

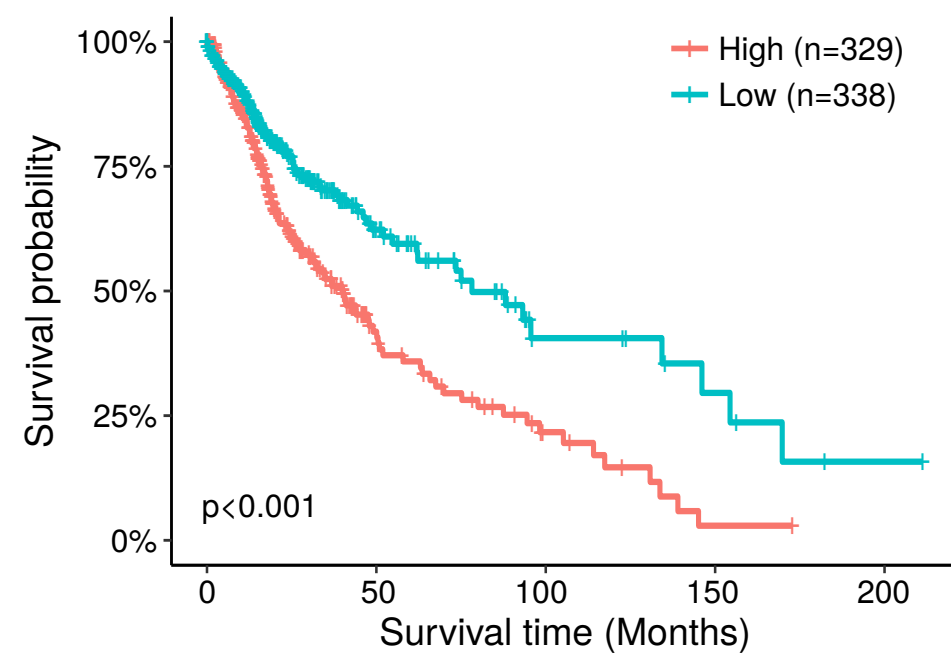
The RNA binding protein SERBP1 functions as a novel oncogenic factor in glioblastoma by bridging cancer metabolism and epigenetic regulation

