

Supplementary Materials

The miR-26b-5p/KPNA2 Axis Is an Important Regulator of Burkitt Lymphoma Cell Growth

Fubiao Niu, Marta Kazimierska, Ilja M. Nolte, Miente Martijn Terpstra, Debora de Jong, Jasper Koerts, Tineke van der Sluis, Bea Rutgers, Ryan M. O'Connell, Klaas Kok, Anke van den Berg, Agnieszka Dzikiewicz-Krawczyk and Joost Kluiver

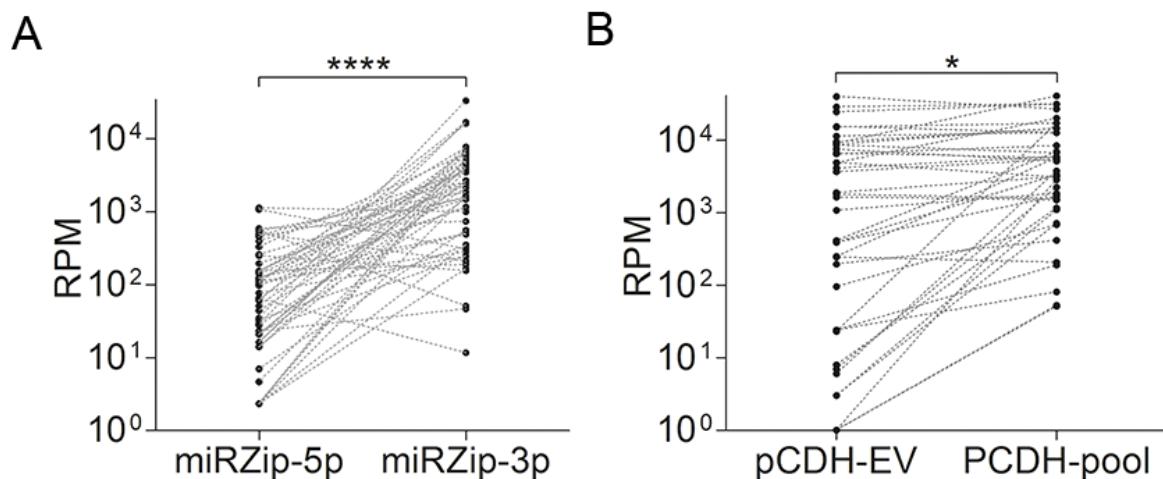


Figure S1. Quality control of miRNA inhibition and overexpression pools. (A) Read counts of the sense (miRZip-5p) and antisense (miRZip-3p, functional strand) strands of the miRZip constructs in ST486 cells infected with the miRNA inhibition library (55/60 constructs). Cells were harvested on day 6 post infection for RNA isolation and library preparation. Almost 16 million reads were generated. (B) Expression of mature miRNAs in HEK-293T cells transduced with the miRNA overexpression library (pCDH-pool, 39/45 constructs) compared to empty vector (pCDH-EV) transduced cells. Cells were harvested on day 6 post infection. Approximately 13 and 16 million reads were obtained from the pCDH-pool and pCDH-EV infected cells, respectively. RPM = reads per million. * $p < 0.05$, *** $p < 0.0001$ (Paired t -test).

