

Table 1. Primers used for PI3KCa, PI3KC2b, and PI3KC3 promoter cloning

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	Step	
<i>PI3KCa</i>	GTCTGTCGTTGCTATGG AGAGC	CCATAGAGCAGTTTCAG GGTA	RT-PCR	
	ACGATGGACTCCAGAG CGGCCGCVVNVNNCC AA	GAGTGTCCAAGCGAAAT AGTAAATACG	Hitail- PCR1	
	ACGATGGACTCCAGAG	TTTCTGTTGAGTAATCCG CTCTCGTG	Hitail- PCR2	
	ACGATGGACTCCAGAG	CATGGTCGTTTAATCCAG GAATGAAGA	Hitail- PCR3	
	<i>PI3KC2b</i>	CGCTGAACTGAGAATCT CGA	TTGATCTGCGGTCCTTAT GA	RT-PCR
		ACGATGGACTCCAGAG CGGCCGCBDBNBNCCG GT	ATCACACATGGGTTCTAA CAACTGC	Hitail- PCR1
ACGATGGACTCCAGAG		ACGACGCTTAAATTCCGC ACTGTATA	Hitail- PCR2	
ACGATGGACTCCAGAG		GAAGGTTTTTCGGTTTGT GCTTTCAG	Hitail- PCR3	
<i>PI3KC3</i>		ATCGATTGCTTATGCCT GTT	CCAGGTCGCAACTGTAA ACAT	RT-PCR
	ACGATGGACTCCAGAG CGGCCGCBNBNNGGT T	GCAGATATTCCTCAGAC TTTGACACA	Hitail- PCR1	
	ACGATGGACTCCAGAG	CACGGTGATTTTATACGG TGTGTGACTTTA	Hitail- PCR2	
	ACGATGGACTCCAGAG	ACAACCTGACTGGAGATG GACACGGAC	Hitail- PCR3	

Table 2. Primers used for 5'-deletion plasmids construction

Primers	Forward primer (5'-3')	Reverse primer (5'-3')
<i>PI3KCa</i>		
pGl-360/+71	ctatcgataggtaccgagctcGCAA TCTAAGCAATTTAT	cagtaccggaatgccaagGCTTGATG GAGACGCTA
pGl-224/+71	ctatcgataggtaccgagctcAAAA GGAATCTGGATGC	cagtaccggaatgccaagGCTTGATG GAGACGCTA
pGl-86/+71	ctatcgataggtaccgagctcATTTG TGTCGCATTACG	cagtaccggaatgccaagGCTTGATG GAGACGCTA
<i>PI3KC2b</i>		
pGl-1848/+79	ctatcgataggtaccgagctcGGCA GTTGAGCCAGCA	cagtaccggaatgccaagcttTCGCAG AAGGTTTTTCG
pGl-1522/+79	ctatcgataggtaccgagctcCTGC	cagtaccggaatgccaagcttTCGCAG

	CGGTGGTCTTCTTCCA	AAGGTTTTTCG
pGl-1263/+79	ctatcgataggtaccgagctcCATG GATTCAAATCCTT	cagtaccggaatgccaaagcttTCGCAG AAGGTTTTTCG
pGl-929/+79	ctatcgataggtaccgagctcGCAC TACCATGCACTTCTC	cagtaccggaatgccaaagcttTCGCAG AAGGTTTTTCG
pGl-623/+79	ctatcgataggtaccgagctcTCCCC AGATGAAGTTCCC	cagtaccggaatgccaaagcttTCGCAG AAGGTTTTTCG
pGl-388/+79	ctatcgataggtaccgagctcAGGG TTTCAATATCG	cagtaccggaatgccaaagcttTCGCAG AAGGTTTTTCG
pGl-144/+79	ctatcgataggtaccgagctcCACG ACAAAAGAAAGCATC	cagtaccggaatgccaaagcttTCGCAG AAGGTTTTTCG
pGl-94/+79	ctatcgataggtaccgagctcAAAT CTCTGATCCGAGC	cagtaccggaatgccaaagcttTCGCAG AAGGTTTTTCG
<i>PI3KC3</i>		
pGl-367/+59	ctatcgataggtaccgagctcTGGT GCTATGTGTTAAAG	cagtaccggaatgccaaagCATAGTGC GTTTCCCGA
pGl-291/+59	ctatcgataggtaccgagctcCATA CTTTCACATGAAAAGC	cagtaccggaatgccaaagCATAGTGC GTTTCCCGA
pGl-85/+59	ctatcgataggtaccgagctcGCGG CTCTAAATCTCT	cagtaccggaatgccaaagCATAGTGC GTTTCCCGA

