K-RAS Acts as a Critical Regulator of CD44 to Promote the Invasiveness and Stemness of GBM in Response to Ionizing Radiation

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Supplementary Information:

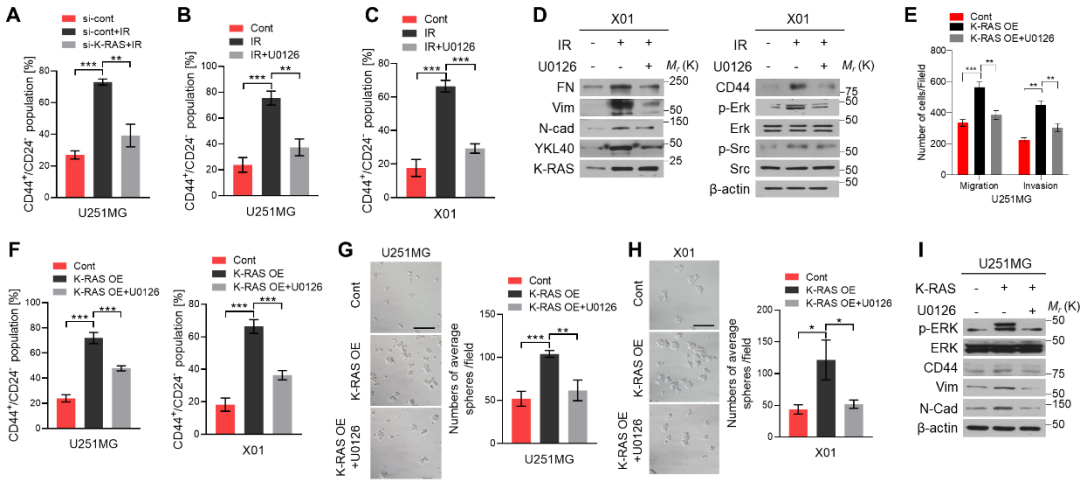


Figure S1. K-RAS/ERK/CD44 axis regulates IR-induced stemness in GBM cells. (A). FACS analysis of the percentage of CD44+/CD24- cells using the IR-treated U251MG cells with or without K-RAS expression. **(B–D).** FACS and western blotting analysis were performed to assess the cell stemness features and mesenchymal markers expression using the IR- treated U251MG and patient-derived X01 GBM cells, respectively, with or without the treatment of U0126. **(E).** Invasion assay were performed to assess the invasiveness in the K-RAS-overexpressing U251MG cells treated with or without U0126. **(F–H).** FACS analysis and sphere formation assay were used to measure the stemness features of K-RAS-overexpressing U251MG cells and patient-derived X01 GBM cells, respectively, with the treatment of U0126 or not. **(I).** Western blotting analysis of p-ERK, ERK, CD44, Vim, and N-Cad expression using the same condition samples of above. β-actin was used as a control for normalization of expression. ** $p < 0.001$, *** $p < 0.0001$, n.s., not significant. A two-tailed Student's t-test was used to compare data between two groups, and ANOVA was used for multiple comparisons.

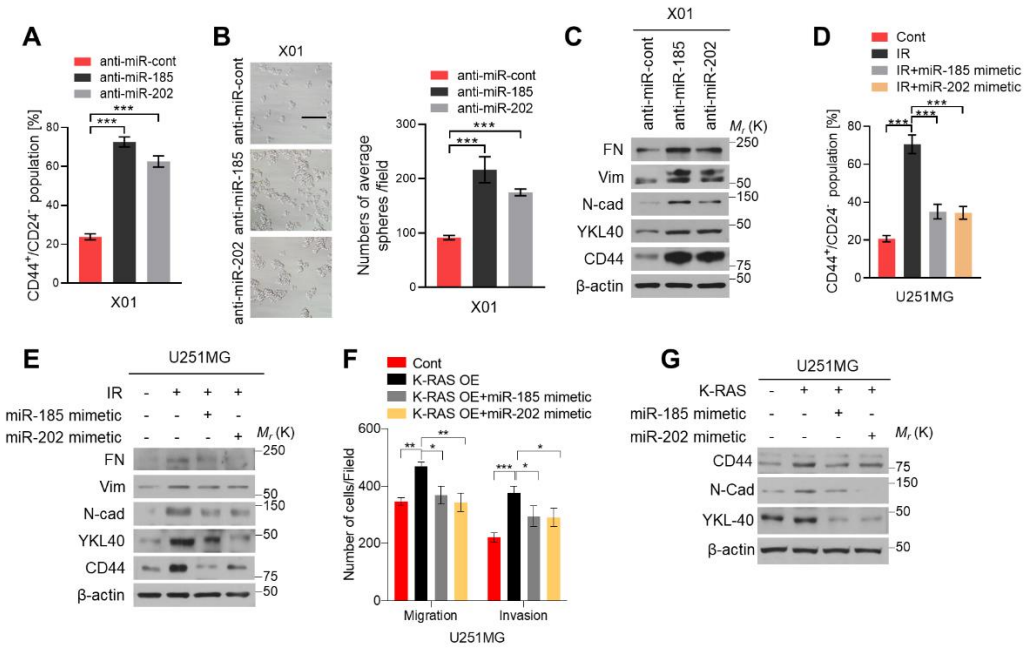


Figure S2. miR-185 and miR202 inhibit stemness of GBM by suppressing CD44 expression. (**A**,**B**). FACS analysis of the percentage of CD44+/CD24- cells in patient-derived X01 GBM cells with the treatment of miR-185 or miR-202 inhibitor. (**C**). Western blotting analysis of mesenchymal markers and CD44 expression using the same condition samples of above. (**D**,**E**). FACS and western blotting analysis were performed to assess the stemness features and the expression of mesenchymal markers in IR-treated U251MG cells, transfected with miR-185 or miR-202 mimetic. (**F**,**G**). Invasion and migration assay and western blotting analysis were performed to measure the invasiveness ability of cells and the mesenchymal markers expression in U251MG cells with overexpression of the K-RAS alone or together with miR-185 and/or miR-202 mimetic. β-actin was used as a control for normalization of expression. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, n.s., not significant. A two-tailed Student's t-test was used to compare data between two groups, and ANOVA was used for multiple comparisons.

Table S1. The list of qRT-PCR primer sequences.

Primer Name	Sequence (5′-3′)	
Fibronectin	Sense:	ACCAACCTACGGATGACTCG
	Antisense:	GCTCATCATCTGGCCATTTT
CDH2	Sense:	GACAATGCCCCTCAAGTGTT
	Antisense:	CCATTAAGCCGAGTGATGGT
Vimentin	Sense:	GAACCAATGAGTCCCTGGAA
	Antisense:	TCCAGCAGCTTCCTGTAGGT
CD133	Sense	GCCACCGCTCTAGATACTGC
	Antisense	TGTTGTGATGGGCTTGTCAT
β-actin	Sense:	CATCCGCAAAGACCTGTACG
	Antisense:	CCTGCTTGCTGATCCACATC