

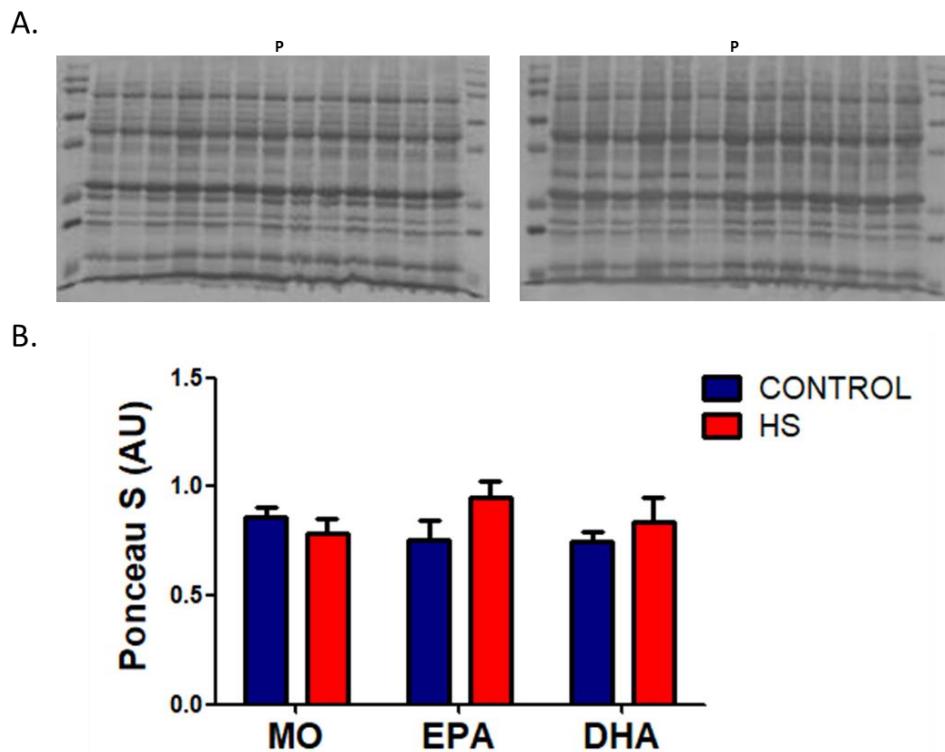
Supplemental Table S1. Composition of fatty acids in g/100 g gastrocnemius muscle wet weight.

