Supplementary Material

Super Resolution Microscopy Reveals DHA-dependent Alterations in Glioblastoma Membrane Remodelling and Cell Migration

Xia Xu[a, 1], Yixiong Wang[a, 1], Won-Shik Choi[1], Xuejun Sun[1], Roseline Godbout[1, *]

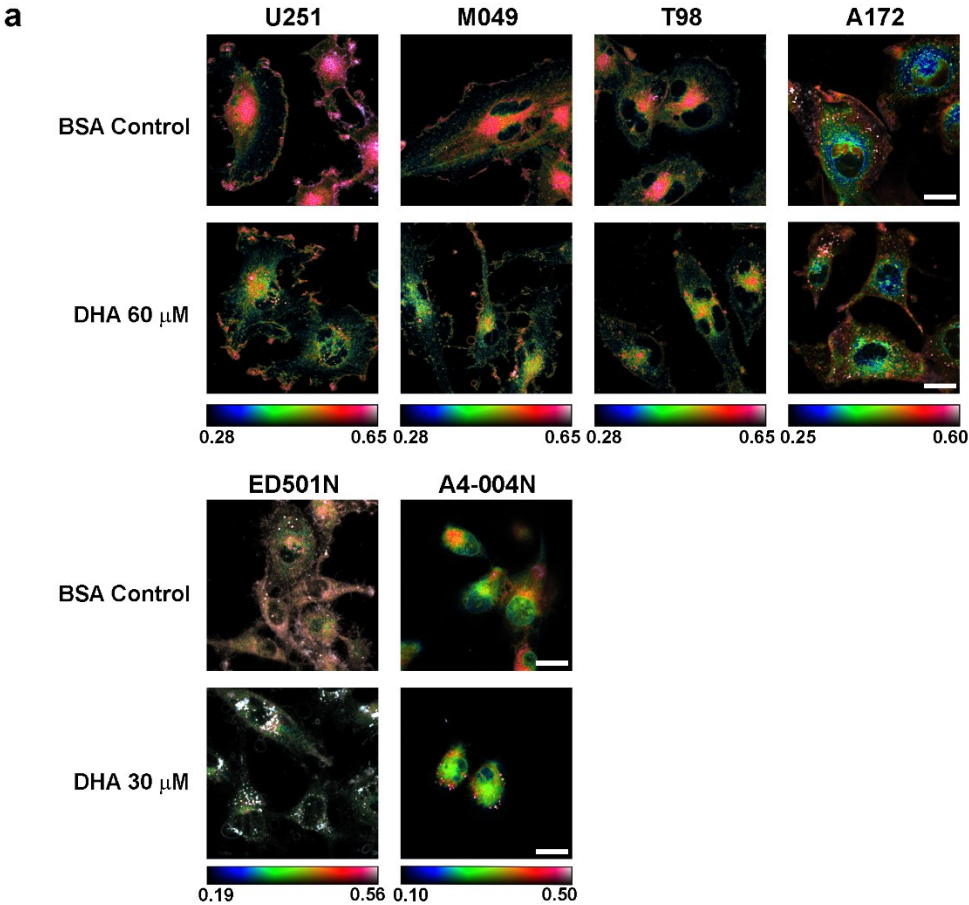[1] Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton,
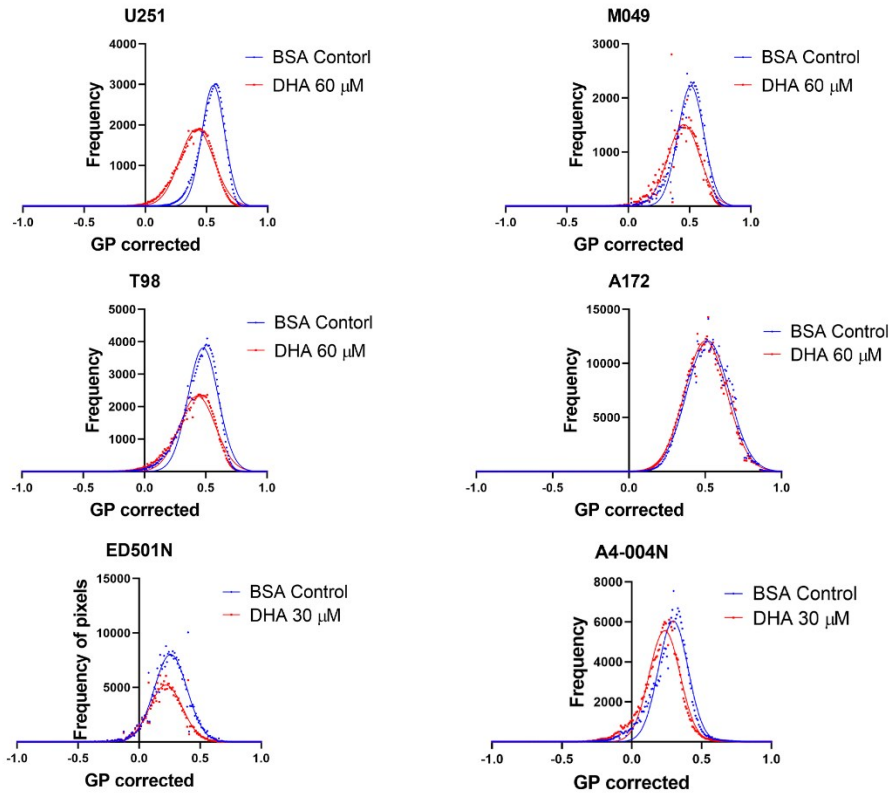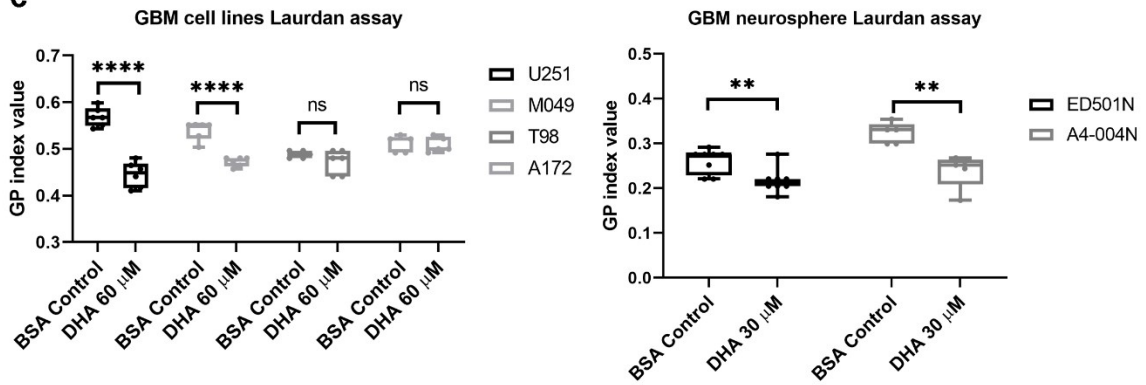

