# Core regions in immunoglobulin heavy chain enhancers essential for survival of non-Hodgkin lymphoma cells are identified by a CRISPR interference screen

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# Core regions in immunoglobulin heavy chain enhancers essential for survival of non-Hodgkin lymphoma cells identified by a CRISPR interference screen

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## Supplementary Methods

### Plasmids

Lenti-dCas9-KRAB-blast[1] (#89567) and lentiGuide-Puro[2] (#52963) were purchased from Addgene (Watertown, MA, USA) and used to establish NHL cell lines with stable expression of catalytically inactive Cas9 fused with KRAB domain and cloning of CRISPR-eIGH library, respectively. LentiGuide-Puro was also used for further validation of individual sgRNAs. pAW12.lentiguide.GFP[3] (#104374) purchased from Addgene was used in the GFP growth competition assays.

For MYC over-expression experiments (OE), the MYC ORF was cloned in the pCW57-MCS1-P2A-MCS2 (GFP) vector[4] (#80924, Addgene). This construct was used to establish DG75-dCas9-KRAB-MYC OE cell line with inducible MYC expression. Cells transduced with empty vector were used as controls.

For the production of lentiviral particles 2nd generation plasmids were used: envelope expressing plasmid pMD2.G (#12259, Addgene) and packaging plasmid psPAX2 (#12260, Addgene).

### Design and generation of the CRISPR-eIGH library

In this study we aimed to target the *IGH* enhancer regions as defined by the H3K27ac histone mark plus 5 kb flanking sequences: Eμ (hg19 chr14:106317800-106336124), 3'RR1 (hg19 chr14:106140100-106181250) and 3'RR2 (hg19 chr14:106019800-106055011). The sequences were searched for the presence of the PAM sequence (5'-NGG-3') on both strands and all possible sgRNAs were listed using a custom Python script. Since 3'RR1 and 3'RR2 are highly homologous, numerous sgRNAs could target both regions, and those were allowed. Off-target binding of sgRNAs was checked using the Cas-OT script[5]. Only sgRNAs with at least three mismatches to the potential off-targets or at least two mismatches including at least one in the seed region (nt 9-20) were retained. We also included in the library 900 non-targeting sgRNAs from the Brunello library as a negative control[6] and 10 sgRNAs targeting regions -50 bp to +100 bp relative to TSS of CD79a and CD79b as positive controls. The list of all sgRNA oligonucleotides is provided in Supplementary Table 1.

### Cloning of the CRISPR-eIGH library

Designed sgRNAs were ordered from Twist Bioscience (San Francisco, CA, USA) as oligonucleotides with the 20 nt sgRNA sequences flanked by sequences complementary to the lentiGuide-Puro vector TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACACCG[sgRNA]GTTTTAGAGCTAGAAATAGCAAGTTAAAA TAAGGCTAGTCCGT. 2 ng of CRISPR-eIGH library was amplified with oligo-F and oligo-R primers (Supplementary Table 4) using NEBNext HiFidelity 2X PCR Master Mix (New England Biolabs, Ipswich,

MA, USA) in 20 x 25 µl PCR reactions. PCR program: 98°C 30 sec; (98°C 10 sec; 63°C 10 sec; 72°C 15 sec) x 6 cycles; 72°C 2 min. Pooled PCR reactions were purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany). Purified PCR product was then run on agarose gel and extracted with QIAquick Gel Extraction Kit (Qiagen). LentiGuide-Puro was digested with BsmBI (New England Biolabs) and purified from agarose gel, same as oligo library. Purified sgRNA CRISPR-eIGH library was cloned into the lentiGuide-Puro vector using the circular polymerase extension cloning (CPEC) method as described previously[7]. Briefly, 20 CPEC reactions were performed with 10:1 insert:vector molar ratio (17 ng amplified oligos, 100 ng vector) and NEB Next HiFidelity 2X PCR Master Mix. PCR program: 98°C 30 sec; (98°C 10 sec; 72°C 4,5 min) x 5 cycles; 72°C 5 min. All PCR reactions were pooled and purified by isopropanol precipitation. 300 ng of the purified CPEC product was used for transformation of electrocompetent Endura cells (Lucigen, Middleton, WI, USA) according to the manufacturer's protocol. Three electroporations were performed giving in total ~7.7 million colonies and resulting in ~710x coverage of the library. Bacteria were spread on 245x245 mm agar plates and grown for 13 h at 37°C. Colonies were scraped off the plates and plasmid DNA was isolated using Plasmid Plus Maxi Kit (Qiagen). Quality of the cloned CRISPR-eIGH library was verified by MiSeq next generation sequencing on Illumina platform (Laboratory of High-Throughput Technologies, Adam Mickiewicz University, Poznań, Poland).

**Cloning of individual sgRNA constructs**

For cloning of individual sgRNAs into the lentiGuide-Puro and pAW12.lentiguide.GFP vectors, sense and antisense oligonucleotides containing overhangs compatible with BsmBI sticky ends (Supplementary Table 5) were synthesized by Genomed (Warsaw, Poland). They were resuspended in annealing buffer (10mM Tris-HCl pH 8, 1mM EDTA pH 8, 50mM NaCl) and annealed in a thermocycler under conditions: 95°C 5min, 95°C (-1°C/cycle) x 70 cycles. Annealed oligos were ligated into the BsmBI-digested lentiGuide-Puro and pAW12.lentiguide.GFP vectors at 1:5 vector:insert molar ratio with T4 DNA ligase (Invitrogen, Carlsbad, CA, USA). 1 µl of ligation reaction was used for transformation of JM109 competent cells (Promega, Madison, WI, USA). Plasmid DNA from single colonies was isolated using Plasmid Plus Maxi Kit (Qiagen) and the sequences were confirmed by Sanger sequencing (Genomed).

**Generation of lentiviral particles**

HEK293T cells were seeded in 6-well plates one day prior to transfection. Next day, ~80% confluent cells were transfected with 2nd generation packaging plasmids psPAX (1.5 µg) and pMD2.G (1 µg), together with 2 µg of the transfer plasmid using Lipofectamine 2000 (Invitrogen) or calcium phosphate method (Invitrogen). One day after transfection medium was replaced with fresh DMEM supplemented with 10% FBS. 48 h and 72 h post-transfection lentiviral supernatants were filtered through 0.45 µm filter and stored at -80°C.

**Establishing cell lines with stable expression of dCas9-KRAB**

1-2 x $10^6$ of optimally dividing cells were transduced with 2nd generation lentiviral particles carrying lenti-dCas9-KRAB-blast. Briefly, cells were seeded on a 12-well plate, cell line-optimized virus amount was added along with 4 µg/ml polybrene and placed in 37°C incubator. For some cell lines spinfection was performed at 33°C, 1000 x g, 2h. After 24 h, transduced cell lines were washed in PBS and seeded for blasticidin (bln; Gibco, Grand Island, NY, USA) selection as indicated in Supplementary Methods Table 1. Antibiotic selection lasted 6 days. Genomic insertion of dCas9-KRAB expressing cassette was

confirmed on DNA level, followed by expression validation on RNA level by qRT-PCR and on protein level by Western Blot.

**Supplementary Methods Table 1.** Seeding densities and antibiotic concentrations used for cells selection after lentiviral transduction.

| Cell line | Seeding density x$10^6$/ml | Puromycin concentration µg/ml | Blasticidin concentration µg/ml |
|---|---|---|---|
| BL41 | 0.25 | 1 | 2.5 |
| CA46 | 0.15 | 2.3 | 5 |
| DG75 | 0.17 | 3 | 20 |
| ST486 | 0.25 | 0.3 | 8 |
| LY91 | 0.3 | 1 | 5 |
| JI | 0.25 | 0.5 | 5 |
| BL2 | 0.15 | 0.5 | 10 |
| BL60 | 0.2 | 0.3 | 15 |
| SUDHL4 | 0.3 | 1 | 10 |
| WSU-DLCL2 | 0.3 | 1 | 15 |
| P493-6 | 0.25 | 1 | 20 |
| HEK293T | 0.2 | NA | 5 |

NA - not applicable

**Determination of virus titer and cell transduction with CRISPR-eIGH library**

For CRISPRi screening experiment, the lentivirus titer was determined in order to transduce ca. 30% cells, which guarantees that approximately 85% of them are infected by a single construct[8]. For this purpose, 2.5 x $10^6$ cells per well were plated in 12-well plate and transduced with various amounts of lentivirus in the presence of 4 µg/ml polybrene. After spinfection at 33°C, 1000 x g, for 2h 1 ml of medium was added. 24 h after transduction cells were washed and plated in duplicate with and without puromycin. Medium was changed after three days. After four days of selection cells were counted and the percentage of cells surviving puromycin treatment relative to cells without puromycin was calculated. Based on this, the amount of virus resulting in ~30% surviving cells was determined.

For the CRISPR screen, 27.5 x $10^6$ cells were transduced in duplicate with CRISPR-eIGH library, with the previously established amount of virus. After four days of selection with puromycin (T0) 8 x $10^6$ cells were collected for DNA isolation. Remaining cells were further cultured for 20 population doublings. At each passage, the number of cells corresponding to 1,000x coverage of the library, that is 8 x $10^6$, were cultured in RPMI medium with 1 µg/ml (DG75) or 0.3 µg/ml (other cell lines) puromycin and collected at the final timepoint (T1).

