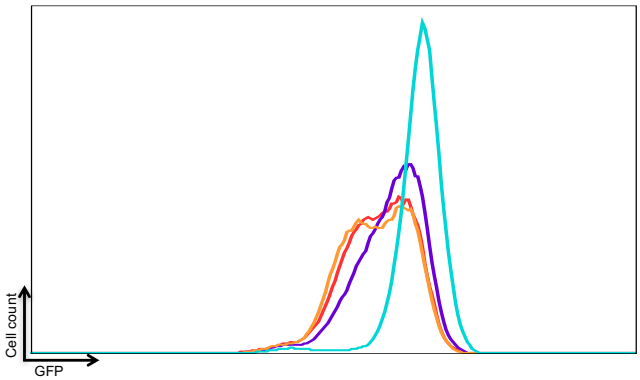
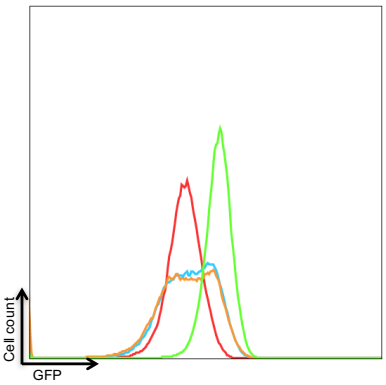
a

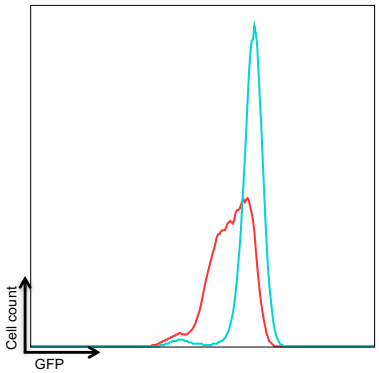


FKBP-Gal4 [CEM23]	% GFP (-)
0nM	3.69
10nM	21.9
50nM	33.2
100nM	37.8

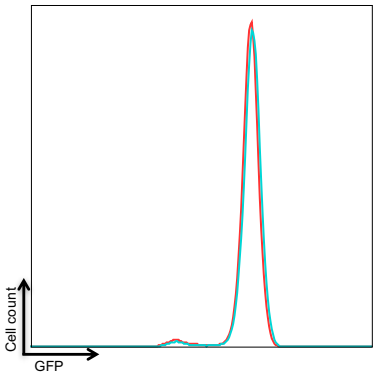


FKBP-Gal4 [CEM23]	% GFP (-)
0nM	2.27
100nM	47.4
1000nM	43.4
10000nM	66.1

b

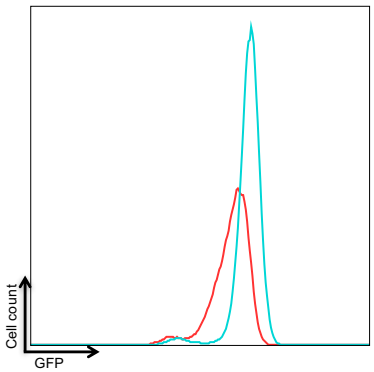


Cell Line	% GFP (-)
FKBP- Gal4	3.8
FKBP-Gal4 +CEM42	41.1

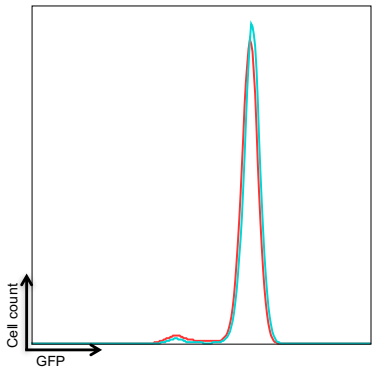


Cell Line	% GFP (-)
Gal4	3.37
Gal4 +CEM42	5.31

c

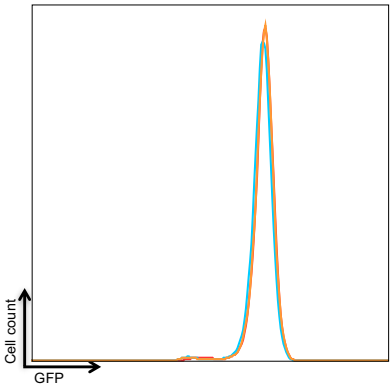


Cell Line	% GFP (-)
FKBP-Gal4	3.8
FKBP-Gal4 +Cem36	29.9

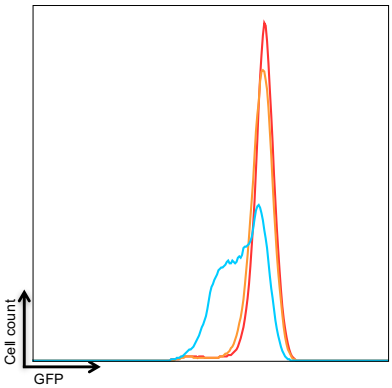


Cell Line	% GFP (-)
Gal4	3.37
Gal4 +Cem36	5.07

Supplemental Figure S1. (a) Overlays of histograms of dose response for CEM23 (0, 10, 50, 100, 1000, 10000nM) with legend showing colors for each dose. (b) Overlays of histograms of CEM42 +/- compound for Gal4 and Gal4-FKBP cell lines. (c) Overlays of histograms of CEM36 +/- compound for Gal4 and Gal4-FKBP cell lines.

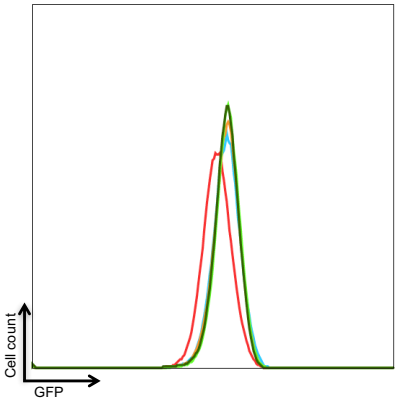


Gal4	% GFP (-)
0nM	2.03
100nM CEM23	2.27
100nM CEM23 + 1μM FK506	2.04



FKBP-Gal4	% GFP (-)
0nM	3.05
100nM CEM23	30.2
100nM CEM23 + 1μM FK506	3.55

Supplemental Figure S1d. Overlays of histograms for the Gal4 cell line and FKBP-Gal4 cell line with 100nM of CEM23, with and without 1μM FK506



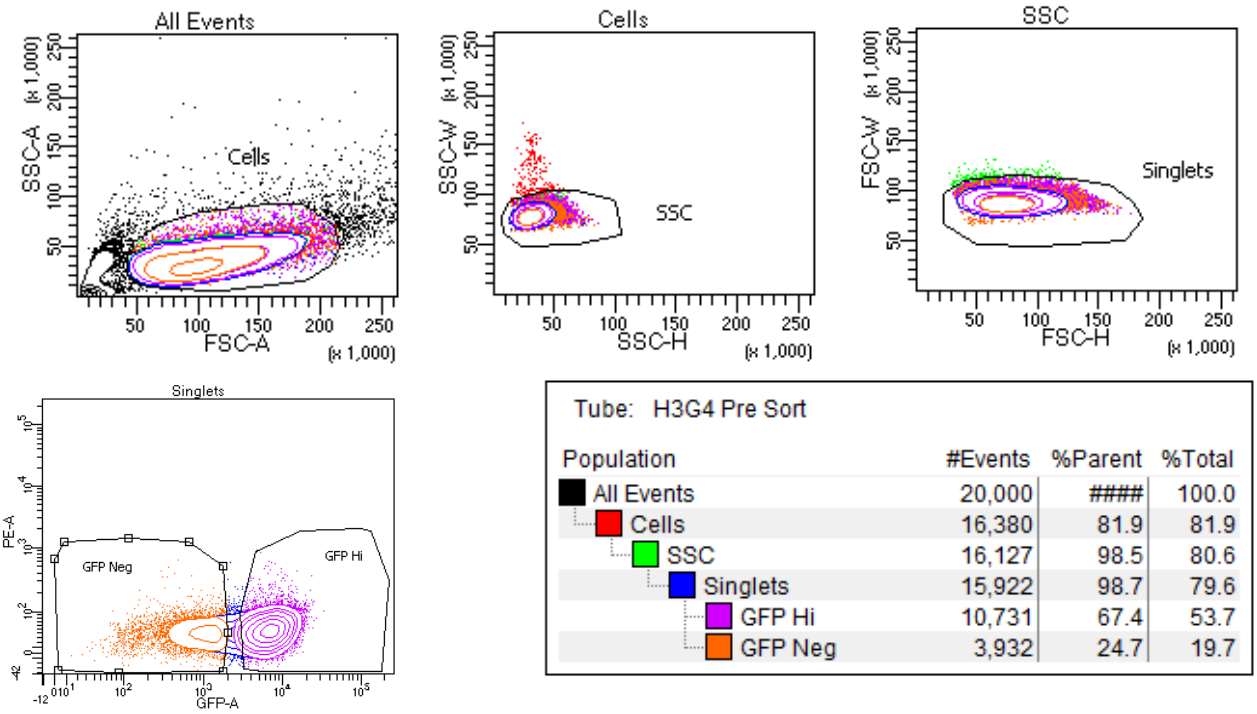
FKBP-Gal4	% GFP (-)
0nM	2.27
100nM FK506	1.84
1000nM FK506	2.21
100nM SAHA	2.37
1000nM SAHA	4.82

Supplemental Figure S1e. Overlays of histograms for the FKBP-Gal4 cell line with FK506 or suberoylanilide hydroxamic acid (SAHA).

