# Discovery of a selective inhibitor for the YEATS domains of ENL/AF9

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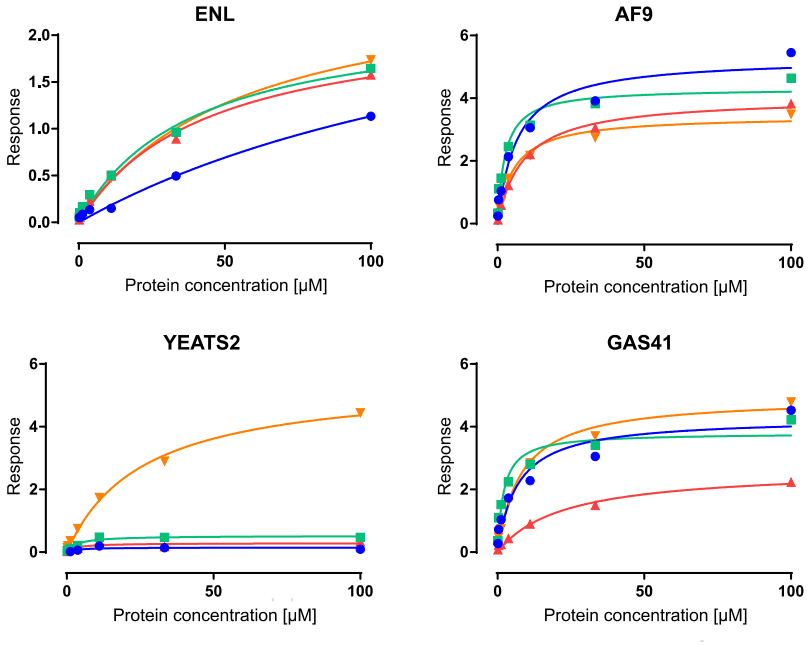
## Supplementary Figures:




$K_d$ in µM

| | H3K9ac | H3K9cro | H3K18ac | H3K27cro |
|---|---|---|---|---|
| MLLT1 | 90.9 | 10.0 | 8.1 | 9.1 |
| MLLT3 | 2.0 | 0.7 | 1.4 | 1.3 |
| YEATS2 | N/D | N/D | N/D | 3.6 |
| YEATS4 | 2.9 | 0.7 | 4.9 | 1.3 |

**Figure S1:** Affinity of the YEATS domains of ENL, AF9, YEATS2 and GAS41 for histone peptides determined with biolayer interferometry.

