

*Supplementary Materials*

## **FABP7 Facilitates Uptake of Docosahexaenoic Acid in Glioblastoma Neural Stem-like Cells**

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### **Table of contents**

Figure S1: Effects of DHA treatment on lipid droplet formation in U251 and ED501N cells with or without FABP7 depletion.

Table S1: Fatty acid composition of total lipids extracted from A4-004N and A4-004Adh cells.

Table S2: Fatty acid composition of total lipids extracted from A4-004N and A4-004Adh cells cultured in media supplemented with BSA (control), 30 µM AA or 30 µM DHA.

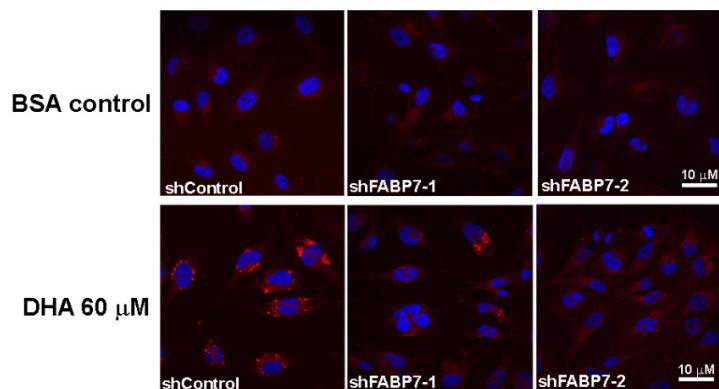
Table S3: Fatty acid composition of total lipids and total phospholipids extracted from A4-004N and A4-004Adh cells cultured under normal culture conditions and in media supplemented with 30 µM BSA.

Table S4: Fatty acid composition of total phospholipids from A4-004N and A4-004Adh cells.

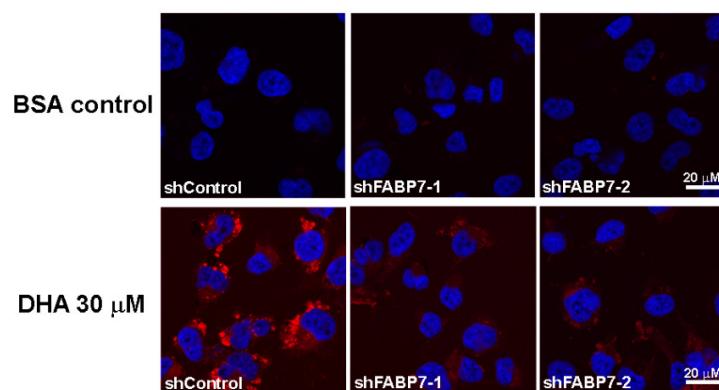
Table S5: Fatty acid composition of total phospholipids from A4-004N and A4-004Adh cells cultured in media supplemented with BSA (control), 30 µM AA or 30 µM DHA.

## Figure S1

U251



ED501N



**Supplementary Figure S1. Effect of DHA treatment and FABP7-knockdown on lipid droplet formation in U251 and ED501N cells.** Stable knockdown of FABP7 in U251 GBM adherent cells and ED501 GBM neural stem-like cells was confirmed as previously described [49]. Cells were cultured in medium supplemented with BSA (control), 60 μM DHA (U251 cells), or 30 μM DHA (ED501N cells). After 24 hours, cells were fixed and stained with Nile Red. Representative confocal images are shown. DAPI was used to stain the nucleus.

