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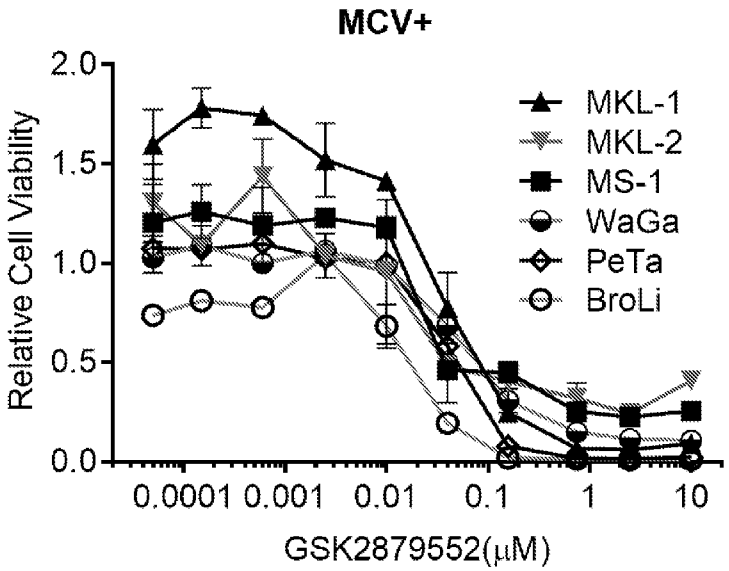



FIG. 22A

(57) Abstract: This disclosure relates to methods and compositions for treating cancers, e.g., Merkel cell carcinoma. The methods involve, e.g., administering to a subject in need thereof an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor.

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# TREATING MERKEL CELL CARCINOMA

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority of U.S. Provisional Appl. No. 62/571,668, filed October 12, 2017, the contents of which are incorporated by reference in their entirety herein.

## FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

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## TECHNICAL FIELD

This disclosure relates to methods and compositions for treating cancers, e.g., Merkel cell carcinoma.

## BACKGROUND

Merkel cell carcinoma (MCC) is a highly aggressive, neuroendocrine carcinoma of the skin. There are two causes of MCC; one resulting from ultraviolet damage from excessive sunlight exposure giving rise to extensive mutation of tumor genome and the other from integration of Merkel cell polyomavirus (MCPyV) DNA into the tumor DNA with sustained expression of the viral oncoproteins Large T antigen (LT) and Small T antigen (ST).

To date, the only FDA approved drug for MCC is Avelumab, a PD-L1 immune checkpoint inhibitor that had a 30% response rate in patients with relapsed MCC (Kaufman, H. L. et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicenter, single-group, open-label, phase 2 trial. The Lancet. Oncology 17, 1374-1385, doi:10.1016/S1470-2045(16)30364-3 (2016)). However, not all patients with MCC respond to checkpoint blockade inhibition and many who do progress after their initial response. Although there are high response rates to standard chemotherapy with cisplatin and etoposide, responses are transient with a median

duration of 3 months (Iyer, J. G. et al. Response rates and durability of chemotherapy among 62 patients with metastatic Merkel cell carcinoma. Cancer medicine 5, 2294-2301, doi:10.1002/cam4.815 (2016)). Therefore, there is an urgent need to develop targeted therapies for MCC.

## SUMMARY

This disclosure relates to methods and compositions for treating Merkel cell polyomavirus (MCPyV)-positive cancers, e.g., MCPyV-positive Merkel cell carcinoma.

In one aspect, the disclosure relates to methods of treating a human subject having, or at risk of developing, a Merkel cell polyomavirus (MCPyV)-positive Merkel cell carcinoma (MCC). The methods include the step of administering to the human subject an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor.

In some embodiments, the LSD1 inhibitor is an antisense molecule, a miRNA, a shRNA, an antibody, or a small molecule.

In some embodiments, the LSD1 inhibitor is GSK2879552, GSK-LSD1, ORY-1001 (RG6016), IMG-7289, CPI-242 or INCB059872.

In some embodiments, the methods also include the step of administering to the human subject a surgery, a radiotherapy or a chemotherapy. In some embodiments, the methods also include the step of administering to the human subject an immune checkpoint inhibitor. In some embodiments, the methods also include the step of administering to the human subject an immune checkpoint inhibitor selected from the group consisting of Avelumab, Pembrolizumab, Nivolumab, and Ipilimumab. In some embodiments, the methods also include the step of administering to the human subject a Myc inhibitor. In some embodiments, the Myc inhibitor is Omomyc.

In another aspect, the disclosure relates to methods of treating a human subject having, or at risk of developing, a Merkel cell polyomavirus (MCPyV)-positive Merkel cell carcinoma (MCC). The methods involve administering to the human subject an effective amount of an inhibitory nucleic acid targeting one or more genes selected from the group consisting of EP400, MYCL, MAX, Small T antigen, LSD1, RCOR2, and INSM1. In some embodiments, the inhibitory nucleic acid targets LSD1, RCOR2, or INSM1.

In one aspect, the disclosure also provides a pharmaceutical composition comprising, consisting of, or consisting essentially of a LSD1 inhibitor and an immune checkpoint inhibitor.

In some embodiments, the LSD1 inhibitor is an antisense molecule, a miRNA, a shRNA, an antibody, or a small molecule.

In some embodiments, the LSD1 inhibitor is GSK2879552, GSK-LSD1, ORY-1001 (RG6016), IMG-7289, CPI-242 or INCB059872.

In some embodiments, the immune checkpoint inhibitor is selected from the group consisting of Avelumab, Pembrolizumab, Nivolumab, and Ipilimumab.

In one aspect, the disclosure relates to methods of determining whether a MCC tumor is Merkel cell polyomavirus (MCPyV)-positive. The methods involve obtaining a tumor tissue sample from a subject; contacting the tumor tissue with an antibody that specifically binds MCPyV T antigen; and determining that the tumor tissue expresses MCPyV T antigen.

In some embodiments, the methods also include the step of contacting the tumor tissue sample with an antibody that binds to one or more antigens selected from the group consisting of RCOR2, LSD1, INSM1, and ATOH1; and determining that the tumor tissue expresses the one or more antigens.

In another aspect, the disclosure relates to methods of determining whether a MCC tumor is Merkel cell polyomavirus (MCPyV)-positive. The methods involve obtaining a tumor tissue sample from a subject; measuring the levels of LSD1+8a mRNA in the tumor tissue sample; and determining that the levels of LSD1+8a mRNA are higher than a control tissue sample from a subject that has a MCPyV-negative tumor. In some embodiments, the levels of LSD1+8a mRNA are measured by RT-qPCR.

In one aspect, the disclosure relates to methods of determining whether a MCC tumor is Merkel cell polyomavirus (MCPyV)-positive. The methods involve obtaining a tumor tissue sample from a subject; contacting the tumor tissue with an antibody that specifically binds LSD1+8a; and determining that the tumor tissue expresses LSD1+8a at a higher level than a control tissue sample from a subject that has a MCPyV-negative tumor.

In another aspect, the disclosure relates to methods of determining whether a MCC tumor is Merkel cell polyomavirus (MCPyV)-positive. The methods involve obtaining a tumor tissue sample from a subject; measuring the levels of one or more mRNA or proteins selected from the group consisting of LSD1, RCOR2, INSM1 and ATOH1 in the tumor tissue sample; and determining that the levels of the one or more mRNA or proteins are higher than a control tissue sample from a subject that has a MCPyV-negative tumor.

In some embodiments, the one or more mRNAs or proteins are selected from the group consisting of LSD1, RCOR2, and INSM1.

In some embodiments, the methods further involve administering an LSD1 inhibitor to the subject.

In some embodiments, the subject is a human.

In one aspect, the disclosure also relates to methods of selecting a human subject having MCC for treatment with an LSD1 inhibitor. The methods involve determining that:

(a) tumor tissue from the human subject comprises DNA from MCPyV;

(b) tumor tissue from the human subject binds an antibody that specifically binds MCPyV T antigen;

(c) tumor tissue from the human subject binds an antibody that specifically binds at least one antigen selected from the group consisting of RCOR2, LSD1, INSM1, and ATOH1 more than a control tissue sample from a subject that has a MCPyV-negative tumor;

(d) tumor tissue from the human subject has increased levels of LSD1+8a mRNA than a control tissue sample from a subject that has a MCPyV-negative tumor;

(e) tumor tissue from the human subject binds an antibody that specifically binds LSD1+8a protein more than a control tissue sample from a subject that has a MCPyV-negative tumor; or

(f) tumor tissue from the human subject has increased levels of mRNAs or proteins selected from the group consisting of LSD1, RCOR2, INSM1, and ATOH1 than a control tissue sample from a subject that has a MCPyV-negative tumor.

In another aspect, the disclosure relates to methods for screening an agent that is useful for treating a MCPyV-positive tumor. The methods involve contacting the agent with a MCPyV-positive tumor cell; determining that (1) the agent inhibits the levels of mRNAs or proteins selected from the group consisting of LSD1, RCOR2, INSM1, and ATOH1 in the tumor cell or (2) the agent increases the levels of mRNAs or proteins selected from the group consisting of ID1 and HES1; and selecting the agent as being useful for treating a MCPyV-positive tumor.

In some embodiments, the agent is a small molecule. In some embodiments, the agent is an antisense molecule, a miRNA, a shRNA, or an antibody.

In one aspect, the disclosure relates to methods of killing a Merkel cell polyomavirus (MCPyV)-positive cancer cell. The methods involve contacting the MCPyV-positive cancer cell with an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor; or contacting the MCPyV-positive cancer cell with an effective amount of an inhibitory nucleic acid targeting one or more genes selected from the group consisting of EP400, MYCL, MAX, Small T antigen, LSD1, RCOR2, and INSM1.

In another aspect, the disclosure relates to methods of inhibiting the growth of a Merkel cell polyomavirus (MCPyV)-positive tumor. The methods involve contacting the MCPyV-positive tumor with an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor; or contacting the MCPyV-positive tumor with an effective amount of an inhibitory nucleic acid targeting one or more genes selected from the group consisting of EP400, MYCL, MAX, Small T antigen, LSD1, RCOR2, and INSM1.

In one aspect, the disclosure also relates to a pharmaceutical composition comprising, consisting of, or consisting essentially of a LSD1 inhibitor and a chemotherapeutic or radiotherapeutic agent.

In one aspect, the disclosure also relates to methods of treating MCPyV-positive MCC in a human subject in need thereof. The methods involve administering to the human subject an effective amount of a LSD1 inhibitor, wherein the human subject has previously been administered at least one of a checkpoint inhibitor, chemotherapy, or radiotherapy.

In one aspect, the disclosure also relates to methods of treating MCPyV-positive MCC in a human subject in need thereof. The methods involve administering to the

human subject an effective amount of a LSD1 inhibitor, wherein the human subject is subsequently administered at least one of a checkpoint inhibitor, chemotherapy, or radiotherapy.

In one aspect, the disclosure also relates to methods of determining that LSD1 inhibition is effective in a human subject undergoing LSD1 inhibitor therapy. The methods involve obtaining a tumor sample from the subject before and after treatment with an LSD1 inhibitor; and determining that the mRNA or protein levels of NICD and/or HES1 is/are elevated in the sample obtained after treatment relative to the mRNA or protein levels of NICD and/or HES1 before treatment.

In some embodiments, the human subject has MCPyV-positive MCC.

In some embodiments, the methods involve continuing treatment with the LSD1 inhibitor.

In any of the aspects or embodiments as described herein, the MCC can be metastatic MCC.

In any of the aspects or embodiments as described herein, the human subject can be a subject that has not received prior chemotherapy or radiotherapy.

In any of the aspects or embodiments as described herein, the MCC can be Stage IA, IB, IIA, IIC, IIIA, IIIB, or IV.

In any of the aspects or embodiments as described herein, the MCC can be relapsed MCC.

In any of the aspects or embodiments described herein, the human subject can be a subject that has previously been determined to not respond to, or weakly respond to, a therapy comprising one of a checkpoint inhibitor, chemotherapy, or radiotherapy.

In any of the aspects or embodiments described herein, the LSD1 inhibitor can be administered after it is determined that a tumor sample from the human subject comprises MCPyV DNA and/or increased levels of a protein selected from the group consisting of LSD1, RCOR2, INSM1, and ATOH1 relative to a MCPyV-negative MCC tumor sample.

In one aspect, the disclosure further provides methods for screening an agent (e.g., a small molecule, a nucleic acid (e.g., siRNA, shRNA, miRNA, antisense), an antibody or antigen-binding fragment thereof) that is useful for treating a MCPyV-positive tumor. The methods involve contacting the agent with a MCPyV-positive tumor cell; and

selecting the agent as being useful for treating a MCPyV-positive tumor if the cells after the treatment have one or more the following characteristics:

(1) the mRNA or protein levels of one or more genes selected from the group consisting of RWDD2A (UniProt ID: Q9UIY3), FAM13C (UniProt ID: Q8NE31), DPF1 (UniProt ID: Q92782), SAT2 (UniProt ID: Q96F10), CREB5 (UniProt ID: Q02930), SMARCA1 (UniProt ID: P28370), LYSMD2 (UniProt ID: Q8IV50), ESRP2 (UniProt ID: Q9H6T0), MANSC1 (UniProt ID: Q9H8J5), LRRC49 (UniProt ID: Q8IUZ0), ATP8B4 (UniProt ID: Q8TF62), GFI1 (UniProt ID: Q99684), DCHS1 (UniProt ID: Q96JQ0), CACNG5 (UniProt ID: Q9UF02), PGBD1 (UniProt ID: Q96JS3), ZNF781 (UniProt ID: Q8N8C0), SMAD9 (UniProt ID: O15198), QPRT (UniProt ID: Q15274), FAM5B (UniProt ID: Q9C0B6), ARPP21 (UniProt ID: Q9UBL0), and RGNEF (UniProt ID: Q8N1W1) are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(2) the mRNA or protein levels of one or more genes selected from the group consisting of CISD3 (UniProt ID: P0C7P0), SLC2A4RG (UniProt ID: Q9NR83), POLR3K (UniProt ID: Q9Y2Y1), ZBTB42 (UniProt ID: B2RXF5), C7orf50 (UniProt ID: Q9BRJ6), DYSF (UniProt ID: O75923), HMGN2 (UniProt ID: P05204), DCK (UniProt ID: P27707), and CLN6 (UniProt ID: Q9NWW5) are decreased in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(3) the mRNA or protein levels of one or more genes selected from the group consisting of FAM5B (UniProt ID: Q9C0B6), ID1 (UniProt ID: P41134), CDH11 (UniProt ID: P55287), CALB2 (UniProt ID: P22676), and PROM1 (UniProt ID: O43490) are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(4) the mRNA or protein levels of ID1 (UniProt ID: P41134) and/or SMARCA1 (UniProt ID: P28370) are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(5) determining increased phosphorylation of SMAD1 (UniProt ID: Q15797), SMAD5 (UniProt ID: Q99717), and/or SMAD9 (UniProt ID: O15198) in the sample obtained after treatment relative to the sample before treatment;

(6) the mRNA or protein levels of one or more genes selected from the group consisting of ATF5 (UniProt ID: Q9Y2D1), F159B (UniProt ID: A6NKW6), TRIB3 (UniProt ID: Q96RU7), AKNA (UniProt ID: Q7Z591), CEBPB (UniProt ID: P17676), and ESRP2 (UniProt ID: Q9H6T0) are decreased in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(7) the mRNA or protein levels of one or more genes selected from the group consisting of GFI1 (UniProt ID: Q99684), MGP (UniProt ID: P08493), LSAMP (UniProt ID: Q13449), DLL1 (UniProt ID: O00548), AMPH (UniProt ID: P49418), PROM1 (CD133) (UniProt ID: O43490), CALB2 (UniProt ID: P22676), BASP1 (UniProt ID: P80723), and BRNP2(FAM5B) (UniProt ID: Q9C0B6) are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment; or

(8) the mRNA or protein levels of one or more genes selected from the group consisting of ID1 (UniProt ID: P41134), ID2 (UniProt ID: Q02363), ZNF781 (UniProt ID: Q8N8C0), HES1 (UniProt ID: Q14469), and DLL1 (UniProt ID: O00548) are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment.

In some embodiments, the selected genes are one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12) of ID1, ID2, ID3 (UniProt ID: Q02535), SMAD9, FAM5B, CDH11, CALB2, PROM1, SMARCA1, DLL1, GFI1, and ZNF781. The mRNA or protein levels of the above are increased relative to prior to treatment with an LSD1 inhibitor. In some embodiments, the selected genes are CALB2, PROM1, FAM5B, DLL1, and/or GFI1. In some embodiments, the selected genes are SMAD9, FAM5B and/or ZNF781. In some embodiments, the selected genes are ID1 and/or SMAD9. In some embodiments, the selected genes are FAM5B, CDH11, CALB2, PROM1 and/or SMARCA1. In some embodiments, the selected genes are CALB2, PROM1, FAM5B, DLL1, and/or GFI1. In

some embodiments, the selected genes are ID1, ID2, SMAD9, ZNF781, HES1 and/or DLL1.

In another aspect, the disclosure also provides methods of determining that LSD1 inhibition is effective in a human subject undergoing LSD1 inhibitor therapy. The methods involve obtaining a tumor sample from the subject before and after treatment with an LSD1 inhibitor; and

(1) determining that the mRNA or protein levels of one or more genes selected from the group consisting of RWDD2A, FAM13C, DPF1, SAT2, CREB5, SMARCA1, LYSMD2, ESRP2, MANSC1, LRRC49, ATP8B4, GFI1, DCHS1, CACNG5, PGBD1, ZNF781, SMAD9, QPRT, FAM5B, ARPP21, RGNEF are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(2) determining that the mRNA or protein levels of one or more genes selected from the group consisting of CISD3, SLC2A4RG, POLR3K, ZBTB42, C7orf50, DYSF, HMGN2, DCK, and CLN6 are decreased in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(3) determining that the mRNA or protein levels of one or more genes selected from the group consisting of FAM5B, ID1, CDH11, CALB2, and PROM1 are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(4) determining that the mRNA or protein levels of ID1 and/or SMARCA1 are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(5) determining increased phosphorylation of SMAD1, SMAD5, and/or SMAD9 in the sample obtained after treatment relative to the sample before treatment;

(6) determining that the mRNA or protein levels of one or more genes selected from the group consisting of ATF5, F159B, TRIB3, AKNA, CEBPB, AND ESRP2 are decreased in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(7) determining that the mRNA or protein levels of one or more genes selected from the group consisting of GFI1, MGP, LSAMP, DLL1, AMPH, PROM1 (CD133), CALB2, BASP1, and BRNP2(FAM5B) are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment; or

(8) determining that the mRNA or protein levels of one or more genes selected from the group consisting of ID1, ID2, ZNF781, HES1, and DLL1 are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment.

In some embodiments, the selected genes are one or more (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12) of ID1, ID2, ID3, SMAD9, FAM5B, CDH11, CALB2, PROM1, SMARCA1, DLL1, GFI1, and ZNF781. The mRNA or protein levels of the above are increased relative to prior to treatment with an LSD1 inhibitor. In some embodiments, the selected genes are CALB2, PROM1, FAM5B, DLL1, and/or GFI1. In some embodiments, the selected genes are SMAD9, FAM5B and/or ZNF781. In some embodiments, the selected genes are ID1 and/or SMAD9. In some embodiments, the selected genes are FAM5B, CDH11, CALB2, PROM1 and/or SMARCA1. In some embodiments, the selected genes are CALB2, PROM1, FAM5B, DLL1, and/or GFI1. In some embodiments, the selected genes are ID1, ID2, SMAD9, ZNF781, HES1 and/or DLL1.

In some embodiments, if the LSD1 inhibitor therapy has been determined to be effective, the methods further involve continuing treating the subject with the LSD inhibitor therapy or another LSD1 inhibitor; otherwise, the LSD1 inhibitor therapy is terminated.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other

references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

Other features and advantages of the invention will be apparent from the following detailed description and figures, and from the claims.

## DESCRIPTION OF DRAWINGS

**FIG. 1A.** MCPyV early region showing nucleotide positions for LT start (5387), ST stop (4827), LT stop (2503), and LT splice donor (5154) and acceptor (4722) and approximate positions of mutations that result in truncated LT found in MCC. LT and ST share an N-terminal J domain. The ST unique domain contains the LSD and Zn fingers. LT splices from J domain to a second exon containing the LXCXE or RB1 binding motif. Antibody Ab3 binds LT only and Ab5 binds both LT and ST.

**FIG. 1B.** Identification of co-precipitating proteins by MudPIT with antibodies Ab3 (LT), Ab5 (LT/ST), EP400 and MAX.

**FIG. 1C.** MKL-1 lysates were immunoprecipitated (IP) with indicated antibodies (top) followed by immunoblotting with indicated antibodies (left). Asterisks indicate non-specific bands in IgG control immunoprecipitation lane.

**FIG. 1D.** MKL-1 lysates (Input) were separated in a Superose 6 column and fractions (#) were blotted with antibodies indicated on left. Protein size markers in kDa indicated at top and right.

**FIG. 1E.** Three MYCL isoforms (i1, i2, i3) are indicated (see also **FIG. 8**). Immunogen of MYCL antibody contained MYCL-i1 residues 16-139.

**FIG. 1F.** Fractions #5, 13 and 21 from **FIG. 1D** were immunoprecipitated with MAX antibody and blotted.

**FIG. 2A.** MKL-1 cells transduced with lentiviral scramble shRNA (shScr) or shRNA specific for LT and ST (shPanT) or ST only (shST) for 1 day followed by selection in puromycin (1 μg/ml) for additional 3 days were lysed (Input) and immunoprecipitated for MAX, EP400 or non-specific IgG and blotted.

**FIG. 2B.** Lysates from HCT116 (lanes 1 and 2) or UISO (lanes 3, 4 and 5) cells stably expressing MCPyV ST (lanes 2 and 5) or a C-terminal epitope tagged ST (lane 4)

were immunoblotted (Input) or immunoprecipitated with antibodies to MAX or non-specific IgG.

**FIG. 2C.** MCPyV ST residues 70 -112 (SEQ ID NO: 1) is shown with corresponding substitution mutations. Residues in the LT stabilization domain (LSD) are indicated.

**FIG. 2D.** HCT116 cells stably expressing wild type (WT) MCPyV ST or indicated mutant constructs. Lysates were immunoprecipitated with Ab5 (ST) or MAX antibodies and blotted. Dashed lines are shown to indicate 11 lanes in ST-Input lanes. Identical panel is also shown in **FIG. 9D** for input.

**FIG. 3A.** CRISPR-Cas9 screen of MKL-1 cells was analyzed in the MAGeCK-VISPR pipeline. Cumulative distribution function of p-values plotted based on 18,493 human genes. EP400 complex components and MYCL were identified in CRISPR screen negative selection with p-values < 0.05 were indicated.

**FIG. 3B.** Lysates from virus-positive MCC cell lines MKL-1, WaGa, MS-1, PeTa, BroLi and MKL-2, virus-negative MCC cell line UISO, and additional lines were immunoblotted. ST-CT are UISO cells stably expressing C-terminal epitope tagged ST.

**FIG. 3C.** Lysates from MKL-1 cell lines containing Dox-inducible shRNA (shMYCL) or miRNA (mirMYCL) specific for MYCL, prepared 2 days after addition of 0.3 μg/ml Dox (Input), were immunoprecipitated for MAX, Ab5, EP400 or non-specific IgG and blotted.

**FIG. 3D.** MKL-1 cells containing Dox-inducible HA tagged Omomyc before (-) or after (+) 5 days of Dox treatment. Dox (0.3 μg/ml) was added every two days. Lysates (Input) were immunoprecipitated with non-specific IgG, MAX, Ab5 and HA antibodies and blotted.

**FIG. 3E.** Viability of MKL-1 Dox-inducible cell lines described in **FIG. 3C** and **FIG. 3D**. 3,000 cells of each line were aliquoted in 96 well plate on day 0. Total days of Dox treatment is indicated on the X axis. Fresh medium or medium with 0.3 μg/ml Dox was supplemented every two days. At the end of time course (day 10), all samples were assessed for viability by CellTiter-Glo (Promega). Values were normalized to untreated samples of each inducible cell line. Three biological replicas were performed. Data are presented as mean (SD).

**FIG. 4A**. MKL-1 cells containing three different Dox-inducible shRNA targeting EP400 (shEP400 -1, shEP400-2, or shEP400-3) or shScramble (shScr) treated with Dox (0.3 μg/ml) every two days for five days. Lysates (Input) were immunoprecipitated with EP400 or control IgG antibodies and blotted for cells before (-) or after (+) 5 days of Dox treatment.

**FIG. 4B**. Same as **FIG. 4A** except lysates were immunoprecipitated with control IgG, MAX or Ab5 antibodies and blotted for cells after (+) 5 days of Dox treatment.

**FIG. 4C**. Cell viability assay of MCPyV positive MCC cell line MKL-1 containing Dox-inducible shRNA targeting EP400 (shEP400) or scramble (shScr). Dox added for indicated number of days. Three biological replicas were performed. Data are presented as mean (SD).

**FIG. 4D**. Lysates from UISO cells containing an inducible scramble shRNA (shScr) or 3 different shRNAs specific for EP400, prepared after 5 days Dox treatment were immunoblotted (Input) or immunoprecipitated with EP400 antibody or control IgG and blotted with indicated antibodies.

**FIG. 4E**. Cell viability assay of MCPyV negative MCC cell line UISO containing Dox-inducible shRNA targeting EP400 (shEP400) or scramble (shScr). Dox added for indicated number of days. Three biological replicas were performed; data are presented as mean (SD).

**FIG. 4F**. Lysates from parental Kelly cells or containing Dox inducible scramble shScr or shEP400-1 prepared after 5 days Dox treatment were immunoblotted (Input) or immunoprecipitated with MAX antibody or non-specific IgG and blotted with antibodies indicated.

**FIG. 4G**. Cell viability assay of Kelly cells containing Dox-inducible shRNA targeting EP400 (shEP400) or scramble (shScr). Three biological replicas were performed; data are presented as mean (SD).

**FIG. 5A**. HFK-hTERT cells were transduced with Dox-inducible OCT4, SOX2 and KLF4 (P) and stably expressed MYCL, 3M or 4M MCPyV ST. Cells were treated with Dox for 31 days and then were immunostained with fluorescent antibodies to TRA-1-60 or TRA-1-81. Light field images demonstrate flat iPSC colonies formed with 3M and MYCL but not from 4M.

**FIG. 5B**. Cells were stained with alkaline phosphatase one day after immunostaining (**FIG. 5 A**).

**FIG. 5C**. Number of iPSC colonies detected after 31 days. Three biological replicas were performed. Data are presented as mean (SD).

**FIG. 5D**. IMR90 cells stably expressing dominant negative p53 and hTERT (PH) were transduced with MYCL (PHL) or tumor derived MCPyV ER region containing truncated LT and wild type ST (PHE) and MYCL (PHEL) or 3M mutant ST (PH3) and 4M mutant ST (PH4). Lysates (Input) were prepared from indicated cells, immunoprecipitated with Ab5 or MAX antibodies followed by immunoblotting with the indicated antibodies.

**FIG. 5E**. Images of soft agar colonies from PHEL cells (4X or 20X magnification).

**FIG. 5F**. Anchorage independent growth of IMR90 cells indicated in D ($10^5$ cells) plated in soft agar and cultured for 4 weeks. Three biological replicas were performed. Data are presented as mean (SD).

**FIG. 6A**. Venn diagram of annotated genes corresponding to peaks identified by ChIP-seq with indicated antibodies. Two biological replicas of MAX and EP400 were performed and shared genes indicated. Shared genes identified with Ab5 and ST-HA are indicated.

**FIG. 6B**. De novo DNA motif identification with indicated antibodies.

**FIG. 6C**. Distribution of peaks by Metagene analysis.

**FIG. 6D**. Heatmaps of H3K4me3, MAX, EP400 and ST (Ab5) ChIP peaks ranked by read density of H3K4me3 and scaled against the 75th percentile of genome-wide read density for each ChIP.

**FIG. 6E**. Meta-track analysis of ChIP-seq read density for MAX, EP400 and ST at all H3K4me3 peaks genome-wide. Regions are centered and ranked for H3K4me3 peaks over input.

**FIG. 7A.** Heatmap depicts average mean-centered and standard-deviation-scaled gene expression profiles for each of 62 clusters created by applying model-based clustering to the differentially expressed genes (DEG) in MKL-1 cells after depletion of

EP400 or MYCL in comparison to shScr control. Merged Clusters (CL1-4) are indicated on the left-hand side.

**FIG. 7B**. Diagram illustrating BETA Activating/Repressing Function Prediction of transcription factors by correlation of distance of peaks from corresponding TSS obtained in ChIP-seq of ST, MAX and EP400 with changes in gene expression by RNA-seq after Dox-induction with shRNA targeting EP400 or MYCL.

**FIG. 7C**. Venn diagram showing common direct target genes of MAX, EP400 and ST identified by BETA based on ChIP-seq of MAX, EP400, ST and RNA-seq of shEP400 -2, -3 and MYCL shRNA (BETA3).

**FIG. 7D**. Heatmap depicts average mean-centered and standard-deviation-scaled gene expression profiles for each of 37 clusters created by applying model-based clustering to the 951 BETA3 target genes in MKL-1 cells after depletion of EP400 or MYCL in comparison to shScr control. Merged Clusters (CL1-4) are indicated on the left-hand side.

**FIG. 7E**. MKL-1 cells containing Dox inducible shRNA for shScr, shMYCL or EP400 (shEP400-2, -3) were treated with dox for 5 days. Lysates were blotted with indicated antibodies. EP400 immunoprecipitations were blotted with EP400 antibody.

**FIG. 8A.** Illustration of MAX and MYC family interacting proteins highlighting interaction of ST with MYCL, MAX and EP400 complex.

**FIG. 8B.** Conserved MYC boxes in MYCL, MYCN and MYC.

**FIG. 8C.** Predicted coding of human MYC (SEQ ID NO: 2), MYCN (SEQ ID NO: 3), MYCL isoform i1 (SEQ ID NO: 4) and MYCL isoform i3 (SEQ ID NO: 5). Conserved MYC box elements are boxed. MB0 is also known as NC1. Note that MB3a is not present in MYCL. Identical residues and conserved residues are in gray color.

**FIG. 9A.** MKL-1 cells transduced with lentiviral shRNA scrambled (shScr), LT and ST (shPanT) or ST only (shST) for 1 day followed by selection in puromycin (1 μg/ml) and cultured for 3 days were immunoblotted with Ab5 (upper panel) and Vinculin.

**FIG. 9B.** Human foreskin fibroblasts (HFF) were stably transduced with lentiviruses expressing MCPyV ST, codon optimized ST (STco) or GFP. Lysates blotted with indicated antibodies.

**FIG. 9C.** Alignment of MCPyV ST residues 61-109 (SEQ ID NO: 6) corresponding to the region between the J domain and the Zn finger domain with ST from Gg1PyV (Gorilla gorilla gorilla 1; SEQ ID NO: 7), LIPyV (Lyon IARC, HPyV14; SEQ ID NO: 8), NJPyV (New Jersey, HPyV13; SEQ ID NO: 9), HPyV9 (SEQ ID NO: 10), TSPyV (Trichodysplasia spinulosa, HPyV8; SEQ ID NO: 11), WUPyV (HPyV4; SEQ ID NO: 12), KIPyV (HPyV3; SEQ ID NO: 13), HPyV6 (SEQ ID NO: 14), HPyV7 (SEQ ID NO: 15), MWPyV (Malawi, HPyV10; SEQ ID NO: 16), STLPyV (Saint Louis, HPyV11; SEQ ID NO: 17), BKPyV (B.K., HPyV1; SEQ ID NO: 18), JCPyV (HPyV2; SEQ ID NO: 19) and HPyV12 (SEQ ID NO: 20). The lysine residue (K61) is the last conserved residue in the N-terminal J domain. The cysteine residue on the right (residue 109 in MCPyV) is the first residue from the conserved Zn fingers for the ST species shown.

**FIG. 9D.** HCT116 cells stably expressing MCPyV ST including wild type (WT) or indicated mutant constructs. Lysates were blotted with indicated antibodies. Input blot for ST is shown again in **FIG.** 2D. Dashed lines are shown to distinguish lanes.

**FIG. 10A.** Gene Set Enrichment Analysis (GSEA) on known human housekeeping genes ranked in MKL-1 CRISPR screen using H1 (left) and H2 (right) sgRNA libraries to illustrate negative correlation of CRISPR screen and housekeeping genes.

**FIG. 10B.** Copy numbers of every 50-kb segment of MKL-1 genome were called from the input of ChIP-seq experiments (see **FIG. 6**) using QDNAseq software. Segmented copy numbers were converted to copy numbers per gene based on gene coordinates.

**FIG. 10C.** Venn diagram analysis of human housekeeping genes and 481 negatively selected CRISPR targets with FDR < 0.05 identified from H1 and H2 sgRNA libraries screen of MKL-1 cells.

**FIG. 10D.** Lysates from HCT116 cells stably expressing C-terminal 3xHA-tagged MYCL constructs with (+) or without (-) ST were immunoprecipitated with HA (MYCL) and Ab5 (ST) antibodies and blotted.

**FIG. 11A.** Venn diagram of biological replicas of ChIP-seq for MAX, EP400, Ab5 and ST-HA for ST.

**FIG. 11B.** Peak Height distribution. All peaks were separated into promoter, intron, and distal intragenic regions. Input Genome legend shown for comparison.

**FIG. 11C.** ChIP-reChIP followed by qPCR was performed. Initial (1st) ChIP was performed with antibodies to MAX (left panel), EP400 (middle), ST (gray bar) and ST-HA (black) followed by re-ChIP with indicated antibody or no IgG. Primers for MCM7 or PCBP1 promoters as indicated.

**FIG. 12A.** Chromatin was prepared from MKL-1 cells containing Dox inducible scrambled shRNA (shScr), MYCL (shMYCL), or Dox inducible miRNAs targeting negative control DNA sequence (mirNRneg) or MYCL (mirMYCL) after 2 days with 0.3 μg/ml Dox addition. ChIP-qPCR performed with Ab5 antibody and primers for MYCL promoter.

**FIG. 12B.** Same as **FIG. 12A** with primers for indicated promoters.

**FIG. 12C.** Overlapped peaks of MAX, EP400, ST and H3K4me3 ChIP-seq at MYCL locus.

**FIG. 12D.** Chromatin from MKL-1 cells with a Dox inducible shRNA targeting EP400 before (Gray bars) and after (black bars) 5 days of Dox addition. ChIP-qPCR was performed with MAX antibody and indicated promoters. 544-545 and 647-648 represent two DNA sites used as negative controls.

**FIG. 13.** Principal components analysis was performed on the data before applying ComBat (but after normalization; left-hand side) and after applying ComBat (right-hand side). Numbers located below each data point indicate the batch in which the experiment was performed.

**FIG. 14A.** BETA Activating/Repressing Function Prediction for MAX, EP400, and ST upon EP400 or MYCL knockdown by combining MAX, EP400, ST ChIP-seq with RNA-seq from MKL-1 cells containing EP400 shRNA -1, -2, -3, shScr after 5 days Dox treatment or shMYCL after 2 days Dox treatment. Genes were Ranked on both ChIP peaks proximity to transcription start site and differential expression upon factor binding, rank product of the two was used to predict direct targets. Top solid line represents genes downregulated upon EP400 knock-down (Down), bottom solid line represents upregulated (Up) and dashed line (in the middle) represents static genes with no change. p values indicated in parentheses.

**FIG. 14B.** Venn diagram showing common direct target genes of MAX, EP400 and ST identified by BETA based on ChIP-seq of MAX, EP400, ST and RNA-seq of shEP400-1, -2, -3 and MYCL shRNA.

**FIG. 14C.** Venn diagram showing common direct target genes of MAX, EP400 and ST identified by BETA based on ChIP-seq of MAX, EP400, ST and RNA-seq of shEP400-1, -2, -3 and MYCL shRNA (BETA4).

**FIG. 15A.** Heatmap shows the logarithm (base 2) of the fold change for each BETA3 gene in each sample relative to the average expression of the same gene in the three shScr replicates in the shEP400 experiment.

**FIG. 15B.** Histogram showing the spread of fold changes across all BETA3 genes in the shEP400 samples relative to the shScr samples. Fold change was computed as $2^{\Delta Y}$, where $\Delta Y$ indicates the average of the log (base 2) expression levels of all six shEP400 samples (shEP400-2, -3 in triplicate) subtracted by the average log (base 2) expression levels in the three shScr (shScr in triplicate) samples from the EP400 experiment.

**FIG. 15C.** Histogram showing the spread of fold changes across all BETA3 genes in the shMYCL samples relative to the shScr samples. Fold change was computed as $2^{\Delta Y}$, where $\Delta Y$ indicates the average of the log (base 2) expression levels of three shMYCL samples subtracted by the average log (base 2) expression levels in the three shScr samples from the MYCL experiment.

**FIG. 16.** Comparison of effect of inducible ST in IMR90 cells with depletion of EP400 and MYCL in MKL-1 cells.

**FIG. 17.** Model of MCPyV ST recruiting MYCL (L-Myc) to EP400 complex and transactivating expression of LSD1 (KDM1A), INSM1 and RCOR2, components of CoREST complex.

**FIG. 18.** LSD1 inhibitors reduce viability of virus-positive MCC. Viability of virus-positive MCC Broli and Peta and virus-negative UISO and MCC-26 cell lines measured after 12-13 days treatment with 2 different LSD1 inhibitors CPI-242 (‘242) and CPI-890 (‘890; also known as CI-664890 or GSK9552).

**FIG. 19.** Merkel cell virus ST induces LSD1 inhibitor sensitivity in IMR90 fibroblasts. IMR90 cells with ST (MCV T+) or without (T-), MKL-1 MCC cell line, and 293T control cells cultured in soft agar were treated with DMSO or LSD1 inhibitor.

Colonies were counted. Error bars indicate standard deviation.

**FIG. 20.** RT-qPCR quantification of mRNA levels in MCC cell lines.

**FIG. 21 s**hows the amino acid sequence and nucleotide sequence for LSD1+8a.

**FIG. 22A.** Virus-positive (MKL-1, MKL-2, MS-1, WaGa, PeTa and BroLi) MCC cell lines are sensitive to LSD1 inhibition.

**FIG. 22B.** Virus –negative (MCC13, MCC26 and UISO) MCC cell lines are not sensitive to LSD1 inhibition.

**FIG. 23A**. Soft agar assay of T-antigen-transformed IMR90 cells and MKL-1 MCC cells with an LSD1 inhibitor (GSK-LSD1, 0.01 μM) shows that transformation of normal cells by MCV T antigens depends on LSD1 activities.

**FIG. 23B.** Quantification of 3 biological replicas of experiment shown in **FIG. 23A**. Data are shown as mean ± SD; * $P<0.05$, ** $P<0.005$, and ***$P<0.0005$.

**FIG. 24.** MKL-1 and WaGa virus-positive MCC cell lines grown as xenografts in SCID mice display sensitivity to LSD1 inhibition (Data are shown as mean ± SD; * (or #) $P<0.05$, ** (or ##) $P<0.005$, and ***$P<0.0005$. $- Day 22 has no measurement).

**FIG. 25.** Strategy used to identify biological processes affected by LSD1 inhibition and EP400 depletion.

**FIG. 26.** Heatmap for the results of RNA-seq of six virus-positive MCC (MKL-1, MKL-2, MS-1, WaGa, PeTa and BroLi) and virus-negative UISO cell lines treated with LSD1 inhibitors (GSK-LSD1 for 3 days or CPI-242 for 1 day).

**FIG. 27A.** RT-qPCR analysis of selected LSD1-coREST target genes MKL-1 and WaGa (virus-positive MCC) and UISO (virus-negative MCC) cell lines treated with CPI-242 for 1 day. The signals were normalized to untreated samples and RPLP0 in each sample. Data are shown as mean ± SD and reflect 3 biological replicas.

**FIG. 27B.** Western blot of MKL-1 and WaGa virus-positive MCC cell lines. LT indicates Merkel cell polyomavirus Large T antigen. ST indicates Merkel cell polyomavirus Small T antigen. VINC indicates Vinculin used as protein loading control.

**FIG. 28**. Changes in differentially expressed proteins for MKL-1 virus-positive MCC cell line treated with GSK-LSD1 for 8 days.

**FIG. 29.** ChIP-qPCR of LSD1 in MKL-1 MCC cells shows that LSD1 enrichment decreases following LSD1 inhibition. Data are shown as mean ± SD; *$P<0.05$

and **P<0.005.

## DETAILED DESCRIPTION

Merkel cell carcinoma (MCC) is a highly aggressive, neuroendocrine cancer of the skin. MCC frequently contains integrated copies of Merkel cell polyomavirus DNA and expresses two viral transcripts including a truncated form of Large T antigen (LT) and an intact Small T antigen (ST). While LT binds the Retinoblastoma protein and inactivates its tumor suppressor function, it is less clear how ST contributes to MCC tumorigenesis. The present disclosure shows that ST specifically recruits the MYC homolog MYCL (L-MYC) to the 15-component EP400 histone acetyltransferase and chromatin remodeling complex. The ST-MYCL-EP400 complex binds to specific gene promoters to activate their expression. Both MYCL and EP400 are required for maintenance of MCC cell line viability and can cooperate with ST to promote gene expression. The present disclosure demonstrates that ST enhances the interaction between MYCL and the EP400 complex interaction and this activity contributes to transcriptional activation, oncogenesis and reprogramming of MCC.

The present disclosure further shows a striking relationship between MYCL and MCPyV ST. MCPyV ST regulates MYCL levels and MYCL are expressed in virus-positive MCC cell lines. Introduction of ST into several naïve cell lines can lead to increased levels of MYCL. Conversely, depletion of ST from MKL-1 cells can lead to decreased levels of MYCL. ST together with EP400 and MAX can bind to the MYCL promoter. In addition, the virus-positive MKL-1 cell line is sensitive to Omomyc expression indicating that the MYCL-MAX heterodimer is required for viability as well as ST interaction. These results in the present disclosure are consistent with a positive feedback loop where ST binding to the MYCL promoter contributes to transcriptional activation of MYCL leading to increased levels of MYCL that in turn binds to ST and the EP400 complex.

MCPyV ST shows a strong preference for recruiting MYCL to the EP400 complex. MYC and MYCL can cooperate with the OSK reprogramming factors to induce a pluripotent state in somatic cells [39, 57]. Comparison of the contributions of MYC to transformation and iPS cell generation show significant overlap with the interaction with

the EP400 complex as a key component [41]. The present disclosure shows that MCPyV ST can substitute for MYCL in iPS cell generation and that this activity is strictly dependent upon ST interaction with the EP400 complex. The data indicate that, at least in part, MCPyV ST functions similarly to MYC by binding to the EP400 complex, recruiting it to specific promoters to transactivate gene expression and thereby promoting the generation of iPS cells. These functions can also prove to be critical in establishing and maintaining the oncogenic state of MCC.

The data further reveal that the ST-MYCL-EP400 complex functions, at least in part, to activate specific gene expression. Depletion of MYCL and EP400 leads to significant changes in gene expression and cell viability. Those genes whose levels are decreased upon MYCL and EP400 depletion are significantly associated with ST, MAX and EP400 binding to their promoters and include classic MYC targets involved in RNA processing, ribosome biogenesis, nitrogen compound and peptide metabolic processes. Additional target genes are involved in cell morphogenesis and signaling in the TNF, WNT, NFκB and DNA damage pathways. Importantly, a large number of metabolic genes are activated by the ST-MYCL-EP4000 complex including a number of transporters including SLC16A1 and SLC7A5 and the MYC-metabolism genes MLX and MLXIP (Mondo) [16, 58]. Factors that promote transcription elongation are also highly enriched including EIF4E, EIF4EBP1, and EIF5A [13].

Interestingly, genes whose levels increased upon MYCL or EP400 depletion were involved in neurogenesis, axon guidance, wound healing and cell-cell adhesion. These results can be interpreted to indicate that ST-MYCL-EP400 complex serves to repress differentiation markers and induce a more primitive, progenitor or embryonic state, consistent with its ability to generate iPS cells.

The MYC family functions to activate gene expression at least in part by interaction with a variety of chromatin factors. In addition to the EP400 complex, MYC can bind to the TRRAP-containing STAGA (SPT3-TAF9-GCN5 acetylase) complex that in turn interacts with Mediator [59]. MYC binds to BRD4 and the pTEFb complex to facilitate transcriptional elongation by release of paused RNA polymerase II [60, 61]. The conserved Myc Boxes contribute to transformation with the Myc Box 3b (MB3b) binding to WDR5 and Myc Box 4 (MB4) binding to HFCF1 (**FIG. 8**) [62, 63]. MB3a, or simply

referred to as MB3, found only in MYC and MYCN and not MYCL, is required for tumorigenic activity of MYC *in vitro* and *in vivo* [64] and contributes to transcriptional repression by recruiting HDAC3 [65]. At oncogenic expression levels, MYC interacts with MIZ-1 (ZBTB17) to repress transcription, which can be disrupted by mutating valine 394 (V394) in the helix-loop-helix (HLH) domain [66]. The present disclosure only detected the EP400 complex and did not detect any of these other MYC binding factors in any of the ST complexes. Both MB1 and MB2 of MYCL contribute to ST and MYCL binding. Of note, it appears that ST and MYCL bind directly to TRRAP as evidenced by co-precipitation of TRRAP with ST and MAX antibodies after EP400 depletion (**FIG. 4B**). In contrast to MCPyV ST, transformation by SV40 ST is strictly dependent on its interaction with PP2A. SV40 ST binding to PP2A perturbs its ability to de-phosphorylate certain substrates including MYC that in turn leads to higher levels of MYC.

Among the downstream targets of the MCPyV ST-MYCL-EP400 complex, components of the CoREST (CoRepressor for Element-1-Silencing Transcription factor) complex were identified. The present disclosure further shows that MCPyV ST can specifically increase levels of LSD1 (lysine specific demethylase 1, KDM1A), the enzymatic component of CoREST, as well as RCOR2 and INSM1, two critical components of the CoREST complex. LSD1 is a histone demethylase that can erase methylation from H3K4me1 and H3K4me2, an activity that has been implicated in iPS cell generation. Very high levels of LSD1 in 6 virus-positive MCC cells lines with little to no expression in virus-negative, UV-damaged, MCC cells. All 6 MCPyV-positive MCC cell lines tested were sensitive to treatment with LSD1 inhibitors *in vitro*. In contrast, all 3 virus-negative MCC cell lines tested were resistant to LSD1 inhibition. Furthermore, introduction of MCPyV ST into a normal cell conferred sensitivity to LSD1 inhibition indicating that the viral oncogene drove the cellular dependency on LSD1. These results provide a rationale for using LSD1 inhibitors in treatment of MCPyV-positive MCC.

These results indicate that the ST-MYCL-EP400 complex not only serves to transactivate key target genes to promote MCC oncogenesis but creates dependencies on downstream target genes such as the CoREST complex. The discovery that virus-positive

MCC is sensitive to LSD1 inhibitors is unexpected and could not have been predicted from prior studies.

In summary, these results highlight an important mechanism for MCPyV ST mediated transformation. The ST-MYCL-EP400 complex functions as a powerful engine to transactivate gene expression and promote oncogenesis. As LSD1 inhibitors can effectively inhibit the growth of MCC cell lines *in vitro*, the present disclosure further provides a useful targeted therapeutic approach for the treatment of MCPyV-positive cancers (e.g., MCPyV-positive Merkel cell carcinoma).

**Merkel cell carcinoma (MCC)**

Merkel cell carcinoma (MCC) is an aggressive skin cancer with a high rate of mortality. Risk factors for developing MCC include immunosuppression and UV-induced DNA damage from excessive exposure to sunlight [1]. MCC can be categorized into different stages, e.g., Stage 0, Stage IA, IB, IIA, IIC, IIIA, IIIB, or IV. The stage of MCC depends on the size and location of the tumor, whether it has grown into nearby tissues or bones, whether it has spread to nearby lymph nodes or any other organs, and certain other factors. The stage is based on the results of physical exams, any biopsies that have been done (including sentinel lymph node biopsy), and any imaging tests (CT, MRI, PET/CT scan, etc.) that have been done.

Recognition of the immunosuppressive risk for MCC prompted a search to identify pathogens and led to the discovery of Merkel cell polyomavirus (MCPyV) [2]. MCPyV-positive MCC tumors contain clonally integrated copies of viral DNA and express small T antigen (ST) (Nucleotide sequence: NCBI GeneBank HM011556.1; amino acid sequence: NCBI GenBank: ADE45417.1) and a truncated form of large T antigen (LT) (Nucleotide sequence: NCBI GeneBank HM011556.1; amino acid sequence: NCBI GenBank: ADE45416.1).

