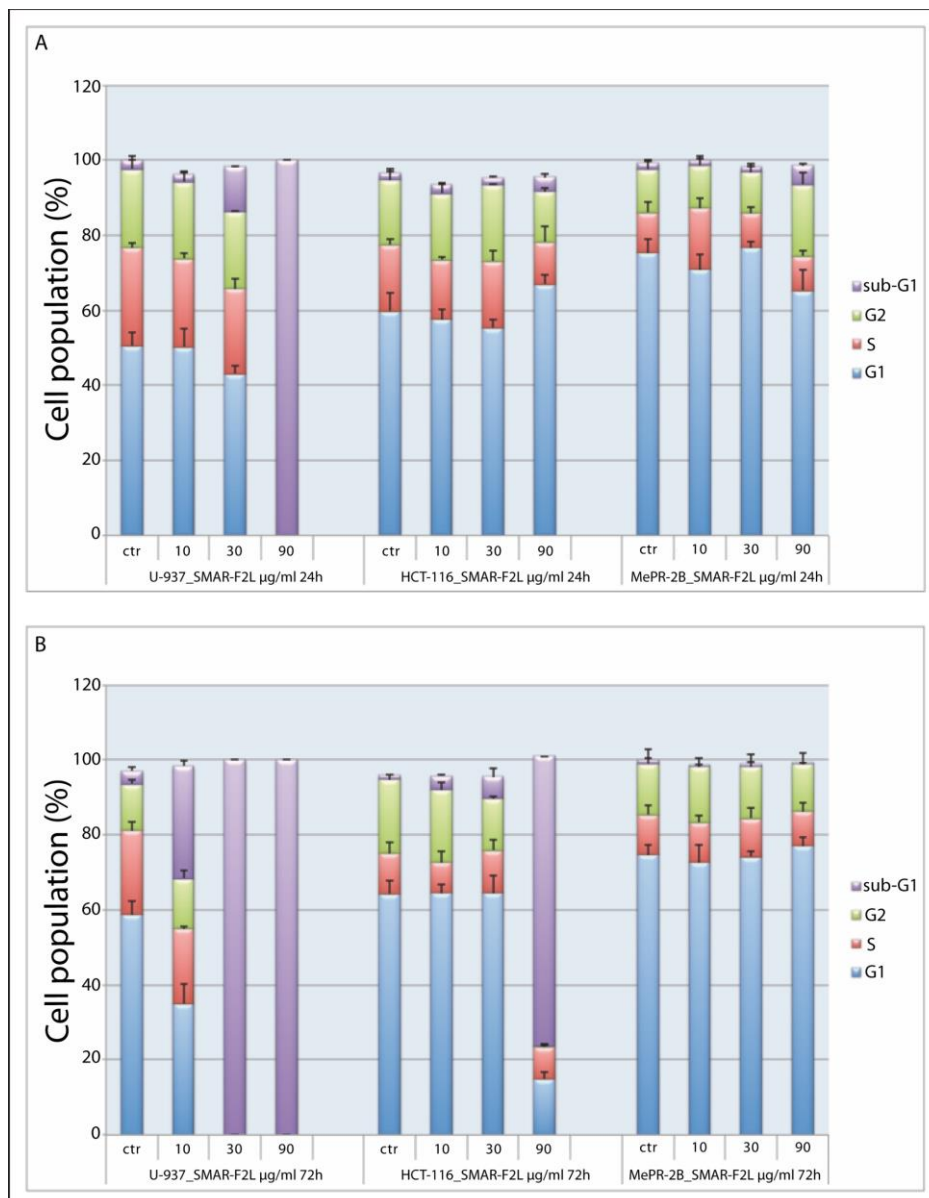


# Monoacylglycerides from the diatom *Skeletonema marinoi* induce selective cell death in cancer cells

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## Supplementary Information



**Figure S1.** Cell cycle distribution in U-937, HCT-116 and MePR-2B cells after treatment for 24 (A) and 72 hours (B) with fraction SMAR-F2L at 10, 30 and 90 µg/ml with the vehicle DMSO (control). All bars represent means  $\pm$  SD with n = 3.