Cell line:	Median Fluorescence :	Referencing from Figure:
FKBP-Gal4 control	47613	Figure 2c & Suppl. Figure 1b and 1c
FKBP-Gal4 + CEM23 24hrs	23968	Figure 2c
FKBP-Gal4 + CEM23 48hrs	15024	Figure 2c
FKBP-Gal4 + CEM23 72hrs	14022	Figure 2c
FKBP-Gal4 + CEM36 48hrs	22098	Suppl. Figure 1c
FKBP-Gal4 + CEM42 48hrs	17964	Suppl. Figure 1b
Gal4 control	48590	Figure 2b & Suppl. Figure 1b and 1c
Gal4 +CEM23 72hrs	44981	Figure 2b
Gal4 +CEM36 72hrs	46091	Suppl. Figure 1c
Gal4 +CEM42 72hrs	44077	Suppl. Figure 1b
Sorted negative population + CEM23 for 3 days	6973	Figure 2d
Sorted negative population - CEM23 for 3 days	51852	Figure 2d
Sorted negative population + CEM23 for 5 days	6377	Figure 2d
Sorted negative population - CEM23 for 5 days	44617	Figure 2d
Sorted negative population - CEM23 for 5 days then + CEM23 for 2 days	8371	Figure 2d
Sorted negative population - CEM23 for 7 days	52916	Figure 2d
Gal4 control (with HDAC3 experiment)	47807	Figure 2a
HDAC3-Gal4	18861	Figure 2a
FKBP-Gal4 control (for lower concentrations)	46656	Suppl. Figure 1a
FKBP-Gal4 + 10nM CEM23	22460	Suppl. Figure 1a
FKBP-Gal4 + 50nM CEM23	15775	Suppl. Figure 1a
FKBP-Gal4 + 100nM CEM23	14194	Suppl. Figure 1a
FKBP-Gal4 control (for higher concentrations)	1766	Suppl. Figure 1a
FKBP-Gal4 + 100nM CEM23	522	Suppl. Figure 1a
FKBP-Gal4 + 1000nM CEM23	588	Suppl. Figure 1a
FKBP-Gal4 + 10-00nM CEM23	561	Suppl. Figure 1a
Gal4 control	52916	Suppl. Figure 1d
Gal4 +100nM CEM23	47807	Suppl. Figure 1d
Gal4 +100nM CEM23 +1μM FK506	52701	Suppl. Figure 1d
FKBP-Gal4 control	51642	Suppl. Figure 1d
FKBP-Gal4 +100nM CEM23	19643	Suppl. Figure 1d
FKBP-Gal4 +100nM CEM23 +1μM FK506	45164	Suppl. Figure 1d
FKBP-Gal4 control	1766	Suppl. Figure 1e
FKBP-Gal4 + 100nM FK506	1827	Suppl. Figure 1e
FKBP-Gal4 + 1000nM FK506	1766	Suppl. Figure 1e
FKBP-Gal4 + 100nM SAHA	1748	Suppl. Figure 1e
FKBP-Gal4 + 1000nM SAHA	1217	Suppl. Figure 1e

Supplemental Figure S1e. Median fluorescences for each cell line and experimental condition.

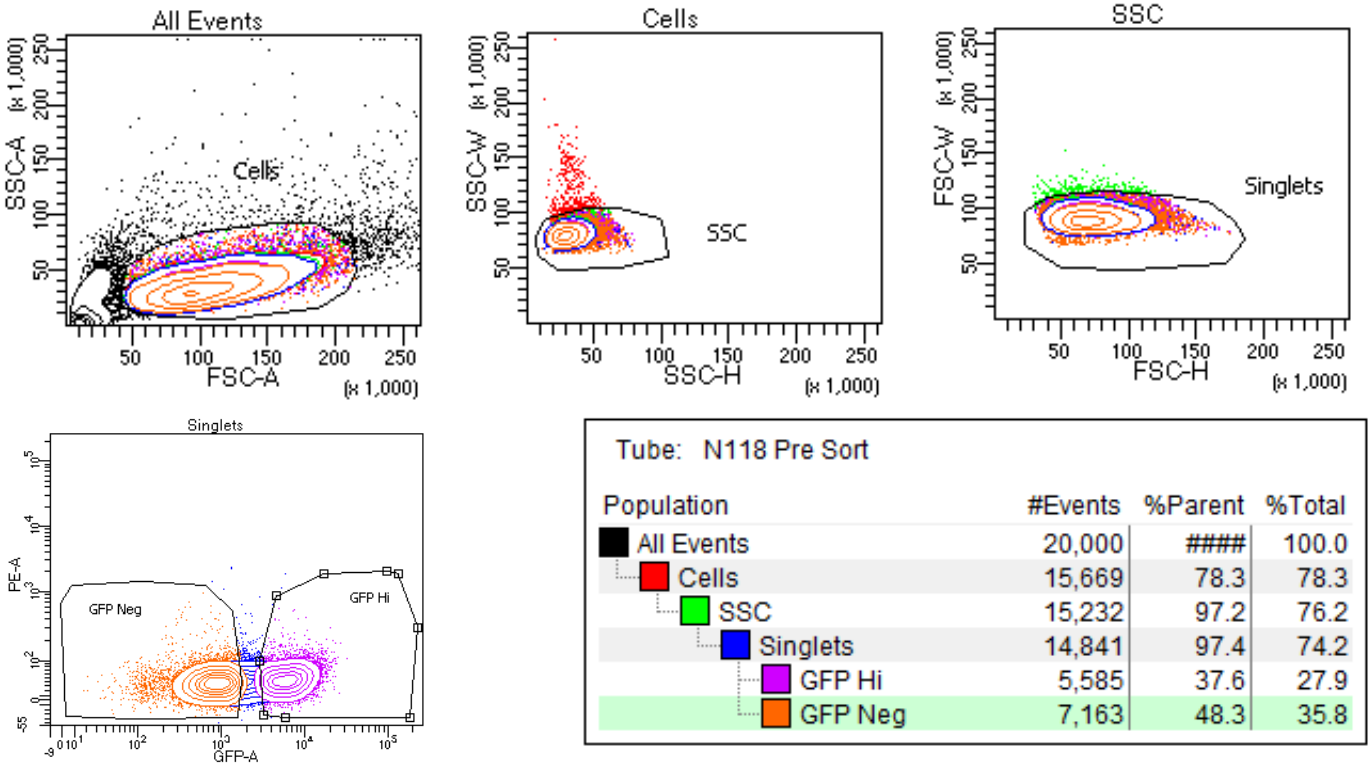
BD FACSDiva 8.0.1



Tube: H3G4 Pre Sort

Population	#Events	%Parent	%Total
All Events	20,000	####	100.0
Cells	16,380	81.9	81.9
SSC	16,127	98.5	80.6
Singlets	15,922	98.7	79.6
GFP Hi	10,731	67.4	53.7
GFP Neg	3,932	24.7	19.7

BD FACSDiva 8.0.1

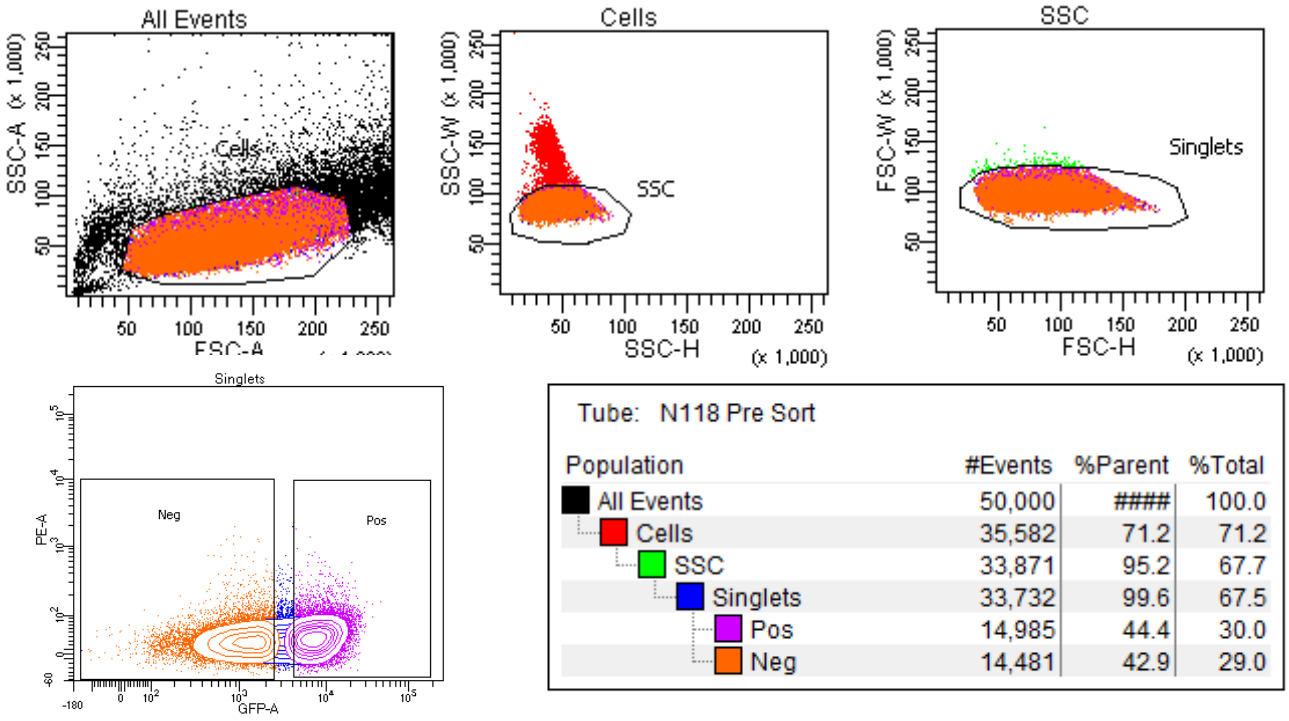


Tube: N118 Pre Sort

Population	#Events	%Parent	%Total
All Events	20,000	####	100.0
Cells	15,669	78.3	78.3
SSC	15,232	97.2	76.2
Singlets	14,841	97.4	74.2
GFP Hi	5,585	37.6	27.9
GFP Neg	7,163	48.3	35.8

Supplemental Figure S2a.The gating of FKBP-Gal4 + 100nM CEM23 cells and gating of HDAC3-Gal4 cells for Fluorescence Activated Cell Sorting (FACS) for replicate 1 of ChIP experiments.

