Supporting Information for:

Identification of bioactive SNM1A inhibitors

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* denotes equal contribution
| Substrate | Oligo | Sequence | Modifications |
|-----------|-------|----------|---------------|
| 5P-FQ     | HTS2  | 5'-[P]-AGC-[dT-F]-A-[dT-Q]-GGTTCGATCAAG-3' | 5' phosphorylation, internal fluorescein, internal BHQ1 |
| 5P-3F     | 5'P   | 5'-[P]-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
| Compound ID | Compound                                              | % Activity |
|------------|-------------------------------------------------------|------------|
| 1          | ACTINOMYCIN D                                         | 36.9       |
| 3          | SULFURETINE                                           | 53.2       |
| 5          | DOXORUBICIN                                           | 48.5       |
| 6          | HARMALOL                                              | 13.9       |
| 7          | EPIGALLOCATECHIN GALLATE                             | 20.3       |
| 8          | WEDELOLACTONE                                         | 26.6       |
| 9          | PATULIN                                               | 22.9       |
| 10         | GOSSYPOL                                              | 38.6       |
| 11         | PELLITORINE                                           | 42.8       |
| 12         | ALOE-EMODINE                                          | 50.2       |
| 13         | AMENTOFлавONE                                        | 35.4       |
| 14         | DIHYDROTANSHINONE                                     | 27.0       |
| 15         | GERALDOL                                              | 47.4       |
| 17         | TETRAHYDROPAPAVERINE                                  | 20.0       |
| 18         | ELLAGIC ACID                                          | 45.0       |
| 19         | OXOKAHWEOL, 16-                                       | 17.7       |
| 20         | AURINTRICARBOXYLIC ACID                               | 16.9       |
| 21         | CALMIDAZOLIUM CHLORIDE                                | 40.6       |
| 22         | AC-93253 IODIDE                                       | 30.8       |
| 23         | BU99006                                               | 51.4       |
| 24         | GW5074                                                | 51.4       |
| 26         | MITOXANTRONE                                          | 42.7       |
| 27         | 6-HYDROXY-DL-DOPA                                     | 42.7       |
| 28         | HISPIDIN                                              | 41.9       |
| 29         | NF 023                                                | 49.6       |
| 30         | REACTIVE BLUE 2                                       | 18.7       |
| 31         | RUTHENIUM RED                                         | 41.6       |
| 32         | PPNDS TETRASODIUM                                     | 51.8       |
| 33         | ME-3,4-DEPHOSTATIN                                    | 45.0       |
| 34         | TYRPHOSTIN 51                                         | 17.3       |
| 35         | GAMBOGIC ACID                                         | 27.3       |
| 39         | KOPARIN                                               | 49.3       |
| 40         | THEAFLAVIN DIGALLATE                                  | 27.2       |
| 41         | BETA-SITOSTEROL                                       | 20.4       |
| 42         | HARMALOL HYDROCHLORIDE                                | 16.6       |
| 43         | HAEMATOPORPHYRIN                                     | 17.7       |
| 44         | 3-OXOURSAN (28-13)OLIDE                              | 42.0       |
| 45         | PHYTOL                                                | 42.8       |
| 46         | HEMATEIN                                              | 18.6       |
| 48         | DAUNORUBICIN                                          | 52.7       |
| 49         | RIBOFLAVIN                                            | 44.7       |
| 50         | MERBROMIN                                             | 16.3       |
| 51         | ACRIFLAVINIUM HYDROCHLORIDE                           | 19.8       |
| 53         | 4,4'-DISOTHIOCYANOSTILBENE-2,2'-SUFONIC ACID SODIUM SALT | 16.8   |
| 54         | FLUORESCENIN                                          | 14.2       |
| 57         | HOMIDIUM BROMIDE                                      | 36.6       |
| 58         | CHLORANIL                                             | 29.5       |
| 60         | 7-DESHYDROXYPYROGALLIN-4-CARBOXYLIC ACID              | 28.8       |
| 61         | METHYL 7-DESHYDROXYPYROGALLIN-4-CARBOXYLATE           | 37.2       |
| 62         | ERYTHROSINE SODIUM                                    | 51.7       |
| 63         | CHLORHEXIDINE                                         | 53.1       |
| 64         | HARMALOL HYDROCHLORIDE                                | 18.2       |

