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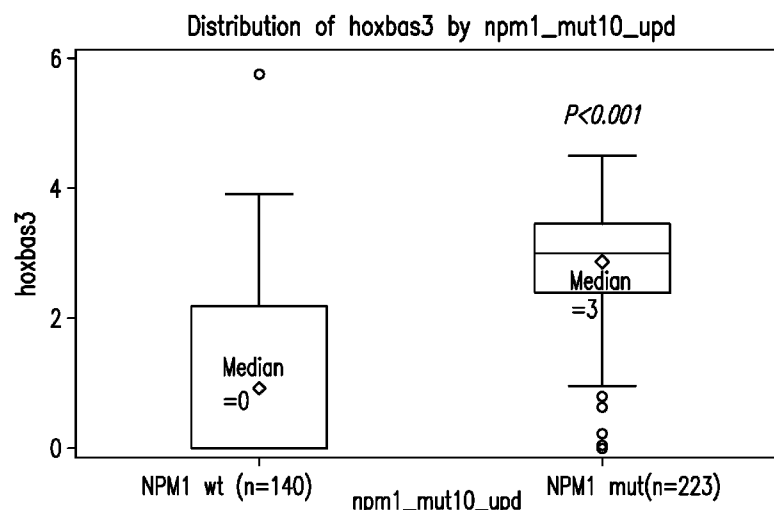


FIG.1A

(57) Abstract: The present disclosure relates to antisense oligomers targeting the long non-coding RNA HOXB-AS3 and methods of treating acute myeloid leukemia.

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ANTISENSE OLIGOMERS TARGETING HOXB-AS3 LONG NON-CODING RNA**CROSS REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Patent Application Serial No. 62/416,993 filed November 3, 2016, which is expressly incorporated herein by reference.

FIELD

The present disclosure relates to antisense oligomers for use in methods of treating acute myeloid leukemia.

BACKGROUND

Acute myeloid leukemia (AML) is a highly heterogeneous disease with regard to its underlying genetic abnormalities and clinical course. Chromosomal abnormalities (e.g., deletions, translocations) are identified in approximately 50% of all de novo AML patients. The remaining 50% of AML patients display no karyotypic aberrations in their leukemic clones when assessed with conventional karyotype analyses, that is they have cytogenetically normal AML (CN-AML). Previous work has identified several recurrent gene mutations which associate with the clinical outcome of CN-AML patients and are used in the clinic to risk-stratify the treatment of this patient population. Among them, mutations of the *NPM1* gene represent one of the most common genetic alterations in CN-AML, as they are detected in approximately 60% of the newly diagnosed CN-AML cases. This gene encodes for a ubiquitously expressed nucleolar protein that shuttles between the nucleus and cytoplasm. *NPM1* mutations specifically result in the inappropriate localization of the NPM1 protein from the nucleus into the cytoplasm. It has been reported that *NPM1*-mutated CN-AML exhibits distinctive and strong mRNA expression profiles characterized by *HOX* gene family overexpression and CD34 negativity. AML with mutant *NPM1* is now recognized as a distinct entity of AML according to the WHO classification of myeloid neoplasms. Mechanistically, over-expression of mutant *NPM1* in mice progenitors induces myeloid proliferation and *HOX* gene overexpression, supporting a critical role of the mutated protein in leukemogenesis.

In addition to genetic alterations in protein coding genes, aberrant expression of noncoding RNAs plays a critical role in leukemogenesis. A novel class of non-coding RNAs, named long noncoding RNAs (lncRNAs), which encompasses all non-protein coding RNA transcripts longer than 200 nucleotides has been recently described. A large body of accumulating data supports the important regulatory role that lncRNAs play during imprinting,

cell differentiation, apoptosis, stem cell function via a plethora of mechanisms. The long intergenic RNA (lincRNA) *HOTAIR*, whose overexpression induces metastatic phenotype in murine models of breast cancer, the lincRNA *SAMSON*, which associates with aggressive phenotype of melanoma and the lincRNA *LUNARI* which regulates *IGF1R* mRNA expression and affects *IGF1* signaling in acute lymphoblastic leukemia are notable examples among many others. In CN-AML, it has previously reported that a small number of lncRNAs strongly associate with clinical outcome and provide independent prognostic information in younger adults and older patients with CN-AML. In addition, it was found that recurrent prognostic gene mutations, including *NPM1* mutations, associate with distinctive lncRNA signatures. Among the most highly upregulated lncRNAs in patients who harbored *NPM1* mutations in both younger and older patient datasets, was a *HOXB* cluster-embedded lncRNA named *HOXB-AS3*.

The *HOX* gene locus contains several lncRNAs that have been shown to play significant roles in malignant diseases, mainly by regulating the expression levels of *HOX* genes. *HOTAIR* is a paradigm of this category, as it has been shown to associate with the Polycomb repressor complex and guide its localization on the genome, thereby silencing the *HOXD* locus. *HOTTIP* is a lncRNA that is implicated in chromosomal looping, whose overexpression results in Polycomb repressor complex-mediated silencing of the *HOXD9*. *HOTAIRMI* is a *HOXA*-antisense lncRNA which is involved in the retinoic acid-mediated granulocytic maturation of healthy hematopoietic and leukemic cells by regulating the expression levels of *HOXA1* and *HOXA4*.

The prognosis of AML is poor, highlighting the urgent need for novel therapeutic approaches. The compounds, compositions, and methods disclosed herein address these and other needs.

SUMMARY

Disclosed herein are novel locked nucleic acid (LNA) oligomers targeting the long non-coding RNA *HOXB-AS3* for treating acute myeloid leukemia. The inventors have determined that there is a causal relationship between expression of *HOXB-AS3* and the presence of *NPM1* mutations in acute myeloid leukemia. In addition, *in vivo* knock down of *HOXB-AS3* using LNA oligomers led to a significant increase in the overall survival of mice xenografted with AML blasts from several patients. In some embodiments, a liposome-based method for packaging and delivering *in vivo* *HOXB-AS3* targeting LNA oligomers is used with no significant toxicities.

In some aspects, disclosed herein is a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and

wherein the oligomer comprises at least one nucleotide analogue having a modified sugar moiety.

In some embodiments, the oligomer is between 10-30 nucleotides. In some embodiments, the oligomer is between 15-25 nucleotides.

5 In some embodiments, the at least one nucleotide analogue having a modified sugar moiety comprises a Locked Nucleic Acid. In some embodiments, the oligomer further comprises a modified phosphodiester linkage. In some embodiments, the modified phosphodiester linkage comprises a phosphorothioate bond.

10 In some embodiments, the *HOXB-AS3* non-coding RNA has the nucleic acid sequence encoded by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.

In some embodiments, the oligomer comprises a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a mixture thereof. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:2. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:3.

15 In some aspects, disclosed herein is a method for treating acute myeloid leukemia comprising administering to a subject in need thereof a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar.

20 In some embodiments, the oligomer inhibits the expression of *HOXB-AS3* non-coding RNA in a cell.

In some embodiments, the acute myeloid leukemia is cytogenetically normal AML (CN-AML).

25 In some aspects, disclosed herein is a method of inhibiting the expression of *HOXB-AS3* in a cell, comprising contacting the cell with an effective amount of a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar moiety.

In some embodiments, the cell is within a tissue of a mammal.

30 In other aspects, disclosed herein is a composition comprising a cationic nanoparticle and a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar moiety.

In some aspects, disclosed herein is a pharmaceutical composition comprising a single stranded oligomer as described herein and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

5 BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying figures, which are incorporated in and constitute a part of this specification, illustrate several aspects described below.

FIG. 1A-1H. Characterization and expression levels of the *HOXB-AS3* long non-coding RNA in acute myeloid leukemia (AML) blasts and healthy hematopoietic cells. (FIG. 1A) Distribution of *HOXB-AS3* expression in younger adults with cytogenetically normal AML (CN-AML) with wild-type *NPM1* (*NPM1*wt; n=168) or with *NPM1* mutations (*NPM1*mut). (FIG. 1B) Genomic location of the four *HOXB-AS3* transcripts, which were identified by 5-end and 3-end rapid amplification of cDNA ends-assays in OCI-AML3 cells. Rectangles indicate exons and lines indicate introns. The detected *HOXB-AS3* transcripts are depicted in red and the neighboring, protein-coding *HOXB5* and *HOXB6* genes are depicted in blue color. The positive strand is depicted in red and the negative strand in blue (FIG. 1C) Expression levels of *HOXB-AS3* as measured by real-time PCR (RT-PCR) in six different AML cell lines. Threshold cycle (Ct) values of *HOXB-AS3* RNA expression were normalized against *GAPDH* Ct values. (FIG. 1D) Comparison of *HOXB-AS3* expression in bone marrow samples from six healthy donors (hBM), AML blasts from six patients with *NPM1*mut and AML blasts from six patients with *NPM1*wt, in aggregate. *HOXB-AS3* RNA measurements were normalized against β -*ACT* Ct values. ** indicates $P \leq 0.002$. (FIG. 1E) *NPM1* mRNA and (FIG. 1F) *HOXB-AS3* RNA abundance in OCI-AML3 cells treated with non-targeting control (Scramble) versus *NPM1*--targeting oligonucleotides (*NPM1*-KD). Threshold cycle (Ct) values of *NPM1* and *HOXB-AS3* expression were normalized against *GAPDH* Ct values. ** indicates $P < 0.01$, * indicates $P < 0.05$. (FIG. 1G) *HOXB-AS3* RNA abundance in CRISPR-engineered OCI-AML3 cells that lack the mutant *NPM1* allele (*NPM1*^{wt/-}). OCI-AML3 cells treated with non-targeting guide RNAs (*NPM1*^{wt/mut}) were used as controls. **** indicates $P < 0.001$. (FIG. 1H) *Hoxb5os* RNA abundance in lineage negative murine cells of *Npm1*^{mut/wt} transgenic mice versus *Npm1*^{wt/wt} controls. *HOXB-AS3* RNA measurements were normalized against β -*act* Ct values.

FIG. 2A-2G. *In vitro* functional significance of the *HOXB-AS3* long non-coding RNA in acute myeloid leukemia cells. (FIG. 2A) Abundance of *HOXB-AS3* RNA in OCI-AML3 cells treated with non-targeting control (Scramble) versus *HOXB-AS3*-targeting oligonucleotides (HBAS3-KD). Threshold cycle (Ct) values of *HOXB-AS3* expression were normalized against

GAPDH Ct values. The ratios of the normalized Ct values to that of the Scramble-treated sample are depicted in the graph. *** indicates $P=0.001$ (FIG. 2B) Cell cycle analysis based on Bromodeoxyuridine (BrdU) and 7-Aminoactinomycin D (7-AAD) incorporation and flow cytometry in Scramble- *versus* HBAS3-KD-treated OCI-AML3 cells. The flow cytometry graphs of one experiment are depicted as an example. (FIG. 2C) Comparison of the per cent of cells at each stage of the cell cycle (G0/G1 Phase, S Phase, G2/M Phase) in OCI-AML3 cells treated with Scramble *versus* HBAS3-KD. * indicates $P=0.02$; NS indicates not significant. Results of three independent experiments are depicted (FIG. 2D) Comparison of the number of colonies formed by Scramble- *versus* HBAS3-KD-treated OCI-AML3 cells in colony-forming unit assays. * indicates $P=0.02$. Results of three independent experiments are depicted. (FIG. 2E) Abundancy of *HOXB-AS3* RNA in K562 cells transfected with empty pcDNA3 vector (Control) *versus* a *HOXB-AS3*-overexpressing pcDNA3 vector. ** indicates $P=0.02$. (FIG. 2F) Cell-cycle analysis based on BrdU and 7-AAD incorporation followed by flow cytometry in Control- *versus* HBAS3-transfected K562 cells. Results of one experiment are depicted as an example. (FIG. 2G) Comparison of the per cent of cells at each stage of the cell cycle (G0/G1 Phase, S Phase, G2/M Phase) in K562 cells transfected with Control *versus* HBAS3. ** indicates $P=0.01$; * indicates $P=0.02$; NS indicates not significant. Results of three independent experiments are depicted.

FIG. 3A-3F. *In vitro* and *in vivo* evaluation of the functional significance of *HOXB-AS3* AML patient blasts. (FIG. 3A) Abundancy of *HOXB-AS3* RNA in AML blasts from three patients with *NPM1* mutations (*NPM1*mut), treated with non-targeting control (Scramble) *versus* *HOXB-AS3*-targeting oligonucleotides (HBAS3-KD). **** indicates $P<0.001$. (FIG. 3B) Number of colonies formed by Scramble *versus* HBAS3-KD-treated OCI-AML3 cells in colony-forming unit assays. * indicates $P<0.05$; *** indicates $P<0.005$. (FIG. 3C) Schematic representation of the study design of *in vivo* HBAS3-KD, in a patient-derived xenograft (PDX) mouse model of NOD Scid gamma mice engrafted with human AML patient blasts (FIG. 3D) Abundancy of *HOXB-AS3* RNA in AML blasts from an *NPM1*mut AML patient treated *in vivo*, in a PDX mouse model, with nanoparticle-formulated Scramble *versus* HBAS3-KD. Human AML blasts were isolated from the bone marrow of treated animals by magnetic bead-based sorting after staining for human CD45. *** indicates $P=0.003$ (FIG. 3E), (FIG. 3F) Kaplan-Meyer curves depicting survival of NOD scid gamma (NSG) mice, which were xenotransplanted with blasts of two different *NPM1*mut AML patients and treated with lipid nanoparticle-formulated Scramble or HBAS3-KD. In FIG. 3E, 10 mice were treated with Scramble and 11 with HBAS3-KD. In FIG. 3F, seven mice were treated with Scramble and eight with HBAS3-KD.

FIG. 4A-4L. Interactions of the *HOXB-AS3* long non-coding RNA with the transcriptome and proteome of OCI-AML3 cells. (FIG. 4A) *HOX* gene expression in scramble versus *HOXB-AS3*-KD treated OCI-AML3 cells, measured by RNA sequencing as aligned fragments per kilobase of transcript per million mapped reads. Abundancy of *HOX* messenger and long non-coding RNAs is depicted. Purification of the *HOXB-AS3* and the *UI* transcripts with biotinylated probes according to the RNA antisense purification (RAP) protocol in OCI-AML3 cell lysates. Yield of (FIG. 4B) *HOXB-AS3* RNA, (FIG. 4C) *UI* RNA and (FIG. 4D) *GAPDH* RNA in the eluate and the flow-through of lysates hybridized with either *HOXB-AS3*- or *UI*-targeting probes. Yield is depicted relative to (i.e., as per cent of) the input sample. (FIG. 4E) List of candidate RNA binding proteins identified to interact with the *HOXB-AS3* or the *UI* transcript by Mass Spectrometry analysis of the *HOXB-AS3*- or the *UI*-specific eluates. Proteins that have previously been reported to interact with *UI* RNA are annotated in yellow color in the *UI*-specific list. Proteins that have previously been reported to form complexes are listed in proximity and annotated in blue color in the *HOXB-AS3*-specific list. The functions that these proteins regulate or the names of the complexes that they form are also annotated. (FIG. 4F) Validation of *HOXB-AS3*-protein interactions via RNA-Immunoprecipitation (RIP) experiments with nine exemplary chosen proteins. **** indicates $P < 0.001$, *** indicates $P < 0.005$, ** indicates $P < 0.01$, * indicates $P < 0.05$, NS indicates not significant. (FIG. 4G)-(FIG. 4I) Direct visualization of the *HOXB-AS3* with custom-designed RNAScope assays (in red), combined with indirect immunofluorescent visualization of NPM1 and EBP1 proteins (in green). Visualization of Nucleolin (in green) served as non-*HOXB-AS3* binding control. (FIG. 4J) Quantitation of the co-localizing NPM1, EBP1 or Nucleolin voxels which co-localize with *HOXB-AS3*. (FIG. 4K) Pearson co-relation coefficient values for the co-localization of NPM1, EBP1 or Nucleolin voxels with the *HOXB-AS3*. (FIG. 4L) Co-Immunoprecipitation experiments with anti-EBP1 or anti-NPM1 antibodies of nuclear lysates of OCI-AML3 cells, or the corresponding controls (Mouse or Rabbit IgG). Western-blot visualization for the EBP1 and NPM1 proteins was subsequently performed.

FIG. 5A-5M. *HOXB-AS3* long non-coding RNA regulates ribosomal RNA transcription and ribosome biogenesis in acute myeloid leukemia blasts. (FIG. 5A) Effect of *HOXB-AS3* depletion on the formation of the EBP1-NPM1 complex in OCI-AML3 cells. In brief protein complexes were immunoprecipitated with anti-EBP1 or the corresponding control (Rabbit IgG). Western-blot visualization for the NPM1 and EBP1 protein was subsequently performed (FIG. 5B) Effect of *HOXB-AS3*-KD on the abundancy of pre-ribosomal RNA (pre-rRNA) in OCI-AML3 cells. Threshold cycle (Ct) values of *HOXB-AS3* RNA expression were normalized against

GAPDH Ct values. *** indicates $P < 0.005$. (FIG. 5C), (FIG. 5D) Effect of *HOXB-AS3* depletion on *de novo* protein synthesis in OCI-AML3 cells, as measured by Alexa Fluor 688-labelled O-Propargyl-Puromycin incorporation into newly synthesized peptides in Scramble *versus* HBAS3-KD treated cells. The mean fluorescent intensity of the FITCH-positive Scramble *versus* HBAS3-KD treated cells is compared and results of three independent experiments are depicted. (FIG. 5E) Sucrose gradient-based ribosome profiling of Scramble (in red) *versus* HBAS3-KD (in black) treated OCI-AML3 cells. (FIG. 5F) Occupancy of ribosomal DNA promoter repeat sequences (28S rDNA) by RNA-Polymerase I in OCI-AML3 cells treated with either non-targeting control (Scramble) *versus* *HOXB-AS3*-targeting oligonucleotides (HBAS3-KD). Rabbit IgG Immunoprecipitations were performed as control for unspecific antibody binding. ***indicates $P < 0.005$, ** indicates $P < 0.001$ (FIG. 5G) Effect of *HOXB-AS3* depletion on abundancy of pre-rRNA transcripts in AML patient blasts, after *in vivo* treatment of patient-derived xenografts. Human AML blasts were isolated from the bone marrow of treated animals by magnetic bead-based sorting after staining for human CD45 prior to RNA isolation. Three scramble and four HBAS3-KD-treated mice were analyzed, in aggregate. *** indicates $P < 0.005$ (FIG. 5H) Effect of *HOXB-AS3* overexpression on the formation of the EBP1- NPM1 complex in K562 cells. In brief protein complexes were immunoprecipitated with anti-EBP1 antibodies, or the corresponding control (Rabbit IgG) in control *versus* *HOXB-AS3*-overexpressing K562 cells. Western-blot visualization for the EBP1 and NPM1 proteins was subsequently performed. (FIG. 5I) Effect of *HOXB-AS3* overexpression (HBAS3) on abundancy of pre-rRNA in K562 cells. Threshold cycle (Ct) values of *HOXB-AS3* RNA expression were normalized against *GAPDH* Ct values. *** indicates $P < 0.005$. (FIG. 5J), (FIG. 5K) Effect of *HOXB-AS3* overexpression on *de novo* protein synthesis in K562 cells xenograft, as measured by GFP-labelled O-Propargyl-Puromycin incorporation into newly synthesized peptides in empty vector control *versus* *HOXB-AS3* overexpressing K562 cells. The mean fluorescent intensity of the FITCH-positive control *versus* *HOXB-AS3* overexpressing cells is compared and results of three independent experiments are depicted. (FIG. 5L) Luciferase/Renilla activity in K562 cells transfected with an empty luciferase reporter vector or an rDNA-promoter containing luciferase reporter vector, a renilla vector and empty pcDNA3 expression vector or a *HOXB-AS3* overexpressing vector. ** indicates $P < 0.01$. (FIG. 5M) Occupancy of ribosomal DNA promoter repeat sequences (28S rDNA) by RNA-Polymerase I in K562 cells transfected with either empty vector control or *HOXB-AS3* overexpressing pcDNA3 vector.

FIG. 6A-6B. Functional characterization of the *HOXB-AS3* long non-coding RNA region which interacts with the EBP1 protein. (FIG. 6A) Wild-type *HOXB-AS3* and five mutant variants

generated by deletion of approximately 100 nucleotides. (FIG. 6B) RNA immunoprecipitation experiments with native EBP1 of K562 cells which were transfected with either wt or mutant variants of *HOXB-AS3*.

FIG. 7A-7C. Expression of the *HOXB-AS3* long non-coding RNA across different populations of healthy hematopoietic cells and acute myeloid leukemia subtypes. (FIG. 7A) *HOXB-AS3* expression between different cytogenetic AML subtypes and bone marrow-isolated healthy hematopoietic cells based on the publicly available dataset of the International Microarray Innovations in Leukemia Study Group. (FIG. 7B) *HOXB-AS3* expression between different cytogenetic AML subtypes in the publicly available dataset of The Cancer Genome Atlas project. (FIG. 7C) *HOXB-AS3* expression between different molecular subtypes of cytogenetically normal AML in the publicly available dataset of The Cancer Genome Atlas project.

FIG. 8A-8C. Targeting of the *HOXB-AS3* long non-coding RNA with RNase H-recruiting, locked nucleic acid-modified oligonucleotides (LNAs). (FIG. 8A) The areas of the *HOXB-AS3* transcripts that are targeted by the different LNAs (LNA#1- #5) are annotated in blue-color boxes. (FIG. 8B), (FIG. 8C) Abundancy of the *HOXB-AS3* RNA in OCI-AML3 cells treated with non-targeting control (Scramble) *versus* different LNAs (LNA#1- LNA#5), as measured by custom-designed real-time PCR assays. The assay used in FIG. 8B measures abundancy of transcripts NR_033202.2 and NR_033203.1. The assay used in FIG. 8C measures abundancy of transcripts NR_033201.2 and uc060gwg.1. **** indicates $P < 0.0001$; *** indicates $P \leq 0.0009$; ** indicates $P = 0.002$.

FIG. 9A-9B. Knock-down of the *HOXB-AS3* long non-coding RNA does not affect apoptosis in OCI-AML3 cells. (FIG. 9A) Apoptosis evaluation in OCI-AML3 cells treated with either non-targeting control (Scramble) or *HOXB-AS3*-targeting oligonucleotides (HBAS3-KD). Apoptosis analysis was conducted with Annexin V and Propidium Iodide (PI) staining and flow cytometry. Results of one experiment are depicted as an example. (FIG. 9B) Comparison of per cent of viable and apoptotic cells in Scramble- *versus* HBAS3-KD-treated cells. Results of three independent experiments are depicted. NS indicates not significant.

FIG. 10A-10B. Engraftment of human AML blasts in NOD Scid Gamma mouse recipients. Per cent of human AML blasts in the peripheral blood of mice engrafted with human AML blasts of patients 1 (FIG. 10A) and 2 (FIG. 10B), who harbor *NPM1* mutations, as evaluated by staining for the human CD45 and the murine CD45.1 antigens and flow cytometric analyses. Mice were randomly assigned to receive scramble non-targeting control or anti-*HOXB-AS3* treatment. NS indicates not significant.

DETAILED DESCRIPTION

Disclosed herein are novel locked nucleic acid (LNA) oligomers targeting the long non-coding RNA *HOXB-AS3* for treating acute myeloid leukemia. The inventors have determined that there is a causal relationship between expression of *HOXB-AS3* and the presence of *NPM1* mutations in acute myeloid leukemia. In addition, *in vivo* knock down of *HOXB-AS3* using LNA oligomers led to a significant increase in the overall survival of mice xenografted with AML blasts from several patients. In some embodiments, a liposome-based method for packaging and delivering *in vivo* *HOXB-AS3* targeting LNA oligomers is used with no significant toxicities.

Reference will now be made in detail to the embodiments of the invention, examples of which are illustrated in the drawings and the examples. This invention may, however, be embodied in many different forms and should not be construed as limited to the embodiments set forth herein.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this disclosure belongs. The term “comprising” and variations thereof as used herein is used synonymously with the term “including” and variations thereof and are open, non-limiting terms. Although the terms “comprising” and “including” have been used herein to describe various embodiments, the terms “consisting essentially of” and “consisting of” can be used in place of “comprising” and “including” to provide for more specific embodiments and are also disclosed.

The following definitions are provided for the full understanding of terms used in this specification.

Terminology

As used in the specification and claims, the singular form “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a cell” includes a plurality of cells, including mixtures thereof.

As used herein, the terms “may,” “optionally,” and “may optionally” are used interchangeably and are meant to include cases in which the condition occurs as well as cases in which the condition does not occur. Thus, for example, the statement that a formulation “may include an excipient” is meant to include cases in which the formulation includes an excipient as well as cases in which the formulation does not include an excipient.

As used here, the terms “beneficial agent” and “active agent” are used interchangeably herein to refer to a chemical compound or composition that has a beneficial biological effect.

Beneficial biological effects include both therapeutic effects, i.e., treatment of a disorder or other undesirable physiological condition, and prophylactic effects, i.e., prevention of a disorder or other undesirable physiological condition. The terms also encompass pharmaceutically acceptable, pharmacologically active derivatives of beneficial agents specifically mentioned
5 herein, including, but not limited to, salts, esters, amides, prodrugs, active metabolites, isomers, fragments, analogs, and the like. When the terms “beneficial agent” or “active agent” are used, then, or when a particular agent is specifically identified, it is to be understood that the term includes the agent per se as well as pharmaceutically acceptable, pharmacologically active salts, esters, amides, prodrugs, conjugates, active metabolites, isomers, fragments, analogs, etc.

10 As used herein, the terms “treating” or “treatment” of a subject includes the administration of a drug to a subject with the purpose of preventing, curing, healing, alleviating, relieving, altering, remedying, ameliorating, improving, stabilizing or affecting a disease or disorder, or a symptom of a disease or disorder. The terms “treating” and “treatment” can also refer to reduction in severity and/or frequency of symptoms, elimination of symptoms and/or
15 underlying cause, prevention of the occurrence of symptoms and/or their underlying cause, and improvement or remediation of damage.

As used herein, the term “preventing” a disorder or unwanted physiological event in a subject refers specifically to the prevention of the occurrence of symptoms and/or their underlying cause, wherein the subject may or may not exhibit heightened susceptibility to the
20 disorder or event.

By the term “effective amount” of a therapeutic agent is meant a nontoxic but sufficient amount of a beneficial agent to provide the desired effect. The amount of beneficial agent that is “effective” will vary from subject to subject, depending on the age and general condition of the subject, the particular beneficial agent or agents, and the like. Thus, it is not always possible to
25 specify an exact “effective amount.” However, an appropriate “effective” amount in any subject case may be determined by one of ordinary skill in the art using routine experimentation. Also, as used herein, and unless specifically stated otherwise, an “effective amount” of a beneficial can also refer to an amount covering both therapeutically effective amounts and prophylactically effective amounts.

30 An “effective amount” of a drug necessary to achieve a therapeutic effect may vary according to factors such as the age, sex, and weight of the subject. Dosage regimens can be adjusted to provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation.

As used herein, a “therapeutically effective amount” of a therapeutic agent refers to an amount that is effective to achieve a desired therapeutic result, and a “prophylactically effective amount” of a therapeutic agent refers to an amount that is effective to prevent an unwanted physiological condition. Therapeutically effective and prophylactically effective amounts of a given therapeutic agent will typically vary with respect to factors such as the type and severity of the disorder or disease being treated and the age, gender, and weight of the subject.

The term “therapeutically effective amount” can also refer to an amount of a therapeutic agent, or a rate of delivery of a therapeutic agent (e.g., amount over time), effective to facilitate a desired therapeutic effect. The precise desired therapeutic effect will vary according to the condition to be treated, the tolerance of the subject, the drug and/or drug formulation to be administered (e.g., the potency of the therapeutic agent (drug), the concentration of drug in the formulation, and the like), and a variety of other factors that are appreciated by those of ordinary skill in the art.