Additional File 1

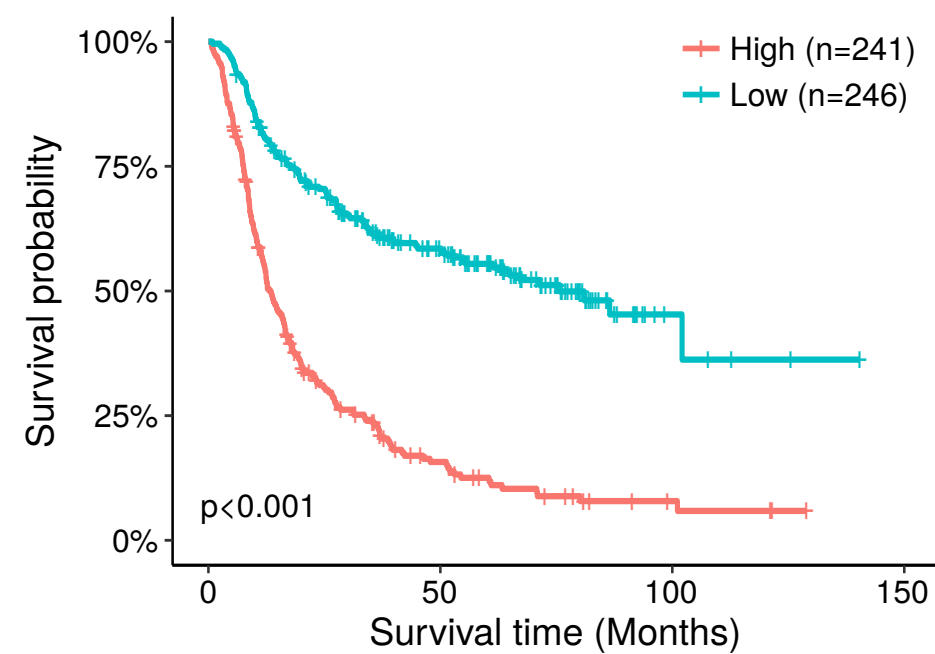
Supplementary Figures

A

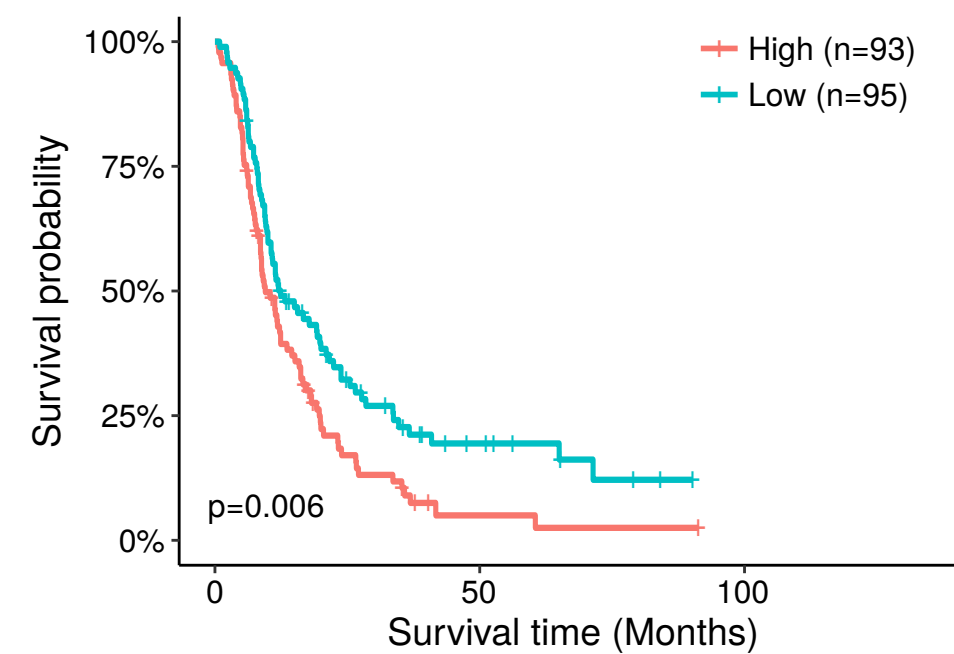
TCGA All glioma



CGGA All Glioma

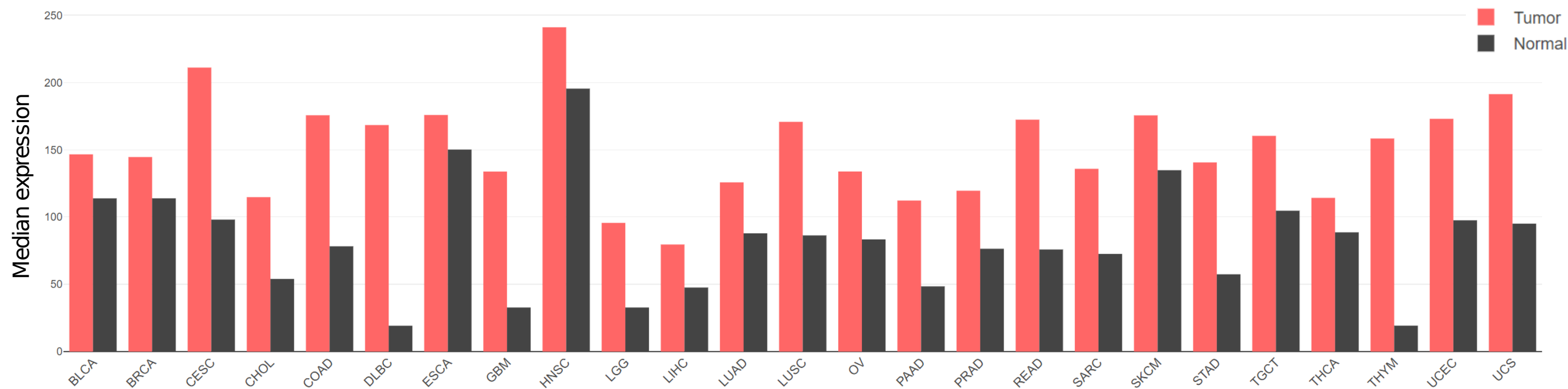


CGGA GBM



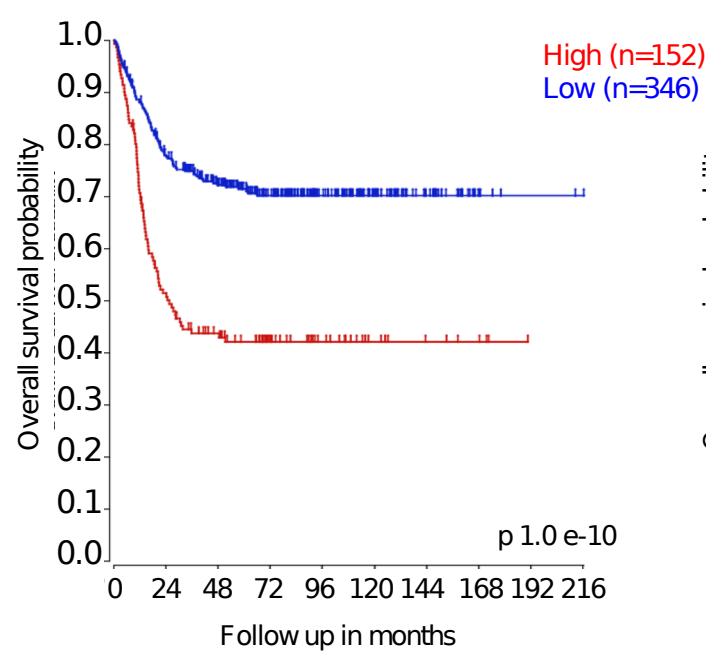
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TCGA Tumors vs. Normal

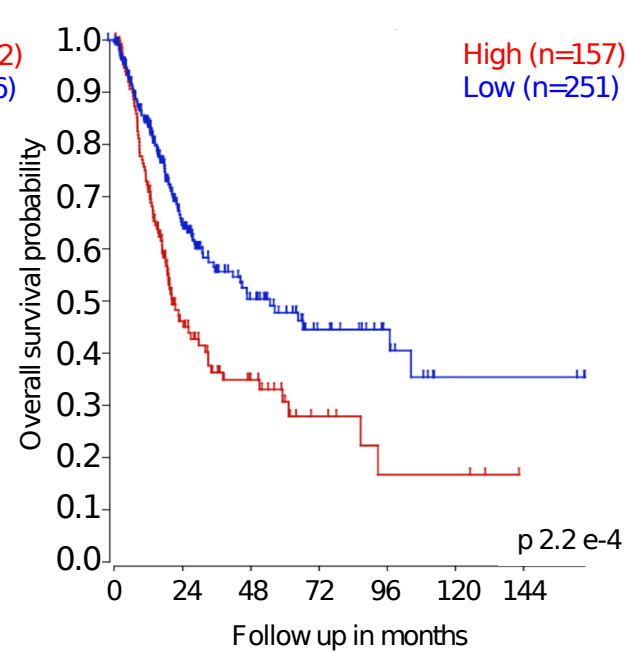


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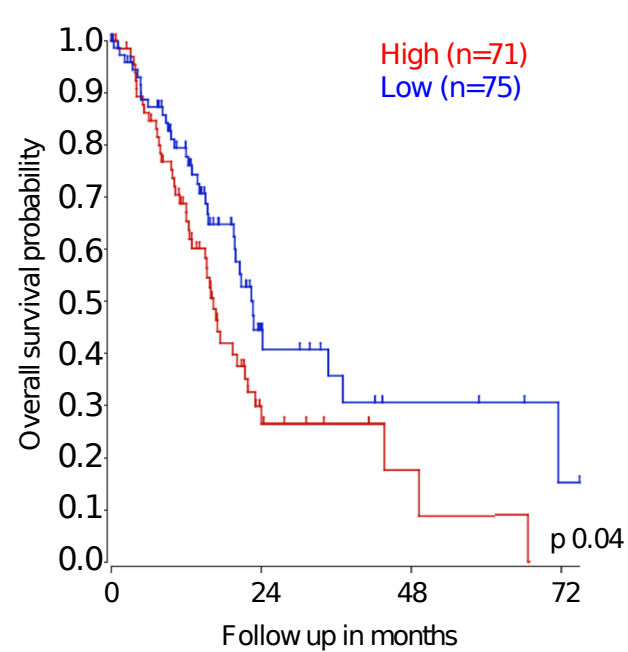
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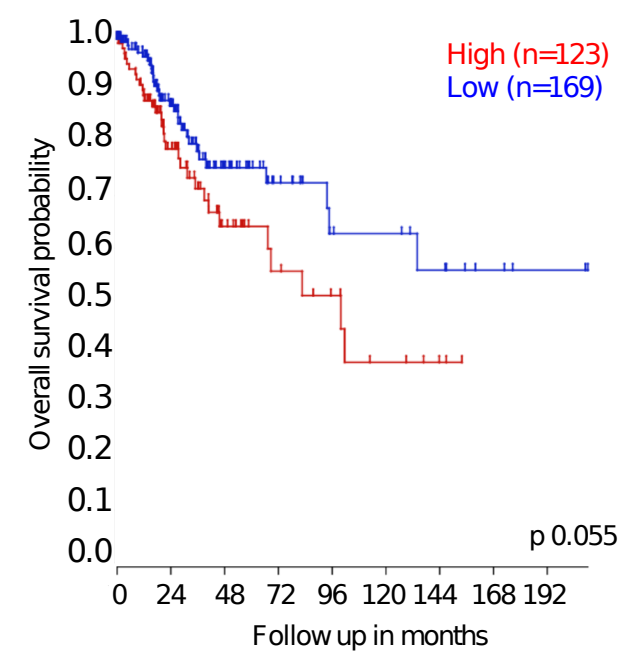
Bladder Urothelial Carcinoma- TCGA



