Supplementary Data

Screening for small molecule modulators of Long non-coding RNA - protein interactions using AlphaScreen®

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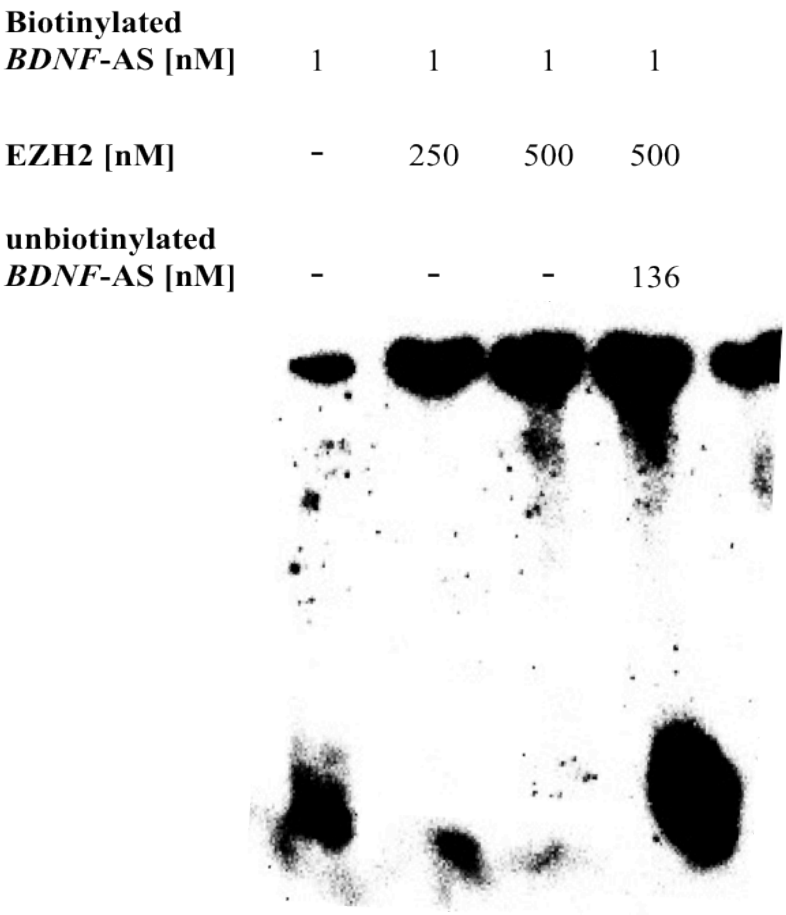
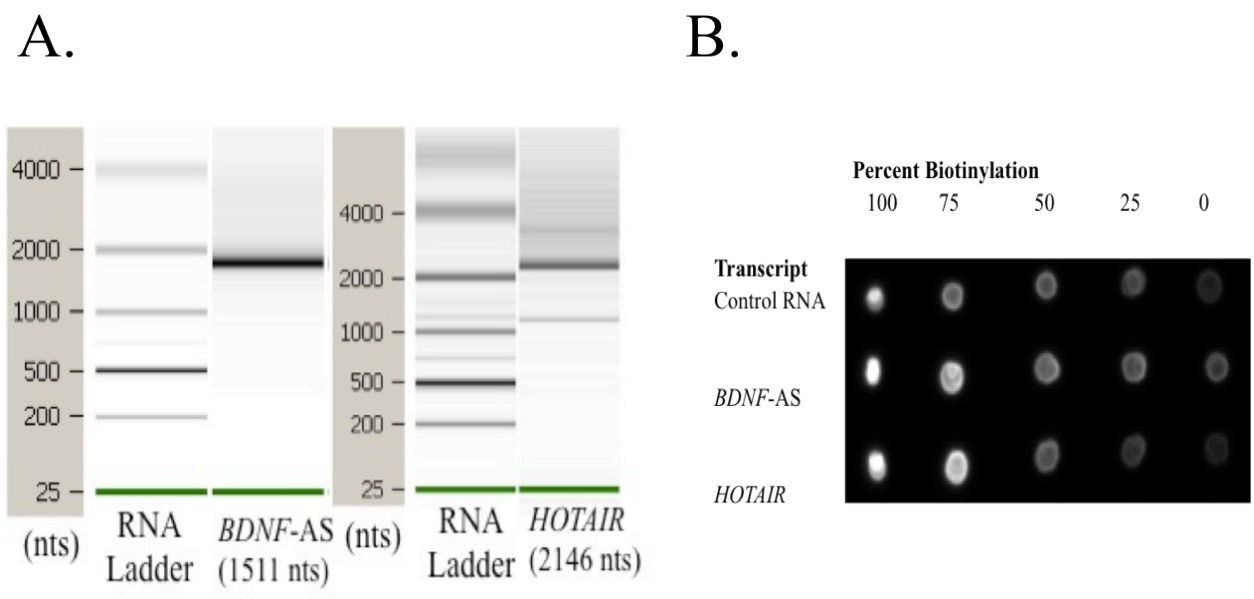
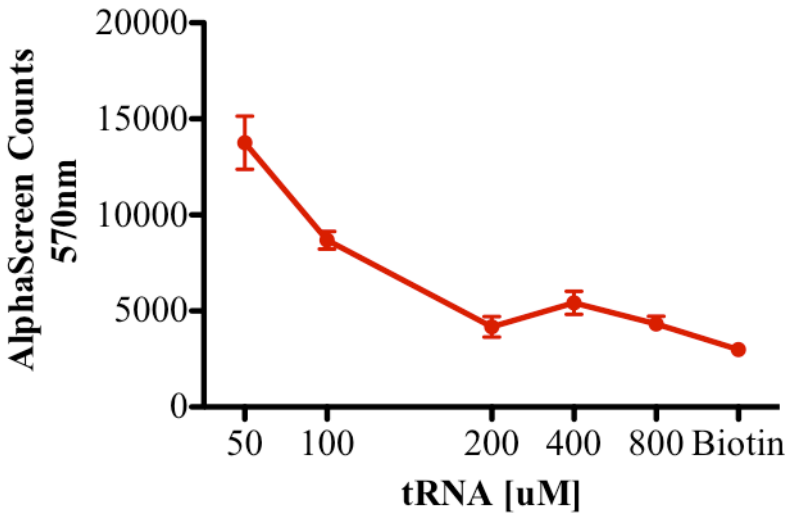


