

CLAIMS

What is claimed is:

1. A method of identifying a compound that modulates a characteristic associated with one or more condensates comprising a RBM20 polypeptide ("RBM20 condensates") in the cytoplasm of a cell and/or the RBM20 polypeptide in the cytoplasm of a cell, the method comprising:
 - (a) admixing the compound and a composition comprising the cell,
wherein (i) the cell comprises the one or more RBM20 condensates, and/or (ii) the one or more RBM20 condensates form in the cell after the compound contacts the composition; and
 - (b) determining the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide,wherein a modulation in the characteristic, as compared to a reference, indicates that the compound modulates the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide.
2. The method of claim 1, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide is based on any one or more of the following:
 - (i) location of the one or more RBM20 condensates;
 - (ii) distribution of the one or more RBM20 condensates and/or the RBM20 polypeptide;
 - (iii) number of the one or more RBM20 condensates;
 - (iv) size of the one or more RBM20 condensates;
 - (v) ratio of the amount of one or more RBM20 condensates and a reference condensate;
 - (vi) a functional activity associated with the one or more RBM20 condensates;
 - (vii) composition of the one or more RBM20 condensates;
 - (viii) co-localization of the one or more RBM20 condensates with a biomolecule;

- (ix) diffusion coefficient of a component of the one or more RBM20 condensates;
 - (x) stability of the one or more RBM20 condensates;
 - (xi) dissolution or reduction in size of the one or more RBM20 condensates;
 - (xii) surface area of the one or more RBM20 condensates;
 - (xiii) sphericity of the one or more RBM20 condensates;
 - (xiv) liquidity of the one or more RBM20 condensates;
 - (xv) solidification of the one or more RBM20 condensates;
 - (xvi) location of the RBM20 polypeptide;
 - (xvii) amount of the RBM20 polypeptide or a precursor thereof;
 - (xviii) condensate partitioning of the RBM20 polypeptide into the one or more RBM20 condensates;
 - (xix) a functional activity associated with the RBM20 polypeptide;
 - (xx) aggregation of the RBM20 polypeptide;
 - (xxi) post-translational modification status of the RBM20 polypeptide; and
 - (xxii) amount of a RBM20 polypeptide degradation product.
3. The method of claim 2, wherein the modulation in the characteristic is based on a decrease in the number of the one or more RBM20 condensates in the cytoplasm of the cell.
 4. The method of claim 2 or 3, wherein the modulation in the characteristic is based on a decrease in the amount of the RBM20 polypeptide or a precursor thereof in the cytoplasm of the cell.
 5. The method of any one of claims 2-4, wherein the modulation in the characteristic is based on dissolution or reduction in size of the one or more RBM20 condensates in the cytoplasm of the cell.
 6. The method of any one of claims 2-5, wherein the modulation in the characteristic is based on a decrease in the functional activity associated with the one or more RBM20 condensates and/or the RBM20 polypeptide in the cytoplasm of the cell.

7. The method of claim 1, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide comprises location of the one or more RBM20 condensates, distribution of the one or more RBM20 condensates and/or the RBM20 polypeptide, number of the one or more RBM20 condensates, size of the one or more RBM20 condensates, and ratio of the amount of one or more RBM20 condensates and a reference condensate.
8. The method of claim 1, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide comprises composition of the one or more RBM20 condensates, and co-localization of the one or more RBM20 condensates with a biomolecule.
9. The method of claim 7 or 8, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide further comprises the functional activity associated with the one or more RBM20 condensates.
10. The method of claim 1, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide comprises stability of the one or more RBM20 condensates, dissolution or reduction in size of the one or more RBM20 condensates, and surface area of the one or more RBM20 condensates.
11. The method of claim 1, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide comprises sphericity of the one or more RBM20 condensates, liquidity of the one or more RBM20 condensates, and solidification of the one or more RBM20 condensates.
12. The method of claim 1, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide comprises location of the RBM20 polypeptide, and amount of the RBM20 polypeptide or a precursor thereof.

