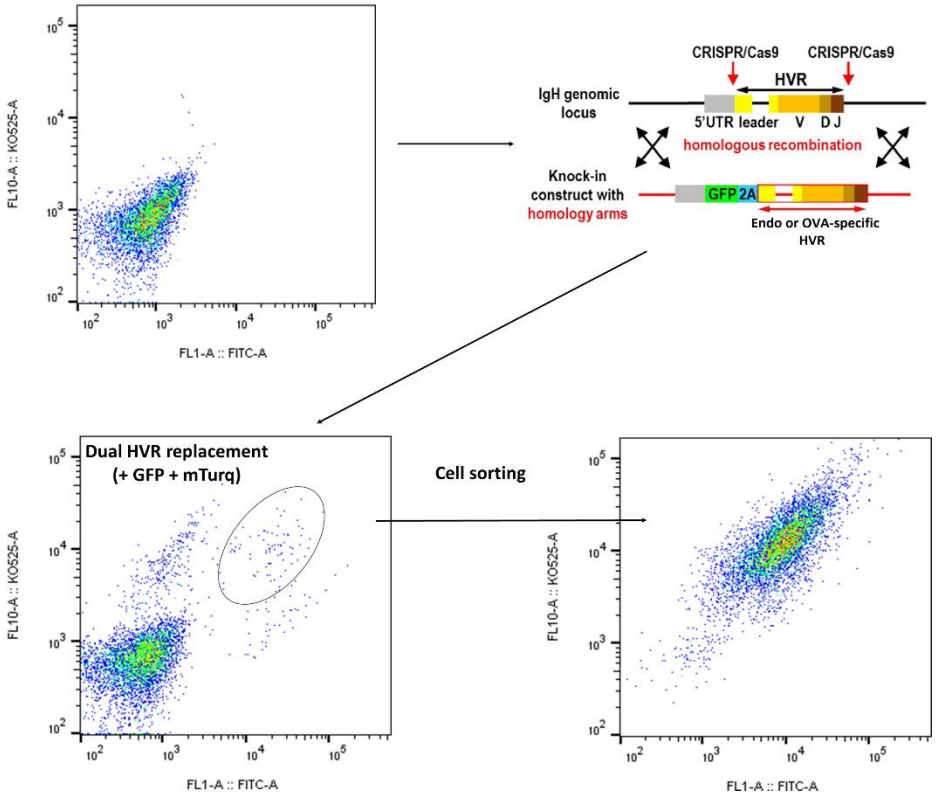
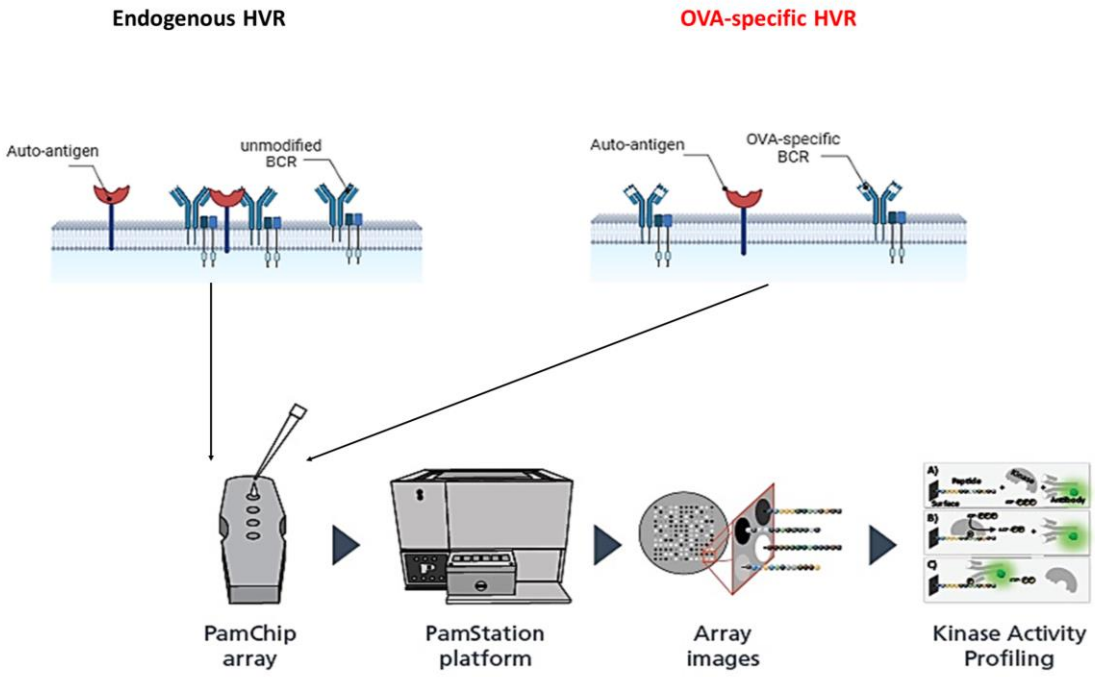
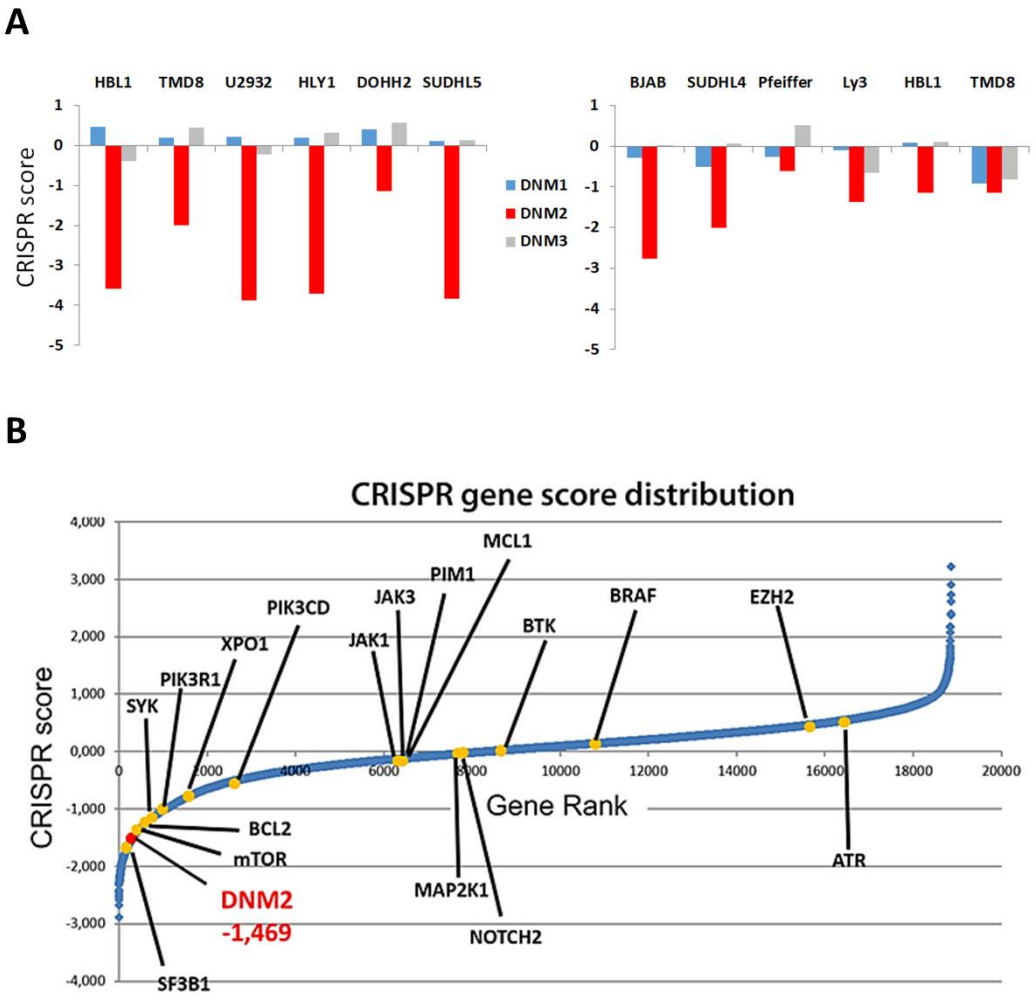
Supplementary Information



