Supplementary Information

Light Controlled Modulation of Gene Expression by Chemical Optoepigenetic Probes

Authors: Surya A. Reis[2,†], Balaram Ghosh[1,2,†,‡], J. Adam Hendricks[1], D. Miklos Szantai-Kis[1], Lisa Törk[1], Kenneth N. Ross[3], Justin Lamb[4], Willis Read-Button[4], Baixue Zheng[4], Hongtao Wang[5], Christopher Salthouse[5], Stephen J. Haggarty[2,6,*], Ralph Mazitschek[1,6,*]

Affiliations:

[1]Center for Systems Biology, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114, USA

[2]Chemical Neurobiology Laboratory, Center for Human Genetic Research, Massachusetts General Hospital, Departments of Neurology & Psychiatry, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114, USA

[3]Center for Cancer Research, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114, USA

[4]Genometry Inc., One Kendall Square, Cambridge, MA 02139, USA

[5]Department of Electrical & Computer Engineering & Center for Personalized Health Monitoring, University of Massachusetts, 100 Natural Resources Rd., Amherst, MA 01003, USA

[6]Broad Institute of Harvard & Massachusetts Institute of Technology, Cambridge, MA 02142, USA

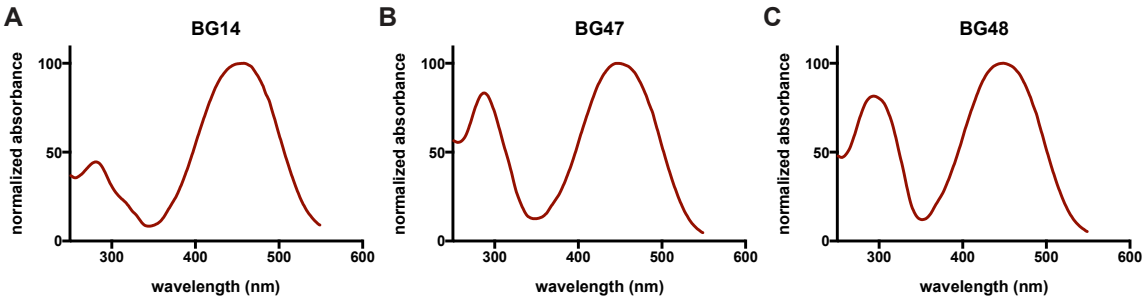
*Correspondence to: shaggarty@mgh.harvard.edu (SJH), rmazitschek@mgh.harvard.edu (RM)

†Contributed equally

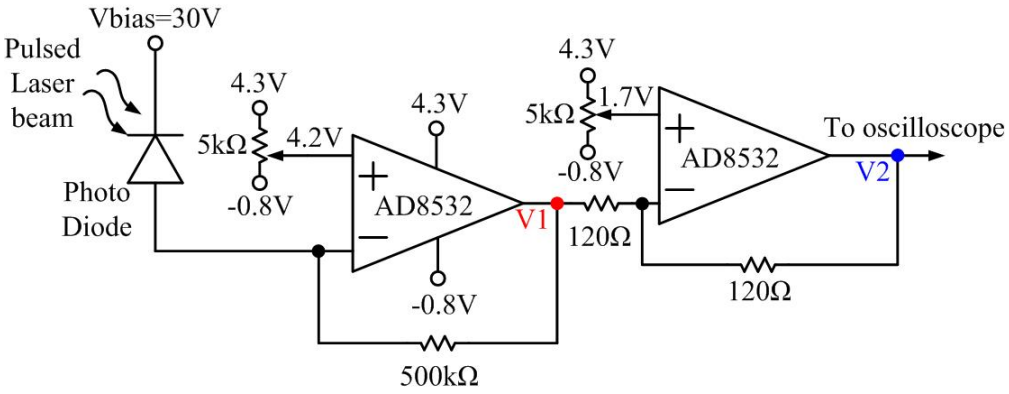
‡ Current affiliation: Department of Pharmacy, BITS-Pilani at Hyderabad Campus, India 500 078