Fatty acid	Name	MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS
14 : 0	Myristic	—	—	—	—	—	—
16 : 0	Palmitic	0.241 ± 0.067	0.182 ± 0.041	0.187 ± 0.040	0.160 ± 0.008	0.179 ± 0.020	0.176 ± 0.028
16 : 1 (n-7)	Hexadecenoic	—	0.009 ± 0.006	0.007 ± 0.006	—	0.006 ± 0.005	0.008 ± 0.007
17 : 0	Margaric	—	—	0.004 ± 0.004	—	—	—
17 : 1 (n-7)	Heptadecenoic	—	—	—	—	—	—
18 : 0	Stearic [#]	0.158 ± 0.028	0.112 ± 0.008	0.114 ± 0.018	0.112 ± 0.011	0.105 ± 0.019	0.102 ± 0.009
18 : 1 (n-9)	Oleic	0.153 ± 0.082	0.108 ± 0.054	0.112 ± 0.027	0.080 ± 0.021	0.099 ± 0.014	0.097 ± 0.016
18 : 1 (n-7)	Vaccenic [#]	0.032 ± 0.007	0.024 ± 0.007	0.021 ± 0.005	0.020 ± 0.002	0.022 ± 0.001	0.021 ± 0.003
18 : 2 (n-6)	Linoleic	0.332 ± 0.142	0.220 ± 0.086	0.208 ± 0.067	0.160 ± 0.009	0.201 ± 0.039	0.183 ± 0.026
20 : 0	Eicosanoic	—	—	—	—	—	—
20 : 1 (n-9)	Eicosenoic	—	—	—	—	—	—
18 : 3 (n-6)	γ -Linolenic	—	—	—	—	0.001 ± 0.000	—
18 : 3 (n-3)	α-Linolenic*	0.040 ± 0.034	0.009 ± 0.005	0.006 ± 0.006	—	0.015 ± 0.012	0.005 ± 0.004
22 : 0	Docosanoic	0.025 ± 0.0024	—	—	—	—	—
20 : 2	Eicosadienoic	—	—	—	0.002 ± 0.002	—	—
20 : 3 (n-6)	Eicosatrienoic	—	—	—	—	—	—
20 : 4 (n-6)	Arachidonic###	0.165 ± 0.015	0.136 ± 0.003	0.079 ± 0.009	0.081 ± 0.011	0.076 ± 0.008	0.074 ± 0.007
20 : 5 (n-3)	Eicosapentaenoic	—	—	—	0.022 ± 0.003	0.007 ± 0.001	0.007 ± 0.002
24 : 1 (n-9)	Nervonic	0.018 ± 0.013	0.002 ± 0.002	—	0.006 ± 0.002	0.007 ± 0.007	0.003 ± 0.003
22 : 5 (n-6)	Docosapentaenoic	0.014 ± 0.003	0.013 ± 0.001	—	0.003 ± 0.003	0.006 ± 0.001	0.006 ± 0.001
22 : 5 (n-3)	Docosapentaenoic###	0.021 ± 0.002	0.017 ± 0.002	0.034 ± 0.006	0.031 ± 0.002	0.011 ± 0.002	0.010 ± 0.002
22 : 6 (n-3)	Docosahexaenoic###	0.071 ± 0.010	0.064 ± 0.003	0.126 ± 0.018	0.138 ± 0.027	0.173 ± 0.034	0.176 ± 0.026
Total	Saturated	0.424 ± 0.111	0.295 ± 0.046	0.305 ± 0.056	0.277 ± 0.017	0.284 ± 0.034	0.279 ± 0.035

	Monounsaturated	0.203 ± 0.099	0.143 ± 0.065	0.140 ± 0.037	0.107 ± 0.025	0.133 ± 0.011	0.129 ± 0.028
	Polyunsaturated	0.642 ± 0.188	0.459 ± 0.097	0.483 ± 0.113	0.437 ± 0.043	0.488 ± 0.081	0.461 ± 0.066
	Omega-6##	0.511 ± 0.152	0.369 ± 0.088	0.287 ± 0.076 ^x	0.247 ± 0.013	0.283 ± 0.044 ^x	0.263 ± 0.034
	Omega-3###	0.132 ± 0.038	0.090 ± 0.009	0.196 ± 0.037	0.191 ± 0.030 ^y	0.206 ± 0.042 ^x	0.198 ± 0.033 ^y
	Fatty %	1.360 ± 0.419	0.963 ± 0.223	0.991 ± 0.220	0.878 ± 0.047	0.969 ± 0.128	0.929 ± 0.131

The determination of fat was calculated from the tridecanoate triglyceride, which was used as internal standard. Values are presented as mean ± SD, n=3 animals. The results were compared using two-way ANOVA. The results were compared using two-way ANOVA. * P<0.05: hindlimb suspension main effect. # P<0.05; ## P<0.01; ### P<0.001: fish oil supplementation main effect. **MO-C:** Mineral oil supplemented group; **MO-HS:** Mineral oil supplemented and hindlimb suspension group; **EPA-C:** High eicosapentaenoic acid fish oil supplemented group; **EPA-HS:** High eicosapentaenoic acid fish oil supplemented and hindlimb suspension group; **DHA-C:** High docosahexaenoic acid fish oil supplemented group; **DHA-HS:** High docosahexaenoic acid fish oil supplemented and hindlimb suspension group; — : not detected.

