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(54) **Title:** COMPOSITIONS AND METHODS FOR TREATING NEOPLASIAS

(57) **Abstract:** The invention provides therapeutic combinations comprising an agent that inhibits Notch signaling and an agent that inhibits B cell receptor signaling, and methods of using such agents to inhibit the survival or proliferation of a neoplastic cell.

COMPOSITIONS AND METHODS FOR TREATING NEOPLASIAS

CROSS-REFERENCE TO RELATED APPLICATION

This application claims priority to U.S. Provisional Patent Application Serial No. 62/383,111, filed on September 2, 2016. The entire content of this application is hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

Chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) are two prevalent lymphoid malignancies that share the phenotype of small, mature, non-germinal center B-cells, but demonstrate distinctive clinical and biological features. Somatic mutations of the *NOTCH1* gene are seen in 8-15% of CLL and MCL patients, while recurrent *NOTCH2* mutations have also been reported in MCL. Notch gene mutations are associated with decreased overall survival and reduced time to treatment in both CLL and MCL, while in CLL, *NOTCH1* mutations also appear to increase the risk of high-grade transformation, and reduce responsiveness to anti-CD20 monoclonal antibody therapy. In recent years, the clinical development of drugs targeting B-cell receptor (BCR) signaling and anti-apoptotic pathways have provided new options for patients with small B-cell lymphomas, but new approaches are still needed to improve response rate and prevent development of secondary drug resistance.

SUMMARY OF THE INVENTION

The invention provides therapeutic combinations comprising an agent that inhibits Notch signaling and an agent that inhibits B cell receptor signaling, and methods of using such agents to inhibit the survival or proliferation of a neoplastic cell.

In one aspect, the invention provides a pharmaceutical composition containing an effective amount of an agent that inhibits the expression or activity of a Notch polynucleotide or polypeptide and an effective amount of an agent that inhibits the expression or activity of a functional component of a B cell receptor polypeptide or polynucleotide.

In another aspect, the invention provides a method of inhibiting the survival or proliferation of a neoplastic cell, the method involving contacting the cell with an agent that inhibits expression or activity of a Notch polynucleotide or polypeptide and an effective

amount of an agent that inhibits expression or activity of a functional component of a B cell receptor polypeptide or polynucleotide

In yet another aspect, the invention provides a method of inhibiting the survival or proliferation of a neoplastic cell, the method involving contacting the cell with a gamma secretase inhibitor and ibrutinib, thereby inhibiting the survival or proliferation of the neoplastic cell.

In still another aspect, the invention provides a method of treating a neoplasia in a subject, the method involving administering to the subject an agent that inhibits the expression or activity of a Notch polynucleotide or polypeptide and an effective amount of an agent that inhibits the expression or activity of a functional component of a B cell receptor polypeptide or polynucleotide, thereby treating cancer in the subject.

In still another aspect, the invention provides a method of treating a subject having a leukemia or lymphoma, the method involving administering to the subject a gamma secretase inhibitor and ibrutinib.

In still another aspect, the invention provides a method of treating a subject having a leukemia or lymphoma that has developed resistance to a B cell receptor signaling inhibitor, the method involving administering a gamma secretase inhibitor and an agent that inhibits expression or activity of a functional component of the B cell receptor.

In various embodiments of any of the above aspects or any other aspect of the invention delineated herein, the agent is a small compound, polypeptide, or polynucleotide. In various embodiments of any of the above aspects or any other aspect of the invention delineated herein, the agent that inhibits Notch expression or activity is a gamma secretase inhibitor (e.g., Compound E, MK-0752, PF03084014, RO-4929097, DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester, tetralin imidazole PF-03084014, LY3039478, and BMS906-024), a Notch signaling pathway inhibitory antibody (e.g., anti-Delta-like-4 antibody), or an anti-Notch1 antibody (e.g., OMP-52M521). In various embodiments of any of the above aspects, the agent that inhibits Notch expression or activity is an inhibitory nucleic acid molecule. In various embodiments of any of the above aspects, the agent that inhibits B cell receptor signaling is a PI3 kinase inhibitor (e.g., idelalisib), BTK inhibitor (e.g., ibrutinib, ACP-196, ONO/GS-4059, BGB-3111, and CC-292), SRC family kinase inhibitor (e.g., Dasatinib), SYK inhibitor (e.g., Fostamatinib), or a protein kinase C inhibitor (e.g., Midostaurin, Enzastuarin, or Sotrasturin). In embodiments of any of the above aspects, the agents are formulated together or are formulated separately for

simultaneous, separate or sequential co-administration. In embodiments of any of the above aspects or any other aspect of the invention delineated herein, a composition of the invention contains an agent that inhibits Notch expression or activity, an agent that inhibits B cell receptor expression or activity, and one or more additional therapeutic agents. In embodiments of any of the above aspects, the Notch activity is signaling. In embodiments of any of the above aspects, B cell receptor activity is signaling. The method further involves administration of one or more additional therapeutic agents. In embodiments of any of the above aspects, the neoplastic cell is derived from a leukemia or lymphoma. In embodiments of any of the above aspects, the leukemia is any one or more of a chronic lymphocytic leukemia, B cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and early T cell acute lymphoblastic leukemia. In embodiments of any of the above aspects, the lymphoma is any one or more of small B-cell lymphomas, mantle cell lymphoma, small lymphocytic lymphoma, diffuse large B cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, splenic red pulp lymphoma, and MALT lymphoma. In embodiments of any of the above aspects, the neoplastic cell is a murine, rat, or human cell. In embodiments of any of the above aspects, the cell is in vitro or in vivo.

Definitions

Unless defined otherwise, all technical and scientific terms used herein have the meaning commonly understood by a person of ordinary skill in the art to which this invention belongs. The following references provide a person of ordinary skill with a general definition of many of the terms used in this invention: Singleton et al., Dictionary of Microbiology and Molecular Biology (2nd ed. 1994); The Cambridge Dictionary of Science and Technology (Walker ed., 1988); The Glossary of Genetics, 5th Ed., R. Rieger et al. (eds.), Springer Verlag (1991); and Hale & Marham, The Harper Collins Dictionary of Biology (1991). As used herein, the following terms have the meanings ascribed to them below, unless specified otherwise.

By "B cell receptor activity" is meant activation of proteins within the B-cell receptor (BCR) pathway that result in B cell activation. Such activation can take the form of tyrosine kinase phosphorylation (e.g., phosphorylation by a Src family kinase, Lyn, spleen tyrosine kinase (Syk), Bruton tyrosine kinase (Btk), Phospholipase C gamma 2 (PLCG2)), as well as activation or modulation of proteins in downstream pathways as a result of BCR signaling (e.g. phosphoinositol-3-kinase (PI3K) / AKT pathway protein phosphorylation, mitogen-

activated protein kinase (MAPK) pathway protein phosphorylation, or protein kinase C / nuclear factor kappa B (NF-κB) phosphorylation, altered proteolysis, altered ubiquitination, or altered subcellular localization). In one embodiment, B cell receptor activity is B cell receptor signaling.

By "Notch activity" is meant activation of proteins within the Notch pathway that results in modifications in cell growth or proliferation. Such protein activation can take the form of proteolytic cleavage of Notch receptor proteins (or chimaeric proteins incorporating a portion of a Notch receptor protein), altered subcellular localization of Notch receptor proteins or a portion therof from cellular membranes to the nucleus, cytoplasm, or other organelles, binding of Notch receptor proteins or a portion thereof to DNA (either directly or via binding of Notch proteins to other DNA-bound proteins), or binding of Notch proteins to transcriptional regulatory proteins independendent of association with DNA. In one embodiment, Notch activity is Notch signaling.

By "B cell receptor" is meant a transmembrane receptor protein complex present on B cells comprising a membrane bound immunoglobulin, CD79A and CD79B as functional components.

By "CD79A protein" is meant a polypeptide having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: P11912, or a fragment thereof, and having signal transduction activity.

```
>sp|P11912|CD79A_HUMAN B-cell antigen receptor complex-associated
protein alpha chain OS=Homo sapiens GN=CD79A PE=1 SV=2
MPGGPGVLQALPATIFLLFLLSAVYLGPGCQALWMHKVPASLMVSLGEDAHFQCPHNSSN
NANVTWWRVLHGNYTWPPEFLGPGEDPNGTLIIQNVNKSHGGIYVCRVQEGNESYQQSCG
TYLRVRQPPPRPFLDMGEGTKNRIITAEGIILLFCAVVPGTLLLFRKRWQNEKLGLDAGD
EYEDENLYEGLNLDDCSMYEDISRGLQGTYQDVGSLNIGDVQLEKP
```

By "CD79A polynucleotide" is meant a nucleic acid molecule encoding the CD79A protein.

By "CD79B protein" is meant a polypeptide having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: P40259, or a fragment thereof, and having signal transduction activity.

```
>sp|P40259|CD79B_HUMAN B-cell antigen receptor complex-associated protein
beta chain OS=Homo sapiens GN=CD79B PE=1 SV=1
MARLALSPVPSHWMVALLLLLSAEPVPAARSEDRYRNPKGSACSRIWQSPRFIARKRGFT
VKMHCYMNSASGNVSWLWKQEMDENPQQLKLEKGRMEESQNESLATLTIQGIRFEDNGIY
FCQQKCNNTSEVYQGCGTELRVMGFSTLAQLKQRNTLKDGIIMIQTLLIILFIIVPIFLL
LDKDDSKAGMEEDHTYEGLDIDQTATYEDIVTLRTGEVKWSVGEHPGQE
```

By "CD79B polynucleotide" is meant a nucleic acid molecule encoding the CD79B protein.

By "Bruton's tyrosine kinase (BTK) polypeptide" is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: Q06187.3, or a fragment thereof, and having tyrosine kinase activity. An exemplary BTK amino acid sequence is provided below:

```
   1 maavilesif lkrsqqkkkt splnfkkrlf lltvhklsyy eydfergrrg skkgsidvek
  61 itcvetvvpe knppperqip rrgeesseme qisiierfpy pfqvvydegp lyvfspteel
 121 rkrwihqlkn virynsdlvq kyhpcfwidg qylccsqtak namgcqilen rngslkpgss
 181 hrktkkplpp tpeedqilkk plppepaaap vstselkkvv alydympmna ndlqlrkgde
 241 yfileesnlp wwrardkngq egyipsnyvt eaedsiemye wyskhmtrsq aeqllkqegk
 301 eggfivrdss kagkytvsvf akstgdpqgv irhyvvcstp qsqyylaekh lfstipelin
 361 yhqhnsagli srlkypvsqq nknapstagl gygsweidpk dltflkelgt gqfgvvkygk
 421 wrgqydvaik mikegsmsed efieeakvmm nlsheklvql ygvctkqrpi fiiteymang
 481 cllnylremr hrfqtqqlle mckdvceame yleskqflhr dlaarnclvn dqgvvkvsdf
 541 glsryvldde ytssvgskfp vrwsppevlm yskfssksdi wafgvlmwei yslgkmpyer
 601 ftnsetaehi aqglrlyrph lasekvytim yscwhekade rptfkillsn ildvmdees
```

By "BTK polynucleotide" is meant a nucleic acid molecule encoding a BTK polypeptide. An exemplary BTK polynucleotide sequence is provided at NCBI Reference Sequence: NM_000061.2, and reproduced herein below.

```
   1 aactgagtgg ctgtgaaagg gtggggtttg ctcagactgt ccttcctctc tggactgtaa
  61 gaatatgtct ccagggccag tgtctgctgc gatcgagtcc caccttccaa gtcctggcat
 121 ctcaatgcat ctgggaagct acctgcatta agtcaggact gagcacacag gtgaactcca
 181 gaaagaagaa gctatggccg cagtgattct ggagagcatc tttctgaagc gatcccaaca
 241 gaaaaagaaa acatcacctc taaacttcaa gaagcgcctg tttctcttga ccgtgcacaa
 301 actctcctac tatgagtatg actttgaacg tgggagaaga ggcagtaaga agggttcaat
 361 agatgttgag aagatcactt gtgttgaaac agtggttcct gaaaaaaatc ctcctccaga
 421 aagacagatt ccgagaagag gtgaagagtc cagtgaaatg gagcaaattt caatcattga
 481 aaggttccct tatcccttcc aggttgtata tgatgaaggg cctctctacg tcttctcccc
 541 aactgaagaa ctaaggaagc ggtggattca ccagctcaaa aacgtaatcc ggtacaacag
 601 tgatctggtt cagaaatatc acccttgctt ctggatcgat gggcagtatc tctgctgctc
 661 tcagacagcc aaaaatgcta tgggctgcca aattttggag aacaggaatg gaagcttaaa
 721 acctgggagt tctcaccgga agacaaaaaa gcctcttccc ccaacgcctg aggaggacca
 781 gatcttgaaa aagccactac cgcctgagcc agcagcagca ccagtctcca caagtgagct
 841 gaaaaaggtt gtggcccttt atgattacat gccaatgaat gcaaatgatc tacagctgcg
 901 gaagggtgat gaatatttta tcttggagga aagcaactta ccatggtgga gagcacgaga
 961 taaaaatggg caggaaggct acattcctag taactatgtc actgaagcag aagactccat
1021 agaaatgtat gagtggtatt ccaaacacat gactcggagt caggctgagc aactgctaaa
1081 gcaagagggg aaagaaggag gtttcattgt cagagactcc agcaaagctg gcaaatatac
1141 agtgtctgtg tttgctaaat ccacagggga ccctcaaggg gtgatacgtc attatgttgt
1201 gtgttccaca cctcagagcc agtattacct ggctgagaag caccttttca gcaccatccc
1261 tgagctcatt aactaccatc agcacaactc tgcaggactc atatccaggc tcaaatatcc
1321 agtgtctcaa caaaacaaga atgcaccttc cactgcaggc ctgggatacg gatcatggga
1381 aattgatcca aaggacctga ccttcttgaa ggagctgggg actggacaat ttggggtagt
1441 gaagtatggg aaatggagag gccagtacga cgtggccatc aagatgatca aagaaggctc
1501 catgtctgaa gatgaattca ttgaagaagc caaagtcatg atgaatcttt cccatgagaa
1561 gctggtgcag ttgtatggcg tctgcaccaa gcagcgcccc atcttcatca tcactgagta
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1621 catggccaat ggctgcctcc tgaactacct gagggagatg cgccaccgct tccagactca
1681 gcagctgcta gagatgtgca aggatgtctg tgaagccatg gaatacctgg agtcaaagca
1741 gttccttcac cgagacctgg cagctcgaaa ctgtttggta aacgatcaag gagttgttaa
1801 agtatctgat ttcggcctgt ccaggtatgt cctggatgat gaatacacaa gctcagtagg
1861 ctccaaattt ccagtccggt ggtccccacc ggaagtcctg atgtatagca agttcagcag
1921 caaatctgac atttgggctt ttggggtttt gatgtgggaa atttactccc tggggaagat
1981 gccatatgag agatttacta acagtgagac tgctgaacac attgcccaag gcctacgtct
2041 ctacaggcct catctggctt cagagaaggt atataccatc atgtacagtt gctggcatga
2101 gaaagcagat gagcgtccca ctttcaaaat tcttctgagc aatattctag atgtcatgga
2161 tgaagaatcc tgagctcgcc aataagcttc ttggttctac ttctcttctc cacaagcccc
2221 aatttcactt tctcagagga aatcccaagc ttaggagccc tggagccttt gtgctcccac
2281 tcaatacaaa aaggcccctc tctacatctg ggaatgcacc tcttctttga ttccctggga
2341 tagtggcttc tgagcaaagg ccaagaaatt attgtgcctg aaatttcccg agagaattaa
2401 gacagactga atttgcgatg aaaatatttt ttaggaggga ggatgtaaat agccgcacaa
2461 aggggtccaa cagctctttg agtaggcatt tggtagagct tgggggtgtg tgtgtggggg
2521 tggaccgaat ttggcaagaa tgaaatggtg tcataaagat gggaggggag ggtgttttga
2581 taaaataaaa ttactagaaa gcttgaaagt c
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By "myc proto-oncogene protein (MYC of c-MYC) polypeptide" is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: NP_002458.2, or a fragment thereof, and having growth regulatory activity. Growth regulatory activity includes, but is not limited to, cell division or increase in cell size. An exemplary MYC amino acid sequence is provided below:

```
  1 mdffrvvenq qppatmplnv sftnrnydld ydsvqpyfyc deeenfyqqq qqselqppap
 61 sediwkkfel lptpplspsr rsglcspsyv avtpfslrgd ndggggsfst adqlemvtel
121 lggdmvnqsf icdpddetfi kniiiqdcmw sgfsaaaklv seklasyqaa rkdsgspnpa
181 rghsvcstss lylqdlsaaa secidpsvvf pyplndsssp kscasqdssa fspssdslls
241 stesspqgsp eplvlheetp pttssdseee qedeeeidvv svekrqapgk rsesgspsag
301 ghskpphspl vlkrchvsth qhnyaappst rkdypaakrv kldsvrvlrq isnnrkctsp
361 rssdteenvk rrthnvlerq rrnelkrsff alrdqipele nnekapkvvi lkkatayils
421 vqaeeqklis eedllrkrre qlkhkleqlr nsca
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By "MYC polynucleotide" is meant a nucleic acid molecule encoding a MYC polypeptide. An exemplary MYC polynucleotide sequence is provided at NCBI Reference Sequence: V00568.1, and reproduced herein below.

```
  1 ctgctcgcgg ccgccaccgc cgggccccgg ccgtccctgg ctcccctcct gcctcgagaa
 61 gggcagggct tctcagaggc ttggcgggaa aaaagaacgg agggagggat cgcgctgagt
121 ataaaagccg gttttcgggg ctttatctaa ctcgctgtag taattccagc gagaggcaga
181 gggagcgagc gggcggccgg ctagggtgga agagccgggc gagcagagct gcgctgcggg
241 cgtcctggga agggagatcc ggagcgaata gggggcttcg cctctggccc agccctcccg
301 cttgatcccc caggccagcg gtccgcaacc cttgccgcat ccacgaaact ttgcccatag
361 cagcgggcgg gcactttgca ctggaactta caacacccga gcaaggacgc gactctcccg
421 acgcggggag gctattctgc ccatttgggg acacttcccc gccgctgcca ggacccgctt
481 ctctgaaagg ctctccttgc agctgcttag acgctggatt tttttcgggt agtggaaaac
541 cagcagcctc ccgcgacgat gcccctcaac gttagcttca ccaacaggaa ctatgacctc
601 gactacgact cggtgcagcc gtatttctac tgcgacgagg aggagaactt ctaccagcag
661 cagcagcaga gcgagctgca gcccccggcg cccagcgagg atatctggaa gaaattcgag
721 ctgctgccca ccccgccccc gtcccctagc cgccgctccg ggctctgctc gccctcctac
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 781 gttgcggtca cacccttctc ccttcgggga gacaacgacg gcggtggcgg gagcttctcc
 841 acggccgacc agctggagat ggtgaccgag ctgctgggag gagacatggt gaaccagagt
 901 ttcatctgcg acccggacga cgagaccttc atcaaaaaca tcatcatcca ggactgtatg
 961 tggagcggct tctcggccgc cgccaagctc gtctcagaga agctggcctc ctaccaggct
1021 gcgcgcaaag acagcggcag cccgaacccc gcccgcggcc acagcgtctg ctccacctcc
1081 agcttgtacc tgcaggatct gagcgccgcc gcctcagagt gcatcgaccc ctcggtggtc
1141 ttcccctacc ctctcaacga cagcagctcg cccaagtcct gcgcctcgca agactccagc
1201 gccttctctc cgtcctcgga ttctctgctc tcctcgacgg agtcctcccc gcagggcagc
1261 cccgagcccc tggtgctcca tgaggagaca ccgcccacca ccagcagcga ctctgaggag
1321 gaacaagaag atgaggaaga aatcgatgtt gtttctgtgg aaaagaggca ggctcctggc
1381 aaaaggtcag agtctggatc accttctgct ggaggccaca gcaaacctcc tcacagccca
1441 ctggtcctca agaggtgcca cgtctccaca catcagcaca actacgcagc gcctccctcc
1501 actcggaagg actatcctgc tgccaagagg gtcaagttgg acagtgtcag agtcctgaga
1561 cagatcagca acaaccgaaa atgcaccagc cccaggtcct cggacaccga ggagaatgtc
1621 aagaggcgaa cacacaacgt cttggagcgc cagaggagga acgagctaaa acggagcttt
1681 tttgccctgc gtgaccagat cccggagttg gaaaacaatg aaaaggcccc caaggtagtt
1741 atccttaaaa aagccacagc atacatcctg tccgtccaag cagaggagca aaagctcatt
1801 tctgaagagg acttgttgcg gaaacgacga gaacagttga aacacaaact tgaacagcta
1861 cggaactctt gtgcgtaagg aaaagtaagg aaaacgattc cttctaacag aaatgtcctg
1921 agcaatcacc tatgaacttg tttcaaatgc atgatcaaat gcaacctcac aaccttggct
1981 gagtcttgag actgaaagat ttagccataa tgtaaactgc ctcaaattgg actttgggca
2041 taaaagaact tttttatgct taccatcttt ttttttttctt taacagattt gtatttaaga
2101 attgttttta aaaaatttta a
```

By "Notch protein" or "Notch receptor" is meant any one of Notch 1, 2, 3, or 4.

By "Neurogenic locus notch homolog protein 1 (Notch1) polypeptide" is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: P46531.4, or a fragment thereof, and having Notch receptor activity. Examples of Notch receptor activity include interaction with Notch ligands at the cell surface, proteolytic cleavage of the Notch protein by ADAM family metalloproteases and / or gamma secretase (either following interaction with Notch ligands, or through ligand-independent mechanisms), altered sub-cellular localization of an intracellular portion of the Notch protein following a proteolytic cleavage event, binding of a Notch protein (or portion thereof) to other transcriptional regulatory proteins in the nucleus or cytoplasm, or binding of a Notch protein (or portion thereof) to DNA-bound chromatin complexes. An exemplary Notch1 amino acid sequence is provided below:

```
   1 mppllapllc lallpalaar gprcsqpget clnggkceaa ngteacvcgg afvgprcqdp
  61 npclstpckn agtchvvdrr gvadyacsca lgfsgplclt pldnacltnp crnggtcdll
 121 tlteykcrcp pgwsgkscqq adpcasnpca nggqclpfea syichcppsf hgptcrqdvn
 181 ecgqkpglcr hggtchnevg syrcvcrath tgpncerpyv pcspspcqng gtcrptgdvt
 241 hecaclpgft gqnceenidd cpgnncknqg acvdgvntyn crcppewtgq yctedvdecq
 301 lmpnacqngg tchnthggyn cvcvngwtge dcseniddca saacfhgatc hdrvasfyce
 361 cphgrtgllc hlndacisnp cnegsncdtn pvngkaictc psgytgpacs qdvdecslga
 421 npcehagkci ntlgsfecqc lqgytgprce idvnecvsnp cqndatcldq igefqcicmp
 481 gyegvhcevn tdecasspcl hngrcldkin efqcecptgf tghlcqydvd ecastpckng
 541 akcldgpnty tcvctegytg thcevdidec dpdpchygsc kdgvatftcl crpgytghhc
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 601 etninecssq pcrhggtcqd rdnaylcfcl kgttgpncei nlddcasspc dsgtcldkid
 661 gyecacepgy tgsmcninid ecagnpchng gtcedgingf tcrcpegyhd ptclsevnec
 721 nsnpcvhgac rdslngykcd cdpgwsgtnc dinnnecesn pcvnggtckd mtsgyvctcr
 781 egfsgpncqt ninecasnpc lnqgtciddv agykcncllp ytgatcevvl apcapspcrn
 841 ggecrqsedy esfscvcptg wqgqtcevdi necvlspcrh gascqnthgg yrchcqagys
 901 grncetdidd crpnpchngg sctdgintaf cdclpgfrgt fceedineca sdpcrnganc
 961 tdcvdsytct cpagfsgihc enntpdctes scfnggtcvd ginsftclcp pgftgsycqh
1021 dvnecdsqpc lhggtcqdgc gsyrctcpqg ytgpncqnlv hwcdsspckn ggkcwqthtq
1081 yrcecpsgwt glycdvpsvs cevaaqrqgv dvarlcqhgg lcvdagnthh crcqagytgs
1141 ycedlvdecs pspcqngatc tdylggysck cvagyhgvnc seeideclsh pcqnggtcld
1201 lpntykcscp rgtqgvhcei nvddcnppvd pvsrspkcfn ngtcvdqvgg ysctcppgfv
1261 gercegdvne clsnpcdarg tqncvqrvnd fhcecraght grrcesving ckgkpckngg
1321 tcavasntar gfickcpagf egatcendar tcgslrclng gtcisgprsp tclclgpftg
1381 pecqfpassp clggnpcynq gtceptsesp fyrclcpakf ngllchildy sfgggagrdi
1441 ppplieeace lpecqedagn kvcslqcnnh acgwdggdcs lnfndpwknc tqslqcwkyf
1501 sdghcdsqcn sagclfdgfd cqraegqcnp lydqyckdhf sdghcdqgcn saecewdgld
1561 caehvperla agtlvvvvlm ppeqlrnssf hflrelsrvl htnvvfkrda hgqqmifpyy
1621 greeelrkhp ikraaegwaa pdallgqvka sllpggsegg rrrreldpmd vrgsivylei
1681 dnrqcvqass qcfqsatdva aflgalaslg slnipykiea vqsetveppp paqlhfmyva
1741 aaafvllffv gcgvllsrkr rrqhgqlwfp egfkvseask kkrreplged svglkplkna
1801 sdgalmddnq newgdedlet kkfrfeepvv lpdlddqtdh rqwtqqhlda adlrmsamap
1861 tppqgevdad cmdvnvrgpd gftplmiasc sgggletgns eeeedapavi sdfiyqgasl
1921 hnqtdrtget alhlaarysr sdaakrllea sadaniqdnm grtplhaavs adaqgvfqil
1981 irnratdlda rmhdgttpli laarlavegm ledlinshad vnavddlgks alhwaaavnn
2041 vdaavvllkn gankdmqnnr eetplflaar egsyetakvl ldhfanrdit dhmdrlprdi
2101 aqermhhdiv rlldeynlvr spqlhgaplg gtptlspplc spngylgslk pgvqgkkvrk
2161 psskglacgs keakdlkarr kksqdgkgcl ldssgmlspv dslesphgyl sdvasppllp
2221 spfqqspsvp lnhlpgmpdt hlgighlnva akpemaalgg ggrlafetgp prlshlpvas
2281 gtstvlgsss ggalnftvgg stslngqcew lsrlqsgmvp nqynplrgsv apgplstqap
2341 slqhgmvgpl hsslaasals qmmsyqglps trlatqphlv qtqqvqpqnl qmqqqnlqpa
2401 niqqqqslqp pppppqphlg vssaasghlg rsflsgepsq advqplgpss lavhtilpqe
2461 spalptslps slvppvtaaq fltppsqhsy sspvdntpsh qlqvpehpfl tpspespdqw
2521 sssphsnvs dwsegvsspp tsmqsqiari peafk
```

By "Notch1 polynucleotide" is meant a nucleic acid molecule encoding a Notch1 polypeptide. An exemplary Notch1 polynucleotide sequence is provided at NCBI Reference Sequence: NM_017617.4, and reproduced herein below.

```
   1 atgccgccgc tcctggcgcc cctgctctgc ctggcgctgc tgcccgcgct cgccgcacga
  61 ggcccgcgat gctcccagcc cggtgagacc tgcctgaatg gcgggaagtg tgaagcggcc
 121 aatggcacgg aggcctgcgt ctgtggcggg gccttcgtgg gcccgcgatg ccaggacccc
 181 aacccgtgcc tcagcacccc ctgcaagaac gccgggacat gccacgtggt ggaccgcaga
 241 ggcgtggcag actatgcctg cagctgtgcc ctgggcttct ctgggcccct ctgcctgaca
 301 cccctggaca atgcctgcct caccaacccc tgccgcaacg ggggcacctg cgacctgctc
 361 acgctgacgg agtacaagtg ccgctgcccg cccggctggt cagggaaatc gtgccagcag
 421 gctgacccgt gcgcctccaa cccctgcgcc aacggtggcc agtgcctgcc cttcgaggcc
 481 tcctacatct gccactgccc acccagcttc catggcccca cctgccggca ggatgtcaac
 541 gagtgtggcc agaagcccgg gctttgccgc cacggaggca cctgccacaa cgaggtcggc
 601 tcctaccgct gcgtctgccg cgccacccac actggcccca actgcgagcg gccctacgtg
 661 ccctgcagcc cctcgccctg ccagaacggg ggcacctgcc gccccacggg cgacgtcacc
 721 cacgagtgtg cctgcctgcc aggcttcacc ggccagaact gtgaggaaaa tatcgacgat
 781 tgtccaggaa acaactgcaa gaacgggggt gcctgtgtgg acggcgtgaa cacctacaac
 841 tgccgctgcc cgccagagtg gacaggtcag tactgtaccg aggatgtgga cgagtgccag
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 901 ctgatgccaa atgcctgcca gaacggcggg acctgccaca acacccacgg tggctacaac
 961 tgcgtgtgtg tcaacggctg gactggtgag gactgcagcg agaacattga tgactgtgcc
1021 agcgccgcct gcttccacgg cgccacctgc catgaccgtg tggcctcctt ctactgcgag
1081 tgtccccatg gccgcacagg tctgctgtgc cacctcaacg acgcatgcat cagcaacccc
1141 tgtaacgagg gctccaactg cgacaccaac cctgtcaatg gcaaggccat ctgcacctgc
1201 ccctcggggt acacgggccc ggcctgcagc caggacgtgg atgagtgctc gctgggtgcc
1261 aacccctgcg agcatgcggg caagtgcatc aacacgctgg gctccttcga gtgccagtgt
1321 ctgcagggct acacgggccc ccgatgcgag atcgacgtca acgagtgcgt ctcgaacccg
1381 tgccagaacg acgccacctg cctggaccag attggggagt tccagtgcat ctgcatgccc
1441 ggctacgagg gtgtgcactg cgaggtcaac acagacgagt gtgccagcag cccctgcctg
1501 cacaatggcc gctgcctgga caagatcaat gagttccagt gcgagtgccc cacgggcttc
1561 actgggcatc tgtgccagta cgatgtggac gagtgtgcca gcacccctg caagaatggt
1621 gccaagtgcc tggacggacc caacacttac acctgtgtgt gcacggaagg gtacacgggg
1681 acgcactgcg aggtggacat cgatgagtgc gaccccgacc cctgccacta cggctcctgc
1741 aaggacggcg tcgccacctt cacctgcctc tgccgcccag gctacacggg ccaccactgc
1801 gagaccaaca tcaacgagtg ctccagccag ccctgccgcc acgggggcac ctgccaggac
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1921 aacctggatg actgtgccag cagcccctgc gactcgggca cctgtctgga caagatcgat
1981 ggctacgagt gtgcctgtga gccgggctac acagggagca tgtgtaacat caacatcgat
2041 gagtgtgcgg gcaacccctg ccacaacggg ggcacctgcg aggacggcat caatggcttc
2101 acctgccgct gccccgaggg ctaccacgac cccacctgcc tgtctgaggt caatgagtgc
2161 aacagcaacc cctgcgtcca cggggcctgc cgggacagcc tcaacgggta caagtgcgac
2221 tgtgaccctg ggtggagtgg gaccaactgt gacatcaaca acaatgagtg tgaatccaac
2281 ccttgtgtca acggcggcac ctgcaaagac atgaccagtg gctacgtgtg cacctgccgg
2341 gagggcttca gcggtcccaa ctgccagacc aacatcaacg agtgtgcgtc caacccatgt
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2821 ttctgtgagg aggacatcaa cgagtgtgcc agtgacccct gccgcaacgg ggccaactgc
2881 acggactgcg tggacagcta cacgtgcacc tgccccgcag gcttcagcgg gatccactgt
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3181 cactggtgtg actcctcgcc ctgcaagaac ggcggcaaat gctggcagac ccacacccag
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3961 acctgcgccg tggcctccaa caccgcccgc gggttcatct gcaagtgccc tgcgggcttc
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4081 ggcacatgca tctccggccc gcgcagcccc acctgcctgt gcctgggccc cttcacgggc
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4201 gggacctgtg agcccacatc cgagagcccc ttctaccgtt gcctgtgccc cgccaaattc
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4321 cccccgccgc tgatcgagga ggcgtgcgag ctgcccgagt gccaggagga cgcgggcaac
4381 aaggtctgca gcctgcagtg caacaaccac gcgtgcggct gggacggcgg tgactgctcc
4441 ctcaacttca atgacccctg gaagaactgc acgcagtctc tgcagtgctg gaagtacttc
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4621 agcgacgggc actgcgacca gggctgcaac agcgcggagt gcgagtggga cgggctggac
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5281 cggcggcagc atggccagct ctggttccct gagggcttca aagtgtctga ggccagcaag
5341 aagaagcggc gggagcccct cggcgaggac tccgtgggcc tcaagccccct gaagaacgct
5401 tcagacggtg ccctcatgga cgacaaccag aatgagtggg gggacgagga cctggagacc
5461 aagaagttcc ggttcgagga gcccgtggtt ctgcctgacc tggacgacca gacagaccac
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5881 ggccgcaccc cgctgcatgc ggctgtgtct gccgacgcac aaggtgtctt ccagatcctg
5941 atccggaacc gagccacaga cctggatgcc cgcatgcatg atggcacgac gccactgatc
6001 ctggctgccc gcctggccgt ggagggcatg ctggaggacc tcatcaactc acacgccgac
6061 gtcaacgccg tagatgacct gggcaagtcc gccctgcact gggccgccgc cgtgaacaat
6121 gtggatgccg cagttgtgct cctgaagaac ggggctaaca aagatatgca gaacaacagg
6181 gaggagacac ccctgtttct ggccgcccgg gagggcagct acgagaccgc caaggtgctg
6241 ctggaccact ttgccaaccg ggacatcacg gatcatatgg accgcctgcc gcgcgacatc
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6421 tcgcccaacg gctacctggg cagcctcaag cccggcgtgc agggcaagaa ggtccgcaag
6481 cccagcagca aaggcctggc ctgtggaagc aaggaggcca aggacctcaa ggcacggagg
6541 aagaagtccc aggacggcaa gggctgcctg ctggacagct ccggcatgct ctcgcccgtg
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6841 ggcaccagca ccgtcctggg ctccagcagc ggaggggccc tgaatttcac tgtgggcggg
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7021 tccctgcagc atggcatggt aggcccgctg cacagtagcc ttgctgccag cgccctgtcc
7081 cagatgatga gctaccaggg cctgcccagc acccggctgg ccacccagcc tcacctggtg
7141 cagacccagc aggtgcagcc acaaaactta cagatgcagc agcagaacct gcagccagca
7201 aacatccagc agcagcaaag cctgcagccg ccaccaccac caccacagcc gcaccttggc

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7261 gtgagctcag cagccagcgg ccacctgggc cggagcttcc tgagtggaga gccgagccag
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7381 agccccgccc tgcccacgtc gctgccatcc tcgctggtcc cacccgtgac cgcagcccag
7441 ttcctgacgc ccccctcgca gcacagctac tcctcgcctg tggacaacac ccccagccac
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7741 gggccgacca gaggagcctt tttaaaacac atgtttttat acaaaataag aacgaggatt
7801 ttaatttttt ttagtattta tttatgtact tttattttac acagaaacac tgccttttta
7861 tttatatgta ctgttttatc tggccccagg tagaaacttt tatctattct gagaaaacaa
7921 gcaagttctg agagccaggg ttttcctacg taggatgaaa agattcttct gtgtttataa
7981 aatataaaca aagattcatg atttataaat gccatttatt tattgattcc ttttttcaaa
8041 atccaaaaag aaatgatgtt ggagaaggga agttgaacga gcatagtcca aaaagctcct
8101 ggggcgtcca ggccgcgccc tttccccgac gcccacccaa ccccaagcca gcccggccgc
8161 tccaccagca tcacctgcct gttaggagaa gctgcatcca gaggcaaacg gaggcaaagc
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8281 tgggttagat gttgccatgt gttttagatg gtttcttgca agcatgcttg tgaaaatgtg
8341 ttctcggagt gtgtatgcca agagtgcacc catggtacca atcatgaatc tttgtttcag
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8461 cggcccccctg cccgttcttg aaatgtaggc atcatgcatg tcaaacatga gatgtgtgga
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8581 gcctgggctg accccggtgg cggccccagc acctcagcct gcacagtgtc ccccaggttc
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8881 ttgcaatacc aagtatagcc tatggcagaa aaaatgtctg taaatatgtt tttaaaggtg
8941 gattttgttt aaaaaatctt aatgaatgag tctgttgtgt gtcatgccag tgagggacgt
9001 cagacttggc tcagctcggg gagccttagc cgcccatgca ctggggacgc tccgctgccg
9061 tgccgcctgc actcctcagg gcagcctccc ccggctctac gggggccgcg tggtgccatc
9121 cccagggggc atgaccagat gcgtcccaag atgttgattt ttactgtgtt ttataaaata
9181 gagtgtagtt tacagaaaaa gactttaaaa gtgatctaca tgaggaactg tagatgatgt
9241 attttttca tctttttgt taactgattt gcaataaaaa tgatactgat ggtgatctgg
9301 cttccaaaaa aaaaaaaaaa aa
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By "Neurogenic locus notch homolog protein 2 (Notch2) polypeptide" is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: AAG37073.1, or a fragment thereof, and having Notch receptor activity. An exemplary Notch2 amino acid sequence is provided below:

```
  1 mpalrpallw allalwlcca tpahalqcrd gyepcvnegm cvtyhngtgy ckcpegflge
 61 ycqhrdpcek nrcqnggtcv aqamlgkatc rcasgftged cqystshpcf vsrpclnggt
121 chmlsrdtye ctcqvgftgk ecqwtdacls hpcangstct tvanqfsckc ltgftgqkce
181 tdvnecdipg hcqhggtcln lpgsyqcqcl qgftgqycds lyvpcapspc vnggtcrqtg
241 dftfecnclp gfegstcern iddcpnhrcq nggvcvdgvn tyncrcppqw tgqfctedvd
301 ecllqpnacq nggtcanrng gygcvcvngw sgddcsenid dcafasctpg stcidrvasf
361 scmcpegkag llchlddaci snpchkgalc dtnplngqyi ctcpqgykga dctedvdeca
421 mansnpceha gkcvntdgaf hceclkgyag prcemdinec hsdpcqndat cldkiggftc
481 lcmpgfkgvh celeinecqs npcvnngqcv dkvnrfqclc ppgftgpvcq ididdcsstp
541 clngakcidh pngyecqcat gftgvlceen idncdpdpch hgqcqdgids ytcicnpgym
601 gaicsdqide cysspclndg rcidlvngyq cncqpgtsgv nceinfddca snpcihgicm
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 661 dginryscvc spgftgqrcn ididecasnp crkgatcing vngfrcicpe gphhpscysq
 721 vneclsnpci hgnctgglsg ykclcdagwv gincevdkne clsnpcqngg tcdnlvngyr
 781 ctckkgfkgy ncqvnideca snpclnqgtc fddisgytch cvlpytgknc qtvlapcspn
 841 pcenaavcke spnfesytcl capgwqgqrc tididecisk pcmnhglchn tqgsymcecp
 901 pgfsgmdcee diddclanpc qnggscmdgv ntfsclclpg ftgdkcqtdm neclsepckn
 961 ggtcsdyvns ytckcqagfd gvhcennine ctesscfngg tcvdginsfs clcpvgftgs
1021 fclheinecs shpclnegtc vdglgtyrcs cplgytgknc qtlvnlcsrs pcknkgtcvq
1081 kkaesqclcp sgwagaycdv pnvscdiaas rrgvlvehlc qhsgvcinag nthycqcplg
1141 ytgsyceeql decasnpcqh gatcsdfigg yrcecvpgyq gvnceyevde cqnqpcqngg
1201 tcidlvnhfk cscppgtrgl lceeniddca rgphclnggq cmdriggysc rclpgfager
1261 cegdinecls npcssegsld ciqltndylc vcrsaftgrh cetfvdvcpq mpclnggtca
1321 vasnmpdgfi crcppgfsga rcqsscgqvk crkgeqcvht asgprcfcps prdcesgcas
1381 spcqhggsch pqrqppyysc qcappfsgsr celytappst ppatclsqyc adkardgvcd
1441 eacnshacqw dggdcsltme npwancsspl pcwdyinnqc delcntvecl fdnfecqgns
1501 ktckydkyca dhfkdnhcdq gcnseecgwd gldcaadqpe nlaegtlviv vlmppeqllq
1561 darsflralg tllhtnlrik rdsqgelmvy pyygeksaam kkqrmtrrsl pgeqeqevag
1621 skvfleidnr qcvqdsdhcf kntdaaaall ashaiqgtls yplvsvvses ltpertqlly
1681 llavavviil fiillgvima krkrkhgslw lpegftlrrd asnhkrrepv gqdavglknl
1741 svqvseanli gtgtsehwvd degpqpkkvk aedeallsee ddpidrrpwt qqhleaadir
1801 rtpslaltpp qaeqevdvld vnvrgpdgct plmlaslrgg ssdlsdeded aedssaniit
1861 dlvyqgaslq aqtdrtgema lhlaarysra daakrlldag adanaqdnmg rcplhaavaa
1921 daqgvfqili rnrvtdldar mndgttplil aarlavegmv aelincqadv navddhgksa
1981 lhwaaavnnv eatllllkng anrdmqdnke etplflaare gsyeaakill dhfanrditd
2041 hmdrlprdva rdhmhhdivr lldeynvtps ppgtvltsal spvicgpnrs flslkhtpmg
2101 kksrrpsaks tmptslpnla keakdakgsr rkkslsekvq lsessvtlsp vdslesphty
2161 vsdttsspmi tspgilqasp npmlataapp apvhaqhals fsnlhemqpl ahgastvlps
2221 vsqllshhhi vspgsgsags lsrlhpvpvp adwmnrmevn etqynemfgm vlapaegthp
2281 giapqsrppe gkhittprep lppivtfqli pkgsiaqpag apqpqstcpp avagplptmy
2341 qipemarlps vafptammpq qdgqvaqtil payhpfpasv gkyptppsqh syassnaaer
2401 tpshsghlqg ehpyltpspe spdqwssssp hsasdwsdvt tsptpggagg gqrgpgthms
2461 epphnnmqvy a
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By "Notch2 polynucleotide" is meant a nucleic acid molecule encoding a Notch2 polypeptide. An exemplary Notch2 polynucleotide sequence is provided at NCBI Reference Sequence: AF315356.1, and reproduced herein below.

```
   1 gcgaccgaga agatgcccgc cctgcgcccc gctctgctgt gggcgctgct ggcgctctgg
  61 ctgtgctgcg cgacccccgc gcatgcattg cagtgtcgag atggctatga accctgtgta
 121 aatgaaggaa tgtgtgttac ctaccacaat ggcacaggat actgcaaatg tccagaaggc
 181 ttcttggggg aatattgtca acatcgagac ccctgtgaga agaaccgctg ccagaatggt
 241 gggacttgtg tggcccaggc catgctgggg aaagccacgt gccgatgtgc ctcagggttt
 301 acaggagagg actgccagta ctcgacatct catccatgct ttgtgtctcg accctgcctg
 361 aatggcggca catgccatat gctcagccgg gatacctatg agtgcacctg tcaagtcggg
 421 tttacaggta aggagtgcca atggaccgat gcctgcctgt ctcatccctg tgcaaatgga
 481 agtacctgta ccactgtggc caaccagttc tcctgcaaat gcctcacagg cttcacaggg
 541 cagaaatgtg agactgatgt caatgagtgt gacattccag gacactgcca gcatggtggc
 601 acctgcctca acctgcctgg ttcctaccag tgccagtgcc ttcagggctt cacaggccag
 661 tactgtgaca gcctgtatgt gccctgtgca ccctcgcctt gtgtcaatgg aggcacctgt
 721 cggcagactg gtgacttcac ttttgagtgc aactgccttc caggttttga agggagcacc
 781 tgtgagagga atattgatga ctgccctaac cacaggtgtc agaatggagg ggtttgtgtg
 841 gatggggtca acacttacaa ctgccgctgt cccccacaat ggacaggaca gttctgcaca
 901 gaggatgtgg atgaatgcct gctgcagccc aatgcctgtc aaaatggggg cacctgtgcc
 961 aaccgcaatg gaggctatgg ctgtgtatgt gtcaacggct ggagtggaga tgactgcagt
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1021 gagaacattg atgattgtgc cttcgcctcc tgtactccag gctccacctg catcgaccgt
1081 gtggcctcct tctcttgcat gtgcccagag gggaaggcag gtctcctgtg tcatctggat
1141 gatgcatgca tcagcaatcc ttgccacaag ggggcactgt gtgacaccaa ccccctaaat
1201 gggcaatata tttgcacctg cccacaaggc tacaaagggg ctgactgcac agaagatgtg
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1381 atcaatgagt gccattcaga cccctgccag aatgatgcta cctgtctgga taagattgga
1441 ggcttcacat gtctgtgcat gccaggtttc aaaggtgtgc attgtgaatt agaaataaat
1501 gaatgtcaga gcaacccttg tgtgaacaat gggcagtgtg tggataaagt caatcgtttc
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1681 cagtgtgcca caggtttcac tggtgtgttg tgtgaggaga acattgacaa ctgtgacccc
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1801 cccgggtaca tgggcgccat ctgcagtgac cagattgatg aatgttacag cagcccttgc
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1921 acgtcagggg ttaattgtga aattaatttt gatgactgtg caagtaaccc ttgtatccat
1981 ggaatctgta tggatggcat taatcgctac agttgtgtct gctcaccagg attcacaggg
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2941 gcaggatttg atggagtcca ttgtgagaac aacatcaatg agtgcactga gagctcctgt
3001 ttcaatggtg gcacatgtgt tgatgggatt aactccttct cttgcttgtg ccctgtgggt
3061 ttcactggat ccttctgcct ccatgagatc aatgaatgca gctctcatcc atgcctgaat
3121 gagggaacgt gtgttgatgg cctgggtacc taccgctgca gctgcccccc gggctacact
3181 gggaaaaact gtcagaccct ggtgaatctc tgcagtcggt ctccatgtaa aaacaaaggt
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3301 tattgtgacg tgcccaatgt ctcttgtgac atagcagcct ccaggagagg tgtgcttgtt
3361 gaacacttgt gccagcactc aggtgtctgc atcaatgctg gcaacacgca ttactgtcag
3421 tgcccccctgg gctatactgg gagctactgt gaggagcaac tcgatgagtg tgcgtccaac
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3541 ccaggctatc agggtgtcaa ctgtgagtat gaagtggatg agtgccagaa tcagccctgc
3601 cagaatggag gcacctgtat tgaccttgtg aaccatttca agtgctcttg cccaccaggc
3661 actcggggcc tactctgtga agagaacatt gatgactgtg cccggggtcc ccattgcctt
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3781 gctggggagc gttgtgaggg agacatcaac gagtgcctct ccaacccctg cagctctgag
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4081 tgtgtgcaca ccgcctctgg accccgctgc ttctgcccca gtccccggga ctgcgagtca
4141 ggctgtgcca gtagcccctg ccagcacggg ggcagctgcc accctcagcg ccagcctcct

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4381 ctcaccatgg agaaccccctg ggccaactgc tcctccccac ttccctgctg ggattatatc
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4501 caggggaaca gcaagacatg caagtatgac aaatactgtg cagaccactt caaagacaac
4561 cactgtgacc aggggtgcaa cagtgaggag tgtggttggg atgggctgga ctgtgctgct
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6121 gacatcacag accatatgga tcgtcttccc cgggatgtgg ctcgggatca catgcaccat
6181 gacattgtgc gccttctgga tgaatacaat gtgaccccaa gccctccagg caccgtgttg
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6361 cctaaccttg ccaaggaggc aaaggatgcc aagggtagta ggaggaagaa gtctctgagt
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7201 gctgctgagc gaacacccag tcacagtggt cacctccagg gtgagcatcc ctacctgaca
7261 ccatccccag agtctcctga ccagtggtca agttcatcac cccactctgc ttctgactgg
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7621 tgattattct aatctaataa gacaagtttg tggaaatgca agatgaatac aagccttggg
7681 tccatgttta ctctcttcta tttggagaat aagatggatg cttattgaag cccagacatt
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7801 tacactagcg tcctgttggg aattatgccc tggaattctg cctgaattga cctacgcatc
7861 tcctcctcct tggacattct tttgtcttca tttggtgctt ttggttttgc acctctccgt
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7981 catgaaagca actttggtct ccttttcccct cctgtcttcc cggtatccct tggagtctca
8041 caaggtttac tttggtatgg ttctcagcac aaacctttca agtatgttgt ttctttggaa
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8161 gaatactttt cttcaacaaa ttttgggggc aggagatccc ttcaagaggc tgcaccttaa
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8941 cattcccttt ggcaactaac gtagaaactc aacagaacat tttcctttcc tagagtcacc
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9781 aaattgcttg tttctttttc ttggactact ttaatttgga tcctttgggt ttggagaaag
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10201 tggggaaggg aaaggtatga gggaaagaga gtctgagaat taaaatattt tagtataagc
10261 aattggctgt gatgctcaaa tccattgcat cctcttattg aatttgccaa tttgtaattt
10321 ttgcataata aagaaccaaa ggtgtaatgt tttgttgaga ggtggtttag ggattttggc
10381 cctaaccaat acattgaatg tatgatgact atttgggagg acacatttat gtacccagag
10441 gcccccacta ataagtggta ctatggttac ttccttgtgt acatttctct taaaagtgat
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10561 aacgtagtaa ggaaaatgca cactttgttt ttacttttcc gtttcattct aaaggtagtt
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10741 tgtcataatt tgctgagaga gcaaattttc ttttctttct gaatcccaca aagcctagca
10801 ccaaacttct ttttttcttc ctttaattag atcataaata aatgatcctg gggaaaaagc
10861 atctgtcaaa taggaaacat cacaaaactg agcactcttc tgtgcactag ccatagctgg
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10981 tatagcaaga attgggaact gggatataag tcataatatt aattatgctg ttatgtaaat
11041 gattggtttg taacattcct taagtgaaat ttgtgtagaa cttaatatac aggattataa
11101 aataatattt tgtgtataaa tttgttataa gttcacattc atacatttat ttataaagtc
11161 agtgagatat ttgaacatga aaaaaaaaa
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By "Neurogenic locus notch homolog protein 3 (Notch3) polypeptide" is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: AAB91371.1, or a fragment thereof, and having Notch receptor activity. An exemplary Notch3 amino acid sequence is provided below:

```
   1 mgpgargrrr rrrpmspppp pppvralpll lllagpgaaa ppcldgspca nggrctqlps
  61 reaaclcppg wvgercqled pchsgpcagr gvcqssvvag tarfscrcpr gfrgpdcslp
 121 dpclsspcah garcsvgpdg rflcscppgy qgrscrsdvd ecrvgepcrh ggtclntpgs
 181 frcqcpagyt gplcenpavp capspcrngg tcrqsgdlty dcaclpgfeg qncevnvddc
 241 pghrclnggt cvdgvntync qcppewtgqf ctedvdecql qpnachnggt cfntlgghsc
 301 vcvngwtges csqniddcat avcfhgatch drvasfycac pmgktgllch lddacvsnpc
 361 hedaicdtnp vngraictcp pgftggacdq dvdecsigan pcehlgrcvn tqgsflcqcg
 421 rgytgprcet dvneclsgpc rnqatcldri gqftcicmag ftgtycevdi decqsspcvn
 481 ggvckdrvng fsctcpsgfs gstcqldvde castpcrnga kcvdqpdgye crcaegfegt
 541 lcdrnvddcs pdpchhgrcv dgiasfscac apgytgtrce sqvdecrsqp crhggkcldl
 601 vdkylcrcps gttgvncevn iddcasnpct fgvcrdginr ydcvcqpgft gplcnveine
 661 casspcgegg scvdgengfr clcppgslpp lclppshpca hepcshgicy dapggfrcvc
 721 epgwsgprcs qslardaces qpcraggtcs sdgmgfhctc ppgvqgrqce llspctpnpc
 781 ehggrcesap gqlpvcscpq gwqgprcqqd vdecagpapc gphgictnla gsfsctchgg
 841 ytgpscdqdi ndcdpnpcln ggscqdgvgs fscsclpgfa gprcardvde clsnpcgpgt
 901 ctdhvasftc tcppgyggfh ceqdlpdcsp sscfnggtcv dgvnsfsclc rpgytgahcq
 961 headpclsrp clhggvcsaa hpgfrctcle sftgpqcqtl vdwcsrqpcq nggrcvqtga
1021 yclcppgwsg rlcdirslpc reaaaqigvr leqlcqaggq cvdedsshyc vcpegrtgsh
1081 ceqevdpcla qpcqhggtcr gymggymcec lpgyngdnce ddvdecasqp cqhggscidl
1141 varylcscpp gtlgvlcein eddcgpgppl dsgprclhng tcvdlvggfr ctcppgytgl
1201 rceadinecr sgachaahtr dclqdpgggf rclchagfsg prcqtvlspc esqpcqhggq
1261 crpspgpggg ltftchcaqp fwgprcerva rscrelqcpv gvpcqqtprg prcacppgls
1321 gpscrsfpgs ppgasnasca aapclhggsc rpaplapffr cacaqgwtgp rceapaaape
1381 vseeprcpra acqakrgdqr cdrecnspgc gwdggdcsls vgdpwrqcea lqcwrlfnns
1441 rcdpacsspa clydnfdcha ggrertcnpv yekycadhfa dgrcdqgcnt eecgwdgldc
1501 asevpallar gvlvltvllp peellrssad flqrlsailr tslrfrldah gqamvfpyhr
1561 pspgseprar relapevigs vvmleidnrl clqspendhc fpdaqsaady lgalsaverl
1621 dfpyplrdvr gepleppeps vpllpllvag avlllvilvl gvmvarrkre hstlwfpegf
1681 slhkdvasgh kgrrepvgqd algmknmakg eslmgevatd wmdtecpeak rlkveepgmg
1741 aeeavdcrqw tqhhlvaadi rvapamaltp pqgdadadgm dvnvrgpdgf tplmlasfcg
1801 galepmptee deaddtsasi isdlicqgaq lgartdrtge talhlaarya radaakrlld
1861 agadtnaqdh sgrtplhtav tadaqgvfqi lirnrstdld armadgstal ilaarlaveg
1921 mveeliasha dvnavdelgk salhwaaavn nveatlallk ngankdmqds keetplflaa
1981 regsyeaakl lldhfanrei tdhldrlprd vaqerlhqdi vrlldqpsgp rsppgphglg
2041 pllcppgafl pglkaaqsgs kksrrppgka glgpqgprgr gkkltlacpg pladssvtls
```

```
2101 pvdsldsprp fggppaspgg fplegpyaaa tatavslaql ggpgraglgr qppggcvlsl
2161 gllnpvavpl dwarlpppap pgpsfllpla pgpqllnpgt pvspqerppp ylavpghgee
2221 ypvagahssp pkarflrvps ehpyltpspe spehwaspsp pslsdwsest pspatatgam
2281 atttgalpaq plplsvpssl aqaqtqlgpq pevtpkrqvl a
```

By "Notch3 polynucleotide" is meant a nucleic acid molecule encoding a Notch3 polypeptide. An exemplary Notch3 polynucleotide sequence is provided at NCBI Reference Sequence: U97669.1, and reproduced herein below.

```
   1 acgcggcgcg gaggctggcc cgggacgcgc ccggagccca gggaaggagg gaggagggga
  61 gggtcgcggc cggccgccat ggggccgggg gcccgtggcc gccgccgccg ccgtcgcccg
 121 atgtcgccgc caccgccacc gccacccgtg cgggcgctgc ccctgctgct gctgctagcg
 181 gggccggggg ctgcagcccc cccttgcctg gacggaagcc cgtgtgcaaa tggaggtcgt
 241 tgcacccagc tgccctcccg ggaggctgcc tgcctgtgcc cgcctggctg ggtgggtgag
 301 cggtgtcagc tggaggaccc ctgtcactca ggcccctgtg ctggccgtgg tgtctgccag
 361 agttcagtgg tggctggcac cgcccgattc tcatgccggt gcccccgtgg cttccgaggc
 421 cctgactgct ccctgccaga tccctgcctc agcagccctt gtgcccacgg tgcccgctgc
 481 tcagtggggc ccgatggacg cttcctctgc tcctgcccac ctggctacca gggccgcagc
 541 tgccgaagcg acgtggatga gtgccgggtg ggtgagccct gccgccatgg tggcacctgc
 601 ctcaacacac ctggctcctt ccgctgccag tgtccagctg gctacacagg gccactatgt
 661 gagaaccccg cggtgccctg tgcgccctca ccatgccgta acgggggcac ctgcaggcag
 721 agtggcgacc tcacttacga ctgtgcctgt cttcctgggt ttgagggtca gaattgtgaa
 781 gtgaacgtgg acgactgtcc aggacaccga tgtctcaatg gggggacatg cgtggatggc
 841 gtcaacacct ataactgcca gtgccctcct gagtggacag gccagttctg cacggaggac
 901 gtggatgagt gtcagctgca gcccaacgcc tgccacaatg ggggtacctg cttcaacacg
 961 ctgggtggcc acagctgcgt gtgtgtcaat ggctggacag gtgagagctg cagtcagaat
1021 atcgatgact gtgccacagc cgtgtgcttc catggggcca cctgccatga ccgcgtggct
1081 tctttctact gtgcctgccc catgggcaag actggcctcc tgtgtcacct ggatgacgcc
1141 tgtgtcagca acccctgcca cgaggatgct atctgtgaca caaatccggt gaacggccgg
1201 gccatttgca cctgtcctcc cggcttcacg ggtggggcat gtgaccagga tgtggacgag
1261 tgctctatcg gcgccaaccc ctgcgagcac ttgggcaggt gcgtgaacac gcagggctcc
1321 ttcctgtgcc agtgcggtcg tggctacact ggacctcgct gtgagaccga tgtcaacgag
1381 tgtctgtcgg ggccctgccg aaaccaggcc acgtgcctcg accgcatagg ccagttcacc
1441 tgtatctgta tggcaggctt cacaggaacc tattgcgagg tggacattga cgagtgtcag
1501 agtagcccct gtgtcaacgg tggggtctgc aaggaccgag tcaatggctt cagctgcacc
1561 tgcccctcgg gcttcagcgg ctccacgtgt cagctggacg tggacgaatg cgccagcacg
1621 ccctgcagga atggcgccaa atgcgtggac cagcccgatg gctacgagtg ccgctgtgcc
1681 gagggctttg agggcacgct gtgtgatcgc aacgtggacg actgctcccc tgacccatgc
1741 caccatggtc gctgcgtgga tggcatcgcc agcttctcat gtgcctgtgc tcctggctac
1801 acgggcacac gctgcgagag ccaggtggac gaatgccgca gccagccctg ccgccatggc
1861 ggcaaatgcc tagacctggt ggacaagtac ctctgccgct gcccttctgg gaccacaggt
1921 gtgaactgcg aagtgaacat tgacgactgt gccagcaacc cctgcacctt tggagtctgc
1981 cgtgatggca tcaaccgcta cgactgtgtc tgccaacctg gcttcacagg gccccttttgt
2041 aacgtggaga tcaatgagtg tgcttccagc ccatgcggcg agggaggttc ctgtgtggat
2101 ggggaaaatg gcttccgctg cctctgcccg cctggctcct tgcccccact ctgcctcccc
2161 ccgagccatc cctgtgccca tgagccctgc agtcacggca tctgctatga tgcacctggc
2221 gggttccgct gtgtgtgtga gcctggctgg agtggccccc gctgcagcca gagcctggcc
2281 cgagacgcct gtgagtccca gccgtgcagg gccggtggga catgcagcag cgatggaatg
2341 ggtttccact gcacctgccc gcctggtgtc cagggacgtc agtgtgaact cctctccccc
2401 tgcaccccga acccctgtga gcatgggggc cgctgcgagt ctgcccctgg ccagctgcct
2461 gtctgctcct gcccccaggg ctggcaaggc ccacgatgcc agcaggatgt ggacgagtgt
2521 gctggccccg caccctgtgg ccctcatggt atctgcacca acctggcagg gagtttcagc
```

2581 tgcacctgcc atggagggta cactggccct tcctgtgatc aggacatcaa tgactgtgac
2641 cccaacccat gcctgaacgg tggctcgtgc caagacggcg tgggctcctt ttcctgctcc
2701 tgcctccctg gtttcgccgg cccacgatgc gcccgcgatg tggatgagtg cctgagcaac
2761 ccctgcggcc cgggcacctg taccgaccac gtggcctcct tcacctgcac ctgcccgccg
2821 ggctacggag gcttccactg cgaacaggac ctgcccgact gcagccccag ctcctgcttc
2881 aatggcggga cctgtgtgga cggcgtgaac tcgttcagct gcctgtgccg tcccggctac
2941 acaggagccc actgccaaca tgaggcagac ccctgcctct cgcggccctg cctacacggg
3001 ggcgtctgca gcgccgccca ccctggcttc cgctgcacct gcctcgagag cttcacgggc
3061 ccgcagtgcc agacgctggt ggattggtgc agccgccagc cttgtcaaaa cgggggtcgc
3121 tgcgtccaga ctggggccta ttgcctttgt ccccctggat ggagcggacg cctctgtgac
3181 atccgaagct tgccctgcag ggaggccgca gcccagatcg gggtgcggct ggagcagctg
3241 tgtcaggcgg gtgggcagtg tgtggatgaa gacagctccc actactgcgt gtgcccagag
3301 ggccgtactg gtagccactg tgagcaggag gtggaccccct gcttggccca gccctgccag
3361 catgggggga cctgccgtgg ctatatgggg ggctacatgt gtgagtgtct tcctggctac
3421 aatggtgata actgtgagga cgacgtggac gagtgtgcct cccagccctg ccagcacggg
3481 ggttcatgca ttgacctcgt ggcccgctat ctctgctcct gtcccccagg aacgctgggg
3541 gtgctctgcg agattaatga ggatgactgc ggcccaggcc caccgctgga ctcagggccc
3601 cggtgcctac acaatggcac ctgcgtggac ctggtgggtg gtttccgctg cacctgtccc
3661 ccaggataca ctggtttgcg ctgcgaggca gacatcaatg agtgtcgctc aggtgcctgc
3721 cacgcggcac acacccggga ctgcctgcag gacccaggcg gaggtttccg ttgcctttgt
3781 catgctggct tctcaggtcc tcgctgtcag actgtcctgt ctccctgcga gtcccagcca
3841 tgccagcatg gaggccagtg ccgtcctagc ccgggtcctg ggggtgggct gaccttcacc
3901 tgtcactgtg cccagccgtt ctggggtccg cgttgcgagc gggtggcgcg ctcctgccgg
3961 gagctgcagt gcccggtggg cgtcccatgc cagcagacgc cccgcgggcc gcgctgcgcc
4021 tgccccccag ggttgtcggg accctcctgc cgcagcttcc cggggtcgcc gccgggggcc
4081 agcaacgcca gctgcgcggc cgcccctgt ctccacgggg gctcctgccg ccccgcgccg
4141 ctcgcgccct tcttccgctg cgcttgcgcg cagggctgga ccgggccgcg ctgcgaggcg
4201 cccgccgcgg cacccgaggt ctcggaggag ccgcggtgcc cgcgcgccgc ctgccaggcc
4261 aagcgcgggg accagcgctg cgaccgcgag tgcaacagcc caggctgcgg ctgggacggc
4321 ggcgactgct cgctgagcgt gggcgacccc tggcggcaat gcgaggcgct gcagtgctgg
4381 cgcctcttca acaacagccg ctgcgacccc gcctgcagct cgcccgcctg cctctacgac
4441 aacttcgact gccacgccgg tggccgcgag cgcacttgca acccggtgta cgagaagtac
4501 tgcgccgacc actttgccga cggccgctgc gaccagggct gcaacacgga ggagtgcggc
4561 tgggatgggc tggattgtgc cagcgaggtg ccggccctgc tggcccgcgg cgtgctggtg
4621 ctcacagtgc tgctgccgcc ggaggagcta ctgcgttcca gcgccgactt tctgcagcgg
4681 ctcagcgcca tcctgcgcac ctcgctgcgc ttccgcctgg acgcgcacgg ccaggccatg
4741 gtcttccctt accaccggcc tagtcctggc tccgaacccc gggcccgtcg ggagctggcc
4801 cccgaggtga tcggctcggt agtaatgctg gagattgaca accggctctg cctgcagtcg
4861 cctgagaatg atcactgctt ccccgatgcc cagagcgccg ctgactacct gggagcgttg
4921 tcagcggtgg agcgcctgga cttcccgtac ccactgcggg acgtgcgggg ggagccgctg
4981 gagcctccag aacccagcgt cccgctgctg ccactgctag tggcgggcgc tgtcttgctg
5041 ctggtcattc tcgtcctggg tgtcatggtg gcccggcgca agcgcgagca cagcaccctc
5101 tggttccctg agggcttctc actgcacaag gacgtggcct ctggtcacaa gggccggcgg
5161 gaacccgtgg gccaggacgc gctgggcatg aagaacatgg ccaagggtga gagcctgatg
5221 ggggaggtgg ccacagactg gatggacaca gagtgcccag aggccaagcg gctaaaggta
5281 gaggagccag gcatgggggc tgaggaggct gtggattgcc gtcagtggac tcaacaccat
5341 ctggttgctg ctgacatccg cgtggcacca gccatggcac tgacaccacc acagggcgac
5401 gcagatgctg atggcatgga tgtcaatgtg cgtggcccag atggcttcac cccgctaatg
5461 ctggcttcct tctgtggggg ggctctggag ccaatgccaa ctgaagagga tgaggcagat
5521 gacacatcag ctagcatcat ctccgacctg atctgccagg gggctcagct tggggcacgg
5581 actgaccgta ctggcgagac tgctttgcac ctggctgccc gttatgcccg tgctgatgca
5641 gccaagcggc tgctggatgc tggggcagac accaatgccc aggaccactc aggccgcact
5701 cccctgcaca cagctgtcac agccgatgcc cagggtgtct tccagattct catccgaaac

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5761 cgctctacag acttggatgc ccgcatggca gatggctcaa cggcactgat cctggcggcc
5821 cgcctggcag tagagggcat ggtggaagag ctcatcgcca gccatgctga tgtcaatgct
5881 gtggatgagc ttgggaaatc agccttacac tgggctgcgg ctgtgaacaa cgtggaagcc
5941 actttggccc tgctcaaaaa tggagccaat aaggacatgc aggatagcaa ggaggagacc
6001 cccctattcc tggccgcccg cgagggcagc tatgaggctg ccaagctgct gttggaccac
6061 tttgccaacc gtgagatcac cgaccacctg gacaggctgc cgcgggacgt agcccaggag
6121 agactgcacc aggacatcgt gcgcttgctg gatcaaccca gtgggccccg cagcccccc
6181 ggtccccacg gcctggggcc tctgctctgt cctccagggg ccttcctccc tggcctcaaa
6241 gcggcacagt cggggtccaa gaagagcagg aggccccccg ggaaggcggg gctggggccg
6301 caggggcccc gggggcgggg caagaagctg acgctggcct gcccgggccc cctggctgac
6361 agctcggtca cgctgtcgcc cgtggactcg ctggactccc cgcggccttt cggtgggccc
6421 cctgcttccc ctggtggctt ccccccttgag gggccctatg cagctgccac tgccactgca
6481 gtgtctctgg cacagcttgg tggcccaggc cgggcaggtc tagggcgcca gccccctgga
6541 ggatgtgtac tcagcctggg cctgctgaac cctgtggctg tgcccctcga ttgggcccgg
6601 ctgcccccac ctgcccctcc aggcccctcg ttcctgctgc cactggcgcc gggaccccag
6661 ctgctcaacc cagggacccc cgtctccccg caggagcggc ccccgcctta cctggcagtc
6721 ccaggacatg gcgaggagta cccggtggct ggggcacaca gcagcccccc aaaggcccgc
6781 ttcctgcggg ttcccagtga gcacccttac ctgaccccat cccccgaatc ccctgagcac
6841 tgggccagcc cctcacctcc ctccctctca gactggtccg aatccacgcc tagcccagcc
6901 actgccactg gggccatggc caccaccact ggggcactgc ctgcccagcc acttcccttg
6961 tctgttccca gctcccttgc tcaggcccag acccagctgg ggccccagcc ggaagttacc
7021 cccaagaggc aagtgttggc ctgagacgct cgtcagttct tagatcttgg gggcctaaag
7081 agacccccgt cctgcctcct ttctttctct gtctcttcct tccttttagt ctttttcatc
7141 ctcttctctt tccaccaacc ctcctgcatc cttgccttgc agcgtgaccg agataggtca
7201 tcagcccagg gcttcagtct tcctttattt ataatgggtg ggggctacca cccaccctct
7261 cagtcttgtg aagagtctgg gacctccttc ttccccactt ctctcttccc tcattccttt
7321 ctctctcctt ctggcctctc atttccttac actctgacat gaatgaatta ttattatttt
7381 tctttttctt ttttttttta cattttgtat agaaacaaat tcatttaaac aaacttatta
7441 ttattatttt ttacaaaata tatatatgga gatgctccct ccccctgtga acccccccagt
7501 gcccccgtgg ggctgagtct gtgggcccat tcggccaagc tggattctgt gtacctagta
7561 cacaggcatg actgggatcc cgtgtaccga gtacacgacc caggtatgta ccaagtaggc
7621 acccttgggc gcacccactg gggccagggg tcgggggagt gttgggagcc tcctccccac
7681 cccacctccc tcacttcact gcattccaga ttggacatgt tccatagcct tgctggggaa
7741 gggcccactg ccaactccct ctgccccagc cccacccttg gccatctccc tttgggaact
7801 agggggctgc tggtgggaaa tgggagccag ggcagatgta tgcattcctt tatgtccctg
7861 taaatgtggg actacaagaa gaggagctgc ctgagtggta ctttctcttc ctggtaatcc
7921 tctggcccag ccttatggca gaatagaggt atttttaggc tatttttgta atatggcttc
7981 tggtcaaaat ccctgtgtag ctgaattccc aagccctgca ttgtacagcc ccccactccc
8041 ctcaccacct aataaaggaa tagttaacac tcaaaaaaaa aaaaaaaaaa a
```

By "Neurogenic locus notch homolog protein 4 (Notch4) polypeptide" is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: AAC32288.1, or a fragment thereof, and having Notch receptor activity. An exemplary Notch4 amino acid sequence is provided below:

```
  1 mqppsllllll lllllllcvsv vrprgllcgs fpepcanggt clslslgqgt cqcapgflge
 61 tcqfpdpcqn aqlcqnggsc qallpaplgl psspspltps flctclpgft gercqakled
121 pcppsfcskr grchiqasgr pqcscmpgwt geqcqlrdfc sanpcvnggv clatypqiqc
181 hcppgfegha cerdvnecfq dpgpcpkgts chntlgsfqc lcpvgqegpr celragpcpp
241 rgcsnggtcq lmpekdstfh lclcppgfig pdcevnpdnc vshqcqnggt cqdgldtytc
301 lcpetwtgwd csedvdecet qgpphcrngg tcqnsagsfh cvcvsgwggt sceenlddci
361 aatcapgstc idrvgsfscl cppgrtgllc hledmclsqp chgdaqcstn pltgstlclc
```

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 421 qpgysgptch qdldeclmaq qgpspcehgg sclntpgsfn clcppgytgs rceadhnecl
 481 sqpchpgstc ldllatfhcl cppglegqlc evetnecasa pclnhadchd llngfqcicl
 541 pgfsgtrcee didecrsspc anggqcqdqp gafhckclpg fegprcqtev declsdpcpv
 601 gascldlpga ffclcpsgft gqlcevplca pnlcqpkqic kdqkdkancl cpdgspgcap
 661 pednctchhg hcqrsscvcd vgwtgpecea elggcisapc ahggtcypqp sgynctcptg
 721 ytgptcseem tachsgpcln ggscnpspgg yyctcppsht gpqcqtstdy cvsapcfngg
 781 tcvnrpgtfs clcamgfqgp rcegklrpsc adspcrnrat cqdspqgprc lcptgytggs
 841 cqtlmdlcaq kpcprnshcl qtgpsfhclc lqgwtgplcn lplsscqkaa lsqgidvssl
 901 chngglcvds gpsyfchcpp gfqgslcqdh vnpcesrpcq ngatcmaqps gylcqcapgy
 961 dgqncskeld acqsqpchnh gtctpkpggf hcacppgfvg lrcegdvdec ldqpchptgt
1021 aachslanaf ycqclpghtg qwceveidpc hsqpcfhggt ceatagsplg fichcpkgfe
1081 gptcshraps cgfhhchhgg lclpspkpgf pprcaclsgy ggpdcltppa pkgcgppspc
1141 lyngscsett glggpgfrcs cphsspgprc qkpgakgceg rsgdgacdag csgpggnwdg
1201 gdcslgvpdp wkgcpshsrc wllfrdgqch pqcdseeclf dgydcetppa ctpaydqych
1261 dhfhnghcek gcntaecgwd ggdcrpedgd pewgpslall vvlsppaldq qlfalarvls
1321 ltlrvglwvr kdrdgrdmvy pypgaraeek lggtrdptyq eraapqtqpl gketdslsag
1381 fvvvmgvdls rcgpdhpasr cpwdpglllr flaamaavga lepllpgpll avhphagtap
1441 panqlpwpvl cspvagvill algallvlql irrrrrehga lwlppgftrr prtqsaphrr
1501 rpplgedsig lkalkpkaev dedgvvmcsg peegeevgqa eetgppstcq lwslsggcga
1561 lpqaamltpp qesemeapdl dtrgpdgvtp lmsavccgev qsgtfqgawl gcpepwepll
1621 dggacpqaht vgtgetplhl aarfsrptaa rrlleaganp nqpdragrtp lhaavaadar
1681 evcqlllrsr qtavdarted gttplmlaar lavedlveel iaaqadvgar dkwgktalhw
1741 aaavnnaraa rsllqagadk daqdnreqtp lflaaregav evaqlllglg aarelrdqag
1801 lapadvahqr nhwdlltlle gagppearhk atpgreagpf prartvsvsv pphgggalpr
1861 crtlsagagp rgggaclqar twsvdlaarg ggayshcrsl sgvgagggpt prgrrfsagm
1921 rgprpnpaim rgrygvaagr ggrvstddwp cdwvalgacg sasnipippp cltpspergs
1981 pqldcgppal qempinqgge gkk
```

By "Notch4 polynucleotide" is meant a nucleic acid molecule encoding a Notch4 polypeptide. An exemplary Notch4 polynucleotide sequence is provided at NCBI Reference Sequence: U95299.1, and reproduced herein below.

```
   1 gccggccgcg tcgaccctgc cccagtgaga gctctgaggg tccctgcctg aagagggaca
  61 gggaccgggg cttggagaag gggctgtgga atgcagcccc cttcactgct gctgctgctg
 121 ctgctgctgc tgctgctatg tgtctcagtg gtcagaccca gagggctgct gtgtgggagt
 181 ttcccagaac cctgtgccaa tggaggcacc tgcctgagcc tgtctctggg acaagggacc
 241 tgccagtgtg cccctggctt cctgggtgag acgtgccagt ttcctgaccc ctgccagaac
 301 gcccagctct gccaaaatgg aggcagctgc caagccctgc ttcccgctcc cctagggctc
 361 cccagctctc cctctccatt gacacccagc ttcttgtgca cttgcctccc tggcttcact
 421 ggtgagagat gccaggccaa gcttgaagac ccttgtcctc cctccttctg ttccaaaagg
 481 ggccgctgcc acatccaggc ctcgggccgc ccacagtgct cctgcatgcc tggatggaca
 541 ggtgagcagt gccagcttcg ggacttctgt tcagccaacc catgtgttaa tggaggggtg
 601 tgtctggcca catacccccа gatccagtgc cactgcccac cgggcttcga gggccatgcc
 661 tgtgaacgtg atgtcaacga gtgcttccag gacccaggac cctgccccaa aggcacctcc
 721 tgccataaca ccctgggctc cttccagtgc ctctgccctg tggggcagga gggtccacgt
 781 tgtgagctgc gggcaggacc ctgccctcct aggggctgtt cgaatggggg cacctgccag
 841 ctgatgccag agaaagactc cacctttcac ctctgcctct gtcccccagg tttcataggc
 901 ccagactgtg aggtgaatcc agacaactgt gtcagccacc agtgtcagaa tgggggcact
 961 tgccaggatg ggctggacac ctacacctgc ctctgcccag aaacctggac aggctgggac
1021 tgctccgaag atgtggatga gtgtgagacc cagggtcccc ctcactgcag aaacgggggc
1081 acctgccaga actctgctgg tagctttcac tgcgtgtgtg tgagtggctg gggcggcaca
1141 agctgtgagg agaacctgga tgactgtatt gctgccacct gtgccccggg atccacctgc
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1201 attgaccggg tgggctcttt ctcctgcctc tgcccacctg gacgcacagg actcctgtgc
1261 cacttggaag acatgtgtct gagccagccg tgccatgggg atgcccaatg cagcaccaac
1321 cccctcacag gctccacact ctgcctgtgt cagcctggct attcggggcc cacctgccac
1381 caggacctgg acgagtgtct gatggcccag caaggcccaa gtccctgtga acatggcggt
1441 tcctgcctca acactcctgg ctccttcaac tgcctctgtc cacctggcta cacaggctcc
1501 cgttgtgagg ctgatcacaa tgagtgcctc tcccagccct gccacccagg aagcacctgt
1561 ctggacctac ttgccacctt ccactgcctc tgcccgccag gcttagaagg gcagctctgt
1621 gaggtggaga ccaacgagtg tgcctcagct ccctgcctga accacgcgga ttgccatgac
1681 ctgctcaacg gcttccagtg catctgcctg cctggattct ccggcacccg atgtgaggag
1741 gatatcgatg agtgcagaag ctctccctgt gccaatggtg ggcagtgcca ggaccagcct
1801 ggagccttcc actgcaagtg tctcccaggc tttgaagggc cacgctgtca aacagaggtg
1861 gatgagtgcc tgagtgaccc atgtcccgtt ggagccagct gccttgatct tccaggagcc
1921 ttcttttgcc tctgcccctc tggtttcaca ggccagctct gtgaggttcc cctgtgtgct
1981 cccaacctgt gccagcccaa gcagatatgt aaggaccaga aagacaaggc caactgcctc
2041 tgtcctgatg gaagccctgg ctgtgcccca cctgaggaca actgcacctg ccaccacggg
2101 cactgccaga gatcctcatg tgtgtgtgac gtgggttgga cggggccaga gtgtgaggca
2161 gagctagggg gctgcatctc tgcaccctgt gcccatgggg ggacctgcta cccccagccc
2221 tctggctaca actgcacctg ccctacaggc tacacaggac ccacctgtag tgaggagatg
2281 acagcttgtc actcagggcc atgtctcaat ggcggctcct gcaaccctag ccctggaggc
2341 tactactgca cctgccctcc aagccacaca gggccccagt gccaaaccag cactgactac
2401 tgtgtgtctg ccccgtgctt caatgggggt acctgtgtga acaggcctgg caccttctcc
2461 tgcctctgtg ccatgggctt ccagggcccg cgctgtgagg gaaagctccg ccccagctgt
2521 gcagacagcc cctgtaggaa tagggcaacc tgccaggaca gccctcaggg tccccgctgc
2581 ctctgcccca ctggctacac cggaggcagc tgccagactc tgatggactt atgtgcccag
2641 aagccctgcc cacgcaattc ccactgcctc cagactgggc cctccttcca ctgcttgtgc
2701 ctccagggat ggaccgggcc tctctgcaac cttccactgt cctcctgcca gaaggctgca
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2821 ggcccctcct atttctgcca ctgcccccct ggattccaag gcagcctgtg ccaggatcac
2881 gtgaacccat gtgagtccag gccttgccag aacggggcca cctgcatggc ccagcccagt
2941 gggtatctct gccagtgtgc cccaggctac gatggacaga actgctcaaa ggaactcgat
3001 gcttgtcagt cccaaccctg tcacaaccat ggaacctgta ctcccaaacc tggaggattc
3061 cactgtgcct gccctccagg ctttgtgggg ctacgctgtg agggagacgt ggacgagtgt
3121 ctggaccagc cctgccaccc cacaggcact gcagcctgcc actctctggc caatgccttc
3181 tactgccagt gtctgcctgg acacacaggc cagtggtgtg aggtggagat agacccctgc
3241 cacagccaac cctgctttca tggagggacc tgtgaggcca cagcaggatc acccctgggt
3301 ttcatctgcc actgccccaa gggttttgaa ggccccacct gcagccacag ggccccttcc
3361 tgcggcttcc atcactgcca ccacggaggc ctgtgtctgc cctcccctaa gccaggcttc
3421 ccaccacgct gtgcctgcct cagtggctat gggggtcctg actgcctgac cccaccagct
3481 cctaaaggct gtggccctcc ctccccatgc ctatacaatg gcagctgctc agagaccacg
3541 ggcttggggg gcccaggctt tcgatgctcc tgccctcaca gctctccagg gccccggtgt
3601 cagaaacccg gagccaaggg gtgtgagggc agaagtggag atggggcctg cgatgctggc
3661 tgcagtggcc cgggaggaaa ctgggatgga ggggactgct ctctgggagt cccagacccc
3721 tggaagggct gcccctccca ctctcggtgc tggcttctct tccgggacgg gcagtgccac
3781 ccacagtgtg actctgaaga gtgtctgttt gatggctacg actgtgagac ccctccagcc
3841 tgcactccag cctatgacca gtactgccat gatcacttcc acaacgggca ctgtgagaaa
3901 ggctgcaaca ctgcagagtg tggctgggat ggaggtgact gcaggcctga agatggggac
3961 ccagagtggg ggccctccct ggccctgctg gtggtactga gccccccagc cctagaccag
4021 cagctgtttg ccctggcccg ggtgctgtcc ctgactctga gggtaggact ctgggtaagg
4081 aaggatcgtg atggcaggga catggtgtac ccctatcctg gggcccgggc tgaagaaaag
4141 ctaggaggaa ctcgggaccc cacctatcag gagagagcag cccctcaaac gcagcccctg
4201 ggcaaggaga ccgactccct cagtgctggg ttcgtggtgg tcatgggtgt ggatttgtcc
4261 cgctgtggcc ctgaccaccc ggcatcccgc tgtccctggg accctgggct tctactccgc
4321 ttccttgctg cgatggctgc agtgggagcc ctggagcccc tgctgcctgg accactgctg

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4381 gctgtccacc ctcatgcagg gaccgcaccc cctgccaacc agcttccctg gcctgtgctg
4441 tgctccccag tggccggggt gattctcctg gccctagggg ctcttctcgt cctccagctc
4501 atccggcgtc gacgccgaga gcatggagct ctctggctgc cccctggttt cactcgacgg
4561 cctcggactc agtcagctcc ccaccgacgc cggcccccac taggcgagga cagcattggt
4621 ctcaaggcac tgaagccaaa ggcagaagtt gatgaggatg gagttgtgat gtgctcaggc
4681 cctgaggagg gagaggaggt gggccaggct gaagaaacag gcccaccctc cacgtgccag
4741 ctctggtctc tgagtggtgg ctgtggggcg ctccctcagg cagccatgct aactcctccc
4801 caggaatctg agatggaagc ccctgacctg gacacccgtg gacctgatgg ggtgacaccc
4861 ctgatgtcag cagtttgctg tggggaagta cagtccggga ccttccaagg ggcatggttg
4921 ggatgtcctg agccctggga acctctgctg gatggagggg cctgtcccca ggctcacacc
4981 gtgggcactg gggagacccc cctgcacctg gctgcccgat tctcccggcc aaccgctgcc
5041 cgccgcctcc ttgaggctgg agccaacccc aaccagccag accgggcagg gcgcacaccc
5101 cttcatgctg ctgtggctgc tgatgctcgg gaggtctgcc agcttctgct ccgtagcaga
5161 caaactgcag tggacgctcg cacagaggac gggaccacac ccttgatgct ggctgccagg
5221 ctggcggtgg aagacctggt tgaagaactg attgcagccc aagcagacgt gggggccaga
5281 gataaatggg ggaaaactgc gctgcactgg gctgctgccg tgaacaacgc ccgagccgcc
5341 cgctcgcttc tccaggccgg agccgataaa gatgcccagg acaacaggga gcagacgccg
5401 ctattcctgg cggcgcggga aggagcggtg gaagtagccc agctactgct ggggctgggg
5461 gcagcccgag agctgcggga ccaggctggg ctagcgccgg cggacgtcgc tcaccaacgt
5521 aaccactggg atctgctgac gctgctggaa ggggctgggc caccagaggc ccgtcacaaa
5581 gccacgccgg gccgcgaggc tgggcccttc ccgcgcgcac ggacggtgtc agtaagcgtg
5641 cccccgcatg ggggcggggc tctgccgcgc tgccggacgc tgtcagccgg agcaggccct
5701 cgtgggggcg gagcttgtct gcaggctcgg acttggtccg tagacttggc tgcgcggggg
5761 ggcggggcct attcgcattg ccggagcctc tcgggagtag gagcaggagg aggcccgacc
5821 cctcgcggcc gtaggttttc tgcaggcatg cgcgggcctc ggcccaaccc tgcgataatg
5881 cgaggaagat acggagtggc tgccgggcgc ggaggcaggg tctcaacgga tgactggccc
5941 tgtgattggg tggccctggg agcttgcggt tctgcctcca acattccgat cccgcctcct
6001 tgccttactc cgtccccgga gcggggatca cctcaacttg actgtggtcc cccagccctc
6061 caagaaatgc ccataaacca aggaggagag ggtaaaaaat agaagaatac atggtaggga
6121 gg
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By "Notch inhibitor" is meant an agent capable of inhibiting the expression or activity of a Notch protein. Notch proteins include, but are not limited to, Notch1, Notch2, Notch3 and/or Notch4. In one embodiment, a Notch inhibitor reduces Notch signaling, for example by disrupting the receptor: ligand interaction or any other signaling event downstream of the Notch1, Notch2, Notch3 and/or Notch4 receptor, such as proteolytic cleavage of the Notch protein. In one embodiment, the Notch inhibitor is a gamma-secretase inhibitor (GSI). Notch inhibitors can include, for example, MK-0752, PF03084014, RO-4929097, DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester, tetralin imidazole PF-03084014, LY3039478 and BMS906-024. In some embodiments, inhibition is by at least about 10%, 25%, 50%, 75% or more. In another embodiment, a Notch inhibitor is any inhibitory nucleic acid that inhibits, for example, the expression of a Notch protein. In another embodiment, a Notch inhibitor is an antibody against Notch that inhibits Notch activity. Exemplary inhibitory Notch antibodies are known in the art, and include, for example, anti-Notch 1 (OMP-52M521) and anti-delta-like-4. In another embodiment, a

Notch inhibitor is a CRISPR-based therapeutic that depletes Notch (e.g., results in the conditional depletion of Notch).

By "B cell receptor inhibitor" is meant an agent capable of reducing B cell receptor signaling, including signaling by downstream pathways that are functionally regulated by B cell receptor signaling. In one embodiment, the B cell receptor inhibitor interrupts the receptor: ligand interaction or any other signaling event downstream of the B cell receptor. In one embodiment, the inhibitor is a Bruton tyrosine kinase (BTK) inhibitor. B cell receptor inhibitors can include, for example, ibrutinib (PCI-32765), acalabrutinib (ACP-196), ONO-4059 (e.g., GS-4059 or NCT02457598), spebrutinib (e.g., AVL-292, CC-292), and BGB-3111. In some embodiments, inhibition is by at least about 10%, 25%, 50%, 75% or more. In another embodiment, a B cell receptor inhibitor is any inhibitory nucleic acid that inhibits, for example, the expression of a B cell receptor component, e.g., any protein that forms a functional part of the B cell receptor. In another embodiment, a B cell receptor inhibitor is an antibody that inhibits B cell receptor activity. In another embodiment, a B cell receptor inhibitor is a CRISPR-based therapeutic that depletes a B cell receptor component (e.g., results in the conditional depletion of a B cell receptor component).