**Table S1. Fatty acid composition of total lipids extracted from A4-004N and A4-004Adh cells.**

Fatty Acids	A4-004N (N = 3)		A4-004Adh (N = 3)		<i>p</i> -value
	Mean	±SD	Mean	±SD	
C14:0	2.14	±0.10	1.71	±0.07	<i>p</i> = 0.53
C15:0	0.13	±0.02	0.52	±0.03	<i>p</i> = 0.0001
C16:0 (PA)	25.75	±0.59	29.22	±1.08	<i>p</i> = 0.008
C17:0	0.89	±0.06	0.67	±0.06	<i>p</i> = 0.01
C18:0 (SA)	15.74	±0.11	17.87	±1.15	<i>p</i> = 0.03
C20:0	0.21	±0.04	0.39	±0.07	<i>p</i> = 0.01
C24:0	4.37	±0.40	0.63	±0.13	<i>p</i> = 0.0001
Total SFA	49.24	±0.27	52.39	±1.48	<i>p</i> = 0.02
C16:1ω-9	7.88	±0.16	6.14	±0.50	<i>p</i> = 0.004
C18:1ω-9 (OA)	27.45	±0.48	21.94	±0.66	<i>p</i> = 0.0003
C18:1ω-7	5.65	±0.09	7.37	±0.25	<i>p</i> = 0.0004
C24:1ω-9	1.42	±0.51	1.15	±0.17	<i>p</i> = 0.44
Total MUFA	42.40	±0.09	36.59	±1.26	<i>p</i> = 0.001
C18:2ω-6 (LA)	0.18	±0.03	0.72	±0.09	<i>p</i> = 0.0005
C20:2ω-6	3.25	±0.24	0.95	±0.14	<i>p</i> = 0.0001
C20:3ω-6 (DGLA)	0.74	±0.10	1.67	±0.07	<i>p</i> = 0.0002
C20:4ω-6 (AA)	1.01	±0.14	3.01	±0.36	<i>p</i> = 0.0008
C22:4ω-6 (ADA)	0.60	±0.01	1.24	±0.13	<i>p</i> = 0.001
C22:5ω-6 (ω-6 DPA)	0.10	±0.03	0.22	±0.03	<i>p</i> = 0.006
Total ω-6	5.88	±0.29	7.82	±0.47	<i>p</i> = 0.004
C18:3ω-3 (ALA)	1.79	±0.09	0.95	±0.08	<i>p</i> = 0.0003
C20:4ω-3	0.06	±0.02	0.16	±0.01	<i>p</i> = 0.001
C20:5ω-3 (EPA)	0.09	±0.03	0.09	±0.02	<i>p</i> = 0.88
C22:5ω-3 (ω-3 DPA)	0.47	±0.04	0.96	±0.11	<i>p</i> = 0.002
C22:6ω-3 (DHA)	0.09	±0.01	1.04	±0.13	<i>p</i> = 0.0002
Total ω-3	2.49	±0.05	3.20	±0.19	<i>p</i> = 0.003
Total PUFA	8.36	±0.34	11.02	±0.65	<i>p</i> = 0.003
C22:6ω-3:C20:4ω-6	0.14	±0.02	0.84	±0.05	<i>p</i> < 0.0001
ω-3 PUFA:ω-6 PUFA	0.42	±0.01	0.41	±0.00	<i>p</i> = 0.13

Data are presented as mean ± standard deviation (SD). *p*-value of <0.05 was considered statistically significant.

**Table S2. Fatty acid composition of total lipids extracted from A4-004N and A4-004Adh cells cultured in media supplemented with BSA (control), 30 µM AA or 30 µM DHA.**

