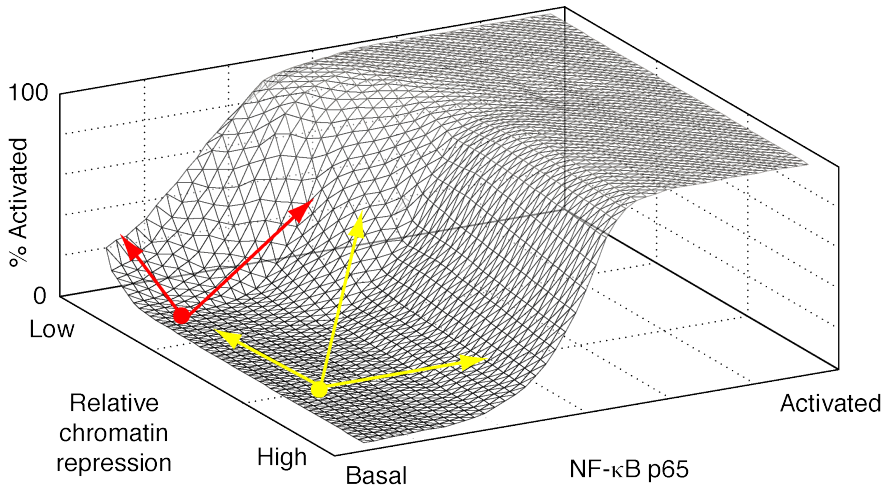
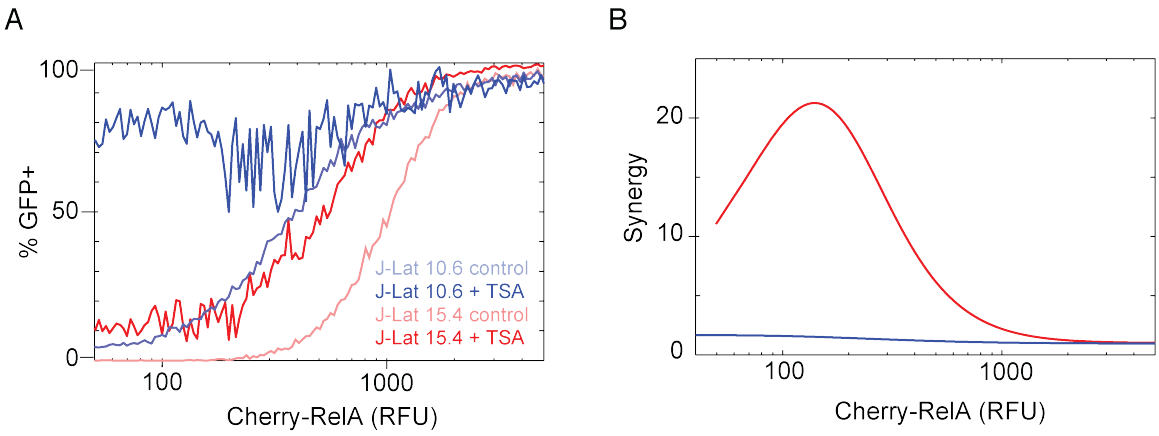
Quantitative evaluation and optimization of co-drugging to improve anti-HIV latency therapy

Victor C. Wong[1+], Linda E. Fong[2+], Nicholas M. Adams[2], Qiong Xue[2], Siddharth S. Dey[3,4] and Kathryn Miller-Jensen[1,2*]

