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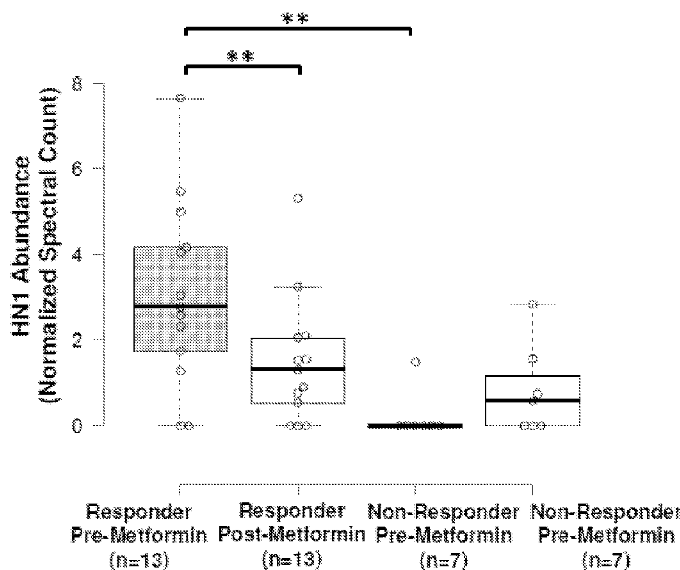

FIG. 2

(57) Abstract: Described herein are methods of identifying a subject that can be a responder to metformin treatment, where the subject has an increased HN1 protein expression in comparison to a suitable control. The subject can have endometrial cancer. The subject can have endometrioid endometrial cancer. In some aspects, the method can include identifying that a subject is a responder to metformin by detecting that the subject has increased HN1 protein expression as compared to a suitable control and administering to the subject a therapeutically effective amount of metformin.

[Continued on next page]

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METHODS AND ASSAYS FOR ENDOMETRIAL DISEASES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of and priority to co-pending U.S. Provisional Patent Application No. 62/644,534, filed on March 18, 2018, entitled "METHODS AND ASSAYS FOR ENDOMETRIAL DISEASES," the contents of which is incorporated by reference herein in its entirety

BACKGROUND

Endometrial cancer (EC) is now the second most prevalent cancer among women in the USA and one of the few cancers for which disease incidence is on the rise, particularly for aggressive histologic subtypes

SUMMARY

Described herein are aspects of a method that can include measuring the amount of HN1 protein in a biological sample obtained from a subject having endometrial cancer. The step of measuring can be performed using an immunological assay or protein sequencing. Thestep of measuring can be performed using an immunological assay using an antibody that specifically binds HN1 protein. The subject can have endometrioid endometrial cancer. The subject can be obese. The method can further include administering a therapeutically effective amount of metformin to the subject. The subject can have increased HN1 protein in the biological sample as compared to a suitable control sample. The method can be performed prior to performing a hysterectomy on the subject. The method can be performed after performing a hysterectomy on the subject. The therapeutically effective amount can be about 850 mg. The therapeutically effective amount can be administered once daily. The therapeutically effective amount can be administered for 1, 2, 3, or 4 weeks.

Also described herein are aspects of a method that can include the steps of obtaining a biological sample from a subject having endometrial cancer; and measuring the amount of HN1 protein in the biological sample. The step of measuring can be performed using an immunological assay or protein sequencing. The step of measuring can be performed using an immunological assay using an antibody that specifically binds HN1 protein. The subject can have endometrioid endometrial cancer. The subject can be obese. The method can further include administering to the subject a therapeutically effective amount of metformin. The subject

can have increased HN1 protein in the biological sample as compared to a suitable control sample. The method can be performed prior to performing a hysterectomy on the subject. The method can be performed after performing a hysterectomy on the subject. The therapeutically effective amount can be about 850 mg. The therapeutically effective amount can be administered once daily. The therapeutically effective amount can be administered for 1, 2, 3, or 4 weeks.

Also described herein are aspects of a method of treating a subject having endometrial cancer and an increased HN1 protein in a biological sample that can include administering a therapeutically effective of metformin to the subject. The subject can have endometrioid endometrial cancer. The subject can be obese. The step of administering can be preformed prior to performing a hysterectomy. The step of administering can be preformed after performing a hysterectomy. The therapeutically effective amount can be about 850 mg. The therapeutically effective amount can be administered once daily. The therapeutically effective amount can be administered for 1, 2, 3, or 4 weeks.

Also described herein is metformin as a medicament in a formulation to treat endometrial cancer in a subject having an increased level of HN1 protein in a biological sample.

Also described herein is the use of metformin for the treatment of endometrial cancer in a subject having an increased level of HN1 protein.

Also described herein are aspects of a kit that can include an amount of an antibody that is capable of specifically binding NH1 protein; and instructions fixed in a tangible medium of expression, wherein the instructions can provide directions for performing an immunoassay on a biological sample from a subject using the antibody and administering a non-antineoplastic agent to a subject in need thereof when the amount of NH1 protein is increased in the biological sample. The instructions can provide that the biological sample is a tissue sample. The instructions can provide that the biological sample is a bodily fluid. The instructions can provide directions to perform a hysterectomy after administering the non-antineoplastic agent. The instructions can identify that the non-antineoplastic can be metformin. The instructions can identify that the amount of the metformin is about 850 mg.

BRIEF DESCRIPTION OF THE DRAWINGS

Further aspects of the present disclosure will be readily appreciated upon review of the detailed description of its various embodiments, described below, when taken in conjunction with the accompanying drawings.

FIG 1. shows heatmap results from a differential proteomic analyses of endometrial cancer (EC) tissues collected from women who responded (n=13) or did not respond (n=7) to metformin treatment. Heatmap details a supervised analyses of 79 proteins (edgeR $p<0.05$) significantly altered between EC patients who did or did not respond to metformin treatment.

5 **FIG 2.** shows a graph demonstrating hematological and neurological expressed 1 (HN1) protein in elevated in metformin responders and decreases following metformin treatment. HN1 was observed as significantly abundant in metformin responder versus non-responder patients and was significantly decreased in abundance following metformin treatment in responders by LC-MS/MS-based proteomic analyses (** edgeR $p<0.05$).

10 **FIGS. 3A-3B** show an erification of altered HN1 protein abundance by immunohistochemical analyses of EEC patients tissues collected from metformin responder (n=13) versus non-responders (n=7). **FIG. 3A**: HN1 IHC staining in EEC tissues harvested from a representative responder versus non-responder patient, pre-metformin treatment. **FIG. 3B**: IHC H Score for HN1 abundance in metformin responders and non-responder, pre and post-
15 Metformin treatment. * p-value = 0.05, ** p=value = 0.011.

FIG 4. shows representative blots that can demonstrate HN1 decreases in response to metformin treatment in endometrial cancer cells. RL95-2 and ACI-181 cells were treated with metformin (20mM) for 96h or 120h and equivalent amounts of protein lysate was immunoblotted for HN1 protein abundance as well as AMPKα, p-AMPKα (Thr172), and Ki-67 proteins.

20 **FIGS. 5A-5F** show blots and graphs that can demonstrate that HN1 knockdown does not alter response of endometrial cancer cell lines to metformin. HN1 expression was knocked down in RL95-2 and ACI-181 cells by small interfering RNA (siRNA) targeting HN1 or with a non-targeting (siNT) control and confirmed at the protein (**FIGS. 5A and 5D**) and mRNA (**FIGS. 5B and 5E**) levels before (72h) and following (168h) dose-response analyses with metformin
25 treatment; p-value was determined by student t-test. **$p < 0.001$, *$p < 0.005$. RL95-2 and ACI-181 cells were treated with metformin 72h following siRNA transfection and dose-response was assessed after an additional 72h by MTS assay.

FIGS. 6A-6D show graphs and blots that can demonstrate that HN1 knockdown does not alter the proliferation of endometrial cancer cell lines. HN1 expression was silenced in RL95-
30 2 and ACI-181 cells by small interfering RNA (siRNA) targeting HN1 or with a non-targeting (siNT) control and confirmed at the protein level (**FIGS. 6B and 6D**) before (72h) and following (168h) daily assessment of cellular proliferation by MTS assay; p-value was determined by student t-test. Each data point reflects triplicate technical replicates measured at two sub-

confluent cell densities per day, error bars reflect standard error. Rep 1 = biological replicate #1, Rep2 = biological replicate 2.

FIGS. 7A-7B show graphs that can demonstrate that HN1 gene expression correlates with Ki-67 expression and was associated with altered overall survival in endometrial cancer patients. **FIG. 7A**: Correlative HN1 and Ki-67 mRNA abundance determined by RNA-seq analyses of tumor tissues from endometrial cancer patients (n=542, The Cancer Genome Atlas); Spearman = 0.37, Pearson = 0.36. **FIG. 7B**: Overall survival was evaluated using Kaplan-Meier method with survival distributions compared using log-rank test in 540 endometrial cancer patients from the Cancer Genome Atlas for HN1 stratified by tertile gene expression levels (log-rank p-value = 0.047).

FIG. 8 show as table that can demonstrate that tumor tissues from endometrial cancer patients in a pre-operative phase 0 window trial were stratified as responders (n=13) or non-responders (n=7) to metformin treatment

FIG. 9 shows tables summarizing results from a pathway analysis that can demonstrate top altered pathways in metformin responders.

FIG. 10 shows a table of Case identifiers, pre- and post-metformin treatment as well as metformin responder and non-responder status for endometrial cancer patient tissue samples analyzed.

FIG. 11 shows a table of clinical characteristics.

FIG. 12 shows a functional analyses of 79 proteins significantly (p<0.05) altered between EC patients among responders and non-responders to Metformin treatment demonstrated marked activation of molecular signaling promoting cell proliferation and inhibition of pathways associated with cell death and apoptosis.

FIGS. 13A-13B shows the top canonical signaling pathways (**FIG. 13A**) or disease and biofunctions activated or inhibited (**FIG. 13B**) amongst 79 proteins significantly altered between Metformin responder vs non-responder.

FIG. 14 shows a table that can demonstrate prioritization of protein alterations predictive of and indicating pharmacodynamic response to Metformin. Proteins significantly co-altered in pre-treated responder vs non-responder and in post-versus pre-metformin treated responders.

FIG. 15 shows hematological and neurological expressed 1 (HN1) protein was observed as significantly abundant in Metformin responder versus non-responder patients and was significantly decreased in abundance following Metformin treatment in responders.

FIGS. 16A-16B shows orthogonal verification of HN1 protein alterations in EEC patients that responded (n=13) or did not respond (n=7) to metformin treatment by immunohistochemical

analyses. **FIG. 16A**: HN1 IHC staining in EEC tissues harvested from a representative responder versus non-responder patient, pre metformin treatment. **FIGS. 16B**: Average IHC H Score for HN1 abundance in metformin responders and non-responder, pre and post-metformin treatment. * p-value = 0.05, ** p=value = 0.011.

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DETAILED DESCRIPTION

Before the present disclosure is described in greater detail, it is to be understood that this disclosure is not limited to particular embodiments described, and as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

10 Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present disclosure, the preferred methods and materials are now described.

15 All publications and patents cited in this specification are cited to disclose and describe the methods and/or materials in connection with which the publications are cited. All such publications and patents are herein incorporated by references as if each individual publication or patent were specifically and individually indicated to be incorporated by reference. Such incorporation by reference is expressly limited to the methods and/or materials described in the 20 cited publications and patents and does not extend to any lexicographical definitions from the cited publications and patents. Any lexicographical definition in the publications and patents cited that is not also expressly repeated in the instant application should not be treated as such and should not be read as defining any terms appearing in the accompanying claims. The citation of any publication is for its disclosure prior to the filing date and should not be construed 25 as an admission that the present disclosure is not entitled to antedate such publication by virtue of prior disclosure. Further, the dates of publication provided could be different from the actual publication dates that may need to be independently confirmed.

As will be apparent to those of skill in the art upon reading this disclosure, each of the individual embodiments described and illustrated herein has discrete components and features 30 which may be readily separated from or combined with the features of any of the other several embodiments without departing from the scope or spirit of the present disclosure. Any recited method can be carried out in the order of events recited or in any other order that is logically possible.

Where a range is expressed, a further aspect includes from the one particular value and/or to the other particular value. Where a range of values is provided, it is understood that each intervening value, to the tenth of the unit of the lower limit unless the context clearly dictates otherwise, between the upper and lower limit of that range and any other stated or
5 intervening value in that stated range, is encompassed within the disclosure. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges and are also encompassed within the disclosure, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure. For example, where the
10 stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the disclosure, e.g. the phrase “x to y” includes the range from ‘x’ to ‘y’ as well as the range greater than ‘x’ and less than ‘y’. The range can also be expressed as an upper limit, e.g. ‘about x, y, z, or less’ and should be interpreted to include the specific ranges of ‘about x’, ‘about y’, and ‘about z’ as well as the ranges of ‘less than x’, less than y’, and ‘less
15 than z’. Likewise, the phrase ‘about x, y, z, or greater’ should be interpreted to include the specific ranges of ‘about x’, ‘about y’, and ‘about z’ as well as the ranges of ‘greater than x’, greater than y’, and ‘greater than z’. In addition, the phrase “about ‘x’ to ‘y’”, where ‘x’ and ‘y’ are numerical values, includes “about ‘x’ to about ‘y’”.

It should be noted that ratios, concentrations, amounts, and other numerical data can be
20 expressed herein in a range format. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as “about” that particular value in addition to the value itself. For example, if the value “10” is disclosed, then “about 10” is also disclosed. Ranges can be
25 expressed herein as from “about” one particular value, and/or to “about” another particular value. Similarly, when values are expressed as approximations, by use of the antecedent “about,” it will be understood that the particular value forms a further aspect. For example, if the value “about 10” is disclosed, then “10” is also disclosed.

It is to be understood that such a range format is used for convenience and brevity, and
30 thus, should be interpreted in a flexible manner to include not only the numerical values explicitly recited as the limits of the range, but also to include all the individual numerical values or sub-ranges encompassed within that range as if each numerical value and sub-range is explicitly recited. To illustrate, a numerical range of “about 0.1% to 5%” should be interpreted to include not only the explicitly recited values of about 0.1% to about 5%, but also include

individual values (e.g., about 1%, about 2%, about 3%, and about 4%) and the sub-ranges (e.g., about 0.5% to about 1.1%; about 5% to about 2.4%; about 0.5% to about 3.2%, and about 0.5% to about 4.4%, and other possible sub-ranges) within the indicated range.

As used in the specification and the appended claims, the singular forms “a,” “an,” and 5 “the” include plural referents unless the context clearly dictates otherwise.

As used herein, "about," "approximately," “substantially,” and the like, when used in connection with a numerical variable, can generally refers to the value of the variable and to all values of the variable that are within the experimental error (e.g., within the 95% confidence interval for the mean) or within +/- 10% of the indicated value, whichever is greater. As used 10 herein, the terms “about,” “approximate,” “at or about,” and “substantially” can mean that the amount or value in question can be the exact value or a value that provides equivalent results or effects as recited in the claims or taught herein. That is, it is understood that amounts, sizes, formulations, parameters, and other quantities and characteristics are not and need not be exact, but may be approximate and/or larger or smaller, as desired, reflecting tolerances, 15 conversion factors, rounding off, measurement error and the like, and other factors known to those of skill in the art such that equivalent results or effects are obtained. In some circumstances, the value that provides equivalent results or effects cannot be reasonably determined. In general, an amount, size, formulation, parameter or other quantity or characteristic is “about,” “approximate,” or “at or about” whether or not expressly stated to be 20 such. It is understood that where “about,” “approximate,” or “at or about” is used before a quantitative value, the parameter also includes the specific quantitative value itself, unless specifically stated otherwise.

Embodiments of the present disclosure will employ, unless otherwise indicated, techniques of molecular biology, microbiology, organic chemistry, biochemistry, physiology, cell 25 biology, cancer biology, and the like, which are within the skill of the art. Such techniques are explained fully in the literature.

Before the embodiments of the present disclosure are described in detail, it is to be understood that, unless otherwise indicated, the present disclosure is not limited to particular materials, reagents, reaction materials, manufacturing processes, or the like, as such can vary. 30 It is also to be understood that the terminology used herein is for purposes of describing particular embodiments only, and is not intended to be limiting. It is also possible in the present disclosure that steps can be executed in different sequence where this is logically possible unless the context clearly dictates otherwise.

Definitions

As used herein, “administering” refers to an administration that is oral, topical, intravenous, subcutaneous, transcutaneous, transdermal, intramuscular, intra-joint, parenteral, intra-arteriole, intradermal, intraventricular, intraosseous, intraocular, intracranial, intraperitoneal, intralesional, intranasal, intracardiac, intraarticular, intracavernous, intrathecal, intravireal, intracerebral, and intracerebroventricular, intratympanic, intracochlear, rectal, vaginal, by inhalation, by catheters, stents or via an implanted reservoir or other device that administers, either actively or passively (e.g. by diffusion) a composition the perivascular space and adventitia. For example a medical device such as a stent can contain a composition or formulation disposed on its surface, which can then dissolve or be otherwise distributed to the surrounding tissue and cells. The term “parenteral” can include subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional, and intracranial injections or infusion techniques.

As used herein, “antibody” can refer to a glycoprotein containing at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, or an antigen binding portion thereof. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. Each light chain is comprised of a light chain variable region and a light chain constant region. The VH and VL regions retain the binding specificity to the antigen and can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR). The CDRs are interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four framework regions, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen.

As used herein, “aptamer” can refer to single-stranded DNA or RNA molecules that can bind to pre-selected targets including proteins with high affinity and specificity. Their specificity and characteristics are not directly determined by their primary sequence, but instead by their tertiary structure.

As used herein, “control” can refer to an alternative subject or sample used in an experiment for comparison purpose and included to minimize or distinguish the effect of variables other than an independent variable. Based on what is being evaluated one of ordinary skill in the art will be able to appreciate suitable controls. Controls can be positive or negative.

As used herein, “negative control” can refer to a “control” that is designed to produce no effect or result, provided that all reagents are functioning properly and that the experiment is

properly conducted. Other terms that are interchangeable with “negative control” include “sham,” “placebo,” and “mock.”

As used herein, “positive control” can refer to a “control” that is designed to produce the desired result, provided that all reagents are functioning properly and that the experiment is
5 properly conducted.

A “suitable control” is a control that will be instantly appreciated by one of ordinary skill in the art as one that is included such that it can be determined if the variable being evaluated an effect, such as a desired effect or hypothesized effect. One of ordinary skill in the art will also instantly appreciate based on inter alia, the context, the variable(s), the desired or hypothesized
10 effect, what is a suitable or an appropriate control needed

As used herein, the term “specific binding” can refer to non-covalent physical association of a first and a second moiety wherein the association between the first and second moieties is at least 2 times as strong, at least 5 times as strong as, at least 10 times as strong as, at least 50 times as strong as, at least 100 times as strong as, or stronger than the association of either
15 moiety with most or all other moieties present in the environment in which binding occurs. Binding of two or more entities may be considered specific if the equilibrium dissociation constant, Kd, is 10^{-3} M or less, 10^{-4} M or less, 10^{-5} M or less, 10^{-6} M or less, 10^{-7} M or less, 10^{-8} M or less, 10^{-9} M or less, 10^{-10} M or less, 10^{-11} M or less, or 10^{-12} M or less under the conditions employed, e.g., under physiological conditions such as those inside a cell or
20 consistent with cell survival. In some embodiments, specific binding can be accomplished by a plurality of weaker interactions (e.g., a plurality of individual interactions, wherein each individual interaction is characterized by a Kd of greater than 10^{-3} M). In some embodiments, specific binding, which can be referred to as “molecular recognition,” is a saturable binding interaction between two entities that is dependent on complementary orientation of functional groups on
25 each entity. Examples of specific binding interactions include primer-polynucleotide interaction, aptamer-aptamer target interactions, antibody-antigen interactions, avidin-biotin interactions, ligand-receptor interactions, metal-chelate interactions, hybridization between complementary nucleic acids, etc.

As used interchangeably herein, “subject,” “individual,” or “patient” can refer to a
30 vertebrate organism, such as a mammal (e.g. human). "Subject" can also refer to a cell, a population of cells, a tissue, an organ, or an organism, preferably to human and constituents thereof.

As used herein, “therapeutically effective amount” can refer to the amount of a compound provided herein that is sufficient to effect beneficial or desired biological, emotional,

medical, or clinical response of a cell, tissue, system, animal, or human. An effective amount can be administered in one or more administrations, applications, or dosages. The term cam also include within its scope amounts effective to enhance or restore to substantially normal physiological function. The “therapeutically effective amount” can refer to the amount of a metformin described herein that can treat endometrial cancer and/or a symptom thereof.

As used herein, the terms "treating" and "treatment" can refer generally to obtaining a desired pharmacological and/or physiological effect. The effect can be, but does not necessarily have to be, prophylactic in terms of preventing or partially preventing a disease, symptom or condition thereof, such as an endometrial cancer. The effect can be therapeutic in terms of a partial or complete cure of a disease, condition, symptom or adverse effect attributed to the disease, disorder, or condition. The term "treatment" as used herein covers any treatment of an endometrial cancer, in a subject, particularly a human, and can include any one or more of the following: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, i.e., arresting its development; and (c) relieving the disease, i.e., mitigating or ameliorating the disease and/or its symptoms or conditions. The term "treatment" as used herein can refer to both therapeutic treatment alone, prophylactic treatment alone, or both therapeutic and prophylactic treatment. Those in need of treatment (subjects in need thereof) can include those already with the disorder and/or those in which the disorder is to be prevented. As used herein, the term "treating", can include inhibiting the disease, disorder or condition, e.g., impeding its progress; and relieving the disease, disorder, or condition, e.g., causing regression of the disease, disorder and/or condition. Treating the disease, disorder, or condition can include ameliorating at least one symptom of the particular disease, disorder, or condition, even if the underlying pathophysiology is not affected, such as treating the pain of a subject by administration of an analgesic agent even though such agent does not treat the cause of the pain.

As used herein, “cDNA” refers to a DNA sequence that is complementary to a RNA transcript in a cell. It is a man-made molecule. Typically, cDNA is made in vitro by an enzyme called reverse-transcriptase using RNA transcripts as templates.

As used herein with reference to the relationship between DNA, cDNA, cRNA, RNA, protein/peptides, and the like “corresponding to” or “encoding” (used interchangeably herein) refers to the underlying biological relationship between these different molecules. As such, one of skill in the art would understand that operatively “corresponding to” can direct them to determine the possible underlying and/or resulting sequences of other molecules given the sequence of any other molecule which has a similar biological relationship with these

molecules. For example, from a DNA sequence an RNA sequence can be determined and from an RNA sequence a cDNA sequence can be determined.

