

SALIVARY BIOMARKERS OF BRAIN INJURY

FIELD

5 [0001] The present invention relates to compositions, kits, systems and methods for diagnosing and/or monitoring brain injury, including, without limitation, traumatic brain injury (TBI). More particularly, the present invention relates to the diagnosis and monitoring of TBI using RNA biomarkers.

BACKGROUND

10 [0002] Traumatic brain injury (TBI) is the leading cause of death and disability under the age of 45 years in Western countries. Its healthcare burden and social costs are expected to continue to rise and, by 2020, the World Health Organization projects TBI to become the third leading cause of disability worldwide.

15 [0003] Despite many studies, no reliable biomarkers have been found to assess the severity of TBI and predict recovery. This is especially true for mild TBI (mTBI), which remains currently difficult to assess in clinical practice. Although TBI patients are initially assessed by the Glasgow Coma Score (GCS) and neuroimaging techniques, which require costly equipment, the current diagnostic tools are lacking in the ability to precisely define and quantify the actual severity of the brain injury, thus leading to an easy detection of severe but
20 not of mTBI, which represents the vast majority of cases (75-90%).

[0004] The correct diagnosis of mTBI is particularly important in patients, such as athletes, soldiers and children, who are at greater risk of repetitive mTBI and a catastrophic form of brain injury known as second impact syndrome (SIS) where the synergistic effects of repeated TBI result in profound damage and even death. Early diagnosis and evaluation of
25 the severity of TBI thus becomes crucial for patients' wellbeing and ultimately saving their life.

[0005] The subject technology was devised with these issues in mind.

SUMMARY

30 [0006] The disclosure provides methods and detection systems relating to diagnosing, monitoring, and treating traumatic brain injury (TBI) including mild traumatic brain injury (mTBI) in a human subject who has suffered an injury to the head by detecting one or more miRNA molecules in a biological sample from the subject. In the context of the following

disclosure, the terms 'level' and 'amount' in reference to the level or amount of an miRNA molecule or molecules in a biological sample are used interchangeably.

[0007] In one aspect, the disclosure relates to a method of diagnosing and treating traumatic brain injury (TBI) in a human subject in need thereof. The method involves:

- 5 obtaining a saliva sample from the subject; contacting the saliva sample with a probe comprising a nucleic acid able to bind to at least one RNA biomarker: (a) selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-
- 10 miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or (b) selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-
- 15 miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof; determining an amount of the at least one RNA biomarker in the saliva sample; identifying the subject as having TBI where the amount of the at least one
- 20 RNA biomarker is increased or decreased relative to a predetermined threshold value or relative to the amount of the RNA biomarker in a control sample; and treating the subject identified as having TBI according to one or more of the following: subjecting the subject to a verbal, cognitive, motor, or optical test, or any combination of the foregoing; subjecting the subject to diagnostic imaging in the form of a CT or MRI, or a combination
- 25 thereof; and/or administering to the subject one or more neuroprotective therapies.

- [0008]** In certain embodiments of this method, the at least one RNA biomarker further comprises one or more miRNA: (a) selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p,
- 30 hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or (b) selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

[0009] In another aspect, the present disclosure relates to a method of diagnosing and/or monitoring traumatic brain injury (TBI) in a subject. This method involves: determining a level of at least one RNA biomarker in a saliva sample obtained from the subject, wherein the at least one RNA biomarker: (a) selected from the group consisting of

5 hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or (b) selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249,

10 U6.375, U6.428, and YRNA-255, or any combination thereof.

[0010] In one embodiment of this method, the at least one RNA biomarker further comprises one or more miRNA: (a) selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or (b) selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

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[0011] In another aspect, the present disclosure relates to a sensor element for a detection system for diagnosing and/or monitoring TBI, the sensor element comprising a substrate functionalized with a probe specific for at least one RNA biomarker: (a) selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or (b) selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-

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miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.

- 5 [0012] In one embodiment of the sensor element, the at least one RNA biomarker further comprises one or more miRNA: (a) selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and
10 hsa-miR-934, or any combination thereof; and/or (b) selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

- [0013] In another aspect, the present disclosure relates to a detection system for diagnosing and/or monitoring TBI, comprising: a sensor element according to the present
15 disclosure, and a detection device capable of detecting the binding of a target RNA biomarker to the probe.

- [0014] In another aspect, the present disclosure relates to a method for determining a course of action for a subject suspected of having TBI, comprising applying a saliva sample obtained from the subject to a detection system according to the present disclosure, and if an
20 upregulated level or a downregulated level of the at least one RNA biomarker is detected, providing a treatment for TBI.

- [0015] In another aspect, the present disclosure relates to a method of treating a subject with suspected TBI. This method involves: determining whether an upregulated level or a downregulated level of at least one RNA biomarker: (a) selected from the group
25 consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG,
30 tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or (b) selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7,

RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof, is detectable in a saliva sample obtained from the subject, and if an upregulated level or a downregulated level of at least one RNA biomarker is detected, providing treatment for TBI to the subject.

- 5 **[0016]** In one embodiment of this method, the at least one RNA biomarker further comprises one or more miRNA: (a) selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-10
10 934, or any combination thereof; and/or (b) selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

- 15 **[0017]** In another aspect, the present disclosure relates to a method of detecting an RNA biomarker in a saliva sample. This method involves: obtaining a saliva sample from a human subject, contacting the saliva sample with at least one oligonucleotide primer complementary to at least one RNA biomarker: (a) selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-
20 miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or (b) selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188,
25 put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof; amplifying the at least one RNA biomarker using a polymerase chain reaction; and detecting the amplified RNA biomarker.

- 30 **[0018]** In one embodiment of this method, the at least one RNA biomarker further comprises one or more miRNA: (a) selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-

934, or any combination thereof; and/or (b) selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

[0019] In another aspect, the present disclosure relates to a kit for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the kit comprising at least one probe specific for at least one RNA biomarker: (a) selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or (b) selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.

[0020] In one embodiment of this kit, the at least one RNA biomarker further comprises one or more miRNA: (a) selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or (b) selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

[0021] In another aspect, the present disclosure relates to a composition for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the composition comprising at least one probe specific for at least one RNA biomarker: (a) selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT,

tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or (b) selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.

[0022] In one embodiment of this composition, the at least one RNA biomarker further comprises one or more miRNA: (a) selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or (b) selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

DETAILED DESCRIPTION

[0023] The present invention provides methods of diagnosing or monitoring traumatic brain injury (TBI) in a subject, as well as methods for treating a subject identified as suffering from TBI.

[0024] The quest for TBI biomarkers has received significant impetus by the increased profile of sport concussion in the media. In the last few years many studies have focused on biomarkers that can support clinical decision making pitch-side or in a sports clinic. However, protein biomarkers reported in the literature lack specificity or sensitivity, or are not detectable for some time after injury. This may be due to the fact that following concussion, which is a form of TBI, brain-derived compounds are only released in very small amounts and the blood-brain barrier remains mostly closed.

[0025] MicroRNAs (miRNAs) are an abundant class of highly conserved, non-coding RNA molecules of approximately 22 nucleotides in length that induce mRNA degradation, translational repression or both via pairing with partially complementary sites in the 3'UTR of target genes. The human genome encodes over 2,000 miRNAs, which may target about 60% of all genes. However, despite the abundance of miRNAs, their biomolecular functions and involvement in pathology remain to be fully elucidated. They play a central role in many biological processes including cell cycle, cell metabolism, apoptosis and immune responses,

and are attracting increasing interest in clinical research as potential biomarkers for the detection, identification and classification of cancers and other disease states including neurodegenerative diseases.

[0026] For the avoidance of doubt, it will be understood that “the at least one miRNA is selected from a group of miRNAs”, as used herein, means that the method in question, whether carried out for a diagnostic, prognostic, or therapeutic purpose, can be carried out with any one of the listed miRNAs or with any plurality of the listed miRNAs (e.g., two, three, four, or more of the listed miRNAs). It follows that any one or more of the listed miRNAs may be explicitly excluded.

[0027] Traumatic brain injury occurs when an external force traumatically injures the brain. There are different systems for classifying TBI based on, for example, severity, type of injury and prognosis. The most commonly used system for classifying TBI is the Glasgow Coma Scale (GCS), which grades a person's level of consciousness on a scale of 3-15 based on verbal, motor, and eye-opening reactions to stimuli. In general, a TBI with a GCS score of 13 or above is defined as mild, 9-12 as moderate and 8 or below as severe. Another system, the Mayo Classification System, has three main classifications including definite moderate-severe TBI, probable mild TBI, and possible TBI. Multiple criteria are used in each diagnosis including loss of consciousness, post-traumatic amnesia, skull fracture, and evidence of neuroradiological abnormalities including subdural haematoma, cerebral contusion, and hemorrhagic contusion. The classification of TBI using the GCS or Mayo systems will be known to those skilled in the art.

[0028] As used herein, references to “mild”, “moderate” and “severe” TBI are made in accordance with the GCS. References herein to “moderate-to-severe” TBI encompass both moderate and severe TBI in accordance with the GCS.

[0029] As used herein, a reference to mild TBI (mTBI) is also a reference to concussion.

[0030] The diagnosis and/or monitoring of TBI using the biomarkers of the present invention is expected to support clinical decision making and treatment regimens in a variety of contexts, including the following situations: as part of the initial assessment by paramedics to determine whether patients should be transported to a facility with neurosurgical expertise, a major trauma centre or a local trauma unit; in the emergency department of hospitals to determine appropriate treatment, including the need for a CT brain scan; pitch-side, to assist decision making as to the removal of a player from play and assessment of the need to take a player to hospital; in sports clinics, to confirm a concussive event and enable decision making

with regard to returning to play; in combat situations, to determine the need to dispatch a rescue team and evacuate a victim. Thus, subjects for whom the present invention provides particular benefit can include, without limitation, accident victims, sports players, and military personnel.

5 **[0031]** In any case, but perhaps particularly where the subject is at greater risk for a TBI (e.g., where the subject is a professional athlete or enlisted in the military), a sample may be obtained from the subject at a time prior to any known or recent trauma (e.g., near the beginning of a sporting career or prior to a military deployment) and any miRNAs of interest can be assessed at that time or later, when the subject has experienced a possible
10 TBI. Such samples may thereby provide an internal reference standard.

[0032] In some embodiments, the subject is human.

[0033] The TBI may be mild TBI (mTBI), moderate TBI or severe TBI (sTBI). In some embodiments, the TBI is moderate-to-severe TBI (m-sTBI).

[0034] The level of the miRNA or of each miRNA in the sample may be determined
15 quantitatively or semi-quantitatively. By “quantitatively”, it will be understood that the absolute amount or concentration of the miRNA or of each miRNA in the sample is determined. The absolute amount of the miRNA or of each miRNA in the sample can then be compared to a predetermined threshold (e.g. a published literature value for expected normal levels), a known level of the same or a reference miRNA in a control sample taken from a
20 healthy subject, or the amount of a reference miRNA in the sample taken from the subject. In some embodiments, the subject is diagnosed as having a TBI when the level of the miRNA is below the predetermined threshold, or decreased relative to a reference or control sample. In other embodiments, the subject is diagnosed as having a TBI when the level of the miRNA is increased compared to the predetermined threshold.

25 **[0035]** By “semi-quantitatively”, it will be understood that the level of the or each miRNA of interest is measured relative to a reference.

[0036] The reference may be an invariant miRNA, i.e. a miRNA having an expression level that remains substantially unchanged between healthy subjects and those having a TBI. A subject may be diagnosed as suffering from a TBI if the level of the miRNA
30 or of each miRNA of interest is increased or decreased relative to that of an invariant miRNA.

[0037] In some embodiments, the level of the miRNA or of each miRNA in the sample obtained from the subject may be about 0.01 times to about 100 times, about 0.05

times to about 50 times, about 0.1 times to about 10 times, about 0.5 times to about 5 times, about 1.0 to about 3 times, or about 1.5 times to about 2.0 times lower or higher than the level in the control sample, the reference level or the published value.

[0038] Where a device or method is employed to generate a value, we may qualify that value with the term “about” in order to capture the stated value and any variation of that value inherent to the device or method employed. Where values or ranges of values are specifically disclosed, “about” may mean plus-or-minus 10% of the stated value or range. For example, about 10 minutes may mean 9-11 minutes.

[0039] The level of the miRNA or of each miRNA of interest can be determined using methods known to those skilled in the art. In some embodiments, determining the level of the miRNA or of each miRNA of interest comprises amplifying the miRNA. In some embodiments, total miRNA may be first isolated from the sample using standard techniques, for example using the miRNeasy mini kit (Qiagen). The amount of the miRNA of interest can then be determined. In some embodiments, the level of the miRNA or of each miRNA of interest in the sample is determined using PCR (polymerase chain reaction). For example, quantitative PCR may be used for quantitative determination of the level of the miRNA or of each miRNA of interest. PCR may also be used for semi-quantitative determination, by comparing the level of the miRNA or of each miRNA of interest in the sample with that of a reference (e.g. an invariant miRNA).

[0040] Suitable techniques for miRNA detection and/or quantification, which will be known to those skilled in the art, include qPCR, miRNA assays, next-generation sequencing (NGS), and multiplex miRNA profiling assays.

[0041] In some embodiments, the level of the miRNA or of each miRNA of interest is determined using in-situ hybridization, for example using a probe (e.g., a labelled probe) specific for the miRNA.

[0042] The level of miRNA may be determined in a sample which was obtained from the subject immediately after injury (i.e. less than 1 hour after injury), and/or in a sample obtained at one or more time points a few hours or days after injury. Thus, changes in the miRNA level can be detected over time to enable monitoring of a TBI. In the event miRNA levels change over time, the methods described herein for monitoring TBI can be expanded to include maintaining or adjusting the subject's treatment regimen accordingly.

[0043] Depending on the specific miRNA and the type of TBI, the level of miRNA in the subject may change significantly over time. In some embodiments, it may therefore be advantageous to measure the miRNA relatively soon after injury to enable an accurate

diagnosis. In some embodiments, the level of miRNA is determined in a sample obtained from the subject no more than 72 hours, no more than 48 hours, no more than 36 hours, no more than 24 hours, no more than 12 hours, no more than 6 hours, no more than 4 hours, no more than 2 hours or no more than 1 hour after injury.

5 **[0044]** The level of some miRNAs is substantially stable over time, thus allowing a diagnosis to be made a few hours, days or even weeks after injury. In some embodiments, the level of miRNA is determined in a sample obtained from the subject up to 20, 18, 15, 12, 10, 8, 5 or 2 days from injury.

10 **[0045]** In some embodiments, the level of miRNA is determined in a sample obtained from the subject immediately after injury (e.g. at T=0 h), at 4-12 hours after injury, at 48-72 hours after injury, or at 15 days after injury.

15 **[0046]** In some embodiments, the level of miRNA is determined in a sample obtained from the subject at least 24 hours after injury. In some embodiments, the level of miRNA is determined in a sample obtained from the subject 15 days or fewer after injury. In some
embodiments, the level of miRNA is determined in a sample obtained from the subject
between 24 hours and 15 days after injury, or between 24 hours and 10 days after injury, or
between 24 hours and 7 days after injury, or between 48 hours and 5 days after injury.

20 **[0047]** In some embodiments, the TBI is mild TBI (mTBI) or moderate-to-severe TBI (m-sTBI).

20 **[0048]** In some embodiments, the TBI is mild TBI (mTBI).

25 **[0049]** Conveniently the sample may be any appropriate fluid or tissue sample obtained from the subject. For example, the biological sample may comprise at least one of the group consisting of: urine, saliva, whole blood, plasma, serum, sputum, semen, faeces, a nasal swab, tears, a vaginal swab, a rectal swab, a cervical smear, a tissue biopsy, and a
urethral swab. In some embodiments the sample is a fluid sample. Suitably, the sample is one that can be readily obtained from the individual, such as urine, saliva, blood and sputum. In some embodiments, the sample comprises saliva, blood, plasma or serum. It will be appreciated that in some embodiments the process of obtaining the sample does not form part of the invention described herein.

30 **[0050]** In some embodiments, the sample comprises or is constituted by serum. Not only does serum have practical advantages, but it is also free of anticoagulants such as heparin, a potential inhibitor of PCR reactions. Serum may also be less affected by haemolysis, compared to plasma.

[0051] In some embodiments, the sample is saliva. Saliva can be easily obtained from the patient (e.g. pitch-side, or in the field), without specialist training or medical equipment.

[0052] The diagnosis of a subject as suffering from a TBI, and in particular diagnosis of mild TBI or moderate-to-severe TBI, may facilitate in the determination of an appropriate treatment. The present invention thus provides a test that enables healthcare workers, such as physicians, clinicians, paramedics, and even non-medical personnel (e.g. teachers, sports coaches, military personnel) to decide on appropriate action for a subject suspected of having a TBI. A subject determined as having a TBI may therefore receive the most appropriate treatment as a result of a diagnosis being made. The method of the invention may thus further comprise directing appropriate therapy to a subject diagnosed with a TBI.

[0053] A subject diagnosed with TBI may be further evaluated, e.g. by CT scanning. In some embodiments, the subject is admitted to hospital. In some embodiments, if moderate-to-severe TBI can be ruled out, the subject may not need to be admitted to hospital for evaluation. A subject diagnosed with moderate-to-severe TBI may be admitted to hospital, or a specialist centre with neurotrauma expertise.

[0054] A subject diagnosed with a TBI (particularly mTBI) outside a hospital environment, for example, at a sporting event, during combat or during play, may be removed from play or combat immediately. The subject may subsequently be started on a graduated return to play or combat.

[0055] In a further aspect, there is provided a method for determining whether it is appropriate to administer to a subject a therapy for alleviating TBI, the method comprising: determining a level of at least one miRNA in a sample from the subject; and determining whether or not it is appropriate to administer a therapy for alleviating TBI, based on the level of the at least one miRNA.

[0056] It will be appreciated that the step of administering the therapy to the subject does not form a part of the claimed method, unless specifically stated.

[0057] In some embodiments the method may further comprise administering to the subject an appropriate treatment. In some embodiments, the treatment may comprise a therapy for alleviating TBI. Accordingly, the invention features methods of diagnosing and treating TBI in a subject, the method comprising the steps of (a) obtaining a sample (e.g., a sample of blood, plasma, urine, or saliva) from the subject; (b) detecting one or more miRNAs (selected from those described herein); diagnosing the patient as having a TBI when the level(s) of the miRNA(s) differ from a reference standard (as described herein); and administering a treatment for the TBI.

- [0058] In a further aspect, the invention provides a method of determining an appropriate treatment to a subject suspected of suffering from a TBI, the method comprising identifying whether or not the subject has a TBI by determining a level of at least one miRNA in a sample from the subject.
- 5 [0059] If a subject is identified as having a TBI, an appropriate treatment may include one or more of the following: further evaluating the subject, for example by further tests (e.g. verbal, cognitive, motor and/or optical tests), CT and/or MRI scanning; removing the subject from activity (e.g. the activity during which the TBI was incurred); admitting the subject to hospital or a specialist clinic; surgery; and administering a therapy for alleviating TBI to the
- 10 subject.
- [0060] The therapy for alleviating TBI may include neuroprotective drugs, e.g. drugs to treat cerebral swelling such as mannitol and hypertonic saline, and/or other neuroprotective measures, such as avoidance of hypertensive resuscitation and the use of sedation.
- [0061] In some embodiments, the subject may be subsequently monitored to track
- 15 their recovery, for example in a hospital or clinic setting.
- [0062] According to a further aspect of the invention, there is provided a method of detecting and/or determining a level of a target miRNA in a subject, the method comprising the steps of (a) obtaining a sample from the subject; and (b) detecting and/or determining the level of the target miRNA in the sample by contacting the sample with a probe that is specific
- 20 for the target miRNA.
- [0063] The sample may be any appropriate fluid or tissue sample obtained from the subject, as defined above. In some embodiments, the sample is blood, serum, plasma, urine or saliva.
- [0064] In some embodiments, the method may comprise determining the level of two
- 25 or more target miRNAs in the sample.
- [0065] According to a further aspect of the invention, there is provided a therapy for alleviating TBI for use in a method of treating a subject in need thereof, wherein said subject is identified as having a TBI by determining a level of at least one miRNA in a sample from the subject.
- 30 [0066] The step of determining the level of the target miRNA may comprise contacting the sample with a substrate functionalized with the probe, for example a chip comprising the probe. The substrate or chip may conveniently include multiple probes, each specific for a different target miRNA.

[0067] The subject may have suffered an injury, in particular a head injury. The subject may be suspected as having a TBI. The subject may be suspected of having mTBI or concussion. In some embodiments, the sample is obtained no more than 72 hours, no more than 48 hours, no more than 36 hours, no more than 24 hours, no more than 12 hours, no more than 6 hours, no more than 4 hours, no more than 2 hours or no more than 1 hour after injury. In some embodiments the sample is obtained 24 hours or more after the injury. In further embodiments the sample is obtained from the subject 15 days or fewer after injury. In some embodiments the sample is obtained from the subject between 24 hours and 15 days after injury, or between 24 hours and 10 days after injury, or between 24 hours and 7 days after injury, or between 48 hours and 5 days after injury.

[0068] In some embodiments, the method further comprises treating the subject. The treatment may include one or more of the following: further evaluating the subject, for example by further tests (e.g. verbal, cognitive, motor and/or optical tests), CT and/or MRI scanning; removing the subject from activity (e.g. the activity during which the TBI was incurred); admitting the subject to hospital or a specialist clinic; and administering a therapy for alleviating TBI to the subject. In some embodiments, the treatment comprises administering an effective amount of a neuroprotective drug.

[0069] Thus, in yet a further aspect the invention provides a method of treating TBI, the method comprising: determining a level of at least one miRNA in a sample from the subject; and if the level of the at least one miRNA is indicative of mTBI, administering a treatment appropriate for mTBI; or if the level of the at least one miRNA is indicative of m-sTBI, administering a treatment appropriate for m-sTBI.

[0070] It will be appreciated by those skilled in the art that different treatment pathways may be used for mTBI and m-sTBI.

[0071] A subject with mTBI may be treated as follows:

[0072] An athlete diagnosed with mTBI would typically be started on a graduated return to play protocol (as defined by the Berlin Consensus Conference on Concussion and individual sport authorities, e.g. RFU, IRB, NFL, NHL, NBA, IMMAF). This would involve a period of rest followed by a gradual increase in level of exertion and exposure to contact. An athlete with mTBI would typically not be able to play for a period that varies between 6 and 23 days, depending on level of medical supervision and age. Conversely, if mTBI was excluded, the athlete would not have any restrictions and could train as normal the following day. If diagnosed in a pre-hospital setting, mTBI would be an indication for a patient to seek

medical attention (e.g. in hospital or in primary care), whereas, if mTBI was excluded no medical review would be indicated.

[0073] A person with mTBI must not be left unsupervised for 24-48 hours and must not drive or operate a fork lift or open machinery until recovered.

- 5 [0074] Military personnel diagnosed with mTBI would be removed from the operational theatre and would be rested, whereas, if mTBI could be excluded, that person would continue on active duties.

- [0075] In the NHS, a person with mTBI may be kept in hospital for observation or may have a CT scan according to the guidance issued by NICE, whereas if mTBI could be
10 excluded, that patient could be discharged straightaway.

[0076] A person diagnosed with mTBI would typically be asked not to return to work or study for a certain period of time.

- [0077] A person diagnosed with mTBI may be referred to a mild TBI clinic or a neurology clinic or a neurosurgery clinic. Typical interventions for mTBI consist of
15 education, advice in regard to work, study and driving, medical management of typical sequelae such as headache or anxiety or mood disorder or post-traumatic stress disorder with medication and/or psychological intervention if necessary, and referral to other services as indicated, e.g. neuropsychology, neurovestibular or ophthalmology.

- [0078] A patient with mTBI would need to be monitored for delayed post-traumatic
20 pituitary dysfunction according to the guidance issued by the Society of British Neurological Surgeons.

- [0079] In the medico-legal context, the diagnosis of mTBI is often unclear and speculative, as the radiological investigations are typically normal and symptoms are non-specific. Objective confirmation of mTBI may lead to an award for damages and care needs.
25 An appropriate treatment for mTBI may include: removing the subject from activity; treatment in situ or in the community; further evaluating the subject in hospital without overnight admission (typically mTBI patients are discharged with promptly with head injury advice); or admission to hospital for a period of observation (typically 1-2 days). The subject may be further evaluated using tests (e.g. verbal, cognitive, motor and/or optical tests). CT
30 scanning is generally only required if certain indications are present, including suspected skull fracture, post-traumatic seizure, focal neurological deficit, repeated vomiting, a GCS score of less than 13 on initial assessment (less than 14 for children, or less than 15 for infants under 1 year), in accordance with NICE guidelines.

