

## Supporting Information

### Discovery of Selective Small Molecule Inhibitors for the ENL YEATS Domain

Xinyu R. Ma,<sup>1,7</sup> Longxia Xu,<sup>2,7</sup> Shiqing Xu,<sup>1</sup> Brianna J. Klein,<sup>3</sup> Hongkuan Wang,<sup>2</sup> Sukant Das,<sup>1</sup> Kuai Li,<sup>2</sup> Kai S. Yang,<sup>1</sup> Sana Sohail,<sup>2</sup> Andrew Chapman,<sup>1</sup> Tatiana G. Kutateladze,<sup>3</sup> Xiaobing Shi,<sup>2</sup> Wenshe Ray Liu,<sup>1,4,5,6,\*</sup> and Hong Wen<sup>2,\*</sup>

<sup>1</sup>Texas A&M Drug Discovery Laboratory, Department of Chemistry, Texas A&M University, College Station, TX 77843, USA

<sup>2</sup>Department of Epigenetics, Van Andel Institute, Grand Rapids, MI, 49503, USA

<sup>3</sup>Department of Pharmacology, University of Colorado School of Medicine, Aurora, CO 80045, USA

<sup>4</sup>Institute of Biosciences and Technology and Department of Translational Medical Sciences, College of Medicine, Texas A&M University, Houston, TX 77030, USA

<sup>5</sup>Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA

<sup>6</sup>Department of Molecular and Cellular Medicine, College of Medicine, Texas A&M University, College Station, TX 77843, USA

<sup>7</sup>These authors contributed equally

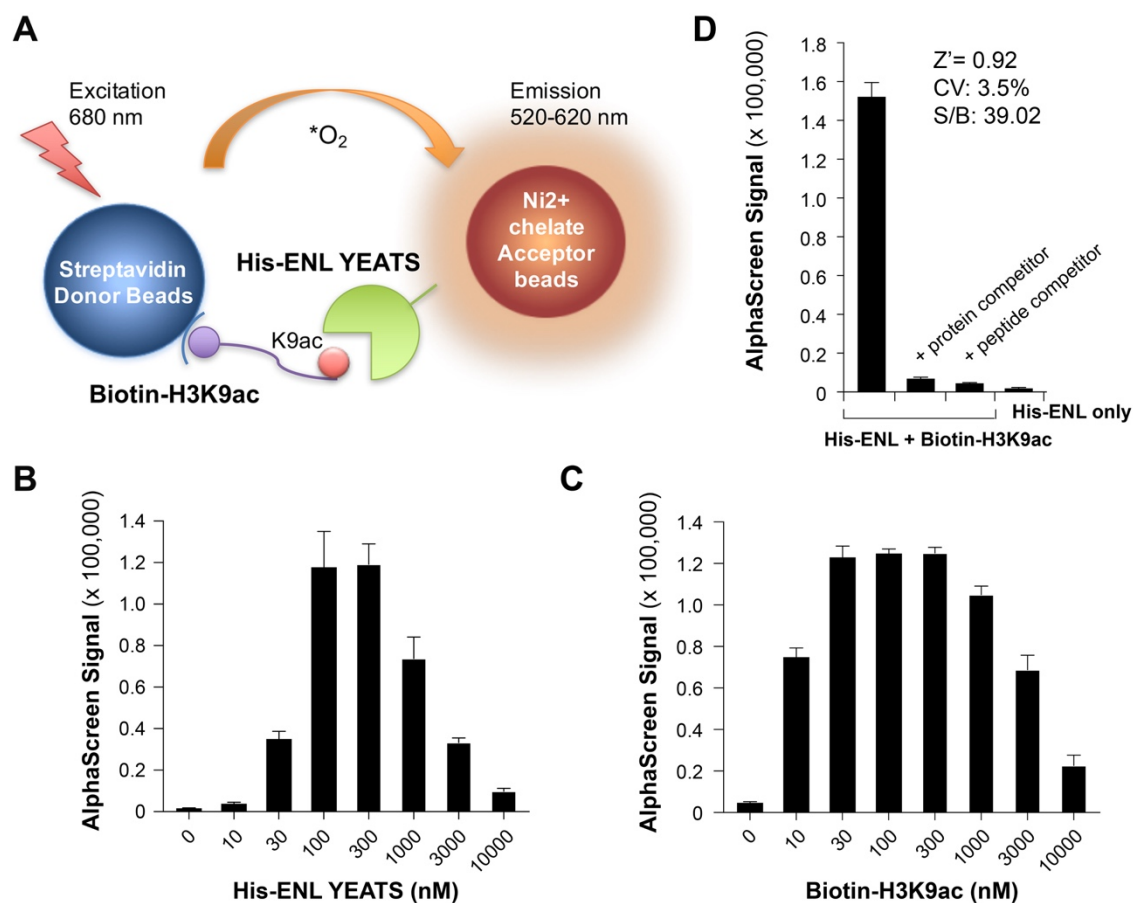
\*Correspondence: wliu@chem.tamu.edu (W.R.L), Hong.Wen@vai.org (H.W.)

