Supplementary Materials and Methods

1. GBM single-cell RNA sequencing (scRNA-seq) data analysis

Data were normalized with LogNormalize function. Cells were clustered using the FindClusters function which applies modularity optimization techniques. The non-linear dimensional reduction technique was used to generate UMAPs and to visualize cell cluster features. Markers of specific clusters were identified using the FindMarkers function. Total number of cells expressing specific stem cell or EMT markers in each of the *FABP7* subset was calculated and plotted in the bar graphs and p-value was calculated using prop.test function in R.

2. Co-immunofluorescence (IF) staining and scoring of scratch assay

The scratch assay[1] combined with co-immunofluorescence assay was used to study the expression of FABP7, EMT marker ZEB1, and GBM neural stem like cells markers SOX2 and nestin, in GBM fast-migrating versus slow-migrating cells. A4-004 cells were cultured in neurosphere medium on laminin-coated glass coverslips until they reached 70%-80% confluence. A top-to-bottom scratch was introduced in the middle of the coverslip with a P20 pipet tip, and cells were incubated for an additional 24 h. Cells were washed with 1X PBS and fixed in 4% paraformaldehyde (PFA), and then incubated with primary antibodies followed by secondary antibodies. Antibody information: mouse anti-FABP7 antibody (Santa Cruz,1:400, sc-374588), rabbit anti-FABP7 antibody (prepared in-house; 1:400), anti-ZEB1 antibody (Invitrogen,1:400, #14-9741-82), anti-SOX2 antibody (Cell Signaling,1:400, #3579), anti-nestin antibody (Abcam,1:400, ab22035), anti-rabbit Alexa-488 or Alexa-555 secondary antibodies (Invitrogen, 1:400), and anti-mouse Alexa-488 or Alexa-555 secondary antibodies (Invitrogen, 1:400).

Immunofluorescence imaging was carried out using confocal microscopy with a Zeiss 20X objective. MetaXpress 6 Software Multi-Wavelength Cell Scoring Module (Molecular Devices, USA) was used to quantitate the immunoreactivity of FABP7 and cell markers (SOX2, Nestin and ZEB1) in fast migrating (in the closing gap) and slow migrating (in areas away from the scratch) cells. We first counted total cell numbers within these areas using DAPI as a nuclei marker. Next, we established the thresholds for the expression of FABP7 and other markers based on immunostaining signal intensity that best matched their optical scanning. The number of positive or negative cells for FABP7 or each of the co-stained markers was determined independently using threshold values specified by the software. The percentage of positive cells for each marker was computed as one hundredth of the total cell number. The percentage of the co-expressing cells (positive for both FABP7 and one of the co-stained markers)was computed as one hundredth of the total number of cells positive for FABP7 alone, the co-stained marker alone and both FABP7/co-stained marker.

3. Orthotopic xenografts generated with U87 cells and A4-007 cells

U87-pREP4 (FABP7-negative) and U87-pREP4-FABP7 were stereotaxically-injected (~10^5 cells in 5 µL) into the right frontal cortex at a depth of 2 mm (1.5 mm lateral from the bregma) of 4-week old female SCID mice (n=3 mice per group). SE T2-weighted images of brains were acquired at weekly intervals starting two weeks after cell injection using a 9.4 Tesla/21.5 cm diameter MRI system. Animals were sacrificed between 4 to 6 weeks after cell injections and brain samples were collected. Brains were embedded and sections containing tumor tissues were immunostained with rabbit anti-FABP7 antibody (prepared in-house; 1:500), followed by EnVision + anti-rabbit HRP-labeled polymer (DakoCytomation, Carpinteria, CA, USA).

A4-007 GSCs (~10^5 cells in 5 µL) were stereotaxically-injected into the brains of 8-week old male NSG mice as described above (n=3 per group). Mice were intraperitoneally injected with either DMSO (control) or FABP7 inhibitor (SBFI-26, Aobious, INC) (15 mg/kg)[2] once a week for three weeks starting one week after tumor injection. Mice were euthanized 8 weeks post-tumor injection. Mouse brains were dissected, processed and the tissue sections were immunostained with rabbit anti-FABP7 as described above.

4. Tissue microarray (TMA) analysis

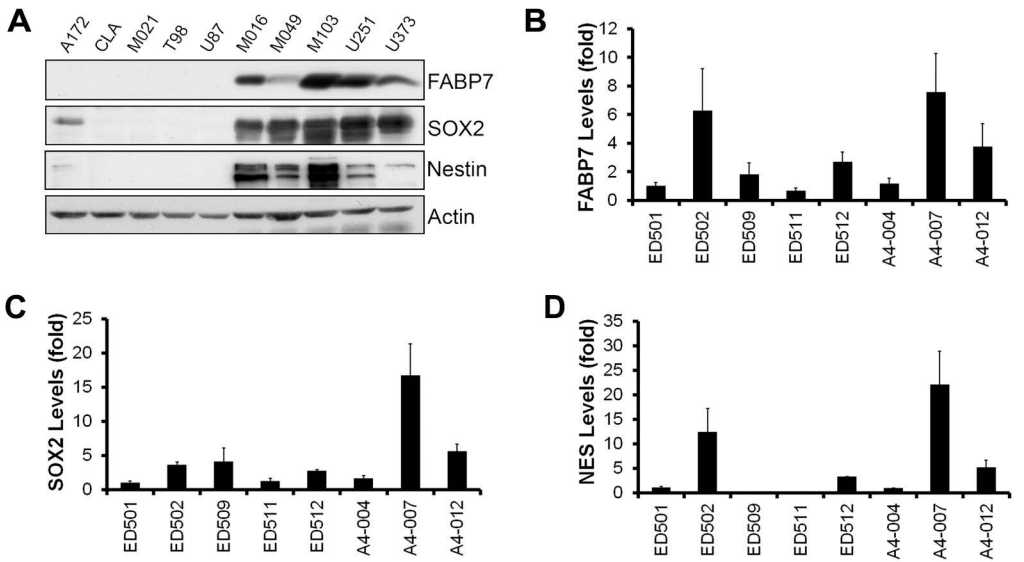
TMA tissues (in triplicate) from a previously described GBM patient cohort[3] were immunostained with anti-FABP7 antibody (prepared in-house; 1:500) and DakoCytomationEnVision+ anti-rabbit secondary system (Dako, CA). Tissues were counterstained with hematoxylin. Cytoplasmic and nuclear FABP7 immunoreactivity were evaluated separately by two professional evaluators independently (blind to each other). The Spearman's coefficient of rank correlation between the two sets of scores was 0.833 ($p<0.0001$) and 0.840 ($p<0.0001$) for cytoplasmic and nuclear FABP7 immunoreactivity, respectively, suggesting strong agreement. Immunostaining intensity was scored on a scale of 0 (negative), 1 (weak), 2 (intermediate) and 3 (strong). For each tumor core, an H-score were calculated based on average staining intensity and percentage of immunostained cells throughout the tumor tissue using the following formula: H-score = [(0 x % negative cells) + (1 x % weakly positive cells) + (2 x % moderately positive cells) + (3 x % strongly positive cells)]. The range of H-scores was 0 to 300 and the scores from different TMA cores of the same tumor sample were averaged. For cut-off point in patient survival analysis, H-scores higher than 150 was classified as "high", and tumor tissues with H-scores equal or less than 150 were classified as "low".