As used herein, “deoxyribonucleic acid (DNA)” and “ribonucleic acid (RNA)” can generally refer to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. RNA can be in the form of non-coding RNA such as tRNA (transfer RNA), snRNA (small nuclear RNA), rRNA (ribosomal RNA), anti-sense RNA, RNAi (RNA interference construct), siRNA (short interfering RNA), microRNA (miRNA), or ribozymes, aptamers, guide RNA (gRNA) or coding mRNA (messenger RNA).

As used herein, “differentially expressed,” refers to the differential production of RNA, including but not limited to mRNA, tRNA, miRNA, siRNA, snRNA, and piRNA transcribed from a gene or regulatory region of a genome or the protein product encoded by a gene as compared to the level of production of RNA or protein by the same gene or regulator region in a normal or a control cell. In another context, “differentially expressed,” also refers to nucleotide sequences or proteins in a cell or tissue which have different temporal and/or spatial expression profiles as compared to a normal or control cell.

As used herein, “dose,” “unit dose,” or “dosage” can refer to physically discrete units suitable for use in a subject, each unit containing a predetermined quantity of the metformin and/or a pharmaceutical formulation thereof calculated to produce the desired response or responses in association with its administration.

As used herein, “expression” refers to the process by which polynucleotides are transcribed into RNA transcripts. In the context of mRNA and other translated RNA species, “expression” also refers to the process or processes by which the transcribed RNA is subsequently translated into peptides, polypeptides, or proteins. In some instances, “expression” can also be a reflection of the stability of a given RNA. For example, when one measures RNA, depending on the method of detection and/or quantification of the RNA as well as other techniques used in conjunction with RNA detection and/or quantification, it can be that increased/decreased RNA transcript levels are the result of increased/decreased transcription and/or increased/decreased stability and/or degradation of the RNA transcript. One of ordinary skill in the art will appreciate these techniques and the relation “expression” in these various contexts to the underlying biological mechanisms.

As used herein, “gene” can refer to a hereditary unit corresponding to a sequence of DNA that occupies a specific location on a chromosome and that contains the genetic instruction for a characteristic(s) or trait(s) in an organism. The term gene can refer to translated and/or untranslated regions of a genome. “Gene” can refer to the specific sequence of DNA that

is transcribed into an RNA transcript that can be translated into a polypeptide or be a catalytic RNA molecule, including but not limited to, tRNA, siRNA, piRNA, miRNA, long-non-coding RNA and shRNA.

As used herein, “identity,” can refer to a relationship between two or more nucleotide or polypeptide sequences, as determined by comparing the sequences. In the art, “identity” can also refers to the degree of sequence relatedness between nucleotide or polypeptide sequences as determined by the match between strings of such sequences. “Identity” can be readily calculated by known methods, including, but not limited to, those described in (Computational Molecular Biology, Lesk, A. M., Ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., Ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., Eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., Eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math. 1988, 48: 1073. Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in publicly available computer programs. The percent identity between two sequences can be determined by using analysis software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, Madison Wis.) that incorporates the Needelman and Wunsch, (J. Mol. Biol., 1970, 48: 443-453,) algorithm (e.g., NBLAST, and XBLAST). The default parameters are used to determine the identity for the polypeptides of the present disclosure, unless stated otherwise.

The term “molecular weight”, as used herein, can generally refer to the mass or average mass of a material. If a polymer or oligomer, the molecular weight can refer to the relative average chain length or relative chain mass of the bulk polymer. In practice, the molecular weight of polymers and oligomers can be estimated or characterized in various ways including gel permeation chromatography (GPC) or capillary viscometry. GPC molecular weights are reported as the weight-average molecular weight (Mw) as opposed to the number-average molecular weight (Mn). Capillary viscometry provides estimates of molecular weight as the inherent viscosity determined from a dilute polymer solution using a particular set of concentration, temperature, and solvent conditions.

As used herein, the terms “optional” or “optionally” means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

As used herein, “pharmaceutical formulation” refers to the combination of an active agent, compound, or ingredient with a pharmaceutically acceptable carrier or excipient, making the composition suitable for diagnostic, therapeutic, or preventive use in vitro, in vivo, or ex vivo.

As used herein, “pharmaceutically acceptable carrier or excipient” refers to a carrier or
5 excipient that is useful in preparing a pharmaceutical formulation that is generally safe, non-toxic, and is neither biologically or otherwise undesirable, and includes a carrier or excipient that is acceptable for veterinary use as well as human pharmaceutical use. A “pharmaceutically acceptable carrier or excipient” as used in the specification and claims includes both one and more than one such carrier or excipient.

10 As used herein, “pharmaceutically acceptable salt” refers to any acid or base addition salt whose counter-ions are non-toxic to the subject to which they are administered in pharmaceutical doses of the salts.

As used herein, “polypeptides” or “proteins” refers to amino acid residue sequences. Those sequences are written left to right in the direction from the amino to the carboxy terminus.
15 In accordance with standard nomenclature, amino acid residue sequences are denominated by either a three letter or a single letter code as indicated as follows: Alanine (Ala, A), Arginine (Arg, R), Asparagine (Asn, N), Aspartic Acid (Asp, D), Cysteine (Cys, C), Glutamine (Gln, Q), Glutamic Acid (Glu, E), Glycine (Gly, G), Histidine (His, H), Isoleucine (Ile, I), Leucine (Leu, L), Lysine (Lys, K), Methionine (Met, M), Phenylalanine (Phe, F), Proline (Pro, P), Serine (Ser, S),
20 Threonine (Thr, T), Tryptophan (Trp, W), Tyrosine (Tyr, Y), and Valine (Val, V). “Protein” and “Polypeptide” can refer to a molecule composed of one or more chains of amino acids in a specific order. The term protein is used interchangeable with “polypeptide.” The order is determined by the base sequence of nucleotides in the gene coding for the protein. Proteins can be required for the structure, function, and regulation of the body’s cells, tissues, and organs.

25 As used herein, the terms “weight percent,” “wt%,” and “wt. %,” which can be used interchangeably, indicate the percent by weight of a given component based on the total weight of a composition of which it is a component, unless otherwise specified. That is, unless otherwise specified, all wt% values are based on the total weight of the composition. It should be understood that the sum of wt% values for all components in a disclosed composition or
30 formulation are equal to 100. Alternatively, if the wt% value is based on the total weight of a subset of components in a composition, it should be understood that the sum of wt% values the specified components in the disclosed composition or formulation are equal to 100.

Discussion

Endometrial cancer is a significant medical condition for women. The American Cancer Society estimated that in 2014 there were at least 50,000 new cases diagnosed. Surgery is typically employed to manage these cases. In some cases, surgery is combined with drug therapy. Although isolated pre-clinical and phase 0 clinical trial data has described the use of non-antineoplastic (e.g. metformin), the use of a non-antineoplastic alone or in combination with current treatment modalities is currently non-routine. Indeed, there is no current approved treatment for endometrial cancer involving, for example, metformin. Further complicating the matter, some patients with endometrial cancer are non-responsive to metformin treatment. Given that metformin has potential side effects, it is important to be able to identify responders from non-responders for optimal patient care.

With that said, described herein are assays that can quantify the amount of HN1 protein and/or gene expression in subjects having endometrial cancer. In some aspects, subjects having endometrial cancer with an increased amount of HN1 protein and/or gene expression as compared to a suitable control can be responsive to metformin. Also described herein, are methods of treating a subject having endometrial cancer and an elevated level of HN1 protein and/or gene expression as compared to a suitable control with a therapeutically effective amount of metformin. Other compositions, compounds, methods, features, and advantages of the present disclosure will be or become apparent to one having ordinary skill in the art upon examination of the following drawings, detailed description, and examples. It is intended that all such additional compositions, compounds, methods, features, and advantages be included within this description, and be within the scope of the present disclosure.

Methods

Described herein are methods that can include the step of measuring the amount of HN1 protein and/or gene expression in a biological sample from a subject having endometrial cancer. The biological sample can be any fluid (e.g. blood, urine, milk, and saliva), tissue, cell population, or a component thereof, that can be collected from the subject. This includes, but is not limited to, biopsied tumor tissue and any other tissue or fluid collected from the subject. The step of measuring can be performed using an immunological assay or protein sequencing. As used herein the terms “immunological assay” or “immunoassay” can refer to any assay that can rely on an antibody or fragment thereof or aptamer that is specific or specifically binds to a protein or portion thereof that is desired to be isolated, measured, or otherwise detected (e.g. a protein of interest), to specifically bind to the protein of interest and thus allow for isolation, measurement, and/or detection of the protein of interest in a sample. Such assays include, but

are not limited to, immunohistology, immunocytochemical analysis, western blot, radioimmunoassays, enzyme-linked immunosorbent assay, immunoprecipitation assays, and immunochormatographic assays. The step of measuring can be performed using an immunological assay using an antibody that specifically binds HN1 protein. The subject can
5 have endometrioid endometrial cancer. The subject can be obese. The method can include the step of administering a therapeutically effective amount of metformin to the subject. The subject can have increased HN1 protein in the biological sample as compared to a suitable control sample. The method can be performed prior to performing a hysterectomy on the subject. The method can be performed after performing a hysterectomy on the subject. Gene expression can
10 be measured using any suitable technique, including but not limited to, any PCR, qPCR, real-time PCR, and transcript sequencing.

Also described herein are methods that can include obtaining a biological sample from a subject having endometrial cancer and measuring the amount of HN1 protein and/or gene expression in the biological sample. As used herein, “obtaining” can refer to the process of
15 collecting a biological sample directly from a subject. As used herein, “obtaining” can refer to the process of receiving a biological sample that was previously collect from a subject, such as when a processing lab or other entity obtains a collected sample from a different entity such as the medical professional who collected the sample or the subject. The step of measuring can be performed using an immunological assay or protein sequencing. The step of measuring can be
20 performed using an immunological assay using an antibody that specifically binds HN1 protein. The subject can have endometrioid endometrial cancer. The subject can be obese. The method can include the step of administering to the subject a therapeutically effective amount of metformin. The therapeutically effective amount can be about 850 mg. In some aspects, the therapeutically effective amount can range from 500 to 750, 850, 1000, 1500, 2000, to about
25 2500 mg. The therapeutically effective amount of metformin can be given 1 or more times per day. Further the total amount to be given daily can be broken up and administered over multiple administrations per day. Administration of metformin can be prior to the subject undergoing a hysterectomy and/or after undergoing a hysterectomy. In some aspects, a daily administration of a therapeutically effect amount of metformin can continue for 1, 2, 3, 4, or more weeks prior
30 to and/or after a hysterectomy. The subject can have increased HN1 protein and/or gene expression in the biological sample as compared to a suitable control sample. The entire or any one or more parts of the method can be performed prior to performing a hysterectomy on the subject. The entire or any one or more parts of the method can be performed after performing a hysterectomy on the subject.

Also described herein are methods of treating a subject having endometrial cancer and an increased HN1 protein and/or gene expression in a biological sample that can include administering a therapeutically effective of metformin to the subject. The therapeutically effective amount can be about 850 mg or as otherwise described herein. The therapeutically effective amount of metformin can be given 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more times per day. Further the total amount to be given daily can be broken up and administered over multiple administrations per day. Administration of the therapeutically effective amount of metformin can be prior to the subject undergoing a hysterectomy and/or after undergoing a hysterectomy. In some aspects, a daily administration of a therapeutically effect amount of metformin can continue for 1, 2, 3, 4, or more weeks prior to and/or after a hysterectomy. The subject has endometrioid endometrial cancer. The subject can be obese. The step of administering the therapeutically effective amount of metformin can be performed prior to performing a hysterectomy. In some aspects, absolute or relative amount HN1 protein and/or gene expression in a responder is increased 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 to 50 or more fold as compared to a suitable control. In some aspects, “responsive to metformin” can be considered when a subject has a statistically significant decrease in Ki-67 protein expression after metformin treatment as determined by a suitable method (e.g. inmunnohistochemistry).

Also described herein is the use of metformin for the use to treat endometrial cancer in a subject having an increased HN1 protein and/or gene expression as compared to a control. Also described herein is the use of metformin as a medicament in a formulation for the treatment of endometrial cancer in a subject having an increased HN1 protein and/or gene expression as compared to a control.

Kits

Also described herein are combination kits that can include an amount of an antibody or fragment thereof and/or an aptamer that can specifically bind the HN1 protein. In some embodiments, the kit can include one or more oligonucleotide primers that can bind the HN1 mRNA and/or cDNA. As used herein, the terms “combination kit” or “kit of parts” refers to t an antibody or fragment thereof or an aptamer that can specifically bind the HN1 protein and pharmaceutical formulations thereof and any additional components that are used to package, sell, market, deliver, and/or administer the combination of elements or a single element, such as the active ingredient, contained therein. Such additional components include but are not limited to, packaging, syringes, blister packages, bottles, and the like. When one or more of the components (e.g. active agents) contained in the kit are administered simultaneously, the

combination kit can contain the active agents in a single pharmaceutical formulation (e.g. a tablet) or in separate pharmaceutical formulations.

The combination kit can contain each agent, compound, pharmaceutical formulation or component thereof described herein, in separate compositions or pharmaceutical formulations. The separate compositions or pharmaceutical formulations can be contained in a single package or in separate packages within the kit. Also provided in some embodiments, are buffers, diluents, solubilization reagents, cell culture media and other reagents. These additional components can be contained in a single package or in separate packages within the kit.

In some embodiments, the combination kit also includes instructions printed on or otherwise contained in a tangible medium of expression. The instructions can provide information regarding the content of the antibody or fragment thereof or an aptamer that can specifically bind the HN1 protein, safety information regarding the of the antibody or fragment thereof or an aptamer that can specifically bind the HN1 protein and/or pharmaceutical formulations thereof, information regarding the dosages, indications for use, and/or recommended treatment regimen(s) of the antibody or fragment thereof or an aptamer that can specifically bind the HN1 protein and pharmaceutical formulations thereof. In some embodiments, the instructions can provide directions for using the of the antibody or fragment thereof or an aptamer that can specifically bind the HN1 protein in an assay that can be used to determine if a subject having endometriosis is going to be a responder to a non-antineoplastic agent, such as metformin. The instructions can also provide directions to contact the of the antibody or fragment thereof or an aptamer that can specifically bind the HN1 protein with a bodily fluid and/or tissue sample from a subject that has endometriosis and determine if there is binding and/or the amount binding that occurs. The instructions can provide to measure HN1 gene expression in the bodily fluid and/or tissue sample from the subject. The instructions can also provide the direction to treat a subject with a therapeutically effective amount of a non-antineoplastic agent, such as metformin. The instructions can provide that the amount of the non-antineoplastic agent, e.g. metformin, can be 850 mg per dose. The instructions can provide that the amount of the non-antineoplastic agent can be administered to a subject before performing a hysterectomy. The instructions can provide that the amount of the non-antineoplastic agent can be administered to a subject before after a hysterectomy.

Without further elaboration, it is believed that one skilled in the art can, based on the description herein, utilize the present disclosure to its fullest extent. It is emphasized that the embodiments of the present disclosure, particularly any “preferred” embodiments, are merely possible examples of the implementations, merely set forth for a clear understanding of the

principles of the disclosure. Many variations and modifications may be made to the disclosed embodiment(s) of the disclosure without departing substantially from the spirit and principles of the disclosure. All such modifications and variations are within the scope of this disclosure.

EXAMPLES

Now having described the embodiments of the present disclosure, in general, the following Examples describe some additional embodiments of the present disclosure. While embodiments of the present disclosure are described in connection with the following examples and the corresponding text and figures, there is no intent to limit embodiments of the present disclosure to this description. On the contrary, the intent is to cover all alternatives, modifications, and equivalents included within the spirit and scope of embodiments of the present disclosure.

Example 1.

In a phase 0 clinical study the preoperative use of metformin in obese women with endometrioid endometrial cancer is associated with reduced tumor proliferation and an inhibition of the mammalian target of rapamycin (mTOR) pathway in some cases. This Example can demonstrate the identification of elevated HN1 in subjects that were responsive to metformin. Briefly, samples were those used in the phase 0 clinical trial described in Schuler et al. Cancer Medicine 2015, 4(2):161-173. Differential proteomic analysis of laser microdissected tumor cells collected from matched, pre-treatment biopsies and post-treatment hysterectomy EC patient tissue specimens (n=20) treated with metformin (850 mg) daily for 1-4 weeks prior to surgical staging was conducted. Thirteen patients responded and seven patients did not respond to metformin treatment based on decrease expression of the proliferation marker Ki-67. Mass spectrometry-based proteomics was performed from tryptic digests of tissue samples. Protein abundance was measured using spectral counting, and functional analyses were performed using Ingenuity Pathway Analysis. HN1 protein expression was also assessed by immunohistochemistry.

Differential proteomic analyses revealed alterations of molecular signaling pathways supporting increased cell proliferation and decreased cellular apoptosis signaling in metformin responder vs. non-responder EC patients. Protein HN1 was significantly elevated in responder vs. non-responder patients and further decreased in comparisons of post- vs. pre-treated responder patients, as evidenced by both proteomic and immunohistochemical analysis. HN1

represents a predictive biomarker candidate of preoperative therapeutic response to metformin in EC patients.

Example 2.

5 *Introduction.*

Endometrial cancer (EC) is now the second most prevalent cancer among women in the USA and one of the few cancers for which disease incidence is on the rise, particularly for aggressive histologic subtypes (1) (2). Clinical management of EC includes total hysterectomy, bilateral salpingo-oopherectomy and pelvic and peri-aortic lymph node dissection and can be 10 followed by adjuvant treatment with chemotherapy and vaginal brachytherapy. Given the increasing incidence and the paucity of effective treatments for advanced and recurrent EC, novel therapeutic agents that could be used alone or in combination with more traditional hormonal and cytotoxic chemotherapy to combat these trends are under active investigation. Emerging therapeutics for EC include the antihyperglycemic drug metformin (3-5). Owing to its 15 effect of inhibiting hepatic gluconeogenesis, metformin is widely used clinically to manage type II diabetes. Metformin activates adenosine monophosphate kinase (AMPK) that, in turn, stimulates a number of catabolic pathways, such as glucose uptake, glycolysis and fatty acid oxidation and inhibits numerous ATP-consuming processes, such as fatty acid, cholesterol and protein synthesis (3,6). In the context of cancer, metformin has been shown to dramatically 20 decrease proliferation of a number of different human cancer cell lines *in vitro* (5,7-9). Metformin has also been shown to effectively repress tumor growth in xenograft models of breast, prostate and colon cancer (10-12). In endometrial cancer cells, metformin-mediated AMPK activation decreases cell growth by inhibiting mTOR signaling (5).

Epidemiologic studies of diabetic patients have revealed those treated with metformin 25 exhibit a significantly reduced risk to develop diverse cancers relative to non-metformin treated patients (13) (14). Focused meta-analyses have suggested that metformin improves disease outcome in diabetic cancer patients, as evidenced by a recent retrospective cohort study of 2,529 diabetic patients with early stage breast cancer that found that women receiving metformin and adjuvant chemotherapy had a higher complete pathologic response rate than 30 those not taking metformin (15). In endometrial cancer, our team recently completed a preoperative phase 0 window study investigating the impact of short-term metformin treatment in women undergoing surgical staging for type I endometrial cancer who were obese (BMI >30) and/or have diabetes, but who were metformin treatment naïve (ClinicalTrials.gov: NCT01911247). We recently reported a comparative metabolomic analysis of serum and tumor

tissues before and after metformin treatment stratified by response as measured by decreased abundance of the proliferation antigen Ki-67 in tumor tissues as measured by IHC staining (4). Metformin responders exhibited significantly decreased levels of phospho-AMPK as well as several downstream mTOR targets in tumor tissues and the serum metabolome reflected activated lipolysis suggesting activation of fatty acid metabolism following metformin treatment. We describe here a quantitative proteomics investigation of matched tumor tissues from the same cohort of pre- and post-metformin treated-patients collected during our phase 0 study of metformin in obese EC patients and identification of hematological and neurological expressed 1 (HN1) as a predictive and pharmacodynamic biomarker of metformin response in endometrial cancer patients.

Methods

Tissue Specimens: Formalin-fixed, paraffin embedded (FFPE) tissues were collected during pre-treatment biopsies or during primary surgery from endometrial cancer patients enrolled in a Phase 0 window trial for metformin treatment under IRB approved protocols (4). Thin (10 µm) tissue sections were cut using a microtome and placed on polyethylene naphthalate membrane slides. After staining with aqueous H&E, laser microdissection (LMD) was used to harvest tumor cells from the thin sections and were collected in 45 uL of LC-MS grade water (mean tumor cell area captured = ~16.1 ± 23 mm^2).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) proteomics: Tissue samples were processed and analyzed by high resolution LC-MS/MS on an Orbitrap Velos MS (Thermo Fisher, San Jose, CA) and HN1 IP samples (preparation described below) were similarly analyzed on a Fusion Lumos MS (Thermo Fisher) as previously described (16,17). Peptide identifications were filtered to include peptide spectrum matches (PSMs) passing an FDR < 1.0%. Proteins included in subsequent quantitative analyses were required to have a minimum of two PSMs. Significantly altered proteins were identified using edgeR (18) based on PSMs normalized to the patient sample exhibiting the lowest total PSM counts (edgeR $p<0.05$). Pathway analyses were performed using Ingenuity Pathway Analysis (Qiagen).

Immunohistochemistry (IHC): Rabbit polyclonal anti-HN1 antibody was purchased from Atlas Antibodies (Sigma-Aldrich - HPA059729, Bromma, Sweden). IHC was carried out in the Bond Autostainer (Leica Microsystems Inc. Norwell MA). Briefly, slides were dewaxed in Bond Dewax solution (AR9222) and hydrated in Bond Wash solution (AR9590). Antigen retrieval was performed for 20 min at 100ºC in Bond-Epitope Retrieval solution 1, pH-6.0 (AR9961). Slides were incubated with primary antibody (1:100) for 2 h. Antibody detection was performed using

the Bond Polymer Refine detection system (DS9800). Stained slides were dehydrated and coverslipped. Positive and negative controls (no primary antibody) were included during the run. *IHC image analysis*: Stained slides were digitally scanned at 20x magnification using Aperio ScanScope-XT (Aperio Technologies, Vista, CA). Digital images were stored and analyzed
5 within an Aperio eSlideManager Database. TMA images were segmented into individual cores using the Tissue Studio TMA portal (Tissue Studio version 2.7 with Tissue Studio Library v4.4.2; Definiens Inc., Munich, Germany). Epithelial cell enriched regions were digitally separated out for analysis using Tissue Studio Composer software (Definiens). Tissue Studio's Nuclear Algorithm was then used to detect and enumerate cells that expressed HN1. The percentage of
10 positive nuclei and an H-score (formula = (% at 1+) * 1 + (% at 2+) * 2 + (% at 3+) * 3) were determined for each TMA core.

