# Supplementary Information

## Temozolomide suppresses *MYC* via activation of TAp63 to inhibit progression of human glioblastoma

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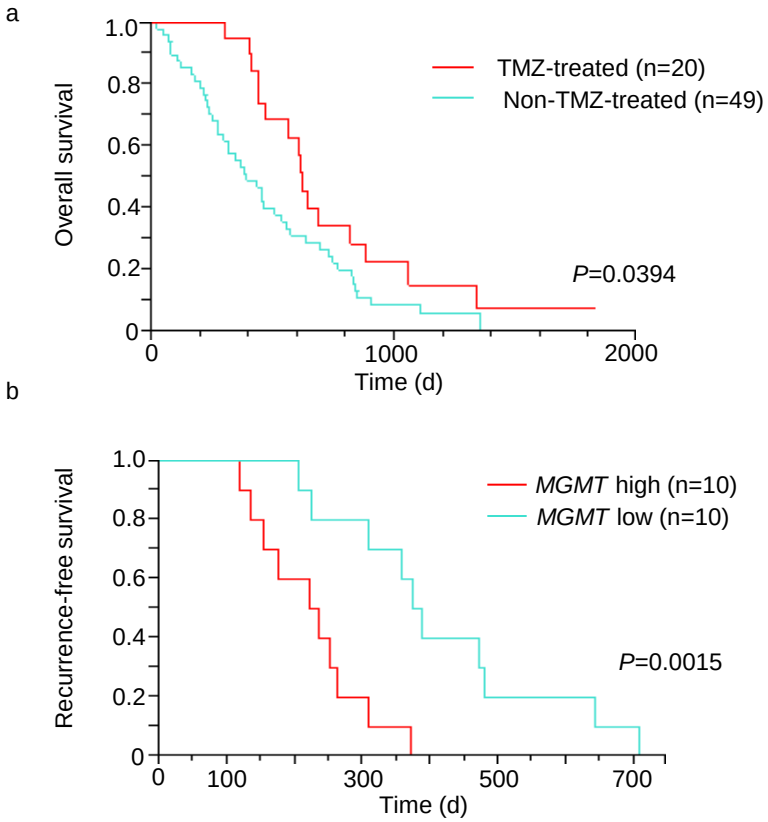
**Supplementary Table 1**
Summary of available RNA data derived from 69 newly-diagnosed malignant glioma patients.

| Classification | *n* | (%) |
|---|---|---|
| **All patients** | 69 | |
| **Average age (years ± s.d.)** | 59.2 ± 12.6 | |
| **Sex** | | |
| Female | 29 | 42 |
| Male | 40 | 58 |
| **Diagnosis** | | |
| Glioblastoma | 59 | 85.5 |
| Anaplastic astrocytoma | 7 | 10.1 |
| Anaplastic oligoastrocytoma | 3 | 4.4 |
| ***MGMT* promoter status** | | |
| Methylated | 17 | 24.6 |
| Methylated/unmethylated | 11 | 15.9 |
| Unmethylated | 34 | 49.3 |
| Not available | 7 | 10.2 |
| **Temozolomide treatment** | | |
| Yes (2006 – 2011) | 20 | 29 |
| No (1994 – 2007) | 49 | 71 |

**Supplementary Figure 1**



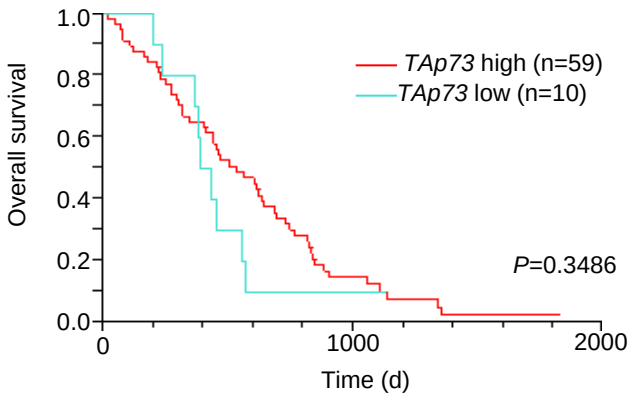

**Supplementary Figure 1**

TMZ treatment and low *MGMT* mRNA expression are associated with favorable prognosis in malignant glioma .