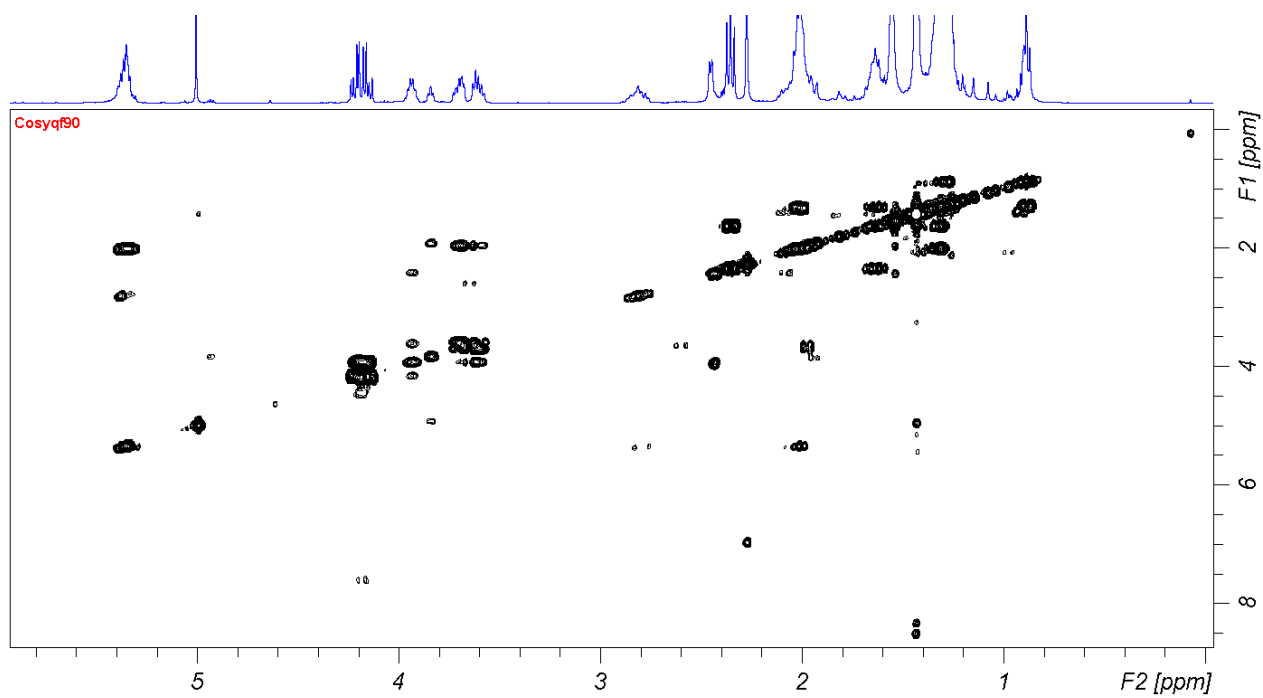


Figure S2. <sup>1</sup>H, <sup>1</sup>H COSY-NMR spectrum (400MHz, CDCl<sub>3</sub>) of fraction SMAR-F2L.

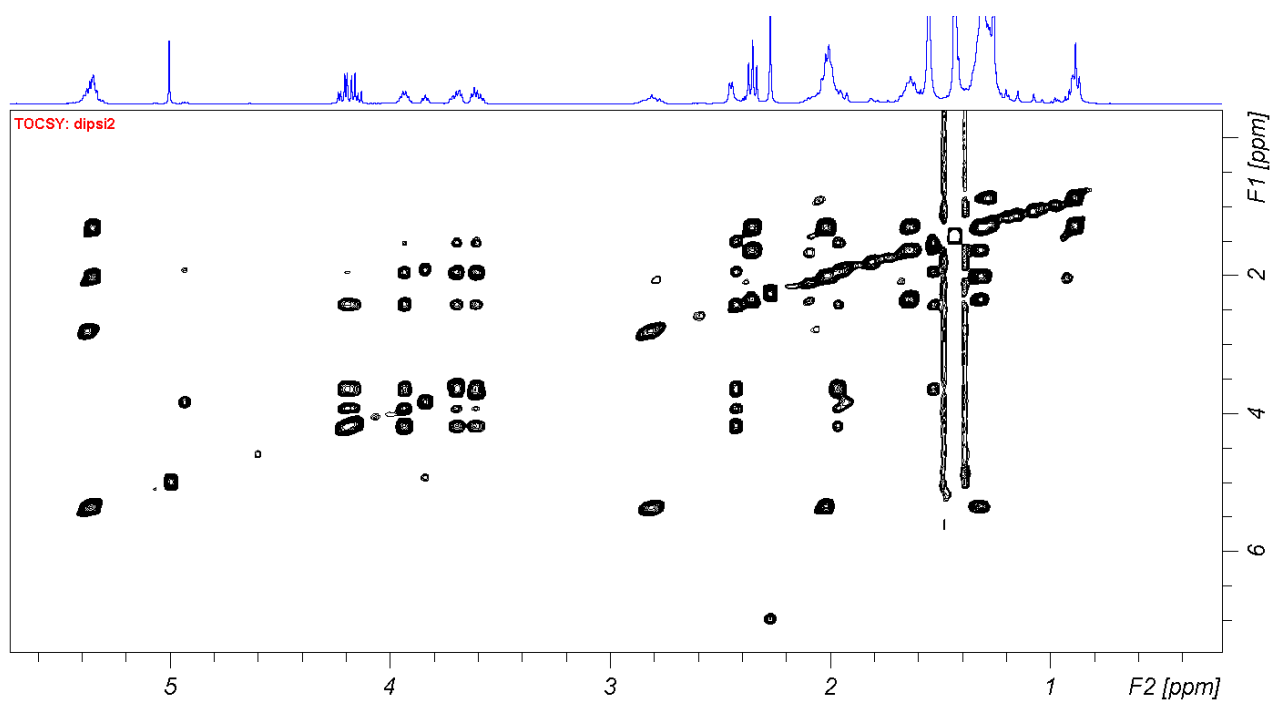


Figure S3. <sup>1</sup>H, <sup>1</sup>H TOCSY-NMR spectrum (400MHz, CDCl<sub>3</sub>) of fraction SMAR-F2L.

