SUPPORTING INFORMATION

For manuscript:

Small Molecule Screen Reveals Regulation of Survival Motor Neuron Protein Abundance by Ras Proteins

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SUPPLEMENTAL TABLES

Supplemental Table S1.

Descriptor	Weight
Number of Rotatable bonds	0.343453
No. Nitrogen atoms	0.478301
logP	0.142011
Molecular refractivity	0.41457
VDW.SURF. AREA+0	1
VDW.SURF. AREA+1	0.467561
VDW.SURF. AREA+2	0.337016
VDW.SURF. AREA+3	0.579149
VDW.SURF. AREA+4	0.356697
VDW.SURF. AREA+5	0.176136
VDW.SURF. AREA+6	0.233498
VDW.SURF. AREA-0	0.787855
VDW.SURF. AREA-1	0.76905
VDW.SURF. AREA-2	0.191082
VDW.SURF. AREA-3	0.208545
VDW.SURF. AREA-4	0.092401
VDW.SURF. AREA-5	0.107985
VDW.SURF. AREA-6	0.03543
logP_VDW.SURF. AREA WEIGHED1	0.106565
logP_VDW.SURF. AREA WEIGHED2	0.149196
logP_VDW.SURF. AREA WEIGHED4	0.073578
logP_VDW.SURF. AREA WEIGHED5	0.460146
logP_VDW.SURF. AREA WEIGHED6	0.055342
logP_VDW.SURF. AREA WEIGHED7	0.381712
logP_VDW.SURF. AREA WEIGHED8	0.314164
logP_VDW.SURF. AREA WEIGHED9	0.41783
SMR_VDW.SURF. AREA WEIGHED0	0.134093
SMR_VDW.SURF. AREA WEIGHED1	0.277358
SMR_VDW.SURF. AREA WEIGHED5	0.25668
SMR_VDW.SURF. AREA WEIGHED6	0.255068

Supplemental Table S2.

Library	No. of cmpds	Hitpicked	Tested by WB	Confirmed by WB
TIC	23,686	595	82	2
ACL	2,337	5	1	0
NINDS	1,040	36	2	0
CGX	20,000	296	0	0
BBC	4,621	49	3	0
BBL	5,240	31	7	0
BBA	12,265	94	10	1
Total	**69,189**	**1,106**	**105**	**3**

Supplemental Table S3.

Cell Line	Cell Line Origin	SMN Increase (%)	SD
Human		*by Western blot*	$n \geq 2$
232	Fibroblast, SMA patient type I	29%	15%
3813	Fibroblast, SMA patient type I	71%	18%
9677	Fibroblast, SMA patient type I	93%	24%
3814	Fibroblast, SMA carrier	73%	38%
10684	Lymphoblast, SMA patient type I	18%	11%
SMA ESC-MN	ESC-derived MN, SMA	27%	3%
ESC-MN	ESC-derived MN, WT	- 1%	8%
HT1080	Fibrosarcoma	32%	14%
293T	Embryonic kidney	10%	6%
A549	Lung carcinoma	21%	7%
Rodent			
WT MEF	Embryonic fibroblast, mouse	2%	18%
PC12	Pheochromocytoma, rat	- 8%	2%
ST14A	Striatal cells, rat	0%	15%
mESC-MN	ESC-derived MN, WT mouse	- 21%	13%

SUPPLEMENTAL LEGENDS

Supplemental Table S1. List of descriptors used for *in silico* calculations

Supplemental Table S2. Summary of screening data

Supplemental Table S3. Genotype-specificity testing of cuspin-1

Table lists designations and origins of each cell line, as well as the mean percent increase of SMN protein levels normalized to actin compared to DMSO-only control after treatment with 5µg/ml (18µM) cuspin-1 for two days, as quantified by Western blot. Standard deviation (SD) for each value is shown in the following column.

Supplemental Figure S1. Validation of *in silico* screening filters for selection of compounds for the BBB libraries

Correlation between experimentally determined logBB values and predicted values of the training set (A) and the validation set (B).

Supplemental Figure S2. SMN-upregulating activity of hit compounds c72 and c81

Graph of relative luminescence (RLU) from dose-response in cytoblot assay quantifying the amount of SMN upregulation by compound 72 (A) and compound 81 (B). To the right of each dose curve is the structure of the active compound and a representative Western blot of #9677 SMA patient fibroblast cells treated with 5µg/ml (12.5µM for c72, 14.6µM for c81) for 2 days. Fold increase of SMN to actin ratio, normalized to control is quantified below each lane.

Supplemental Figure S3. ^{1}H NMR of cuspin-1

^{1}H NMR of cuspin-1 confirming the structure. ^{1}H NMR ($CDCl_3$, 400MHz) 8.89 (t, *J* = 2.2 Hz, 2H), 8.25 (t, *J* = 2.0 Hz, 1H), 7.74 (d, *J* = 8.2 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 2.49 (s, 3H).