**FIG. 1A** shows nucleotide positions for LT start (5387), ST stop (4827), LT stop (2503), and LT splice donor (5154) and acceptor (4722) and approximate positions of mutations that result in truncated LT found in MCC. LT and ST share an N-terminal J domain. The ST unique domain contains the LSD and Zn fingers. LT splices from J

domain to a second exon containing the LXCXE or RB1 binding motif. Antibody Ab3 binds LT only and Ab5 binds both LT and ST.

Genome sequencing of virus-negative MCC revealed an extremely high number of single nucleotide polymorphisms containing the C>T transition consistent with UV damage [3, 4]. In contrast, MCPyV positive tumors contain very few somatic mutations suggesting that MCPyV ST and LT contribute the major oncogenic activity to MCC development.

In all virus-positive MCC cases reported to date, LT has undergone truncations that disrupt viral replication activities but leave the LXCXE, RB-binding, motif intact [5]. While LT can bind and inactivate RB, prior to the present disclosure, it was not clear how ST contributes to MCC tumorigenesis.

The EP400 histone acetyltransferase complex is involved in multiple biological events including transcription, stem cell maintenance and DNA damage response. The mammalian EP400 complex contains at least 15 distinct components including the large subunits EP400 (also known as p400) and TRRAP plus ACTL6A, BRD8, DMAP1, EPC1 (and its homologue EPC2), ING3, KAT5 (also known as Tip60), MBTD1, MEAF6, MORF4L1 (and MORFL2), MRGBP, RUVBL1 (and RUVBL2), VPS72 and YEATS4 [17-20]. The EP400 complex contains several intrinsic enzymatic activities including EP400 chaperone activity for histone variants H3.3 and H2AZ, KAT5 mediated acetylation of histones H2A and H4, and the DNA helicase activity of RUVBL1 and RUVBL2. TRRAP can bind directly to MYC and bind equally well to the homologue MYCN and poorly to MYCL (L-MYC).

The present disclosure demonstrates that MCPyV ST recruits MYCL to the EP400 complex to activate specific gene expression, promote cellular transformation and contribute to its oncogenic potential. The present disclosure further demonstrates that MCPyV ST specifically recruits the MYCL and MAX heterodimer to the 15-component EP400 complex. These interactions are essential for the transforming function of MCPyV ST, the viability of virus-positive MCC cells and likely to be a major contributor to the oncogenic potential of MCPyV in MCC. Consistent with this model, a genome-wide CRISPR-Cas9 screen revealed that MYCL and several components of the EP400 complex were essential for viability of the virus-positive MCC cell line MKL-1. The

interaction of MCPyV ST with MYCL and the EP400 complex is unique to the family of polyomaviruses. Prior to the present disclosure, it is believed that no other polyomavirus ST has been reported to bind the EP400 complex or a MYC homolog.

**Lysine Specific Demethylase 1 (LSD1)**

Lysine specific demethylase 1 (LSD1) (also known as Lysine-specific histone demethylase 1A or KDM1A; NCBI Reference Sequence: NM_001009999.2; NP_055828.2) is a flavin-dependent monoamine oxidase, which can demethylate mono- and di-methylated lysines, specifically histone 3, lysines 4 and 9 (H3K4 and H3K9). LSD1 has roles critical in embryogenesis and tissue-specific differentiation, as well as oocyte growth.

The LSD1 gene contains 19 exons that are highly conserved among vertebrates. Through RNA alternative splicing, two additional exons, exon E2a and exon E8a, can be included in the mature mRNA, generating four possible LSD1 isoforms, namely the conventional LSD1, LSD1 plus exon E2a (LSD1+2a), exon E8a (LSD1+8a; NP_001009999.1), or both (LSD1+2a+8a).

The alternative splicing of LSD1 (e.g., LSD1+8a) is known in the art, and is described, e.g., in Jotatsu, et al. "LSD1/KDM1 isoform LSD1+ 8a contributes to neural differentiation in small cell lung cancer." Biochemistry and Biophysics Reports 9 (2017): 86-94, which is incorporated herein by reference in its entirety.

The present disclosure shows that the MCPyV Small T antigen (ST) can specifically increase the expression of LSD1 as well as associated proteins RCOR2 and INSM1 that form the coREST complex. LSD1 demethylates histones H3K4me1 and H3K4me2 resulting in transcriptional repression and H3K9me1 and H3K9me2 leading to gene activation.

As shown in the present disclosure, it has been determined that small molecule inhibitors of LSD1 can cause a growth arrest and cell death of MCPyV-positive MCC cell lines, and introduction of MCPyV ST into a naive normal cell induces the expression of LSD1 and RCOR2, and induces a sensitivity to LSD1 inhibitors.

Therefore, the present disclosure provides methods of treating a subject having, or at risk of developing, a Merkel cell polyomavirus (MCPyV)-positive cancer, (e.g.,

Merkel cell carcinoma). The methods include the steps of administering to the subject an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor.

**Methods of treatments**

The present disclosure provides methods of treating a subject having, or at risk of developing, a Merkel cell polyomavirus (MCPyV)-positive cancer or a Merkel cell polyomavirus (MCPyV)-positive tumor. The methods include the steps of administering to the subject an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor, or an effective amount of compositions as described herein (e.g., EP400, MAX, MYCL, Small T antigen, LSD1, RCOR2, ATOH1, or INSM1 inhibitors).

As used herein, the term "Merkel cell polyomavirus (MCPyV)-positive tumor" or "Merkel cell polyomavirus (MCPyV)-positive cancer" refers to a tumor or a cancer that is infected by Merkel cell polyomavirus. Similarly, the term "MCPyV-negative tumor" or "MCPyV-negative cancer" refers to a tumor or a cancer that is not infected by Merkel cell polyomavirus. Methods of determining whether a tumor or a cancer is infected by Merkel cell polyomavirus are described in the present disclosure, e.g., by determining whether the cancer cell or the tumor cell has Merkel cell polyomavirus nucleic acids, or by determining whether the cancer cell or the tumor cell expresses MCPyV ST or LT.

As used herein, the term "cancer" refers to cells having the capacity for autonomous growth. Examples of such cells include cells having an abnormal state or condition characterized by rapidly proliferating cell growth. The term is meant to include cancerous growths, e.g., tumors; oncogenic processes, metastatic tissues, and malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Also included are malignancies of the various organ systems, such as respiratory, cardiovascular, renal, reproductive, hematological, neurological, hepatic, gastrointestinal, and endocrine systems; as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, and cancer of the small intestine. Cancer that is "naturally arising" includes any cancer that is not experimentally induced by implantation of cancer cells into a subject, and includes, for example, spontaneously arising cancer, cancer caused by exposure of a patient to a carcinogen(s), cancer resulting

from insertion of a transgenic oncogene or knockout of a tumor suppressor gene, and cancer caused by infections, e.g., viral infections. The term "carcinoma" is art recognized and refers to malignancies of epithelial or endocrine tissues. The term also includes carcinosarcomas, which include malignant tumors composed of carcinomatous and sarcomatous tissues. An "adenocarcinoma" refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures. The term "sarcoma" is art recognized and refers to malignant tumors of mesenchymal derivation. The term "hematopoietic neoplastic disorders" includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin. A hematopoietic neoplastic disorder can arise from myeloid, lymphoid or erythroid lineages, or precursor cells thereof. In some embodiments, the cancer as described here are caused by or characterized by Merkel cell polyomavirus infection. In some embodiments, the Merkel cell polyomavirus (MCPyV)-positive cancer is MCPyV-positive Merkel cell carcinoma.

As used herein, the terms "subject" and "patient" are used interchangeably throughout the specification and can describe an animal, human or non-human, to whom treatment according to the methods of the present disclosure is provided. Veterinary and non-veterinary applications are contemplated. Human patients can be adult humans or juvenile humans (e.g., humans below the age of 18 years old). In addition to humans, patients include but are not limited to mice, rats, hamsters, guinea-pigs, rabbits, ferrets, cats, dogs, and primates. Included are, for example, non-human primates (e.g., monkey, chimpanzee, gorilla, and the like), rodents (e.g., rats, mice, gerbils, hamsters, ferrets, rabbits), lagomorphs, swine (e.g., pig, miniature pig), equine, canine, feline, bovine, and other domestic, farm, and zoo animals.

As used in this context, to "treat" means to ameliorate at least one symptom of cancers or tumors. Often, the treatment results in ameliorate, stop, stabilize, reverse, inhibit, slow and/or delay progression of a cancer in a subject. Thus, administration of a therapeutically effective amount of the composition as described herein can result in a decrease of tumor size or tumor volume, a decrease of tumor growth, a reduction of the increase rate of tumor volume in a subject (e.g., as compared to the rate of increase in tumor volume in the same subject prior to treatment or in another subject without such treatment), a decrease in the risk of developing a metastasis or the risk of developing one

or more additional metastasis, an increase of survival rate, and an increase of life expectancy, etc. The tumor volume in a subject can be determined by various methods, e.g., as determined by direct measurement, MRI or CT.

In some embodiments, an LSD1 inhibitor is administered to the subject. In some embodiments, the LSD1 inhibitor is GSK2879552, GSK-LSD1, ORY-1001 (RG6016), IMG-7289, CPI-242 or INCB059872.

In some embodiments, inhibitors of EP400, MAX, MYCL, Small T antigen, LSD1, RCOR2, ATOH1, or INSM1 are administered to a subject. These inhibitors can be an antisense molecule, a miRNA, a shRNA, an antibody, or a small molecule.

The antibody can specifically bind to the proteins as described herein. As used herein, when referring to an antibody, the phrases "specifically binding" and "specifically binds" mean that the antibody interacts with its target molecule preferably to other molecules, because the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the target molecule; in other words, the reagent is recognizing and binding to molecules that include a specific structure rather than to all molecules in general. An antibody that specifically binds to the target molecule may be referred to as a target-specific antibody. For example, an antibody that specifically binds to a LSD1 molecule may be referred to as a LSD1-specific antibody or an anti-LSD1 antibody.

The present disclosure also provides methods of killing a Merkel cell polyomavirus (MCPyV)-positive cancer cell or tumor cell. The methods involve contacting the MCPyV-positive cancer cell or tumor cell an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor; or contacting the MCPyV-positive cancer cell or tumor cell an effective amount of an inhibitory nucleic acid (e.g., an shRNA or an antisense molecule) targeting one or more of target genes as described herein (e.g., EP400, MAX, MYCL, Small T antigen, LSD1, RCOR2, ATOH1, or INSM1).

In another aspect, the present disclosure provides methods of inhibiting the growth a Merkel cell polyomavirus (MCPyV)-positive tumor. The methods include the steps of contacting the MCPyV-positive tumor an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor; or contacting the MCPyV-positive tumor an effective

amount of an shRNA or an antisense molecule targeting one or more of target genes as described herein (e.g., EP400, MYCL, Small T antigen, LSD1, RCOR2, or INSM1).

The MCPyV-positive cancer cell or tumor cell can be a cultured cell, or a cell in a subject having MCPyV-positive cancer.

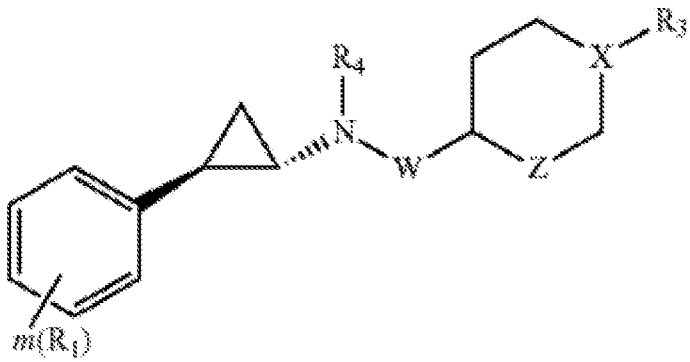
**LSD1 inhibitors**

The present disclosure shows that LSD1 inhibitors can effectively inhibit the growth of MCPyV-positive cells or kill MCPyV-positive cancer cells.

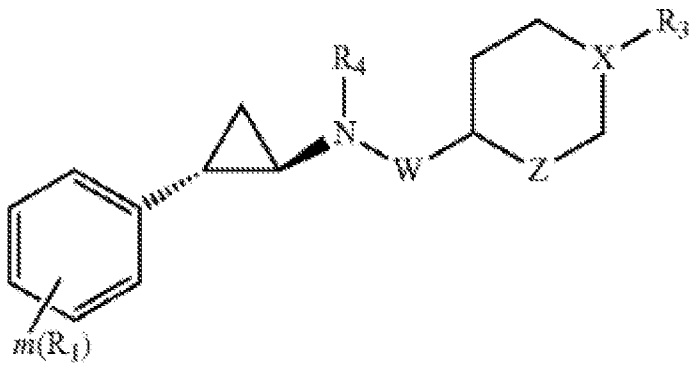
Numerous LSD1 inhibitors are known in the art. These LSD1 inhibitors can be antisense molecules, miRNA, shRNA, antibodies, or small molecules. In some embodiments, the LSD1 inhibitor is an antisense molecule, a miRNA, or a shRNA. In some embodiments, the LSD1 inhibitor is a small molecule.

As used herein, the term "small molecules" refers to small organic or inorganic molecules of molecular weight below about 3,000 Daltons. In general, small molecules useful for the purpose have a molecular weight of less than 3,000 Daltons (Da). The small molecules can be, e.g., from at least about 100 Da to about 3,000 Da (e.g., between about 100 to about 3,000 Da, about 100 to about 2500 Da, about 100 to about 2,000 Da, about 100 to about 1,750 Da, about 100 to about 1,500 Da, about 100 to about 1,250 Da, about 100 to about 1,000 Da, about 100 to about 750 Da, about 100 to about 500 Da, about 200 to about 1500, about 500 to about 1000, about 300 to about 1000 Da, or about 100 to about 250 Da).

In some embodiments, the LSD1 inhibitor can be a compound having the structure of Formula I, or Formula II, or a pharmaceutically acceptable salt thereof:



(Formula I)




(Formula II)

Wherein $R_1$ is selected from the group consisting of $C_1$-$C_6$ alkyl, —$NHSO_2Me$, —$NHSO_2Ph$, arylalkoxy, $C_3$-$C_7$ cycloalkyl, —$NHC(O)R_a$, 1-methyl-1H-pyrazol-4-yl, hydroxyl, $C_1$-$C_4$alkoxy, halogen, amino, substituted amino, and —$C(O)OR_a$;

$R_3$ is selected from the group consisting of aryl, heteroaryl —$SO_2R_a$, —$NHC(O)R_a$, —$CH_2C(O)OR_a$, —$C(O)OR_a$, —$C(O)R_a$, —$C(O)NR_aR_b$, amino, substituted amino, arylalkyl, and heteroarylalkyl;

each $R_a$ is independently hydrogen, phenyl, phenylmethyl, 3,5-dimethylisoxazol-4-yl, 1,2-dimethyl-1H-imidazol-4-yl, $C_3$-$C_7$cycloalkyl, or $C_1$-$C_6$alkyl;

$R_b$ is hydrogen or $C_1$-$C_3$alkyl; or

$R_a$ and $R_b$ together form a 5- or 6-membered heterocycloalkyl ring;

$R_4$ is H;

W is —$(CH_2)_{1\text{-}4}$ or —$CH(R_c)(CH_2)_{0\text{-}3}$, in which $R_c$ is —CN or $C_1$-$C_4$alkyl;

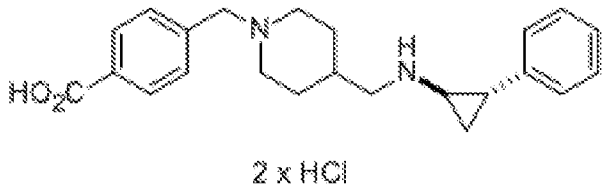
X is N;

Z is $(CH_2)_q$, wherein q is 0-2, and wherein when q is 0, Z represents a bond; and m is 0-3; or a pharmaceutically acceptable salt thereof. A detailed description regarding these LSD1 inhibitors can be found, e.g., in US Patent No. 9346840, which is incorporated herein by reference in its entirety.

In some embodiments, the LSD1 inhibitors is GSK2879552 (GlaxoSmithKline, Brentford, UK).

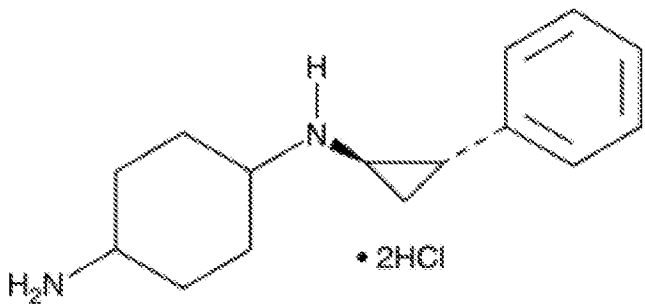
In some embodiments, the LSD1 inhibitor is CPI-890 (also known as CI-664890 or GSK9552; GlaxoSmithKline, Brentford, UK).

In some embodiments, the LSD1 inhibitor can be a compound having the structure of Formula III (GSK2879552) or a pharmaceutically acceptable salt thereof:



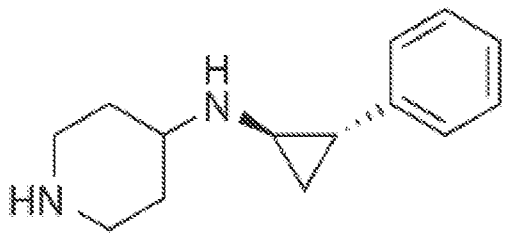

(Formula III)

In some embodiments, the LSD1 inhibitor is ORY-1001 (or RG6016; Oryzon/Roche, Basel, Switzerland).




(Formula IV)

In some embodiments, the LSD1 inhibitor can be a compound having the structure of Formula V (GSK-LSD1) or a pharmaceutically acceptable salt thereof.




(Formula V)

In some embodiments, the LSD1 inhibitor is IMG-7289 (Imago BioSciences, San Francisco, CA).

In some embodiments, the LSD1 inhibitor is CPI-242 (Constellation Pharmaceuticals, Cambridge, MA).

In some embodiments, the LSD1 inhibitor is CC-90011 (Celgene, Summit, NJ).

In some embodiments, the LSD1 inhibitor is INCB059872 (Incyte, Wilmington, DE).

In some embodiments, the LSD1 inhibitor is an LSD1 inhibitor know in the art. Numerous LSD1 inhibitors are known in the art, and are described, see e.g., in US 20150225401, US 20170129857, US20170281567, US20170281566, US20170183308, US20170283397, US20170209432, US20170044101, US 9493442, US 9346840, WO/2016/007736, WO/2016/161282, US 20160009711, and Fu et al., Advances toward LSD1 inhibitors for cancer therapy, *Future Medicinal Chemistry*, vol. 9, no. 11 (2017)|; each of which is incorporated herein by reference in its entirety.

**Inhibitory Nucleic Acids**

The present disclosure provides inhibitory nucleic acids for any genes, and/or the RNA product of these genes as described in the present disclosure. For example, the present disclosure provides inhibitory nucleic acids for E1A binding protein p400 (EP400; NM_015409.4 →NP_056224.3), MYC associated factor X (MAX; NM_001271068.1 → NP_001257997.1 ), MYCL proto-oncogene, bHLH transcription factor (MYCL; NM_001033081.2 → NP_001028253.1), Small T antigen, LSD1 (including LSD1+8a), REST corepressor 2 (RCOR2; NM_173587.3 → NP_775858.2), atonal bHLH transcription factor 1 (ATOH1; NM_005172.1 → NP_005163.1 ), hes family bHLH transcription factor 1 (HES1; NM_005524.3 → NP_005515.1 ), inhibitor of DNA binding 1, HLH protein (ID1; NM_002165.3 → NP_002156.2), INSM transcriptional repressor 1 (INSM1; NM_002196.2 → NP_002187.1), and/or NOTCH (e.g., NOTCH1, NM_017617.4 → NP_060087.3, or Notch intra-cellular domain (NICD)), etc. These inhibitory nucleic acids can be used in various methods as described herein, e.g., treating a subject having, or at risk of developing, a Merkel cell

polyomavirus (MCPyV)-positive cancer, or killing a Merkel cell polyomavirus (MCPyV)-positive cancer cell.

These inhibitory nucleic acids useful in the present methods and compositions include, e.g., antisense oligonucleotides, ribozymes, external guide sequence (EGS) oligonucleotides, siRNA compounds, single- or double-stranded RNA interference (RNAi) compounds such as siRNA compounds, modified bases/locked nucleic acids (LNAs), peptide nucleic acids (PNAs), and other oligomeric compounds or oligonucleotide mimetics which hybridize to at least a portion of the target nucleic acid and modulate its function. In some embodiments, the inhibitory nucleic acids include antisense molecules, antisense RNA, antisense DNA, chimeric antisense oligonucleotides, antisense oligonucleotides comprising modified linkages, interference RNA (RNAi), short interfering RNA (siRNA); a micro, interfering RNA (miRNA); a small, temporal RNA (stRNA); or a short, hairpin RNA (shRNA); small RNA-induced gene activation (RNAa); small activating RNAs (saRNAs), or combinations thereof.

In some embodiments, the inhibitory nucleic acids are 10 to 50, 10 to 20, 10 to 25, 13 to 50, or 13 to 30 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies inhibitory nucleic acids having complementary portions of 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 nucleotides in length, or any range therewithin. In some embodiments, the inhibitory nucleic acids are 15 nucleotides in length. In some embodiments, the inhibitory nucleic acids are 12 or 13 to 20, 25, or 30 nucleotides in length. One having ordinary skill in the art will appreciate that this embodies inhibitory nucleic acids having complementary portions of 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides in length, or any range therewithin (complementary portions refer to those portions of the inhibitory nucleic acids that are complementary to the target sequence).

The inhibitory nucleic acids useful in the present methods are sufficiently complementary to the target RNA, i.e., hybridize sufficiently well and with sufficient specificity, to give the desired effect. "Complementary" refers to the capacity for pairing, through hydrogen bonding, between two sequences comprising naturally or non-naturally occurring bases or analogs thereof. For example, if a base at one position of an inhibitory

nucleic acid is capable of hydrogen bonding with a base at the corresponding position of a RNA, then the bases are considered to be complementary to each other at that position. 100% complementarity is not required.

In some embodiments, the inhibitory nucleic acid molecules can be designed to target a specific region of the RNA sequence. For example, a specific functional region can be targeted, e.g., a region comprising a known RNA localization motif (i.e., a region complementary to the target nucleic acid on which the RNA acts). Alternatively, or in addition, highly conserved regions can be targeted, e.g., regions identified by aligning sequences from disparate species such as primate (e.g., human) and rodent (e.g., mouse) and looking for regions with high degrees of identity. Percent identity can be determined routinely using basic local alignment search tools (BLAST programs) (Altschul et al., J. Mol. Biol., 1990, 215, 403-410; Zhang and Madden, Genome Res., 1997, 7, 649-656), e.g., using the default parameters.

For further disclosure regarding inhibitory nucleic acids, please see US2010/0317718 (antisense oligos); US2010/0249052 (double-stranded ribonucleic acid (dsRNA)); US2009/0181914 and US2010/0234451 (LNAs); US2007/0191294 (siRNA analogues); US2008/0249039 (modified siRNA); and WO2010/129746 and WO2010/040112 (inhibitory nucleic acids); each of which is incorporated herein by reference in its entirety.

*Antisense*

In some embodiments, the inhibitory nucleic acids are antisense molecules or antisense oligonucleotides. Antisense oligonucleotides are typically designed to block expression of a DNA or RNA target by binding to the target and halting expression at the level of transcription, translation, or splicing. Antisense oligonucleotides of the present invention are complementary nucleic acid sequences designed to hybridize under stringent conditions to an RNA. Thus, oligonucleotides are chosen that are sufficiently complementary to the target, i.e., that hybridize sufficiently well and with sufficient specificity, to give the desired effect.

*siRNA/shRNA*

In some embodiments, the nucleic acid sequence that is complementary to an RNA as described herein can be an interfering RNA, including but not limited to a small interfering RNA ("siRNA") or a small hairpin RNA ("shRNA"). Methods for constructing interfering RNAs are well known in the art. For example, the interfering RNA can be assembled from two separate oligonucleotides, where one strand is the sense strand and the other is the antisense strand, wherein the antisense and sense strands are self-complementary (i.e., each strand comprises nucleotide sequence that is complementary to nucleotide sequence in the other strand; such as where the antisense strand and sense strand form a duplex or double stranded structure); the antisense strand comprises nucleotide sequence that is complementary to a nucleotide sequence in a target nucleic acid molecule or a portion thereof (i.e., an undesired gene) and the sense strand comprises nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. Alternatively, interfering RNA is assembled from a single oligonucleotide, where the self-complementary sense and antisense regions are linked by means of nucleic acid based or non-nucleic acid-based linker(s). The interfering RNA can be a polynucleotide with a duplex, asymmetric duplex, hairpin or asymmetric hairpin secondary structure, having self-complementary sense and antisense regions, wherein the antisense region comprises a nucleotide sequence that is complementary to nucleotide sequence in a separate target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof. The interfering can be a circular single-stranded polynucleotide having two or more loop structures and a stem comprising self-complementary sense and antisense regions, wherein the antisense region comprises nucleotide sequence that is complementary to nucleotide sequence in a target nucleic acid molecule or a portion thereof and the sense region having nucleotide sequence corresponding to the target nucleic acid sequence or a portion thereof, and wherein the circular polynucleotide can be processed either *in vivo* or *in vitro* to generate an active siRNA molecule capable of mediating RNA interference.

In some embodiments, the interfering RNA coding region encodes a self-complementary RNA molecule having a sense region, an antisense region and a loop

region. Such an RNA molecule when expressed desirably forms a "hairpin" structure, and is referred to herein as a "shRNA." The loop region is generally between about 2 and about 10 nucleotides in length. In some embodiments, the loop region is from about 6 to about 9 nucleotides in length. In some embodiments, the sense region and the antisense region are between about 15 and about 20 nucleotides in length. Following post-transcriptional processing, the small hairpin RNA is converted into a siRNA by a cleavage event mediated by the enzyme Dicer, which is a member of the RNase III family. The siRNA is then capable of inhibiting the expression of a gene with which it shares homology. For details, see Brummelkamp et al., Science 296:550-553, (2002); Lee et al, Nature Biotechnol., 20, 500-505, (2002); Miyagishi and Taira, Nature Biotechnol 20:497-500, (2002); Paddison et al. Genes & Dev. 16:948-958, (2002); Paul, Nature Biotechnol, 20, 505-508, (2002); Sui, Proc. Natl. Acad. Sd. USA, 99(6), 5515-5520, (2002); Yu et al. Proc Natl Acad Sci USA 99:6047-6052, (2002).

The target RNA cleavage reaction guided by siRNAs is highly sequence specific. In general, siRNA containing a nucleotide sequences identical to a portion of the target nucleic acid are preferred for inhibition. However, 100% sequence identity between the siRNA and the target gene is not required. Thus, the methods as described herein have the advantage of being able to tolerate sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence. For example, siRNA sequences with insertions, deletions, and single point mutations relative to the target sequence have also been found to be effective for inhibition. Alternatively, siRNA sequences with nucleotide analog substitutions or insertions can be effective for inhibition. In general, the siRNAs must retain specificity for their target, i.e., must not directly bind to, or directly significantly affect expression levels of, transcripts other than the intended target.

*Making and Using Inhibitory Nucleic Acids*

The nucleic acid sequences used to practice the methods described herein, whether RNA, cDNA, genomic DNA, vectors, viruses or hybrids thereof, can be isolated from a variety of sources, genetically engineered, amplified, and/or expressed/ generated recombinantly. Recombinant nucleic acid sequences can be individually isolated or

cloned and tested for a desired activity. Any recombinant expression system can be used, including e.g. *in vitro*, bacterial, fungal, mammalian, yeast, insect or plant cell expression systems.

Nucleic acid sequences can be inserted into delivery vectors and expressed from transcription units within the vectors. The recombinant vectors can be DNA plasmids or viral vectors. Generation of the vector construct can be accomplished using any suitable genetic engineering techniques well known in the art, including, without limitation, the standard techniques of PCR, oligonucleotide synthesis, restriction endonuclease digestion, ligation, transformation, plasmid purification, and DNA sequencing, for example as described in Sambrook et al. Molecular Cloning: A Laboratory Manual. (1989)), Coffin et al. (Retroviruses. (1997)) and "RNA Viruses: A Practical Approach" (Alan J. Cann, Ed., Oxford University Press, (2000)). As will be apparent to one of ordinary skill in the art, a variety of suitable vectors are available for transferring nucleic acids of the invention into cells. The selection of an appropriate vector to deliver nucleic acids and optimization of the conditions for insertion of the selected expression vector into the cell, are within the scope of one of ordinary skill in the art without the need for undue experimentation. Viral vectors comprise a nucleotide sequence having sequences for the production of recombinant virus in a packaging cell. Viral vectors expressing nucleic acids can be constructed based on viral backbones including, but not limited to, a retrovirus, lentivirus, adenovirus, adeno-associated virus, pox virus or alphavirus. The recombinant vectors capable of expressing the nucleic acids of the invention can be delivered as described herein, and persist in target cells (e.g., stable transformants).

Nucleic acid sequences used to practice this invention can be synthesized *in vitro* by well-known chemical synthesis techniques, as described in, e.g., Adams (1983) J. Am. Chem. Soc. 105:661; Belousov (1997) Nucleic Acids Res. 25:3440-3444; Frenkel (1995) Free Radic. Biol. Med. 19:373-380; Blommers (1994) Biochemistry 33:7886-7896; Narang (1979) Meth. Enzymol. 68:90; Brown (1979) Meth. Enzymol. 68:109; Beaucage (1981) Tetra. Lett. 22:1859; U.S. Patent No. 4,458,066.

Nucleic acid sequences of the invention can be stabilized against nucleolytic degradation such as by the incorporation of a modification, e.g., a nucleotide modification. For example, nucleic acid sequences of the invention include a

phosphorothioate at least the first, second, or third internucleotide linkage at the 5' or 3' end of the nucleotide sequence. As another example, the nucleic acid sequence can include a 2'-modified nucleotide, e.g., a 2'-deoxy, 2'-deoxy-2'-fluoro, 2'-O-methyl, 2'-O-methoxyethyl (2'-O-MOE), 2'-O-aminopropyl (2'-O-AP), 2'-O-dimethylaminoethyl (2'-O-DMAOE), 2'-O-dimethylaminopropyl (2'-O-DMAP), 2'-O-dimethylaminoethyloxyethyl (2'-O-DMAEOE), or 2'-O--N-methylacetamido (2'-O--NMA). As another example, the nucleic acid sequence can include at least one 2'-O-methyl-modified nucleotide, and in some embodiments, all of the nucleotides include a 2'-O-methyl modification. In some embodiments, the nucleic acids are "locked," i.e., comprise nucleic acid analogues in which the ribose ring is "locked" by a methylene bridge connecting the 2'-O atom and the 4'-C atom (see, e.g., Kaupinnen et al., Drug Disc. Today 2(3):287-290 (2005); Koshkin et al., J. Am. Chem. Soc., 120(50):13252–13253 (1998)). For additional modifications see US 20100004320, US 20090298916, and US 20090143326.

Techniques for the manipulation of nucleic acids used to practice this invention, such as, e.g., subcloning, labeling probes (e.g., random-primer labeling using Klenow polymerase, nick translation, amplification), sequencing, hybridization and the like are well described in the scientific and patent literature, see, e.g., Sambrook et al., Molecular Cloning; A Laboratory Manual 3d ed. (2001); Current Protocols in Molecular Biology, Ausubel et al., eds. (John Wiley & Sons, Inc., New York 2010); Kriegler, Gene Transfer and Expression: A Laboratory Manual (1990); Laboratory Techniques In Biochemistry And Molecular Biology: Hybridization With Nucleic Acid Probes, Part I. Theory and Nucleic Acid Preparation, Tijssen, ed. Elsevier, N.Y. (1993).

**Biomarkers**

The present disclosure provides methods of determining whether a cancer, a tumor, a cancer cell or a tumor cell is MCPyV-positive. There are several methods to distinguish virus-positive from virus-negative cells from each other. For example, Merkel cell polyomavirus infection can be determined by assaying a sample from a subject for the presence of one or more Merkel cell polyomavirus molecules. These molecules can be, e.g., Merkel cell polyomavirus DNA, Merkel cell polyomavirus RNA, and Merkel

cell polyomavirus polypeptides. In some embodiments, the molecule is DNA, RNA, or polypeptides of Merkel cell polyomavirus small T antigen and large T antigen.

In one aspect, the methods include the steps of obtaining a tumor tissue sample from a subject; contacting the tumor tissue with an antibody that specifically binds MCPyV T antigen; and determining that the tumor tissue expresses MCPyV T antigen. In some embodiments, the method involves determining that the tumor tissue expresses MCPyV T antigen more than a control tumor tissue from a MCPyV-negative MCC subject. As used herein, the term "MCPyV T antigen" includes Merkel cell polyomavirus small T antigen (ST) and large T antigen (LT). In some embodiments, the MCPyV T antigen is small T antigen.

In some embodiments, the methods involve contacting the tumor tissue sample with an antibody that binds to one or more antigens selected from the group consisting of RCOR2, LSD1, INSM1 and ATOH1; and determining that the tumor tissue expresses the one or more antigens. In some embodiments, the method involves determining that the tumor tissue expresses one or more antigens selected from the group consisting of RCOR2, LSD1, INSM1 and ATOH1 more than a control tumor tissue from a MCPyV-negative MCC subject.

In another aspect, the methods involve obtaining a tumor tissue sample from a subject; measuring the levels of LSD1+8a mRNA in the tumor tissue sample; and determining that the levels of LSD1+8a mRNA are higher than a control tissue sample from a subject that has a MCPyV-negative tumor.

In one aspect, the methods involve obtaining a tumor tissue sample from a subject; contacting the tumor tissue with an antibody that specifically binds LSD1+8a protein; and determining that the tumor tissue expresses LSD1+8a. In some embodiments, the method involves determining that the tumor tissue expresses LSD1+8a more than a control tumor tissue from a MCPyV-negative MCC subject.

In one aspect, the methods involve obtaining a tumor tissue sample from a subject; measuring the levels of one or more mRNA or proteins selected from the group consisting of LSD1, RCOR2, INSM1 and ATOH1 in the tumor tissue sample; and determining that the levels of the one or more mRNA or proteins are higher than a control tissue sample from a subject that has a MCPyV-negative tumor.

These biomarkers will also be a useful way to identify virus-positive tumors that will likely respond to LSD1 inhibitors. Thus, in one aspect, the present disclosure also provides methods of determining whether a cancer, a tumor, a cancer cell or a tumor cell is sensitive to LSD1 inhibitors. These biomarkers can be used to predict the sensitivity of tumors to LSD1 inhibition. These biomarkers can be obtained from, e.g., sequencing information, immunohistochemistry (IHC) staining for MCPyV T antigen and INSM1, a component of the CoREST complex, and a virus hybrid capture sequencing platform that can specifically identify MCPyV DNA from tumors.

Thus, in one aspect, the present disclosure provides methods of selecting a subject having a cancer for treatment with an LSD1 inhibitor or determining whether a subject having a cancer is likely to respond to an LSD1 inhibitor. The methods involve determining that:

(a) tumor tissue from the human subject comprises DNA from MCPyV;

(b) tumor tissue from the human subject binds an antibody that specifically binds MCPyV T antigen;

(c) tumor tissue from the human subject binds an antibody that specifically binds RCOR2, LSD1, INSM1, and ATOH1;

(d) tumor tissue from the human subject has increased levels of LSD1+8a mRNA than a control tissue sample from a subject that has a MCPyV-negative tumor;

(e) tumor tissue from the human subject binds an antibody that specifically binds LSD1+8a protein; or

(f) tumor tissue from the human subject has increased levels of mRNAs or proteins selected from the group consisting of LSD1, RCOR2, INSM1, and ATOH1 than a control tissue sample from a subject that has a MCPyV-negative tumor.

In addition to determining if a tumor is MCPyV-positive, and likely sensitive to LSD1 inhibition, there are several genes whose expression increases significantly after treatment with LSD1 inhibitors. These genes as provided in the present disclosure (e.g., ID1 and HES1) can represent a useful biomarker for determining the response of the cells to LSD1 inhibitors.

The present disclosure also provides several biomarkers that can be used to identify virus-positive tumors that will likely respond to LSD1 inhibitors. As shown in

FIGS. 26, 27A, 27B, 28, and 29, in tumors that can be effectively treated by LSD1 inhibition, the mRNA or protein levels of certain genes (e.g., RWDD2A, FAM13C, DPF1, SAT2, CREB5, SMARCA1, LYSMD2, ESRP2, MANSC1, LRRC49, ATP8B4, DCHS1, CACNG5, PGBD1, ZNF781, SMAD9, QPRT, FAM5B, ARPP21, RGNEF, ID1, CDH11, CALB2, PROM1, GFI1, MGP, LSAMP, DLL1, AMPH, BASP1, ID2, and HES1) will increase, the phosphorylation of certain proteins (e.g., SMAD1, SMAD5, and/or SMAD9) will increase, and/or mRNA or protein levels of certain genes (e.g., CISD3, SLC2A4RG, POLR3K, ZBTB42, C7orf50, DYSF, HMGN2, DCK, CLN6, ATF5, F159B, TRIB3, AKNA, CEBPB, and ESRP2) will decrease after LSD1 inhibition. In tumors that cannot be effectively treated by LSD1 inhibition, LSD1 inhibition will not induce these changes of mRNA or protein levels in these genes or induce the changes in a different direction.

Thus, the present disclosure further provides method of determining that LSD1 inhibition is effective in a human subject undergoing LSD1 inhibitor therapy or whether a particular LSD1 inhibitor is effective for treating a subject have tumors. In some embodiments, a tumor sample from the subject before and after treatment with an LSD1 inhibitor is obtained. In some embodiments, the methods involve:

(1) determining that the mRNA or protein levels of one or more genes selected from the group consisting of RWDD2A, FAM13C, DPF1, SAT2, CREB5, SMARCA1, LYSMD2, ESRP2, MANSC1, LRRC49, ATP8B4, GFI1, DCHS1, CACNG5, PGBD1, ZNF781, SMAD9, QPRT, FAM5B, ARPP21, and RGNEF are elevated in the sample obtained after treatment relative to a control sample (e.g., a sample before treatment);

(2) determining that the mRNA or protein levels of one or more genes selected from the group consisting of CISD3, SLC2A4RG, POLR3K, ZBTB42, C7orf50, DYSF, HMGN2, DCK, and CLN6 are decreased in the sample obtained after treatment relative to a control sample (e.g., a sample before treatment);

(3) determining that the mRNA or protein levels of one or more genes selected from the group consisting of FAM5B, ID1, CDH11, CALB2, and PROM1 are elevated in the sample obtained after treatment relative to a control sample (e.g., a sample before treatment);

(4) determining that the mRNA or protein levels of ID1 and/or SMARCA1 are elevated in the sample obtained after treatment relative to a control sample (e.g., a sample before treatment);

(5) determining increased phosphorylation of SMAD1, SMAD5, and/or SMAD9 in the sample obtained after treatment relative to a control sample (e.g., a sample before treatment);

(6) determining that the mRNA or protein levels of one or more genes selected from the group consisting of ATF5, F159B, TRIB3, AKNA, CEBPB, and ESRP2 are decreased in the sample obtained after treatment relative to a control sample (e.g., a sample before treatment);

(7) determining that the mRNA or protein levels of one or more genes selected from the group consisting of GFI1, MGP, LSAMP, DLL1, AMPH, PROM1 (CD133), CALB2, BASP1, and BRNP2(FAM5B) are elevated in the sample obtained after treatment relative to a control sample (e.g., a sample before treatment); or

(8) determining that the mRNA or protein levels of one or more genes selected from the group consisting of ID1, ID2, ZNF781, HES1, and DLL1 are elevated in the sample obtained after treatment relative to a control sample (e.g., a sample before treatment).

**Combination therapy**

The methods as described herein can also be used in combination with some other therapies, e.g., surgeries, radiotherapies, chemotherapies, and/or immunotherapies. The LSD1 inhibitor and the other therapy may be administered before, after, or at substantially the same time as one another.

In some embodiments, the immunotherapy involves administering an effective amount of an immune checkpoint inhibitor (e.g., anti-PD-1 antibody, anti-PD-L1 antibody, or anti-CTLA-4 antibody) to the subject. The current FDA approved drug for MCC is Avelumab, a PD-L1 immune checkpoint inhibitor that had a 30% response rate in patients with relapsed MCC. Pembrolizumab (anti-PD-1 antibody), Nivolumab (anti-PD-L1 antibody), Ipilimumab (anti-CTLA-4 antibody) also have activity in MCC and may receive approval in the near future. Thus, the compositions as described herein (e.g.,

LSD1 inhibitors) can be used in combination with a checkpoint inhibitor, e.g., an anti-PD-1 antibody (e.g., Pembrolizumab), and anti-PD-L1 antibody (e.g., Avelumab, or Nivolumab), or an anti-CTLA-4 antibody (e.g., Ipilimumab) to treat Merkel cell polyomavirus (MCPyV)-positive cancers (e.g., Merkel cell carcinoma).

Furthermore, the present disclosure shows that the viability of cells was decreased when the MYCL-MAX heterodimer was disrupted by a Myc inhibitor (e.g., Omomyc). Thus, in some embodiments, the methods as described herein can also be used in combination with a Myc inhibitor, e.g., Omomyc. Omomyc is a modified fragment of MYC that can bind to MAX and disrupt endogenous MYC-MAX heterodimers. Omomyc is described, e.g., in US 20160122415, which is incorporated herein by reference in its entirety.

The methods can also include the step of administering to the subject one or more chemotherapeutic agents, one or more forms of ionizing radiation, or one or more immunomodulatory agents. The one or more forms of ionizing radiation can be gamma-irradiation, X-irradiation, or beta-irradiation. The one or more chemotherapeutic agents can be selected from the group consisting of cisplatin, carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, adriamycin, ifosfamide, melphalan, chlorambucil, bisulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide, verampil, podophyllotoxin, tamoxifen, taxol, thalidomide, lenalidomide, a proteosome inhibitor (e.g., bortezomib), an hsp90 inhibitor (e.g., tenespinmycin), transplatinum, 5- flurouracil, vincristin, vinblastin, methotrexate, or an analog of any of the aforementioned. Immunomodulatory agents include, e.g., a variety of chemokines and cytokines such as Interleukin 2 (IL-2), granulocyte/macrophage-colony stimulating factor (GM-CSF), and Interleukin 12 (IL-12). In some embodiments, the chemotherapeutic agent is cisplatin or etoposide.

In some embodiments, the methods described herein can be used alone or in combination with some other methods known in the art, including, e.g., treating the subject with chemotherapy. Alternatively or in addition, the methods can include performing surgery on the subject to remove at least a portion of the cancer, e.g., to remove a portion of or all of a tumor(s), from the patient.

**Methods of Screening**

Included herein are methods for screening test compounds, e.g., polypeptides, polynucleotides, inorganic or organic large or small molecule test compounds, to identify agents useful in the treatment of Merkel cell polyomavirus (MCPyV)-positive cancer, e.g., Merkel cell carcinoma. The methods involve contacting a MCPyV-positive tumor cell (or cancer cell) with an agent; determining that (1) the agent inhibits the levels of mRNAs or proteins selected from the group consisting of LSD1, RCOR2, INSM1, and ATOH1 in the tumor cell, or (2) the agent increases the levels of mRNAs or proteins selected from the group consisting of NICD, ID1 and HES1; and selecting the agent as being useful for treating a MCPyV-positive tumor.

In some embodiments, the methods involve contacting a MCPyV-positive tumor with an agent; determining that the agent inhibits the growth of the MCPyV-positive tumor or kills the MCPyV-positive tumor cell; and selecting the agent as being useful for treating a MCPyV-positive tumor.

In some embodiments, the agent is a small molecule. In some embodiments, the agent is an antisense molecule, a miRNA, a shRNA, or an antibody.

The test agents can be, e.g., natural products or members of a combinatorial chemistry library. A set of diverse molecules should be used to cover a variety of functions such as charge, aromaticity, hydrogen bonding, flexibility, size, length of side chain, hydrophobicity, and rigidity. Combinatorial techniques suitable for synthesizing small molecules are known in the art, e.g., as exemplified by Obrecht and Villalgordo, Solid-Supported Combinatorial and Parallel Synthesis of Small-Molecular-Weight Compound Libraries, Pergamon-Elsevier Science Limited (1998), and include those such as the "split and pool" or "parallel" synthesis techniques, solid-phase and solution-phase techniques, and encoding techniques (see, for example, Czarnik, Curr. Opin. Chem. Bio. 1:60-6 (1997)). In addition, a number of small molecule libraries are commercially available. A number of suitable small molecule test compounds are listed in U.S. Patent No. 6,503,713, incorporated herein by reference in its entirety.

Libraries screened using the methods of the present invention can comprise a variety of types of test compounds. A given library can comprise a set of structurally related or unrelated test compounds. In some embodiments, the test compounds are

peptide or peptidomimetic molecules. In some embodiments, the test compounds are nucleic acids.

In some embodiments, the test compounds and libraries thereof can be obtained by systematically altering the structure of a first test compound, e.g., a first test compound that is structurally similar to a known natural binding partner of the target polypeptide, or a first small molecule identified as capable of binding the target polypeptide, e.g., using methods known in the art or the methods described herein, and correlating that structure to a resulting biological activity, e.g., a structure-activity relationship study. As one of skill in the art will appreciate, there are a variety of standard methods for creating such a structure-activity relationship. Thus, in some instances, the work may be largely empirical, and in others, the three-dimensional structure of an endogenous polypeptide or portion thereof can be used as a starting point for the rational design of a small molecule compound or compounds. For example, in some embodiments, a general library of small molecules is screened, e.g., using the methods described herein.

In some embodiments, a test compound is applied to a test sample, e.g., a cell or living tissue or organ, and one or more effects of the test compound is evaluated. In a cultured or primary cell for example, the ability of the test compound to inhibit the growth of the tumor, kill the tumor cell, modulate gene expression (e.g., LSD1, RCOR2, INSM1, ATOH1, LSD1+8a, ID1 and/or HES1) is evaluated.

Methods for evaluating each of these effects are known in the art. For example, ability to modulate expression of a protein can be evaluated at the gene or protein level, e.g., using quantitative PCR or immunoassay methods. In some embodiments, high throughput methods, e.g., protein or gene chips as are known in the art (see, e.g., Ch. 12, Genomics, in Griffiths et al., Eds. Modern genetic Analysis, 1999,W. H. Freeman and Company; Ekins and Chu, Trends in Biotechnology, 1999, 17:217-218; MacBeath and Schreiber, Science 2000, 289(5485):1760-1763; Simpson, Proteins and Proteomics: A Laboratory Manual, Cold Spring Harbor Laboratory Press; 2002; Hardiman, Microarrays Methods and Applications: Nuts & Bolts, DNA Press, 2003), can be used to detect the effect of the test agents.

