

B-CATENIN DEPOT DETECTION AND MODULATION ASSAYS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority to and benefit of U.S. Provisional Patent Application No. 63/512,560, filed on July 7, 2023, the contents of which are hereby incorporated herein by reference in their entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The content of the electronic sequence listing (185992001940seqlist.xml; Size: 2,666 bytes; and Date of Creation: July 1, 2024) is herein incorporated by reference in its entirety.

TECHNICAL FIELD

[0003] The present application relates to the field of biological condensates.

BACKGROUND

[0004] Conventional disease research and application to therapeutic drug discovery focuses on identifying individual biomolecules (molecular targets) that cause or mediate disease-relevant biology, such as via known cellular pathways. This “single target” approach to disease research and therapeutic drug discovery has significant limitations as *in vivo* systems are extraordinarily complex and our understanding of cellular pathways and biomolecular actions and interactions are often incomplete. Moreover, not all individual biomolecules, when viewed singularly, are suitable drug targets. Such challenges have contributed to the current shortage of new therapeutic drugs to treat unmet medical needs. In the search new therapeutic drugs, biological condensates are currently being evaluated as a drug discovery target and/ or tool. Certain initial studies in the field of biological condensates observed chemical entities capable of indiscriminately dissolving and/ or preventing the formation of biological condensates. However, chemical entities with such a broad impact may lead to undesirable drug toxicity and off-target properties. Thus, there remains a need for the identification of new biological condensates to target for therapeutic purposes, and for new ways to identify therapeutic candidates that selectively modulate such condensates and/ or condensate components.

BRIEF SUMMARY

[0005] In certain aspects, provided is a method of identifying a compound that modulates β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with the compound; (b) assaying the composition comprising the cell, or a derivative of the composition, for the β -catenin depots; and (c) identifying the compound that modulates β -catenin depots in the cell by comparison of the assayed β -catenin depots in the cell to a reference.

[0006] In some embodiments, comparison of the assayed β -catenin depots in the cell to the reference is based on one or more of the following attributes of the β -catenin depots: (i) presence of the β -catenin depots; (ii) number of the β -catenin depots; (iii) size of the β -catenin depots; (iv) surface area of the β -catenin depots; (v) cellular location of the β -catenin depots; (vi) distribution of the β -catenin depots; (vii) ratio of the amount of the β -catenin depots and a reference condensate; or (viii) composition of the β -catenin depots. In some embodiments, modulation of the β -catenin depots is based on increasing the β -catenin depots in the cell as compared to the reference. In some embodiments, modulation of the β -catenin depots is based on an increase in the number of the β -catenin depots in the cell as compared to the reference. In some embodiments, modulation of the β -catenin depots in the cell is based on an increase in the number of the β -catenin depots in the nucleus of the cell as compared to the reference. In some embodiments, modulation of the β -catenin depots in the cell is based on an increase in the presence of the β -catenin depots in the cell as compared to the reference. In some embodiments, modulation of the β -catenin depots in the cell is based on an increase in the presence of the β -catenin depots in the nucleus of the cell as compared to the reference.

[0007] In some embodiments, the assaying for the β -catenin depots comprises detecting an inactive form of a β -catenin polypeptide. In some embodiments, the detecting the inactive form of the β -catenin polypeptide comprises contacting the composition comprising the cell, or the derivative of the composition, with a detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide. In some embodiments, the detectable reagent is an antibody specifically recognizing the inactive form of the β -catenin polypeptide. In some embodiments, the assaying comprises detecting the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide. In some embodiments, the detecting comprises imaging at least a portion of the cell. In some embodiments, the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide is a fluorescently labeled antibody

specifically recognizing the inactive form of the β -catenin polypeptide. In some embodiments, the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide is detected using a fluorescently labeled secondary antibody that specifically recognizes the detectable reagent. In some embodiments, the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide specifically recognizes a phosphorylation at one or more of S33, S37, T41, or S45 of the inactive form of the β -catenin polypeptide, and wherein the amino acid position is relative to amino acid sequence of SEQ ID NO:1. In some embodiments, the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide specifically recognizes a phosphorylation at T41 and/ or S45. In some embodiments, the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide specifically recognizes phosphorylations at T41 and S45. In some embodiments, the detectable reagent that specifically recognizes the β -catenin polypeptide is the 23H16L13 antibody clone.

[0008] In some embodiments, the comparison of the assayed β -catenin depots in the cell to the reference is based on one or more of the following attributes of an inactive form of the β -catenin polypeptide associated with the β -catenin depots: (i) location of the inactive form β -catenin polypeptide; (ii) amount of the inactive form β -catenin polypeptide; (iii) distribution of the inactive form β -catenin polypeptide; (iv) condensate partitioning of the inactive form β -catenin polypeptide into the β -catenin depots and/ or one or more other condensates; (v) a functional activity associated with the inactive form β -catenin polypeptide; (vi) aggregation of the inactive form β -catenin polypeptide; (vii) post-translational modification status of the inactive form β -catenin polypeptide; or (viii) amount of a degradation product of the inactive form β -catenin polypeptide.

[0009] In some embodiments, the modulation, such as increase, in the β -catenin depots in the cell is based on an increase in the amount of the inactive form of the β -catenin polypeptide in the β -catenin depots as compared to the reference.

[0010] In some embodiments, the assaying for the β -catenin depots comprises detecting a phospho-H2A.X (P-2A.X) and/ or mediator of DNA damage checkpoint protein 1 (MDC1).

[0011] In some embodiments, the cell is a model for aberrant Wnt/ β -catenin signaling. In some embodiments, the cell is a model for a proliferative pathology. In some embodiments, the cell is a model for a cancer. In some embodiments, the cell is a cancer cell. In some embodiments, the cancer cell is a human osteosarcoma U2OS cell or human colon adenocarcinoma DLD-1 cell.

[0012] In some embodiments, the cell is a model for autosomal dominant polycystic kidney disease (ADPKD), hereditary familial adenomatous polyposis (FAP), Alzheimer's, or a metabolic disease, such as obesity and/ or insulin resistance.

[0013] In some embodiments, the inactive form of the β -catenin polypeptide is a wildtype β -catenin polypeptide. In some embodiments, the inactive form of the β -catenin polypeptide is a mutant β -catenin polypeptide.

[0014] In some embodiments, the assaying further comprises contacting the composition comprising the cell, or the derivative of the composition, with a second detectable reagent that specifically recognizes an active form of a β -catenin polypeptide comprising a phosphorylation at S552 and/or S675, and wherein the amino acid position is relative to amino acid sequence of SEQ ID NO:1.

[0015] In some embodiments, the method further comprises contacting the composition comprising the cell, or the derivative of the composition, with a fixative agent before the assaying of step (b).

[0016] In some embodiments, the method further comprises determining one or more cellular features of the cell.

[0017] In some embodiments, the reference is based on: (i) a composition comprising the cell not contacted with the compound; (ii) a composition comprising the cell admixed with a control vehicle and not contacted with the compound; (iii) a desired β -catenin profile; (iv) a reference condensate or a reference polypeptide within the cell; and/ or (v) a reference composition comprising a cell.

[0018] In some embodiments, the method further comprises subjecting the composition comprising the cell to a condition that promotes formation of the β -catenin depots prior to the contacting the composition comprising the cell with the compound.

[0019] In some embodiments, the method further comprises assessing the identified compound using a second cell-based assay and/or an *in vitro* assay. In some embodiments, the method further comprises assessing the identified compound using a second cell-based assay.

[0020] In certain other aspects, provided herein is a method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45; and (b) detecting the detectable reagent, wherein the detection of the detectable reagent within the cell (*e.g.*, within a condensate)

identifies the β -catenin depots in the cell. In some embodiments, the detectable reagent is an antibody specifically recognizing the inactive form of the β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45. In some embodiments, the detectable reagent is an antibody specifically recognizing the inactive form of the β -catenin polypeptide comprising phosphorylations at T41 and S45. In some embodiments, the detecting comprises imaging at least a portion of the cell. In some embodiments, the detectable reagent is a fluorescently labeled antibody specifically recognizing the inactive form of the β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45. In some embodiments, the detectable reagent is detected using a fluorescently labeled secondary antibody that specifically recognizes the detectable reagent. In some embodiments, the method further comprises contacting the composition comprising the cell with a fixative agent before contacting with the detectable reagent. In some embodiments, the method further comprises measuring a signal of the detectable reagent. In some embodiments, the method further comprises comparing the signal with a reference. In some embodiments, the reference is based on: (i) a composition comprising the cell not contacted with the detectable reagent; (ii) a composition comprising the cell admixed with a control vehicle and not contacted with the detectable reagent; (iii) a reference signal; (iv) a reference condensate or a reference polypeptide within the cell; and/ or (v) a reference composition comprising a cell.

[0021] In certain other aspects, provided herein is a method of identifying a compound useful for treating a β -catenin-associated disease, wherein the method comprises identifying a compound according to a method described herein. In some embodiments, the β -catenin-associated disease is selected from the group consisting of a cancer, autosomal dominant polycystic kidney disease (ADPKD), hereditary familial adenomatous polyposis (FAP), Alzheimer's, or a metabolic disease.

[0022] In certain other aspects, provided herein is a compound identified by a method described herein.

[0023] In certain other aspects, provided herein is a method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a first detectable reagent that specifically recognizes a β -catenin polypeptide; (b) contacting the composition comprising the cell with a second detectable reagent that specifically recognizes a phospho-H2A.X (P-2A.X) or mediator of DNA damage checkpoint protein 1 (MDC1); and (c) detecting the first detectable reagent and the second detectable reagent; wherein the

detection of a colocalization of the first detectable reagent and the second detectable reagent within the cell identifies the β -catenin depots in the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] **FIG. 1** shows images of cells expressing Halo- β -catenin or labeled with an antibody specifically binding to inactive β -catenin or active β -catenin (“ABC”). The antibody specifically binding to inactive β -catenin allows for the visualization of β -catenin depots while the antibody specifically binding to active β -catenin does not due to high background noise. DAPI was used to stain for nuclei.

[0025] **FIG. 2** shows a dose-response curve for Compound A tested against wt U2OS cells.

[0026] **FIG. 3** shows immunofluorescence (IF) images of healthy versus malignant colon cells upon treatment with a small molecule condensate modulator B (compound B) which was identified and optimized from a screen of 20,000 compounds. C-mod treatment of malignant cells results in sequestration of β -catenin into condensates.

[0027] **FIG. 4** shows selective killing of cancerous colon cells over healthy colon cells upon treatment of compound B which was identified and optimized from a screen of 20,000 compounds.

[0028] **FIG. 5** shows gene set enrichment analysis (GSEA) plots revealing selective down-regulation of Wnt/ β -Catenin and LEF1 target genes in colorectal cancer cells when treated with the β -catenin depot-inducing compound B.

[0029] **FIG. 6** shows results from assay where 99 cancer cell lines were subjected to treatment with compound B. These results demonstrate that compound B is active in a broad range of Wnt-associated cancers.

[0030] **FIG. 7** shows compound B causes stasis in tumor growth in a patient-derived xenograft (PDX) model derived from heavily pre-treated CRC patients with high β -Catenin expression. FOLFOX is a combination of chemotherapy drugs which represents standard-of-care in patients with metastatic colorectal cancer. QW = weekly dosing; BID = twice daily dosing; QD = dosed every day.

