SUPPORTING INFORMATION

Evaluation of EED Inhibitors as a Class of PRC2-Targeted Small Molecules for HIV Latency Reversal

Anne-Marie W. Turner[1,2], Raghuvar Dronamraju[3], Frances Potjewyd[4], Katherine M. Sholtis[1], Daniel K. Winecoff[1], Jennifer L. Kirchherr[1,] Nancie M. Archin[1,2], Edward P. Browne[1,2], Brian D. Strahl[3], David M. Margolis[1,2,5,6,*], Lindsey I. James[1,4,*]

[1] UNC HIV Cure Center, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, 27599 USA

[2] Department of Medicine, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, 27599 USA

[3] Department of Biochemistry & Biophysics, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, 27599 USA

[4] Center for Integrative Chemical Biology and Drug Discovery, Division of Chemical Biology and Medicinal Chemistry, Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599 USA

[5] Department of Epidemiology, University of North Carolina at Chapel Hill School of Public Health, Chapel Hill, NC, 27599 USA

[6] Department of Microbiology and Immunology, University of North Carolina at Chapel Hill School of Medicine, Chapel Hill, NC, 27599 USA

Corresponding authors:

* David M. Margolis - 120 Mason Farm Road, 2016 Genetic Medicine Building/CB 7042, Chapel Hill, NC 27599-7042, US. (919) 966-6388. dmargo@med.unc.edu

* Lindsey I. James - 125 Mason Farm Road, 3202 Marsico Hall/CB 7363, Chapel Hill, NC 27599-7363. 919-962-4870. ingerman@email.unc.edu