Supplementary Results

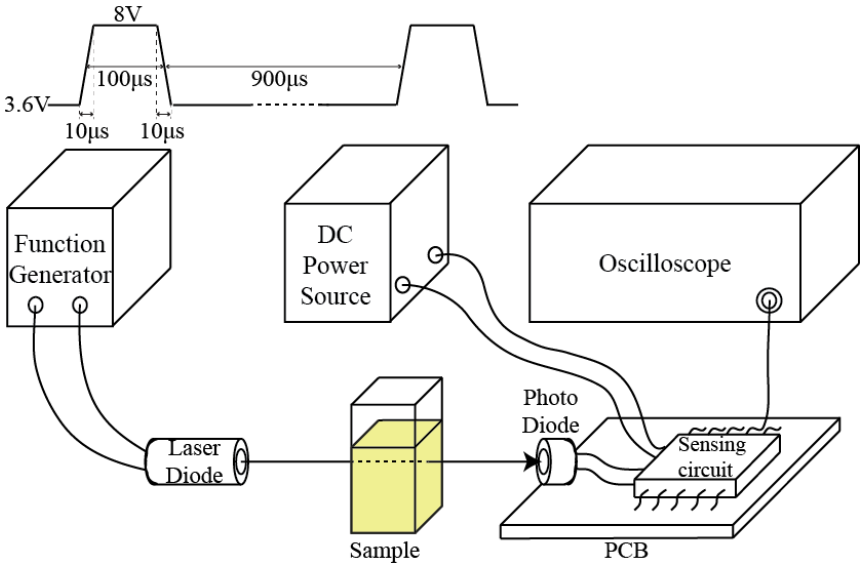
Supplementary Figures



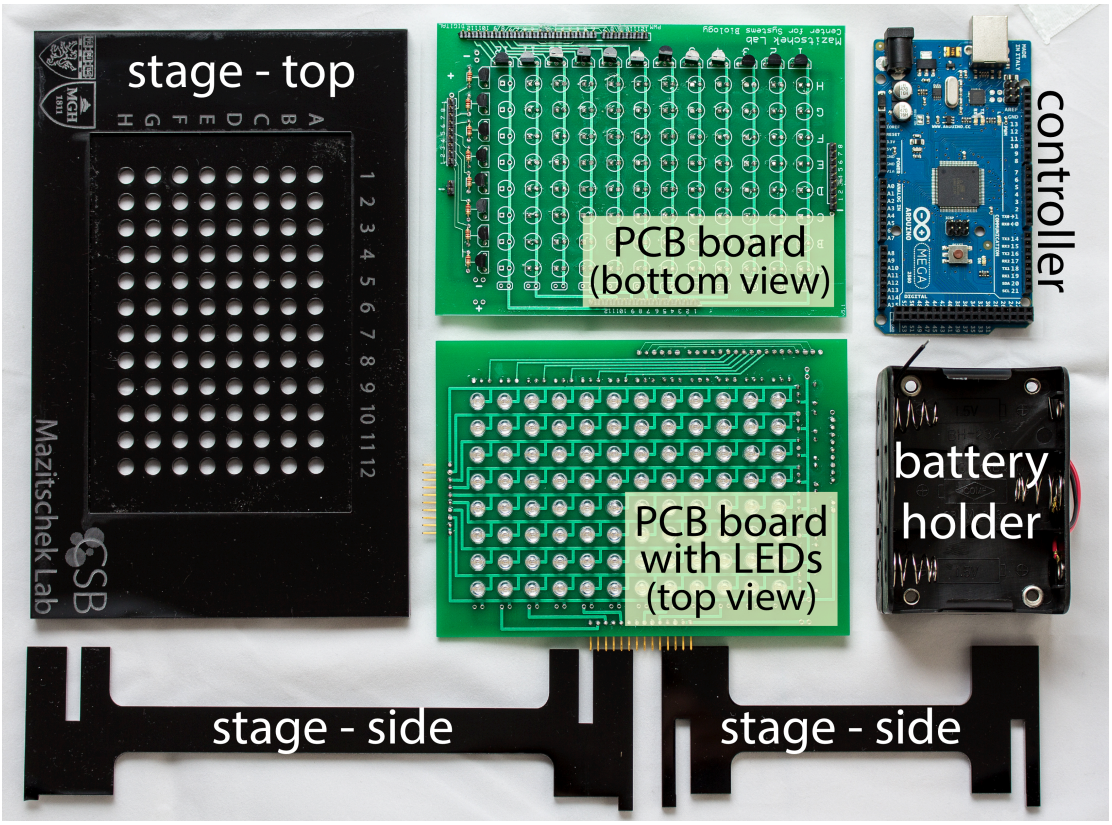
Supplementary Figure 1. UV-Vis absorbance spectra of COMET probes in PBS. BG14 **(A)**, BG47 **(B)** and BG48**(C)**.



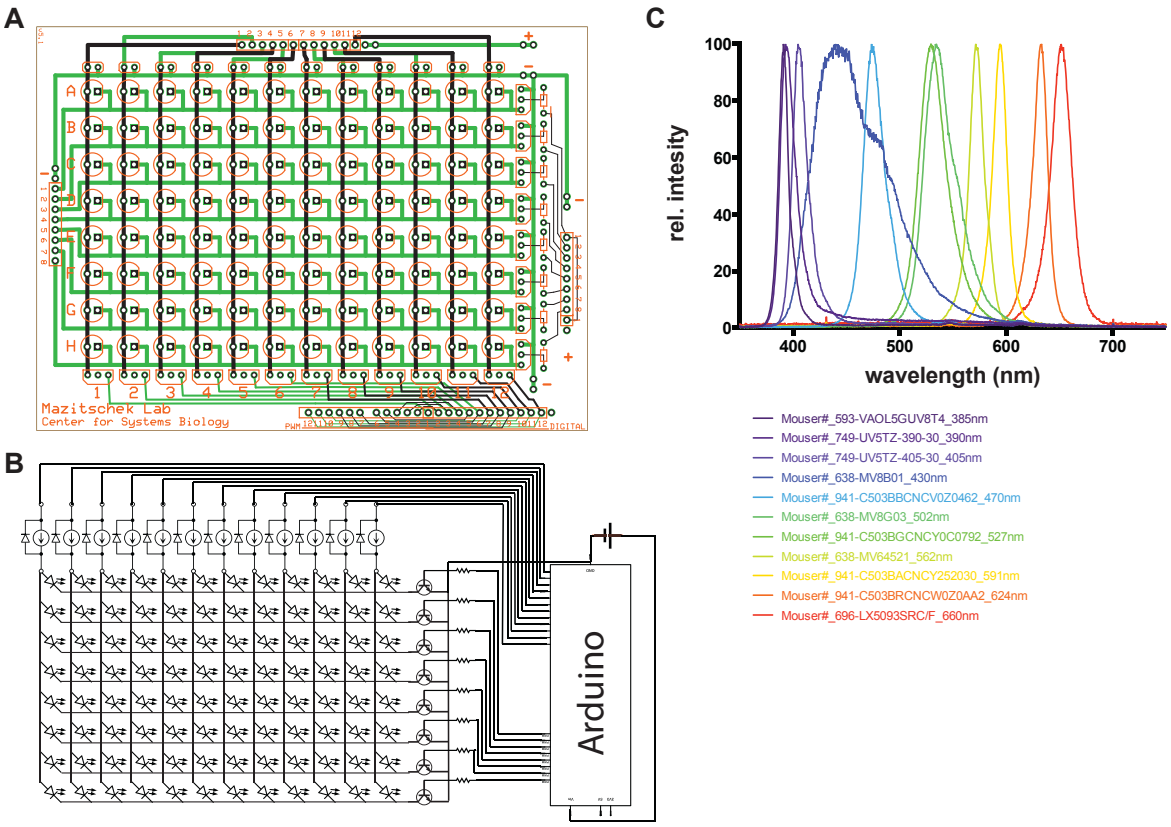
Supplementary Figure 2. Schematic of custom designed sensing circuit for photophysical measurements.



