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Supplemental Information

DGCR8/ZFAT-AS1 Promotes CDX2 Transcription in a PRC2 Complex-Dependent Manner to Facilitate the Malignant Biological Behavior of Glioma Cells

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Figure S1

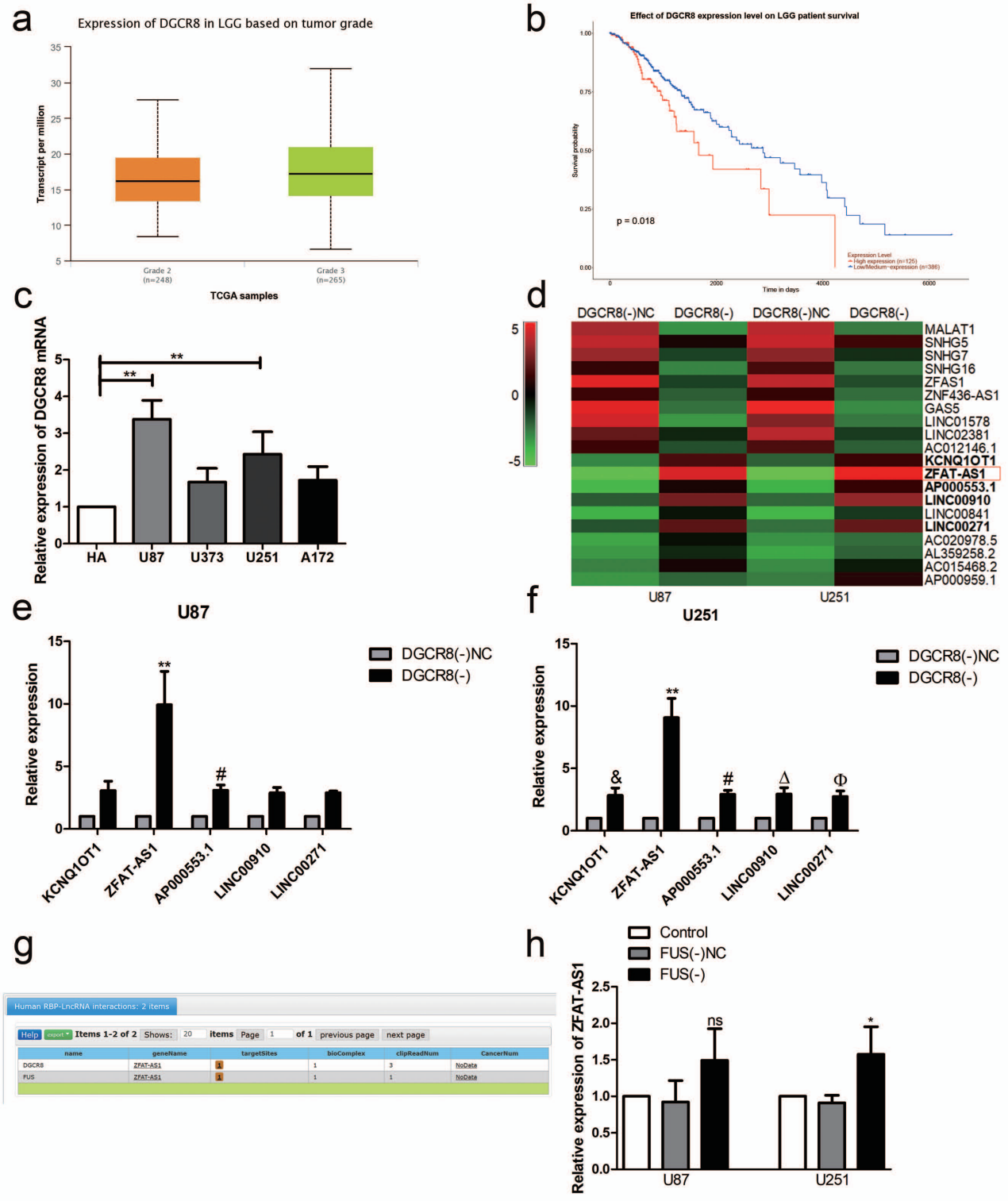

Figure S1. Selected DGCR8 and ZFAT-AS1 as candidates. **a** Expression of DGCR8 in low-grade gliomas (LGG) from TCGA samples. **b** Effect of DGCR8 expression level on LGG patient survival from database TCGA. **c** Expression of DGCR8 in different glioma cell lines. Data were presented as the mean ± standard deviation (SD) (n=3, each group). $^{**}P < 0.01$ by Student's t-test. **d** LncRNAs gene expression profiles as obtained from samples of three groups in DGCR8 knockdown. Group treated with the short-hairpin RNA against DGCR8 (DGCR8(−)), Group treated with negative control empty vector plasmid with non-targeting sequence (DGCR8(−)NC). **e f** qRT-PCR was performed to validate the selected molecules. Data were presented as the mean ± SD (n=3, each group). $^{**}P < 0.01$ vs. DGCR8(−)NC group; $^{\&/\#/\Delta/\Phi/*}P < 0.05$ vs. DGCR8(−)NC group by one-way ANOVA. **g** RBPs interacted with ZFAT-AS1 were DGCR8 and FUS according to a bioinformatics database (starBase v2.0). **h** Influence of FUS on the expression of ZFAT-AS1. Data were presented as the mean ± SD (n=3, each group). $^{*}P < 0.05$ vs. FUS(−)NC group by one-way ANOVA.