Cell culture and metformin treatment: RL95-2 was purchased from ATCC (Manassas, VA) and maintained in DMEM:F-12 medium supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin and 0.005 mg/mL insulin (Sigma-Aldrich, St. Louis, MO). ACI-181 (19), a
15 model of endometrioid endometrial cancer was a gift from Dr. John Risinger (Department of Obstetrics, Gynecology and Reproductive Biology, Michigan State University) and cultured in DMEM:F12 supplemented with 10% FBS 1% Pen/Strep. Both cell lines were maintained at 37 °C and 5% CO_2. Metformin was purchased from Sigma-Aldrich.

Cell Proliferation Assay: Cells were trypsinized with 0.25% trypsin-EDTA (ATCC),
20 counted with 0.4% Trypan Blue using an automated cell counter (TC10, Bio-Rad, Hercules, CA) and viable cells were plated in 96-well plates at equivalent densities for each cell lines. For the metformin dose response assay, media was removed 24 hrs later and replaced with fresh media containing metformin (0, 1.56, 3.13, 6.25, 12.5, 25, 50, 100 mM) followed by incubation for 72h. Cell viability was assessed using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-
25 carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega, Madison, WI) according to manufacturer's instructions. Briefly, after a 3 h incubation at 37 °C, absorbance was measured at 490 nm using a microplate spectrophotometer (xMark, Bio-Rad). Two biological replicates were performed for each cell line with three technical replicates per metformin dose. For the cell proliferation assay,
30 cells were plated in 96-well plates 72 h following transfection with siNT or siHN1 siRNA and cell viability was assessed daily by MTS assay as described above each day for four consecutive days for both the RL95-2 and ACI-181 cell lines.

HN1 knockdown by siRNA: ON-TARGE Tplus Human JPT1 (HN1) siRNA SMART pool and ON-TARGET plus Human Non Targeting Control pool were purchased from Dharmacon.

TransIT-siQUEST transfection reagent (Mirus Bio) was used for the siRNA transfections of the RL95-2 and ACI-181 cell lines. Cells were transfected with 75 nM siRNA according to the manufacturer's protocol. Seventy-two hours post-transfection, cells were trypsinized, counted and seeded for cell viability assay. Cells were also harvested at 72 and 168 h after transfection to assess HN1 knockdown by immunoblotting and qPCR analyses. Two biological replicates were performed for each cell line.

Quantitative PCR:

cDNA was prepared from equivalent amounts of total RNA by reverse-transcription using the Superscript VILO cDNA synthesis kit (Invitrogen). HN1 (Hs00602957_m1) and GAPDH (Hs99999905_m1) TAQMAN assays were obtained from Applied Biosystems (Thermo Fisher). Quantitative PCR was performed using TAQMAN gene expression master mix of equivalent amounts of total cDNA for forty cycles (ABI GeneAmp 9700 DNA thermal cycler). Endpoint data was assembled by comparison of Delta-Ct values for HN1 versus corresponding GAPDH Delta-Ct values for each cell line. Triplicate technical replicates were performed.

Immunoblot analyses:

Cells were washed with cold PBS and lysed in lysis buffer containing 1% SDS and 150 mM NaCl (pH 7.4). Equivalent amounts of protein lysates were resolved via 4-15% mini-PROTEIN TGX gels (Bio-Rad) and transferred to PVDF membranes. Membranes were blocked for 1 hr using 5% non-fat dry milk (Bio-Rad) and incubated with primary antibody overnight at 4 °C, secondary antibody for 3 hat ambient temperature, and SuperSignal West Chemiluminescent Substrate (Thermo Scientific) for 5 min. Images were acquired using a ChemiDoc XRS+ system (Bio-Rad). Anti-Ki67 and anti-GAPDH were purchased from Abcam. Anti-MYC was purchased from Santa Cruz. Anti-HN1 was purchased from GeneTex. Anti-pAMPKα Thr172, anti-AMPKα, anti-pAKT1 Ser473, anti-AKT (pan) and goat anti-rabbit IgG HRP-linked were purchased from Cell Signaling Technologies.

IP-MS analyses of the HN1: Twenty million RL95-2 cells were used per immunoprecipitation. For each immunoprecipitation, 30 µL of protein G sepharose 4 fast flow (GE Healthcare) was bound to 5 µL of anti-HN1 (GeneTex) for 2.5 h at 4ºC with end-over-end rotation. Cells were lysed in 50 mM Tris (pH 7.4), 137 mM NaCL, 10% glycerol and 0.1% Triton X supplemented with 1 X Halt protease and phosphatase inhibitor (ThermoFisher Scientific) and 5mM EDTA for thirty min on ice. Lysate was spun at 16,000 x g for 20min at 4 ºC and the supernatant was incubated with protein G sepharose 4 fast flow and anti-HN1 for 3 h at 4ºC with end-over-end rotation. Sample was resolved on a 4-15% mini-PROTEAN TGX gel (Bio-Rad) and ten gel bands per lane were cut. Gel bands were digested using trypsin/LysC Mix

(Promega) overnight and samples were cleaned up using Reversed-Phase ZipTip (Millipore) according to manufacturer's protocol prior to LC-MS/MS analysis. Immunoprecipitation with anti-HN1 with protein G sepharose 4 fast flow was performed in duplicate. Immunoprecipitation with protein G sepharose 4 fast flow alone was used as a control.

5 *Public-Data Analysis:* Normalized RNA-seq data (TCGA V2, (20) for HN1 and Ki-67 was downloaded from the https://gdac.broadinstitute.org for n=542 EC patients and transcript abundance was directly compared by Spearman correlation. Clinical characteristics were extracted from cgdsr (version 1.2.5) and a Kaplan-Meier analysis with log-rank testing was performed to assess the relationship between HN1 abundance and patient outcome using 10 survival (version 2.37-7) package in R (version 3.1.2). For Kaplan-Meier curves, high versus low transcript expression was defined by the median cut-point capped at 60 months.

Results

Proteomic analysis of endometrial cancer (EEC) tumor tissues collected from pre and post-15 treated patient can demonstrate conserved protein alterations between metformin responders and non-responders.

Tumor tissues from endometrial cancer patients in a pre-operative phase 0 window trial were stratified as responders (n=13) or non-responders (n=7) to metformin treatment (**FIG. 8**). Response was defined as a decrease in IHC staining for Ki-67 when comparing pre- versus 20 post-treatment endometrial cancer tissue as previously described (4). Quantitative LC-MS/MS-based global proteomic analyses of pathologically-defined, tumor cell populations harvested by laser microdissection from FFPE endometrial biopsies and endometrial cancer surgical tumor tissues identified 1,289 proteins by at least two peptide spectral matches (PSMs) across patients (**FIG. 10**). Seventy-nine proteins were identified to be significantly altered (edgeR p-25 value ≤ 0.05) in pre-treatment tumor cells from metformin responder and non-responder patients (**FIG. 1** and **Table 1**). Pathway analysis revealed top altered pathways to include adenosine monophosphate kinase (AMPK) signaling (**FIG. 9**), along with those related to activating cellular signaling, regulating cellular proliferation, and inhibiting cell death and apoptosis in tissues in metformin responders (**FIG. 9**). Protein alterations in pre-treatment tissues from responder 30 versus non-responder patients (**Table 2**) were correlated with significant alterations in post versus pre-treated tissues from metformin responders (edgeR p-value ≤ 0.05), revealing 11 co-altered proteins between these groups (Uniprot Accession * and bolded, **Table 2**). Further analyses of metformin responders revealed activation of cell death and apoptosis signaling, but inhibition of viral infection as well as molecular transport in response to metformin. Additional

analyses of significant protein alterations in metformin non-responders also revealed activation of cell death as well as organ hypoplasia signaling, inhibition of T cell proliferation and leukocyte viability signaling in post versus pre-metformin treated tissues.

Table 1. Proteins significantly altered between responder versus non-responder patient tissue samples, pre-metformin treatment.

Protein	Uniprot Accession	Non-Responders - AverageNormPSMs	Responder versus Non-Responder (Ratio)	Responders - AverageNormPSMs	Responder versus Non-Responder (LogFC, edgeR)	Pvalue (edgeR)
Hematological and neurological expressed 1 protein OS=Homo sapiens GN=HN1 PE=1 SV=3	Q9UK76	0.2	14.36	3.5	3.42119541	9.78E-06
Echinoderm microtubule-associated protein-like 4 OS=Homo sapiens GN=EML4 PE=1 SV=3	Q9HC35	1.2	3.84	4.6	1.7869206	0.000683034
Carbonyl reductase [NADPH] 1 OS=Homo sapiens GN=CBR1 PE=1 SV=3	P16152	20.3	0.63	12.7	-0.729345416	0.00097424
Tubulin-folding cofactor B OS=Homo sapiens GN=TBCB PE=1 SV=2	Q99426	2.4	2.2	5.4	1.046665164	0.001001156
Keratin, type I cytoskeletal 13 OS=Homo sapiens GN=KRT13 PE=1 SV=4	P13646	44.4	0.35	15.5	-1.64436746	0.002806686
DNA replication licensing factor MCM3 OS=Homo sapiens GN=MCM3 PE=1 SV=3	P25205	0.5	7.68	3.6	2.66307444	0.003587382
Protein-glutamine gamma-glutamyltransferase 2 OS=Homo sapiens GN=TGM2 PE=1 SV=2	P21980	26.1	0.47	12.2	-1.194263825	0.004302032
Nucleoprotein TPR OS=Homo sapiens GN=TPR PE=1 SV=3	P12270	7.8	1.71	13.4	0.696980828	0.004667673
Segment polarity protein dishevelled homolog DVL-2 OS=Homo sapiens GN=DVL2 PE=1 SV=1	O14641	0.6	3.23	1.9	1.551665634	0.004967764
Mitotic checkpoint protein BUB3 OS=Homo sapiens GN=BUB3 PE=1 SV=1	O43684	2.1	2.23	4.7	1.062568634	0.007648084
Thyroid hormone receptor-associated protein 3 OS=Homo sapiens GN=THRAP3 PE=1 SV=2	Q9Y2W1	4.1	2.16	8.7	1.026258573	0.007886741

Keratin, type II cytoskeletal 1 OS=Homo sapiens GN=KRT1 PE=1 SV=6	P04264	43	0.49	21.1	-1.132199401	0.007970925
Fatty acid synthase OS=Homo sapiens GN=FASN PE=1 SV=3	P49327	14.5	1.76	25.5	0.757445385	0.009110922
Cytoplasmic aconitate hydratase OS=Homo sapiens GN=ACO1 PE=1 SV=3	P21399	10.8	0.59	6.4	-0.827573624	0.009194801
Programmed cell death protein 10 OS=Homo sapiens GN=PDCD10 PE=1 SV=1	Q9BUL8	0.7	3.1	2.1	1.36602404	0.009602186
KH domain-containing, RNA-binding, signal transduction-associated protein 1 OS=Homo sapiens GN=KHDRBS1 PE=1 SV=1	Q07666	2.2	1.97	4.2	0.8972794	0.01014147
Golgin subfamily A member 2 OS=Homo sapiens GN=GOLGA2 PE=1 SV=3	Q08379	2.8	1.88	5.3	0.806267902	0.0109574
Protein FAM49B OS=Homo sapiens GN=FAM49B PE=1 SV=1	Q9NUQ9	5.2	0.55	2.9	-0.913189699	0.011207273
RNA-binding protein FUS OS=Homo sapiens GN=FUS PE=1 SV=1	P35637	4.4	1.74	7.7	0.726107862	0.011213898
Keratin, type I cytoskeletal 10 OS=Homo sapiens GN=KRT10 PE=1 SV=6	P13645	38.6	0.48	18.7	-1.13607543	0.011510681
Protein RCC2 OS=Homo sapiens GN=RCC2 PE=1 SV=2	Q9P258	1	3.7	3.8	1.727199105	0.012474484
Splicing factor U2AF 35 kDa subunit OS=Homo sapiens GN=U2AF1 PE=1 SV=3	Q01081	1.7	2.23	3.7	1.069399611	0.012723149
NADH dehydrogenase [ubiquinone] iron-sulfur protein 2, mitochondrial OS=Homo sapiens GN=NDUFS2 PE=1 SV=2	O75306	7.6	0.49	3.7	-1.080494803	0.013276056
Very-long-chain (3R)-3-hydroxyacyl-CoA dehydratase 3 OS=Homo sapiens GN=HACD3 PE=1 SV=2	Q9P035	2.7	0.45	1.2	-1.241035785	0.013726468
Hemoglobin subunit alpha OS=Homo sapiens GN=HBA1 PE=1 SV=2	P69905	56.2	0.48	27.2	-1.136766161	0.014828474
Methyl-CpG-binding protein 2 OS=Homo sapiens GN=MECP2 PE=1 SV=1	P51608	4.3	0.36	1.5	-1.50453064	0.015489259

Ig kappa chain C region OS=Homo sapiens GN=IGKC PE=1 SV=1	P01834	29.3	0.63	18.6	-0.748160187	0.016298364
Dipeptidyl peptidase 3 OS=Homo sapiens GN=DPP3 PE=1 SV=2	Q9NY33	7.9	1.63	12.9	0.635102062	0.016529296
Transformer-2 protein homolog alpha OS=Homo sapiens GN=TRA2A PE=1 SV=1	Q13595	1.9	2.02	3.8	0.861072237	0.019084465
RNA-binding protein Raly OS=Homo sapiens GN=RALY PE=1 SV=1	Q9UKM9	1.3	2.78	3.6	1.313117553	0.019753446
Sorbitol dehydrogenase OS=Homo sapiens GN=SORD PE=1 SV=4	Q00796	10.8	0.47	5.1	-1.134427811	0.02013638
Far upstream element-binding protein 1 OS=Homo sapiens GN=FUBP1 PE=1 SV=3	Q96AE4	7.4	1.58	11.7	0.587945555	0.020185803
Membrane-associated progesterone receptor component 1 OS=Homo sapiens GN=PGRMC1 PE=1 SV=3	O00264	3.7	2.28	8.4	1.083792737	0.020617618
SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily E member 1 OS=Homo sapiens GN=SMARCE1 PE=1 SV=2	Q969G3	1	2.42	2.5	1.110997135	0.020680311
26S proteasome non-ATPase regulatory subunit 9 OS=Homo sapiens GN=PSMD9 PE=1 SV=3	O00233	1.2	2.34	2.9	1.139705334	0.022226598
Nuclease-sensitive element-binding protein 1 OS=Homo sapiens GN=YBX1 PE=1 SV=3	P67809	23.3	1.39	32.5	0.421481798	0.022487857
Heat shock protein HSP 90-alpha OS=Homo sapiens GN=HSP90AA1 PE=1 SV=5	P07900	27.2	1.35	36.8	0.371840368	0.022689058
Actin, aortic smooth muscle OS=Homo sapiens GN=ACTA2 PE=1 SV=1	P62736	59.9	0.81	48.6	-0.376938727	0.022789263
Exportin-2 OS=Homo sapiens GN=CSE1L PE=1 SV=3	P55060	5.7	1.67	9.6	0.670247487	0.022941548
Cytochrome b-c1 complex subunit 7 OS=Homo sapiens GN=UQCRB PE=1 SV=2	P14927	3.6	0.62	2.3	-0.776245621	0.025237088
Proliferating cell nuclear antigen OS=Homo sapiens GN=PCNA PE=1 SV=1	P12004	1.6	2.63	4.2	1.293616403	0.025380008

UPF0568 protein C14orf166 OS=Homo sapiens GN=C14orf166 PE=1 SV=1	Q9Y224	9.4	0.69	6.5	-0.577595826	0.025660125
Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens GN=KRT2 PE=1 SV=2	P35908	28	0.57	16	-0.908034494	0.025701653
Flap endonuclease 1 OS=Homo sapiens GN=FEN1 PE=1 SV=1	P39748	2.2	2.13	4.6	0.997799904	0.026626564
Alanine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=AARS PE=1 SV=2	P49588	4.5	2.5	11.3	1.223545474	0.027032834
Heat shock cognate 71 kDa protein OS=Homo sapiens GN=HSPA8 PE=1 SV=1	P11142	44.3	1.26	55.7	0.263836201	0.0281985
Cadherin-1 OS=Homo sapiens GN=CDH1 PE=1 SV=3	P12830	8	1.53	12.3	0.537673104	0.028185932
Serine hydroxymethyltransferase, mitochondrial OS=Homo sapiens GN=SHMT2 PE=1 SV=3	P34897	1.1	3.77	4	1.773389533	0.029575119
Myosin-11 OS=Homo sapiens GN=MYH11 PE=1 SV=3	P35749	26.4	0.57	15.1	-0.924900844	0.030536622
Luc7-like protein 3 OS=Homo sapiens GN=LUC7L3 PE=1 SV=2	O95232	3.7	2.1	7.7	0.986625226	0.031210343
Cold-inducible RNA-binding protein OS=Homo sapiens GN=CIRBP PE=1 SV=1	Q14011	13.5	0.77	10.4	-0.422921152	0.031316514
Splicing factor 3A subunit 3 OS=Homo sapiens GN=SF3A3 PE=1 SV=1	Q12874	5.6	1.77	9.9	0.726161832	0.032251328
Collagen alpha-1(VI) chain OS=Homo sapiens GN=COL6A1 PE=1 SV=3	P12109	8.6	0.41	3.5	-1.407260955	0.032259219
Agrin OS=Homo sapiens GN=AGRN PE=1 SV=5	O00468	4.5	0.5	2.2	-1.0316362	0.03280252
Thymosin beta-4 OS=Homo sapiens GN=TMSB4X PE=1 SV=2	P62328	1.9	2.68	5.2	1.349658228	0.03301636
Nuclear autoantigenic sperm protein OS=Homo sapiens GN=NASP PE=1 SV=2	P49321	1.4	3.04	4.3	1.47639945	0.0359411
Serine--tRNA ligase, cytoplasmic OS=Homo sapiens GN=SARS PE=1 SV=3	P49591	4.2	1.53	6.5	0.547289839	0.036970353

Eukaryotic translation initiation factor 3 subunit I OS=Homo sapiens GN=EIF3I PE=1 SV=1	Q13347	0.9	2.58	2.4	1.26836554	0.037032143
Actin-related protein 2/3 complex subunit 5 OS=Homo sapiens GN=ARPC5 PE=1 SV=3	O15511	2	0.48	1	-1.076189121	0.037083055
Transcription factor BTF3 OS=Homo sapiens GN=BTF3 PE=1 SV=1	P20290	1	3.35	3.3	1.563835364	0.037453589
Transketolase OS=Homo sapiens GN=TKT PE=1 SV=3	P29401	47.7	0.82	39	-0.350320999	0.038077703
Serine/arginine repetitive matrix protein 2 OS=Homo sapiens GN=SRRM2 PE=1 SV=2	Q9UQ35	2.6	2.06	5.3	0.962233871	0.038330601
Glial fibrillary acidic protein OS=Homo sapiens GN=GFAP PE=1 SV=1	P14136	70.6	0.4	28.4	-1.409290933	0.038989635
Charged multivesicular body protein 4b OS=Homo sapiens GN=CHMP4B PE=1 SV=1	Q9H444	2.8	1.8	5	0.757935936	0.039165691
RNA-binding protein 3 OS=Homo sapiens GN=RBM3 PE=1 SV=1	P98179	3.4	1.88	6.4	0.828682694	0.039509349
Aconitate hydratase, mitochondrial OS=Homo sapiens GN=ACO2 PE=1 SV=2	Q99798	17.8	0.69	12.3	-0.583922008	0.039877776
Fidgetin-like protein 1 OS=Homo sapiens GN=FIGNL1 PE=1 SV=2	Q6PIW4	0.8	2.49	2	1.164254596	0.040415685
Erythrocyte band 7 integral membrane protein OS=Homo sapiens GN=STOM PE=1 SV=3	P27105	5.8	0.54	3.1	-0.942687746	0.041315275
Microtubule-associated protein 4 OS=Homo sapiens GN=MAP4 PE=1 SV=3	P27816	5.9	1.64	9.6	0.619665091	0.04132938
Lipoma-preferred partner OS=Homo sapiens GN=LPP PE=1 SV=1	Q93052	1	2.23	2.2	0.961332686	0.041689169
26S proteasome non-ATPase regulatory subunit 11 OS=Homo sapiens GN=PSMD11 PE=1 SV=3	O00231	2.2	1.86	4.1	0.759079498	0.042886534
Ubiquitin carboxyl-terminal hydrolase 10 OS=Homo sapiens GN=USP10 PE=1 SV=2	Q14694	0.6	2.82	1.6	1.344954018	0.043058971

Solute carrier family 2, facilitated glucose transporter member 1 OS=Homo sapiens GN=SLC2A1 PE=1 SV=2	P11166	1.7	2.01	3.4	0.912231429	0.043676105
Transcription activator BRG1 OS=Homo sapiens GN=SMARCA4 PE=1 SV=2	P51532	1.9	2.26	4.2	1.052927408	0.044955154
Retinal dehydrogenase 1 OS=Homo sapiens GN=ALDH1A1 PE=1 SV=2	P00352	9.8	0.6	5.8	-0.806690579	0.045206283
Trifunctional enzyme subunit alpha, mitochondrial OS=Homo sapiens GN=HADHA PE=1 SV=2	P40939	29.7	0.79	23.5	-0.398752775	0.045463408
Protein phosphatase 1G OS=Homo sapiens GN=PPM1G PE=1 SV=1	O15355	1.3	3.15	4	1.563039068	0.04761446
Vesicle-fusing ATPase OS=Homo sapiens GN=NSF PE=1 SV=3	P46459	5.6	0.57	3.2	-0.856934035	0.048618009
Histone H2B type 1-A OS=Homo sapiens GN=HIST1H2BA PE=1 SV=3	Q96A08	6.4	0.73	4.7	-0.561865917	0.049577183

Table 2. Proteins significantly altered between post versus pre-metfomrin treated tissues collected from metformin responders.

