Supplementary Information

Extended Supplementary Methods

**Cell Growth.** Laminin cultures were maintained using standard tissue culture techniques, except that instead of trypsin, they were dislodged with accutase (Sigma-Aldrich A6964), which was removed by centrifugation prior to replating and direct addition of laminin.[1]

**Screening.** Each screening sample was begun with freshly dissociated neurospheres or freshly detached laminin-supplemented cells. Dissociated neurospheres were passed through a cell strainer to remove clumps. Cells were counted on a Beckman Coulter Vi-Cell Cell Viability Analyzer. Cells were diluted to 0.038 x $10^6$ cells/mL, for a final amount of 1,000 cells/well in 26 µL. With a Biomek FX liquid handling system, tips were pre-wetted and cells were seeded in white, opaque, tissue-culture-treated plates (PerkinElmer 6007688). Tips in wells C3 to M3 were removed, so no cells were seeded in those wells. NMGF (neurosphere media with growth factors) was manually added, and the luminescence reading from those wells was later averaged and set to 0% viability.

Twenty-four hours later, the inhibitors were added. Generally, except in some instances of low solubility, each inhibitor was added in a 17-point, 2-fold, dilution series with a final concentration range of 0.8 nM – 50 µM. Seventy-two hours later, viability was assessed by the addition of a 50% DMEM/F12:50% CellTiter-Glo (CTG) mixture in accordance with CTG manufacturer's instructions and a PerkinElmer Victor X5 plate reader. Percent viability was calculated with wells containing only NMGF as 0% viability, and cells treated with DMSO as 100% viability. The final concentration of DMSO was 0.5%. All experiments were performed in technical triplicate, and $EC_{50}$ values were determined using GraphPad Prism 6.0e with the following equation: Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)). Fits were generally constrained to 0% viability for the bottom. For compounds that did not kill the entire cell population, the bottom of the curve was not constrained to 0% viability and instead was a fitted parameter. Therefore, in these cases, the $EC_{50}$ was the 50% effective concentration. In cases in which every concentration of drug affected viability, the top was constrained to 100% viability. In general, constraints were chosen to have the fit most representative of the data. Compounds that were not potent within the concentration range tested are denoted as $EC_{50} > 50$ µM. To compute fold change (Figure 3b and Table S4) and calculate the z-scores used for the heatmap in Figure 2, the value 100 µM was assigned to cases of $EC_{50} > 50$ µM.

**Comparison of Inhibitor Sensitivity in Neurosphere and Laminin Growth Conditions.** HF2303 cells, grown since being defrosted as neurospheres, were tested against a set of ten inhibitors. A subset of HF2303 cells were switched to laminin and allowed to grow for three weeks. For the HF2876 sample, the cells were originally defrosted and neurosphere growth was attempted. After the cells failed to expand, laminin was added and cell growth increased. For HF2876, inhibitor sensitivity for neurospheres and laminin were tested on the same day, where one set of plates was seeded with laminin and the other without.

**Heatmap Generation.** The heatmap in Figure 2, with clustering, was generated in R using the heatmap.2 function in the gplots package. The log10 of the $EC_{50}$ values and the z-score was

computed for each compound within the cell lines to adjust for the general potency of the compound.

**a. HF2303 Tested as Spheres and on Laminin**

| Drug | $EC_{50}$ Sphere (μM) | 95% Confidence Interval | $EC_{50}$ Laminin (μM) | 95% Condfidence Interval |
|---|---|---|---|---|
| Phenylarsine Oxide | 0.01914 | 0.01667 to 0.02199 | 0.03731 | 0.03334 to 0.04176 |
| Lovastatin | 45.16 | 32.12 to 63.49 | 51.17 | 32.95 to 79.45 |
| Bortezomib | 0.006577 | 0.005965 to 0.007252 | 0.02057 | 0.01912 to 0.02214 |
| Melatonin | > 50.00 | NA | > 50.00 | NA |
| AGI-5198 | > 50.00 | NA | > 50.00 | NA |
| Decitabine | > 50.00 | NA | > 50.00 | NA |
| JIB-04 | 5.814 | Very Wide | 39.52 | 36.98 to 42.24 |
| Lenvatinib (E7080) | > 50.00 | NA | > 50.00 | NA |
| Lenalidomide | > 50.00 | NA | > 50.00 | NA |
| Pifithrin-u | 3.353 | 3.061 to 3.673 | 5.804 | 5.371 to 6.271 |