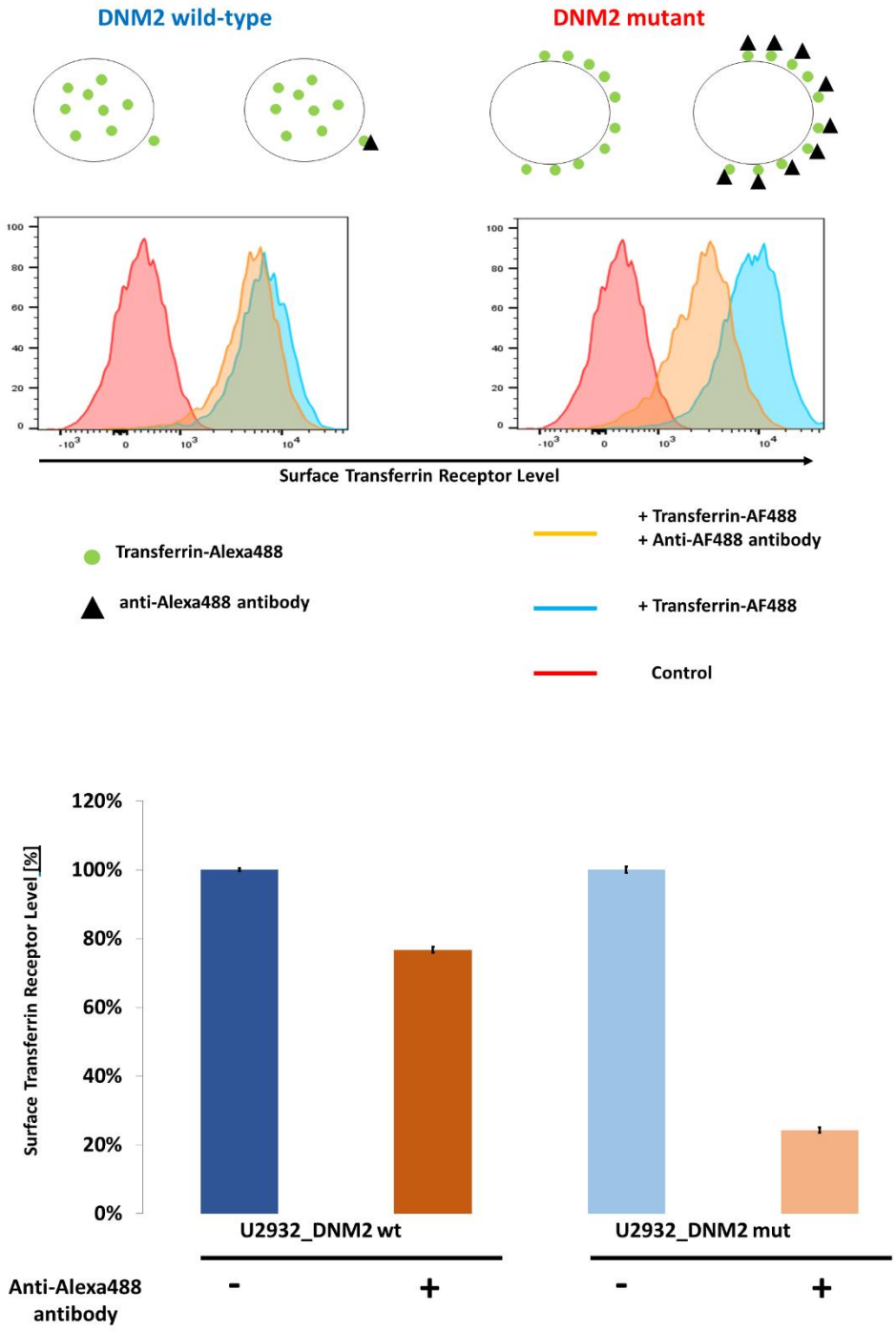
Supplementary Figure 1. Scheme illustrating the replacement of IgH hypervariable region (HVR) fragments in DLBCL cells and the associated flow cytometry gating strategy. Complementary DNA encoding a fluorescent protein (GFP for IgH), followed by sequences for a 2A peptide (which creates a break during translation), a signal peptide, and either an OVA-specific HVR or an endogenous HVR, was knocked in at the start of the immunoglobulin heavy (IgH) translation site using CRISPR-Cas9 methodology. The design for replacing the IgL HVR is similar, utilizing a CFP variant (mTurquoise2) with no D segment. Five days post-electroporation, GFP and mTurquoise2 double-positive cells were sorted for further experiments.