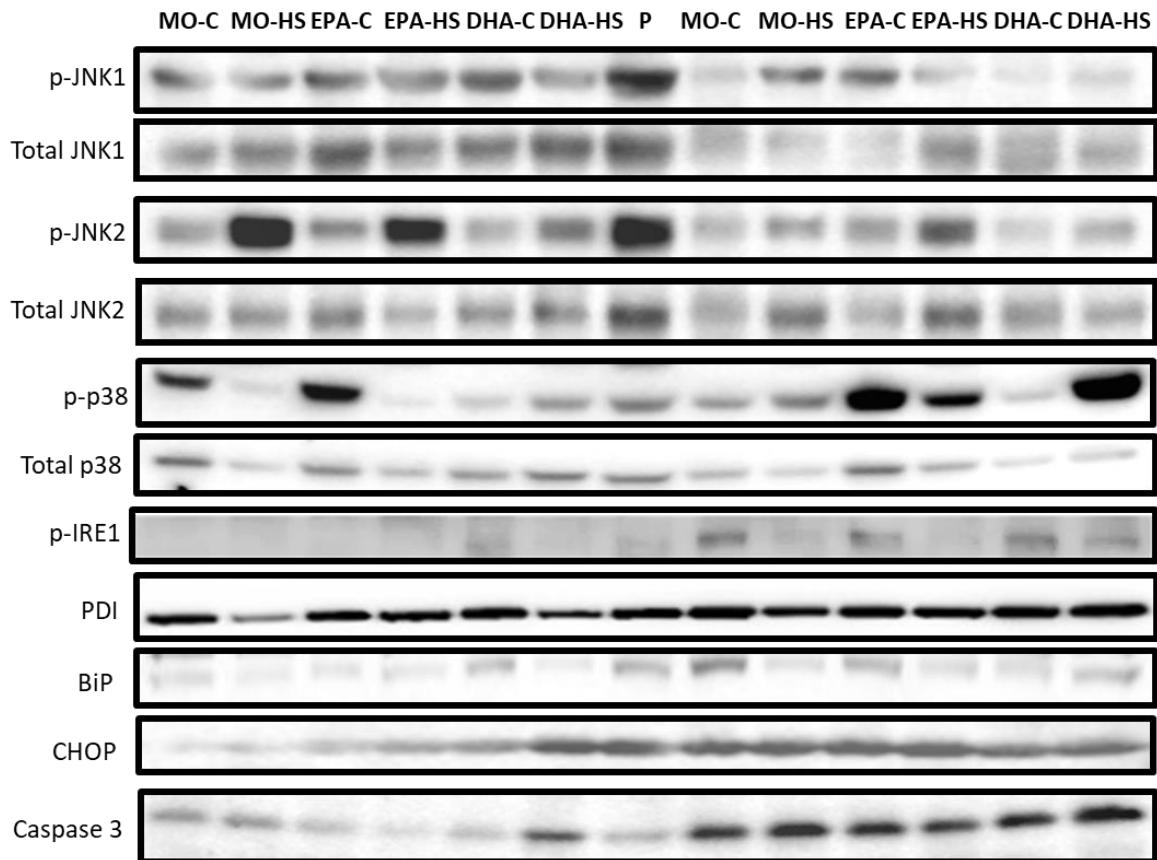
Supplemental Figure S1



Supplemental Figure S1. Ponceau S quantification of western blot membranes.

(A) Representative images of the western blot membranes stained with Ponceau S used in this study. (B) Average quantitative analysis of Ponceau S staining. No significant differences were observed. Results were compared using two-way ANOVA (hindlimb suspension effect) and the Bonferroni post-hoc test. The six groups included mineral oil supplemented control (MO-C), MO with hind limb suspension (MO-HS), high EPA fish oil control (EPA-C), EPA with HS (EPA-HS), high DHA fish oil control (DHA-C) and DHA with HS (DHA-HS). P, pool containing a mixture with equal parts of all samples – used to normalize Ponceau S quantitative results; AU, arbitrary units.

