

Supplementary Materials

Table S1 Primers used for q-PCR.

Primers	Sequences (5'-3')	Annealing temperature (°C)	Product size (bp)	Accession number
<i>Bad</i>	F:TCGGAGGAGATAGAGGAAGC	60°C	156 bp	XM_020095653.1 (accessed on 28 August 2022)
	R:TCGTGAGACAGTGGAGGCGT			
<i>EPAS1</i>	F:ATGAGTGGCACAACCAGAAG	60°C	131 bp	XM_020096630.1 (accessed on 28 August 2022)
	R:AGACAGGAGGGCTCGAACGAG			
18S	F:ATTGACGGAAGGGCACCAC	60°C	134 bp	EF126037.1
	R:ATGCACCACCACCCACAGA			

Table S2 System used for q-PCR.

Component	Volume
2 × ChamQ SYBR Color qPCR Master Mix (High ROX Premixed)	10.0 µl
Forward Primer	0.4 µl
Reverse Primer	0.4 µl
cDNA	2.0 µl
ddH ₂ O	7.2 µl

*The RNA concentration of the samples was 500ng/ul, and the concentration of cDNA obtained by reverse transcription of RNA was 1000ng/ul.

Table S3 Procedure used for q-PCR.

Stage	Repeat	Temperature	Time
Stage 1	Rep: 1	95 °C	30 sec
Stage 2	Rep: 40	95 °C	10 sec
		60 °C	30 sec
Stage 3	Rep: 1	95 °C	15 sec
		60 °C	60 sec
		95 °C	15 sec

Table S4 System and procedure used for double digestion.

Component	Volume
Restriction Enzyme (KpnI / HindIII / BamHI / XhoI)	1.0 μ l
DNA	1.0 μ g
10X NEBuffer	5 μ l
ddH ₂ O	To 50.0 μ l
Incubation Time	3 hours
Incubation Temperature	37°C

Table S5 Primers of plasmid construction for dual-luciferase reporter assay.

Primers	Sequences (5'-3')	Annealing temperature (°C)	Product size (bp)	Accession number
pc3.1~ <i>EPAS1</i>	F:cttggtaccgagctcggtaccCGTGCAGAGGAGGAAACAT	54°C	2982 bp	XM_020096630.1 (accessed on 28 August 2022)
	R:aacggggccctctagactcgagACACCAAAAGACCCGAGTT			
pGL~ <i>Bad</i>	F:atttctctatcgataggtaccACGGTGGTTTTATGGCTTA	51°C	2013 bp	109634873
	R:cagtaccggaatgccaagcttGGCTTGACTTTTCTGTGTG			
pGL~Bf1	F:atttctctatcgataggtaccCGTCAGTGTATGAAAGGCT	52°C	1790 bp	109634873
	R:cagtaccggaatgccaagcttGGCTTGACTTTTCTGTGTG			
pGL~Bf2	F:atttctctatcgataggtaccTCAGGGGATGAGAAACACT	52°C	1404 bp	109634873
	R:cagtaccggaatgccaagcttGGCTTGACTTTTCTGTGTG			
pGL~Bf3	F:atttctctatcgataggtaccGGGCCTTAAAAAGCCTGAA	52°C	830 bp	109634873
	R:cagtaccggaatgccaagcttGGCTTGACTTTTCTGTGTG			
pGL~Bf4	F:atttctctatcgataggtaccGCCTCACCCCTACAAAAAA	52°C	679 bp	109634873
	R:cagtaccggaatgccaagcttGGCTTGACTTTTCTGTGTG			
pGL~Bf5	F:atttctctatcgataggtaccACGAGGTAGGTGTTCCTC	52°C	529 bp	109634873
	R:cagtaccggaatgccaagcttGGCTTGACTTTTCTGTGTG			
pGL~Bmp3	F:TAAGTGCTTG GTTGAAACTGGCTGAATAAATCG	53°C	2004 bp	109634873
	R:GTTTCAACCAAGCACTTATAAATTAGATGAATAA TATTA AAAATATTATAATCATT CAGG			
pGL~Bm3	F:TAAGTGCTTG GTTGAAACTGGCTGAATAAATCG	53°C	821 bp	109634873
	R:GTTTCAACCAAGCACTTATAAATTAGATGAATAA TATTA AAAATATTATAATCATT CAGG			
pGL~Bmp5	F:TTCTTCATCTTTGATTCATTAATGGCAAGATGTAA CAAAG	53°C	2001 bp	109634873
	R:TGAATCAAAGATGAAGAACAACCTACCTCGT			
pGL~Bm5	F:TTCTTCATCTTTGATTCATTAATGGCAAGATGTAA CAAAG	53°C	517 bp	109634873
	R:TGAATCAAAGATGAAGAACAACCTACCTCGT			

Table S6 System used for dual-luciferase reporter assay.