**Preparation of libraries for next-generation sequencing of the plasmid pool or genomic DNA**

sgRNA sequences from the CRISPR-eIGH library plasmids were amplified as described previously[9]. Briefly, PCR reactions were performed using High-Fidelity MasterMix 2x (New England Biolabs, #M0541L) and primers incorporating Illumina adaptors (Supplementary Table 6). PCR products were purified with QIAquick PCR Purification Kit (Qiagen, #28106), then analyzed by DNA electrophoresis and extracted from agarose gel with QIAquick Gel Extraction Kit (Qiagen, #28706). Prior to NGS, the

quality and quantity of library were determined using KAPA Library Quantification Kit (Roche, #07960140001).

To analyze the sgRNA representation in the screening experiments, genomic DNA from cell lines was isolated using GENTRA Puregene Kit (Qiagen, #158722) and then sgRNA sequences were amplified as described above. DNA from 8 x $10^6$ cells was amplified in 30 (BL41, DG75) or 40 (SUDHL4) individual 50 µl PCR reactions per sample with 2-2.5 µg DNA input.

**NGS and data analysis**

NGS was performed on Illumina X-Ten platform at BGI (Hong-Kong). Adaptor sequences were removed, and reads were split for individual samples based on specific barcodes. Supplementary Table 2 summarizes the number of reads obtained for each sample. A Python script[10] was used for sgRNA enumeration. Only reads with no mismatches to sgRNA sequences were counted. Next, fold change (FC) between the beginning and end of screen was calculated for each sgRNA. FC values for both screen replicates were averaged. To identify regions whose targeting significantly affected lymphoma cell growth, we applied the sliding window approach[11]. Average FC values were calculated for 20 consecutive sgRNAs (Supplementary Table 3). Significance was calculated with t-test comparing FC values for 20 sgRNAs in each window with the negative control sgRNAs. We considered as significant windows which were at least 1.5-fold depleted (log2FC -0.585) and with FDR<0.001.

**Cellular fractionation**

20 x $10^6$ cells (30 x $10^6$ for SUDHL4) were used for cellular fractionation as described previously[12]. All steps were performed on ice. Buffers were supplemented with DTT (if noted), 1x EDTA-free protease inhibitors and 40U/ml RNaseOUT (Invitrogen) directly before use and kept on ice. Briefly, cells were washed in ice-cold PBS. Then cell pellet was resuspended in 500 µl Buffer W (300 mM sucrose, 10 mM Tris-HCl pH 8.0, 10 mM NaCl, 2 mM $MgAc_2$ 0.5 mM DTT), followed by addition of 500 µl Buffer L (Buffer W supplemented with 6 mM $CaCl_2$, 0.2 % IGEPAL CA-630, 0.5 mM DTT). Lysates were centrifuged 1000 x g, 4°C, 10 min. Resulting supernatant was saved as cytoplasmic fraction, while nuclear pellet was processed further. Nuclei were washed in Buffer G (50 mM Tris-HCL pH 8.0, 25% glycerol, 5 mM $MgAc_2$, 0.1 mM EDTA, 5 mM DTT), then lysed with Buffer U (1 M urea, 20 mM HEPER pH 7.5, 7.5 mM $MgCl_2$, 0,1 mM EGTA, 300 mM NaCl, 1 mM DTT), vortexed and centrifuged 20 000 x g, 4°C, 10 min. Resulting supernatant was saved as nuclear fraction, while chromatin pellet was washed twice in Chromatin Wash Buffer (50 mM Tris-HCl pH 8.0 supplemented with 40 U/ml RNaseOUT). Chromatin pellet was then sonicated twice [5 sec on, 30 sec off] (Misonix 3000; Misonix, Farmingdale, NY, USA) on ice in 300 µl Buffer G for RNA isolation or in 500 µl RIPA buffer (Sigma, Saint Louis, MO, USA) supplemented with protease inhibitors for protein extraction. RNA was isolated from each fraction using Trizol method. Additionally, 10 µl 500 mM EDTA was added to chromatin fraction and heated 10 min 65°C with 600 rpm shaking on thermoblock. RNA and protein samples were stored in -80°C until analysis. To confirm successful separation of the fractions on RNA level, 2 marker genes were used per each fraction: RPPH1 and DANCER for cytoplasm, U3SNO and MIAT for nuclei and TBP_intron and KTN1_AS1_intron for chromatin (primers listed in Supplementary Table 4). To additionally confirm that the fractionation was successful, Western Blot was performed with fraction-specific antibodies (Supplementary Table 7), as described in Supplementary Methods section **Western Blot**.

**Chromatin-enriched RNA-Seq and data analysis**

RNA from chromatin fraction of two biological replicates per each cell line was tested on 2% agarose gel for integrity. Prior to sequencing, RNA was treated with TURBO-DNase (Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer instructions, followed by purification using RNA Clean & Concentrator (Zymo Research, Irvine, CA, USA) with additional DNase treatment on the column. RNA-Seq stranded library preparation, pair-end sequencing and bioinformatic data analysis were performed by Novogene (Beijing, China). Ribo-zero rRNA Removal Kit (Epicentre, Paris, France) and NEBNext Ultra Directional RNA Library Prep Kit for Illumina (NEB) were used for library preparation. Sequencing was performed on Illumina X-Ten platform. Quality control of raw data in FASTQ format was performed, clean data were obtained by removing reads containing adapter and poly-N sequences and reads with low quality.. Approximately 77-92 x $10^6$ clean reads were obtained per sample. All downstream analyses were based on clean data with high quality. Reads were mapped to the human reference genome version GRCh37.87 using HISAT 2 software. Reads mapping to the *IGH* enhancer regions (see **Design of the CRISPR-eIGH library**) were selected for further study. Using Galaxy[13] BAM files provided by Novogene were sliced by genomic region chr14:106019800-106337000 and filtered by second mate to retrieve information about the strand from which the transcript was derived. Next, using Integrative Genome Browser[14], BedGraph files were generated and visualized in UCSC Genome Browser (http://genome.ucsc.edu)[15].

**RNA isolation, cDNA synthesis and RT-qPCR**

RNA was isolated from 1-2 x $10^6$ cells using RNA MiniPrep (Zymo Research, Irvine, CA, USA) according to the manufacturer's instructions. DNase treatment was performed on the column. To obtain RNA from cellular fractions Trizol method was applied. Briefly, samples were resuspended in TRI Reagent (Sigma), chromatin fraction was additionally heated as described in Supplementary Methods section **Cellular fractionation.** Samples were then mixed with chloroform and centrifuged according to manufacturer's instructions. Next, RNA was precipitated with 96-99.9 % ethanol, 300 mM NaCl and 15 µg GlycoBlue Coprecipitant (Invitrogen) at -20$^o$C over-night. Next day, the RNA pellet was washed in 75% ethanol and resuspended in UltraPure RNAse-free water. Chromatin fraction was additionally treated with TURBO-DNase (Invitrogen) according to the manufacturer's instructions. Next, RNA from all fractions was purified and DNase treated on the column using RNA Clean and Concentrator (ZYMO Research). Reverse transcription was performed using 300-1,000 ng of total RNA or RNA fractions with QuantiTect Reverse transcription Kit (Qiagen) or SuperScript III and random primers (both Thermo Fisher Scientific).

qPCR analysis was performed using 5-15 ng cDNA with PowerUp SYBR Green Master Mix (Thermo Fisher Scientific) on CFX96 Touch qPCR System (Bio-Rad, Hercules, CA, USA). Gene expression was normalized relative to the housekeeping gene. For eRNA expression validation in larger panel of B-cell cell lines and in FFPE patient samples qPCR was done with 2X SYBR Green Master Mix (Applied Biosystems B.V., Bleiswijk, The Netherlands) on Light Cycler 480 (F. Hoffmann-La Roche, Basel, Switzerland). All primer sequences used in this study are available in Supplementary Table 4.

**Validation of enhancer RNA expression**

To validate eRNA expression and cellular localization, primers specific for transcriptionally active core *IGH* regions, based on chromatin-enriched RNA-seq, were designed. Primers detected transcripts from

both the plus and minus strands. eRNA expression was verified in a panel of B-cell lines and in patient-derived samples (described in Supplementary Methods section **Patient samples**). Cell panel consisted of 9 BL cell lines: EBV-negative - DG75, CA46, ST486, BL41, Ramos and EBV-positive - BL65, Namalwa, Jijoye, Raji; 12 DLBCL: GCB type - SUDHL16, SUDHL10, SUDHL6, SUDHL5, SUDHL4, SC1, WSU-DLCL2 and ABC type - U2932, Ocily3, RI-1, Nu-dul1, SUDHL2; 8 Hodgkin lymphoma (HL) cell lines: L428, L1236, KMH2, DEV, L540, L591, SUPHD1, HDLM2 and 4 germinal center B-cell samples as controls[16].