By "Neural precursor cell expressed developmentally down-regulated protein 9 (Nedd9) polypeptide" is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: AAH40207.1, or a fragment thereof, and having cell cycle or growth regulatory activity. An exemplary Nedd9 amino acid sequence is provided below:

```
  1 mkyknlmara lydnvpecae elafrkgdil tvieqntggl egwwlcslhg rqgivpgnrv
 61 klligpmqet assheqpasg lmqqtfgqqk lyqvpnpqaa prdtiyqvpp syqnqgiyqv
121 ptghgtqeqe vyqvppsvqr siggtsgphv gkkvitpvrt ghgyvyeyps ryqkdvydip
181 pshttqgvyd ippssakgpv fsvpvgeikp qgvydipptk gvyaippsac rdeaglrekd
241 ydfpppmrqa grpdlrpegv ydipptctkp agkdlhvkyn cdipgaaepv arrhqslspn
301 hpppqlgqsv gsqndaydvp rgvqfleppa etsekanpqe rdgvydvplh nppdakgsrd
361 lvdginrlsf sstgstrsnm stsstsskes slsaspaqdk rlfldpdtai erlqrlqqal
421 emgvsslmal vttdwrcygy merhineirt avdkvelflk eylhfvkgav anaaclpeli
481 lhnkmkrelq rvedshqils qtshdlnecs wslnilaink pqnkcddldr fvmvaktvpd
541 dakqltttin tnaealfrpg pgslhlkngp esimnsteyp hggsqgqllh pgdhkaqahn
601 kalppglske qapdcsssdg serswmddyd yvhlqgkeef erqqkellek enimkqnkmq
661 lehhqlsqfq lleqeitkpv endiskwkps qslpttnsgv saqdrqllcf yydqcethfi
721 sllnaidalf scvssaqppr ifvahskfvi lsahklvfig dtltrqvtaq dirnkvmnss
781 nqlceqlkti vmatkmaalh ypsttalqem vhqvtdlsrn aqlfkrslle matf
```

By "Nedd9 polynucleotide" is meant a nucleic acid molecule encoding a Nedd9 polypeptide. An exemplary Nedd9 polynucleotide sequence is provided at NCBI Reference Sequence BC040207.1, and reproduced herein below.

```
   1 agtgacttga gggaggcgct gcgactgaca agcggctctg cccgggacct tctcgctttc
  61 atctagcgct gcactcaatg gaggggcggg caccgcagtg cttaatgctg tcttaactag
 121 tgtaggaaaa cggctcaacc caccgctgcc gaaatgaagt ataagaatct tatggcaagg
 181 gccttatatg acaatgtccc agagtgtgcc gaggaactgg cctttcgcaa gggagacatc
 241 ctgaccgtca tagagcagaa cacaggggga ctggaaggat ggtggctgtg ctcgttacac
 301 ggtcggcaag gcattgtccc aggcaaccgg gtgaagcttc tgattggtcc catgcaggag
 361 actgcctcca gtcacgagca gcctgcctct ggactgatgc agcagacctt tggccaacag
 421 aagctctatc aagtgccaaa cccacaggct gctccccgag acaccatcta ccaagtgcca
 481 ccttcctacc aaaatcaggg aatttaccaa gtccccactg gccacggcac ccaagaacaa
 541 gaggtatatc aggtgccacc atcagtgcag agaagcattg ggggaaccag tgggccccac
 601 gtgggtaaaa aggtgataac ccccgtgagg acaggccatg gctacgtata cgagtaccca
 661 tccagatacc aaaaggacgt ctatgatatc cctccttctc ataccactca aggggtatac
 721 gacatccctc cctcatcagc aaaaggccct gtgttttcag ttccagtggg agagataaaa
 781 cctcaagggg tgtatgacat cccgcctaca aaaggggtat atgccattcc gccctctgct
 841 tgccgggatg aagcagggct tagggaaaaa gactatgact tccccctcc catgagacaa
 901 gctggaaggc cggacctcag accggagggg gtttatgaca ttcctccaac ctgcaccaag
 961 ccagcaggga aggaccttca tgtaaaatac aactgtgaca ttccaggagc tgcagaaccg
1021 gtggctcgaa ggcaccagag cctgtccccg aatcacccac ccccgcaact cggacagtca
1081 gtgggctctc agaacgacgc atatgatgtc ccccgaggcg ttcagtttct tgagccacca
1141 gcagaaacca gtgagaaagc aaacccccag gaaagggatg gtgtttatga tgtccctctg
1201 cataacccgc cagatgctaa aggctctcgg gacttggtgg atgggatcaa ccgattgtct
1261 ttctccagta caggcagcac ccggagtaac atgtccacgt cttccacctc ctccaaggag
1321 tcctcactgt cagcctcccc agctcaggac aaaaggctct tcctggatcc agacacagct
1381 attgagagac ttcagcggct ccagcaggcc cttgagatgg gtgtctccag cctaatggca
1441 ctggtcacta ccgactggcg gtgttacgga tatatggaaa gacacatcaa tgaaatacgc
1501 acagcagtgg acaaggtgga gctgttcctg aaggagtacc tccactttgt caagggagct
1561 gttgcaaatg ctgcctgcct cccggaactc atcctccaca acaagatgaa gcgggagctg
1621 caacgagttg aagactccca ccagatcctg agtcaaacca gccatgactt aaatgagtgc
1681 agctggtccc tgaatatctt ggccatcaac aagccccaga acaagtgtga cgatctggac
1741 cggtttgtga tggtggcaaa gacggtgccc gatgacgcca agcagctcac cacaaccatc
1801 aacaccaacg cagaggccct cttcagaccc ggccctggca gcttgcatct gaagaatggg
1861 ccggagagca tcatgaactc aacggagtac ccacacggtg gctcccaggg acagctgctg
1921 catcctggtg accacaaggc ccaggcccac aacaaggcac tgcccccagg cctgagcaag
1981 gagcaggccc ctgactgtag cagcagtgat ggttctgaga ggagctggat ggatgactac
2041 gattacgtcc acctacaggg taaggaggag tttgagaggc aacagaaaga gctattggaa
2101 aaagagaata tcatgaaaca gaacaagatg cagctggaac atcatcagct gagccagttc
2161 cagctgttgg aacaagagat tacaaagccc gtggagaatg acatctcgaa gtggaagccc
2221 tctcagagcc tacccaccac aaacagtggc gtgagtgctc aggatcggca gttgctgtgc
2281 ttctactatg accaatgtga gacccatttc atttcccttc tcaacgccat tgacgcactc
2341 ttcagttgtg tcagctcagc ccagcccccg cgaatcttcg tggcacacag caagtttgtc
2401 atcctcagtg cacacaaact ggtgttcatt ggagacacgc tgacacggca ggtgactgcc
2461 caggacattc gcaacaaagt catgaactcc agcaaccagc tctgcgagca gctcaagacc
2521 atagtcatgg caaccaagat ggccgccctc cattacccca gcaccacggc cctgcaggaa
2581 atggtgcacc aagtgacaga cctttctaga aatgcccagc tgttcaagcg ctctttgctg
2641 gagatggcaa cgttctgaga agaaaaaaaa gaggaagggg actgcgttaa cggttactaa
2701 ggaaaactgg aaatactgtc tggtttttgt aaatgttatc tatttttgta gatattttat
2761 ataaaaatga aatattttaa cattttatgg gtcagtcaac tttcagaaat tcagggagct
2821 ggagagggaa atctttttttt ttccccctga gtggttctta tgtacataga ggtatctgag
2881 acataaactg tacagaaaac ttgtccacgt gcttttgtat gcccatgtat tcatgtttgt
2941 ttgtagatgt ttgtctgatg catttcatta aaaaaaaaac catgaattac gaagcacctt
3001 agtaagcacc tcctaatgct gcatttttttt tgttgttgtt aaaaacatac cagctggtta
3061 taatattgtt ctccacgtcc ttgtgatgat tctgagcctg gcactcccaa atctgggaag
3121 catagtttat ttgcaagtgt tcaccttcca aatcatgagg catagcatga cttattcttg
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3181 tttggaaaac tcttttcaaa actgaccatc ttaaacacat gatggccaag tgcccaaaag
3241 ccctcttgcg gagcaaattt cagaatatat atgtggatcc aagctctgat agttcaggtg
3301 ctggagggaa gagagacctg tgtgtttaga ggccaggacc acagttagga ttgggttgtt
3361 tcaatactga gagacagcta caataaaagg agagcaattg cctccctggg gctgttcaat
3421 cttctgcatt tgtgagtggt tcagtcatga ggttttccaa aagatgtttt tagagttgta
3481 aaaaccatat ttgcagcaaa gatttacaaa ggcgtatcag actatgattg ttcaccaaaa
3541 taggggaatg gtttgatccg ccagttgcaa gtagaggcct ttctgactct taatattcac
3601 tttggtgcta ctacccccat tacctgaggg aaactggcca ggtccttgat catggaacta
3661 tagagctacc aggacatatc ctgctctcta agggaattta ttgctatctt gcaccttctt
3721 taaaactcac atatgcagac ctgacactca agagtggcta gctacacaga gtccatctaa
3781 tttttgcaac ttcctgtggc cagtgtgtat aacccccttcc actatctcac agatagtcac
3841 agcgtccatt ccatagtctg tctcctcaca tctgttagta ttgacacagc acagacacca
3901 caagccatca ggttcttcat ggggcaggtg aaatacttct accccatggg taaatgtatt
3961 cacatattac caagagaaga agcacattat ctatgatctt ttggcccagt tcttatttag
4021 catttttatt ccagcctact tggaaacatg tttttatttg caatatatgc ctgactgaat
4081 taagcttgct tgttttaaac aaccaaatca ttggaacaga aaaggattta aaaaacaaga
4141 atgcatgatc tcagagtgat taaaaaaaaa tcagtggaaa taaatgatca tagaaggtgc
4201 ttttcaaaac aactgctatt ataattctca aagtcctact ctgccaaaag aagattaaaa
4261 gtcatacatt acattacaag gaaatgttca tgtgggaaga gggttgctga aaatcaacaa
4321 cgcttgaagt taaaaagtgt gtctttgtag atttcattgt ataatgtgta tttcttagga
4381 gatggctgac ttgattgatc tacgctaagt ggagacattt cacattttta aaaccaaatg
4441 ttcaatctgt attactcttt gccgtcttgt atgtagaggc tatttttaaa tcattaaatt
4501 tttagatctc tgttttcaaa aaaaaaaaaa aa
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By "Phospholipase C Gamma 2, (PLCG2, 1-Phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-2) polypeptide" is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: AAQ76815.1, or a fragment thereof, and having phospholipase activity. An exemplary PLCG2 amino acid sequence is provided below:

```
   1 msttvnvdsl aeyeksqikr alelgtvmtv fsfrkstper rtvqvimetr qvawsktadk
  61 iegfldimei keirpgknsk dferakavrq kedccftily gtqfvlstls laadskedav
 121 nwlsglkilh qeamnastpt iieswlrkqi ysvdqtrrns islrelktil plinfkvssa
 181 kflkdkfvei gahkdelsfe qfhlfykklm feqqksilde fkkdssvfil gntdrpdasa
 241 vylhdfqrfl iheqqehwaq dlnkvrermt kfiddtmret aepflfvdef ltylfsrens
 301 iwdekydavd mqdmnnplsh ywissshnty ltgdqlrses speayirclr mgcrcieldc
 361 wdgpdgkpvi yhgwtrttki kfddvvqaik dhafvtssfp vilsieehcs veqqrhmaka
 421 fkevfgdlll tkpteasadq lpspsqlrek iiikhkklgp rgdvdvnmed kkdehkqqge
 481 lymwdsidqk wtrhycaiad aklsfsddie qtmeeevpqd ipptelhfge kwfhkkvekr
 541 tsaekllqey cmetggkdgt flvresetfp ndytlsfwrs grvqhcrirs tmeggtlkyy
 601 ltdnlrfrrm yaliqhyret hlpcaefelr ltdpvpnpnp heskpwyyds lsrgeaedml
 661 mriprdgafl irkregsdsy aitfrargkv khcrinrdgr hfvlgtsayf eslvelvsyy
 721 ekhslyrkmr lrypvtpell erynterdin slydvsrmyv dpseinpsmp qrtvkalydy
 781 kakrsdelsf crgalihnvs kepggwwkgd ygtriqqyfp snyvedista dfeelekqii
 841 ednplgslcr gildlntynv vkapqgknqk sfvfilepke qgdppvefat drveelfewf
 901 qsireitwki dskennmkyw eknqsiaiel sdlvvyckpt sktkdnlenp dfreirsfve
 961 tkadsiirqk pvdllkynqk gltrvypkgq rvdssnydpf rlwlcgsqmv alnfqtadky
1021 mqmnhalfsl ngrtgyvlqp esmrtekydp mppesqrkil mtltvkvlga rhlpklgrsi
1081 acpfveveic gaeygnnkfk ttvvndngls piwaptqekv tfeiydpnla flrfvvyeed
1141 mfsdpnflah atypikavks gfrsvplkng ysedielasl lvfcemrpvl eseeelyssc
1201 rqlrrrqeel nnqlflydth qnlrnanrda lvkefsvnen hssctrrnat rg
```

By "PLCG2 polynucleotide" is meant a nucleic acid molecule encoding a PLCG2 polypeptide. An exemplary PLCG2 polynucleotide sequence is provided at NCBI Reference Sequence: NM_002661.4, and reproduced herein below.

```
   1 gaggatcacg tggcgcggcg ccgcggccga agcagaagta gcgagcgccg gcggcggagg
  61 gcgtgagcgg cgctgagtga cccgagtcgg gacgcgggct gcgcgcgcgg gaccccggag
 121 cccaaacccg gggcaggcgg gcagctgtgc ccgggcggca cggccagctt cctgatttct
 181 cccgattcct tccttctccc tggagcggcc gacaatgtcc accacggtca atgtagattc
 241 ccttgcggaa tatgagaaga gccagatcaa gagagccctg gagctgggga cggtgatgac
 301 tgtgttcagc ttccgcaagt ccacccccga gcggagaacc gtccaggtga tcatggagac
 361 gcggcaggtg gcctggagca agaccgctga caagatcgag ggcttcttgg atatcatgga
 421 aataaaagaa atccgcccag ggaagaactc caaagatttc gagcgagcaa aagcagttcg
 481 ccagaaagaa gactgctgct tcaccatcct atatggcact cagttcgtcc tcagcacgct
 541 cagcttggca gctgactcta aagaggatgc agttaactgg ctctctggct tgaaaatctt
 601 acaccaggaa gcgatgaatg cgtccacgcc caccattatc gagagttggc tgagaaagca
 661 gatatattct gtggatcaaa ccagaagaaa cagcatcagt ctccgagagt tgaagaccat
 721 cttgcccctg atcaacttta aagtgagcag tgccaagttc cttaaagata agtttgtgga
 781 aataggagca cacaaagatg agctcagctt tgaacagttc catctcttct ataaaaaact
 841 tatgtttgaa cagcaaaaat cgattctcga tgaattcaaa aaggattcgt ccgtgttcat
 901 cctggggaac actgacaggc cggatgcctc tgctgtttac ctgcatgact tccagaggtt
 961 tctcatacat gaacagcagg agcattgggc tcaggatctg aacaaagtcc gtgagcggat
1021 gacaaagttc attgatgaca ccatgcgtga aactgctgag cctttcttgt ttgtggatga
1081 gttcctcacg tacctgtttt cacgagaaaa cagcatctgg gatgagaagt atgacgcggt
1141 ggacatgcag gacatgaaca acccccctgtc tcattactgg atctcctcgt cacataacac
1201 gtaccttaca ggtgaccagc tgcggagcga gtcgtcccca gaagcttaca tccgctgcct
1261 gcgcatgggc tgtcgctgca ttgaactgga ctgctgggac gggcccgatg ggaagccggt
1321 catctaccat ggctggacgc ggactaccaa gatcaagttt gacgacgtcg tgcaggccat
1381 caaagaccac gcctttgtta cctcgagctt cccagtgatc ctgtccatcg aggagcactg
1441 cagcgtggag caacagcgtc acatggccaa ggccttcaag gaagtatttg gcgacctgct
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1561 gaagatcatc atcaagcata agaagctggg cccccgaggc gatgtggatg tcaacatgga
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1681 gaaatggact cggcactact gcgccattgc cgatgccaag ctgtccttca gtgatgacat
1741 tgaacagact atggaggagg aagtgcccca ggatataccc cctacagaac tacattttgg
1801 ggagaaatgg ttccacaaga aggtggagaa gaggacgagt gccgagaagt tgctgcagga
1861 atactgcatg gagacggggg gcaaggatgg caccttcctg gttcgggaga gcgagacctt
1921 ccccaatgac tacaccctgt ccttctggcg gtcaggccgg gtccagcact gccggatccg
1981 ctccaccatg gagggcggga ccctgaaata ctacttgact gacaacctca ccttcagcag
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2101 gcggctcacg gaccctgtgc ccaaccccaa cccccacgag tccaagccgt ggtactatga
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2281 ggtaaagcat tgtcgcatca accgggacgg ccggcacttt gtgctgggga cctccgccta
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2461 aaactccctc tacgacgtca gcagaatgta tgtggatccc agtgaaatca atccgtccat
2521 gcctcagaga accgtgaaag ctctgtatga ctacaaagcc aagcgaagcg atgagctgag
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2701 tgcagacttc gaggagctag aaaagcagat tattgaagac aatcccttag ggtctctttg
2761 cagaggaata ttggacctca atacctataa cgtcgtgaaa gcccctcagg gaaaaaacca
2821 gaagtccttt gtcttcatcc tggagcccaa gcagcagggc gatcctccgg tggagtttgc
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2881 cacagacagg gtggaggagc tctttgagtg gtttcagagc atccgagaga tcacctggaa
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3001 gctctctgac ctggttgtct actgcaaacc aaccagcaaa accaaggaca acttagaaaa
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3121 gaagcccgtc gacctcctga agtacaatca aaagggcctg acccgcgtct acccaaaggg
3181 acaaagagtt gactcttcaa actacgaccc cttccgcctc tggctgtgcg gttctcagat
3241 ggtggcactc aatttccaga cggcagataa gtacatgcag atgaatcacg cattgttttc
3301 tctcaatggg cgcacgggct acgttctgca gcctgagagc atgaggacag agaaatatga
3361 cccgatgcca cccgagtccc agaggaagat cctgatgacg ctgacagtca aggttctcgg
3421 tgctcgccat ctccccaaac ttggacgaag tattgcctgt ccctttgtag aagtggagat
3481 ctgtggagcc gagtatgaca acaacaagtt caagacgacg gttgtgaatg ataatggcct
3541 cagccctatc tgggctccaa cacaggagaa ggtgacattt gaaatttatg acccaaacct
3601 ggcatttctg cgctttgtgg tttatgaaga agatatgttc agcgatccca actttcttgc
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3721 tgggtacagc gaggacatag agctggcttc cctcctggtt ttctgtgaga tgcggccagt
3781 cctggagagc gaagaggaac tttactcctc ctgtcgccag ctgaggaggc ggcaagaaga
3841 actgaacaac cagctctttc tgtatgacac acaccagaac ttgcgcaatg ccaaccggga
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3961 caacaagagg ttaagagaga agagagtcag caacagcaag ttttactcat agaagctggg
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4081 ctattcacac tctgggaaga cgctaatctg tgacatcttt tcttcaagcc tgccatcaag
4141 gacatttctt aagacccaac tggcatgagt tggggtaatt tcctattatt ttcatcttgg
4201 acaactttct taacttatat tctttataga ggattcccca aaatgtgctc ctcatttttg
4261 gcctctcatg ttccaaacct cattgaataa aagcaatgaa aaccttgatc aattaagcct
4321 tctgttgcac gacctgtgca gtgaacagga tttcttttct ggccaagaag attctacctc
4381 taatgatcca ggtaactgat gtccatggag gatgagctgg aaatgtaaga aactattcat
4441 gagattctga aaaggatttt aactcaaagg caaatgattc cataagggcc caaagagaag
4501 ccctacccac aggcagcctg ctcagttcaa tgtactttaa ctaccaccgg ctgcctgctg
4561 cagtccacaa gaaaatggct gagtgatggg atctgttcat taagacaatt tctaattaat
4621 ggtgacagct tgttttgtga ctagagttac tgggatggag ggtaggaatc ttggggcctc
4681 tttgttttaa aaagcccatc agagagacca gagccgtgct gcaggggcag gttctcactt
4741 gcccctggct ctgccagctg ctgggaggct ctggccccac tagtccctca tggccctact
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4861 caactgctgc ttgaaagagg tagacaaaag ttaggttgat ggcgaaatgt ctctgggtta
4921 cccagtcttc tggagcagca agctgagctt taatgggcta agcattaggg tgttacagaa
4981 aatttcaaat gcagccatct cccttggggc agatctacct agttcatgac agtatgtgcg
5041 gctggccagg gctttacacc tctgcatctt aagttgttaa tacataccaa taatgtaata
5101 tggcttttta aaggagagga gagtgctggg ttgggaaggg aggtggttgg tagagtcaca
5161 acttctcaat gagtgaattt acagctgatg ggaaaaggag tgtaactgtg aaaaacgatg
5221 gctgtggtgg ggaagaacaa accagcagta agcctgatgt ttgatgtgga tggaactggc
5281 ccctagaaac ccatctgacc ctcctcttgt tacccgaaat gctgggctta gtatgcatgt
5341 actgctgaaa agcagggcag aacaaatcag gctctgacca gaagatcctt ctggtccctt
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5461 tgtggatgtt taaacaaaaa gctgtgggtc tcatcaatca tctccatcca caagctccta
5521 aaagaaagcc atttacctcg cttgaagcca ggaacacagg gaacagcagt ctggccaagg
5581 aagggctgtt atctggtgct atcactccag ttactcctcc aactgggagc tgctatttta
5641 tttggcagtc agcaactgaa gaaagaacat tcctcttagt ggcagatgtt caaagcaact
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5761 ggccccatta gcctttggcc aggtagccac cagaacctat ttattgcacc tggcatctcc
5821 cccaacccct ctcagctctg ttaggacttc cacacagcag agctcaggtg ttgctgtcat
5881 tacctccttt cagctcctca cttcattcta ctttaaagcc acagtgctaa ggcctgcatc
5941 ccctttctgc ccaaatgggt tttttgctac catatcaaag aacctgacat atggcggcat
6001 aggaagcaga agctaagcct ctctccagct gctgctgtgt aaaatccatg cgtggccaaa

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6061 gagaagtcag gggattatga cataaatggt gctgggaaga accctctgcc taaaactgtc
6121 tccttctcct ggtgctacaa ccggaatcca ccatgagaga gtactttctt cggttctttc
6181 ctcctgtcct tgacagagta acacgttaat ctggttcttg gtggtgttag ggactgattc
6241 tctcaggaaa ggcacacatg gtatgatggc tcttcccaga gtctatgtga tgctacataa
6301 cttcagtatc tagctgagac atgcttccta catgactgtt aaagcacagc caatccaggc
6361 caagaagact agtaacaggc acattctgaa agatggaagc agcactgata gatcaaaacc
6421 accactgcat atgtattaca ctgtttttgt tcaccatttt cctaagtgtg ttatttagaa
6481 tattggttat tacaaggaaa aataaagtgg ggaggctggt taggccttgt gagtttggga
6541 aacttaggtt ataaaaacta aataaagttt ttctactgtg agactagatg tgcaggagtg
6601 aaaggtgtag agggtcttgt tttccaaatt cgatctcaga atctttttgc cagaagtgtc
6661 tcatgggact tatctatagt ggaacacatt tgaagaccta ctgctctatt aagaaggcag
6721 ccggacaaca tgttctaata cttcgtatgc tttgtgacct agttaaaatc taaacttaag
6781 tcgccatggc cagtggcctt tagattaagc tagccttacc cctgggagta taccagagct
6841 ttccaaggaa tacacagact ccagtactct caggggagca gtgttcagag cctcatcttc
6901 ctgttatatt cttctctaag attcatctgc ctgagaaaat gccctttttct caccttacaa
6961 aagaaaatat ggctgtctcc acctctagtc ttactgtaga gcatgtccca aggtgtaaaa
7021 attcaaaatg tggatatttg gaaagtgaaa gacttatcaa cagggcacaa atctttttgc
7081 aaatggattt tccaagtttt tctggtggtt ccaaattttt tgctttcaac aaagtgggag
7141 gaacagcctg tagatttctg agtctcttag catgtaacta caaaggggtt ggaagaattc
7201 agtgattctg ctatcataaa gcttccgttc ccattgatgt atctgtgtga acaaggatca
7261 acatctccat aaatgaaatt gaaaacggaa aatagaattg atgatgaact ttggctcaat
7321 cttaagatgt tatcaatcta catagatgaa ataattgtgg agaaaagccc tctttatctc
7381 attaagtgat acatttccaa agaagtttta ctatgtttaa taatttagtg aaatttgggc
7441 tatgtgttta ttgattcagc tcaatccaga ggaaaatttt aaaggcttac agccttagga
7501 ttataggata ctatataata cttttggtac agagatagaa ttaaataaca taaaaatcaa
7561 aaatttatta ggctaaaatt ttgagggaga agtggtatga aaatacaaat tcaaggagta
7621 aaaggaaaag tggggcattc cttgctacta aaaattgcct tgttccaggt aagactgatc
7681 ataaaaaaat ggccctgttc ataaaatttt taaaaagatc atagtatcta tcaaataact
7741 tatattaaga acctcctggg ctaaatttaa aaagtaatac aacagtttta tttaaacatg
7801 tagtgtctac ggtatgccag cactttgcag ctatttataa tgagaaattt tagatgtcaa
7861 tatagcaatg tgcaagaaga tagagatttt caaaattcac ttaagagtat ctgagcataa
7921 aatgttaaga ttgctgatcg gatgtgaggg cgatctggct gcgacatctg tcaccccatt
7981 gatcgccagg gttgattcgg ctgatctggc tggctaggtg ggtgtcccct tcctacctca
8041 ccgctccatg tgcgtccctc ccgaagctgc gcgctccgtc gaagaggacg accaaccccg
8101 atagaggagg accggtcttc ggtcaagggt atacgagtag ctgcgctccc ctgctggaac
8161 ctccaaacaa gctctcaaga ttgctgatct agggccacta agtgatgaat tgtatttgga
8221 agcaaaaagg atggctaaaa aggacctcaa ccctttttgac tttaaaagga aaatagctta
8281 accttcaacc tgtgtgacat ttaacttttt gaacccaacc gtaaaagcta tcttctaacc
8341 aacaaaaagt taataattag atttggaatt atacagaatt agaaaattgg catttaaaaa
8401 tactcaataa tttgtccctg gtttttaatt ttcaaaatat tttctttttg aagagccaga
8461 ttccagtgat cctgcctctc agaaatttcc acatttctta tttttcatta ggccttaaga
8521 agctgcattt gtaaacttgt gtttcattat taaagcttaa tttatttttt atataaatag
8581 tatgtgcttt gtgtacatag agaattaagt gaatgagtca cacagatgtt ggctgttgtt
8641 aatgtgaaaa ttaaacagct gtatcacatt ttgaaaaata aaagtttcat ctgaatgaat
8701 atagcaa
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By "recombining binding protein suppressor of hairless isoform 1 (RBPJ) polypeptide" is meant a protein having at least about 85% amino acid identity to the sequence provided at NCBI Reference Sequence: NP_005340.2, or a fragment thereof, and having transcriptional regulatory activity. An exemplary RBPJ amino acid sequence is provided below:

```
   1 mdhtegspae eppahapspg kfgerpppkr ltreamrnyl kergdqtvli lhakvaqksy
  61 gnekrffcpp pcvylmgsgw kkkkeqmerd gcseqesqpc afigignsdq emqqlnlegk
 121 nyctaktlyi sdsdkrkhfm lsvkmfygns ddigvflskr ikviskpskk kqslknadlc
 181 iasgtkvalf nrlrsqtvst rylhveggnf hassqqwgaf fihlldddes egeeftvrdg
 241 yihygqtvkl vcsvtgmalp rliirkvdkq talldaddpv sqlhkcafyl kdtermylcl
 301 sqeriiqfqa tpcpkepnke mindgaswti istdkaeytf yegmgpvlap vtpvpvvesl
 361 qlngggdvam leltgqnftp nlrvwfgdve aetmyrcges mlcvvpdisa fregwrwvrq
 421 pvqvpvtlvr ndgiiystsl tftytpepgp rphcsaagai lranssqvpp nesntnsegs
 481 ytnastnsts vtsstatvvs
```