**b. HF2876 Tested as Spheres and on Laminin**

| Drug | $EC_{50}$ Sphere (μM) | 95% Confidence Interval | $EC_{50}$ Laminin (μM) | 95% Condfidence Interval |
|---|---|---|---|---|
| Phenylarsine Oxide | 0.03519 | 0.03292 to 0.03762 | 0.06563 | 0.06010 to 0.07168 |
| Crizotinib | 3.921 | 3.533 to 4.351 | 7.659 | 6.647 to 8.825 |
| Vemurafenib | 10.40 | 9.068 to 11.92 | 12.59 | 10.85 to 14.61 |
| Dasatinib | 41.38 | 32.17 to 53.22 | 59.85 | 36.10 to 99.21 |
| Sunitinib | 10.17 | 8.284 to 12.49 | 28.22 | 25.50 to 31.24 |
| Temsirolimus | > 50.00 | NA | > 50.00 | NA |
| AZD-8055 | 0.04241 | 0.03330 to 0.05400 | 0.1260 | 0.09133 to 0.1738 |
| Selumetinib | 40.40 | 29.57 to 55.19 | 74.41 | 48.93 to 113.1 |
| Temozolomide | > 50.00 | NA | > 50.00 | NA |
| Afatinib | 0.4502 | 0.2690 to 0.7535 | 1.324 | 0.7998 to 2.192 |
| Axitinib | 5.131 | 3.796 to 6.936 | 37.38 | 26.89 to 51.97 |

Table S2: Neurosphere and Laminin Grown Inhibitor Sensitivity. HF2303 cells grown as neurospheres were tested as neurospheres and after being switch to cell growth with laminin for three weeks. HF2876 grows on laminin, but not as neurospheres, and thus inhibitor sensitivity of HF2876 as neurospheres was tested after immediate removal from laminin growth conditions. $EC_{50}$s were obtained from technical triplicate and 95% confidence intervals were generated from curve fitting.