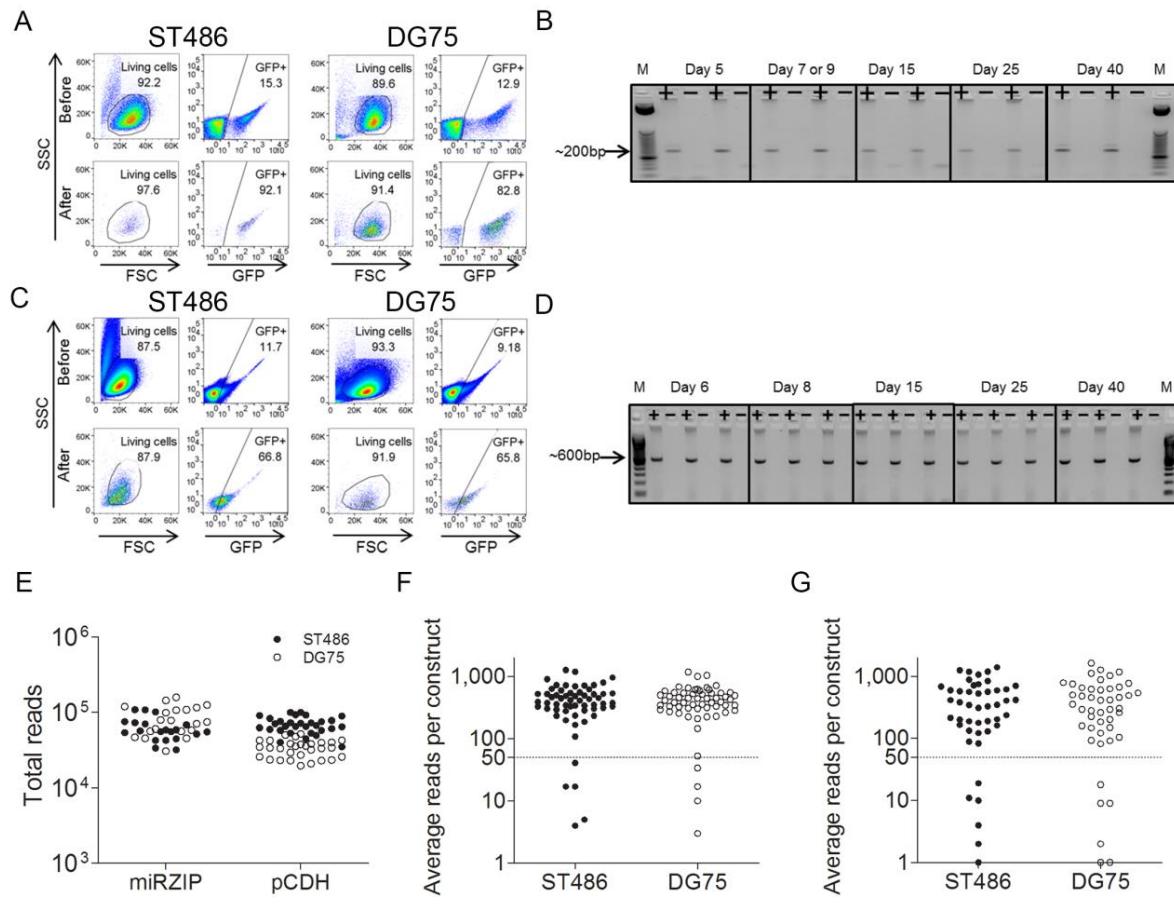


Figure S2. Sorting of infected BL cells, agarose gel electrophoresis of the PCR products, and sequencing reads overview of miRZip and pCDH pools. GFP+ cells were sorted at day 5 for (A) miRZip pool infected samples and day 6 for (C) pCDH pool infected samples. (B) Sizes of miRZip PCR products range from 172 to 194 bp. (D) Sizes of pCDH PCR products range from 572 to 602 bp. +, PCR reaction with genomic DNA; -, no template control; M, 25 bp or 100bp molecular weight DNA ladder. (E) Total reads of 40 miRZip and 60 pCDH samples. Average read counts per construct in (F) miRZip and (G) pCDH samples on sorting day. Constructs with a minimum of 50 reads in both BL cell lines were used for further analysis.