Protein	Uniprot Accession	Pre-Metformin - AverageNormPSMs	Post versus Pre-Metformin (Ratio)	Post versus Pre-Metformin (LogFC)	Post-Metformin - AverageNormPSMs	Pvalue (edgeR)
Mimecan OS=Homo sapiens GN=OGN PE=1 SV=1	P20774	0.7	6.82	2.77	4.6	0.004065169
Serine/arginine-rich splicing factor 5 OS=Homo sapiens GN=SRSF5 PE=1 SV=1	Q13243	0.3	4.40	2.14	1.4	0.011265334
Biglycan OS=Homo sapiens GN=BGN PE=1 SV=2	P21810	0.4	4.11	2.04	1.6	0.002551019
Transmembrane protein 205 OS=Homo sapiens GN=TMEM205 PE=1 SV=1	Q6UW68	0.6	3.40	1.77	2.2	0.014957502
Collagen alpha-1(XIV) chain OS=Homo sapiens GN=COL14A1 PE=1 SV=3	Q05707	2.4	2.93	1.55	6.9	0.002401681
Transgelin OS=Homo sapiens GN=TAGLN PE=1 SV=4	Q01995	3.2	2.81	1.49	9.0	0.023301132
Galactokinase OS=Homo sapiens GN=GALK1 PE=1 SV=1	P51570	0.9	2.67	1.42	2.5	0.009653633
Periostin OS=Homo sapiens GN=POSTN PE=1 SV=2	Q15063	2.3	2.54	1.34	5.7	0.000760662
EMILIN-1 OS=Homo sapiens GN=EMILIN1	Q9Y6C2	2.2	2.40	1.26	5.4	0.00839

PE=1 SV=2						8609
Amine oxidase [flavin-containing] A OS=Homo sapiens GN=MAOA PE=1 SV=1	P21397	0.9	2.36	1.24	2.0	0.02435 3098
Superoxide dismutase [Cu-Zn] OS=Homo sapiens GN=SOD1 PE=1 SV=2	P00441	0.8	2.29	1.20	1.9	0.04635 3732
Putative high mobility group protein B1-like 1 OS=Homo sapiens GN=HMGB1P1 PE=5 SV=1	B2RPK0	1.2	2.26	1.18	2.8	0.04695 0064
Nidogen-1 OS=Homo sapiens GN=NID1 PE=1 SV=3	P14543	1.4	2.26	1.18	3.1	0.02386 2654
Plakophilin-3 OS=Homo sapiens GN=PKP3 PE=1 SV=1	Q9Y446	1.2	2.22	1.15	2.7	0.04606 0597
DnaJ homolog subfamily A member 2 OS=Homo sapiens GN=DNAJA2 PE=1 SV=1	O60884	1.6	2.21	1.14	3.5	0.02882 0516
Lumican OS=Homo sapiens GN=LUM PE=1 SV=2	P51884	2.6	2.15	1.10	5.6	0.00543 1087
Lon protease homolog, mitochondrial OS=Homo sapiens GN=LONP1 PE=1 SV=2	P36776	3.6	2.12	1.08	7.6	0.00083 8604
DnaJ homolog subfamily A member 1 OS=Homo sapiens GN=DNAJA1 PE=1 SV=2	P31689	1.8	2.07	1.05	3.8	0.04118 2585
Collagen alpha-1(I) chain OS=Homo sapiens GN=COL1A1 PE=1 SV=5	P02452	7.1	2.04	1.03	14.4	0.01599 0285
Small acidic protein OS=Homo sapiens GN=SMAP PE=1 SV=1	O00193	1.9	2.00	1.00	3.7	0.02556 5365
Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial OS=Homo sapiens GN=ALDH6A1 PE=1 SV=2	Q02252	6.4	1.96	0.97	12.6	0.00120 239
COP9 signalosome complex subunit 7b OS=Homo sapiens GN=COPS7B PE=1 SV=1	Q9H9Q2	1.2	1.86	0.90	2.2	0.03819 6266
Ubiquitin carboxyl-terminal hydrolase isozyme L3 OS=Homo sapiens GN=UCHL3 PE=1 SV=1	P15374	1.5	1.85	0.89	2.7	0.01760 2232
3-hydroxyisobutyrate dehydrogenase, mitochondrial OS=Homo sapiens GN=HIBADH PE=1 SV=2	P31937	4.2	1.84	0.88	7.6	0.00753 494
Hydroxysteroid dehydrogenase-like protein 2 OS=Homo sapiens GN=HSDL2 PE=1 SV=1	Q6YN16	1.5	1.80	0.85	2.7	0.03065 436
Cytochrome b-c1 complex subunit 1, mitochondrial OS=Homo sapiens GN=UQCRC1 PE=1 SV=3	P31930	3.9	1.78	0.83	6.9	0.01384 9871
Deoxynucleoside triphosphate triphosphohydrolase SAMHD1 OS=Homo sapiens GN=SAMHD1 PE=1 SV=2	Q9Y3Z3	12.2	1.78	0.83	21.7	0.01094 3811
Tropomyosin beta chain OS=Homo sapiens GN=TPM2 PE=1 SV=1	P07951	3.1	1.71	0.77	5.3	0.00873 2377
Collagen alpha-2(I) chain OS=Homo sapiens GN=COL1A2 PE=1 SV=7	P08123	8.5	1.68	0.75	14.3	0.00602 9516
Cytochrome b-c1 complex subunit 2,	P22695	3.8	1.66	0.73	6.3	0.01534

mitochondrial OS=Homo sapiens GN=UQCRC2 PE=1 SV=3						5154
MICOS complex subunit MIC60 OS=Homo sapiens GN=IMMT PE=1 SV=1	Q16891	4.5	1.66	0.73	7.4	0.00689 7591
Ras GTPase-activating-like protein IQGAP2 OS=Homo sapiens GN=IQGAP2 PE=1 SV=4	Q13576	4.1	1.64	0.71	6.8	0.02686 7261
Desmoglein-2 OS=Homo sapiens GN=DSG2 PE=1 SV=2	Q14126	6.6	1.62	0.70	10.6	0.00938 2364
Catenin delta-1 OS=Homo sapiens GN=CTNND1 PE=1 SV=1	O60716	4.6	1.61	0.69	7.3	0.01845 7785
Cysteine-rich protein 2 OS=Homo sapiens GN=CRIP2 PE=1 SV=1	P52943	3.1	1.61	0.69	5.0	0.01070 3618
Neuroblast differentiation-associated protein AHNAK OS=Homo sapiens GN=AHNAK PE=1 SV=2	Q09666	28.1	1.61	0.69	45.1	0.01398 5857
Nuclear protein localization protein 4 homolog OS=Homo sapiens GN=NPLOC4 PE=1 SV=3	Q8TAT6	2.6	1.55	0.63	4.0	0.01690 7691
Scaffold attachment factor B1 OS=Homo sapiens GN=SAFB PE=1 SV=4	Q15424	3.9	1.53	0.61	6.0	0.01332 5683
PRA1 family protein 3 OS=Homo sapiens GN=ARL6IP5 PE=1 SV=1	O75915	6.7	1.50	0.58	10.0	0.00249 0547
Glutathione S-transferase kappa 1 OS=Homo sapiens GN=GSTK1 PE=1 SV=3	Q9Y2Q3	4.1	1.49	0.58	6.2	0.02884 3983
Endoplasmic reticulum resident protein 44 OS=Homo sapiens GN=ERP44 PE=1 SV=1	Q9BS26	2.9	1.45	0.54	4.1	0.02251 7109
Protein ERGIC-53 OS=Homo sapiens GN=LMAN1 PE=1 SV=2	P49257	3.2	1.44	0.53	4.7	0.04710 1342
NADH-cytochrome b5 reductase 3 OS=Homo sapiens GN=CYB5R3 PE=1 SV=3	P00387	6.6	1.42	0.51	9.4	0.03615 2341
Actin, aortic smooth muscle OS=Homo sapiens GN=ACTA2 PE=1 SV=1	**P62736***	42.3	1.42	0.51	60.2	0.02017 5029
Tripeptidyl-peptidase 1 OS=Homo sapiens GN=TPP1 PE=1 SV=2	O14773	3.8	1.41	0.50	5.3	0.02865 7792
Ubiquitin-conjugating enzyme E2 variant 1 OS=Homo sapiens GN=UBE2V1 PE=1 SV=2	Q13404	3.2	1.41	0.50	4.6	0.04182 8244
Serine/arginine-rich splicing factor 1 OS=Homo sapiens GN=SRSF1 PE=1 SV=2	Q07955	9.0	1.40	0.49	12.6	0.02413 3052
Calnexin OS=Homo sapiens GN=CANX PE=1 SV=2	P27824	18.6	1.38	0.46	25.6	0.00068 6389
Succinyl-CoA ligase [GDP-forming] subunit beta, mitochondrial OS=Homo sapiens GN=SUCLG2 PE=1 SV=2	Q96I99	5.6	1.37	0.45	7.7	0.02471 2178
Isoaspartyl peptidase/L-asparaginase OS=Homo sapiens GN=ASRGL1 PE=1 SV=2	Q7L266	5.1	1.35	0.43	6.9	0.04353 6771
Protein SETSIP OS=Homo sapiens GN=SETSIP PE=1 SV=1	P0DME0	6.8	1.34	0.42	9.1	0.02271 5086

Cytosolic non-specific dipeptidase OS=Homo sapiens GN=CNDP2 PE=1 SV=2	Q96KP4	26.0	1.33	0.41	34.7	0.049613069
Spliceosome RNA helicase DDX39B OS=Homo sapiens GN=DDX39B PE=1 SV=1	Q13838	9.0	1.32	0.40	11.9	0.012612719
Alpha-enolase OS=Homo sapiens GN=ENO1 PE=1 SV=2	P06733	100.9	1.29	0.37	130.1	0.012841916
60 kDa heat shock protein, mitochondrial OS=Homo sapiens GN=HSPD1 PE=1 SV=2	P10809	48.5	1.29	0.37	62.9	0.043923629
14-3-3 protein epsilon OS=Homo sapiens GN=YWHAE PE=1 SV=1	P62258	22.2	1.28	0.36	28.6	0.004720709
Apoptosis-inducing factor 1, mitochondrial OS=Homo sapiens GN=AIFM1 PE=1 SV=1	O95831	10.4	1.26	0.33	13.2	0.045477884
Transmembrane emp24 domain-containing protein 10 OS=Homo sapiens GN=TMED10 PE=1 SV=2	P49755	11.4	1.25	0.32	14.2	0.039802185
Glucosidase 2 subunit beta OS=Homo sapiens GN=PRKCSH PE=1 SV=2	P14314	14.1	1.23	0.30	17.4	0.043500155
Heterogeneous nuclear ribonucleoprotein A3 OS=Homo sapiens GN=HNRNPA3 PE=1 SV=2	P51991	15.2	1.23	0.30	18.7	0.037242637
Actin, cytoplasmic 1 OS=Homo sapiens GN=ACTB PE=1 SV=1	P60709	73.7	1.21	0.28	89.3	0.014836203
Fructose-bisphosphate aldolase A OS=Homo sapiens GN=ALDOA PE=1 SV=2	P04075	24.1	0.79	-0.34	19.0	0.024070871
Moesin OS=Homo sapiens GN=MSN PE=1 SV=3	P26038	18.0	0.79	-0.34	14.3	0.049604988
T-complex protein 1 subunit beta OS=Homo sapiens GN=CCT2 PE=1 SV=4	P78371	38.1	0.78	-0.36	29.6	0.017235245
Complement C3 OS=Homo sapiens GN=C3 PE=1 SV=2	P01024	42.9	0.75	-0.42	32.0	0.036880487
Macrophage-capping protein OS=Homo sapiens GN=CAPG PE=1 SV=2	P40121	12.9	0.75	-0.42	9.7	0.041825834
Nucleoprotein TPR OS=Homo sapiens GN=TPR PE=1 SV=3	**P12270***	11.7	0.74	-0.43	8.6	0.020272302
Transforming growth factor-beta-induced protein ig-h3 OS=Homo sapiens GN=TGFBI PE=1 SV=1	Q15582	9.7	0.74	-0.43	7.1	0.01988812
Cullin-associated NEDD8-dissociated protein 1 OS=Homo sapiens GN=CAND1 PE=1 SV=2	Q86VP6	15.1	0.74	-0.43	11.2	0.041406005
Annexin A7 OS=Homo sapiens GN=ANXA7 PE=1 SV=3	P20073	6.6	0.72	-0.47	4.7	0.033712311
40S ribosomal protein S18 OS=Homo sapiens GN=RPS18 PE=1 SV=3	P62269	8.3	0.71	-0.49	5.9	0.014266509
40S ribosomal protein S4, X isoform OS=Homo sapiens GN=RPS4X PE=1 SV=2	P62701	7.7	0.71	-0.49	5.4	0.025643617
26S proteasome non-ATPase regulatory subunit 2 OS=Homo sapiens GN=PSMD2	Q13200	9.8	0.71	-0.49	6.9	0.032105994

PE=1 SV=3						
Complement C4-A OS=Homo sapiens GN=C4A PE=1 SV=2	P0C0L4	10.3	0.70	-0.51	7.2	0.021669956
Microtubule-associated protein 4 OS=Homo sapiens GN=MAP4 PE=1 SV=3	**P27816***	8.4	0.70	-0.51	5.9	0.026676481
Coatomer subunit beta' OS=Homo sapiens GN=COPB2 PE=1 SV=2	P35606	9.4	0.70	-0.51	6.6	0.04482518
Ubiquitin-60S ribosomal protein L40 OS=Homo sapiens GN=UBA52 PE=1 SV=2	P62987	17.2	0.70	-0.51	12.1	0.045934496
Extended synaptotagmin-1 OS=Homo sapiens GN=ESYT1 PE=1 SV=1	Q9BSJ8	12.8	0.70	-0.51	9.0	0.028900202
Eukaryotic translation initiation factor 3 subunit H OS=Homo sapiens GN=EIF3H PE=1 SV=1	O15372	5.3	0.69	-0.54	3.7	0.046732141
Kinesin-1 heavy chain OS=Homo sapiens GN=KIF5B PE=1 SV=1	P33176	12.4	0.69	-0.54	8.5	0.032480468
Delta-1-pyrroline-5-carboxylate synthase OS=Homo sapiens GN=ALDH18A1 PE=1 SV=2	P54886	17.3	0.69	-0.54	12.0	0.005696722
Hemoglobin subunit alpha OS=Homo sapiens GN=HBA1 PE=1 SV=2	**P69905***	23.7	0.69	-0.54	16.3	0.025779999
Hemoglobin subunit delta OS=Homo sapiens GN=HBD PE=1 SV=2	P02042	8.2	0.68	-0.56	5.6	0.018327596
Cytosol aminopeptidase OS=Homo sapiens GN=LAP3 PE=1 SV=3	P28838	11.8	0.68	-0.56	8.0	0.049744867
Twinfilin-1 OS=Homo sapiens GN=TWF1 PE=1 SV=3	Q12792	6.0	0.68	-0.56	4.1	0.043479767
40S ribosomal protein S15 OS=Homo sapiens GN=RPS15 PE=1 SV=2	P62841	11.6	0.66	-0.60	7.6	0.022062916
Lysine--tRNA ligase OS=Homo sapiens GN=KARS PE=1 SV=3	Q15046	6.4	0.66	-0.60	4.2	0.010352552
Glycerol-3-phosphate dehydrogenase, mitochondrial OS=Homo sapiens GN=GPD2 PE=1 SV=3	P43304	4.8	0.65	-0.62	3.1	0.019949385
40S ribosomal protein S16 OS=Homo sapiens GN=RPS16 PE=1 SV=2	P62249	6.5	0.64	-0.64	4.2	0.035333037
HLA class I histocompatibility antigen, A-1 alpha chain OS=Homo sapiens GN=HLA-A PE=1 SV=1	P30443	13.4	0.63	-0.67	8.4	0.009207411
Lysosome-associated membrane glycoprotein 1 OS=Homo sapiens GN=LAMP1 PE=1 SV=3	P11279	4.5	0.62	-0.69	2.8	0.014970655
Fibrinogen gamma chain OS=Homo sapiens GN=FGG PE=1 SV=3	P02679	4.2	0.61	-0.71	2.6	0.015022403
Leucine-rich repeat flightless-interacting protein 1 OS=Homo sapiens GN=LRRFIP1 PE=1 SV=2	Q32MZ4	5.2	0.61	-0.71	3.2	0.020290275
Protein transport protein Sec24C OS=Homo sapiens GN=SEC24C PE=1 SV=3	P53992	7.5	0.60	-0.74	4.5	0.027104957

UTP--glucose-1-phosphate uridylyltransferase OS=Homo sapiens GN=UGP2 PE=1 SV=5	Q16851	4.1	0.60	-0.74	2.5	0.03525 6935
26S proteasome non-ATPase regulatory subunit 11 OS=Homo sapiens GN=PSMD11 PE=1 SV=3	**O00231***	3.6	0.59	-0.76	2.1	0.02059 7956
SUN domain-containing protein 2 OS=Homo sapiens GN=SUN2 PE=1 SV=3	Q9UH99	4.3	0.59	-0.76	2.5	0.03882 7632
Solute carrier family 2, facilitated glucose transporter member 1 OS=Homo sapiens GN=SLC2A1 PE=1 SV=2	**P11166***	3.0	0.58	-0.79	1.7	0.03757 1195
Syntaxin-7 OS=Homo sapiens GN=STX7 PE=1 SV=4	O15400	3.2	0.57	-0.81	1.8	0.03555 6598
Lysosomal alpha-glucosidase OS=Homo sapiens GN=GAA PE=1 SV=4	P10253	9.3	0.55	-0.86	5.1	0.02287 0579
Tryptophan--tRNA ligase, cytoplasmic OS=Homo sapiens GN=WARS PE=1 SV=2	P23381	7.8	0.54	-0.89	4.2	0.04241 0158
ATP-citrate synthase OS=Homo sapiens GN=ACLY PE=1 SV=3	P53396	9.3	0.52	-0.94	4.9	0.00016 687
Gamma-glutamyl hydrolase OS=Homo sapiens GN=GGH PE=1 SV=2	Q92820	5.1	0.52	-0.94	2.7	0.04622 3609
Serine/arginine repetitive matrix protein 2 OS=Homo sapiens GN=SRRM2 PE=1 SV=2	**Q9UQ35***	4.7	0.52	-0.94	2.4	0.00503 1683
Transmembrane protein 33 OS=Homo sapiens GN=TMEM33 PE=1 SV=2	P57088	2.4	0.51	-0.97	1.2	0.02146 7387
Lysophosphatidylcholine acyltransferase 1 OS=Homo sapiens GN=LPCAT1 PE=1 SV=2	Q8NF37	3.3	0.50	-1.00	1.7	0.02220 8333
Cytoplasmic dynein 1 light intermediate chain 1 OS=Homo sapiens GN=DYNC1LI1 PE=1 SV=3	Q9Y6G9	5.2	0.50	-1.00	2.6	0.00100 4272
Cytoplasmic dynein 1 light intermediate chain 2 OS=Homo sapiens GN=DYNC1LI2 PE=1 SV=1	O43237	7.7	0.49	-1.03	3.8	0.00028 2087
Signal recognition particle subunit SRP72 OS=Homo sapiens GN=SRP72 PE=1 SV=3	O76094	2.6	0.49	-1.03	1.3	0.00919 0082
Casein kinase I isoform alpha OS=Homo sapiens GN=CSNK1A1 PE=1 SV=2	P48729	2.3	0.49	-1.03	1.1	0.03922 8087
Proteasome subunit beta type-3 OS=Homo sapiens GN=PSMB3 PE=1 SV=2	P49720	7.8	0.48	-1.06	3.8	0.00076 1731
Cytoplasmic FMR1-interacting protein 1 OS=Homo sapiens GN=CYFIP1 PE=1 SV=1	Q7L576	4.9	0.48	-1.06	2.4	0.02652 2425
Peflin OS=Homo sapiens GN=PEF1 PE=1 SV=1	Q9UBV8	3.9	0.48	-1.06	1.9	0.04862 6625
Hematological and neurological expressed 1 protein OS=Homo sapiens GN=HN1 PE=1 SV=3	**Q9UK76***	3.1	0.48	-1.06	1.5	0.02010 3534
Signal transducer and activator of transcription 1-alpha/beta OS=Homo sapiens GN=STAT1 PE=1 SV=2	P42224	10.1	0.47	-1.09	4.7	0.00336 4244

Golgi reassembly-stacking protein 2 OS=Homo sapiens GN=GORASP2 PE=1 SV=3	Q9H8Y8	3.2	0.47	-1.09	1.5	0.013949895
Reticulon-4 OS=Homo sapiens GN=RTN4 PE=1 SV=2	Q9NQC3	2.9	0.46	-1.12	1.3	0.046779278
Protein PRRC1 OS=Homo sapiens GN=PRRC1 PE=1 SV=1	Q96M27	3.0	0.45	-1.15	1.3	0.019343021
U4/U6 small nuclear ribonucleoprotein Prp31 OS=Homo sapiens GN=PRPF31 PE=1 SV=2	Q8WWY3	2.3	0.44	-1.18	1.0	0.04798099
Probable 28S rRNA (cytosine(4447)-C(5))-methyltransferase OS=Homo sapiens GN=NOP2 PE=1 SV=2	P46087	2.4	0.43	-1.22	1.0	0.02759675
Splicing factor U2AF 35 kDa subunit OS=Homo sapiens GN=U2AF1 PE=1 SV=3	**Q01081***	3.3	0.42	-1.25	1.4	0.007388295
Proteasome-associated protein ECM29 homolog OS=Homo sapiens GN=ECM29 PE=1 SV=2	Q5VYK3	2.0	0.39	-1.36	0.8	0.006577553
Calcyclin-binding protein OS=Homo sapiens GN=CACYBP PE=1 SV=2	Q9HB71	1.5	0.39	-1.36	0.6	0.017289193
Transferrin receptor protein 1 OS=Homo sapiens GN=TFRC PE=1 SV=2	P02786	4.5	0.38	-1.40	1.7	0.00022614
Branched-chain-amino-acid aminotransferase, cytosolic OS=Homo sapiens GN=BCAT1 PE=1 SV=3	P54687	4.1	0.38	-1.40	1.5	0.015432545
ADP-ribosylation factor GTPase-activating protein 3 OS=Homo sapiens GN=ARFGAP3 PE=1 SV=1	Q9NP61	3.0	0.38	-1.40	1.2	0.007545223
V-type proton ATPase subunit B, brain isoform OS=Homo sapiens GN=ATP6V1B2 PE=1 SV=3	P21281	4.2	0.37	-1.43	1.5	0.005113738
Thymosin beta-4 OS=Homo sapiens GN=TMSB4X PE=1 SV=2	**P62328***	4.5	0.36	-1.47	1.6	0.00044808
Alpha-2-macroglobulin OS=Homo sapiens GN=A2M PE=1 SV=3	P01023	4.0	0.35	-1.51	1.4	0.00145374
40S ribosomal protein S29 OS=Homo sapiens GN=RPS29 PE=1 SV=2	P62273	1.6	0.35	-1.51	0.6	0.045926544
EF-hand domain-containing protein D2 OS=Homo sapiens GN=EFHD2 PE=1 SV=1	Q96C19	1.8	0.35	-1.51	0.6	0.025455993
Fructose-2,6-bisphosphatase TIGAR OS=Homo sapiens GN=TIGAR PE=1 SV=1	Q9NQ88	1.6	0.35	-1.51	0.6	0.047436912
Ubiquilin-2 OS=Homo sapiens GN=UBQLN2 PE=1 SV=2	Q9UHD9	2.1	0.35	-1.51	0.7	0.029580088
Proteasome subunit alpha type-3 OS=Homo sapiens GN=PSMA3 PE=1 SV=2	P25788	2.1	0.32	-1.64	0.7	0.002057122
Segment polarity protein dishevelled homolog DVL-2 OS=Homo sapiens GN=DVL2 PE=1 SV=1	**O14641***	1.6	0.31	-1.69	0.5	0.012031531
Ras-related protein Rab-6A OS=Homo sapiens	P20340	4.3	0.22	-2.18	1.0	0.00077

GN=RAB6A PE=1 SV=3						929
Golgi-specific brefeldin A-resistance guanine nucleotide exchange factor 1 OS=Homo sapiens GN=GBF1 PE=1 SV=2	Q92538	2.1	0.19	-2.40	0.4	0.00542274
Coiled-coil-helix-coiled-coil-helix domain-containing protein 2 OS=Homo sapiens GN=CHCHD2 PE=1 SV=1	Q9Y6H1	1.6	0.18	-2.47	0.3	0.002860882

Hematological and Neurological Expressed 1 (HN1) - a predictive and pharmacodynamic biomarker of metformin response in endometrial cancers.