Pancreatic adenocarcinoma- TCGA



Cervical Squamous cell carcinoma - TCGA



Sarcoma - TCGA

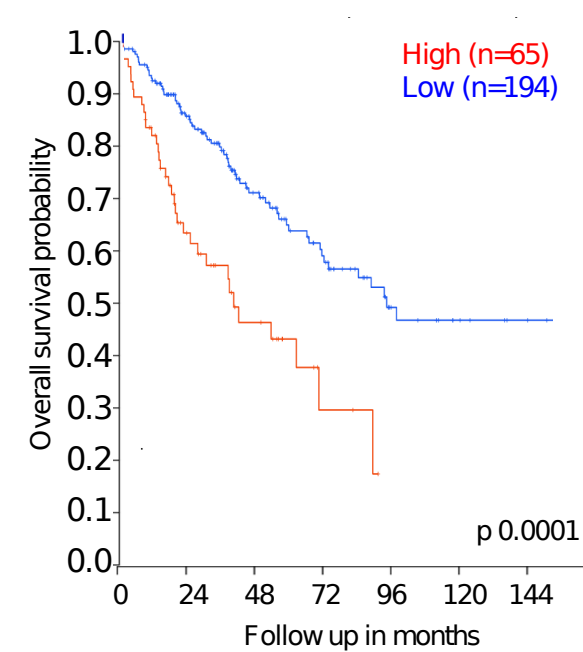
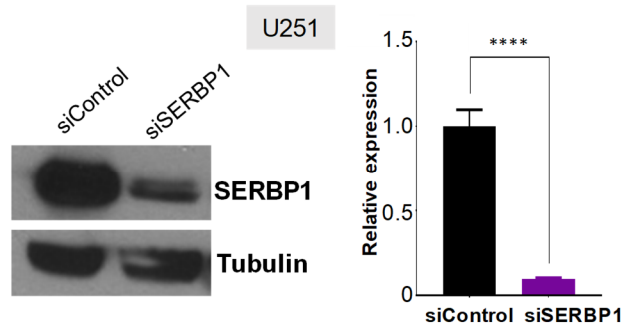
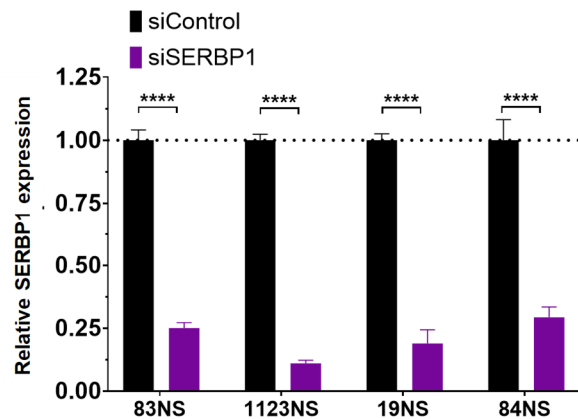


Fig. S1. SERBP1 expression in normal vs. normal tissue and impact on survival. A) Kaplan–Meier curves show the survival of 667 glioma patients in the TCGA cohort, 487 glioma patients in the CGGA cohort and 188 glioblastoma patients in the CGGA cohort displaying low and high SERBP1 levels. **B)** SERBP1 expression in tumor (TCGA) vs. normal tissue (GTEx). Data was generated using Gepia ²⁴. ACC-Adrenocortical carcinoma, BLCA-Bladder Urothelial Carcinoma, BRCA-Breast invasive carcinoma, CESC-Cervical squamous cell carcinoma and endocervical adenocarcinoma, CHOL-Cholangio carcinoma, COAD-Colon adenocarcinoma, DLBC-Lymphoid Neoplasm Diffuse Large B-cell Lymphoma, ESCA-Esophageal carcinoma, GBM-GBM multiforme, HNSC-Head and Neck squamous cell carcinoma, LGG-Lower Grade Glioma, LIHC-Liver hepatocellular carcinoma, LUAD-Lung adenocarcinoma, LUSC-Lung squamous cell carcinoma, OV-Ovarian serous cystadenocarcinoma, PAAD-Pancreatic adenocarcinoma, PRAD-Prostate adenocarcinoma, READ-Rectum adenocarcinoma, SARC-Sarcoma, SKCM-Skin Cutaneous Melanoma, STAD-Stomach adenocarcinoma, TGCT-Testicular Germ Cell Tumors, THCA-Thyroid carcinoma, THYM-Thymoma, UCEC-Uterine Corpus Endometrial Carcinoma, UCS-Uterine Carcinosarcoma. **C)** Examples of tumor types in which high expression levels of SERBP1 are associated with a poorer prognosis. Datasets were obtained from R2 Genomics Analysis and Visualization Platform. Kaplan–Meier curves indicate the survival of patients displaying low and high SERBP1 levels; default parameters were used, Kaplan Scan (KaplanScan) established optimum survival cut-off based on statistical testing.

