

Supplementary Materials: Timing of Novel Drug 1A-116 to Circadian Rhythms Improves Therapeutic Effects against Glioblastoma

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Primers for exon 1, 2 and 3 of *p53* gene to detect Pro98Leu mutation, and primers for exon 1, 2, 3 and 4 of *PTN* gene were designed. Oligos for *cyclin dependent kinase inhibitor 2A*, that encodes for many transcriptional variants, including *p14ARF* and *p16* genes, were also designed.

P53

Alignment: Global DNA alignment against reference molecule
Parameters: Scoring matrix: Linear (Mismatch 2, OpenGap 4, ExtGap 1)

Reference molecule: mRNA p53var1, Region 1 to 2591
Number of sequences to align: 3
Total length of aligned sequences with gaps: 2591 bps
Settings: Similarity significance value cutoff: >= 60%

Summary of Percent Matches:

Ref:	mRNA p53var1	1 to	2591	(2591 bps)	--
2:	ex3p53	1 to	253	(253 bps)	9%
3:	exlp53	1 to	67	(67 bps)	2%

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mRNA p53var1      1 gatgggattgggggttttccctcccatgtgctcaagactggcgctaaaagtgttgagctt
ex3p53            -----
exlp53            -----

mRNA p53var1     61 ctcaaaagtctagagccacgctccagggagcaggtagctgctgggctccggggacacttt
ex3p53            -----
exlp53            -----

mRNA p53var1    121 gcgttcgggctgggagcgtgctttccacgacggtgacacgcttccctggattggcagcca
ex3p53            -----
exlp53            -----

mRNA p53var1    181 gactgccttcgggtcactgccatggaggagcgcagtcagatcctagcgtcgagccccc Ex1
ex3p53            -----
exlp53            -----
                    1 atggaggagcgcagtcagatcctagcgtcgagccccc

mRNA p53var1    241 tctgagtcaggaaacattttcagacctatggaaactacttctctgaaaacaacgttctgtc Ex2
ex3p53            -----
exlp53            -----
                    39 tctgagtcaggaaacattttcagacctat

mRNA p53var1    301 ccccttgccgtcccaagcaatggatgatttgatgctgtccccggacgatattgaacaatg Ex3
ex3p53            -----
exlp53            -----
                    1 gatgatttgatgctgtccccggacgatattgaacaatg

mRNA p53var1    361 gttcactgaagacccaggtccagatgaagctcccagaatgccagaggctgctccccccgt Ex3
ex3p53            -----
exlp53            -----
                    39 gttcactgaagacccaggtccagatgaagctcccagaatgccagaggctgctccccccgt

mRNA p53var1    421 ggccccctgcaccagcagctcctacacggggggccccctgcaccagccccctcctggcccc Ex3
ex3p53            -----
exlp53            -----
                    99 ggccccctgcaccagcagctcctacacggggggccccctgcaccagccccctcctggcccc

mRNA p53var1    481 gtcattcttctgtccttccagaaaaacctaccagggcagctacggtttccgtctgggctt Ex3
ex3p53            -----
exlp53            -----
                    159 gtcattcttctgtccttccagaaaaacctaccagggcagctacggtttccgtctgggctt

mRNA p53var1    541 cttgcattctgggacagccaagtctgtgacttgcaactccctgcctcaacaagat Ex3
ex3p53            -----
exlp53            -----
                    219 cttgcattctgggacagccaagtctgtgacttgca

mRNA p53var1    601 gttttgccaaactggccaagacctgcctgtgcagctgtgggttgattccacacccccgcc
ex3p53            -----
exlp53            -----

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In red, mutated codon 98 CCT-CTT