As used herein, the term “pharmaceutically acceptable” component can refer to a component that is not biologically or otherwise undesirable, i.e., the component may be incorporated into a pharmaceutical formulation of the invention and administered to a subject as described herein without causing any significant undesirable biological effects or interacting in a deleterious manner with any of the other components of the formulation in which it is contained. When the term “pharmaceutically acceptable” is used to refer to an excipient, it is generally implied that the component has met the required standards of toxicological and manufacturing testing or that it is included on the Inactive Ingredient Guide prepared by the U.S. Food and Drug Administration.

Also, as used herein, the term “pharmacologically active” (or simply “active”), as in a “pharmacologically active” derivative or analog, can refer to a derivative or analog (e.g., a salt, ester, amide, conjugate, metabolite, isomer, fragment, etc.) having the same type of pharmacological activity as the parent compound and approximately equivalent in degree.

The term “subject” or “host” refers to any individual who is the target of administration or treatment. The subject can be a vertebrate, for example, a mammal. Thus, the subject can be a human or veterinary patient. The term “patient” refers to a subject under the treatment of a clinician, e.g., physician. The subject can be either male or female.

The terms “peptide,” “protein,” and “polypeptide” are used interchangeably to refer to a natural or synthetic molecule comprising two or more amino acids linked by the carboxyl group of one amino acid to the alpha amino group of another.

The term “nucleic acid” refers to a natural or synthetic molecule comprising a single nucleotide or two or more nucleotides linked by a phosphate group at the 3’ position of one nucleotide to the 5’ end of another nucleotide. The nucleic acid is not limited by length, and thus the nucleic acid can include deoxyribonucleic acid (DNA) or ribonucleic acid (RNA). In some
5 embodiments, the DNA and RNA sequences are used interchangeably to refer to the RNA sequence encoded by the underlying DNA (gene) sequence. For example, antisense oligomers targeting the corresponding cDNA sequence of a non-coding RNA, are understood to hybridize to the analogous RNA sequence encoded by the DNA sequence provided. It is understood that for DNA sequences disclosed herein, the corresponding RNA sequences are also disclosed
10 herein (wherein all thymine (T) nucleotides are replaced by a uracil (U) nucleotide, and all deoxyribose moieties in the DNA are replaced with the ribose moieties present in RNA).

“Complementary” or “substantially complementary” refers to the hybridization or base pairing or the formation of a duplex between nucleotides or nucleic acids, such as, for instance, between the two strands of a double stranded DNA molecule or between an oligonucleotide
15 primer and a primer binding site on a single stranded nucleic acid. Complementary nucleotides are, generally, A and T/U, or C and G. Two single-stranded RNA or DNA molecules are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 80% of the nucleotides of the other strand, usually at least about 90% to 95%, and more preferably from
20 about 98 to 100%. Alternatively, substantial complementarity exists when an RNA or DNA strand will hybridize under selective hybridization conditions to its complement. Typically, selective hybridization will occur when there is at least about 65% complementary over a stretch of at least 14 to 25 nucleotides, at least about 75%, or at least about 90% complementary. See Kanehisa (1984) Nucl. Acids Res. 12:203.

“Hybridization” refers to the process in which two single-stranded oligonucleotides bind non-covalently to form a stable double-stranded oligonucleotide. The term “hybridization” may also refer to triple-stranded hybridization. The resulting (usually) double-stranded oligonucleotide is a “hybrid” or “duplex.” “Hybridization conditions” will typically include salt concentrations of less than about 1 M, more usually less than about 500 mM and even more
30 usually less than about 200 mM. Hybridization temperatures can be as low as 5° C., but are typically greater than 22° C., more typically greater than about 30° C., and often in excess of about 37° C. In certain exemplary embodiments, hybridization takes place at room temperature. The term “stringent hybridization conditions” as used herein is the binding which occurs within a range from about T_m 5° C. (5° C. below the melting temperature T_m of the probe) to about 20°

C. to 25° C. below T_m. The term “highly stringent hybridization conditions” as used herein refers to conditions of: at least about 6×SSC and 1% SDS at 65° C., with a first wash for 10 minutes at about 42° C. with about 20% (v/v) formamide in 0.1×SSC, and with a subsequent wash with 0.2×SSC and 0.1% SDS at 65° C.

5 The term “oligomer” as used herein refers to a molecule formed by covalent linkage of two or more nucleotides (i.e. an oligonucleotide). Herein, a single nucleotide (unit) may also be referred to as a monomer or unit. In some embodiments, the oligomer consists or comprises of a contiguous nucleotide sequence of between 10-50, such as 10-30 nucleotides in length.

10 The term “LNA” refers to a bicyclic nucleotide analogue, known as “Locked Nucleic Acid”. It may refer to an LNA monomer, or, when used in the context of an “LNA oligonucleotide”, “LNA oligomer”, or “LNA gapmer”, LNA refers to an oligonucleotide containing one or more such bicyclic nucleotide analogues. LNA nucleotides are characterized by the presence of a biradical ‘bridge’ between C2’ and C4’ of the ribose sugar ring (LNA bases have a modification to the ribose backbone that locks the base in the C3'-endo-position). See for
15 example, US8563528, US9506060.

The terms “about” and “approximately” are defined as being “close to” as understood by one of ordinary skill in the art. In one non-limiting embodiment the terms are defined to be within 10%. In another non-limiting embodiment, the terms are defined to be within 5%. In still another non-limiting embodiment, the terms are defined to be within 1%.

20 Antisense Oligomers and Methods

In some aspects, disclosed herein is a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar
25 moiety.

In some embodiments, the oligomer is between 10-30 nucleotides. In some embodiments, the oligomer is between 15-25 nucleotides. In some embodiments, the oligomer is selected from the following number of nucleotides: 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30.

30 In some embodiments, the at least one nucleotide analogue having a modified sugar moiety comprises a Locked Nucleic Acid. In some embodiments, the oligomer further comprises a modified phosphodiester linkage. In some embodiments, the modified phosphodiester linkage comprises a phosphorothioate bond.

In some embodiments, at least one nucleotide analogue comprises a locked nucleic acid (for example, at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10) or more nucleotide analogues comprising a locked nucleic acid.

In some embodiments, the *HOXB-AS3* non-coding RNA is encoded by the nucleic acid sequence encoded by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10. In some
5 embodiments, the *HOXB-AS3* non-coding RNA is encoded by the nucleic acid sequence SEQ ID NO:7. In some embodiments, the *HOXB-AS3* non-coding RNA is encoded by the nucleic acid sequence SEQ ID NO:8. In some embodiments, the *HOXB-AS3* non-coding RNA is encoded by the nucleic acid sequence SEQ ID NO:9. In some embodiments, the *HOXB-AS3* non-coding
10 RNA is encoded by the nucleic acid sequence SEQ ID NO:10. In some embodiments, the *HOXB-AS3* non-coding RNA encoded by a nucleic acid sequence comprising at least 60% (for example, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%) similarity to SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10. The *HOXB-AS3* transcripts are shown in Figure 8 and the DNA sequences
15 encoding the RNA transcripts are disclosed in the Sequences section below.

In some embodiments, the oligomer comprises a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a mixture thereof. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:1. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:2. In some
20 embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:3. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:4. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:5. In some embodiments, the oligomer comprises a mixture of the nucleic acid sequence SEQ ID NO:2 and SEQ ID NO:3.

25 In some embodiments, the oligomer comprises a nucleic acid sequence with at least 60% (for example, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 99%) similarity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5.

In some embodiments, the oligomer targets a continuous sequence within the non-coding
30 RNA. In some embodiments, the oligomer targets a split (non-continuous) sequence within the non-coding RNA (wherein the oligomer can hybridize to different portions of the non-coding RNA or may target different splice variants of the non-coding RNA).

In some aspects, disclosed herein is a single stranded oligomer, wherein the oligomer is substantially complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and

wherein the oligomer comprises at least one nucleotide analogue having a modified sugar moiety.

In some embodiments, the at least one nucleotide analogue comprises a chemically modified nucleobase, a modified sugar moiety, a chemically modified phosphodiester linkage, or a combination thereof. In one embodiment, the chemically modified phosphodiester linkage is selected from Phosphorothioate (PS), Boranophosphate, phosphodithioate (PS₂), 3',5'-amide, N3'-phosphoramidate (NP), Phosphodiester (PO), or 2',5'-phosphodiester (2',5'-PO). In one embodiment, the chemically modified phosphodiester linkage is phosphorothioate.

In some aspects, disclosed herein is a method for treating acute myeloid leukemia comprising administering to a subject in need thereof a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar.

In some embodiments, the oligomer is between 10-30 nucleotides. In some embodiments, the oligomer is between 15-25 nucleotides.

In some embodiments, the at least one nucleotide analogue having a modified sugar moiety comprises a Locked Nucleic Acid. In some embodiments, the oligomer further comprises a modified phosphodiester linkage. In some embodiments, the modified phosphodiester linkage comprises a phosphorothioate bond.

In some embodiments, the *HOXB-AS3* non-coding RNA has the nucleic acid sequence encoded by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.

In some embodiments, the oligomer comprises a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a mixture thereof. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:2. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:3.

In some embodiments, the oligomer inhibits the expression of *HOXB-AS3* non-coding RNA in a cell.

In some embodiments, the acute myeloid leukemia is cytogenetically normal AML (CN-AML).

In some aspects, disclosed herein is a method of inhibiting the expression of *HOXB-AS3* in a cell, comprising contacting the cell with an effective amount of a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar moiety.

In some embodiments, the oligomer is between 10-30 nucleotides. In some embodiments, the oligomer is between 15-25 nucleotides.

In some embodiments, the at least one nucleotide analogue having a modified sugar moiety comprises a Locked Nucleic Acid. In some embodiments, the oligomer further comprises a modified phosphodiester linkage. In some embodiments, the modified phosphodiester linkage comprises a phosphorothioate bond.

In some embodiments, the *HOXB-AS3* non-coding RNA has the nucleic acid sequence encoded by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.

In some embodiments, the oligomer comprises a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a mixture thereof. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:2. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:3.

In some embodiments, the cell is within a tissue of a mammal.

In some embodiments, the acute myelogenous leukemia (AML) is cytogenetically normal acute myelogenous leukemia (CN-AML). In some embodiments, the acute myelogenous leukemia (AML) is *NPM1*mut (comprises an *NPM1* mutation).

Compositions

In some aspects, disclosed herein is a composition comprising a cationic nanoparticle and a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar moiety.

In some embodiments, the oligomer is between 10-30 nucleotides. In some embodiments, the oligomer is between 15-25 nucleotides.

In some embodiments, the at least one nucleotide analogue having a modified sugar moiety comprises a Locked Nucleic Acid. In some embodiments, the oligomer further comprises a modified phosphodiester linkage. In some embodiments, the modified phosphodiester linkage comprises a phosphorothioate bond.

In some embodiments, the *HOXB-AS3* non-coding RNA has the nucleic acid sequence encoded by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.

In some embodiments, the oligomer comprises a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a mixture thereof. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:2. In some embodiments, the oligomer comprises the nucleic acid sequence SEQ ID NO:3.

In some aspects, disclosed herein is a pharmaceutical composition comprising the oligomer as described herein and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

Compositions, as described herein, comprising an active compound and an excipient of some sort may be useful in a variety of applications. For example, pharmaceutical compositions comprising an active compound and an excipient can be useful for the treatment or prevention of acute myeloid leukemia. In one embodiment, disclosed herein is a pharmaceutical composition comprising: an oligomer as disclosed herein and a pharmaceutically acceptable excipient.

“Excipients” include any and all solvents, diluents or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, lubricants and the like, as suited to the particular dosage form desired. General considerations in formulation and/or manufacture can be found, for example, in *Remington's Pharmaceutical Sciences*, Sixteenth Edition, E. W. Martin (Mack Publishing Co., Easton, Pa., 1980), and *Remington: The Science and Practice of Pharmacy*, 21st Edition (Lippincott Williams & Wilkins, 2005).

Exemplary excipients include, but are not limited to, any non-toxic, inert solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. Some examples of materials which can serve as excipients include, but are not limited to, sugars such as lactose, glucose, and sucrose; starches such as corn starch and potato starch; cellulose and its derivatives such as sodium carboxymethyl cellulose, ethyl cellulose, and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients such as cocoa butter and suppository waxes; oils such as peanut oil, cottonseed oil; safflower oil; sesame oil; olive oil; corn oil and soybean oil; glycols such as propylene glycol; esters such as ethyl oleate and ethyl laurate; agar; detergents such as Tween 80; buffering agents such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; and phosphate buffer solutions, as well as other non-toxic compatible lubricants such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, releasing agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the composition, according to the judgment of the formulator. As would be appreciated by one of skill in this art, the excipients may be chosen based on what the composition is useful for. For example, with a pharmaceutical composition or cosmetic composition, the choice of the excipient will depend on the route of administration, the agent being delivered, time course of delivery of the agent, etc., and can be administered to humans and/or to animals, orally, rectally, parenterally, intracisternally, intravaginally, intranasally,

intraperitoneally, topically (as by powders, creams, ointments, or drops), buccally, or as an oral or nasal spray.

Exemplary diluents include calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, etc., and combinations thereof.

Exemplary granulating and/or dispersing agents include potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinyl-pyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (Veegum), sodium lauryl sulfate, quaternary ammonium compounds, etc., and combinations thereof.

Exemplary surface active agents and/or emulsifiers include natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and Veegum [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g. stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (e.g. carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate [Tween 20], polyoxyethylene sorbitan [Tween 60], polyoxyethylene sorbitan monooleate [Tween 80], sorbitan monopalmitate [Span 40], sorbitan monostearate [Span 60], sorbitan tristearate [Span 65], glyceryl monooleate, sorbitan monooleate [Span 80]), polyoxyethylene esters (e.g. polyoxyethylene monostearate [Myrij 45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and Solutol), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. Cremophor), polyoxyethylene ethers, (e.g. polyoxyethylene lauryl ether [Brij 30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, Pluronic F 68, Poloxamer 188,

cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof.

Exemplary binding agents include starch (e.g. cornstarch and starch paste), gelatin, sugars (e.g. sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol, etc.), natural and synthetic gums (e.g. acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, 5 mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (Veegum), and larch arabogalactan), alginates, polyethylene oxide, polyethylene glycol, 10 inorganic calcium salts, silicic acid, polymethacrylates, waxes, water, alcohol, etc., and/or combinations thereof.

Exemplary preservatives include antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and other preservatives.

Exemplary antioxidants include alpha tocopherol, ascorbic acid, acorbyl palmitate, 15 butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and sodium sulfite.

Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA) and salts 20 and hydrates thereof (e.g., sodium edetate, disodium edetate, trisodium edetate, calcium disodium edetate, dipotassium edetate, and the like), citric acid and salts and hydrates thereof (e.g., citric acid monohydrate), fumaric acid and salts and hydrates thereof, malic acid and salts and hydrates thereof, phosphoric acid and salts and hydrates thereof, and tartaric acid and salts and hydrates thereof. Exemplary antimicrobial preservatives include benzalkonium chloride, 25 benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride, chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and thimerosal.

Exemplary antifungal preservatives include butyl paraben, methyl paraben, ethyl paraben, 30 propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and sorbic acid.

Exemplary alcohol preservatives include ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and phenylethyl alcohol.

Exemplary acidic preservatives include vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and phytic acid.

Other preservatives include tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisol (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, Glydant Plus, Phenonip, methylparaben, Germall 115, Germaben II, Neolone, Kathon, and Euxyl. In certain embodiments, the preservative is an anti-oxidant. In other embodiments, the preservative is a chelating agent.

Exemplary buffering agents include citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium gluconate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, etc., and combinations thereof.

Exemplary lubricating agents include magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, etc., and combinations thereof.

Exemplary natural oils include almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba, castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mint, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary synthetic oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl

sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and combinations thereof.

Additionally, the composition may further comprise a polymer. Exemplary polymers contemplated herein include, but are not limited to, cellulosic polymers and copolymers, for example, cellulose ethers such as methylcellulose (MC), hydroxyethylcellulose (HEC),
 5 hydroxypropyl cellulose (HPC), hydroxypropyl methyl cellulose (HPMC), methylhydroxyethylcellulose (MHEC), methylhydroxypropylcellulose (MHPC), carboxymethyl cellulose (CMC) and its various salts, including, e.g., the sodium salt, hydroxyethylcarboxymethylcellulose (HECMC) and its various salts,
 10 carboxymethylhydroxyethylcellulose (CMHEC) and its various salts, other polysaccharides and polysaccharide derivatives such as starch, dextran, dextran derivatives, chitosan, and alginic acid and its various salts, carageenan, various gums, including xanthan gum, guar gum, gum arabic, gum karaya, gum ghatti, konjac and gum tragacanth, glycosaminoglycans and proteoglycans such as hyaluronic acid and its salts, proteins such as gelatin, collagen, albumin, and fibrin, other
 15 polymers, for example, polyhydroxyacids such as polylactide, polyglycolide, poly(lactide-co-glycolide) and poly(epsilon-caprolactone-co-glycolide)-, carboxyvinyl polymers and their salts (e.g., carbomer), polyvinylpyrrolidone (PVP), polyacrylic acid and its salts, polyacrylamide, polyacilic acid/acrylamide copolymer, polyalkylene oxides such as polyethylene oxide, polypropylene oxide, poly(ethylene oxide-propylene oxide), and a Pluronic polymer,
 20 polyoxyethylene (polyethylene glycol), polyanhydrides, polyvinylalcohol, polyethyleamine and polypyridine, polyethylene glycol (PEG) polymers, such as PEGylated lipids (e.g., PEG-stearate, 1,2-Distearoyl-sn-glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-1000], 1,2-Distearoyl-sn-glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-2000], and 1,2-Distearoyl-sn-glycero-3-Phosphoethanolamine-N-[Methoxy(Polyethylene glycol)-5000]), copolymers and salts thereof.

Additionally, the composition may further comprise an emulsifying agent. Exemplary emulsifying agents include, but are not limited to, a polyethylene glycol (PEG), a polypropylene glycol, a polyvinyl alcohol, a poly-N-vinyl pyrrolidone and copolymers thereof, poloxamer nonionic surfactants, neutral water-soluble polysaccharides (e.g., dextran, Ficoll, celluloses),
 30 non-cationic poly(meth)acrylates, non-cationic polyacrylates, such as poly(meth)acrylic acid, and esters amide and hydroxyalkyl amides thereof, natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and Veegum [magnesium aluminum silicate]), long chain amino acid derivatives, high

molecular weight alcohols (e.g. stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl polymer), carrageenan, cellulosic derivatives (e.g. carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate [Tween 20], polyoxyethylene sorbitan [Tween 60], polyoxyethylene sorbitan monooleate [Tween 80], sorbitan monopalmitate [Span 40], sorbitan monostearate [Span 60], sorbitan tristearate [Span 65], glyceryl monooleate, sorbitan monooleate [Span 80]), polyoxyethylene esters (e.g. polyoxyethylene monostearate [Myrj 45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and Solutol), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. Cremophor), polyoxyethylene ethers, (e.g. polyoxyethylene lauryl ether [Brij 30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, Pluronic F 68, Poloxamer 188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof. In certain embodiments, the emulsifying agent is cholesterol.

Liquid compositions include emulsions, microemulsions, solutions, suspensions, syrups, and elixirs. In addition to the active compound, the liquid composition may contain inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and perfuming agents.

Injectable compositions, for example, injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing or wetting agents and suspending agents. The sterile injectable preparation may also be a injectable solution, suspension, or emulsion in a nontoxic parenterally acceptable diluent or solvent, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents for pharmaceutical or cosmetic compositions that may be employed are water, Ringer's solution, U.S.P. and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. Any bland fixed oil can be employed including synthetic mono- or

diglycerides. In addition, fatty acids such as oleic acid are used in the preparation of injectables. In certain embodiments, the particles are suspended in a carrier fluid comprising 1% (w/v) sodium carboxymethyl cellulose and 0.1% (v/v) Tween 80. The injectable composition can be sterilized, for example, by filtration through a bacteria-retaining filter, or by incorporating
5 sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

Compositions for rectal or vaginal administration may be in the form of suppositories which can be prepared by mixing the particles with suitable non-irritating excipients or carriers such as cocoa butter, polyethylene glycol, or a suppository wax which are solid at ambient
10 temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the particles.

Solid compositions include capsules, tablets, pills, powders, and granules. In such solid compositions, the particles are mixed with at least one excipient and/or a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol, and silicic acid, b) binders such as, for
15 example, carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose, and acacia, c) humectants such as glycerol, d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, e) solution retarding agents such as paraffin, f) absorption accelerators such as quaternary ammonium compounds, g) wetting agents such as, for example, cetyl alcohol and glycerol monostearate, h)
20 absorbents such as kaolin and bentonite clay, and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof. In the case of capsules, tablets, and pills, the dosage form may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight
25 polyethylene glycols and the like.

Tablets, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally contain opacifying agents and can also be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a
30 delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes.

Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

Compositions for topical or transdermal administration include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants, or patches. The active compound is admixed with an excipient and any needed preservatives or buffers as may be required.

The ointments, pastes, creams, and gels may contain, in addition to the active compound, excipients such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc, and zinc oxide, or mixtures thereof.

Powders and sprays can contain, in addition to the active compound, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates, and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants such as chlorofluorohydrocarbons.

Transdermal patches have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms can be made by dissolving or dispensing the nanoparticles in a proper medium. Absorption enhancers can also be used to increase the flux of the compound across the skin. The rate can be controlled by either providing a rate controlling membrane or by dispersing the particles in a polymer matrix or gel.

The active ingredient may be administered in such amounts, time, and route deemed necessary in order to achieve the desired result. The exact amount of the active ingredient will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the infection, the particular active ingredient, its mode of administration, its mode of activity, and the like. The active ingredient, whether the active compound itself, or the active compound in combination with an agent, is preferably formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the total daily usage of the active ingredient will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the active ingredient employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific active ingredient employed; the duration of the treatment; drugs used in combination or coincidental with the specific active ingredient employed; and like factors well known in the medical arts.

The active ingredient may be administered by any route. In some embodiments, the active ingredient is administered via a variety of routes, including oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, subcutaneous, intraventricular,

transdermal, interdermal, rectal, intravaginal, intraperitoneal, topical (as by powders, ointments, creams, and/or drops), mucosal, nasal, bucal, enteral, sublingual; by intratracheal instillation, bronchial instillation, and/or inhalation; and/or as an oral spray, nasal spray, and/or aerosol. In general the most appropriate route of administration will depend upon a variety of factors including the nature of the active ingredient (e.g., its stability in the environment of the gastrointestinal tract), the condition of the subject (e.g., whether the subject is able to tolerate oral administration), etc.

The exact amount of an active ingredient required to achieve a therapeutically or prophylactically effective amount will vary from subject to subject, depending on species, age, and general condition of a subject, severity of the side effects or disorder, identity of the particular compound(s), mode of administration, and the like. The amount to be administered to, for example, a child or an adolescent can be determined by a medical practitioner or person skilled in the art and can be lower or the same as that administered to an adult.

EXAMPLES

The following examples are set forth below to illustrate the compounds, compositions, methods, and results according to the disclosed subject matter. These examples are not intended to be inclusive of all aspects of the subject matter disclosed herein, but rather to illustrate representative methods and results. These examples are not intended to exclude equivalents and variations of the present invention which are apparent to one skilled in the art.

Example 1. The long non-coding RNA *HOXB-AS3* regulates ribosome biogenesis in *NPM1*-mutated acute myeloid leukemia

Acute myeloid leukemia (AML) is a highly heterogeneous disease with regard to its underlying genetic abnormalities and clinical course. The prognosis of AML is poor, highlighting the urgent need for novel therapeutic approaches. Chromosomal abnormalities (e.g., deletions, translocations) are identified in approximately 50% of all de novo AML patients. The remaining 50% of AML patients display no karyotypic aberrations in their leukemic clones when assessed with conventional karyotype analyses, that is they have cytogenetically normal AML (CN-AML). Previous work has identified several recurrent gene mutations which associate with the clinical outcome of CN-AML patients and are used in the clinic to risk-stratify the treatment of this patient population. Among them, mutations of the *NPM1* gene represent one of the most common genetic alterations in CN-AML, as they are detected in approximately 60 % of the

newly diagnosed CN-AML cases. This gene encodes for a ubiquitously expressed nucleolar protein that shuttles between the nucleus and cytoplasm. *NPM1* mutations specifically result in the inappropriate localization of the NPM1 protein from the nucleus into the cytoplasm. It has been reported that *NPM1*-mutated CN-AML exhibits distinctive and strong mRNA expression profiles characterized by *HOX* gene family overexpression and CD34 negativity. AML with mutant *NPM1* is now recognized as a distinct entity of AML according to the WHO classification of myeloid neoplasms. Mechanistically, over-expression of mutant *NPM1* in mice progenitors induces myeloid proliferation and *HOX* gene overexpression supporting a critical role of the mutated protein in leukemogenesis.

In addition to genetic alterations in protein coding genes, aberrant expression of noncoding RNAs plays a critical role in leukemogenesis. A novel class of non-coding RNAs, named long noncoding RNAs (lncRNAs), which encompass all non-protein coding RNA transcripts longer than 200 nucleotides has been recently described. A large body of accumulating data supports the important regulatory role that lncRNAs play during imprinting, cell differentiation, apoptosis, stem cell function via a plethora of mechanisms. The long intergenic RNA (lincRNA) *HOTAIR*, whose overexpression induces metastatic phenotype in murine models of breast cancer, the lincRNA *SAMSON*, which associates with aggressive phenotype of melanoma and the lincRNA *LUNARI* which regulates *IGF1R* mRNA expression and affects *IGF1* signaling in acute lymphoblastic leukemia are notable examples among many others. In CN-AML, it has previously reported that a small number of lncRNAs strongly associate with clinical outcome and provide independent prognostic information in younger adults and older patients with CN-AML. In addition, it was found that recurrent prognostic gene mutations, including *NPM1* mutations, associate with distinctive lncRNA signatures. Among the most highly upregulated lncRNAs in patients who harbored *NPM1* mutations in both younger and older patient datasets, was a *HOXB* cluster-embedded lncRNA named *HOXB-AS3*.

The *HOX* gene locus contains several lncRNAs that have been shown to play significant roles in malignant diseases, mainly by regulating the expression levels of *HOX* genes. *HOTAIR* is a paradigm of this category, as it has been shown to associate with the Polycomb repressor complex and guide its localization on the genome, thereby silencing the *HOXD* locus. *HOTTIP* is a lncRNA that is implicated in chromosomal looping, whose overexpression results in Polycomb repressor complex-mediated silencing of the *HOXD9*. *HOTAIRMI* is a *HOXA*-antisense lncRNA which is involved in the retinoic acid-mediated granulocytic maturation of healthy hematopoietic and leukemic cells by regulating the expression levels of *HOXA1* and

HOXA4. In the example here, the functional significance of *HOXB-AS3* was examined in *NPM1mut* AML.

Methods

5 AML patient samples and cell lines

For association analyses of *HOXB-AS3* expression with clinical characteristics, gene mutations and clinical outcome, a previously published cohort of 377 younger adults with CN-AML, who were treated on frontline Alliance CALGB protocols was analyzed. All patients had provided informed consent for the use of their biologic specimens for research purposes
 10 according to the Declaration of Helsinki. Cytogenetic analyses were performed with standard banding technique after stimulation of cells with for 48h. Gene mutations, which have previously been described to be prognostic in AML were analyzed by means of a targeted DNA sequencing panel (Miseq; *ASXL1*, *DNMT3A*, *IDH1*, *IDH2*, *FLT3-TKD*, *NPM1*, *RUNX1*, *TET2*, *WT1*), Sanger sequencing (double *CEBPA*) and fragment analyses (*FLT3-ITD*), as previously
 15 described. Expression levels of prognostic mRNA (*BAALC*, *MNI*, *ERG*) and microRNA (miR-155, miR-181) genes were evaluated with total RNAseq and small RNAseq, respectively.