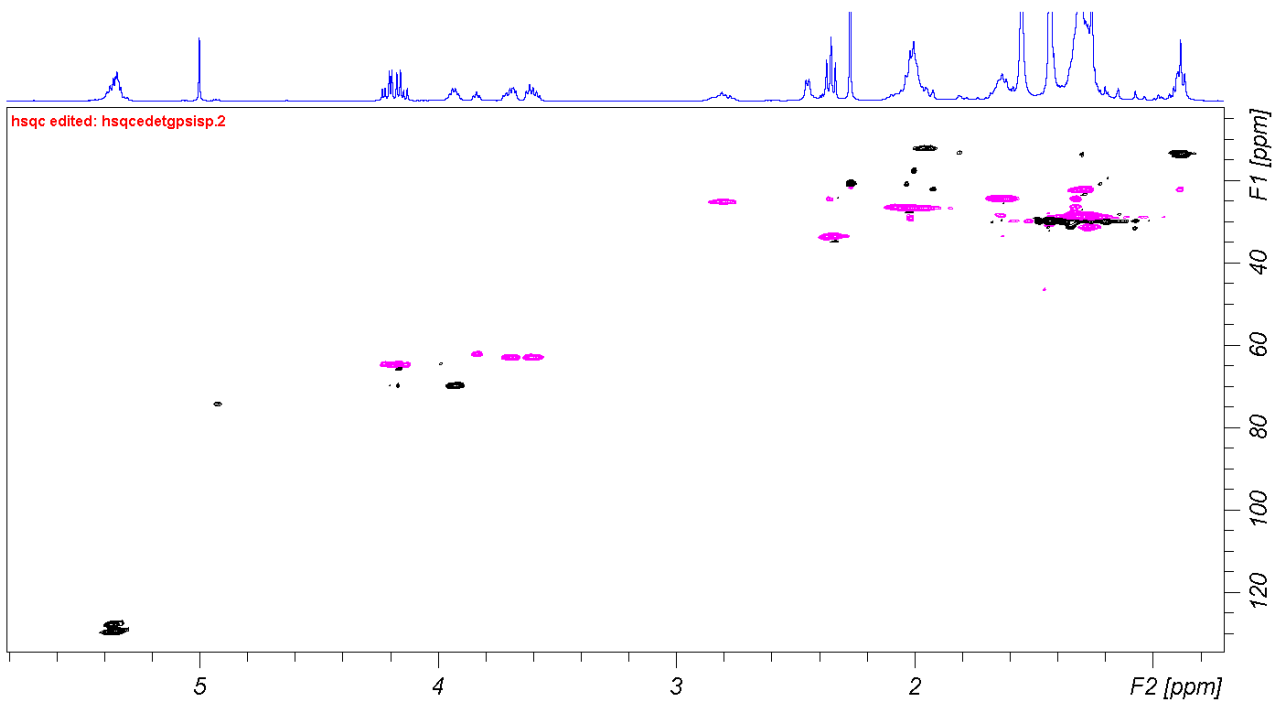


Figure S4. Edited-HSQC NMR spectrum (400MHz, CDCl<sub>3</sub>) of fraction SMAR-F2L.