Supplemental Figures

Figure S1

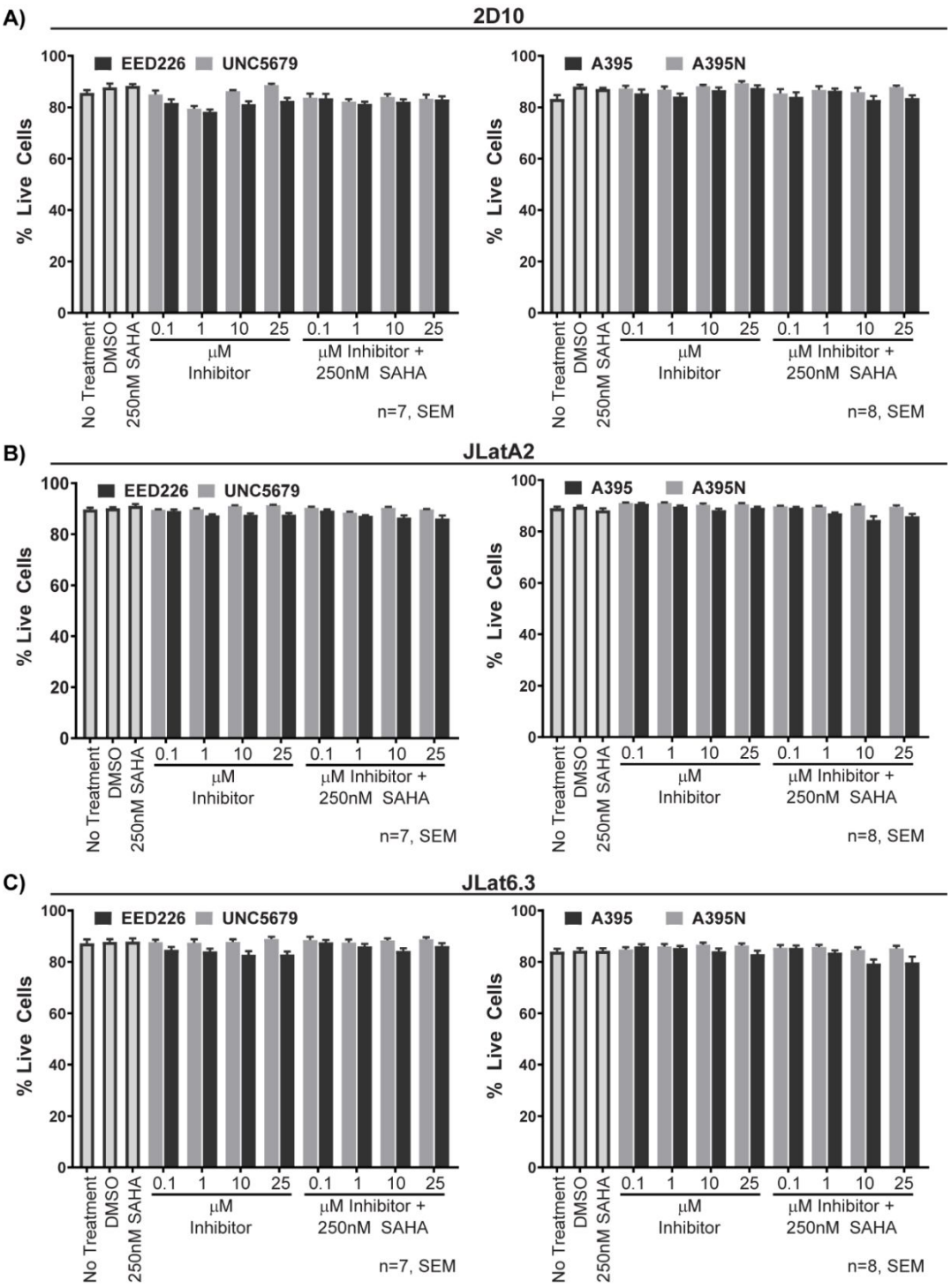


Figure S1. *Viability of Jurkat models when treated with EEDi* – Cell viability was measured using a fixable live/dead stain and was assessed for all EED inhibitor experiments by flow cytometry. The viability data provided for 2D10 **(A)**, JLatA2 **(B)**, and JLat6.3 **(C)** lines is associated with the experimental data provided in Figures 1 and 2, as well as Supplementary Figures 2 and 3.

Figure S2

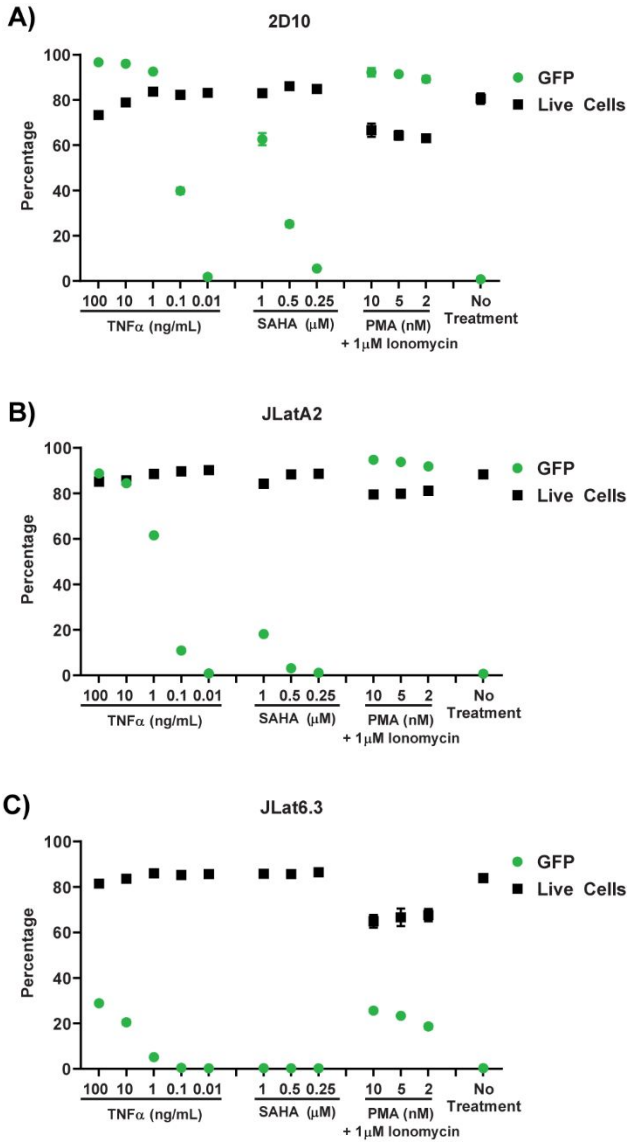


Figure S2. *Response to various LRAs by Jurkat Models* – Three Jurkat-derived latency models, **(A)** 2D10, **(B)** JLatA2, and **(C)** JLat6.3 cells exhibit differential latency reactivation to LRAs TNFα, HDAC inhibitor SAHA, and PMA/Ionomycin. Percentage on the Y-axis represents either percent of cells GFP positive (green circles) or percent of live cells determined via viability stain (black squares) from the same samples as measured by flow cytometry. Error bars represent an n=6, SEM.