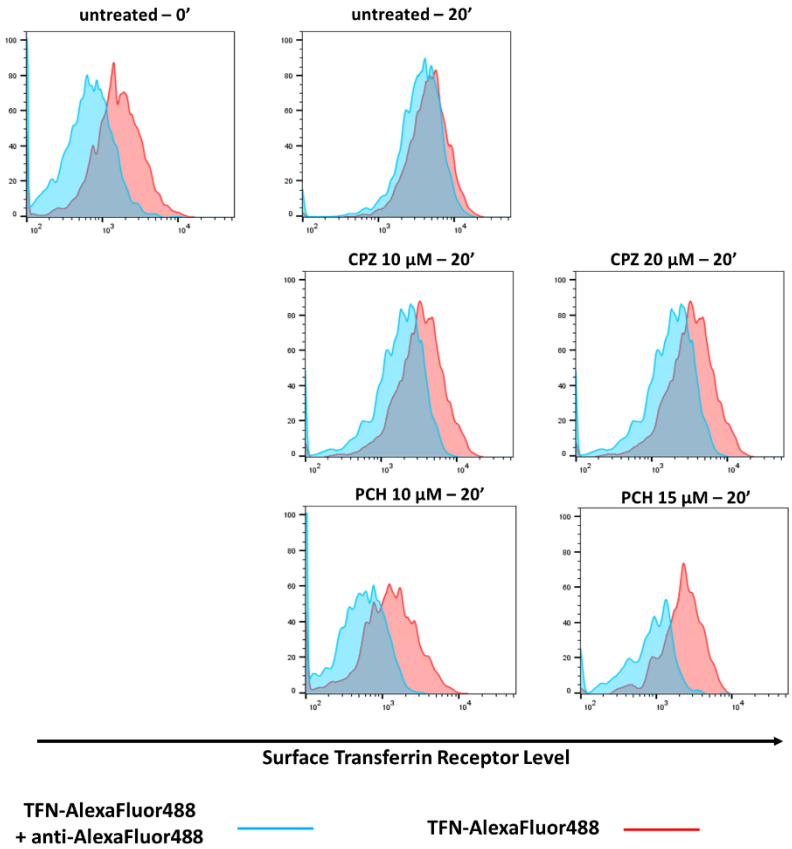
Supplementary Figure 2. Schematic presentation of experimental setup for the identification of autoantigen-dependent signaling using dual HVR-replaced DLBCL models and the PamGene platform. This fluorescent platform measures the ability of active kinases in a specimen to phosphorylate specific peptides imprinted on multiplex chip arrays. Each chip contains four arrays, with the STK and PTK arrays displaying 144 serine/threonine and 196 tyrosine immobilized peptides, respectively. Each peptide represents a 15-amino-acid sequence from putative phosphorylation sites in human proteins, derived from the literature and correlated with one or more upstream kinases. In the presence of ATP, the kinases in the sample actively phosphorylate substrates on the PamChip. Phosphorylation is detected using an antibody, while a second FITC-conjugated antibody quantifies the signal.

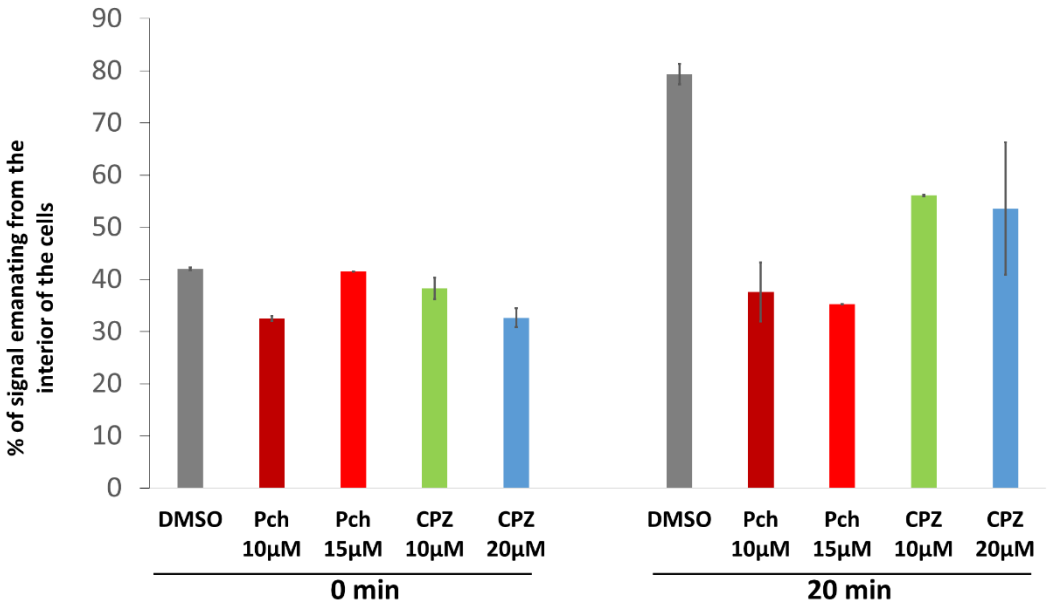


Supplementary Figure 3. CRISPR screen in DLBCL cell lines. (A) CRISPR scores for DNM1/2/3 in DLBCL cell lines, utilizing datasets from Phelan et al. (2018) for the left panel and Reddy et al. (2017) for the right panel. (B) Ranked list of CRISPR scores for the 19,032 genes targeted in the screen. Genes targeted by currently used inhibitors are highlighted in yellow, with DNM2 shown in red (data from Reddy et al., 2017). A CRISPR score < 0 indicates that CRISPR-mediated gene depletion is lethal.