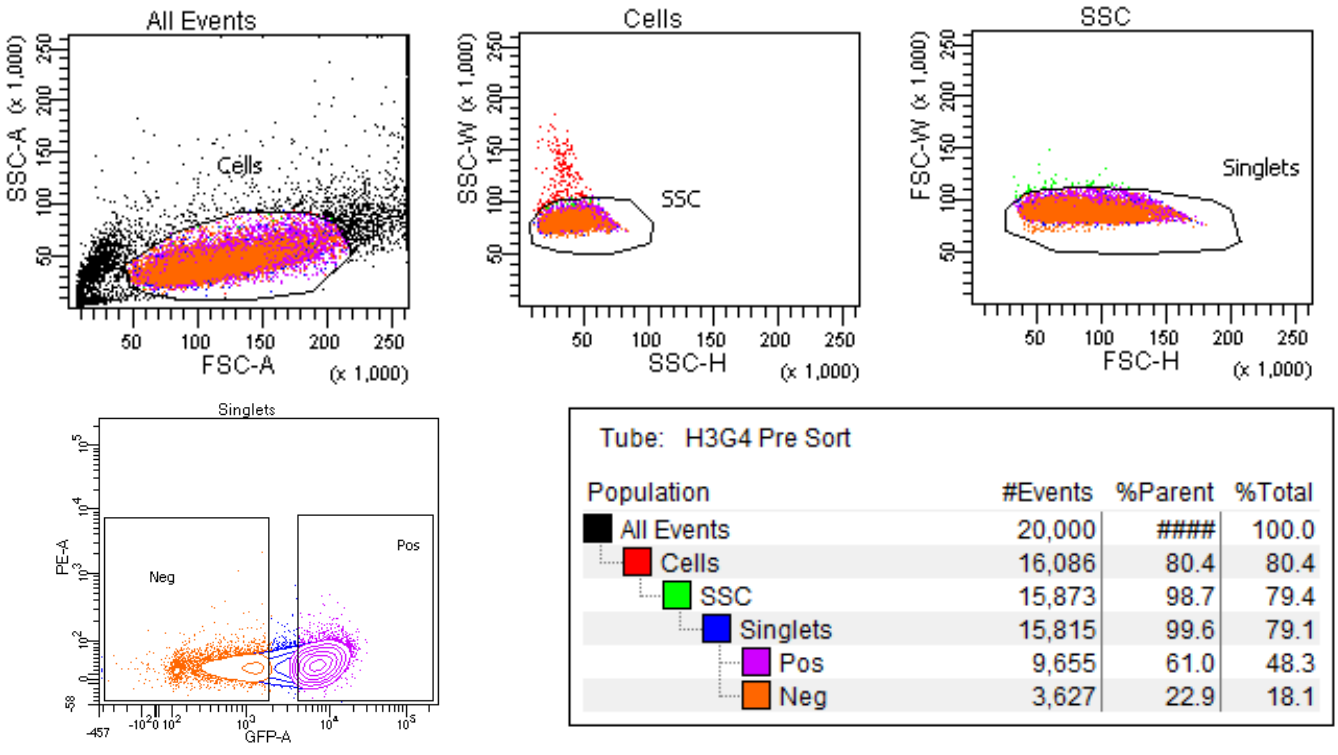
BD FACSDiva 8.0.1



Tube: N118 Pre Sort

Population	#Events	%Parent	%Total
All Events	50,000	####	100.0
Cells	35,582	71.2	71.2
SSC	33,871	95.2	67.7
Singlets	33,732	99.6	67.5
Pos	14,985	44.4	30.0
Neg	14,481	42.9	29.0

BD FACSDiva 8.0.1

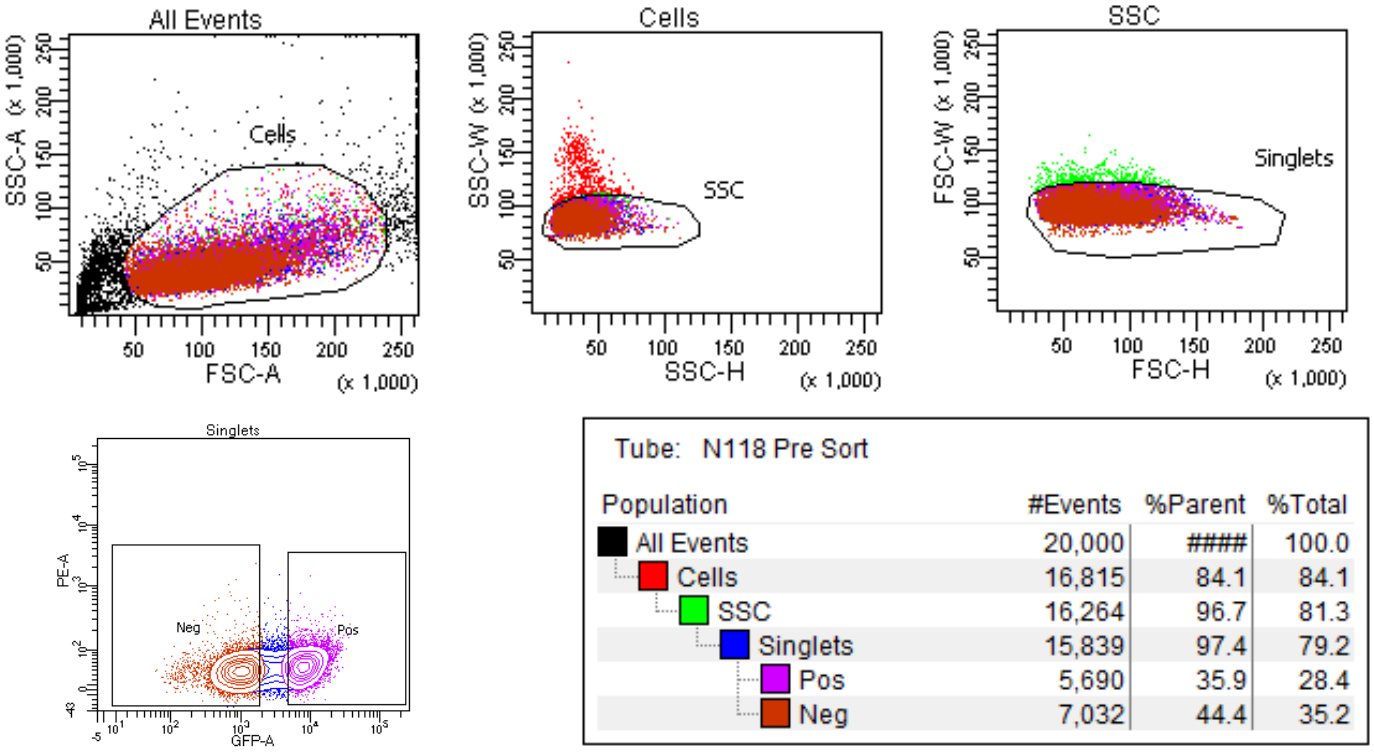


Tube: H3G4 Pre Sort

Population	#Events	%Parent	%Total
All Events	20,000	####	100.0
Cells	16,086	80.4	80.4
SSC	15,873	98.7	79.4
Singlets	15,815	99.6	79.1
Pos	9,655	61.0	48.3
Neg	3,627	22.9	18.1

Supplemental Figure S2b.The gating of FKBP-Gal4 + 100nM CEM23 cells and gating of HDAC3-Gal4 cells for Fluorescence Activated Cell Sorting (FACS) for replicate 2 of ChIP experiments.

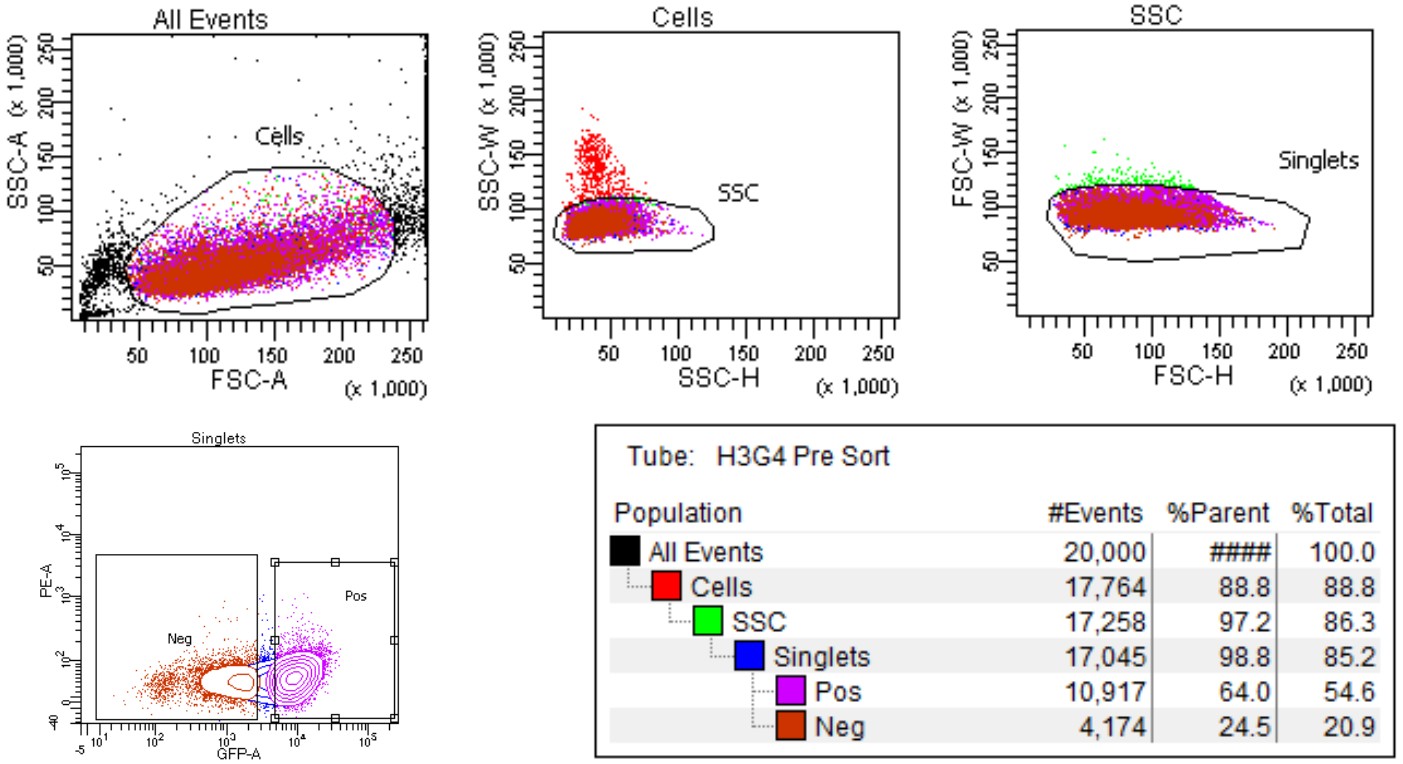
BD FACSDiva 8.0.1



Tube: N118 Pre Sort

Population	#Events	%Parent	%Total
All Events	20,000	####	100.0
Cells	16,815	84.1	84.1
SSC	16,264	96.7	81.3
Singlets	15,839	97.4	79.2
Pos	5,690	35.9	28.4
Neg	7,032	44.4	35.2