[0080] An appropriate treatment for m-sTBI may include: MRI or CT scanning (particularly within 1 hour of injury); admission to hospital (which may include admission to intensive care and/or transfer to a specialist clinic or major trauma centre with neurosurgical facilities); neuromonitoring; surgery; administering a therapy for alleviating TBI, such as
5 administering neuroprotective drugs, e.g. drugs to treat cerebral swelling such as mannitol and hypertonic saline, and/or other neuroprotective measures, such as avoidance of hypertensive resuscitation and the use of sedation.

[0081] Thus, the present invention enables subjects with a TBI to be clinically and quickly stratified into mTBI or m-sTBI, so that they may receive the most appropriate
10 treatment.

[0082] According to a further aspect of the invention, there is provided a detection system for diagnosing and/or monitoring TBI, the detection system comprising a sensor element comprising a substrate functionalized with a probe specific for a target miRNA. The detection system can further comprise a detection device that is capable of detecting the
15 binding of a target miRNA to the probe.

[0083] According to a yet further aspect of the invention, there is provided a sensor element for use in a detection system for diagnosing and/or monitoring TBI, the sensor element comprising a substrate functionalized with a probe specific for a target miRNA.

[0084] The sensor element may further comprise a sample addition zone for receiving
20 a sample (e.g. a fluid sample) thereon.

[0085] The probe is capable of selectively binding the miRNA of interest. The substrate may be functionalized with a plurality of probes. The probes may all be the same, or two or more different probes may be provided. For example, in some embodiments, the substrate may be functionalized with a first probe specific for a first miRNA, and a second
25 probe specific for a second miRNA. The first and second probes may be grouped together, for example on different portions of the sensor element.

[0086] In a further aspect of the invention, there is provided a composition for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in a subject, the composition comprising a probe specific for a target miRNA. The composition may comprise
30 any one of the listed miRNAs or with any plurality of the listed miRNAs (e.g., two, three, four, or more of the listed miRNAs).

[0087] The probe may comprise a biological molecule such as a protein (e.g. an antibody) or a nucleic acid. In some embodiments, the probe comprises a nucleic acid. The nucleic acid may comprise a sequence which is at least 70%, at least 75%, at least 80%, at

least 85%, at least 90% or at least 95% identical to a sequence which is the complement of the full-length sequence of the target miRNA. In some embodiments, the nucleic acid comprises a sequence which is 100% identical to a sequence which is the complement of the sequence of the target miRNA (i.e. the receptor comprises a nucleic acid sequence which is the exact complement of the target miRNA sequence).

5 [0088] The probes may be attached to a surface of the substrate by any suitable means, such as by coupling chemistry known to those skilled in the art. In some embodiments, each probe is attached to a surface of the substrate via a linker. In some embodiments, the probe comprises a moiety for immobilizing the probe on the substrate, or for attaching the probe to a linker immobilized on the substrate.

10 [0089] Alternatively or in addition, the probe may comprise a detectable label. The detectable label may be, for example, radioactive, fluorescent, luminescent, or antibody-based (e.g., it may constitute a conventional tetrameric antibody or a detectable fragment thereof).

15 [0090] The substrate of the sensor element may be formed from any suitable material. In some embodiments, the substrate comprises or is formed from metal, plastic, glass, silica, silicon, graphite, graphene, or any combination thereof. In some embodiments, the substrate comprises multiple layers. For example, a substrate may be prepared by forming a surface or layer of graphene on a layer of silicon carbide or silica. The graphene surface may be chemically modified, for example to graphene-oxide (GO) or graphene-amine (GA). Methods for forming graphene layers, such as epitaxial growth and sublimation growth, will be known to those skilled in the art.

20 [0091] Conveniently, probes comprising or constituted by a nucleic acid can be attached to a GO surface via a linker, using an amide coupling reagent (e.g. (O-(7-azabenzotriazole-1-yl)-N,N,N,N'-tetramethyluronium hexafluorophosphate (HATU)). A sensor element comprising a surface functionalized with a nucleic acid probe can then be used to selectively detect its complementary miRNA.

25 [0092] Suitable linkers may comprise an aniline moiety (or a derivative thereof), a benzoic acid moiety (or a derivative thereof) or an ethendiamine moiety (or a derivative thereof). An aniline linker may be formed by attaching a nitrobenzene molecule (or derivative) to a graphene surface (e.g. using a diazonium salt), and reducing the nitrobenzene to aniline. The amine group of the aniline may then be used to attach to the probe. Similarly, a diazonium salt (e.g. 4-benzoic acid diazonium tetrafluoroborate) can be used to attach a

benzoic acid or benzoic acid derivative to a graphene surface. An ethanediamine moiety may be attached to carboxylated graphene or graphene oxide.

[0093] The sensor element may be comprised within a test strip. The test strip may be disposable.

5 [0094] The detection device may be configured to detect the binding of a target miRNA to the receptor by any suitable means known to those skilled in the art, for example by detecting changes in electrical impedance, hydrogen ion concentration, or conformational changes resulting from hybridisation.

10 [0095] The detection device may further include a user interface to output data to a user.

[0096] In some embodiments, the detection device includes a database of treatment information. The device may be capable of identifying suitable treatment options from the database depending on the levels of the miRNA of interest. The treatment information may be provided to the user via the user interface.

15 [0097] Conveniently, the detection device may be portable, e.g. hand-held. The detection device may comprise a data storage unit for storing miRNA levels and other information relating to the subject. In some embodiments, the device comprises a data communication means for communicating data to other devices. For example, the device may communicate data wirelessly through WiFi, 3G, 4G, Bluetooth, or through a mobile app. This
20 may conveniently enable the data to be easily accessed by medical professionals if necessary.

[0098] It is thus envisaged that the detection device of the invention provides an affordable, portable, point of care means for diagnosing and monitoring TBI non-invasively. The device may be used by ambulance crews, the military, schools, sports clubs and healthcare professionals, enabling the correct assessment and triage of patients suspected to
25 have a TBI.

[0099] A further aspect of the present invention is a system for detecting and/or monitoring mTBI in a subject.

[00100] A type of detection system is based on complementarity between a target miRNA and a nucleic acid probe, generally an oligonucleotide probe. The complementary or
30 base pairing region can be 7 or 8 or more nucleotides in length. In embodiments the complementary or base pairing region can be 9, 10, 12, 15 or more nucleotides in length or the complementary or base pairing region can be the full length of the miRNA. The substrate functionalised with an oligonucleotide can be a bead or a nanoparticle. The detection system

can have further components capable of converting bound nucleic acid probe-miRNA into a detectable signal.

[00101] Another type of detection system is based on RT-PCT. Therefore the present invention embraces a detection system for detecting and/or monitoring mTBI in a subject comprising a primer pair designed for amplification of a cDNA complement of at least one miRNA described herein.

[00102] In a further aspect there is provided a kit for use in the present methods. The kit may comprise at least probe (e.g. a protein, such as an antibody, or a nucleic acid) which is capable of selectively binding the miRNA of interest. In some embodiments, the kit comprises an array comprising a plurality of probes. In some embodiments, the at least one probe is a primer for carrying out PCR. The kit may further comprise instructions for use, for example instructions for use in the diagnosis and/or monitoring of TBI. The kit may further comprise suitable buffers and reagents, such as amplification primers and enzymes (e.g. DNA polymerase, reverse transcriptase for conversion of miRNA to cDNA).

Additional Aspects and Embodiments

[00103] In further aspects, the present disclosure provides, *inter alia*, methods, sensor elements, detection systems, kits, and compositions involving various differentially expressed RNA biomarkers suitable for use in diagnosing, monitoring, and treating traumatic brain injury (TBI), including mild traumatic brain injury (mTBI), in a human subject.

[00104] The sequences and accession numbers for these RNA biomarkers are provided in Table 1 below:

Table 1

RNA Biomarker	Sequence	Accession No.
put-miR-410	ggagaatagaacatgctgatt (SEQ ID No. 70)	
put-miR-1306	ataacgtcatctagtgtg (SEQ ID No. 71)	
put-miR-806	cacgagagaacgcacacc (SEQ ID No. 72)	
put-miR-71	gacctgggtgggtcgtggt (SEQ ID No. 73)	
put-miR-468	ataaggaaactgctcttc (SEQ ID No. 74)	
put-miR-476	gtggtcaggtagagaa (SEQ ID No. 75)	
put-miR-293	gggtaaaactgcagtggcggtgtag (SEQ ID No. 76)	
put-miR-742	cagggtgtgctaact (SEQ ID No. 77)	
put-miR-6	gcggacctgtctcaagg (SEQ ID No. 78)	
put-miR-1207	gtgaaaagacataggggg (SEQ ID No. 79)	
put-miR-1084	aggaccattgcgtgcc (SEQ ID No. 80)	
put-miR-92	agagactgactttgagta (SEQ ID No. 81)	
put-miR-188	aatggcgatactcagg (SEQ ID No. 82)	

RNA Biomarker	Sequence	Accession No.
put-miR-1352	tggattgtggggaacc (SEQ ID No. 83)	
put-miR-1146	caactgtaagtcatt (SEQ ID No. 84)	
put-miR-209	ggtcctcgatcgcc (SEQ ID No. 85)	
put-miR-961	tagcttgatccagttg (SEQ ID No. 86)	
put-miR-1080	tgtggattgatgctct (SEQ ID No. 87)	
put-miR-750	caagagatgaggaatg (SEQ ID No. 88)	
put-miR-1003	tggacttcagaacagc (SEQ ID No. 89)	
put-miR-1098	aaggcgaaggatatgtg (SEQ ID No. 90)	
hsa-miR-934	tgtctactctggagacactgg (SEQ ID No. 91)	
hsa-miR-142-5p	cataaagtagaaagcactact (SEQ ID No. 92)	
hsa-miR-6748-3p	tctgtccctgtctctacag (SEQ ID No. 93)	MIMAT0027397
hsa-miR-1271-5p	cttgccacntagcaagcactca (SEQ ID No. 94)	MIMAT0005796
hsa-miR-34b-3p	caatcactaactccactgccat (SEQ ID No. 95)	MIMAT0004676
hsa-miR-449a	tggcagtgtattgttagctgtg (SEQ ID No. 96)	MIMAT0001541
hsa-miR-143-3p	tgagatgaagcactgtagctc (SEQ ID No. 48)	MIMAT0000435
hsa-miR-135b-5p	tatggcttttcattcctatgtga (SEQ ID No. 40)	MIMAT0000758
U6.428	aagattagcatgaggatgacacgcaaattcgtgaagcgttccatttc ttt (SEQ ID No. 97)	
RNU6-4	ggcccctgcacaggatgacacgcaaattcgtgaagcgttccatat ttt (SEQ ID No. 98)	
RNU6-7	ggcccctgcgcaaggatgacatgcaaattcgtgaagcgttccatatt ttt (SEQ ID No. 99)	
RNU6-6	ggcccctgtgcaaggatgacacgcaaattcgtgaagcgttccatatt ttt (SEQ ID No. 100)	
RNU6-45	ggcccctgtgcaaggatgacacgcaaattcgtgaagcgttccatatt ttt (SEQ ID No. 101)	
RNU6-73	ggcccctgtgcaaggatgacatgcaaattcgtgaagcgttccatatt ttt (SEQ ID No. 102)	
Y_RNA.255	gugucaccaacguugguauacaacccccacacuaauuug acuggcuu (SEQ ID No. 103)	
U6.375	ggcccctgtgcaagaatgactcgcaaattcgtgaagcgttccatatt ttt (SEQ ID No. 104)	
U6.1249	gatggcatgaccctgatcaaggacggcatgcaaatttgtgaagta tttc (SEQ ID No. 105)	

[00105] According to an aspect of the present disclosure, there is provided a method of diagnosing and treating traumatic brain injury (TBI) in a human subject in need thereof, the method comprising: obtaining a saliva sample from the subject; contacting the saliva sample with a probe comprising a nucleic acid able to bind to at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-

188, put-miR-1352, put-miR-1080, put-miR-750, put-miR-1003, put-miR-1098, hsa-miR-934, and hsa-miR-142-5p; determining an amount of the at least one RNA biomarker in the saliva sample; identifying the subject as having TBI where the amount of the at least one RNA biomarker is increased or decreased relative to a predetermined threshold value or relative to the amount of the RNA biomarker in a control sample; and
5 treating the subject identified as having TBI according to one or more of the following: subjecting the subject to a verbal, cognitive, motor, or optical test, or any combination of the foregoing; subjecting the subject to diagnostic imaging in the form of a CT or MRI, or a combination thereof; and/or administering to the subject one or more neuroprotective
10 therapies.

[00106] Examples of neuroprotective therapies that may be chosen by a clinician for treating TBI can include, without limitation, statins, progesterone, corticosteroids, cell cycle inhibitors (such as flavopiridol, a semi-synthetic flavonoid, and purine analogs roscovitine and olomoucine), autophagy inhibitors including caspase inhibitors (such as the tetrapeptide
15 caspase-3 inhibitor (z-DEVD-fmk), the pan-caspase peptide inhibitor Boc-aspartyl fluoromethylketone, and Boc-aspartyl fluoromethylketone), inhibitors of poly (ADP-ribose) polymerase (PARP), anti-inflammatory agents (such as minocycline and anti-inflammatory cytokines (e.g., IL-10) or interleukin-1 receptor antagonist (IL-1ra)), sulfonylurea receptor 1 (SUR1)-regulated calcium channel inhibitors (such as glibenclamide), Substance P (SP)
20 antagonists, diketopiperazines, and cyclosporine A.

[00107] In some embodiments, the saliva sample is obtained during a period of time after injury selected from immediately after the injury to any period post-injury.

[00108] In some embodiments, the saliva sample is obtained during a period of time after injury selected from immediately after the injury until the expression level of at least
25 one of the RNA biomarkers returns to the predetermined threshold value or to the amount of the RNA biomarker in the control sample.

[00109] In some embodiments, the saliva sample is obtained during a period of time after injury selected from immediately after the injury to up to 1 year, up to 10 months, up to 8 months, up to 6 months, up to 5 months, up to 4 months, up to 3 months, up to 2 months, up
30 to 1 month, up to 25 days, up to 20 days, up to 15 days, up to 7 days, up to 5 days, up to 3 days, up to 2 days, and/or up to 24 hours post-injury.

[00110] In some embodiments, the saliva sample is obtained during a period of time after injury selected from immediately after the injury to 15 days, from 1 hour to 15 days, from 24 hours to 15 days, from 24 hours to 7 days, and from 2 to 5 days.

- [00111] In some embodiments, the method further comprises obtaining one or more additional saliva samples from the subject at one or more additional times after the injury and repeating the detecting and amplifying steps for each additional sample.
- 5 [00112] In some embodiments, the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury to any period post-injury.
- [00113] In some embodiments, the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury until the expression level of at least one of the RNA biomarkers returns to the predetermined
- 10 threshold value or to the amount of the RNA biomarker in the control sample.
- [00114] In some embodiments, the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury to up to 1 year, up to 10 months, up to 8 months, up to 6 months, up to 5 months, up to 4 months, up to 3 months, up to 2 months, up to 1 month, up to 25 days, up to 20 days, up to 15 days, up to 7
- 15 days, up to 5 days, up to 3 days, up to 2 days, and/or up to 24 hours post-injury.
- [00115] In some embodiments, the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury to 15 days, from 1 hour to 15 days, from 24 hours to 15 days, from 24 hours to 7 days, and from 2 to 5 days.
- 20 [00116] In some embodiments, the one or more additional saliva samples is obtained at day 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 after the injury.
- [00117] In some embodiments, the detecting an amount of the at least one RNA biomarker is performed using a PCR-based assay, a light array assay, a laminar flow chip assay, or any assay suitable for detecting that at least one RNA biomarker.
- 25 [00118] In some embodiments, when using the PCR-based assay the predetermined threshold is equivalent to a fold change of 1.5 or more using the 2-delta delta CT (2- $\Delta\Delta$ CT) method.
- [00119] In some embodiments, the predetermined threshold is equivalent to a fold change of 2 or more using the 2-delta delta CT (2- $\Delta\Delta$ CT) method.
- 30 [00120] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.
- [00121] In some embodiments, the method further comprises identifying the human subject as being fit for normal activity after undergoing successful treatment for TBI.

[00122] In another aspect of the present disclosure, there is provided a method of diagnosing and/or monitoring traumatic brain injury (TBI) in a subject, the method comprising determining a level of at least one RNA biomarker in a saliva sample obtained from the subject, wherein the at least one RNA biomarker is selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, put-miR-1352, put-miR-1080, put-miR-750, put-miR-1003, put-miR-1098, hsa-miR-934, and hsa-miR-142-5p, or any combination thereof.

[00123] In some embodiments, either an upregulated level or a downregulated level of the at least one RNA biomarker is indicative of TBI.

[00124] In some embodiments, the subject is diagnosed as having TBI if the level of the at least one RNA biomarker is either above or below a predetermined threshold or increased or decreased relative to a control.

[00125] In some embodiments, the method further comprises identifying the human subject as being fit for normal activity after undergoing successful treatment for TBI.

[00126] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00127] In another aspect of the present disclosure, there is provided a sensor element for a detection system for diagnosing and/or monitoring TBI, the sensor element comprising a substrate functionalized with a probe specific for at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, put-miR-1352, put-miR-1080, put-miR-750, put-miR-1003, put-miR-1098, hsa-miR-934, and hsa-miR-142-5p.

[00128] In some embodiments, the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.

[00129] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.

5 [00130] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, and 105.

10 [00131] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00132] In another aspect of the present disclosure, there is provided a detection system for diagnosing and/or monitoring TBI, comprising a sensor element according to the present disclosure, and a detection device capable of detecting the binding of a target RNA biomarker to the probe.

15 [00133] In some embodiments, the detection system further comprises means to determine whether the target RNA biomarker is upregulated or downregulated.

[00134] In another aspect of the present disclosure, there is provided a method for determining a course of action for a subject suspected of having TBI, comprising applying a saliva sample obtained from the subject to a detection system according to the present disclosure, and if an upregulated level or a downregulated level of the at least one RNA biomarker is detected, providing a treatment for TBI.

20 [00135] In another aspect of the present disclosure, there is provided a method of treating a subject with suspected TBI, the method comprising determining whether an upregulated level or a downregulated level of at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, put-miR-1352, put-miR-1080, put-miR-750, put-miR-1003, put-miR-1098, hsa-miR-934, and hsa-miR-142-5p is detectable in a saliva sample obtained from the subject, and if an upregulated level or a downregulated level of at least one RNA biomarker is detected, providing treatment for TBI to the subject.

[00136] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00137] In another aspect of the present disclosure, there is provided a method of
5 detecting an RNA biomarker in a saliva sample, the method comprising obtaining a saliva sample from a human subject, contacting the saliva sample with at least one oligonucleotide primer complementary to at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-
10 miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, put-miR-1352, put-miR-1080, put-miR-750, put-miR-1003, put-miR-1098, hsa-miR-934, and hsa-miR-142-5p; amplifying the at least one RNA biomarker using a polymerase chain reaction; and detecting the amplified RNA
15 biomarker.

[00138] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00139] In another aspect of the present disclosure, there is provided a kit for use in a
20 method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the kit comprising at least one probe specific for at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-
25 449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, put-miR-1352, put-miR-1080, put-miR-750, put-miR-1003, put-miR-1098, hsa-miR-934, and hsa-miR-142-5p.

[00140] In some embodiments, the probe comprises a nucleic acid able to bind to the at
30 least one RNA biomarker.

[00141] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.

[00142] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, and 105.

5 [00143] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00144] In another aspect of the present disclosure, there is provided a composition for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a
10 human subject, the composition comprising at least one probe specific for at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-
15 1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, put-miR-1352, put-miR-1080, put-miR-750, put-miR-1003, put-miR-1098, hsa-miR-934, and hsa-miR-142-5p.

[00145] In some embodiments, the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.

20 [00146] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.

[00147] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 70, 71, 72, 73, 74, 75, 76,
25 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, and 105.

[00148] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

30 [00149] It will be appreciated that statements made herein in relation to any aspect of the invention may equally apply to any other aspect of the invention, as appropriate. Furthermore, the various aspects and embodiments of the present disclosure can also be used in combination with various techniques, methods, systems, compositions, devices, and/or

disclosures as described in WO2017/153710A1 and WO2018/138468A1, the entire disclosures of which are hereby incorporated by reference herein.

Further Additional Aspects and Embodiments

5 [00150] In further aspects, the present disclosure provides, *inter alia*, methods, sensor elements, detection systems, kits, and compositions involving various differentially expressed RNA biomarkers suitable for use in diagnosing, monitoring, and treating traumatic brain injury (TB), including mild traumatic brain injury (mTBI), in a human subject.

[00151] As shown in **Example 1** (below), in certain embodiments, the suitable RNA
10 biomarkers were identified according to an MiRNA qPCR validation study performed in 176 saliva baseline samples (B); 42 samples from concussed players after injury (group Ca); 52 samples from concussed players post-match (group Cb) and 54 concussed players after 36-48h (group Cc); 61 uninjured players post-match (group Ub); 45 uninjured players 36-48h (group Uc); 30 musculoskeletal injury players post-match (group Mb); 24 musculoskeletal
15 injury players 36-48h (group Mc).

