Supplementary Information

A small molecule targeting TDP-43's RNA recognition motifs reduces locomotor defects in a *Drosophila* model of ALS

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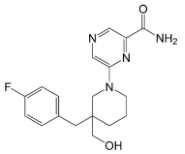
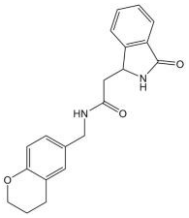
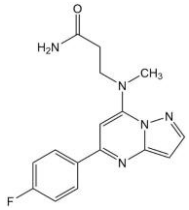
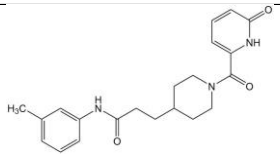
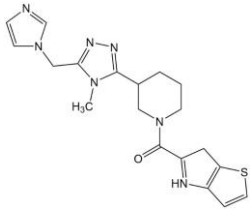
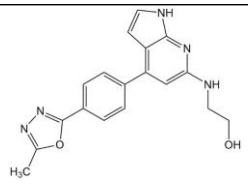
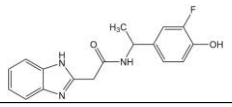
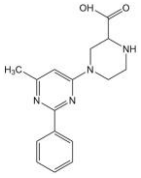
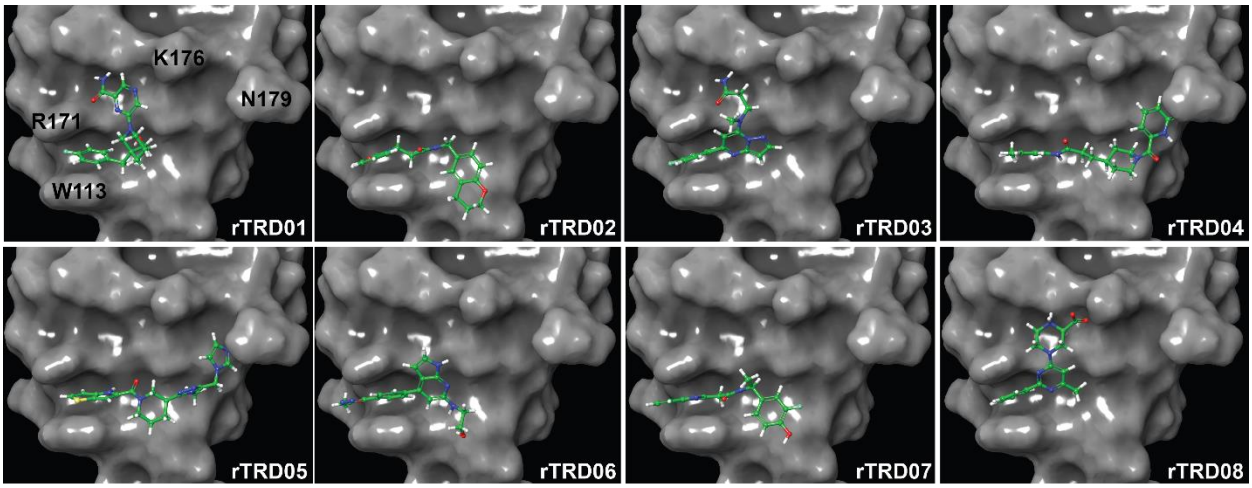
Supplementary methods

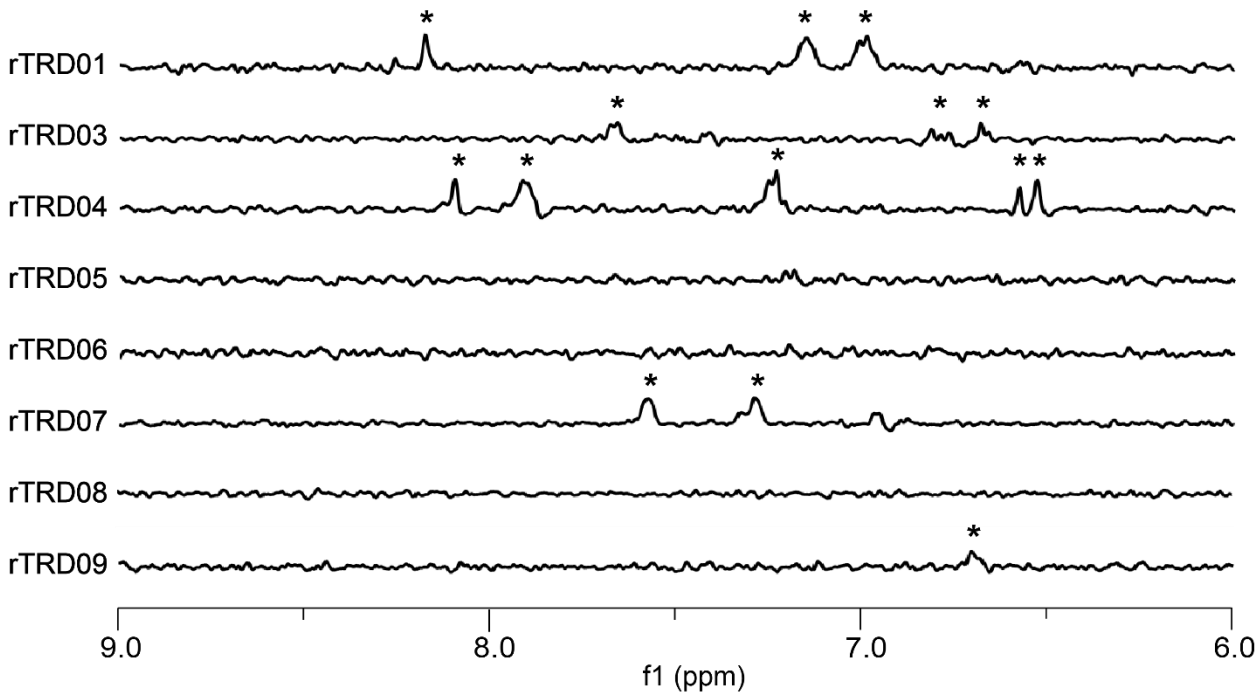
Cytotoxicity assay. Pierce LDH Cytotoxicity Assay was used per manufacturer's protocol (Pierce Biotechnology, Rockford, lL) to determine the cytotoxicity of rTRD01. Mouse Motor Neuron cells (NCS-34) were seeded in a 96 well plate in DMEM media supplemented with 10% FBS and 1% penicillin-streptomycin antibiotics at 37°C with 5% CO2, at a density of 2 x 10 cells/well and were incubated for 24 hours. The cells at 80% confluence were treated with 50 µM rTRD01 and incubated for overnight. Additional cells were plated for Spontaneous LDH Activity Controls (water) and Maximum LDH Activity Controls (10X Lysis Buffer). The LDH released into the medium is transferred to a new plate and mixed with Reaction Mixture. After a 30-minute room temperature incubation, reactions are stopped by adding Stop Solution. Absorbances at 490nm and 680nm were measured using a plate-reading spectrophotometer (Synergy HT Multi-Mode Microplate Reader) to determine LDH activity.