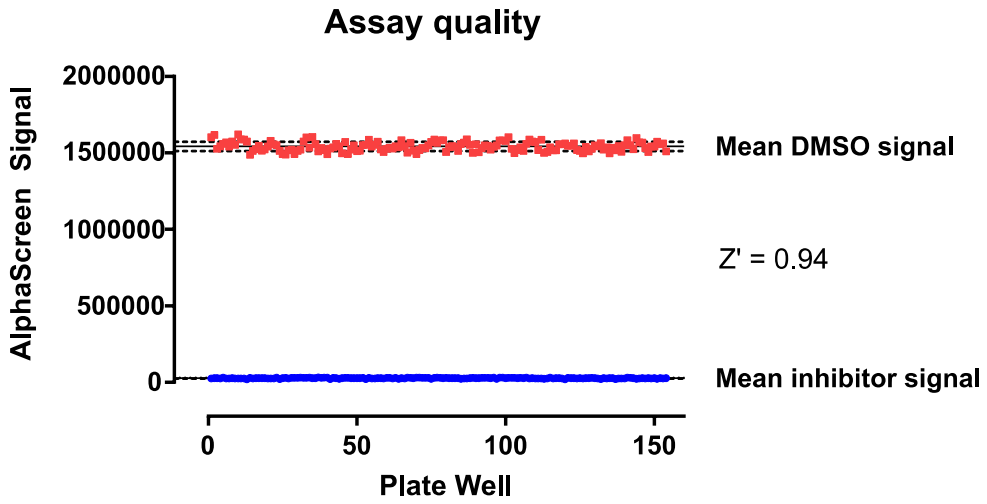



**Figure S2:** AlphaScreen® assay quality.

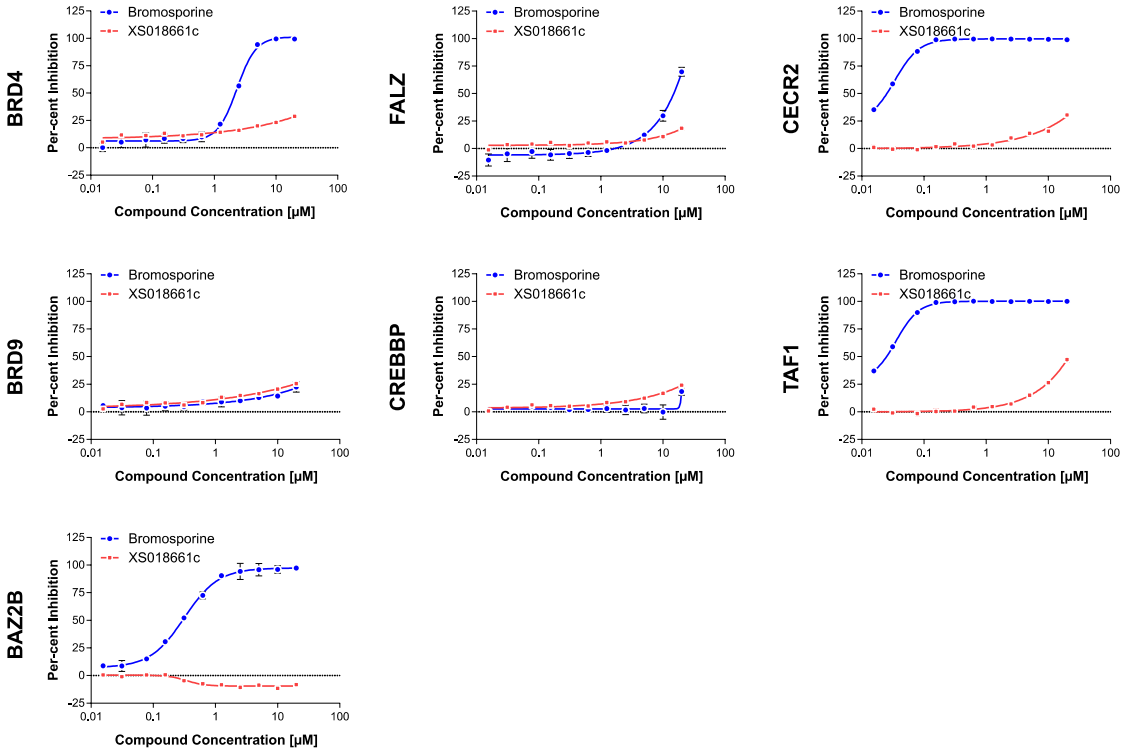



**Figure S3:** Dose response curves for XS018661 against selected bromodomains

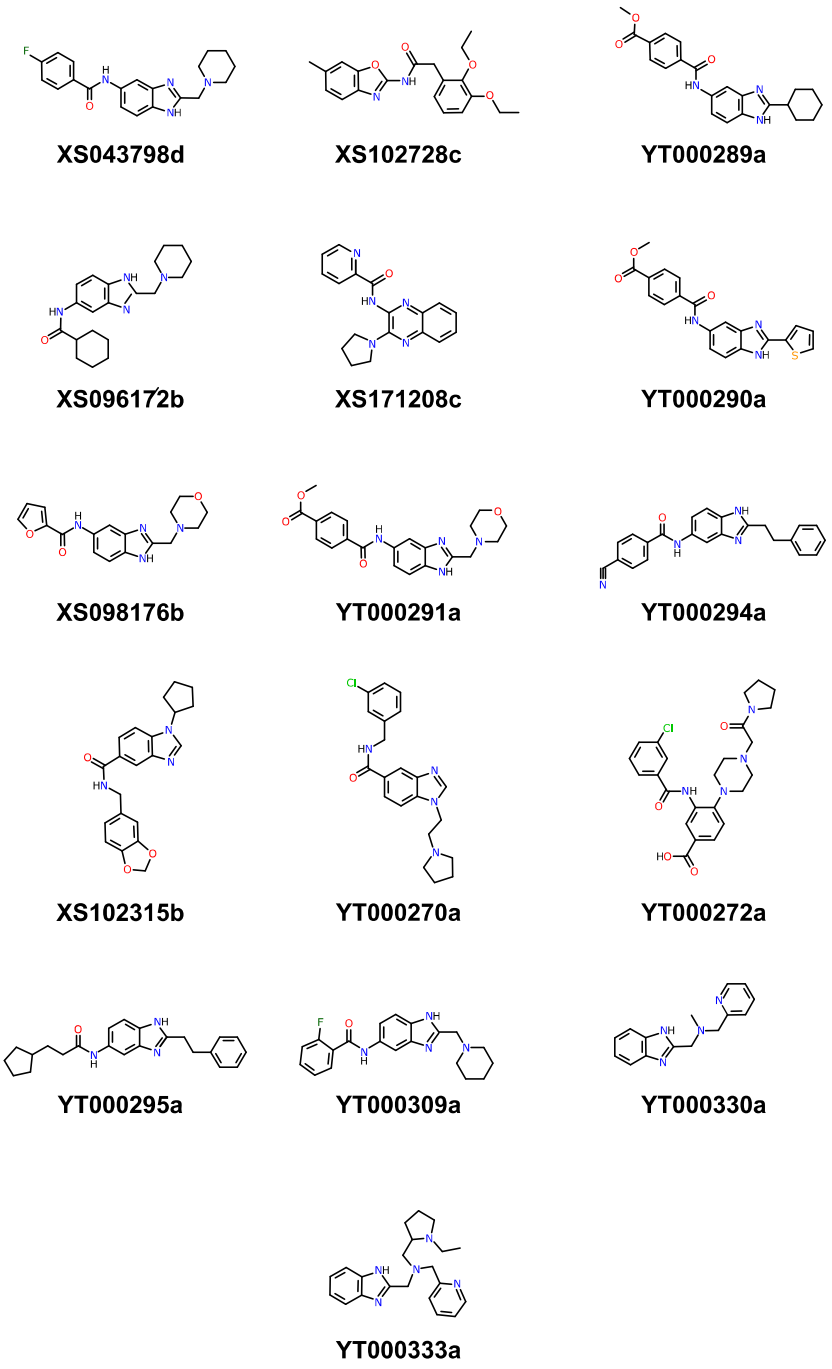



**Figure S4:** Structures of purchased follow up compounds

## Supplementary Tables:

**Table S1:** Constructs and vectors used

| Protein | Vector | Start | End | Hexahistidyl tag | Use |
|---|---|---|---|---|---|
| ENL | pET28a | D2 | M144 | N-terminal | AS |
| | pNIC-CH | M1 | A148 | C-terminal | AS, ITC, TM |
| AF9 | pNIC-Bsa4 | M1 | D141 | N-terminal | AS |
| | pNIC-CH | M1 | D141 | C-terminal | AS, ITC, TM |
| YEATS2 | pNIC-Bsa4 | S202 | E345 | N-terminal | AS, TM |
| GAS41 | pNIC-CH | V16 | K225 | C-terminal | AS, TM |

AS: AlphaScreen
ITC: Isothermal Titration Calorimetry
TM: Thermal shift assays

**Table S2:** DMSO effect on AlphaScreen® signal in peptide displacement assay. Signal of no-compound-addition wells ("buffer") expressed as per-cent signal of DMSO-only-addition wells (0.5 % v/v) is shown alongside the number of well pairs and number of corresponding plates.

| Protein | DMSO relative to buffer (%) | Well pairs | Assay plates |
