

SUPPLEMENTARY INFORMATION

Ubiquitin-specific protease 6 n-terminal-like protein (USP6NL) and epidermal growth factor receptor (EGFR) signaling axis regulates the ubiquitin-mediated DNA repair and temozolomide-resistance in glioblastoma

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Supplementary Table

Supplementary Table S1. Baseline clinical characteristics and outcome of GBM patients in SHH-GBM cohort.

Characteristics (n=60)		Cases	Percentage (%)
Age	Less than or equal 60 y.o	34	52.3%
	More than 60 y.o	31	47.7%
Gender	Male	32	49.2%
	Female	33	50.8%
Tumor Location	Frontal	17	26.2%
	Parietal	6	9.2%
	Temporal	21	32.3%
	Occipital	1	1.5%
	Others	20	30.8%
Recurrences	Yes	3	4.6%
	No	62	95.4%

y.o: years old

Supplementary Table S2. List of primary antibodies used for immunoblotting and immunostaining.

Primary antibody	Company	Host species	Clonality	Concentration	Catalogue Number
USP6NL	Creative	Rabbit	Monoclonal	1:1,000 (WB)	DCABH-17363
	Diagnostics			1:100 (IHC)	
EGFR	Abcam	Rabbit	Monoclonal	1:1,000 (WB)	ab52894
				1:20 (IP)	
CD44	Cell Signaling	Mouse	Monoclonal	1:1,000 (WB)	3570
ABCG2	Cell Signaling	Rabbit	Monoclonal	1:5,000 (WB)	3579
Nanog	Cell Signaling	Rabbit	Monoclonal	1:5,000 (WB)	4903
CD133	Proteintech	Rabbit	Polyclonal	1:1,000 (WB)	18495-1-AP
SOX2	GeneTex	Mouse	Monoclonal	1:1,000 (WB)	GTX627405
E-cadherin	Cell Signaling	Rabbit	Monoclonal	1:100 (IF)	3195
N-cadherin	Cell Signaling	Rabbit	Monoclonal	1:100 (IF)	13116
γH2AX	Cell Signaling	Rabbit	Monoclonal	1:100 (IF)	7631
RAD51	Abcam	Rabbit	Polyclonal	1:100 (IF)	ab63801
BRCA2	ThermoFisher	Rabbit	Polyclonal	1:100 (IF)	PA5-96128
ATG5	Abcam	Rabbit	Polyclonal	1:100 (IF)	ab228668
				1:2,000 (WB)	
ATG7	Abcam	Rabbit	Monoclonal	1:100 (IF)	ab133528
				1:2,000 (WB)	
LC3A/B	Cell Signaling	Rabbit	Polyclonal	1:100 (IF)	4108
				1:1,000 (WB)	
Beclin-1	Abcam	Rabbit	Polyclonal	1:100 (IF)	ab62557
				1:2,000 (WB)	
GAPDH	PROTEINTECH	Mouse	Monoclonal	1:10,000 (WB)	60004-1-Ig

WB: western blot; IF: immunofluorescence; IHC: immunohistochemistry; IP: immunoprecipitation

Supplementary Table S3. Cohen's Kappa values for each pathologist, assessing expression Q-score of USP6L in glioma tissue.

Pathologist_B	Pathologist_A				
	0-75	76-150	151-225	226-300	
0-75	2	0	0	0	2 (6.7%)
76-150	1	8	0	0	9 (30.0%)
151-225	0	5	5	1	11 (36.7%)
226-300	0	0	0	8	8 (26.7%)
	3 (10.0%)	13 (43.3%)	5 (16.7%)	9 (30.0%)	30