Supplementary Figure 3. Schematic diagram of the measurement system for photophysical profiling.

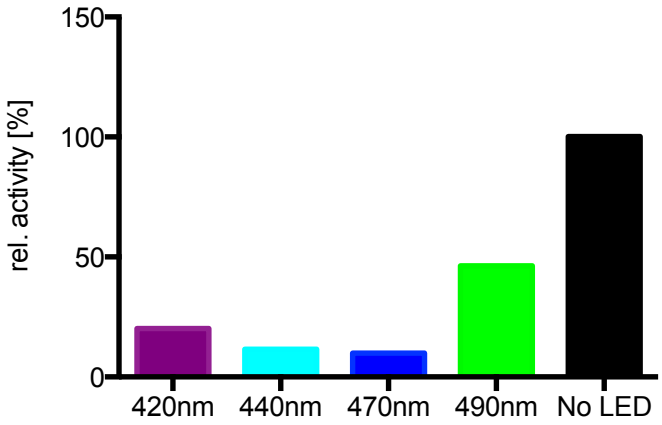


Supplementary Figure 4. Components of LED-array assembly.



Supplementary Figure 5. Design of microprocessor-controlled 12x8 LED-arrays compatible with 96-well microtiter plates. (**A**) LED 12x8 matrix PCB design based upon the open-source Arduino platform (www.arduino.cc). (top layer – black, bottom layer - green, silkscreen - orange). (**B**) Integrated circuit schematic. (**C**) Normalized LED emission profiles**.**

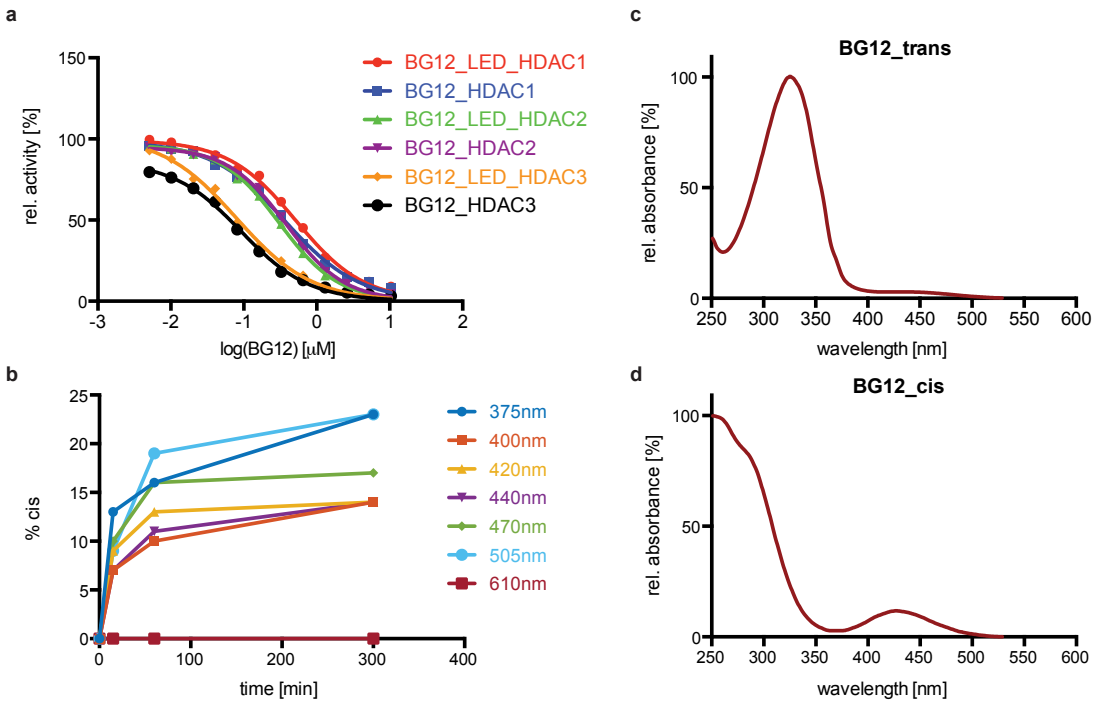
A



B

	Rep A	Rep B	Mean
420nm	73	104	88.5
440nm	31	71	51
470nm	52	35	43.5
490nm	187	220	203.5
no light	369	510	439.5