In one aspect, the disclosure also provides methods for screening an agent that is useful for treating a tumor (e.g., MCPyV-positive tumor) or killing a tumor cell. In some embodiments, the methods involve contacting the agent with a tumor cell (e.g., MCPyV-positive tumor cell) and determining that the cell after treatment show one or more of the following characteristics:

(1) the mRNA or protein levels of one or more genes selected from the group consisting of RWDD2A, FAM13C, DPF1, SAT2, CREB5, SMARCA1, LYSMD2, ESRP2, MANSC1, LRRC49, ATP8B4, GFI1, DCHS1, CACNG5, PGBD1, ZNF781, SMAD9, QPRT, FAM5B, ARPP21, RGNEF are elevated in the sample obtained after treatment relative to a control sample (e.g., the sample before the treatment or a sample without being treated with the agent);

(2) the mRNA or protein levels of one or more genes selected from the group consisting of CISD3, SLC2A4RG, POLR3K, ZBTB42, C7orf50, DYSF, HMGN2, DCK, and CLN6 are decreased in the sample obtained after treatment relative to a control sample (e.g., the sample before the treatment or a sample without being treated with the agent);

(3) the mRNA or protein levels of one or more genes selected from the group consisting of FAM5B, ID1, CDH11, CALB2, and PROM1 are elevated in the sample obtained after treatment relative to a control sample (e.g., the sample before the treatment or a sample without being treated with the agent);

(4) the mRNA or protein levels of ID1 and/or SMARCA1 are elevated in the sample obtained after treatment relative to a control sample (e.g., the sample before the treatment or a sample without being treated with the agent);

(5) determining increased phosphorylation of SMAD1, SMAD5, and/or SMAD9 in the sample obtained after treatment relative to a control sample (e.g., the sample before the treatment or a sample without being treated with the agent);

(6) the mRNA or protein levels of one or more genes selected from the group consisting of ATF5, F159B, TRIB3, AKNA, CEBPB, and ESRP2 are decreased in the sample obtained after treatment relative to a control sample (e.g., the sample before the treatment or a sample without being treated with the agent);

(7) the mRNA or protein levels of one or more genes selected from the group consisting of GFI1, MGP, LSAMP, DLL1, AMPH, PROM1 (CD133), CALB2, BASP1, and BRNP2(FAM5B) are elevated in the sample obtained after treatment relative to a control sample (e.g., the sample before the treatment or a sample without being treated with the agent); or

(8) the mRNA or protein levels of one or more genes selected from the group consisting of ID1, ID2, ZNF781, HES1, and DLL1 are elevated in the sample obtained after treatment relative to a control sample (e.g., the sample before the treatment or a sample without being treated with the agent).

If the cells show one or more of the following characteristics, the agent can be determined to be useful for treating a tumor (e.g., a MCPyV-positive tumor) or killing a tumor cell; otherwise, the agent is unlikely to be useful for treating a tumor (e.g., a MCPyV-positive tumor) or killing a tumor cell.

**Dosage**

An "effective amount" is an amount sufficient to effect beneficial or desired results. For example, a therapeutic amount is one that achieves the desired therapeutic effect. This amount can be the same or different from a prophylactically effective amount, which is an amount necessary to prevent onset of disease or disease symptoms. An effective amount can be administered in one or more administrations, applications or dosages. A therapeutically effective amount of a therapeutic agent (i.e., an effective dosage) depends on the therapeutic agent selected. The compositions can be administered one from one or more times per day to one or more times per week; including once every other day. The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of the therapeutic compounds described herein can include a single treatment or a series of treatments.

Dosage, toxicity and therapeutic efficacy of the therapeutic compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental

animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit high therapeutic indices are preferred. While compounds that exhibit toxic side effects may be used, care should be taken to design a delivery system that targets such compounds to the site of affected tissue in order to minimize potential damage to uninfected cells and, thereby, reduce side effects.

**Pharmaceutical Compositions and Methods of Administration**

The methods described herein include the use of pharmaceutical compositions comprising LSD1 (e.g., LSD1+8a), EP400, MAX, MYCL, Small T antigen, RCOR2, ATOH1, HES1, ID1, NICD and/or INSM1 inhibitors as active ingredients.

Pharmaceutical compositions typically include a pharmaceutically acceptable carrier. As used herein the language "pharmaceutically acceptable carrier" includes saline, solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration.

Pharmaceutical compositions are typically formulated to be compatible with its intended route of administration. Examples of routes of administration include parenteral, e.g., intravenous, intradermal, subcutaneous, oral (e.g., inhalation), transdermal (topical), transmucosal, and rectal administration.

Methods of formulating suitable pharmaceutical compositions are known in the art, see, e.g., Remington: The Science and Practice of Pharmacy, 21st ed., 2005; and the books in the series Drugs and the Pharmaceutical Sciences: a Series of Textbooks and Monographs (Dekker, NY). For example, solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or

dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

Pharmaceutical compositions suitable for injectable use can include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringability exists. It should be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle, which contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying, which yield a

powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Oral compositions generally include an inert diluent or an edible carrier. For the purpose of oral therapeutic administration, the active compound can be incorporated with excipients and used in the form of tablets, troches, or capsules, e.g., gelatin capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash. Pharmaceutically compatible binding agents, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating agent such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening agent such as sucrose or saccharin; or a flavoring agent such as peppermint, methyl salicylate, or orange flavoring.

Systemic administration of a therapeutic compound as described herein can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the active compounds are formulated into ointments, salves, gels, or creams as generally known in the art.

Therapeutic compounds that are or include nucleic acids can be administered by any method suitable for administration of nucleic acid agents, such as a DNA vaccine. These methods include gene guns, bio injectors, and skin patches as well as needle-free methods such as the micro-particle DNA vaccine technology disclosed in U.S. Patent No. 6,194,389, and the mammalian transdermal needle-free vaccination with powder-form vaccine as disclosed in U.S. Patent No. 6,168,587. Additionally, intranasal delivery is possible, as described in, *inter alia,* Hamajima et al., Clin. Immunol. Immunopathol., 88(2), 205-10 (1998). Liposomes (e.g., as described in U.S. Patent No. 6,472,375) and

microencapsulation can also be used. Biodegradable targetable microparticle delivery systems can also be used (e.g., as described in U.S. Patent No. 6,471,996).

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration.

## EXAMPLES

The invention is further described in the following examples, which do not limit the scope of the invention described in the claims.

### EXAMPEL 1. Materials and methods

The following materials and methods were used in the following examples.

*Cell lines*

MCC cell lines MKL-1, MKL-2 and MS-1 were obtained from University of Pittsburgh, PA; MCC cell lines WaGa and UISO were obtained from Medical University Graz, Austria; MCC cell lines PeTa and BroLi were obtained from University of Wuerzburg, Germany. Kelly neuroblastoma cell line was obtained from Dana-Farber Cancer Institute, MA. 293T, HCT116 and IMR90 cells were obtained from ATCC. HFK-hTERT cells were obtained from Tufts University, MA.

*DNA*

MCPyV early region was PCR amplified from DNA extracted from a Merkel cell carcinoma sample. The cDNA for ST was modified to eliminate the LT splice donor by introducing silent mutations (GAG|GTCAGT to GAa|GTCtcc). Additional ST mutants were generated using QuikChange Lightning Site-Directed Mutagenesis Kit (Agilent, Santa Clara, CA).

The EP400, MYCL shRNA target sequence was designed using Block-iT RNAi Designer (Life Technologies, Carlsbad, CA) and annealed forward and reverse oligos of hairpin sequence were cloned between AgeI/EcoRI sites of the doxycycline inducible shRNA vector Tet-pLKO-puro (Addgene #21915). The MYCL miRNA target sequence

was designed using Block-iT RNAi Designer and cloned into pcDNA 6.2-GW/EmGFP-miR vector (Life Technologies, Carlsbad, CA) and the pre-miRNA expression cassette targeting MYCL was transferred to pLIX_402 Dox-inducible expression vector via consecutive BP and LR recombination reactions to generate pLIX-mirMYCL plasmid. shRNAs constitutively expressed from lentiviral PLKO vector targeting MCPyV LT/ST (shPanT), ST (shST) or scramble (shScr) are described, e.g., in Shuda M, Kwun HJ, Feng H, Chang Y, Moore PS. Human Merkel cell polyomavirus small T antigen is an oncoprotein targeting the 4E-BP1 translation regulator. J Clin Invest. 2011;121(9):3623-34. Epub 2011/08/16. doi: 46323; Houben R, Shuda M, Weinkam R, Schrama D, Feng H, Chang Y, et al. Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. J Virol. 2010;84(14):7064-72. Epub 2010/05/07. doi: JVI.02400-09; Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science. 2005;307(5712):1098-101. doi: 10.1126/science.1106148.

pMXs-Hu-L-Myc (Addgene # 26022) was used. MYCL was PCR amplified with C-terminal 3xHA tag or with original stop codon and cloned into pLenti-CMV gateway vector. Omomyc was obtained from Sapienza University of Rome, Italy, modified by PCR amplification to include C-terminal HA tag and cloned into pLIX_402. The OCT4-2A-SOX2-2A-KLF4 polycistronic coding sequence was PCR amplified from pKP332 Lenti-OSK1 (Addgene #21627) and cloned into pLIX_402.

Expression vectors include pLenti-CMV (Addgene #17451) [70], doxycycline inducible lentiviral gateway expression vector pLIX_402 (Addgene #41394). Lentiviral packaging plasmid psPAX2 and envelope plasmid pMD2.G were also used in the experiments (Addgene #12260, #12259). Retroviral packaging plasmid pUMVC3 (Addgene # 8449) and envelope plasmid pHCMV-AmphoEnv (Addgene # 15799) were used in the experiments as well. Retroviral plasmids pBabe-neo-p53DD and pBabe-hygro-hTERT were previously described, e.g., in Hahn WC, Dessain SK, Brooks MW, King JE, Elenbaas B, Sabatini DM, et al. Enumeration of the simian virus 40 early region elements necessary for human cell transformation. Mol Cell Biol. 2002;22(7):2111-23.

Packaging and envelope plasmids were co-transfected with lentiviral or retroviral expression vectors into 293T cells using Lipofectamine 2000 (Life Technologies,

Carlsbad, CA). Two days after transfection, 293T cell supernatant was purified with 0.45 μm filter and supplemented with 4 μg/ml polybrene before transducing recipient cells. Stable cell lines were generated after selection with 1-2 μg/ml puromycin, 5-10 μg/ml blasticidin, 500 μg/mL neomycin, and 100 μg/mL hygromycin as required by each vector.

*Cell viability assay*

CellTiter-Glo Luminescent Cell Viability Assay was performed according to the protocol from Promega (Madison, WI). Basically, 3000 MKL-1 parental or dox-inducible cells were plated in 96 well plate. Fresh medium was supplemented every two days with or without doxycycline. The number of days that cells had been treated with doxycycline was labelled on X-axis. At the end of time course, CellTiter-Glo reagents were added to lyse cells. For each cell line, doxycycline treated samples were normalized to untreated samples.

*Anchorage independent growth assay*

Anchorage independent growth was performed using 6-well dishes with SeaPlaque Agarose (Lonza, Basel, Switzerland) at concentrations of 0.3% top and 0.6% bottom layers. Agarose was diluted with 2X MEM (Gibco, Thermo Fisher Scientific) supplemented with 2X Gluta-max (Gibco, Thermo Fisher Scientific), 2X pen-strep (Gibco, Thermo Fisher Scientific), and 30% FBS. IMR90 cells ($10^5$) were seeded in triplicate in the top agarose layer. Wells were fed with top agarose twice per week. After 4 weeks, cells were stained with 0.005% crystal violet (Sigma, St. Louis, MO) in PBS and colonies were counted. Statistical significance was determined by ordinary one-way ANOVA for multiple comparisons with $p < 0.05$.

*iPS cell generation*

HFK-hTERT cells were transduced with pLIX-OSK and selected with puromycin to establish the parental cell line (P) followed by transduction with MYCL or ST in pLenti-CMV vector and selection with blasticidin. 200,000 cells were seeded in Matrigel (BD Biosciences, Woburn, MA) coated 6-well plate in triplicate on day 0 in

Keratinocyte-SFM medium (Gibco, Thermo Fisher Scientific) supplemented with 0.5 μg/ml doxycycline. On day 3, medium was changed to mTeSR1 (Stemcell Technologies, Vancouver, Canada) supplemented with doxycycline. iPS colonies were visible under microscope after 3 weeks and stained with StainAlive TRA-1-60 or TRA-1-81 antibodies (Stemgent, Lexington, MA) and Alkaline Phosphatase Detection Kit (Millipore, Billerica, MA).

*Immunoprecipitation and Immunoblotting*

The following antibodies were used: Ab5 and Ab3 [23, 73]; HA (Abcam, Cambridge, MA); EP400, RUVBL2 (Bethyl, Montgomery, TX); MAX, KAT5, DMAP1, MNT (Santa Cruz); MYCL (R&D Systems, Minneapolis, MN); ING3 (Sigma, St. Louis, MO); PPP2CA (BD Biosciences, Woburn, MA); and H3K4me3 (Millipore, Billerica, MA; 07-473).

Cell lysates were prepared in EBC Lysis buffer (50 mM Tris pH 8.0, 150 mM NaCl, 0.5% NP-40, 0.5 mM EDTA, 1 mM β-Mercaptoethanol and freshly added protease inhibitor and phosphatase inhibitor cocktail). Immunoprecipitations were performed with protein G Dynabeads (Life Technologies, Carlsbad, CA) mixed with immunoprecipitation antibodies or anti-HA magnetic beads (Pierce Biotechnology, Waltham, MA). After overnight incubation on a rotating apparatus at 4°C, magnetic beads were washed with high salt wash buffer (50 mM Tris pH 7.4, 300 mM NaCl, 0.5% NP-40, 0.5 mM EDTA) five times. Bound proteins were eluted from magnetic beads with 2x Laemmli sample buffer (Bio-Rad, Hercules, CA). After electrophoresis, the separated proteins were transferred to PVDF membrane and blotted. Immunoblots were developed using Clarity Western ECL substrate (Bio-Rad, Hercules, CA) and imaged with G:BOX Chemi system (Syngene).

*MudPIT*

MudPIT was performed with MKL-1 or WaGa suspension cells (30 x 15-cm diameter plates) harvested in 30 ml EBC lysis buffer. Clarified cell extract (100-300 mg) was incubated overnight at 4°C with 30 μg antibodies crosslinked to 30 mg protein G agarose beads by dimethyl pimelimidate (DMP). Beads were washed with high salt wash

buffer five times, then eluted with 0.2 M glycine pH 3 and neutralized with 1 M Tris pH 8.0. Proteins were precipitated with 1/5 TCA overnight at 4°C and washed with cold acetone twice and analyzed by MudPIT. The triple-phase fused-silica microcapillary column was packed with 8-9 cm of 5-μm C18 Reverse Phase (Aqua, Phenomenex, Torrance, CA), followed by 3 to 4 cm of 5-μm Strong Cation Exchange material (Partisphere SCX, Whatman, Maidstone, UK) and 2 to 3 cm of C18 RP and equilibrated with Buffer A (5% ACN, 0.1% Formic Acid). A10-step chromatography run was performed with the last two chromatography steps consisting of a high salt wash with 100% Buffer C (500mM Ammonium Acetate, 5% ACN, 0.1% Formic Acid) followed by an acetonitrile gradient to 100% Buffer B (80% ACN, 0.1% Formic Acid). 2.5 kV voltage was applied distally to electrospray the eluting peptides. Full MS spectra were recorded on the peptides over a 400 to 1,600 m/z range, followed by five tandem mass (MS/MS) events sequentially generated in a data-dependent manner on the first to fifth most intense ions selected from the full MS spectrum (at 35% collision energy).

*Gel filtration*

A frozen pellet of MKL-1 cells was resuspended in mammalian cell lysis buffer (MCLB; 50mM Tris pH 7.8, 150 mM NaCl, 0.5% NP40) in the presence of protease and phosphatase inhibitors (Roche Complete, EDTA-free Protease Inhibitor Cocktail and 25 mM sodium fluoride, 1 mM sodium orthovanadate, 5 mM β-glycerophosphate). The lysate was incubated on ice for 15 minutes then clarified by centrifugation in a refrigerated microfuge for 10 minutes at top speed. The supernatant was further clarified using 0.45 μM Durapore PVDF spin filters (Millipore, Billerica, MA). Approximately 7 mg of total cellular protein was applied to a Superose 6 10/300 GL column run in an AKTA pure FPLC (GE Healthcare, Chicago, IL) with MCLB as the running buffer. The injection volume was 500 μl, the flow rate was 0.5 ml/minute, and 0.5 mL fractions were collected from 0.2 column volumes to 1.5 column volumes. The molecular weights were estimated by loading 1 mg of individual protein standards from the Gel Filtration Markers Kit for Protein Molecular Weights 29,000 - 700,000 Da (Sigma-Aldrich, St. Louis, MO).

*RNAi knockdown of MCV T antigens*

MCV T antigens were knocked down in MKL-1 cells using shRNAs as described in Shuda M, Kwun HJ, Feng H, Chang Y, Moore PS. Human Merkel cell polyomavirus small T antigen is an oncoprotein targeting the 4E-BP1 translation regulator. J Clin Invest. 2011;121(9):3623-34. Epub 2011/08/16. doi: 46323; and Houben R, Shuda M, Weinkam R, Schrama D, Feng H, Chang Y, et al. Merkel cell polyomavirus-infected Merkel cell carcinoma cells require expression of viral T antigens. J Virol. 2010;84(14):7064-72. Epub 2010/05/07. doi: JVI.02400-09. The shRNAs were cloned into pLKO.Puro vectors, lentivirus was generated in 293T cells using psPax2 and pVSV.G vectors, and MKL-1 cells were infected using spinoculation (centrifugation at 800g for 30 mins with viral supernatants) followed by infection overnight in the presence of 1 μg/ml Polybrene. 24 hours post infection, MKL1 cells were spun down and resuspended in medium containing puromycin (1 μg/ml). Cells were harvested after 72 hours and processed for immunoblotting and immunoprecipitation.

*Genome-wide CRISPR screen*

CRISPR lentiviral libraries H1 and H2 each contain 92,817 pooled sgRNAs targeting 18,493 human genes. CRISPR screen was performed. Briefly, $2x10^8$ MKL-1 cells were transduced with H1 and H2 CRISPR libraries separately at MOI 0.3 to ensure single sgRNA incorporation per cell. After 6 days of 1 μg/ml puromycin selection, surviving cells from each sgRNA library transduction were split in half, $3x10^7$ cells were saved as initial state controls, the rest were cultured for a month with at least $3x10^7$ cells maintained and used as final state samples. Genomic DNA was extracted and 200 μg from each sample were used to PCR amplify integrated sgRNAs and to generate 4 libraries for next generation sequencing. 50 million reads were obtained for each sequencing library. To filter out false positive targets due to strong correlation between decreased cell viability and increased gene copy number in CRISPR/cas9 screens [32], copy numbers of every 50-kb segment of MKL-1 genome were called from the input of ChIP-seq experiments using QDNAseq software. Segmented copy numbers were converted to copy numbers per gene based on gene coordinates. MAGeCK-VISPR

pipeline was used to assess data quality, correct copy number variation effect and identify statistically significant targets [75].

*ChIP-seq*

MKL-1 cells or a derivative stably expressing MCPyV ST with a C-terminal 3xHA tag were used for ChIP. For MAX, EP400, Ab5 and HA antibodies, ChIP was performed as described in an early protocol with the modification that cells were dual cross-linked with 2 mM disuccinimidyl glutarate (DSG) and 1% formaldehyde [77] and sonicated at 4 °C with a Branson Sonifier 250 at 20% duty cycle for 1 minute with 1 minute rest in between for 15 cycles. The early protocol was described in Schmidt D, Wilson MD, Spyrou C, Brown GD, Hadfield J, Odom DT. ChIP-seq: using high-throughput sequencing to discover protein-DNA interactions. Methods. 2009;48(3):240-8. doi: 10.1016/j.ymeth.2009.03.001.

ChIP- reChIP was performed using the Re-ChIP-IT kit (Active Motif, Carlsbad, CA). For ChIP-seq, 10 ng of DNA from ChIP experiments or input DNA were prepared for sequencing with NEBNext ChIP-seq Library Prep Reagent Set for Illumina (New England BioLabs, Ipswich, MA). Amplified libraries were cleaned up using AMPure XP beads (Beckman Coulter, Brea, CA) and checked on a Bioanalyzer (Agilent, Santa Clara, CA) to confirm a narrow distribution with a peak size around 275 bp. Diluted libraries were used for 50 cycles single-end sequencing on HiSeq 2000 system (Illumina, San Diego, CA) following the manufacturer's protocol.

H3K4me3 ChIP-seq was performed as described with minor changes [78]. 0.5xE06 MKL-1 cells were cross-linked with 1.1% formaldehyde and sonicated at 4°C with a Bioruptor (Diagenode, Denville, NJ). Samples were sonicated on the high setting for 30 seconds with 30 seconds rest in between. Libraries for Illumina sequencing were prepared using the ThruPlex FD DNA-seq kit (Rubicon Genomics, Ann Arbor, MI). Amplified libraries size-selected using a 2% gel cassette in the Pippin Prep system (Sage Science, Beverly, MA) to capture fragments between 200 - 700 basepairs. Libraries were run in Illumina Nextseq.

ChIP-seq mapping was performed using Bowtie (version 0.12.7) against human genome version hg19 allowing only uniquely mapping reads. Peak calling was done

using MACS2 (version 2.1.0.20140616) on either single replicate mapped files or replicates merged as mapped bam files using the samtools (version 0.1.18-dev (r982:313)) merge function. Top ranking peaks (5000 most significant, p-val as reported by MACS2) and the Macs2 generated tag pileup output was visualized using the Meta Gene signal distribution function of the CEAS (version 0.9.9.7) analysis package [79].

Significantly enriched transcription factor binding element motifs where found using the Cistrome SeqPos tool using the 1000 most significant (Macs2 p-value) peaks [80].

To calculate genome-wide overlap, all enriched H3K4me3 peaks were extended 5kb in each direction, divided into 250 bins and the read density was calculated in each bin. Density was normalized to the largest value observed in each experiment genome-wide and plotted either as an average of all regions (meta plot) or as a heat map.

*RNA-seq*

MKL-1 cells containing tet-PLKO-shEP400, tet-PLKO-shMYCL and tet-PLKO-shScramble were used to perform RNA-seq. Cells ($10^7$) were collected before and 5 days after dox addition. Total RNA was purified using RNeasy Plus Mini Kit (Qiagen, Hilden, Germany). mRNA was isolated with NEBNext Poly(A) mRNA Magnetic Isolation Module (New England BioLabs, Ipswich, MA). Sequencing libraries were prepared with NEBNext mRNA library Prep Master Mix Set for Illumina (New England BioLabs, Ipswich, MA) and passed Qubit, Bioanalyzer and qPCR QC analyses. 50 cycles single-end sequencing was performed on HiSeq 2000 system.

Reads were mapped to the Hg19 genome by TOPHAT. HTSeq was used to create a count file containing gene names [81]. The R package DESeq2 was used to normalize counts and calculate total reads per million (TPM), and determine differential gene expression. QC was performed to generate a MA plot to display differentially expressed genes.

To create the heatmaps, counts were normalized separately for the two experiments (shEP400 and shMYCL) using "voom" from the R/Bioconductor package *limma*. Data were then corrected for batch effect using ComBat from the R/Bioconductor package *sva*. In ComBat, the normalized gene expression data were fit to a linear model

capturing the effects of basal expression, sample conditions, batch variation, and noise [46]. In this case, the sample conditions corresponded to shScr, shEP400, and shMYCL, and the batch variable corresponded to the experiment in which the data were measured. In the final step of ComBat, the best-fit parameters from the linear model were used to subtract only the effect of the batch variable from the data. Principal Components Analysis (PCA) plots of the data before and after ComBat show that shScr samples from the two different batches cluster together after ComBat (**FIG. 14**). The batch-adjusted data were subsetted using the "BETA3" list, defined as previously described in the section on BETA analysis of the ChIP-Seq data, or a "DEG" list of genes that were differentially expressed with $P_{adj} < 0.001$ in all three comparisons: shP400-2 vs. shScramble, shEP400-3 vs. shScramble, and shMYCL vs. shScramble. Differential expression was determined using DESeq2. Note that the DEG list includes both up- and down-regulated genes, whereas the BETA3 list includes only down-regulated genes.

For each gene list, the batch-adjusted expression values were first standardized across all 15 samples by mean-centering and scaling so that standard deviations are all set to 1. Genes were then clustered using model-based clustering as implemented in the R package *mclust.* An average profile was created for each gene cluster by taking the mean over the standardized expression values for all the genes in the cluster. Next, the average profiles were merged using complete linkage hierarchical clustering with a Euclidean distance metric. By cutting the tree at a height of 3.5 (for the BETA3 list) or 5 (for the DEG list), the model-based clusters were merged into larger patterns of gene expression. Gene Ontology (GO) term enrichment was run on the final merged clusters using the R/Bioconductor package *GOstats* with the following parameters: the background set consisted of all the genes from the original RNA-seq alignment, the Benjamini-Hochberg method was applied for multiple testing correction, and the conditional hypergeometric test was used to take into account relationships between GO terms. Heatmaps depict the average standardized expression profiles and were created using the "heatmap.2" function from the R package *gplots*.

The IMR90 ST and GFP RNA-seq data is available from the Gene Expression Omnibus (GEO) with accession number GSE79968. The IMR90 data were processed using Tophat and Bowtie, and the log-transformed FPKM values were used for all

analysis, as described [16]. The genes in the DEG list that also had non-zero expression values across all IMR90 expression profiles were used to create the final heatmap. To visualize both datasets in the same setting, the IMR90 profiles were each subtracted by a corresponding control, which was defined as the average expression level in the IMR90 GFP cell line at the same time point. The MKL-1 shEP400 profiles were subtracted by the average expression level in the shScr samples from the shEP400 batch. Likewise, the shMYCL profiles were subtracted by the average expression level in the shScr samples from the shMYCL batch. Finally, for each gene, all its expression values across both IMR90 and MKL-1 datasets were centered and scaled to the same standard deviation to create the final heatmap. Complete linkage hierarchical clustering with Euclidean distance was used to create the row dendrogram.

*Direct targets prediction*

MAX, EP400, ST ChIP-seq data were integrated with individual differential expression data from shEP400 -1, -2, -3 and shMYCL RNA-seq using Binding and Expression Target Analysis (BETA) software package, which infers activating or repressive function of MAX, EP400, ST and predict the target genes based on rank product of binding potential and differential expression [48]. Shared targets of all three factors were termed shEP400-1 BETA, shEP400-2 BETA, shEP400-3 BETA and shMYCL BETA respectively. Common targets of all four aforementioned datasets were termed BETA4, or BETA3 if shEP400-1 BETA was excluded.

*Data availability*

All next generation sequencing reads and processed data of MKL-1 ChIP-seq and RNA-seq have been deposited in the NCBI Gene Expression Omnibus (GEO) with accession number GSE69878. The IMR90 ST and GFP RNA-seq data is available from the NCBI GEO with accession number GSE79968. Mass spectrometry data can be accessed from the Stowers Original Data Repository.

**EXAMPEL 2. MCPyV ST binds MYCL and the EP400 complex**

To understand how MCPyV T antigens contribute to MCC oncogenesis, a large-scale immunoprecipitation was performed with a monoclonal antibody (Ab5) specific for the shared N-terminal region of LT and ST to identify associated cellular proteins from lysates of virus-positive MCC cell lines MKL-1 and WaGa (**FIG. 1A**) [23]. Identification of the immunoprecipitated proteins by multi-dimensional protein identification technology (MudPIT) revealed MCPyV LT and ST (**FIG. 1B** ) [24]. RB1 and VPS39 were detected as expected given their previously reported association with LT [5, 25]. Both homologues of the PP2A scaffold (PPP2R1A, PPP2R1B) and catalytic (PPP2CA, PPP2CB) subunits were also detected, likely due to association with ST [10, 14]. Unexpectedly, Ab5 also co-precipitated MYCL and MAX as well as all known subunits of the EP400 complex listed above including ACTL6B, a homologue of ACTL6A, and the recently reported MBTD1 [20]. In contrast, MudPIT using Ab3, specific for LT only, identified LT, RB1 and VPS39 and none of the components of the EP400 complex.

To validate the interactions with endogenous proteins, MKL-1 cell lysates were immunoprecipitated with antibodies to MAX, EP400, ACTL6A, EPC1 and VPS72. Each of these antibodies co-precipitated ST, PPP2CA and MYCL as well as several components of the EP400 complex (**FIG. 1C**). MudPIT with antibodies for EP400 identified all 15 subunits including homologs of the EP400 complex plus MYCL, MAX, ST and PP2A (**FIG. 1B**). MudPIT with antibodies for MAX enriched for MYCL, ST, PP2A, all components of the EP400 complex plus several MAD and MAD-associated proteins [26, 27]. MudPIT with an IgG control antibody detected small amounts of RUVBL1, RUVBL2, MEAF6 and ACTL6B but none of the other EP400 complex components. Therefore, antibodies for MAX, EP400 and MCPyV ST each specifically co-precipitated MYCL, the EP400 complex, ST and PP2A (**FIGS. 1B and 8A**).

To determine if ST could form a single complex with MYCL and the EP400 complex, gel filtration of MKL-1 cell lysates was performed [28]. Fractions #5-7 contained protein complexes of $\geq$ 2 MDa with ST, MYCL, MAX, several EP400 complex components and EP400 itself (**FIG. 1D**). EP400 was only detected in the large complex fraction while other subunits of the complex including TRRAP, KAT5, RUVBL2, DMAP1 and ING3 were present in the large complex and in fractions with smaller sized complexes. Of note, MYCL1 isoform 1 (i1) was present in the ST-

containing fractions #5-7 whereas the larger MYCL i3 was detected in intermediate sized fractions and the shortest form (i2) in smaller size fractions (**FIGS. 1D, 1E, 8B-8C**). An immunoprecipitation for MAX with lysates from fraction #5 co-precipitated EP400, TRRAP and ST (**FIG. 1F**). In contrast, MAX co-precipitated TRRAP and ST but not EP400 from fraction #13 and neither TRRAP or EP400 from fraction #21. This indicates that a specific fraction of MAX binds to EP400, a key component of the ST-MYCL complex [29].

**EXAMPEL 3. MCPyV ST binds specifically to the EP400 complex**

To determine the contribution of MCPyV ST binding to MYCL and the EP400 complex in MCC, MKL-1 cells were transduced with lentiviral shRNAs targeting both LT and ST (shPanT) or ST only (shST) [13, 30]. Expression of either shRNA but not scrambled shRNA (shScr) led to reduced levels of ST and MYCL (**FIGS. 2A, 9A**). Reduced levels of ST led to decreased ability of MAX to co-precipitate EP400, TRRAP, DMAP1 and YEATS4 and reduced the ability of EP400 to co-precipitate MYCL and MAX. Of note, when ST levels were reduced, EP400 retained the ability to bind to other components of the EP400 complex including TRRAP, DMAP1 and YEATS4.

To determine if MCPyV ST could increase the ability of MAX to bind to the EP400 complex, ST or C-terminal HA-tagged ST was introduced into HCT116 cells and a virus-negative MCC cell line UISO. Immunoprecipitation for MAX from parental HCT116 and UISO cell lysates readily co-precipitated MYC but not any EP400 complex components (**FIG. 2B**). However, in the presence of MCPyV ST, MAX efficiently co-precipitated TRRAP, EP400, DMAP1 and KAT5. Of note, MYCL levels increased in HCT116 and UISO cells when ST was expressed (**FIG. 2B**, Input). Stable expression of ST in primary human foreskin fibroblasts (HFF) also increased levels of MYCL (**FIG. 9B**).

To determine if ST interaction with the EP400 complex was specific, a series of ST mutants were generated and stably expressed in HCT116 cells. Experiments focused on the mutagenesis on a region of MCPyV ST within the unique domain that is not well conserved with ST from other human polyomaviruses and includes the LSD motif (residues 91-95) (**FIGS. 2C and 9C**). Immunoprecipitation for ST containing alanine

substitutions of residues 83 to 88 (83-88A) showed decreased binding to EP400 complex components while retaining strong binding to PP2A (**FIGS. 2D and 9D**). Within this region, substitution of E86 and E87 with serine (E86S, E87S referred to as 2M) led to reduced EP400 complex binding yet retained some PP2A binding. In contrast, alanine substitution of residues 83 to 95 (83-88A, 90-95A, 93-95A) or 102 to 105 (102-105A) led to increased levels of ST relative to WT ST (**FIGS. 2D and 9D**). Within this region, substitution of K92 and D93 with serine (K92S, D93S, referred to as 3M) led to increased levels of MCPyV ST (**FIGS. 2D and 9C**). Combining the 2M and 3M mutants to create 4M (E86S, E87S, K92S, D93S) resulted in a ST construct that expressed at levels higher than WT ST and retained PP2A binding, but was unable to co-precipitate the EP400 complex. To test the ability of these ST constructs to promote MAX binding to the EP400 complex, we performed an IP with MAX antibodies. WT, 102-105A, and 3M ST led to increased ability of MAX to co-precipitate the EP400 complex and PP2A, while the 2M and 4M mutants were not co-precipitated by MAX and did not enable MAX to co-precipitate the EP400 complex or PP2A (**FIG. 2D**).

**EXAMPEL 4. ST requires MYCL and MAX heterodimers to sustain MCC viability**

It was observed that MCPyV ST binds specifically to MYCL and the EP400 complex. However, it was not clear if any of these factors were required for proliferation. To identify essential genes in MKL-1 cells, a CRISPR-Cas9 screen of 18,493 genes was performed using two pooled sgRNA libraries H1 and H2, each containing 5 unique sgRNAs for each gene. Using the MAGeCK-VISPR analysis pipeline, Gene Set Enrichment Analysis (GSEA) of known human housekeeping genes revealed that these genes were significantly negatively correlated with the results of the CRISPR screen [31] (**FIG. 10A**). After accounting for copy number variations in MKL-1 cells, 481 genes that were negatively selected in the CRISPR-Cas9 screen were identified with a false discovery rate (FDR) < 0.05 [32], of which 276 have been classified as housekeeping genes (**FIGS. 10B-10C**) [33]. Among the 205 genes not classified as housekeeping genes, 79 genes were identified with FDR < 0.01 and the remaining 126 genes were identified with FDR > 0.01 but < 0.05. MYCL, EP400 and RUVBL2 were identified as essential (FDR < 0.05, **FIG. 3A**). Additional components of the EP400 complex were

identified in the CRISPR-Cas9 negative selection screen with p-values < 0.05 but with higher FDR values and included KAT5, TRRAP, DMAP1, ING3 and YEATS4. Furthermore, the copy number of MYCL was 3.5 copies in MKL-1 cells based on analyzing ChIP-seq input DNA (**FIG. 10B**).

Given the requirement of MYCL for viability of MKL-1 cells in the CRISPR-Cas9 screen and the presence of MYCL in the ST-EP400 complex in both MKL-1 and WaGa cell lines (**FIG. 1B**), the levels of the three MYC family members were examined in MCC cell lines. Six different virus-positive MCC cell lines that expressed ST and LT also expressed MYCL while some had low levels of MYCN and none expressed full length MYC (**FIG. 3B**). For controls, HCT116 cells were tested that predominantly expressed MYC and Kelly neuroblastoma cells that expressed MYCN. The virus-negative MCC cell line UISO did not have detectable levels of MYCL until a C-terminal epitope tagged ST (ST-CT) was introduced (**FIG. 3B**).

To determine if MYCL was required for ST to binding to the EP400 complex, MKL-1 cells that contained doxycycline (Dox) inducible shRNA (shMYCL) or miRNA (mirMYCL) that specifically targeted MYCL were generated. Expression of shMYCL or mirMYCL led to reduced levels of MYCL and decreased MAX co-precipitation of EP400, TRRAP, KAT5 and ST (**FIG. 3C**). Notably, depletion of MYCL reduced the ability of ST to co-precipitate the EP400 complex and reduced EP400 binding to ST (**FIG. 3C**).

Omomyc is a modified fragment of MYC that can bind to MAX and disrupt endogenous MYC-MAX heterodimers [34]. To test if MYCL-MAX heterodimers were necessary for ST interaction with the EP400 complex, a Dox-inducible, HA-tagged, Omomyc construct was introduced into MKL-1 cells. When expressed, HA-Omomyc co-precipitated MAX as expected but not MYCL, ST or subunits of the EP400 complex and led to decreased levels of both MAX and MYCL (**FIG. 3D**). However, ST retained the ability to co-precipitate components of the EP400 complex but not MYCL or MAX when Omomyc was expressed, indicating that ST can bind to the EP400 complex independent of the MYCL/MAX heterodimer. The viability of MKL-1 cells was decreased when MYCL levels were depleted by shMYCL or mirMYCL and when the MYCL-MAX heterodimer was disrupted by Omomyc (**FIG. 3E**).

To determine regions of MYCL that contributed to ST and EP400 binding, a series of HA tagged C-terminal constructs of MYCL was stably expressed in HCT116 cells in the presence or absence of ST. When ST was present, MYCL robustly co-precipitated TRRAP, EP400, YEATS4, DMAP1 as well as ST (**FIG. 10D**). In the absence of ST, WT MYCL co-precipitated MAX but only weakly bound to EP400 complex components. In contrast, the N-terminal 165 residues of MYCL, unable to bind MAX, could co-precipitate ST and the EP400 complex in the presence or absence of ST (**FIG. 10D**). The N-terminal 165 residues of MYCL contains several highly conserved MYC homology boxes (MB) that function to bind MYC modifying proteins [35]. MB1 binds to FBXW7 and MB2 contributes to TRRAP binding (**FIG. 8**) [21, 36]. HCT116 cells that stably expressed HA-tagged MYCL full length constructs with small in-frame deletions of MB1 or MB2 were generated. An HA IP for ΔMB2 MYCL co-precipitated ST and MAX but not the EP400 complex, while the ΔMB1 MYCL co-precipitated MAX but neither ST nor the EP400 complex (**FIG. 10D**). These data indicate that MB1 and MB2 of MYCL contribute to ST and EP400 complex binding.

**EXAMPEL 5. ST binds TRRAP and MYCL in absence of EP400**

To test the requirement for EP400 in virus-positive MCC, MKL-1 cell lines containing three different dox-inducible shRNAs targeting EP400 were generated (**FIG. 4A**). In the presence of dox, levels of EP400 were reduced and an immunoprecipitation for EP400 was unable to co-precipitate DMAP1 or MAX (**FIG. 4A**). Of note, knockdown with shEP400-1 led to decreased levels of ST and MYCL in addition to lower levels of EP400 (**FIG. 4B**). In contrast, shEP400-2 and shEP400-3 reduced EP400 levels but did not affect ST and MYCL levels. When EP400 levels were reduced by shEP400-2 or -3, MAX and ST retained the ability to co-precipitate each other, as well as MYCL and TRRAP but not DMAP1 or YEATS 4 **(FIG. 4B)**. The viability of MKL-1 cells decreased significantly upon depletion of EP400 with each of the 3-inducible shRNA (**FIG. 4C**).

To determine if the effect of EP400 depletion was specific to virus-positive MCC cell lines, the 3 inducible shEP400 constructs were introduced into the virus-negative MCC line UISO. It was confirmed that knockdown of EP400 led to reduced levels of EP400 in UISO cells (**FIG. 4D**). In contrast to the MCPyV-positive MCC cell line MKL-

1, the viability of UISO cells was unaffected by EP400 knock-down (**FIG. 4E**). Given that the Kelly neuroblastoma cell line is dependent on continued expression of MYCN, its sensitivity to EP400 depletion was tested [37]. Depletion of EP400 in Kelly cells by shEP400-1 led to reduced binding of MAX to DMAP1 and YEATS4 while retaining binding to MYCN and TRRAP (**FIG. 4F**). Of note, shEP400-1 did not affect MYCN levels in Kelly cells. As shown in **FIG. 4G**, Kelly cells had reduced viability when EP400 levels were reduced.

**EXAMPEL 6. MCPyV ST, MYCL and EP400 complex cooperate to reprogram and transform cells**

Expression of MYC or MYCL together with OCT4, SOX2 and KLF4 (OSK) can generate induced pluripotent stem (iPS) cells from a variety of somatic cell types [38, 39]. Furthermore, MYC interaction with the EP400 complex has been implicated in the generation and maintenance of embryonic stem (ES) and iPS cells [40, 41]. Given that MCPyV ST can bind to MYCL and the EP400 complex, the ability of MCPyV ST to contribute to iPS cell generation was tested. Since keratinocytes have higher reprogramming efficiency compared to other cell types due to lower p53 and p21 protein levels [42], hTERT-immortalized human keratinocytes were generated with an inducible OSK expression vector and MYCL, ST or ST mutants were stably introduced. Expression of OSK in the presence of MYCL, ST or 3M led to the appearance of flat human ES cell-like colonies with defined borders that could be stained by alkaline phosphatase and ES cell surface markers TRA-1-60 and TRA-1-81 (**FIGS. 5A-5C**) [43]. In contrast, the ST EP400-binding defective 2M and 4M mutants were unable to generate iPS cells. These results indicate that ST binding to MYCL and the EP400 complex was able to cooperate with OSK to promote the generation of iPS cells.

Experiments were preformed to determine if ST mediated transformation was dependent on interaction with MYCL and the EP400 complex. When a MCC tumor-derived MCPyV early region (E) that encoded truncated LT and wild type ST was expressed in IMR90 human diploid fibroblasts, it was observed a senescent phenotype with elevated levels of p53 and p21 [23]. To suppress this phenotype, a dominant negative form of p53 (p53DD abbreviated as P) and hTERT (H) were expressed in

IMR90 cells to generate PH cells [44]. The PH cells tolerated MCPyV early region with wild type ST (PHE), 3M ST (PH3) or 4M (PH4) mutant ST, and exogenous MYCL (PHL) without undergoing senescence. Immunoprecipitation of ST with Ab5 from PHE cell lysates revealed a weak interaction with DMAP1, a component of the EP400 complex (**FIG. 5D**). However, when MYCL was co-expressed with wild type ST in PHEL cells, ST and MAX readily co-precipitated the EP400 complex. The 3M ST mutant could efficiently co-precipitate the EP400 complex even without exogenous MYCL expression (PH3). In contrast, the 4M ST mutant (PH4) was unable to co-precipitate the EP400 complex.

The ability of these fibroblasts to grow in an anchorage-independent manner was tested when cultured in soft agar. IMR90 cells expressing p53DD and hTERT (PH) alone or with MYCL (PHL) were unable to form colonies. Cells expressing the MCPyV early region (PHE) formed a few colonies, while co-expression of MYCL (PHEL) led to an increased number of soft agar colonies (**FIGS. 5E-5F**). The highly expressed 3M ST mutant (PH3) could induce anchorage-independent growth while the 4M mutant (PH4) failed to form soft agar colonies. The number of colonies formed for each cell type reflected the relative binding of ST and MAX to the EP400 complex in the presence of the various ST constructs (compare **FIGS.** 5D and 5F).

**EXAMPEL 7. MAX, EP400 and MCPyV ST bind to actively transcribed promoters**

Given the known chromatin binding activities of MYCL and the EP400 complex, experiments were performed to determine if MCPyV ST could bind specifically to DNA. Chromatin immunoprecipitation was performed followed by sequencing (ChIP-seq) with the mass spectrometry-validated antibodies to EP400 and ST. Since no antibody suitable for immunoprecipitation or ChIP was available for MYCL, ChIP-seq was performed with the MAX antibody. Although it has been reported that MCPyV truncated LT does not bind to chromatin, it is possible that ChIP with Ab5 might also enrich for chromatin-bound LT [6]. To account for this possibility, a MCC cell line (MKL-1) derivative was generated. The cell line derivative stably expressed MCPyV ST with a C-terminal HA epitope tag and performed ChIP with an HA antibody. Replicas of MAX and EP400 ChIP-seq identified many peaks that were also identified by anti-ST (Ab5) and anti-HA

ChIP-seq. Common gene targets were identified by assigning peaks to the nearest genes (**FIGS. 6A and 11A**). *De novo* DNA motif analysis identified the MYC target E-box sequence CACGTG as the most frequently observed motif with Z-scores -42.1726, -20.0773, -23.9634, -19.137 for MAX, EP400, ST-HA and Ab5 antibodies respectively (**FIG. 6B**). Peaks were highly enriched for promoters and 5'UTR sequences (**FIGS. 6C and 11B**). ChIP for MAX, EP400 or ST followed by re-ChIP for these three factors indicated that they could bind simultaneously to DNA (**FIG. 11C**).

Given the strong enrichment for promoters, ChIP-seq was performed with antibodies to histone H3 modified by lysine 4 trimethylation (H3K4me3), a histone mark enriched at actively transcribed gene promoters [45]. H3K4me3 ChIP-seq identified 20,222 peaks with MAX, EP400 and ST centered on the same peaks (**FIGS. 6D, 6E**). These results indicate that MAX, EP400 and MCPyV ST bind as a complex specifically to E boxes near the transcription start sites (TSS) of actively expressed genes.

ChIP-seq experiments on several promoters were validated. Chromatin from MKL-1 cells after transduction with vectors expressing shMYCL, mirMYCL or controls was prepared and ChIP with Ab5 was performed. As shown in **FIGS. 12A-12C**, it was observed ST binding to the MYCL gene as well as three additional gene promoters that were significantly reduced by MYCL depletion. Chromatin from MKL-1 cells containing the inducible shEP400-1 before and after dox addition was also prepared. Strong MAX binding to several gene promoters that was reduced upon EP400 depletion was observed (**FIG. 12D**).

**EXAMPEL 8. Specific gene regulation by ST-MYCL-EP400**

To identify genes and associated biological functions that are controlled by the ST-MYCL-EP400 complex, RNA-seq was performed for MKL-1 cells containing inducible shMYCL, shEP400-2, shEP400-3 and shScr with RNA isolated from cells treated with dox for 5 days. The differentially expressed genes (DEG) list consists of 2157 genes that passed the cutoff $P_{adj} < 0.001$ in all three comparisons (shEP400-2, shEP400-3 and shMYCL vector, relative to shScr control). To create heatmaps, counts were normalized separately for the two experiments (shEP400 and shMYCL) and then corrected for batch effect using ComBat (**FIG. 13**) [46]. These genes were first grouped

into 62 clusters using model-based clustering [47]. The average expression profiles of each cluster were then merged into four general patterns of behavior using hierarchical clustering (**FIG. 7A**). The genes in each of the four merged clusters were evaluated for statistical enrichment in Gene Ontology (GO) biological process terms. Cluster membership and all results of the GO term analysis were performed. It was observed that genes upregulated by shEP400 and shMYCL fell into the cluster DEG-CL2 and were enriched in neurogenesis, skin development and hair cycle. DEG-CL4 contained genes downregulated by EP400 and MYCL and were enriched in cellular component biogenesis, RNA processing and amide biosynthetic process. Two smaller clusters represent genes that behaved differently under shEP400 and shMYCL conditions. DEG-CL1 genes were decreased by shEP400, increased by shMYCL and enriched for actin cytoskeleton and regulation of signaling. DEG-CL3 exhibited the opposite pattern of expression and was enriched in nerve development and liposaccharide biosynthesis. These results show that both MYCL and EP400 support cell growth by upregulating bulk synthesis of biomolecules including ribosomes and proteins while simultaneously repressing cell adhesion and developmental programs in neurogenesis and skin.

To integrate expression profiling with the aforementioned ChIP-seq experiments (**FIG. 6**), Binding and Expression Target Analysis (BETA) that links the proximity of the ChIP-seq binding peaks to the TSS with expression level changes in the corresponding genes was performed to predict activating and repressive activities of transcription factors [48] (**FIG. 7B**). BETA analysis for MAX, EP400 and ST ChIP-seq studies with RNA-seq analysis for shEP400-2, shEP400-3 and shMYCL were performed. It was observed that the genes whose levels decreased (downregulate) upon EP400 or MYCL depletion were significantly enriched for MAX, EP400 and ST chromatin binding (**FIG. 14A**). In contrast, genes whose levels increased (upregulate) with EP400 depletion were not significantly associated with the MAX, EP400 and ST ChIP-peaks. This indicates that the ST, MYCL/MAX and EP400 complex binding contributes to specific gene activation. The target genes identified for each ChIP-seq analysis were compared with the RNA-seq analysis for shEP400-2, shEP400-3 and shMYCL and 951 shared target genes of MAX, EP400 and ST whose levels went down upon EP400 or MYCL depletion and had significant evidence for direct ChIP binding by BETA analysis (BETA3, **FIGS. 7C** and

**14B**). When the RNA-seq data for shEP400-1 was also included in the analysis, a total of 379 target genes were identified (BETA4, **14**A-C).

951 genes identified in BETA3 that were downregulated by shEP400 and shMYCL with evidence of direct binding according to BETA analysis of the ChIP-seq data were examined. It is noted that these genes exhibited a wide range of fold changes upon depletion of EP400 and MYCL, with 136 of the 951 genes showing greater than 2-fold downregulation due to shEP400 (shEP400 inverse fold change), and 62 out of 951 genes showing greater than 2-fold downregulation due to shMYCL (**FIG. 15**). To find global patterns of expression that reflected functional regulation, their expression profiles were centered and scaled and model-based clusters and merged clusters were created, using the same procedure as in the analysis of the DEG list. The final merged clusters were then evaluated for GO term enrichment. If was found that these genes naturally divide into two groups: genes that were more strongly affected by shEP400 (BETA3-CL1 and 2) and genes that were more strongly affected by shMYCL (BETA3-CL3 and 4) (**FIG. 7D**). The shEP400 clusters are enriched for nucleobase-containing compound metabolic process and translation initiation and elongation whereas the shMYCL clusters are involved in RNA processing and peptide metabolic processes.