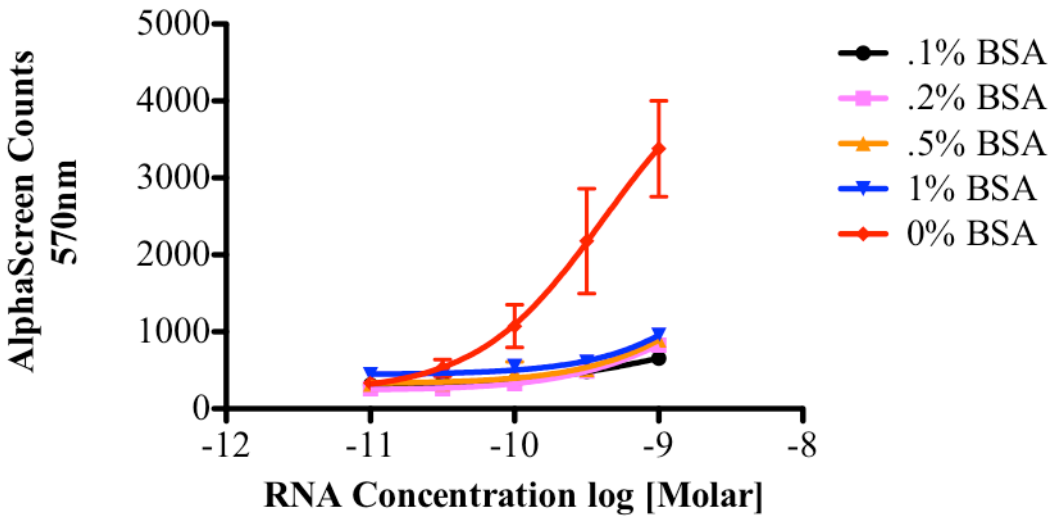
Supplementary Figure 1. RNA EMSA shows that BDNF-AS interacts directly with EZH2. Lane 1 (left) shows the migration of free biotinylated *BDNF*-AS. This migration is markedly reduced upon addition of increasing concentrations of EZH2 protein (lanes 2 and 3). The effect of EZH2 on migration of biotinylated *BDNF*-AS is reversed upon addition of unbiotinylated *BDNF*-AS (lane 4, right).



