Supplementary Table 1. qPCR primers used in the gene expression study

| Gene	Forward primer	Reverse primer
ATP1B2	GAGGACGCACCAGTTTATGGG	GGGGTATGGTCGGAGACAGT
Nestin	GCAGCAGGAAATATGGGAAG	TCTCATGGCTCTGGTTTTCC
SOX2	CAAGATGCACAACTCGGAGA	GCTTAGCCTCGTCGATGAAC
ATP1A1	AAAGGTGTGGGCATCATCTC	GCTTGCTTGGACACATCTGA
18S rRNA	GTAACCCGTTGAACCCCATT	CCATCCAATCGGTAGTAGCG

Supplementary Table 2. The target sequences of short hairpin RNA for ATP1B2

| shRNA	Target sequence
shATP1B2-1#	GAATGTAGAATGTCGCATCAA
shATP1B2-2#	GAACCTTGATGTCATTGTCAA
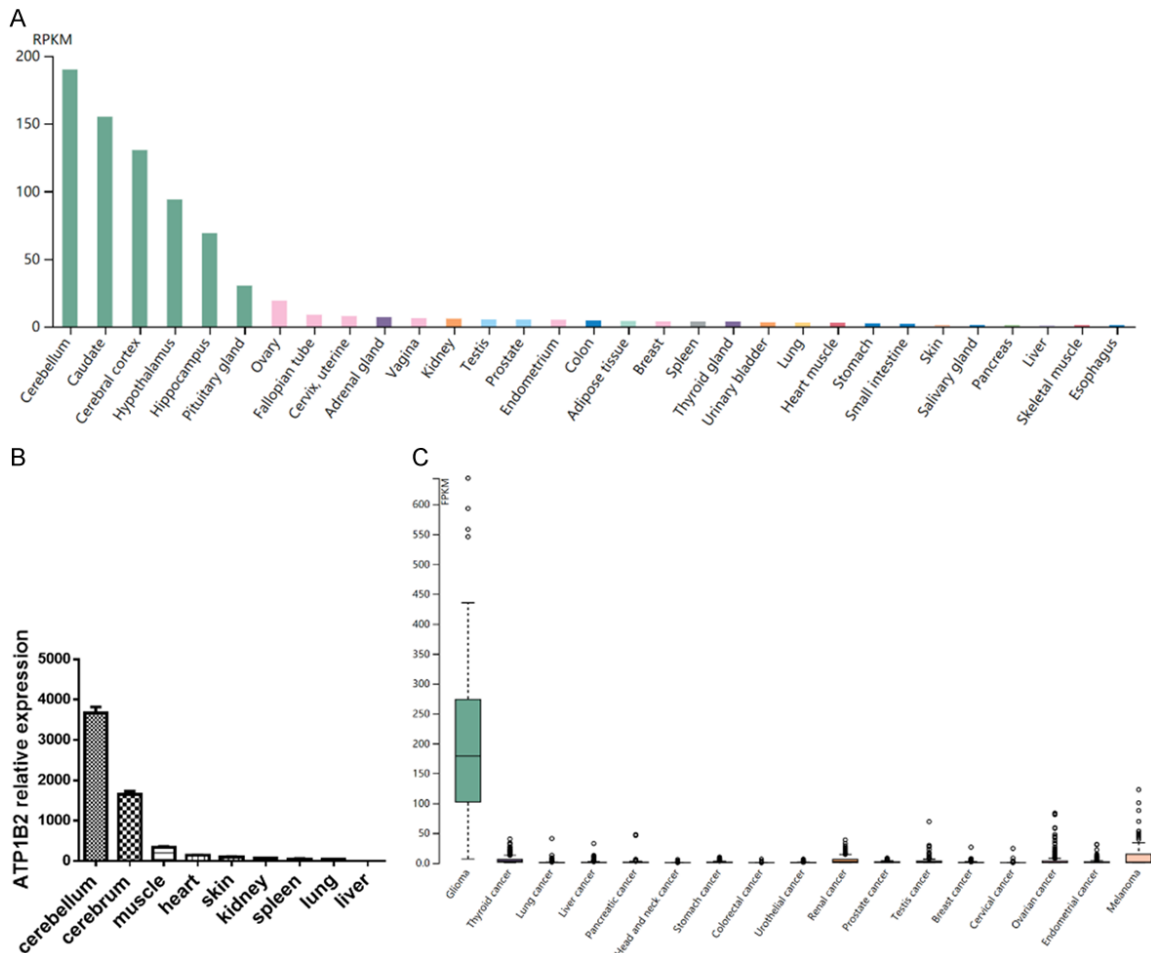
Supplementary Figure 1. ATP1B2 is expressed exclusively in human and mouse nervous system and GBM. A. ATP1B2 RNA expression levels from Human Protein Atlas (HPA). B. Quantitative