DETAILED DESCRIPTION

[0031] The present application provides, in certain aspects, methods of identifying a compound that modulates β -catenin depots in a cell. The present disclosure is based, at least in

part, on the inventors' identification of, and ability to measure, specific β -catenin depots, a type of condensate comprising certain inactive forms of β -catenin, useful for identifying compounds that impart a desired result in cells exhibiting aberrant Wnt/ β -catenin signaling causing growth-related pathologies such as cancer and other human conditions. The ability to measure, such as via imaging techniques, said β -catenin depots enables high throughput compound screening methods for identifying compounds that can regulate aberrant Wnt/ β -catenin signaling. As also described herein, the identification of said β -catenin depot enabled the discovery of co-localizing macromolecules, which further expands macromolecules that can be used to assess β -catenin depots. Moreover, it has been found that various Wnt/ β -catenin signaling pathway mutations are relevant to cancer and lead to increased β -catenin signaling, however, in a panel of cell lines it was found that regardless of what mutations are present (including the β -catenin hot spot mutation) that a single phenotype is relevant to identifying therapeutically useful compounds, namely, β -catenin depot formation. The findings described herein regarding the ability to specifically measure β -catenin depots is a significant advancement in the field of condensates and drug discovery methodologies, especially considering, *e.g.*, the failure of certain methodology to measure said β -catenin depots.

[0032] Thus, in some aspects, provided herein is a method of identifying a compound that modulates β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with the compound; (b) assaying the composition comprising the cell, or a derivative of the composition, for the β -catenin depots; and (c) identifying the compound that modulates β -catenin depots in the cell by comparison of the assayed β -catenin depots in the cell to a reference.

[0033] In other aspects, provided herein is a method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45; and (b) detecting the detectable reagent, wherein the detection of the detectable reagent within the cell (*e.g.*, within a condensate) identifies the β -catenin depots in the cell. In certain aspects described herein, the detectable reagent that specifically recognized an inactive form of a β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45 is a detectable reagent that is determinative of the presence of the target in the presence of a heterogeneous population of molecules. In some embodiments,

the detectable reagent may also bind other molecules, including biomolecules, to a certain degree and/ or affinity.

[0034] In other aspects, provided herein is a method of identifying a compound useful for treating a β -catenin-associated disease, wherein the method comprises identifying a compound according to a method described herein. In some embodiments, the β -catenin-associated disease is selected from the group consisting of a cancer, autosomal dominant polycystic kidney disease (ADPKD), hereditary familial adenomatous polyposis (FAP), Alzheimer's, or a metabolic disease.

[0035] In certain other aspects, provided herein is a compound identified by a method described herein.

[0036] In certain other aspects, provided herein is a method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a first detectable reagent that specifically recognizes a β -catenin polypeptide; (b) contacting the composition comprising the cell with a second detectable reagent that specifically recognizes a phospho-H2A.X (P-2A.X) or mediator of DNA damage checkpoint protein 1 (MDC1); and (c) detecting the first detectable reagent and the second detectable reagent; wherein the detection of a colocalization of the first detectable reagent and the second detectable reagent within the cell identifies the β -catenin depots in the cell.

[0037] It will also be understood by those skilled in the art that changes in the form and details of the implementations described herein may be made without departing from the scope of this disclosure. In addition, although various advantages, aspects, and objects have been described with reference to various implementations, the scope of this disclosure should not be limited by reference to such advantages, aspects, and objects.

[0038] All references cited herein, including patent applications and publications, are incorporated herein by reference in their entirety.

[0039] The section headings used herein are for organizational purposes only and are not to be construed as limiting the subject matter described. For example, some aspects of the disclosure are presented in a modular fashion, and such presentation is not to be construed as limited the possible combinations of approaches taught herein.

I. Definitions

[0040] For purposes of interpreting this specification, the following definitions will apply and, whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth shall control.

[0041] As used herein, “condensate” means a non-membrane-encapsulated compartment formed by phase separation of one or more proteins and/or other macromolecules such as nucleic acids (including all stages of phase separation).

[0042] The terms “polypeptide” and “protein,” as used herein, may be used interchangeably to refer to a polymer comprising amino acid residues, and are not limited to a minimum length. Such polymers may contain natural or non-natural amino acid residues, or combinations thereof, and include, but are not limited to, peptides, polypeptides, oligopeptides, dimers, trimers, and multimers of amino acid residues. Full-length polypeptides or proteins, and fragments thereof, are encompassed by this definition. The terms also include modified species thereof, *e.g.*, post-translational modifications of one or more residues, including but not limited to, methylation, phosphorylation glycosylation, sialylation, or acetylation.

[0043] The term “antibody,” and grammatical equivalents thereof, includes full-length antibodies and antigen-binding fragments thereof. A full-length antibody comprises two heavy chains and two light chains. The term “antigen-binding fragment” as used herein refers to an antibody fragment including, for example, a diabody, a Fab, a Fab', a F(ab')₂, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multi-specific antibody formed from a portion of an antibody comprising one or more CDRs, a camelized single domain antibody, a nanobody, a domain antibody, a bivalent domain antibody, any other antibody fragment that binds to an antigen but does not comprise a complete full-length antibody structure, or an antibody mimetic (*e.g.*, designed ankyrin repeat proteins (DARPin), affimer, or monobody (ADNECTINS®)). An antigen-binding fragment is capable of binding to the same antigen to which the parent antibody or a parent antibody fragment (*e.g.*, a parent scFv) binds.

[0044] As use herein, the terms “specifically binds” or “is specific for,” or grammatical equivalents thereof, refer to measurable and reproducible interactions, such as binding between a target and an antibody or antibody moiety that is determinative of the presence of the target in

the presence of a heterogeneous population of molecules, including biological molecules. For example, an antibody or antibody moiety that specifically binds to a target (which can be an epitope) is an antibody or antibody moiety that binds this target with greater affinity, avidity, more readily, and/or with greater duration than its bindings to other targets. In some embodiments, an antibody or antibody moiety that specifically binds to an antigen reacts with one or more antigenic determinants of the antigen (for example β -catenin or a portion thereof, including β -catenin species comprising post-translation modification such as one or more phosphorylations) with a binding affinity that is at least about 10 times its binding affinity for other targets.

[0045] The terms “comprising,” “having,” “containing,” and “including,” and other similar forms, and grammatical equivalents thereof, as used herein, are intended to be equivalent in meaning and to be open ended in that an item or items following any one of these words is not meant to be an exhaustive listing of such item or items, or meant to be limited to only the listed item or items. For example, an article “comprising” components A, B, and C can consist of (*i.e.*, contain only) components A, B, and C, or can contain not only components A, B, and C but also one or more other components. As such, it is intended and understood that “comprises” and similar forms thereof, and grammatical equivalents thereof, include disclosure of embodiments of “consisting essentially of” or “consisting of.”

[0046] 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 dictate otherwise, between the upper and lower limit of that range and any other stated or intervening value in that stated range, is 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.

[0047] Reference to “about” a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to “about X” includes description of “X.”

[0048] As used herein, including in the appended claims, the singular forms “a,” “or,” and “the” include plural referents unless the context clearly dictates otherwise.

II. Methods of assaying for β -catenin depots and/ or identifying β -catenin depot modulators

[0049] Provided herein, in certain aspects, are methods of assaying a composition comprising a cell, or a derivative of the composition, for β -catenin depots, and uses thereof such as for the identification of β -catenin depot modulators (*e.g.*, small molecule therapeutic compounds that modulate, such as increase, β -catenin depots in a cell as compared to a reference).

[0050] In certain aspects, provided herein is a method of identifying (such as visualizing and/ or measuring) a β -catenin depot in a composition comprising a cell, the method comprising contacting the composition comprising the cell, or a derivative thereof, with a detectable reagent (*e.g.*, antibody or antigen-binding fragment thereof) that specifically binds to a macromolecule in a β -catenin depot (such as to allow for a sufficient signal-to-baseline-noise value to image the β -catenin depot). As taught herein, the detectable reagent that specifically binds to a macromolecule in a β -catenin depot is a detectable reagent that is determinative of the presence of the target in the presence of a heterogeneous population of molecules. In some embodiments, the detectable reagent may also bind other molecules, including macromolecules (*e.g.*, biomolecules), to a certain degree and/ or affinity. In some embodiments, the macromolecule is also located outside of a β -catenin depot. In some embodiments, the macromolecule is a polypeptide or a species thereof, such as a polypeptide characterized by one or more specific post-translation modifications (*e.g.*, as defined by the site of polypeptide attachment and/ or composition). In some embodiments, the polypeptide comprises one or more phosphorylations. In some embodiments, the macromolecule is a β -catenin polypeptide, *e.g.*, an inactive β -catenin polypeptide comprising one or more phosphorylations at one or more of S33, S37, T41, or S45, and wherein the amino acid position is relative to amino acid sequence of SEQ ID NO:1. In some embodiments, the detecting of an inactive β -catenin polypeptide (*e.g.*, comprising one or more phosphorylations at one or more of S33, S37, T41, or S45), such as via a detectable reagent, above a threshold level identifies the β -catenin depot. In some embodiments, the threshold level is the background noise (*e.g.*, fluorescent noise signal from immunofluorescent staining or imaging). In some embodiments, the threshold level is the average or median fluorescent signal not in a condensate, *e.g.*, surrounding a condensate, or in cytoplasm. As described herein, general techniques for condensate identification are known in the art (such as described in 2020/0150107, WO 2022/035989, and WO 2022/187225, which are hereby

incorporated herein by reference), and can be combined with the teachings herein to identify β -catenin depots. In some embodiments, the inactive β -catenin polypeptide comprises a phosphorylation at T41 and/ or S45, including T41 and S45, wherein the amino acid position is relative to amino acid sequence of SEQ ID NO:1. In some embodiments, the detectable reagent comprises the 23H16L13 antibody clone, or a fragment thereof having substantially the same binding specificity as the 23H16L13 antibody clone. In some embodiments, the detectable reagent comprises an antibody or antigen-binding fragment thereof comprising all 6 CDRs of 23H16L13, such as a humanized version of 23H16L13 antibody clone. In some embodiments, the macromolecule is phospho-H2A.X (P-2A.X). In some embodiments, the macromolecule is DNA damage checkpoint protein 1 (MDC1). In some embodiments, the method comprises detecting more than one macromolecule, such as one or more of an inactive β -catenin polypeptide, P-2A.X, or MDC1. In some embodiments, the method comprises detecting co-localization of more than one macromolecule, such as two or more of any of an inactive β -catenin polypeptide, P-2A.X, or MDC1.

[0051] In certain aspects, provided herein is a method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a detectable reagent (e.g., antibody or antigen-binding fragment thereof) that specifically recognizes an inactive form of a β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45; and (b) detecting the detectable reagent, wherein the detection of the detectable reagent in the cell (e.g., within a condensate) identifies the β -catenin depots in the cell. In some embodiments, the detectable reagent is an antibody specifically recognizing the inactive form of the β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45, including a phosphorylation at T41 and S45. In some embodiments, the detectable reagent comprises the 23H16L13 antibody clone, or a fragment thereof having substantially the same binding specificity as the 23H16L13 antibody clone. In some embodiments, the detectable reagent comprises an antibody or antigen-binding fragment thereof comprising all 6 CDRs of 23H16L13, such as a humanized version of 23H16L13 antibody clone.

[0052] In certain aspects, provided herein is a method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a detectable reagent (e.g., antibody or antigen-binding fragment thereof) that specifically recognizes an inactive form of β -catenin polypeptide (e.g., comprising phosphorylation at one or more of S33, S37, T41, or S45); (b) detecting a condensate or component thereof (e.g., by contacting the

composition comprising the cell with a second detectable reagent (e.g., antibody or antigen-binding fragment thereof) that specifically recognizes the condensate component, or using a fluorescently labeled condensate component); and (c) detecting the detectable reagent and the condensate or component thereof; wherein the detection of a colocalization of the detectable reagent and the condensate or component thereof within the cell identifies the β -catenin depots in the cell.