Figure S3

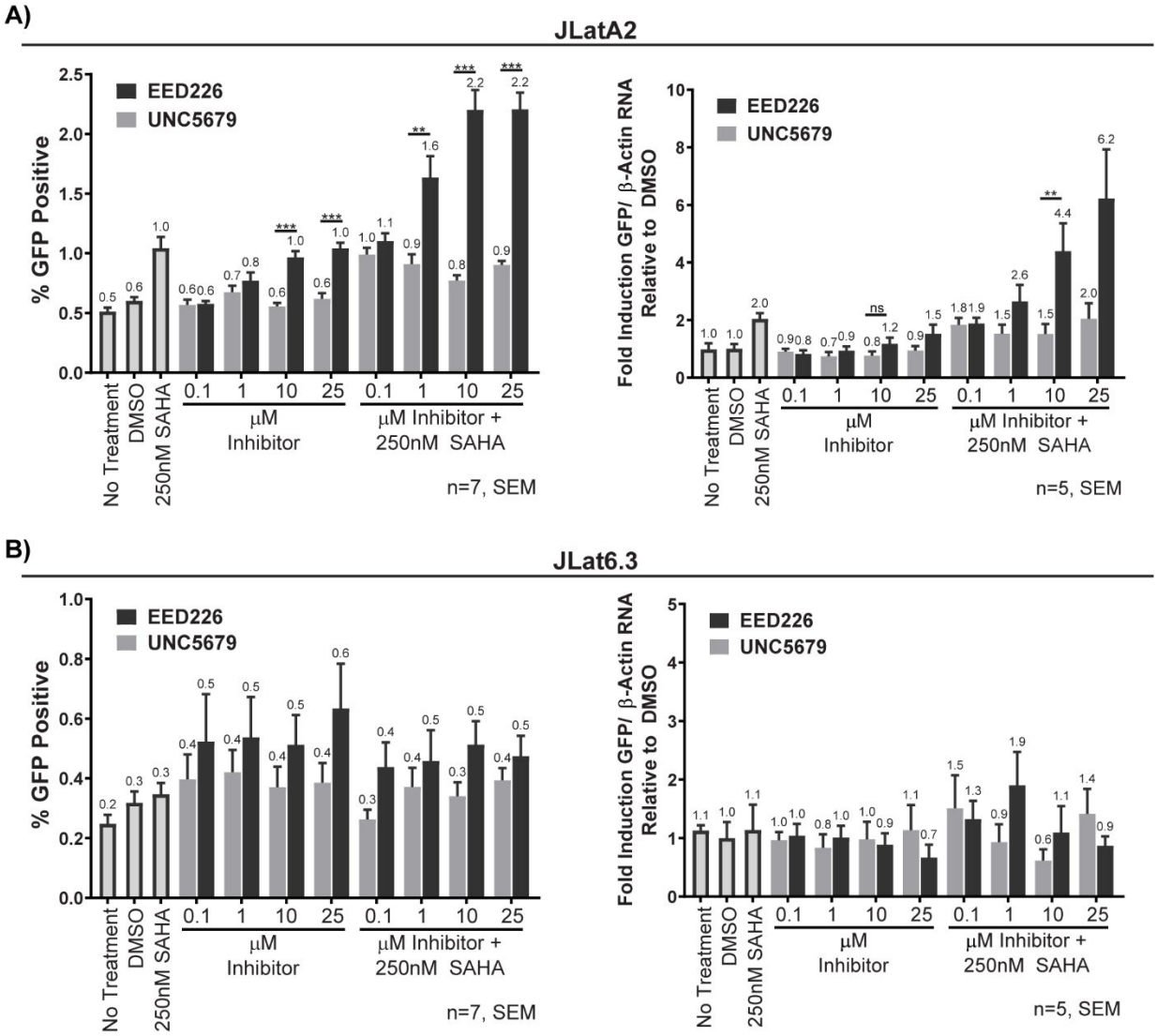


Figure S3. *Dose response of latency reactivation to EED226 in JLat Models* – Dose response for latency reactivation in JLatA2 **(A)** and JLat6.3 **(B)** cells in response to treatment with EED226 at both the protein (GFP) and RNA (GFP transcript) level. (* - p<0.05, ** - p<0.01, *** - p<0.001, Mann-Whitney Test)