[00152] The sequences and accession numbers for these RNA biomarkers are provided in **Table 2** below:

Table 2

RNA Biomarker	Sequence	miRBase Accession No.	SEQ ID NO.
hsa-let-7a-5p	ugagguaguagguuguauaguu	MIMAT0000062	106
hsa-let-7b-5p	ugagguaguagguuguguguu	MIMAT0000063	107
hsa-let-7f-5p	ugagguaguagauuguauaguu	MIMAT0000067	108
<i>hsa-let-7i-5p</i>	ugagguaguaguugugcuguu	MIMAT0000415	29
hsa-miR-103a-3p	agcagcauuguacagggcuauga	MIMAT0000101	109
<i>hsa-miR-107</i>	agcagcauuguacagggcuauc	MIMAT0000104	39
hsa-miR-1246	aauggauuuuuggagcagg	MIMAT0005898	110
<i>hsa-miR-126-3p</i> (<i>miR-126*</i>)	ucguaccgugaguaauaauagcg	MIMAT0000445	111
<i>hsa-miR-126-5p</i> (= <i>miR-126*</i>)	cauuauuacuuuugguacgcg	MIMAT0000444	14
<i>hsa-miR-135b-5p</i>	uauggcuuuuacauuccuauugga	MIMAT0000758	40
<i>hsa-miR-142-3p</i>	uguaguguuuuccuacuuuauugga	MIMAT0000434	26
	cauaaaguagaaagcacuacu		112
hsa-miR-142-5p		MIMAT0000433	
<i>hsa-miR-143-3p</i> (= <i>miR-143</i>)	ugagaugaagcacuguagcuc	MIMAT0000435	48
<i>hsa-miR-144-3p</i> (<i>miR-144*</i>)	uacaguauagaugauguacu	MIMAT0000436	113

RNA Biomarker	Sequence	miRBase Accession No.	SEQ ID NO.
<i>hsa-miR-144-5p</i> (= <i>miR-144*</i>)	ggauaucaucuauacuguaag	MIMAT0004600	16
<i>hsa-miR-148a-3p</i>	ucagugcacuacagaacuuugu	MIMAT0000243	27
<i>hsa-miR-16-1-3p</i>	ccaguauuaacugugcugcuga	MIMAT0004489	114
<i>hsa-miR-206</i>	uggaauuaaggaagugugugg	MIMAT0000462	115
<i>hsa-miR-21-5p</i>	uagcuuauacagacugauguuga	MIMAT0000076	1
<i>hsa-miR-29c-3p</i>	uagcaccauuugaaaucgguua	MIMAT0000681	116
<i>hsa-miR-339-5p</i>	ucccuguccuccaggagcucacg	MIMAT0000764	117
<i>hsa-miR-34b-3p</i>	caaucacuaacuccacugccau	MIMAT0004676	118
<i>hsa-miR-425-5p</i>	aaugacacgaucacuccguuga	MIMAT0003393	2
<i>hsa-miR-449a</i>	uggcaguguaauuguuagcuggu	MIMAT0001541	119
<i>hsa-miR-497-5p</i>	cagcagcacacuguguuugu	MIMAT0002820	120
<i>hsa-miR-671-3p</i>	uccgguucucagggcuccacc	MIMAT0004819	23
<i>hsa-miR-6748-3p</i>	uccugucccugucuccuacag	MIMAT0027397	121
<i>hsa-miR-92a-3p</i>	uauugcacuuguccggcugu	MIMAT0000092	122
<i>hsa-miR-934</i>	ugucuacuacuggagacacugg	MIMAT0004977	123
put-miR-1003	tggacttcagaacagc		89
put-miR-1080	tgtggattgatgctct		87
put-miR-1084	aggaccattgcgttgcc		80
put-miR-1146	caactgtaagtccatt		84
put-miR-1204	agggctgggcacggggg		124
put-miR-1207	gtgaaaagacataggggg		79
put-miR-1306	ataacgtcatctagtgtg		71
put-miR-188	aaatggcgatactcagg		82
put-miR-209	ggtcctcgatcgcc		85
put-miR-323	ataaatggcgcttgagg		125
put-miR-325	ttcaaatcccacttctgacacca		126
put-miR-444	ctgaaaaggggacggattgggaa		127
put-miR-465	ccagttgtcgtgggtttt		128
put-miR-468	ataaggaaactgctctctc		74
put-miR-469	gcgggggattagctcagctggg		129
put-miR-476	gtggtcaggtagagaa		75
put-miR-594	tgatttttttggcgaa		130
put-miR-6	gcggacctgtctcaagg		78
put-miR-71	gaccttgggttgggtcgtggt		73
put-miR-742	cagggtctgtgctaact		77
put-miR-806	cacgagagaacgcacacc		72
put-miR-856	aaggataggaggtattt		131
put-miR-893	accatcctctgctacca		132
put-miR-92	agagactgactttgagta		81
put-miR-958	acagggtgtgcaaaaa		133
put-miR-961	tagcttgatccagttg		86
RNU4-6p	tatcgtagccaatgaggttatccgag gcgtgattattgctaattgaaaa		134
RNU6-4	ggcccctgcacagggtacacgca aatcgtgaagcgttccatattttt		98

RNA Biomarker	Sequence	miRBase Accession No.	SEQ ID NO.
RNU6-45	ggcccttgcaaggatgacacgcaa attcgtgaagcggtccatatttt		101
RNU6-6	ggcccttgcaaggatgacacgca aattcgtgaagcggtccatatttt		100
RNU6-7	ggccctgcgcaaggatgacatgca aattcgtgaagcggtccatatttt		99
RNU6-73	ggcccttgcaaggatgacatgcaa attcgtgaagcggtccatatttt		102
SNORA57	tgctggcggttcccatccgtgttct atcctcaaacgcccggacaccg		135
SNORD3B-2	cttctcctgattggggagttagagg gagagaacgcggtctgagtggt		136
tRNA120- AlaAGC	gcgcatgcttagcatgcatgaggtccc gggttcgatccccagcatctcca		137
tRNA18-ArgCCT	gcactggcctcctaagccaggattgt gggttcgagtcacacgtgggta		138
tRNA27-MetCAT	gcgtcagttcctaatactgaaggtcct gagttcgagcctcagagagggca		139
tRNA2-LeuTAA	gcataaaacttaaaatttataatcaga gggtcaactcctctcttaaca		140
tRNA73-ArgCCG	gcgtctgattccggatcagaagattga gggttcgagtccttcgtggtcg		141
tRNA8-ThrAGT	gcgcctgtctagtaaacaggagatcct gggttcgaatcccagcgtgcct		142
tRNA9-TyrGTA	cttttgactgtagagcaagaggtccct gggtcaaatccaggttctccct		143
U2.3	tcacttcacgcatgcatctggtattgca gtacctccaggaacagtgcacc		144
U4.64	gtatcgtagccaatgaggtttatccaa gggtcgattattgctaattgaaa		145
U6.1249	gatggcatgaccctgatcaaggacg gcatgcaaatttggaagtatttc		105
U6.168	atggccctgcgcaaggatgacacg caaatttggaaggattccatattt		146
U6.375	ggcccttgcaagaatgactcgcaa attcgtgaagcggtccatatttt		104
U6.428	aagattagcatgaggatgacacgcaa attcgtgaagcggtccattcttt		97
U6.601	ggccctgcgcaaggatgacatgca aatttggaagtgtccatatttt		147
UC022CJG1	cattgatcatgacacttcgaacgcac ttg		148
YRNA-245	gtctttgtgaactcttccctcttctcat tactgtacttgaccagtct		149
YRNA-255	gugucaccaacguugguauacaac ccccacaacuaauuugacuggc uu		103

RNA Biomarker	Sequence	miRBase Accession No.	SEQ ID NO.
YRNA-684	gcttcttttactctttcccttcattctcact actgtacctgattcgtctt		150

[00153] As discussed in more detail in **Example 1** (below), these biomarkers were found to be differentially expressed and statistically significant between the different group comparisons and the different time points (Ca vs Ub; Cb vs Ub; Cc vs Uc; Ca vs Mb; Cb vs Mb; Cc vs Mc; Ca vs U+M b; Cb vs U+M b; Cc vs U+M c; Ca vs B; Cb vs B; Cc vs B).

[00154] According to an aspect of the present disclosure, there is provided a method of diagnosing and treating traumatic brain injury (TBI) in a human subject in need thereof, the method comprising: obtaining a saliva sample from the subject; contacting the saliva sample with a probe comprising a nucleic acid able to bind to at least one RNA biomarker selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-let-7i-5p, hsa-miR-103a-3p, hsa-miR-107, hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-148a-3p, hsa-miR-16-1-3p, hsa-miR-206, hsa-miR-21-5p, hsa-miR-29c-3p, hsa-miR-339-5p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-497-5p, hsa-miR-671-3p, hsa-miR-6748-3p, hsa-miR-92a-3p, hsa-miR-934, put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1204, put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-468, put-miR-469, put-miR-476, put-miR-594, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-856, put-miR-893, put-miR-92, put-miR-958, put-miR-961, RNU4-6p, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.1249, U6.168, U6.375, U6.428, U6.601, UC022CJG1, YRNA-245, YRNA-255, and YRNA-684; determining an amount of the at least one RNA biomarker in the saliva sample; identifying the subject as having TBI where the amount of the at least one RNA biomarker is increased or decreased relative to a predetermined threshold value or relative to the amount of the RNA biomarker in a control sample; and treating the subject identified as having TBI according to one or more of the following: administering to the subject one or more neuroprotective therapies.

[00155] Examples of neuroprotective therapies that may be chosen by a clinician for treating TBI can include, without limitation, statins, progesterone, corticosteroids, cell cycle

inhibitors (such as flavopiridol, a semi-synthetic flavonoid, and purine analogs roscovitine and olomoucine), autophagy inhibitors including caspase inhibitors (such as the tetrapeptide caspase-3 inhibitor (z-DEVD-fmk), the pan-caspase peptide inhibitor Boc-aspartyl fluoromethylketone, and Boc-aspartyl fluoromethylketone), inhibitors of poly (ADP-ribose) polymerase (PARP), anti-inflammatory agents (such as minocycline and anti-inflammatory cytokines (e.g., IL-10) or interleukin-1 receptor antagonist (IL-1ra)), sulfonylurea receptor 1 (SUR1)-regulated calcium channel inhibitors (such as glibenclamide), Substance P (SP) antagonists, diketopiperazines, and cyclosporine A.

[00156] In some embodiments, the method further comprises identifying the human subject as being fit for normal activity after undergoing successful treatment for TBI.

[00157] In another aspect of the present disclosure, there is provided a method of diagnosing and/or monitoring traumatic brain injury (TBI) in a subject, the method comprising determining a level of at least one RNA biomarker in a saliva sample obtained from the subject, wherein the at least one RNA biomarker is selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-let-7i-5p, hsa-miR-103a-3p, hsa-miR-107, hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-148a-3p, hsa-miR-16-1-3p, hsa-miR-206, hsa-miR-21-5p, hsa-miR-29c-3p, hsa-miR-339-5p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-497-5p, hsa-miR-671-3p, hsa-miR-6748-3p, hsa-miR-92a-3p, hsa-miR-934, put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1204, put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-468, put-miR-469, put-miR-476, put-miR-594, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-856, put-miR-893, put-miR-92, put-miR-958, put-miR-961, RNU4-6p, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG SNORD3B-2, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.1249, U6.168, U6.375, U6.428, U6.601, UC022CJG1, YRNA-245, YRNA-255, and YRNA-684, or any combination thereof.

[00158] In some embodiments, either an upregulated level or a downregulated level of the at least one RNA biomarker is indicative of TBI.

[00159] In some embodiments, the subject is diagnosed as having TBI if the level of the at least one RNA biomarker is either above or below a predetermined threshold or increased or decreased relative to a control.

[00160] In some embodiments, the method further comprises identifying the human subject as being fit for normal activity after undergoing successful treatment for TBI.

[00161] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00162] In another aspect of the present disclosure, there is provided a sensor element for a detection system for diagnosing and/or monitoring TBI, the sensor element comprising a substrate functionalized with a probe specific for at least one RNA biomarker selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-let-7i-5p, hsa-miR-103a-3p, hsa-miR-107, hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-148a-3p, hsa-miR-16-1-3p, hsa-miR-206, hsa-miR-21-5p, hsa-miR-29c-3p, hsa-miR-339-5p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-497-5p, hsa-miR-671-3p, hsa-miR-6748-3p, hsa-miR-92a-3p, hsa-miR-934, put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1204, put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-468, put-miR-469, put-miR-476, put-miR-594, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-856, put-miR-893, put-miR-92, put-miR-958, put-miR-961, RNU4-6p, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.1249, U6.168, U6.375, U6.428, U6.601, UC022CJG1, YRNA-245, YRNA-255, and YRNA-684.

[00163] In some embodiments, the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.

[00164] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.

[00165] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 1, 2, 14, 16, 23, 26, 27, 29, 39, 40, 48, 71, 72, 73, 74, 75, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 89, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150.

[00166] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00167] In another aspect of the present disclosure, there is provided a detection system for diagnosing and/or monitoring TBI, comprising a sensor element according to the present disclosure, and a detection device capable of detecting the binding of a target RNA biomarker to the probe.

[00168] In some embodiments, the detection system further comprises means to determine whether the target RNA biomarker is upregulated or downregulated.

[00169] In another aspect of the present disclosure, there is provided a method for determining a course of action for a subject suspected of having TBI, comprising applying a saliva sample obtained from the subject to a detection system according to the present disclosure, and if an upregulated level or a downregulated level of the at least one RNA biomarker is detected, providing a treatment for TBI.

[00170] In another aspect of the present disclosure, there is provided a method of treating a subject with suspected TBI, the method comprising determining whether an upregulated level or a downregulated level of at least one RNA biomarker selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-let-7i-5p, hsa-miR-103a-3p, hsa-miR-107, hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-148a-3p, hsa-miR-16-1-3p, hsa-miR-206, hsa-miR-21-5p, hsa-miR-29c-3p, hsa-miR-339-5p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-497-5p, hsa-miR-671-3p, hsa-miR-6748-3p, hsa-miR-92a-3p, hsa-miR-934, put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1204, put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-468, put-miR-469, put-miR-476, put-miR-594, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-856, put-miR-893, put-miR-92, put-miR-958, put-miR-961, RNU4-6p, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.1249, U6.168, U6.375, U6.428, U6.601, UC022CJG1, YRNA-245, YRNA-255, and YRNA-684 is detectable in a saliva sample obtained from the subject, and if an upregulated level or a downregulated level of at least one RNA biomarker is detected, providing treatment for TBI to the subject.

[00171] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00172] In another aspect of the present disclosure, there is provided a method of
 5 detecting an RNA biomarker in a saliva sample, the method comprising obtaining a saliva sample from a human subject, contacting the saliva sample with at least one oligonucleotide primer complementary to at least one RNA biomarker selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-let-7i-5p, hsa-miR-103a-3p, hsa-miR-107, hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-126-5p (=miR-126*), hsa-miR-135b-
 10 5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-148a-3p, hsa-miR-16-1-3p, hsa-miR-206, hsa-miR-21-5p, hsa-miR-29c-3p, hsa-miR-339-5p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-497-5p, hsa-miR-671-3p, hsa-miR-6748-3p, hsa-miR-92a-3p, hsa-miR-934, put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1204, put-
 15 miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-468, put-miR-469, put-miR-476, put-miR-594, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-856, put-miR-893, put-miR-92, put-miR-958, put-miR-961, RNU4-6p, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA,
 20 tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.1249, U6.168, U6.375, U6.428, U6.601, UC022CJG1, YRNA-245, YRNA-255, and YRNA-684; amplifying the at least one RNA biomarker using a polymerase chain reaction; and detecting the amplified RNA biomarker.

[00173] In some embodiments, the at least one RNA biomarker further comprises one
 25 or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00174] In another aspect of the present disclosure, there is provided a kit for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the kit comprising at least one probe specific for at least one RNA biomarker selected
 30 from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-let-7i-5p, hsa-miR-103a-3p, hsa-miR-107, hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-148a-3p, hsa-miR-16-1-3p, hsa-miR-206, hsa-miR-21-5p, hsa-miR-29c-3p, hsa-miR-339-5p, hsa-miR-34b-

- 3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-497-5p, hsa-miR-671-3p, hsa-miR-6748-3p, hsa-miR-92a-3p, hsa-miR-934, put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1204, put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-468, put-miR-469, put-miR-476, put-miR-594, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-856, put-miR-893, put-miR-92, put-miR-958, put-miR-961, RNU4-6p, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.1249, U6.168, U6.375, U6.428, U6.601, UC022CJG1, YRNA-245, YRNA-255, and YRNA-684.
- [00175]** In some embodiments, the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.
- [00176]** In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.
- [00177]** In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 1, 2, 14, 16, 23, 26, 27, 29, 39, 40, 48, 71, 72, 73, 74, 75, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 89, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150.
- [00178]** In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.
- [00179]** In another aspect of the present disclosure, there is provided a composition for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the composition comprising at least one probe specific for at least one RNA biomarker selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-let-7i-5p, hsa-miR-103a-3p, hsa-miR-107, hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-148a-3p, hsa-miR-16-1-3p, hsa-miR-206, hsa-miR-21-5p, hsa-miR-29c-3p, hsa-miR-339-5p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-497-5p, hsa-miR-671-3p, hsa-miR-6748-3p, hsa-miR-92a-3p, hsa-miR-934, put-miR-1003, put-miR-1080, put-miR-

1084, put-miR-1146 (2), put-miR-1204, put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-468, put-miR-469, put-miR-476, put-miR-594, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-856, put-miR-893, put-miR-92, put-miR-958, put-miR-961, RNU4-6p, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.1249, U6.168, U6.375, U6.428, U6.601, UC022CJG1, YRNA-245, YRNA-255, and YRNA-684.

[00180] In some embodiments, the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.

[00181] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.

[00182] In some embodiments, the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 1, 2, 14, 16, 23, 26, 27, 29, 39, 40, 48, 71, 72, 73, 74, 75, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 89, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150.

[00183] In some embodiments, the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00184] It will be appreciated that statements made herein in relation to any aspect of the invention may equally apply to any other aspect of the invention, as appropriate.

EXAMPLES

[00185] Embodiments of the invention will now be described by way of example.

Example 1

Salivary Non-Coding RNA:

Next Generation Biomarkers in Concussed Rugby Football Union Players

Study design

- [00186] The Study of Concussion in Rugby Union through MicroRNAs (SCRUM study) is a prospective observational study of players (up to 1100) in the two highest tiers of senior professional domestic rugby competition in England, the Premiership and Championship. The study is embedded within the ongoing RFU Injury Surveillance Programme for the 2017-2018 seasons.
- [00187] All players, previous written consent, were asked to provide a single baseline samples of approximately 2mL of saliva during the preseason preparations (group B), before the training sessions if possible, at their clubs as well as completing a consent form detailing the processing and handling of their samples, the anonymisation of their data and their right to withdraw their voluntary consent at any time. The investigators recorded the timing of these baseline extractions in relation to preseason training sessions, other injuries, or supplement/medication use.
- [00188] During the season each time a player entered the Head Injury Assessment (HIA) process (group C), he was asked to provide a saliva sample. Players who had been removed during the match for a pitch side assessment, provided a sample during this medical evaluation, called HIA1, and constituted group Ca. If players exhibited clear signs of concussion, also known as category 1 signs, no pitch side evaluation was necessary - in accordance with World Rugby guidance – and the player was immediately and permanently removed from the match. These players formed the “Immediate Permanent Removal” group (IPR). All players evaluated pitch-side or immediately and permanently removed for concussion were subsequently assessed immediately after the match (HIA2) and at 36-48h post-match (HIA3). Samples were collected at each of these assessments. Therefore, we obtained saliva samples for groups C and IPR immediately post-match (group Cb and IPRb respectively) and 36h-48h HIA (group Cc and IPRc respectively).
- [00189] The HIAs at each time point take the form of a modified Sport Concussion Assessment Tool 5th Edition (SCAT5) which has been well studied as a diagnostic tool in the context of sports-related concussion and is complemented by baseline performance data in these assessments collected by the clubs during their preseason preparations. In this way, we investigated the presence of concussion diagnostic miRNAs noninvasively at multiple time points, which were matched to a standardised clinical assessment and diagnostic threshold for concussion performed by a clinician with experience in rugby head injuries. All IPR players were deemed to have been concussed. Group C players were deemed to be concussed if they failed the HIA at any time point (HIA1, HIA2 or HIA3); otherwise they were cleared of

concussion. Players who entered the HIA process for suspected concussion but were cleared were analysed separately.

[00190] Players diagnosed with concussion were also asked for a sample of saliva at the point at which they were cleared by their team physician to return to competitive play (Stage VI) (group Cd and IPRd), as per the Berlin Consensus Graduated Return-to-Play (GRTP) protocol.

[00191] Whenever a player was removed for an HIA, club medical teams also approached an “uninjured control” (group U) player for saliva samples after the game (group Ub) and at 36-48 hours later (group Uc). An “uninjured control” is a non-concussed player who has played approximately a matched number of minutes in the same game and same playing position of the concussed player whenever possible. This allows for the description of miRNA profiles in response to the physical stress of athletic activity and exposure to the likely subconcussive blows, which come from participation in a collision sport.

[00192] To rule out contamination from non-neurological injuries and musculoskeletal trauma, club medics collected samples from players removed from the field of play due to musculoskeletal injuries (orthopaedic controls) (group M) at the same time points as the “uninjured control” group (group Mb and group Mc).

Ethics and dissemination

[00193] The SCRUM study runs under University of Birmingham (UoB) ethics (ERN_11-0429AP28) as part of the Repetitive Concussion in Sport (RECOS) study. This study was approved by the East of England - Essex Research Ethics Committee (REC) on 22 September 2017 – REC 17/EE/0275; IRAS 216703.

Saliva collection

[00194] The samples were collected in Oragene®-RNA RE-100 saliva self-collection kit (DNA Genotek) which contains a RNA stabilizing solution for a total of 8 weeks. Saliva was collected from each participant at enrollment following orally rinsing with tap water. The samples were then transported to the University of Birmingham where they were processed in line with the sample pot manufacturer’s guidance to allow freezing and storage.

Materials and Methods

Next Generation Sequencing- discovery phase

[00195] All experiments were conducted at QIAGEN Genomic Services, Germany.

RNA preparation

[00196] RNA preparation was performed according to Oragene recommendations, except the use of the miRNeasy kit (Qiagen, Germany) instead of the RNeasy kit.

5

Next Generation SequencingLibrary preparation and sequencing

[00197] The library preparation was done using the QIAseq miRNA Library Kit (QIAGEN). A total of 5ul total RNA was converted into microRNA NGS libraries. Adapters containing UMIs were ligated to the RNA. Then RNA was converted to cDNA. The cDNA as amplified using PCR (22 cycles) and during the PCR indices were added. After PCR the samples were purified. Library preparation QC was performed using either Bioanalyzer 2100 (Agilent) or TapeStation4200 (Agilent). Based on quality of the inserts and the concentration measurements the libraries were pooled in equimolar ratios. The library pools were quantified using the qPCR ExiSEQ LNA™ Quant kit (Exiqon). The library pools were then sequenced on a NextSeq500 sequencing instrument according to the manufacturer instructions (NEBNext Multiplex Small RNA Library Prep Set for Illumina) to make approximately 163-175 base-pair sized libraries. Raw data as demultiplexed and FASTQ files for each sample were generated using the bcl2fastq software (Illumina inc.). FASTQ data were checked using the FastQC tool (www.bioinformatics.babraham.ac.uk/projects/fastqc/).

15
20Trimming

[00198] Cutadapt was used to extract information of adapter and UMI in raw reads, and output from cutadapt was used to remove adapter sequences and to collapse reads by UMI with inhouse script.

25

UMI correction

[00199] According to the experiment protocol, each raw read was expected to contain, starting from 5' end, an insert sequence, the adapter sequence, 12nt-long UMI sequence, and other ligated sequence. Depending on the read length and insert length, not all parts were present in all reads. To correct PCR bias with UMI information, raw reads were processed as described below:

30

[00200] 1. Use cutadapt on raw reads with provided adapter sequence to acquire output with information about the presence of adapter for each read.

[00201] 2. Parse cutadapt output and keep only reads that fulfil all of the following requirements:

- Reads contain adapters.
- Insert sequence should be equal or larger than minimal insert length (default 16nt).
- UMI sequence should be equal or longer than minimal UMI length (default 10nt).

[00202] 3. Extract insert sequences from reads which do not contain full length UMI sequence from step 2 output as partial-UMI reads.

10 [00203] 4. Examine reads with full length UMI in step 2 output and identify all unique insert+UMI combinations. Extract insert sequences from unique insert + UMI combinations as full-UMI reads.

[00204] 5. Combine partial-UMI reads and full-UMI reads as output of UMI correction.

15

Mapping

[00205] A reference profile of sequencing data for each sample was obtained using the whole human genome sequence GRCh37, downloaded from the Genome Reference Consortium and mirbase_20 as annotation reference. Reads were aligned to the miRbase using Bowtie2. The mapping criteria for aligning reads to spike-ins, abundant sequence and miRBase were the reads have to have perfect match to the reference sequences. For mapping to the genome the restriction of one mismatch was allowed in the first 32 bases of the read. No indels were allowed in mapping. Unaligned reads were mapped against the host reference genome and used as input for mirPara and miRbase to predict putative miRNAs.

25

Differential Expression

[00206] Aligned reads were counted and differential expression analysis, p-values for significantly differentially expressed microRNAs and false discovery rate according to Benjamini-Hochberg were performed with EdgeR. For normalisation, the trimmed mean of M-values (TMM) method based on log-fold and absolute gene-wise changes in expression levels between samples was used.

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Unsupervised analysis

[00207] Principal component analysis is performed with R using TMM-normalized quantifications from defined collections of samples as input.

MiRNA qPCR Data analysis - validation study

- 5 [00208] All experiments were conducted at QIAGEN Genomic Services, Germany.
 [00209] MiRNA qPCR validation was performed in 176 saliva baseline samples (B); 42 samples from concussed players after injury (group Ca); 52 samples from concussed players post-match (group Cb) and 54 concussed players after 36-48h (group Cc); 61 uninjured players post-match (group Ub); 45 uninjured players 36-48h (group Uc); 30
 10 musculoskeletal injury players post-match (group Mb); 24 musculoskeletal injury players 36-48h (group Mc).

RNA extraction and qPCR

- [00210] Total RNA was extracted from the samples using miRNeasy Serum/Plasma kit
 15 (QIAGEN) after manufacturer's instructions. The purified total RNA was eluted in a final volume of 32 µl. 14 µl RNA was reverse transcribed in 70 µl reactions using the miRCURY LNA RT Kit (QIAGEN). cDNA was diluted 50 x and assayed in 10 µl PCR reactions according to the protocol for miRCURY LNA miRNA PCR; each miRNA was assayed once by qPCR on the miRNA Ready-to-Use PCR, Custom panel using miRCURY LNA SYBR
 20 Green master mix. Negative controls excluding template from the reverse transcription reaction was performed and profiled like the samples. The amplification was performed in a LightCyclerp 480 Real-Time PCR System (Roche) in 384 well plates. The amplification curves were analyzed using the Roche LC software, both for determination of Cq (by the 2nd derivative method) and for melting curve analysis. The amplification efficiency was
 25 calculated using algorithms similar to the LinReg software. All assays were inspected for distinct melting curves and the Tm was checked to be within known specifications for the assay. Furthermore assays must be detected with 0 Cq less than the negative control, and with Cq<37 to be included in the data analysis. Data that did not pass these criteria were omitted from any further analysis. Cq was calculated as the 2nd derivative. Normalization was
 30 performed based on the average of hsa-miR-29c-3p and hsa-let-7b-5p (custom normalizer assays), the two more stable miRs identified across all samples by Normofinder software (33). The formula used to calculate the normalized Cq values is the difference between the custom normalizer assays mean Cq and the assay Cq (miRNA of interest). A higher value indicates that the miRNA is more abundant in that sample.

Statistical Analyses

[00211] All analyses were carried out using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).

5 *Group comparisons*

[00212] We used the two-tailed Independent-Samples T Test procedure to compare means for two groups of cases. The two-tailed Paired-Samples T Test procedure compares the means of two variables for a single group. The procedure computes the differences between values of the two variables for each case and tests whether the average differs from
10 0. Bootstrapping is a method for deriving robust estimates of standard errors and confidence intervals for estimates. Bootstrapping is most useful as an alternative to parametric estimates when the parametric inference is impossible.