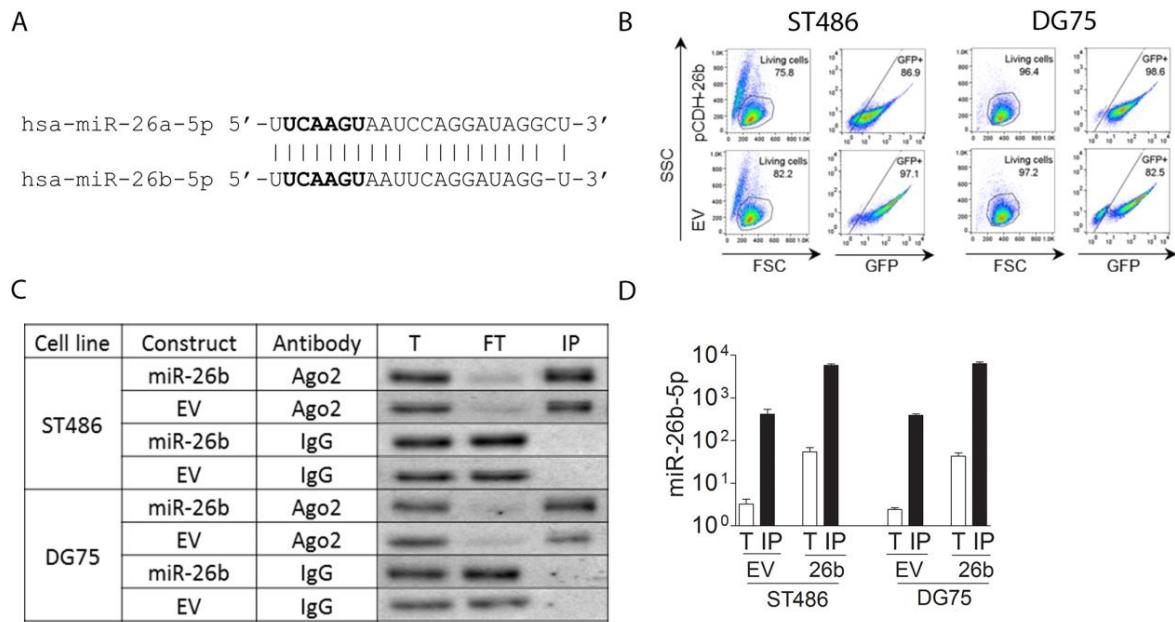


Figure S3. Ago2-IP experiment upon miR-26b-5p overexpression. **(A)** Sequences and alignment of miR-26a-5p and miR-26b-5p. The nucleotides in bold represent seed region. **(B)** Infection of ST486 and DG75 cells with miR-26b overexpression and negative control constructs. Cells were harvested at day 9 (ST486) and day 11 (DG75) post infection. **(C)** Western blot of Ago2 protein in total (T), flow through (FT), and immunoprecipitated (IP) fractions after Ago2 or control IgG IP shows Ago2 enrichment in the Ago2-IP fraction but not in the IgG-IP control fraction. **(D)** Overexpression efficiency and enrichment of miR-26b-5p in the IP fraction. MiR-26b-5p levels were 16.8- and 17.9-fold upregulated in ST486 and DG75, respectively, indicating an effective overexpression. In line with the western blot, miRNA qRT-PCR showed strong enrichment of miR-26b-5p in the Ago2-IP fractions, further indicating effective pull down of the miRNA-containing Ago2-RISC complexes.