Figure S2

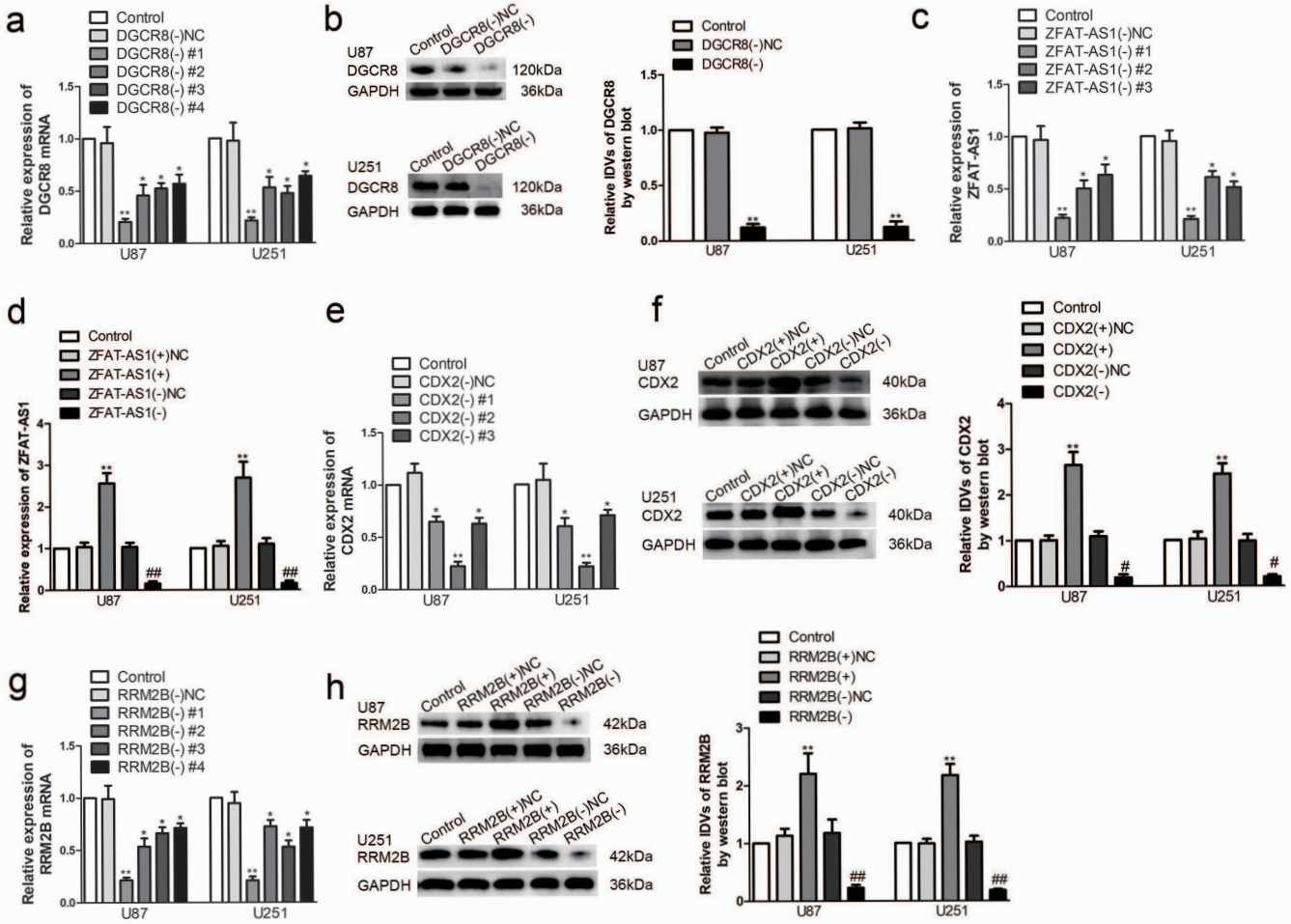

Figure S2. Transfection efficiency of DGCR8, ZFAT-AS1, CDX2 and RRM2B. **a** Efficiency of transient knockdown of DGCR8 four different sites. **b** Efficiency of stable transfection of DGCR8 site #1. Group treated with the short-hairpin RNA against DGCR8 (DGCR8(−)), Group treated with negative control empty vector plasmid with non-targeting sequence (DGCR8(−)NC). The integrated density values (IDVs) of the blot bands were statistic analyzed. Data were presented as the mean ± standard deviation (SD) (n=3, each group). $^{*}P < 0.05$ vs. DGCR8(−)NC group; $^{**}P < 0.01$ vs. DGCR8(−)NC group by one-way ANOVA. **c** Efficiency of transient knockdown of ZFAT-AS1 three different sites. Data were presented as the mean ± SD (n=3, each group). $^{*}P < 0.05$ vs. ZFAT-AS1(−)NC group; $^{**}P < 0.01$ vs. ZFAT-AS1(−)NC group by one-way ANOVA. **d** Efficiency of stable transfection of ZFAT-AS1 over-expression and knockdown site #1. Data were presented as the mean ± SD (n=3, each group). $^{**}P < 0.01$ vs. ZFAT-AS1(+)NC group; $^{\#\#}P < 0.01$ vs. ZFAT-AS1(−)NC group by one-way ANOVA. **e** Efficiency of transient knockdown of CDX2 three different sites. Data were presented as the mean ± SD (n=3, each group). $^{*}P < 0.05$ vs. CDX2(−)NC group; $^{**}P < 0.01$ vs. CDX2(−)NC group by one-way ANOVA. **f** Efficiency of stable transfection of CDX2 over-expression or knockdown site #2. Data were presented as the mean ± SD (n=3, each group). $^{**}P < 0.01$ vs. CDX2(+)NC group; $^{\#}P < 0.05$ vs. CDX2(−)NC group; $^{\#\#}P < 0.01$ vs. CDX2(−)NC group by one-way ANOVA. **g** Efficiency of transient knockdown of RRM2B four different sites. Data were presented as the mean ± SD (n=3, each group). $^{*}P < 0.05$ vs. RRM2B(−)NC group; $^{**}P < 0.01$ vs. RRM2B(−)NC group by one-way ANOVA. **h** Efficiency of stable transfection of RRM2B over-expression or knockdown site #1. Data were presented as the mean ± SD (n=3, each group). $^{**}P < 0.01$ vs. RRM2B(+)NC group; $^{\#\#}P < 0.01$ vs. RRM2B(−)NC group by one-way ANOVA.

