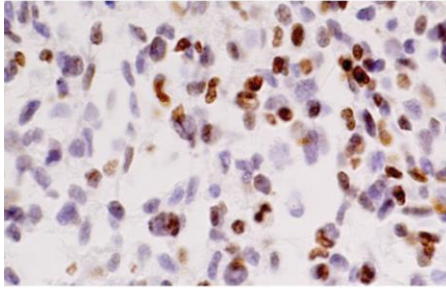


Supplementary Figure 1

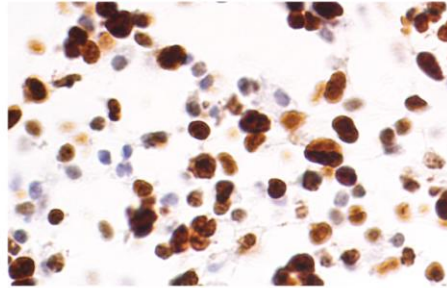
Sphere forming assay of BT145 GBM PDCL

(a) Sphere forming potential of single BT145 GBM cells isolated from bulk culture or SMR post-MAR measurement. p-values reflect output of Pearson's chi-squared test. **(b)** Representative single-cell trajectories paired with images of sphere forming potential 2-weeks after measurement. Note that even cells with minimal or negative growth over the 15-minute period may retain tumorsphere-forming potential (bottom right).