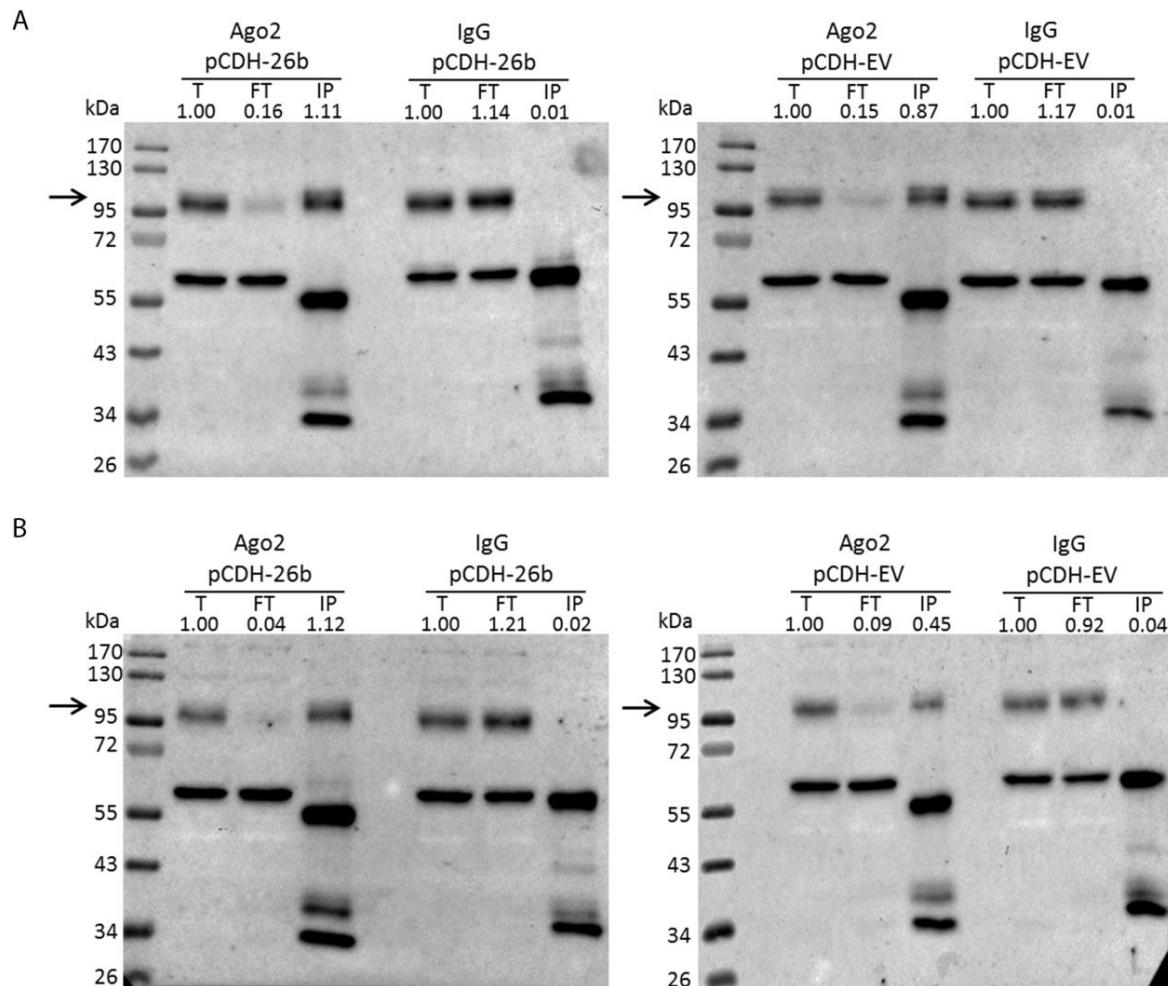


Figure S4. Uncropped western blot results for Ago2 protein in Total fraction (T), flow through fraction (FT), and Ago2-immunoprecipitation fraction (IP) samples using the anti-Ago2 antibody or the control anti-IgG antibody for miR-26b overexpression and the negative control (pCDH-EV) in (A) ST486 and (B) DG75 (see also Figure S3C). Arrows indicate the AGO2 protein. The T and FT lanes contain an aspecific band at ~60 kDa and the IP lanes also contain bands for the heavy and light chain of the antibody used for IP. For each IP experiment the total fraction is set to 1.