polymerase chain reaction (qPCR) analysis of ATP1B2 expression levels in mouse tissues. ATP1B2 expression level was normalized to 18S rRNA. Data are means ± SD. C. RNA-seq data of ATP1B2 in 17 cancer types reported as median fragments per kilobase of exon per million reads (FPKM), generated by The Cancer Genome Atlas (TCGA).
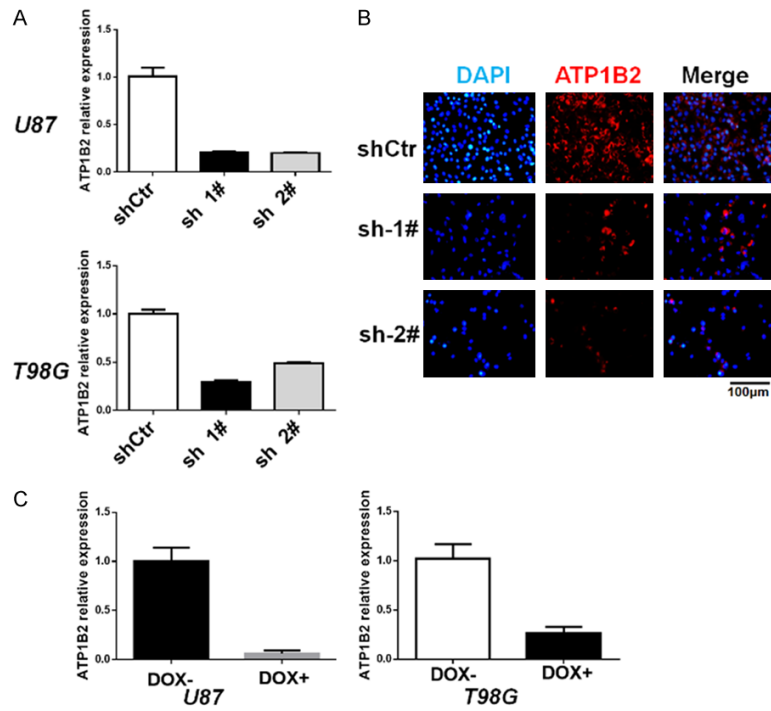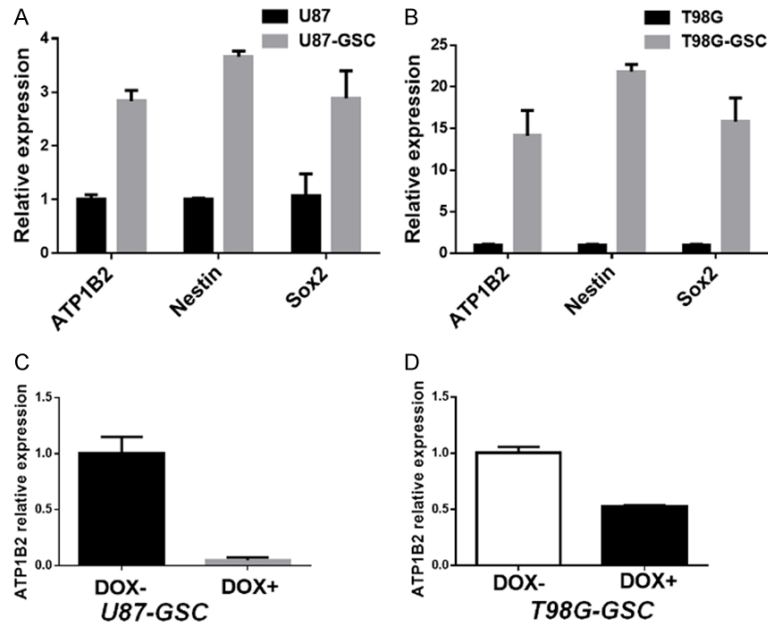
Supplementary Figure 2. Knockdown efficiency of ATP1B2 using short hairpin RNAs (shRNAs). (A, B) sh-1# and sh-2# knockdown efficiency in U87 and T98G cells using (A) qPCR and (B) immunofluorescence. (C) Doxycycline-inducible sh-1# knockdown efficiency in U87 and T98G by qPCR.
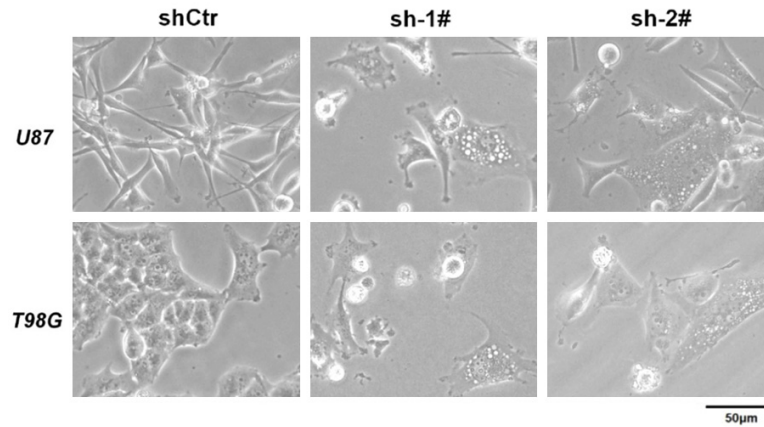