Alberta, T6G 1Z2 Canada

[a] These authors contributed equally to the manuscript

* Corresponding author: Roseline Godbout; email: rgodbout@ualberta.ca

Emails: Xia Xu: xia5@ualberta.ca

Yixiong Wang: yixiong@ualberta.ca

Wonshik Choi: wonshik@ualberta.ca

Xuejun Sun: xjsun@ualberta.ca

Fig. S1

a

	U251	M049	T98	A172

BSA Control

DHA 60 μM

0.28   0.65   0.28   0.65   0.28   0.65   0.25   0.60

ED501N   A4-004N

BSA Control

DHA 30 μM

0.19   0.56   0.10   0.50

b

U251

M049

T98

A172

ED501N

A4-004N

**c**

GBM cell lines Laurdan assay

GBM neurosphere Laurdan assay

**Fig. S1. DHA decreases membrane lipid order in FABP7-expressing GBM cells.**
Laurdan imaging analysis of membrane lipid order in 4 GBM cell lines (U251, M049, T98 and A172) and 2 patient-derived GBM neurosphere cultures (ED501N and A4-004N) treated with BSA (control) or 60 μM (GBM cell lines)/30 μM (GBM neurosphere cultures) DHA for 24 hours, respectively. (**a**) Representative merged pseudo-colored GP images are shown, with color range indicated by the color bar. Scale bars = 20 μm. (**b**) Histograms of FABP7-expressing GBM cells (U251, M049, ED501N and A4-004N cells) are shifted to the left (lower GP value) upon DHA treatment, whereas histograms of FABP7-negative GBM cells (T98 and A172 cells) show no shift upon DHA treatment. (**c**) Average GP index from several images similar to the ones shown in panel a (n=6-8). Statistical analysis was performed with multiple *t*-test the Holm-Sidak method, with alpha = 0.05. Center line, median; box limits, 25th and 75th percentiles; whiskers, minimum to maximum with all points shown. ** indicates p<0.01, **** indicates p<0.001, and ns indicates p>0.05. GP, generalized polarization.

Fig. S2

a

b

c
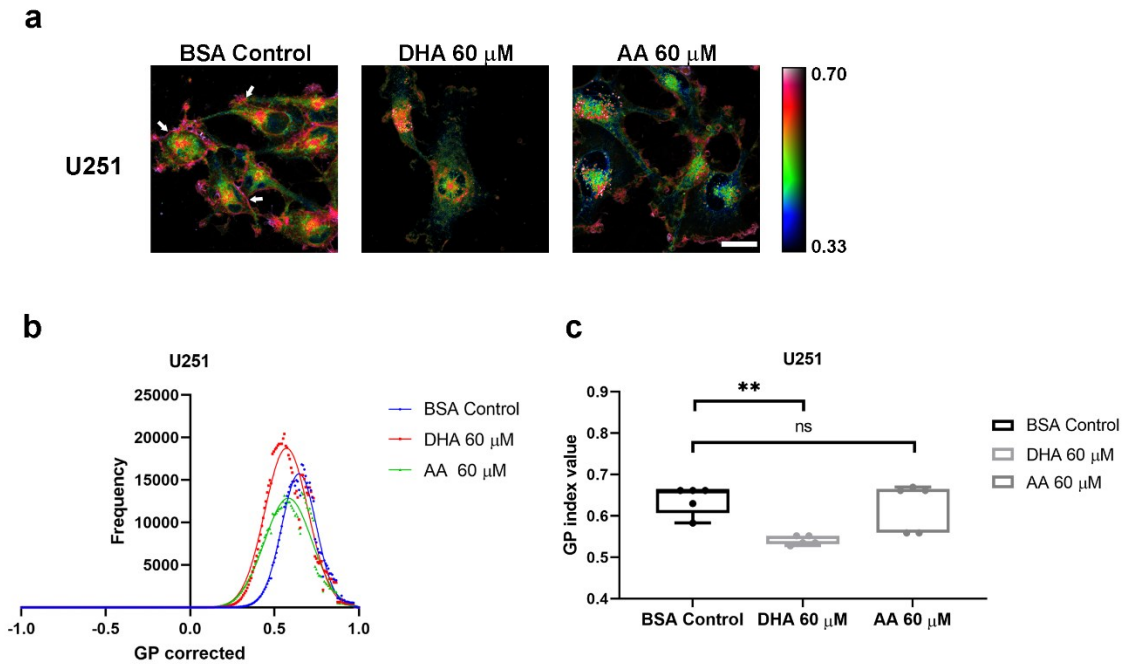
Fig. S2. AA has no effect on membrane lipid order in GBM cells. Laurdan imaging analysis of membrane lipid order in U251 cells cultured in BSA (control) or medium supplemented with different fatty acids (60 μM DHA/AA) for 24 hours. (**a**) Representative merged pseudo-colored GP images are shown, with color range indicated by the color bar. Scale bar = 20 μm. (**b**) Histograms of U251 cells are shifted to the left (lower GP value) in cells cultured in DHA-supplemented medium but show no shift in cells cultured in AA. (**c**) Average GP index from several images similar to those shown in panel a (n=5). Statistical analysis was performed with one-way ANOVA and Dunnett multiple comparisons test. Center line, median; box limits, 25th and 75th percentiles; whiskers, minimum to maximum with all points shown. ** indicates p<0.01, and ns indicates p>0.05. GP, generalized polarization.