PTN

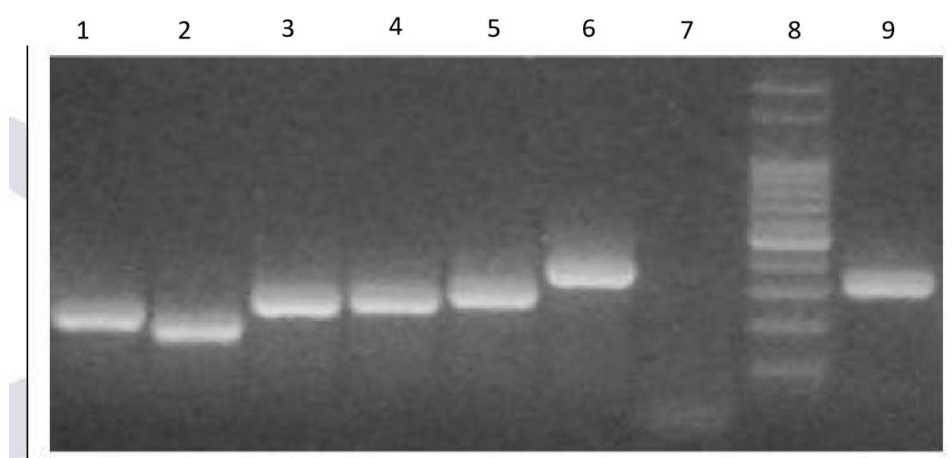
Alignment: Global DNA alignment against reference molecule
Parameters: Scoring matrix: Linear (Mismatch 2, OpenGap 4, ExtGap 1)

Reference molecule: PTN mRNA, Region 297 to 803
Number of sequences to align: 5
Total length of aligned sequences with gaps: 507 bps
Settings: Similarity significance value cutoff: >= 60%

Summary of Percent Matches:

Ref:	PTN mRNA	297 to	803	(507 bps)	--
2:	ex1PTN	1 to	59	(59 bps)	11%
3:	ex2PTN	1 to	49	(49 bps)	9%
4:	ex3PTN	1 to	140	(140 bps)	27%
5:	ex4PTN	1 to	56	(56 bps)	11%

PTN mRNA	297	atgcaggctcaacagtaccagcagcagcgtcgaaaatttgcagctgccttcttggcattc	Ex1
ex1PTN	1	-----attc	
ex2PTN		-----	
ex3PTN		-----	
ex4PTN		-----	
PTN mRNA	357	attttcatactggcagctgtggatactgctgaagcaggggaagaaagagaaaccagaaaaa	
ex1PTN	5	attttcatactggcagctgtggatactgctgaagcaggggaagaaagagaaaccag-----	
ex2PTN	1	-----aaaaa	
ex3PTN		-----	
ex4PTN		-----	
PTN mRNA	417	aaagtgaagaagtctgactgtggagaatggcagtgagtggtgtgtgtgccaccagtgga	Ex2
ex1PTN		-----	
ex2PTN	6	aaagtgaagaagtctgactgtggagaatggcagtgagtggtgtgtg-----	
ex3PTN		-----	
ex4PTN		-----	
PTN mRNA	477	gactgtgggctgggcacacgggagggcactcggactggagctgagtgcagcaaacatg	
ex1PTN		-----	
ex2PTN		-----	
ex3PTN		-----	
ex4PTN		-----	
PTN mRNA	537	aagaccagagatgtaagatcccctgcaactggaagaagcaatttggcgaggagtgcaaa	
ex1PTN		-----	
ex2PTN		-----	
ex3PTN		-----	
ex4PTN		-----	
PTN mRNA	597	taccagttccaggcctggggagaatgtgacctgaacacagccctgaagaccagaactgga	Ex3
ex1PTN		-----	
ex2PTN		-----	
ex3PTN	1	-----ggcctggggagaatgtgacctgaacacagccctgaagaccagaactgga	
ex4PTN		-----	
PTN mRNA	657	agtctgaagcgagccctgcacaatgccgaatgccagaagactgtcaccatctccaagccc	
ex1PTN		-----	
ex2PTN		-----	
ex3PTN	50	agtctgaagcgagccctgcacaatgccgaatgccagaagactgtcaccatctccaagccc	
ex4PTN		-----	
PTN mRNA	717	tgtggcaaaactgaccaagcccaaacctcaagcagaatctaagaagaagaaaaaggaaggc	Ex4
ex1PTN		-----	
ex2PTN		-----	
ex3PTN	110	tgtggcaaaactgaccaagcccaaacctcaag-----	
ex4PTN	1	-----cagaatctaagaagaagaaaaaggaaggc	
PTN mRNA	777	aagaaacaggagaagatgctggattaa	
ex1PTN		-----	
ex2PTN		-----	
ex3PTN		-----	
ex4PTN	30	aagaaacaggagaagatgctggattaa	

P14 and p16

1: Exon 4 PTN 2: Exon 1 PTN 3: Exon 3 PTN 4: Exon 2 PTN 5: Ex1-Ex2 – P53
 6: Ex3 –P53 **7: p16/p14ARF** 8: Ladder 100 pb 9: Human dystrophin gene fragment (Ctrl +)

Genetic profile of LN229 cell line evaluated by STR.

