

CRISPR/Cas9-mediated editing of $\Delta 5$ and $\Delta 6$ desaturases impairs $\Delta 8$ -desaturation and docosahexaenoic acid synthesis in Atlantic salmon (*Salmo salar* L.)

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	Total number of albinos screened	Number of fish with CRISPR-indels in Δ6 & Δ5 <i>fads2</i> genes	Number of albinos with unedited <i>fads2</i> gene
Δ6abc/5 ^{Mt}	56	56	0
Δ6bc ^{Mt}	55	53	2

Supplemental table 1. Correlation between albinos and CRISPR-induced indels in Atlantic salmon *fads2* genes

	% of total fatty acids				
	Standard diet	DHA-1	DHA-2	Low LC-PUFA	High LC-PUFA
Saturates					
C14:0	4.98	5.93	6.21	3.74	7.69
C16:0	14.99	21.78	20.31	11.75	17.82
C18:0	3.06	3.82	3.80	3.18	3.15
Monoenes					
C16:1n7	5.46	4.97	5.84	2.90	7.43
C18:1n7	2.66	3.45	3.70	2.56	3.45
C18:1n9	22.14	14.32	13.95	23.93	12.54
C22:1n9	0.37	0.00	0.00	0.14	0.25
n-6 fatty acids					
C18:2n6	7.56	9.78	10.34	20.69	8.69
C20:2n6	0.17	0.19	0.20	0.08	0.15
C20:4n6	0.74	0.83	0.91	1.04	0.74
n-3 fatty acids					
C18:3n3	3.11	1.36	1.54	16.36	2.04
C20:3n3	0.09	0.13	0.13	0.05	0.11
C20:4n3	0.56	0.45	0.56	0.06	0.47
C20:5n3	9.05	7.61	9.28	1.63	10.10
C22:5n3	1.37	1.16	1.33	0.17	1.29
C22:6n3	7.90	12.44	10.98	2.13	8.64

Supplemental table 2. Fatty acid composition (% of total fatty acids) in the standard commercial diet, the two diets relatively rich in docosahexaenoic acid (22:6n-3, DHA), namely DHA-1 and DHA-2, and low LC-PUFA and high LC-PUFA diets

Name	Sequence (5' - 3')	Targeted gene(s)
$\Delta 6abc/5^{Mt}$ target	GGCACCGACAGAGCCCAGCCAGG**	<i>\Delta 6fads2-a</i> , <i>\Delta 6fads2-b</i> , <i>\Delta 6fads2-c</i> , <i>\Delta 5fads2</i>
$\Delta 6bc^{Mt}$ target	** <u>CCA</u> AGGGTGGCGTGGTTGGGCCC	<i>\Delta 6fads2-b</i> , <i>\Delta 6fads2-c</i>
<i>slc45a2</i> target	GGGGAACAGGCCGATAAGACTGG**	<i>slc45a2</i>
$\Delta 6abc/5$ -F	TAGGCACCGACAGAGCCCAGCC	
$\Delta 6abc/5$ -R	AAACGGCTGGGCTCTGTCTGGTG	
$\Delta 6bc$ -F	TAGGGCCCAACCACGCCACCCT	
$\Delta 6bc$ -R	AAACAGGGTGGCGTGGTTGGGC	
<i>slc45a2</i> -F	TAGGGGAACAGGCCGATAAGAC	
<i>slc45a2</i> -R	AAACGTCTTATCGGCCTGTTCC	