**GFP competition assay**

0.5-1 x $10^6$ cells were transduced with individual sgRNA constructs in pAW12.lentiguide.GFP and cultured for 22 days. Cells were analyzed three times a week by flow cytometry using CytoFLEX S (Beckman Coulter, Indianapolis, IN, USA). The percentage of GFP-positive cells was calculated in reference to the start of the experiment (day 4 or day 6) set as 100%.

**B-cell receptor (BCR) immunostaining**

1 x $10^6$ cells per sample were collected and washed with PBS, then with ice-cold staining buffer (2% FBS in PBS). Cells were resuspended in 95 µl of ice-cold staining buffer with addition of 5 µl FcR blocking reagent, human, (MiltenyiBiotec, Bergisch Gladbach, Germany, #130-059-901) and incubated 10 min in a fridge. Then, 20 µl of APC Mouse Anti-Human IgM (BD Biosciences, Franklin Lakes, NJ, USA, #551062) or 0.1 µg of Goat F(ab')2 Anti-Human IgG-AF647 (Southern Biotech, Birmingham, AL, USA, 2042-31) was added and cells were incubated 25 min on ice, protected from light. Next, cells were washed twice with 2 ml of staining buffer and finally resuspended in 100 µl of this buffer for flow cytometry analysis, performed on CytoFLEX S (Beckman Coulter).

**MYC overexpression**

1 x $10^6$ DG75-dCas9-KRAB cells were transduced with 2$^{nd}$ generation lentiviral particles carrying pCW57-MCS1-P2A-MCS2-MYC OE (GFP) or empty vector (EV). GFP-positive cells were sorted in PBS supplemented with 1% FBS and collected in culture medium supplemented with 50% FBS in 5 ml cytometric tubes. DG75-dCas9-KRAB-MYC OE cells were sorted on SH800S cell sorter (Sony Biotechnology, San Jose, CA, USA) using 100 µM nozzle, normal mode. DG75-dCas9-KRAB-EV cells sorting was carried out using the BD FACS AriaIII (Becton Dickinson, Franklin Lakes, NJ, USA) cell sorter using 100 µM nozzle, 20 psi (0,138 MPa), 4-way sorting purity mode was selected for gaining highest purity level. After sorting cells were seeded at high density 1 x $10^6$ cells/ml in complete RPMI medium and then split depending on cell fitness. MYC overexpression upon addition of doxycycline (Merck, Darmstadt, Germany) was evaluated on RNA and protein level. To assess cell survival upon MYC overexpression, DG75-MYC-OE cells were mixed 1:1 with DG75-dCas9 (no GFP, no ectopic MYC overexpression) and GFP growth competition assay was performed for 3 weeks. For the rescue experiment, 1 x $10^6$ DG75-dCas9-KRAB-MYC OE and EV cells were transduced with 2$^{nd}$ generation lentiviral particles carrying individual sgRNAs cloned into lentiGuidePuro, followed by puromycin selection.

**Cell viability assay**

Cell viability was measured using CellTiter-Glo Luminescent Cell Viability Assay (Promega, Madison, WI, USA). Briefly, at day 6 post-infection, 2000 cells per well were seeded on 96-well plates in triplicate. Doxycycline was added to the final concentration of 0.1 µg/ml. As control, wells without doxycycline

were prepared. CellTiter-Glo reagent was mixed 1:2 in PBS and 100 µl per well was added. Luminescence was measured on GloMax-Multi Detection System (Promega) at 1h (baseline) and 72h after seeding cells. Cell viability was determined as the relative luminescence (RLU) calculated relative to the first measurement (1h). For each experiment three biological replicates were made. Change in cell growth upon doxycycline administration in cells infected with sgRNAs targeting *IGH* enhancers was calculated relative to the non-targeting controls.

**Western Blot**

5-20 x $10^6$ cells were washed in PBS, then lysed in RIPA buffer (Sigma, Saint Louis, MO, USA), supplemented with protease inhibitors. Protein concentration was determined using Bicinchoninic Acid Kit (Sigma). 20 µg of total protein was mixed with the Laemmli 4X sample buffer (Sigma), heat-denatured and separated at 120V on the 8% (for Cas9 detection) or 12% polyacrylamide gel (PAA, acrylamide:bisacrylamide, 49:1) supplemented with 2,2,2-trichloroetanol (Merck, Kenilworth, NJ, USA) to allow for stain-free total protein detection. For cellular fractionation control, an equal portion of each faction was loaded on 12 % PAA. Proteins were transferred onto PVDF (Bio-Rad, #1620177) or Low Fluorescence PVDF (Bio-Rad, #1620264) membrane for 1.5h 75V at room temperature with cooling, blocked for 1h in 5% milk in TBST and incubated with primary antibody at 4°C overnight. Next membranes were washed in TBST, followed by TBS wash and incubated with secondary HRP-conjugated antibody for 1h at room temperature. Signal was detected by chemiluminescence using Clarity Western ECL Substrate (Bio-Rad) with ChemiDoc Imaging Systems (Bio-Rad). Quantitative analysis was performed using Image Lab Software (Bio-Rad). For expression analysis bands were normalized using total protein method. Full list of antibodies used in this study can be found in Supplementary Table 7.

**Statistical analysis**

In eRNA expression validation, difference in expression between tested groups was evaluated with ANOVA Kruskall-Wallis with Dunn's Multiple Comparison Post-Test. For the other qRT-PCR results the Mann-Whitney test was applied. BCR staining was analyzed with Student's two-tailed t-test and cell viability change upon MYC overexpression with Student's paired one-tailed t-test. Those statistical analyses were calculated with GraphPad Prism version 5.0.0 (GraphPad Software Inc., San Diego, CA, USA) with a P-value significance cut-off $P<0.05$. Statistics for GFP-growth competition assay was calculated as described previously[17] using SPSS software (IBM, Armonk, NY, USA). Mixed model analysis was used for comparison of GFP-positive cells decrease over time between controls and samples with blocked *IGH*-enhancers essential regions, time and the interaction of time and given sgRNA were treated as fixed effects and the measurement repeat for a given sgRNA as random effect.

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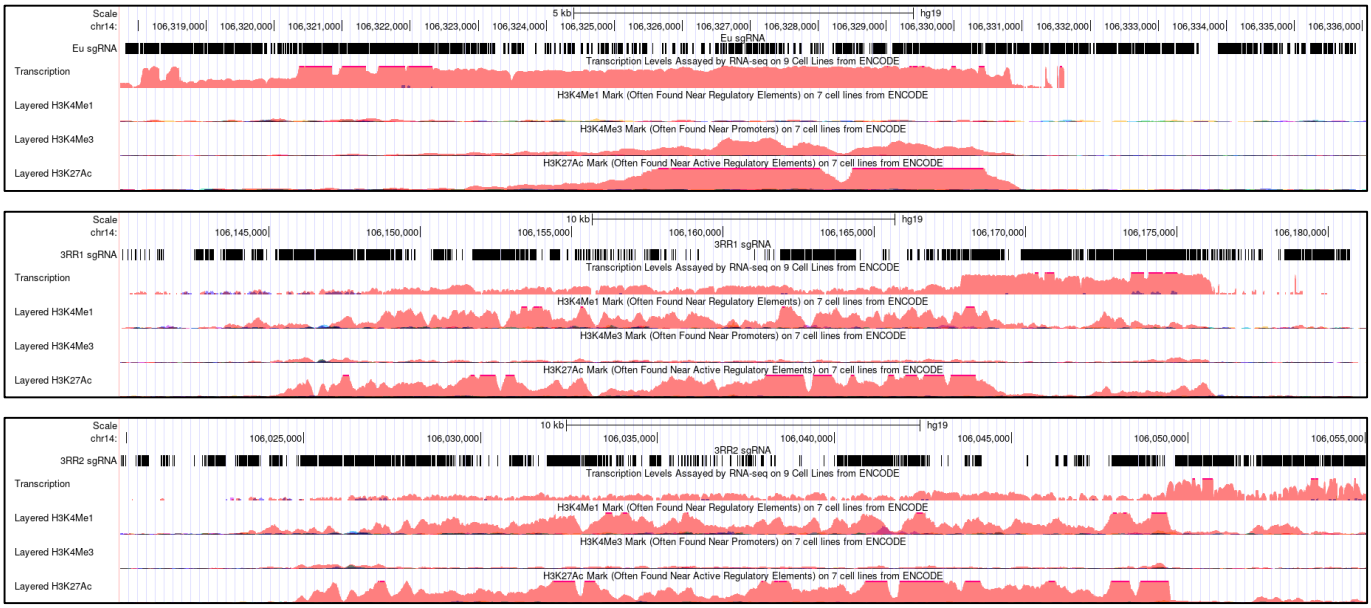
**Supplementary Materials**