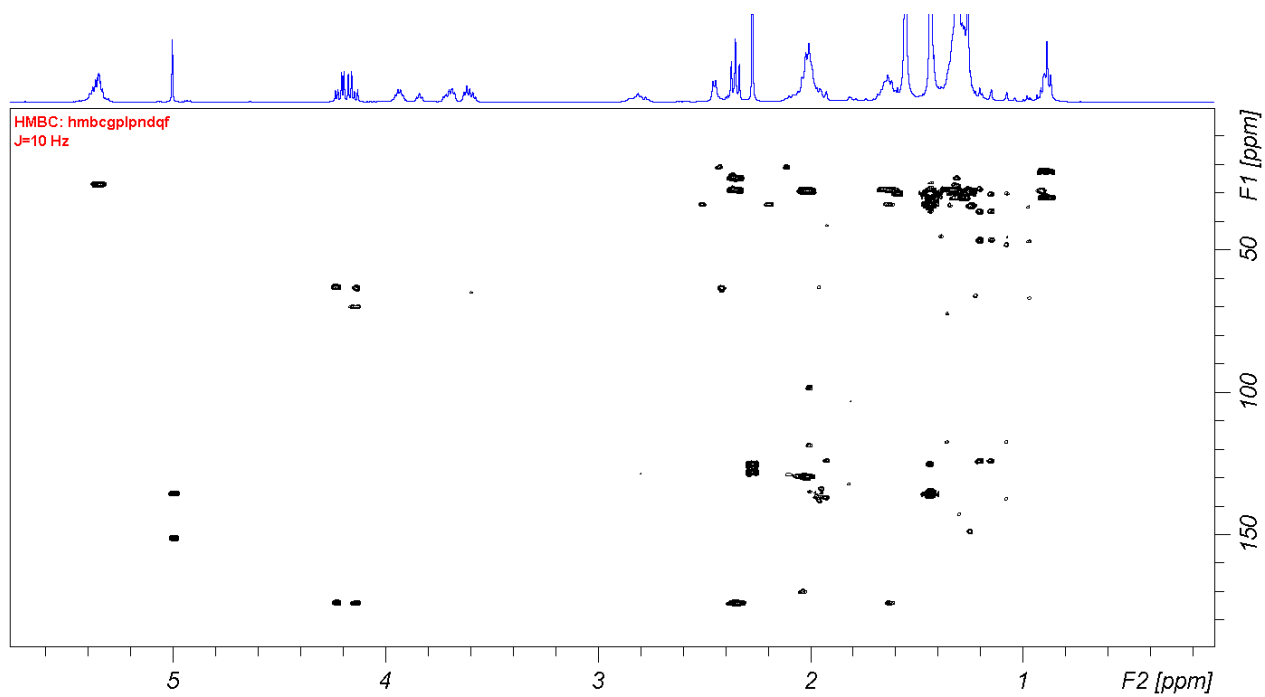
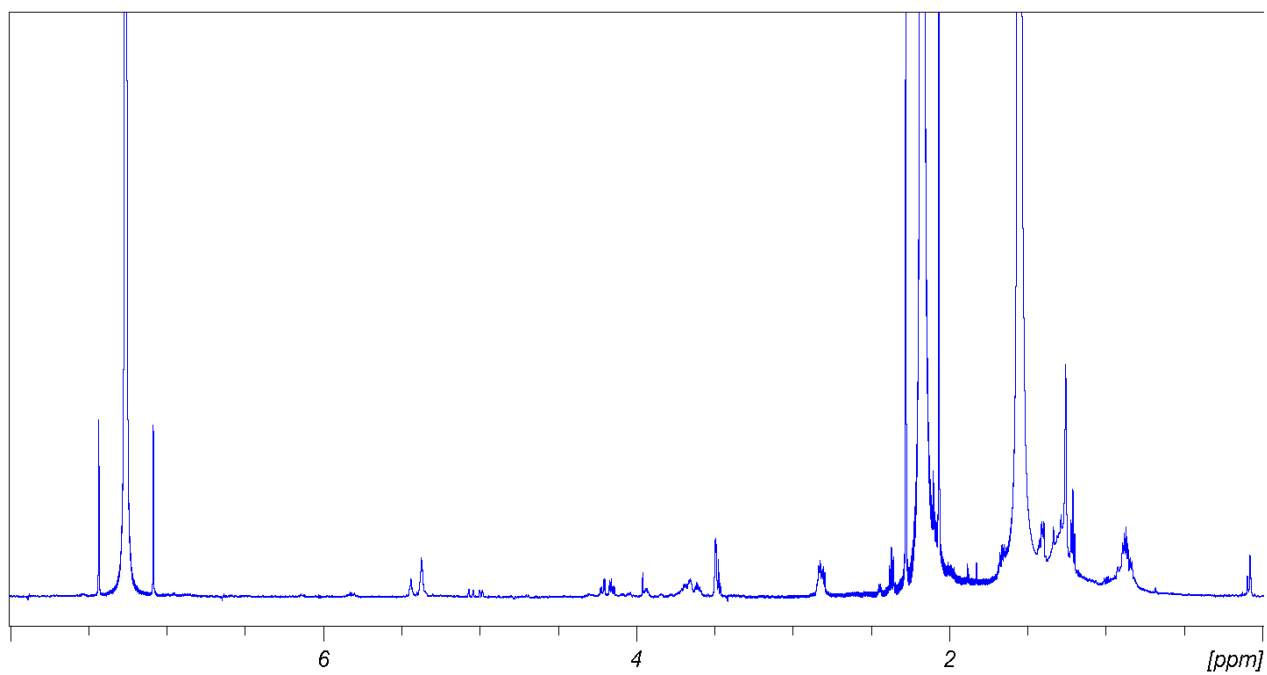
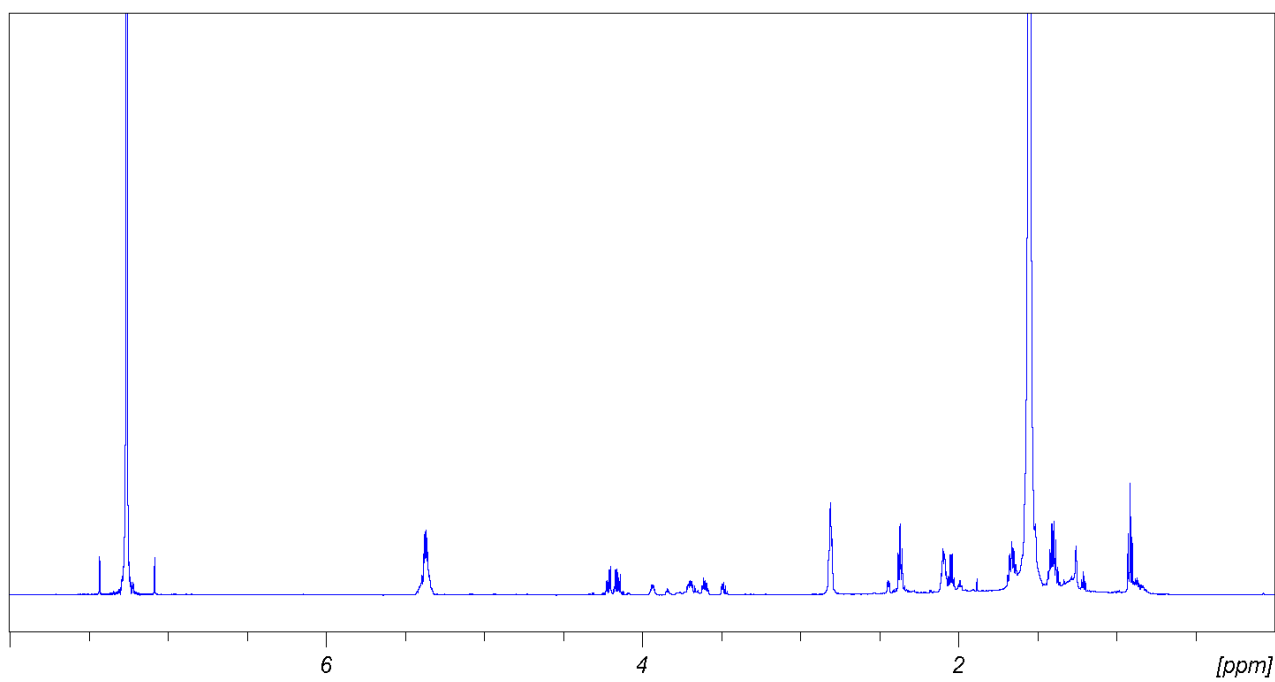