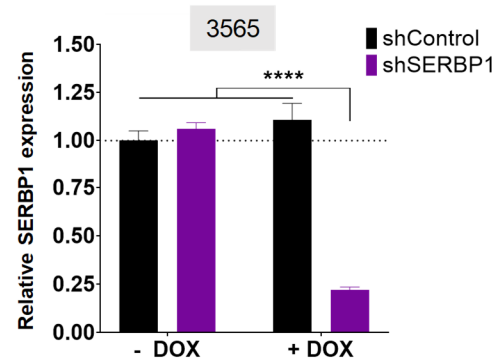
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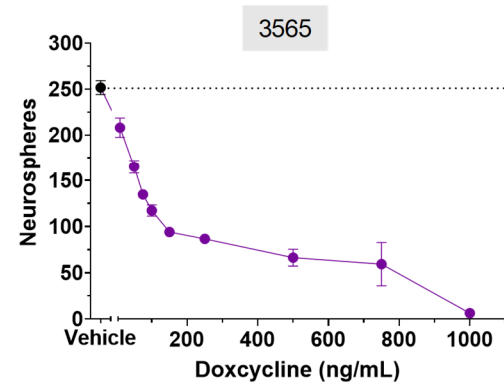
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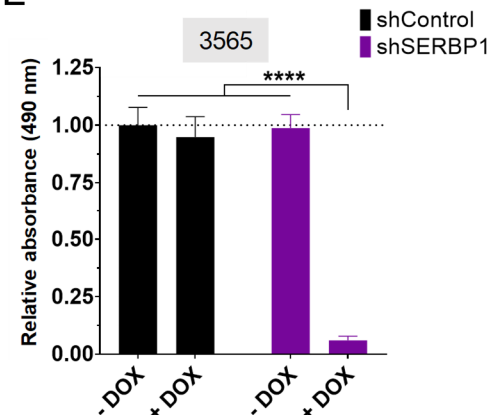
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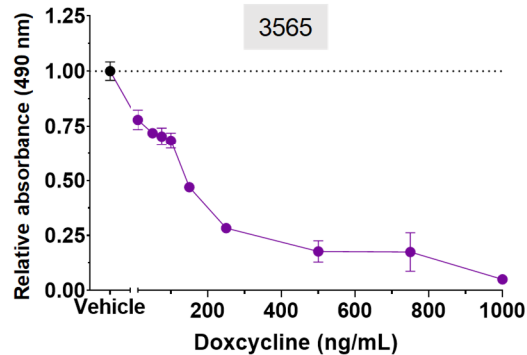
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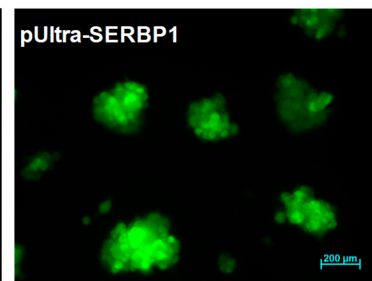
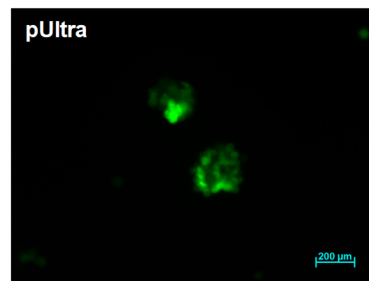
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G



H



Fig. S2. SERBP1 expression in knockdown and over expressing cell lines used in the study. **A)** Western shows SERBP1 knockdown levels 72 hours post siRNA transfection in U251 and U343 cells. **B)** SERBP1 knockdown levels 72 hours post siRNA transfection in different GSC lines was measured by qRT-PCR. **C)** 3565 shControl and shSERBP1 GSC lines were exposed to Doxycycline to induce shRNA expression and SERBP1 knockdown levels were evaluated 24 hours later by qRT-PCR. **D)** Reduction in 3565 shSERBP1 neurosphere formation was proportional to the amount of Doxycycline used to trigger SERBP1 knockdown. **E)** 3565 shControl and shSERBP1 GSC lines were exposed to Doxycycline to induce shRNA expression; impact of SERBP1 reduced expression on cell viability was measured by MTS. **F)** Reduction in 3565 shSERBP1 viability was proportional to the amount of Doxycycline used to trigger SERBP1 knockdown. **G)** Aspect of control and SERBP1 Overexpression (OE) U343 cells grown as neurospheres. **H)** Western showing levels of SERBP1 expression in control (pUltra) and SERBP1 OE lines.

