Supplemental Information

MIR93 (*MicroRNA -93*) Regulates Tumorigenicity and Therapy Response of Glioblastoma through Targeting Autophagy

Tianzhi Huang, Xuechao Wan, Angel A. Alvarez, C. David James, Xiao Song, Yongyong Yang, Namratha Sastry, Ichiro Nakano, Erik P. Sulman, Bo Hu and Shi-Yuan Cheng

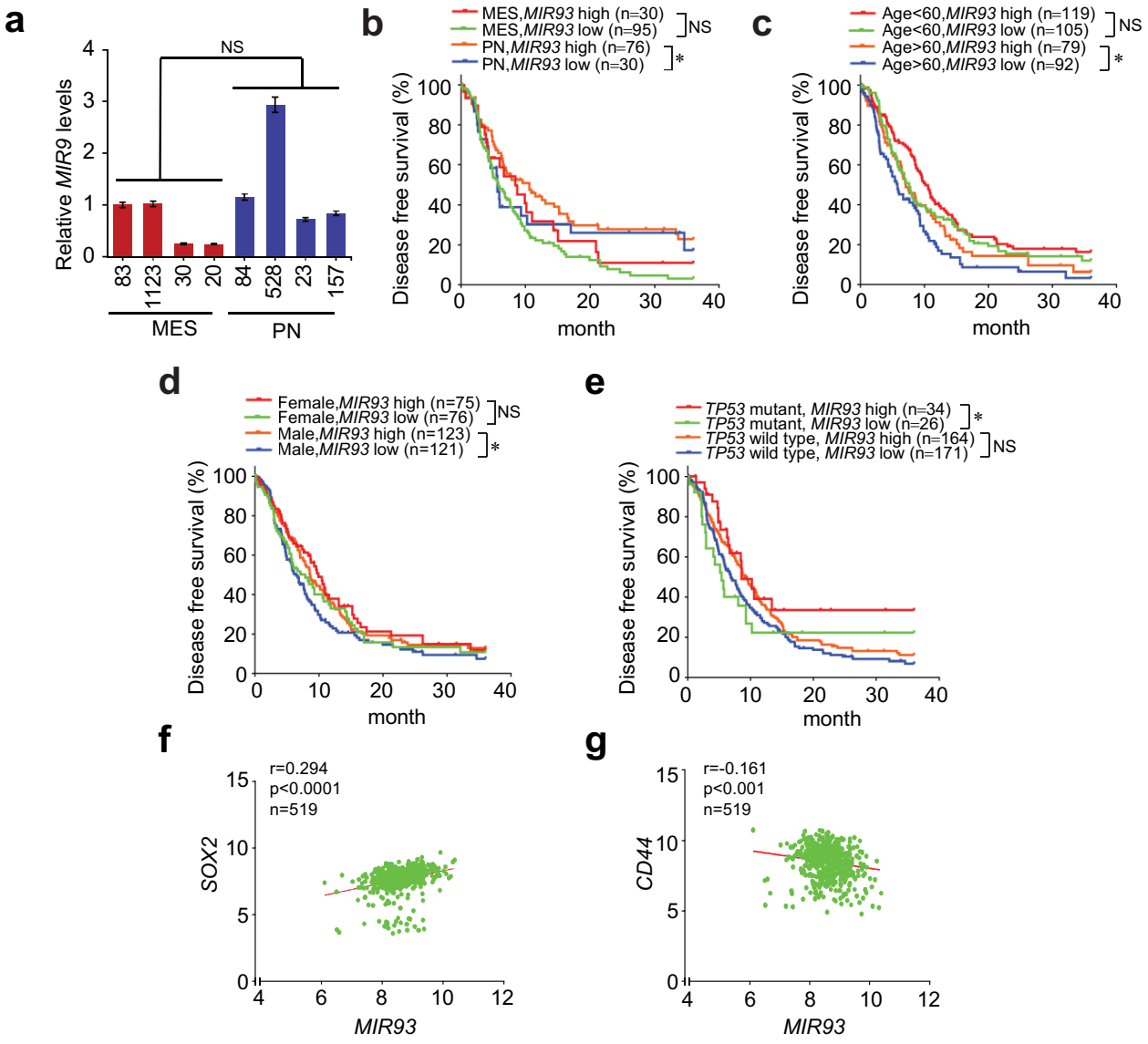


Figure S1. Analysis of *MIR9* expression in GSCs and *MIR93* expression in GBM prognosis and correlation with GBM subtype markers. (**a**) Relative expression levels of *MIR9* in 4 PN and 4 MES GSCs as indicated were determined with quantitative RT-PCR (qRT-PCR) assays. Data are representative from 3 independent experiments with similar results. (**b**-d), Multivariate analyses of Kaplan-Meier survival for *MIR93* expression versus GBM subtype of MES or PN (**b**), age (**c**, < or > age 60), sex (**d**), and *TP53* status (**e**) were performed using the TCGA miR dataset. (**e**) Correlation of expression levels between *MIR93* and PN marker *SOX2* (**f**), or MES marker *CD44* (**g**) in the TCGA datasets. NS, not significant, *, p<0.05.