Fig. S3
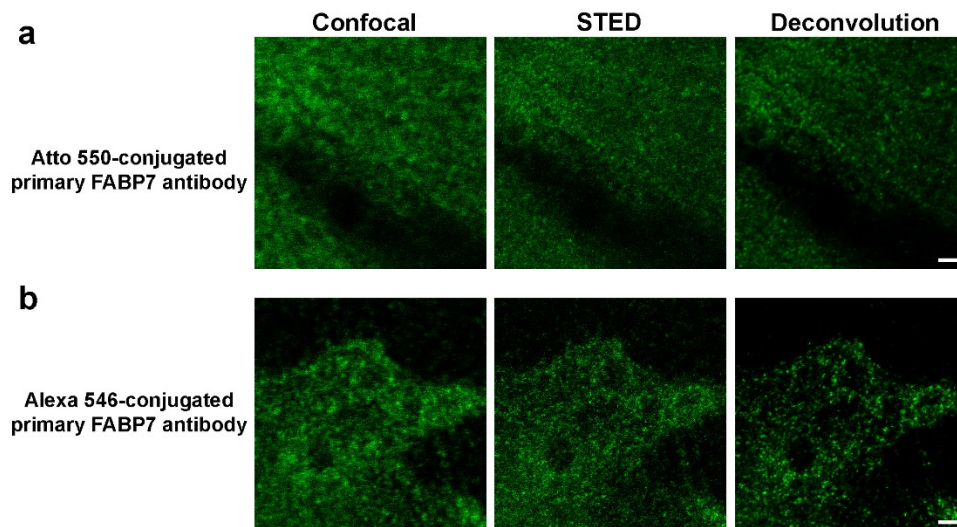
Fig. S3. STED images reveal the distribution of FABP7 membrane nanoscale domains in GBM cells. U251 GBM cells were labelled with either Atto 550-conjugated primary anti-FABP7 antibody (**a**) or Alexa 546-conjugated primary anti-FABP7 antibody (**b**). Images were acquired using a Leica confocal microscope, a super-resolution STED microscope and STED microscope with deconvolution (Lightning). Images are maximum intensity projections of Z-stacks. Scale bars = 1 μm.

