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(54) Title: B CELL DISORDER CLASSIFICATION AND SUSCEPTIBTY TO INHIBITORS

(57) Abstract: In embodiments of the invention, the invention provides methods of classifying lymphomas with respect to treatment sensitivity in order to provide better prognosis and treatment.

B CELL DISORDER CLASSIFICATION AND SUSCEPTIBILITY TO INHIBITORS**CROSS-REFERENCE TO A RELATED APPLICATION**

[0001] This patent application claims the benefit of U.S. Provisional Patent Application No. 62/518,994, filed June 13, 2017, which is incorporated by reference herein in its entirety.

**STATEMENT REGARDING
FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT**

[0002] This invention was made with Government support under project number Z01 ZIA BC 011010 by the National Institutes of Health, National Cancer Institute. The Government has certain rights in the invention.

**INCORPORATION-BY-REFERENCE OF MATERIAL SUBMITTED
ELECTRONICALLY**

[0003] Incorporated by reference in its entirety herein is a computer-readable nucleotide/amino acid sequence listing submitted concurrently herewith and identified as follows: One 7,994 Byte ASCII (Text) file named "737957_ST25.txt" created on March 30, 2018.

BACKGROUND OF THE INVENTION

[0004] There is an unmet need in the art for better methods of classifying lymphomas with respect to treatment sensitivity in order to provide better prognosis and treatment. The present invention provides such methods.

BRIEF SUMMARY OF THE INVENTION

[0005] In certain embodiments, the invention provides a method of detecting an association of a first protein and a second protein, the method comprising: (A) obtaining a sample comprising one or more B cells; (B) contacting the sample with a first antibody and a second antibody, wherein the first antibody binds the first protein and does not bind the second protein and the second antibody binds the second protein and does not bind the first protein, wherein the first antibody is of a species different than the first protein and the second antibody is of a species different than the second protein, and wherein the first

antibody is of a species different than the second antibody; (C) performing a proximity ligation assay using the first antibody and the second antibody and a detectable probe to produce a PLA signal of one or more detectable puncta; (D) contacting the sample with a third antibody, wherein the third antibody binds to a protein on the B cell surface, and wherein the third antibody is labeled with a detectable label; and (E) detecting the proximity ligation assay signal of (C) wherein a positive PLA signal indicates association of the first and second proteins in one or more of the B cells comprised in the sample. The items (C) and (D) may be performed in any order or simultaneously.

[0006] In certain embodiments, the invention provides a method of identifying a B cell disorder having a greater probability of responding to treatment using an inhibitor, the method comprising: (a) obtaining a B cell disorder sample; (b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex, wherein the two proteins are different from one another and are components of different complexes; (b') detecting in the sample the presence of the protein complex of (b); and (c) identifying the sample having the presence of the protein complex of (b) as a B cell disorder having a greater probability of responding to treatment using an inhibitor of the BCR complex, an inhibitor of the MYD88 complex, or an inhibitor of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b).

[0007] In certain embodiments, the invention provides a method of identifying an activated B-cell-like diffuse large B cell lymphoma (ABC DLBCL) tumor having a greater probability of responding to treatment using an inhibitor of the B cell receptor (BCR) signaling pathway, the method comprising obtaining an ABC DLBCL tumor sample; analyzing the sample for the presence of an activated component of the BCR signaling pathway; detecting in the sample the presence of an activated component of the BCR signaling pathway; and identifying the sample having the presence of an activated component of the BCR signaling pathway as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway compared to a sample having an absence of an activated component of the BCR signaling pathway.

[0008] In certain embodiments, the invention provides a method of identifying germinal center B-cell-like diffuse large B cell lymphoma (GCB DLBCL) or Burkitt lymphoma tumor having a greater probability of responding to treatment using an inhibitor of the B cell

receptor (BCR) signaling pathway proximal component LYN, SYK, or CD19, the method comprising obtaining a GCB DLBCL tumor sample; analyzing the sample for survival dependence on LYN, SYK, or CD19; detecting in the sample survival dependence on LYN, SYK, or CD19; and identifying the sample having survival dependence on LYN, SYK, or CD19 as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19 compared to a tumor sample not detected to have a survival dependence on LYN, SYK, or CD19.

[0009] In certain embodiments, the invention provides a method of identifying a tumor having a greater probability of responding to treatment using an inhibitor of the B cell receptor (BCR), CD45, PI3 kinase pathway, or mTOR, the method comprising obtaining a tumor sample; analyzing the sample for the presence of a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins; detecting in the sample the presence of a complex of BCR, MYD88, and TLR9 proteins; and identifying the sample having the presence of a complex of BCR, MYD88, and TLR9 proteins as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR compared to a sample having an absence of a complex of BCR, MYD88, and TLR9 proteins.

[0010] In certain embodiments, the invention provides a method of identifying a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway, the method comprising obtaining a tumor sample; analyzing the sample for the presence of a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins; detecting in the sample the presence of a complex of BCR, MYD88, and TLR9 proteins; and identifying the sample having the presence of a complex of BCR, MYD88, and TLR9 proteins as a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway compared to a sample having an absence of a complex of BCR, MYD88, and TLR9 proteins.

[0011] In certain embodiments, the invention provides a method of classifying a lymphoma tumor, the method comprising obtaining a lymphoma tumor sample; analyzing the sample for survival dependence on LYN, SYK, or CD19; detecting in the sample the presence of survival dependence on LYN, SYK, or CD19; and classifying the sample as germinal center B-cell-like diffuse large B cell lymphoma (GCB DLBCL) or Burkitt lymphoma.

[0012] In certain embodiments, the invention provides a method of treating or preventing an activated B-cell-like diffuse large B cell lymphoma (ABC DLBCL) in a human subject,

the method comprising administering to the subject an effective amount of an inhibitor of CD45. In certain embodiments, the inhibitor of CD 45 is 2-(4-acetylanilino)-3-chloronaphthoquinone. In certain embodiments, the inhibitor is an inhibitor of mTOR and is selected from the group of OSI-027, AZD-8055, AZD2014, SIROLIMUS, BEZ-235, and everolimus. In certain embodiments, the inhibitor is an inhibitor of PI3 kinase pathway and is selected from the group of IPI-145, BYL-719, BKM-120, Idelalisib, GDC-0980, and Copanlisib.

[0013] In certain embodiments, the invention provides a method of treating or preventing a germinal center B-cell-like diffuse large B cell lymphoma (GCB DLBCL) or Burkitt lymphoma in a human subject, the method comprising administering to the subject an effective amount of an inhibitor of LYN, SYK, or CD19.

[0014] Additional embodiments are described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The accompanying drawings, which are incorporated into and form a part of the specification, illustrate several embodiments of the present invention and, together with the description, serve to explain the principles of the invention. The drawings are only for the purpose of illustrating (an) embodiment(s) of the invention and are not to be construed as limiting the invention.

[0016] Figure 1A presents a schematic of CRISPR-Cas9 screens in lymphoma cell lines.

[0017] Figure 1B presents graphs showing negative control (top) or positive control (bottom) sgRNAs displayed for each cell line with indicated metrics.

[0018] Figure 1C presents graphs showing cumulative CRISPR screen scores (CSS) of selected transcription factors.

[0019] Figures 1D and 1E present graphs showing cumulative CSS of significantly different genes between BCR-dependent ABC or GCB lines compared to all others.

[0020] Figure 1F is a schematic wiring diagram of pathways important to survival of DLBCL subtypes.

[0021] Figure 2A presents graphs showing toxicity of sgRNA mediated depletion of BCR signaling genes or control RPL6. DLBCL lines were transduced and percentages of sgRNA-expressing green fluorescent ("GFP") (GFP+) cells were monitored by FACS. GFP was normalized to day 0 and followed for two weeks. Average and SEM of replicates is displayed, $n \geq 3$

[0022] Figure 2B presents graphs showing correlation of sgRNA toxicity 14 days post sgRNA induction with GRmax of SYK inhibitor PRT062607 or BTK inhibitor, ibrutinib four days after treatment.

[0023] Figure 2C is a bar graph showing calculated GRmax from dose response of PRT062607 in 44 DLBCL cell lines.

[0024] Figure 3A is graph showing DLBCL lines transduced and percentages of sgRNA-expressing (GFP+) cells monitored by FACS. GFP was normalized to day 0 and followed for two weeks; average and SEM of replicates is displayed, $n \geq 3$.

[0025] Figure 3B shows overlap and frequency of copy number gains or amplifications in 290 ABC DLBCL patients for indicated genes (top). Amplification of the UNC93B1 and CNPY3 loci in ABC DLBCL biopsies (black lines, below chromosome ideogram). Minimal shared regions are bracketed and genes displayed below (bottom).

[0026] Figure 3C is a dot plot showing the essential TLR9 interactome in HBL1. TLR9-BioID2 interactome determined by SILAC-based mass spectrometry (y-axis) plotted by the CRISPR screen score (CSS, x-axis). Bait (TLR9) is labeled using a square. Essential interactors are labeled using a labeled dot, with those interactors shared with TMD8 labeled using a circle (see Fig. 11A-C).

[0027] Figure 3D shows gels. Lysates of indicated DLBCL cell lines were immunoprecipitated with anti-IgM or isotype control antibodies before being immunoblotted with TLR9 or IgM antibodies, representative blot; $n=3$.

[0028] Figure 3E is a confocal image of PLA reaction between TLR9 and IgM counterstained with DAPI and wheat germ agglutinin (left), and normalized quantification (PLA Score) of IgM:TLR9 proximal spots, data from five independent experiments in HBL1 cells. White scale bar is 10 μ m. Statistical analysis performed using one-way ANOVA with Tukey's post test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

[0029] Figure 3F is a PLA image of TLR9 and MYD88 counterstained with DAPI. PLA score of IgM and TLR9 after shRNA knockdown of indicated genes, data from four experiments. White scale bar is 10 μ m. Statistical analysis performed using one-way ANOVA with Tukey's post test, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

[0030] Figure 4 presents dot plots showing gene expression values (Log_2 FPKM) values of MYD88 are shown by DLBCL subtype, ABC ($n=294$), GCB ($n=164$) and unclassified (Unc) ($n=115$). Gene expression data were correlated with DNA copy number and linear regression calculated for ABC samples (right). One-way

ANOVA and Tukey's post test * $p < 0.05$ *** $p < 0.001$ (left), linear regression * $p < 0.05$, *** $p < 0.0001$ (right).

[0031] Figure 5 presents a summary model of My-T-BCR signaling (left) and constitutive GC BCR signaling (right).

[0032] Figure 6 presents a bar graph showing cumulative CRISPR screen scores (CSS) of indicated lymphoma cell lines for MCL1, BCL2, and BCL-XL (BCL2L1).

[0033] Figures 7A-7G present dot plots showing correlation of genome-wide and replication CRISPR screens. A subset of lymphoma cell lines were rescreened with replication libraries containing approximately 10 sgRNAs targeting each of the displayed genes. Depletion scores of the genome-wide screen are shown on the x-axis while the z-score of the average $\log_2$ fold change of all sgRNAs targeting a given gene is shown on the y-axis for the replication screen. Pearson correlations and linear regressions are displayed for each of the overlapping datasets. Genes displayed are the same as in Fig. 1F.

[0034] Figure 8 presents a bar graph showing cumulative CRISPR screen scores (CSS) displayed for indicated lymphoma cell lines from a replication screen containing approximately 10 sgRNAs targeting each of the indicated TLR family genes.

[0035] Figures 9A-9F show toxicity profiles of lymphoma cell lines treated with PRT062607. ABC and GCB cell lines were counted, plated and treated with various doses of the SYK inhibitor, PRT062607, or DMSO control. MTS assays were performed after plating and again four days later to monitor growth inhibition. Normalized MTS readings were used to calculate the GRmetrics (see methods) and GR value was plotted against PRT062607 dose. Results are grouped by DLBCL subtype and GRmax values.

[0036] Figure 10 presents a bar graph. A panel of 67 lymphoma cell lines was transduced with an shRNA targeting CD19. Shown is the percent of live, shRNA-containing (GFP+) cells at the last time point (10-12 days) after shRNA induction normalized to the level GFP before induction (day 0). Average and SEM are displayed, $n \geq 3$.

[0037] Figure 11A shows the essential TLR9 interactome in TMD8. TLR9-BioID2 interactome determined by SILAC-based mass spectrometry (y-axis) plotted by the CRISPR screen score (CSS, x-axis). Bait (TLR9) is labeled using a square. Essential interactors are labeled using a labeled dot, those shared with HBL1 (see Fig. 3C) are labeled using a circle.

[0038] Figure 11B is a Venn diagram of the overlap of SILAC mass spectrometry TLR9-BioID2 interactors in experiments performed in TMD8 and HBL1 ABC lines. The 47 overlapping proteins are listed.

[0039] Figure 11C is a bar graph showing the enrichment of 47 overlapping TLR9-bioID2 proximal proteins is shown (upper) relative to their CRISPR screen score (lower). Gene names labeled in red are enriched and toxic to both HBL1 and TMD8.

[0040] Figure 12A presents gels showing whole cell lysates of indicated DLBCL cell lines immunoprecipitated with anti-IgM or isotype control antibodies before being immunoblotted with IgM or indicated TLR antibodies, representative blots, n=3.

[0041] Figure 12B presents gels showing ABC DLBCL cell lines HBL1 and TMD8 incubated on ice with IgM or isotype control antibodies and lysed. Lysates were immunoprecipitated (plasma membrane) with IgM or isotype control. Unbound lysates (cytosolic) were then immunoprecipitated with IgM or isotype control antibodies before all IP lysates were immunoblotted with indicated antibodies, representative blots, n=2.

[0042] Figure 12C presents confocal images of PLA reaction between IgM and TLR9 in HBL1 and TMD8 cells transduced with control SC4, CD79A or TLR9 shRNAs. Cells were puromycin selected and shRNAs induced with dox for two days before being fixed and imaged (left); quantification right, data from 3 separate experiments. One-way ANOVA and Tukey's post test **p< 0.01 ***p< 0.001 (left)

[0043] Figure 12D presents images. An IgM:TLR9 PLA was performed in a panel of ABC and GCB DLBCL cell lines and the presence of chronic active BCR signaling (+ = present), MYD88 mutational status and IgH isotype (μ =IgM, γ =IgG).

[0044] Figure 12E shows the number of puncta per cell of IgM:TLR9 PLA quantitated. Box and whisker plots display mean and interquartile data, outliers represent 10% of total dataset, n $\geq$ 3.

[0045] Figure 12F shows the data from Fig. 12E plotted as a box and whisker plot segregated by ABC (left) and GCL (right) lines. Mann-Whitney unpaired t-test **p< 0.01.

[0046] Figure 12G shows images. To define the cytoplasmic location of the BCR-TLR9 interaction, ABC cells were counterstained for LAMP1, a marker of late endolysosomes, where TLR9 resides, and PLA was performed between IgM:TLR9, IgM:LAMP1 and IgM:SYK.

[0047] Figure 12H is a dot plot showing Pearson's correlation coefficients. To quantify the association between PLA signals and LAMP1 staining, the Pearson's correlation coefficients was calculated across all pixels in each imaged cell. The highest correlation was between an IgM:LAMP1 PLA and LAMP1 staining (R=0.471), whereas the correlation between an IgM:SYK PLA signal and LAMP1 was much lower

($R=0.153$). The correlation between the IgM:TLR9 PLA signal and LAMP1 staining was intermediate ($R=0.310$), indicating that a significant component of the IgM:TLR9 interaction is in LAMP1+ vesicles.

[0048] Figure 12I shows quantitation of IgM:TLR9 PLA signal following ectopic expression of either empty vector, TLR9, wild type MYD88 or MYD88^{L265P}.

[0049] Figure 13A is a graph showing TLR9 shRNA is rescued by overexpression of TLR9. HBL1 cells were transduced with empty vector (EV) or wild type TLR9 expressing dsRedExpress2 vectors and then with shRNA vectors marked by GFP targeting a control (SC4), MYD88 or TLR9. The percent of double positive cells was monitored by FACS and normalized to day 0.

[0050] Figure 13B is a heatmap of gene expression values showing the global phenocopy of MYD88-dependent genes after shRNA-mediated knockdown of TLR9 or MYD88 in HBL1 at indicated time points.

[0051] Figure 13C is a bar graph showing gene signatures enriched in downregulated genes from HBL1 or TMD8 after shRNA-mediated knockdown of TLR9.

[0052] Figure 13D is a bar graph showing normalized $\text{IkB}\alpha$ -luciferase reporter levels at indicated time points after knockdown of TLR9 with indicated shRNAs; $n=3$.

[0053] Figure 14A shows the essential MYD88 interactome in TMD8. MYD88^{L265P}-BioID interactome determined by SILAC-based mass spectrometry (y-axis) plotted by the CRISPR screen score (CSS, x-axis). Bait (MYD88) is labeled in a square. Essential interactors are labeled using a labeled dot, with those shared between at least two ABC lines (see Fig. 15) are labeled in a circle.

[0054] Figure 14B is a bar graph showing the common MYD88 interactome. Abundance of proteins detected in two or more MYD88^{L265P}-BioID interactome experiments in the indicated ABC lines (upper) with their corresponding CRISPR screen scores (lower).

[0055] Figures 14C-14F are confocal images (left) of a PLA of (C) MALT1 with MYD88 (D) CARD11 with BCL10 (E) IgM with phospho- $\text{IkB}\alpha$ (F) TLR9 with phospho- $\text{IkB}\alpha$. Quantitation (right) of a normalized PLA score is shown for all cells collected in at least 3 independent experiments in HBL1 ABC cells. White scale bar is 10 μm . Statistical analysis performed using one-way ANOVA with Tukey's post test, *** $p < 0.0001$.

[0056] Figure 14G shows MYD88^{L265P}-BioID2 selected cells imaged with indicated antibodies, white scale bar is 10 μm .

[0057] Figure 14H shows MYD88^{L265P}-BioID2 selected cells transduced with indicated shRNAs and BioID2 signal was measured by streptavidin binding via FACS.

[0058] Figure 15A shows the essential MYD88^{L265P} interactome in HBL1. MYD88^{L265P} -BioID2 interactome from SILAC-based mass spectrometry (y-axis) plotted by the CRISPR screen score (CSS, x-axis). Bait (MYD88) is labeled in a square. Essential interactors are a labeled dot, with those shared with either TMD8 or OCI-Ly10 labeled in a circle.

[0059] Figure 15B is a Venn diagram of the overlap of MYD88^{L265P} -BioID2 interactors in TMD8, OCI-Ly10 and HBL1 ABC lines. Proteins found in two or more experiments are listed.

[0060] Figure 15C shows gels. Lysates of TMD8, HBL1 and U2932 cells transduced with empty vector or MYD88^{L265P} -BioID2, selected and treated with 50 uM biotin for 24 hours. Lysates were prepared and immunoprecipitated with streptavidin before being immunoblotted with CARD11 and MYD88 antibodies.

[0061] Figure 15D shows gels. Lysates of TMD8 cells transduced with empty vector, MYD88^{L265P} or wild type (WT) BioID2-fusion proteins, selected and treated with 50 uM biotin for 24 hours. Lysates were prepared and immunoprecipitated with streptavidin before being immunoblotted with CARD11, MALT1 or MYD88 antibodies; representative blot; n=3.

[0062] Figure 15E shows a confocal image (left) of a PLA of BCL10 with MYD88. Quantitation (right) of a normalized PLA score is shown for all cells collected in at least 3 independent experiments in HBL1 ABC cells. White scale bar is 10 um. *** p < 0.0001.

[0063] Figure 15F shows a bar graph of BCL10:MYD88 and MALT1:MYD88 PLA in ABC (left) and GCB (right) lines, Mann-Whitney unpaired t-test, **p < 0.01.

[0064] Figure 16A is a bar graph showing ABC lines expressing MYD88^{L265P} -BioID2 treated with DMSO or 10 nM ibrutinib for 24 hours, and the numbers of biotin puncta were quantified from confocal images (representative experiment, n=3).

[0065] Figure 16B is a graph showing SILAC-based mass spectrometry comparison of MYD88^{L265P} -BioID2 interactome in TMD8 cells treated with DMSO (x-axis) vs. 10 nM ibrutinib (y-axis). Proteins reduced upon ibrutinib treatment are shown in circles, those similarly decreased in two separate cell lines (see Fig. 17A) are labeled in circles. Bait (MYD88^{L265P}) is labeled in a square. Venn diagram showing overlap of proteins decreased by $\geq 30\%$ in OCI-Ly10 cells (Fig. 17A) is shown as an inset.

[0066] Figure 16C shows a graph showing synergistic killing.

[0067] Figure 17A shows SILAC mass spectrometry comparison of MYD88^{L265P}-BioID2 interactome in DMSO (x-axis) vs. 10nM ibrutinib (y-axis). Proteins reduced upon ibrutinib treatment in two separate experiments (Fig. S9) are labeled in circles, while bait (MYD88) is labeled in a square.

[0068] Figure 17B presents confocal images of mTOR, LAMP1 and MYD88^{L265P}-BioID2 in the HBL1 and TMD8 ABC lines.

[0069] Figure 17C shows quantitation of normalized PLA score for the indicated PLA pairs shown for ABC lines treated with DMSO or 10 nM ibrutinib, n=3 independent experiments.

[0070] Figure 17D shows ABC lines expressing MYD88^{L265P}-BioID2 treated with DMSO, 10 nM ibrutinib, 200 nM AZD2014 or both for 24 hours. Intensity of the biotin signal was quantified from confocal images; n≥2.

[0071] Figure 17E shows immunoblot analysis of phospho-Ser176/180-IKKα/β, phospho-Ser371-p70S6K, phospho-Thr37/46-4E-BP1 and total MYD88 in HBL1 and TMD8 cells treated with either ibrutinib (10 nM), AZD2014 (200 nM) or both drugs in combination for 24 hours.

[0072] Figure 17F shows a matrix drug screen. Left: Representative matrix viability data from a 10-fold dose titration of ibrutinib vs. the mTORC1/2 inhibitor AZD2014 in the TMD8 ABC line. Middle: ΔBliss score, with negative values indicating synergy. Right: ΔBliss negative sum (synergism metric) for TMD8 cells treated for the indicated times with combinations of the BTK inhibitors ibrutinib or acalabrutinib with the indicated mTOR, PI3 kinase, or chemotherapy drugs.

[0073] Figure 17G is a graph showing TMD8 cells established as s.c. tumors in NSG mice and treated daily by i.p. injection with vehicle or BTK inhibitor ibrutinib (5 mg/kg), orally with AZD2014 (15mg/kg) twice per day or with a combination of ibrutinib and AZD2014. Mice were treated for 12 days and tumor growth was measured as a function of tumor volume ($1/2 \times \text{length} \times \text{width}^2$). Error bars represent SEM, n=10 mice per group for vehicle, ibrutinib, combination, n=9 mice per group AZD2014. ***p≤0.001, Day 12, two-way ANOVA, Tukey's post test.

[0074] Figure 18A is a graph showing IgM:TLR9 PLA performed on an FFPE fixed tissue microarray of lymphoma cell lines. PLA puncta were quantified and plotted as the absolute number of spots per cell from at least 2 images. Cell lines are divided by putative lymphoma subtype for presentation. PMBL = Primary Mediastinal B cell

lymphoma, HL = Hodgkin lymphoma, BPDC = Blastic Plasmacytoid Dendritic Cell neoplasm, BL = Burkitt lymphoma, MZL = Marginal Zone lymphoma, GCB = Germinal Center DLBCL, WM = Waldenström's macroglobulinemia, ABC = Activated B cell-like DLBCL.

[0075] Figure 18B shows representative confocal fluorescent image of a germinal center from a reactive lymph node.

[0076] Figure 19A shows IgM:TLR9 PLA puncta per cell in ABC and GCB FFPE patient biopsies. All data were normalized to an FFPE TMD8 sample stained concurrently.

[0077] Figure 19B shows IgM:TLR9 PLA measured in FFPE primary patient material from the indicated lymphoma types. All data were normalized to an FFPE TMD8 sample stained concurrently.

[0078] Figure 19C shows PLA performed between IgM and TLR9 on DLBCL patient biopsy samples, shown in bright field (top) or fluorescence (bottom). White scale bar is 10 um.

[0079] Figure 19D shows quantification of IgM:TLR9 PLA puncta per cell from confocal images of DLBCL FFPE patient biopsy samples. Patients were grouped by ibrutinib response: Responders (left, CR, PR, SD) included 3 ABC and 1 unclassified patient, Non-Responders (right, PD,) included 3 ABC, and 1 GCB patient.

[0080] Figure 20A shows shRNA-mediated toxicity of indicated genes in two WM cell lines. Control (SC4), CD79A, TLR9 or MYD88 shRNAs were expressed in tandem with GFP and the relative level of GFP was followed over time by FACS. Data from 3 independent experiments.

[0081] Figures 20B and 20C show (B) confocal images of PLA reaction between IgM and TLR9 counterstained with DAPI and wheat germ agglutinin, and (C) normalized quantification (PLA Score) of IgM:TLR9. Data from 3 independent experiments. White scale bar is 10 um.

DETAILED DESCRIPTION OF THE INVENTION

[0082] In certain embodiments, the invention provides a method of identifying an activated B-cell-like diffuse large B cell lymphoma (ABC DLBCL) tumor having a greater probability of responding to treatment using an inhibitor of the B cell receptor (BCR) signaling pathway, the method comprising obtaining an ABC DLBCL tumor sample; analyzing the sample for the presence of an activated component of the BCR signaling

pathway; detecting in the sample the presence of an activated component of the BCR signaling pathway; and identifying the sample having the presence of an activated component of the BCR signaling pathway as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway compared to a sample having an absence of an activated component of the BCR signaling pathway.

[0083] In certain embodiments, the activated component of the BCR signaling pathway is (i) a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins, (ii) CARD11, (iii) BCL10, (iv) MALT1, (v) IkappaB kinase (IKK) alpha, beta, or gamma subunits, (vi) phosphorylated IKK beta, (vii) IkappaB alpha, or (viii) phosphorylated IkappaB alpha, or any combination of (i) – (viii).

[0084] In certain embodiments, the inhibitor of the BCR signaling pathway is ibrutinib, acalabrutinib, or ONO/GS-4059. In certain embodiments, the inhibitor of the BCR signaling pathway is ibrutinib.

[0085] As defined herein, an inhibitor is a substance that decreases the function or expression of a protein or the signaling of a protein in a pathway. An inhibitor may be a small molecule, an siRNA, an shRNA, CRISPR, an antibody, etc.

[0086] In certain embodiments, the activated component of the BCR signaling pathway is a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins. In certain embodiments, none of these proteins are mutated. In certain embodiments, the MYD88 protein in the complex has an L265P mutation and the CD79A or CD79B subunit of the BCR protein in the complex has an activating mutation.

[0087] In certain embodiments, the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample, which may be any suitable sample, e.g., derived from any type of tissue, liquid (e.g., blood, plasma, urine, etc.). In certain embodiments, analyzing the sample comprises performing a proximity ligation assay on the sample. In certain embodiments, the tumor sample is from a human.

[0088] Proximity ligation assay signal is dependent upon the distance between two species-specific secondary antibodies that each bind to a target-specific primary antibody, where the primary antibodies are each bound to a target protein/antigen. When the primary antibody target proteins/antigens are within tens of nanometers of one another, i.e., when the two target proteins are associated with one another (e.g., within 10 nm of each other, within 20 nm of each other, within 30 nm of each other, within 40 nm of each other, within a distance that will permit a detectable PLA signal), a double-stranded PCR template is formed

from complementary oligos bound to the species-specific secondary antibodies. This double-stranded PCR template is then ligated, amplified, and detected by a probe. Any suitable detectable signal may be used, including, for example, an oligonucleotide probe conjugated to a detectable tag, wherein the detectable tag is a FLAG tag, a poly-histidine tag (e.g. 6xHis), a SNAP tag, a Halo tag, a cMyc tag, a glutathione-S-transferase tag, an avidin tag, a biotin tag, an enzyme, a fluorescent protein, a luminescent protein, a chemiluminescent protein, a bioluminescent protein, a phosphorescent protein, use of horseradish peroxidase, etc. The signal is dependent upon the specificity of the primary antibodies, the distance between the two target proteins, and importantly, the absence of additional antibodies that might be bound by the oligo-bound secondary PLA antibody. A positive PLA signal is indicated by an increase of at least 5%, at least 10%, at least 15%, at least 20% or more in the number, size, and/or intensity of the detectable puncta compared to a PLA signal obtained from a negative control sample. The PLA signal can be normalized to a positive control.

[0089] The ability to co-stain patient samples when performing PLA is an aspect of the invention. Using antibodies against surface antigens on tumor enriched populations 1) identifies the cells of interest and 2) filters out cells from complex tissue samples which can have high levels of non-specific background PLA signal. This is the case in FFPE tissue samples from lymph node biopsies. Previous methods for co-staining have been for chemical dyes and other non-antibody stains such as phalloidin, but these methods are inadequate for tumor samples. Previous methods were to perform the complete PLA protocol, wash the samples, e.g., back into TBS with 0.5% tween-20, and stain with antibodies against surface antigens. However, this destroys antibody binding, which is perhaps due to degradation of epitopes. One embodiment of the invention provides co-staining with B cell-specific, fluorescently-tagged antibodies (e.g., mouse anti-CD20 labeled with Alexa488) while staining with, e.g., anti-IgM (e.g., goat) and, e.g., anti-TLR9 (e.g., rabbit). A PLA between these antibodies, e.g., goat and rabbit antibodies, can then be performed. As shown in the Example, by selecting anti-B-cell specific antibodies from a third species (e.g., mouse anti-human CD20), the PLA signal between IgM and TLR9 was unaffected and the surface epitopes were detected before their degradation.

[0090] In certain embodiments, the invention provides a method of treating an ABC DLBCL tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway, the method comprising identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling

pathway according to a method as described herein; and administering to the tumor an effective amount of an inhibitor of the BCR signaling pathway. In certain embodiments, the inhibitor of the BCR signaling pathway is ibrutinib, acalabrutinib, or ONO/GS-4059. In certain embodiments, the inhibitor of the BCR signaling pathway is ibrutinib. In certain embodiments, the tumor is in a human.

[0091] In certain embodiments, the invention provides a method of treating a human subject having an ABC DLBCL tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway, the method comprising identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway according to a method as described herein; and administering to the human subject an effective amount of an inhibitor of the BCR signaling pathway. In certain embodiments, the inhibitor of the BCR signaling pathway is ibrutinib, acalabrutinib, or ONO/GS-4059. In certain embodiments, the inhibitor of the BCR signaling pathway is ibrutinib.

[0092] In certain embodiments, the invention provides a method of identifying germinal center B-cell-like diffuse large B cell lymphoma (GCB DLBCL) or Burkitt lymphoma tumor having a greater probability of responding to treatment using an inhibitor of the B cell receptor (BCR) signaling pathway proximal component LYN, SYK, or CD19, the method comprising obtaining a GCB DLBCL tumor sample; analyzing the sample for survival dependence on LYN, SYK, or CD19; detecting in the sample survival dependence on LYN, SYK, or CD19; and identifying the sample having survival dependence on LYN, SYK, or CD19 as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19 compared to a tumor sample not detected to have a survival dependence on LYN, SYK, or CD19.

[0093] In certain embodiments, the lymphoma is a GCB DLBCL. In certain embodiments, the lymphoma is a Burkitt lymphoma. In certain embodiments, the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample, which may be any suitable sample, e.g., derived from any type of tissue, liquid (e.g., blood, plasma, urine, etc.). In certain embodiments, the tumor sample is from a human.

[0094] In certain embodiments, the inhibitor of the BCR signaling pathway proximal component is an inhibitor of LYN and is dasatinib or ponatinib. In certain embodiments, the inhibitor of the BCR signaling pathway proximal component is an inhibitor of SYK and is entospletinib or PRT-060318 or PRT-062607.

[0095] In certain embodiments, the invention provides a method of treating a GCB DLBCL tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19, the method comprising identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19 according to a method described herein; and administering to the tumor an effective amount of an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19. In certain embodiments, the inhibitor of the BCR signaling pathway proximal component is an inhibitor of LYN and is dasatinib or ponatinib. In certain embodiments, the inhibitor of the BCR signaling pathway proximal component is an inhibitor of SYK and is entospletinib, PRT-062607, or PRT-060318. In certain embodiments, the tumor is in a human.

[0096] In certain embodiments, the invention provides a method of treating a human subject having a GCB DLBCL tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19, the method comprising identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19 according to a method described herein; and administering to the human subject an effective amount of an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19. In certain embodiments, the inhibitor of the BCR signaling pathway proximal component is an inhibitor of LYN and is dasatinib or ponatinib. In certain embodiments, the inhibitor of the BCR signaling pathway proximal component is an inhibitor of SYK and is entospletinib, PRT-062607, or PRT-060318.

[0097] In certain embodiments, the invention provides a method of identifying a tumor having a greater probability of responding to treatment using an inhibitor of the B cell receptor (BCR), CD45, PI3 kinase pathway, or mTOR, the method comprising obtaining a tumor sample; analyzing the sample for the presence of a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins; detecting in the sample the presence of a complex of BCR, MYD88, and TLR9 proteins; and identifying the sample having the presence of a complex of BCR, MYD88, and TLR9 proteins as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR compared to a sample having an absence of a complex of BCR, MYD88, and TLR9 proteins.

[0098] In certain embodiments, the tumor is a lymphoma. In certain embodiments, the lymphoma is a diffuse large B cell lymphoma (DLBCL), a primary central nervous system lymphoma (PCNSL), or a Waldenström macroglobulinemia/ lymphoplasmacytic lymphoma (WM/LPL). In certain embodiments, the DLBCL is an activated B-cell–like (ABC) DLBCL.

[0099] In certain embodiments, none of these proteins are mutated. In certain embodiments, the MYD88 protein in the complex has an L265P mutation and the CD79A or CD79B subunit of the BCR protein in the complex has an activating mutation.

[0100] In certain embodiments, the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample, which may be any suitable sample, e.g., derived from any type of tissue, liquid (e.g., blood, plasma, urine, etc.). In certain embodiments, analyzing the sample comprises performing a proximity ligation assay on the sample.

[0101] In certain embodiments, the inhibitor is an inhibitor of CD45 and is 2-(4-acetylanilino)-3-chloronaphthoquinone (Perron et al., Mol. Pharm., 85: 553-63 (2014), incorporated by reference herein). In certain embodiments, the inhibitor is an inhibitor of mTOR and is selected from the group of OSI-027, AZD-8055, AZD2014, SIROLIMUS, BEZ-235, and everolimus. In certain embodiments, the inhibitor is an inhibitor of PI3 kinase pathway and is selected from the group of IPI-145, BYL-719, BKM-120, Idelalisib, GDC-0980, and Copanlisib. In certain embodiments, the tumor sample is from a human.

[0102] In certain embodiments, the invention provides a method of treating a tumor having a greater probability of responding to treatment using an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR, the method comprising identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR according to a method described herein; and administering to the tumor an effective amount of an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR. In certain embodiments, the inhibitor is an inhibitor of CD45 and is 2-(4-acetylanilino)-3-chloronaphthoquinone. In certain embodiments, the inhibitor is an inhibitor of mTOR and is selected from the group of OSI-027, AZD-8055, AZD2014, SIROLIMUS, BEZ-235, and everolimus. In certain embodiments, the inhibitor is an inhibitor of PI3 kinase pathway and is selected from the group of IPI-145, BYL-719, BKM-120, Idelalisib, GDC-0980, and Copanlisib. In certain embodiments, the tumor is in a human.

[0103] In certain embodiments, the invention provides a method of treating a human subject having a tumor having a greater probability of responding to treatment using an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR, the method comprising

identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR according to a method described herein; and administering to the human subject an effective amount of an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR. In certain embodiments, the inhibitor is an inhibitor of CD45 and is 2-(4-acetylanilino)-3-chloronaphthoquinone. In certain embodiments, the inhibitor is an inhibitor of mTOR and is selected from the group of OSI-027, AZD-8055, AZD2014, SIROLIMUS, BEZ-235, and everolimus. In certain embodiments, the inhibitor is an inhibitor of PI3 kinase pathway and is selected from the group of IPI-145, BYL-719, BKM-120, Idelalisib, GDC-0980, and Copanlisib.

[0104] In certain embodiments, the invention provides a method of identifying a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway, the method comprising obtaining a tumor sample; analyzing the sample for the presence of a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins; detecting in the sample the presence of a complex of BCR, MYD88, and TLR9 proteins; and identifying the sample having the presence of a complex of BCR, MYD88, and TLR9 proteins as a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway compared to a sample having an absence of a complex of BCR, MYD88, and TLR9 proteins.

[0105] In certain embodiments, the tumor is a lymphoma. In certain embodiments, the lymphoma is a diffuse large B cell lymphoma (DLBCL), a primary central nervous system lymphoma (PCNSL), or a Waldenström macroglobulinemia/ lymphoplasmacytic lymphoma (WM/LPL). In certain embodiments, the DLBCL is an activated B-cell-like (ABC) DLBCL. In certain embodiments, none of these proteins are mutated. In certain embodiments, the MYD88 protein in the complex has an L265P mutation and the CD79A or CD79B subunit of the BCR protein in the complex has an activating mutation.

[0106] In certain embodiments, the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample, which may be any suitable sample, e.g., derived from any type of tissue, liquid (e.g., blood, plasma, urine, etc.). In certain embodiments, analyzing the sample comprises performing a proximity ligation assay on the sample.

[0107] In certain embodiments, the inhibitor of the NF- κ B pathway is ibrutinib, acalabrutinib, or ONO/GS-4059. In certain embodiments, the inhibitor of the NF- κ B pathway is ibrutinib. In certain embodiments, the tumor sample is from a human.

[0108] In certain embodiments, the invention provides a method of treating a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway, the method comprising identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway according to a method described herein; and administering to the tumor an effective amount of an inhibitor of the NF- κ B pathway. In certain embodiments, the inhibitor of the NF- κ B pathway is ibrutinib, acalabrutinib, or ONO/GS-4059. In certain embodiments, the inhibitor of the NF- κ B pathway is ibrutinib. In certain embodiments, the tumor is in a human.

[0109] In certain embodiments, the invention provides a method of treating a human subject having a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway, the method comprising identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway according to a method described herein; and administering to the human subject an effective amount of an inhibitor of the NF- κ B pathway. In certain embodiments, the inhibitor of the NF- κ B pathway is ibrutinib, acalabrutinib, or ONO/GS-4059. In certain embodiments, the inhibitor of the NF- κ B pathway is ibrutinib.

[0110] In certain embodiments, the invention provides a method of classifying a lymphoma tumor, the method comprising obtaining a lymphoma tumor sample; analyzing the sample for survival dependence on LYN, SYK, or CD19; detecting in the sample the presence of survival dependence on LYN, SYK, or CD19; and classifying the sample as germinal center B-cell-like diffuse large B cell lymphoma (GCB DLBCL) or Burkitt lymphoma.

[0111] In certain embodiments, the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample, which may be any suitable sample, e.g., derived from any type of tissue, liquid (e.g., blood, plasma, urine, etc.). In certain embodiments, analyzing the sample comprises performing a proximity ligation assay on the sample. In certain embodiments, the tumor sample is from a human.

[0112] In certain embodiments, the invention provides a method of treating or preventing an activated B-cell-like diffuse large B cell lymphoma (ABC DLBCL) in a human subject, the method comprising administering to the subject an effective amount of an inhibitor of CD45. In certain embodiments, the inhibitor of CD 45 is 2-(4-acetylanilino)-3-chloronaphthoquinone. In certain embodiments, the inhibitor is an inhibitor of mTOR and is

selected from the group of OSI-027, AZD-8055, AZD2014, SIROLIMUS, BEZ-235, and everolimus. In certain embodiments, the inhibitor is an inhibitor of PI3 kinase pathway and is selected from the group of IPI-145, BYL-719, BKM-120, Idelalisib, GDC-0980, and Copanlisib.

[0113] In certain embodiments, the invention provides a method of treating or preventing a germinal center B-cell-like diffuse large B cell lymphoma (GCB DLBCL) or Burkitt lymphoma in a human subject, the method comprising administering to the subject an effective amount of an inhibitor of LYN, SYK, or CD19. In certain embodiments, the inhibitor of the BCR signaling pathway proximal component is an inhibitor of LYN and is dasatinib or ponatinib. In certain embodiments, the inhibitor of the BCR signaling pathway proximal component is an inhibitor of SYK and is entospletinib, PRT-062607, or PRT-060318.

[0114] In certain embodiments, the present invention provides a method of determining a CRISPR score of a gene, which method comprises

- (a) normalizing raw counts by a total read counts using the equation:

$$N_{irjd} = 1 + (X_{irjd} * 4 \times 10^7) / \sum_i X_{irjd};$$

- (b) eliminating sgRNAs with low counts across all experimental conditions using the equation:

$$m_i = \text{Max}_{jd} \left(\min_r N_{irjd} \right)$$

and eliminating those sgRNAs for which $m_i < 100$;

- (c) calculating log ratios using the equation:

$$LR_{irj} = \log_2(N_{irj21}/N_{irj0});$$

- (d) transforming log-ratios using the equation:

$$Z_{irj} = (LR_{irj} - \text{Mean}_i(LR_{irj})) / \text{Standard deviation}_i(LR_{irj});$$

- (e) averaging across replicates using the equation:

$$S_{ij} = \text{Mean}_r(Z_{ijr});$$

- (f) calculating signal variance for each sgRNA using the equations:

$$\text{Total Variance: } TV_i = \text{Var}_j(S_{ij})$$

$$\text{Error Variance: } EV_i = \text{Mean}_j \left(\frac{\text{Var}_r(Z_{irj})}{R_i} \right)$$

$$\text{Signal Variance: } SV_i = TV_i - EV_i$$

- (g) calculating the maximal pair wise correlation between any two sgRNAs in this set by, for each gene g , letting G_g be the set of sgRNAs that represent it, and using the equation

$$C_g = \text{Max}_{k,l \in G_g} \left(\text{Correlation}_j(S_{kj}, S_{lj}) \right)$$

where, if for a given gene $C_g < 0.45$, then letting i_g be the index of the sgRNA representing that gene that has the highest signal variance and using that as the sole representative of gene j using the equation

$$CSS_{gj} = S_{i_g j}$$

where, if $C_g \geq 0.45$, then proceeding;

- (h) averaging the two sgRNAs which were most correlated within gene g using the equation

$$M_{gj} = \frac{1}{2}(S_{kj} + S_{lj}),$$

where $k, l \in G_g$ are such that

$$\text{Correlation}_j(S_{kj}, S_{lj}) = C_g,$$

- (i) using the equation

$$V_i = \text{Correlation}_j(S_{ij}, M_{gj}) / C_g$$

to calculate for each $i \in G_g$, and averaging together those sgRNAs for which $V_i > 0.85$;

- (j) calculating the CSS for gene g using the equation

$$CSS_{gj} = \text{mean}_{i \in G_g, V_i > 0.85} (S_{ij});$$

wherein

i is an index indicating sgRNA,

j is an index indicating cell line,

R_j is the number of replicates for cell line j ,

r is $\{1 - R_j\}$ and is an index indicating replicate,

d is an index indicating time point, and

X_{irjd} indicates the raw sequencing counts for sgRNA i , in replicate r of cell line j at time d .

[0115] The terms “lymphoma” and “lymphoid malignancy” as used herein refer to malignant tumors derived from lymphocytes and lymphoblasts. In some embodiments, when required, the lymphoma will have My-T-BCR involvement. The phrase may refer to a broad lymphoma class (e.g., DLBCL, FL, MCL, etc.) or to a subtype or subgroup falling within a broad lymphoma class (e.g., GCB DLBCL, ABC DLBCL). Examples of lymphomas include, but are not limited to, follicular lymphoma (FL), Burkitt lymphoma (BL), mantle cell lymphoma (MCL), follicular hyperplasia (FH), small cell lymphocytic lymphoma (SLL), mucosa-associated lymphoid tissue lymphoma (MALT), splenic lymphoma, multiple myeloma, lymphoplasmacytic lymphoma, post-transplant lymphoproliferative disorder (PTLD), lymphoblastic lymphoma, nodal marginal zone lymphoma (NMZ), germinal center B cell-like diffuse large B cell lymphoma (GCB), activated B cell-like diffuse large B cell lymphoma (ABC) and primary mediastinal B cell lymphoma (PMBL), primary central nervous system lymphoma (PCNSL) and Waldenstrom’s macroglobulinemia (WM).

[0116] The inventive method can comprise analyzing a sample from a subject, such as from fresh tissue, a snap-frozen sample from a subject, or a formalin-fixed and paraffin-embedded (FFPE) sample from a subject. The sample may be any suitable sample, e.g., derived from any type of tissue, liquid (e.g., blood, plasma, urine, etc.). As understood by one of ordinary skill in the art, the phrase “a snap-frozen sample from a subject” means that a sample is first taken from a subject and afterwards snap-frozen, and the phrase “obtaining or providing a formalin-fixed and paraffin-embedded (FFPE) sample from the subject” means that a sample is first taken from a subject and afterwards fixed with formalin and embedded in paraffin. The sample may be a biopsy sample. For lymphoma samples, for example, since the tumor can be in any anatomic location, from any tissue, including, but not limited to needle cores of lymph nodes, whole lymph nodes, skin and liver.

[0117] One or more biopsy samples may be obtained from a patient that has been diagnosed as having a tumor, and the biopsy samples can be formalin-fixed and paraffin-embedded using protocols that are known in the art or are commercially available (see, e.g., Keirnan, J. (ed.), *Histological and Histochemical Methods: Theory and Practice*, 4th edition, Cold Spring Harbor Laboratory Press (2008), incorporated herein by reference.

[0118] In certain embodiments, the invention provides an inhibitor of the NF- κ B pathway for use in treating a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway, wherein the tumor is identified as a tumor having a greater

probability of responding to treatment using an inhibitor of the NF- κ B pathway according to methods described herein.

[0119] In certain embodiments, the invention provides an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR for use in treating a tumor having a greater probability of responding to treatment using an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR, wherein the tumor is identified as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR, CD45, PI3 kinase pathway, or mTOR according to methods described herein.

[0120] The terms “treat,” “inhibit,” and “prevent” as well as words stemming therefrom, as used herein, do not necessarily imply 100% or complete treatment, inhibition, or prevention. Rather, there are varying degrees of treatment, inhibition, or prevention of which one of ordinary skill in the art recognizes as having a potential benefit or therapeutic effect. In this respect, the inventive methods can provide any amount of any level of treatment, inhibition, or inhibition of a protein, protein complex, or condition in a mammal. Furthermore, the treatment or prevention provided by the inventive method can include treatment or prevention of one or more conditions or symptoms of the condition, e.g., cancer, being treated or prevented. For example, treatment or prevention can include promoting the regression of a tumor. Also, for purposes herein, “prevention” can encompass delaying the onset of the condition, or a symptom or condition thereof.

[0121] An “effective amount” or “an amount effective to treat” refers to a dose that is adequate to prevent or treat cancer in an individual. Amounts effective for a therapeutic or prophylactic use will depend on, for example, the stage and severity of the disease or disorder being treated, the age, weight, and general state of health of the patient, and the judgment of the prescribing physician. The size of the dose will also be determined by the active selected, method of administration, timing and frequency of administration, the existence, nature, and extent of any adverse side-effects that might accompany the administration of a particular active, and the desired physiological effect. It will be appreciated by one of skill in the art that various diseases or disorders could require prolonged treatment involving multiple administrations, perhaps using the inhibitors in each or various rounds of administration.

[0122] The subject referred to herein may be any mammal. As used herein, the term “mammal” refers to any mammal, including, but not limited to, mammals of the order Rodentia, such as mice and hamsters, and mammals of the order Logomorpha, such as rabbits. The mammals may be from the order Carnivora, including Felines (cats) and

Canines (dogs). The mammals may be from the order Artiodactyla, including Bovines (cows) and Swines (pigs) or of the order Perssodactyla, including Equines (horses). The mammals may be of the order Primates, Ceboids, or Simoids (monkeys) or of the order Anthropoids (humans and apes). Preferably, the mammal is a human.

[0123] In certain embodiments, the invention provides a method of detecting an association of a first protein and a second protein, the method comprising: (A) obtaining a sample comprising one or more B cells; (B) contacting the sample with a first antibody and a second antibody, wherein the first antibody binds the first protein and does not bind the second protein and the second antibody binds the second protein and does not bind the first protein, wherein the first antibody is of a species different than the first protein and the second antibody is of a species different than the second protein, and wherein the first antibody is of a species different than the second antibody; (C) performing a proximity ligation assay using the first antibody and the second antibody and a detectable probe to produce a PLA signal of one or more detectable puncta; (D) contacting the sample with a third antibody, wherein the third antibody binds to a protein on the B cell surface, and wherein the third antibody is labeled with a detectable label; and (E) detecting the proximity ligation assay signal of (C) wherein a positive PLA signal indicates association of the first and second proteins in one or more of the B cells comprised in the sample. The items (C) and (D) may be performed in any order or simultaneously.

[0124] In certain embodiments, the B cell surface protein is IgM, IgD, IgA, IgE, IgG, IgK@, IgL@, CD79A, CD79B, CD20, CD10, CD19, CD81, CR2, CD38, CD138, CD21, CD22, CD23, CD24, CD27, CD40, CD43, CD5, CD72, CD95, CD180, CD45/B220, TACI/TNFRSF13B, IL10RA, or IL10RB.

[0125] In certain embodiments, a proximity ligation assay signal is utilized. In certain embodiments, a proximity ligation assay signal of fluorescent puncta (formed from detection of two secondary antibodies, conjugated to unique complementary oligonucleotides, with specificity for different species-specific primary antibodies, such that when in proximity of less than 40 nM of one another, the conjugated oligos hybridize allowing a rolling circle DNA amplification to proceed, which can be detected by a fluorescently-labeled probe oligo) indicates association of the first and second proteins. In certain embodiments, the third antibody is fluorescently labeled. In certain embodiments, the first antibody and the second antibody each bind to a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex.

[0126] In certain embodiments, the invention provides a method of identifying a B cell disorder having a greater probability of responding to treatment using an inhibitor, the method comprising: (a) obtaining a B cell disorder sample; (b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex, wherein the two proteins are different from one another and are components of different complexes, and wherein the analysis comprises detecting an association of the two proteins according to any suitable method described herein; and (c) identifying the sample having the presence of the protein complex of (b) as a B cell disorder having a greater probability of responding to treatment using an inhibitor of the BCR complex, an inhibitor of the MYD88 complex, or an inhibitor of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b).

[0127] In certain embodiments, the invention provides a method of identifying a B cell disorder having a greater probability of responding to treatment using an inhibitor, the method comprising: (a) obtaining a B cell disorder sample; (b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex, wherein the two proteins are different from one another and are components of different complexes; (b') detecting in the sample the presence of the protein complex of (b); and (c) identifying the sample having the presence of the protein complex of (b) as a B cell disorder having a greater probability of responding to treatment using an inhibitor of the BCR complex, an inhibitor of the MYD88 complex, or an inhibitor of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b).

[0128] In certain embodiments, the sample is identified as a B cell disorder having a greater probability of responding to treatment (i) using an inhibitor of the BCR complex, when the protein complex of (b) includes a protein component of the BCR complex, (ii) using an inhibitor of the MYD88 complex, when the protein complex of (b) includes a protein component of the MYD88 complex, or (iii) using an inhibitor of the mTORC1 complex, when the protein complex of (b) includes a protein component of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b).

[0129] In certain embodiments, the sample is identified as a B cell disorder having a greater probability of responding to treatment (i) using an inhibitor of the BCR complex, when the protein complex of (b) includes a protein component of the BCR complex, (ii) using an inhibitor of the MYD88 complex, when the protein complex of (b) includes a protein component of the MYD88 complex, and (iii) using an inhibitor of the mTORC1 complex, when the protein complex of (b) includes a protein component of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b).

[0130] Herein, the term “B cell disorder” refers to a disease or disorder characterized by aberrant or abnormal proliferation and/or function of B cells. B cell disorders can include autoimmune diseases, autoinflammatory diseases, cancers, immunodeficiency disorders, infectious diseases, and the like. In certain embodiments, the B cell disorder is diffuse large B-cell lymphoma (DLBCL); follicular lymphoma; marginal zone B-cell lymphoma (MZL) or Mucosa-Associated Lymphatic Tissue lymphoma (MALT); small lymphocytic lymphoma (also known as chronic lymphocytic leukemia, CLL); mantle cell lymphoma (MCL); DLBCL variants or sub-types of primary mediastinal (thymic) large B cell lymphoma, T cell/histiocyte-rich large B-cell lymphoma, primary cutaneous diffuse large B-cell lymphoma, leg type (Primary cutaneous DLBCL, leg type), EBV positive diffuse large B-cell lymphoma of the elderly, diffuse large B-cell lymphoma associated with inflammation; Burkitt's lymphoma; lymphoplasmacytic lymphoma, which may manifest as Waldenström's macroglobulinemia; nodal marginal zone B cell lymphoma (NMZL); splenic marginal zone lymphoma (SMZL); intravascular large B-cell lymphoma; primary effusion lymphoma; lymphomatoid granulomatosis; primary central nervous system lymphoma; ALK-positive large B-cell lymphoma; plasmablastic lymphoma; large B-cell lymphoma arising in HHV8-associated multicentric Castleman's disease; B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma; B-cell lymphoma, unclassifiable with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma.

[0131] In certain embodiments, the B cell disorder is a lymphoma. In certain embodiments, the lymphoma is a diffuse large B cell lymphoma (DLBCL), a primary central nervous system lymphoma (PCNSL), or a Waldenström macroglobulinemia/lymphoplasmacytic lymphoma (WM/LPL). In certain embodiments, the lymphoma is a DLBCL and is an activated B-cell-like (ABC) DLBCL. In certain embodiments, the protein

complex comprises the proteins of BCR, MYD88, and mTOR. In certain embodiments, the B cell disorder sample is a formalin-fixed and paraffin-embedded (FFPE) sample, which may be any suitable sample, e.g., derived from any type of tissue, liquid (e.g., blood, plasma, urine, etc.). In certain embodiments, the inhibitor is OSI-027, AZD-8055, AZD2014, SIROLIMUS, BEZ-235, everolimus, or ibrutinib. In certain embodiments, the inhibitor is ibrutinib.

[0132] In certain embodiments, the invention provides an inhibitor of the BCR complex, MYD88 complex, or mTORC1 complex for use in treating a B cell disorder having a greater probability of responding to treatment using an inhibitor, wherein the B cell disorder is identified as a B cell disorder having a greater probability of responding to treatment using an inhibitor according to any method described herein.

[0133] In an embodiment, the invention provides a method of identifying a patient suffering from a B cell disorder that is responsive to treatment with one or more inhibitors comprising: (a) obtaining a sample comprising one or more B cells from the patient; (b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises at least two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex, wherein each of the two proteins are different from one another and are components of different complexes, and wherein the analysis comprises detecting an association of the two proteins according to any suitable method described herein; and (c) identifying the sample having the presence of the protein complex of (b), wherein detection of the protein complex in the patient sample identifies the patient as having a greater likelihood of responsiveness to treatment with one or more inhibitors of the BCR complex, the MYD88 complex, and/or the mTORC1 complex as compared to a patient in which the presence of the protein complex was not detected.

[0134] In an embodiment, the invention provides a method of identifying a patient suffering from a B cell disorder that is responsive to treatment with one or more inhibitors comprising: (a) obtaining a sample comprising one or more B cells from the patient; (b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises at least two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex, wherein the two proteins are different from one another and are components of different complexes; (b') detecting in the sample the presence of the protein complex of (b),

wherein detection of the protein complex in the patient sample identifies the patient as having a greater likelihood of responsiveness to treatment with one or more inhibitors of the BCR complex, the MYD88 complex, and/or the mTORC1 complex as compared to a patient in which the presence of the protein complex was not detected.

[0135] The My-T-BCR complex is a complex of proteins. The My-T-BCR complex comprises at least two different proteins, where each of these two different proteins are of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex. The two proteins are of different complexes. As an example, a “protein A” may be from the BCR complex and a “protein B” may be from the MYD88 complex; a protein A from the BCR complex and a protein B from the mTORC1 complex; a protein A may be from the nTORC1 complex and a protein B may be from the MYD88 complex;). The My-T-BCR complex may comprise more than two different proteins from the BCR complex, the MYD88 complex, or the mTORC1 complex. As an example, the My-T-BCR complex may comprise several proteins, where at least two are different such that there are several of a protein A and one of a protein B; equal numbers of a protein A and a protein B; one or more of a protein A and one or more of a protein B and one or more of a protein C, etc.

