FigureS1. Validation of *asb5a* gene knockout efficiencyin zebrafish. The expression level of *asb5a*-mRNA in 48hpf homozygous embryos was identified by qPCR. Plotted data represent means ± standard error of the mean (SEM; n = 10, N=3).** $p < 0.01$.

A *asb5b*

Intron1

5'... TTCTATGATTTTGGCCAAGGGCACAGTAAGTAGGCACACATTGTGGTTATTTATTTTGGAGTC ...3'

asb5b-ATGmo-target

B

M 1 2 3 4 5 6 7 8

10kb
3kb
1kb
500bp
100bp

C Knock down efficiency
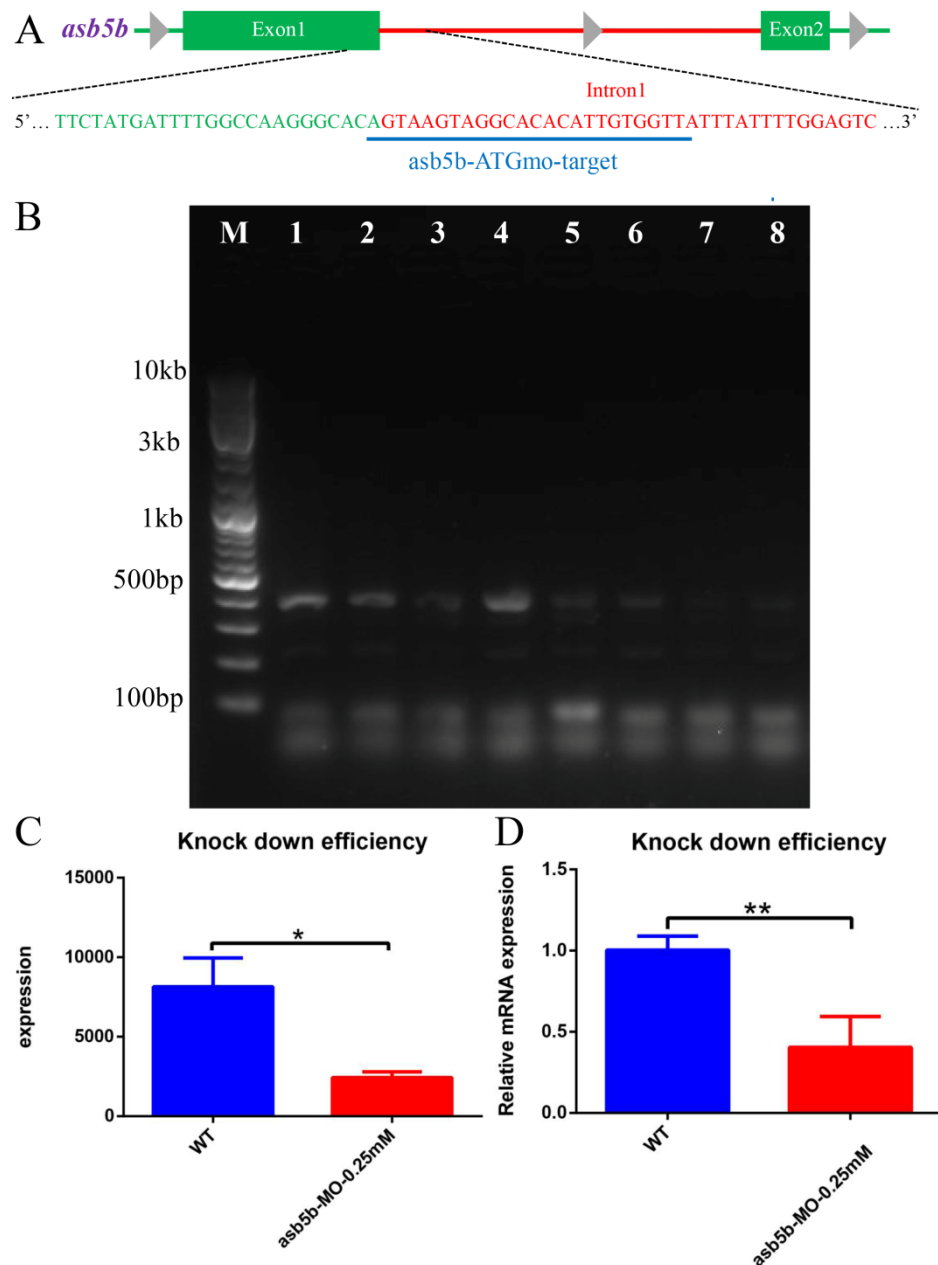
D Knock down efficiency