Fatty Acids	A4-004N						A4-004Adh							
	BSA Control (N = 3)		AA 30 µM (N = 3)		DHA 30 µM (N = 3)		BSA Control (N = 3)		AA 30 µM (N = 3)		DHA 30 µM (N = 3)			
	% Abundance of fatty acids in total lipids													
C14:0	Average	±SD	Average	±SD	Average	±SD	ANOVA	Average	±SD	Average	±SD	ANOVA		
	2.21a	±0.08	2.65b	±0.05	2.18a	±0.03	<i>p</i> = 0.0001	1.89a	±0.02	1.73ab	±0.07	1.68b	±0.11	<i>p</i> = 0.036
C15:0	0.16	±0.02	0.14	±0.02	0.12	±0.03	<i>p</i> = 0.14	0.55a	±0.01	0.48b	±0.02	0.50b	±0.02	<i>p</i> = 0.0066
C16:0 (PA)	25.54a	±0.24	25.53a	±0.40	24.23b	±0.42	<i>p</i> = 0.0065	29.38a	±0.46	30.52b	±0.36	29.61ab	±0.41	<i>p</i> = 0.033
C17:0	0.81a	±0.06	0.83a	±0.04	0.55b	±0.07	<i>p</i> = 0.0012	0.60	±0.12	0.64	±0.04	0.70	±0.04	<i>p</i> = 0.38
C18:0 (SA)	16.39a	±0.46	17.74b	±0.48	17.47ab	±0.54	<i>p</i> = 0.034	17.30a	±0.40	18.45b	±0.07	17.91ab	±0.29	<i>p</i> = 0.027
C20:0	0.29	±0.05	0.24	±0.03	0.24	±0.03	<i>p</i> = 0.17	0.36a	±0.03	0.29b	±0.02	0.29b	±0.01	<i>p</i> = 0.014
C24:0	3.91a	±0.44	2.52b	±0.29	2.72b	±0.06	<i>p</i> = 0.0082	0.77a	±0.03	0.34b	±0.06	0.39b	±0.06	<i>p</i> = 0.0001
Total SFA	49.30	±0.71	49.65	±0.45	47.79	±1.27	<i>p</i> = 0.10	50.85	±0.98	52.22	±0.22	51.08	±0.73	<i>p</i> = 0.23
C16:1ω-9	7.43a	±0.14	5.75b	±0.05	5.46b	±0.49	<i>p</i> = 0.0004	5.76a	±0.25	3.74b	±0.19	4.10b	±0.23	<i>p</i> < 0.0001
C18:1ω-9 (OA)	27.27a	±0.37	20.67b	±0.24	23.39c	±0.52	<i>p</i> < 0.0001	22.31a	±0.47	15.64b	±0.54	18.70c	±0.56	<i>p</i> < 0.0001
C18:1ω-7	5.85a	±0.03	4.33b	±0.13	3.98b	±0.41	<i>p</i> = 0.0002	7.25a	±0.16	5.81b	±0.13	5.76b	±0.19	<i>p</i> < 0.0001
C24:1ω-9	1.25	±0.16	0.98	±0.10	0.95	±0.13	<i>p</i> = 0.058	1.47a	±0.05	0.78b	±0.22	1.05b	±0.02	<i>p</i> = 0.0018
Total MUFA	41.80a	±0.62	31.73b	±0.46	34.22c	±0.73	<i>p</i> < 0.0001	36.79a	±0.90	25.97b	±0.98	29.60c	±0.95	<i>p</i> < 0.0001
C18:2ω-6 (LA)	0.34a	±0.01	0.16b	±0.03	0.79c	±0.20	<i>p</i> < 0.0001	1.20a	±0.14	0.68b	±0.07	1.81c	±0.08	<i>p</i> < 0.0001
