Differential DNA Methylation Patterns in Primary and Recurrent Glioblastoma : A Protumorigenic Role for the TEM8 Gene

Paramita Kundu
Indian Institute of Science
Ruchi Jain
Indian Institute of Science
Nandaki Nag Kanuri
National Institute of Mental Health and Neurosciences
A. Arivazhagan
National Institute of Mental Health and Neurosciences
Vani Santosh
National Institute of Mental Health and Neurosciences
Paturu Kondaiah (paturu@iisc.ac.in)
Indian Institute of Science

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Abstract

Introduction : Glioblastomas are infiltrative malignant brain tumors which mostly recur within a year's time, following surgical resection and chemo-radiation therapy. Studies on glioblastoma cells following radio-chemotherapies, have been demonstrated to induce trans-differentiation, cellular plasticity, activation of DNA damage response and stemness.

Methods : Utilizing the Illumina 450K BeadChip Arrays, we compared the DNA methylation profile of primary (first occurrence) tumors with recurrent (relapsed) disease, in order to delineate the contribution of epigenetic changes associated with therapy exposure, therapy resistance, and relapse of the disease.

Results : Our data revealed 1224 hypermethylated- and 526 hypomethylated-probes in recurrent glioblastomas compared to primary disease. We found differential methylation of solute carrier and ion channel genes, interleukin receptor/ligand genes, tumor-suppressor genes and genes associated with metastasis. We functionally characterized one such hypomethylated-upregulated gene, namely ANTXR1/TEM8 (Anthrax Toxin Receptor 1/Tumor Endothelial Marker 8), whose RNA was validated to be significantly upregulated in recurrent glioblastoma compared to primary tumors and confirmed by immunohistochemistry. Using overexpression and knockdown approaches, we show TEM8 induced proliferation, invasion, migration, and chemo-radioresistance in glioma cells. Mechanistically, we demonstrate β-catenin stabilization and activation of the β-catenin transcriptional program due to TEM8 over-expression. Inhibitor experiments revealed involvement of Src/PI3K/AKT/GSK3β/β-catenin pathway on TEM8 activation.

Conclusion : Overall, we report DNA methylation changes in recurrent GBM, and suggest the involvement of TEM8 in the progression of GBM.

Introduction

A major bottleneck in Glioblastoma (GBM, Grade IV glioma) treatment is the problem of recurrence. Recurrence in glioblastoma is inevitable [1] and invariably fatal. Owing to their highly infiltrative nature, it is virtually impossible to attain complete surgical debulking in GBM, in spite of gross total resection (GTR) of all contrast-enhancing areas on pre-operative MRIs. Tumor cells microscopically infiltrate beyond the contrast enhancing areas and to the contralateral hemisphere via the corpus callosum. Additionally, GBMs contain a peri-tumoral zone defined by tumor cell infiltration into the normal brain parenchyma. The extent of this zone is variable and therefore GTR is incapable of eliminating all residual tumor cells. Indeed, two-thirds of all recurrences occur locally, within 3 cm [2] at a median time of 59.5 weeks [3].

Standard protocol for glioblastoma management entails a combination of maximal safe resection, chemotherapy with Temozolomide, and fractionated ionizing radiation (IR) to the tumor bed [4]. While this regimen definitely improves survival compared to surgery alone, it is increasingly implicated that both IR and Temozolomide may result in generation of therapy resistance. For instance, therapeutic

concentrations of Temozolomide exposure led to increase in stem-cells and more diffuse tumors [5, 6], trans-differentiation of bulk tumor cells to endothelial cells showing vasculogenic mimicry [7], and induced HIF1α signaling [6] in glioma cells. Exposure to therapeutically relevant (2–3 Grays) doses of IR showed an increase in stem-cell content and accelerated DNA-damage response [8], a marked decrease in differentiation markers [9], subtype plasticity from proneural to mesenchymal phenotypes [10], and induced HIF1α signaling [6]. Much older studies [3] also show that radiographic implants at tumor beds accelerated the detection of new lesions in glioblastomas.

To explain the phenomena of inevitable recurrence in GBMs, it was proposed that infiltrated and residual tumor cells in the tumor-bed, termed Recurrence initiating stem-cells (RISCs), serve as seeds for the recurrent disease [11]. Given that GBM cells are inherently plastic, these cells are postulated to undergo adaptive responses to genotoxic therapy, resulting in therapy resistance commonly seen in recurrent GBMs.

IR and Temozolomide, apart from inducing genetic aberrations such as mutations [12], may also result in epigenetic changes such as DNA methylation or histone modifications [13, 14]. Additionally, the hypoxic nature of GBMs poses a dual challenge, it initiates proangiogenic signaling and dampens the IR response due to lack of O_2 radicals to ‘fix’ double-strand breaks. Interestingly, hypoxia also influences DNA methylation kinetics [15, 16]. It is plausible that in this hypoxic, genotoxic GBM microenvironment, residual tumor cells undergo DNA methylation changes which may translate into clinically more aggressive and therapy-refractory tumors. In this study, utilizing Illumina’s 450K Methylation Bead Array, we have compared the DNA methylome of glioblastoma (first occurrences/primary tumors) with recurrent tumors to understand if there exists DNA methylation changes that may impact the aggressiveness of recurrent tumors.

Utilizing the data generated from our cohort, we report that several genes are hyper- or hypomethylated in recurrent tumors, resulting in transcriptional changes. We have functionally characterized one such hypomethylated-upregulated gene in recurrent tumors, ANTXR1/TEM8, and delineated the signaling mechanism responsible for its protumorigenic actions in glioblastomas.

Materials And Methods

Patient characteristics.