Table.3 Primers used for site-mutation analysis

Primers	Forward primer (5'-3')	Reverse primer (5'-3')
aMut- HNF1-1	TAAAAGgggggAAAAAATTT ATATCAGATCCAGAGTTTG	TTTTTtccccCTTTTAATAAA AAAAAGAAAACCTTATATTC AAG
aMut- HNF1-2	AATTATGgggAAGCTCCATTA AAAGGAATCTGGA	GGAGCTTcccCATAATTCATA GTCTGACATTATGACTGAA
2bMut- FOXO1-1	TTTGTGggggCACACAAACAG GCTCACAAATGC	TTTGTGTGccccCACAAACA GTGACCAGTTGCGC
2bMut- FOXO1-2	ATTCAGTAgggggAAGCCATGG TTCACAGCCAA	GCTTccccTACTGAATTTAC GGAAGGATTTGAAT
2bMut- FOXO1-3	AAACTGgggggACTTCGACAT GCCAAGGAAGAT	CGAAGTccccCAGTTTGGCT GTGAACCATGG
2bMut- FOXO1-4	TTGTACgggggCAATACTGTTA TTTGCACACTACCATGC	GTATTGccccGTACAATATG CAGCAGCAGCAGT
2bMut- IK1	ATGAAgggggATGGAAAGTTT CCGGCCCCTT	TTCCATccccTTCATCTGGG GAGAGCTCGG
3Mut- FOXO1-1	AAAGTAggggAGAAAACCTTT AAAAATTCTAAGCTAAGAT	GGTTTTCTcccTACTTTAAC ACATAGCACCAGAGCTCG
3Mut- FOXO1-2	GACTTATTAGATggggTATTAA TTCAAATACAATTAATAATA TGAATAATAAA	TAccccATCTAATAAGTCATA GTTTAAACTAGAATGTGTT G

3Mut- FOXO1-3	TAAgggggACATAAAGGACTG AGACGGGGTGA	CCTTTATGTccccTTATTATT CATATTATTTAATTGTATTTG AATTAATAA
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Table 4 Primers used for electrophoretic mobility-shift assay

Primers	Forward primer (5'-3')	Reverse primer (5'-3')
B-PI3KCa- HNF1	TATGA <u>AATT</u> TATGTAAAGC TCCA	TGGAGCTTTAACATA <u>AATTCAT</u> A
Mut-PI3KCa- HNF1	TATGGGGTATGTAAAG CTCCA	TGGAGCTTTAACATA <u>CCCCAT</u> A
B-PI3KC2b- FOXO1-1	CACTGTTTGTGA <u>ACACA</u> CACAA	TTGTGTG <u>TGTT</u> CACAAACAG TG
Mut-PI3KC2b- FOXO1-1	CACTGTTTGTGGGGGCA CACAA	TTGTGTG <u>CCCC</u> CACAAACAG TG
B-PI3KC2b- FOXO1-2	GCCAAACTG <u>AAAAA</u> AC TTCGAC	GTCGAAGT <u>TTTTT</u> CAGTTTG GC
Mut-PI3KC2b- FOXO1-2	GCCAAACTGGGGGGAC TTCGAC	GTCGAAGT <u>CCCCC</u> CAGTTTG GC
B-PI3KC2b- FOXO1-3	TGTACA <u>AAAA</u> CAATACT GTTAT	ATAACAGTATTG <u>TTTTT</u> GTAC A
Mut-PI3KC2b- FOXO1-3	TGTACGGGGGCAATACT GTTAT	ATAACAGTATTG <u>CCCCC</u> GTAC A
B-PI3KC2b- IK1	CAGATGAAGT <u>TCCC</u> ATG GAA AG	CTTTCCATGGGA <u>ACTTCATCT</u> G
Mut-PI3KC2b -IK1	CAGATGAAGGGGGGATG GAA AG	CTTTCCAT <u>CCCCC</u> CTTCATCT G
B-PI3KC3- FOXO1	TATTAGAT <u>TGTTT</u> TAT TAATTCA	TGAATTAATA <u>ACAATCTAAT</u> A
Mut-PI3KC3- FOXO1	TATTAGATGGGGTAT TAATTCA	TGAATTAATA <u>CCCCATCTAAT</u> A

Table 5. Primers used for q-PCR from yellow catfish

Primers	Forward primer (5'-3')	Reverse primer (5'-3')
β -Actin	GGACTCTGGTGATG GTGTGA	CTGTAGCCTCTCTCG GTCAG
18srRNA	CTGCCGGTGGTCTTC TTCCA	ATTCAGCGGGTCGTC TCGTC
RPL17	GGCAAATGTACAGGA GCGAG	GCCTTGTTGAGCTTG ACGAA
GAPDH	TTTCAGCGAGAGAGA CCCAG	ATGACTCTCTTGGA CCTCC
HPRT	ATGCTTCTGACCTGG AACGT	TTGCGGTTTCAGTGCT TTGAT