|---|---|---|---|
| ENL | 96.1 ± 8.4 | 206 | 26 |
| AF9 | 98.1 ± 5.3 | 204 | 26 |
| YEATS2 | 94.6 ± 6.2 | 208 | 26 |
| GAS41 | 97.0 ± 5.8 | 207 | 26 |

**Table S3:** Number of replicate $IC_{50}$ experiments

| | Replicate experiments | | | |
|---|---|---|---|---|
| Compound | ENL | AF9 | YEATS2 | GAS41 |
| Bromosporine | 1 | 1 | 1 | 1 |
| XS018661 | 9 | 4 | 5 | 8 |
| XS003366 | 4 | 3 | 2 | 2 |
| XS009468 | 5 | 3 | 2 | 2 |
| XS003960 | 4 | 3 | 2 | 2 |
| XS009757 | 3 | 3 | 2 | 2 |
| XS166760 | 2 | 1 | 2 | 2 |
| XS180723 | 4 | 3 | 2 | 2 |
| XS000739 | 4 | 3 | 2 | 2 |
| XS043798 | 8 | 3 | 5 | 5 |
| XS096172 | 4 | 2 | 1 | 1 |
| XS098176 | 4 | 3 | 2 | 2 |
| XS102315 | 4 | 3 | 2 | 1 |
| XS102728 | 4 | 3 | 2 | 1 |
| XS171208 | 4 | 3 | 2 | 2 |
| YT000270 | 4 | 3 | 2 | 1 |
| YT000272 | 2 | 1 | 2 | 1 |
| YT000289 | 4 | 3 | 2 | 2 |
| YT000290 | 4 | 3 | 2 | 2 |
| YT000291 | 4 | 3 | 2 | 2 |
| YT000294 | 4 | 3 | 2 | 2 |
| YT000295 | 4 | 3 | 1 | 2 |
| YT000309 | 6 | 3 | 4 | 4 |
| YT000330 | 5 | 2 | 1 | 1 |
| YT000333 | 4 | 2 | 1 | 1 |