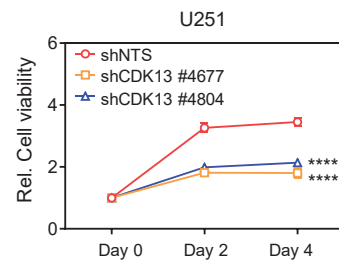
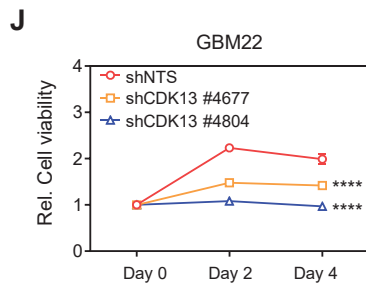
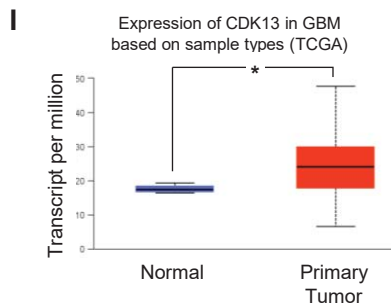
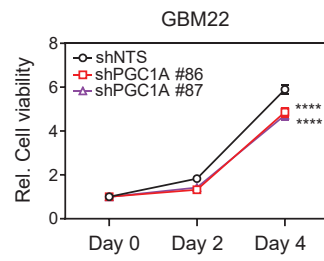
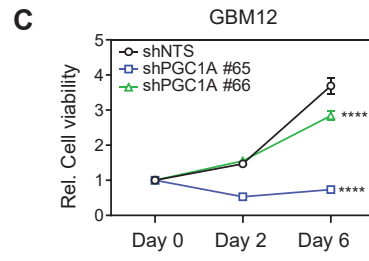
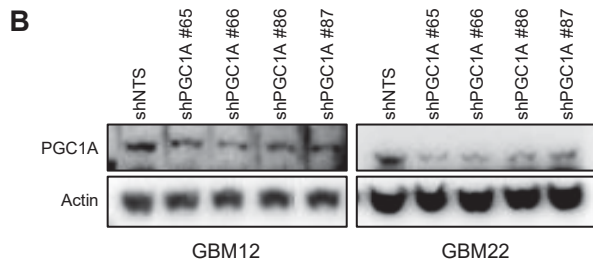
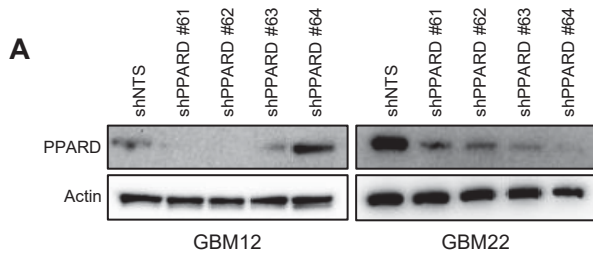


H IC₅₀ values for SR in various GBM cell lines.