Fig. S4

Supplementary figure 4
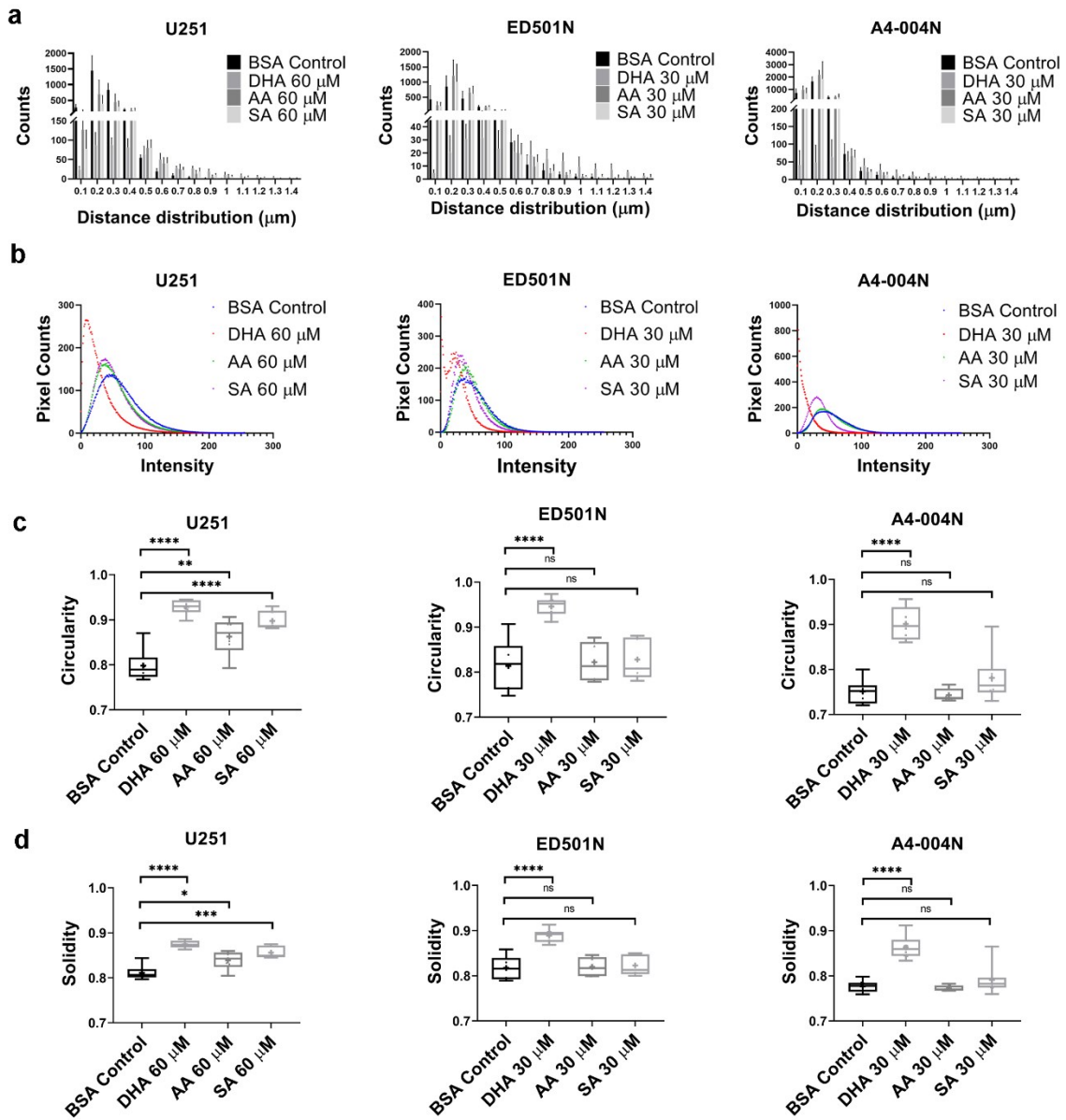
Fig. S4. DHA disrupts FABP7 membrane nanoscale domains in GBM cells. Nearest neighbour distance analysis (NND) was performed to determine the distance of an individual FABP7 nanoscale domain to its five nearest neighbors. (**a**) The NDD distribution curve is shifted to the right (increased inter-domain distance) upon DHA supplementation for all three GBM cell lines tested. Error bars represent standard deviation. (**b**) Quantitative intensity distribution shows that the FABP7 nanoscale domain intensity curve shifts to the left (reduced intensity counts) upon DHA treatment for all three GBM cell lines tested. Particle shape analysis shows that DHA-treated FABP7 nanoscale domains are more circular (**c**) and have a significantly higher solidity (**d**) compared to either BSA control or cells cultured in medium supplemented with AA or SA ($P<0.0001$, n=7 for all three GBM cell lines tested). Each data point in (**c**) and (**d**) represents the average circularity or solidity value, respectively, of all the particles in one image. Statistical analysis of (**c**) and (**d**) was performed with one-way ANOVA and Dunnett multiple comparisons test. Center line, median; box limits, 25th and 75th percentiles; whiskers, min to max with all data points shown; mean values are labelled as "+". * indicates $p<0.05$, ** indicates $p<0.01$, *** indicates $p<0.001$, **** indicates $p<0.0001$, and ns indicates $p>0.05$.

