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Supplemental information

LRRC4 mediates the formation of circular RNA CD44 to inhibit GBM cell proliferation

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Supplementary Figures and Legends

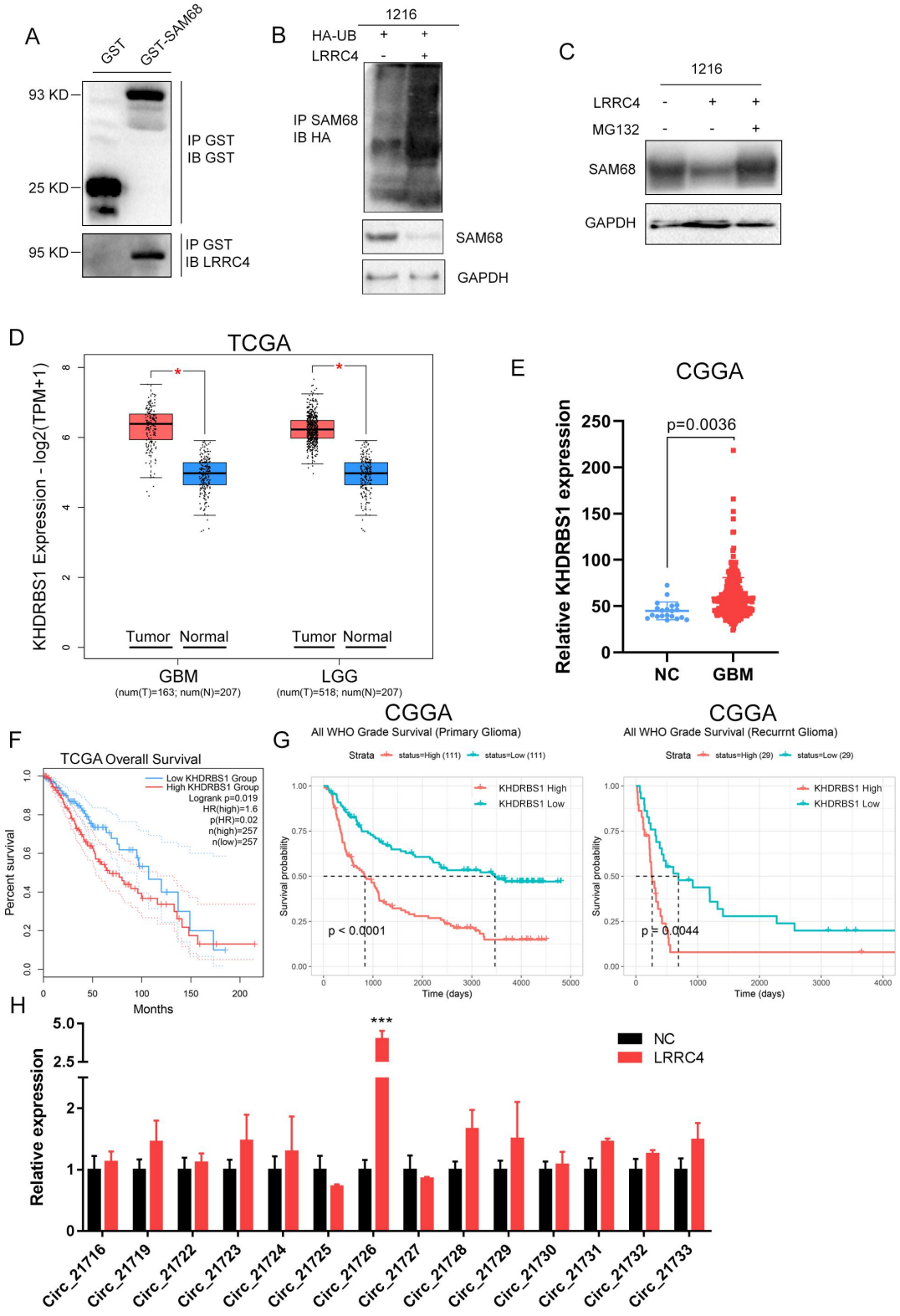

Figure S1 SAM68 directly binding with LRRC4 and up-regulated in glioma

A:GST pull-down assay revealed that LRRC4 was pulled down by GST-fused SAM68. **B**:Ubiquitin modifications assay revealed that the ubiquitin modifications of SAM68 were also increased by LRRC4. **C**: Western blotting showed that the

inhibition effect of LRRC4 on SAM68 was blocked when treatment with MG132. **D**: By TCGA dataset analysis, SAM68 is up-regulated both in GBM and LGG group. **E**: By CGGA dataset analysis, SAM68 is up-regulated in GBM. **F**: By TCGA dataset analysis, patients with higher SAM68 expression level have a worse prognosis than those patients with lower SAM68 expression level. **G**: In CGGA primary and recurrent glioma dataset, patients with higher SAM68 expression level have a worse prognosis than those patients with lower SAM68 expression level. **H**: RT-qPCR analyses showing that LRRC4 increases the expression of hsa_circ_0021726 in 1104 cells.