[0136] Genes known to be involved in the BCR signaling pathway, which comprises the BCR complex, include, but are not limited to, IgM, CD79A, CD79B, SYK, BTK, BLNK, PLCG2, BANK1, CARD11, BCL10, MALT1, CD22, LYN, MS4A1, PTPN1. Additional genes involved in the BCR signaling pathway and may be suitable for use in the methods described herein include BLK, CBL, CBLB, CD19, CD72, CD81, CR2, CSK, FYN, GRB2, HCK, LAPTM5, LASP1, LAT2, LCK, LRMP, MAP4K1, MAPK1, PRKCB, PRKCD, PTPN22, PTPN6, PTPRC, RAB3GAP2, SIGLEC10, SLA, TEC, VAV1, VAV2, VAV3. Genes known to be involved in the MyD88 signaling pathway include, but are not limited to, TLR9, MYD88, IRAK1, IRAK4, CNPY3, UNC93B1, STAT3, pIkBa, NFKB2. Additional genes involved in the MyD88 signaling pathway and may be suitable for use in the methods described herein include CHUK, cREL, IKBKB, IKBKG, IRAK2, IRAK3, IRF3, IRF4, IRF5, IRF7, MAP3K7, NFKB1, NFKBIA, NFKBIE, NLRP2, RELA, RELB, TAB1, TAB2, TBK1, TIRAP, TOLLIP, TRAM, TRAF3, TRAF6, TRIF, UBE2M. Genes known to be involved in the mTOR signaling pathway include, but are not limited to, mTOR, LAMTOR1, LAMTOR3, FKBP4, RUVBL1, RUVBL2, MAP4K4, PIK3C3. Additional genes involved in the mTOR signaling pathway and may be suitable for use in the methods described herein include AKT1, AKT2, AKT3, AKT1S1, ATP6V0A1, ATP6V0D1, ATP6V0D2, ATP6V1A,

ATP6V1B2, ATP6V1E1, ATP6V1H, FKBP1A, FKBP1B, LAMTOR2, LAMTOR4, LAMTOR5, MIOS, MLST8, NPRL2, NPRL3, PDPK1, PIK3CA, PIK3CB, PIK3CD, PIK3CG, PTEN, TSC1, TSC2, TBC1D7, RHEB, RPS6KB1, RPS6KB2, RPTOR, RRAGA, RRAGB, RRAGC, RRAGD.

[0137] Preferred proteins of the BCR complex include: IgM, CD79A, CD79B, SYK, BTK, BLNK, PLCG2, BANK1, CARD11, BCL10, MALT1, CD22, LYN, MS4A1, PTPN1. Preferred proteins of the MYD88 complex include: TLR9, MYD88, IRAK1, IRAK4, CNPY3, UNC93B1, STAT3, pIkBa, NFkB2. Preferred proteins of the mTORC1 complex include: mTOR, LAMTOR1, LAMTOR3, FKBP4, RUVBL1, RUVBL2, MAP4K4, PIK3C3.

[0138] As used herein, “a greater probability” means an increased likelihood of an event. The increase may be any level of increase that is statistically significant. Methods of determining an increased likelihood and statistical significance are known in the art and are described herein.

[0139] The following includes certain aspects of the invention.

[0140] 1. A method of identifying an activated B-cell-like diffuse large B cell lymphoma (ABC DLBCL) tumor having a greater probability of responding to treatment using an inhibitor of the B cell receptor (BCR) signaling pathway, the method comprising:

- (a) obtaining an ABC DLBCL tumor sample;
- (b) analyzing the sample for the presence of an activated component of the BCR signaling pathway;
- (c) detecting in the sample the presence of an activated component of the BCR signaling pathway; and
- (d) identifying the sample having the presence of an activated component of the BCR signaling pathway as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway compared to a sample having an absence of an activated component of the BCR signaling pathway.

[0141] 2. The method of aspect 1, wherein the activated component of the BCR signaling pathway is (i) a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins, (ii) CARD11, (iii) BCL10, (iv) MALT1, (v) IkappaB kinase (IKK) alpha, beta, or gamma subunits, (vi) phosphorylated IKK beta, (vii) IkappaB alpha, or (viii) phosphorylated IkappaB alpha, or any combination of (i) – (viii).

[0142] 3. The method of aspect 1 or 2, wherein the inhibitor of the BCR signaling pathway is ibrutinib, acalabrutinib, or ONO/GS-4059.

[0143] 4. The method of any one of aspects 1-3, wherein the inhibitor of the BCR signaling pathway is ibrutinib.

[0144] 5. The method of aspect 2, wherein the activated component of the BCR signaling pathway is a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins.

[0145] 6. The method of aspect 5, wherein (i) the MYD88 protein in the complex has an L265P mutation and (ii) the CD79A or CD79B subunit of the BCR protein in the complex has an activating mutation.

[0146] 7. The method of any one of aspects 1-6, wherein the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample.

[0147] 8. The method of any one of aspects 1-7, wherein analyzing the sample comprises performing a proximity ligation assay on the sample.

[0148] 9. The method of any one of aspects 1-8, wherein the tumor sample is from a human.

[0149] 10. A method of treating an ABC DLBCL tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway, the method comprising:

(1) identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway according to the method of any one of aspects 1-9; and

(2) administering to the tumor an effective amount of an inhibitor of the BCR signaling pathway.

[0150] 11. The method of aspect 10, wherein the inhibitor of the BCR signaling pathway is ibrutinib, acalabrutinib, or ONO/GS-4059.

[0151] 12. The method of aspect 10 or 11, wherein the inhibitor of the BCR signaling pathway is ibrutinib.

[0152] 13. The method of any one of aspects 10-12, wherein the tumor is in a human.

[0153] 14. A method of treating a human subject having an ABC DLBCL tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway, the method comprising:

(1) identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway according to the method of any one of aspects 1-9; and

(2) administering to the human subject an effective amount of an inhibitor of the BCR signaling pathway.

[0154] 15. The method of aspect 14, wherein the inhibitor of the BCR signaling pathway is ibrutinib, acalabrutinib, or ONO/GS-4059.

[0155] 16. The method of aspect 14 or 15, wherein the inhibitor of the BCR signaling pathway is ibrutinib.

[0156] 17. A method of identifying germinal center B-cell-like diffuse large B cell lymphoma (GCB DLBCL) or Burkitt lymphoma tumor having a greater probability of responding to treatment using an inhibitor of the B cell receptor (BCR) signaling pathway proximal component LYN, SYK, or CD19, the method comprising:

- (a) obtaining a GCB DLBCL tumor sample;
- (b) analyzing the sample for survival dependence on LYN, SYK, or CD19;
- (c) detecting in the sample survival dependence on LYN, SYK, or CD19; and
- (d) identifying the sample having survival dependence on LYN, SYK, or CD19 as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19 compared to a tumor sample not detected to have a survival dependence on LYN, SYK, or CD19.

[0157] 18. The method of aspect 17, wherein the lymphoma is a GCB DLBCL.

[0158] 19. The method of aspect 17, wherein the lymphoma is a Burkitt lymphoma.

[0159] 20. The method of any one of aspects 17-19, wherein the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample.

[0160] 21. The method of any one of aspects 17-20, wherein the inhibitor of the BCR signaling pathway proximal component is an inhibitor of LYN and is dasatinib or ponatinib.

[0161] 22. The method of any one of aspects 17-20, wherein the inhibitor of the BCR signaling pathway proximal component is an inhibitor of SYK and is entospletinib, PRT-062607, or PRT-060318.

[0162] 23. The method of any one of aspects 17-22, wherein the tumor sample is from a human.

[0163] 24. A method of treating a GCB DLBCL tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19, the method comprising:

(1) identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19 according to the method of any one of aspects 17-23; and

(2) administering to the tumor an effective amount of an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19.

[0164] 25. The method of aspect 24, wherein the inhibitor of the BCR signaling pathway proximal component is an inhibitor of LYN and is dasatinib or ponatinib.

[0165] 26. The method of aspect 24, wherein the inhibitor of the BCR signaling pathway proximal component is an inhibitor of SYK and is entospletinib, PRT-062607, or PRT-060318.

[0166] 27. The method of any one of aspects 24-26, wherein the tumor is in a human.

[0167] 28. A method of treating a human subject having a GCB DLBCL tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19, the method comprising:

(1) identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19 according to the method of any one of aspects 17-23; and

(2) administering to the human subject an effective amount of an inhibitor of the BCR signaling pathway proximal component LYN, SYK, or CD19.

[0168] 29. The method of aspect 28, wherein the inhibitor of the BCR signaling pathway proximal component is an inhibitor of LYN and is dasatinib or ponatinib.

[0169] 30. The method of aspect 28, wherein the inhibitor of the BCR signaling pathway proximal component is an inhibitor of SYK and is entospletinib, PRT-062607, or PRT-060318.

[0170] 31. A method of identifying a tumor having a greater probability of responding to treatment using an inhibitor of the B cell receptor (BCR) or CD45, the method comprising:

(a) obtaining a tumor sample;

(b) analyzing the sample for the presence of a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins;

(c) detecting in the sample the presence of a complex of BCR, MYD88, and TLR9 proteins; and

(d) identifying the sample having the presence of a complex of BCR, MYD88, and TLR9 proteins as a tumor having a greater probability of responding to treatment using

an inhibitor of the BCR or CD45 compared to a sample having an absence of a complex of BCR, MYD88, and TLR9 proteins.

[0171] 32. The method of aspect 31, wherein the tumor is a lymphoma.

[0172] 33. The method of aspect 32, wherein the lymphoma is a diffuse large B cell lymphoma (DLBCL).

[0173] 34. The method of aspect 33, wherein the DLBCL is an activated B-cell-like (ABC) DLBCL.

[0174] 35. The method of any one of aspects 31-34, wherein (i) the MYD88 protein in the complex has an L265P mutation and (ii) the CD79A or CD79B subunit of the BCR protein in the complex has an activating mutation.

[0175] 36. The method of any one of aspects 31-35, wherein the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample.

[0176] 37. The method of any one of aspects 31-36, wherein analyzing the sample comprises performing a proximity ligation assay on the sample.

[0177] 38. The method of any one of aspects 31-37, wherein the inhibitor is an inhibitor of CD45 and is 2-(4-acetylanilino)-3-chloronaphthoquinone.

[0178] 39. The method of any one of aspects 31-38, wherein the tumor sample is from a human.

[0179] 40. A method of treating a tumor having a greater probability of responding to treatment using an inhibitor of the BCR or CD45, the method comprising:

(1) identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR or CD45 according to the method of any one of aspects 31-39; and

(2) administering to the tumor an effective amount of an inhibitor of the BCR or CD45.

[00100] 41. The method of aspect 40, wherein the inhibitor is an inhibitor of CD45 and is 2-(4-acetylanilino)-3-chloronaphthoquinone.

[0180] 42. The method of aspect 40 or 41, wherein the tumor is in a human.

[0181] 43. A method of treating a human subject having a tumor having a greater probability of responding to treatment using an inhibitor of the BCR or CD45, the method comprising:

(1) identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the BCR or CD45 according to the method of any one of aspects 31-39; and

(2) administering to the human subject an effective amount of an inhibitor of the BCR or CD45.

[0182] 44. The method of aspect 43, wherein the inhibitor is an inhibitor of CD45 and is 2-(4-acetylanilino)-3-chloronaphthoquinone.

[0183] 45. A method of identifying a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway, the method comprising:

(a) obtaining a tumor sample;

(b) analyzing the sample for the presence of a complex of BCR, MYD88, and Toll-like receptor 9 (TLR9) proteins;

(c) detecting in the sample the presence of a complex of BCR, MYD88, and TLR9 proteins; and

(d) identifying the sample having the presence of a complex of BCR, MYD88, and TLR9 proteins as a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway compared to a sample having an absence of a complex of BCR, MYD88, and TLR9 proteins.

[0184] 46. The method of aspect 45, wherein the tumor is a lymphoma.

[0185] 47. The method of aspect 46, wherein the lymphoma is a diffuse large B cell lymphoma (DLBCL).

[0186] 48. The method of aspect 47, wherein the DLBCL is an activated B-cell-like (ABC) DLBCL.

[0187] 49. The method of any one of aspects 45-48, wherein (i) the MYD88 protein in the complex has an L265P mutation and (ii) the CD79A or CD79B subunit of the BCR protein in the complex has an activating mutation.

[0188] 50. The method of any one of aspects 45-49, wherein the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample.

[0189] 51. The method of any one of aspects 45-50, wherein analyzing the sample comprises performing a proximity ligation assay on the sample.

[0190] 52. The method of any one of aspects 45-51, wherein the inhibitor of the NF- κ B pathway is ibrutinib, acalabrutinib, or ONO/GS-4059.

[0191] 53. The method of any one of aspects 45-52, wherein the inhibitor of the NF- κ B pathway is ibrutinib.

[0192] 54. The method of any one of aspects 45-53, wherein the tumor sample is from a human.

[0193] 55. A method of treating a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway, the method comprising:

(1) identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway according to the method of any one of aspects 45-54; and

(2) administering to the tumor an effective amount of an inhibitor of the NF- κ B pathway.

[0194] 56. The method of aspect 55, wherein the inhibitor of the NF- κ B pathway is ibrutinib, acalabrutinib, or ONO/GS-4059.

[0195] 57. The method of aspect 55 or 56, wherein the inhibitor of the NF- κ B pathway is ibrutinib.

[0196] 58. The method of any one of aspects 55-57, wherein the tumor is in a human.

[0197] 59. A method of treating a human subject having a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway, the method comprising:

(1) identifying the tumor as a tumor having a greater probability of responding to treatment using an inhibitor of the NF- κ B pathway according to the method of any one of aspects 45-54; and

(2) administering to the human subject an effective amount of an inhibitor of the NF- κ B pathway.

[0198] 60. The method of aspect 59, wherein the inhibitor of the NF- κ B pathway is ibrutinib, acalabrutinib, or ONO/GS-4059.

[0199] 61. The method of aspect 59 or 60, wherein the inhibitor of the NF- κ B pathway is ibrutinib.

[0200] 62. A method of classifying a lymphoma tumor, the method comprising:

(a) obtaining a lymphoma tumor sample;

(b) analyzing the sample for the presence of a complex of B cell receptor (BCR), MYD88, and Toll-like receptor 9 (TLR9) proteins;

(c) detecting in the sample the presence of a complex of BCR, MYD88, and TLR9 proteins; and

(d) classifying the sample as activated B-cell–like diffuse large B cell lymphoma (ABC DLBCL).

[0201] 63. A method of classifying a lymphoma tumor, the method comprising:

(a) obtaining a lymphoma tumor sample;

(b) analyzing the sample for survival dependence on LYN, SYK, or CD19;

(c) detecting in the sample the presence of survival dependence on LYN, SYK, or CD19; and

(d) classifying the sample as germinal center B-cell–like diffuse large B cell lymphoma (GCB DLBCL) or Burkitt lymphoma.

[0202] 64. The method of aspect 62 or 63, wherein the tumor sample is a formalin-fixed and paraffin-embedded (FFPE) sample.

[0203] 65. The method of any one of aspects 62-64, wherein analyzing the sample comprises performing a proximity ligation assay on the sample.

[0204] 66. The method of any one of aspects 62-65, wherein the tumor sample is from a human.

[0205] 67. A method of treating or preventing an activated B-cell–like diffuse large B cell lymphoma (ABC DLBCL) in a human subject, the method comprising administering to the subject an effective amount of an inhibitor of CD45.

[0206] 68. The method of aspect 67, wherein the inhibitor of CD 45 is 2-(4-acetylanilino)-3-chloronaphthoquinone.

[0207] 69. A method of treating or preventing a germinal center B-cell–like diffuse large B cell lymphoma (GCB DLBCL) or Burkitt lymphoma in a human subject, the method comprising administering to the subject an effective amount of an inhibitor of LYN, SYK, or CD19.

[0208] 70. The method of aspect 69, wherein the inhibitor is dasatinib or ponatinib.

[0209] 71. The method of aspect 69, wherein the inhibitor is entospletinib, PRT-062607, or PRT-060318.

[0210] 72. A method of detecting an association of a first protein and a second protein, the method comprising:

(A) obtaining a sample comprising one or more B cells;

(B) contacting the sample with a first antibody and a second antibody, wherein the first antibody binds the first protein and does not bind the second protein and the second antibody binds the second protein and does not bind the first protein, wherein the first antibody is of a species different than the first protein and the second antibody is of a species different than the second protein, and wherein the first antibody is of a species different than the second antibody;

(C) performing a proximity ligation assay using the first antibody and the second antibody and a detectable probe to produce a PLA signal of one or more detectable puncta;

(D) contacting the sample with a third antibody, wherein the third antibody binds to a protein on the B cell surface, and wherein the third antibody is labeled with a detectable label; and

(E) detecting the proximity ligation assay signal of (C) wherein a positive PLA signal indicates association of the first and second proteins in one or more of the B cells comprised in the sample.

[0211] 73. The method of aspect 72, wherein a proximity ligation assay signal of fluorescent puncta indicates association of the first and second proteins.

[0212] 74. The method of aspect 72 or 73, wherein the third antibody is fluorescently labeled.

[0213] 75. The method of any one of aspects 72-74, wherein the first antibody and the second antibody each bind to a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex.

[0214] 76. A method of identifying a B cell disorder having a greater probability of responding to treatment using an inhibitor, the method comprising:

(a) obtaining a B cell disorder sample;

(b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex,

wherein the two proteins are different from one another and are components of different complexes,

and wherein the analysis comprises detecting an association of the two proteins according to any one of aspects 72-75; and

(c) identifying the sample having the presence of the protein complex of (b) as a B cell disorder having a greater probability of responding to treatment using an inhibitor of the BCR complex, an inhibitor of the MYD88 complex, or an inhibitor of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b).

[0215] 77. A method of identifying a B cell disorder having a greater probability of responding to treatment using an inhibitor, the method comprising:

- (a) obtaining a B cell disorder sample;
- (b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex,

wherein the two proteins are different from one another and are components of different complexes;

- (b') detecting in the sample the presence of the protein complex of (b); and
- (c) identifying the sample having the presence of the protein complex of (b) as a B cell disorder having a greater probability of responding to treatment using an inhibitor of the BCR complex, an inhibitor of the MYD88 complex, or an inhibitor of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b).

[0216] 78. The method of aspect 76 or 77, wherein the sample is identified as a B cell disorder having a greater probability of responding to treatment

- (i) using an inhibitor of the BCR complex, when the protein complex of (b) includes a protein component of the BCR complex,
 - (ii) using an inhibitor of the MYD88 complex, when the protein complex of (b) includes a protein component of the MYD88 complex, or
 - (iii) using an inhibitor of the mTORC1 complex, when the protein complex of (b) includes a protein component of the mTORC1 complex,
- as compared to a sample having an absence of the protein complex of (b).

[0217] 79. The method of any one of aspects 76-78, wherein the sample is identified as a B cell disorder having a greater probability of responding to treatment

- (i) using an inhibitor of the BCR complex, when the protein complex of (b) includes a protein component of the BCR complex,
- (ii) using an inhibitor of the MYD88 complex, when the protein complex of (b) includes a protein component of the MYD88 complex, and

(iii) using an inhibitor of the mTORC1 complex, when the protein complex of (b) includes a protein component of the mTORC1 complex,

as compared to a sample having an absence of the protein complex of (b).

[0218] 80. The method of any one of aspects 76-79, wherein the B cell disorder is a lymphoma.

[0219] 81. The method of aspect 80, wherein the lymphoma is a diffuse large B cell lymphoma (DLBCL), a primary central nervous system lymphoma (PCNSL), or a Waldenström macroglobulinemia/ lymphoplasmacytic lymphoma (WM/LPL).

[0220] 82. The method of aspect 81, wherein the lymphoma is a DLBCL and is an activated B-cell-like (ABC) DLBCL.

[0221] 83. The method of any one of aspects 76-82, wherein the protein complex comprises the proteins of BCR, MYD88, and mTOR.

[0222] 84. The method of any one of aspects 76-83, wherein the B cell disorder sample is a formalin-fixed and paraffin-embedded (FFPE) sample.

[0223] 85. The method of any one of aspects 76-84, wherein the inhibitor is OSI-027, AZD-8055, AZD,2014, SIROLIMUS, BEZ-235, everolimus, or ibrutinib.

[0224] 86. The method of any one of aspects 76-85, wherein the inhibitor is ibrutinib.

[0225] 87. An inhibitor of the BCR complex, MYD88 complex, or mTORC1 complex for use in treating, in a subject in need thereof, a B cell disorder having a greater probability of responding to treatment using an inhibitor, wherein the B cell disorder is identified as a B cell disorder having a greater probability of responding to treatment using an inhibitor according to the method of any one of aspects 76-86.

[0226] It shall be noted that the preceding are merely examples of embodiments. Other exemplary embodiments are apparent from the entirety of the description herein. It will also be understood by one of ordinary skill in the art that each of these embodiments may be used in various combinations with the other embodiments provided herein.

[0227] The following example further illustrates the invention, including embodiments of the invention, but, of course, should not be construed as in any way limiting its scope.

EXAMPLE

[0228] This example demonstrates embodiments of the invention.

Cell culture

[0229] Cell lines were grown at 37°C in the presence of 5% CO₂ and maintained in RPMI supplemented with fetal bovine serum (Tet tested, Atlanta Biologics, Atlanta, GA, USA) and 1% pen/strep and 1% L-glutamine (Invitrogen, Carlsbad, CA, USA), except for OCI-Ly10 and OCI-Ly3 which were grown in IMDM supplemented with 20% heparinized human plasma, 1% pen/strep and 55uM β -mercaptoethanol. All cell lines were regularly tested for mycoplasma using the MycoAlert Mycoplasma Detection Kit (Lonza, Basel, Switzerland) and DNA fingerprinted by examining 16 regions of copy number variants.

Cas9 vector construction

[0230] pRetroCMV/TO-Cas9-Hygro was created by ligating the tetracycline-inducible CMV promoter from pcDNA4/TO (Invitrogen) with MfeI/XbaI and blunt cloned into the XhoI/EcoRI digested pRetrosuper vector (Brummelkamp et al., Cancer Cell, 2: 243-247 (2002), incorporated by reference herein). The puromycin resistance gene from pRetroCMV/TO was removed with StuI/ClaI and replaced with PGK-hygromycin which was isolated from pMSCV Hygro (Clontech, Mountain View, CA, USA) with AgeI/HindIII and similarly cloned into pRetroCMV/TO. Cas9 was isolated from the LentiCrispr v2 (Addgene #52961) and blunt cloned into pRetroCMV/TO-hygro digested with AgeI/BamHI. pCW-Cas9-Blasticidin was generated from pCW-Cas9-puro which was purchased from Addgene, Cambridge, MA, USA (#50661) and digested with BamHI and XbaI to remove the puromycin resistance gene. A g-block (IDT) containing the blasticidin resistance gene was Gibson cloned into the cut vector with 12 basepair overlaps.

Cas9-clone generation

[0231] Cell lines were transduced multiple times with either pTO-Cas9-hygro or pCW-Cas9, selected and dilution cloned. Single cell clones were picked and tested for functional Cas9 cutting after transduction with sgRNAs targeting surface markers including CD20 or ICAM1. Clones were selected based upon loss of surface expression within the transduced population as measured by FACS 8-14 days after addition of doxycycline.

sgRNA vector and cloning

[0232] The pLKO-based sgRNA vector was purchased from Addgene (#52628). The puromycin gene was removed and replaced with a puro-GFP fusion protein previously

described (Ngo et al., Nature, 441: 106-110 (2006), incorporated by reference herein) using Gibson assembly. The resulting plasmid was digested with BfuAI and incubated with shrimp alkaline phosphatase before isolating the backbone. Complementary sgRNA sequences flanked by ACCG (SEQ ID NO: 1) on the 5' end, and CTTT (SEQ ID NO: 2) on the 3' of the reverse strand, were annealed, diluted, and ligated into the cut vector with T4 ligase according to the manufacturer's instructions. All transformations were performed in *Stbl3* bacteria and grown at 30° C.

sgRNA Library Construction

[0233] The genome-wide Brunello sgRNA library (Doench et al., Nat. Biotechnol., 34: 184-191 (2016), incorporated by reference herein) was purchased from Addgene and transformed in *Stbl4* bacteria from Invitrogen. Sequences for the follow up library of 12,472 sgRNAs were chosen from published sgRNA libraries (Wang et al., Science, 350: 1096-1101 (2015), incorporated by reference herein; and Shalem et al., Science, 343: 84-87 (2014), incorporated by reference herein) or were designed using the online tool at <http://crispr.mit.edu>. The library (CustomArray Inc., Bothell, WA, USA) of 74-mer of the sgRNA sequence prepended with the oligo sequence GGAAAGGACGAAACACCG (SEQ ID NO: 3) and followed by GTTTTAGAGCTAGAAATAGCAAGTTAAAATAAGGC (SEQ ID NO: 4). The oligo library was PCR amplified with Herculanase II Fusion DNA polymerase (Agilent, Santa Clara, CA, USA) using ArrayF and ArrayR (Shalem et al., Science, 343: 84-87 (2014), incorporated by reference herein). The subsequent PCR product was gel extracted using an eGel from Invitrogen and 20ng of library was Gibson cloned into BfuAI-cut sgRNA vector following the manufacturer's instructions. Transformations were grown at 30°C overnight on 24.5cm² bioassay plates maintaining at least 100X coverage. Colonies were scrapped, spun and DNA was isolated with Blood and Cell Culture DNA Maxi kits (Qiagen, Hilden, Germany).

Virus production and transduction

[0234] Lentiviruses were produced in 293FT cells by cotransfecting sgRNA vectors with packaging vectors pPAX2 (Addgene #12260) and pMD2.g (Addgene #12259) in a 4:3:1 ratio in serum-free Opti-MEM. Trans-IT 293T (Mirus, Madison, WI, USA) was added and incubated for 15 minutes before adding dropwise to cells. Supernatants were harvested 24, 48, and 72 hours later, spun at 1000g to pellet any virus producing cells and then incubated

with Lenti-X concentrator (ClonTech). Virus was concentrated according to manufacturer's instructions, aliquoted and frozen. Virus titration was performed on target cell populations and GFP was measured 3-4 days later. When GFP was not present in the backbone of the sgRNA plasmids, transduced cells were split and incubated with or without puromycin until untransduced control cells were dead. The percentage of viable cells was then measured by FACS and percent transduction was calculated as the ratio of viable cells in treated versus untreated wells.

Pooled sgRNA screening

[0235] For both genome-wide and targeted follow-up screens, individual replicates were transduced such that an average of 500 copies of each sgRNA were present after selection. Cultures were carried for the duration of the 21-day screen maintaining 500X coverage. Antibiotic selection was started 3-4 days after transduction and carried out until untransduced control cells were dead, approximately 4-5 days later. Cells were then harvested for a day 0 time point and doxycycline was added to the culture media at 200ng/mL final concentration. Transduced cells were counted and passaged every two days with fresh media containing dox until day 21 when cells were again harvested for DNA extraction. DNA was isolated from frozen cell pellets using Qiagen QIAmp DNA Blood Midi and Maxi kits.

Library amplification, sequence extraction and PCR primers

[0236] For both screens, sgRNA sequences were amplified using a nested PCR to first isolate the sgRNA sequence from genomic DNA and then to add nextgen sequencing adapters compatible with NextSeq500 from Illumina (San Diego, CA, USA). Products were amplified using ExTaq (Takara, Shiga, Japan) for 18 cycles in both rounds of amplification. Products were size selected using an eGel (Invitrogen) and libraries were quantitated using an Illumina specific Kapa quantification kit according to the manufacturer's instructions or by Qubit (Thermo Fisher Scientific, Waltham, MA, USA). All libraries were sequenced using a high output single-read 75 cycle read flow cell. An average of 400X (200-700X) sequencing depth was achieved. Libraries were multiplexed using indexes compatible with the Illumina TrueSeq HT kit with the primers below where x denotes an eight basepair index and y represents a variable length adapter inserted to prevent monotemplate. Eight forward primers and 12 reverse primers were used following this format such that 96 samples could be multiplexed. Basespace.com was used to evaluate sequencing quality measures and to

demultiplex sequencing reads. Sequences were aligned to the sgRNAs library allowing for a one basepair mismatch using custom scripts and Bowtie 2 version 2.2.9.

CRISPR Screen primers

First PCR forward primer:

AATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCG (SEQ ID NO: 5)

First PCR reverse primer:

GTAATTCTTTAGTTTGTATGTCTGTTGCTATTATG (SEQ ID NO: 6)

Second PCR forward primer:

AATGATACGGCGACCAACCGAGATCTACACxACAACCTTTCCCTACACGACGCTCT
TCCGATCTyTCTTGTGGAAAGGACGAAACACCG (SEQ ID NO: 7)

Second PCR reverse primer:

CAAGCAGAAGACGGCATAACGAGATxGTGACTGGAGTTCAGACGTGTGCTCTTCCG
ATCTCTACTATTCTTTCCCCTGCACTGT (SEQ ID NO: 8)

PCR amplification for sgRNA library construction

ARRAY-F:

TAACTTGAAAGTATTTTCGATTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAA
CACCG (SEQ ID NO: 9)

ARRAY-R:

ACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTA
AAAC (SEQ ID NO: 10)

Pooled sgRNA screen analysis

[0237] Crispr screen scores (CSS) were calculated using the following formulas:

Notation:

$i = \{1 - 77,441\}$ Index indicating sgRNA

$j = \{1 - 11\}$ Index indicating cell line

R_j = Number of replicates for cell line j

$r = \{1 - R_j\}$ Index indicating replicate

$d = \{0, 21\}$ index indicating time point

X_{ijd} = Indicates the raw sequencing counts for sgRNA i , in replicate r of cell line j at time d

[0238] Step 1: Normalize raw counts by total read counts

$$N_{irjd} = 1 + (X_{irjd} * 4 \times 10^7) / \sum_i X_{irjd}$$

Step 2: Eliminate sgRNAs with low counts across all experimental conditions by calculating

$$m_i = \text{Max}_{jd} \left(\min_r N_{irjd} \right)$$

and eliminating those sgRNAs for which $m_i < 100$.

Step 3: Calculate log ratios

$$LR_{irj} = \log_2(N_{irj21}/N_{irj0})$$

Step 4: Z transform log-ratios

$$Z_{irj} = (LR_{irj} - \text{Mean}_i(LR_{irj})) / \text{Standard deviation}_i(LR_{irj})$$

Step 5a: Average across replicates

$$S_{ij} = \text{Mean}_r(Z_{ijr})$$

Step 5b: Calculate signal variance for each sgRNA

$$\text{Total Variance: } TV_i = \text{Var}_j(S_{ij})$$

$$\text{Error Variance: } EV_i = \text{Mean}_j \left(\text{Var}_r(Z_{irj}) / R_i \right)$$

$$\text{Signal Variance: } SV_i = TV_i - EV_i$$

Step 6: For each gene g , let G_g be the set of sgRNAs that represent it, and calculate the maximal pair wise correlation between any two sgRNAs in this set.

$$C_g = \text{Max}_{k,l \in G_g} \left(\text{Correlation}_j(S_{kj}, S_{lj}) \right)$$

If for a given gene $C_g < 0.45$, then let i_g be the index of the sgRNA representing that gene that has the highest signal variance and use that as the sole representative of gene j

$$CSS_{gj} = S_{i_g j}$$

If $C_g \geq 0.45$, then proceed to steps 7 and 8.

Step 7: Average the two sgRNAs which were most correlated within gene g .

$$M_{gj} = \frac{1}{2} (S_{kj} + S_{lj})$$

where $k, l \in G_g$ are such that

$$\text{Correlation}_j(S_{kj}, S_{lj}) = C_g$$

Step 8: For each $i \in G_g$ calculate

$$V_i = \text{Correlation}_j(S_{ij}, M_{gj})/C_g$$

Average together those sgRNAs for which $V_i > 0.85$ to arrive at final CSS for gene g

$$CSS_{gj} = \text{mean}_{i \in G_g, V_i > 0.85} (S_{ij})$$

[0239] For the replication sgRNA library, in which most genes had 9-10 sgRNAs per gene, it was found this could be simplified by using the z-scores of the averaged $\log_2$ fold change of all sgRNAs per gene. As described above, using the best correlated sgRNAs per gene excluded many poor performing sgRNAs, it also excluded many high performing sgRNAs that shared expected subtype-specific toxicities.

Statistical significance

[0240] The statistical significance in Figure 1 of the CSS of ABC BCR-dependent, or GCB DLBCL, compared to all other cell lines, was calculated with a Random Variance T-test (Wright et al., Bioinformatics, 19: 2448-2455 (2003), incorporated by reference herein) for individual genes. Genes in Figures 1D and 1E were filtered as follows: p-value of < 0.01 , a subtype-specific CSS of < -1.5 , a CSS of > -0.6 in control lymphoid lines, were not present in the ABC-specific Chr19 amplicon, and were not previously reported as essential (Hart et al., Cell 163: 1515-1526 (2015), incorporated by reference herein).

FACS analysis

[0241] Cell lines were transduced with sgRNA vectors marked by GFP. Three to four days after transduction, GFP levels were measured by flow cytometry on a BD FACS Calibur. Cells were split every other day into doxycycline containing media and GFP levels were followed for 14 days and normalized to the day 0 measurement. All sgRNA and shRNA sequences used in the Example are listed below in Table 1.

Table 1: shRNA and sgRNA sequences used in functional assays

Construct	Sequence
shSC4 (MSMO1 ex5)	CTCTCAACCCTTTAAATCTGA (SEQ ID NO: 17)
shCD79A (5' UTR)	GGGGCTTCCTTAGTCATATTC (SEQ ID NO: 18)
shTLR9 #2 (5' UTR)	GCACGGTGCCACCTCCACACT (SEQ ID NO: 19)
shMYD88 #2 (ex3)	GGCATATGCCTGAGCGTTTC (SEQ ID NO: 20)
shBCL10 #2 (5' UTR)	CTGACATTGTCTCCTATATA (SEQ ID NO: 21)
shCARD11 (5' UTR)	GGGGTGTGTACCAGGCTATGA (SEQ ID NO: 22)
sgIgM #11	GCTTCCCATCAGTCCTGAGA (SEQ ID NO: 23)
sgIgG #1	GGCAAGGAGTACAAGTGCA (SEQ ID NO: 24)
sgCD79A #8	GAACAGGAGGATGATCCCCT (SEQ ID NO: 25)
sgSYK #5	GTAATCTTCTGCCTCCTCCC (SEQ ID NO: 26)
sgBTK #2	GATGCTCTCCAGAATCACTG (SEQ ID NO: 27)
sgPLCg2 #2	GCTGTTGACGAAGCCCACGG (SEQ ID NO: 28)
sgBCAP #3	TGTAAGGATGGAAGCCAAGG (SEQ ID NO: 29)
sgCARD11 #4	GAAGCTGGTGAAGGACATGG (SEQ ID NO: 30)
sgTLR9 #8	GACCAGGCTCCCGAAGGAAG (SEQ ID NO: 31)
sgMYD88 #10	CCGGCAACTGGAGACACAAG (SEQ ID NO: 32)
sgUNC93B1 #B73	TGTTGCCATACTTCACCTCG (SEQ ID NO: 33)
sgCNPY3 #9	TCAGCACGTGGTTGGCGCAG (SEQ ID NO: 34)
sgCD19 #4	GCCGAGCAGTGATCTCCAGG (SEQ ID NO: 35)
sgLYN #2	AATGAGTACACAGCAAGGGA (SEQ ID NO: 36)
sgAAVS1	GGGGCCACTAGGACAGGAT (SEQ ID NO: 37)
sgRPL6	GGTACTCTCACCTTGCCCT (SEQ ID NO: 38)

[0242] When surface proteins were targeted, knockout was validated by flow cytometry by spinning cells down, washing in FACS buffer (PBS plus 2% (vol/vol) FBS, 1mM EDTA), and stained at 4°C for 30 minutes in FACS buffer with fluorescently labeled antibodies from Biolegend, San Diego, CA, USA: mouse anti-human CD19-APC (Biolegend SJ25C1, 1:500), mouse anti-human CD81-PE (Biolegend 5A6, 1:500), mouse anti-human IgM-APC (MHM-88, 1:400); or from Southern Biotech, Birmingham, AL, USA: goat anti-human IgG-PE (1:200).

MTS Growth Assays

[0243] DLBCL cell lines were enumerated and 10,000 cells were seeded in triplicate in a 96-well plate in fresh media. Ibrutinib (Selleck Chem, Houston, TX, USA) and AZD2014

(AstraZeneca, Cambridge, UK) were dissolved in DMSO and equal volumes of diluted drug were added to cells to reach the indicated final concentration. Cells were cultured with drugs, which were replenished after 48 hours. Metabolic activity was measured at day 4 by adding 10 μ l of MTS reagent (Promega, Madison, WI, USA) and incubating at 37°C for 4 hours. Absorbance was measured at 490 nm using a 96-well Tecan Infinite 200 Pro plate reader. Absorbance from media-only wells were subtracted and data were normalized to DMSO control unless otherwise stated. Absorbance from media-only wells were subtracted and data were normalized to DMSO control unless otherwise stated. GR50 was calculated using the online tool GRcalculator (<http://www.grcalculator.org/>) (Hafner et al., Nat. Methods, 13: 521-527 (2016), incorporated by reference herein). Drug matrix screens and Δ bliss calculations were performed as previously described (Mathews Griner, et al., Proc. Natl. Acad. Sci. U. S. A., 111: 2349-2354 (2014), incorporated by reference herein).

Gene Expression Profiling and Signature Enrichment

[0244] Cells were transduced with shRNAs, puro-selected and harvested at indicated times after shRNA induction. RNA was isolated using RNEasy mini kits (Qiagen). Gene expression was assessed using two-color human Agilent 4 $\times$ 44K gene expression arrays following the manufacturers protocol. Briefly, control shSC4 (control, Cy3-labelled) RNA was compared to RNA from cells with shRNAs targeting TLR9 (C4), TLR9 (D7), MyD88 (A7), MyD88 (B3) (Cy5-labelled) at each of the indicated time points. Array elements were filtered for spot quality using Agilent Feature Extraction software, specific genes were determined to be downregulated if the log₂ fold change (comparing control shSC4 to shRNA for TLR9) was less than -0.3 for at least 3 of the 4 time points (shTLR9) per cell line. Gene expression data have been deposited in Gene Expression Omnibus (GEO) under accession GSE99276. Signature enrichment was performed as previously described (Ceribelli et al., Proc. Natl. Acad. Sci. USA, 111: 11365-11370 (2014), incorporated by reference herein). Briefly, downregulated genes were tested for overlap with published gene signatures in a 2x2 contingency table using a Fisher's exact test.

DNA Copy Number analysis

[0245] DLBCL DNA samples were analyzed with the Affymetrix SNP6.0 array (Affymetrix, Santa Clara, CA, USA). Probe log ratios were calculated using Affymetrix Genotyping Console, and were collected into segments of similar value using circular binary

segmentation (<https://www.bioconductor.org/packages/devel/bioc/manuals/.../man/DNACopy.pdf>). These segments were assigned copy number values as previously described (G. Lenz et al., Proc. Natl. Acad. Sci. USA, 105: 13520-13525 (2008), incorporated by reference herein). DNA copy number was correlated to sample gene expression using linear regressions calculated with Prism 7. Overlapping DNA segments of amplified regions were identified and bedgraph files generated using bedtools2.19.1 before being visualized on the UCSC genome browser, Hg19.

NF- κ B reporters

[0246] The generation of the I κ B α luciferase reporter cell line has been previously described (Lam et al., Clin. Cancer Res, 11: 28-40 (2005), incorporated by reference herein). Briefly, TMD8-I κ B α cell line was transduced with indicated shRNAs, puro-selected and induced with doxycycline. Cells were harvested at the indicated time points and luciferase was measured with the Dual Luciferase Reporter Assay System (Promega) on a Tecan Infinite 200 Pro plate reader.

IgM co-immunoprecipitation

[0247] HBL1, TMD8, OCI-Ly10 and OCI-Ly19 cells were lysed at 10^7 cells per ml in a modified RIPA buffer (0.5% Triton X-100, 0.25% deoxycholate, 0.025% SDS, 10 mM Tris, pH 8.0, 100 mM NaCl, 10 mM EDTA, 1 mM Na₃VO₄, 30 mM pyrophosphate, 10 mM glycerophosphate, 1 mM AEBSF, 0.02 U ml⁻¹ aprotinin and 0.01% NaN₃) for 10 min. on ice. Lysates were cleared by centrifugation at 14,000xg for 20 min. at 4°C. IgM was immunoprecipitated by incubating lysates on ice for 1 hour with 10 μ g of biotin-labeled goat anti-human IgM (Jackson ImmunoResearch, West Grove, PA, USA), followed by the addition of 35 μ l of pre-washed streptavidin-agarose beads (Invitrogen) and rotated for 30 min. at 4°C. Beads were washed 3X with cold 1X RIPA buffer, then solubilized by adding 2X LDS sample buffer (Invitrogen) with 1% β -mercaptoethanol and boiled for 5 min. Samples were separated on a 10% polyacrylamide gel and transferred to Immobilon-p PVDF membrane (Millipore, Billerica, MA, USA) for western blot analysis. Membranes were probed with rabbit anti-TLR9 monoclonal XP, rabbit anti-TLR7 (Cell Signaling Technologies, Danvers, MA, USA), rabbit anti-TLR4 (Santa Cruz Biotechnology, Dallas, TX, USA) and goat anti-IgM-HRP (Bethyl, Montgomery, TX, USA).

Proximity Ligation Assay

[0248] DLBCL cell lines were either left untreated, treated with 10nM ibrutinib or equivalent volumes of DMSO, or transduced with either control shRNA (SC4) or shRNAs targeting CD79A, TLR9, MYD88, CARD11, BCL10 or MALT1, followed by puromycin (Invitrogen) selection as previously described (Ngo et al., Nature, 470: 115-119 (2011), incorporated by reference herein). Cells were plated onto a 15 well μ -Slide Angiogenesis ibiTreat chamber slide (Ibidi, Martinsried, Germany) and allowed to adhere to the surface for 30 min at 37°C. Cells were then fixed with 4% paraformaldehyde (Electron Microscopy Sciences, Hatfield, PA, USA) for 20 min at room temperature and then washed in PBS (Invitrogen). Cellular membranes were labeled with 5 μ g/ml wheat germ agglutinin conjugated to Alexa Fluor 488 (Thermo Fisher Scientific) for 10 min at room temperature. Cells were permeabilized in cold methanol for 10 min, washed in PBS and then blocked in Duolink Blocking buffer (Sigma, St. Louis, MO, USA) for 30 min at room temperature. Primary antibodies were diluted in Duolink Antibody Diluent (Sigma) and incubated overnight at 4°C (See Table 2).

Table 2

Pair	Rabbit antibody	Goat antibody	Mouse antibody
IgM:TLR9	anti-TLR9 CST #2254 1:200	anti-IgM Jackson 109-005-129 1:1000	
TLR9:MYD88	anti-TLR9 CST #2254 1:200		anti-MYD88 (B-1) sc-136970 1:200
IgM:pIkBa	anti--phospho-IkBa (ser32) (14D4) CST #2859 1:200	anti-IgM Jackson 109-005-129 1:1000	
TLR9:pIkBa	anti-TLR9 CST #2254 1:200		anti--phospho-IkBa (ser32) (5A5) CST #9246 1:200
BCL10:MYD88	anti-BCL10 (H-197) sc-5611 1:100	anti-MYD88 (N-19) sc-8196 1:100	
MALT1:MYD88	anti-MALT1 (H-300) sc-28246 1:100		anti-MYD88 (B-1) sc-136970 1:200
CARD11:BCL10	anti-BCL10 (H-197) sc-5611 1:100	anti-CARD11 (N-20) sc-20458 1:100	

[0249] Where appropriate, cells were counterstained with mouse anti-Lamp1 conjugated to Alexa Fluor 488 (Santa Cruz Biotechnology) with the primary antibodies. The following morning, cells were washed for 20 min in a large volume of PBS with 1% BSA, followed by addition of the appropriate Duolink secondary antibodies (Sigma), diluted and mixed according to the manufacturer's instructions. Cells were incubated for 1 hour at 37°C, after which cells were washed in TBST with 0.5% tween-20 for 10 min. Ligation and amplification steps of the PLA were performed using the Duolink *in situ* Detection Reagents Orange kit (Sigma) according to the manufacturer's instructions. Following the PLA, cells were mounted in Prolong Gold mounting media with DAPI (Invitrogen). Images were

acquired on a Zeiss LSM 880 Confocal microscope. Images for display and Pearson's correlation coefficients values were calculated with NIH ImageJ/FIJI software (Schindelin et al., Nat. Methods, 9: 676-682 (2012), incorporated by reference herein). PLA spots were counted in cell lines using Blobfinder (Allalou et al., Comput. Methods Programs Biomed., 94: 58-65 (2009), incorporated by reference herein).

[0250] The PLA was performed on formalin-fixed, paraffin-embedded (FFPE) tissue microarrays or biopsy samples in a similar manner. FFPE microarrays (7 μ m) and patient tissue sections (4 μ m) were deparaffinized in xylene and rehydrated in graded alcohol and distilled water. Heat induced antigen retrieval was performed on TMAs and tissue sections at pH 6.0 for 30 minutes. Slides were then placed in tris-buffered solution and prepared for proximity ligation assay, as described above, samples were costained with mouse anti-human CD20-eFluor660 (L26, eBioscience/Thermo Fisher Scientific). Tissue microarrays were prepared by fixing cells in neutral buffer formalin for 24 hours, pelleting, and resuspending in an equal volume of low-melt agarose in a 10 ml conical tube. The resulting pellet was paraffin embedded by standard protocol (Hewitt, Methods in Enzymology, 410: 400-415 (2006), incorporated by reference herein). The resultant blocks were used to construct a cell line array (CMA) using the same approach used for construction of a tissue microarray, with 1.00 mm needles, using a Beecher MTA-1 instrument (Beecher Instruments, Silver Spring, MD, USA). Sample identifiers were removed and blinded before pathology review for PLA signal. Samples were called positive if the presence of ≥ 2 fluorescent puncta/cell in $>25\%$ of the malignant cells was observed. After all data were collected, sample identifiers were revealed and samples were grouped by response to ibrutinib.

BioID2 constructs

[0251] BioID2 (Addgene #80899) was appended to the c-terminus of TLR9 and MYD88^{L265P} using Gibson cloning techniques. MYD88^{L265P}-13X-BioID2 was cloned by removing GFP from the previously described pBMN-MYD88^{L265P}-VD-GFP⁹ by restriction digest with StuI and NotI. BioID2 was PCR amplified with a 13X N-terminal linker and Gibson cloned as above from Addgene #80899 with the following primers:

MYD88-Cterm/13X

CTGGACTCGCCTTGCCAAGGCCTTGTCCTGCCCGGTGGAGGCGGGTCTGGAGGC
(SEQ ID NO: 15)

pBMN-NotI-BioID2-Cterm

CCTCTAGTGCGGCCGCTTATGCGTAATCCGGTACATC (SEQ ID NO: 16)

BioID2 was also appended to the c-terminus of both wild type and mutant isoforms of MYD88 with a two-amino acid linker (VD). First, BioID2 and MYD88 were PCR amplified with Primestar (Takara) using the following primers:

BioID FWD

TTGTCCCTGCCCCGTCGACTTCAAGAACCTGATCTGGCTG (SEQ ID NO: 11)

BioID REV

CGCCGGCCCTCGAGGCTATGCGTAATCCGGTACATCG (SEQ ID NO: 12)

MYD88 FWD

AATTCGAATTCTGAAGGGCCACCATGCGACCCGACCGCGC (SEQ ID NO: 13)

MYD88 REV

AGATCAGGTTCTTGAAGTCGACGGGCAGGGACAAGGC (SEQ ID NO: 14)

[0252] TLR9 was cloned into a modified version of pBMN that expresses a 10X linker followed by BioID2 with the following oligos.

TLR9 C-BioID FWD

CTGCCGGATCCGAATTCTA GCC ACA atgggtttctgccgcagcg (SEQ ID NO: 39)

TLR9 C-BioID REV

CCCGACCCGCCTCCACCTAC ttcggccgtgggtccctggc (SEQ ID NO: 40)

[0253] The PCR products were separated on a 1% agarose gel and column purified (Qiagen). Purified PCR products were mixed and added to pBMN-LYT2 vector that was linearized with *StuI* (New England Biolabs, Ipswich, MA, USA) and subjected to a Gibson reaction (New England Biolabs) following the manufacturer's protocol.

Imaging MYD88-13X-BioID2

[0254] MYD88^{L265P}-13X-BioID2 was retrovirally transduced into TMD8 cells, and then purified with anti-LYT2 beads as described above. Cells were first cultured for 16 hr in 50µM biotin. Next, cells were incubated with 1µg/ml goat anti-human IgM FAB conjugated

to Alexa Fluor 488 (Jackson ImmunoResearch) for 90 min at 37°C. During this incubation period, cells were plated onto a 15 µ-Slide 8 well IbiTreat chamber slides (Ibidi) for 30 min and allowed to stick to the slides. Cells were washed 2X with PBS and then fixed with 4% paraformaldehyde (Electron Microscopy Sciences) for 20 min and then permeabilized with cold methanol for 10 min at -20°C. Fixed and permeabilized cells were blocked with Duolink blocking buffer (Sigma) for 30 min at room temperature. Cells were then incubated with rabbit mAb anti-phospho-IKKα/β (Ser176/180) (Cell Signaling Technology) diluted 1:200 in PBS with 1% BSA for 2 hour at room temperature, followed by 2X washes with PBS:BSA. Cells were then incubated with anti-rabbit F(ab')₂ conjugated to Alexa Fluor 555 (Cell Signaling Technology) at 1:1000, mouse anti-Lamp1 conjugated to Alexa Fluor 405 (Santa Cruz Biotechnology) at 1:50 and streptavidin conjugated to Alexa Fluor 647 (Biolegend) at 1:1000, all diluted into PBS:BSA and allowed to incubate for 1 hour at room temperature. Cells were then washed for 15 min in a large volume of PBS:BSA and mounted with Prolong Diamond mounting media (Invitrogen). Images were acquired on a Zeiss LSM 880 Confocal microscope. Images for display were prepared with NIH ImageJ/FIJI (73) and animations were prepared using the Imaris 3D rendering software (Bitplane, Belfast, Northern Ireland).

[0255] In certain instances, TMD8, OCI-Ly10 and/or HBL1 cells expressing MYD88^{L265P}-13X-BioID2 were additionally transduced with either control shRNA (SC4) or shRNAs targeting CD79A, TLR9 or MYD88, as described above. Following puromycin (Invitrogen) selection, cells stained with streptavidin conjugated to Alexa Fluor 555 (Thermo Fisher Scientific) and anti-LYT2 conjugated to Alexa Fluor 647 (Biolegend). Cells were either subjected to FACS analysis, as described above, or were imaged as described above. Biotin spots or blobs were counted using Blobfinder, as for the PLA above. Likewise, these cell lines were either left untreated, treated with 10nM ibrutinib or equivalent volumes of DMSO, then stained and analyzed in the same manner.

Mass spectrometry and western blot analysis of BioID2 constructs

[0256] TLR9-10X-BioID2 pBMN-LYT2 and MYD88-13X-BioID2 pBMN-LYT2 constructs were retrovirally transduced into DLBCL cell lines, as described above. Infected cells were enriched by positive selection with LYT2 magnetic beads (Invitrogen). Cells were then grown in SILAC media, containing amino acids labeled with stable isotopes or arginine and lysine, for 2 weeks prior to expansion to 100x10⁶ cells. 16 hours prior to lysis, biotin

(Sigma) was added to a final concentration of 50 μ M to transduced cells. Cells were then lysed at 2.5×10^7 cells per ml in RIPA buffer modified for MS analysis (1% NP-40, 0.5% deoxycholate, 50 mM Tris, pH 7.5, 150 mM NaCl, 1 mM Na₃VO₄, 5mM NaF, 1 mM AEBSF) for 10 min. on ice. Lysates were cleared by centrifugation at 14,000xg for 20 min. at 4°C. 35 μ l of Pre-washed streptavidin agarose beads were added to each sample; samples were then rotated at 4°C for 2 hours, then washed four times in 1X RIPA buffer, then solubilized with 4X LDS sample buffer (Invitrogen) with 1% β -mercaptoethanol, and boiled for 5 min. Lysates were then subjected to western blot analysis as described above. Western blots were probed with rabbit anti-CARD11 and rabbit anti-MALT1 (Cell Signaling Technologies) and mouse anti-MYD88 (Santa Cruz Biotechnology). A fraction of lysates were also subjected to western blot analysis as described above. Western blots were probed with rabbit anti-CARD11 and rabbit anti-MALT1 (Cell Signaling Technologies) and mouse anti-MYD88 (Santa Cruz Biotechnology).

[0257] For MS analysis, proteins were separated by one-dimensional gel electrophoresis (4–12% NuPAGE Bis-Tris Gel, Invitrogen) and the entire lane of a Coomassie blue-stained gel was cut into 23 slices. All slices were processed as described previously (Oellerich et al., Blood, 121: 3889-3899, S3881-3866 (2013)). After tryptic digestion of the proteins the resulting peptides were resuspended in sample loading buffer (2% acetonitrile and 0.05% trifluoroacetic acid) and were separated by an UltiMate 3000 RSLCnano HPLC system (Thermo Fisher Scientific) coupled online to a Q Exactive HF mass spectrometer (Thermo Fisher Scientific). First, peptides were desalted on a reverse phase C18 precolumn (Dionex 5 mm length, 0.3 mm inner diameter) for 3 minutes. After 3 minutes the precolumn was switched online to the analytical column (30cm length, 75 mm inner diameter) prepared in-house using ReproSil-Pur C18 AQ 1.9 mm reversed phase resin (Dr. Maisch GmbH, Ammerbuch, Germany). Buffer A consisted of 0.1 % formic acid in H₂O, and buffer B consisted of 80% acetonitrile and 0.1% formic acid in H₂O. The peptides eluted from buffer B (5 to 42 % gradient) at a flow rate of 300 nl/min over 76 min. The temperature of the precolumn and the analytical column was set to 50°C during the chromatography. The mass spectrometer was operated in a TopN data-dependent mode, where the 30 most intense precursors from survey MS1 scans were selected with an isolation window of 1.6 Th for MS2 fragmentation under a normalized collision energy of 28. Only precursor ions with a charge state between 2 and 5 were selected. MS1 scans were acquired with a mass range from 350 to 1600 m/z at a resolution of 60,000 at 200 m/z. MS2 scans were acquired with a starting mass

of 110 Th at a resolution of 15,000 at 200 m/z with maximum IT of 54ms. AGC targets for MS1 and MS2 scans were set to 1E6 and 1E5, respectively. Dynamic exclusion was set to 20 seconds.

MS data analysis

[0258] MS data analysis was performed using the software MaxQuant (version 1.6.0.1, Max Planck Institute of Biochemistry, Germany) linked to the UniProtKB/Swiss-Prot human database containing 155990 protein entries and supplemented with 245 frequently observed contaminants via the Andromeda search engine (Cox et al., J. Proteome Res., 10:1794-1805 (2011)). Precursor and fragment ion mass tolerances were set to 6 and 20 ppm after initial recalibration, respectively. Protein biotinylation, N-terminal acetylation and methionine oxidation were allowed as variable modifications. Cysteine carbamidomethylation was defined as a fixed modification. Minimal peptide length was set to 7 amino acids, with a maximum of two missed cleavages. The false discovery rate (FDR) was set to 1% on both the peptide and the protein level using a forward-and-reverse concatenated decoy database approach. For SILAC quantification, multiplicity was set to two or three for double (Lys+0/Arg+0, Lys+8/Arg+10) or triple (Lys+0/Arg+0, Lys+4/Arg+6, Lys+8/Arg+10) labeling, respectively. At least two ratio counts were required for peptide quantification. The “re-quantify” option of MaxQuant was enabled..

Xenograft

[0259] All mouse experiments were approved by the National Cancer Institute Animal Care and Use Committee (NCI-ACUC) and were performed in accordance with NCI-ACUC guidelines and under approved protocols. Female NSG (non-obese diabetic/severe combined immunodeficient/common gamma chain deficient) mice were obtained from NCI Fredrick Biological Testing Branch and used for the xenograft experiments between 6-8 weeks of age. TMD8 tumors were established by subcutaneous injection of 10×10^6 cells in a 1:1 Matrigel/PBS suspension. Treatments were initiated when tumor volume reached a mean of 200mm^3 . Ibrutinib was prepared in PBS with 50% (v/v) DMSO and administered i.p. once per day (6mg/kg/day). AZD2014 was prepared in deionized water with 1% (v/v) Tween 80 and administered p.o. twice per day on days 0, 1, 7 and 8 (10mg/kg/day). For ADZ2014/ibrutinib combination, drugs were given at the same concentration and schedule as single agents. Tumor growth was monitored every other day by measuring tumor size in two

orthogonal dimensions and tumor volume was calculated by the following equation: tumor volume = (length x width²)/2.

