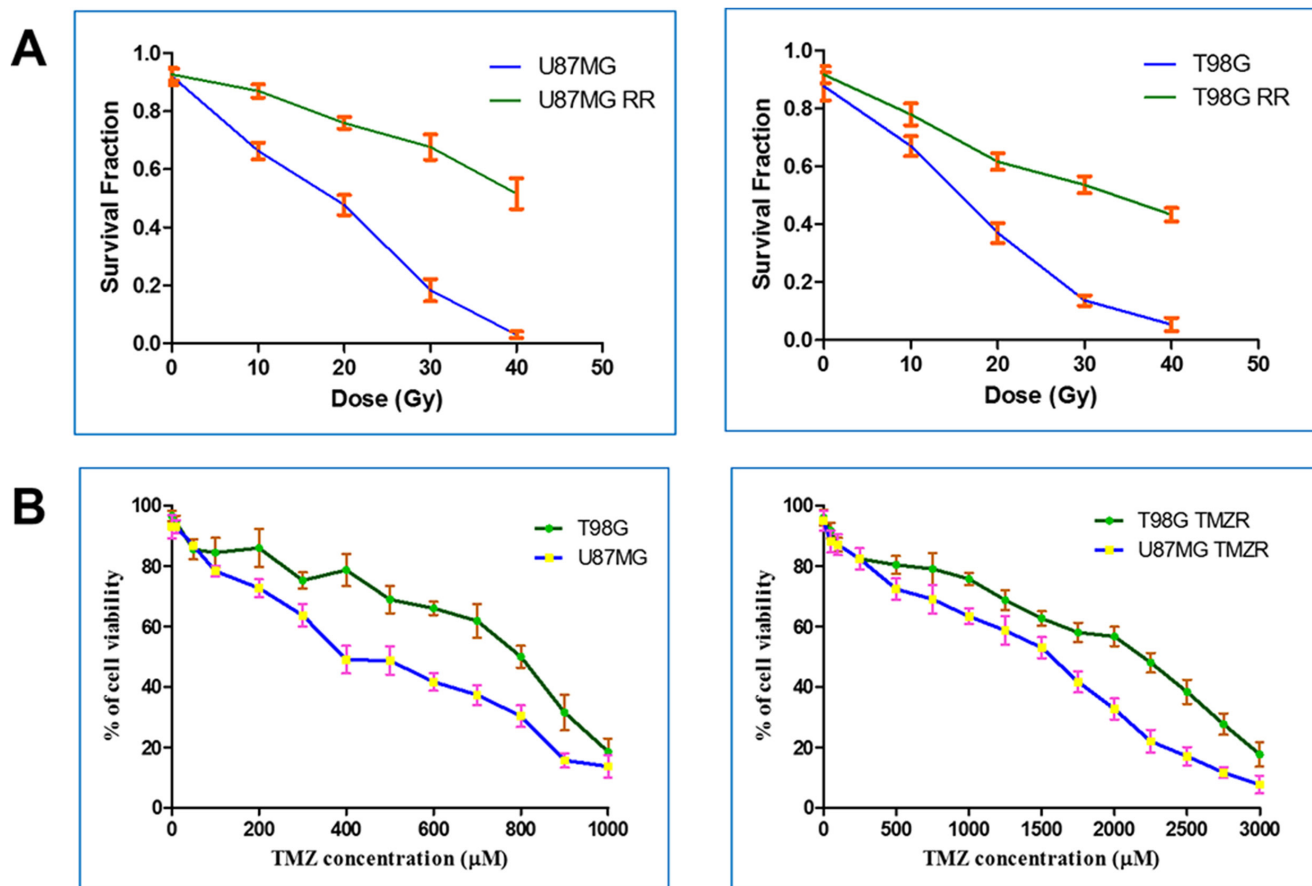


Transcriptional regulation of HSPB1 by Friend leukemia integration-1 factor modulates radiation and temozolomide resistance in glioblastoma

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: (A) Determination of survival fraction in parental U87MG and T98G cells and radio-resistant U87MG RR and T98G RR cells. (B) Determination of IC50 of temozolomide (TMZ) in parental U87MG and T98G cells and TMZ-resistant U87MG TMZR and T98G TMZR cells.