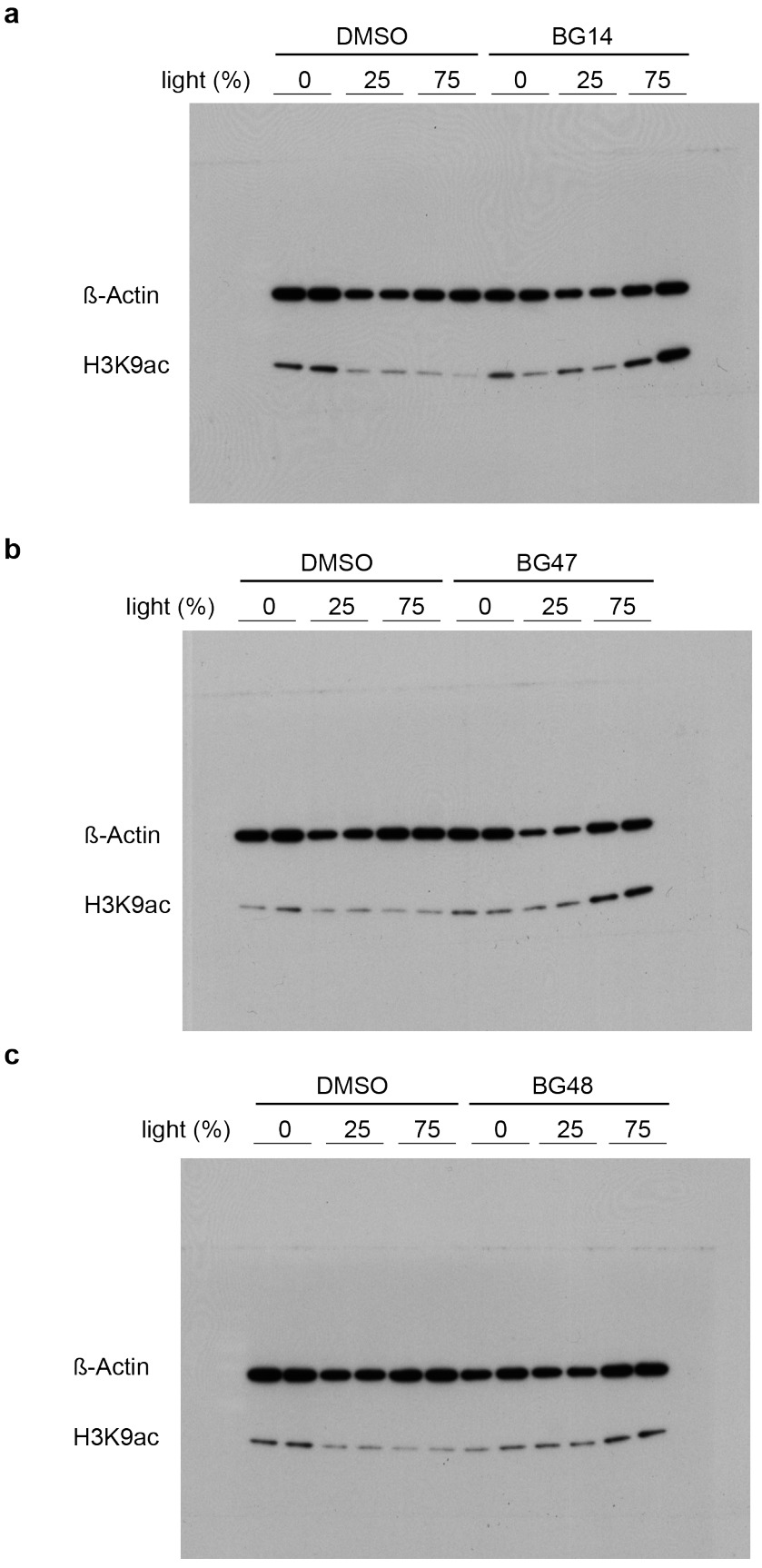
Supplementary Figure 6. Wavelength dependent activity of BG14 against HDAC3. **(A)** HDAC3 was incubated with BG14 (20 μM) and exposed to LEDs with varying wavelengths for 15 min prior to addition of HDAC substrate and trypsin. Values represent means of duplicate measurements. (**B**) Source data for panel (A).



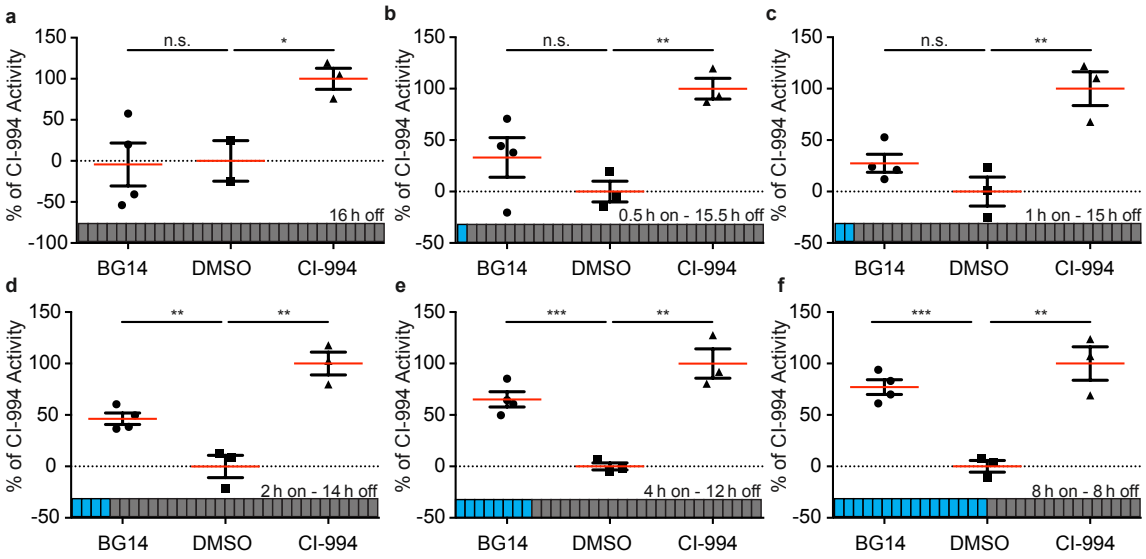
e

concentration (assay) [uM]	BG12_LED_HDAC1		BG12_HDAC1		BG12_LED_HDAC2		BG12_HDAC2		BG12_LED_HDAC3	
10.41667	8.768	9.451	7.882	8.732	4.573	4.376	4.341	4.601	3.931	2.930
5.208333	12.292	11.800	11.511	12.588	5.500	5.280	7.498	7.190	3.837	3.379
2.604167	15.351	15.802	14.403	15.452	8.462	7.384	11.145	10.568	4.961	3.922
1.302083	26.714	27.315	21.604	22.994	16.500	15.216	18.548	17.625	9.004	7.918
0.6510417	45.343	44.947	34.760	36.177	30.021	28.988	34.334	32.550	17.278	14.704
0.3255208	61.677	60.967	52.650	53.656	49.119	46.103	53.024	50.632	25.776	23.923
0.1627604	78.694	76.058	67.364	69.179	66.667	63.112	71.445	68.399	35.567	33.245
0.08138021	81.399	80.961	74.906	78.776	76.937	74.233	77.523	77.223	49.381	47.669
0.04069011	90.986	89.211	81.285	86.786	88.909	85.605	90.356	87.278	73.492	65.452
0.02034505	93.335	91.983	91.634	93.108	91.796	89.547	91.098	89.480	77.610	73.071
0.01017253	99.317	96.613	92.541	95.093	95.047	93.087	92.551	91.674	89.815	85.229
0.005086263	100.628	98.593	94.412	97.318	96.187	94.850	95.518	94.713	94.982	90.536