Fig. S5
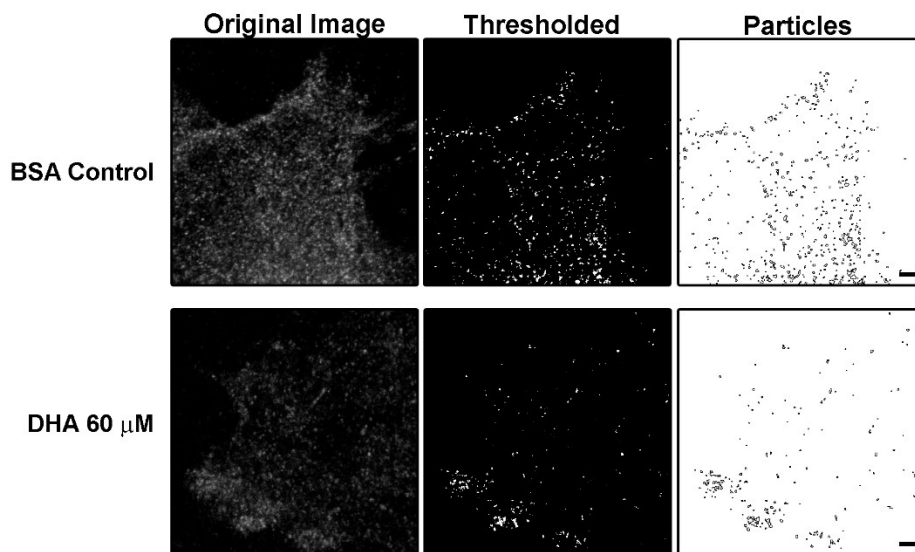
Fig. S5. Particle analysis of U251 Cells BSA (control) and DHA-supplemented.

U251 cells were cultured in BSA (control) or medium supplemented with 60 μM DHA. Images were exported as 16-bit images to Photoshop and thresholded with threshold level 2. The thresholded images were then imported into ImageJ for particle analysis. Particles are defined as areas with more than two pixels. Scale bars = 1 μm.
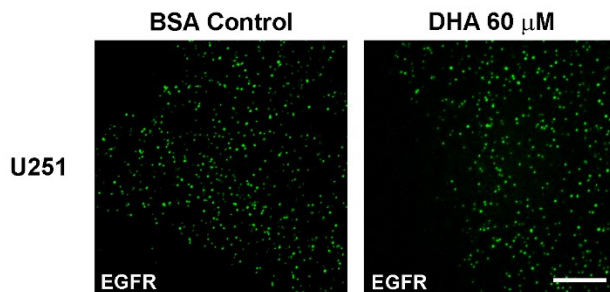
Fig. S6

Fig. S6. DHA treatment has no effect on EGFR membrane nanodomains distribution in U251 cells. U251 GBM cells were immunostained with anti-EGFR antibody, then labelled with Alexa 555 secondary antibody. Images were acquired using a STED microscope with deconvolution (Lightning). Images are maximum intensity projections of Z-stacks. Scale bar = 5 μm.