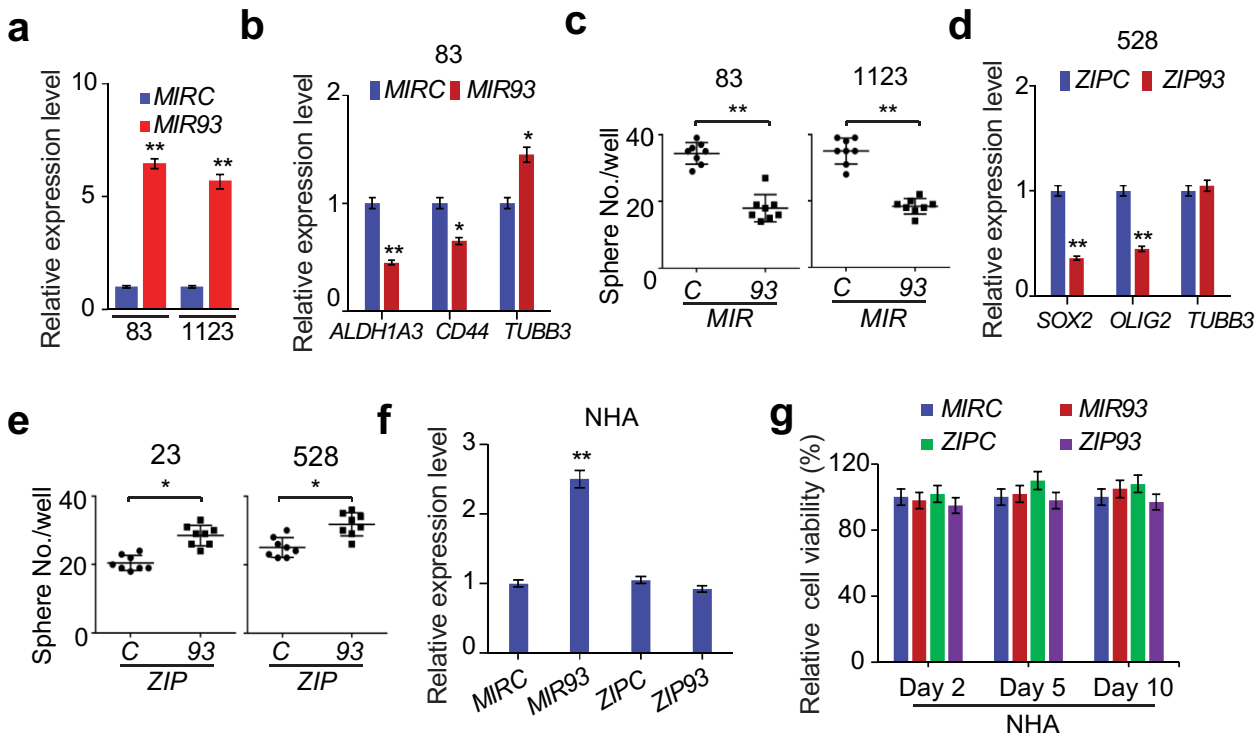


Figure S2. *MIR93* regulates stem-like properties of GSCs, but has no effects on normal human astrocytes (NHA). (**a**) qRT-PCR analyses for ectopic expression levels of *MIR93* in the indicated GSCs. (**b**) qRT–PCR analysis of MES GSC markers *ALDH1A3*, *CD44*, and differentiation marker *TUBB3*/*Tuj1* in GSC 83 with or without *MIR93* overexpression. (**c**) Sphere formation assays for GSC 83 and 1123 with or without overexpression of *MIR93*. (**d**) qRT-PCR analysis of PN markers *SOX2*, *OLIG2*, and the differentiation marker *TUBB3*/*Tuj1* in GSC 528 with or without *ZIP93* overexpression. (**e**) Sphere formation assays for GSC 23 and 528 with or without *ZIP93* (inhibition of *MIR93*). (**f**) qRT-PCR analyses for *MIR93* in NHA with indicated modifications. (**g**) Cell viability assays for NHA with indicated modifications. *MIRC*, a control miRNA. *ZIPC*, a control ZIP. *, $p<0.05$, **, $p<0.01$. Data are representative from 3 independent experiments with similar results.

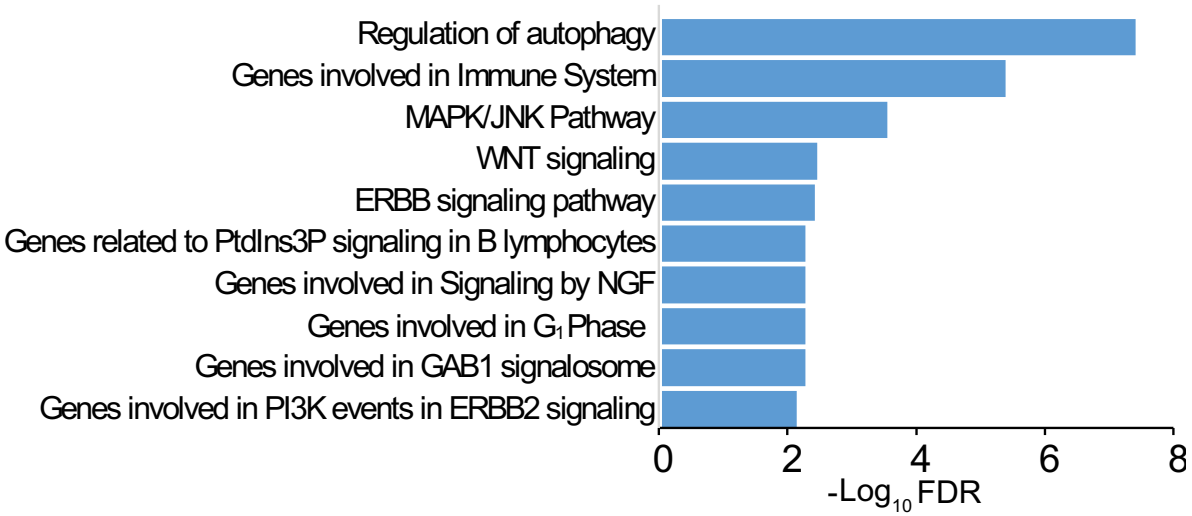


Figure S3. *MIR93* regulates autophagy signaling. Analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) for pathway enrichment for predicted *MIR93* target genes. FDR, false discovery rate.