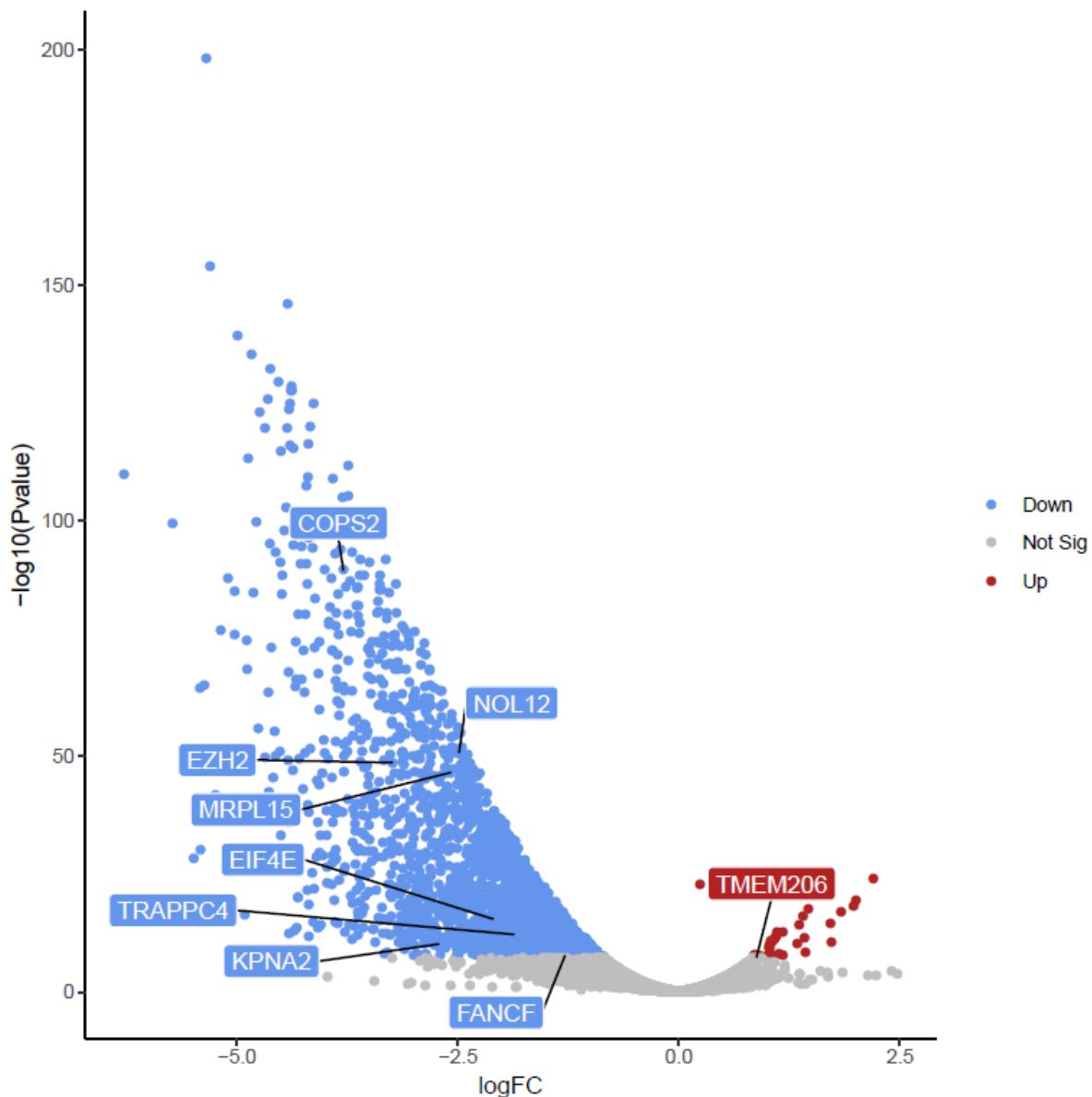


Figure S5. Volcano plot visualizing the results of the Brunello screen in ST486. The gene names indicated in the figure were identified as targets of miR-26b-5p by the Ago2-IP that showed significant changes in abundance in the Brunello screen.

Table S1. An overview of the miRZip pool infected samples and NGS raw read counts.

ST486 1 st Experiment	GFP+ (%)	Mapped Read Counts and Percentage	
		PCR 1 (%)	PCR 2 (%)
5	93	31,795 (92)	56,673 (92)
9	93	108,732 (93)	101,591 (93)
15	93	51,459 (92)	44,457 (92)
25	92	58,474 (92)	70,032 (92)
40	92	68,249 (92)	53,461 (92)
ST486 2nd Experiment			
5	92	107,316 (92)	75,083 (93)
9	88	58,643 (93)	55,291 (92)
15	90	55,313 (92)	42,473 (92)
25	89	33,564 (91)	42,457 (92)
40	89	72,573 (91)	54,945 (91)
DG75 1st Experiment			
5	83	157,866 (92)	125,162 (92)
7	82	56,140 (92)	45,032 (93)
15	79	79,209 (92)	119,128 (92)
25	77	98,804 (92)	108,243 (92)
40	75	106,658 (93)	58,098 (92)
DG75 2nd Experiment			
5	82	143,826 (93)	115,756 (92)
7	82	78,086 (93)	59,933 (93)
15	80	70,552 (92)	74,650 (93)
25	78	45,146 (92)	56,168 (92)
40	77	46,606 (92)	30,684 (92)

Table S2. An overview of the pCDH pool infected samples and NGS raw read counts.