---

#### Content of Supporting Information

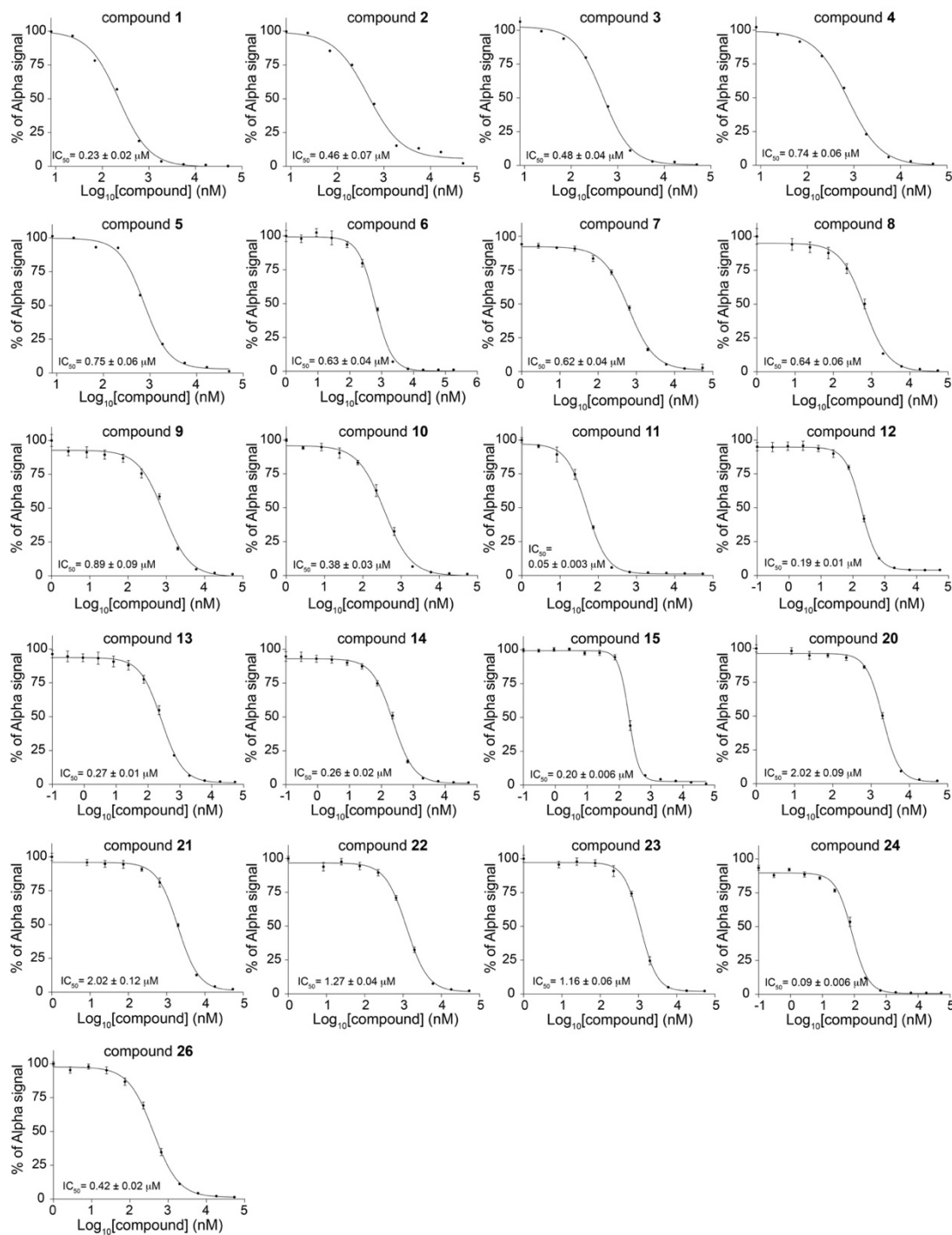
---

	Pages
<b>Figure S1.</b> Development of an AlphaScreen assay detecting the interaction between His-ENL YEATS domain and biotinylated H3K9ac peptide.	S2
<b>Figure S2.</b> IC <sub>50</sub> determination of compounds <b>1-15</b> , <b>20-24</b> and <b>26</b> by AlphaScreen assay.	S3
<b>Figure S3.</b> SPR and NMR analysis of compound <b>11</b> , <b>24</b> or <b>7</b> .	S4
<b>Figure S4.</b> ENL inhibition by compound <b>7</b> in <i>MLL</i> -rearranged leukemia cells.	S5
<b>Figure S5.</b> The triazolopyridine pharmacophore of compounds <b>1</b> , <b>7</b> , <b>11</b> and <b>24</b> adopt conformations to form stronger pi-pi interactions with H56 residue in ENL than in AF9 YEATS domain.	S6
<b>Figure S6.</b> HPLC chromatograph, <sup>1</sup> H-NMR and <sup>13</sup> C-NMR of compound <b>7</b> .	S7
<b>Figure S7.</b> HPLC chromatograph, <sup>1</sup> H-NMR and <sup>13</sup> C-NMR of compound <b>11</b> .	S8
<b>Figure S8.</b> HPLC chromatograph, <sup>1</sup> H-NMR and <sup>13</sup> C-NMR of compound <b>12</b> .	S9
<b>Figure S9.</b> HPLC chromatograph, <sup>1</sup> H-NMR and <sup>13</sup> C-NMR of compound <b>24</b> .	S10
<b>Table S1.</b> Structure and IC <sub>50</sub> of compounds from HTS with IC <sub>50</sub> below 5 $\mu$ M.	S11-12
<b>Molecular Formula Strings</b>	uploaded
<b>PDB files of Docking Models</b>	uploaded