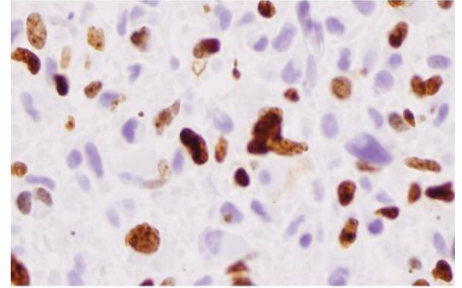
BT145 Ki67+: 30.0%



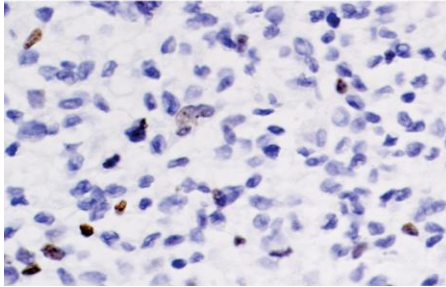
BT159 Ki67+: 80.5%



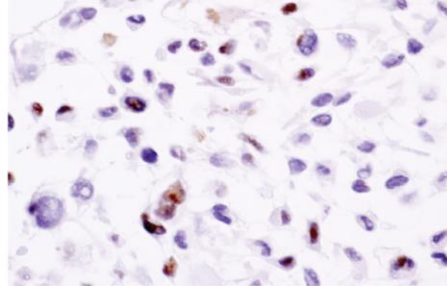
BT179 Ki67+: 45.5%



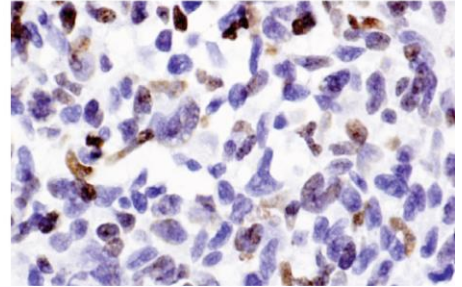
BT240 Ki67+: 4.8%



BT320 Ki67+: 29.1%



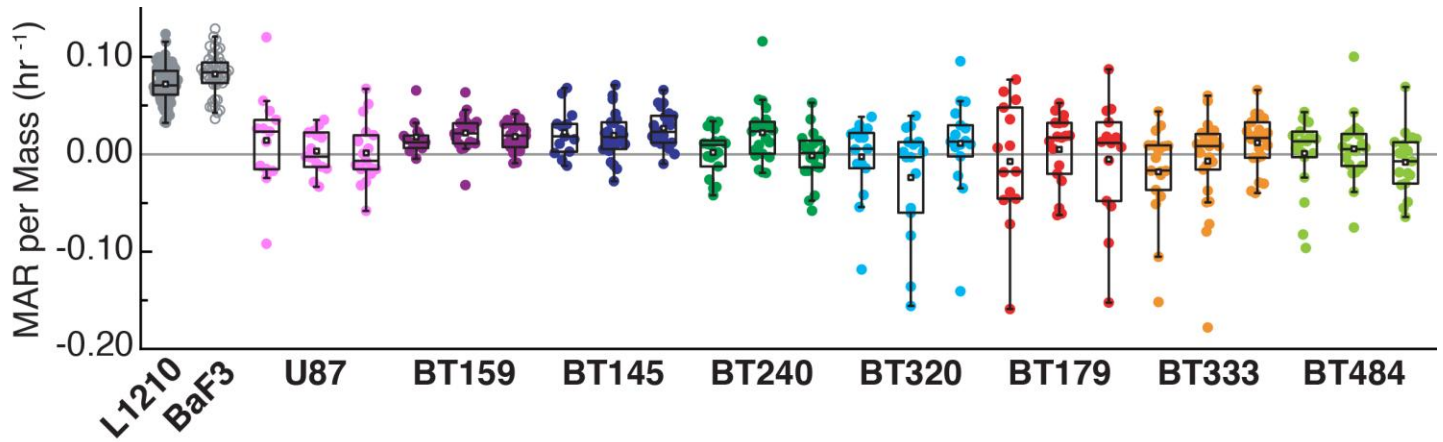
BT333 Ki67+: 30.0%



Supplementary Figure 2

Growth heterogeneity maintained across multiple passages of PCDLs

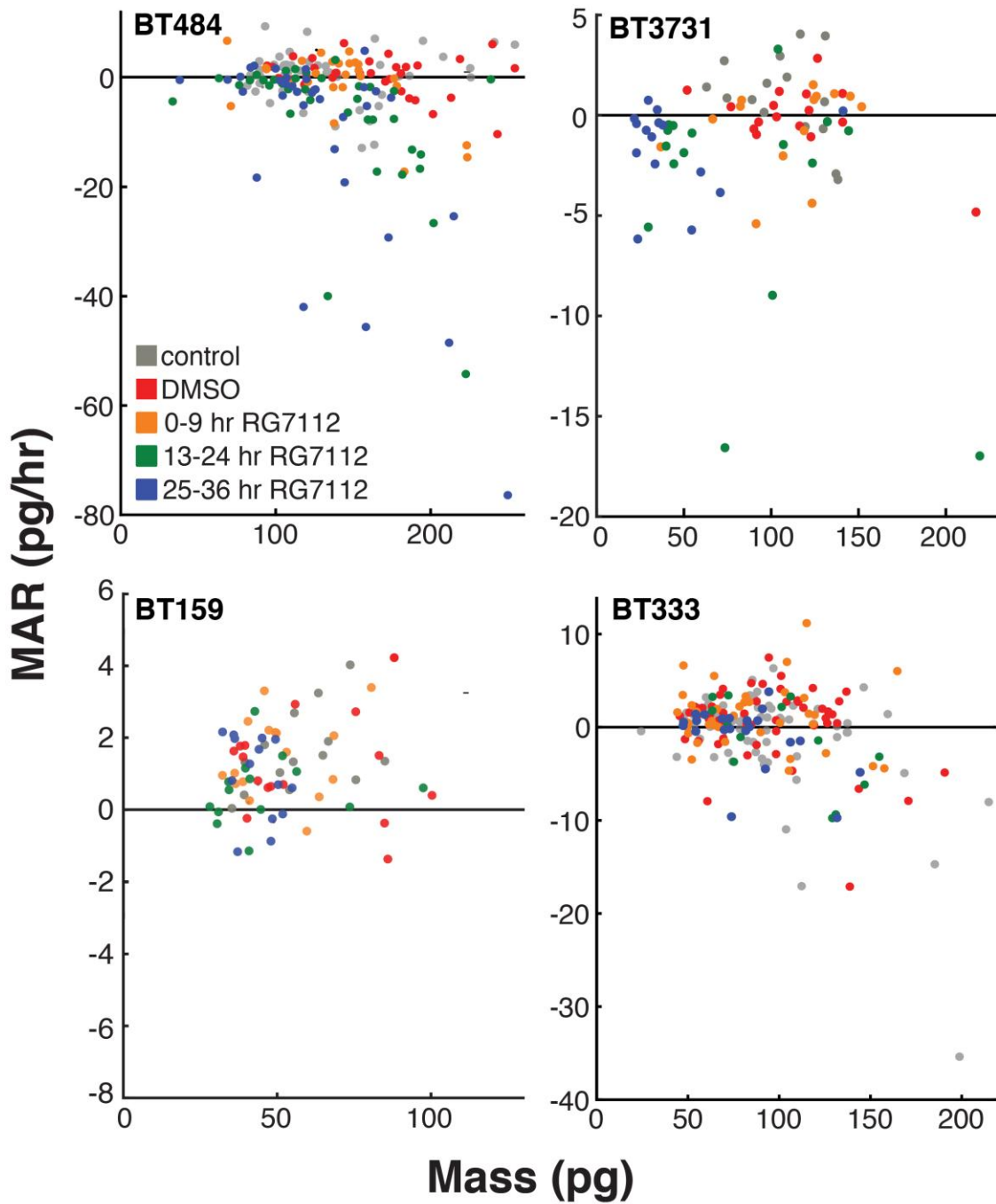
Representative staining results from immunohistochemistry for Ki67 on BT145, BT159, BT179, BT240, BT320, and BT333 cell lines. Results quantified as percentage of total cells stained positive.