[0053] In certain aspects, provided herein is a method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a detectable reagent (e.g., antibody or antigen-binding fragment thereof) that specifically recognizes an inactive form of β -catenin polypeptide (e.g., comprising phosphorylation at one or more of S33, S37, T41, or S45); (b) detecting a condensate or component thereof (e.g., by contacting the composition comprising the cell with a second detectable reagent (e.g., antibody or antigen-binding fragment thereof) that specifically recognizes the condensate component, or using a fluorescently labeled condensate component); and (c) detecting the detectable reagent and the condensate or component thereof; wherein the detection of a colocalization of the detectable reagent and the condensate or component thereof within the cell identifies the β -catenin depots in the cell. In some embodiments, the condensate is associated with prolonged DSB DNA damage. In some embodiments, the condensate associated with prolonged DSB DNA damage comprises P-2A.X and/ or MDC1. In some embodiments, the condensate component to be detected is P-2A.X and/ or MDC1. In some embodiments, the condensate associated with prolonged DSB DNA damage further comprise one or more of: 53BP1, RIF1, p-TEFb, DCP2, or FOXO3a. Hence in some embodiments, the condensate component to be detected can be one or more of: 53BP1, RIF1, p-TEFb, DCP2, or FOXO3a.

[0054] In certain aspects, provided herein is a method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a detectable reagent (e.g., antibody or antigen-binding fragment thereof) that specifically recognizes P-2A.X; and (b) detecting the detectable reagent, wherein the detection of the detectable reagent in the cell (e.g., within a condensate) within the cell identifies the β -catenin depots in the cell.

[0055] In certain aspects, provided herein is a method of identifying β -catenin depots in a cell, the method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a detectable reagent (e.g., antibody or antigen-binding fragment thereof) that specifically recognizes MDC1; and (b) detecting the detectable reagent,

wherein the detection of the detectable reagent within the cell (*e.g.*, within a condensate) identifies the β -catenin depots in the cell.

[0056] In certain aspects, provided herein is a method of identifying β -catenin depots in a cell, the method comprising: (a) contacting a composition comprising the cell with a first detectable reagent (*e.g.*, antibody or antigen-binding fragment thereof) that specifically recognizes a β -catenin polypeptide; (b) contacting the composition comprising the cell with a second detectable reagent (*e.g.*, antibody or antigen-binding fragment thereof) that specifically recognizes P-2A.X or MDC1; and (c) detecting the first detectable reagent and the second detectable reagent; wherein the detection of a colocalization of the first detectable reagent and the second detectable reagent within the cell identifies the β -catenin depots in the cell. As described herein, identification of the β -catenin depots is assessed by observing areas of higher intensity signal as compared to the background and/ or a threshold value. The first detectable reagent can specifically recognize any form of a β -catenin polypeptide, *e.g.*, active form, inactive form, or pan- β -catenin. In some embodiments, the first detectable reagent is an antibody specifically recognizing an inactive form of the β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45, including a phosphorylation at T41 and S45. In some embodiments, the first detectable reagent comprises the 23H16L13 antibody clone, or a fragment thereof having substantially the same binding specificity as the 23H16L13 antibody clone. In some embodiments, the first detectable reagent comprises an antibody or antigen-binding fragment thereof comprising all 6 CDRs of 23H16L13, such as a humanized version of 23H16L13 antibody clone. In some embodiments, the first detectable reagent comprises an antibody or antigen-binding fragment thereof that recognizes a β -catenin polypeptide regardless of its phosphorylation status.

[0057] In certain aspects, provided herein is a method of identifying a compound that modulates β -catenin depots (*e.g.*, any of the β -catenin depots identified herein, or β -catenin depots identified by any of the methods described herein) in a cell, the method comprising: (a) contacting a composition comprising the cell with the compound; and (b) identifying the compound that modulates β -catenin depots in the cell by comparison of the β -catenin depots in the cell to a reference. In some embodiments, the method further comprises assaying for the β -catenin depots in the cell, such as using any of the β -catenin depots identification methods described herein. Hence in certain aspects, provided herein is a method of identifying a compound that modulates β -catenin depots in a cell, the method comprising: (a) contacting a

composition comprising the cell with the compound; (b) assaying the composition comprising the cell, or a derivative of the composition, for the β -catenin depots; and (c) identifying the compound that modulates β -catenin depots in the cell by comparison of the assayed β -catenin depots in the cell to a reference. In some embodiments, the assaying for the β -catenin depots comprises detecting an inactive form of a β -catenin polypeptide using a detectable reagent. In some embodiments, the detectable reagent is an antibody specifically recognizing the inactive form of the β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45, including a phosphorylation at T41 and S45. In some embodiments, the detectable reagent comprises the 23H16L13 antibody clone, or a fragment thereof having substantially the same binding specificity as the 23H16L13 antibody clone. In some embodiments, the detectable reagent comprises an antibody or antigen-binding fragment thereof comprising all 6 CDRs of 23H16L13, such as a humanized version of 23H16L13 antibody clone. In some embodiments, the assaying for the β -catenin depots comprises detecting a colocalization of a β -catenin polypeptide (e.g., any form of β -catenin polypeptide) and a P-2A.X and/ or MDC1. In some embodiments, comparison of the assayed β -catenin depots in a cell to a reference is based on one or more of the following attributes of the β -catenin depots: (i) presence of the β -catenin depots; (ii) number of the β -catenin depots; (iii) size of the β -catenin depots; (iv) surface area of the β -catenin depots; (v) cellular location of the β -catenin depots; (vi) distribution of the β -catenin depots; (vii) ratio of the amount of the β -catenin depots and a reference condensate; or (viii) composition of the β -catenin depots. In some embodiments, modulation of the β -catenin depots is based on increasing the β -catenin depots in the cell as compared to the reference. In some embodiments, modulation of the β -catenin depots is based on an increase in the number of the β -catenin depots in the cell as compared to the reference. In some embodiments, modulation of the β -catenin depots in the cell is based on an increase in the number of the β -catenin depots in the nucleus of the cell as compared to the reference. In some embodiments, modulation of the β -catenin depots in the cell is based on an increase in the presence of the β -catenin depots in the cell as compared to the reference. In some embodiments, modulation of the β -catenin depots in the cell is based on an increase in the presence of the β -catenin depots in the nucleus of the cell as compared to the reference.

[0058] In certain aspects described herein, the term “ β -catenin depot,” or grammatical equivalents thereof, refers to a nuclear condensate characterized by the presence of one or more inactive forms of β -catenin. In some embodiments, the one or more inactive forms of β -catenin

are associated with a reduced or absent activity in transcription as compared to an active form of β -catenin (*e.g.*, β -catenin having a phosphorylation at S552 and/ or S675). In some embodiments, the inactive form of β -catenin comprises a phosphorylation at one or more of S33, S37, T41, or S45. In some embodiments, the inactive form of β -catenin comprises a phosphorylation at T41 and S45. In some embodiments, the inactive form of β -catenin is not phosphorylated at S552 and/ or S675. *See, e.g.*, Verheyen & Gottardi, *Dev Dyn*, 239, 2010. In some embodiments, the β -catenin depot further comprises one or more active forms of β -catenin. In some embodiments, the β -catenin depot further comprises another macromolecule, *e.g.*, a polypeptide or a specific species thereof, *e.g.*, a post-translationally modified species. In some embodiments, the β -catenin depot selectively comprises another macromolecule, *e.g.*, the other macromolecule is not present in another condensate, or the other macromolecule is not present in another condensate comprising β -catenin (such as active form of β -catenin). In some embodiments, the β -catenin depot is not a transcriptional condensate. In some embodiments, the β -catenin depot does not comprise one or more mediator components. In some embodiments, the β -catenin depot does not comprise DNA and/ or RNA. In some embodiments, the β -catenin depot does not reside at transcription start site of a gene. In some embodiments, the presence of β -catenin depots is indicative of a specific biological function desired to be provided by a small molecule therapeutic compound, such as reduced activity in β -catenin associated transcription as compared to an active form of β -catenin. In some embodiments, the formation (including increase) of β -catenin depots is not correlated with an increase in cell death. In some embodiments, the β -catenin depots cannot be rescued (release inactive β -catenin from a β -catenin depots to be converted to active forms of β -catenin) by transcription factor LEF1 and/or TCF4. In some embodiments, the β -catenin depot is not associated with closed chromatin. In some embodiments, the β -catenin depot does not comprise PRMT5.

[0059] Methodology, and elements thereof, for assaying for β -catenin depots, and uses thereof, are described in more detail throughout the application, including the sections below. The modular discussion of such methodology and elements is not intended to limit the scope of the teachings provided herein, and one of ordinary skill in the art will readily appreciate combinations thereof in light of the description provided herein.

A. Assaying for and detecting β -catenin depots

[0060] In certain aspects of the methods described herein, the methods comprise assaying a composition comprising cells, or a derivative of the composition, to detect β -catenin depots (or an absence thereof, such as a sub-measurable quantity). As taught herein, assaying for a β -catenin depot comprises detecting a component (or an absence thereof), such as a macromolecule, *e.g.*, a polypeptide or a nucleic acid such as DNA or RNA, of the β -catenin depot. One of ordinary skill in the art will readily appreciate that there are numerous techniques to perform such assay techniques, and the description of exemplary techniques below is not intended to limit the scope of the teachings provided herein.

[0061] In some embodiments, the assaying for β -catenin depots comprises detecting an inactive form of a β -catenin polypeptide. In some embodiments, the detecting an inactive form of a β -catenin polypeptide comprises contacting a composition comprising a cell, or the derivative of the composition, with a detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide. In some embodiments, the detectable reagent is an antibody specifically recognizing an inactive form of a β -catenin polypeptide. In some embodiments, the assaying comprises detecting a detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide. In some embodiments, the detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide is a labeled (such as fluorescently or colorimetrically, *e.g.*, using an enzyme) antibody specifically recognizing the inactive form of the β -catenin polypeptide (*e.g.*, a labeled primary antibody). In some embodiments, the detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide is detected using a labeled (such as fluorescently or enzymatically) secondary antibody that specifically recognizes the detectable reagent. In some embodiments, the detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide specifically recognizes a phosphorylation at one or more of S33, S37, T41, or S45 of the inactive form of the β -catenin polypeptide, and wherein the amino acid position is relative to amino acid sequence of SEQ ID NO:1 (**Table 1**). In some embodiments, the detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide specifically recognizes a phosphorylation at T41 and/or S45. In some embodiments, the detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide specifically recognizes phosphorylations at T41 and S45. In some embodiments, the detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide is the 23H16L13 antibody clone, or a humanized version thereof.

[0062] In some embodiments, the assaying for β -catenin depots comprises (including further comprises) detecting an active form of a β -catenin polypeptide (or an absence thereof), such as a β -catenin polypeptide comprising one or more C-terminal phosphorylations of β -catenin polypeptide. In some embodiments, the assaying for β -catenin depots comprises (including further comprises) detecting an active form of a β -catenin polypeptide (or an absence thereof), such as a β -catenin polypeptide comprising phosphorylation at S552 and/ or S675, wherein the amino acid position is relative to amino acid sequence of SEQ ID NO:1. In some embodiments, the detectable reagent that specifically recognizes an active form of a β -catenin polypeptide is a labeled (such as fluorescently or enzymatically) antibody specifically recognizing the active form of the β -catenin polypeptide (*e.g.*, a labeled primary antibody). In some embodiments, the detectable reagent that specifically recognizes an active form of a β -catenin polypeptide is detected using a labeled (such as fluorescently or enzymatically) secondary antibody that specifically recognizes the detectable reagent.