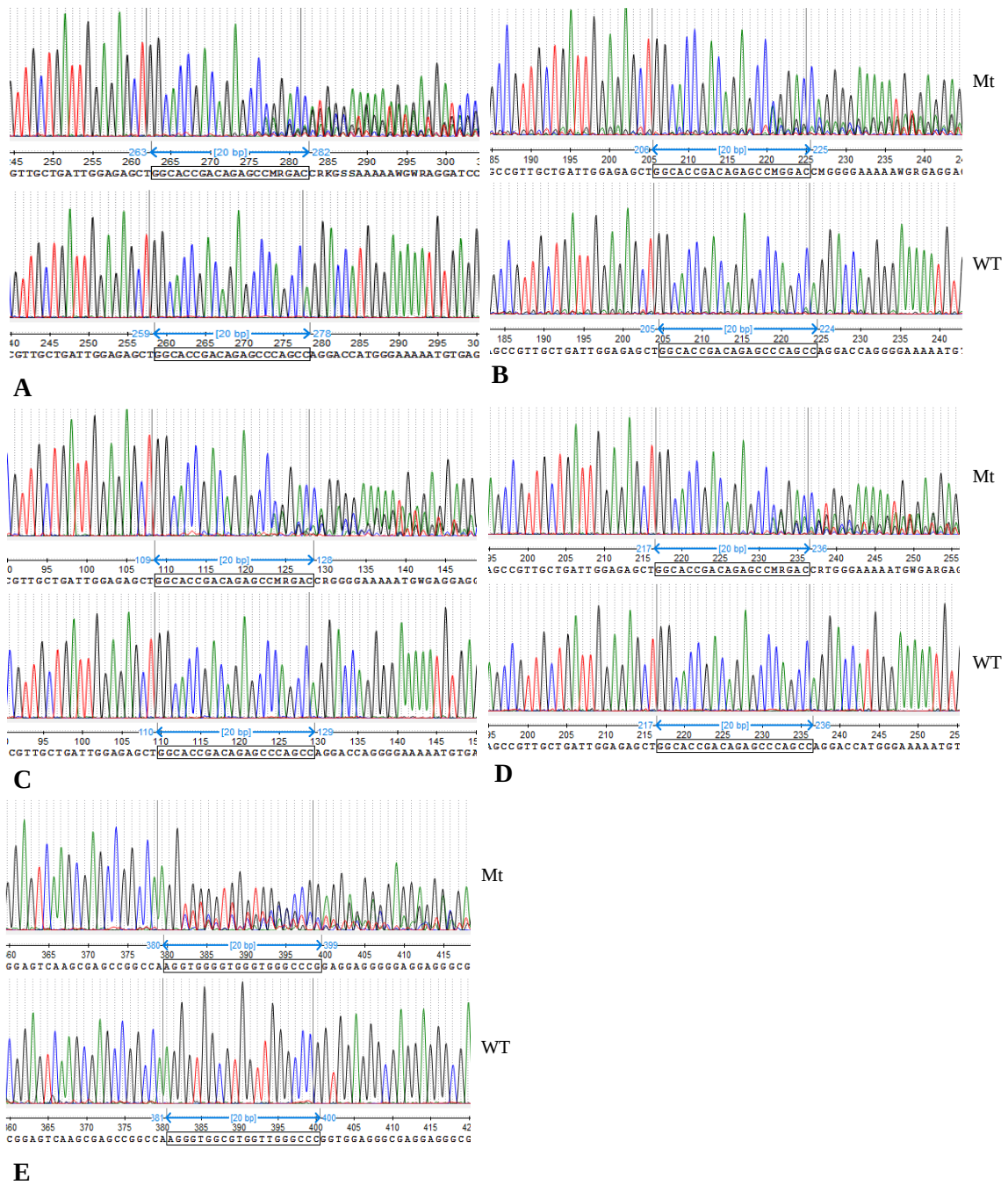
Supplemental table 3. CRISPR-target sequences and oligonucleotides, **Underlined trinucleotides are the CRISPR protospacer adjacent motif (PAM) sites: -F and -R indicate the respective forward and reverse oligonucleotide.

Primer name	Sequence (5' – 3')	Target gene(s) amplified
$\Delta 6abc/5-1F$	CGGTAAACCCCTGTTAGTAGAT	<i>$\Delta 6fads2-a$</i>
$\Delta 6abc/5-1R$	AGACGCTCTAGGCTTCACATTC	
$\Delta 6abc/5-2F$	GTTAGTGGATTGTACATTACTGG	<i>$\Delta 5fads2$</i>
$\Delta 6abc/5-2R$	ATATCTCCATCCACATCATACT	
$\Delta 6abc/5-3F$	TTTACTGAGAGGACATTTGTATG	<i>$\Delta 6fads2-c$</i>
$\Delta 6abc/5-3R$	TGTCTGAGAAGCATGGAGAAAG	
$\Delta 6abc/5-4F$	AACAACCAGGAAATGGCAGAGC	<i>$\Delta 6fads2-b$</i>
$\Delta 6abc/5-4R$	AGAGACGCTCTAGGCTTCACAT	
$\Delta 6bc-1F$	ACAGTCACAGTCATCAAATCAG	<i>$\Delta 6fads2-b$ and $\Delta 6fads2-c$</i>
$\Delta 6bc-1R$	TTCCTGTCTGATGACCAACCACT	