Figure S3

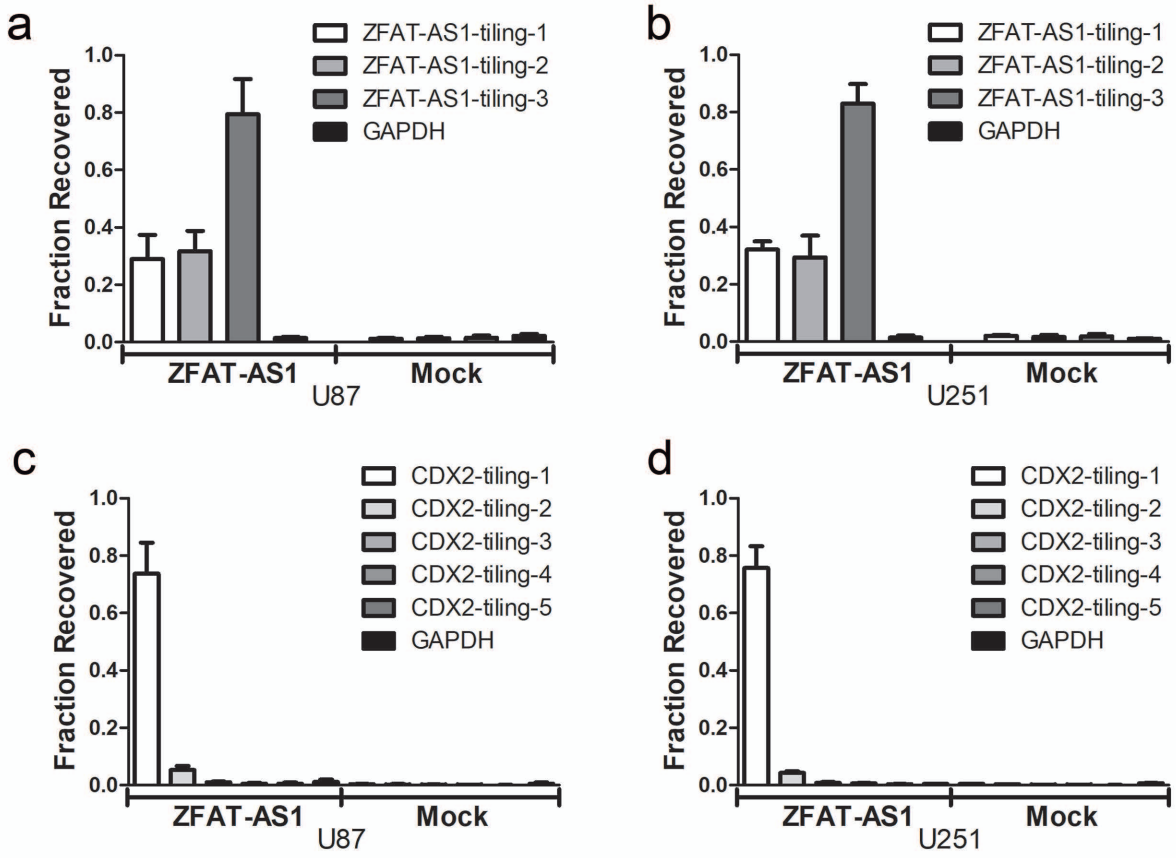

Figure S3. ZFAT-AS1 was present at the promoter region of CDX2 in Chromatin immunoprecipitation assay.
a b ZFAT-AS1 obtained 35-87% retrieval using tiling probes from the RNA fraction recovered in chromatin isolation by RNA purification (ChIRP). **c d** The promoter region 0-500bp of CDX2 (CDX2-tiling-1) obtained ~80% retrieval and the promoter region 500-1000bp (CDX2-tiling-1) obtained ~4% retrieval from the DNA fraction recovered ChIRP. LacZ RNA used as the mock control.

Table S1. The primers of DGCR8 mRNA, ZFAT-AS1, CDX2 mRNA, RRM2B mRNA in quantitative real-time PCR (qRT-PCR).

Primers	Forward	Reverse
DGCR8：	5′-CGATGTGGATGCTCTGCTGGAAG-3′	5′-TCGGACGGATGGTCGCTGTC-3′
ZFAT-AS1:	5′-GGCTTGCTACTTACCAGTGCTGTG-3′	5′-CCGATGGCTGTGACTACTCAACTC-3′
CDX2:	5′-GGAGGACTGGAATGGCTACG-3′	5′-CCTGGTTTTCACTTGGCTGC-3′
RRM2B:	5′-GCCTGGCTTCGTCGTTGCG-3′	5′-TTCGTTGGTGTCTGAAGATGATCTCC-3′

Table S2. The primers for each PCR set, annealing temperatures, and the sizes of PCR products in Chromatin immunoprecipitation assay, to confirm the existence of H3K27me3 modification in CDX2 promoter region.