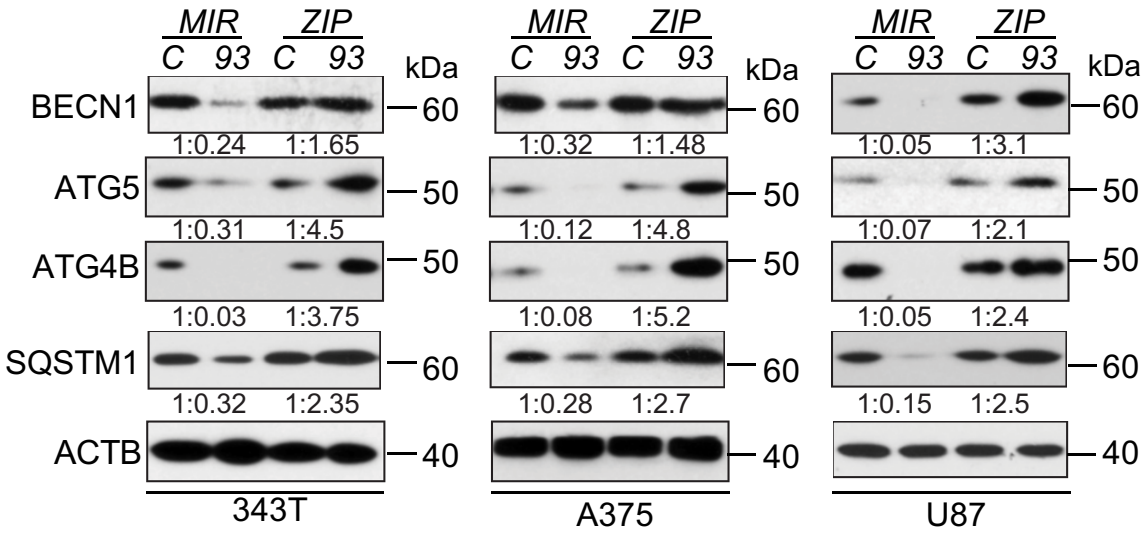


Figure S4. IB for BECN1, ATG5, ATG4B, and SQSTM1 in MIR- or ZIP-transduced lung cancer (343T), melanoma (A375), and glioma (U87) cells. Quantification in ratios of BECN1, ATG5, ATG4B, and SQSTM1 relative to ACTB are shown. Data are representative from 3 independent experiments with similar results.