**Supplementary Figures:**

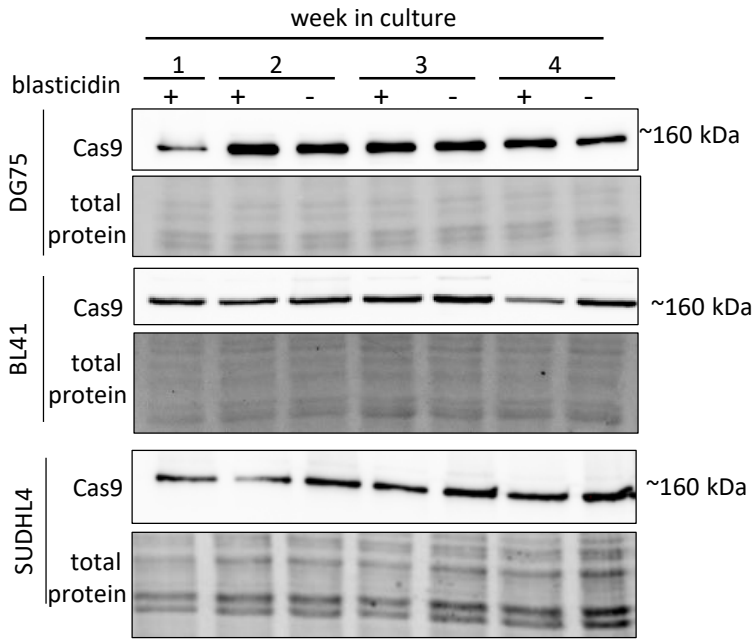


**Supplementary Tables:**




**Supplementary Figure 1. IGH regions used for the design of the CRISPR-eIGH library.** Black lines indicate localization of sgRNAs oligonucleotides. From the top: Eμ, 3'RR1 and 3'RR2. Peaks represent UCSC tracks : transcription, H3K4me1, H3K4me3, H3K27ac of GM12878 B-cells from ENCODE.

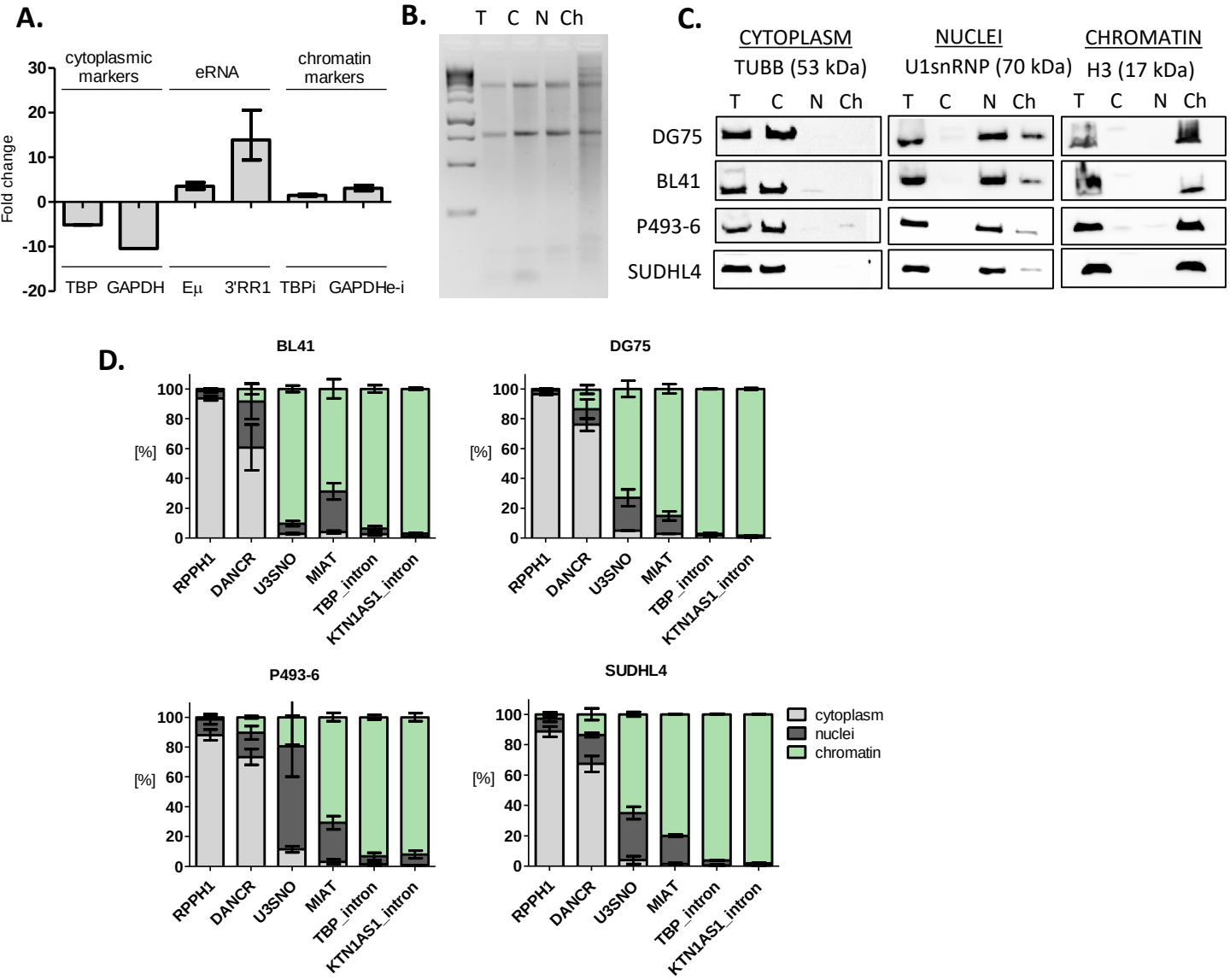
**A.**



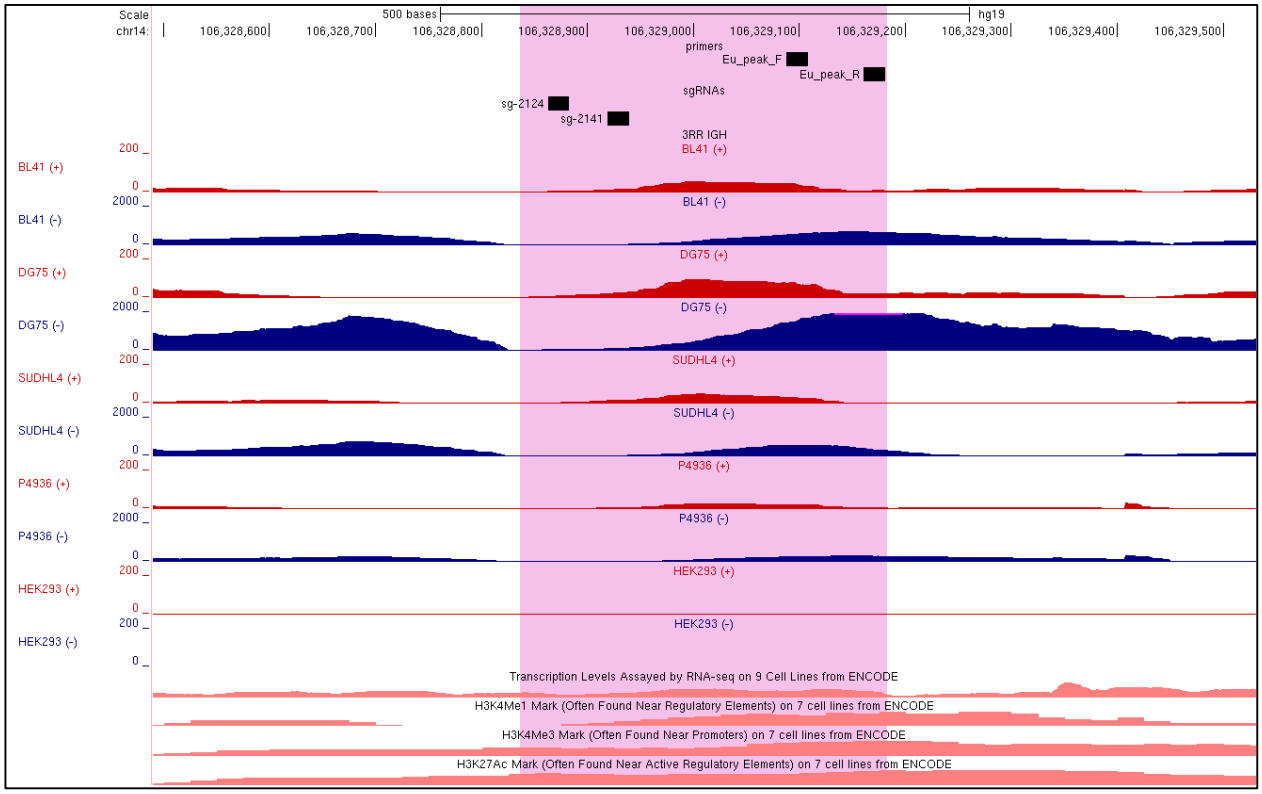

**B.**

| Experiment | Percent of transduced cells | Number of transduced cells | CRISPR-eIGH library coverage |
|---|---|---|---|
| BL41 screen #1 | 25.1 % | 6.9 M | 860x |
| BL41 screen #2 | 28.1 % | 7.7 M | 965x |
| DG75 screen #1 | 30.4 % | 8.4 M | 1045x |
| DG75 screen #2 | 21.8 % | 6 M | 750x |
| SUDHL4 screen #1 | 28.4 % | 7.8 M | 975x |
| SUDHL4 screen #2 | 22.6 % | 6.2 M | 775x |

**Supplementary Figure 2**. **A.** Verification of dCas9 expression on protein level over time with or without addition of blasticidin in cell lines used in CRISPRi screens. **B.** CRISPRi screens details for each replicate.