Supplementary Table 1: Structure, chemical name and docking scores of the top 8 hits from the *in-silico* docking experiment.

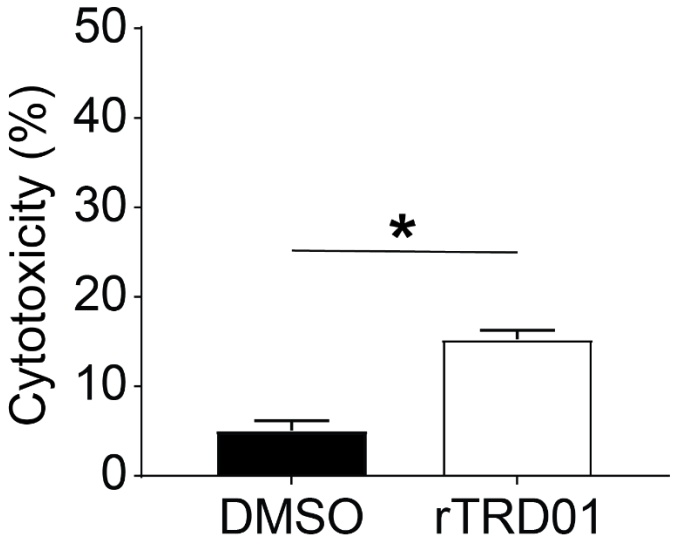
	Chemical name	Structure	Glide XP Score
rTRD01	6-[3-(4-fluorobenzyl)-3-(hydroxymethyl) piperidin-1-yl]pyrazine-2-carboxamide		-5.117
rTRD02	N-(3,4-dihydro-2H-chromen-6-ylmethyl)-2-(3-oxo-2,3-dihydro-1H-isoindol-1-yl)acetamide		-5.009
rTRD03	N~3~-[5-(4-fluorophenyl)pyrazolo[1,5-a] pyrimidin-7-yl]-N~3~-methyl-beta-alaninamide		-4.994
rTRD04	N-(3-methylphenyl)-3-{1-[(6-oxo-1,6-dihydropyridin-2-yl) carbonyl]piperidin-4-yl}propanamide		-4.894
rTRD05	5-({3-[5-(1H-imidazol-1-ylmethyl)-4-methyl-4H-1,2,4-triazol-3-yl]piperidin-1-yl}carbonyl)-4H-thieno[3,2-b]pyrrole		-4.884
rTRD06	2-({4-[4-(5-methyl-1,3,4-oxadiazol-2-yl) phenyl]-1H-pyrrolo[2,3-b]pyridin-6-yl}amino)ethanol		-4.88
rTRD07	2-(1H-benzimidazol-2-yl)-N-[1-(3-fluoro-4-hydroxyphenyl)ethyl]acetamide		-4.853
rTRD08	4-(6-methyl-2-phenylpyrimidin-4-yl) piperazine-2-carboxylic acid		-4.845



Supplementary Figure 1: Top hits from *in silico* docking poses. RRM TDP-43 RNA Disruptor (rTRD) 1 to 09 docking poses (*stick and ball representation, green)* on 4iuf (*surface representation, gray*) are shown.



Supplementary Figure 2: Direct binding of small molecules obtained from docking of TDP-43-RRM. 1D ^{1}H STD NMR showing on-resonance difference spectrum of 1 mM small molecules with 5 µM TDP-43$_{102-269}$. The aromatic region of the NMR spectrum (6-9ppm) is shown. Asterisks correspond to protons that found in independent experiments.



Supplementary Figure 3: LDH measured cytotoxicity in NSC34 cells following overnight treatment with either DMSO or 50 µM of rTRD01. Asterisk denotes statistical difference as assessed by Mann-Whitney test ($p < 0.05$, $n=6$).