Among proteins correlating with response, we prioritized Hematological and Neurological
5 Expressed 1 (HN1) for further validation as this protein exhibited the greatest fold-change difference in pre-metformin treatment biopsies from responders versus non-responders ($\log_2$ ratio = 3.84, edgeR p-value = 9.78E-6), and was decreased in abundance in post versus pre-treated tumor tissues in metformin responders ($\log_2$ ratio = -1.06, edgeR p-value = 0.02), but remaining largely unaltered in non-responders (**FIG. 2**). Immunohistochemical staining for HN1
10 in the same set of patient tissues verified the proteomics data (**FIGS. 3A-3B**, **Table 3**) These results confirmed that HN1 expression is significantly elevated in responder versus non-responder tissues, pre-metformin treatment (p-value = 0.05) and that HN1 expression was decreased in responders, but unaltered in non-responders, post-metformin treatment (p-value = 0.011) (**FIG. 3B**).

Table 3. HN1 IHC verification analyses.

CASE ID	1=Metformin Responder, 2=Metformin Non-Responder	pre-Metformin (HN1 H-Score IHC Intensity)	post-Metformin (HN1 H-Score IHC Intensity)
H0001	1	122.3	75.3
H2494	2	75	124.3
H2495	2	133.7	121.7
H2497	2	92.3	104
H2499	2	113.3	111
H2501	1	196.5	116.7
H2505	2	103	56.3
H2507	2	125.7	133
H2511	1	105.3	68.3
H2513	1	41.7	127.3

H2515	1	143.3	113.3
H2517	1	210.7	59.3
H2519	1	134.3	65.3
H2521	1	158	69.3
H2523	1	143	128.3
H3094	2	141.7	93.7
H3692	1	165	74
H3693	1	184.3	65.7
H3694	1	159	102.3
H3695	1	196	135.3

Hematological and Neurological Expressed 1 (HN1) expression is decreased following metformin treatment of endometrial cancer cells, but HN1 is not necessary for response to metformin or for endometrial cancer cell proliferation. The endometrial cancer cell lines RL95-2 and ACI-181 were treated with 20 mM metformin (~LD50) for 96 and 120 h and HN1, AMPKα, p-AMPKα (T172), and Ki-67 protein abundance was assessed by immunoblotting. Metformin induced activation of AMPKα, as evidenced by increase in p-AMPKα (T172) abundance and further mediated a decrease in Ki-67 and HN1 abundance (**FIG. 4**). We further assessed the impact of HN1 on response to metformin in EC cells in which HN1 expression was silenced by small interfering RNAs targeting HN1 mRNA (**FIGS. 5A, 5B, 5D, and 5E**). These analyses revealed that loss of HN1 expression did not alter the response of EC cells to metformin treatment (**FIGS. 5C and 5F**). As AKT has previously been noted to be hyperactivated in low HN1 backgrounds (21), we further assessed activation of AKT in HN1 silenced cells. However, we did not observe alterations of p-AKT (S473) abundance in EC cells transfected with HN1-specific versus non-targeting siRNAs (**FIGS. 5A-5D**). Further, recent evidence has shown that HN1 knockdown results in decreased abundance of the MYC oncogene (22). We further assessed MYC abundance in HN1 silenced endometrial cancer cells, but did not reproduce this finding (**FIGS. 5A-5D**). We further assessed the impact of silencing HN1 on the proliferation rate of RL95-2 and ACI-181 cells (**FIGS. 6A-6D**). These analyses revealed that loss of HN1 expression does not significantly alter the proliferation of endometrial cancer cells.

Hematological and Neurological Expressed 1 (HN1) is correlated with Ki-67 expression and associated with altered overall survival in endometrial cancer patients. As Ki-67 and HN1 abundance were both decreased following metformin treatment, we assessed correlation trends of HN1 protein abundance (**Table 3**) relative to Ki-67 protein abundance previously assessed in

these tissues (4). We found that HN1 and Ki-67 exhibit concordant abundance trends in comparisons of both pre- (Spearman = 0.44) as well as in post- (Spearman=0.58) metformin treated tissues. We further assessed correlation of HN1 and Ki-67 transcript expression using a public RNA-seq dataset established from n=542 endometrial cancer patient tumor tissues (20)
5 and found that these genes were positively correlated in endometrial cancer patient tissues (Spearman = 0.37, **FIG. 7A**). We also assessed whether HN1 abundance was directly correlated with disease outcome in endometrial cancer patients and found that elevated expression of HN1 was associated with poor overall survival (n=540 EC patients, Log-rank p-value = 0.047, **FIG. 7B**) but not with disease-free survival (n=499 EC patients, Log-Rank p-
10 value = 0.14, data not shown).

Hematological and Neurological Expressed 1 (HN1) exhibits binding partners regulating metabolic signaling pathways in endometrial cancer cells. We performed an immunoprecipitation and mass spectrometry (IP-MS)-based analyses of HN1 in subconfluent RL95-2 cells to identify putative binding partners of HN1. HN1 was the most abundantly
15 identified protein in HN1 IP-MS biological replicates analyses, i.e. 61 ± 8 PSMs, following removal of proteins identified in a companion control IP-MS analyses of sepharose beads alone that contained no anti-HN1 antibody. Top functional pathways enriched following analyses of proteins abundantly identified with HN1, i.e. ≥ 10 PSMs in both HN1 IP-MS analyses were associated with metabolic pathways such as purine nucleotides de novo biosynthesis, glycolysis
20 and gluconeogenesis as well as fatty acid biosynthesis signaling (**Table 4**).

Table 4. Functional pathway analyses of HN1 immunoprecipitation mass spectrometry analyses – canonical pathways enriched.

Function Annotation - Post Vs. Pre Metformin Responders	-log(p-value)	Activation z-score	Molecules
Purine Nucleotides De Novo Biosynthesis II	5.48E+00	2.73E-01	IMPDH2,ATIC,GART
Tetrahydrofolate Salvage from 5,10-methenyltetrahydrofolate	4.12E+00	4.00E-01	MTHFD1,GART
Glycolysis I	2.63E+00	7.69E-02	PGK1,GPI
Gluconeogenesis I	2.63E+00	7.69E-02	PGK1,GPI
Acetyl-CoA Biosynthesis III (from Citrate)	2.56E+00	1.00E+00	ACLY
RhoA Signaling	2.31E+00	2.42E-02	EZR,SEPT7,SEPT2
Signaling by Rho Family GTPases	2.28E+00	1.59E-02	RELA,EZR,SEPT7,SEPT2
Choline Degradation I	2.26E+00	5.00E-01	ALDH7A1
Palmitate Biosynthesis I (Animals)	2.26E+00	5.00E-01	FASN
Fatty Acid Biosynthesis Initiation II	2.26E+00	5.00E-01	FASN
Protein Ubiquitination Pathway	2.20E+00	1.51E-02	PSME1,DNAJB11,PSMD1,UBA1
Aryl Hydrocarbon Receptor Signaling	2.15E+00	2.13E-02	RELA,ALDH7A1,MCM7

5-aminoimidazole Ribonucleotide Biosynthesis I	2.08E+00	3.33E-01	GART
Inosine-5'-phosphate Biosynthesis II	2.08E+00	3.33E-01	ATIC
Cell Cycle Control of Chromosomal Replication	1.98E+00	3.57E-02	MCM3,MCM7
Arginine Degradation I (Arginase Pathway)	1.96E+00	2.50E-01	OAT
Regulation of Cellular Mechanics by Calpain Protease	1.88E+00	3.17E-02	EZR,CAPN1
Lysine Degradation II	1.86E+00	2.00E-01	ALDH7A1
Lysine Degradation V	1.86E+00	2.00E-01	ALDH7A1
Folate Polyglutamylation	1.86E+00	2.00E-01	MTHFD1
Arginine Biosynthesis IV	1.78E+00	1.67E-01	OAT
Proline Biosynthesis II (from Arginine)	1.78E+00	1.67E-01	OAT
Arginine Degradation VI (Arginase 2 Pathway)	1.78E+00	1.67E-01	OAT
GDP-mannose Biosynthesis	1.78E+00	1.67E-01	GPI
Caveolar-mediated Endocytosis Signaling	1.78E+00	2.82E-02	ARCN1,COPA
Pentose Phosphate Pathway (Non-oxidative Branch)	1.72E+00	1.43E-01	TALDO1
Histidine Degradation III	1.66E+00	1.25E-01	MTHFD1
Citrulline Biosynthesis	1.61E+00	1.11E-01	OAT
Folate Transformations I	1.61E+00	1.11E-01	MTHFD1
Actin Cytoskeleton Signaling	1.57E+00	1.29E-02	DIAPH1,EZR,GIT1
Calcium Transport I	1.56E+00	1.00E-01	ANXA5
Apoptosis Signaling	1.54E+00	2.08E-02	RELA,CAPN1
Pentose Phosphate Pathway	1.52E+00	9.09E-02	TALDO1
UDP-N-acetyl-D-galactosamine Biosynthesis II	1.52E+00	9.09E-02	GPI
CDK5 Signaling	1.46E+00	1.90E-02	CAPN1,LAMB1
Urate Biosynthesis/Inosine 5'-phosphate Degradation	1.45E+00	7.69E-02	IMPDH2
Colanic Acid Building Blocks Biosynthesis	1.42E+00	7.14E-02	GPI
Superpathway of Citrulline Metabolism	1.39E+00	6.67E-02	OAT
Pancreatic Adenocarcinoma Signaling	1.36E+00	1.67E-02	RELA,RALBP1
LXR/RXR Activation	1.35E+00	1.65E-02	RELA,FASN
Sirtuin Signaling Pathway	1.32E+00	1.03E-02	PGK1,RELA,ACLY
Purine Nucleotides Degradation II (Aerobic)	1.29E+00	5.26E-02	IMPDH2
Histamine Degradation	1.29E+00	5.26E-02	ALDH7A1
Oxidative Ethanol Degradation III	1.25E+00	4.76E-02	ALDH7A1
Polyamine Regulation in Colon Cancer	1.23E+00	4.55E-02	PSME1
Fatty Acid α-oxidation	1.23E+00	4.55E-02	ALDH7A1
Putrescine Degradation III	1.21E+00	4.35E-02	ALDH7A1
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	1.19E+00	4.17E-02	RELA
Glutathione Redox Reactions I	1.19E+00	4.17E-02	PRDX6
TCA Cycle II (Eukaryotic)	1.19E+00	4.17E-02	FH
IL-17A Signaling in Gastric Cells	1.17E+00	4.00E-02	RELA
Tryptophan Degradation X (Mammalian, via Tryptamine)	1.17E+00	4.00E-02	ALDH7A1
Ethanol Degradation IV	1.17E+00	4.00E-02	ALDH7A1
IL-15 Production	1.13E+00	3.57E-02	RELA
Regulation of eIF4 and p70S6K Signaling	1.12E+00	1.23E-02	EIF3B,EIF3A

TNFR2 Signaling	1.10E+00	3.33E-02	RELA
4-1BB Signaling in T Lymphocytes	1.07E+00	3.12E-02	RELA
TWEAK Signaling	1.03E+00	2.86E-02	RELA
MIF-mediated Glucocorticoid Regulation	1.03E+00	2.86E-02	RELA
IL-17A Signaling in Fibroblasts	1.03E+00	2.86E-02	RELA
PPARα/RXRα Activation	1.03E+00	1.08E-02	RELA,FASN
Interferon Signaling	1.02E+00	2.78E-02	RELA
Dopamine Degradation	1.01E+00	2.70E-02	ALDH7A1
Ethanol Degradation II	1.01E+00	2.70E-02	ALDH7A1
April Mediated Signaling	9.89E-01	2.56E-02	RELA
tRNA Charging	9.89E-01	2.56E-02	GARS
Noradrenaline and Adrenaline Degradation	9.78E-01	2.50E-02	ALDH7A1
NRF2-mediated Oxidative Stress Response	9.77E-01	1.01E-02	STIP1,DNAJB11
Role of PKR in Interferon Induction and Antiviral Response	9.68E-01	2.44E-02	RELA
B Cell Activating Factor Signaling	9.68E-01	2.44E-02	RELA
Mechanisms of Viral Exit from Host Cells	9.68E-01	2.44E-02	PDCD6IP
mTOR Signaling	9.49E-01	9.66E-03	EIF3B,EIF3A
MIF Regulation of Innate Immunity	9.49E-01	2.33E-02	RELA
Role of RIG1-like Receptors in Antiviral Innate Immunity	9.39E-01	2.27E-02	RELA
Stearate Biosynthesis I (Animals)	9.39E-01	2.27E-02	FASN
Osteoarthritis Pathway	9.32E-01	9.43E-03	RELA,ANXA5
IL-9 Signaling	9.30E-01	2.22E-02	RELA
iNOS Signaling	9.30E-01	2.22E-02	RELA
Role of IL-17F in Allergic Inflammatory Airway Diseases	9.21E-01	2.17E-02	RELA
nNOS Signaling in Neurons	9.12E-01	2.13E-02	CAPN1
Integrin Signaling	9.09E-01	9.13E-03	CAPN1,GIT1
TNFR1 Signaling	8.87E-01	2.00E-02	RELA
EIF2 Signaling	8.84E-01	8.81E-03	EIF3B,EIF3A
Amyloid Processing	8.79E-01	1.96E-02	CAPN1
Systemic Lupus Erythematosus Signaling	8.65E-01	8.58E-03	SNRNP70,SNRNP200
CD27 Signaling in Lymphocytes	8.64E-01	1.89E-02	RELA
Triacylglycerol Degradation	8.56E-01	1.85E-02	PRDX6
Huntington's Disease Signaling	8.17E-01	8.00E-03	PSME1,CAPN1
Induction of Apoptosis by HIV1	8.07E-01	1.64E-02	RELA
Activation of IRF by Cytosolic Pattern Recognition Receptors	7.94E-01	1.59E-02	RELA
Wnt/Ca+ pathway	7.94E-01	1.59E-02	RELA
PXR/RXR Activation	7.82E-01	1.54E-02	RELA
Mitotic Roles of Polo-Like Kinase	7.76E-01	1.52E-02	CAPN1
Lymphotoxin β Receptor Signaling	7.70E-01	1.49E-02	RELA
IL-10 Signaling	7.58E-01	1.45E-02	RELA
Role of IL-17A in Arthritis	7.58E-01	1.45E-02	RELA

TREM1 Signaling	7.25E-01	1.33E-02	RELA
Agrin Interactions at Neuromuscular Junction	7.25E-01	1.33E-02	LAMB1
Heparan Sulfate Biosynthesis (Late Stages)	7.20E-01	1.32E-02	PRDX6
Toll-like Receptor Signaling	7.20E-01	1.32E-02	RELA
Serotonin Degradation	7.15E-01	1.30E-02	ALDH7A1
IL-17A Signaling in Airway Cells	7.10E-01	1.28E-02	RELA
CD40 Signaling	7.05E-01	1.27E-02	RELA
Role of PI3K/AKT Signaling in the Pathogenesis of Influenza	7.05E-01	1.27E-02	RELA
Xenobiotic Metabolism Signaling	7.03E-01	6.76E-03	RELA,ALDH7A1
IL-15 Signaling	6.91E-01	1.22E-02	RELA
BMP signaling pathway	6.91E-01	1.22E-02	RELA
Heparan Sulfate Biosynthesis	6.86E-01	1.20E-02	PRDX6
Angiopoietin Signaling	6.81E-01	1.19E-02	RELA
Small Cell Lung Cancer Signaling	6.77E-01	1.18E-02	RELA
Estrogen-Dependent Breast Cancer Signaling	6.72E-01	1.16E-02	RELA
Regulation of IL-2 Expression in Activated and Anergic T Lymphocytes	6.72E-01	1.16E-02	RELA
Erythropoietin Signaling	6.68E-01	1.15E-02	RELA
Renal Cell Carcinoma Signaling	6.63E-01	1.14E-02	FH
Crosstalk between Dendritic Cells and Natural Killer Cells	6.59E-01	1.12E-02	RELA
JAK/Stat Signaling	6.59E-01	1.12E-02	RELA
Altered T Cell and B Cell Signaling in Rheumatoid Arthritis	6.55E-01	1.11E-02	RELA
IL-17 Signaling	6.51E-01	1.10E-02	RELA
OX40 Signaling Pathway	6.51E-01	1.10E-02	RELA
IL-1 Signaling	6.46E-01	1.09E-02	RELA
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	6.42E-01	1.08E-02	EZR
LPS-stimulated MAPK Signaling	6.42E-01	1.08E-02	RELA
NF-κB Activation by Viruses	6.42E-01	1.08E-02	RELA
PEDF Signaling	6.42E-01	1.08E-02	RELA
Death Receptor Signaling	6.42E-01	1.08E-02	RELA
TR/RXR Activation	6.22E-01	1.02E-02	FASN
Ceramide Signaling	6.19E-01	1.01E-02	RELA
Acute Myeloid Leukemia Signaling	6.19E-01	1.01E-02	RELA
PPAR Signaling	6.11E-01	9.90E-03	RELA
RANK Signaling in Osteoclasts	6.07E-01	9.80E-03	RELA
Glucocorticoid Receptor Signaling	6.06E-01	5.80E-03	RELA,FKBP4
Prostate Cancer Signaling	6.04E-01	9.71E-03	RELA
FAK Signaling	5.96E-01	9.52E-03	CAPN1
PAK Signaling	5.89E-01	9.35E-03	GIT1
Antioxidant Action of Vitamin C	5.86E-01	9.26E-03	RELA

Amyotrophic Lateral Sclerosis Signaling	5.76E-01	9.01E-03	CAPN1
Type I Diabetes Mellitus Signaling	5.76E-01	9.01E-03	RELA
Chronic Myeloid Leukemia Signaling	5.72E-01	8.93E-03	RELA
T Cell Receptor Signaling	5.63E-01	8.70E-03	RELA
iCOS-iCOSL Signaling in T Helper Cells	5.38E-01	8.13E-03	RELA
Rac Signaling	5.38E-01	8.13E-03	RELA
NGF Signaling	5.32E-01	8.00E-03	RELA
PTEN Signaling	5.32E-01	8.00E-03	RELA
FXR/RXR Activation	5.29E-01	7.94E-03	FASN
Atherosclerosis Signaling	5.26E-01	7.87E-03	RELA
Molecular Mechanisms of Cancer	5.25E-01	5.08E-03	RELA,RALBP1
Renin-Angiotensin Signaling	5.23E-01	7.81E-03	RELA
fMLP Signaling in Neutrophils	5.20E-01	7.75E-03	RELA
PI3K/AKT Signaling	5.15E-01	7.63E-03	RELA
CD28 Signaling in T Helper Cells	5.12E-01	7.58E-03	RELA
IL-6 Signaling	5.07E-01	7.46E-03	RELA
GP6 Signaling Pathway	5.07E-01	7.46E-03	LAMB1
PI3K Signaling in B Lymphocytes	5.01E-01	7.35E-03	RELA
14-3-3-mediated Signaling	4.99E-01	7.30E-03	PDCD6IP
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	4.99E-01	7.30E-03	RELA
Androgen Signaling	4.99E-01	7.30E-03	RELA
Corticotropin Releasing Hormone Signaling	4.94E-01	7.19E-03	IVL
HMGB1 Signaling	4.94E-01	7.19E-03	RELA
P2Y Purigenic Receptor Signaling Pathway	4.91E-01	7.14E-03	RELA
Gα12/13 Signaling	4.89E-01	7.09E-03	RELA
IL-12 Signaling and Production in Macrophages	4.76E-01	6.85E-03	RELA
Insulin Receptor Signaling	4.74E-01	6.80E-03	ACLY
Phagosome Maturation	4.71E-01	6.76E-03	PRDX6
Type II Diabetes Mellitus Signaling	4.57E-01	6.49E-03	RELA
Relaxin Signaling	4.49E-01	6.33E-03	RELA
Hepatic Cholestasis	4.44E-01	6.25E-03	RELA
Gαq Signaling	4.42E-01	6.21E-03	RELA
PKCθ Signaling in T Lymphocytes	4.34E-01	6.06E-03	RELA
Tight Junction Signaling	4.29E-01	5.99E-03	RELA
Cdc42 Signaling	4.29E-01	5.99E-03	DIAPH1
Aldosterone Signaling in Epithelial Cells	4.27E-01	5.95E-03	DNAJB11
Tec Kinase Signaling	4.23E-01	5.88E-03	RELA
GNRH Signaling	4.21E-01	5.85E-03	RELA
Acute Phase Response Signaling	4.12E-01	5.68E-03	RELA
RhoGDI Signaling	4.10E-01	5.65E-03	EZR
Granulocyte Adhesion and Diapedesis	4.02E-01	5.52E-03	EZR
Hepatic Fibrosis / Hepatic Stellate Cell Activation	3.91E-01	5.35E-03	RELA

NF-κB Signaling	3.91E-01	5.35E-03	RELA
RAR Activation	3.86E-01	5.26E-03	RELA
Role of NFAT in Regulation of the Immune Response	3.83E-01	5.21E-03	RELA
Agranulocyte Adhesion and Diapedesis	3.81E-01	5.18E-03	EZR
Dendritic Cell Maturation	3.79E-01	5.15E-03	RELA
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	3.79E-01	5.15E-03	RELA
B Cell Receptor Signaling	3.79E-01	5.15E-03	RELA
Regulation of the Epithelial-Mesenchymal Transition Pathway	3.77E-01	5.13E-03	RELA
ILK Signaling	3.74E-01	5.08E-03	RELA
Adrenomedullin signaling pathway	3.69E-01	5.00E-03	RELA
IL-8 Signaling	3.64E-01	4.93E-03	RELA
Thrombin Signaling	3.53E-01	4.76E-03	RELA
Leukocyte Extravasation Signaling	3.52E-01	4.74E-03	EZR
AMPK Signaling	3.44E-01	4.63E-03	FASN
LPS/IL-1 Mediated Inhibition of RXR Function	3.36E-01	4.50E-03	ALDH7A1
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	3.21E-01	4.29E-03	RELA
Phospholipase C Signaling	3.06E-01	4.10E-03	RELA
Colorectal Cancer Metastasis Signaling	2.94E-01	3.94E-03	RELA
G-Protein Coupled Receptor Signaling	2.64E-01	3.55E-03	RELA
Neuroinflammation Signaling Pathway	2.36E-01	3.22E-03	RELA
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	2.28E-01	3.12E-03	RELA
Axonal Guidance Signaling	0.00E+00	2.19E-03	GIT1
Protein Kinase A Signaling	0.00E+00	2.49E-03	RELA

This Example identifies proteomic alterations in tumor tissues harvested from endometrial cancer patients before and after daily treatment with metformin (850mg) for 4 weeks prior to surgical staging. Women who responded to metformin exhibited a significant
5 decrease (7 to 50%) in expression of the cellular proliferation marker Ki-67 in post-treated tumor tissues, whereas non-responders exhibited a slight increase in Ki-67 IHC staining (~2-12%), as described previously (4). A global quantitative LC-MS/MS-based proteomics analysis identified discrete protein alterations in tumor cells harvested by LMD from pre-treated endometrial biopsies that correlate with canonical cellular pathways that include AMPK signaling, a kinase
10 regulating cellular energy homeostasis activated by metformin (6), as well as pathways involved in activating cellular proliferation and inhibiting cell death signaling in metformin responders. Among the proteins altered, Hematological and Neurological Expressed 1 (HN1) was the most significantly elevated protein in pre-treated endometrial biopsies from metformin responders

(responder versus non-responder logFC=3.42, edgeR p-value = 9.78E-06). We cross-correlated HN1 in pre and post-treatment biopsies in metformin responders and found that HN1 was also significantly decreased following metformin treatment (post-versus pre-treatment logFC = -1.06, edgeR p-value = 0.02) – a finding that was independently verified by IHC.