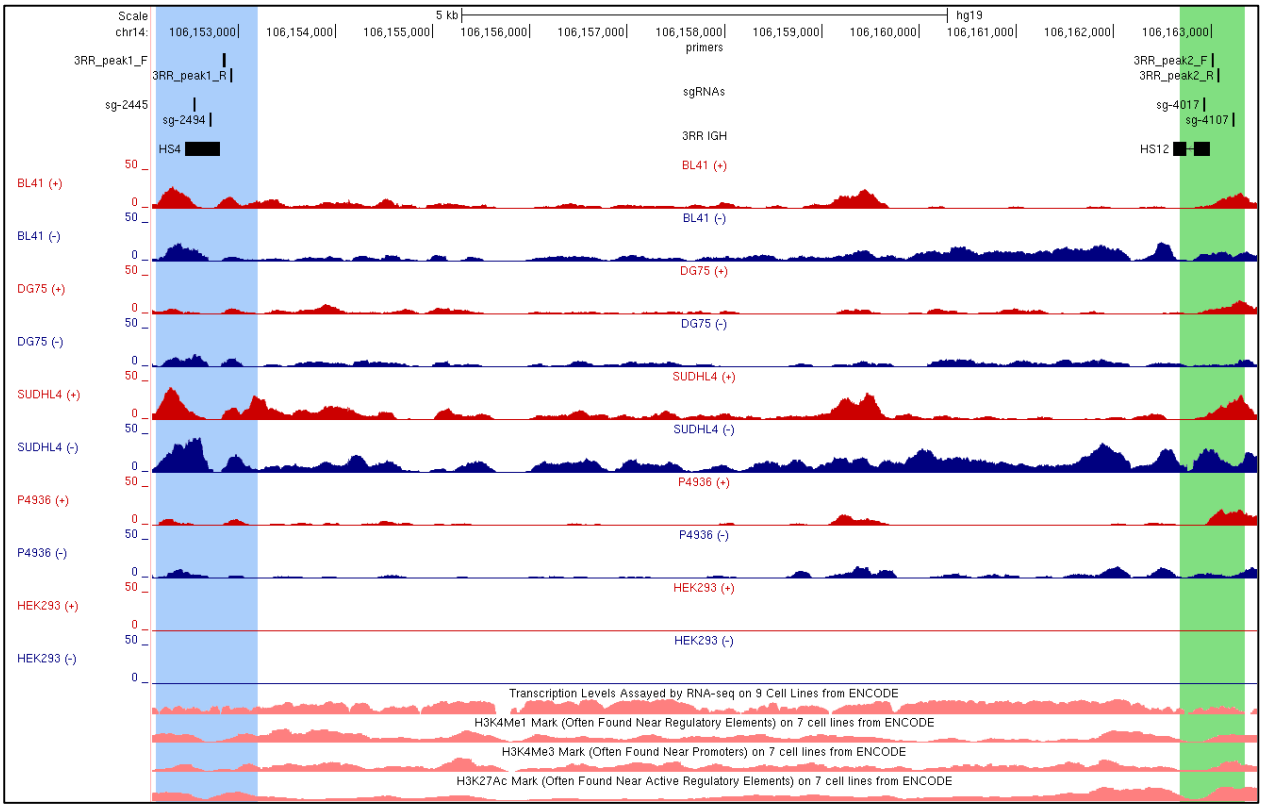



**Supplementary Figure 3**. **Subcellular RNA fractionation. A.** Abundance of transcripts in the chromatin fraction relative to total RNA. Cytoplasmic markers target spliced transcripts, while chromatin markers target unspliced transcripts. TBPi – intron, GAPDHe-i – transcript from exon-intron boundary. *IGH* enhancer RNA transcripts were predicted based on available transcription data from GM12878 cells (ENCODE). **B.** RNA from cellular fractionation on 2% agarose gel. Enrichment of small RNAs is visible in the cytoplasmic fraction. In chromatin fraction the pattern of observed ribosomal RNAs differs due to presence of rRNA precursors. T – total RNA, C – cytoplasmic fraction, N – nuclear fraction, Ch – chromatin fraction. **C.** Cellular fractionation control on protein level. T – total protein, C – cytoplasmic fraction, N – nuclear fraction, Ch – chromatin fraction. Fraction markers: cytoplasm – TUBB, nuclei – U1 snRNP 70, chromatin – H3. A representative blot is shown. **D.** Cellular fractionation control on RNA level. Fraction markers: cytoplasm – RPPH1, DANCR, nuclei – U3SNO, MIAT, chromatin – introns of TBP and KTN1_AS1. Average and SD from two independent biological replicates is shown.

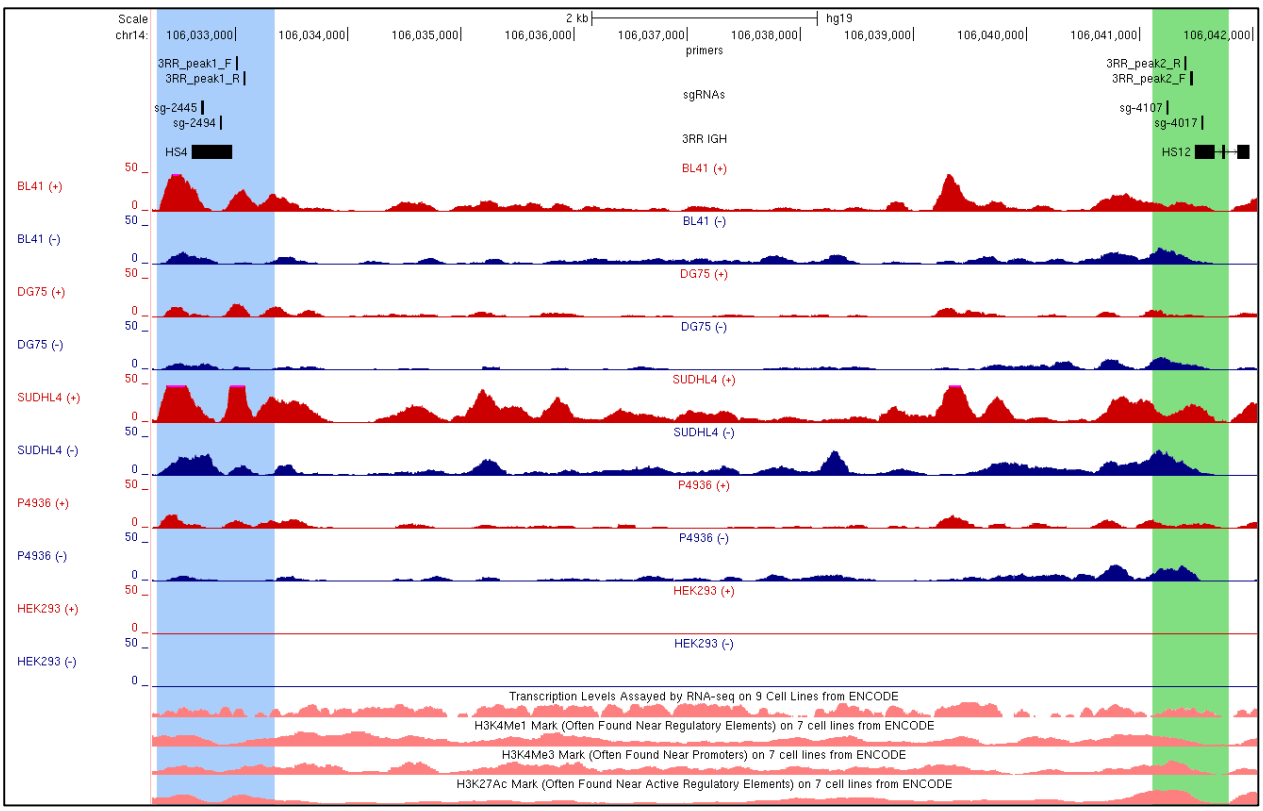



**Supplementary Figure 4. UCSC Genome Browser zoom in on IGH Eμ enhancer.** CRISPRi-identified enhancer-essential region is highlighted in pink. Localization of sgRNAs and primers used in CRISPRi screens and eRNA expression validation is marked as black boxes. Presented coverage are reads obtained from chromatin-enriched RNA-Seq. Red – reads from the plus strand, blue – reads from the minus strand obtained from chromatin-enriched RNA-Seq. Bottom tracks represent transcription and layered histone marks of GM12878 cells from ENCODE.