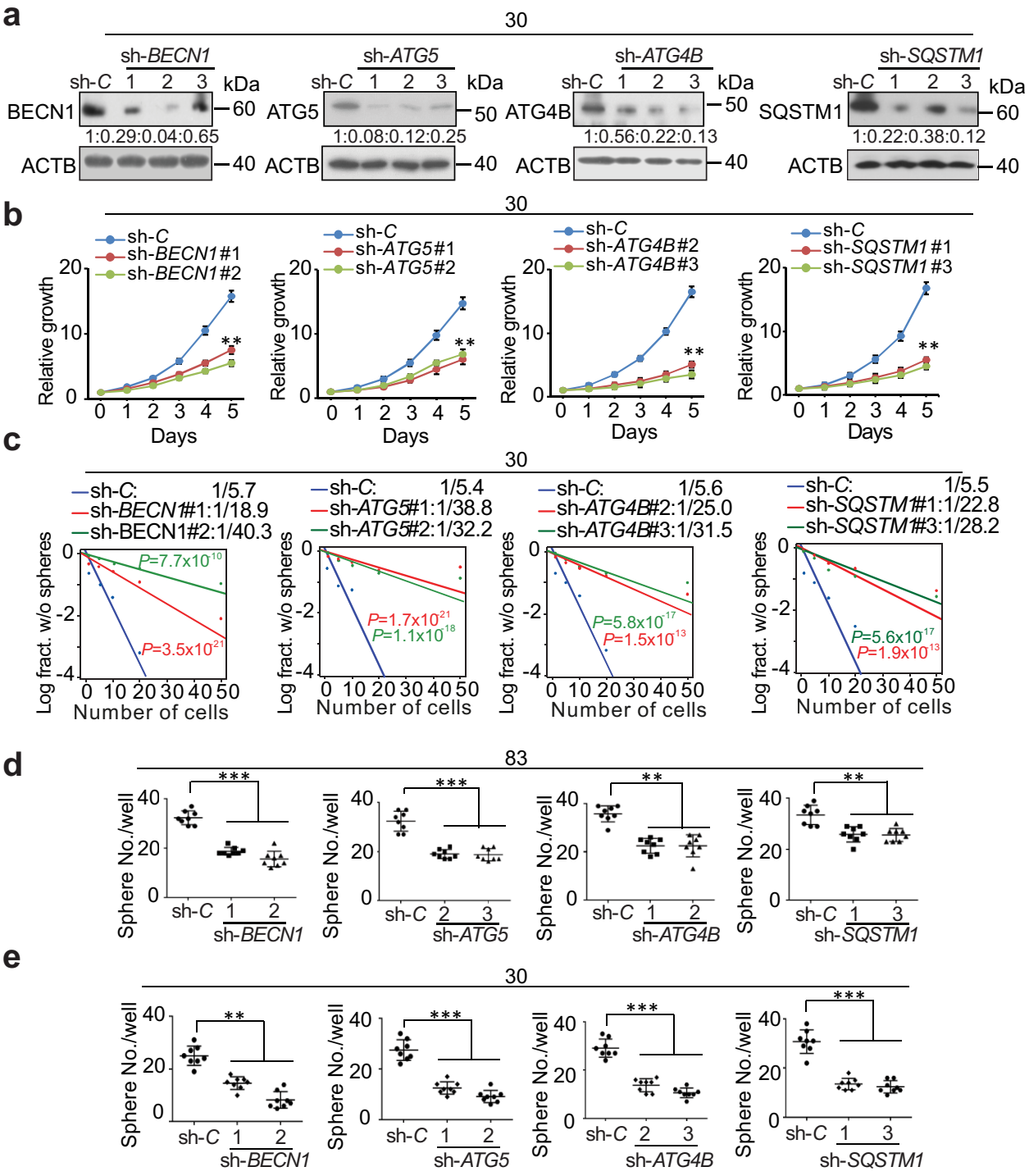
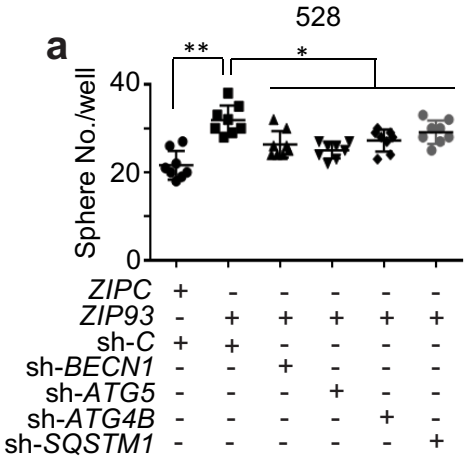


Figure S5. BECN1, ATG5, ATG4B, and SQSTM1 are downstream effectors of *MIR93* in GSCs. (**a**) IB for BECN1, ATG5, ATG4B, and SQSTM1 in GSC 30 with indicated shRNAs or a control shRNA (sh-*C*). Quantifications in ratios of BECN1, ATG5, ATG4B, and SQSTM1 relative to ACTB are shown. (**b** and **c**) Knockdown of *BECN1*, *ATG5*, *ATG4B* or *SQSTM1* by specific shRNAs or a control (sh-*C*) in GSC 30 decreased the cell growth (**b**) and sphere-forming frequencies (**c**) *in vitro*. (**d** and **e**) Sphere formation assays for

GSC 83 (**d**) and 30 (**e**) with the indicated shRNAs. **p<0.01, ***p<0.001. Data are representative from 3 independent experiments with similar results.



b

528

Group 1	Group 2	P value
ZIPC	*ZIP93*+sh-*C*	5.63E-09
ZIP93+sh-*C*	*ZIP93*+sh-*BECN1*	0.000883
ZIP93+sh-*C*	*ZIP93*+sh-*ATG5*	3.45E-05
ZIP93+sh-*C*	*ZIP93*+sh-*ATG4B*	8.01E-08
ZIP93+sh-*C*	*ZIP93*+sh-*SQSTM1*	0.00599

Figure S6. BECN1, ATG5, ATG4B, and SQSTM1 are essential for *MIR*93 function in GSCs. (**a**) Sphere formation assays for GSC 528 with the indicated modifications. (**b**) Overall test for differences in sphere-forming frequency among the indicated GSC 528 cells. *p<0.05, **p<0.01. Data are representative from 3 independent experiments with similar results.