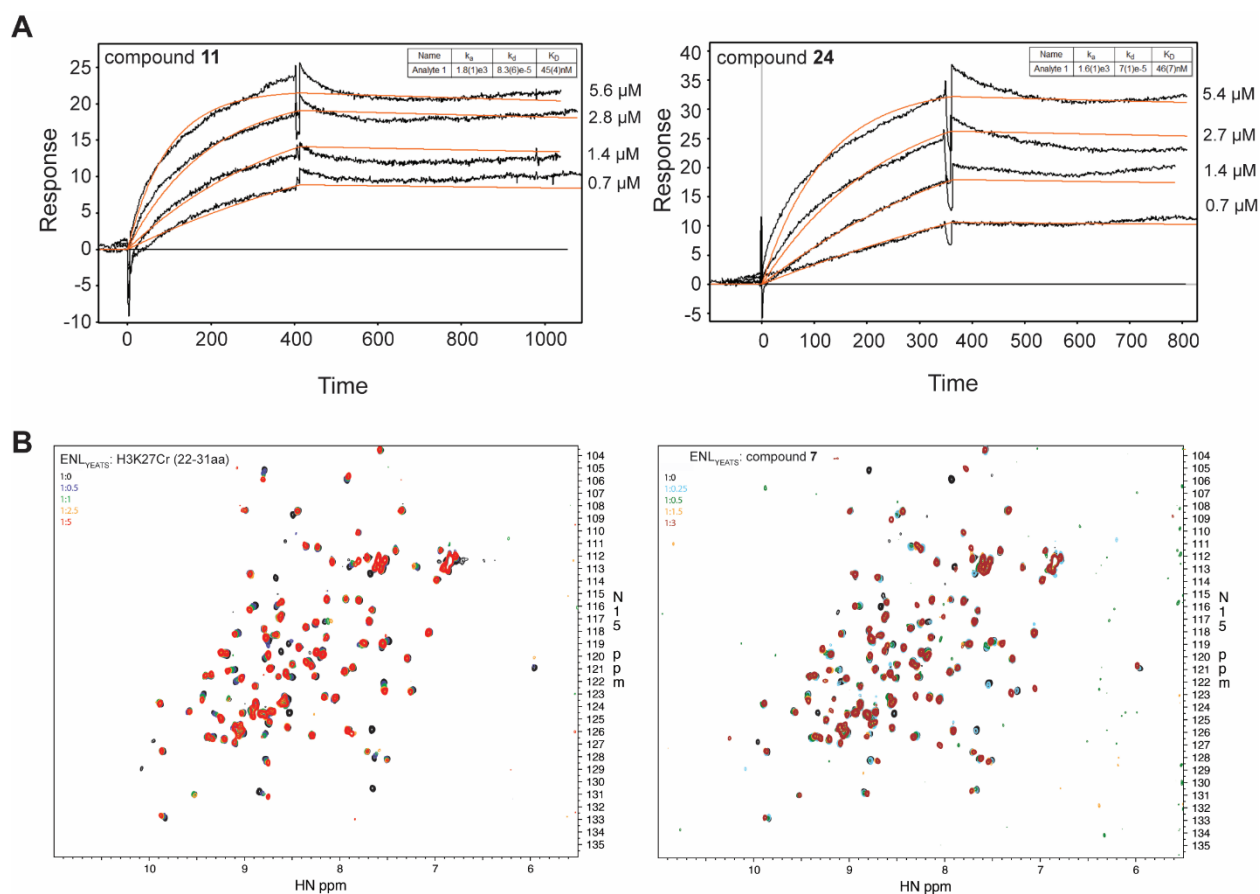


**Figure S1. Development of an AlphaScreen assay detecting the interaction between His-ENL YEATS domain and biotinylated H3K9ac peptide.**

(A) A schematic representation of the developed AlphaScreen assay. (B) Alpha signals when different concentrations of His-ENL YEATS were titrated into 30 nM of H3K9ac peptide. (C) Alpha signals when different concentrations of the H3K9ac peptide were titrated into 100 nM of His-ENL YEATS. (D) The developed AlphaScreen assay produces robust and highly reproducible signals in the detection of the interaction between His-ENL YEATS and H3K9ac peptide. Data in B-D represent mean  $\pm$  SEM,  $n \geq 4$  in B and C, and  $n = 192$  in D.