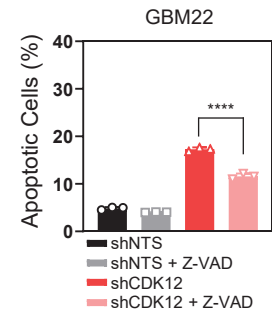
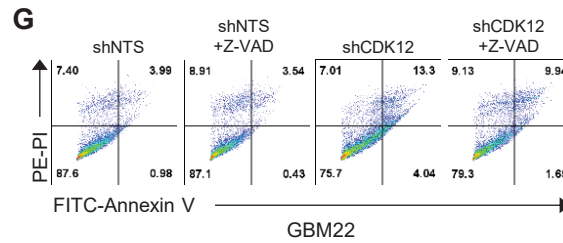
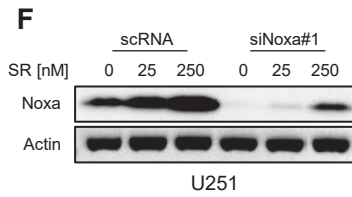
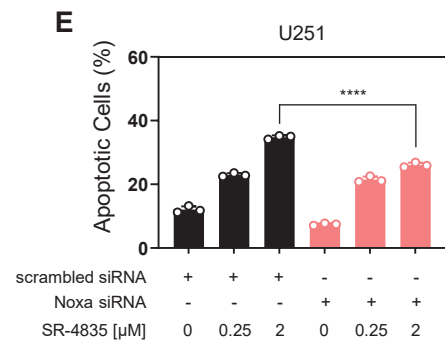
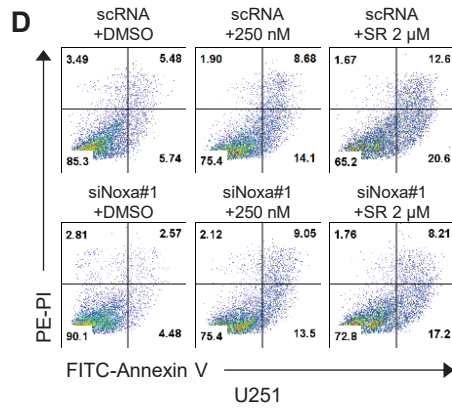
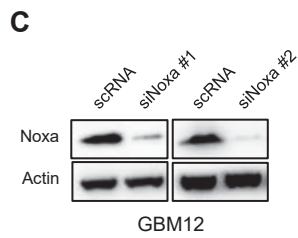
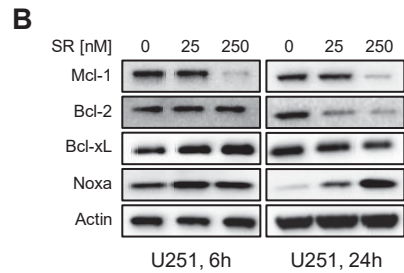
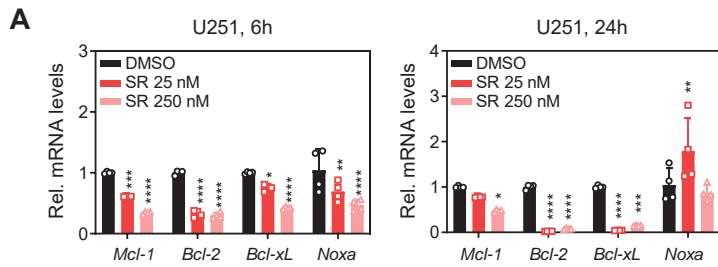
Cell lines	IC ₅₀ [nM]
GBM12	20.01
GBM22	861
GBM39	513
GBM43	224.6
GBM67	43.1
KNS42	402.6
U251	259.2
NCH644	53.84