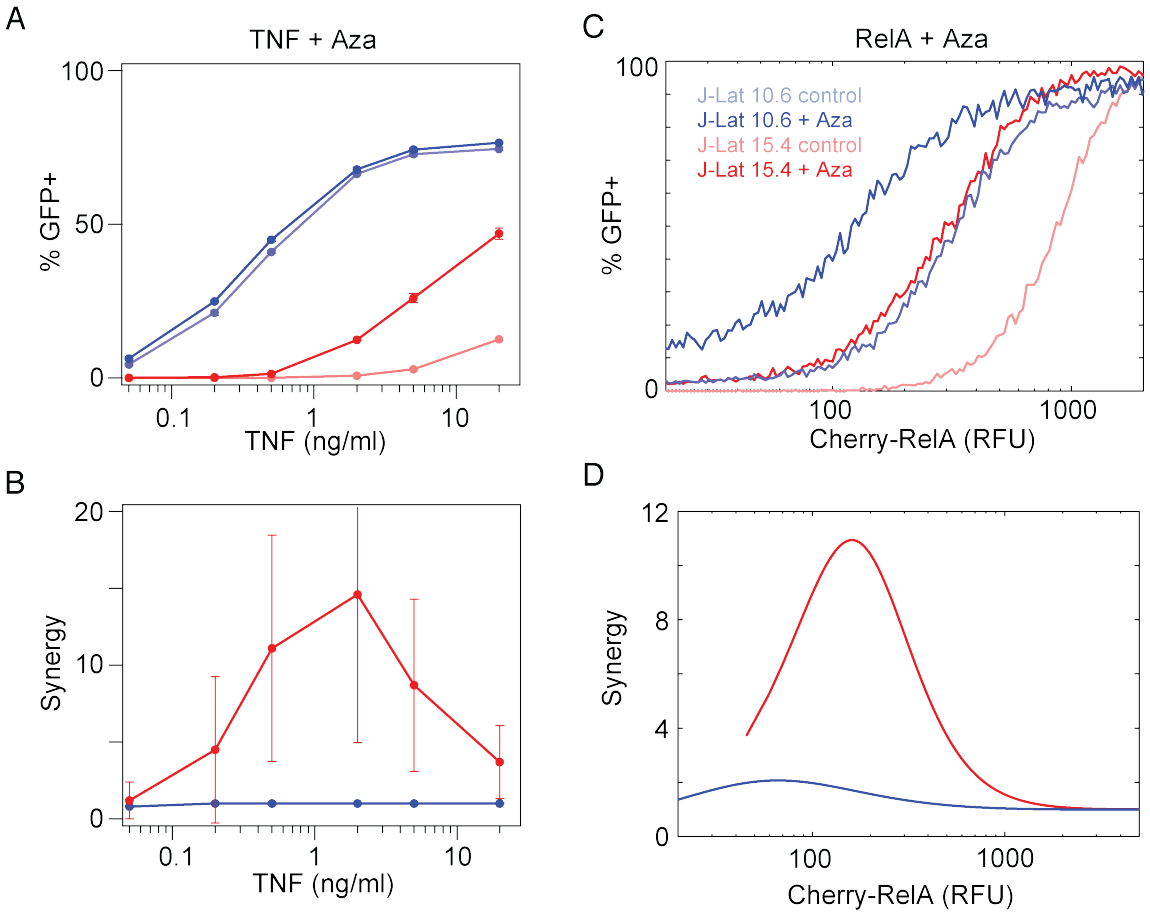
Supplementary Figures



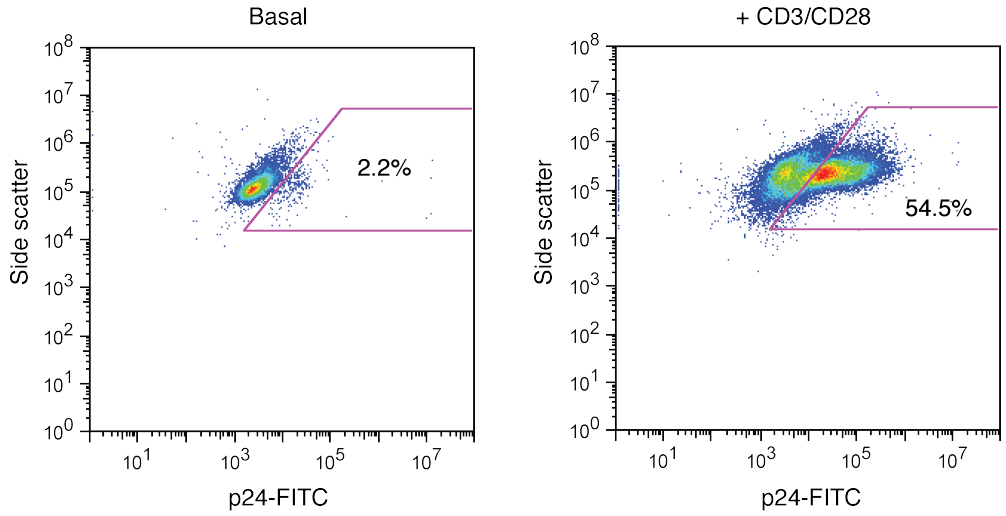
Supp. Fig. S1. 3-D surface plot demonstrates HIV activation as a function of NF-κB p65 and chromatin repression for two hypothetical genomic integrations. Yellow point represents a promoter with high chromatin repression that would demonstrate synergistic drug interactions for a drug that both activates NF-κB p65 and lowers chromatin repression but would not be activated by either drug individually. Red point represents a promoter with low chromatin repression that would be efficiently activated by either drug.



