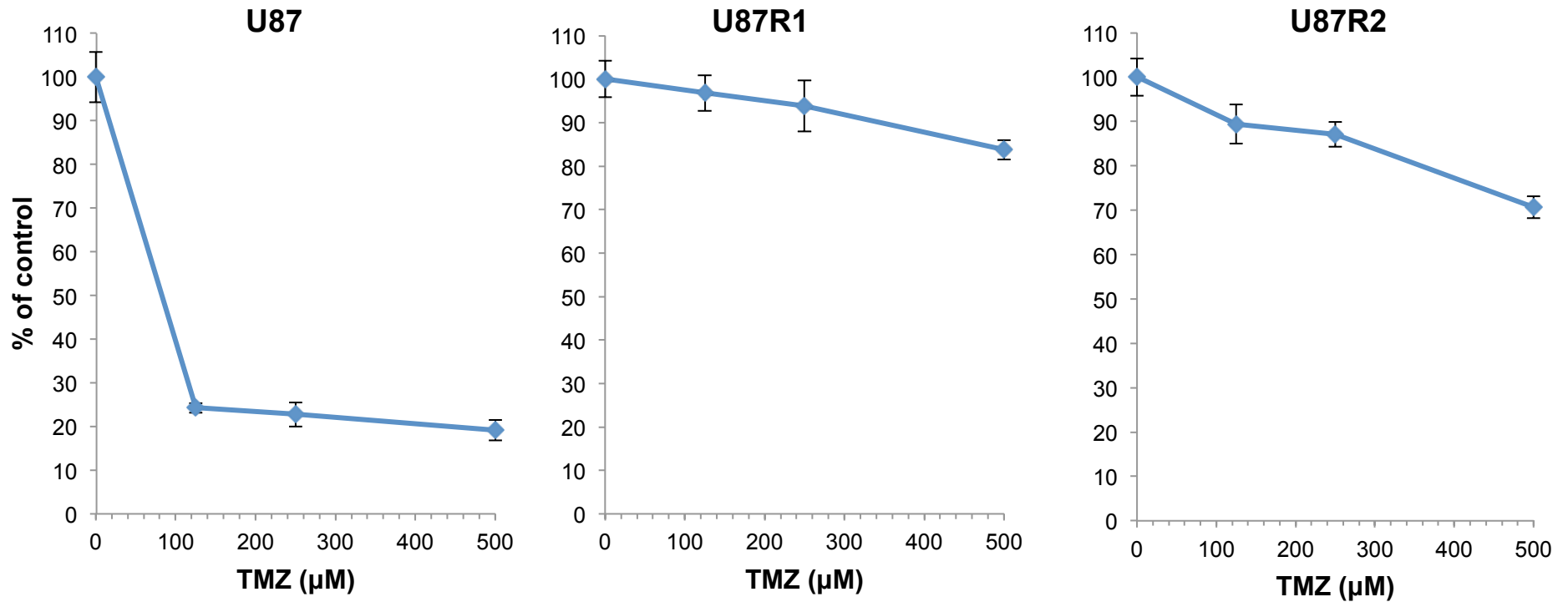
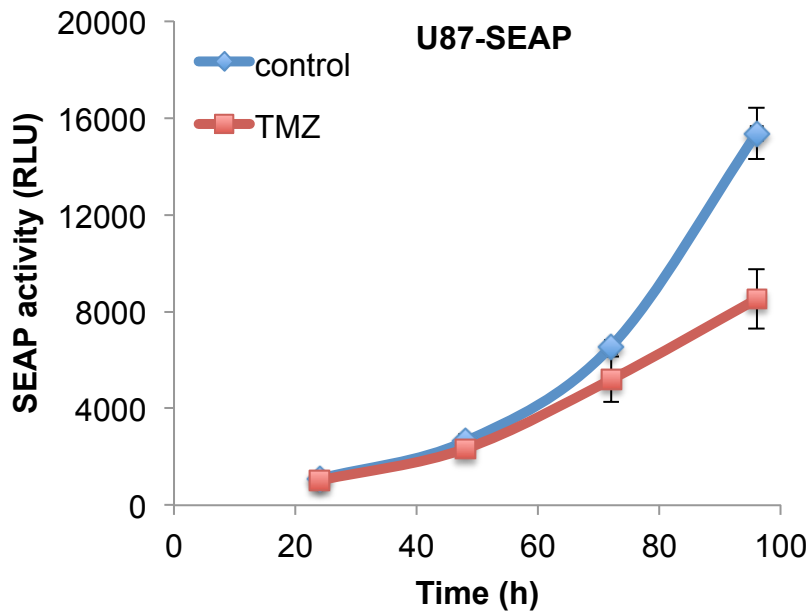
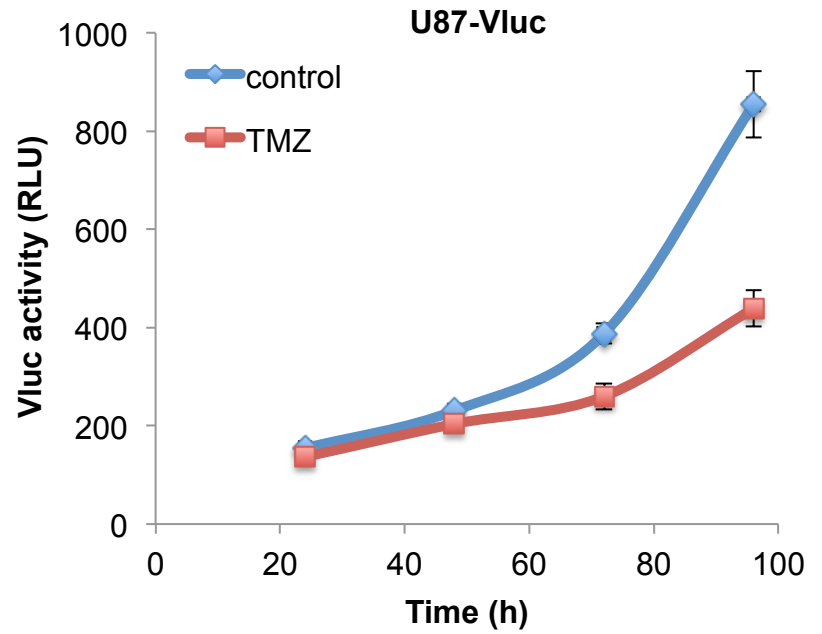
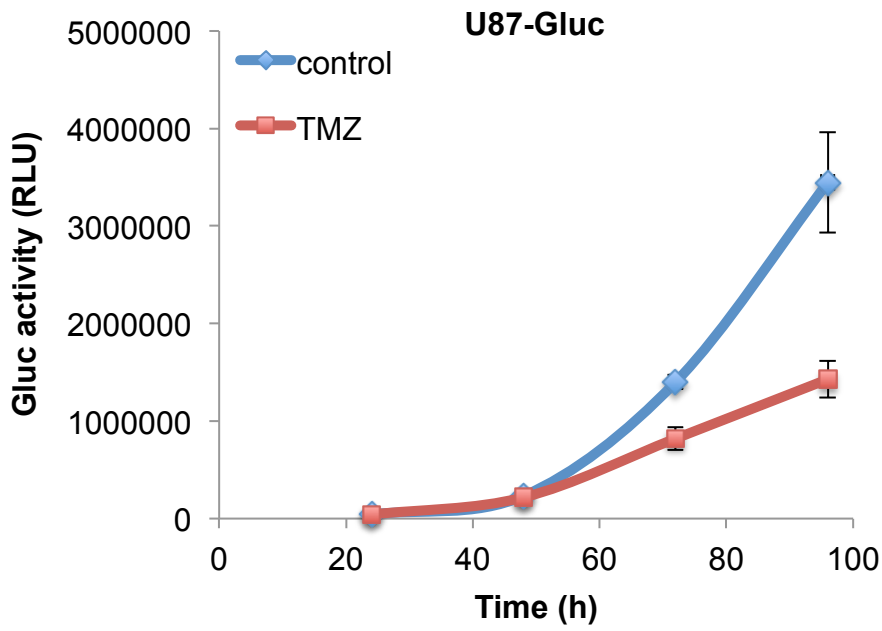