Supplemental Figure S2



Supplemental Figure S2. Images used for quantitative analysis of the western blot assays in this study. **MO-C:** Mineral oil supplemented group; **MO-HS:** Mineral oil supplemented and hindlimb suspension group; **EPA-C:** High eicosapentaenoic acid fish oil supplemented group; **EPA-HS:** High eicosapentaenoic acid fish oil supplemented and hindlimb suspension group; **DHA-C:** High docosahexaenoic acid fish oil supplemented group; **DHA-HS:** High docosahexaenoic acid fish oil supplemented and hindlimb suspension group; **P:** pool containing a mixture of equal parts of all samples – used to normalize Ponceau S quantitative results.

Supplemental Figure S3

Supplemental Figure S3. Results of the analysis of fatty acids composition in the gastrocnemius muscle used in the Supplemental Table 1 of this study. **MO-C:** Mineral oil supplemented group; **MO-HS:** Mineral oil supplemented and hindlimb suspension group; **EPA-C:** High eicosapentaenoic acid fish oil supplemented group; **EPA-HS:** High eicosapentaenoic acid fish oil supplemented and hindlimb suspension group; **DHA-C:** High docosahexaenoic acid fish oil supplemented group; **DHA-HS:** High docosahexaenoic acid fish oil supplemented and hindlimb suspension group.

