

Supplementary Materials: CircAgtbp1 Acts as a Molecular Sponge of miR-543-5p to Regulate the Secretion of GH in Rat Pituitary Cells

ZeWen Yu [†], WenZhi Ren [†], Tian Wang, WeiDi Zhang, ChangJiang Wang, HaoQi Wang, Fei Gao ^{*} and Bao Yuan ^{*}

Table S1. Primers and sequences used in this study.

Primers for qPCR		
U6	RT	CGCTTCACGAATTTGCGTGTCAT
U6	F	GCTTCGGCAGCACATATACTAAAAT
	R	CGCTTCACGAATTTGCGTGTCAT
miR-543-5p	RT	CTCAACTGGTGTCTGGAGTCGGCAATTCAGTT-GAGCGAAAAAC
	F	ACACTCCAGCTGGGAAGTTGCCCGCGTGT
	R	Universal Reverse Primer (CTCAAGTGTCTGGAGTCGGCAA)
GADPH	F	GGAAACCCATCACCATCTTC
	R	GTGGTTCACACCCATCACAA
Gh1	F	CATGCCCTTGTCCAGTCTGT
	R	AATGTAGGCACGCTCGAACT
CircAgtbp1	F	TGACCTCATTCTGAACTCTGACA
	R	TCTCGACTTCATTTTCAGCTTCT
Agtbp1	F	TTACCATGCGAAGGGGCTAC
	R	GTCTTCCACAACATCGCAGG
SiRNAs		
CircAgtbp1 si-1		GCCAGTTTAACTACGTGGA
CircAgtbp1 si-2		AGTTTAACTACGTGGACGA
CircAgtbp1 si-3		TTAACTACGTGGACGACGT
Probes for FISH		
Cy3-circAgtbp1		RiboBio
MiRNA sequences		
Mimic NC		RiboBio
miR-543-5p mimic		RiboBio
Inhibitor NC		RiboBio
MiR-543-5p inhibitor		RiboBio

File S1. The full sequence of circAgtbp1.

The results of sequencing the full sequence of circAgtbp1 was as follows:

TGGACGACGTGGTGGACGAGAGTGACGACAACGATGACATTGATTAGAA
GCTGAAAATGAAGTCGAGAATGAAGATGACCTAGATCAAAGTTTTAAGAATGA
TGATATTGAAACAGATATTAATAAATTAAGACCCCAGCAAGTACCAGGACGAA
CAATAGAAGAACTAAAAATGTATGAGCACCTTTTCCCTGAGCTTGTTGATGATT
TCAGGACTATGAATTAATCGCTAAAGAACCCAAACCTTTTGTGTTTGAGGGGAA
AGTTCGGGGCCCGATTGTAGTTCCACAGCTGGAGAGGAAGTGCCTGGGAATCC
AGGTAACGTAAGGAAAGGAGCTGCAGTGAAGGAGAAAGCGAGTCCTAAAGGA
GAGGAAGTCAAGGAAGATGCCAAGGGCCATGACAAAACACCGCCGTGGCAGCT
GGGTGGCCAGAACAGAGCGGCCGCTTCAGCCCACAGCTCCAACAACGATCTTGT

GAAGGCCTTAGACCGAATCACACTGCAGAGTACCCCTTCACAAGTAGCCGCGG
 GCTTGACTGCAGGAATGAGGAAGGACTACGGCTCCCTCTCACTGTCCTCTCATG
 CACGAAAGCGTGTCTCACGTGGCTAAGTGCACAAGTGCCCTTTTCGAAGGGCG
 GACAGTACATCTTGGTAAACTGTGTTGTACTGGAGTTGAAACGGAAGATGATGA
 AGACTTTGAGTCCCACTCATCAGCAGAGCAGGTCTCCTCTGTTGAAGCCTCTGAT
 GGACCACCAACACTGCATGACCCAGACCTCTACATCGAGATTGTGAAAAATACA
 AAGTCTGTTCCCGAGTACTCAGAGGTGGCCTATCCTGATTATTTTGGACACATTC
 CACCTCCCTTCAAAGAGCCTATTTTAGAAAGGCCTTATGGTGTACAAAGGACAA
 AAATTGCCCAAGATATCGAGAGGCTGATACACCAGAATGATATCATTGACCGGG
 TGGTGTATGACTTAGATAACCCTAACTATACCACTCCAGAAGAAGGAGATATTT
 TGAAGTTTAACTCAAAATTTGAATCTGGGAATCTGCGCAAAGTAATTCAAATTA
 GAAAAAGCGAGTATGACCTCATTCTGAACTCTGACATAAACAGTAACCATTACC
 ATCAGTGGTTCTACTTTGAAGTCAGTGGGATGCGGCCGGGTGTGGCATAACAGGTT
 CAACATCATCAACTGTGAGAAGTCCAACAGCCAGTTTAACTACG