Figure S4

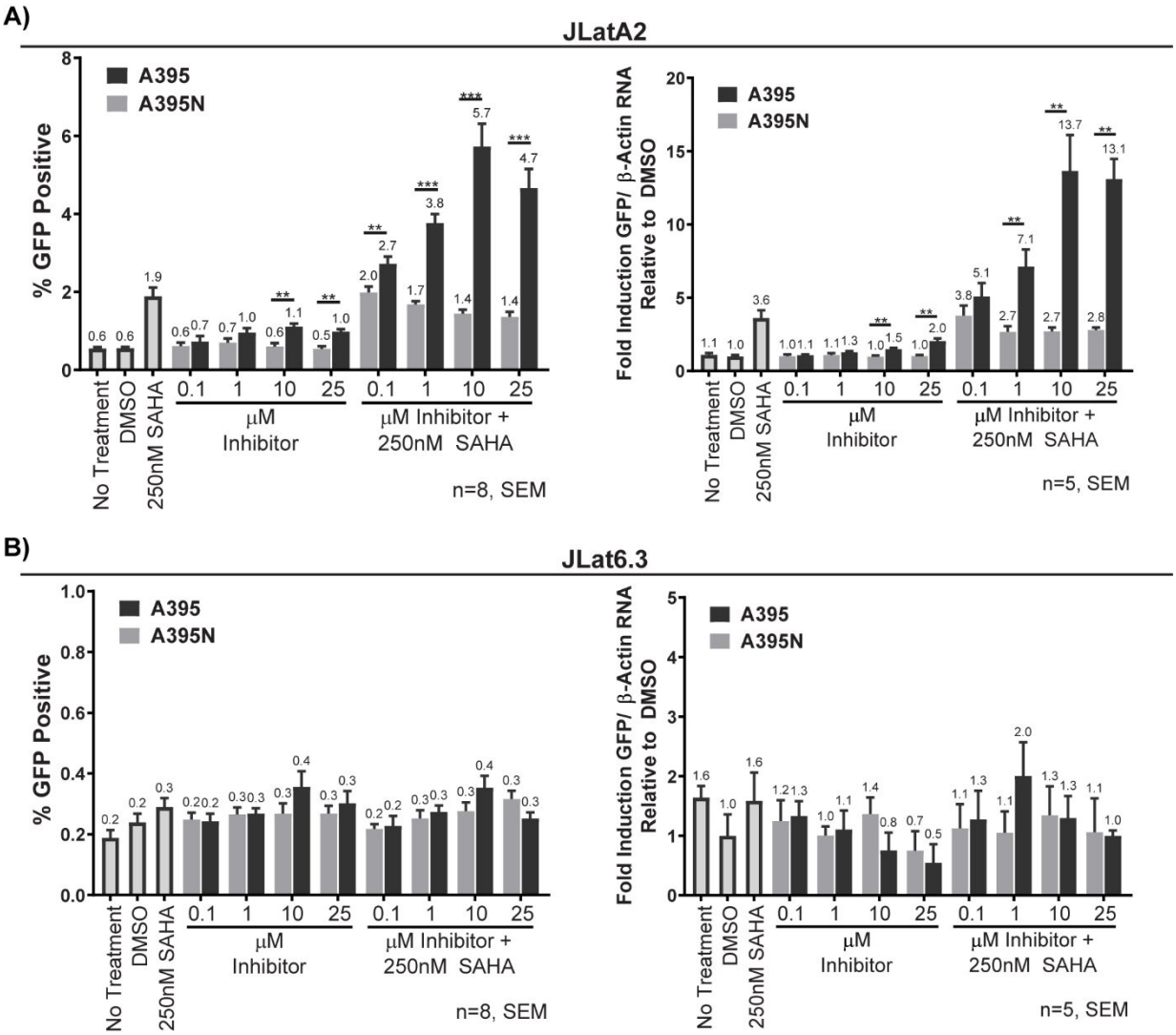


Figure S4. *Dose response of latency reactivation to A-395 in JLat Models* – Dose response for latency reactivation in JLatA2 **(A)** and JLat6.3 **(B)** cells in response to treatment with A-395 at both the protein (GFP) and RNA (GFP transcript) level. (* - p<0.05, ** - p<0.01, *** - p<0.001, Mann-Whitney Test)