Among the target genes identified in the shEP400-2, -3 and shMYCL depletion analyses was the translational control factor 4EBP1 that has been reported to be upregulated by ST [13]. To test this effect, lysates were generated from MKL-1 cells before or after depletion of EP400 and MYCL were blotted for 4EBP1. As expected, levels of MYCL were depleted by shMYCL and EP400 by shEP400-2 and -3 (**FIG. 7E**). Of note, levels of 4EBP1 and the phosphorylated serine residue 65 form (pS65-4EBP1) were reduced upon EP400 or MYCL knockdown. In addition, we have recently reported that levels of the lactate transporter MCT1 (SLC16A1) increase upon expression of ST [16]. Levels of MCT1 were also decreased upon depletion of EP400 or MYCL (**FIG. 7E**).

The effect of depleting EP400 or MYCL in the virus-positive MKL-1 cells was compared to the effect of expressing ST in normal cells. RNA-seq profiles from IMR90 human fibroblasts with inducible expression of GFP or MCPyV ST over the course of 4 days was examined [16].

In **FIG. 16**, Heatmap illustrating comparison of all 2157 DEG genes in IMR90 cells with inducible expression of GFP or MCPyV ST with all DEG genes in MKL-1 cells after depletion of EP400 and MYCL and shScr. The IMR90 profiles were each subtracted by a corresponding control, which was defined as the average expression level in the IMR90 GFP cell line at the same time point. The MKL-1 shEP400 profiles were subtracted by the average expression level in the shScramble samples from the shEP400 batch. Likewise, the shMYCL profiles were subtracted by the average expression level in the shScramble samples from the shMYCL batch. Finally, for each gene, all its log-transformed expression values across both IMR90 and MKL-1 datasets were centered and scaled to the same standard deviation to create the final heatmap. Complete linkage hierarchical clustering with Euclidean distance was used to create the row dendrogram. As shown in the heatmap in **FIG. 16**, the genes that were downregulated by shEP400 and shMYCL in MKL-1 cells tend to be upregulated by ST in IMR90 cells consistent with the model that ST activates functional interactions with EP400 and MYCL and their transcriptional targets.

**EXAMPEL 9. LSD1 is a downstream target of MCPyV ST-MYCL-EP400 complex**

Among the downstream targets of the MCPyV ST-MYCL-EP400 complex, it was determined that MCPyV ST can specifically increase levels of LSD1 (also known as lysine specific demethylase 1 or KDM1A) as well as RCOR2 and INSM1 that together form the CoREST (CoRepressor for Element-1-Silencing Transcription factor) complex (**FIG. 17**). When levels of EP400, MYCL or ST in virus-positive MCC cell lines were reduced by shRNA, the levels of RCOR2, INSM1 and LSD1 also decreased implying that the ST-MYCL-EP400 complex functions to increase levels of CoREST components.

LSD1 is a histone demethylase that can erase methylation from H3K4me1 and H3K4me2, an activity that have been implicated in iPS cell generation. An alternatively spliced form of LSD1+8a functions to activate gene expression by de-methylating H3K9me1 and H3K9me2. Very high levels of LSD1+8a was detected by RT-qPCR in 6 virus-positive MCC cells lines with little to no expression in virus-negative, UV-damaged, MCC cells. The results suggest that the CoREST complex may function to repress as well as activate specific gene expression in virus-positive MCC.

The presence of a CoREST complex in several virus-positive MCC cells was confirmed by performing RT-qPCR, immunoprecipitation followed by mass spectrometry identification of associated proteins, and western blotting. In addition, 6 different virus-positive MCC cell lines had significant sensitivity to small molecule inhibitors to LSD1 with a strong cytostatic effect and complete inhibition of anchorage independent growth (**FIG. 18**). Treating virus-positive MCC cell lines with several different LSD1 inhibitors resulted in a significant growth arrest, changes in cellular morphology and changes in gene expression. Remarkably, it was observed that genes whose levels increased upon EP400 depletion were also upregulated by inhibition of the histone demethylase activity of LSD1. These results imply that ST-MYCL-EP400 can indirectly repress specific gene expression through CoREST activity.

When MCPyV ST and MYCL were expressed in IMR90 human diploid fibroblasts, significantly increased levels of RCOR2 and LSD1 were observed. In addition, ST, MYCL-MAX and EP400 binding were detected by ChIP to the promoters of RCOR2 and LSD1 in the ST-transformed fibroblasts. These results indicate that the ST-MYCL-EP400 complex can transactivate expression of key CoREST components in MCC cells as well as in normal fibroblasts. This was an unexpected result because RCOR2 expression is typically restricted to embryonic stem cells. LSD1 inhibitors also inhibited the ability of ST transformed IMR90 cells to grow in an anchorage independent manner (**FIG. 19**). These results indicate that the ST-MYCL-EP400 complex not only serves to transactivate key target genes to promote MCC oncogenesis but creates dependencies on downstream target genes such as the CoREST complex. The discovery that virus-positive MCC is sensitive to LSD1 inhibitors is unexpected and could not have been predicted from any prior studies. Thus, LSD1 inhibitors can be an effective targeted approach for the treatment of MCPyV-positive MCC.

**EXAMPEL 10. Biomarkers predictive for sensitivity to LSD1 inhibition**

Experiments were performed to determine specific biomarkers predictive for sensitivity to LSD1 inhibition. The results of RT-qPCR quantification of mRNA levels in MCC cell lines show that the components of the CoREST complex were highly expressed in virus-positive MCC cell lines and poorly expressed in virus-negative cell

lines. Virus-positive MCC (MKL-1, MKL-2, MS-1, Waga, Peta, Broli) had higher levels of LSD1, RCOR2, INSM1 and ATOH1 compared to virus-negative MCC lines (UISO, MCC13 and MCC26). Human foreskin fibroblasts (HFF) served as control. Particularly, RCOR2, INSM1 and LSD1 were expressed at 10 to 100-fold higher levels in virus-positive MCC cell lines compared to virus-negative cells (**FIG. 20**).

In addition to biomarkers predictive for LSD1 response, experiments were performed to identify biomarkers that can measure the response to LSD1 inhibition. Several components indicative of activated Notch including the Notch intra-cellular domain (NICD) and HES1 were elevated in MCC cell lines after treatment. ID1, downstream of the BMP pathway was also identified as significantly increased in cells after LSD1 inhibition.

**EXAMPEL 11. Test efficacy of LSD1 inhibitors against MCC cell lines *in vitro***

Experiments are performed to determine LSD1 inhibition reduces growth rate of virus-positive MCC cell lines.

*Design*

The activity of two LSD1 inhibitors that are currently being tested in Phase 1 studies and have shown activity *in vitro* and in rodent tumor models are tested. GSK2879552 and ORY-1001 are tested (Mohammad, H. P. et al. A DNA Hypomethylation Signature Predicts Antitumor Activity of LSD1 Inhibitors in SCLC. Cancer Cell 28, 57-69, 2015.06.002 (2015); Hosseini, A. & Minucci, S. A comprehensive review of lysine-specific demethylase 1 and its roles in cancer. Epigenomics 9, 1123-1142, doi:10.2217/epi-2017-0022 (2017)). In addition, the clinical candidate LSD1 inhibitor CPI-242 from Constellation Pharmaceuticals is also tested.

IC50 levels of 3 different clinical compounds in a total of 9 previously established MCC cell lines are determined. These MCC cell lines include 6 virus-positive lines including MKL-1, MKL-2, MS-1, WaGa, BroLi and PeTa are virus positive MCC cell lines and 3 virus-negative MCC cell lines UISO, MCC13 and MCC26. Newly derived MCC cell lines include DFMC 275, 277, 282, 290, 2314, 301, 336, and 350. The virus status for all cell lines and PDX models is being determined by hybrid capture DNA

sequencing. In addition, these cell lines are being sequenced by Profile Oncopanel version 3 (POPv3) or by whole exome sequencing. The sequencing studies are integrated to provide definitive characterization regarding their viral status as well as the mutation statues of relevant oncogenes and tumor suppressor genes. For example, most virus-negative MCC tumors and cell lines have inactivating mutations in TP53, RB1, NOTCH1 and NOTCH2 while most virus-positive MCC are wild type for these genes. While viral status appears to predict sensitivity to LSD1 inhibition, it is not known if mutation of any oncogene or tumor suppressor affects this activity.

The data indicated that sensitivity to LSD1 inhibitors took several days of treatment. LSD1 inhibitors typically did not yield detectable differences in cell viability until at least 6 days of treatment. The experiments indicated the clearest evidence for decrease in viability at 12 days. This extended treatment response may reflect changes in the epigenome that may take more than one or more cell divisions to become evident.

The number of cell divisions that cells undergo during treatment with LSD1 inhibitors can be determined using BrdU staining for DNA and CFSE staining for total protein levels.

As a positive control for treatment, the sensitivity of MCC cell lines to MDM2 inhibitors such as Nutlin-3a is also tested. It has been confirmed that MCC cell lines expressing wild type p53 are highly sensitive to Nutlin-3a undergoing apoptosis using BH3 profiling. BH3 profiling of the MCC cell lines is preformed upon treatment with LSD1 inhibitors to determine the degree of apoptosis.

In addition, RNA-seq of the MCC cell lines is performed after LSD1 inhibition. In part, the goal of the RNA-seq is to characterize the effect of LSD1 inhibitors n virus-positive and virus-negative MCC cells as well as to provide data for identification of biomarkers.

*Rationale*

Data support the model that virus-positive MCC cell lines are sensitive to LSD1 inhibition. The data indicate that MCPyV ST cooperates with MYCL and the EP400 complex to transactivate LSD1, INSM1 and RCOR2 and appears to create a cell type dependency on LSD1 activity. The goal is to determine if this is expandable to a larger

cohort of cell lines including newly generated and previously uncharacterized MCC cell lines.

*Expected Results*

It is expected that all virus-positive MCC cell lines show decreased growth in response to LSD1 inhibition, and LSD1 inhibition contributes to apoptotic cell death in sensitive cell lines. RNA-seq data will be used to identify and validate biomarkers indicative of sensitivity or response to LSD1 inhibition.

**EXAMPEL 12. Test efficacy of LSD1 inhibitors against MCC cell lines and PDX *in vivo***

Experiments are performed to determine LSD1 inhibition reduces growth of virus-positive MCC xenografts.

*Design*

The sensitivity of MCC cell lines grown as xenografts in NSG mice and of PDX MCC tumors is assessed. The goal is to determine if LSD1 inhibitors given to tumor bearing mice are capable of reducing the growth rate and eliminating the tumor entirely. All animals are dosed daily using these drugs in mouse models. Tumors are measured and body weight is measured every 3 days for the duration of the trial, typically 2 to 3 weeks.

The experiments are performed in a 3-step process. First, the sensitivity of the MCC cell lines MKL-1 and WaGa grown as xenografts is tested. Second, the sensitivity of newly generated MCC cell lines that are generated directly from patient derived tumor material or from tissue first propagated as a patient derived xenograft in mice is tested. Third, the sensitivity of MCC PDX tumors that have never been previously cultured *in vitro* is tested. In all three experiments, the presence of MCPyV viral DNA integrated into the tumor genome is confirmed using hybrid capture sequencing. In addition, RNA-seq data are analyzed to detect expression of MCPyV LT and ST mRNA. Furthermore, RT-qPCR and western blot are performed to detect expression of MCPyV LT and ST as well as LSD1, INSM1 and RCOR2, components of the CoREST complex.

The sensitivity of the MKL-1 and WaGa cell lines grown as xenografts to 3 LSD1 inhibitors is tested *in vivo*. 4 tumor bearing mice for each cell line with 3 drugs and vehicle control for a total of 32 mice are tested. The goal of these initial *in vivo* studies is to determine the efficacy of all three drugs in two well characterized cell lines. The experiments can be further expanded to include at least additional MCC cell lines that were generated directly from patient derived tumor material or from tissue first propagated as PDX in mice. Newly derived MCC cell lines include DFMC 275, 277, 282, 290, 2314, 301, 336, and 350. At least two additional MCC cell lines are tested *in vivo*. At least two mice are inoculated with each of the 8 newly generated MCC cell line for a total of 16 mice, two of the cell lines capable of growing are selected as xenografts for testing with the 3 LSD1 inhibitors plus vehicle control. 4 mice for each cell line and each drug for a total of 32 mice are tested.

Two different virus-positive MCC PDX models for sensitivity to the 3 LSD1 inhibitors are tested. Two independent PDX models that have not been previously cultured *in vitro* are selected. 4 mice each will be tested for each LSD1 inhibitor plus vehicle control. Each PDX model has been shown to be capable of being grown when injected subcutaneously. Available MCC PDX lines include DFMC-96712, 33043, 14161, 63632, 11112 (Virus-negative), 87346, 48396 and 40067.

*Rationale*

The goal is to determine the MCC tumor response *in vivo* to LSD1 inhibition. By staggering the approach using well characterized MCC cell lines before testing newly generated cell lines and PDX models, the responses *in vitro* can be correlated with *in vivo* responses.

*Expected Results*

It is expected that tumor growth is specifically inhibited by each of the 3 clinical compounds in virus-positive MCC xenografts in all models tested.

**EXAMPEL 13. Identify and validate biomarkers predictive and reflective of response to LSD1 inhibition.**

Experiments are performed to show that viral-positive status can predict sensitivity to LSD1 inhibition and specific genes that contribute to LSD1 sensitivity are reflective and predictive of response to LSD1 inhibition.

*Design*

RNA-seq is performed for treated cell lines and xenograft cell lines and PDX material treated with each LSD1 inhibitor or control. Peripheral blood mononuclear cells (PBMCs) are obtained from treated mice and RNA-seq is performed. Gene expression changes can be determined and differentially expressed genes are identified with a focus on identifying shared target genes. Analysis of differentially expressed genes is performed to search for common targets affected under all inhibitors. RNA-seq data are integrated with the previously obtained ChIP-seq data performed with LSD1 and RCOR2 antibodies to determine if any target genes are directly regulated by the CoREST complex. 9 established cell lines, 8 newly established cells lines and 8 MCC PDX are tested for expression of these various markers by RT-qPCR, western blot and immunohistochemistry for the PDX samples.

*Expected Results*

It is expected that INSM1, LSD1 and RCOR2 are strongly detected in virus-positive MCC cell lines and are correlated with sensitivity to LSD1 inhibition. In addition, candidate biomarkers HES1 and ID1 are useful markers of LSD1 inhibition activity in virus-positive MCC. It is expected that a large number of differentially expressed genes are detected in RNA-seq experiments with cell lines and xenografts. By restricting the number to those differentially expressed in all samples and integrating with ChIP-seq data, the total number of genes can be limited to a reasonable number that can be validated by RT-qPCR and western blotting.

**EXAMPEL 14. LSD1 inhibition causes death of virus-positive MCC cell lines.**

To test whether MCC cell lines are sensitive to LSD1 inhibition, six virus-positive (MKL-1, MKL-2, MS-1, WaGa, PeTa, and BroLi) and three virus-negative (UISO, MCC13 and MCC26) MCC cell lines were treated with various LSD1 inhibitors

including GSK2879552, and performed the XTT cell viability assay at 12 days treatment. Interestingly, all the virus-positive MCC cell lines were sensitive to LSD1 inhibition, whereas the virus-negative cell lines were not. MCV T antigens may confer LSD1 dependency in MCC by activating the LSD1 complex.

**FIGS. 22A-22B** show that virus-positive (MKL-1, MKL-2, MS-1, WaGa, PeTa and BroLi), but not virus –negative (MCC13, MCC26 and UISO), MCC cell lines are sensitive to LSD1 inhibition. Cells were treated with varying concentration of LSD1 inhibitor GSK2879552 for 12 days. Relative viability was measured at 12 days of treatment by the XTT assay. Data are shown as mean ± SD and reflect 3 biological replicas. MKL-1 MCC cells were also found to be sensitive to several different LSD1 inhibitors in a dose-dependent manner (data not shown).

**EXAMPEL 15. LSD1 inhibition abrogates MCV T antigen dependent transformation of normal human cells *in vitro*.**

To determine whether transformation of normal cells by MCV T antigens requires LSD1 activities, a soft agar transformation assay was performed using MKL-1 MCC cells and the IMR90 normal cells transformed with MCV T antigens. It has been found that addition of an LSD1 inhibitor, GSK-LSD1, abrogates transformation by MCV T antigens in soft agar. Likewise, MKL-1 MCC cells could sustain growth in soft agar, but addition of the LSD1 inhibitor completely blocked it. The results indicate that transformation by MCV T antigens depend on LSD1 activities.

**FIG. 23A** shows that Soft agar assay of T-antigen-transformed IMR90 cells and MKL-1 MCC cells with an LSD1 inhibitor (GSK-LSD1, 0.01 μM) shows that transformation of normal cells by MCV T antigens depends on LSD1 activities. 293T cell line growth in soft agar did not get affected by LSD1 inhibition. **FIG. 23B** shows quantification of 3 biological replicas of experiments.

**EXAMPEL 16. LSD1 inhibition decreases growth of virus-positive MCC cell lines *in vivo*.**

To test the efficacy of LSD1 inhibition *in vivo*, MKL-1 and WaGa virus-positive MCC cells were implanted as xenografts in severe combined immunodeficient (SCID)

mice. When the tumor reached the size of 200 $mm^3$, the mice were treated with an LSD1 inhibitor CPI-670242 (CPI-242). It has been determined that LSD1 inhibition significantly decreased tumor growth from two distinct MCC cell lines in SCID mice.

**FIG. 24** shows that MKL-1 and WaGa virus-positive MCC cell lines grown as xenografts in SCID mice display sensitivity to LSD1 inhibition. The CPI-242 (40 mg/kg) was administered orally once a week starting when xenograft tumors were 200$mm^3$. The study was terminated when the tumor volume reached maximum permissible size.

The table below shows xenograft efficacy study statistics for data shown in **FIG. 24**.

**Table 1**

| Body Weight (g) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Group | F | M | T | Mean | StdDev | Min | Max | % Diff |
| 1. Vehicle | 8 | 0 | 8 | 22.24 | 2.42 | 19.20 | 25.90 | 2.78 |
| 2. CPI-242, 40 mg/kg | 8 | 0 | 8 | 21.08 | 1.68 | 19.00 | 23.80 | 2.59 |
| 3.Vehicle PD | 3 | 0 | 3 | 21.53 | 0.68 | 21.00 | 22.30 | 0.48 |
| 4. CPI-242, 40 mg/kg PD | 3 | 0 | 3 | 21.70 | 0.61 | 21.00 | 22.10 | 0.29 |
| Brown-Forsythe: | | W = 4.0693 | | P = 0.0227 | | | | |
| ANOVA: | | F = 0.5233 | | P = 0.6718 | | | | |
| Tumor Volume ($mm^3$) | | | | | | | | |
| Group | F | M | T | Mean | StdDev | Min | Max | % Diff |
| 1. Vehicle | 8 | 0 | 8 | 726.26 | 307.81 | 377.60 | 1218.43 | 28.14 |
| 2. CPI-242, 40 mg/kg | 8 | 0 | 8 | 362.00 | 147.82 | 211.42 | 598.04 | 36.13 |
| 3.Vehicle PD | 3 | 0 | 3 | 647.35 | 327.39 | 275.70 | 893.10 | 14.22 |
| 4. CPI-242, 40 mg/kg PD | 3 | 0 | 3 | 531.47 | 148.05 | 377.58 | 672.89 | 6.23 |
| Brown-Forsythe: | | W = 0.9866 | | P = 0.4212 | | | | |
| ANOVA: | | F = 3.1263 | | P = 0.0515 | | | | |

**EXAMPEL 17. Comparison of Pathways repressed by LSD1-coREST and upregulated by EP400 depletion in MKL-1 virus-positive MCC cell line.**

To elucidate the mechanism behind LSD1 inhibition sensitivity in MCC, experiments were performed to determine LSD1 targets in MCC. RNA-seq of six virus-positive (MKL-1, MKL-2, MS-1, WaGa, Peta and BroLi) and UISO virus-negative MCC cell lines were performed following one or three days of LSD1 inhibition. The RNA-seq data were correlated with ChIP-seq of LSD1 to define LSD1 targets in MCC. It has been

found that pathways upregulated by EP400 depletion, such as neuron development and BMP and TGF pathways are also enriched with genes repressed MCC.

The table below shows selected GOTERM biological processes. Values are –log10 of p-values. Comparison between pathways repressed by LSD1 and induced by EP400 depletion shows overlap in GO Biological Terms associated with Pathways in neuronal differentiation and in TGF Beta WNT, MAPK, and BMP/SMAD signaling,

**Table 2. GOTERM Biological Terms (-Log10 of p-value)**

| Pathways | Repressed by LSD1 | Induced by EP400 depletion |
|---|---|---|
| synaptic transmission | 8.903396995 | 6.671620397 |
| axon guidance | 6.634514331 | 2.632343718 |
| axonogenesis | 4.803845395 | 6.607303047 |
| neuron migration | 3.962077846 | 2.143875919 |
| liver development | 3.05122549 | 1.610631156 |
| neuron projection morphogenesis | 3.036644378 | 5.684029655 |
| Wnt signaling pathway | 3.02296652 | 2.995883782 |
| neuron projection development | 2.726421353 | 5.508638306 |
| cell migration | 2.623860488 | 6.002176919 |
| negative regulation of cell migration | 2.453514826 | 3.207608311 |
| mechanoreceptor differentiation | 2.371971989 | 1.798421766 |
| receptor kinase signaling pathway | 2.287073075 | 5.210419288 |
| muscle cell differentiation | 2.274847561 | 2.923787873 |
| angiogenesis | 2.241596712 | 3.88941029 |
| SMAD protein signal transduction | 2.020570924 | 1.125652817 |
| axon regeneration | 1.921813703 | 1.149073646 |
| TGF beta receptor signaling pathway | 1.65995231 | 2.483267817 |
| activation of MAPK activity | 1.56315665 | 2.827394442 |
| negative regulation of cell proliferation | 1.562948452 | 2.199919093 |
| glial cell differentiation | 1.35952149 | 1.297741337 |
| ion transport | 1.35151362 | 4.031517051 |
| BMP signaling pathway | 1.278256637 | 2.155120651 |
| positive regulation of transcription | 1.2661817 | 3.301899454 |
| cell adhesion | 1.138785637 | 15.12726117 |
| regulation of neurogenesis | 1.127816811 | 3.296708622 |
| blood vessel development | 1.101934868 | 7.315154638 |

**EXAMPEL 18. Integrated ChIP- and RNA-seq LSD1-coREST targetome analysis reveals that LSD1-coREST regulates neuronal differentiation pathways in MCC.**

By performing RNA-seq of MCC cell lines, it has been determined that similar gene expression changes occur in all six virus-positive cell lines but not in UISO virus-negative cell line during LSD1 inhibition. For example, genes such as SMAD9, FAM5B and ZNF781 were upregulated by LSD1 inhibition in the virus-positive cell lines but not in UISO. This suggests that LSD1 inhibition causes gene expression changes associated growth inhibition in virus-positive but not in virus-negative MCC cells.

RNA-seq was performed for six virus-positive MCC (MKL-1, MKL-2, MS-1, WaGa, PeTa and BroLi) and virus-negative UISO cell lines that were treated with LSD1 inhibitors (GSK-LSD1 for 3 days or CPI-242 for 1 day). Heatmap reveals that similar gene expression changes occur in all virus-positive cell lines but not in UISO during LSD1 inhibition (**FIG. 26**).

**EXAMPEL 19. LSD1 inhibition specifically activates gene and protein expression in virus-positive MCC cells.**

Bone morphogenetic proteins (BMPs) regulate essential processes governing embryonic patterning and neural and skin development. BMP proteins, as soluble factors, bind to their receptors to initiate active BMP signaling. This promotes phosphorylation of SMAD1, SMAD5 and SMAD9, which in turn oligomerize with SMAD4 to transactivate specific genes such as Inhibitors of DNA binding (IDs).

The LSD1 targetome analysis predicted that ID1, ID2 and ID3, along with SMAD9 BMP signaling genes are prominent LSD1 targets in MCC cancer cell lines. It has been confirmed by RT-qPCR and western blotting following LSD1 inhibition of various time periods that ID1 and SMAD9 along with FAM5B, CDH11, CALB2, PROM1 and SMARCA1 are targets of LSD1. LSD1 inhibition in MKL-1 and WaGa MCC cell lines dramatically increased phosphorylation of SMAD1, 5 and 9 and ID1 protein levels, suggesting that LSD1 inhibition activates the BMP pathway.

**FIG. 27A** shows RT-qPCR analysis of selected LSD1-coREST target genes MKL-1 and WaGa (virus-positive MCC) and UISO (virus-negative MCC) cell lines treated with CPI-242 for 1 day. Western blot of MKL-1 and WaGa virus-positive MCC

cell lines. Cells were treated with LSD inhibitor GSK-LSD1 (0.05 μM) for 3 or 6 days. SMAD9 total protein levels do not change with LSD1 inhibition. LSD1 inhibition activates BMP pathway as assessed by increased levels of phosphorylated SMAD1/5/9 (P-SMAD1/5/9). LSD1 inhibition increases levels of LSD1 targets SMARCA1 and ID1 (**FIG. 27B**).

**EXAMPEL 20. Multiplexed Isobaric Tag-Based Profiling of MKL-1 virus-positive MCC cell line treated with GSK-LSD1 for 8 days.**

To examine changes in protein expression during LSD1 inhibition, multiplexed isobaric tag-based profiling was performed for MKL-1 virus-positive MCC cell line treated with GSK-LSD1 for 8 days. It has been determined that LSD1 inhibition led to major perturbations in protein expression shown by the volcano plot. Also notably, protein levels of predicted LSD1 targets such as CALB2, PROM1, FAM5B, DLL1, and GFI1 increased significantly.

**FIG. 28** shows changes in differentially expressed proteins for MKL-1 virus-positive MCC cell line treated with GSK-LSD1. Levels of proteins identified with arrows were significantly changed in levels (fold changed) and reflect neuronal differentiation. Proteomic analysis was performed with 5 biological replicas.

**EXAMPEL 21. ChIP-qPCR of LSD1 in MKL-1 MCC cells shows that LSD1 enrichment decreases following LSD1 inhibition.**

It has been determined that LSD1 enrichment to the LSD1 target genes decreased following LSD1 inhibition. It has been suggested that LSD1 inhibitors evict LSD1 from chromatin in acute myelogenous leukemia (AML) cells (McGrath, John P., et al. "Pharmacological inhibition of the histone lysine demethylase KDM1A suppresses the growth of multiple acute myeloid leukemia subtypes." Cancer research (2016)). The results provide evidence for the model in which LSD1 inhibitors decrease LSD1 DNA occupancy and rapidly activate expression of target genes.

In this example, MKL-1 cells were treated with GSK-LSD1 for 3 days. Chromatin was harvested and prepared for Chromatin Immunoprecipitation (ChIP) with LSD1 antibodies followed by quantitative PCR (qPCR) assessment of binding to specific target

gene promoters. The experiment was performed in triplicate. Levels of LSD1 binding to the promoters of ID1, ID2, SMAD9, ZNF781, HES1 and DLL1 are significantly decreased after treatment with LSD1 inhibitor (**FIG. 29**).

## OTHER EMBODIMENTS

It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

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**WHAT IS CLAIMED IS:**

1. A method of treating a human subject having, or at risk of developing, a Merkel cell polyomavirus (MCPyV)-positive Merkel cell carcinoma (MCC), the method comprising administering to the human subject an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor.

2. The method of claim 1, wherein the LSD1 inhibitor is an antisense molecule, a miRNA, a shRNA, an antibody, or a small molecule.

3. The method of claim 1, wherein the LSD1 inhibitor is GSK2879552, GSK-LSD1, ORY-1001 (RG6016), IMG-7289, CPI-242 or INCB059872.

4. The method of claim 1, further comprising administering to the human subject a surgery, a radiotherapy or a chemotherapy.

5. The method of claim 1, further comprising administering to the human subject an immune checkpoint inhibitor.

6. The method of claim 1, further comprising administering to the human subject an immune checkpoint inhibitor selected from the group consisting of Avelumab, Pembrolizumab, Nivolumab, and Ipilimumab.

7. The method of claim 1, further comprising administering to the human subject a Myc inhibitor.

8. The method of claim 7, wherein the Myc inhibitor is Omomyc.

9. A method of treating a human subject having, or at risk of developing, a Merkel cell polyomavirus (MCPyV)-positive Merkel cell carcinoma (MCC), the method comprising

administering to the human subject an effective amount of an inhibitory nucleic acid targeting one or more genes selected from the group consisting of EP400, MYCL, MAX, Small T antigen, LSD1, RCOR2, and INSM1.

10. The method of claim 9, wherein the inhibitory nucleic acid targets LSD1, RCOR2, or INSM1.

11. A pharmaceutical composition comprising a LSD1 inhibitor and an immune checkpoint inhibitor.

12. The composition of claim 11, wherein the LSD1 inhibitor is an antisense molecule, a miRNA, a shRNA, an antibody, or a small molecule.

13. The composition of claim 11, wherein the LSD1 inhibitor is GSK2879552, GSK-LSD1, ORY-1001 (RG6016), IMG-7289, CPI-242 or INCB059872.

14. The composition of claim 11, wherein the immune checkpoint inhibitor is selected from the group consisting of Avelumab, Pembrolizumab, Nivolumab, and Ipilimumab.

15. A method of determining whether a MCC tumor is Merkel cell polyomavirus (MCPyV)-positive, the method comprising:
obtaining a tumor tissue sample from a subject;
contacting the tumor tissue with an antibody that specifically binds MCPyV T antigen; and
determining that the tumor tissue expresses MCPyV T antigen.

16. The method of claim 15, further comprising contacting the tumor tissue sample with an antibody that binds to one or more antigens selected from the group consisting of RCOR2, LSD1, INSM1, and ATOH1; and
determining that the tumor tissue expresses the one or more antigens.

17. A method of determining whether a MCC tumor is Merkel cell polyomavirus (MCPyV)-positive, the method comprising:
obtaining a tumor tissue sample from a subject;
measuring the levels of LSD1+8a mRNA in the tumor tissue sample; and
determining that the levels of LSD1+8a mRNA are higher than a control tissue sample from a subject that has a MCPyV-negative tumor.

18. The method of claim 17, wherein the levels of LSD1+8a mRNA are measured by RT-qPCR.

19. A method of determining whether a MCC tumor is Merkel cell polyomavirus (MCPyV)-positive, the method comprising:
obtaining a tumor tissue sample from a subject;
contacting the tumor tissue with an antibody that specifically binds LSD1+8a; and
determining that the tumor tissue expresses LSD1+8a at a higher level than a control tissue sample from a subject that has a MCPyV-negative tumor.

20. A method of determining whether a MCC tumor is Merkel cell polyomavirus (MCPyV)-positive, the method comprising:
obtaining a tumor tissue sample from a subject;
measuring the levels of one or more mRNA or proteins selected from the group consisting of LSD1, RCOR2, INSM1 and ATOH1 in the tumor tissue sample; and
determining that the levels of the one or more mRNA or proteins are higher than a control tissue sample from a subject that has a MCPyV-negative tumor.

21. The method of claim 20, wherein the one or more mRNAs or proteins are selected from the group consisting of LSD1, RCOR2, and INSM1.

22. A method of selecting a human subject having MCC for treatment with an LSD1 inhibitor, the method comprising determining that:

(a) tumor tissue from the human subject comprises DNA from MCPyV;

(b) tumor tissue from the human subject binds an antibody that specifically binds MCPyV T antigen;

(c) tumor tissue from the human subject binds an antibody that specifically binds at least one antigen selected from the group consisting of RCOR2, LSD1, INSM1, and ATOH1 more than a control tissue sample from a subject that has a MCPyV-negative tumor;

(d) tumor tissue from the human subject has increased levels of LSD1+8a mRNA than a control tissue sample from a subject that has a MCPyV-negative tumor;

(e) tumor tissue from the human subject binds an antibody that specifically binds LSD1+8a protein more than a control tissue sample from a subject that has a MCPyV-negative tumor; or

(f) tumor tissue from the human subject has increased levels of mRNAs or proteins selected from the group consisting of LSD1, RCOR2, INSM1, and ATOH1 than a control tissue sample from a subject that has a MCPyV-negative tumor.

23. A method for screening an agent that is useful for treating a MCPyV-positive tumor, the method comprising:
contacting the agent with a MCPyV-positive tumor cell;
determining that (1) the agent inhibits the levels of mRNAs or proteins selected from the group consisting of LSD1, RCOR2, INSM1, and ATOH1 in the tumor cell or (2) the agent increases the levels of mRNAs or proteins selected from the group consisting of ID1 and HES1; and
selecting the agent as being useful for treating a MCPyV-positive tumor.

24. The method of claim 23, wherein the agent is a small molecule.

25. The method of claim 23, wherein the agent is an antisense molecule, a miRNA, a shRNA, or an antibody.

26. A method of killing a Merkel cell polyomavirus (MCPyV)-positive cancer cell, the method comprising:
contacting the MCPyV-positive cancer cell with an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor; or
contacting the MCPyV-positive cancer cell with an effective amount of an inhibitory nucleic acid targeting one or more genes selected from the group consisting of EP400, MYCL, MAX, Small T antigen, LSD1, RCOR2, and INSM1.

27. A method of inhibiting the growth of a Merkel cell polyomavirus (MCPyV)-positive tumor, the method comprising:
contacting the MCPyV-positive tumor with an effective amount of a lysine specific demethylase 1 (LSD1) inhibitor; or
contacting the MCPyV-positive tumor with an effective amount of an inhibitory nucleic acid targeting one or more genes selected from the group consisting of EP400, MYCL, MAX, Small T antigen, LSD1, RCOR2, and INSM1.

28. The method of any one of claims 1-10 or 15-27, wherein the MCC is metastatic MCC.

29. The method of any one of claims 1-10, wherein the human subject has not received prior chemotherapy or radiotherapy.

30. The method of any one of claims 1-10, wherein the MCC is Stage IA, IB, IIA, IIC, IIIA, IIIB, or IV.

31. The method of any one of claims 1-10, wherein the MCC is relapsed MCC.

32. The method of any one of claims 1-10, wherein the human subject has previously been determined to not respond to, or weakly respond to, a therapy comprising one of a checkpoint inhibitor, chemotherapy, or radiotherapy.

33. A pharmaceutical composition comprising a LSD1 inhibitor and a chemotherapeutic or radiotherapeutic agent.

34. A method of treating MCPyV-positive MCC in a human subject in need thereof, the method comprising administering to the human subject an effective amount of a LSD1 inhibitor, wherein the human subject has previously been administered at least one of a checkpoint inhibitor, chemotherapy, or radiotherapy.

35. A method of treating MCPyV-positive MCC in a human subject in need thereof, the method comprising administering to the human subject an effective amount of a LSD1 inhibitor, wherein the human subject is subsequently administered at least one of a checkpoint inhibitor, chemotherapy, or radiotherapy.

36. The method of any one of claims 1-10, wherein the LSD1 inhibitor is administered after it is determined that a tumor sample from the human subject comprises MCPyV DNA and/or increased levels of a protein selected from the group consisting of LSD1, RCOR2, INSM1, and ATOH1 relative to a MCPyV-negative MCC tumor sample.

37. The method of any one of claims 15-21, further comprising administering an LSD1 inhibitor to the subject.

38. The method of claim 37, wherein the subject is human.

39. A method of determining that LSD1 inhibition is effective in a human subject undergoing LSD1 inhibitor therapy, the method comprising:
obtaining a tumor sample from the subject before and after treatment with an LSD1 inhibitor; and
determining that the mRNA or protein levels of NICD and/or HES1 is/are elevated in the sample obtained after treatment relative to the mRNA or protein levels of NICD and/or HES1 before treatment.

40. The method of claim 39, wherein the human subject has MCPyV-positive MCC.

41. The method of claim 39 or 40, further comprising continuing treatment with the LSD1 inhibitor.

42. A method for screening or identifying an agent that is useful for treating a MCPyV-positive tumor, the method comprising:
contacting the agent with a MCPyV-positive tumor cell;
determining that
(1) the mRNA or protein levels of one or more genes selected from the group consisting of RWDD2A, FAM13C, DPF1, SAT2, CREB5, SMARCA1, LYSMD2, ESRP2, MANSC1, LRRC49, ATP8B4, GFI1, DCHS1, CACNG5, PGBD1, ZNF781, SMAD9, QPRT, FAM5B, ARPP21, RGNEF are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;
(2) the mRNA or protein levels of one or more genes selected from the group consisting of CISD3, SLC2A4RG, POLR3K, ZBTB42, C7orf50, DYSF, HMGN2, DCK, and CLN6 are decreased in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;
(3) the mRNA or protein levels of one or more genes selected from the group consisting of FAM5B, ID1, CDH11, CALB2, and PROM1 are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;
(4) the mRNA or protein levels of ID1 and/or SMARCA1 are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;
(5) determining increased phosphorylation of SMAD1, SMAD5, and/or SMAD9 in the sample obtained after treatment relative to the sample before treatment;
(6) the mRNA or protein levels of one or more genes selected from the group consisting of ATF5, F159B, TRIB3, AKNA, CEBPB, AND ESRP2 are decreased

in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(7) the mRNA or protein levels of one or more genes selected from the group consisting of GFI1, MGP, LSAMP, DLL1, AMPH, PROM1 (CD133), CALB2, BASP1, and BRNP2(FAM5B) are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment; or

(8) the mRNA or protein levels of one or more genes selected from the group consisting of ID1, ID2, ZNF781, HES1, and DLL1 are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment, and

selecting the agent as being useful for treating a MCPyV-positive tumor, and optionally, administering the agent to a human subject in need of treatment for a MCPyV-positive tumor.

43. A method of determining that LSD1 inhibition is effective in a human subject undergoing LSD1 inhibitor therapy, the method comprising:

obtaining a tumor sample from the subject before and after treatment with an LSD1 inhibitor; and

(1) determining that the mRNA or protein levels of one or more genes selected from the group consisting of RWDD2A, FAM13C, DPF1, SAT2, CREB5, SMARCA1, LYSMD2, ESRP2, MANSC1, LRRC49, ATP8B4, GFI1, DCHS1, CACNG5, PGBD1, ZNF781, SMAD9, QPRT, FAM5B, ARPP21, RGNEF are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(2) determining that the mRNA or protein levels of one or more genes selected from the group consisting of CISD3, SLC2A4RG, POLR3K, ZBTB42, C7orf50, DYSF, HMGN2, DCK, and CLN6 are decreased in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(3) determining that the mRNA or protein levels of one or more genes selected from the group consisting of FAM5B, ID1, CDH11, CALB2, and PROM1 are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(4) determining that the mRNA or protein levels of ID1 and/or SMARCA1 are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(5) determining increased phosphorylation of SMAD1, SMAD5, and/or SMAD9 in the sample obtained after treatment relative to the sample before treatment;

(6) determining that the mRNA or protein levels of one or more genes selected from the group consisting of ATF5, F159B, TRIB3, AKNA, CEBPB, AND ESRP2 are decreased in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment;

(7) determining that the mRNA or protein levels of one or more genes selected from the group consisting of GFI1, MGP, LSAMP, DLL1, AMPH, PROM1 (CD133), CALB2, BASP1, and BRNP2(FAM5B) are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment; or

(8) determining that the mRNA or protein levels of one or more genes selected from the group consisting of ID1, ID2, ZNF781, HES1, and DLL1 are elevated in the sample obtained after treatment relative to the mRNA or protein levels of the one or more selected genes before treatment.

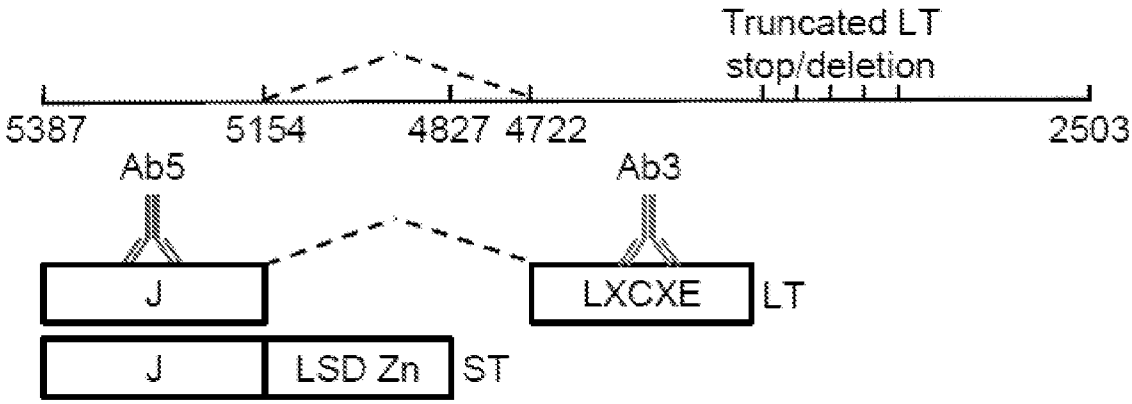

Truncated LT
stop/deletion
5387
5154
4827 4722
2503
Ab5
Ab3
J
LXCXE
LT
J
LSD Zn
ST

FIG. 1A

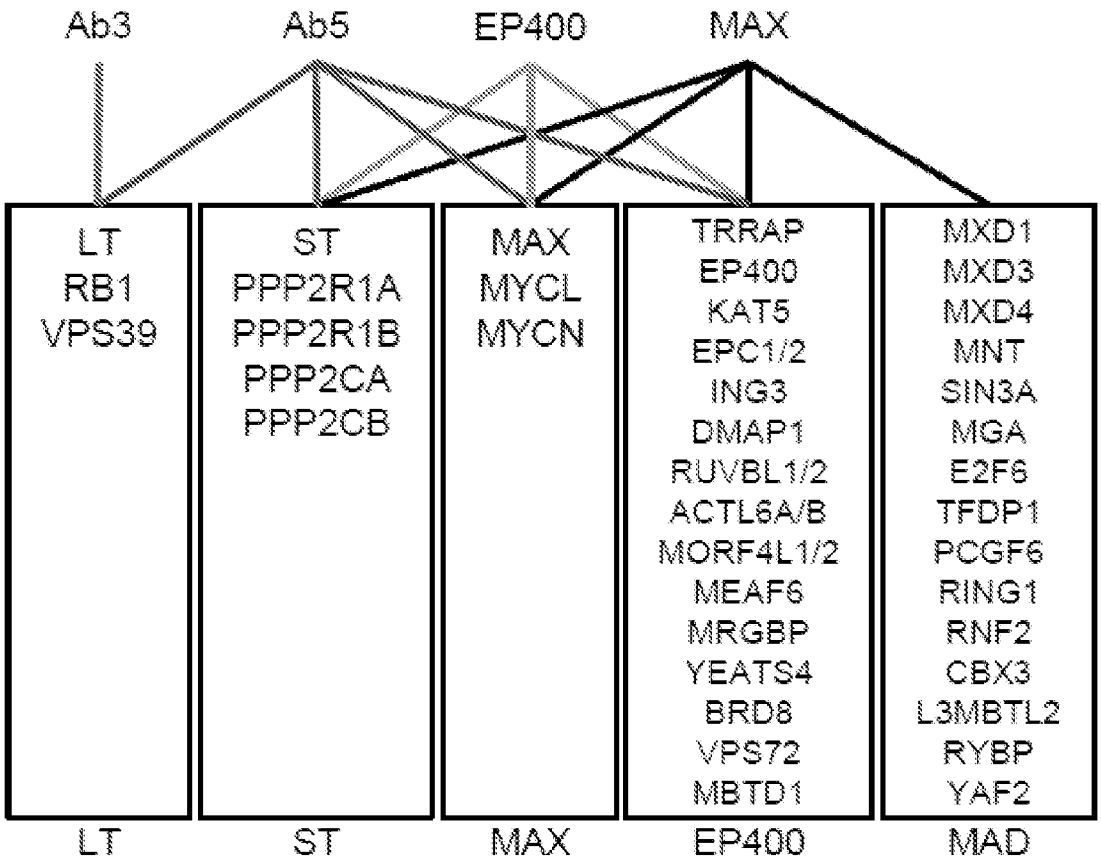

Ab3
Ab5
EP400
MAX
LT
RB1
VPS39
ST
PPP2R1A
PPP2R1B
PPP2CA
PPP2CB
MAX
MYCL
MYCN
TRRAP
EP400
KAT5
EPC1/2
ING3
DMAP1
RUVBL1/2
ACTL6A/B
MORF4L1/2
MEAF6
MRGBP
YEATS4
BRD8
VPS72
MBTD1
MXD1
MXD3
MXD4
MNT
SIN3A
MGA
E2F6
TFDP1
PCGF6
RING1
RNF2
CBX3
L3MBTL2
RYBP
YAF2
LT
ST
MAX
EP400
MAD

