

# Supplementary materials: A nutraceutical rich in docosahexaenoic acid improves portal hypertension in a preclinical model of advanced chronic liver disease

Zoe Boyer-Diaz, Joan Carles Domingo, Estefanía de Gregorio, Nicolò Manicardi, Peio Aristu-Zabalza, Begoña Cordobilla, Laia Abad-Jorda, Martí Ortega-Ribera, Anabel Fernández-Iglesias, Montserrat Mari, Jaime Bosch and Jordi Gracia-Sancho

Supplementary Table S1. List of primer sequences for quantitative PCR studies.

<i>Rattus norvegicus</i> genes	Forward (5'→3')	Reverse (3'→5')	Product size (bp)
IL-1 $\beta$	GCTTCCTTGTGCAAGTGTCT	TCTGGACAGCCCAAGTCAAG	160
IL-6	TCCGGAGAGGAGACTTCACA	GCCATTGCACAACCTCTTTTCT	128
IL-10	CCTGGTAGAAGTGATGCCCC	AGACACCTTTGTCTTGGAGCTTAT	194
$\alpha$ -SMA	CTCATGCCATCATGCGTCTG	CACGCTCAGCAGTAGTCACG	105
collagen-1 $\alpha$ 1	GTACATCAGCCCAAACCCCA	TCGCTTCCATACTCGAACTGG	87
gapdh	GGCATCGTGGAAGGGCTCAT	AGGGATGATGTTCTGGGCTGC	120
<i>Mus musculus</i> genes	Forward (5'→3')	Reverse (3'→5')	Product size (bp)
IL-6	CCGGAGAGGAGACTTCACAG	CAGAATTGCCATTGCACAAC	136
tnf $\alpha$	CTGAACTTCGGGGTGATCGGT	ACGTGGGCTACAGGCTTGCA	134
Arg1	TCGGAACTCAACGGGAGGGT	TGCTTTGCTGTGATGCCCC A	108
$\beta$ -actin	GACGGCCAGGTCATCACTAT	CGGATGTCAACGTCACACTT	140

**Supplementary Table S2.** List of antibodies used for western blot studies.

<b>Primary antibodies</b>	<b>Supplier</b>	<b>Species</b>	<b>Type</b>	<b>Dilution</b>	<b>Reference</b>
$\alpha$ -SMA	Sigma	Mouse	Monoclonal	1:1000	A2547
Collagen-1 $\alpha$ 1	Cell Signaling	Rabbit	Polyclonal	1:1000	84336S
IL-1 $\beta$	Abcam	Rabbit	Polyclonal	1:1000	ab9722
IL-6	Abcam	Mouse	Monoclonal	1:1000	ab9324
TGF $\beta$	Santa Cruz	Rabbit	Polyclonal	1:1000	SC-398
PDGFR $\beta$	Santa Cruz	Goat	Polyclonal	1:1000	SC-1627
p65-NF $\kappa$ B	Santa Cruz	Rabbit	Polyclonal	1:200	SC-372
GAPDH	Santa Cruz	Mouse	Monoclonal	1:1000	SC-32233
Lamin A/C	Santa Cruz	Goat	Polyclonal	1:200	SC-6215
<b>Secondary antibodies</b>	<b>Supplier</b>	<b>Species</b>	<b>Type</b>	<b>Dilution</b>	<b>Reference</b>
Anti-Mouse IgG (HRP conjugate)	Sigma	Rabbit	Polyclonal	1:10000	A9044
Anti-Rabbit IgG (HRP conjugate)	Enzo Life Sciences	Goat	Polyclonal	1:10000	ADI-SAB-300-J
Anti-Goat IgG (HRP conjugate)	Santa Cruz	Mouse	Monoclonal	1:10000	SC-2354

**Supplementary Table S3.** Effects of DHA administration on body and organ weight in a rat model of ACLD.

Parameter	Vehicle	DHA	p value
Body weight (g)	475 ± 11	506 ± 16	0.13
Liver weight (g)	15.19 ± 0.97	15.26 ± 0.90	> 0.20
Spleen weight (g)	1.39 ± 0.08	1.32 ± 0.09	> 0.20

Results are expressed as mean ± S.E.M. n=11 and n=14, respectively, for vehicle and DHA.