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Supplemental information

Low-intensity pulsed ultrasound-generated singlet oxygen induces telomere damage leading to glioma stem cell awakening from quiescence

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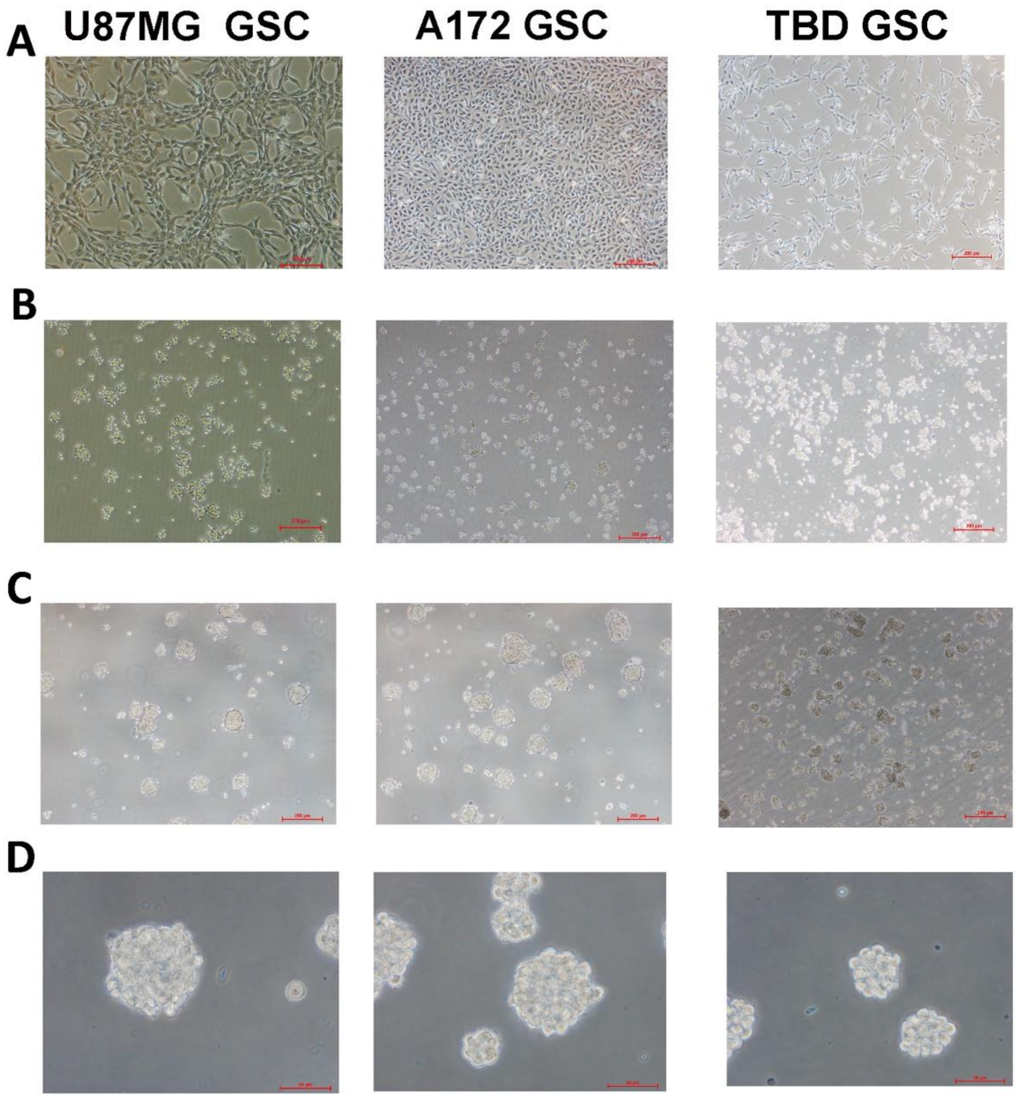
Supplemental Information

