PARP Inhibitor Counteracts Temozolomide Resistance in Glioblastoma Multiforme

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CONFLICTS OF INTEREST

The authors declare no potential conflicts of interest.

ABSTRACT

Background:
Glioblastoma multiforme (GBM) is the most common malignant primary brain tumour in adults and is invariably associated with poor prognosis. Resistance to Temozolomide (TMZ), the standard chemotherapeutic agent, remains a major clinical challenge, particularly due to DNA mismatch repair (MMR) deficiencies. The aim of this study was to determine whether combining TMZ with the poly(ADP-ribose) polymerase inhibitor Olaparib (OLA) could overcome TMZ resistance in GBM.

Methods:
We conducted *in vitro* experiments using U251 cell-line, including a TMZ-resistant derivative, and primary GBM cultures derived from patient tumours. A CRISPR/Cas9 knockout screen was employed to identify genes involved in TMZ resistance. Cell viability, proliferation, and morphology were assessed following treatment with TMZ, OLA, or their combination.

Results:
The CRISPR screen identified inactivation of MMR pathway genes as key mediators of TMZ resistance. Co-treatment with OLA and TMZ demonstrated synergistic cytotoxicity in both parental and TMZ-resistant U251 cells, as well as in primary GBM cultures at diagnosis or relapse. Notably, OLA restored sensitivity to TMZ in MMR-deficient contexts and in tumours expressing O6-methylguanine-DNA-methyltransferase (MGMT). The combination treatment induced persistent DNA damage, cell cycle disruption, and cell death.

Conclusions:
These findings provide strong preclinical evidence that combining TMZ with OLA can effectively overcome key mechanisms of TMZ resistance in GBM. This approach offers a promising therapeutic strategy warranting further clinical investigation.

KEY WORDS
Glioblastoma, Temozolomide, CRISPR screen, MMR, Olaparib.

48 **KEY POINTS**

49 - PARP inhibitor Olaparib restores temozolomide sensitivity in resistant GBM cells
50 - Combination therapy overcomes resistance driven by MMR deficiency or MGMT
51 expression
52 - Dual treatment induces persistent DNA damage and apoptosis in glioblastoma primary
53 cultures

54

55 **IMPORTANCE OF THE STUDY**

56 Temozolomide (TMZ) resistance remains a major therapeutic obstacle in glioblastoma (GBM),
57 often driven by MMR deficiency or MGMT expression. While poly(ADP-ribose) polymerase
58 (PARP) inhibitors have shown potential in other cancers, their role in overcoming TMZ
59 resistance in GBM has remained unclear. In this study, a CRISPR screen identified MMR
60 deficiency as a key driver of TMZ resistance. We further demonstrate that co-treatment with
61 the PARP inhibitor Olaparib (OLA) restores TMZ sensitivity in both MMR-deficient and MGMT-
62 expressing GBM cells and patient-derived cultures. These findings provide strong preclinical
63 evidence supporting PARP inhibition as a promising therapeutic strategy to overcome
64 chemoresistance in GBM and justify further clinical investigation.

65 **INTRODUCTION**

66 Glioblastoma, a WHO classification 4 grade tumour, is the most common and most
67 aggressive malignant brain tumour in adults[1,2]. These highly proliferative gliomas mainly arise
68 in the supratentorial brain[3] and are characterized histologically by high cellularity, high mitotic
69 activity, vascular proliferation, and necrosis[4]. GBM displays high inter and intra tumoral
70 heterogeneity, which has been suggested to contribute to resistance to treatments [5]. Indeed,
71 tumour cells may differ in molecular, phenotypic, and functional characteristics, potentially
72 enabling less sensitive tumour cell subpopulations to drive resistance to cancer drugs [6].

73 The standard of care for GBM involves surgical resection of the tumour if feasible,
74 followed by a post-operative combination of concomitant radiotherapy and chemotherapy
75 with Temozolomide (TMZ), known as the Stupp protocol[7]. Complete surgical resection is rarely
76 achievable due to the GBM's diffuse and infiltrative nature, as well as the risks of damaging
77 surrounding brain tissue. This makes mandatory the use of complementary treatments[3]. TMZ
78 is a monofunctional alkylating agent which induces cytotoxic lesions, including O^6-
79 methylguanine (O^6MeG), N^7-methylguanine (N^7MeG) and N3-methyladenine (N3MeA)
80 adducts [8]. The DNA mismatch repair pathway (MMR), comprised of several proteins (MSH2,
81 MSH3, MSH6, MLH1 and PMS2), is essential to mediate cytotoxicity of TMZ: mispairing of
82 O6MeG with another thymine leads to repetitive rounds of MMR, known as "futile cycling",
83 ultimately inducing double-strand breaks and triggering p53-dependent cell cycle arrest and
84 apoptosis[9,10]. These lesions are repaired by the O6-Methylguanine-DNA-Methyl Transferase
85 (MGMT) enzyme, expressed by approximately 40% GBM tumours, leading to resistance to
86 TMZ [11–13]. However, in some GBM, the MGMT enzyme is silenced by promoter methylation,
87 resulting in persistent O^6MeG lesions and potential cytotoxicity. On the other hand, N^7MeG
88 and N^3MeA adducts, which account for approximately 90% of methylation events induced by
89 TMZ, are usually repaired by the base-excision repair (BER) pathway and contribute minimally
90 to the overall cytotoxicity of TMZ[8,9]. The action of the BER pathway is coordinated by the
91 Poly(ADP-Ribose) Polymerase (PARP) enzymes, which bind to the damaged DNA to recruit BER
92 components[14,15].

93

94 Despite aggressive treatment of GBM, including surgery and adjuvant radio-
95 chemotherapy as discussed above, prognosis remains dismal with a median overall survival of
96 14-15 months and a five-year survival rate of only 5% [16,17]. Almost all TMZ-sensitive GBM

97 become drug resistant over time, resulting in tumour recurrence and clinical progression [18].
98 Unfortunately, all the treatments used in tumour recurrence such as chemotherapy or
99 targeted therapy failed to show significant clinical efficacy and survival improvement [3].
100 Development of new therapeutic modalities therefore seems essential to improve care for
101 patients with GBM.

102 One mechanism of acquired resistance to TMZ is the gain of mutations that inactivate
103 the MMR pathway, thus inducing tolerance to DNA mispairing via O^6MeG adducts, leading to
104 cell survival [19–21]. A way to overcome alkylating chemotherapy failure could be a targeting of
105 the BER pathway, the inactivation of which could increase cytotoxicity of N^7MeG and N^3MeA
106 adducts, otherwise repaired in normal cells and tumour cells with an intact BER pathway [8,22].
107 The use of small molecule inhibitors of the PARP enzymes has shown promising results in
108 preclinical models [18,23–25], through a potentiation of TMZ therapy by BER disrupting and other
109 mechanisms such as destabilization of replication forks [26]. Furthermore, it has been suggested
110 that PARP inhibitors could also be effective in cancer cells expressing MGMT (without MGMT
111 promoter methylation) [27], due to the necessary PARylation of MGMT by PARP for repairing
112 TMZ-induced O^6MeG adducts [28].

113 PARP1-inhibitors (PARP1i) have been marketed with specific indications with respect
114 to homologous recombination repair (HR) status, which is considered a predictive biomarker
115 in response to PARP1i[29]. In cells which have homologous repair deficiency (HRD), non-
116 homologous end joining (NHEJ) would result from double strand break (DSB) formation, but
117 with respect to homologous repair (HR), considered "error-free", NHEJ affords DSBs repair
118 with almost no consequence for sequence homology. The error-prone NHEJ promotes
119 accumulation of DNA damage, and its activity is a major driver for PARP1i synthetic lethality
120 in HR-defective cells[30].

121 We present the results of *in vitro* experiments studying the use of the
122 inhibitor of the enzyme Poly(ADP-ribose) polymerase 1 (PARP1), Olaparib (OLA), in
123 combination with TMZ in the treatment of GBM tumour cells. Experiments were done on U251
124 cell lines, with no prior exposure to treatment, or on U251 resistant cell line due to prolonged
125 exposure to TMZ. Then, experiments were replicated on several patient-derived cells at
126 diagnosis or relapse.

127

128 **MATERIALS AND METHODS**

129 *Materials:* Temozolomide (LSC711) and Olaparib were obtained from Interchim (Montluçon,
130 France), all other drugs used in this study were from Sigma (Saint Louis, MO). All cell culture
131 products were obtained from Life Technologies (Carlsbad, CA).
132
133 *Cell culture:* After informed consent, tumour samples classified as Glioblastoma type 4 (GBM)
134 based on WHO criteria[31], were obtained from patients undergoing surgical intervention at the
135 "CHU de Nantes" and the "Tumorothèque IRCNA". Within 4 h after surgical removal, GBM
136 cells were recuperated after mechanical dissociation as described in *Brocard et al.*[32]. All
137 procedures involving human participants were in accordance with the ethical standards of the
138 ethic national research committee and with the 1964 Helsinki declaration and its later
139 amendments or comparable ethical standards. GBM cells were cultured in defined medium
140 (DMEM/F12 supplemented with 2 mM L-glutamine, N2 and B27 supplement, 2 µg/ml heparin,
141 20 ng/ml EGF, 40 ng/ml bFGF, 100 U/ml penicillin and 100 μg/ml streptomycin). All the
142 experiments with primary GBM cells were performed at early passages. Cells were regularly
143 analysed for mycoplasma.
144 U251 cells were cultured in DMEM (Gibco, ref. 21969035) enriched with 10% FCS with
145 100 U/ml penicillin, 100 µg/ml streptomycin and 2 mM L-glutamine. U251 cell line was
146 certified by Eurofins Genomics (Ebersberg, Germany). Cells were cultured at 37°C with 5% CO_2
147 and 95% humidity. The cell medium was replaced every 2-3 days with fresh medium and the
148 cells were reseeded in a new dish when the cell layer was approximately 80% confluent.
149
150 *CRISPR KO screen (cell culture)*. We generated Cas9-expressing U251 clones by lentiviral
151 transduction (Addgene, ref. 52962-LV) and blasticidine selection. We checked Cas9 expression
152 by Western-Blot and selected the clone with the most efficient Cas9 nuclease activity using a
153 Cas9 activity reporter (Addgene, ref. 67983 and ref. 67984). The Cas9-expressing U251 clone
154 was expanded, transduced with the Brunello lentiviral library at MOI 0.3 (gift from David Root
155 and John Doench; Addgene, ref. 73178-LV)[33] and selected with puromycin. After expansion to
156 3 x 10^8 cells: 10^8 cells were harvested and frozen down, 10^8 cells were treated with 50 µM
157 TMZ and 10^8 cells were treated with DMSO (vehicle condition). For the TMZ-treated cells, the
158 medium was changed every 3 days with fresh TMZ. A minimum of 10^8 DMSO-treated cells
159 were replated every 3 days in fresh medium. The cells were cultured during 18 days, until the

