

miRNA-Dependent Regulation of AKT1 Phosphorylation

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Supplementary Materials:

Table S1. Oligonucleotides used in this study.

Oligonucleotide (5'-3')	SOURCE
Synthetic miRNA: let-7a pUGAGGUAGUAGGUUGUAUAGUU	[1,2]
Synthetic miRNA: let-7b pUGAGGUAGUAGGUUGUGUGUU	[1,2]
Synthetic miRNA: let-7g pUGAGGUAGUAGUUUGUACAGUU	[1,2]
Primer: RT-PCR, let-7a/d/e/f: GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGCATACGACAACTAT	This paper
Primer: RT-PCR, let-7b: GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGCATACGACAACCAC	This paper
Primer: RT-PCR, let-7c: GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGCATACGACAACCAT	This paper
Primer: RT-PCR, let-7g: GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGCATACGACAACTGT	This paper
Primer: RT-PCR, let-7i: GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGCATACGACAACAGC	This paper
Primer: RT-PCR, SNORD47 GTCGTATGCAGAGCAGGGTCCGAGGTATTCGCACTGCATACGACAACCTC	[3]
Primer: qPCR, let-7a/b/c, Forward: AGGCTGAGGTAGTAGTTG	This paper
Primer: qPCR, let-7d, Forward: AGGCAGAGGTAGTAGTTG	This paper
Primer: qPCR, let-7e, Forward: AGGCTGAGGTAGGAGTTG	This paper
Primer: qPCR, let-7f, Forward: AGGCTGAGGTAGTAGATTG	This paper
Primer: qPCR, let-7g/i, Forward: AGGCTGAGGTAGTAGTTG	This paper
Primer: qPCR, SNORD47, Forward: ATCACTGTAAAACCGTTCCA	[3]
Primer: qPCR, let-7 & SNORD47, Reverse: GAGCAGGGTCCGAGGT	[3]
Primer: qPCR, PIK3C2A, Forward: TGAATAGTTCATTAGTGCAATTCCTT	[4]
Primer: qPCR, PIK3C2A, Reverse:	[4]

GGCATCTTTGAGAAGCCAAT	
Primer: qPCR, GAPDH, Forward:	
GAAGGTGAAGGTCGGAGTCAAC	[5]
Primer: qPCR, GAPDH, Reverse:	
CAGAGTTAAAAGCAGCCCTGGT	[5]

Table S2. Antibodies used in this study.

ANTIBODY	SOURCE	IDENTIFIER
anti-pan-AKT	Cell Signaling Technology	Cat#2920; RRID:AB_1147620
anti-AKT1	Cell Signaling Technology	Cat#2938; RRID:AB_915788
anti-AKT2	Cell Signaling Technology	Cat#3063; RRID:AB_2225186
anti-AKT3	Cell Signaling Technology	Cat#4059; RRID:AB_2225351
anti-pAKT ^{T308}	Cell Signaling Technology	Cat#9275; RRID:AB_329828
anti-pAKT ^{S473}	Cell Signaling Technology	Cat#9271; RRID:AB_329825
anti-GAPDH	Millipore	Cat#MAB374; RRID:AB_2107445
anti-GSK-3 α /b	Santa Cruz Biotechnology	Cat#sc-7291; RRID:AB_2279451
anti-pGSK-3 α /b	Cell Signaling Technology	Cat#9331; RRID:AB_329830
anti-PDK1	Cell Signaling Technology	Cat#3062; RRID:AB_2236832
anti-pPDK1	Cell Signaling Technology	Cat#3438; RRID:AB_2161134
anti-PP2A	BD Biosciences	Cat#610555; RRID:AB_397909
anti-PIK3C2A	Cell Signaling Technology	Cat#12402; RRID:AB_2797900
anti-RICTOR	Bethyl Laboratories	Cat#A300-459A; RRID:AB_2179967
anti-mTOR	Cell Signaling Technology	Cat#2972; RRID:AB_330978
anti-vinculin	Cell Signaling Technology	Cat#13901; RRID:AB_2728768

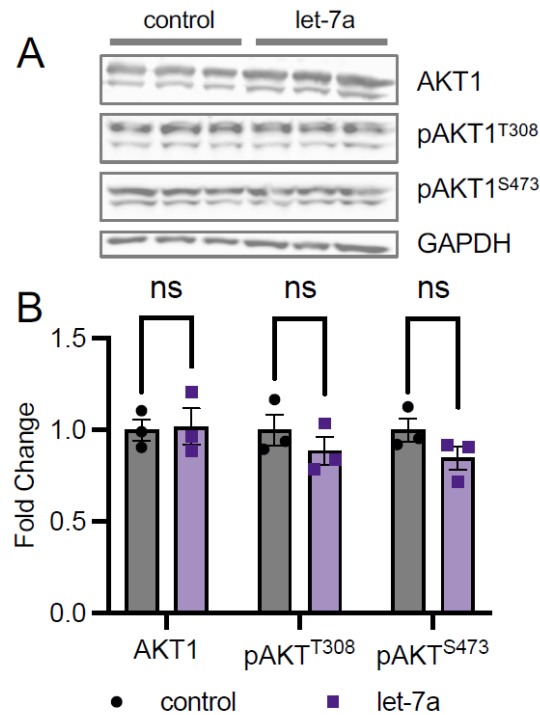


Figure S1. AKT1 phosphorylation is not changed in unstimulated cells. (A) Western blots and (B) quantification of mCherry-AKT1 and mCherry-pAKT1 levels in HEK 293T cells co-transfected with 5 μ g mCherry-mAKT1 plasmid and 60 fmol let-7a (purple) or a control lacking RNA (black) for 24 hours. The data include N = 3 biological replicates. Error bars show \pm 1 SEM. ns = not significant.

References

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