For the *in vitro* and *in vivo* functional experiments leukapheresis samples of AML patients deposited in the biobank of The Ohio State University were used. For *in vitro* experiments patient blasts were cultured in SFEM media (Stem cell technologies), supplemented
 20 with 10% BIT media and cytokines (hSCF, hTPO, hFLT3-ligand, hIL-3 and hIL-6- Peprotech). For colony formation unit assays, cells were electroporated (see below), cultured for 24 h and then mixed with Methocult, Stem Cell technologies, according to the instructions of the manufacturer. For *in vivo* experiments NOD-Scid Gamma (NSG) mice, pretreated with Busulfan (20 mg/kg weight) were engrafted with 10 million AML blasts, as previously described.

25 Kasumi-1, KG1-A, MV4-11, MOLM-13, K562, OCI-AML3 and the THP1 AML cell lines were grown in RPMI media, supplemented with 10% FBS. For colony formation unit assays, cells were electroporated (see below), cultured for 24 h and then mixed with plain Methocult (without cytokines), Stem Cell technologies, according to the instructions of the manufacturer

30 RNA isolation and cDNA transcription

RNA isolations were performed using the Trizol reagent according to the instructions of the manufacturer. Reverse transcription was performed with the Superscript III single strand RT kit. All custom real time PCR experiments were conducted in the standard conditions, suggested

by Applied Biosystems. The sequences of the real-time assays used to interrogate *HOXB-AS3* expression were the following:

Primer #1: CCA TTC TCG ATC TTT TCA AGC G (SEQ ID NO:11)

Primer #2: AGG TTG CTT GTC TGG AGA TG (SEQ ID NO:12)

5 Probe: /56-FAM/CGC CTC ATC /ZEN/GCT CTT ATC TAA GCC C/3IABkFQ/ (SEQ ID NO:13)

Rapid amplification of cDNA ends was performed with the Generacer Assay Kit, Invitrogen, following the instructions of the manufacturer. The primer sequences used for the
10 RACE assays are the following:

For the 5-end-RACE:

Rv.Primer#1: 5'- GCC GGC GAG GGA GAG GAA AC -3' (SEQ ID NO:14)

Rv.Primer#2: 5'- CTT GGT TGG TGG GTC CGT GGT G -3' (SEQ ID NO:15)

15 For the 3-end-RACE:

Fw primer#1: 5'- CGT TTC CTC TCC CTC GCC G -3' (SEQ ID NO:16)

Fw primer#2: 5'- CAC CAA CCA AGG AGC TGG C -3' (SEQ ID NO:17)

Gene knock-downs and overexpression

20 For the knock-down of *HOXB-AS3* in OCI-AML3 cells and samples from *NPM1*mut AML patients, custom-designed locked nucleic acid modified RNaseH recruiting oligonucleotides were used. For the in vitro delivery of the oligos, the Amaxa Nucleofector device and corresponding reagents (Solution T for OCI-AML3 cells and human Monocyte Solution for AML blasts) were used according to the instructions of the manufacturer.

25 For the *in vivo* knock down of the lncRNA, *HOXB-AS3*-targeting, LNA-modified Gapmers or non-targeting controls were packaged in a cationic liposomal nanoparticle formulation and were conjugated with human transferrin. Human AML xenografts were treated and followed for survival as previously described.

30 For the overexpression of *HOXB-AS3*, RACE identified transcripts were cloned into TOPO-Blunt end vectors (Invitrogen) and were then excised with double digestion with the enzymes Hind III and BamHI. Standard pcDNA3 LIC 6A vectors were acquired from Addgene and were also digested with the same restriction enzymes in parallel. Digestion products were weight separated by agarose gel electrophoresis and visible bands were excised, purified with the

Qiagen gel extraction kit and ligated into the pcDNA3 vectors with T4 DNA ligase. Sequences were verified by Sanger sequencing.

Flow cytometry analyses

Cell cycle and apoptosis analyses were performed with the BrDU and the Annexin/PI staining kits of BD Biosciences. Experiments were analyzed with flow cytometry on an LSRII instrument, according to the instructions of the manufacturers.

RNA Antisense purification experiments

RNA-protein complex pull down experiments were conducted according to a modified version of the RAP protocol, as published by McHugh et al. (McHugh CA, Chen CK, Chow A1 et al. The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3. *Nature*, 2015;521(7551):232-236). In brief, batches of 20×10^6 cells were crosslinked directly with 8000 Joules/m² of UV irradiation. Cells were lysed in lysis buffer (10 mM Tris-HCl, 500 mM LiCl, 0.5% dodecyl maltoside (DDM), 0.2% sodium dodecyl sulfate (SDS), 0.1% sodium deoxycholate), sonicated and treated with DNase I (Norgen Biotek). Lysates were mixed with hybridization buffer (10 mM Tris-HCl, 5 mM EDTA, 500 mM LiCl, 0.5% DDM, 0.2% SDS, 0.1% sodium deoxycholate, 4 M urea, 2.5 mM TCEP) and spun down in a table top centrifuge (10 minutes at 16000 Gs at 4°C). The supernatants were pre-cleaned, by incubation (37 degrees for 30 minutes, shaking) with streptavidin-coated magnetic beads (Life Technologies, Dynabeads MyOne, Streptavidin C1). After removal of beads the lysates were incubated with biotinylated probes (2 µgs per 20 million cells) that were complementary to either the *HOXB-AS3* or the *UI* transcripts. The sequences of the biotinylated probes are provided in the supplemental material. Probes and lysates were allowed to hybridize by incubation at 67°C for 2 hours, shaking. Streptavidin-coated magnetic beads were used for the purification of complexes. The purified complexes were either treated with Proteinase K (New England Biolabs) (to digest protein residues) or with benzonase nuclease (EMD Millipore) (to digest probes and captured RNA and release RNA-bound protein residues from the streptavidin beads). Proteinase K-treated eluates were mixed with an N-lauroylsarcosine-rich (NLS, Sigma) buffer, heated for 2 minutes at 95°C and released from the magnetic beads. Eluted nucleic acids were subsequently treated with DNase I and purified by use of magnetic SILANE beads (Invitrogen, Dynabeads MyOne). The isolated RNA was reversed transcribed into cDNA and analyzed with RT-PCR. Polypeptides in the benzonase endonuclease treated samples were precipitated by overnight with trichloroacetic acid (Ricca Chemical Company) and centrifugation. Polypeptides were then digested with Trypsin (Promega) and Endopeptidase Lys-C (Wako), purified with HiPPR detergent removal columns (Life Technologies) and analyzed with tandem mass spectrometry. The results were

filtered for number and quality of identified peptides (for more information see data supplement) and analyzed comparatively to identify putative *HOXB-AS3* and *UI*-specific protein interactors.

RNA immunoprecipitations and protein-protein co-immunoprecipitation experiments

RNA immunoprecipitation experiments to validate the predicted *HOXB-AS3*-protein interactions by the RAP screening experiments and co-immunoprecipitation experiments to study protein-protein interactions were conducted in nuclear lysates as previously described. In brief 10x 10⁶ cells were crosslinked by exposure to UV irradiation (8000 Joules/m²) prior to cell lysis. Cells were then re-suspended in 10 ml of lysis Buffer consisting of 6 ml of water, 2 ml of PBS and 2 ml of Nuclear Isolation Buffer (1.28 M sucrose, 40 mM Tris-HCl pH 7.5, 20 mM MgCl₂, 4% Triton X-100). Nuclei were pelleted by centrifugation at 2500 Gs at 4°C for 15 minutes and were then re-suspended in 1 ml of RIP Buffer (150 mM KCl, 25 mM Tris pH 7.4, 5 mM EDTA, 0.5 mM DTT, 0.5% NP40 plus RNAase and Protease inhibitors). Cell pellets were sonicated with a microtip sonicator (pulse sonication of 5 Watts for 10 seconds followed by a 50-second interval, three repeats) and spun down for 10 minutes at 16,000 Gs at 4°C. Supernatants were mixed with magnetic A/G beads, pre-coated and cross-linked with antibodies as previously described and incubated overnight at 4°C. Beads were then magnetically separated, washed and mixed with either Proteinase K-containing solution (for RIP analyses) or beta-mercaptoethanol containing buffer (for Co-immunoprecipitation analyses).

Immunoprecipitates used for RIP analysis were treated with proteinase K (one hour at 52°C) and were then mixed with 1 ml of Trizol reagent. Standard Trizol-based RNA isolations were subsequently performed. For Co-IPs, the beads were denatured at 99 °C for 10 mins. Following magnetic separation, the eluates were directly loaded onto SDS-PAGE gels and separated by electrophoresis.

Chromatin Immunoprecipitation experiments

Chromatin immunoprecipitation experiments were conducted according to previously published protocols. To isolate nucleolar chromatin cells, the experimental approach described by O'Sullivan et al. was used (O'Sullivan AC, Sullivan GJ, McStay B. UBF binding in vivo is not restricted to regulatory sequences within the vertebrate ribosomal DNA repeat. *Mol Cell Biol.* 2002;22(2):657-668). Briefly, cells cross-linked by formaldehyde (0.25% for 10 min) and washed in phosphate buffered saline (PBS). After centrifugation at 200 Gs for 5 minutes, cell pellets were re-suspended in 1.0 ml of high-magnesium buffer (10 mM HEPES [pH 7.5], 0.88 M sucrose, 12 mM MgCl₂, and 1 mM dithiothreitol [DTT], plus protease inhibitors). Nucleoli were released by sonication on ice (two to three bursts of 10 s each at full power) using a Soniprep 150 (MSE) with a fine probe. Nucleoli were pelleted by centrifugation in a microfuge (15,000 Gs

for 20 s), and the pellets were resuspended in 1.0ml of low-magnesium buffer (10 mM HEPES [pH 7.5], 0.88 M sucrose, 1 mM MgCl₂, and 1 mM DTT, plus protease inhibitors). Nucleoli were subject to further sonication on ice (10 s at full power) and pelleted as before. Nucleoli were resuspended in 0.2 ml of 20/2TE (20 mM Tris [pH 8.0], 2 mM EDTA) and a 1/10 volume of 20% sodium dodecyl sulfate (SDS) was added. Following incubation at 37°C for 15 min, 20/2TE (0.8 ml) was added, and the solutions were sonicated (three to four bursts of 5 s each at full power). The resulting sheared nucleolar chromatin was centrifuged in a microfuge (15,000 Gs for 10 min), and the nucleolar chromatin supernatant was used immediately in ChIP assays.

RNA Pol I or EBP1 (Cell signaling) antibodies were incubated with magnetic Agarose beads for one hour at room temperature and washed twice with PBS. Agarose beads were blocked by incubation in 2% BSA and salmon sperm containing solution. Cell lysates were pre-cleaned with blocked agarose beads. Antibody-coated beads were incubated overnight with nucleolar lysates. Chromatin immunoprecipitates were analyzed with the Simple Chip 28S rDNA assay (Cell signaling) and/or the custom primers for Nucleolar Chip as described by O; Sullivan et al.

rRNA transcription reporter and assay

To further study the effect of *HOXB-AS3* on the expression of rRNA transcription by RNA polymerase I, the pHrD-IRES-Luc (human rRNA promoter-luciferase reporter) was used. In this plasmid, Kozak sequence of the pGL3-basic vector has replaced by the internal ribosome entry site (IRES) of encephalomyocarditis viral genome to optimize pol I-driven reporter gene expression. K562 cells were concomitantly transfected with i) the Renilla, ii) the pGL3-IRES-Luc or the pHrD-IRES-Luc and iii) the pcDNA3 or the pcDNA3-HOXB-AS3 vectors. Transfected cells were incubated for 72 hours and were then lysed in lysis buffer (Promega), according to the instructions of the manufacturer. Luciferase activity was measured using the Dual Luciferase Assay kit (Promega) in a Luminometer (Lumat LB 9507; EG&G Berthold, Oak Ridge, TN).

Results

Associations of *HOXB-AS3* expression status with pre-treatment characteristics and clinical outcome of CN-AML patients

The association of *HOXB-AS3* expression with clinical features was evaluated in a dataset of 377 younger (aged <60 years) adults with CN-AML patients, whose samples were analyzed with RNA sequencing. Patients with *NPM1* mutations (*NPM1*mut) had approximately a three-fold increase in *HOXB-AS3* expression when compared to patients with wild-type *NPM1*

(*NPM1*wt; Figure 1A, $P<0.001$). Next, the median *HOXB-AS3* expression value was used to divide the patient cohort into high or low *HOXB-AS3* expressers. With regard to pretreatment characteristics, younger adult CN-AML patients with high *HOXB-AS3* expression were more likely to be female than those with low *HOXB-AS3* expression ($P=0.007$). Patients with high *HOXB-AS3* expression were also more likely to have higher white blood cell counts ($P<0.001$), and higher percent of blasts in peripheral blood ($P<0.001$) and in bone marrow (BM) ($P<0.001$) at the time of diagnosis. Other than *NPM1* mutations, patients with high *HOXB-AS3* were more likely to harbor internal tandem duplications of the *FLT3* gene (*FLT3*-ITD, $P<0.001$), as well as mutations in the *DNMT3A* ($P=0.004$) and *WT1* ($P=0.02$) genes and to be high expressers of miR-155 ($P<0.001$). In contrast, patients with low *HOXB-AS3* expression were more likely to have mutations in the *CEBPA* ($P<0.001$), *ASXL1* ($P=0.006$) and *RUNX1* ($P<0.001$) genes and to be high expressers of *MNI* ($P<0.001$), *BAALC* ($P<0.001$), miR-3151 ($P<0.001$) and miR-181a ($P=0.05$) genes. With regard to clinical outcome, patients with low *HOXB-AS3* expression showed a trend towards higher complete remission rates when compared to patients with high *HOXB-AS3* expression (87% versus 80%, $P=0.07$). There was no significant difference in other survival end points.

Characterization of *HOXB-AS3* transcripts and expression profiling in healthy hematopoietic cells and leukemic blasts

The *HOXB-AS3* locus shows striking transcriptional complexity as 24 transcript variants of this lncRNA have been identified and annotated in genomic databases (UCSC Genome Browser). To characterize the transcripts that are expressed in leukemic blasts, 5-end and 3-end rapid amplification of cDNA ends (RACE) assays were performed in the *NPM1*mut harboring OCI-AML3 cells. Primers were designed based on the transcripts previously identified in a cohort of 148 older CN-AML patients, analyzed with the Arraystar microarray assay. Four different transcript variants of *HOXB-AS3* were identified ((NR_033201.2, NR_033202.2, NR_033203.1, and uc60gwg.1; Figure 1B).

The expression levels of *HOXB-AS3* were then analyzed in six different AML cell lines (Kasumi-1, KG1a, MOLM13, MV4-11, OCI-AML3 and THP1: all are *NPM1*wt except OCI-AML3) by real-time PCR, custom designed to interrogate the expression of RACE-identified transcripts. Detectable levels of *HOXB-AS3* were only found in the *NPM1*mut OCI-AML3 cells (Figure 1C). Furthermore, samples from *NPM1*mut and *NPM1*wt AML patients were screened, as well as samples of whole BM cells from healthy donors ($n=6$ in each group). It was found that *HOXB-AS3* was expressed at higher levels in *NPM1*mut AML patients compared to patients with *NPM1*wt ($P<0.01$) and healthy BM cells (Figure 1D; $P<0.01$). There was no significant

difference in *HOXB-AS3* expression between healthy BM cells and the *NPM1*/wt samples. In addition, publicly available microarray and RNA sequencing datasets of AML samples and normal hematopoietic cells were also queried (as analyzed by the International Microarray Innovations in Leukemia Study Group and The Cancer Genome Atlas Project. Consistent with these findings, these analyses showed that *HOXB-AS3* is not expressed in healthy BM cells and that it is primarily detected in CN-AML samples (Figure 7A and 7B). With regard to molecular subsets of CN-AML, *HOXB-AS3* was highly expressed in patients who harbored *NPM1* mutations independently of the concomitant presence or absence of *FLT3*-ITD (Figure 7C).

***HOXB-AS3* overexpression in AML blasts is driven by the presence of *NPM1* mutations.**

To further examine the association between *HOXB-AS3* expression and presence of *NPM1* mutations, knock-down (KD) experiments of *NPM1* were performed in OCI-AML3 cells. Electroporation-mediated delivery of *NPM1*-targeting, locked nucleic acid (LNA)-modified oligonucleotides led to significant downregulation of *NPM1* mRNA (Figure 1E; $P < 0.001$). At 48 hours post electroporation, when no differences in apoptosis between cells treated with scramble or anti-*NPM1* oligos could be detected KD of *NPM1* led to a decrease in *HOXB-AS3* expression (Figure 1F; $P = 0.003$). To further study this association, CRISPRi technology was used to generate genetically modified OCI-AML3 cells, which lack the *NPM1*mut and express one *NPM1*wt allele (*NPM1*^{wt}). Genetic removal of the mutated *NPM1* allele led to a decrease in the expression of *HOXB-AS3* as well as that of the flanking *HOXB5* and *HOXB6* genes (Figure 1G; $P < 0.001$).

Finally, lineage marker negative progenitor cells were analyzed, which were harvested from bone marrow of *NPM1*mut transgenic mice and age-matched controls. The expression of a murine lncRNA transcript was measured, which is antisense to the *Hoxb5* and *Hoxb6* genes and displays a 25% sequence identity to the human *HOXB-AS3*, named *Hoxb5os*. As shown in Figure 1H, *Hoxb5os* was significantly upregulated in the *NPM1*mut mice ($P < 0.001$), when compared to the wt controls. Taken together, these data indicate that *HOXB-AS3* overexpression in AML blasts is driven by the presence of *NPM1* mutations.

***HOXB-AS3* expression regulates cell proliferation in AML cells**

To evaluate the functional significance of *HOXB-AS3* expression in *NPM1* mutated AML cells, *HOXB-AS3*-KD experiments were conducted using RNaseH recruiting, LNA-modified gapmers. Delivery of five different oligonucleotides that targeted distinct regions of the four *HOXB-AS3* variants to OCI-AML3 cells via electroporation led to significant concomitant downregulation of all *HOXB-AS3* transcripts (Figure 8), suggesting that all variants stem from one common precursor transcript. In all subsequent KD experiments, a mixture of the Gapmers

#2 and #3 were used to deplete *HOXB-AS3* (Figure 2A, $P<0.001$). Cell-cycle analysis based on Bromodiuridine (BrdU) incorporation and 7-Actinomycin D staining revealed a decrease of the proliferating fraction of OCI-AML3 cells upon *HOXB-AS3*-KD (Figure 2B and 2C; $P=0.02$; S-phase average % in control *versus* KD: 24% *versus* 16%,). Annexin V/ Propidium Iodide (PI) staining analysis showed no difference in apoptosis between the scramble and the *HOXB-AS3*-KD treated cells (Supplemental Figure 9. Colony formation unit assays in soft agar revealed a decrease in the colony forming capacity of OCI-AML3 cells after *HOXB-AS3*-KD (Figure 2D; $P=0.02$; mean of colonies in scramble *versus* *HOXB-AS3*-KD; 37 *versus* 18).

In contrast, overexpression of the *HOXB-AS3* in K562 cells (Figure 2E; $P=0.008$) led to an increase in the fraction of the proliferating blasts as measured by BrdU-based cell cycle analysis (Figure 2G; $P=0.02$; S-phase average % in control *versus* *HOXB-AS3* overexpression: 32% *versus* 43%,), with a concomitant decrease in the percent of cells in the G0-G1 phase (Figure 2G; $P=0.008$; G0-G1-phase average % in control *versus* *HOXB-AS3* overexpression: 22% *versus* 16%, $P<0.01$). The proliferative phenotype was specifically mediated by overexpression of the transcript variant NR_033202.2. Overexpression of *HOXB-AS3* did not affect the viability of K562 cells (Data not shown).

Knock down (KD) experiments were then conducted in blasts of AML patients, who harbored *NPM1*mut. Delivery of anti-*HOXB-AS3* oligonucleotides via electroporation efficaciously targeted and down-regulated *HOXB-AS3* expression (Figure 3A; $P<0.001$ in all three cases). *HOXB-AS3*-KD led to a significant decrease in the number of colonies formed by the leukemic blasts in CFU-assays in soft agar, compared to non-targeting scramble control (Figure 3B).

***In vivo* depletion of *HOXB-AS3* prolongs survival of mice xenotransplanted with *NPM1*mut patient AML blasts**

In order to evaluate the *in vivo* effect of *HOXB-AS3*-KD in AML blasts, experiments were conducted in patient-derived xenograft mouse models. In brief, a modification of a previously described protocol was used to transplant Busulfan-pre-treated Nod Scid Gamma (NSG) mice with unselected leukemic blasts of two AML patients, who harbored *NPM1*mut. Mice were randomly divided into a group which received nanoparticle-formulated LNA-modified anti-*HOXB-AS3* oligos or similarly formulated non-targeting scramble control. A schematic overview of the treatment protocol and experimental design is provided in Figure 3C. A small cohort of mice engrafted with blasts of patient #2 was treated for two weeks and was then sacrificed to evaluate the efficacy of the *in vivo* *HOXB-AS3*-KD. Treatment with anti-*HOXB-AS3* oligos led to significant down-regulation of *HOXB-AS3* expression in human-CD45

selected AML blasts, which were isolated from the bone marrow of the treated mice (Figure 3D; $P < 0.001$). Larger cohorts of mice engrafted with blasts of patient #1 and patient #2 were treated and monitored for survival. Mice were evaluated for engraftment on day 42 post-transplant by flow cytometry analysis of peripheral blood and evaluation of human CD45 and murine CD45.1 expression. In the xenografts of both patients there was no significant difference in the engraftment of human leukemia in the scramble treated *versus* the *HOXB-AS3*-KD treated groups (Figure 10). For both *NPM1*mut xenograft models, *in vivo* *HOXB-AS3*-KD led to significant prolongation of the survival of the treated mice compared to the non-targeting scramble control. In xenografts of patient #1, the median survival of the scramble treated mice ($n=10$) was 62 days whereas the median survival of the *HOXB-AS3*-KD treated groups ($n=11$) was not reached after 100 days of treatment (Figure 3E, $P < 0.001$). In xenografts of patient #2 the median survival of the scramble treated arm ($n=7$) was 42 days and that of the *HOXB-AS3*-KD arm ($n=8$) was 58 days (Figure 3F $P = 0.02$).

Interaction of *HOXB-AS3* with the protein-coding transcriptome of *NPM1*mut AML blasts.

To study the molecular pathways that are regulated by *HOXB-AS3*, additional analyses were performed in the cohort of 377 younger CN-AML patients who were previously studied with RNAseq. The initial focus was on the *NPM1* mutated subpopulation and correlation analysis of *HOXB-AS3* expression with protein-coding mRNA transcripts was performed. Pathway analysis revealed that genes involved in RNA-processing, ribosome biogenesis and DNA repair response are enriched in patients with high *HOXB-AS3* expression. Furthermore, RNA sequencing was performed in scramble *versus* *HOXB-AS3*-KD treated OCI-AML3 cells as well as blasts of three *NPM1*mut AML patients. Pathway analyses revealed that genes involved in DNA damage response were upregulated.

Since several *HOX*-loci-embedded lncRNAs have been shown to play a significant role in cancer by regulating (in cis or trans) expression levels of *HOX* genes, it was specifically examined whether *HOXB-AS3*-KD affected the expression of *HOX* genes, in particular the *HOXA* and *HOXB* clusters, which are upregulated in *NPM1*mut CN-AML. It was found that *HOXB-AS3*-KD had no effect on the expression levels of any of the *HOXB* and *HOXA* loci that were overexpressed in the OCI-AML3 cells (Figure 4A). Since *HOXA9* is highly expressed in *NPM1*mut CN-AML and is relevant for leukemogenesis, *HOXA9* protein expression was also assessed by western blotting after *HOXB-AS3* KD in OCI-AML3 cells. In concordance with the RNA seq data, no difference between controls and *HOXB-AS3* KD was detected.

Interaction of *HOXB-AS3* with the proteome of *NPM1*mut AML cells

To further study the mechanism by which *HOXB-AS3* modulates cell proliferation, RNA-protein complex pull-down experiments were performed to identify the interactions of *HOXB-AS3* with the proteome of *NPM1*mut AML cells. A modified version of the RNA-antisense purification technique was used, as previously described by McHught et al. (McHugh CA, Chen CK, Chow A1 et al. The Xist lncRNA interacts directly with SHARP to silence transcription through HDAC3. *Nature*, 2015;521(7551):232-236). In brief, biotinylated probes that are complementary to the *HOXB-AS3* lncRNA or the *U1* transcripts were incubated with UV-cross-linked lysates of OCI-AML3 cells and allowed to hybridize. Streptavidin-coated magnetic beads were used for the purification of the RNA-protein complexes, which were then subjected either to RNA isolation and real-time PCR (RT-PCR) or mass-spectrometry analyses. RT-PCR analyses showed that a high percent of the targeted RNA transcripts could be purified from the hybridized lysates with high specificity (Figures 4B-4D). Comparative analysis of the putative *U1* and *HOXB-AS3* interacting proteins, as identified by mass spectrometry analyses was conducted and the results are listed in Figure 4E. Four proteins, previously reported to form the *U1*-complex (SNRPA, SMD2, SMD3 and RUXE) were included in the list of the identified *U1*-interactors, validating the efficacy of the approach.

Twenty-two RNA-binding proteins were identified as putative *HOXB-AS3* interactors. Validation experiments were performed with nine of the 22 identified proteins by RNA immunoprecipitations. Significant enrichment of *HOXB-AS3* was found in the respective immunoprecipitates in seven of the nine proteins (Figure 4F). The validated *HOXB-AS3*-binding proteins showed specificity with regard to the *HOXB-AS3* transcripts, with which they interacted (EBP1, NONO, DHX9, and PARK7 interacted with the NR_033202.2 and/or the NR_033202.1 transcripts, whereas MATR3, SRSF2 and PHB1 interacted with the NR_033201.2 and/or the uc060gwg.1 transcripts). EBP1 was shown to bind to *HOXB-AS3* most avidly, as it bound to approximately 80% of the measured *HOXB-AS3* in input samples. In addition, *in vitro* KD of *EBP1* in OCI-AML3 cells generated a similar phenotype to *HOXB-AS3*-KD; a decrease in the proliferating fraction of the cells with no significant effect on apoptosis.

EBP1 is an RNA binding protein which regulates a variety of cellular functions, such as proliferation, cell cycle progression and response to cellular stress. EBP1 has been reported to interact with NPM1 and regulate transcription of ribosomal RNA species transcription and ribosome biogenesis in AML cells. The interaction of EBP1 and NPM1 proteins in the presence of *NPM1* mutations was investigated by co-immunoprecipitation experiments in nuclear lysates of OCI-AML3 cells. It was found that EBP1 and NPM1 interact as shown by

immunoprecipitation experiments of either of the two proteins, followed by western blotting (Figure 4L. As both wt and mutant NPM1 proteins are expressed in OCI-AML3 cells, similar immunoprecipitation experiments were performed with an antibody which specifically recognizes the C-terminus of the mutated NPM1 protein. Only a small amount of the mutated protein could be isolated from nuclear lysates and this was not found to interact with the EBP1 protein (Figure 5A).

To further study the interaction of *HOXB-AS3* with its binding proteins and to dissect the cellular localization of the lncRNA, direct visualization experiments were performed using the RNAScope assay and custom designed probes, which specifically targeted the NR_033202.2 transcript. Co-staining with DAPI showed that the *HOXB-AS3* lncRNA is located in the nucleus. Concomitant immuno-staining was performed of the EBP1 and the NPM1 proteins, as well as of the RNA-binding protein Nucleolin, which was not predicted to interact with *HOXB-AS3*, as a control. Analyses of the generated images showed a high degree of intra-nuclear interaction of *HOXB-AS3* with the NPM1 and the EBP1 proteins, in contrast to Nucleolin. The number of visualized voxels co-localizing with *HOXB-AS3* was 60000 for NPM1, 42000 for EBP1 and 169 for Nucleolin. The Pearson correlation co-efficient of co-localization was 0.72 for NPM1, 0.82 for EBP1 and 0.19 with Nucleolin (Figure 4K).