a) Kaplan–Meier survival curves of overall survival. Subgroups of TMZ-treated (n=20) and non-TMZ-treated (n=49) patients among 69 individual samples of newly diagnosed malignant gliomas (GBM, 59; anaplastic astrocytoma, 7; anaplastic oligoastrocytoma, 3). *P* value by log-rank test.

b) Kaplan–Meier survival curves of recurrence-free survival of 20 malignant glioma patients (GBM, 17; anaplastic oligoastrocytoma, 3) according to tumor sample *MGMT* mRNA expression from patients who were TMZ-treated after resection. *MGMT* expression was normalized by *ACTB,* and then designated high (n=10) or low (n=10) according to the median value. High: n=10, mean *MGMT* expression = 15.46 ± 2.45 s.d. Low: n=10, mean *MGMT* expression = 5.45 ± 0.70 s.d. *P* value by log-rank test.

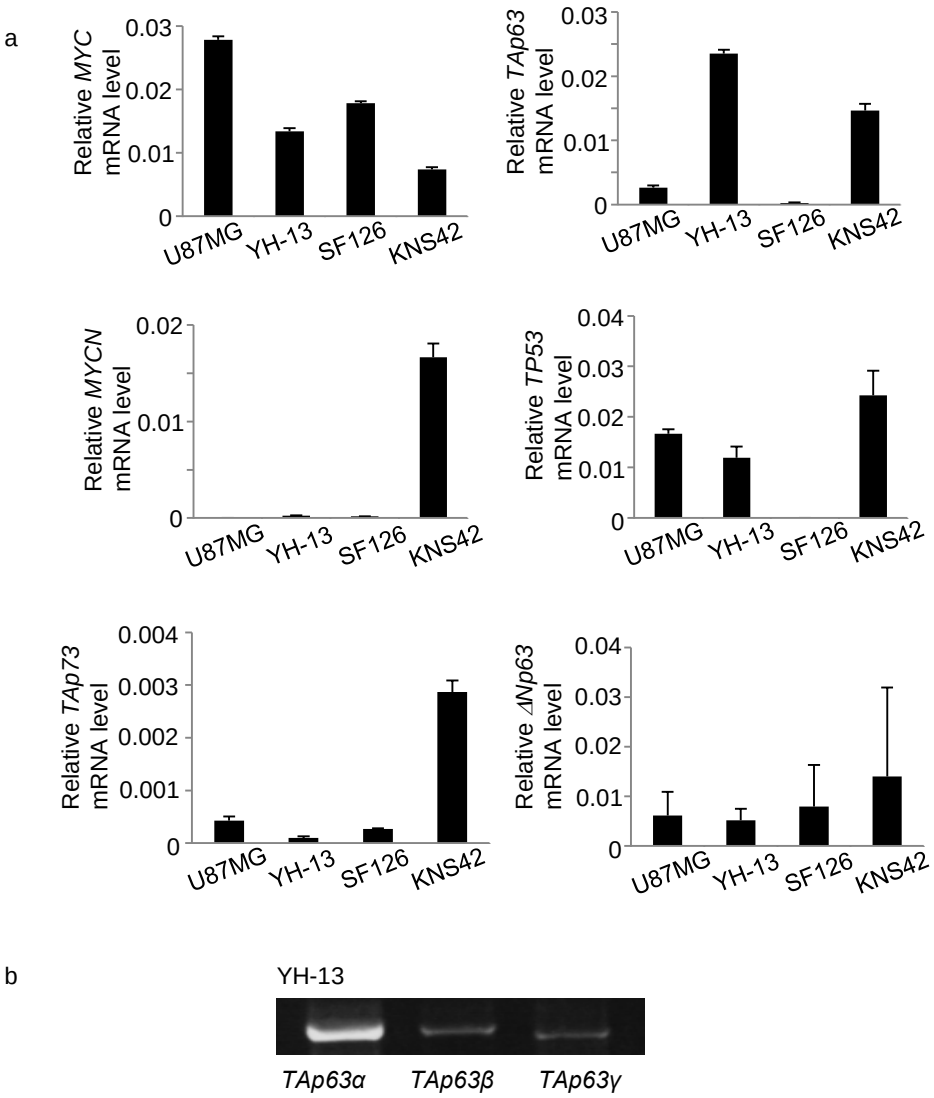
**Supplementary Figure 2**




**Supplementary Figure 2**

Kaplan–Meier survival curves of overall survival time in subjects with newly diagnosed malignant gliomas according to the relative expression levels of *TAp73* before chemotherapy (n=69). *TAp73* was normalized to *ACTB*, and then designated high or low based on the normal human brain expression. High: n=59, mean *TAp73* expression = 0.93 ± 0.17 s.d. Low: n=10, mean *TAp73* expression = 0.01 ± 0.002 s.d. *P* value by log-rank test.