[00213] The One-Way ANOVA procedure produces a one-way analysis of variance for a quantitative dependent variable by a single factor (independent) variable. Analysis of
15 variance is used to test the hypothesis that several means are equal. This technique is an extension of the two-sample *t* test. In addition to determining that differences exist among the means, you may want to know which means differ. There are two types of tests for comparing means: a priori contrasts and post hoc tests. Contrasts are tests set up *before* running the experiment, and post hoc tests are run *after* the experiment has been conducted.

20 [00214] General Linear Model Repeated Measures analyzes groups of related dependent variables that represent different measurements of the same attribute. It is possible to define one or more within-subjects factors for use in GLM Repeated Measures.

Discriminant Analysis

25 [00215] The Discriminant analysis (DA) was applied to the Cq values provided by Qiagen.

[00216] The main purpose of DA was to find the dimension(s) along which groups differ and to find classification function(s) to predict group membership from a combination of variables (predictors). In the present case, the two groups were concussed (group C+IPR)
30 and uninjured subjects (group U), and the predictors were a set of biomarkers selected from the NGS study.

[00217] DA works with data that is already classified into groups to derive rules for classifying new (and as yet unclassified) individuals on the basis of their observed variable values.

- [00218] In univariate terms (t-test, Oneway Anova) a significant difference among groups implies that, given a score, you can predict which group it comes from. In DA, there is often an effort to interpret the pattern of differences among the predictors as a whole in an attempt to understand the dimensions along which groups differ reliably.
- 5 [00219] With only two groups, there is only one linear combination of predictors that best separates them. The separation of two group *centroids* (multivariate version of means), represents the best linear combination of predictors that separate the groups 1 and 2. A line parallel that connects the two centroids represents the linear combination or *discriminant function*, a pattern of scores that discriminates between the two groups.
- 10 [00220] There are two facets of DA, and one or both may be emphasized in any given research application. The researcher may be interested in a decision rule for classifying cases where the number of dimensions and their meaning is irrelevant. Or the emphasis may be on interpreting the results of DA in terms of the combinations of predictors (*discriminant functions*) that separate various groups from each other.
- 15 [00221] Classification functions are used to predict group membership for new cases and to check the adequacy of classification for cases in the same sample through cross-validation.
- [00222] The canonical correlation explains the percent of variance accounted by the scores for the discriminant function between these groups. A canonical correlation is a
- 20 multiple-multiple correlation because there are multiple variables on both sides of the regression equation, and for each discriminant function, when squared, indicates the proportion of variance shared between groups and predictors on that function.
- [00223] In **Standard (direct)** DA, like standard multiple regression, all predictors enter the equations at once and each predictor is assigned only the unique association it has
- 25 with groups. Variance shared among predictors contributes to the total relationship, but not to any one predictor.
- [00224] In **Stepwise** DA statistical criteria can be used to produce a reduced set of predictors.
- [00225] All analyses were carried out using SPSS 20.0 (SPSS Inc., Chicago, IL, USA).
- 30

ROC curve analyses

- [00226] The diagnostic performance of a test, or the accuracy of a test to discriminate experimental cases from normal cases is evaluated using Receiver Operating Characteristic

(ROC) curve analysis. ROC curves can also be used to compare the diagnostic performance of two diagnostic tests.

[00227] When one considers the results of a particular test in two populations, one population with a characteristic and the other population without the characteristic, you will rarely observe a perfect separation between the two groups. Indeed, the distribution of the test results will overlap.

[00228] In a ROC curve the true positive rate (Sensitivity) is plotted in function of the false positive rate (100-Specificity) for different cut-off points of a parameter. Each point (cut-off) on the ROC curve represents a sensitivity/specificity pair corresponding to a particular decision threshold. The area under the ROC curve (AUC) is a measure of how well a parameter can distinguish between two diagnostic groups (experimental/normal). A test with perfect discrimination (no overlap in the two distributions) has a ROC curve that passes through the upper left corner (100% sensitivity, 100% specificity). Therefore the closer the ROC curve is to the upper left corner, the higher the overall accuracy of the test.

15

ROC statistics

[00229] *Sensitivity*: probability that a test result will be positive when the characteristic is present (true positive rate, expressed as a percentage).

[00230] *Specificity*: probability that a test result will be negative when the characteristic is not present (true negative rate, expressed as a percentage).

[00231] *Positive likelihood ratio*: ratio between the probability of a positive test result given the presence of the characteristic and the probability of a positive test result given the absence of the characteristic, i.e. = True positive rate / False positive rate = Sensitivity / (1-Specificity)

[00232] *Negative likelihood ratio*: ratio between the probability of a negative test result given the presence of the characteristic and the probability of a negative test result given the absence of the characteristic, i.e. = False negative rate / True negative rate = (1-Sensitivity) / Specificity

30

Results

Sample size

[00233] A total of 906 players were recruited to the study between July 2017 and April 2018. This included players from 11 clubs playing in The Premiership and 11 clubs playing in the IPA Championship. One club in The Premiership chose not to take part after

consultation with the RFU as they were already participating in a concussion research project involving saliva sampling for different purposes run by another organisation. One club in the IPA Championship chose not to take part as they felt they would struggle to fulfil the requirements of the study due to limited numbers of medical staff while another club in the

5 IPA Championship felt decided to withdraw from the study for similar reasons but after their players had provided baseline samples. This made a total of 11 clubs in The Premiership and 10 clubs in the IPA Championship participating in collecting samples through the season from head injury events.

[00234] Overall, a total of 229 head injury incidents took place across both

10 competitions. This figure is an amalgamation of incidents in which the player was immediately and permanently removed from play due to exhibiting “Category 1” signs and symptoms, as well as all of the incidents in which players were temporarily removed from play for an off field HIA1 assessment. Samples were received from a total of 129 of these incidents making overall compliance 56%.

15 [00235] Samples were received from 73 of a possible 120 incidents in which the on field or subsequent diagnosis was concussion (61%). Within this group 26 (36%) were incidents in which the player was immediately and permanently removed from play due to exhibiting “Category 1” signs and symptoms. Samples were received from 42 of a possible 109 incidents in which the on field or subsequent diagnosis excluded concussion (39%).

20 [00236] Matched uninjured control samples were received in 71 cases (55%). Matched musculoskeletal injury control samples were received in 39 cases (30%).

Next generation sequencing – discovery phase

[00237] MicroRNA/small RNA Next Generation Sequencing (NGS) was performed in

25 the initial discovery phase using 15 saliva baseline samples (B), 15 samples from concussed players (consisting of 10 concussed (C) and 5 IPR) and 20 controls (consisting of 10 musculoskeletal injuries (M) and 10 uninjured players (U)). All samples analysed in this phase were collected post-match (time point b).

[00238] On average 16.3 million reads were obtained per sample. A detailed

30 microRNA/small RNA NGS data analysis report was obtained. The differential expression analysis step attempts to distinguish biological variation from technical variation within the experiment, assuming that this varies amongst microRNAs.

[00239] P-values for significantly differentially expressed microRNAs are estimated by an exact test on the negative binomial distribution. A list of microRNAs predicted to be

differentially expressed between the given experimental conditions was created. This list includes different RNA fragments, among these known microRNA, other small non-coding RNAs, and predicted microRNA (described as put-miR) differentially expressed between groups C+IP and M+U, B and C+IPR, and B and M+U.

5

Selection for validation

[00240] A panel of microRNA/small RNA/put microRNA, differentially expressed in NGS analysis was selected in the group C+IPR and M+U according the following parameters: FDR<0.5 or p-value <0.05. The Panel consisting of 168 small RNAs, 38 known microRNAs and 233 put-miRs was used for the validation study by qPCR. Following this test, the most significantly altered biomarkers were reduced to 30 known microRNAs; 34 putative miRs; 28 small non-codingRNA, which were then analysed in a further 405 samples. The Cq values obtained from the 2 qPCR validation sets were finally merged and collected in a total of 598 samples.

15

Statistical analysis - validation study

SncRNAs differentially expressed in group C vs U+M

[00241] Concussion samples were compared to uninjured and musculoskeletal injury at different time points. **Table 5** shows the sncRNAs survived to a p value <0.05 among the different comparisons: Ca vs U+M b (no samples were collected at time point a for the category U and M, for this reason the group Ca was compared to the closest time point, U+M b), Cb vs U+M b; Cc vs U+M c. The table also includes: AUC, CI, count, CT average, SD, ddcq, fold change and power analysis for each individual biomarker.

[00242] The biomarkers selected from the STEPWISE analysis are shown in grey cells of **Table 5** and presented an AUC of 0.86; 0.86 and 0.97 in Ca vs U+M b; Cb vs U+M b; Cc vs U+M c comparisons respectively.

SncRNAs differentially expressed in group C vs U

[00243] Concussion samples were compared to uninjured control group only at different time points. **Table 6** shows the sncRNAs survived to a p value <0.05 among the different comparisons: Ca vs Ub; Cb vs Ub; Cc vs Uc. The table also includes: AUC, CI, count, CT average, SD, ddcq, fold change and power analysis for each individual biomarker. The biomarkers selected from the STEPWISE analysis are shown in grey cells of **Table 6**

and presented an AUC of 0.83; 0.91 and 0.97 in the following comparisons Ca vs Ub; Cb vs Ub; Cc vs Uc respectively.

SncRNAs differentially expressed in group C vs B

- 5 [00244] Concussion samples were compared to the baseline data of the same subjects. **Table 7** shows the sncRNAs survived to a p value <0.05 in the different comparisons: Ca vs B Cb vs B; Cc vs B. The table also includes: count, CT average, SD, ddcq, fold change and power analysis for each individual biomarker.

10 *SncRNAs differentially expressed in group C vs M*

[00245] Concussion samples were compared to musculoskeletal injury control group only at different time points. **Table 8** shows the sncRNAs survived to a p value <0.05 among the different comparisons: Ca vs Mb; Cb vs Mb; Cc vs Mc. The table also includes: AUC, CI, count, CT average, SD, ddcq, fold change and power analysis for each individual biomarker.

- 15 The biomarkers selected from the STEPWISE analysis are shown in grey cells of **Table 8** and presented an AUC 0.96; 0.83 and 0.94 in the following comparisons Ca vs Mb; Cb vs Mb; Cc vs Mc respectively.

[00246] MicroRNAs differentially expressed between Cb and U+M b groups (FDR<0.5 or p-value <0.05), selected by NGS analysis and used for the first qPCR validation

- 20 step are shown in **Table 3A**, below.

Table 3A

microRNAs	logFC	logCPM	p-Value	FDR
hsa-miR-199a-5p	-3.79380184	5.744164606	8.15823E-06	0.004797041
hsa-miR-133a-3p	-4.95698696	10.40020121	7.04586E-05	0.020714819
hsa-miR-206	-4.6959152	9.788488822	0.000180043	0.03528849
hsa-miR-1273h-3p	2.629420889	3.481729832	0.001297451	0.190725263
hsa-miR-133b	-4.20181151	6.465178373	0.002184888	0.256942851
hsa-miR-561-5p	1.579551555	4.525850808	0.003970504	0.348018465
hsa-miR-126-3p	-1.41838668	8.449681001	0.004143077	0.348018465
hsa-miR-16-1-3p	1.43849327	4.458454478	0.005764959	0.352449414
hsa-miR-449b-3p	3.467958972	3.218975	0.005920497	0.352449414
hsa-miR-449c-5p	2.749952622	5.534179256	0.006033974	0.352449414
hsa-miR-6748-3p	4.143101791	3.188086662	0.007073212	0.352449414
hsa-miR-133a-5p	-3.92797466	4.752927061	0.008472679	0.352449414
hsa-miR-34b-3p	2.1437498	4.339967792	0.008637715	0.352449414
hsa-miR-6824-3p	2.731145621	3.076936275	0.008970282	0.352449414
hsa-miR-5195-5p	5.376105018	4.09767258	0.009313959	0.352449414

microRNAs	logFC	logCPM	p-Value	FDR
hsa-miR-5096	2.906908779	4.723794715	0.00959046	0.352449414
hsa-miR-4488	3.491520052	3.097320079	0.012515423	0.409279535
hsa-miR-92a-3p	-0.60902412	10.49283489	0.012885103	0.409279535
hsa-miR-1	-2.36876254	10.80951287	0.013692259	0.409279535
hsa-miR-885-5p	1.507019382	4.182851751	0.013921073	0.409279535
hsa-miR-548h-5p	2.055544755	3.480848725	0.015148132	0.42414769
hsa-miR-1246	0.980484408	14.78271469	0.017085283	0.445746733
hsa-miR-4492	-3.18487947	3.53612341	0.017435672	0.445746733
hsa-miR-484	1.274418725	6.148369199	0.019071294	0.449461809
hsa-miR-449a	2.203777576	6.338766334	0.019109771	0.449461809
hsa-miR-3122	3.142476669	3.078975177	0.02000366	0.452390465
hsa-miR-1180-3p	1.165471248	3.889331639	0.021316281	0.457201951
hsa-miR-619-5p	2.792045377	3.207604869	0.021771521	0.457201951
hsa-miR-2277-5p	1.067026052	4.057818901	0.023836409	0.474930011
hsa-miR-5699-3p	1.876788488	2.955683933	0.024231123	0.474930011
hsa-miR-125b-2-3p	-0.96587973	4.933520984	0.025679562	0.487083301
hsa-miR-4449	2.06863176	3.047374886	0.027679506	0.503997815
hsa-miR-671-3p	1.125735449	3.934232773	0.02867908	0.503997815
hsa-miR-33a-3p	0.959071217	4.091785492	0.029142731	0.503997815
hsa-miR-193b-5p	-1.14025756	4.138245693	0.031271072	0.522692794
hsa-miR-34c-3p	1.690242737	4.489423918	0.0320016	0.522692794
hsa-miR-339-5p	1.057419584	7.902671962	0.034354837	0.545963351
hsa-miR-6813-3p	2.813650185	2.933287004	0.035310016	0.546376034
hsa-miR-671-5p	0.838663749	5.600518309	0.047339286	0.598988904
hsa-miR-1537-5p	1.129462606	4.117629615	0.048138455	0.598988904
hsa-miR-193a-3p	-1.5845588	3.995294293	0.048897053	0.598988904
hsa-miR-130a-3p	-0.88824466	6.580760266	0.051263632	0.604409433
hsa-miR-126-5p	-1.22112275	8.006357503	0.051621005	0.604409433

[00247] Other sncRNAs differentially expressed between Cb and U+M b groups (FDR<0.5 or p-value <0.05), selected by NGS analysis and used for the first qPCR validation step are shown in **Table 3B**, below.

Table 3B

small non-coding RNAs	logFC	logCPM	p-Value	FDR
tRNA138-ArgACG	-2.27538237	7.674009775	0.000243882	0.204980883
RNU6-11	-1.67718785	4.732492163	0.000245185	0.204980883
tRNA11-ArgACG	-2.21044709	7.59261144	0.000408122	0.204980883
RNU6-36	-1.84798113	4.63862015	0.000436362	0.204980883
tRNA175-SerGCT	-1.84762954	8.390549302	0.001054009	0.290869197
U3.39	4.655316415	4.424357984	0.001147835	0.290869197
RNU4-6P	-1.71160894	4.864935322	0.001317818	0.290869197
U6.375	-1.86391998	4.231358228	0.001366655	0.290869197
snoU13.63	4.286830869	4.29762358	0.001552935	0.290869197
tRNA8-ThrAGT	-1.65324248	9.428427256	0.001556007	0.290869197

small non-coding RNAs	logFC	logCPM	p-Value	FDR
RNU6-1	-1.37118776	4.843026936	0.001713194	0.290869197
RNU6-31	-1.53604238	4.741724921	0.002351551	0.290869197
tRNA4-ArgTCG	-1.70070174	10.2139807	0.002452994	0.290869197
tRNA130-ValCAC	-2.21562973	4.853113645	0.002797565	0.290869197
U3.3	-1.72401526	5.267608648	0.002991025	0.290869197
U4.57	-1.55456476	4.807782758	0.003084965	0.290869197
tRNA84-GluTTC	-1.39818637	10.32369425	0.003223874	0.290869197
U6.428	-1.83108856	4.169870903	0.003289229	0.290869197
U3.4	-1.80036039	5.421618508	0.00346512	0.290869197
tRNA95-AsnGTT	-2.24304788	4.752212371	0.003469246	0.290869197
uc003oif.3	-2.59243747	4.028384043	0.003838286	0.290869197
U3.2	-1.73251154	5.414684824	0.003880282	0.290869197
U3.42	2.126244843	4.003126274	0.003910676	0.290869197
uc002tgp.1	2.810368883	4.510093789	0.004221387	0.290869197
tRNA105-PseudoTTC	-1.59015632	7.983774255	0.004475105	0.290869197
tRNA4-GluCTC	-1.60952388	8.107675756	0.00453217	0.290869197
RNU6-14	-1.86375965	4.058373711	0.004721183	0.290869197
tRNA4-ThrAGT	-1.34446936	8.68050744	0.004730298	0.290869197
U6.97	-2.76317407	3.745083257	0.004815026	0.290869197
tRNA120-AlaAGC	-1.31815493	9.587766586	0.004875694	0.290869197
snoU13.348	2.256666202	4.075754187	0.005036202	0.290869197
U1.105	-2.32216301	4.207692971	0.005058512	0.290869197
tRNA27-MetCAT	-2.34782418	6.706470837	0.0051084	0.290869197
U1.10	-2.59329176	4.816351388	0.005540106	0.306172302
tRNA134-GluTTC	-1.3431528	10.39664851	0.006091168	0.32697053
RNU6-6	-1.54430018	4.19348578	0.00626447	0.32697053
Y_RNA.75	1.904869354	5.50161645	0.006713926	0.340251318
tRNA8-Undet	1.964251986	4.197405842	0.006905386	0.340251318
SNORA25	-2.35025006	3.740784336	0.007062161	0.340251318
RNU6-39	-1.60601357	4.333253034	0.007621329	0.3538182
tRNA119-AlaCGC	-1.25397272	9.514833568	0.007808074	0.3538182
tRNA85-PseudoTTC	1.126764653	5.8624175	0.008322426	0.3538182
U6.254	-1.80163898	3.954386151	0.008365638	0.3538182
tRNA3-ArgCCT	-1.40848109	10.46544955	0.008483646	0.3538182
SNORD114-2	-3.1187271	3.929437693	0.008576479	0.3538182
tRNA162-MetCAT	-2.31337858	6.829504657	0.008700411	0.3538182
Y_RNA.661	-2.13613309	3.924521406	0.008896949	0.3538182
tRNA8-AlaTGC	-1.03417608	10.23229845	0.009262877	0.3538182
tRNA56-ThrTGT	-1.43137722	10.38511882	0.009580922	0.3538182
tRNA26-AsnGTT	-1.2260783	8.800207822	0.009587969	0.3538182
tRNA73-ArgCCG	-0.87676773	7.646480047	0.009603368	0.3538182
tRNA156-ArgACG	-1.38659506	6.417990561	0.009974671	0.36043089
tRNA28-IleAAT	-0.96475102	8.290575814	0.010463073	0.370945534
RNU6-33	-1.41177741	4.713635974	0.010694162	0.372117242
tRNA3-ProTGG	2.564031745	13.63247318	0.01106572	0.37247176
tRNA19-ArgTCG	-1.57386692	9.439040466	0.011100808	0.37247176
tRNA164-MetCAT	-2.24158666	6.824859219	0.011731788	0.372701442
tRNA13-AlaCGC	-1.05980195	9.619304583	0.011862589	0.372701442

small non-coding RNAs	logFC	logCPM	p-Value	FDR
U6atac.4	-2.29659983	4.169967341	0.012205483	0.372701442
tRNA9-AlaAGC	-1.76859219	4.913678407	0.012206837	0.372701442
tRNA132-PseudoCAC	-2.28282945	4.122549605	0.012249051	0.372701442
RNU6-7	-1.54429239	4.25135459	0.012297759	0.372701442
tRNA20-MetCAT	-1.09889413	8.557399735	0.012787128	0.376829052
U3.24	-1.49142526	5.576968961	0.01283505	0.376829052
U6atac.29	-2.38352866	3.785989635	0.013059629	0.377523747
tRNA2-LeuTAA	-1.66936742	3.974107686	0.013474016	0.383601155
tRNA1-AsnGTT	-0.93147558	7.962088764	0.014090452	0.39410967
tRNA40-ThrAGT	-1.20252417	8.00746198	0.014483817	0.39410967
Y_RNA.684	2.380168702	3.849210771	0.014510103	0.39410967
snoU13.86	3.047260829	3.887450563	0.014700195	0.39410967
uc021qtn.1	-2.42558461	4.054546636	0.015164662	0.39410967
RNU6-9	-1.27226823	4.707463894	0.01590456	0.39410967
tRNA13-AlaTGC	-1.07694323	9.510390292	0.016125256	0.39410967
tRNA36-ArgACG	-1.42222279	6.510745574	0.016234233	0.39410967
Y_RNA.719	-1.87621292	4.049552581	0.016340695	0.39410967
RNU6-10	-1.35736219	4.333057065	0.016389921	0.39410967
SCARNA4	0.900162218	5.772147414	0.016691546	0.39410967
tRNA10-AlaCGC	-1.08617369	9.364295675	0.016698714	0.39410967
tRNA12-SerAGA	-2.17129695	3.960150632	0.016746309	0.39410967
uc011ley.2	-1.1603733	7.787151849	0.01677955	0.39410967
U1.116	-2.53057666	4.143384152	0.017351703	0.3975759
tRNA7-SerGCT	-1.22744148	8.048077286	0.017683906	0.3975759
U6.266	-1.68215765	4.162672228	0.018006863	0.3975759
tRNA136-AsnGTT	-1.18546894	6.252578135	0.018057145	0.3975759
uc031qaf.1	3.07982529	4.21672176	0.018100789	0.3975759
RNU4-9P	1.971224737	3.892961999	0.018196662	0.3975759
RNU6-48	-1.41494718	4.179417866	0.018610429	0.40194249
U4.64-201	-1.46842052	4.573943777	0.019052174	0.405290215
SNORA70.5	-2.3066839	3.705457206	0.019552672	0.405290215
tRNA83-AsnGTT	-0.95893311	7.650753844	0.019695599	0.405290215
tRNA21-ArgCCT	-1.3243702	9.463647642	0.019740822	0.405290215
RNU6-26	-1.26772628	4.674223977	0.019843906	0.405290215
tRNA4-AsnGTT	-0.95216219	7.630343605	0.020477143	0.409060595
U1.28	-1.66671858	4.666167958	0.020920233	0.409060595
U6.168	-1.75957771	3.988195609	0.021394709	0.409060595
RNU6-41-201	-1.2429829	4.599263654	0.021413125	0.409060595
tRNA15-ThrCGT	-0.94643604	8.011751983	0.021715336	0.409060595
tRNA88-PseudoCCT	1.406009411	4.775992477	0.021727046	0.409060595
SNORD116-19-201	-2.08108498	3.769046556	0.022072958	0.409060595
uc031qtw.1	-1.59586877	3.698957807	0.022111714	0.409060595
U6.422-201	-2.18510267	3.771206474	0.022136152	0.409060595
U2.3-201	-1.09052752	5.567176812	0.022205525	0.409060595
U3.20-201	-1.55295135	5.225744713	0.022487766	0.410237987
tRNA165-IleAAT	-0.81674152	8.525161575	0.023067622	0.416176914
snoU13.160-201	-1.88380693	3.690235583	0.023604159	0.416176914
tRNA36-ThrAGT	-1.09203025	7.99788872	0.023653935	0.416176914