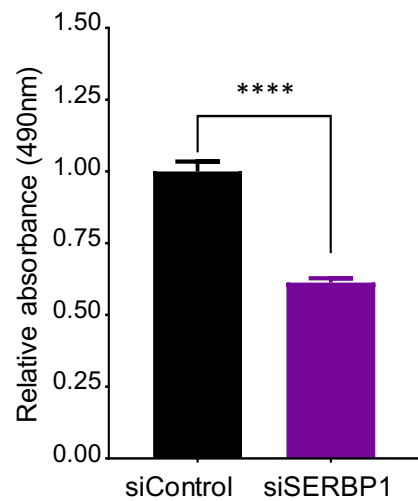
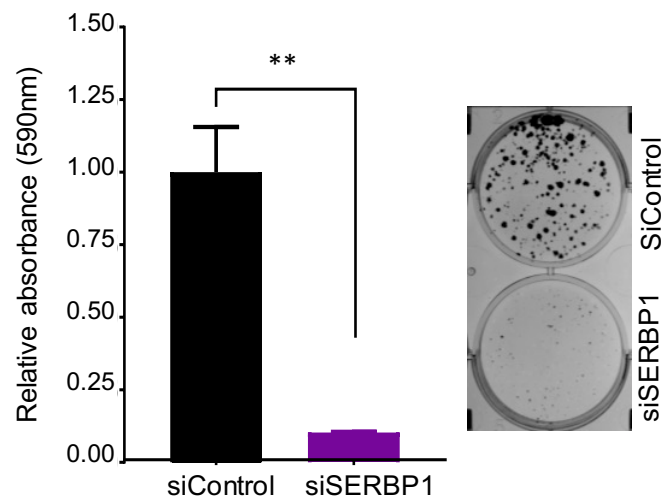
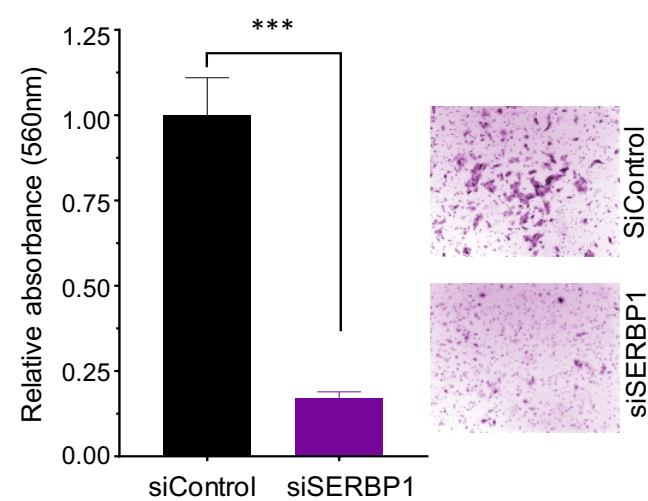
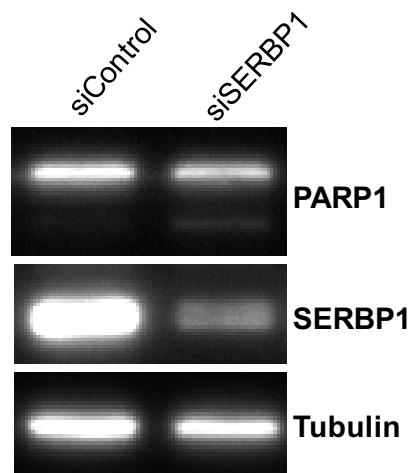
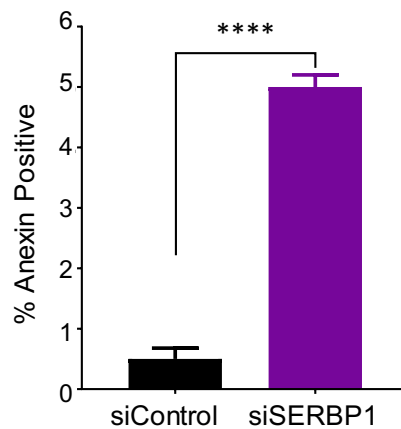
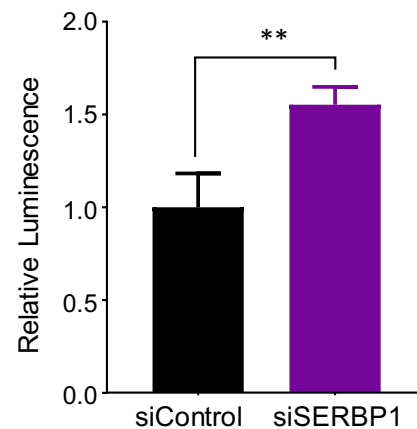
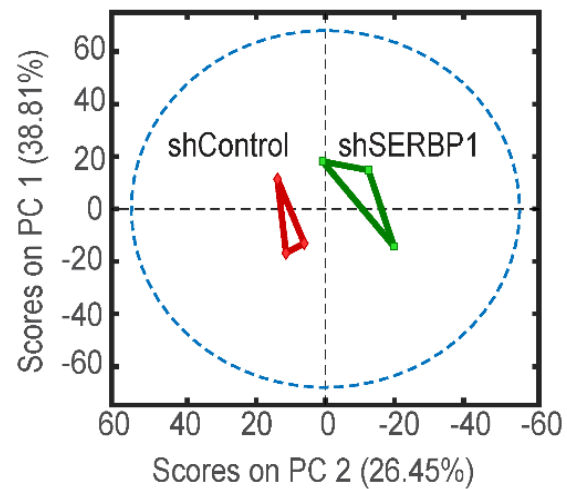
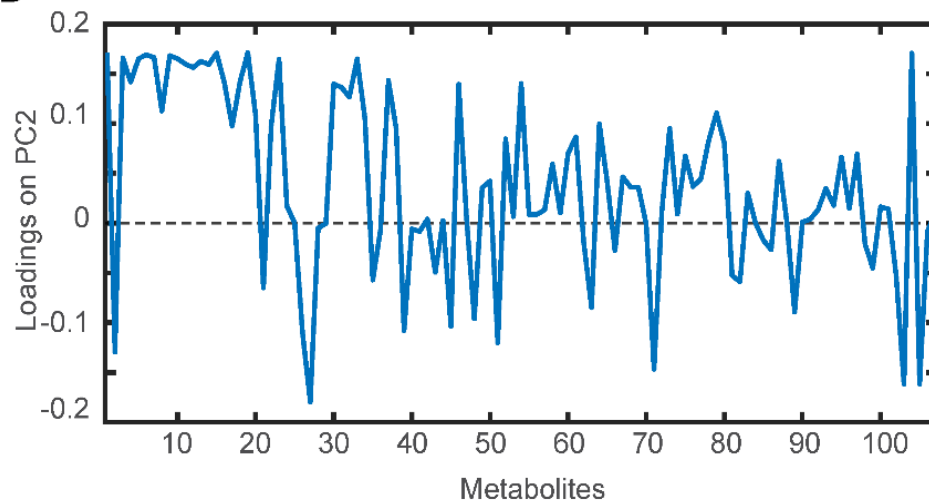
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Fig. S3. SERBP1 affects cancer related phenotypes. **A)** Knockdown of SERBP1 in U343 cells decreased viability (MTS assay). **B)** SERBP1 KD in U343 cells diminished clonogenic potential, as measured by colony formation assay. **C)** The Boyden chamber assay was used to evaluate SERBP1 impact on invasion; values of crystal violet absorbance showed that SERBP1 KD decreased invasion potential. **D-E)** SERBP1 silencing increased apoptosis as indicated by PARP1 cleavage (D) and Annexin staining (E). Data were analyzed with Students' t-test and presented as mean $\pm$ standard deviation. Bonferroni-correction was used for multiple-comparisons. * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** $p \leq 0.0001$.