**Supplementary Figure 3**



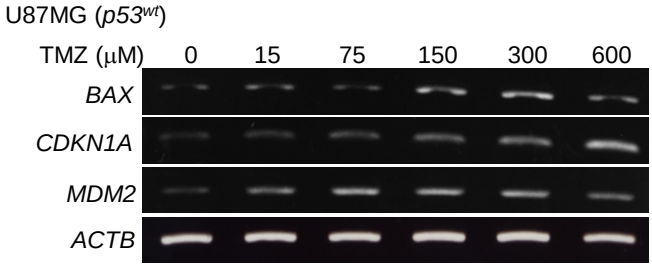

**Supplementary Figure 3**

Basal mRNA expression levels of p53 and Myc family members in 4 GBM cell lines.

a) qRT-PCR analyses of the relative steady-state mRNA expression of p53 family members (*TP53*, *TAp63*, *ΔNp63* and *TAp73*) and Myc family members (*MYC*, *MYCN*) in GBM cell lines (U87MG, YH-13, SF126 and KNS42), normalized by *ACTB*.

b) RT-PCR analysis of *TAp63* isoforms α, β and γ in YH-13 cells.

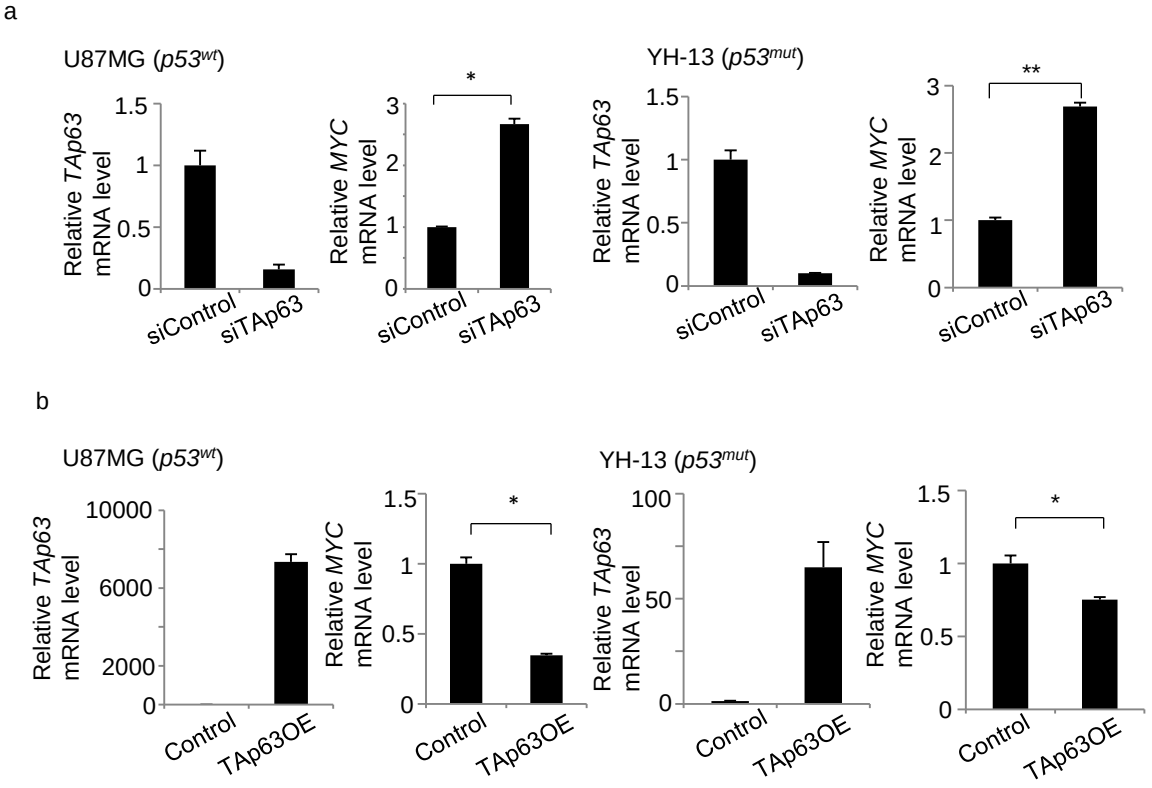
**Supplementary Figure 4**




**Supplementary Figure 4**

RT-PCR analyses of p53 downstream target genes (*BAX*, *CDKN1A* and *MDM2*) in a TMZ-dependent manner.