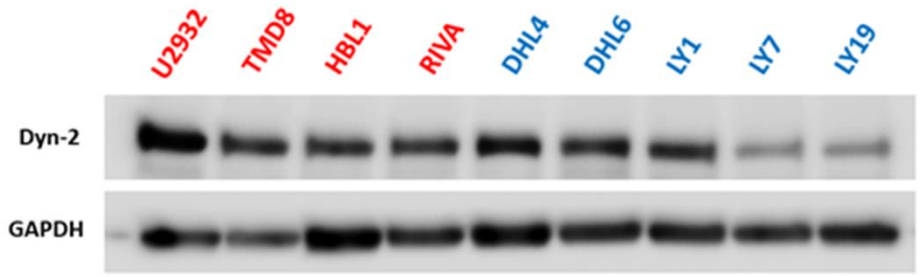


Supplementary Figure 4. Expression of DNM2mut inhibits transferrin receptor internalization in DLBCL cells. To induce DNM2 wild-type (DNM2wt) or mutant (DNM2mut) versions, U2932 cells were incubated with doxycycline (DOX) (100 ng/mL) for 24 hours. Flow cytometry-based transferrin assays were then performed, and results are presented as histograms and MFI plots (means ± SD of 2 independent replicates. Transferrin-AF488 internalization in cells expressing DNM2wt is not impaired, therefore most of the transferrin-AF488 is located inside the cell. Addition of anti-AF488 quenching antibody, does not significantly affect the signal. In cells expressing DNM2mut, transferrin-AF488 internalization is inhibited. Therefore, most of the transferrin-AF488 is located on the cell surface, and addition of anti-AF488 antibody significantly inhibits the AF488 signal.

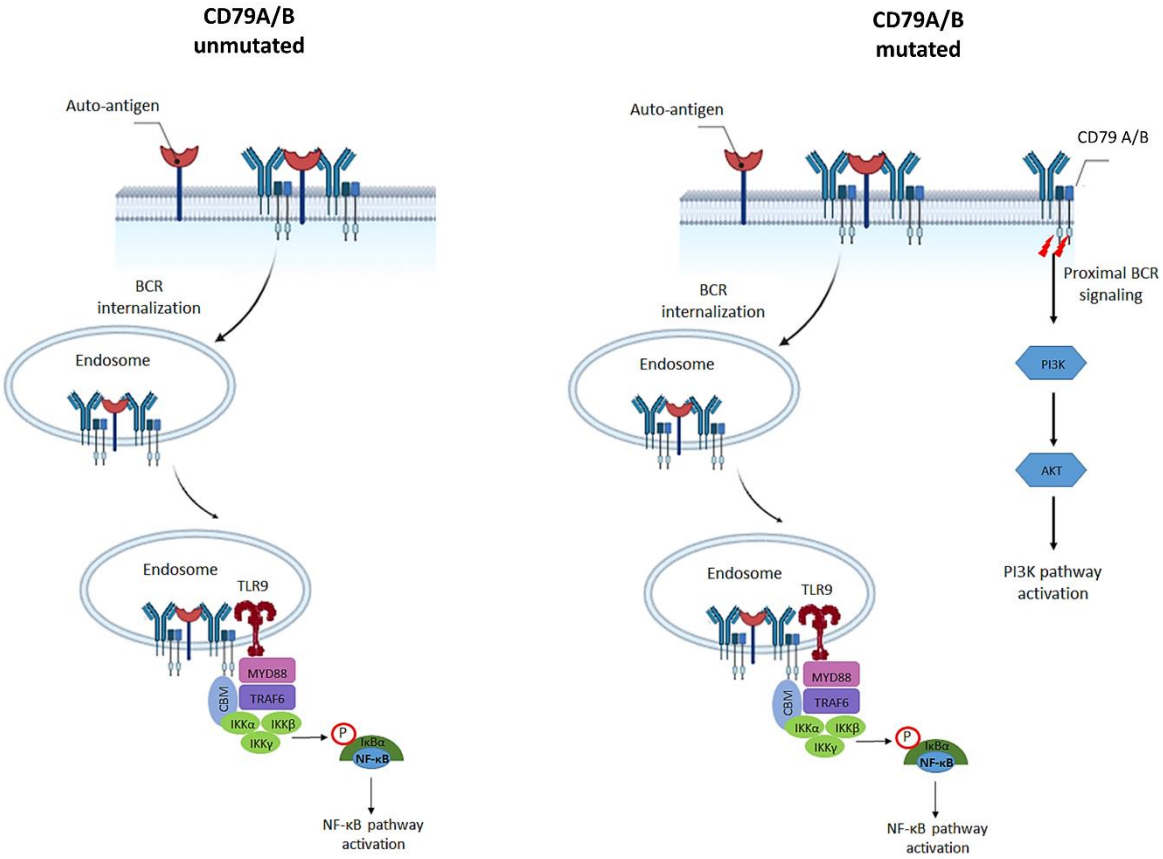




Supplementary Figure 5. Phenothiazine derivatives inhibit transferrin receptor internalization in DLBCL cells. U2932 cells were incubated with DMSO, prochlorperazine (PCH) (10 and 15 µM), or chlorpromazine (CPZ) (10 and 20 µM) for 16 hours, followed by flow cytometry-based transferrin assays. Results are presented as histograms and MFI plots (mean ± SD of 2 independent replicates).



Supplementary Figure 6. Western blot analysis demonstrating the expression of Dynamin-2 in DLBCL cell lines. GAPDH was used as a loading control.



Supplementary Figure 7. Hypothetical modes of BCR signaling in DLBCL. In the ABC-DLBCL subtype, mutations in CD79A/B disrupt BCR internalization, resulting in two distinct pools of BCRs in cells harboring heterozygous mutations: a completely wild-type BCR capable of autoantigen-induced internalization, leading to the assembly of the oncogenic BCR-TLR9 complex, and a BCR with mutated CD79A/B that remains on the cell surface, sustaining tonic BCR signaling. In ABC-DLBCL cells with wild-type CD79A/B, all BCRs can respond to autoantigens; thus, BCR signaling is predominantly reliant on internalization and the assembly of the BCR-TLR9 complex. In contrast, autoantigen-independent (GCB-type) cells primarily utilize tonic BCR signaling, with PI3K (phosphatidylinositol 3-kinase) serving as the major effector.

Supplementary Table 1. Sequences of genomic target sites used for knock-in (KI) experiments.

