

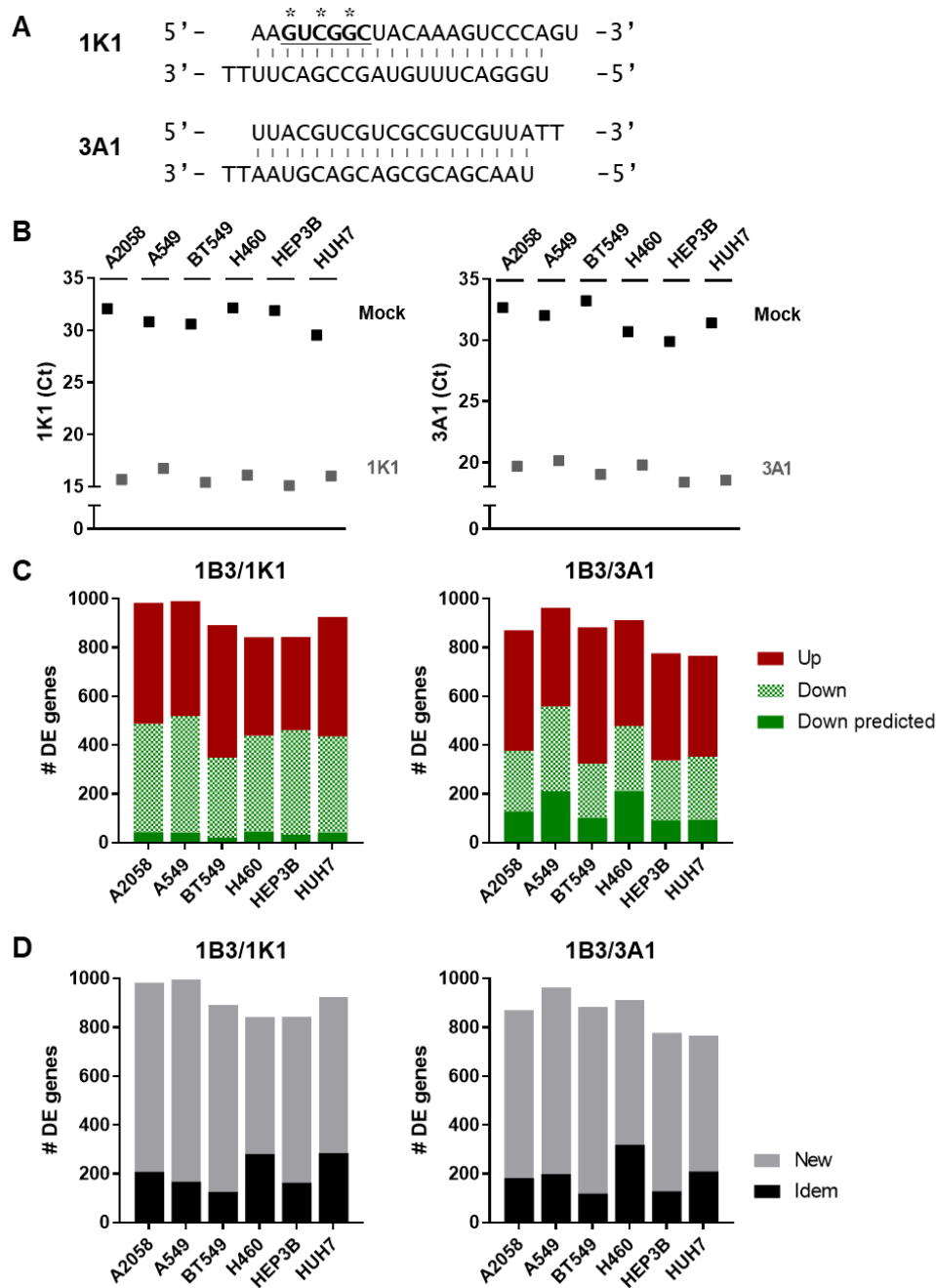
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## Supplemental Information

**Transcriptome-wide analysis reveals insight  
into tumor suppressor functions of 1B3, a novel  
synthetic miR-193a-3p mimic**