By "RBPJ polynucleotide" is meant a nucleic acid molecule encoding a RBPJ polypeptide. An exemplary RBPJ polynucleotide sequence is provided at NCBI Reference Sequence NM_014276.3, and reproduced herein below.

```
   1 gtgtgcaggg ttccagcgac agcagcactg gactcgtcca gagggcggcg ggtgagcggc
  61 tggggccccg tggagccacc atggaccccg caggggcagc agacccctca gtgcctccca
 121 atcctttgac tcacctgagc ctgcaggaca gatcagagat gcagctgcag agcgaagccg
 181 acaggcggag cctcccgggc acttggacca ggtcatcccc agagcacacc accattctga
 241 ggggaggcgt gcgcaggtgc ctgcagcaac agtgtgaaca gactgtgcgg atcctgcatg
 301 ccaaggtggc ccagaaatca tacggaaatg agaagcggtt cttctgcccc ccgccctgtg
 361 tctacctctc ggggcctggc tggagggtga agccagggca ggatcaagct caccaggcgg
 421 gggaaacggg gcccacggtc tgcggttaca tgggactgga cagcgcgtcc ggcagcgcca
 481 ctgagacgca gaagctgaat ttcgagcagc agccggactc cagggaattc ggctgcgcca
 541 agaccctgta catctcagat gcagacaaga ggaagcactt tcggctggtg ctgcggctgg
 601 tgctgcgcgg gggccgggag ctgggtacct tccacagccg ccttatcaag gtcatctcga
 661 agccctcgca gaagaagcag tcgctgaaaa acaccgatct gtgcatatcc tccggctcaa
 721 aggtctccct cttcaaccgc ctgcgctctc agacggtctc cacacgctac ctctctgtgg
 781 aggatggggc ctttgtggcc agtgcacgac agtgggctgc cttcacgctc cacctggctg
 841 atgggcactc tgcccaagga gacttcccac cgcgagaggg ctacgttcgc tatggctccc
 901 tggtgcagct cgtctgcacg gtcaccggca tcacactacc tcccatgatc atccgtaaag
 961 tagcaaaaca gtgtgcgctc cttgatgtgg atgagcccat ctcccagctg cacaagtgtg
1021 cattccagtt tccaggcagt cccccaggag ggggtggcac ctacttatgc cttgccacag
1081 agaaggtggt gcaatttcag gcctctccct gccccaagga ggcgaacagg gctctgctta
1141 acgacagctc ttgctggacc atcatcggca ccgagtcggt ggaattttcc ttcagcacca
1201 gcctggcgtg taccctggag ccggtcactc cggtgcctct catcagcacc ctagagctga
1261 gcggcggggg cgacgtggcc acgctggagc tccacggaga gaacttccac gcggggctca
1321 aggtgtggtt tggggacgtg gaggcagaaa ccatgtacag gagcccgcgg tccctggtgt
1381 gcgtggtgcc ggacgtggcg gccttctgca gcgactggcg ctggctgcgc gctcccatca
1441 caatccccat gagcctggtg cgcgccgacg ggctcttcta ccctagtgcc ttctccttca
1501 cctacacccc ggaatacagc gtgcggccgg gtcaccccgg cgtccccgag cccgccaccg
1561 acgccgacgc gctcctggag agcatccatc aggagttcac gcgcaccaac ttccacctct
1621 tcatccagac ttaggcgcgc ccggtagccc cggctgccca ccctggaggg ctgcgcccgc
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1741 aagggcagct gaaggctcac cctagaaacc gggcctggtg ggtcttaccc ggctcactcc
1801 ctcccttgtc cttacacata caggaagaca agacctgagt ggtgctgtct ttgtgtccgt
1861 cgtgtatggc tctccctgtc ttcatttctt ctcactctgt ctctaaacct ctctctctct
1921 cccttccccc tcagtactta gtctacagac ctatgtgcgt gtccctatcc ttctgtcctt
1981 ttctctcttc agctctccct gcctctcaca cacaatttta catgccccga ggagccaagt
2041 ttgggacatt taccctccag gcatctgtgt cccctcttga agagaaaaca cacagcttca
2101 cacatccagg catagggggc aagctcttgg ggcatcagga ccctggagca ccaggtcctt
2161 cctggaatat tagatccacc tggagcaccg ggtctctcta agtctcacct ggggaattcg
2221 gtcccacctg gggcaccagt tcccacctag agcactgtgt cctgccctag agcacaaaga
2281 cctgctcctc ccgagactct ctctgactgc agccaggcat agtacctttg cctgtgtttg
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2341 ctccctggtc cacagatttg gtggctgggc aggtgcctgg acagtgatga ggtcttgccg
2401 ccttaactgt ccccccagt cacttctccc acaggcccag caggacgcag tcctgaggat
2461 cagggattct acagctgcat taaaatcaat cctatccaa
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By “agent” is meant a small compound, polynucleotide, or polypeptide.

By “ameliorate” is meant decrease, suppress, attenuate, diminish, arrest, or stabilize the development or progression of a disease.

By "alteration" is meant a change (increase or decrease) in the expression levels or activity of a gene or polypeptide as detected by standard art known methods such as those described herein. As used herein, an alteration includes a 10% change in expression or activity levels, a 25% change, a 40% change, a 50% change, or an even greater change in expression or activity levels (i.e., 75%, 80%, 85%, 90%).

By "analog" is meant a molecule that is not identical, but has analogous functional or structural features. For example, a polypeptide analog retains the biological activity of a corresponding naturally-occurring polypeptide, while having certain biochemical modifications that enhance the analog's function relative to a naturally occurring polypeptide. Such biochemical modifications could increase the analog's protease resistance, membrane permeability, or half-life, without altering, for example, ligand binding. An analog may include an unnatural amino acid.

The term "co-administration" or "combined administration" as used herein is defined to encompass the administration of the selected therapeutic agents to a single patient, and are intended to include treatment regimens in which the agents are not necessarily administered by the same route of administration or at the same time.

In this disclosure, "comprises," "comprising," "containing" and "having" and the like can have the meaning ascribed to them in U.S. Patent law and can mean " includes," "including," and the like; "consisting essentially of" or "consists essentially" likewise has the meaning ascribed in U.S. Patent law and the term is open-ended, allowing for the presence of more than that which is recited so long as basic or novel characteristics of that which is recited is not changed by the presence of more than that which is recited, but excludes prior art embodiments.

“Detect” refers to identifying the presence, absence or amount of the analyte to be detected.

By "disease" is meant any condition or disorder that damages, or interferes with the normal function of a cell, tissue, or organ. Examples of diseases include cancer, including but not limited to small B-cell lymphomas, such as mantle cell lymphoma, or chronic

lymphocytic leukemia (e.g., small lymphocytic lymphoma), diffuse large B cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, splenic red pulp lymphoma, MALT lymphoma and leukemias such as chronic lymphocytic leukemia, B cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and early T cell acute lymphoblastic leukemia).

By "effective amount" is meant the amount of an agent required to ameliorate the symptoms of a disease relative to an untreated patient. In one embodiment, an effective amount of an agent of the invention reduces or stabilizes the growth or proliferation of a neoplastic cell. In other embodiments, an effective amount of an agent of the invention reduces the survival of a neoplastic cell. The effective amount of active compound(s) used to practice the present invention for therapeutic treatment of a disease varies depending upon the manner of administration, the age, body weight, and general health of the subject. Ultimately, the attending physician or veterinarian will decide the appropriate amount and dosage regimen. Such amount is referred to as an "effective" amount.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule. This portion contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 nucleotides or amino acids.

"Hybridization" means hydrogen bonding, which may be Watson-Crick, Hoogsteen or reversed Hoogsteen hydrogen bonding, between complementary nucleobases. For example, adenine and thymine are complementary nucleobases that pair through the formation of hydrogen bonds.

By "inhibitory nucleic acid" is meant a double-stranded RNA, siRNA, shRNA, or antisense RNA, or a portion thereof, or a mimetic thereof, that when administered to a mammalian cell results in a decrease (e.g., by 10%, 25%, 50%, 75%, or even 90-100%) in the expression of a target gene. Typically, a nucleic acid inhibitor comprises at least a portion of a target nucleic acid molecule, or an ortholog thereof, or comprises at least a portion of the complementary strand of a target nucleic acid molecule. For example, an inhibitory nucleic acid molecule comprises at least a portion of any or all of the nucleic acids delineated herein.

The terms "isolated," "purified," or "biologically pure" refer to material that is free to varying degrees from components which normally accompany it as found in its native state. "Isolate" denotes a degree of separation from original source or surroundings. "Purify"

denotes a degree of separation that is higher than isolation. A "purified" or "biologically pure" protein is sufficiently free of other materials such that any impurities do not materially affect the biological properties of the protein or cause other adverse consequences. That is, a nucleic acid or peptide of this invention is purified if it is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Purity and homogeneity are typically determined using analytical chemistry techniques, for example, polyacrylamide gel electrophoresis or high performance liquid chromatography. The term "purified" can denote that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. For a protein that can be subjected to modifications, for example, phosphorylation or glycosylation, different modifications may give rise to different isolated proteins, which can be separately purified.

By "isolated polynucleotide" is meant a nucleic acid (e.g., a DNA) that is free of the genes which, in the naturally-occurring genome of the organism from which the nucleic acid molecule of the invention is derived, flank the gene. The term therefore includes, for example, a recombinant DNA that is incorporated into a vector; into an autonomously replicating plasmid or virus; or into the genomic DNA of a prokaryote or eukaryote; or that exists as a separate molecule (for example, a cDNA or a genomic or cDNA fragment produced by PCR or restriction endonuclease digestion) independent of other sequences. In addition, the term includes an RNA molecule that is transcribed from a DNA molecule, as well as a recombinant DNA that is part of a hybrid gene encoding additional polypeptide sequence.

By an "isolated polypeptide" is meant a polypeptide of the invention that has been separated from components that naturally accompany it. Typically, the polypeptide is isolated when it is at least 60%, by weight, free from the proteins and naturally-occurring organic molecules with which it is naturally associated. Preferably, the preparation is at least 75%, more preferably at least 90%, and most preferably at least 99%, by weight, a polypeptide of the invention. An isolated polypeptide of the invention may be obtained, for example, by extraction from a natural source, by expression of a recombinant nucleic acid encoding such a polypeptide; or by chemically synthesizing the protein. Purity can be measured by any appropriate method, for example, column chromatography, polyacrylamide gel electrophoresis, or by HPLC analysis.

The term "jointly therapeutically active" or "joint therapeutic effect" as used herein means that the therapeutic agents may be given separately (in a chronologically staggered manner, especially a sequence-specific manner) in such time intervals as are preferable, in the subject, especially human subject, to be treated, and show an additive or greater effect. In a preferred embodiment, the joint therapeutic effect is an effect greater than the combined effect that each of the compounds would be expected to provide when administered on its own.

By “marker” is meant any protein or polynucleotide having an alteration in expression level or activity that is associated with a disease or disorder.

By “neoplasia” is meant abnormal cell proliferation. A neoplasm is a collection of cells characterized by increased cell division, poor cellular differentiation, and that is potentially cancerous.

As used herein, “obtaining” as in “obtaining an agent” includes synthesizing, purchasing, or otherwise acquiring the agent.

By “reduces” is meant a negative alteration of at least 10%, 25%, 50%, 75%, or 100%.

By “reference” is meant a standard or controlled condition.

A "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset of or the entirety of a specified sequence; for example, a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence. For polypeptides, the length of the reference polypeptide sequence will generally be at least about 16 amino acids, preferably at least about 20 amino acids, more preferably at least about 25 amino acids, and even more preferably about 35 amino acids, about 50 amino acids, or about 100 amino acids. For nucleic acids, the length of the reference nucleic acid sequence will generally be at least about 50 nucleotides, preferably at least about 60 nucleotides, more preferably at least about 75 nucleotides, and even more preferably about 100 nucleotides or about 300 nucleotides or any integer thereabout or therebetween.

By "siRNA" is meant a double stranded RNA. Optimally, a siRNA is 18, 19, 20, 21, 22, 23 or 24 nucleotides in length and has a 2 base overhang at its 3' end. These dsRNAs can be introduced to an individual cell or to a whole animal; for example, they may be introduced systemically via the bloodstream. Such siRNAs are used to downregulate mRNA levels or promoter activity.

By "specifically binds" is meant a compound or antibody that recognizes and binds a polypeptide of the invention, but which does not substantially recognize and bind other molecules in a sample, for example, a biological sample, which naturally includes a polypeptide of the invention.

Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. Nucleic acid molecules useful in the methods of the invention include any nucleic acid molecule that encodes a polypeptide of the invention or a fragment thereof. Such nucleic acid molecules need not be 100% identical with an endogenous nucleic acid sequence, but will typically exhibit substantial identity. Polynucleotides having "substantial identity" to an endogenous sequence are typically capable of hybridizing with at least one strand of a double-stranded nucleic acid molecule. By "hybridize" is meant pair to form a double-stranded molecule between complementary polynucleotide sequences (e.g., a gene described herein), or portions thereof, under various conditions of stringency. (See, e.g., Wahl, G. M. and S. L. Berger (1987) Methods Enzymol. 152:399; Kimmel, A. R. (1987) Methods Enzymol. 152:507).

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and more preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and more preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30° C, more preferably of at least about 37° C, and most preferably of at least about 42° C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those of ordinary skill in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred: embodiment, hybridization will occur at 30° C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will occur at 37° C in 500 mM NaCl, 50 mM trisodium citrate,

1% SDS, 35% formamide, and 100 μg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42° C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50% formamide, and 200 μg/ml ssDNA. Useful variations on these conditions will be readily apparent to a person of ordinary skill in the art.

For most applications, washing steps that follow hybridization will also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate. Stringent temperature conditions for the wash steps will ordinarily include a temperature of at least about 25° C, more preferably of at least about 42° C, and even more preferably of at least about 68° C. In a preferred embodiment, wash steps will occur at 25° C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42 C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 68° C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to a person of ordinary skill in the art. Hybridization techniques are well known to a person of ordinary skill in the art and are described, for example, in Benton and Davis (Science 196:180, 1977); Grunstein and Hogness (Proc. Natl. Acad. Sci., USA 72:3961, 1975); Ausubel et al. (Current Protocols in Molecular Biology, Wiley Interscience, New York, 2001); Berger and Kimmel (Guide to Molecular Cloning Techniques, 1987, Academic Press, New York); and Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, New York.

By "substantially identical" is meant a polypeptide or nucleic acid molecule exhibiting at least 50% identity to a reference amino acid sequence (for example, any one of the amino acid sequences described herein) or nucleic acid sequence (for example, any one of the nucleic acid sequences described herein). Preferably, such a sequence is at least 60%, more preferably 80% or 85%, and more preferably 90%, 95% or even 99% identical at the amino acid level or nucleic acid to the sequence used for comparison.

Sequence identity is typically measured using sequence analysis software (for example, Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, BESTFIT, GAP, or PILEUP/PRETTYBOX programs). Such software matches

identical or similar sequences by assigning degrees of homology to various substitutions, deletions, and/or other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid, asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. In an exemplary approach to determining the degree of identity, a BLAST program may be used, with a probability score between e^{-3} and e^{-100} indicating a closely related sequence.

By "subject" is meant a mammal, including, but not limited to, a human or non-human mammal, such as a bovine, equine, canine, ovine, or feline.

The term "synergistic effect" as used herein refers to action of two therapeutic agents such as, for example, an agent that inhibits Notch signaling and an agent that inhibits B cell receptor signaling producing an effect, for example, slowing the symptomatic progression of a proliferative disease, particularly cancer, or symptoms thereof, which is greater than the simple addition of the effects of each drug administered by themselves. A synergistic effect can be calculated, for example, using suitable methods such as the Sigmoid-Emax equation (Holford, N. H. G. and Scheiner, L. B., Clin. Pharmacokinet 6: 429-453 (1981)), the equation of Loewe additivity (Loewe, S. and Muischnek, H., Arch. Exp. Pathol Pharmacol. 114: 313-326 (1926)) and the median-effect equation (Chou, T. C. and Talalay, P., Adv. Enzyme Regul. 22: 27-55 (1984)). Each equation referred to above can be applied to experimental data to generate a corresponding graph to aid in assessing the effects of the drug combination. The corresponding graphs associated with the equations referred to above are the concentration-effect curve, isobologram curve and combination index curve, respectively.

Ranges provided herein are understood to be shorthand for all of the values within the range. For example, a range of 1 to 50 is understood to include any number, combination of numbers, or sub-range from the group consisting 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

As used herein, the terms “treat,” treating,” “treatment,” and the like refer to reducing or ameliorating a disorder and/or symptoms associated therewith. It will be appreciated that, although not precluded, treating a disorder or condition does not require that the disorder, condition or symptoms associated therewith be completely eliminated.

Unless specifically stated or obvious from context, as used herein, the term "or" is understood to be inclusive. Unless specifically stated or obvious from context, as used herein, the terms "a", "an", and "the" are understood to be singular or plural.

Unless specifically stated or obvious from context, as used herein, the term “about” is understood as within a range of normal tolerance in the art, for example within 2 standard deviations of the mean. About can be understood as within 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, 0.1%, 0.05%, or 0.01% of the stated value. Unless otherwise clear from context, all numerical values provided herein are modified by the term about.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an embodiment for a variable or aspect herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

Any compositions or methods provided herein can be combined with one or more of any of the other compositions and methods provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1A depicts in schematic form a transcript identified using RNASeq analysis, where the transcript includes the first exon of *HLA-DMB* and exons 24-30 of *NOTCH4*.

FIG. 1B provides a Western blot showing free (i.e., gamma secretase-cleaved) ICN-1 expression in MCL cell lines grown in the presence or absence of immobilized recombinant Notch ligand ($DLL1^{ext}$-IgG) or control protein (IgG) at various times following exposure.

FIG. 2 provides graphs showing the effect of a gamma secretase inhibitor (GSI) on four clones (numbered 3, 4, 5, and 7) engineered to express GFP and tet activator from a constitutive transgene promoter, and MYC from a doxycycline-inducible promoter. The construct is called pINDUCER-22-MYC. in the presence of doxycycline.

FIG. 3A provides a schematic diagram of wild-type and mutants Notch proteins expressed in specific MCL cell lines (indicated in bold type).

FIG 3B provides a western blot for cleaved ICN-1 in Mino cells plated on $DLL1^{ext}$-IgG-coated plates for the indicated time period.

FIG. 3C provides a schematic diagram of GSI-washout experiments in MCL lines with ligand-independent (top) and ligand-dependent (bottom) Notch signaling.

FIG. 3D provides a Western blot showing modulation of ICN-1 levels by GSI-washout in Mino and Rec-1 cells.

FIG. 4 provides a graph showing that myc enhancers are bound in enhancer 1 and enhancer RBPJ.

FIG. 5A shows the targeted epigenetic repression of 5' enhancers inhibits MYC expression in Notch-dependent and EBV and MCL lines.

FIG. 5B shows flow cytometry quantification of the ratio of mCherry+ versus GFP+ cells relative to cells infected with a control gRNA

FIG. 5C shows a graph indicating decreased proliferation of the dCas9-KRAB-E2F-mCherry population for Granta-519, but little effect was seen for SP-49.

FIGs. 6A-6F show that GSI-sensitive MCL is driven by a Notch-dependent *MYC* program shared with other Notch-dependent cancers. FIG. 6A shows heatmaps indicating significantly up-regulated genes identified in GSI-washout versus mock-washout experiments in at least 2 of 3 MCL lines (Mino, Sp-49 and Rec-1). Heatmap clusters were defined and numbered as shown in the Venn diagram at the lower right of the figure, and are sorted within clusters by mean change in expression in GSI-washout experiments conducted in T-cell acute lymphoblastic leukemia (T-ALL) cell line CUTLL1 and TNBC cell line HCC-1599. Canonical Notch target genes are labeled in grey text (NRARP, HES1, HEY1, NOTCH3, HES4, HEY2, and DTX1).

FIG. 6B shows gene sets from the MSigDB Hallmark ('H') and Reactome ('R') databases enriched in genes activated by GSI-washout in both GSI-sensitive and GSI-insensitive MCL cell lines (Figure 6A, groups 1-3). FDR q-values are for combined analysis of both gene set collections.

FIG. 6C shows gene sets from the MSigDB Hallmark ('H') and Reactome ('R') databases enriched in genes activated by GSI-washout in GSI-sensitive MCL cell lines only (FIG. 6A, group 4). FDR q-values are for combined analysis of both gene set collections.

FIG. 6D provides a western blot for Notch and MYC proteins in MCL cell lines treated for three days with GSI or DMSO. It should be noted that the NOTCH4 band in GSI-treated SP-49 has a slightly increased molecular weight.

FIG. 6E provides a Western blot showing rescue of *MYC* expression in single-cell-derived clones of SP-49 transduced with pINDUCER-22-*MYC*, or parental SP-49, treated with GSI or GSI + 100 ng/ml doxycycline.

FIG. 6F provides a graph showing growth of parental SP-49 and pINDUCER-22-*MYC* clones treated with GSI or GSI + doxycycline. Doxycycline doses were as follows: Clones 3 & 7 – 33.6 ng/ml, Clone 4 and parental – 100 ng / ml.

FIGs. 7A-7E show data illustrating that Notch-rearranged and EBV+, but not *MYC*-rearranged MCL/CLL lines show acetylation and RBPJ binding at B cell-specific 5' *MYC* enhancers.

FIG. 7A shows H3K27ac ChIP-Seq data showing mutually exclusive acetylation of 5' *MYC* enhancers in Notch-dependent MCL and 3' *MYC* enhancer in Notch-dependent T-ALL cell lines. Arrows indicate previously described looping interactions with the *MYC* promoter in MCL (Ryan et al., 2015) and T-ALL (Herranz et al., 2014; Yashiro-Ohtani et al., 2014).

FIG. 7B shows H3K27ac ChIP-Seq data for 5' MYC enhancers and CD79A promoter regions in CLL (Me) and MCL (Jv, Gr, Re, Sp, Mi, Je, Z1, Ma, Hb, and Up) cell lines. The cell line abbreviations used are: Me = Mec-1, Jv = JVM2, Gr = Granta-519, Re = Rec-1, Sp = SP-49, Mi = Mino, Je = Jeko-1, Z1 = Z138, Ma = MAVER1, Hb = HBL-2, and Up = UPN-1.

FIG. 7C provides a Western blot showing expression of EBNA2 and c-MYC in nuclear extracts from CLL and MCL lines.

FIG. 7D provides a graph showing ChIP-PCR showing binding of RBPJ at 5' *MYC* enhancer E-2 in CLL and MCL cell lines.

FIG. 7E provides a graph showing ChIP-PCR showing binding of EBNA2 at 5' *MYC* enhancer E-2 in CLL and MCL cell lines.

FIGs 8A-8E provide data showing that ChIP-Seq and CRISPR-Cas9 validation of Notch-dependent 5' *MYC* enhancers confirms the role of Notch in *MYC* expression and MCL proliferation.

FIG. 8A provides ChIP-Seq data showing the dynamics of ICN-1 and RBPJ binding, and H3K27ac modification at the 5' B cell Notch-dependent MYC enhancers (BNDME) sites. Mino cells in the top two rows were plated on $DLL1^{ext}$-IgG for 48 hours. The bottom six rows depict ChIP-Seq data for the indicated marker after GSI-washout experiments conducted as in figure 1C. Washout = 'on', grey track; Mock washout = 'off', black overlay track.

FIG. 8B shows ICN-1 and RBPJ binding at BNDME sites after GSI-washout, as well as Phastcons 46-vertebrate conservation score ('conservation'). Consensus RBPJ logos are aligned to the position of conserved RBPJ motifs in each enhancer. The positions of specific gRNAs are indicated.

FIG. 8C provides a graph showing qRT-PCR measurement of *MYC* expression after transduction of dCAS9-KRAB:E2A:mCherry-expressing EBV+ (Granta-519), Notch-

rearranged (SP-49), and *MYC*-rearranged / amplified (Jeko-1) MCL cell lines with guideRNAs targeting the BNDME sites, or non-targeting controls (GFP).

FIG. 8D provides a series of graphs showing qRT-PCR measurement of *MYC* expression after transduction of Cas9 nuclease-expressing MCL lines with gRNAs against BNDME sites, or non-targeting controls.

FIG. 8E provides a series of graphs showing growth of indicated Cas9 nuclease-expressing MCL cell lines after transduction with gRNAs as in (FIG. 8D).

FIGs 9A-9E shows genes activated by Notch independently of *MYC* are highly enriched for direct Notch regulatory targets, and include B cell signaling pathway regulators.

FIG. 9A provides a graph showing fraction of Notch-activated genes identified in MCL models that show ICN-1 binding in Rec-1 to the gene promoter, or to a distal site linked to the gene promoter by 3D looping in EBV+ B cells (GM12878 Pol2 ChIA-PET). Gene groups are defined as in Figure 6A, with genes in groups 1-3 showing activation in a cell line (Mino) that lacks Notch-dependent *MYC* activation ("*MYC*-independent"). "Rnd" is a randomly selected group of expressed genes that do not show Notch-dependent differential expression.

FIG. 9B shows representative known and novel direct Notch target genes with promoter-proximal ICN-1 binding in Rec-1. H3K27 acetylation shown for Rec-1 and for *NOTCH1*-mutant MCL and CLL lymph node biopsies.

