

Supplemental information

Supplemental Table 1. Grading of p-Smad2 immunostaining, along with the histopathology and molecular pathology of HGG specimens used in Figure 1.

Supplemental Table 2. Molecular characteristics of specimens used for explant culture. WGS, whole genome sequencing; NGS, next generation sequencing; N/A: not available.

Supplemental Table 3. Table summarizing the time-points and experiments performed on each explant. (IF: Immunofluorescence, N/A: not available)

Supplemental Figure 1. Copy number variation analysis of the specimens used for explant cultures. 450K DNA methylation array analysis of the explants used in the study reveals various copy number variations.

Supplemental Figure 2. Fraction of p-Smad2+ cells in individual explant. Raw data for the percentage of p-Smad2+ nuclei upon RIKI, TGF- β or combined treatments in 6 HGG explants. ns, not significant; *, $P < 0.05$; **, $P < 0.01$.

Supplemental Figure 3. Characterization of nuclei on the basis of γ -H2AX immunoreactivity. **A.** IR significantly increased the percentage of γ -H2AX+ cells (nuclei) at both the 30-min and the 60-min marks, while RIKI had no effect ($F_{(5,34)}=5.662$, $P<0.001$). **B.** There was no correlation between the percentage of γ -H2AX+ cells and the number of γ -H2AX+ foci/positive nucleus (Spearman coefficient $r=0.061$, $P=0.897$). Each point represents averaged data from the 30-min and 60-min marks after IR. The line obtained with linear regression analysis represents the best-fit linear representation of the relationship between the two variables. **C.** The size of γ -H2AX- nuclei was significantly smaller than that of γ -H2AX+ nuclei (two-tailed t-test, $P<0.001$). A total of 524 nuclei were counted for this analysis. **D.** Representative microscopic images

of explant cultures 60 min following IR demonstrate that intratumoral leukocytes (CD45+) and tumor stem cells (Sox2+) both respond to IR with induction of γ -H2AX foci. Arrows indicate γ -H2AX- nuclei. Nuclei were stained with DAPI (blue). ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Supplemental Figure 4. Nuclear Sox2 immunoreactivity in individual explants. Raw data for the intensity of nuclear Sox2 immunoreactivity upon RIKI, TGF- β or combined treatments in HGG explants L49 (i), L50 (ii), L52 (iii), L53 (iv), L54 (v), and L55 (vi). ANOVA for each explant showed significant effect of treatment (all $P < 0.02$). Pertinent *post hoc* comparisons are shown in the figure. A.U., arbitrary units; ns, not significant; *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.001$.

Patient	Age	Sex	Diagnosis	IDH1 status	1p/19q status	Prior radiation	p-Smad2 /Observer 1	p-Smad2 /Observer 2
1	40	M	AO (WHO III)	mutant	co-deleted	No	+++ /++++	++++
2	24	F	GBM with oligodendroglial component (WHO IV)	mutant	co-deleted	Yes	++ /+++	++ /+++
3	30	F	GBM (WHO IV)	mutant		No	+++ /++++	++++
4	57	F	GBM (WHO IV)	wt		No	- /+++	- /+++
5	54	F	GBM (WHO IV)	wt		No	+++ /++++	++++
6	63	F	GBM (WHO IV)	wt		No	++ /+++	+++
	65	F	GBM (WHO IV) - recurrent	wt		Yes	+++ /++++	++++
7	70	M	GBM (WHO IV)	wt		No	+ /+++	+++
	71	M	GBM (WHO IV) - recurrent	wt		Yes	- /++	++
8	77	M	GBM (WHO IV)	wt		No	+ /+++	+++
9	62	F	GBM (WHO IV) - recurrent	wt		Yes	+++ /++++	+++ /++++
10	82	F	GBM (WHO IV)	wt		No	++ /++++	++++
11	48	F	GBM (WHO IV)	wt		No	- /++++	- /++++
12	42	F	GBM (WHO IV)	wt		No	++ /++++	++++