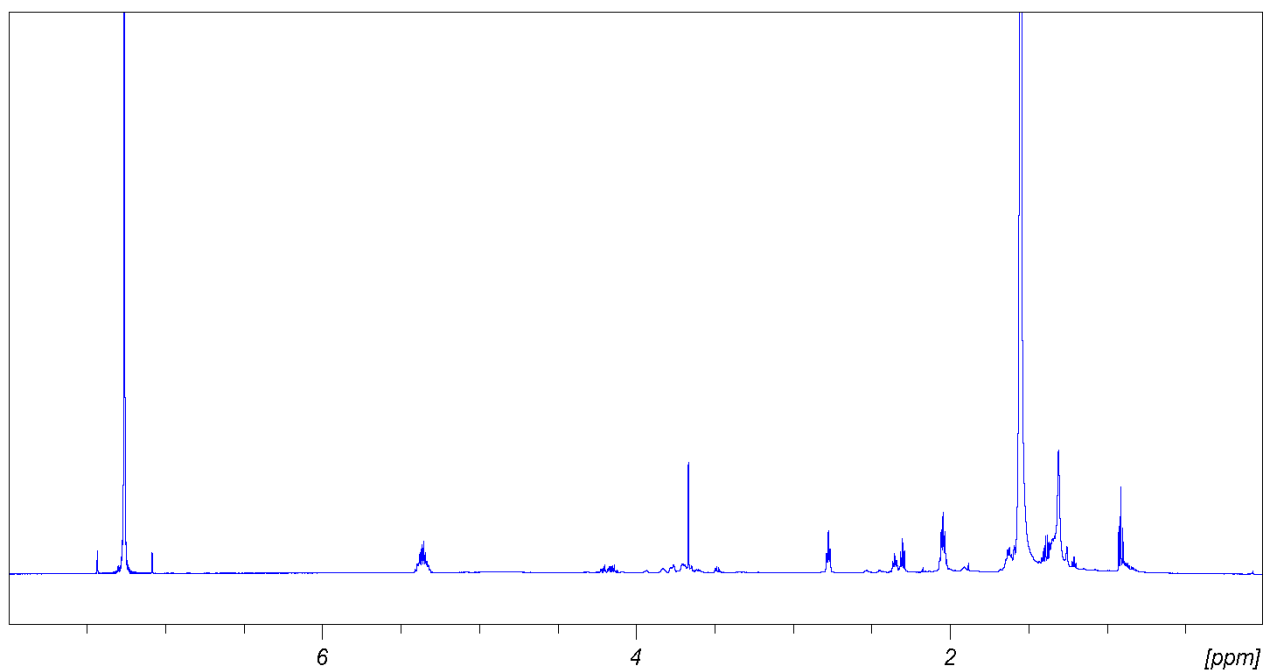
Figure S5. HMBC-NMR spectrum (400MHz, CDCl<sub>3</sub>) of fraction SMAR-F2L.