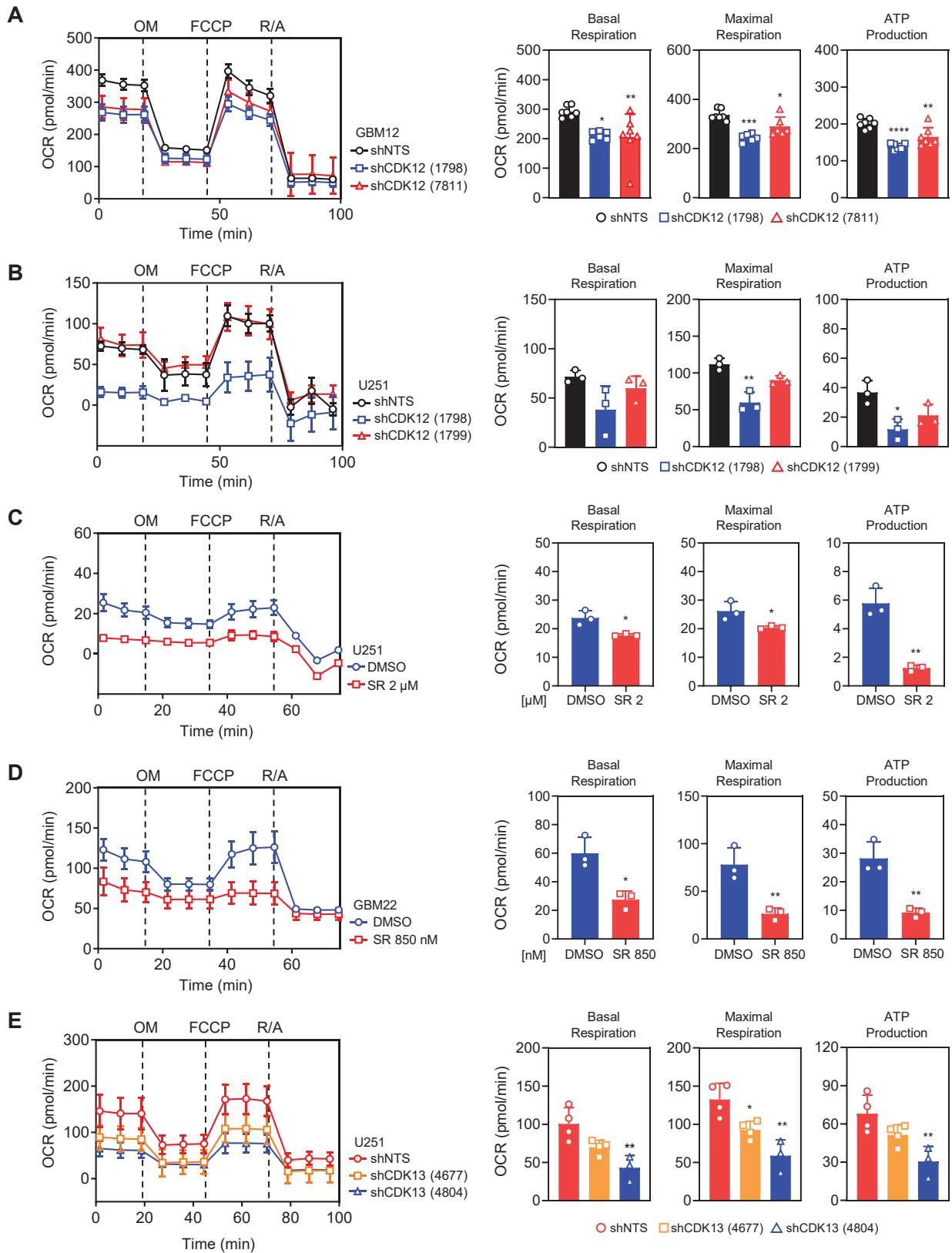
Supplemental Figure 1. CDK12 is a therapeutic target in GBM. (A) Kaplan-Meier curve in GBM TCGA cohort (TCGA_GBM, RNA-SEQ, CDK12, GBM, Mesenchymal, Gender: all, IDH: All, Recurrence: all, MGMT: all) comparing survival rates of GBM patient samples divided into high-CDK12 and low-CDK12 based on CDK12 mRNA expression (HR = 0.61, 95% CI: 0.32–1.17; log-rank $P = 0.1296$; Wilcoxon $P = 0.2184$). High-CDK12 levels correlate with a worse overall survival in GBM patients. (B) CDK12 mRNA levels were quantified using qPCR and compared between astrocytes and multiple GBM cells. Data are presented as mean \pm SD. *** $p < 0.001$; **** $p < 0.0001$; ns. not significant, one-way ANOVA with Dunnett's multiple comparisons test. (C) CDK12 expression validation in TCGA database on GBM samples by UALCAN (<https://ualcan.path.uab.edu/cgi-bin/ualcan-res-cbttc.pl>). (D) GBM12, GBM22, and NCH644 cells expressing shNTS or shCDK12 cells were analyzed by flow cytometry following staining with Annexin V/PI. (E-F) KNS42, NCH644, and GBM39 cells were treated with SR for 72 h and were analyzed by flow cytometry with Annexin V/PI staining. Bar graph shows percentage of apoptosis cells. Data are presented as mean \pm SD. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons test. (G) Western blot shows CDK12 protein levels after SR treatment in GBM12 cells. Actin was used as a loading control. (H) Multiple GBM cells; GBM12, GBM22, GBM39, GBM43, GBM67, KNS42, U251, and NCH644 were treated with SR for 72 h, then performed to cell viability assays. There are listed IC₅₀ values, respectively. (I) CDK13 expression validation in TCGA database on GBM samples by UALCAN. (J) Stable shCDK13 cells (no. 4677, 4804), or shNTS cells were assessed for cell viability for 0, 2, and 4 days. Data are presented as mean \pm SD. **** $p < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons test.