Supplementary Figure 3

Growth heterogeneity maintained across multiple passages of PCDLs

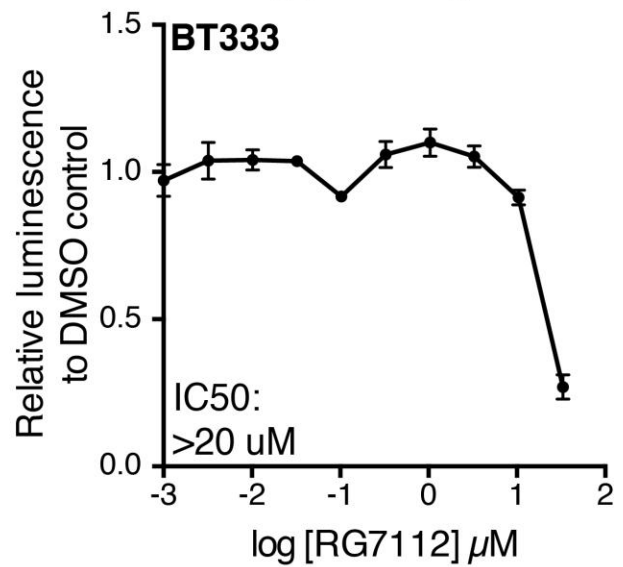
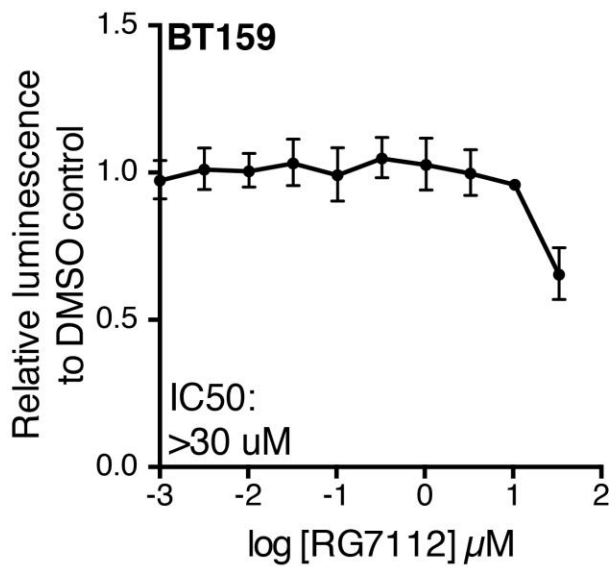
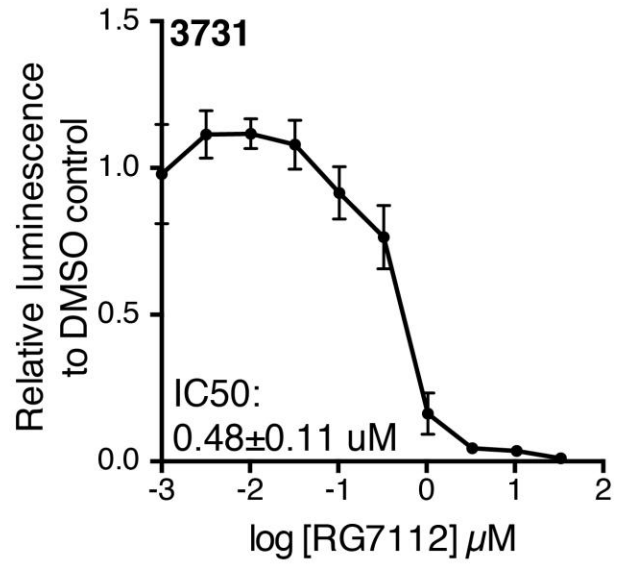
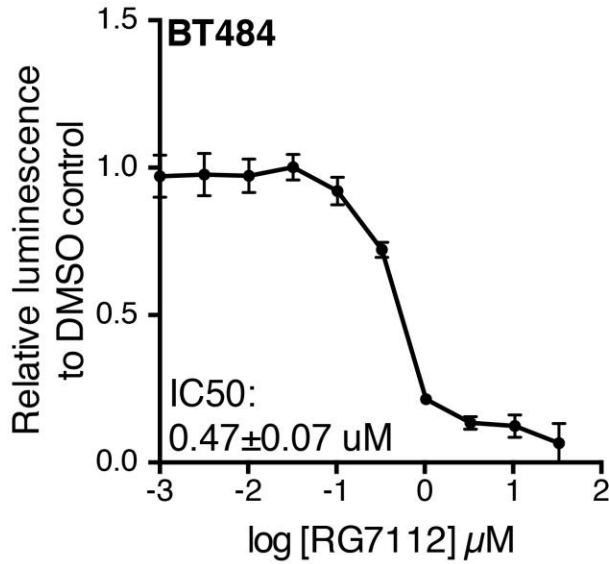
Box plot comparison of MAR normalized to mass for the same cell lines as in Figure 1c with three passages of GBM-PDLs shown separately. Boxes represent the inter-quartile range and white squares the average of all measurements. From left to right, n = 84; 46; 13, 14, 17; 12, 21, 18; 21, 18, 13; 19, 21, 21; 16, 16, 16; 14, 18, 14; 18, 25, 21; 19, 22, 18.