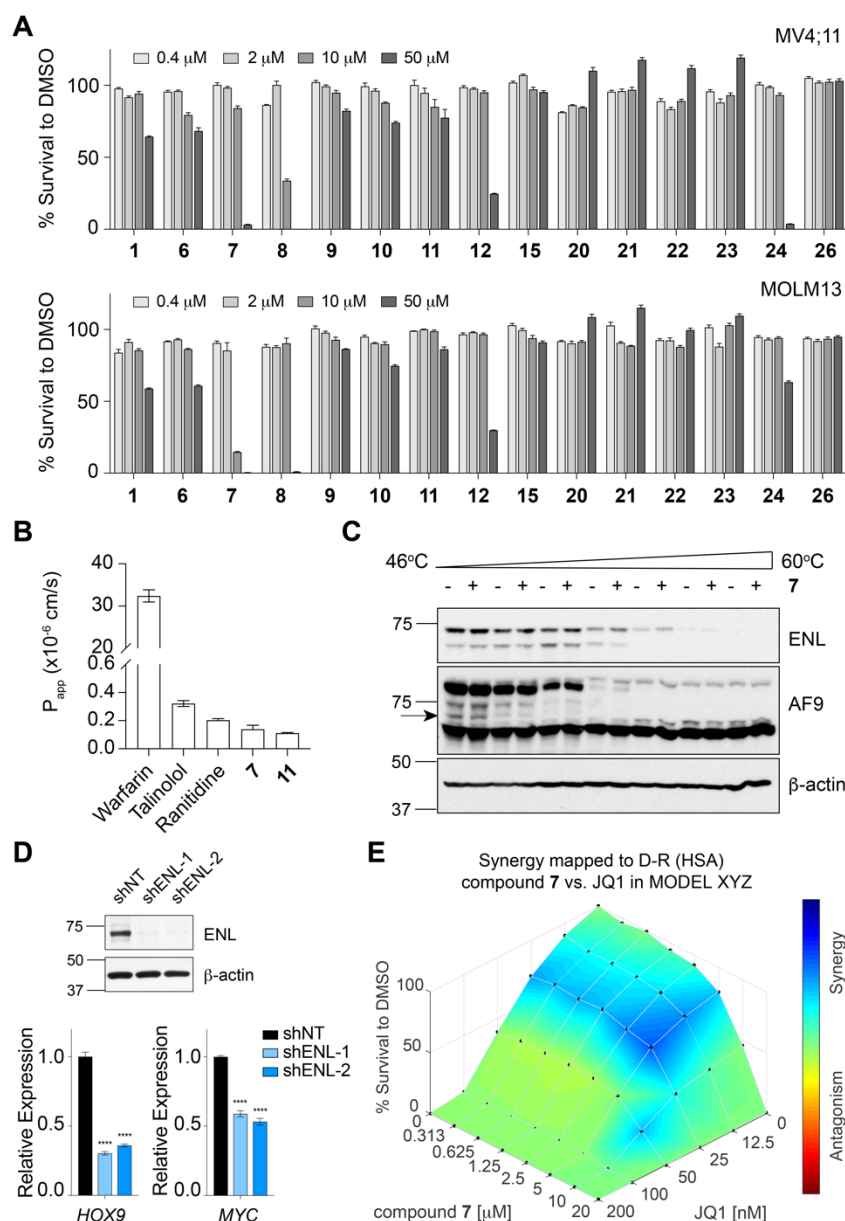


**Figure S2. IC<sub>50</sub> determination of compounds 1-15, 20-24 and 26 by AlphaScreen assay.** Compounds were subjected to a series of 3-fold dilutions from 54 μM for dose response curve AlphaScreen assays. IC<sub>50</sub> values were determined from the plot using nonlinear regression of variable slope (four parameters) and curve fitting performed by the GraphPad Prism software. Error bars show ± SEM, n ≥ 4.



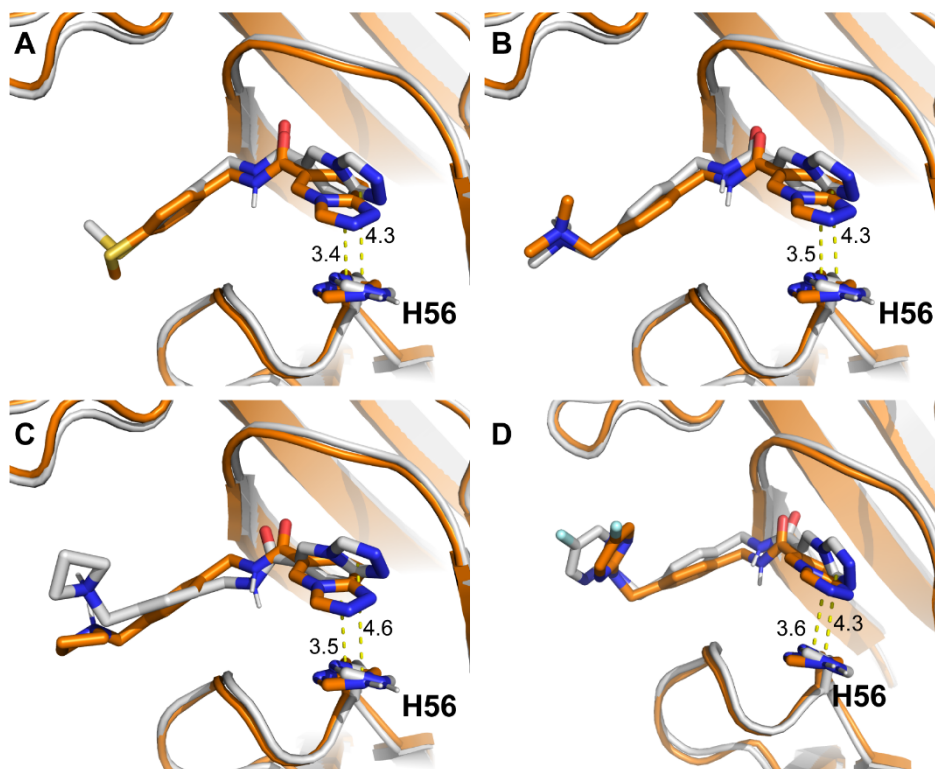
**Figure S3. SPR and NMR analysis of compound 11, 24 or 7.**

**(A)** Sensorgrams of SPR experiments and the fitted Langmuir 1:1 binding kinetic model with compound **11** (left panel) and **24** (right panel). **(B)** Overlay of  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC spectra of  $^{15}\text{N}$ -labeled ENL YEATS domain collected before and after the H3K27cr (aa 22-31 of H3) peptide (left panel) or compound **7** (right panel) was added stepwise. Spectra are color coded according to the protein-peptide molar ratio as indicated.