ST486 1 st Experiment	GFP+ (%)	Mapped Read Counts and Percentage		
		PCR 1 (%)	PCR 2 (%)	PCR 3 (%)
6	63	71,329 (97)	65,326 (98)	70,674 (98)
8	68	62,557 (98)	40,014 (98)	49,125 (96)
15	56	57,815 (96)	76,006 (98)	60,922 (97)
25	48	52,953 (98)	64,646 (99)	57,869 (98)
40	47	89,435 (96)	93,227 (97)	72,239 (97)
ST486 2nd Experiment				
6	57	72,609 (97)	99,881 (98)	91,069 (97)
8	62	99,923 (97)	91,916 (97)	83,151 (97)
15	53	59,275 (94)	64,382 (94)	64,716 (98)
25	45	75,033 (97)	77,715 (99)	66,511 (99)
40	46	52,951 (99)	44,555 (97)	34,878 (99)
DG75 1st Experiment				
6	64	34,832 (98)	42,598 (98)	51,590 (98)
8	73	35,737 (98)	38,406 (97)	35,330 (98)
15	72	38,055 (98)	33,745 (95)	33,306 (98)
25	66	47,685 (98)	50,028 (99)	42,360 (99)
40	62	37,446 (99)	27,186 (99)	29,223 (95)
DG75 2nd Experiment				
6	62	34,044 (94)	39,928 (95)	39,546 (92)
8	79	23,505 (97)	42,419 (87)	33,133 (98)
15	78	22,791 (98)	31,935 (98)	25,679 (93)
25	72	25,839 (96)	19,569 (70)	20,839 (79)
40	69	23,144 (98)	22,828 (90)	23,210 (97)

Table S3. Overview of the 58 miRNAs included in the miRZip pool.

Construct	Top 30 in BL [19]	Increased in BL vs. GC-B [19]	MYC-Induced in P493-6 [12]	Increased in BL vs. CLL [12]	Increased in eBL vs. GC-B [11]	Increased in BL vs. DLBCL [10]	Increased in BL vs. DLBCL-FL [9]	Increased in BL vs. other Lymphomas [8]	Depleted in Screen
miRZip-let-7b-5p									
miRZip-let-7e-5p									✓
miRZip-let-7f-2-3p		✓							✓
miRZip-9-5p						✓			✓
miRZip-15a-5p									
miRZip-16-5p	✓								
miRZip-17-5p		✓	✓	✓					
miRZip-18a-5p			✓	✓					✓
miRZip-19a-3p			✓	✓			✓		✓
miRZip-19b-3p	✓		✓			✓			✓
miRZip-20a-5p	✓	✓	✓						
miRZip-21-3p					✓				
miRZip-21-5p		✓							✓
miRZip-23a-3p									
miRZip-23b-3p									
miRZip-24-3p									
miRZip-25-3p	✓								
miRZip-26a-5p	✓				✓				
miRZip-27a-3p						✓			
miRZip-27b-3p							✓		
miRZip-29a-3p									
miRZip-30e-5p	✓								✓
miRZip-34a-5p									
miRZip-92-3p	✓		✓		✓		✓		✓
miRZip-99b-5p					✓				
miRZip-100-5p									
miRZip-106a-5p			✓	✓					
miRZip-106b-5p							✓		✓
miRZip-125a-5p									
miRZip-142-3p									
miRZip-142-5p	✓								
miRZip-144-3p									
miRZip-146a-5p	✓								
miRZip-146b-5p					✓				

miRZip-150-5p					
miRZip-155-5p					
miRZip-181a-5p	✓		✓		✓
miRZip-182-5p	✓	✓		✓	
miRZip-183-5p	✓	✓		✓	✓
miRZip-190a-5p		✓			✓
miRZip-196a-5p					
miRZip-205-5p					
miRZip-301b					
miRZip-320a		✓			
miRZip-324-3p					
miRZip-330-5p		✓			
miRZip-345-5p					
miRZip-378a-3p	✓	✓	✓		
miRZip-431-star					
miRZip-449a-5p					✓
miRZip-494-3p			✓		✓
miRZip-500a-5p				✓	
miRZip-615-3p					✓
miRZip-615-3p					
miRZip-625-5p		✓			
miRZip-892b					
miRZip-4454		✓			
miRZip-4455					