Regulation of ribosomal RNA (rRNA) transcription and ribosome biogenesis by the *HOXB-AS3*-EBP1-NONO-NPM1 ribonucleoprotein complex

As mentioned previously, EBP1 has been shown to interact with NPM1 in the nucleus and to regulate rRNA transcription and ribosome biogenesis in AML cells. To investigate whether *HOXB-AS3* modulates ribosome biogenesis in *NPM1*mut CN-AML, it was investigated how the *HOXB-AS3* levels affect the formation of the EBP1-NPM1 complex. As shown in Figure 5A, depletion of *HOXB-AS3* in OCI-AML3 cells led to a decrease in the interacting amount of EBP1 and NPM1, as evaluated by co-immunoprecipitation experiments. The effect of *HOXB-AS3* KD in rRNA transcription and ribosome biogenesis was also investigated. *HOXB-AS3*-KD led to a decrease in the abundance of the transcribed rRNA in OCI-AML3 cells (Figure 5B, $P < .001$). In addition, chromatin-immunoprecipitation experiments with an RNA Polymerase I (POL1RA) binding antibody showed that *HOXB-AS3*-KD led to a reduction in the occupancy of the rDNA promoter by POL1RA (Figure 5F; $P < .01$). With regard to ribosome biogenesis, sucrose gradient-mediated polysome profiling in OCI-AML3 cells showed a reduction in the formed polysomes upon *HOXB-AS3*-KD. This reduction primarily concerned the 80S fraction of the formed polysomes (Figure 5E). Consistently, *in vitro* labelling of the *de novo* synthesized polypeptides in OCI-AML3 cells by incorporation of O-propargyl-puromycin (OPP) and

fluorochrome-conjugation, showed that *HOXB-AS3*-KD led to a reduction of protein synthesis in the OCI-AML3 cells, when compared to scramble control (Figure 5D; $P=0.02$). Finally, short-term *in vivo* treatment of human AML blasts in murine PDX models revealed that *HOXB-AS3*-KD led to a decrease in the amount of transcribed rRNA species and a decrease (Figure 5B; $P<0.001$) in the *de novo* protein synthesis in these cells (Figure 5G).

Inversely, overexpression of the *HOXB-AS3* in K562 led to an increase in the formed EBP1-NPM1 complex, as shown by co-immunoprecipitation experiments (Figure 5H). In addition, overexpression of *HOXB-AS3* led to an increase in the transcribed rRNA species, in comparison to empty vector control (Figure 5I; $P<0.05$). Overexpression of *HOXB-AS3* also led to increased occupancy of the rDNA promoter by POL1RA when compared to control (Figure 5L; $P<0.001$). To further validate these observations, experiments were performed with a luciferase reporter vector of POL1RA activity as described by Ghoshal et al. (Ghoshal K, Majumder S, Datta J et al. Role of Human Ribosomal RNA (rRNA) Promoter Methylation and of Methyl-CpG-binding Protein MBD2 in the Suppression of rRNA Gene Expression, *J Biol Chem.* 2004;279(8):6783-6793). In brief, a region of 800 nucleotides of the ribosomal DNA promoter sequence was ligated into a luciferase reporter of promoter activity, which was modified to minimize interference by RNA-polymerase II-mediated transcription. K562 cells transfected with the rDNA promoter-containing luciferase vector showed an approximately 10-fold increase in the luciferase-to-renilla ratio compared to cells transfected with empty vector controls. This increase was significantly higher when the K562 cells were concomitantly transfected with a *HOXB-AS3* overexpressing vector *versus* a control empty vector (Figure 5M; $P<0.01$). Finally, *HOXB-AS3* overexpression led to an increase in the *de novo* protein synthesis of transfected K562 cells, as measured by OPP incorporation and subsequent fluorochrome labelling of the newly synthesized peptides (Figure 5K; $P=0.01$).

To study whether the proliferation inducing effect of *HOXB-AS3* overexpression is primarily mediated by its interaction with the EBP1-NPM1 protein complex, EBP1 (or NPM1) was concomitantly knocked down and *HOXB-AS3* was overexpressed in K562 cells. NPM1 was shown to be indispensable for the proliferative phenotype that is induced by *HOXB-AS3* overexpression, as its depletion abrogated this effect.

Identification of the specific *HOXB-AS3* regions which interact with the EBP1 and NONO proteins

To identify the specific regions of *HOXB-AS3* that interact with the EBP1 protein, overlapping PCR experiments were performed to generate mutant variants of the *HOXB-AS3* lncRNA. Specifically, 5 variants of the *HOXB-AS3* lncRNA were generated, each of which was

lacking 100 nucleotides when compared to the wt sequence (Figure 6A). The primers used to generate the constructs are the following:

Primer Pair A: Fw: 5'-CAAGCTGGAATGGAGGAGGGGA-3' (SEQ ID NO:18)

Rev: 5'-CGTCAGTTCCACTCGGTTGTC-3' (SEQ ID NO:19)

Primer Pair B: Fw#1: 5'-CAAGCTGGAATGGAGGAGGGGA-3' (SEQ ID NO:20)

Rev #1: 5'-GAGCGAGGAGGAAGCCGAG-3' (SEQ ID NO:21)

Fw#2: 5'-CGGCTTCCTCCTCGCTCTTATCTAAGC-3' (SEQ ID NO:22)

Rev#2: 5'-TACGCTACGTAACGGCATGACAG-3' (SEQ ID NO:23)

Primer Pair C: Fw#1: 5'-CAAGCTGGAATGGAGGAGGGGA-3' (SEQ ID NO:24)

Rev#1: 5'-GGTTAGTGGCTCCATCTCCAGAC-3' (SEQ ID NO:25)

Fw#2: 5'-GATGGAGCCACTAACCAACCAGGAG-3' (SEQ ID NO:26)

Rev#2: 5'-TACGCTACGTAACGGCATGACAG-3' (SEQ ID NO:27)

Primer Pair D: Fw#1: 5'-CAAGCTGGAATGGAGGAGGGGA-3' (SEQ ID NO:28)

Rev#1: 5'-GGTTGTGCAAGTGAGTGCCGA-3' (SEQ ID NO:29)

Fw#2 5'-CACTTGCACAACCGAGTGGAAGT-3' (SEQ ID NO:30)

Rev#2: 5'-TACGCTACGTAACGGCATGACAG-3' (SEQ ID NO:31)

Primer Pair E: Fw: 5'-CTCGGCTTCCTCCTCACCAGC-3' (SEQ ID NO:32)

Rev: 5'-TACGCTACGTAACGGCATGACAG-3' (SEQ ID NO:33)

Primer pairs A, B, C, D and E were used for the generation of the mutant variants 1,2,3,4 and 5 respectively. Overexpression experiments were performed with the wt and mutant *HOXB-AS3* variants in K562 cells followed by RIPs with an EBP1-binding antibody and RT-PCRs assays specifically designed to measure the expression levels of each *HOXB-AS3* variant. Deletion of the region between nucleotides 200 and 300 of the *HOXB-AS3* lncRNA significantly reduced the amount of the lncRNA that is bound by EBP1 and identified this region as the primary site of the EBP1-*HOXB-AS3* interaction. Consistently, transfection of K562 cells with the mutant variants of *HOXB-AS3* did not affect the proliferation rate of K562 cells or the transcription of rRNA species.

Discussion

Deregulation of lncRNA expression is gaining gradual recognition for its important role in leukemogenesis. Previous work in cohorts of younger adults and older patients with CN-AML has identified a lncRNA which strongly associates with the presence of NPM1 mutations, named *HOXB-AS3*. In the example here, a series of experiments were performed to show that there is a causal relationship of the *HOXB-AS3* and the presence of *NPM1* mutations. In fact, transcript

quantification by RNA sequencing analyses showed that *HOXB-AS3* is the most aberrantly overexpressed gene of the HOXB cluster in OCI-AML3 cells and is expressed at similar levels with the protein coding HOXA9 and HOXA10.

Despite the association with the aberrant activation of the *HOXB* locus and the functional role of other HOX-locus embedded lncRNAs, depletion of *HOXB-AS3* did not have an effect on the of *HOX* mRNA or HOX protein levels at the time when its effect on cell proliferation was observed.

The interactions of *HOXB-AS3* with the proteome of OCI-AML3 cells were investigated and it was found that the *HOXB-AS3* strongest interactor was the EBP1 protein. EBP1 has been shown to interact with NPM1 and to regulate transcription of rRNA species and ribosome biogenesis. It was shown here that *HOXB-AS3* affects the interaction of EBP1 with NPM1; when OCI-AML3 cells were depleted of *HOXB-AS3*, a lesser amount of NPM1 was found to interact and could be co-immunoprecipitated with EBP1 and vice versa. Furthermore, it was found that the depletion of *HOXB-AS3* impacts on the rRNA transcription, the ribosome biogenesis and, subsequently to the protein synthesis capacity of the cells. This is consistent with the impact of *HOXB-AS3* manipulations on the proliferating capacity of the leukemic blasts as the amounts of transcribed rRNA has been shown to affect cell cycle progression of cells. In this sense, the functional role of *HOXB-AS3*, in the context of *NPM1* mutations could be regarded as a compensatory mechanism which allows the leukemic blasts to maintain adequate amounts of ribosomal RNA and maximize the efficiency of the protein translating machinery in the metabolically demanding state of constant proliferation. Compatible with this scenario are unpublished observation in CRISPRi-modified OCI-AML3 cells; the deletion of the *NPM1* mutant allele, which results to a frank *NPM1* haplo-insufficiency and downregulation of *HOXB-AS3* expression, leads to decreased rRNA transcription and ribosome biogenesis.

EBP1 is the protein most avidly binding to *HOXB-AS3* in OCI-AML3 cells. Additional proteins which mediate key cellular functions were also identified and validated as *HOXB-AS3* interactors. RNAseq analyses focused on the protein-coding fraction of the transcriptome revealed that the pathways of DNA-damage response could be affected by aberrant *HOXB-AS3* expression. While it is likely that *HOXB-AS3* exerts more than one function, depletion of either EBP1 or NPM1 abrogated the proliferative phenotype that is induced by *HOXB-AS3* overexpression. These findings indicate that the effects of *HOXB-AS3* manipulation on the proliferating capacity of leukemic blasts is primarily mediated by its interaction with EBP1 and NPM1 protein complex and the subsequent regulation of rRNA abundance and ribosome biogenesis.

As *in vitro* experiments delineate the significance of *HOXB-AS3* expression in *NPM1*mut AML, *HOXB-AS3* was also targeted in preclinical *in vivo* models for therapeutic use. An additional reason for this is the validated absence of *HOXB-AS3* expression in healthy hematopoietic BM cells. A liposome-based method for packaging and delivering *in vivo* *HOXB-AS3* targeting gapmers was used with no significant toxicities. Importantly *in vivo* knock down of *HOXB-AS3* led to significant prolongation of the overall survival of mice xenografted with AML blasts of two different patients. In particular, in xenografts of one patient, *HOXB-AS3* depletion led to eradication of the disease in a significant number of treated mice. This is the first known patient derived xenograft model in which *in vivo* depletion of a lncRNA shows therapeutic effects against AML as a single agent therapy.

Sequences

The symbols used in the sequences are as follows:

iMe-dC: This refers to a deoxyCytidine that is substituted with a Methyl-deoxyCytidine.

+: Locked nucleic acid-modified bases.

5 *: Phosphorothioated bonds among bases.

LNA#1 (SEQ ID NO:1)

+C*+G*G*/iMe-dC/G*C*A*T*/iMe-dC/G*A*G*A*T*C*+G*+C

10 LNA#2 (SEQ ID NO:2)

+G*+G*A*G*G*G*A*A*T*T*G*T*A*G*+C*+G*+A

LNA#3 (SEQ ID NO:3)

+T*+G*/iMe-dC/G*T*T*G*T*A*T*T*G*G*T*A*T*G*+G*+G

15

LNA#4 (SEQ ID NO:4)

+G*+G*G*G*A*G*G*T*T*A*T*T*T*C*+G*+T*+T

LNA#5 (SEQ ID NO:5)

20 +T*+T*+C*T*A*T*T*A*C*T*T*G*/iMe-dC/*G*T*T*G*+T*+A

Negative Control (SEQ ID NO:6)

+C*+G*+A*A*T*A*G*T*T*A*G*T*A*+G*+C*+G

25 NR_033201.2 (SEQ ID NO:7)

1 gtcatagcga cttttgggat agtttgctat cgacaaaggg agacaaagtc aaggggtgaa
 61 gggaaaggag ggccaagtag agcctccacg accctcggct tcctcctcac cagctcccc
 121 tcctccaag tccagtaaga agttgggcca agctggaagg gattgaccgg ccgtttcctc
 181 tcctcgcgcg gcctcggcgg agattccagg ccctatagaa accaggacgt cccttagcgc
 30 241 caccgcctca catgccagtgc ctgcggggaa ccagcgata tccgcaccag cggagaaggt
 301 tccaggctgc cggcggcggc gcagagagcg ggaagagagg ctcgaggaa gccccgggcg
 361 tggcgtggtc aggctccgag agcggccggg atgcggccac accggcctgg taaactcgca
 421 cctcttagga tcttgctccc ggaactatc cctccccac cccctatattt aaagttttat
 481 ttgggtcgtc tgtatcaatt tagaacgaga taaattaaga caaagaaagt aaaataaatc
 35 541 gaaataaaat ataggaatag ctcttggcga aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa
 601 aaaaaaaaaa a

NR_033202.2 (SEQ ID NO:8)

1 gtcatagcga cttttgggat agtttgctat cgacaaaggg agacaaagtc aaggggtgaa
 61 gggaaaggag ggccaagtag agcctccacg accctcggct tcctcctcac cagctccccc
 5 121 tccttccaag tccagtaaga agttgggcca agctggaagg gattgaccgg gttgcttgct
 181 tggagatgga gccactacag gcgggcctgg ggcctggag tcgggcatga aagaaaatag
 241 cgcctcatcg ctcttatcta agcccagagg tagacttcgc ttgaaaagat cgagaatgga
 301 ggaggggato ggcactcact tgcagacatc aacagtttcc agaaaattcg gttcaattcc
 361 ttcaccacgg accaccaaac caaggagctg gcaaaaccca ctaaccaacc aggagaacac
 10 421 agaccgggtt tgtcttttga caaccgagtg gaactgacgg ggccggcgcc tgcctgcgg
 481 gtccctccc ttggctgaga agaaaaacaa ataaaccttg aacagccttg gcttcgaaaa
 541 aaaaaaaaa

NR_033203.1 (SEQ ID NO:9)

1 ggaaccagat cttgatctgg cgctcggaca ggcagagtc gtgggcgac tcgatgcgcc
 61 gtgcgcgggt caggttgctt gtctggagat ggagccacta caggcgggcc tgggcgcctg
 121 gagtcgggca tgaaagaaaa tagcgctca tcgctcttat ctaagcccag aggtagactt
 181 cgcttgaaaa gatcgagaat ggaggagggg atcggcactc acttgacagac atcaacagtt
 241 tccagaaaat tcggttcaat tccttcacca cggaccacc aaccaaggag ctggcaaac
 20 301 ccactaacca accaggagaa cacagaccg gtttgtcttt tgacaaccga gtggaactga
 361 cggggccggc gcctgcctg cgggtccct ccttggtg agaaagaaac caaataaacc
 421 ttgaacagcc ttggttcga aaaaaaaaa aa

uc60gwg.1 (SEQ ID NO:10)

25 cagtttccagaaaattcggttcaattccttcaccacggaccaccaaccaaggagctggcaaaacccactaaccaac
 caggagaacacagaccgcggttctctcctcgcggcctcggcggagattccaggccctatagaaaccaggacg
 tcccttagcgcaccgcctcacatgccagtgcgcgggaaccacgcgatatccgcaccagcggagaaggttccagg
 ctgcccggcgcgcgagagagcggaagagaggctcggaggaagccccggcggtggcgtggtcaggctccgagagc
 ggccgggatgcggccacaccggcctggtaaacctgcacctcttaggatcttgctcccggaactcattccctccccac
 30 cccctatttttaaagttttatttgggtcgtctgtatcaatttagaacgagataaattaagacaaagaaagtaaaataa
 atcgaaataaaatata

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of skill in the art to which the disclosed invention belongs. Publications cited herein and the materials for which they are cited are specifically incorporated by reference.

5 Those skilled in the art will appreciate that numerous changes and modifications can be made to the preferred embodiments of the invention and that such changes and modifications can be made without departing from the spirit of the invention. It is, therefore, intended that the appended claims cover all such equivalent variations as fall within the true spirit and scope of the invention.

10

CLAIMS

What is claimed is:

1. A single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar moiety.
2. The oligomer of claim 1, wherein the oligomer is between 10-30 nucleotides.
3. The oligomer of claim 2, wherein the oligomer is between 15-25 nucleotides.
4. The oligomer of any one of claims 1 to 3, wherein the at least one nucleotide analogue having a modified sugar moiety comprises a Locked Nucleic Acid.
5. The oligomer of any one of claims 1 to 4, wherein the oligomer further comprises a modified phosphodiester linkage.
6. The oligomer of claim 5, wherein the modified phosphodiester linkage comprises a phosphorothioate bond.
7. The oligomer of any one of claims 1 to 6, wherein the *HOXB-AS3* non-coding RNA has the nucleic acid sequence encoded by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.
8. The oligomer of any one of claims 1 to 7, wherein the oligomer comprises a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a mixture thereof.
9. The oligomer of claim 8, wherein the oligomer comprises the nucleic acid sequence SEQ ID NO:2.
10. The oligomer of claim 8, wherein the oligomer comprises the nucleic acid sequence SEQ ID NO:3.
11. A method for treating acute myeloid leukemia comprising administering to a subject in need thereof a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar.
12. The method of claim 11, wherein the oligomer is between 10-30 nucleotides.
13. The method of claim 12, wherein the oligomer is between 15-25 nucleotides.
14. The method of any one of claims 11 to 13, wherein the at least one nucleotide analogue having a modified sugar moiety comprises a Locked Nucleic Acid.
15. The method of any one of claims 11 to 14, wherein the oligomer further comprises a modified phosphodiester linkage.

16. The method of claim 15, wherein the modified phosphodiester linkage comprises a phosphorothioate bond.
17. The method of any one of claims 11 to 16, wherein the *HOXB-AS3* non-coding RNA has the nucleic acid sequence encoded by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.
18. The method of any one of claims 11 to 17, wherein the oligomer comprises a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a mixture thereof.
19. The method of claim 18, wherein the oligomer comprises the nucleic acid sequence SEQ ID NO:2.
20. The method of claim 18, wherein the oligomer comprises the nucleic acid sequence SEQ ID NO:3.
21. The method of any one of claims 11 to 20, wherein the oligomer inhibits the expression of *HOXB-AS3* non-coding RNA in a cell.
22. The method of any one of claims 11 to 21, wherein the acute myeloid leukemia is cytogenetically normal AML (CN-AML).
23. A method of inhibiting the expression of *HOXB-AS3* in a cell, comprising contacting the cell with an effective amount of a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar moiety.
24. The method of claim 23, wherein the oligomer is between 10-30 nucleotides.
25. The method of claim 24, wherein the oligomer is between 15-25 nucleotides.
26. The method of any one of claims 23 to 25, wherein the at least one nucleotide analogue having a modified sugar moiety comprises a Locked Nucleic Acid.
27. The method of any one of claims 23 to 26, wherein the oligomer further comprises a modified phosphodiester linkage.
28. The method of claim 27, wherein the modified phosphodiester linkage comprises a phosphorothioate bond.
29. The method of any one of claims 23 to 28, wherein the *HOXB-AS3* non-coding RNA has the nucleic acid sequence encoded by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.

30. The method of any one of claims 23 to 29, wherein the oligomer comprises a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a mixture thereof.
31. The method of claim 30, wherein the oligomer comprises the nucleic acid sequence SEQ ID NO:2.
32. The method of claim 30, wherein the oligomer comprises the nucleic acid sequence SEQ ID NO:3.
33. The method of any one of claims 23 to 32, wherein the cell is within a tissue of a mammal.
34. A composition comprising a cationic nanoparticle and a single stranded oligomer, wherein the oligomer is perfectly complementary to a corresponding region of *HOXB-AS3* non-coding RNA, and wherein the oligomer comprises at least one nucleotide analogue having a modified sugar moiety.
35. The composition of claim 34, wherein the oligomer is between 10-30 nucleotides.
36. The composition of claim 35, wherein the oligomer is between 15-25 nucleotides.
37. The composition of any one of claims 34 to 36, wherein the at least one nucleotide analogue having a modified sugar moiety comprises a Locked Nucleic Acid.
38. The composition of any one of claims 34 to 37, wherein the oligomer further comprises a modified phosphodiester linkage.
39. The composition of claim 38, wherein the modified phosphodiester linkage comprises a phosphorothioate bond.
40. The composition of any one of claims 34 to 39, wherein the *HOXB-AS3* non-coding RNA has the nucleic acid sequence encoded by SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, or SEQ ID NO:10.
41. The composition of any one of claims 34 to 40, wherein the oligomer comprises a nucleic acid sequence selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, or a mixture thereof.
42. The composition of claim 41, wherein the oligomer comprises the nucleic acid sequence SEQ ID NO:2.
43. The composition of claim 41, wherein the oligomer comprises the nucleic acid sequence SEQ ID NO:3.
44. A pharmaceutical composition comprising the oligomer according to any one of claims 1 to 10 and a pharmaceutically acceptable diluent, carrier, salt or adjuvant.