5 Hematological and Neurological Expressed 1 (HN1), also known as Jupiter microtubule associated homolog 1 (JPT1), is a ~16 kDa protein (Q9UK76, uniprot.org) initially identified as an abundant transcript in both murine hematopoietic and brain cells (23). As HN1 was significantly elevated in patients who responded to metformin, we assessed the impact of silencing HN1 expression on response to metformin using two endometrial cancer cell line 10 models, i.e. RL95-2 and ACI-181 (**FIGS. 5A-5F**). It was found that loss of HN1 did not alter cellular response to metformin treatment *in vitro*. In cancer, HN1 has been shown to inhibit the growth of androgen-sensitive, but promote the growth of androgen-insensitive, prostate cancer cells through altered androgen receptor stability and by modulating cell metabolism and cell cycle progression through protein kinase B (AKT) and downstream glycogen synthase kinase-3 15 beta (GSK3β)/ β-catenin (CTNNB1)-dependent mechanisms (21,24). Specifically, silencing of HN1 expression increased activation of AKT via upregulation of p-AKT (S473) in androgen-dependent and independent prostate cancer cell lines (21). The modulation of p-AKT (S473) was similarly assesedfollowing knockdown of HN1 in endometrial cancer cell lines, though we did not reproduce this finding (**FIG. 4**). Recent evidence has also shown that elevated HN1 20 expression is directly correlated with poor prognosis in breast cancer patients (22). Further investigations revealed that HN1 increases the migratory and invasive potential of breast cancer cells by upregulating the MYC oncogene and downstream effectors *in vitro* and further increases breast tumorigenesis *in vivo* (22). There were no observed alterations in MYC protein abundance following silencing of HN1 in endometrial cancer cells (**FIGS. 5A-5F**). It was found 25 that metformin treatment inhibited endometrial cancer cell proliferation *in vitro*, as evidence by decreased Ki-67 levels, and that this response was further accompanied by loss of HN1 abundance (**FIGS. 5A-5F**). Pathway analyses of significantly altered proteins in metformin pre-treatment tumors from responders revealed that cellular proliferation signaling was altered in metformin responders as evidenced by elevation of proliferating cell nuclear antigen in 30 responder patients (PCNA, edgeR LogFC=1.29, p-value = 0.025). PCNA regulates DNA replication via interactions with DNA polymerase delta and is a marker of cellular proliferation often assessed during routine histopathology analyses of tumor tissues along with Ki-67 to determine the proliferative indices of tumor cells *in situ* (25). Indeed, it was previously observed (4) that pre-treatment tumor tissues from metformin responders exhibited elevated Ki-67

expression levels versus non-responders (47.3% vs. 24.9%, p-value = 0.004), providing suggestive evidence that these patients may harbor tumor cells with a greater proliferative capacity. No observation of an association between HN1 and response to metformin and as HN1 levels closely parallel PCNA levels in responders versus non-responders, HN1 abundance
5 may also serve as a surrogate measure of proliferative activity in endometrial cancer cells. Similarly to our findings with HN1, recent assessments of Ki-67 have identified that this hallmark biomarker of cellular proliferation does not directly contribute to tumor cell proliferation (26,27), but rather functions to maintain cancer stem cell populations (27) and facilitates chromosomal motility and mitotic spindle interactions (28). To gain further insight into the possible biologic
10 roles of HN1 in endometrial cancer cells, an immunoprecipitation-mass spectrometry based analyses of HN1 in sub-confluent RL95-2 cells was performed to identify functional binding partner candidates. These analyses revealed putative HN1 binding proteins to be predominantly associated with metabolic signaling pathways such as nucleotide and aerobic glycolysis and fatty acid signaling.

15 Immunohistochemical markers of metformin response used in clinical trials to date include assessments of AMPK abundance and activation state (p-AMPK, T172), mTOR signaling, such as p-S6 kinase as well as p-EIF4-BP1, as well as assessing markers of cellular proliferation (29). Activation of AMPK via increased levels of p-AMPK (T172) in endometrial cancer cells treated with metformin *in vitro* was observed (**FIG. 4**). Elevated p-AMPK (T172) by
20 IHC in endometrial cancer tissues following metformin treatment (4) was not observed, though Ki-67 was altered these same tissues, recapitulating the findings *in vitro*. Comparative analyses of Ki-67 and HN1 protein abundance by IHC analyses in pre and post-metformin treated patient tissues revealed these protein abundances to be positively correlated. The correlation of HN1 and Ki-67 mRNA abundance in public data for over 500 endometrial cancer patients was
25 assessed and found that HN1 and Ki-67 expression are positively correlated (R=~0.365). Furthermore, it was also identified that elevated HN1 is associated with poor overall survival, underscoring that high HN1 expressing patients may experience a survival benefit from metformin treatment. These data can support that IHC assessment of HN1 in pre-surgical endometrial tissue biopsies may aid to prioritize patients for neoadjuvant treatment with
30 metformin, and that this protein can further function as a pharmacodynamic surrogate of therapeutic response.

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Example 3.

The preoperative use of metformin in obese women with endometrioid endometrial
30 cancer (EEC) is associated with reduced tumor proliferation and an inhibition of the mammalian target of rapamycin (mTOR) pathway in select cases.

This Example evaluates whether a proteomic signature exists to explain the inhibition of the mTOR pathway and to identify a biomarker that may predict response to preoperative

metformin, using samples from a previously completed Phase 0 study of metformin in obese EEC patients.

Differential proteomic analysis of laser microdissected tumor cells collected from matched, pre-treatment biopsies and post-treatment hysterectomy EEC patient tissue
5 specimens (n=20) treated with metformin (850 mg) daily for 1-4 weeks prior to surgical staging was conducted. Thirteen patients responded and seven patients did not respond to metformin treatment based on decreased expression of the proliferation marker Ki-67. Mass spectrometry-based proteomics was performed on tryptic digests of tissue samples. Protein abundance was measured using spectral counting and functional analyses were performed using Ingenuity
10 Pathway Analysis. HN1 alterations was verified by immunohistochemistry analyses in patient tissues.

Results are shown in **FIGS. 11** through **16B**.

Differential proteomic analyses revealed alterations of molecular signaling pathways supporting increased cell proliferation and decreased cellular apoptosis signaling in metformin
15 responder vs. non-responder EEC patients. Protein HN1 was significantly elevated in responder vs. non-responder patients and further decreased in comparisons of post- vs. pre-treated responder patients. HN1 represents a predictive biomarker candidate of preoperative therapeutic response to metformin in EEC patients.

We claim:

1. A method comprising:

measuring the amount of HN1 protein in a biological sample obtained from a subject having endometrial cancer.

2. The method of claim 1, wherein the step of measuring is performed using an immunological assay or protein sequencing.

3. The method of any one of claims 1-2, wherein the step of measuring is performed using an immunological assay using an antibody that specifically binds HN1 protein.

4. The method of any one of claims 1-3, wherein the subject has endometrioid endometrial cancer.

5. The methods of any one of claims 1-4, wherein the subject is obese.

6. The methods of any one of claims 1-5, further comprising administering a therapeutically effective amount of metformin to the subject.

7. The method of any one of claims 1-6, wherein the subject has increased HN1 protein in the biological sample as compared to a suitable control sample.

8. The method of any one of claims 1-7, wherein the method is performed prior to performing a hysterectomy on the subject.

9. The method of any one of claims 1-8, wherein the method is performed after performing a hysterectomy on the subject.

10. The method of any one of claims 6-9, wherein the therapeutically effective amount is about 850 mg.

11. The method of any one of claims 6-10, wherein the therapeutically effective amount is administered once daily.

12. The method of any one of claims 6-11, wherein the therapeutically effective amount is administered for 1, 2, 3, or 4 weeks.

13. A method comprising:
obtaining a biological sample from a subject having endometrial cancer; and
measuring the amount of HN1 protein in the biological sample.

14. The method of claim 13, wherein the step of measuring is performed using an immunological assay or protein sequencing.

15. The method of any one of claims 13-14, wherein the step of measuring is performed using an immunological assay using an antibody that specifically binds HN1 protein.

16. The method of any one of claims 13-15, wherein the subject has endometrioid endometrial cancer.

17. The method of any one of claims 13-16, wherein the subject is obese.

18. The method of any one of claims 13-17, further comprising administering to the subject a therapeutically effective amount of metformin.

19. The method of any one of claims 13-18, wherein the subject has increased HN1 protein in the biological sample as compared to a suitable control sample.

20. The method of any one of claims 13-19, wherein the method is performed prior to performing a hysterectomy on the subject.

21. The method of any one of claims 13-20, wherein the method is performed after performing a hysterectomy on the subject.

22. The method of any one of claims 18-21, wherein the therapeutically effective amount is about 850 mg.

23. The method of any one of claims 18-22, wherein the therapeutically effective amount is administered once daily.

24. The method of any one of claims 18-23, wherein the therapeutically effective amount is administered for 1, 2, 3, or 4 weeks.

25. A method of treating a subject having endometrial cancer and an increased HN1 protein in a biological sample comprising:

administering a therapeutically effective of metformin to the subject.

26. The method of claim 25, wherein the subject has endometrioid endometrial cancer.

27. The method of any one of claims 25-26, wherein the subject is obese.

28. The method of any one of claims 25-27, wherein the step of administering is preformed prior to performing a hysterectomy.

29. The method of any one of claims 25-28, wherein the step of administering is preformed after performing a hysterectomy.

30. The method of any one of claims 25-29, wherein the therapeutically effective amount is about 850 mg.

31. The method of any one of claims 25-30, wherein the therapeutically effective amount is administered once daily.

32. The method of any one of claims 25-31, wherein the therapeutically effective amount is administered for 1, 2, 3, or 4 weeks.

33. Metformin as a medicament in a formulation to treat endometrial cancer in a subject having an increased level of HN1 protein in a biological sample.

34. The use of metformin for the treatment of endometrial cancer in a subject having an increased level of HN1 protein.

35. A kit comprising:

an amount of an antibody that is capable of specifically binding NH1 protein; and

instructions fixed in a tangible medium of expression, wherein the instructions provide

performing an immunoassay on a biological sample from a subject using the antibody and administering a non-antineoplastic agent to a subject in need thereof when the amount of NH1 protein is increased in the biological sample.

36. The kit of claim 35, wherein the instructions provide that the biological sample is a tissue sample.

37. The kit of any one of claims 35-36, wherein the instructions provide that the biological sample is a bodily fluid.

38. The kit of any one of claims 35-37, wherein the instructions provide directions to perform a hysterectomy after administering the non-antineoplastic agent.

39. The kit of any one of claims 36-38, wherein the non-antineoplastic is metformin.

40. The kit of claim 39, wherein the amount of the metformin is about 850 mg.

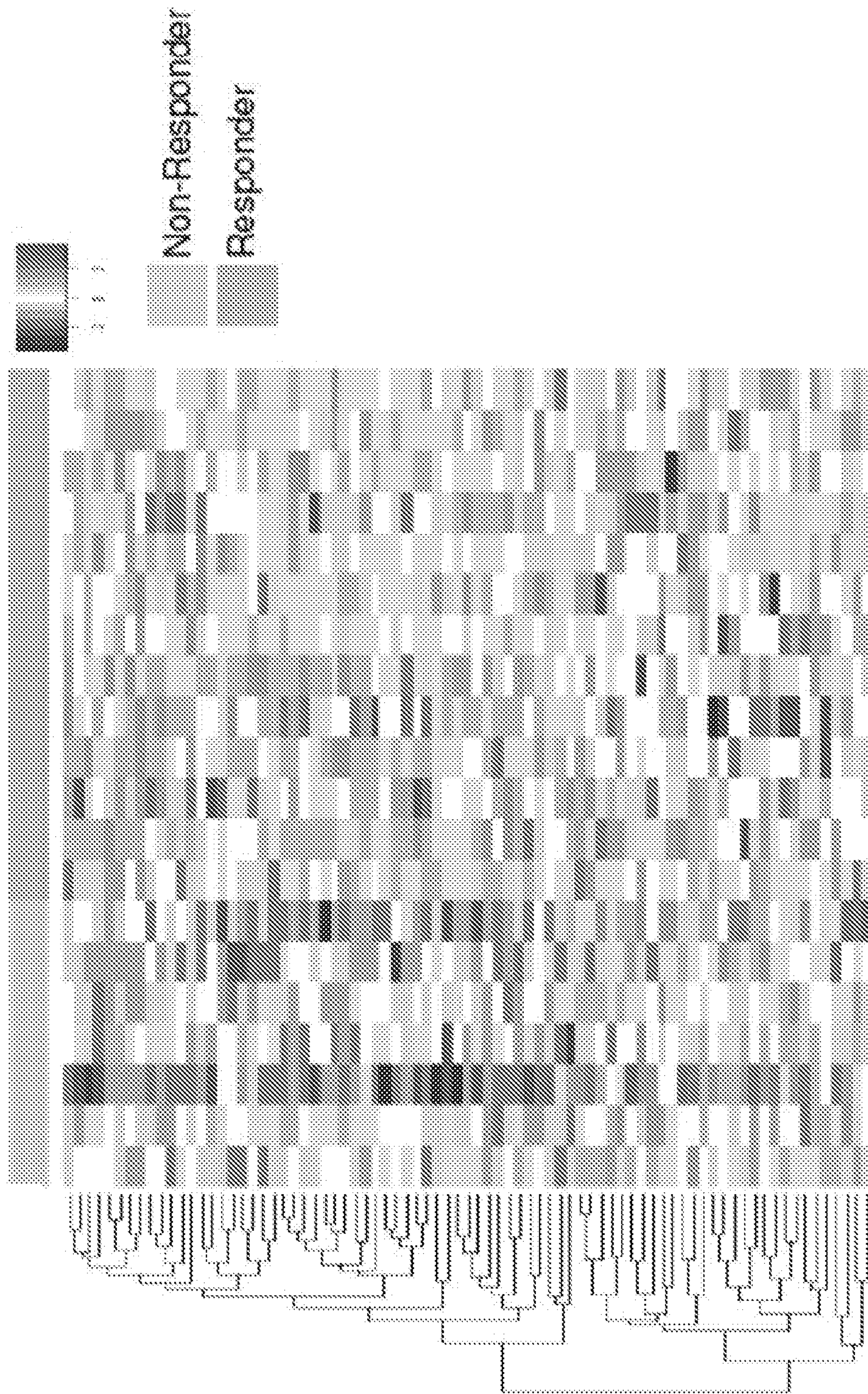


FIG. 1

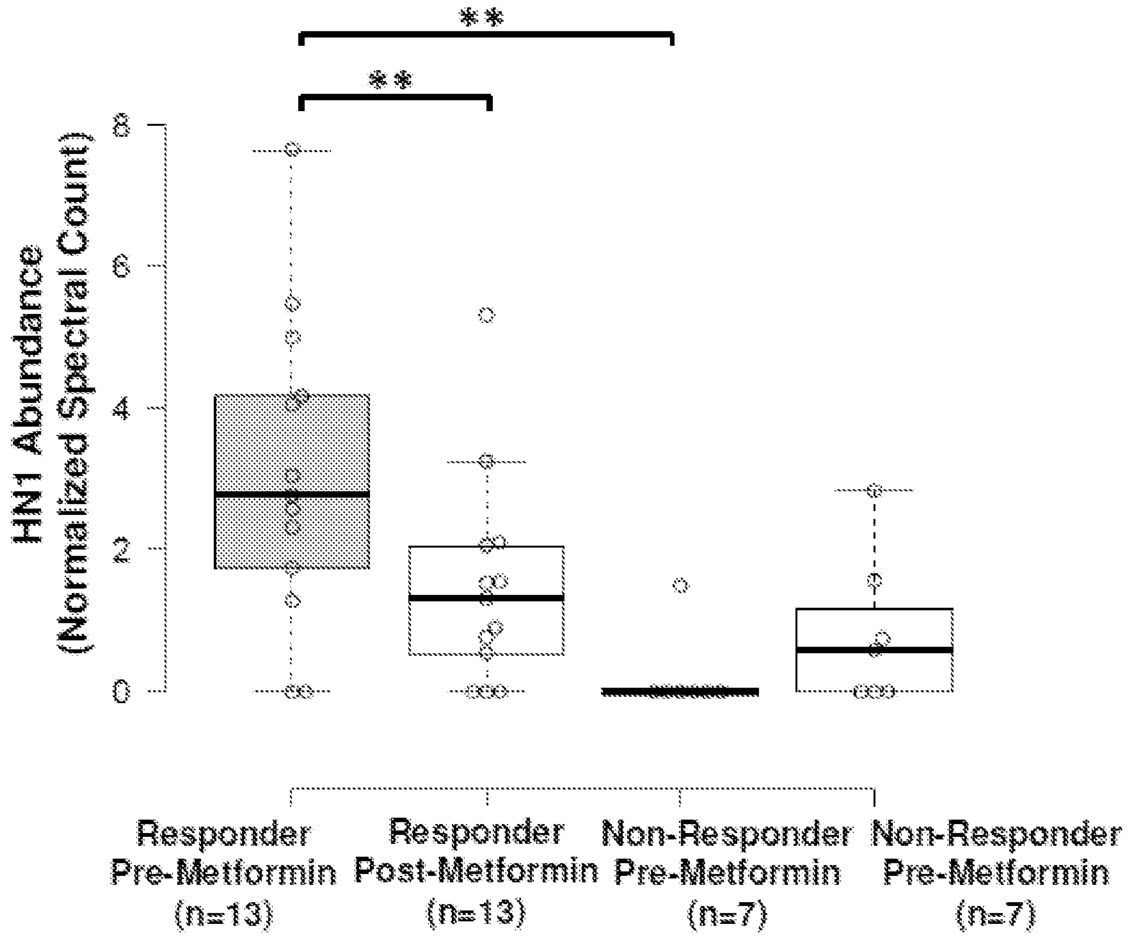
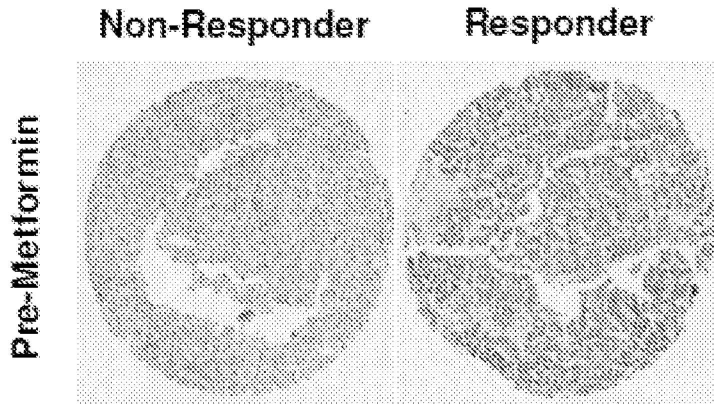


FIG. 2



Hematological and neurological expressed 1 (HN1) protein IHC - *Representative TMA Spot*