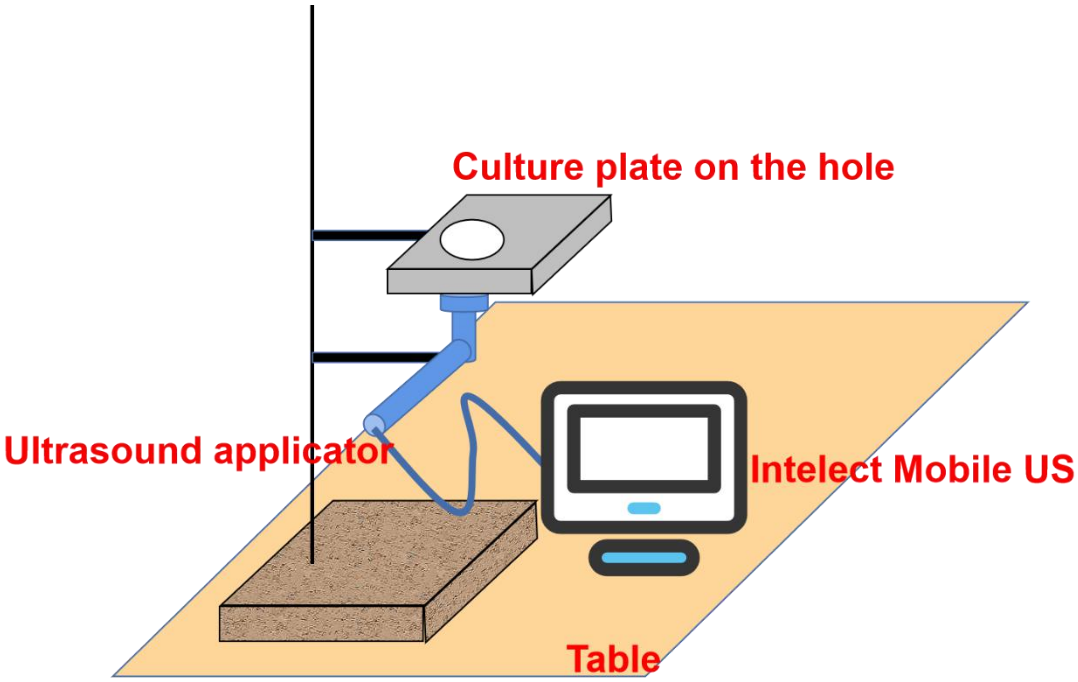
Supplemental Figures:

Figure S1 U87MG glioma stem cell (GSC)，A172 GSC culture and TBD GSC. Related to STAR Methods.