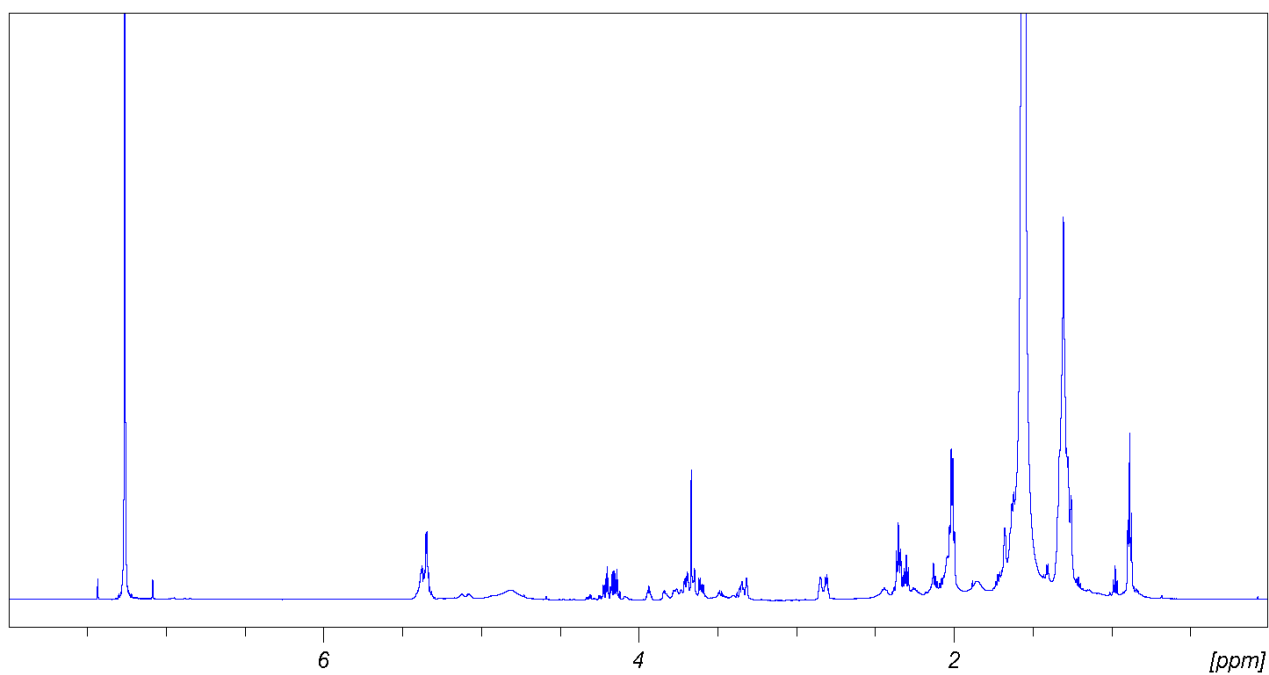
**Figure S6.** <sup>1</sup>H-NMR spectrum (600MHz, CDCl<sub>3</sub>) of MAG-C16:4.



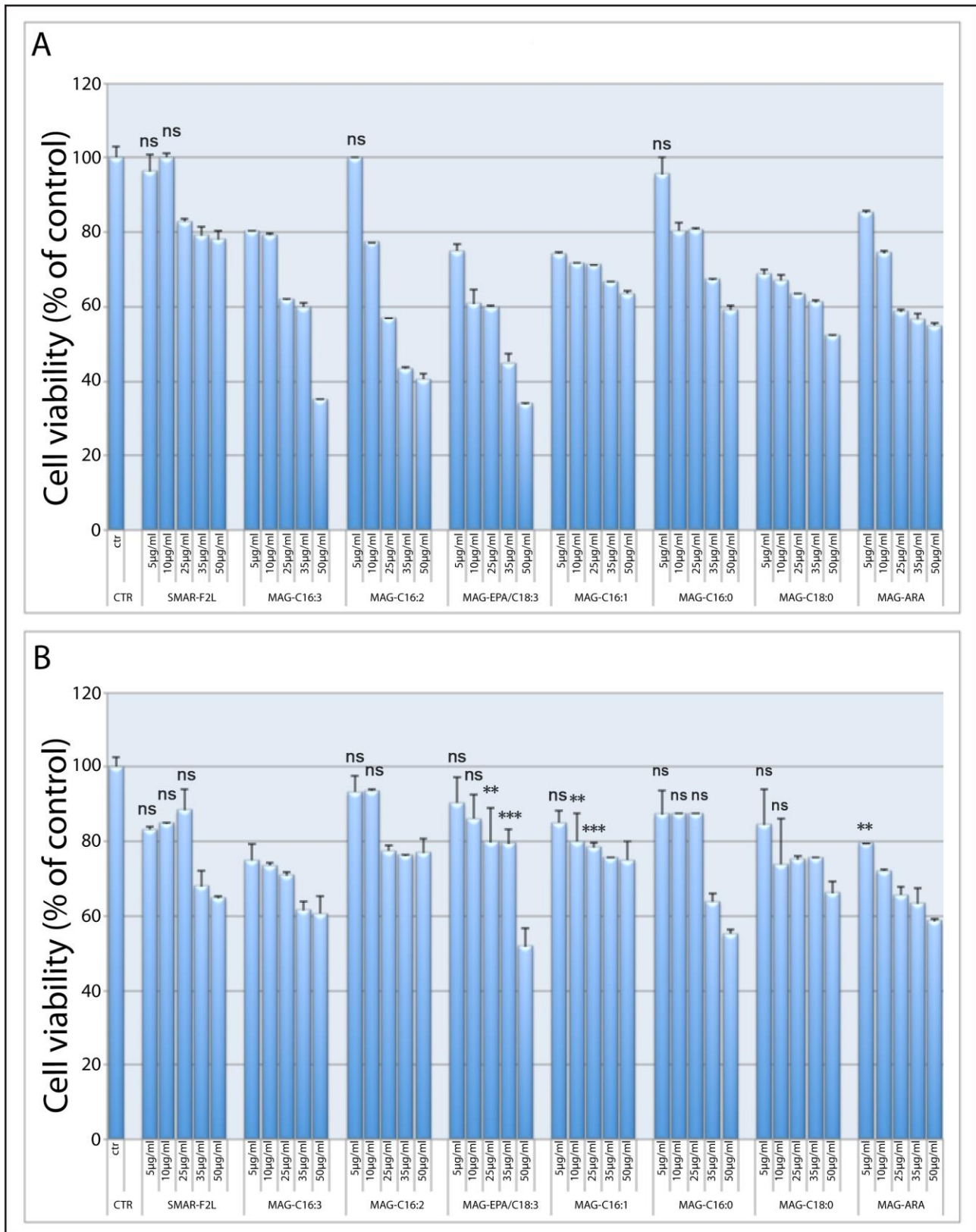
**Figure S7.** <sup>1</sup>H-NMR spectrum (600MHz, CDCl<sub>3</sub>) of MAG-C16:3