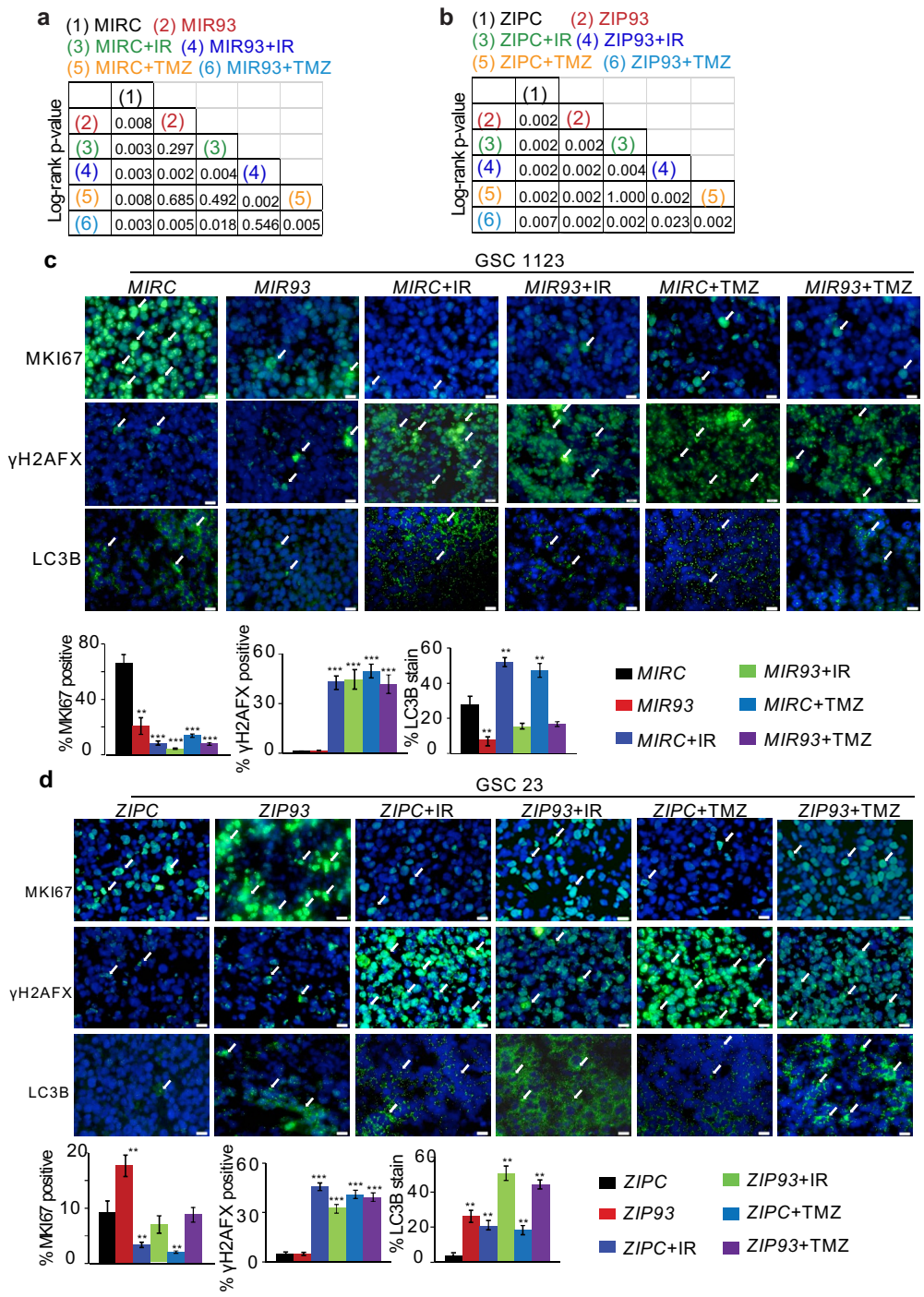


Figure S7. *MIR93* enhances anti-tumor activities of radiation (IR) and TMZ and on GSC brain tumor xenografts in mice. (**a** and **b**) Log-rank p value analyses of mice bearing GSC 1123 (A) or 23 (B) orthotopic xenografts with indicated treatments. (**c** and **d**) IF staining for MKI67, γH2AFX, and LC3B expression in brain sections with GSC 1123 (**c**) or 23 (**d**) brain tumor xenografts with indicated treatments (upper). Quantification of IF data (bottom). Scale bars: 20 μm. n = 3 sections.