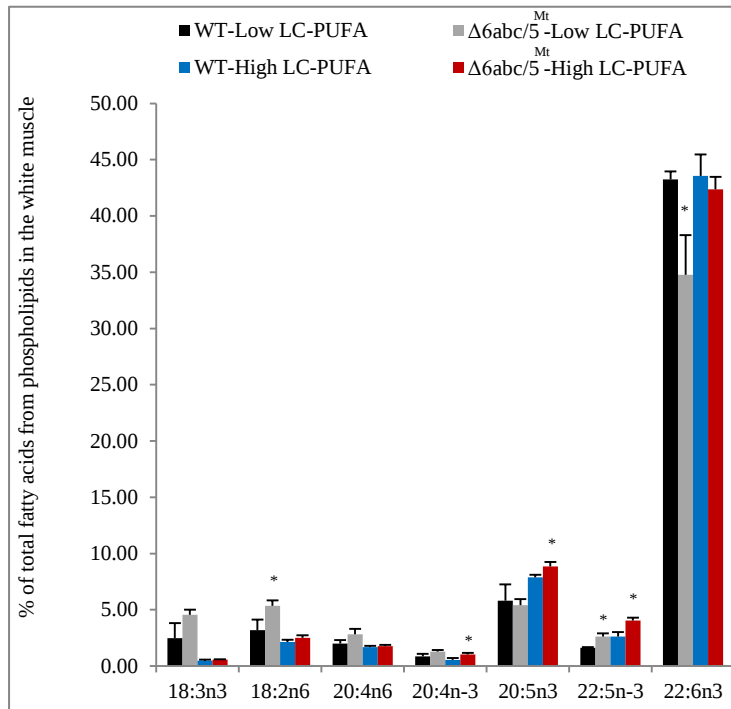
Supplemental table 4. Primers used in CRISPR-target site PCR amplification

Primer	Forward primer 5'-3'	Reverse primer 5'-3'	Accession #**
<i>srebp-1</i>	GCCATGCGCAGGTTGTTTCTCA	TCTGGCCAGGACGCATCTCACACT	HM561860.1
<i>$\Delta 5fads2$</i>	GTGAATGGGGATCCATAGCA	AAACGAACGGACAACCAGA	AF478472.3
<i>$\Delta 6fads2-a$</i>	CCCCAGACGTTTGTGTCAG	CCTGGATTGTTGCTTTGGAT	AY458652.3
<i>$\Delta 6fads2-b$</i>	CAGTCTGGTTGTCCGTTCTGT	GATATTTCACTGGCTGTGTCC	NM_001172281.1
<i>$\Delta 6fads2-c$</i>	CCTGCGAACAGAGATAGATTGA	GATTACGCTTGAGCTAACATC	GU207401.1
<i>fas-a</i>	GTATGGCACTACCCAAATACTG	AGGTACGAGCTGAATCTATCCA	XM_014179803.1
<i>fas-b</i>	GGTAGTGCCATACAAGTGATGT	TCAGTGGGCACCAAACATGAAC	XM_014209529.1
<i>elf1a-b</i>	TGCCCCCTCAGGATGTCTAC	CACGGCCCCACAGGTACTG	BG933853.1
<i>srebp-2</i>	TCGCGGCCTCCTGATGATT	AGGGCTAGGTGACTGTTCTGG	NM_001195819.1