**Figure S8.** <sup>1</sup>H-NMR spectrum (600MHz, CDCl<sub>3</sub>) of MAG-C16:2

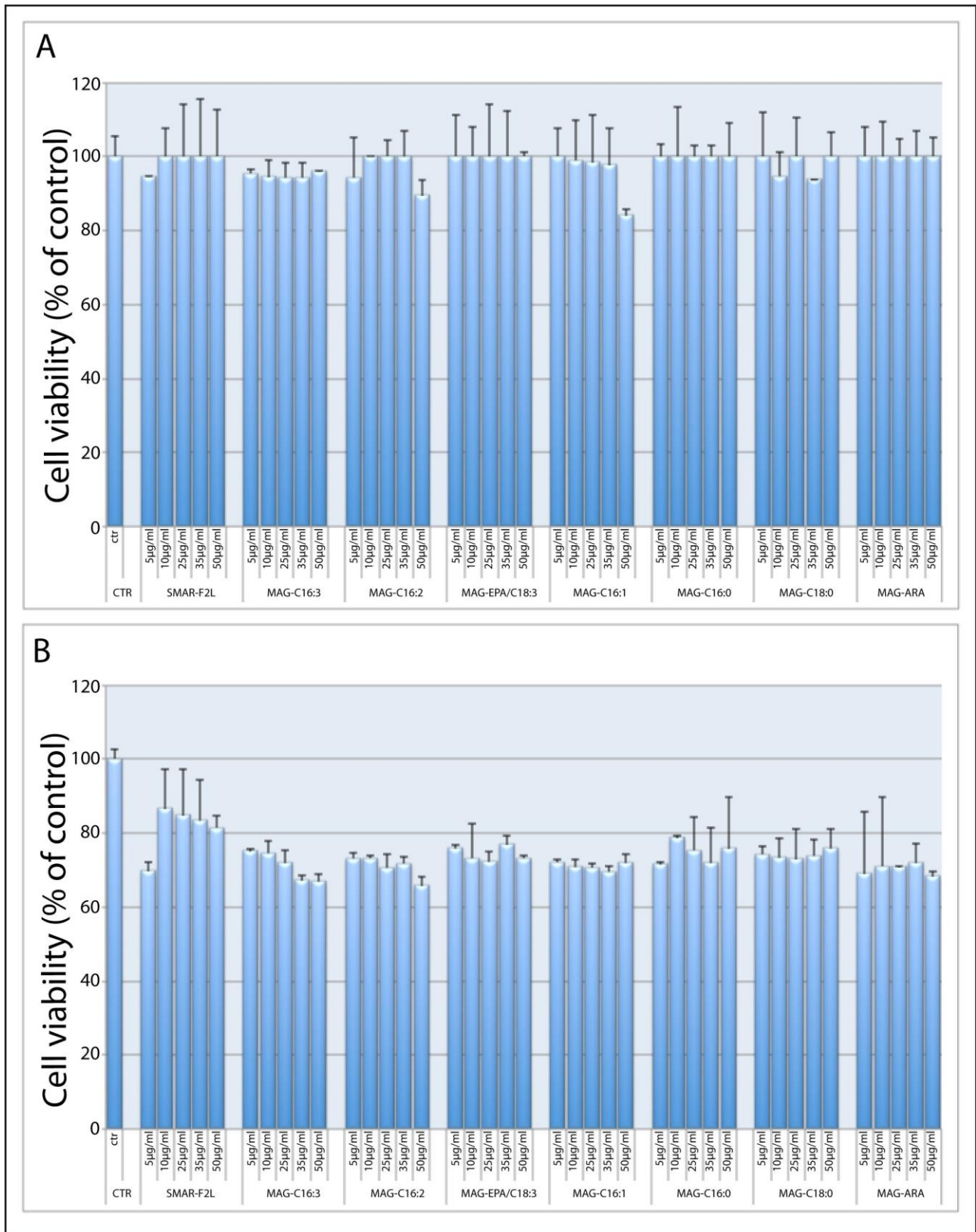


**Figure S9.** <sup>1</sup>H-NMR spectrum (600MHz, CDCl<sub>3</sub>) of MAG mixture EPA/C18:3.

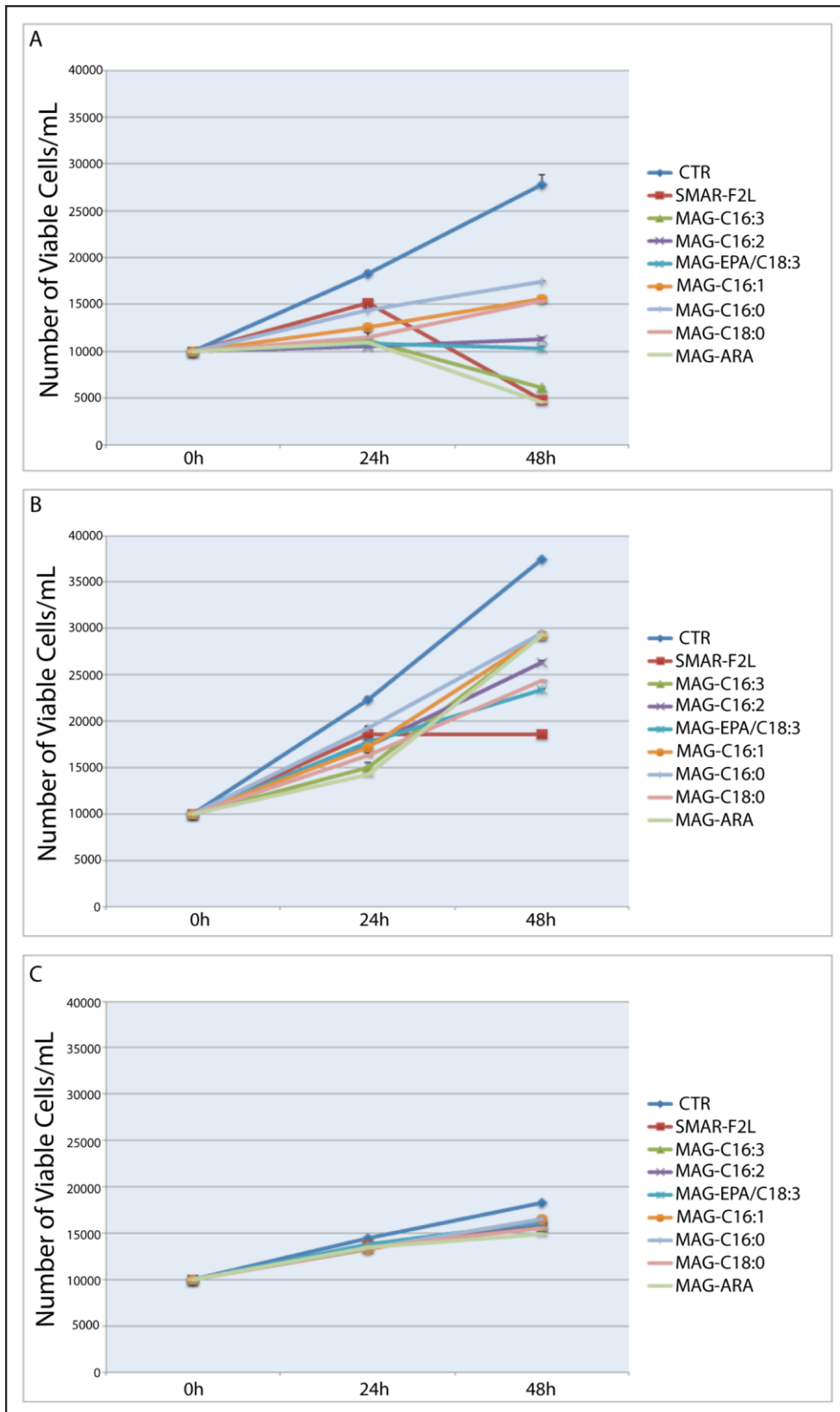


**Figure S10.** Cell viability assay (MTT) on U-937 (A) and HCT-116 (B) cell lines after 24 hours of treatment with purified and commercial MAGs at increasing concentrations of 5, 10, 25, 35 and 50 µg/ml. All bars represent means ± SD with n = 3.

Only differences non-significant with respect to the control (ns) and difference with p-value < 0.05 (\*), p < 0.01 (\*\*), and p < 0.001 (\*\*\*) are indicated. Extremely significant differences (p < 0.0001), which are the majority, are not indicated to avoid overcrowding of symbols (ANOVA, with Tukey's test; p < 0.01 was considered significant).



**Figure S11.** Cell viability assay (MTT) on MePR-2B cells after 24 (A) and 48 hours (B) of treatment with purified and commercial MAGs at increasing concentrations of 5, 10, 25, 35 and 50 µg/ml. All bars represent means ± SD with n = 3. No significant difference was observed (ANOVA, with Tukey's test; p<0.01 was considered significant)



**Figure S12.** Cell proliferation assay on U-937 (A), HCT-116 (B) and MePR-2B (C) cells after 24 and 48 hours of treatment with purified and commercial MAGs at the concentration of 25 $\mu$ g/ml. All points represent means  $\pm$  SD with n = 3. Statistical results of Two-Way ANOVA with Tukey's post-test are reported in supplementary table S2.



**Table S1.** List of species selected from the culture collection of the Stazione Zoologica with relative identification code.

<b>Code</b>	<b>Species</b>
FE7	<i>Skeletonema marinoi</i>
FE102	<i>Amphidinium carterae</i>
FE80	<i>Thalassiosira rotula</i>
FE205	<i>Tetraselmis suecica</i>
FE107	<i>Alexandrium tamarense</i>
FE1320	<i>Dunaliella salina</i>
FE21	<i>Chaetoceros affinis</i>
FE32	<i>Skeletonema japonicum</i>