FIG. 9C-1-9C-6 shows representative direct Notch target genes with ICN-1 binding to promoter-distal sites. GM12878 Pol2 ChIA-PET data shows loop interactions between ICN1-bound distal sites and Notch-activated gene promoters.

FIG. 9D shows CRISPR-Cas9-mediated validation of representative ICN1+ regulatory sites for CR2 and IL6R.

FIGs 10A-10F show Notch-dependent activation of target genes and pathways in primary CLL cells.

FIG. 10A shows immunohistochemistry for ICN-1 in representative cases of ICN1-high and ICN-1-low CLL.

FIG. 10B shows a heatmap indicating relative expression of genes (RNA-Seq) significantly upregulated by gamma-secretase inhibitor-washout in MCL, and in ICN1-high versus ICN1-low MCL.

FIG. 10C shows ChIP-Seq data from MCL cell lines and primary CLL and MCL samples, demonstrating ICN-1 and RBPJ binding at enhancers of genes validated as direct Notch targets in MCL cell lines and primary CLL samples.

FIG. 10D shows a schematic diagram of primary CLL / HS-5 co-culture experiments.

FIG. 10E provides a graph showing the relative expression of MYC (qRT-PCR) in CD19+ CD5+ CLL cells sorted following three-day HS-5-DLL-1 co culture in the presence of GSI or vehicle.

FIG. 10F provides a series of a graphs showing the phosphorylation-specific flow analysis of specified epitopes in primary CLL cells (CLL-015) co-cultured for three days with HS-5-DLL1 cells in the presence of GSI or vehicle. Indicated samples were treated for the stated time with $F(ab)_2$ anti-IgG/IgM to crosslink B-cell receptors. Dotted line marks the mode of fluorescence intensity in the un-stimulated / GSI-treated sample for each epitope.

FIG 11 shows a schematic wherein Notch drives potentiation of B-cell receptor and cytokine signaling via *MYC*-independent targets, as well as a *MYC*-dependent metabolic shift. The diagram depicts direct Notch target gene products as well as their relationship to B cell-receptor signaling and other pathways. Solid lines indicate direct regulatory relationships, while dotted lines indicate presence of one or more intermediaries. Phosphorylation of active B-cell receptor (BCR) signaling mediators is potentiated by Notch-dependent increases in expression of SRC-family kinases and signaling adaptor proteins, while another direct Notch target gene product, c-MYC, controls expression of critical metabolic regulators. Both the BCR and MYC pathways drive signaling events that regulate mTORC1 activity. NF-KB activation downstream of BCR signaling may activate additional genes in the setting of Notch activation, or may confer synergistic activation of direct Notch target genes.

FIG. 12A shows a schematic of CLL HS-5 co-culture experiments performed in the presence of CpG-rich oligodideoxynucleotides.

FIG. 12B shows quantification of CLL HS-5 co-culture experiments.

FIG. 12C shows quantification of Notch target cell surface proteins in MCL cells within the spleen, bone marrow and blood.

DETAILED DESCRIPTION OF THE INVENTION

The invention generally provides therapeutic compositions comprising a combination of an agent that inhibits the activity of or decreases the levels of a Notch protein and an agent that inhibits B-cell receptor (BCR) signalling, and methods of using such combinations to

treat cancer (e.g., small B-cell lymphomas, such as mantle cell lymphoma, or chronic lymphocytic leukemia (e.g., small lymphocytic lymphoma), diffuse large B cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, splenic red pulp lymphoma, MALT lymphoma and leukemias, such as chronic lymphocytic leukemia, B cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and early T cell acute lymphoblastic leukemia).

Recurrent gain-of-function mutations in genes encoding Notch receptors are associated with poor clinical outcome in two small B-cell lymphoma subtypes, mantle cell lymphoma (MCL) and chronic lymphocytic leukemia (CLL; also known as small lymphocytic lymphoma, SLL), but functional targets of Notch signaling in B cells have not been systematically characterized. As described herein, a gamma-secretase washout strategy was used to rapidly activate Notch signaling in Notch-dependent and -independent MCL lines, and to identify direct Notch regulatory targets through genome-wide expression profiling and chromatin immunoprecipitation (ChIP-Seq) of Notch transcriptional complex (NTC) components.

The invention is based, at least in part, on the discovery that proliferation of Notch-dependent mantle cell lymphoma (MCL) lines was driven by activation of the oncogene *MYC* via Notch transcriptional complex binding at B-cell-specific 5' enhancer elements, resulting in secondary activation of *MYC* target genes and a metabolic program associated with mTORC1 activation. These studies identified novel Notch regulatory targets in B-cell lymphomas associated with NTC binding to proximal and distal regulatory elements, that activate genes encoding cytokine receptors (*IL6R, IL10R, IL21R*), as well as SRC-family kinases (*FYN, LYN, BLK*) and signaling adaptor proteins (*BLNK, NEDD9, SH2B2, PIK3AP1*) involved in activation of pathways downstream of B-cell receptor (BCR) signaling. Genome-wide profiling analysis of lymphoma biopsies, plus functional studies of patient-derived lymphoma cells *in vitro* and *in vivo* were utilized to validate Notch-dependent regulation of *MYC* and oncogenic BCR signaling in primary human CLL and MCL.

Genome-wide profiling of mRNA, histone acetylation, and NTC binding in MCL was used to identify differential regulation of enhancers and genes that represent the direct targets of Notch signaling in B cell lymphoma. The findings indicated that Notch signaling drives two distinct oncogenic programs in lymphoma cell lines and primary tumors. First, ICN binds and activates B-cell-specific 5' *MYC* enhancers, resulting in activation of a *MYC*-dependent metabolic program that is shared with other Notch-dependent tumor types. Second, Notch

directly activates the expression of cytokine receptors and B cell receptor signaling intermediates, thus potentiating the response of lymphoma cells to activating stimuli. Notably, the data indicated a Notch-dependent increase in B cell-receptor-dependent phosphorylation of PLC2G and downstream activation of NF-KB, a pathway that is known to be central to the proliferation and survival of small B cell lymphomas.

Building on these findings, the invention provides novel therapeutic compositions and methods combining direct B cell receptor inhibition (expected to block B cell receptor signaling and to drive cancerous B cells towards apoptosis and/or disrupts tumor formation) with Notch inhibition (expected to both cease the activation of MYC and to also cease B cell receptor potentiation). In taking both approaches towards B cell inhibition in concert, cancerous B cells are specifically targeted and have increased difficulty escaping the treatment by mutation.

Accordingly, the invention provides therapeutic compositions comprising an agent (e.g., polypeptides, inhibitory nucleic acids, and small molecules) that inhibits a Notch polypeptide (e.g., Notch1, Notch2, Notch3, Notch4) expression or activity and an agent that inhibits B Cell Receptor (BCR) signaling, and methods of using such compositions to inhibit the growth or proliferation of a neoplastic cell. Compositions of the invention are useful for the treatment of cancer (e.g., e.g., small B-cell lymphomas, such as mantle cell lymphoma, or chronic lymphocytic leukemia (e.g., small lymphocytic lymphoma), diffuse large B cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, splenic red pulp lymphoma, MALT lymphoma and leukemias such as chronic lymphocytic leukemia, B cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and early T cell acute lymphoblastic leukemia).

Notch

Notch proteins are expressed as trans-membrane receptors that undergo sequential proteolytic cleavage upon interaction with Notch ligands expressed on neighboring cells, resulting in gamma secretase-dependent release of the intracellular notch (ICN) fragment. ICN then traffics to the nucleus, where it binds to transcriptional regulatory elements in a Notch transcriptional complex (NTC) with the DNA sequence-specific transcription factor RBPJ, mastermind-like (MAML) proteins, and other co-factors. Nearly all Notch gene mutations reported in CLL and MCL result in frameshift-mediated truncation of the C-terminal PEST domain, which mediates ubiquitination and degradation of ICN. Notch PEST

domain truncations have been extensively studied in T-cell acute lymphoblastic leukemia (T-ALL), where they enhance the nuclear accumulation of ICN, but do not confer active signaling in the absence of ligand. This contrasts with Notch gene heterodimerization domain mutations and rearrangements, which do confer ligand-independent signaling, and are common in T-ALL, but are extremely rare in CLL and MCL patients. Immunohistochemistry (IHC) with an antibody that specifically recognizes the gamma-secretase-cleaved NOTCH1 ICN (ICN-1) was previously used to demonstrate NOTCH1 activation in >80% of CLL lymph node biopsies. Strong and diffuse ICN-1 staining was significantly, but not exclusively, associated with cases bearing *NOTCH1* PEST mutations. These findings suggested that activation of Notch signaling in lymphoma cells via interaction with ligand-presenting cells in the lymph node microenvironment may be a broadly important feature of this disease.

In vitro models for the study of Notch signaling in B-cell lymphoma have been limited. Two MCL cell lines, Rec-1 and SP-49, were reported to show marked growth inhibition upon treatment with gamma-secretase inhibitors (GSI) or expression of a Notch-inhibiting transgene, suggesting dependency of these lines on ligand-independent Notch signaling (Kridel et al., 2012). Subsequently, ICN-1 activation in Rec-1 was found to be due to a genomic deletion encompassing most of the exons encoding the *NOTCH1* extracellular domain, and that this allele confers ligand-independent Notch signaling that is sensitive to GSI inhibition.

Therapeutic Compositions Comprising Notch and B Cell Receptor Inhibitors

The present invention features compositions comprising one or more agents that inhibit Notch signaling and one or more agents that inhibit B cell receptor signaling. Such agents include small molecules, polypeptides, and polynucleotides described herein.

Small molecules capable of inhibiting Notch include gamma-secretase inhibitors (GSI). Exemplary gamma-secretase inhibitors are known in the art, and include, for example, Compound E, MK-0752, PF03084014, RO-4929097, DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester, tetralin imidazole PF-03084014, LY3039478 and BMS906-024.

Further examples of compounds suitable as Notch inhibitors can include the compounds listed in U.S. Patent Nos. 8,377,886, 6,756,511, 6,890,956, 6,984,626, 7,049,296,

7,101,895, 7,138,400, 7,144,910, and 7,183,303, incorporated by reference herein in their entirety.

Other Notch inhibitors include antibodies that specifically bind Notch and inhibit or disrupt its activity, or deplete its levels. Exemplary inhibitory Notch antibodies are known in the art, and include, for example, anti-Notch 1 (OMP-52M521) and anti-delta-like-4.

Further examples of antibodies suitable for inhibiting Notch and Notch signaling pathway include the antibodies listed in U.S. Patent Nos. 9,090,690, 8,945,547, 8,945,873, 7,534,868 and International Patent Application Nos. WO 2008150525, WO 2010059543, WO 2011041336 , incorporated by reference herein in their entirety.

Examples of compounds suitable as B cell receptor (BCR) inhibitors can include Bruton tyrosine kinase (BTK) inhibitors, SRC family kinase inhibitors, SYK inhibitors, or protein kinase C inhibitors, and PI3 Kinase inhibitors.

Exemplary B cell receptor inhibitors include, for example, ibrutinib (PCI-32765), acalabrutinib (ACP-196), ONO-4059 (e.g., GS-4059 or NCT02457598), spebrutinib (e.g., AVL-292, CC-292), and BGB-3111.

Further examples of compounds suitable as BCR inhibitors can include the compounds listed in U.S. Patent Nos. 8,227,433, 6,306,897, 8,999,999 and International Patent Application Nos. WO2015110923, WO1999054286 (incorporated by reference in their entirety).

Small molecules capable of inhibiting signaling mediated by B cell receptors or Notch can include SRC family kinase inhibitors. Exemplary SRC family kinase inhibitors are known in the art, and include, for example, dasatinib (BMS-354825), KX2-391, bosutinib (SKI-606), and saracatinib (AZD-0530).

Small molecules capable of inhibiting signaling mediated by B cell receptors or Notch can include spleen tyrosine kinase (SYK) inhibitors. Exemplary SYK inhibitors are known in the art, and include, for example, fostamatinib (R788), piceatannol, entospletinib (GS-9973), and GSK2646264.

Small molecules capable of inhibiting signaling mediated by B cell receptors or Notch can include protein kinase C (PKC) inhibitors. Exemplary PKC inhibitors are known in the art, and include, for example, midostaurin (PKC412), enzastaurin (LY317615), sotrastaurin (AEB071), and ruboxistaurin (LY333531).

Small molecules capable of inhibiting signaling mediated by B cell receptors or Notch can include phosphoinositol-3-kinase (PI3K) inhibitors. Exemplary PI3K inhibitors are

known in the art, and include, for example, idelalisib (e.g., zydelig, GS-1101, CAL-101), alpelisib (BYL719), AEZS-136, buparlisib (BKM120), copanlisib (BAY 80-6946), CAL263, CUDC-907, dactolisib (e.g., NVP-BEZ235, BEZ-235), duvelisib (IPI-145), GNE-477, GSK1059615, IC87114, IPI-549, INK1117, palomid 529, perifosine (KRX-0401), pictilisib (GDC-0941), ME-401, PI-103, PWT33597, PX-866, RP6503, RP6530, SF1126, TGR 1202, wortmannin, demethoxyviridin, XL147 (SAR245408), XL765 (SAR245409), ZSTK474.

Further examples of compounds suitable as PI3K inhibitors can include the compounds listed in U.S. Patent Nos. 9,403,779, 9,150,579, 9,126,948, 8,940,752, 8,759,359, 8,440,651, U.S. Patent Application Nos. 20140364447, 20100056523, 20100029693, and International Patent Application Nos. WO 2016051374, WO 2015181728, WO 2015160986, WO 2014195888, WO 2011123751 (incorporated by reference herein in their entirety).

In accordance with the present invention, a therapeutically effective amount of each of the combination partners (e.g., an agent that inhibits Notch signaling and an agent that inhibits B cell receptor signaling) may be administered simultaneously or sequentially and in any order, and the components may be administered separately or as a fixed combination. For example, the method of treating a neoplasia according to the invention may comprise (i) administration of the first agent (a) in free or pharmaceutically acceptable salt form and (ii) administration of an agent (b) in free or pharmaceutically acceptable salt form, simultaneously or sequentially in any order, in jointly therapeutically effective amounts, preferably in synergistically effective amounts, e.g. in daily or intermittently dosages corresponding to the amounts described herein. The individual combination partners may be administered separately at different times during the course of therapy or concurrently in divided or single combination forms. Furthermore, the term "administering" also encompasses the use of a pro-drug of a combination partner that converts in vivo to the combination partner as such. The invention is therefore to be understood as embracing all such regimens of simultaneous or alternating treatment and the term "administering" is to be interpreted accordingly.

The effective dosage of each of the combination partners employed in the methods of the invention may vary depending on the particular compound or pharmaceutical composition employed, the mode of administration, the condition being treated, and the severity of the condition being treated. Thus, the dosage regimen is selected in accordance with a variety of factors including the route of administration and the renal and hepatic function of the patient. A clinician or physician of ordinary skill in the art can readily determine and prescribe the

effective amount of the single therapeutic agents required to alleviate, counter or arrest the progress of the condition.

The optimum ratios, individual and combined dosages, and concentrations of the combination partners that yield efficacy without toxicity are based on the kinetics of the therapeutic agents' availability to target sites, and are determined using methods known to those of skill in the art.

The effective dosage of each of the combination partners may require more frequent administration of one of the agents in the combination. Therefore, to permit appropriate dosing, packaged pharmaceutical products may contain one or more dosage forms that contain the combination of compounds, and one or more dosage forms that contain one of the combination of compounds, but not the other compound(s) of the combination.

When the combination partners are employed or as marketed as single drugs, their dosage and mode of administration can be in accordance with the information provided on the package insert of the respective marketed drug, if not mentioned herein otherwise.

The optimal dosage of each combination partner for treatment of a proliferative disease can be determined empirically for each individual using known methods and will depend upon a variety of factors, including, though not limited to, the degree of advancement of the disease; the age, body weight, general health, gender and diet of the individual; the time and route of administration; and other medications the individual is taking optimal dosages may be established using routine testing and procedures that are well known in the art.

The amount of each combination partner that may be combined with the carrier materials to produce a single dosage form will vary depending upon the individual treated and the particular mode of administration. In some embodiments the unit dosage forms containing the combination of agents as described herein will contain the amounts of each agent of the combination that are typically administered when the agents are administered alone.

Frequency of dosage may vary depending on the compound used and the particular condition to be treated or prevented. In general, the use of the minimum dosage that is sufficient to provide effective therapy is preferred. Patients may generally be monitored for therapeutic effectiveness using assays suitable for the condition being treated or prevented, which will be familiar to those of ordinary skill in the art.

The present invention relates to a method of treating a subject having a proliferative disease comprising administering to said subject a combination of an agent that inhibits Notch signaling and an agent that inhibits B cell receptor signaling in a quantity which is jointly therapeutically effective against a neoplastic disease. In particular, the neoplastic disease to be treated is a leukemia or lymphoma.

The present invention further provides a commercial package comprising as therapeutic agents an agent that inhibits Notch signaling and an agent that inhibits B cell receptor signaling, optionally together with instructions for simultaneous, separate or sequential administration thereof for use in the delay of progression or treatment of a proliferative disease in a subject in need thereof.

Inhibitory Nucleic Acids

The invention further provides inhibitory nucleic acids (e.g., antisense molecules, siRNA, shRNA) that inhibit the expression of a Notch polypeptide (e.g., Notch 1, Notch 2, Notch 3, Notch4). In addition, the invention provides inhibitory nucleic acids (e.g., antisense molecules, siRNA, shRNA) that inhibit the expression of a functional component of the B cell receptor. Such oligonucleotides include single and double stranded nucleic acid molecules (e.g., DNA, RNA, and analogs thereof) that bind a nucleic acid molecule that encodes a Notch polypeptide, as well as nucleic acid molecules that bind directly to the polypeptide to modulate its biological activity (e.g., aptamers).

siRNA

Short twenty-one to twenty-five nucleotide double-stranded RNAs are effective at down-regulating gene expression (Zamore et al., Cell 101: 25-33; Elbashir et al., Nature 411: 494-498, 2001, hereby incorporated by reference). The therapeutic effectiveness of a siRNA approach in mammals was demonstrated *in vivo* by McCaffrey et al. (Nature 418: 38-39.2002).

Given the sequence of a target gene, siRNAs may be designed to inactivate that gene. Such siRNAs, for example, could be administered directly to an affected tissue, or administered systemically. The nucleic acid sequence of a gene can be used to design small interfering RNAs (siRNAs). The 21 to 25 nucleotide siRNAs may be used, for example, as therapeutics to treat cancer (e.g., small B-cell lymphomas, such as mantle cell lymphoma, or chronic lymphocytic leukemia (e.g., small lymphocytic lymphoma), diffuse large B cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, splenic red pulp

lymphoma, MALT lymphoma and leukemias such as chronic lymphocytic leukemia, B cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and early T cell acute lymphoblastic leukemia).

The inhibitory nucleic acid molecules of the present invention may be employed as double-stranded RNAs for RNA interference (RNAi)-mediated knock-down of expression of a Notch polypeptide. RNAi is a method for decreasing the cellular expression of specific proteins of interest (reviewed in Tuschl, Chembiochem 2:239-245, 2001; Sharp, Genes & Devel. 15:485-490, 2000; Hutvagner and Zamore, Curr. Opin. Genet. Devel. 12:225-232, 2002; and Hannon, Nature 418:244-251, 2002). The introduction of siRNAs into cells either by transfection of dsRNAs or through expression of siRNAs using a plasmid-based expression system is increasingly being used to create loss-of-function phenotypes in mammalian cells.

In one embodiment of the invention, a double-stranded RNA (dsRNA) molecule is made that includes between eight and nineteen consecutive nucleobases of a nucleobase oligomer of the invention. The dsRNA can be two distinct strands of RNA that have duplexed, or a single RNA strand that has self-duplexed (small hairpin (sh)RNA). Typically, dsRNAs are about 21 or 22 base pairs, but may be shorter or longer (up to about 29 nucleobases) if desired. dsRNA can be made using standard techniques (e.g., chemical synthesis or *in vitro* transcription). Kits are available, for example, from Ambion (Austin, Tex.) and Epicentre (Madison, Wis.). Methods for expressing dsRNA in mammalian cells are described in Brummelkamp et al. Science 296:550-553, 2002; Paddison et al. Genes & Devel. 16:948-958, 2002. Paul et al. Nature Biotechnol. 20:505-508, 2002; Sui et al. Proc. Natl. Acad. Sci. USA 99:5515-5520, 2002; Yu et al. Proc. Natl. Acad. Sci. USA 99:6047-6052, 2002; Miyagishi et al. Nature Biotechnol. 20:497-500, 2002; and Lee et al. Nature Biotechnol. 20:500-505 2002, each of which is hereby incorporated by reference.

Small hairpin RNAs (shRNAs) comprise an RNA sequence having a stem-loop structure. A "stem-loop structure" refers to a nucleic acid having a secondary structure that includes a region of nucleotides which are known or predicted to form a double strand or duplex (stem portion) that is linked on one side by a region of predominantly single-stranded nucleotides (loop portion). The term "hairpin" is also used herein to refer to stem-loop structures. Such structures are well known in the art and the term is used consistently with its known meaning in the art. As is known in the art, the secondary structure does not require exact base-pairing. Thus, the stem can include one or more base mismatches or bulges.

Alternatively, the base-pairing can be exact, i.e. not include any mismatches. The multiple stem-loop structures can be linked to one another through a linker, such as, for example, a nucleic acid linker, a miRNA flanking sequence, other molecule, or some combination thereof.

As used herein, the term "small hairpin RNA" includes a conventional stem-loop shRNA, which forms a precursor miRNA (pre-miRNA). While there may be some variation in range, a conventional stem-loop shRNA can comprise a stem ranging from 19 to 29 bp, and a loop ranging from 4 to 30 bp. "shRNA" also includes micro-RNA embedded shRNAs (miRNA-based shRNAs), wherein the guide strand and the passenger strand of the miRNA duplex are incorporated into an existing (or natural) miRNA or into a modified or synthetic (designed) miRNA. In some instances the precursor miRNA molecule can include more than one stem-loop structure. MicroRNAs are endogenously encoded RNA molecules that are about 22-nucleotides long and generally expressed in a highly tissue- or developmental-stage-specific fashion and that post-transcriptionally regulate target genes. More than 200 distinct miRNAs have been identified in plants and animals. These small regulatory RNAs are believed to serve important biological functions by two prevailing modes of action: (1) by repressing the translation of target mRNAs, and (2) through RNA interference (RNAi), that is, cleavage and degradation of mRNAs. In the latter case, miRNAs function analogously to small interfering RNAs (siRNAs). Thus, one can design and express artificial miRNAs based on the features of existing miRNA genes.

shRNAs can be expressed from DNA vectors to provide sustained silencing and high yield delivery into almost any cell type. In some embodiments, the vector is a viral vector. Exemplary viral vectors include retroviral, including lentiviral, adenoviral, baculoviral and avian viral vectors, and including such vectors allowing for stable, single-copy genomic integrations. Retroviruses from which the retroviral plasmid vectors can be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus. A retroviral plasmid vector can be employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which can be transfected include, but are not limited to, the PE50l, PA3l7, R-2, R-AM, PA12, T19-14x, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector can transduce the

packaging cells through any means known in the art. A producer cell line generates infectious retroviral vector particles which include polynucleotide encoding a DNA replication protein. Such retroviral vector particles then can be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express a DNA replication protein.

Examples of delivery methods suitable to deliver siRNA and shRNA molecules of the present invention are disclosed in Nature Materials Vol 12, 2013, pages 967-977, incorporated by reference in its entirety.

Catalytic RNA molecules or ribozymes that include an antisense sequence of the present invention can be used to inhibit expression of a nucleic acid molecule *in vivo* (e.g., a nucleic acid encoding any component of the Notch signaling pathway (e.g., Notch 1, Notch 2, Notch 3, Notch, 4, canonical Notch signaling modalities) and B Cell receptor (BCR) signaling (e.g. phospholipase C gamma 2, LYN, FYN, PI3K, NF-KB transcription factor pathway). The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs. The design and use of target RNA-specific ribozymes is described in Haseloff et al., Nature 334:585-591. 1988, and U.S. Patent Application Publication No. 2003/0003469 A1, each of which is incorporated by reference.