160 TMZ-treated cells reached the sub-confluence. At this point, DMSO-treated cells and TMZ-
161 treated cells were harvested and frozen down.
162
163 *CRISPR KO screen (library preparation)*. Genomic DNA was purified from cell pellets using
164 Qiagen Blood & Cell Culture DNA Maxi Kit (ref. 13362). SgRNA sequences were amplified by
165 PCR on genomic DNA using Q5 (NEB, ref. M0492S) and P5/P7 primers as recommended by
166 Doench JG *et al.*[33]. Illumina libraries were quality-controlled by Agilent TapeStation, quantified
167 by qPCR and submitted for HiSeq sequencing at the genomics core facility GenoA in Nantes,
168 France.
169
170 *PARP1 inhibitor treatment.* The PARP1 inhibitor, Olaparib (OLA) was dissolved in DMSO to give
171 a stock concentration of 10 mM. In all experiments, the final working concentration of OLA
172 was 1 µM (Supplementary Figure S8). The medium in the dishes was replaced every 2-3 days.
173 DMSO was used as a vehicle control in all experiments (**Figure S1**).
174
175 *Transfection of U251 cells with siRNA.* Cells were plated at 10^5 cells/well in 6-well plates and
176 24h later transfected with 600 ng/ml siLuc, siMSH2, siMSH6 or siPMS2 (MISSION® esiRNA,
177 Sigma-Aldrich, Saint-Louis, MI) using the using the Lipofectamine RNAiMax transfection
178 reagent (Thermo-Fisher Scientific, Waltham, MA) for 48h and then used in experiments
179 (**Figure S2**).
180
181 *Microscopy:* Images of the cells were taken using an Axio Observer optical microscope (Zeiss®,
182 Iéna, Germany) at 10x magnification. The images were then processed using ImageJ2
183 and Adobe Photoshop 8.0 software.
184
185 *Statistical analyses:* Statistical analyzes were performed using one-way and two-way ANOVA
186 tests with Prism 8 software. A p-value less than 0.05 is considered significant. The p-value of
187 significant values is indicated by * < 0.05, ** < 0.01 and *** < 0.001.
188
189 **RESULTS**
190 **Discovery of TMZ-resistance genes by CRISPR screen knock-out.**

191 Based on our observations from Rabé *et al*.[34,35], we first conducted a CRISPR screen
192 knock-out to identify genes involved in TMZ resistance. We generated a Cas9-expressing U251
193 clone and transduced it with the Brunello lentiviral library (gift from David Root and John
194 Doench[33]). The cell library was expanded and treated with vehicle or with a therapeutic
195 concentration of TMZ. About 95% of the cells died upon this TMZ insult. We harvested the
196 cells once the surviving cells reached confluence after 18 days. After library preparation and
197 sequencing of the sgRNA carried by the vehicle- and TMZ-treated cells, we analyzed the data
198 using MAGeCKFlute [36] and found gene knock-outs significantly enriched in the TMZ-treatment
199 condition, suggesting that these gene invalidations would confer resistance against TMZ.
200 Several genes from the DNA MMR pathway were found: EXO1, MLH1, MSH2, MSH6, PMS1
201 and PMS2. We also found 3 other invalidations: BCL11A, CLHC1 and GPR75 involved
202 respectively in neurone maturation, mitochondria and neurotransmission (**Figure 1A**). We did
203 not find any genes whilst KO confers a higher sensitivity to the TMZ treatment.
204 We decided to proceed by validating the genes of interest by knock-down in U251 cells.
205 We performed siRNA against the MMR members MSH2, MSH6, PMS2 and against BCL11A,
206 CLHC1 and GPR75, using siRNA against Luciferase as control (**Figure 1B**). Only the knock-down
207 of the MMR members MSH2, MSH6 and PMS2 conferred resistance against TMZ. This result
208 suggests that, in the context of loss-of-function mutations, MMR deficiency is the main driver
209 of TMZ resistance in GBM in recurrences.
210
211 **Combination of the PARPi OLA and TMZ counteracts TMZ-resistance in U251 cells.**
212 We tested the hypothesis that a PARPi could counteract TMZ resistance driven by MMR
213 deficiency.
214 We found that 1 uM OLA had no effect on U251 cells in terms of cell proliferation, viability
215 and morphology even after 16 days culture. In contrast, TMZ induced about a 90% cell death
216 until day 12, after which the cells resumed proliferation as shown in Rabé *et al*.[34], while the
217 cells treated with OLA plus TMZ showed a marked decrease in cell number and there was no
218 increase in cell number even after 16 days (**Figure 2A-B**).
219 We have shown that prolonged exposure to TMZ selects for resistant U251 by expressing
220 MGMT *de novo* through promoter demethylation. These cells which we call U251-R cells were
221 also treated with OLA and TMZ, and as shown in **Figure 2C-D,** we were able to induce greater

222 cell death with OLA plus TMZ than with TMZ alone. OLA seems to have a synergistic effect
223 when combined with TMZ.
224 We then questioned whether this was still true in a MMR-deficient context. We knocked-
225 down MSH6 in U251 cells and treated the cells with control, OLA-only, TMZ-only or OLA plus
226 TMZ (**Figure 3A-B**). We confirmed that MSH6 knock-down confers a strong resistance against
227 TMZ. However, when combined to the TMZ, the OLA strongly reduces cell growth in MSH6-
228 depleted cells. These results suggest that TMZ plus OLA have a synergistic effect that is able
229 to counteract the TMZ-resistance induced by MSH6 deficiency.
230
231 **The combination of the PARPi OLA and TMZ counteracts TMZ-resistance in GBM primary**
232 **cultures.**
233 We then tested the combination of TMZ plus OLA on glioblastoma primary cultures. We
234 observed a synergistic effect of TMZ plus OLA in several patient cell cultures in GBM3, GBMG5,
235 GBM69, GBM81, GBM120 (primary cultures at diagnosis), and GBMA1 and GBM96 (primary
236 cultures at relapse) (**Figure 4A-B** and **Supplementary Figures S3-9**). In many cases after 17
237 days, the TMZ plus OLA treatment was able to eradicate all cells while the TMZ-only failed to
238 do so. For the GBM86 patient, we cultured the GBM surgical excisions at diagnosis and at
239 relapse, and treated them with TMZ-only, OLA-only or TMZ plus OLA (**Figure 4C-D**). Again, the
240 combination of TMZ plus OLA had a stronger repressive effect on cell growth than TMZ alone
241 both at diagnosis and relapse. It should be noted that the combination TMZ + OLA is also
242 efficient in the context of MGMT expression like in U251-R, GBM3, GBMG5, GBM81, GBM96
243 and GBM120.
244
245 **DISCUSSION**
246
247 Despite the use of TMZ as the standard chemotherapeutic agent for glioblastoma
248 multiforme (GBM), resistance to this alkylating agent remains a major challenge, particularly
249 at recurrence. In this study, we employed a genome-wide CRISPR/Cas9 knockout screen and
250 identified loss of function in key mismatch repair (MMR) genes—*MSH2/6, MLH1*, *PMS1/2*—
251 as the primary mechanism of resistance to TMZ. Notably, we show that co-treatment with the
252 PARP inhibitor OLA can overcome this resistance not only in a TMZ-resistant GBM cell line but
253 also in patient-derived GBM primary cultures. These findings unveil a therapeutically

254 actionable vulnerability in TMZ-resistant GBM and support a new rationale for combining
255 alkylating agents with PARP inhibitors in clinically relevant contexts.

256 Our CRISPR screen uncovered MMR gene disruption as the predominant hit conferring
257 TMZ resistance. This finding corroborates prior work suggesting that functional MMR is
258 essential for the cytotoxicity of TMZ-induced O^6-methylguanine lesions[37]. During DNA
259 replication, DNA polymerase mispairs O^6-methylguanine with thymine, prompting futile
260 MMR cycles removing mismatched thymine and ultimately double-strand breaks and
261 apoptosis[38]. Loss of MMR function abrogates this process, allowing cells to tolerate genotoxic
262 insults. Indeed, Johnson *et al*.[39] observed *MSH6* mutations in recurrent GBM following TMZ
263 therapy, associating this with hypermutation and therapeutic failure. Our unbiased screen
264 thus confirms in a systematic fashion that MMR loss is a major driver of TMZ resistance.

265 A key innovation of this study is the demonstration that OLA, a PARP1 inhibitor
266 approved for clinical use in ovarian and breast cancers[40], restores TMZ sensitivity in MMR-
267 deficient cells. Previous studies have shown that PARP1 is essential for base excision repair
268 (BER) and responds primarily to N7-methylguanine and N3-methyladenine lesions. These
269 lesions comprise about 90% of TMZ-induced adducts but are usually non-cytotoxic[41]. Curtin[42]
270 proposed that blocking BER could render these adducts more deleterious, especially in cells
271 that tolerate O^6MeG due to MGMT or MMR loss. Our results confirm this: OLA synergizes
272 with TMZ and significantly reduces proliferation of MMR-deficient U251 cells, including those
273 depleted of *MSH2, MSH6 or PMS2* as well as of a TMZ-resistant cells (U251-R). This synergy is
274 consistent with prior findings by Higuchi et al. (2020)[43], who showed that PARP inhibition
275 could partially re-sensitize glioma cells lacking *MSH6* to TMZ. Moreover, our study extends
276 these observations to patient-derived GBM cultures, including those from relapsed tumours
277 with known therapy resistance. To our knowledge, this is the first demonstration of TMZ re-
278 sensitization by a PARP inhibitor in *ex vivo* GBM cultures harbouring acquired resistance.

279 Our study reveals that PARP inhibition may represent a viable strategy to overcome
280 MGMT-mediated resistance. Wu et al.[44] recently reported that MGMT requires PARylation by
281 PARP1 for its activity in repairing O^6MeG lesions. They showed that PARP inhibition impairs
282 MGMT function, sensitizing otherwise resistant tumour cells. Erice et al.[45] similarly found that
283 melanoma cells with high MGMT expression were rendered TMZ-sensitive by PARP inhibition.
284 In line with these reports, we show that OLA enhances TMZ efficacy in MGMT-expressing GBM
285 models.