REFERENCES

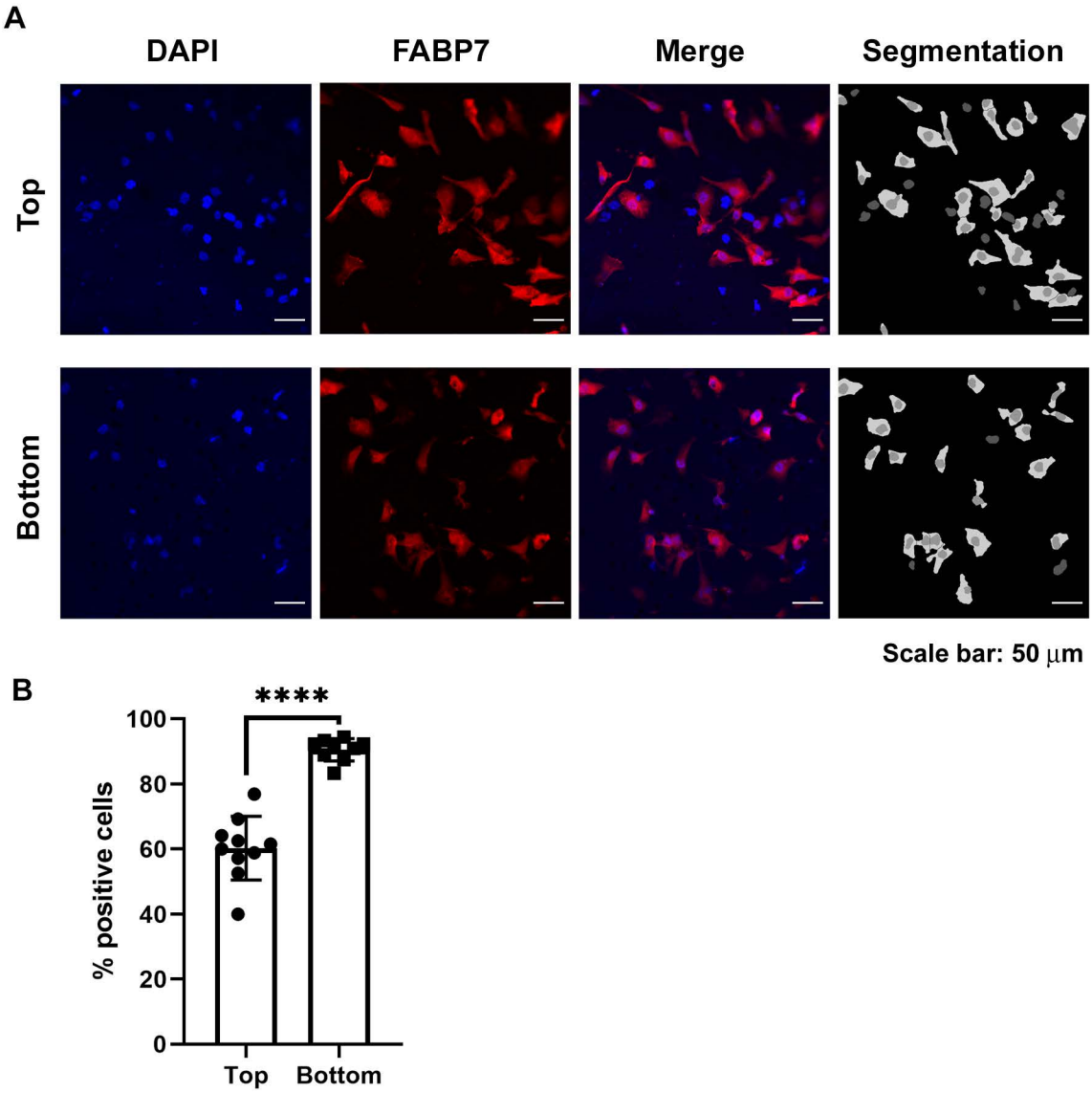
1. Liang CC, Park AY, Guan JL. In vitro scratch assay: a convenient and inexpensive method for analysis of cell migration in vitro. *Nat Protoc.* 2007; 2(2):329-333.

2. Hoang-Minh LB, Siebzehnrubl FA, Yang C, et al. Infiltrative and drug-resistant slow-cycling cells support metabolic heterogeneity in glioblastoma. *EMBO J.* 2018; 37(23).