Accordingly, the invention also features a catalytic RNA molecule that includes, in the binding arm, an antisense RNA having between eight and nineteen consecutive nucleobases. In preferred embodiments of this invention, the catalytic nucleic acid molecule is formed in a hammerhead or hairpin motif. Examples of such hammerhead motifs are described by Rossi et al., Aids Research and Human Retroviruses, 8:183, 1992. Example of hairpin motifs are described by Hampel et al., "RNA Catalyst for Cleaving Specific RNA Sequences," filed Sep. 20, 1989, which is a continuation-in-part of U.S. Ser. No. 07/247,100 filed Sep. 20, 1988, Hampel and Tritz, Biochemistry, 28:4929, 1989, and Hampel et al., Nucleic Acids Research, 18: 299, 1990. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention is that it has a specific substrate binding site which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

Essentially any method for introducing a nucleic acid construct into cells can be employed. Physical methods of introducing nucleic acids include injection of a solution containing the construct, bombardment by particles covered by the construct, soaking a cell, tissue sample or organism in a solution of the nucleic acid, or electroporation of cell membranes in the presence of the construct. A viral construct packaged into a viral particle can be used to accomplish both efficient introduction of an expression construct into the cell and transcription of the encoded shRNA. Other methods known in the art for introducing nucleic acids to cells can be used, such as lipid-mediated carrier transport, chemical mediated transport, such as calcium phosphate, and the like. Thus the shRNA-encoding nucleic acid construct can be introduced along with components that perform one or more of the following activities: enhance RNA uptake by the cell, promote annealing of the duplex strands, stabilize the annealed strands, or otherwise increase inhibition of the target gene.

For expression within cells, DNA vectors, for example plasmid vectors comprising either an RNA polymerase II or RNA polymerase III promoter can be employed. Expression of endogenous miRNAs is controlled by RNA polymerase II (Pol II) promoters and in some cases, shRNAs are most efficiently driven by Pol II promoters, as compared to RNA polymerase III promoters (Dickins et al., 2005, Nat. Genet. 39: 914-921). In some embodiments, expression of the shRNA can be controlled by an inducible promoter or a conditional expression system, including, without limitation, RNA polymerase type II promoters. Examples of useful promoters in the context of the invention are tetracycline-inducible promoters (including TRE-tight), IPTG-inducible promoters, tetracycline transactivator systems, and reverse tetracycline transactivator (rtTA) systems. Constitutive promoters can also be used, as can cell- or tissue-specific promoters. Many promoters will be ubiquitous, such that they are expressed in all cell and tissue types. A certain embodiment uses tetracycline-responsive promoters, one of the most effective conditional gene expression systems in in vitro and in vivo studies. See International Patent Application PCT/US2003/030901 (Publication No. WO 2004-029219 A2) and Fewell et al., 2006, Drug Discovery Today 11: 975-982, for a description of inducible shRNA.

Delivery of Polynucleotides

Naked polynucleotides, or analogs thereof, are capable of entering mammalian cells and inhibiting expression of a gene of interest. Nonetheless, it may be desirable to utilize a formulation that aids in the delivery of oligonucleotides or other nucleobase oligomers to

cells (see, e.g., U.S. Pat. Nos. 5,656,611, 5,753,613, 5,785,992, 6,120,798, 6,221,959, 6,346,613, and 6,353,055, each of which is hereby incorporated by reference). Inhibitory nucleic acid molecule can be delivered using a nanoparticle. Nanoparticle compositions suitable for use with inhibitory nucleic acid molecules are known in the art and described for example by Kanasty et al., Nature materials 12: 967-977, 2013, which is incorporated herein by reference. Such nanoparticle delivery compositions include cyclodextrin polymer (CDP)-based nanoparticles, lipid nanoparticles, cationic or ionizable lipid, lipid-anchored PEG, PEGylated nanoparticles, oligonucleotide nanoparticles (ONPs), and siRNA–polymer conjugate delivery systems (e.g., Dynamic PolyConjugate, Triantennary GalNAc–siRNA).

Chemotherapeutic Agents

The invention further provides for the use of a combination of the invention (e.g., an agent that inhibits Notch signaling and an agent that inhibits B cell receptor signaling) in combination with another therapeutic agent, such as a conventional chemotherapeutic agent, or agent that mitigates a side effect associated with an agent of the invention. Chemotherapeutic agents can be used with the methods of the present invention including, but are not limited to alkylating agents. Without intending to be limited to any particular theory, alkylating agents directly damage DNA to keep the cell from reproducing. Alkylating agents work in all phases of the cell cycle and are used to treat many different cancers (e.g., small B-cell lymphomas, such as mantle cell lymphoma, or chronic lymphocytic leukemia (e.g., small lymphocytic lymphoma), diffuse large B cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, splenic red pulp lymphoma, MALT lymphoma and leukemias such as chronic lymphocytic leukemia, B cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and early T cell acute lymphoblastic leukemia). Alkylating agents are divided into different classes, including, but not limited to: (i) nitrogen mustards, such as, for example mechlorethamine (nitrogen mustard), chlorambucil, cyclophosphamide (Cytoxan®), ifosfamide, and melphalan; (ii) nitrosoureas, such as, for example, streptozocin, carmustine (BCNU), and lomustine; (iii) alkyl sulfonates, such as, for example, busulfan; (iv) riazines, such as, for example, dacarbazine (DTIC) and temozolomide (Temodar®); (v) ethylenimines, such as, for example, thiotepa and altretamine (hexamethylmelamine); and (v) platinum drugs, such as, for example, cisplatin, carboplatin, and oxalaplatin.

Uses of Notch and B Cell Receptor Inhibitors

The invention features methods for inhibiting the proliferation, growth, or viability of a neoplastic cell by contacting the cell with a Notch inhibitor and an agent that inhibits B Cell Receptor signaling. In general, the method includes a step of contacting a neoplastic cell with an effective amount of a compound of the invention. The present method can be performed on cells in culture, e.g., in vitro or ex vivo, or can be performed on cells present in an animal subject, e.g., as part of an in vivo therapeutic protocol. The therapeutic regimen can be carried out on a human or other subject.

The compounds of the invention or otherwise described herein can be tested initially in vitro for their inhibitory effects on the proliferation or survival of neoplastic cells. Examples of cell lines that can be used are any of the MCL cell lines described herein or any other suitable cell line known in the art. Alternatively, the antineoplastic activity of compounds of the invention can be tested in vivo using various animal models known in the art. For example, xenographs of human neoplastic cells or cell lines are injected into immunodeficient mice (e.g., nude or SCID) mice. Compounds of the invention are then administered to the mice and the growth and/or metastasis of the tumor is compared in mice treated with a compound of the invention relative to untreated control mice. Agents that reduce the growth or metastasis of a tumor or increase mice survival are identified as useful in the methods of the invention.

The methods discussed herein can be used to inhibit the proliferation of virtually any neoplastic cell. The invention provides methods for treating a subject having a neoplasia by administering to the subject an effective amount of an agent that inhibits Notch signaling and an agent that inhibits B cell receptor signaling as described herein. In certain embodiments, the subject is a mammal, in particular a human.

Agents which are determined to be effective for the prevention or treatment of neoplasias in animals, *e.g.,* dogs, rodents, may also be useful in treatment of neoplasias in humans. Those skilled in the art of treating neoplasias in humans will know, based upon the data obtained in animal studies, the dosage and route of administration of the compound to humans. In general, the dosage and route of administration in humans is expected to be similar to that in animals.

The identification of those patients who are in need of prophylactic treatment for hyperplastic/neoplastic disease states is well within the ability and knowledge of one skilled in the art. Certain of the methods for identification of patients who are at risk of developing

neoplastic disease states which can be treated by the subject method are appreciated in the medical arts, such as family history of the development of a particular disease state and the presence of risk factors associated with the development of that disease state in the subject patient. A clinician skilled in the art can readily identify such candidate patients, by the use of, for example, clinical tests, physical examination and medical/family history.

Pharmaceutical Compositions

The invention provides pharmaceutical compositions for the treatment of a neoplasia, comprising an effective amount of an agent that inhibits Notch activity or decreases Notch levels, an agent that inhibits B Cell Receptor signaling and a pharmaceutically acceptable carrier. In particular embodiments, compositions of the invention comprise an agent or combination of agents described herein in combination with a conventional chemotherapeutic agent. In still other embodiments, such compositions are labeled for the treatment of cancer. In a further embodiment, the effective amount is effective to reduce the growth, proliferation, or survival of a neoplastic cell or to otherwise treat or prevent a neoplasia in a subject, as described herein.

In an embodiment, the agent is administered to the subject using a pharmaceutically-acceptable formulation. In certain embodiments, these pharmaceutical compositions are suitable for oral or parenteral administration to a subject. In still other embodiments, as described in detail below, the pharmaceutical compositions of the present invention may be specially formulated for administration in solid or liquid form, including those adapted for the following: (1) oral administration, for example, drenches (aqueous or non-aqueous solutions or suspensions), tablets, boluses, powders, granules, pastes; (2) parenteral administration, for example, by subcutaneous, intramuscular or intravenous injection as, for example, a sterile solution or suspension; (3) topical application, for example, as a cream, ointment or spray applied to the skin; (4) intravaginally or intrarectally, for example, as a pessary, cream or foam; or (5) aerosol, for example, as an aqueous aerosol, liposomal preparation or solid particles containing the compound. In certain embodiments, the subject is a mammal, *e.g.,* a primate, *e.g.,* a human.

The methods of the invention further include administering to a subject a therapeutically effective amount of a compound in combination with a pharmaceutically acceptable excipient. The phrase "pharmaceutically acceptable" refers to those compounds of the invention, compositions containing such compounds, and/or dosage forms which are,

within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

The phrase "pharmaceutically-acceptable excipient" includes pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, carrier, solvent or encapsulating material, involved in carrying or transporting the subject compound from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate and magnesium stearate, as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Examples of pharmaceutically-acceptable antioxidants include: (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Compositions containing a compound(s) include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal, aerosol and/or parenteral administration.

The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated, the particular mode of administration. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will generally be that amount of the compound which produces a therapeutic effect. Generally, out of one hundred per cent, this amount will range from about 1 per cent to about ninety-nine percent of active ingredient, preferably from about 5 per cent to about 70 per cent, most preferably from about 10 per cent to about 30 per cent.

Methods of preparing these compositions include the step of bringing into association a agent(s) with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

Compositions of the invention suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound(s) as an active ingredient. A compound may also be administered as a bolus, electuary or paste.

In solid dosage forms of the invention for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate; (5) solution retarding agents, such as paraffin; (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof;

and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered active ingredient moistened with an inert liquid diluent.

The tablets, and other solid dosage forms of the pharmaceutical compositions of the present invention, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The active ingredient can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compound(s) include pharmaceutically-acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol,

tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof.

In addition to inert diluents, the oral compositions can include adjuvants, such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

Suspensions, in addition to the active compound(s) may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Pharmaceutical compositions of the invention for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compound(s) with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active agent.

Compositions of the present invention which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

Dosage forms for the topical or transdermal administration of a compound(s) include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active compound(s) may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or propellants which may be required.

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

Powders and sprays can contain, in addition to a compound(s), excipients, such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

The compound(s) can be alternatively administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the

compound. A nonaqueous (*e.g.,* fluorocarbon propellant) suspension could be used. Sonic nebulizers are preferred because they minimize exposing the agent to shear, which can result in degradation of the compound.

Ordinarily, an aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically-acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids, such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

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

Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

Pharmaceutical compositions of this invention suitable for parenteral administration comprise one or more compound(s) in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

These compositions may also contain adjuvants, such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material having poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle.

Injectable depot forms are made by forming microencapsule matrices of compound(s) in biodegradable polymers, such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissue.

When the compound(s) are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1 to 99.5% (more preferably, 0.5 to 90%) of active ingredient in combination with a pharmaceutically-acceptable carrier.

Regardless of the route of administration selected, the compound(s), which may be used in a suitable hydrated form, and/or the pharmaceutical compositions of the present invention, are formulated into pharmaceutically-acceptable dosage forms by conventional methods known to those of skill in the art.

Actual dosage levels and time course of administration of the active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. An exemplary dose range is from about about 0.1μg to 20 milligram per kilogram of

body weight per day (mg/kg/day) (e.g., 0.1μg/kg to 10mg/kg, 0.1-10μg/kg, 0.1-1 mg/kg). In other embodiments, the amount varies from about 0.1 mg/kg/day to about 100 mg/kg/day. In still other embodiments, the amount varies from about 0.001 μg to about 100 μg/kg (e.g., of body weight). Ranges intermediate to the above-recited values are also intended to be part of the invention.

Kits

The invention provides kits for the treatment or prevention of cancer. In some embodiments, the kit includes a therapeutic or prophylactic composition containing an effective amount of an agent that inhibits the activity of or decreases the levels of a Notch protein and an effective amount of an agent that inhibits B cell receptor signaling. In one embodiment, the invention provides a commercial package comprising as therapeutic agents a combination comprising a first agent (e.g., an agent that inhibits Notch signaling) or a pharmaceutically acceptable salt thereof, and at least one second agent (e.g., an agent that inhibits B cell receptor signaling) or a pharmaceutically acceptable salt thereof, together with instructions for simultaneous, separate or sequential administration thereof for use in the delay of progression or treatment of a neoplasia.

In particular embodiments, each agent is provided in unit dosage form in a sterile container. Such containers can be boxes, ampoules, bottles, vials, tubes, bags, pouches, blister-packs, or other suitable container forms known in the art. Such containers can be made of plastic, glass, laminated paper, metal foil, or other materials suitable for holding medicaments.

The kit optionally includes instructions for administering the pharmaceutical composition to a subject having or at risk of contracting or developing cancer. The instructions will generally include information about the use of the composition for the treatment or prevention of cancer. In other embodiments, the instructions include at least one of the following: description of the therapeutic/prophylactic agent; dosage schedule and administration for treatment or prevention of cancer or symptoms thereof; precautions; warnings; indications; counter-indications; over dosage information; adverse reactions; animal pharmacology; clinical studies; and/or references. The instructions may be printed directly on the container (when present), or as a label applied to the container, or as a separate sheet, pamphlet, card, or folder supplied in or with the container.

The practice of the present invention employs, unless otherwise indicated, conventional techniques of molecular biology (including recombinant techniques), microbiology, cell biology, biochemistry and immunology, which are well within the purview of the skilled artisan. Such techniques are explained fully in the literature, such as, "Molecular Cloning: A Laboratory Manual", second edition (Sambrook, 1989); "Oligonucleotide Synthesis" (Gait, 1984); "Animal Cell Culture" (Freshney, 1987); "Methods in Enzymology" "Handbook of Experimental Immunology" (Weir, 1996); "Gene Transfer Vectors for Mammalian Cells" (Miller and Calos, 1987); "Current Protocols in Molecular Biology" (Ausubel, 1987); "PCR: The Polymerase Chain Reaction", (Mullis, 1994); "Current Protocols in Immunology" (Coligan, 1991). These techniques are applicable to the production of the polynucleotides and polypeptides of the invention, and, as such, may be considered in making and practicing the invention. Particularly useful techniques for particular embodiments will be discussed in the sections that follow.

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the assay, screening, and therapeutic methods of the invention, and are not intended to limit the scope of what the inventors regard as their invention.

EXAMPLES

Example 1: A novel HLA-DMB / NOTCH4 rearrangement in the MCL cell line SP-49.

Rec-1 and SP-49 are the only known MCL cell lines that demonstrate substantial growth inhibition upon treatment with GSI (Kridel et al., 2012) (FIG. 2). To understand the basis of GSI-sensitivity in SP-49, paired-end RNA-Seq data was analyzed from that line. The analysis detected a highly expressed, aberrant transcript consisting of the first exon of *HLA-DMB* and exons 24-30 of *NOTCH4* (**FIG. 1A**) resulting from an approximately 700 kb deletion on chromosome 6 that juxtaposes the corresponding portions of the *HLA-DMB* and *NOTCH4* genes. Exon 1 of *HLA-DMB* encodes a signal peptide similar to that found at the N-terminal of normal Notch precursor proteins and the truncated Rec-1 *NOTCH1* allele, while exons 24-30 of *NOTCH4* encode the trans-membrane and intracellular portions of NOTCH4, as well as the gamma-secretase protease site that is required for release of the intracellular NOTCH4 transcription factor from the membrane (**FIG. 3A**). Thus, the predicted protein product of this fusion transcript resembles other constitutively active aberrant Notch proteins,

such as those reported in Rec-1 and T-cell acute lymphoblastic leukemia (T-ALL). Indeed, western blot of CLL and MCL cell line nuclear extracts with a NOTCH4 antibody revealed a band at the predicted size of intracellular NOTCH4 (ICN-4) that was exclusive to SP-49 (Fig. 6D).

Example 2: Genome-wide identification of functional Notch target genes

To model ligand-dependent Notch activation, MCL cell lines on immobilized recombinant Notch ligand ($DLL1^{ext}$-IgG) or control protein (IgG) were grown. Analysis by Western blot with an antibody specific for free (gamma secretase-cleaved) ICN-1 demonstrated a time-dependent accumulation of ICN-1 expression in both Mino (**FIG. 1B** and **FIG. 3B**), and Jeko-1 (**FIG. 1B**). ICN-1 accumulation was stronger and more rapid in Mino, consistent with the predicted stabilizing effects of the PEST-truncating mutation in that line (*NOTCH1* Q2487*)(**FIG. 3B**).

To identify Notch-regulated genes and enhancers genome-wide, a GSI-washout strategy in three MCL cell lines was employed (**FIG. 3C**). Rec-1 and SP-49 were treated for three days with GSI (1 μM compound E), to eliminate intracellular Notch proteins. Subsequently, the media was replaced and a four-hour incubation was performed with media containing vehicle only (washout), or GSI (mock-washout). To rapidly activate Notch in the Mino line, Mino cells were grown in the presence of both $DLL1^{ext}$-IgG stimulation and GSI over a 48-hour period, during which time Notch receptors on the cell surface can undergo ligand- and ADAM-protease-dependent S2 cleavage, but not the gamma-secretase-dependent S3 cleavage event that releases ICN. This was then followed by a four-hour GSI-washout or mock-washout procedure identical to that employed for Rec-1 and SP-49. Both the ligand-independent and ligand-dependent procedures lead to rapid Notch activation as measured by ICN-1 accumulation in the *NOTCH1*-mutant cell lines (**FIG. 3D**).

Analysis of triplicate RNA-Seq datasets in each state for the three MCL lines revealed primarily gene activation rather than gene repression, consistent with the known role of intracellular Notch proteins as transcriptional activators (**FIG. 5**). A total of 377 genes showed independently significant activation in at least two of the three lines (**FIG. 6A**). Significant Notch-activated genes were further clustered into genes up-regulated in all three, or only two of three MCL lines, and were compared to RNA-Seq data from comparable GSI-washout experiments performed in two other Notch-dependent cancer lines: the T-ALL cell line CUTLL1 and the triple-negative breast cancer line HCC-1599 (Stoeck et al., 2014). Most

targets showed less activation in SP-49 compared in Mino and Rec-1, possibly due to altered dynamics or transactivation potential of ICN-4 compared to PEST-truncated ICN-1.

The set of genes up-regulated in all three MCL lines (n=142) included many canonical Notch target genes (*HES1*, *HES4*, *HEY1*, *HEY2*, *NRARP*, and *NOTCH3*), which were also strongly up-regulated in CUTLL1 and HCC-1599. However, a large proportion of genes up-regulated by Notch activation in all MCL lines showed unchanged, or even reduced expression upon Notch activation in CUTLL1 and HCC-1599, indicating that these may represent context-specific Notch targets. A similar pattern was seen in the set of activated genes common to Mino and Rec-1, but not SP-49 (n=56), which included the canonical Notch target gene *DTX1* as well as many apparently tissue-specific target genes. Gene set analysis of all genes activated by Notch in at least one GSI-sensitive MCL line and the GSI-insensitive Mino line revealed significant enrichment for gene sets associated with Notch signaling in the mSigDB Hallmark and Reactome collections (**FIG. 6B**), but also for gene sets related to lymphocyte or B-cell biology, including interleukin, interferon, and B-cell receptor signaling, as well as a signature of NF-KB target gene activation.

In contrast, a very different pattern was observed in the large set of genes (n=151) that were activated by Notch signaling in both of the GSI-sensitive MCL lines SP-49 and Rec-1, but not in GSI-insensitive Mino. The vast majority of these genes were also Notch-activated in CUTLL1 and HCC-1599 (**FIG. 6A**), indicating that these may represent a gene expression module associated with Notch-dependent growth across cancer types. Indeed, the most strongly up-regulated of these genes in all four GSI-sensitive lines was the oncogene *MYC*, which is known to be a critical direct Notch target in T-ALL. Furthermore, comparison of genes uniquely activated in GSI-sensitive MCL to the curated mSigDB Hallmark and Reactome collections (**FIG. 6C**) revealed strong enrichment for *MYC* target genes, and *MYC*-regulated biological processes, including nucleotide metabolism, transcriptional processing, protein synthesis, and cell cycle control, indicating that many genes in this set may be secondarily or cooperatively activated by Notch-dependent *MYC* activation. Genes associated with mTORC1 activation were also enriched in this set, consistent with prior data linking mTORC1 to *MYC* upregulation in T-ALL (Chan et al., 2007) and in mature T cell activation (Wang et al., 2011).

Treatment of MCL cell lines with GSI revealed a substantial decrease in c-Myc protein levels for Rec-1 and SP-49 only (**FIG. 6D**), supporting *MYC* as a Notch-activated target in GSI-sensitive MCL. Given the broad role of *MYC* in normal and neoplastic

lymphocyte proliferation, these findings indicated that loss of *MYC* expression might explain the proliferation defect seen in GSI-treated Rec-1 and SP-49. To test this, single-cell clones were derived from SP-49 transduced with a lentiviral vector encoding a *MYC* transgene under the control of a doxycycline-inducible promoter (pINDUCER-22-*MYC*). Indeed, clones that demonstrated effective *MYC* induction showed a doxycycline dose-dependent rescue of cell growth in the presence of GSI (**FIGs. 6E -6F**). Thus, Notch-dependent regulation of *MYC* expression explains much of the dependency of Rec1 and SP-49 on constitutive Notch signaling. Interestingly, expression of *MYC* at levels higher than that seen in parental SP-49 cells was associated with reduced cell viability, indicating that Notch-dependent MCL cells are highly sensitive to either excessive or insufficient *MYC* levels.