Tumor tissues were obtained from patients availing surgical treatment at the National Institute of Mental Health and Neurosciences (NIMHANS), Bangalore, after obtaining written informed consent and approval by the Institutional Ethics Committee of NIMHANS, dated NIMHANS/3rd IEC (BS & NS Div.)/2016. The study was performed according to the Declaration of Helsinki guidelines. 24 fresh-frozen tissues were utilized in the study, consisting of 11 treatment-naïve (primary) GBMs and 13 relapsed (recurrent) GBMs, of which 10 samples belonged to patient-matched pairs (5 pairs). The inclusion criteria was treatment-naïve and relapsed Isocitrate Dehydrogenase-wildtype (IDH-wt) glioblastomas, while the exclusion criteria

was glioblastomas of IDH-mutant origin. Tumors were verified to be grade IV glioblastoma by neuropathologist, and wildtype-IDH status was confirmed by PCR for R132H.

Methodological details on cell lines, plasmids, DNA and protein isolation, methylation array analyses, immunoblotting, PCRs, immunohistochemistry, *in-vitro* assays, and detailed statistical analyses are mentioned in Supplementary Methods File.

Results

DNA methylation changes in primary and recurrent glioblastomas

A brief overview of methylation data analysis is depicted in Fig. 1A. Genomic DNA were subjected to Infinium 450K Methylation Bead Array for assessing differentially methylated regions (DMRs) with a Δβ-value cutoff of |0.2| and $p \leq 0.05$, revealing 1224 hypermethylated and 526 hypomethylated probes (Supplementary Table 2). We performed a gene-level annotation for these commonly regulated DMRs for probes localizing to 5'-Untranslated Regions, Transcription Start Site (TSS) 200 and TSS1500, and 1st Exon regions. The top 50 hypo/hypermethylated probes with their gene annotation is represented in Supplementary Table 3.

We observed several interesting candidates among our list of differentially regulated genes, categorized according to reported functions in Supplementary Figure S1A. Several genes such as CAV2, HTATIP2, DSC3 for which epigenetic inactivation in different cancers is reported [17–19], came up as hypermethylated genes in recurrent tumors. Genes associated with histological grade such as ZHX2, ASAP1 and genes with reported protumorigenic functions such as ANTRX1/TEM8, FAM46D, AZIN1, CNOT7 were hypomethylated. Several genes encoding solute carrier ion channels or potassium channels (KCNN3, KCNT1, KCNQ1) were dysregulated (hypo-/hypermethylated) in recurrent glioblastomas. This is interesting because involvement of ion channels in gliomagenesis is increasingly being noted [20], and this data may suggest that there could be unexplored contribution of ion channel dysregulation in tumor progression. The β-values for genes showing most dysregulated hypo- and hypermethylated probes, are shown in Supplementary Figure S1B.

We validated randomly selected hypomethylated genes in our patient cohort, and found them to be upregulated in recurrent glioblastomas (Fig. 1B). These include stem-cell biomarker ANTXR1/TEM8, transcriptional co-repressor CNOT7. We decided to functionally characterize the ANTXR1/TEM8 (Anthrax Toxin Receptor 1/Tumor Endothelial Marker 8) gene as it was hypomethylated at promoter-associated CpG island at TSS200, suggesting that hypomethylation could potentially regulate its transcription, apart from the fact that it is characterized as a stem-cell biomarker in triple negative breast cancer [21], and is pro-tumorigenic in osteosarcomas, colorectal and gastric cancer [22–24]. However, significance of its expression or role in glioblastoma is not yet reported.

ANTXR1/TEM8 expression in paired sequential glioblastomas.

As the ANTXR1/TEM8 gene was found to be hypomethylated (Supplementary Figure S2A) and transcriptionally upregulated in recurrent glioblastomas, we assessed whether TEM8 protein is overexpressed in paired recurrent glioblastomas compared to primary tumors. To this end, a separate retrospective cohort of 29 paired primary and recurrent glioblastomas was utilized to determine protein expression via immunohistochemistry (Fig. 1C,D). A semi-quantitative labeling index (LI) approach for 2 + staining revealed significant upregulation of protein expression (Wilcoxon paired signed-rank test, p-value 0.001) in recurrent tumors (mean LI_{rec} : 34.5 compared to mean LI_{pri} : 27, Supplementary Figure S2B). Additionally, we utilized a control brain tissue microarray to determine TEM8 protein expression in non-neoplastic brain tissues (Fig. 1E) and there was no detectable staining in control brains.

In glioblastomas, the role or consequence of TEM8 expression is unknown. Analysis of transcript expression in REMBRANDT database (Repository of Molecular Brain Neoplasia Data) revealed TEM8 upregulation in lower-grade gliomas (LGGs) and glioblastomas (Supplementary Fig. 2D). This correlated with upregulation in other datasets such as TCGA, Bredel Brain and Sun Brain *(*Supplementary Figure S2E*).* Survival analysis via Gliovis [25] revealed that higher TEM8 expression conferred poor survival in glioma (Fig. 1F).

TEM8 expression regulates proliferation, invasion, migration, chemoresistance and radiation resistance in glioblastoma cells.