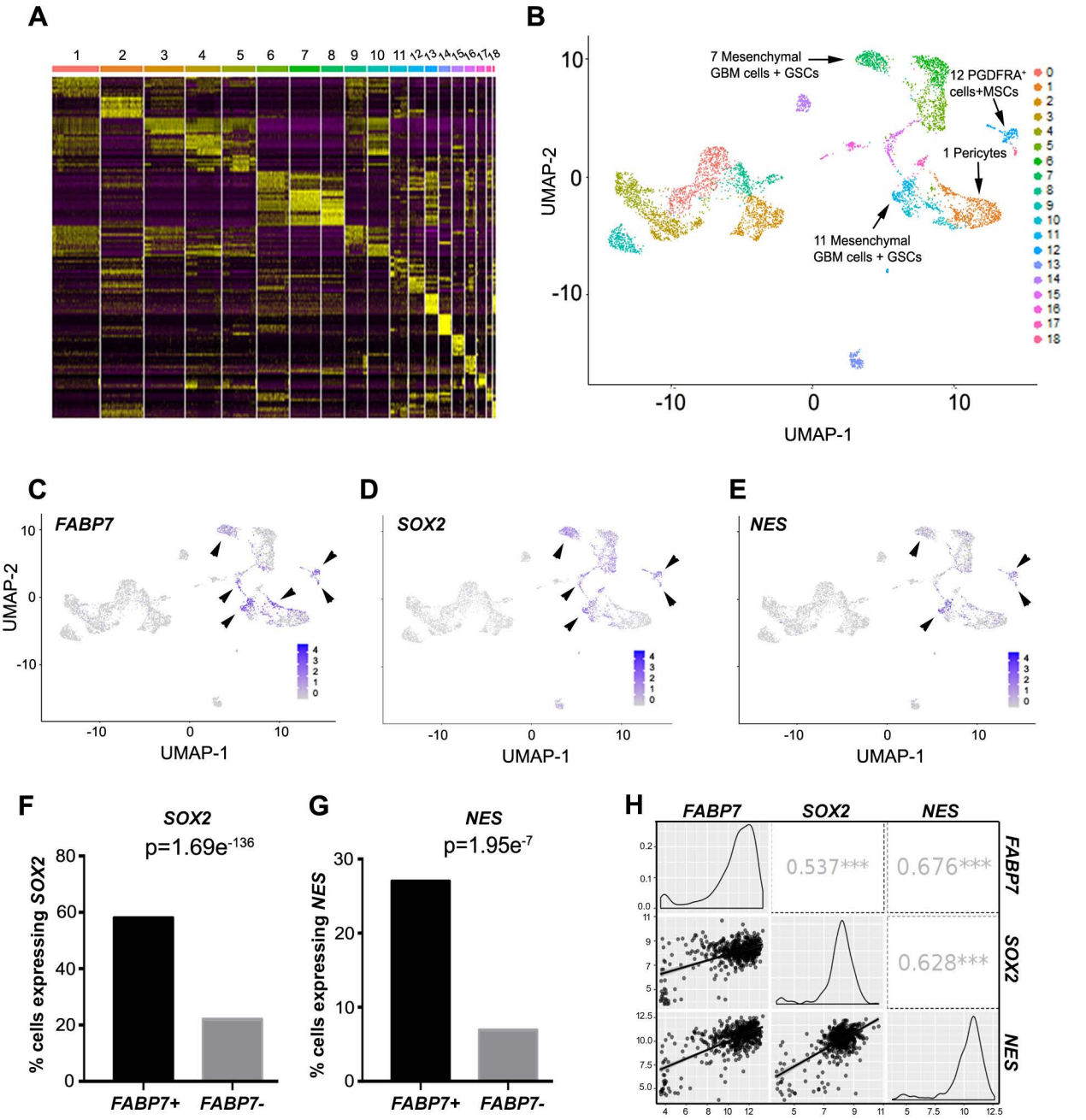
3. Liu RZ, Li S, Garcia E, et al. Association between cytoplasmic CRABP2, altered retinoic acid signaling, and poor prognosis in glioblastoma. *Glia.* 2016; 64(6):963-976.



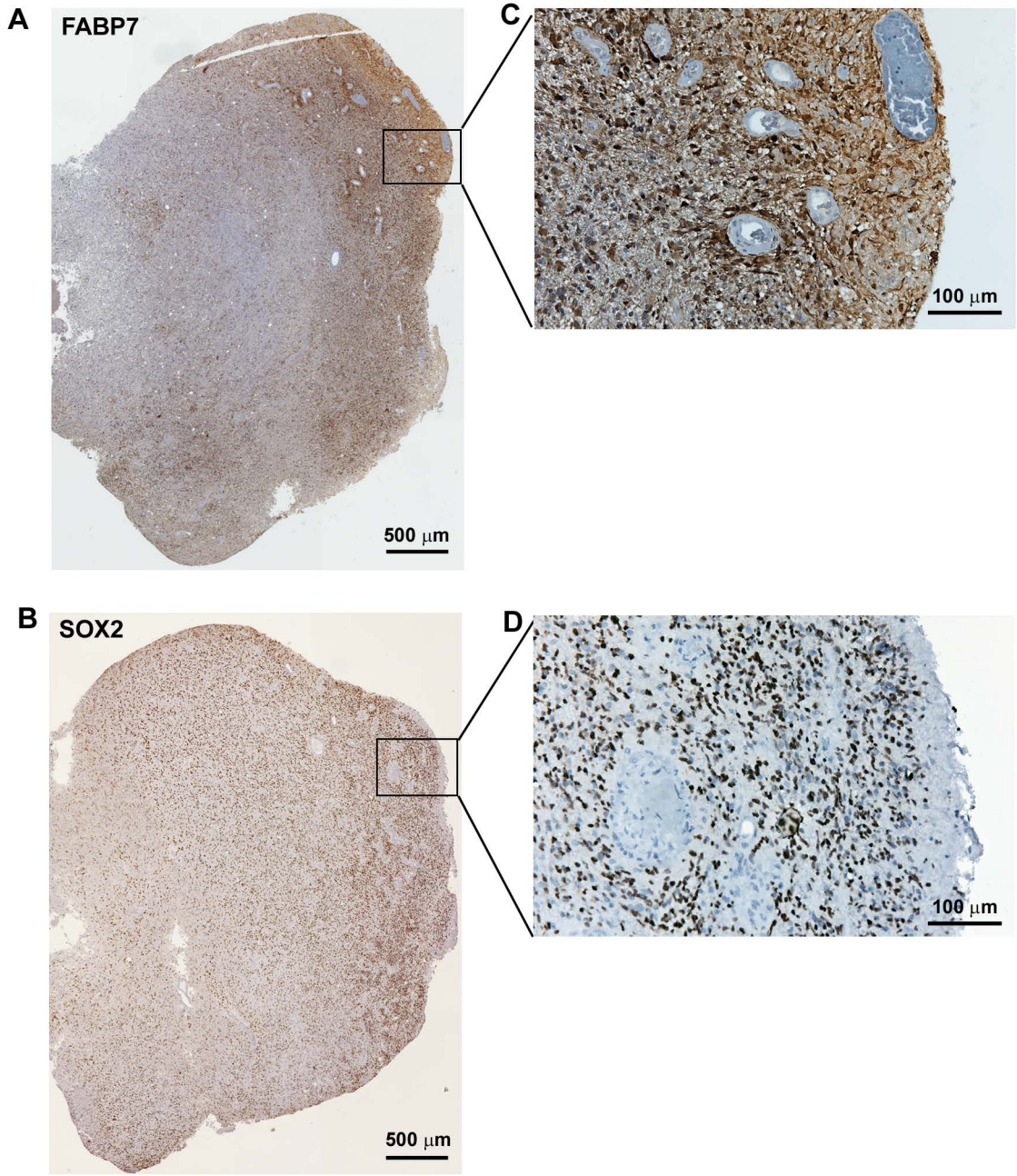
Supplementary Figure 1. Co-expression of FABP7 with GBM stem cell markers SOX2 and nestin in established GBM cell lines and patient-derived stem-like cell lines. (**A**) Western blot showing a general correlation between FABP7 expression and expression of neural stem cell markers SOX2 and nestin in ten established GBM cell lines. (**B-D**) RT-qPCR showing *FABP7*, *SOX2* and nestin (*NES*) RNA levels in eight patient-derived GBM stem-like cell lines.