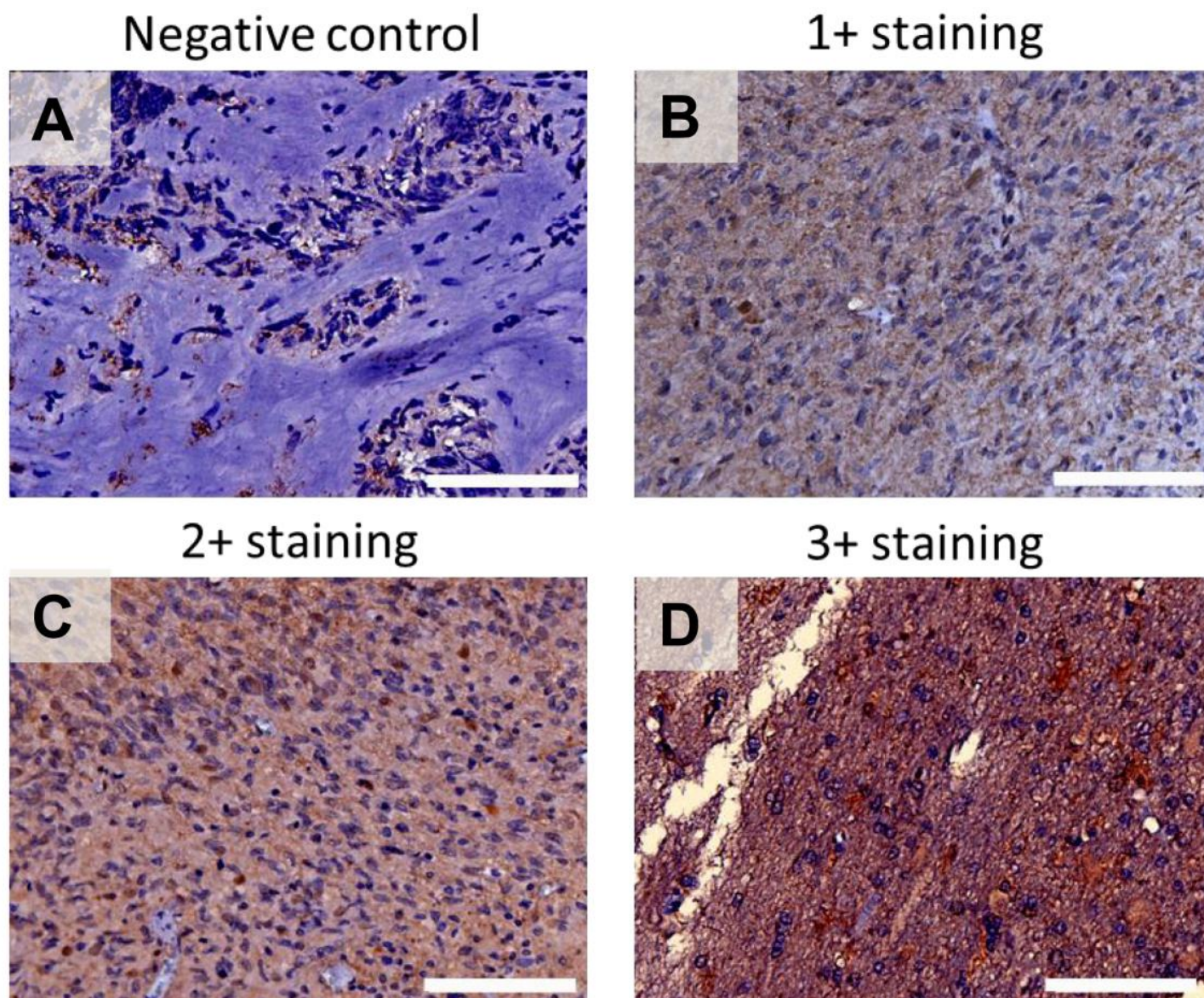
Weighted Kappa^a	0.78170
Standard error	0.07644
95% CI	0.63188 to 0.93153
P-value	1.07e-09

^a Linear weights

Supplementary Table S4. List of primers used for qRT-PCR amplification of each gene.

Gene	Strand	Sequence of Primers
USP6NL	Forward	5'-TACTCAGCCTTTCAACTC-3'
	Reverse	5'-GCAAGTACACGTCAAATC-3
CD44	Forward	5'-CAACACAAATGGCTGGTACG-3'
	Reverse	5'-GTGTGGTTGAAATGGTGCTG-3'
ABCG2	Forward	5'-TTTCAGCCGTGGAAGTCTTT-3'
	Reverse	5'-TGAGTCCTGGGCAGAAAGTTT-3'
Nanog	Forward	5'-GTGATTTGTGGGCCTGAAGA-3'
	Reverse	5'-ACACAGCTGGGTGGAAGAGA-3'
CD133	Forward	5'-TCTTGACCGACTGAGACCCAAC-3'
	Reverse	5'-ACTTGATGGATGCACCAAGCAC-3'
SOX2	Forward	5'-GACAGTTACGCGCACATGAA-3'
	Reverse	5'-TAGGTCTGCGAGCTGGTCAT-3'
β-actin	Forward	5'-CCCTAAGGCCAACCCTGAA-3'
	Reverse	5'-CCAGAGGCATACAGGGACAAC-3'

Supplementary Figure



Supplementary Figure S1. Representative staining of USP6L and control in glioma FFPE tissue sections. Panel (A) is demonstrating a representative picture of negative control (glioma tissue stained with isotype rabbit IgG antibody), panel (B) is a representative picture of a 1+ positive glioma sample, panel (C) a representative picture of a 2+ positive glioma sample, and panel (D) a representative picture of a 3+ positive or the highest intensity of USP6L expression in glioma sample. Scale bar = 200 μ m.