Component	Volume
2 × Phanta Max Master Mix (Dye Plus)	25.0 µl
Forward Primer	2.0 µl
Reverse Primer	2.0 µl
cDNA (<i>EPAS1</i>) / DNA(<i>Bad</i>)	1.0 µl
ddH ₂ O	20.0 µl

Table S7 Procedure used for dual-luciferase reporter assay.

Stage	Repeat	Temperature	Time
Stage 1	Rep: 1	95 °C	3 min
Stage 2	Rep: 30	95 °C	15 sec
		60 °C*	15 sec
		72 °C	30 - 60 sec/kb
Stage 3	Rep: 1	72 °C	5 min

*Set annealing temperature according to melting temperature (T_m) value of primer.

Table S8 Primers of methylation status detecting in *EPAS1* and *Bad* promoter by MS-PCR.

Primers	Sequences (5'-3')	Annealing temperature (°C)	Product size (bp)	Accession number
<i>EPAS1</i> - M	F:ATTTTATAATGTAAGAAAAGTTGGTTGTGT	50°C	193bp	109635465
	R:TTAAATCATTTCAAAAACAAATCTC			
<i>Bad</i> -M	F:ATAGAAAAGTTAAGTTTGATTTGTGA	48.8°C	400bp	109634873
	R:ACTAAAAAACAACAAAAAAGCTTCCTA			

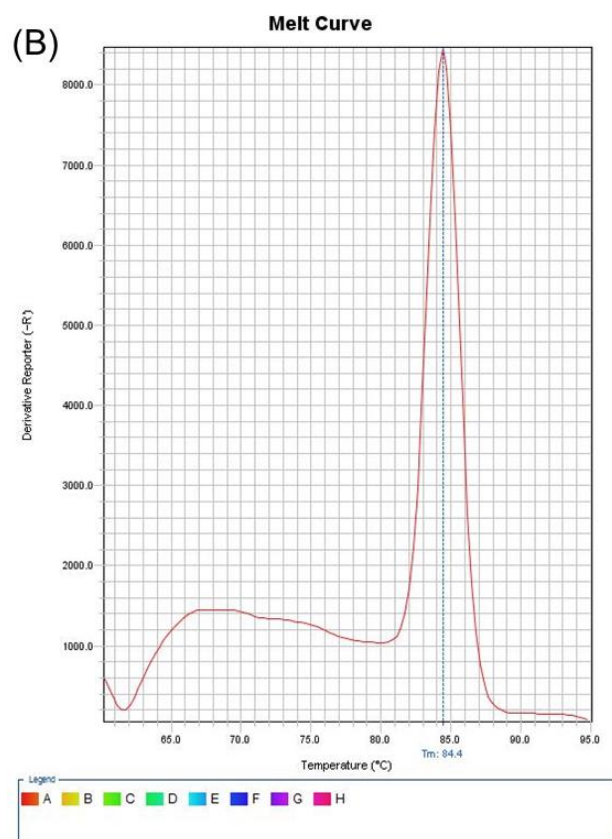
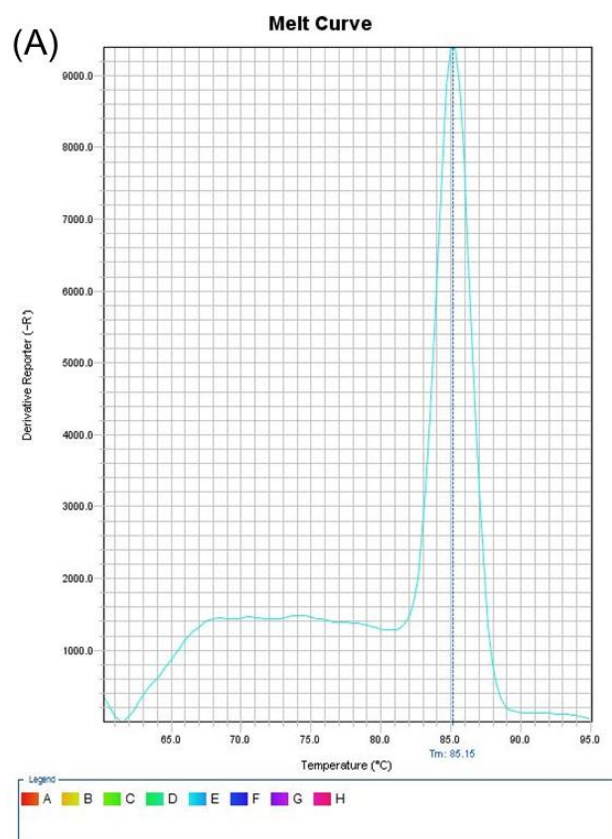
Table S9 The accession numbers of Bad protein.