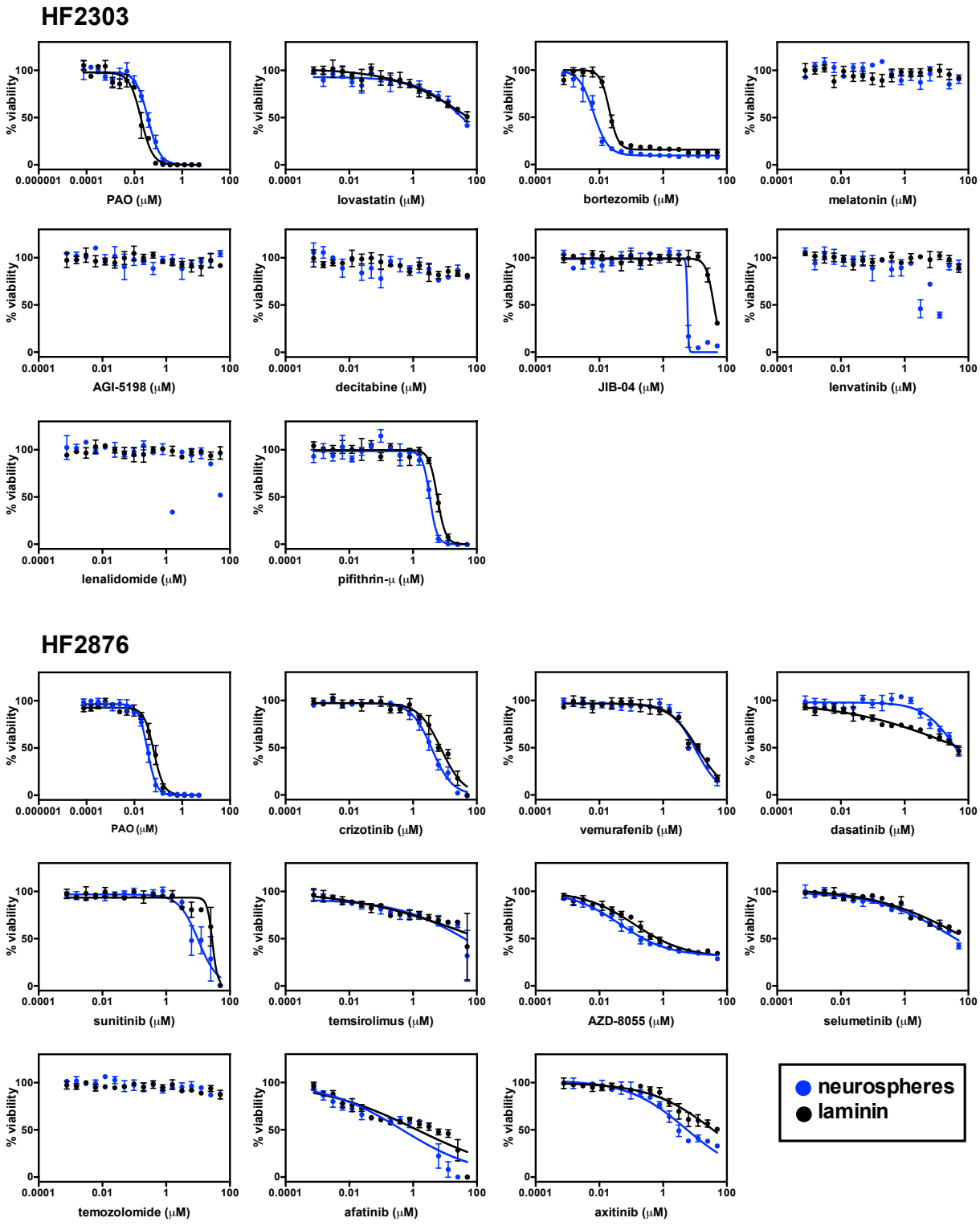


Figure S1: Inhibitor Sensitivity Comparison of Neurosphere and Laminin Growth Method. Error bars are the standard deviation of technical triplicates. Data was fit using GraphPad Prism 6.0e log(inhibitor) vs. response – variable slope (four parameters) equation: Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)).

**a. HF2476 Tested with CellTiter-Glo and PrestoBlue**

| | PrestoBlue $EC_{50}$ (μM) | 95% Confidence Interval | CellTiter-Glo $EC_{50}$ (μM) | 95% Confidence Interval |
|---|---|---|---|---|
| Phenylarsine Oxide | 0.003573 | 0.003310 to 0.003858 | 0.01044 | 0.009524 to 0.01145 |
| Crizotinib | 1.037 | 0.7716 to 1.394 | 1.320 | 1.126 to 1.547 |
| Vemurafenib | 0.8927 | 0.8066 to 0.9881 | 3.384 | 2.931 to 3.907 |
| Dasatinib | 3.823 | 3.282 to 4.453 | 2.858 | 2.173 to 3.760 |
| Sunitinib | 2.038 | 1.874 to 2.215 | 3.420 | 3.131 to 3.735 |
| Temsirolimus | 1.291 | 1.143 to 1.458 | 7.096 | 4.865 to 10.35 |
| AZD-8055 | 0.01305 | 0.006667 to 0.02555 | 0.03276 | 0.02608 to 0.04116 |
| Selumetininb | 17.64 | 15.74 to 19.78 | 15.10 | 11.17 to 20.40 |
| Temozolomide | > 50.00 | NA | > 50.00 | NA |
| Afatinib | 1.825 | 1.687 to 1.974 | 2.207 | 1.555 to 3.132 |
| Axitinib | 13.99 | 10.62 to 18.42 | 0.4933 | 0.3886 to 0.6262 |