[0063] In some embodiments, the assaying for β -catenin depots comprises (including further comprises) detecting phospho-H2A.X (P-2A.X) using a detectable reagent. In some embodiments, the detectable reagent that specifically recognizes P-2A.X is a labeled (such as fluorescently or enzymatically) antibody specifically recognizing P-2A.X (*e.g.*, a labeled primary antibody). In some embodiments, the detectable reagent that specifically recognizes P-2A.X is detected using a labeled (such as fluorescently or enzymatically) secondary antibody that specifically recognizes the detectable reagent. Any anti-P-2A.X antibodies or antigen-binding fragments thereof can be used herein, such as 20E3 antibody clone.

[0064] In some embodiments, the assaying for β -catenin depots comprises (including further comprises) detecting mediator of DNA damage checkpoint protein 1 (MDC1) using a detectable reagent. In some embodiments, the detectable reagent that specifically recognizes MDC1 is a labeled (such as fluorescently or enzymatically) antibody specifically recognizing MDC1 (*e.g.*, a labeled primary antibody). In some embodiments, the detectable reagent that specifically recognizes MDC1 is detected using a labeled (such as fluorescently or enzymatically) secondary antibody that specifically recognizes the detectable reagent. Any anti-MDC1 antibodies or antigen-binding fragments thereof can be used herein, such as P2B11 or MDC1-50 antibody clone.

[0065] In some embodiments, the assaying for β -catenin depots comprises (including further comprises) detecting using a detectable reagent of one or more macromolecules of: p53-binding

protein 1 (53BP1), RIF1, positive transcription elongation factor (p-TEFb), mRNA-decapping enzyme 2 (DCP2), and FOXO3a. In some embodiments, the detectable reagent that specifically recognizes such macromolecule is a labeled (such as fluorescently or enzymatically) antibody specifically recognizing such macromolecule (*e.g.*, a labeled primary antibody). In some embodiments, the detectable reagent that specifically recognizes such macromolecule is detected using a labeled (such as fluorescently or enzymatically) secondary antibody that specifically recognizes the detectable reagent.

[0066] In some embodiments, the assaying for β -catenin depots comprises (including further comprises) detecting a β -catenin polypeptide (or an absence thereof), such as any form of a β -catenin polypeptide, *e.g.*, non-phosphorylated β -catenin, β -catenin phosphorylated at N-terminus (*e.g.*, phosphorylation at one or more of S33, S37, T41, or S45), β -catenin phosphorylated at C-terminus (*e.g.*, phosphorylation at S552 and/or S675), or any combination thereof. In some embodiments, the assaying for β -catenin depots comprises detecting a colocalization (or an absence thereof) of a β -catenin polypeptide of any form and a macromolecule (*e.g.*, MDC1 and/or P-2A.X). In some embodiments, the assaying for β -catenin depots further comprises detecting a colocalization (or an absence thereof) of any of 53BP1, RIF1, p-TEFb, DCP2, or FOXO3a with a colocalized signal of a β -catenin polypeptide and MDC1 and/or P-2A.X. Antibodies or antigen-binding fragments thereof that can specifically bind to various forms of β -catenin are well known in the art, including but not limited to, 12F7 antibody clone.

[0067] In some embodiments, the detectable reagent comprises a label such as a fluorescent label, *e.g.*, GFP or mCherry. In some embodiments, the detectable reagent comprises a detectable feature inherent to the detectable reagent, *e.g.*, a fluorophore that is a native feature of the detectable reagent. In some embodiments, the detectable reagent comprises an antibody, aptamer, or a nucleic acid, which provides for indirect detection and/or direct measure of a macromolecule of a β -catenin depot. In some embodiments, the detectable reagent comprises a radioactive label, a colorimetric label, a luminescent label, a chemically-reactive label (such as a component moiety used in click chemistry), or a fluorescent label.

[0068] In some embodiments, the macromolecule, *e.g.*, a polypeptide or a nucleic acid such as DNA or RNA, of a β -catenin depot used for detecting the β -catenin depot is a fusion molecule comprising a label, such as a fluorescent label. In some embodiments, the fusion molecule comprises a Halo, Dendra2, GFP, RFP, or mCherry moiety. In some embodiments, the

macromolecule of a β -catenin depot used for detecting the β -catenin depot comprises a detectable feature inherent to the macromolecule.

[0069] In some embodiments, the detection reagent is not a fusion protein, *e.g.*, not a fusion macromolecule associated with a β -catenin depot. In some embodiments, the detection reagent directly binds to a β -catenin polypeptide, such as an inactive form of a β -catenin polypeptide.

[0070] One of ordinary skill in the art will readily understand methodology for performing the assaying and detecting described herein, *e.g.*, using immunofluorescent (IF) staining techniques. In some embodiments, the methods comprise detecting more than one macromolecule, wherein such methods comprise the use of more than one detectable reagent (*e.g.*, one detectable reagent for each macromolecule). In some embodiments, the detectable reagent is detectable via an imaging technique, *e.g.*, microscopy such as fluorescent microscopy. In some embodiments, the more than one detectable reagents are configured for simultaneous use (*e.g.*, a composition comprising a cell, or a derivative of the composition, is contacted with a first detectable reagent and a second detectable reagent, wherein the first detectable reagent and the second detectable reagent specifically bind to different macromolecule species). In some embodiments, the more than one detectable reagents are configured for serial use (*e.g.*, a composition comprising a cell, or a derivative of the composition, is contacted with a first detectable reagent that is stripped away before the composition, or the derivative of the composition, is contacted with a second detectable reagent, wherein the first detectable reagent and the second detectable reagent specifically bind to different macromolecule species). In some embodiments, when the more than one detectable reagents are configured for serial use, the composition comprising a cell, or a derivative of the composition, is contacted with a first detectable reagent, then with a second detectable reagent, without stripping away the first detectable reagent. In some embodiments, when more than one macromolecules of a β -catenin depot is assessed it is helpful to utilize antibodies from different species, *e.g.*, a mouse antibody specific to a first macromolecule and a rabbit antibody specific to a second macromolecule. Such approaches enable co-staining. In some embodiments, different labeling techniques or co-staining are used to enable co-localization measurements. In some embodiments, co-localization measurements are performed by overlaying images, wherein each image comprises staining of one or more aspect therein. In some embodiments, the detection method, *e.g.*, IF staining technique, involves washing away unbound detectable reagent prior to imaging.

[0071] In some embodiments, the detecting comprises imaging at least a portion of a cell, such as a specific cell location, *e.g.*, the nucleus or a portion thereof, or a field of view comprising at least a portion of a cell. In some embodiments, the imaging comprises a fluorescent imaging technique. In some embodiments, the imaging technique comprises any one or more of immunofluorescence (IF), *in situ* hybridization (ISH, such as FISH), gene fusion (*e.g.*, GFP labeling), or a dye that is specific a macromolecule of a β -catenin depot. In some embodiments, the imaging technique comprises use of an agent to visualize a cellular feature, such as a cell membrane or an organelle, such as nucleus (*e.g.*, by DAPI staining). In some embodiments, the methods provided herein further comprise measuring (such as quantifying) a signal of the detectable reagent.

[0072] In some embodiments, the imaging technique comprises use of a microscopy technique (and associated microscopy instrumentation). In some embodiments, the microscopy technique comprises a confocal microscopy technique. In some embodiments, the microscopy technique comprises a fluorescence microscopy technique. In some embodiments, the microscopy technique comprises a high-resolution microscopy technique. In some embodiments, the microscopy technique comprises a stimulated emission depletion (STED) microscopy technique. In some embodiments, the microscopy technique comprises a SoRa super-resolution spinning-disk microscopy technique. In some embodiments, the microscopy technique comprises an electron microscopy technique (such as cryo-EM or cryo-ET). In some embodiments, the microscopy technique comprises a total internal reflection fluorescence (TIRF) microscopy technique.

[0073] In some embodiments, the method comprises assessing the distribution of a macromolecule of a β -catenin depot. In some embodiments, the distribution of a macromolecule is based on a pixel analysis. In some embodiments, the pixel analysis is performed on a region of a cell, such as the nucleus and/ or cytosol, or a region thereof. In some embodiments, the pixel analysis is performed without identification of individual condensates or features thereof (such as the boundary of a condensate). For example, a pixel having a signal above a threshold (such as above a background level) is indicative of a condensate, such as a β -catenin depot. In some embodiments, a condensate is identified using another agent, such as a dye, that specifically partitions in the condensate. The pixels used for pixel analyses described herein can be any shape and size with respect to constraints imparted by the analyzed region. For example, in some embodiments, the region analyzed contains at least 2, such as at least any of 10, 100, 1,000,

5,000, 10,000, 20,000, 30,000, 40,000, or 50,000, pixels, such as within the boundary of the nucleus or cytosol of an image of a cell. In some embodiments, the pixels are uniformly sized. In some embodiments, the pixels are uniformly shaped.

[0074] In some embodiments, the methods provided herein further comprise contacting a composition comprising a cell, or the derivative of the composition, with a fixative agent (e.g., formaldehyde) before an assaying or detecting step is performed. In some embodiments, the methods provided herein further comprise determining one or more cellular features of a cell, such as by staining the cell with a dye.

[0075] In some embodiments, the methods provided herein further comprise subjecting a composition comprising a cell to a condition that promotes formation of β -catenin depots prior to the contacting the composition comprising the cell with a compound, such as used in a compound screening assay. In some embodiments, formation of β -catenin depots comprises expressing (such as expressing in greater quantities than endogenously observed for a cell or a population thereof) β -catenin polypeptide in a cell. In some embodiments, the condition that promotes formation of β -catenin depots comprises activating or up-regulating Wnt signaling in the cell, such as inducing nuclear translocation of β -catenin.

[0076] One of ordinary skill in the art will readily understand the scope of detectable reagents encompassed herein. For example, techniques are well known in the art for assessing that a reagent is a detectable reagent useful for the methods described herein, such as a detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide. In some embodiments, a reagent can be confirmed to be a detectable reagent useful for the methods described herein using ELISA or use of a cell model for which the β -catenin depot phenotype is well characterized.

B. Comparisons and identification of β -catenin depot modulators

[0077] In some embodiments, the methods provided herein comprise comparisons between an assayed composition comprising a cell, or a derivative of the composition, for a β -catenin depots and a reference. For example, in certain aspects, provided herein are methods of identifying a compound that modulates β -catenin depots in a cell, the method comprising: identifying the compound that modulates β -catenin depots in a cell by comparison of assayed β -catenin depots in the cell to a reference. In some embodiments, the detection of β -catenin depots

(*e.g.*, presence) in a cell treated with a compound identifies the compound as modulating β -catenin depots in the cell.

[0078] In some embodiments, comparison of assayed β -catenin depots in a cell to a reference is based on one or more of the following attributes of the β -catenin depots: (i) presence (including the absence thereof) of the β -catenin depots; (ii) number of the β -catenin depots (including number counts restricted by cell location, *e.g.*, β -catenin depots in the nucleus); (iii) size of the β -catenin depots; (iv) surface area of the β -catenin depots; (v) cellular location of the β -catenin depots; (vi) distribution of the β -catenin depots; (vii) ratio of the amount of the β -catenin depots and a reference condensate; or (viii) composition of the β -catenin depots. In some embodiments, the size of β -catenin depots is assessed by one or more of a largest crossing dimension (*e.g.*, diameter), perimeter (*e.g.*, circumference), a cross-sectional area (*e.g.*, the largest cross-section area), or volume.