Species	Accession numbers
human (<i>Homo sapiens</i>)	NP_004313.1
goat (<i>Capra hircus</i>)	XP_017898279.1
pig (<i>Sus scrofa</i>)	XP_020938542.1
Green sea turtle (<i>Chelonia mydas</i>)	XP_007072774.3
tropical clawed frog (<i>Xenopus tropicalis</i>)	XP_031756504.1
large yellow croaker (<i>Larimichthys crocea</i>)	XP_010741574.1
Atlantic salmon (<i>Salmo salar</i>)	XP_014051932.1
Japanese medaka (<i>Oryzias latipes</i>)	XP_011485704.1
Nile tilapia (<i>Oreochromis niloticus</i>)	XP_003452465.1
climbing perch (<i>Anabas testudineus</i>)	XP_026208848.1
Japanese flounder (<i>Paralichthys olivaceus</i>)	XP_019951212.1

Table S10 The accession numbers of EPAS1 protein.

Species	Accession numbers
Nile tilapia (<i>Oreochromis niloticus</i>)	XP_003438301.1
Japanese medaka (<i>Oryzias latipes</i>)	XP_023819367.1
large yellow croaker (<i>Larimichthys crocea</i>)	XP_019116438.1
ballan wrasse (<i>Labrus bergylta</i>)	XP_020511317.1
turbot (<i>Scophthalmus maximus</i>)	XP_035461545.2
Japanese flounder (<i>Paralichthys olivaceus</i>)	XP_019952189.1
tongue sole (<i>Cynoglossus semilaevis</i>)	XP_008320789.1
zebrafish (<i>Danio rerio</i>)	XP_695262.6
goldfish (<i>Carassius auratus</i>)	XP_026132160.1
chicken (<i>Gallus gallus</i>)	XP_046794384.1
Green sea turtle (<i>Chelonia mydas</i>)	XP_043399726.1
human (<i>Homo sapiens</i>)	XP_011531000.1

Figure S1 The melt curves of primers for three genes *EPAS1* (A), *Bad* (B), 18S (C) in qPCR.



(C)