Supplementary Figure 4

PDCLs following treatment with MDM2 inhibitor RG7112

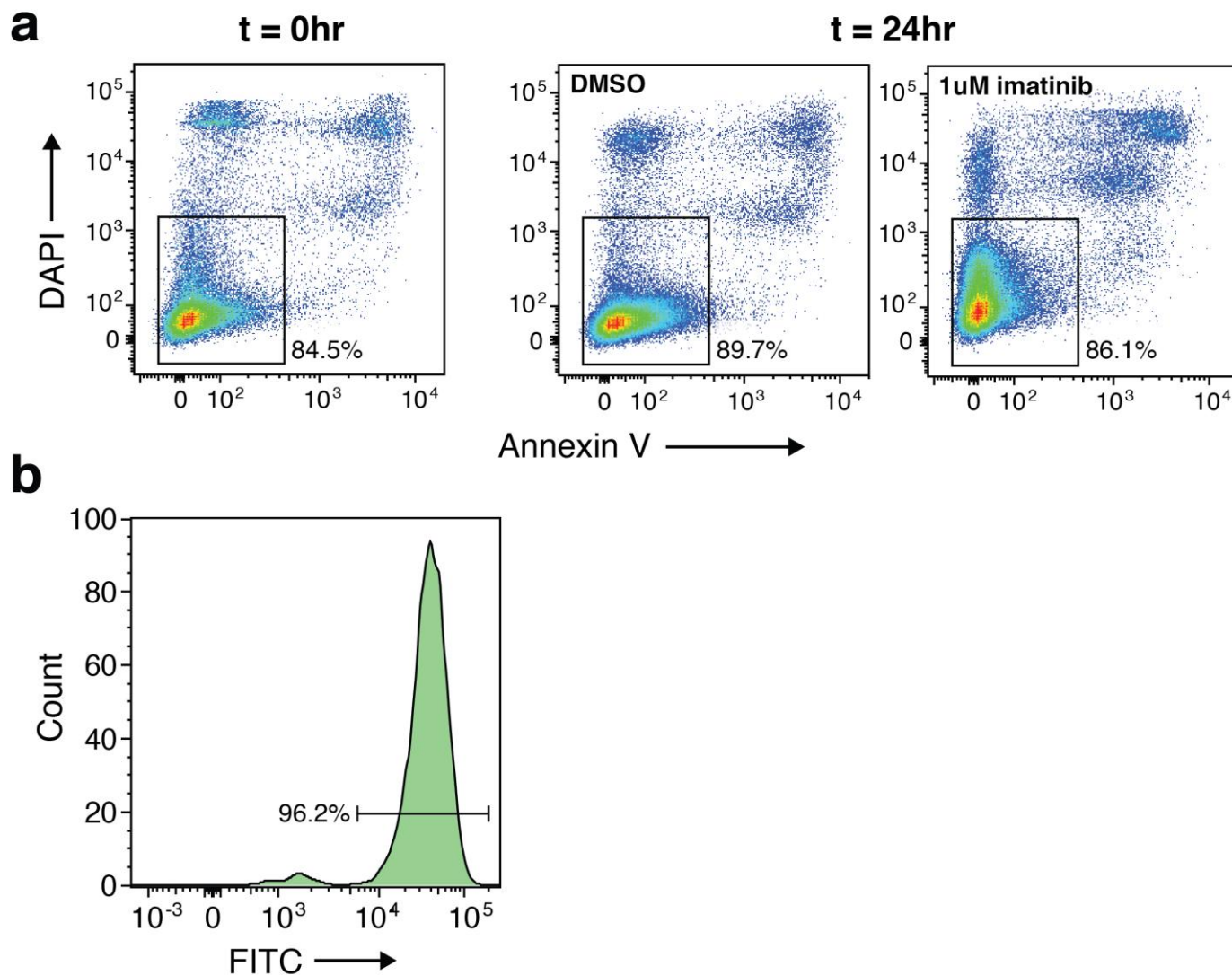
Scatter plots of MAR versus mass for BT484, BT3731, BT159, BT333 cells following treatment with 1 μ M RG7112.