[0079] In some embodiments, the comparison of assayed β -catenin depots in a cell to a reference is based on (including further based on) one or more of the following attributes: (viii) a functional activity associated with the one or more β -catenin depots; (ix) composition of the one or more β -catenin depots; (x) co-localization of the one or more β -catenin depots with a macromolecule; (xi) diffusion coefficient of a component of the one or more β -catenin depots; (xii) stability of the one or more β -catenin depots; (xiii) sphericity of the one or more β -catenin depots; (xiv) liquidity of the one or more β -catenin depots; or (xv) solidification of the one or more β -catenin depots.

[0080] Generally speaking, the methods described herein enable the identification of one or more compounds that modulate a β -catenin depot in a desired manner. In some embodiments, the modulation of β -catenin depots is based on increasing the β -catenin depots in a cell as compared to a reference. In some embodiments, modulation of β -catenin depots is based on an increase in the number of the β -catenin depots in a cell as compared to a reference. In some embodiments, modulation of β -catenin depots in a cell is based on an increase in the number of the β -catenin depots in the nucleus of the cell as compared to a reference. In some embodiments, modulation of β -catenin depots in a cell is based on an increase in the presence of the β -catenin depots in the cell as compared to a reference. In some embodiments, modulation of β -catenin depots in a cell is based on an increase in the presence of the β -catenin depots in the nucleus of the cell as compared to a reference.

[0081] In certain aspects of the methods provided herein, a characteristic of β -catenin depots in a cell can be assessed (including further assessed) via a macromolecule, such as a macromolecule associated with the β -catenin depots. The assaying and detection methods described above can also be used to assess such macromolecules. Additionally, other techniques are available to assess such macromolecules, including techniques for protein measurement such as Western blot, ELISA, and mass spectrometry. In some embodiments, comparison of assayed β -catenin depots in a cell to a reference is based (including further based) on one or more of the following attributes of an inactive form of the β -catenin polypeptide associated with the β -catenin depots: (i) location of the inactive form β -catenin polypeptide; (ii) amount of the inactive form β -catenin polypeptide; (iii) distribution of the inactive form β -catenin polypeptide; (iv) condensate partitioning of the inactive form β -catenin polypeptide into the β -catenin depots and/or one or more other condensates; (v) a functional activity associated with the inactive form β -catenin polypeptide; (vi) aggregation of the inactive form β -catenin polypeptide; (vii) post-translational modification status of the inactive form β -catenin polypeptide; or (viii) amount of a degradation product of the inactive form β -catenin polypeptide. In some embodiments, modulation in β -catenin depots in a cell, as compared to a reference, is based on one or more of: (i) an increase in the amount of the β -catenin polypeptide with a phosphorylation at one or more of S33, S37, T41, and S45 in the cell; (ii) the presence of the β -catenin polypeptide with a phosphorylation at one or more of S33, S37, T41, and S45 in the cell; (iii) an increase in the amount of the β -catenin polypeptide with a phosphorylation at one or more of S33, S37, T41, and S45 in one or more condensates (e.g., β -catenin depots, or condensates associated with prolonged double-strand break (DSB) DNA damage); (iv) the presence of the β -catenin polypeptide with a phosphorylation at one or more of S33, S37, T41, and S45 in one or more condensates (e.g., β -catenin depots, or condensates associated with prolonged double-strand break (DSB) DNA damage); (v) a decrease in the amount of the β -catenin polypeptide with a phosphorylation at S552 and/or S675 in the cell (e.g., in nucleus); (vi) the absence of the β -catenin polypeptide with a phosphorylation at S552 and/or S675 in the cell (e.g., in nucleus); (vii) a decrease in the amount of the β -catenin polypeptide with a phosphorylation at S552 and/or S675 in one or more condensates; (viii) the absence of the β -catenin polypeptide with a phosphorylation at S552 and/or S675 in one or more condensates; (ix) an increase in the amount of the β -catenin polypeptide with a phosphorylation at S552 and/or S675 in one or more β -catenin depots; and (x) the presence of the β -catenin polypeptide with a phosphorylation at S552

and/or S675 in one or more β -catenin depots. In some embodiments, modulation in β -catenin depots in a cell, as compared to a reference, is based on an increase in the amount of the inactive form of the β -catenin polypeptide in the β -catenin depots as compared to a reference. In some embodiments, the inactive form of a β -catenin polypeptide is a wildtype β -catenin polypeptide. In some embodiments, the inactive form of a β -catenin polypeptide is a mutant β -catenin polypeptide.

[0082] In some embodiments, comparison of assayed β -catenin depots in a cell to a reference is based (including further based) on: i) one or more of attributes of a β -catenin polypeptide (such as any form of a β -catenin polypeptide, e.g., β -catenin polypeptide associated with the β -catenin depots) and ii) one or more of attributes of a macromolecule (e.g., MDC1 and/or P-2A.X). Such one or more attributes include: (i) location of the β -catenin polypeptide and/or macromolecule; (ii) amount of the β -catenin polypeptide and/or macromolecule; (iii) distribution of the β -catenin polypeptide and/or macromolecule; (iv) condensate partitioning of the β -catenin polypeptide and/or macromolecule into the β -catenin depots and/or one or more other condensates; (v) a functional activity associated with the β -catenin polypeptide and/or macromolecule; (vi) aggregation of the β -catenin polypeptide and/or macromolecule; (vii) post-translational modification status of the β -catenin polypeptide and/or macromolecule; or (viii) amount of a degradation product of the β -catenin polypeptide and/or macromolecule.

[0083] In some embodiments, modulation in β -catenin depots in a cell, as compared to a reference, is based on an increase of active β -catenin sequestered into β -catenin depots. In some embodiments, modulation in β -catenin depots in a cell, as compared to a reference, is based on a decrease of active β -catenin in transcriptionally active condensates, such as a condensate comprising one or more mediator or active transcription factor (e.g., active Pol II). In some embodiments, the active form of a β -catenin polypeptide is a wildtype β -catenin polypeptide. In some embodiments, the active form of a β -catenin polypeptide is a mutant β -catenin polypeptide.

[0084] In some embodiments, modulation in β -catenin depots in a cell, as compared to a reference, is based on: (i) an increase in the amount of the β -catenin polypeptide with a phosphorylation at one or more of S33, S37, T41, and S45 in one or more condensates; (ii) the presence of the β -catenin polypeptide with a phosphorylation at one or more of S33, S37, T41, and S45 in one or more condensates. In some embodiments, the one or more condensates are β -catenin depots, such as β -catenin depots identified using any of the methods described herein. In

some embodiments, the one or more condensates are condensates not associated with any β -catenin before contacting the composition comprising the cell with the compound. In some embodiments, the one or more condensates are condensates not associated with an inactive form of β -catenin (such as with a phosphorylation at one or more of S33, S37, T41, and S45) before contacting the composition comprising the cell with the compound. In some embodiments, the one or more condensates are condensates associated with an active form of β -catenin (e.g., β -catenin polypeptide with a phosphorylation at S552 and/or S675) or a non-phosphorylated form of β -catenin, before contacting the composition comprising the cell with the compound. In some embodiments, the one or more condensates are associated with prolonged DSB DNA damage. In some embodiments, the one or more condensates associated with prolonged DSB DNA damage comprises P-2A.X and/ or MDC1. In some embodiments, the one or more condensates associated with prolonged DSB DNA damage further comprise one or more of: 53BP1, RIF1, p-TEFb, DCP2, or FOXO3a. Hence in some embodiments, any of the methods described herein, such as assaying β -catenin depots, assaying colocalization of β -catenin and a macromolecule, assaying colocalization of β -catenin and a condensate, and/or identifying β -catenin depots, can comprise (including further comprise) detecting with a detectable reagent that specifically recognizes the one or more condensates (e.g., those associated with prolonged DSB DNA damage) or components thereof described herein. Methods of detecting condensates and components thereof have been described in, e.g., US 2020/0150107, WO 2022/035989, and WO 2022/187225, which are hereby incorporated herein by reference.

[0085] In some embodiments, the reference is based on: (i) a composition comprising the cell not contacted with the compound; (ii) a composition comprising the cell admixed with a control vehicle and not contacted with the compound; (iii) a desired β -catenin profile (including a theoretical profile, e.g., no nuclear β -catenin depots, or a nuclear β -catenin depot profile induced by a desired control compound); (iv) a reference condensate or a reference polypeptide within the cell (e.g., a condensate not comprising β -catenin and/ or not modulated by the compound); and/ or (v) a reference composition comprising a cell (e.g., a drug resistant cell line, e.g., a cancer cell line, or healthy cells not having a disease such as cancer). In some embodiments, the compounds identified using any of the methods described herein modulate β -catenin depots in a diseased cell (e.g., a cell with aberrant Wnt/ β -catenin signaling, such as cancer), but do not modulate β -catenin depots in a healthy cell.

[0086] In some embodiments, comparisons described herein comprise comparing the signal of one or more detectable reagents used to measure a β -catenin depot in a cell subjected to a compound with a reference. In some embodiments, a characteristic is calculated for each cell and averaged for the number of assayed cells, *e.g.*, a mean number of nuclear β -catenin depots per cell. In some embodiments, the methods comprise determining a Z-factor (Z') to score modulation of β -catenin depots in a composition comprising a cell. The use of Z-factors (Z') in high-throughput screening is well known in the art, *e.g.*, Zhang & Oldenburg, *Z-Factor*; *Encyclopedia of Cancer*, pp 3227-3228, 2008.

C. Compositions comprising a cell and assay formats

[0087] In certain aspects, the methods provided herein involve one or more composition comprising a cell (also referred to herein as a cellular composition), assay formats, and elements thereof.

1. Cellular compositions

[0088] In certain aspects, the methods described herein involve one or more cellular compositions. The cellular compositions may comprise any cell type or any mixture of cell types. In some embodiments, the composition comprises a cell. In some embodiments, the cell is a model for aberrant Wnt/ β -catenin signaling. In some embodiments, the cell is a model for a proliferative pathology. In some embodiments, the cell is a model for a cancer. In some embodiments, the cell is a cancer cell. In some embodiments, the cancer cell is a human osteosarcoma U2OS cell or human colon adenocarcinoma DLD-1 cell. In some embodiments, the cell comprises an APC truncation. In some embodiments, the cell is a model for autosomal dominant polycystic kidney disease (ADPKD), hereditary familial adenomatous polyposis (FAP), Alzheimer's, or a metabolic disease, such as obesity and/ or insulin resistance.

[0089] In some embodiments, the composition comprises at least 1 cell (*e.g.*, the methods described herein are single-cell assay). In some embodiments, the composition comprises at least about 100 cells, such as at least about any of 1,000 cells, 5,000 cells, 1×10^4 cells, 5×10^4 , 1×10^5 , 5×10^5 , or 1×10^6 cells of a cell type.

[0090] In some embodiments, the composition comprises a single cell type. In some embodiments, the composition comprises at least about 1,000 cells of a single cell type. In some embodiments, the composition comprises a plurality of cell types, such as any of 2, 3, 4, or 5 cell

types. In some embodiments, the composition is aliquoted for use in the methods described herein, such as aliquots containing at least about 100 cells, such as at least about any of 200 cells, 300 cells, 400 cells, 500 cells, 600 cells, 700 cells, 800 cells, 900 cells, 1,000 cells, 1250 cells, 1,500 cells, 1,750 cells, or 2,000 cells.