Example 3: Intracellular Notch or viral surrogates drive *MYC* via 5' enhancers in MCL cell lines.

Additional studies to understand the genomic mechanism by which Notch signaling regulates *MYC* expression in MCL were undertaken. Prior studies across diverse tissues and cancer types have implicated highly tissue-specific distal enhancer elements in *MYC* activation, including the Notch-dependent 3' *MYC* enhancer identified in immature T cells and T-lymphoblastic leukemia (hereafter TNDME). Lymph node biopsies from CLL and MCL showed no evidence of T-NDME acetylation, but do show strong acetylation of enhancer-like elements on the 5' side of the *MYC* gene (Ryan et al., 2015). ChIP-Seq was performed for histone H3 Lysine 27 acetylation (H3K27ac) in one CLL and ten MCL cell lines, and noted strong acetylation at the 5' *MYC* enhancers in only five lines, including the two Notch gene-rearranged lines Rec-1 and SP-49 **(FIGs. 7A-7B)**. EBV+-transformed human B cells show acetylation of these same elements, which are bound by RBPJ and the EBV-encoded RBPJ cofactor EBNA2 (Zhao et al., 2011). Three of the CLL and MCL cell lines are known to be positive for EBV infection and showed EBNA2 protein expression by Western blot (**FIG. 7C and FIG. 4**), and all three show strong 5' enhancer acetylation. Thus, all CLL / MCL lines showing acetylation of 5' enhancers express either constitutively active intracellular Notch, or a viral Notch surrogate protein, indicating that these elements represent B cell-specific Notch-dependent *MYC* enhancers (hereafter BNDME sites E1 and E2). Indeed, ChIP-PCR demonstrated binding of EBNA2 at the two 5' enhancers in the EBV+ lines, while RBPJ was exclusively bound to 5' enhancers in the EBV+ and Notch-rearranged lines (**FIGs. 7D-7E**). Importantly, analysis of all 11 cell lines with *MYC* break-

apart and *MYC* / *IGH* dual fusion FISH, as well as published conventional karyotyping and other analyses convincingly demonstrate the presence of genomic *MYC* locus rearrangements in all six MCL lines that lack both EBNA2 expression and an activating Notch gene rearrangement, thus explaining the high levels of Notch-independent *MYC* expression in these lines, including Mino (**FIG. 7C**).

To directly evaluate enhancer regulation by Notch transcription complex, ChIP-Seq was performed for H3K27ac, RBPJ, and ICN-1 in Notch-rearranged MCL cell lines following GSI-washout and mock-washout experiments. Specific peaks of RBPJ and (in Rec-1) ICN-1 binding at the BNDME sites were noted in the washout ('notch-on') samples which were absent or markedly reduced in the mock-washout ('notch off') state (**FIG. 8A**). BNDME sites also showed markedly stronger acetylation in the Notch-on state. Mino cells stimulated with recombinant DLL1 also showed binding of NTC proteins and activation of BDME acetylation, despite decoupling of *MYC* expression from Notch activity in the setting of a *MYC-IGH* genomic rearrangement. Motif analysis of DNA sequence within each BNDME site revealed the presence of one evolutionarily conserved RBPJ motif in E1 and two conserved motifs in E2 (**FIG. 8B**). Importantly, no evidence of ICN-1 or RBPJ binding at the T-NMDE was observed in any MCL line, while conversely, published RBPJ binding data in CUTLL1 showed strong binding at the T-NDME, but not at the B-NDME sites, indicating that additional tissue-specific factors must be necessary to facilitate tissue-specific binding of the NTC to each enhancer in a tissue-specific manner.

To prove that the BNDME sites are *bona fide MYC* enhancers, lentiviral guideRNA constructs targeting 15 distinct sites across the *MYC* locus were designed, including the *MYC* promoter, RBPJ motifs with the T-NDME and both B-NDME sites, as well as the *MYC* promoter and other intergenic sites (**FIG. 8B and FIG. 5A**), plus a non-targeting control guideRNA. Populations were generated of SP-49 (Notch-rearranged), Granta-519 (EBV+), and Jeko-1 (*MYC*-rearranged and amplified) stably expressing a dCas9-KRAB-E2A-mCherry transgene, which encodes a nuclease-dead Cas9-KRAB fusion protein that mediates local epigenetic repression. Transduction of dCas9-KRAB-E2A-mCherry stable lines with *MYC* locus gRNAs led to a substantial decrease in *MYC* expression in Granta-519 and SP-49 for guides targeting the *MYC* promoter or central RBPJ of E1, a modest but significant decrease for gRNAs targeting the E2 RBPJ sites, and no change in MYC expression for guides targeting the T-NDME or intergenic regions (**FIG. 5C**). Next, dCas9-KRAB-E2A-mCherry stable lines were simultaneously infected with E1- and E2-targeting guideRNA lentiviruses

encoding distinct fluorescent proteins, sorted doubly-transduced cells, and measured MYC expression, revealing a substantially greater decrease in *MYC* expression for Granta-519 and SP-49 (**FIG. 8C**) when both enhancers were targeted compared to targeting of E1 or E2 alone. To test the effect of these guides on MCL proliferation, the original 16 guideRNAs were utilized to infect a mixture of dCas9-KRAB-E2F-mCherry-expressing cells and cells transduced with a vector expressing GFP alone (FIG. 5B). After 7 days, flow cytometry was used to measure the ratio of mCherry+ versus GFP+ cells relative to cells infected with a control gRNA. Guides targeting the MYC promoter and E1 were associated with decreased proliferation of the dCas9-KRAB-E2F-mCherry population for Granta-519, but little effect was seen for SP-49 (**FIG. 5C**). However, both MYC expression (**FIG. 8D**) and proliferation (**FIG. 8E**) markedly suppressed in both Granta-519 and SP-49 (but not Jeko-1) with a combination of E1- and E2-targeting guides in cells stably expressing Cas9 nuclease. Together, these findings demonstrate that the BNDME sites drive *MYC* expression and proliferation in EBV+ and Notch-dependent MCL lines.

Example 4: Direct Notch targets include regulators of B cell signaling and differentiation

Additional studies were undertaken to identify other direct Notch target genes that might play an important role in MCL and CLL biology. Only a small fraction of Notch-activated genes identified in the GSI-washout analysis showed ICN-1 and RBPJ binding, raising the possibility that many of these genes, like *MYC*, might be activated by Notch-dependent distal elements. To identify such elements, published genome-wide maps were utilized of 3-dimensional genomic interactions associated with RNA Polymerase II via Chromatin Interaction Analysis by Paired-End Tag sequencing (PolII ChIA-PET) in the EBV-immortalized B-lymphoblastoid cell line (LCL) GM12878 (Tang et al., 2015). In support of this approach, strong interactions between both B-NDME sites and the *MYC* promoter were observed in the GM12878 PolII ChIA-PET data (**FIG. 7A**). Strikingly, the majority of genes activated by GSI-washout in both GSI-sensitive and –insensitive MCL models showed either ICN-1-bound enhancers linked via ChIA-PET analysis or ICN-1 bound promoters (**FIG. 9A**), strongly supporting these genes as direct Notch regulatory targets. This association was highly significant compared to randomly selected gene sets, or to the set of genes activated by Notch in GSI-sensitive MCL only, consistent with most of the latter genes being secondary targets up-regulated via Notch-dependent *MYC* activation. Because the

regulatory state of some true Notch target genes in MCL might be different in EBV+ LCLs, a secondary linkage analysis was performed based on the presence on a gene promoter and ICN-1 binding site within the same CTCF-mediated chromatin contact domains (CCD), which are thought to be relatively invariant between related cell types. This analysis yielded an even higher proportion of candidate direct Notch targets among Notch-activated genes in GSI-sensitive and –insensitive MCL, and highly significant enrichment over GSI sensitive-only and random gene sets. Notch-activated enhancers identified in these analyses showed properties consistent with Notch target enhancers in other tissues, including dynamic ICN-1 and RBPJ binding in the presence or absence of GSI, and increased H3K27ac signal in the notch-on state.

In total, the combined functional and epigenetic analysis revealed high-confidence direct Notch target genes with linked regulatory elements in the MCL models presented herein. Only a minority of these genes also showed Notch-dependent activation in T-ALL (CUTLL-1) and TNBC (HCC-1599) cell lines, and most have not been previously identified as Notch target genes in any tissue, although all of the canonical Notch target genes identified in the gene expression analysis presented herein was correctly supported as direct ICN-1 targets via promoter binding or ChIA-PET linkage. The positions of ICN-1 peaks with respect to novel target gene promoters were diverse, reflecting a similar diversity seen in canonical Notch target genes (**FIG. 9B, FIGs. 9C-1-9C-6, FIG. 9D**). Some targets showed only a single ICN-1 peak at or just proximal to the gene promoter (e.g. *HES4*, *BLK*, *BLNK*), while a substantial number of genes showed an ICN-1 peak within the proximal first intron (*NOTCH3*, *CD300A*, *IL6R*, *NEDD9*) a region often associated with regulation of RNA polymerase pause-release. Other genes showed ChIA-PET-linked ICN-1 binding sites more distally within the gene body (*SH2B2, MYBL2, LYN*), at intergenic sites upstream (*RUNX3, CR2*) or downstream (*SEMA7A, IL10RA, IKZF3*) of the target gene, or within the gene body of an adjacent gene (*NRARP*, *CDK5R1*). Some genes showed both strong promoter-proximal and –distal ICN-1 peaks (*HES1, IL21R*), while others showed multiple distal peaks (*BATF, POU2AF1, PAX5, PIK3AP1*). Finally, there were several loci that contained multiple Notch-activated genes commonly linked to adjacent ICN-1 binding sites, likely representing multi-gene regulatory units (*DNASE1L3* / *ABHD6* and *PLAC8* / *COQ2*). To validate the linkage analysis, three strongly Notch-regulated genes were selected, that encode cell surface proteins that were associated with a first intron ICN-1 binding site (*IL6R*), a 5' distal enhancer (*CR2*), and a 3' distal enhancer (*SEMA7A*) and demonstrated knockdown of cell surface expression

in SP-49 by dCas9-KRAB using guideRNAs designed to target the corresponding regulatory sites (**FIG. 9D**).

Next, the set of identified direct Notch target genes for association with pathways identified in the gene set analysis of the RNA-Seq data was examined. Notably, genes involved in cytokine / interleukin signaling (*IL6R, IL10RA, IL21R*) and B cell receptor activation (*FYN, LYN, BLK, BLNK, PIK3AP1, SH2B2, NEDD9*) were identified as direct Notch targets, indicating that these pathways may be directly modulated by Notch-dependent gene activation. Functional analysis of the set of direct Notch targets with the Ingenuity system predicted a significant activatory effect of Notch-regulated genes on B cell receptor signaling. The large number of transcription factor genes that were predicted to be direct Notch targets was striking, indicating a broad effect of Notch in activating or reinforcing diverse transcriptional regulatory programs in MCL lines. Interestingly, the NF-KB target gene signature noted in the Notch-activated genes was substantially driven by genes that were not associated with ICN1 peaks, indicating that secondary activation of NF-KB and NF-KB target genes may be an early feature of Notch activation in B-cell lymphoma cells, similar to the phenomenon observed with MYC.

Example 5: Direct targets are regulated by Notch in primary CLL and MCL

Since rapidly proliferating MCL cell lines show important biological differences from relatively low-grade MCL and CLL cells *in vivo*, experiments were conducted to validate the activity of Notch target genes and enhancers in primary CLL and MCL cells. RNA-Seq was performed on CLL lymph node biopsies with strong, diffuse ICN-1 staining by IHC and compared it to data from CLL lymph node biopsies with low ICN-1 staining (0 of 4 with *NOTCH1* PEST domain mutations). Genome-wide analysis revealed significantly increased expression in the ICN1-high biopsies of many of the strongest Notch target genes identified in the cell line analysis (**FIG. 10A**), including genes implicated in B-cell receptor (BCR) signaling (*FYN*) and cytokine (*IL6R*) signaling, or associated with B cell activation (*SEMA7A*). As in the cell line models, GSEA analysis revealed up-regulation of MYC and NF-KB target gene signatures in ICN1-high versus ICN1-low CLL lymph nodes (Suppl), although *MYC* itself did not show a significant difference in expression.

Next, ChIP-Seq was performed for ICN1, RBPJ, and H3K27ac in CLL and MCL biopsies. One CLL (CLL-013) and one MCL (MCL-010) biopsy yielded a dramatically higher number of significant RBPJ peaks compared to the others, and both contained

NOTCH1 PEST domain mutations (**FIG. 7B**). ICN1 enrichment was relatively poor in the primary samples, but again, the largest number of peaks were seen in CLL-013 and MCL-010. Both cases showed enrichment for ICN1 and RBPJ binding at enhancers linked to *MYC* and other Notch target genes (**FIG. 10C and FIG. 7B**). Furthermore, enhancers linked to Notch-regulated genes were acetylated in most primary CLL and MCL lymph node biopsies, but showed reduced acetylation in peripheral blood CLL samples, consistent with microenvironment-dependent activation.

To functionally demonstrate Notch-dependent activation of Notch target genes in primary CLL and MCL cells, a co-culture model with the immortalized human bone-marrow stromal cell line HS-5 was utilized, which has been widely employed to support the survival of CLL cells *in vitro* (**FIG. 10D**). Peripheral blood mononuclear cells from CLL patients were co-cultured for three days with HS-5 cells stably transduced with a DLL1-IRES-GFP transgene (HS5-DLL1) in the presence of GSI or vehicle, and then sorted CD19+ CD5+ CLL cells for analysis. Co-cultured CLL cells showed a significant and reproducible, albeit modest, increase in expression of *MYC* and other Notch target genes by qRT-PCR (**FIG. 10E**), while flow analysis showed a significant increase in cell surface proteins encoded by Notch target genes.

Next, the same model was used to evaluate the effect of Notch activation on the activity of signaling pathways linked to lymphoma proliferation and survival. CLL PBMC's were harvested following three days of co-culture with HS5-DLL1 with or without GSI, and then performed an additional brief incubation in the presence of absence of B-cell receptor (BCR)-crosslinking antibodies, followed by flow cytometric analysis of phosphoepitopes associated with BCR signaling and downstream pathways (**FIG. 10F and FIG. 12A**). As expected, BCR crosslinking was associated with a rapid increase in phosphorylation of proximal signaling mediators (p-SYK, p-PLCg2), MAP kinases (p-ERK, p-p38), pSTAT5, and mediators downstream of PI3 kinase and mTOR (pAKT, p-S6). Of all phospho-proteins evaluated, only ribosomal protein S6, a target of p70-S6 kinase downstream of mTORC1, showed a substantial notch-dependent increase in phosphorylation in the absence of BCR signaling. This Notch-dependent increase in S6 phosphorylation was still maintained in the setting of a 10-fold increase in S6 phosphorylation seen at 15 minutes after BCR crosslinking. A Notch-dependent difference in AKT phosphorylation was not detected either at rest or upon PI3K-AKT activation by BCR crosslinking, indicating that Notch activates S6 phosphorylation through a pathway independent of BCR signaling or PI3K-AKT activation.

Proximal BCR signaling mediators did not show a notch-dependent difference in phosphorylation in the absence of stimulation, but significantly greater phosphorylation of SYK and PLCg2 were noted in Notch-on CLL cells upon BCR crosslinking. These findings indicate that Notch potentiates BCR signaling via up-regulation of proximal pathway regulators, resulting in increased NF-KB activity upon initiation of BCR signaling (**FIG. 10F, FIG. 11**).

NF-KB is known to be a strong activator of enhancer-mediated gene expression, and in fact, published ChIP-Seq datasets from LCLs show NF-KB protein binding at many ICN-1 bound enhancers, indicating that NF-KB and Notch may act cooperatively to activate many target genes. To test this, additional CLL HS-5 co-culture experiments were performed in the presence of CpG-rich oligodideoxynucleotides, which act as a strong agonist of Toll-like receptor 9 (TLR9) signaling (**FIG. 12A).** The toll-like receptor signaling pathway activates NF-KB independent of the BCR signaling pathway, and is mutationally activated in a minority of CLL cases. CLL surface expression of CD300A was increased by Notch signaling, but unaffected by TLR activation, while SEMA7A showed additive increases in expression due to Notch and TLR signaling, and the activation of IL6R expression by Notch was detectable only in the presence of concomitant TLR activation, indicating a synergistic effect (FIG. **12B)**

Example 6: Notch target genes show microenvironment-specific activation in MCL *in vivo*

Implicit in the present investigation of CLL and MCL lymph node biopsies, as well as co-culture model described herein, is the assumption that Notch activation occurs due to interaction of lymphoma cells with Notch ligand-expressing cells within the lymph node microenvironment. To support this *in vivo*, a patient-derived xenograft (PDX) model derived from a case of MCL with a *NOTCH1* PEST domain mutation was utilized.

Immunohistochemistry showed strong expression of ICN1 in MCL cells within the spleen, but minimal staining in three different, NOTCH1 wild-type MCL PDX models. PDX-XXX mice were treated for five days with either the gamma-secretase inhibitor DBZ or vehicle. Flow cytometry revealed the highest expression of Notch target cell surface proteins in MCL cells within the spleen compared to bone marrow or blood, with substantially decreased expression seen in GSI-treated animals (**FIG. 12C).**

Since the initial discovery of recurrent Notch gene mutations in CLL and MCL, it has been clear that aberrant Notch signaling plays a role in the etiology of small B cell lymphomas, but the specific mechanisms by which Notch signaling drives B cell lymphoma growth, and its interaction with other oncogenic signaling pathways have remained largely obscure. The present study reported herein represents a substantial advance by defining a set of direct Notch regulatory targets in B cell lymphoma that is distinct from those identified in other tissue types, indicating unique mechanisms by which small B-cell lymphomas may utilize this pathway to drive malignant biology.

The data presented herein provides the first demonstration of *MYC* as a critical and direct regulatory target of enhancer activation by ICN/RBPJ in small B cell lymphomas, and the findings reported herein are consistent with other recent data linking Notch signaling to *MYC* activation in CLL. The BNDME sites are recurrently amplified in a small subset of CLL cases, and an enhancer-like element immediately adjacent to BNDME1 contains a germline polymorphism linked by genome-wide association studies (GWAS) to hereditary risk for CLL, further supporting the central role of these elements in CLL pathogenesis. *MYC* is a pivotal regulator of cellular growth, directly activating genes responsible for nutrient import, metabolic pathway activation, nucleotide synthesis and core components of the transcriptional and translational machinery. *MYC* is essential for the proliferation of normal mature B and T cells, as well as most, if not all B-cell lymphomas, and activating genomic rearrangements of the *MYC* locus are frequently seen in aggressive B cell lymphomas, including blastic transformation of MCL and large-cell transformation of CLL (Richter syndrome), where *NOTCH1* mutations and *MYC*-activating genomic lesions show near-complete mutual exclusivity. Notch-dependent activation of *MYC* and *MYC* target genes appears to be a common feature of Notch-dependent cell lines across at least three cancer types (B-cell lymphoma, T-ALL, and TNBC), although the specific distal regulatory elements through which Notch activates *MYC* in B-cell lymphomas are not utilized in T-ALL. The data presented herein indicates that inhibition of Notch-dependent *MYC* expression is the primary mechanism by which GSI inhibits growth of Notch-dependent MCL cell lines, since a similar loss of *MYC* expression and proliferation could be demonstrated via direct CRISPR-Cas9 targeting of the 5' BNDME sites, while conversely, GSI sensitivity could be largely rescued via expression of a *MYC* transgene **(FIG. 2)**.

CLL and MCL are considered to be low-grade lymphomas, and it is important to note that the growth cycle of these tumors *in vivo* is different from that of the rapidly proliferating

MCL cell lines utilized in the present study (doubling time 24-36 hours). Clinical and biological observations demonstrate that most cases of MCL show slow tumor growth for years after initial presentation, while the majority of CLL cells in most patients are in a quiescent state in both peripheral blood and secondary lymphoid organs, with bursts of proliferation limited to a small subset of cells in proliferation centers. However, the data presented herein, and the findings others, supports an important role for Notch-dependent *MYC* activation in driving a shift toward anabolic metabolism in primary CLL cells, which may facilitate subsequent cellular growth and proliferation. Co-culture of CLL cells with Notch ligand-expressing stromal cells has been shown to activate expression of hexokinase II and other *MYC*-activated metabolic regulators, resulting in activation of glycolysis. During activation of normal T cells, *MYC* is required for initiation of glycolysis and altered amino acid transport and metabolism, resulting in activation of p70-S6 kinase and other mTORC-regulated drivers of protein synthesis. The data presented herein from both proliferating cell lines and non-proliferating primary CLL cells is consistent with an analogous model in which Notch-dependent *MYC* activation leads to up-regulation of nutrient transporters, as well as HK2 and other metabolic gatekeepers, leading to activation of mTORC1 and S6 phosphorylation. This mechanism could play an important role in the growth of CLL and MCL cells during either proliferation or a pre-proliferative state.

In addition to activating *MYC*, the data indicated that Notch directly activates genes that encode regulators of B-cell receptor (BCR) signaling, including all three of the SRC family kinases implicated in proximal BCR activation (*LYN*, *BLK*, and *FYN*), as well as signaling adaptor proteins associated with PI3 kinase (*PIK3AP*; encodes BCAP) and phospholipase C gamma 2 (*BLNK*). While many details about the oncogenic role of BCR signaling in CLL and MCL are still unclear, phosphorylation of PLCγ2 by Bruton tyrosine kinase (BTK) appears to be a critical step, since treatment with the BTK inhibitor ibrutinib drives sustained clinical remission in many CLL and MCL patients, while acquired ibrutinib resistance in lymphoma is often associated with mutations in *BTK* or *PLCG2*. A reproducibly stronger increase was observed in PLCγ2 phosphorylation upon BCR signaling activation in "notch on" versus GSI-treated CLL cells from HS-5-DLL1 co-cultures, demonstrating that Notch activation potentiates this step of the BCR signaling cascade, likely through increased expression of one or more of the Notch target genes described above.

The validation studies were focused on the MYC and BCR signaling pathways, this work also identified genes encoding a striking array of cell surface signaling receptors as

direct Notch targets, including receptors for IL6, IL10, and IL21, interferon gamma, TNF, and others, indicating that Notch may also potentiate signaling through these pathways. IL6R is a particularly strong Notch target, and has been implicated in the pathogenesis of both small B cell lymphomas and several autoimmune disorders. IL6R was among the Notch target genes that showed significantly increased expression in ICN1-high CLL (FIG. 10B), and given the availability of an FDA-approved antibody inhibitor of IL6R, the potential value of anti-IL6R therapy in Notch-mutant CLL could be worth further investigation. It is likely that many of the direct Notch target genes identified in this study may be regulated by Notch in normal immunity or autoimmune disease, and in this context it is interesting to note that several direct Notch target genes lie in loci that have been linked by genome wide association studies to immunological disorders. Notch is known to play a critical role in the development of specific B cell subsets, since B cell-specific deletion of *Rbpj* or *Notch2* results in absence of splenic marginal zone B cells (MZB) in mice. Interestingly, mice with homozygous inactivation of *Nedd9*, the human homolog of which was identified as a direct Notch target in this study, also results in absence of MZB, indicating that Notch-dependent activation of *Nedd9* may play a critical role in development of this subset. The protein product of *Nedd9* (also known as HEF1 or CAS-L) encodes a signaling adaptor known to play an important role in motility and mitosis. In B cells, *NEDD9* associates with *LYN* or *FYN* to convey active integrin- or B-cell receptor signals to CRKL, which activates downstream effectors involved in cytoskeletal regulation and motility. Interference with BCR- and integrin-mediated trafficking signals has been cited as an important therapeutic mechanism of action for ibrutinib in CLL (De Rooij et al., 2012). Given that the data presented herein identification of *NEDD9* and *FYN* as strong direct Notch targets in MCL cell lines, and as significantly up-regulated genes in ICN1-high CLL, the role of Notch signaling in regulation of lymphoma adhesion and trafficking merits further study.

The findings presented herein have important implications for the potential use of Notch inhibitors in the treatment of small B cell lymphomas. Notch signaling in lymphomas with wild-type or PEST domain-mutated Notch receptors is predicted to be largely or entirely ligand-dependent, and thus Notch inhibitors might be expected to have little effect on circulating lymphoma cells outside of secondary lymphoid organs, or other microenvironments that support Notch signaling activation. However, there is precedent for selectively targeting lymphoma within a tissue niche, as clinically efficacious agents that inhibit BCR-related signaling, including ibrutinib and the PI3Kδ inhibitor idelalisib, show