FIG. 1B

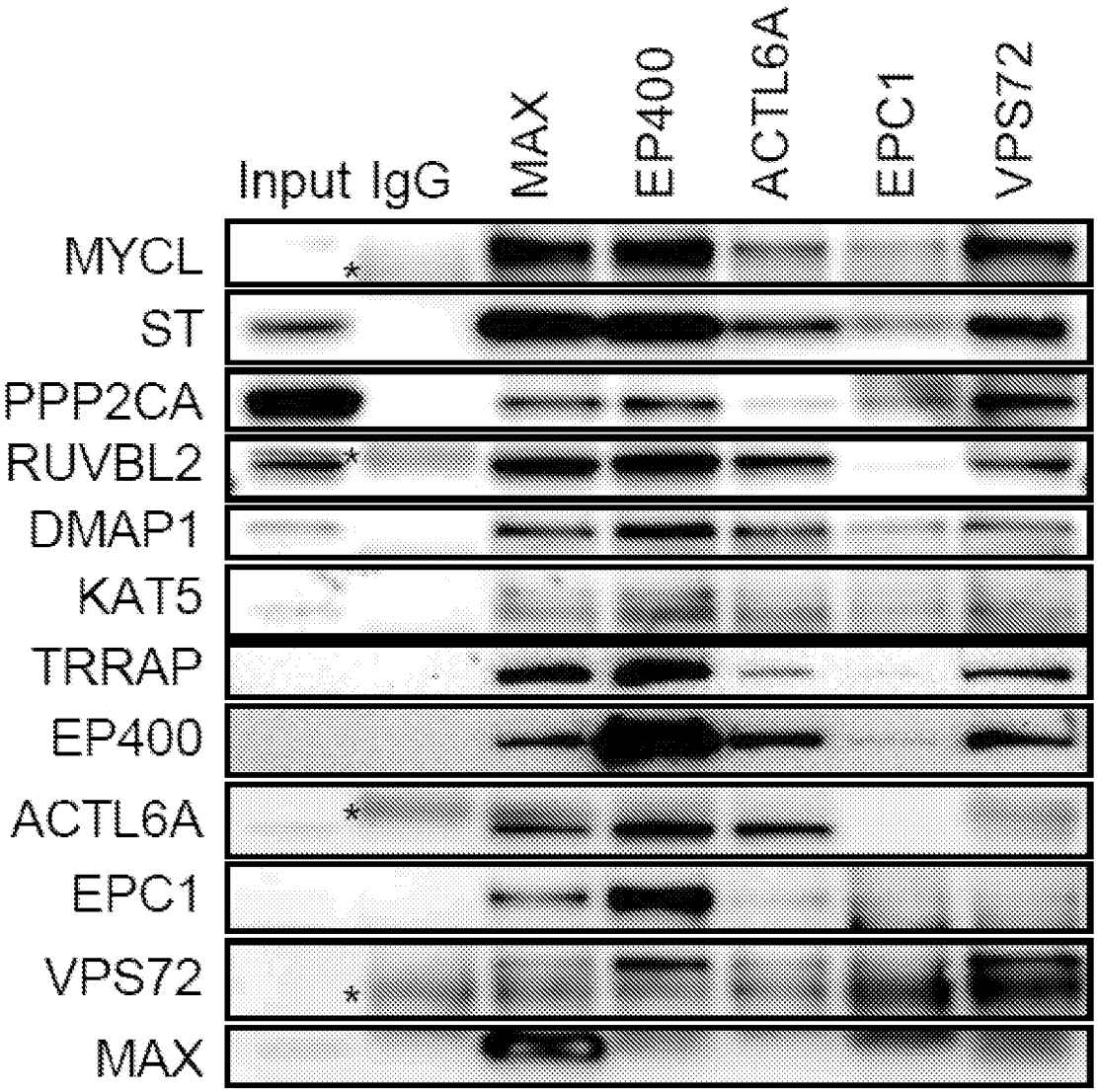

Input IgG
MAX
EP400
ACTL6A
EPC1
VPS72
MYCL
ST
PPP2CA
RUVBL2
DMAP1
KAT5
TRRAP
EP400
ACTL6A
EPC1
VPS72
MAX

FIG. 1C

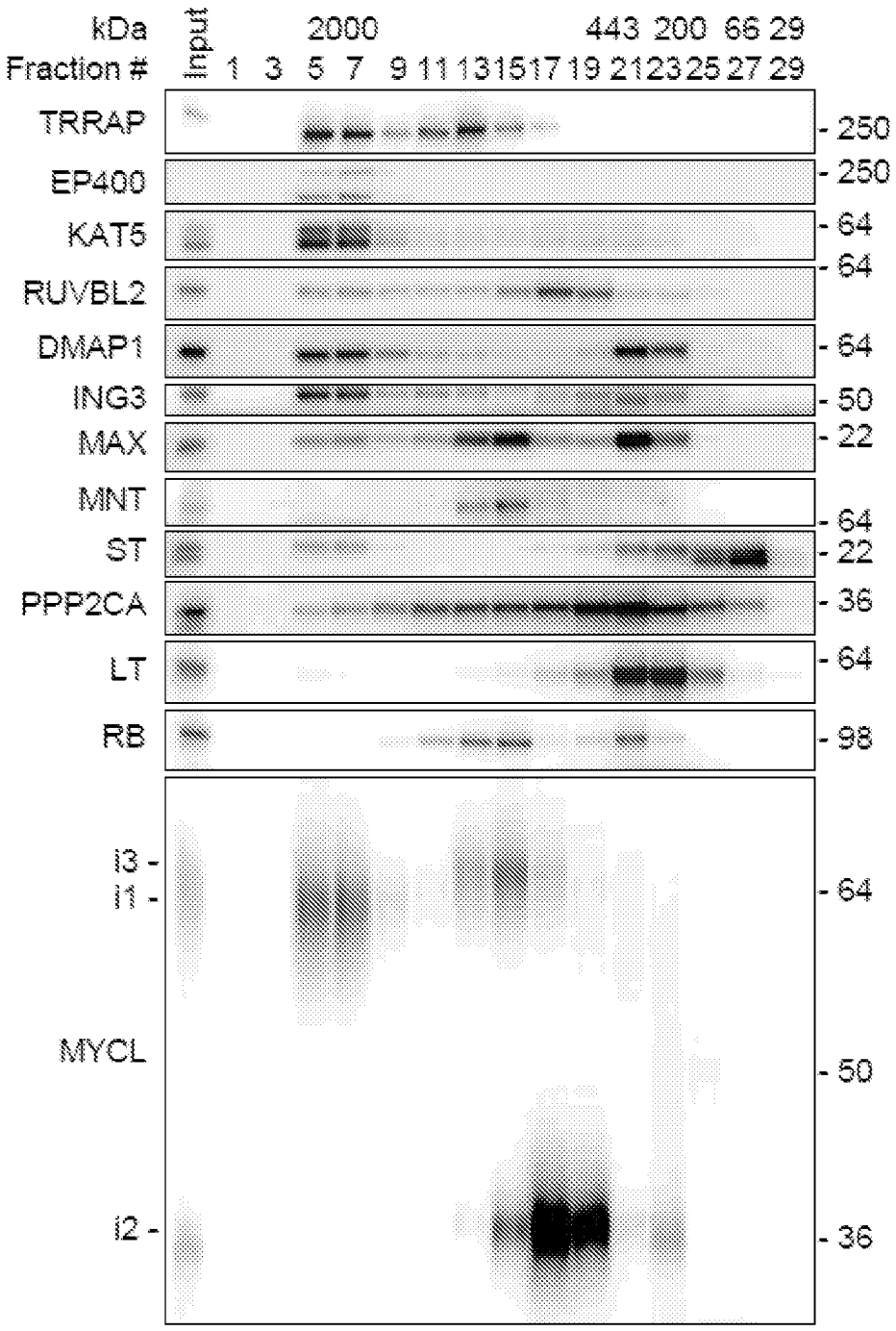

kDa
Fraction #
input
2000
443 200 66 29
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
TRRAP
EP400
KAT5
RUVBL2
DMAP1
ING3
MAX
MNT
ST
PPP2CA
LT
RB
I3 -
I1 -
MYCL
I2 -
- 250
- 250
- 64
- 64
- 64
- 50
- 22
- 64
- 22
- 36
- 64
- 98
- 64
- 50
- 36

FIG. 1D

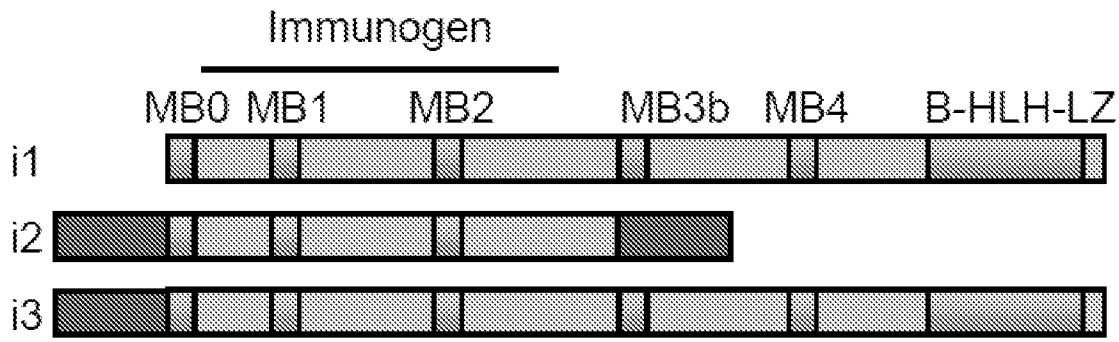

Immunogen
MB0 MB1 MB2 MB3b MB4 B-HLH-LZ
i1
i2
i3

FIG. 1E

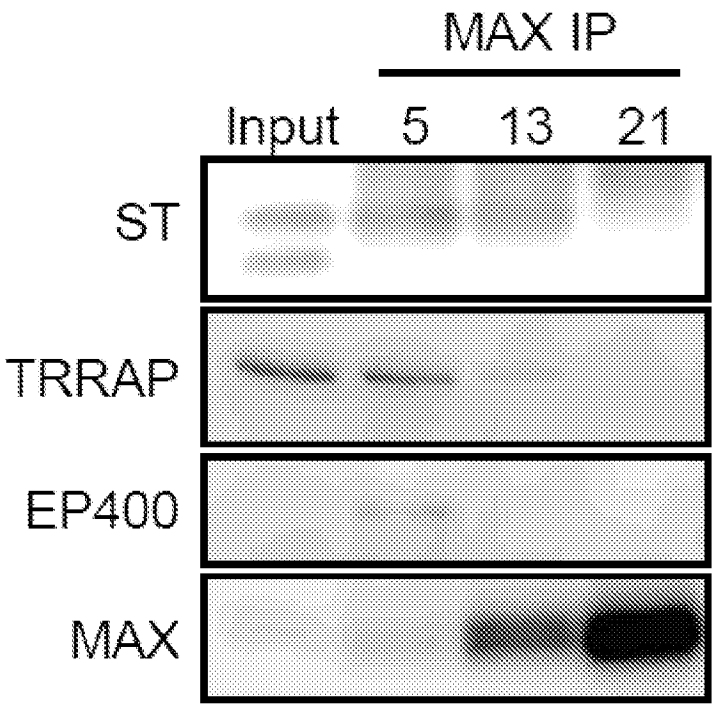

MAX IP
Input 5 13 21
ST
TRRAP
EP400
MAX

FIG. 1F

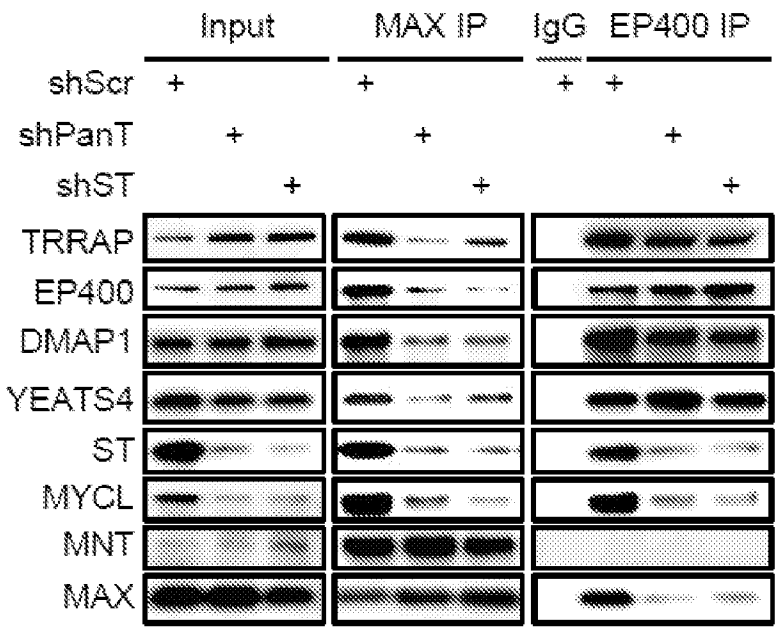

Input
MAX IP
IgG
EP400 IP
shScr
shPanT
shST
TRRAP
EP400
DMAP1
YEATS4
ST
MYCL
MNT
MAX

FIG. 2A

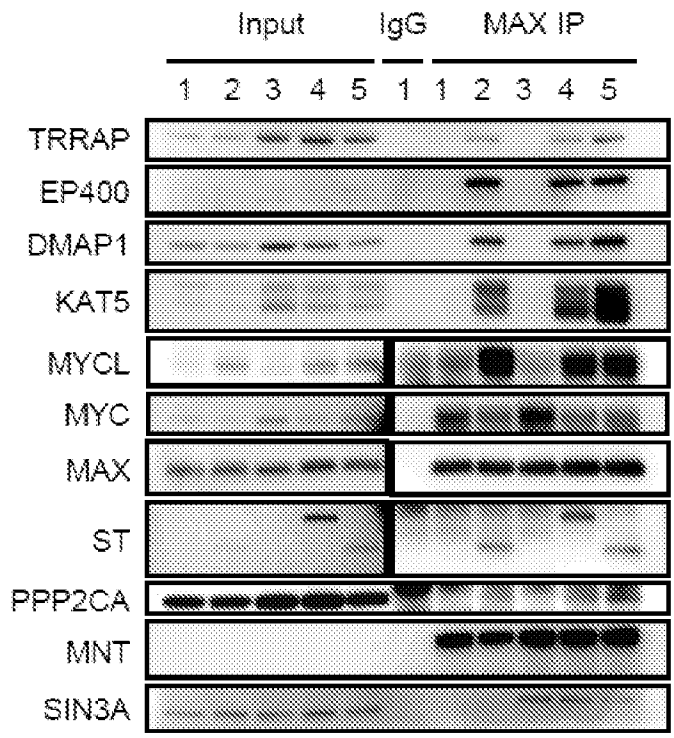

Input
IgG
MAX IP
1 2 3 4 5 1 1 2 3 4 5
TRRAP
EP400
DMAP1
KAT5
MYCL
MYC
MAX
ST
PPP2CA
MNT
SIN3A

FIG. 2B

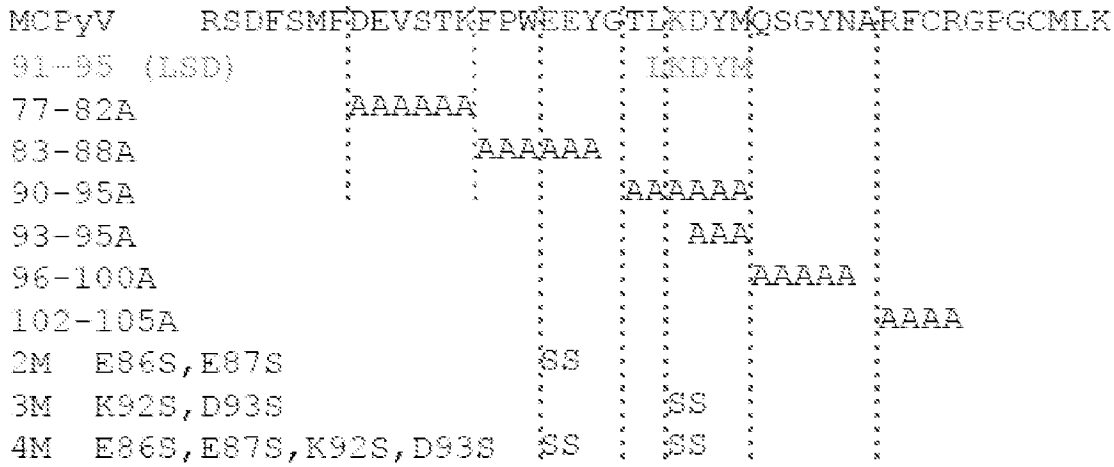

MCPyV RSDFSMFDEVSTKFPWEEYGTLKDYMQSGYNARFCRGPGCMLK
91-95 (LSD) LKDYM
77-82A AAAAAA
83-88A AAAAAA
90-95A AAAAAA
93-95A AAA
96-100A AAAAA
102-105A AAAA
2M E86S,E87S SS
3M K92S,D93S SS
4M E86S,E87S,K92S,D93S SS SS

FIG. 2C

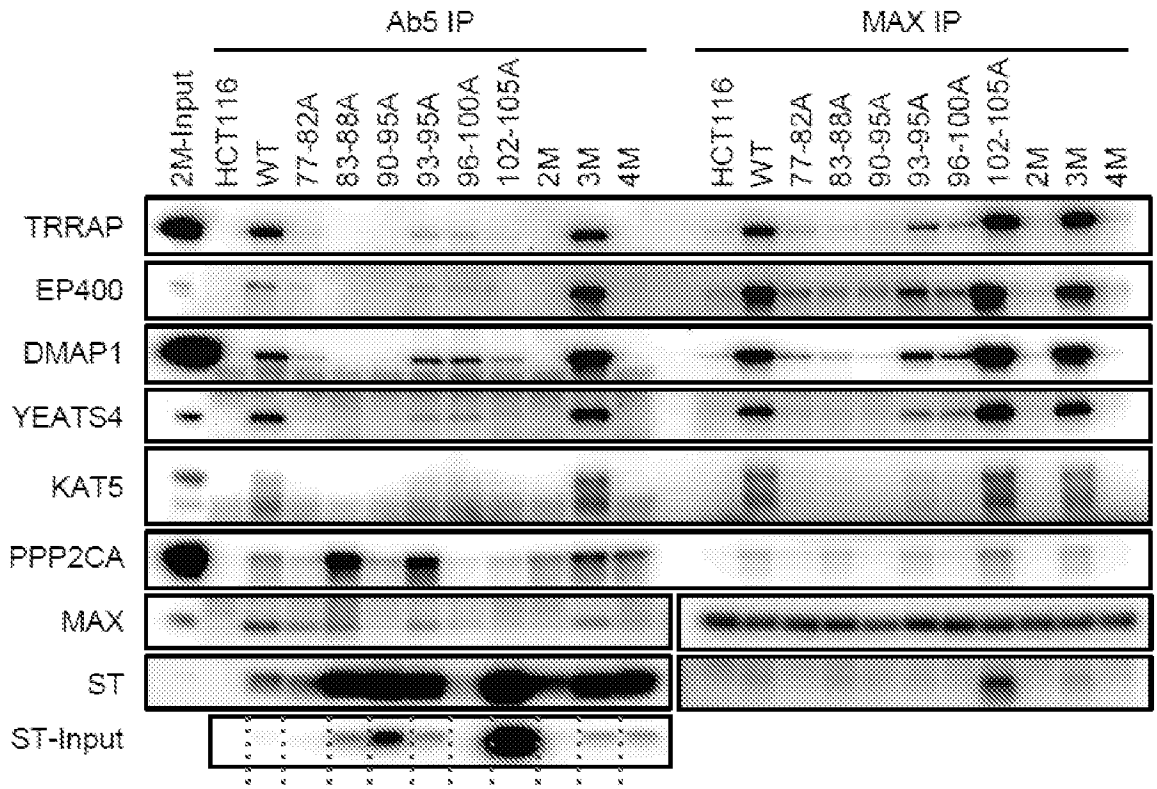

Ab5 IP
MAX IP
2M-Input
HCT116
WT
77-82A
83-88A
90-95A
93-95A
96-100A
102-105A
2M
3M
4M
TRRAP
EP400
DMAP1
YEATS4
KAT5
PPP2CA
MAX
ST
ST-Input

FIG. 2D

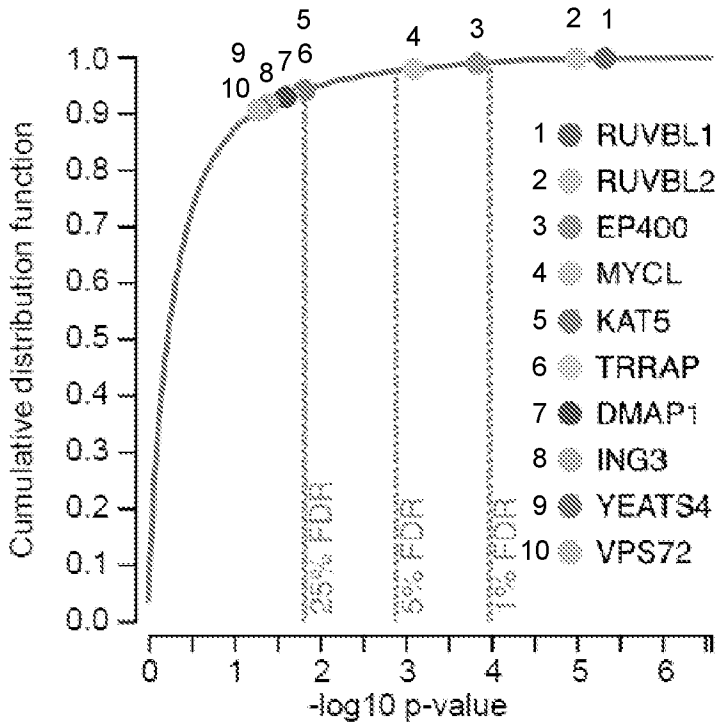

Cumulative distribution function
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
1
2
3
4
5
6
-log10 p-value
25% FDR
5% FDR
1% FDR
1 RUVBL1
2 RUVBL2
3 EP400
4 MYCL
5 KAT5
6 TRRAP
7 DMAP1
8 ING3
9 YEATS4
10 VPS72

FIG. 3A

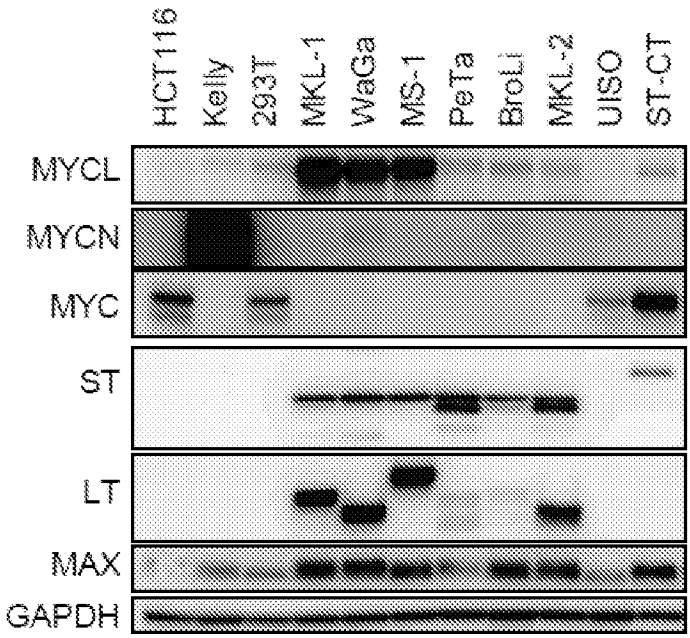

HCT116
Kelly
293T
MKL-1
WaGa
MS-1
PeTa
BroLi
MKL-2
UISO
ST-CT
MYCL
MYCN
MYC
ST
LT
MAX
GAPDH

FIG. 3B

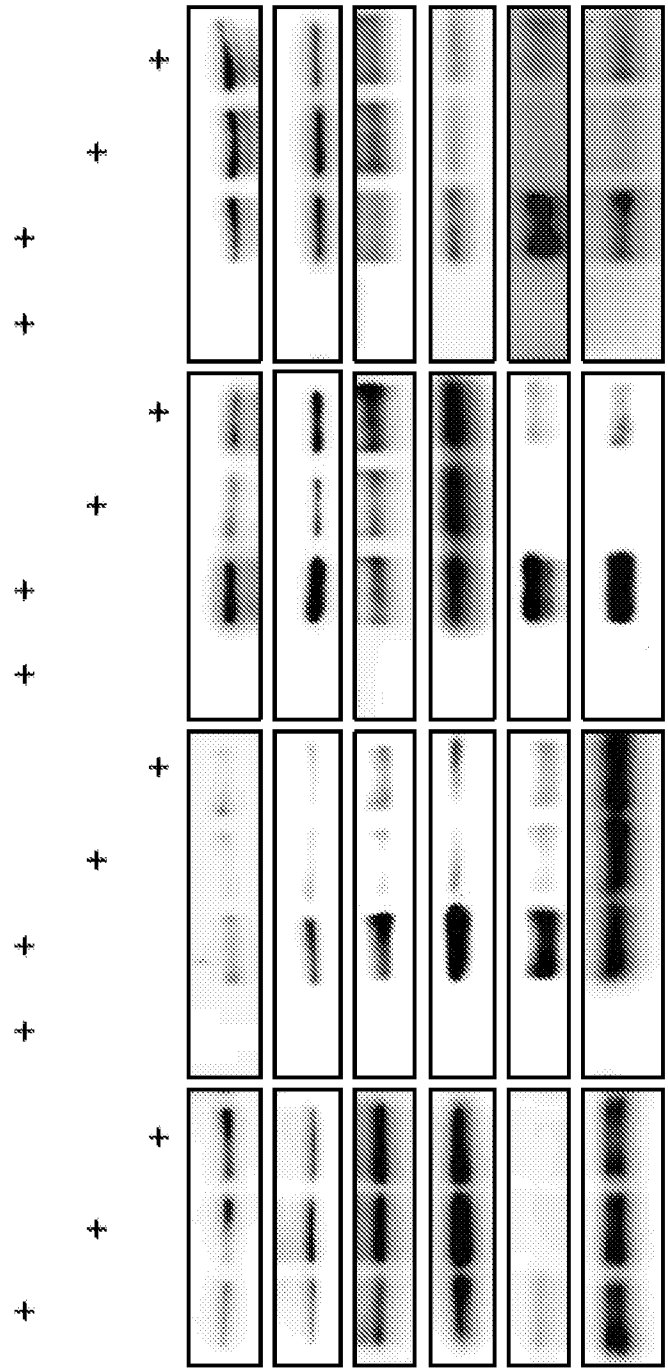

Input
IgG
MAX IP
IgG
Ab5 IP
IgG
EP400 IP
parental
shMYCL
mirMYCL
EP400
TRRAP
KAT5
ST
MYCL
MAX

FIG. 3C

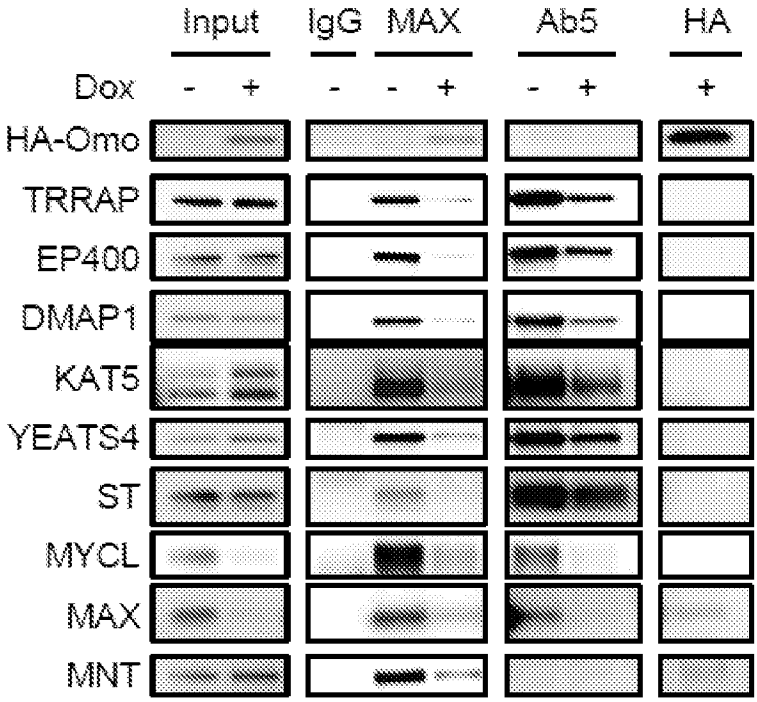

Input
IgG
MAX
Ab5
HA
Dox
- + - - + - + +
HA-Omo
TRRAP
EP400
DMAP1
KAT5
YEATS4
ST
MYCL
MAX
MNT

FIG. 3D

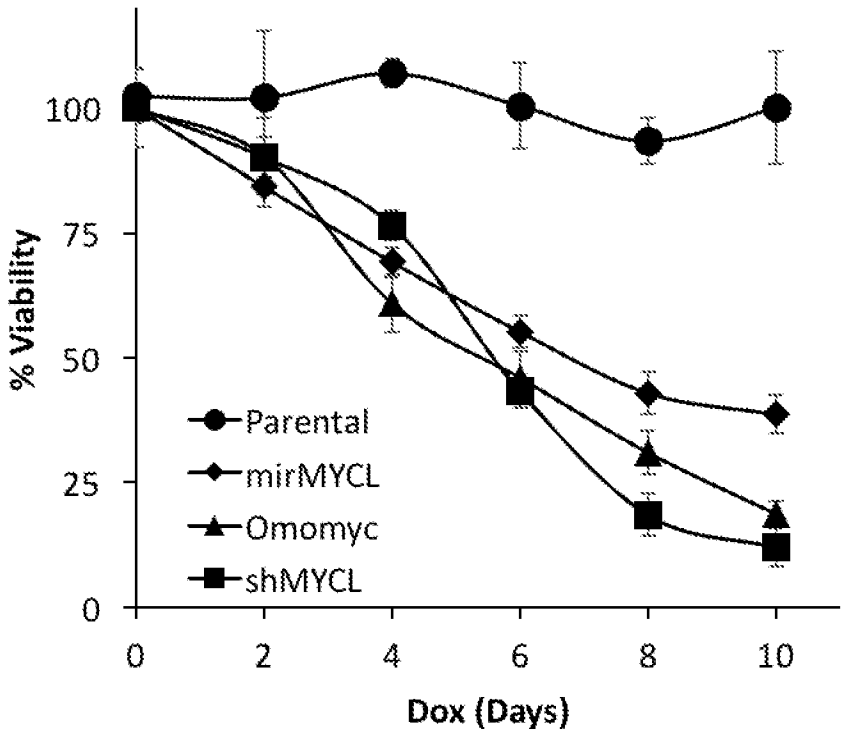

% Viability
100
75
50
25
0
0
2
4
6
8
10
Dox (Days)
Parental
mirMYCL
Omomyc
shMYCL

FIG. 3E

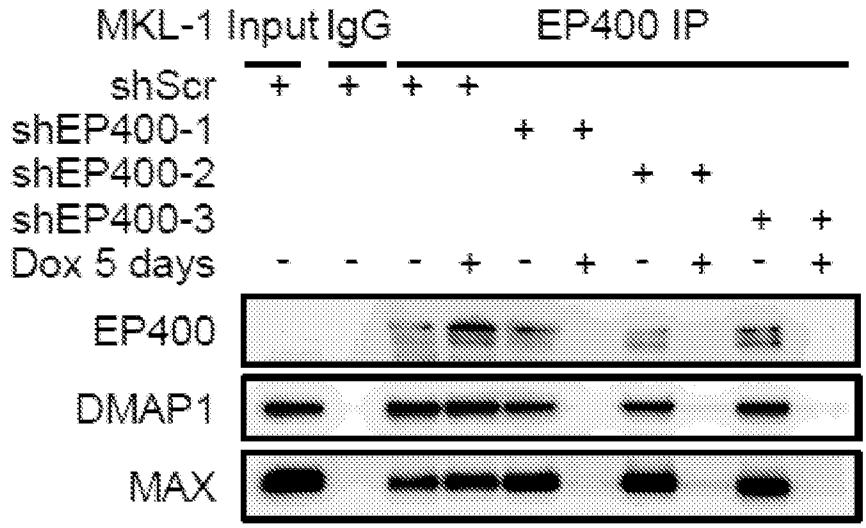

MKL-1 Input IgG EP400 IP
shScr + + + +
shEP400-1 + +
shEP400-2 + +
shEP400-3 + +
Dox 5 days - - - + - + - + - +
EP400
DMAP1
MAX

FIG. 4A

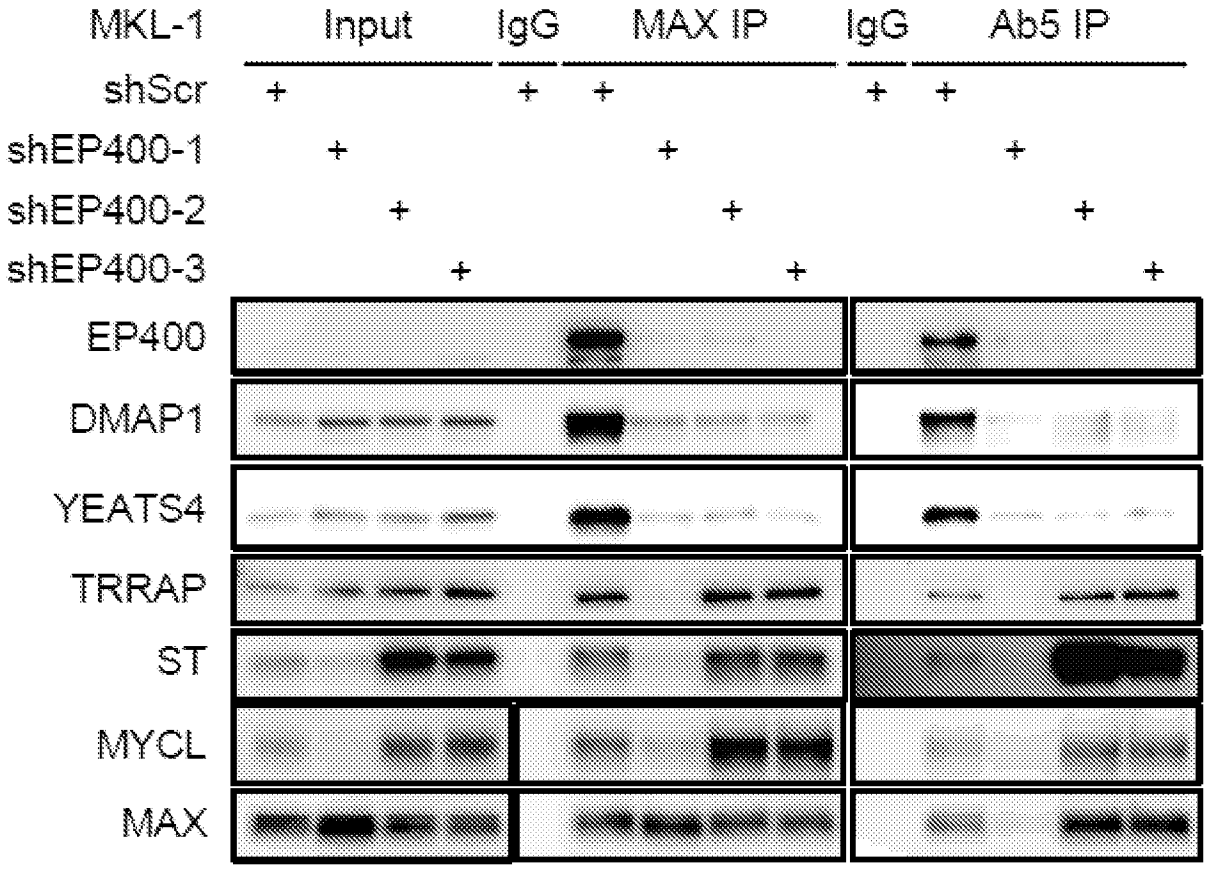

MKL-1 Input IgG MAX IP IgG Ab5 IP
shScr + + + + +
shEP400-1 + + +
shEP400-2 + + +
shEP400-3 + + +
EP400
DMAP1
YEATS4
TRRAP
ST
MYCL
MAX

FIG. 4B

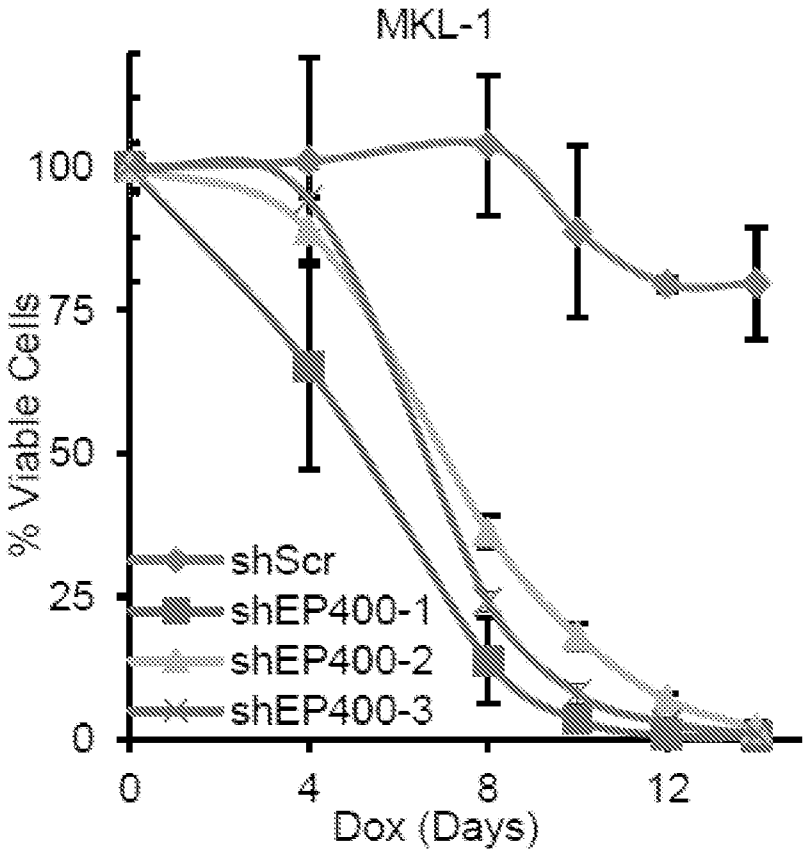

MKL-1
100
75
50
25
0
% Viable Cells
shScr
shEP400-1
shEP400-2
shEP400-3
0
4
8
12
Dox (Days)

FIG. 4C

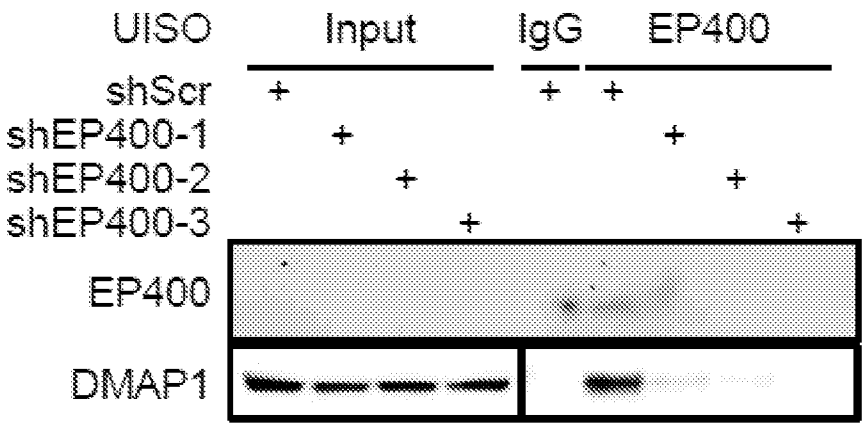

UISO
Input
IgG
EP400
shScr
shEP400-1
shEP400-2
shEP400-3
EP400
DMAP1

FIG. 4D

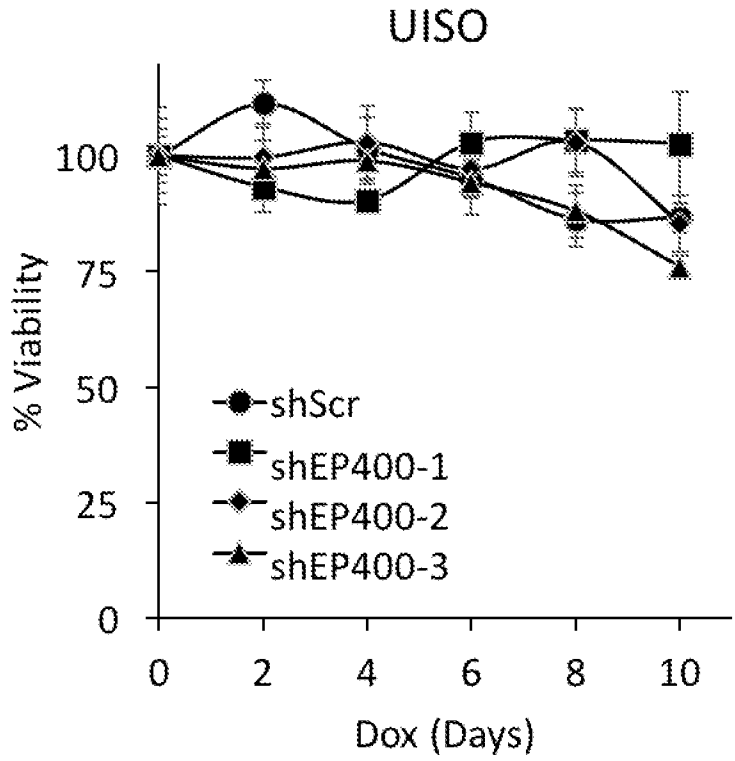

UISO
% Viability
100
75
50
25
0
shScr
shEP400-1
shEP400-2
shEP400-3
0
2
4
6
8
10
Dox (Days)

FIG. 4E

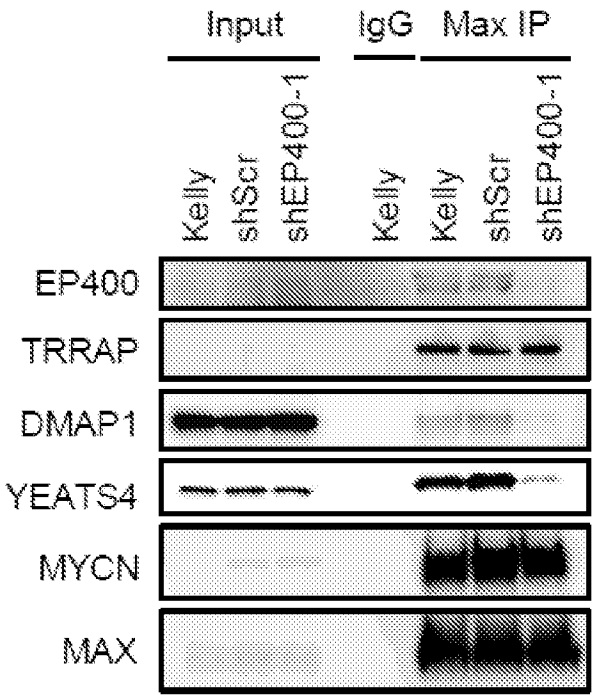

Input
IgG
Max IP
Kelly
shScr
shEP400-1
Kelly
Kelly
shScr
shEP400-1
EP400
TRRAP
DMAP1
YEATS4
MYCN
MAX

FIG. 4F

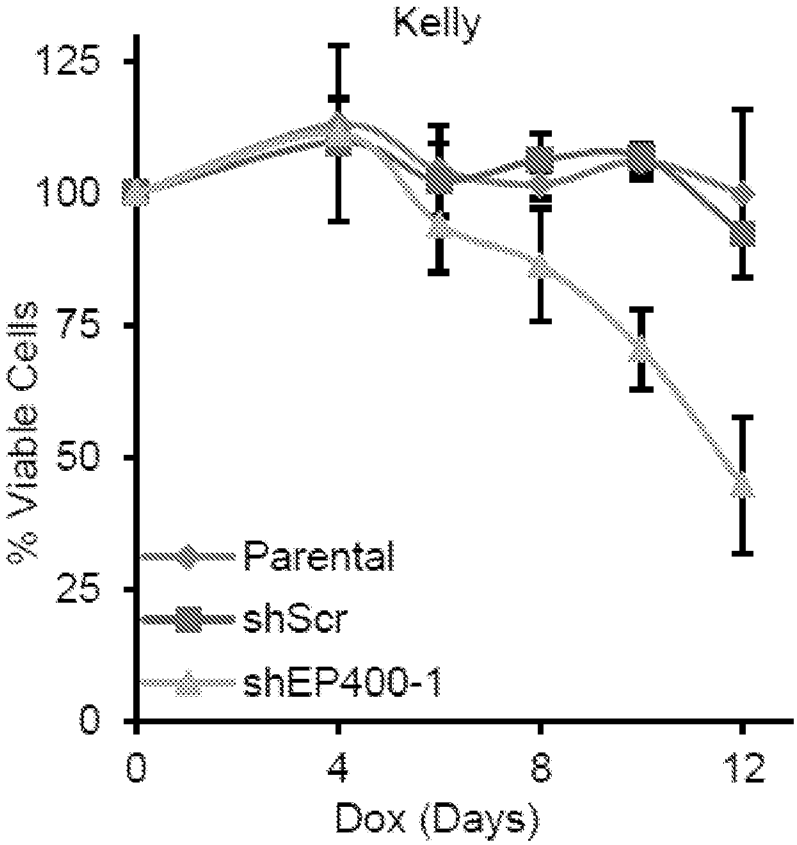

Kelly
125
100
75
50
25
0
% Viable Cells
Parental
shScr
shEP400-1
0
4
8
12
Dox (Days)

FIG. 4G

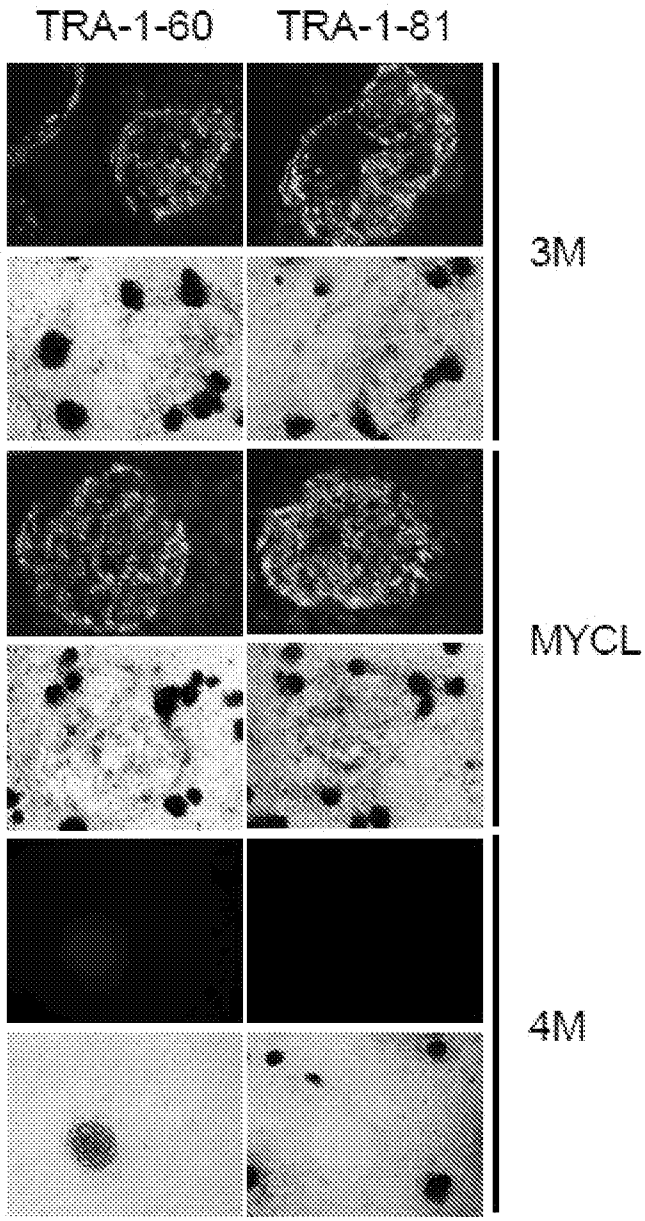



FIG. 5A

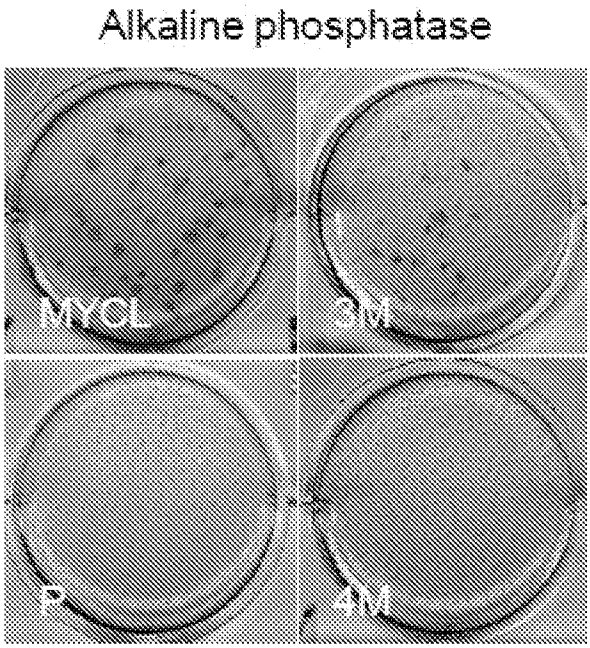

Alkaline phosphatase
MYCL
3M
P
4M

FIG. 5B

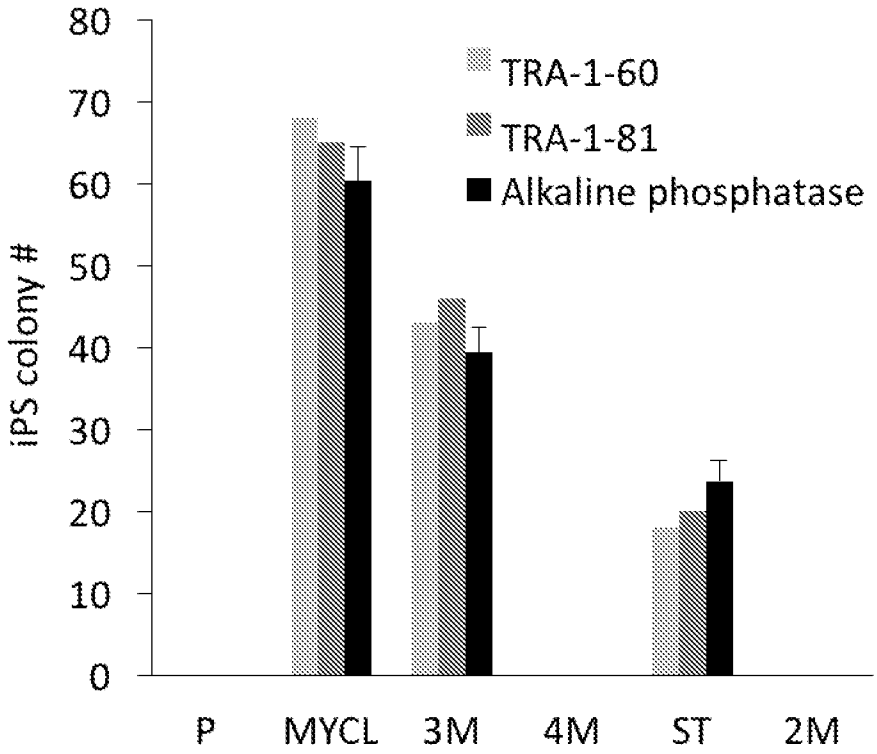

80
70
60
50
40
30
20
10
0
iPS colony #
TRA-1-60
TRA-1-81
Alkaline phosphatase
P
MYCL
3M
4M
ST
2M