1/30

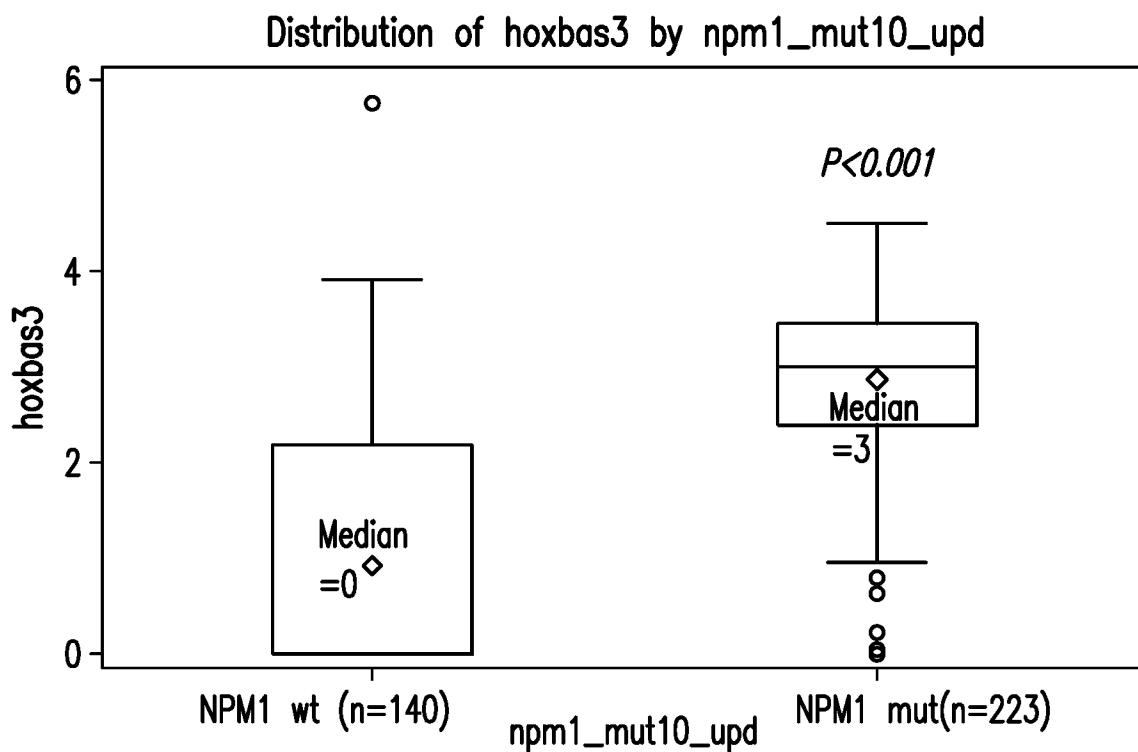


FIG. 1A

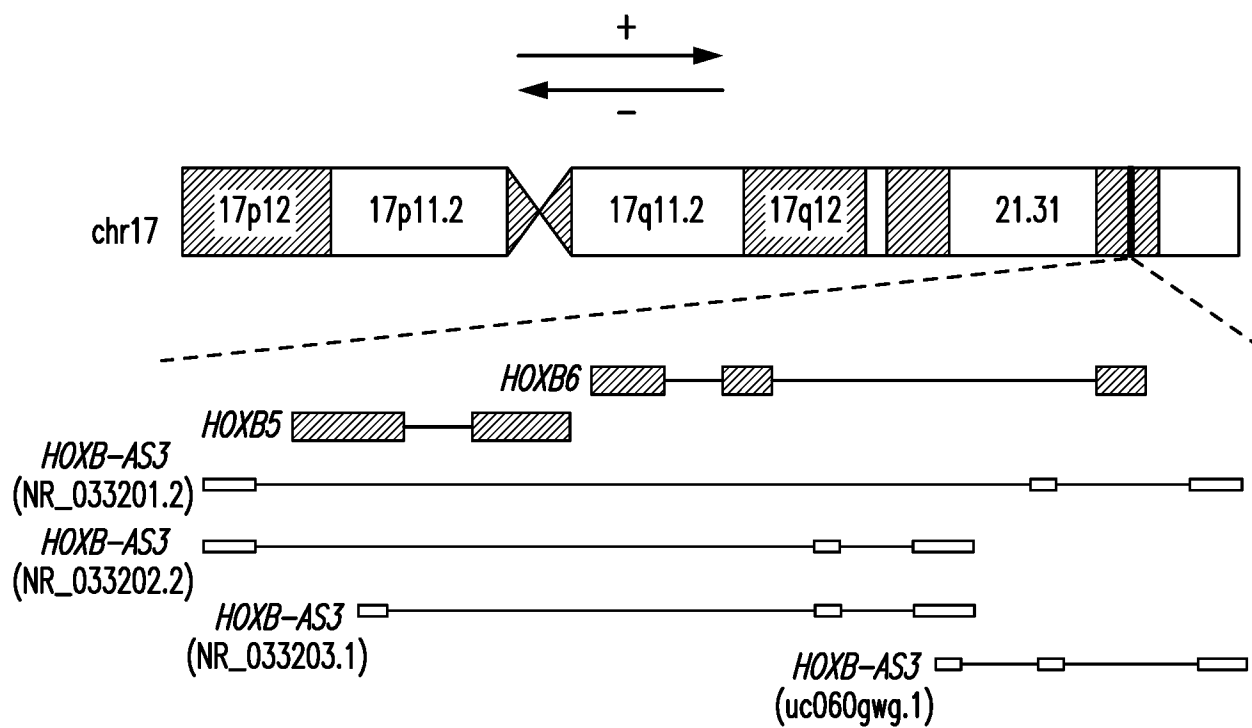


FIG. 1B

2/30

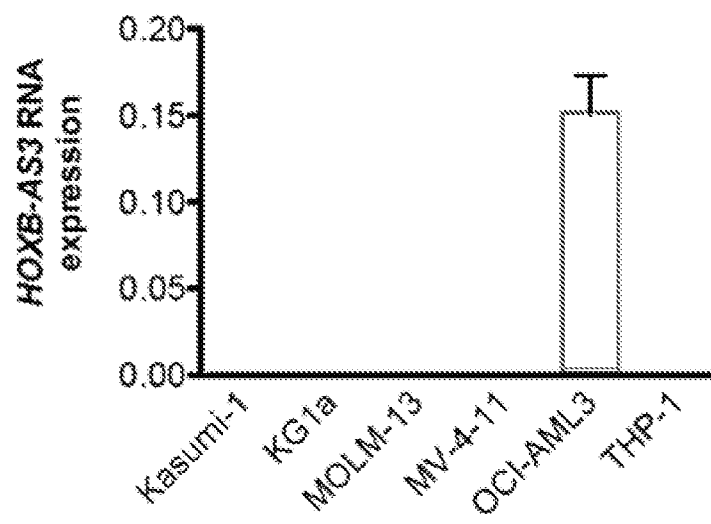


FIG. 1C

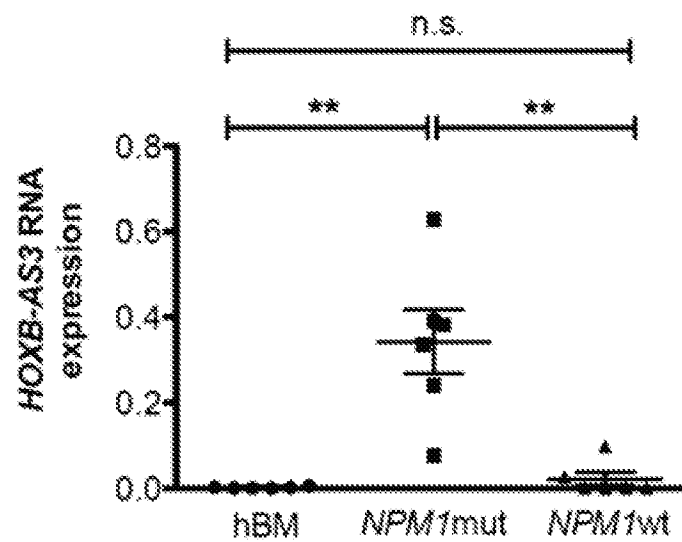


FIG. 1D

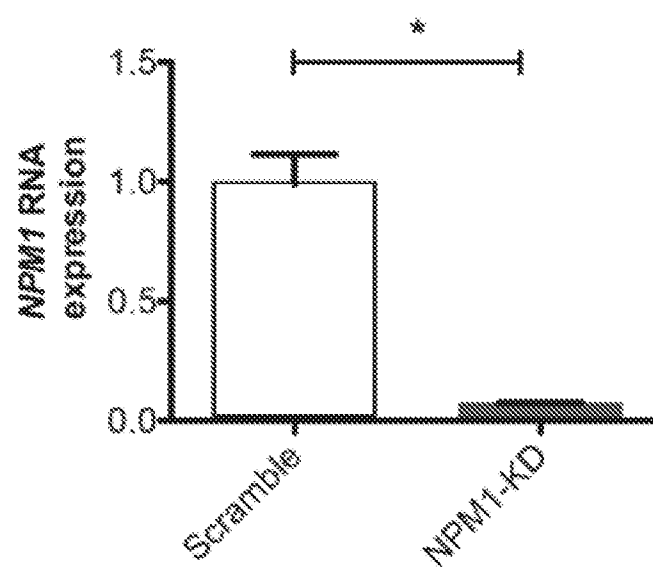


FIG. 1E

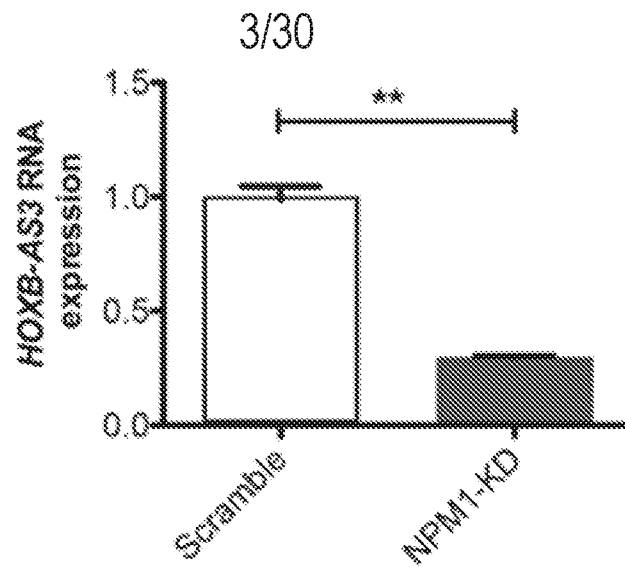


FIG. 1F

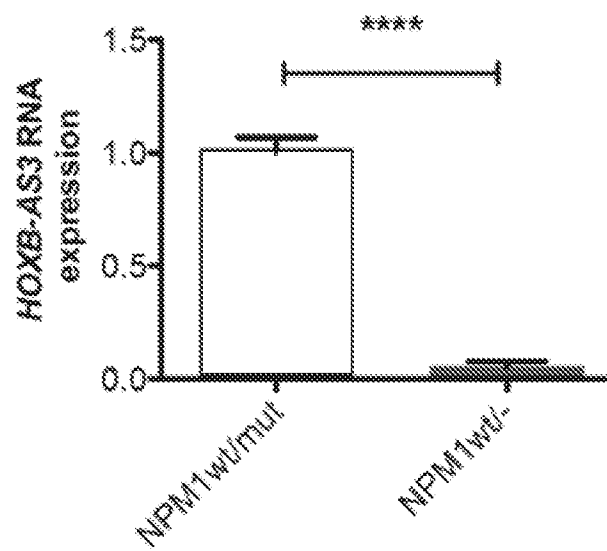


FIG. 1G

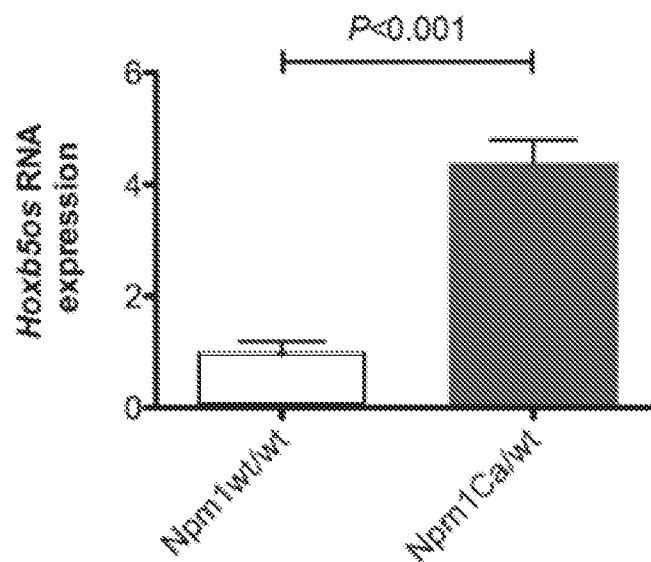


FIG. 1H

4/30

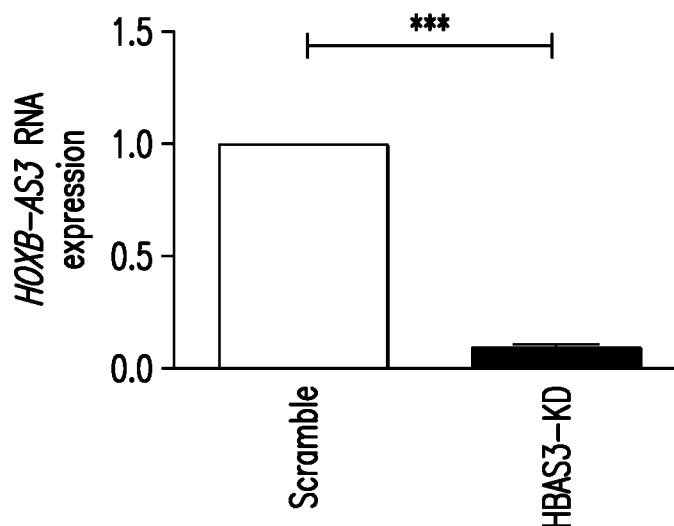


FIG. 2A

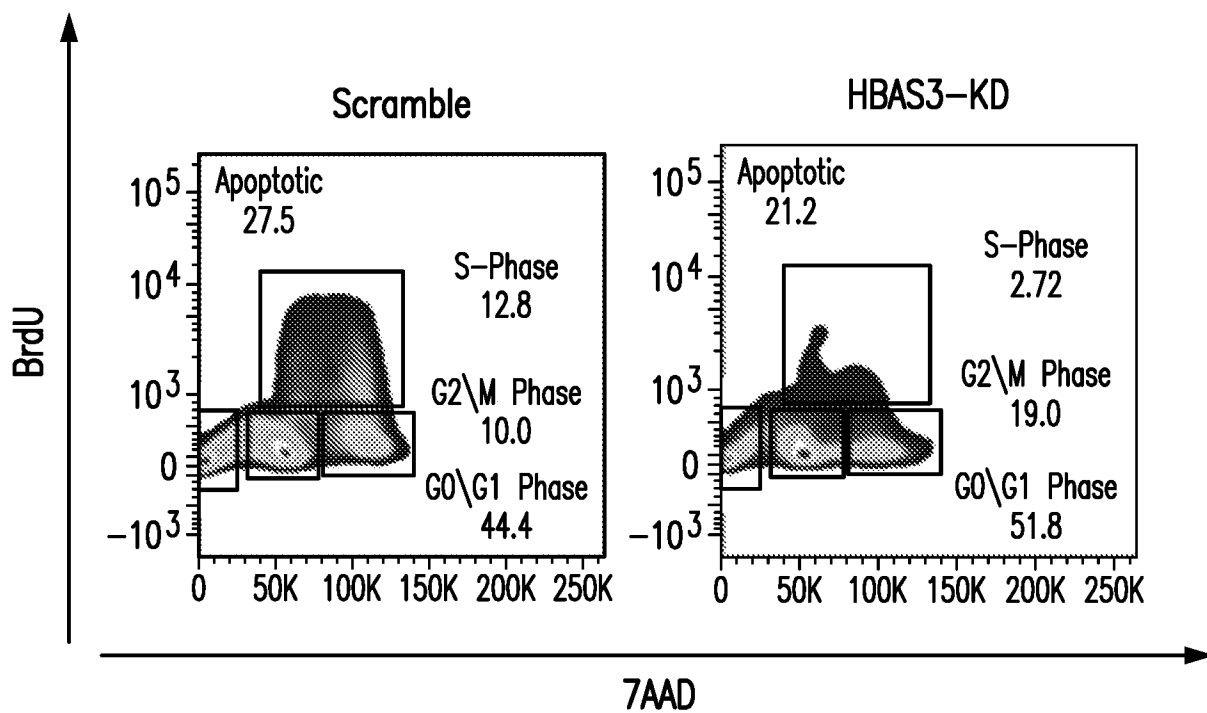


FIG. 2B

5/30

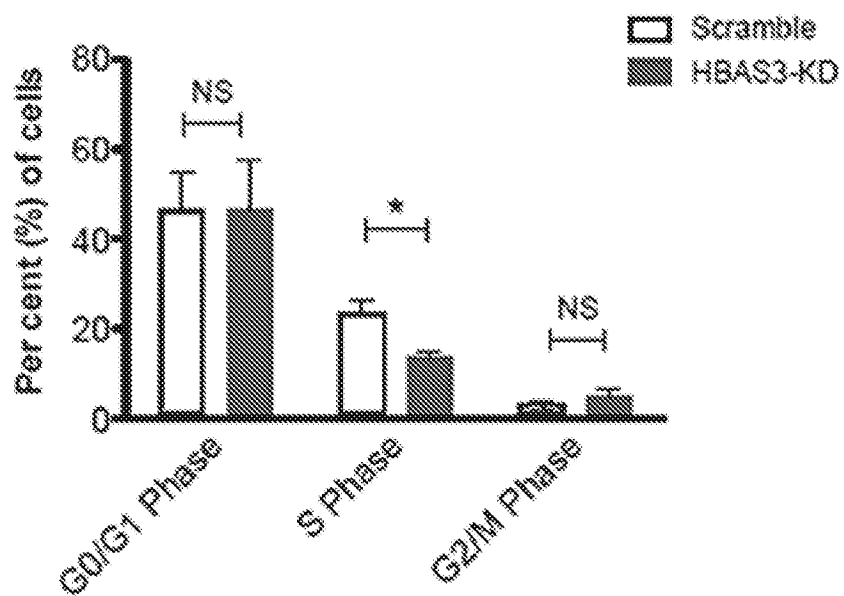


FIG. 2C

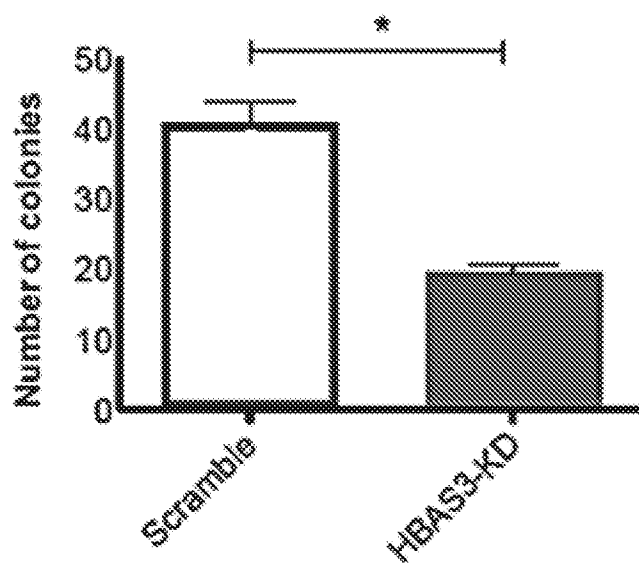


FIG. 2D

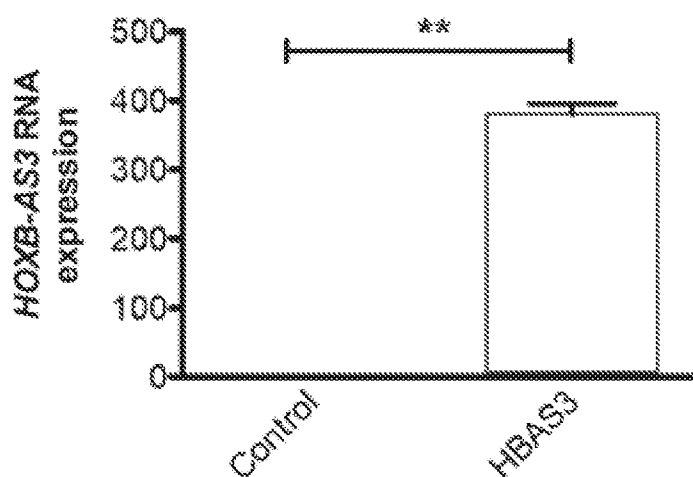


FIG. 2E

6/30

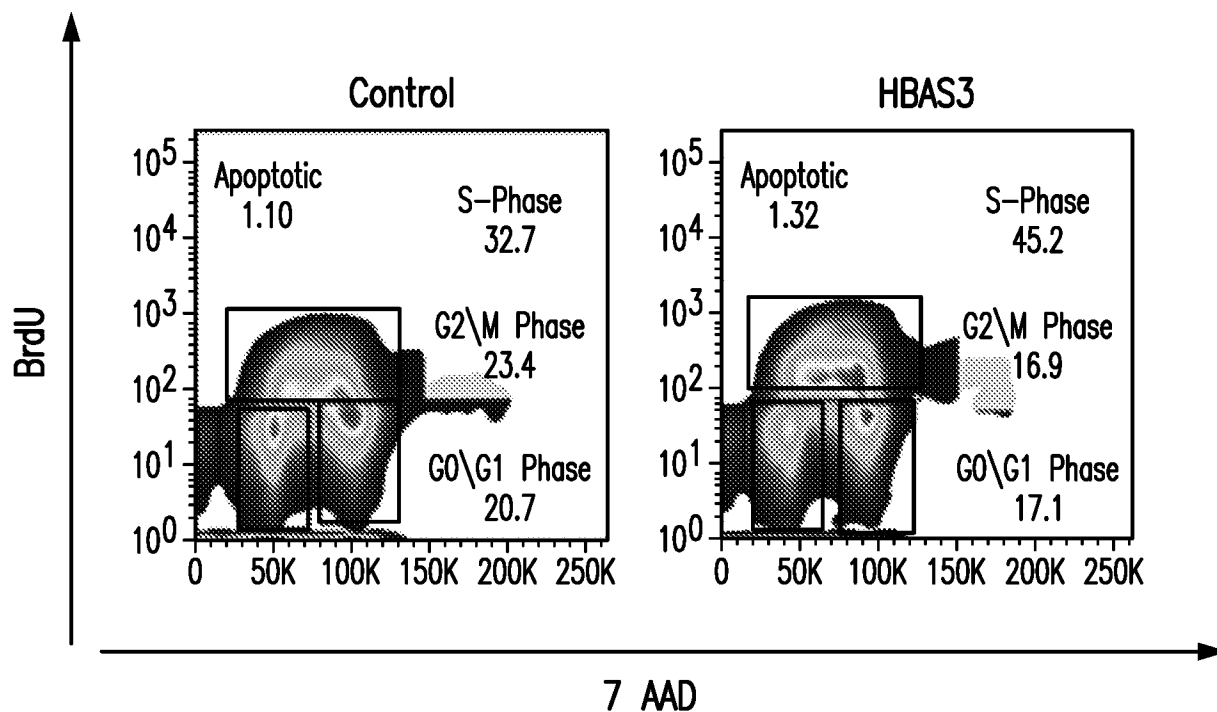


FIG. 2F

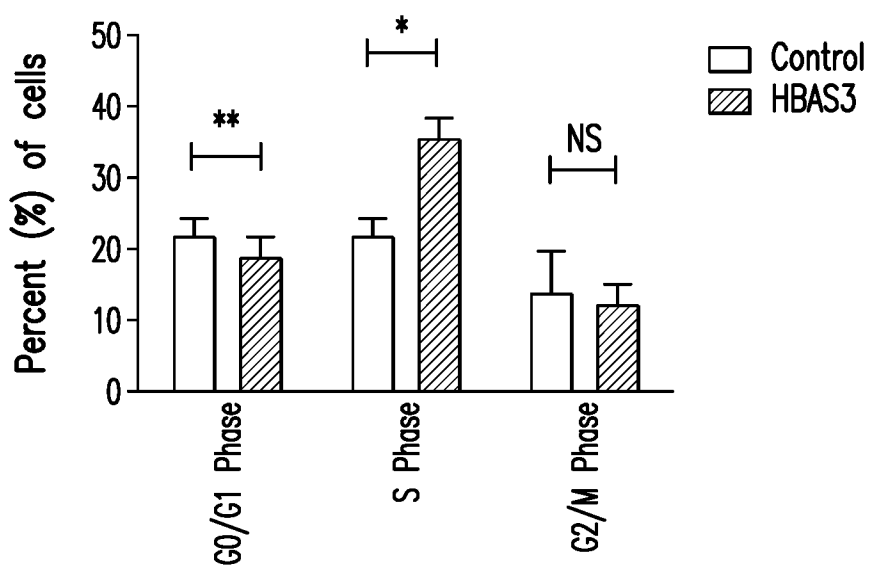


FIG. 2G

7/30

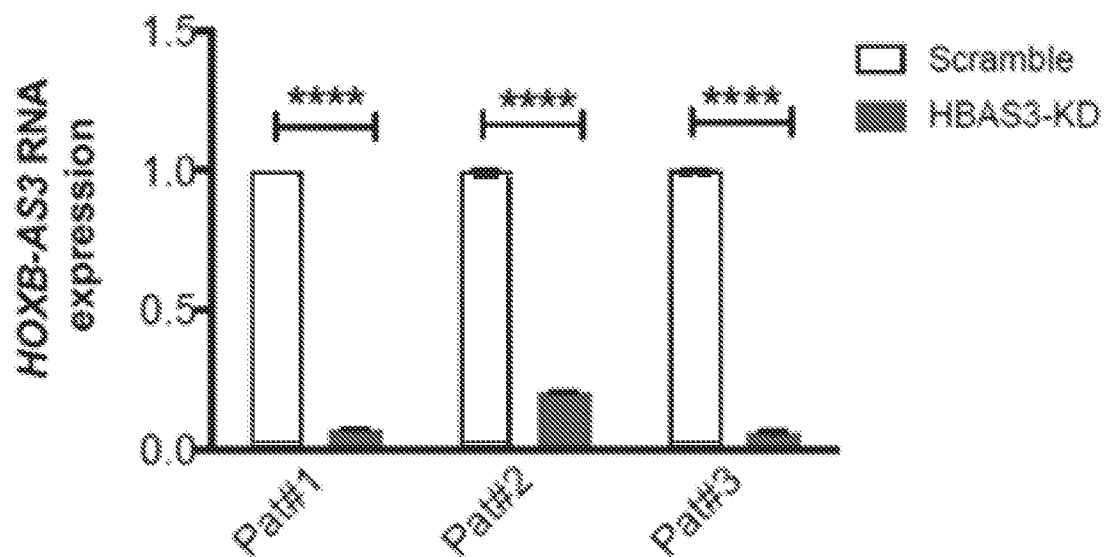