**b. HF2876 Tested with CellTiter-Glo and PrestoBlue**

| | PrestoBlue $EC_{50}$ (μM) | 95% Confidence Interval | CellTiter-Glo $EC_{50}$ (μM) | 95% Confidence Interval |
|---|---|---|---|---|
| Phenylarsine Oxide | 0.006545 | 0.005820 to 0.007361 | 0.06563 | 0.06010 to 0.07168 |
| Crizotinib | 1.257 | 1.078 to 1.465 | 7.659 | 6.647 to 8.825 |
| Vemurafenib | 1.246 | 1.084 to 1.433 | 12.59 | 10.85 to 14.61 |
| Dasatinib | 7.228 | 6.348 to 8.231 | 59.85 | 36.10 to 99.21 |
| Sunitinib | 3.261 | (Very wide) | 28.22 | 25.50 to 31.24 |
| Temsirolimus | 2.087 | 1.888 to 2.307 | > 50.00 | NA |
| AZD-8055 | 0.01503 | 0.01145 to 0.01972 | 0.126 | 0.09133 to 0.1738 |
| Selumetininb | > 50.00 | NA | 76.65 | 50.91 to 115.4 |
| Temozolomide | > 50.00 | NA | > 50.00 | NA |
| Afatinib | 1.445 | 1.259 to 1.659 | 1.324 | 0.7998 to 2.192 |
| Axitinib | 26.89 | 18.87 to 38.32 | 37.38 | 26.89 to 51.97 |

Table S5: Confirmation of $EC_{50}$ Values with PrestoBlue for a) HF2476 and b) HF2876. Using identical cell culture, cell seeding, and inhibitor addition conditions, inhibitor sensitivity was confirmed using PrestoBlue (Life A-13262) as the reagent to measure cell viability.