Supplemental Figure S4

p-IRE1						p-JNK1					
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS	MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS
0.6153585	0.5497207	0.4044116	0.7700508	1.630951	1.999162	0.3871916	0.3975725	0.4863653	0.4059652	0.5834132	0.3501655
0.8997738	1.164103	1.014536	1.857821	2.355515	1.460809	0.4874083	0.2735124	0.8336885	0.6581348	0.6120651	1.243006
0.9194162	2.687632	3.556192	0.7280849	0.8740129	5.567465	1.624972	1.25753	2.101955	0.9493784	1.18002	1.450953
1.565451	1.006157	1.698187	0.9563621	1.482459	2.452181	1.500428	0.7043236	0.4065093	0.4190323	1.033029	0.6854423
0.2083717	0.4683085	0.390644	1.048939	0.7234957	0.6945592	0.3809046	0.8976814	0.8587769	0.3268104	0.2172374	0.2996055
0.9089412	0.5989404	0.7149263	0.4225054	1.034033	0.9780508	1.180888	1.595899	0.9892865	1.006252	1.553147	0.6189446
1.557801	0.7225244	1.363589	0.9749871	1.166191	0.8963407	1.645584	0.4753423	0.1841554	1.05153	0.5587942	1.506965
1.324886	0.7502297	1.619482	1.015114	1.070303	1.51302	0.7926243	1.194505	0.7764287	1.758858	0.6268156	1.316148
Total JNK1						p-JNK1/total JNK1					
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS	MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS
0.7023358	0.7807512	1.126911	1.489938	1.576421	1.182366	0.439367	0.4058354	0.3439688	0.2171535	0.2949514	0.2360302
1.706282	1.268036	1.684552	1.260394	1.379447	1.035032	0.2276609	0.1719062	0.394426	0.4161545	0.3536217	0.9571183
0.6138797	0.9206573	1.157587	0.9704418	1.127453	1.07681	2.109643	1.088596	1.447158	0.7796795	0.8341371	1.073892
0.9775026	1.011782	1.209735	0.9547787	1.279452	0.8558885	1.223329	0.5547938	0.2678099	0.3497768	0.6434799	0.6382633
1.357799	1.310717	1.44964	1.136579	1.228455	0.980612	0.25225267	0.6165098	0.5332699	0.258835	0.159185	0.2750295
0.9753177	0.9942121	1.022756	0.7702624	0.6786119	0.6312598	1.089907	1.444951	0.8707169	1.175966	2.060241	0.8826133
0.8130485	0.5443257	0.54624	1.479133	1.327633	1.106339	1.821924	0.786094	0.3034784	0.6399434	0.3788791	1.226145
0.8538344	0.8728133	1.030575	0.8667182	0.9188889	1.070962	0.8356426	1.231951	0.6781856	1.826753	0.6140499	1.10626
p-p38						Total p38					
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS	MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS
0.7290294	0.3726717	1.368305	0.09549447	0.1567636	0.4370244	0.9365178	0.6888247	0.7310548	0.7069595	1.184724	1.065191
0.8461534	0.551604	0.31547	0.5616307	1.199858	0.603361	0.3101498	0.1740624	0.7106556	0.5097835	0.7819099	0.569061
0	0.2226684	0.05338126	0.1117527	0.1276871	0.7128024	0.6018504	0.6179724	0.7864144	0.3008575	0.3931499	1.115617
1.424817	0.3007653	0.6838436	1.012428	0.6256877	1.744648	2.151482	0.71633	1.266323	1.231333	1.116805	1.179705
0	0.4798277	1.046334	0.6451332	0.1218611	1.359512	1.271954	0.6087031	1.358421	1.720866	1.389281	0.3969254
1.114661	0.6737167	0.4442618	0.6915135	1.611775	0.04808569	1.276328	0.5658719	0.6607621	0.5914145	1.477918	0.8968603