Figure S2. Identification of *asb5b* gene morpholino knockdown in zebrafish. **(A)** Target site design schematic of *asb5b*-ATGmo. Green represents the exons region, red represents the introns region, and blue represents the target site region. **(B)** The expression of *asb5b*-mRNA in 48hpf embryos was identified by RT-PCR. M: DNA marker bands；1-4: Four biological replicates of WT, 5-8: Four biological replicates of *asb5b*-ATGmo-0.25 mM were injected at the WT embryo one-cell stage. **(C)** Results of gray scale analysis electrophoresis. The results showed that at a low concentration of 0.25 mM, effectiveness knockdown of *asb5b*-mRNA expression levels compared with the WT group. **(D)** qPCR identified *asb5b*-mRNA expression levels in 48hpf WT embryos after injection of 0.25 mm low concentration *asb5b*-ATGmo. Plotted data represent means ± standard error of the mean (SEM; n = 10, N=4). * p< 0.05; ** p< 0.01.
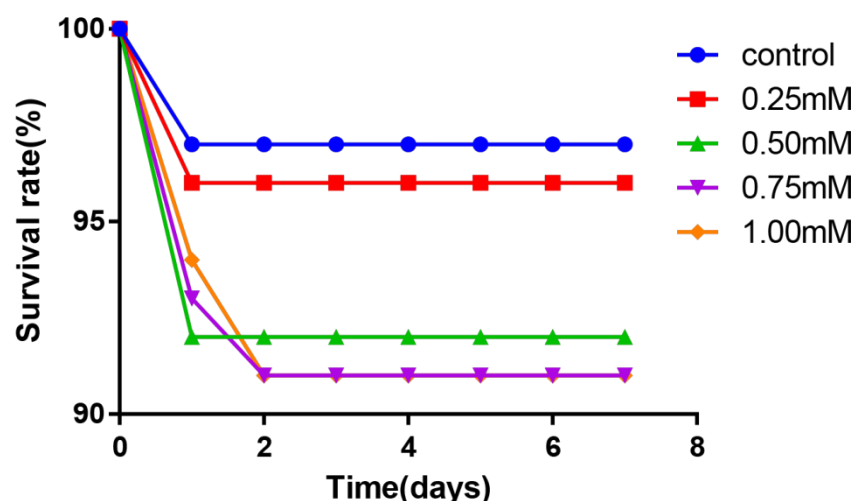
Figure S3. Survival curve of zebrafish with *asb5b*-ATGmo knockdown. Compared with WT Group, all the embryos with different injection concentration were broken membrane, normal body shape and heart development after 3dpf. Overall survival rates were above 90%, as shown above.

Table S1: Primers used for gene knockout and their sequences.

Primer	Sequences (5'→3')
sgRNA-F1	tgTAATACGACTCACTATAGGgaatggcctgtactcctctgGTTTTAGAGCTAGAAATAGC
sgRNA-F2	tgTAATACGACTCACTATAGGagttctatgacaatggtcaaGTTTTAGAGCTAGAAATAGC
sgRNA-R	aaGCACCGACTCGGTGCCACT
asb5a -KO-F	ACTAGACATTTTGTATATAAACTTAT
asb5a -KO-R	GTGCCCTTTAAATCTATTTTTG

Note: tg was the protective base, and the sequence of T7 promoter was TAATACGACTCACTATA. GG was added to improve the transcription efficiency of T7 promoter. The horizontal part of the base is the target site sequence,GTTTTAGAGCTAGAAATAGC is the upstream of the sgRNA backbone sequence, and aais the protective base.

Table S2: *asb5b*-ATGmo concentration gradient design.

asb5b-ATGmoNuclease-Free Water	Final concentration
Original fluid	
1μL3μL	0.25milliMolar(About 1.2 ng/embryo)
1μL1μL	0.50milliMolar(About 2.5 ng/embryo)
3μL1μL	0.75milliMolar(About 3.6 ng/embryo)
1μL0μL	1.00milliMolar(About 4.9 ng/embryo)

Note: *asb5b*-MO powder 300 nanomoles (about 2.5 mg) . ADD 0.3 ml Nuclease-Free

Water (about 8.33 ug/ul) to the powder. About 1,700 embryos can be injected per 1 ul of solution.

Table S3: Primers used for WISH and their sequences.

Primer	Sequences (5′→3′)
asb5a–WISH-F	agttcctatgtgcccgcaagctg
asb5a–WISH-T7R	CCCTAATACGACTCACTATAGGGttatctgtattgtagaaagttcttc
asb5b–WISH-F	ggcctttcggctctaatacca
asb5b–WISH-T7R	CCCTAATACGACTCACTATAGGGttatctgtattgaaggaagc

Table S4: Primers used for qPCR and their sequences.

Primer	Sequences (5'→3')
actb1–qPCR-F	TCCAGCCTTCCTTCCTGGGTAT
actb1–qPCR- R	GTGGAAGGAGCAAGAGAGGTG
cox6a2–qPCR-F	ACTGTCCTTCGTGTTAGCCC
cox6a2–qPCR-R	AGTGGGCAGAGCGTTTGTAT
cox7c–qPCR-F	ACAAGCTGTTCGTCGGTTTG
cox7c–qPCR-R	ATGAAGGGGAAGGCGAATCC
tnnc1a–qPCR-F	GGATGTTAGGCCAGAACCCT
tnnc1a–qPCR-R	TGGCTTCACCTGTAGCTTCC
tnnc1b–qPCR-F	CAGCGGTGGAGAACTTGACA
tnnc1b–qPCR-R	CGCACCATCATGACCAAGAAC
tnnt2a–qPCR-F	GATCGACGCAAACCTCTGGA
tnnt2a–qPCR-R	GGTCACTGACTCTGTTCCTCA
acta1a–qPCR-F	ATGCCATCATGCGTTTGGAC
acta1a–qPCR-R	GGAAGCTCGTAGCTCTTTTCG
actc1a–qPCR-F	GGAAATGAGCGTTTCCGCTG

