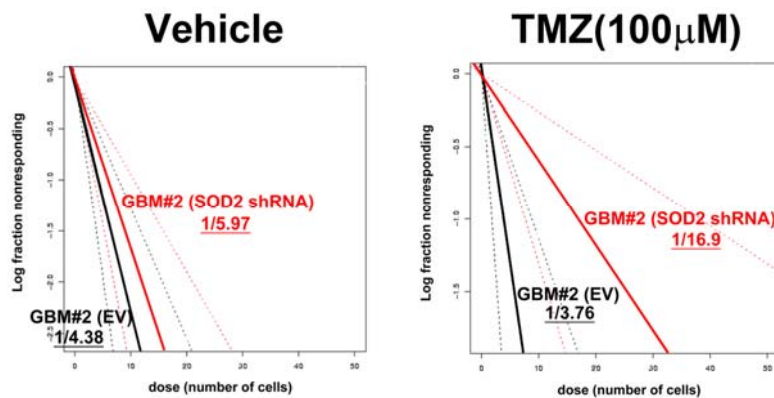


**A**

Cells per injection	Tumor culture	28 days	35 days	42 days	49 days	56 days	Days to growth at $\geq 0.1 \text{ cm}^3$ tumor
50	U87MG	(0/5)	(0/5)	(0/5)	(0/5)	(0/5)	>56
	U87-r#10	(0/5)	(0/5)	(3/5)	(3/5)	(3/5)	$56 \pm 3$
100	U87MG	(0/4)	(0/4)	(1/4)	(1/4)	(1/4)	>56
	U87-r#10	(0/4)	(2/4)	(4/4)	(4/4)	(4/4)	$48 \pm 3$
250	U87MG	(0/4)	(0/4)	(0/4)	(2/4)	(3/4)	$56 \pm 4$
	U87-r#10	(0/4)	(0/4)	(3/4)	(4/4)	(4/4)	$46 \pm 2$
500	U87MG	(0/4)	(0/4)	(1/4)	(2/4)	(4/4)	$49 \pm 2$
	U87-r#10	(0/4)	(2/4)	(4/4)	(4/4)	(4/4)	$42 \pm 2$
1000	U87MG	(0/3)	(1/3)	(2/3)	(3/3)	(3/3)	$46 \pm 2$
	U87-r#10	(1/3)	(2/3)	(3/3)	(3/3)	(3/3)	$39 \pm 2$

**B**



**Additional file 5: Figure S5.** The assays of tumor-initiating cell (TIC) properties. (A) Tumor formation analysis with limiting dilution (50~1000 cells per injection) of U87-parental and TMZ-resistant (r#10) cells in subcutaneous flank area of NOD-SCID mice was performed and recorded. Tumor formation was defined as the measurement to reach  $0.1 \text{ cm}^3$  or larger. The number of tumor formation in total implanted mice was shown in the brackets. (B) The frequency of the TIC-featuring population of GBM#2 (derived from another recurrent GBM patient) with or without TMZ treatment was estimated using the *in vitro* extreme limiting dilution assay.