Table S4. Overview of the 44 miRNA precursors included in the pCDH pool.

pCDH Construct	Strand	Decreased in BL vs. GC-B [19]	MYC-Repressed [12]	Decreased in BL vs. CLL [12]	Decreased in eBL vs. GCB [11]	Decreased in BL vs. DLBCL [10]	Decreased in BL vs. DLBCL-FL [9]	Decreased in BL vs. other Lymphomas [8]	Depleted/Enriched in Screens
let-7a	5p		✓	✓	✓			✓	
miR-9	5p					✓			
	3p					✓			
miR-10a	5p							✓	
miR-19b-1									
miR-21	5p	✓		✓	✓				
	3p	✓							
miR-23a	5p					✓			
	3p		✓	✓					
miR-24	3p		✓	✓					
miR-26a	5p		✓	✓		✓		✓	✓
miR-26b	5p		✓	✓		✓		✓	✓
miR-27a	3p			✓					
miR-27b	3p			✓					
miR-28	5p	✓		✓	✓				
	3p	✓							
miR-29a	3p		✓	✓	✓			✓	
miR-29b	3p		✓	✓		✓		✓	
miR-29c	5p						✓		
	3p		✓	✓	✓			✓	
miR-30a	5p	✓		✓		✓			
	3p	✓		✓					
miR-34a	5p		✓	✓					✓
miR-34b	5p			✓		✓			
	3p				✓				
miR-34c	5p								✓
miR-101	3p		✓	✓					
miR-106b									
miR-125a	5p	✓		✓					
miR-125b	5p		✓	✓		✓			
miR-141	3p	✓		✓					
miR-142	5p			✓		✓			
	3p		✓	✓					
miR-145	5p			✓					

miR-146a	5p	✓	✓		✓		✓
miR-148	3p		✓	✓			
miR-150	5p	✓	✓	✓		✓	✓
	3p	✓			✓		✓
miR-151a	5p	✓					✓
	3p	✓					✓
miR-155	5p	✓	✓	✓	✓	✓	✓
miR-181a-2							
miR-181b-2							
miR-200a							
miR-200c	3p	✓		✓			
miR-205							
miR-221	5p	✓					
	3p	✓		✓	✓	✓	✓
miR-222	3p	✓	✓	✓	✓		✓
miR-363	3p	✓					✓
miR-429							
miR-449a							
miR-451			✓		✓		✓
miR-486	5p	✓					
miR-577							

Table S5. Sequences of the single guide RNAs.

NO.	sgRNA	Sense (S)/ Anti-Sense (AS)	5' to 3'
1	COPS2_sg1	S	CACCGGTGGTGGTAAAATGCACTTG
		AS	AAACCAAGTGCATTACACCACC
2	COPS2_sg2	S	CACCGCCAGTTACATCAGTCGTGCC
		AS	AAACGGCACGACTGATGTAAGTGGC
3	KPNA2_sg1	S	CACCGGGATTATGTTGTCTATGGG
		AS	AAACCCCATAAGACAACATAATCCGC
4	KPNA2_sg2	S	CACCGAAATGAACGAATTGGCATGG
		AS	AAACCCATGCCAATTGTTCAATTTC
5	MRPL15_sg1	S	CACCGAAGTTGGCCAGGCTCACACG
		AS	AAACCGTGTGAGCCTGGCCAACCTC
6	MRPL15_sg2	S	CACCGGTTAAGTCAATAGGTTGACT
		AS	AAACAGTCAACCTATTGACTTAACC
7	NOL12_sg1	S	CACCGGCTCGTTCTTAGCTTCGAGC
		AS	AAACCGTCGAAGCTAACGAGGCC
8	NOL12_sg2	S	CACCGACCTGACAGGCTTCCACAAG
		AS	AAACCTTGGAAGCCTGTCAGGTC
9	NTCR1	S	CACCGACGGAGGCTAACGCGCAA
		AS	AAACTTGCACGCTAACGCTCCGTC
10	NTCR2	S	CACCGATCGTTCCGCTAACGGCG
		AS	AAACGCCGTTAACGCGAACGATC