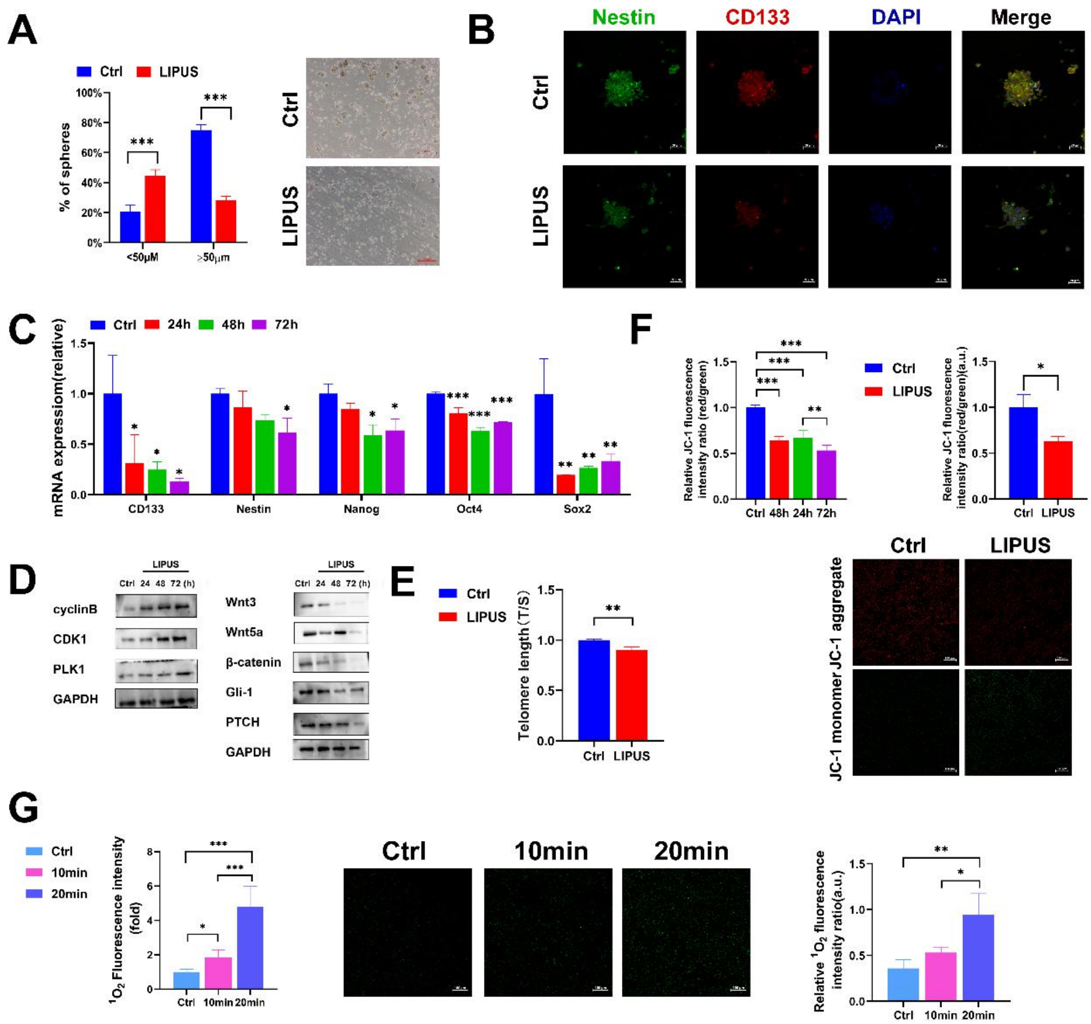
(A)U87MG, A172 and TBD cells were attached to the wall. (B) On the first day, U87MG, A172 and TBD cells started to be suspended and several small cells started to grow in clusters. (C-D) After the second passaging, U87MG GSCs, A172 GSCs and TBD GSCs grew uniformly into spheres.

Figure S2 The concrete set up of the ultrasound treatment. Related to STAR Methods.



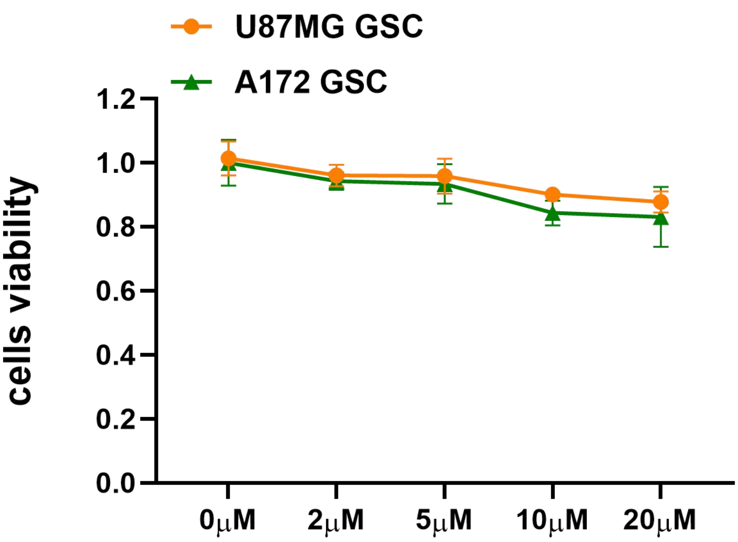
Ultrasound stimulation was delivered by 2776 Intelect Mobile US, DJO GLOBAL Chattanooga. The 6-well plate was placed directly above the wells when the cells were sonicated.