FIG. 3A

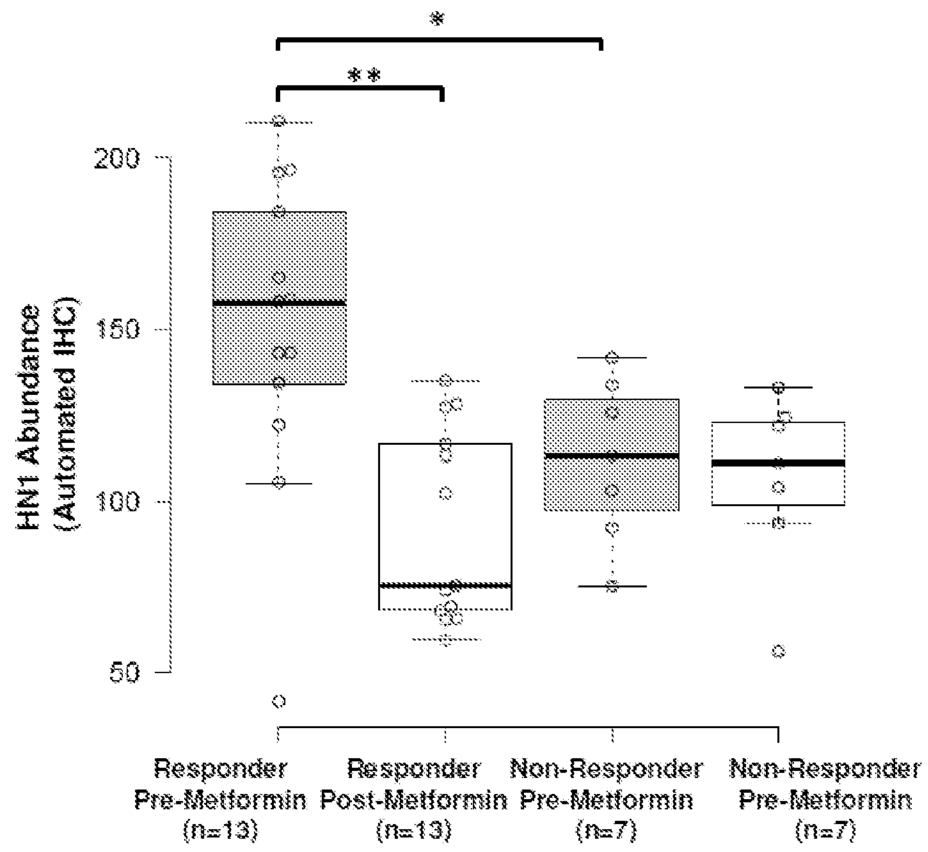


FIG. 3B

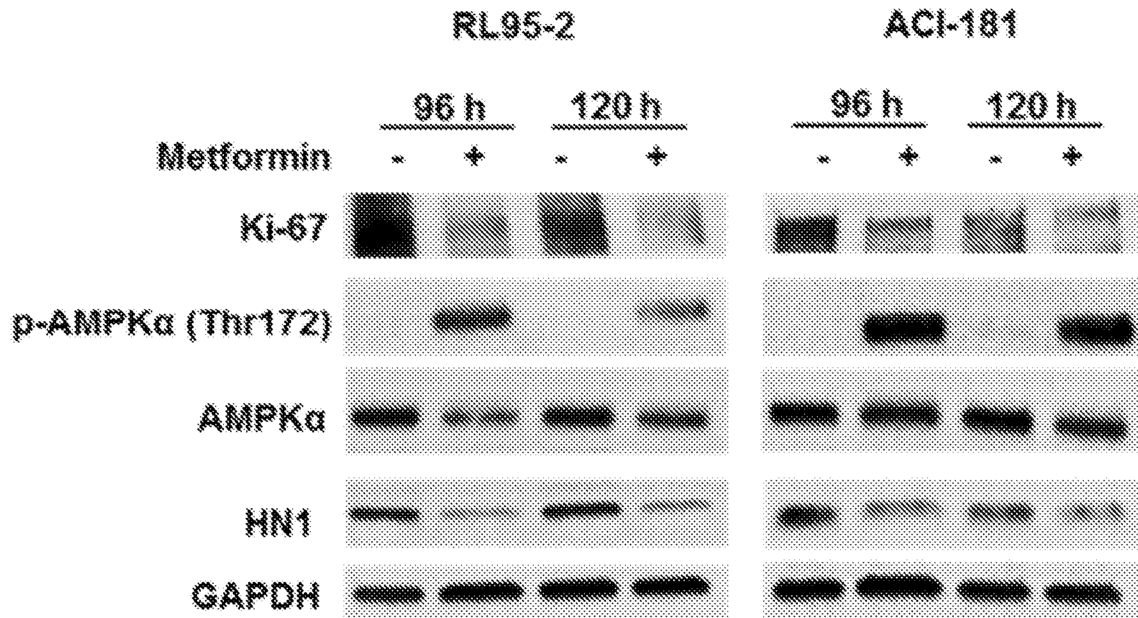


FIG. 4

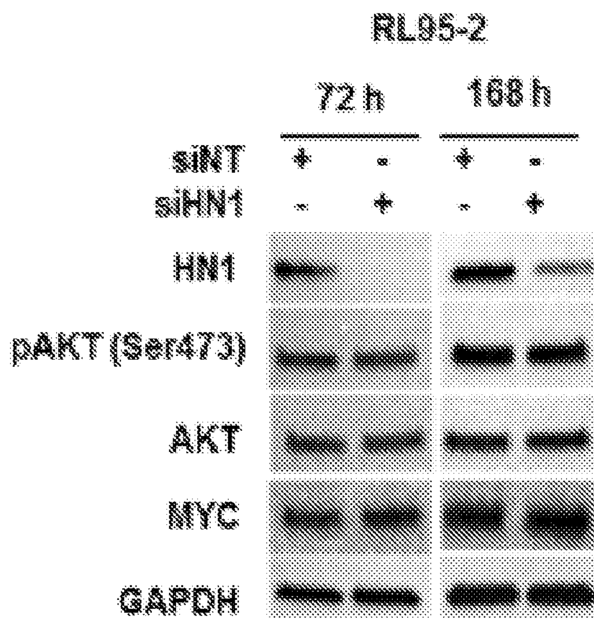


FIG. 5A

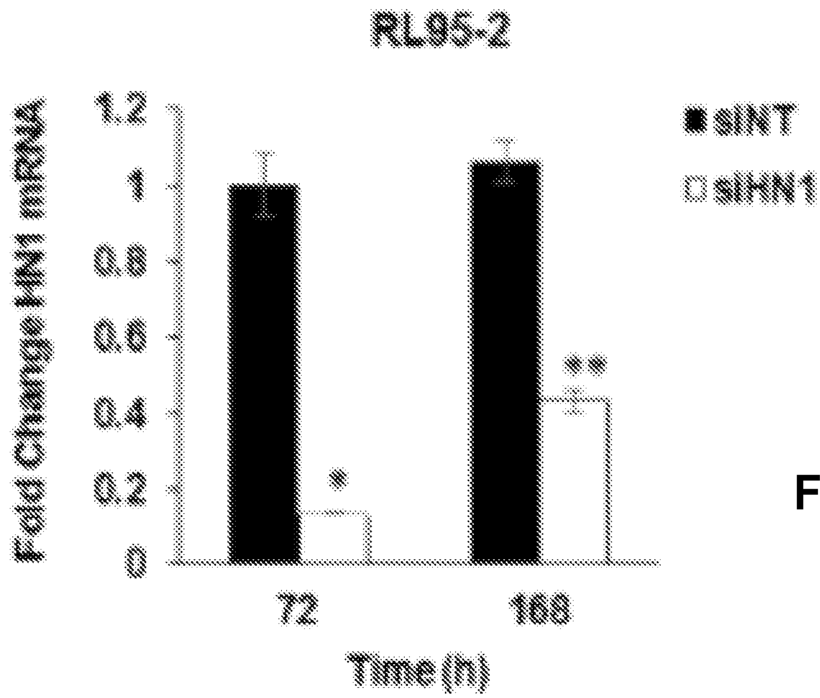


FIG. 5B

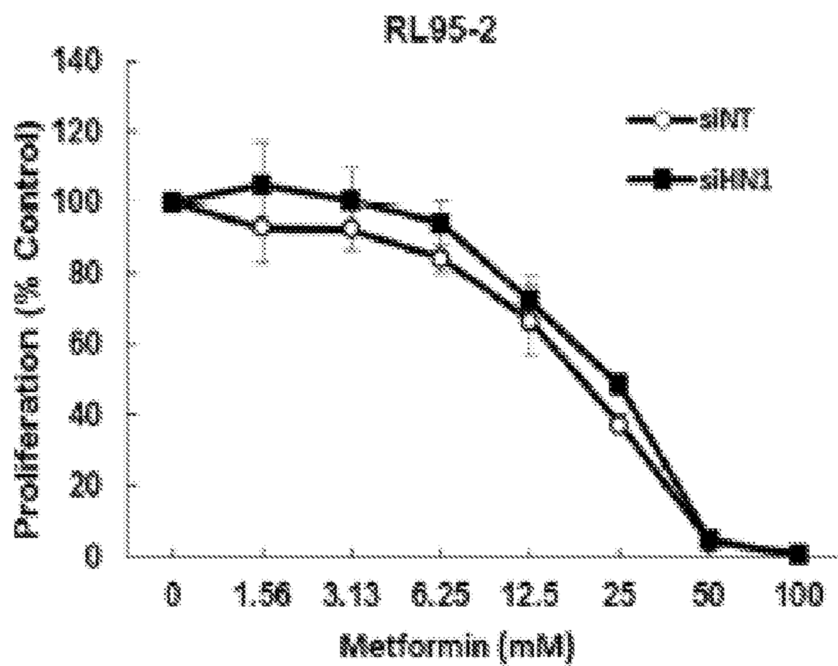


FIG. 5C

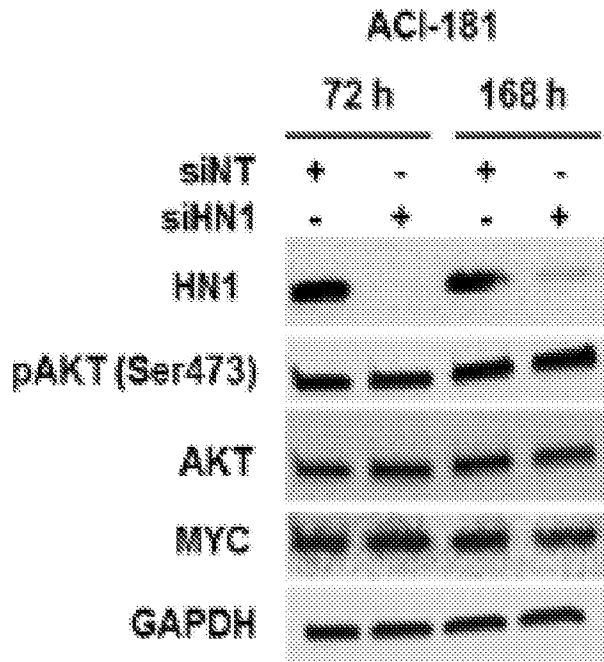


FIG. 5D

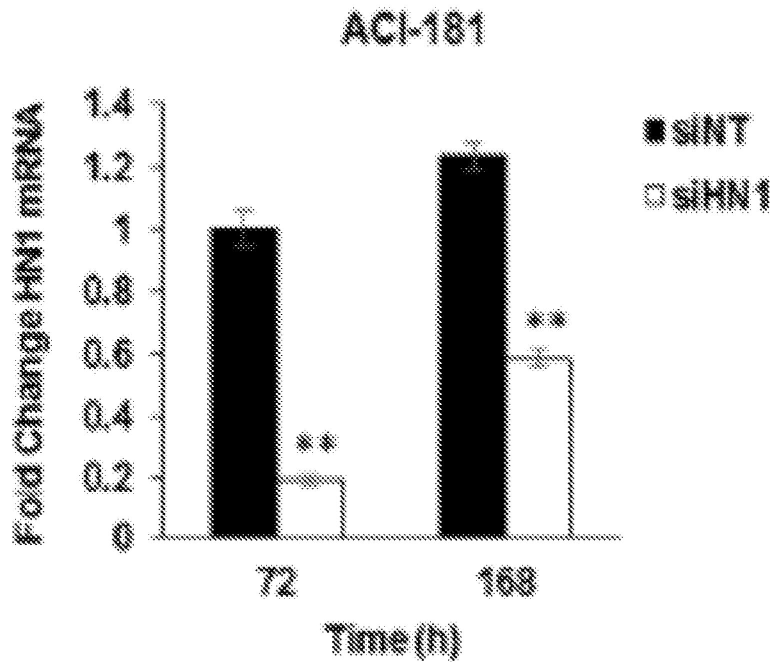


FIG. 5E

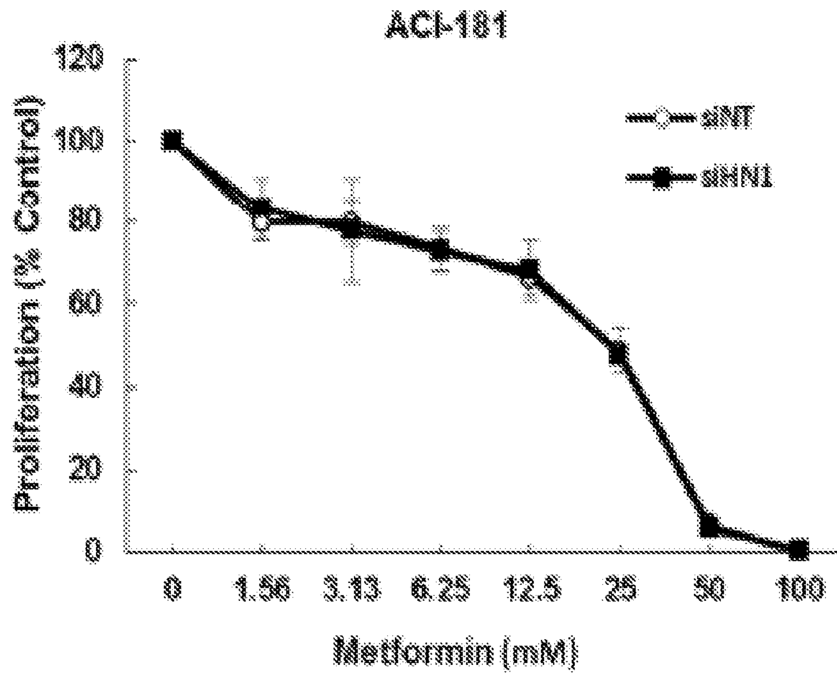


FIG. 5F

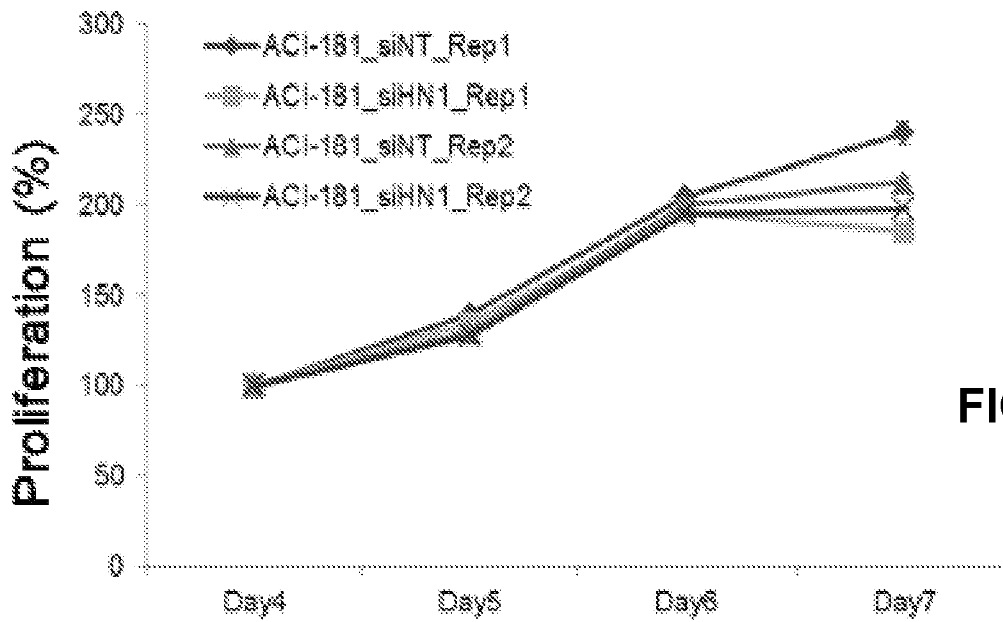


FIG. 6A

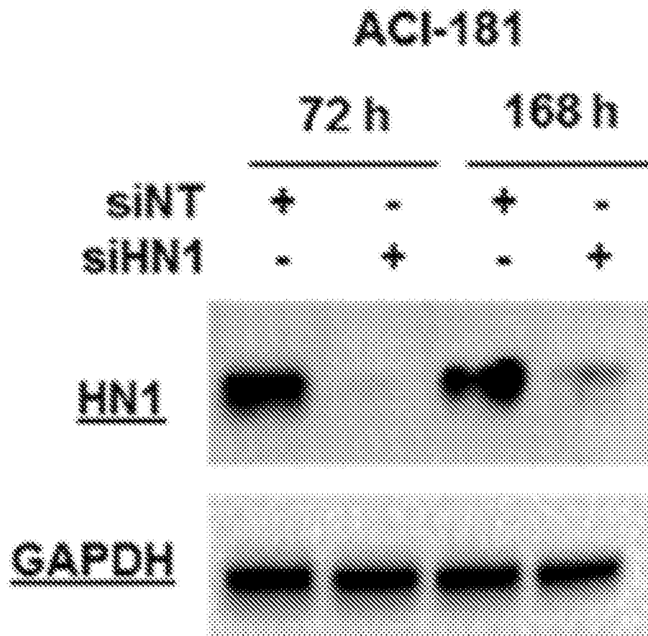


FIG. 6B

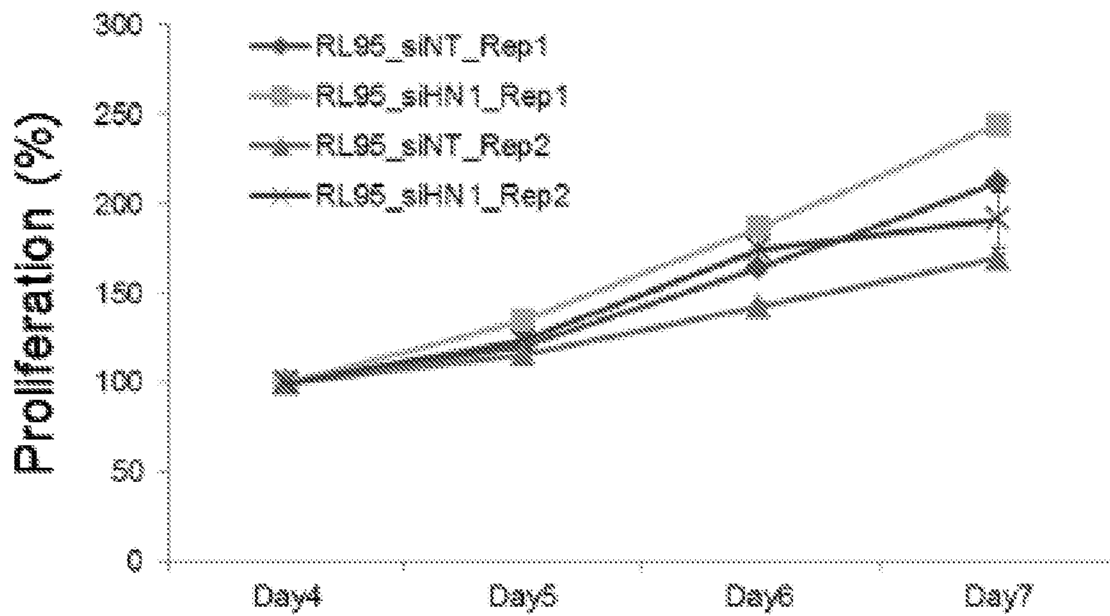


FIG. 6C

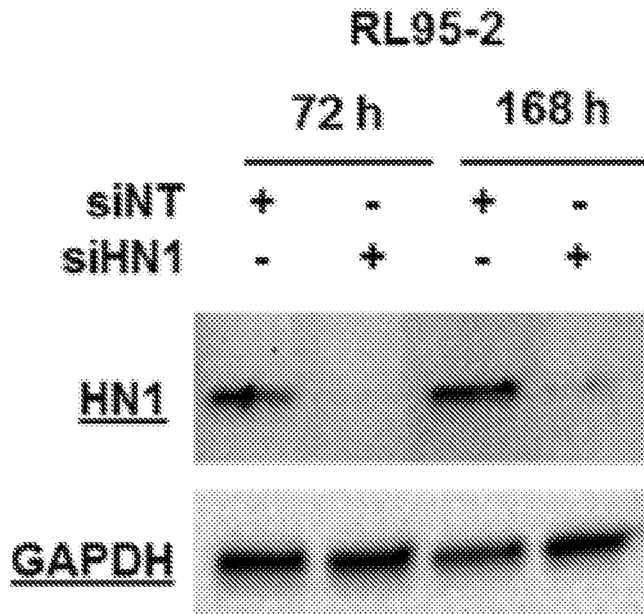


FIG. 6D

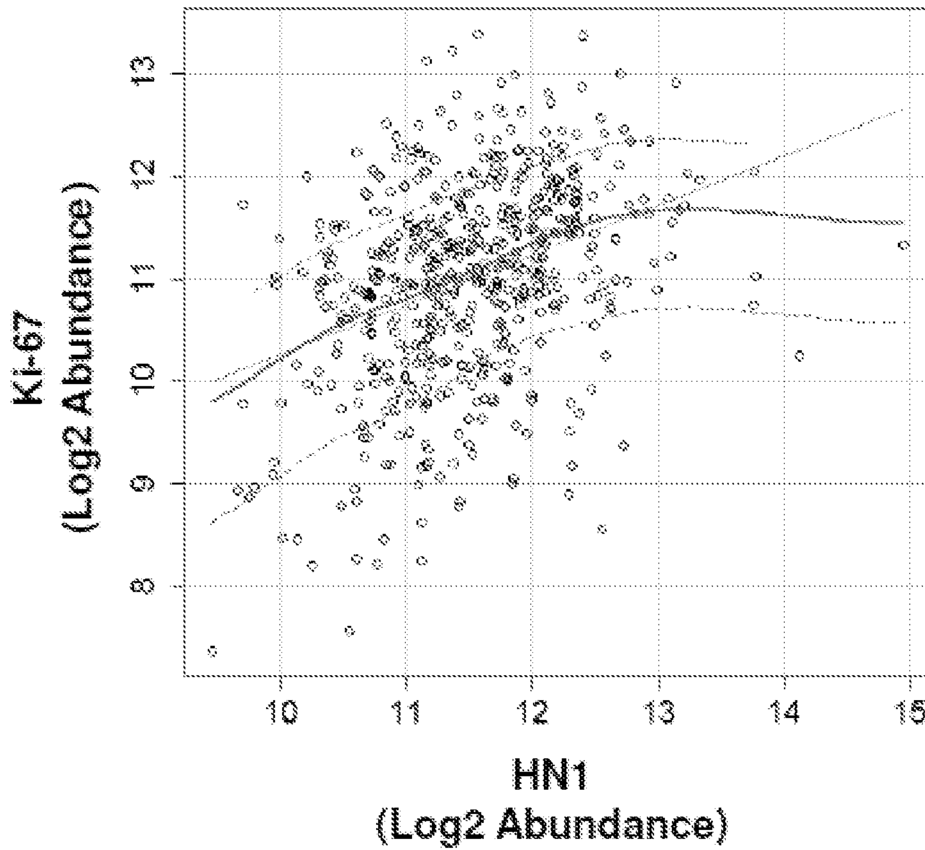


FIG. 7A

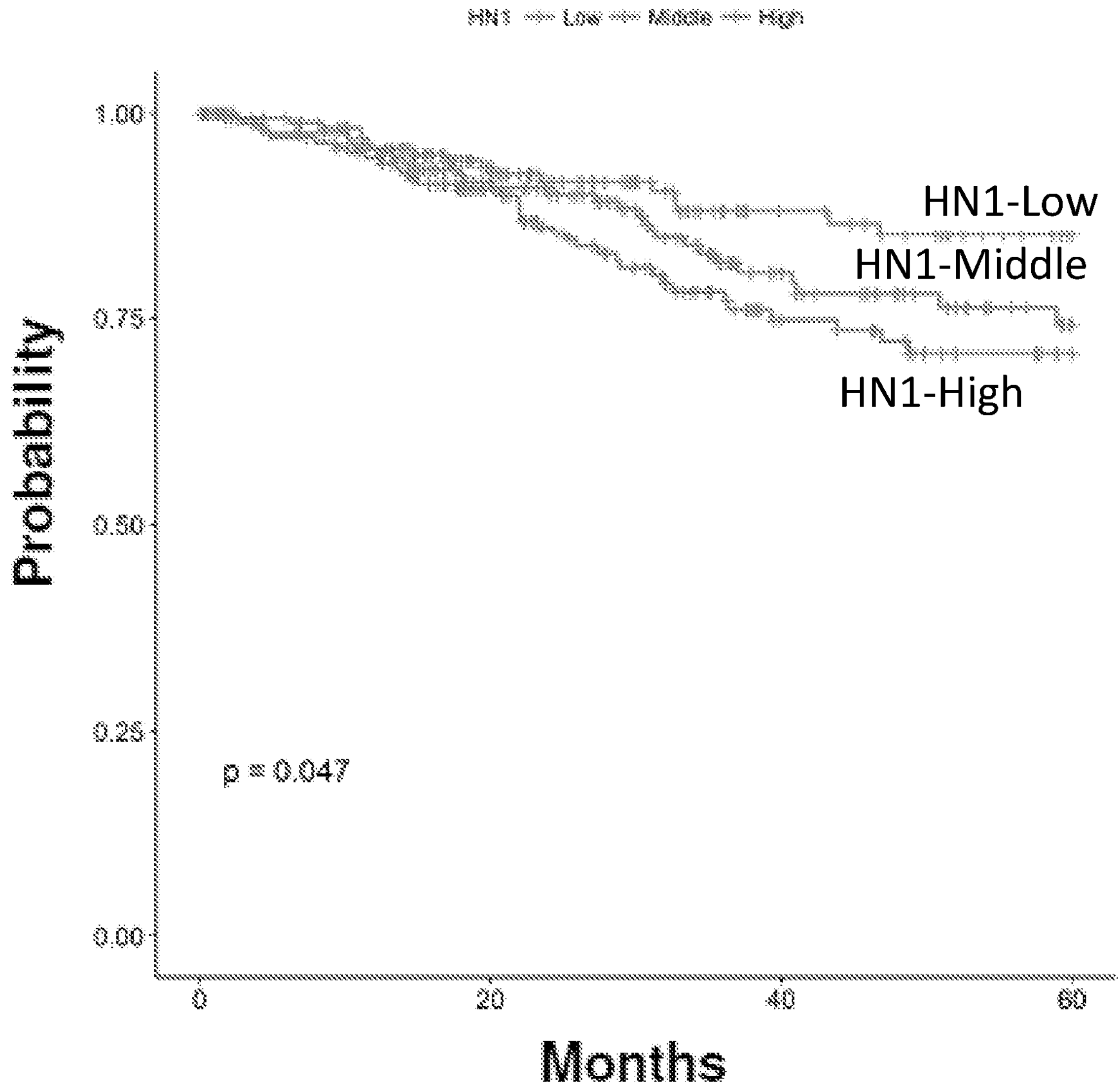


FIG. 7B

Demographic	Responders (n=13)	Non-Responders (n=7)	P
Age (years)	60 (9.6)	60 (9.6)	NS
BMI	38.4 (5.4)	41.8 (7.2)	NS
HgbA1c	5.6 (0.4)	5.6 (0.5)	NS
Duration of metformin treatment (days)	13.1 (5.3)	17.4 (7.7)	NS
Stage			
1A	8	7	NS
1B	4	0	NS
2	1	0	NS
Grade			
1	8	2	NS
2	4	3	NS
3	1	2	NS
Underwent nodal dissection	11	7	NS

FIG. 8

Canonical Pathways	p-value	Proteins (#)
Glucocorticoid Receptor Signaling	2.75E-05	8
BER pathway	8.13E-04	2
AMPK Signaling	1.05E-03	5
Mismatch Repair in Eukaryotes	1.45E-03	2
Aryl Hydrocarbon Receptor Signaling	1.62E-03	4

Functional Annotation	p-value	Activation z-score	Proteins (#)
Viral Infection	1.18E-03	2.776	18
Cell proliferation of tumor cell lines	1.00E-06	2.702	25
Repair of DNA	1.04E-04	2.599	8
Migration of cells	7.75E-06	2.265	26
Proliferation of prostate cancer cell lines	1.63E-04	2.157	8
Death of embryo	2.87E-06	-1.937	7
Neural tube defect	3.74E-03	-1.949	4
Apoptosis	2.15E-07	-2.164	34
Cell death of central nervous system cells	2.39E-03	-2.173	6
Cell death	1.28E-10	-2.288	45

FIG. 9

CASE ID	1=Pre-Metformin Treatment , 2=Post-Metformin Treatment	1=Metformin Responder, 2=Metformin Non-Responder
H0001	1	1
H2501	1	1
H2511	1	1
H2513	1	1
H2515	1	1
H2517	1	1
H2519	1	1
H2521	1	1
H2523	1	1
H3692	1	1
H3693	1	1
H3694	1	1
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H2499	1	2
H2505	1	2
H2507	1	2
H3094	1	2
H2502	2	1
H2504	2	1
H2510	2	1
H2512	2	1
H2514	2	1
H2516	2	1
H2518	2	1
H2520	2	1
H2522	2	1
H2524	2	1
H3095	2	1
H3096	2	1
H3097	2	1
H2492	2	2
H2496	2	2
H2498	2	2
H2500	2	2
H2506	2	2
H2508	2	2
H3093	2	2

FIG. 10

Clinical Characteristics[1]			
Demographic	Responders (N=13)	Non-Responders (N=7)	*P*
Age (years)	60 (9.6)	60 (9.6)	NS
BMI	38.4 (5.4)	41.8 (7.2)	NS
HgbA1c	5.6 (0.4)	5.6 (0.5)	NS
Duration of metformin treatment (days)	13.1 (5.3)	17.4 (7.7)	NS
Grad 1 adverse effects	1	2	NS
Grade 2-4 adverse effects	0	0	NS
Stage			
1A	8	7	NS
1B	4	0	NS
2	1	0	NS
Grade			
1	8	2	NS
2	4	3	NS
3	1	2	NS
Underwent nodal dissection	11	7	NS

[1] Schuler KM, Rambally BS, DiFurio MJ, Sampey BP, Gehrig PA, Makowski L, et al. Antiproliferative and metabolic effects of metformin in a preoperative window clinical trial for endometrial cancer. Cancer Med. 2015;4(2):161-73

FIG. 11

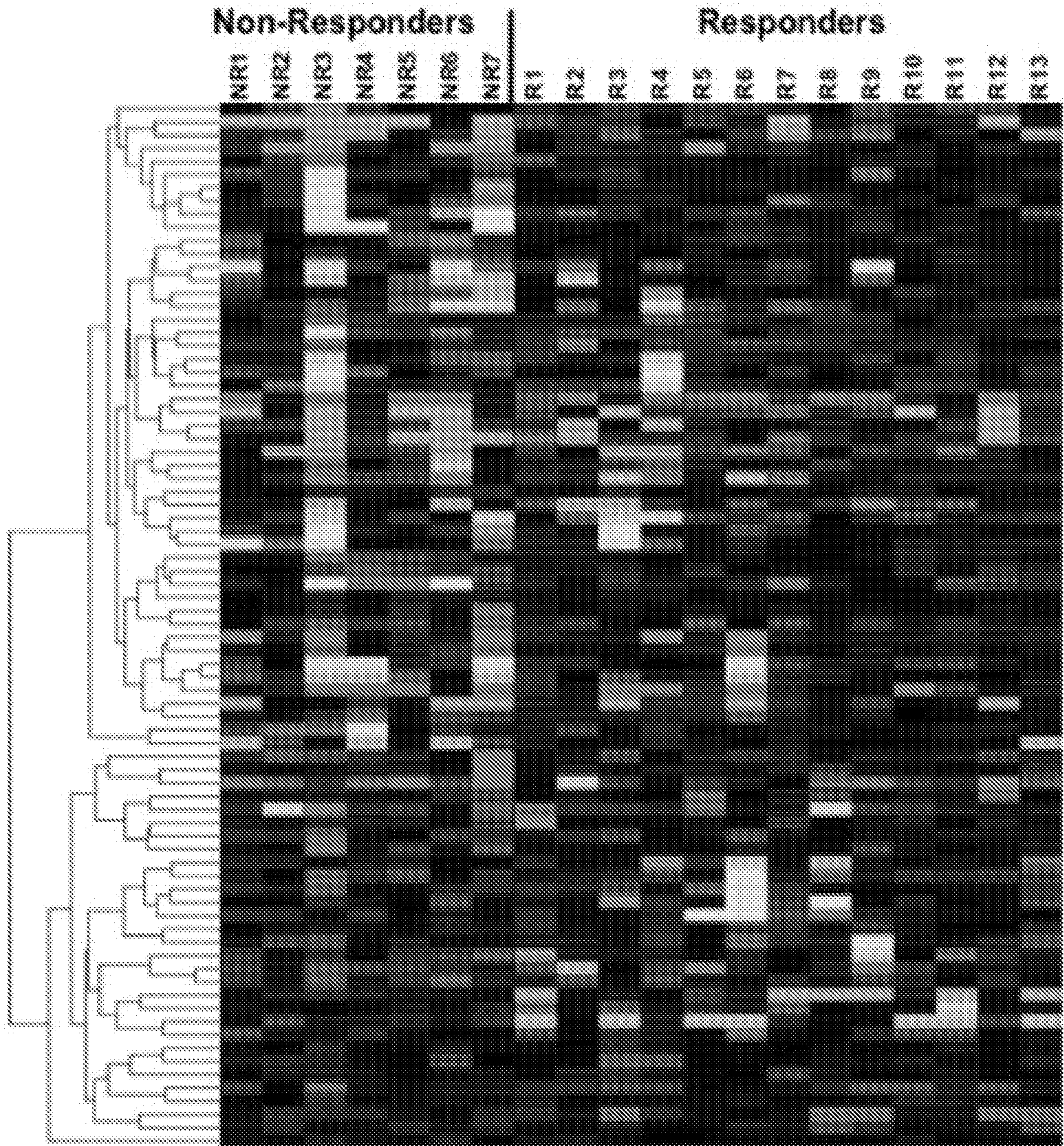

FIG. 12

Canonical Pathway	*P*-Value	# Genes
AMPK Signaling	8.13E-04	5
BER Pathway	9.33E-04	2
Aryl Hydrocarbon Receptor Signaling	1.78E-03	4