A



B



C

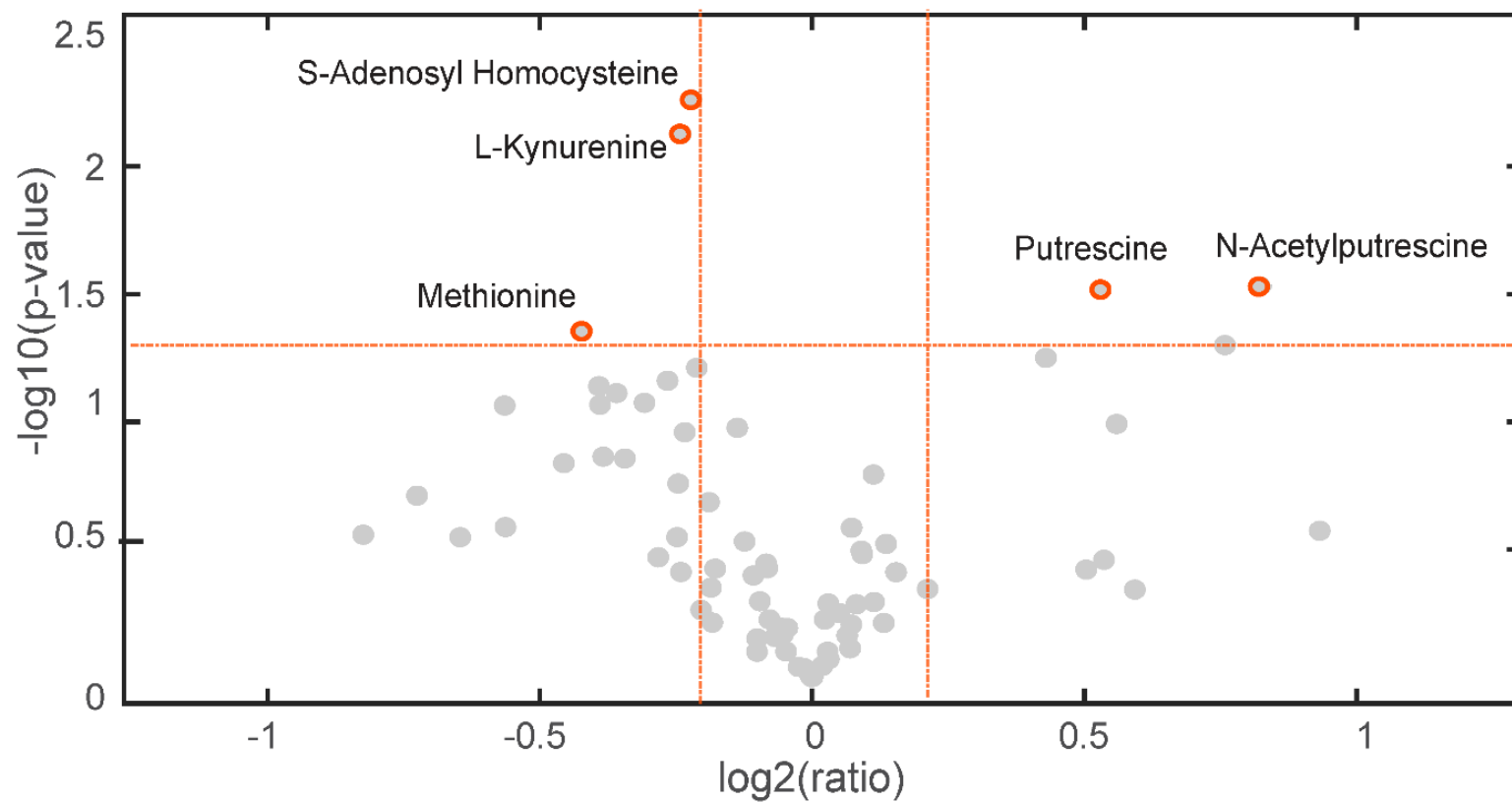
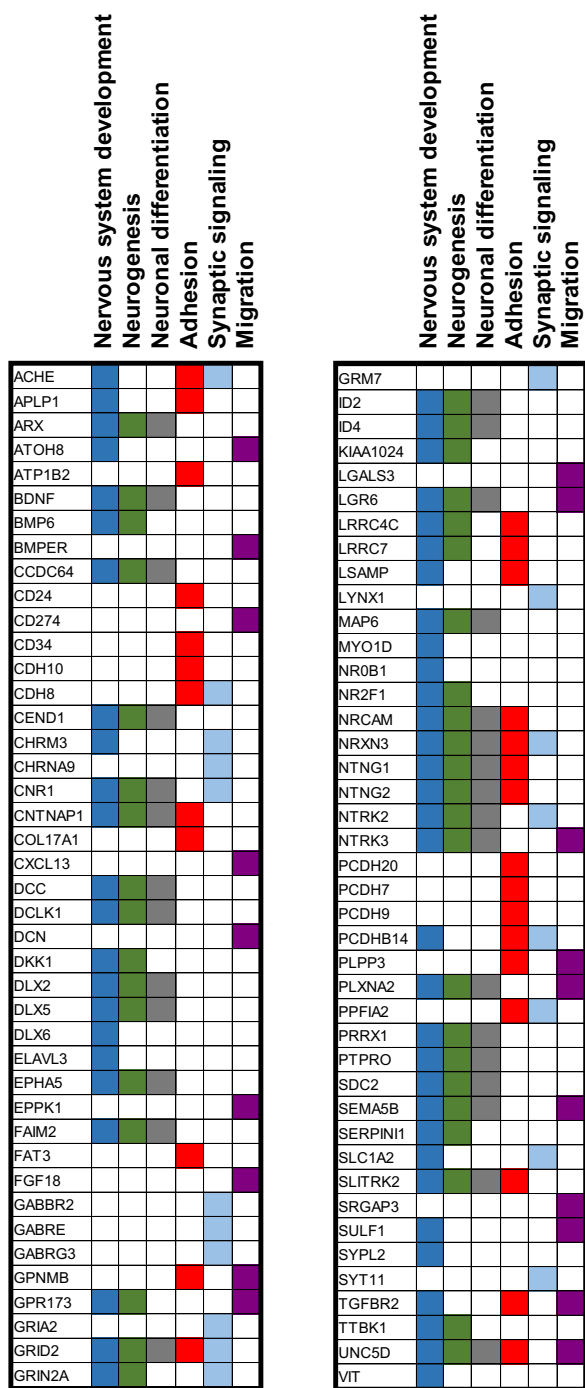


Fig. S4. Metabolomic analysis of the impact of SERBP1 knockdown. **A)** Principal Component Analysis (PCA) score plot performed on validated metabolites assigned using in-house small molecule compounds library, shows a distinct separation when comparing control to silenced SERBP1 groups. **B)** The loadings plot for principle component 2 shows the contribution of several metabolites to the variation between groups. **C)** The volcano plot highlights statistically significant metabolites which are shown in metabolomics data (Fig. 5). Statistical significance was set to $p=0.05$, fold change=0.4.

A



B

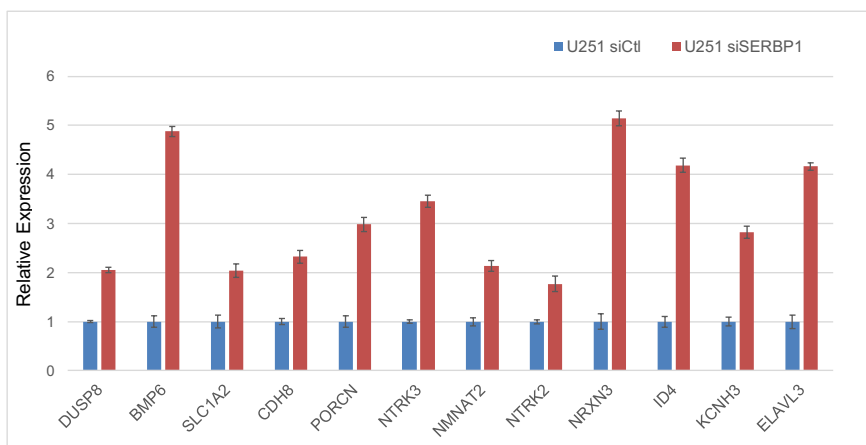


Fig. S5. Upregulated genes in SERBP1 knockdown cells. A) Main biological functions associated with genes upregulated in SERBP1 knockdown U251 cells. **B)** Validation of expression variation induced by SERBP1 knockdown by qRT-PCR.