Supplementary Figure 1. Optimization of Vluc assay. (a) Blood was withdrawn from mice bearing subcutaneous U87-Vluc tumors and 5 μl blood or serum (in triplicates) was assayed for Vluc activity using different concentration of vargulin (in 100 μl). (b) conditioned medium from U87 cells expressing Vluc or Fluc (control) were assayed for Vluc activity in the presence and absence of EDTA.

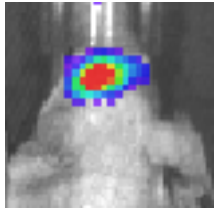


Supplementary Figure 2. Characterization of U87R1 and U87R2 TMZ-resistant clones. U87, U87R1 and U87R2 cells were plated in a 96-well plate and treated with different concentrations of TMZ or DMSO control. Four days later, cells were fixed with 4% paraformaldehyde and stained with DAPI (0.3 μg/ml diluted in PBS) . Cell number was assessed by counting the number of DAPI-stained cells using the Acumen Ex3 laser scanning cytometer (TTP LabTech, Royston, UK). Data presented as Average ± SD in which the DMSO control is set at 100%.



Supplementary Figure 3. Gluc, Vluc and SEAP as reporters for cell viability. U87 parent glioma cells (sensitive to TMZ) expressing either Gluc, Vluc or SEAP were plated in a 96-well plate and treated with 100 μ M TMZ or DMSO (control). At different time points, aliquots of conditioned medium were assayed for each reporter activity.

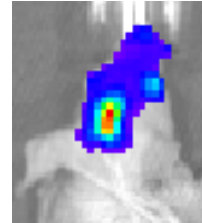
Gluc imaging with
coelenterazine



Imaging 4 hours
post-coelenterazine



Vluc imaging
with vargulin



Imaging 24 hours
post-vargulin



Supplementary Figure 4. Sequential in vivo imaging. U87-Gluc, U87R1-Vluc and U87R2-SEAP/Fluc were mixed equally (20,000 from each line) and implanted in the brain of nude mice. One week later, mice were first imaged for Gluc after injection of coelenterazine. Four hours later, the same mice were imaged without injection of any substrate which gave no signal showing that this waiting period is sufficient for the Gluc signal to reach background levels. Then, mice were injected with vargulin and imaged for Vluc. Twenty-four hours later, mice were imaged again without injection of any substrate which also showed no signal proving that this waiting period is also sufficient for Vluc signal to reach background levels.