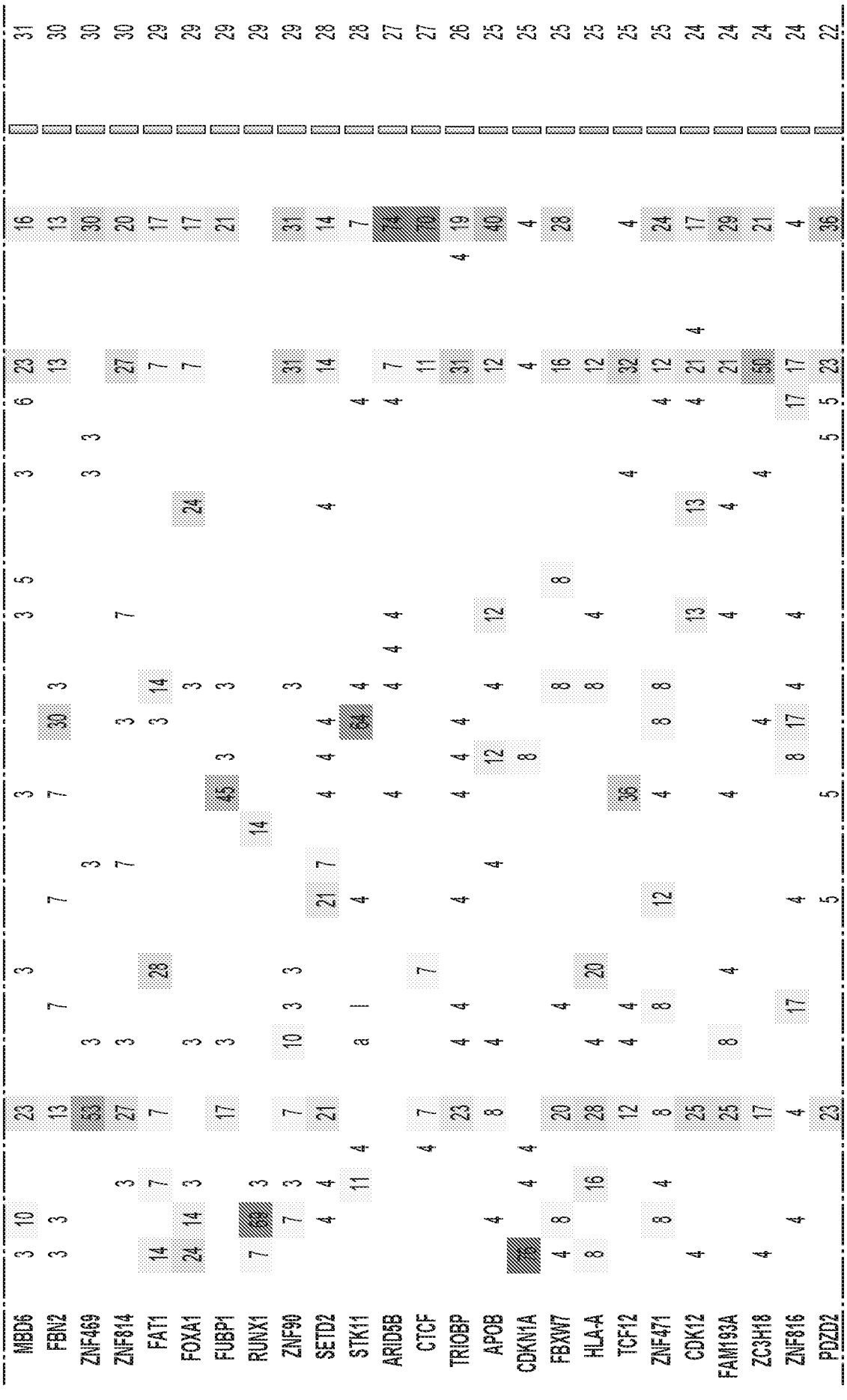


Fig. 8 (Cont. III)