13. The method claim 12, wherein the characteristic associated with the one or more RBM20 condensate and/or the RBM20 polypeptide further comprises post-translational modification status of the RBM20 polypeptide.
14. The method of claim 12 or 13, wherein the characteristic associated with the one or more RBM20 condensate and/or the RBM20 polypeptide further comprises the functional activity associated with the RBM20 polypeptide.
15. The method of claim 1, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide comprises co-localization of the one or more RBM20 condensates with a biomolecule, and diffusion coefficient of a component of the one or more RBM20 condensates.
16. The method of claim 1, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide comprises stability of the one or more RBM20 condensates, and dissolution or reduction in size of the one or more RBM20 condensates.
17. The method of claim 1, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide comprises surface area of the one or more RBM20 condensates, sphericity of the one or more RBM20 condensates, liquidity of the one or more RBM20 condensates, and solidification of the one or more RBM20 condensates.
18. The method of any one of claims 1-17, wherein the RBM20 polypeptide is a wild type RBM20 polypeptide.
19. The method of any one of claims 1-17, wherein the RBM20 polypeptide is a mutant RBM20 polypeptide.
20. The method of claim 19, wherein the mutant RBM20 polypeptide comprises a mutation in an intrinsically disorder region (IDR).
21. The method of claim 19 or 20, wherein the mutant RBM20 polypeptide comprises a mutation in an RS-rich region.

22. The method of any one of claims 19-21, wherein the mutant RBM20 polypeptide comprises a mutation in one or more of the following positions: arginine 634, serine 635, arginine 636, serine 637, and proline 638.
23. The method of claim 22, wherein the mutant RBM20 polypeptide comprises one or more of the following mutations: R636S, R636C, R636H, R634Q, S637G, P638L, S635A, S635E, and S637E.
24. The method of claim 19, wherein the mutant RBM20 polypeptide comprises one or more of the following mutations: E913K, R716Q, and V535L.
25. The method of any one of claims 1-24, wherein the RBM20 polypeptide is heterologously expressed in the cell.
26. The method of any one of claims 1-24, wherein the RBM20 polypeptide is homologously expressed in the cell.
27. The method of any one of claims 1-26, wherein the cell is a model of a cardiac cell type.
28. The method of any one of claims 1-27, wherein the cell is a cardiomyocyte.
29. The method of claim 27 or 28, wherein the cell is a Rat H9C2 cell.
30. The method of claim 27 or 28, wherein the cell is a human AC-16 cell, a patient-derived cardiomyocyte, a human induced pluripotent stem cell differentiated to a cardiomyocyte, or a stem cell differentiated to a cardiomyocyte.
31. The method of any one of claims 1-26, wherein the cell is selected from the group consisting of: a HeLa cell, a U2OS cell, a human embryonic kidney cell, a human induced pluripotent stem cell, and a stem cell.
32. The method of any one of claims 1-31, wherein the cell is homozygous for alleles encoding the RBM20 polypeptide.

33. The method of any one of claims 1-31, wherein the cell is heterozygous for alleles encoding the RBM20 polypeptide.
34. The method of any one of claims 1-33, wherein the reference comprises an aliquot of the composition comprising the cell admixed with a control agent.
35. The method of any one of claims 1-34, further comprising imaging at least a portion of the composition or the cell.
36. The method of any one of claims 1-35, wherein the method further comprises determining one or more cellular features of the cell.
37. The method of any one of claims 1-36, further comprising contacting at least a portion of the composition or the cell with a fixative.
38. The method of any one of claims 1-37, further comprising contacting at least a portion of the composition or the cell with a stain.
39. The method of any one of claims 1-38, further comprising assessing the identified compound using a second cell-based assay.
40. The method of any one of claim 1-39, further comprising assessing the identified compound using an *in vitro* assay.
41. A method of identifying a compound that reduces the size and/or number of condensates comprising a RBM20 polypeptide ("RBM20 condensates") in the cytoplasm of a cell, the method comprising:
 - (a) determining the size and/or number of the RBM20 condensates in at least a portion of the cytoplasm of the cell subjected to the compound; and
 - (b) comparing the size and/or number of the RBM20 condensates with a reference, thereby identifying the compound that reduces the size and/or number of the RBM20 condensates in the cytoplasm of the cell.