Supplementary Figure 5

Dose-response curves for GBM PDCLs treated with RG7112

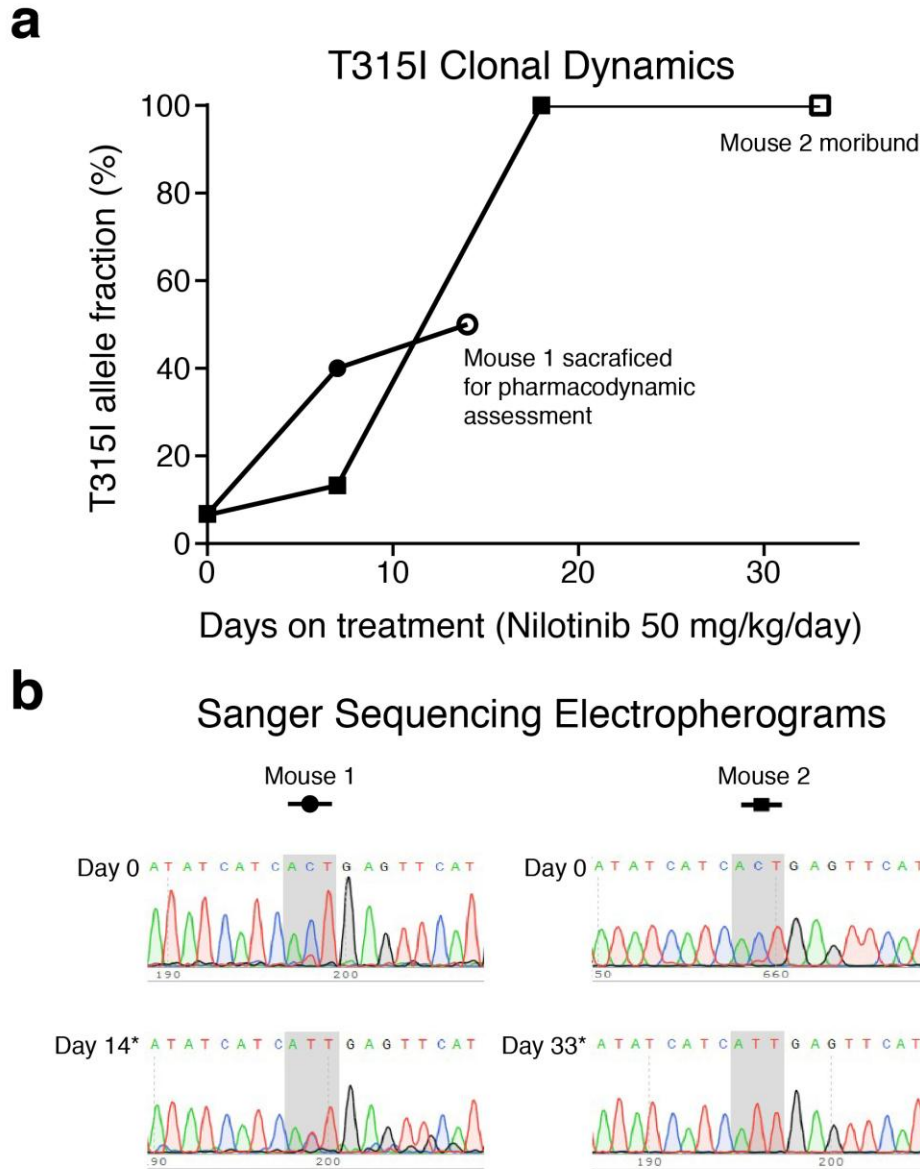
Curves from PDCLs were generated using CellTiter-Glo at 72 hrs, following treatment with 1 μM RG7112. IC50 values embedded in each graph reflect the output of a four parameter nonlinear regression model +/- the range of the 95% confidence interval.