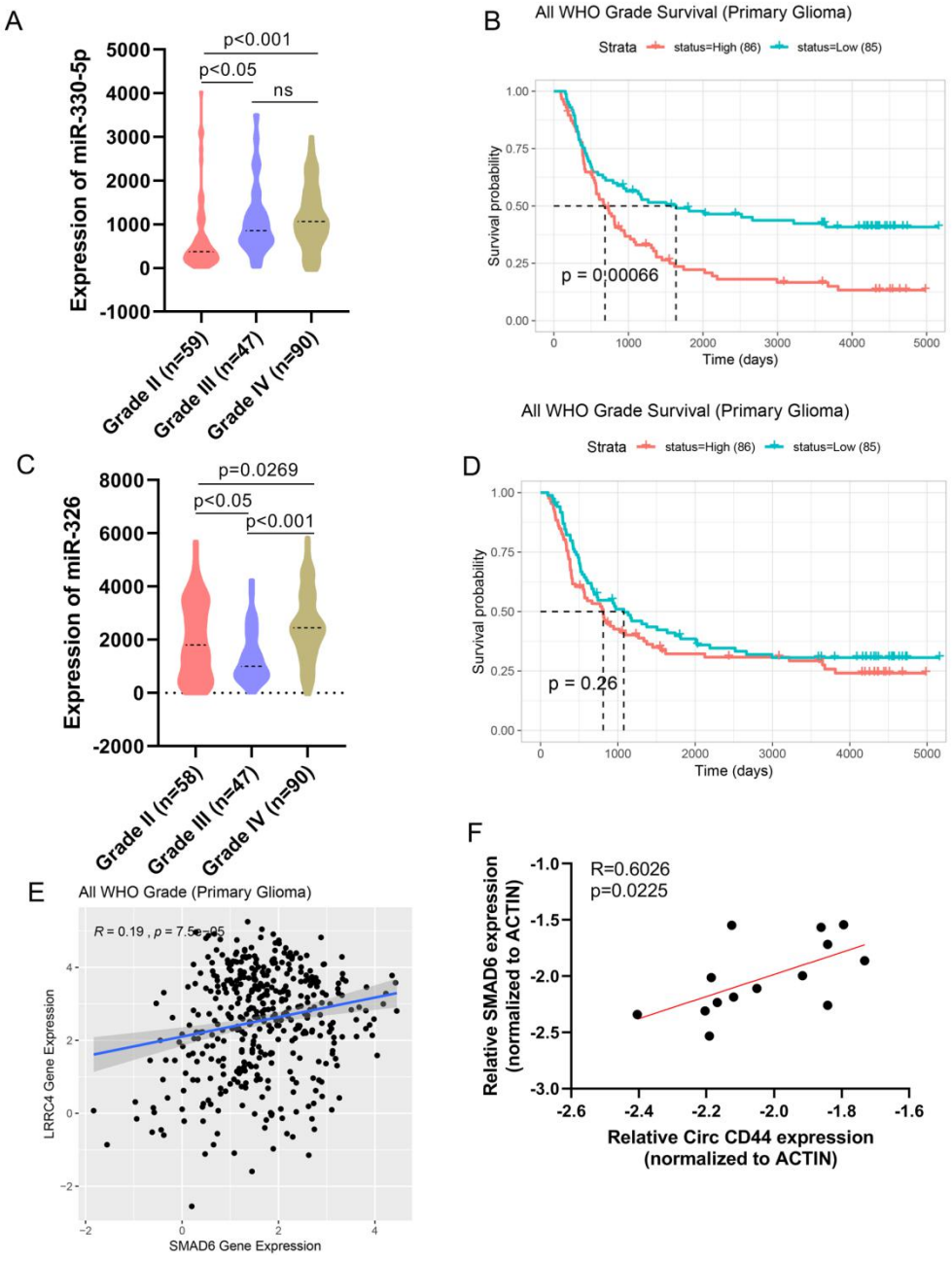

Figure S2 database analysis of miR-326, miR-330-5p and LRRC4 in CGGA

A: In CGGA database, the relative expression of miR-326 in different grade gliomas. **B**: Kaplan–Meier survival curves of glioma patients with higher or lower expression of miR-326. **C**: In CGGA database, the relative expression of miR-330-5p in different grade gliomas. **D**: Kaplan–Meier survival curves of glioma patients with higher or lower expression of miR-330-5p. **E**: Correlation between the expression of LRRC4 and SMAD6 in CGGA dataset was evaluated by Pearson's correlation test. **F**: Correlation between the expression of circCD44 and SMAD6 in GBM samples was evaluated by Pearson's correlation test.