Supplementary Figure 7. BG12 Optical properties and absence of light-dependent inhibitory activity toward recombinant HDACs. (**a**) Dose dependent but absence of light dependent (470 nm, 17 mW/cm^2) activity of BG12, a close structural analog of BG14 lacking only an electron donating dimethylamine substituent, against HDAC1-3 in both *trans*-geometry and *cis*-geometry. The absence of differential potency upon photoisomerization supports the conclusion of the importance of electronic properties of COMET probes for light dependent HDAC inhibition. Data represent mean values of duplicates. (**b**) Photoisomerization kinetics of BG12 to *cis*-geometry as a function of wavelength for BG12.. Values represent individual measurements. (**c**) Relative absorbance spectra of BG12 in *trans*-geometry (**d**) Relative absorbance spectra of BG12 in *cis*-geometry. (**e**) Source data for panel (a).

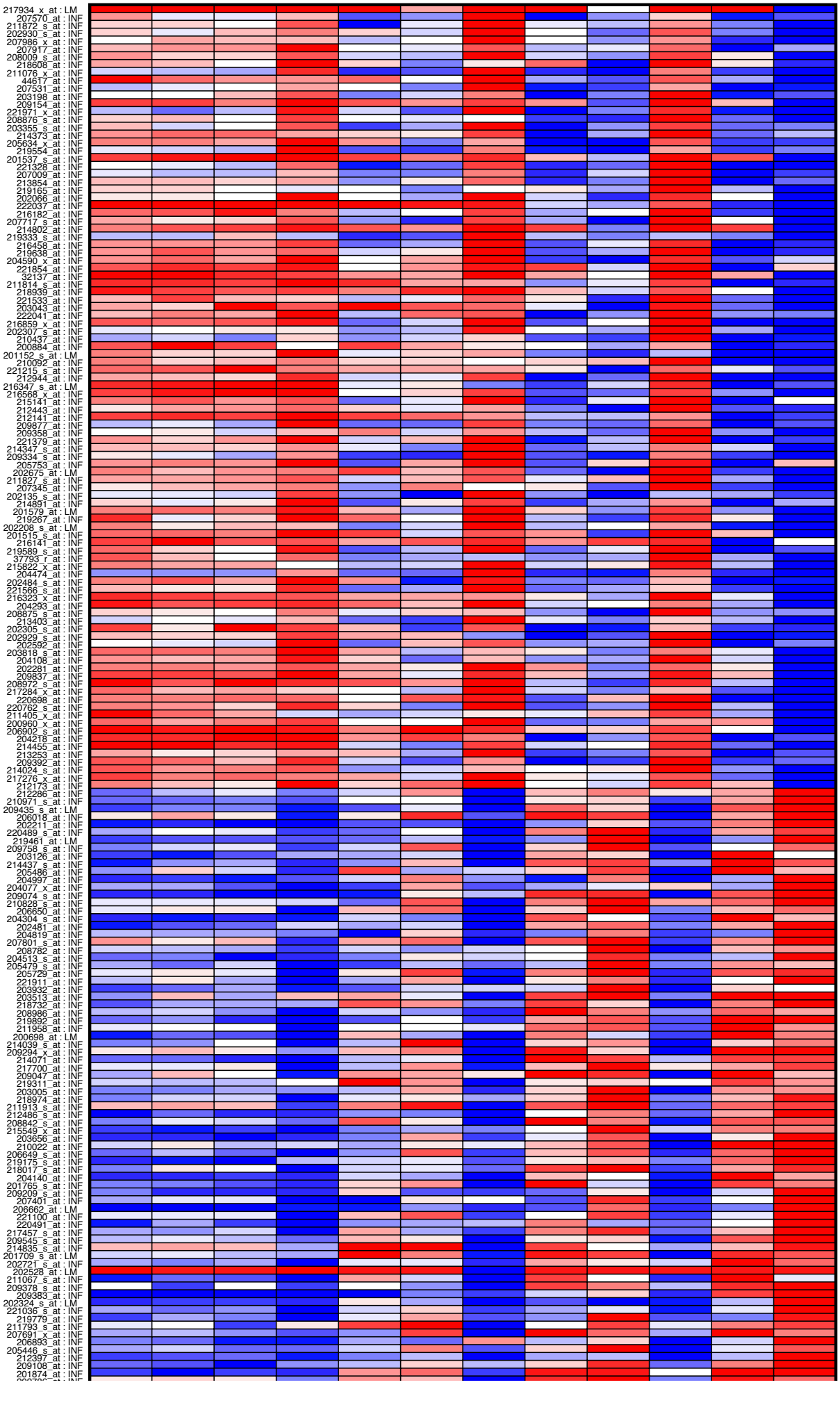


Supplementary Figure 8. Full Western Blots of Histone H3K9 acetylation

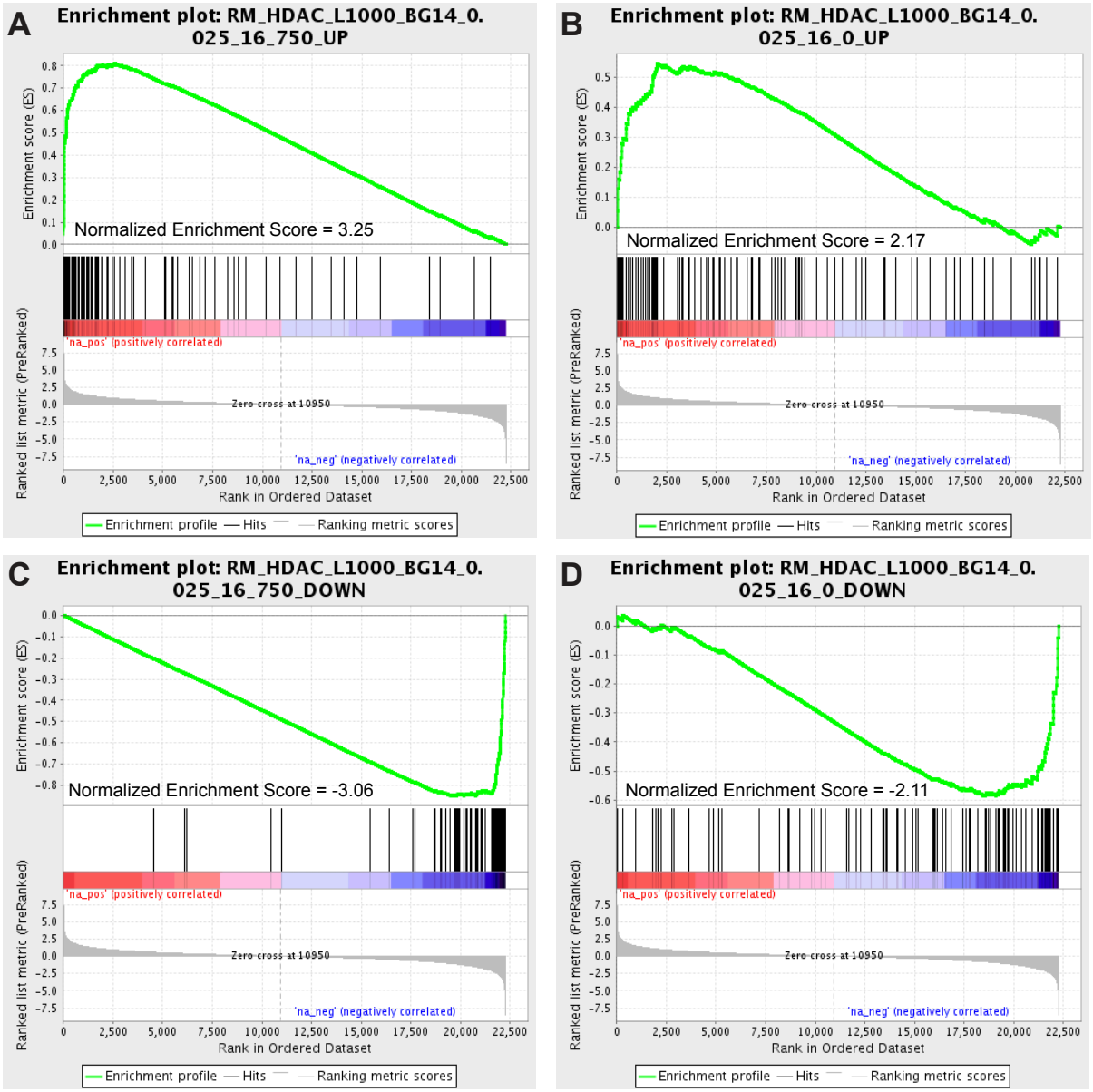