Table 6. Sequences of the miR-26b-5p binding sites from selected genes cloned in the reporter vector.

miR-26b-5p Binding Site (MBS)	Sense(S)/Anti-Sense (AS)	5' to 3'
COPS2_MBS1	WT S	TCGATTACCTGCAGAAATTCTCTCACAAATAACCTGCAATA <ins>ACTTGA</ins> AATGCATACCC
	WT AS	GGCCGGGTATGCATTCAAGTTAGTATTGAGAGAATTCTGCAGGTA
	MUT S	TCGATTACCTGCAGAAATTCTCTCACAAATAACCTGCAATA <ins>AGTAGT</ins> AATGCATACCC
	MUT AS	GGCCGGGTATGCATTACTACTTATTGAGAGAATTCTGCAGGTA
COPS2_MBS2	WT S	TCGACAAAAGCGGCATTAAGCAGTTCAAAGGTTGGA <ins>ACTTGA</ins> GGTAAAAAG
	WT AS	GGCCCTTTCACCTCAAGTCCAAAACCTTGGAAACTGCTTAATGCCGTTTG
	MUT S	TCGACAAAAGCGGCATTAAGCAGTTCAAAGGTTGGA <ins>AGTAGT</ins> GGTAAAAAG
	MUT AS	GGCCCTTTCACCTACTACTTCCAAAACCTTGGAAACTGCTTAATGCCGTTTG
KPNA2	WT S	TCGATGGTTTACTGTAGCACTTTACACTGAAACTA <ins>TACTTGA</ins> CAGTCCAAC
	WT AS	GGCCGTTGGAACTGTTCAAGTATAGTTCAGTGTAAAAGTGTACAGTAACAAACCA
	MUT S	TCGATGGTTTACTGTAGCACTTTACACTGAAACTA <ins>AGTAGT</ins> CAGTCCAAC
	MUT AS	GGCCGTTGGAACTGTTACTATAGTTCAGTGTAAAAGTGTACAGTAACAAACCA
MRPL15	WT S	TCGAGGTACCTGGGGATCCTGCCAATTCTGAAGCACG <ins>ACTTGA</ins> CTGCCAGGA
	WT AS	GGCCTCTGGCGAGTCAACTCGCTTCAGGAAATTGCCAGGATCCGCCAGGTACC
	MUT S	TCGAGGTACCTGGGGATCCTGCCAATTCTGAAGCACG <ins>AGTAGT</ins> CTGCCAGGA
	MUT AS	GGCCTCTGGCGAGTACTACTCGCTTCAGGAAATTGCCAGGATCCGCCAGGTACC
NOL12_MBS1	WT S	TCGATAATCCCAGCTACTGGGAGGCTGAGGTGGAAGAAC <ins>ACTTGA</ins> CCCTGGAGGC
	WT AS	GGCCGCCTCCAGGGTTCAAGTATTCTCACCTCAGCCTCCAAAGTAGCTGGGATTA
	MUT S	TCGATAATCCCAGCTACTGGGAGGCTGAGGTGGAAGAAC <ins>AGTAGT</ins> CCCTGGAGGC
	MUT AS	GGCCGCCTCCAGGGTACTACTGATTCTCACCTCAGCCTCCAAAGTAGCTGGGATTA
NOL12_MBS2	WT S	TCGAGAGCAGAGGAAGCTCGGGAGGAGGCCACCAGGA <ins>TACTTGA</ins> GATGCTGGCA
	WT AS	GGCCTGCCAGCATCTCAAGTATTCTGGCGCTCCCGAAGCTCCTCTGCTC
	MUT S	TCGAGAGCAGAGGAAGCTCGGGAGGAGGCCACCAGGA <ins>AGTAGT</ins> GATGCTGGCA
	MUT AS	GGCCTGCCAGCATCTACTATTCCTGGCGCTCCCGAAGCTCCTCTGCTC

Green letters indicate miR-26b-5p binding sites and red letters indicate the mismatches introduced in the mutants.



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