**Supplementary Figure 5**



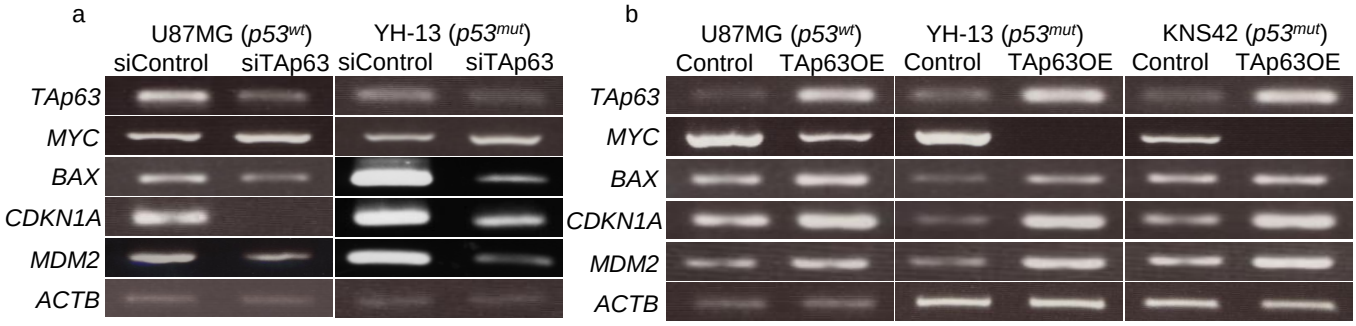

**Supplementary Figure 5**

*MYC* levels are affected by *TAp63* expression.

a) qRT-PCR analysis of relative *MYC* expression in U87MG and YH-13 cells after siRNA-mediated knockdown of *TAp63,* normalized by *ACTB*. *P<0.005, **P<0.0005 by two-tailed t-test.

b) qRT-PCR analysis of relative *MYC* expression in U87MG and YH-13 cells after lentiviral *TAp63* overexpression (TAp63OE), normalized by *ACTB*. *$P$<0.005 by two-tailed *t*-test.