Identifier	Position	Mismatches	Score	Binding Factor	Sequence
ASSETS1_10	2219 (+)	0	100.00	c-Ets-1	ACAGGAAGTC
ELK1\$CONS_02	2219 (+)	0	90.91	Elk-1	ACMGGAAGTNC
ASSCETS1P54_07	2219 (+)	0	100.00	c-Ets-1 54	ACAGGAAGTC
TCF2ALPHA\$CONS	2220 (+)	0	87.50	TCF-2alpha	SAGGAAGY
FLI1\$CONS_01	2220 (+)	0	93.75	Fli-1	CMGGAAGT
MOUSE\$TIMP1_02	2220 (+)	0	100.00	c-Ets-1	CAGGAAG
ASSELK1_13	2220 (-)	0	100.00	Elk-1 , SAP-1a , SAP-1b , SRF	TTCCTG
PEA3\$CONS	2221 (+)	0	91.67	c-Ets-1 54 , c-Ets-1 68 , c-Ets-2 58-64 ,	AGGAAR

```
seq( 2220.. 2279)      aggaagtccagactaattcaagtttgcggcatttgaggttcctggccagtgtttctttt
Segments:
1.1.3.0      2212 2221  p=
3.1.1.9      2217 2226  =K-2a==
3.5.2.0      2217 2226  Ets-1 =
1.6.1.0      2259 2268
1.3.1.2      2260 2269  =AP-2alph=
9.9.539      2261 2270  =====USF===
9.9.428      2270 2279  =====NF-1==
3.1.1.2      2272 2281  ===ISGF-3=
1.1.1.5      2273 2282  =====Antp
=====GCN=====
```

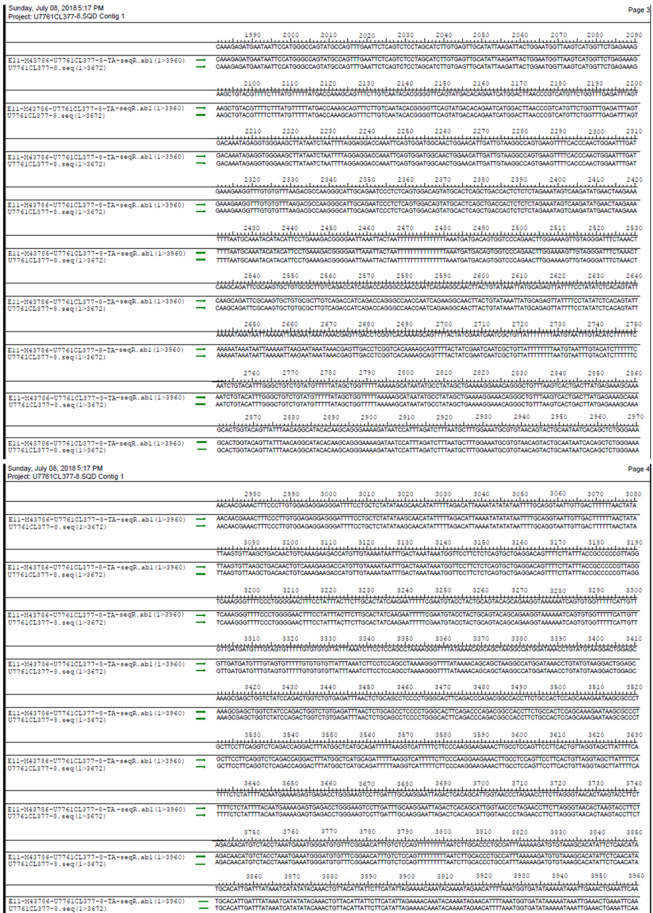
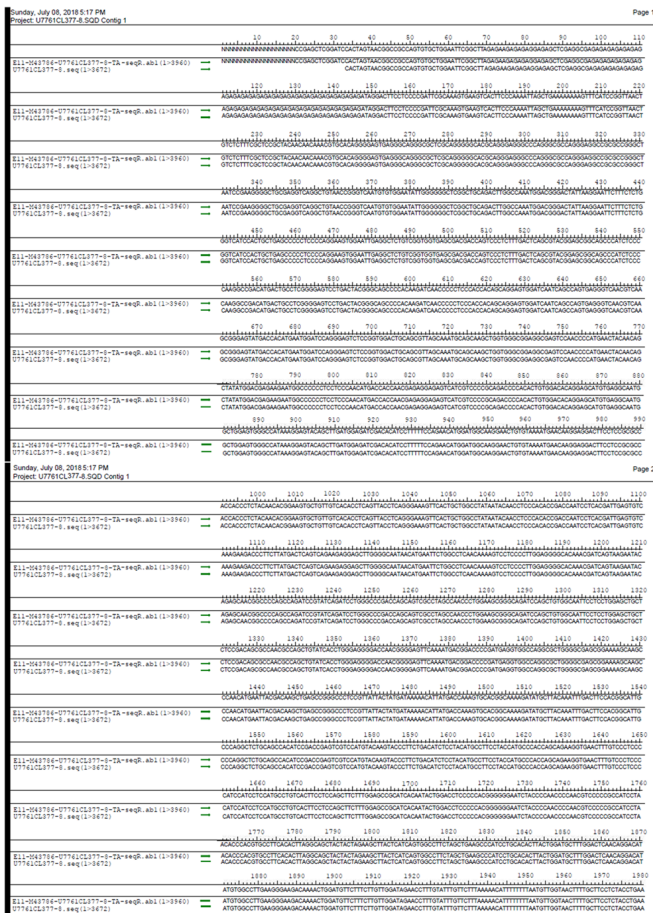
```
seq( 1740.. 1799)      atcccagccacttgggagctctgaggcaggaggatcgcttgaaccaggaggcggaggttg
Segments:
9.9.539      1732 1741  ==
1.1.3.0      1738 1747  /EBPalp=
1.1.1.1      1758 1767  ===c-Jun==
1.1.1.2      1759 1768  ===c-Fos==
9.9.29       1760 1769  ===AP-1==
1.2.1.0      1762 1771  =====E1===
1.2.2.0      1762 1771  ===MyoD==
3.5.2.0      1762 1771  =c-Ets-1 =
2.3.1.0      1786 1796  =====Sp1===
2.2.1.1      1797 1806  ==
```

```
seq( 4800.. 4859)      cctgtctggctctgtcctccttaacagaaggacggccttggccacgggcccacagccagca
Segments:
2.2.1.1      4817 4826  ===GATA-1=
3.5.2.0      4817 4826  ==c-Ets-1=
2.3.1.0      4828 4841  =====Sp1=====
2.3.1.0      4835 4848  =====Sp1=====
1.3.1.2      4836 4845  =====USF===
1.3.2.1      4836 4845  ===c-Myc==
1.3.2.2      4836 4845  ===Max1==
1.6.1.0      4836 4845  =AP-2alph=
9.9.539      4838 4847  =====NF-1==
2.3.1.0      4847 4856  =====Sp1=====
1.1.1.1      4852 4861  ===c-Jun
1.5.1        4852 4861  =====EF-C
```