CRISPR screens reveal essential pathways that distinguish DLBCL subtypes

[0260] To gain new insights into the pathogenesis of DLBCL, pooled, loss-of-function CRISPR-Cas9 screens were performed for essential genes in ABC and GCB DLBCL cell lines. These screens employed libraries of lentiviral vectors to express short guide RNAs (sgRNAs) that direct Cas9 to distinct genes, leading to their inactivation by repeated cycles of cleavage and error-prone repair. A library of 77,441 lentiviral vectors that express sgRNAs targeting unique loci in most (19,114) protein-coding genes in the human genome was used, with approximately four sgRNAs per gene (Doench et al., Nat. Biotechnol., 34: 184-191 (2016), incorporated by reference herein). Cell lines were engineered to express Cas9 in a doxycycline-inducible fashion, and selected clones of each line with optimal sgRNA-mediated deletion (see above). For ABC DLBCL, three BCR-dependent and ibrutinib-sensitive lines were studied, two of which harbor both *CD79B* and *MYD88* L265P mutations (TMD8, HBL1), and one (U2932) that lacks both mutations (Davis et al., Nature, 463: 88-92 (2010), incorporated by reference herein; and Kelly et al., J. Exp. Med., 212: 2189-2201 (2015), incorporated by reference herein). For comparison, one BCR-independent, ibrutinib-resistant line (HLY1) was included, that line being *MYD88*-dependent and having a less prevalent *MYD88* S219C mutation (Ngo et al., Nature, 470: 115-119 (2011), incorporated by reference herein). For GCB DLBCL, four ibrutinib-resistant lines with an unknown dependence on BCR signaling (DOHH2, SUDHL4, SUDHL5, WSU-DLCL2) were studied. As controls, parallel screens were performed in two multiple myeloma lines (ARPC1, KMS11) and one T cell lymphoma line (DEL).

[0261] After lentiviral transduction, duplicate cultures for each line were passed for three weeks before measuring the abundance of each sgRNA in the initial and final cultures by next-generation sequencing (Fig. 1A). For each gene, the two sgRNAs that displayed the most correlated toxicity across the 11 cell lines were identified, and averaged their effect to derive a gene-level depletion/enrichment value (see above). For the minority of genes without correlated sgRNAs, the sgRNA with the greatest variance across the cell lines as the gene exemplar were chosen. For each gene in each sample, a "CRISPR screen score" (CSS) was defined that measures the effect of its CRISPR-mediated inactivation as the number of standard deviations away from the average effect of inactivating any gene (see above). Little

evidence of non-specific toxicity among control, non-gene targeting sgRNAs was observed, whereas sgRNAs targeting essential genes (Hart et al., *Cell*, 163: 1515-1526 (2015), incorporated by reference herein) were depleted in all lines (Fig. 1B). As expected from previous work (Wang et al., *Science*, 350: 1096-1101 (2015), incorporated by reference herein), sgRNAs targeting genomic regions with high-level amplifications were non-specifically toxic and were removed from functional analyses.

[0262] sgRNAs depleted in the final cultures frequently targeted essential genes previously identified in CRISPR loss-of-function screens in non-lymphoma cancer cell lines (Fig. 1B) (Hart et al., *Cell*, 163: 1515-1526 (2015), incorporated by reference herein). Dependence of both GCB and ABC lines on the anti-apoptotic BCL2 family members BCL2, BCL-X_L (BCL2L1) and MCL1 was also observed, with each member contributing variably to the survival of individual cell lines (Fig. 6). In keeping with the derivation of the DLBCL subtypes from distinct stages of normal B cell differentiation (Shaffer et al., *Annu. Rev. Immunol.*, 30: 565-610 (2012), incorporated by reference herein), several B cell-restricted transcription factors were selectively essential in ABC DLBCL (IRF4, SPIB, BATF) or GCB DLBCL (MEF2B, TCF3, IRF8, SPI1), along with others (BCL6, PAX5, EBF, POU2F2, POU2AF1) that were required in the majority of DLBCL lines but were variably essential in the myeloma and T cell lymphoma lines (Fig. 1C).

Distinct modes of BCR signaling

[0263] Next, identity of genes that were specifically essential in the three BCR-dependent ABC DLBCL lines were sought. Twenty-eight genes were identified whose inactivation produced a significantly greater depletion ($p < 0.01$, $\text{CSS} < -1.5$) in BCR-dependent ABC lines than in all others, and that were not reported to be essential in previous CRISPR-Cas9 screens (Hart et al., *Cell*, 163: 1515-1526 (2015), incorporated by reference herein) (Figs. 1D and 1E). In keeping with the known pro-survival role of NF- κ B in these cell lines, NF- κ B subunits p50 (NFKB1) and c-Rel (REL) were essential, as was the IKK catalytic β subunit (IKKBK), the defining kinase of the classical NF- κ B pathway. Also essential were components of the BCR-dependent signaling cascade known to activate IKK in these ABC DLBCL cells (Davis et al., *Nature*, 463: 88-92 (2010), incorporated by reference herein; Yang et al., *Cancer Discov.*, 4: 480-493 (2014), incorporated by reference herein; and Ngo et al., *Nature*, 441: 106-110 (2006), incorporated by reference herein), including the kinase BTK and its substrate phospholipase C γ 2 (PLCG2), protein kinase C β (PRKCB), components

of the CBM signaling adapter complex (CARD11, BCL10, MALT1), and the RBCK1 subunit of the linear ubiquitin chain assembly complex (LUBAC) that facilitates recruitment of IKK to the CBM complex (Yang et al., *Cancer Discov.*, 4: 480-493 (2014), incorporated by reference herein). Additional NF- κ B regulators that were essential in ABC lines include TRAF6, TAK1 (MAP3K7) and NFKBIZ.

[0264] Analogous selection criteria were used to identify genes that were selectively essential in the GCB DLBCL lines versus all others (Figs. 1D and 1E). Surprisingly, two of the 12 genes identified encode proteins involved in proximal BCR signaling, LYN and CD19. LYN, one of several SRC-family kinases (SFKs) that can initiate BCR signaling (Gross et al., *J. Immunol.*, 182: 5382-5392 (2009), incorporated by reference herein), was essential in all GCB lines. Among ABC lines, LYN was only moderately essential in HBL1 and behaved as a tumor suppressor in TMD8 cells (i.e. LYN sgRNAs were enriched at day 21). CD19 is a BCR co-receptor that participates in PI3K activation (reviewed in Fearon et al., *Annu. Rev. Immunol.*, 13: 127-149 (1995), incorporated by reference herein). CD19 was essential in all GCB lines but behaved as a tumor suppressor in ABC lines. Given the essentiality of LYN and CD19 in GCB DLBCL, the function of other proximal BCR components in these lines was investigated. Surprisingly, the BCR subunits CD79A and CD79B were not only essential in ABC lines, but also in GCB lines, a finding that was not appreciated in previous studies (Davis et al., *Nature*, 463: 88-92 (2010), incorporated by reference herein), most likely due to incomplete knockdown of these highly expressed genes by RNA interference.

[0265] These observations prompted an investigation into whether oncogenic signaling pathways were differentially engaged in ABC and GCB DLBCL. Figure 1F depicts the average CSS scores from the whole-genome CRISPR screens of three BCR-dependent ABC and four GCB lines in the proximal BCR, PI3K, NF- κ B, TLR and JAK/STAT signaling pathways. A validation screen using ~10 sgRNAs per gene demonstrated strong correlations between screens of overlapping cell lines (Pearson's correlation coefficients averaging 0.79; 0.68-0.84, $p < 0.0001$; Figs. 7A-7G).

[0266] Proximal BCR signaling in developing B cells is initiated by the redundant SFKs LYN, BLK and FYN (Saijo et al., *Nat. Immunol.* 4: 274-279 (2003), incorporated by reference herein). In addition to the already mentioned requirement for LYN in all GCB lines, two lines depended on the SFK BLK (DOHH2, SUDHL4) and one depended on LCK (DOHH2). By contrast, no single SFK was essential in ABC lines, possibly due to functional redundancy. The phosphatase CD45, a regulator of SFKs, was essential in GCB lines but had

the opposite effect in ABC lines. In this regard, it is notable that CD45 positively regulates BCR signaling during B cell development but has less effect on antigen-induced active BCR signaling (Cyster et al., *Nature*, 381: 325-328 (1996), incorporated by reference herein). CSK, another regulator of SFKs, was essential in both BCR-dependent ABC and GCB lines. While this was unexpected, given the fact that CSK inhibits SFK function in T cells (Zikherman et al., *Immunity*, 32: 342-354 (2010), incorporated by reference herein), this finding may fit with the observation that a CSK polymorphism that increases its expression predisposes to systemic lupus erythematosus and is associated with increased BCR signaling (Manjarrez-Orduno et al., *Nat. Genet.*, 44: 1227-1230 (2012), incorporated by reference herein).

[0267] The proximal BCR tyrosine kinase SYK was essential in both ABC and GCB lines, which is notable given that small molecule SYK inhibitors are in clinical trials (see below). Nonetheless, the consequences of SYK activity appear to differ between the DLBCL subtypes. SYK mediates PI3K signaling in normal B cells by several mechanisms, including direct interaction with the PI3K p85 subunit as well as phosphorylation of CD19 and BCAP (PIK3AP1), enabling them to also recruit p85 (reviewed in Kulathu et al., *Immunological Reviews*, 232: 286-299 (2009), incorporated by reference herein). As mentioned, CD19 was selectively essential in GCB lines, as was CD81, which facilitates CD19 localization to the plasma membrane (Maecker et al., *J. Exp. Med.*, 185: 1505-1510 (1997), incorporated by reference herein). By contrast, PIK3AP1, was required only in BCR-dependent ABC DLBCL lines. Although CD19 and BCAP are functionally redundant in normal mouse B cells (Aiba et al., *Blood*, 111: 1497-1503 (2008), incorporated by reference herein), these results indicate that that is not the case in DLBCL and suggest that each DLBCL subtype uses a distinct mechanism to engage PI3K (Id.). ABC and GCB lines shared a dependence on the delta catalytic subunit of PI3K (PIK3CD) and the downstream kinases PDPK1, mTOR and RPS6KB1. However, PTEN, a negative regulator of PI3K signaling, functioned as a tumor suppressor in GCB but not ABC lines, in keeping with the fact that inactivation of PTEN is a recurrent genetic event in GCB but not ABC DLBCL tumors (Lenz et al., *Proc. Natl. Acad. Sci. USA*, 105: 13520-13525 (2008), incorporated by reference herein; and Pfeifer et al., *Proc. Natl. Acad. Sci. USA*, 110: 12420-12425 (2013), incorporated by reference herein).

[0268] As already discussed, multiple components of the NF- κ B pathway downstream of BTK were essential in ABC but not GCB lines (Fig. 1F). BLNK is a signaling adapter that is phosphorylated by SYK and partners with BTK to form a plasma membrane-associated

complex that activates NF- κ B. BLNK was selectively essential in ABC lines, in keeping with their chronic active form of BCR signaling. Two negative regulators of NF- κ B – A20 (TNFAIP3) and its associated protein TNIP1 – behaved as tumor suppressors in ABC but not GCB lines, in keeping with the frequent inactivation of A20 by a variety of genetic and epigenetic means in ABC DLBCL tumors (Compagno et al., *Nature*, 459: 717-721 (2009), incorporated by reference herein). Conversely, the NF- κ B negative regulator I κ B α (NFKBIA) functioned as a tumor suppressor only in GCB lines, presumably because the chronic active BCR signaling in ABC DLBCL triggers IKK β phosphorylation of I κ B α , leading to its degradation.

[0269] A parallel means to activate NF- κ B in ABC DLBCL involves MYD88 and its associated kinases IRAK1 and IRAK4 (Ngo et al., *Nature*, 470: 115-119 (2011), incorporated by reference herein), which were selectively essential in the ABC lines with MYD88 mutations (TMD8, HBL1, HLY1). Unexpectedly, the MYD88-dependent lines further required the Toll-like receptor TLR9, and its associated chaperone proteins UNC93B1 and CNPY3 (reviewed in Saitoh et al., *Immunological reviews* 227, 32-43 (2009), incorporated by reference herein), uncovering a new facet of MYD88-dependent NF- κ B activation in these lines that were examined in detail below. Of note, none of the other nine TLRs were found to be essential in ABC DLBCL lines (Fig. 8). NF- κ B activation by oncogenic MYD88 isoforms directs the expression of the cytokines IL-10 and IL-6 (Ngo et al., *Nature*, 470: 115-119 (2011), incorporated by reference herein). The alpha and beta subunits of the IL-10 receptor were selectively essential in ABC lines, while the components of the IL-6 receptor were not. The role of JAK1 and its target STAT3 in MYD88-mutant ABC DLBCL has been described recently (Rui et al., *Proc. Natl. Acad. Sci. USA*, 113: E7260-E7267 (2016), incorporated by reference herein), and both genes were essential in the MYD88-mutant ABC lines (Fig. 1F).

BCR signaling in germinal center-derived lymphomas

[0270] Although previous studies have not identified a role for the BCR in the pathogenesis of GCB DLBCL (Davis et al., *Nature*, 463: 88-92 (2010), incorporated by reference herein; Young et al., *Proc. Natl. Acad. Sci. USA*, 112: 13447-13454 (2015), incorporated by reference herein; and Schmitz et al., *Nature*, 490: 116-120 (2012), incorporated by reference herein), the CRISPR screens suggested that a subset of GCB DLBCL lines require BCR signaling for survival, but in a fashion that is mechanistically

distinct from chronic active BCR signaling in ABC DLBCL. To validate these results, a larger set of Cas9-expressing GCB and ABC lines was transduced with vectors expressing individual sgRNAs targeting BCR pathway components along with GFP, which allowed use of GFP to track sgRNA toxicity over a two-week period by FACS. As a positive control, transduction of an sgRNA targeting the ribosomal protein RPL6 caused a time-dependent decrease in GFP+ cells in all lines (Fig. 2A). Genetic depletion of CD79A or SYK was toxic to five of seven ABC lines and four of six GCB lines (Fig. 2A). BCR-dependent ABC lines uniquely required BTK, PLCG2, CARD11 and PIK3AP1, whereas the BCR-dependent GCB lines uniquely required CD19.

[0271] These genetic data suggested that the toxicity of small molecule inhibitors of BCR signaling would depend on the mode of BCR signaling in each DLBCL line. To examine this issue, MTS viability assays were performed using a large panel of DLBCL lines treated with small molecule inhibitors of SYK (PRT062607 (Coffey et al., *J. Pharmacol. Exp. Ther.*, 340: 350-359 (2012), incorporated by reference herein)) or BTK (ibrutinib), and used the GR_{max} calculation to measure toxicity since it is less susceptible to changes in cell density than the IC_{50} metric (Hafner et al., *Nat. Methods*, 13: 521-527 (2016), incorporated by reference herein). Most ABC lines were sensitive to ibrutinib, while GCB lines were insensitive, and GR_{max} values for ibrutinib correlated with the degree of depletion of a BTK sgRNA after 14 days ($R^2=0.602$). In contrast, a subset of both GCB and ABC lines were sensitive to the SYK inhibitor, again with a good correlation between the GR_{max} and the genetic toxicity of SYK deletion ($R^2=0.849$; Fig. 2B). Across 44 DLBCL lines, a spectrum of sensitivity to the SYK inhibitor was observed: 16 lines had a GR_{max} below 0, signifying a cytotoxic effect, 17 had a GR_{max} between 0 and 0.75, suggesting a cytostatic effect, and 11 lines were insensitive to SYK inhibition (Fig. 2C, Figs. 9A-9F).

[0272] The dependence of GCB DLBCL lines on SYK and the PI3K pathway is shared by Burkitt lymphoma, another GC-derived malignancy (Schmitz et al., *Leukemia*, 19: 1452-1458 (2005), incorporated by reference herein). Interestingly, the CD19-dependence of GCB DLBCL lines was also shared by Burkitt lymphoma lines, as judged by the toxicity of a CD19 shRNA (Fig. 10). “Constitutive GC BCR signaling” is a term that can be used to characterize the shared mode of BCR signaling in these two GC-derived lymphoma subtypes (see below).

TLR9 is essential for BCR signaling in ABC DLBCL

[0273] The survival of BCR-dependent ABC lines relied on TLR9, which coordinates MYD88 signaling in innate immune cells, and on two chaperones that regulate the subcellular localization of TLR9, CNPY3 and UNC93B1. TLR9 was the only essential TLR, and its essentiality was confined to MYD88^{L265P}-mutant ABC lines (Fig. 8). These findings were validated using time-dependent toxicity assays in 12 DLBCL lines transduced with vectors co-expressing sgRNAs and GFP (Fig. 3A). ABC lines expressing mutant isoforms of MYD88 were sensitive to MYD88 deletion. In contrast, TLR9, UNC93B1 and CNPY3 were only essential in ABC lines with MYD88^{L265P} and either a *CD79B* or *CD79A* mutation. These double-mutant lines were also particularly sensitive to BTK depletion.

[0274] ABC tumors had recurrent single copy gains or amplifications involving *MYD88* (31.0%), *TLR9* (26.9%), *CNPY3* (17.9%), and *UNC93B1* (15.9%), and these occurred more frequently in ABC than in GCB tumors. All four genes were more highly expressed in ABC tumors, and expression correlated with copy number (Fig. 3B, Fig. 4). *MYD88* and *TLR9* are located ~15 Mb from each other on chromosome 3, which is notable since trisomy or amplification of chromosome 3 is present in more than 25% of ABC cases. Amplification of *CNPY3* was observed in 14 ABC biopsies (4.8%) as well as the ABC line HBL1, with a minimal common amplified region of 1.1 Mb containing 24 protein coding genes (Fig. 3B). *UNC93B1* was amplified in 9 ABC biopsies (3.1%), with a 277 kb minimal common amplified region containing only 3 genes (Fig. 3B). Altogether, 49.7% of ABC DLBCL tumors had increased copy number of one or more of these TLR9 module genes (Fig. 3B), providing genetic evidence that this pathway contributes to the ABC DLBCL phenotype.

The My-T-BCR supercomplex

[0275] To elucidate the function of TLR9 in ABC DLBCL, a fusion protein was expressed, linking TLR9 to BioID2, a promiscuous biotin ligase that biotinylates proteins within ~10 nm. Biotinylated proteins in transduced HBL1 and TMD8 ABC cells were purified using streptavidin and compared to proteins from control cells expressing empty vector by a SILAC-based quantitative mass spectrometry (MS) approach. To define the subset of the TLR9 interactome that is essential in ABC DLBCL, the TLR9- BioID2 enrichment of each protein was compared with its respective CRISPR CSS metric (Fig. 3C, Fig. 11A). The TLR9-essential interactome shared by both HBL1 and TMD8 cells confirmed

association of TLR9 with MYD88 and CNPY3, but also revealed interactions with the BCR subunits CD79A and CD79B (Fig. 3C, Fig. 11A-C). It was confirmed that immunoprecipitated IgM was associated with TLR9 in three ABC lines, but to a much lesser extent in the GCB line OCI-Ly19 (Fig. 3D). By contrast, neither TLR4 nor TLR7 co-immunoprecipitated with IgM (Fig. 12A). Sub-cellular fractionation revealed that TLR9 was associated with IgM in an intracellular fraction rather than a plasma membrane fraction (Fig. 12B), suggesting that the BCR may cooperate with TLR9 in signaling at an intracellular location.

[0276] To visualize where TLR9 and the BCR interact, the proximity ligation assay (PLA) was employed (Soderberg et al., *Methods*, 45: 227-232 (2008), incorporated by reference herein), which identifies interactions between proteins within tens of nanometers of each other. An IgM:TLR9 PLA produced fluorescent puncta in the cytoplasm of ABC cells (Fig. 3E). Depletion of the BCR component CD79A or TLR9 substantially reduced the PLA signal, demonstrating its specificity (Fig. 3E, Fig. 12C). IgM:TLR9 PLA signal was present across a panel of BCR-dependent ABC lines, with higher signals in double-mutant lines, whereas BCR-independent ABC and GCB lines had substantially lower signals (Fig. 12D-F). The IgM:TLR9 PLA signal co-localized with the endolysosomal marker LAMP1 (Fig. 12G-H), consistent with the dependence of these ABC lines on UNC93B1 and CNPY3, which facilitate entry of TLR9 into LAMP1⁺ endolysosomes.¹⁹ Ectopic expression of TLR9, MYD88^{WT} or MYD88^{L265P} markedly increased the IgM:TLR9 PLA signal (Fig. 12I), suggesting that TLR9/MYD88 copy number gains in ABC tumors may augment this signaling complex (Fig. 3B).

[0277] MYD88 mediates TLR9-dependent NF- κ B activation, and accordingly, knockdown of MYD88 or TLR9 with small hairpin RNAs (shRNAs) produced similar changes in gene expression and I κ B α levels in two ABC lines with MYD88^{L265P} (Fig. 13A-D). A TLR9:MYD88 PLA produced a strong signal in the cytoplasm of ABC lines, which was diminished by knockdown of TLR9 or MYD88. Knockdown of CD79A also decreased the TLR9:MYD88 PLA signal, suggesting that the BCR facilitates recruitment of MYD88 to TLR9 (Fig. 3F). These results suggest that TLR9 coordinates signaling between the BCR and MYD88. It was hypothesized that the BCR, TLR9 and MYD88 nucleate a signalosome that activates NF- κ B, which we will term the MyD88-TLR9-BCR (My-T-BCR) supercomplex. To identify additional My-T-BCR components, a MYD88^{L265P}-BioID2 fusion protein was expressed in TMD8, HBL1 and OCI-Ly10 ABC cells and performed SILAC-MS analysis of

MYD88-proximal biotinylated proteins. Proteins were identified as biotinylated in all 3 MYD88^{L265P}-BioID2 lines and used their corresponding CRISPR CSS scores to define the essential MYD88 interactome, which included the BCR (CD79B), mTOR, PLC γ 2, and the CBM complex (CARD11, MALT1) (Fig. 14A-B, Fig. 15A-B). It was confirmed that both CARD11 and MALT1 are biotinylated by MYD88^{L265P}-BioID2 in ABC cells using streptavidin pulldown and immunoblot analysis (Fig. 15C,D). Finding the CBM complex in close proximity to MYD88 was unexpected since these adaptors are thought to independently promote NF- κ B activation. Both MALT1:MYD88 and BCL10:MYD88 PLAs yielded robust cytoplasmic puncta in ABC cells, confirming the association of endogenous MYD88 with the CBM complex (Fig. 14C, Fig. 15E-F). MYD88:MALT1 and MYD88:BCL10 puncta were reduced by knockdown of CD79A, TLR9, and CARD11, suggesting that BCR and TLR9 signaling cooperate to assemble MYD88 and the CBM into a supercomplex. It was further hypothesized that CBM assembly might depend not only on BCR signaling, but also, on TLR9 and MYD88. Indeed, the puncta observed using a CARD11:BCL10 PLA were reduced by knockdown of TLR9 or MYD88, as well as by knockdown of CARD11, BCL10, and CD79A (Fig. 14D).

[0278] The classical NF- κ B pathway is activated by IKK-dependent phosphorylation of I κ B α , making phospho-I κ B α a marker of NF- κ B activation. By PLA, we observed association of IgM and phospho-I κ B α in the cytoplasm of ABC cells, which was reduced by knockdown of CD79A, TLR9 or MYD88 (Fig. 14E). Phospho-I κ B α was also associated with TLR9, and knockdown of CD79A, TLR9 or MYD88 reduced this signal (Fig. 14F). Thus, NF- κ B activation is closely associated with the My-T-BCR complex.

[0279] The subcellular location of the My-T-BCR complex was visualized by staining ABC cells bearing MYD88^{L265P}-BioID2 with fluorescently-labeled streptavidin. The MYD88-BioID2 signal defined large cytoplasmic structures that colocalized with phospho-IKK, consistent with active NF- κ B signaling at these sites (Fig. 14G). These complexes extended into the cytoplasmic space from the surface of LAMP1⁺ vesicles. BCR internalization was visualized by cell surface labelling of IgM with a fluorescent Fab on ice, followed by brief warming of the cells to allow BCR internalization. The LAMP1⁺ vesicles with MYD88-BioID2 signals also contained IgM, suggesting a dynamic shuttling of the BCR from the plasma membrane to the intracellular site of My-T-BCR complex formation. Knockdown of CD79A or TLR9 decreased the MYD88-BioID2 signal (Fig. 14H),

demonstrating that these complexes were similar to those detected by the PLA method in their dependence on TLR9 and the BCR. Taken together, these data provide evidence that IKK-dependent activation of classical NF- κ B signaling is coordinated in ABC cells by the My-T-BCR supercomplex.

My-T-BCR and ibrutinib responses

[0280] Given that the My-T-BCR complex coordinates pro-survival signaling in ABC DLBCL, it was hypothesized that inhibition of BTK activity by ibrutinib might disrupt this signaling complex. Indeed, ibrutinib treatment of ABC lines bearing MYD88^{L265P}-BioID2 reduced My-T-BCR-dependent biotinylated puncta (Fig. 16A). To globally assess the influence of ibrutinib on the MYD88 interactome, SILAC-MS analysis of biotinylated proteins was performed in OCI-Ly10 and TMD8 ABC lines bearing MYD88^{L265P}-BioID2, in the presence or absence of ibrutinib. This analysis revealed that interactions of MYD88 with the CBM complex (CARD11), PLC γ 2, and mTOR were disrupted by ibrutinib treatment in both ABC lines (Fig. 17A, Fig. 16B).

[0281] The ibrutinib-sensitive association of mTOR with MYD88 was surprising and raised the possibility that signaling by the My-T-BCR complex might affect essential downstream pathways controlled by mTOR. Of note, components of the Ragulator complex (LAMTOR1, LAMTOR3, LAMTOR4, RRAGA), which regulates mTORC1 activity at the lysosomal membrane, were biotinylated by TLR9-BioID2, as were components of the lysosomal V-ATPase (ATP6V1B2, ATP6V0D1), which regulates the mTORC1 response to amino acids (Fig. 3C, Fig. 11C). In ABC lines expressing MYD88^{L265P}-BioID2, mTOR was localized to LAMP1⁺ vesicles, often in proximity to the My-T-BCR (Fig. 17B). PLA in three ABC lines confirmed that ibrutinib decreased the association of endogenous MYD88 with mTOR, MALT1, and BCL10 (Fig. 17C). Ibrutinib also decreased the association of IgM and phospho-I κ B α but had mixed effects on IgM association with TLR9. These findings suggest that IgM trafficking to TLR9-containing endolysosomes is constitutive in these lines, but interaction of the My-T-BCR complex with mTOR, the CBM complex, and NF- κ B is controlled by BCR signaling in an ibrutinib-sensitive manner.

[0282] Given the proximity of MYD88 and mTOR, the effect of mTOR inhibition on the My-T-BCR was investigated. In three ABC cells bearing MYD88^{L265P}-BioID2, the My-T-BCR was reduced following ibrutinib treatment, but was further

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attenuated by the addition of AZD2014, an mTORC1/2 inhibitor (Fig. 17D). Dual mTOR and BTK inhibition also cooperatively decreased MYD88 protein levels and blocked mTOR activity, as assessed by phospho-4E-BP1 and phospho-S6 kinase, as well as NF- κ B activation, as assessed by phospho-IKK (Fig. 17E). These data provide mechanistic insights into the synergistic toxicity of combinations of BTK inhibitors and drugs targeting mTOR or PI3 kinase in ABC models growing *in vitro* (Fig. 17F) and *in vivo* (Fig. 17G) (Mathews Griner et al., PNAS, 111: 2349-2354 (2014) and Ezell et al., Oncotarget, 5: 4990-5001 (2014), both incorporated by reference herein).

mTOR inhibitors: OSI-027, AZD-8055, AZD2014, SIROLIMUS, BEZ-235, and everolimus

PI3 kinase pathway inhibitors: IPI-145, BYL-719, BKM-120, Idelalisib, GDC-0980, andCopanlisib

Figure 17F has the following tables:

Table 3: Viability (48 hrs)

4.7	6.3	5.2	7.7	7.6	6.1	4.9	6.4	5.9	29	2000	AZD2014 (nM)
9.9	7.7	10.2	13.6	13.5	14.7	10.1	12.1	14.7	34.7	1000	
13	12.2	16.4	16.8	15.3	17.6	16.7	18.8	20.4	40.3	500	
15.4	23.2	22.7	24.7	22.3	21	26	26.1	33.5	55.1	250	
17.5	32.8	32	35.9	42.7	32.7	36.1	37.1	38.8	60.8	125	
29.6	35.5	34.7	48.1	45.4	58.8	44.6	53.6	55.5	78	62	
32.8	48.8	50.4	52.9	50.1	55.2	52.3	54.1	57.2	89.7	31	
35.3	54.2	46.6	53.8	53.7	61.5	58.3	56.2	58.5	92.9	16	
45	56.6	60.1	51.2	66.9	62.6	65.6	64.5	80.1	100	8	
47.8	52.1	54	54.9	55.8	56.6	56.8	60.9	69	100	0	
500	250	125	62	31	16	8	4	2	0		
Ibrutinib (nM)											

Table 4: Δ Bliss (48 hrs)

-0.09	-0.09	-0.1	-0.08	-0.09	-0.1	-0.12	-0.11	-0.14	0	2000	AZD2014 (nM)
-0.07	-0.1	-0.09	-0.05	-0.06	-0.05	-0.1	-0.09	-0.09	0	1000	
-0.06	-0.09	-0.05	-0.05	-0.07	-0.05	-0.06	-0.06	-0.07	0	500	
-0.11	-0.05	-0.07	-0.06	-0.08	-0.1	-0.05	-0.07	-0.05	0	250	
-0.12	0.01	-0.01	0.03	0.09	-0.02	0.02	0	-0.03	0	125	
-0.08	-0.05	-0.07	0.05	0.02	0.15	0	0.06	0.02	0	62	
-0.1	0.02	0.02	0.04	0	0.04	0.01	0	-0.05	0	31	
-0.09	0.06	-0.04	0.03	0.02	0.09	0.06	0	-0.06	0	16	
-0.03	0.04	0.06	-0.04	0.11	0.06	0.09	0.04	0.11	0	8	
0	0	0	0	0	0	0	0	0	0	0	
500	250	125	62	31	16	8	4	2	0		
Ibrutinib (nM)											

[0283] It was examined whether the My-T-BCR complex is detectable in primary lymphoma patient biopsy samples, and if its presence might predict response to ibrutinib. The PLA was optimized for use in formalin-fixed and paraffin-embedded (FFPE) samples using a tissue microarray prepared from FFPE pellets of 81 lymphoma cell lines. The highest IgM:TLR9 PLA signals were in ABC lines with chronic active BCR signaling, whereas little if any PLA signal was observed in GCB lines, Burkitt lymphoma lines, or in normal B cells present in tonsils or reactive lymph nodes (Fig. 18A-B). Among DLBCL FFPE biopsy samples, ABC cases had significantly more IgM:TLR9 puncta than GCB cases (Fig. 19A). High IgM:TLR9 PLA signals were also observed in FFPE biopsies of primary central nervous system lymphoma (PCNSL), Waldenström macroglobulinemia (WM), and its relative lymphoplasmacytic lymphoma (LPL) (Fig. 19B). These malignancies commonly have MYD88^{L265P} and/or *CD79A* or *CD79B* mutations, and respond frequently to ibrutinib. Of two WM lines tested, one had My-T-BCR complexes, and knockdown of either the BCR (*CD79A*) or TLR9 was selectively toxic for this line (Fig. 20A-C). My-T-BCR complexes were not evident in mantle cell lymphoma (MCL) or chronic lymphocytic leukemia (CLL) samples (Fig. 19B), suggesting that these malignancies rely on a qualitatively distinct form of BCR signaling.

[0284] Eight available biopsies were examined from patients with relapsed or refractory DLBCL enrolled on a clinical trial of ibrutinib monotherapy. The IgM:TLR9 PLA methodology was adapted to allow the malignant lymphoma cells to be identified by anti-CD20 immunohistochemical staining (Fig. 19C). Three ABC cases and one unclassified DLBCL case scored positive in the IgM:TLR9 PLA while three other ABC cases and one GCB case were negative. The percentage of IgM:TLR9 PLA-positive malignant cells was significantly higher in tumors that responded to ibrutinib than in those that progressed on treatment (Fig. 19D, $p < 0.0286$). In this series, two responding cases with IgM:TLR9 puncta had *CD79B* or *CD79A* mutations, respectively, but lacked MYD88^{L265P}, while two other responders were wild type for these genes (Table 5). These findings demonstrate that the My-T-BCR exists in ABC DLBCL tumors that respond to ibrutinib, even in those lacking the MYD88^{L265P}/*CD79B* double-mutant genotype.

Table 5

Patient ID	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8
Subtype	ABC	UNCLASS.	ABC	ABC	ABC	GCB	ABC	ABC
Ibrutinib clinical response	CR	CR	PD	SD	PD	PD	PD	PR
Ibrutinib response group	+	+	-	+	-	-	-	+
Cells counted	452	492	395	425	427	381	779	337
Average number of IgM:TLR 9 puncta/cell	1.31	0.58	0.45	9.27	0.37	0.18	0.09	4.81
Standard Deviation	2.21	1.12	0.77	9.29	0.80	0.59	0.37	5.29
% Cells with ≥ 2 puncta/cell	27.43	13.41	7.59	88.24	7.49	2.89	1.67	71.22
CD79B mutation	Y196C	WT	WT	WT	WT	WT	WT	WT
CD79A mutation	WT	CD79A_c.568-2_610delAGGGCCTG AACCTGGACGACTG CTCCATGTATGAGG ACATCTCCC_p.splice	WT	WT	WT	WT	WT	WT
MYD88 mutation	WT	WT	WT	WT	WT	WT	G214R	WT

CR = Complete Responder, PR = Partial Responder, SD = Stable disease; PD = Progressive Disease

Response to ibrutinib = "+", Non-response = "-"

[0285] The CRISPR-Cas9 genetic screens revealed two distinct modes of BCR signaling in lymphomas, which will help guide the development of therapies targeting components of the BCR pathway (Fig. 5). One BCR signaling mode, termed herein as “constitutive GC BCR signaling,” is shared by GCB DLBCL and Burkitt lymphoma lines. This mode of signaling utilizes the BCR, CD19, and SYK to engage PI3K as its principle downstream survival pathway (including AKT and mTOR). By contrast, a subset of ABC DLBCLs with mutations targeting both the BCR and MYD88 are sustained by a form of chronic active BCR signaling in which the My-T-BCR complex in endolysosomes engages NF- κ B to promote pathological survival.

[0286] The first unexpected insight from the CRISPR-Cas9 screens was the strong dependence of some GCB DLBCL lines on the BCR, which was likely missed in previous

studies because RNA interference cannot fully eliminate expression of the BCR subunits (Davis et al., *Nature*, 463: 88-92 (2010), incorporated by reference herein). Previous studies used RNA interference to show that SYK is essential in some GCB lines (Cheng et al., *Blood*, 118: 6342-6352 (2011), incorporated by reference herein; and Chen et al., *Cancer Cell*, 23: 826-838 (2013), incorporated by reference herein), but these studies did not directly assess the role of the BCR, leaving considerable uncertainty since SYK can be activated by a wide variety of receptors and signaling pathways (Mocsai et al., *Nat. Rev. Immunol.*, 10: 387-402 (2010), incorporated by reference herein). In the CRISPR-Cas9 screens, the BCR-dependent GCB lines were not dependent on BTK or the NF- κ B pathway, thus distinguishing BCR signaling in GCB DLBCL from chronic active BCR signaling in ABC DLBCL and explaining why ibrutinib yielded only a 5% response rate among GCB DLBCL cases in a phase II clinical trial (Wilson et al., *Nat. Med.*, 21: 922-926 (2015), incorporated by reference herein).

[0287] Superficially, BCR signaling in GCB DLBCL resembles “tonic” BCR signaling, which sustains the survival of naïve mature mouse B cells, since PI3K is the principle downstream survival mechanism engaged by the BCR in both cases (Lam et al., *Cell*, 90: 1073-1083 (1997), incorporated by reference herein; Kraus et al., *J. Exp. Med.*, 194: 455-469 (2001), incorporated by reference herein; and Srinivasan et al., *Cell*, 139: 573-586 (2009), incorporated by reference herein). Indeed, BCR signaling in Burkitt lymphoma was previously characterized as “tonic” based on similar considerations (Schmitz et al., *Nature*, 490: 116-120 (2012), incorporated by reference herein). However, certain mechanistic aspects of BCR signaling in GCB DLBCL and Burkitt lymphoma appear to be distinct from tonic BCR signaling in naïve mature mouse B cells. First, the CRISPR screens revealed that GCB lines rely on the BCR co-receptor CD19 and its associated membrane protein CD81 for survival, and similarly, knockdown of CD19 in BCR-dependent Burkitt lymphoma lines is toxic. CD19^{-/-} mice have only a modest decrease in naïve mature B cell numbers, largely due to loss of the marginal zone and B1-B subpopulations, in contrast to the dramatic loss of all mature B cells following conditional deletion of the BCR (Rickert et al., *Nature*, 376: 352-355 (1995), incorporated by reference herein; and Engel et al., *Immunity*, 3: 39-50 (1995), incorporated by reference herein). Both CD19^{-/-} and CD81^{-/-} mice are severely impaired in their antibody responses to T cell-dependent antigens, which require a germinal center response, but responses to T cell-independent antigens are intact (Maecker et al., *J. Exp. Med.*, 185: 1505-1510 (1997), incorporated by reference herein; Rickert et al., *Nature*, 376:

352-355 (1995), incorporated by reference herein; and Engel et al., *Immunity*, 3: 39-50 (1995), incorporated by reference herein). Indeed, CD19^{-/-} mice do not form germinal centers upon immunization with T cell-dependent antigens (Rickert et al., *Nature*, 376: 352-355 (1995), incorporated by reference herein). Knock-in mice lacking the CD19 tyrosine residues necessary for PI3K recruitment can support early steps in germinal center B cell formation, but the proliferation of these cells and the expansion of the germinal center are dramatically attenuated (Wang et al., *Immunity*, 17: 501-514 (2002), incorporated by reference herein).

[0288] A second aspect of BCR signaling in GCB DLBCL lines that is distinct from tonic BCR signaling is the dependence of GCB lines on the kinase LYN. Mature B cells from LYN^{-/-} mice are in fact increased in number and are hyperresponsive to BCR crosslinking, ultimately leading to autoimmune glomerulonephritis (Wang et al., *J. Exp. Med.*, 184: 831-838 (1996), incorporated by reference herein; and Hibbs et al., *Cell*, 83: 301-311 (1995), incorporated by reference herein). LYN^{-/-} mice, like CD19^{-/-} mice, fail to form germinal centers in response to T cell-dependent antigens (Kato et al., *J. Immunol.*, 160: 4788-4795 (1998), incorporated by reference herein). It thus seems likely that the dependence of GCB lines on CD19 and LYN reflects their required role in normal GC B cell function.

[0289] For these reasons, “constitutive GC BCR signaling” is a term used herein to characterize the mode of BCR signaling in GCB DLBCL and Burkitt lymphoma, which engages PI3K, but not NF-κB, to promote malignant cell survival. In this regard, it is notable that PI3K-dependent AKT activation is a feature of normal GC B cells residing in the GC “light zone”, where the B cells engage antigen on the surface of follicular dendritic cells (Sander et al., *Immunity*, 43: 1075-1086 (2015), incorporated by reference herein). By contrast, BCR-dependent NF-κB activation is impaired in normal GC B cells, apparently because PKCβ cannot be efficiently recruited to the immune synapse on the cell surface in response to antigen engagement of the BCR (Nowosad, *Nat. Immunol.*, 17: 870-877 (2016), incorporated by reference herein).

[0290] The second insight from the CRISPR-Cas9 screens is that chronic active BCR signaling in ABC DLBCL is mechanistically heterogeneous, with a subset of these lymphomas utilizing a novel TLR9-dependent mechanism to promote strong addiction to BCR signaling. Among ABC lines with chronic active BCR signaling, some depend only on the BCR (U2932 and RIVA) while others depend on the BCR and TLR9 (TMD8, HBL1, OCI-Ly10). Cooperative signaling between the BCR and TLRs has been reported previously

in the context of normal and pathological immune responses in the mouse. BCR ablation in mouse B cells impairs their proliferative response to TLR9 stimulation (Otipoby et al., Proc. Natl. Acad. Sci. USA, 112: 12145-12150 (2015), incorporated by reference herein). The antibody response to virus-like particles containing TLR9 ligands depends on MYD88 in GC B cells (Hou et al., Immunity, 34: 375-384 (2011), incorporated by reference herein). In a murine model of systemic lupus erythematosus, pathological B cell proliferation depends on endosomal TLR7 and/or TLR9 engaging ligands in complex self-antigens that are internalized by the BCR (Leadbetter et al., Nature, 416: 603-607 (2002), incorporated by reference herein). Finally, MYD88 L265P augments self-antigen-driven proliferation of mouse B cells in a TLR9-dependent fashion, especially in the presence of a Bcl2 transgene (Wang et al., J. Exp. Med., 211: 413-426 (2014), incorporated by reference herein).

[0291] Here, a mechanistic basis is provided for cooperation between TLR9 and the BCR in the pathological survival of ABC DLBCL cells. The ABC lines that depend on the BCR and TLR9 form a multi-protein, My-T-BCR complex on endolysosomal membranes that includes all components known to activate NF- κ B in ABC DLBCL. The My-T-BCR complex provides an unexpected nexus between distinct biochemical mechanisms that activate IKK and trigger classical NF- κ B signaling in lymphoma. BCR signaling has long been known to promote formation of the CBM complex, a large signaling assembly that recruits and activates IKK (Thome et al., Cold Spring Harbor Perspectives in Biology, 2: a003004 (2010), incorporated by reference herein; and Qiao et al., Mol. Cell, 51: 766-779 (2013), incorporated by reference herein). Separately, a variety of innate immune receptors recruit MYD88, allowing it to coordinate a signalosome with IRAK1 and IRAK4 that activates IKK (Lin et al., Nature, 465: 885-890 (2010), incorporated by reference herein). This work shows that these seemingly separate molecular machines coalesce into a signaling complex on the surface of endolysosomal membranes in ABC DLBCL cells. The formation of the My-T-BCR complex depended on both the BCR and TLR9, and was the site of active NF- κ B signaling, as judged by the phosphorylation of IKK and its substrate I κ B α in this complex. Importantly, ibrutinib treatment decreased the recruitment of the CBM components, MYD88 and p-I κ B α into this complex. Interestingly, ibrutinib did not disrupt the IgM-TLR9 interaction, which is shown as a hallmark of a subset of ABC DLBCL cell lines and patient samples. It is speculated that the IgM-TLR9 interaction in endolysosomes may be related to the cell of origin of ABC DLBCL rather than the product of oncogenic signaling.

[0292] In survival assays, ABC lines with My-T-BCR complexes were particularly sensitive to ibrutinib, consistent with ibrutinib-induced reduction in My-T-BCR complexes. All ABC lines with this phenotype had a “double mutant” genotype consisting of the MYD88 L265P mutation in conjunction with an activating mutation targeting CD79B or CD79A. In a recent clinical trial, such double mutant tumors had an 80% response rate to ibrutinib compared to only 30% in other ABC DLBCL tumors (Wilson et al., *Nat. Med.*, 21: 922-926 (2015), incorporated by reference herein). Analysis of biopsies from patients on this trial revealed that tumors that responded to ibrutinib had significantly more My-T-BCR complexes than tumors that progressed on treatment. Among nodal, systemic ABC DLBCL tumors, the double mutant genotype is observed in 10% of cases (Ngo et al., *Nature*, 470: 115-119 (2011), incorporated by reference herein). The prevalence of this genotype is much higher in extranodal tumors with an ABC-like gene expression profile, such as primary central nervous system lymphoma (PCNSL), in which 37% of tumors are double mutant (Kraan et al., *Leukemia*, 28: 719-720 (2014), incorporated by reference herein; Chapuy et al., *Blood*, 127: 869-881 (2016), incorporated by reference herein; and Taniguchi et al., *The American Journal of Surgical Pathology*, 40: 324-334 (2016), incorporated by reference herein). Notably, a recent clinical trial in PCNSL reported that ibrutinib induced objective responses in 94% of cases, consistent with an extreme and uniform dependence on BCR signaling. Thus, we examined PCNSL samples for the presence of the MY-T-BCR and found relatively high expression in contrast to CLL and MCL patient biopsies.

[0293] The distinct modes of BCR signaling revealed by the CRISPR-Cas9 screens – constitutive GC BCR signaling and My-T-BCR-dependent chronic active BCR signaling – will inform the development of therapeutic agents targeting components of the BCR pathway and will help shape the design of precision medicine trials in aggressive lymphomas. Both types of BCR-dependent DLBCLs are sensitive to inhibition of the proximal BCR kinase SYK and the downstream PI3K pathway, but other aspects of proximal BCR signaling differ between these two BCR signaling modes. GCB DLBCLs with constitutive GC BCR signaling are uniquely dependent on LYN, supporting the development of LYN-selective SRC-family kinase inhibitors. Conversely, the phosphatase CD45 emerges as a therapeutic target in ABC but not GCB DLBCL. This work suggests that agents targeting BCR-dependent NF- κ B activity – including inhibitors of BTK, PKC β , PLC γ 2, TAK1 (MAP3K7), and IKK β – could be developed and evaluated in ABC but not GCB DLBCL. These findings suggest that these agents would have their best opportunity to produce therapeutic responses

in tumors with the CD79A/B-MYD88 double mutant genotype because of hyperaddiction to NF- κ B fostered by My-T-BCR complexes.

[0294] In further summary, herein is provided genetic, proteomic and cell biological evidence for a pro-survival signaling hub – termed the My-T-BCR supercomplex – that coordinates NF- κ B activation in DLBCL and identifies tumors that respond to therapeutic inhibition of NF- κ B by ibrutinib. This supercomplex is present in a subset of ABC DLBCL lines and tumors, but is generally absent from GCB DLBCL. Instead, GCB lines are sustained by an alternative “constitutive GC” BCR signaling mode that engages PI3K but not NF- κ B (Fig. 5). The My-T-BCR supercomplex integrates signals from the BCR-dependent CBM complex and MYD88, which were previously thought to be parallel mechanisms to activate NF- κ B in ABC DLBCL. BCR signaling controls CBM complex formation in normal B cells, but these studies demonstrate that TLR9 and MYD88 also influence CBM assembly in ABC DLBCL. Further, it has been proposed that MYD88 mutant isoforms activate NF- κ B by dimerizing spontaneously, but these studies show that TLR9 is required for NF- κ B activation by MYD88 and the BCR. The dependence of ABC DLBCL cells on TLR9 revealed that NF- κ B activation by the My-T-BCR supercomplex occurs on the surface of endolysosomal vesicles. Consequently, the My-T-BCR supercomplex is co-localized with the mTORC1 complex, allowing the proposal that pro-survival, signaling cross-talk between mTOR and NF- κ B takes place at this intracellular location (Fig. 5).

[0295] While the My-T-BCR supercomplex was readily detected in a variety of malignant B cell types, it was not apparent in normal B cells in tonsils or reactive lymph nodes. Nonetheless, this signaling mechanism may play a role in non-transformed B-cells since the BCR is necessary for proliferative responses of normal B cells to TLR ligands. Moreover, in a murine model of systemic lupus erythematosus, B cell proliferation depends on endosomal TLR7 and/or TLR9 recognition of ligands present in complex self-antigens that are internalized by the BCR. Although self-antigens also drive BCR signaling in ABC DLBCL, they have no known role in activating TLR9. Thus, it is not clear whether ligand recognition by TLR9 is required for oncogenic signaling in ABC DLBCL.

[0296] The proteogenomic study revealed a new form of molecular heterogeneity among ABC DLBCL tumors that has implications for precision medicine approaches to these

often recalcitrant cancers. The My-T-BCR supercomplex was a feature of some, but not all, ABC DLBCL tumors. To some extent, this heterogeneity tracks with genetic aberrations. Among ABC cell lines, those with *MYD88*^{L265P} and a *CD79B* or *CD79A* mutation had My-T-BCR supercomplexes and were particularly sensitive to ibrutinib. This double-mutant genotype was previously linked to frequent responses to ibrutinib in a trial of relapsed/refractory DLBCL. However, these results suggest that analysis of the My-T-BCR could provide information regarding oncogenic pathway dependence that is complementary to tumor genetics. Specifically, the My-T-BCR was detected in ibrutinib-responsive cases that lacked the double-mutant genotype, including one case that was wild type for *MYD88*, *CD79B* and *CD79A*. Similarly, the prevalence of My-T-BCR supercomplexes in PCNSL (82% of cases) fits with their high response rate to ibrutinib (80-90%), which can not be explained by the prevalence of the double-mutant genotype (37%). These considerations suggest that the My-T-BCR supercomplex could serve as a biomarker to identify ibrutinib-responsive ABC tumors.

[0297] It was observed that small molecule inhibitors of BTK and mTOR cooperated in decreasing My-T-BCR supercomplex formation, which tracked with the synergism of these drugs in suppressing NF- κ B activation and the growth of ABC lines in vitro and in vivo. Thus, My-T-BCR supercomplex formation could be used to assess the mechanism of action of drug combinations in ABC DLBCL, which could help in prioritizing certain combinations for clinical evaluation.

[0298] All references, including publications, patent applications, and patents, cited herein are hereby incorporated by reference to the same extent as if each reference were individually and specifically indicated to be incorporated by reference and were set forth in its entirety herein.

[0299] The use of the terms “a” and “an” and “the” and “at least one” and similar referents in the context of describing the invention (especially in the context of the following claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. The use of the term “at least one” followed by a list of one or more items (for example, “at least one of A and B”) is to be construed to mean one item selected from the listed items (A or B) or any combination of two or more of the listed items (A and B), unless otherwise indicated herein or clearly contradicted by context. The terms “comprising,” “having,” “including,” and “containing”

are to be construed as open-ended terms (i.e., meaning “including, but not limited to,”) unless otherwise noted. Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., “such as”) provided herein, is intended merely to better illuminate the invention and does not pose a limitation on the scope of the invention unless otherwise claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the invention.

[0300] Preferred embodiments of this invention are described herein, including the best mode known to the inventors for carrying out the invention. Variations of those preferred embodiments may become apparent to those of ordinary skill in the art upon reading the foregoing description. The inventors expect skilled artisans to employ such variations as appropriate, and the inventors intend for the invention to be practiced otherwise than as specifically described herein. Accordingly, this invention includes all modifications and equivalents of the subject matter recited in the claims appended hereto as permitted by applicable law. Moreover, any combination of the above-described elements in all possible variations thereof is encompassed by the invention unless otherwise indicated herein or otherwise clearly contradicted by context.

CLAIMS:

1. A method of detecting an association of a first protein and a second protein, the method comprising:
 - (A) obtaining a sample comprising one or more B cells;
 - (B) contacting the sample with a first antibody and a second antibody, wherein the first antibody binds the first protein and does not bind the second protein and the second antibody binds the second protein and does not bind the first protein, wherein the first antibody is of a species different than the first protein and the second antibody is of a species different than the second protein, and wherein the first antibody is of a species different than the second antibody;
 - (C) performing a proximity ligation assay using the first antibody and the second antibody and a detectable probe to produce a PLA signal of one or more detectable puncta;
 - (D) contacting the sample with a third antibody, wherein the third antibody binds to a protein on the B cell surface, and wherein the third antibody is labeled with a detectable label; and
 - (E) detecting the proximity ligation assay signal of (C) wherein a positive PLA signal indicates association of the first and second proteins in one or more of the B cells comprised in the sample.
2. The method of claim 1, wherein a proximity ligation assay signal of fluorescent puncta indicates association of the first and second proteins.
3. The method of claim 1 or 2, wherein the third antibody is fluorescently labeled.
4. The method of any one of claims 1-3, wherein the first antibody and the second antibody each bind to a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex.
5. A method of identifying a B cell disorder having a greater probability of responding to treatment using an inhibitor, the method comprising:
 - (a) obtaining a B cell disorder sample;

(b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex,

wherein the two proteins are different from one another and are components of different complexes,

and wherein the analysis comprises detecting an association of the two proteins according to any one of claims 1-4; and

(c) identifying the sample having the presence of the protein complex of (b) as a B cell disorder having a greater probability of responding to treatment using an inhibitor of the BCR complex, an inhibitor of the MYD88 complex, or an inhibitor of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b).

6. A method of identifying a B cell disorder having a greater probability of responding to treatment using an inhibitor, the method comprising:

(a) obtaining a B cell disorder sample;

(b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex,

wherein the two proteins are different from one another and are components of different complexes;

(b') detecting in the sample the presence of the protein complex of (b); and

(c) identifying the sample having the presence of the protein complex of (b) as a B cell disorder having a greater probability of responding to treatment using an inhibitor of the BCR complex, an inhibitor of the MYD88 complex, or an inhibitor of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b).

7. The method of claim 5 or 6, wherein the sample is identified as a B cell disorder having a greater probability of responding to treatment

(i) using an inhibitor of the BCR complex, when the protein complex of

(b) includes a protein component of the BCR complex,

(ii) using an inhibitor of the MYD88 complex, when the protein complex of (b) includes a protein component of the MYD88 complex, or

(iii) using an inhibitor of the mTORC1 complex, when the protein complex of (b) includes a protein component of the mTORC1 complex,

as compared to a sample having an absence of the protein complex of (b).

8. The method of any one of claims 5-7, wherein the sample is identified as a B cell disorder having a greater probability of responding to treatment

(i) using an inhibitor of the BCR complex, when the protein complex of (b) includes a protein component of the BCR complex,

(ii) using an inhibitor of the MYD88 complex, when the protein complex of (b) includes a protein component of the MYD88 complex, and

(iii) using an inhibitor of the mTORC1 complex, when the protein complex of (b) includes a protein component of the mTORC1 complex,

as compared to a sample having an absence of the protein complex of (b).

9. The method of any one of claims 5-8, wherein the B cell disorder is a lymphoma.

10. The method of claim 9, wherein the lymphoma is a diffuse large B cell lymphoma (DLBCL), a primary central nervous system lymphoma (PCNSL), or a Waldenström macroglobulinemia/ lymphoplasmacytic lymphoma (WM/LPL).

11. The method of claim 10, wherein the lymphoma is a DLBCL and is an activated B-cell-like (ABC) DLBCL.

12. The method of any one of claims 5-11, wherein the protein complex comprises the proteins of BCR, MYD88, and mTOR.

13. The method of any one of claims 5-12, wherein the B cell disorder sample is a formalin-fixed and paraffin-embedded (FFPE) sample.

14. The method of any one of claims 5-13, wherein the inhibitor is OSI-027, AZD-8055, AZD,2014, SIROLIMUS, BEZ-235, everolimus, or ibrutinib.
15. The method of any one of claims 5-14, wherein the inhibitor is ibrutinib.
16. An inhibitor of the BCR complex, MYD88 complex, or mTORC1 complex for use in treating, in a subject in need thereof, a B cell disorder having a greater probability of responding to treatment using an inhibitor, wherein the B cell disorder is identified as a B cell disorder having a greater probability of responding to treatment using an inhibitor according to the method of any one of claims 5-15.