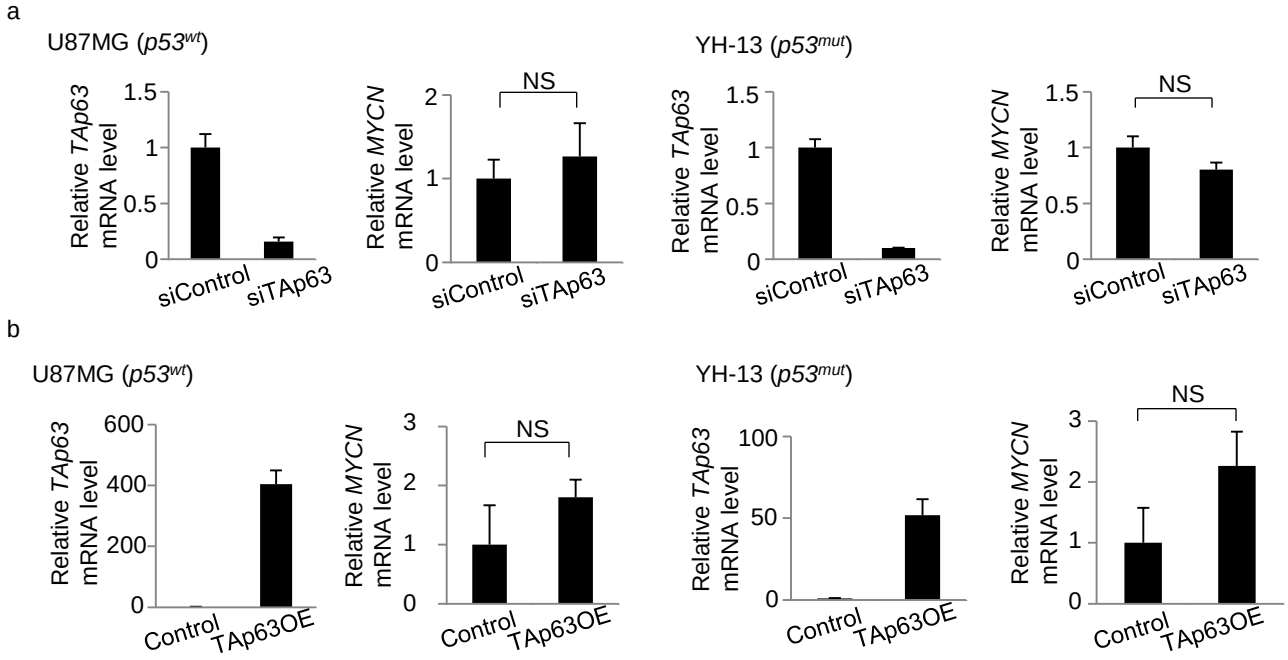
**Supplementary Figure 6**




**Supplementary Figure 6**

p53 downstream genes are regulated in a *TAp63*-dependent manner. RT-PCR analyses of p53 downstream target genes (*BAX*, *CDKN1A* and *MDM2*) in (a) *TAp63* siRNA-transfected cells and (b)*TAp63*-overexpressing cells after lentivirus infection. *ACTB* was used for internal control.

**Supplementary Figure 7**



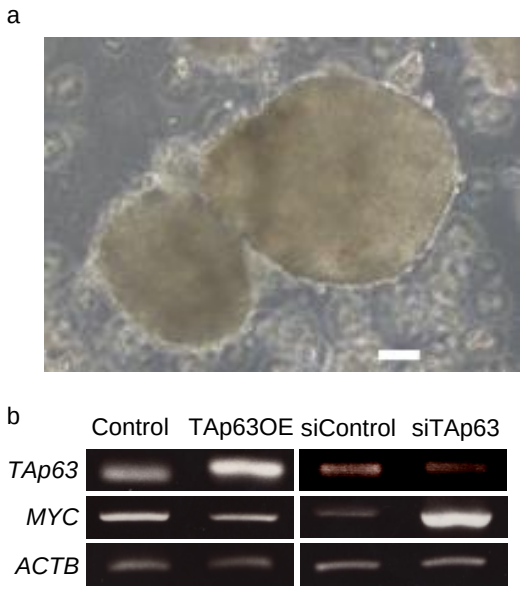

**Supplementary Figure 7**

*MYCN* levels are not affected by *TAp63* expression.

a) qRT-PCR analysis of relative *MYCN* expression in U87MG and YH-13 cells after siRNA-mediated knockdown of *TAp63,* normalized by *ACTB*. NS, not significant, by two-tailed *t*-test.

b) qRT-PCR analysis of relative *MYCN* expression in U87MG and YH-13 cells after lentiviral *TAp63* overexpression (TAp63OE), normalized by *ACTB*. NS, not significant, by two-tailed *t*-test.

**Supplementary Figure 8**



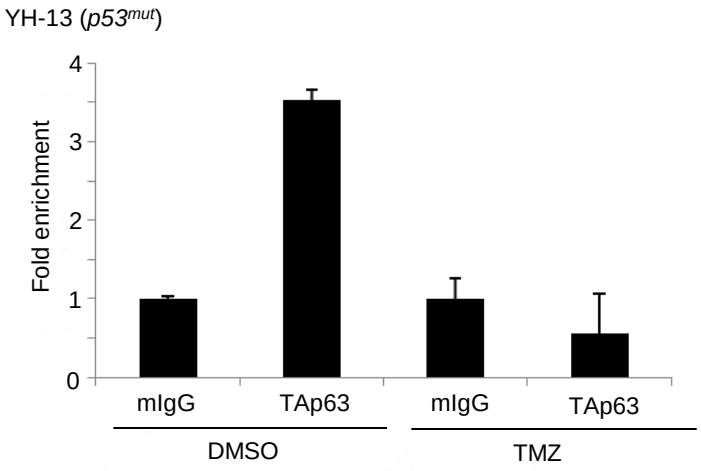

**Supplementary Figure 8**

TAp63-*MYC* pathway was intact in the cancer tissue-derived spheroids (CTOS).

a) Representative images of CTOS with RT-PCR analysis of overexpressed or knocked-down *TAp63*. Scale bar, 40 μm.

b) RT-PCR analyses of *TAp63*, *MYC* and *β-actin* in*TAp63* siRNA-transfected CTOS and *TAp63*-overexpressing CTOS after lentivirus infection. *ACTB* was used for internal control.