small non-coding RNAs	logFC	logCPM	p-Value	FDR
tRNA6-TrpCCA	-1.06582891	10.59444161	0.023699271	0.416176914
SNORA57-201	-1.08263716	5.14915277	0.024108905	0.416665441
tRNA10-PseudoTGA	-1.42087364	5.662435588	0.024173287	0.416665441
uc001myu.3	-2.42408101	4.681561536	0.02449142	0.416665441
Y_RNA.428-201	-1.2545658	6.369684888	0.024614084	0.416665441
SCARNA1-201	-1.61719768	3.852134167	0.025631096	0.427567583
tRNA95-AlaAGC	-1.73027899	5.825175769	0.02592783	0.427567583
RNU6-27-201	-1.6140049	3.926299889	0.025940769	0.427567583
SNORA79.1-201	-1.15165538	4.196182085	0.026538748	0.433620064
tRNA9-IleAAT	-0.73350055	8.536774944	0.027547951	0.446033785
tRNA9-TyrGTA	-1.6487492	3.959996969	0.027773259	0.446033785
tRNA23-ArgCCG	-1.31033598	11.27930104	0.029044541	0.450180862
snoU13.318-201	-1.91587082	3.698261411	0.029045067	0.450180862
snoU13.328-201	2.684496203	4.280709032	0.029064976	0.450180862
RNU4-4P-201	-1.2311376	4.615863264	0.029137817	0.450180862
Y_RNA.633-201	-1.30999509	4.118055921	0.029229412	0.450180862
RNU5F-4P-201	-1.48557387	4.239223966	0.03016703	0.460844298
tRNA7-AsnGTT	-0.88097126	7.861805832	0.031177808	0.472444358
tRNA2-ArgCCT	-1.14460252	9.768619156	0.031689267	0.476353057
tRNA18-ArgCCT	-1.24884324	9.421599432	0.032541851	0.484021239
SNORD116-25-201	1.724675764	6.034751745	0.032861609	0.484021239
uc021ybm.1	-0.9249247	7.924193472	0.033450576	0.484021239
tRNA8-SerGCT	-0.98753133	8.589816708	0.033481121	0.484021239
tRNA11-IleAAT	-0.72451906	8.545784821	0.033487366	0.484021239
Y_RNA.597-201	1.732733959	3.760735382	0.034265836	0.491492414
snoU13.120-201	2.312826375	3.740373282	0.034550843	0.491826017
uc031qpu.1	2.324532842	3.768342356	0.035349099	0.49676233
RNU6-32-201	-1.18812391	4.327815738	0.035426372	0.49676233
tRNA100-PseudoGAA	-1.02843913	9.329302032	0.036035102	0.501555229
SNORD116-21-201	2.33201432	3.747426753	0.039146195	0.532853476
tRNA5-IleTAT	-1.08777911	7.207879458	0.039415342	0.532853476
Y_RNA.522-201	-1.81515534	4.101934504	0.039799275	0.532853476
tRNA12-ArgCCT	-1.27295081	9.216801318	0.039946538	0.532853476
U6atac.20-201	-1.94596283	4.036085302	0.040551585	0.532853476
RNU6-4-201	-1.52406279	4.105411071	0.040667896	0.532853476
SNORD3B-2-201	-0.79576357	8.540015612	0.040838684	0.532853476
tRNA11-AsnGTT	-1.30462038	4.970141103	0.040850618	0.532853476
tRNA31-AsnGTT	-0.87710624	7.603607761	0.041107699	0.532853476
Y_RNA.727-201	-1.25639747	4.115195415	0.041225337	0.532853476
RNU6-73-201	-1.46456929	4.007858615	0.042069664	0.532853476
SNORA65-201	-0.88817274	4.89950806	0.042220138	0.532853476
tRNA7-LeuAAG	-0.71492427	8.880350642	0.042319347	0.532853476
tRNA144-AspGTC	-0.82964134	11.54055138	0.042320413	0.532853476
U6atac.25-201	-1.77774451	3.762252674	0.042537531	0.532853476
tRNA1-ArgCCG	-0.76847131	7.276226466	0.043525106	0.535998445
SNORA7.4-201	-1.13362614	3.738511771	0.043665668	0.535998445
tRNA125-ThrCGT	-1.08045128	5.458664529	0.043842913	0.535998445
RNU6-45-201	-1.22266691	4.217769003	0.043929622	0.535998445

small non-coding RNAs	logFC	logCPM	p-Value	FDR
tRNA131-GlnCTG	-1.07262477	7.700551934	0.04461864	0.538855811
U6.1249-201	1.668615649	3.756761746	0.044737364	0.538855811
Y_RNA.245	2.25187274	3.754483152	0.045943642	0.545496798
U6.601	-1.35324491	3.970443965	0.046003943	0.545496798
RNU5E-4P	-1.0837447	5.498269856	0.046159655	0.545496798
tRNA66-AlaTGC	-0.81125784	9.226831102	0.046988475	0.545729532
tRNA32-MetCAT	-0.82679514	8.99856138	0.047086999	0.545729532
U4.79	-1.25353509	4.433132105	0.047164353	0.545729532
tRNA4-HisGTG	-1.61899083	3.790696889	0.047553836	0.545729532
U3.13	-1.51261982	3.797771351	0.047790048	0.545729532
RNU6-5	-1.02972321	4.777615387	0.048416122	0.545729532
SNORD36A	-1.75616298	3.84054929	0.048459133	0.545729532
tRNA1-LeuAAG	-0.6699966	8.883794181	0.048648388	0.545729532
RNU6-29	-1.13048434	4.36662749	0.048793274	0.545729532

[00248] Putative microRNAs differentially expressed between Cb and U+M b groups (FDR<0.5 or p-value <0.05), selected by NGS analysis and used for the first qPCR validation

5 step are shown in **Table 3C**, below.

Table 3C

putative microRNAs	Sequences	logFC	logCPM	PValue	FDR
put-miR-718	TTCAGGTTACCGCAGG	- 6.825255	9.009089	1.21E-05	0.01609
put-miR-594	TGATTTTTTTTGCGCAA	- 8.122089	13.62184	4.24E-05	0.028197
put-miR-703	GACTGCTCGAGCTGCTT	- 4.582706	9.340091	0.000177	0.065276
put-miR-477	TGGGCAGCAAGAATGG	- 6.350308	8.65459	0.00021	0.065276
put-miR-727	GGAGGATTTCAATTGG	- 4.944239	7.848316	0.000246	0.065276
put-miR-646	TCTGTCATGGCTGAGC	- 6.551056	8.808979	0.000322	0.065276
put-miR-675	TACCTCAATTCTCTAGG	- 5.756095	8.531088	0.000387	0.065276
put-miR-319	TGTCGGCGGGGGCCCCGAC	- 3.557921	10.21937	0.000409	0.065276
put-miR-327	CATTCTCTGAAACTACA	- 6.062301	8.473821	0.000441	0.065276
put-miR-338	GACTTGTGATTAGCGG	- 5.013978	10.59887	0.000571	0.067661
put-miR-1187	GAGCATGTTGACTGGAGA	- 6.150807	8.514764	0.000634	0.067661
put-miR-6	GCGGACCTTGCTCAAGG	- 8.830503	11.10951	0.000659	0.067661
put-miR-341	TGAGAAAACATTTGAGG	- 5.709943	8.260643	0.000661	0.067661
put-miR-750	CAAGAGATGAGGAATG	- 5.585098	8.184314	0.000814	0.07204
put-miR-183	CCAGGCTGGTCGTGATGA	- 5.716867	8.806919	0.000855	0.07204

putative microRNAs	Sequences	logFC	logCPM	PValue	FDR
put-miR-980	TCGGAAGAAGAAGCTGACCCA	-			
		5.533531	8.158978	0.000866	0.07204
put-miR-798	TAACTCTTGGAATCTTGG	-			
		3.422593	9.962536	0.001016	0.075645
put-miR-765	AAAAAAGAGGGACAGAAATG	-			
		5.173073	7.963608	0.001123	0.075645
put-miR-71	GACCTTGGGTTGGGTCGTGGT	-			
		3.989796	8.732315	0.001131	0.075645
put-miR-1327	GAAAAGCAATCGTCACAG	-			
		7.093524	9.887535	0.001158	0.075645
put-miR-371	AATATTCAGCAGTCAGACTGG	-			
		6.778311	9.768186	0.001224	0.075645
put-miR-354	CAGGGTGATGACTTCTG	-			
		4.795128	7.781813	0.001302	0.075645
put-miR-688	GTGATGTCGGCTCATCGAACCT	-			
		2.199185	10.66612	0.001307	0.075645
put-miR-756	TGAGGGATGTTTGATGCTCGCTTTGA	-			
		2.032426	8.603553	0.001428	0.079215
put-miR-1146	CAACTGTAAGTCCATT	-			
		4.770832	9.196252	0.00158	0.079645
put-miR-1333	TAATTGTCCTCTTGGGG	-			
		4.794112	7.786171	0.001619	0.079645
put-miR-511	AAGCAGGCGTTACAATG	-			
		7.089632	9.54354	0.001631	0.079645
put-miR-1199	TGAACTCGGGAGAAGGAAGT	-			
		2.994051	11.94621	0.001702	0.079645
put-miR-705	CCTGTCTGACTGGGTCTCC	-			
		6.097356	9.460857	0.00175	0.079645
put-miR-1275	AAGAAGTAGACTGGATTGG	-			
		4.175643	7.541711	0.001817	0.079645
put-miR-1204	AGGGCTGGGCACGGGGG	-			
		3.329219	8.43806	0.00195	0.079645
put-miR-857	TAAGTGGGAGTGCTCATG	-			
		5.519775	8.389269	0.001957	0.079645
put-miR-806	CACGAGAGAACGCACACC	-			
		7.044016	9.996928	0.002048	0.079645
put-miR-680	GTTAAGTATGCACAGG	-			
		6.207669	9.293799	0.002048	0.079645
put-miR-576	TGGCATCCTGTCTCTTGC	-			
		5.455074	8.34397	0.002094	0.079645
put-miR-444	CTGAAAAGGGGACGGATTGGGAA	-			
		4.481465	8.254479	0.002327	0.083951
put-miR-1132	CACGCTGTCTTTTGTCTCTCT	-			
		5.505609	11.5441	0.002357	0.083951
put-miR-1025	TGGAAGAGGGGAAAGGAGA	-			
		4.492628	7.660657	0.002418	0.083951
put-miR-496	CTAAGAGTATGAGTAGC	-			
		8.320903	10.63469	0.00246	0.083951
put-miR-657	TCAGGACATTGGACTCT	-			
		4.875422	9.301419	0.002633	0.087604
put-miR-625	GAGAAGACTGAATGCTCTTCTC	-			
		5.988262	8.702871	0.002699	0.087604
put-miR-40	GCTGGAATAGCTCAGTTGC	-			
		4.339713	10.82143	0.00295	0.093474
put-miR-666	CAGCATCATGATCATTATGG	-			
		6.351541	8.967721	0.003077	0.095259
put-miR-1098	AAGGCGAAGGATATGTTG	-			
		7.117298	9.563651	0.003171	0.095916
put-miR-538	CATTGTGCTTCGTGGGAGAGTAGGGCA	-			
		1.614431	8.767576	0.003422	0.101205
put-miR-952	GCCGACAGGTCCGGGTAA	-			
		5.048736	10.09864	0.003556	0.101506
put-miR-572	TAGATTCAGGAACTGCCA	-			
		7.280517	9.715353	0.003598	0.101506
put-miR-972	TTTAGTAGAATTGTGTTGGGT	-			
		3.277557	9.430308	0.003661	0.101506

putative microRNAs	Sequences	logFC	logCPM	PValue	FDR
put-miR-650	AAAAGCAAGGCGAAGAGG	-			
		2.912473	9.278157	0.003775	0.102538
put-miR-134	CAGACTGCTCGAGCTGCTGC	-			
		3.339267	9.218851	0.003866	0.102908
put-miR-975	TTTGCAGACTGGAACT	5.625435	8.451316	0.004252	0.109356
put-miR-161	TGTCTGTAGCAATGTGCT	-			
		6.214205	9.449224	0.004553	0.109356
put-miR-1265	TGGGGATAAAGTTAGGT	3.433066	12.55135	0.004559	0.109356
put-miR-968	TGGCTGTTGCACTTCT	8.03171	10.36384	0.004661	0.109356
put-miR-1125	TTGAAGACTGGCTCTCA	-			
		4.530734	8.355286	0.00501	0.109356
put-miR-892	CAACGGTCTTGAAAACA	3.239723	8.81171	0.005015	0.109356
put-miR-1306	ATAACGTCATCTAGTGTG	-			
		4.744221	10.56016	0.005065	0.109356
put-miR-948	TGGAAGAAAACGAGGAG	5.807023	8.569858	0.005204	0.109356
put-miR-925	ATTTTGGTCTGTTGGTT	4.384338	8.643659	0.005342	0.109356
put-miR-259	ATGTAACCGGGCTTTTGTGCT	-			
		1.786601	8.598848	0.005378	0.109356
put-miR-209	GGTCCTCGGATCGGCC	-			
		4.053427	9.542155	0.005417	0.109356
put-miR-773	CAGGCAGAAGGGAGCTTGT	-2.64537	7.45467	0.005514	0.109356
put-miR-910	CACGTTCTTCTCATGGT	-			
		3.449747	7.34067	0.00564	0.109356
put-miR-664	AAAGTTGTGTTAGCTGA	5.719212	8.51182	0.005786	0.109356
put-miR-1068	TATGATTGTAACTCTGA	5.890866	8.622725	0.005843	0.109356
put-miR-265	CACATGGGGGTAGAGCACTGACTGGG	2.110742	12.54113	0.005876	0.109356
put-miR-490	CTGCAGGAGTGTGTGAGA	2.057932	11.07639	0.005969	0.109356
put-miR-1155	CAAATGATCAAAGCAGG	2.494173	8.354137	0.006008	0.109356
put-miR-893	ACCATCCTCTGCTACCA	-			
		2.575152	8.358309	0.006075	0.109356
put-miR-1342	ATCGGAAAATGTGGGAA	5.585468	8.418657	0.006226	0.109356
put-miR-967	AAATGCATTGGATATGG	7.085615	9.549591	0.006355	0.109356
put-miR-811	GTTGTAAAGCTCTGTTG	1.99226	9.063173	0.006362	0.109356
put-miR-812	GTTCTGAGTTCTTTGGTTGGA	7.523344	9.907158	0.006484	0.109356
put-miR-1084	AGGACCATTGCGTTGCC	6.454871	9.04042	0.006577	0.109356
put-miR-626	TTCACTACTTTATCTCTTTT	2.849801	9.706572	0.006577	0.109356
put-miR-127	AGACTGTGATGACTGGGAGAGCGGGCT	-			
		2.251745	7.719415	0.006585	0.109356
put-miR-219	GAGGCTCGAGAGCAATGGC	4.972872	8.410708	0.006604	0.109356
put-miR-863	AAGTTGGAGTATGTTTAGG	4.174184	8.270046	0.006626	0.109356
put-miR-33	CCATGACTGCAGATGG	-			
		4.437318	8.062018	0.006671	0.109356
put-miR-1246	CTGGAGATCTGTTGGGC	6.389535	8.993512	0.00675	0.109356
put-miR-126	GTGGGGCTTAGTGCTGA	-			
		4.160086	8.05272	0.006849	0.109356
put-miR-459	GGGATCAACCTGACAA	7.00545	9.47012	0.00689	0.109356
put-miR-907	CCAACAGCTTCTGAGTTG	-			
		5.599664	9.28021	0.006923	0.109356
put-miR-171	GTCCTGTGTCTGTACGG	2.359948	10.86341	0.006942	0.109356
put-miR-1163	AGAGGACAGGGAAGCTT	6.420538	9.017069	0.006996	0.109356

putative microRNAs	Sequences	logFC	logCPM	PValue	FDR	
put-miR-204	AGAAGTCAGAACCTCTAT	6.610111	9.154177	0.007138	0.109356	
put-miR-498	GACAGATTTTAGCTTGTC	2.214527	8.621153	0.007148	0.109356	
put-miR-286	TAACAGGCGTTGAAATTGT	6.754071	9.266989	0.007468	0.112951	
put-miR-223	GATGGGGGTAGAGCACTGC	2.25836	12.07399	0.007601	0.113671	
put-miR-75	TGGGGATTGTGGGTCTC	-	2.009287	9.598075	0.00774	0.114174
	AGAGGACAGGGAAGCT	6.104866	8.784136	0.007923	0.114174	
put-miR-1118	AGAGGGTTCCTGTAGACCTAGGGAGGA	-	2.053777	10.22693	0.007925	0.114174
	AGCCAGAGTTCTGATTGTGAGTG	-	4.954652	9.727141	0.007978	0.114174
put-miR-313	TGAGTTCTTTGGTCAGAA	6.693723	9.211486	0.00821	0.114289	
put-miR-659	AGTAGCTGGTCGATTTGGC	6.996383	11.79431	0.008416	0.114289	
put-miR-210	CTTCAGACTGTGAACTGA	-	2.997776	9.522779	0.008481	0.114289
	TAGAGATAGAGCTTATG	5.529267	8.376117	0.008489	0.114289	
put-miR-563	TGGAGACATTAACTATGA	5.915791	8.643491	0.008604	0.114289	
put-miR-1140	TGGGAAGGGCTGCCGG	-	5.145788	9.310603	0.008606	0.114289
	TTGGTAGACTCTCACTT	-	4.014605	9.04369	0.008664	0.114289
put-miR-573	TGGATAACTCTTTTGTGA	6.440895	9.021507	0.008788	0.114289	
put-miR-906	TATGGGAAGAATCTGGG	6.054559	8.747883	0.008793	0.114289	
put-miR-231	TATGTCATGGTGGCTTTGG	-2.9467	8.39848	0.008918	0.114289	
put-miR-85	GAATCCCACTTCTGACACCA	-	2.952626	7.663083	0.00901	0.114289
	ATGGTCTAGAGCTACAGGT	5.870477	8.628199	0.00911	0.114289	
put-miR-918	CAGCTTCTCCGCTTCTT	5.860051	8.60284	0.009174	0.114289	
put-miR-985	GTAATGCATCTCTGCA	5.898607	8.628545	0.009188	0.114289	
put-miR-320	ACTGGATCCAAGAAAAG	-	2.920156	8.226536	0.009571	0.11795
	AGGACGTTGGTCAGAGC	-	2.895467	7.636799	0.009857	0.119419
put-miR-221	TCTCTCAATCCTCTTGG	5.954163	8.658651	0.009869	0.119419	
put-miR-471	AGAAGCAGAGAACGAGG	-	3.987795	8.44957	0.009973	0.119585
	TGGATTGTGGGGGAACC	5.478958	8.36409	0.010086	0.119857	
put-miR-610	AGGAGATATAGCTCTGT	5.722079	8.518132	0.010392	0.122408	
put-miR-48	AAAATGGATTGCAACCA	5.632443	8.450136	0.010602	0.123785	
put-miR-529	TGACCTTTGCCTTCTGC	5.426419	8.338572	0.010802	0.125018	
put-miR-424	TAAAAAGTTCTCTGTTTTTC	-2.78953	7.208748	0.010937	0.12549	
put-miR-24	TTGGTGCATCTGTAGTCCAAC	-	4.932824	10.24045	0.011113	0.126418
	TGAAATTCTAAATATTGCA	5.725561	8.51014	0.011372	0.126957	
put-miR-229	TGGGATTTAGCTCAGC	3.799609	8.12666	0.011424	0.126957	
put-miR-525	AAGTGGGAAGGCCAGA	-	3.723862	8.317629	0.011446	0.126957
	TTCAAATCCCACTTCTGACACCA	-	2.927094	7.781125	0.012443	0.136691
put-miR-188	AAATGGCGATACTCAGG	6.335286	9.481014	0.012559	0.136691	

putative microRNAs	Sequences	logFC	logCPM	PValue	FDR
put-miR-1066	TGCTTTGATCGTAGCCC	-			
		3.205461	7.447675	0.012632	0.136691
put-miR-1295	GAGGTTAGGATATCTGGCT	-			
		3.324069	7.580857	0.012893	0.13839
put-miR-588	TTTTGAACGTTCTTTCTT	1.985563	8.910298	0.013328	0.141683
put-miR-1273	AAGGGGAGGAATTTACGTG	4.845324	8.579749	0.013413	0.141683
put-miR-783	AAGGAAAAAGCGGATA	-			
		3.722555	7.559427	0.013621	0.14275
put-miR-37	TGGTGGAGTGAAGACG	-			
		4.836261	8.547591	0.014174	0.14647
put-miR-1317	TGGAGTGTGGATTGGGG	1.625507	8.870365	0.014196	0.14647
put-miR-97	CTGCGTGGCTCTGACAC	3.27664	9.296956	0.014347	0.146889
put-miR-1016	AGGTAAAGCTCATGAGG	-			
		2.141009	9.304303	0.014567	0.148003
put-miR-546	CCAAGGGGTGTAGGGCCACT	3.880018	10.64815	0.015027	0.15152
put-miR-1099	AGACTCCTTTATCGTA	4.881265	8.359673	0.015178	0.151894
put-miR-874	ATGAGCATTGATTTAGG	-			
		4.133563	8.481847	0.015742	0.15277
put-miR-701	TTGAGATTGAGGGGGCCT	2.361165	8.446935	0.015782	0.15277
put-miR-279	CTGTGAAGCCTGTTGGTTTGCTGCTG	-			
		2.002787	8.036423	0.015783	0.15277
put-miR-184	TGCTGAACCTCTGTATGT	5.624113	9.598097	0.015798	0.15277
put-miR-495	CAGGGAGTGAAAGAGAATT	-			
		2.791411	9.973484	0.015923	0.15277
put-miR-90	GAAGTTAAATCCTTGGG	3.643929	8.886684	0.016047	0.15277
put-miR-468	ATAAGGAACTGCTCTCTC	4.936942	8.333361	0.016099	0.15277
put-miR-469	GCGGGGGATTAGCTCAGCTGGG	1.223156	14.10575	0.016186	0.15277
put-miR-1195	ATTCTTTGACATGCAGAT	2.561114	9.286221	0.016299	0.15277
put-miR-250	CTGGTGTAGAATTGAGG	-			
		4.411037	8.166635	0.016733	0.153408
put-miR-1264	CAGGTTTCAGACTTTAGG	2.452989	9.271027	0.0168	0.153408
put-miR-465	CCAGTTGTCGTGGGTTTTT	2.030242	8.962972	0.016867	0.153408
put-miR-1243	TGTGGATTTTGTGCATGT	4.743252	8.639084	0.016871	0.153408
put-miR-771	CCTTGATCTGACTGGGGGCC	-			
		1.886812	8.602159	0.016943	0.153408
put-miR-534	CTGTTGAGAAATTTGGAATATTAGGT	-4.34848	8.658536	0.01741	0.155563
put-miR-80	GAAGAGGGAGTGGTCTGTAAATGCG	6.380693	9.533096	0.017415	0.155563
put-miR-347	AAAGGGAAACAAGAATTCCT	1.937026	10.57284	0.017748	0.156927
put-miR-410	GGAGAATAGAACATGCTGATT	6.652715	9.666187	0.017824	0.156927
put-miR-891	TAAATGTTGGTTTGTGTTGT	1.858836	8.369573	0.017921	0.156927
put-miR-189	GAGCGAAACGGCAGGAT	-			
		2.368257	8.305643	0.018423	0.160266
put-miR-842	ACCCGGAGAACTGAACT	-			
		2.045068	9.280359	0.01862	0.160514
put-miR-81	ATTTGAAAGAATGCTTG	2.468711	8.296721	0.018693	0.160514
put-miR-824	CTAGCTGAACCTCTGTAT	5.277761	8.805933	0.018935	0.161554
put-miR-958	ACAGGGCTGTGCAAAAA	3.475245	8.121044	0.019332	0.163891
put-miR-742	CAGGGCTGTGCTAACT	2.255055	7.794269	0.020414	0.171266
put-miR-605	TGAGCTTTGGAAGAAGGACCA	-			
		2.303646	8.016644	0.020459	0.171266

