**Supplemental Material**

Stearoyl-CoA desaturase (*scd1a*) is epigenetically regulated by broodstock nutrition in gilthead sea bream (*Sparus aurata*)

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**Running title:** Epigenetic regulation of stearoyl-CoA desaturase in fish

**A (*fads2*)**

**NF-Y**

Gilthead sea bream TGAGCCGTTTATTGAGCCTATTGCACATCAGCCGGTG-GTCCAGGATATA -129

Seriola TGAGCAGCTTGTTGAGCCCATTGCACATGAGCCCGTCTGTCCAGGATATA -34

Stegastes TGAGCAGCTTGTTGAGCCTATGGCACGTTGGCCAGTG-GTCCAGAATATA -149

Croaker TGAGCTGTTGATTGAGCCTATTGCACATCAGCCAGTG-GTCCAGCATGTA -149

Salmon GAAGGGCTTTTTTGAACCAATTGCAGATATGCCAGGG-GTCTA----TTG -223

Japanese sea bass TAAGCTGTTTATTGAGCCTATTGCACATGAGCCAGTG-GTCCAGGATATA -849

European sea bass TGAGCTGTTTATTGAGCCTATTGCACATCAGCCAGCG-GTCTAGGATATA -504

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**NF-Y SRE**

Gilthead sea bream C----TGTACGCCGATTGGCCCAGAAACCCTCGAATGATCGGCT- -89

Seriola C----TGTTCGCCGATTGGCCCAGAAACCCTCGAATGATCAGCT- +7

Stegastes C----TGTGCGCCGATTGGCCCAGACACCCTCGAATGATCTGCT- -109

Croaker C----TGTACTCCGATTGGCTCAGAAACCCTCGAATGATCGGCA- -109

Salmon A----AATAACCCCATTGGACTAGAGACCCTCGAATGATCTGCT- -183

Japanese sea bass CCTACTGTGCGCCGATTGGCCCAGAAAGCCTCGAATGATCAGCT- -805

European sea bass C----TGTACGCCGATTGGCCCAGAAACCCTCGAATGATCGGCTC -463

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**PUFA response region**

**B (*scd1*)**

**SREB NF-Y/NF-1**

Gilthead sea bream AAAGGTGAACAGCAGATAAACAGGCAGCCAATCAGAGGGCGAGGTTG-AGGCGGCATCCG -137

Cod GAACGTGAACAGCAGATAGAGCAGCAGCCAATAGGAGGCCGCGCCTA-AGGTGGTACCAA -451

Turbot GAAGGTGAACAGCAGATAAAGAGGCGGCCAATCAGAGGCCGAGCTCG-CGGCGAGCTACG +449

Clownfish AAAGGTGAACAGCAGATAAACAAGCAGCCAATCAGAGCCCGAGTTTG-AGGCGGCATCCG -2

Yellow catfish CCGCCTGAACAGCAGATGATTAT-CAGCCAATCAGAAGCGCAGAAACCGGGTGGCATCA- -27

Chanel catfish CTGCCTGAACAGCAGATGATTAT-CAGCCAATCAGAACCACCGAACC-AGGTGGCATCA- +89

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**PUFA response region**

**Supplementary Figure 1.** Most conserved TFBS (shaded) in proximal promoters of *fads2* (A) and *scd1* (B) in fish. Numbers indicate position relative to TSS (+1), assumed to be the first base of first exon. Sequence alignment was performed by Pro-Coffee from ExPASy (<https://tcoffee.vital-it.ch/apps/tcoffee/do:procoffee>). Prediction of TFBS was done by ConTra v3 (<http://bioit2.irc.ugent.be/contra/v3/#/step/1>) using the TRANSFAC database, with sensitivity and accuracy set at core match = 0.95 and matrix match = 0.85. Gilhead sea bream sequences were extracted from the genomic database <http://nutrigroup-iats.org/seabreamdb>, and were uploaded to GenBank under the accession numbers MN061683 (*fads2*) and MN061682 (*scd1a*). Seriola (*Seriola dumerili*) *fads2,* 111229900; Stegastes (*Stegastes partitus*) *fads2*, 103371510; Croaker (*Larimichthys crocea*) *fads2,*  104937137; Salmon (*Salmo salar*) *fads2*, AY736067.2; Japanese sea bass (*Lateolabrax japonicus*) *fads2*, KT781410.1; European sea bass (*Dicentrarchus labrax*) *fads2*, FP671139.1; Atlantic Cod (*Gadus morhua*) scd1, ENSGMOG00000000395; Turbot (*Scophthalmus maximus*) scd1, ENSSMAG00000002283; Clownfish (*Amphiprion percula*) *scd1*, ENSAPEG00000019881; Yellow catfish (*Tachysurus fulvidraco*) *scd1*, 113636202; Channel catfish (*Ictalurus punctatus*) *scd1*, ENSIPUG00000001348.