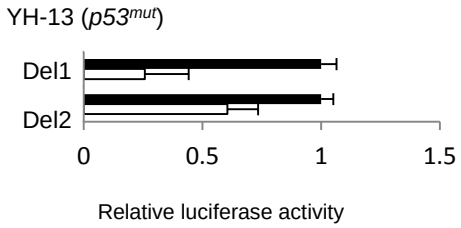
**Supplementary Figure 9**




**Supplementary Figure 9**

ChIP-qPCR analyses of the amount of TAp63 recruited onto the *MYC* intron (R3).

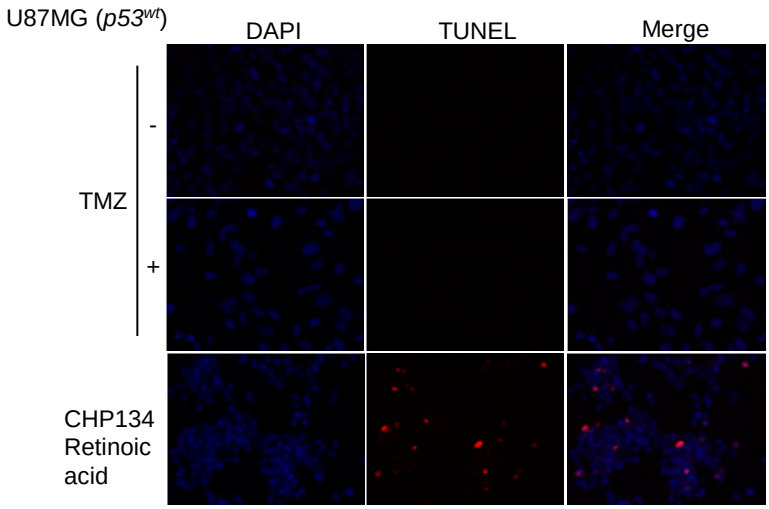
**Supplementary Figure 10**




**Supplementary Figure 10**

Luciferase activity of *MYC* promoter reporters after *TAp63* overexpression in YH-13 cells. Data shown as the relative activity of the firefly luciferase compared with that in control YH-13 cells.

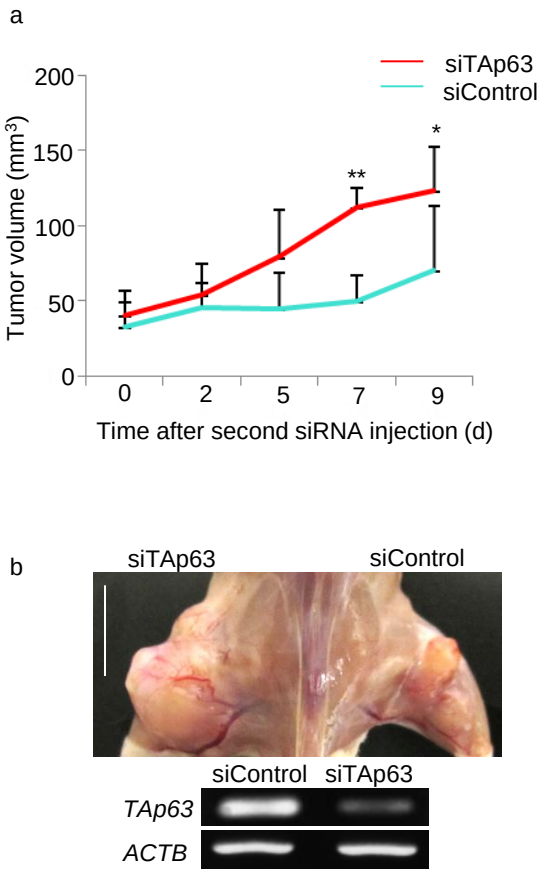
**Supplementary Figure 11**




**Supplementary Figure11**

TMZ suppresses invasion of U87MG cell lines by day 5 of exposure without inducing apoptosis. TUNEL assay revealing no apoptotic cells in the control and TMZ-treated U87MG cells. CHP134 cells treated with 5 μM retinoic acid for 48 h served as a positive control.