**A.**




**B.**




**Supplementary Figure 5**. **UCSC Genome Browser zoom in on *IGH* 3'RR enhancers regions.** Presented coverage are reads obtained from chromatin-enriched RNA-Seq. Red – reads from the plus strand, blue – reads from the minus strand obtained from chromatin-enriched RNA-Seq. Localization of primers and sgRNAs used in CRISPRi screens and eRNA expression validation is marked as black boxes. **A.** 3'RR1 enhancer and enhancer-essential region 1 (peak 1) highlighted in blue and region 2 (peak 2) in green. **B.** 3'RR2 enhancer and enhancer-essential region 1 (peak 1) highlighted in blue and region 2 (peak 2) in green. Bottom tracks represent transcription and layered histone marks of GM12878 cells from ENCODE

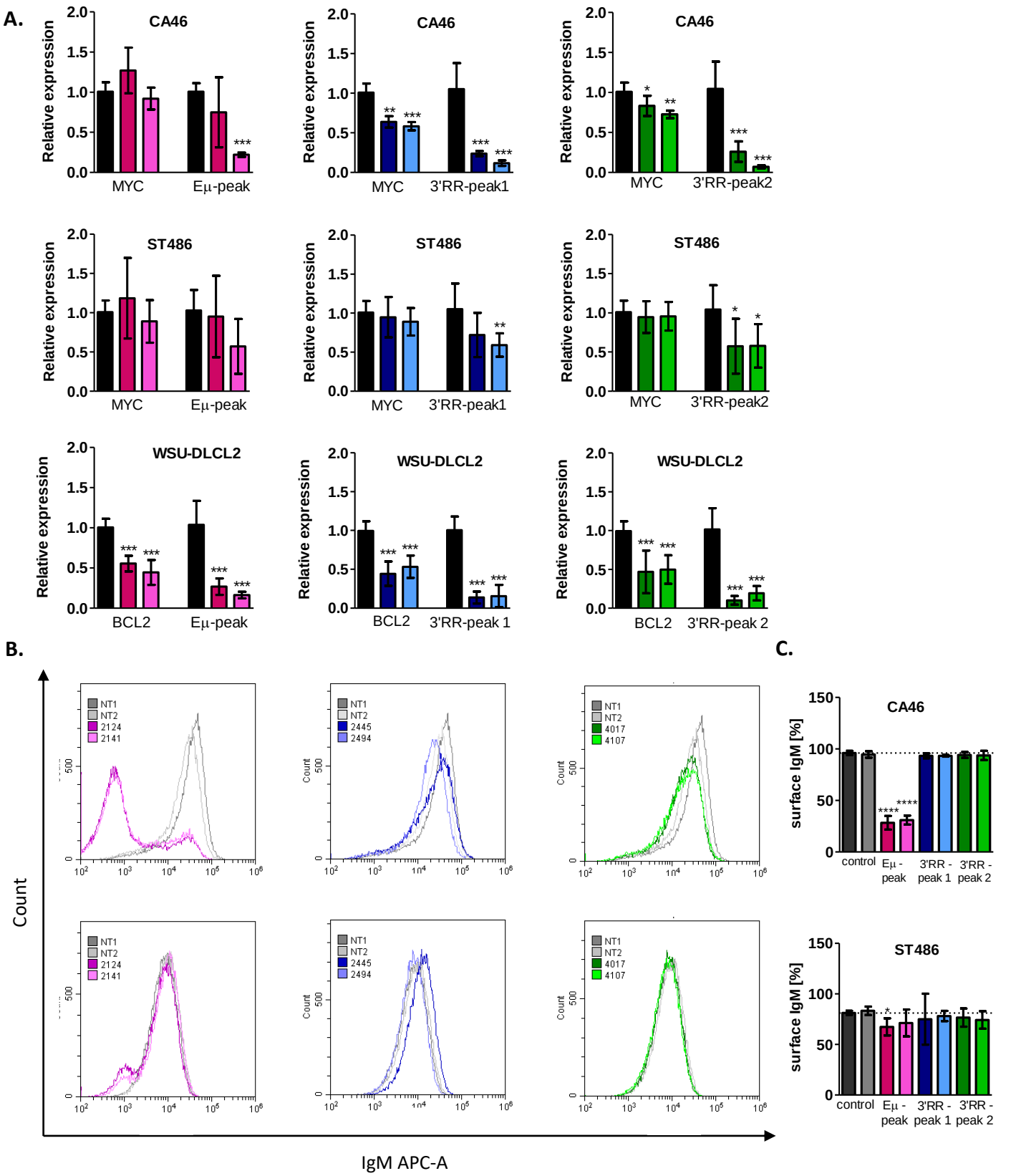
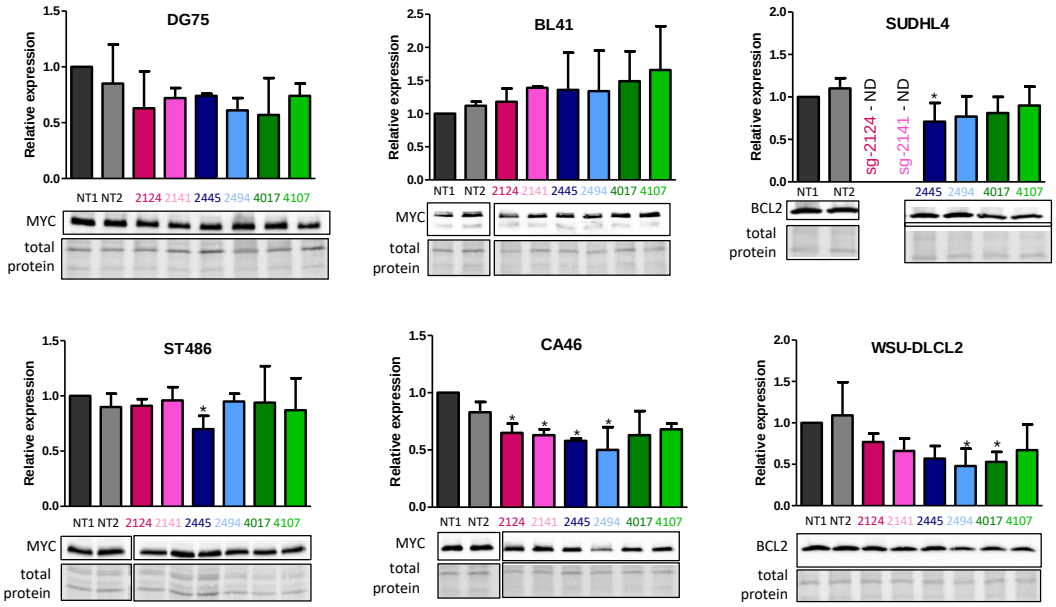
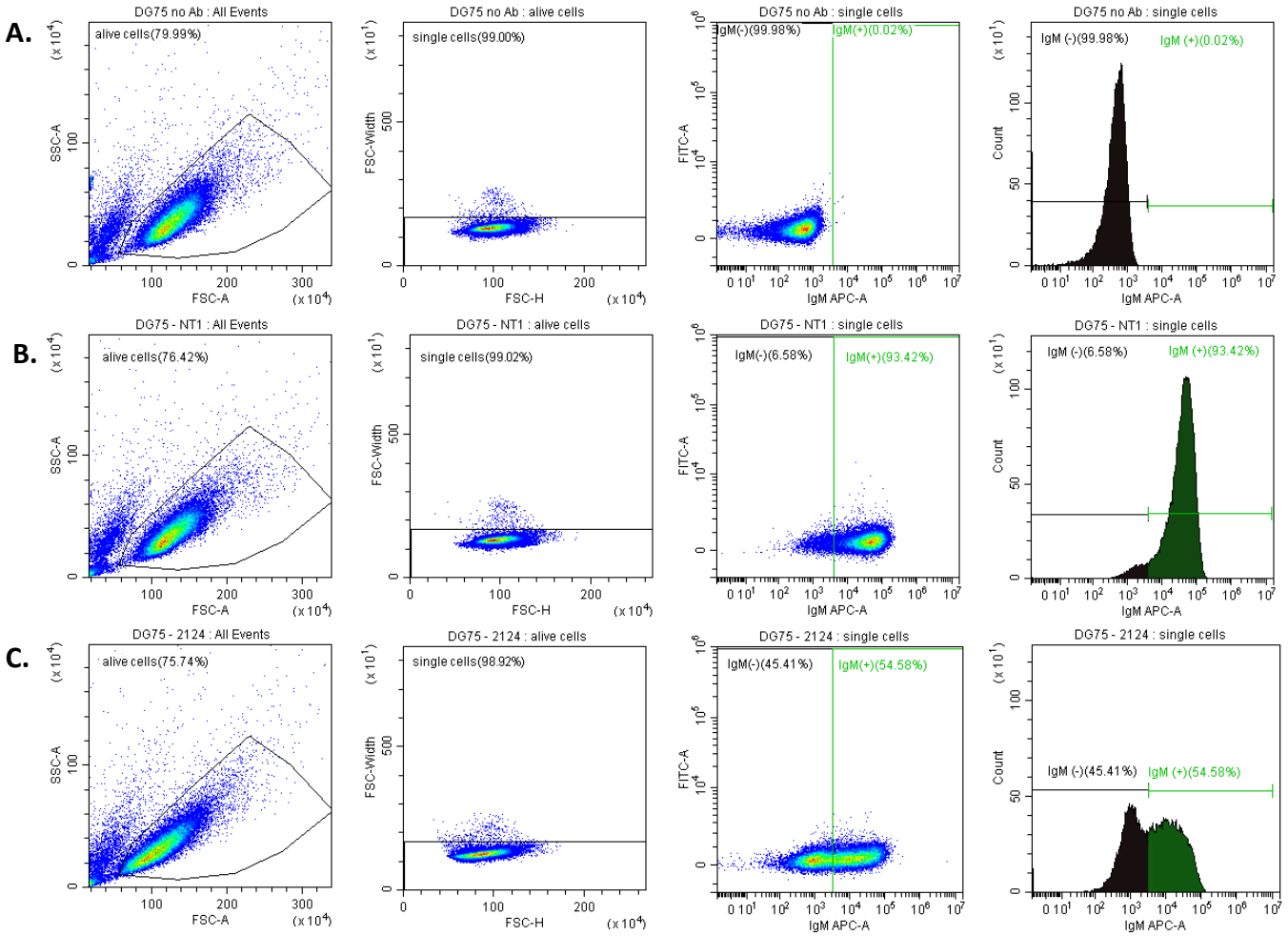