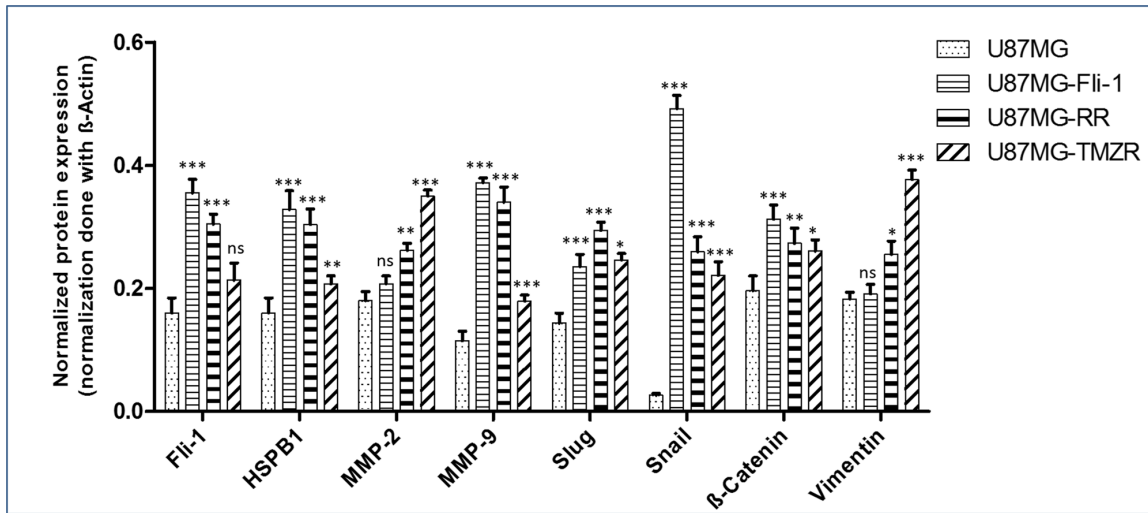
Supplementary Figure 3: Prediction of Fli-1 transcription factor in 5 KB upstream region of HSPB1 using ALIBABA and PATCH detection software.