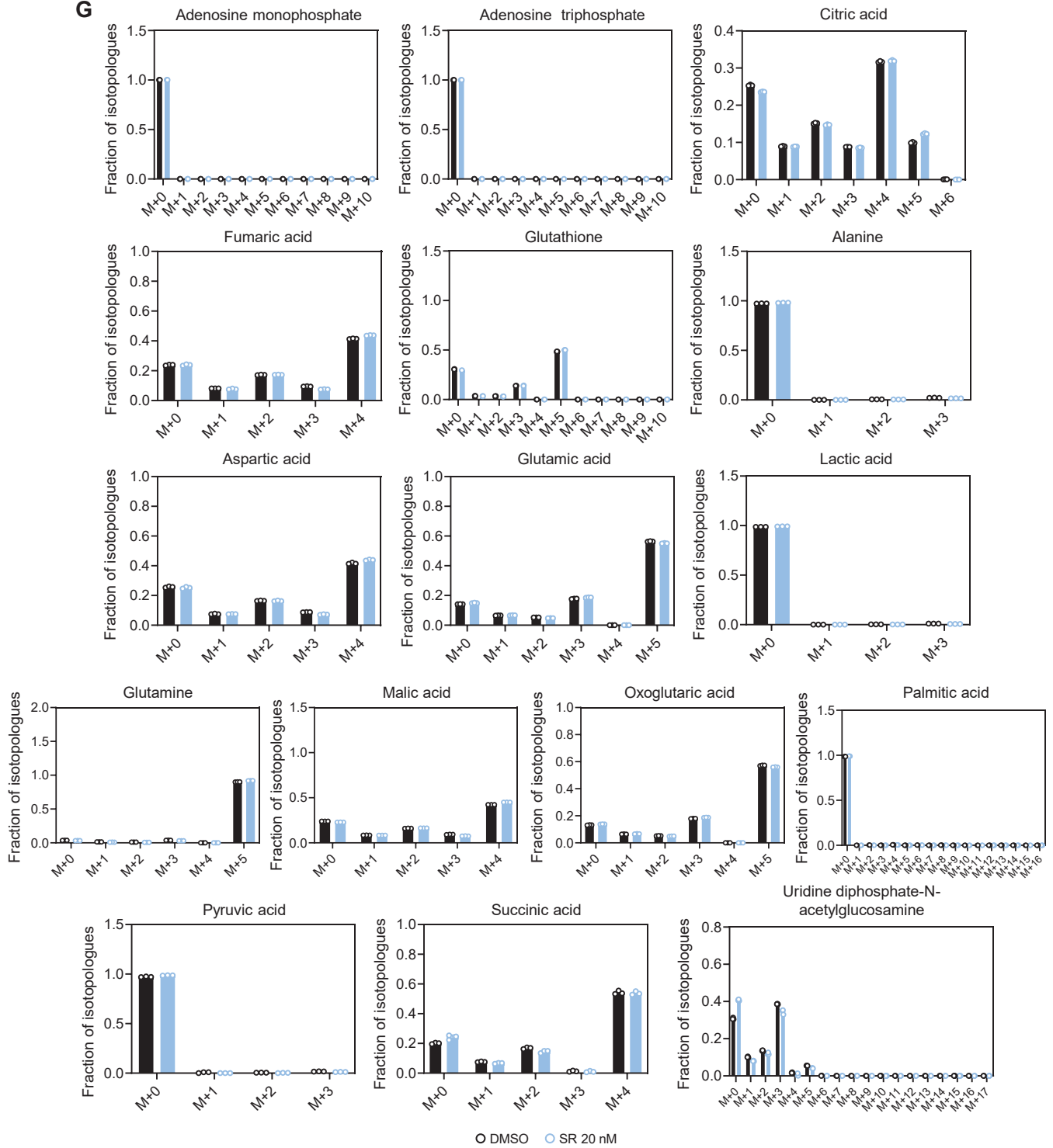


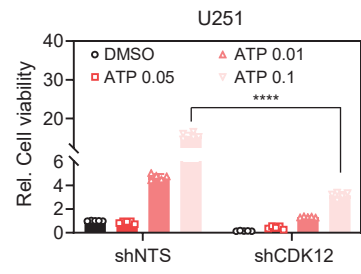
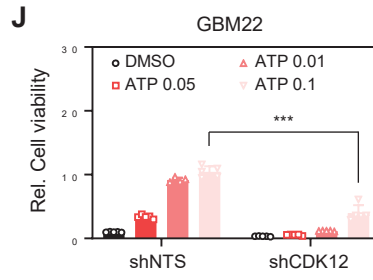
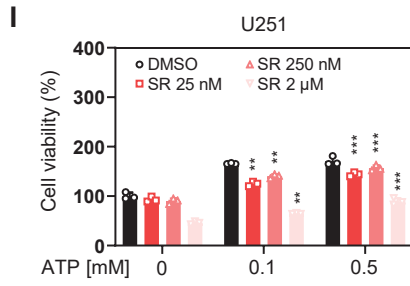
Supplemental Figure 2. PGC1A is a driver of proliferation in PDX GBM cultures. (A) Western blot shows PPARD protein levels in shPPARD (no. 61,62,63,64) cells compared to shNTS cells in GBM12, and GBM22 cells. (B) Western blot shows PGC1A protein levels in shPGC1A (no. 65,66,86,87) cells compared to shNTS cells in GBM12, and GBM22 cells. (C) Cell viability of GBM12, GBM22-shPGC1A cells compared to shNTS cells was assessed for 0, 2, 4, or 6 days. Data are presented as mean \pm SD. ****p < 0.0001, one-way ANOVA with Dunnett's multiple comparisons test.