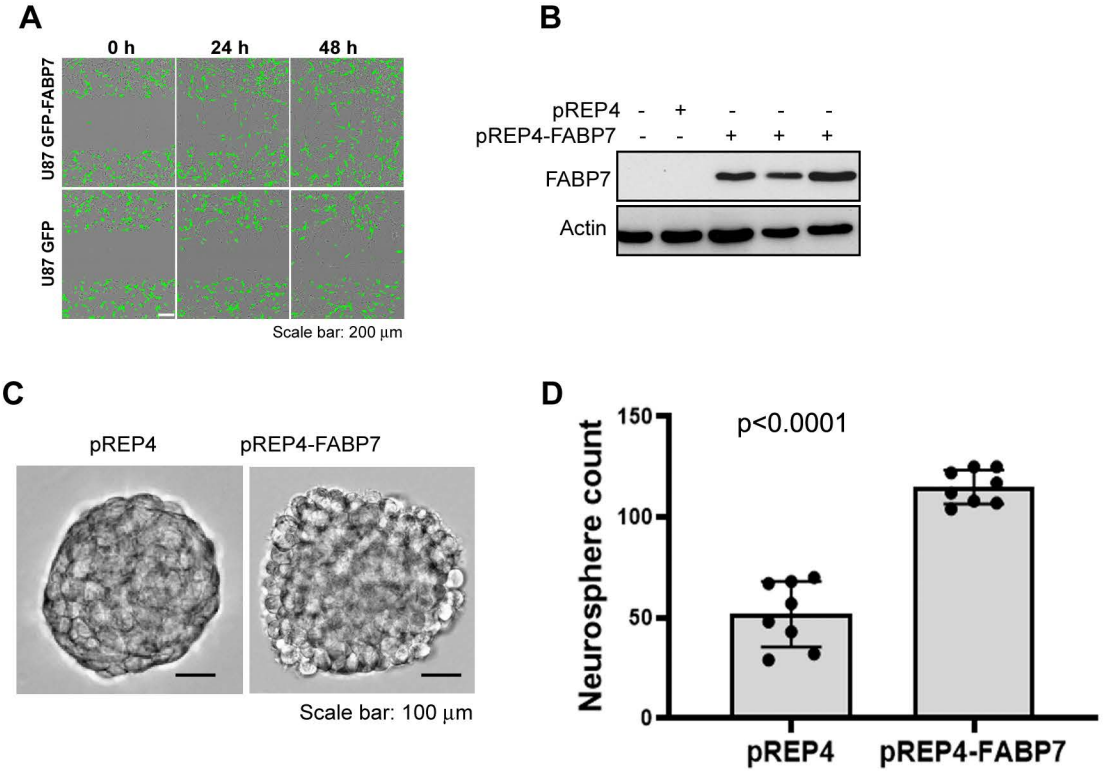
Supplementary Figure 2. FABP7 is enriched in migrating patient-derived GSCs. Thirty thousand A4-004 cells were seeded in Transwell chambers with DMEM/F12 medium in the top chamber and neurosphere medium in the bottom chamber. After 30 hours of incubation, cells on the top and bottom sides of duplicate Transwell membranes were immunostained with anti-FABP7 antibody. FABP7-positive cells were detected and analyzed with the MetaXpress 6 Software Multi-Wavelength Cell Scoring Module. (**A**) Representative images of FABP7 immunofluorescence staining. (**B**) Quantification and statistical analysis of FABP7 immunoreactivity in migrated (bottom) and non-migrated (top) cell populations. Each circle or square represents a separate microscopic field. **** denotes $p<0.0001$.