FIG. 5C

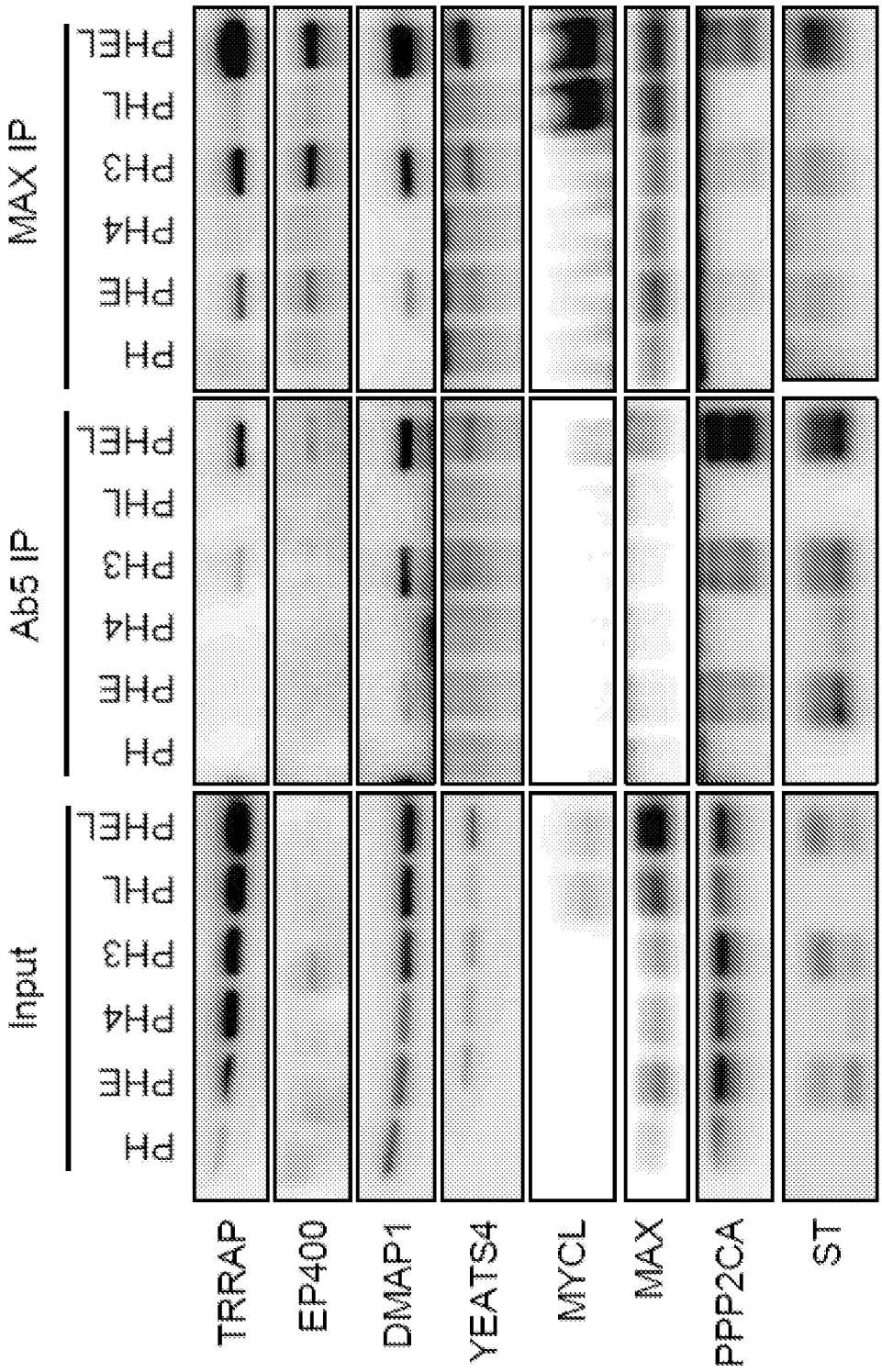

Input
Ab5 IP
MAX IP
pH
pHE
pH4
pH3
pHL
pHEL
TRRAP
EP400
DMAP1
YEATS4
MYCL
MAX
PPP2CA
ST

FIG. 5D

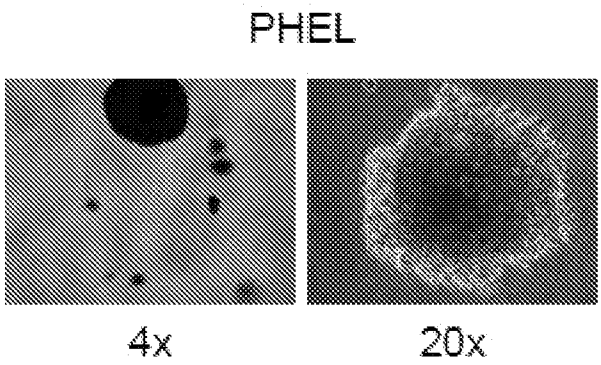

PHEL
4x
20x

FIG. 5E

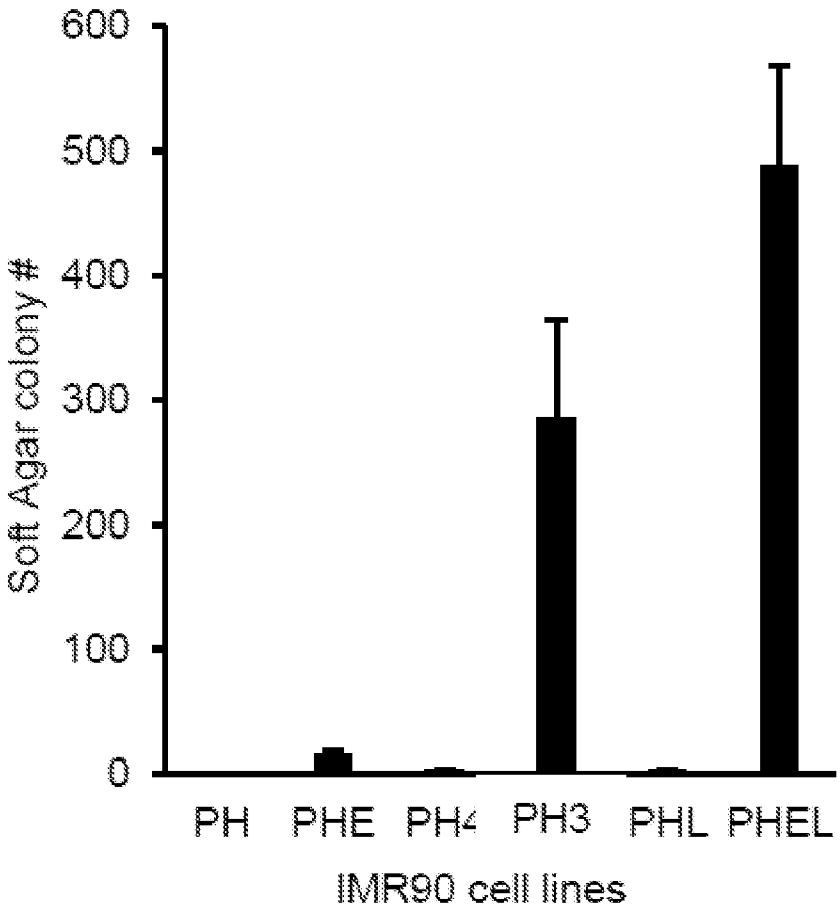

600
500
400
300
200
100
0
Soft Agar colony #
PH
PHE
PH4
PH3
PHL
PHEL
IMR90 cell lines

FIG. 5F

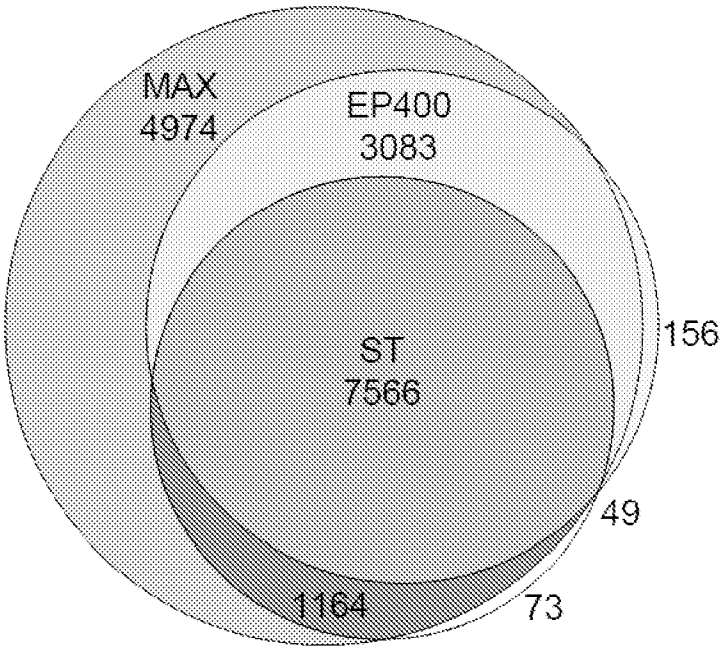



FIG. 6A

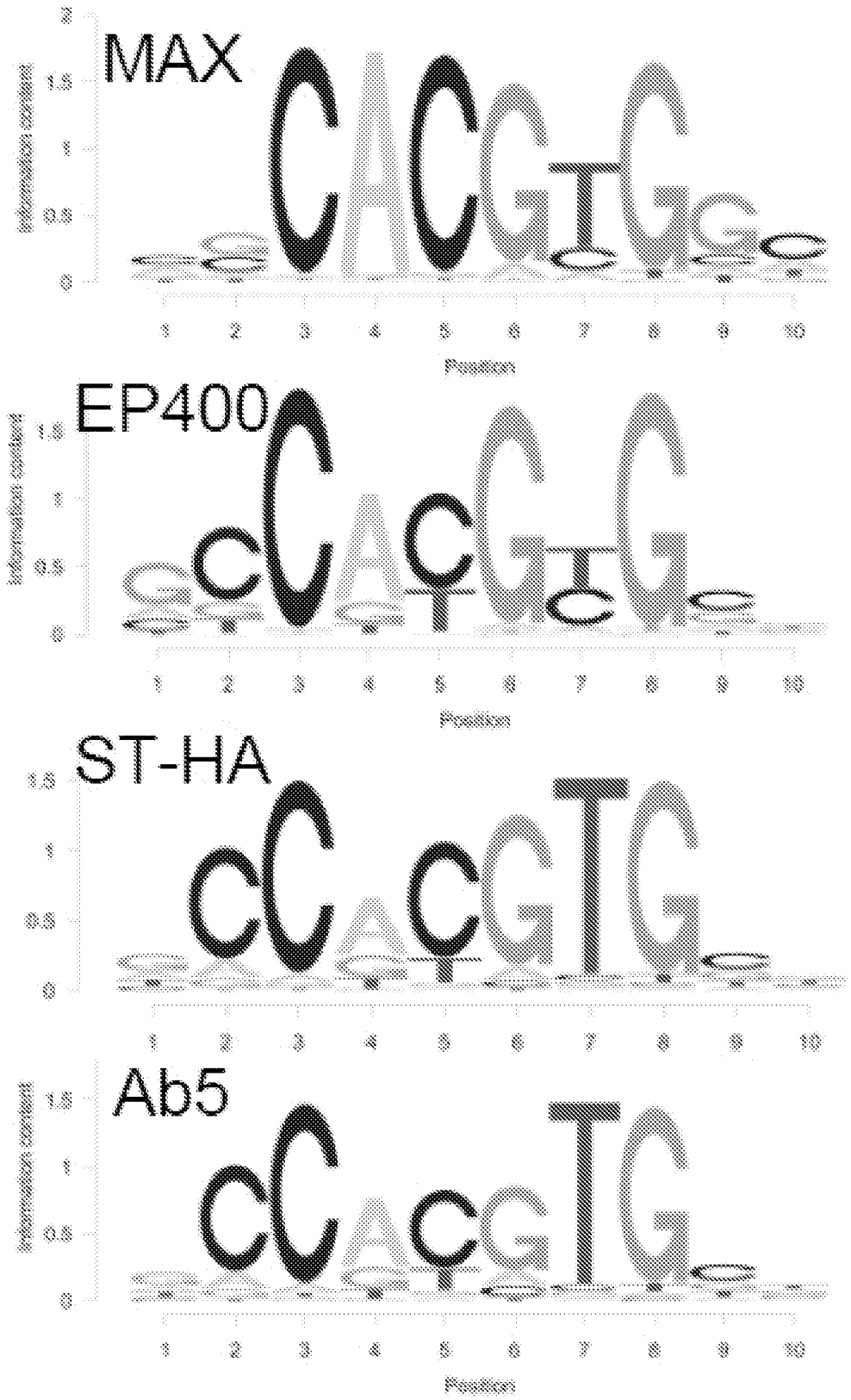



FIG. 6B

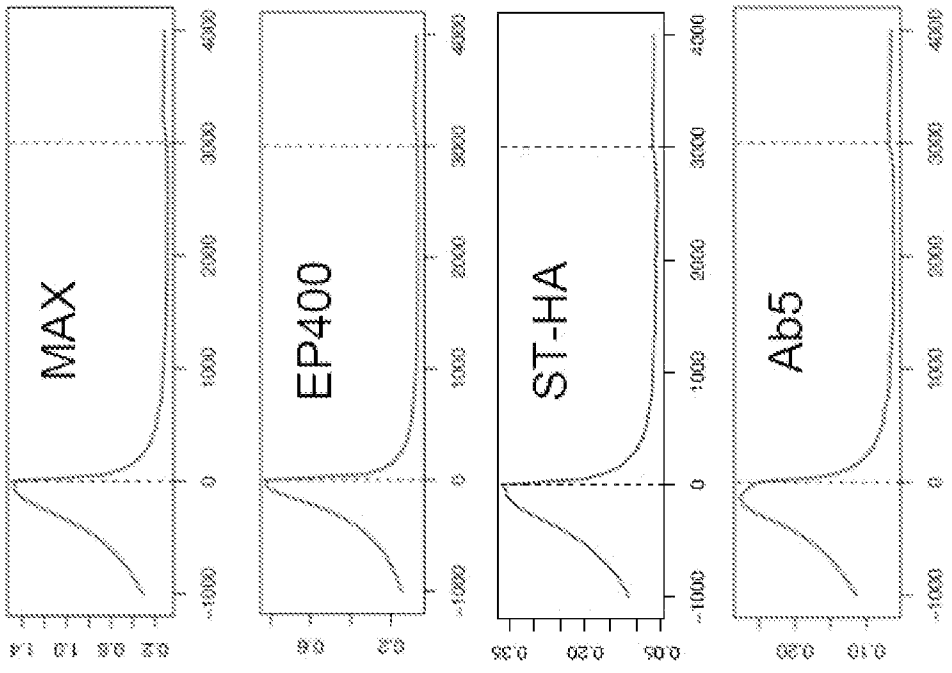



FIG. 6C

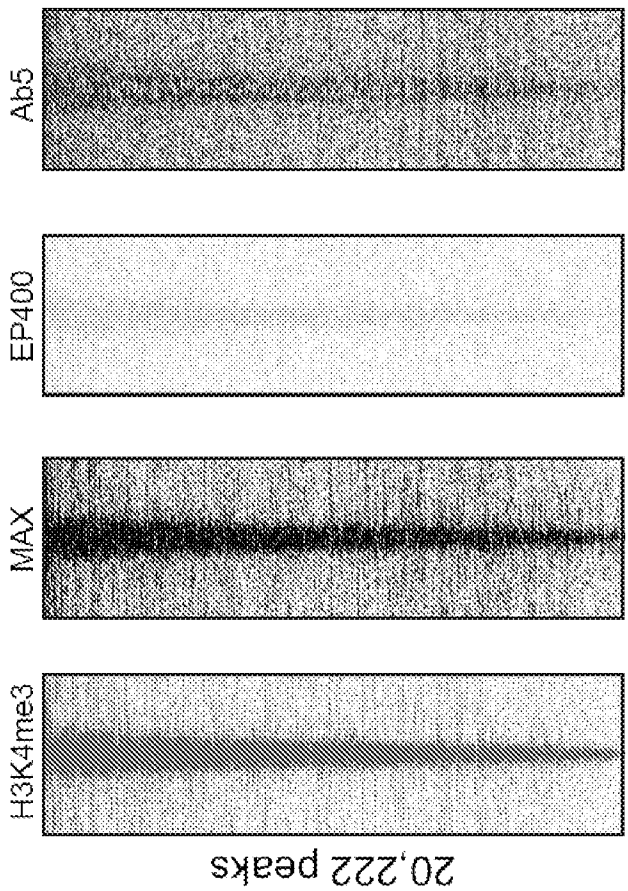



FIG. 6D

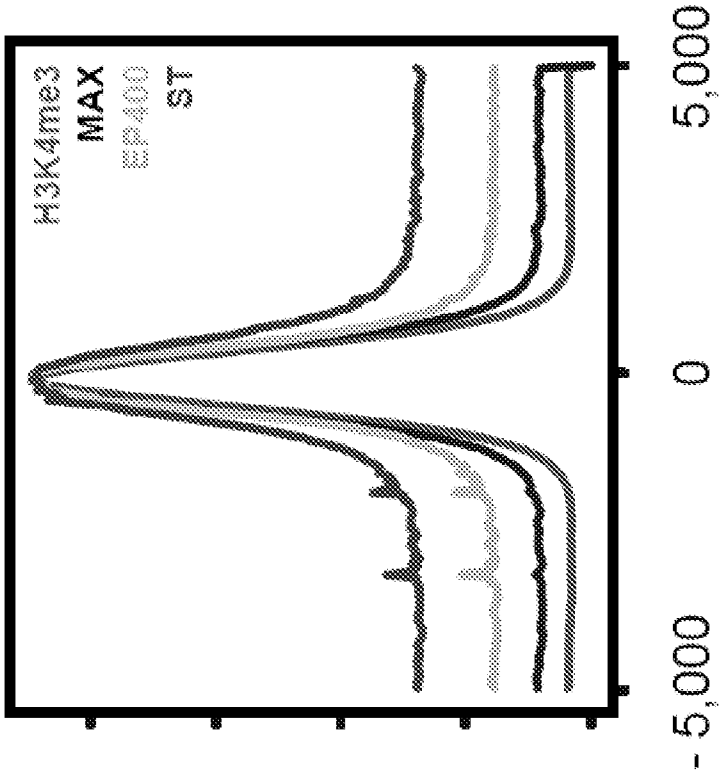



FIG. 6E

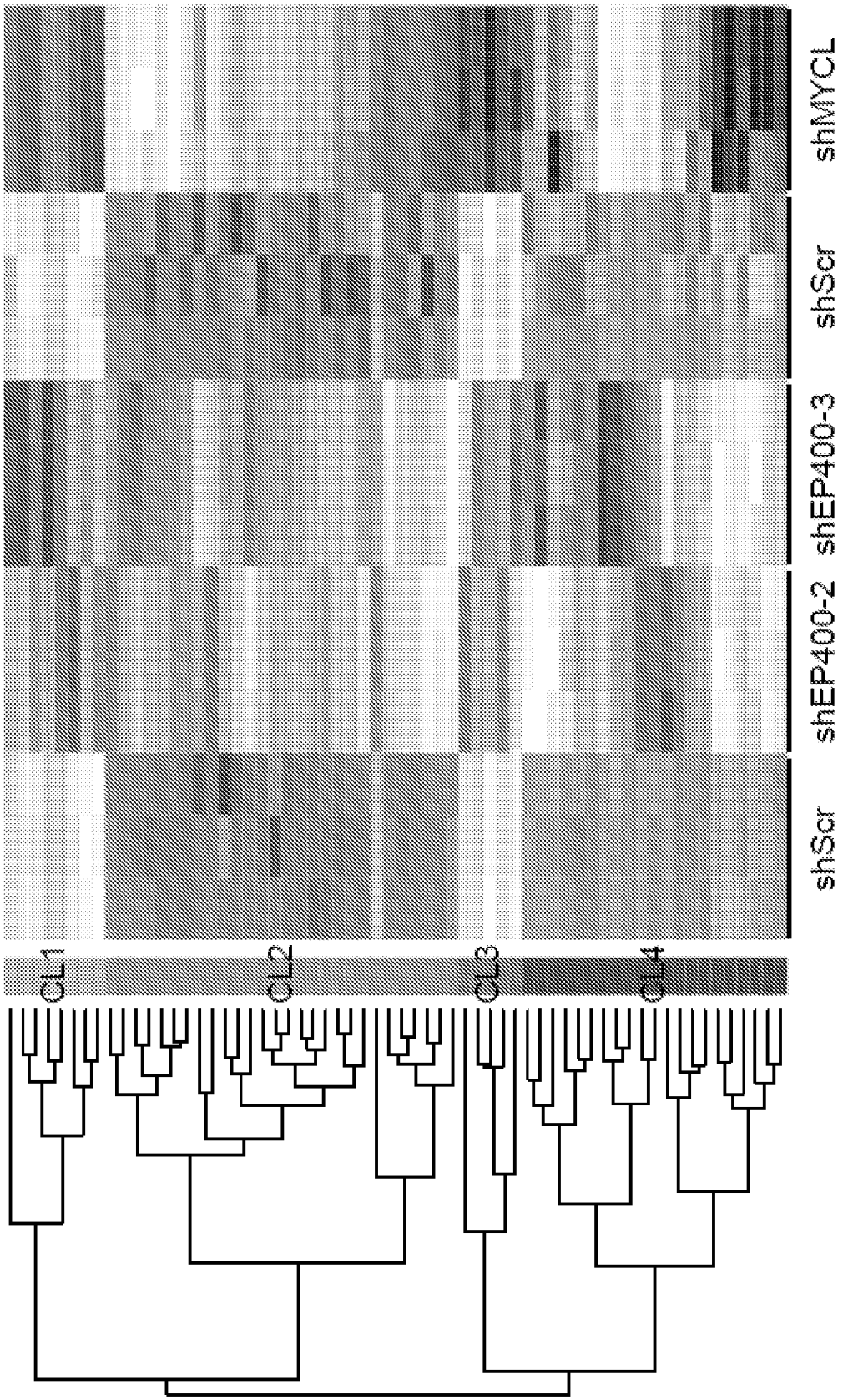



FIG. 7A

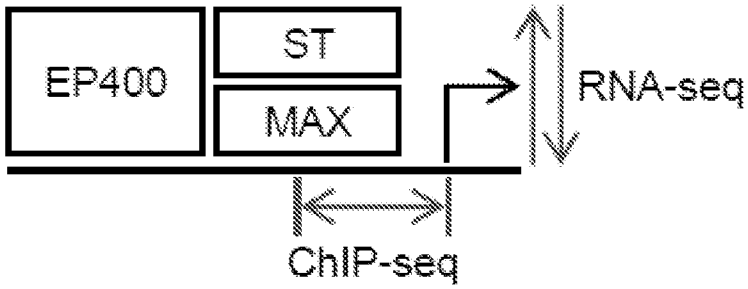

EP400
ST
MAX
RNA-seq
ChIP-seq

FIG. 7B

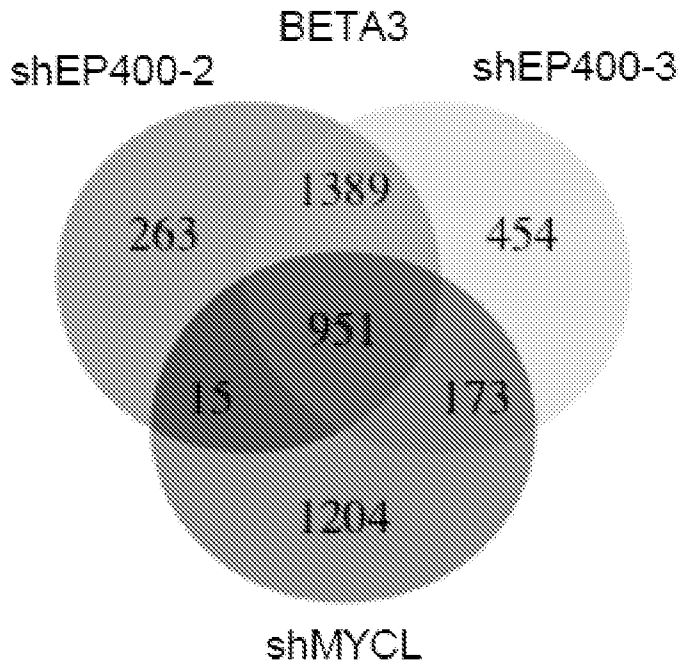

BETA3
shEP400-2
shEP400-3
263
1389
454
951
15
173
1204
shMYCL

FIG. 7C

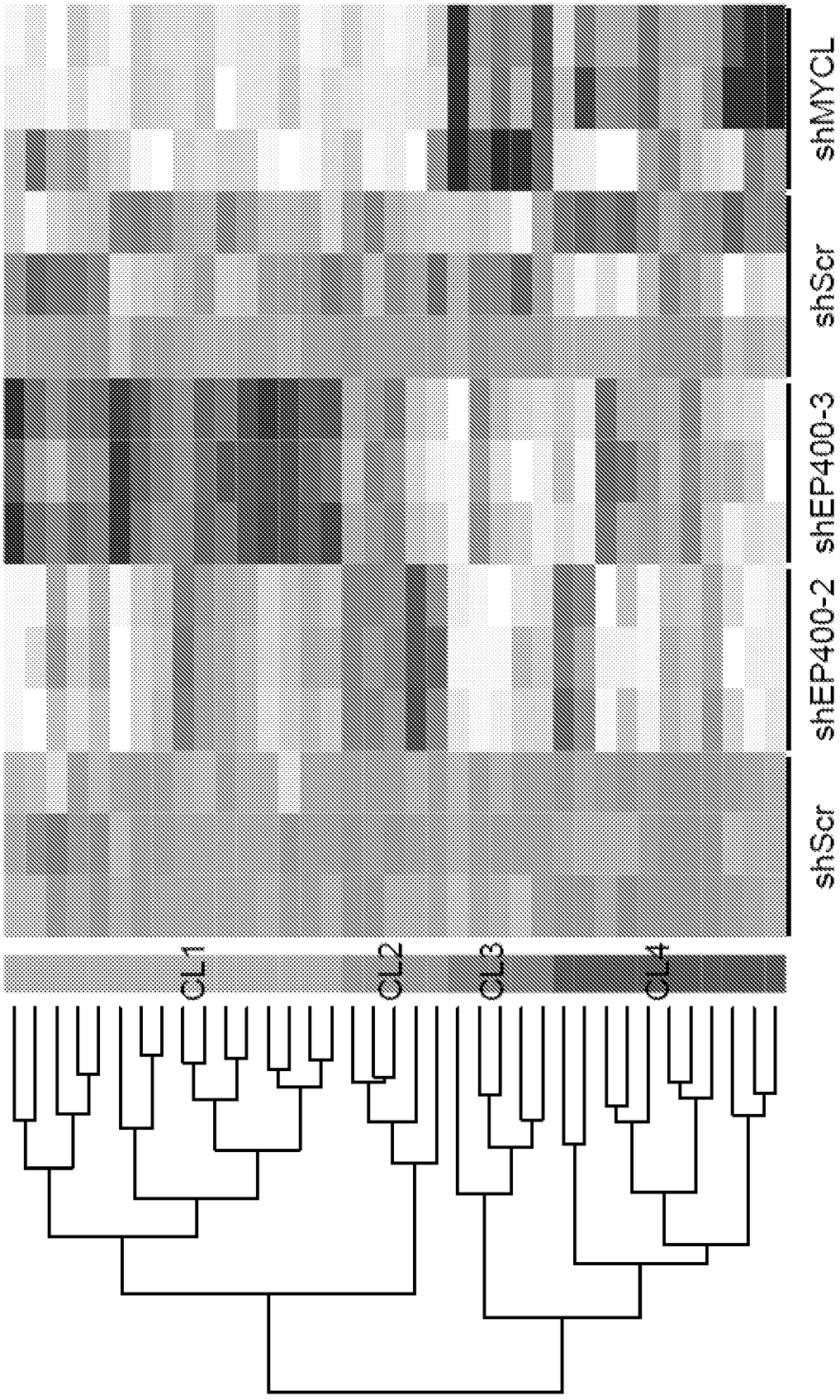



FIG. 7D

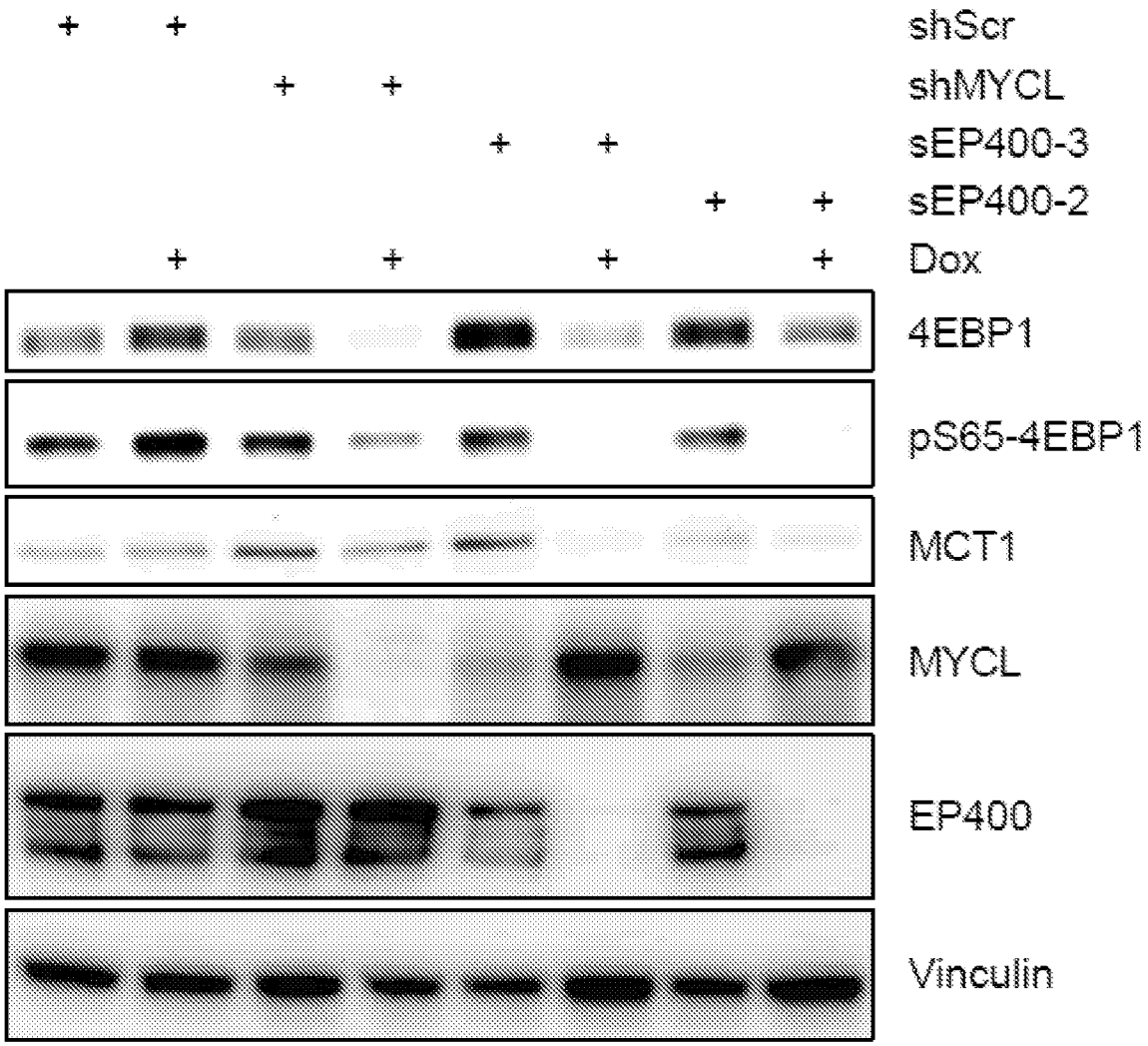

shScr
shMYCL
sEP400-3
sEP400-2
Dox
4EBP1
pS65-4EBP1
MCT1
MYCL
EP400
Vinculin

FIG. 7E

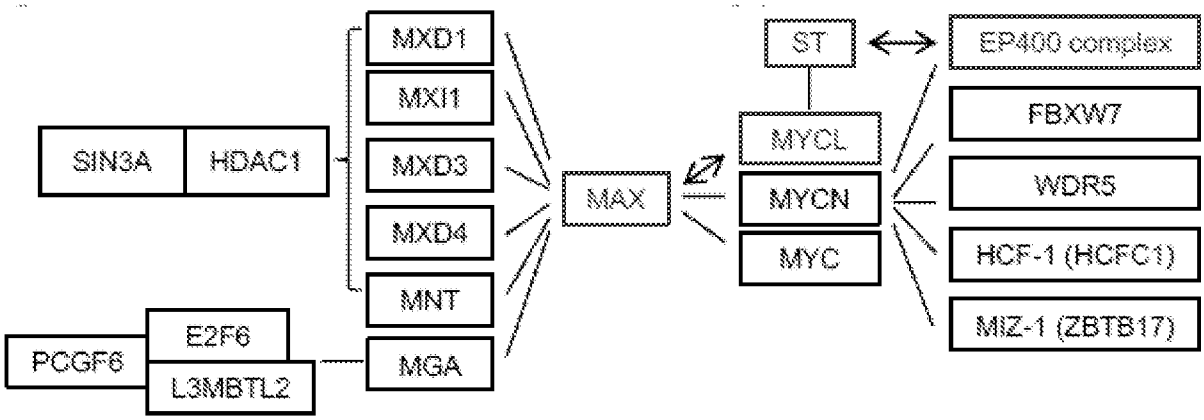

MXD1
MXI1
SIN3A
HDAC1
MXD3
MXD4
MAX
MNT
E2F6
PCGF6
L3MBTL2
MGA
ST
EP400 complex
FBXW7
MYCL
MYCN
WDR5
MYC
HCF-1 (HCFC1)
MIZ-1 (ZBTB17)

FIG. 8A

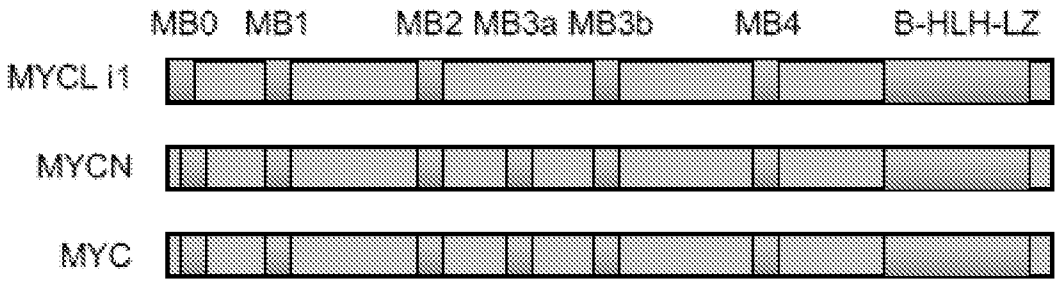

MB0
MB1
MB2
MB3a
MB3b
MB4
B-HLH-LZ
MYCL i1
MYCN
MYC

FIG. 8B

MB0
MB1
MB2
MB3a
MB3b
MB4
165
166
Basic Region
Helix1
Loop
Helix2
Zipper

FIG. 8C

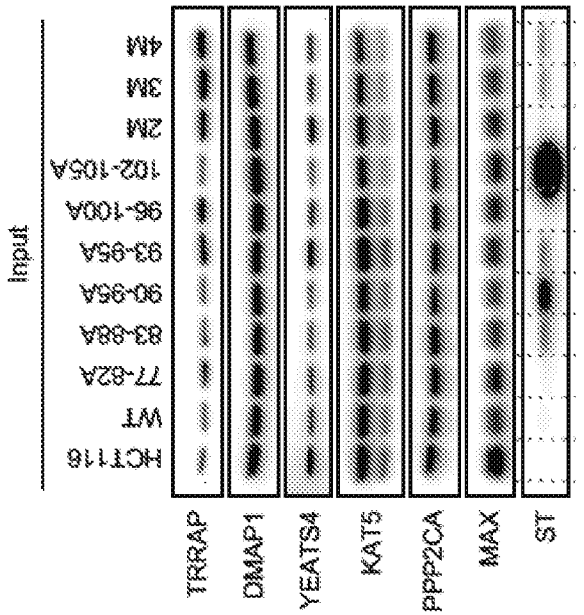



FIG. 9D

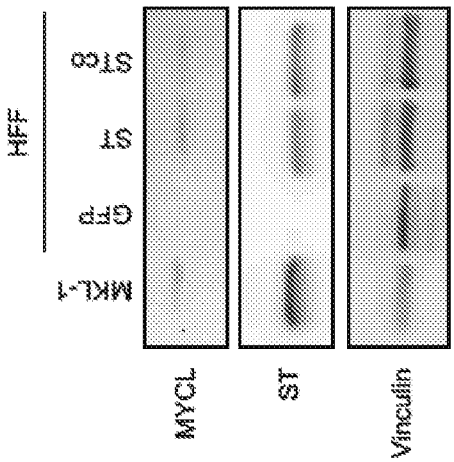



FIG. 9B

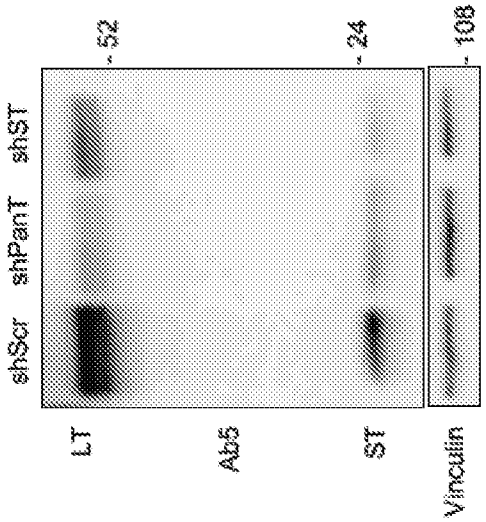



FIG. 9A

```
                61                                  86  87                92,93                                 109
    MCPyV KFQ--Q-NIH KL-R-S-DF- S-MF--D--E V-STKF-PWE ---E-------Y -GTL-KD-YM QSG----Y-N AR-FCRG--S -K-C
Gorilla KFQ--R-MIH KL-R-S-EF- T-IF--D--E V-SIKF-PWE ---E-------Y -GTL-KD-YM QSG----Y-R AI-FCKG--P -G-C
   LIPyV KFT--T-SLT EA-R-A-S-- T-Y-------Q A-STLF--WE I--D------- -NPL-KN-LL --GP---VIK -RPFLKS--P -H-C
   NJPyV KFQ--E-GLI EV-RDS-E-- V--C-------Q V-S--F---S ---DC-----Y DSSLLK--CC -S-PK--VFH EL-FLRS--P -Q-C
   HPyV9 KLQ--V-TLL EI-R-S-NCG S---S-SS-Q V-A-WYF-WD E--N-------F -RTL-GA-FL --GEK--F-N QR-IIGG-YP -D-C
   TSPyV KLQ--E-GIY NA-R-Q-EFP T---SFSS-Q VGS-WY--WE A--N-------L -ISL-KE-YF --GKK-Y-D EN-VIKH-WP -Q-C
   WUPyV KLQECVSTVH QL-N-E-EED E-VWS-SS-Q V-----EC-TE LCCNFPPRKY -R-LVGEVY- --GDV--F-E EY-ILKD-W- -DIC
   KIPyV KLQDSVSSVH DL-N-E-EED N-IWQ-SS-Q V-----YC-KD LCCN-----KF -R-LVGAIY- --GDY--Y-E AY-IMKQ-W- -DVC
   HPyV6 KLN--A-T-- -L-RDQ-MSS SPTWCFSS-E V-S--D-DWG I--P-------L --TV-GE-FL --GPE--FHK RK-V----WD FRLC
   HPyV7 KLE--A-T-- -L-RDQ-RSG SPMWHYSSDE V-S--F--WD I--E-------L --TV-GE-FL --GPE--FNR KK-V----WN YNLC
   MWPyV KMY--T-TIE KL-R---REG E-VY-FPA-K V-G-YFID-D V---------V --TL-GD-VL --GPS--F-E EK-II-YIWP --LC
STLPyV RMQ--N-TLQ NL-RSS-NEN ENMY-PPV-R M---LLLT-D T---------F --TL-GE-LL --GPQ--F-E SK-VI-FIWP --TC
   BKPyV KME--Q-DV- KVAH-QPDPG T--WS-SS-E V-----CA-D ---------F --PL------C ---P-----D TL-YCKE-WP -I-C
   JCPyV KME--Q-GV- KVAH-QPDPG T--WN-SS-E V-G---C--D ---------F --P--------- ---PN--S-D TL-YCKE-WP -N-C
   HPyV12 KFK--D-GIY NL-R-EVK-P S-LH--PV-- V-T---C--- ---------- --TV-----L --GARNIF-N -L-ITNS--S -Q-C
```

FIG. 9C

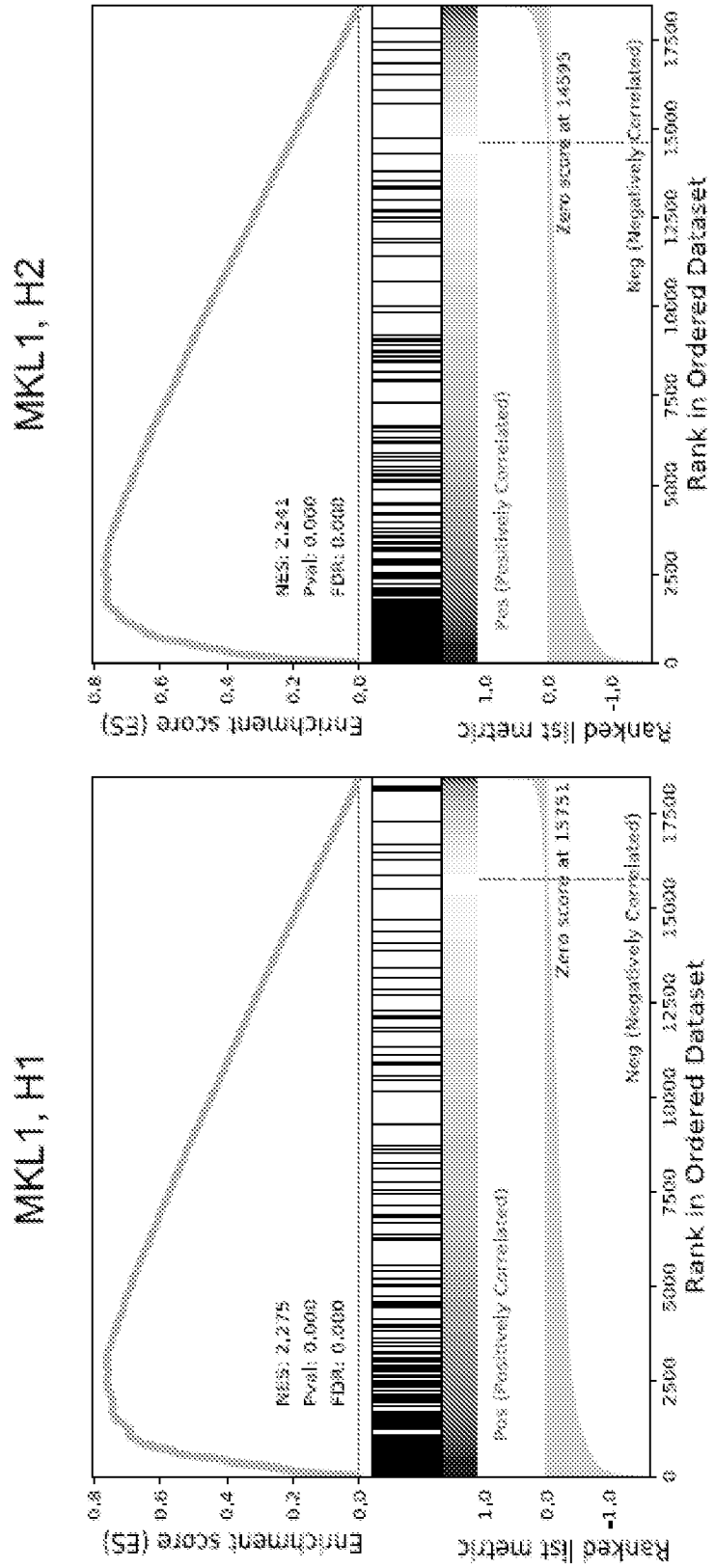

MKL1, H1
MKL1, H2
Enrichment score (ES)
NES: 2.275
Pval: 0.000
FDR: 0.000
NES: 2.261
Pval: 0.000
FDR: 0.000
Ranked list metric
Pos (Positively Correlated)
Neg (Negatively Correlated)
Zero score at 13751
Zero score at 14595
Rank in Ordered Dataset