Supplemental Figure 3. Noxa, a mediator of cell death, plays a crucial role in the response of GBM cells to the treatment of SR-4835. (A) The qPCR analysis in apoptosis related genes of U251 cells treated with increasing concentrations of SR for 6 h and 24 h. qPCR data are presented as mean \pm SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons test. (B) U251 cells were treated with SR for 6 h or 24 h cell lysates were subjected to standard western blotting with the indicated antibodies the anti-apoptotic Bcl-2 family members (Mcl-1, Bcl-xL, and Bcl-2) and pro-apoptotic protein (Noxa). (C) GBM12 cells were transfected with scRNA or siNoxa#1, 2, and detected by western blot with anti-Noxa. (D-E) U251 cells were transfected with siNoxa and treated with SR for 72 h and analyzed by flow cytometry using Annexin V/PI staining. Bar graph shows percentage of apoptotic cells. Data are presented as mean \pm SD. **** $p < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons test. (F) U251 cells were transfected with scRNA or siNoxa#1, and treated with SR, and detected by western blot with anti-Noxa. (G) GBM22-shNTS and GBM22-shCDK12 (1798) cells were treated with Z-VAD (50 μ M). Apoptosis was assessed by flow cytometry using Annexin V/PI staining. The percentage of apoptotic cells was quantified. Data are presented as mean \pm SD. **** $p < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons test.



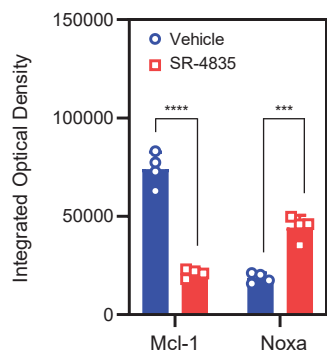
G



Supplemental Figure 4. CDK12 regulates the oxygen consumption rate in GBM22 and U251 cells.

(A) Seahorse mitochondrial stress assay was conducted on GBM12-shNTS, shCDK12 (1798, 7811) cells and (B) U251-shNTS, shCDK12 (1798, 1799) cells. Data are presented as mean \pm SD. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$, one-way ANOVA with Dunnett's multiple comparisons test. (C) Seahorse mitochondrial stress assay of U251 cells treated DMSO or SR 2 μ M for 24 h. Data are presented as mean \pm SD. * $p < 0.05$; ** $p < 0.01$, unpaired 2-tailed t test. (D) Seahorse mitochondrial stress assay of GBM22 cells treated DMSO or SR-4835 850 nM for 24 h. Data are presented as mean \pm SD. * $p < 0.05$; ** $p < 0.01$, unpaired 2-tailed t test. (E) Seahorse mitochondrial stress assay was conducted on U251-shNTS, shCDK13 (4677, 4804) cells. Data are presented as mean \pm SD. * $p < 0.05$; ** $p < 0.01$, one-way ANOVA with Dunnett's multiple comparisons test. (F) For glucose tracing, GBM12 cells were treated with SR 20 nM in DMEM containing 25 mM U-¹³C₆-glucose, 4 mM glutamine, and 1.5% dialyzed FBS for 24 h. Shown are fractions of the isotopologues for each metabolite (n = 3, each group). (G) For glutamine tracing, GBM12 cells were treated with SR 20 nM in DMEM containing 25 mM glucose, 4 mmol/L U-¹³C₅ Glutamine, and 1.5% dialyzed FBS for 24 h. Shown are fractions of the isotopologues for each metabolite (n = 3, each group). (H) For glucose tracing, GBM22-shNTS and shCDK12 (1798) cell lines conducted glucose tracing in DMEM containing 25 mM U-¹³C₆-glucose, 4 mM glutamine, and 1.5% dialyzed FBS for 24 h. Shown are fractions of the isotopologues for each metabolite (n = 3, each group). (I) U251 cells were treated with SR at various concentrations and were evaluated for proliferation in the presence or absence of ATP for a 48-hour period. Data are presented as mean \pm SD. ** $p < 0.01$; *** $p < 0.001$, two-way ANOVA with Turkey's multiple comparisons test. (J) GBM22-shNTS and shCDK12 (1798) cells were evaluated for proliferation in the presence or absence of ATP for a 48-hour period. Loss of CDK12 in GBM22 or U251 cells was treated with ATP at various concentrations. Data are presented as mean \pm SD. *** $p < 0.001$; **** $p < 0.0001$, two-way ANOVA with Turkey's multiple comparisons test.