0.8575584	0.2499453	1.011951	0.8550895	1.113358	0.7644187	0.7052578	0.8355139	0.916728	0.3089169	1.040816	1.017373
1.027781	0.7969056	1.15758	2.215019	0.1058038	1.038571	0.7464595	0.4569152	1.44857	0.6780984	0.1911304	0.3776686
p-p38/total p38						BiP					
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS	MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS
0.5601808	0.3893292	1.34689	0.09720374	0.09521984	0.2952415	0	0.2807142	1.065777	1.357345	0.8471891	0.2648359
1.963256	2.280456	0.3194465	0.7928013	1.104263	0.762988	0.8775132	0.2957402	0.2632837	0.2643753	0.4639175	0.1775585
0	0.238455	0.04884686	0.2672984	0.2337159	0.4597833	1.153148	0.4495475	0.9144222	0.3425869	0.4699619	0.3920566
0.4765634	0.302144	0.3886077	0.5916811	0.4031619	1.064225	0.9693384	0.2669658	0.7816318	0.2923143	0.6335202	0.6537158
0	0.6822644	0.666667	0.3244705	0.07591854	2.96447	0.9757515	0.9718959	1.811129	1.52732	1.313757	0.9616829
0.7558805	1.030462	0.5819246	1.012002	0.9439021	0.04640491	1.101882	0.3417288	0.8086839	0.4733619	0.583824	0.8605221
1.052419	0.2589191	0.9554148	2.395757	0.9258346	0.650315	1.147827	1.171393	0.9317048	0.8206658	1.086263	0.8239077
1.191701	1.509538	0.6916466	2.827207	0.47912	2.380115	0.7745399	0.487431	1.064199	0.3442866	0.7676737	0.2835129
PDI						CHOP					
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS	MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS
0.5315657	0.2042233	0.50086	0.5072151	0.573514	0.3573191	0.4054731	0.796629	1.319978	1.741857	1.872081	4.209004
1.23878	1.05621	0.7158358	0.965629	0.6405954	0.4574019	2.03895	2.229763	1.574215	2.273082	2.152474	0.2562622
1.778726	1.496685	1.02199	0.3641724	0.596355	0.505756	1.321636	2.180821	2.257548	2.222811	2.935706	2.720378
0.4509286	0.414589	0.5634969	0.3337305	0.4737948	0.4734475	0.2339406	0.3375282	0.9856287	1.187397	2.77637	2.846222
1.070917	0.8171548	0.9285584	0.9124311	1.114486	1.289647	0.8396529	0.7395914	0.709124	0.6809554	0.4247961	0.5880466
1.11959	0.9437391	1.259529	1.034163	1.514888	2.060867	1.282733	1.169081	2.049561	1.155932	1.026089	0.891471
0.9281873	1.726753	1.950918	1.54825	2.292979	3.048619	0.7202581	0.7898347	0.7333388	0.5047874	0.5038145	0.1126229
0.8813055	0.8296435	0.9381829	0.8741558	1.10552	1.100499	1.157356	0.4675514	0.4577951	0.2489748	0.268135	0.3043923
Caspse 3											
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS						
0.8130626	1.110164	1.02688	1.76136	0.9353209	0.9968339						
1.342096	1.580731	1.133577	1.584083	0.6919156	0.8467507						
0.8448417	1.613304	1.359702	2.266006	2.427004	1.853052						
0	3.092324	1.182628	1.019917	1.080899	2.841983						
0.9440381	1.35222	1.284026	1.480267	0.9387398	0.8509735						
0.8446205	1.180406	0.909142	1.072325	1.151763	1.748046						
1.010927	1.577311	1.56993	2.006764	1.814891	2.278161						
1.200414	1.408836	1.216581	1.194373	1.151727	1.620704						