Supplementary Figure 9 . Light exposure time-dependent activity of BG14 in human cells. Quantification of relative immunofluorescence intensity of H3K9ac upon treatment of MCF-7 cells with DMSO, CI-994 (25 μM) and BG14 (25 μM) for 16 h under varying duration of light exposure (470 nm, 8.5 mW/cm^2, 1Hz, 50% duty cycle) at the beginning of the treatment period. No light (**a**), 0.5 h (**b**), 1 h (**c**), 2 h (**d**), 4 h (**e**), 8 h (**f**). The light/dark treatment is indicated by the timeline in each subpanel, each box represents a 30 min time increment. Blue – light on; Gray – light off. Cellular H3K9 acetylation levels are normalized within each group to the positive control CI-994 = 100% and DMSO = 0. A total of 9 fields/well were captured per treatments; treatments were done with n≥ 3. Significance values (n.s. – not significant, ** $p < 0.01$, *** $p < 0.001$) were calculated using unpaired t-tests (Graphpad PRISM).

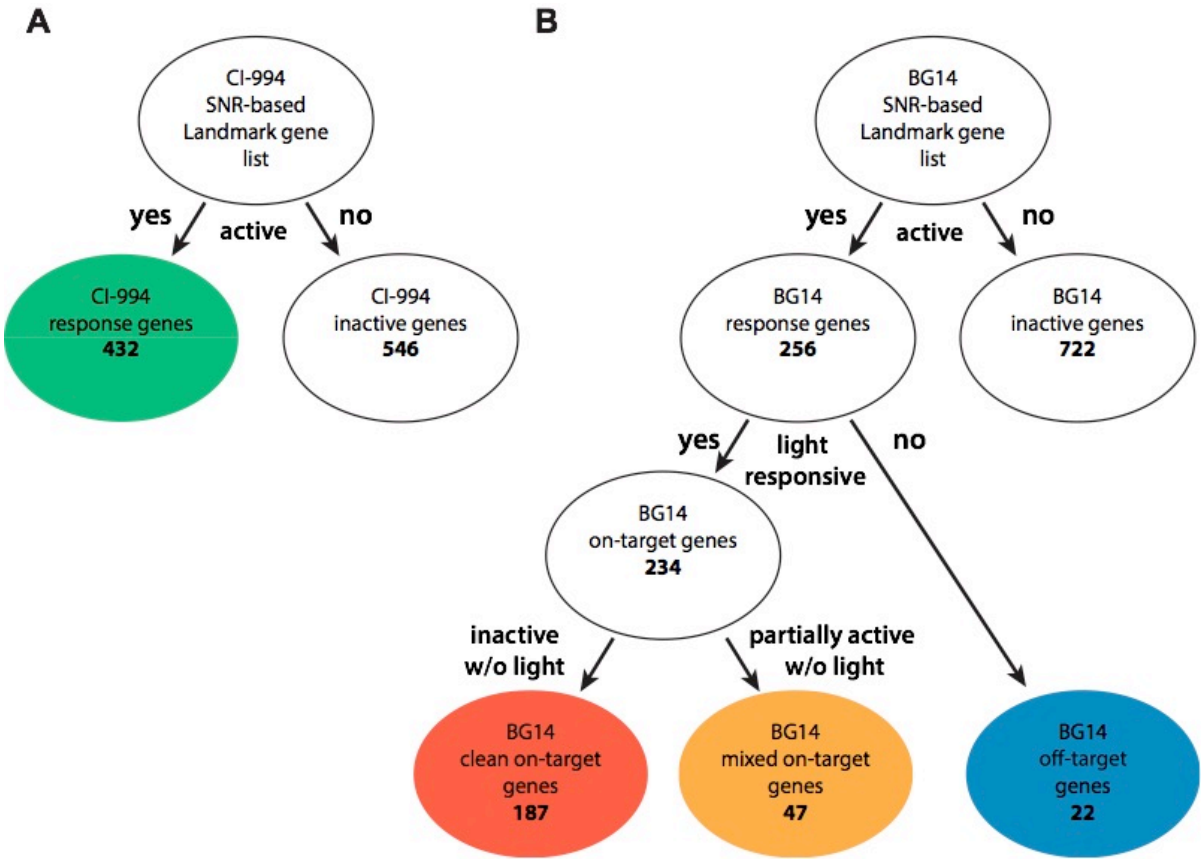
L1000 BG14 16 hours (top 100 genes Cosine Distance – Sample Means)