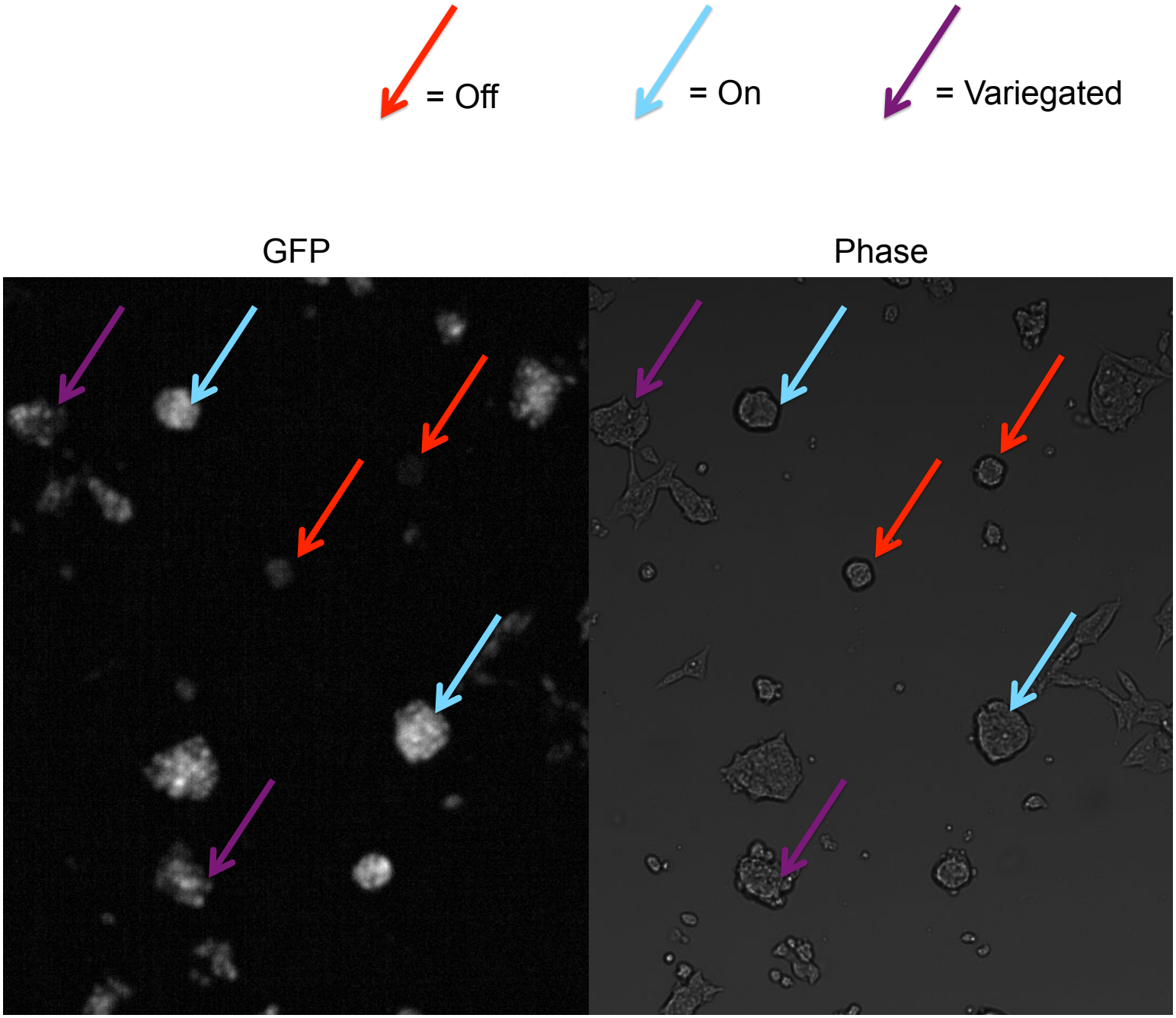
BD FACSDiva 8.0.1



Tube: H3G4 Pre Sort

Population	#Events	%Parent	%Total
All Events	20,000	####	100.0
Cells	17,764	88.8	88.8
SSC	17,258	97.2	86.3
Singlets	17,045	98.8	85.2
Pos	10,917	64.0	54.6
Neg	4,174	24.5	20.9

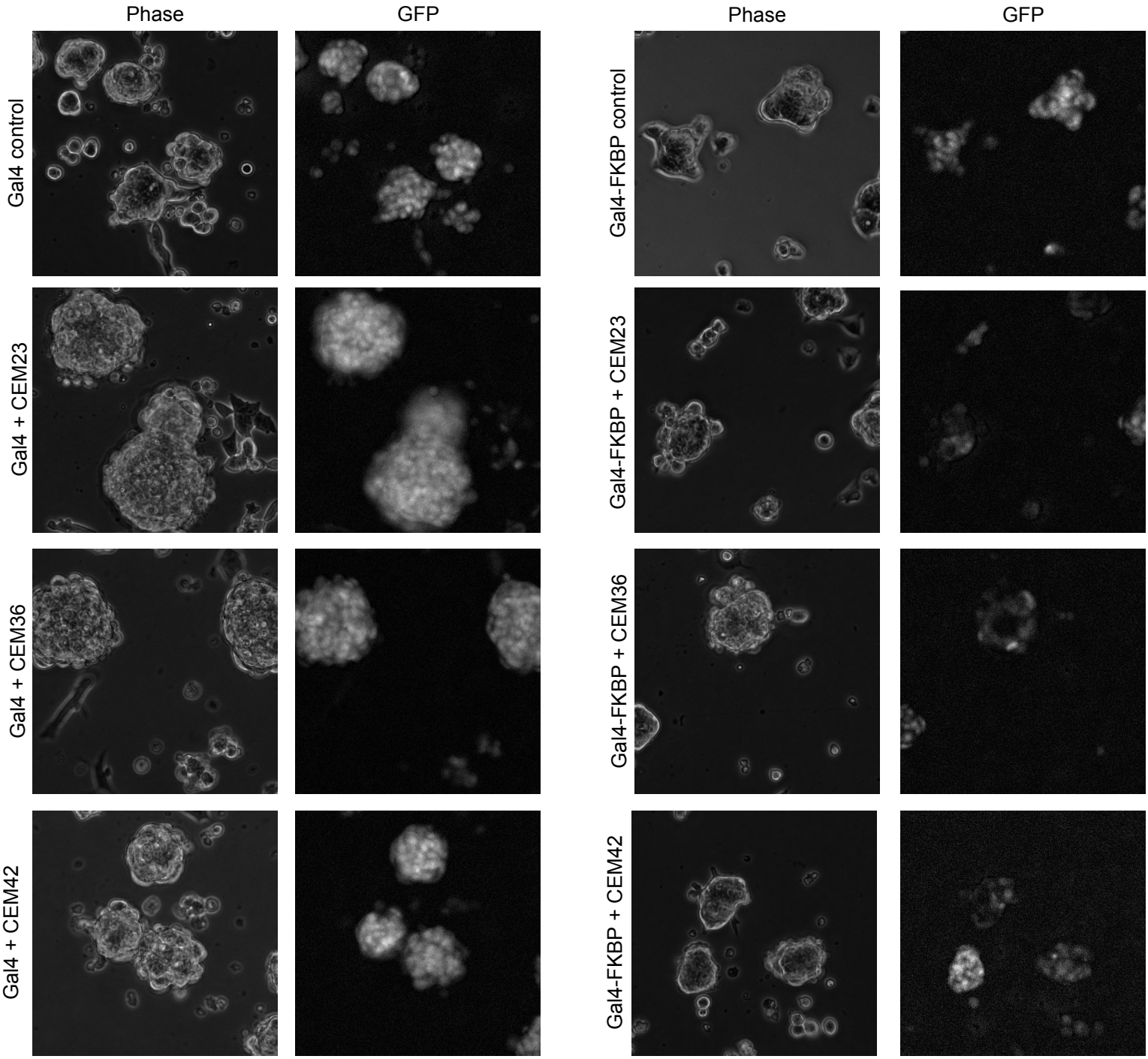
Supplemental Figure S2c.The gating of FKBP-Gal4 + 100nM CEM23 cells and gating of HDAC3-Gal4 cells for Fluorescence Activated Cell Sorting (FACS) for replicate 3 of ChIP experiments.



Supplemental Figure S3a. Representative images of colonies characterized as GFP-Off, GFP-On, or GFP-Variegated.