SUPPLEMENTAL METHODS

Preparation of Compound Libraries for Primary Screening

Compound libraries were either obtained at a concentration of 4 mg/mL in dimethyl sulfoxide (DMSO) or were solubilized in DMSO and plated at this concentration in 384-well, clear, polypropylene, 13mm deep "mother" plates (Greiner/#781280). These stocks were diluted into media to create "daughter" plates as follows: 148 µL of Dulbecco's Modified Eagle's Medium (Invitrogen/#11995-040) were dispensed using a Biomek FX into each well of a 384-well, clear, polypropylene, 22mm deep daughter plate (Greiner/#781270). Two µL from the mother plate were transferred to the daughter plates and thoroughly mixed using a Beckman Biomek FX workstation. This resulted in a compound concentration of 53.33 µg/mL in each well of the daughter plates.

Cytoblot Cell Seeding into 384-Well Plates and Primary Compound Screening

GM03813 cells (herein referred to as "#3813"), GM09677 cells ("#9677"), and GM00232 cells ("#232") were trypsinized, centrifuged at 214g (1000rpm with 19.2cm rotor radius) for five minutes and resuspended in culture media at a concentration of 420,000 cells/mL. Thirty-six μL of cell suspension were dispensed into each well of a 384-well, opaque bottom, white, tissue culture-treated, 13mm deep assay plate (Perkin Elmer/#6007680) using a Biomek FX, for a concentration of 15,000 cells/well. Four μL from the daughter plate was transferred to each assay plate using a Biomek FX, resulting in a final compound concentration of 5.33 μg/mL per well. The plate was covered with the supplied lid and incubated at 37°C and 5% carbon dioxide for 48 hours. All compounds were tested in triplicate.

Cytoblot Fixation, Permeablization and Antibody Staining

After 48 hours incubation with small molecules, the growth medium was aspirated out of each assay plate, and 30 μL of ice cold (-20°C) methanol was added to each well for fixation and permeabilization using a Biomek FX. The plates were incubated for 5 minutes on ice, after which the methanol was aspirated and the primary antibody solution was immediately added. Twenty μL of 10% (v/v) goat serum (Invitrogen/#16210-072) in PBS with 0.1% Tween® 20 (Aldrich/#274348) containing primary antibody (see below for dilutions) was dispensed to each well using a Biomek FX. Plates were sealed using a Velocity11 PlateLoc and incubated overnight at 4°C. The following day, the seals were removed and the primary antibody solution was aspirated out of each assay plate. Twenty uL of secondary antibody in the same blocking buffer was dispensed into each well using

a Biomek FX. Plates were incubated for one hour at room temperature prior to signal detection. HRP SMN cytoblot: mouse anti-SMN (BD Transduction Laboratories/#610647) (1:3,000 dilution) and goat anti-mouse-HRP (Santa Cruz Biotechnology/#sc-2031) (1:9,000 dilution)

Cytoblot Signal Detection

After the one hour incubation period, the secondary antibody solution was aspirated out of each assay plate. Fifty µL of 10% (v/v) goat serum in PBS with 0.1% Tween® 20 were added to each well using a Biomek FX, to rinse out unbound secondary antibody. After 10 minute incubation on ice, the solution was aspirated. Twenty µL of enhanced chemiluminescence (ECL) reagent were added to each well using a Biomek FX. ECL reagent is composed of two solutions mixed together immediately prior to use – Solution A: 5 mL Tris (pH 8.5) (Roche/#0604205) and 4 µL H_2O_2 (Sigma/#H1009); Solution B: 5 mL Tris (pH 8.5) (Roche/#0604205), 22 µL 90 mM coumaric acid (Sigma/#C9008), and 50 µL 250 mM luminol (Fluka/#09253). Luminescence was read using a Perkin Elmer Victor 3 microtiter platereader (#1420-041) equipped with an infrared filter, using a 0.8 s integration time per well. Data was exported and evaluated in Excel.

Dose-response experiments

Compounds showing activity in the primary screen were retested in dilution series, daughter plates of the hit-containing mother plates were recreated (as described above). For the "step-daughter" plates, fifty-eight μL of DMEM (as diluent) were dispensed using a Biomek FX into every well (except for columns 8 and 15) of an empty, 384-well, clear, polypropylene, 22mm deep plate (Greiner/#781270). One hundred and sixteen (116) μL of hit compound in media (53.33μg/ml) were transferred using the Span-8 head of a Biomek FX from the daughter plate to the step-daughter plate. Picked hits were dispensed in columns 8 and 15 using rows C through N (one well per compound), allowing 24 hits per step-daughter plate. A two-fold, six-point serial dilution was performed by the Biomek FX Span-8 head: compounds in column 8 were diluted through column 13, and compounds in column 15 were diluted through column 20. All compounds were tested in triplicate.

Robotic Scripts and Settings

BioMek FX (software version 2.5e) method files are available upon request.