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Figure 1A

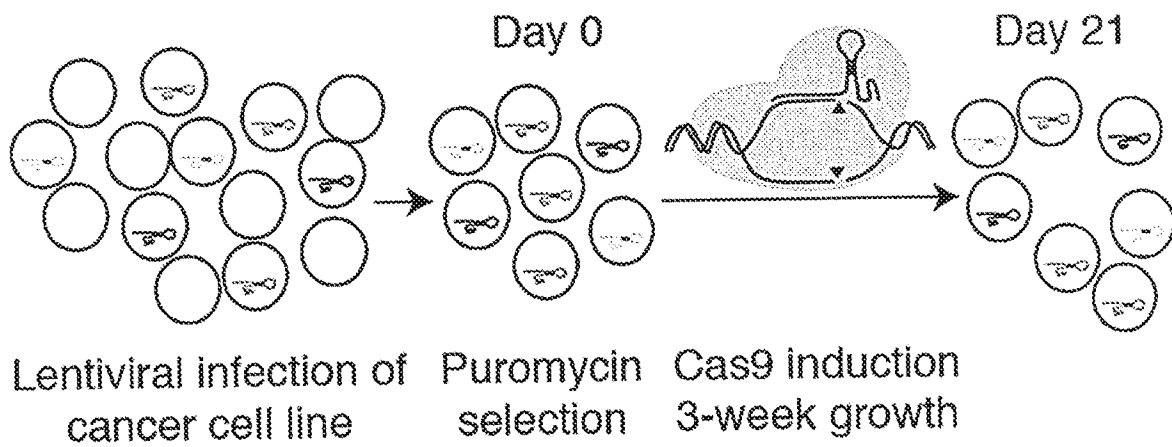


Figure 1B

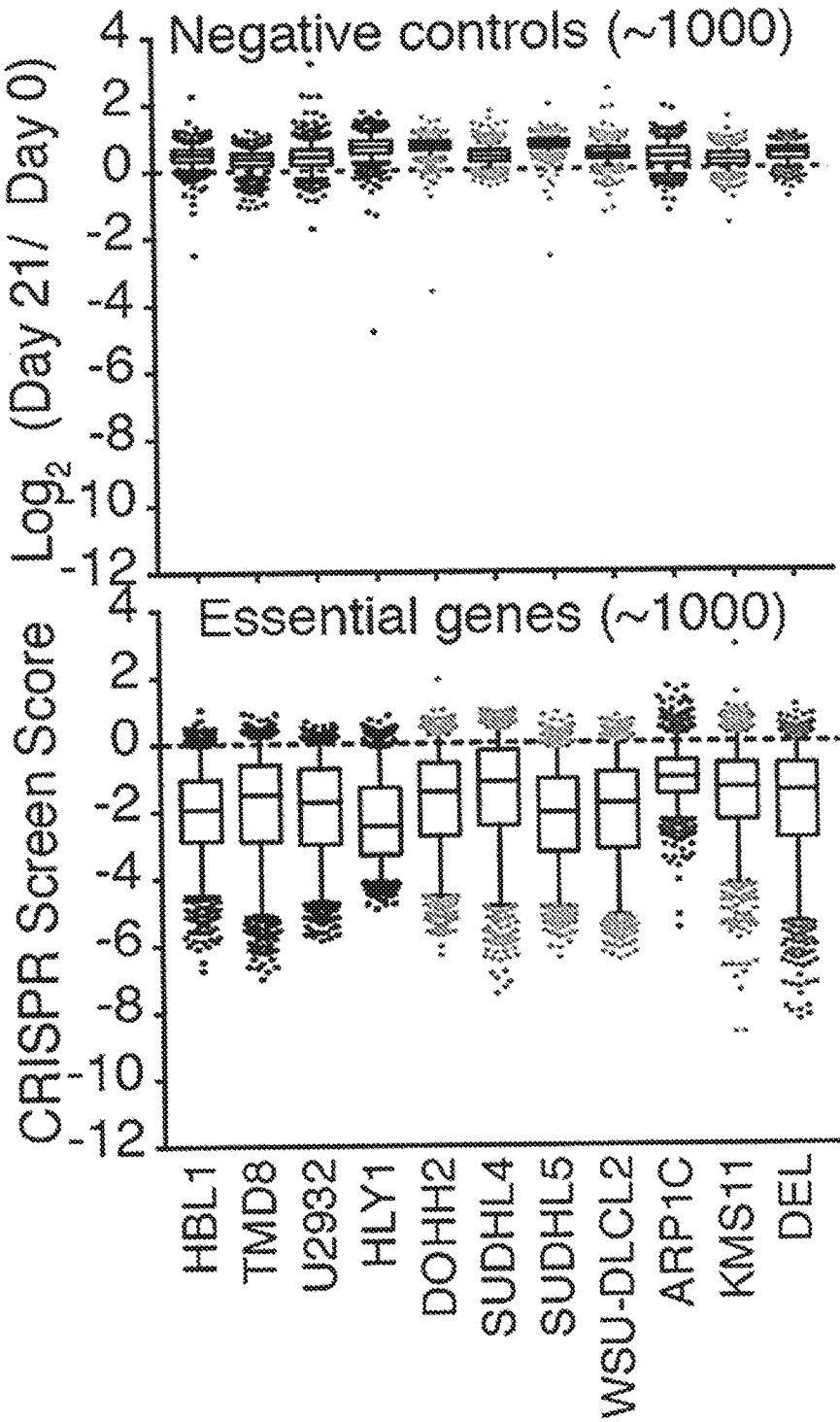


Figure 1C

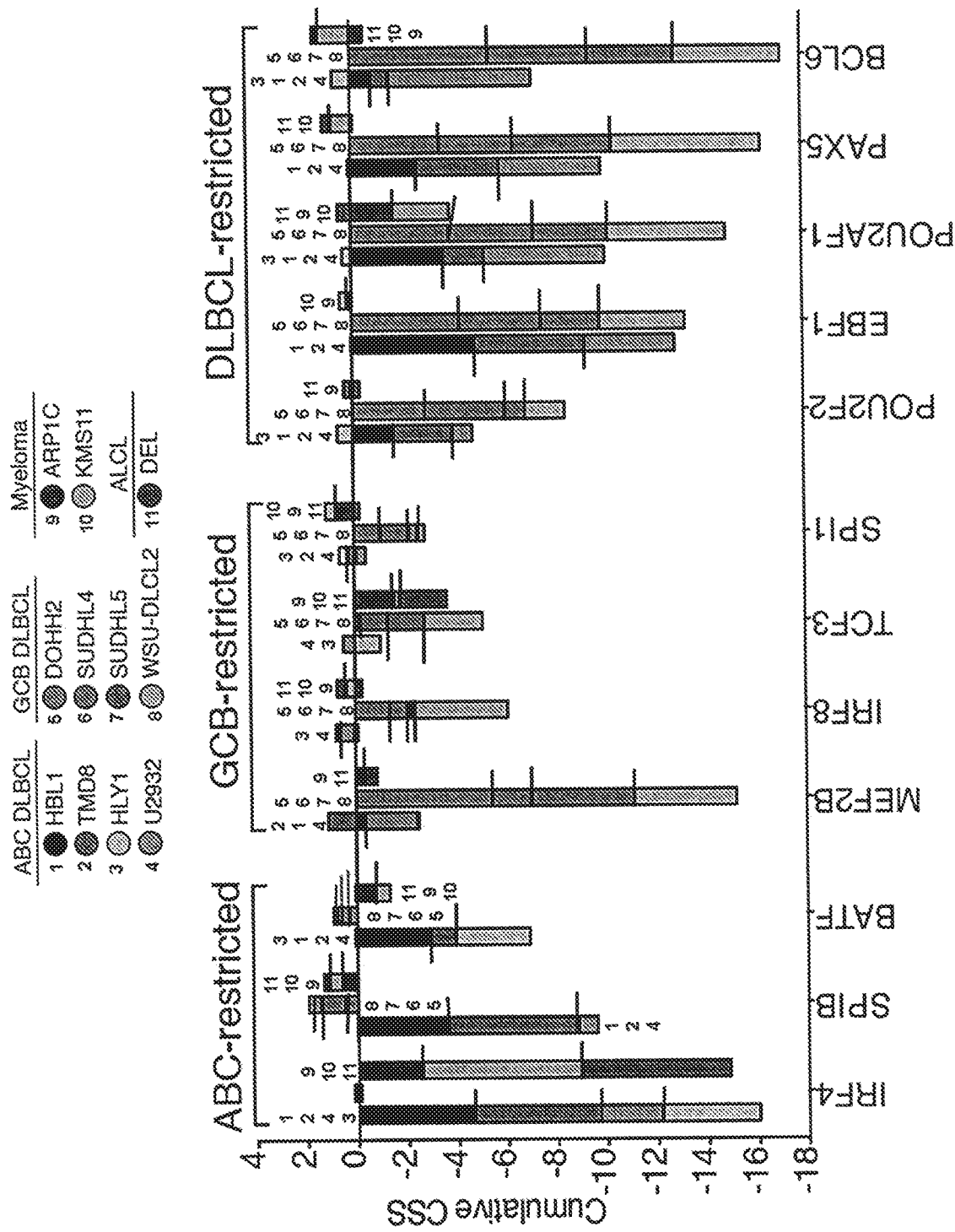
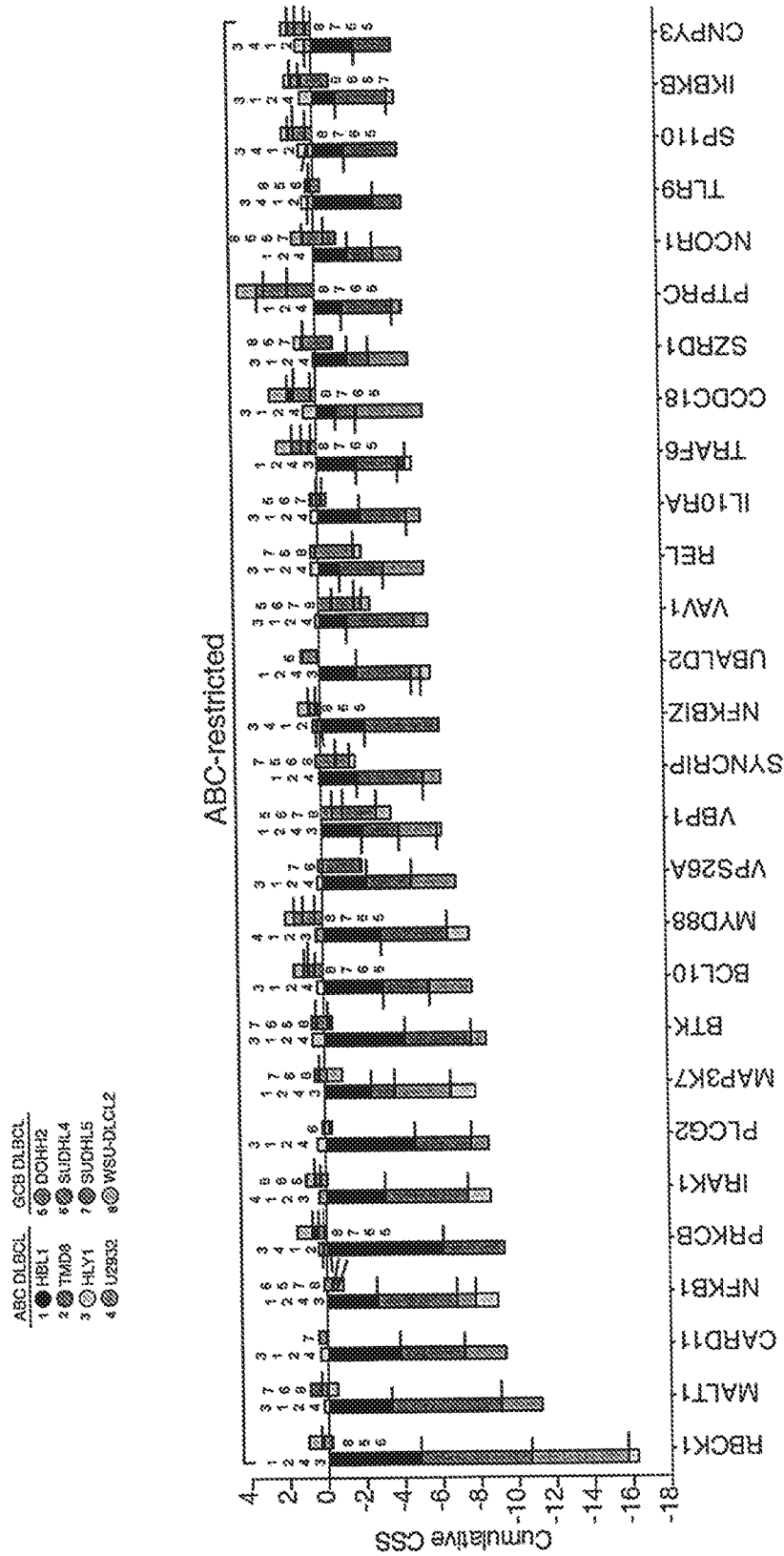
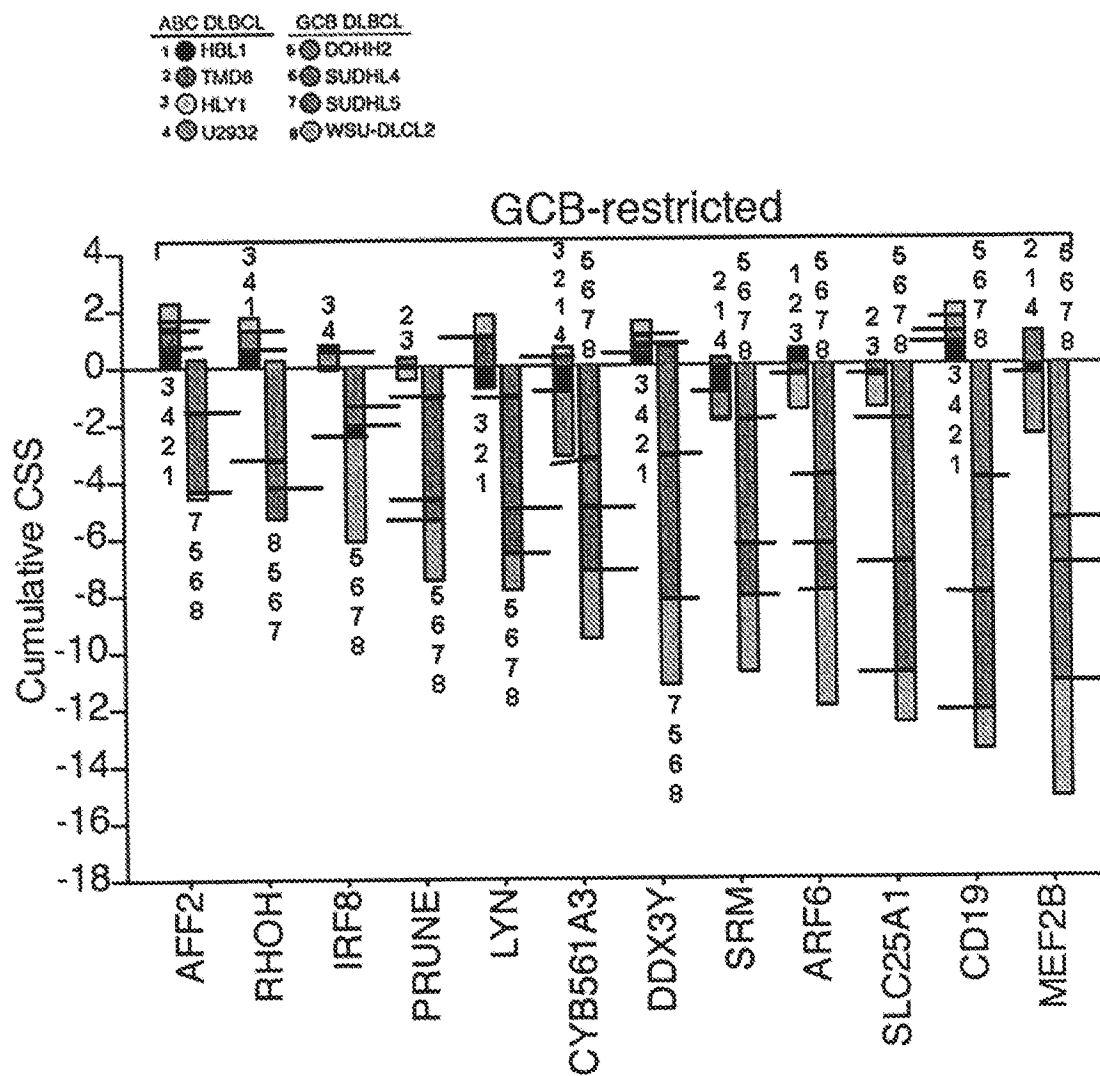


Figure 1D



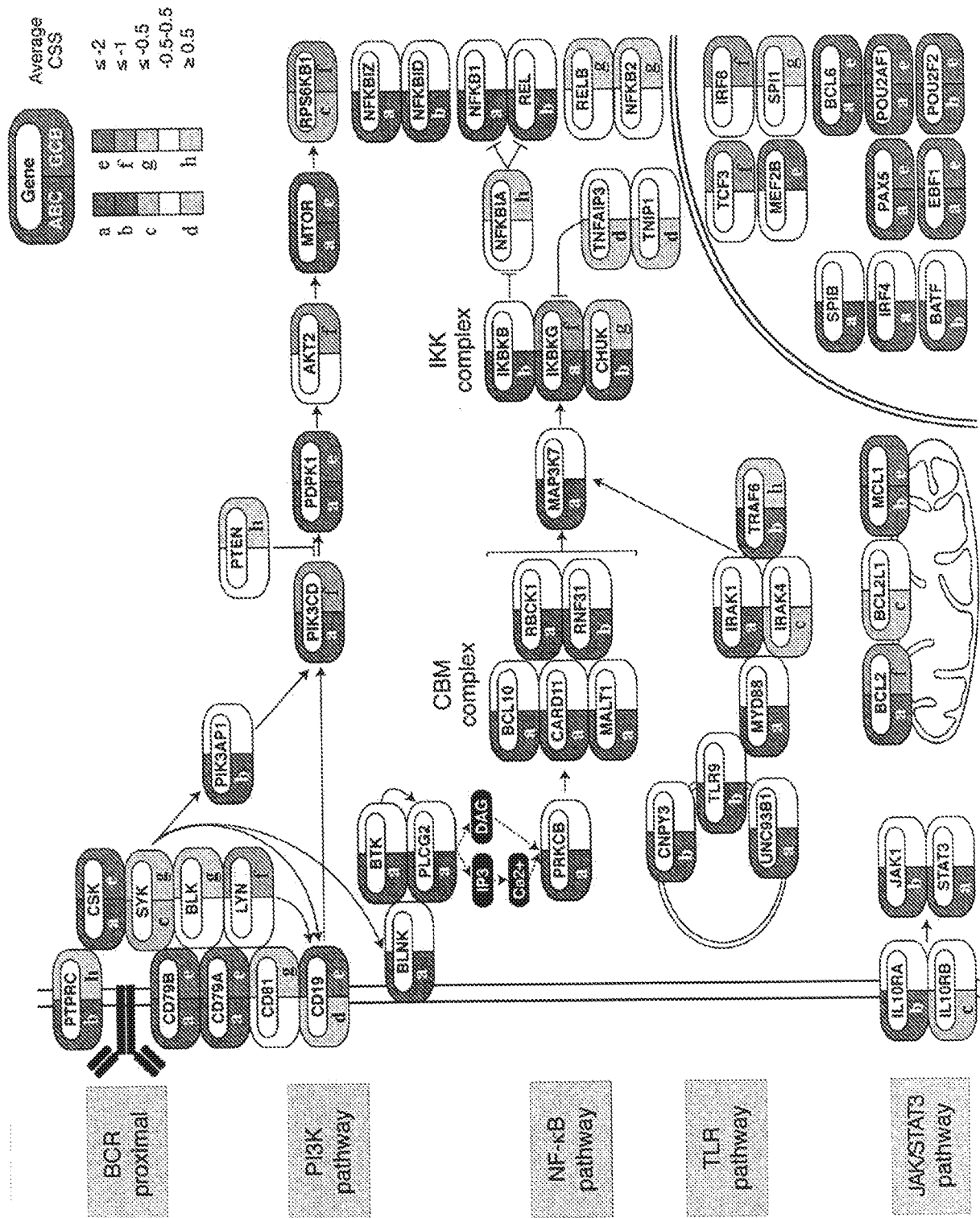
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Figure 1E



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Figure 1F



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Figure 2A

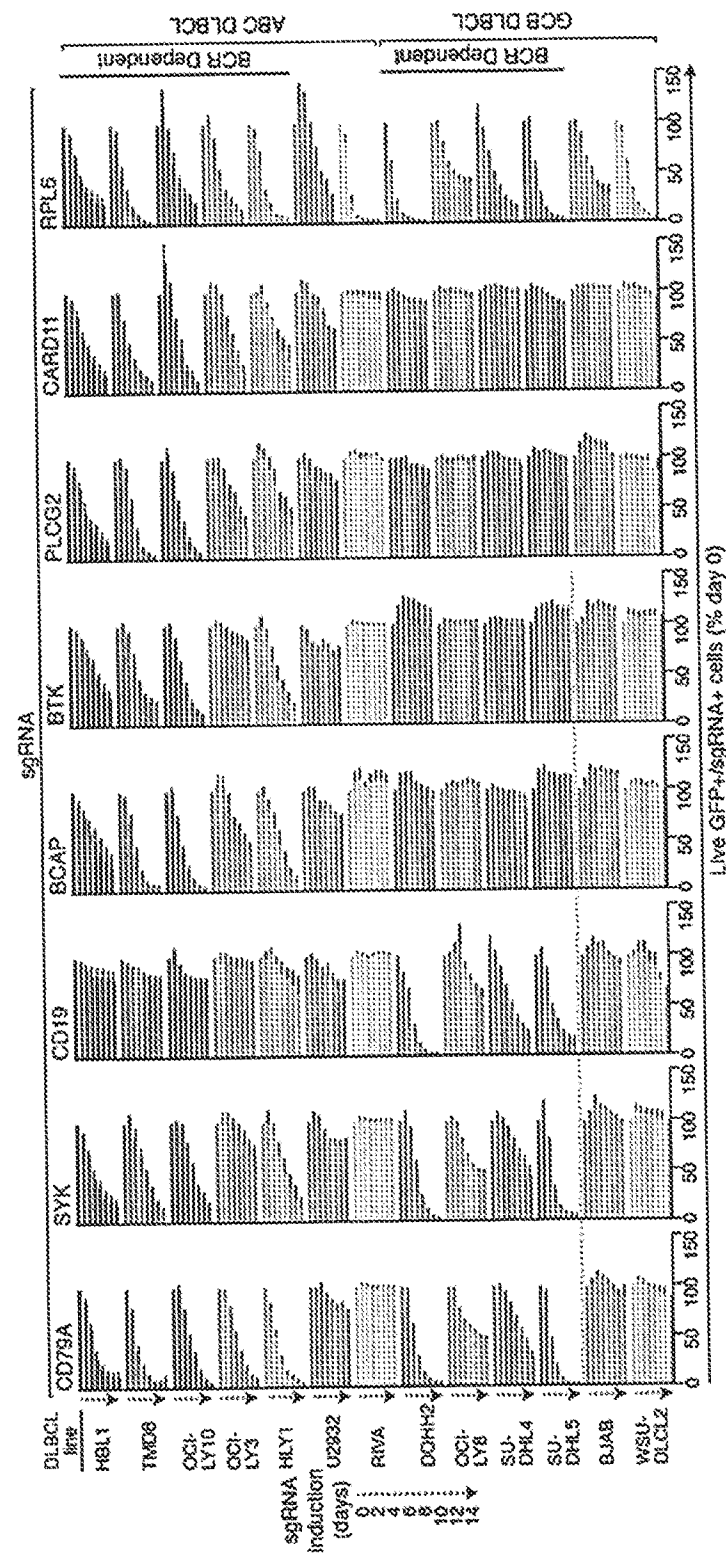
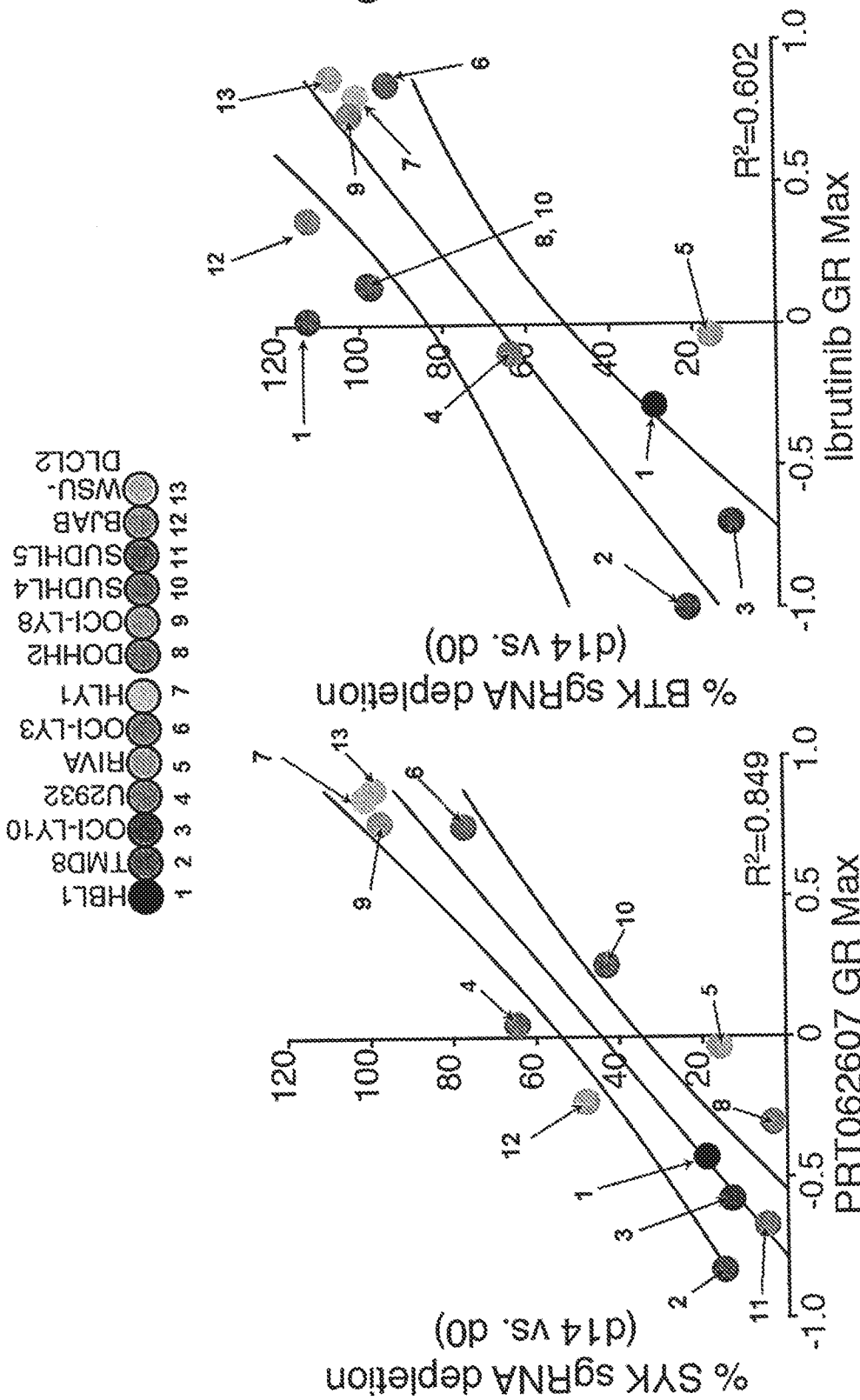
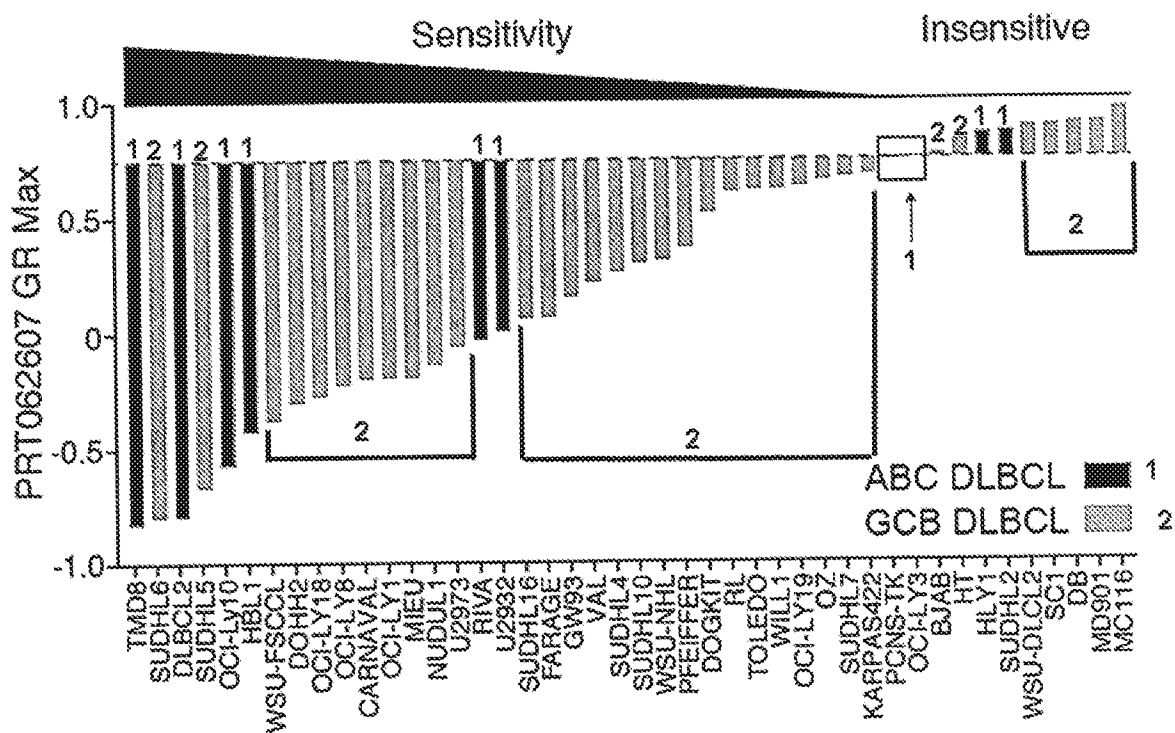


Figure 2B



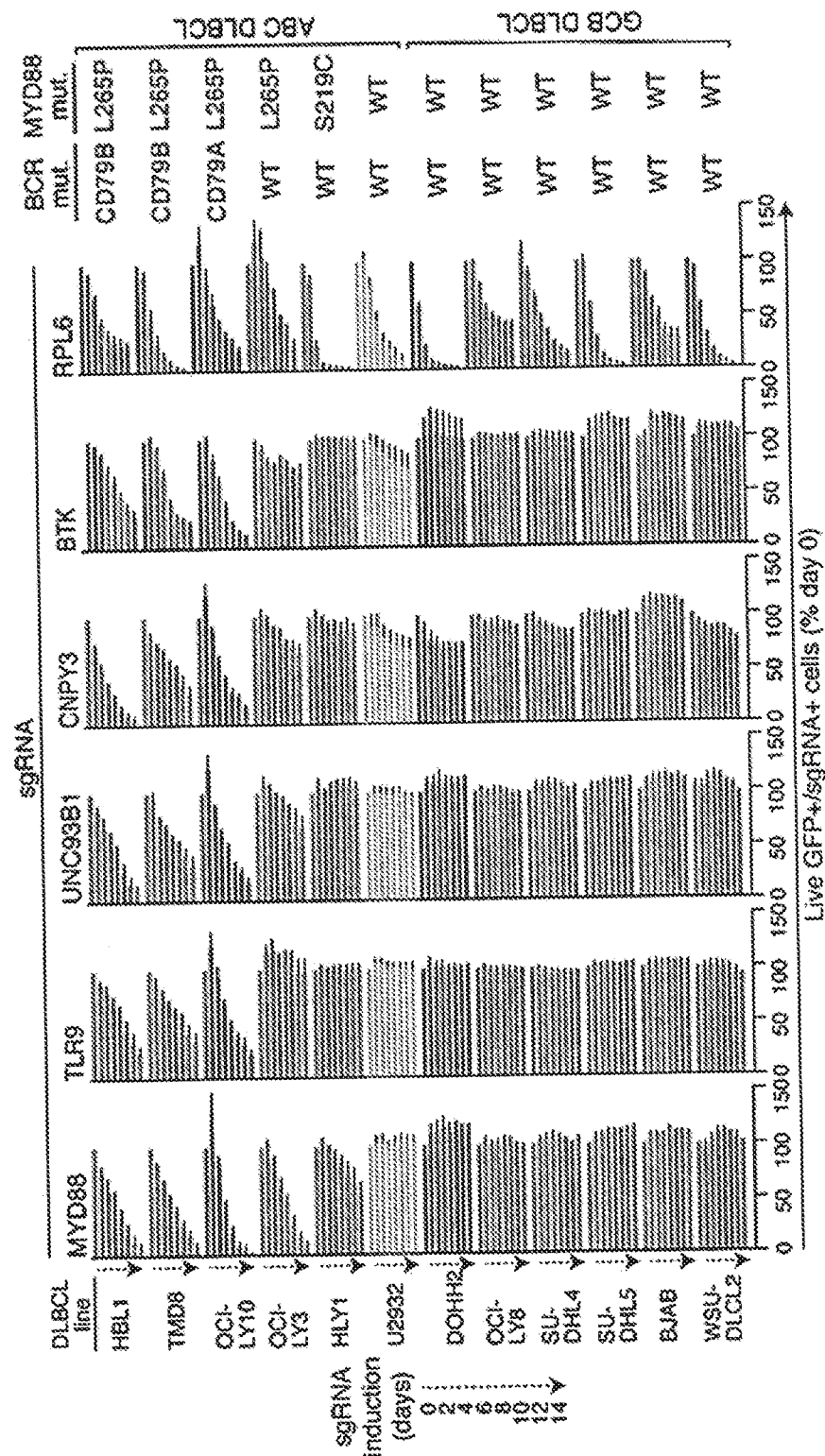
9/82

Figure 2C



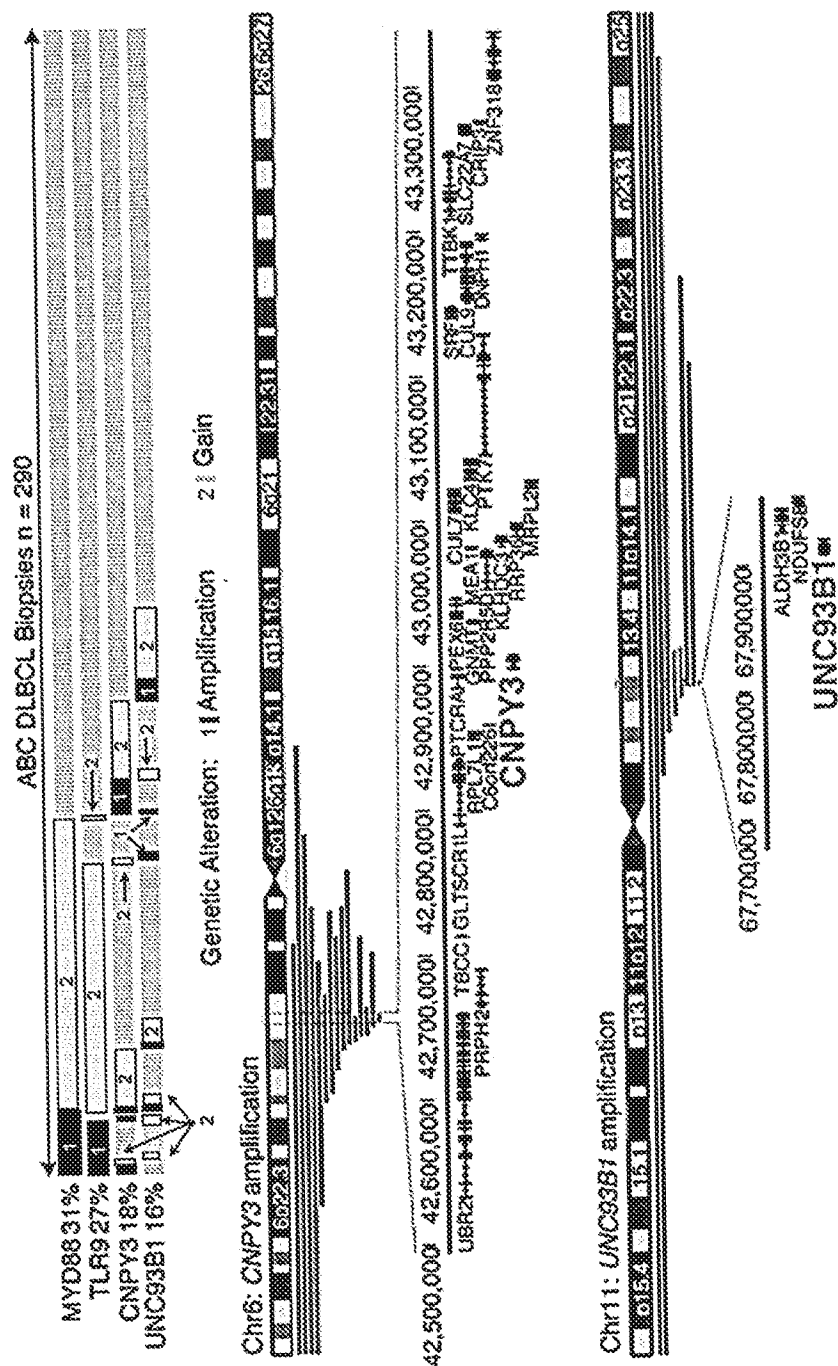
10/82

Figure 3A



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Figure 3B



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Figure 3C

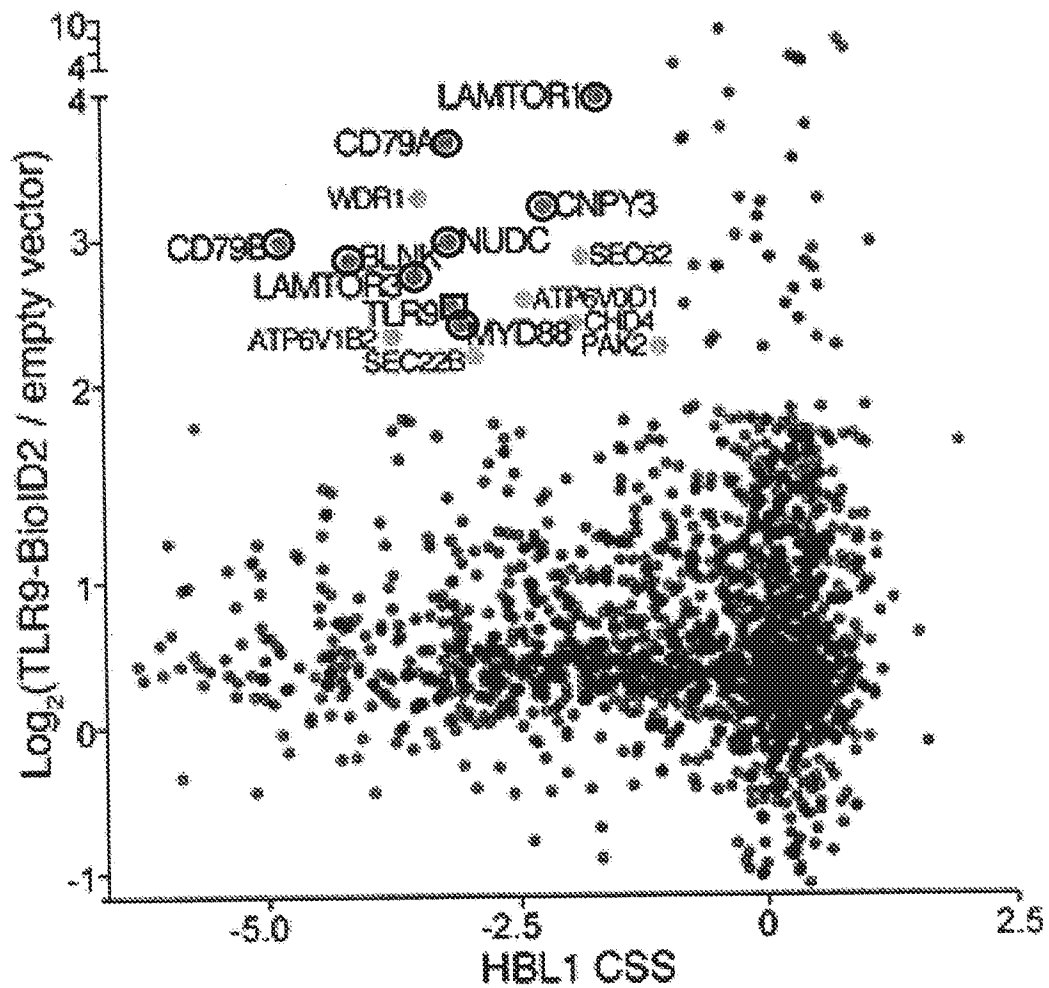


Figure 3D

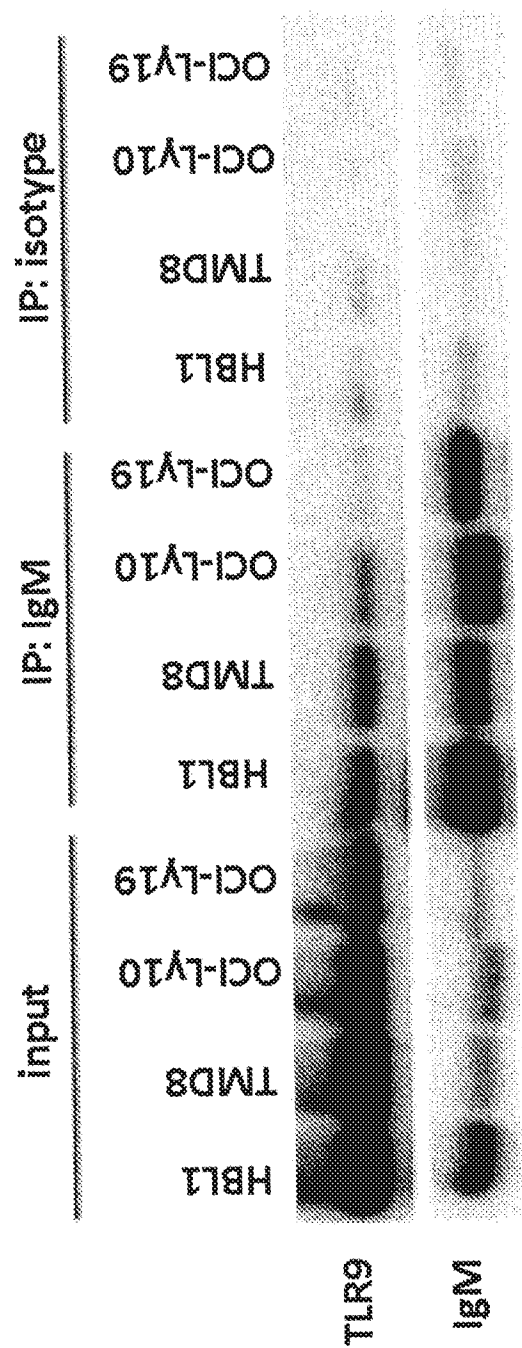
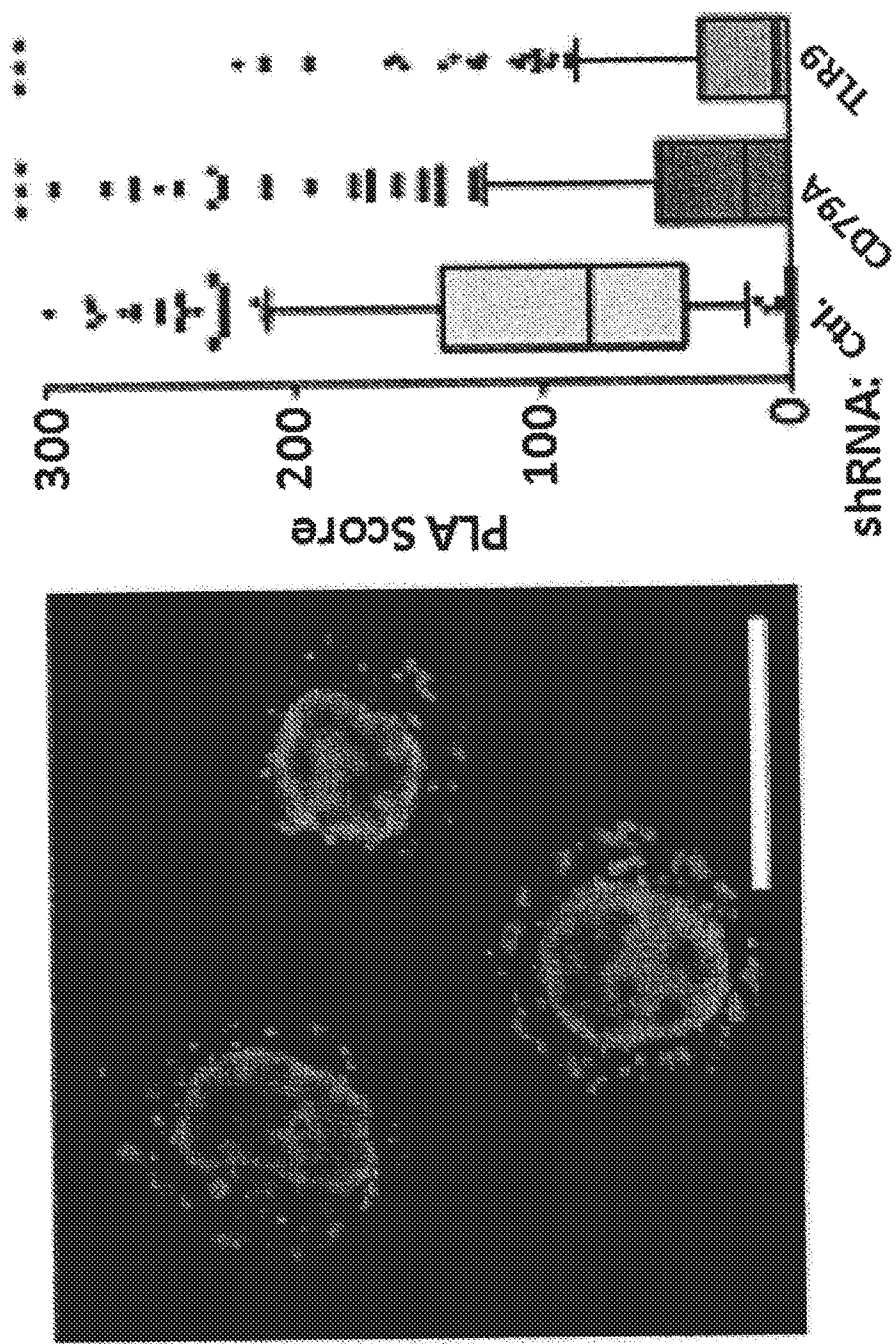
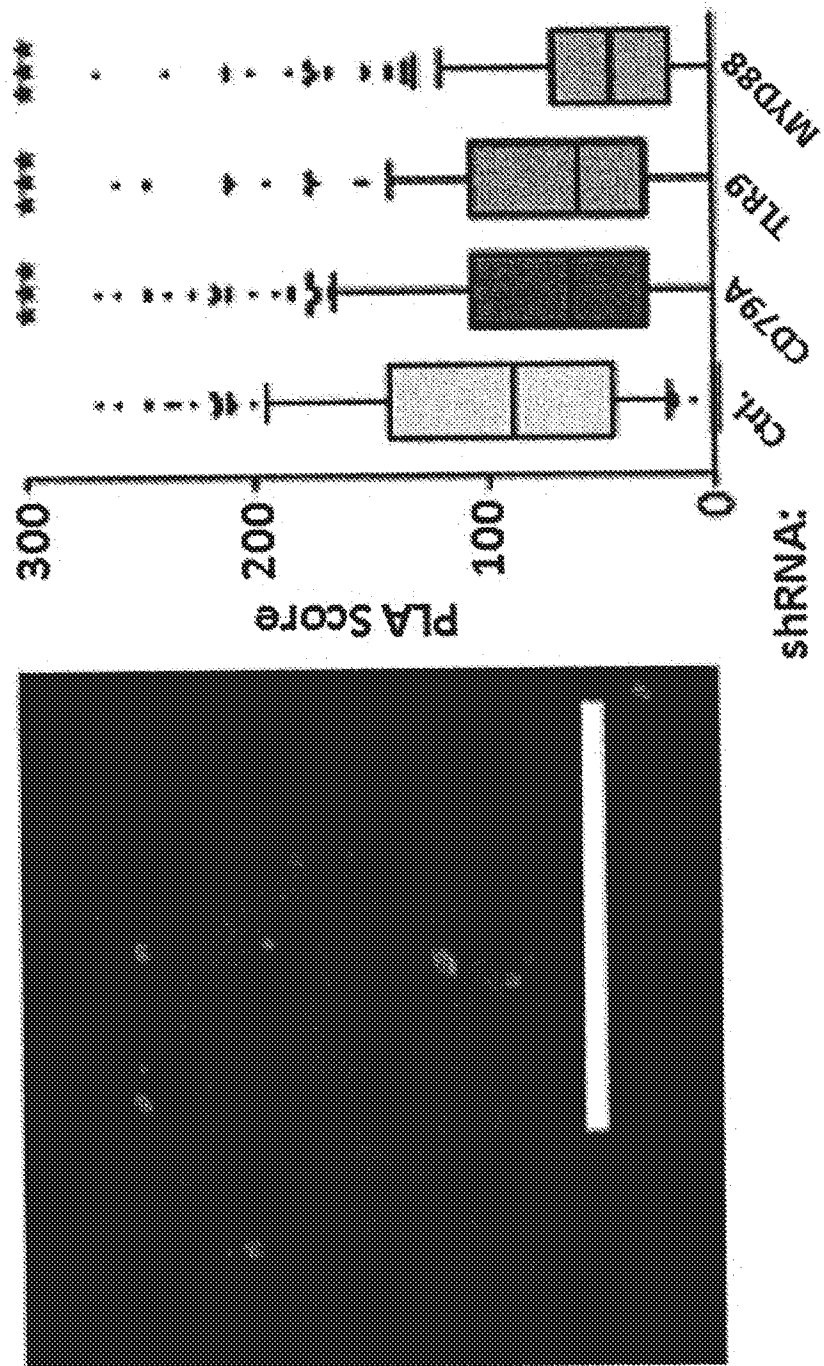


Figure 3E



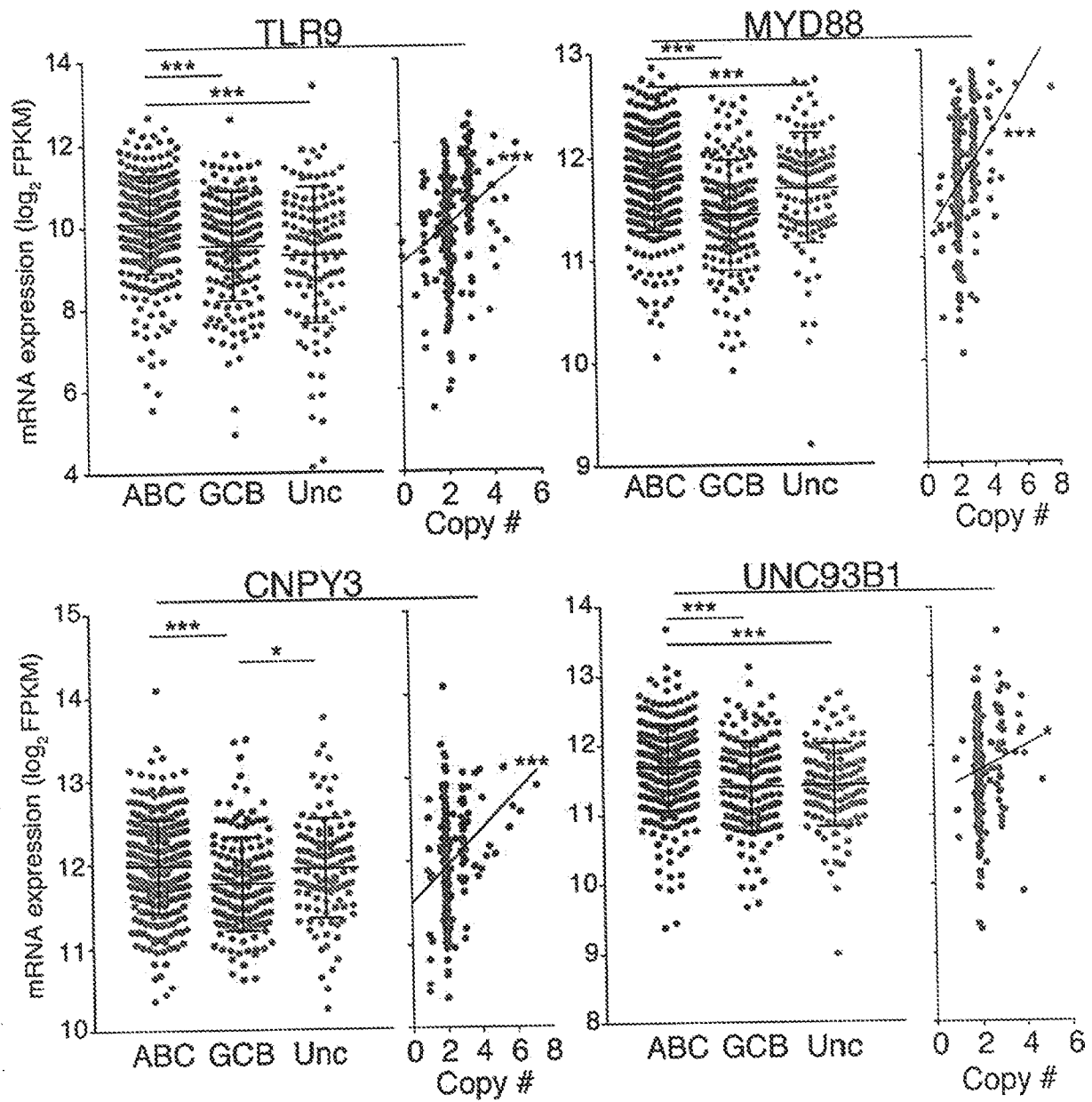
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Figure 3F