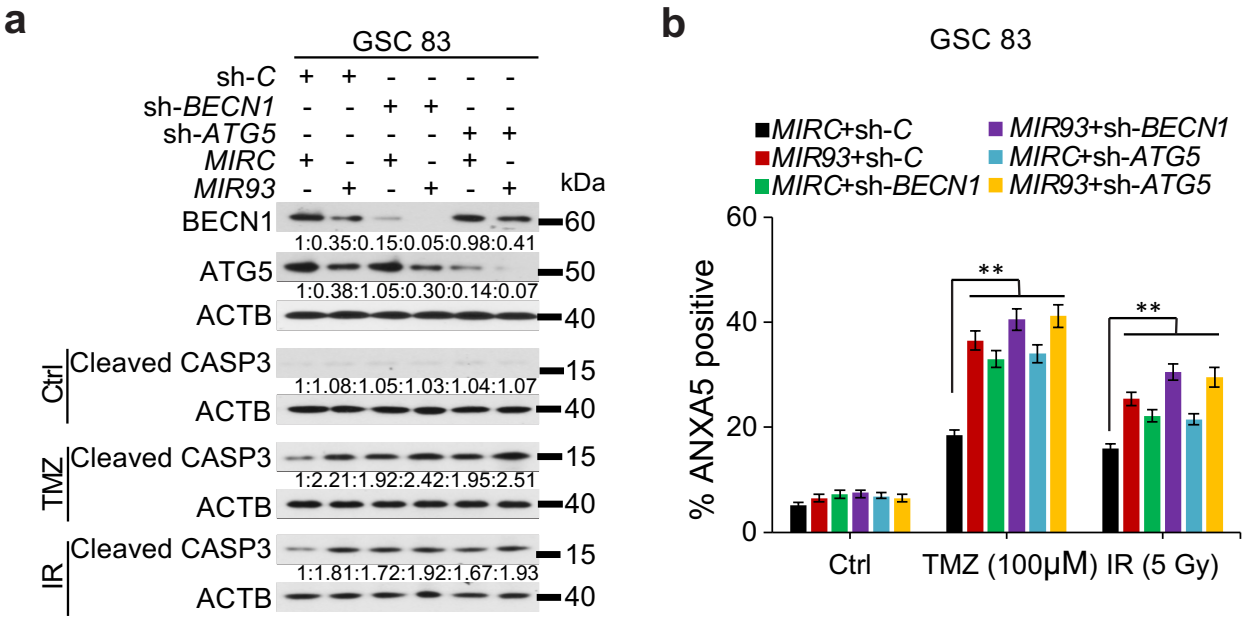


Figure S8. *MIR93* exerts additional proapoptotic effects on GSCs. (**a**) IB for BECN1, ATG5, cleaved CASP3 and ACTB in GSC 83 cells with the indicated treatments. Quantification in ratios of BECN1, ATG5, cleaved CASP3 relative to ACTB are shown. (**b**) Cell apoptosis with ANXA5 positive for GSC 83 cells with the indicated treatments for 72 h. **p < 0.01, ***p < 0.001. Error bar: ± SEM. Data are representative from 2 independent experiments with similar results.

Table S1. Primers for real-time PCR.