FIG. 3A

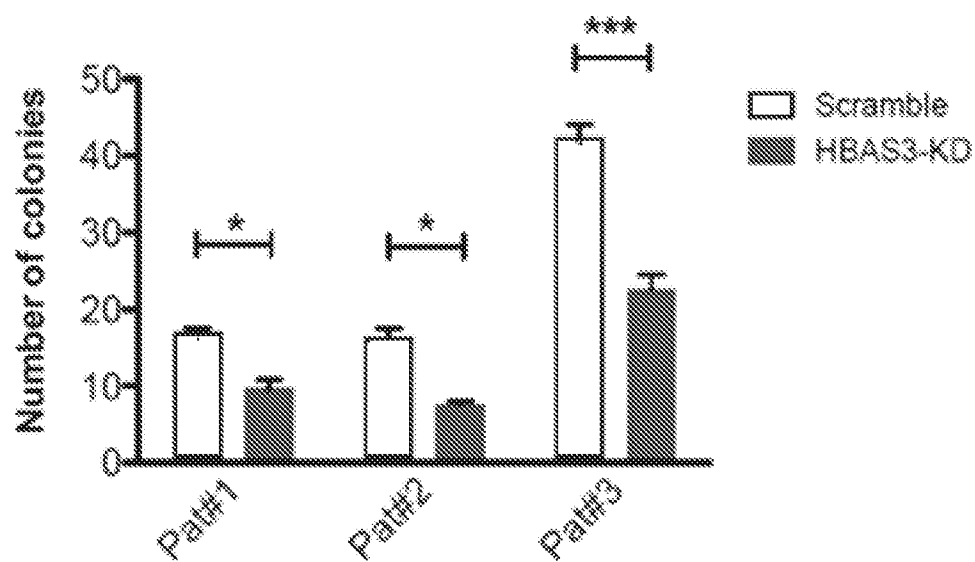


FIG. 3B

8/30

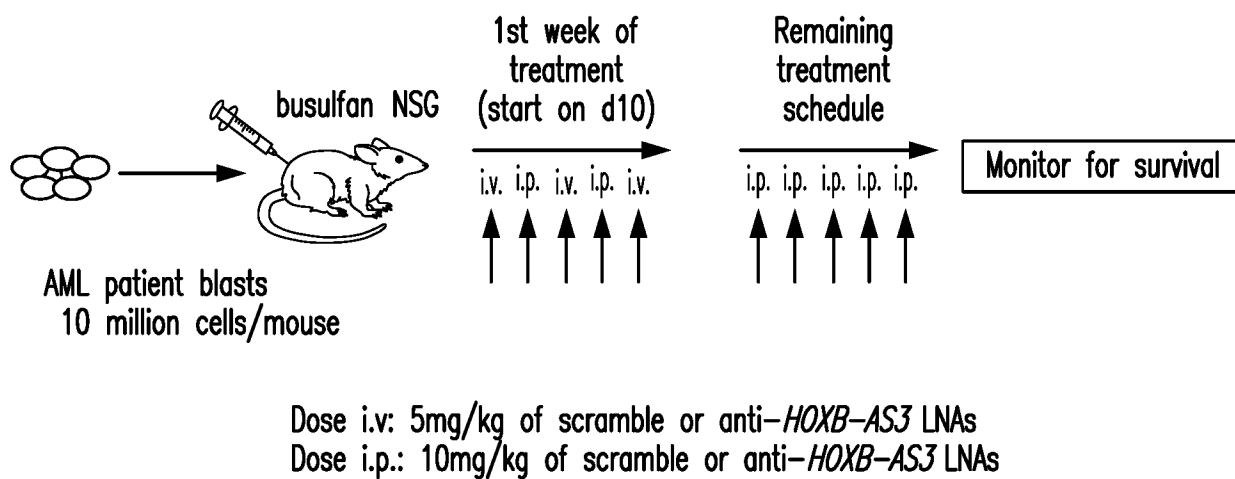


FIG. 3C

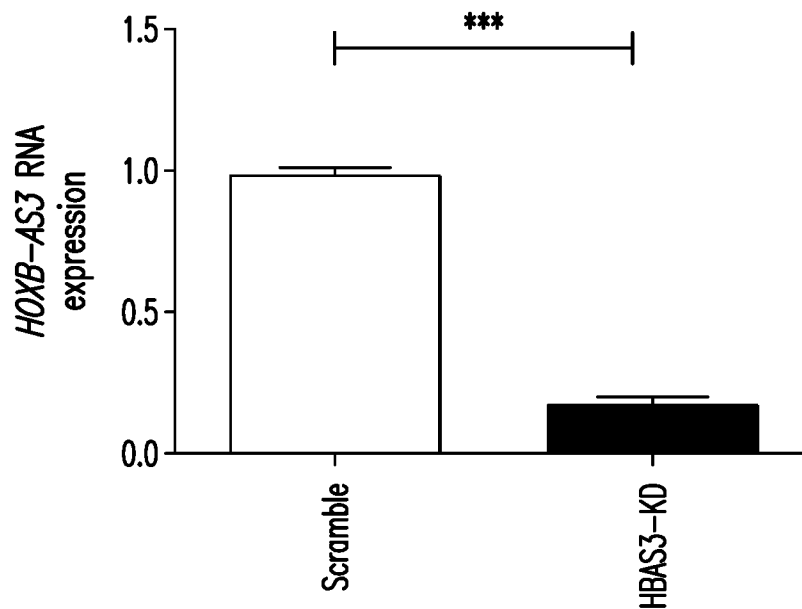


FIG. 3D

9/30

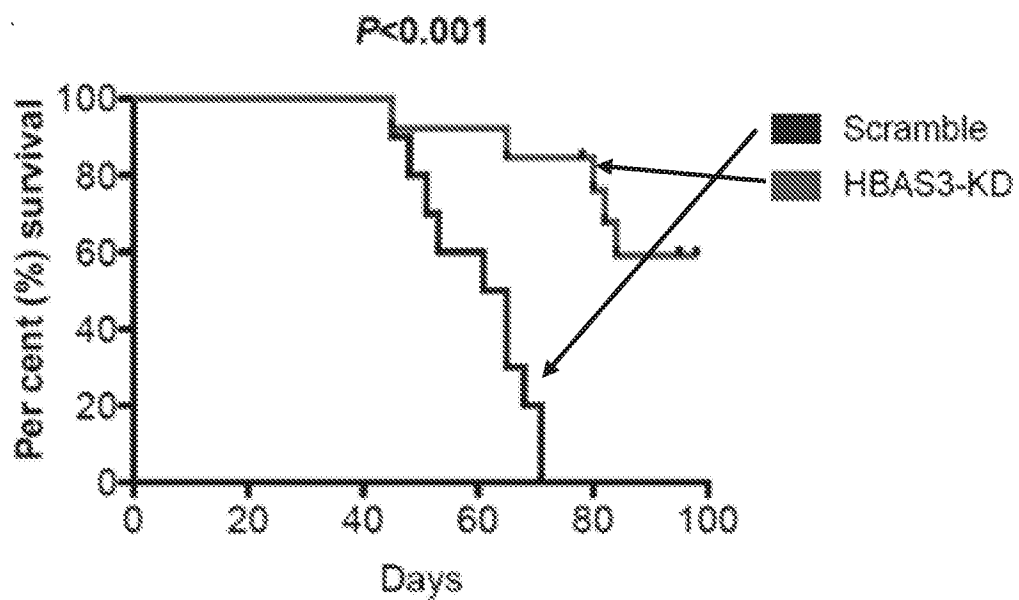


FIG. 3E

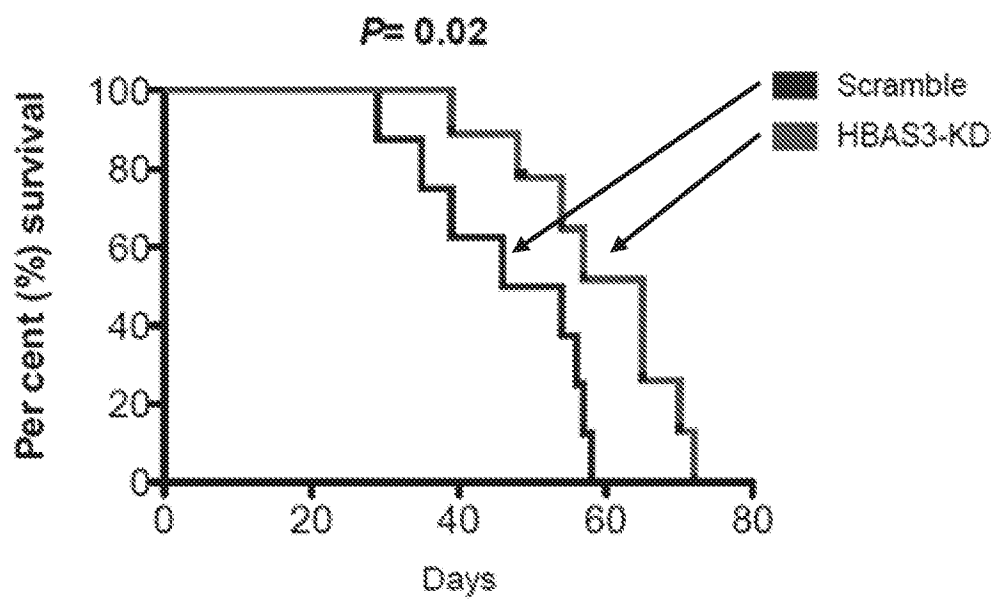


FIG. 3F

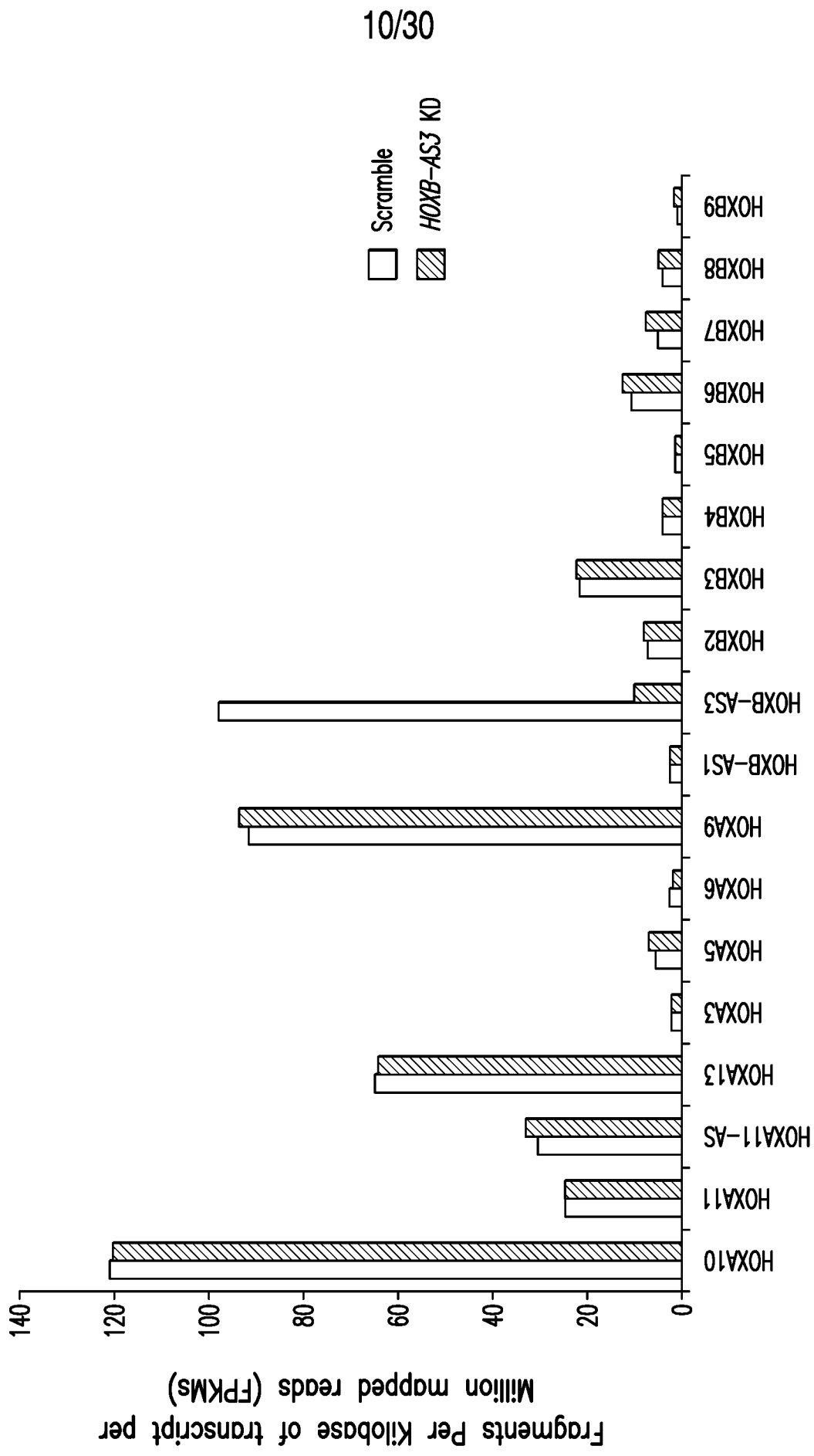


FIG. 4A

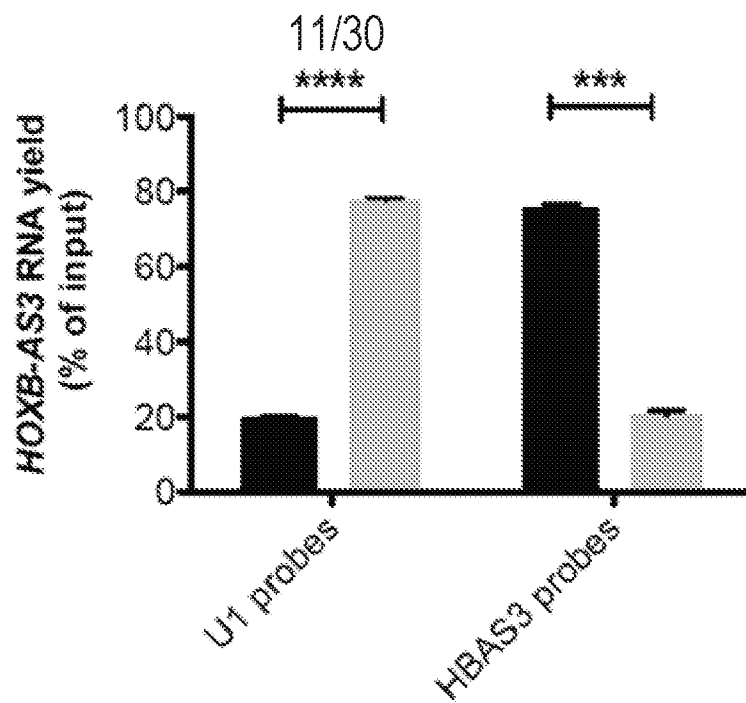


FIG. 4B

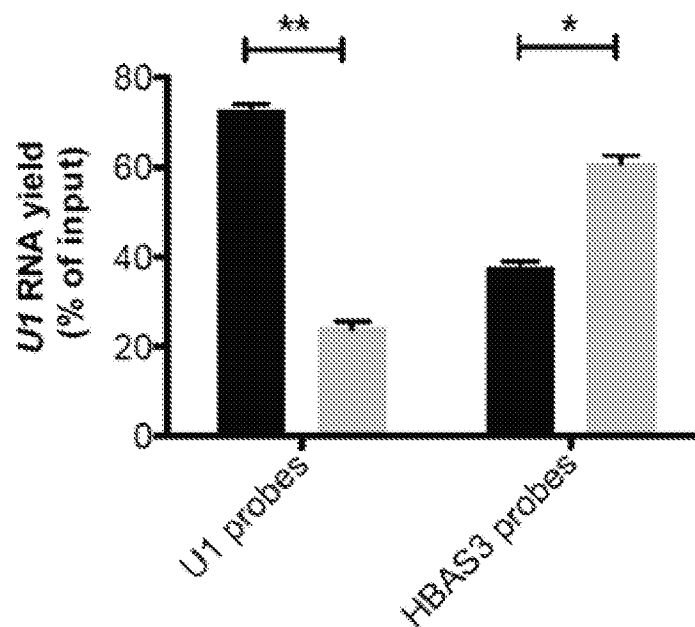


FIG. 4C

12/30

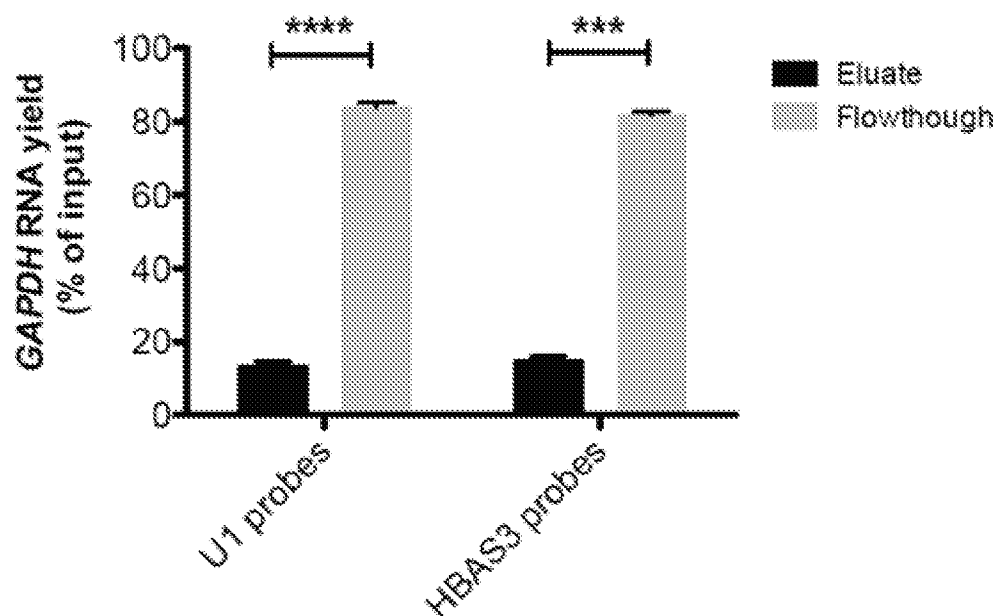


FIG. 4D

	Putative <i>HOXB-AS3</i> interactors	Putative <i>U1</i> interactors	
mRNA stability regulation	DXH9 HNRPU	DDX3X FLNB PLEC PPIB	
Ribosomal biogenesis	HMGB2 MATR3 EBP1 PARK7 PCBP1 PHB1 PRDX3 PTMA SF3B3 NONO SFPQ SRSF2 XRCC6 HNRPC HNRPM HNRH1 HNRH3 HNRPF STIP1 TCEA1	RUXE SMD2 SMD3 SNRPA	U1 Interacting Proteins
Drosophila behavior/human splicing (DBHS) complex			