A



Supplemental Figure 5. Immunohistochemical quantification of Mcl-1 and Noxa following SR-4835 treatment. (A) Quantification of IHC staining intensity with Mcl-1, and Noxa. A presented as a bar graph. Data are presented as mean \pm SD. *** $p < 0.001$; **** $p < 0.0001$, unpaired two-tailed t test.

Supplemental Table 1. Primer sequences for qPCR.

Gene list	5' -> 3'
qPCR primer: <i>18S</i> forward	ACCCGTTGAACCCCATTCGTGA
qPCR primer: <i>18S</i> reverse	GCCTCACTAAACCATCCAATCGG
qPCR primer: <i>CDK12</i> forward	CGAACTCAGCAAAATGGCTCCTC
qPCR primer: <i>CDK12</i> reverse	TTGGATGGAGGTGGCTCTTCGA
qPCR primer: <i>CPT1A</i> forward	GATCCTGGACAATACCTCGGAG
qPCR primer: <i>CPT1A</i> reverse	CTCCACAGCATCAAGAGACTGC
qPCR primer: <i>PGC1A</i> forward	CCAAAGGATGCGCTCTCGTTCA
qPCR primer: <i>PGC1A</i> reverse	CGGTGTCTGTAGTGGCTTGACT
qPCR primer: <i>PPARD</i> forward	GGCTTCCACTACGGTGTTTCATG
qPCR primer: <i>PPARD</i> reverse	CTGGCACTTGTTGCGGTTCTTC
qPCR primer: <i>ACADSB</i> forward	GCCACCTATTTGCCTCAGCTCA
qPCR primer: <i>ACADSB</i> reverse	GCTCAGCACTGCTGATCCACAT
qPCR primer: <i>ACADVL</i> forward	TAGGAGAGGCAGGCAAACAGCT
qPCR primer: <i>ACADVL</i> reverse	CACAGTGGCAAACCTGCTCCAGA
qPCR primer: <i>CPT2</i> forward	GCAGATGATGGTTGAGTGCTCC
qPCR primer: <i>CPT2</i> reverse	AGATGCCGCAGAGCAAACAAGTG
qPCR primer: <i>ETFDH</i> forward	GGAAACACCATCCTAGCATTCCGG
qPCR primer: <i>ETFDH</i> reverse	CCACCAGGAAAGGTGAGTTTTGG
qPCR primer: <i>HADHB</i> forward	CACAGTCTAGCCAAGAAGGCAC
qPCR primer: <i>HADHB</i> reverse	CATCTGCTCCAGTGAGGAAGGA
qPCR primer: <i>Mcl-1</i> forward	CCAAGAAAGCTGCATCGAACCAT
qPCR primer: <i>Mcl-1</i> reverse	CAGCACATTCCTGATGCCACCT
qPCR primer: <i>Bcl-2</i> forward	ATCGCCCTGTGGATGACTGAGT
qPCR primer: <i>Bcl-2</i> reverse	GCCAGGAGAAATCAAACAGAGGC
qPCR primer: <i>Bcl-xL</i> forward	GCCACTTACCTGAATGACCACC
qPCR primer: <i>Bcl-xL</i> reverse	AACCAGCGGTTGAAGCGTTCCT
qPCR primer: <i>Noxa</i> forward	CTGGAAGTCGAGTGTGCTACTC
qPCR primer: <i>Noxa</i> reverse	TGAAGGAGTCCCCTCATGCAAG