**Supplementary Figure 12**



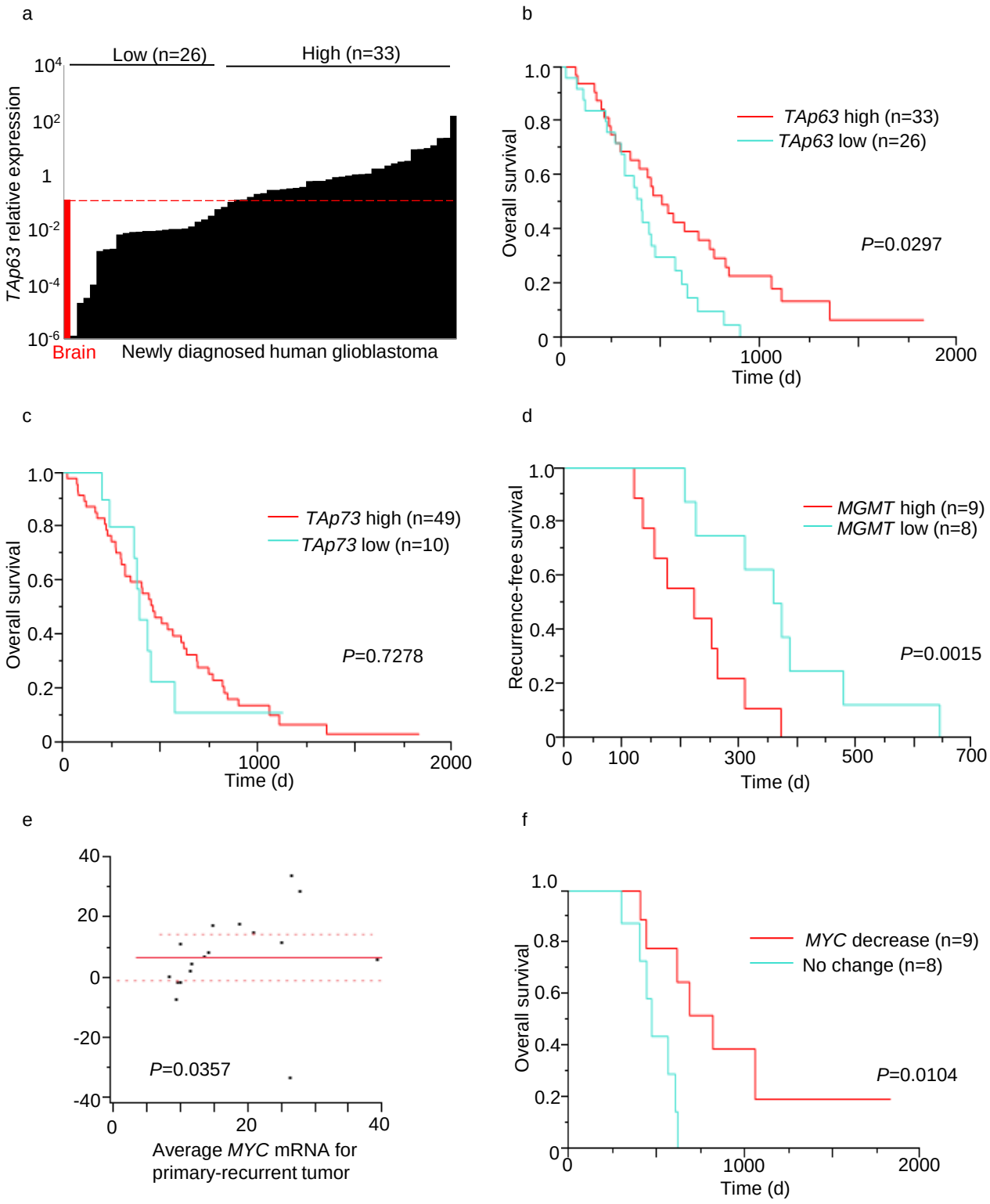

**Supplementary Figure 12**

TAp63 functions as a tumor suppressor in GBM *in vivo*.

a) U87MG cells were inoculated into the hind legs of mice and *TAp63* knockdown was performed by injecting small interference RNA (siRNA) against *TAp63* (siTAp63) into the palpable tumors once a week. Data are representative of two independent experiments. * *P*=0.0483, ** *P*=0.0002 by two-tailed *t*-tests (n=5).

b) Representative photographs of subcutaneous xenograft tumors derived from siControl- and siTAp63-transfected U87MG cells. Corresponding RT-PCR analysis of xenograft-derived RNA. Scale bar, 10 mm.