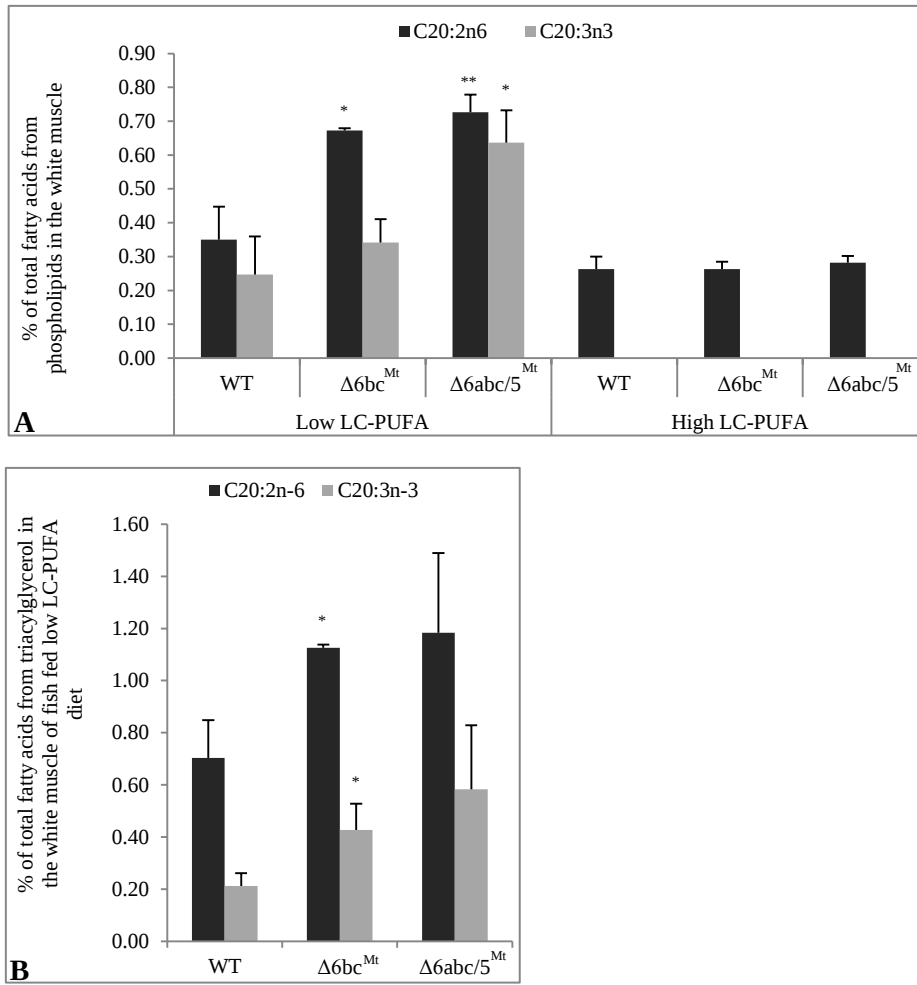
Supplemental table 5. List of primers used for RT-qPCR, ** GenBank (<http://www.ncbi.nlm.nih.gov/>)