Name	Target Sequence (5' to 3')	Location
HBL1_HV_07	GAAGTGCTTTCTGAGAGTCATGG	V 5' UTR
HBL1_HV_04	AAACCAGGAGAGACGTTGTGAGG	beginning of post J intron
HBL1_LV_01	TGGAGAAGAGCTGCTCAGTTAGG	V 5' UTR
HBL1_LV_04	TCGTGAGATTTTAGTGCCATTGG	beginning of post J intron
Ly19_HV_08	CAGAGGACTCACCATGAAGTTGG	beginning of V
Ly19_HV_06	AAAGTAAATGAGACGTTGTGAGG	beginning of post J intron
Ly19_LV_01	AGAGATTTTCCCTGAAGTTCCGG	V intron
Ly19_LV_03	ATATATCACTTCATAGACACAGG	beginning of post J intron
U2932_HV_02	GCAAGAAAATGAAGCACCTGTGG	beginning of V
U2932_HV_03	AAAGCAGGAGAGAGGTCGTGAGG	beginning of post J intron
U2932_LV_01	TGGAGAAGAGCTGCTCAGTTAGG	V 5' UTR
U2932_LV_04	TAGATCACTTCATAGACACAGGG	beginning of post J intron

Supplementary Table 2. Basic characteristics of DLBCL cell lines used.

Cell line	Ig Heavy isotype	Ig light isotype
HBL1	M	κ
U2932	M	κ
OCI-Ly19	M	κ

Supplementary Table 3. Neon electroporation conditions.

Cell line	Electroporationconditions
HBL1	1200 V, 20 ms, 2 Pulses, R buffer
U2932	1400 V, 20 ms, 1 Pulse, R buffer
OCI-Ly19	1500 V, 10 ms, 3 Pulses, R buffer

Supplementary Table 4. Primers used in gene expression analysis.

TNAIFP3_ For	5'-ATGATACTCGGAACTGGAATG-3'
TNAIFP3_ Rev	5' - ATGACAATGATTGGCCTTCTG-3'
BCL2L1_For	5' - GGATTTGAATCTCTTTCTCTCC-3'
BCL2L1_Rev	5' - CAACCACCAGCTCCCGGT- 3'
CD40_For	5'- AACAGGCAGGCACAAACAAGACTG-3'
CD40_Rev	5'- TGGCAAACAGGATCCCGAAGATGA-3'
BCL2A1-For	5' - CAGAAGATGACAGACTGTGAA-3'
BCL2A1-Rev	5' - TCCAAGCATGACTTCAGATTC-3'
NFkBiz_For	5' - ATGGTGACACGTTCCTTCATA- 3'
NFkBiz_Rev	5' - CTGCACAATGAGATGCTGATT- 3'
GAPDH_For	5' - AGCCTCCCGCTTCGCTCTCT-3'
GAPDH_Rev	5' - CGACCAAATCCGTTGACTCCGAC-3'

Supplementary Table 5. Antibodies used.

Antigen	Source	Catalog no.	Application
Anti-Human Kappa-APC	Thermo Fisher Scientific	MH10515	Flow cytometry
Anti-Mouse CD8a-PE	BD Pharmingen	553032	Flow cytometry
Mouse IgG3-APC	R&D systems	IC007A	Flow cytometry
Rat IgG2A- PE	R&D systems	IC006P	Flow cytometry
4G10 Platinum Anti-Phospho tyrosine	Millipore	05-1050	Western Blot
Anti phospho-IκBα (S32)	Cell Signaling	2859S	Western Blot/PLA
Anti phospho-CD79A (Y182)	Cell Signaling	5173	Western Blot
Anti phospho-SRC family (Y416)	Cell Signaling	2101	Western Blot
Anti phospho-AKT (S473)	Cell Signaling	9271	Western Blot
Anti-Dynamin-2	Abcam	AB65556	Western Blot
Anti-GAPDH	Millipore	MAB 374	Western Blot
Goat Anti-Human IgM, $Fc_{5\mu}$ fragment specific	Jackson Immunoresearch	109-005-129	PLA
anti-LAMP1	Santa Cruz Biotechnology	sc-20011	PLA
Anti-TLR9	Cell Signaling	2254S	PLA

Supplementary File 1. Comparison of tyrosine and serine/threonine kinase activities in HBL1 OVA-specific vs. HBL1 control cells using the chip-based phosphoproteomic PamGene platform.

Supplementary File 2. Comparison of tyrosine and serine/threonine kinase activities in K44A-DNM2 mutant vs. wild-type DNM2-expressing HBL1 cells using the chip-based phosphoproteomic PamGene platform.

SEQUENCES

Homology arms for H-HVR replacement with inserted GFP and F2A sequences

Sequences of homology arms with inserted GFP and F2A sequences were previously published by Havranek et al. Blood 2017.

Fragments of DNA were inserted into the pSC-B-amp/kan plasmid (Agilent Technologies) and used for ligation mediated assembly of repair template plasmids for H-HVR and L-HVR replacement.

The sequences are marked as follows:
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Exons in bold: **CCTTAAA**
GFP: ATGGTGA
F2A: GTGACAG
Splice donor and acceptor sites: GT AG
Silent changes to prevent re-targeting of repair template plasmid and modified genomic locus: ACTGG
BsmBI restriction cassette: GGAGACGCCCTCGTCTCC
BsmBI restriction sites: GAGACG and CGTCTC
Cas9/sgRNA sites: underlined

HBL-1_H_HA_GFP_F2A

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OCI-Ly19_H_HA_GFP_F2A

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F2A: GTGACAG
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Silent changes to prevent re-targeting of repair template plasmid and modified genomic locus: ACTGG
BsmBI restriction cassette: GGAGACGCCCTCGTCTCC
BsmBI restriction sites: GAGACG and CGTCTC
Cas9/sgRNA sites: underlined

HBL-1_L_HA_mTruquoise2_F2A

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OCI-Ly19_L_HA_mTruquoise2_F2A