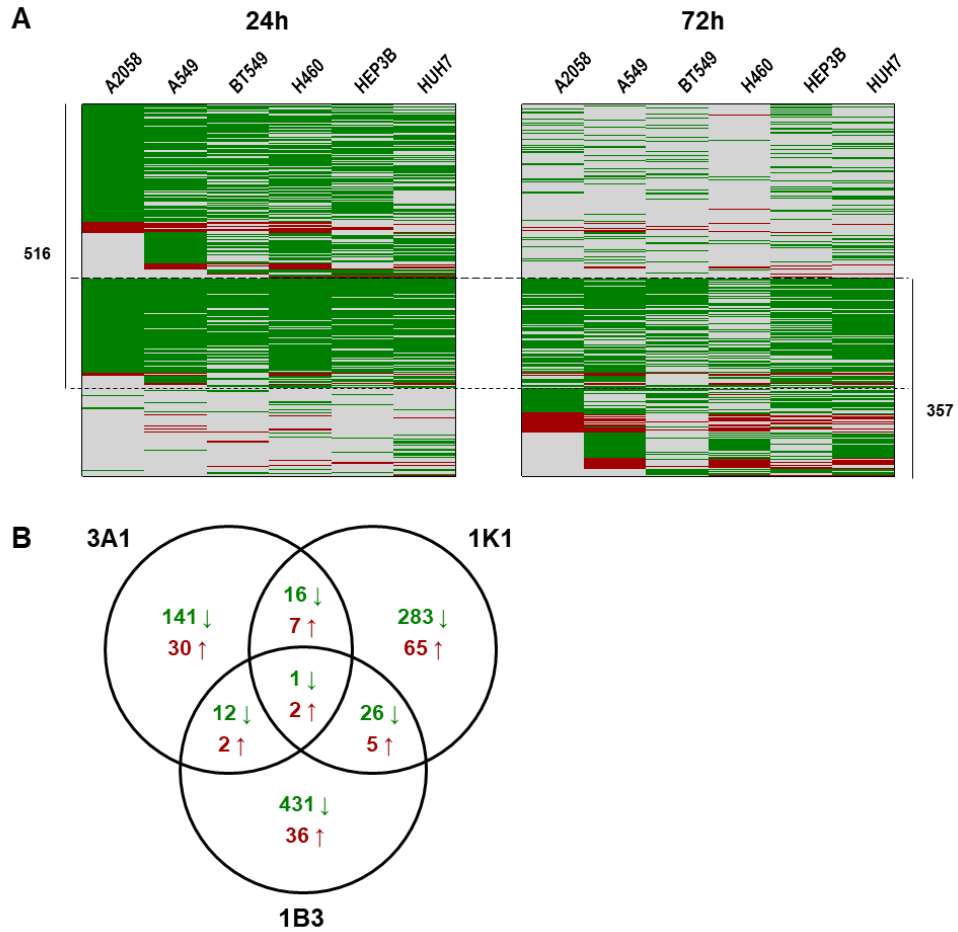
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Supplemental Figures



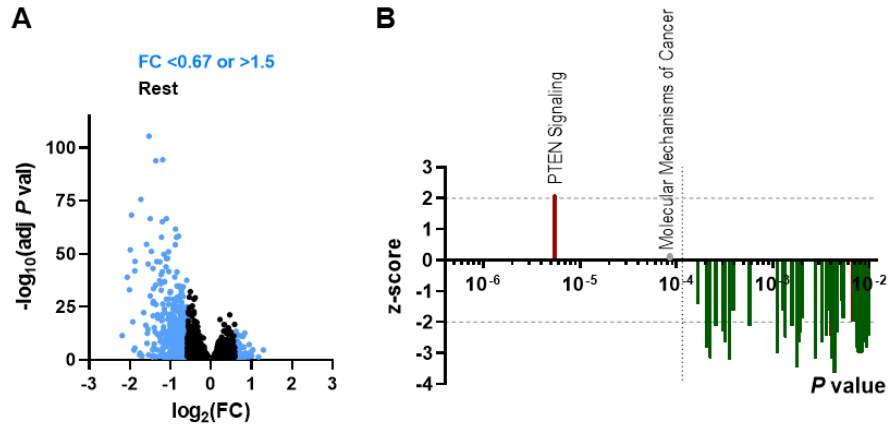
**Figure S1: Transfection of human tumor cells with miRNA controls.**