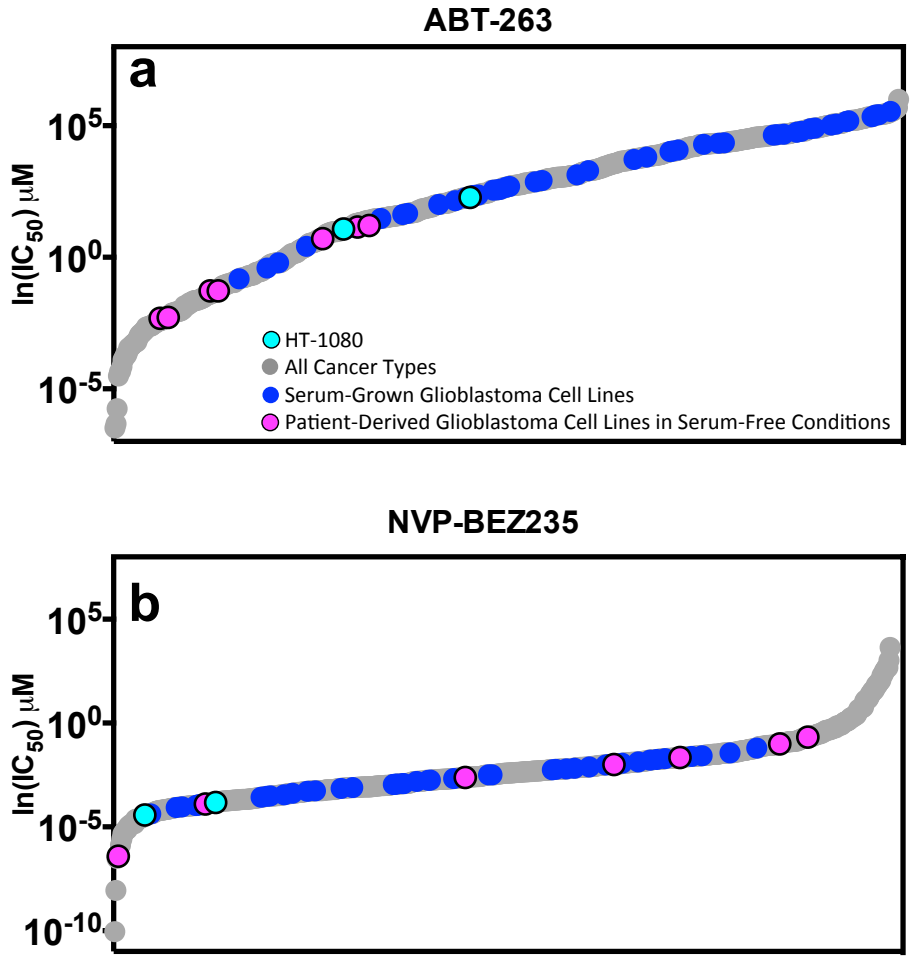


Figure S2: Comparison of Patient-Derived GBM Cell Line Inhibitor Sensitivity to www.CancerRxGene.org Data.[2] Comparison of all inhibitor sensitivity data from www.CancerRxGener.org for a) ABT-263 and b) NVP-BEZ235 to the patient-derived culture conditions. The teal HT-1080 point is a control, which is the HT-1080 inhibitor sensitivity in the Stockwell lab (point on the left in both cases) against the indicated compound, and the inhibitor sensitivity of HT-1080 against the indicated compound in the www.CancerRxGene.org screen (point of the right). Inhibitor sensitivity data was plotted from most sensitive to least sensitive. In panel a, sensitivity ranking of the patient-derived GBM cell line data was shifted slightly for overlapping points to allow for visualization.

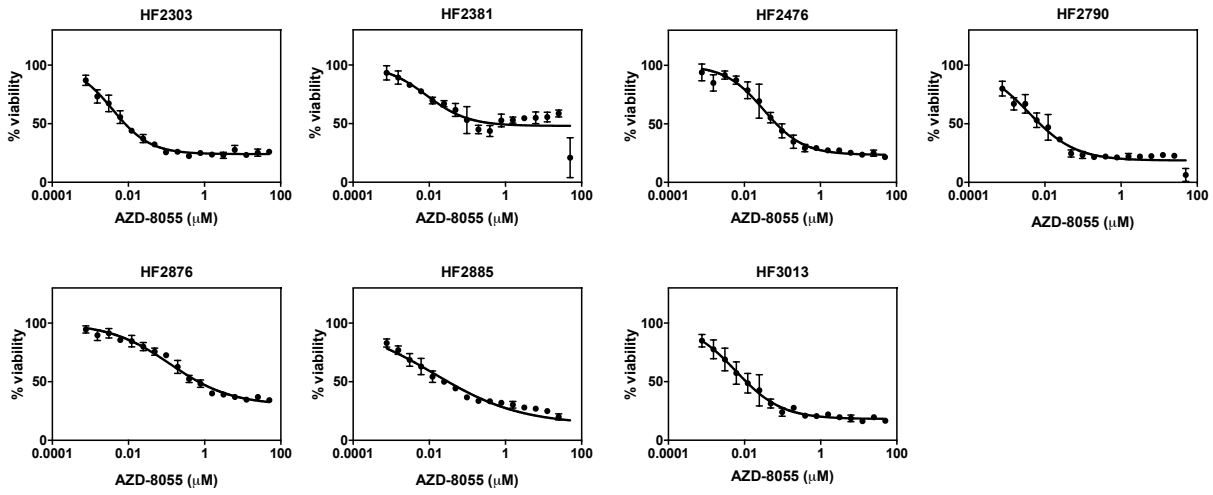


Figure S3: Inhibition by AZD-8055 Across All Cell Lines. Error bars are the standard deviation of technical triplicate experiments. When fitting AZD-8055 data, fitting was constrained with a "Top" value of 100.