286 These findings have significant clinical implications. The majority of GBM patients with
287 MGMT-unmethylated tumours do not benefit from TMZ therapy and face limited therapeutic
288 options. Recent clinical trials of veliparib, a less potent PARP inhibitor, in combination with
289 TMZ in newly diagnosed GBM failed to improve survival[46]. However, those studies did not
290 stratify patients based on MMR status, nor did they include agents with stronger PARP
291 trapping activity such as OLA or niraparib. Our data suggest that future trials should selectively
292 enroll patients with MMR-deficient or MGMT-expressing tumours and employ more potent
293 PARP inhibitors.
294 Additionally, there is growing interest in combining PARP inhibitors with radiotherapy.
295 The PARADIGM-2 trial has explored OLA combined with radiation in newly diagnosed GBM
296 and demonstrated good tolerability[47]. Combining OLA, TMZ, and radiotherapy could therefore
297 represent a next-generation Stupp-like regimen tailored to MGMT-unmethylated or MMR-
298 deficient GBM.
299 For patients with recurrent GBM, particularly those previously treated with TMZ, re-
300 treatment is often ineffective due to resistance[48]. However, MMR mutations have been
301 detected in such recurrences[37,39], suggesting that our approach could benefit this large and
302 underserved population. By showing efficacy in recurrent tumour cultures, we provide a
303 strong preclinical rationale for evaluating OLA+TMZ in the recurrent setting.
304 The permeability of the Blood-Brain Barrier (BBB) remains a concern. Hanna *et al.*[49]
305 showed that OLA concentrations in brain tumours can vary, and optimizing delivery remains
306 a challenge. Novel formulations or transient BBB disruption may be required to achieve
307 therapeutic efficacy in patients. Finally, the hematologic toxicity of combining PARP inhibitors
308 with TMZ must be carefully managed. Previous trials have shown manageable toxicity with
309 adjusted dosing[50], but further pharmacokinetic studies will be necessary to identify optimal
310 dosing regimens that balance efficacy with tolerability.
311
312 **CONCLUSION**
313
314 In summary, our study identifies MMR deficiency as the dominant mechanism of TMZ
315 resistance in GBM via an unbiased CRISPR/Cas9 screen and demonstrates that PARP inhibition
316 with OLA restores TMZ sensitivity in both MMR-deficient and MGMT-expressing tumours.
317 These results redefine the landscape of TMZ resistance and suggest that a combination of TMZ

318 and PARP inhibitors may have broad applicability in GBM therapy. Given the established
319 clinical use of OLA and the limited options for resistant GBM, our findings justify immediate
320 translational efforts and clinical evaluation of this strategy.
321
322
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462

463
464 **FIGURE CAPTIONS**

465 **Figure 1: Genes involved in TMZ-resistance in U251 cells.**

466 **A.** CRISPR knock-out screen of U251 cells challenged with TMZ.

467 **B.** SiRNA validation of genes identified by CRISPR screen. SiLuc = siLuciferase = control.
468 Silencing of MSH2, MSH6 and PMS2 induce TMZ-resistance but not the silencing of CLHC1,
469 BCL11A and GPR75.

470

471 **Figure 2: Effect of treatment of U251 and U251-R cells with Olaparib, TMZ or Olaparib + TMZ**
472 **for 16 days.**

473

474 **Figure 3: Effect of Olaparib treatment on U251 cells resisting TMZ.**

475 U251 cells were depleted in MSH6. This induces its resistance against TMZ. When Olaparib is
476 added in combination with TMZ, cell death is restored.

477

478 **Figure 4: Effect of OLA, TMZ and OLA + TMZ on the GBM primary culture GBM86 at diagnosis**
479 **and at relapse.**

480

481 **Supplementary Figure S1: Effect of different OLA concentrations on U251 cells for 72 hours.**

482

483 **Supplementary Figure S2: Effect of different siRNA concentration targeting MSH2 and CLHC1**
484 **on U251 cells for 72 hours.**

485

486 **Supplementary Figure S3: Effect of OLA, TMZ and OLA + TMZ on the GBM primary culture**
487 **GBMA1 at relapse.**

488

489 **Supplementary Figure S4: Effect of OLA, TMZ and OLA + TMZ on the GBM primary culture**
490 **GBM3.**

491

492 **Supplementary Figure S5: Effect of OLA, TMZ and OLA + TMZ on the GBM primary culture**
493 **GBM96 at relapse.**

494

495 **Supplementary Figure S6: Effect of OLA, TMZ and OLA + TMZ on the GBM primary culture**
496 **GBMG5.**
497
498 **Supplementary Figure S7: Effect of OLA, TMZ and OLA + TMZ on the GBM primary culture**
499 **GBM69.**
500
501 **Supplementary Figure S8: Effect of OLA, TMZ and OLA + TMZ on the GBM primary culture**
502 **GBM81.**
503
504 **Supplementary Figure S9: Effect of OLA, TMZ and OLA + TMZ on the GBM primary culture**
505 **GBM120.**
506
507
508 **Supplementary Table I:**
509 A. Table describing the GBM primary cultures used in this study
510 B. Oligonucleotide sequences used to produce the CRISPR screen Illumina libraries.
511 C. Provider and references of the siRNA used.
512
513 **ABBREVIATIONS LIST**
514 BER Base Excision Repair
515 GBM Glioblastoma Multiforme
516 HR Homology-directed Repair
517 MGMT O^6-Methylguanine-DNA-Methyl Transferase
518 N^3MeA N^3-methyladenine
519 N^7MeG N^7-methylguanine
520 NHEJ Non-Homologous End Joining
521 O^6MeG O^6-methylguanine
522 OLA Olaparib
523 PARP Poly(ADP-Ribose) Polymerase
524 TMZ Temozolomide
525
526 **ACKNOWLEDGEMENTS**

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AUTHOR CONTRIBUTIONS

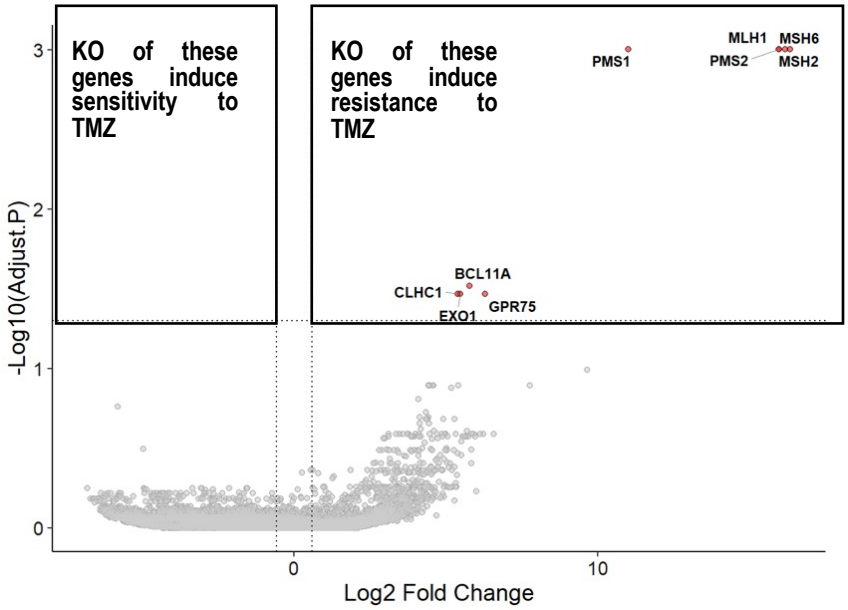
Samarut Edouard, Tabourel Gaston and Briand Joséphine contributed equally to the experimental work. Olivier Lisa and Sérandour Aurélien designed the project and wrote the manuscript with the involvement of Vallette François, Salaud Céline and Cartron Pierre-François. Landais Yuna, Gratas Catherine, Loussouarn Delphine and Bougras-Cartron Gwenola analysed the sequencing, histologic, clinical and qPCR data.

DATA AVAILABILITY.

The CRISPR screen sequencing data are available in ENA PRJEB73760.

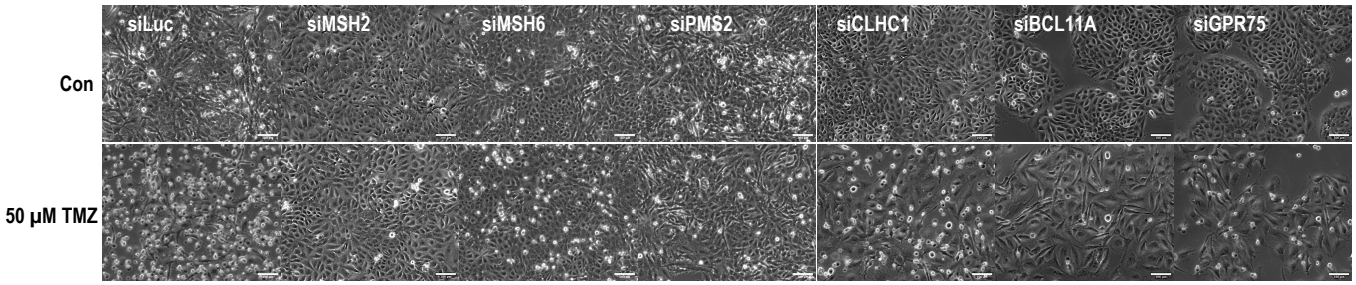
Other data will be made available upon reasonable request.

A



B

U251



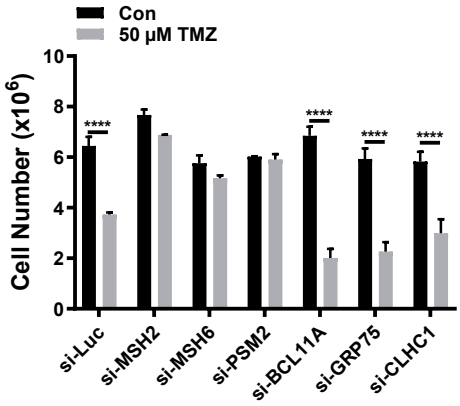
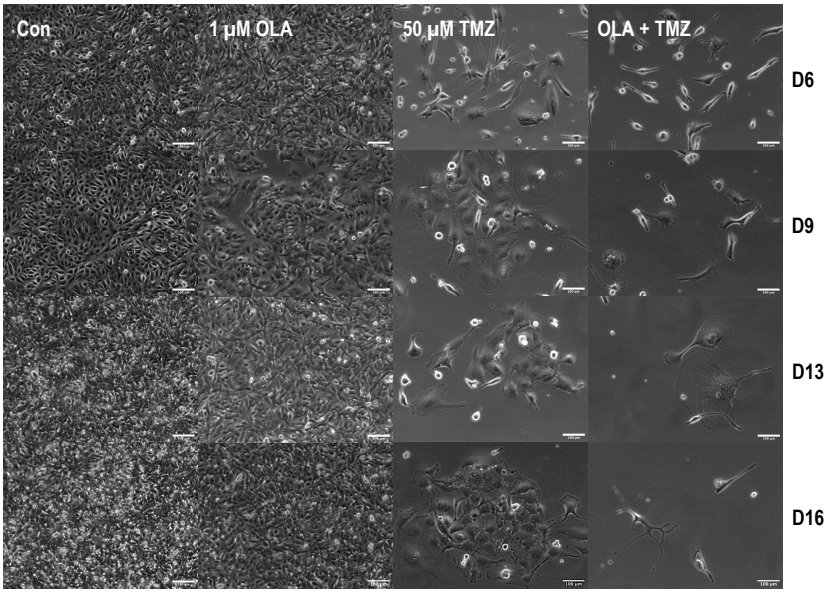