FIG. 4E

13/30

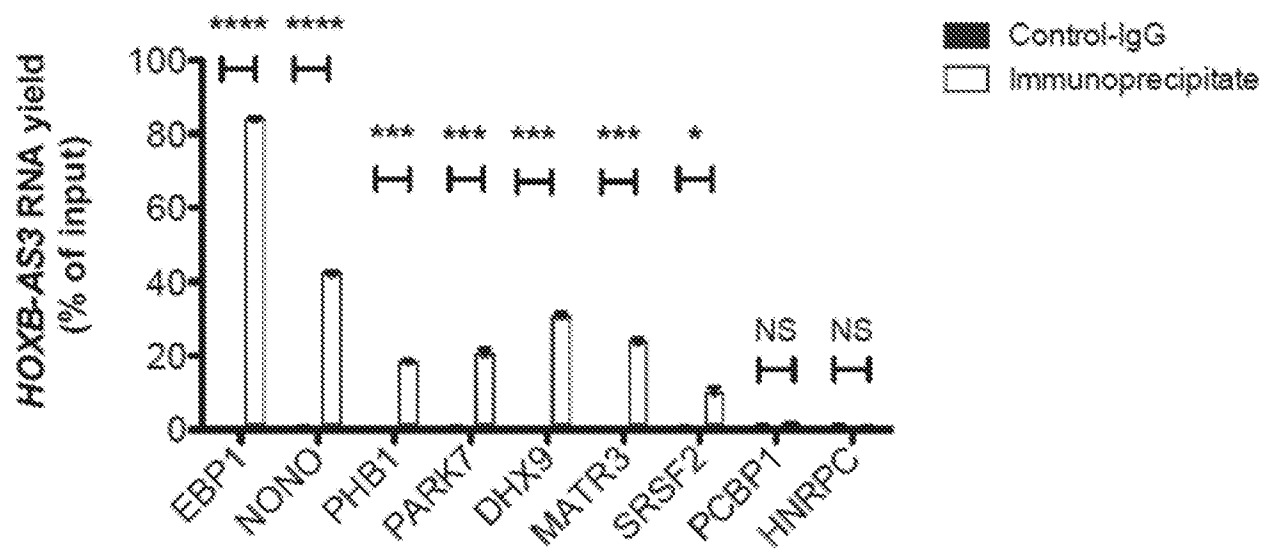


FIG. 4F

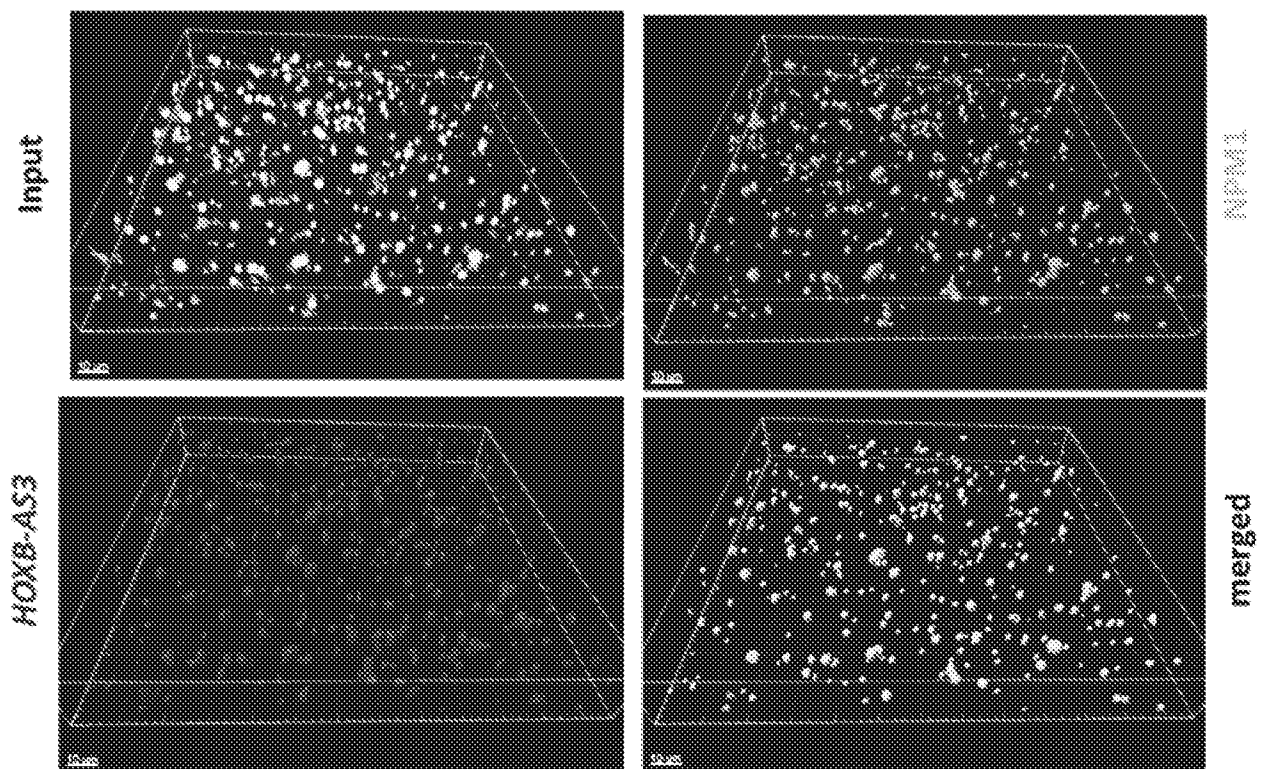


FIG. 4G

14/30

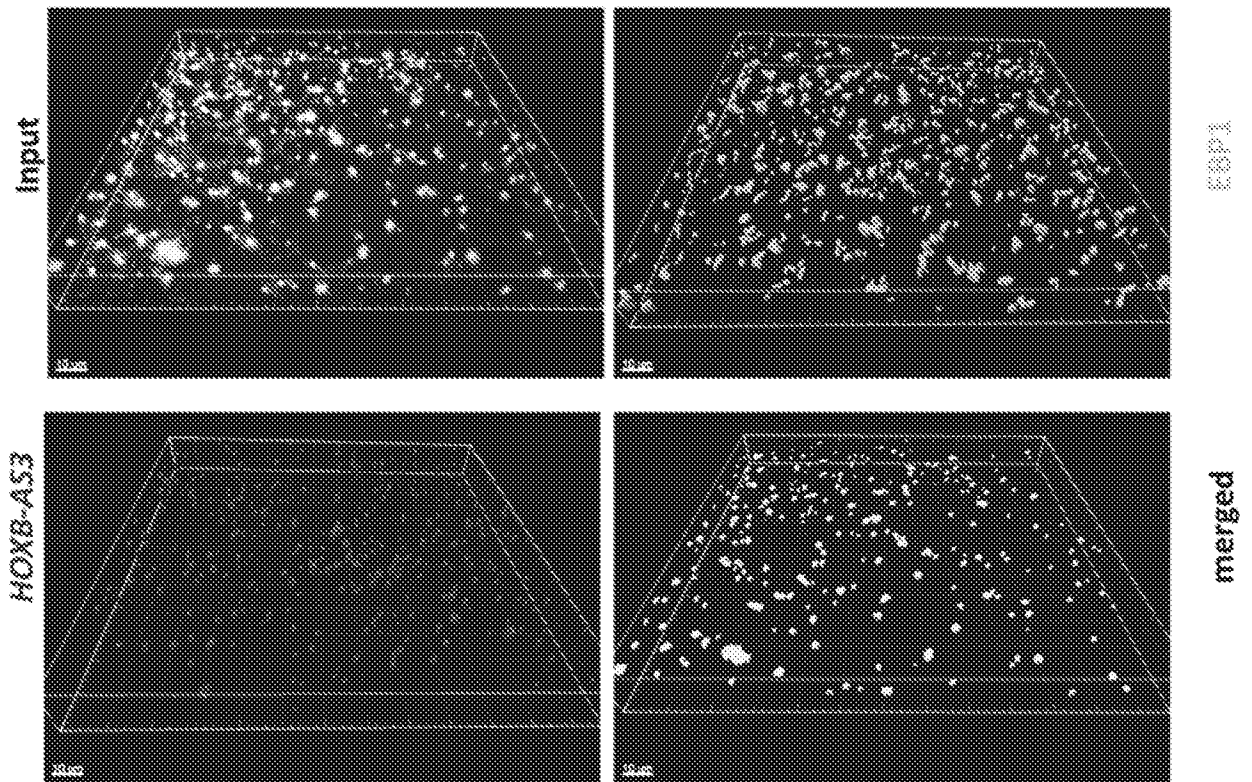


FIG. 4H

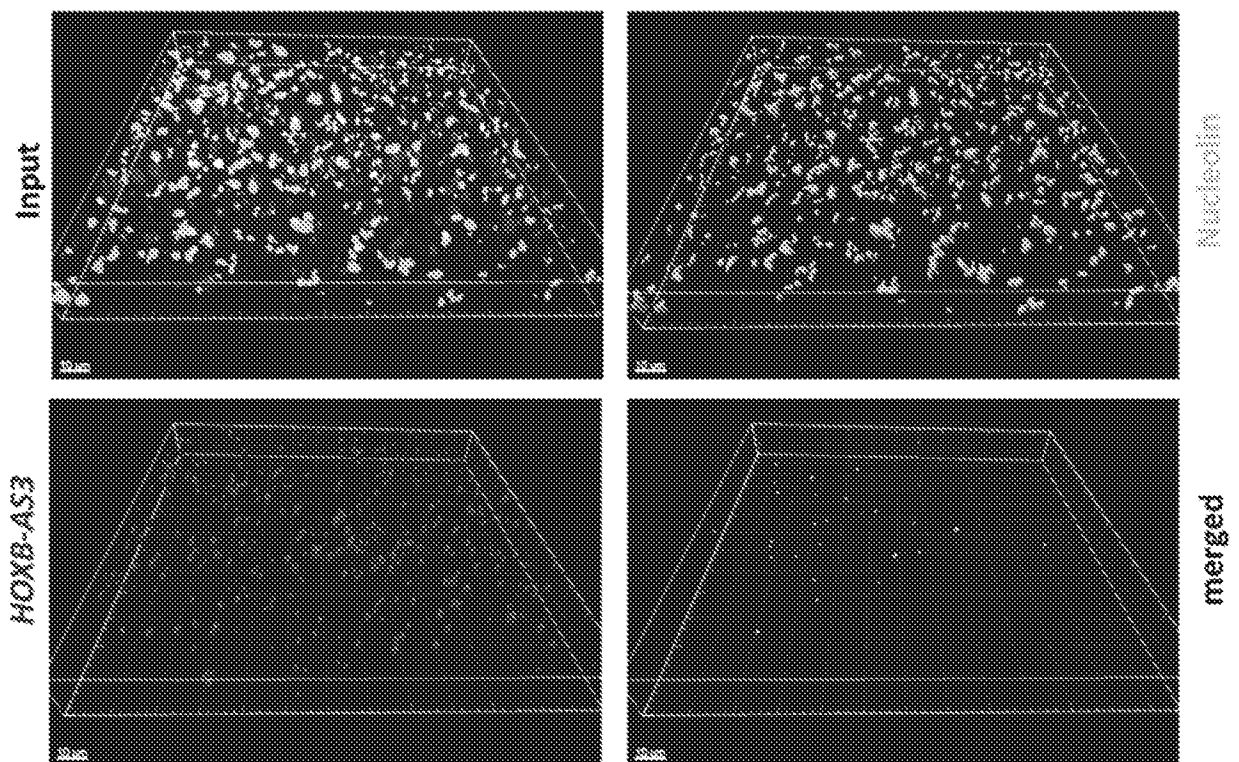


FIG. 4I

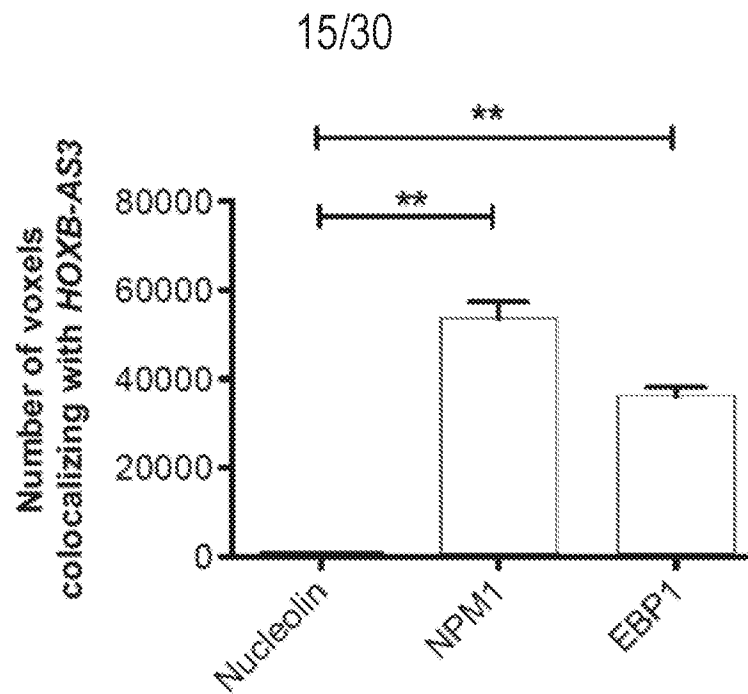


FIG. 4J

Protein	Pearson's Correlation Coefficient
Nucleolin	0.19
NPM1	0.72
EBP1	0.83

FIG. 4K

16/30

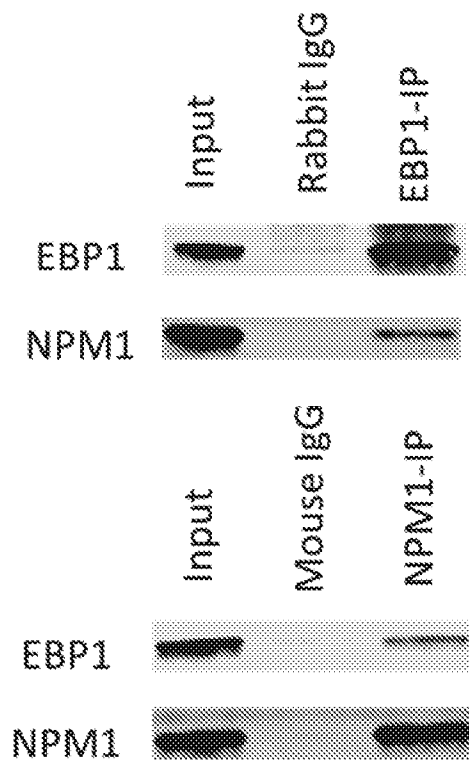


FIG. 4L

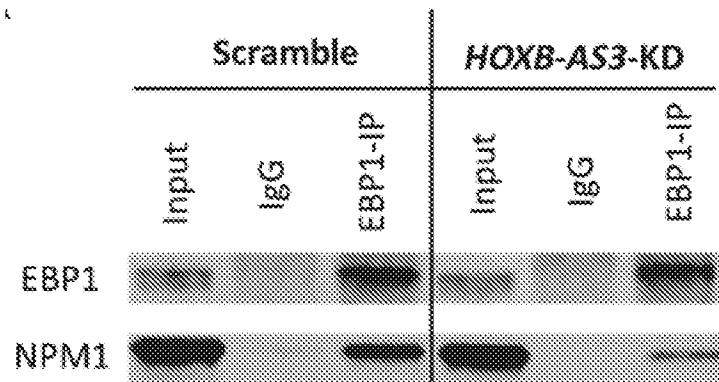


FIG. 5A

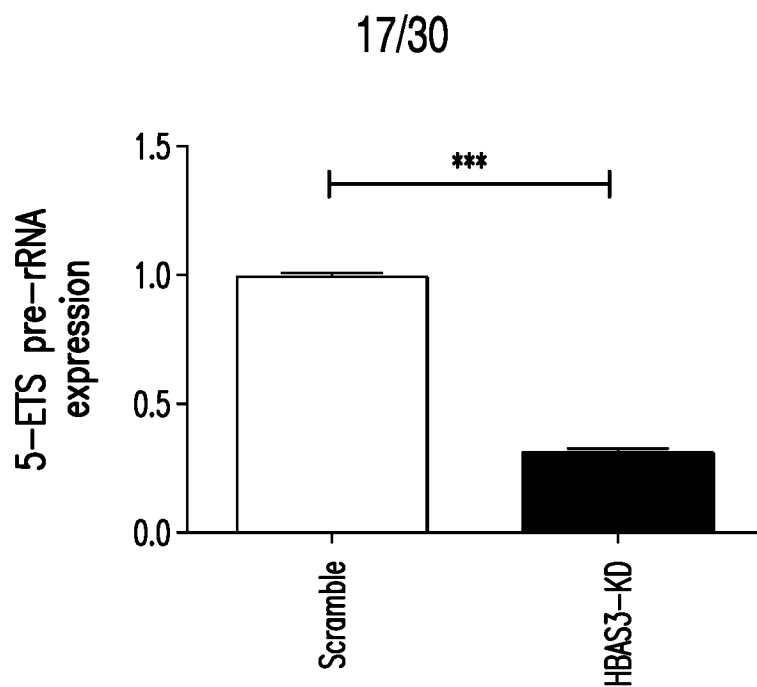


FIG. 5B

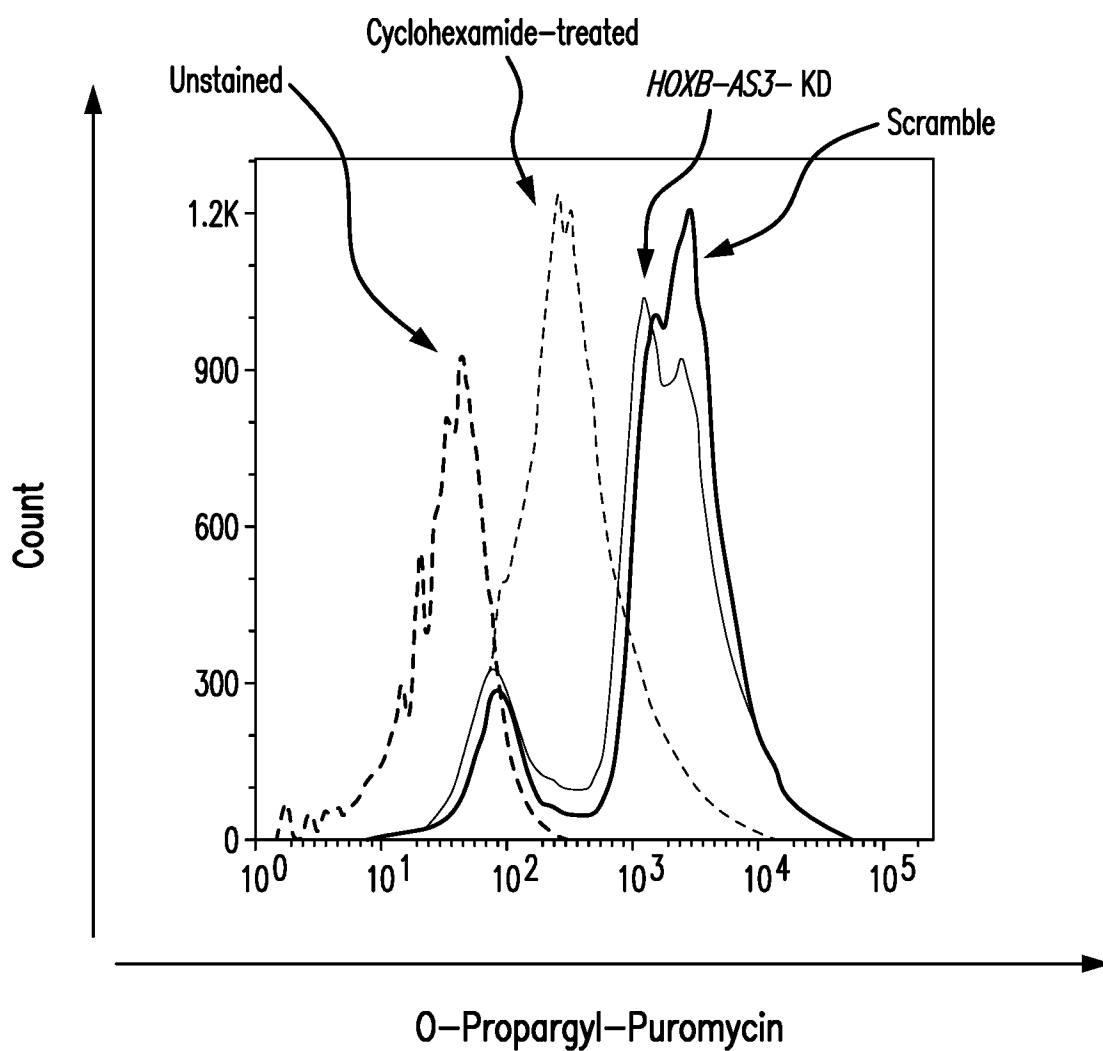


FIG. 5C

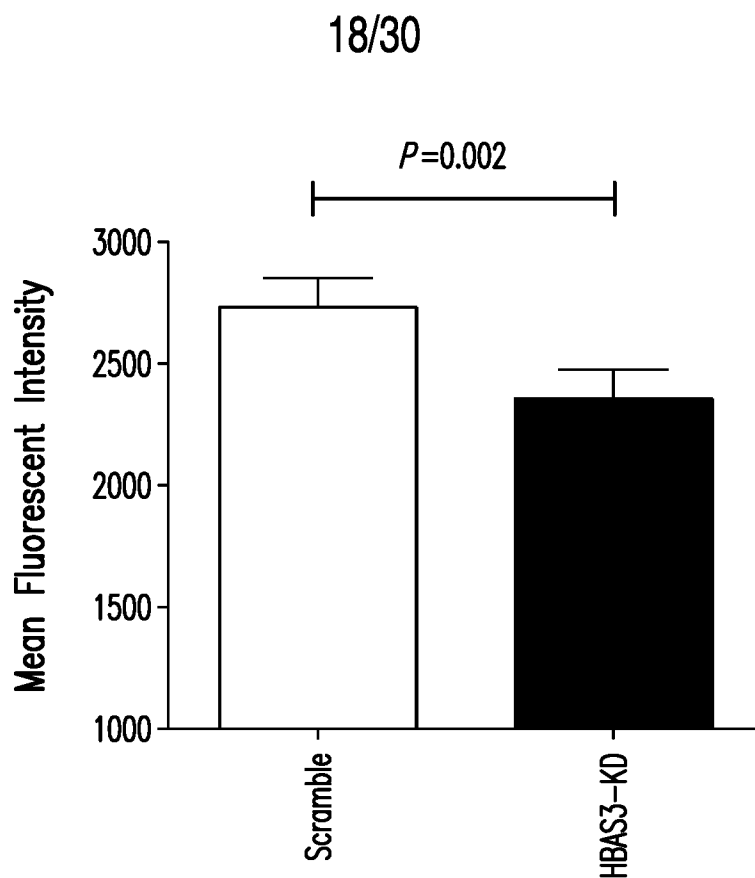


FIG. 5D

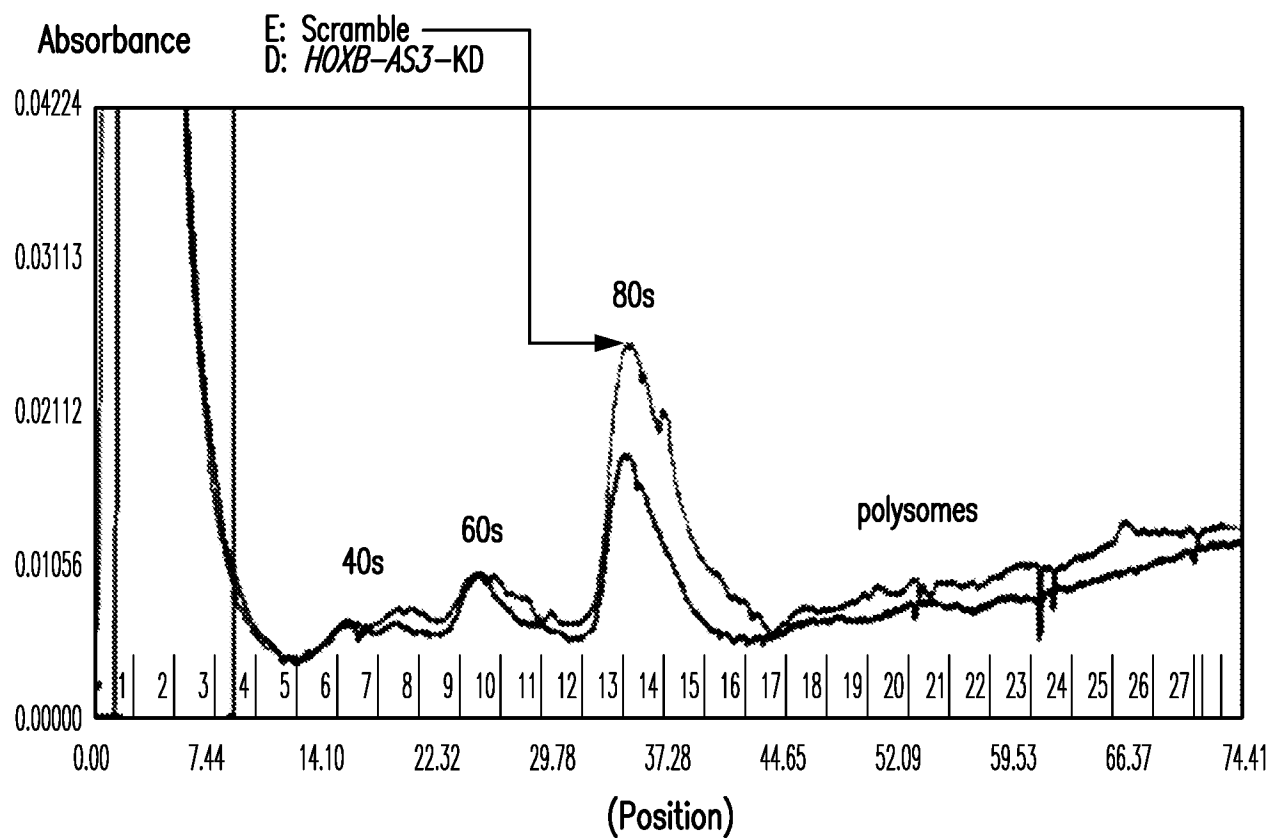


FIG. 5E

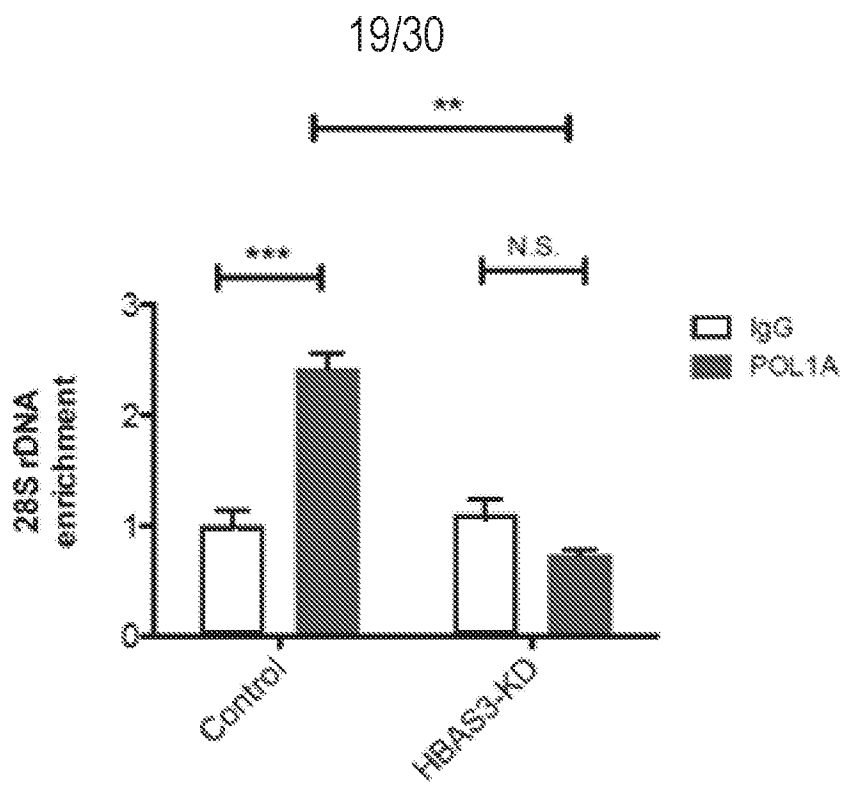


FIG. 5F

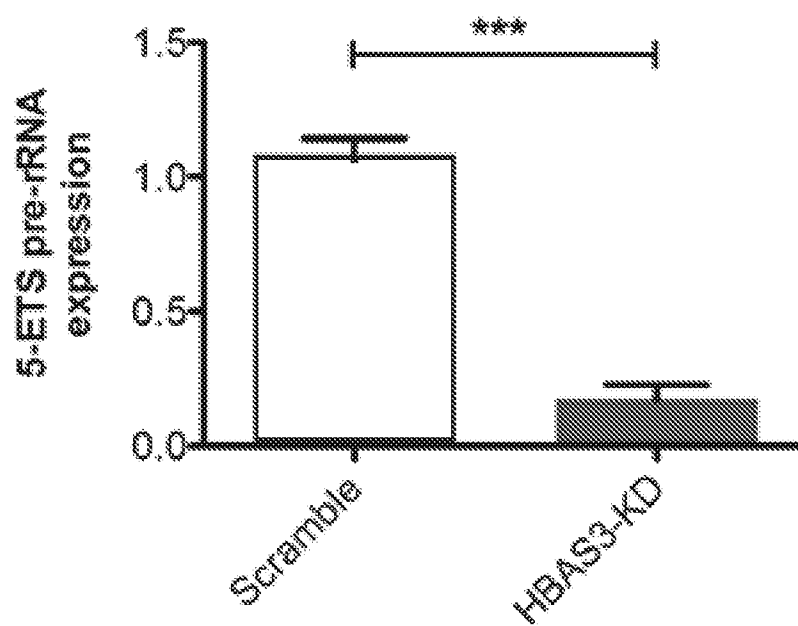


FIG. 5G

20/30

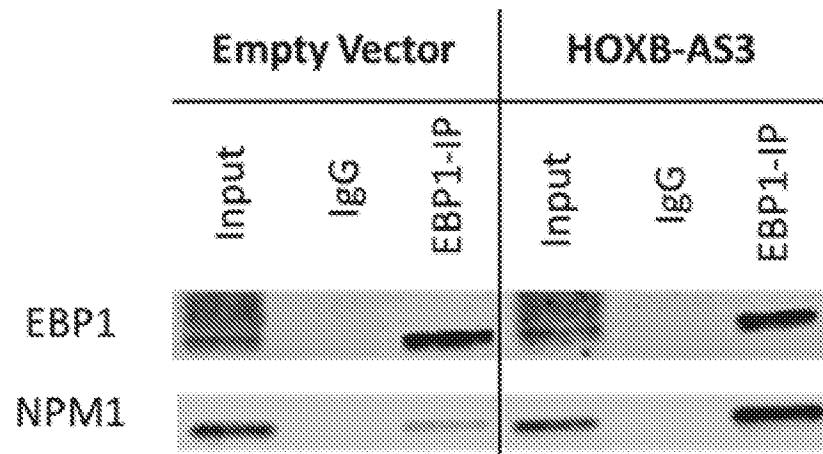


FIG. 5H

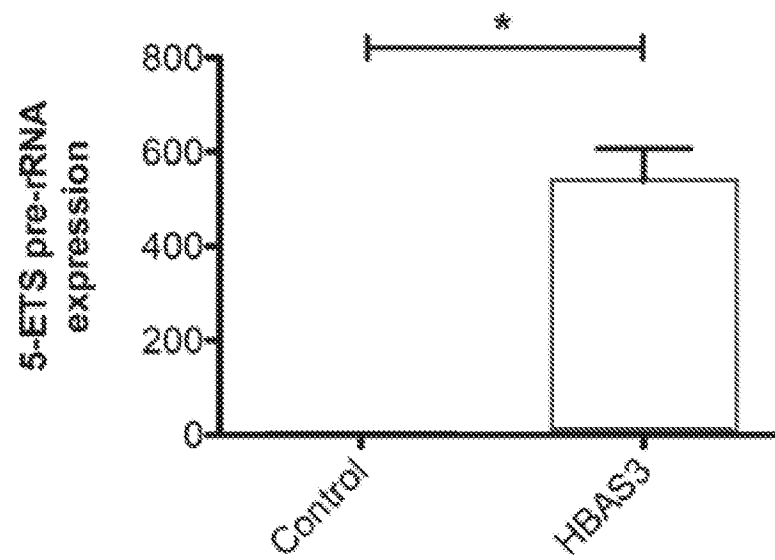


FIG. 5I

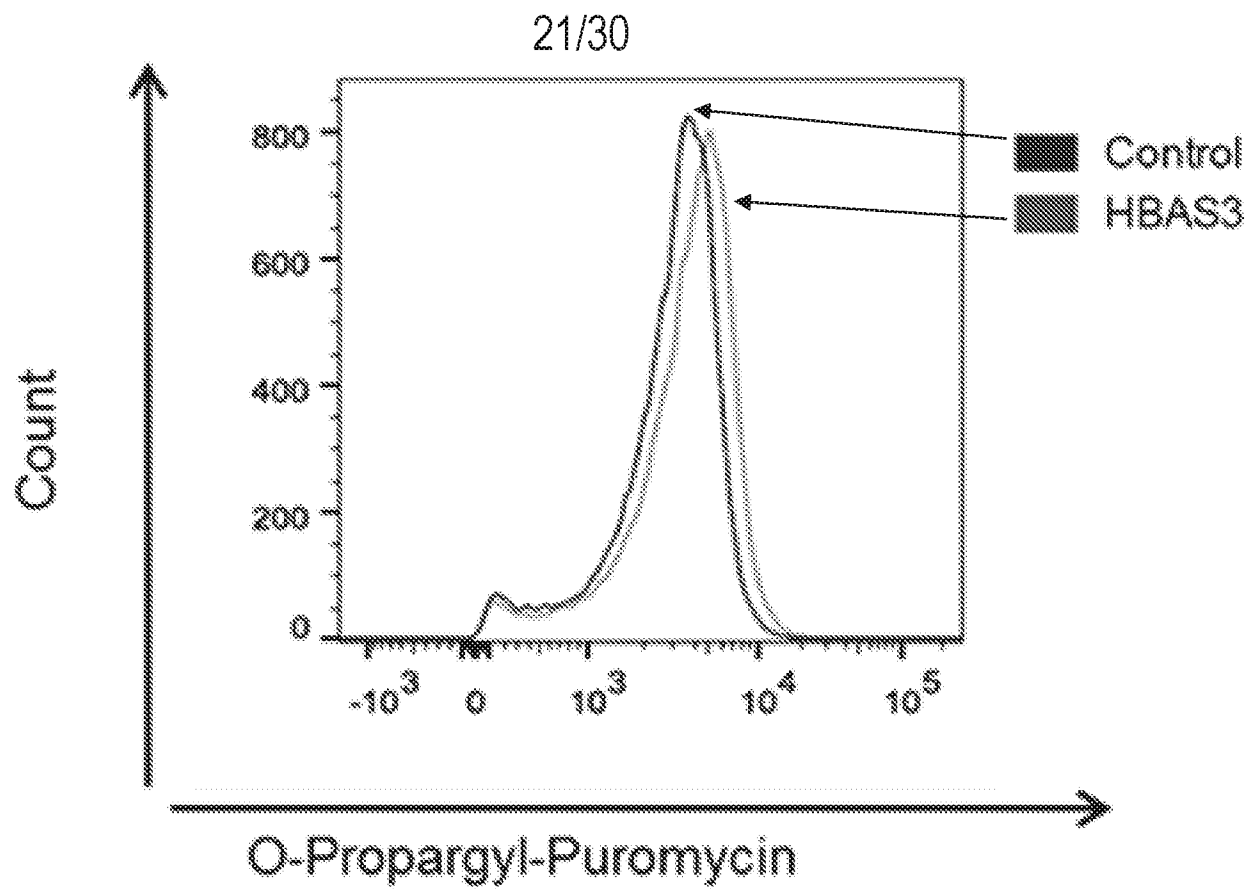


FIG. 5J

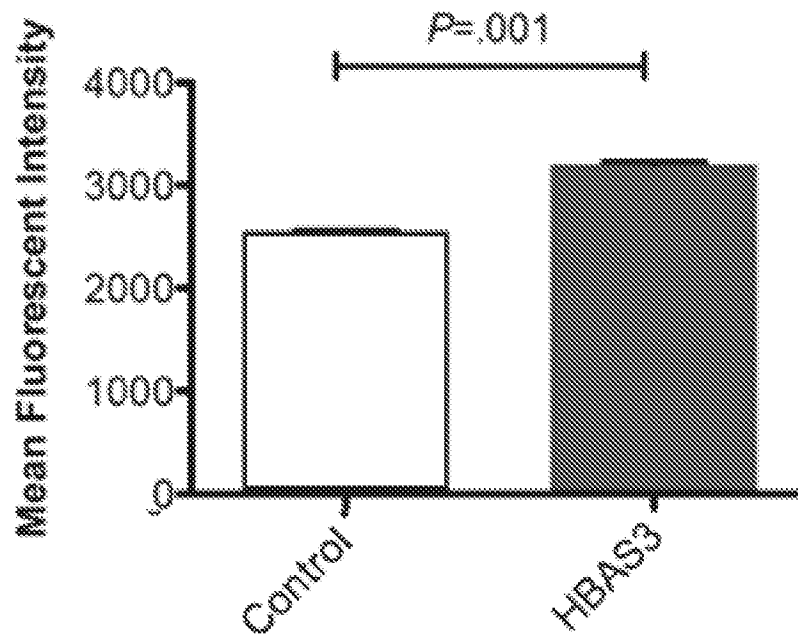


FIG. 5K

22/30

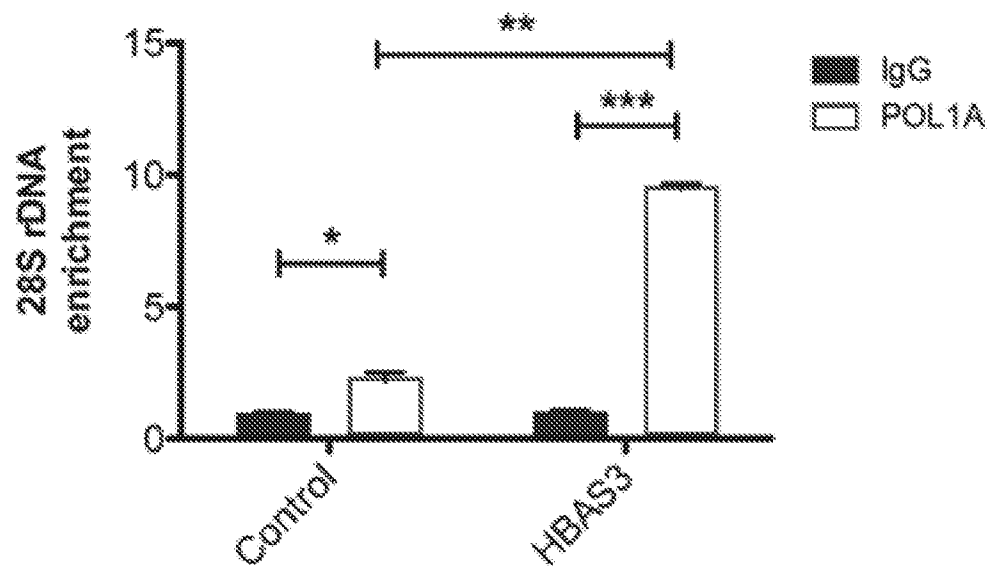


FIG. 5L

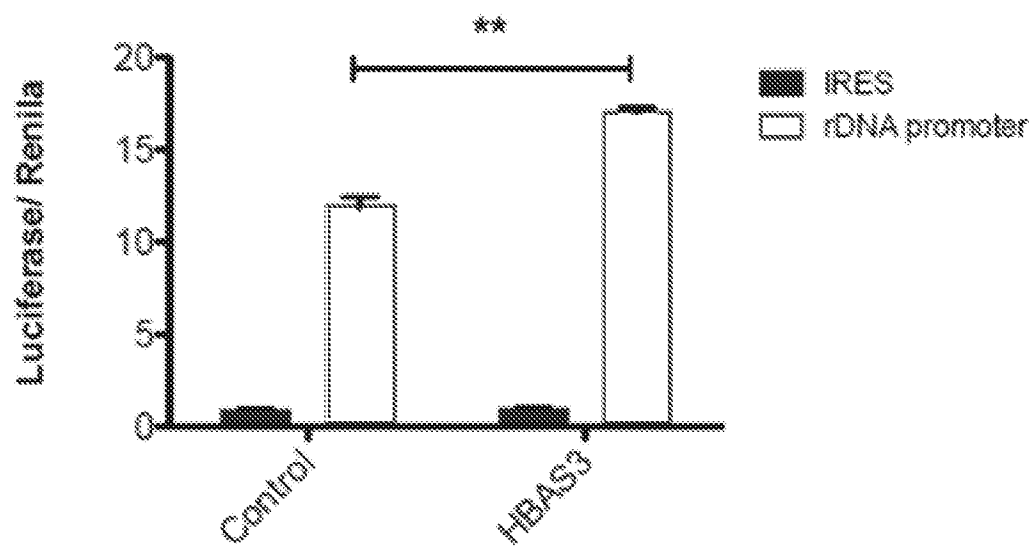


FIG. 5M

23/30

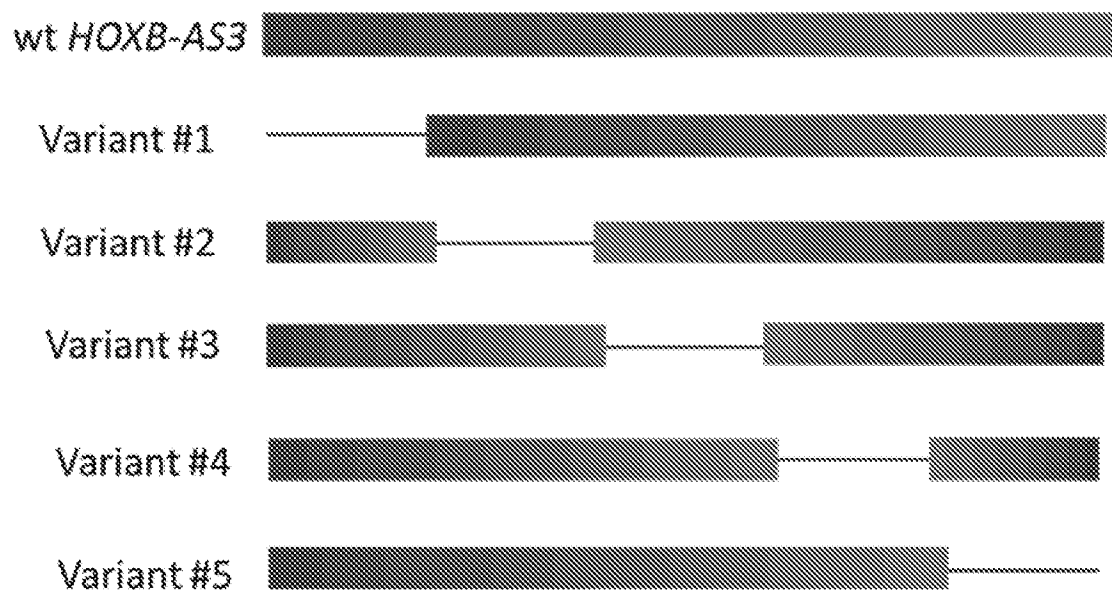


FIG. 6A

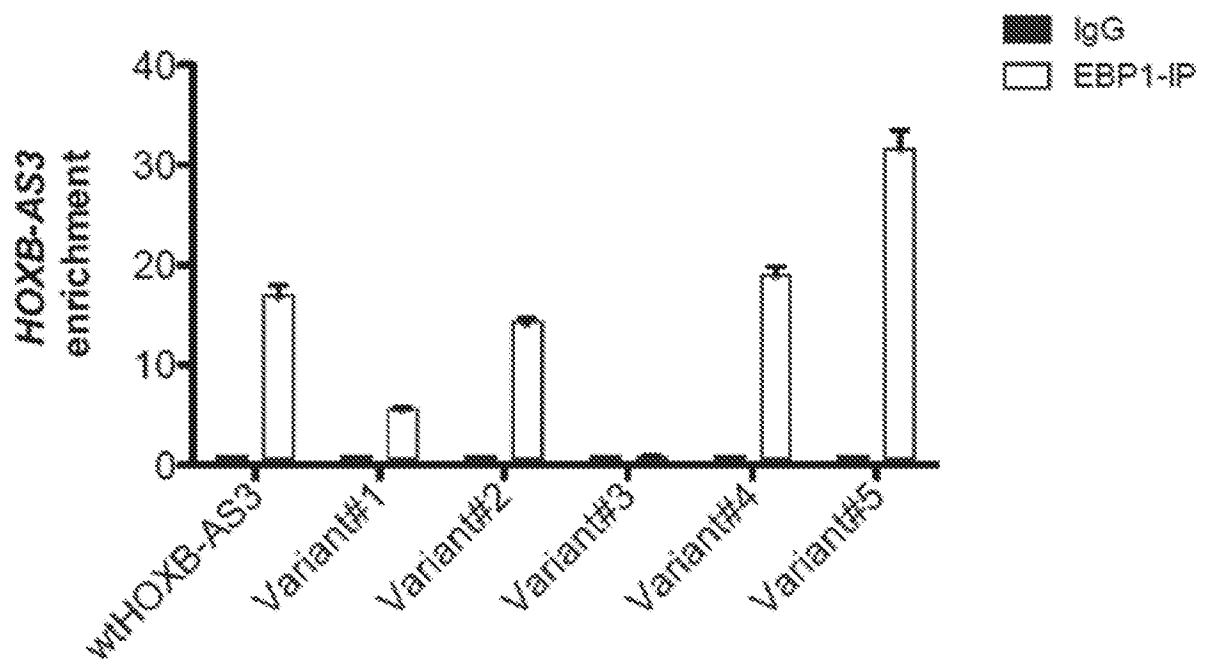


FIG. 6B

24/30

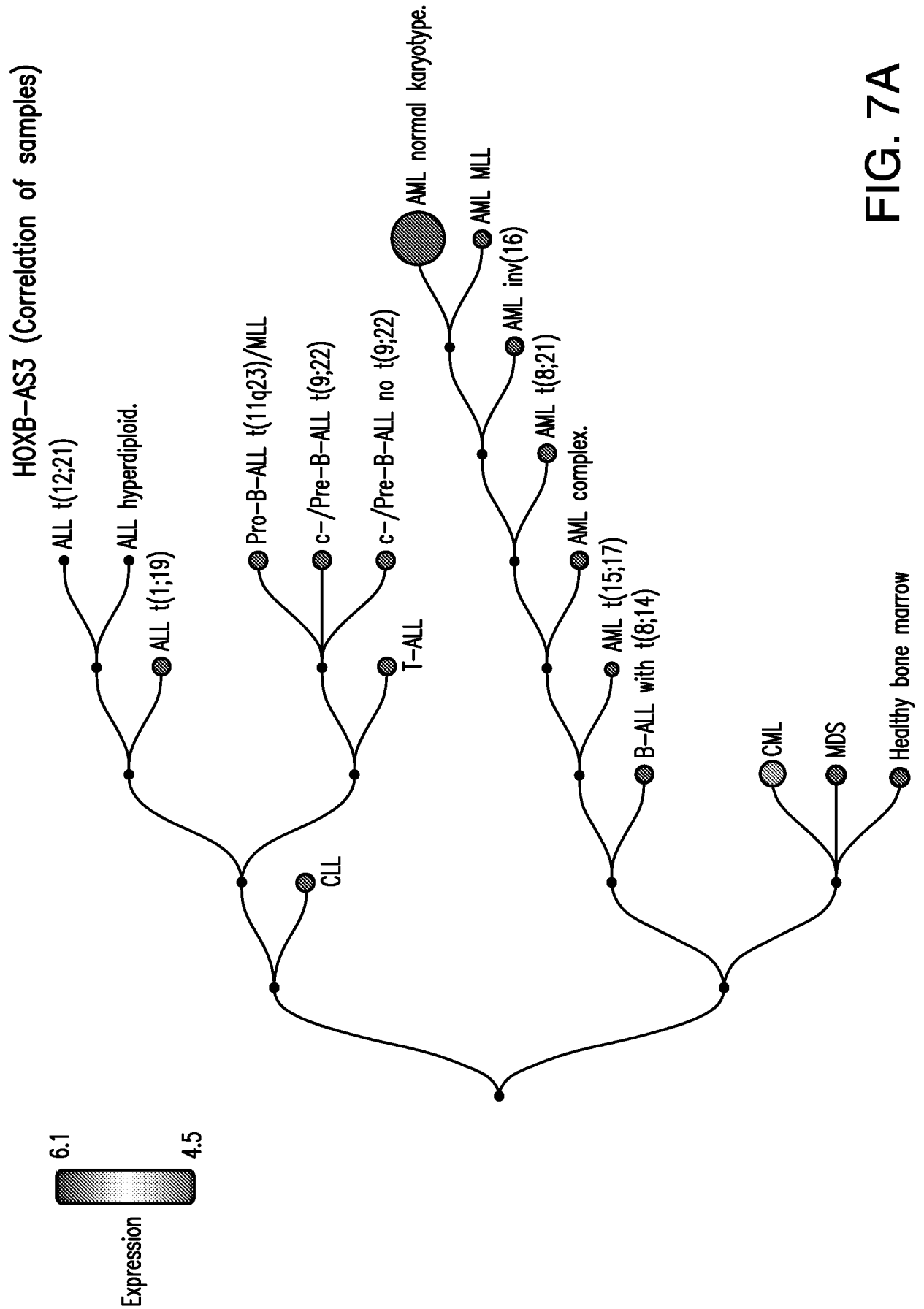


FIG. 7A

25/30

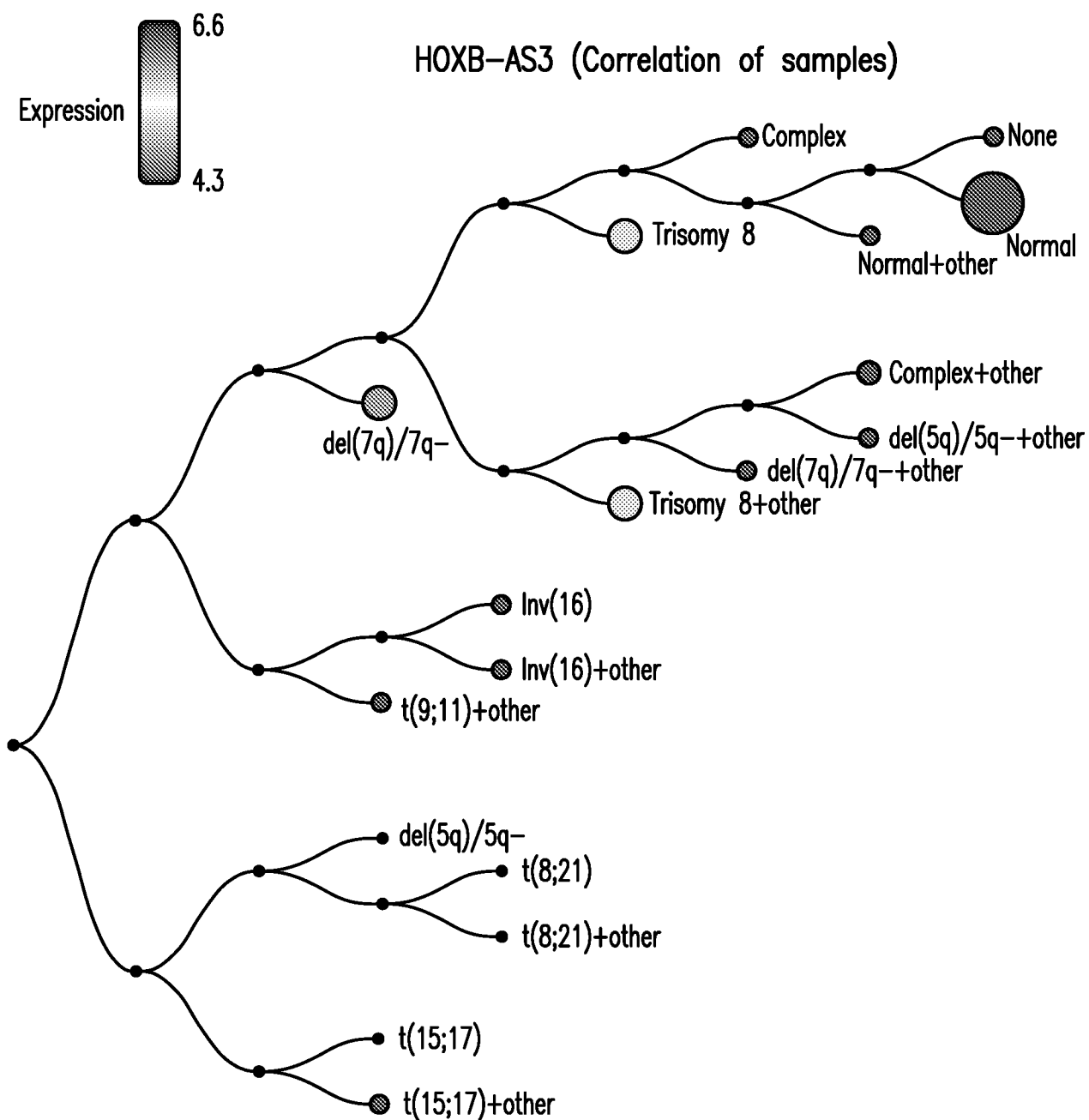


FIG. 7B

26/30

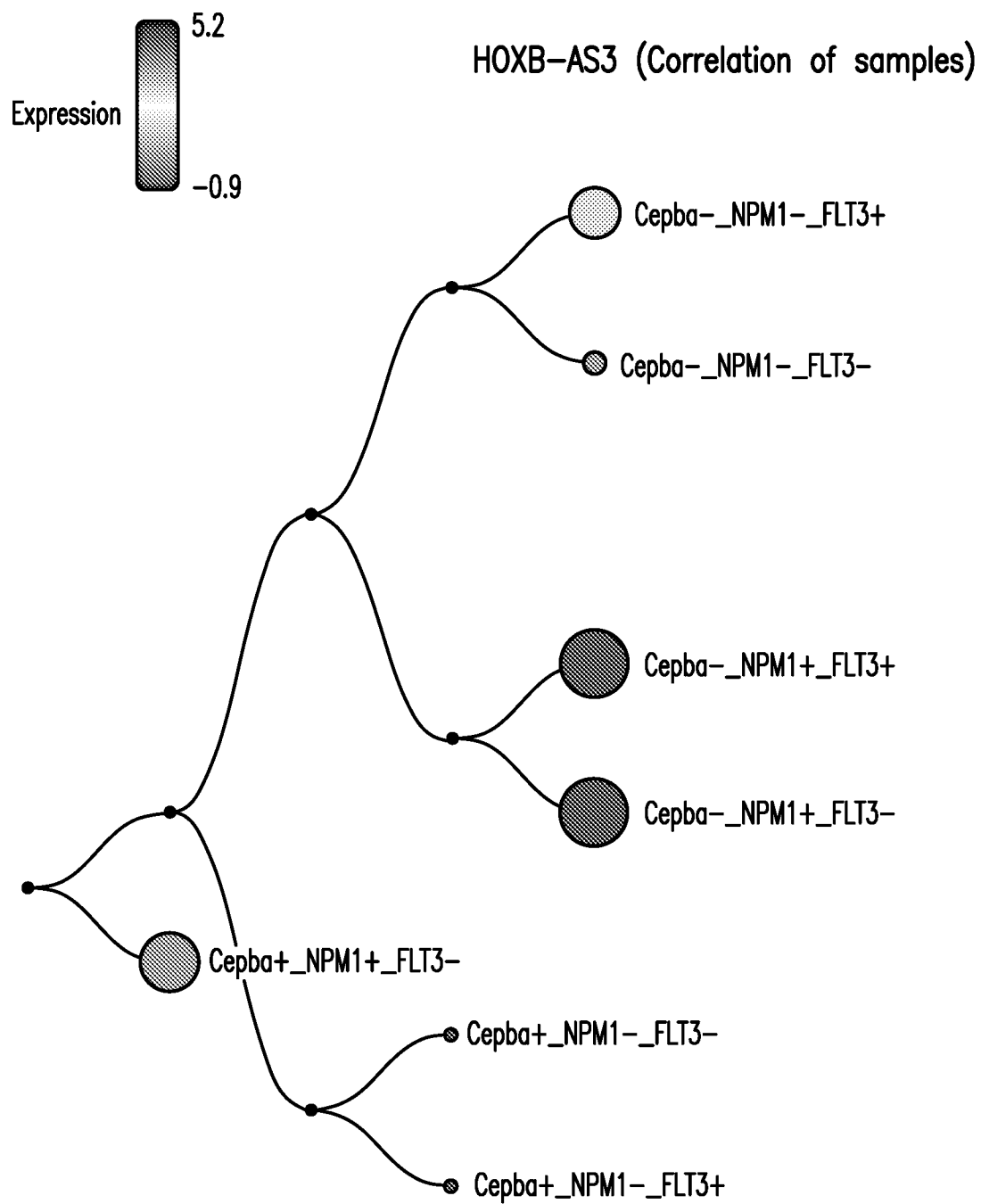


FIG. 7C

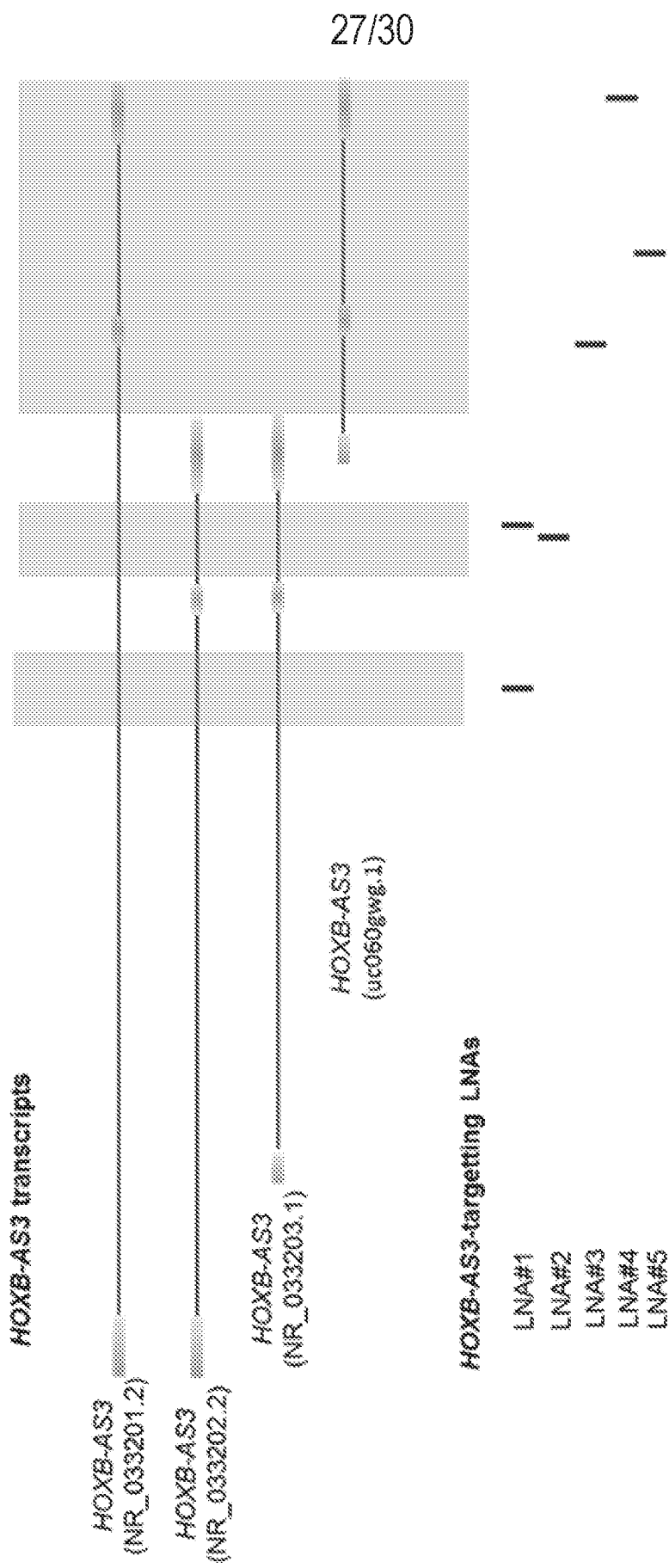


FIG. 8A

28/30

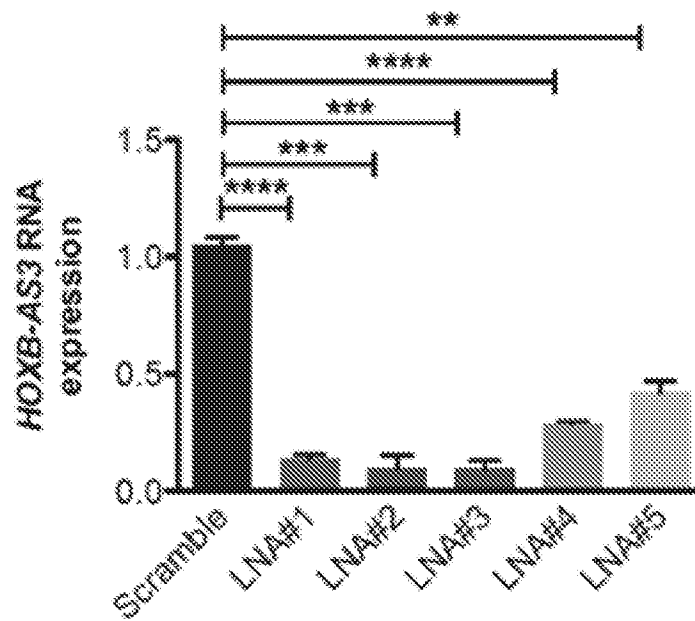


FIG. 8B

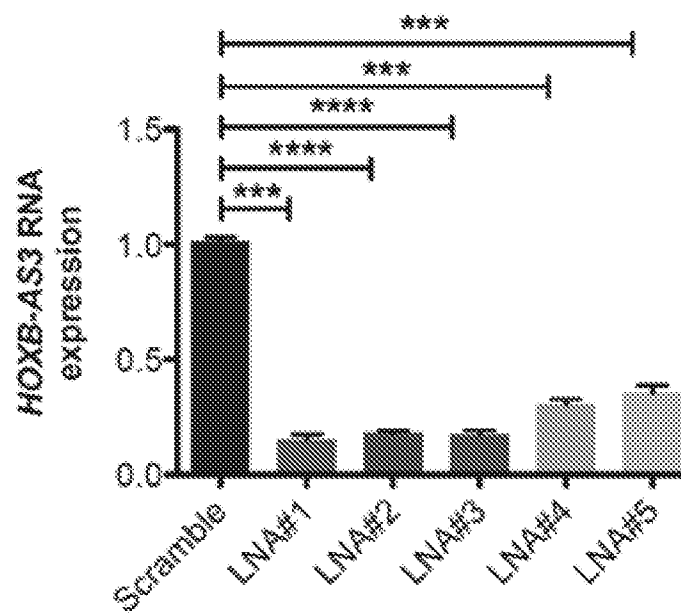


FIG. 8C

29/30

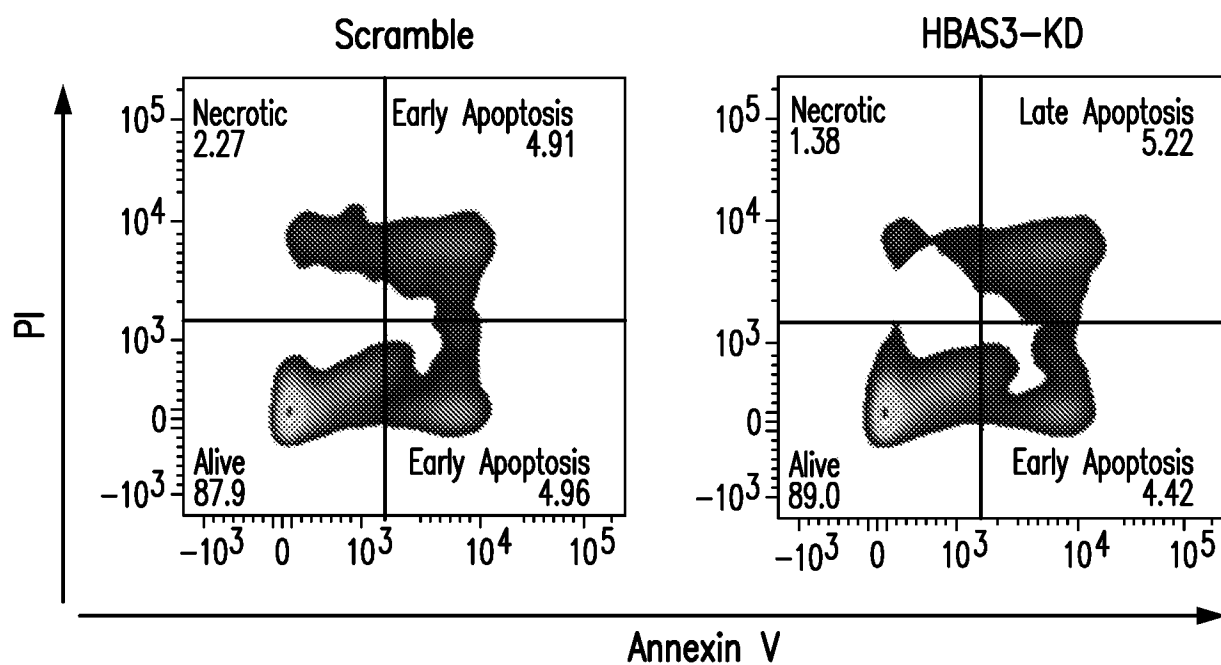