**Supplementary Figure 13**




**Supplementary Figure 13**

*TAp63* expression and *MYC* suppression are good prognosis factors in only GBM samples.

qRT-PCR for *TAp63* (a, b), *TAp73* (c), *MGMT* (d) and *MYC* (e, f)

**Supplementary Materials**

**Primer sequences**

**RT-PCR**

*TAp63*-sense GACCTGAGTGACCCCATGTG

*TAp63*-antisense GAGGAGCCGTTCTGAATCTG

*TAp63α*-sense GTCCCAGAGCACACAGACAA

*TAp63α*-antisense CACTCCCCCTCCTCTTTGAT

*TAp63β*-sense GTCCCAGAGCACACAGACAA

*TAp63β*-antisense AGACTTGCCAGATCCTGACAATGCT

*TAp63γ*-sense GTCCCAGAGCACACAGACAA

*TAp63γ*-antisense CGGGGCTCCACAAGCTCATTC

*MYC*-sense AGAGAAGCTGGCCTCCTACC

*MYC*-antisense CGCCTCTTGACATTCTCCTC

*CDKN1A*-sense ATGAAATTCACCCCCTTTCC

*CDKN1A*-antisense CCCTAGGCTGTGCTCACTTC

*BAX*-sense AGAGGATGATTGCCGCCGT

*BAX*-antisense CAACCACCCTGGTCTTGGAT

*MDM2*-sense GGTGGGAGTGATCAAAAGGA

*MDM2*-antisense ACACAGAGCCAGGCTTTCAT

*ACTB* -sense CAAGAGATGGCCACGGCTGCT

*ACTB* -antisense TCCTTCTGCATCCTGTCGGCA

**qRT-PCR**

*MYC*-sense AGGGTCAAGTTGGACAGTGTCA

*MYC*-antisense TGGTGCATTTTCGGTTGTTG

*MYCN*-sense CCTTCGGTCCAGCTTTCTCA

*MYCN*-antisense GGCCTTCTCATTCTTTACCAACTC

*TP53*-sense GCCTGAGGTTGGCTCTGACT

*TP53*-antisense CCATGCAGGAACTGTTACACATG

*TAp63*-sense TGGTGCGACAAACAAGATTG

*TAp63*-antisense ATAGGGACTGGTGGACGAGG

*ΔNp63*-sense CAATGCCCAGACTCAATTTAGTG

*ΔNp63*-antisense TGCTGGTCCATGCTGTTCAG

*TAp73*-sense CACCACGTTTGAGCACCTC

*TAp73*-antisense TGCTCAGCAGATTGAACTGG

*ΔNp73*-sense AAAAGCGAAAATGCCAACAAA

*ΔNp73*-antisense AGAGGCTCCGCAGCTAGTGA

*MGMT*-sense CCGTTTGCGACTTGGTACTTG

*MGMT*-antisense CCAGTGTGGTGCGTTTCATT

**ChIP-PCR assay**

R1-sense TAAAATGCCTTTGGGTGAGG

R1-antisense GCCCCACACATGATTTGTTT

R2-sense CCCTTTATAATGCGAGGGTCT

R2-antisense TGCCTCTCGCTGGAATTACT

R3-sense CTTGGAGTAGGGACCGCATA

R3-antisense AAAAGCCAAATGCCAACTTC

R4-sense AGCGACTCTGGTAAGCGAAG

R4-antisens AAACGCTAAAGCCCAAGGTT

*GAPDH*-sense TTCCCTCTTCTTGACTCACC

*GAPDH*-antisense CACAAAGGCACTCCTGGAAA

**ChIP-qPCR assay**

R1-sense TGCTATACACGCACCCCTTTC

R1-antisense TCCCTCCACCACCTCCAA

R3-sense TTTGCGGTGGGCAGAAA

R3-antisense GCGGTCCCTACTCCAAGGA