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U2932_L_HA_mTruquoise2_F2A

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AAGCTGGAGTACAACTACTTTAGCGACAACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAA
GGCCAACTTCAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGCCGACCACTACCAGCAGA
ACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCAAGCTG
AGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCGGGATCAC
TCTCGGCATGGACGAGCTGTACAAGGTGACAGAGTTGCTGTACAGGATGAAGCGGGCCGAGACCTACT
GTCCAAGGCCTCTGCTGGCAATTCACCCAACGGAGGCTCGGCATAAGCAAAAGATTGTGGCCCCTGTC
AAGCAGACTCTGAACTTCGATTTGCTCAAACTGGCCGGCGATGTGGAGTCCAATCCGGGACCCGGAGA
CGCCCTCGTCTCC**AC**GTGAGTAGAATTTAAAGTTTGCTTACTTAGTTGTCTGTGTCTTCTGCTTCCGG
TGTCTATGAAGTGATCTATAAACTGACTCTGCAATCAGCCTCTGATATCCTTCAGGGAAAAGAAAAAG
ATAAGTCTGTAGTCAAACTCGAGAATTGATTGCACATTTTCTTTGAAGAGCAAGCAAGATTCAGTCAT
TGGGTGAGAATAACTTGTCTAAGTAATAGCTTCAGAAATGTCCTGGGGAACATAACATGTTCTGGACA
GAGCCTTGGTCAATTGTCAGAAAGGGAGTTTTTGTATAGGAGGGAAGTTAAGAGGAACCATTGTGTGT
ACACTTTTGGCCAGGGGACCAAGCTGGAGATCAAACGTAAGTACTTTTTTCCACTGATTCTTCACTGT
TGCTA

Endogenous H-HVR and L-HVR fragments

Sequences of H-HVR and L-HVR were previously published by Havranek et al. Blood 2017.

Fragments of DNA were inserted into the pSC-B-amp/kan plasmid (Agilent Technologies) and used for ligation mediated assembly of repair template plasmids for H-HVR and L-HVR replacement.

The sequences are marked as follows:
Introns: GTTATAT
Exons in bold: **CCTTAAA**
F2A: GTGACAG
Splice donor and acceptor sites: GT AG
Silent changes to prevent re-targeting of repair template plasmid and modified genomic locus: ACTGG
BsmBI restriction sites: GAGACG and CGTCTC
BbsI restriction sites: GAAGAC and GTCTTC
Cas9/sgRNA sites: underlined

HBL-1_H-HVR

GGACACTGAGAAGACCTACCC**AAACACCTGTGGTTCTTCCTCCTCCTGGTGGCAGCTCCCGGATGT**GA
GTGTCTCAGGAATGCGGATATGAAGATATGAGATGCTGCCTCTGATCCCAGGACTCACTGTGGGTTTC
TCTGTTCACAGGGGTCCTGTCCCAGGTGCAGCTACAGCAGTGGGGCGCAGGACTGTTGAAGCCTTCGG
AGACCCTGTCCCTCACTTG**CGCTGTCTATGGTGGGTCCTTCAGTGATTACTACTGGACCTGGATCCGT**
CAGTCCCCAGGAAAGGGGCTGGAGTGGATTGGGGAAATCAATCGTAGTGGAAGTACCGACTACAACCC
GTCCCTCAAGAGTCGAGTCACCATATCACTAGACACGTCCAAGAACCAATTCTCCCTGCATCTGACCT
CTGTGACCGCCGCGGACACGGCTCTATATTACTGTGCGGGGGGACAAGACTACGGTGACTATGTTAGG
GGGGGTAGTGACTACTGGGGCCAGGGAAACCTGGTCACCGTCTCCTCAGGTGGGTCTTCGACC

HBL-1_L-HVR

GGAACGTCTCCACCC**GAGACACCCGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAG**
GTGAGGGGAACATGGGATGGTTTTGCATGTCAGTGAAAACCCTCTCAAGTCCTGTTACCTGGCAACTC
TGCTCAGTCAATACAATAATTAAAGCTCTGTATAAAGCAATAATTCTGGCTCTTCTGGGAAGACAATG
GGTTTGATTTAGATTACATGGGTGACTTTTCTGTTTTATTTCCAATCTCAG**ATACCACCGGAGAAATT**
GTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGC
CAGTCAGAGTATTAGCAGCAACTACTTAGCCTGGTTCCAGCTGAAAGGTGGCCAGGCTCCCAGGCTCC
TCATCTTTGGTGCATCCAACAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACA
GACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATGG
TAGCTCACCGATCACTTTCGGCCCTGGCACAAAGGTGGATATCAAACGTGGAGACGAAACC

OCI-Ly19_H-HVR

GGATGTCGTCTCCACCC**AAGTTGGGGCTGTGCTGGGTTTTCCTTGTTGGTATTTTAGAAG**GTGATTCA
TGGAAAACTAGAGAGATTTAGTGTGTGTGGATATGAATGAGACAAACAGTGGATATGTGTGGCAGTTT
CTGATTTTGGTGTCTCTTTGTTTGC**AGGTGTCCAGTGTGAAGTTGAGTTGGTGGAGTCTGGGGGAGGG**
TTGGTACAGCCTGGGGGGTCCTTGAGACTCTCCTGTGAAGTCTCTGGATTCACCTTCAATACCTATAC
TATGAGCTGGGTCCGCCAGGCTCCAGGTAAGGGGCTGGAGTGGGTTTCAAATATTAGTAGTAGTAGTA

GTGCCATATACTATGCAGGCTCTGTGAAGGGCCGATTCATCATCTCCAGAGACAATGCCAAAAACTCA
TTATATCTGCAAATGAACAACCTGAGAGCCGAGGACACGGCTGTCTATTTCTGTGCGCGAGCGTCTTA
TGATTCGGGGACTTATTTCCACGACTACTGGGGCCAGGGAACACTCGTCACAGTCTCCTCAGGTGGAG
ACGACC