Supplementary Figure 6

Viability and purity of primary murine B-ALL by flow cytometry

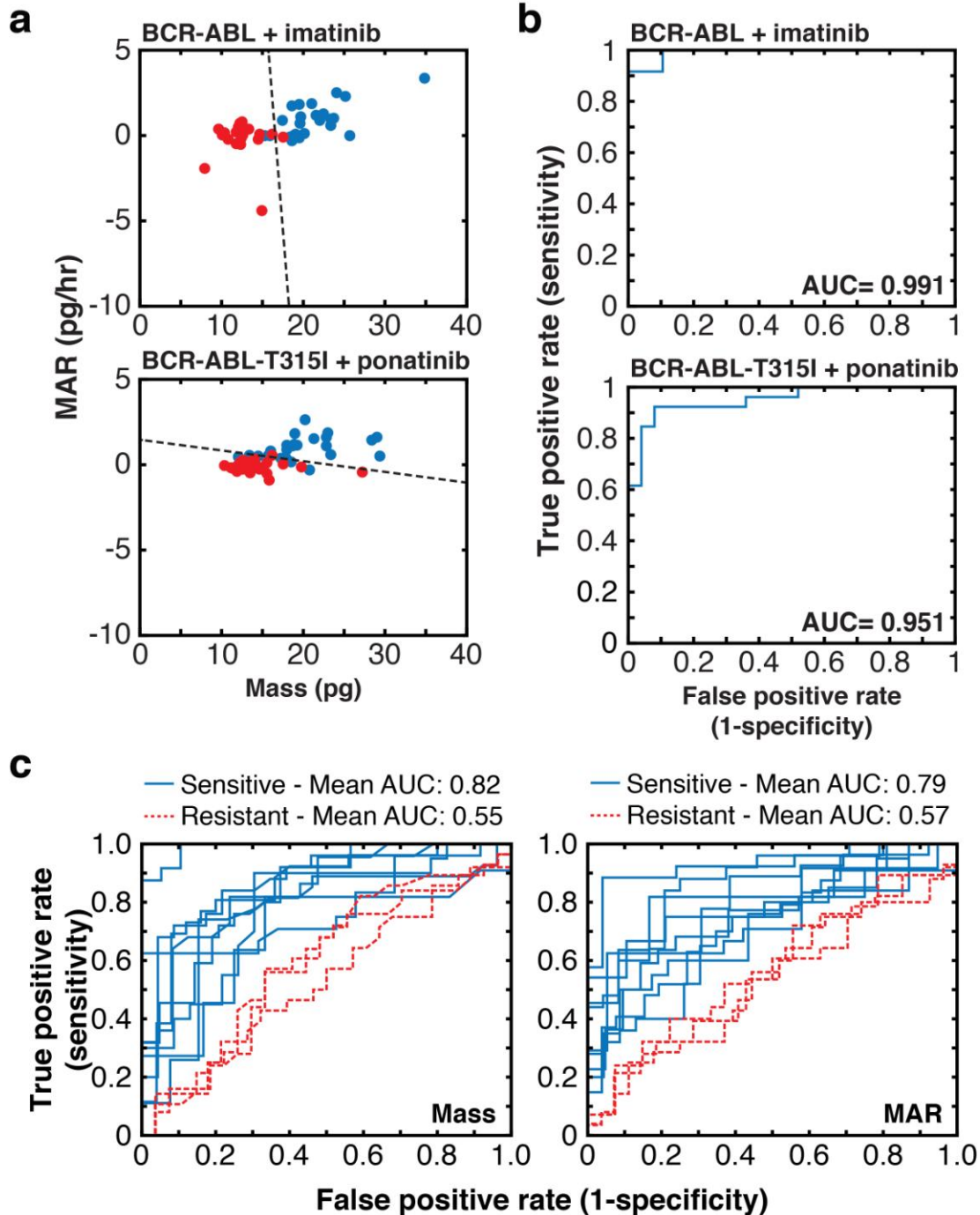
(a) Representative dot plots of cells stained with DAPI and Annexin V, as markers of viability. **(b)** Representative histogram of GFP expression after cell sorting. Leukemia cells in this model uniquely express GFP. FACS analysis was performed on all primary murine splenocyte samples.