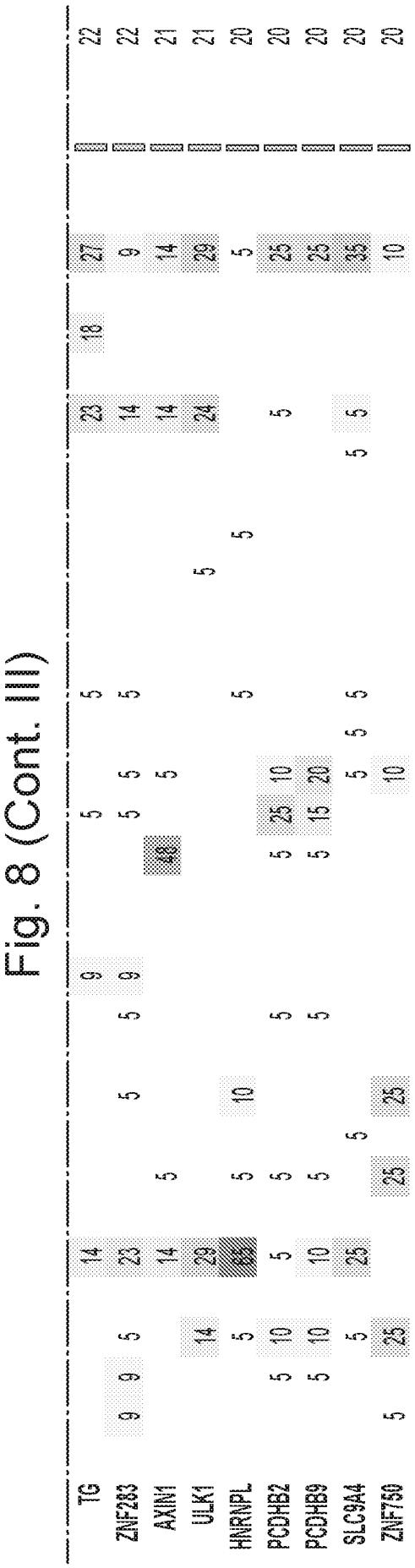


Fig. 9a

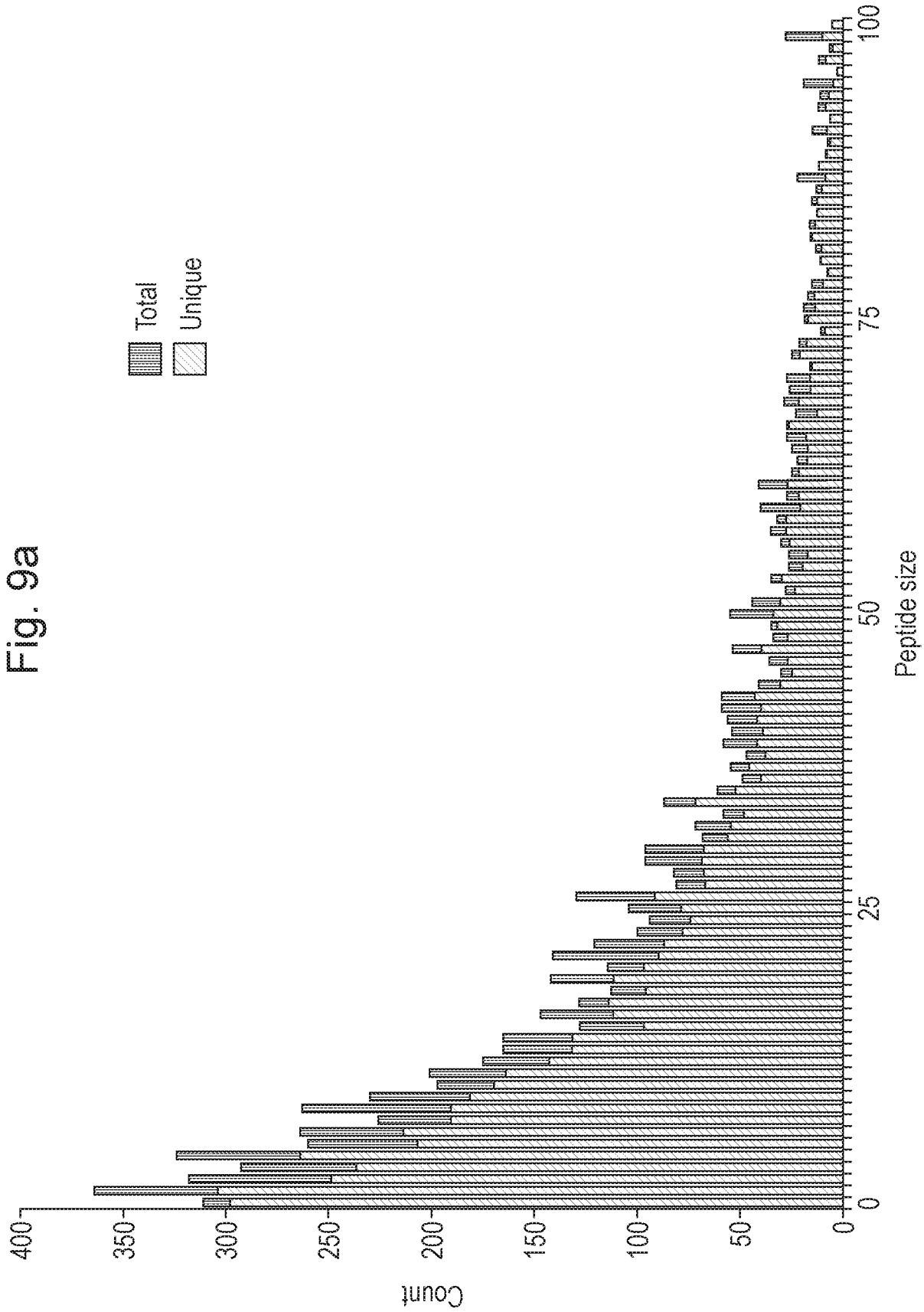

Total
Unique
Count
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350
300
250
200
150
100
50
0
0
25
50
75
100
Peptide size