Figure S5

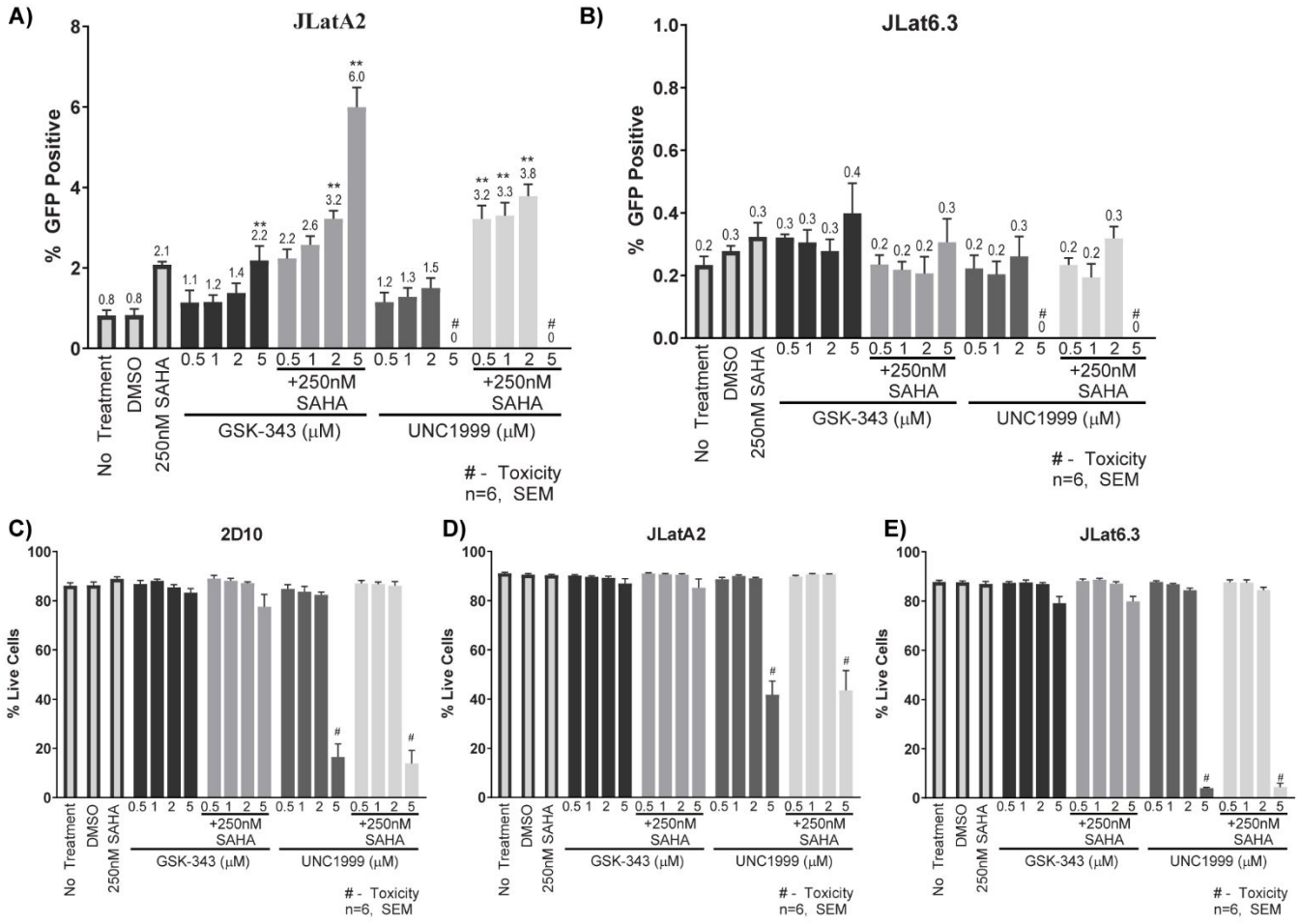


Figure S5. *Dose Response and Viability of Jurkat models when treated with EZH2i* – Dose response for latency reactivation as measured by GFP protein expression in JLatA2 **(A)** and JLat6.3 **(B)** cells in response to treatment with EZH2 inhibitors GSK343 and UNC1999 alone and in combination with SAHA (* - p<0.05, ** - p<0.01, *** - p<0.001, Mann-Whitney Test, single treatments relative to DMSO control and combo treatment relative to 250nM SAHA control). Cell viability was measured using a fixable live/dead stain and was assessed for all EZH2 inhibitor experiments by flow cytometry. Viability data shown for 2D10 **(C)**, JLatA2 **(D)**, and JLat6.3 **(E)** lines is associated with the experimental data provided in Figure 4 and this figure.