**Table S2.** Fatty acid composition of *Skeletonema marinoi* lipids by GC-MS analysis of FAME derivatives.

<b>FAME</b>	<b>Apex RT</b>	<b>%Area</b>		
<b>C14:0</b>	<b>8,96</b>	<b>4,79</b>		
<b>C16:4w1</b>	<b>13,06</b>	<b>2,16</b>		
<b>C16:3w4</b>	<b>13,22</b>	<b>16,23</b>		
<b>C16:1+C16:2</b>	<b>13,59</b>	<b>34,06</b>		
<b>C16:0</b>	<b>14,17</b>	<b>5,1</b>		
<b>C18:3</b>	<b>18,39</b>	<b>0,05</b>		
<b>C18:4w3</b>	<b>18,53</b>	<b>6,81</b>		
<b>C18:2w6</b>	<b>18,85</b>	<b>0,76</b>		
<b>C18:1w9</b>	<b>19,03</b>	<b>0,52</b>		
<b>C18:1w7</b>	<b>19,21</b>	<b>0,4</b>		
<b>C18:0</b>	<b>19,82</b>	<b>1,59</b>		
<b>ARA</b>	<b>23,35</b>	<b>0,06</b>		

**Table S3.** Statistical results for Two-Way ANOVA, Tukey post-test ( $\alpha = 0.01$ ) performed on proliferation assay for U-937, HCT-116 and MePR-2B cells treated with 25  $\mu\text{g/ml}$  of purified and commercial MAGs (Supplementary Figure S12). P-values $<0.01$  were considered significant.

Tukey's multiple comparisons test	Mean	95,00% CI of diff	Summary	Adjusted p-Value
<b>U-937 24 h</b>				
ctr dms0 vs. SMAR-F2L	1475	123,6 to 2826	*	0,0229
ctr dms0 vs. MAG-C16:3	5070	3719 to 6421	****	<0,0001
ctr dms0 vs. MAG-C16:2	5790	4439 to 7141	****	<0,0001
ctr dms0 vs. MAG-EPA/C18:3	5495	4144 to 6846	****	<0,0001
ctr dms0 vs. MAG-C16:1	3715	2364 to 5066	****	<0,0001
ctr dms0 vs. MAG-C16:0	1905	553,6 to 3256	**	0,0011
ctr dms0 vs. MAG-C18:0	4775	3424 to 6126	****	<0,0001
ctr dms0 vs. MAG-ARA	5345	3994 to 6696	****	<0,0001
<b>U-937 48 h</b>				
ctr dms0 vs. SMAR-F2L	19190	17839 to 20541	****	<0,0001
ctr dms0 vs. MAG-C16:3	17888	16536 to 19239	****	<0,0001
ctr dms0 vs. MAG-C16:2	12745	11394 to 14096	****	<0,0001
ctr dms0 vs. MAG-EPA/C18:3	13645	12294 to 14996	****	<0,0001
ctr dms0 vs. MAG-C16:1	8383	7032 to 9734	****	<0,0001
ctr dms0 vs. MAG-C16:0	6598	5247 to 7949	****	<0,0001
ctr dms0 vs. MAG-C18:0	8583	7231 to 9934	****	<0,0001
ctr dms0 vs. MAG-ARA	19459	18108 to 20810	****	<0,0001
<b>HCT-116 24 h</b>				
ctr dms0 vs. SMAR-F2L	1700	1047 to 2353	****	<0,0001
ctr dms0 vs. MAG-C16:3	5300	4647 to 5952	****	<0,0001
ctr dms0 vs. MAG-C16:2	2969	2316 to 3622	****	<0,0001
ctr dms0 vs. MAG-EPA/C18:3	2539	1886 to 3191	****	<0,0001
ctr dms0 vs. MAG-C16:1	3050	2397 to 3702	****	<0,0001
ctr dms0 vs. MAG-C16:0	1007	354,0 to 1660	***	0,0004
ctr dms0 vs. MAG-C18:0	3997	3344 to 4650	****	<0,0001
ctr dms0 vs. MAG-ARA	6015	5362 to 6668	****	<0,0001
<b>HCT-116 48 h</b>				
ctr dms0 vs. SMAR-F2L	17960	17307 to 18613	****	<0,0001
ctr dms0 vs. MAG-C16:3	7260	6607 to 7913	****	<0,0001
ctr dms0 vs. MAG-C16:2	10210	9557 to 10863	****	<0,0001
ctr dms0 vs. MAG-EPA/C18:3	13210	12557 to 13863	****	<0,0001
ctr dms0 vs. MAG-C16:1	7270	6617 to 7923	****	<0,0001
ctr dms0 vs. MAG-C16:0	7078	6425 to 7730	****	<0,0001
ctr dms0 vs. MAG-C18:0	12193	11540 to 12845	****	<0,0001
ctr dms0 vs. MAG-ARA	7255	6602 to 7908	****	<0,0001
<b>MePR-2B 24 h</b>				
ctr dms0 vs. SMAR-F2L	-245	-1145 to 654,9	ns	0,9917
ctr dms0 vs. MAG-C16:3	35	-864,9 to 934,9	ns	>0,9999
ctr dms0 vs. MAG-C16:2	91,5	-808,4 to 991,4	ns	>0,9999
ctr dms0 vs. MAG-EPA/C18:3	-238	-1138 to 661,9	ns	0,9932
ctr dms0 vs. MAG-C16:1	236,5	-663,4 to 1136	ns	0,9934
ctr dms0 vs. MAG-C16:0	198	-701,9 to 1098	ns	0,9981

ctr dmso vs. MAG-C18:0	77,5	-822,4 to 977,4	ns	>0,9999
ctr dmso vs. MAG-ARA	98	-801,9 to 997,9	ns	>0,9999
<b>MePR-2B 48 h</b>				
ctr dmso vs. SMAR-F2L	1649	748,6 to 2548	****	
ctr dmso vs. MAG-C16:3	1715	815,1 to 2615	****	<0,0001
ctr dmso vs. MAG-C16:2	1694	794,1 to 2594	****	<0,0001
ctr dmso vs. MAG-EPA/C18:3	1233	332,6 to 2132	**	<0,0001
ctr dmso vs. MAG-C16:1	983	83,06 to 1883	*	0,0019
ctr dmso vs. MAG-C16:0	950	50,06 to 1850	*	0,0235
ctr dmso vs. MAG-C18:0	1904	1004 to 2804	****	0,0319
ctr dmso vs. MAG-ARA	2605	1705 to 3505	****	<0,0001

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