Fig. 9b

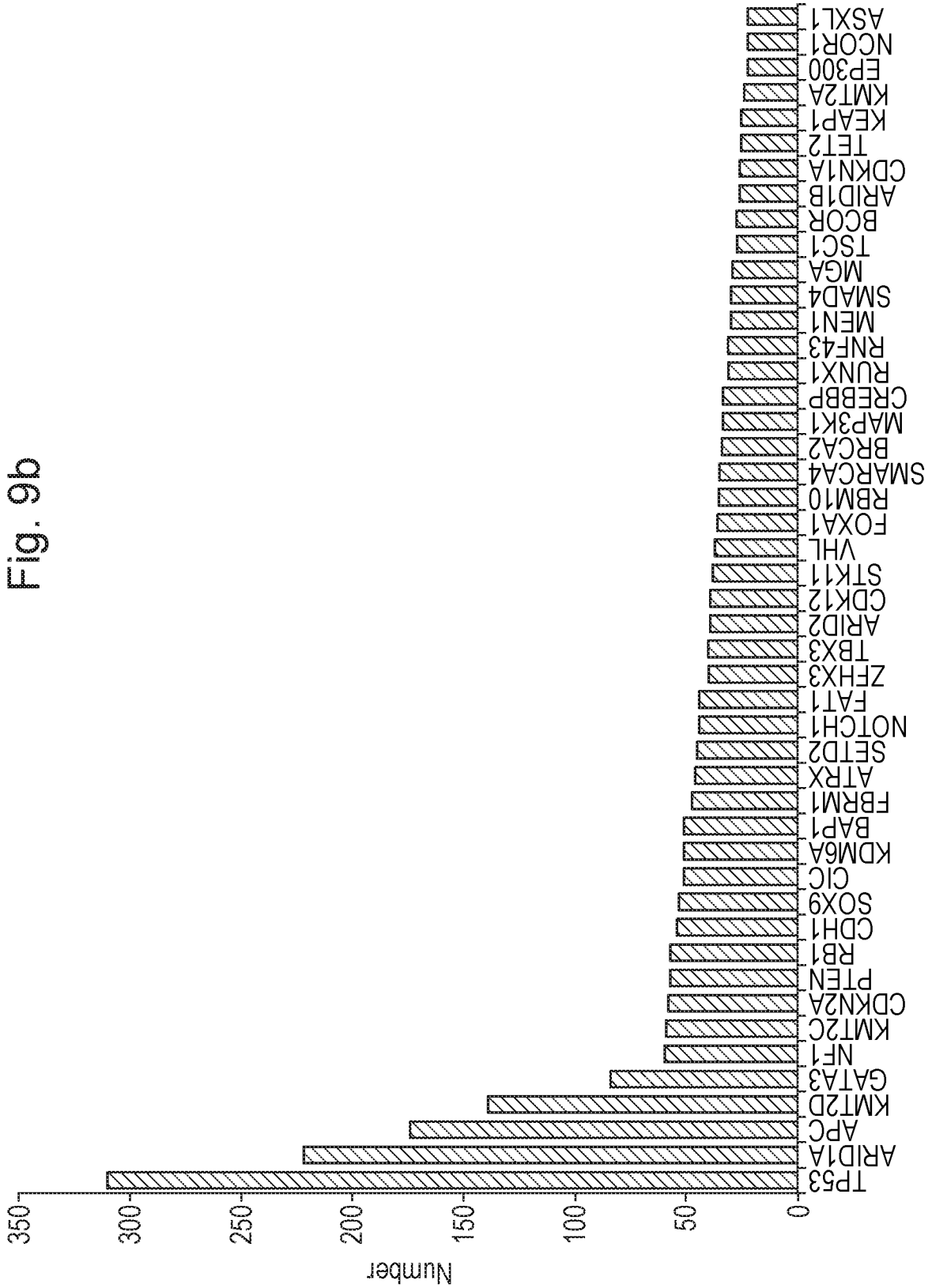
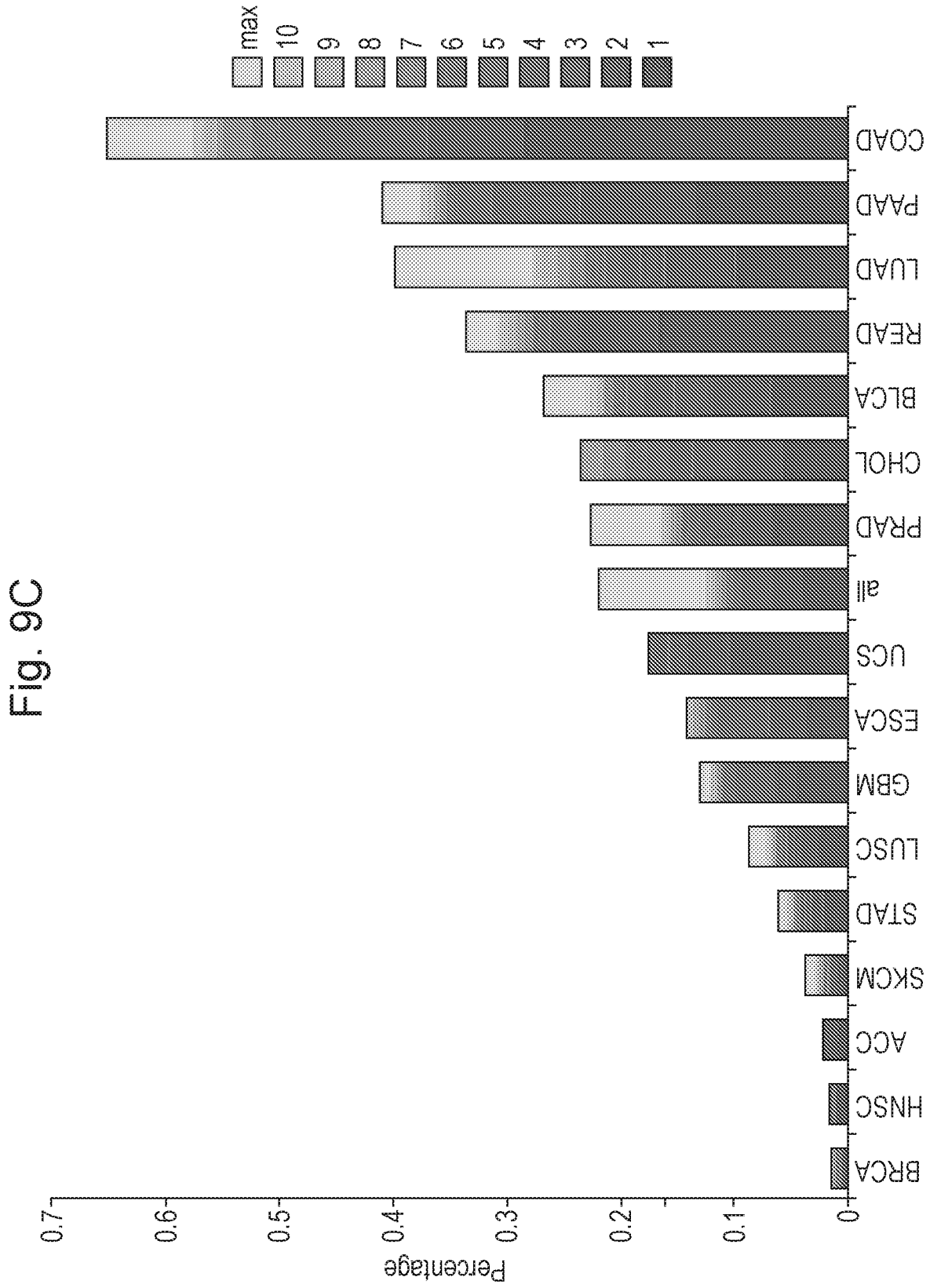