Figure S6

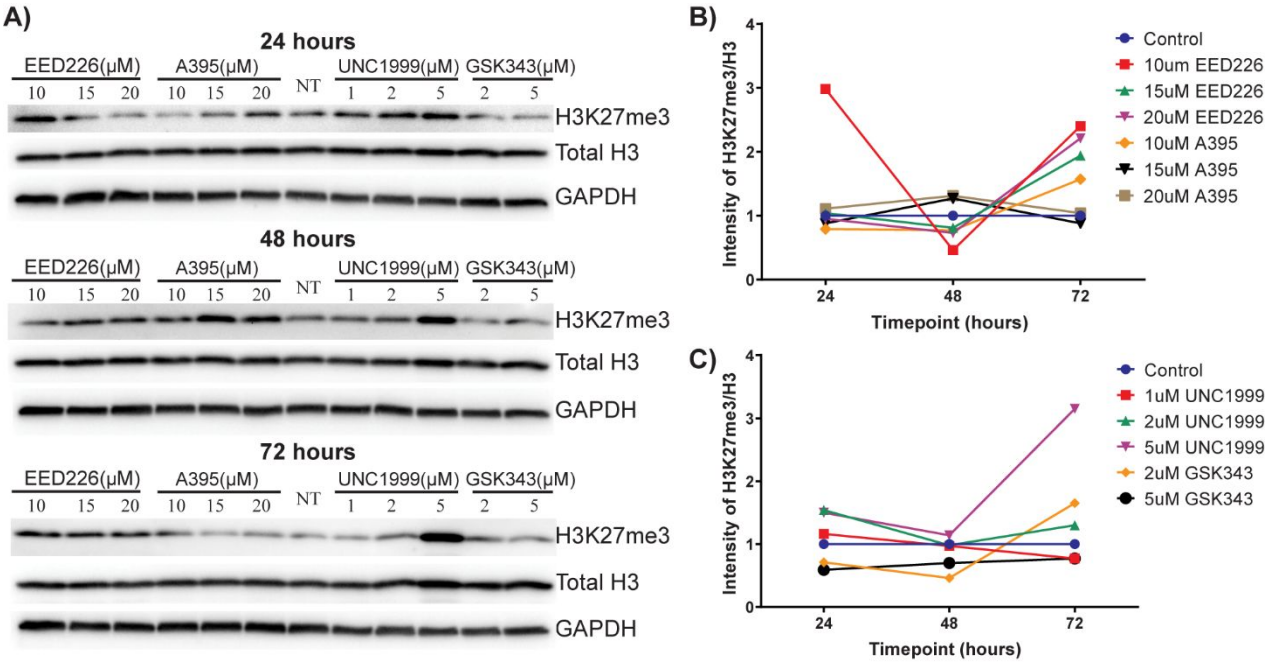
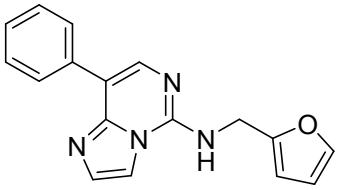


Figure S6. Effects of *EEDi on H3K27me3 levels in primary CD4+ T-cells* – **(A)** Western blot of global H3K27me3 levels in total CD4+ T-cells isolated from a second healthy donor treated with various concentrations of EEDi (A-395 and EED226) and EZH2i (UNC1999 and GSK343) for 24, 48, and 72hrs. **(B)** Quantification of global H3K27me3 levels relative to total histone H3 for tCD4 cells treated with EEDi for data shown in **(A)**. **(C)** Quantification of global H3K27me3 levels relative to total histone H3 for tCD4 cells treated with EZH2i for data shown in **(A)**.

Supplementary Experimental Section

General Chemistry Procedures

All reagents and solvents were obtained from commercial suppliers and were used without further purification unless otherwise stated. Thin layer chromatography was carried out using Merck silica plates coated with fluorescent indicator UV254 and analyzed under 254 nm UV light. Analytical LCMS data were acquired using an Agilent 6110 Series system with the UV detector set to 220 and 254 nm. Samples were injected (<10 µL) onto an Agilent Eclipse Plus 4.6 × 50 mm, 1.8 µm, C18 column at room temperature. Mobile phases A (H_2O + 0.1% acetic acid) and B (MeOH + 0.1% acetic acid) were used with a linear gradient from 10% to 100% B in 5.0 min, followed by a flush at 100% B for another 2 minutes with a flow rate of 1.0 mL/min. Mass spectra (MS) data were acquired in positive ion mode using an Agilent 6110 single quadrupole mass spectrometer with an electrospray ionization (ESI) source. Normal phase column chromatography was performed with a Teledyne ISCO CombiFlash®R_f 200 using RediSep®R_f SILICA columns with the UV detector set to 254 nm and 280 nm. ^{1}H and ^{13}C NMR spectra were obtained on a Bruker AV 400 at 400 MHz, 101 MHz respectively. Chemical shifts are reported in ppm and coupling constants are reported in Hz with $CDCl_3$ referenced at 7.26 (^{1}H) and 77.1 ppm (^{13}C). All compounds that were evaluated in biochemical and biophysical assays had >95% purity as determined by LCMS and ^{1}H and NMR.