Figure 1

A

U251 (TMZ sensitive, MGMT-)



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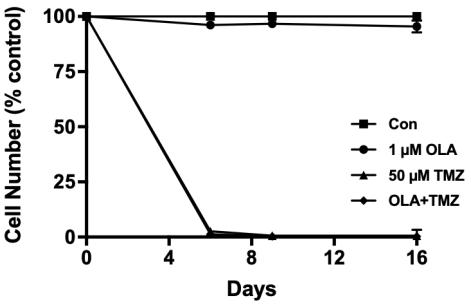

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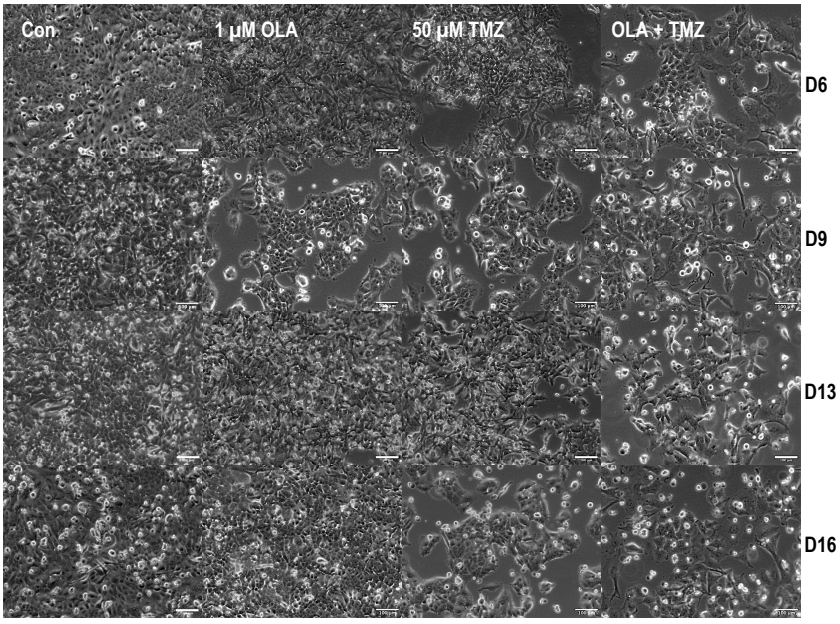
U251-R (TMZ resistant, MGMT+)



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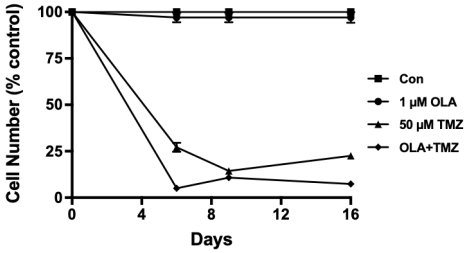


Figure 2

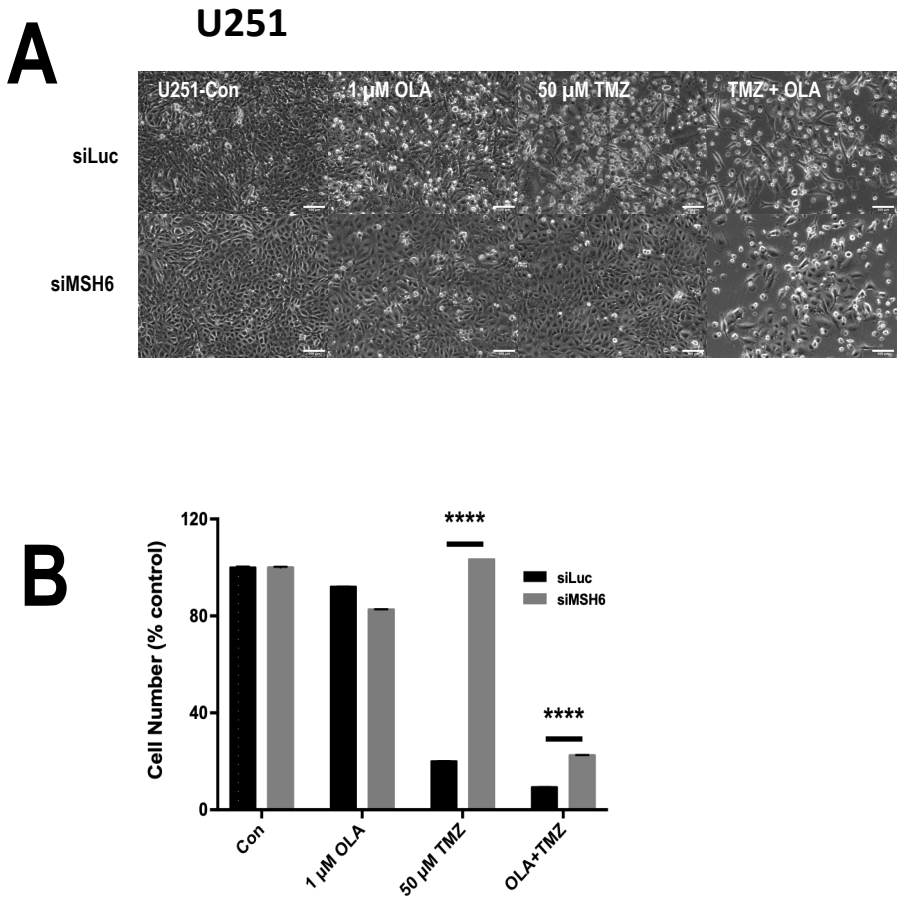
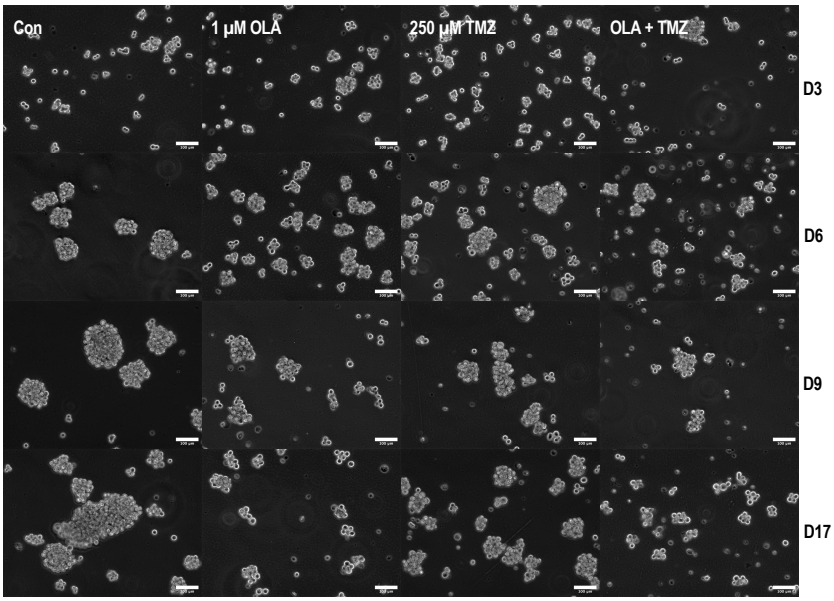

A
U251
U251-Con
1 μM OLA
50 μM TMZ
TMZ + OLA
siLuc
siMSH6
B
Cell Number (% control)
120
80
40
0
****
****
siLuc
siMSH6
Con
1 μM OLA
50 μM TMZ
OLA+TMZ

Figure 3

A

GBM86 at Diagnosis



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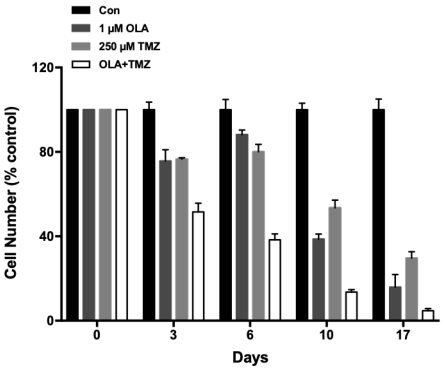
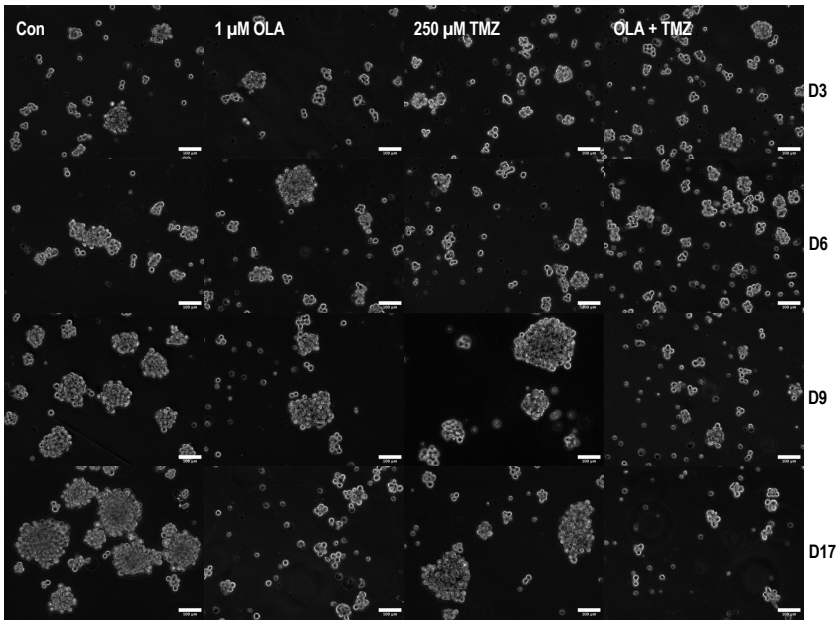