C20:2ω-6	2.72a	±0.22	1.75b	±0.20	1.83b	±0.28	<i>p</i> = 0.0042	0.82a	±0.15	0.35b	±0.05	0.39b	±0.05	<i>p</i> = 0.0017
C20:3ω-6 (DGLA)	0.94	±0.09	1.06	±0.07	1.07	±0.11	<i>p</i> = 0.22	2.20a	±0.11	1.54b	±0.05	2.05a	±0.09	<i>p</i> = 0.0002
C20:4ω-6 (AA)	1.47a	±0.04	6.31b	±0.19	1.18a	±0.09	<i>p</i> < 0.0001	3.43a	±0.09	7.87b	±0.75	3.01a	±0.16	<i>p</i> < 0.0001
C22:4ω-6 (ADA)	0.87a	±0.06	6.77b	±0.12	0.58c	±0.07	<i>p</i> < 0.0001	1.40a	±0.03	6.81b	±0.79	1.12a	±0.08	<i>p</i> < 0.0001
C22:5ω-6 (ω-6 DPA)	0.10a	±0.01	0.76b	±0.07	0.11a	±0.03	<i>p</i> < 0.0001	0.19a	±0.01	1.04b	±0.09	0.24a	±0.04	<i>p</i> < 0.0001
Total ω-6	6.45a	±0.29	16.80b	±0.07	5.66c	±0.09	<i>p</i> < 0.0001	9.24a	±0.35	18.30b	±1.55	8.63a	±0.20	<i>p</i> < 0.0001
C18:3ω-3 (ALA)	1.68a	±0.04	1.19b	±0.06	1.13b	±0.12	<i>p</i> = 0.0003	0.91a	±0.04	0.60b	±0.08	0.63b	±0.03	<i>p</i> = 0.0006
C20:4ω-3	0.08	±0.02	0.05	±0.01	0.04	±0.01	<i>p</i> = 0.12	0.19a	±0.02	0.11b	±0.03	0.10b	±0.02	<i>p</i> = 0.014
C20:5ω-3 (EPA)	0.07a	±0.01	0.07a	±0.01	0.41b	±0.08	<i>p</i> = 0.0001	0.09a	±0.01	0.07a	±0.01	0.45b	±0.03	<i>p</i> < 0.0001
C22:5ω-3 (ω-3 DPA)	0.52a	±0.06	0.42a	±0.03	1.21b	±0.18	<i>p</i> = 0.0003	0.92a	±0.05	0.86a	±0.10	1.42b	±0.09	<i>p</i> = 0.0003
C22:6ω-3 (DHA)	0.10a	±0.02	0.07a	±0.01	11.20b	±2.76	<i>p</i> = 0.0002	1.01a	±0.09	0.90a	±0.11	8.09b	±0.29	<i>p</i> < 0.0001
Total ω-3	2.45a	±0.06	1.81a	±0.06	13.99b	±2.91	<i>p</i> = 0.0002	3.12a	±0.07	2.54a	±0.15	10.69b	±0.41	<i>p</i> < 0.0001
Total PUFA	8.90a	±0.35	18.62b	±0.01	18.00b	±0.54	<i>p</i> < 0.0001	12.36a	±0.37	20.84b	±1.69	19.31b	±0.58	<i>p</i> = 0.0001
C22:6ω-3:C20:4ω-6	0.07a	±0.01	0.01a	±0.00	9.65b	±3.13	<i>p</i> = 0.0009	0.30a	±0.02	0.11b	±0.01	2.69c	±0.12	<i>p</i> < 0.0001
ω-3 PUFA:ω-6 PUFA	0.38a	±0.01	0.11b	±0.00	2.18c	±0.14	<i>p</i> < 0.0001	0.34a	±0.01	0.14b	±0.00	1.24c	±0.03	<i>p</i> < 0.0001