42. The method of claim 41, wherein the compound reduces the number of RBM20 condensates.
43. The method of claim 41 or 42, wherein the compound reduces the size of RBM20 condensates.
44. A method of identifying a compound that prevents formation or growth of one or more condensates comprising a RBM20 polypeptide ("RBM20 condensates") in the cytoplasm of a cell, the method comprising:
- (a) combining the compound and a composition comprising the cell,
wherein (i) the cell comprises the one or more RBM20 condensates, and/or (ii) the one or more RBM20 condensates form after the compound contacts the composition; and
 - (b) obtaining a first measurement of the size and/or number of the one or more RBM20 condensates in at least a portion of the cytoplasm of the cell; and
 - (c) comparing the first measurement to a reference, thereby identifying a compound that prevents formation or growth of the one or more RBM20 condensates in the cytoplasm of the cell.
45. The method of claim 44, wherein the reference comprises an aliquot of the composition comprising the cell admixed with a control agent.
46. The method of claim 44, wherein the reference is a second measurement of the size and/or number of the one or more RBM20 condensates in at least a portion of the cytoplasm of the cell, and wherein the second measurement is taken at a different time than the first measurement.
47. The method of any one of claims 44-46, further comprising subjecting the cell to a condition that promotes formation of the one or more RBM20 condensates.
48. A method of identifying a compound that decreases the amount of a RBM20 polypeptide in the cytoplasm of a cell, the method comprising:
- (a) combining the compound and a composition comprising the cell; and

- (b) obtaining a first measurement of the amount of the RBM20 polypeptide in at least a portion of the cytoplasm of the cell; and
 - (c) comparing the first measurement to a reference, thereby identifying a compound that decreases the amount of the RBM20 polypeptide in the cytoplasm of the cell.
- 49. The method of claim 48, wherein the reference comprises an aliquot of the composition comprising the cell admixed with a control agent.
- 50. The method of claim 49, wherein the reference is a second measurement of the amount of the RBM20 polypeptide in at least a portion of the cytoplasm of the cell, and wherein the second measurement is taken at a different time than the first measurement.
- 51. A method of identifying a compound that modulates a characteristic associated with one or more condensates comprising a RBM20 polypeptide ("RBM20 condensates"), the method comprising:
 - (a) admixing the compound and a solution comprising the one or more RBM20 condensates and an extra-condensate solution; and
 - (b) determining the characteristic associated with the one or more RBM20 condensates, wherein a modulation in the characteristic, as compared to a reference, indicates that the compound modulates the characteristic associated with the one or more RBM20 condensates.
- 52. A method of identifying a compound that modulates a characteristic associated with one or more condensates comprising a RBM20 polypeptide ("RBM20 condensates") and/or the RBM20 polypeptide, the method comprising:
 - (a) combining an agent and a solution comprising the RBM20 polypeptide in the presence of the compound,
 - wherein the agent is capable of causing the formation of the one or more RBM20 condensates and the one or more RBM20 condensates form after the agent contacts the solution; and

- (b) determining the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide,
wherein a modulation in the characteristic, as compared to a reference, indicates that the compound modulates the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide.
53. The method of claim 51 or 52, wherein the characteristic associated with the one or more RBM20 condensates and/or the RBM20 polypeptide is based on any one or more of the following:
- (i) number of the one or more RBM20 condensates;
 - (ii) composition of the one or more RBM20 condensates;
 - (iii) size of the one or more RBM20 condensates;
 - (iv) stability of the one or more RBM20 condensates;
 - (v) dissolution or reduction in size of the one or more RBM20 condensates;
 - (vi) surface area of the one or more RBM20 condensates;
 - (vii) sphericity of the one or more RBM20 condensates;
 - (viii) liquidity of the one or more RBM20 condensates;
 - (ix) solidification of the one or more RBM20 condensates;
 - (x) amount of the RBM20 polypeptide not in the one or more RBM20 condensates;
 - (xi) partitioning of the RBM20 polypeptide into the one or more RBM20 condensates; and
 - (xii) aggregation of the RBM20 polypeptide.
54. A method of identifying a compound that modulates the partitioning of a biomolecule for a condensate comprising a RBM20 polypeptide ("RBM20 condensate"), the method comprising:
- (a) admixing the compound and a composition comprising a cell,
wherein (i) the cell comprises the RBM20 condensate, and/or (ii) the RBM20 condensate forms in the cell after the compound contacts the composition;
and

(b) determining the partitioning of the biomolecule for the RBM20 condensates.

55. The method of claim 54, wherein the biomolecule is a non-RBM20 polypeptide.
56. The method of claim 54, wherein the biomolecule is a wild type RBM20 polypeptide.
57. The method of any one of claims 54-56, wherein the RBM20 condensate comprising the RBM20 polypeptide comprises a mutant RBM20 polypeptide.
58. A method of identifying a compound useful for treating a RBM20-associated disease, the method comprising identifying a compound according to any one of the methods of claims 1-57.
59. The method of claim 58, wherein the RBM20-associated disease is a cardiomyopathy.
60. The method of claim 59, wherein the cardiomyopathy is dilated cardiomyopathy.