Supplementary Figure 3. The morphology of U87 and T98G cells after ATP1B2 was down-regulated.

Supplementary Figure 4. ATP1B2 and GSC markers are upregulated in GSCs and knockdown efficiency of ATP1B2 in GSCs. (A, B) Quantitative polymerase chain reaction (qPCR) analysis of ATP1B2 and GSC marker expression levels in (A) U87-GSC and (B) T98G-GSC cells. The expression level was normalized to 18S rRNA. Data are means ± SD. (C, D) Doxycycline-inducible shATP1B2-1# knockdown efficiency in (C) U87-GSC and (D) T98G-GSC using qPCR.
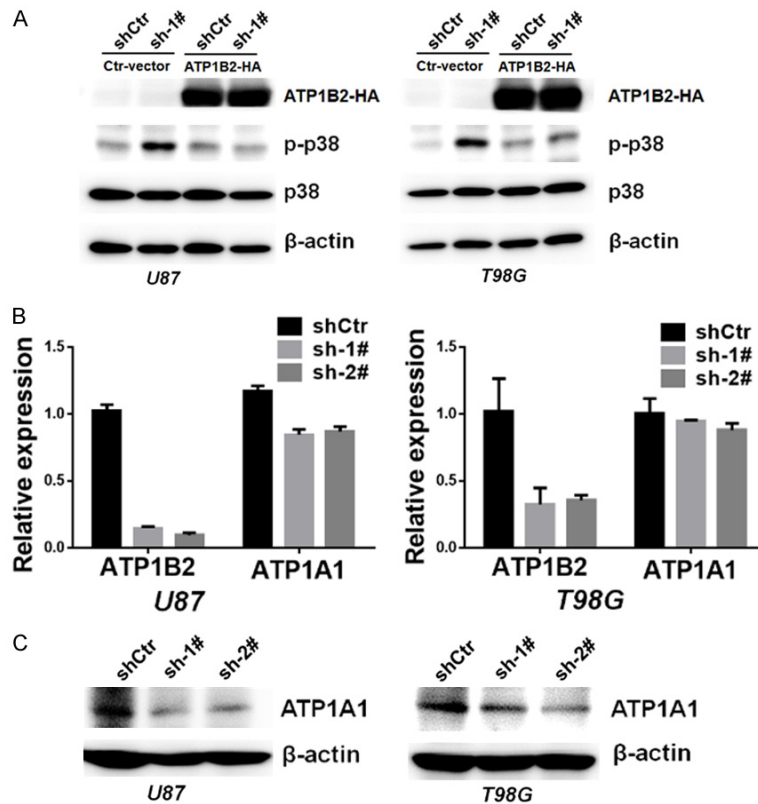
Supplementary Figure 5. ATP1B2 downregulation increases activates p38 mitogen-activated protein kinase (MAPK) pathway and decreases ATP1A1 expression. A. Downregulation of p38 activation in U87 and T98G cells with HA-tagged mutated human ATP1B2, which is not targeted for degradation by sh-1#. B. Quantitative PCR analysis of ATP1A1 expression after ATP1B2 downregulation in U87 and T98G cells. C. ATP1A1 expression were detected by western blotting using specific antibodies in U87 and T98G cells.