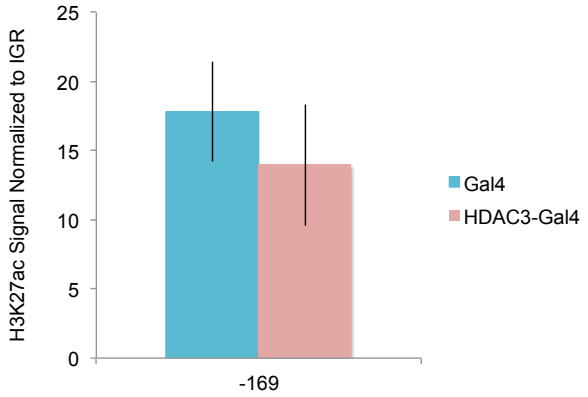
15-Nov																		
	FKBP-Gal4 48hrs																	
	control-on	control-off	control-var	cem23-on	cem23-off	cem23-var	cem42-on	cem42-off	cem42-var	cem36-on	cem36-off	cem36-var	Fk506-on	Fk506-off	Fk506-var			
Total for each category	163	5	5	64	53	35	76	43	31	96	44	20	154	6	5			
Total for the cell line			173			152			150			160			165			
%	0.942	0.029	0.029	0.421	0.349	0.230	0.507	0.287	0.207	0.600	0.275	0.125	0.933	0.036	0.030			
%x100	94.22	2.89	2.89	42.11	34.87	23.03	50.67	28.67	20.67	60.00	27.50	12.50	93.33	3.64	3.03			
	Gal4 48hrs																	
	Fk506-on	Fk506-off	Fk506-var	H3G4-on	H3G4-off	H3G4-var	control-on	control-off	control-var	cem42-on	cem42-off	cem42-var	cem36-on	cem36-off	cem36-var	cem23-on	cem23-off	cem23-var
Total for each category	179	7	5	82	56	55	176	2	9	200	5	8	182	5	8	171	4	8
Total for the cell line			191			193			187			213			195			183
%	0.937	0.037	0.026	0.425	0.290	0.285	0.941	0.011	0.048	0.939	0.023	0.038	0.933	0.026	0.041	0.934	0.022	0.044
%x100	93.72	3.66	2.62	42.49	29.02	28.50	94.12	1.07	4.81	93.90	2.35	3.76	93.33	2.56	4.10	93.44	2.19	4.37
15-Nov	Gal4 48hrs																	
	cem23-on	cem23-off	cem23-var	Fk506-on	Fk506-off	Fk506-var	control-on	control-off	control-var	cem42-on	cem42-off	cem42-var	H3G4-on	H3G4-off	H3G4-var	cem36-on	cem36-off	cem36-var
Total for each category	168	7	7	170	5	7	138	5	6	189	4	10	90	46	73	176	4	9
Total for the cell line			182			182			149			203			209			189
%	0.923	0.038	0.038	0.934	0.027	0.038	0.926	0.034	0.040	0.931	0.020	0.049	0.431	0.220	0.349	0.931	0.021	0.048
%x100	92.31	3.85	3.85	93.41	2.75	3.85	92.62	3.36	4.03	93.10	1.97	4.93	43.06	22.01	34.93	93.12	2.12	4.76
19-Nov	FKBP-Gal4 48hrs																	
	Fk506-on	Fk506-off	Fk506-var	cem23-on	cem23-off	cem23-var	cem42-on	cem42-off	cem42-var	cem36-on	cem36-off	cem36-var	control-on	control-off	control-var			
Total for each category	159	2	8	77	53	47	88	52	34	90	28	35	138	3	6			
Total for the cell line			169			177			174			153			147			
%	0.941	0.012	0.047	0.435	0.299	0.266	0.506	0.299	0.195	0.588	0.183	0.229	0.939	0.020	0.041			
%x100	94.08	1.18	4.73	43.50	29.94	26.55	50.57	29.89	19.54	58.82	18.30	22.88	93.88	2.04	4.08			
Combined Data for Average and Standard Deviation Calculation																		
Gal4 construct	**Gal4: Control**	**Gal4: FK506**	**Gal4: Cem23**	**Gal4: Cem36**	**Gal4: Cem42**	**HDAC3-Gal4**			**FKBP-Gal4**	**F-G4: Control**	**F-Gl4: Fk506**	**F-G4: Cem23**	**F-G4: Cem36**	**F-G4: Cem42**				
GFP On	94.12	93.72	93.44	93.33	93.90	42.49			**GFP On**	93.88	94.08	43.50	58.82	50.57				
GFP On	92.62	93.41	92.31	93.12	93.10	43.06			**GFP On**	94.22	93.33	42.11	60.00	50.67				
Average	93.37	93.57	92.88	93.23	93.50	42.78			**Average**	94.05	93.71	42.81	59.41	50.62				
Std Dev	1.06	0.22	0.80	0.15	0.57	0.40			**Std Dev**	0.24	0.53	0.98	0.83	0.07				
GFP Off	1.07	3.66	2.19	2.56	2.35	29.02			**GFP Off**	2.04	1.18	29.94	18.30	29.89				
GFP Off	3.36	2.75	3.85	2.12	1.97	22.01			**GFP Off**	2.89	3.64	34.87	27.50	28.67				
Average	2.22	3.21	3.02	2.34	2.16	25.52			**Average**	2.47	2.41	32.41	22.90	29.28				
Std Dev	1.62	0.64	1.17	0.31	0.27	4.96			**Std Dev**	0.60	1.74	3.48	6.50	0.86				
Variegated	4.81	2.62	4.37	4.10	3.76	28.50			**Variegated**	4.08	4.73	26.55	22.88	19.54				
Variegated	4.03	3.85	3.85	4.76	4.93	34.93			**Variegated**	2.89	3.03	23.03	12.50	20.67				
Average	4.42	3.24	4.11	4.43	4.35	31.72			**Average**	3.49	3.88	24.79	17.69	20.11				
Std Dev	0.55	0.87	0.37	0.47	0.83	4.55			**Std Dev**	0.84	1.20	2.49	7.34	0.80				

Supplemental Figure S3b. The raw data of colony characterization into GFP-Off, GFP-On, GFP-Variegated of 10 experimental conditions for two replicates.

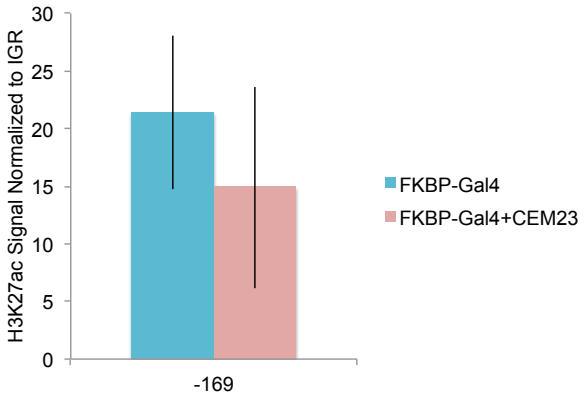


Supplemental Figure S3c. Representative images of Phase and GFP Fluorescence for Gal4 and FKBP-Gal4 cell lines exposed to CEMs.

