Electronic Supplementary Material (ESI) for Integrative Biology

Supporting Information

Response of Single Leukemic Cells to Peptidase Inhibitor Therapy Across Time and Dose Using a Microfluidic Device

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As noted in previous experiments with Tosedostat,[1] treated cells exhibited reduced viability and proliferation. Trypan blue staining showed that a larger percentage of cells were dead in drug-treated cultures compared to vehicle control cells (Figure S-1a). This effect became more pronounced as dose increased, and experiments were terminated due to poor viability at a dose of 16 μM. The reduction in viability was accompanied by reduced proliferation, as evidenced by the cell density relative to control cultures (Figure S-1b) and cell cycle data (Figure 1c). Cell cycle results demonstrated that drug treatment increased the proportion of cells in a gap or resting phase of the cell cycle (i.e., G0 or G1 phases).

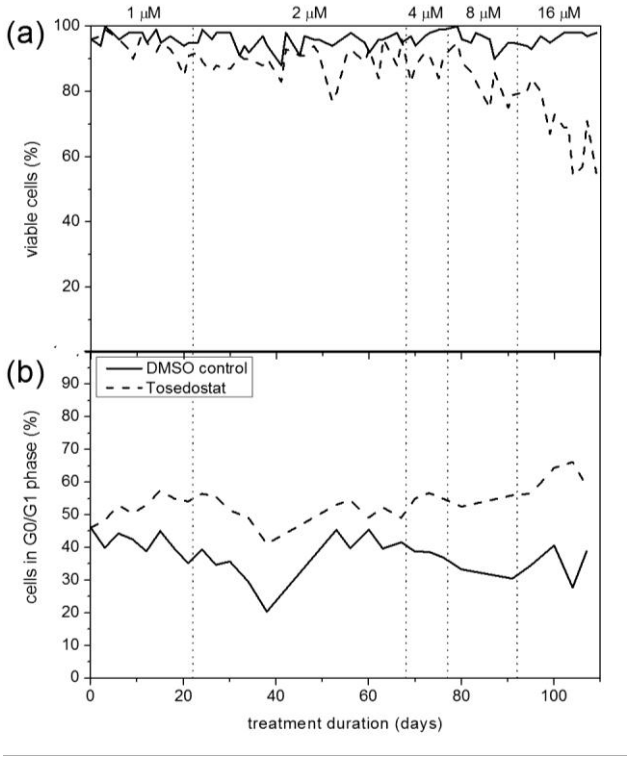

Figure S-1. (a) Percent of cells that were viable based on trypan blue staining over the course of the experiment. (b) Percent of cells in either G0 or G1 phase based on propidium iodide staining and flow cytometry over the course of the experiment. The drug dose during each phase of the experiment is given at the top of the figure.

Table S-1. Dimensions of microchannels at the intersection on the chemical cytometry device.

Channel	Width (μm)	Depth (μm)
cell channel	60	25
focusing channel	120	25
electrophoresis channel	20	25
waste channel	240	25

Table S-2. Detailed log of single-cell experiments.

Day	Dose (μM)	Duration of Data Collection (min)	Number of Cells Analyzed	Number of Cells with 3 Fragment Peaks
0	0	95	43	0
2	1	64	36	21
3	1	82	33	14
6	1	76	25	18
15	1	92	48	27
18	1	93	77	37
21	1	84	43	24
27	2	63	27	13
30	2	88	28	13
34	2	80	30	23
56	2	45	9	7
60	2	77	43	17
63	2	56	43	26
67	2	83	31	16
70	4	43	27	6
73	4	75	28	13
76	4	27	24	0
80	8	71	23	13
83	8	26	20	5
91	8	50	25	2
95	16	33	15	3

Table S-3. Estimated coefficients for the proportional odds model.

	Adjusted Model			Unadjusted Model		
Coefficient	Estimate	SE	*p*-value	Estimate	SE	*p*-value
β_1 (Effect of Time)	-0.006	0.003	0.036			
β_2 (Effect of Area)	0.022	0.003	<0.001			
β_1 (Effect of Dose Level 2 µM)	0.024	0.180	0.900	-0.079	0.178	0.660
β_1 (Effect of Dose Level 4 µM)	-0.984	0.239	<0.001	-0.987	0.234	<0.001
β_1 (Effect of Dose Level 8 µM)	-1.190	0.258	<0.001	-0.809	0.246	0.001
β_1 (Effect of Dose Level 16 µM)	-1.417	0.487	0.004	-1.504	0.482	0.002
α_1	-1.797	0.230		-1.773	0.144	
α_2	-0.298	0.217		-0.352	0.124	
α_3	3.208	0.294		2.873	0.211	
α_4	6.762	1.053		6.252	1.004	

Table S-4. Results of Wald's test comparing dose levels in the adjusted proportional odds model.