(A) Nucleotide sequences of miRNA controls. 1K1 represents a mutated version of 1B3 (three mutations in the seed sequence) and 3A1 is a commercially available random miRNA. (B) RNA was harvested 24 h after transfection of six tumor cell lines with 10 nM of either 1K1 or 3A1, and RNA samples from three independent experiments were pooled. The miRNA expression levels in samples submitted for RNA-sequencing were analyzed by stem-loop qRT-PCR. Shown are the Cycle threshold (Ct) values. (C) DE gene analysis of 1B3 *versus* either miRNA control (estimated expression in the 1B3 sample divided by the estimated expression in the 1K1 or 3A1 sample) in each cell line. The number of downregulated genes that are predicted miR-193a-3p targets is indicated. (D) For both the 1B3/1K1 and 1B3/3A1 analyses, the number of genes that were also identified in the 1B3/mock analysis (idem) and the number of genes that were not identified in the 1B3/mock analysis (new) are shown.



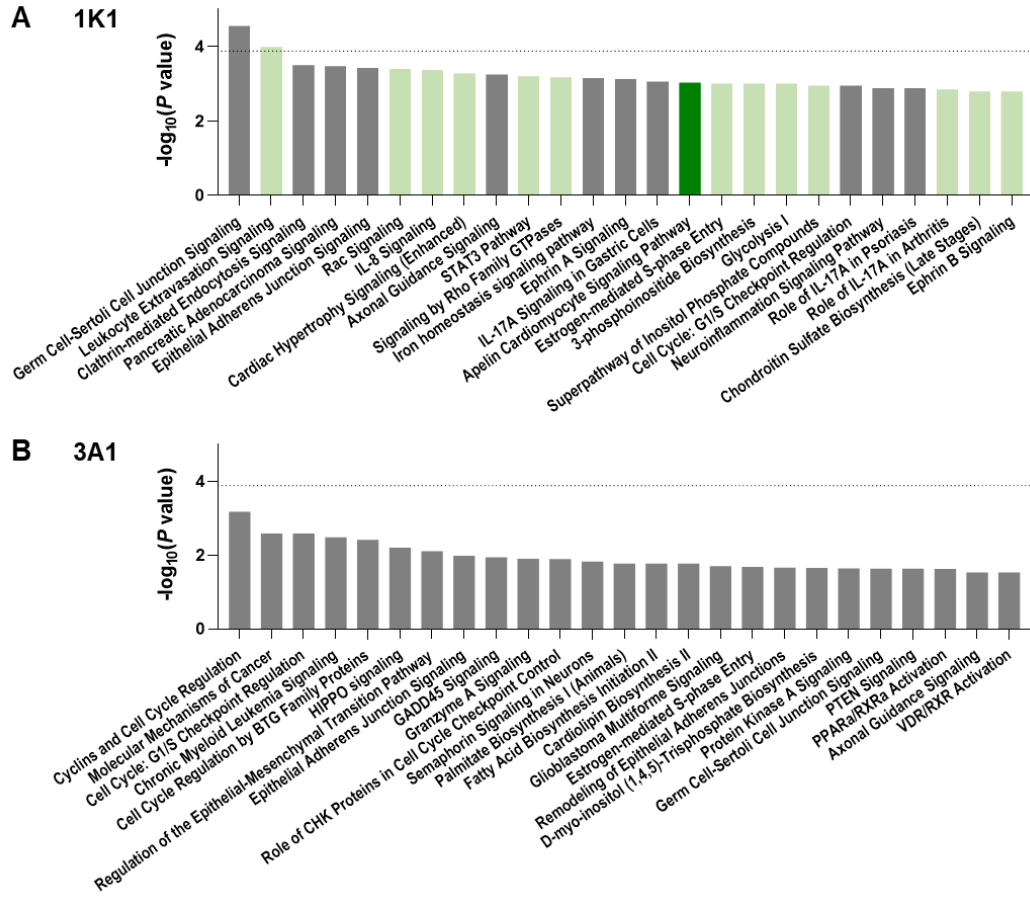
**Figure S2: Genes submitted to IPA and overlap with miRNA controls.**

(A) Heat maps showing differential expression changes of common genes in at least three cell lines at 24 h and 72 h post-transfection with 1B3. All DE genes are on the y-axis. Green means downregulated and red means upregulated as compared to mock. Grey means no DE. (B) Venn diagram demonstrating genes DE in at least three cell lines common between 1B3, 1K1 and 3A1. Downward arrow indicates downregulation and upward arrow indicates upregulation, as compared to mock.