Supplementary Figure 10. Heatmap of differentially regulated genes from L1000 mRNA profiles. Top 100 up- and down-regulated transcripts (landmark and inferred genes) that are specifically modulated by BG14 in light dependent fashion. No significant transcriptional changes are observed by BG14 treatment alone or by light in the absence of inhibitor (control, 1 μM, 5 μM, 25 μM; average n = 4 measurements).



Supplementary Figure 11. Gene Set Enrichment Analysis (GSEA) of CI-994 regulated genes and BG14. Correlation of (**a**) BG14 upregulated genes (25 µM, 16 h, light), (**b**) BG14 upregulated genes (25 µM, 16 h, no light), (**c**) BG14 downregulated genes (25 µM, 16 h, light), (**d**) BG14 downregulated genes (25 µM, 16 h, no light). The more positive the nES the greater the gene set enrichment from BG14 at the top of the ranked list from CI-994; the more negative nES the greater the gene set enrichment from BG14 at the bottom of the ranked list from CI-994. $p < 0.001$ and FDR $q < 0.001$.



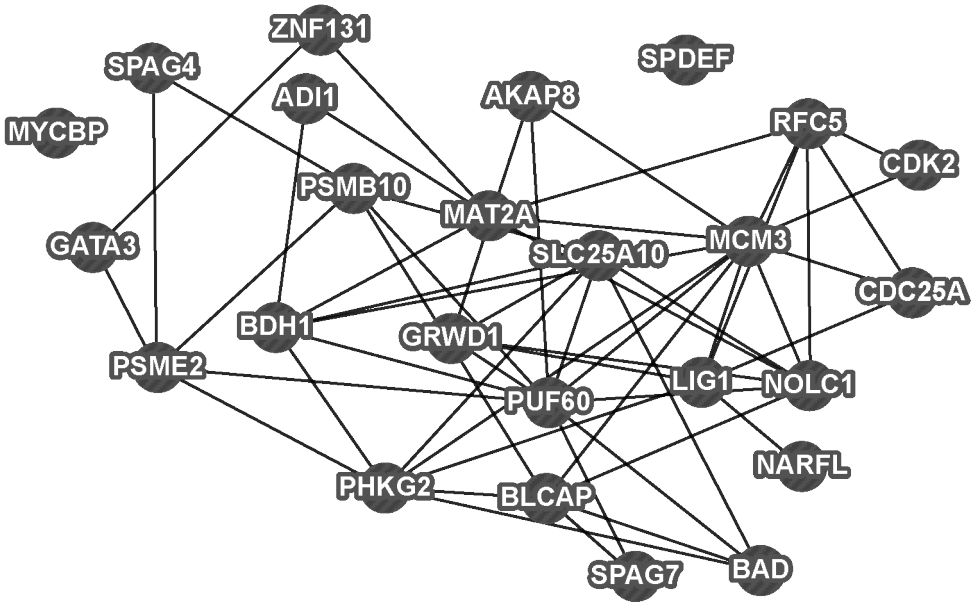
Supplementary Figure 12. Summary schematic of L1000 gene signature classifications to determine 'on' and 'off' target genes. On-target genes are defined experimentally as those that show light responsiveness with off-target genes defined as those that are affected by BG14 treatment but are not light responsive.