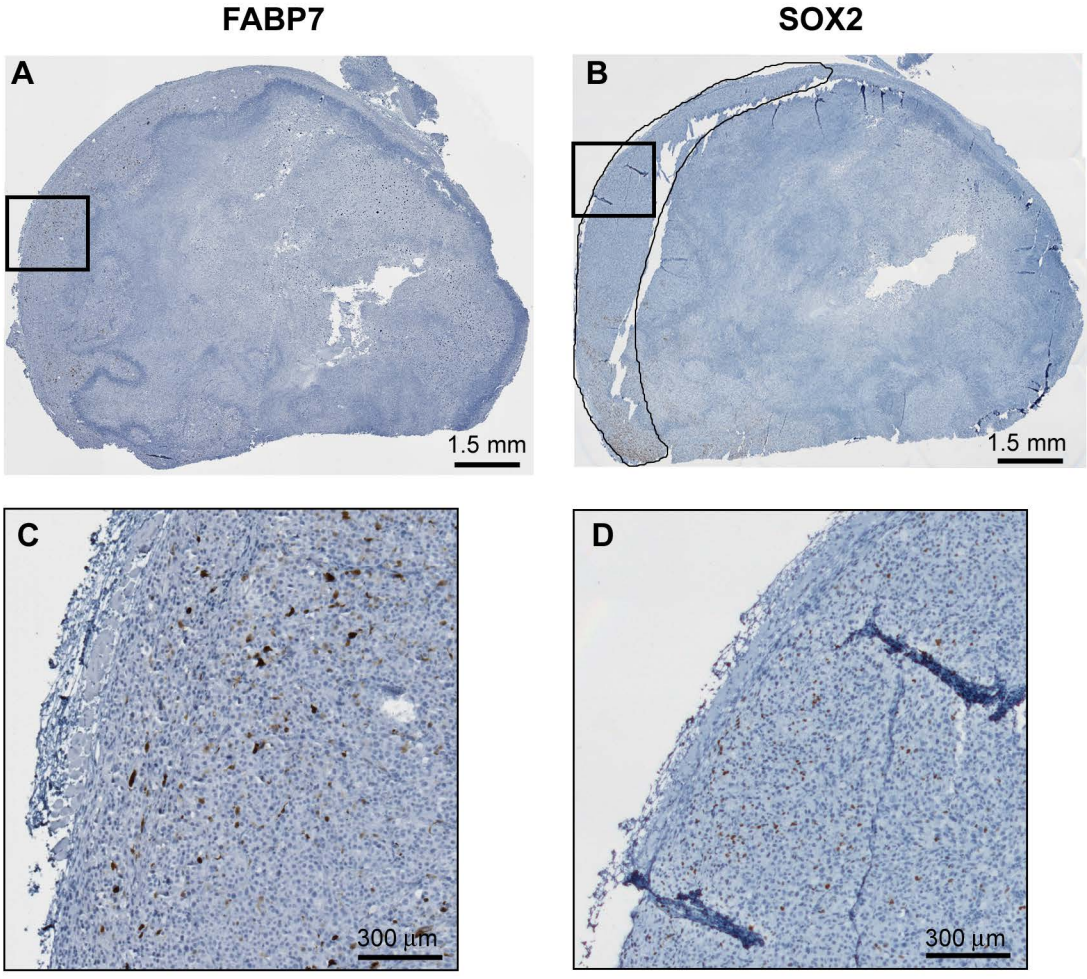
Supplementary Figure 3. Co-expression of FABP7 with stem cell markers revealed by scRNA-seq and GBM patient cohort analysis. (**A**) Heatmap depicting the scaled expression of the top 10 signature gene markers for each of the 18 identified cell clusters. (**B**) UMAP visualization of single cell transcriptome community/similarity structure, with color-coding of all 18 discrete cell clusters shown in (A). Cell clusters associated with *FABP7* transcript enrichment are numbered and labelled based on gene expression signature. (**C-E**) UMAP showing similar expression patterns for *FABP7* (C), *SOX2* (D) and *NES* (E) in GBM cell clusters. (**F, G**) Analysis of the scRNA-seq data showing significantly higher percentages of cells expressing *SOX2* (F) and *NES* (G) in FABP7-positive cells compared to FABP7-negative cells. (**H**) Correlation of *FABP7* with *SOX2* and *NES* RNA expression in a TCGA dataset (HG U-133A) comprised of 453 GBM patients. Numbers in squares denote correlation coefficient. ***, p<0.001.



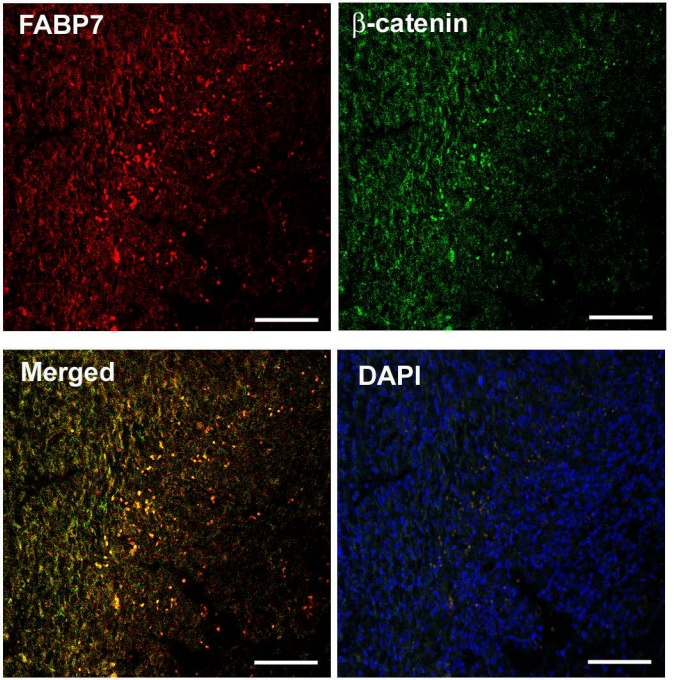
Supplementary Figure 4. Immunohistochemical staining showing similar patterns of FABP7 and SOX2 immunoreactivity. **(A, B)** FABP7 (A) and SOX2 (B) immunostaining of human GBM tissue sections from patient ID-670 obtained from the Tumour Tissue Bank, London Health Sciences Centre, London, Ontario, Canada. Enrichment of FABP7 and SOX2 positive cells are observed in the invasive front of the tumor. **(C, D)** Magnifications of the squared regions are shown in the left panels. Tissue sections were counterstained with hematoxylin.