Number
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100
150
200
250
300
350
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APC
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GATA3
NF1
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CDKN2A
PTEN
RB1
CDH1
SOX9
CIC
KDM6A
BAP1
FBRM1
ATRX
SETD2
NOTCH1
FAT1
ZFHX3
TBX3
ARID2
CDK12
STK11
VHL
FOXA1
RBM10
SMARCA4
BRCA2
MAP3K1
CREBBP
RUNX1
RNF43
MEN1
SMAD4
MGA
TSC1
BCOR
ARID1B
CDKN1A
TET2
KEAP1
KMT2A
EP300
NCOR1
ASXL1

Fig. 9C


max
10
9
8
7
6
5
4
3
2
1
COAD
PAAD
LUAD
READ
BLCA
CHOL
PRAD
all
UCS
ESCA
GBM
LUSC
STAD
SKCM
ACC
HNSC
BRCA
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
Percentage

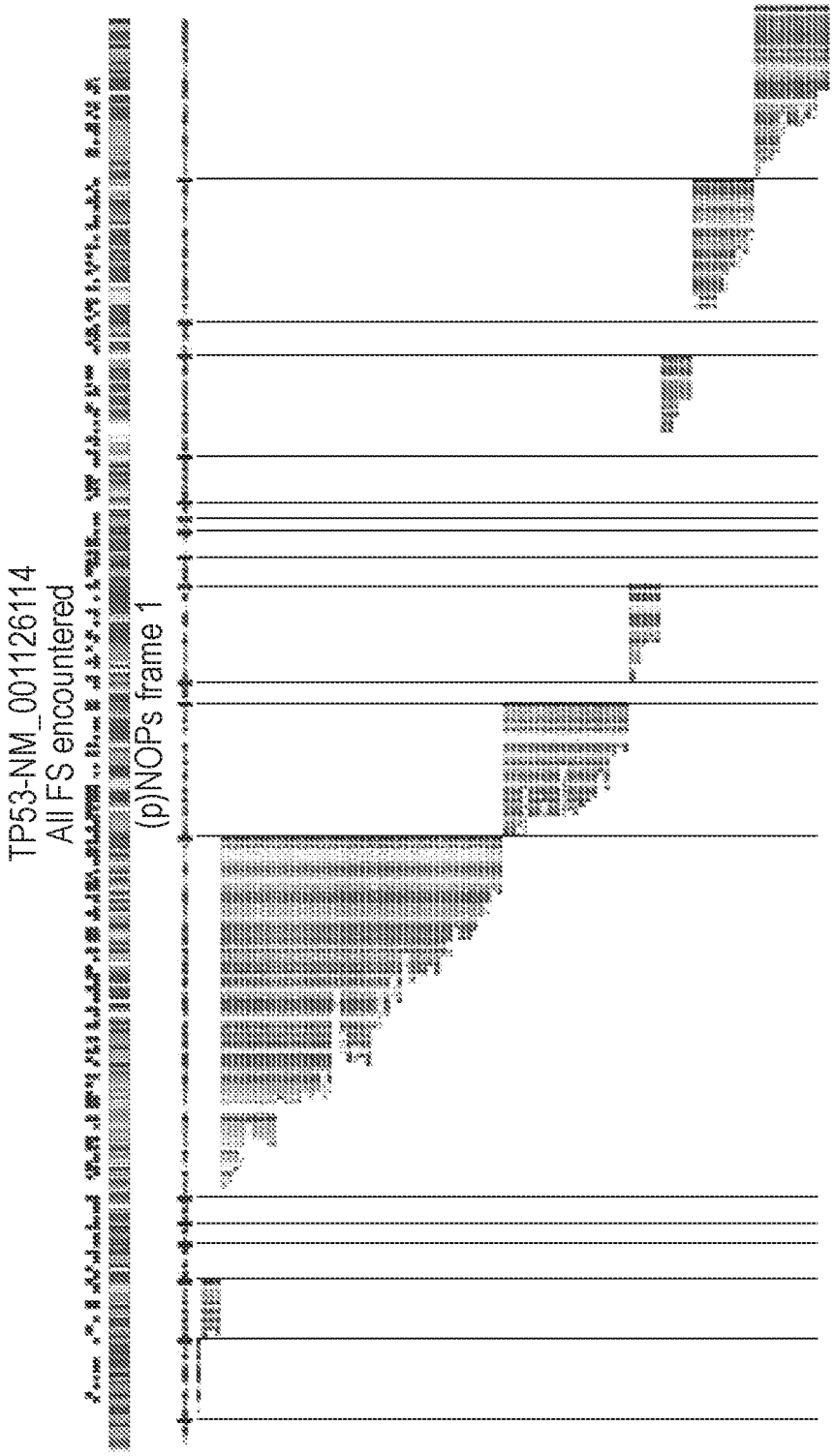


Fig. 9D

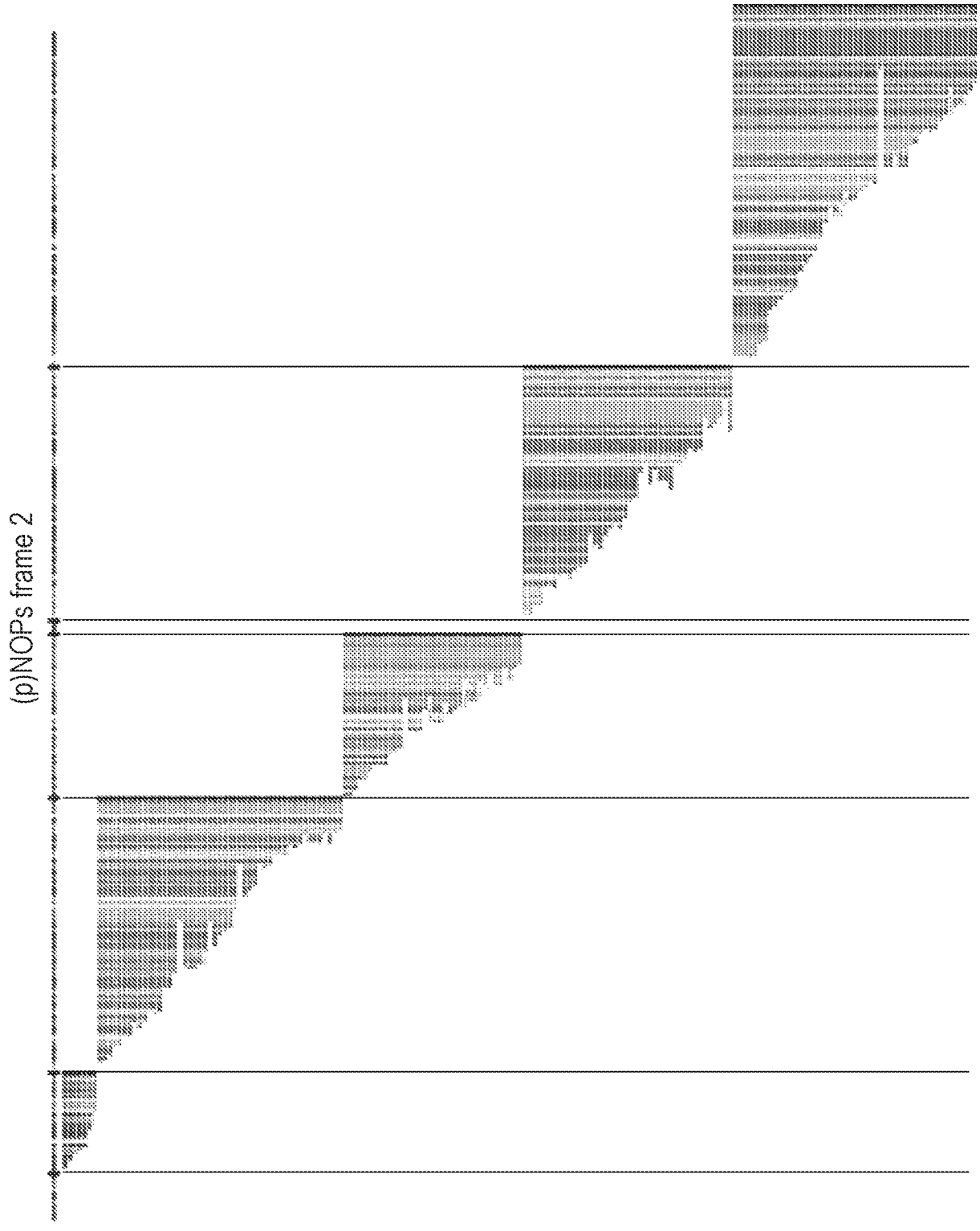


Fig. 9D
(Cont. I)

Fig. 9D. (Cont II)