Remodeling of Epithelial Adherens Junctions	1.82E-03	3
Mismatch Repair in Eukaryotes	1.82E-03	2

FIG. 13A

Diseases and Bio Function	*P*-Value	Activation z-score	# Genes
Viral Infection	3.07E-03	2.903	17
Proliferation of Cells	3.72E-11	2.489	48
Cell Proliferation of Tumor Cell Lines	6.37E-06	2.471	23
Cell Survival	3.22E-04	2.298	18
Proliferation of Prostate Cancer Cell Lines	1.52E-04	2.157	8
Death of Embryo	2.46E-06	-1.937	7
Neural Tube Defect	3.24E-03	-1.949	4
Cell Death	1.67E-10	-2.113	45
Cell Death of Central Nervous System Cells	3.84E-03	-2.173	6
Apoptosis	2.84E-07	-2.286	34

FIG. 13B

Protein	Responder vs Non-Responder (Log2 Ratio)	Responder Post vs Pre-Metformin (Log2 Ratio)	Cellular Localization	Type(s)
HN1	3.84	-1.05	Nucleus	other
DVL2	1.69	-1.69	Cytoplasm	other
TMSB10/ TMSB4X	1.42	-1.49	Cytoplasm	other
U2AF1/ U2AF1L5	1.16	-1.26	Nucleus	other
SRRM2	1.04	-0.93	Nucleus	other
SLC2A1	1.01	-0.77	Plasma Membrane	transporter
PSMD11	0.90	-0.77	Cytoplasm	other
TPR	0.78	-0.44	Nucleus	transporter
MAP4	0.71	-0.51	Cytoplasm	other
ACTA2	-0.30	0.51	Cytoplasm	other

FIG. 14

Protein	Responder vs Non-Responder (Log2 Ratio)	Responder vs Non-Responder (edgeR p-value)	Responder Post vs Pre-Metformin (Log2 Ratio)	Responder Post vs Pre-Metformin (edgeR p-value)
HN1	3.84	9.78E-06	-1.05	0.02

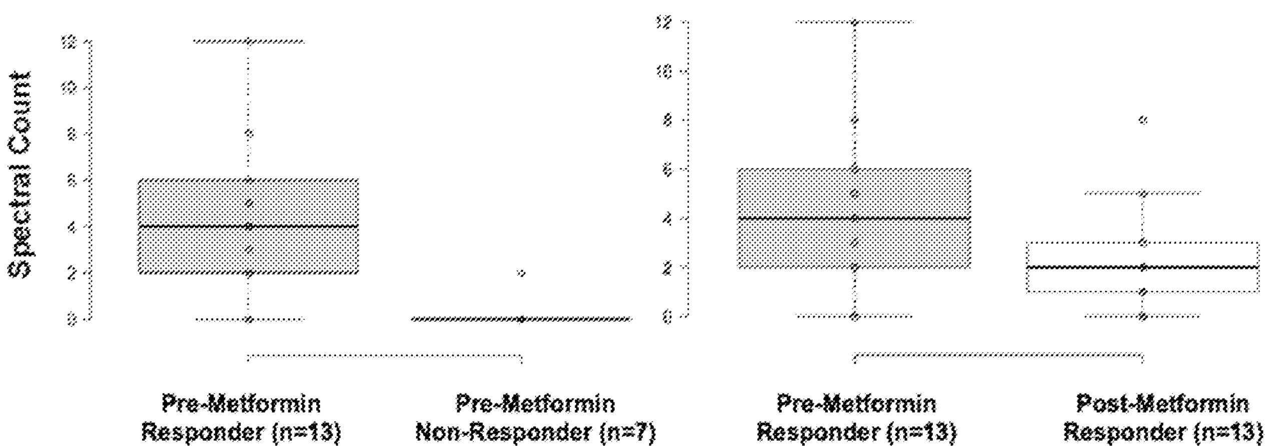

FIG. 15

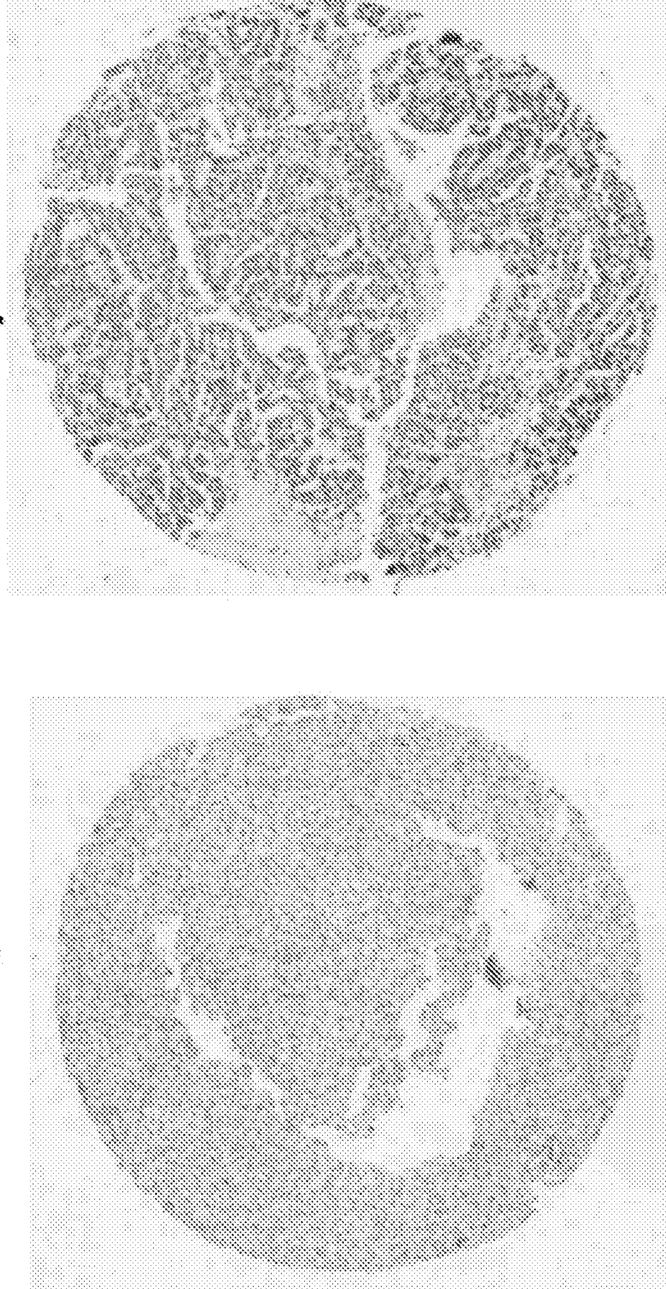

FIG. 16A

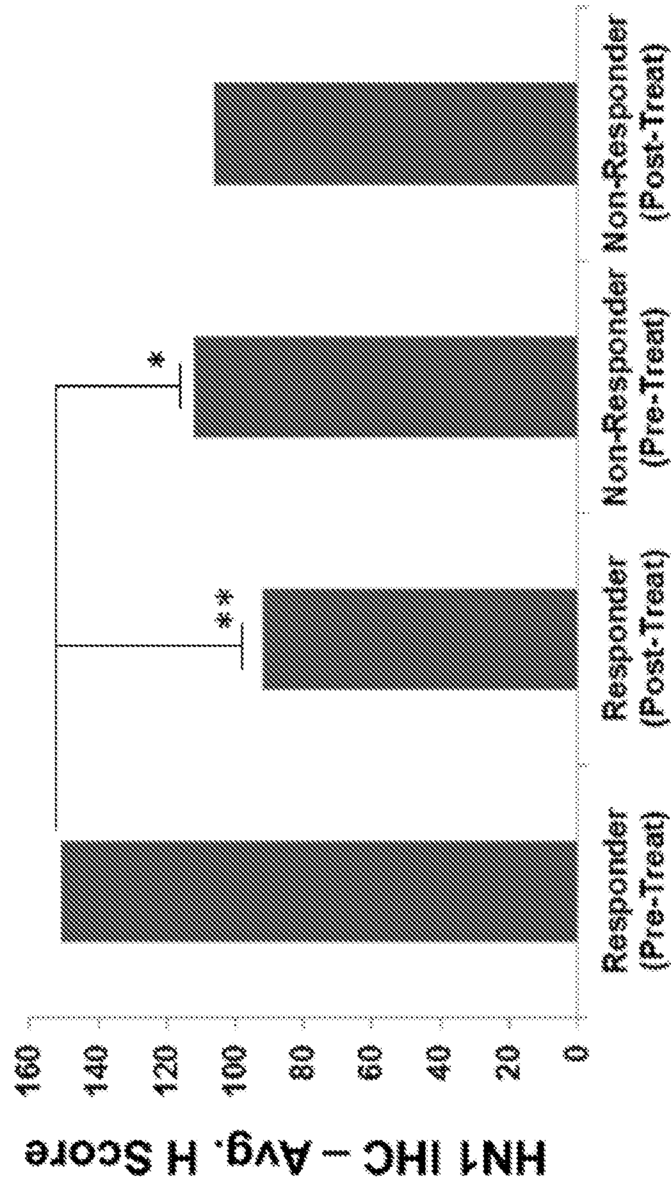

FIG. 16B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/022823

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 4-12, 16-24, 28-32, 38-40
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2019/022823

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 15/09; C12Q 1/68; G01N 33/5; G01N 33/53; G01N 33/574 (2019.01)
CPC - C12Q 1/6886; C12Q 2600/118; C12Q 2600/158 (2019.05)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/6.12 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOPE et al. "Differential Proteomic Analysis of Metformin Response in a Preoperative-Window Clinical Trial for Endometrial Cancer," Gynecologic Oncology, 01 June 2017 (01.06.2017), Vol. 145, Supplement 1, Pg. 32. entire document	25-27, 34
X	US 2012/0220664 A1 (STRUHL et al) 30 August 2012 (30.08.2012) entire document	33
X	US 2011/0159498 A1 (KAO et al) 30 June 2011 (30.06.2011) entire document	1-3, 13-15, 35-37
A	US 2018/0015093 A1 (INFINITY PHARMACEUTICALS, INC) 18 January 2018 (18.01.2018) entire document	1-3, 13-15, 25-27, 33-37
A	SCHULER et al. "Antiproliferative and Metabolic Effects of Metformin in a Preoperative Window Clinical Trial for Endometrial Cancer," Cancer Medicine, 21 November 2014 (21.11.2014), Vol. 4, Iss. 2, Pgs. 161-173. entire document	1-3, 13-15, 25-27, 33-37
T	BATEMAN et al. "Hematological and Neurological Expressed 1 (HN1): A Predictive and Pharmacodynamic Biomarker of Metformin Response in Endometrial Cancers," bioRxiv, 19 April 2019 (19.04.2019), Pgs. 1-35. entire document	1-3, 13-15, 25-27, 33-37

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 May 2019	13 JUN 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774