Immunoblotting for TEM8 on glioblastoma tissue lysates revealed a predominant ~ 63 kDa band, suggesting that this is an important isoform expressed in glioblastomas (Supplementary Figure S2F). To understand the functional consequences of TEM8 expression in glioblastomas, we overexpressed a 3X Flag-hTEM8/pcDNA3.1 construct in U87-MG, A172 and LN229 cell lines (Supplementary Fig S2G). We observed increased proliferation in TEM8 overexpressing (OE) cells compared to vector controls (VC) as seen by Trypan-blue based viability assays (Fig. 2A) and BromodeoxyUridine uptake for 4 hours (Supplementary Figure S3A). We assessed differences in cell cycle progression by stimulating cells with 10% FBS for 12 hours after prolonged (72h) serum-starvation induced synchronization in U87 cells. At 12 hours of growth-stimulation, we observed 8.83% cells in S phase in OE cells compared to 4.58% in VC cells (Fig. 2B, Supplementary Figure S3C), suggesting that TEM8 OE cells had basally increased S phase cells in the absence of growth factors, which progressed faster on additional serum-stimulation, contributing to the proliferation advantage. We also observed increased invasion (Fig. 2C), migration (Fig. 2D, Supplementary Figure S3B) in OE cells; while gelatin zymography using conditioned media revealed expression of active MMP-2 (Fig. 2E) suggesting a possible role in the increased invasion. Using the MTT assay we explored if TEM8 enhanced chemo-resistance in cells towards four drugs viz. Temozolomide, Cisplatin, Etoposide, and 5-FluoroUracil (Fig. 2F). Relative 50% Inhibitory Concentration (IC_{50}) calculations by non-linear regression curve fitting revealed increased IC50 values for all the above drugs in TEM8 OE cells except in the case of 5-FluoroUracil, which showed a marginal increase

(Supplementary Figure S3E). Additionally, TEM8 OE cells generated bigger and about 7 fold increase in neurospheres than compared to control cells (Fig. 2G, Supplementary Figure S3D)

When TEM8 was stably knocked-down in U251 glioma cells with two shRNAs, pLKO.1-D6 and D9, we observed a growth lag in TEM8 knock down cells (Fig. 3A,B) compared to scrambled-shRNA transfected cells (SCR). We used a similar approach of 72h serum-starvation induced synchronization and release with 10% FBS-media. After 72h (i.e. at 0h timepoint) we observed more cells (76%) being arrested in G1 phase in D6 and D9 compared to 61% cells in SCR controls (Supplementary Figure S4A,B). To ascertain if parental U251 cells showed similarly low levels of G1-synchronization as SCR cells, we performed the same experiment with U251-parental cells which revealed that ~ 66% cells were G1 arrested (Supplementary Figure S4C). This suggested that TEM8 knockdown led to more efficient G1-arrest in D6 and D9 cells on prolonged serum-starvation. Further, at 12h of growth-stimulation, we observed 26.3% cells in G2/M phase in SCR, compared to 15 and16% in D6 and D9 cells respectively. The experiment was repeated thrice and is quantified in Fig. 3C.

We also demonstrate reduced invasion (Fig. 3D) and migration (Fig. 3F) in D6 and D9 cells when compared to SCR, and gelatin zymography with conditioned media from these cells revealed reduced MMP2 levels in knockdown cells compared to SCR controls (Supplementary Figure S4D). To assess whether TEM8 knockdown led to reduced chemo- and radioresistance, we performed MTT and clonogenic assays respectively. In response to Temozolomide and Cisplatin, we found relative IC_{50} concentrations were reduced in D6 and D9 cells compared to SCR (Fig. 3E, Supplementary Figure S4E). Additionally, clonogenic assays after γ-radiation exposure (0–10 Gray) revealed that TEM8 knockdown-D6 and D9 cells had attenuated radioresistance compared to SCR cells (Fig. 3G), as evidenced by lesser area-under-curve (SCR : 2.67, D6 : 1.98, D9 : 2.5) and surviving fraction (also see Supplementary Figure S4F).

The TEM8 gene regulates β-catenin signaling in glioblastoma cells.

Few reports suggests that ANTXR1 can interact with LRP6 [26, 27], and engaging ANTXR1 with the C5 fragment of Collagen VI or with Anthrax toxin component Protective Antigen, lead to induction of Wnt target genes such as Axin2 or Zeb1 in triple-negative breast cancer or endothelial cells [21, 28]. We therefore explored if TEM8 activation led to enhanced β-catenin signaling in glioblastoma cells. In U87 TEM8 OE cells, we observed induction of Wnt target genes Zeb1, Axin2, Nanog, Twist1 and α-SMA, although β-catenin or LRP6 transcript levels were unchanged (Fig. 4A). In three glioma cell lines U87, LN229 and A172, we found induction of Zeb1, Twist, Vimentin, Oct4 and α-SMA, all of which are induced by β-catenin (Fig. 4B). To determine if this association held true in patients, we assessed the co-expression of TEM8 with β-catenin target genes in glioblastoma patients from the Chinese Glioma Genome Atlas (CGGA) dataset in Gliovis. The expression of Zeb1, Axin1, Axin2, CyclinD1, c-myc and Vimentin were all positively correlated (Pearson's correlation co-efficient r^2: 0.33–0.77) to ANTXR1/TEM8 expression (Supplementary Figure S5A).

We observed no induction of canonical (Wnt1,Wnt2) or non-canonical (Wnt4,Wnt11) Wnt ligands (Supplementary Figure S5B) in OE cells, suggesting that β-catenin induction by TEM8 is likely Wnt-ligand independent. We further confirmed enhanced nuclear β-catenin accumulation in U87 and LN229 OE cells via nuclear-cytoplasmic fractionation (Fig. 4C). Next, we utilized a dual-luciferase assay to gauge nuclear β-catenin activity in overexpressing and knockdown cells. β-catenin responsive 7X TCF containing luciferase reporter construct, Super8X pTOPFlash, and its negative control with 7X mutated TCF, pFOPFlash, was transfected into these cells. We observed enhanced luciferase induction in TEM8 OE cells (Fig. 4D) and reduced induction in D6 and D9 knockdown cells compared to SCR cells (Fig. 4E). Additionally, we found reduced levels of β-catenin target proteins such as Zeb1, Twist1, α-SMA, Vimentin in U251-D6 and -D9 knockdown cells (Fig. 4F).

TEM8 regulation of β-catenin is via Src/PI3K/AKT/GSK3β cascade in glioblastoma cells.