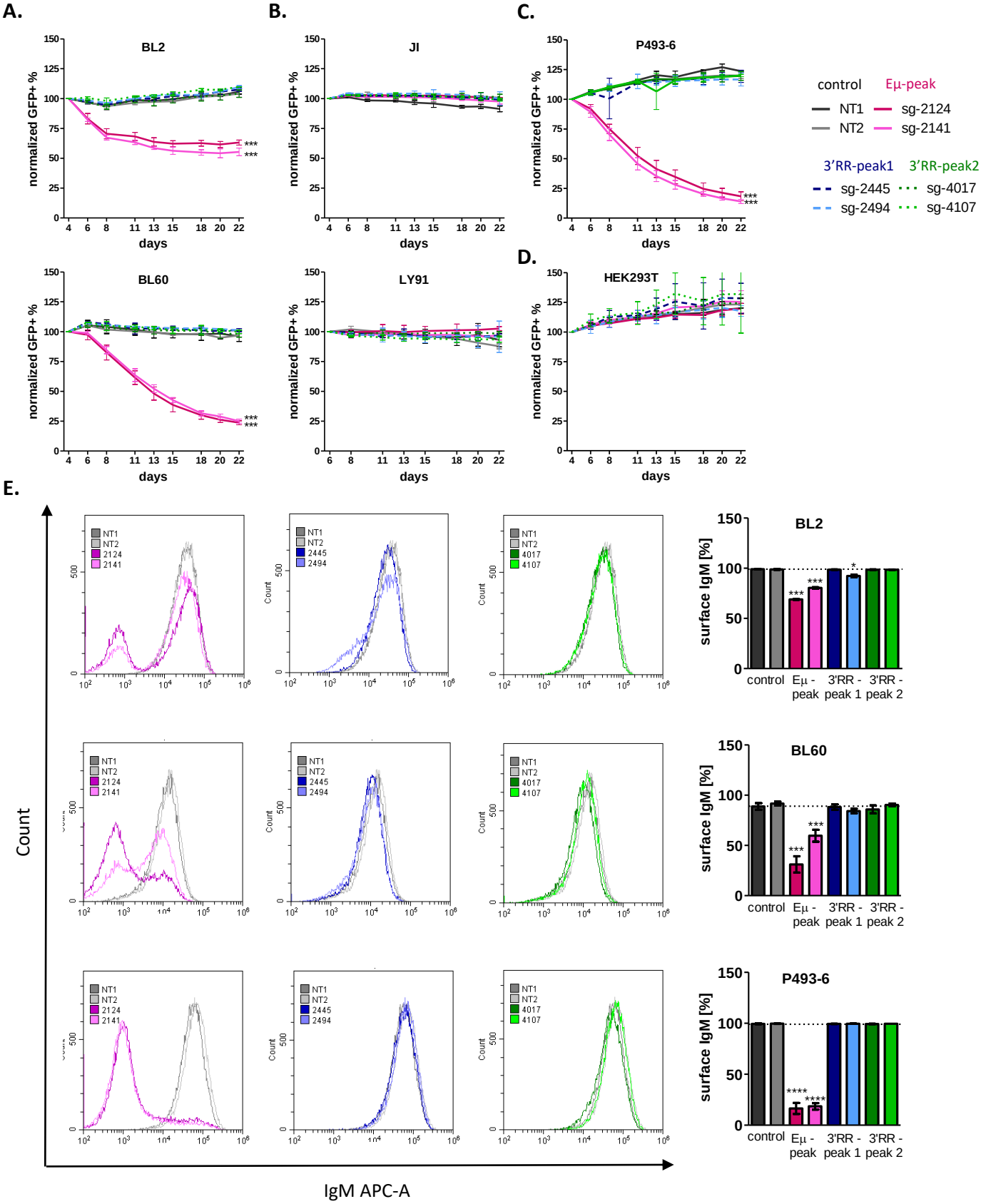

**Figure 6. Downstream effects of targeting *IGH* enhancers. A.** Expression of oncogenes involved in *IGH* translocation – MYC (CA46, ST486) or BCL2 (WSU-DLCL2) and expression of eRNAs upon blocking of *IGH* enhancers essential regions on RNA level determined by qRT-PCR. Mean and SD of three independent biological replicates is shown. Expression normalized to HPRT. *, P ≤ 0.05; **, P ≤ 0.01, ***, P ≤ 0.001, Mann-Whitney test. **B.** Immunostaining of B-cell receptor (BCR) on cell surface CA46 and ST486 (IgM) (WSU-DLCL2 do not express neither IgM nor IgG). Representative histograms of overlaid data for non-targeting controls (grey) and sgRNAs targeting *IGH*-enhancers essential regions (pink, Eμ peak; blue, 3'RR peak 1; green, 3'RR peak 2). **C.** Average and SD of percentage of BCR-positive cells (surface IgM) from two biological replicates. *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001; ****, P ≤ 0.0001, Student's two-tailed t-test.



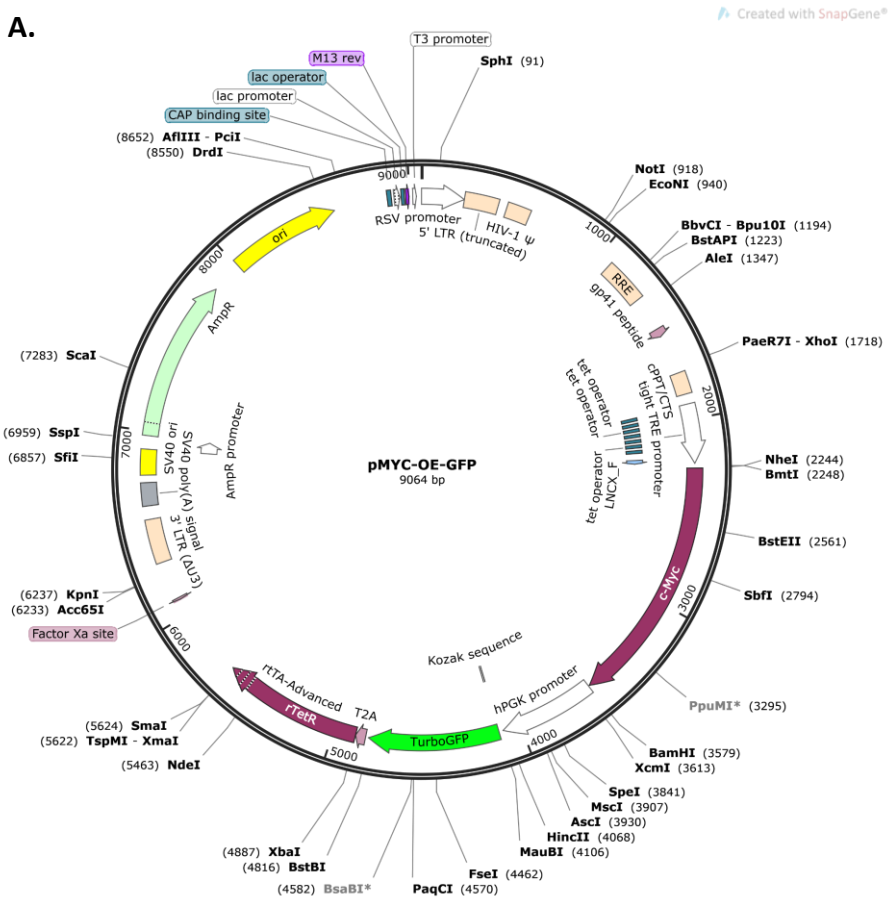

**Supplementary Figure 7. Expression of oncogenes (MYC or BCL2) on protein level.** Mean and SD from two independent biological replicates is shown. Protein bands were normalized to the total protein. ND – not determined due to insufficient cell number.