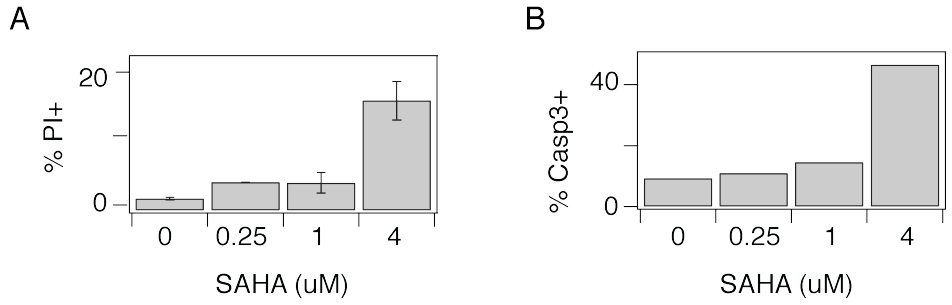
Supp. Fig. S2. (A) Combined flow cytometry data for J-Lat 15.4 (red) and J-Lat 10.6 (blue) infected with a lentiviral vector carrying a doxycycline (dox)-inducible NF-κB-Cherry fusion protein. The clones were treated with a range of dox concentrations in the presence and absence of TSA. More than 50,000 single cell events were divided into 256 bins of mCherry fluorescence, and the fraction of GFP+ cells was calculated and plotted for each bin. (B) Synergy was calculated at each bin according to the Bliss independence model of drug interactions.



Supp. Fig. S3. (A) Dose response of activation by TNF of J-Lat 15.4 (red) and J-Lat 10.6 (blue) in the presence and absence of 1 μM Aza. GFP expression was measured by flow cytometry. Data are reported as the mean ± standard deviation of 3 biological replicates (note that some error bars are not visible). (B) Quantification of drug synergy between TNF and Aza using the Bliss independence model of drug interactions. (C) Combined flow cytometry data for J-Lat 15.4 (red) and J-Lat 10.6 (blue) infected with a lentiviral vector carrying a doxycycline (dox)-inducible NF-κB-Cherry fusion protein. The clones were treated with a range of dox concentrations in the presence and absence of TSA. More than 50,000 single cell events were divided into 256 bins of mCherry fluorescence, and the fraction of GFP+ cells was calculated and plotted for each bin. (D) Synergy was calculated at each bin according to the Bliss independence model of drug interactions.



Supp. Fig. S4. Flow cytometry scatter plots of cultured primary memory CD4+ T_{CM} cells latently infected with a full-length, replication-defective HIV virus in the basal state (left) and activated by anti-CD3/anti-CD28 antibodies (right).



Supp. Fig. S5. Toxicity induced by SAHA in primary cultured memory T_{CM} cells as measured by (A) propidium iodide staining and (B) active caspase 3 staining and analyzed by flow cytometry.

Supp. Table S1. Average data points comprising J-Lat 8.4 Activation Matrix (Fig. 6C)

TNF dose (ng/ml)	SAHA dose (uM)				
	0	**0.25**	**0.5**	**1**	**4**
0	0.0	0.1	0.1	0.7	14.1
1	0.6	0.8	3.2	10.6	41.9
5	3.6	8.2	19.8	36.3	64.4
20	12.7	23.6	42.3	60.6	73.2

Supp. Table S2. Average data points comprising Jurkat Toxicity Matrix (Fig. 6D)

TNF dose (ng/ml)	SAHA dose (uM)				
	0	**0.25**	**0.5**	**1**	**4**
0	0.0	1.2	7.6	32.9	35.9
1	1.5	2.6	13.0	38.8	38.5
5	1.5	5.8	17.6	41.0	45.4
20	2.6	6.9	18.3	43.4	47.1