C

GBM86 at Relapse



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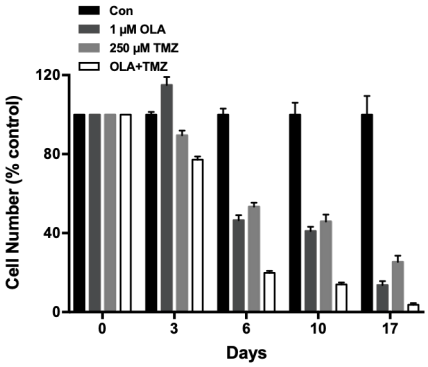
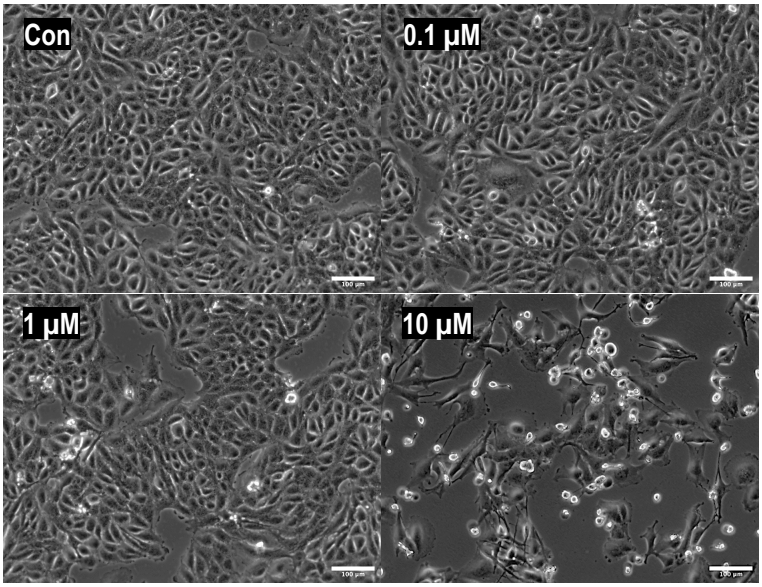


Figure 4

A

U251



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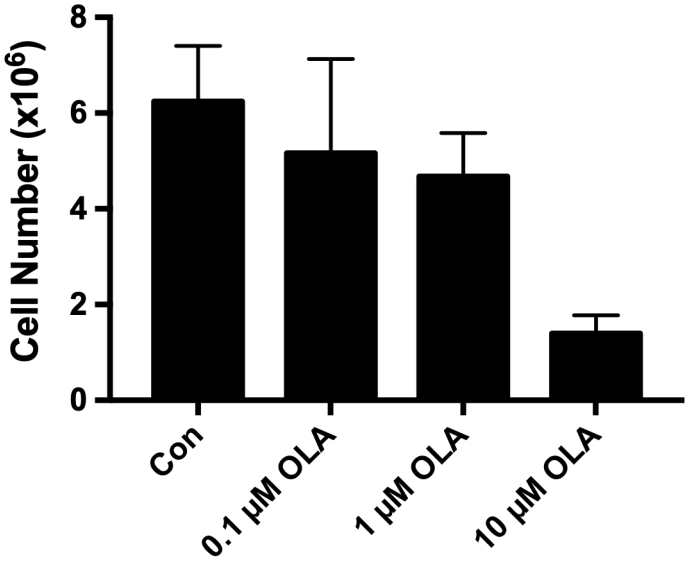

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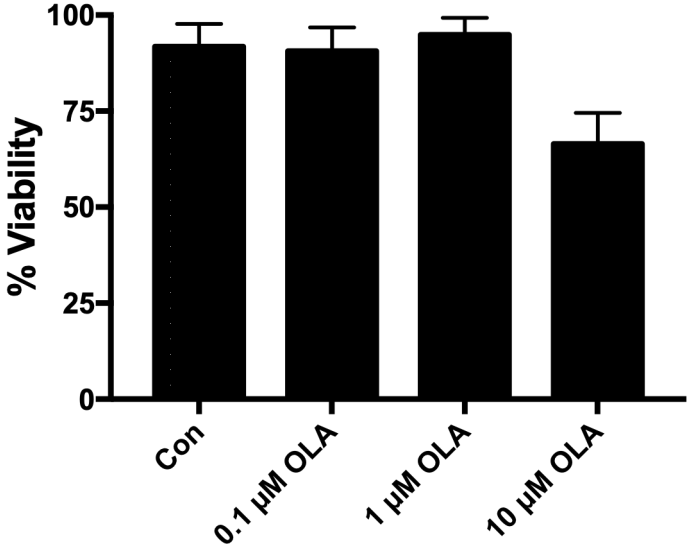
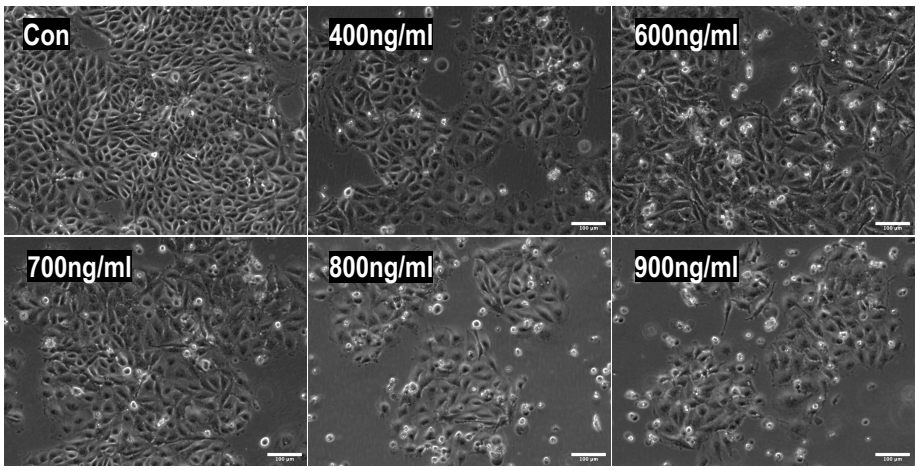
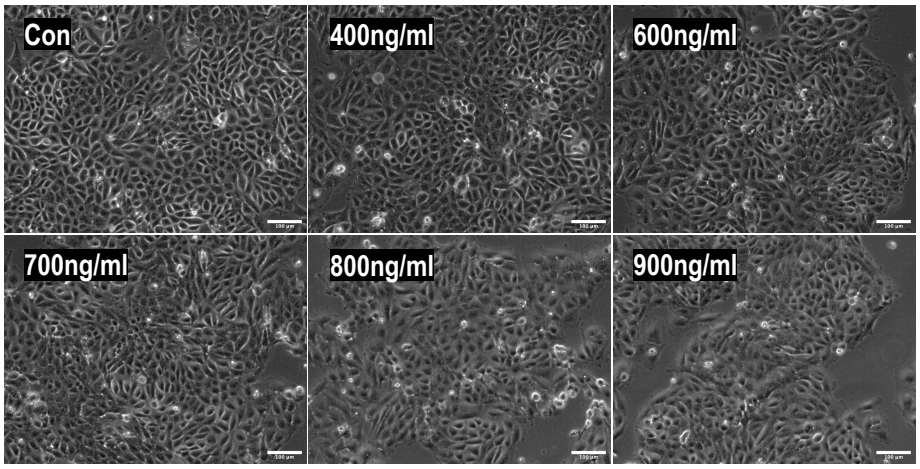