minimal toxicity to circulating CLL cells, and in fact, treatment with these agents is frequently associated with sustained tumor lymphocytosis, despite dramatic shrinkage of lymphadenopathy and eventual clinical remission. BCR signaling-mediated activation of NF-κB, as well as up-regulation of *MYC* and *MYC* target genes, are believed to be critical drivers of lymphoma proliferation and survival in the lymph node microenvironment. The potential of Notch inhibitor therapy to target both of these pathways by a single unique mechanism may provide an advantage over existing agents, either alone or in combination therapy. Mutations or rearrangements predicted to yield ligand-independent Notch signaling, as observed in Notch-dependent MCL lines, are essentially absent in low-grade CLL and MCL, although development of a *NOTCH1* heterodimerization domain mutation has been observed following large cell (Richter) transformation of CLL. Such patients might represent particularly appealing candidates for Notch-targeting therapy. However, the data presented herein indicates that *MYC*-activating genomic rearrangements, which are relatively common following high-grade transformation of CLL or MCL, would be likely to show Notch-independent *MYC* expression and thus reduced susceptibility to Notch inhibitor therapy, indicating that clinical investigators might consider excluding such patients from future trials of Notch-targeting drugs.

The results described herein above, were obtained using the following methods and materials.

Cell lines and specimen collection

MCL-derived cell lines were kindly provided by Dr. Randy Gascoyne, BC Cancer Agency, Canada (Z-138, Maver-1, JVM-2, Granta-519, HBL-2, and UPN-1). The cell lines SP-49, Jeko-1 and Mino were kind gift of Dr. Mariusz Wasik, University of Pennsylvania. Rec-1 and HEK293T cell lines were purchased from the American Type Culture Collection. Mec-1 cells were obtained. All cell lines were authenticated by short tandem repeat (STR) profiling analysis. This study was approved by the Institutional Review Board and MCL and CLL patient samples were collected.

Cell culture and GSI washout assay

All cell lines were grown in RPMI medium 1640 (Invitrogen) supplemented with 10% FCS, 100 IU per 100 μg per mL penicillin/streptomycin, 1% nonessential amino acids, 1 mM sodium pyruvate and 5 μM 2-mercaptoethanol. In GSI washout studies, Rec-1, Mino and SP-49 cells were treated with the GSI compound E (1 μM) (Shelton et al., 2009) for 48-72 hours,

washed, and then replated in either 1 μM GSI (washout control) or in DMSO for 4 h (washout) as described in Weng et al., 2006. To activate Notch signaling Mino and Jeko-1 cells were cultured on either immobilized recombinant Notch ligand ($DLL1^{ext}$-IgG) or control protein (IgG) for 48 hours supplemented with either DMSO or 1 μM GSI, following mock or GSI washout for 4 hours.

Western Blotting

Cells were lysed in 50 mM Tris, pH 8.0, containing 150 mM NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA and supplemented with protease inhibitors. Total protein was determined. Samples were mixed with sample buffer containing 5% β-mercaptoethanol, separated by 4% to 12% NuPAGE Tris-Acetate gel (Life technologies) and transferred to a nitrocellulose membrane that was blocked for 1 hour in 5% non fat dry milk/BSA in TBST (20 mmol/L Tris–HCl, 0.5 mol/L NaCl, and 0.1% Tween 20). The membrane was probed and incubated with a primary antibody overnight at 4°C. Following washes with TBST, the membrane was incubated with horseradish peroxidase–conjugated secondary antibody (Ref) and detected with ECL developing solution (Thermo Scientific). Primary antibodies used are a monoclonal rabbit antibody against the cleaved Notch1 (Val1744, CST; #4147) in 1:1000 dilution, c-MYC and TBP.

Quantitative Real-Time PCR

RNA was isolated using the RNeasy Plus Mini Kit (Qiagen). cDNA was synthetized with the SuperScript III kit (Invitrogen). qRT-PCR was carried out using 1 μL cDNA, SYBR Green PCR Master Mix (ABI) and gene-specific primers (supplementary table 1) on an ABI ViiA 7 real-time PCR System. cDNA was used as template for each pair of primers in triplicate PCR reactions and resulting qPCR data were analyzed using the $\Delta\Delta C_t$ relative quantification protocol.

Chromatin immunoprecipitation assay

ChIP-qPCR and ChIP-Seq were performed as previously described (Ref). Briefly, chromatin samples prepared from fixed cells were immunoprecipitated with rabbit IgG (Santa Cruz Biotechnology, sc-3888), rabbit monoclonal anti-Rbpj (CST, #5313), rabbit polyclonal anti-H3K27ac (Active Motif, #39133) and mouse monoclonal anti-EBNA2(PE2) antibody (Abcam, ab90543). Antibody-chromatin complexes were captured with protein G–conjugated

agarose beads, washed several times, and eluted. Following reversal of cross-links, RNase and proteinase K treatment, DNA was purified with QIAquick PCR Purification Kit (Qiagen). Input sample was prepared in parallel without immunoprecipitation. Real-time PCR was performed in triplicates for indicated regions using primers listed in supplementary table 2. For ChIP-Seq two replicates were used per experimental condition and libraries were prepared using NEBNext® Ultra™ DNA Library Prep Kit for Illumina according to the manufacturer's instructions. Indexed libraries were validated for quality and size distribution using the Agilent 2100 Bioanalyzer. High-throughput sequencing was performed by using the HiSeq 2500 Illumina Genome Analyzer. ChIP-Seq reads were aligned to the human genome (hg19).

Lentiviral infection and cell sorting

Lentiviral particles were generated with the use of standard procedures (Ref). Briefly, lentivirus was produced in HEK293T cells that were transfected with transfection mix containing 3.9 μg of gRNA expression vectors (Addgene, #57822, #57823, #52963) or pHR-SFFV-KRAB-dCas9-P2A-mCherry (Addgene, #60954), 1.3 μg of pCMV-VSV-G and 2.6 μg pCMV-delta and FuGENE HD (Promega). Viral supernatant was harvested 48 hours post-transfection. Cell lines were transduced with lentiviral supernatants by spinfection for 90 minutes in the presence of 12 μg/ml of polybrene at 37°C. 3 days after infection, transduced cells were selected either with puromycin (3 days), or were selected by fluorescent marker with cell sorting on a BD FACSAria II SORP. Selected cells were used for RNA extraction and proliferation assay.

RNA-Seq

RNA-Seq was performed using three replicates per experimental condition. RNA was isolated with RNeasy Plus Mini Kit (Qiagen) from SP-49 cells treated with GSI for 3 days to establish a Notch-off state or cells where Notch was re-activated by GSI washout as described in GSI washout assay or from Mino cells that were cultured with the following modification: supplemented with either immobilized recombinant Notch ligand ($DLL1^{ext}$-IgG) or control protein (IgG) for 48 hours of purified mRNA was used as template for cDNA synthesis and library construction. Indexed libraries were validated for quality and size distribution using the Agilent 2100 Bioanalyzer and were sequenced on the HiSeq 2500 Illumina Genome Analyzer.

MYC rescue experiment

SP-49 cells were stably transduced with pINDUCER-22-MYC (Ref) and single cell clones were isolated by limiting dilution with plating 0.3 cells/well in 96 well plates. Selected clones were treated with DMSO or GSI for 5 days and then MYC expression was induced by increasing concentration of doxycycline for 2 days and cell growth was measured using the CellTiter-Glo Luminescent Cell viability assay (Promega) as recommended by the manufacturer.

Proliferation assay after silencing CR2 and CD300A regulatory elements

SP-49 and Granta-519 were engineered to stably express SFFV-KRAB-dCas9-P2A-mCherry or pLX-304-GFP. GFP+ and dCas9-KRAB-mCherry+ cells derived from SP-49 or Granta-519 were mixed in 1:1 ratio and transduced with gRNA lentiviruses designed against CD300A and CR2 regulatory regions (gRNA sequences are provided in supplementary table 3), following the puromycin selection for 3 days. Flow antibodies against CR2 and CD300A (Ref) were used to detect the expression in GFP+ (negative control) and dCas9-KRAB-mCherry+ populations following the epigenetic silencing of CR2 and CD300A.

Other Embodiments

From the foregoing description, it will be apparent that variations and modifications may be made to the invention described herein to adopt it to various usages and conditions. Such embodiments are also within the scope of the following claims.

The recitation of a listing of elements in any definition of a variable herein includes definitions of that variable as any single element or combination (or subcombination) of listed elements. The recitation of an embodiment herein includes that embodiment as any single embodiment or in combination with any other embodiments or portions thereof.

All patents and publications mentioned in this specification are herein incorporated by reference to the same extent as if each independent patent and publication was specifically and individually indicated to be incorporated by reference.

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What is claimed is:

1. A pharmaceutical composition comprising an effective amount of an agent that inhibits the expression or activity of a Notch polynucleotide or polypeptide and an effective amount of an agent that inhibits the expression or activity of a functional component of a B cell receptor polypeptide or polynucleotide.

2. The composition of claim 1, wherein the agent is a small compound, polypeptide, or polynucleotide.

3. The composition of claim 1, wherein the agent that inhibits Notch expression or activity is a gamma secretase inhibitor, a Notch signaling pathway inhibitory antibody, or an anti-Notch1 antibody.

4. The composition of claim 3, wherein the gamma secretase inhibitor is selected from the group consisting of Compound E, MK-0752, PF03084014, RO-4929097, DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester, tetralin imidazole PF-03084014, LY3039478, and BMS906-024.

5. The composition of claim 3, wherein the anti-Notch1 antibody is OMP-52M521 and the Notch signaling pathway inhibitory antibody is an anti-Delta-like-4 antibody.

6. The composition of claim 2, wherein the agent that inhibits Notch expression or activity is an inhibitory nucleic acid molecule.

7. The composition of claim 1, wherein the agent that inhibits B cell receptor expression or activity is a PI3 kinase inhibitor, BTK inhibitor, SRC family kinase inhibitor, SYK inhibitor, or a protein kinase C inhibitor.

8. The composition of claim 6, wherein the BTK inhibitor is selected from the group consisting of ibrutinib, ACP-196, ONO/GS-4059, BGB-3111, and CC-292.

9. The composition of claim 6, wherein the SRC family kinase inhibitor is Dasatinib and the PI3 kinase inhibitor is idelalisib.

10. The composition of claim 6, wherein the SYK inhibitor is Fostamatinib.

11. The composition of claim 6, wherein the protein kinase C inhibitor is Midostaurin, Enzastuarin, or Sotrasturin.

12. The composition of claim 2, wherein the agent that inhibits the expression or activity of a functional component of the B cell receptor is an inhibitory nucleic acid molecule.

13. The composition of claim 1, wherein the agents are formulated together or are formulated separately for simultaneous, separate or sequential co-administration.

14. The composition of any one of claims 1-11, further comprising one or more additional therapeutic agents.

15. The composition of any one of claims 1-11, wherein the Notch activity is signaling.

16. The composition of any one of claims 1-11, wherein the B cell receptor activity is signaling.

17. A method of inhibiting the survival or proliferation of a neoplastic cell, the method comprising contacting the cell with an agent that inhibits expression or activity of a Notch polynucleotide or polypeptide and an effective amount of an agent that inhibits expression or activity of a functional component of a B cell receptor polypeptide or polynucleotide

18. The method of claim 17, wherein the agent that inhibits Notch expression or activity is a gamma secretase inhibitor, a Notch signaling pathway inhibitory antibody, or an anti-Notch1 antibody.

19. The method of claim 17, wherein the gamma secretase inhibitor is selected from the group consisting of Compound E, MK-0752, PF03084014, RO-4929097, DAPT, N-[N-(3,5-

difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester, tetralin imidazole PF-03084014, LY3039478, and BMS906-024.

20. The method of claim 17, wherein the anti-Notch1 antibody is OMP-52M521 and the Notch signaling pathway inhibitory antibody is an anti-Delta-like-4 antibody.

21. The method of claim 17, wherein the agent that inhibits Notch expression or activity is an inhibitory nucleic acid molecule.

22. The method of claim 17, wherein the agent that inhibits B cell receptor expression or activity is a PI3 kinase inhibitor, inhibitory nucleic acid molecule, BTK inhibitor, SRC family kinase inhibitor, SYK inhibitor, or a protein kinase C inhibitor.

23. The method of claim 22, wherein the BTK inhibitor is selected from the group consisting of ibrutinib, ACP-196, ONO/GS-4059, BGB-3111, and CC-292.

24. The method of claim 22, wherein the SRC family kinase inhibitor is Dasatinib and the PI3 kinase inhibitor is idelalisib.

25. The method of claim 22, wherein the SYK inhibitor is Fostamatinib.

26. The method of claim 16, wherein the protein kinase C inhibitor is Midostaurin, Enzastuarin, or Sotrasturin.

27. The method of claim 16, further comprising administration of one or more additional therapeutic agents.

28. A method of inhibiting the survival or proliferation of a neoplastic cell, the method comprising contacting the cell with a gamma secretase inhibitor and ibrutinib, thereby inhibiting the survival or proliferation of the neoplastic cell.

29. The method of any one of claims 17-28, wherein the neoplastic cell is derived from a leukemia or lymphoma.

30. The method of claim 29, wherein the leukemia is selected from the group consisting of a chronic lymphocytic leukemia, B cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and early T cell acute lymphoblastic leukemia.

31. The method of claim 29, wherein the lymphoma is selected from the group consisting of small B-cell lymphomas, mantle cell lymphoma, small lymphocytic lymphoma, diffuse large B cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, splenic red pulp lymphoma, and MALT lymphoma.

32. The method of claim 28, wherein the gamma secretase inhibitor is selected from the group consisting of Compound E, MK-0752, PF03084014, RO-4929097, and DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester, and tetralin imidazole PF-03084014.

33. The method of any one of claims 29, wherein the neoplastic cell is a murine, rat, or human cell.

34. The method of claim 33, wherein the cell is in vitro or in vivo.

35. A method of treating a neoplasia in a subject, the method comprising administering to the subject an agent that inhibits the expression or activity of a Notch polynucleotide or polypeptide and an effective amount of an agent that inhibits the expression or activity of a functional component of a B cell receptor polypeptide or polynucleotide, thereby treating cancer in the subject.

36. The method of claim 35, wherein the agent that inhibits Notch expression or activity is a gamma secretase inhibitor.

37. The method of claim 36, wherein the gamma secretase inhibitor is selected from the group consisting of Compound E, MK-0752, PF03084014, RO-4929097, DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester, tetralin imidazole PF-03084014, LY3039478, BMS906-024

38. The method of claim 35, wherein the agent that inhibits B cell receptor expression or activity is a PI3 kinase inhibitor, inhibitory nucleic acid molecule, BTK inhibitor, SRC family kinase inhibitor, SYK inhibitor, or a protein kinase C inhibitor.

39. The method of claim 38, wherein the BTK inhibitor is selected from the group consisting of ibrutinib, ACP-196, ONO/GS-4059, BGB-3111, and CC-292.

40. The method of claim 38, wherein the SRC family kinase inhibitor is Dasatinib and the PI3 kinase inhibitor is idelalisib.

41. The method of claim 38, wherein the SYK inhibitor is Fostamatinib.

42. The method of claim 38, wherein the protein kinase C inhibitor is Midostaurin, Enzastuarin, or Sotrasturin.

43. The method of claim 38, wherein the BTK inhibitor is selected from the group consisting of ibrutinib, ACP-196, ONO/GS-4059, BGB-3111, and CC-292.

44. The method of claim 35, wherein the neoplasia is leukemia or lymphoma.

45. The method of claim 44, wherein the leukemia is selected from the group consisting of chronic lymphocytic leukemia, B cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and early T cell acute lymphoblastic leukemia.

46. The method of claim 44, wherein the lymphoma is selected from the group consisting of small B-cell lymphomas, mantle cell lymphoma, small lymphocytic lymphoma, diffuse large B cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, splenic red pulp lymphoma, and MALT lymphoma.

47. A method of treating a subject having a leukemia or lymphoma, the method comprising administering to the subject a gamma secretase inhibitor and ibrutinib.

48. The method of claim 47, wherein the leukemia is selected from the group consisting of chronic lymphocytic leukemia, B cell acute lymphoblastic leukemia, T-cell acute lymphoblastic leukemia, and early T cell acute lymphoblastic leukemia.

49. The method of claim 40, wherein the lymphoma is selected from the group consisting of small B-cell lymphomas, mantle cell lymphoma, small lymphocytic lymphoma, diffuse large B cell lymphoma, splenic marginal zone lymphoma, follicular lymphoma, splenic red pulp lymphoma, and MALT lymphoma.

50. A method of treating a subject having a leukemia or lymphoma that has developed resistance to a B cell receptor inhibitor, the method comprising administering a gamma secretase inhibitor and an agent that inhibits expression or activity of a functional component of the B cell receptor.

51. The method of claim 50, wherein the agent that inhibits B cell receptor expression or activity is a PI3 kinase inhibitor, inhibitory nucleic acid molecule, BTK inhibitor, SRC family kinase inhibitor, SYK inhibitor, or a protein kinase C inhibitor.

52. The method of claim 60, wherein the BTK inhibitor is selected from the group consisting of ibrutinib, ACP-196, ONO/GS-4059, BGB-3111, and CC-292.

53. The method of claim 50, wherein the SRC family kinase inhibitor is Dasatinib and the PI3 kinase inhibitor is idelalisib.

54. The method of claim 50, wherein the SYK inhibitor is Fostamatinib.

55. The method of claim 50, wherein the protein kinase C inhibitor is Midostaurin, Enzastuarin, or Sotrasturin.

56. The method of claim 50, wherein the BTK inhibitor is selected from the group consisting of ibrutinib, ACP-196, ONO/GS-4059, BGB-3111, and CC-292.

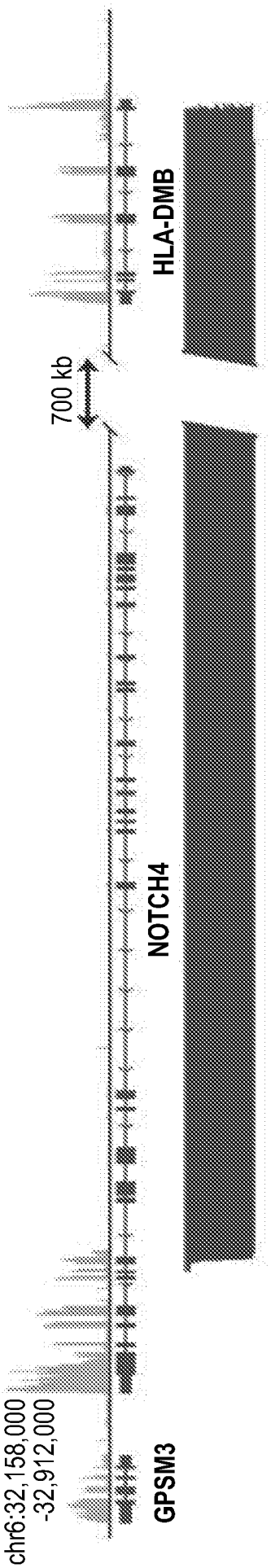

chr6:32,158,000
-32,912,000
GPSM3
NOTCH4
700 kb
HLA-DMB

FIG. 1A

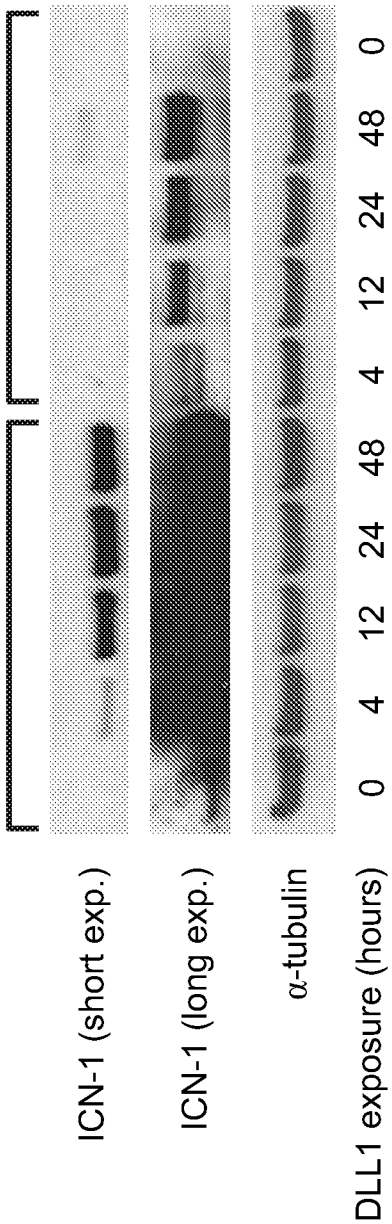

Mino
Jeko-1
ICN-1 (short exp.)
ICN-1 (long exp.)
α-tubulin
DLL1 exposure (hours)
0 4 12 24 48 4 12 24 48 0

FIG. 1B

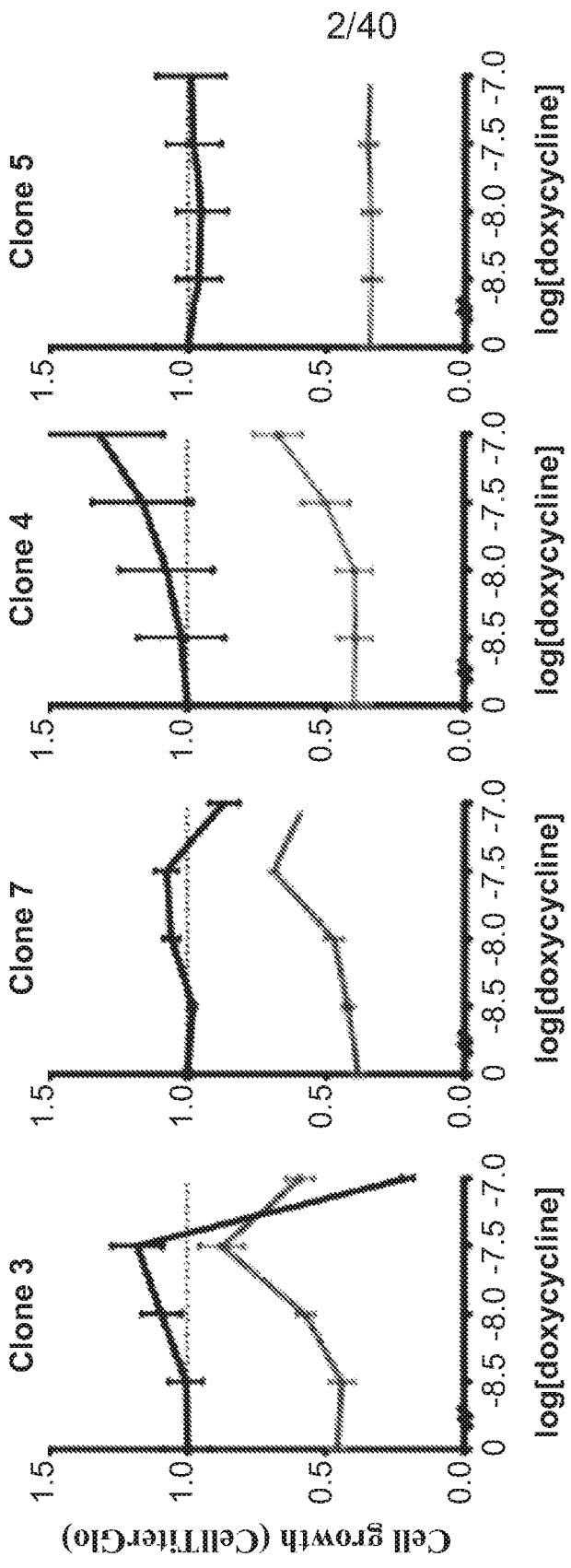

Clone 3
Clone 7
Clone 4
Clone 5
Cell growth (CellTiterGlo)
log[doxycycline]
0
-8.5
-8.0
-7.5
-7.0
0.0
0.5
1.0
1.5
DMSO
GSI

FIG. 2

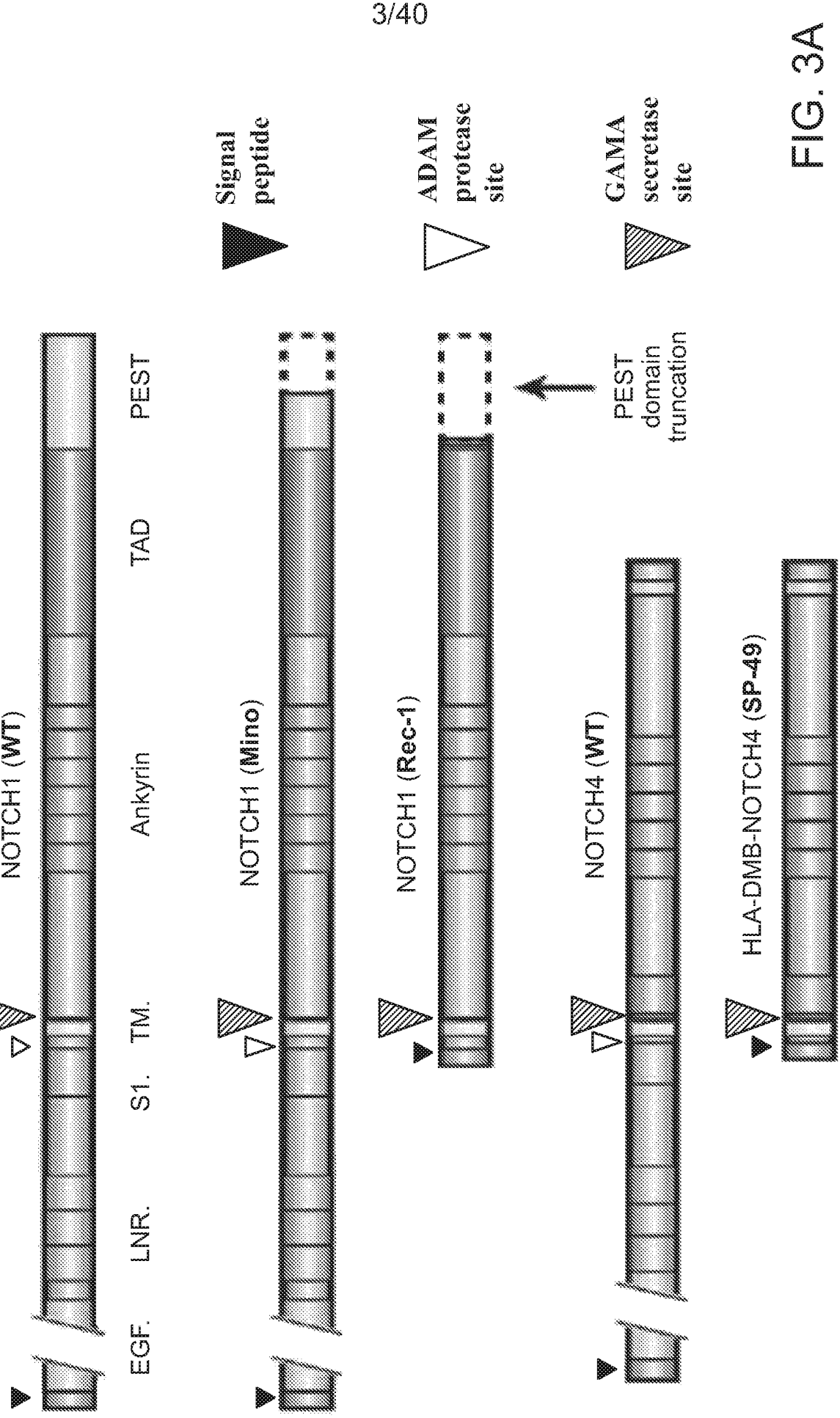

Signal peptide
ADAM protease site
GAMA secretase site
PEST domain truncation
NOTCH1 (WT)
NOTCH1 (Mino)
NOTCH1 (Rec-1)
NOTCH4 (WT)
HLA-DMB-NOTCH4 (SP-49)
EGF.
LNR.
S1.
TM.
Ankyrin
TAD
PEST

FIG. 3A

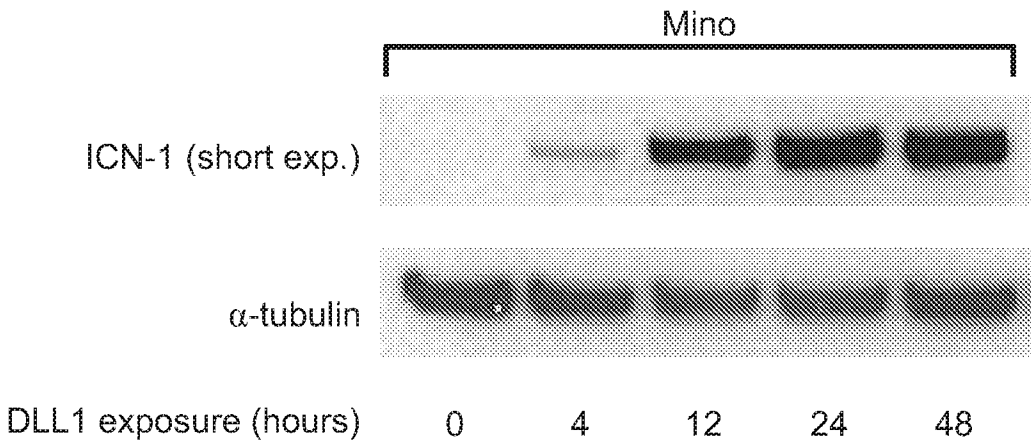


FIG. 3B

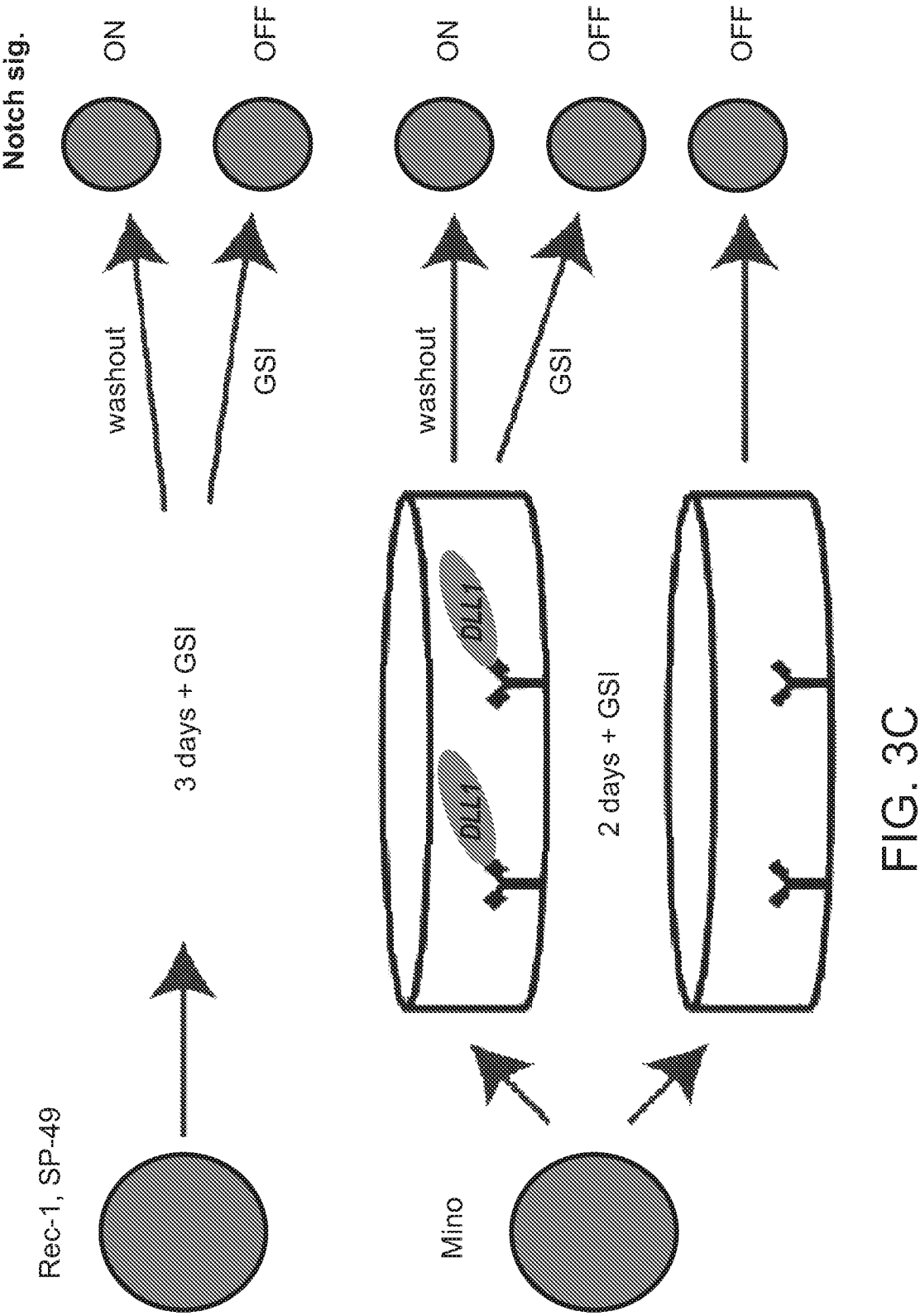

Notch sig.
ON
OFF
ON
OFF
OFF
washout
GSI
washout
GSI
3 days + GSI
2 days + GSI
DLL1
DLL1
Rec-1, SP-49
Mino

FIG. 3C

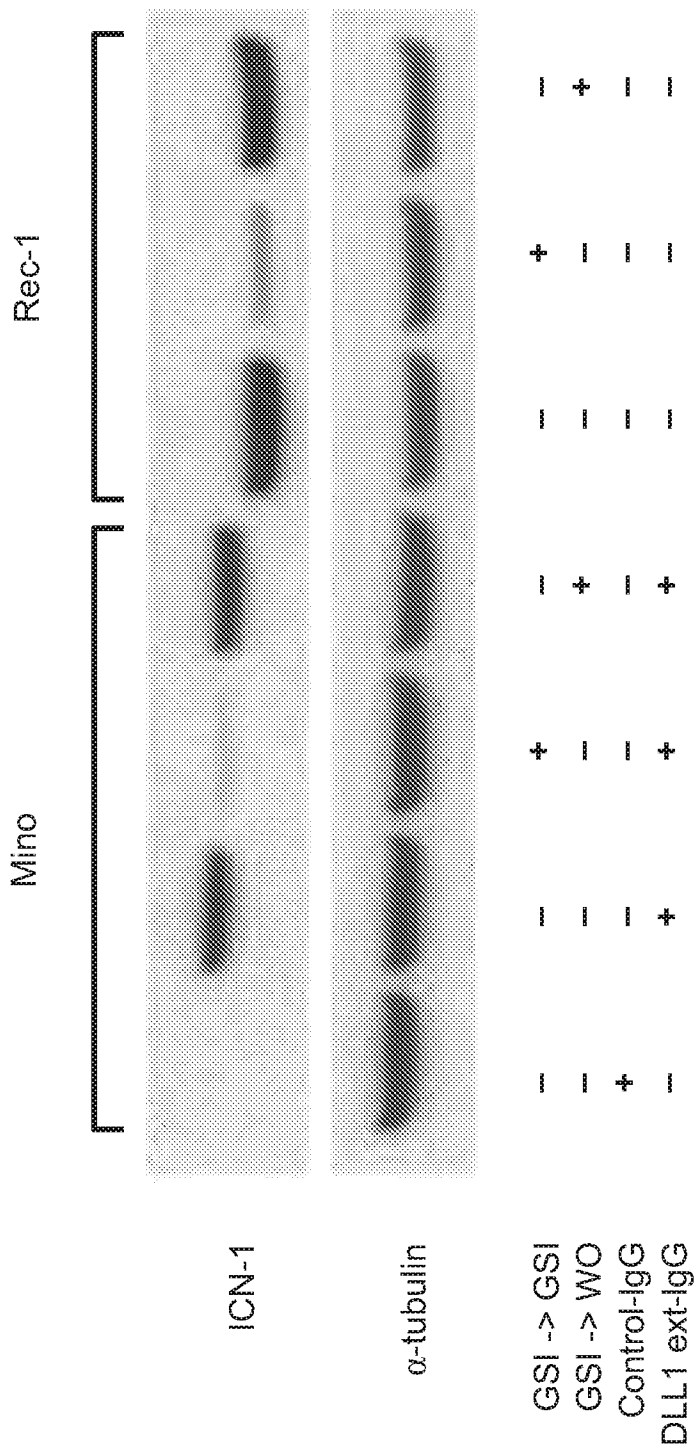

Mino
Rec-1
ICN-1
α-tubulin
GSI -> GSI
GSI -> WO
Control-IgG
DLL1 ext-IgG
- - + -
- - - +
+ - - -
- + - -
- - + -
- + - +
+ - - -
- + - -

FIG. 3D

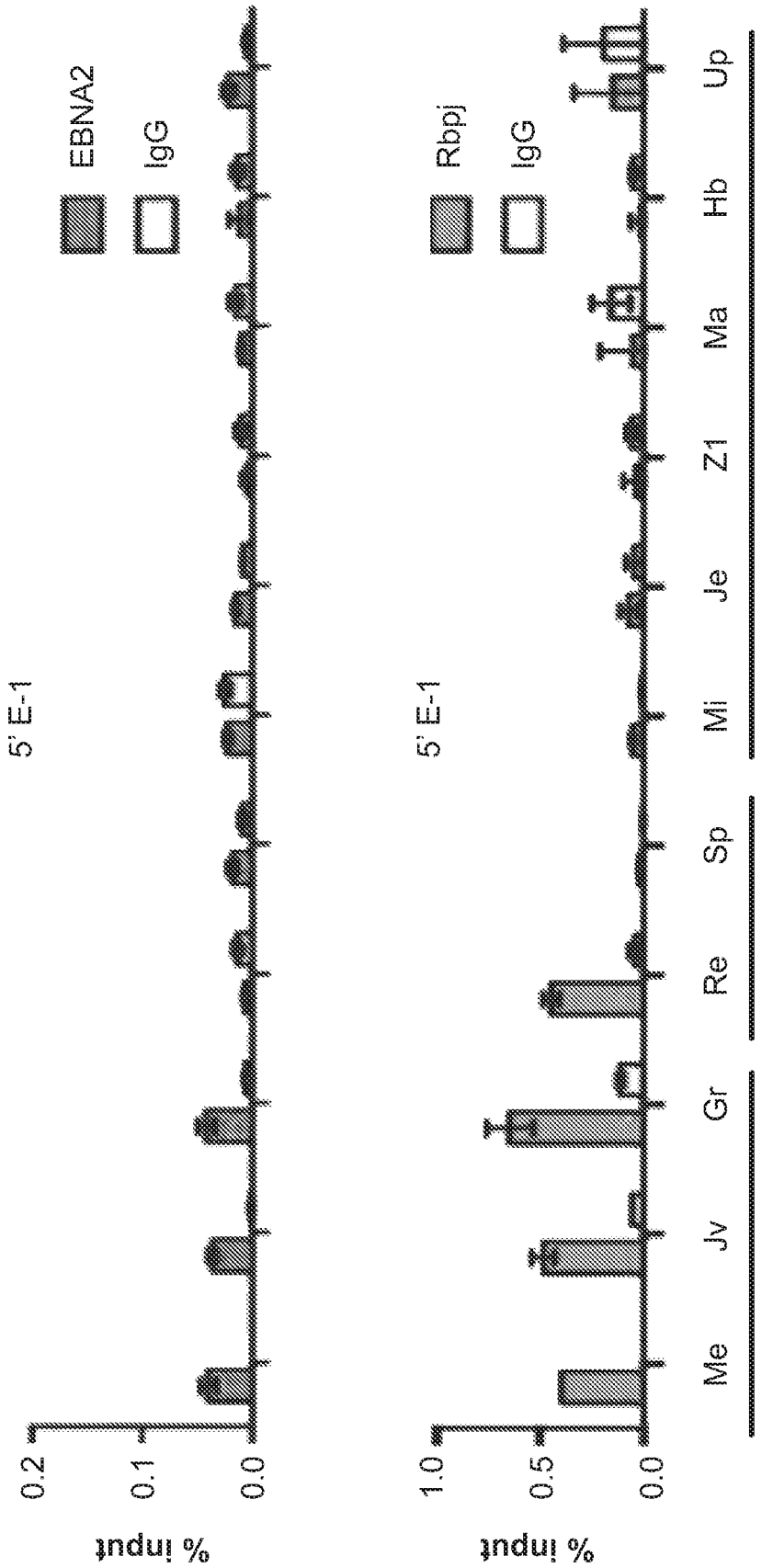

EBNA2
IgG
5' E-1
0.2
0.1
0.0
% input
Rbpj
IgG
5' E-1
1.0
0.5
0.0
% input
Me
Jv
Gr
Re
Sp
Mi
Je
Z1
Ma
Hb
Up
EBV+
Notch-rearranged
MYC-rearranged

FIG. 4

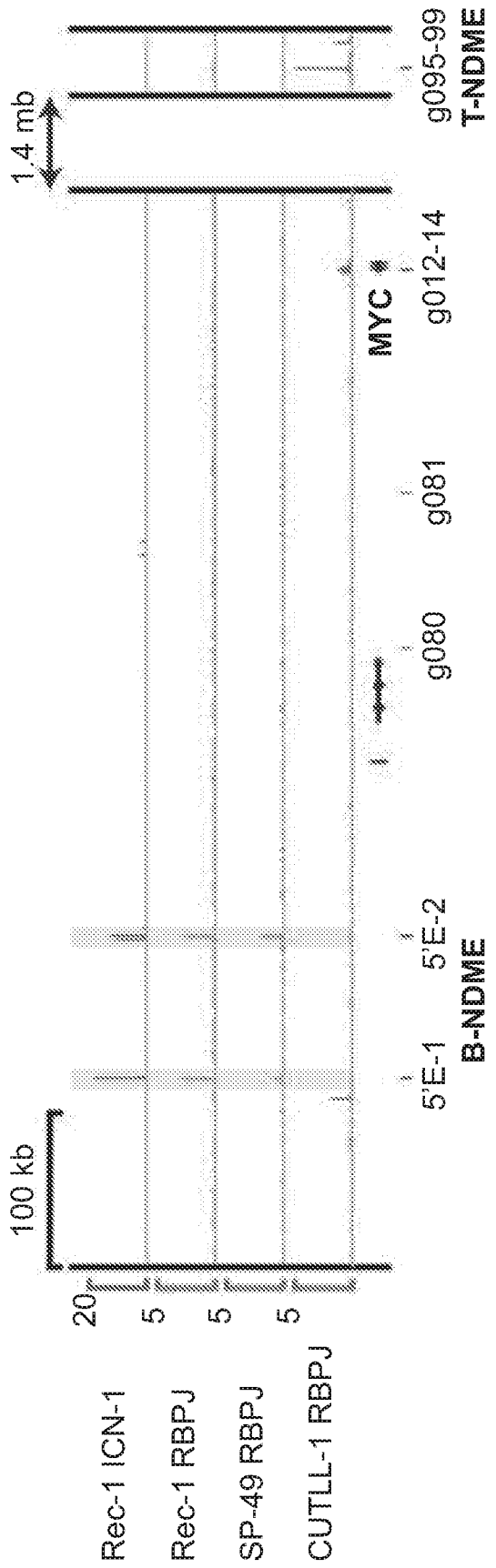

100 kb
1.4 mb
Rec-1 ICN-1
Rec-1 RBPJ
SP-49 RBPJ
CUTLL-1 RBPJ
20
5
5
5
5'E-1
5'E-2
B-NDME
g080
g081
MYC
g012-14
g095-99
T-NDME

FIG. 5A

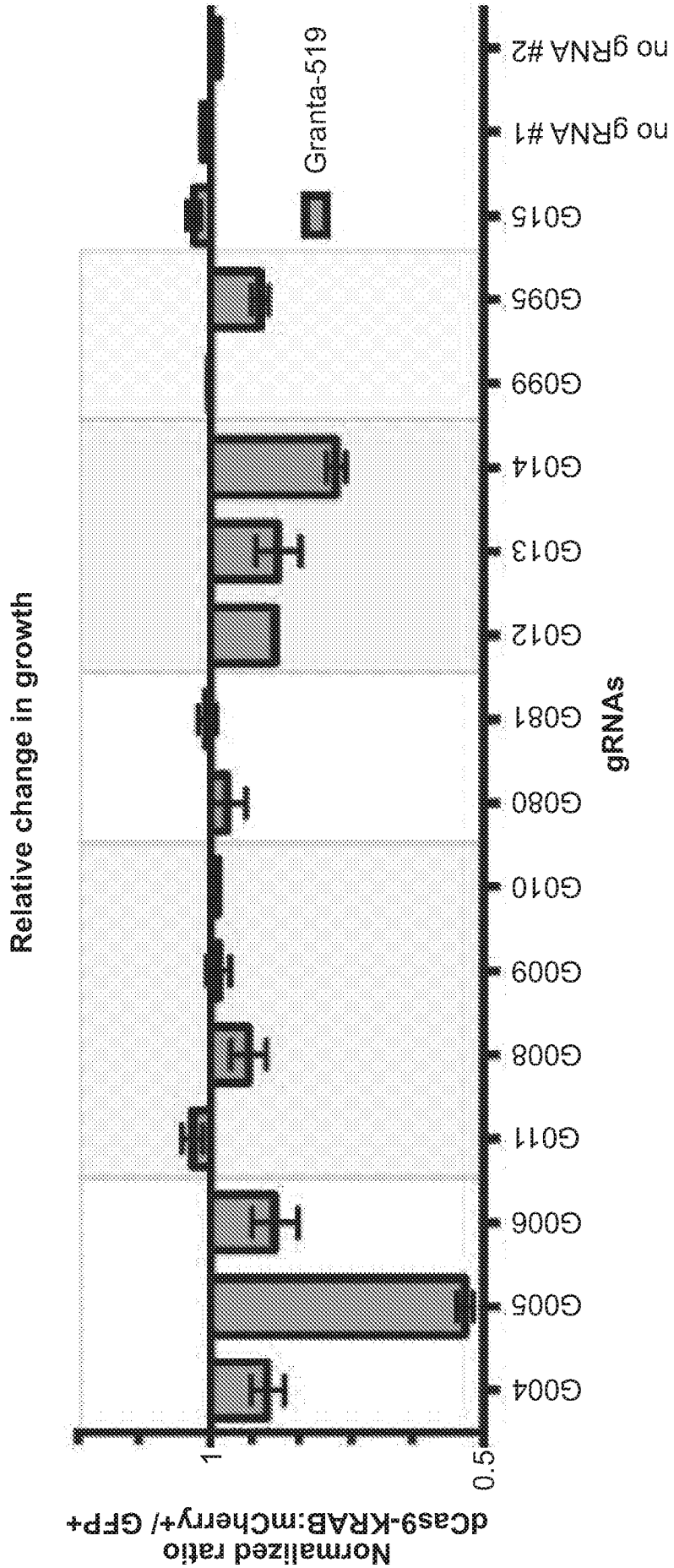

Granta-519
no gRNA #1
no gRNA #2
G015
G095
G099
G014
G013
G012
G081
G080
G010
G009
G008
G011
G006
G005
G004
gRNAs
Relative change in growth
Normalized ratio
dCas9-KRAB:mCherry+/ GFP+
1
0.5

FIG. 5B

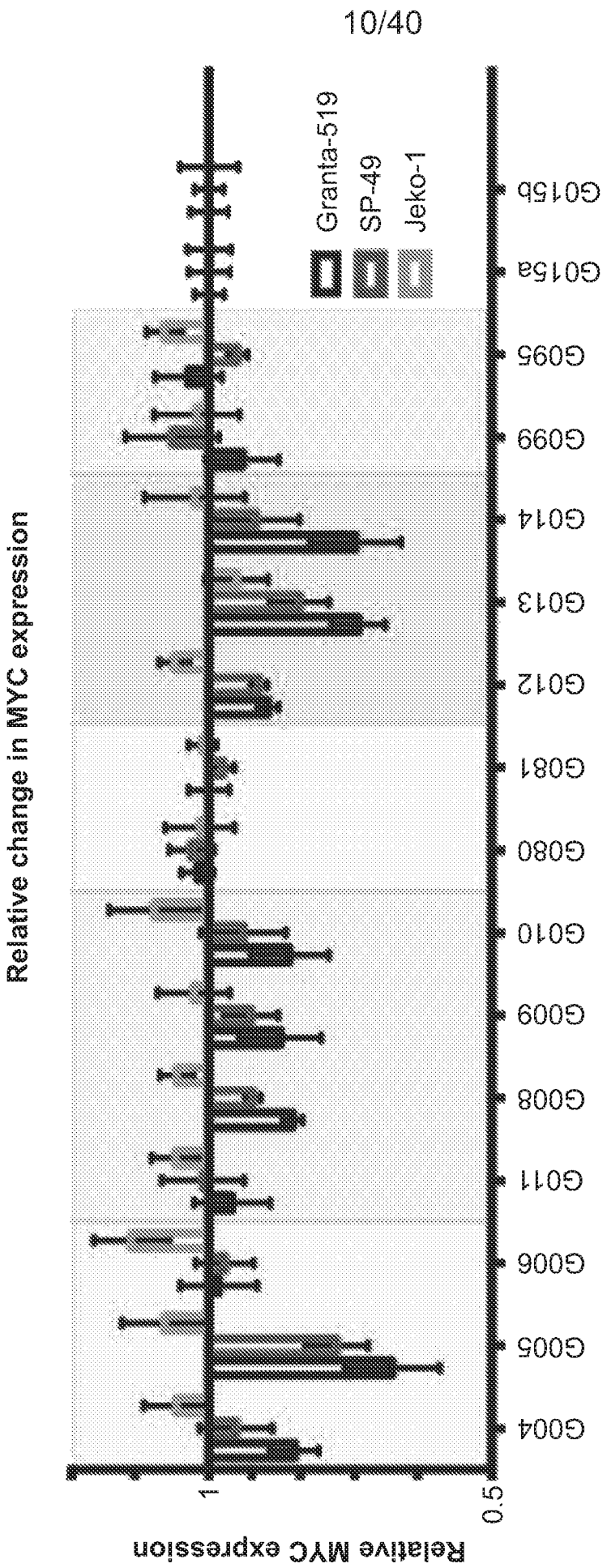

Relative change in MYC expression
Granta-519
SP-49
Jeko-1
G004
G005
G006
G011
G008
G009
G010
G080
G081
G012
G013
G014
G099
G095
G015a
G015b
gRNAs
Relative MYC expression
1
0.5

FIG. 5C

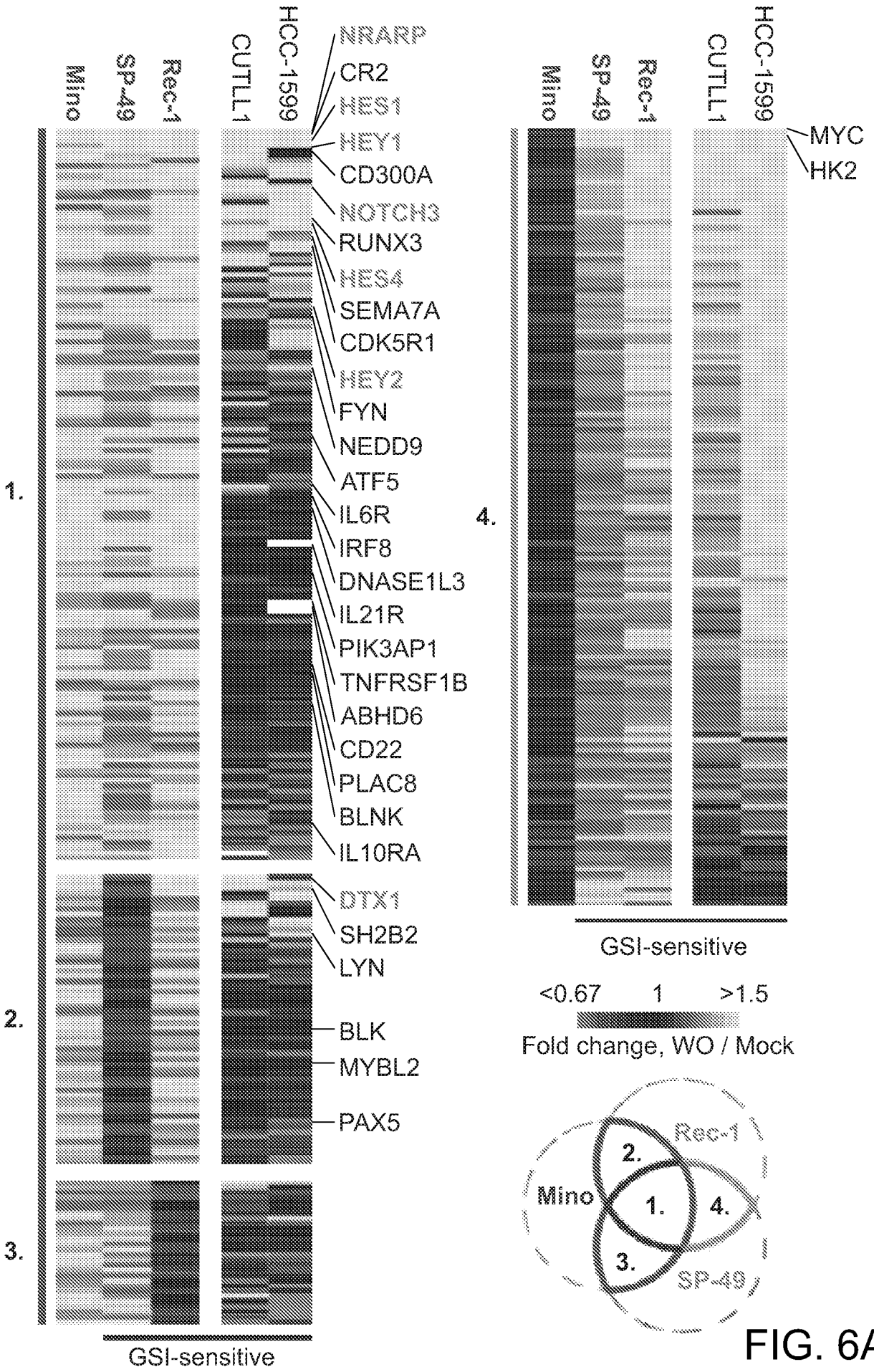

Mino
SP-49
Rec-1
CUTLL1
HCC-1599
NRARP
CR2
HES1
HEY1
CD300A
NOTCH3
RUNX3
HES4
SEMA7A
CDK5R1
HEY2
FYN
NEDD9
ATF5
IL6R
IRF8
DNASE1L3
IL21R
PIK3AP1
TNFRSF1B
ABHD6
CD22
PLAC8
BLNK
IL10RA
DTX1
SH2B2
LYN
BLK
MYBL2
PAX5
1.
2.
3.
4.
GSI-sensitive
MYC
HK2
<0.67
1
>1.5
Fold change, WO / Mock
Rec-1
Mino
SP-49

FIG. 6A

Gene Set	k / K	FDR q-value
H: Regulated by TNF vai NF-kB	**25 / 200**	**2.75e-25**
H: Up-regulated in allograft rejection	**15 / 200**	**1.80e-11**
H: Up-regulated by IL-2 via STAT5	**10 / 200**	**3.43e-06**
H: Up-regulated by IFN-g	**10 / 200**	**3.43e-06**
H: p53 pathway	**10 / 200**	**3.43e-06**
H: Up-regulated by IL6 via STAT3	**7 / 87**	**1.12e-05**
H: Up-regulated by Notch signaling	**5 / 32**	**1.84e-05**
R: Immune system	27 / 933	1.80e-11
R: Cytokine signaling	16 / 270	5.34e-11
R: Signaling by Notch	11 / 103	3.98e-10
R: Signaling by interleukins	8 / 107	3.43e-06
R: Signaling by Rho GTPases	8 / 113	4.59e-06
R: Interferon signaling	9 / 159	4.59e-06
R: Activation of B cell receptor	5 / 29	1.26e-05

FIG. 6B

Gene Set	k / K	FDR q-value
H: Regulated by MYC	**23 / 58**	**2.79e-40**
H: Up-regulated via mTORC1 activation	**15 / 200**	**3.13e-14**
H: Regulated by E2F (cell-cycle related)	**11 / 200**	**8.86e-09**
H: Up-regulated by IL-2 via STAT5	**9 / 200**	**2.05e-06**
H: Up-regulated by unfolded prot. response	**6 / 113**	**1.37e-04**
H: G2 / M checkpoint	**7 / 200**	**2.43e-04**
R: Metabolism of proteins	13 / 518	1.77e-06
R: tRNA aminoacylation	5 / 42	2.34e-05
R: Metabolism of nucleotides	5 / 72	2.43e-04
R: Processing of capped pre-mRNA	6 / 140	3.56e-04

FIG. 6C

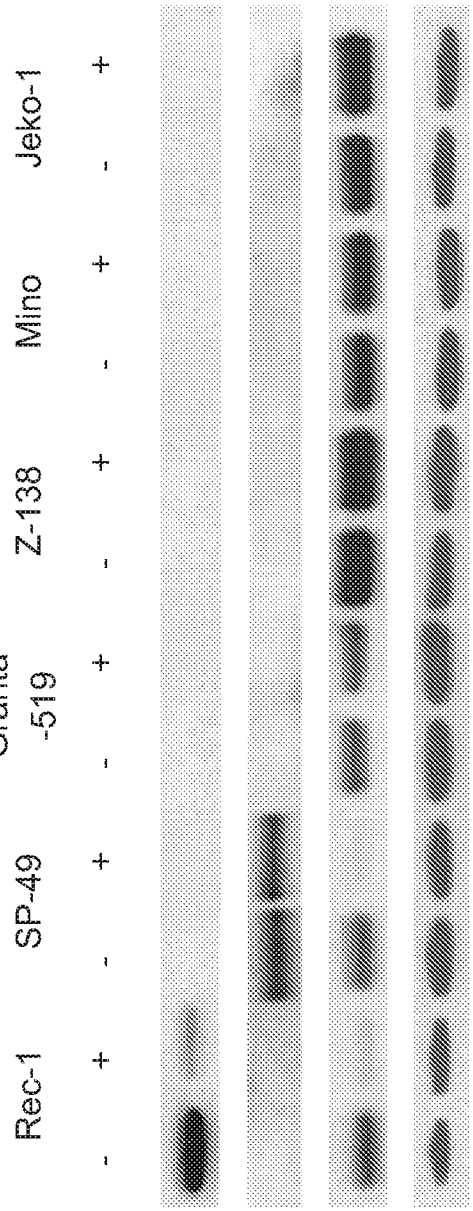

GSI
Rec-1
SP-49
Granta -519
Z-138
Mino
Jeko-1
ICN-1
NOTCH4
c-MYC
TBP

FIG. 6D

SP-49 + pINDUCER-22-MYC clones
Clone 3
Clone 7
Clone 4
Parental SP-49
c-MYC
GSI
Dox

FIG. 6E

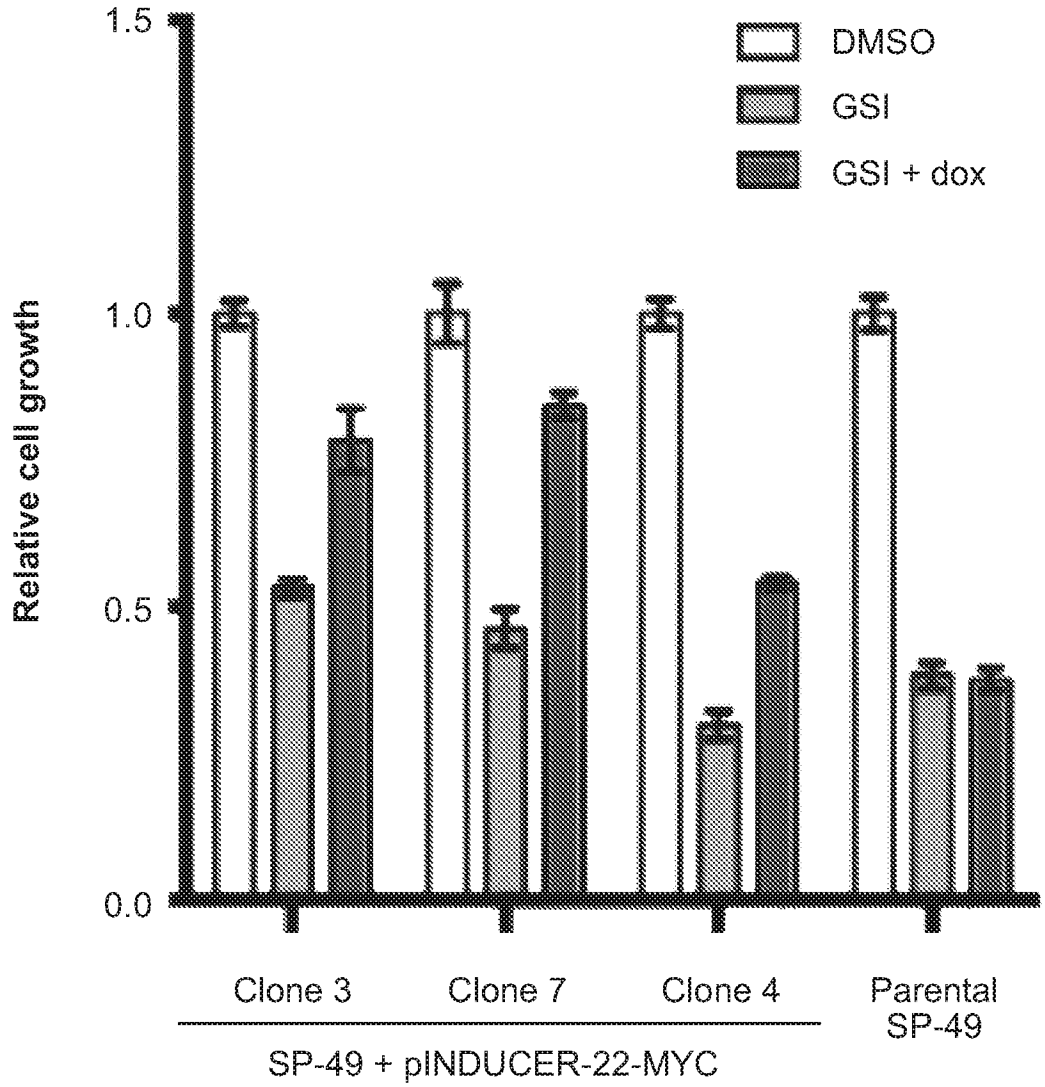

1.5
1.0
0.5
0.0
Relative cell growth
DMSO
GSI
GSI + dox
Clone 3
Clone 7
Clone 4
Parental SP-49
SP-49 + pINDUCER-22-MYC

FIG. 6F

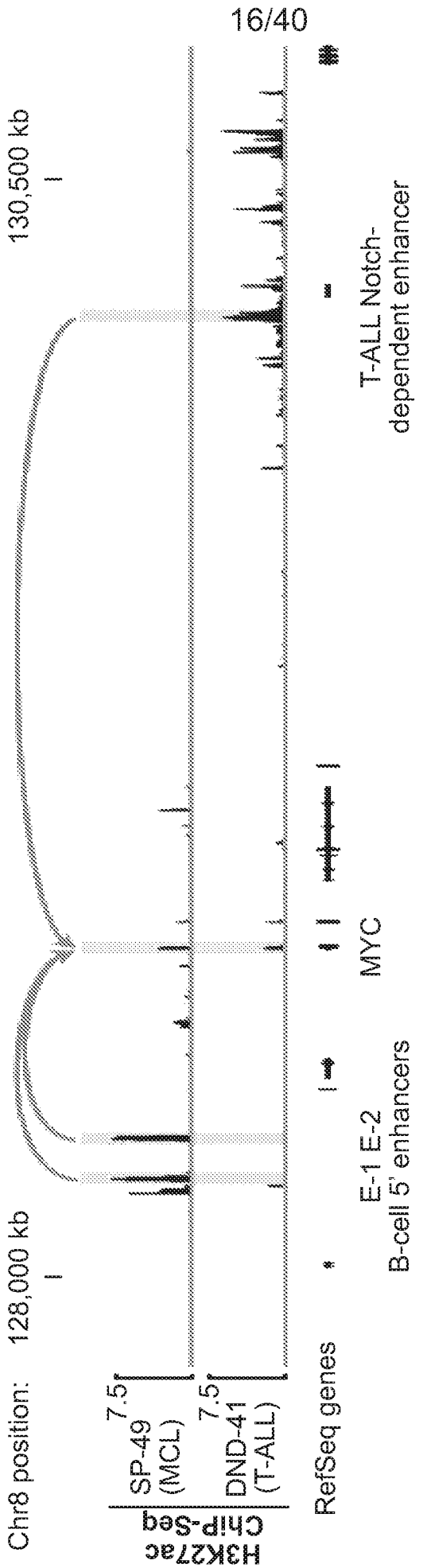

16/40
130,500 kb
Chr8 position: 128,000 kb
H3K27ac ChIP-Seq
SP-49 (MCL)
7.5
DND-41 (T-ALL)
7.5
RefSeq genes
E-1 E-2
B-cell 5' enhancers
MYC
T-ALL Notch-dependent enhancer

FIG. 7A

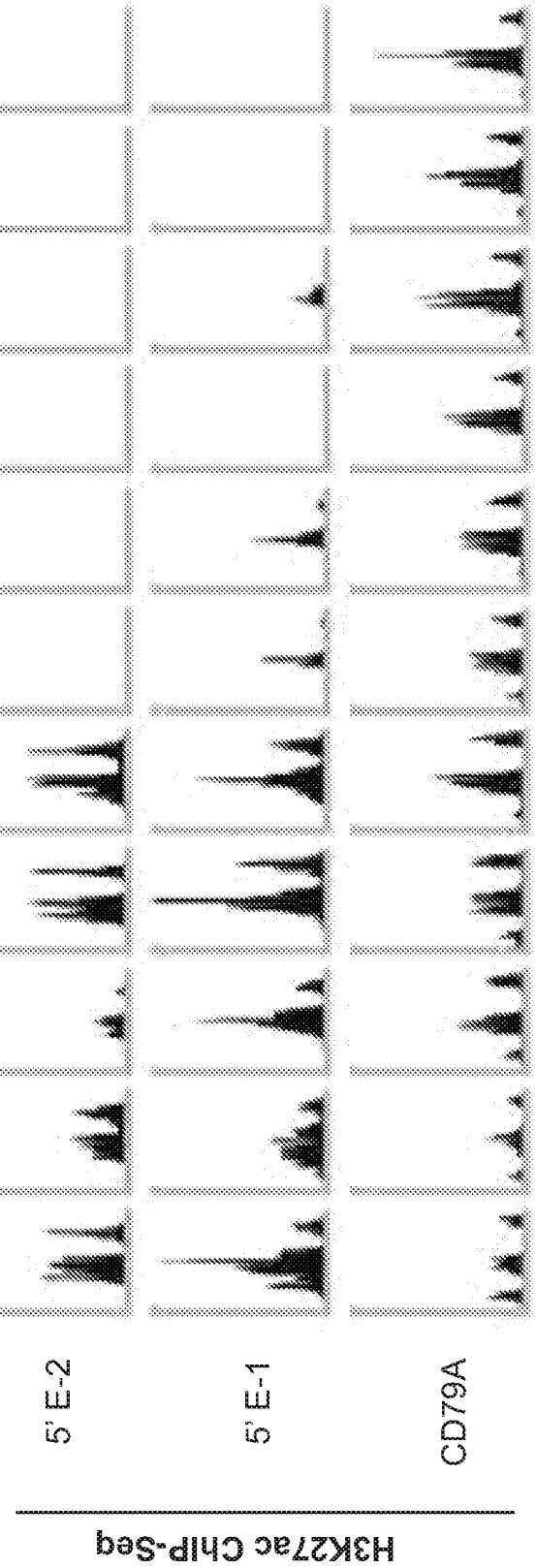

H3K27ac ChIP-Seq
5' E-2
5' E-1
CD79A
Me
Jv
Gr
Re
Sp
Mi
Je
Z1
Ma
Hb
Up
EBV+
Notch-rearranged
MYC-rearranged

FIG. 7B

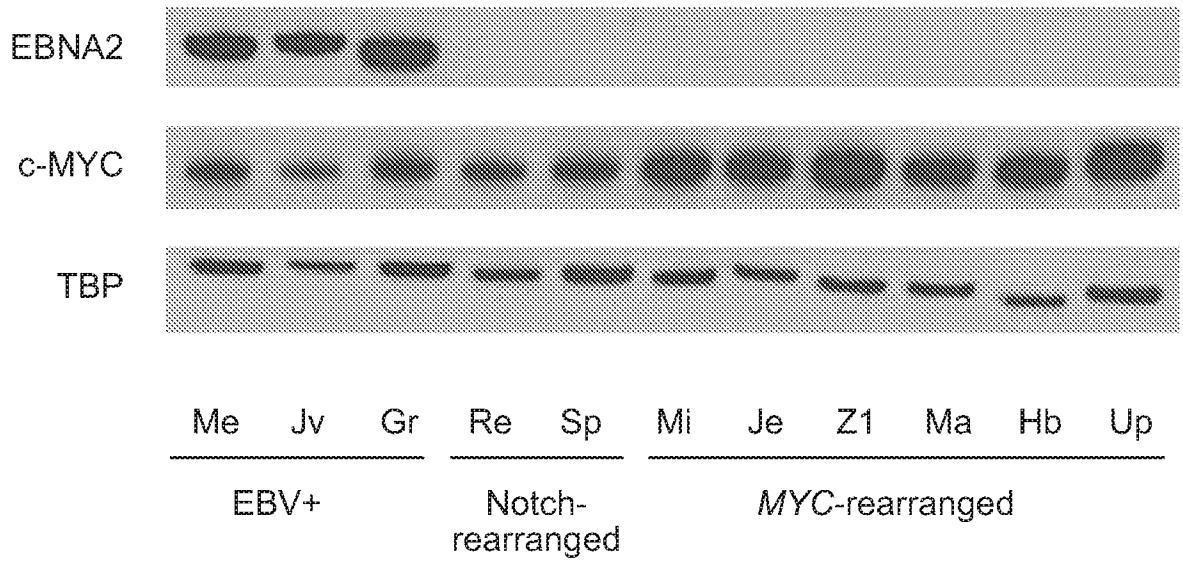

EBNA2
c-MYC
TBP
Me
Jv
Gr
Re
Sp
Mi
Je
Z1
Ma
Hb
Up
EBV+
Notch-rearranged
MYC-rearranged

FIG. 7C

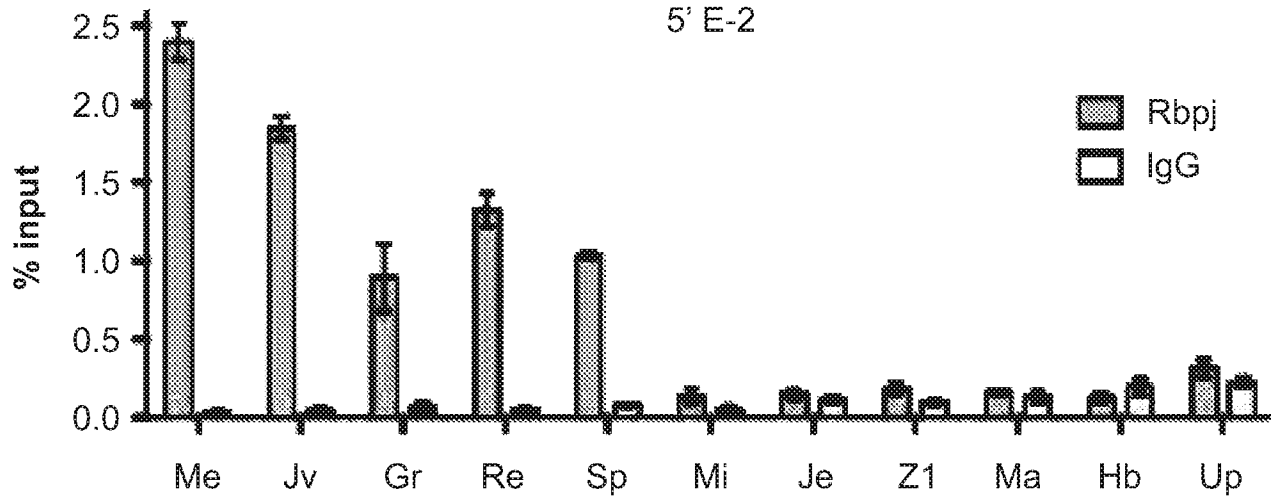

5' E-2
% input
2.5
2.0
1.5
1.0
0.5
0.0
Rbpj
IgG
Me
Jv
Gr
Re
Sp
Mi
Je
Z1
Ma
Hb
Up

FIG. 7D

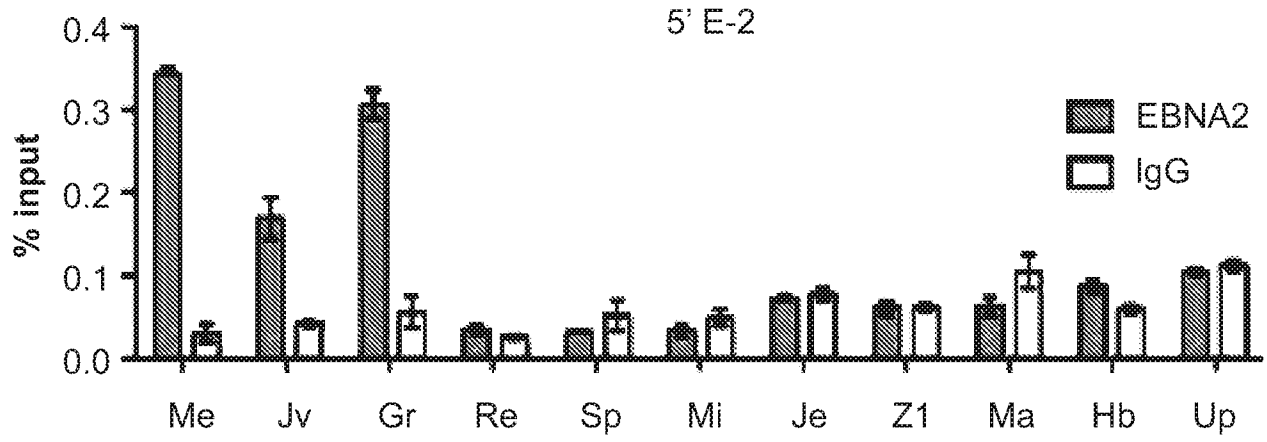

5' E-2
% input
0.4
0.3
0.2
0.1
0.0
EBNA2
IgG
Me
Jv
Gr
Re
Sp
Mi
Je
Z1
Ma
Hb
Up

FIG. 7E

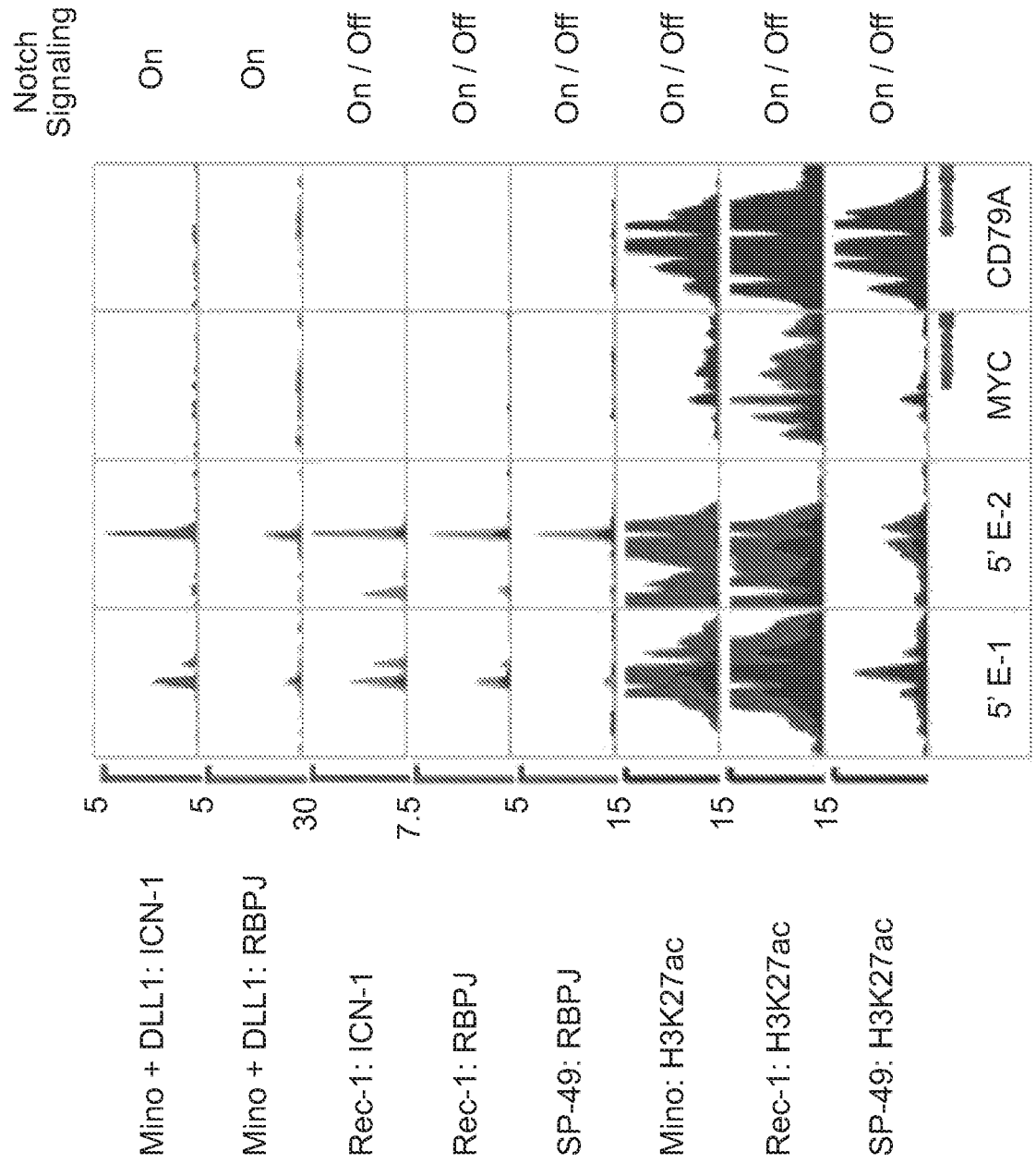

Notch Signaling
On
On
On / Off
On / Off
On / Off
On / Off
On / Off
On / Off
5
5
30
7.5
5
15
15
15
Mino + DLL1: ICN-1
Mino + DLL1: RBPJ
Rec-1: ICN-1
Rec-1: RBPJ
SP-49: RBPJ
Mino: H3K27ac
Rec-1: H3K27ac
SP-49: H3K27ac
5' E-1
5' E-2
MYC
CD79A

FIG. 8A

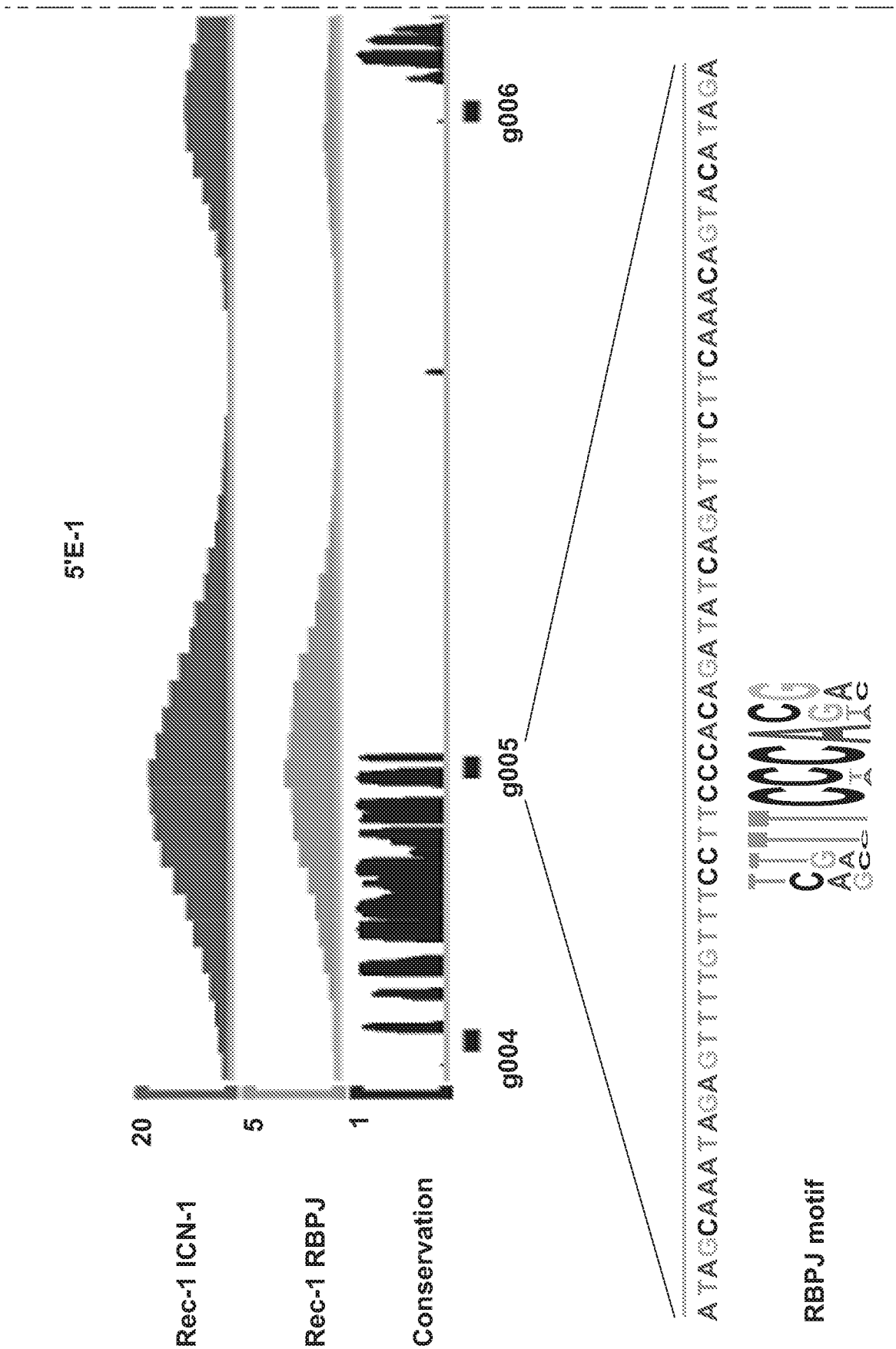
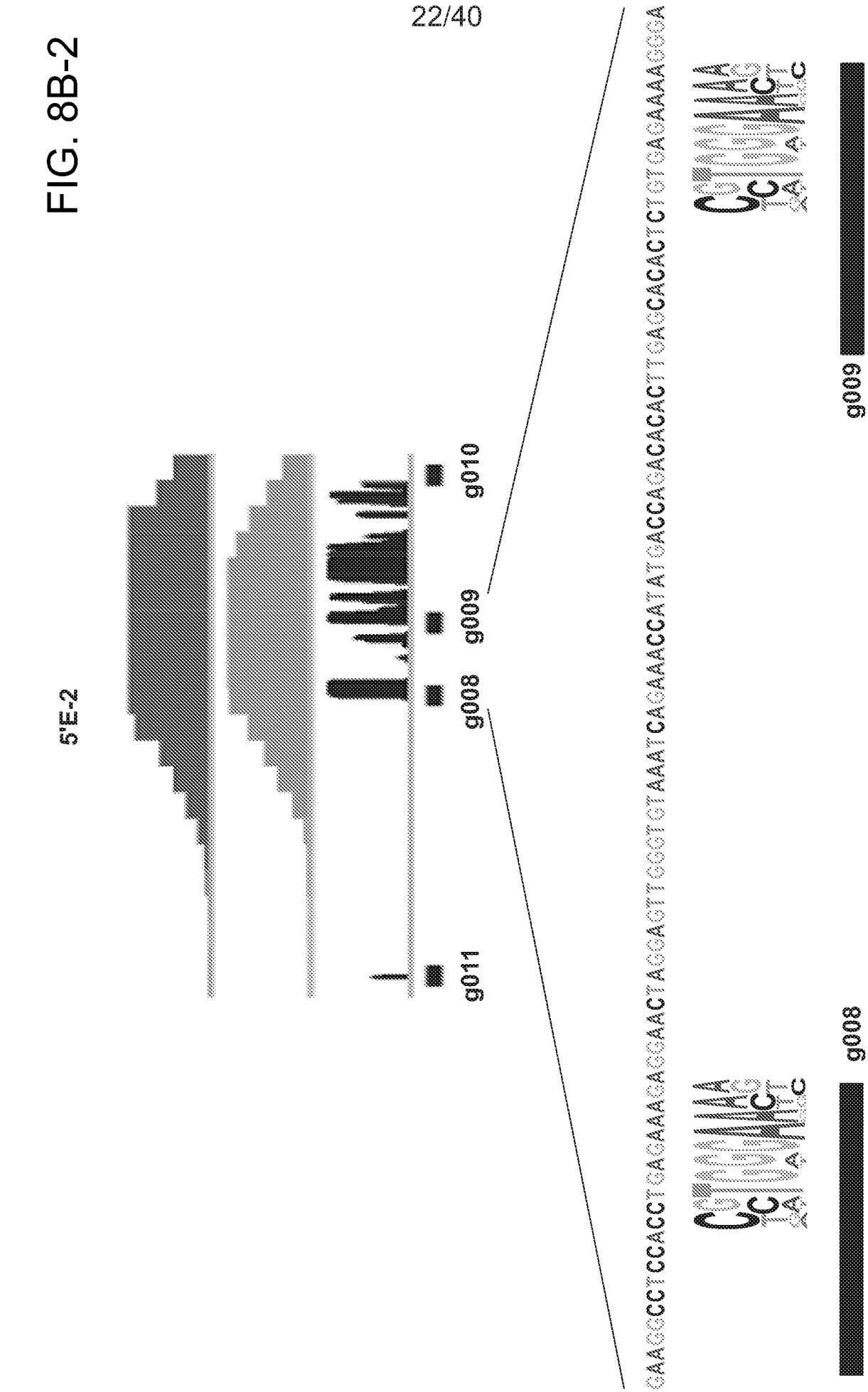

5'E-1
Rec-1 ICN-1
Rec-1 RBPJ
Conservation
20
5
1
g004
g005
g006
ATAGCAAATAGAGTTTTGTTTTCCTTCCCACACATATCAGATTTTCTTCAAACAGTACATAGA
RBPJ motif
g005

FIG. 8B-1

FIG. 8B-2


5'E-2
g008
g009
g010
g011
CAAGCCTCCACCTCAGAAACAGCAACTAGCAGTTGGGTGTAAATCAGAAACCATATCACCAGACACACTTCAGCACACTCTGTCAGAAAAGGGA
g008
g009

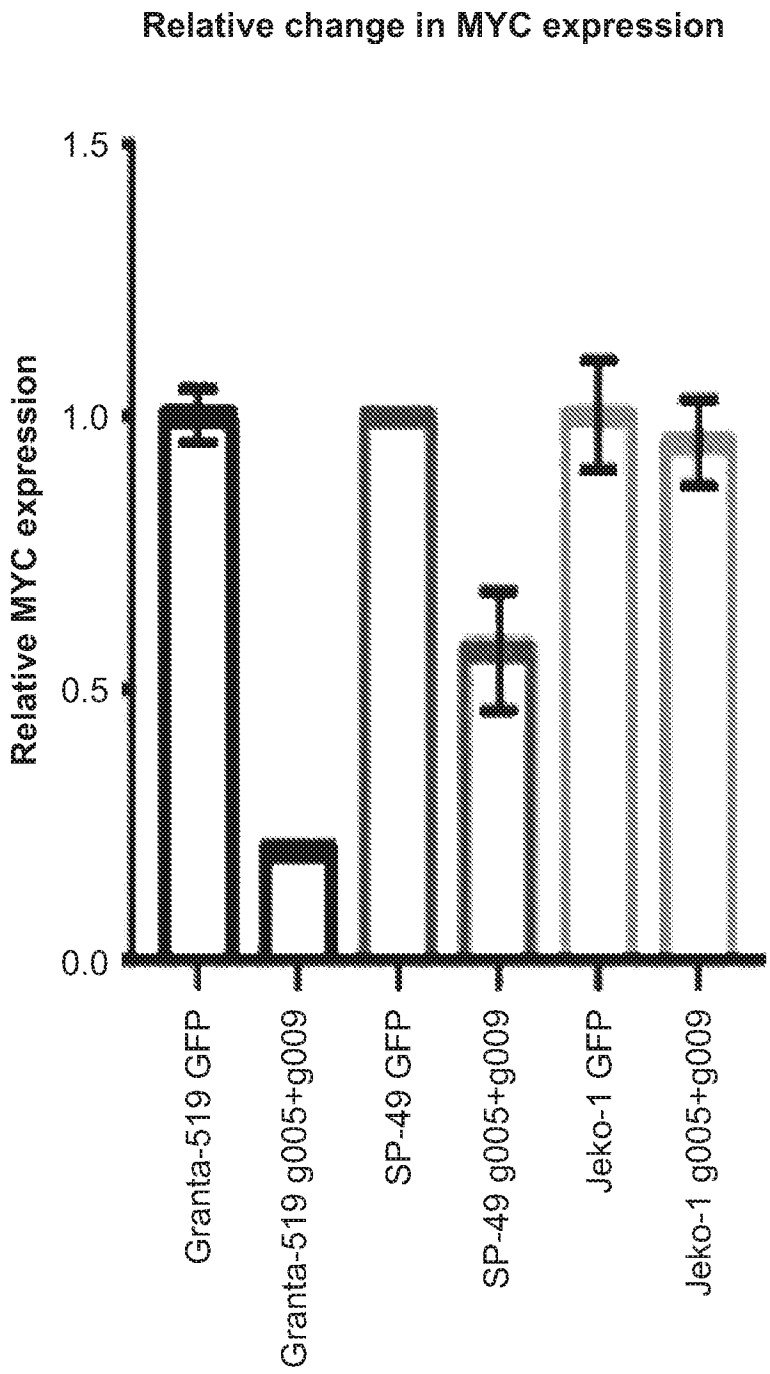

Relative change in MYC expression
Relative MYC expression
1.5
1.0
0.5
0.0
Granta-519 GFP
Granta-519 g005+g009
SP-49 GFP
SP-49 g005+g009
Jeko-1 GFP
Jeko-1 g005+g009

FIG. 8C

FIG. 8D

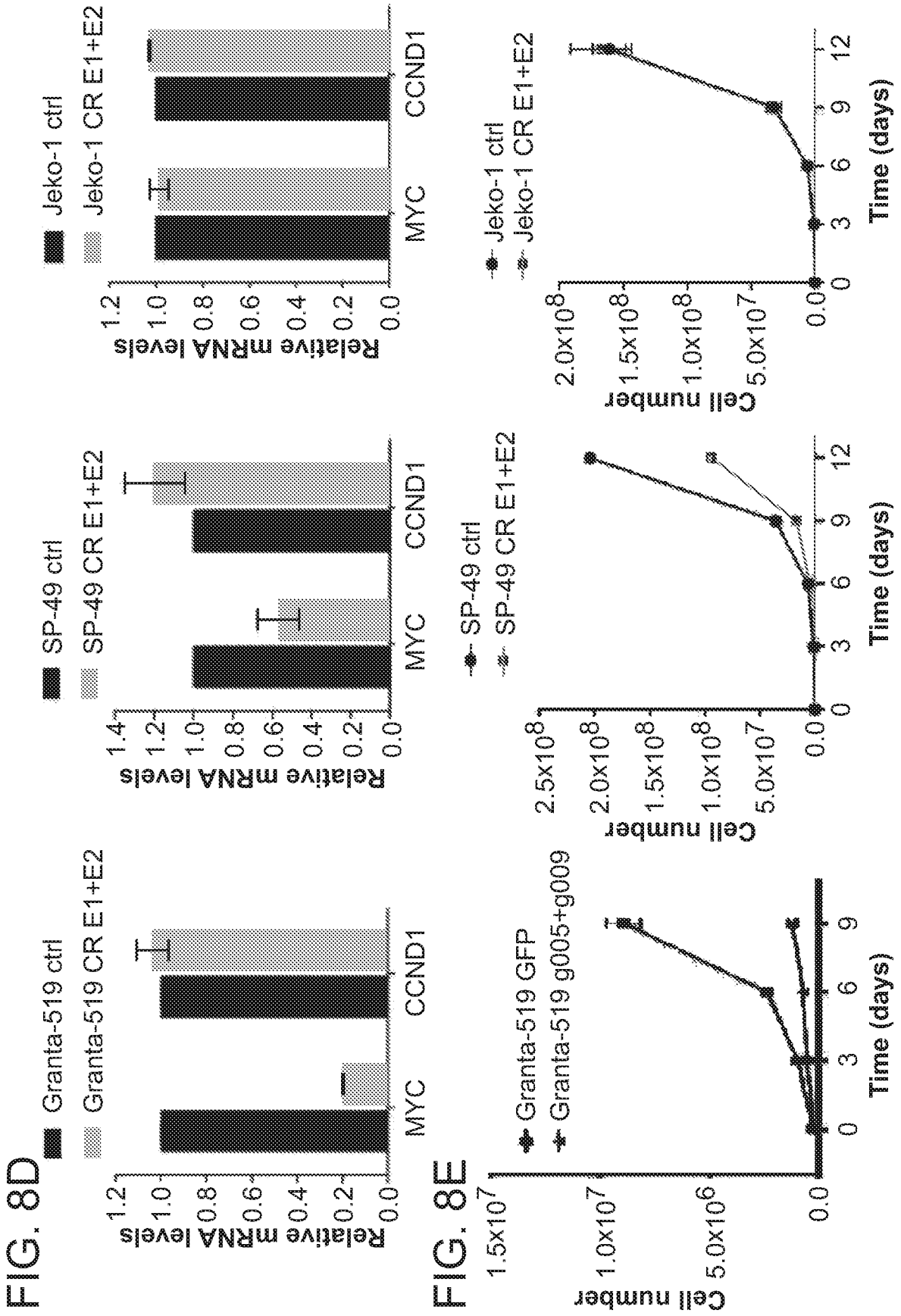

Granta-519 ctrl
Granta-519 CR E1+E2
Relative mRNA levels
1.2
1.0
0.8
0.6
0.4
0.2
0.0
MYC
CCND1
SP-49 ctrl
SP-49 CR E1+E2
1.4
Jeko-1 ctrl
Jeko-1 CR E1+E2
Granta-519 GFP
Granta-519 g005+g009
Cell number
2.5x10^8
2.0x10^8
1.5x10^8
1.0x10^8
5.0x10^7
1.5x10^7
1.0x10^7
5.0x10^6
0.0
0
3
6
9
12
Time (days)

FIG. 8E

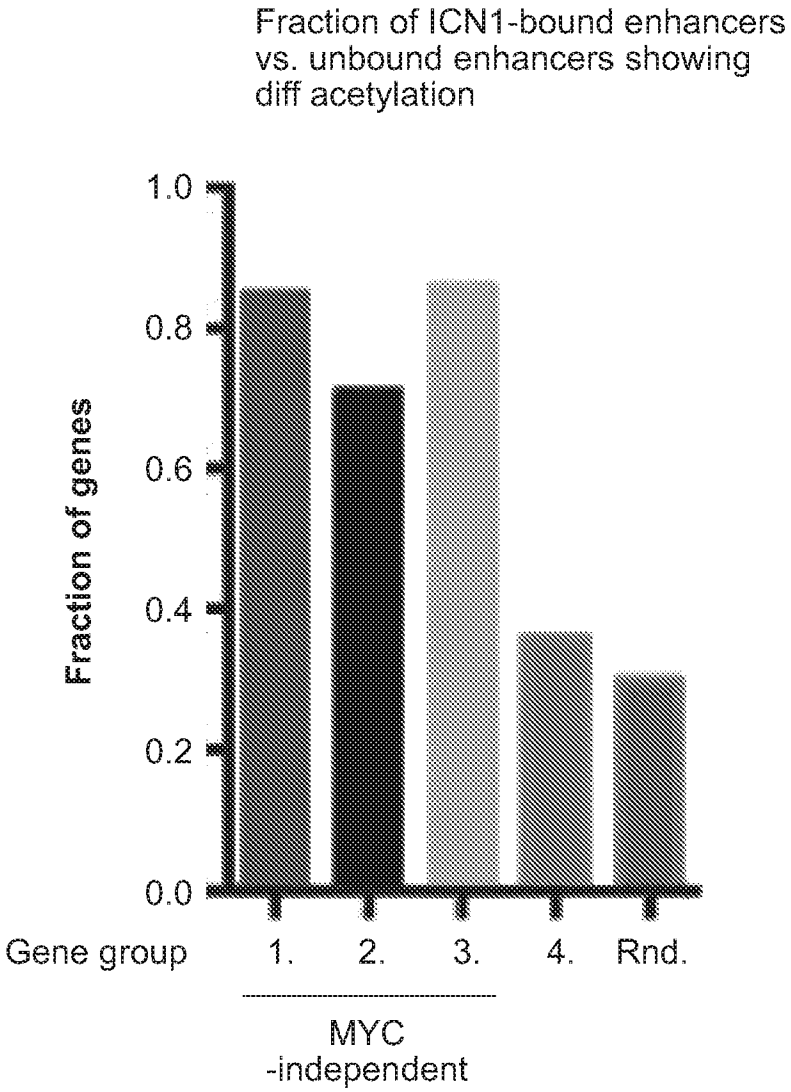

Fraction of ICN1-bound enhancers vs. unbound enhancers showing diff acetylation
Fraction of genes
1.0
0.8
0.6
0.4
0.2
0.0
Gene group
1.
2.
3.
4.
Rnd.
MYC -independent

FIG. 9A

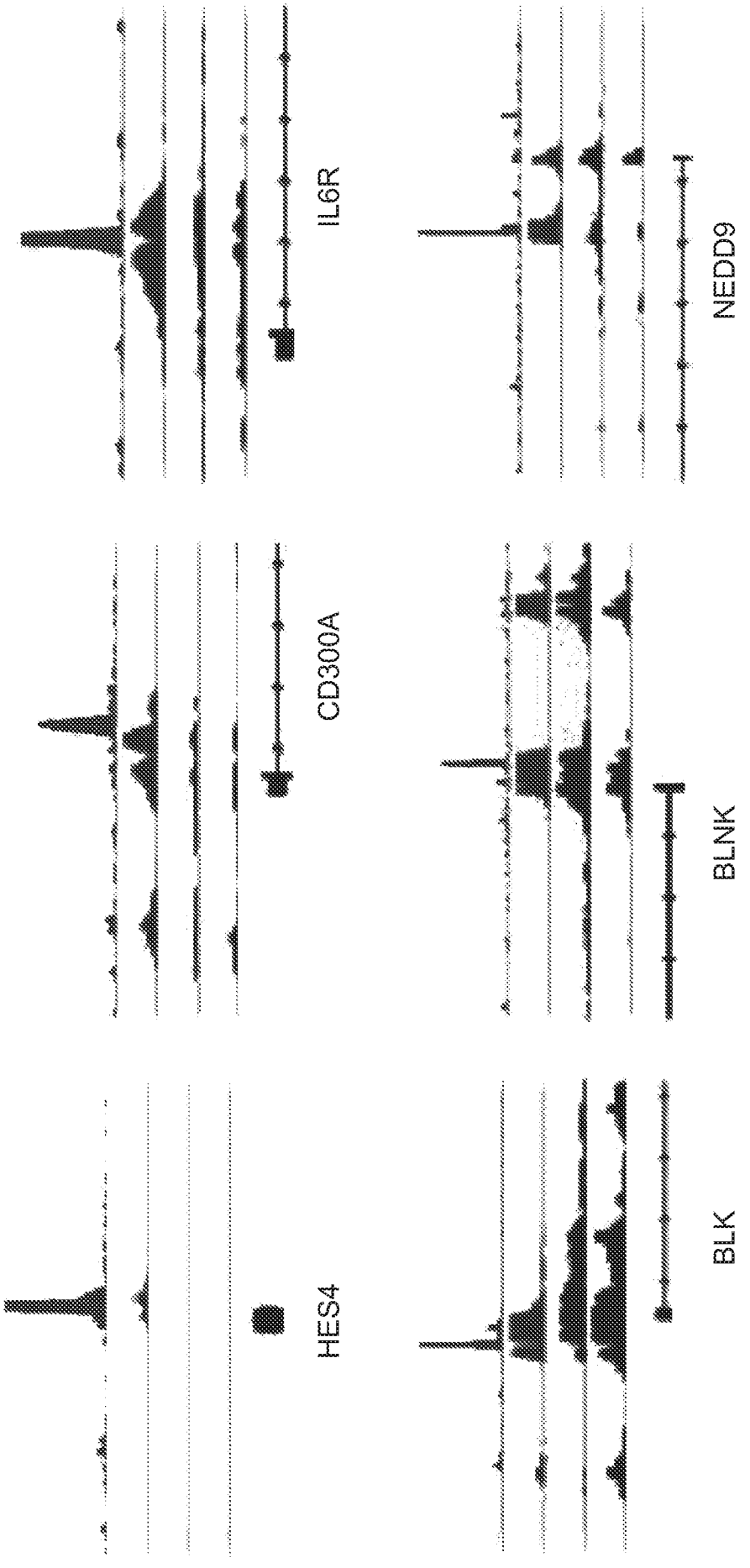


FIG. 9B

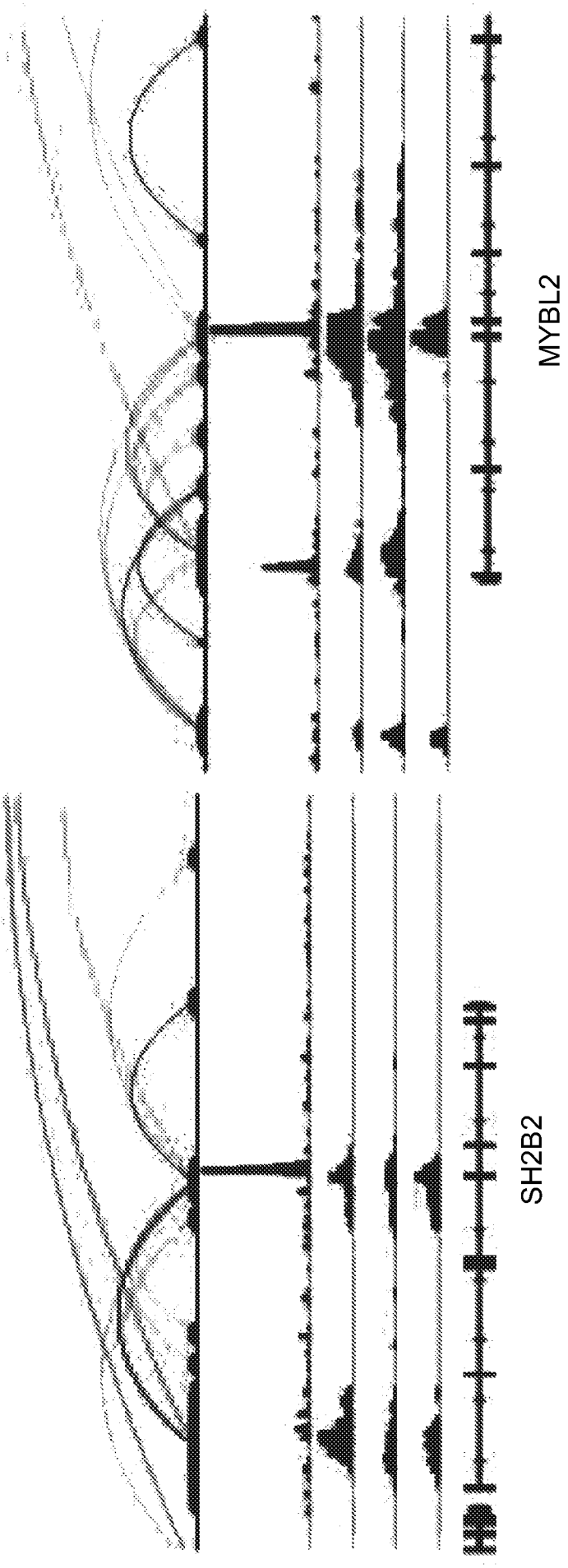


FIG. 9C-1

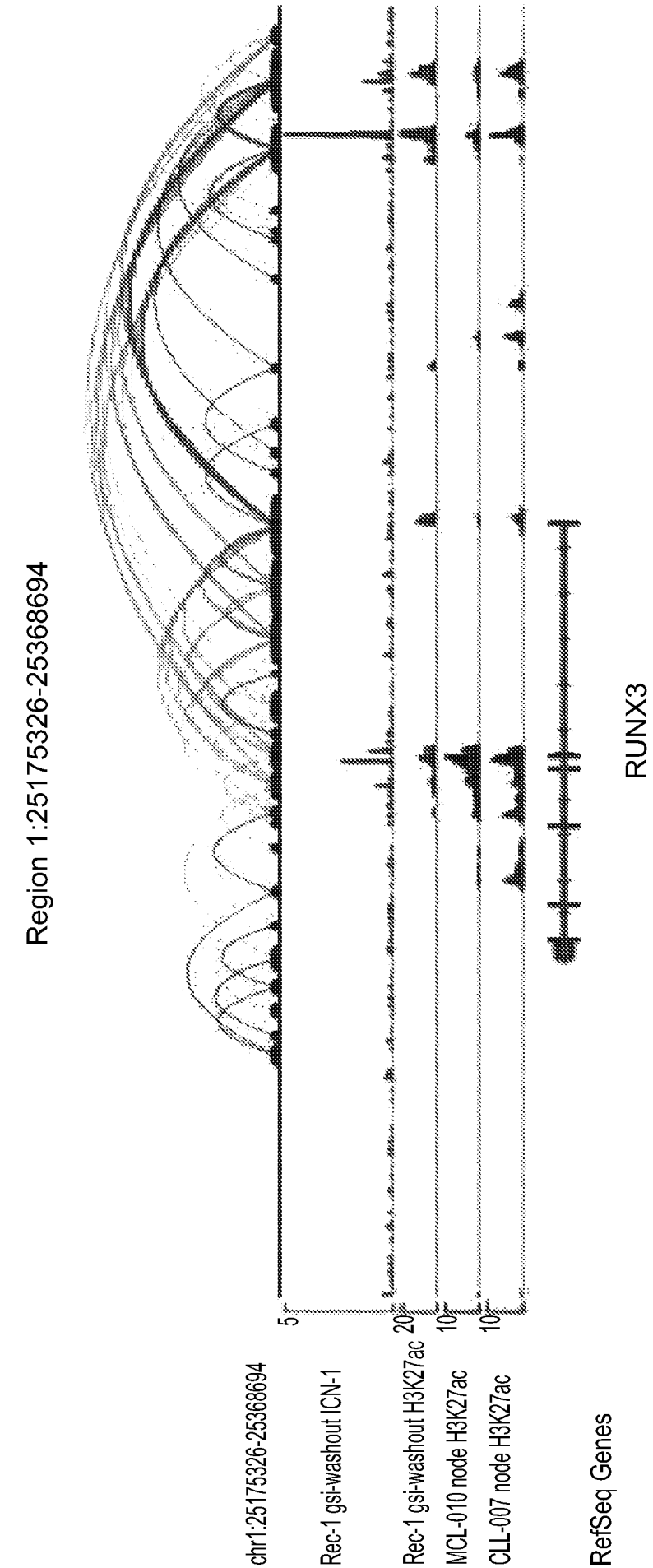

Region 1:25175326-25368694
chr1:25175326-25368694
Rec-1 gsi-washout ICN-1
Rec-1 gsi-washout H3K27ac
MCL-010 node H3K27ac
CLL-007 node H3K27ac
RefSeq Genes
5
20
10
10
RUNX3

FIG. 9C-2

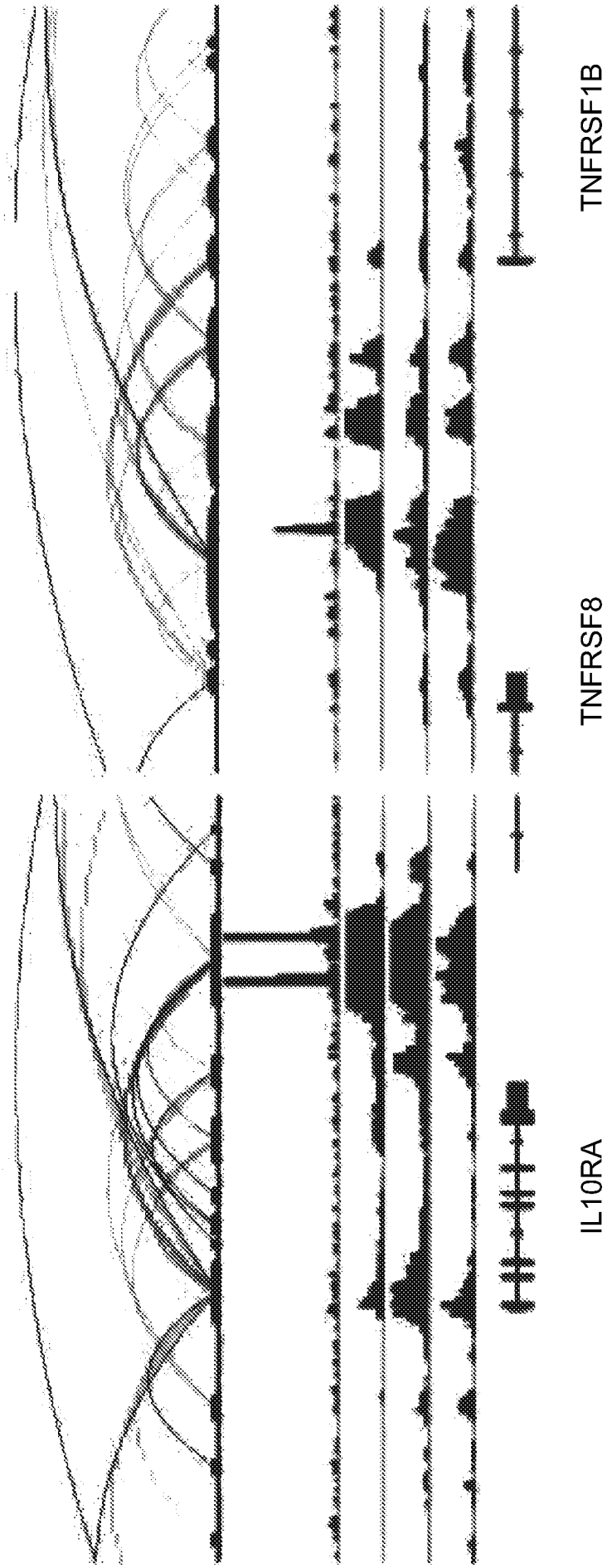


FIG. 9C-3

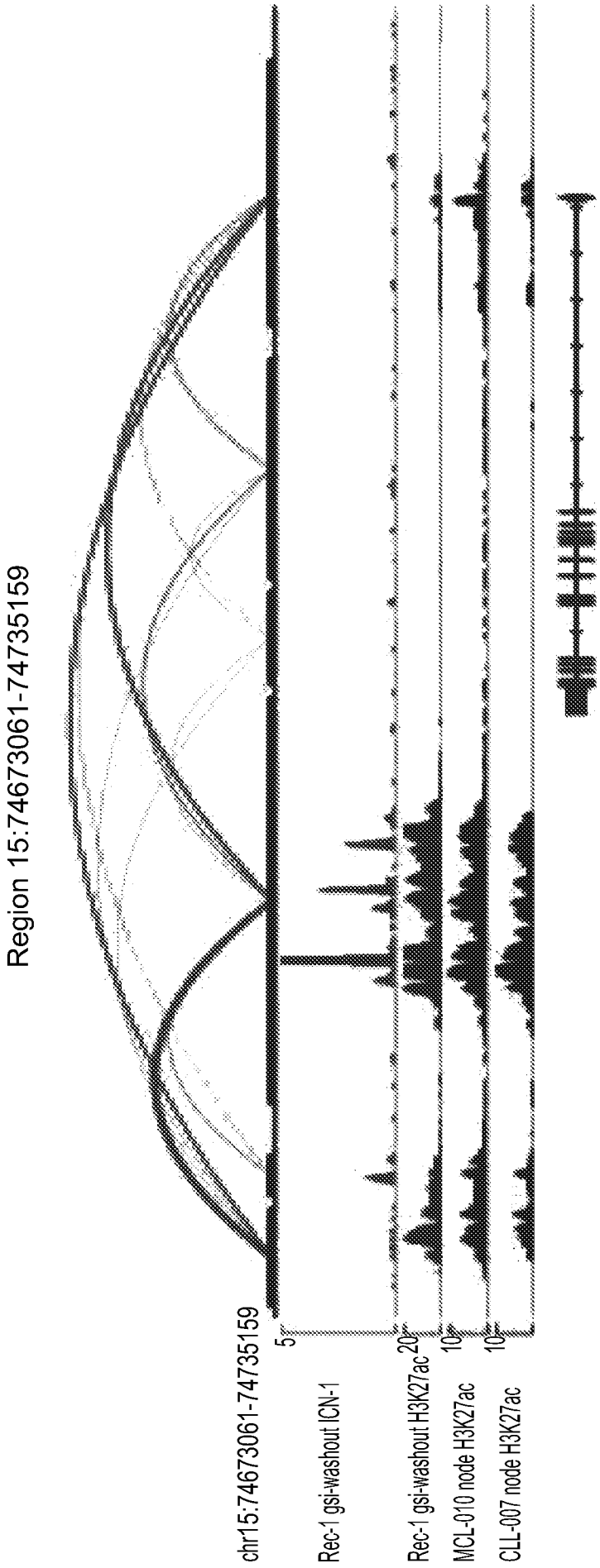

Region 15:74673061-74735159
chr15:74673061-74735159
5
Rec-1 gsi-washout ICN-1
20
Rec-1 gsi-washout H3K27ac
10
MCL-010 node H3K27ac
10
CLL-007 node H3K27ac
RefSeq Genes
SEMA7A

FIG. 9C-4

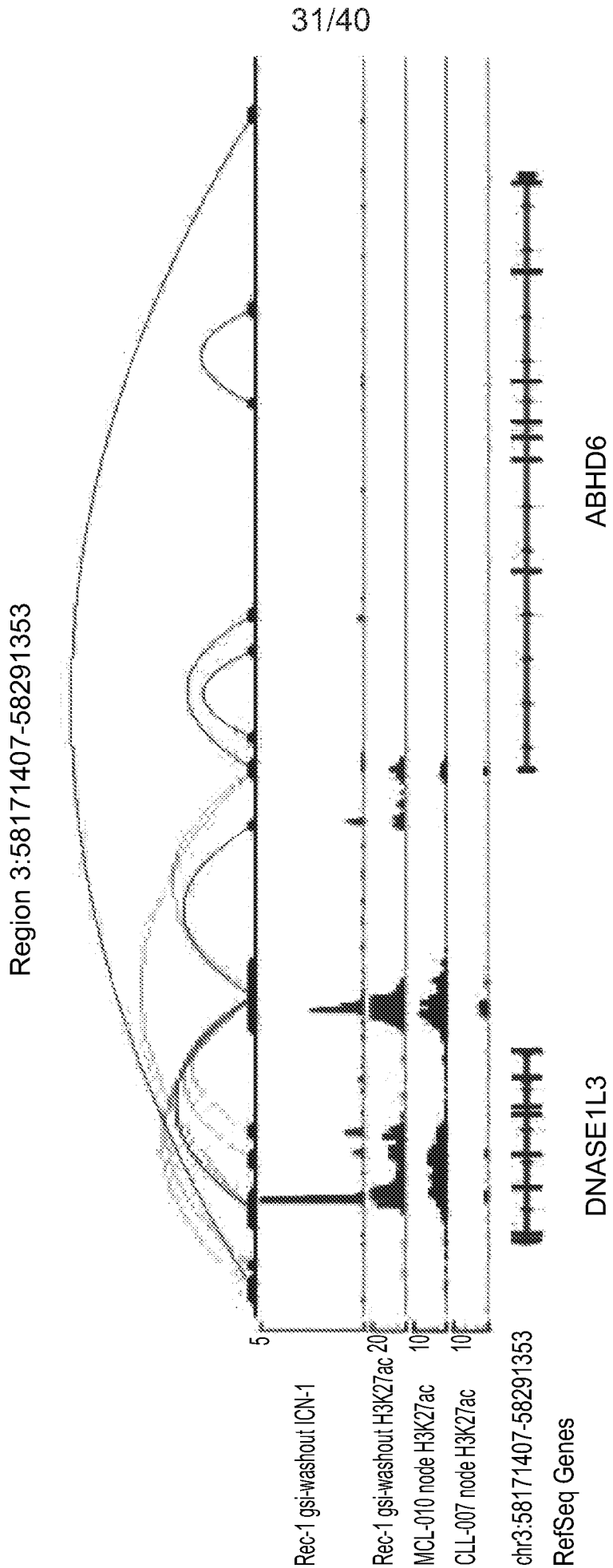

Region 3:58171407-58291353
Rec-1 gsi-washout ICN-1
Rec-1 gsi-washout H3K27ac
MCL-010 node H3K27ac
CLL-007 node H3K27ac
chr3:58171407-58291353
RefSeq Genes
DNASE1L3
ABHD6
5
20
10
10

FIG. 9C-5

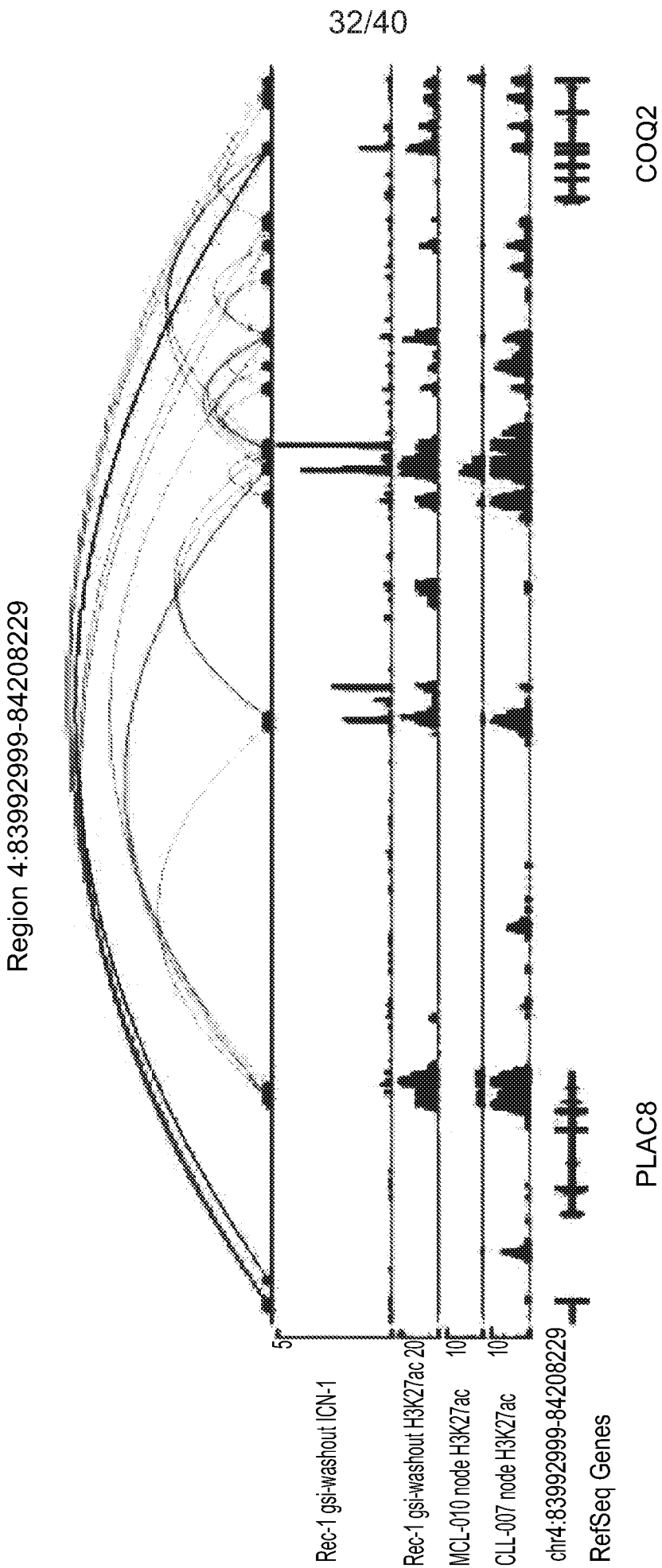

Region 4:83992999-84208229
Rec-1 gsi-washout ICN-1
Rec-1 gsi-washout H3K27ac
MCL-010 node H3K27ac
CLL-007 node H3K27ac
chr4:83992999-84208229
RefSeq Genes
PLAC8
COQ2
5
20
10
10

FIG. 9C-6

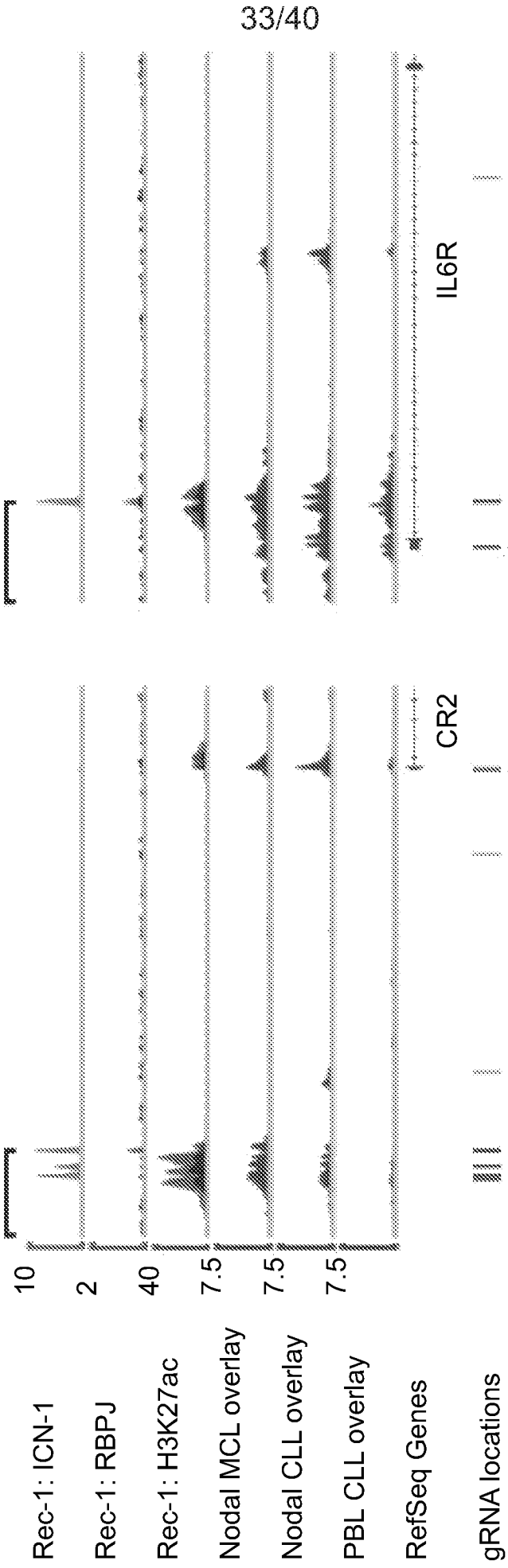

5 kb
5 kb
Rec-1: ICN-1
Rec-1: RBPJ
Rec-1: H3K27ac
Nodal MCL overlay
Nodal CLL overlay
PBL CLL overlay
RefSeq Genes
gRNA locations
10
2
40
7.5
7.5
7.5
CR2
IL6R

FIG. 9D

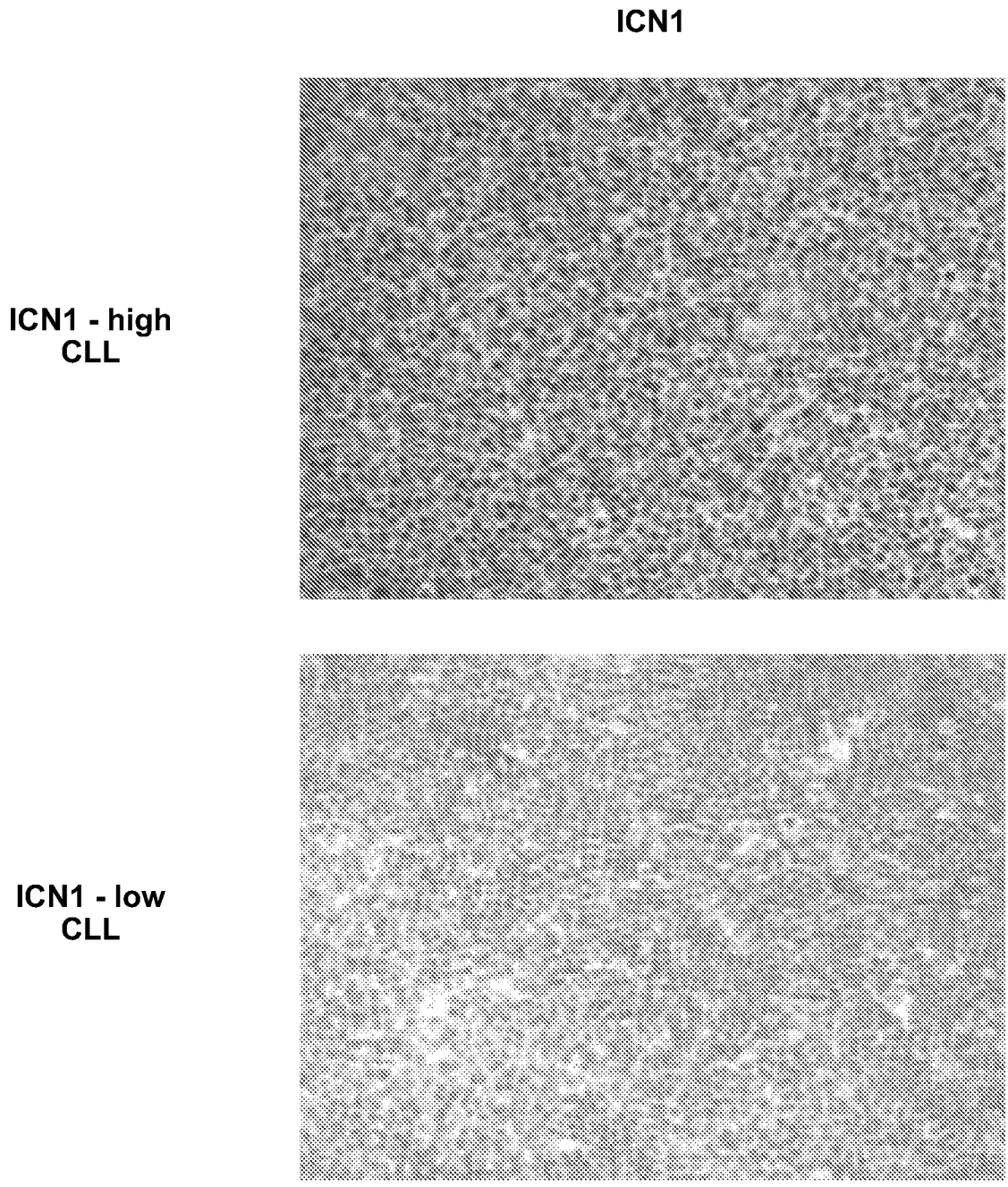


FIG. 10A

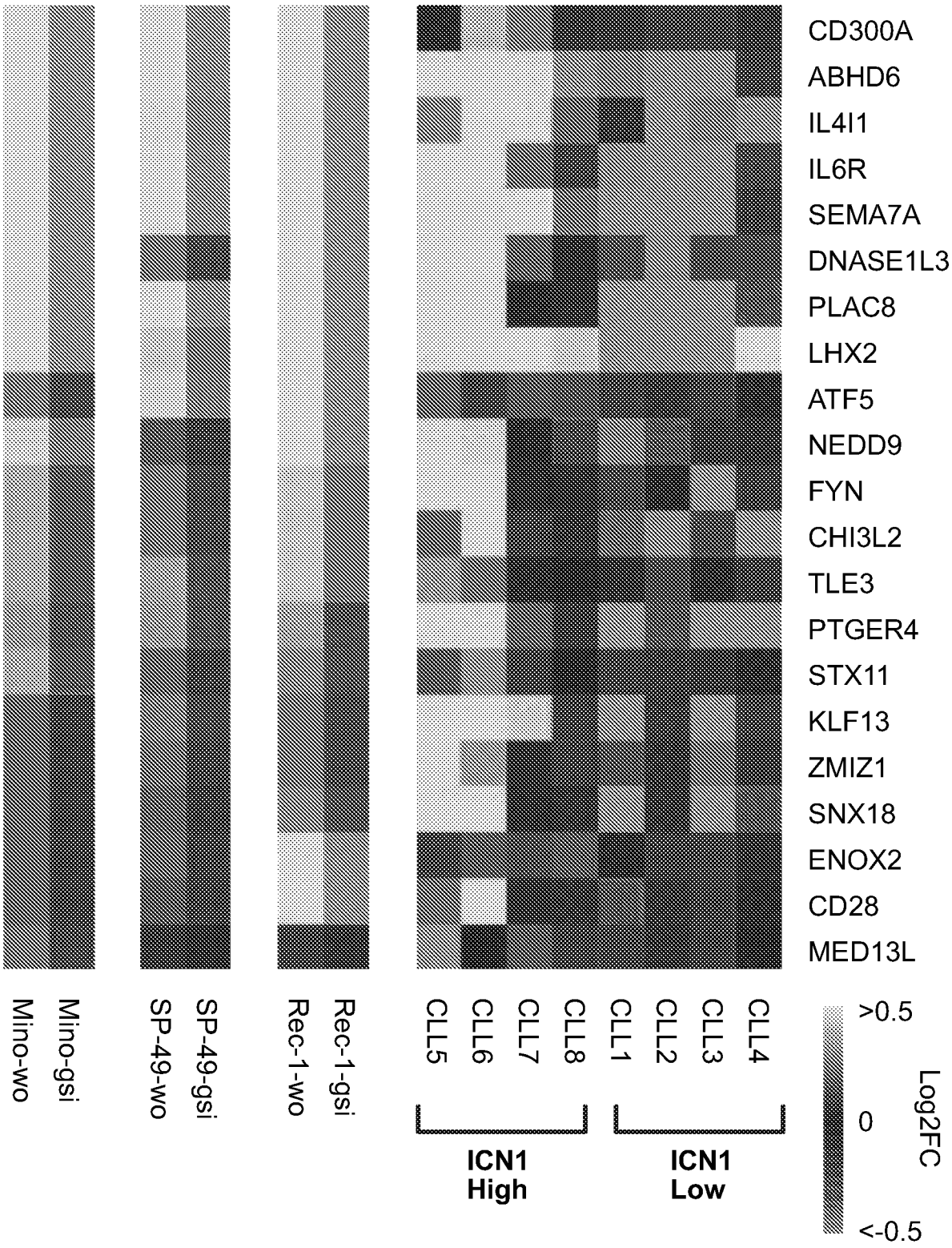

CD300A
ABHD6
IL4I1
IL6R
SEMA7A
DNASE1L3
PLAC8
LHX2
ATF5
NEDD9
FYN
CHI3L2
TLE3
PTGER4
STX11
KLF13
ZMIZ1
SNX18
ENOX2
CD28
MED13L
Mino-wo
Mino-gsi
SP-49-wo
SP-49-gsi
Rec-1-wo
Rec-1-gsi
CLL5
CLL6
CLL7
CLL8
CLL1
CLL2
CLL3
CLL4
ICN1 High
ICN1 Low
>0.5
0
<-0.5
Log2FC

FIG. 10B

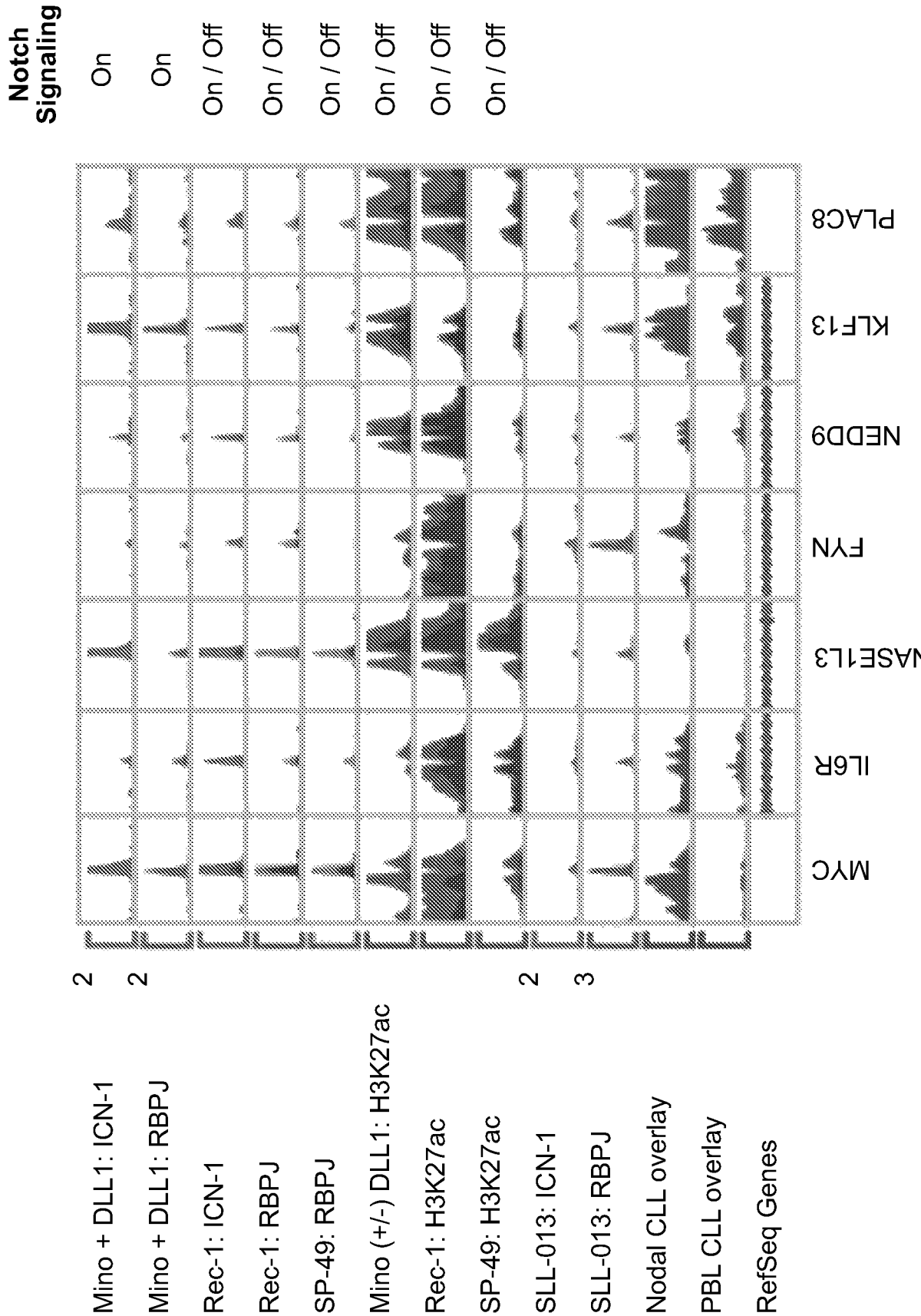

Notch Signaling
On
On
On / Off
On / Off
On / Off
On / Off
On / Off
On / Off
MYC
IL6R
DNASE1L3
FYN
NEDD9
KLF13
PLAC8
2
2
2
3
Mino + DLL1: ICN-1
Mino + DLL1: RBPJ
Rec-1: ICN-1
Rec-1: RBPJ
SP-49: RBPJ
Mino (+/-) DLL1: H3K27ac
Rec-1: H3K27ac
SP-49: H3K27ac
SLL-013: ICN-1
SLL-013: RBPJ
Nodal CLL overlay
PBL CLL overlay
RefSeq Genes

FIG. 10C

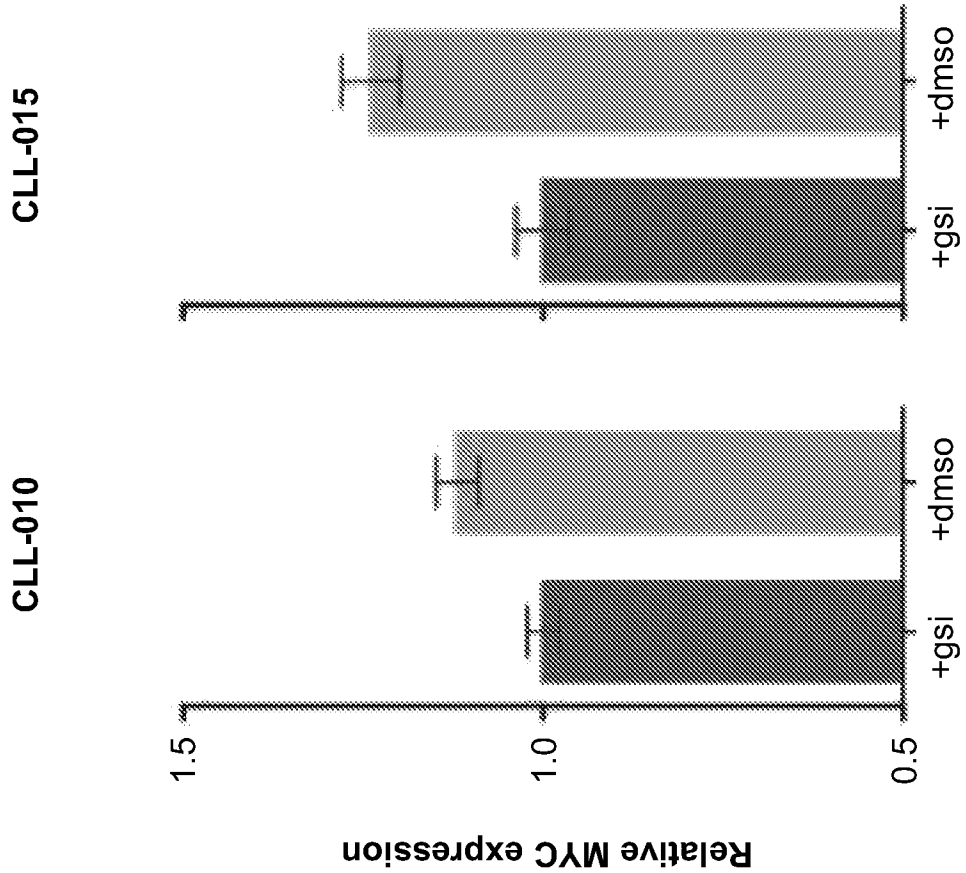


FIG. 10E

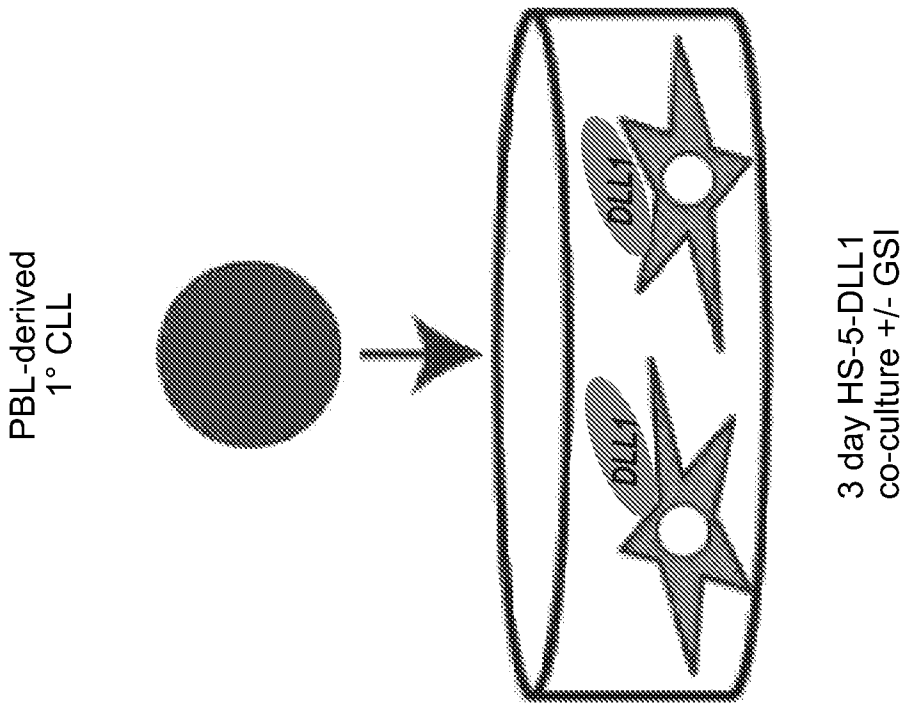


FIG. 10D

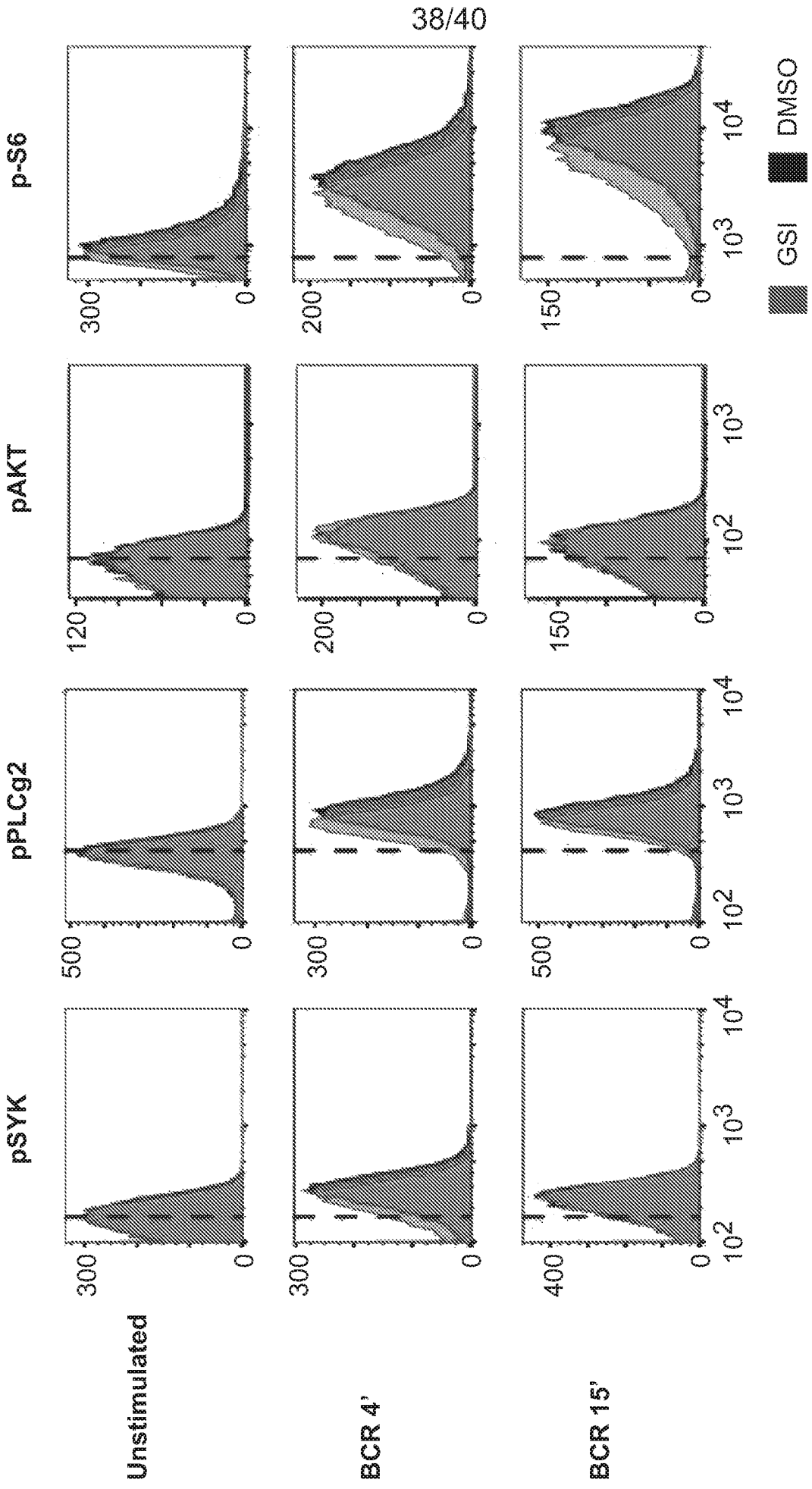


FIG. 10F

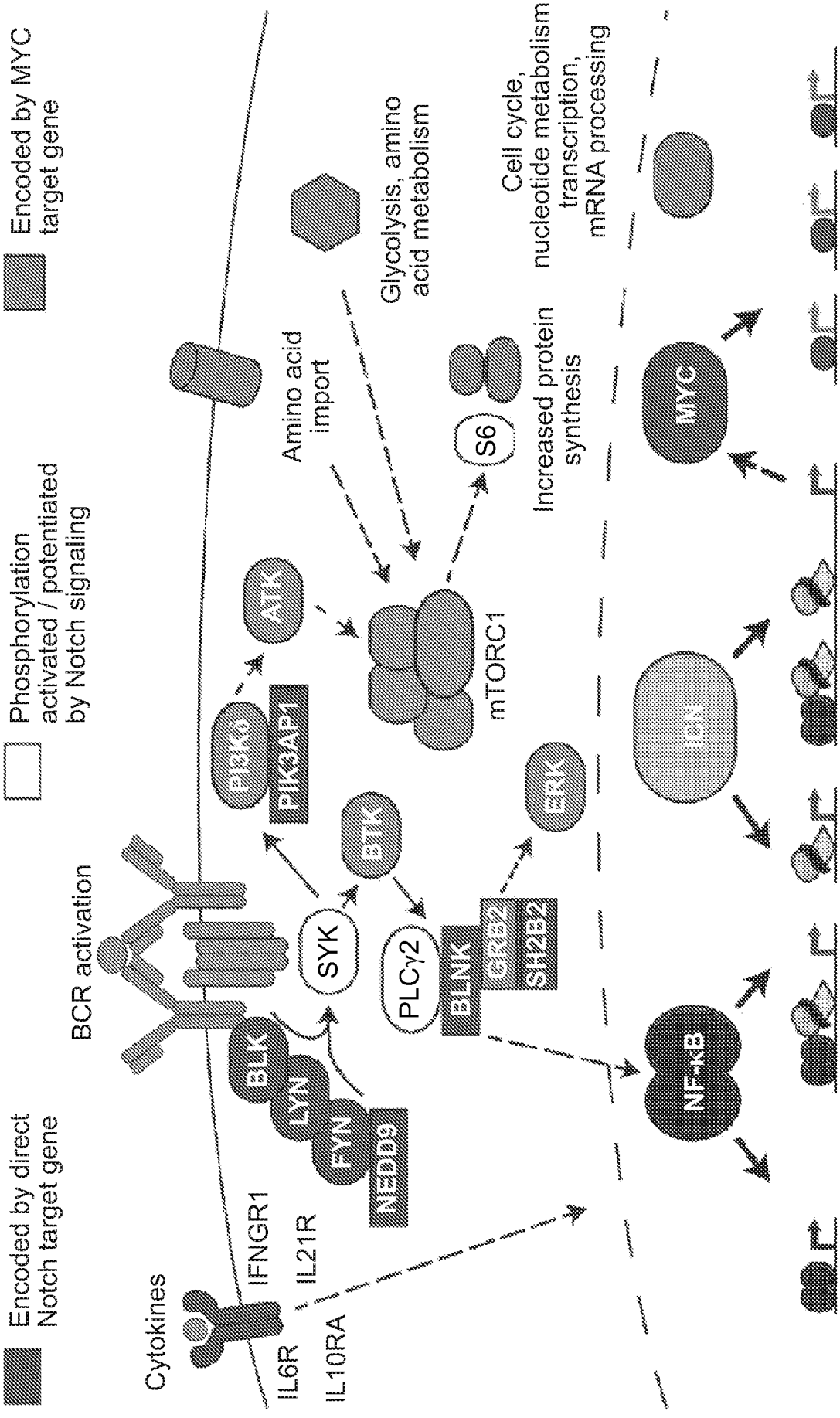

Encoded by direct Notch target gene
Phosphorylation activated / potentiated by Notch signaling
Encoded by MYC target gene
Cytokines
IL6R
IL10RA
IFNGR1
IL21R
BCR activation
BLK
LYN
FYN
NEDD9
SYK
BTK
PLCγ2
BLNK
GRB2
SH2B2
PIK3CD
PIK3AP1
AKT
ERK
Amino acid import
Glycolysis, amino acid metabolism
mTORC1
S6
Increased protein synthesis
Cell cycle, nucleotide metabolism, transcription, mRNA processing
MYC
IgM
NF-κB
E1
E2
MYC
NF-κB targets
Notch target genes
MYC target genes

FIG. 11

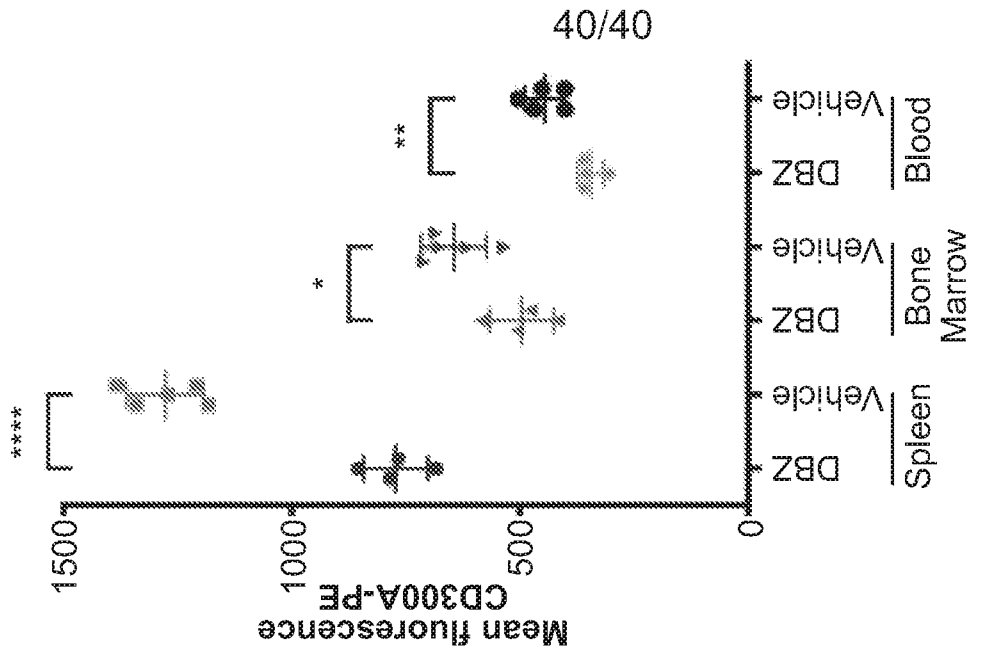


FIG. 12C

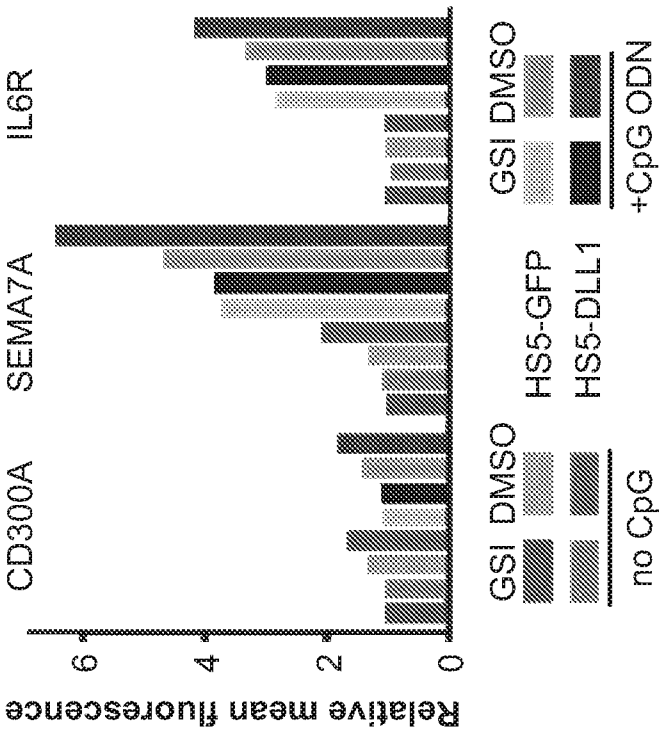


FIG. 12B

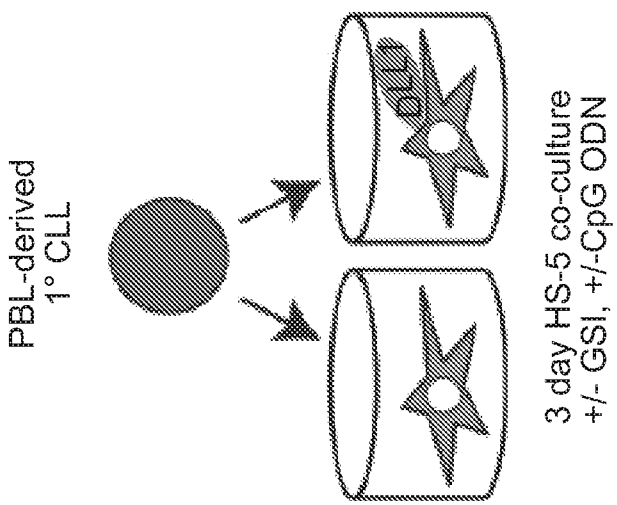


FIG. 12A