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Figure 4



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Figure 5

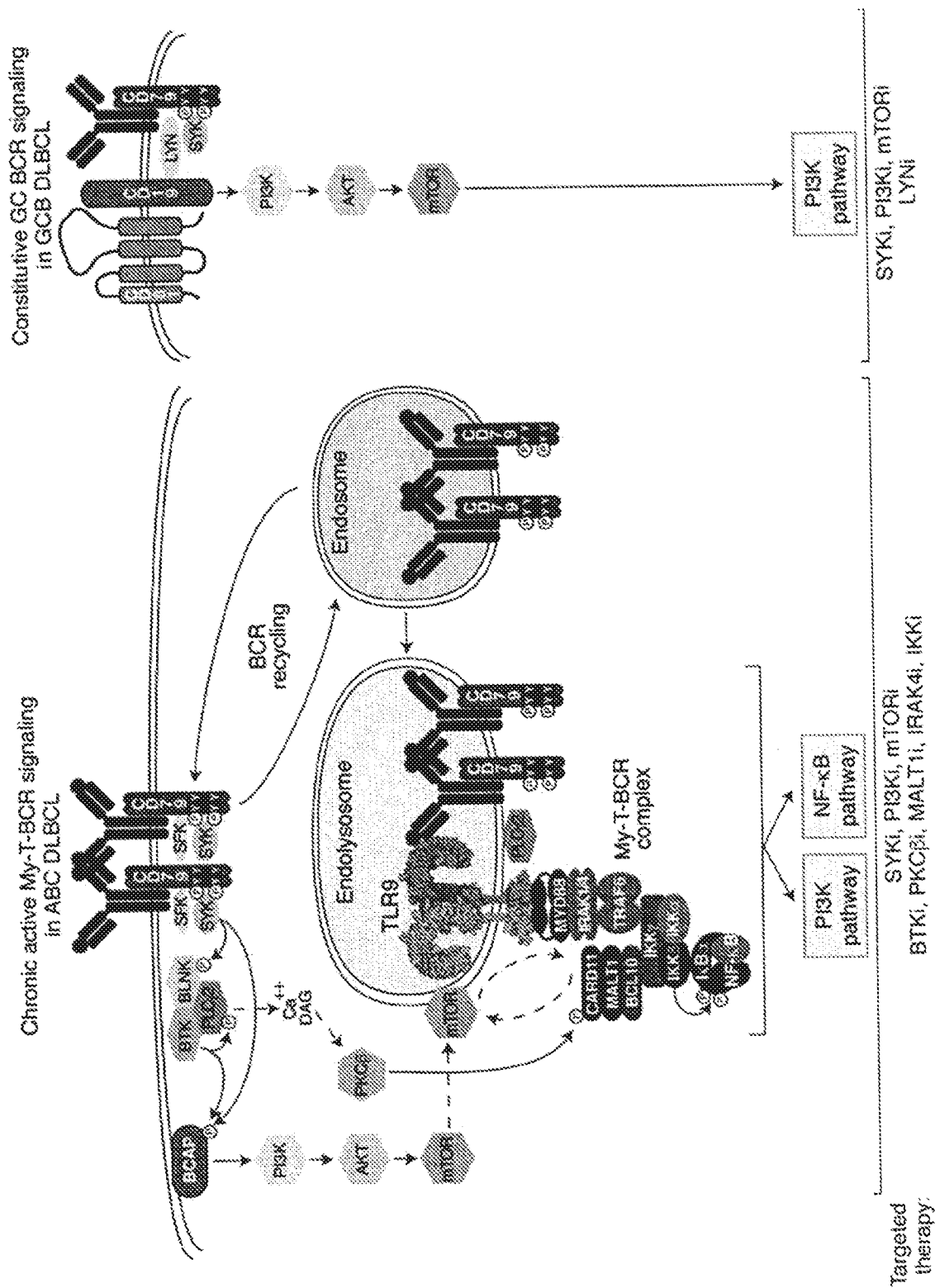
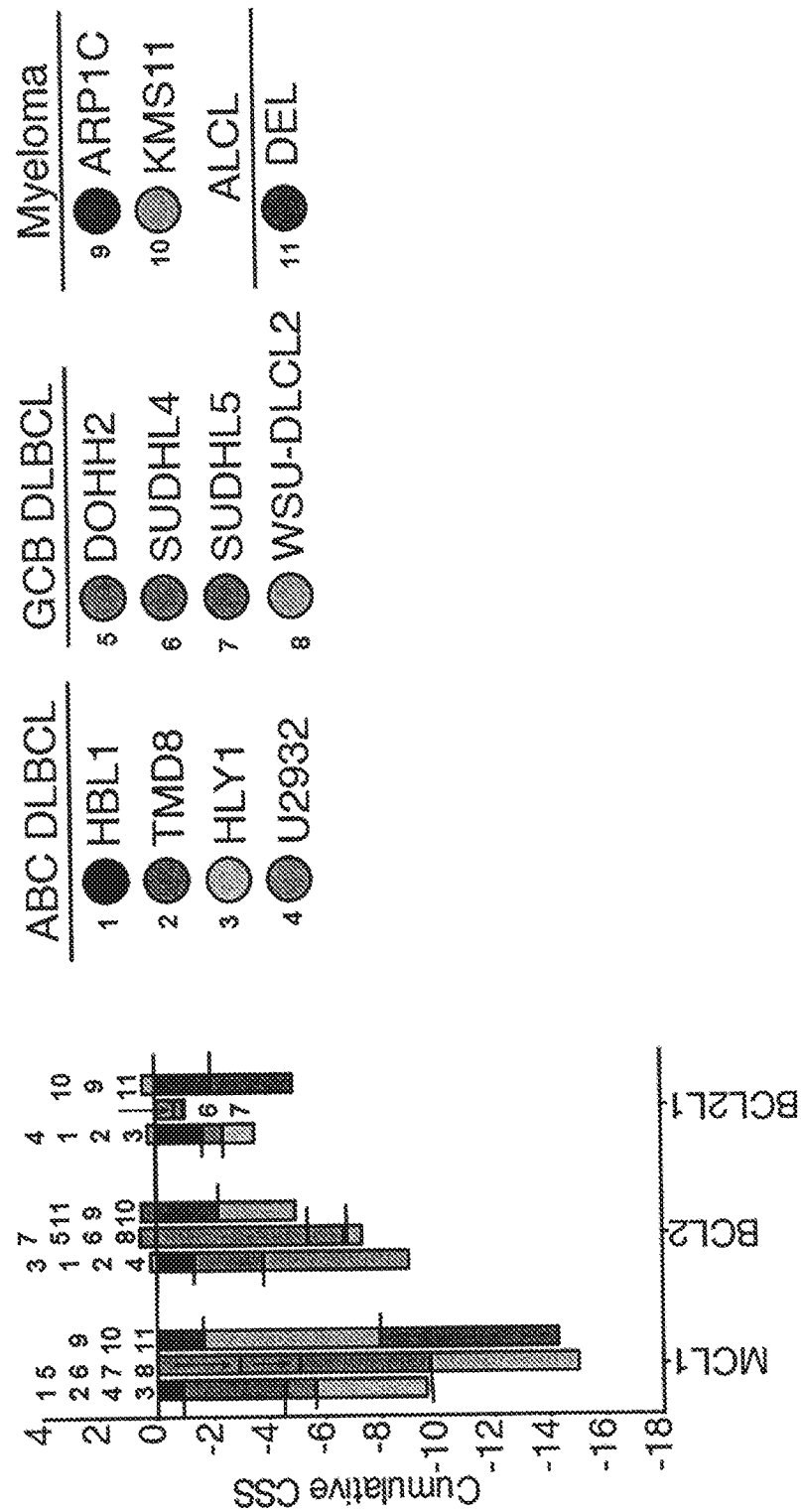
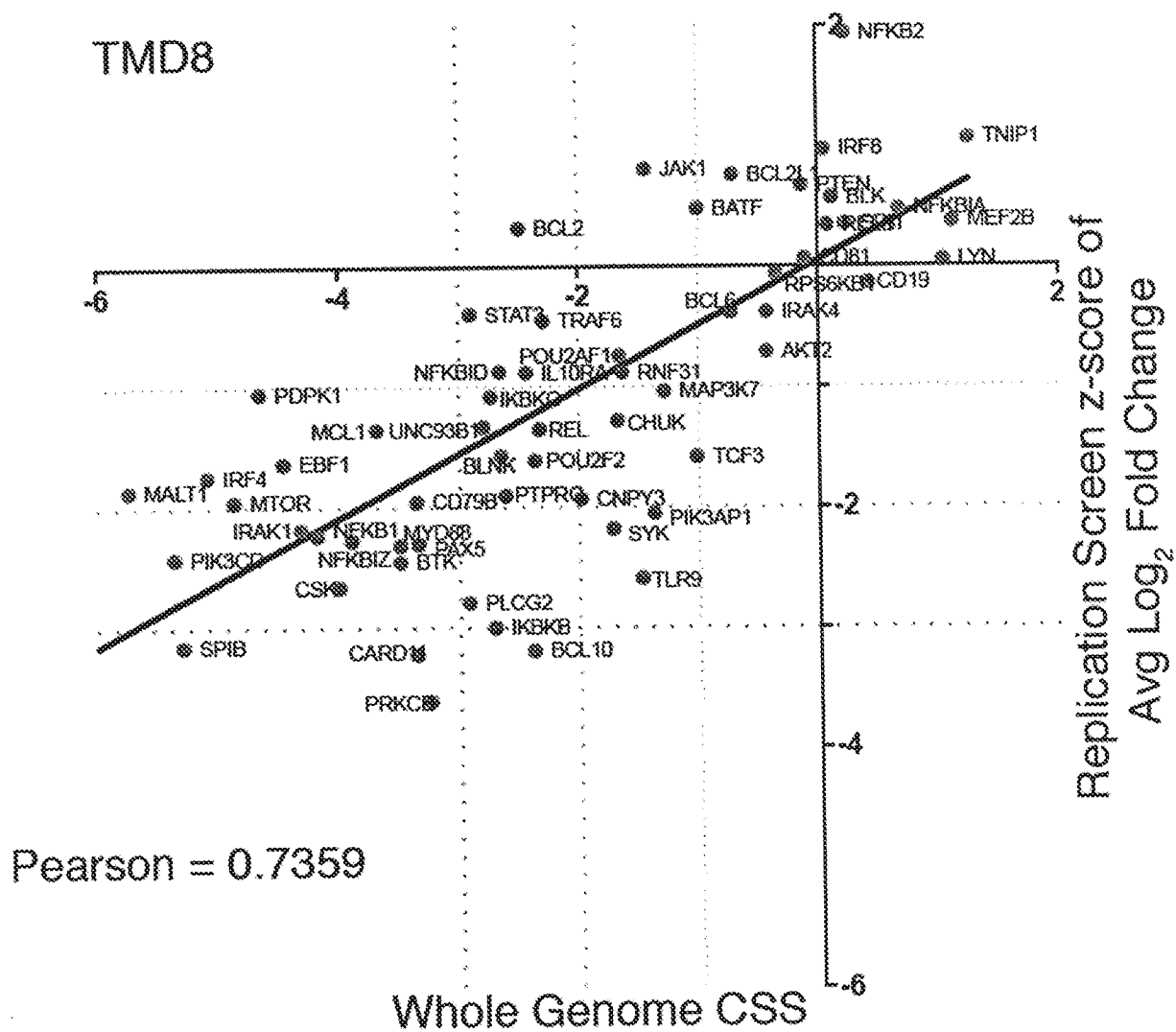


Figure 6



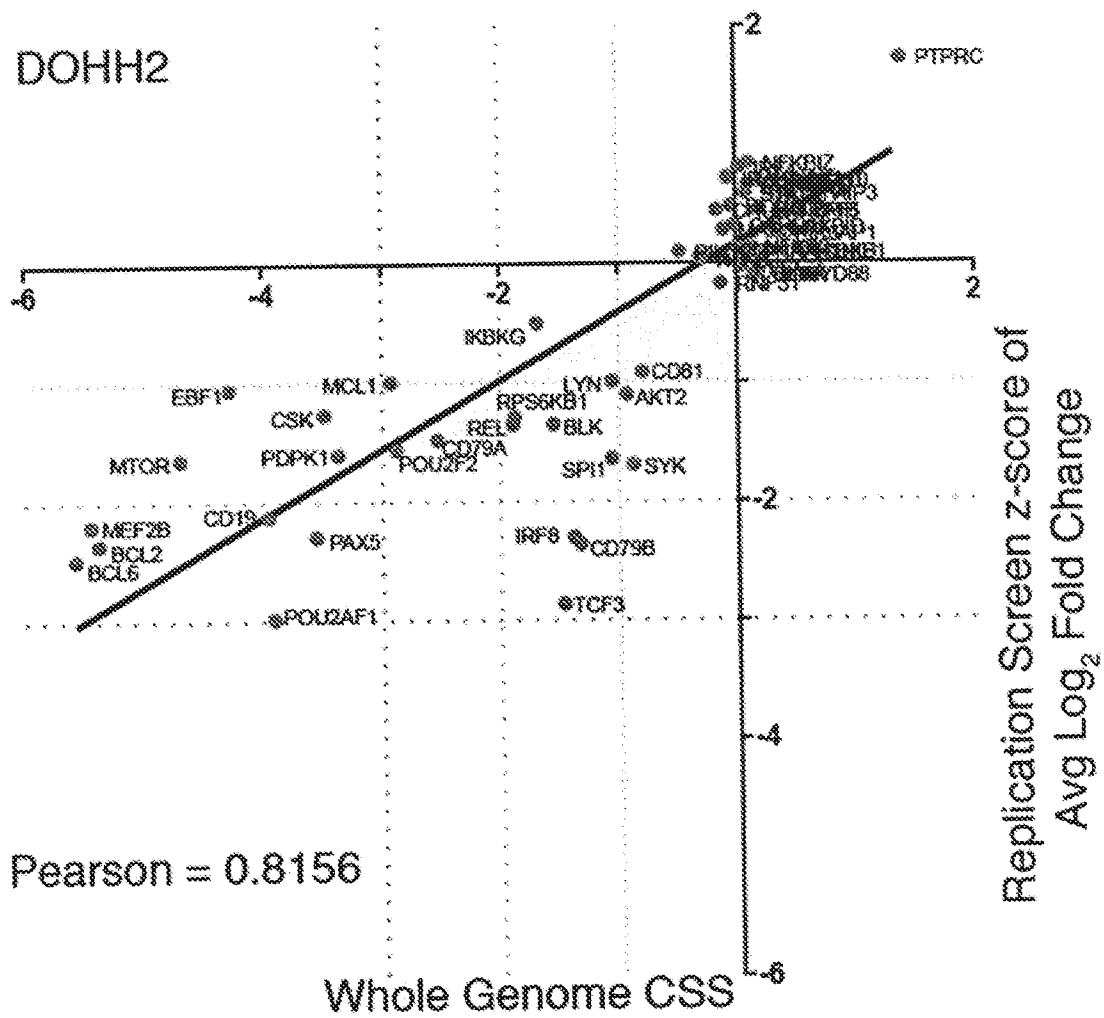
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Figure 7A



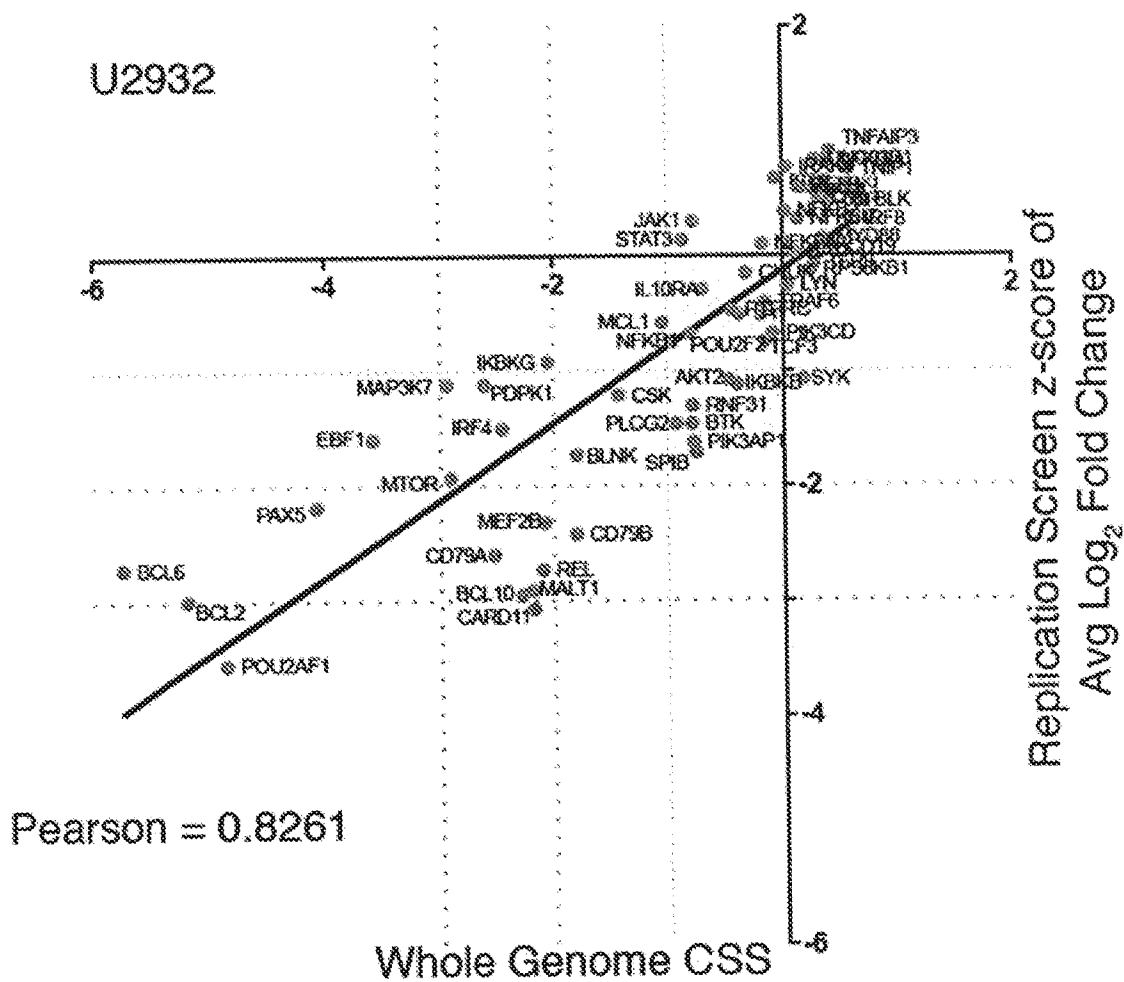
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Figure 7B



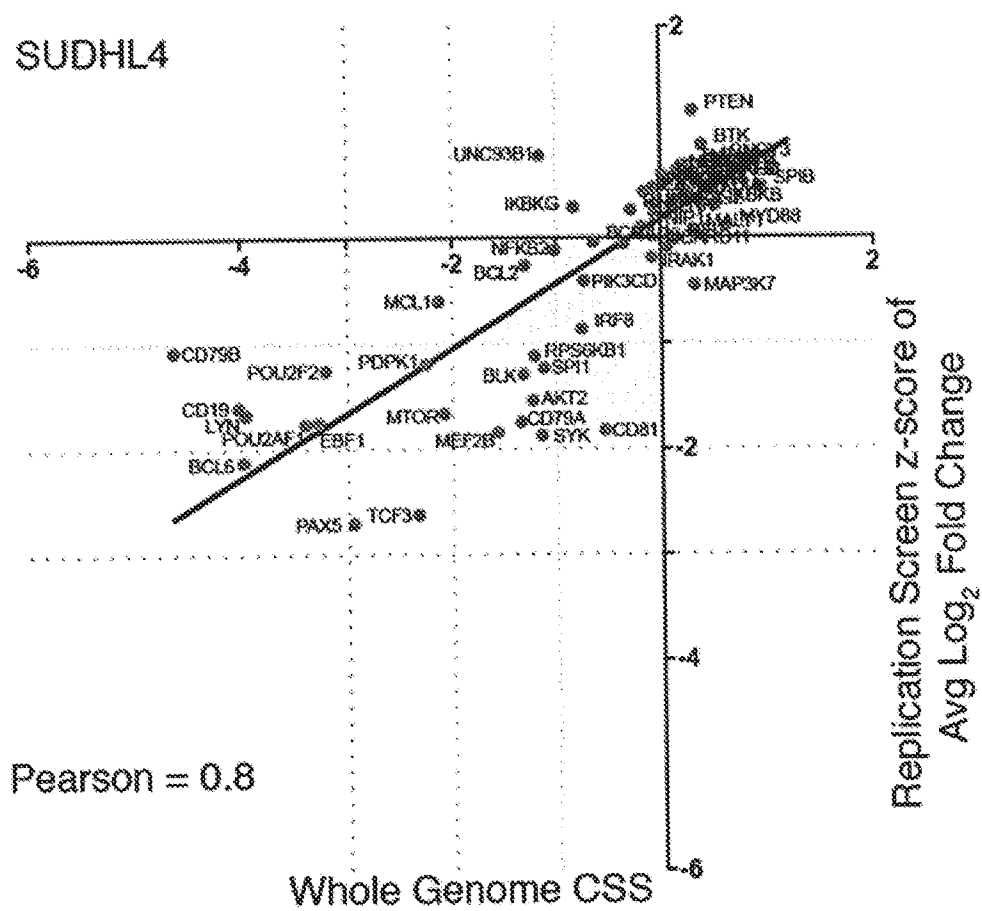
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Figure 7C



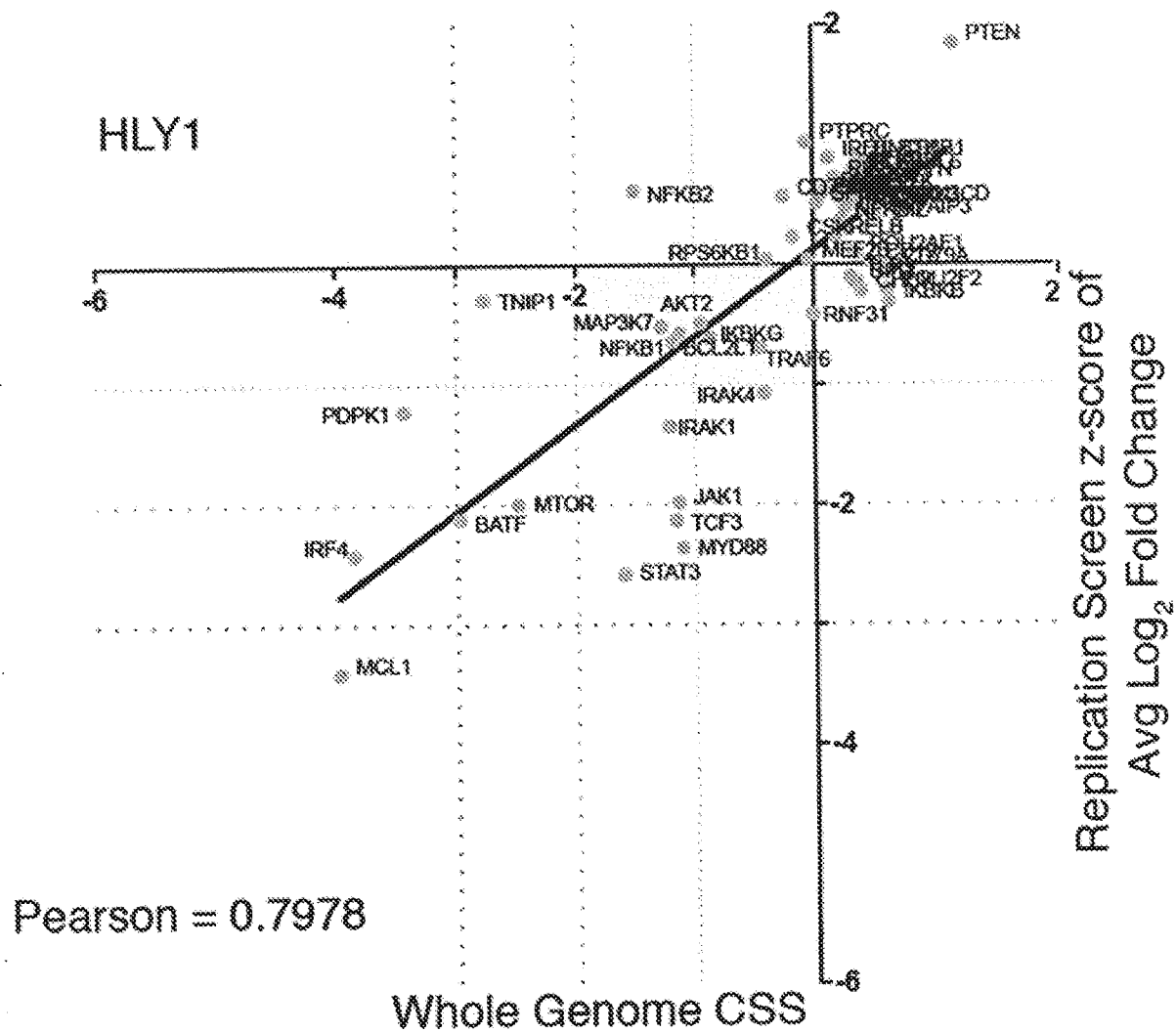
22/82

Figure 7D



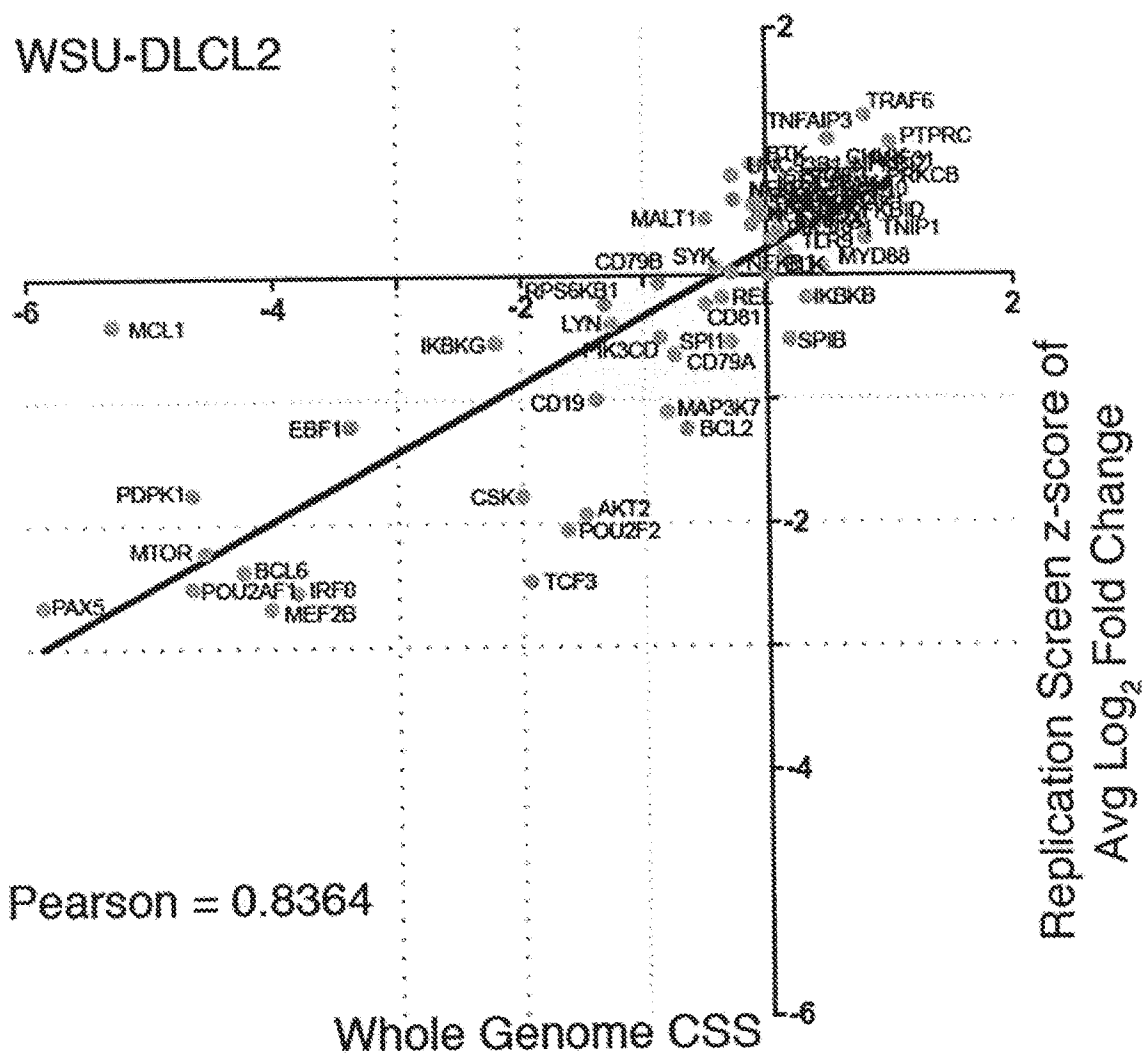
23/82

Figure 7E



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Figure 7F



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Figure 7G

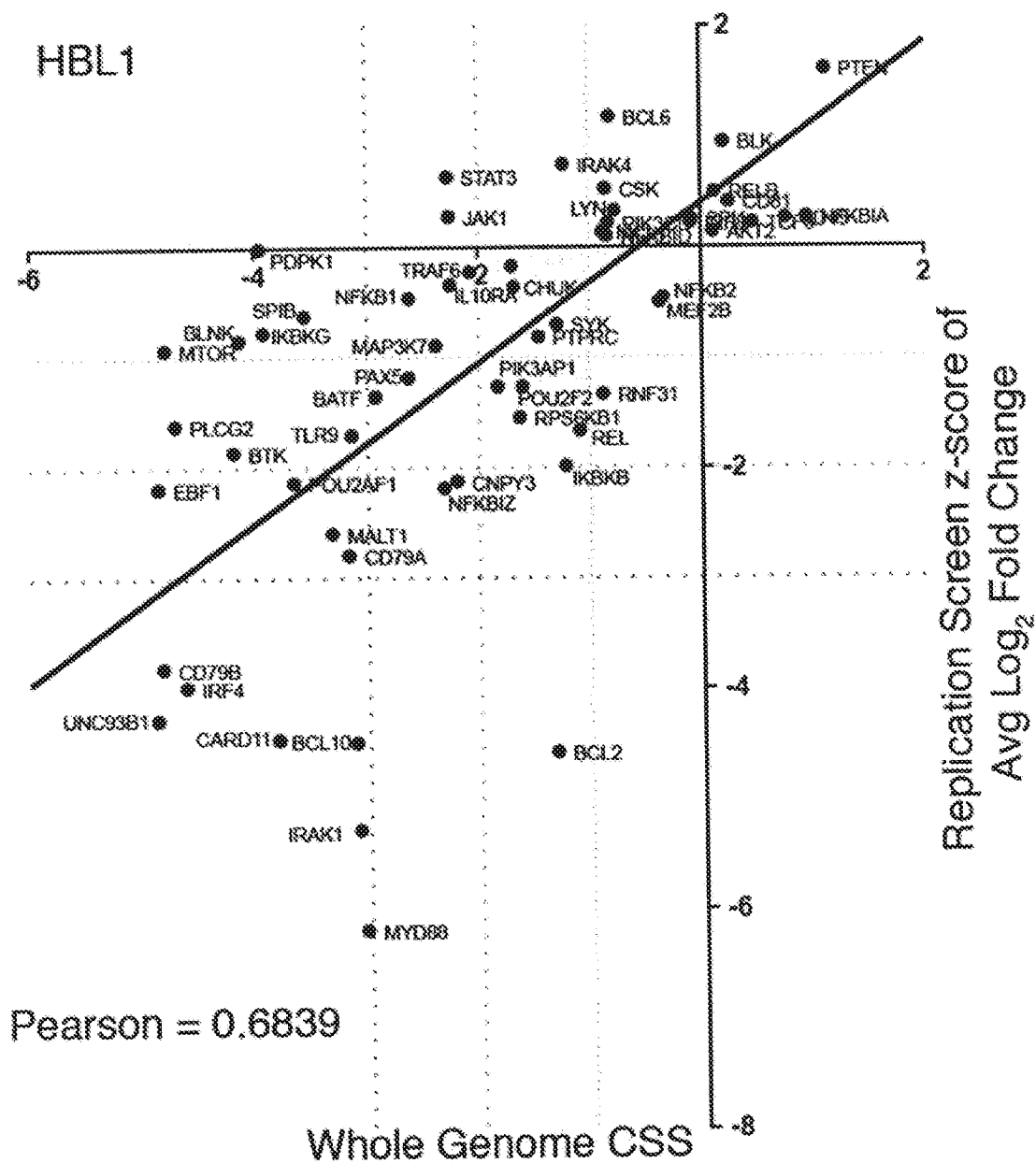


Figure 8

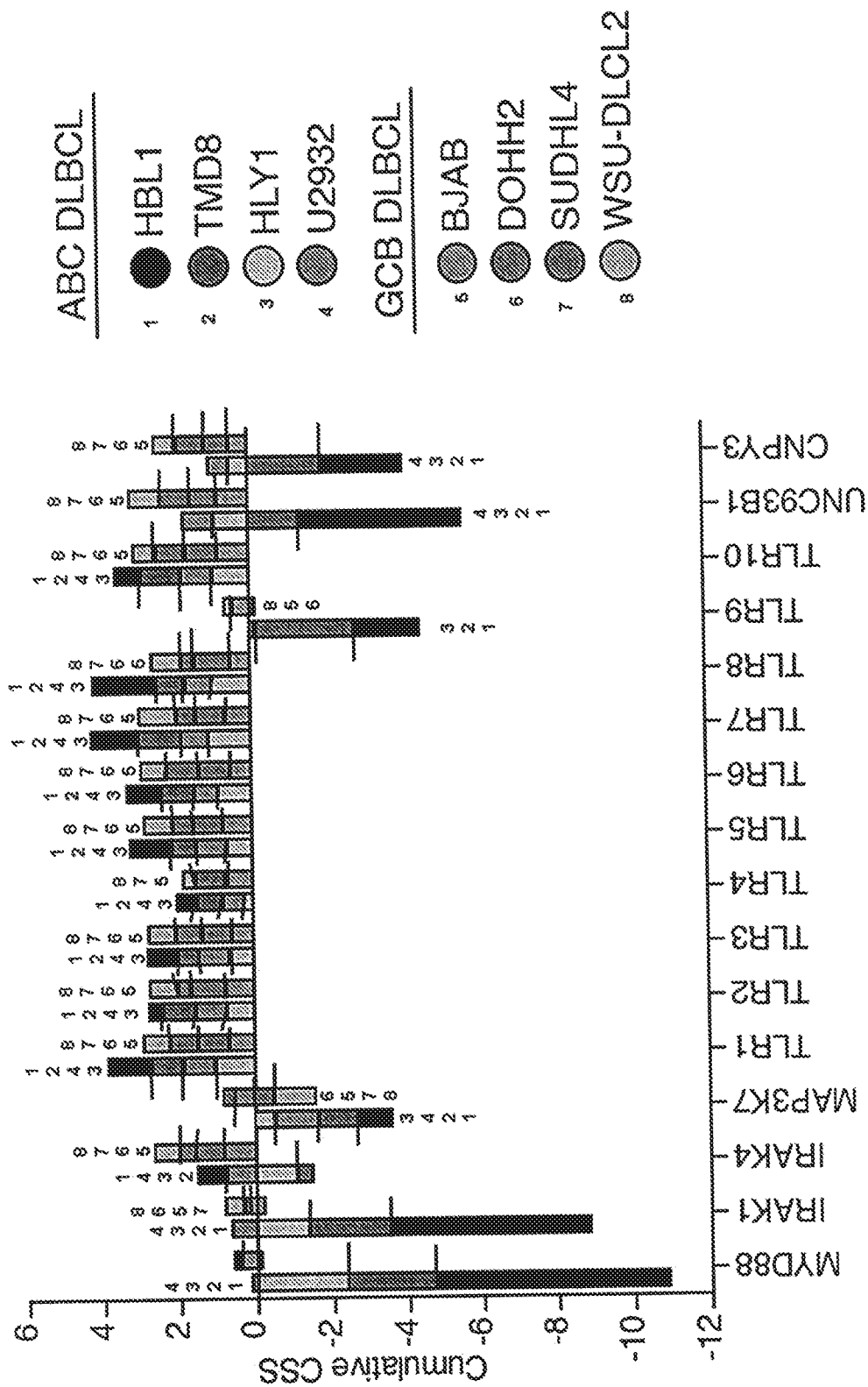
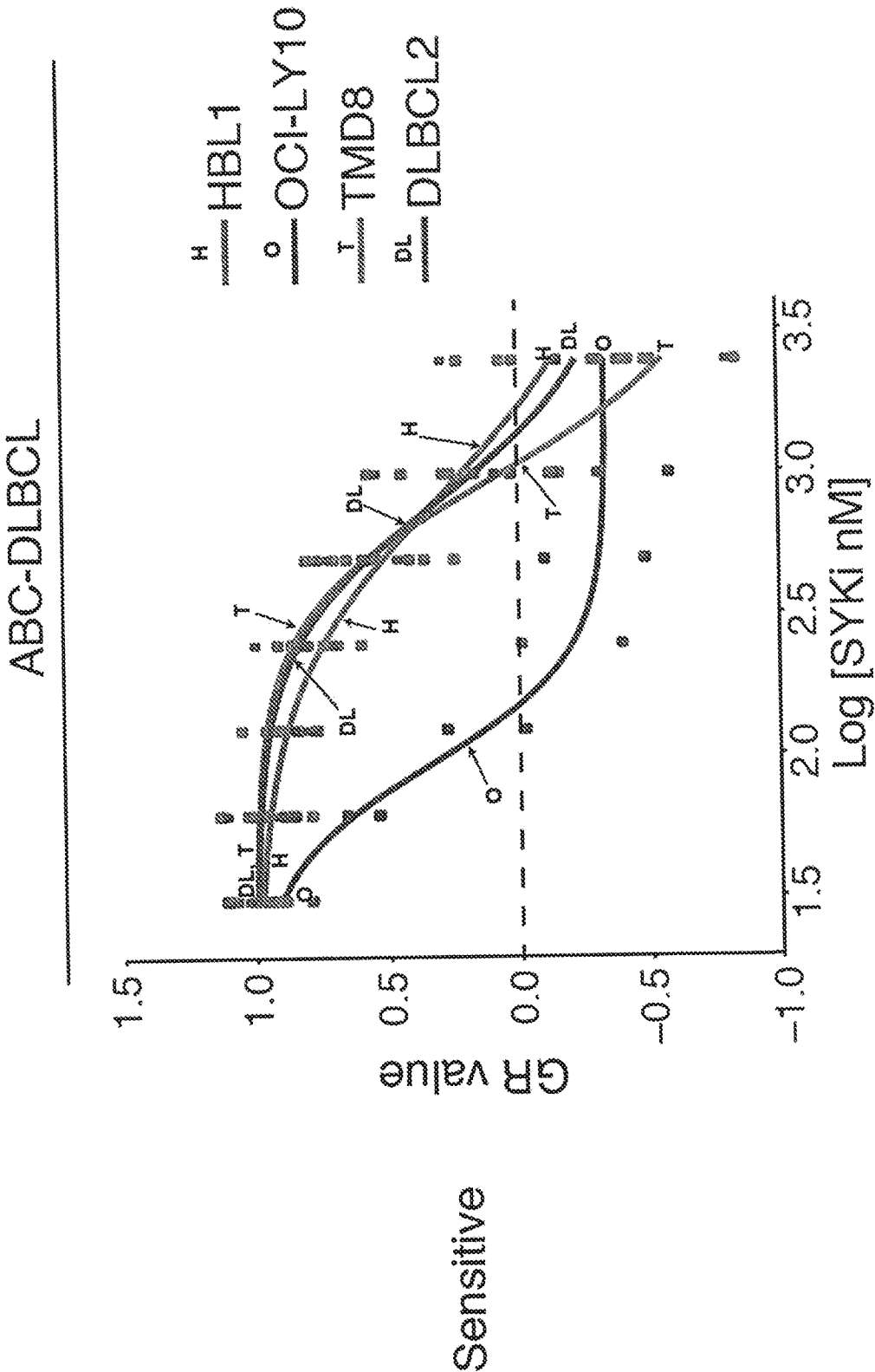
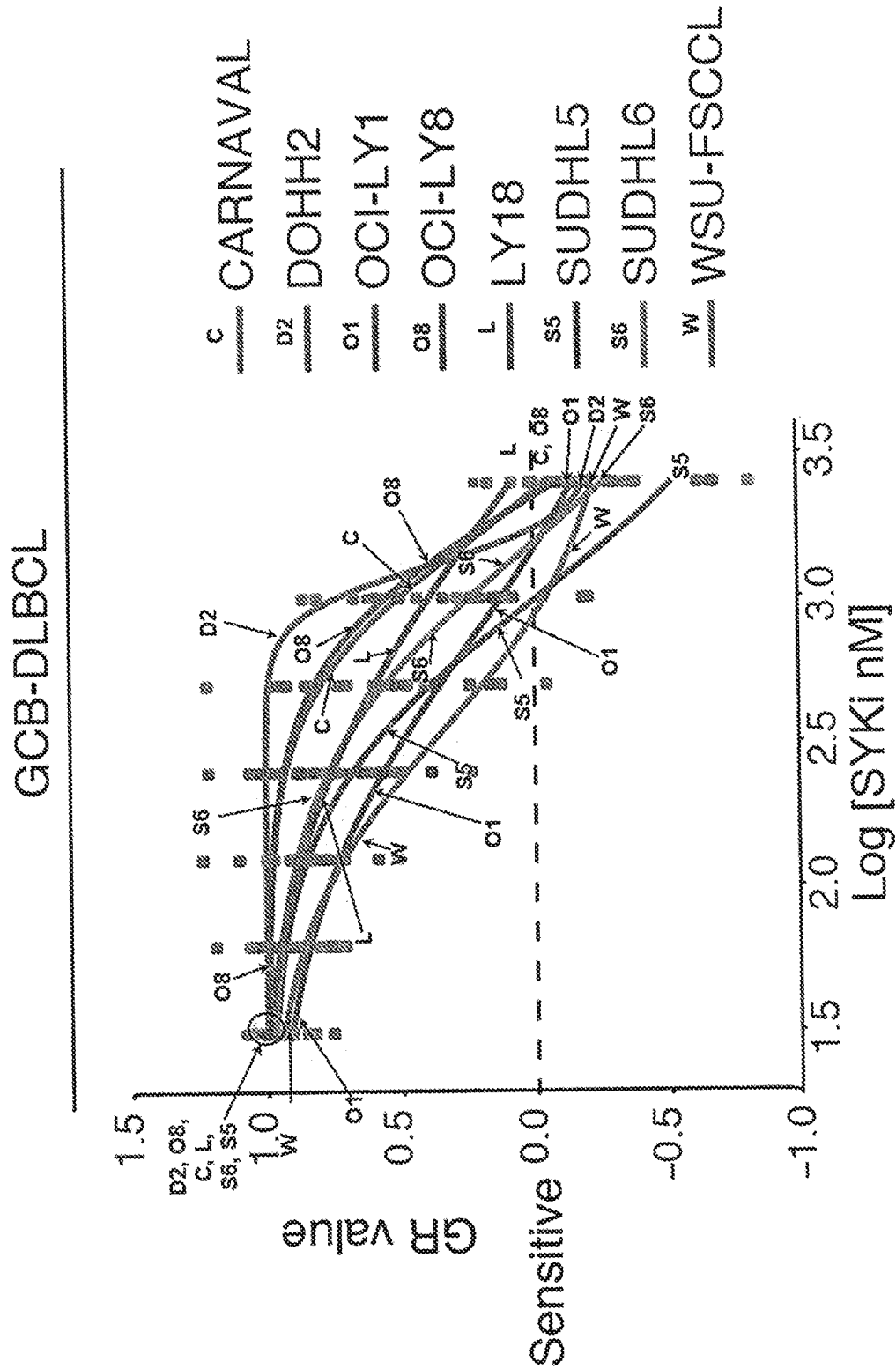


Figure 9A



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Figure 9B



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Figure 9C

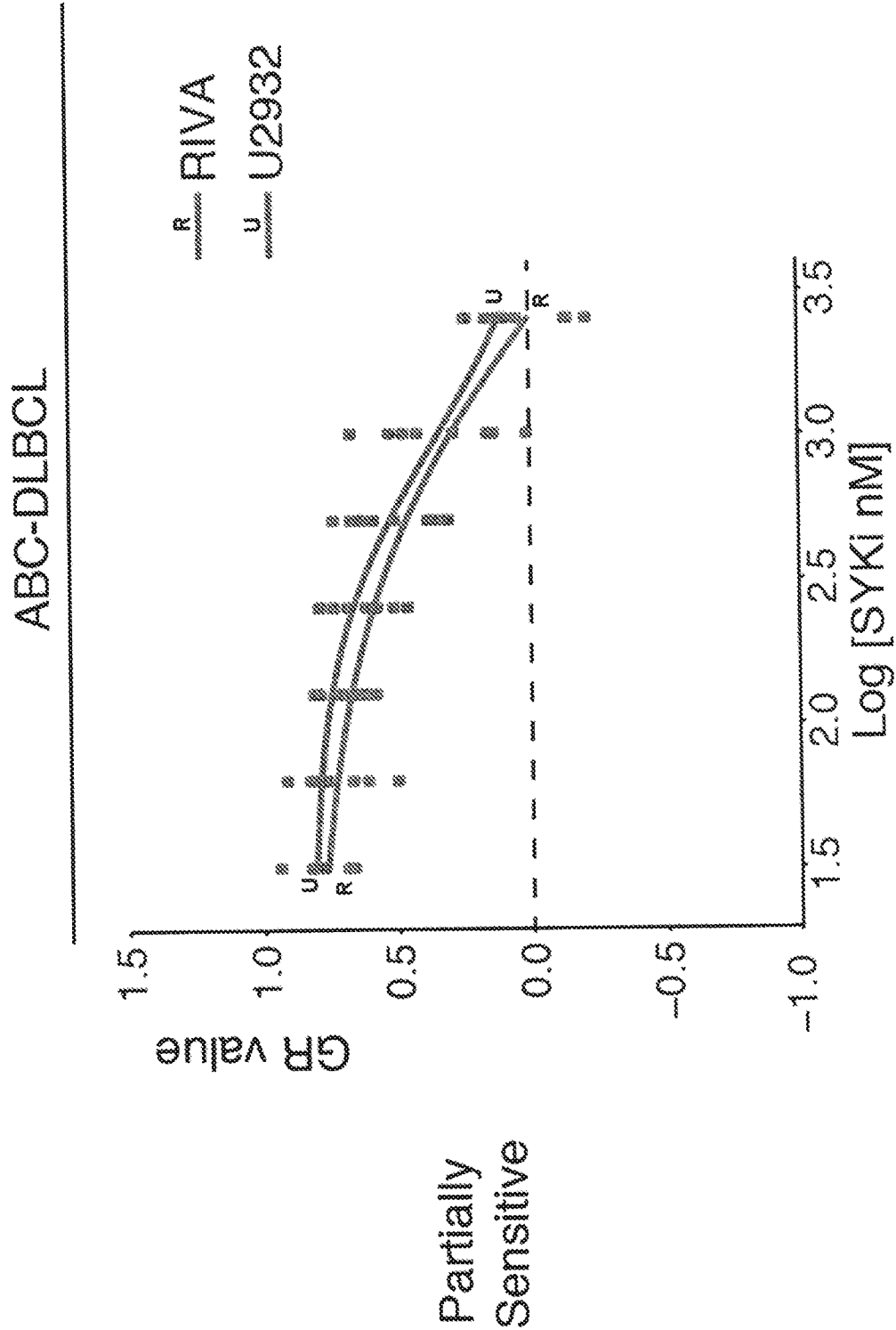


Figure 9D

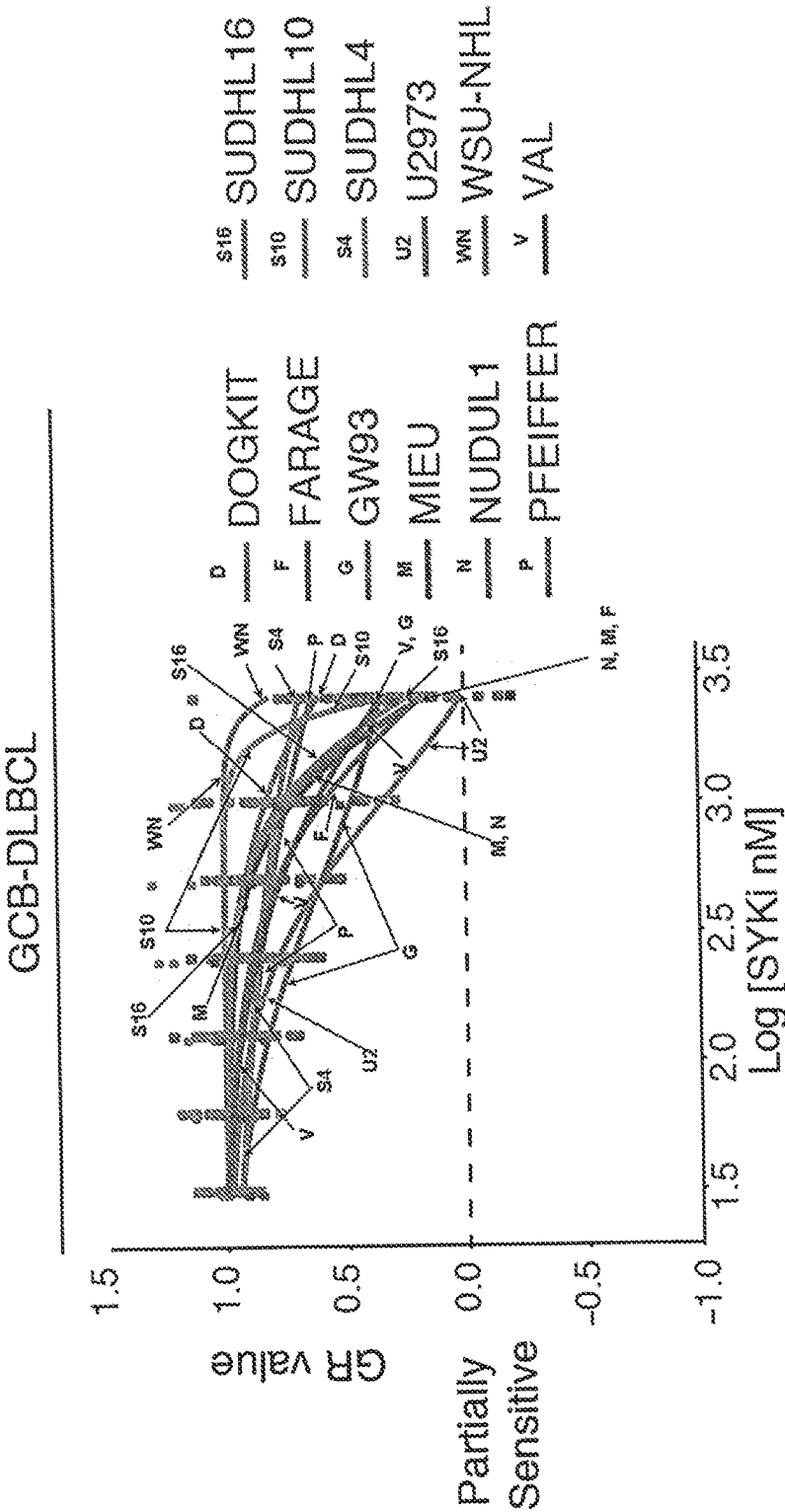
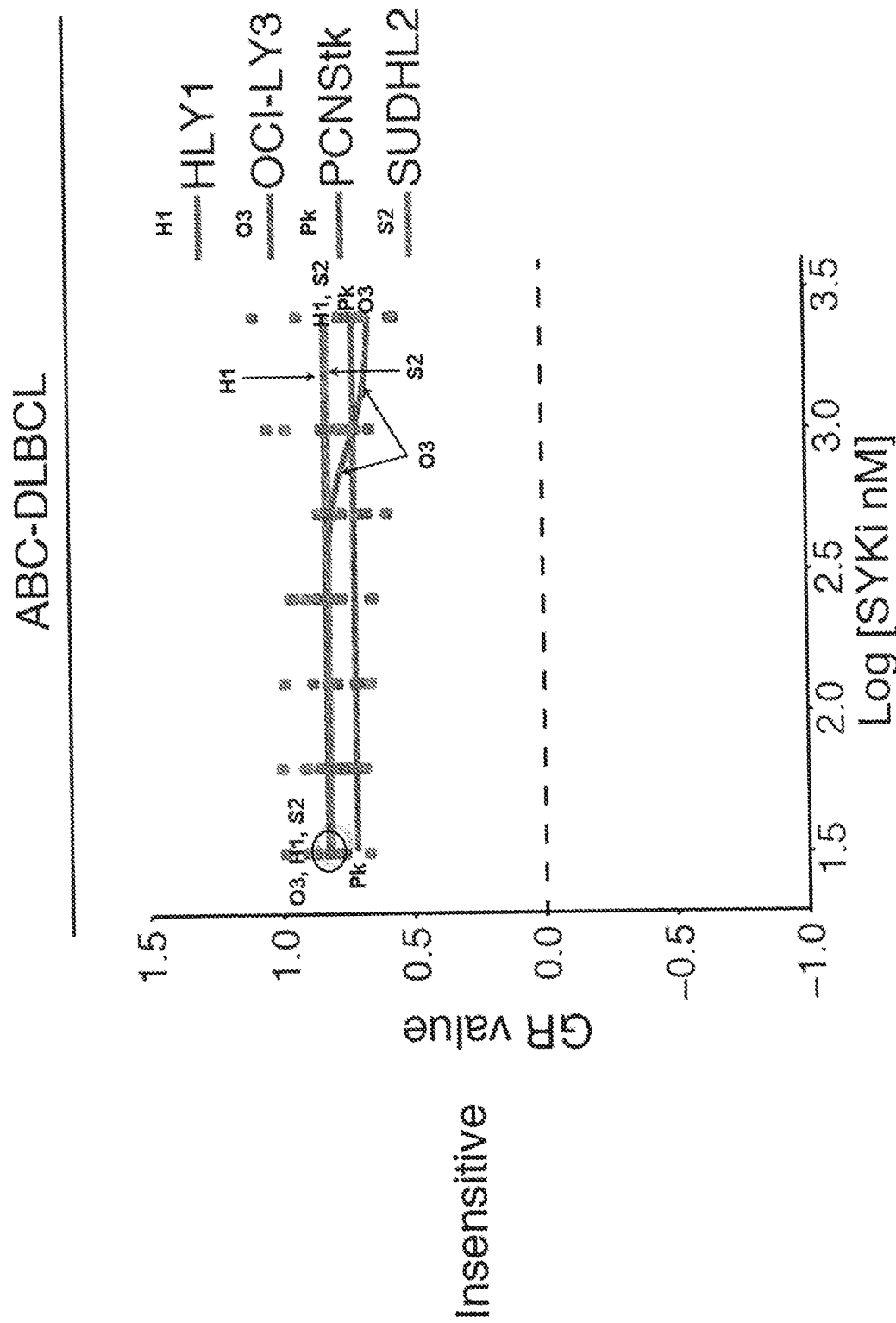
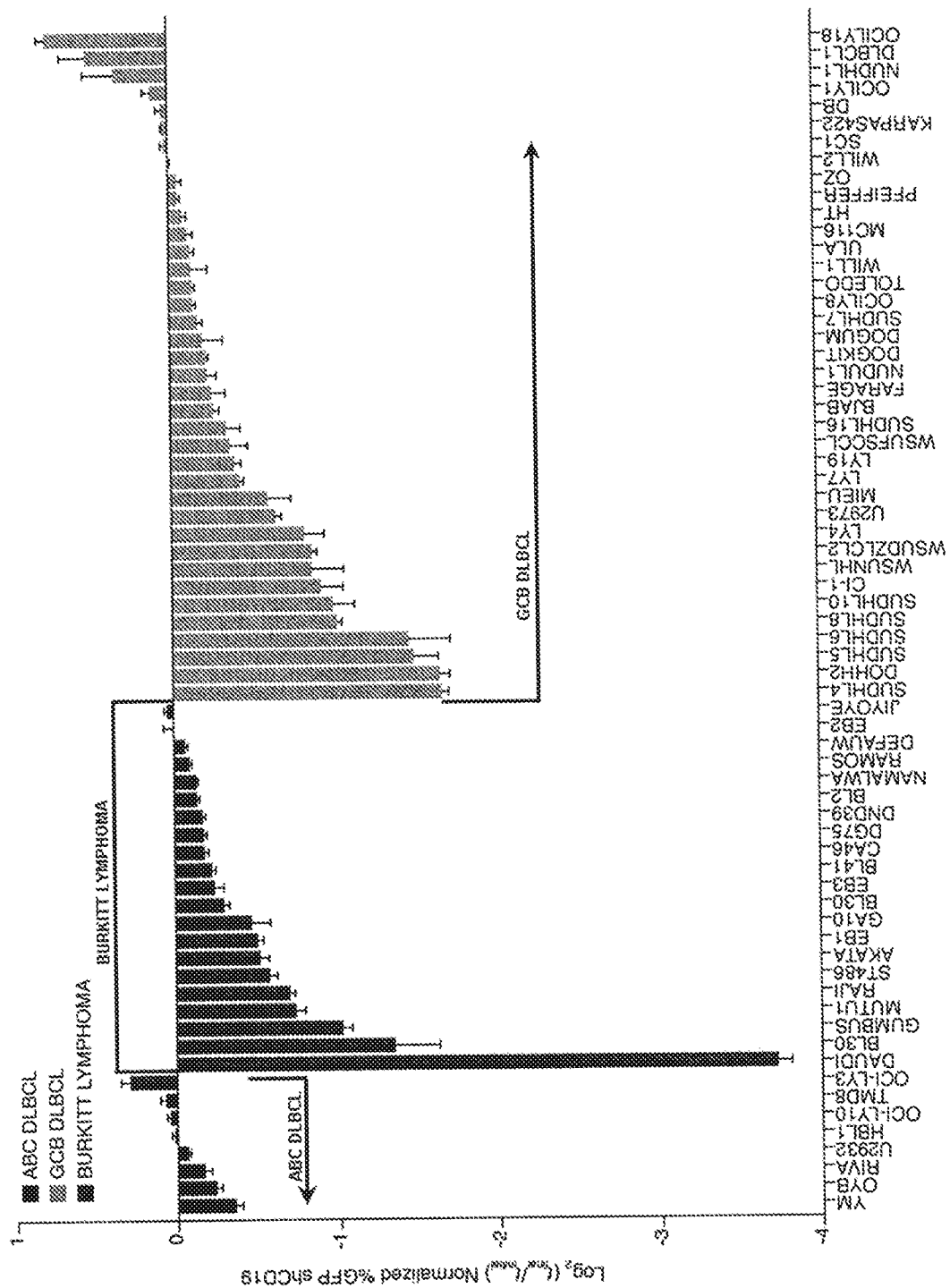