Supplementary Figure 7

In vivo clonal dynamics in a transgenic murine model of BCR-ABL B-ALL

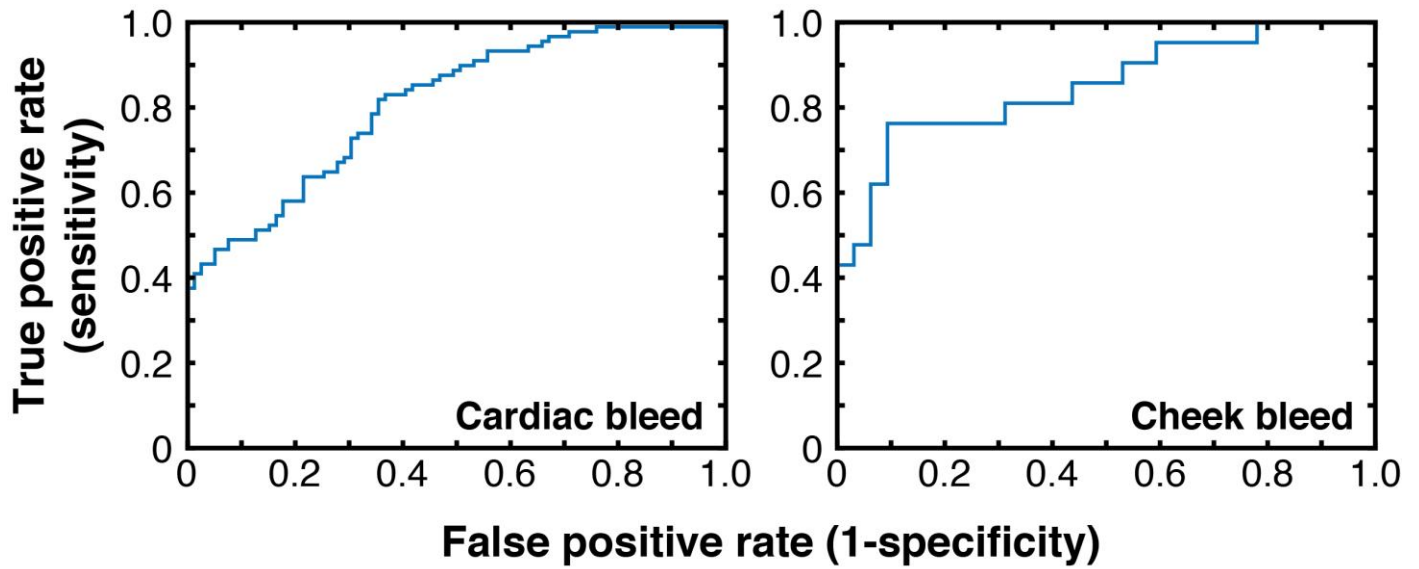
(a) The allelic frequency of BCR-ABL T315I in two mice treated with nilotinib, a surrogate for imatinib. Allelic frequencies were calculated by visual measurement of the relative heights of the electropherogram peaks; values from the paired forward and reverse sequencing phases were averaged to produce the allelic frequencies shown. Mouse 1 (closed circles) was sacrificed on day 14 for routine pharmacodynamic assessment (open circle). Mouse 2 (closed squares) was sacrificed after developing clinical signs of advanced leukemia on day 33 (open square). **(b)** Representative electropherograms showing ABL codon 315 (in gray; ACT indicates wild type T315, and ATT indicates the point mutation T315I). As demonstrated, the mutant subclone expands in relation to WT during treatment with nilotinib, to which WT but not T315I BCR-ABL is sensitive.