Supplemental Figure S4. Results of the quantitative analysis of western blot assays used in the Figures 1-4 of this study. **MO-C:** Mineral oil supplemented group; **MO-HS:** Mineral oil supplemented and hindlimb suspension group; **EPA-C:** High eicosapentaenoic acid fish oil supplemented group; **EPA-HS:** High eicosapentaenoic acid fish oil supplemented and hindlimb suspension group; **DHA-C:** High docosahexaenoic acid fish oil supplemented group; **DHA-HS:** High docosahexaenoic acid fish oil supplemented and hindlimb suspension group. Grubb's test was used to exclude outliers (blue numbers).

Supplemental Figure S5

BiP						CHOP					
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS	MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS
0.6757534	0.5694513	0.9928343	0.2054358	0.4982093	0.3826492	0.902231	1.128686	0.8625171	0.1564485	1.095678	1.03605
0.4247056	0.5221807	0.6894848	0.2418308	0.7078679	0.708095	0.6248297	1.337576	1.206302	0.8461994	0.5817826	2.127287
1.089224	0.2590941	1.911067	0.319735	1.074549	0.2052097	0.7957022	0.978435	1.258215	0.5111961	1.338606	0.2977495
0.5166567	0.4915888	0.5461434	0.2666981	0.5829486	0.3818604	0.8090371	1.267973	0.03885789	0.392368	1.282039	0.8815518
0.9318824	0.3833502	1.235712	0.3624342	0.3421474	0.2354435	0.8095656	0.6171679	0.9153166	0.9745384	0.5624084	1.454429
1.401891	0.390218	0.6872534	0.2030624	1.681313	0.1557699	1.314373	1.101411	0.7932865	0.5498063	1.785822	0.4276458
0.9047857	0.4948895	2.013075	0.5426381	0.7209339	0.2864181	0.9218782	0.3396636	2.065371	1.010495	0.630662	0.4840388
0.9242067		0.6383536		0.4627544	0.2282302	1.023341		0.9456825		0.8921255	0.7348703
2.227358		0.6398183		0.9528923	0.8931368	1.497269		0.8542523		0.6273685	1.733819
2.544628		0.4627929		0.8575469		1.833315		1.759833		0.9561359	
IRE1						PERK					
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS	MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS
0.4732153	2.738991	0.5454901	0.3985299	0.4063582	0.7120716	0.5047247	1.598417	0.6322753	0.7452311	0.504814	0.9815231
1.087138	1.691873	0.6875758	0.7621092	0.8690747	1.969753	1.059611	1.755183	0.8781813	0.6741164	0.7323231	0.9733393
0.4115959	0.8997199	1.042739	1.214994	0.716844	0.4668953	0.4770791	0.7425437	1.200286	0.9753208	0.8601922	0.5881153
0.7543353	3.297846	0.3128085	1.034748	0.7670756	1.502242	0.7052848	2.557127	0.7201411	0.7281357	0.7171966	0.6830757
1.105133	1.285861	0.5290012	1.339589	1.838705	1.47371	0.937714	0.9564323	0.4551275	0.8980023	0.4971283	0.8903552
0.8665618	1.147387	0.9558883	1.046807	4.157131	0.5409204	0.8387859	1.302562	0.5277454	0.7894914	3.996087	0.4312177
0.5554188	0.8017245	1.003751	4.904351	0.8490603	1.158449	0.5229149	0.855108	0.7363181	5.087871	1.033071	0.760592
1.182867		0.9648842		0.6946309	0.576168	1.046293		0.8653933		0.5348916	0.7513523
2.397172		0.8188858		0.6908216	5.216033	2.048167		1.060492		0.476116	5.641008
4.150988		9.090999		0.3800569		6.304817		6.715239		0.6817372	
BECLIN						LC3II					
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS	MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS
0.8739173	0.8880078	0.2775126	0.6496614	0.7050629	0.4219354	1.116179	0.7964437	0.8788111	0.5628256	0.6919904	1.336161
0.6486602	0.4455382	0.803623	0.3063111	0.9218169	0.4941479	1.4325	1.666269	1.187223	2.213101	1.608317	1.439946
0.4073386	0.5405679	0.3699439	0.2545373	0.7293742	0.2617336	0.6229984	1.261857	1.88999	1.877677	1.53446	1.221938
0.6454055	1.861575	0.4532414	0.3595537	0.4451738	0.7695662	1.165766	0.7843252	0.3466019	1.130749	1.315344	1.664533
0.9788908	0.1904831	0.4987341	0.3968844	0.4978179	0.770813	1.126791	1.803419	1.056537	2.203491	1.76136	3.452049
0.8457919	0.3306444	0.312064	0.32556	3.946539	0.4001155	1.080257	2.542684	1.583277	2.134644	0.8727183	2.481975
1.146033	0.2216228	0.9204435	2.280045	0.426004	0.1669161	1.188917	0.8231221	1.477749	0.4389298	0.8133448	3.227003
1.178774		0.5717387		0.4350697	0.2660117	1.495147		1.511968		1.368237	1.051582
2.022772		0.8160527		0.9469664	1.714696	0.8641379		1.78972		1.234892	0.08082543
2.965868		3.835564		0.9587905		0.4605579		1.001692		0.9345136	
ATG14											
MO-C	MO-HS	EPA-C	EPA-HS	DHA-C	DHA-HS						
0.4709248	2.292059	0.6871157	1.700265	0.4394661	0.9747432						
1.143562	2.623733	0.7698187	0.8956889	0.6785618	1.475307						
0.467261	1.569764	0.922345	0.675463	0.9092388	0.5374382						
0.8271827	2.402479	0.9502319	0.6746818	0.9268711	2.602895						
1.122893	2.035996	0.4519837	1.296652	1.095576	2.717826						
0.6445547	2.315546	0.7259358	1.393773	2.426014	1.501587						
0.6305348	2.076535	0.4170814	2.406711	0.9246241	1.735312						
1.113644		0.8475837		0.8451729	0.5538478						
1.897807		1.038668		0.5622895	3.520921						
4.981066		5.0892		0.5853159							

Supplemental Figure S5. RT-PCR results after $2^{-\Delta\Delta CT}$ calculation for the relative expression of genes used in the Figures 5 and 6 of this study. **MO-C:** Mineral oil supplemented group; **MO-HS:** Mineral oil supplemented and hindlimb suspension group; **EPA-C:** High eicosapentaenoic acid fish oil supplemented group; **EPA-HS:** High eicosapentaenoic acid fish oil supplemented and hindlimb suspension group; **DHA-C:** High docosahexaenoic acid fish oil supplemented group; **DHA-HS:** High docosahexaenoic acid fish oil supplemented and hindlimb suspension group. Grubb's test was used to exclude outliers (blue numbers).