Melt Curve

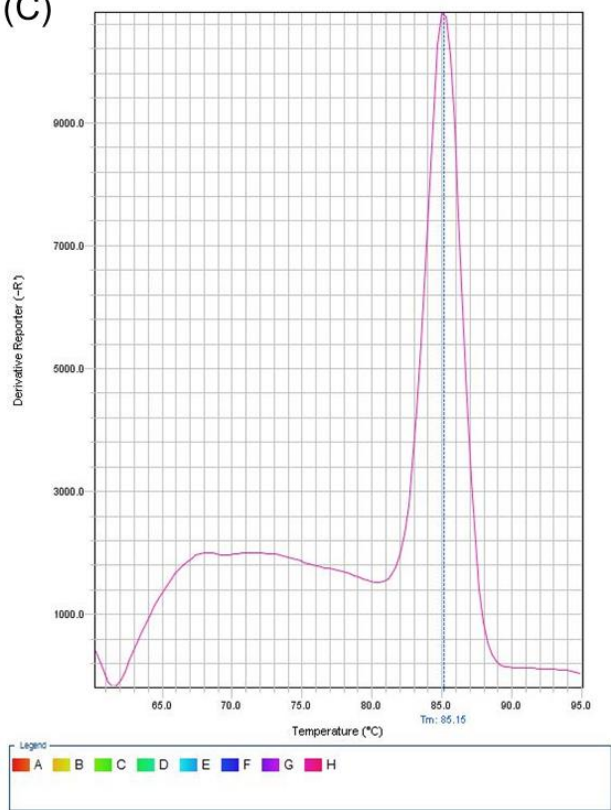
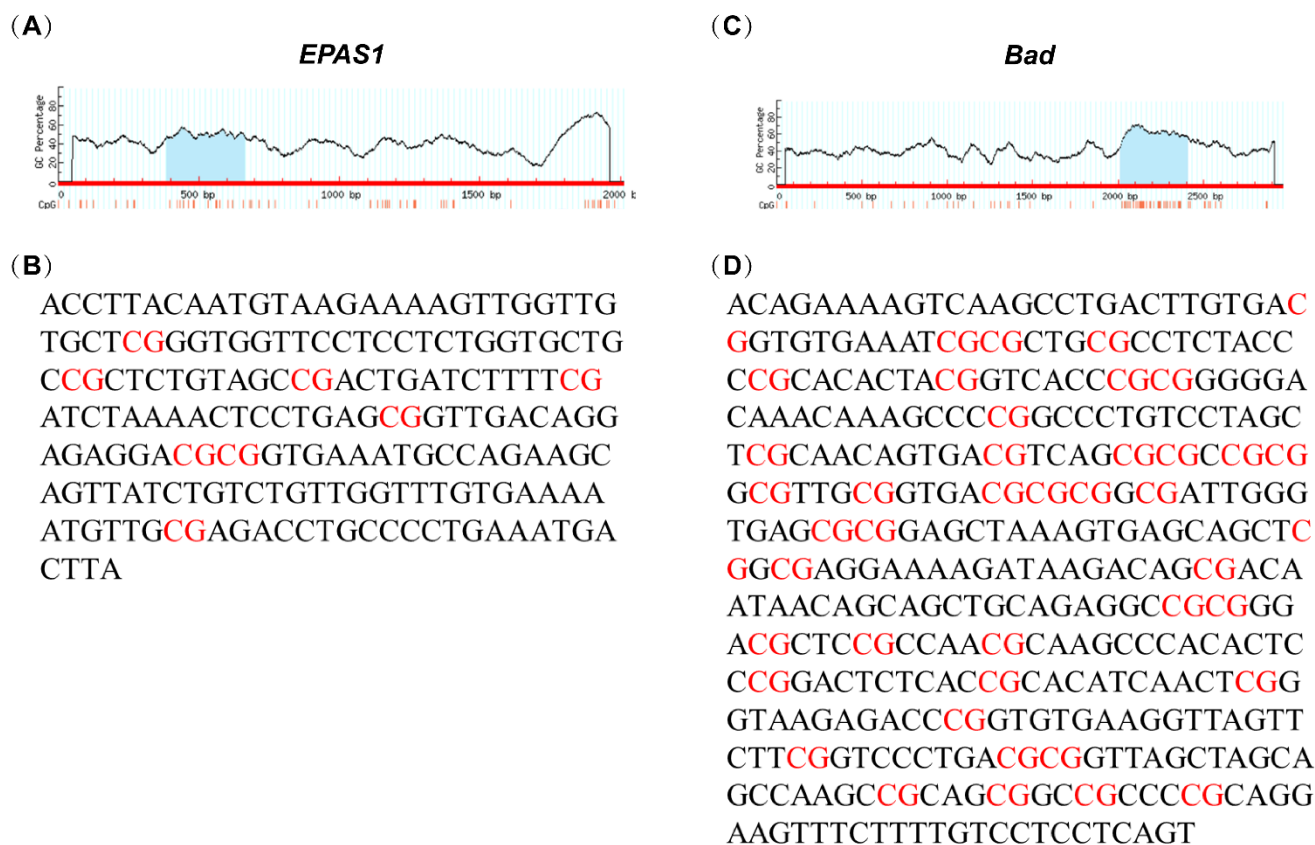


Figure S2 The predicted binding site of EPAS1 on the *Bad* promoter.