putative microRNAs	Sequences	logFC	logCPM	PValue	FDR
put-miR-374	TGTGAGGATGTTCTGTAAGGAGTGTT	4.607626	8.644756	0.020848	0.173428
put-miR-135	TGAATTACGGAAGTGTGGTTAAT	-	-	-	-
put-miR-323	ATAAAATGGGCGTTGAGG	3.311813	7.404022	0.021756	0.179859
put-miR-506	AAGAGGGGCTTTTAGAACC	3.262325	8.71569	0.022489	0.184771
put-miR-293	GGGTAAACTGCAGTGGGCGTTGGTAG	6.174059	11.22955	0.022884	0.186859
put-miR-524	CGTGGTGATGCTCTGACA	-	-	-	-
put-miR-517	TTGGAAGGGCTGCCGGA	1.800636	10.99339	0.02336	0.189584
put-miR-391	ATCTCGATCCAGTAGTC	2.434924	7.973262	0.023561	0.189632
put-miR-92	AGAGACTGACTTTGAGTA	-	-	-	-
put-miR-435	TTTAGACCGTTTTATGTC	4.206993	8.774926	0.023651	0.189632
put-miR-1080	TGTGGATTGATGCTCT	1.801909	7.884667	0.024454	0.194897
put-miR-587	TTTGTAGAAGAGGAAGCG	5.755643	9.030306	0.025075	0.198658
put-miR-1220	TAGAATGGGGCTTGTTC	-	-	-	-
put-miR-1134	AAAGAATGAAGTTGGTCTGG	3.735696	9.202301	0.027033	0.212767
put-miR-352	TAATGAATGACTGTTTG	-	-	-	-
put-miR-1162	AAAGAACGTTCAAAGG	1.707634	8.549845	0.027175	0.212767
put-miR-377	AAAGGGCTTTGACTATTT	2.497802	8.421284	0.029781	0.231807
put-miR-294	TGCTTCCTCAATCGGT	3.980805	8.446505	0.030296	0.233459
put-miR-1229	ACAGTAGCAATGTTCTGC	-	-	-	-
put-miR-1091	GATTATGATTGTGATTGTAGC	1.684102	8.848135	0.030344	0.233459
put-miR-419	AAAAAGTTAGACTTAGG	1.774841	8.080119	0.030753	0.23524
put-miR-249	GCCAGGATGTTGGCTTA	2.807352	8.476403	0.030994	0.235731
put-miR-122	GGAACTCATGATTGTTGACTTTGG	1.681479	13.05617	0.031488	0.237918
put-miR-1320	TTAGAGGCCACTCAAT	3.603279	8.466728	0.031639	0.237918
put-miR-335	TATTTAAATGAGAACTTTGAAGC	3.278472	7.88184	0.032091	0.238095
put-miR-166	CGTTTTGGTGTGTTGGTTG	4.964642	9.493873	0.032144	0.238095
put-miR-1172	AGAACCAAGAAGCTCTGG	1.437457	9.015693	0.032199	0.238095
put-miR-1207	GTGAAAAGACATAGGGGG	-	-	-	-
put-miR-1130	TAGAAGAGGGAGCTTCTTT	1.617688	9.173125	0.03244	0.238442
put-miR-247	AGAGCTAGAATCCAGG	1.814811	8.353927	0.032687	0.238442
put-miR-246	TTTATTAGAGACGGGACTTT	4.820365	8.331847	0.032994	0.238442
put-miR-870	AAAGGATGTAGACAAGGGA	2.147361	9.043008	0.033061	0.238442
put-miR-704	CAAATGAATATCTGGGA	-3.72297	8.052795	0.033142	0.238442
put-miR-266	TTAGGTACTCTGAACAA	2.612818	8.384166	0.034733	0.246677
put-miR-928	CACAGAAGGAACGTTTAGA	1.882735	9.817827	0.034763	0.246677
put-miR-959	GAAAGAGAGTGAGACTCA	2.429349	9.717761	0.034842	0.246677
put-miR-676	TGGTGGTTGGTTTTGGG	1.990242	8.786356	0.035469	0.249784
put-miR-1095	ATTGTGAATGATCTGG	4.838701	8.30995	0.035789	0.250379

putative microRNAs	Sequences	logFC	logCPM	PValue	FDR
put-miR-550	GCACTCTGGACTCTGAATCC	-			
		1.460704	9.782298	0.038501	0.258799
put-miR-835	TTAGGAGTAGGTTACT	1.888177	8.674219	0.038693	0.258799
put-miR-686	TGACTGTTTTATATGAGTAA	1.693385	10.42287	0.038917	0.258993
put-miR-32	ATCCCGGACGAGCCCCCATTA	-			
		1.878608	11.03059	0.039456	0.260368
put-miR-151	TTGAAACTGATATACTGTCTTAGG	-			
		2.781416	7.308382	0.039515	0.260368
put-miR-726	GATGGGCTGATTGAGGGAT	3.117005	10.49059	0.039816	0.261058
put-miR-256	ATGAGGCTGAGATTGTCC	1.962535	8.272489	0.040595	0.264863
put-miR-368	AATGAGAACTTTGAAGGCCGAAG	2.363048	14.35762	0.041097	0.266314
put-miR-1232	ACAGACTGTCTTTGGG	2.165428	8.620526	0.041263	0.266314
put-miR-1219	GAAATTATTGATCCAGTCACGA	1.298803	9.720145	0.041418	0.266314
put-miR-1276	GAAGAGTTGATCCATG	-			
		1.907277	9.309298	0.041682	0.266725
put-miR-1119	GTTTGGGTTGAGTGAGGA	-			
		2.455642	8.629875	0.042052	0.267807
put-miR-901	GTCTATTGGATTATCGTC	1.728483	8.113002	0.042255	0.267816
put-miR-356	GTTTGAATTGGGAAGCTGGGGG	-			
		1.750022	8.007003	0.043232	0.272707
put-miR-856	AAGGATAGGGAGGTATTT	2.182062	8.400665	0.044086	0.276787
put-miR-1244	CAGTTGGTAGAGCACCTGAC	-1.24774	10.46658	0.045019	0.281317
put-miR-476	GTGGTCAGGTAGAGAA	-			
		1.919551	8.047207	0.046618	0.288241
put-miR-142	CAGTATAATTAGGGGTTAATTGTGGGA	1.249016	8.70991	0.046684	0.288241
put-miR-639	TGGAGTGTGGATTGGG	1.474835	8.945467	0.046777	0.288241
put-miR-622	TGAGTGGTCAATGGGGG	-			
		1.336255	8.590784	0.047597	0.291931
put-miR-337	TGGGCTCAAGCTTTCTCT	-			
		2.374139	8.27944	0.047999	0.291931
put-miR-422	TTTAGGGTTTTAAGGTGTTA	1.290525	11.98963	0.048034	0.291931
put-miR-1339	GGACTGAGAGCTCTTTCTG	3.091573	10.60449	0.048448	0.293113
put-miR-1356	GTGCTGCATGGCTGTCA	-			
		1.345358	8.820822	0.049044	0.295237
put-miR-29	GAGATTTACGACCTAGG	1.337942	9.63023	0.049243	0.295237
put-miR-128	TGGCTCTGACACTAGTA	2.165955	8.272955	0.049525	0.295593
put-miR-359	GAAGTGGAAGGACTATGAA	2.152809	8.475685	0.050666	0.301056
put-miR-1003	TGGACTTCAGAACAGC	4.029771	7.874318	0.050924	0.301242
put-miR-238	AGAGACAGGAACTTGATTTT	3.690637	8.383615	0.051316	0.302217
put-miR-961	TAGCTTGATCCAGTTG	2.181109	9.760789	0.052445	0.307507
put-miR-751	AGGCTGAGACTGGACAGAAAGACCT	-			
		1.317479	8.022761	0.053486	0.312239
put-miR-1184	GCCTGCCGGTGCTGGT	1.707305	8.999909	0.053825	0.312845
put-miR-379	GCGTGTGCGCGGGAGG	2.352869	7.584474	0.054246	0.313921
put-miR-361	CAGGTGAAATCGTGATGT	2.224017	10.34793	0.05535	0.318896
put-miR-1111	TGATGCGTTGGGATGTAGC	-			
		1.424105	8.543294	0.055717	0.318896
put-miR-938	TTAGGAAGCTGCTGATT	-			
		1.317019	9.281869	0.055825	0.318896

[00249] MicroRNAs ID selected for the second qPCR validation step are shown in Table 4A, below.

Table 4A

microRNAs	Gene Number
hsa-let-7f-5p	MIMAT0000067
hsa-miR-1246	MIMAT0005898
hsa-miR-126-5p	MIMAT0000444
hsa-miR-135b-5p	MIMAT0000758
hsa-miR-21-5p	MIMAT0000076
hsa-miR-425-5p	MIMAT0003393
hsa-miR-497-5p	MIMAT0002820
hsa-miR-148a-3p	MIMAT0000243
hsa-let-7a-5p	MIMAT0000062
hsa-let-7i-5p	MIMAT0000415
hsa-miR-143-3p	MIMAT0000435
hsa-miR-34b-3p	MIMAT0004676
hsa-miR-449a	MIMAT0001541
hsa-miR-144-3p	MIMAT0000436
hsa-miR-144-5p	MIMAT0004600
hsa-miR-16-1-3p	MIMAT0004489
has-miR-934	MIMAT0004977
hsa-miR-206	MIMAT0000462
hsa-miR-103a-3p	MIMAT0000101
hsa-miR-671-3p	MIMAT0004819
hsa-miR-92a-3p	MIMAT0000092
hsa-let-7b-5p	MIMAT0000063
hsa-miR-142-3p	MIMAT0000434
hsa-miR-142-5p	MIMAT0000433
hsa-miR-29c-3p	MIMAT0000433
hsa-miR-6748-3p	MIMAT0027397
has-miR-339-5p	MIMAT0000764
has-miR-107	MIMAT0000104
hsa-miR-126-3p	MIMAT0000445

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[00250] Genome position and sequence of putative microRNAs selected for the second qPCR validation step are shown in Table 4B, below.

Table 4B

putative microRNAs	Chr	Strand	Start	Stop	Sequence
put-miR-1204	4	+	1661740	1661756	AGGGCTGGGCACGGGGG
put-miR-1207	X	+	12647513	12647530	GTGAAAAGACATAGGGGG
put-miR-444	6	+	20984875	20984897	CTGAAAAGGGGACGGATTGGGAA

putative microRNAs	Chr	Strand	Start	Stop	Sequence
put-miR-465	2	+	29475435	29475453	CCAGTTGTCGTGGGTTTTT
put-miR-468	5	+	62391321	62391338	ATAAGGAACGTCTCTCTC
put-miR-6	16	+	67911172	67911188	GCGGACCTTGCTCAAGG
put-miR-958	16	+	51873548	51873564	ACAGGGCTGTGCAAAAA
put-miR-71	10	+	132351412	132351432	GACCTTGGGTTGGGTCGTGGT
put-miR-1003	X	+	96505351	96505366	TGGACTTCAGAACAGC
put-miR-1080	12	+	131562933	131562948	TGTGGATTGATGCTCT
put-miR-188	2	+	96290475	96290491	AAATGGCGATACTCAGG
put-miR-323	12	+	54420991	54421008	ATAAAATGGGCGTTGAGG
put-miR-92	13	+	28849073	28849090	AGAGACTGACTTTGAGTA
put-miR-209	18	+	13603389	13603404	GGTCCTCGGATCGGCC
put-miR-1084	14	+	51649977	51649993	AGGACCATTGCGTTGCC
put-miR-1306	13	+	100000004	100000021	ATAACGTCATCTAGTGTG
put-miR-325	3	+	193330821	193330843	TTCAAATCCCACTTCTGACACCA
put-miR-1146	5	+	67892184	67892199	ATAGGAGACTTCTATC
put-miR-594	17	+	46822348	46822364	TGATTTTTTTTGCGCAA
put-miR-961	7	+	150352937	150352952	TAGCTTGATCCAGTTG
put-miR-742	16	+	49579869	49579884	CAGGGCTGTGCTAACT
put-miR-469	1	+	22441119	22441140	GCGGGGGATTAGCTCAGCTGGG
put-miR-806	16	+	964420	964437	CACGAGAGAACGCACACC
put-miR-476	4	+	100244534	100244549	GTGGTCAGGTAGAGAA
put-miR-856	16	+	50873777	50873794	AAGGATAGGGAGGTATTT
put-mir-893	5	+	58317814	58317830	ACCATCCTCTGCTACCA

[00251] Genome position and sequence of other sncRNAs selected for the second qPCR validation step are shown in Table 4C, below.

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Table 4C

small non-coding RNAs	Chr	Strand	Start	Stop	Sequence
RNU4-6p	X	-	16893269	16893390	TATCGTAGCCAATGAGGTTTATCCGA GGCGTGATTATTGCTAATTGAAAA
tRNA18Arg CCT	17	+	73030001	73030073	GCACTGGCCTCCTAAGCCAGGGATTG TGGGTTCCGAGTCCCACCTGGGGTA
U6.428	1	+	180727858	180727953	AAGATTAGCATGAGGATGACACGCA AATTCGTGAAGCGTTCCATTTCTTT
RNU6-45	11	+	63737942	63738048	GGCCCTTGTGCAAGGATGACACGCA AATTCGTGAAGCGTTCCATATTTTT
RNU6-4	1	-	31970419	31970525	GGCCCTTGCACAGGGATGACACGCA AATTCGTGAAGCGTTCCATATTTTT
RNU6-6	2	+	201694732	201694839	GGCCCTTGTGCAAGGATGACACGCA AATTCGTGAAGCGTTCCATATTTTT
RNU6-7	3	+	194935516	194935622	GGCCCTTGTGCAAGGATGACATGCA AATTCGTGAAGCGTTCCATATTTTT
RNU6-73	13	+	28402900	28403006	GGCCCTTGTGCAAGGATGACATGCA AATTCGTGAAGCGTTCCATATTTTT

small non-coding RNAs	Chr	Strand	Start	Stop	Sequence
SNORD3B	17	+	18965225	18965440	CTTCTCTCCGTATTGGGGAGTGAGAG GGAGAGAACGCGGTCTGAGTGGTT
tRNA120-AlaAGC	6	-	28626014	28626085	GCGCATGCTTAGCATGCATGAGGTCC CGGGTTCGATCCCCAGCATCTCCA
tRNA73Arg CCG	6	+	28849165	28849237	GCGTCTGATTCCGGATCAGAAGATTG AGGGTTCGAGTCCCTTCGTGGTCC
U6.168	6	-	18307204	18307310	ATGGCCCCTGCGCAAGGATGACACG CAAATTTGTGAAGGATTCCATATTT
U6.375	4	+	109573306	109573412	GGCCCCTGTGCAAGAATGACTCGCA AATTCGTGAAGCGTTCATATTTTT
YRNA-684	18	+	20604559	20604666	GCTTCTTTTACTCTTTCCCTTCATTCT CACTACTGTACCTGATTCTGCTCTT
U6.601	19	-	39287642	39287749	GGCCCCTGCGCAAGGATGACATGCA AATTTGTGAAGTGTCCATATTTTT
YRNA-255	17	+	80375102	80375197	GUGUACCAACGUUGGUUAACAACC CCCCACAACUAAAUUUGACUGGCUU
tRNA9-TyrGTA	7	+	149255133	149255205	CTTTTTGACTGTAGAGCAAGAGGTCC CTGGTTCAAATCCAGGTTCTCCCT
U2.3	1	+	150209315	150209504	TCACTTCACGCATCGATCTGGTATTG CAGTACCTCCAGGAACAGTGCACC
U4.64	9	+	36267780	36267919	GTATCGTAGCCAATGAGGTTTATCCA AGGTGCGATTATTGCTAATTGAAA
SNORA57	10	+	27077946	27078086	TGCTGGCGGCTTCCCATCCGCTGGTT CTATCCTCAAACGCCGGGACACCG
UC022CJG 1	Y	+	10037846	10037870	CATTGATCATCGACACTTCGAACGCA CTTG
tRNA27-MetCAT	6	+	26766444	26766516	GCGTCAGTCTCATAATCTGAAGGTCC TGAGTTCGAGCCTCAGAGAGGGCA
tRNA8-ThrAGT	17	+	8090478	8090551	GCGCCTGTCTAGTAAACAGGAGATCC TGGGTTTCAATCCCAGCGGTGCCT
tRNA2-LeuTAA	4	-	156384978	156385052	GCATAAACTTAAATTTTATAATCA GAGGTTCAACTCCTCTTCTTAACA
tRNA9-TyrGTA	7	+	149255133	149255205	CTTTTTGACTGTAGAGCAAGAGGTCC CTGGTTCAAATCCAGGTTCTCCCT
YRNA-245	2	-	25919945	25920057	GTCTTTGTTGAAGTCTTTCCCTCCTTC TCATTACTGTACTTGACCAGTCT
U6.1249	1	+	67661823	67661926	GATGGCATGACCCCTGATCAAGGAC GGCATGCAAATTTGTGAAGTATTTT

[00252] SncRNAs differentially expressed in group C vs U+M at the 3 time points are shown in **Table 5**, below. This table also includes: AUC, CI, count, CT average, SD, ddcq, fold change and power analysis for each individual biomarker and STEPWISE analysis.

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Table 5

Ca vs U+M b	AUC	95% Confidence interval	Count	Ave. ddcq Ca	Ave. ddcq U+M b	SD Ca	SD U+M b	ddcq Ca - U+M b	Fold change Ca / U+M b	t-test p-value	Power analysis (p = 0.05)
hsa-let-7f-5p	0.71	0.63-0.79	134	-	-16.95	0.74	1.47	-1.07	-2.09	0.00	78
hsa-miR-1246	0.69	0.61-0.77	135	-	-21.17	0.75	1.11	-0.49	-1.41	0.00	221

Ca vs U+M b	AUC	95% Confi dence interv al	Count	Ave. dcq Ca	Ave. dcq U+M b	SD Ca	SD U+M b	ddcq Ca - U+M b	Fold change Ca / U+M b	t-test p- valu e	Power analysis (p = 0.05)
hsa-miR-135b-5p	0.66	0.58- 0.75	133	15.34	-14.68	0.64	1.06	-0.66	-1.58	0.00	111
hsa-miR-21-5p	0.66	0.57- 0.74	135	22.44	-22.00	0.66	0.87	-0.43	-1.35	0.00	183
RNU4-6p	0.67	0.59- 0.75	135	17.44	-16.78	1.12	1.35	-0.66	-1.58	0.00	200
hsa-miR-126-5p	0.76	0.68- 0.83	76	-8.25	-9.12	1.23	2.02	0.87	1.83	0.01	231
put-miR-1207	0.66	0.57- 0.74	132	15.59	-13.83	3.27	2.68	-1.76	-3.39	0.01	141
put-miR-444	0.70	0.61- 0.77	100	12.05	-11.06	1.70	1.84	-0.99	-1.98	0.01	174
U6.428	0.68	0.59- 0.76	122	12.69	-13.43	1.51	1.60	0.74	1.67	0.01	237
hsa-miR-497-5p	0.71	0.63- 0.79	99	15.22	-14.69	1.09	1.14	-0.52	-1.44	0.02	243
put-miR-465	0.81	0.73- 0.87	67	16.09	-14.64	2.07	2.28	-1.45	-2.74	0.02	125
put-miR-1204	0.63	0.54- 0.71	132	18.25	-17.26	2.39	2.37	-0.99	-1.98	0.03	305
hsa-miR-425-5p	0.63	0.54- 0.71	135	18.65	-18.93	0.83	0.82	0.28	1.21	0.05	457
tRNA18Arg CCT	0.62	0.59- 0.75	135	22.56	-23.05	1.67	1.63	0.50	1.41	0.05	571
put-miR-468 *	0.84	0.77- 0.90	50	14.10	-13.02	1.33	1.77	-1.08	-2.12	0.07	129
hsa-miR-148a-3p *	0.61	0.52- 0.69	135	19.97	-19.55	0.88	1.08	-0.41	-1.33	0.08	323
STEPWISE	0.86										
Cb vs U+M b	AUC	95% Confi dence interv al	Count	Average dcq Cb	Average dcq U+M b	SD Cb	SD U+M b	ddcq Cb - U+M b	Fold change Cb / U+M b	t-test p- valu e	Power analysis (p = 0.05)
tRNA18Arg CCT	0.66	0.573- 0.736	145	22.31	-23.05	1.80	1.63	0.74	1.67	0.00	273
hsa-let-7a-5p	0.68	0.591- 0.751	145	20.09	-20.33	0.49	0.51	0.23	1.18	0.00	244
RNU6-45	0.67	0.584- 0.745	145	18.86	-20.02	2.19	2.20	1.16	2.23	0.00	189
RNU6-4	0.68	0.593- 0.753	144	19.90	-21.05	2.19	1.89	1.15	2.22	0.00	161
RNU6-6	0.66	0.580- 0.741	144	19.79	-20.79	2.01	1.90	1.01	2.01	0.00	194
RNU6-7	0.66	0.576- 0.738	145	19.82	-20.85	2.01	1.78	1.02	2.03	0.00	175
RNU6-73	0.66	0.577- 0.739	145	19.76	-20.72	1.87	1.86	0.96	1.95	0.00	197
hsa-let-7f-5p	0.65	0.556- 0.720	144	16.24	-16.95	1.30	1.47	0.71	1.64	0.01	207
hsa-miR-135b-5p	0.64	0.555- 0.719	141	14.17	-14.68	0.90	1.06	0.51	1.43	0.01	205
hsa-let-7i-5p	0.64	0.558- 0.722	145	18.49	-18.69	0.42	0.54	0.20	1.15	0.01	328
hsa-miR-14b-3p	0.71	0.630- 0.785	102	12.33	-13.57	2.02	2.26	1.24	2.36	0.01	164
tRNA120-AlaAGC	0.76	0.677- 0.824	99	16.28	-17.76	2.95	2.61	1.48	2.79	0.01	179
U6.601	0.65	0.560- 0.724	145	18.17	-19.01	1.96	1.86	0.83	1.79	0.01	268
YRNA 255	0.64	0.558- 0.722	145	23.71	-24.55	1.77	1.65	0.84	1.79	0.01	214
U6.168	0.63	0.542- 0.707	143	16.74	-17.36	1.67	1.59	0.62	1.54	0.02	361
hsa-miR-143-3p	0.62	0.529- 0.695	145	18.17	-17.88	0.92	1.01	-0.29	-1.22	0.03	612

Ca vs U+M b	AUC	95% Confidence interval	Count	Ave. dcq Ca	Ave. dcq U+M b	SD Ca	SD U+M b	ddcQ Ca - U+M b	Fold change Ca / U+M b	t-test p-value	Power analysis (p = 0.05)
put-miR-6	0.90	0.839-0.944	46	14.02	-15.23	1.82	2.12	1.21	2.31	0.03	142
SNORD3B	0.63	0.544-0.709	145	19.25	-19.79	1.62	1.33	0.53	1.45	0.03	383
uc022cjl	0.61	0.524-0.690	145	22.04	-22.40	1.05	1.00	0.36	1.28	0.03	421
tRNA73Arg CCG	0.60	0.516-0.683	145	16.65	-17.20	2.31	1.69	0.56	1.47	0.04	634
put-miR-958	0.73	0.650-0.802	73	10.56	-11.37	2.04	1.75	0.81	1.76	0.05	273
U6.375	0.65	0.560-0.724	144	16.38	-17.13	1.77	1.49	0.75	1.68	0.05	237
put-miR-71	0.81	0.730-0.867	49	-8.76	-9.86	1.97	2.19	1.09	2.13	0.05	196
YRNA-684 *	0.61	0.520-0.687	126	12.30	-11.25	2.68	2.76	-1.05	-2.07	0.08	355
STEPWISE	0.86										
Cc vs U+M c	AUC	95% Confidence interval	Count	Average dcq Cb	Average dcq U+M b	SD Cb	SD U+M b	ddcQ Cb - U+M b	Fold change Cb / U+M b	t-test p-value	Power analysis (p = 0.05)
hsa-miR-144-3p	0.75	0.659-0.821	98	12.54	-14.12	1.94	2.52	1.59	3.00	0.00	110
put-miR-1084	0.85	0.770-0.906	53	-8.30	-10.33	1.87	2.70	2.03	4.07	0.00	73
hsa-miR-144-5p	0.89	0.821-0.940	50	10.11	-12.06	2.34	2.15	1.95	3.87	0.01	70
put-miR-188	0.87	0.796-0.924	41	11.85	-10.13	2.23	1.91	-1.72	-3.31	0.01	80
put-miR-323	0.93	0.863-0.965	27	-6.46	-7.95	1.37	1.44	1.49	2.81	0.01	49
hsa-miR-16-1-3p	0.63	0.540-0.718	124	13.80	-14.45	1.59	1.46	0.65	1.57	0.02	281
put-miR-92	0.88	0.809-0.932	35	-7.25	-9.07	1.94	2.98	1.82	3.53	0.02	98
put-miR-1003	0.88	0.797-0.925	41	-8.33	-9.96	1.79	2.36	1.64	3.11	0.03	92
put-miR-1080	0.84	0.765-0.902	45	-9.87	-11.22	2.42	2.20	1.35	2.55	0.04	152
RNU6-4	0.61	0.515-0.695	125	20.26	-20.92	1.88	1.99	0.66	1.58	0.05	453
tRNA120-AlaAGC	0.67	0.576-0.751	97	17.21	-18.21	2.63	2.52	1.00	2.00	0.05	343
hsa-miR-206 *	0.79	0.701-0.854	51	12.09	-10.79	3.41	3.02	-1.30	-2.46	0.06	321
put-miR-6 *	0.84	0.763-0.901	39	13.85	-15.03	1.71	2.37	1.17	2.26	0.08	168
STEPWISE	0.97										

[00253] SncRNAs differentially expressed in group C vs U at the 3 time points are shown in Table 6, below. This table also includes: AUC, CI, count, CT average, SD, ddcq, fold change and power analysis for each individual biomarker and STEPWISE analysis.