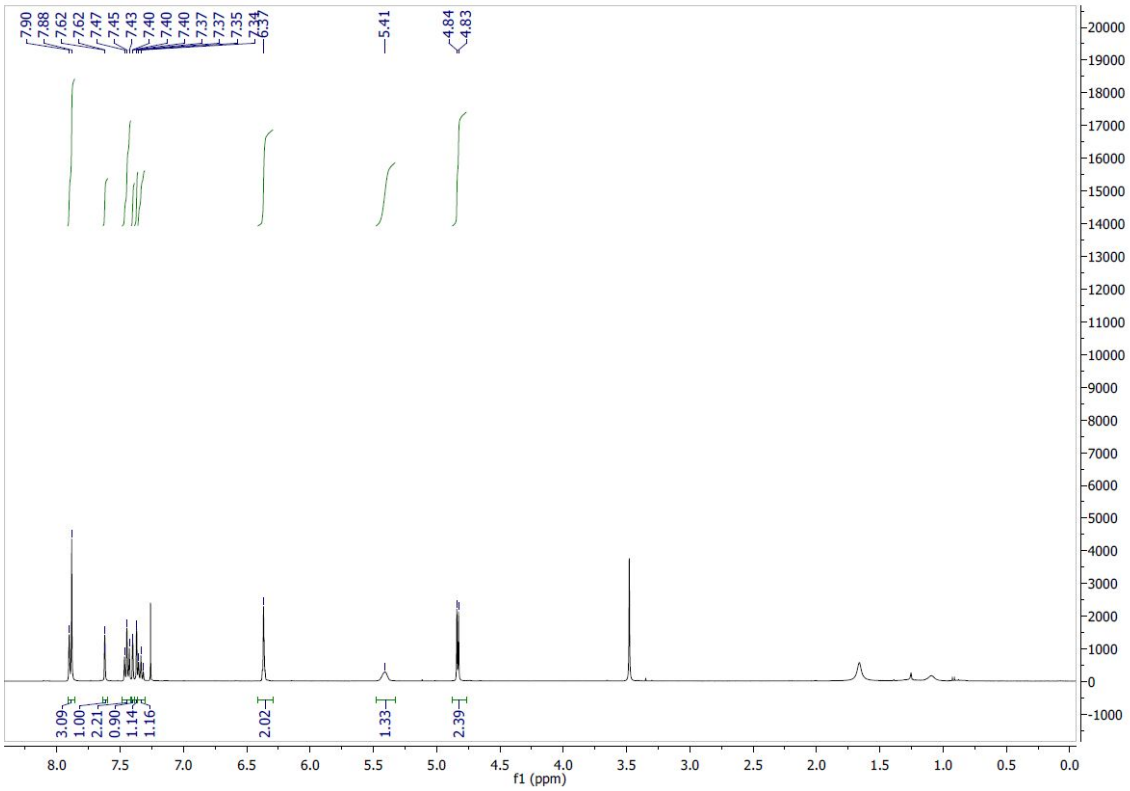
UNC5679 (*N*-(furan-2-ylmethyl)-8-phenylimidazo[1,2-*c*]pyrimidin-5-amine) was synthesized as previously reported [1] to yield the desired product as a white solid (6.6 mg).

^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.91-7.87 (m, 3H), 7.63-7.61 (m, 1H), 7.45 (t, *J* = 7.6 Hz, 2H), 7.41-7.39 (m, 1H), 7.38-7.36 (m, 1H), 7.34 (t, *J* = 7.4 Hz, 1H), 6.38-6.34 (m, 2H), 5.41 (s, 1H), 4.83 (d, *J* = 5.4 Hz, 2H).