Figure 9E



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Figure 10



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Figure 11A

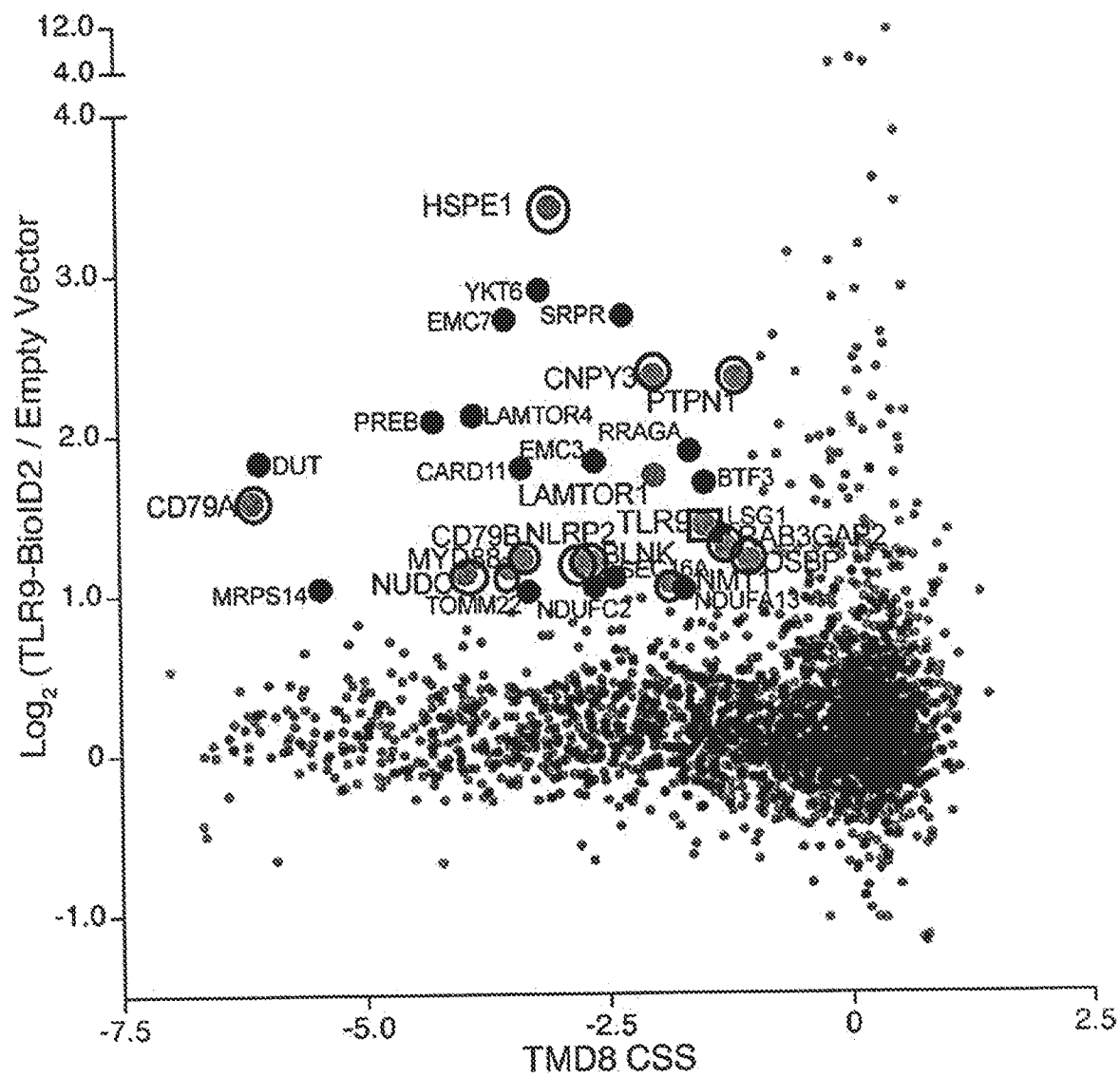


Figure 11B

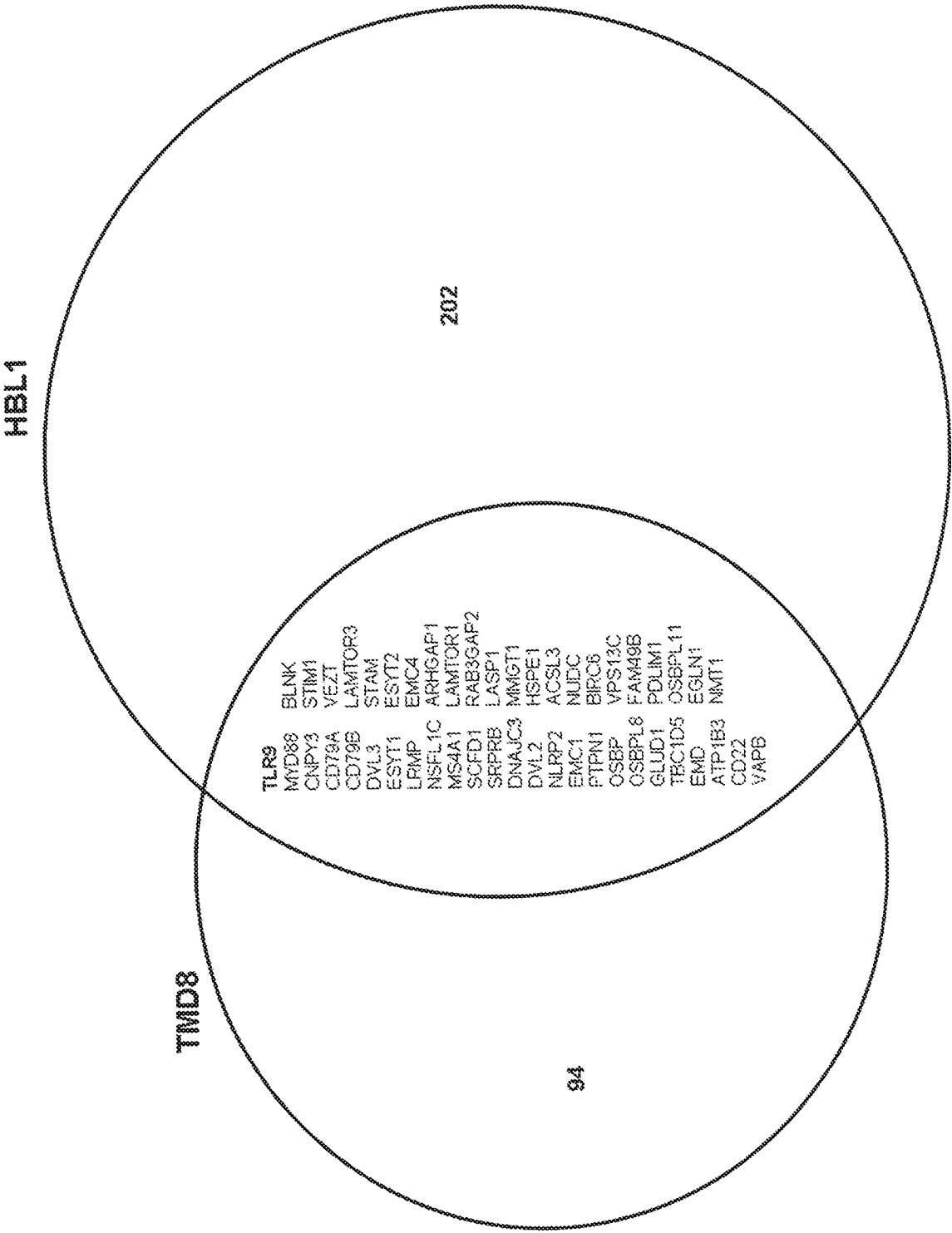


Figure 11C

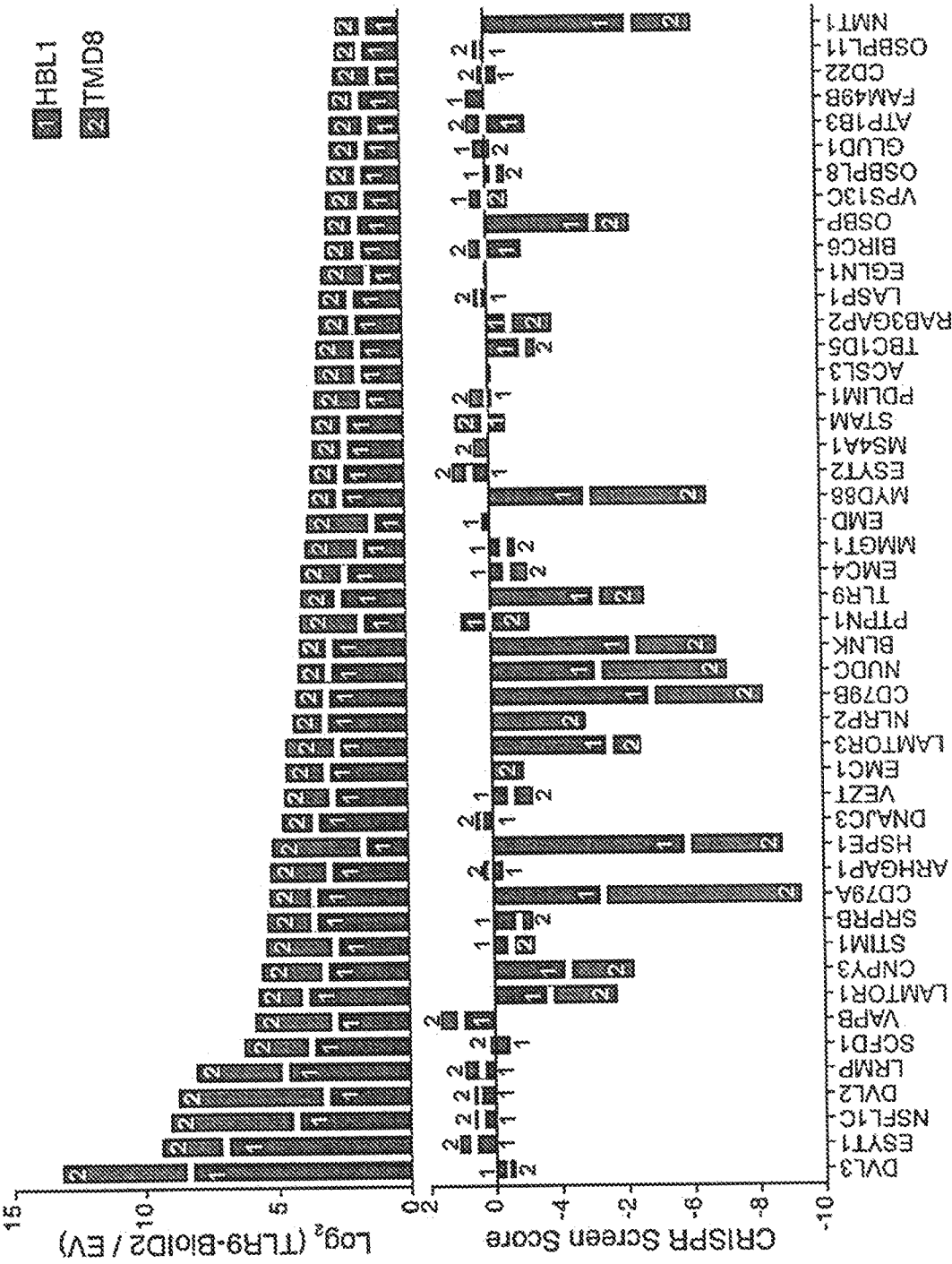


Figure 12A

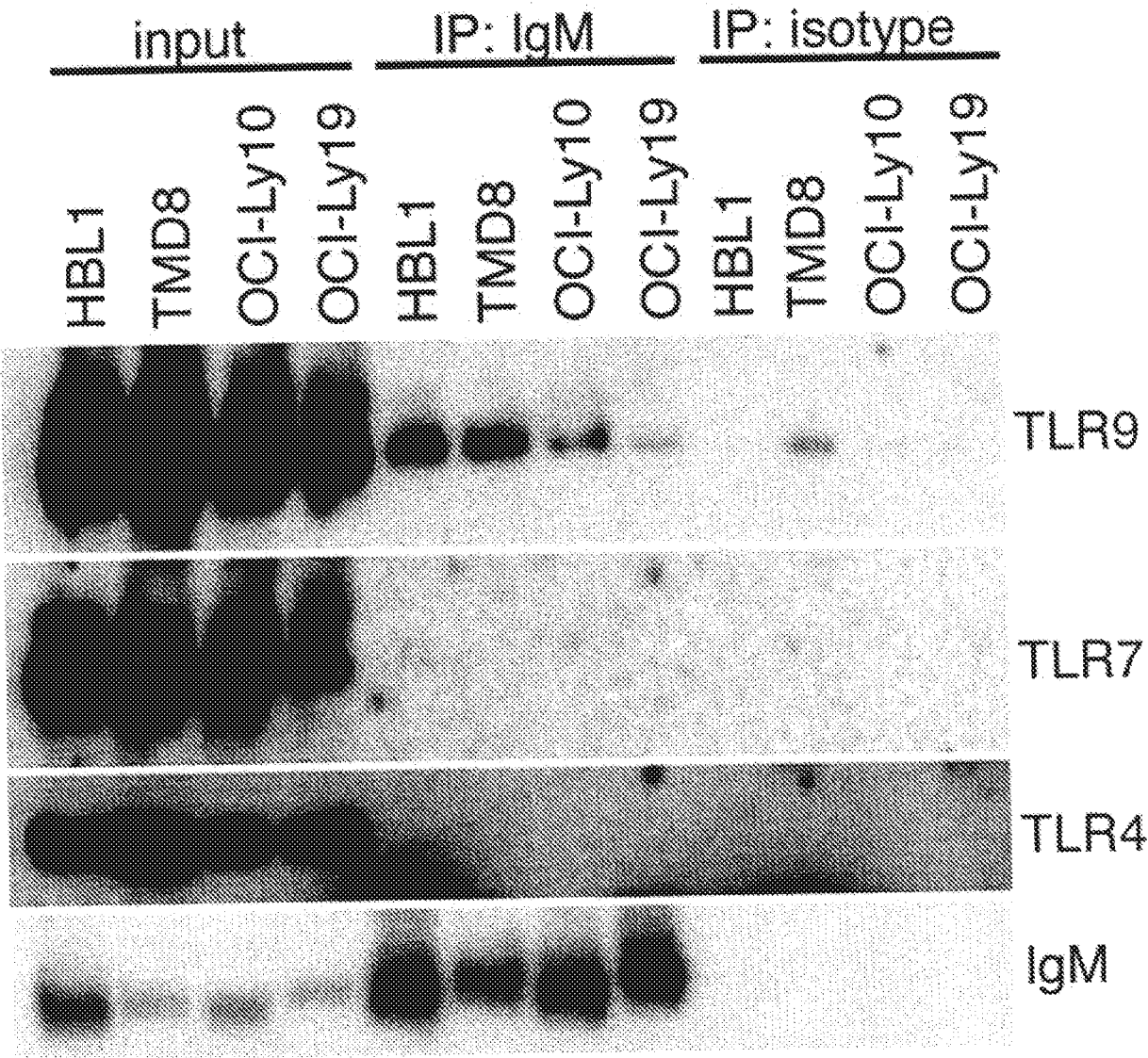


Figure 12B

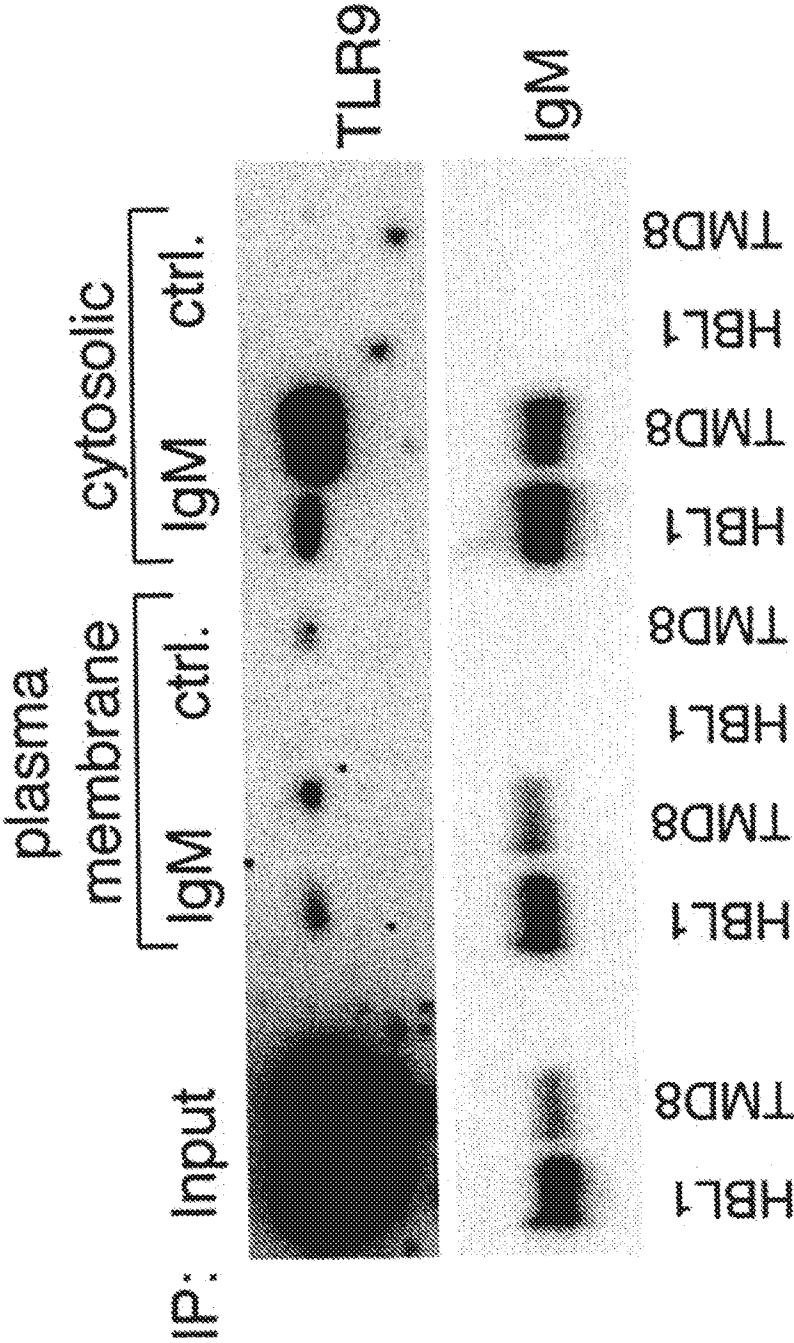
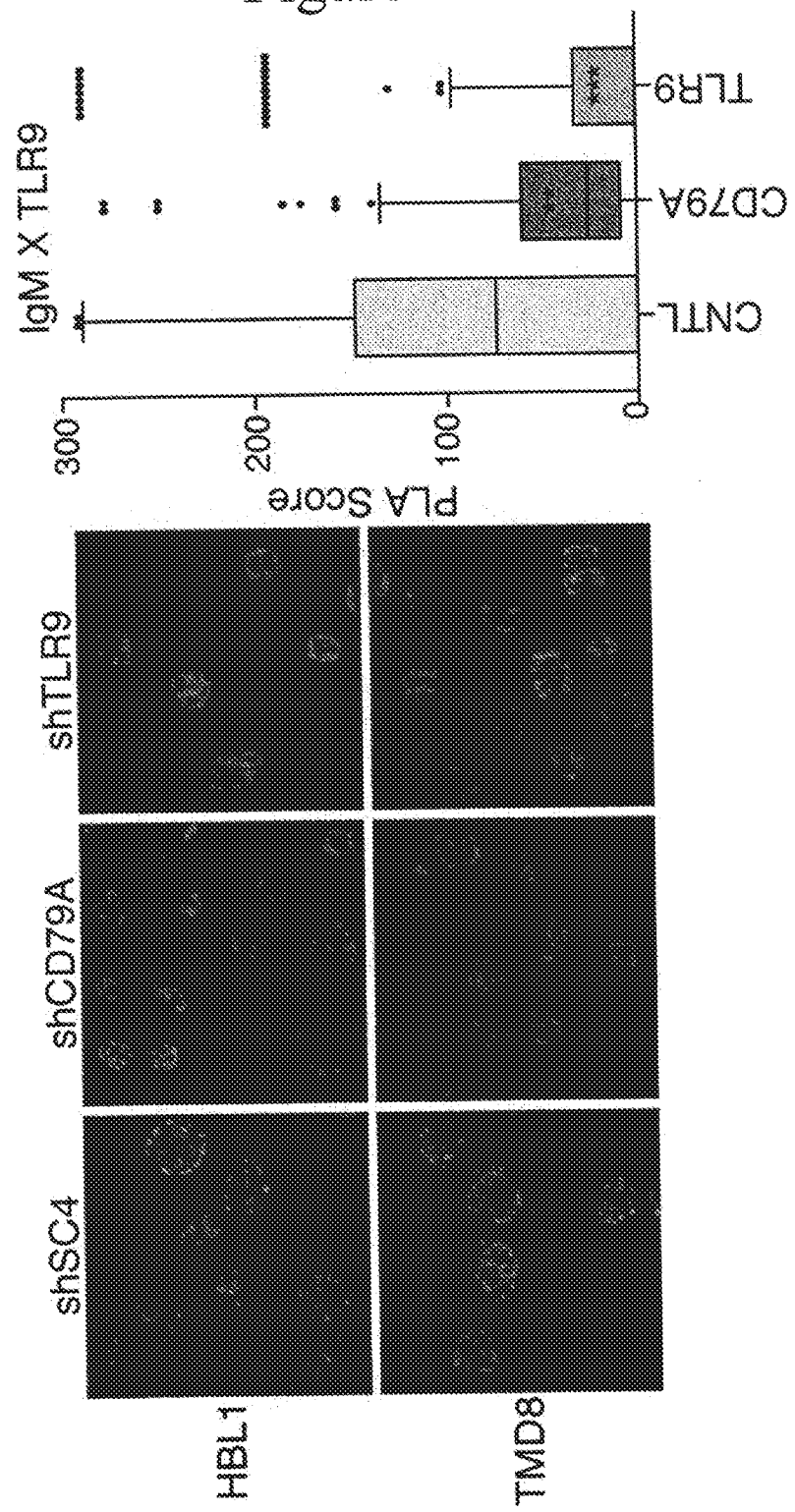


Figure 12C



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Figure 12E

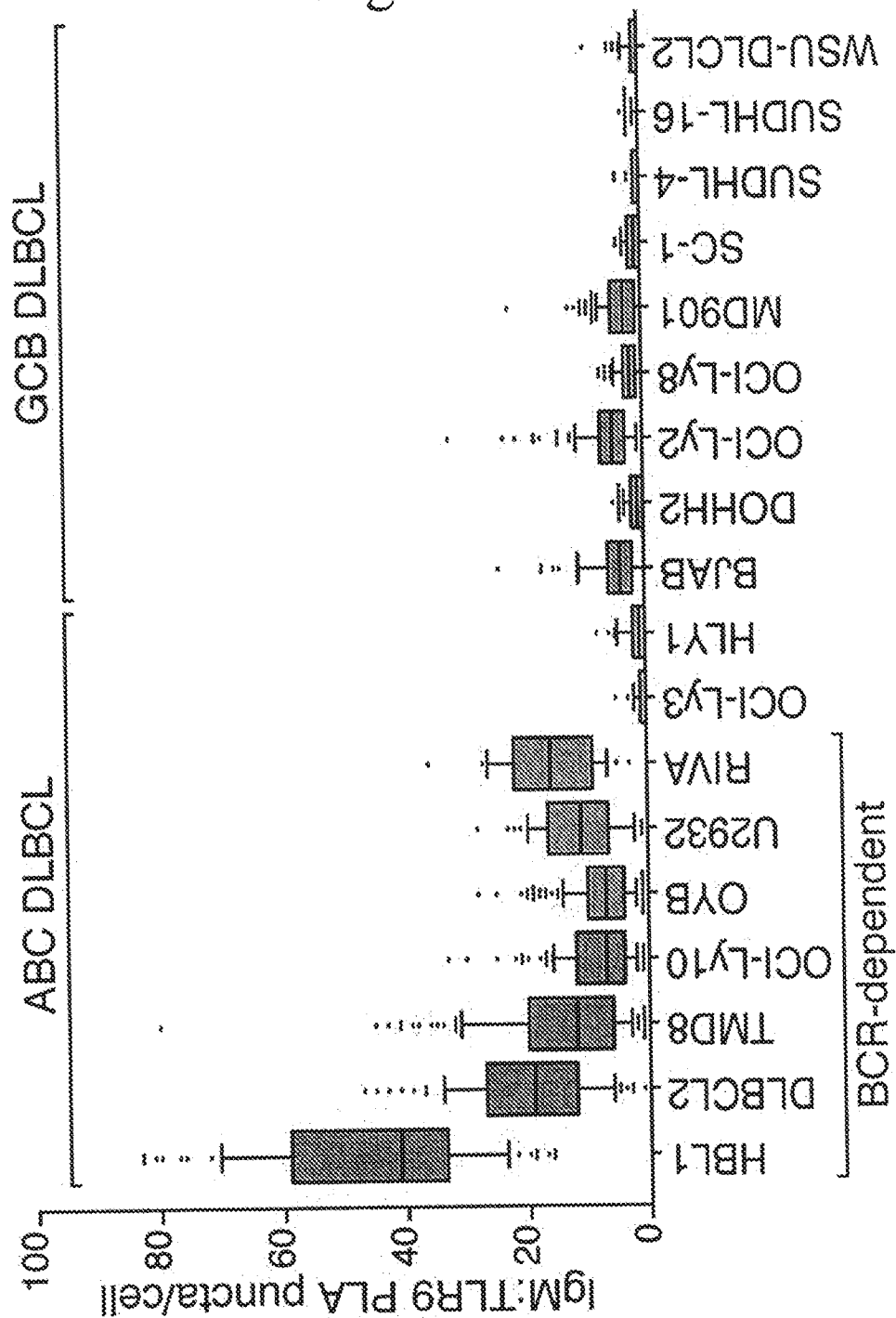


Figure 12F

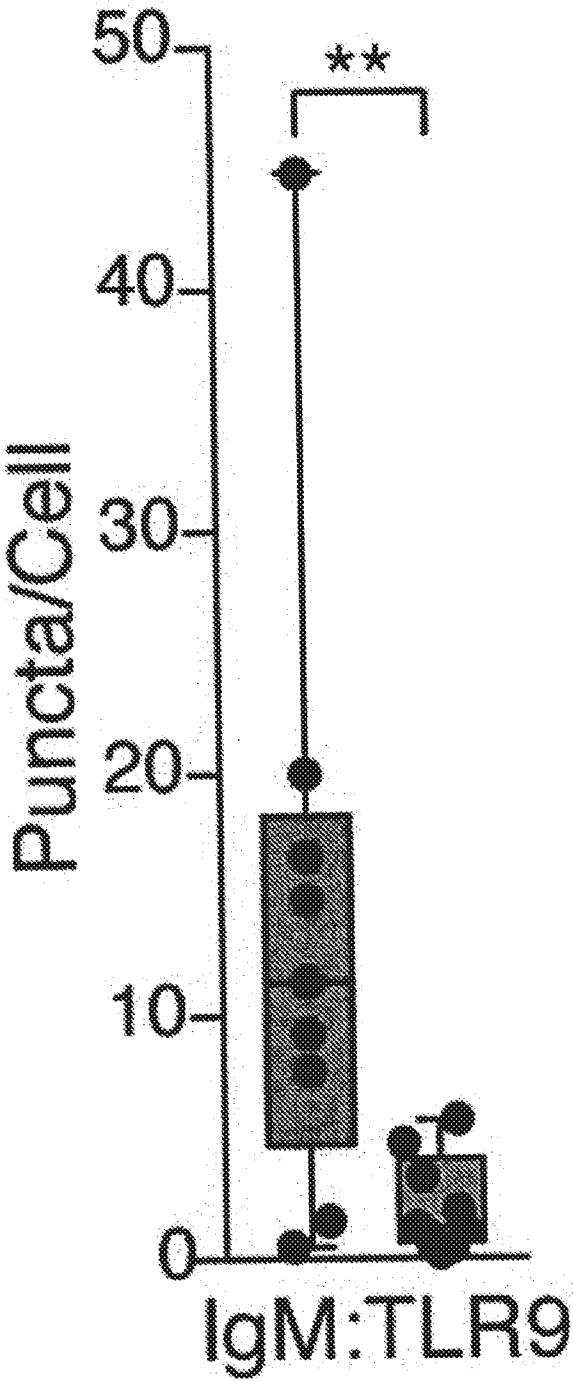
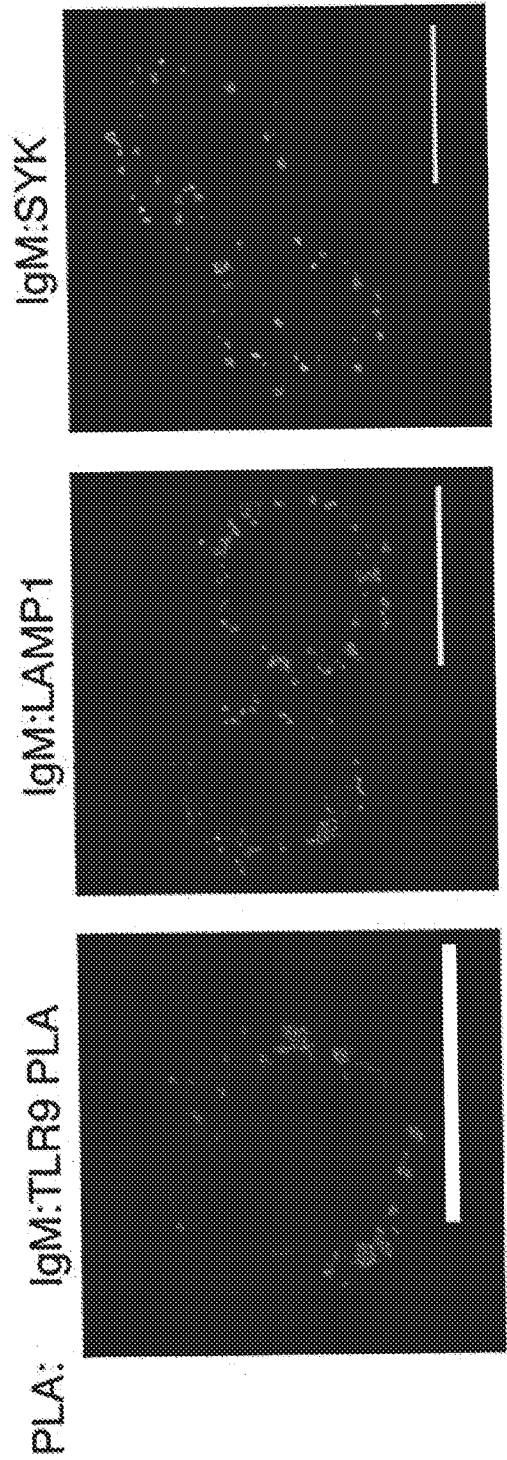


Figure 12G



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Figure 12H

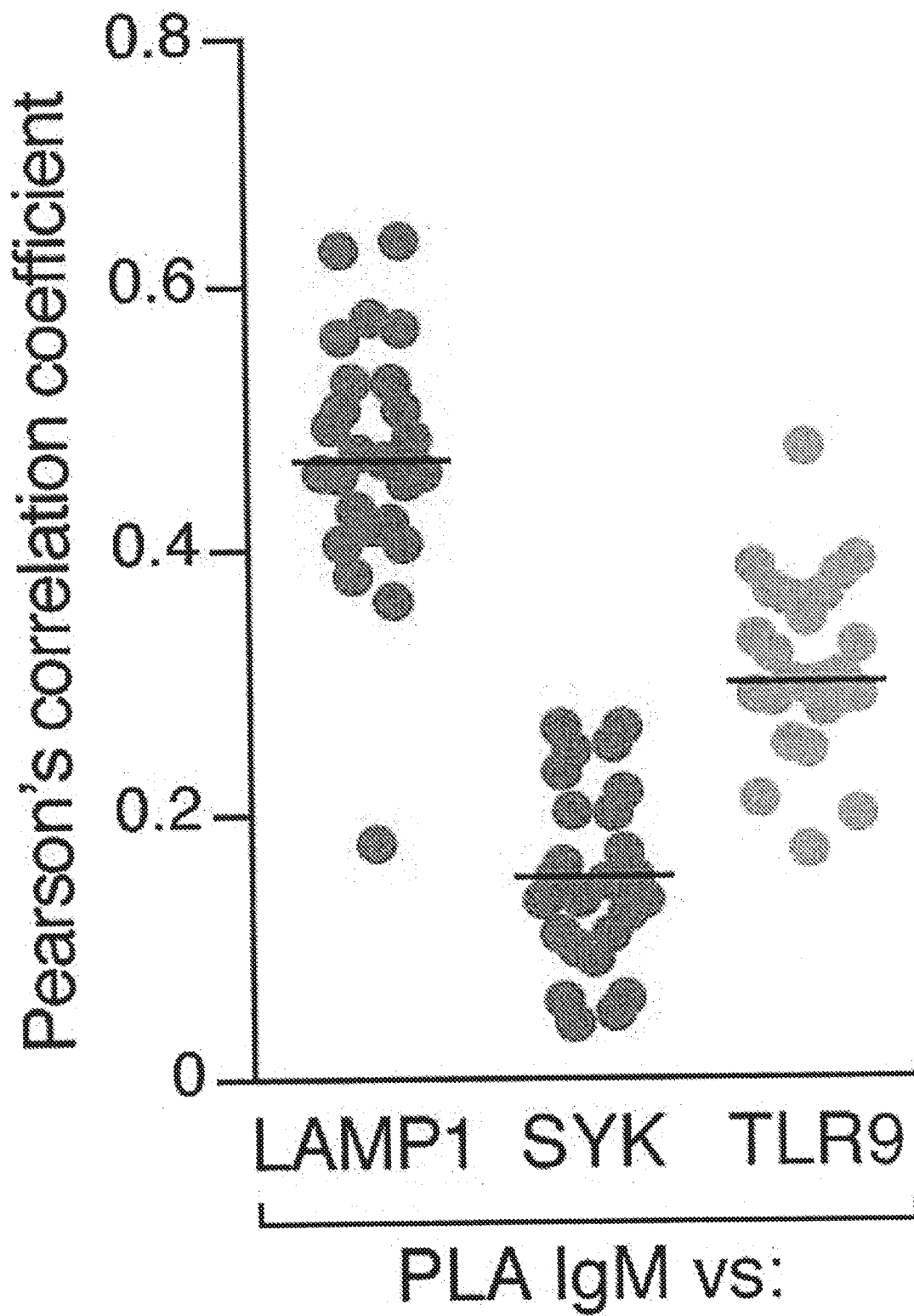
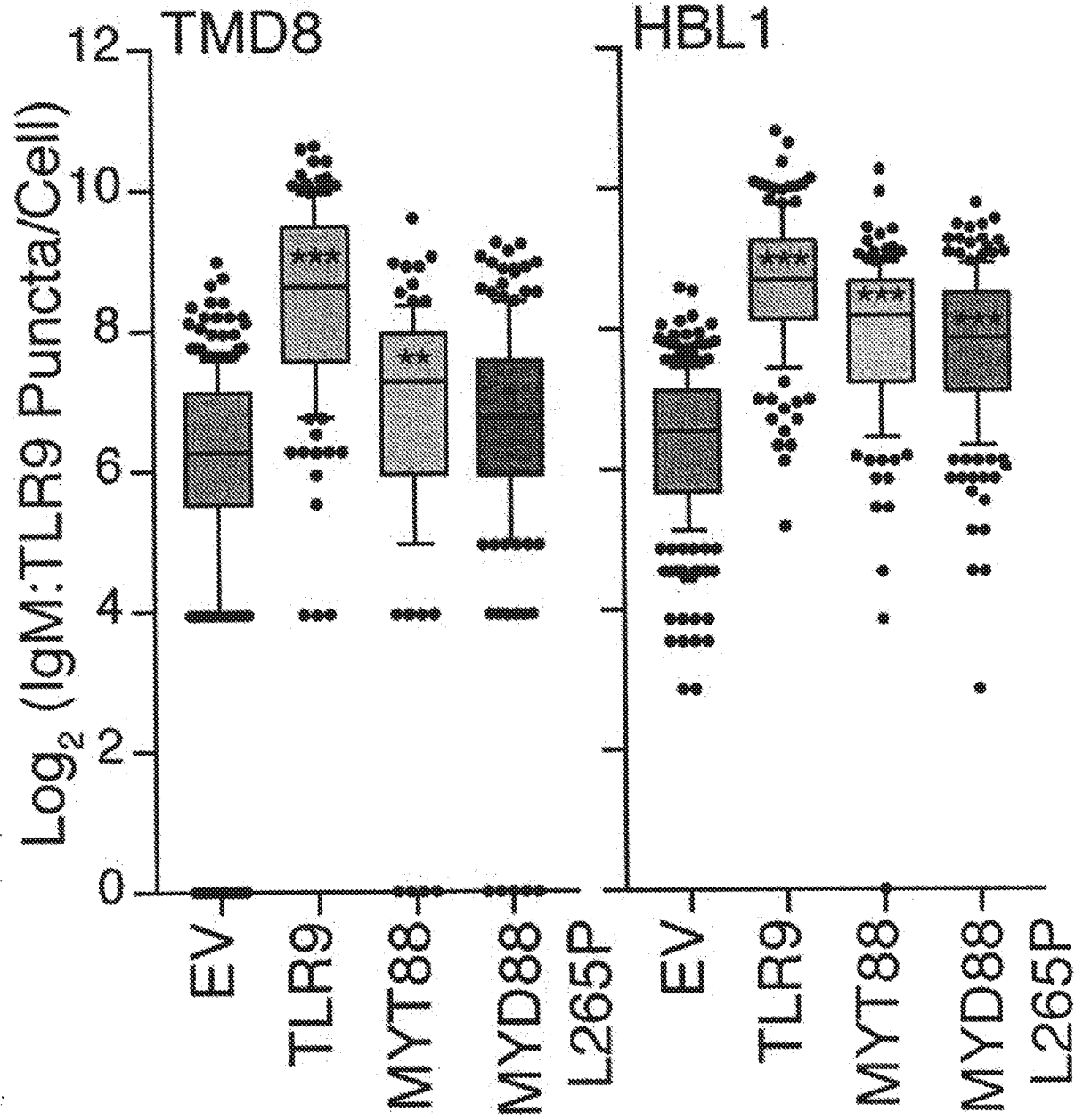


Figure 12I



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Figure 13A

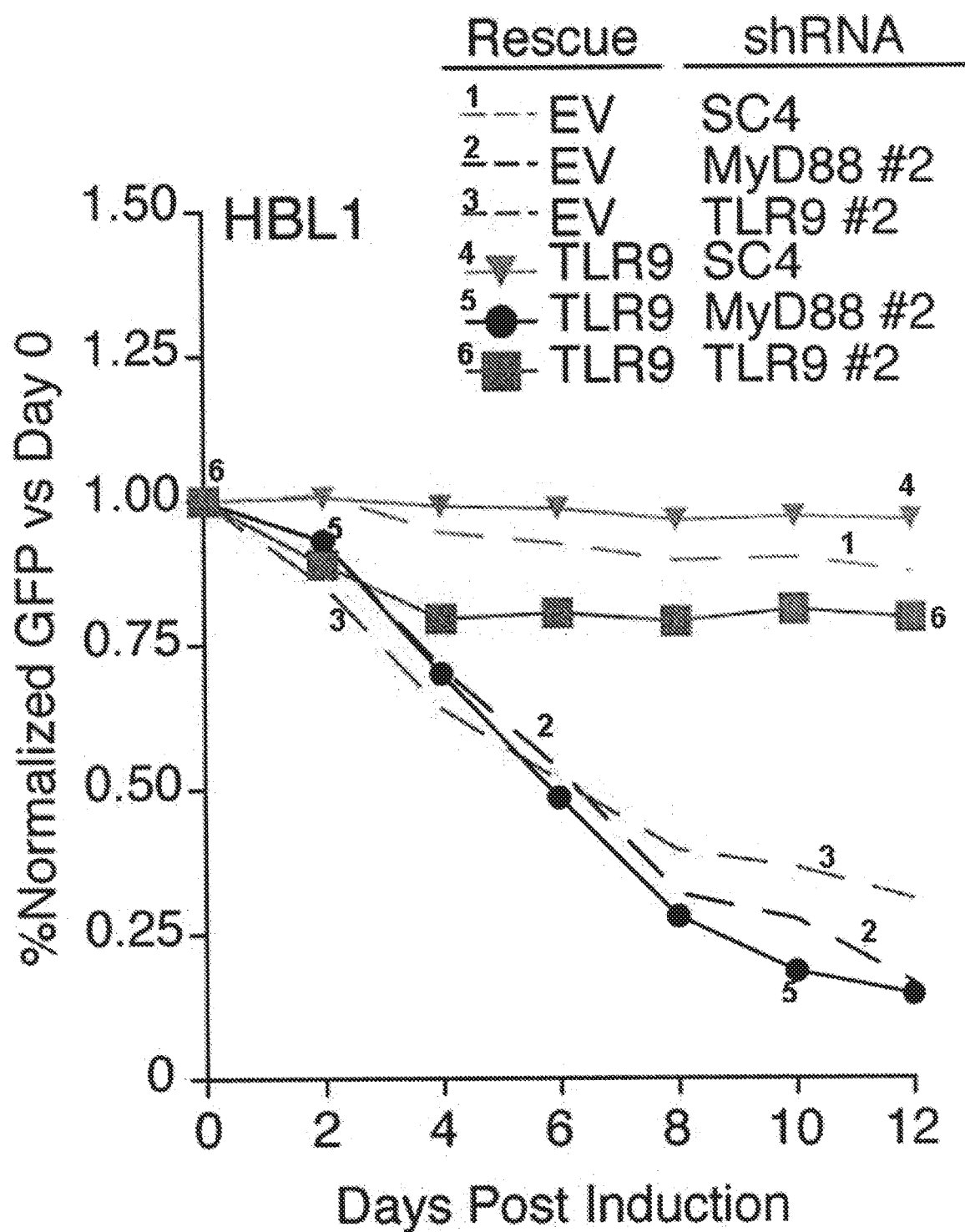
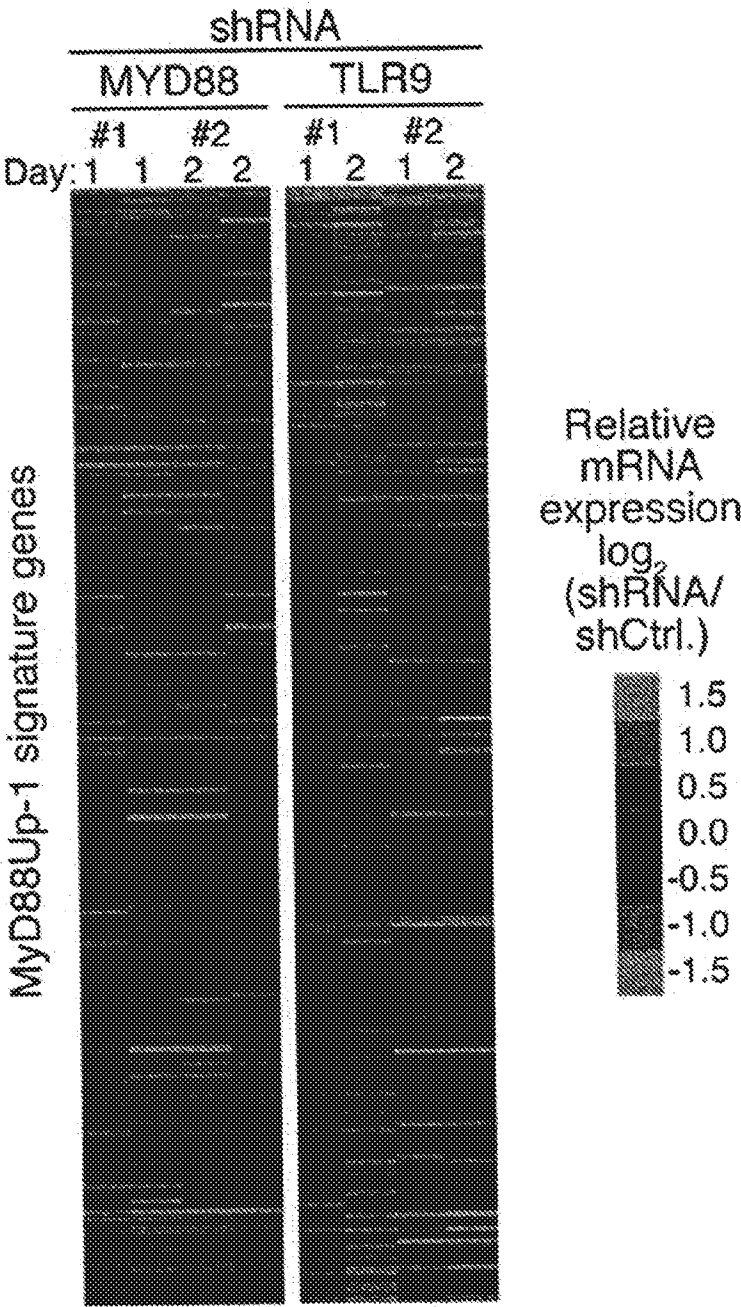
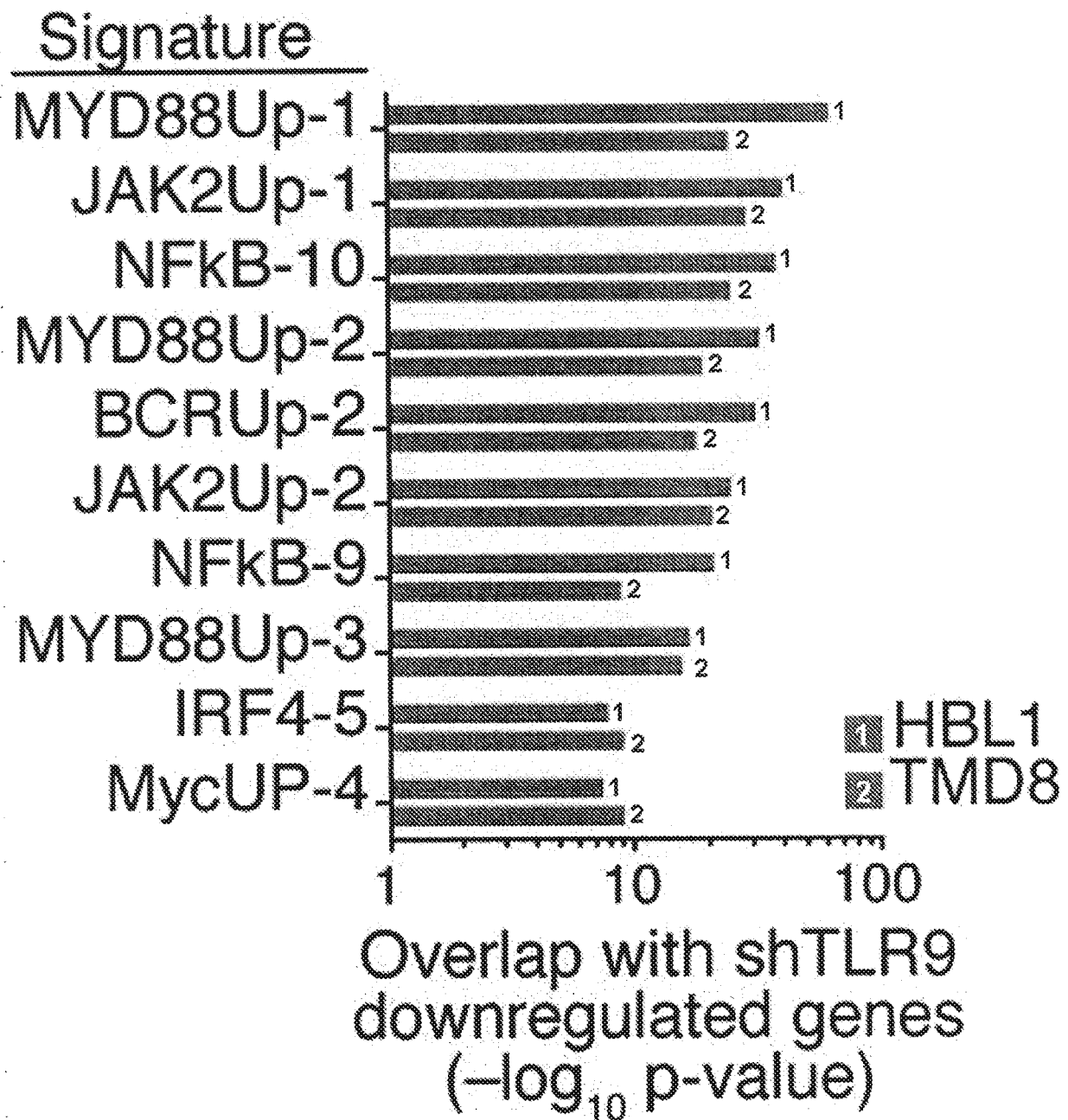


Figure 13B



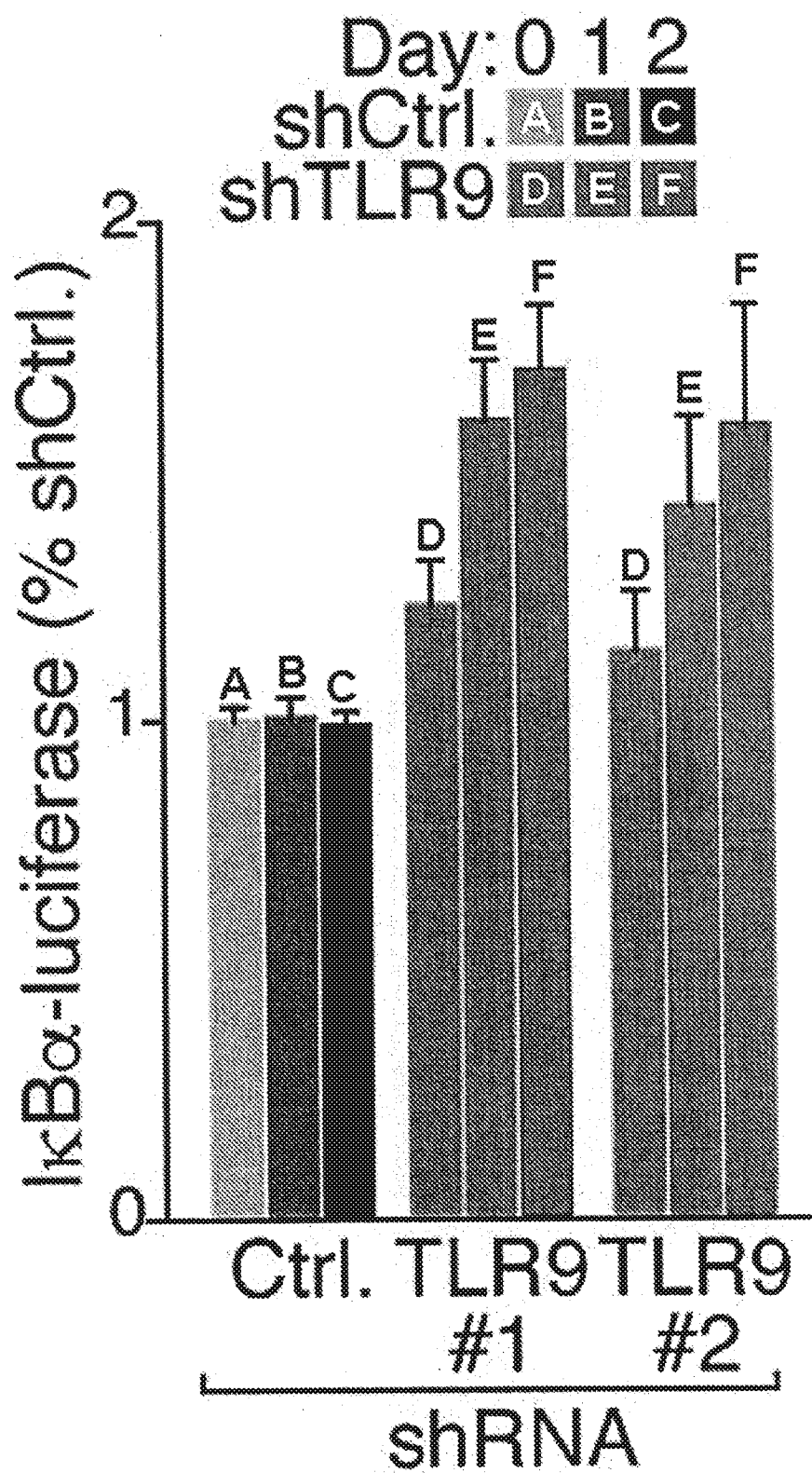
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Figure 13C



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Figure 13D



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Figure 14A

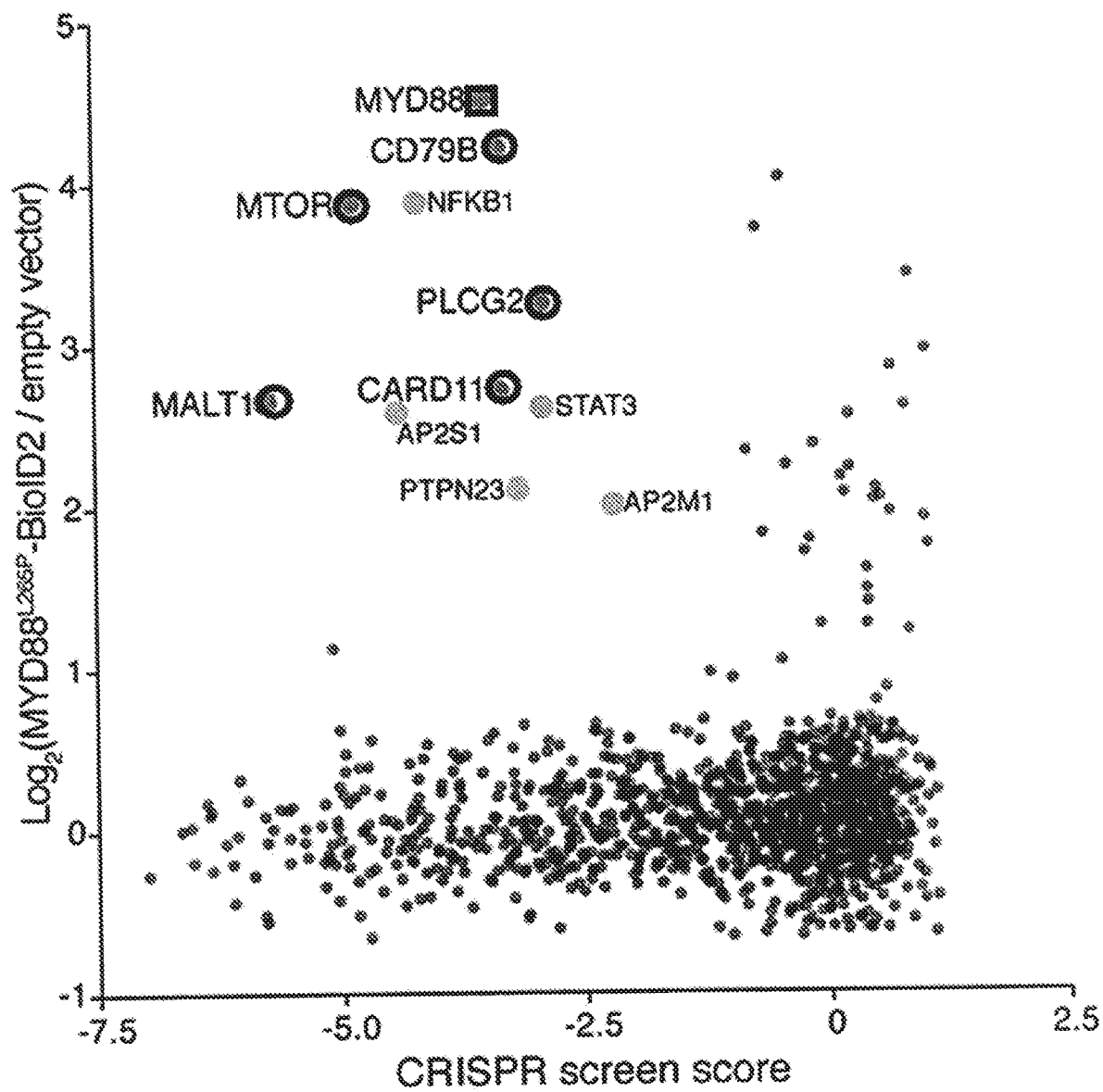
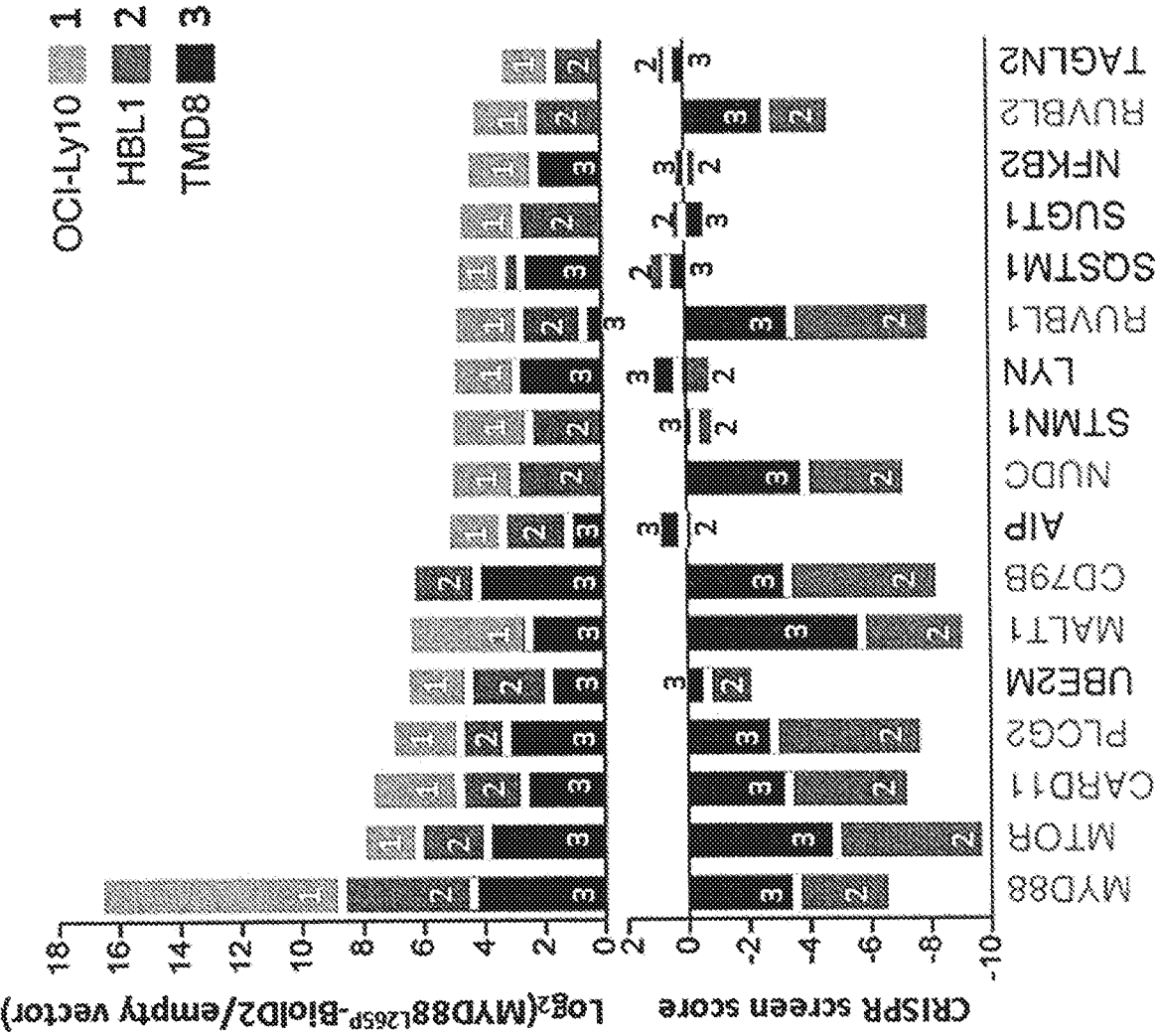