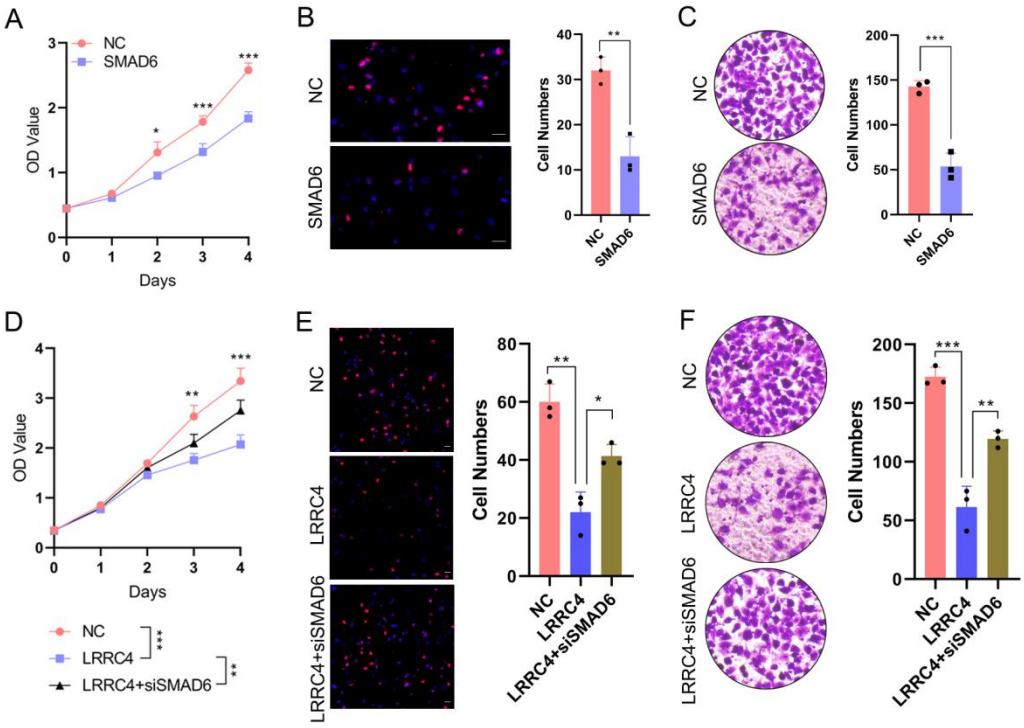

Figure S3 The effect of SMAD6 on GBM cells proliferation and invasion

A: The effect of ectopic SMAD6 expression on 1124C cells. proliferation was assessed by the CCK-8 cell growth assay. **B**: The effect of ectopic SMAD6 expression on 1124C cells was evaluated by EDU assay. **C**: The effect of ectopic SMAD6 expression on 1124C cells. Invasion was assessed by the transwell assay. **D**: 1124C cells were transfected with indicated vectors and siRNA, CCK-8 assays were performed to assess the proliferation ability of the transfected cells. **E**: 1124C cells were transfected with indicated vectors and siRNA, EDU assays were performed to assess the proliferation ability of the transfected cells. **F**: 1124C cells were transfected with indicated vectors and siRNA, transwell assays were performed to assess the invasion ability of the transfected cells.

Supplementary Table 1 A total of 41 miRNAs were found to potentially bind circCD44 in Circular RNA Interactome online database

41 miRNAs were showed as follow:			
hsa-miR-1184	hsa-miR-145	hsa-miR-562	hsa-miR-1270
hsa-miR-1208	hsa-miR-326	hsa-miR-577	hsa-miR-620
hsa-miR-1231	hsa-miR-330-5p	hsa-miR-579	hsa-miR-636
hsa-miR-1236	hsa-miR-370	hsa-miR-580	hsa-miR-640
hsa-miR-1248	hsa-miR-382	hsa-miR-583	hsa-miR-644
hsa-miR-1257	hsa-miR-433	hsa-miR-599	hsa-miR-649
hsa-miR-1287	hsa-miR-488	hsa-miR-593	hsa-miR-661
hsa-miR-1289	hsa-miR-502-5p	hsa-miR-609	hsa-miR-665
hsa-miR-1290	hsa-miR-556-5p	hsa-miR-615-5p	hsa-miR-873
hsa-miR-1322	hsa-miR-558	hsa-miR-616	hsa-miR-924
			hsa-miR-934