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Table 6

Ca vs Ub	AUC	95% Confidence interval	Count	Average dcq Ca	Average dcq Ub	SD Ca	SD Ub	ddcQ Ca - Ub	Fold change Ca / Ub	t-test p-value	Power analysis (p = 0.05)
hsa-lnc-7f-5p	0.66	0.56-0.75	104	-18.01	-17.22	0.74	1.32	-0.80	-1.74	0.00	105

Ca vs Ub	AUC	95% Confidence interval	Count	Average dcq Ca	Average dcq Ub	SD Ca	SD Ub	ddcq Ca - Ub	Fold change Ca / Ub	t-test p-value	Power analysis (p = 0,05)
hsa-miR-1246	0.69	0.59-0.78	104	-21.66	-21.17	0.75	1.04	-0.49	-1.41	0.00	189
hsa-miR-135b-5p	0.65	0.55-0.74	102	-15.34	-14.79	0.64	0.97	-0.55	-1.47	0.00	126
put-miR-465	0.84	0.75-0.90	48	-16.09	-14.27	2.07	2.37	-1.82	-3.53	0.01	83
put-miR-444	0.69	0.58-0.77	79	-12.05	-11.06	1.70	1.88	-0.99	-1.98	0.02	177
RNU4-6P	0.65	0.55-0.74	104	-17.44	-16.90	1.12	1.37	-0.54	-1.46	0.02	287
U6.428	0.67	0.57-0.76	93	-12.69	-13.33	1.51	1.56	0.64	1.56	0.02	298
hsa-miR-497-5p	0.71	0.61-0.79	80	-15.22	-14.70	1.09	1.28	-0.52	-1.44	0.03	280
put-miR-1204	0.64	0.53-0.73	101	-18.25	-17.26	2.39	2.35	-0.99	-1.98	0.03	301
hsa-miR-21-5p	0.63	0.52-0.72	104	-22.44	-22.09	0.66	0.84	-0.35	-1.27	0.04	256
put-miR-742 *	0.79	0.69-0.86	46	-21.16	-20.30	1.62	1.27	-0.86	-1.82	0.06	144
hsa-miR-126-5p *	0.74	0.65-0.83	60	-8.25	-9.06	1.23	2.05	0.81	1.76	0.07	254
put-miR-468 *	0.83	0.74-0.89	38	-14.10	-12.93	1.33	1.89	-1.17	-2.26	0.07	118
STEPWISE	0.83										
Cb vs Ub	AUC	95% Confidence interval	Count	Average dcq Ub	Average dcq Cb	SD Ub	SD Cb	ddcq Ub - Cb	Fold change Ub / Cb	t-test p-value	Power analysis (p = 0,05)
hsa-let-7f-5p	0.69	0.60-0.78	114	-17.22	-16.24	1.32	1.30	-0.98	-1.97	0.00	96
hsa-let-7a-5p	0.70	0.59-0.77	114	-20.36	-20.09	0.37	0.49	-0.27	-1.20	0.00	139
hsa-let-7i-5p	0.65	0.55-0.73	114	-18.71	-18.49	0.49	0.42	-0.22	-1.16	0.00	234
hsa-miR-135b-5p	0.68	0.58-0.76	110	-14.79	-14.17	0.97	0.90	-0.62	-1.53	0.00	122
hsa-miR-34b-3p	0.75	0.66-0.83	78	-13.97	-12.33	2.48	2.02	-1.64	-3.12	0.00	104
RNU6-4	0.67	0.57-0.76	113	-20.97	-19.90	1.78	2.19	-1.07	-2.10	0.00	181
RNU6-45	0.66	0.56-0.75	114	-19.86	-18.86	2.20	2.19	-1.00	-2.01	0.01	250
RNU6-6	0.65	0.56-0.74	113	-20.70	-19.79	1.79	2.01	-0.91	-1.88	0.01	228
RNU6-7	0.64	0.55-0.73	114	-20.72	-19.82	1.78	2.01	-0.89	-1.85	0.01	236
RNU6-73	0.65	0.55-0.73	114	-20.62	-19.76	1.80	1.87	-0.86	-1.82	0.01	238
SNORD3B-2	0.65	0.55-0.74	114	-19.83	-19.25	1.25	1.62	-0.58	-1.49	0.01	321
YRNA-255	0.63	0.53-0.72	114	-24.44	-23.71	1.65	1.77	-0.73	-1.66	0.01	287

Ca vs Ub	AUC	95% Confidence interval	Count	Average dcq Ca	Average dcq Ub	SD Ca	SD Ub	ddcq Ca - Ub	Fold change Ca / Ub	t-test p-value	Power analysis (p = 0,05)
hsa-miR-103a-3p	0.61	0.52-0.70	112	-20.08	-19.70	0.77	0.88	-0.37	-1.30	0.02	255
U6.375	0.63	0.53-0.72	114	-17.01	-16.38	1.47	1.77	-0.63	-1.54	0.02	349
U6.601	0.62	0.53-0.72	114	-18.91	-18.17	1.75	1.96	-0.74	-1.67	0.02	329
hsa-miR-449a	0.74	0.65-0.82	65	-13.41	-11.88	3.01	2.60	-1.53	-2.89	0.03	181
hsa-miR-671-3p	0.67	0.58-0.76	78	-11.81	-12.62	1.57	1.83	0.82	1.76	0.03	222
YRNA-684	0.63	0.53-0.72	101	-11.07	-12.30	2.73	2.68	1.23	2.35	0.04	254
put-miR-1306	0.90	0.83-0.95	27	-7.93	-10.51	2.29	3.34	2.58	5.99	0.05	65
put-miR-958	0.73	0.64-0.81	59	-11.49	-10.56	1.73	2.04	-0.94	-1.91	0.05	211
tRNA120-AlaAGC	0.72	0.62-0.79	80	-17.35	-16.28	2.66	2.95	-1.07	-2.10	0.05	355
put-miR-6 *	0.90	0.82-0.95	39	-15.14	-14.02	2.21	1.82	-1.12	-2.17	0.06	169
put-miR-469 *	0.85	0.77-0.91	23	-7.16	-9.23	1.72	2.39	2.06	4.18	0.06	61
put-miR-806 *	0.82	0.73-0.8	38	-9.55	-8.60	1.49	1.81	-0.95	-1.93	0.08	159
STEPWISE	0.91										
Cc vs Uc	AUC	95% Confidence interval	Count	Average dcq Uc	Average dcq Cc	SD Uc	SD Cc	ddcq Uc - Cc	Fold change Uc/ Cc	t-test p-value	Power analysis (p = 0,05)
hsa-miR-144-3p	0.72	0.62-0.80	83	-13.95	-12.54	2.54	1.94	-1.41	-2.66	0.00	133
put-miR-1084	0.83	0.74-0.89	45	-10.29	-8.30	2.54	1.87	-1.99	-3.97	0.01	67
put-miR-183	0.87	0.79-0.93	38	-10.50	-11.85	1.36	2.23	1.35	2.55	0.01	110
put-miR-423	0.95	0.88-0.98	23	-8.07	-6.46	1.27	1.37	-1.61	-3.04	0.01	37
hsa-let-7f-5p	0.63	0.52-0.72	101	-16.95	-16.33	1.41	1.44	-0.62	-1.54	0.03	279
hsa-miR-144-5p	0.88	0.79-0.94	39	-11.85	-10.11	2.36	2.34	-1.74	-3.34	0.03	97
put-miR-92	0.96	0.89-0.99	28	-9.86	-7.25	3.06	1.94	-2.61	-6.11	0.03	44
hsa-miR-206 *	0.78	0.69-0.86	45	-10.74	-12.09	3.03	3.41	1.34	2.54	0.07	306
put-miR-476 *	0.84	0.75-0.91	37	-15.04	-16.43	1.79	2.45	1.38	2.60	0.08	131
STEPWISE	0.97										

[00254] SncRNAs differentially expressed in group C vs B at the 3 time points are shown in **Table 7**, below. This table also includes: count, CT average, SD, ddcq, fold change and power analysis for each individual biomarker.

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Table 7

Ca vs B	Count	Average dcq Ca	Average dcq B	SD Ca	SD B	ddcq Ca - B	Fold change Ca / B	Pair sample test Sig. (2-tailed)	Power analysis (p = 0.05)
hsa-miR-1246	219	-21.66	-20.79	0.75	1.04	-0.87	-1.83	0.00	69
put-miR-1207	207	-15.59	-12.28	3.27	2.22	-3.30	-9.88	0.00	31
RNU4-6p	219	-17.44	-16.85	1.12	1.09	-0.59	-1.51	0.00	178
hsa-miR-143-3p	114	-18.00	-18.17	0.99	0.92	0.16	1.12	0.00	1821
hsa-miR-34b-3p	182	-13.93	-12.33	1.51	1.87	-1.60	-3.04	0.00	69
hsa-miR-449a	155	-13.48	-11.38	1.89	2.62	-2.10	-4.28	0.00	75
U4.64	107	-11.33	-11.90	2.36	2.64	0.57	1.49	0.00	991
hsa-let-7b-5p	219	-20.07	-20.26	0.58	0.58	0.19	1.14	0.00	480
hsa-miR-142-3p	114	-23.48	-23.56	0.77	0.87	0.08	1.06	0.00	5191
hsa-miR-142-5p	219	-20.83	-20.14	0.90	1.03	-0.69	-1.61	0.00	114
hsa-miR-29c-3p	219	-19.93	-19.74	0.58	0.58	-0.19	-1.14	0.00	480
put-miR-1146	122	-12.59	-11.17	2.39	2.97	-1.43	-2.69	0.00	210
tRNA9-TyrGTA	206	-13.04	-11.65	2.42	2.07	-1.39	-2.61	0.00	127
put-miR-444	133	-12.05	-10.06	1.70	2.27	-1.99	-3.96	0.01	64
has-miR-148a-3p	219	-19.97	-19.67	0.88	0.66	-0.29	-1.22	0.01	305
U2.3	219	-27.18	-27.13	1.32	1.31	-0.05	-1.03	0.01	43231
tRNA27-MetCAT	112	-18.60	-18.36	1.48	1.88	-0.24	-1.18	0.01	2548
tRNA2-LeuTAA	219	-16.87	-17.65	1.30	1.47	0.78	1.71	0.01	181
YRNA-245	219	-15.50	-14.70	1.42	1.36	-0.80	-1.74	0.01	155
hsa-let-7f-5p	219	-18.01	-17.67	0.74	1.27	-0.35	-1.27	0.02	598
hsa-miR-6748-3p	94	-14.95	-12.10	3.29	3.04	-2.85	-7.20	0.02	64
put-miR-468	64	-14.10	-12.25	1.33	1.70	-1.85	-3.60	0.05	44
put-miR-594	190	-15.15	-13.30	4.79	3.42	-1.85	-3.61	0.05	212
Cb vs B	Count	Average dcq Cb	Average dcq B	SD Cb	SD B	ddcq Cb - B	Fold change Cb / B	Pair sample test Sig. (2-tailed)	Power analysis (p = 0.05)
hsa-miR-135b-5p	226	-14.17	-15.45	0.90	0.96	1.28	2.43	0.00	31
hsa-miR-425-5p	229	-18.87	-18.37	0.71	0.86	-0.50	-1.41	0.00	146
hsa-let-7a-5p	229	-20.09	-20.65	0.49	0.41	0.56	1.47	0.00	33
hsa-miR-143-3p	229	-18.17	-17.60	0.92	1.18	-0.57	-1.48	0.00	206
YRNA-684	196	-12.30	-10.58	2.68	2.12	-1.72	-3.29	0.00	93
U4.64	221	-11.90	-10.76	2.64	1.89	-1.14	-2.20	0.00	176
hsa-miR-142-3p	229	-23.56	-22.94	0.87	1.11	-0.63	-1.55	0.00	151
hsa-miR-142-5p	229	-20.82	-20.14	0.97	1.03	-0.68	-1.61	0.00	117

Ca vs B	Count	Average dcq Ca	Average dcq B	SD Ca	SD B	ddcq Ca - B	Fold change Ca / B	Pair sample test Sig. (2-tailed)	Power analysis (p = 0.05)
tRNA9-TyrGTA	213	-13.06	-11.65	2.84	2.07	-1.41	-2.65	0.00	137
YRNA-245	229	-15.18	-14.70	1.83	1.36	-0.48	-1.40	0.00	493
put-miR-961	144	-12.72	-11.03	3.34	2.55	-1.68	-3.21	0.00	143
hsa-let-7f-5p	229	-16.24	-17.67	1.30	1.27	1.43	2.69	0.01	44
hsa-miR-1246	229	-21.39	-20.79	1.33	1.04	-0.60	-1.52	0.01	179
hsa-miR-126-5p	144	-9.35	-8.15	1.87	1.60	-1.20	-2.30	0.01	101
hsa-miR-144-3p	208	-14.19	-13.70	2.60	2.06	-0.49	-1.41	0.01	1021
hsa-let-7b-5p	229	-20.21	-20.26	0.90	0.58	0.05	1.04	0.01	8840
hsa-miR-29c-3p	229	-19.79	-19.74	0.90	0.58	-0.05	-1.04	0.01	8840
SNORD3B	229	-19.25	-20.20	1.62	1.03	0.94	1.92	0.02	86
hsa-miR-671-3p	157	-12.62	-11.09	1.83	1.35	-1.53	-2.89	0.02	49
UC022CJG1	229	-22.04	-22.64	1.05	0.94	0.60	1.51	0.02	139
tRNA2-LeuTAA	227	-16.87	-17.65	1.99	1.47	0.78	1.72	0.02	220
hsa-miR-339-5p	229	-15.89	-15.50	1.60	1.30	-0.39	-1.31	0.02	639
hsa-miR-21-5p	229	-21.86	-22.45	0.99	0.74	0.59	1.51	0.03	98
hsa-miR-34b-3p	180	-12.33	-12.33	2.02	1.87	0.00	-1.00	0.03	11336078
U6.168	228	-16.74	-16.82	1.67	1.56	0.07	1.05	0.03	25072
hsa-miR-16-1-3p	226	-14.16	-13.71	1.41	1.49	-0.45	-1.36	0.03	564
put-miR-1207	217	-13.48	-12.28	3.36	2.22	-1.20	-2.30	0.04	234
put-miR-468	69	-13.67	-12.25	1.68	1.70	-1.42	-2.67	0.04	77
put-miR-893	229	-28.04	-27.27	2.65	1.94	-0.77	-1.71	0.04	396
SNORA57	229	-20.82	-20.63	1.15	1.06	-0.20	-1.15	0.04	1593
Cc vs B	Count	Average dcq Cc	Average dcq B	SD Cc	SD B	ddcq Cc - B	Fold change Cc / B	Pair sample test Sig. (2-tailed)	Power analysis (p = 0.05)
hsa-miR-135b-5p	228	-14.95	-15.45	0.96	0.96	0.50	1.41	0.00	194
hsa-miR-1246	231	-21.19	-20.79	1.21	1.04	-0.40	-1.32	0.01	378
hsa-miR-21-5p	231	-21.88	-22.45	0.92	0.74	0.57	1.48	0.01	101
hsa-let-7f-5p	231	-16.33	-17.67	1.44	1.27	1.34	2.52	0.02	52
U6.428	215	-13.10	-12.76	1.53	1.69	-0.34	-1.26	0.02	1253
YRNA-684	201	-11.24	-10.58	3.03	2.12	-0.66	-1.58	0.02	681
U4.64	231	-16.98	-16.85	1.49	1.09	-0.13	-1.09	0.02	4432
U6.1249	160	-10.16	-8.96	3.17	2.18	-1.20	-2.30	0.02	223
RNU4-6P	231	-16.98	-16.85	1.49	1.08	-0.13	-1.09	0.03	4432
U6.168	231	-16.78	-16.82	1.57	1.56	0.04	1.03	0.04	84454
hsa-miR-92a-3p	231	-20.47	-20.50	0.68	0.46	0.03	1.02	0.04	11846
put-miR-209	97	-8.94	-8.42	2.79	2.89	-0.52	-1.43	0.04	1580

[00255] SncRNAs differentially expressed in group C vs M at the 3 time points are shown in **Table 8**, below. This table also includes: AUC, CI, count, CT average, SD, ddcq, fold change and power analysis for each individual biomarker and STEPWISE analysis.

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Table 8

Ca vs Mb	AUC	95% Confidence Interval	Count	Average dcq Ca	Average dcq Mb	SD Ca	SD Mb	ddcq Ca - Mb	Fold change Ca/ Mb	t-test p-value	Power analysis (p = 0.05)
hsa-let-7f-5p	0.81	0.69-0.92	63	-17.93	-16.40	0.763	1.62	-1.53	-2.89	0.00	37
hsa-miR-135b-5p	0.71	0.58-0.84	63	-15.26	-14.48	0.63	1.22	-0.78	-1.72	0.00	81
hsa-miR-21-5p	0.71	0.59-0.83	64	-22.36	-21.84	0.69	0.90	-0.52	-1.43	0.00	127
put-miR-1207	0.74	0.63-0.86	62	-15.73	-13.08	3.33	2.63	-2.66	-6.26	0.00	69
RNU4-6P	0.71	0.58-0.84	64	-17.37	-16.56	0.94	1.32	-0.81	-1.75	0.00	105
hsa-miR-148a-3p	0.70	0.58-0.82	64	-19.90	-19.31	0.93	1.06	-0.59	-1.50	0.01	151
hsa-miR-34b-3p	0.72	0.60-0.84	52	-14.01	-12.84	1.49	1.58	-1.18	-2.26	0.01	90
tRNA18-ArgCCT	0.68	0.55-0.80	64	-22.65	-23.57	1.69	1.45	0.92	1.89	0.01	157
U6.428	0.67	0.54-0.79	58	-12.61	-13.60	1.39	1.69	0.99	1.99	0.01	128
hsa-miR-425-5p	0.66	0.53-0.79	64	-18.68	-19.09	0.83	0.81	0.41	1.33	0.02	208
U6.168	0.65	0.52-0.78	63	-17.03	-17.78	1.30	1.62	0.75	1.68	0.03	201
hsa-miR-103a-3p	0.65	0.51-0.78	63	-20.05	-19.82	0.597	0.79	-0.23	-1.17	0.04	490
hsa-miR-1246	0.68	0.55-0.82	64	-21.64	-21.17	0.72	1.24	-0.47	-1.38	0.04	242
hsa-miR-16-1-3p	0.65	0.53-0.79	63	-14.44	-14.94	1.26	1.36	0.50	1.41	0.04	362
put-miR-323	0.91	0.82-0.99	15	-8.42	-6.72	1.85	1.66	-1.70	-3.25	0.04	58
put-miR-594	0.71	0.59-0.84	54	-15.24	-12.40	4.90	3.97	-2.84	-7.18	0.04	131
hsa-miR-126-5p	0.80	0.68-0.92	34	-8.18	-9.25	1.12	1.99	1.06	2.09	0.05	119
hsa-miR-143-3p	0.64	0.50-0.78	64	-17.96	-17.63	0.86	1.03	-0.32	-1.25	0.05	445
hsa-miR-497-5p	0.68	0.55-0.80	42	-15.28	-14.69	1.22	0.72	-0.59	-1.50	0.05	161
put-miR-444*	0.72	0.59-0.84	44	-12.21	-11.06	1.71	1.78	-1.15	-2.21	0.06	123
STEPWISE	0.96										
Cb vs Mb	AUC	95% Confidence Interval	Count	Average dcq Cb	Average dcq Mb	SD Cb	SD Mb	ddcq Cb - Mb	Fold change Cb/ Mb	t-test p-value	Power analysis (p = 0.05)
tRNA18ArgCCT	0.73	0.62-0.83	64	-22.54	-23.57	1.66	1.45	1.03	2.04	0.00	123
RNU6-7	0.69	0.57-0.81	64	-20.44	-21.11	1.54	1.77	0.67	1.59	0.00	322
tRNA120-AlaAGC	0.84	0.74-0.93	42	-16.44	-18.71	2.90	2.27	2.27	4.83	0.00	72
U6.168	0.69	0.57-0.81	63	-17.17	-17.78	1.46	1.62	0.61	1.52	0.00	335
hsa-miR-143-3p	0.68	0.56-0.81	64	-18.25	-17.64	0.87	1.03	-0.61	-1.53	0.01	128

Ca vs Mb	AUC	95% Confidence Interval	Count	Average dcq Ca	Average dcq Mb	SD Ca	SD Mb	ddcq Ca - Mb	Fold change Ca/ Mb	t-test p-value	Power analysis (p = 0.05)
RNU6-45	0.69	0.57-0.81	64	-19.49	-20.32	1.68	2.21	0.83	1.78	0.01	290
RNU6-4	0.69	0.57-0.86	64	-20.55	-21.20	1.47	2.09	0.65	1.57	0.01	396
RNU6-73	0.69	0.57-0.82	64	-20.34	-20.93	1.45	2.01	0.59	1.50	0.01	458
U6.375	0.67	0.55-0.79	63	-16.88	-17.39	1.43	1.50	0.51	1.42	0.01	433
hsa-miR-16-1-3p	0.68	0.56-0.79	62	-14.19	-14.94	1.28	1.36	0.75	1.68	0.01	166
YRNA-255	0.67	0.55-0.79	64	-24.05	-24.77	1.52	1.67	0.71	1.64	0.01	260
RNU6-6	0.69	0.57-0.81	64	-20.40	-20.98	1.48	2.10	0.59	1.50	0.02	499
tRNA73ArgCCG	0.63	0.51-0.75	64	-16.84	-17.44	2.15	1.42	0.60	1.51	0.02	495
U6.601	0.69	0.56-0.81	64	-18.76	-19.21	1.62	2.07	0.45	1.37	0.02	868
UC022CJG1	0.66	0.54-0.78	64	-22.05	-22.65	0.84	0.99	0.59	1.51	0.02	127
SNORA57	0.63	0.50-0.76	64	-20.98	-21.33	1.13	1.13	0.36	1.28	0.05	516
put-miR-6 *	0.91	0.82-0.99	22	-13.78	-15.46	1.62	2.03	1.68	3.21	0.06	59
put-miR-856 *	0.81	0.71-0.92	24	-12.97	-14.65	1.46	1.65	1.68	3.21	0.08	46
STEPWISE	0.83										
Cc vs Mc	AUC	95% Confidence Interval	Count	Average dcq Cc	Average dcq Mc	SD Cc	SD Mc	ddcq Cc - Mc	Fold change Cc / Mc	t-test p-value	Power analysis (p = 0.05)
hsa-let-7f-5p	0.71	0.59-0.83	61	-16.44	-15.49	1.51	1.06	-0.95	-1.93	0.00	107
hsa-miR-135b-5p	0.73	0.61-0.86	58	-15.06	-14.08	0.99	1.30	-0.99	-1.98	0.00	72
hsa-miR-425-5p	0.71	0.53-0.85	61	-18.60	-19.20	0.84	1.09	0.60	1.51	0.00	134
hsa-miR-16-1-3p	0.74	0.63-0.86	60	-13.75	-15.06	1.68	1.33	1.30	2.47	0.00	75
U6.428	0.71	0.58-0.83	53	-13.29	-14.26	1.64	1.59	0.97	1.96	0.01	147
tRNA120-AlaAGC	0.76	0.64-0.87	46	-17.20	-19.02	2.30	2.47	1.82	3.54	0.01	90
hsa-miR-144-3p	0.80	0.69-0.91	45	-12.65	-14.57	2.17	2.49	1.92	3.80	0.01	75
put-miR-1003	0.95	0.89-1.00	17	-8.45	-11.23	2.00	1.74	2.78	6.86	0.01	28
YRNA-255	0.70	0.58-0.82	61	-24.38	-25.06	1.71	1.47	0.69	1.61	0.01	292
SNORA57	0.68	0.55-0.81	61	-20.75	-21.42	1.25	1.05	0.68	1.60	0.01	159
hsa-miR-21-5p	0.67	0.53-0.80	61	-21.96	-21.40	0.87	1.03	-0.56	-1.47	0.02	151
hsa-miR-144-5p	0.91	0.83-1.00	22	-11.26	-12.45	2.21	1.73	1.19	2.28	0.02	148
put-miR-1080	0.86	0.75-0.96	24	-10.51	-12.11	2.45	2.06	1.60	3.04	0.02	108
tRNA27-MetCAT	0.65	0.53-0.78	61	-18.80	-19.42	1.66	1.53	0.63	1.54	0.02	346
tRNA18ArgCCT	0.64	0.52-0.77	61	-23.00	-23.74	2.12	1.60	0.74	1.67	0.03	358
tRNA8-ThrAGT	0.64	0.51-0.76	61	-24.04	-24.37	2.50	2.31	0.33	1.26	0.03	2778
tRNA73ArgCCG	0.63	0.51-0.76	61	-17.19	-17.74	2.32	1.66	0.54	1.46	0.04	758

Ca vs Mb	AUC	95% Confidence Interval	Count	Average dcq Ca	Average dcq Mb	SD Ca	SD Mb	ddcq Ca - Mb	Fold change Ca/ Mb	t-test p-value	Power analysis (p = 0.05)
put-miR-325	0.63	0.51-0.76	61	-18.96	-19.78	2.67	1.98	0.82	1.77	0.04	448
RNU4-6P	0.61	0.47-0.74	61	-17.00	-16.23	1.57	1.09	-0.77	-1.71	0.05	173
U6.168	0.64	0.49-0.78	61	-17.09	-17.60	1.64	1.92	0.51	1.42	0.05	619
hsa-miR-671-3p	0.76	0.64-0.87	37	-11.40	-12.77	1.74	1.50	1.38	2.60	0.05	76
YRNA-245	0.62	0.49-0.74	61	-14.91	-15.30	1.79	1.07	0.39	1.31	0.05	801
put-miR-6	0.86	0.75-0.96	17	-14.22	-15.33	1.78	2.15	1.10	2.15	0.07	167
STEPWISE	0.94										

First Set of Examples of Embodiments of the Present Disclosure

- [00256] A1. A method of diagnosing and treating traumatic brain injury (TBI) in a human subject in need thereof, the method comprising:
- 5 obtaining a saliva sample from the subject;
 - contacting the saliva sample with a probe comprising a nucleic acid able to bind to at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410,
 - 10 put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, and put-miR-1352;
 - determining an amount of the at least one RNA biomarker in the saliva
 - 15 sample;
 - identifying the subject as having TBI where the amount of the at least one RNA biomarker is increased or decreased relative to a predetermined threshold value or relative to the amount of the RNA biomarker in a control sample; and
 - treating the subject identified as having TBI according to one or more of
 - 20 the following:
 - subjecting the subject to a verbal, cognitive, motor, or optical test, or any combination of the foregoing;
 - subjecting the subject to diagnostic imaging in the form of a CT or MRI, or a combination thereof; and/or
 - 25 administering to the subject one or more neuroprotective therapies.