Dose Levels Compared	**Null Hypothesis**	**Test Statistic**	***p*-value**
1 µM and 2 µM	$\beta_3 = 0$	0.02	0.90
2 µM and 4 µM	$\beta_4 - \beta_3 = 0$	17.1	<0.01
4 µM and 8 µM	$\beta_5 - \beta_4 = 0$	0.47	0.49
8 µM and 16 µM	$\beta_6 - \beta_5 = 0$	0.19	0.66
4 µM and 16 µM	$\beta_6 - \beta_4 = 0$	0.73	0.39

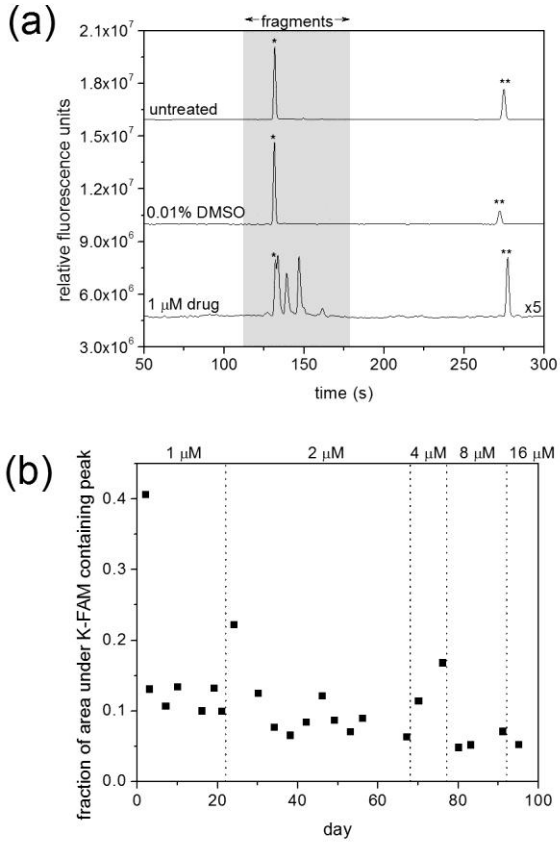

Figure S-2. (a) Sample capillary electropherograms for ensemble samples of untreated, DMSO control, and drug-treated cells. Peaks corresponding to fragments of the reporter peptide are highlighted in gray. Each sample was composed of 10^7 intact cells loaded with the reporter peptide and lysed after 1 h. Addition of standards was used to identify the peaks co-migrating with the labeled lysine (*, K-FAM) and the internal standard, carboxyfluorescein (**).Note that the migration order of the peaks differs from that of samples run on microchips (Figures 3a and 5 in the main text) due to the difference in run buffer and surface coating. Fluorescence signal for the drug-treated sample was multiplied by 5 for clarity. (b) The fraction of the total fragment peak area under the peak co-migrating with the labeled lysine for samples taken throughout the experiment. The Tosedostat dose for each day is given across the top axis.

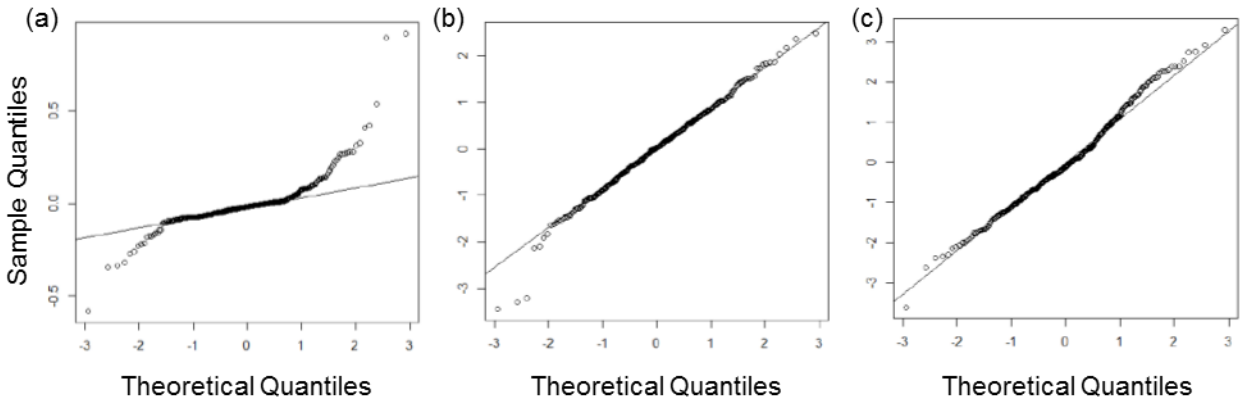

Figure S-3. QQ plots for linear regression models of (a) the rate proxy compared to the log of the rate proxy in (b) the adjusted and (c) unadjusted models.

Table S-5. Estimated coefficients for the linear models.

	Adjusted Model			Unadjusted Model		
Coefficient	Estimate	Conf Int	*p*-value	Estimate	Conf Int	*p*-value
β_0 (Intercept)	0.05	(0.044,0.062)		0.09	(0.072,0.106)	
$\exp(\beta_1)$ (Effect of Area)	1.02	(1.019,1.026)	< 0.01			
$\exp(\beta_2)$ (Dose Level 2 µM)	0.41	(0.331,0.521)	< 0.01	0.38	(0.284,0.501)	< 0.01
$\exp(\beta_3)$ (Dose Level 4 µM)	0.31	(0.196,0.478)	< 0.01	0.38	(0.216,0.659)	< 0.01
$\exp(\beta_4)$ (Dose Level 8 µM)	0.34	(0.221,0.531)	< 0.01	0.69	(0.398,1.181)	0.17

Table S-6. Results of Wald's test comparing dose levels in the adjusted linear model of degradation rate.

Dose Levels Compared	**Null Hypothesis**	**Test Statistic**	***p*-value**
1 µM and 2 µM	$\beta_2 = 0$	57.7	<0.01
2 µM and 4 µM	$\beta_3 - \beta_2 = 0$	1.7	0.19
4 µM and 8 µM	$\beta_4 - \beta_3 = 0$	0.16	0.69
2 µM and 8 µM	$\beta_4 - \beta_2 = 0$	0.64	0.42

References

(1) D. Krige, et al., *Cancer Res.* **2008**, *68*. 6669-6679.