Supplementary Figure S1

U251



MSH2 – 72H

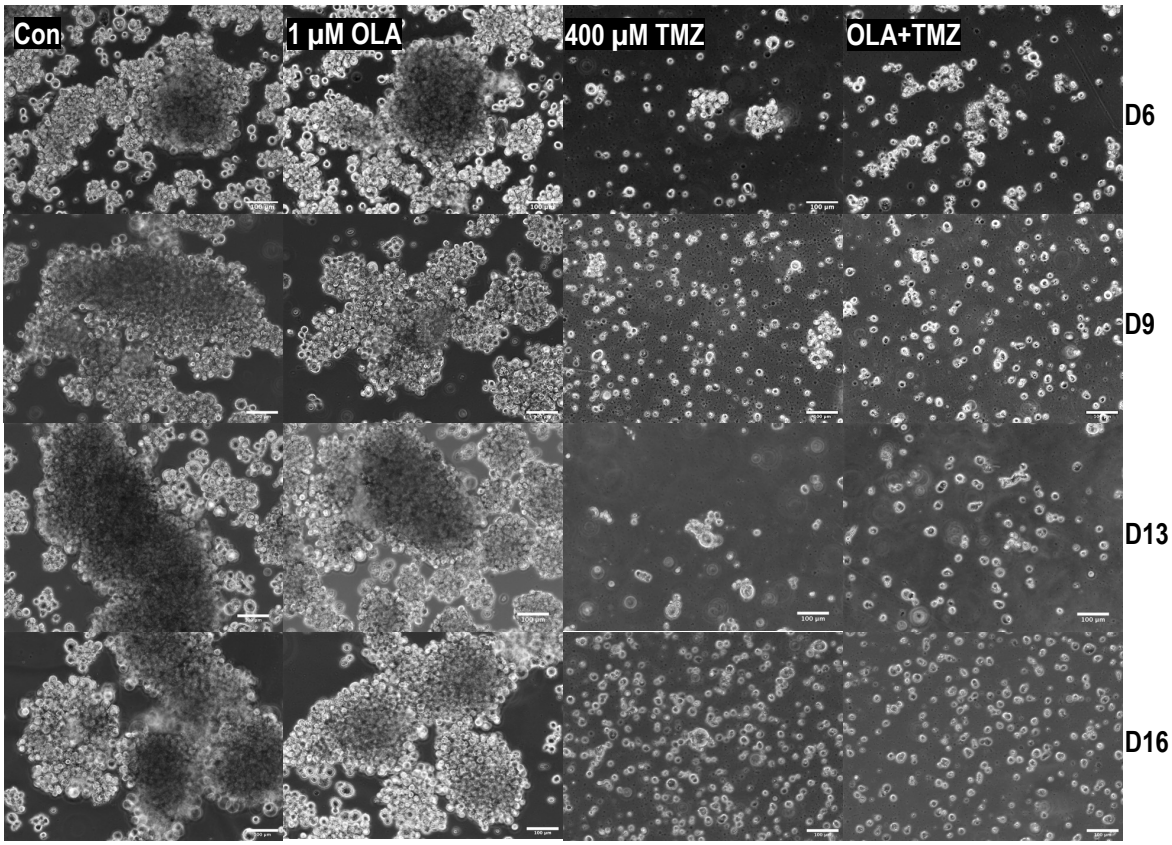


CLHC1 – 72H

CONCLUSION: Use 600 ng/ml siRNA

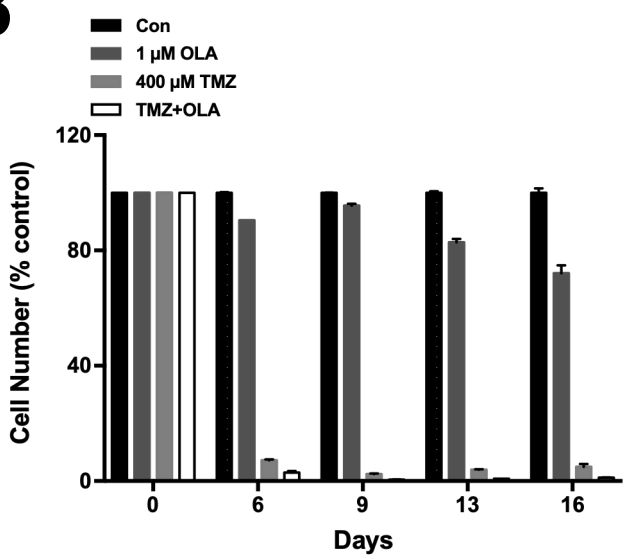
Supplementary Figure S2

A



GBMA1 relapse

B



Supplementary Figure S3

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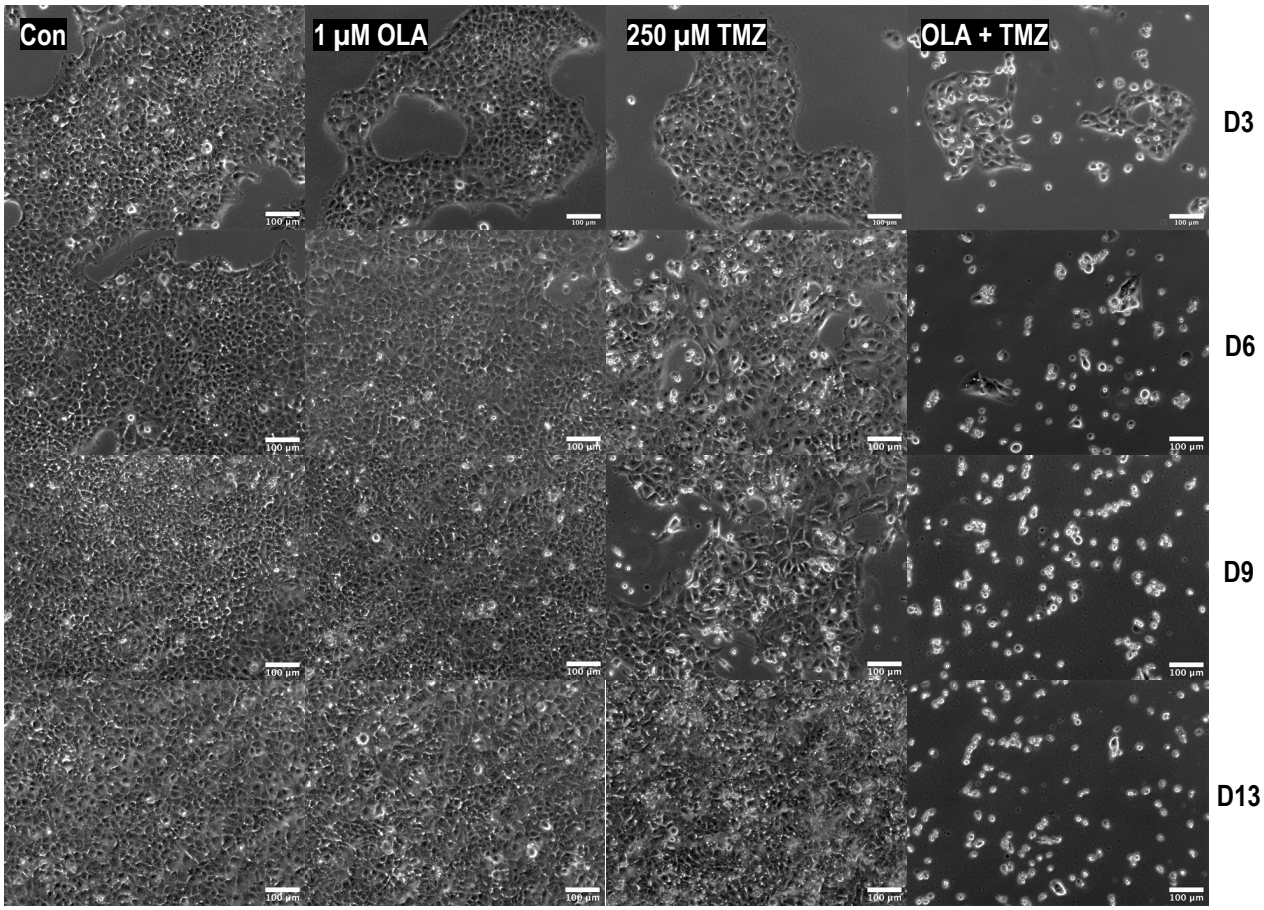
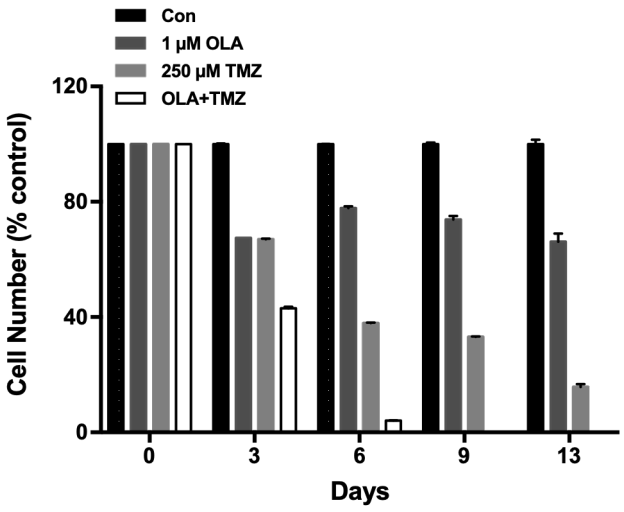


GBM3

B



Supplementary Figure S4

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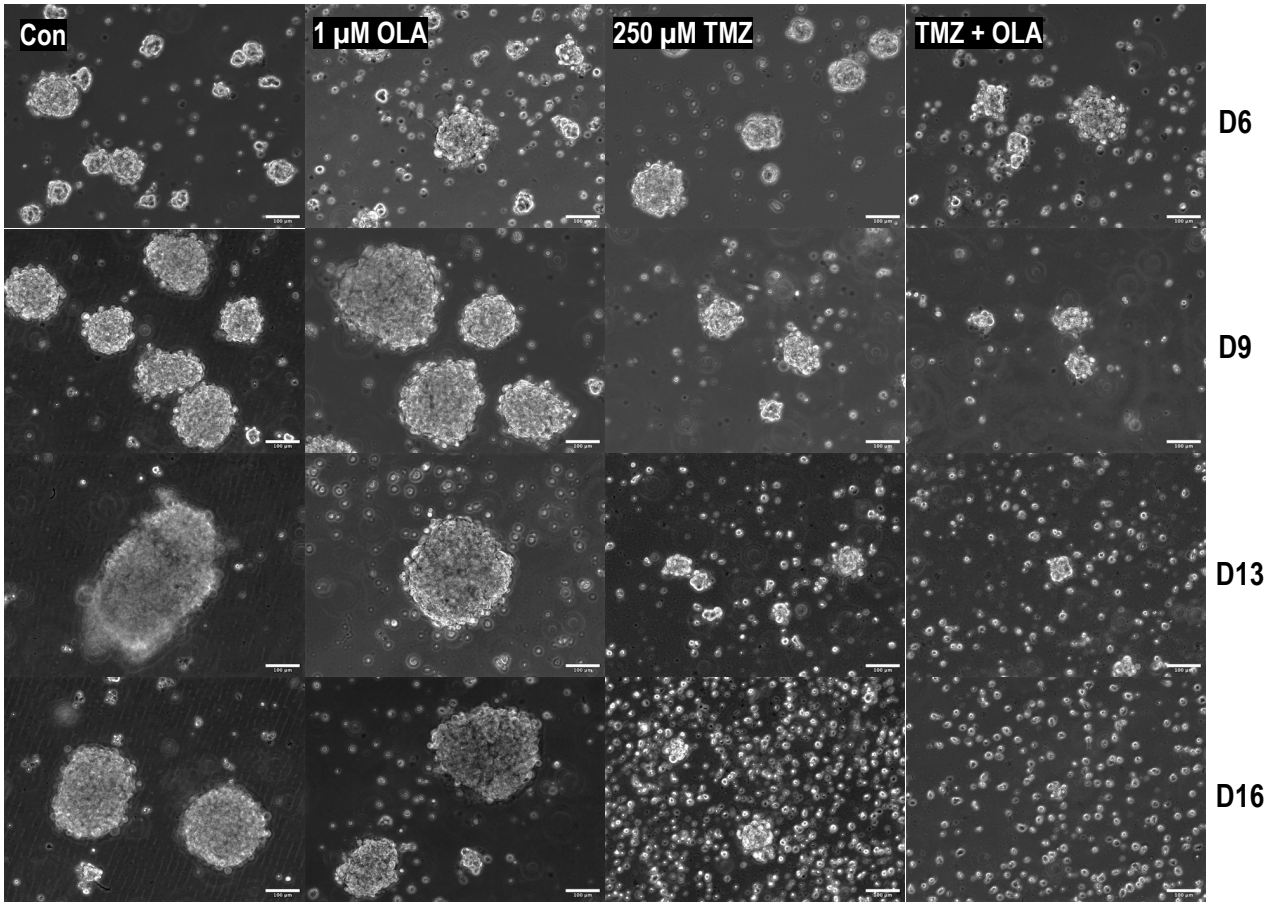
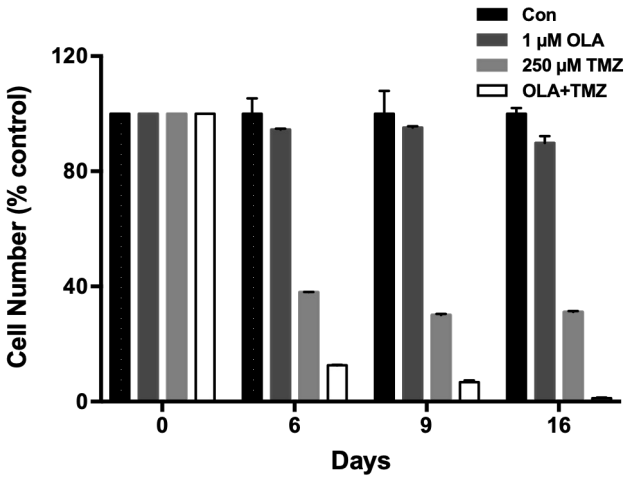