actc1a–qPCR-R　　　　　GGCCAGAGCAGTGATTTCCT

actc1b–qPCR-FCAATGGCTCTGGCTTGGTCA

actc1b–qPCR-R　　　　　ACTTCAGGGTCAAGATACCTCTC

actc1c–qPCR-FTATGTGGGAGACGAGGCTCA

actc1c–qPCR-R　　　　　CGGGAACGTTGAAGGTCTCA

cacng1a–qPCR-FTGAGTCCTCGAGTGGAAACAAT

cacng1a–qPCR-RGAAGAGGGCTATGGCAGCAC

cacng2a–qPCR-FATGGCTGAAATGGTGGGTGT

cacng2a–qPCR-R　　　　GAAGGTAGAGCCCCGAAACC

cacna1fb–qPCR-FACCCTACTGCTCTACAGGCG

cacna1fb–qPCR-R　　　　GGCTTCTGCTTAAGGGCTCA

cacna2d1a–qPCR-FCCCATCGATTGGAGCCATCA

cacna2d1a–qPCR-R　　　GGACCCAGGTTCTTAGGCTT

cacna2d2b–qPCR-FACACCACAGTACAAGCTCGG

cacna2d2b–qPCR-R　　　ATGTAGCGACTTGCCTCGTC

cacna2d3–qPCR-FGGGCAAACTGGTGTCAATGG

cacna2d3–qPCR-R　　　　GTGAGCTGCGGAGAAAAGATT

cacna2d4a–qPCR-FCAATTGGCCAGATCGCAAGG

cacna2d4a–qPCR-R　　　GCACACTGTCCATGTAAGCC

cacna2d4b–qPCR-FGGCCTGACAAGAAGGTACGA

cacna2d4b–qPCR-R　　　　CTTGAACACCACACTGTCCATA

cacnb1–qPCR-FGTCCTCCTGAGCTGTTTGACA

cacnb1–qPCR-R　　　　　GAAGTGAGCACCTGTACCTGC

cacnb2a–qPCR-FAACAACATTGGCGGTTCAGC

cacnb2a–qPCR-R　　　　ATGAGATTCCCGTTCCAGGC

tpma–qPCR-FAGTACTCAGCTAAGGAGGACAAAT

tpma–qPCR-R　　　　　CTCGCTGATGGCTTTGTACTTG

cox7b–qPCR-FAGTCTCGACCGCTCATTTCC

cox7b–qPCR-RCTTCTGTCGAACAGCCACCT

myh7–qPCR-FGGAGCAGCAGCTTCTTACCT

myh7–qPCR-RCTGGGGGTGAATGTCAGCTT

cox5b–qPCR-FGAGCTGTGGTTTTGCGTCAC

cox5b–qPCR-RTGAGGGTCTTGTTTGGAGCC

cox8b–qPCR-FATGTCCGGCTTCAATCGCT

cox8b–qPCR-R   GGATGGTCACAAACATGACCG

uqcrc1–qPCR-FCATCACATTCGGGGGTGGAA

uqcrc1–qPCR-R   TCGGTCACTGTGGTGCATAC

uqcrfs1–qPCR-FGGGTCTACCCTTGTGGTTGG

uqcrfs1–qPCR-R   TTCTCTGTTCTGTGGCGGAC

myl4–qPCR-FACAGAACGCCAGCAAGTGAA

myl4–qPCR-R   TGTGCCCTGATCTTTGGACC

uqcr10–qPCR-FATGGCGCTCTCTAAAACGGT

uqcr10–qPCR-R   GTTTCCAGAGTTTCCCCCGA

cox7a2l–qPCR-FAAGGGCAAATGTACCCTCCG

cox7a2l–qPCR-R   CTGTCTGGAACACCTCGCTT

cox6b2–qPCR-FGATGCCCGTTTCCCAAACAC

cox6b2–qPCR-R   CCCACCCAGCTAATTGGACA

asb5a–qPCR-FTCATGAGGCCTGCCAATGTAA

asb5a–qPCR-R   TGCCCCAAATTCCAGCAAGA

asb5b–qPCR-FCGTGGTTAAAGGAAATCGTAAGG

asb5b–qPCR-R   TTTGCACTGTAACCCTGTGA

Note: Red highlights and yellow highlights are *asb5a⁻ᐟ⁻*:*asb5b*-ATGmo double knockout group compared with the WT group analyzed the enriched top 20hub genes using STRING database and Cytoscape software; Red highlights and green highlights are *asb5a⁻ᐟ⁻*:*asb5b*-ATGmo double knockout group compared with the *asb5a⁻ᐟ⁻* group analyzed the enriched top 20hub genes using STRING database and Cytoscape software.