As TEM8 could upregulate β-catenin and its effector genes, and as per our data Wnt ligands may not be involved, we explored the signaling pathway responsible for β-catenin translocation into the nucleus. We therefore determined the phosphorylation of protein kinases such as GSK3β, whose inactivating phosphorylation at Ser9 leads to β-catenin stabilization. Expectedly, p-GSK3β Ser 9 levels in TEM8 OE cells was upregulated (Fig. 5A) in three cell lines : U87, LN229 and A172, concomitant with upregulated β-catenin levels. GSK3β is regulated by varied upstream kinases, such as PI3 Kinase induced AKT [29, 30], Integrin-linked kinase [31, 32] or focal adhesion kinase [33] in glioblastomas. We observed elevated levels of phospho-AKT S473 and T308 in TEM8 OE cells. Additionally, we observed elevated phospho-ILK (S246) and phospho-FAK (Y397) levels in TEM8 OE cells.

In TEM8 knockdown cells, we observed a concomitant decrease in p-AKT (S473, T308), p-FAK (Y397) and p-GSK3β (S9) levels (Fig. 5B). Interestingly, the total levels of FAK were reduced in knockdown cells, suggesting destabilization of focal adhesions and reduction of focal adhesion-mediated survival signals in these cells.

Since multiple kinases were induced, we utilized a panel of small-molecule inhibitors to identify the exact signaling cascade involved in β-catenin stabilization in the presence of TEM8 : LY294002 (PI3K inhibitor), CPD-22 (ILK inhibitor), FAK inhibitor 1 (324877), RGD peptide (GRDGNP), PP2 (Src inhibitor) and PP3, a non-functional analog of PP2 was used as a negative control. We observed that phosphorylation on AKT (both T308 and S473) and GSK3β (S9) was abrogated in presence of PI3 kinase inhibitor LY294002 (Fig. 5C), suggesting PI3 kinase activation was responsible for induction of p-AKT in TEM8 OE cells. Additionally, we observed that PP2 (Src kinase family inhibitor) abrogated p-AKT and p-GSK3β induction as well, suggesting that Src-dependent activation of PI3 Kinase/AKT was responsible for inactivating phosphorylation of GSK3β and stabilization of β-catenin in TEM8 OE cells. We therefore surmise that a Src/PI3K/AKT/GSK3β/β-catenin pathway is activated in TEM8 expressing glioma cells (Fig. 5D) which leads to enhanced proliferation, invasion, migration, chemo- and radioresistance.

Discussion

In this study, we report that ANTXR1/TEM8 is a hypomethylated and upregulated gene in recurrent GBMs, and we demonstrate increased protein expression in a retrospective cohort of paired primary-recurrent glioblastomas. *In-vitro* studies in multiple glioma cell lines revealed that TEM8 expression conferred proliferation advantage, invasion, migration, chemoresistance and radioresistance in these cells. TEM8 knockout in multiple cell lines such as melanoma, lung, colon was demonstrated to lead to a drastic reduction in subcutaneous tumor growth [34]. To our knowledge, this is the first study demonstrating TEM8's protumorigenic actions in glioblastoma. We demonstrate that the 63 kDa TEM8 isoform (sv1, membrane-bound receptor) is predominantly expressed in glioblastoma patients and cell lines. It can potently regulate proliferation, invasion (perhaps via MMP2) and migration in glioma cells, which is clinically relevant as one of the major causes of glioblastoma relapse and the tumor's ability to invade far away from the tumor bed. TEM8 expression was also found to influence chemo- and radioresistance of glioma cell lines. We also demonstrate the involvement of β-catenin in this protumorigenic phenotype, and implicate a Src/PI3K/AKT/GSK3β/β-catenin pathway in TEM8 expressing glioblastoma cells. Similar regulation as this, i.e. Src/PI3K/AKT/GSK3β/β-catenin was found in transmembrane-bound IL15 receptor signaling in renal cancer [35], suggesting that this might be a non-canonical pathway for β-catenin stabilization. The stimulus for signaling through Src/PI3K/AKT/GSK3β/β-catenin by TEM8 is not known from our study. We speculate that TEM8 is activated by endotrophin (cleaved C5 domain of Collagen α3(VI)). This is because Col α3(VI) was demonstrated to bind the TEM8 extracellular domain [36] and is predominantly expressed in glioblastoma perivascular regions [37].

Interestingly, we found enhanced phosphorylation of Focal Adhesion Kinase (FAK) and Integrin-linked kinase (ILK) in TEM8 expressing glioma cells. It is reported that TEM8 mediates cellular adhesion [38], however the mechanism for this regulation is not clear. The finding from our study that FAK/ILK are activated by TEM8 may explain how TEM8 regulates cellular adhesion and positively impacts migration and invasion [24, 39].

We have restricted this study to IDH-wildtype GBMs as IDH mutation confers a distinct CpG-hypermethylated phenotype [40], which may complicate data interpretation. In the recent past, Klughammer and Keisel et al. [41] and Kraboth et al. [42] reported exploring DNA methylation patterns in sequential glioblastomas. Both these studies utilized formalin-fixed paraffin embedded (FFPE) patient tissues, and a modified reduced representation bisulfite sequencing (RRBS) approach to probe DNA methylation at CpG-rich regions. The Klughammer study reported methylation patterns related to immune cell infiltration and loss of methylation at Wnt signaling related gene promoters. This has parallels with our study wherein we see differential methylation of immune system related genes like Interleukin receptors and ligands, and also hypomethylation of the TEM8 gene leading to non-canonical stabilization of β-catenin levels in glioma cells. Although needs validation, one important aspect revealed from this study is that several different potassium-based voltage-gated ion-channels and solute-carrier channels were differentially methylated in recurrent compared to primary glioblastomas. Potassium channel dysregulation in cancer progression is well-documented [43], and in glioblastomas in particular, they are known to modulate cellular osmolarity and consequently cellular volume; essential for tumor cell invasion

in restricted spaces [44]. Hence, the contribution of such genes in glioblastoma progression needs further investigation.