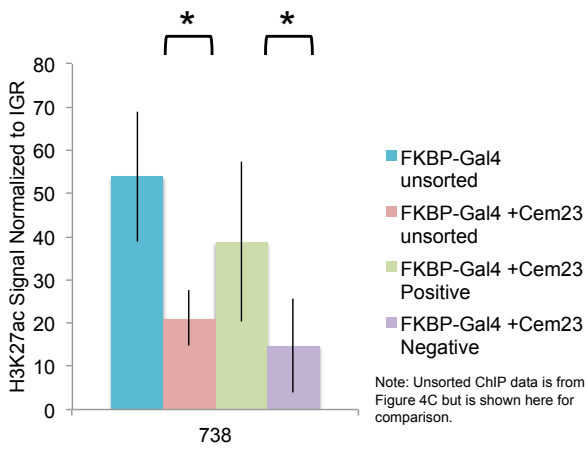
a



b



c

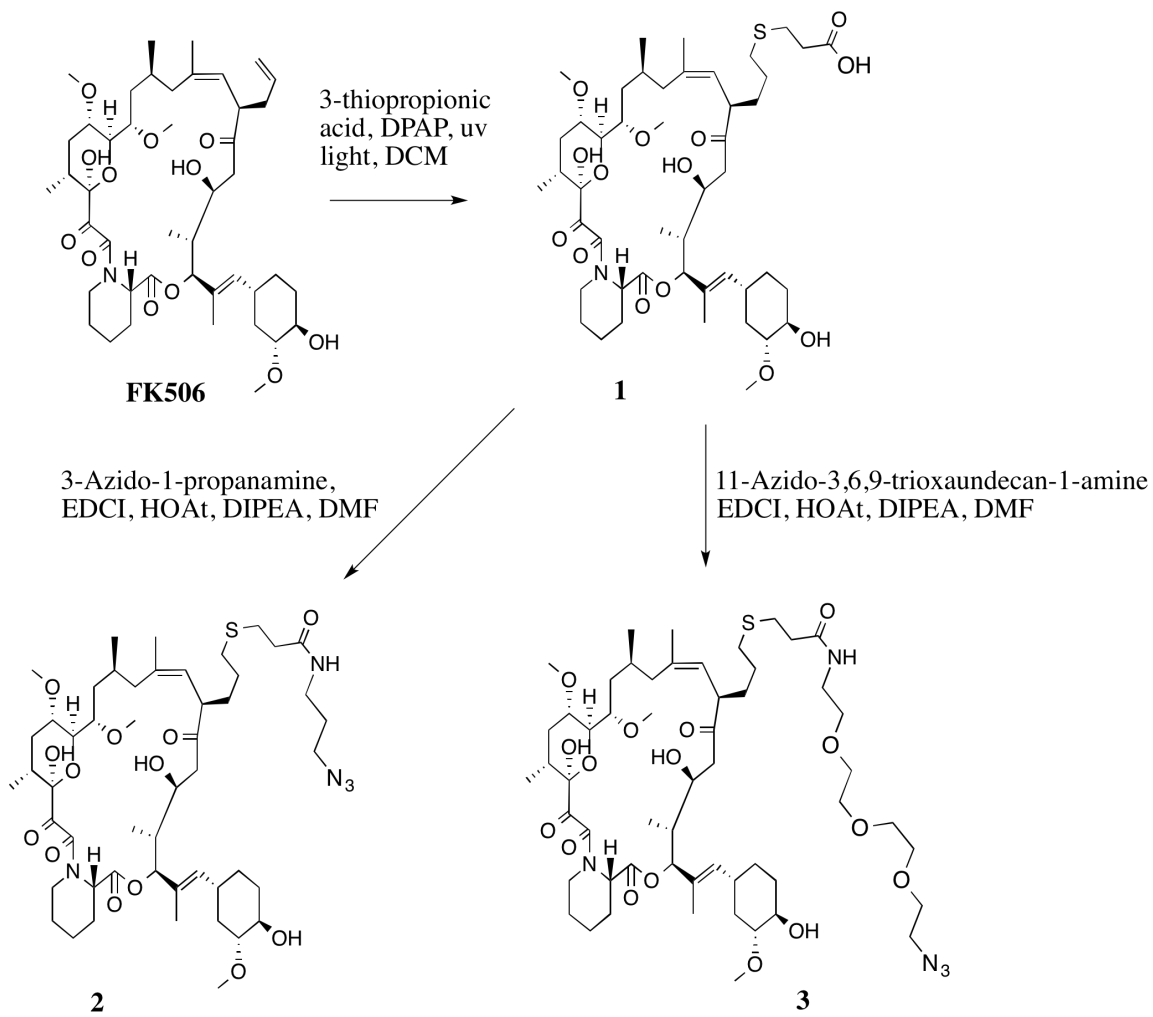


d

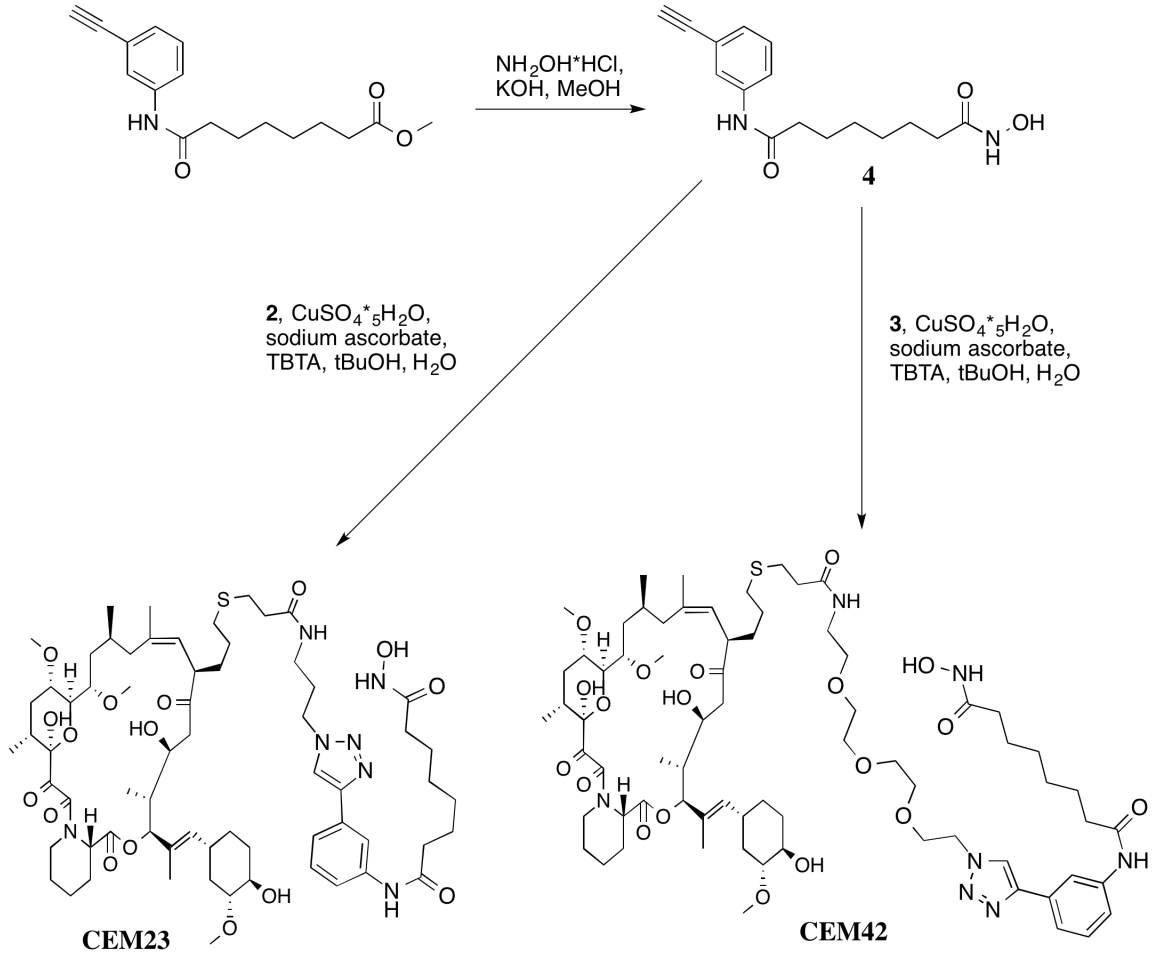
Primer:	Forward:	Reverse:
-169	CTAGAGGATCCGAGGACCAATTG	ACCTTCAAGGTCCTCTCACC
489	CACATGAAGCAGCACGACTT	CCTTGAAGAAGATGGTGCGC
738	CACATGGTCCTGCTGGAGTT	ATCTAGAGTCGCGGCCGG
1199	GTGATGGGTCAGCAGGGCT	TCCGATTCCAGGCCCACCT
IGR	TTACTTCGTGTCTGTCGGGG	GAGAGTAAAGTCAGAGAGGCCA

Supplemental Figure S4. Quantitative RT-PCR comparing Gal4 to HDAC3-Gal4 cell lines (a) and Gal4-FKBP cells to Gal4-FKBP with 100nM CEM23 (b) using an H3K27ac antibody and qPCR primers 169 basepairs upstream of the transcriptional start site. Quantitative RT-PCR compared Gal4-FKBP cells, Gal4-FKBP with 100nM CEM23, and Gal4-FKBP with 100nM CEM23 sorted into positive and negative populations, using an H3K27ac antibody and qPCR primers 738 basepairs downstream of the transcriptional start site (c). Primer sequences used for qPCR in Figure 4 and Supplemental Figure 4A-C (d).

Supplemental Text 1. Chemistry General Procedures. FK506 was purchased from Selleck. All other chemicals were purchased from Sigma-Aldrich or Fisher Scientific. HPLC spectra for all compounds were acquired using an Agilent 1200 Series system with DAD detector. Analytical HPLC chromatography was performed on a 2.1×150 mm Zorbax 300SB-C18 5 µm column with water containing 0.1% formic acid as solvent A and acetonitrile containing 0.1% formic acid as solvent B at a flow rate of 0.4 mL/min. The gradient program was as follows: 1% B (0−1 min), 1−99% B (1−4 min), and 99% B (4−8 min). High resolution mass spectra (HRMS) data were acquired in positive ion mode using an Agilent G1969A API-TOF with an electrospray ionization (ESI) source. Flash column chromatography was performed on a Teledyne ISCO CombiFlash Rf system equipped with a variable wavelength UV detector and a fraction collector using RediSep Rf normal phase silica columns. Microwave reactions were performed using a Discover SP CEM. Nuclear Magnetic Resonance (NMR) spectra were acquired on a Bruker DRX-600 spectrometer with 600 MHz for proton (^{1}H NMR) and 150 MHz for carbon (^{13}C NMR); chemical shifts are reported in ppm (δ). Preparative HPLC was performed on Agilent Prep 1200 series with UV detector set to 254 nm. Samples were injected onto a Phenomenex Luna 75 x 30 mm, 5 µm, C18 column at room temperature. The flow rate was 30 mL/min. A linear gradient was used with 10% (or 50%) of MeOH (A) in H2O (with 0.1 % TFA) (B) to 100% of MeOH (A). HPLC was used to establish the purity of target compounds. All final compounds had > 95% purity using the HPLC methods described above. NMR integration marked as n.d. indicates that integration could not be determined due to large numbers of protons overlapping with solvent peaks.