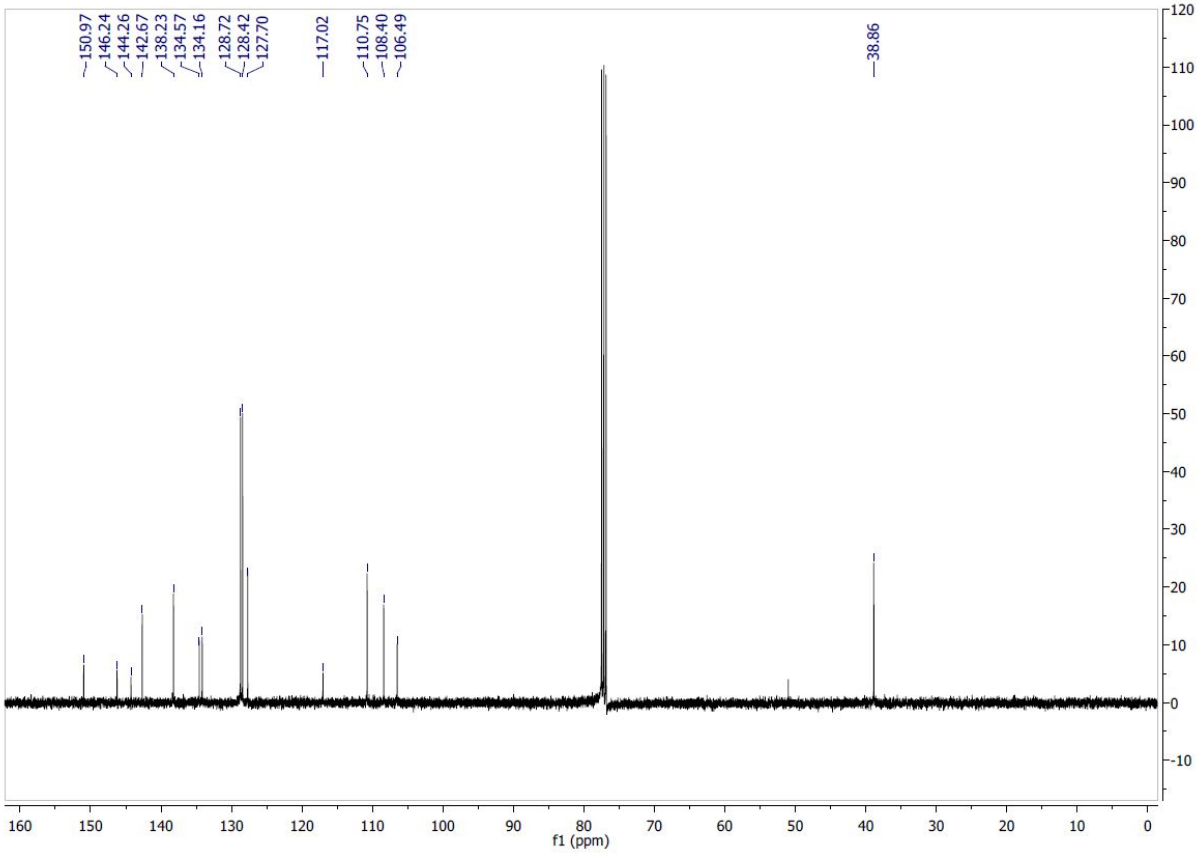
^{13}C NMR ($CDCl_3$, 101 MHz): δ 151.0, 146.2, 144.3, 142.7, 138.2, 134.6, 134.2, 128.7, 128.4, 127.7, 117.0, 110.8, 108.4, 106.5, 38.9.

MS (ESI): $C_{17}H_{14}N_4O$ + H expected: 291.12; $[M+H]^+$ found: 291.15.

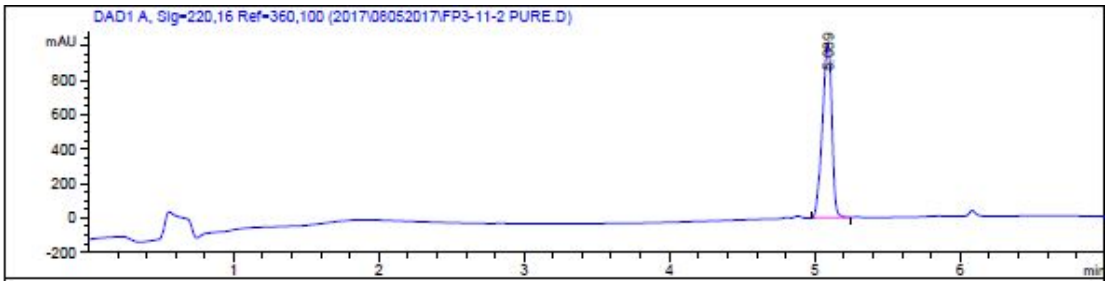
UNC5679 ^{1}H NMR spectra



UNC5679 ^{13}C NMR spectra



UNC5679 LCMS trace at 220 nm



Supplementary References

(1) Huang, Y., Zhang, J., Yu, Z., Zhang, H., Wang, Y., Lingel, A., Qi, W., Gu, J., Zhao, K., Shultz, M. D., Wang, L., Fu, X., Sun, Y., Zhang, Q., Jiang, X., Zhang, J., Zhang, C., Li, L., Zeng, J., Feng, L., Zhang, C., Liu, Y., Zhang, M., Zhang, L., Zhao, M., Gao, Z., Liu, X., Fang, D., Guo, H., Mi, Y., Gabriel, T., Dillon, M. P., Atadja, P., Oyang, C. Discovery of First-in-Class, Potent, and Orally Bioavailable Embryonic Ectoderm Development (EED) Inhibitor with Robust Anticancer Efficacy. J. Med. Chem. (2017). 60, 2215-2226. doi:10.1021/acs.jmedchem.6b01576