When the sequences was aligned in NCBI, 100% identity was observed. A total sequence of 1227 bp was identified, which showed 100% identity.

File S2. Construction of pmiR-circAgtbbp1-WT reporter plasmid and pmiR-circAgtbbp1-MUT reporter plasmid.

About 200 sequences upstream and 200 sequences downstream of circAgtbbp1 targeted binding to miR-543-5p was cloned between the XhoI and NotI sites in the pmirGLO plasmid, forming the pmiR-circAgtbbp1-WT plasmid. The target sequence AAAACAC was mutated into GGGGTGT, forming the pmiR- circAgtbbp1-MUT plasmid

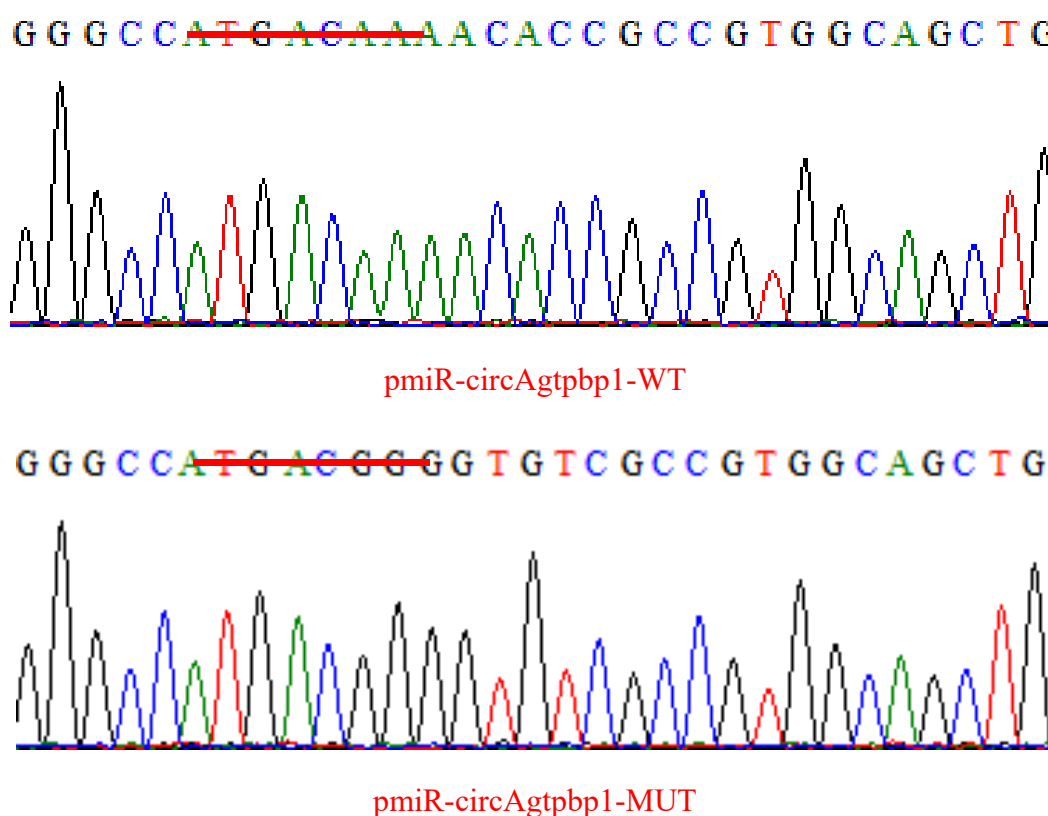


Figure S1. DNA sequence peak map. Sequence of the extracted plasmid; the target sequence AAAACAC was mutated into GGGGTGT.