One of the limitations of this study is the smaller cohort of paired primary and recurrent patient samples. This was due to scarce availability of fresh-frozen paired samples. To mitigate this limitation, we have utilized a separate cohort of retrospective paired FFPE tissues wherever necessary.

To conclude, this study highlights the importance of epigenetic changes that promotes aggressive growth and invasion of glioma cells. We demonstrate the importance of one such gene, TEM8 and there could be several other genes which may individually and/or collectively contribute to the recurrence phenomena. Our study is in concordance with a growing body of literature suggesting that ionizing radiation and chemotherapy with alkylating agents as Temozolomide on highly heterogenous and adaptive tumors as glioblastomas may bring about complex and unpredictable responses, therefore newer and more efficient treatment modalities are urgently required for glioblastoma treatment.

Declarations

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Data availability. The data is deposited in Gene Expression Omnibus with accession number GSE190953.

Conflicts of interest

None declared.

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Figures

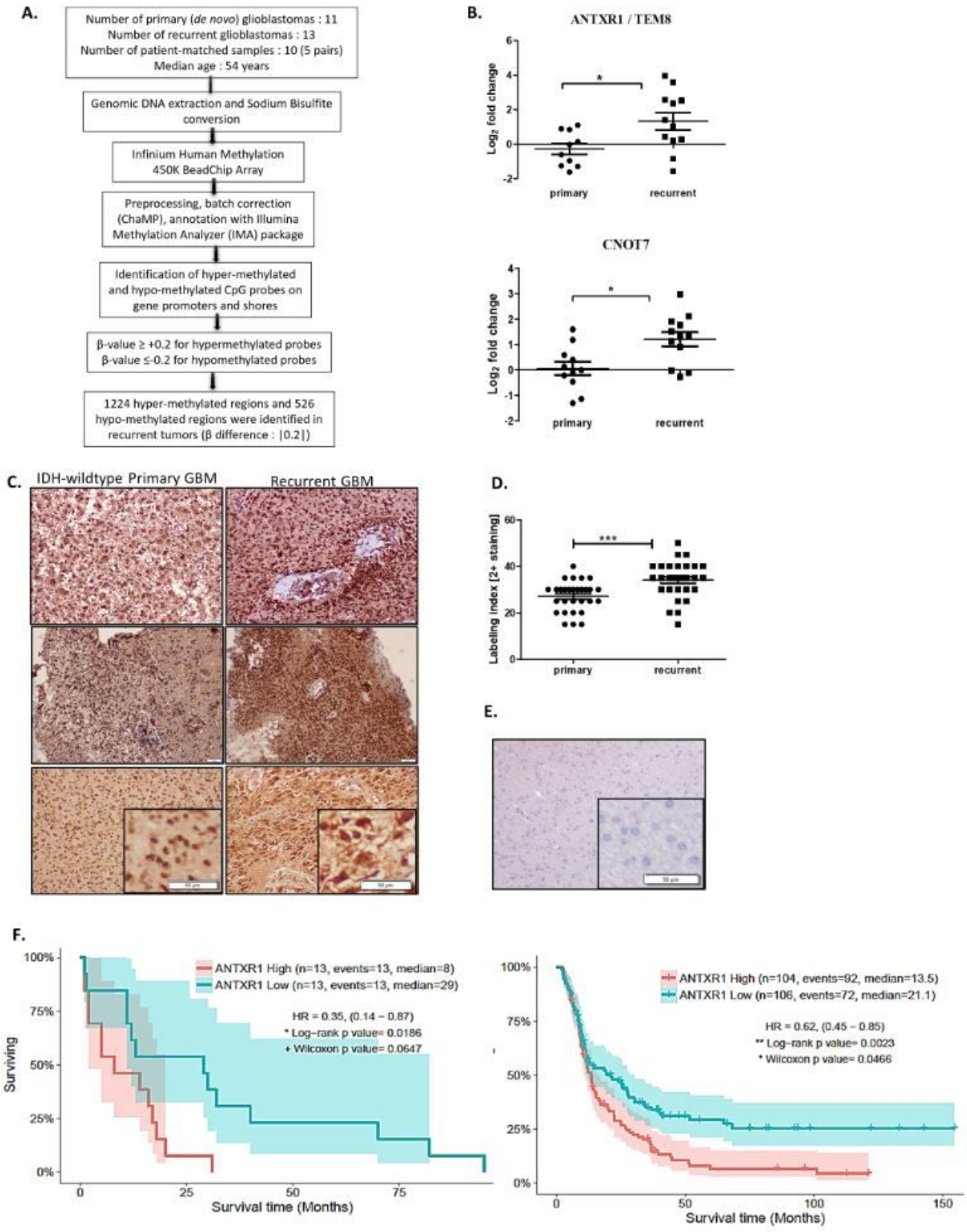
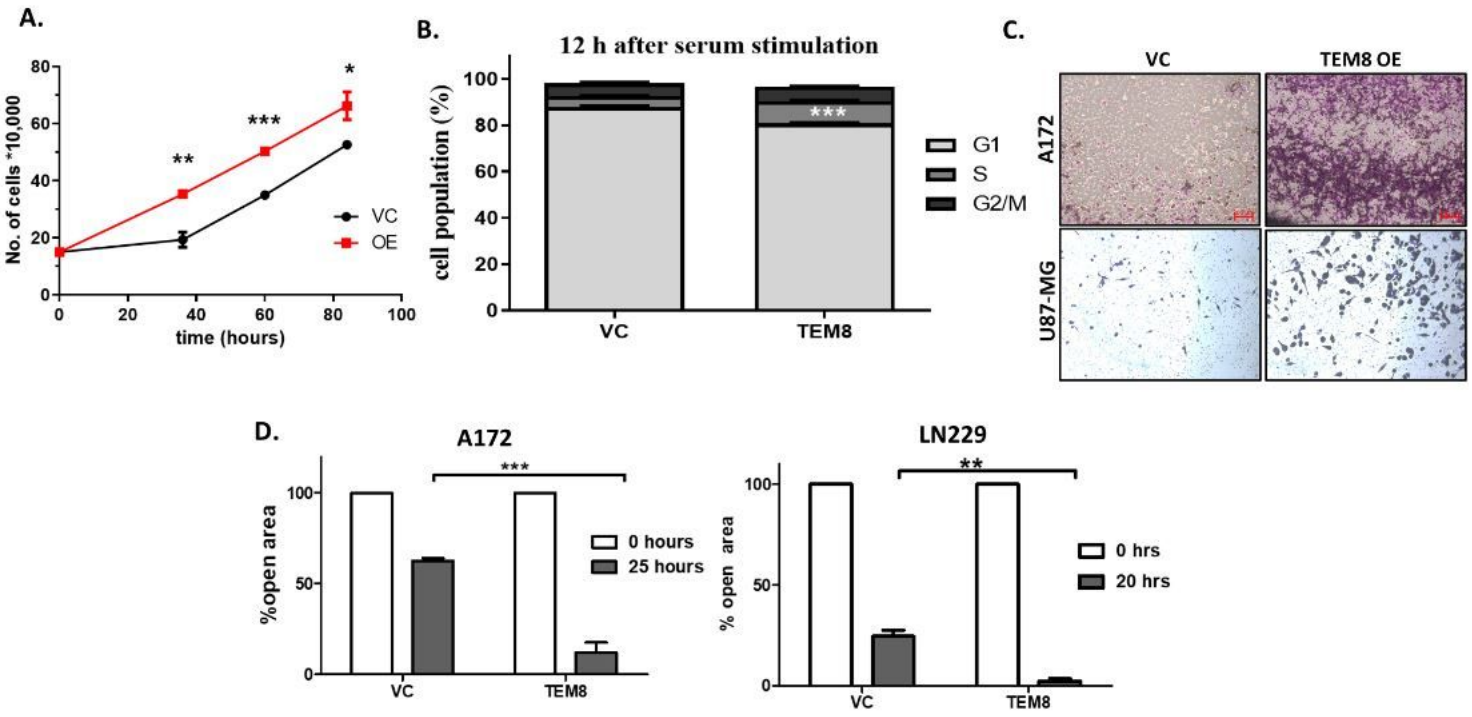
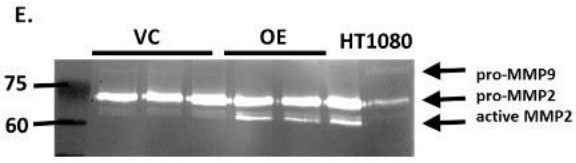
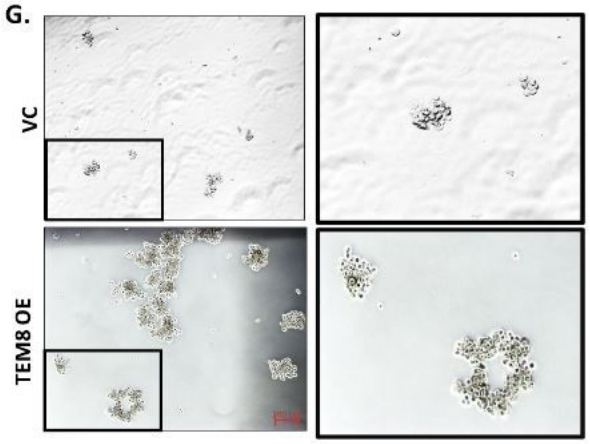