FIG. 10A

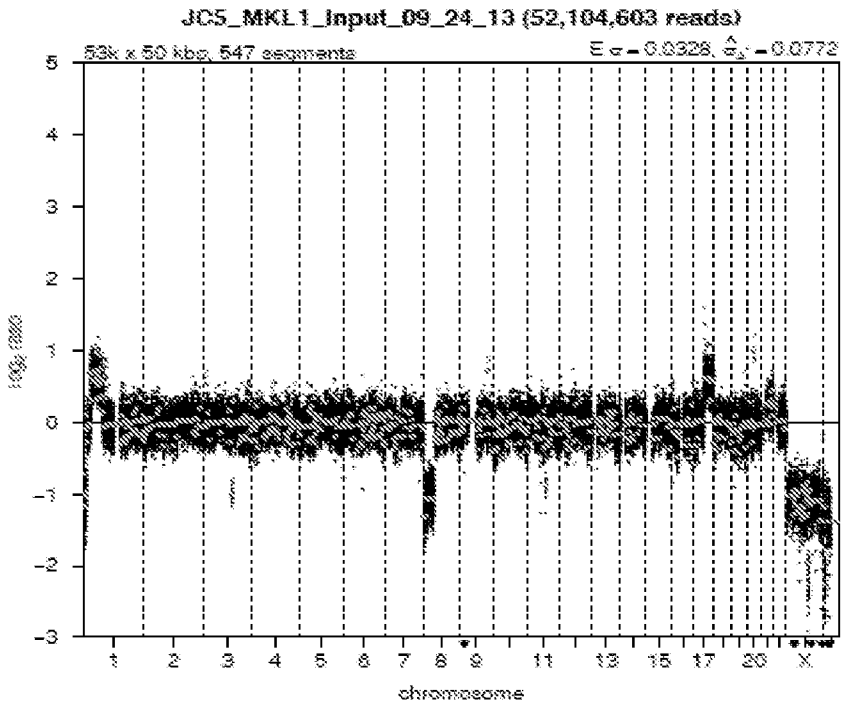



FIG. 10B

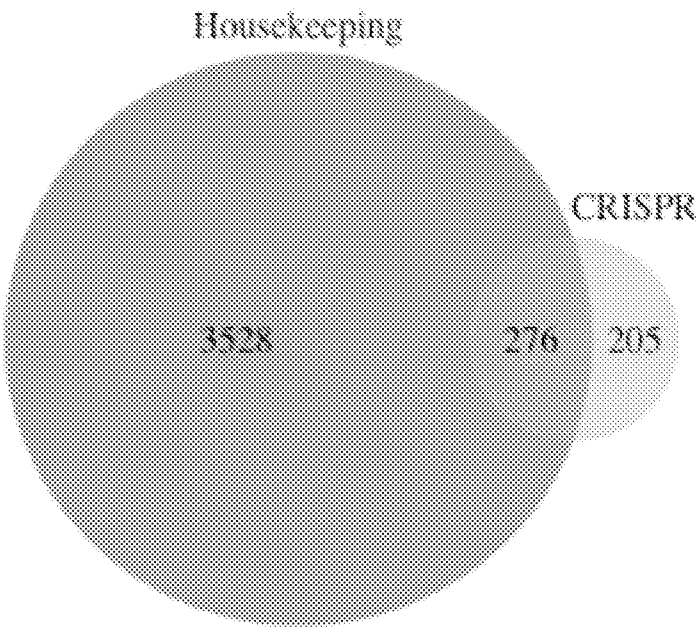



FIG. 10C

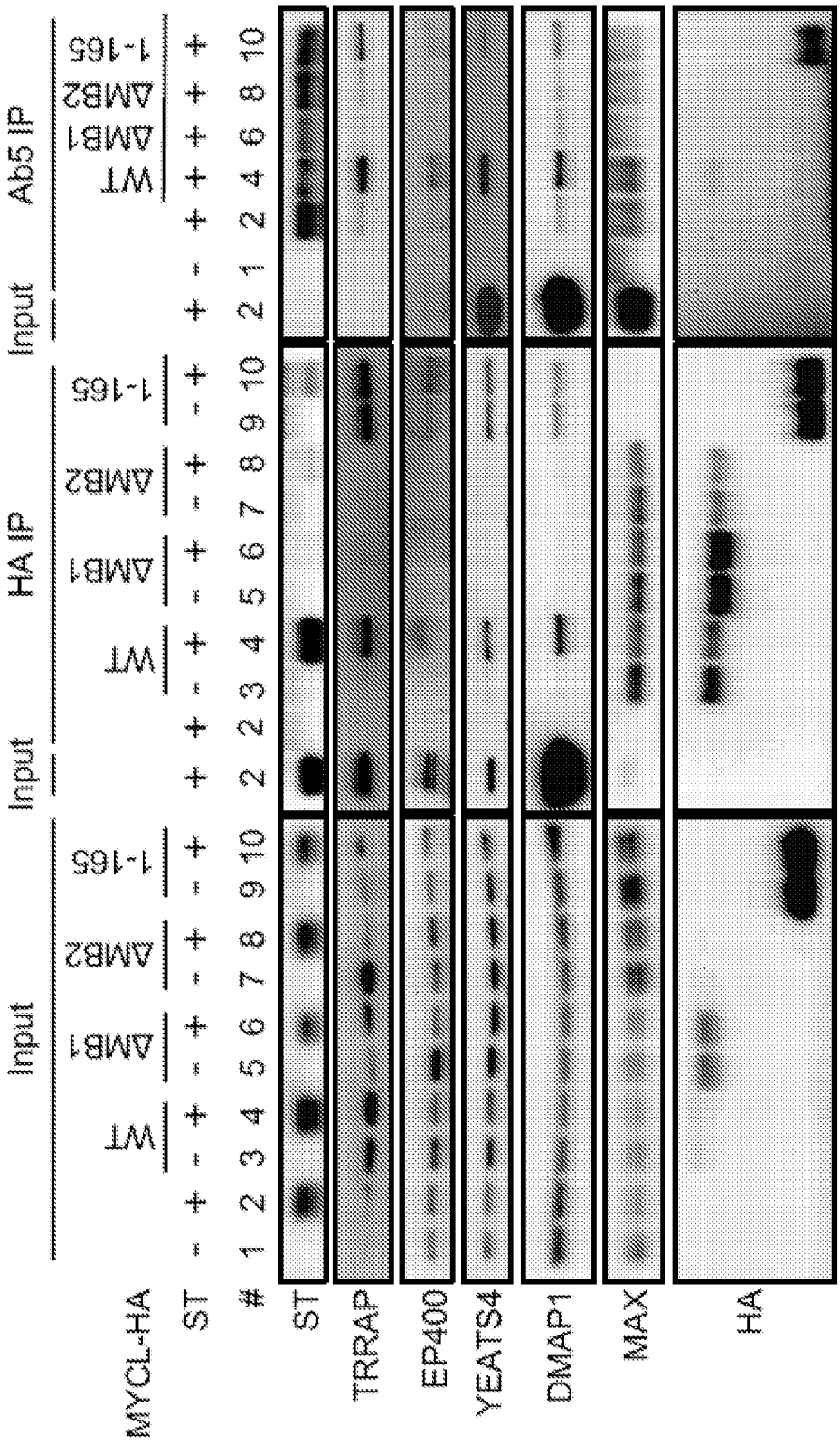

MYCL-HA
ST
#
1 2 3 4 5 6 7 8 9 10
Input
WT
ΔMB1
ΔMB2
1-165
HA IP
Input
Ab5 IP
ST
TRRAP
EP400
YEATS4
DMAP1
MAX
HA

FIG. 10D

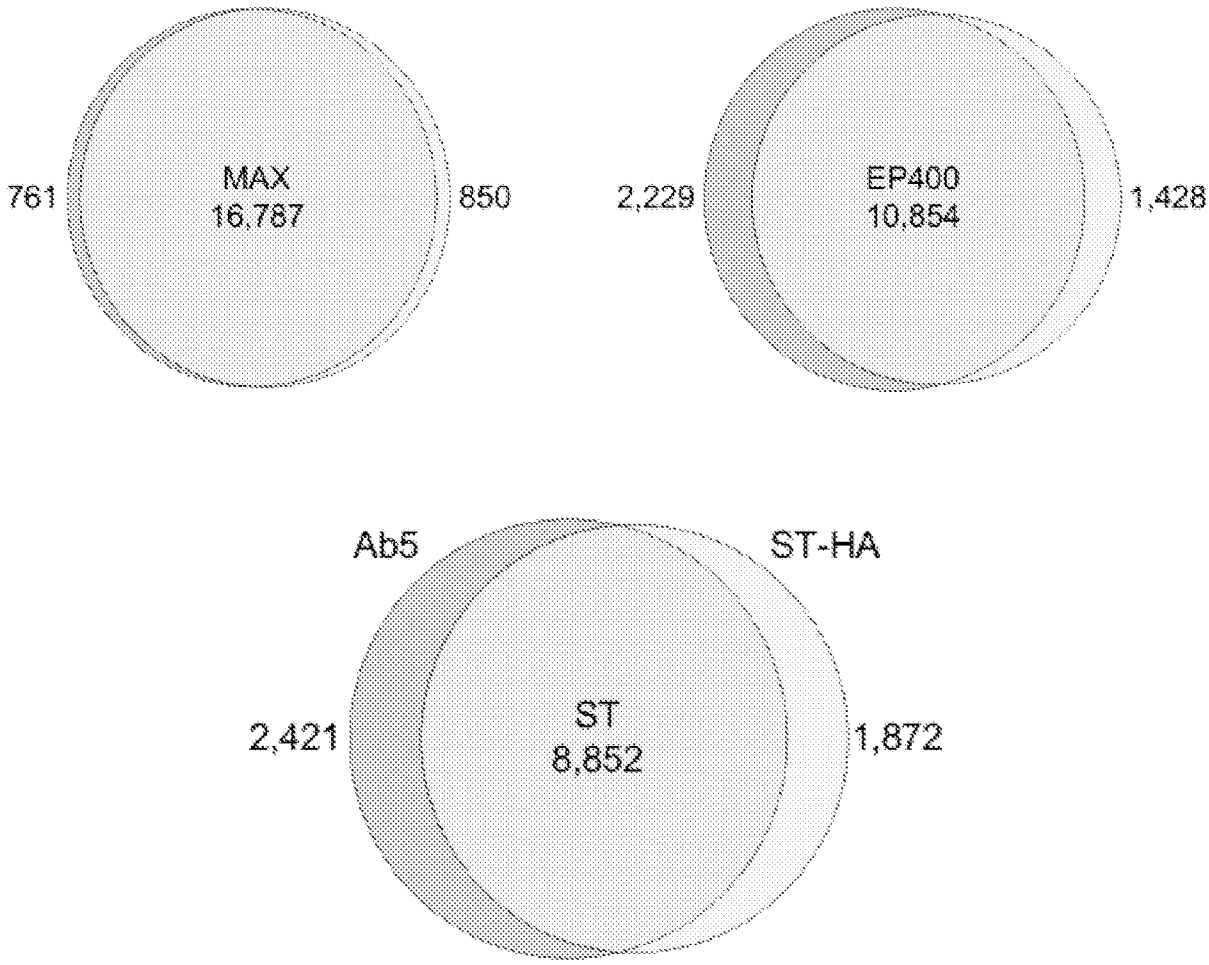

761
MAX
16,787
850
2,229
EP400
10,854
1,428
Ab5
ST-HA
2,421
ST
8,852
1,872

FIG. 11A

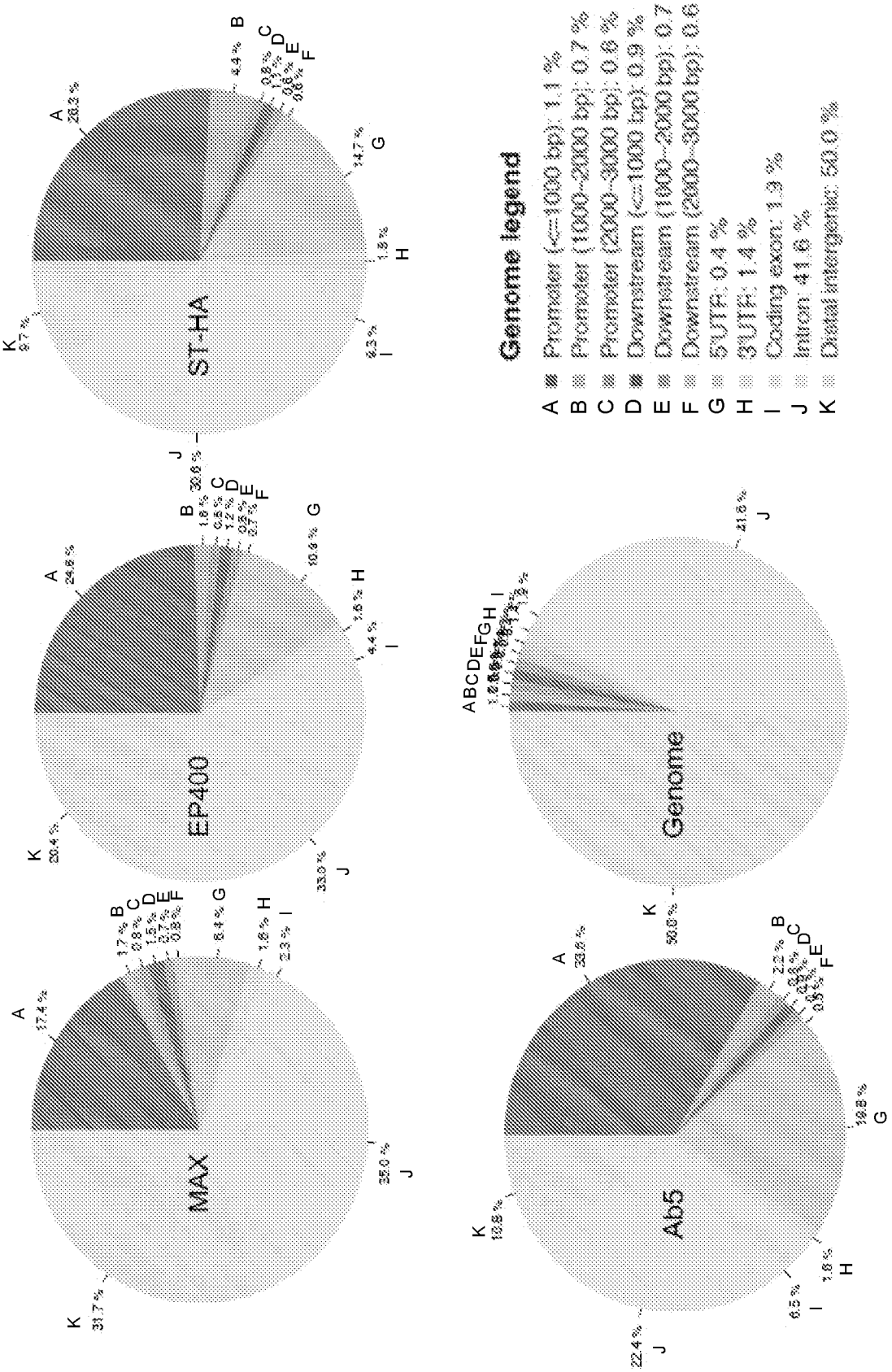

MAX
EP400
ST-HA
Ab5
Genome
Genome legend
A Promoter (<=1000 bp): 1.1 %
B Promoter (1000–2000 bp): 0.7 %
C Promoter (2000–3000 bp): 0.6 %
D Downstream (<=1000 bp): 0.9 %
E Downstream (1000–2000 bp): 0.7 %
F Downstream (2000–3000 bp): 0.6 %
G 5UTR: 0.4 %
H 3UTR: 1.4 %
I Coding exon: 1.3 %
J Intron: 41.6 %
K Distal intergenic: 50.0 %

FIG. 11B

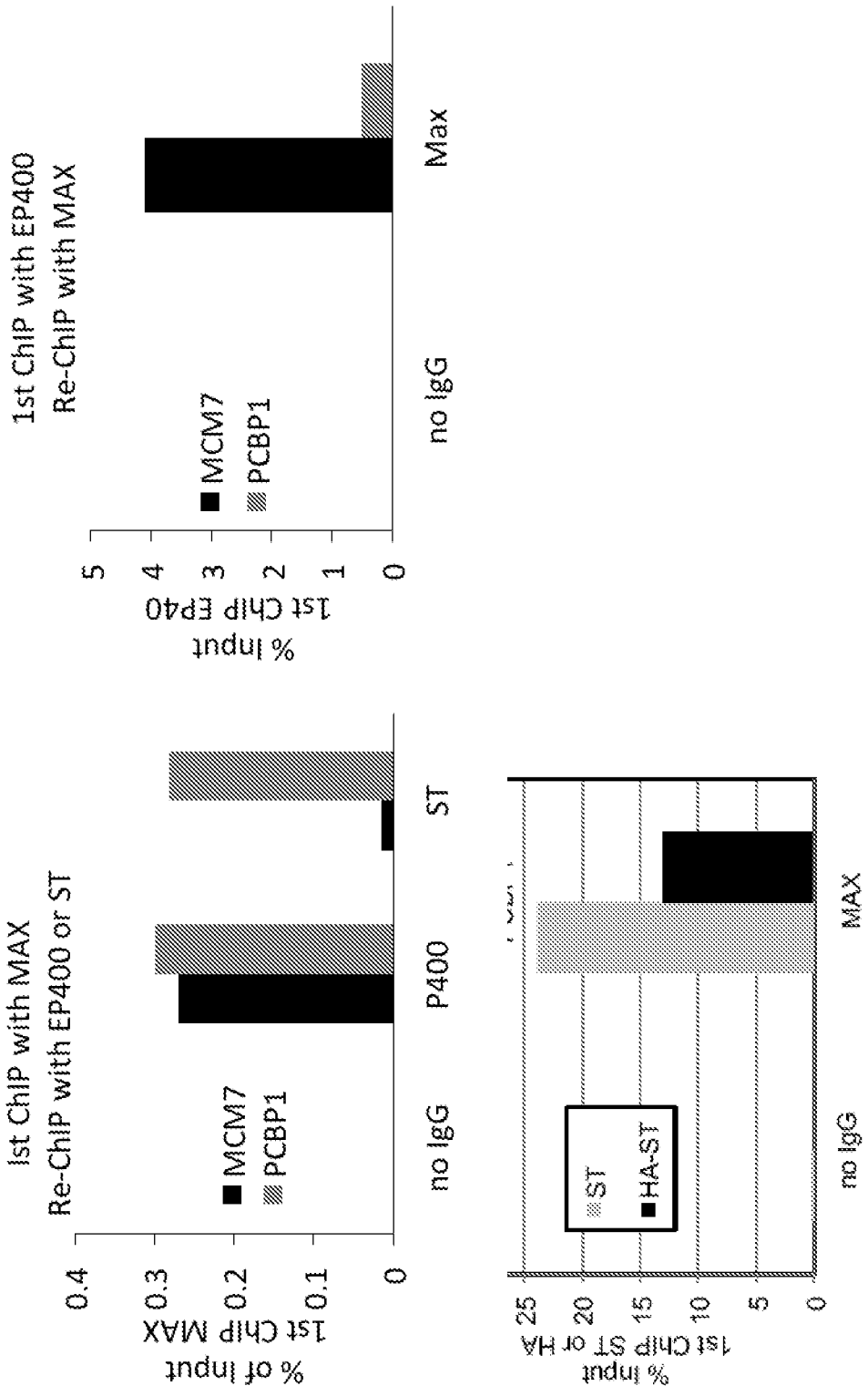

1st ChIP with MAX
Re-ChIP with EP400 or ST
% of Input
1st ChIP MAX
0.4
0.3
0.2
0.1
0
MCM7
PCBP1
no IgG
P400
ST
1st ChIP with EP400
Re-ChIP with MAX
% Input
1st ChIP EP40
5
4
3
2
1
0
MCM7
PCBP1
no IgG
Max
% Input
1st ChIP ST or HA
25
20
15
10
5
0
ST
HA-ST
no IgG
MAX

FIG. 11C

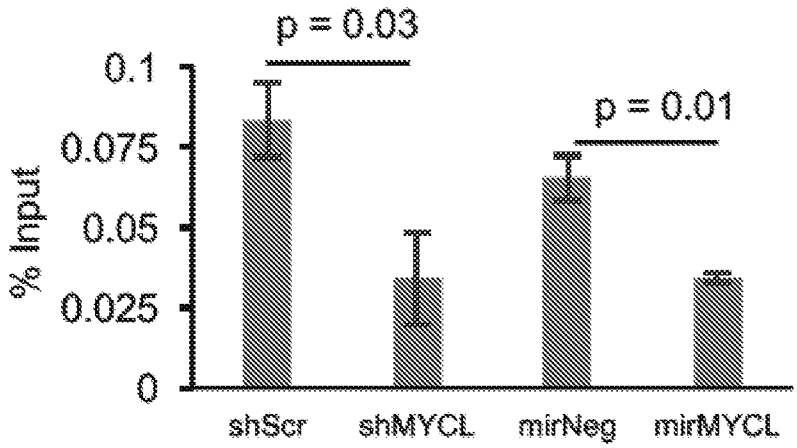



FIG. 12A

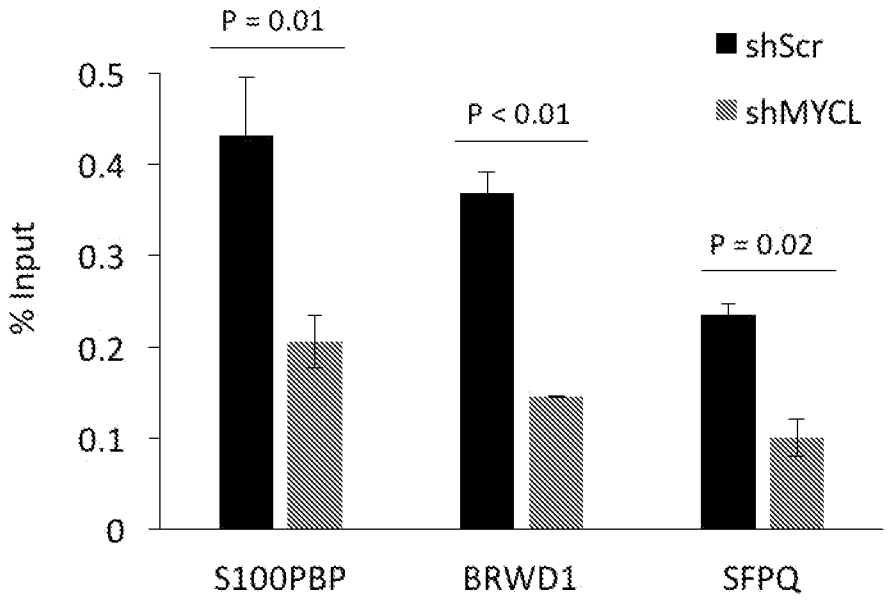



FIG. 12B

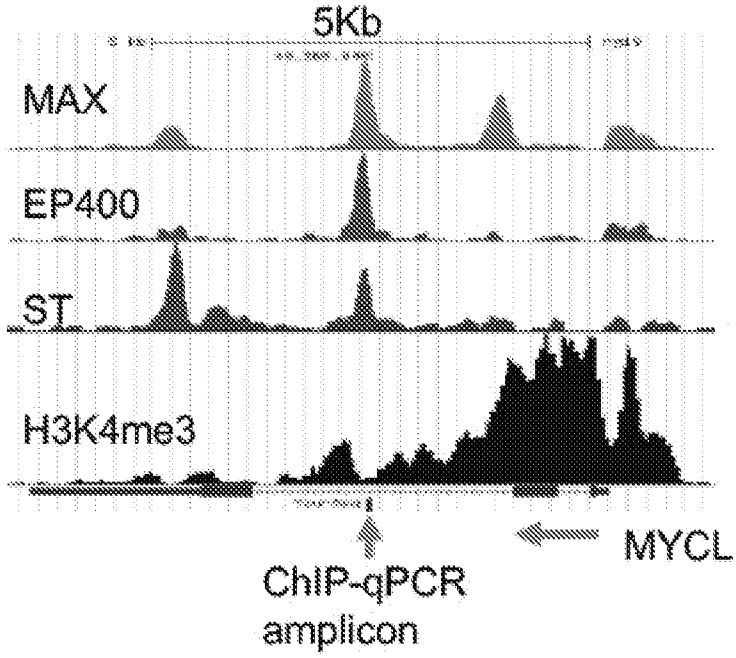



FIG. 12C

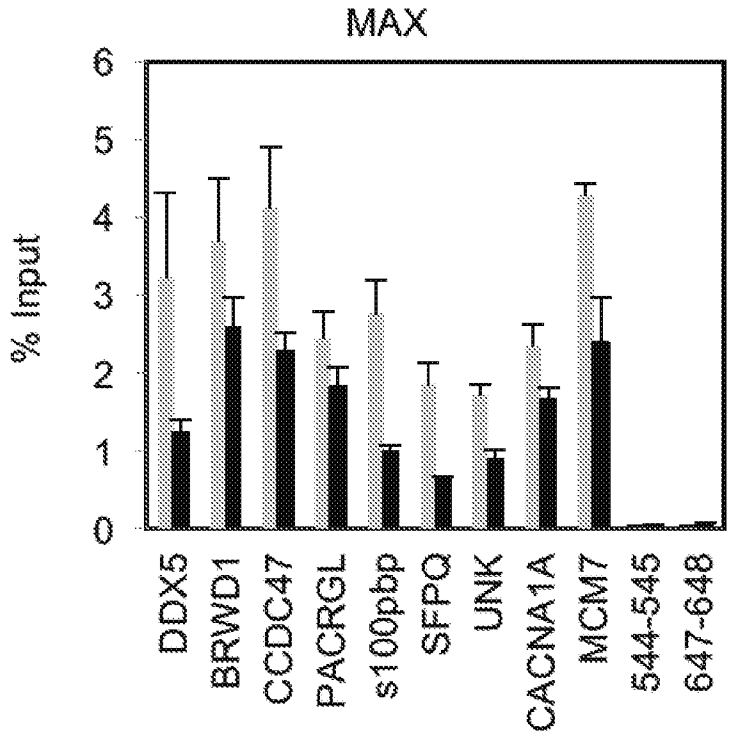



FIG. 12D

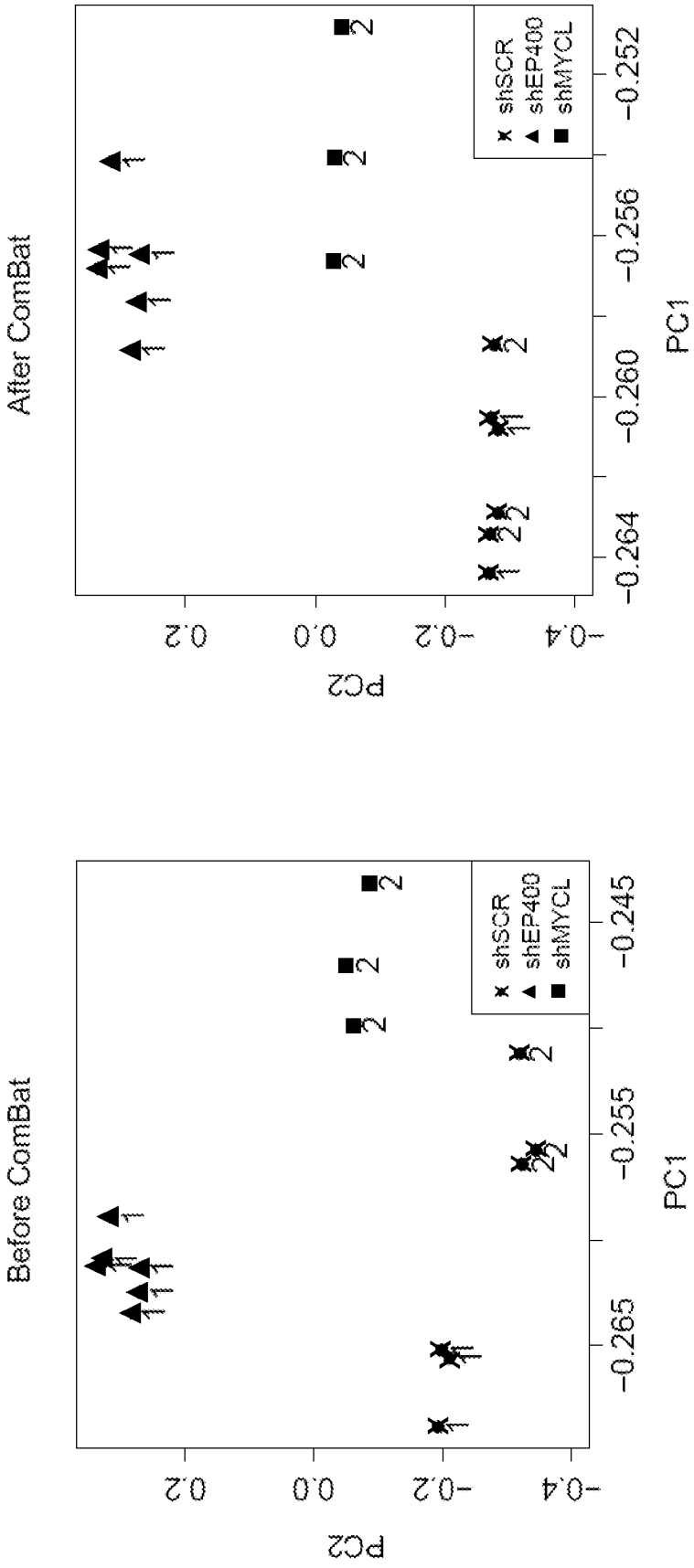

After ComBat
PC1
PC2
shSCR
shEP400
shMYCL
−0.264
−0.260
−0.256
−0.252
−0.4
−0.2
0.0
0.2
Before ComBat
PC1
PC2
shSCR
shEP400
shMYCL
−0.265
−0.255
−0.245
−0.4
−0.2
0.0
0.2

FIG. 13

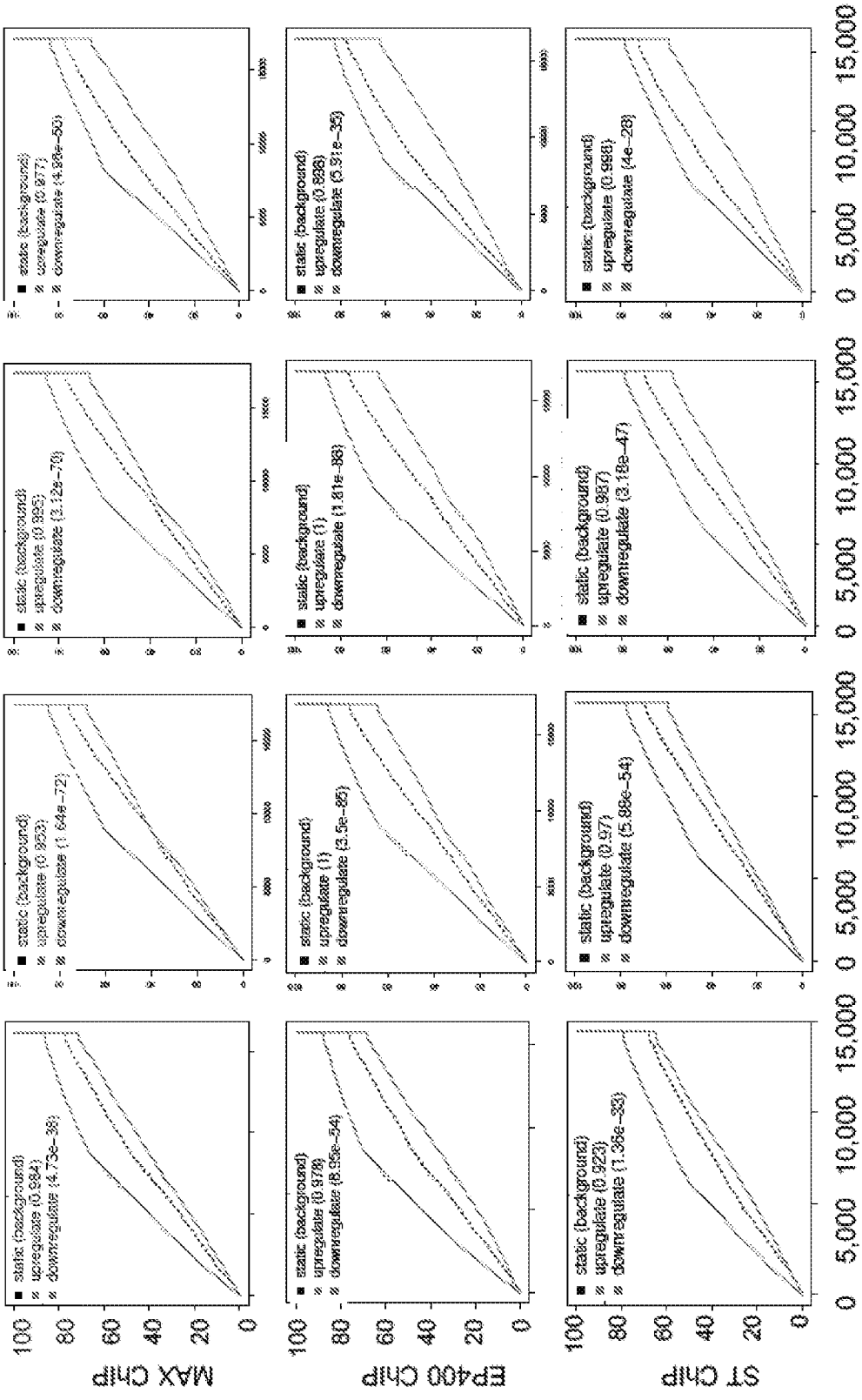

activating/repressive function prediction
shEP400-1
shEP400-2
shEP400-3
shMYCL
MAX ChIP
EP400 ChIP
ST ChIP
Cumulative fraction of genes (%)
Rank of genes based on regulatory potential score (from high to low)
0
5,000
10,000
15,000

FIG. 14A

FIG. 14B




FIG. 14C

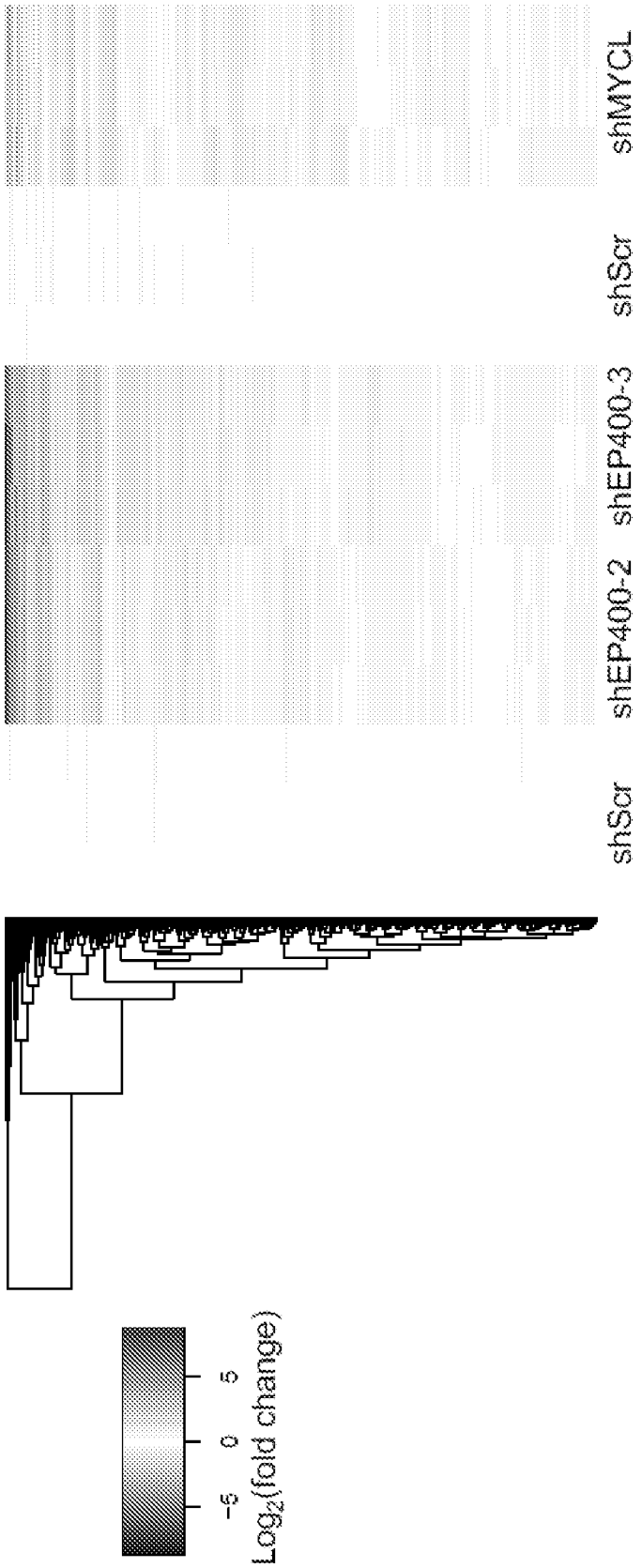



FIG. 15A

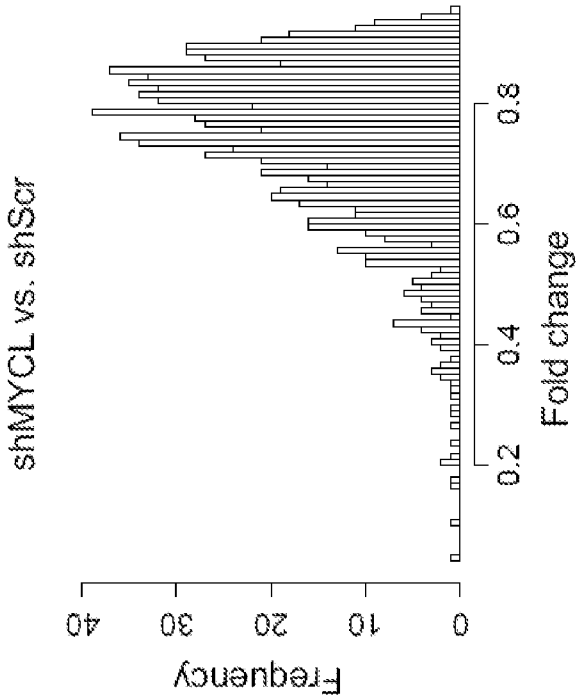



FIG. 15C

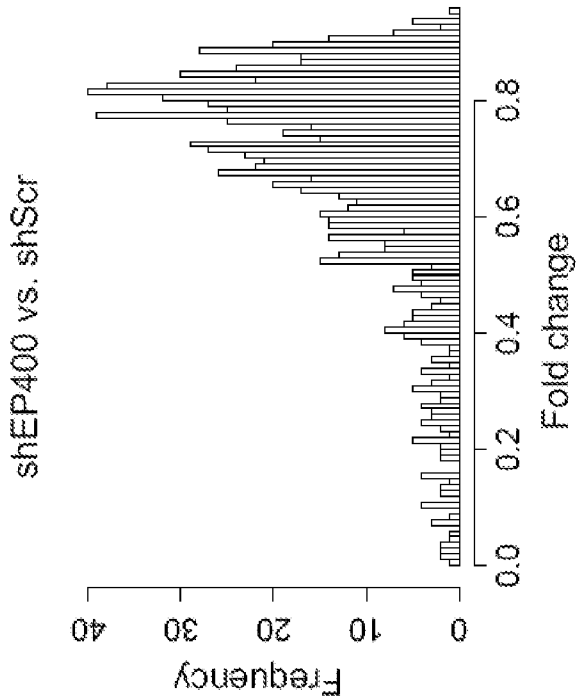



FIG. 15B

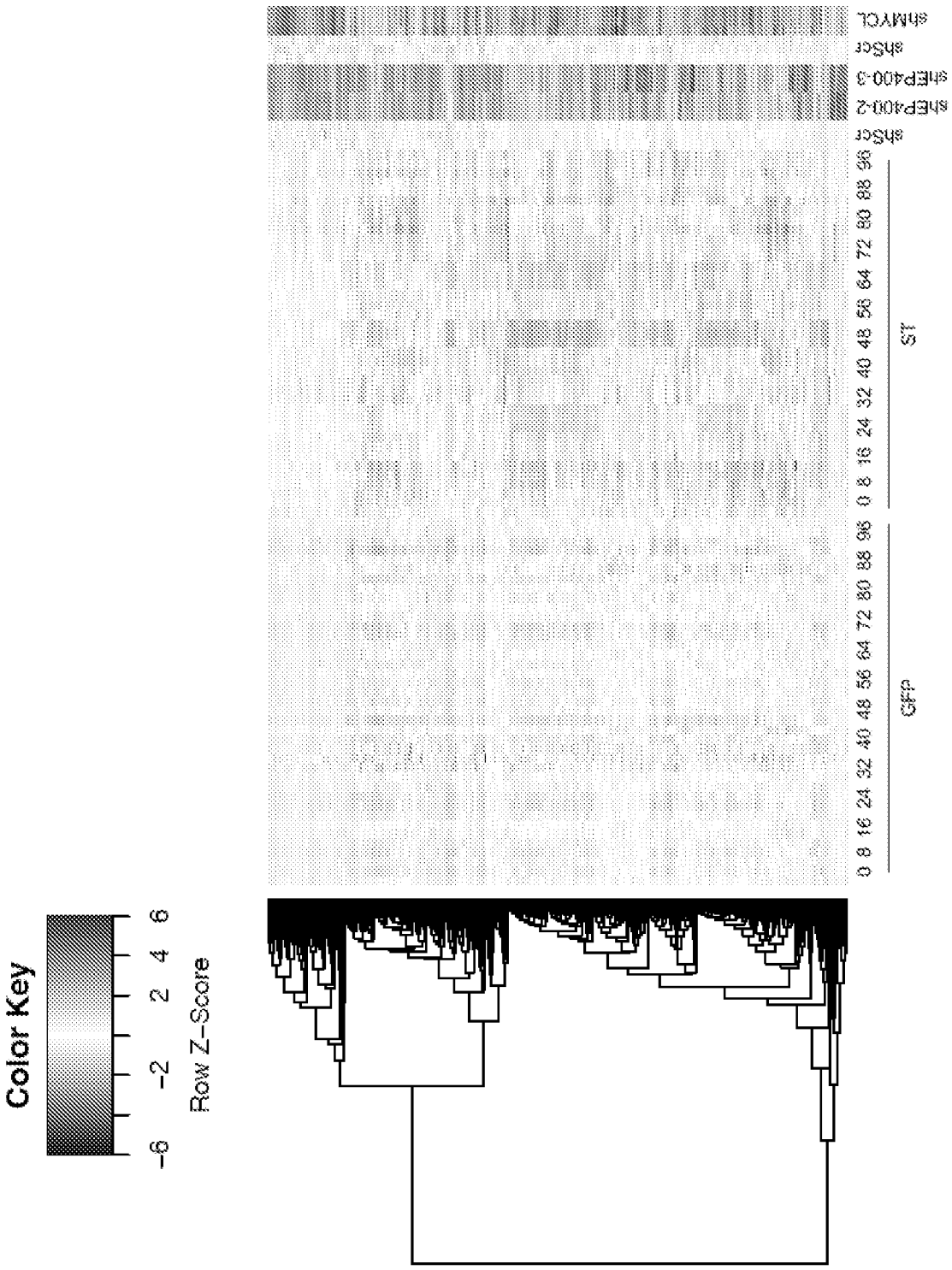

Color Key
−6
−2
2
4
6
Row Z−Score
shMYCL
shScr
shEP400-3
shEP400-2
shScr
0 8 16 24 32 40 48 56 64 72 80 88 96
0 8 16 24 32 40 48 56 64 72 80 88 96
ST
GFP

FIG. 16

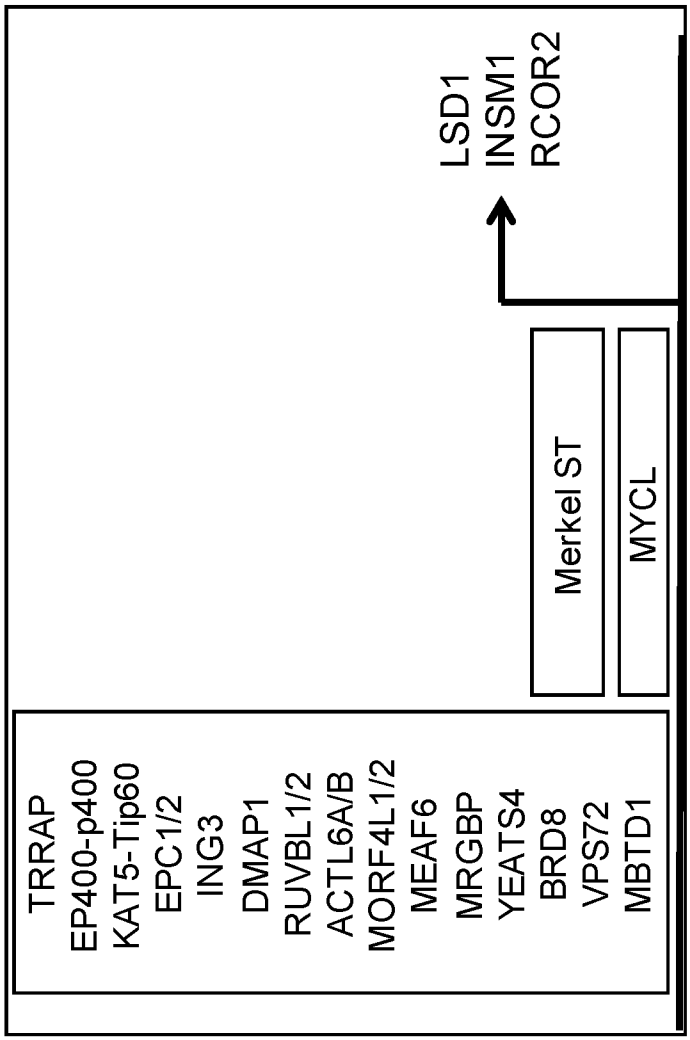

TRRAP
EP400-p400
KAT5-Tip60
EPC1/2
ING3
DMAP1
RUVBL1/2
ACTL6A/B
MORF4L1/2
MEAF6
MRGBP
YEATS4
BRD8
VPS72
MBTD1
Merkel ST
MYCL
LSD1
INSM1
RCOR2

FIG. 17

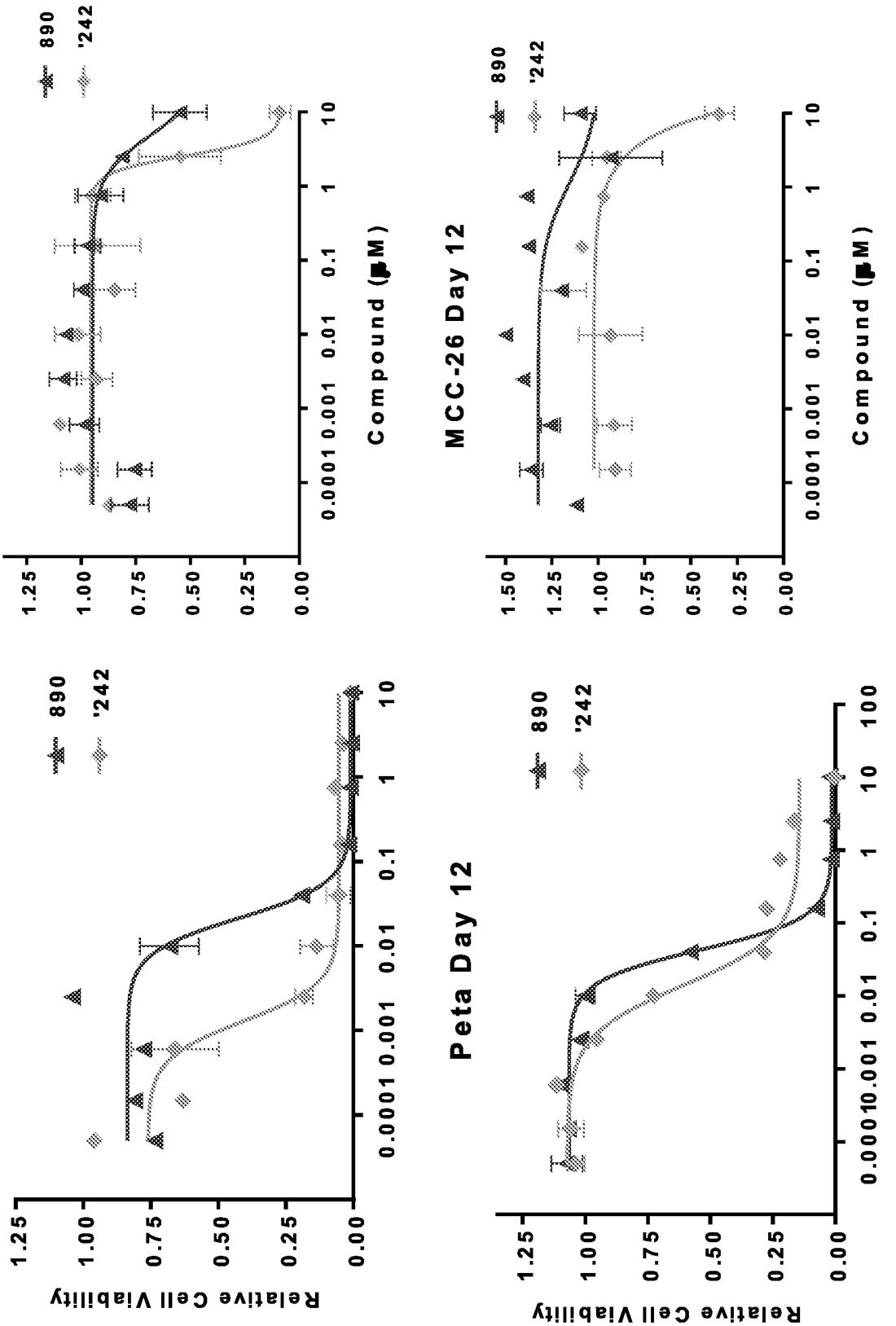

UISO Day 12
Broli Day 13
MCC-26 Day 12
Peta Day 12
890
'242
Relative Cell Viability
Compound (μM)
0.00
0.25
0.50
0.75
1.00
1.25
1.50
0.0001
0.001
0.01
0.1
1
10
100