Primers of CDX2 promoter region in Chromatin immunoprecipitation assay	Forward	annealing temperatures	Reverse	annealing temperatures	products size
CDX2-PCR5:	5′-AGTTTGCTTGGGAAGAAAATATGCTTCACT-3′	74.0℃	5′-AGTCCTTCTATACCTTGCACAGGAAACTC-3′	76.0℃	500bp
CDX2-PCR4:	5′-ATATTATTTGGATAGTTTTATTTTCCTTTTTTCAAAT-3′	76.3℃	5′-AGCTCCCTCCCTGTTCAA-3′	56.0℃	500bp
CDX2-PCR3:	5′-GTGTCAGTGATGTCTGGATTTCAGGAG-3′	70.0℃	5′-GAGCCTTTTGGCCTGCACTGA-3′	66.5℃	500bp
CDX2-PCR2:	5′-CTAAGAAAGACGCCAAGCACCTGG-3′	69.0℃	5′-GAGTGCTCCAGCCATGGAAAGGA-3′	67.0℃	500bp
CDX2-PCR1:	5′-CAAGTGAGCCTCGGGCTTCC-3′	66.2℃	5′-AAACCCCGTTACAAAATCCGAATCATTG-3′	70.0℃	500bp

Table S3. The primers of 500 bp-TSS of CDX2 amplified with the DNA precipitated by H3K27me3 to detect the percentage of relative to its corresponding input in qRT-PCR.

Primers	Forward	Reverse	products size
CDX2：–500 bp-TSS	5′-TGGAGGTTAAAGTGCACCAGGT-3′	5′-GACACCAATGGTTGGAGACG-3′	187bp

Table S4. The primers for each PCR set, annealing temperatures, and the sizes of PCR products in Chromatin immunoprecipitation assay, to confirm the binding sits for CDX2 in the promoter region of RRM2B and DGCR8.

Primers in Chromatin immunoprecipitation assay	Forward	annealing temperatures	Reverse	annealing temperatures	products size
PCR2 (Control-RRM2B)	5′-CATGTTGGTCAGGCTGGTCT-3′	60.0℃	5′-AGATAACATTGGGGCCAGGC-3′	60.1℃	113bp
PCR1 (RRM2B-putative binding site)	5′-GGCTTTCAGCATTTTCAACGC-3′	59.5℃	5′-TGACGGGATCTTGCCATGAC-3′	60.1℃	108bp
PCR3 (Control-DGCR8)	5′-GAAGATGGCTCCTCAGTCCA-3′	58.8℃	5′-TGAAGAGCAGACAAACCCCA-3′	59.2℃	107bp
PCR2 (DGCR8-putative binding site 2)	5′-ATCACTGCAGCCTGGAACTC-3′	60.0℃	5′-AAGTGCTGGGATTACAGGCA-3′	59.3℃	148bp
PCR1 (DGCR8-putative binding site 1)	5′-GATACCAGCCTGGCCAACAT-3′	60.1℃	5′-AAGCGATTCTCCTGGCTCAC-3′	60.1℃	121bp

Table S5. The probes of ZFAT-AS1 and CDX2 in Chromatin Isolation by RNA Purification (ChIRP).

Probe name	Probe sequence (5'-> 3')	Position of first nucleotide of probe
ZFAT-AS1_1	GGTCATTAACAGCAACCACA	19
ZFAT-AS1_2	TTCTGAGAGGCTTATCTCTT	315
ZFAT-AS1_3	TGTCAGGAATGTGCACTGAG	426
ZFAT-AS1_4	TTCCTTTCACAGCAAGAGTC	507
ZFAT-AS1_5	TCACACAGCACTGGTAAGTA	679
ZFAT-AS1_6	AACATCTTGCTGAAGTGTCC	759
ZFAT-AS1_7	GGCAAACTTTTTTGGTACCA	900

Probe name	Probe sequence (5'-> 3')	Position of first nucleotide of probe
ZFAT-AS1_tiling-1	CACATGGTTCTTGGTAGGT	4
ZFAT-AS1_tiling-2	GGAGTGTCAGGAATGTGCA	431
ZFAT-AS1_tiling-3	ACATCTTGCTGAAGTGTCC	759
CDX2_tiling-1	AATCATTGAGAGAAGGGCAGTA	42
CDX2_tiling-2	CATTGTGAACACGAATGTTTCC	548
CDX2_tiling-3	AGAGGCAAACTTGTTTGGGAAG	1158
CDX2_tiling-4	TTCAAAGGTCCTAATATTTGCT	1535
CDX2_tiling-5	CTCAATAATGGTTGGTTTGACC	2243