Fig S3 LIPUS reduced the stemness of TBD GSCs. Related to Figure 1-4.



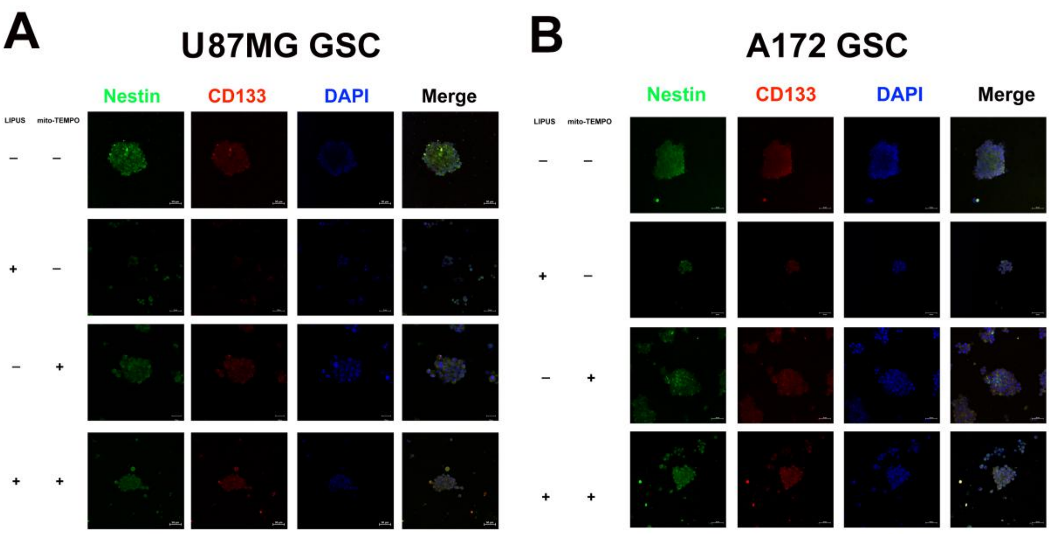
(A) GSC sphere formation ratio with LIPUS treatment. (B) Images of GSC spheres which expressed stem cell markers Nestin (green) and CD133 (red) by immunofluorescence. DAPI (blue) for nuclei. (C) qPCR analysis of mRNA expressions in GSCs with LIPUS treatment in 24 h, 48 h and 72 h respectively. (D) Western blotting analysis expression of the key proteins of the G2/M checkpoint and the key nodes of the Hedgehog (Hh) and Wnt/β-catenin pathways. (E) qPCR analysis of the telomere length (T/S) in GSCs with LIPUS treatment. (F) Mitochondrial membrane potential analysis of GSCs with LIPUS treatment. (G) $^1O_2$ generation in GSCs with LIPUS treatment for 10 minutes and 20 minutes.

Figure S4 IC50 of mito-TEMPO. Related to Figure 5.



The growth rate of GSCs was determined by the cell counting kit-8 (CCK8) assay.