FIG. 18

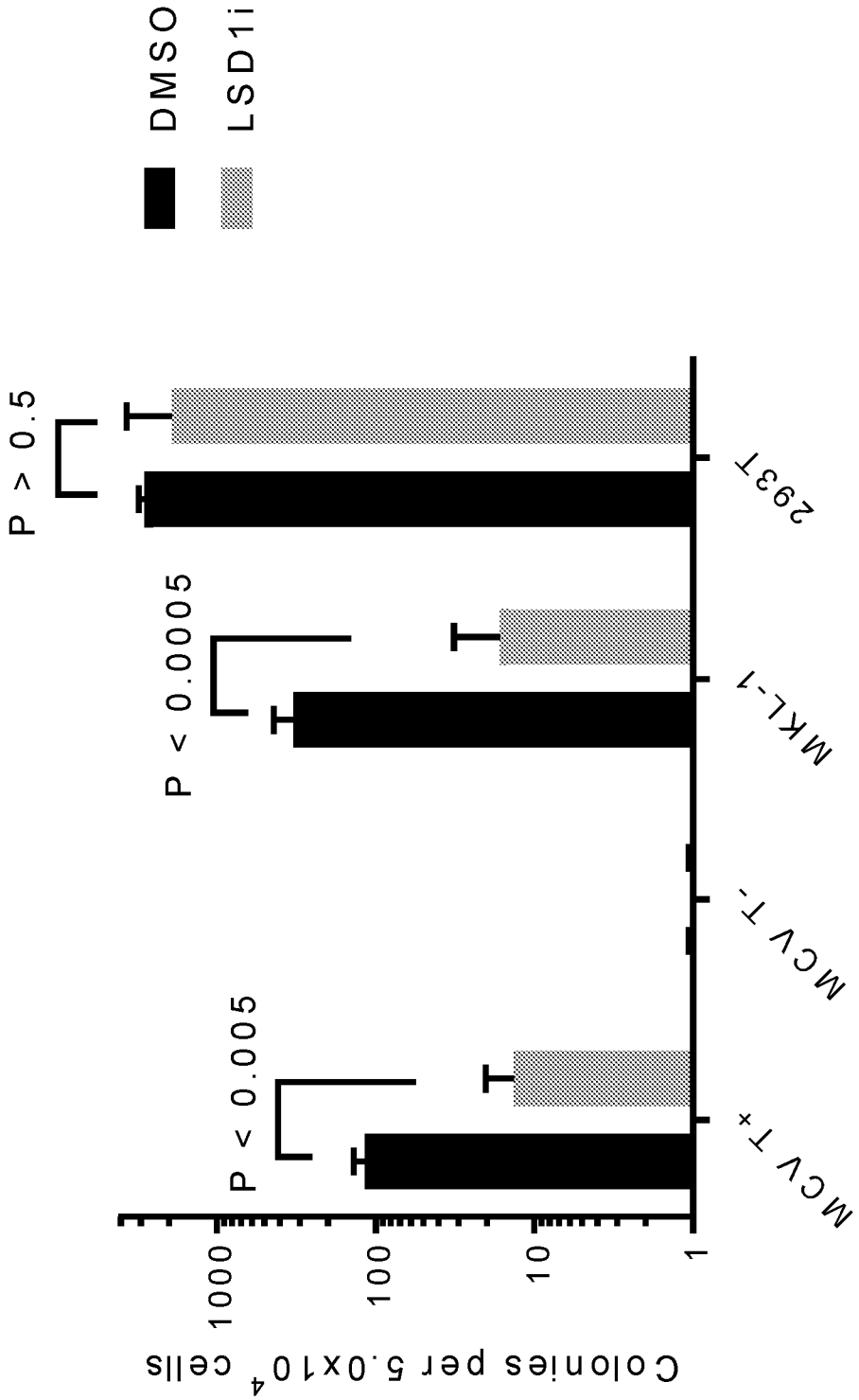

DMSO
LSD1i
Colonies per 5.0x10^4 cells
1000
100
10
1
P < 0.005
P < 0.0005
P > 0.5
MCV T+
MCV T-
MKL-1
293T

FIG. 19

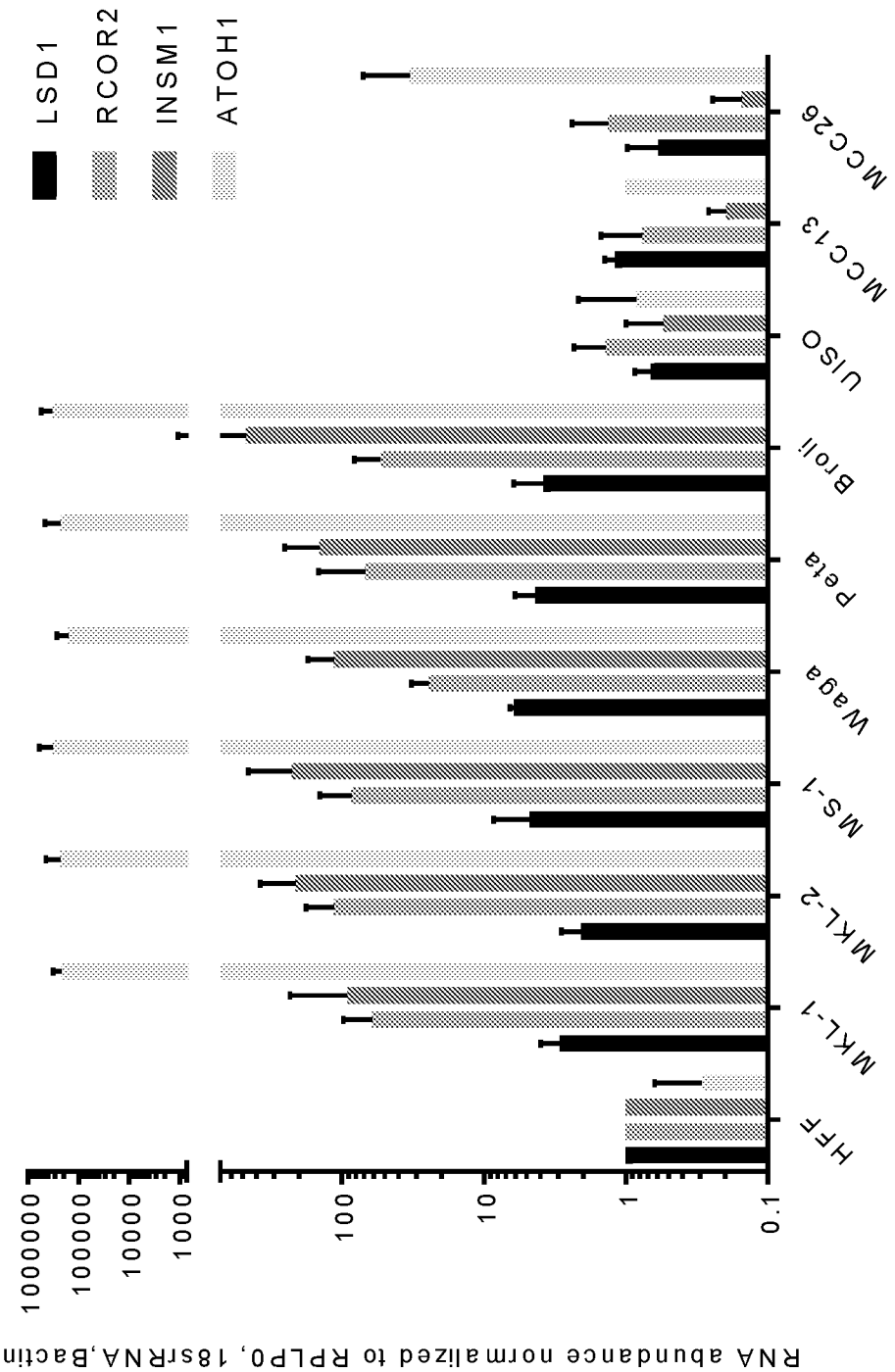

LSD1
RCOR2
INSM1
ATOH1
RNA abundance normalized to RPLP0, 18srRNA,Bactin
1000000
100000
10000
1000
100
10
1
0.1
HFF
MKL-1
MKL-2
MS-1
Waga
Peta
Broii
UISO
MCC13
MCC26

FIG. 20

# FIG. 21

HUMAN Isoform 2 of Lysine-specific histone demethylase 1A OS=Homo sapiens GN=KDM1A Isoform 2 (identifier: O60341-2)
The sequence of this isoform differs from the canonical sequence as follows:

173-173: G → GQAGGLQDDSSGGYGDGQASG (SEQ ID NO: 23)
369-369: A → ADTVK (SEQ ID NO: 24)

MLSGKKAAAAAAAAAAAATGTEAGPGTAGGSENGSEVAAQPAGLSGPAEVGPGAVGERTP
RKKEPPRASPPGGLAEPPGSAGPQAGPTVVPGSATPMETGIAETPEGRRTSRRKRAKVEY
REMDESLANLSEDEYYSEEERNAKAEKEKKLPPPPPQAPPEEENESEPEEPS**QAGGLQD**
**DSSGGYGDGQASG**VEGAAFQSRLPHDRMTSQEAACFPDIISGPQQTQKVFLFIRNRTLQL
WLDNPKIQLTFEATLQQLEAPYNSDTVLVHRVHSYLERHGLINFGIYKRIKPLPTKKTGK
VIIIGSGVSGLAAARQLQSFGMDVTLLEARDRVGGRVATFRKGNYVADLGAMVVTGLGGN
PMAVVSKQVNMELAKIKQKCPLYEANGQ**ADTVK**VPKEKDEMVEQEFNRLLEATSYLSHQL
DFNVLNNKPVSLGQALEVVIQLQEKHVKDEQIEHWKKIVKTQEELKELLNKMVNLKEKIK
ELHQQYKEASEVKPPRDITAEFLVKSKHRDLTALCKEYDELAETQGKLEEKLQELEANPP
SDVYLSSRDRQILDWHFANLEFANATPLSTLSLKHWDQDDDFEFTGSHLTVRNGYSCVPV
ALAEGLDIKLNTAVRQVRYTASGCEVIAVNTRSTSQTFIYKCDAVLCTLPLGVLKQQPPA
VQFVPPLPEWKTSAVQRMGFGNLNKVVLCFDRVFWDPSVNLFGHVGSTTASRGELFLFWN
LYKAPILLALVAGEAAGIMENISDDVIVGRCLAILKGIFGSSAVPQPKETVVSRWRADPW
ARGSYSYVAAGSSGNDYDLMAQPITPGPSIPGAPQPIPRLFFAGEHTIRNYPATVHGALL
SGLREAGRIADQFLGAMYTLPRQATPGVPAQQSPSM (SEQ ID NO: 21)

atg tta tct ggg aag aag gcg gca gcc gcg gcg gcg gcg gct gca gcg gca gca acc ggg acg gag gct ggc
cct ggg aca gca ggc ggc tcc gag aac ggg tct gag gtg gcc gcg cag ccc gcg ggc ctg tcg ggc cca gcc
gag gtc ggg ccg ggg gcg gtg ggg gag cgc aca ccc cgc aag aaa gag cct ccg cgg gcc tcg ccc ccc ggg
ggc ctg gcg gaa ccg ccg ggg tcc gca ggg cct cag gcc ggc cct act gtc gtg cct ggg tct gcg acc ccc
atg gaa act gga ata gca gag act ccg gag ggg cgt cgg acc agc cgg cgc aag cgg gcg aag gta gag tac
aga gag atg gat gaa agc ttg gcc aac ctc tca gaa gat gag tat tat tca gaa gaa gag aga aat gcc aaa
gca gag aag gaa aag aag ctt ccc cca cca ccc cct caa gcc cca cct gag gaa gaa aat gaa agt gag cct
gaa gaa cca tcg ggg caa gca gga gga ctt caa gac gac agt tct gga ggg tat gga gac ggc caa gca tca
ggt gtg gag ggc gca gct ttc cag agc cga ctt cct cat gac cgg atg act tct caa gaa gca gcc tgt ttt
cca gat att atc agt gga cca caa cag acc cag aag gtt ttt ctt ttc att aga aac cgc aca ctg cag ttg
tgg ttg gat aat cca aag att cag ctg aca ttt gag gct act ctc caa caa tta gaa gca cct tat aac agt

gat act gtg ctt gtc cac cga gtt cac agt tat tta gag cgt cat ggt ctt atc aac ttc ggc atc tat aag
agg ata aaa ccc cta cca act aaa aag aca gga aag gta att ata ggc tct ggg gtc tca ggc ttg gca
gca gct cga cag tta caa agt ttt gga atg gat gtc aca ctt ttg gaa gcc agg gat cgt gtg ggt gga cga
gtt gcc aca ttt cgc aaa gga aac tat gta gct gat ctt gga gcc atg gtg gta aca ggt ctt gga ggg aat
cct atg gct gtg gtc agc aaa caa gta aat atg gaa ctg gcc aag atc aag caa aaa tgc cca ctt tat gaa
gcc aac gga caa gct gac act gtc aag gtt cct aaa gag aaa gat gaa atg gta gag caa gag ttt aac cgg
ttg cta gaa gct aca tct tac ctt agt cat caa cta gac ttc aat gtc ctc aat aat aag cct gtg tcc ctt
ggc cag gca ttg gaa gtt gtc att cag tta caa gag aag cat gtc aaa gat gag cag att gaa cat tgg aag
aag ata gtg aaa act cag gaa gaa ttg aaa gaa ctt ctt aat aag atg gta aat ttg aaa gag aaa att aaa
gaa ctc cat cag caa tac aaa gaa gca tct gaa gta aag cca ccc aga gat att act gcc gag ttc tta gtg
aaa agc aaa cac agg gat ctg acc gcc cta tgc aag gaa tat gat gaa tta gct gaa aca caa gga aag cta
gaa gaa aaa ctt cag gag ttg gaa gcg aat ccc cca agt gat gta tat ctc tca tca aga gac aga caa ata
ctt gat tgg cat ttt gca aat ctt gaa ttt gct aat gcc aca cct ctc tca act ctc tcc ctt aag cac tgg
gat cag gat gat gac ttt gag ttc act ggc agc cac ctg aca gta agg aat ggc tac tcg tgt gtg cct gtg
gct tta gca gaa ggc cta gac att aaa ctg aat aca gca gtg cga cag gtt cgc tac acg gct tca gga tgt
gaa gtg ata gct gtg aat acc cgc tcc acg agt caa acc ttt att tat aaa tgc gac gca gtt ctc tgt acc
ctt ccc ctg ggt gtg ctg aag cag cag cca cca gcc gtt cag ttt gtg cca cct ctc cct gag tgg aaa aca
tct gca gtc caa agg atg gga ttt ggc aac ctt aac aag gtg gtg ttg tgt ttt gat cgg gtg ttc tgg gat
cca agt gtc aat ttg ttc ggg cat gtt ggc agt acg act gcc agc agg ggt gag ctc ttc ctc ttc tgg aac
ctc tat aaa gct cca ata ctg ttg gca cta gtg gca gga gaa gct gct ggt atc atg gaa aac ata agt gac
gat gtg att gtt ggc cga tgc ctg gcc att ctc aaa ggg att ttt ggt agc agt gca gta cct cag ccc aaa
gaa act gtg gtg tct cgt tgg cgt gct gat ccc tgg gct cgg ggc tct tat tcc tat gtt gct gca gga tca
tct gga aat gac tat gat tta atg gct cag cca atc act cct ggc ccc tcg att cca ggt gcc cca cag ccg
att cca cga ctc ttc ttt gcg gga gaa cat acg atc cgt aac tac cca gcc aca gtg cat ggt gct ctg ctg
agt ggg ctg cga gaa gcg gga aga att gca gac cag ttt ttg ggg gcc atg tat acg ctg cct cgc cag gcc
aca cca ggt gtt cct gca cag cag tcc cca agc atg tga (SEQ ID NO: 22)

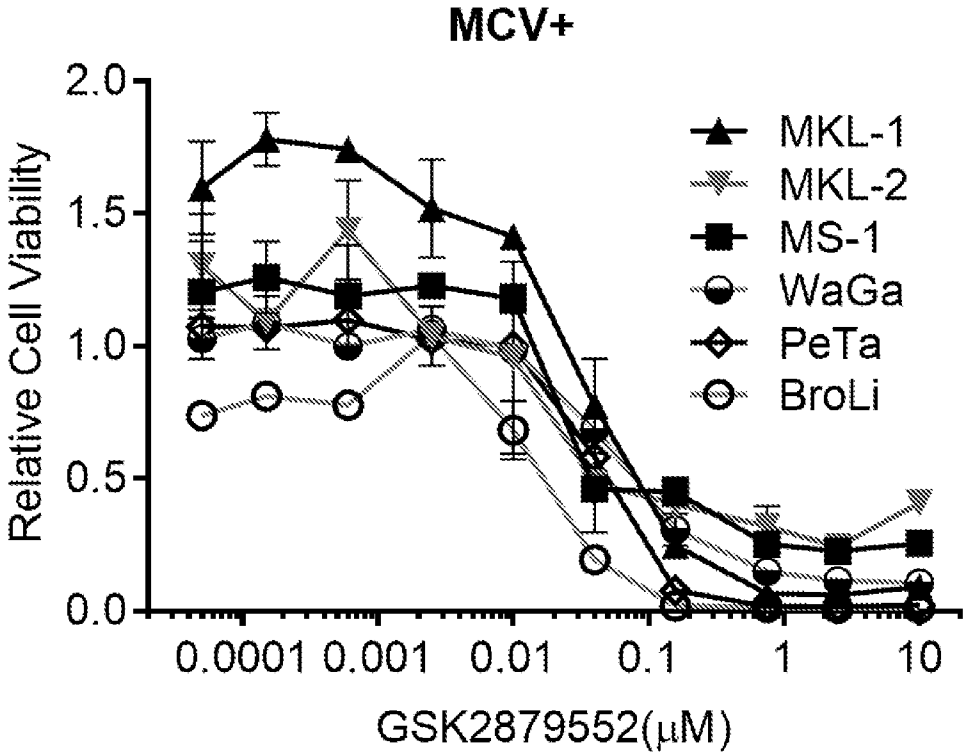



FIG. 22A

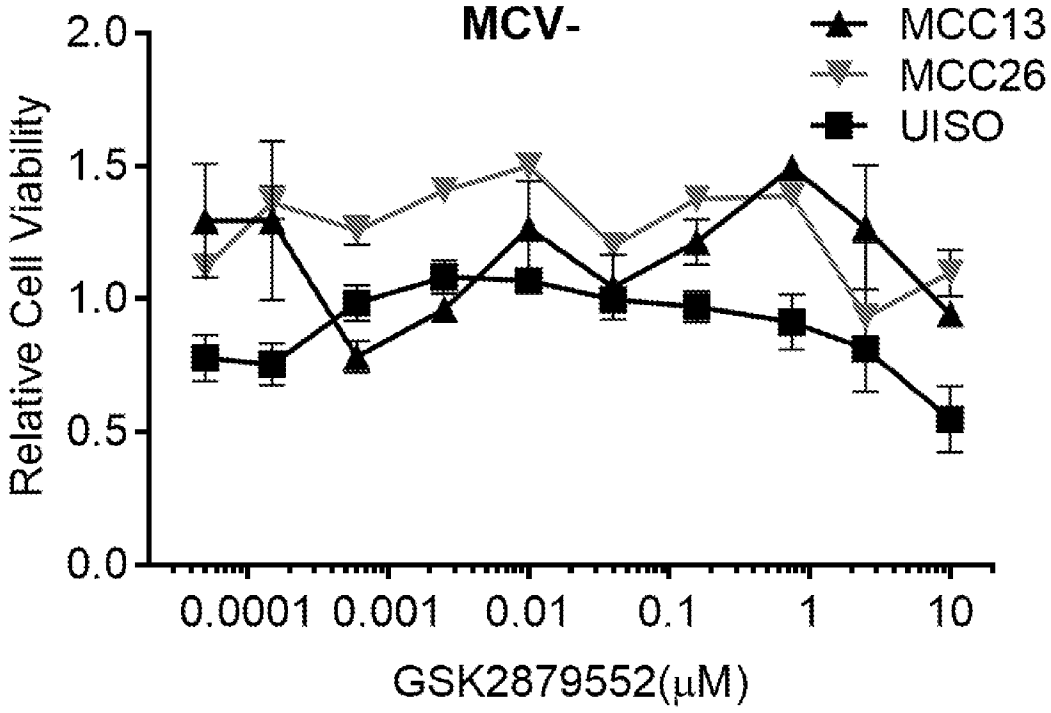



FIG. 22B

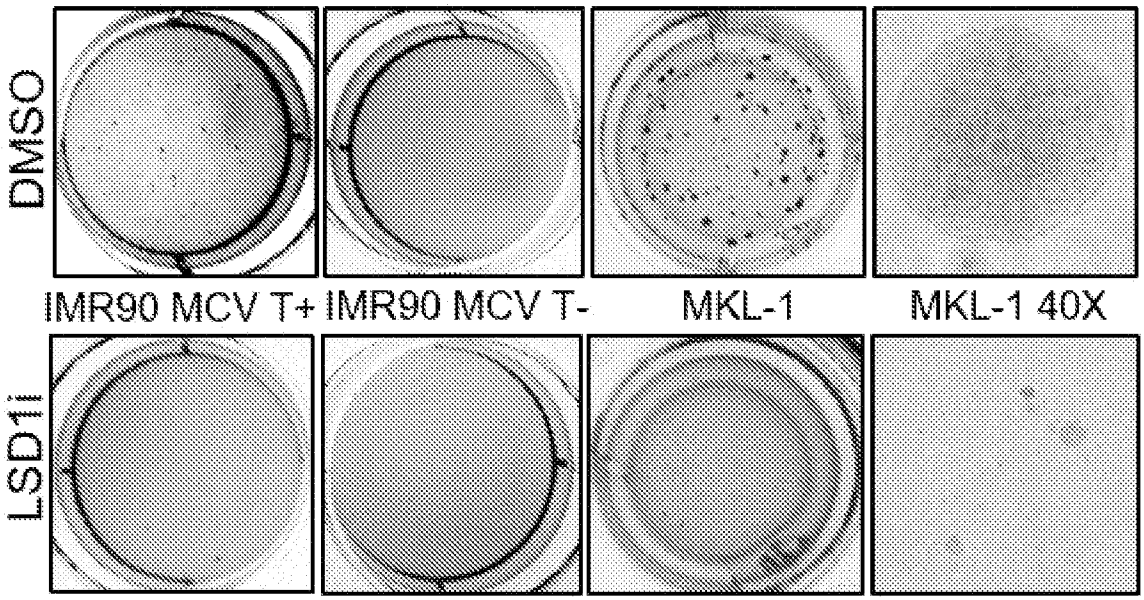



FIG. 23A

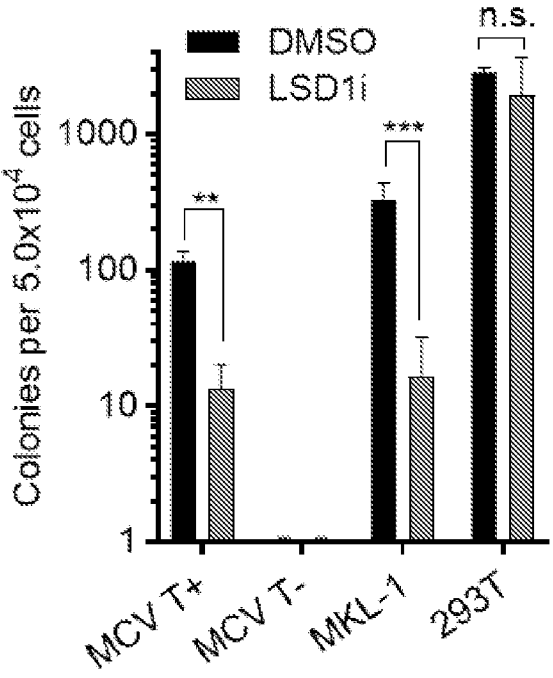



FIG. 23B

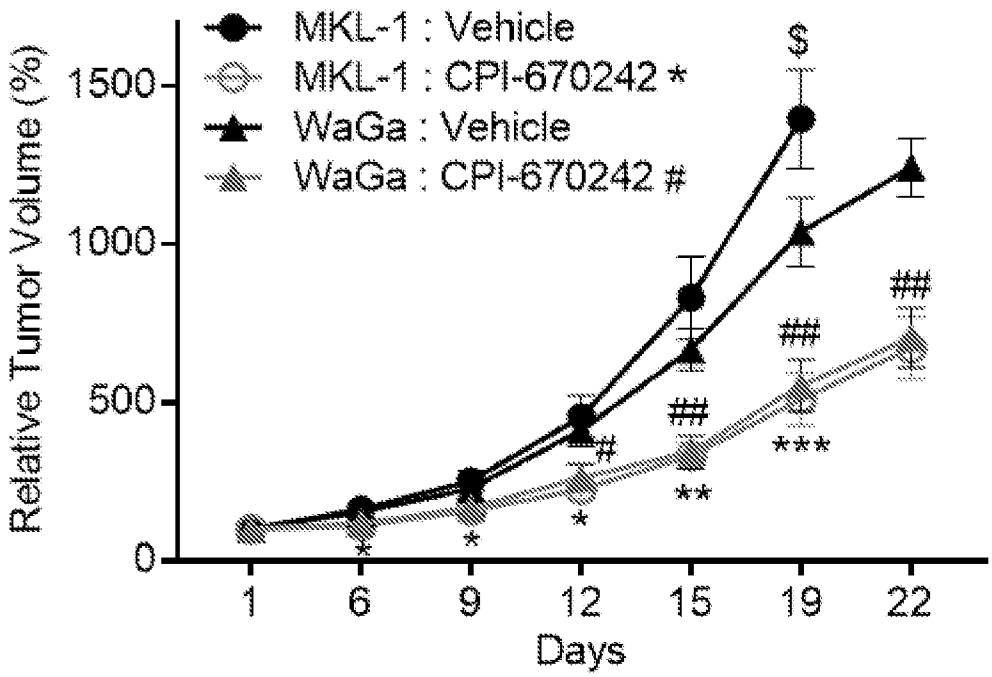

MKL-1 : Vehicle
MKL-1 : CPI-670242 *
WaGa : Vehicle
WaGa : CPI-670242 #
Relative Tumor Volume (%)
1500
1000
500
0
1
6
9
12
15
19
22
Days
$
##
##
##
#
*
*
*
**
***

FIG. 24

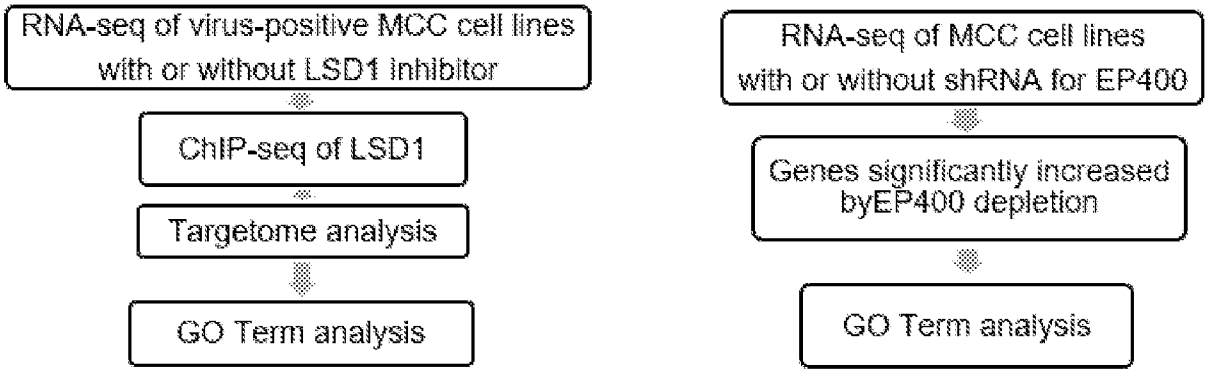

RNA-seq of virus-positive MCC cell lines with or without LSD1 inhibitor
ChIP-seq of LSD1
Targetome analysis
GO Term analysis
RNA-seq of MCC cell lines with or without shRNA for EP400
Genes significantly increased byEP400 depletion
GO Term analysis

FIG. 25

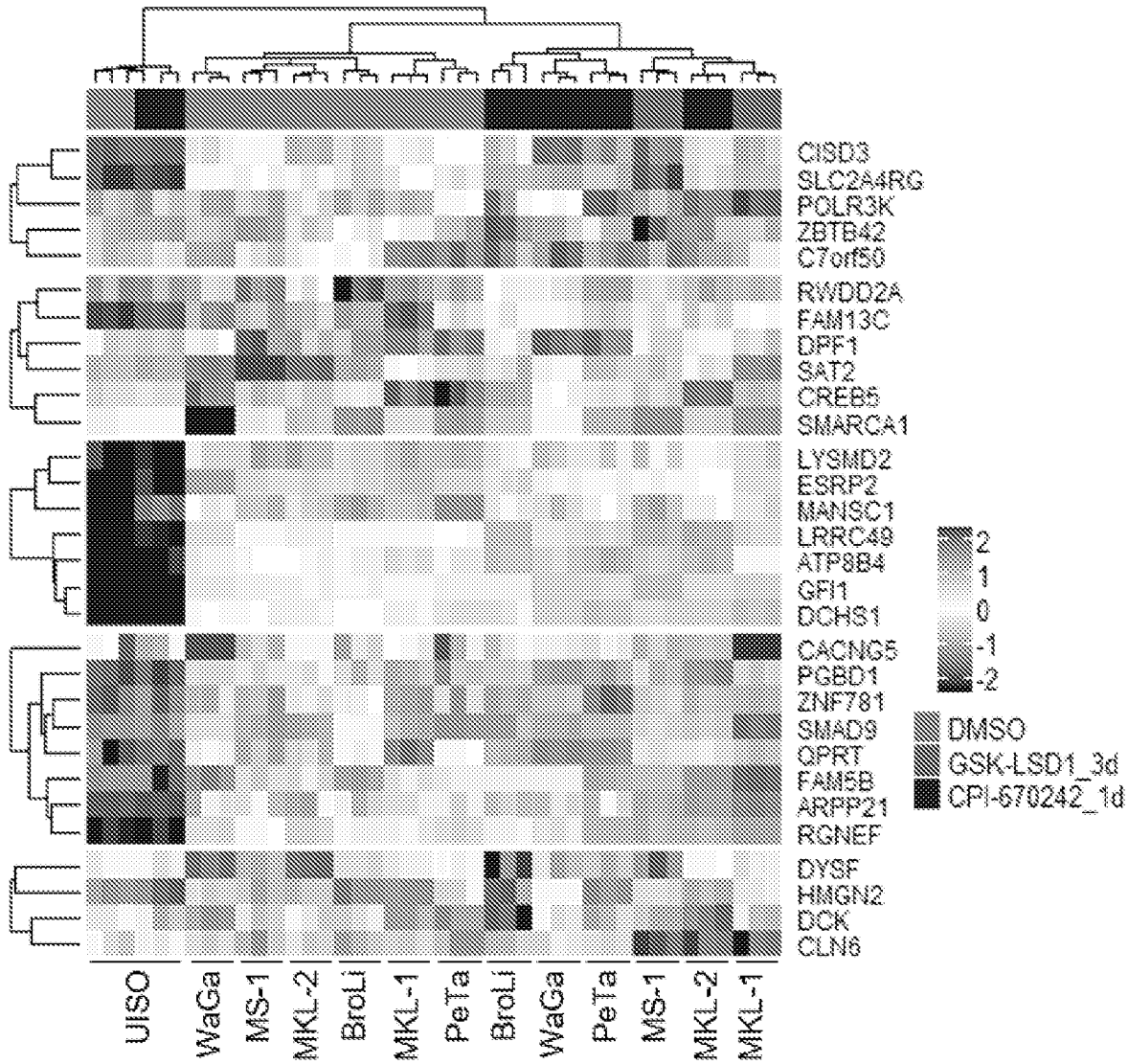

CISD3
SLC2A4RG
POLR3K
ZBTB42
C7orf50
RWDD2A
FAM13C
DFF1
SAT2
CREB5
SMARCA1
LYSMD2
ESRP2
MANSC1
LRRC49
ATP8B4
GFI1
DCHS1
CACNG5
PGBD1
ZNF781
SMAD9
QPRT
FAM5B
ARPP21
RGNEF
DYSF
HMGN2
DCK
CLN6
2
1
0
-1
-2
DMSO
GSK-LSD1_3d
CPI-670242_1d
UISO
WaGa
MS-1
MKL-2
BroLi
MKL-1
PeTa
BroLi
WaGa
PeTa
MS-1
MKL-2
MKL-1

FIG. 26

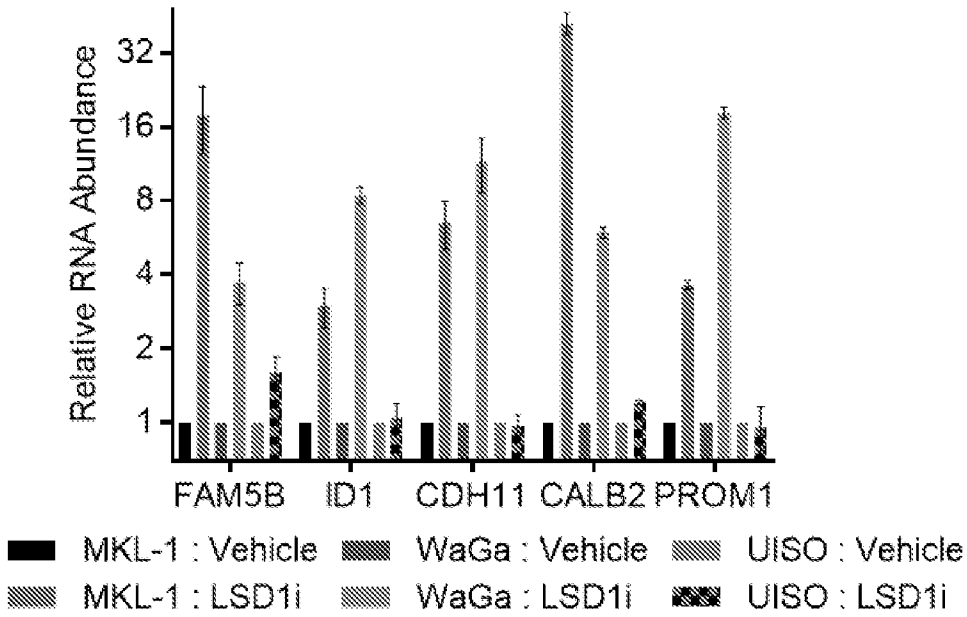



FIG. 27A

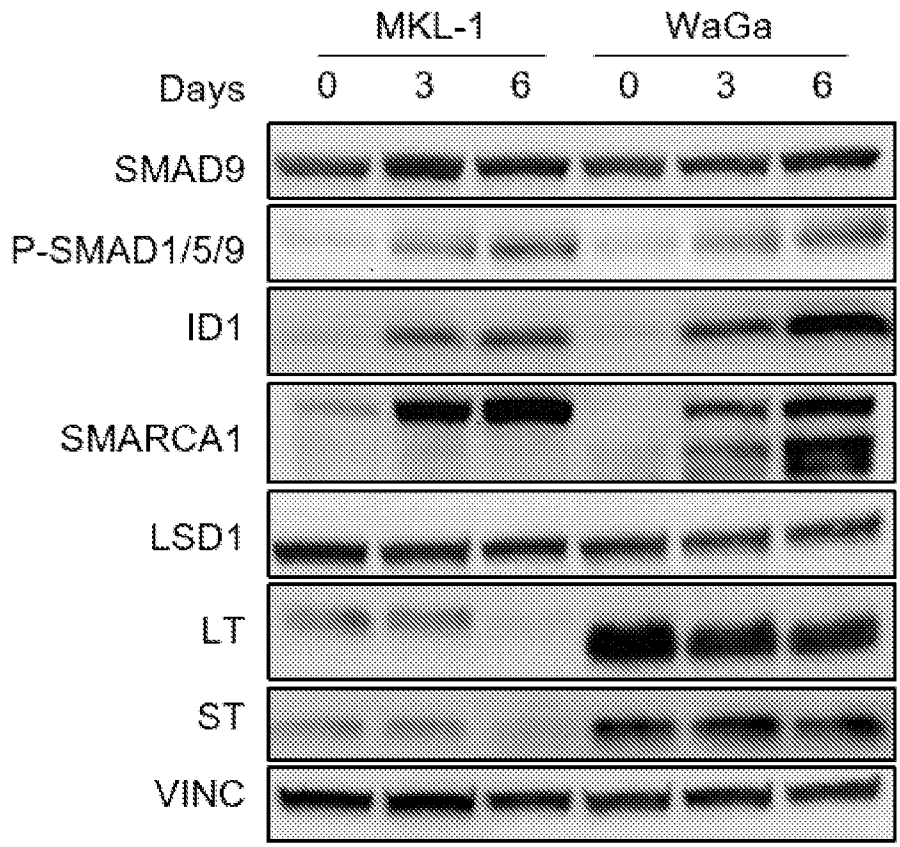



FIG. 27B

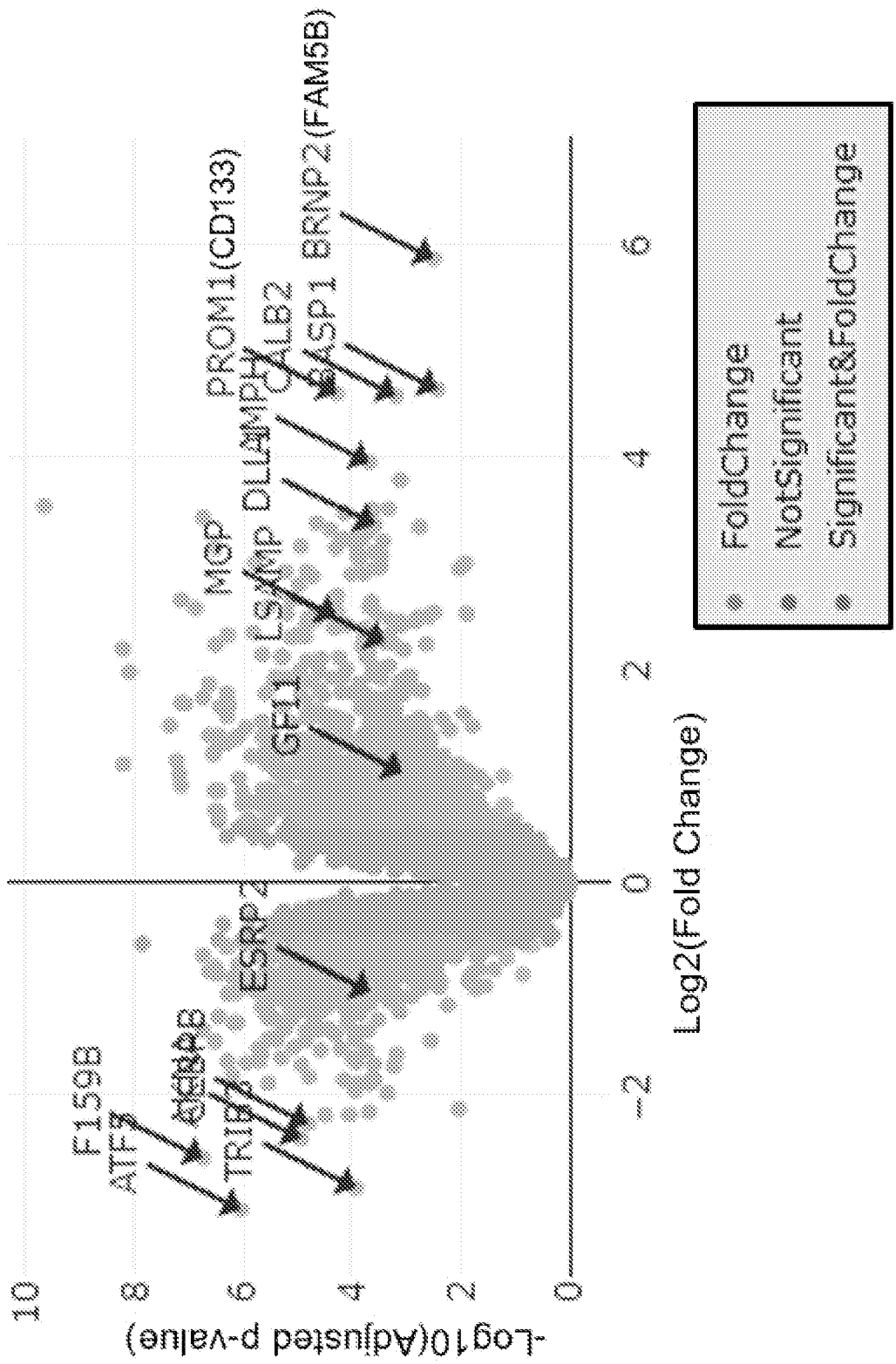

-Log10(Adjusted p-value)
10
8
6
4
2
0
Log2(Fold Change)
−2
0
2
4
6
F159B
ATF3
TRIB3
ESRP1
GFI1
LSAMP
MGP
DLLAMPH
CALB2
PROM1(CD133)
ASP1
BRNP2(FAM5B)
FoldChange
NotSignificant
Significant&FoldChange

FIG. 28

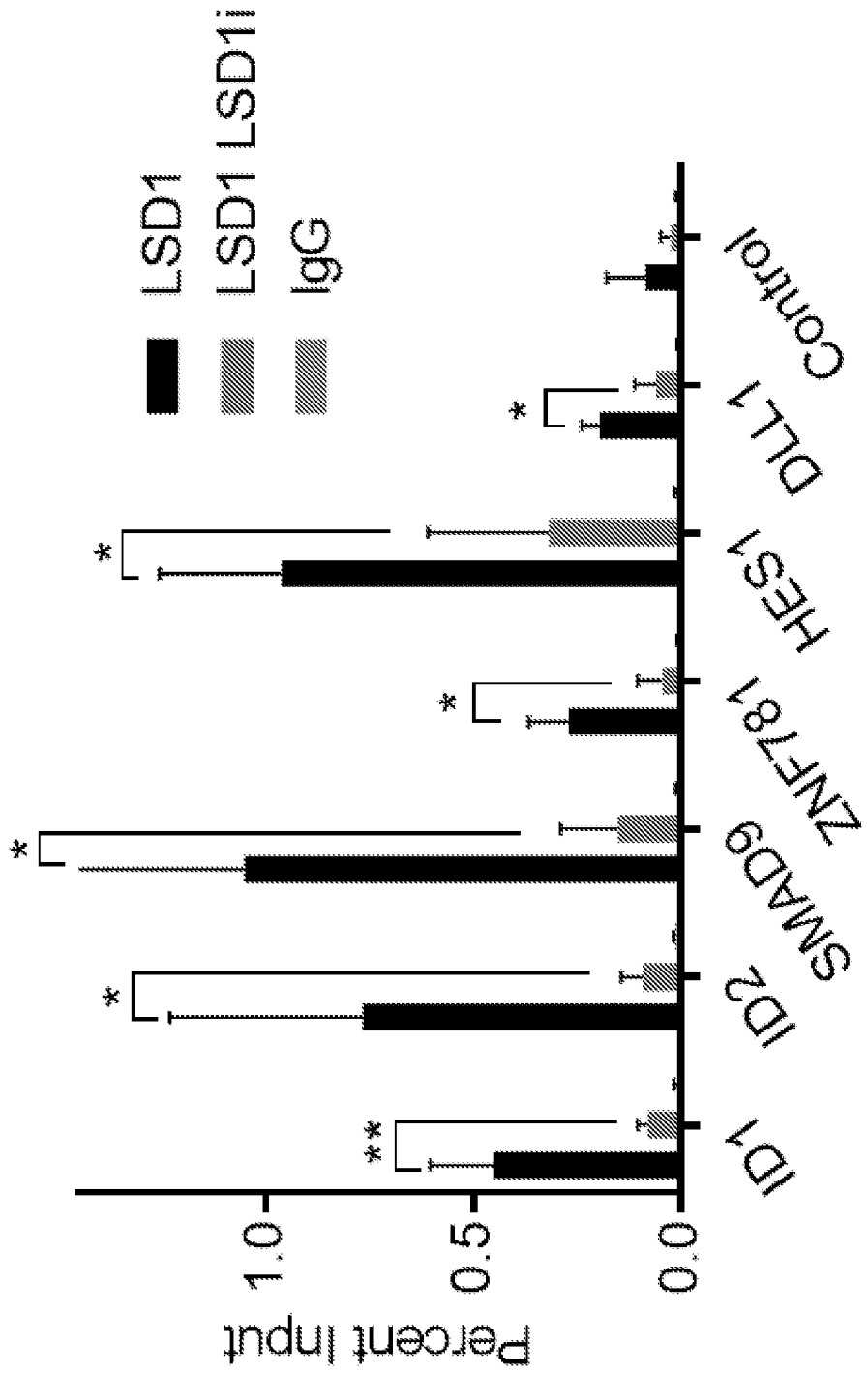

LSD1
LSD1 LSD1i
IgG
Percent Input
1.0
0.5
0.0
ID1
ID2
SMAD9
ZNF781
HES1
DLL1
Control
**
*
*
*
*
*

FIG. 29

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/55611

A. CLASSIFICATION OF SUBJECT MATTER

IPC - C07K 16/08; C12N 15/113; G01N 33/574 (2016.01)

CPC - C07K 16/084; C12N 9/0022, 15/1337; G01N 33/5008, 33/5743, 33/57484

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. |
|---|---|---|
| X | US 2011/0182901 A1 (PETER, M et al.) 28 July 2011; abstract; paragraphs [0039], [0087], [0091], [0092], [0095], [0097]-[0098], [0099]-[0100], [0124], [0134], [0135], [0139]; claims 1, 12-13 | 15, 28/15 |
| A | US 2013/210888A1 (SHI, Y et al.) 15 August 2013; paragraphs [0015], [0093] | 1-2, 9-10, 20-23, 25, 26-27, 28/1-2, 28/9-10, 28/20-23, 28/25-27, 29/1-2, 29/9-10, 30/1-2, 30/9-10, 31/1-2, 31/9-10, 32/1-2, 32/9-10, 34, 36/1-2, 36/9-10, 37/15, 37/20-21, 38/37/15, 38/37/20-21 |
| A | US 2017/0224633 A1 (MCCORD, DE et al.) 10 August 2017; paragraphs [0061], [0147] | 1-2, 9-10, 20-23, 25, 26-27, 28/1-2, 28/9-10, 28/20-23, 28/25-27, 29/1-2, 29/9-10, 30/1-2, 30/9-10, 31/1-2, 31/9-10, 32/1-2, 32/9-10, 34, 36/1-2, 36/9-10, 37/15, 37/20-21, 38/37/15, 38/37/20-21 |

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

* Special categories of cited documents:

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

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

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

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

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

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

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

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

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 23 January 2019 (23.01.2019) | 26 MAR 2019 |
| Name and mailing address of the ISA/<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer<br>Shane Thomas<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (January 2015)

**INTERNATIONAL SEARCH REPORT**

International application No.
PCT/US18/55611

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | (TAKAGI, S et al.) LSD1 inhibitor T-3775440 inhibits SCLC cell proliferation by disrupting LDS1 interactions with SNAG domain proteins INSM1 and GFI1B. Cancer Research. 1 September 2017, Epub 30 June 2017, Vol. 77, No. 17, pages 4652-4662; abstract; DOI: 10.1158/0008-5472.CAN-16-3502 | 1-2, 9-10, 20-23, 25, 26-27, 28/1-2, 28/9-10, 28/20-23, 28/25-27, 29/1-2, 29/9-10, 30/1-2, 30/9-10, 31/1-2, 31/9-10, 32/1-2, 32/9-10, 34, 36/1-2, 36/9-10, 37/15, 37/20-21, 38/37/15, 38/37/20-21 |
| P, X | WO 2018/106984 A1 (CONSTELLATION PHARMACEUTICALS, INC.) 14 June 2018; entire document | 1-2, 9-10, 15, 20-23, 25, 26-27, 28/1-2, 28/9-10, 28/15, 28/20-23, 28/25-27, 29/1-2, 29/9-10, 30/1-2, 30/9-10, 31/1-2, 31/9-10, 32/1-2, 32/9-10, 34, 36/1-2, 36/9-10, 37/15, 37/20-21, 38/37/15, 38/37/20-21 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/55611

**Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)**

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US18/55611

**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.:
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:

-***-Please See Supplemental Page-***-

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.:
Claims 1, 2 (in-part), 9 (in-part), 10 (in-part), 15, 20-23 (each in-part), 25 (in-part), 26-32 (each in-part), 34, and 36-38 (each in-part); an antisense LSD1 inhibitor (treatment), MCPyV T antigen (MCPyV marker), and LSD1 (marker)

**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.

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-43, an antisense LSD1 inhibitor (treatment), MCPyV T antigen (MCPyV marker), and LSD1 (marker) are directed toward methods and pharmaceutical compositions for selecting and treating a subject having a Merkel cell polyomavirus positive cancer, killing or inhibiting the growth of cancer cells, determining whether a tumor is MCPyV positive, determining the efficacy of a treatment, and screening agents useful for treatment.

The methods and pharmaceutical compositions will be searched to the extent they encompass an antisense LSD1 inhibitor (first exemplary treatment), MCPyV T antigen (first exemplary MCPyV marker), and LSD1 (first exemplary marker). Applicant is invited to elect additional treatment(s), and/or MCPyV marker(s) and/or marker(s) to be searched. Additional treatment(s), and/or MCPyV marker (s) and/or marker(s) will be searched upon the payment of additional fees. It is believed that claims 1, 2 (in-part), 9 (in-part), 10 (in-part), 15, 20-23 (each in-part), 25 (in-part), 26-32 (each in-part), 34, and 36-38 (each in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass an antisense LSD1 inhibitor (treatment), MCPyV T antigen (MCPyV marker), and LSD1 (marker). Applicants must specify the claims that encompass any additionally elected treatment(s) and/or MCPyV marker(s) and/or antigen(s) or marker(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be an miRNA LSD1 inhibitor (treatment).

No technical features are shared between the treatments and/or MCPyV markers and/or antigens or markers of Groups I+ and, accordingly, these groups lack unity a priori.