Amelogenina	X
D3S1358	16, 1/
D1S1656	14, 17
D2S441	10, 11
D10S1248	16
D13S317	10, 11
PENTA E	7, 16
D16S539	12
D18S51	13, 15
D2S1338	19, 20
CSF1PO	12
PENTA D	10, 11
TH01	9.3
VWA	16, 19
D21S11	29, 30
D7S820	8, 11
D5S818	11
TPOX	8
D8S1179	13, 14
D12S391	20, 22
D19S433	12, 17.2
SE33	29.2
D22S1045	15, 16
FGA	23
DYS391	NA
DYS576	NA

Figure S1. LN229 cell line authentication.

Sequence alignment performed with Blast, Query: sequence corresponding to a fragment of exon 2 of *bmal1*, Subject: sequence corresponding to *bmal1* WT. In red: site of Crispr/Cas9 editing. Identity: 254/256 (99%).

Sequence ID: Query_6344/ Length: 320
Range 1: 39 to 293

Score:460 bits(249), Expect:1e-134,
Identities:254/256(99%), Gaps:2/256(0%), Strand: Plus/Plus

```

Query 5   ACAGTT-CATGGGTACTGAGAGGAGGGGTGCCTGGCCTGCCTGGAACACTGGGGAGGCT 63
          ||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 39   ACAGTTCATGGGTACTGAGAGGAGGGGTGCCTGGCCTGCCTGGAACACTGGGGAGGCT 98
|
Query 64   TACAGGGAAAACTGTCCAATGGATTTAAAGGACAAGCAGGGATTTTCTAGGCAAAGAAG 123
          ||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 99   TACAGGGAAAACTGTCCAATGGATTTAAAGGACAAGCAGGGATTTTCTAGGCAAAGAAG 158

Query 124  CAGAATGGGCATCCCAGGAGTATGGTAAATCAAACTTATTGGGTGCTATGAATTTAAAG 183
          ||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 159  CAGAATGGGCATCCCAGGAGTATGGTAAATCAAACTTATTGGGTGCTATGAATTTAAAG 218

Query 184  CTAACTTTTATTTTATTGGCTTTTAGGTGGTGGCTAGAGTGATACCGTTTGGACC 243
          ||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 219  CTAACTTTTATTTTATTGGCTTTTAGGTGGTCTGGCTAGAGTGATACCGTTTGGACC 277
                                     CTGGCTAGAGTGATA-CG|

Query 244  CAAGCTTAACCTTTTCC 259
          ||||| |||||||
Sbjct 278  CAAGCTTAACCTTTTCC 293

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Sequence alignment performed with Blast, Query: sequence corresponding to a fragment of exon 2 of *bmal1*, Subject: sequence corresponding to *bmal1* E1. In red: site of Crispr/Cas9 editing. Identity: 256/262 (98%)

Sequence ID: Query_12599 Length: 320
Range 1: 39 to 299

Score:449 bits(243), Expect:3e-131,
Identities:256/262(98%), Gaps:2/262(0%), Strand: Plus/Plus

```

Query 7   ACAGTT-CATGGGTACTGAGAGGAGGGGTGCCTGGCCTGCCTGGAACACTGGGGAGGCT 65
          ||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 39   ACAGTTCATGGGTACTGAGAGGAGGGGTGCCTGGCCTGCCTGGAACACTGGGGAGGCT 98

Query 66   TACAGGGAAAACTGTCCAATGGATTTAAAGGACAAGCAGGGATTTTCTAGGCAAAGAAG 125
          ||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 99   TACAGGGAAAACTGTCCAATGGATTTAAAGGACAAGCAGGGATTTTCTAGGCAAAGAAG 158

Query 126  CAGAATGGGCATCCCAGGAGTATGGTAAATCAAACTTATTGGGTGCTATGAATTTAAAG 185
          ||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 159  CAGAATGGGCATCCCAGGAGTATGGTAAATCAAACTTATTGGGTGCTATGAATTTAAAG 218

Query 186  CTAACTTTTATTTTATTGGCTTTTAGGTGGTGGCTAGAGTGATACCGATTGGACC 245
          ||||| ||||||||||||||||||||||||||||||||||||||||||||
Sbjct 219  CTAACTTTTATTTTATTGGCTTTTAGGTGGTCTGGCTAGAGTGATACCGATTGGACC 277
                                     CTGGCTAGAGTGATA-CG|

Query 246  CAAGCTTATCTTTTGCATGTG 267
          ||||| ||||| | |||||
Sbjct 278  CAAGCTTAACCTTTCCAATGTG 299

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Figure S2. Confirmation of genomic edition of Exon2 of *bmal1* by Crispr/Casp9.