-2013
 ACGGTGGTTTTATGGCTTAAGTTGGTGCATACATGGCTTGACTTTG**CCACACACACACGCACGCACACACA**GA
 TTAATAATGGATAATAATTGCATGGTTCTCAAACATGCATCCAAATCCAACCAAAAGGAGAACTCCAGTGTATT
 ATCTCTATAATCCCTCCATAAATACAGTTCAAAAGAAGCAACTCTCAACAATGATCTTCAAGAGCTGCTTCAT
 GCATACGTCAGTGTATGAAAGGCTGACTGTGCAAACCTTTGCACTACATGTTAGTCTAAGCTTAGAAACTCACA
 TTTGTTCCATTTGGATGCACTGAATTAGAAAAAGCCCTTTGACATTGAATACATCACAGCATTGTTTCAATGA
 CTGACTTTGCCAAATTAATTCAGTTTATTTCTAGTGTCTAATATAATAGGGGGAGAGAGCAGAATGACATTGG
 GGAGGTGAAGAACAGGCTCTGGATTAAAAATGTTAGATAATTACATGGCTGAGTCTCCTGTGTTGACGTGAG
 GAAAGTGCAAGAGAGCAAAGCAAAGCACATACCCATACAGTGCTGCTTACTGTAACGGTTCTGTATAAAGA
 -1432 **Binding site 2**
AGAGGAATGTAACCCATTAATCAGCTTTTCAGGGGATGAGAAACACTTTTTTCTGATGATGTCACAATGCAAT
 TTTTCATGCCTGACACTATGACACGTTTTTGCAGCAATAATGCTACTAGAGTAATCAACTGCAATTGAAAGACA
 TTCAAAATAAGCTATTTGCTCGTATTGTTTCCAAGAAAGCTCACAACTGTGACAGGAGGTAGGAGATGAGG
 CAGTGCGACCAAAGATAAACACTCAGTAAAACACACACACAAGTGAATCAGTTTGTAGTCTACCTCCCCAC
 CTGCTGCTCATGCGAGGTATGACAGCTAACAAACAGCCTGGAGGATTTTCTGTCTTCCATGTGTCCAGCCATA
 GGGTCCCAGCACTAGAGAAAGCAGAGAAGATAAAAGAAGAGCTTAACCTTAAGACCTTCGAGGAACTGAAT
 GTTAATGTGATTCCTCCTCGATATGAATCAATTAATAAATAAAGTTTACCGGATGATTATTACATGTCATGTAAT
 GACAAGAGCAAATCTCACATAAACATTTATCACAGAAACATTATCATAGTGGGGGGGAA**-866 Binding site 3**
GAGTGGAAACGGA
 CTTCCAAAGGCCCAAAGGGGAGGGGGGCCTTAAAAAGCCTGAATGATTATAATATTTTTAATATTATTCATCT
 AATTTATAAGTGCTT**-766 Binding site 4**
AATGGAAACGGTTGAAACTGGCTGAATAAATCGACTGATTGACTGAAATTTATATCTA
 AAAATCGTAGAGGCACCCTGAGGTACCTGCCTCACCCCTACAAAAAACGGTTTAGTCGAA**-646 Binding site 5**
GTGACTGACT
 GACTACTGGAGAAGTGGTGTTCAGCATGTCAAGAAAGCAAACGGAAAGTCTGGGAATCAGAAAAATAAAAAA
 TATAAAAAATAAATACTAGTTAGTTAGTTGGTTCTTACGAGGTAGGTGTTCTTCATCT**-507 Binding site 6**
TCTGTGTGTCAGTTTG
 ATTCATTAATGGCAAGATGTAACAAAGTGAGAGATTACACCCCATTTATAAATGAATACTTAATGGGAGGAA
 AGCTGTATTTTAAAAATGACTTAGGGGGTTAGAAAATAAGATGGTATTTTTAAGGTTTGTTTTATACTTGAG
 AAATAATAAAGTAACTGTAGGTAAAAGTCTGAGAAAGGAGGACAGAAATGCAGCGAGATTTTCATATTGTGC
 ATAATTAAGCATATCAGATCATTATTACTTATACCAGATGTGGTATGTACTGTTTATTAGTGAGACATGGGGG
 CCCACAGATACTGCTTGTGTATAGGGCCCAGAATCTTGTGCTACGCCCCCTGGTGGTGAGTAGATAATGAGTG
 AATGTTTCATGTTTTGGGTGAATTATCCCTTCAATTTGCAGTTGAGTGAATTTGGCTTCACTTTTGGGGATCTGT
 CATATTTATATATCAATCATTATATCAATTCTACCACACACAGAAAAGTCAAGCC⁻¹

The bases under the blue shadow are the predicted binding site sequence.

Figure S3 Methylation status of *EPAS1* promoter and *Bad* promoter.



(A) The methylation status measuring region of the *EPAS1* gene. The abscissa indicates a part of the *EPAS1* gene; ordinate denotes CG percentage; the blue area shows the CpG island (CG percentage more than 50%, -1626~-1349, 278 bp). (B) The 193 bp sequence of methylation status measurement in the *EPAS1* gene. The 8 CpG dinucleotides are indicated in red letters. (C) The methylation status measuring region of the *Bad* gene. The abscissa indicates a part of the *Bad* gene; ordinate denotes CG percentage; the blue area shows the CpG island (CG percentage more than 50%, -953~-555, 399 bp). (D) The 400 bp sequence of methylation status measurement in the *Bad* gene. The 42 CpG dinucleotides are indicated in red letters.