Fig. S7

Supplementary figure 7
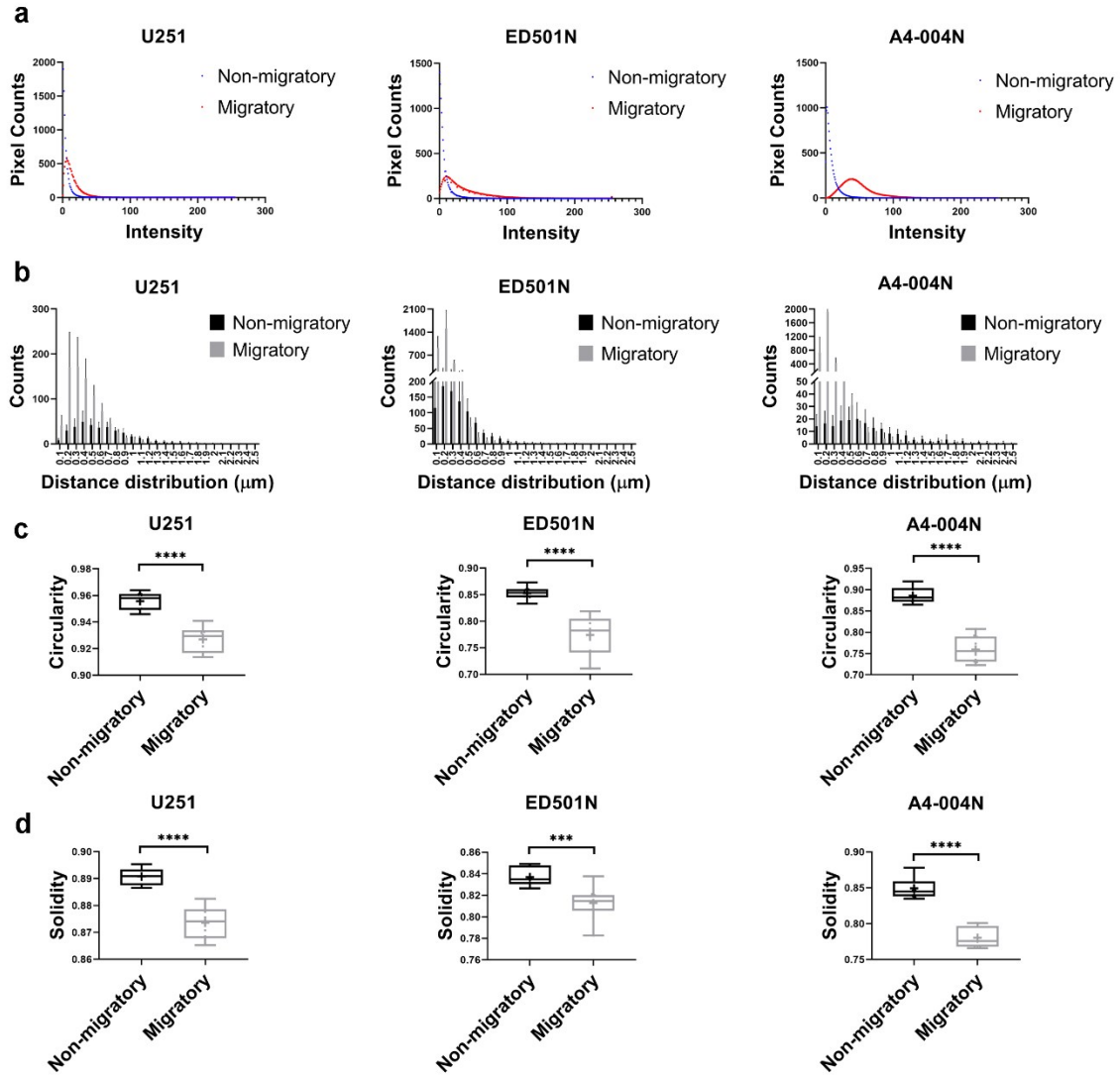
Fig. S7. FABP7 membrane nanoscale domains are differentially distributed in migratory and non-migratory GBM cells. (**a**) Quantitative intensity distribution of FABP7 nanoscale domain intensity curves show a shift to the left (reduced intensity counts) in non-migratory cells compared to migratory cells in all three GBM cell lines tested. (**b**) Nearest neighbour distance analysis (NND) was performed to determine the distance between individual FABP7 nanoscale domains and their five nearest neighbors. Results show that the NND distribution curves are shifted to the right (increased inter-domain distance) in non-migratory cells compared to migratory cells. Error bars represent standard deviation. Particle shape analysis shows that FABP7 nanoscale domains in migratory cells are significantly less circular (**c**) and have a significantly lower solidity (**d**) than control area cells (P<0.0001, n=10) in all three GBM cell lines tested. Each data point in (**c**) and (**d**) represents the average circularity or solidity value, respectively, of all the particles in one image. Statistical analysis was performed with two-tailed unpaired *t*-test. Center line, median; box limits, 25th and 75th percentiles; whiskers, min to max with all data points shown; mean values are labelled as "+". *** indicates p<0.001, **** indicates p<0.0001.
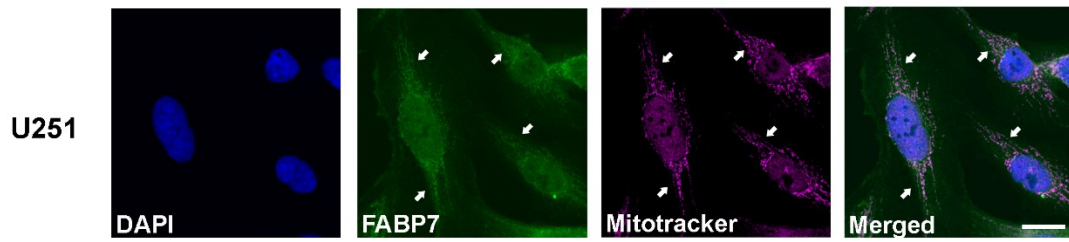
Fig. S8