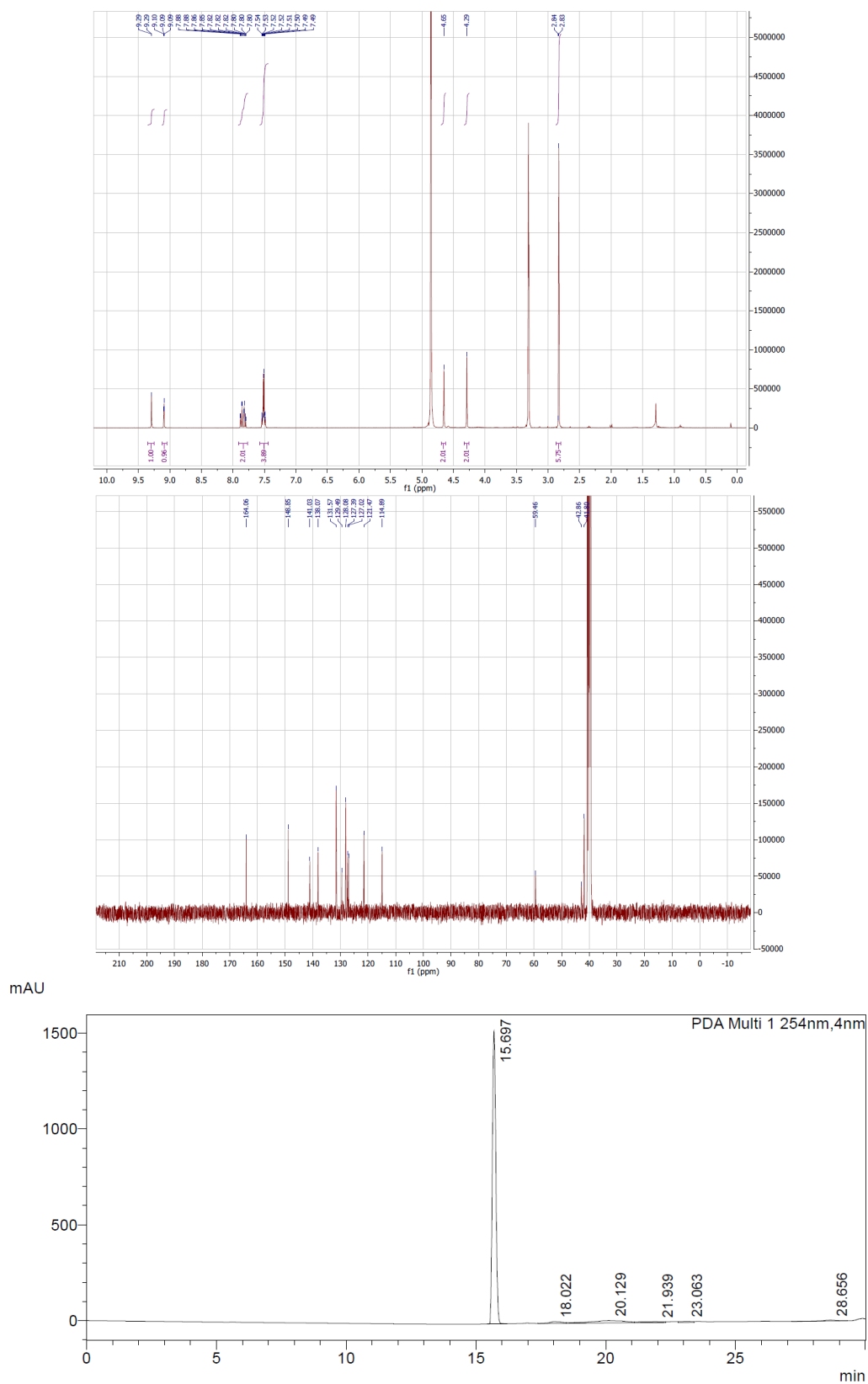
**Figure S4. ENL inhibition by compound 7 in *MLL*-rearranged leukemia cells.**

(A) Cell growth inhibition of ENL inhibitors at the indicated concentrations in MV4;11 and MOLM13 cells. Survived cells were calculated as % relative to DMSO treated cells. Data represent mean  $\pm$  SEM,  $n = 3$ . (B) Caco-2 cell permeability analysis of compound 7 and 11. Warfarin, Talinolol and Ranitidine are control compounds with varied permeability rates used for comparisons by the Charles River Laboratory. Data represent mean  $\pm$  SEM,  $n \geq 2$ . (C) CETSA in HeLa cells treated with 20  $\mu$ M compound 7 at the indicated temperatures. (D) qRT-PCR analysis of *HOXA9* and *MYC* gene expression in ENL knockdown MOLM13 cells. Data represent mean  $\pm$  SEM ( $n = 3$ ), two-tailed Student's  $t$  test. \*\*\*\*  $P < 0.0001$ . Western blot shows efficient knockdown of ENL. (E) 7 shows a synergistic effect with JQ1 in MV4;11 cells. Cells were treated with indicated doses of 7 and JQ1 or DMSO for 6 days.