Figure 1



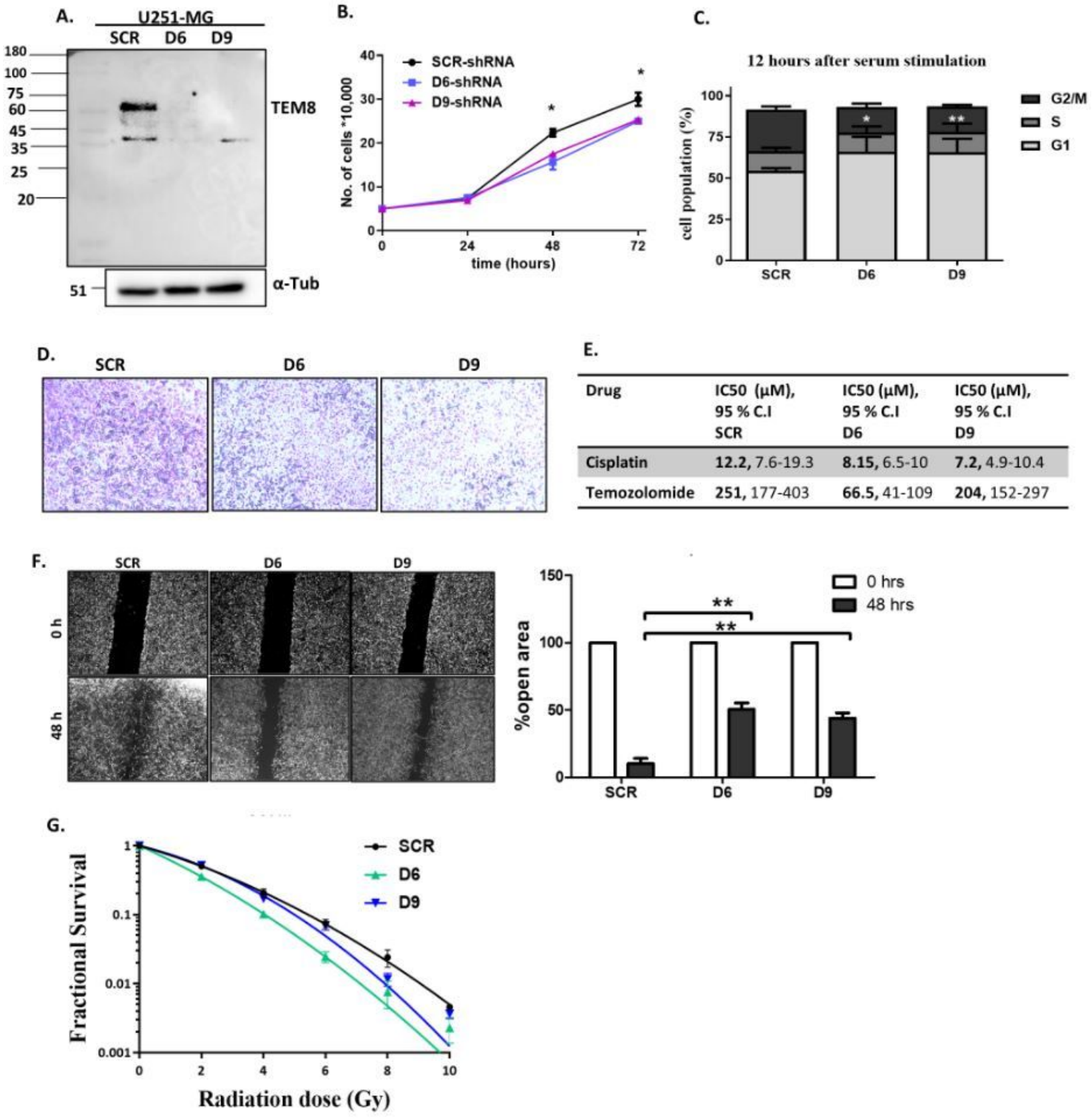




F.

U87-MG	VC	TEM8 OE
Drug	IC50 (μM), 95% C.I	IC50 (μM), 95% C.I
Cisplatin	**1.6,** 0.6-4.4	**2.31,** 0.9-6.0
5-FluoroUracil	**15.5,** 8.6-28	**18,** 7.9-41
Etoposide	**0.3,** 0.04-2.15	**0.63,** 0.05-7.0
Temozolomide	**63,** 51-77.5	**94,** 74-119

Figure 2



Drug	IC50 (μM), 95 % C.I SCR	IC50 (μM), 95 % C.I D6	IC50 (μM), 95 % C.I D9
Cisplatin	**12.2,** 7.6-19.3	**8.15,** 6.5-10	**7.2,** 4.9-10.4
Temozolomide	**251,** 177-403	**66.5,** 41-109	**204,** 152-297