Figure 14B



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Figure 14C

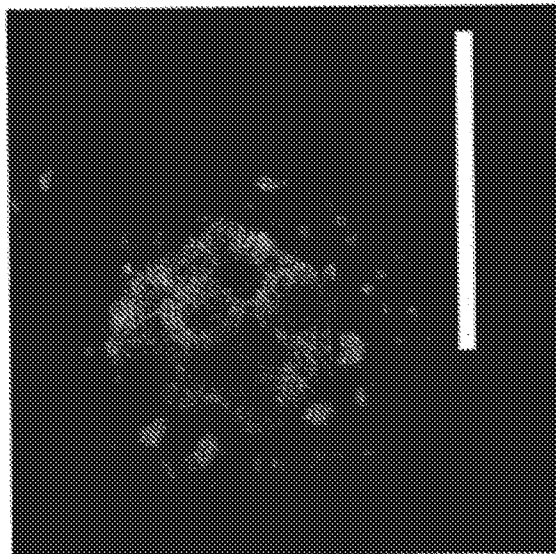
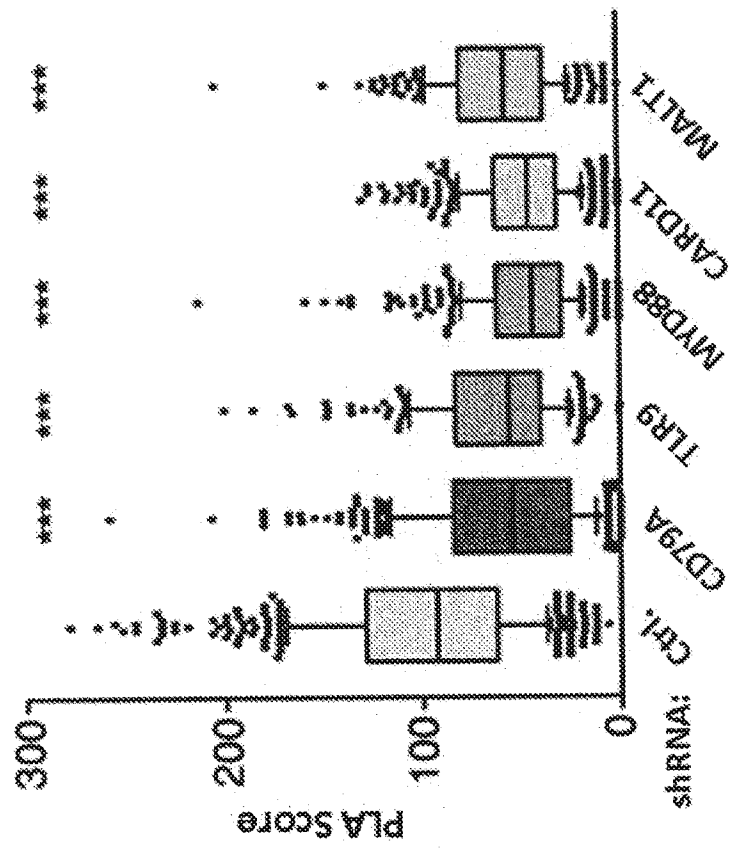


Figure 14D

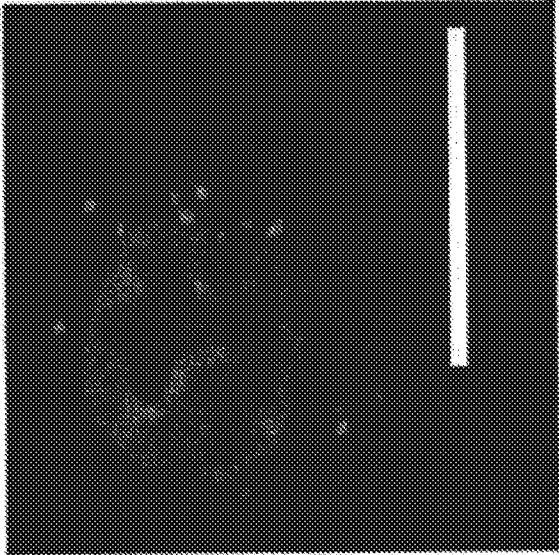
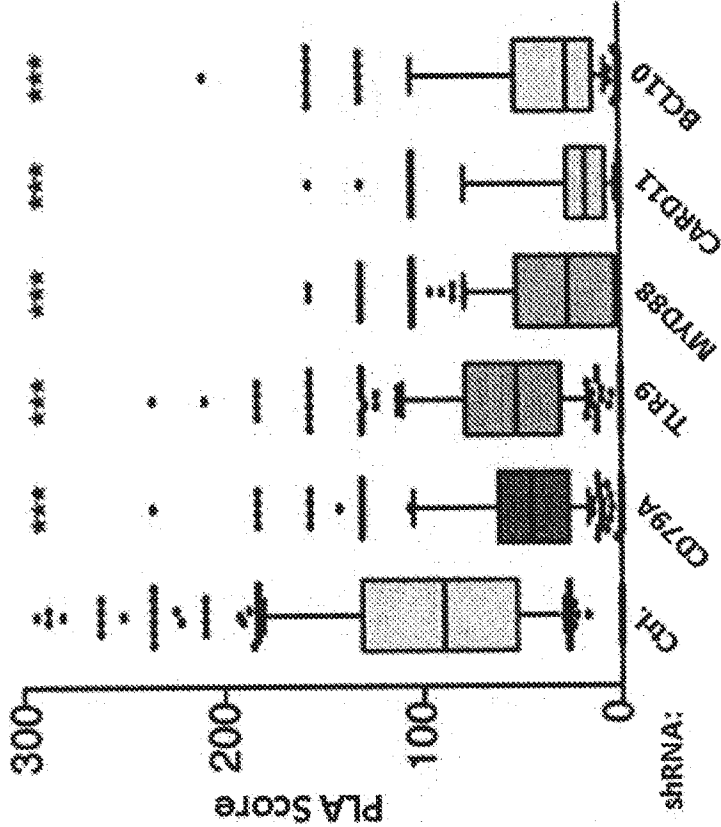


Figure 14E

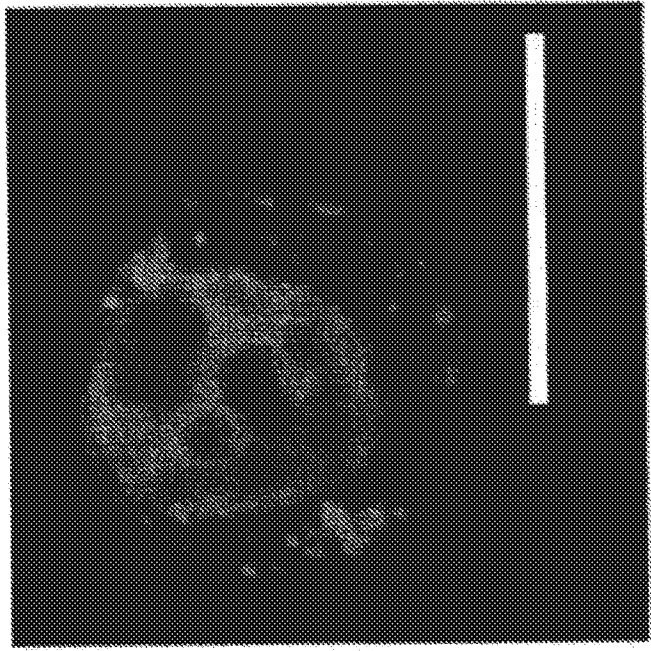
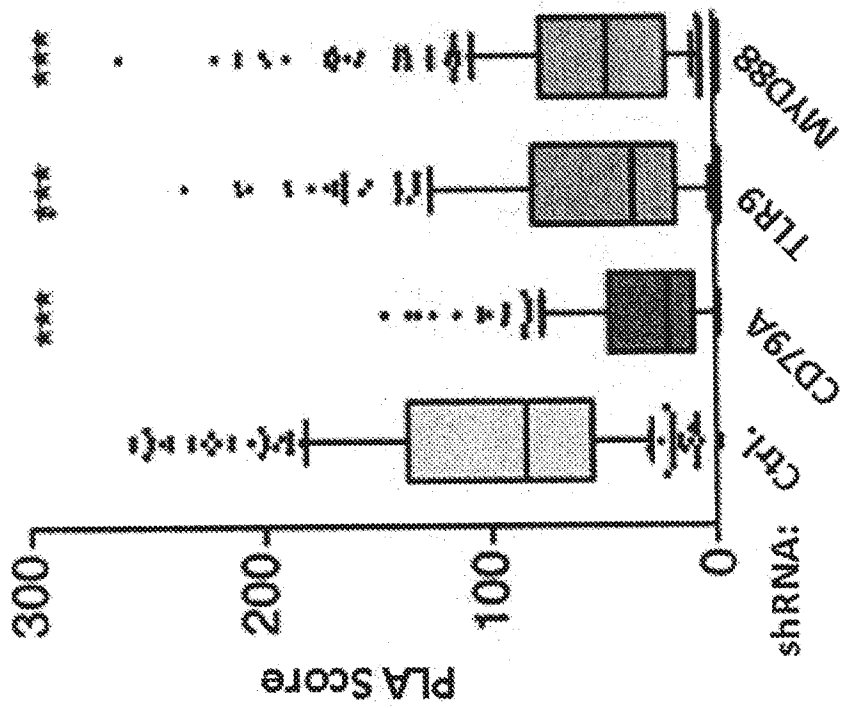


Figure 14F

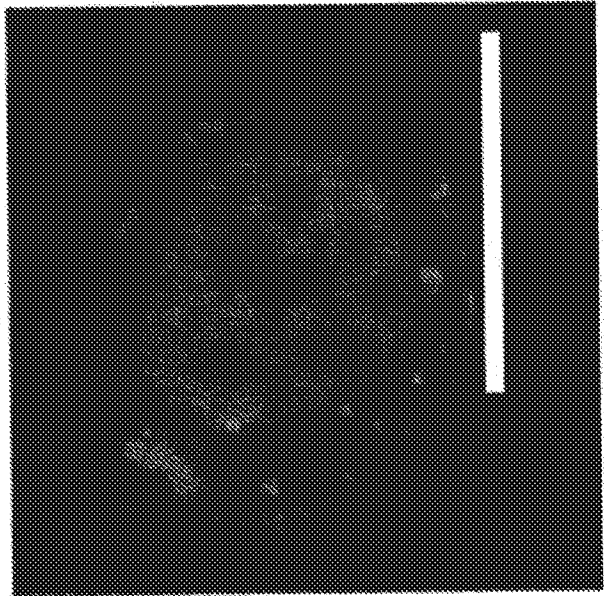
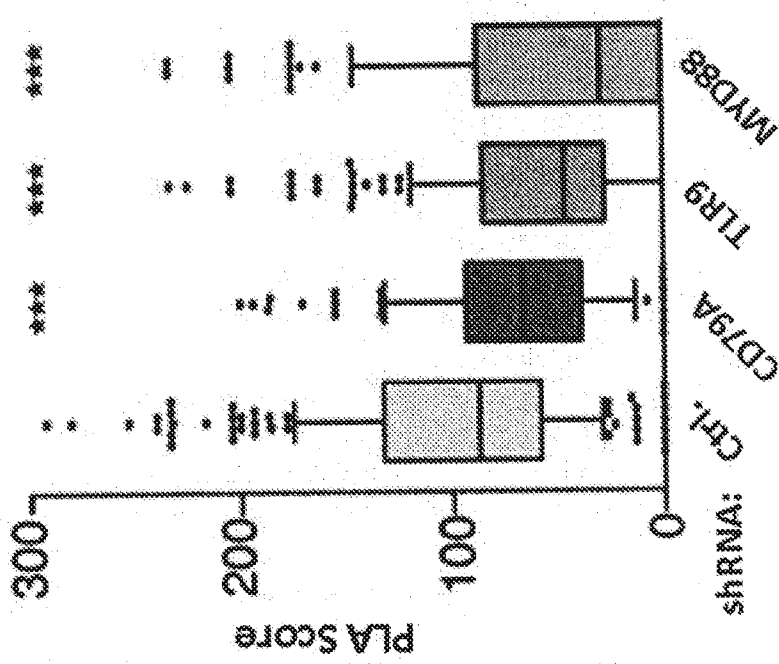
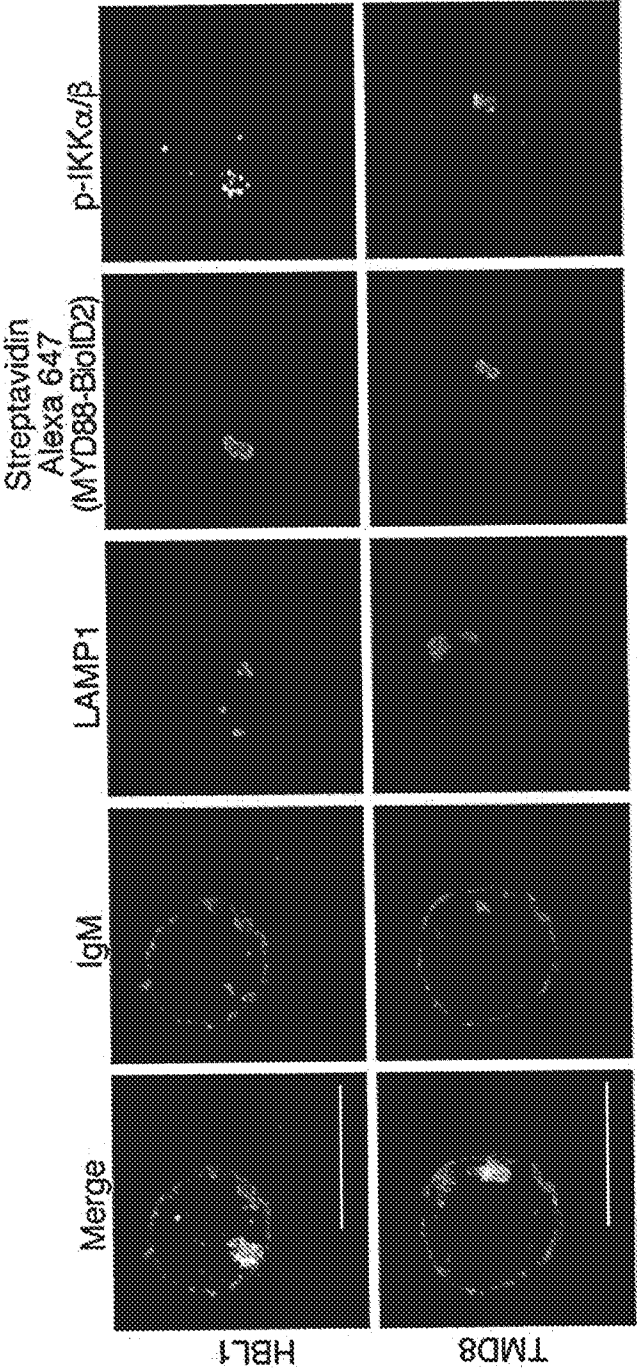
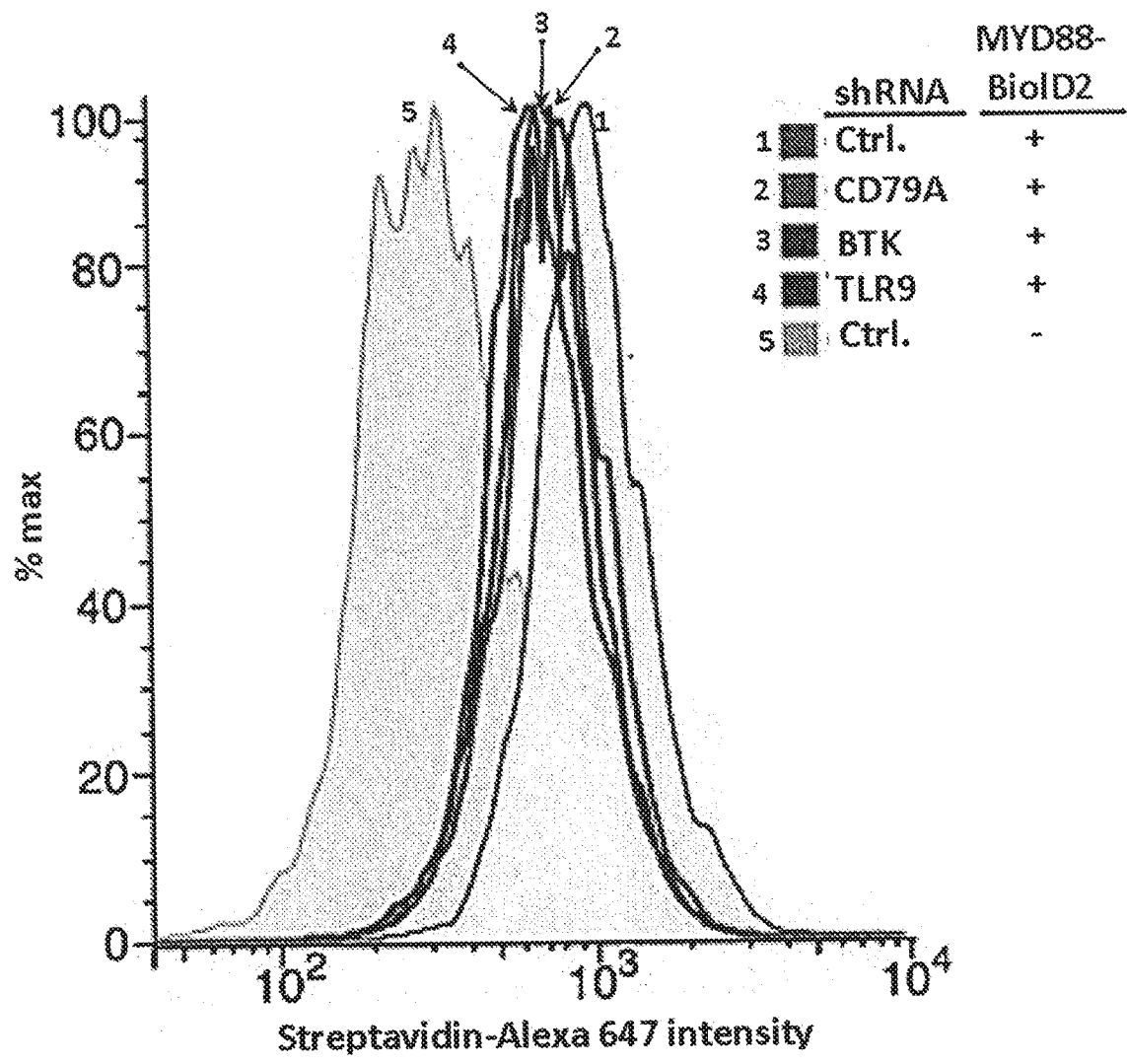


Figure 14G



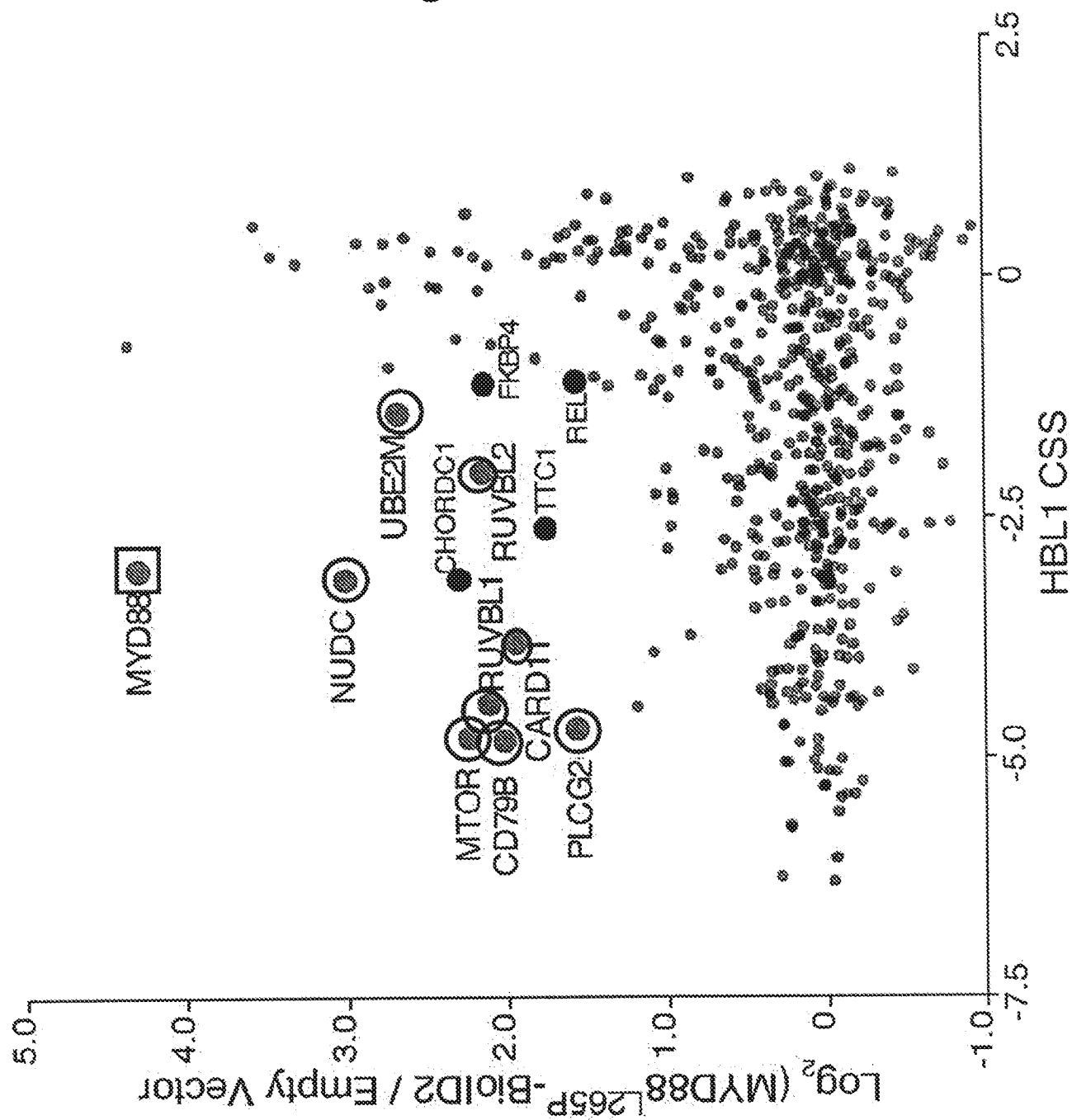
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Figure 14H



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Figure 15A



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Figure 15B

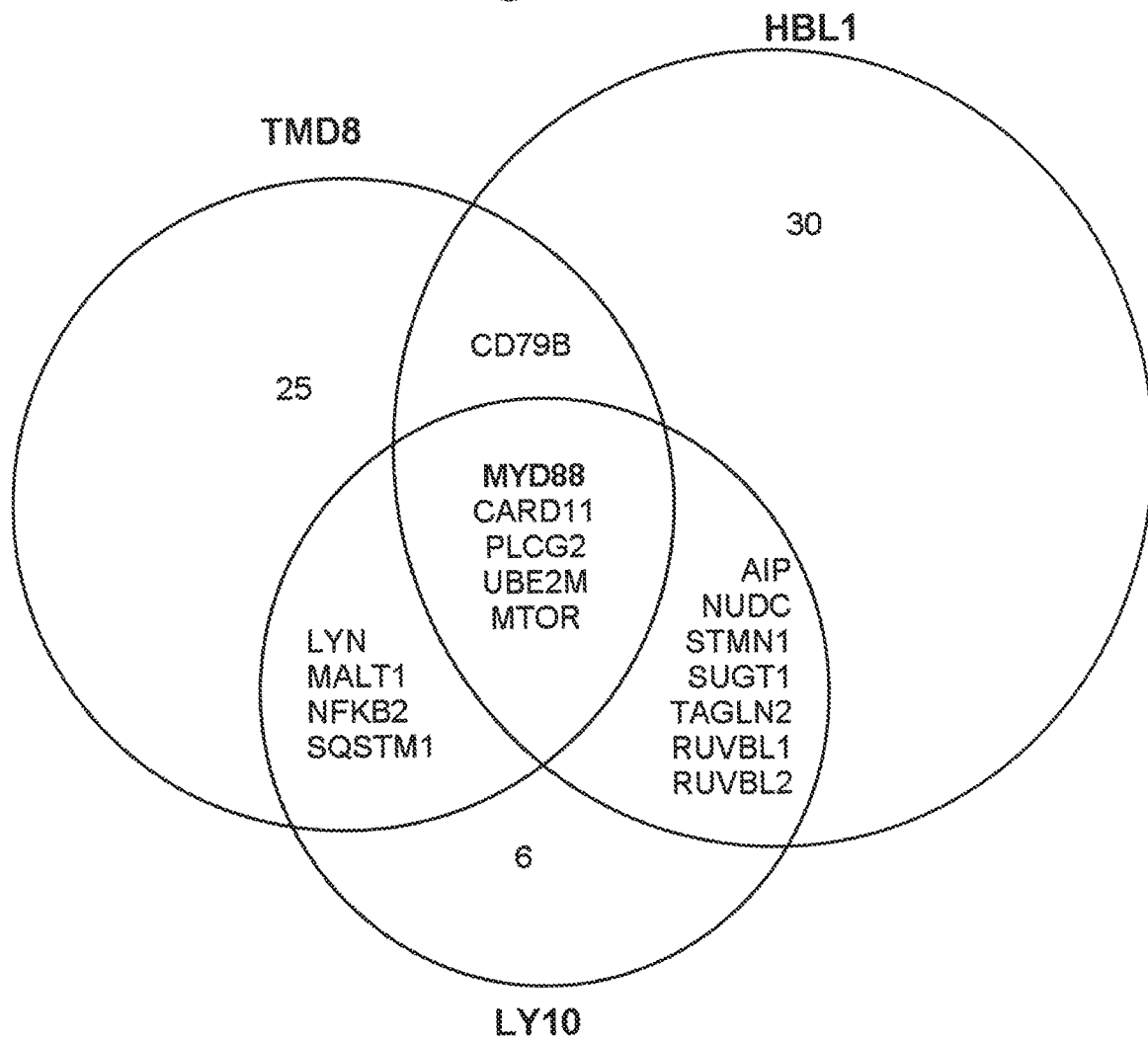


Figure 15C

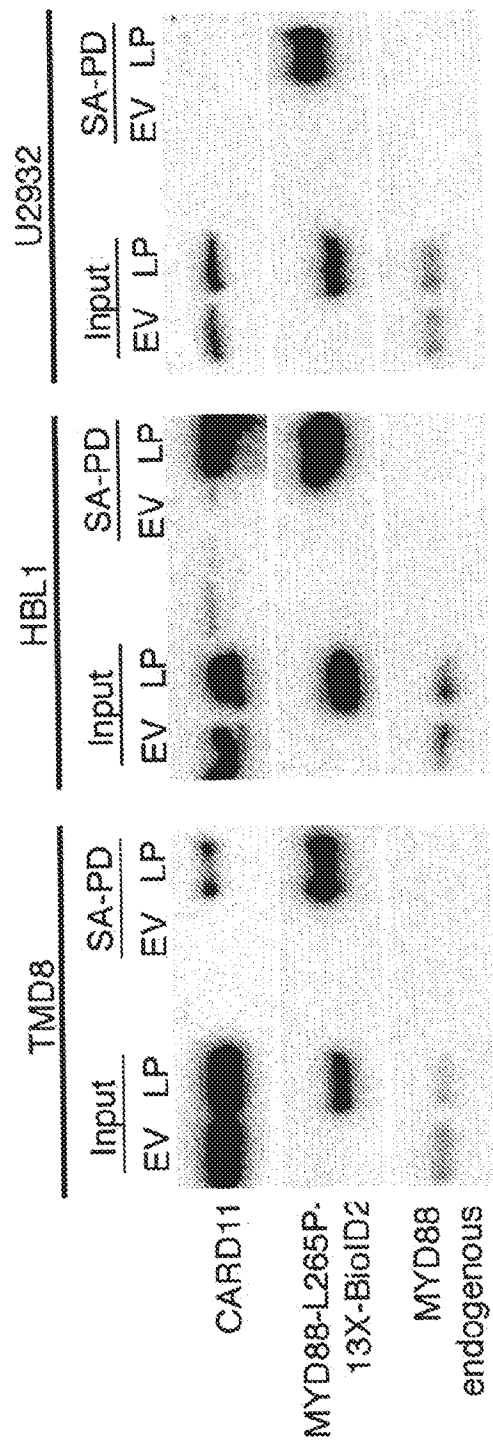
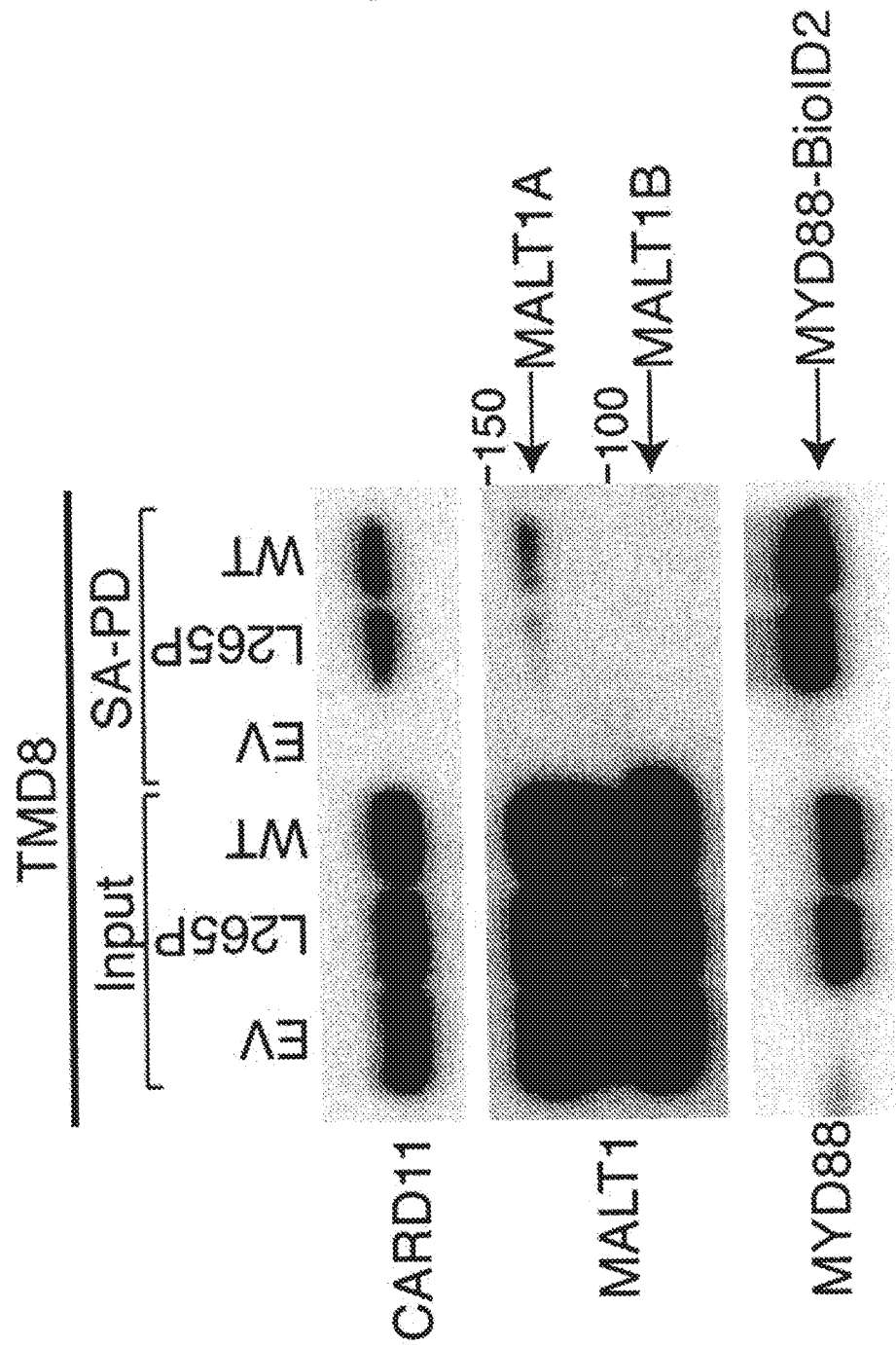
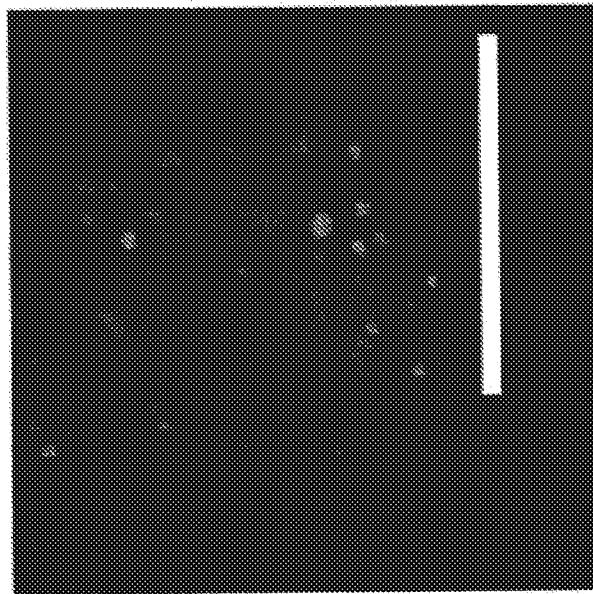
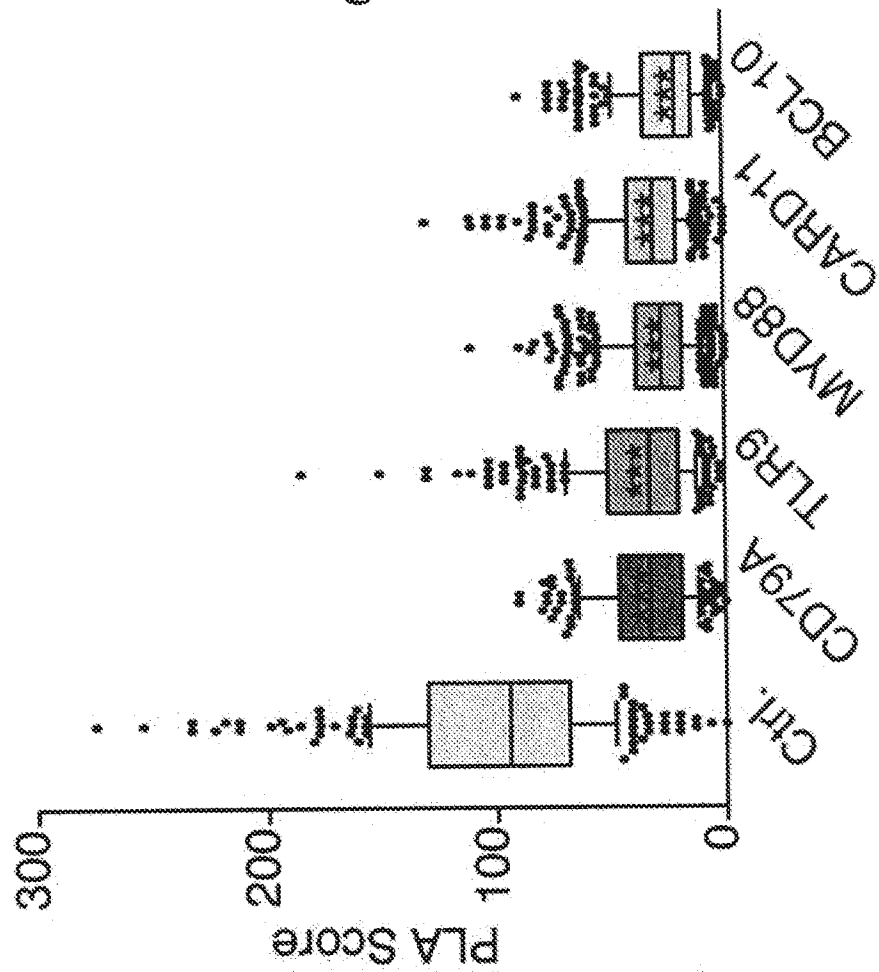


Figure 15D



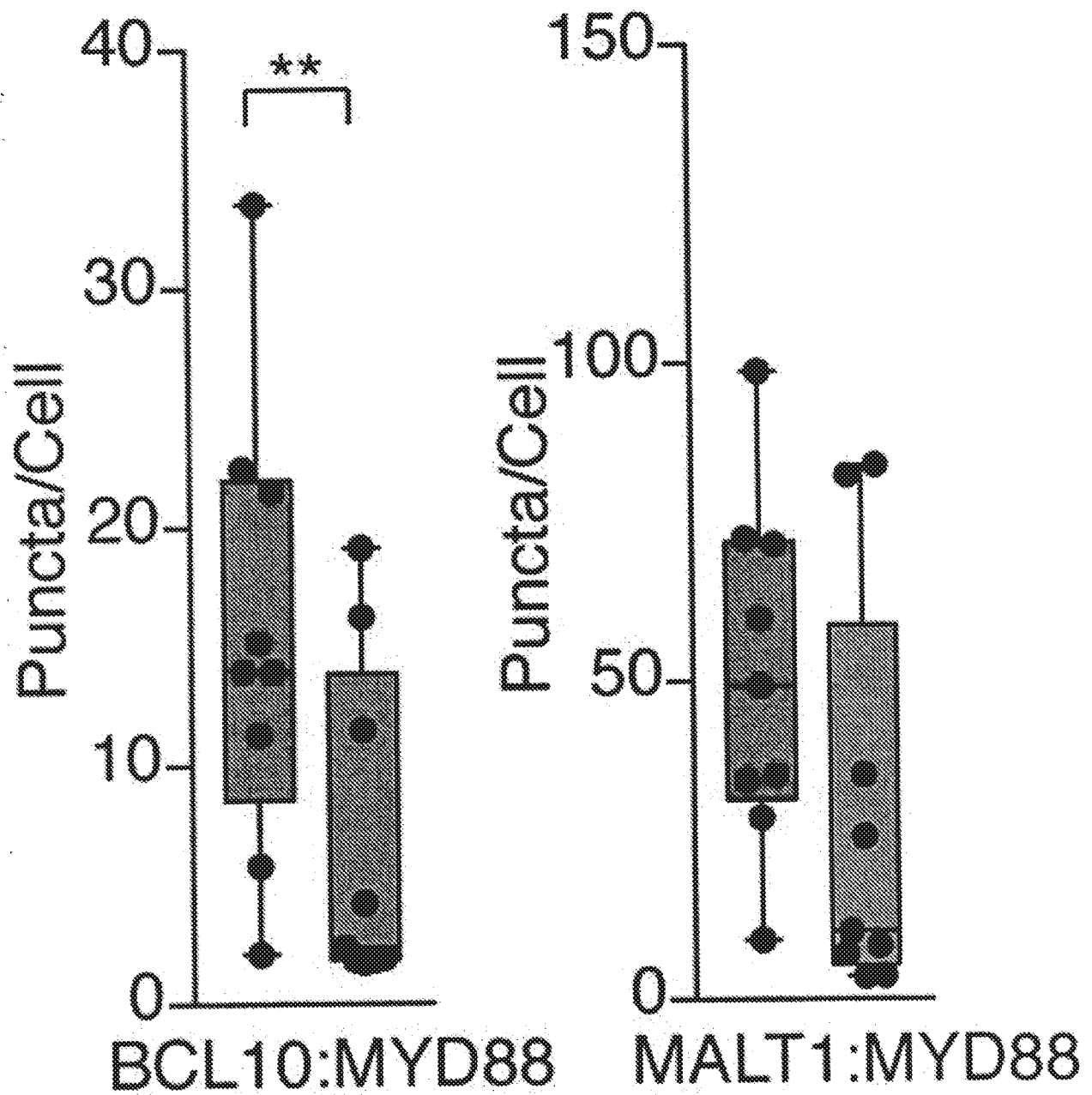
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Figure 15E



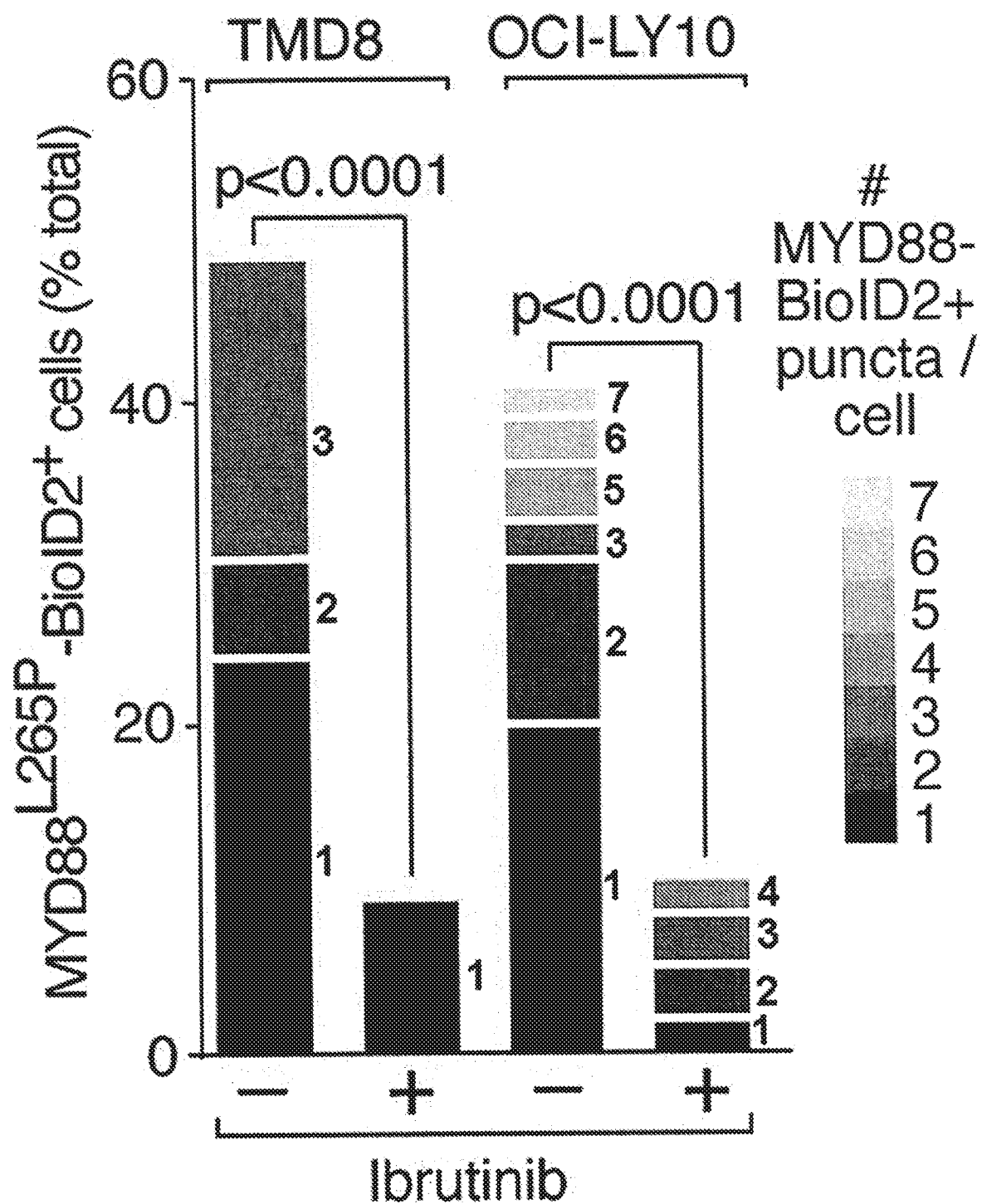
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Figure 15F



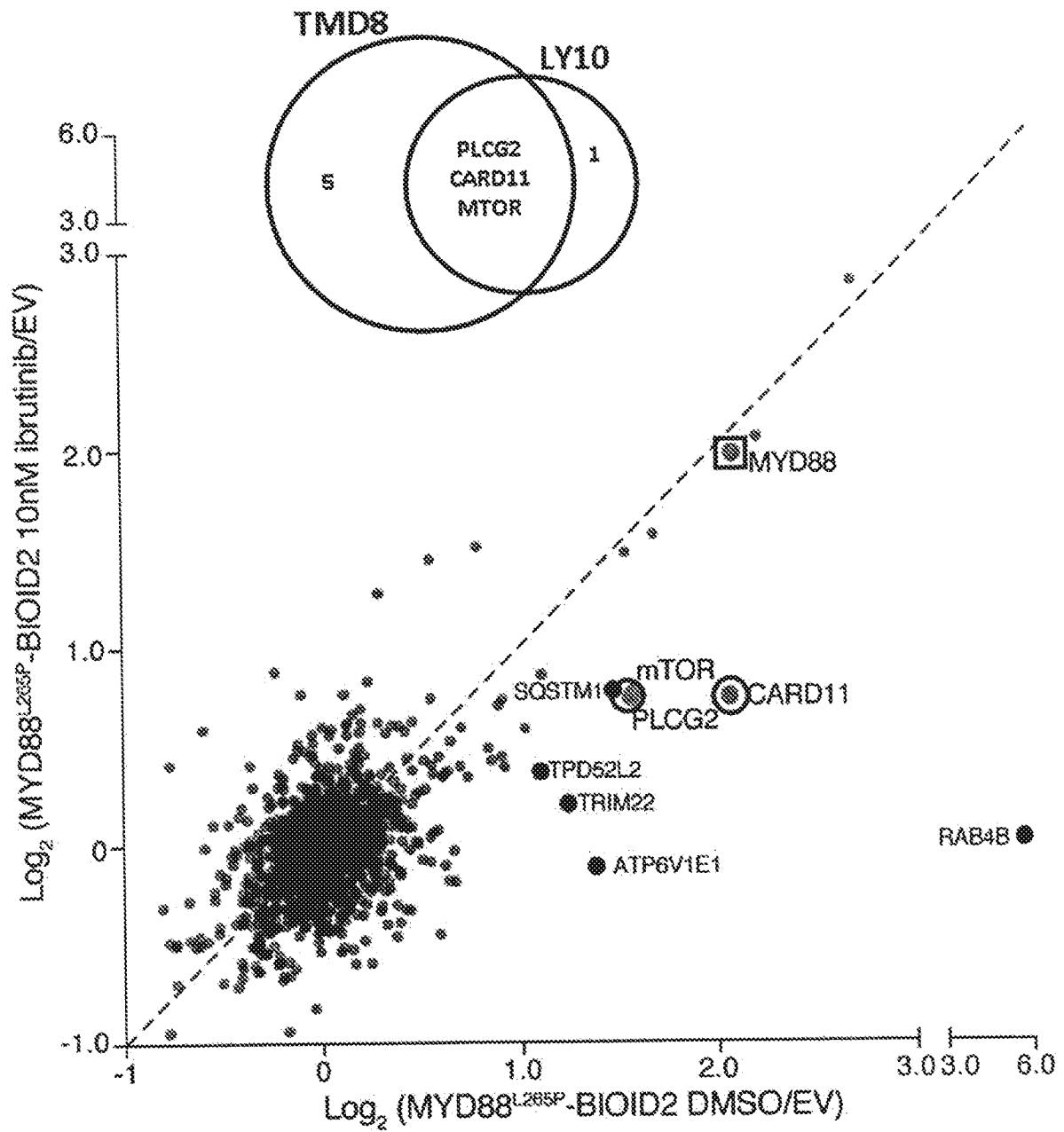
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Figure 16A



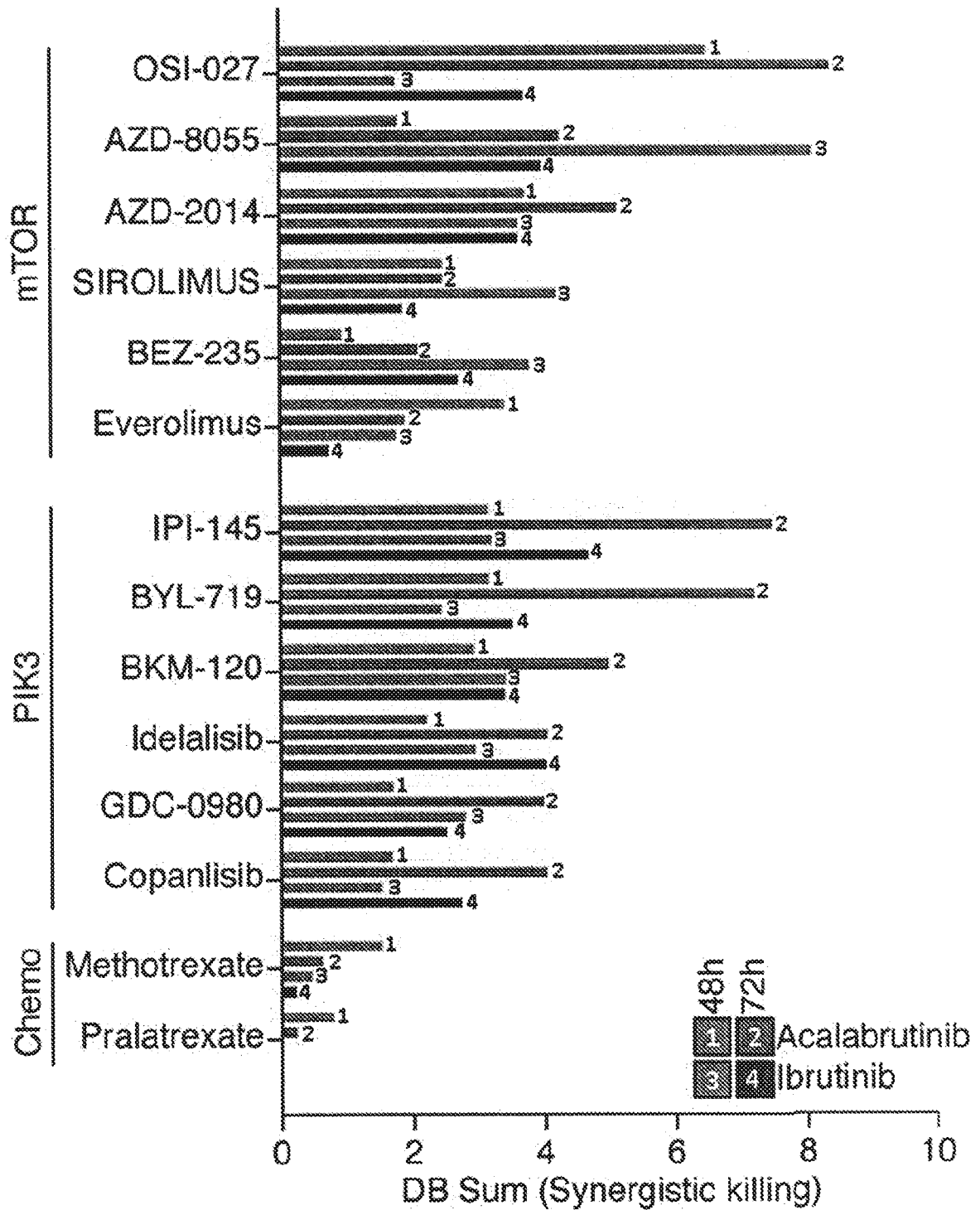
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Figure 16B