**A (*fads2*)**

**SRE ACTCTCATT**TCCTAAAGTTTATTCTGATGCAGATTTTGAATGCAACACAACAGACCACTGTGGGATTTGCTATTTTTACTCGAGCAGCCTACTTCTTCCA

**CpG1 CpG2 CpG3 CpG4 CpG5**

CCGCTGTGAGGCTGTAATTGAGGGAAAGTGTAGAAG**C**GA**C**GTGCTCC**C**GCAGACTGTTTC**C**GTCTGAG**C**GCACAGGGCTCTGTGTAATATTCAGATGA

**NF-Y NF-Y SRE**

GCCGTTTATTGAG**CCTAT**TGCACATCAGCCGGTGGTCCAGGATATACTGTA**C**GC**C**G**ATTGG**CCCAGAAACC**CTCGAATGATC**GGCT**C**GGAATTTGT

**CpG6 CpG7 CpG8 TSS CpG9 CpG10**

ACTGAATGAGTGGGTGAATGAGTCCGTGAACACACTGGGGGCAGGACAGAGGATGAATTTTGGGGATGTGCACGATTT**A**AAATGGACAGTGAACGC

ATCGGGGGCGCATAAAAGTGAGGCGGTGTGTTAATGTGAGTGAAAGAAAAACAACCCGATCA

**B (*scd1a*)**

**PPARα**

GAGCAGTTGGATT**TGTCCTG**AAATGACAATATTATGACATACATTAGCTGGAATGATAAACCAGCTAGCTAGTGTGCTAGCTATCACAGGCCCCGGCT

ATATGCGCCGCAGAGCTCATCCGGGATGCAGGTGATGCTGCCGGTGATGCTGCTGCGTACATCCCCAGCCGAAGCGCCCCTGACTTCGAACGGCTCGG

**SRE SP1**

CCATTTTAACT**CACCCTTCGC**TTTCTCTGTCGGGAAGAGTCTCTGCGCCTTCTCCAGGAACCGCTGCGCCTTGT**CCGGCTGGTT**GTTGGTTAGCGCC

**SP1 HNF4α**

GCGGTCGCGATGTCAATGCAGCGCTCCGCTTCGTCCCTGTTTACTTCCAT**CGCGGCGGGCG**AG**C**GG**CGGCACA**G**C**GGCAG**C**GG**C**GAACTGAACTG

**CpG1 CpG2 CpG3 CpG4/5 CpG6 CpG7 CpG8**

GACCACAAGTAAATAAAGTCGCCGGCCGATGACGTCATGAGGATGCTGCGAATAGATGCATCAACTGGTCTCGCGAATTTTGTTCGCATGTGGGCTCT

**PPARα**

TATTTTATACTTTTTTTGCTGCAAGTGTCTTAAGACCTAGACATTTATGAAATAGGT**TGTCCTG**TTCGCCAGTTATGAAGCCGGCGCAGGCTCCATGTG

**Supplementary Figure 2.** CpG islands (shaded in grey) in promoter regions of *fads2* (A) and *scd1a* (B) genes of gilthead sea bream. Putative TFBS and TSS (for *fads2*) are in bold and underlined. Examined CpG positions for cytosine methylation are shaded in black and were numbered from 5′-to-3′ as CpG1 to CpG10 for *fads2*, and CpG1 to CpG8 for *scd1a*.