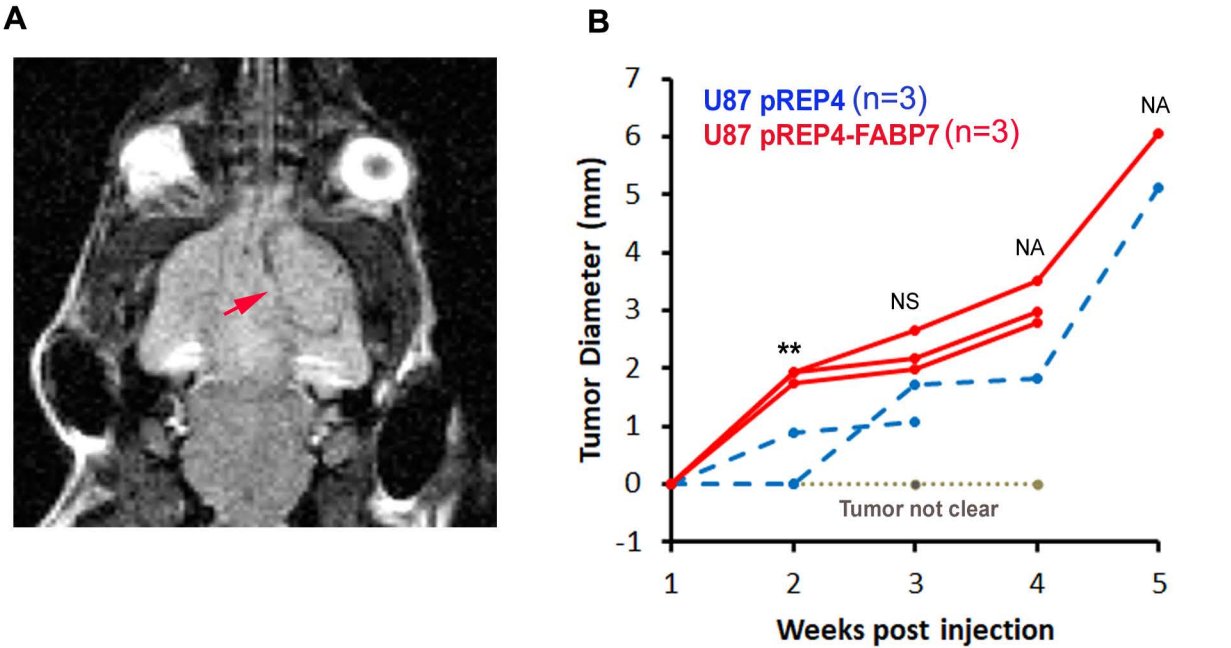
Supplemental Figure 5. FABP7 promotes cell migration and neurosphere formation in U87 cells. (A) Representative images showing distribution of U87-GFP and U87-GFP-FABP7 cells in the scratch assay (Incucyte). **(B)** Western blot showing ectopic expression of FABP7 in U87 transfectants. **(C)** Representative images showing the distinct morphologies of neurospheres generated from U87 control cells (pREP4) and stable FABP7-expressing cells (pREP4-FABP7). **(D)** Histogram showing increased neurosphere formation capacity of U87 cells with stable FABP7 expression relative to control U87 cells. Cells were plated in 24-well ultra-low attachment plates (1000 cells/well) and cultured in neurosphere medium for 6 days. Neurospheres were then counted and analyzed with student's t-test.



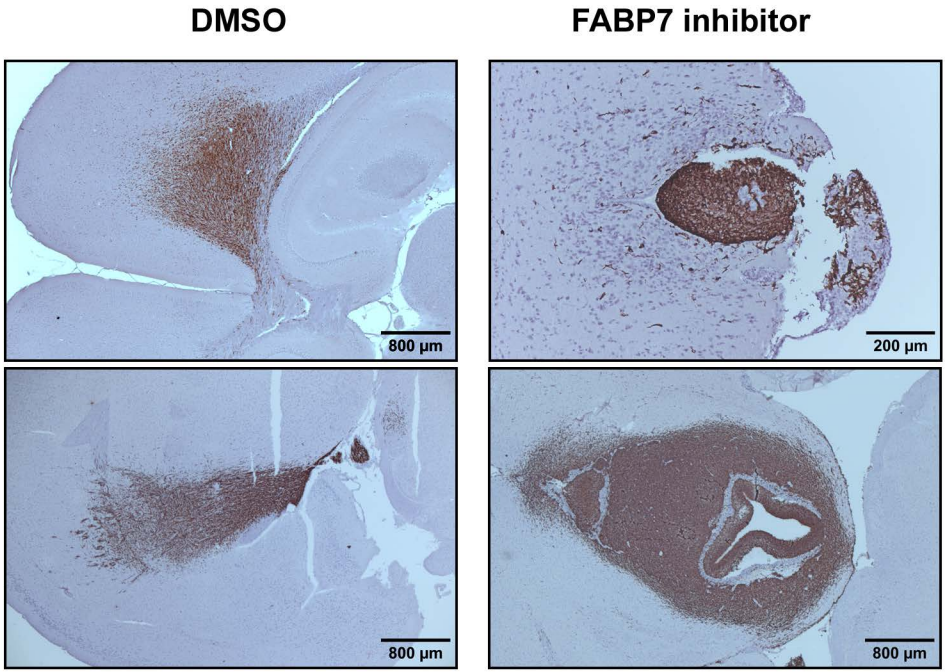
Supplementary Figure 6. (A,B) FABP7 (A) and SOX2 (B) immunoreactivity in cells located in the leading front of the tumor adjacent to the pseudopalisading cell layers (area delineated by the drawn lines) of a xenograft tumor generated from FABP7-transfected U87 cells. **(C, D)** Magnifications of the squared regions shown in the upper panels. Tissue sections were counterstained with hematoxylin.



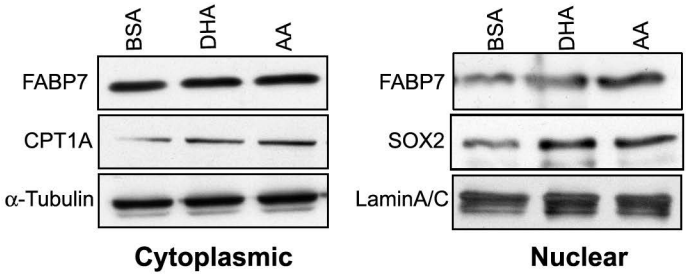
Supplementary Figure 7. FABP7 co-localizes with β-Catenin in the nucleus of a subset of cells in GBM xenograft tumor tissues. Cells were co-immunostained with anti-FABP7 and anti-β-catenin antibodies. DAPI was used to stain nuclei.