a

b

c
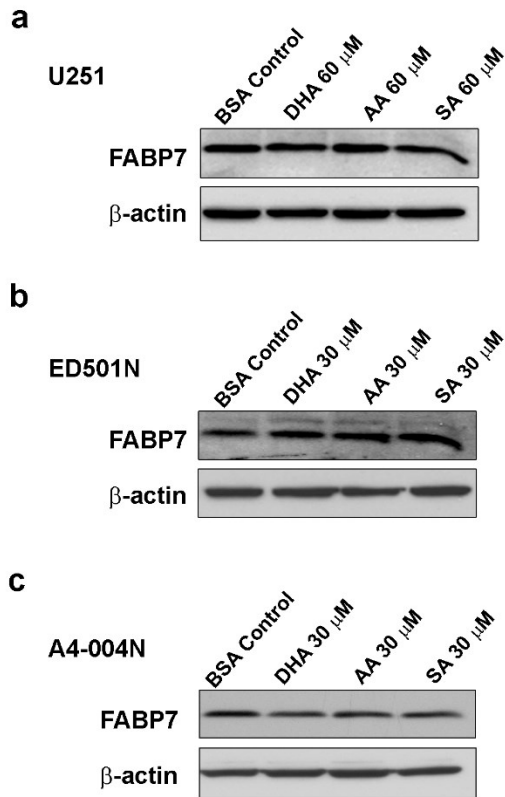
Fig. S8. FABP7 levels in GBM cells are not altered by fatty acid supplementation. U251 (a), ED501N (b) and A4-004N (c) were cultured in control medium (BSA), or in medium supplemented with DHA, AA or SA 60 μM (U251) or 30 μM (ED501N and A4-004N) for 24 hours.

Fig. S9

U251 | DAPI | FABP7 | Mitotracker | Merged

Fig. S9. FABP7 localizes to mitochondria in U251 cells. U251 cells fixed with 4% PFA were co-stained with Atto 550-conjugated primary anti-FABP7 antibody and MitoTracker® Deep Red. Arrows point to mitochondria. Scale bar = 20 μm.