a

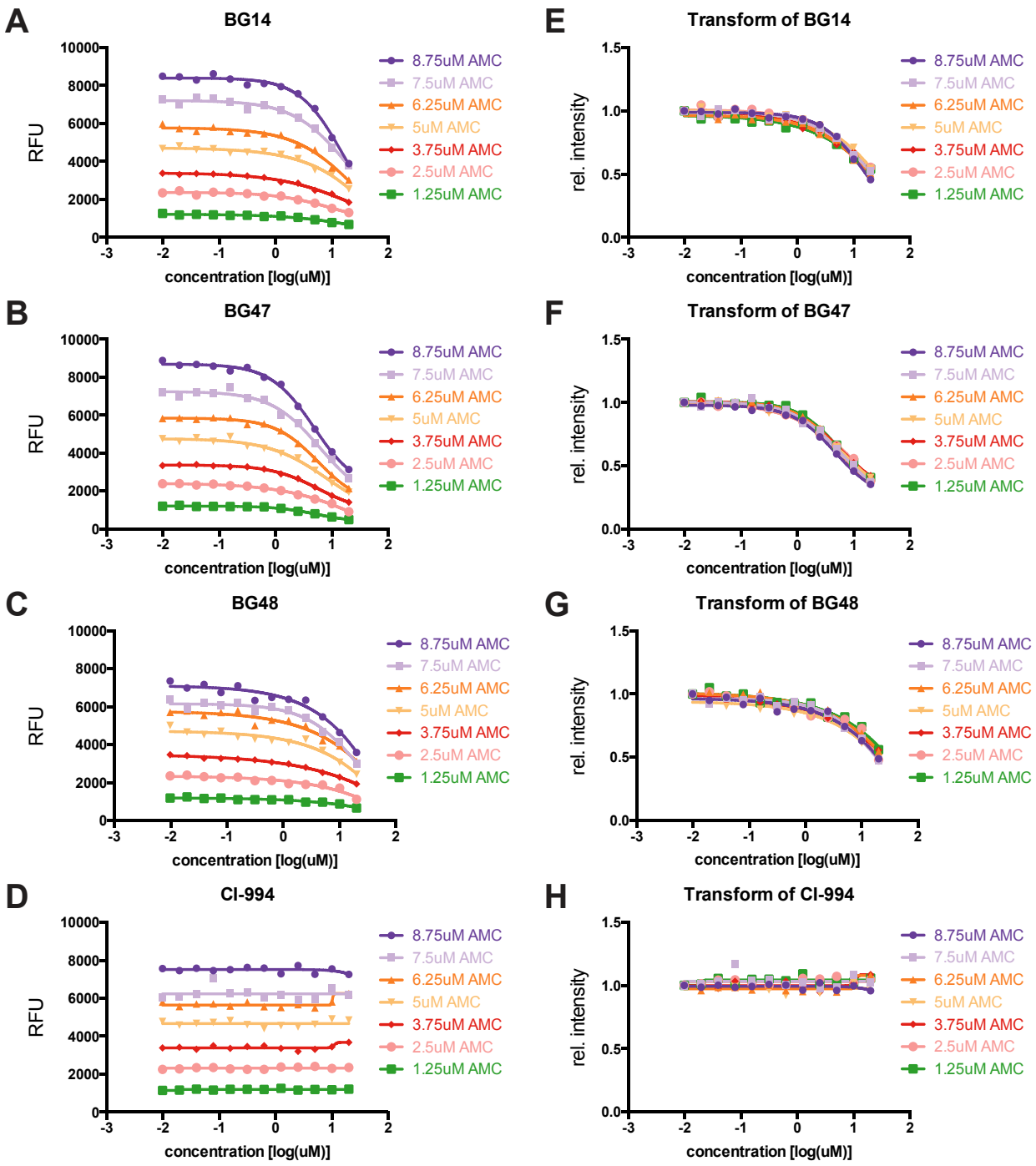
GeneMANIA analysis of core gene set

Feature/Network	FDR	Genes in Network/ Genome(Landmark Genes)
interphase of mitotic cell cycle	5.90E-05	13/258(49)
interphase	5.90E-05	13/263(51)
S phase of mitotic cell cycle	5.90E-05	10/132(34)
S phase	7.25E-05	10/139(36)
G1/S transition of mitotic cell cycle	7.43E-05	11/184(32)
cell cycle checkpoint	6.05E-04	11/230(32)
positive regulation of release of cytochrome c from mitochondria	1.38E-02	4/19(4)
regulation of mitochondrion organization	1.60E-02	5/43(5)
positive regulation of cell cycle arrest	2.02E-02	6/79(17)
signal transduction by p53 class mediator	2.79E-02	7/128(19)
positive regulation of mitochondrion organization	2.79E-02	4/25(4)
regulation of cell cycle arrest	3.43E-02	6/91(18)
regulation of release of cytochrome c from mitochondria	3.76E-02	4/28(5)

b



Supplementary Figure 13. Characterization of COMET-mediated gene expression signatures in human cells. **(a)** Statistically significant (FDR < 0.05) cellular networks identified by GeneMANIA analysis of the Core Gene Set comprising 120 genes shared between CI-994 and BG14 (on-target) in the presence of light. **(b)** GeneMANIA network analysis of protein-protein interactions between the top 25 down-regulated genes within the Core Gene Set.



Supplementary Figure 14. Reduction of 7-AMC fluorescence in the presence of COMET probes. Since relative signal decrease is linearly correlated with the azobenzene concentration, but independent from the concentration of the substrate and the amount of the generated fluorescent product 7-AMC, standard curves were acquired to corrected raw intensity values of HDAC biochemical assays. Each data point represents a single measurement.

a)

Weight	-1	-1	-1
Compound	DMSO	DMSO	DMSO
Dose	0	0	0
Light	0	250	750

Weight	-1	1	2	-1	2	3	-1	3	4
Compound	Cmpd	Cmpd	Cmpd	Cmpd	Cmpd	Cmpd	Cmpd	Cmpd	Cmpd
Dose	0.001	0.001	0.001	0.005	0.005	0.005	0.025	0.025	0.025
Light	0	250	750	0	250	750	0	250	750

b)

Weight	0	1	2	3
Compound	DMSO	Cmpd	Cmpd	Cmpd
Dose	0	0.001	0.005	0.025
Light	0	0	0	0

Supplementary Figure 15. Templates for Transcriptional analysis. (**a**)Template prior to normalization for combined light level and compound concentration dose response. (**b**) Template prior to normalization for no light dose response.

	Trans		***Cis***		**CI-994**	
ESP for:	**N**	**O**	**N**	**O**	**N**	**O**
lowest energy conf.	-0.809	-0.674	-0.743	-0.653	-0.721	-0.664
Boltzmann weighted	-0.883	-0.635	-0.784	-0.664	-0.774	-0.642

Supplementary Figure 16. ESP of the zinc binding nitrogen and oxygen for CI-994, *trans*-BG14 and *cis*-BG14. The ESP values are given for the lowest energy conformer as well as the Boltzmann weights ESP values are given.

Supplementary Data Set 1: Gene expression SNR scores; BG14, CI-994, and C60 response gene IDs

Data set provides Gene IDs for BG14, CI-994, and C60 response genes as well as their SNR scores in the L1000 assay. Gene lists for each section in the Venn diagrm (Fig 5e) are in separate tabs.

Supplementary Video 1: LED array

Video shows footprint of LED array in an incubator and exposure of 470nm light (8.5 mW/cm^2) modulating at 1 Hz (1s on/7s off) per row both with and without a 96-well plate.