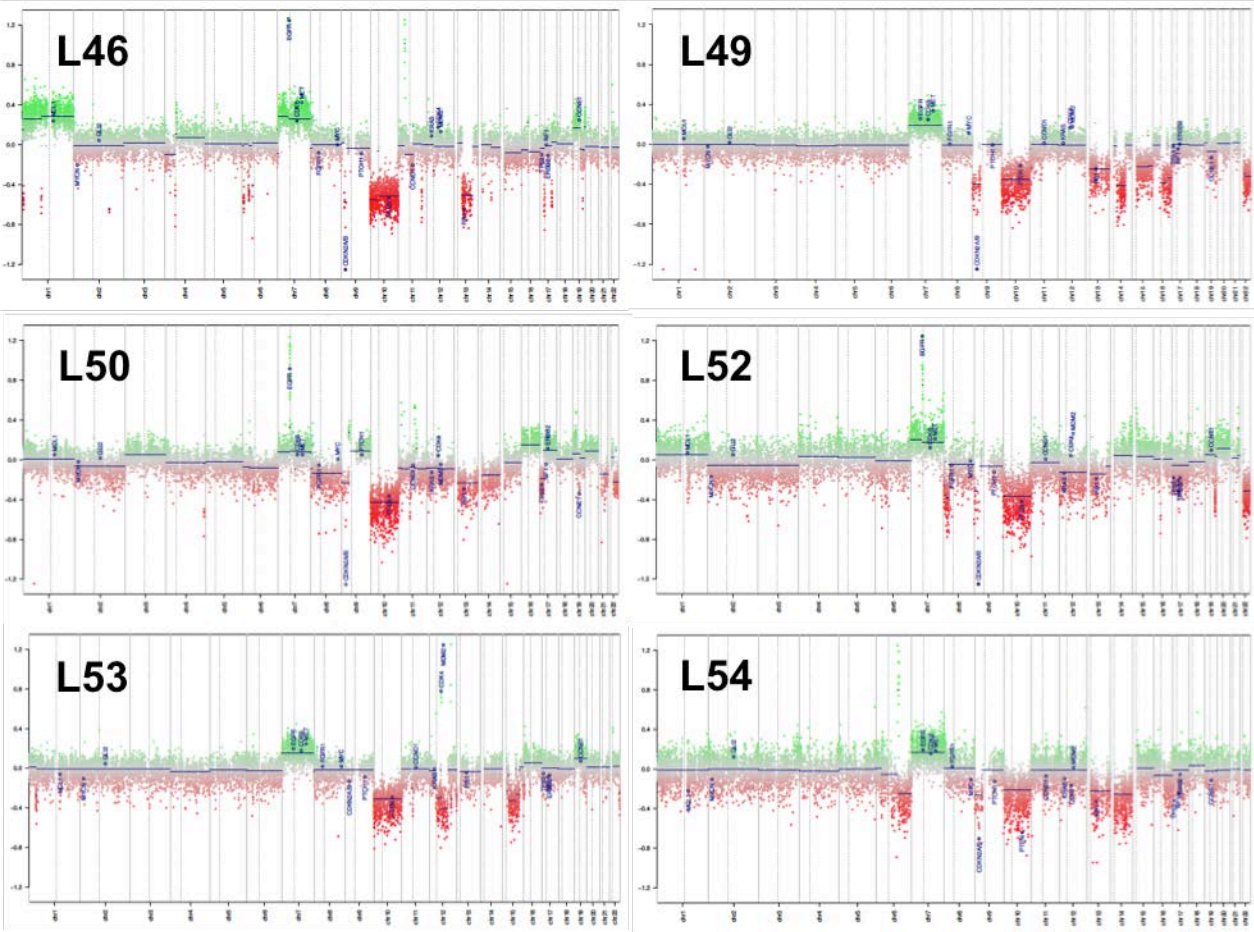
Supplemental Table 1

Patient sample number	Subtype by DNA Methylation	Histology	IDH1 status	MGMT promoter methylation	EGFR amplification	1p/19q co-deletion	Other changes inferred by WGS or focused NGS	Radiation status
L46	Non-RTK1 GBM	GBM	wild-type	Methylated	Amplified			Naïve
L49	Non-RTK1 GBM	GBM with sarcomatous features	wild-type	Methylated	Not amplified	Co-deleted		Naïve
L50	RTK1 GBM (proneural)	GBM	wild-type	Not methylated	Amplified			Naïve
L52	Non-RTK1 GBM	GBM	wild-type	Not methylated	Amplified		TP53 mutation, PTEN mutation	Naïve
L53	Non-RTK1 GBM	GBM	wild-type	Methylated	Not amplified		Mdm2 and CDK4 amplification; PTEN loss	Naïve
L54	Non-RTK1 GBM	GBM	wild-type	Methylated	Not amplified	Intact	PTEN mutation	Naïve
L55	N/A	Anaplastic astrocytoma	wild-type	Not methylated	Amplified			Radiated

	L46	L49	L50	L52	L53	L54	L55
Time points post-radiation	1 hour	30 min & 1 hour	30 min & 1 hour	30 min & 1 hour	30 min & 1 hour	30 min & 1 hour	30 min & 1 hour
γ-H2AX IF	Valid	Valid	Valid	Valid	Valid	Valid	Valid
p-Smad2 IF	Valid	Valid	N/A	Valid	Valid	Valid	Valid
Sox2 IF	N/A	Valid	Valid	Valid	Valid	Valid	Valid

Supplemental
Table 3

Copy number variation profiles by DNA Methylation array

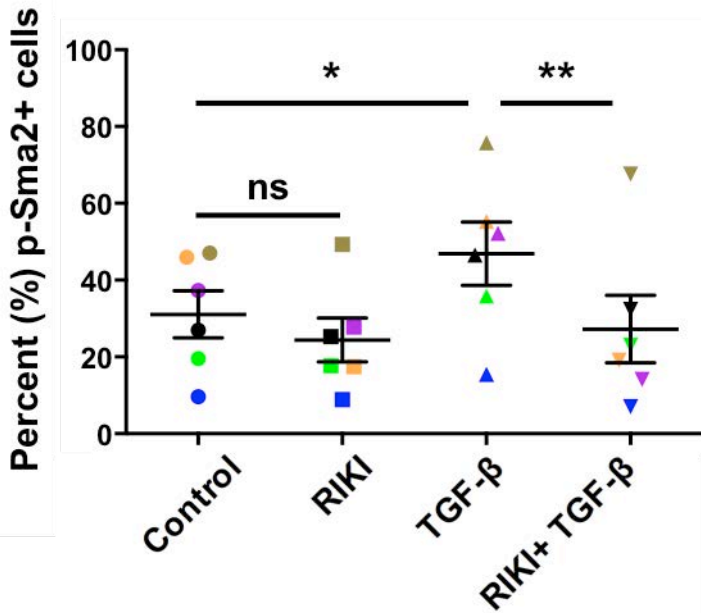


→
Chromosome number (1-22)

Supplemental
Figure 1

Specimen numbers

L46 L49 L52 L53 L54 L55



Supplemental
Figure 2