Figure 3

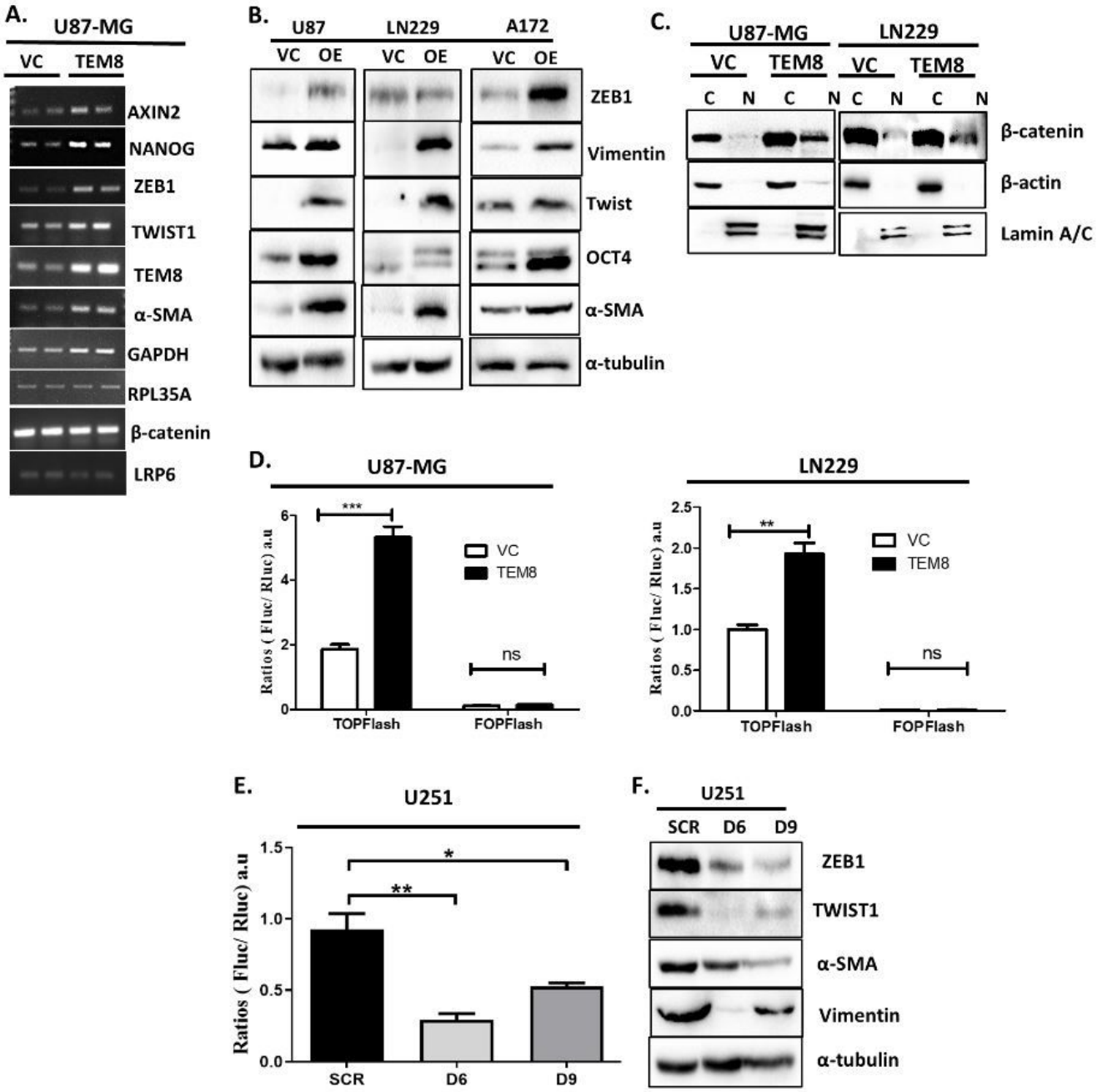

A.
U87-MG
VC
TEM8
AXIN2
NANOG
ZEB1
TWIST1
TEM8
α-SMA
GAPDH
RPL35A
β-catenin
LRP6
B.
U87
LN229
A172
VC OE VC OE VC OE
ZEB1
Vimentin
Twist
OCT4
α-SMA
α-tubulin
C.
U87-MG
LN229
VC
TEM8
VC
TEM8
C N C N C N C N
β-catenin
β-actin
Lamin A/C
D.
U87-MG
Ratios (Fluc/ Rluc) a.u
***
VC
TEM8
ns
TOPFlash
FOPFlash
LN229
Ratios (Fluc/ Rluc) a.u
**
VC
TEM8
ns
TOPFlash
FOPFlash
E.
U251
Ratios (Fluc/ Rluc) a.u
*
**
SCR
D6
D9
F.
U251
SCR D6 D9
ZEB1
TWIST1
α-SMA
Vimentin
α-tubulin

Figure 4

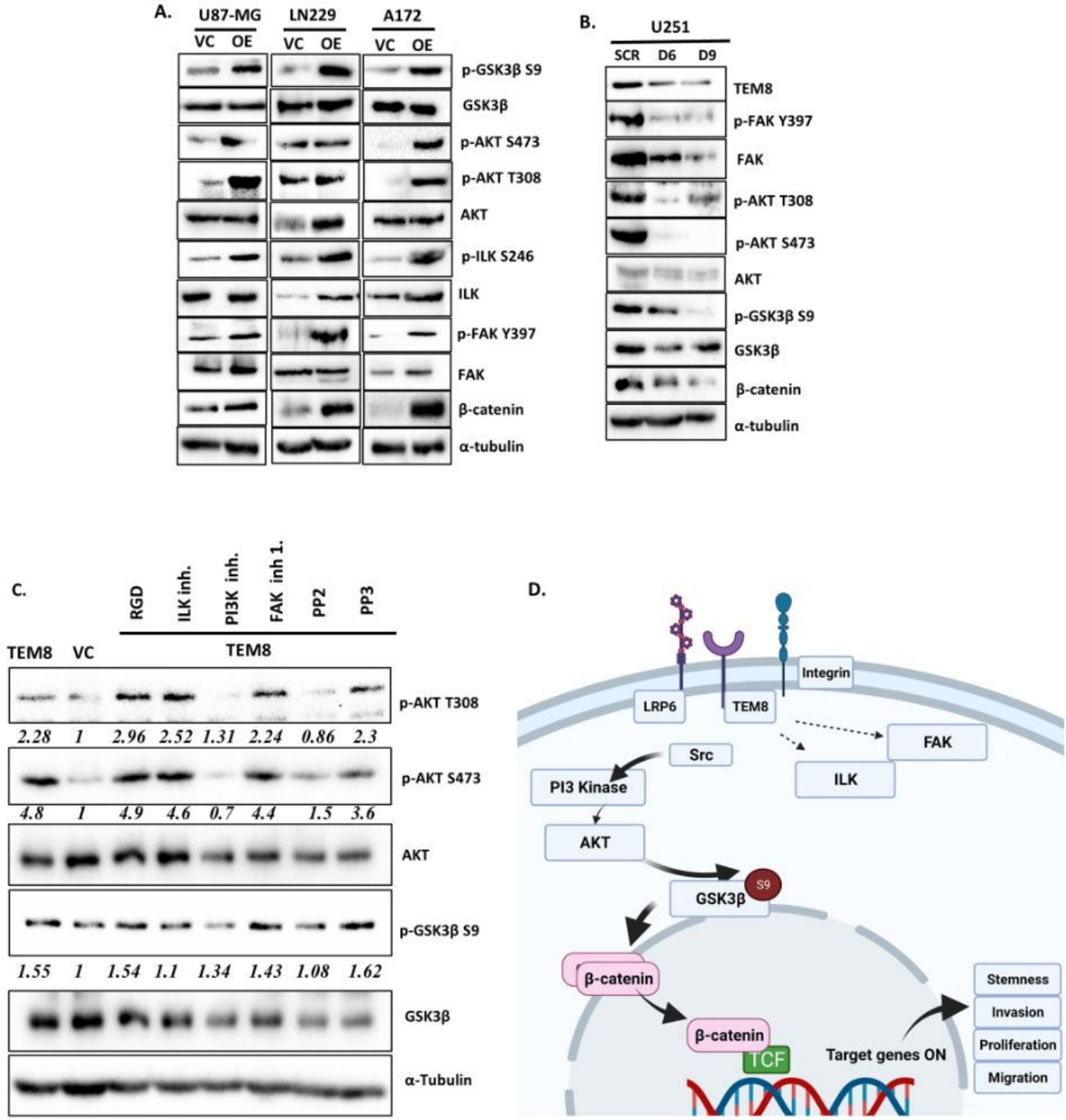


Figure 5

Supplementary Files

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- SupplementaryTable2.xlsx
- SupplementaryTable3.xlsx