**Figure S3: Multiple replicate analysis of 1B3-regulated transcriptome.**

Six cell lines were analyzed as replicates to compare the gene expression profiles between 1B3 and mock at 24 h post-transfection. **(A)** Volcano plot shows genes that were at least 1.5-fold down- or upregulated (blue) in the population of all DE genes (black). Adj  $P$  val: multiple testing-adjusted  $P$  value; FC: fold change. **(B)** Canonical pathways regulated by 1B3 ( $p < 0.01$ ) according to IPA of the genes shown in blue in panel A. Directionality is indicated by z-score ( $< -2$ : inhibition;  $> 2$ : activation). The significance threshold for multiple testing is indicated by a black dotted line.



**Figure S4: Gene pathway analysis in miRNA control samples.**

Top 25 canonical pathways regulated 24 h after transfection with 1K1 (A) or 3A1 (B), ranked based on  $p$  value (all  $p < 0.01$ ). The significance threshold for multiple testing is indicated by a black dotted line. Grey: z-score  $> -1 < 1$  or unknown, light green: z-score  $< -1$ , dark green: z-score  $< -2$ .

## Supplemental Tables

**Table S1: Numbers of differentially expressed genes in miRNA control samples.**

	1K1				3A1			
	DOWN (predicted)	∩ 1B3	UP	∩ 1B3	DOWN (predicted)	∩ 1B3	UP	∩ 1B3
<b>A2058</b>	464 (67)	28	265	17	561 (66)	20	131	7
<b>A549</b>	457 (86)	29	266	20	396 (67)	27	284	19
<b>BT549</b>	538 (130)	38	195	9	522 (108)	29	196	6
<b>H460</b>	412 (68)	32	250	20	431 (76)	29	292	19
<b>HEP3B</b>	519 (88)	27	208	10	491 (75)	22	116	4
<b>HUH7</b>	565 (89)	37	234	8	369 (61)	17	331	3

Down: estimated expression relative to mock < 1, up: estimated expression relative to mock > 1. Between brackets is the number of down-regulated genes that are predicted miR-193a-3p targets. The number of genes overlapping (∩) with 1B3 at 24 h is indicated.

**Table S2: Genes downregulated at 24 h by 1B3 in all six cell lines.**

**Table S3: Genes regulated at 24 h by 1B3 in at least three cell lines.**

**Table S4: Genes regulated at 72 h by 1B3 in at least three cell lines.**

**Table S5: Genes regulated at 24 h by 1K1 in at least three cell lines.**

**Table S6: Genes regulated at 24 h by 3A1 in at least three cell lines.**

**Table S7: Canonical pathways affected by 1B3 in at least three cell lines at 24h.**

**Table S8: Multiple replicate analysis of genes regulated at 24 h by 1B3.**

**Table S9: Quantification of western blot data.**

		<b>PTK2</b>	<b>RPS6KB2</b>	<b>PIK3R1</b>	<b>TGFBR3</b>	<b>PDPK1</b>	<b>pSer473 AKT</b>
<b>A2058</b>	<b>Mock</b>	1.00	1.00	1.00	1.00	1.00	1.00
	<b>3A1</b>	0.75	0.56	0.66	0.89	0.65	1.48
	<b>1B3</b>	0.38	0.69	0.13	0.06	0.49	0.30
<b>A549</b>	<b>Mock</b>	1.00	1.00	1.00	1.00	1.00	1.00
	<b>3A1</b>	0.99	1.12	1.88	1.13	2.14	0.40
	<b>1B3</b>	0.60	1.11	0.45	0.18	0.76	0.21
<b>BT549</b>	<b>Mock</b>	1.00	1.00	1.00	1.00	1.00	1.00
	<b>3A1</b>	1.12	1.27	1.10	2.04	1.66	1.02
	<b>1B3</b>	0.65	1.13	0.49	0.78	1.28	0.88
<b>H460</b>	<b>Mock</b>	1.00	1.00	1.00	1.00	1.00	1.00
	<b>3A1</b>	1.18	0.95	0.74	1.18	0.97	0.56
	<b>1B3</b>	0.56	0.49	0.17	0.06	0.09	0.09
<b>HEP3B</b>	<b>Mock</b>	1.00	1.00	1.00	-	1.00	1.00
	<b>3A1</b>	1.30	1.18	2.93	-	1.39	1.18
	<b>1B3</b>	0.76	1.09	0.74	-	0.69	0.99
<b>HUH7</b>	<b>Mock</b>	1.00	1.00	1.00	-	1.00	1.00
	<b>3A1</b>	1.05	1.21	1.68	-	0.67	2.05
	<b>1B3</b>	0.39	0.55	0.44	-	0.11	0.24