[0091] In some embodiments, the composition comprises a cell type from an animal, such as a human, rat, or mouse. In some embodiments, the cell type from the animal has one or more features of a disease, such as a neurodegenerative, proliferative, immunological, cardiac, infection, or metabolic disease. In some embodiments, the composition is useful for studying a disease or an aspect or factor thereof. In some embodiments, the composition comprises a HeLa cell, a HEK293 cell, a DLD-1 cell, a U2OS cell, a H9C2 cell, an induced pluripotent stem cell (iPSC cell), a cardiomyocyte, a myocyte, a stem cell-derived cell, a neuron, a cancer cell, an immune cell, or an adipocyte. In some embodiments, the cell type is derived from a biopsy or tissue sample, such as from a patient sample, *e.g.*, from a healthy or disease biopsy or tissue sample. In some embodiments, the composition is a tissue sample, such as a tissue sample, cell smear, or secretion. When describing a cell type, it is understood to include cells derived from that type of cell unless explicitly stated otherwise. For example, a HeLa cell comprising a heterologous transgene would be considered a HeLa cell unless explicitly stated otherwise. In some embodiments, the composition comprises a cell type that is modified, such as stably or transiently transfected.

[0092] In some embodiments, the composition is a cultured cellular composition, such as a composition contained in a sample well or culture dish. In some embodiments, the method involves a plurality of aliquot of a composition, such as a composition aliquoted in two or more wells of a multi-well plate, *e.g.*, a 1536-well plate.

[0093] In other aspects, provided herein are methods comprising identifying, obtaining, and/or making a composition comprising a cell. In some embodiments, the method comprises producing a composition having a cell type. In some embodiments, the cell type of a composition is treated and/or engineered to adjust (such as increase or decrease) the expression of one or more polypeptides. Techniques for making compositions comprising a cell are well known in the art. In some embodiments, the cell type is produced from a precursor cell of a cell type via modulating an aspect of the precursor. For example, the cell type can be generated by subjecting a precursor cell to stress, such as oxidative stress, or treating a precursor cell with a small molecule compound or hormone. In some embodiments, the cell type is obtained by

subjecting a precursor cell to infection, such as an infection by virus, bacteria, fungus, or parasite. In some embodiments, the cell type is produced via knock-down or knock-out of a genetic feature or expression product thereof, such as by any methods known in the art, *e.g.*, siRNA, RNAi, TALEN, ZFN, or CRISPR/Cas. In some embodiments, the cell type is produced via knock-in. In some embodiments, the cell type is produced via transfection. In some embodiments, the cell type is transfected with a fusion polypeptide, such as a polypeptide fused to a label, *e.g.*, GFP. In some embodiments, the cell type is transfected with a wild type polypeptide. In some embodiments, the cell type is transfected with a variant polypeptide, such as a mutant polypeptide. In some embodiments, the cell type is transfected to express a level of a gene expression product. In some embodiments, the expression level variant cell type is produced and used in the methods described herein when a gene expression product reaches a pre-determined level.

[0094] In some embodiments, the cell type is transfected to express a polypeptide, such as a wild type polypeptide or a mutant polypeptide (*e.g.*, β -catenin), at a near endogenous level. In some embodiments, the cell type does not express a polypeptide, such as a wild type polypeptide, and the near endogenous level is based on a level of expression of the polypeptide in another cell type. In some embodiments, the cell type is transfected to express a polypeptide with a label, such as a labeled wild type polypeptide, at a near endogenous level, wherein the near endogenous level is based on the level of expression of a respective unlabeled version of the polypeptide. In some embodiments, the cell type has reduced expression of an unlabeled polypeptide, *e.g.*, the cell type comprises a knockout of the unlabeled polypeptide. In some embodiments, the cell type is transfected to express a variant polypeptide, such as a mutant polypeptide (*e.g.*, point mutation, truncation mutation, frameshift mutation, or termination mutation), at a level that is substantially similar to the endogenous expression level of a respective wild type polypeptide of the variant polypeptide. As described herein, in some embodiments, the terms “near endogenous level” or “substantially similar” refer to polypeptide expression levels that are within a 2-fold difference of a measured endogenous level of a polypeptide as measured in a population of cells, such as 1×10^6 cells.

2. Treatments of compositions comprising a cell

[0095] In certain aspects, the methods provided herein comprise subjecting a composition comprising a cell to a stimulus, such as contacting a composition comprising a cell with a

compound. The methods provided herein enable the evaluation of any stimulus. For ease of describing the subject matter provided herein, a composition is generally described as being subjected to a singular stimulus (although this is not a limitation to the description provided herein), and the singular stimulus may encompass numerous factors. For example, a stimulus may be described as a compound, and may also implicitly include any light, heat, O₂ concentration and/ or CO₂ concentration factors to which the cellular composition is subjected. In some embodiments, a stimulus is described as a concentration of a compound, wherein two or more cellular composition are subjected to the compound at different concentrations (or, *e.g.*, one concentration of the compound and one vehicle control). One of ordinary skill in the art will readily understand how to evaluate one or more factors of a stimulus by setting up adequate experimental procedures and references.

[0096] In some embodiments, the stimulus comprises an exogenous compound. In some embodiments, the stimulus comprises an exogenous peptidic material, such as a hormone. In some embodiments, the stimulus comprises an exogenous genetic material. In some embodiments, the stimulus comprises a stressor. In some embodiments, the stimulus comprises an environmental stimulus. In some embodiments, the stimulus comprises any combination of an exogenous compound, an exogenous peptidic material, an exogenous genetic material, a stressor, or an environmental stimulus.

[0097] In some embodiments, the stimulus, such as an exogenous compound, is a small molecule therapeutic candidate or a precursor thereof (*e.g.*, a prodrug). In some embodiments, the stimulus is a known concentration of a small molecule therapeutic candidate. In some embodiments, the stimulus is a mixture of small molecule therapeutic candidates (applied to the cellular composition via a single or multiple compositions). In some embodiments, the stimulus is a small molecule therapeutic candidate and another agent (applied to the cellular composition via a single or multiple compositions). In some embodiments, the stimulus is a polypeptide, a peptidomimetic, a lipid, a nucleic acid, or any combination thereof.

[0098] Any number of stimuli may be assessed using the methods described herein. For example, in the context of drug screening, any number of therapeutic candidates may be assessed, *e.g.*, at least about any of 1, 2, 5, 10, 50, 100, 1,000, 10,000, 25,000, 50,000, 100,000, 250,000, 500,000, 1,000,000, 2,500,000, or 5,000,000.

[0099] As used herein, small molecule therapeutic candidate encompasses any small molecule being evaluated for purposes of drug discovery, such as would be involved in efforts to

identify a therapeutic for a human disease. In some embodiments, the small molecule therapeutic is a member of a small molecule library, such as a chemical compound library. In some embodiments, there may exist some knowledge (obtained before or after performing the methods described herein) that a particular small molecule is not suitable for human therapeutic use – in such instances, said small molecule is still considered a small molecule therapeutic candidate for purposes of this description.

[0100] In some embodiments, the small molecule therapeutic candidate has a molecular weight of about 5,000 Da or less, such as about any of 4,500 Da or less, 4,000 Da or less, 3,500 Da or less, 3,000 Da or less, 2,500 Da or less, 2,000 Da or less, 1,500 Da or less, 1,000 Da or less, or 500 Da or less. In some embodiments, the small molecule therapeutic candidate satisfies one or more of Lipinski's rule of five – no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, a molecular weight of less than 500 Da, and an octanol-water partition coefficient (log P) that does not exceed 5. In some embodiments, the small molecule therapeutic candidate has a desired partitioning characteristic for a condensate type (*e.g.*, a β -catenin depot). For example, in some embodiments, the small molecule therapeutic candidate partitions into a condensate type (*e.g.*, the concentration of the small molecule therapeutic candidate is higher in the condensate type than outside the condensate type), such as when it is desired to deliver the small molecule therapeutic candidate to the condensate type. In some embodiments, the small molecule therapeutic candidate does not substantially partition into a condensate type (*e.g.*, the concentration of the small molecule therapeutic candidate is lower in the condensate type than outside the condensate type), such as when it is desired to avoid having the condensate type serve as a sink for the small molecule therapeutic candidate.

[0101] In some embodiments, the small molecule therapeutic candidate comprises a nucleic acid. In some embodiments, the small molecule therapeutic candidate comprises RNA, such as a siRNA, miRNA, mRNA, or lncRNA, or an analog thereof. In some embodiments, the small molecule therapeutic candidate comprises DNA, or an analog thereof. In some embodiments, the small molecule therapeutic candidate is a non-naturally occurring compound. In some embodiments, the small molecule therapeutic candidate is an exogenous compound. In some embodiments, the small molecule therapeutic candidate comprises a polypeptide. In some embodiments, the small molecule therapeutic candidate is a therapeutic compound approved by a regulatory agency, such as an agent approved for medical treatment by the United States Food

and Drug Administration (FDA). In some embodiments, the small molecule therapeutic candidate is a new chemical entity.

[0102] In some embodiments, the small molecule therapeutic candidate, or a portion thereof, is charged. In some embodiments, the small molecule therapeutic candidate, or a portion thereof, is hydrophobic. In some embodiments, the small molecule therapeutic candidate, or a portion thereof, is hydrophilic. In some embodiments, the small molecule therapeutic candidate, or a portion thereof, comprises an alkaloid, a glycoside, a phenazine, a phenol, a polyketide, a terpene, or a tetrapyrrole.

[0103] In some embodiments, the small molecule therapeutic candidate comprises a label. In some embodiments, the label is a radioactive label, a colorimetric label, a luminescent label, a chemically-reactive label (such as a component moiety used in click chemistry), or a fluorescent label. In some embodiments, the small molecule therapeutic candidate contains a measurable signal, *e.g.*, has a fluorescent characteristic without further modification. In some embodiments, the small molecule therapeutic candidate comprises a fluorophore. In some embodiments, the small molecule therapeutic candidate is a polypeptide comprising a label. In some embodiments, the small molecule therapeutic candidate is a polypeptide comprising a fluorophore. In some embodiments, the small molecule therapeutic candidate is a nucleic acid comprising a label. In some embodiments, the small molecule therapeutic candidate is a nucleic acid comprising a fluorophore. The label can be associated with the small molecule therapeutic candidate covalently or non-covalently.

[0104] In some embodiments, the stimulus is a reference control, such as describe herein.

[0105] The methods provided herein are amenable to any treatment regimen involving subjecting the cellular composition to a stimulus. In some embodiments, subjecting the cellular composition to a stimulus comprises subjecting the cellular composition to the stimulus in a single instance, such as a heat treatment or application of a chemical composition, such as a small molecule therapeutic candidate. Such single treatments may comprise any length of duration or incubation, *e.g.*, application of a chemical composition for a set time before the cellular composition media is removed and replaced with a media not comprising the chemical composition. In some embodiments, subjecting the cellular composition to a stimulus comprises subjecting the cellular composition to the stimulus for a plurality of instances, such as to reflect chronic exposure or a chronic disease.

3. Assay formats

[0106] In certain aspects, the methods provided herein comprise independently subjecting any number of compositions comprising a cell to a stimulus, wherein the methods enable the evaluation of a plurality of stimuli across one or more cell types. In some embodiments, the method comprises performing replicates of subjecting a cellular composition comprising a cell type to a stimulus. The methods described herein can be performed in a diverse array of formats, including formats amenable to high throughput and commercial scale drug discovery.

[0107] In some embodiments, the method provided herein comprises aliquoting a composition comprising a cell into a plurality of sample wells, and subjecting each defined subset of the aliquoted cellular composition to a stimulus. In some embodiments, the cellular composition is aliquoted into wells of a multi-well plate, such as a multi-well plate having any of 6, 12, 24, 48, 96, 384, or 1,536 wells. In some embodiments, the multi-well plate is amenable to other steps of the methods described herein, *e.g.*, suitable for the stimulus and any processing and analytical steps performed on the cell type of the cellular composition.

[0108] In some embodiments, the method provided herein comprises evaluating a plurality of cellular compositions, wherein each cellular composition comprises a different cell type or derivative thereof. In some embodiments, each cellular composition can be independently subjected to a plurality of stimuli such as via subjecting different aliquots of the cellular composition.