Different letters indicate that groups are significantly different. Differences were assessed for significance using one-way analysis of variance followed by post-hoc Tukey's test. *p*-value of <0.05 was considered statistically significant.



**Table S4. Fatty acid composition of total phospholipids from A4-004N and A4-004Adh cells.**

Fatty Acids	A4-004N (N = 3)		A4-004Adh (N = 3)		<i>p</i> -value
	Mean	±SD	Mean	±SD	
% Abundance of fatty acids in total phospholipids					
C14:0	1.92	±0.15	1.47	±0.10	<i>p</i> = 0.011
C15:0	0.21	±0.14	0.47	±0.04	<i>p</i> = 0.038
C16:0 (PA)	24.59	±0.04	26.23	±0.88	<i>p</i> = 0.09
C17:0	0.72	±0.11	0.58	±0.06	<i>p</i> = 0.12
C18:0 (SA)	14.86	±0.58	16.40	±0.55	<i>p</i> = 0.057
C20:0	0.50	±0.05	0.43	±0.11	<i>p</i> = 0.38
C24:0	4.43	±0.15	0.82	±0.22	<i>p</i> = 0.0003
Total SFA	47.23	±0.13	47.20	±0.10	<i>p</i> = 0.84
C16:1ω-9	8.22	±0.15	7.46	±0.39	<i>p</i> = 0.08
C18:1ω-9 (OA)	27.29	±0.22	23.66	±0.41	<i>p</i> = 0.0015
C18:1ω-7	6.18	±0.10	6.96	±0.19	<i>p</i> = 0.0035
C24:1ω-9	1.35	±0.29	1.11	±0.33	<i>p</i> = 0.40
Total MUFA	42.85	±0.22	39.18	±0.92	<i>p</i> = 0.013
C18:2ω-6 (LA)	0.39	±0.30	0.97	±0.08	<i>p</i> = 0.032
C20:2ω-6	3.91	±0.28	1.27	±0.21	<i>p</i> = 0.0012
C20:3ω-6 (DGLA)	1.21	±0.37	2.00	±0.06	<i>p</i> = 0.021
C20:4ω-6 (AA)	1.14	±0.07	3.80	±0.12	<i>p</i> = 0.0001
C22:4ω-6 (ADA)	0.56	±0.02	1.36	±0.05	<i>p</i> = 0.0002
C22:5ω-6 (ω-6 DPA)	0.24	±0.04	0.45	±0.08	<i>p</i> = 0.049
Total ω-6	7.07	±0.10	9.86	±0.36	<i>p</i> = 0.002
C18:3ω-3 (ALA)	1.65	±0.27	1.15	±0.10	<i>p</i> = 0.041
C20:4ω-3	0.16	±0.07	0.36	±0.07	<i>p</i> = 0.020
C20:5ω-3 (EPA)	0.20	±0.08	0.22	±0.02	<i>p</i> = 0.028
C22:5ω-3 (ω-3 DPA)	0.51	±0.01	1.04	±0.08	<i>p</i> = 0.003
C22:6ω-3 (DHA)	0.23	±0.07	1.78	±0.14	<i>p</i> = 0.0008
Total ω-3	2.86	±0.01	4.55	±0.17	<i>p</i> = 0.0009
Total PUFA	9.93	±0.09	14.41	±0.47	<i>p</i> = 0.0010
C22:6ω-3:C20:4ω-6	0.24	±0.04	0.47	±0.02	<i>p</i> = 0.0035
ω-3 PUFA:ω-6 PUFA	0.40	±0.01	0.46	±0.02	<i>p</i> = 0.025