- [00257] A2. The method according to claim A1 further comprising identifying the human subject as being fit for normal activity after undergoing successful treatment for TBI.
- [00258] B1. A method of diagnosing and/or monitoring traumatic brain injury (TBI) in a subject, the method comprising determining a level of at least one RNA biomarker
 5 in a saliva sample obtained from the subject, wherein the at least one RNA biomarker is selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146,
 10 put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, and put-miR-1352, or any combination thereof.
- [00259] B2. The method of claim B1, wherein either an upregulated level or a downregulated level of the at least one RNA biomarker is indicative of TBI.
- [00260] B3. The method of claim B1, wherein the subject is diagnosed as having
 15 TBI if the level of the at least one RNA biomarker is either above or below a predetermined threshold or increased or decreased relative to a control.
- [00261] B4. The method according to claim B1 further comprising identifying the human subject as being fit for normal activity after undergoing successful treatment for TBI.
- [00262] B5. The method of any one of claims B1-B4, wherein the at least one RNA
 20 biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.
- [00263] C1. A sensor element for a detection system for diagnosing and/or monitoring TBI, the sensor element comprising a substrate functionalized with a probe specific for at least one RNA biomarker selected from the group consisting of Y_RNA.255,
 25 RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, and put-miR-1352.
- 30 [00264] C2. The sensor element of claim C1, wherein the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.
- [00265] C3. The sensor element of claim C1 or C2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.

- [00266] C4. The sensor element of claim C1 or C2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, and 99.
- 5 [00267] C5. The sensor element of any one of claims C1-C4, wherein the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.
- [00268] D1. A detection system for diagnosing and/or monitoring TBI, comprising a sensor element according to the present disclosure, and a detection device capable of
10 detecting the binding of a target RNA biomarker to the probe.
- [00269] D2. The detection system according to claim D1, further comprising means to determine whether the target RNA biomarker is upregulated or downregulated.
- [00270] E1. A method for determining a course of action for a subject suspected of having TBI, comprising applying a saliva sample obtained from the subject to a detection
15 system according to the present disclosure, and if an upregulated level or a downregulated level of the at least one RNA biomarker is detected, providing a treatment for TBI.
- [00271] F1. A method of treating a subject with suspected TBI, the method comprising
determining whether an upregulated level or a downregulated level of at least
20 one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, and put-miR-1352 is detectable in
25 a saliva sample obtained from the subject, and
if an upregulated level or a downregulated level of at least one RNA biomarker is detected, providing treatment for TBI to the subject.
- [00272] F2. The method of claim F1, wherein the at least one RNA biomarker
30 further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.
- [00273] G1. A method of detecting an RNA biomarker in a saliva sample, the method comprising
obtaining a saliva sample from a human subject,

contacting the saliva sample with at least one oligonucleotide primer complementary to at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, and put-miR-1352;

amplifying the at least one RNA biomarker using a polymerase chain reaction;

and

detecting the amplified RNA biomarker.

[00274] G2. The method of claim G1, wherein the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

[00275] H1. A kit for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the kit comprising at least one probe specific for at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, and put-miR-1352.

[00276] H2. The kit of claim H1, wherein the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.

[00277] H3. The kit of claim H1 or H2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.

[00278] H4. The kit of claim H1 or H2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, and 99.

[00279] H5. The kit of any one of claims H1-H4, wherein the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

- [00280] I1. A composition for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the composition comprising at least one probe specific for at least one RNA biomarker selected from the group consisting of Y_RNA.255, RNU6-7, RNU6-4, RNU6-6, RNU6-73, RNU6-45, U6.375, put-miR-1207, U6.428, put-miR-742, hsa-miR-6748-3p, put-miR-6, put-miR-410, put-miR-476, put-miR-293, hsa-miR-34b-3p, hsa-miR-1271-5p, hsa-miR-449a, put-miR-806, put-miR-71, put-miR-468, put-miR-1306, put-miR-1146, put-miR-1084, put-miR-92, put-miR-209, put-miR-961, U6.1249, put-miR-188, and put-miR-1352.
- [00281] I2. The composition of claim I1, wherein the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.
- [00282] I3. The composition of claim I1 or I2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.
- [00283] I4. The composition of claim I1 or I2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, and 99.
- [00284] I5. The composition of any one of claims I1-I4, wherein the at least one RNA biomarker further comprises one or more miRNA selected from the group consisting of hsa-miR-143-3p and hsa-miR-135b-5p.

Second Set of Examples of Embodiments of the Present Disclosure

- [00285] A1. A method of diagnosing and treating traumatic brain injury (TBI) in a human subject in need thereof, the method comprising:
- obtaining a saliva sample from the subject;
- contacting the saliva sample with a probe comprising a nucleic acid able to bind to at least one RNA biomarker:
- a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG,

tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or

- 5 b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof;

10 determining an amount of the at least one RNA biomarker in the saliva sample;

 identifying the subject as having TBI where the amount of the at least one RNA biomarker is increased or decreased relative to a predetermined threshold value or relative to the amount of the RNA biomarker in a control sample; and

15 treating the subject identified as having TBI according to one or more of the following:

 subjecting the subject to a verbal, cognitive, motor, or optical test, or any combination of the foregoing;

20 subjecting the subject to diagnostic imaging in the form of a CT or MRI, or a combination thereof; and/or

 administering to the subject one or more neuroprotective therapies.

[00286] A2. The method of claim A1, wherein the at least one RNA biomarker further comprises one or more miRNA:

- 25 a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or

- 30 b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

[00287] A3. The method according to claim A1 further comprising identifying the human subject as being fit for normal activity after undergoing successful treatment for TBI.

- [00288] B1. A method of diagnosing and/or monitoring traumatic brain injury (TBI) in a subject, the method comprising determining a level of at least one RNA biomarker in a saliva sample obtained from the subject, wherein the at least one RNA biomarker is:
- a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
 - b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.
- [00289] B2. The method of claim B1, wherein either an upregulated level or a downregulated level of the at least one RNA biomarker is indicative of TBI.
- [00290] B3. The method of claim B1, wherein the subject is diagnosed as having TBI if the level of the at least one RNA biomarker is either above or below a predetermined threshold or increased or decreased relative to a control.
- [00291] B4. The method of any one of claims B1-B3, wherein the at least one RNA biomarker further comprises one or more miRNA:
- a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or

- b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
- [00292] B5. The method according to claim B1 further comprising identifying the
 5 human subject as being fit for normal activity after undergoing successful treatment for TBI.
- [00293] B6. The method of any one of claims B1-B7, wherein the at least one RNA biomarker further comprises one or more miRNA:
- a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
- 10
- b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
- 15
- [00294] C1. A sensor element for a detection system for diagnosing and/or monitoring TBI, the sensor element comprising a substrate functionalized with a probe specific for at least one RNA biomarker:
- 20
- a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958,
- 25
- RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
- 30
- b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961,

RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.

[00295] C2. The sensor element of claim C1, wherein the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.

5 [00296] C3. The sensor element of claim C1 or C2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.

[00297] C4. The sensor element of claim C1 or C2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ
10 ID NO: 1, 2, 14, 16, 23, 26, 27, 29, 39, 40, 48, 71, 72, 73, 74, 75, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 89, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150.

15 [00298] C5. The sensor element of any one of claims C1-C4, wherein the at least one RNA biomarker further comprises one or more miRNA:

- a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
 - b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
- 20
25

[00299] D1. A detection system for diagnosing and/or monitoring TBI, comprising a sensor element according to the present disclosure, and a detection device capable of detecting the binding of a target RNA biomarker to the probe.

[00300] D2. The detection system according to claim D1, further comprising means
30 to determine whether the target RNA biomarker is upregulated or downregulated.

[00301] E1. A method for determining a course of action for a subject suspected of having TBI, comprising applying a saliva sample obtained from the subject to a detection system according to the present disclosure, and if an upregulated level or a downregulated level of the at least one RNA biomarker is detected, providing a treatment for TBI.

[00302] F1. A method of treating a subject with suspected TBI, the method comprising

determining whether an upregulated level or a downregulated level of at least one RNA biomarker:

- 5 a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958,
 - 10 RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
 - 15 b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961,
 - 20 RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof,
- is detectable in a saliva sample obtained from the subject, and
if an upregulated level or a downregulated level of at least one RNA biomarker is detected, providing treatment for TBI to the subject.

[00303] F2. The method of claim F1, wherein the at least one RNA biomarker further comprises one or more miRNA:

- 25 a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
- 30 b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

- [00304] G1. A method of detecting an RNA biomarker in a saliva sample, the method comprising
- obtaining a saliva sample from a human subject,
- contacting the saliva sample with at least one oligonucleotide primer
- 5 complementary to at least one RNA biomarker:
- a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-
- 10 miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
- 15 b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961,
- 20 RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof;
- amplifying the at least one RNA biomarker using a polymerase chain reaction;
- and
- detecting the amplified RNA biomarker.
- 25 [00305] G2. The method of claim G1, wherein the at least one RNA biomarker further comprises one or more miRNA:
- a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-
- 30 miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or

- b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
- [00306]** H1. A kit for use in a method of diagnosing and/or monitoring traumatic
 5 brain injury (TBI) in saliva from a human subject, the kit comprising at least one probe specific for at least one RNA biomarker:
- a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-
 10 1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601,
 15 UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
- b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961,
 20 RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.
- [00307]** H2. The kit of claim H1, wherein the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.
- [00308]** H3. The kit of claim H1 or H2, wherein the probe comprises a nucleic acid
 25 having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.
- [00309]** H4. The kit of claim H1 or H2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 1, 2,
 30 14, 16, 23, 26, 27, 29, 39, 40, 48, 71, 72, 73, 74, 75, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 89, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150.

[00310] H5. The kit of any one of claims H1-H4, wherein the at least one RNA biomarker further comprises one or more miRNA:

- 5 a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
- 10 b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

[00311] I1. A composition for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the composition comprising at least one probe specific for at least one RNA biomarker:

- 15 a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958,
- 20 RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
- 25 b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375,
- 30 U6.428, and YRNA-255, or any combination thereof.

[00312] I2. The composition of claim I1, wherein the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.

[00313] I3. The composition of claim I1 or I2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.

[00314] I4. The composition of claim I1 or I2, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 1, 2, 14, 16, 23, 26, 27, 29, 39, 40, 48, 71, 72, 73, 74, 75, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 89, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150.

[00315] I5. The composition of any one of claims I1-I4, wherein the at least one RNA biomarker further comprises one or more miRNA:

- a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
- b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

[00316] Other advantages that are obvious and/or inherent to the disclosure will be evident to one skilled in the art. It will be understood that certain features and sub-combinations are of utility and may be employed without reference to other features and sub-combinations. This is contemplated by and is within the scope of the claims. Since many possible embodiments may be made of the disclosure without departing from the scope thereof, it is to be understood that all matter herein set forth or shown in the accompanying drawings is to be interpreted as illustrative and not in a limiting sense.

WHAT IS CLAIMED IS:

1. A method of diagnosing and/or monitoring traumatic brain injury (TBI) in a subject, the method comprising determining a level of at least one RNA biomarker in a saliva sample obtained from the subject, wherein the at least one RNA biomarker:
 - a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
 - b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.
2. The method of claim 1, wherein either an upregulated level or a downregulated level of the at least one RNA biomarker is indicative of TBI.
3. The method of claim 1, wherein the subject is diagnosed as having TBI if the level of the at least one RNA biomarker is either above or below a predetermined threshold or increased or decreased relative to a control.
4. The method of claim 1, wherein the saliva sample is obtained during a period of time after injury selected from immediately after the injury to any period post-injury.
5. The method of claim 4, wherein the saliva sample is obtained during a period of time after injury selected from immediately after the injury until the expression level of at

least one of the RNA biomarkers returns to the predetermined threshold value or to the amount of the RNA biomarker in the control sample.

6. The method of claim 4, wherein the saliva sample is obtained during a period of time after injury selected from immediately after the injury to up to 1 year, up to 10 months, up to 8 months, up to 6 months, up to 5 months, up to 4 months, up to 3 months, up to 2 months, up to 1 month, up to 25 days, up to 20 days, up to 15 days, up to 7 days, up to 5 days, up to 3 days, up to 2 days, and/or up to 24 hours post-injury.
7. The method of claim 6, wherein the saliva sample is obtained during a period of time after injury selected from immediately after the injury to 15 days, from 1 hour to 15 days, from 24 hours to 15 days, from 24 hours to 7 days, and from 2 to 5 days.
8. The method of claim 1, further comprising obtaining one or more additional saliva samples from the subject at one or more additional times after the injury and repeating the detecting and amplifying steps for each additional sample.
9. The method of claim 8, wherein the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury to any period post-injury.
10. The method of claim 8, wherein the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury until the expression level of at least one of the RNA biomarkers returns to the predetermined threshold value or to the amount of the RNA biomarker in the control sample.
11. The method of claim 8, wherein the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury to up to 1 year, up to 10 months, up to 8 months, up to 6 months, up to 5 months, up to 4 months, up to 3 months, up to 2 months, up to 1 month, up to 25 days, up to 20 days, up to 15 days, up to 7 days, up to 5 days, up to 3 days, up to 2 days, and/or up to 24 hours post-injury.

12. The method of claim 11, wherein the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury to 15 days, from 1 hour to 15 days, from 24 hours to 15 days, from 24 hours to 7 days, and from 2 to 5 days.
13. The method of claim 8, wherein the one or more additional saliva samples is obtained at day 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 after the injury.
14. The method of any one of claims 1-13, wherein the detecting an amount of the at least one RNA biomarker is performed using a PCR-based assay, a light array assay, a laminar flow chip assay, or any assay suitable for detecting that at least one RNA biomarker.
15. The method of claim 14, wherein when using the PCR-based assay the predetermined threshold is equivalent to a fold change of 1.5 or more using the 2-delta delta CT (2- $\Delta\Delta$ CT) method.
16. The method of claim 14, wherein the predetermined threshold is equivalent to a fold change of 2 or more using the 2-delta delta CT (2- $\Delta\Delta$ CT) method.
17. The method of any one of claims 1-16, wherein the at least one RNA biomarker further comprises one or more miRNA:
 - a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
 - b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
18. The method according to claim 33 further comprising identifying the human subject as being fit for normal activity after undergoing successful treatment for TBI.

19. The method of any one of claims 1-18, wherein the at least one RNA biomarker further comprises one or more miRNA:
 - a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
 - b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

20. A sensor element for a detection system for diagnosing and/or monitoring TBI, the sensor element comprising a substrate functionalized with a probe specific for at least one RNA biomarker:
 - a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
 - b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.

21. The sensor element of claim 20, wherein the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.

22. The sensor element of claim 20 or 21, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.
23. The sensor element of claim 20 or 21, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 1, 2, 14, 16, 23, 26, 27, 29, 39, 40, 48, 71, 72, 73, 74, 75, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 89, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150.
24. The sensor element of any one of claims 21-23, wherein the at least one RNA biomarker further comprises one or more miRNA:
- a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
 - b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
25. A detection system for diagnosing and/or monitoring TBI, comprising a sensor element according to any one of claims 20-24, and a detection device capable of detecting the binding of a target RNA biomarker to the probe.
26. The detection system according to claim 25, further comprising means to determine whether the target RNA biomarker is upregulated or downregulated.
27. A method for determining a course of action for a subject suspected of having TBI, comprising applying a saliva sample obtained from the subject to a detection system

according to claim 25 or 26, and if an upregulated level or a downregulated level of the at least one RNA biomarker is detected, providing a treatment for TBI.

28. A method of treating a subject with suspected TBI, the method comprising
 - determining whether an upregulated level or a downregulated level of at least one RNA biomarker:
 - a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
 - b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof,
 - is detectable in a saliva sample obtained from the subject, and
 - if an upregulated level or a downregulated level of at least one RNA biomarker is detected, providing treatment for TBI to the subject.
29. The method of claim 28, wherein the at least one RNA biomarker further comprises one or more miRNA:
 - a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or

- b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
30. A method of detecting an RNA biomarker in a saliva sample, the method comprising
 - obtaining a saliva sample from a human subject,
 - contacting the saliva sample with at least one oligonucleotide primer complementary to at least one RNA biomarker:
 - a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
 - b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof;
 - amplifying the at least one RNA biomarker using a polymerase chain reaction;
 - and
 - detecting the amplified RNA biomarker.
31. The method of claim 30, wherein the at least one RNA biomarker further comprises one or more miRNA:
 - a. selected from the group consisting of has-let-7i-5hashsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135hasp, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=mhas143), hsa-mhas148a-3phassa-miR-206hassa-miR-

- 29chas, hsa-miR-has-3p, hsahasR-425-5p, has-miR-449a, hsa-has-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
- b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-has hsa-let-7f-5hashsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
32. A kit for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the kit comprising at least one probe specific for at least one RNA biomarker:
- a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
- b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.
33. The kit of claim 32, wherein the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.
34. The kit of claim 32 or 33, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.
35. The kit of claim 32 or 33, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 1, 2, 14, 16,

23, 26, 27, 29, 39, 40, 48, 71, 72, 73, 74, 75, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 89, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150.

36. The kit of any one of claims 32-35, wherein the at least one RNA biomarker further comprises one or more miRNA:
 - a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
 - b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.

37. A composition for use in a method of diagnosing and/or monitoring traumatic brain injury (TBI) in saliva from a human subject, the composition comprising at least one probe specific for at least one RNA biomarker:
 - a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
 - b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6,

RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof.

38. The composition of claim 37, wherein the probe comprises a nucleic acid able to bind to the at least one RNA biomarker.
39. The composition of claim 37 or 38, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of the sequence of the target RNA biomarker.
40. The composition of claim 37 or 38, wherein the probe comprises a nucleic acid having at least 70% identity with a sequence which is the complement of SEQ ID NO: 1, 2, 14, 16, 23, 26, 27, 29, 39, 40, 48, 71, 72, 73, 74, 75, 77, 78, 79, 80, 81, 82, 84, 85, 86, 87, 89, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, and 150.
41. The composition of any one of claims 37-40, wherein the at least one RNA biomarker further comprises one or more miRNA:
 - a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
 - b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
42. A method of diagnosing and treating traumatic brain injury (TBI) in a human subject in need thereof, the method comprising:
 - obtaining a saliva sample from the subject;
 - contacting the saliva sample with a probe comprising a nucleic acid able to bind to at least one RNA biomarker:

- a. selected from the group consisting of hsa-miR-1246, hsa-miR-126-3p (miR-126*), hsa-miR-144-3p (miR-144*), hsa-miR-144-5p (=miR-144*), hsa-miR-16-1-3p, hsa-miR-339-5p, hsa-miR-497-5p, put-miR-1204, put-miR-323, put-miR-325, put-miR-444, put-miR-465, put-miR-469, put-miR-594, put-miR-856, put-miR-893, put-miR-958, RNU4-6p, SNORA57, SNORD3B-2, tRNA120-AlaAGC, tRNA18-ArgCCT, tRNA27-MetCAT, tRNA2-LeuTAA, tRNA73-ArgCCG, tRNA8-ThrAGT, tRNA9-TyrGTA, U2.3, U4.64, U6.168, U6.601, UC022CJG1, YRNA-245, and YRNA-684, or any combination thereof; and/or
- b. selected from the group consisting of put-miR-1003, put-miR-1080, put-miR-1084, put-miR-1146 (2), put-miR-1207, put-miR-1306, put-miR-188, put-miR-209, put-miR-468, put-miR-476, put-miR-6, put-miR-71, put-miR-742, put-miR-806, put-miR-92, put-miR-961, RNU6-4, RNU6-45, RNU6-6, RNU6-7, RNU6-73, U6.1249, U6.375, U6.428, and YRNA-255, or any combination thereof;

determining an amount of the at least one RNA biomarker in the saliva sample;

identifying the subject as having TBI where the amount of the at least one RNA biomarker is increased or decreased relative to a predetermined threshold value or relative to the amount of the RNA biomarker in a control sample; and

treating the subject identified as having TBI according to one or more of the following:

administering to the subject one or more neuroprotective therapies.

- 43. The method of claim 42, wherein the saliva sample is obtained during a period of time after injury selected from immediately after the injury to any period post-injury.
- 44. The method of claim 43, wherein the saliva sample is obtained during a period of time after injury selected from immediately after the injury until the expression level of at least one of the RNA biomarkers returns to the predetermined threshold value or to the amount of the RNA biomarker in the control sample.

45. The method of claim 43, wherein the saliva sample is obtained during a period of time after injury selected from immediately after the injury to up to 1 year, up to 10 months, up to 8 months, up to 6 months, up to 5 months, up to 4 months, up to 3 months, up to 2 months, up to 1 month, up to 25 days, up to 20 days, up to 15 days, up to 7 days, up to 5 days, up to 3 days, up to 2 days, and/or up to 24 hours post-injury.
46. The method of claim 45, wherein the saliva sample is obtained during a period of time after injury selected from immediately after the injury to 15 days, from 1 hour to 15 days, from 24 hours to 15 days, from 24 hours to 7 days, and from 2 to 5 days.
47. The method of claim 42, further comprising obtaining one or more additional saliva samples from the subject at one or more additional times after the injury and repeating the detecting and amplifying steps for each additional sample.
48. The method of claim 47, wherein the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury to any period post-injury.
49. The method of claim 47, wherein the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury until the expression level of at least one of the RNA biomarkers returns to the predetermined threshold value or to the amount of the RNA biomarker in the control sample.
50. The method of claim 47, wherein the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury to up to 1 year, up to 10 months, up to 8 months, up to 6 months, up to 5 months, up to 4 months, up to 3 months, up to 2 months, up to 1 month, up to 25 days, up to 20 days, up to 15 days, up to 7 days, up to 5 days, up to 3 days, up to 2 days, and/or up to 24 hours post-injury.
51. The method of claim 50, wherein the one or more additional saliva sample is obtained during a period of time after injury selected from immediately after the injury to 15 days, from 1 hour to 15 days, from 24 hours to 15 days, from 24 hours to 7 days, and from 2 to 5 days.

52. The method of claim 47, wherein the one or more additional saliva samples is obtained at day 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 after the injury.
53. The method of any one of claims 42-52, wherein the detecting an amount of the at least one RNA biomarker is performed using a PCR-based assay, a light array assay, a laminar flow chip assay, or any assay suitable for detecting that at least one RNA biomarker.
54. The method of claim 53, wherein when using the PCR-based assay the predetermined threshold is equivalent to a fold change of 1.5 or more using the 2-delta delta CT ($2^{-\Delta\Delta CT}$) method.
55. The method of claim 53, wherein the predetermined threshold is equivalent to a fold change of 2 or more using the 2-delta delta CT ($2^{-\Delta\Delta CT}$) method.
56. The method of any one of claims 42-55, wherein the at least one RNA biomarker further comprises one or more miRNA:
- a. selected from the group consisting of hsa-let-7i-5p, hsa-miR-107, hsa-miR-126-5p (=miR-126*), hsa-miR-135b-5p, hsa-miR-142-3p, hsa-miR-142-5p, hsa-miR-143-3p (=miR-143), hsa-miR-148a-3p, hsa-miR-206, hsa-miR-29c-3p, hsa-miR-34b-3p, hsa-miR-425-5p, hsa-miR-449a, hsa-miR-671-3p, hsa-miR-6748-3p, and hsa-miR-934, or any combination thereof; and/or
 - b. selected from the group consisting of hsa-let-7a-5p, hsa-let-7b-5p, hsa-let-7f-5p, hsa-miR-103a-3p, hsa-miR-21-5p, and hsa-miR-92a-3p, or any combination thereof.
57. The method according to claim 42 further comprising identifying the human subject as being fit for normal activity after undergoing successful treatment for TBI.