**Figure S5.** The triazolopyridine pharmacophore of compounds **1**, **7**, **11** and **24** adopt conformations to form stronger pi-pi interactions with H56 residue in ENL than in AF9 YEATS domain.

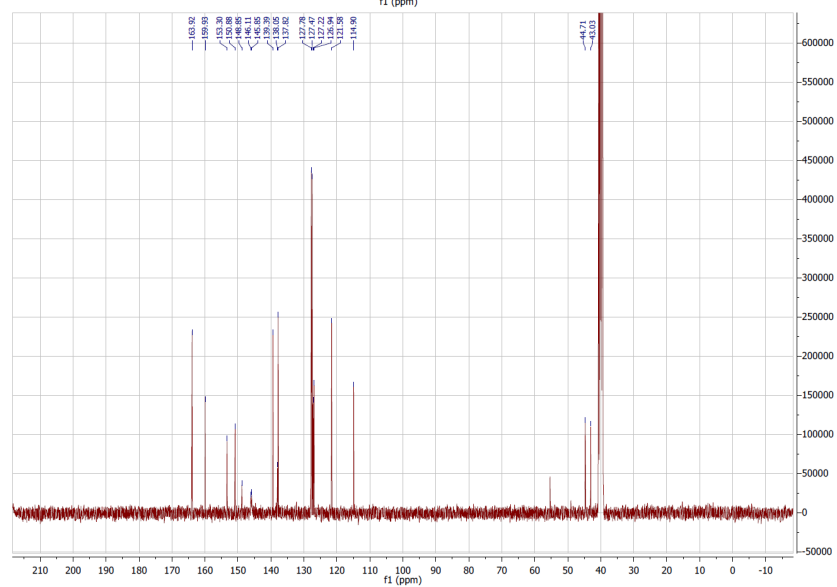
The molecular docking models comparison of compounds **1** (A), **7** (B), **11** (C), and **24** (D) bound to the YEATS domain of AF9 (white colored) and ENL (orange colored). Modeling was based on the PDB entries 5j9s (ENL) and 4tmp (AF9).



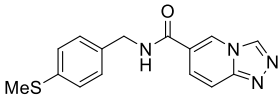
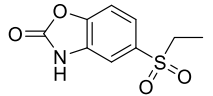
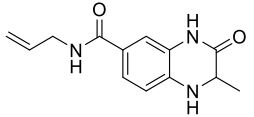
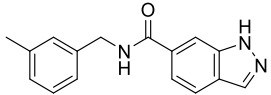
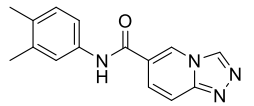
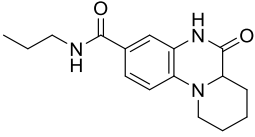
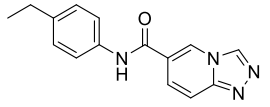
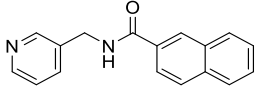
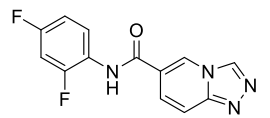
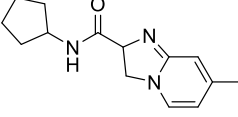
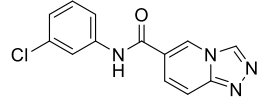
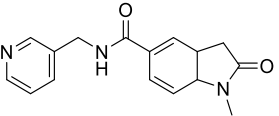
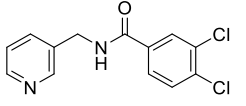
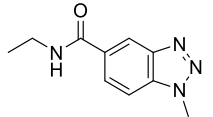
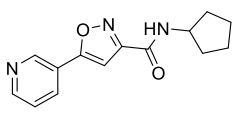
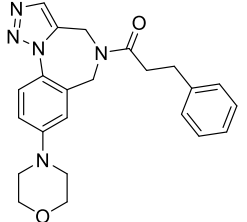
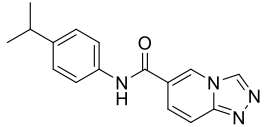
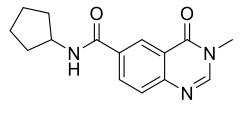
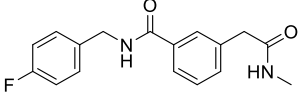
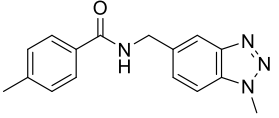
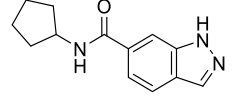
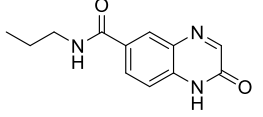