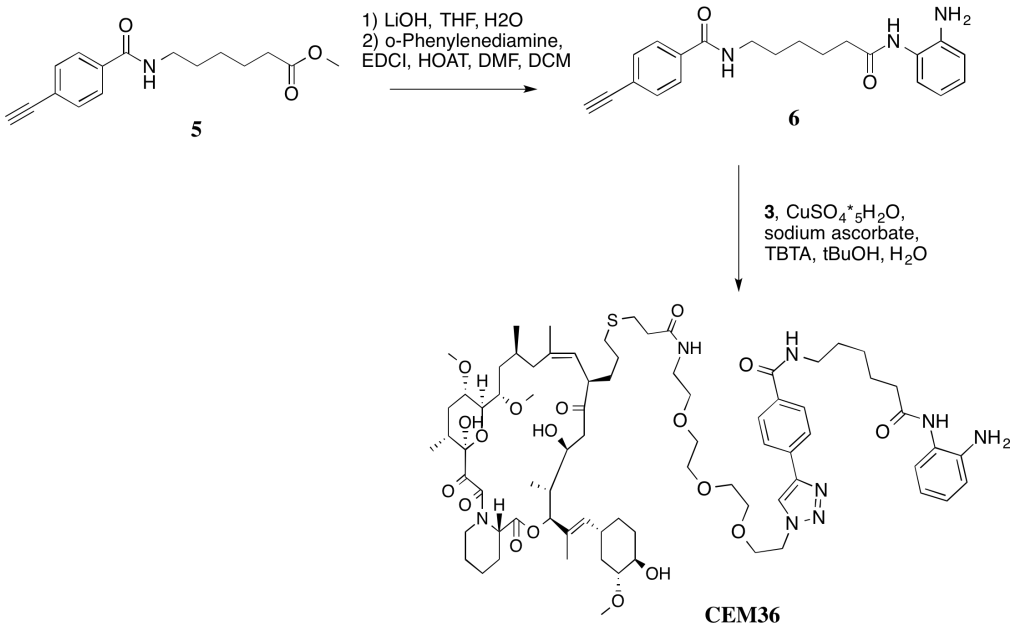


Supplemental Figure 5. Synthesis of compounds 1-3. (2) FK506-mercaptopropanamide propylazide**:** FK506-mercaptopropionic acid (Compound **1**) was prepared as described.[1] Compound **1** (182 mg, 0.2 mmol) in DMF (1 ml) was treated sequentially with EDCI-HCl (25 mg, 0.25 mmol), HOAT (41 mg, 0.3 mmol), 3-Azido-1-propanamine (25 mg, 0.25 mmol), and DIPEA (70 ul, 0.4 mmol). This was stirred for 24 h and purified by HPLC. Yield: 82 mg, 41%. TOF-HRMS (m/z) found (calcd.) for $[C_{50}H_{81}N_5O_{13}S + H]^+$: 992.5634 (992.5630). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.09 (s, 1H), 5.37 (s, 1H), 5.14 - 4.95 (m, 2H), 4.63 (m, 1H), 4.44 (d, *J* = 13.7 Hz, 1H), 3.94 (d, *J* = 10.3 Hz, 1H), 3.70 (d, *J* = 9.6 Hz, 1H), 3.65 - 3.54 (m, 1H), 3.46 - 3.25 (m, 17H), 3.07 - 2.97 (m, 2H), 2.86 - 2.73 (m, 3H), 2.54 (dt, *J* = 12.5, 7.0 Hz, 3H), 2.49 - 2.22 (m, 7H), 2.22 - 1.85 (m, 11H), 1.85 - 1.74 (m, 6H), 1.74 - 1.23 (m, 20H), 1.16 - 0.81 (m, 13H). **(3)** Compound **1** (200 mg, 0.27 mmol), 11-Azido-3,6,9-trioxaundecan-1-amine (51 ul, 0.26 mmol), EDCI-HCl (53 mg, 0.28 mmol), DIPEA (0.094 ml, 0.54 mmol), and HOAT (51 mg, 0.33 mmol) were stirred in 2 ml DCM for 24 h. The product was purified by HPLC. Yield: 143 mg, 48%. TOF-HRMS (m/z) found (calcd.) for $[C_{55}H_{91}N_5O_{16}S + H]^+$: 1110.6260 (1110.6260). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 6.43 (d, *J* = 15.8 Hz, 1H), 5.36 (s, 1H), 5.26 - 4.91 (m, 3H), 4.61 (s, 1H), 4.44 (d, *J* = 13.8 Hz, 1H), 4.04 - 3.84 (m, 1H), 3.71 - 3.57 (m, 12H), 3.48 - 3.29 (m, 14H), 3.02 (d, *J* = 11.7 Hz, 2H), 2.81 (t, *J* = 7.3 Hz, 2H), 2.75 (d, *J* = 15.9 Hz, 1H), 2.54 (s, 2H), 2.47 (t, *J* = 7.4 Hz, 2H), 2.31 (s, 2H), 2.19 - 1.26 (m, n.d.), 1.11 - 0.82 (m, 13H).



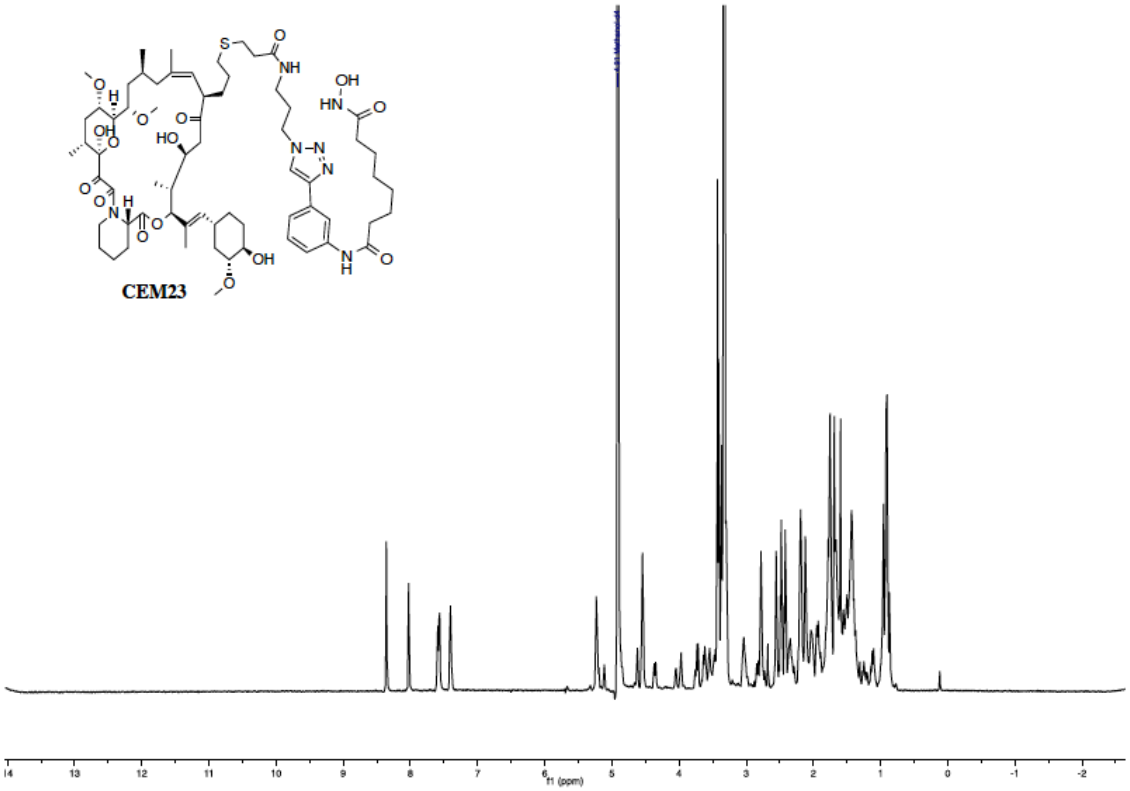
Supplemental Figure 6. Synthesis of compounds 4, CEM23, CEM42. (4) N^1-(3-Ethynylphenyl)-N^8-hydroxyoctanediamide: Methyl 8-((3-ethynylphenyl)amino)-8-oxooctanoate[2] (250 mg, 0.87 mmol) was dissolved in 3 ml of MeOH and 3 ml of THF, then treated with KOH (97 mg, 1.74 mmol, dissolved in a minimal amount of water) and NH_2OH (50% aqueous solution, 0.57 ml, 8.7 mmol), and stirred 24 h. The reaction was neutralized with 1M HCl and the product was extracted with DCM. Purification by HPLC gave the product. Yield: 43 mg, 17%. TOF-HRMS (m/z) found (calcd.) for $[C_{16}H_{20}N_2O_3 + H]^+$: 289.1655 (289.1552). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.34 (s, 1H), 9.98 (s, 1H), 8.67 (s, 1H), 7.79 (s, 1H), 7.55 (d, J = 8.2 Hz, 1H), 7.30 (t, J = 7.9 Hz, 1H), 7.13 (d, J = 7.6 Hz, 1H), 4.17 (s, 1H), 2.29 (t, J = 7.5 Hz, 2H), 1.94 (t, J = 7.4 Hz, 2H), 1.57 (t, J = 7.4 Hz, 2H), 1.52 - 1.45 (m, 2H), 1.27 (s, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.93, 169.49, 139.92, 129.56, 126.58, 122.34, 122.20, 119.97, 83.84, 80.89, 40.44, 36.78, 32.64, 28.80, 25.43, 25.33. **General procedure for click reactions with FK506-azides:** The azide (1 equiv, 0.02 mmol) and the alkyne (1 equiv) were dissolved in 1 ml tBuOH. This was treated sequentially with TBTA (2 mg), copper sulfate pentahydrate (0.05 equiv of a 0.1M solution in water), and sodium ascorbate (0.2 equiv of a 0.1M solution in water). The reaction was stirred for 24 h then purified by HPLC. **(CEM23)** The product was prepared from **10** and **3** by the general procedure for click reactions. Yield: 13 mg, 56%. TOF-HRMS (m/z) found (calcd.) for $[C_{66}H_{101}N_7O_{16}S + H]^+$: 1280.7011 (1280.7104). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.36 (s, 1H), 8.03 (s, 1H), 7.58 (dd, J = 16.2, 7.9 Hz,

2H), 7.40 (t, *J* = 7.9 Hz, 1H), 5.29 - 5.09 (m, 2H), 4.54 (m, 2H), 4.36 (d, *J* = 13.5 Hz, 1H), 3.97 (m, 1H), 3.79 - 3.68 (m, 1H), 3.63 (m, 3H), 3.50 - 3.39 (m, 6H), 3.36 (m, 3H), 3.02 (m, 2H), 2.89 - 2.65 (m, 4H), 2.55 (m, 2H), 2.52 - 2.25 (m, 7H), 2.25 - 1.98 (m, 10H), 1.94 (m, 3H), 1.88 - 1.71 (m, 9H), 1.71 - 1.18 (m, 23H), 1.18 - 1.06 (m, 1H), 1.06 - 0.80 (m, 10H). HPLC Purity: >95%, t_R = 4.78 min. **(CEM42)** The product was prepared from **4** and **3** by the general procedure for click reactions. Yield: 13 mg, 56%. TOF-HRMS (m/z) found (calcd.) for $[C_{71}H_{111}N_7O_{19}S + H]^+$: 1398.7731 (1398.7734). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.37 (s, 1H), 8.06 (s, 1H), 7.57 (d, *J* = 7.8 Hz, 2H), 7.41 (t, *J* = 7.9 Hz, 1H), 5.27 - 5.19 (m, 2H), 4.69 - 4.61 (m, 2H), 3.97 (t, *J* = 5.0 Hz, 2H), 3.78 - 3.71 (m, 1H), 3.69 - 3.51 (m, 9H), 3.49 - 3.39 (m, 7H), 3.39 - 3.34 (m, overlaps with solvent), 3.04 (s, 1H), 2.73 (t, *J* = 7.2 Hz, 2H), 2.55 - 2.25 (m, 9H), 2.23 - 1.86 (m, 10H), 1.82 - 1.03 (m, 31H), 1.02 - 0.80 (m, 10H). HPLC Purity: >95%, t_R = 5.08 min.