UBCE	TCAAGAAGAGCCAGT GGAGG	TAGGGGTAGTCGATG GGGAA
TUBA	TCAAAGCTGGAGTTC TCGGT	AATGGCCTCGTTATCCACC A
PI3KCa	CCAGGGAAACGGGA TACA	TCCACTTTACAGCAAGGAG AA
PI3KCb	CTGATGCTGACTTCTG GGTTA	TTATGGAGGTGTTAGGAGG GA
PI3KCg	CCGATTTTGTCTGAG GGT	TTCTCCACTTTTCAGGCAC T
PI3KCd	GACACCCAAAAGAAC CACG	TTCCCACATTGAGCCACC
PI3KC2a	TGAATCAGAACCCAAG AAGAAG	GGATGCGAAATGTAACGGA
PI3KC2b	GCGGAATTTAAGCGTC GTC	GGGGATTTGGAGGGATAAG A
PI3KC3	AAAGCAGGCACTGGA GTTATT	GCGTAGCGTCTGACTGTAG G
FAS	AACTAAAGGCTGCTGGTTGC TA	CACCTTCCCGTCACAAACC TC
G6PD	CAGGAATGAACGCTGGGATG	TCTGCTACGGTAGGTCAGG TCC
6PGD	GCTCTGATGTGGCGAGGTGG	CGTAGAAGGACAGTGCAG TGGTAAA
CPT IA	ATTTGAAGAAGCACCCAGAG TATGT	CCCTTTTATGGACGGAGAC AGA
PPAR α	CGAGGATGGGATGCTGGTG	CGTCTGGGTGGTTCGTCTG C
PPAR γ	ACGCCCCGTTTCGTTATCC	TGAGCAGAGTCACCTGGT CATTG
ACCa	AATGGCTATGACTGGCAAGG G	TACCGTCGTTCCACTAGTG ATT
ACCb	AAGTGCTGTCTGAGCCCTGG AG	CGCGGATCCTCCACTAGTG ATTTCACTATAGG
ATGL	TTGCGGAAATGTGATTGAGG T	CACGGAAGGCAGGAGGGA
LPL	AGCGATTGGTGGGAGGATTA T	TGAGCACGGTCCAGTTTCC T
CD36	GATCGTTCTGATTTTCGGTTG G	TTATTGTCGTACTIONTCGGCA CTG
FABP	GAAAGTGGACCGCAATGAG A	GTCAGTGTTTTGCCGTTGT CTT

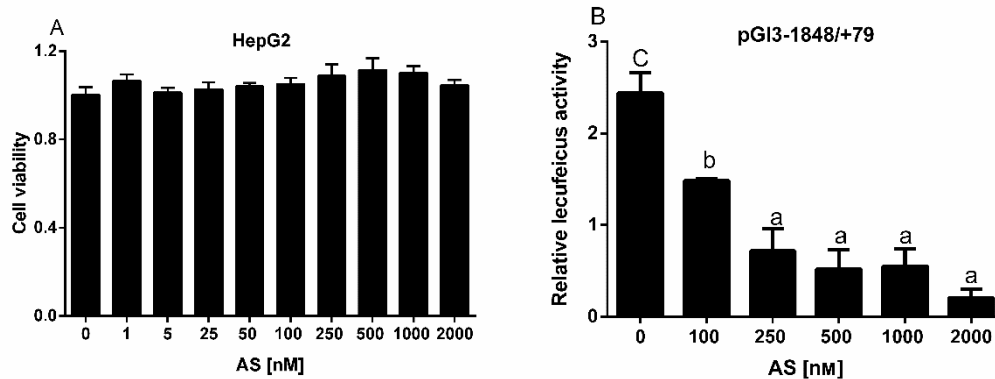


Fig.1. (A) Effects of AS1842856 (AS) on cell viability on HepG₂ cells, and no significant differences were found on the cell viability after incubation for 24 h. (B) Effects of AS on the relative luciferase activity of pGI3-1848/+79 of PI3KC2b-vectors in HepG₂ cells. Values are expressed as means+s.e.m. (N=3). Different letters indicate significant differences among groups (P<0.05).

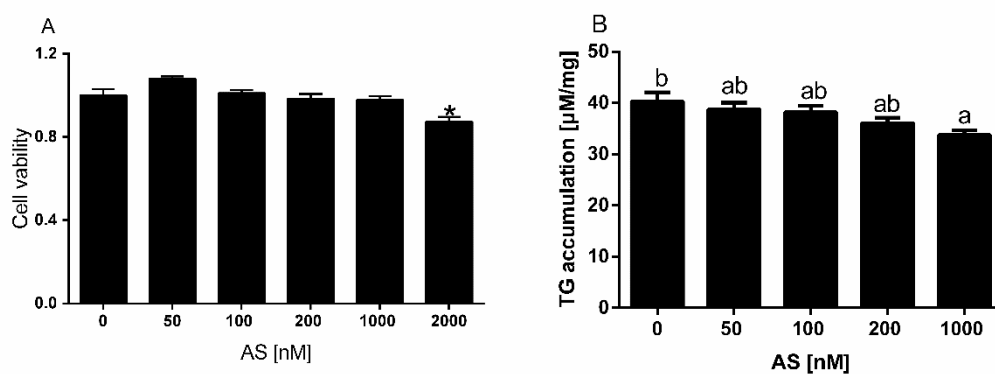


Fig.2 (A) Effects of AS on cell viability on primary hepatocytes from yellow catfish after incubation for 48 h. (B) Effects of AS on triglyceride content in primary hepatocytes from yellow catfish. Symbol (*) indicates significant differences between the treatment group and the control group (P<0.05).