Binding Site 3:
 TTAGCCGGTATGGTGCCACATGCCTGTAATCCAGCCACTTGGGAGTCT**GAGGCAGAG**GATCGCTGAACCCAGGAGCGGAGTTGCAGTGAAGCTGAGATTGCGCCA
Mutation:
 TTAGCCGGTATGGTGCCACATGCCTGTAATCCAGCCACTTGGGAGTCT**TGAAGTACAG**ATCGCTGAACCCAGGAGCGGAGTTGCAGTGAAGCTGAGATTGCGCCA
Binding Site 6:
 AGGCTGGAGTGCAGTGGCGCATCTCAGCTCCCAGCAACCTCGCCCTCT**GGAAT**GATCGCTCGCTCAGCTTCCCAGTAGCCGGGATTACAGGCCCCATC
Mutation:
 AGGCTGGAGTGCAGTGGCGCATCTCAGCTCCCAGCAACCTCGCCCTCT**TAGCT**GATCGCTCGCTCAGCTTCCCAGTAGCCGGGATTACAGGCCCCATC
Binding Site 7:
 TGCTTCTAA**GGA**AGATACATATAGTAGGAGATGTACAGTAATCTTCTCCTGGCCCCCTCAGCACTGTGCCATGGAGTCTGATCTGGGCCAGCTGCGCTGTTAGGGGAAGGG**GGA**AATGGCCAAA
Mutation:
 TGCTTCTAA**TACG**AGATACATATAGTAGGAGATGTACAGTAATCTTCTCCTGGCCCCCTCAGCACTGTGCCATGGAGTCTGATCTGGGCCAGCTGCGCTGTTAGGGGAAGGG**TACG**AATGGCCAAA

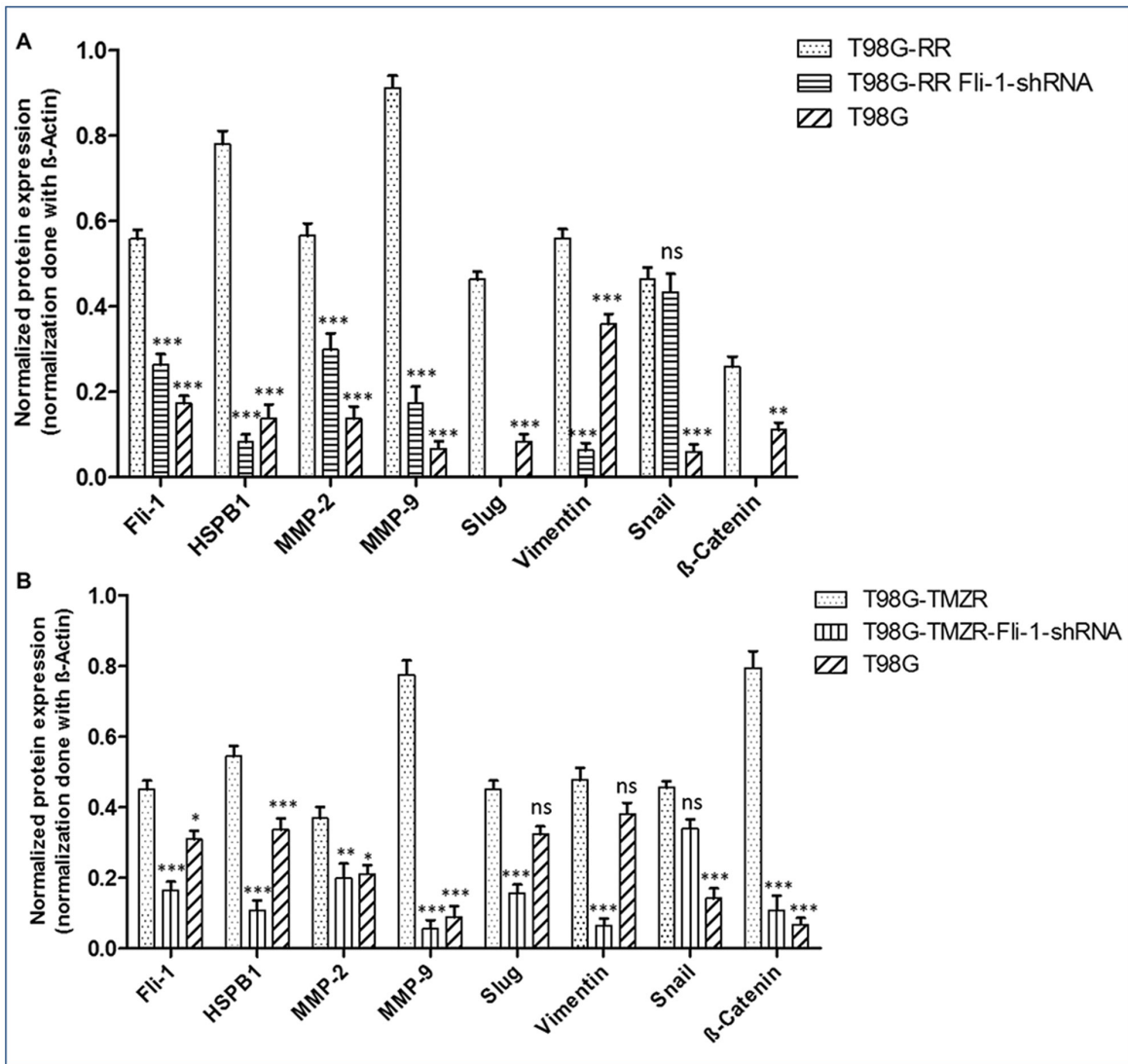
Supplementary Figure 4: Sequence of ~120-bp upstream region of HSPB1 containing the mutated binding sites 3, 6 and 7 and the respective control oligonucleotides without mutations.