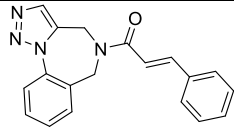
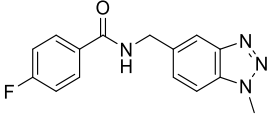
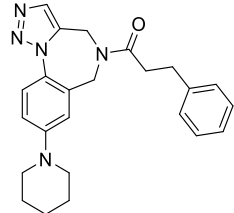
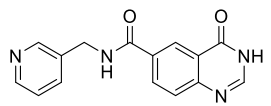
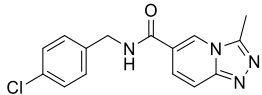
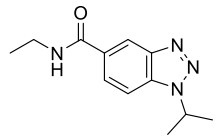
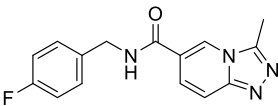
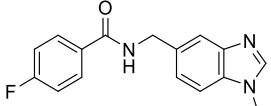
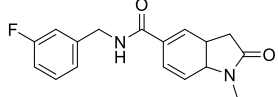
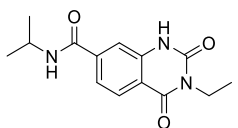
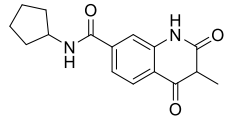
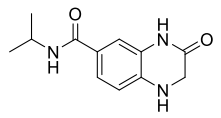
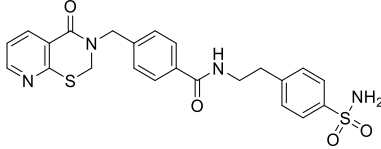
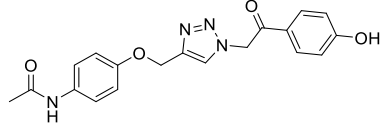
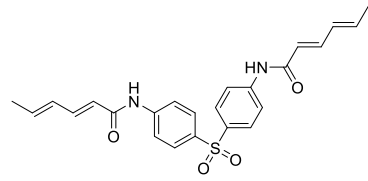






**Table S1. Structure and IC<sub>50</sub> of compounds from HTS with IC<sub>50</sub> below 5 μM.**

Structure	IC <sub>50</sub> (μM)	Structure	IC <sub>50</sub> (μM)
	0.23 ± 0.02		2.57 ± 1.88
	0.39 ± 0.04		2.57 ± 0.25
	0.46 ± 0.07		2.63 ± 0.92
	0.48 ± 0.04		2.68 ± 0.18
	0.74 ± 0.06		2.76 ± 0.44
	0.75 ± 0.06		3.34 ± 0.20
	0.80 ± 0.09		3.32 ± 0.40
	0.99 ± 0.15		3.52 ± 0.21
	1.02 ± 0.08		3.56 ± 0.75
	1.32 ± 0.11		3.62 ± 0.37
	1.33 ± 0.10		3.74 ± 0.27

	$1.34 \pm 0.07$		$3.81 \pm 0.46$
	$1.51 \pm 0.13$		$1.83 \pm 0.22$
	$1.60 \pm 0.26$		$4.86 \pm 0.56$
	$1.93 \pm 0.30$		$4.89 \pm 0.45$
	$1.68 \pm 0.21$		$4.89 \pm 0.48$
	$1.80 \pm 0.11$		$1.96 \pm 0.21$
		$2.02 \pm 0.37$	
		$4.40 \pm 1.26$	
		$1.61 \pm 0.10$	