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Figure 16C



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Figure 17A

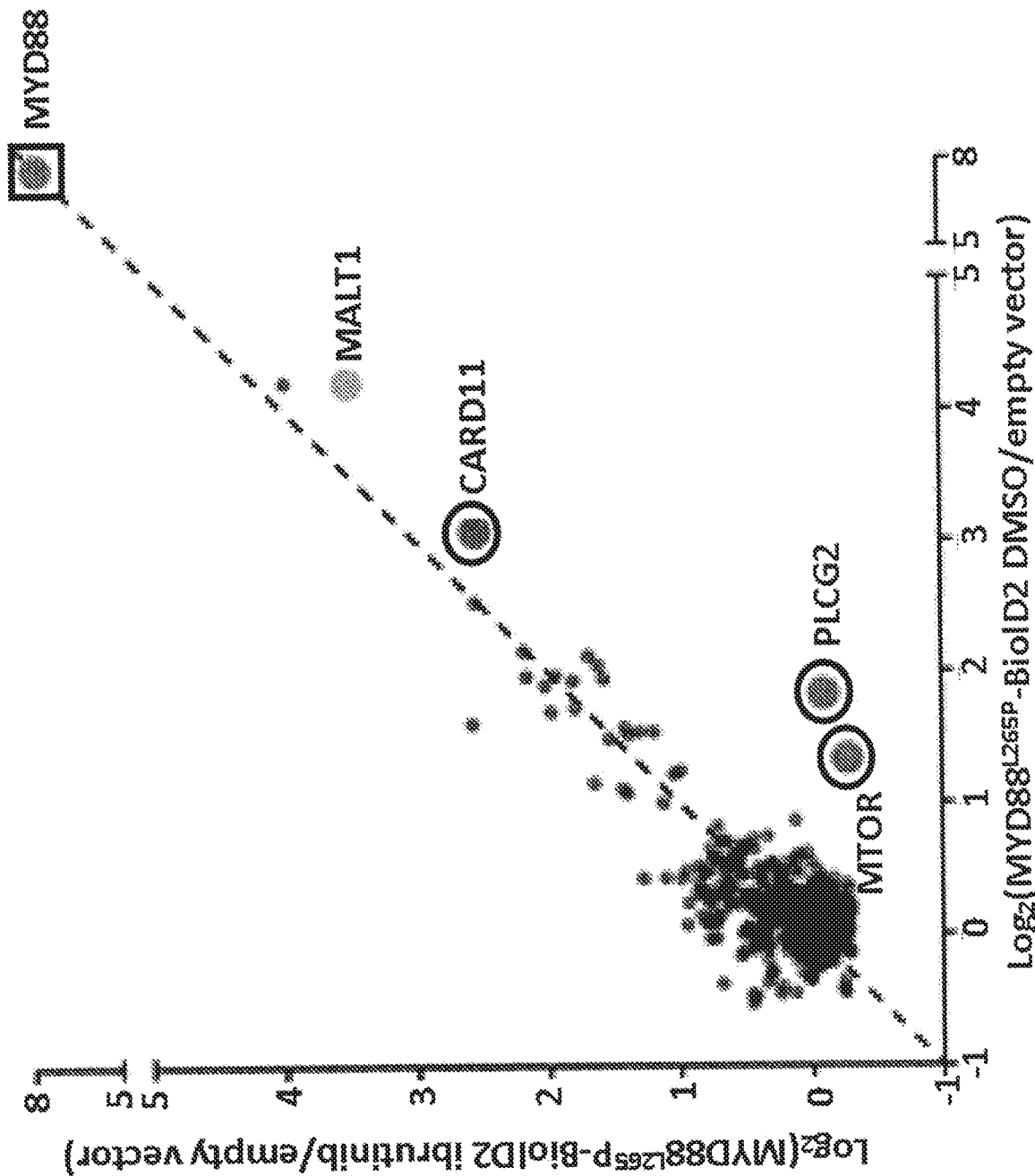
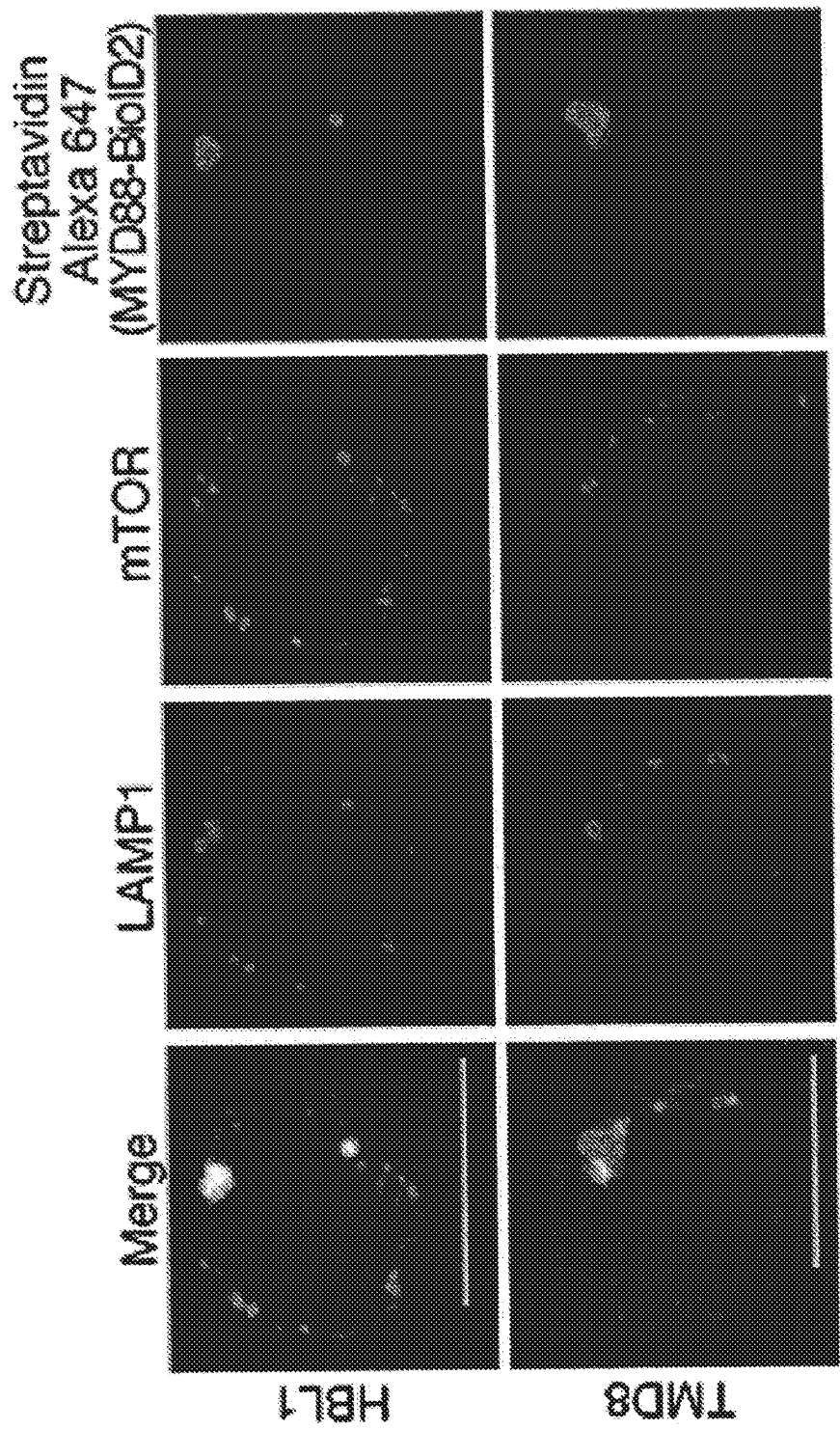
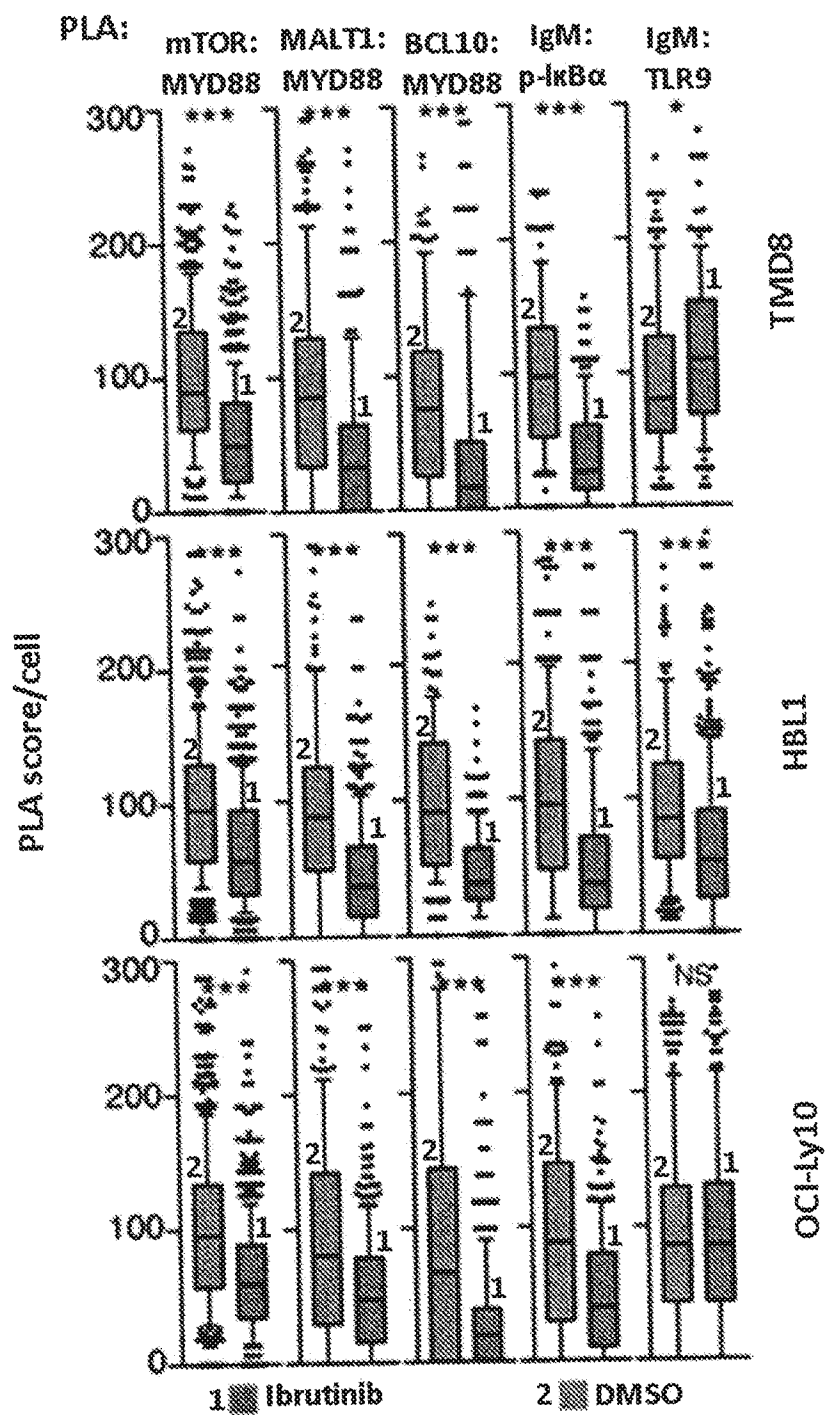


Figure 17B



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Figure 17C



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Figure 17D

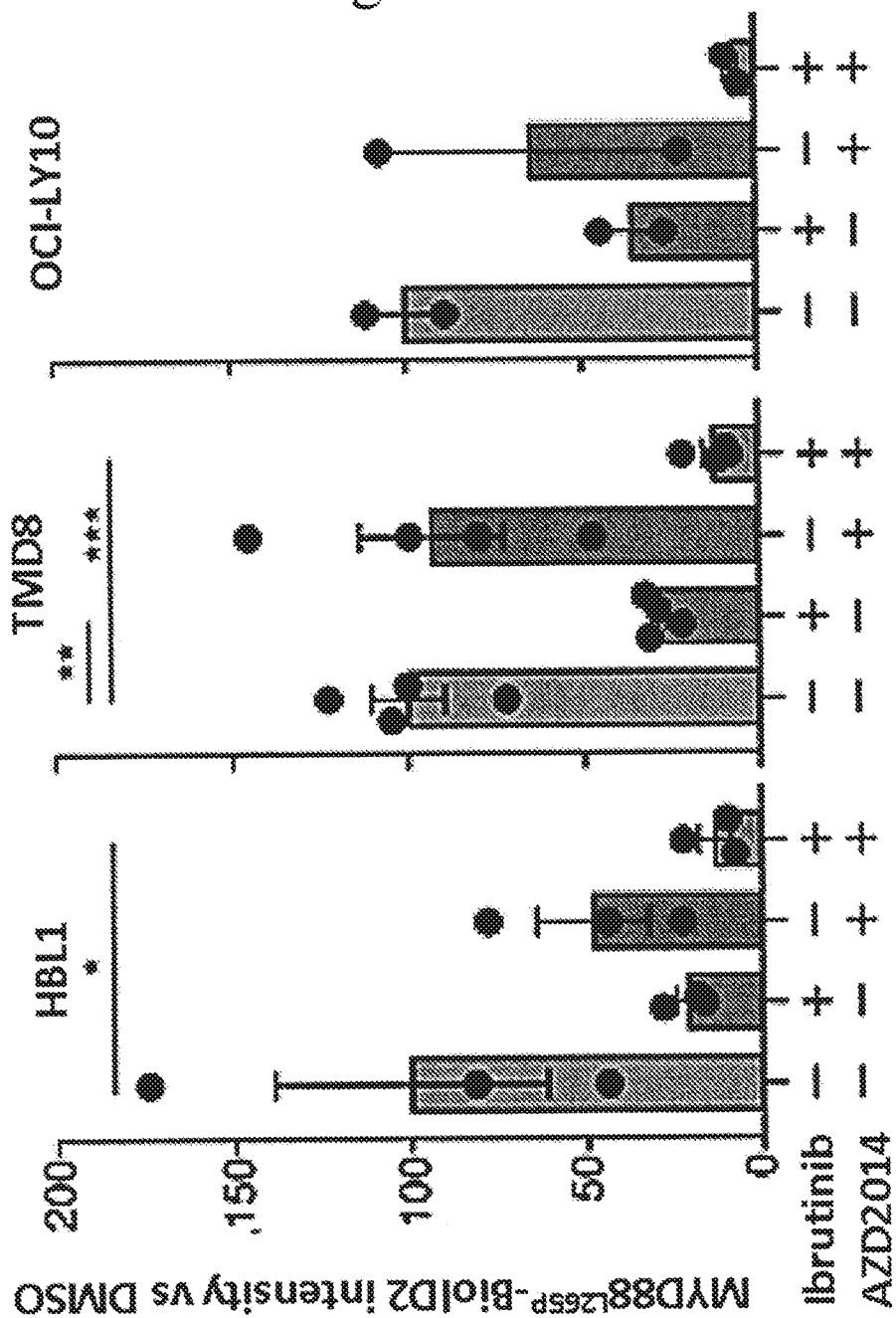
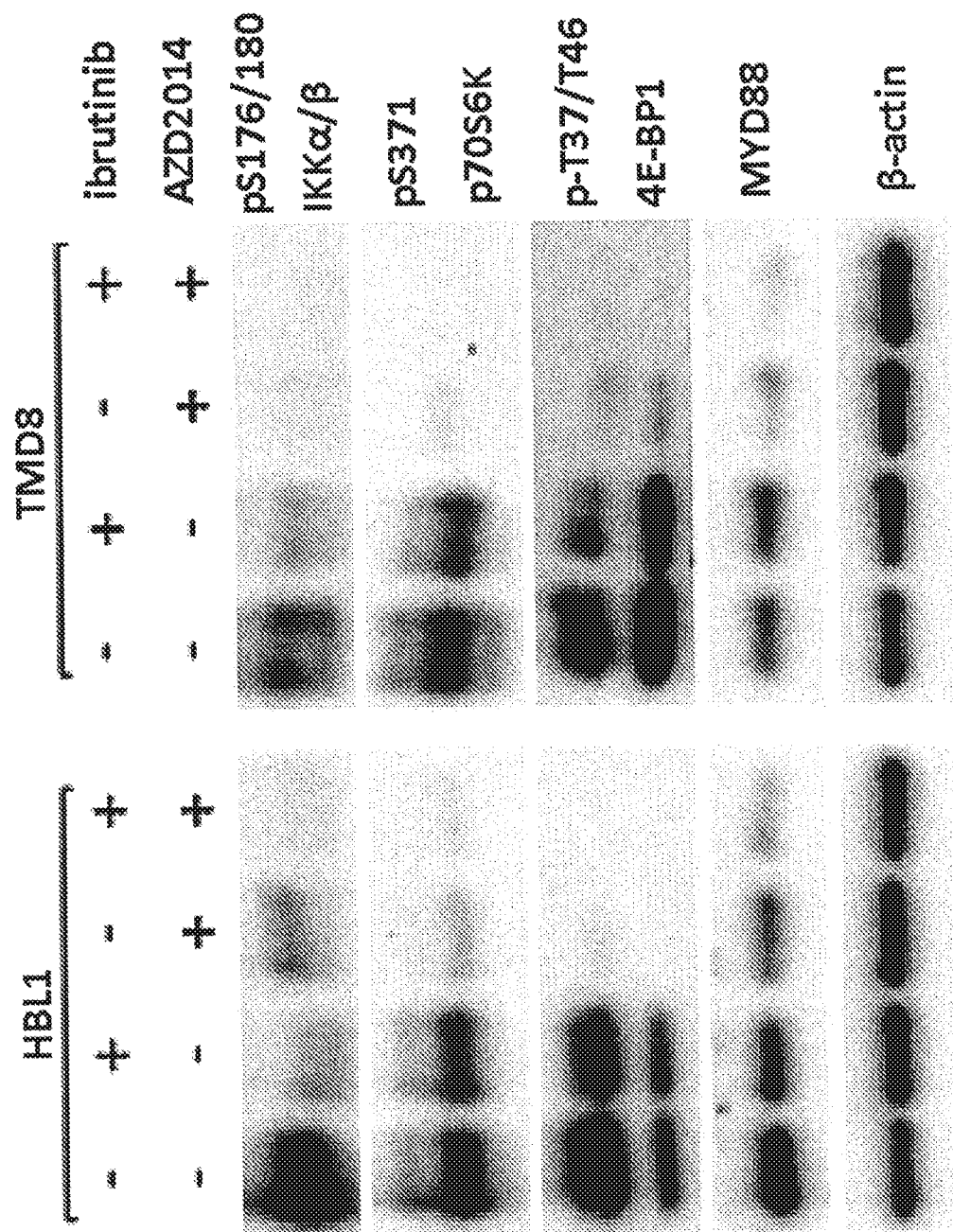
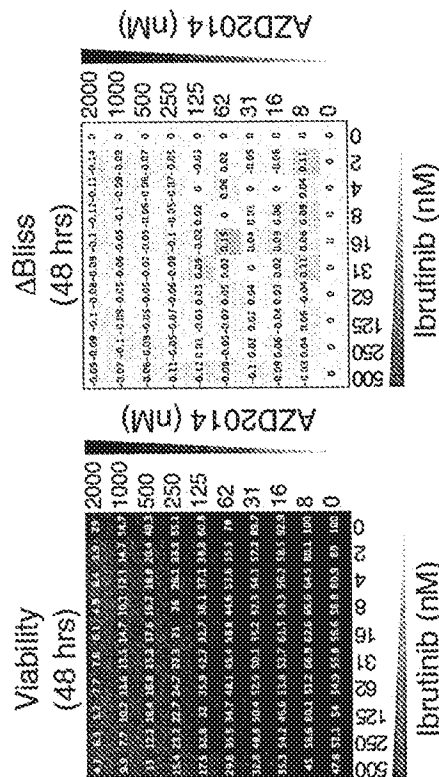
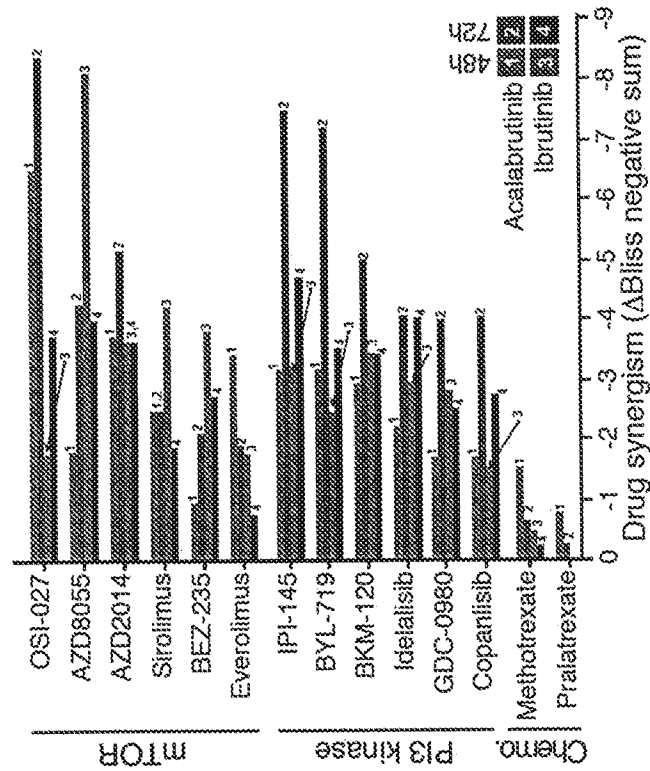


Figure 17E



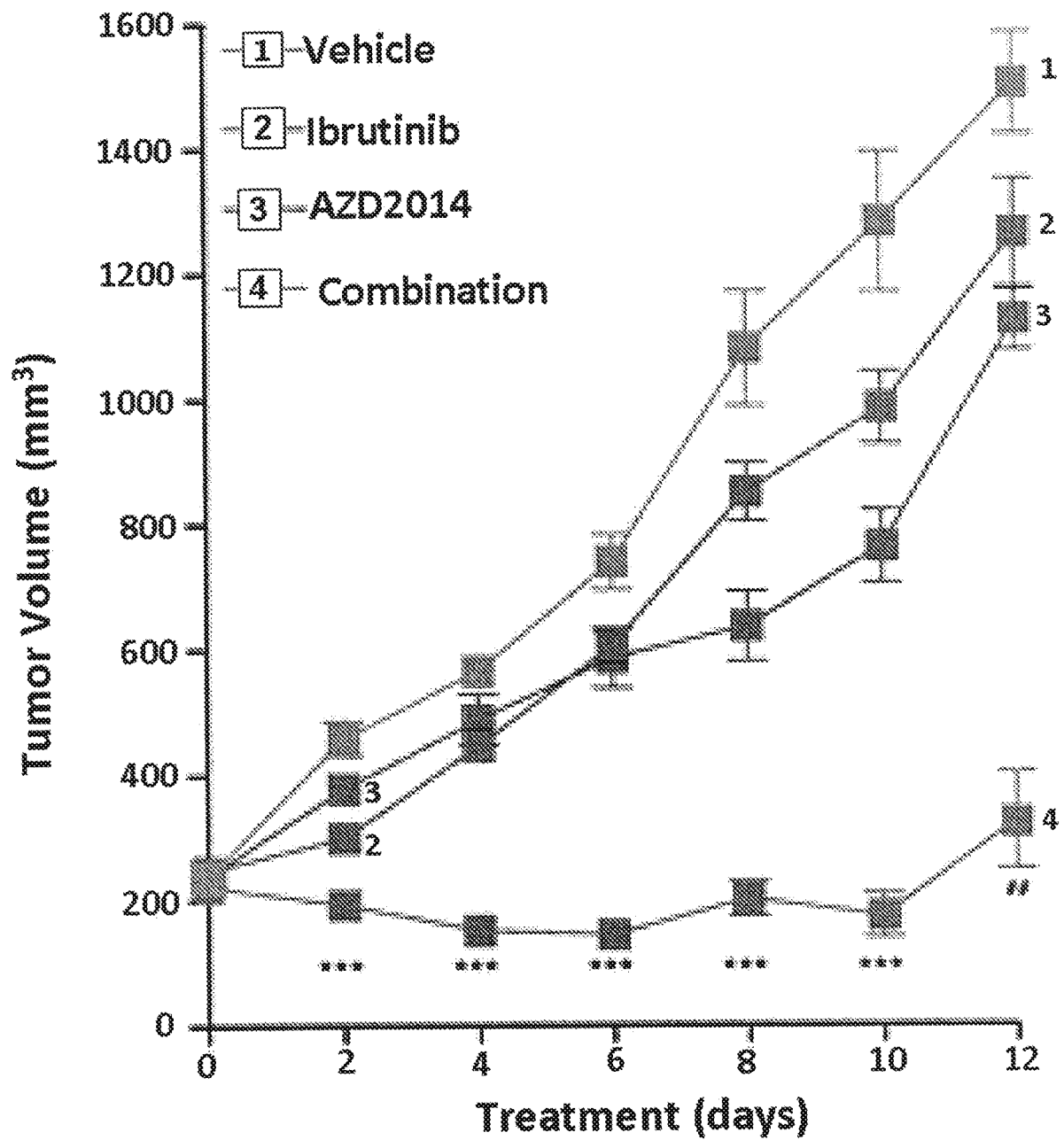
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Figure 17F



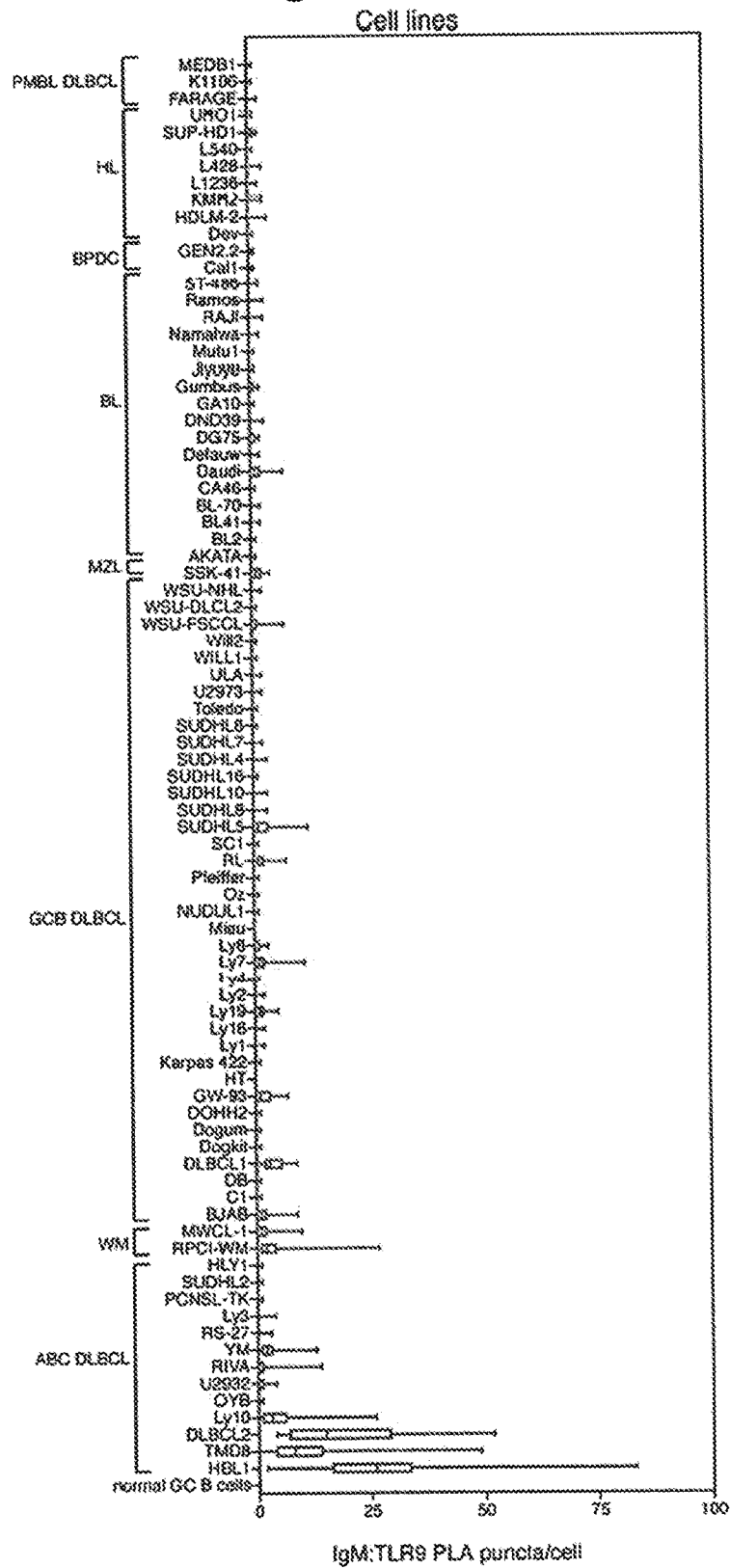
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17G



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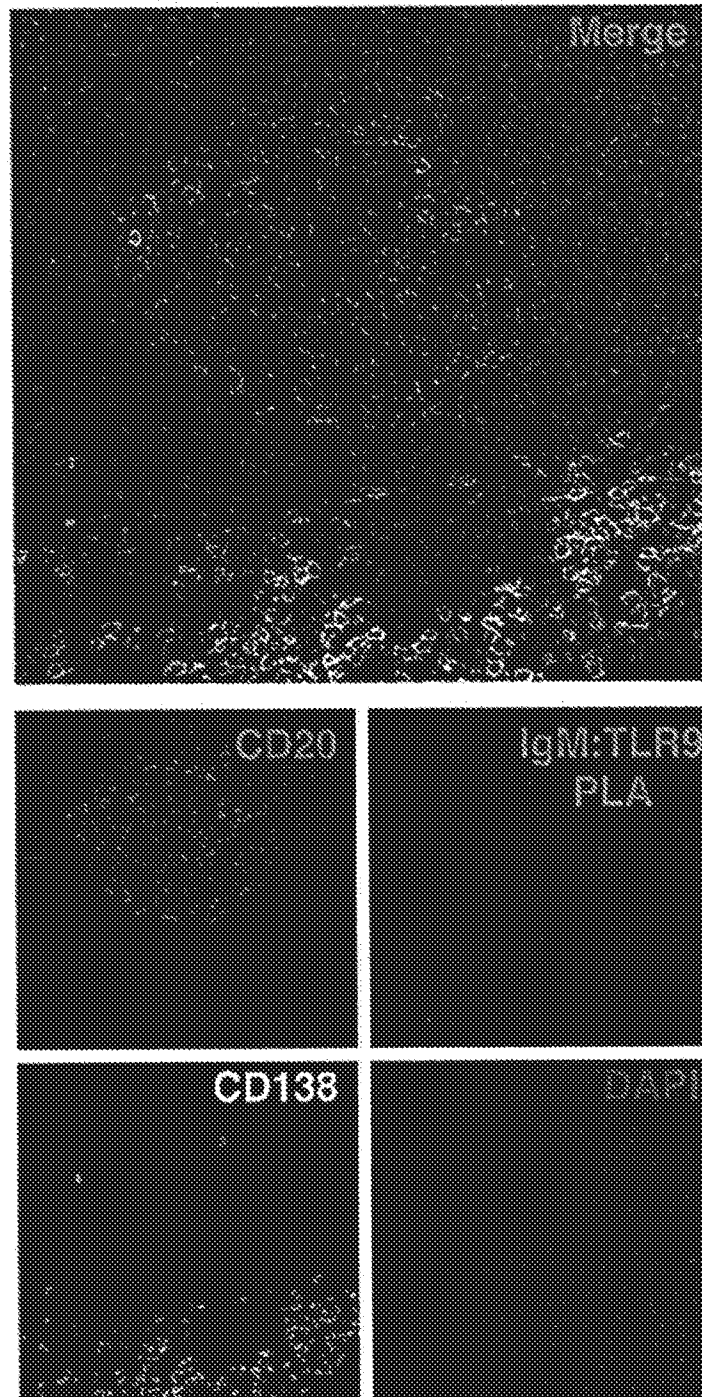
Figure 18A



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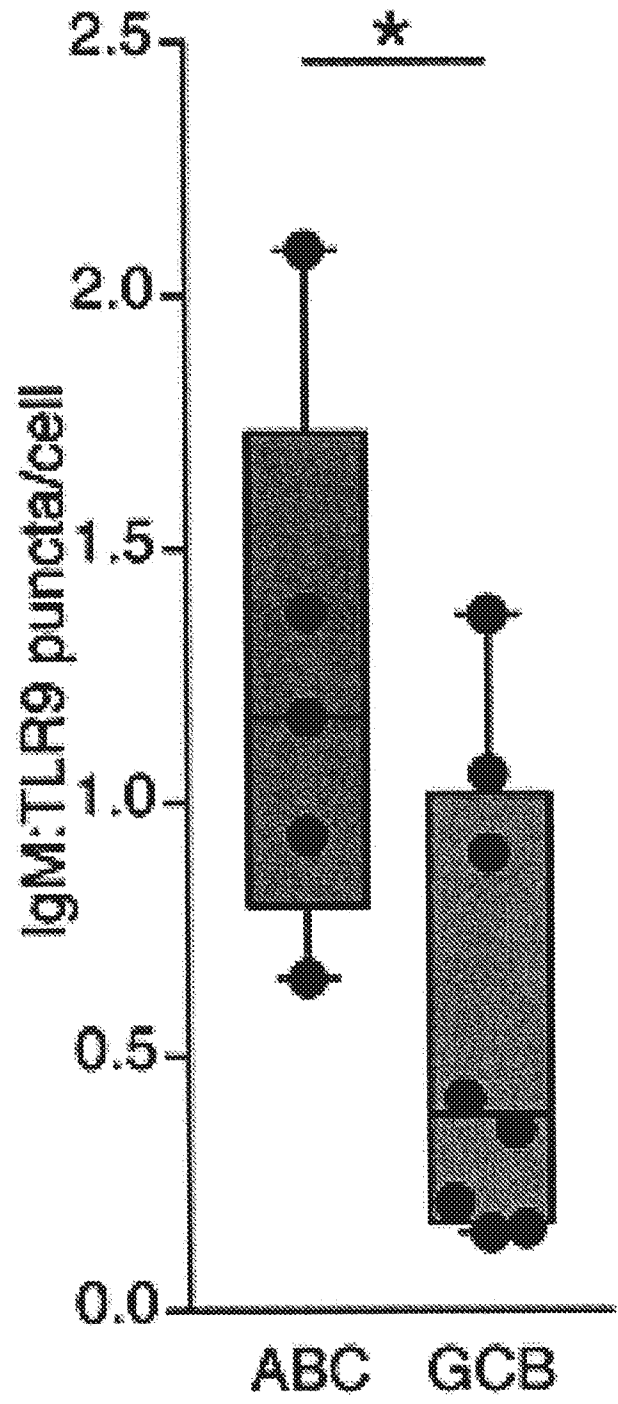
Figure 18B

Reactive lymph node



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Figure 19A



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Figure 19B

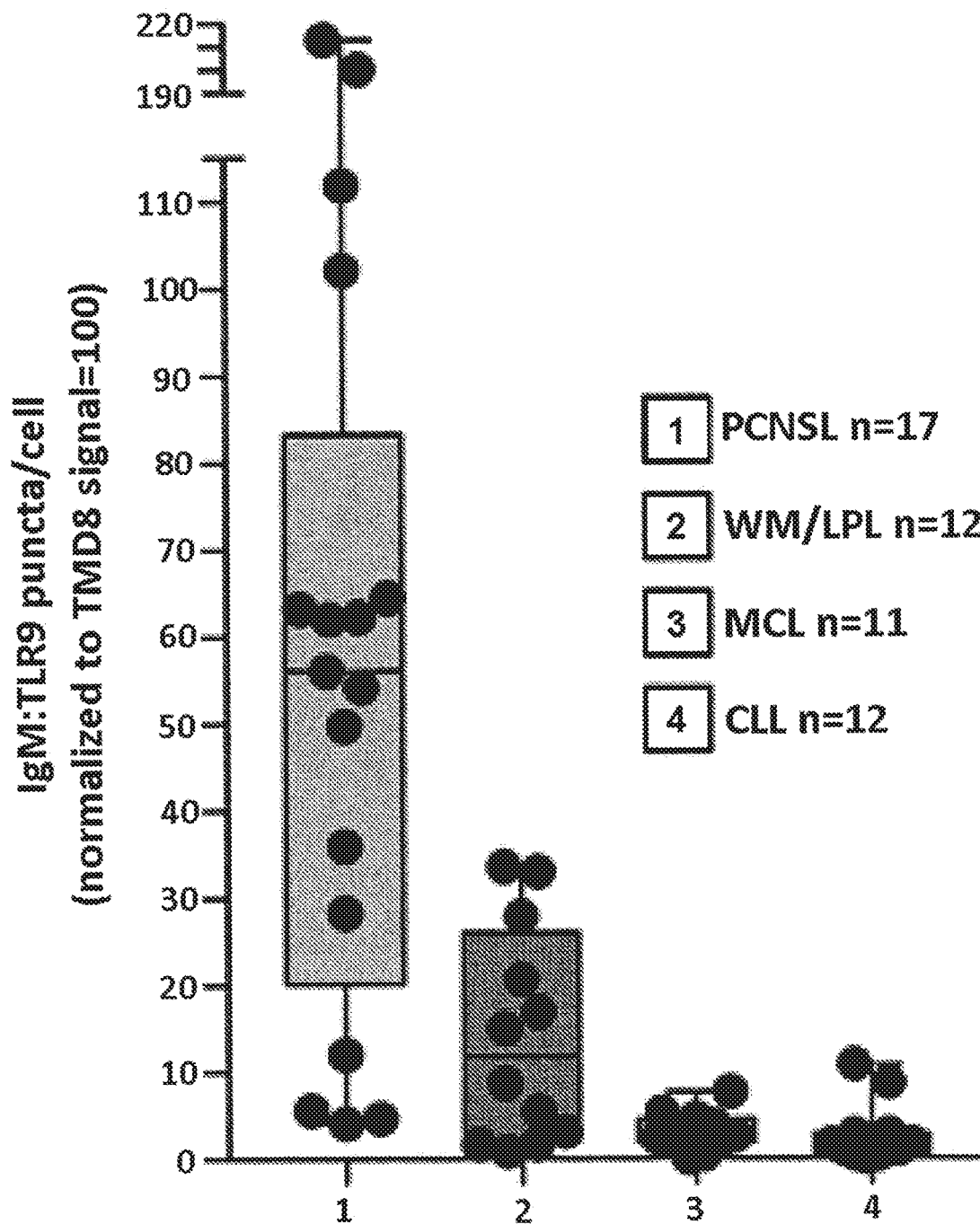
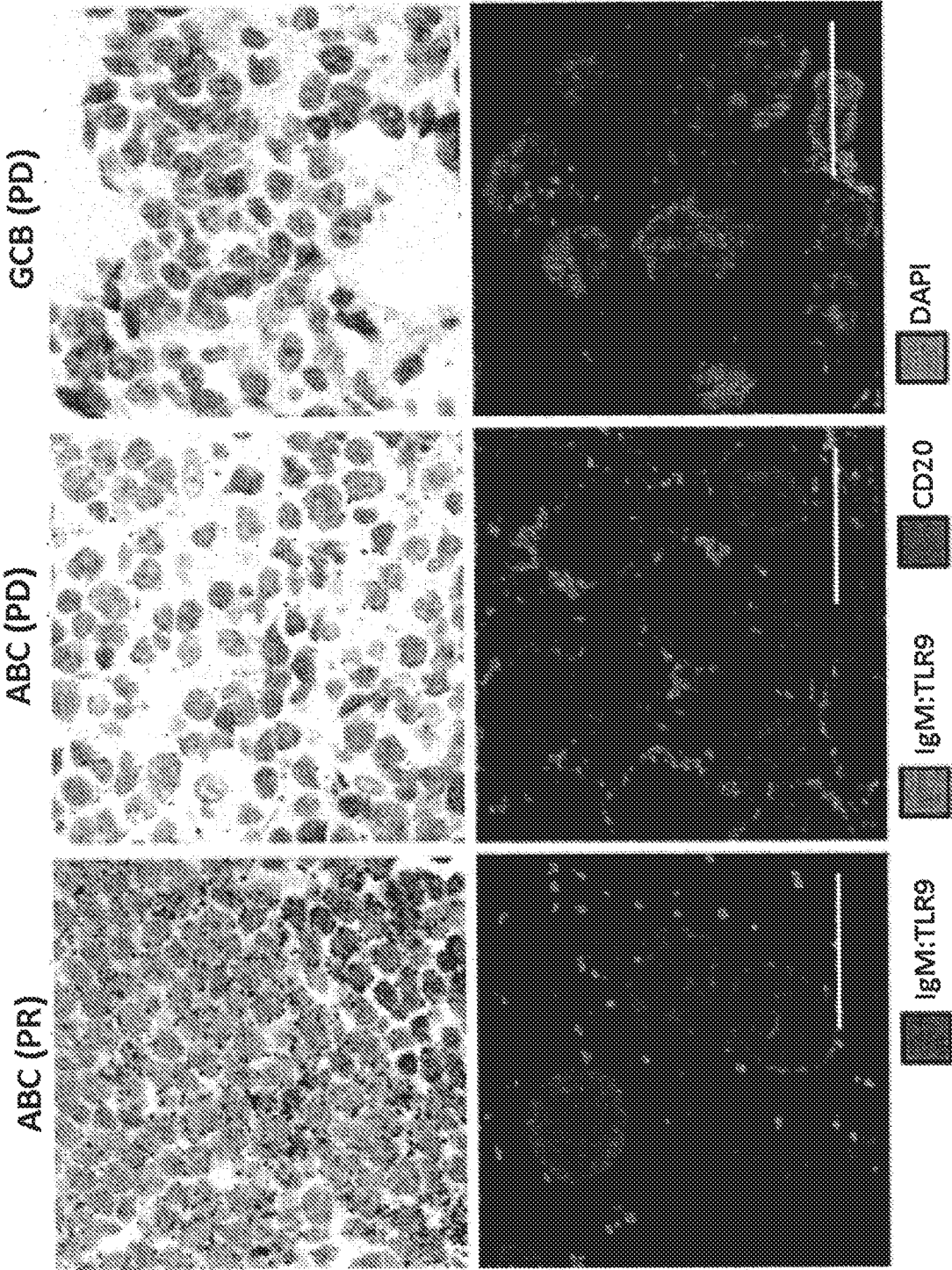
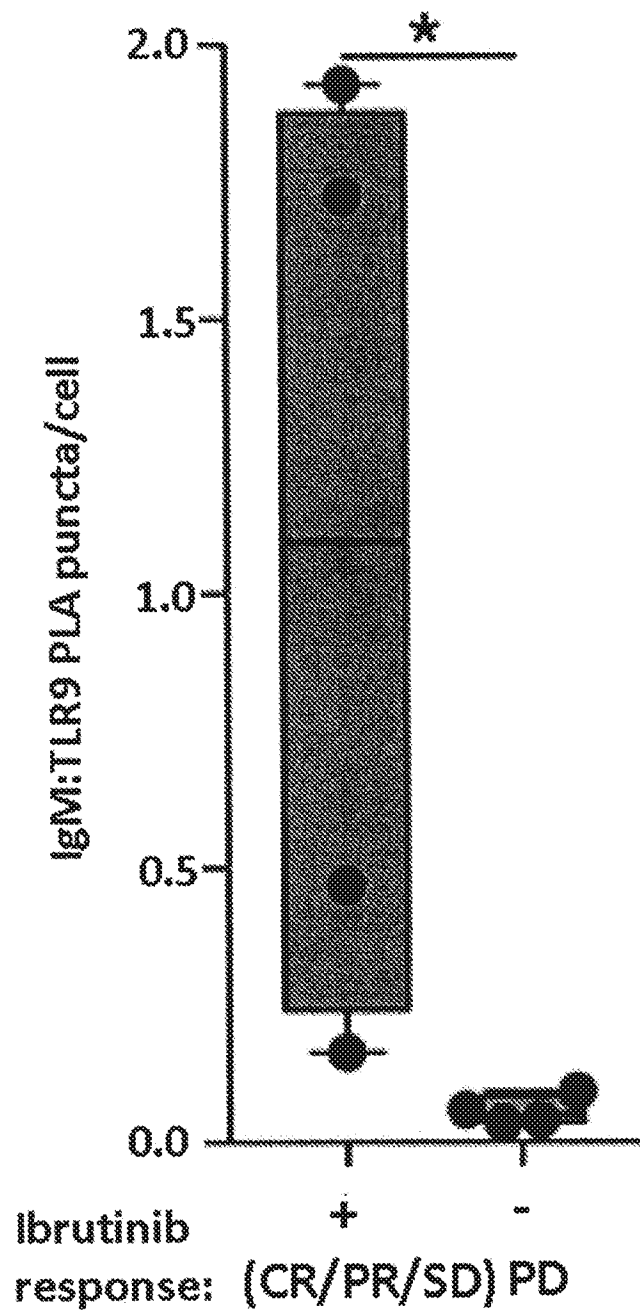


Figure 19C



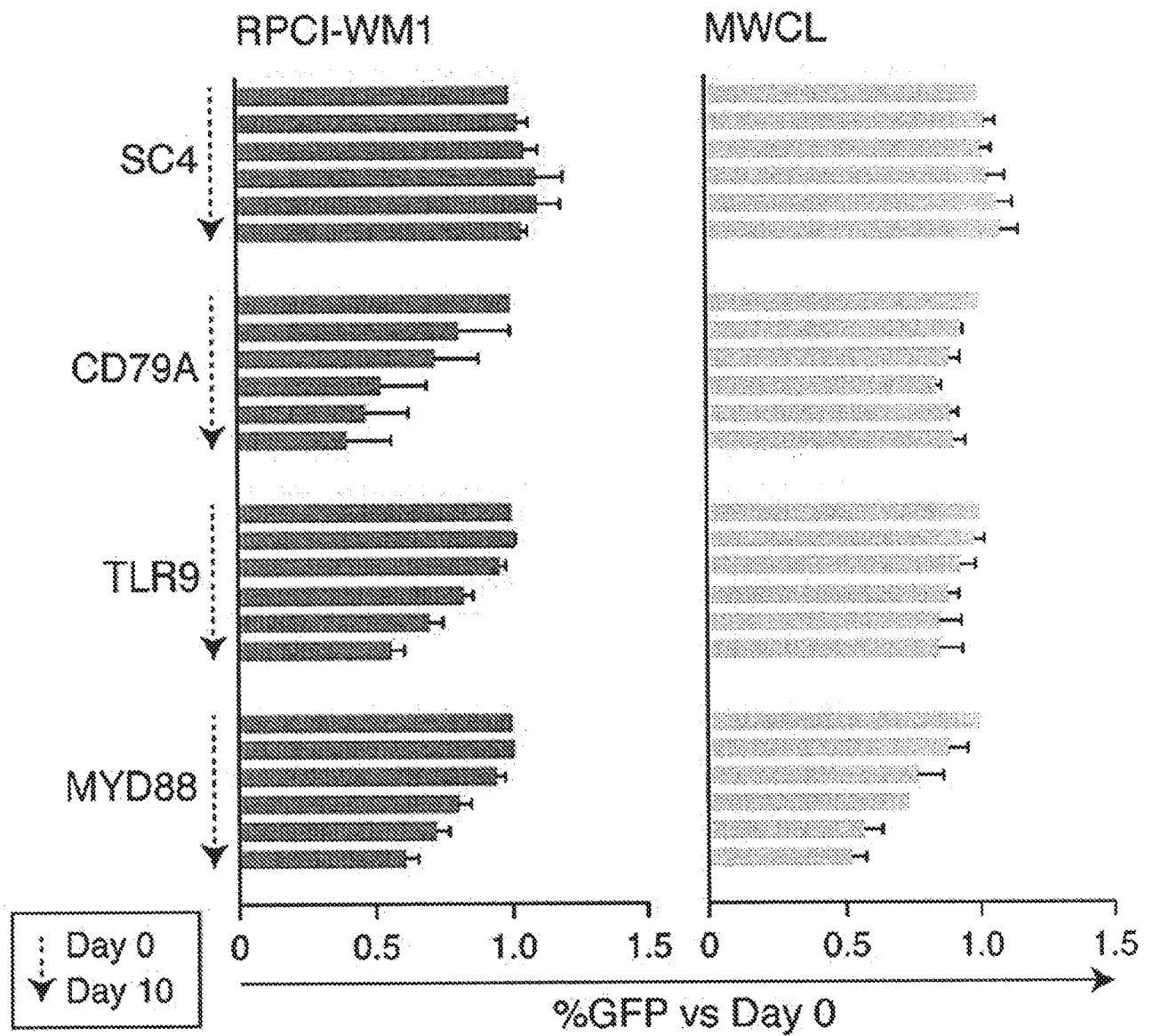
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Figure 19D



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Figure 20A

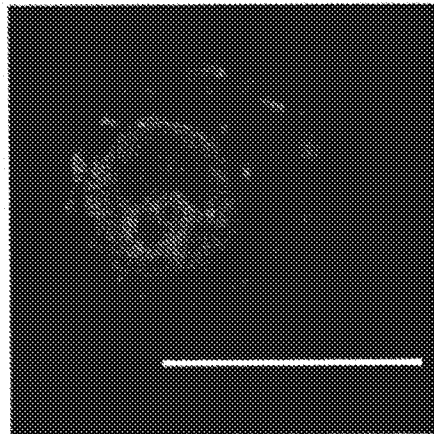


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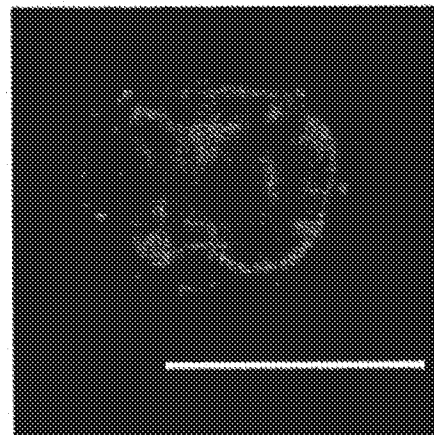
Figure 20B

IgM:TLR9
PLA

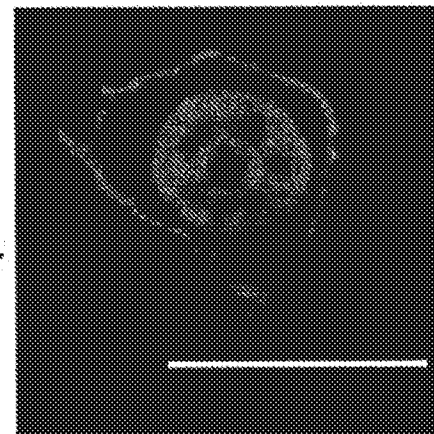
HBL1



RPCI-WM1

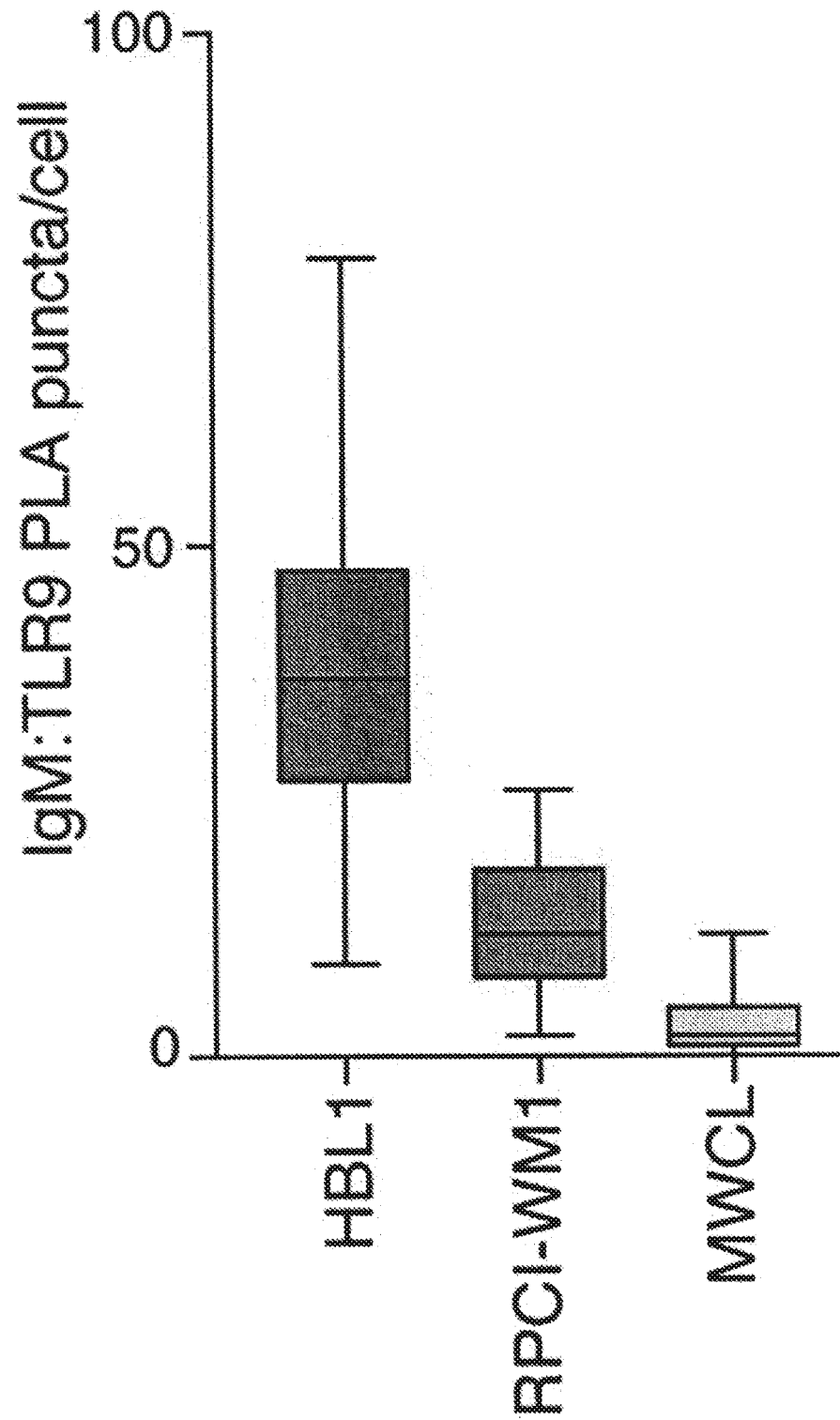


MWCL



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Figure 20C



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2018/025377

A. CLASSIFICATION OF SUBJECT MATTER
INV. G01N33/574 A61K31/00 G01N33/68
ADD.

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)
G01N A61K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2014/122275 A1 (FUNDACIÓN PÚBLICA ANDALUZA PROGRESO Y SALUD [ES]) 14 August 2014 (2014-08-14)	1-4
Y	whole document, in particular p. 6, l. 25-31; p. 8, l. 20 - p. 9, l. 11; p. 10, l. 16-25; p. 11, l. 10-31; p. 12, l. 3-27; p. 15, l. 8-14 p. 45, l. 14 - p. 46, l. 5; example 1, 2, 4 ----- -/--	1-4



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

12 June 2018

Date of mailing of the international search report

20/08/2018

Name and mailing address of the ISA/

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Authorized officer

Chrétien, Eva Maria

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2018/025377

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LM Staudt: "NIH Wednesday afternoon lecture - Therapy of lymphoma inspired by functional and structural genomics", 1 February 2017 (2017-02-01), XP054978411, Retrieved from the Internet: URL:https://videocast.nih.gov/Summary.asp?Live=21795&bhcp=1 [retrieved on 2018-06-11] whole documentation, in particular time points 33:20 min, 35:38 min, 36:45 min, 36:59 min, 37:21 min, 37:54 min -----	1-4
A	KATHRIN KLÄSENER; PALASH C MAITY; ELIAS HOBEIKA; JIANYING YANG; MICHAEL RETH: "B cell activation involves nanoscale receptor reorganizations and inside-out signaling by Syk", ELIFE, vol. 3, 24 June 2014 (2014-06-24), pages 1-17, XP055184372, the whole document -----	1-4
A	WO 2014/016401 A1 (CT LEON BERARD [FR]; CENTRE NAT RECH SCIENT [FR]; INST NAT SANTE RECH) 30 January 2014 (2014-01-30) the whole document -----	1-4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/025377

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:

see additional sheet

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 an 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.:

1-4

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-4

A method of detecting an association of a first protein and a second protein, the method comprising: (A) obtaining a sample comprising one or more B cells; (B) contacting the sample with a first antibody and a second antibody, wherein the first antibody binds the first protein and does not bind the second protein and the second antibody binds the second protein and does not bind the first protein, wherein the first antibody is of a species different than the first protein and the second antibody is of a species different than the second protein, and wherein the first antibody is of a species different than the second antibody; (C) performing a proximity ligation assay using the first antibody and the second antibody and a detectable probe to produce a PLA signal of one or more detectable puncta; (D) contacting the sample with a third antibody, wherein the third antibody binds to a protein on the B cell surface, and wherein the third antibody is labeled with a detectable label; and (E) detecting the proximity ligation assay signal of (C) wherein a positive PLA signal indicates association of the first and second proteins in one or more of the B cells comprised in the sample.

2. claims: 5(completely); 7-16(partially)

A method of identifying a B cell disorder having a greater probability of responding to treatment using an inhibitor, the method comprising: (a) obtaining a B cell disorder sample; (b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC1 complex, wherein the two proteins are different from one another and are components of different complexes, and wherein the analysis comprises detecting an association of the two proteins according to any one of claims 1-4; and (c) identifying the sample having the presence of the protein complex of (b) as a B cell disorder having a greater probability of responding to treatment using an inhibitor of the BCR complex, an inhibitor of the MYD88 complex, or an inhibitor of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b). Respective BCR inhibitor identified with the method.

3. claims: 6(completely); 7-16(partially)

A method of identifying a B cell disorder having a greater probability of responding to treatment using an inhibitor,

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the method comprising: (a) obtaining a B cell disorder sample; (b) analyzing the sample for the presence of a protein complex, wherein the protein complex comprises two proteins, wherein each protein is a protein component of at least one of the following complexes: the BCR complex, the MYD88 complex, and the mTORC 1 complex, wherein the two proteins are different from one another and are components of different complexes; (b') detecting in the sample the presence of the protein complex of (b); and (c) identifying the sample having the presence of the protein complex of (b) as a B cell disorder having a greater probability of responding to treatment using an inhibitor of the BCR complex, an inhibitor of the MYD88 complex, or an inhibitor of the mTORC1 complex, as compared to a sample having an absence of the protein complex of (b). Respective BCR inhibitor identified with the method.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2018/025377

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2014122275	A1	14-08-2014	NONE
WO 2014016401	A1	30-01-2014	AU 2013294967 A1 19-02-2015
		CA 2880105 A1 30-01-2014	
		CN 104823053 A 05-08-2015	
		EP 2877853 A1 03-06-2015	
		JP 2015525881 A 07-09-2015	
		US 2015212087 A1 30-07-2015	
		WO 2014016401 A1 30-01-2014	