Supplementary Figure 5: The sequencing data of the overexpressing Fl-1 plasmid.



Supplementary Figure 6: Densitometry plot of Figure 5C. Western blotting analysis of Fli-1, HSPB1, MMP-2, MMP-9 and EMT proteins in U87MG, U87MG RR, TMZ-resistant U87MG and U87MG Fli-1 cells.



Supplementary Figure 7: (A, B) Densitometry plot of Figure 6E and 6F. Western blotting analysis of Fli-1, HSPB1, MMP-2, MMP-9 and EMT proteins in parental T98G, radio-resistant T98G RR, TMZ-resistant T98G, T98G RR Fli-1 shRNA and T98G TMZR Fli-1 shRNA cells. Each bar represents the average of three independent experiments. The level of significance is indicated by * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplementary Table 1: The primer sequences for qRT-PCR

	Primer Sequence (5'-3')
HSPB1	F GAGACTGCCGCAAGTAAAG R TTTGACAGGTGGTTGCTTTG
HSP 40	F GGAAGGGACCCATTTTCATT R CCACTTCCAAAAGACGGAAA
HSP 70	F CCAGCTGAAGAAGGGTCAAG R CAGGATCTGGATAGGGCAA
HSP 90	F AGACCCAGTCTTGTGGATGG R ACTCCCCTTTCCCCCTAAAT
β-Catenin	F TGCAGTTCGCCTTCACTATG R ACTAGTCGTGGAATGGCACC
Slug	F TGTGTGGACTACCGCTGC R TCCGAAAGAGGAGAGAGG
Snail	F TGCAGGACTCTAATCCAGAGTTTACC R GTGGGATGGCTGCCAGC
Vimentin	F TGTCCAAATCGATGTGGATGTTTC R TTGTACCATTCTTCTGCCTCCTG

Supplementary Table 2: The ChIP primer sequences for Fli-1 binding sites in the 5 kb upstream of HSPB1

Binding Site	Primer Sequence (5'-3')
1	Forward: GTGTCTACAAAGGGCTGGCA Reverse: CTGCTCACCTTGGCCCAG
2	Forward: GGAGAGAGAGAAAGGCATTTATGA Reverse: CCACTGAAGTCTTGCCCTG
3	Forward: CGAAACCCCGTTTCTACTAAAA Reverse: CTCACTCTATCACTCAGGCTGG
4	Forward: CAACAATAAAACAGGGACCTAAGTC Reverse: GGCTCCTATCTCCCAGCTTG
5	Forward: TCTCCCTCTGTGATGTGGG Reverse: GAAACACTGGCCAGGAACC
6	Forward: GAGACAGAGTCTCGTTCTGTCAC Reverse: TACAAAAAATTAGCCAGGTGTGAT
7	Forward: AACCAGTGCTTCTTAAGGAAAGATAC Reverse: CCTGGGATTGAAGAGAGGG
8	Forward: TGCAATGCATGACTGTTACCA Reverse: TAGGCGTGTGGACTTTGAA
9	Forward: TCACACGCGTGTGGT Reverse: TGGAGCCGTGCCACG