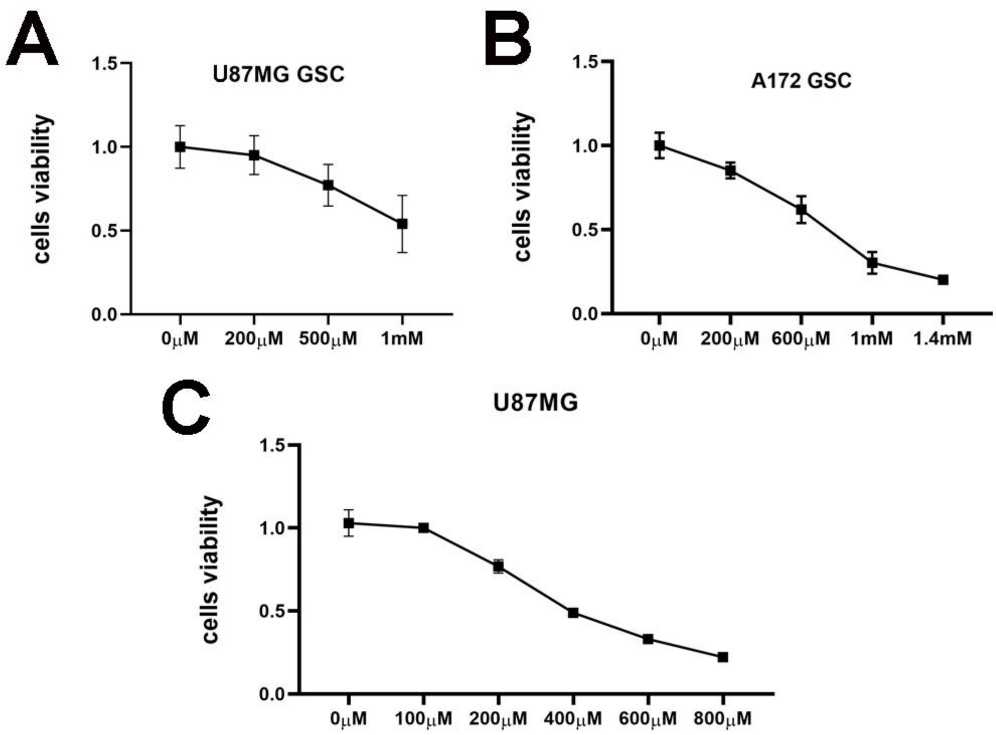
Figure S5 Mito-TEMPO restored the expression of GSC stem cell surface markers. Related to Figure 5.



Images of GSC spheres which expressed stem cell markers Nestin (green) and CD133 (red) by immunofluorescence. DAPI (blue) for nuclei. (A) Images of U87MG GSC spheres. (B)

Images of A172 GSC spheres.

Figure S6 IC50 of TMZ. Related to Figure 6.



The growth rate of GSCs was determined by the CCK8 assay. (A) The growth rate of U87MG GSCs. (B) The growth rate of A172 GSCs. (C) The growth rate of U87MG cells.

Table S1: qPCR and RT-PCR primer sequence. Related to STAR Methods.

Gene	**Primer sequence**
Tel-F	GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT
Tel-R	TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCT
36B4-F	CAGCAAGTGGGAAGGTGTAATCC
36B4-R	CCCATTCTATCATCAACGGGTACAA

human *Nanog*-F	AGTCCCAAAGGCAAACAACCCACTTC
human *Nanog*-R	TGCTGGAGGCTGAGGTATTTCTGTCTC
human *OCT4*-F	GACAGGGGGAGGGGAGGAGCTAGG
human *OCT4*-R	CTTCCCTCCAACCAGTTGCCCCAAAC
human *sox2*-F	GGGAAATGGGAGGGGTGCAAAAGAGG
human *sox2*-R	TTGCGTGAGTGTGGATGGGATTGGTG
human CD133 F	CAGAGTACAACGCCAAACCA
human CD133 R	AAATCACGATGAGGGTCAGC
human nestin F	GAAACAGCCATAGAGGGCAAA
human nestin R	TGGTTTTCCAGAGTCTTCAGTGA
human β-actin F	CTCCATCCTGGCCTCGCTGT
human β-actin R	GCTCGTACCTTCACCGTTCC
human Wnt3 F	GTGTTAGTGTCCAGGGAGTTC
human Wnt3 R	CATTTGAGGTGCATGTGGTC
human Wnt5 F	TCGCCCAGGTTGTAATTGAAG
human Wnt5 R	TGAGAAAGTCCTGCCAGTTG