OCI-Ly19_L-HVR

GGAACGTCTCCACCC**GACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCTGGCTCCGAGG**
TAAGGATGGAGAACACTAGGAATTTACTCAGCCAGTGTACTCAGTACTGATCGAAGCTTCAGGGAAAA
TCTCTGATAACATGATTAGTAGTAAAAATCTTTGTTTTTATGTTTTCACTTTCAG**GTGCCAGATGTGA**
CATCCAGATGACCCAGTCTCCCTCGTCCCTGTCAGCATCTGTAGGAGACAGAGTCACTATCACTTGCC
GGGCAAGTCAGAATATTAGGACCAATTTAAATTGGTATCAACAAAAACCAGGGAGGGCCCCTAAGGTC
CTGATCTATGCCGCTTCCAGTTTGCAAAGTGGAGTCCCATCAAGATTCAGTGGCAGTGGATCTGGGAC
ATATTTCACTCTCACCATTAGCAGTCTGCAGCCTGAGGATTTTGCAACTTTCTATTGTCAACAGACGT
ACAGTTCGTCGTGGACGTTCGGCCAAGGCACAAAAGTGGAAATCAGACGTGGAGACGAAACC

U2932_H-HVR

GGACGAAGACCTACCC**AAGCACCTGTGGTTCTTCCTCCTGCTGGTGGCGGCTCCCAGATGT**GAGTGTT
TCTAGGATGCAGACATGGAGATATGGGAGACTGCCTCTGATCCCAGGGCTCACTGTGGGTTTTTCTGT
TCAC**AG**GGGTCCTGTCCCAGCTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACC
CTGTCCCTCACCTGCACTGTCTCTCGTGTCTCCATCAGCAGTAGTAATTACTACTGGGGCTGGATCCG
CCAGCCCCCAGGGAAGGGGCTGGAATGGATTGGGAGTGACTATTATGGTGGCAGTACCTCCTACAACC
CGTCCCTCAAGGGTCGAGTCATTATATCCGTAGACACGTCCAAGAACCACTTCTCCCTGAAACTGACC
TCTGTGACCGCCGCAGACACGGCTCTATATTACTGTGCGAGAGCGCTGTCTTACTATGATACTGGTGG
TTTCAGGTACTTCTTCGATTATTGGGGCCAGGGAACACTGGTCACAGTGTCCTCAGGTGGGTCTTCGA
CC

U2932_L-HVR

GGAACGTCTCCACCC**GAAACCCCAGCGCAGCTTCTCTTCCTCCTGCTACTCTGGCTCCCAGGT**GAGGG
GAACATGGGATGGTTTTGCATGTCAGTGAAAACCCTCTCAAGTCCTGTTACCTGGCAACTCTGCTCAG
TCAATTCAATAATTAAAGCTCAATATAAAGCAATAATTCTGGCTCTTCTGGGGAGACAATGGGTTTGA
TTTAGATTACATGGGTGACTTTTCTGTTTTATTTCCAATCTC**AGATACCACCGGAGAAATTGTGTTGA**
CGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCAGTCAG
AGTGTTAGCAGCAGCTACTTAACCTGGTACCAGCAGAAACCTGGCCAGGCTCCCAGGCTCCTCATCTA
TGGTGCGTCCAACAGGGCCACTGGCATCCCAGACAGGTTCAGTGGCAGTGGGTCTGGGACAGACTTCA
CTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGTATCGTAGCTCA
CCTCCGACGTGGACGTTCGGCCAAGGGACAAAAGTCGAGATCAAACGTGGAGACGAAACC

Ovalbumin (OVA) recognizing HVR fragments

Fragments of DNA were inserted into the pSC-B-amp/kan plasmid (Agilent Technologies) and used for ligation mediated assembly of repair template plasmids for H-HVR and L-HVR replacement.

The sequences are marked as follows:

Introns: GTTATAT
Exons in bold: **CCTTAAA**
F2A: GTGACAG
Published sequence that used to re-create the HVR underlined **GGCTGT**
Splice donor and acceptor sites: GT AG
Changes to disrupt low GC window for better gBlock Synthesis C
BsmBI restriction sites: GAGACG and CGTCTC

OVA-H-HVR

GGAACGTCTCCACCC**GGATGGAGCTGTATCATCCTCTTTTTGGTAGCAACAGCTACAGGT**AAGGGGCT
CACAGTAGCAGGCTTGAGATCTGGCAATACACTTGGGTGACAATGACATCCACTCTCTCTTTCTCTCC
ATAG**GTGTCCACTCCCAGGTCCAACTGCAGCAGCCTGGGGCTGTGTTGGTGAGGCCTGGGGCTTCAGT**
GAAGCTGTCCTGTAAGGCTTCTGGCTACATCTTCACCAGTTACTGGATGAATTGGGTGAAACAGAGGC
CTGGACAAGGCCTTGAATGGATTGGTATGATTGATTGTTCAGACAGAAAAACTCACTACAATCAAATG
TTCAAAGACAAGGCCACATTGACTGTTGACAAGTCCTCCAATATAGCCTACATTCAGCTCATCAGTCT
GACATCTGAGGACTCTGCGGTCTATTACTGTTCAAGGGGGAGTAAATACTGGGGCCAAGGGACTCTGG
TCACTGTCTCTTCAGGTGGAGACGAAACC

OVA-L-HVR

GGAACGTCTCCACCC**ATGAGTCCTGCCCAGTTCCTGTTTCTGTTAGTGCTCTGGATTCGGGGT**AAGGA
GTTCTGGAATGGGAGGGATGAGAATGGGGATGGAGGGTGATCTCTGGATGCCTATGTGTGCTGTTTAT
TTGTGGTGGGGCAGGTCATATCTTCTAGGATGTGAGGTTTTGTTACATCCTAATGAGATATTCCAGAT
GGAACAGTAGCTGTACTAAGATCAATATTCTGACATAGATTGGATGGAGTGGTATAGACTCTGATGTT
TAGAACCTTCAACATTTGTTTTATGACAAGATATTTGATATATCATATCTTTAAATCTGAAAAACTGC
TAGGATCTTACTTGAAAGGAATAGCATTTTCAAGTAAGATTTCAAGTAGATTTTCAAGTAGATTTCAC
AAAGGTTGCTCAGGACCTTTGCACATGATTTTCCACTATTGTATTGTAATTTCAG**AAACCAACGGTGA**
TGTTGTGATGACCCAGACTCCACTCACTTTGTCGGTTACCATTGGACAACCAGCCTCCATCTCTTGCA
AGTCAAGTCAGAGCCTCTTAGATAGTGATGGAAAGACATATTTGAATTGGTTGTTACAGAGGCCAGGC
CAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTAAACTGGACTCTGGAGTCCCTGACAGGTTCAGTGG
CAGTGGGTCTGGAACGGATTTCACACTGAAAATCAGCAGAGTGGAGGCTGAGGATTTGGGAGTTTATT
ATTGCTGGCAAGCTACACATTTTCCTCAGACGTTCGGTGGAGGTACCAAGTTGGAAATCAAACGTGGA
GACGAAACC