[0109] In some embodiments, the number of individual cellular compositions (including aliquots of a cellular composition) used in a method described herein may be determined based on one or more aspects of the desired analysis, including, but not limited to, the number of stimuli to be evaluated, the number of cell types to be evaluated, the number of replicates to be evaluated, and a level of statistical power.

4. Post-stimulus processing

[0110] In some embodiments, the methods provided herein comprise a processing technique performed after subjecting a composition comprising a cell to a stimulus. Such processing techniques are useful for preserving biological context following treatment with a stimulus and prior to certain downstream analyzes (such as staining and/ or imaging) and/ or preparing the cellular composition or components thereof for certain downstream analyzes.

[0111] For example, in some embodiments, the following subjecting a composition comprising a cell to a stimulus, the cellular composition, or a component thereof such as the cell type, is fixed and/ or permeabilized. In some embodiments, the fixing is performed to maintain a cell in a set place, such as accomplished using a crosslinking reagent, *e.g.*, formaldehyde or an analog thereof. In some embodiments, the permeabilizing is performed to enable a marker, such as an antibody, to penetrate a cell, such as accomplished using solvents (*e.g.*, acetone) and/ or detergents (*e.g.*, Triton, NP-40, Tween 20, Saponin, Digitonin, and/or Leucoperm). In some embodiments, the following subjecting a cellular composition comprising a cell type to a stimulus, the cellular composition, or a component thereof such as the cell type, is frozen.

D. Additional method steps

[0112] In certain aspects, the methods described herein further comprise one or more orthogonal secondary assays performed to assess a compound identified to modulate β -catenin depots, such as a second transcriptional assay to examine transcription activity of downstream target(s) of β -catenin. In some embodiments, the methods further comprise assessing the identified compound using a second cell-based assay and/ or an *in vitro* assay. In some embodiments, the methods further comprise assessing the identified compound using a second cell-based assay, including by not limited to a cytotoxicity assay.

III. Further aspects enable by the methods described herein

[0113] In other aspects, provided herein are further methods and embodiments enabled by the disclosure herein.

[0114] In certain aspects, provided herein is a method of identifying a compound useful for treating a β -catenin-associated disease, wherein the method comprises identifying a compound according to a method described herein. In some embodiments, the β -catenin-associated disease is a cancer.

[0115] In certain aspects, provided herein is a compound identified by a method described herein.

[0116] In certain aspects, provided herein are kits and composition useful for the methods described herein. In some embodiments, provided herein is a kit comprising one or more detection reagents, such as an antibody, useful for measuring, such as visualizing, a β -catenin

depot. In some embodiments, the kit comprises one or more cellular composition useful for the methods described herein.

[0117] Those skilled in the art will recognize that several embodiments are possible within the scope and spirit of the disclosure of this application. The disclosure is illustrated further by the examples below, which are not to be construed as limiting the disclosure in scope or spirit to the specific procedures described therein.

VI. Sequences

[0118] Sequences referenced herein are provided in **Table 1**.

Table 1. Sequences.

SEQ ID NO:	Sequence
1	Human β-catenin polypeptide sequence (catenin beta-1 isoform 1 [Homo sapiens]) BI Reference Sequence: NP_001895.1 MATQADLMELDMAMEPDRKAAVSHWQQQSYLDSGIHSGATTTAPSL SGKGNPEEEDVDTSQVLYEWEQGFSQSFTQEQVADIDGQYAMTRAQR VRAAMFPETLDEGMQIPSTQFDAAHPTNVQRLAEPSQMLKHAVVNLI NYQDDAELATRAIPELTKLLNDEDQVVVNKAAVMVHQLSKKEASRH AIMRSPQMVSIVRTMQNTNDVETARCTAGTLHNLSHHREGLLAIFKS GGIPALVKMLGSPVDSVLFYAITTLHNLLLHQEGAKMAVRLAGGLQK MVALLNKTNVKFLAITTDCLQILAYGNQESKLILASGGPQALVNIMRT YTYEKLLWTTSRVLKVLSSCNKPAIVEAGGMQALGLHLLTDPSSRL VQNCLWTLRNLSDAATKQEGMEGLLGTLVQLLGSDDINVVTCAGIL SNLTCNNYKNKMMVCQVGGIEALVRTVLRAGDREDITEPAICALRHL TSRHQEAEMAQNAVRLHYGLPVVVKLLHPPSHWPLIKATVGLIRNLA LCPANHAPLREQGAIPRLVQLLVRAHQDTQRRTSMGGTQQQFVEGVR MEEIVEGCTGALHILARDVHNIRIVIRGLNTIPLFVQLLYSPIENIQRVAA GVLCELAQDKEAAEAIEAEGATAPL TELLHSRNEG VATYAAAVLFRM SEDKPQDYKKRLSVELTSSLFRTEPMAWNETADLGLDIGAQGEPLGY RQDDPSYRSFHSGGYGQDALGMDPMMEHEMGGHHPGADYPVDGLP DLGHAQDLMDGLPPGDSNQLAWFDTDL

EXAMPLES

Example 1

[0119] This example demonstrates the transient transfection of fluorophore- β -catenin constructs.

[0120] Initial attempts to establish a screening assay for chemical compounds capable of silencing β -catenin transcriptional activity involved transient transfection of cells with fluorophore- β -catenin constructs to visualize β -catenin incorporation into nuclear depots. Previously, Bernkopf *et al.* (*Oncotarget*, 9, 2018) demonstrated that green fluorescent protein tagged β -catenin which had been transiently transfected into cells could be incorporated into nuclear puncta upon treatment with sulforaphane. However, as noted below, transient transfection as a technique was not sufficiently robust for a compound screen or detection of β -catenin depots.

[0121] Transient transfection and expression of fluorescent protein- β -catenin constructs in HCT116 cells resulted in few cells exhibiting visible β -catenin nuclear puncta. Furthermore, those cells which were found to sufficiently express such β -catenin constructs for imaging resulted in generally high baseline noise and an exceedingly small window for detection of potential changes puncta morphology.

[0122] Transient transfection and expression of fluorescent protein- β -catenin constructs in HEK293T cells resulted in dramatically different cellular phenotypes dependent on the type of fluorescent tag used. The use of these constructs in a compound screen was further complicated by inefficient levels of transfection and the fact that overexpression of such constructs resulted in variable depot formation dependent on cell cycle stage. Finally, cell apoptosis was also observed over durations of time necessary for performing screens of large libraries of compounds.

Example 2

[0123] This example demonstrates the detection of β -catenin constructs in nuclear depots via immunofluorescence.

[0124] Given the myriad of challenges described in the previous example associated with transient transfection of labeled β -catenin constructs in the context of a compound screen, an attempt was made to utilize immunofluorescence to detect β -catenin depots in the nucleus of a cell and explore use of such detection in a compound drug screen.

[0125] U2OS cells were reverse transfected with plasmids encoding HaloTag- β -catenin and then seeded into plates at 2500 cells per well. Cells were treated with either sulforaphane or daunorubicin for either 6 or 24 hours, after which point HaloTag labelling was achieved by exposure to Janelia Fluor® 646. Cells were fixed using 3% (v/v) formaldehyde, washed with phosphate buffered saline (PBS), permeabilized with 0.2% (v/v) triton, washed again with PBS, and then blocked with 0.4% (v/v) FSG in PBS.

[0126] After blocking, individual wells of cells were exposed to one of several different staining solutions individually comprising different primary antibodies which target different forms of β -catenin. Cells were then washed with PBS, and then exposed to another antibody staining solution comprising Alexa Fluor® 488 labeled secondary antibodies, a blocking solution, and 4',6-diamidino-2-phenylindole (DAPI) for DNA staining. Cells were washed a final time with PBS.

[0127] This experiment revealed a surprising result where primary antibodies which targeted a specific phosphorylated epitope and biologically inactive form of β -catenin provided a sufficiently high signal-to-noise ratio to measure and count β -catenin nuclear depots accurately (FIG. 1). Cells exposed to primary antibody directed to inactive forms of β -catenin revealed distinct nuclear puncta which co-localized with HaloTag- β -catenin. The same was not true for primary antibodies which targeted active forms of β -catenin. Moreover, β -catenin expressed from fluorescent protein- β -catenin constructs would suffer from the same limitations as active forms of β -catenin would also be labeled and contribute to detectable signal.

Example 3

[0128] This Example demonstrates label free detection of β -catenin in nuclear depots and pilot screen of β -catenin depots.

[0129] With the surprising discovery described in the preceding example, the next steps were to: i) compare the extent of β -catenin nuclear depot formation in wildtype U2OS cells (wt U2OS) and U2OS (Halo U2OS) cells transfected with Halo- β -catenin, and ii) perform a pilot screen using a selected set of chemical compounds. In the prior experiment, daunorubicin was shown to induce β -catenin nuclear depot formation in a similar manner as sulforaphane. Therefore, daunorubicin was selected as a positive control treatment for this assay and dimethylsulfoxide (DMSO) as a negative control treatment.

[0130] wt U2OS cells or Halo U2OS cells were seeded into plates at 2500 cells per well. Cells were then treated with daunorubicin at various concentrations. After daunorubicin treatment, Halo U2OS cells were incubated with Janelia Fluor® 646. All cells were then fixed using 3% (v/v) formaldehyde, washed with PBS, permeabilized with 0.2% (v/v) triton, washed again with PBS, and then blocked with 0.4% (v/v) FSG in PBS.

[0131] After blocking, cells were exposed to a staining solution comprising a primary antibody that targets a specific inactive form of β -catenin. Cells were then washed with PBS, and then exposed to another antibody staining solution comprising Alexa Fluor® 488 labeled secondary antibodies, a blocking solution, and DAPI. Cells were then washed a final time with PBS. Plates were then imaged for mean number of nuclear depots per cell. The Z-factor across both wt U2OS and Halo U2OS plates was greater than 0.5.

[0132] Compound dose-responses resulted in similar EC50s between wt U2OS and Halo U2OS cell thus obviating the need to transiently transfect Halo- β -catenin (as well as the associated challenges described above) when screening for compounds which modulate β -catenin nuclear depot. An exemplary dose-response curve for a single compound tested against wt U2OS cells is depicted in **FIG. 2**.

Example 4

[0133] Using label-free detection of β -catenin depots as described above in Example 3, a larger 20,000 compound phenotypic screen was performed to discover chemical matter capable of reversing the hyperactive function of β -catenin in colorectal cancer by entrapping it into β -catenin depot condensates. U2OS cells were treated with 30 μ M of compound in DMSO for 24 hrs. At 24 hrs after treatment, the cells were fixed with formaldehyde and immunofluorescence was performed by incubating the phosphorylated β -catenin antibody with the fixed samples. After PBS washes, AlexaFluor 488-conjugated goat anti-rabbit antibody was applied to detect antibody-bound antigen. Processed cells were imaged on the Phenix high throughput imaging system and phosphorylated β -catenin levels were quantified using the Phenix-associated Harmony analysis software. Compounds that induced a significant enhancement of depot formation above DMSO-treated cells were determined to be hits and selected for further assessment and development.

[0134] The identified condensate modulators (c-mods) were further optimized, such as via conventional structure-activity relationship SAR techniques, and then assessed for the ability to

sequester of β -Catenin into depots, induce selective cancer cell killing, and reverse oncogenic β -Catenin specific gene expression programs.