Supplementary Figure 8

MAR or mass can be used individually as a classifier for drug susceptibility

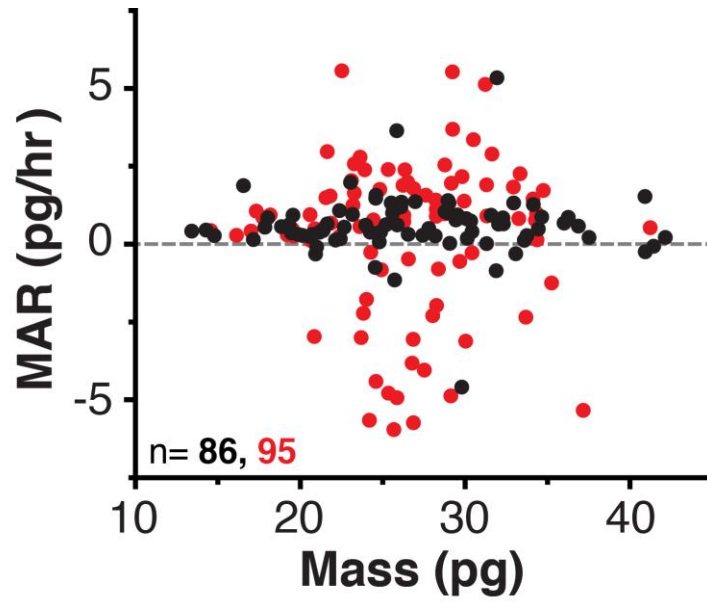
Primary murine BCR-ABL ALL and BCR-ABL T315I ALL cells treated with 1 μ M imatinib, or 100 nM ponatinib, respectively. **(a)** MAR versus mass plot with overlay of an orthogonal vector (dotted line) designating the threshold resulting from LDA. Cells treated with drug are in red, and DMSO control cells are blue **(b)** ROC curves from same paired control and treatment data following LDA of MAR per mass plot. **(c)** Overlaid ROC curves of paired control and treatment data for all treatment replicates using only mass or MAR parameter. Cells treated with therapy to which they are sensitive or resistant are shown with blue solid lines or red dotted lines, respectively.



Supplementary Figure 9

Predictive power of MAR for cells isolated from circulation

Primary murine BCR-ABL T315I cells isolated from circulation, treated with DMSO or 100 nM ponatinib. ROC curves of paired control and treatment data for each replicate following LDA.



Supplementary Figure 10

Patient sample treated *in vivo* shows consistent reduction in MAR

MAR versus Mass plot for blasts from peripheral blood samples of AML. Pre-treatment sample shown in black (n=86), and sample obtained after the patient received 48 hrs of treatment with an experimental MDM2 inhibitor shown in red (n=95).

PDCL	Diagnosis	EGFR	CDK4	CDK6	PTEN	PDGFRA	MET	CDKN2A	NF1	MDM2	RB1	TP53
BT145	GBM Astrocytoma IV	WT	WT	WT	WT	WT	WT	HOMDEL	WT	WT	WT	WT
BT159	GBM Astrocytoma IV	WT	WT	WT	WT	WT	WT	HOMDEL	MUT p.D176E	WT	WT	WT
BT179	GBM Astrocytoma IV	AMP	WT	WT	L70 splice	WT	WT	HOMDEL	WT	WT	WT	MUT p.I255T
BT239	GBM Astrocytoma IV	Mut p.R521K	WT	WT	WT	WT	WT	HOMDEL	MUT L249fs	WT	WT	WT
BT240	GBM Astrocytoma IV	WT	WT	WT	WT	WT	WT	HOMDEL	WT	WT	WT	MUT p.S227Y
BT320	GBM Astrocytoma IV	WT	WT	WT	MUT p.G132A	WT	WT	HOMDEL	WT	WT	WT	HOMDEL
BT333	GBM Astrocytoma IV	AMP, MUT p.F254I	WT	WT	WT	WT	WT	HOMDEL	MUT p.D176E	WT	WT	MUT p.V173M
BT484	GBM Astrocytoma IV				MUT p.C136Y					AMP		WT
3731	GBM Astrocytoma IV									AMP		WT

Supplementary Table 1

GBM PDCL mutation analysis

Abbreviations: AMP, amplification; MUT, mutation; HOMDEL, homozygous deletion.