Data are presented as mean ± standard deviation (SD). *p*-value of <0.05 was considered statistically significant.

**Table S5. Fatty acid composition of total phospholipids from A4-004N and A4-004Adh cells cultured in media supplemented with BSA (control), 30 μM AA or 30 μM DHA.**

Fatty Acids	A4-004N						A4-004Adh											
	BSA Control (N = 3)			AA 30 μM (N = 3)			DHA 30 μM (N = 3)			BSA Control (N = 3)			AA 30 μM (N = 3)			DHA 30 μM (N = 3)		
	% Abundance of fatty acids in total phospholipids																	
C14:0	Average	±SD	Average	±SD	Average	±SD	ANOVA	Average	±SD	Average	±SD	Average	±SD	Average	±SD	Average	±SD	ANOVA
C14:0	1.56	±0.56	2.08	±0.54	1.89	±0.36	p = 0.47	1.48	±0.17	1.68	±0.20	1.63	±0.16	p = 0.49				
C15:0	0.20	±0.09	0.18	±0.09	0.22	±0.10	p = 0.85	0.46	±0.07	0.46	±0.06	0.49	±0.05	p = 0.85				
C16:0 (PA)	24.50	±0.54	24.06	±0.15	25.01	±0.23	p = 0.15	26.18a	±0.97	29.56b	±0.20	28.20ab	±0.85	p = 0.030				
C17:0	0.70	±0.19	0.58	±0.17	0.49	±0.13	p = 0.33	0.57	±0.08	0.66	±0.07	0.73	±0.07	p = 0.17				
C18:0 (SA)	14.53	±0.54	18.29	±2.10	19.20	±1.55	p = 0.064	16.24a	±0.85	17.26ab	±0.36	18.44b	±0.49	p = 0.017				
C20:0	0.56	±0.14	0.35	±0.04	0.48	±0.01	p = 0.20	0.55	±0.11	0.42	±0.02	0.37	±0.00	p = 0.061				
C24:0	3.92	±0.57	2.78	±0.19	2.52	±0.30	p = 0.070	0.78a	±0.15	0.42b	±0.04	0.45b	±0.02	p = 0.017				
Total SFA	46.17a	±0.21	47.41b	±0.10	49.14c	±0.31	p = 0.0021	46.27	±2.10	50.81	±0.15	50.04	±1.36	p = 0.062				
C16:1ω-9	8.00a	±0.06	5.41b	±0.89	5.56b	±0.71	p = 0.022	7.33	±0.38	4.22	±0.26	4.53	±0.40	p = 0.0004				
C18:1ω-9 (OA)	28.14a	±0.36	21.62b	±1.57	23.72ab	±1.39	p = 0.028	23.56a	±0.46	15.99b	±0.80	18.84c	±0.63	p = 0.0002				
C18:1ω-7	6.26a	±0.20	4.95b	±0.08	4.61c	±0.04	p < 0.0001	6.93a	±0.40	5.33b	±0.19	5.21b	±0.14	p = 0.0010				
C24:1ω-9	1.40	±0.04	1.18	±0.32	1.01	±0.30	p = 0.24	1.23	±0.17	1.22	±0.28	1.08	±0.15	p = 0.69				
Total MUFA	43.88a	±0.20	33.46b	±2.04	35.32b	±2.02	p = 0.016	39.05a	±1.41	26.76b	±1.03	29.67b	±1.29	p = 0.0003				
C18:2ω-6 (LA)	0.33a	±0.08	0.24a	±0.02	1.08b	±0.02	p = 0.0007	1.00a	±0.26	0.91a	±0.10	2.17b	±0.27	p = 0.0016				
C20:2ω-6	3.99a	±0.09	2.39b	±0.03	2.44b	±0.02	p = 0.0001	1.27a	±0.27	0.42b	±0.14	0.42b	±0.07	p = 0.0028				
C20:3ω-6 (DGLA)	1.02	±0.07	1.34	±0.13	1.31	±0.06	p = 0.059	1.97ab	±0.20	1.77a	±0.11	2.13b	±0.04	p = 0.035				
C20:4ω-6 (AA)	1.01a	±0.02	6.44b	±0.44	1.70a	±0.87	p = 0.0020	3.96a	±0.18	8.65b	±0.28	3.76a	±0.35	p < 0.0001				
C22:4ω-6 (ADA)	0.49a	±0.02	6.64b	±0.92	0.77a	±0.31	p = 0.0001	1.42a	±0.14	6.71b	±0.22	1.22a	±0.18	p < 0.0001				
C22:5ω-6 (ω-6 DPA)	0.34a	±0.23	1.03b	±0.28	0.45a	±0.14	p = 0.019	0.54a	±0.29	1.32b	±0.07	0.47a	±0.15	p = 0.0032				
Total ω-6	7.04a	±0.21	17.26b	±1.32	7.02a	±0.50	p = 0.0017	10.18a	±0.24	19.77b	±0.62	10.17a	±0.45	p < 0.0001				
C18:3ω-3 (ALA)	2.01a	±0.11	1.23b	±0.17	1.13b	±0.10	p = 0.0003	0.98	±0.21	0.89	±0.03	0.93	±0.17	p = 0.81				
C20:4ω-3	0.19	±0.09	0.13	±0.01	0.10	±0.003	p = 0.29	0.38a	±0.09	0.18b	±0.03	0.20ab	±0.08	p = 0.045				
C20:5ω-3 (EPA)	0.14a	±0.00	0.11a	±0.06	0.41b	±0.06	p = 0.0026	0.26a	±0.14	0.16a	±0.06	0.62b	±0.09	p = 0.0044				
C22:5ω-3 (ω-3 DPA)	0.34a	±0.02	0.84b	±0.05	0.90b	±0.11	p = 0.0036	1.08	±0.14	0.97	±0.10	1.22	±0.11	p = 0.095				
C22:6ω-3 (DHA)	0.33a	±0.18	0.27a	±0.07	6.01b	±0.68	p < 0.0001	1.81a	±0.15	1.52a	±0.29	6.88b	±0.73	p < 0.0001				
Total ω-3	2.85a	±0.10	2.53a	±0.13	8.53b	±0.86	p < 0.0001	4.51a	±0.45	3.71a	±0.42	9.85b	±0.64	p < 0.0001				
Total PUFA	9.84a	±0.25	19.80b	±1.19	15.54b	±1.71	p = 0.0086	14.69a	±0.69	23.48b	±1.01	20.02c	±1.08	p = 0.0006				
C22:6ω-3:C20:4ω-6	0.23a	±0.09	0.04a	±0.01	4.92b	±0.06	p < 0.0001	0.46a	±0.02	0.16b	±0.00	1.76c	±0.03	p < 0.0001				
ω-3 PUFA:ω-6 PUFA	0.40a	±0.01	0.15b	±0.02	1.21c	±0.09	p = 0.0005	0.44a	±0.03	0.18b	±0.00	0.97c	±0.02	p < 0.0001				

Different letters indicate that groups are significantly different. Differences were assessed for significance using one-way analysis of variance followed by post-hoc Tukey's test. p-value of <0.05 was considered statistically significant.