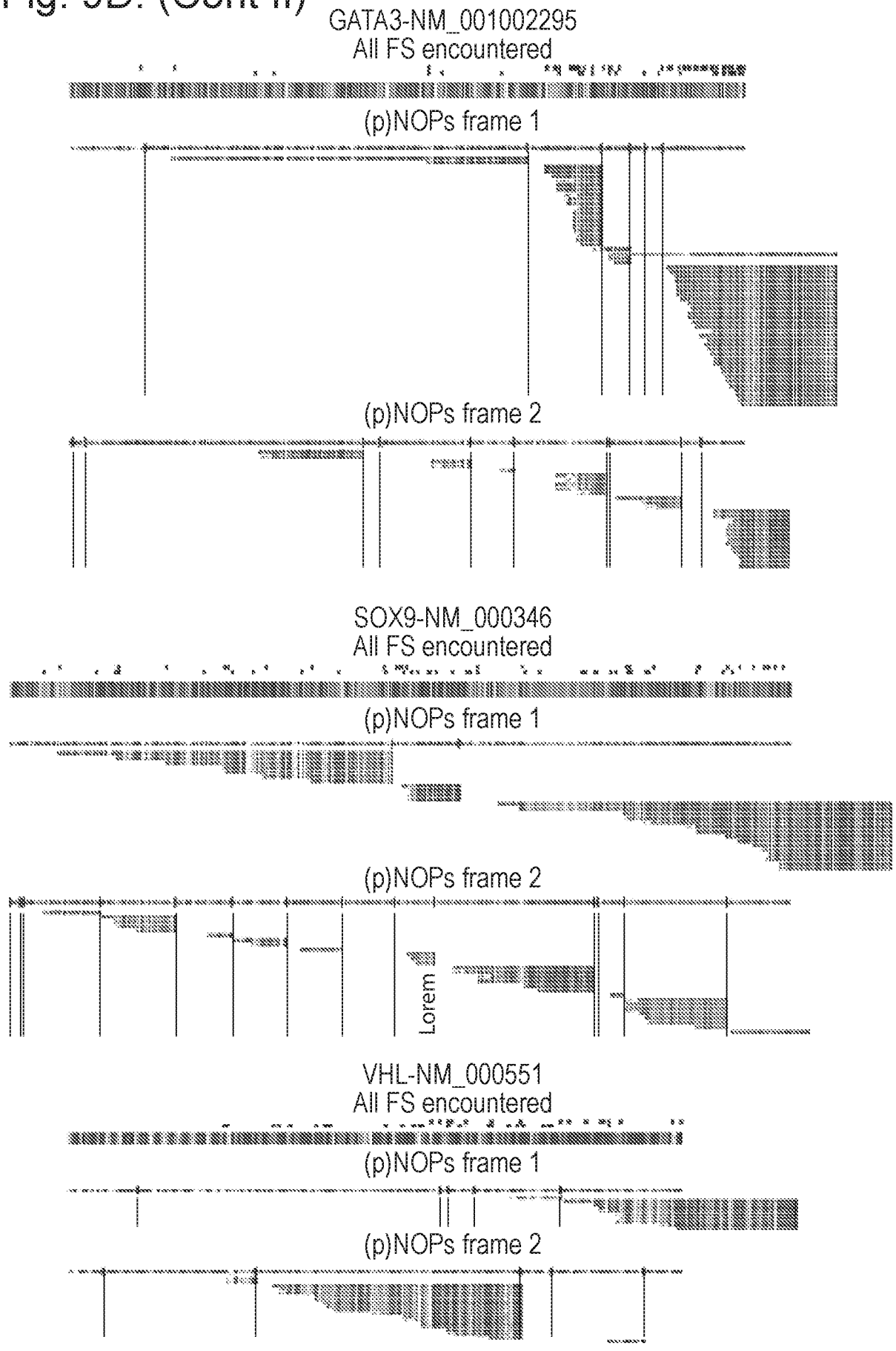
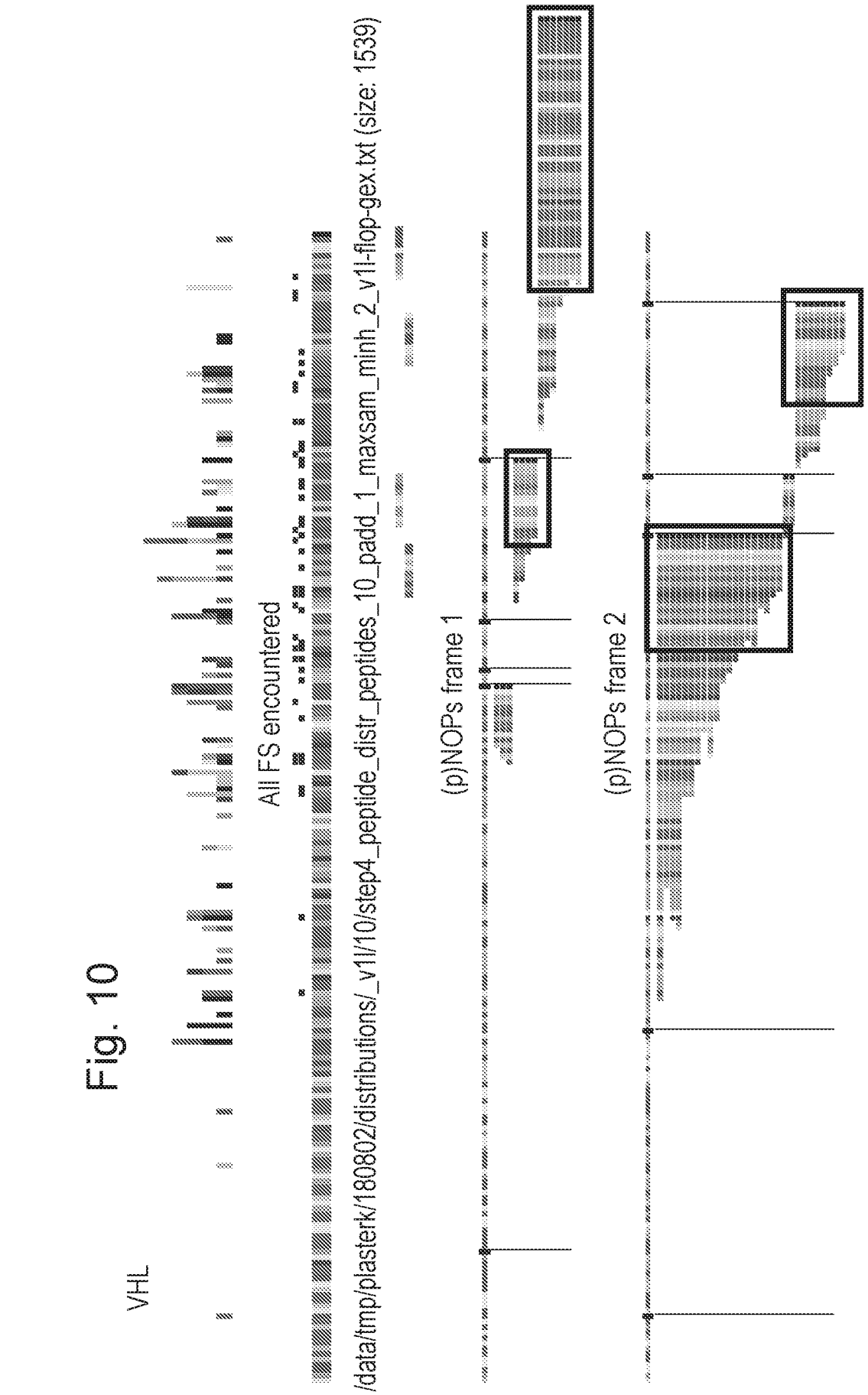