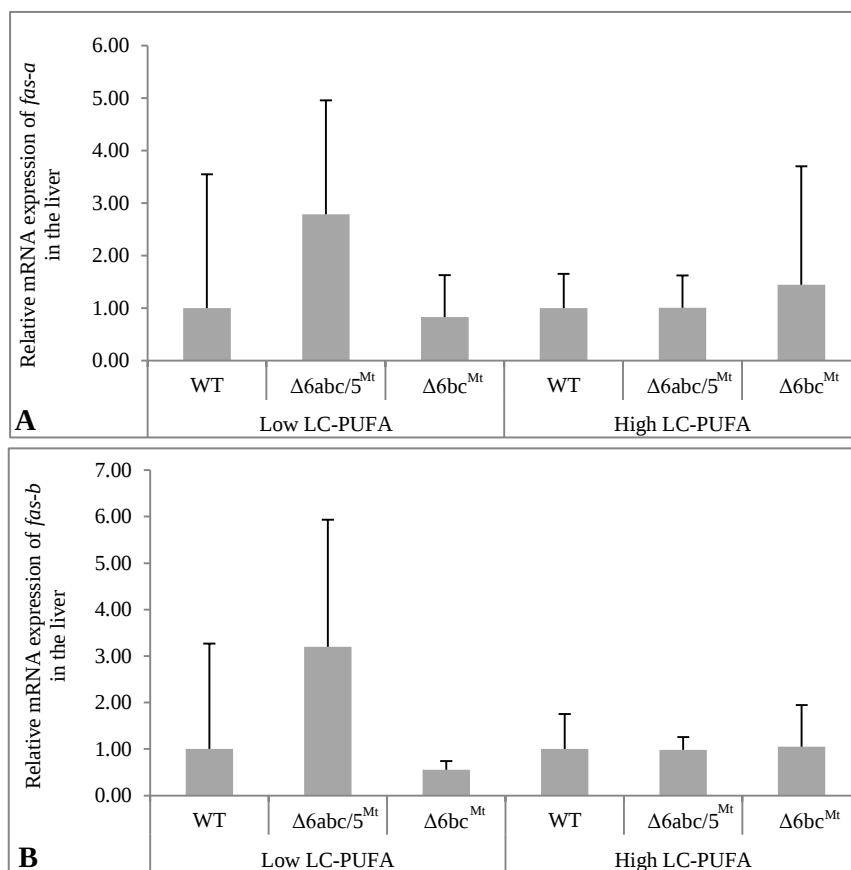
Supplemental figure 1. Confirmation of CRISPR/Cas9-induced mutations in *Δ6fads2-a* (A), *Δ6fads2-b* (B), *Δ6fads2-c* (C) and *Δ5fads2* (D) from a *Δ6abc/5^{Mt}* individual, and in *Δ6fads2-b* and *Δ6fads2-c* (E) from *Δ6bc^{Mt}*, shown as scrambled peaks at CRISPR-target sites in DNA sequencing chromatograms obtained by direct sequencing of gel-purified PCR products flanking regions around the target sites. CRISPR-target sequences are highlighted in boxes below the DNA sequencing chromatograms. CRISPR-target site in *Δ6fads2-b* and *Δ6fads2-c* of *Δ6bc^{Mt}* were co-amplified due to high level of sequence homology between *Δ6fads2-b* and *Δ6fads2-c*. Mutant DNA chromatograms from CRISPR-mutated fish (Mt) are compared to that of wildtypes (WT) that were used as controls in the experiments. Chromatograms were analyzed using the Unipro UGENE ⁴⁴.



Supplemental figure 2. Polyunsaturated fatty acid composition of phospholipid pool in the white muscle of wildtypes (WT) and $\Delta 6abc/5^{Mt}$ salmon fed low LC-PUFA and high LC-PUFA diets for 54 days. Phospholipids were separated on high performance thin layer chromatography silica gel 60 plates. Fatty acid methyl esters (FAMES) were prepared by acid-catalyzed transesterification and quantified by gas chromatography coupled with mass spectroscopy. Results are shown as mean $\pm$ standard deviation of 3 fish. Statistical differences between WT and CRISPR-mutated fish were determined using two-tailed t-test with unequal variance and are denoted as asterisks (* $p \leq 0.05$).



Supplemental figure 3. White muscle 20:2n-6 and 20:3n-3 composition in phospholipids (A) and triacylglycerol (B) in wildtypes (WT), $\Delta 6bc^{Mt}$ and $\Delta 6abc/5^{Mt}$ fed low LC-PUFA and high LC-PUFA diets for 54 days. The composition of 20:3n-3 in white muscle phospholipids of fish fed high LC-PUFA diet was very low and could not be measured in all biological replicates. Phospholipids and triacylglycerol were separated on high performance thin layer chromatography silica gel 60 plates. Fatty acid methyl esters (FAMES) were prepared by acid-catalyzed transesterification and quantified by gas chromatography coupled with mass spectroscopy. Results are shown as mean $\pm$ standard deviation of 3 fishes except samples from $\Delta 6bc^{Mt}$ with N = 2. Statistical differences between WT and CRISPR-mutated fish were determined using two-tailed t-test with unequal variance and are denoted as asterisks (* $p \leq 0.05$, ** $p < 0.01$).



Supplemental figure 4. The expression of liver fatty acid synthase-a, *fas-a* (A) and *fas-b* (B) in $\Delta 6abc/5^{Mt}$ and $\Delta 6bc^{Mt}$ relative to wildtypes (WT) fed low LC-PUFA and high LC-PUFA diets for 54 days. Results are presented as mRNA expression in CRISPR-mutated fish relative to WT, with WT set to 1. All qPCR data were analyzed using qBase⁺ 51 which determined statistical differences between WT and CRISPR-mutated fish using unpaired Mann-Whitney test with two-sided significance. Data are presented as means $\pm$ confidence interval with N = 5 per dietary treatment. Normalization was performed using elongation factor 1 α -b (*ef1 α -b*).