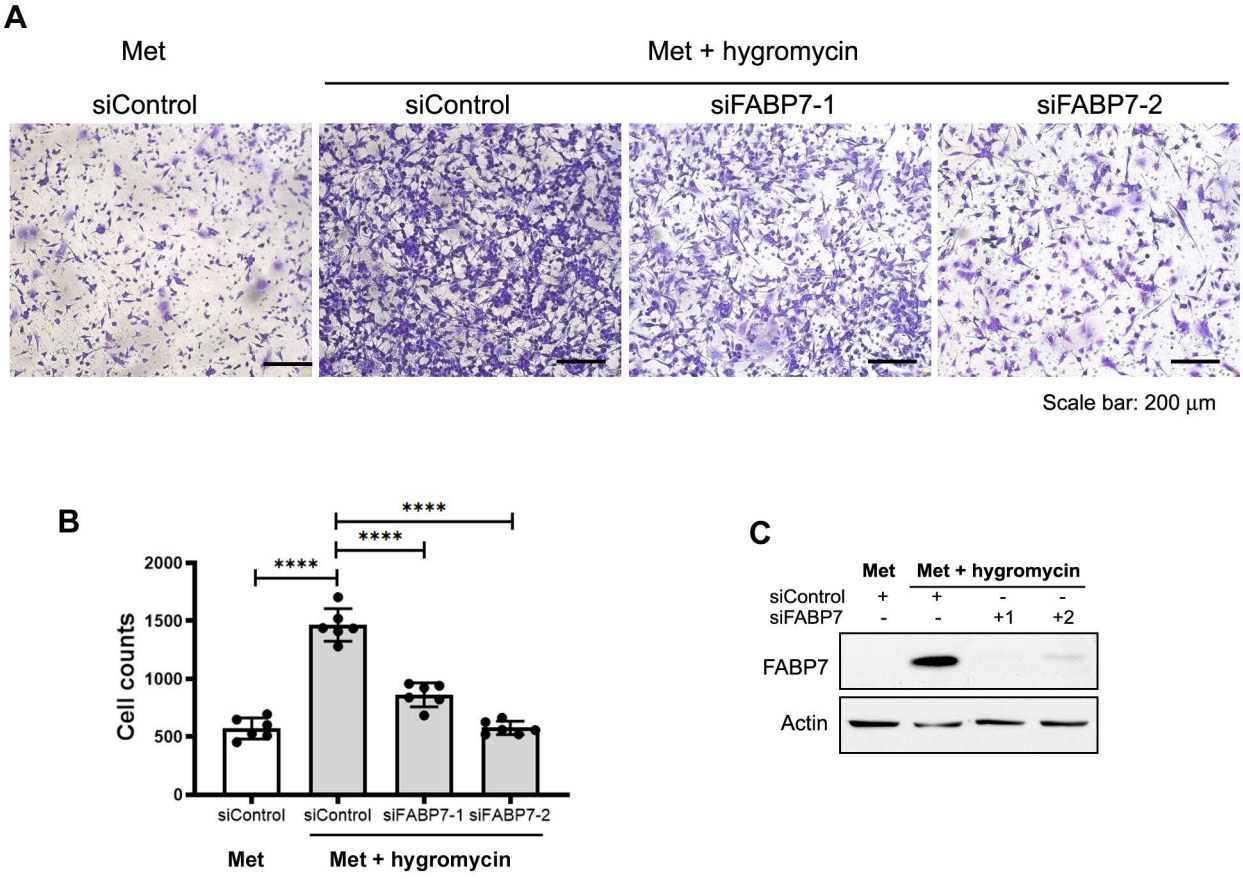
Supplementary Figure 8. Orthotopic tumors generated from U87 control and FABP7-expressing cells. (**A)** 9.4T Magnetic Resonance Imaging **(**MRI) axial scan showing the location of a brain tumor (arrow) from a mouse injected with U87-pREP4-FABP7-expressing cells at five weeks post-injection. The image was created with T2-weighted MRI. **(B)** Graph showing tumor growth curve at specified time points. "**" denotes $p<0.01$; NS, not significant; NA, not applicable.



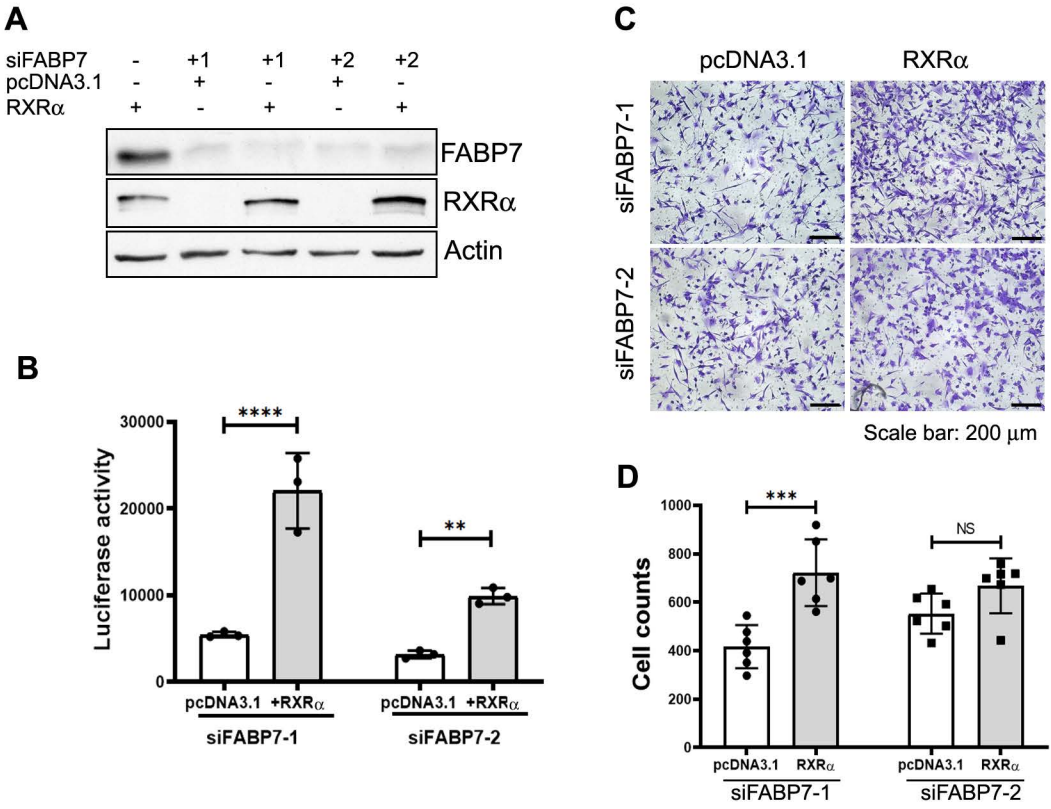
Supplementary Figure 9. Orthotopic xenograft tumors generated with the human GBM patient-derived stem-like cell line A4-007. ~10^5 cells in 5 µL PBS were stereotaxically injected into the brains of 8-week-old male NSG mice. Mice were intraperitoneally injected with either DMSO (left panel) or FABP7 inhibitor (SBFI-26, right panel) at 15 mg/kg once a week for three weeks starting one week post injection. Mice were euthanized 8 weeks post-tumor injection. Mouse brains were dissected, processed and tissue sections immunostained with rabbit anti-FABP7 antibody. Control (DMSO) cells have a typical infiltrative appearance. FABP7 inhibitor-treated mice show a small encapsulated tumor in one case, and a large partially-encapsulated and dense tumor in another case likely reflecting partial response to the FABP7 inhibitor.