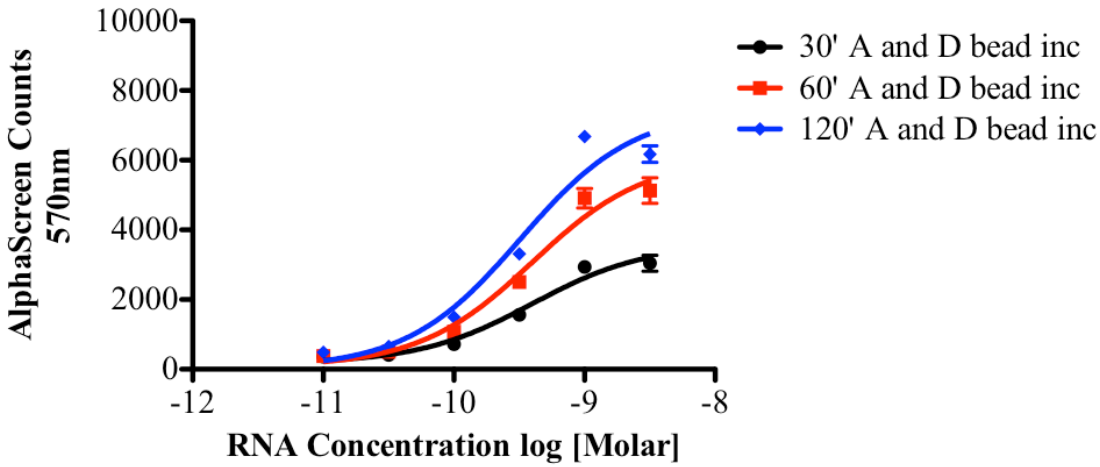
Supplementary figure 2. Confirmation of RNA and protein products used for assay development. (A) Agilent RNA 6000 Nano kit bioanalyzer results confirming the size, concentration and purity of RNAs in vitro transcribed RNAs studied. (B) Dot blot following in vitro transcription of RNAs confirms the presence of biotin tag on synthesized long non-coding RNAs.



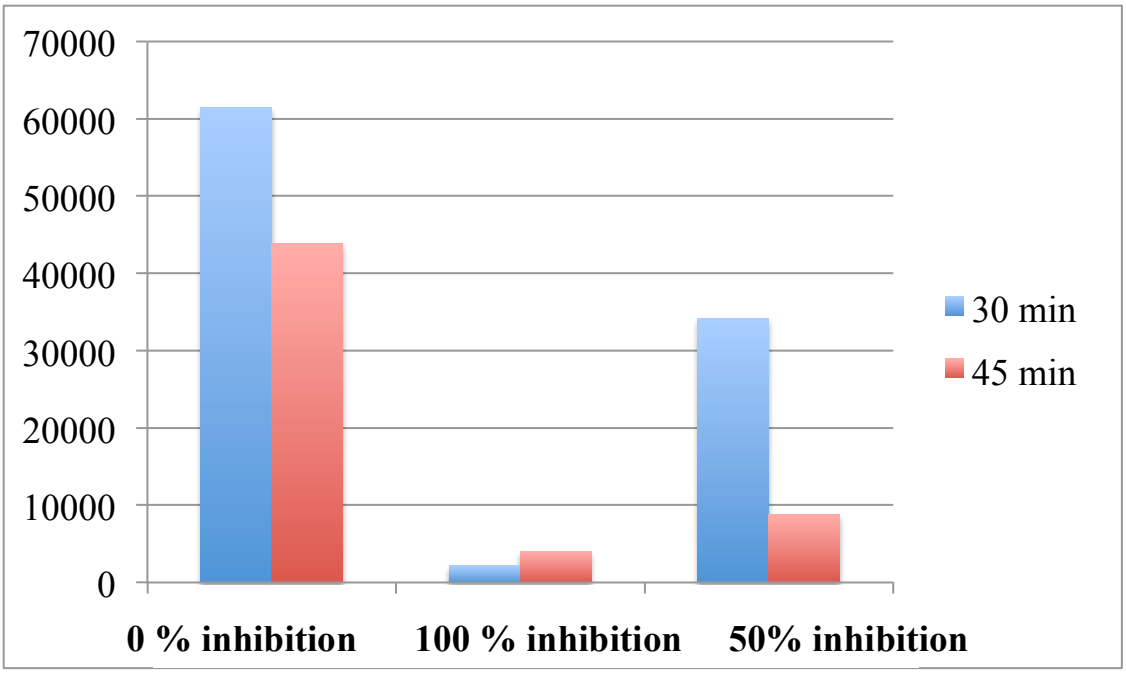
Supplementary figure 3. Assay optimization: *E. coli* tRNA concentration. The tRNA concentration in the assay buffer was titrated and tested with EZH2 [4 nM] and *BDNF*-AS [0.3 nM]. A final tRNA concentration of 50 μM was used for future testing.