[0135] As detailed herein, it was found that one such c-mod, compound B, was effective in genetically diverse colorectal cancers and a range of Wnt-associated cancers, thus demonstrating its ability to treat a broad patient population (**FIGS. 3-7**). **FIG. 3** shows immunofluorescence (IF) images of healthy versus malignant colon cells upon treatment with a small molecule condensate modulator B (compound B). As shown in **FIG. 3**, Compound B treatment of malignant cells results in sequestration of β -catenin into condensates. **FIG. 4** shows selective killing of cancerous colon cells over healthy colon cells upon treatment of compound B. **FIG. 5** shows gene set enrichment analysis (GSEA) plots revealing selective down-regulation of Wnt/ β -Catenin and LEF1 target genes in colorectal cancer cells when treated with the β -catenin depot-inducing compound B. **FIG. 6** shows results from assay where 99 cancer cell lines were subjected to treatment with compound B. These results demonstrate that compound B is active in a broad range of Wnt-associated cancers.

FIG. 7 shows compound B causes stasis in tumor growth in a patient-derived xenograft (PDX) model derived from heavily pre-treated CRC patients with high β -Catenin expression. FOLFOX is a combination of chemotherapy drugs which represents standard-of-care in patients with metastatic colorectal cancer. QW = weekly dosing; BID = twice daily dosing; QD = dosed every day.

[0136] Furthermore, oral dosing of the c-mod demonstrated competitive tumor growth inhibition as a single agent in xenograft and PDX models of colorectal cancer (**Table 2**). Taken together, these results highlight the novelty and inventiveness of methods of identifying compounds that modulate β -catenin depots via depot induction and/or screening in cells. In summary, the utility of compound B described herein demonstrates that the methods of identifying a compound that modulates β -catenin depots in a cell taught herein provide a significant advancement to the field of drug discovery and identification of new therapeutic compositions.

Table 2. Compound B response in *in vivo* models of cancer, including colorectal cancer.

Model	β -Catenin pathway alteration	Response
CRC PDX	High wt β -Catenin; APC mut; Axin2 high	Y
HCT116	β -Catenin/KRAS GoF, Axin2 LoF	Y
COLO320	APC/TP53 LoF	Y
DLD1	APC mut, KRAS GoF	Y
CRC PDX	High wt β -Catenin; APC mut; KRAS GoF	Y
CRC PDX	High wt β -Catenin; APC mut; KRAS GoF	Y
NCI H460	KRAS GoF	Y

*CRC = colorectal; LoF = loss of function; GoF = gain of function; wt = wild type; mut = mutant.

CLAIMS

What is claimed is:

1. A method of identifying a compound that modulates β -catenin depots in a cell, the method comprising:
 - (a) contacting a composition comprising the cell with the compound;
 - (b) assaying the composition comprising the cell, or a derivative of the composition, for the β -catenin depots; and
 - (c) identifying the compound that modulates β -catenin depots in the cell by comparison of the assayed β -catenin depots in the cell to a reference.
2. The method of claim 1, wherein comparison of the assayed β -catenin depots in the cell to the reference is based on one or more of the following attributes of the β -catenin depots:
 - (i) presence of the β -catenin depots;
 - (ii) number of the β -catenin depots;
 - (iii) size of the β -catenin depots;
 - (iv) surface area of the β -catenin depots;
 - (v) cellular location of the β -catenin depots;
 - (vi) distribution of the β -catenin depots;
 - (vii) ratio of the amount of the β -catenin depots and a reference condensate; or
 - (viii) composition of the β -catenin depots.
3. The method of claim 1 or 2, wherein modulation of the β -catenin depots is based on increasing the β -catenin depots in the cell as compared to the reference.
4. The method of any one of claims 1-3, wherein modulation of the β -catenin depots is based on an increase in the number of the β -catenin depots in the cell as compared to the reference.
5. The method of any one of claims 1-4, wherein modulation of the β -catenin depots in the cell is based on an increase in the number of the β -catenin depots in the nucleus of the cell as compared to the reference.

6. The method of any one of claims 1-3, wherein modulation of the β -catenin depots in the cell is based on an increase in the presence of the β -catenin depots in the cell as compared to the reference.
7. The method of any one of claims 1-3 and 6, wherein modulation of the β -catenin depots in the cell is based on an increase in the presence of the β -catenin depots in the nucleus of the cell as compared to the reference.
8. The method of any one of claims 1-7, wherein the assaying for the β -catenin depots comprises detecting an inactive form of a β -catenin polypeptide.
9. The method of claim 8, wherein the detecting the inactive form of the β -catenin polypeptide comprises contacting the composition comprising the cell, or the derivative of the composition, with a detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide.
10. The method of claim 9, wherein the detectable reagent is an antibody specifically recognizing the inactive form of the β -catenin polypeptide.
11. The method of claim 9 or 10, wherein the assaying comprises detecting the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide.
12. The method of any one of claims 8-11, wherein the detecting comprises imaging at least a portion of the cell.
13. The method of any one of claims 9-12, wherein the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide is a fluorescently labeled antibody specifically recognizing the inactive form of the β -catenin polypeptide.
14. The method of any one of claims 9-12, wherein the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide is detected using a fluorescently labeled secondary antibody that specifically recognizes the detectable reagent.
15. The method of any one of claims 9-14, wherein the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide specifically recognizes a

phosphorylation at one or more of S33, S37, T41, or S45 of the β -catenin polypeptide, and wherein the amino acid position is relative to amino acid sequence of SEQ ID NO:1.

16. The method of claim 15, wherein the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide specifically recognizes a phosphorylation at T41 and/ or S45.
17. The method of claim 16, wherein the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide specifically recognizes phosphorylations at T41 and S45.
18. The method of claim 17, wherein the detectable reagent that specifically recognizes the inactive form of the β -catenin polypeptide is the 23H16L13 antibody clone.
19. The method of any one of claims 1-18, wherein the comparison of the assayed β -catenin depots in the cell to the reference is based on one or more of the following attributes of an inactive form of a β -catenin polypeptide associated with the β -catenin depots:
 - (i) location of the inactive form of the β -catenin polypeptide;
 - (ii) amount of the inactive form of the β -catenin polypeptide;
 - (iii) distribution of the inactive form of the β -catenin polypeptide;
 - (iv) condensate partitioning of the inactive form of the β -catenin polypeptide into the β -catenin depots and/ or one or more other condensates;
 - (v) a functional activity associated with the inactive form of the β -catenin polypeptide;
 - (vi) aggregation of the inactive form of the β -catenin polypeptide;
 - (vii) post-translational modification status of the inactive form of the β -catenin polypeptide; or
 - (viii) amount of a degradation product of the inactive form of the β -catenin polypeptide.
20. The method of claim 19, wherein modulation of the β -catenin depots in the cell is based on an increase in the amount of the inactive form of the β -catenin polypeptide in the β -catenin depots as compared to the reference.

21. The method of any one of claims 1-20, wherein the assaying for the β -catenin depots comprises detecting a phospho-H2A.X (P-2A.X) and/ or mediator of DNA damage checkpoint protein 1 (MDC1).
22. The method of any one of claims 1-21, wherein the cell is a model for aberrant Wnt/ β -catenin signaling.
23. The method of any one of claims 1-22, wherein the cell is a model for a proliferative pathology.
24. The method of any one of claims 1-23, wherein the cell is a model for a cancer.
25. The method of any one of claims 1-24, wherein the cell is a cancer cell.
26. The method of claim 25, wherein the cancer cell is a human osteosarcoma U2OS cell or human colon adenocarcinoma DLD-1 cell.
27. The method of any one of claims 1-26, wherein the inactive form of the β -catenin polypeptide is a wildtype β -catenin polypeptide.
28. The method of any one of claims 1-26, wherein the inactive form of the β -catenin polypeptide is a mutant β -catenin polypeptide.
29. The method of any one of claims 1-28, wherein the assaying further comprises contacting the composition comprising the cell, or the derivative of the composition, with a second detectable reagent that specifically recognizes an active form of a β -catenin polypeptide comprising a phosphorylation at S552 and/or S675, and wherein the amino acid position is relative to amino acid sequence of SEQ ID NO:1.
30. The method of any one of claims 1-29, further comprising contacting the composition comprising the cell, or the derivative of the composition, with a fixative agent before the assaying of step (b).
31. The method of any one of claims 1-30, further comprising determining one or more cellular features of the cell.
32. The method of any one of claims 1-31, wherein the reference is based on:

- (i) a composition comprising the cell not contacted with the compound;
 - (ii) a composition comprising the cell admixed with a control vehicle and not contacted with the compound;
 - (iii) a desired β -catenin profile;
 - (iv) a reference condensate or a reference polypeptide within the cell; and/ or
 - (v) a reference composition comprising a cell.
33. The method of any one of claims 1-32, further comprising subjecting the composition comprising the cell to a condition that promotes formation of the β -catenin depots prior to the contacting the composition comprising the cell with the compound.
34. The method of any one of claims 1-33, further comprising assessing the identified compound using a second cell-based assay and/or an *in vitro* assay.
35. The method of any one of claims 1-34, further comprising assessing the identified compound using a second cell-based assay.
36. A method of identifying β -catenin depots in a cell, the method comprising:
- (a) contacting a composition comprising the cell with a detectable reagent that specifically recognizes an inactive form of a β -catenin polypeptide comprising a phosphorylation at T41 and/ or S45; and
 - (b) detecting the detectable reagent,
- wherein the detection of the detectable reagent within the cell identifies the β -catenin depots in the cell.
37. The method of claim 36, wherein the detectable reagent is an antibody specifically recognizing the inactive form of the β -catenin polypeptide comprising the phosphorylation at T41 and/ or S45.
38. The method of claim 37, wherein the detectable reagent is an antibody specifically recognizing the inactive form of the β -catenin polypeptide comprising phosphorylations at T41 and S45.
39. The method of any one of claims 36-38, wherein the detecting comprises imaging at least a portion of the cell.

40. The method of any one of claims 36-39, wherein the detectable reagent is a fluorescently labeled antibody specifically recognizing the inactive form of the β -catenin polypeptide comprising the phosphorylation at T41 and/ or S45.
41. The method of any one of claims 36-39, wherein the detectable reagent is detected using a fluorescently labeled secondary antibody that specifically recognizes the detectable reagent.
42. The method of any one of claims 36-41, wherein the method further comprises contacting the composition comprising the cell with a fixative agent before contacting with the detectable reagent.
43. The method of any one of claims 36-42, further comprising measuring a signal of the detectable reagent.
44. The method of any one of claims 36-43, further comprising comparing the signal with a reference.
45. The method of claim 44, wherein the reference is based on:
 - (i) a composition comprising the cell not contacted with the detectable reagent;
 - (ii) a composition comprising the cell admixed with a control vehicle and not contacted with the detectable reagent;
 - (iii) a reference signal;
 - (iv) a reference condensate or a reference polypeptide within the cell; and/ or
 - (v) a reference composition comprising a cell.
46. A method of identifying a compound useful for treating a β -catenin-associated disease, wherein the method comprises identifying a compound according to the method of any one of claims 1-35.
47. The method of claim 46, wherein the β -catenin-associated disease is selected from the group consisting of a cancer, autosomal dominant polycystic kidney disease (ADPKD), hereditary familial adenomatous polyposis (FAP), Alzheimer's, or a metabolic disease.
48. A compound identified by the method of any one of claims 1-35, 46, and 47.

49. A method of identifying β -catenin depots in a cell, the method comprising:
- (a) contacting a composition comprising the cell with a first detectable reagent that specifically recognizes a β -catenin polypeptide;
 - (b) contacting the composition comprising the cell with a second detectable reagent that specifically recognizes a phospho-H2A.X (P-2A.X) or mediator of DNA damage checkpoint protein 1 (MDC1); and
 - (c) detecting the first detectable reagent and the second detectable reagent;
- wherein the detection of a colocalization of the first detectable reagent and the second detectable reagent within the cell identifies the β -catenin depots in the cell.