ULK1-F	AGCACGATTTGGAGGTCGC
ULK1-R	GCCACGATGTTTTCATGTTTCA
BECN1-F	GGTGTCTCTCGCAGATTCATC
BECN1-R	TCAGTCTTCGGCTGAGGTTCT
ATG14-F	TTCAGAGGCATAATCGCAAACT
ATG14-R	CCAGACGCTCATAATGACTTCTT
UVRAG-F	CTTGGGTCAGCAGATTCATGC
UVRAG-R	CATCGTAAGAATTGCGAACACAG
ATG16L1-F	TCTGGGACATTCGATCAGAGAG
ATG16L1-R	CCTTTCTGGGTTTAAGTCCAGG
ATG12-F	TAGAGCGAACACGAACCATCC
ATG12-R	CACTGCCAAAACACTCATAGAGA
ATG5-F	AGAAGCTGTTTCGTCCTGTGG
ATG5-R	AGGTGTTTCCAACATTGGCTC
ATG7-F	ATGATCCCTGTAACTTAGCCCA
ATG7-R	CACGGAAGCAAACAACTTCAAC
ATG4B-F	GGTGTGGACAGATGATCTTTGC
ATG4B-R	CCAACTCCCATTTGCGCTATC
SQSTM1-F	GACTACGACTTGTGTAGCGTC
SQSTM1-R	AGTGTCCGTGTTTCACCTTCC
TUBB3-F	GGCCAAGGGTCACTACACG
TUBB3-R	GCAGTCGCAGTTTTCACACTC
ALDH1A3-F	GCATGAGCCCATTGGTGTCT
ALDH1A3-R	CGCAGGCTTCAGGACCAT
CD44-F	TGGCACCCGCTATGTCCAG
CD44-R	GTAGCAGGGATTCTGTCTG
SOX2-F	CCCTGCTGAGAATAGGACAT
SOX2-R	CCCTGCAGTACAACTCTATG
OLIG2-F	CTCCTCAAATCGCATCCAGA
OLIG2-R	AGAAAAAGGTCATCGGGCTC

Table S2. Primers for psi-Check reporter constructs.

BECN1-WT-S	TCGAGAGAAAAAATCCACAAAAGCCACTTTATTTTAAAATGC
BECN1-WT-AS	GGCCGCATTTTAAAATAAAGTGGCTTTTGTGGATTTTTTCTC
SQSTM1-WT-S	TCGAGAATTAAATGGCATCAGCACTTTAACCAATGACGTTTGC
SQSTM1-WT-AS	GGCCGCAAACGTCATTGGTTAAAGTGCTGATGCCATTTAATTC
ATG4B-WT-S	TCGAGCTTGGGGAGCGTGGGCACTTTTCTCATGAGCAGCGC
ATG4B-WT-AS	GGCCGCGCTGCTCATGAGAAAAGTGCCCACGCTCCCCAAGC
ATG5-WT-S	TCGAGCAATATAAGGAAAGTTTGCTGCACTTTATTACCAAGCCGC
ATG5-WT-AS	GGCCGCGGCTTGGTAATAAAGTGCAGCAAACTTTCCTTATATTGC
BECN1-mutant-S	TCGAGAGAAAAAATCCACAAAAGCACAGGGCTTTTAAAATGC
BECN1-mutant-AS	GGCCGCATTTTAAAAGCCCTGTGCTTTTGTGGATTTTTTCTC
SQSTM1-mutant-S:	TCGAGAATTAAATGGCATCAACAGGGCACCAATGACGTTTGC
SQSTM1-mutant-AS	GGCCGCAAACGTCATTGGTGCCCTGTTGATGCCATTTAATTC
ATG4B-mutant-S	TCGAGCTTGGGGAGCGTGGACAGGGCCTCATGAGCAGCGC
ATG4B-mutant-AS	GGCCGCGCTGCTCATGAGGCCCTGTCCACGCTCCCCAAGC
ATG5-mutant-AS	GGCCGCGGCTTGGTAAGCCCTGTAGCAAACTTTCCTTATATTGC
ATG5-mutant-S	TCGAGCAATATAAGGAAAGTTTGCTACAGGGCTTACCAAGCCGC