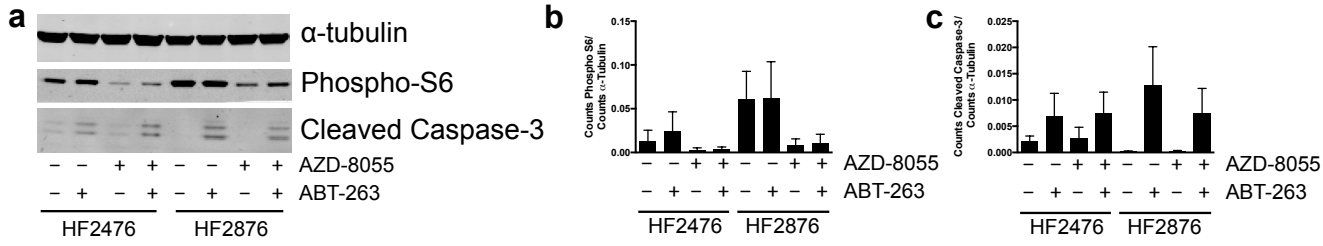


Figure S4: Western Blot of AZD-8055 and ABT-263 Treated Cells. HF2476 and HF2876 patient-derived GBM cells were treated with vehicle (DMSO), AZD-8055, ABT-263, or both AZD-8055 and ABT-263. a) A western blot showing the levels of phospho-S6, phosphorylation of ribosomal protein S6 at Ser 235 and Ser 236 (CST 4858), which is indicative of mTOR activity, and levels of cleaved caspase-3 (CST 9664), an indicator of apoptosis. Quantification of band intensity normalized to the α-tubulin control is shown b) for phospho-S6 protein levels and c) for cleaved caspase-3 protein levels. For bar graphs N = 2 and error bars are the standard deviation. Imaging and quantification were performed with the LI-COR system.

References:

(1) Danovi, D.; Folarin, A. A.; Baranowski, B.; Pollard, S. M. High Content Screening of Defined Chemical Libraries Using Normal and Glioma-Derived Neural Stem Cell Lines. *Methods Enzymol* **2012**, *506*, 311–329.

(2) Garnett, M. J.; Edelman, E. J.; Heidorn, S. J.; Greenman, C. D.; Dastur, A.; Lau, K. W.; Greninger, P.; Thompson, I. R.; Luo, X.; Soares, J.; Liu, Q.; Iorio, F.; Surdez, D.; Chen, L.; Milano, R. J.; Bignell, G. R.; Tam, A. T.; Davies, H.; Stevenson, J. A.; Barthorpe, S.;

Lutz, S. R.; Kogera, F.; Lawrence, K.; McLaren-Douglas, A.; Mitropoulos, X.; Mironenko, T.; Thi, H.; Richardson, L.; Zhou, W.; Jewitt, F.; Zhang, T.; O'Brien, P.; Boisvert, J. L.; Price, S.; Hur, W.; Yang, W.; Deng, X.; Butler, A.; Choi, H. G.; Chang, J. W.; Baselga, J.; Stamenkovic, I.; Engelman, J. A.; Sharma, S. V; Delattre, O.; Saez-Rodriguez, J.; Gray, N. S.; Settleman, J.; Futreal, P. A.; Haber, D. a; Stratton, M. R.; Ramaswamy, S.; McDermott, U.; Benes, C. H. Systematic Identification of Genomic Markers of Drug Sensitivity in Cancer Cells. *Nature* **2012**, *483* (7391), 570–575.