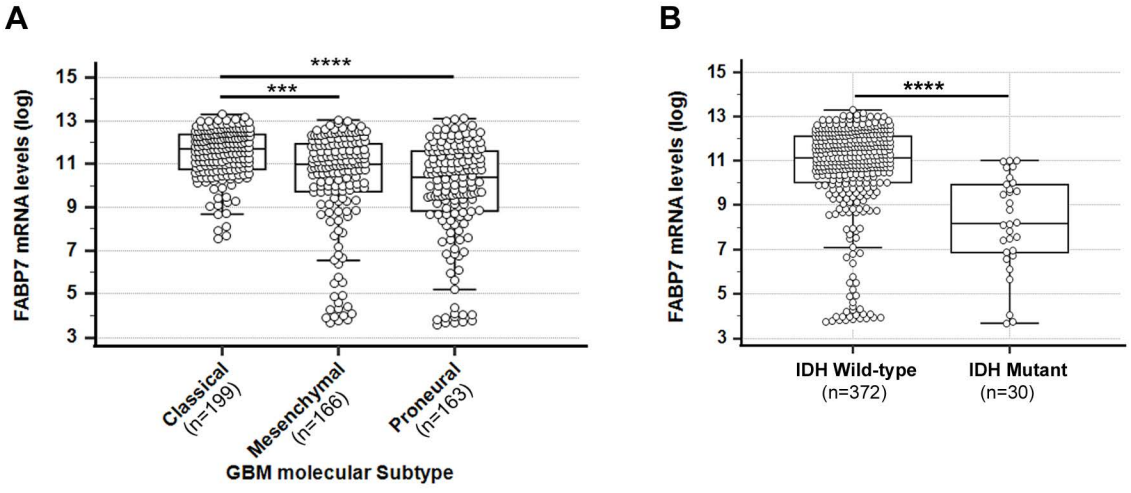
Supplementary Figure 10. Western blot of subcellular FABP7 and CPT1A upon treatment with DHA and AA in A4-007 cells. Patient-derived GBM stem-like cells A4-007 were treated with 30 μM DHA or AA for 24 h. Cytoplasmic and nuclear proteins were extracted using the NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo Fisher Scientific) according to manufacturer's instructions. Forty μg/lane cytoplasmic extract and twenty μg/lane nuclear extract were loaded, with α-tubulin and lamin A/C serving as cytoplasmic and nuclear loading controls, respectively.



Supplementary Figure 11. Effect of hygromycin selection and FABP7 depletion on cell migration in mouse xenograft-derived cells. Cell lines were generated from intestinal metastasis obtained from mice injected with U87 pREP4-FABP7 cells. Cells were cultured in the absence (Met) or presence (Met + hygromycin) of hygromycin used for pREP4 episomal vector selection. Hygromycin-selected cells were transfected with scrambled siRNAs (siControl) or siRNAs targeting FABP7 (siFABP7-1, FABP7-2). Cell migration was measured using 8 μm Transwell inserts. (**A**) Representative images showing the cells that have migrated through the Transwell membranes. (**B**) Bar chart showing the number of cells that have migrated through the Transwell membranes. Statistical significance was tested with one-way ANOVA. (**C**) Western blot showing FABP7 levels in intestinal metastasis cell lines. **** denotes $p<0.0001$



Supplementary Figure 12. Rescue of RXRα transcriptional activity and cell migration upon overexpression of RXRα in FABP7-depleted cells. Hygromycin-selected cells from a xenograft metastasis generated from U87 pREP4-FABP7 cells were transfected with siRNAs targeting FABP7 (siFABP7-1 and siFABP7-2). After 24 h, cells were co-transfected with a luciferase reporter vector driven by DR1/PPRE and either empty vector (pcDNA3.1) or a pcDNA3.1-hRXRα expression construct (RXRα, Addgene). Luciferase activity was measured with the Luciferase Assay System (Promega) according to the manufacturer's directions. For the migration assay, 50,000 cells transfected with either pcDNA3.1 or pcDNA3.1-hRXRα were seeded in triplicate in the upper chamber of Transwell inserts and allowed to migrate towards the bottom chamber containing fetal calf serum over a 24 h period. **(A)** Western blot showing levels of FABP7 and RXRα after the indicated transfections. **(B)** Dot plots showing significant elevation of luciferase activity upon RXRα overexpression. **(C)** Representative images showing the density of cells that had migrated through the Transwell membranes for each transfection as indicated. **(D)** Dot plots showing statistical analysis of cell numbers on Transwell membranes. N=2 in triplicates. NS denotes "not significant" ; **, p<0.01; ***, p<0.001; ****, p<0.0001.



Supplementary Figure 13. Differential expression of FABP7 in GBM subtypes. Statistical analysis of *FABP7* RNA levels based on GBM molecular subtypes (**A**) and IDH status (**B**) using one-way ANOVA. Microarray gene profiling and phenotype data of the TGCA HA-U133A dataset were obtained from GlioVis (http://gliovis.bioinfo.cnio.es/).