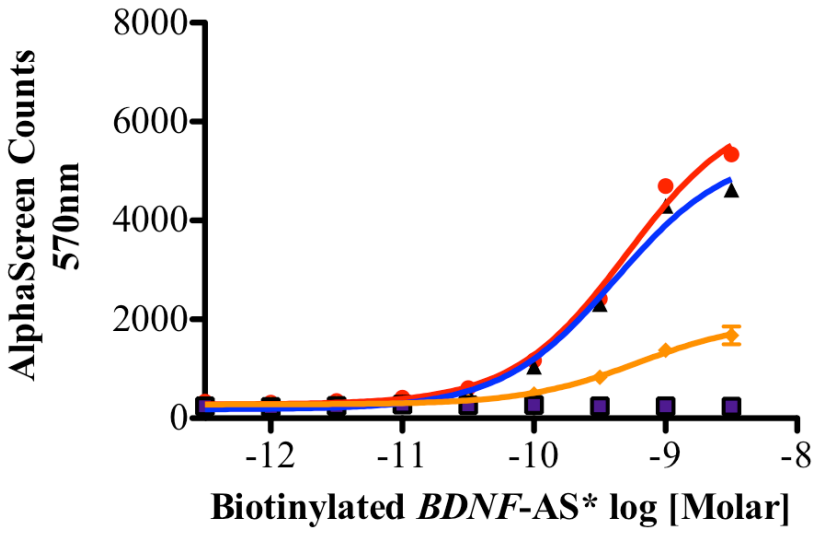
Supplementary figure 4. Assay optimization: bovine serum albumin (BSA). A range of BSA concentrations were tested in the assay buffer to determine optimal concentration of this nonspecific blocking agent. EZH2 protein [4 nM] is purified and not from a cell lysate; the addition of any BSA completely inhibited the assay signal.



Supplementary figure 5. Assay optimization: AlphaScreen bead incubation time. *BDNF*-AS was titrated into EZH2 [4 nM] and following a 30 minute incubation, AlphaScreen acceptor (A) and donor (D) beads were added simultaneously (10 μg/ml) to each well, before incubation for 30, 60 or 120 minutes at room temperature. Two hour bead incubations produced the optimal assay signal and this condition was used for future experiments.



Supplementary Figure 6. Assay optimization: RNA-protein incubation times. RNA-protein incubation times were tested at 30 and 45 minutes to determine which incubation time produced maximal signal counts in our AlphaScreen assay. Conditions tested include: 0% inhibition (*BDNF*-AS [0.3 nM], EZH2 [4 nM] in 0.1% DMSO), 100% inhibition (*BDNF*-AS [0.3 nM], EZH2 [4 nM], 10 μM biotin), and 50 % inhibition (*BDNF*-AS [0.3 nM], EZH2 [4 nM], 10 μM ellipticine). Thirty minute RNA-protein interactions were used for future experiments due to the high signal to noise ratio observed.



Supplementary figure 7. Biotinylated *BDNF*-AS was titrated in 4 nM fixed final concentration of EZH2 protein (red). Addition of fixed concentrations of non-biotinylated *BDNF*-AS (1- , 10-, 100-fold increase, blue, yellow, and purple points, respectively) indicate that biotinylated *BDNF*-AS is competitively inhibited by non-biotinylated *BDNF*-AS and indicates that our lncRNA-protein interaction of interest is specific. Red points/ curve indicate 0 nM non-biotinylated *BDNF*-AS addition. Asterisk denotes biotinylated RNA.

Supplementary table 1. List of initial hits from the primary screen for *BDNF*-AS-EZH2. Compounds with >70% inhibition were considered hits and were selected for follow-up studies. Compounds in blue were not further tested due to limited availability.

***BDNF*-AS-EZH2 Compound Hits**	**% Inhibition**	**Molecular weight (g/mol)**
Solanine alpha	102.9	868.06
Syrosingopine	72.4	666.7
Myricetin	69.4	318.24
Roseoflavin	76.37	404.42
Curcumin	97.2	368.38
9-Methoxyellipticine	73.89	276.34
Gossypol	94.26	518.56
Biotin	129.43	244.31
Ellipticine	116.7	246.34
Ergotamine tartrate	95.96	656.7

Supplementary table 2. List of initial hits from the *HOTAIR*-EZH2 screen. Compounds with >70% inhibition were considered hits and were selected for follow-up studies. All of these compounds underwent concentration-response validation testing.

***HOTAIR*-EZH2 Compound Hits**	**% Inhibition**	**Molecular weight (g/mol)**
Coraline chloride hydrate	84.01	417.89
Ellagic Acid	61.9	302.197
Camptothecin (S, +)	74.35	348.4
Biotin	107.02	244.31