Fig. 10


VHL
All FS encountered
/data/tmp/plasterk/180802/distributions/_v11/10/step4_peptide_distr_peptides_10_padd_1_maxsam_minh_2_v11-flop-gex.txt (size: 1539)
(p)NOPs frame 1
(p)NOPs frame 2

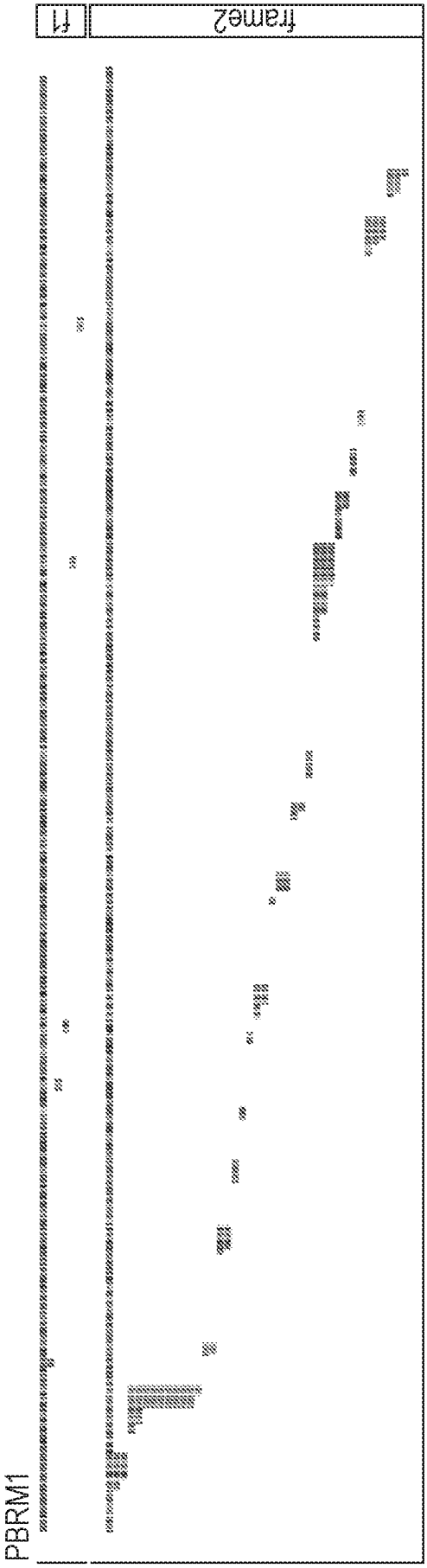
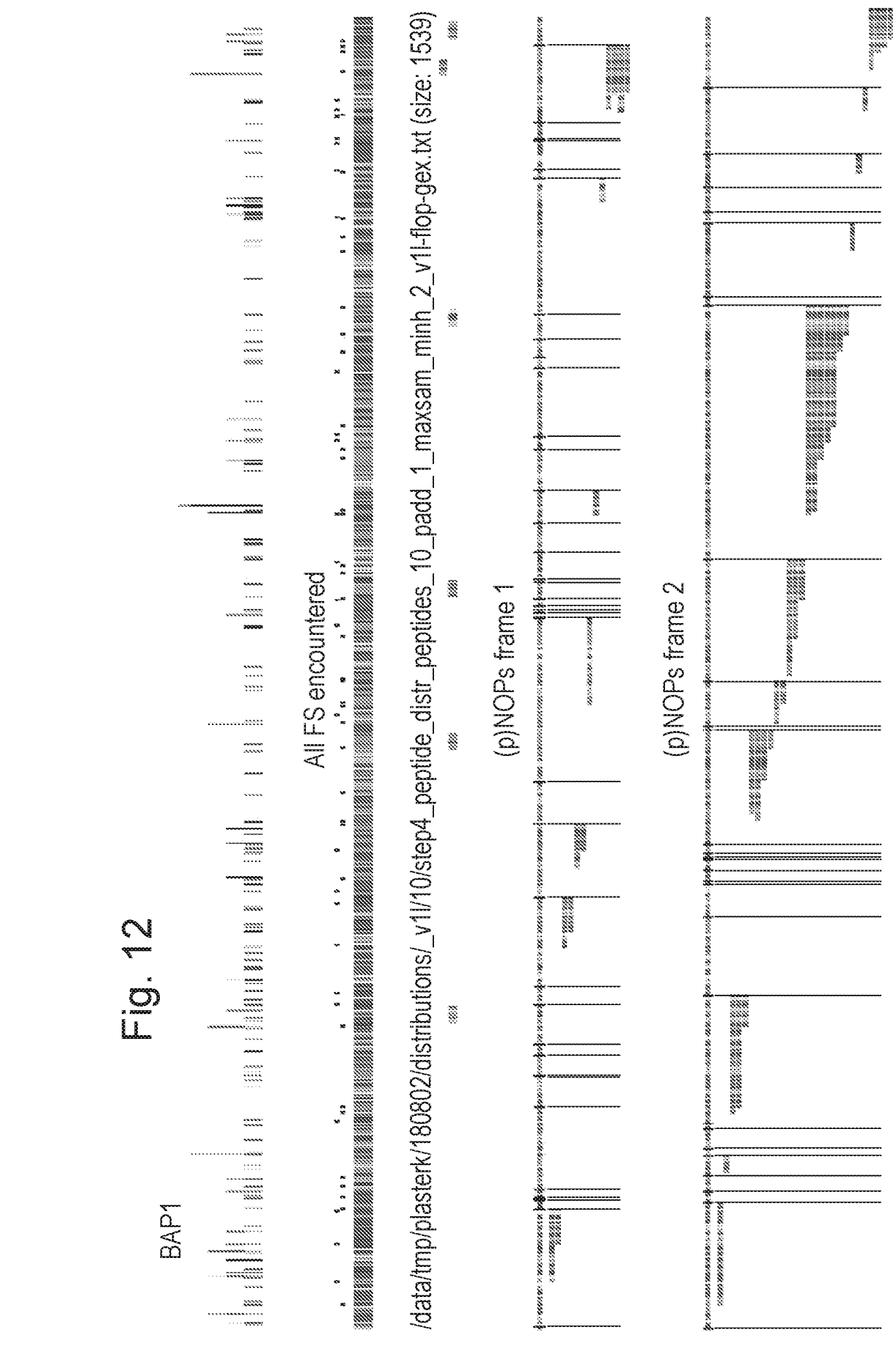