GBM96 relapse

B



Supplementary Figure S5

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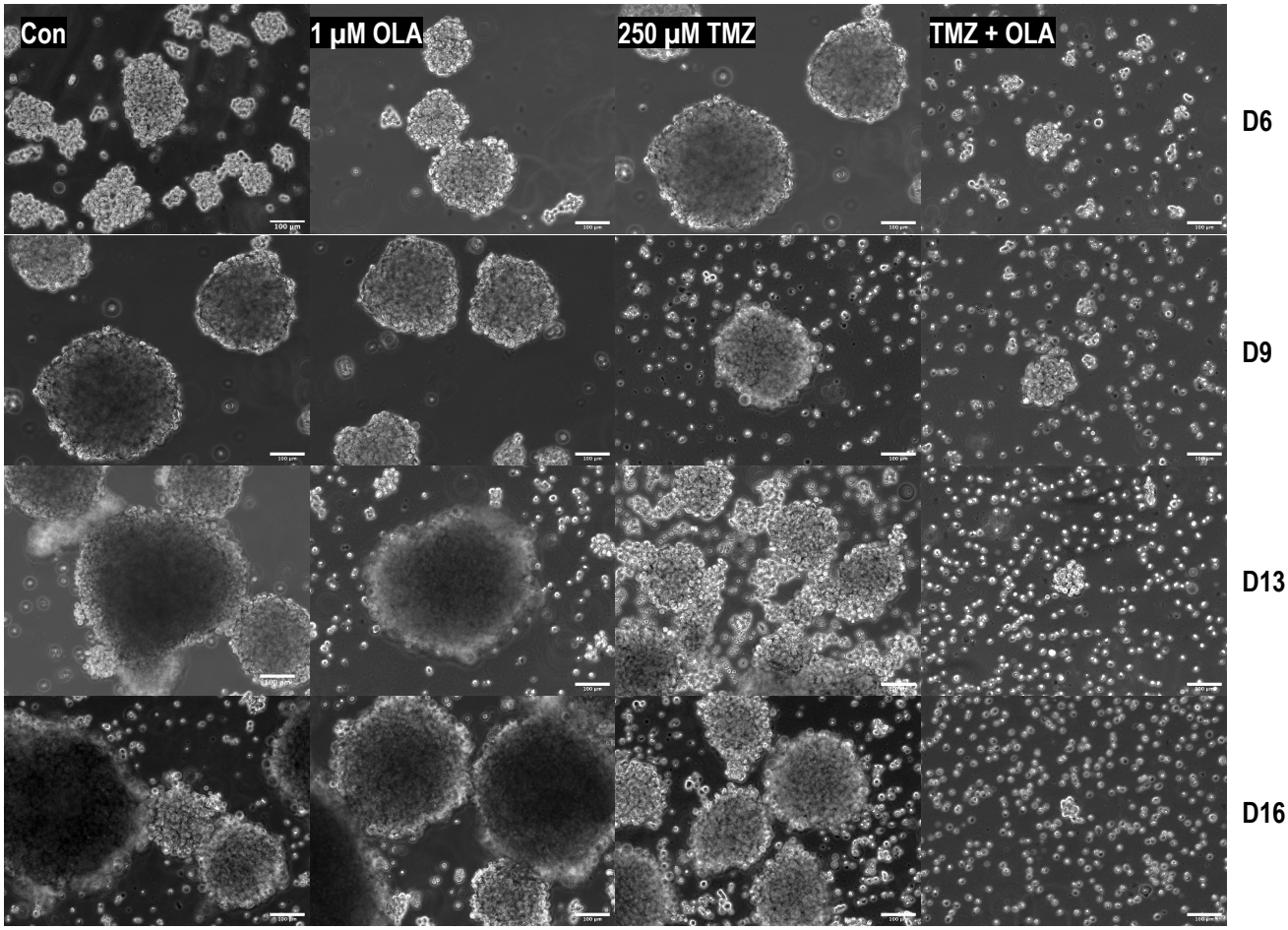

GBMG5

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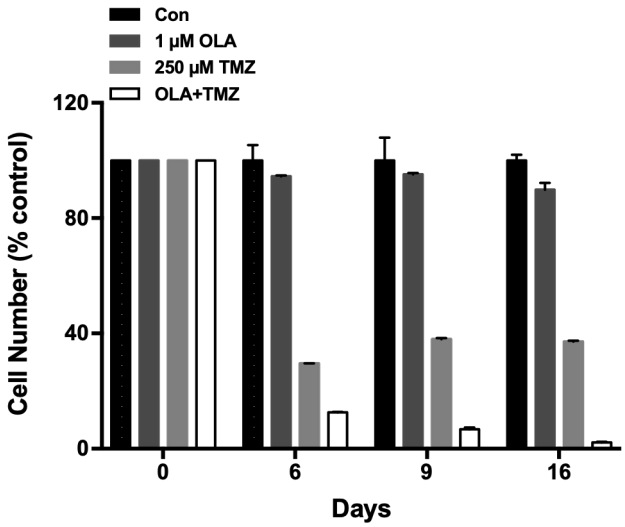

Supplementary Figure S6

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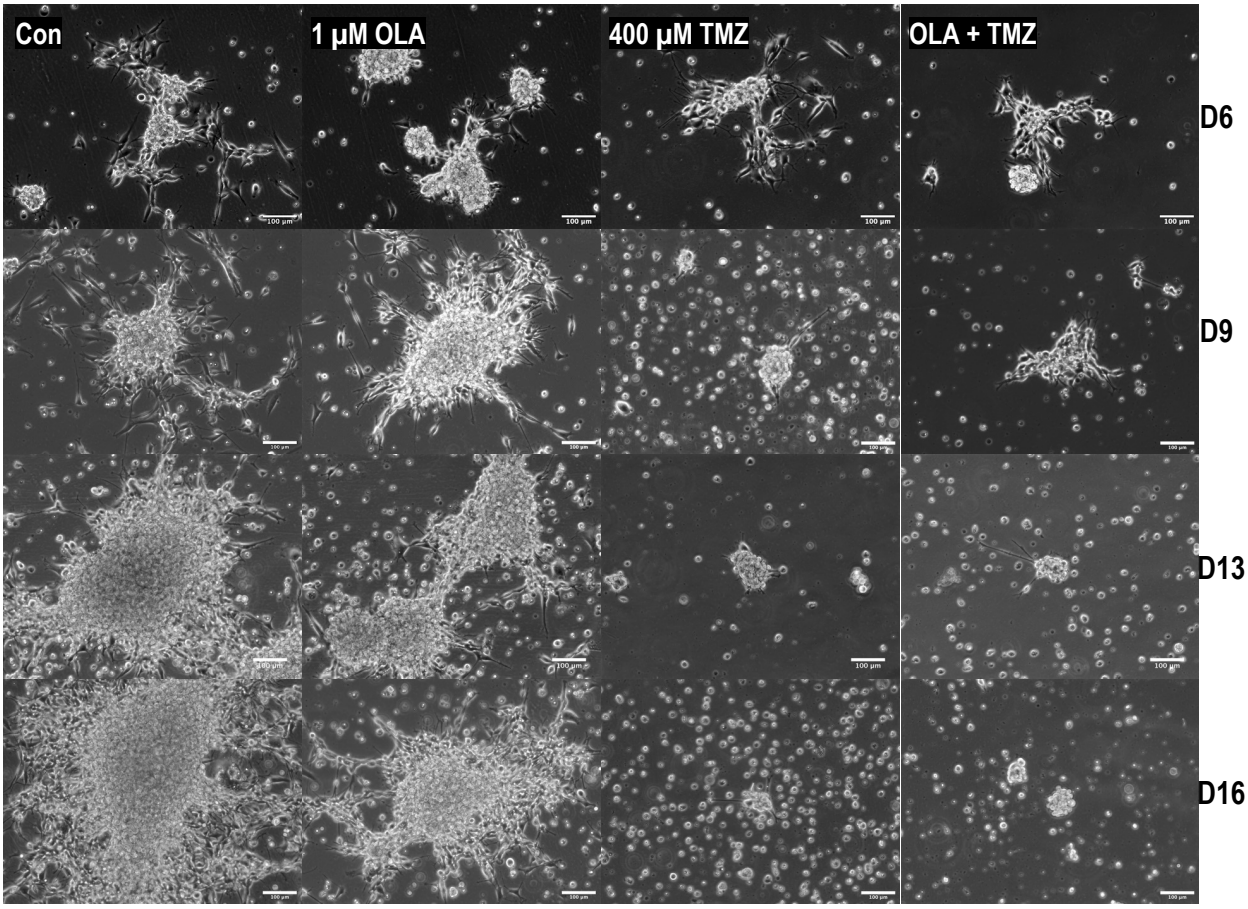

GBM69

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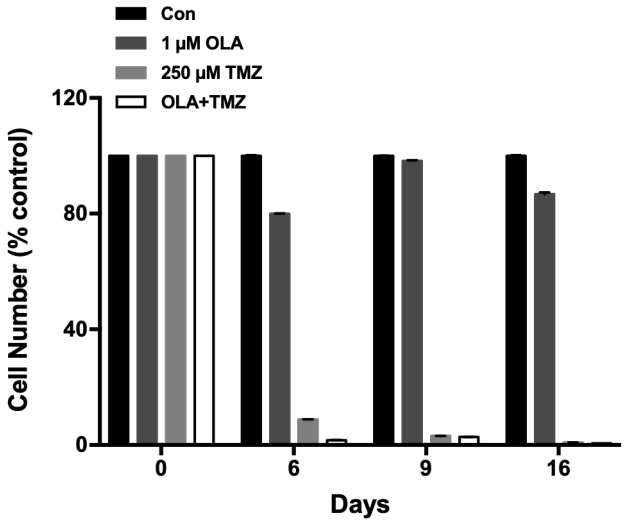