FIG. 9A

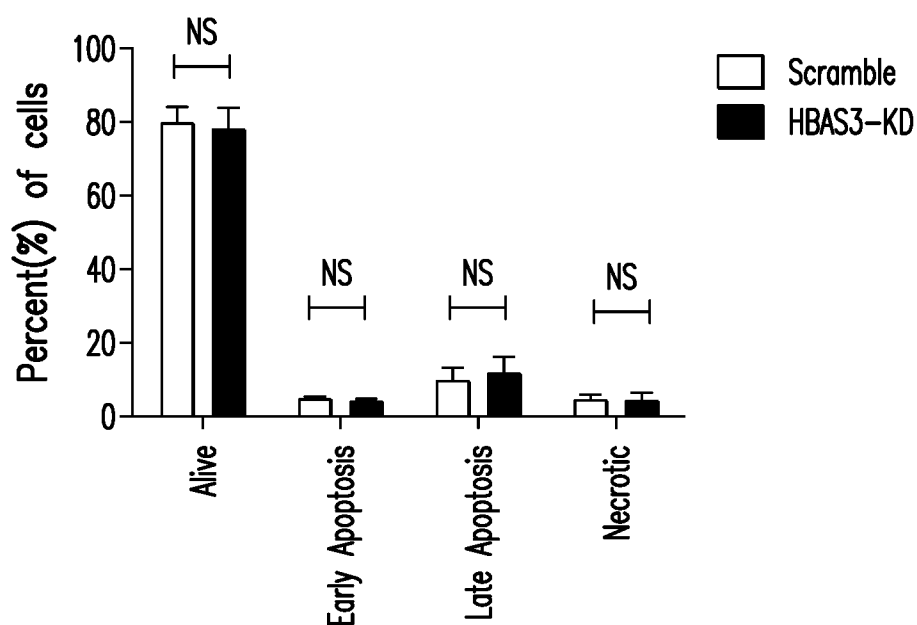


FIG. 9B

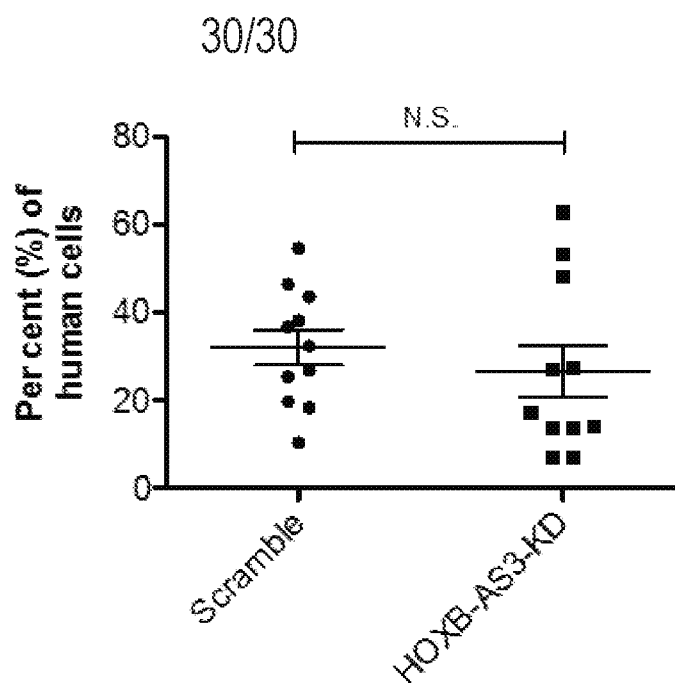


FIG. 10A

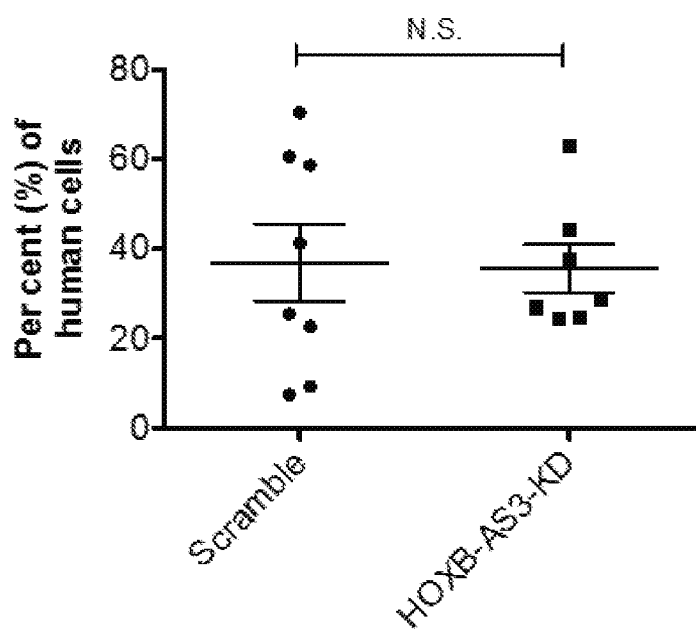


FIG. 10B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/59932

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

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

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

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

3. ☒ Claims Nos.: 5-10, 15-22, 27-33, 38-44
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/59932

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/712, C12N 15/113, A61P 35/02 (2018.01)

CPC - A61K 31/712, C12N 15/113, C12N 2310/113, C12N 2310/3231

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

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

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2014/0142160 A1 (LEE et al.) 22 May 2014 (22.05.2014) para [0019], [0041], [0048], [0049], [0050], [0054], [0565], [0686], [0688], [0689], [0802]	1-4, 11-14, 23-26, 34-37
Y	GARZON et al., Expression and prognostic impact of lncRNAs in acute myeloid leukemia. Proc Natl Acad Sci U S A. 30 December 2014, Vol 111, No 52, pp 18679-18684. Especially p 18681, col 1, para 1	1-4, 11-14, 23-26, 34-37

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 January 2018

Date of mailing of the international search report

06 FEB 2018

Name and mailing address of the ISA/US

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P.O. Box 1450, Alexandria, Virginia 22313-1450

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PCT OSP: 571-272-7774