*Figure S2. Compound ID number.*
Figure S3. Dose-response curves of HTS hits. All compounds from SI.2 were tested for dose-response inhibition. SNM1A (3nM) was incubated with 62.5nM-25µM of compound before the addition of DNA (8nM). Duplicate assays were performed using a Biomek FX workstation (Beckman Coulter) equipped with a BioRAPTR (Beckman Coulter) liquid dispensing system and measured with the Envision Plate Reader (Perkin Elmer) at 535nm.
Figure S4. IC$_{50}$ determination of SNM1A exonuclease activity. SNM1A (0.2nM) was incubated with substrate (110nM) for 60 min. Products were resolved using denaturing PAGE and quantified with ImageLab (BioRad). IC$_{50}$ was determined using GraphPad PRISM. Error bars are standard deviation from triplicate experiments.
Figure S5. IC\textsubscript{50} determination of SNM1A endonuclease activity. SNM1A (200nM) was incubated with substrate (30nM) for 150 min. Products were resolved using denaturing PAGE and quantified with ImageLab (BioRad). IC\textsubscript{50} was determined using GraphPad PRISM. Error bars are standard deviation from triplicate experiments.
Figure S6. Ethidium bromide (EtBr) displacement by SNM1A inhibitors. Compound concentrations at the exonuclease IC$_{50}$ of SNM1A (see Figure 4C) were added to EtBr-bound DNA (15pmole EtBr, 3pmol DNA) for 10 minutes. Fluorescence was measured (Exi: 360nm, Emi: 595nm) before and after addition of inhibitor with a Synergy H1 plate reader (BioTek). Fluorescence values were normalized between no inhibitor (high) and no DNA (low) controls. Error bars are standard error from four independent experiments.
Figure S7. SNM1B cross-inhibition by SNM1A inhibitors. A. Nuclease reactions of SNM1A (4nM) and SNM1B (13nM) were preincubated with compounds at the IC$_{50}$ of SNM1A. Nuclease reactions were initiated with addition of 5P-1F (100nM) and incubated at 37°C for 5 or 30 minutes (SNM1A or SNM1B, respectively). Products of triplicate reactions were resolved using 23% denaturing PAGE. B. Reactions were quantified using ImageLab (BioRad). Activity was normalized to uninhibited reaction. Error bars are standard deviation from triplicate experiments.
Supplementary Methods

Ethidium displacement assay
Fluorescence reactions were completed in phosphate buffered saline at 37°C using 384-well plates (Corning 3575). EtBr (BioBasic) was incubated with double-stranded DNA (EtBr-DS, see SI-1). Inhibitor dissolved in DMSO was added and the fluorescence (exi: 360nm, emi: 595nm) was measured after a 10 minute incubation. Fluorescence values were determined using no DNA (low) and no inhibitor (high) controls. All measurements were carried out using Synergy H1 plate reader (BioTek).

SNM1B purification
Human SNM1B (Q9H816, residues 1-335) was expressed recombinantly in Star pRARE pLysS E. coli (Invitrogen). Cells were grown in Terrific Broth, and induced at 1.5 OD_{600} with 1mM IPTG at 25°C overnight. Cells were resuspended in Nickel A buffer (50mM Tris pH 7.5, 500mM NaCl, 0.5mM TCEP, 10% glycerol, 0.05% Triton X-100) and protease inhibitors, then lysed with three passes through a cell disruptor at 10,000 psi. The lysate was clarified by centrifugation at 48,000 x g for 60 minutes and filtered. The sample was loaded onto a HisTrap HP nickel-chelating column (GE Healthcare) and step eluted with Nickel A buffer containing 210mM imidazole. SNM1B-containing elutions were TEV digested at 4°C for 24 hours in 500mM NaCl, 50mM Tris pH 7.5, 10% glycerol, 0.5mM TCEP. Sample was reloaded onto HisTrap column and SNM1B was eluted with Nickel A buffer with 15mM imidazole. SNM1B was concentrated, flash-frozen in liquid nitrogen, and stored at -80°C.

SNM1B assay
All reactions were performed at 37°C in buffer containing 50mM Tris-acetate pH 7.2, 10mM magnesium acetate, 75mM potassium acetate, 1mM DTT, and 100ug/ml of BSA. Exonuclease activity was measured with DNA substrate 5P-1F. Reactions were stopped with the addition of formamide loading buffer. All gels were resolved with 23% denaturing PAGE and imaged with the ChemiDoc XRS (Bio-Rad) at 526nm.