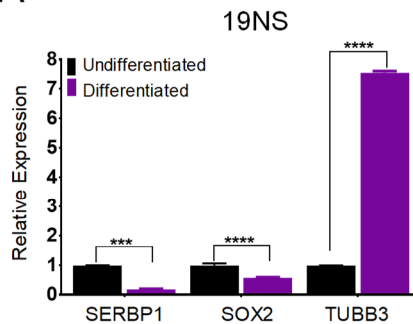
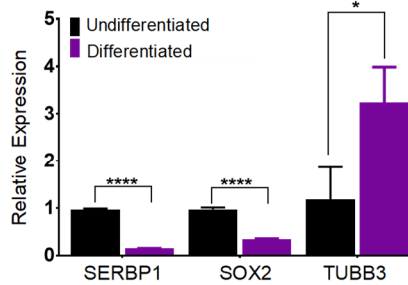
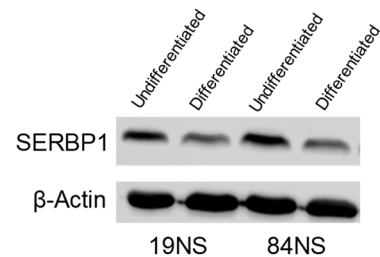
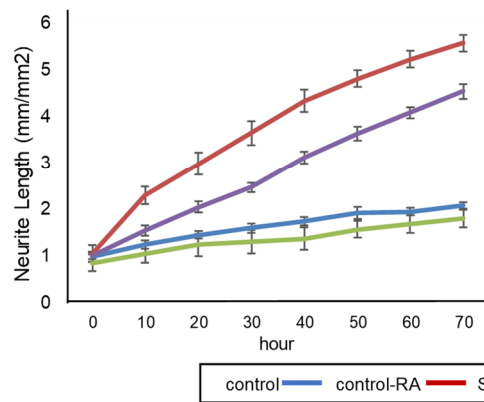
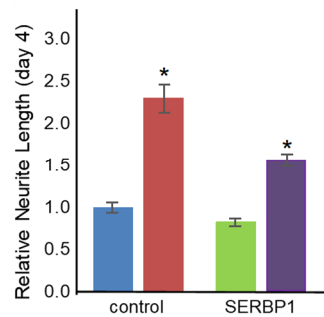
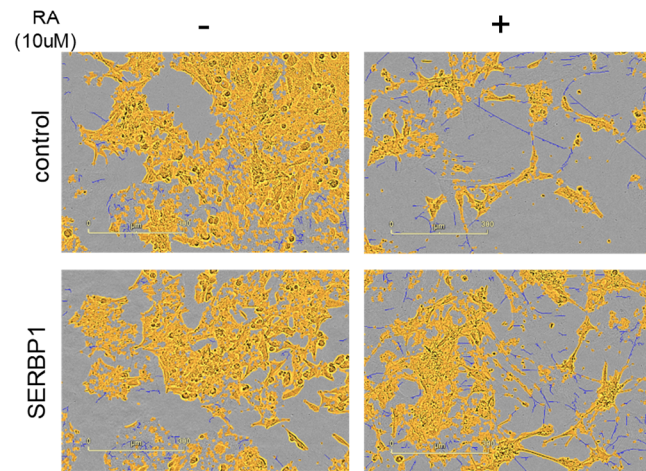
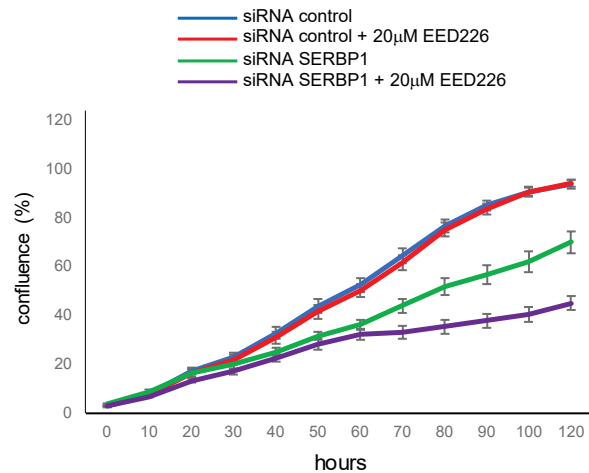
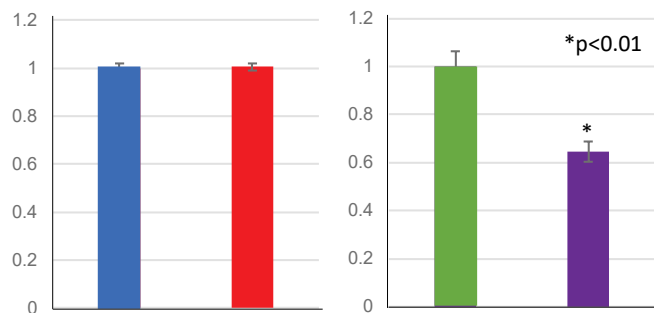
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Fig. S6. SERBP1 promotes “stemness”. **A-B)** GSC lines were transferred to differentiation media and SERBP1 expression levels in undifferentiated and differentiated cells were measured by (A) qRT-PCR and (B) Western blots. Sox2 and Tubb3 were used as markers of stem and differentiated cells, respectively. **C-E)** BE(2)C neuroblastoma cells were infected with control or SERBP1 expression lentivirus or control and treated with or without 10 μ M retinoic acid. IncuCyte was used to measure neurite outgrowth as a marker of neuronal differentiation. (C) Length of neurites over time. (D) Untreated and retinoic acid-treated cells at 70 hours post-treatment showing effects of SERBP1 on neuronal differentiation. (E) Aspects of control and SERBP1 OE cells at 0 and 70 hour post-treatment. Neurites are highlighted in blue.