Fig. 11

Fig. 12


BAP1
All FS encountered
/data/tmp/plasterk/180802/distributions/_v11/10/step4_peptide_distr_peptides_10_padd_1_maxsam_minh_2_v11-flop-gex.txt (size: 1539)
(p)NOPs frame 1
(p)NOPs frame 2

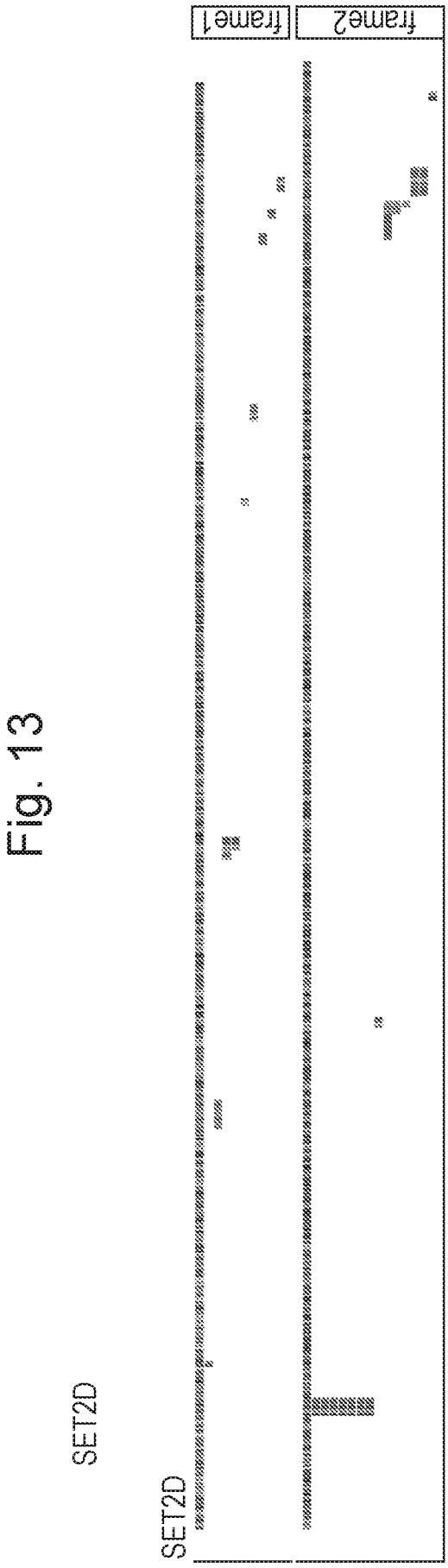


Fig. 13