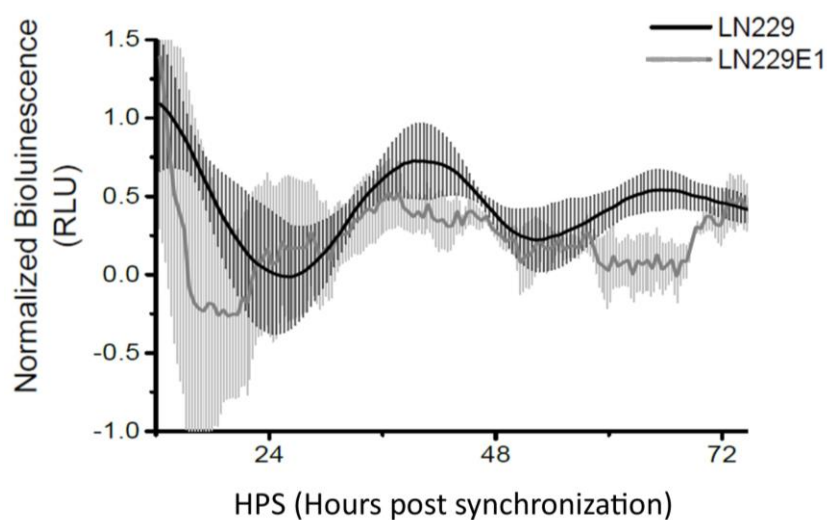


Figure S3. Circadian expression of Bmal1 is maintained for over 72 h.

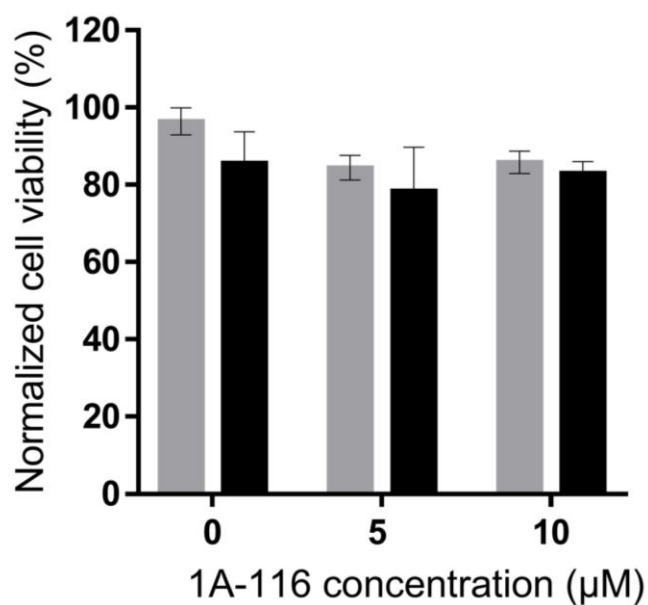


Figure S4. Cell viability.

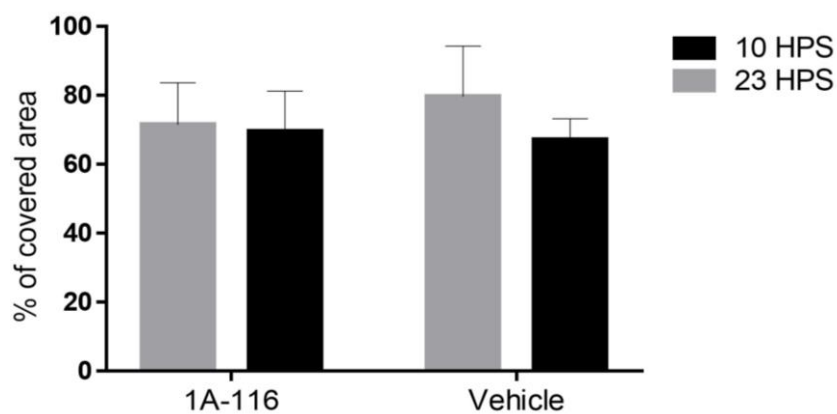


Figure S5. Number of cells outside of the migration area.