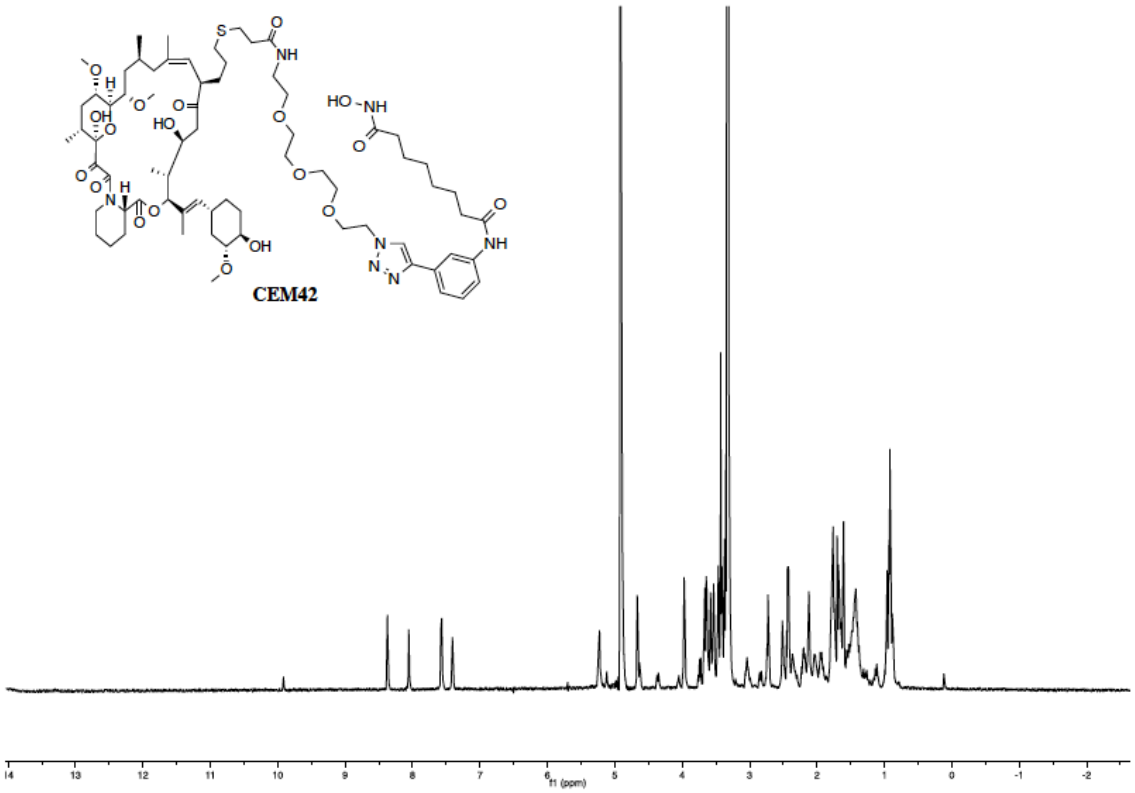


Supplemental Figure 7. Synthesis of compounds 5, 6, CEM36. (5) Methyl 6-(4-ethynylbenzamido)hexanoate: 4-Ethynylbenzoic acid (500 mg, 3.42 mmol), methyl 6-aminohexanoate HCl salt (743 mg, 4.11 mmol), EDCI-HCl (795 mg, 5.13 mmol), HOAT (697 mg, 5.13 mmol), and DIPEA (1.49 ml, 8.55 mmol) were stirred in 12 ml of a 1:3 mixture of DMF: DCM. Aqueous workup followed by silica gel purification (20 to 100% gradient of EtOAc in hexane) gave the product. Yield: 487 mg, 52%. TOF-HRMS (m/z) found (calcd.) for $[C_{16}H_{19}NO_3 + H]^+$: 274.2439 (274.1443). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.75 (d, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 2H), 6.24 (s, 1H), 3.69 (s, 3H), 3.49 (q, *J* = 6.7 Hz, 2H), 3.21 (s, 1H), 2.36 (t, *J* = 7.3 Hz, 2H), 1.71 - 1.65 (m, 4H), 1.44 (t, *J* = 7.8 Hz, 2H). **(6)** *N*-(6-((2-Aminophenyl)amino)-6-oxohexyl)-4-ethynylbenzamide**:** Compound 4 (340 mg, 1.25 mmol) was dissolved in THF (20 ml). To this was added aqueous LiOH (355 mg LiOH dissolved in 20 ml H2O), and the mixture was stirred 24 h. The mixture was acidified with 2N HCl and the product was extracted with EtOAc. The EtOAc extracts were concentrated to give 6-(4-ethynylbenzamido)hexanoic acid. Yield: 93%, 301 mg. TOF-HRMS (m/z) found (calcd.) for C15H17NO3 (M): [M+H]+, 260.1286 (260.1287). 6-(4-ethynylbenzamido)hexanoic acid (100 mg, 0.38 mmol), o-phenylenediamine (206 mg, 1.90 mmol), EDCI (66 mg, 0.42 mmol), and HOAT (58 mg, 0.42 mmol) were stirred in 8 ml of a 1:3 mixture of DMF:DCM for 24 h. The reaction was taken up in sat. aq. NaHCO3 and the product was extracted with EtOAc. The product was purified by silica gel chromatography (eluting first with a gradient of 50 to 100% EtOAc in hexane, followed by 0 to 20% MeOH in DCM). Yield: 50 mg, 38% for final step. TOF-HRMS (m/z) found (calcd.) for $[C_{21}H_{23}N_3O_2 + H]^+$: 350.1954 (350.1869). ^{1}H NMR (600 MHz, Chloroform-*d*) δ 7.74 (d, *J* = 8.4 , 2H), 7.47 (m, 2H), 7.21 (d, 1H), 7.04 (q, *J* = 8.0 Hz, 1H), 6.88 - 6.77 (m, 2H), 3.50 - 3.44 (m, 2H), 3.20 (s, 1H), 2.45 (t, *J* = 7.1 Hz, 2H), 1.78 (p, *J* = 7.2 Hz, 2H), 1.66 (m, 2H), 1.48 (m, *J* = 7.8 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 171.49, 165.64, 142.31, 135.15, 132.01, 127.84, 126.10, 125.71, 124.62, 123.94, 116.55, 116.27, 83.33, 83.13, 40.45, 36.12, 29.30, 26.59, 25.49. **(CEM36)** The product was prepared from **2** and **5** by the general procedure for click reactions, except that the product was purified by silica gel

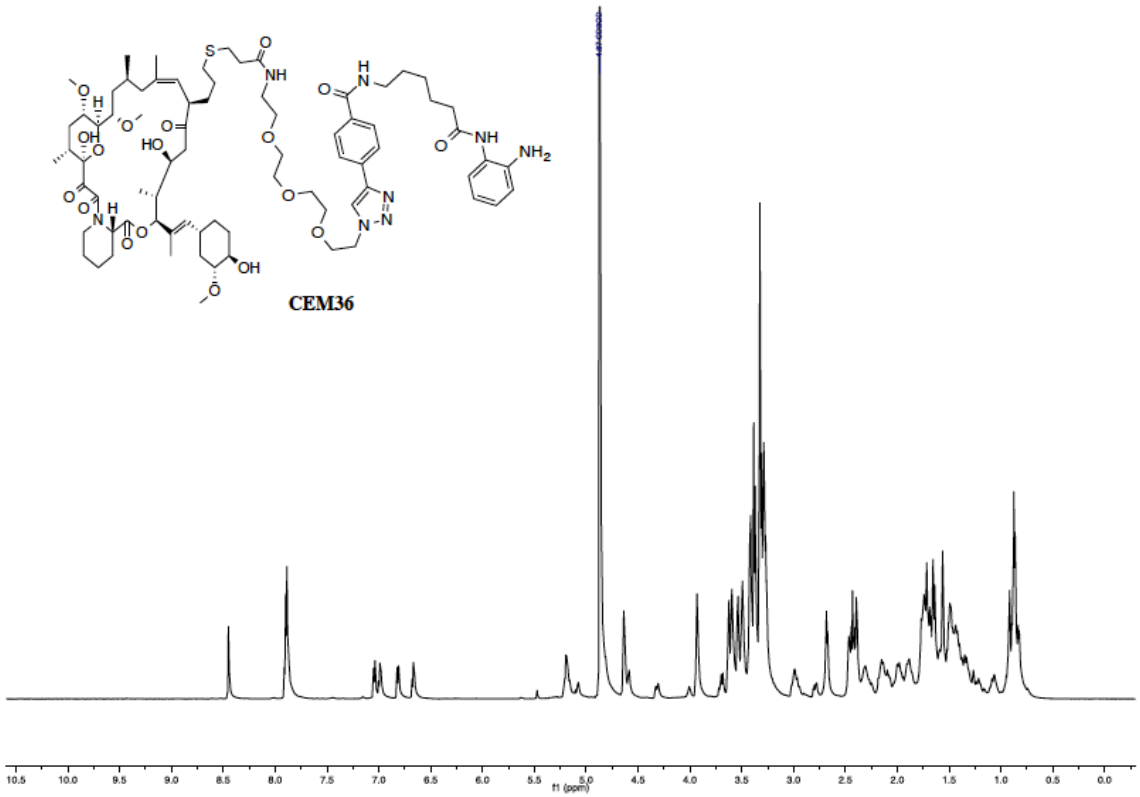
chromatography (gradient of 0 to 20% MeOH in DCM) rather than by HPLC. Exposure to acid used in HPLC buffer causes the product to decompose. Yield: 41 mg, 78%. TOF-HRMS (m/z) found (calcd.) for $[C_{76}H_{114}N_8O_{18}S + H]^+$: 1459.8060 (1459.8050). HPLC Purity: >95%, t_R = 4.88 min. ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.51 - 8.46 (m, 1H), 7.93 (q, *J* = 8.0 Hz, 4H), 7.11 - 7.06 (m, 1H), 7.05 - 7.00 (m, 1H), 6.85 (s, 1H), 6.73 - 6.67 (m, 1H), 5.22 (s, 2H), 4.65 (dd, *J* = 26.9, 5.5 Hz, 3H), 4.36 (d, *J* = 13.7 Hz, 1H), 4.00 - 3.94 (m, 3H), 3.68 - 3.50 (m, 13H), 3.48 - 3.28 (m, n.d.), 3.02 (d, *J* = 17.0 Hz, 2H), 2.72 (t, *J* = 7.2 Hz, 3H), 2.47 (ddd, *J* = 28.7, 14.1, 6.8 Hz, 7H), 2.41 - 1.89 (m, 13H), 1.82 - 1.37 (m, 31H), 0.98 - 0.85 (m, 11H).



Supplemental Figure 8. ^{1}H NMR spectra of CEM23.



Supplemental Figure 9. ^{1}H NMR spectra of CEM42.



Supplemental Figure 10. ^{1}H NMR spectra of CEM36.

References:

1. Guo, Z.-F., Zhang, R. & Liang, F.-S. (2014) Facile functionalization of FK506 for biological studies by the thiol–ene 'click' reaction. *RSC Adv.* **4,** 11400.
2. Chen, Y. *et al.* (2008) A series of potent and selective, triazolylphenyl-based histone deacetylases inhibitors with activity against pancreatic cancer cells and Plasmodium falciparum. *J. Med. Chem.* **51,** 3437–3448.