**Supplementary Figure 8. Flow cytometry analysis of B-cell receptor (BCR) immunostaining.** APC-based gating strategy for surface BCR analysis, from left: living cells selection, single cells selection, surface BCR positive (IgM or IgG) cells selection and histogram visualization. Examples are given for **A.** sample without anti-BCR antibody, **B.** control sample NT1, **C.** sample in which Eμ enhancer is targeted with sgRNA




**Supplementary Figure 9. Effect of targeting IGH enhancers in cells without IGH translocations. A-D.** GFP growth competition assay in **A.** BL cell lines with MYC-IGL t(8;22) translocation (BL2, BL60); **B.** BL cell lines with MYC-IGK t(2;8) translocation (JI, LY91); **C.** B-cell cell line without MYC translocation (P493-6); **D.** Embryonic kidney HEK293T cell line. Assay performed with individual sgRNAs over the course of 3 weeks. Average and standard deviation from 2-3 independent biological replicates is shown. ***, P≤0.001, mixed model analysis. **E.** Immunostaining of B-cell receptor (BCR) on cell surface in BL2, BL60 and P4936 cells (all IgM, JI and LY91 are BCR-negative). Representative histograms of overlaid data for non-targeting controls (grey) and sgRNAs targeting *IGH*-enhancers essential regions (pink, Eμ peak; blue, 3'RR peak 1; green, 3'RR peak 2). Column graphs show average percentage and SD of BCR-positive cells (surface IgM) from two biological replicates. *, P ≤ 0.05; ***, P ≤ 0.001; ****, P ≤ 0.0001, Student's two-tailed t-test.




**Supplementary Figure 10. Establishment of MYC-overexpressing DG75 cells. A.** Map of plasmid pMYC-OE-GFP used for establishment of DG75-MYC-OE cell line. **B-C.** Validation of doxycycline-induced MYC overexpression in DG75- MYC-OE cell line on **B.** RNA level, expression normalized to HPRT and **C.** on protein level, normalized to total protein. **D.** DG75-MYC-OE cells (expressing GFP) survival upon doxycycline-induced MYC overexpression over 3 weeks of culture determined by GFP growth competition assay.

**Supplementary Table 4.** List of primers used in this study.

| Name | Sequence - Fwd 5'-3’ | Sequence - Rev 5'-3’ |
|---|---|---|
| **Gene expression analysis** | | |
| HPRT | GGCAGTATAATCCAAAGATGGTCAA | GTCTGGCTTATATCCAACACTTCGT |
| TBP | GCCCGAAACGCCGAATAT | CCGTGGTTCGTGGCTCTCT |
| GAPDH | GAGTCCACTGGCGTCTTCAC | TGATGACCCTTTTGGCTCCC |
| Eμ-peak | TCCTACAGACACCGCTCCTG | GGCTTGGGGAGCCACATTT |
| 3'RR peak 1 | TGACCCCCGATGAGTGTGAG | TGGATAACGCTCAGGACGGG |
| 3'RR peak 2 | GCCCAGAGATGCCGAAAACT | CTAGGGGCAAGCTGGTGAG |
| MYC | CACCAGCAGCGACTCTGA | ATCCAGACTCTGACCTTTTGC |
| BCL2 | TGAACTGGGGGAGGATTGTG | CGTACAGTTCCACAAAGGCA |
| TBP_intron | TTTGTCTGAAGCCCTGATGTGT | CTGTGAAGAGAGCGCAGTGT |
| GAPDH_exon-intron | AATCCCATCACCATCTTCCAG | GAGCCACACCATCCTAGTTG |
| **Fractionation controls** | | |
| TBP_intron | TTTGTCTGAAGCCCTGATGTGT | CTGTGAAGAGAGCGCAGTGT |
| KTN1_AS1_intron | TTGGCTGCTATTTACTACCCTCC | GCTGGGTGTGTTGCTAATCC |
| RPPH1 | AGCTTGGAACAGACTCACGG | AATGGGCGGAGGAGAGTAGT |
| DANCR | CGTCTCTTACGTCTGCGGAA | TGGCTTGTGCCTGTAGTTGT |
| U3 snoRNA | AACCCCGAGGAAGAGAGGTA | CACTCCCCAATACGGAGAGA |
| MIAT | TGGAGGCATCTGTCCACCCATGT | CCCTGTGATGCCGACGGGGT |
| **CRISPR-eIGH library amplification** | | |
| oligo-F/R | GTAACTTGAAAGTATTTCGATTTCTTGGCTTTA TATATCTTGTGGAAAGGACGAAACACC | ACTTTTTCAAGTTGATAACGGACTAGCCTTAT TTTAACTTGCTATTTCTAGCTCTAAAAC |

**Supplementary Table 5.** List of sgRNA oligonucleotides used in CRISPRi screens validation. Bold– overhangs for cloning.

| Name | Target region | Sequence - Sense 5'-3' | Sequence - Antisens 5'-3' |
|---|---|---|---|
| sg-2124 | Eμ peak | **CACCG**TCCCTAAGCCCCTGTCAGGA | **AAAC**TCCTGACAGGGGCTTAGGGA**C** |
| sg-2141 | Eμ peak | **CACCG**CCCTGCTCTCATCAAGACCG | **AAAC**CGGTCTTGATGAGAGCAGGG**C** |
| sg-2445 | 3'RR_peak 1 | **CACCG**TGGGGGGAAGGCTGGCACCC | **AAAC**GGGTGCCAGCCTTCCCCCCA**C** |
| sg-2494 | 3'RR_peak 1 | **CACCG**GCTGCGGCCCGGTGCCCATG | **AAAC**CATGGGCACCGGGCCGCAGC**C** |
| sg-4017 | 3'RR_peak 2 | **CACCG**TGACTCATTCTGGGCAGACT | **AAAC**AGTCTGCCCAGAATGAGTCA**C** |
| sg-4107 | 3'RR_peak 2 | **CACCG**CCCGAGGCTAGGCTGTGGGA | **AAAC**TCCCACAGCCTAGCCTCGGG**C** |
| sg-NT1 | non-targeting control | **CACCG**ACGGAGGCTAAGCGTCGCAA | **AAAC**TTGCGACGCTTAGCCTCCGT**C** |
| sg-NT2 | non-targeting control | **CACCG**ATCGTTTCCGCTTAACGGCG | **AAAC**CGCCGTTAAGCGGAAACGAT**C** |

**Supplementary Table 6.** List of primers used in preparation of CRISPR-eIGH library for NGS.

| Name | Sequence 5'-3' |
|---|---|
| Fwd-1 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTAAGTAGAGGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Fwd-2 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCATGCTTAGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Fwd-3 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATGCACATCTGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Fwd-4 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATTGCTCGACGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Fwd-5 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTCGATAGCAATTCGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Fwd-6 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTATCGATAGTTGCTTGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Fwd-7 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGATCGATCCAGTTAGGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Fwd-8 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCGATCGATTTGAGCCTGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Fwd-9 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTACGATCGATACACGATCGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Fwd-10 | AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTACGATCGATGGTCCAGAGCTTTATATATCTTGTGGAAAGGACGAAACACC |
| Rev-1 | CAAGCAGAAGACGGCATACGAGATTCGCCTTGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACTCGGTGCCACTTTTTCAA |
| Rev-2 | CAAGCAGAAGACGGCATACGAGATATAGCGTCGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACTCGGTGCCACTTTTTCAA |
| Rev-3 | CAAGCAGAAGACGGCATACGAGATGAAGAAGTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACTCGGTGCCACTTTTTCAA |
| Rev-4 | CAAGCAGAAGACGGCATACGAGATATTCTAGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACTCGGTGCCACTTTTTCAA |
| Rev-5 | CAAGCAGAAGACGGCATACGAGATCGTTACCAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACTCGGTGCCACTTTTTCAA |
| Rev-6 | CAAGCAGAAGACGGCATACGAGATGTCTGATGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACTCGGTGCCACTTTTTCAA |
| Rev-7 | CAAGCAGAAGACGGCATACGAGATTTACGCACGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACTCGGTGCCACTTTTTCAA |
| Rev-8 | CAAGCAGAAGACGGCATACGAGATTTGAATAGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACTCGGTGCCACTTTTTCAA |

**Supplementary Table 7.** List of antibodies used in this study. WB – Western Blot; IC – Immunostaining.

| Name | Host species | Company | Catalog number | Amount / dliution used | Purpose |
|---|---|---|---|---|---|
| **Primary Antibodies** | | | | | |
| Cellular Fractionation | | | | | |
| H3 | Rabbit | Abcam | ab18521 | 1:1000 | WB |
| Beta tubulin | Mouse | Abcam | ab131205 | 1:5000 | WB |
| U1 snRNP 70 | Mouse | Santa Cruz Biotechnology | sc-390899 | 1:500 | WB |
| dCas9 expression validation | | | | | |
| Cas9 | Mouse | Cell Signaling | #14697 | 1:1000 | WB |
| Oncogene expression | | | | | |
| Bcl2 | Rabbit | Abcam | ab32124 | 1:5000 | WB; SU-DHL-4 |
| Bcl2 | Mouse | BD Biosciences | 610538 | 1:1000 | WB; WSU-DLCL2 |
| Myc | Rabbit | Abcam | ab32072 | 1:10 000 | WB |
| BCR immunostaining | | | | | |
| IgG | Goat | Sothern Biotech | 2042-31 | 0.1 µg | IC |
| IgM | Mouse | BD Biosciences | 551062 | 20 µl | IC |
| **Secondary Antibodies** | | | | | |
| Anti-Mouse | Goat | Santa Cruz Biotechnology | sc-2005 | 1:10 000 | WB |
| Anti-Rabbit | Goat | Abcam | ab6721 | 1:5000 | WB |