Supplementary Figure S7

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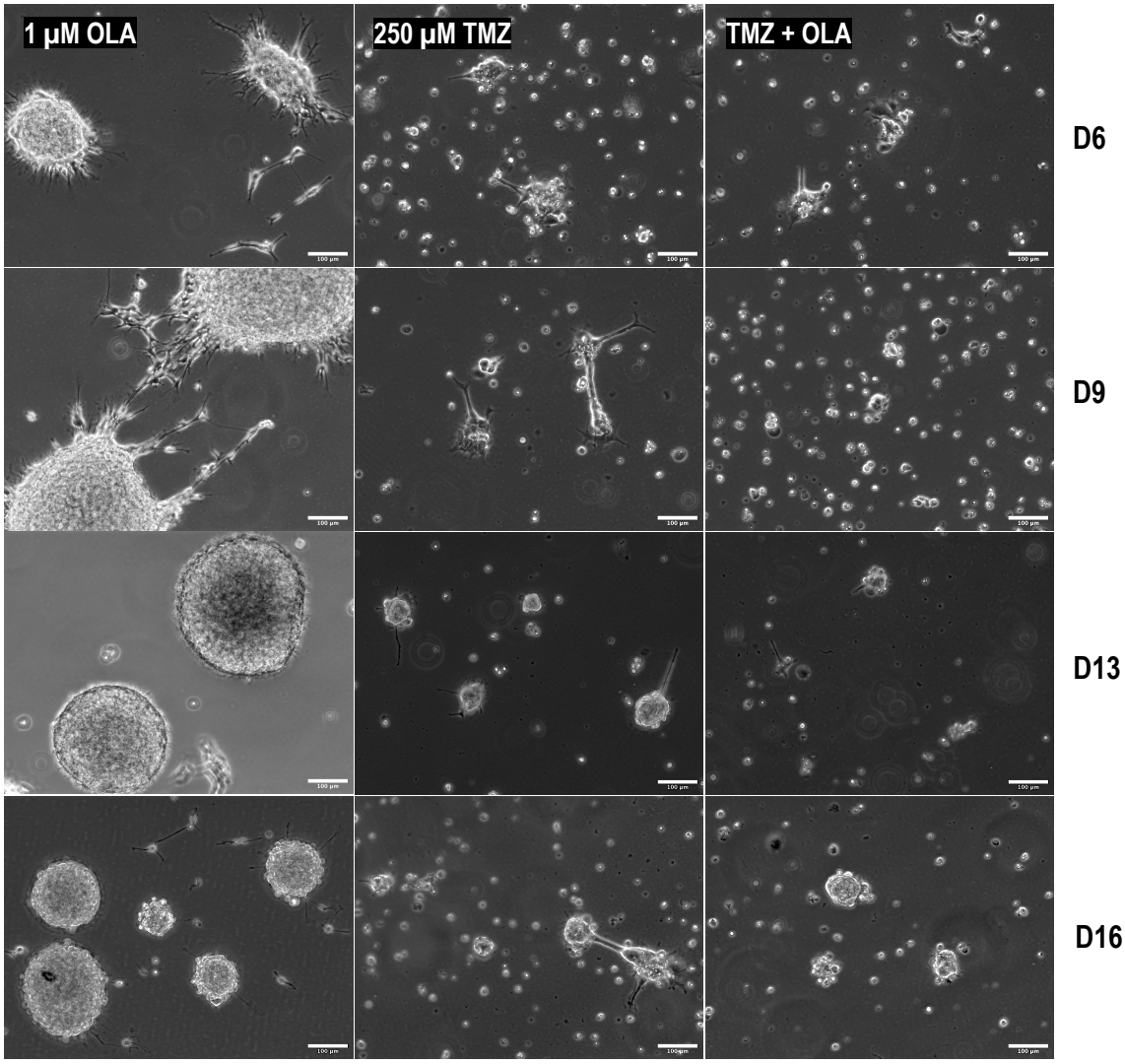

GBM81

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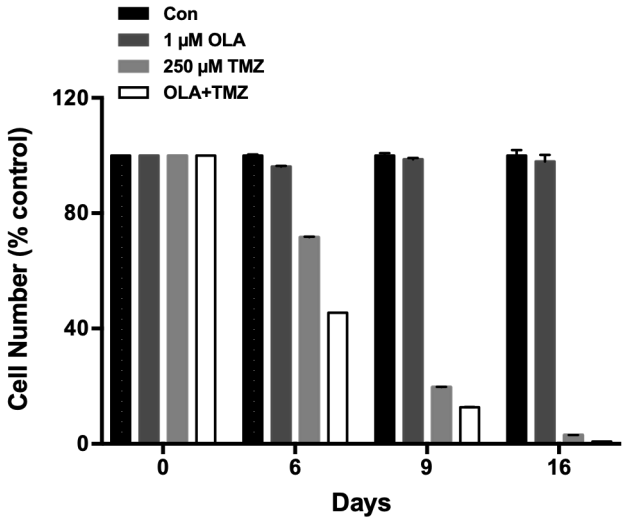

Supplementary Figure S8

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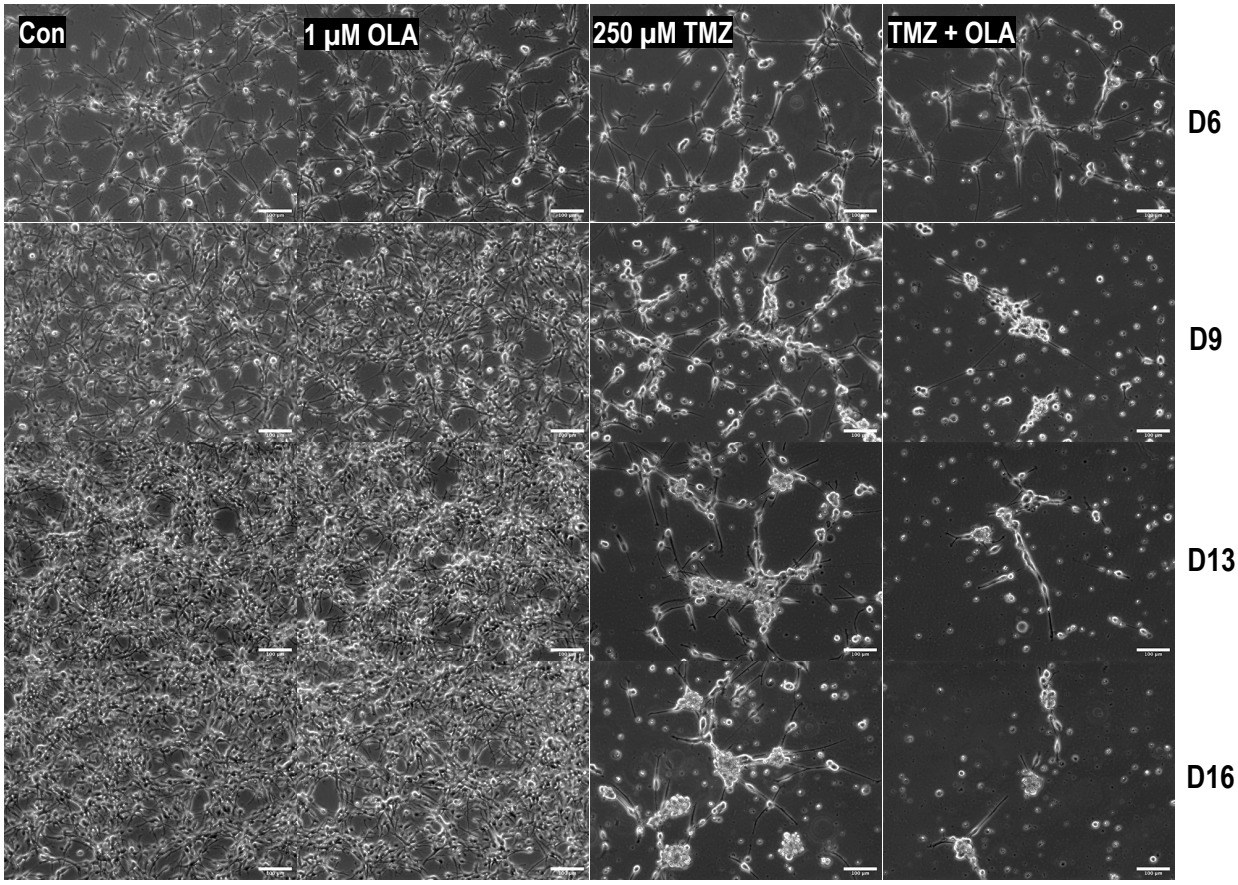

GBM120

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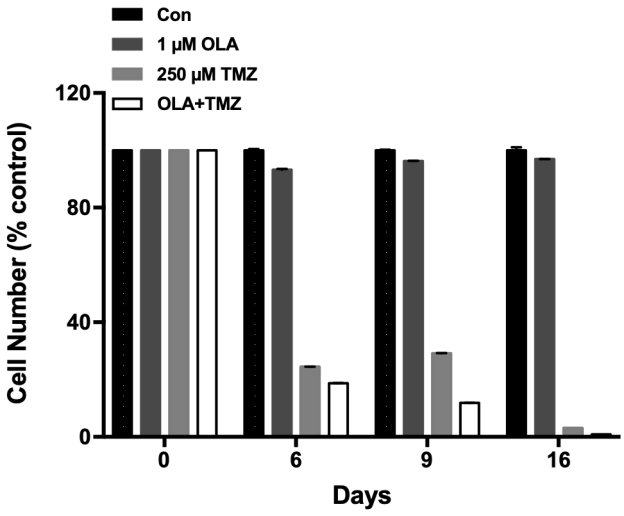

Supplementary Figure S9

A

Patients	Sex	Age at diagnosis	Survival (months)	Localization	Tumor	IDH	Tx	MGMT status
GBM3	F	66	-	Right temporal	primary	wt	Stupp	+
GBM69	M	50			primary	wt	Stupp	-
GBM96	M	71	12	right frontal	recurrent	wt	Stupp	+
GBM120	F	78		left frontal	primary		Stupp	+
GBM81	M	52	20	right frontal	primary	wt	Stupp	+
GBMA1	M	54		left temporal	recurrent	wt	Stupp	-
GBMG5	F	56		right frontal	primary	wt	Stupp	+

Distribution sex: F = 43%, M = 57%

Mean age at diagnosis: 61 years

B

P5_0_nt_stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTTTGTGGAAAGGACGAAACACC*G
P5_1_nt_stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTGTGGAAAGGACGAAACACC*G
P5_2_nt_stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTTGTGGAAAGGACGAAACACC*G
P5_3_nt_stagger	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAGCTTGTGGAAAGGACGAAACACC*G
P7_A01	CAAGCAGAAGACGGCATACGAGATCGGTTCAAGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTG*T
P7_A02	CAAGCAGAAGACGGCATACGAGATGCTGGATTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTG*T
P7_A03	CAAGCAGAAGACGGCATACGAGATTAACTCGGGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTG*T
P7_A04	CAAGCAGAAGACGGCATACGAGATTAACAGTTGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTACTATTCTTTCCCCTGCACTG*T

Custom primers for CRISPR screen library preparation.

C

siRNA target name	Provider	Reference
BCL11A	Sigma	EHU145981-20UG
C2ORF63 (= CLHC1)	Sigma	EHU027921-20UG
GPR75	Sigma	EHU121351-20UG
MSH2	Sigma	EHU013561-20UG
MSH6	Sigma	EHU049441-20UG
PMS2	Sigma	EHU011451-20UG
Rluc (= luciferase)	Sigma	EHURLUC-20UG

siRNA references.

Supplementary Table I