Sequences of murine CD8a (mCD8) protein and truncated mCD8a, fused to 17mer-OVA peptide

mCD8A sequence

ATGGCCTCACCGTTGACCCGCTTTCTGTCGCTGAACCTGCTGCTGCTGGGTGAGTCGATTATCCTGGGGAGTGGA
GAAGCTAAGCCACAGGCACCCGAACTCCGAATCTTTCCAAAGAAAATGGACGCCGAACTTGGTCAGAAGGTGGAC
CTGGTATGTGAAGTGTTGGGGTCCGTTTCGCAAGGATGCTCTTGGCTCTTCCAGAACTCCAGCTCCAAACTCCCC
CAGCCCACCTTCGTTGTCTATATGGCTTCATCCCACAACAAGATAACGTGGGACGAGAAGCTGAATTCGTCGAAA
CTGTTTTCTGCCATGAGGGACACGAATAATAAGTACGTTCTCACCCTGAACAAGTTCAGCAAGGAAAACGAAGGC
TACTATTTCTGCTCAGTCATCAGCAACTCGGTGATGTACTTCAGTTCTGTCGTGCCAGTCCTTCAGAAAGTGAAC
TCTACTACTACCAAGCCAGTGCTGCGAACTCCCTCACCTGTGCACCCTACCGGGACATCTCAGCCCCAGAGACCA
GAAGATTGTCGGCCCCGTGGCTCAGTGAAGGGGACCGGATTGGACTTCGCCTGTGATATTTACATCTGGGCACCC
TTGGCCGGAATCTGCGTGGCCCTTCTGCTGTCCTTGATCATCACTCTCATCTGCTACCACAGGAGCCGAAAGCGT
GTTTGCAAATGTCCCAGGCCGCTAGTCAGACAGGAAGGCAAGCCCAGACCTTCAGAGAAAATTGTGTAA

Signal peptide, extracellular, transmembrane, and intracellular.

OVA 17-mer

TTCGACAAGTTGCCCGGCTTCGGAGATTCCATCGAAGCCCAGGGGGGCAAG

Translated: FDKLPGFGDSIEAQGGK

mCD8a-flag-2xOVA-17-mer

GGAAGGCCTCTGAGGCCACCATGGCCTCACCGTTGACCCGCTTTCTGTCGCTGAACCTGCTGCTGCTGGGTGA
GTCGATTATCCTGGGGAGTGGAGAAGCTAAGCCACAGGCACCCGAACTCCGAGACTATAAAGACGACGACGAC
AAAGACTATAAGGACGACGATGACAAGGATTACAAGGACGACGACGATAAGTTCGACAAGTTGCCCGGCT
TCGGAGATTCCATCGAAGCCCAGGGGGGCAAGGGAGGAGGCAGCGGTGGTGGAAGTGGATTCGACAAGTTGC
CCGGCTTCGGAGATTCCATCGAAGCCCAGGGGGGCAAGGATTGTCGGCCCCGTGGCTCAGTGAAGGGGACCGGAT
TGGACTTCGCCTGTGATATTTACATCTGGGCACCCTTGGCCGGAATCTGCGTGGCCCTTCTGCTGTCCTTGATCA
TCACTCTCATCTGCTACCACAGGAGCCGATAAGGCCTGTCAGGCCAACC

SfiI site, Signal peptide, part of extracellular domain, 3x flag, OVA-17-mer, linker, OVA-17-mer, part of extracellular domain, transmembrane domain, part of intracellular domain, STOP, SfiI site.

Translated correctly:
MASPLTRFLSLNLLLLGESIILGSGEAKPQAPELRDYKDDDDKDYKDDDDKDYKDDDDKFDKLPGFGD
SIEAQGGKGGGSGGGSGFDKLPGFGDSIEAQGGKDCRPRGSVKGTGLDFACDIYIWAPLAGICVALLL
SLIITLICYHRSR*

Sequences of wild-type and K44A-mutant DNM2

dymamin 2 WT

ACCACTTCCTACCCTCGAAAGGCCTCTGAGGCCACCatgggcaaccgcgggatggaagagct
gatcccgctggtcaacaaactgcaggacgccttcagctccatcggccagagctgccacctgg
acctgccgcagatcgctgtagtgggcggccagagcgccggcaagagctcggtgctggagaac
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agaccattcgcaacctggtggactcatacgtggccatcatcaacaagtccatccgcgacctc
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atccgcccagccgagccatccctgctcgacTACGGCCTGTCAGGCCAAGCTTCCATCGATAG
ACATGATAAGATACATTGATGAGTTTGGACAAACCACAACAAGAATG

dymamin 2 K44A

ACCACTTCCTACCCTCGAAAGGCCTCTGAGGCCACCatgggcaaccgcgggatggaagagct
gatcccgctggtcaacaaactgcaggacgccttcagctccatcggccagagctgccacctgg
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gctgaacaagaagagagccatccccaatcaggtgatccgcaggggctggctgaccatcaaca

acatcagcctgatgaaaggcggctccaaggagtactggtttgtgctgactgccgagtcactg
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tgccaacagtgacctcttcccagccccgcctcagatcccatctcggccagttcggatccccc
cagggattcccccaggagtgcccagcagaagacccccctgctgcgcccagccggcccaccatt
atccgcccagccgagccatccctgctcgacTAGGGCCTGTCAGGCCAAGCTTCCATCGATAG
ACATGATAAGATACATTGATGAGTTTGGACAAACCACAACAAGAATG

START, STOP, Mutated codon

PCR primers for insertion via in-fusion reaction into Sleeping Beauty pSB-tet-Bla plasmid :

Dynamin_01_F ACCCTCGAAAGGCCTCTGAGGCCACCatgggcaaccgcgggatg
Dynamin_01_R ATGGAAGCTTGGCCTGACAGGCCCTAGTCGAGCAGGGATGGCTCGG