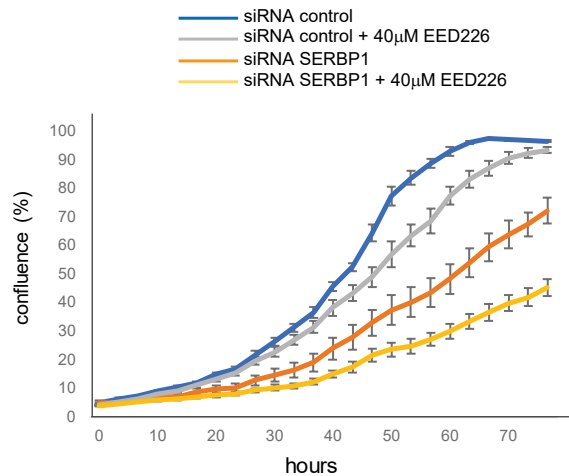
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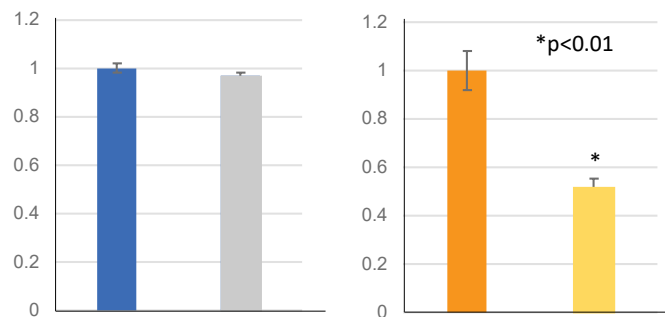


Fig. S7. SERBP1 knockdown increases sensitivity to PRC2 inhibitor, EED226. U251 cells were transfected with control or SERBP1 siRNA and treated with PRC2 inhibitor EED226 to determine if a reduction in SERBP1 expression increases sensitivity to the drug. **A)** Proliferation curve obtained with Incucyte; cells were treated with 20 μ M EED226. **B)** Graph displays difference in proliferation between control and treated cells at a single time point (120 h). **C)** Proliferation curve obtained with Incucyte, cells were treated with 40 μ M EED226. **D)** Differences in proliferation between control and treated cells at 70 hours.

Fig. S8. SERBP1's impact on the AKT pathway. A) KEGG AKT pathway; genes showing expression changes after SERBP1 knockdown in U251 cells are shown in purple. **B)** Western analysis showing that SERBP1 knockdown affected AKT and p-AKT levels in U251 and U343 cells. **C)** Association of AKT1 staining intensity scores levels with SERBP1 staining intensity scores using pairwise combinations in samples from all patients with GBM in the Shanghai Changzheng Hospital cohort (n=177).

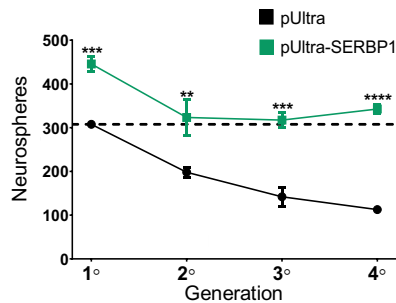
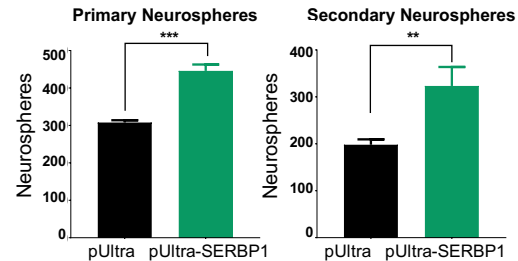
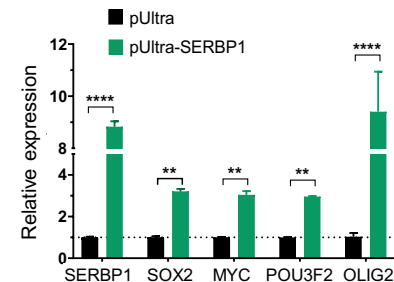
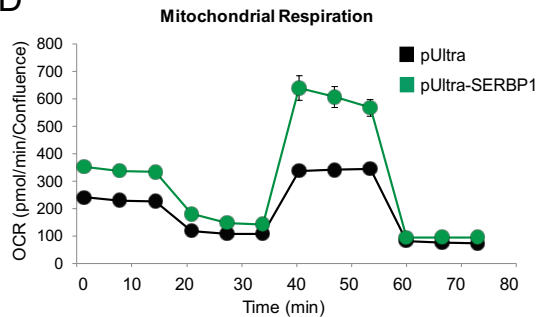
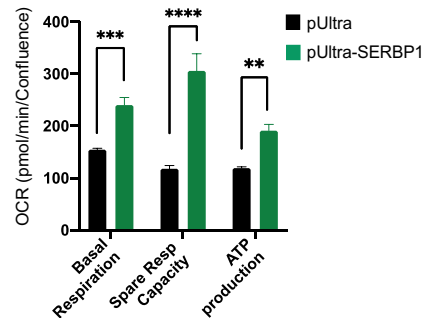
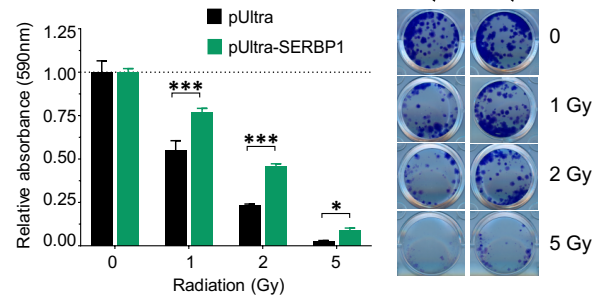
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Fig. S9. SERBP1 over expression promotes “glioma stem cell” phenotypes. **A)** U343 control (pUltra) and U343 SERBP1 OE lines were grown in stem cell media. The graph shows the number of neurospheres in passages 1-4. **B)** Graphs show the number of primary and secondary neurospheres of control and U343 SERBP1 OE lines. **C)** Expression of stem cell markers in neurospheres of control and SERBP1 OE lines. **D)** Graphs show mitochondria respiration in control and U343 SERBP1 OE cells measured over time with a Seahorse XFe96. **E)** Graphs show basal respiration, and ATP production, spare respiration capacity in control and U343 SERBP1 OE cells measured with a Seahorse XFe96. **F)** U343 control (pUltra) and U343 SERBP1 OE lines were exposed to increased amount of radiation and the impact on cell proliferation was measured via colony formation assay. Left: relative absorbance (590nm) as measurement of colony quantification. Right: aspect of plates. Data were analyzed with Student’s t-test and presented as mean $\pm$ standard deviation. Bonferroni-correction was used for multiple-comparisons. * = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$; **** $p \leq 0.0001$.