Densitometry was performed to quantify band intensities. Non-phosphoproteins were normalized to the loading control (Vinculin), whereas phosphoprotein pSer473 AKT was normalized to AKT total protein. Data are presented as ratio over mock.

**Table S10: Canonical pathways affected by 1B3 and miRNA controls in at least three cell lines at 24h.**

Canonical pathway	Regulated by	p value	z-score	DE genes				
Rac Signaling	1K1	0.00040	-1.667	FGFR1 PIK3CD	ITGA5 PIK3R3	MAP3K11 PIP5K1C	NCF2 RHOA	PAK4
	1B3	0.00046	-3.162	ABI2 PAK4	ARPC2 PARD6A	ARPC5 PIP4K2C	KRAS PIK3R1	MAPK8 PTK2
Signaling by Rho Family GTPases	1K1	0.00068	-2.111	CIT RHOA PIK3CD	FGFR1 ROCK1 PIK3R3	GNA11 MAP3K11 PIP5K1C	GNAI2 NCF2	ITGA5 PAK4
	1B3	0.00158	-2.496	ARHGEF12 PTK2 PAK4	ARPC2 RHOU PARD6A	ARPC5 STMN1 PIK3R1	CDC42EP2 MAPK8 PIP4K2C	GNAI3 MYLK
Molecular Mechanisms of Cancer	3A1	0.00040		CCND1 LRP5	CCND3 RBPJ	CDK6 RHOB	CRK SYNGAP1	E2F2 TGFB2
	1B3	0.00046		ADCY9 CCND1 GNAI3 MAX PSEN1	APH1B CDK2 HHAT MDM2 PTK2	ARHGEF12 CDK6 KRAS PAK4 RHOU	CASP9 CHEK1 LEF1 PIK3R1 SOS2	CBL CREBBP MAPK8 PRKCA

Canonical pathways ( $p < 0.01$ ) genes and differentially expressed (DE) genes that belong to each pathway are shown. Directionality is indicated by z-score (negative: inhibition; positive: activation).



**Table S11: Primer sequences for stem-loop qRT-PCR and antibody details.**

<b>SL-RT primers</b>		
<b>1B3 and 1K1 3A1</b>	GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACACTGGGA GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGATACGACAATAACC	
<b>qPCR primers</b>	<b>Forward</b>	<b>Reverse</b>
<b>1B3/miR-193a-3p 1K1 3A1</b>	TGCCCGAACTGGCCTACAAAGT TGCCCGAAGTCGGCTACAAAGT TGCTAGTTACGTCGTCGCGT	GTGCAGGGTCCGAGGT GTGCAGGGTCCGAGGT GTGCAGGGTCCGAGGT
<b>Antigen</b>	<b>Antibody product #</b>	<b>Manufacturer</b>
<b>Rabbit IgG-HRP</b>	7074	Cell Signaling
<b>Mouse IgG-HRP</b>	7076S	Cell Signaling
<b>pSer473 AKT</b>	4060	Cell Signaling
<b>AKT</b>	4691	Cell Signaling
<b>TGFBR3</b>	2519S	Cell Signaling
<b>PIK3R1</b>	4257S	Cell Signaling
<b>PTK2</b>	13009S	Cell Signaling
<b>PDPK1</b>	3062	Cell Signaling
<b>Vinculin</b>	4650S	Cell Signaling
<b>RPS6KB2</b>	8418	Santa Cruz