**Supplementary Figure 3.** Response of hepatic fatty acid elongases and desaturases to deficiencies (**A**) and low dietary levels (**B**) of *n*-3 LC-PUFAs in gilthead sea bream juveniles. \*, P < 0.05; \*\*, P<0.01 (Student t-test). Gene expression was assessed by qPCR in fish from previous studies with increased liver fat deposition (Ballester-Lozano et al., 2015; Benedito-Palos et al., 2016). At the transcriptional level, *elovl6*, *scd1a*, *scd1b* exhibited the highest response in fish with signs of *n*-3 LC-PUFA deficiencies, which was especially evident in the case of *scd1a* (**A**). At the lower extent, this feature was also evidenced with a reduced dietary supply of *n*-3 LC-PUFA, though the relative response of *fads2* was magnified in the absence of signs of deficiencies in terms of growth performance (**B**). Detailed experimental design, diets formulation, and phenotypic outcomes are provided in the specified papers. Briefly: **A**) Different isoproteic and isolipidic semi-purified diets were formulated to assess specific nutrient deficiencies (Ballester-Lozano et al., 2015). In one of such diets, FO was totally replaced by a blend of vegetable oils (VO) in order to reduce the EPA and DHA contents to trace levels (*n*-3 LC-PUFA deficient diet). This diet significantly reduced weight gain and increased both hepatosomatic index (HSI) and hepatic fat accumulation. **B**) Different isoproteic and isolipidic practical diets were formulated to assess the effects of low FM/FO (Benedito-Palos et al., 2016). FM was included at 23% in the control diet and at 3% in the low FM/FO diet. Added oil was FO in the control diet and a blend of VO replacing 84% of FO in the low *n*-3 LC-PUFA diet. EPA (20:5n-3) plus DHA (22:6n-3) content (as % DM) decreased from 2.9% in the control diet to 0.6% in the low *n*-3 LC-PUFA diet. The low *n*-3 LC-PUFA diet reduced weigh gain and increased HSI.

**Supplementary Table 1.** Forward (F) and reverse (R) primers (5' to 3') for pyrosequencing of *fads2* and *scd1a* promoters of gilthead sea bream are in the upper half of the table. Primers for (RT-qPCR) of *fads2, scd1a* and transcription factors are in the lower half of the table.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene | Region | Use | CpGs | Sequence |
| *fads2* | Left | F |  | GTTGTAATTGAGGGAAAGTGTAGAA |
|  | Left | R |  | [btn]CACCCACTCATTCAATACAAATTC |
|  | Left | Seq | 5 | AGGGAAAGTGTAGAAG |
|  | Right | F |  | GTTGTAATTGAGGGAAAGTGTAGAAG |
|  | Right | R |  | [btn]TCATTCACCCACTCATTCAATACAAATTC |
|  | Right | Seq | 5 | GGTGGTTTAGGATATATTG |
| *scd1a* |  | F |  | [btn]GGGAAGAGTTTTTGYGTTTTTTTTAGGAAT |
|  |  | R |  | ATACATCTATTCRCAACATCCTCATAAC |
|  |  | Seq | 8 | ACTTTATTTACTTATAATCCA |
| *fads2* |  | F |  | GCAGGCGGAGAGCGACGGTCTGTTCC |
|  |  | R |  | AGCAGGATGTGACCCAGGTGGAGGCAGAAG |
| *scd1a* |  | F |  | CGGAGGCGGAGGCGTTGGAGAAGAAG |
|  |  | R |  | AGGGAGACGGCGTACAGGGCACCTATATG |
| *sp1* |  | F |  | CTTCTACAGCAATCACAACATC |
|  | R |  | GAGTTTGGGTCATTCCGTAA |
| *nf-y* |  | F |  | GAGTGTGTGAGCGAGTTCATCA |
|  | R |  | TGGTCTTCCTCTTCTCCTGGT |
| *srebp1* |  | F |  | CGCAGCAGAGAGACTTTACC |
|  | R |  | TGTGTTCAGACATGCCTTAGG |
| *lxrα* |  | F |  | GCACTTCGCCTCCAGGACAAG |
|  | R |  | CAGTCTTCACACAGCCACATCAGG |
| *pparα* |  | F |  | TCTCTTCAGCCCACCATCCC |
|  | R |  | ATCCCAGCGTGTCGTCTCC |
| *pparβ* |  | F |  | AGGCGAGGGAGAGTGAGGATGAGGAG |
|  | R |  | CTGTTCTGAAAGCGAGGGTGACGATGTTTG |
| *pparγ* |  | F |  | CGCCGTGGACCTGTCAGAGC |
|  | R |  | GGAATGGATGGAGGAGGAGGAGATGG |