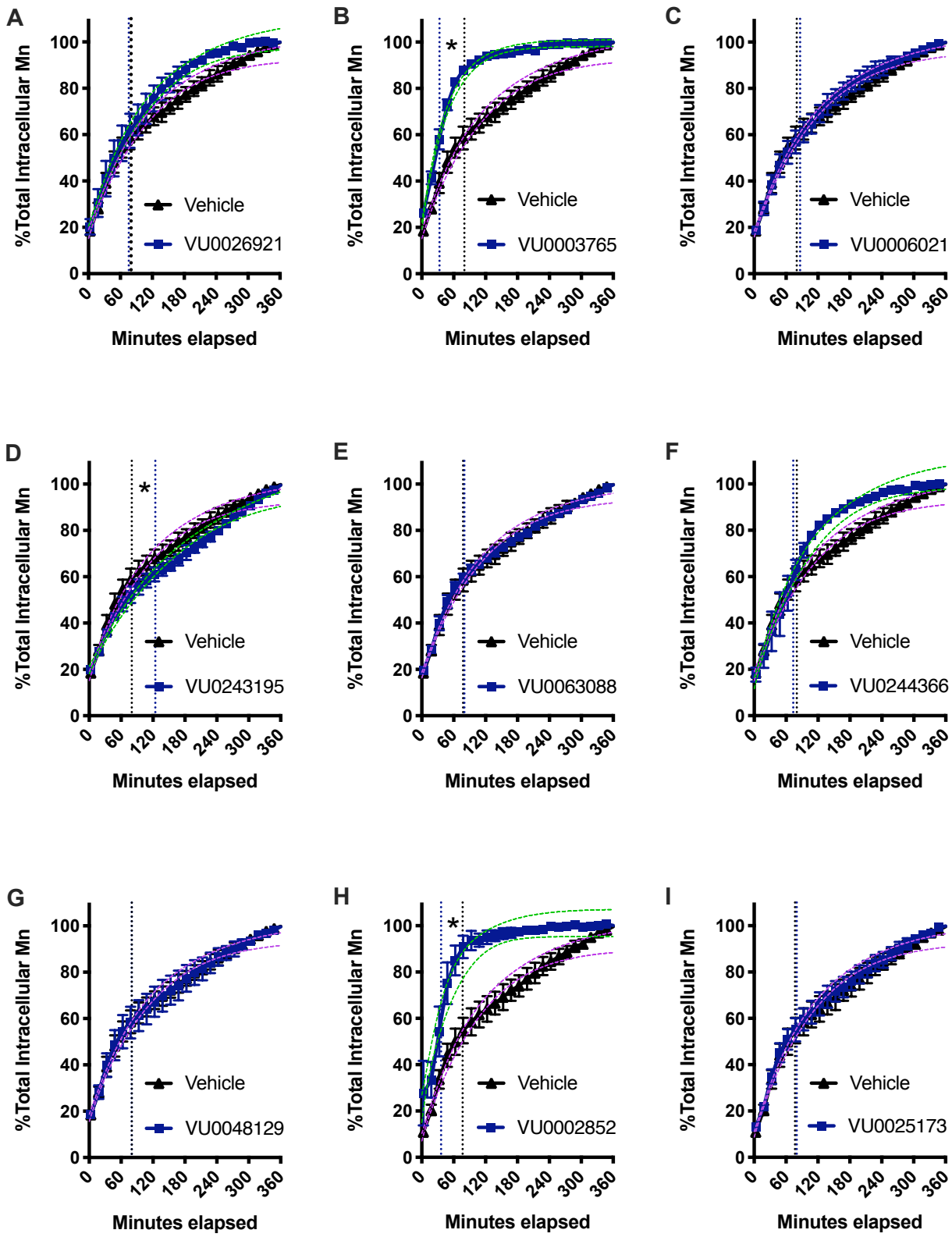
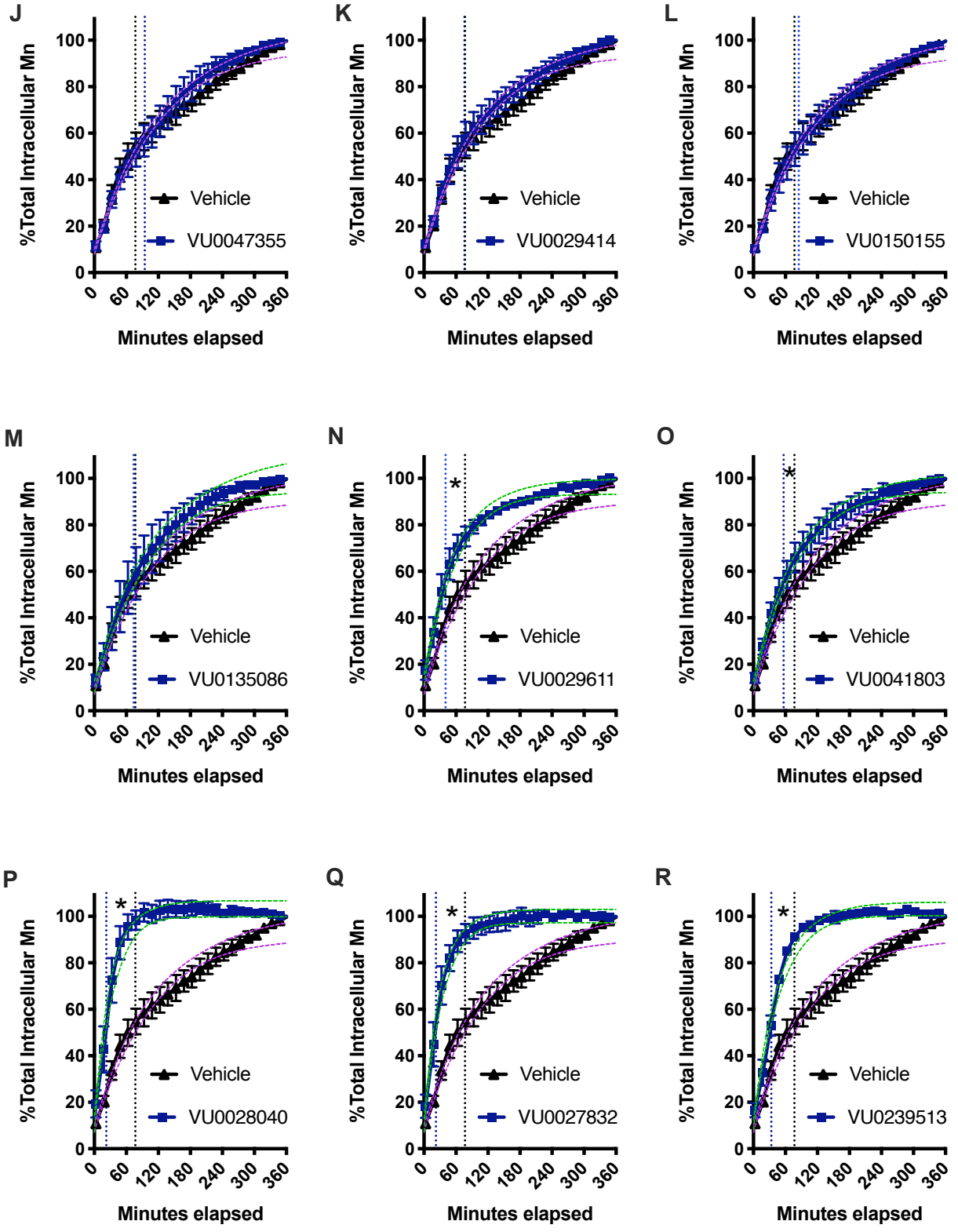
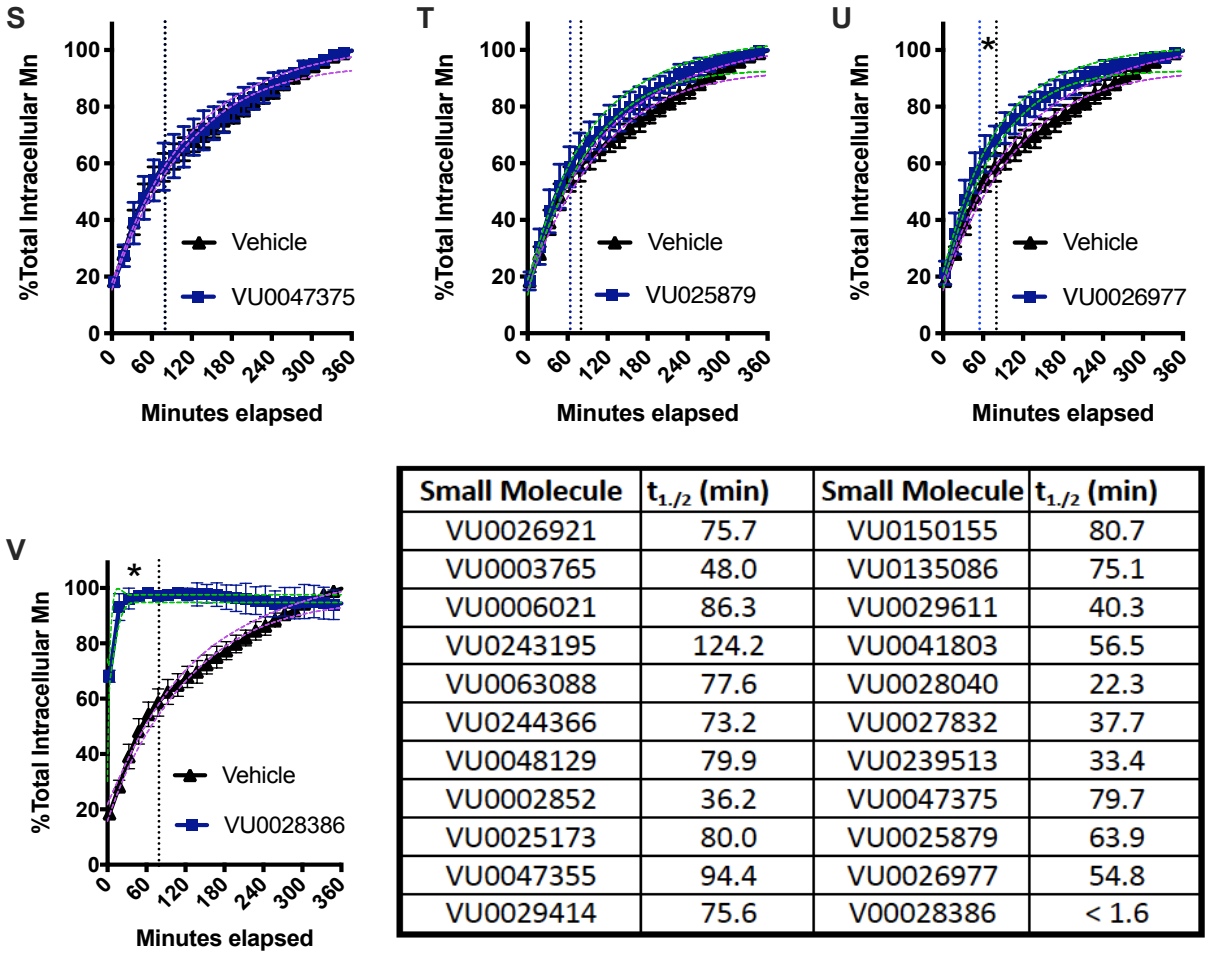
SUPPORTING INFORMATION



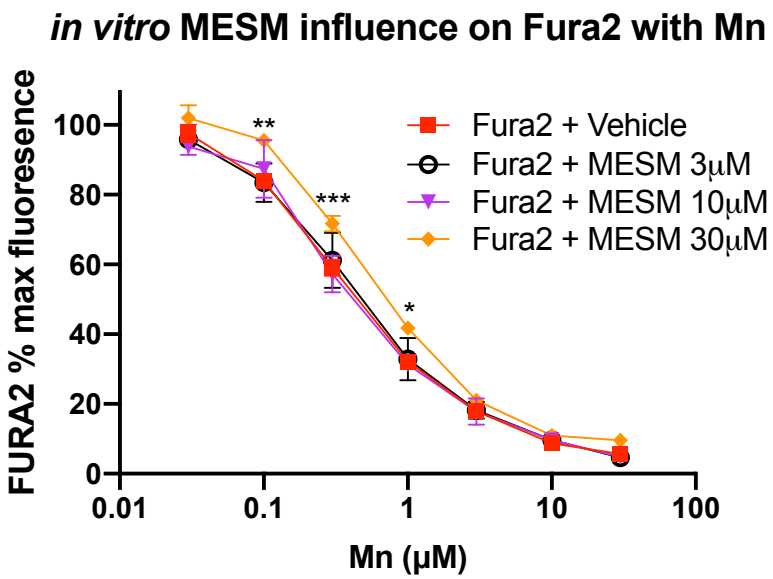

J
K
L
M
N
O
P
Q
R
%Total Intracellular Mn
Minutes elapsed
Vehicle
VU0047355
VU0029414
VU0150155
VU0135086
VU0029611
VU0041803
VU0028040
VU0027832
VU0239513
*



Small Molecule	$t_{1./2}$ (min)	Small Molecule	$t_{1./2}$ (min)
VU0026921	75.7	VU0150155	80.7
VU0003765	48.0	VU0135086	75.1
VU0006021	86.3	VU0029611	40.3
VU0243195	124.2	VU0041803	56.5
VU0063088	77.6	VU0028040	22.3
VU0244366	73.2	VU0027832	37.7
VU0048129	79.9	VU0239513	33.4
VU0002852	36.2	VU0047375	79.7
VU0025173	80.0	VU0025879	63.9
VU0047355	94.4	VU0026977	54.8
VU0029414	75.6	V00028386	< 1.6

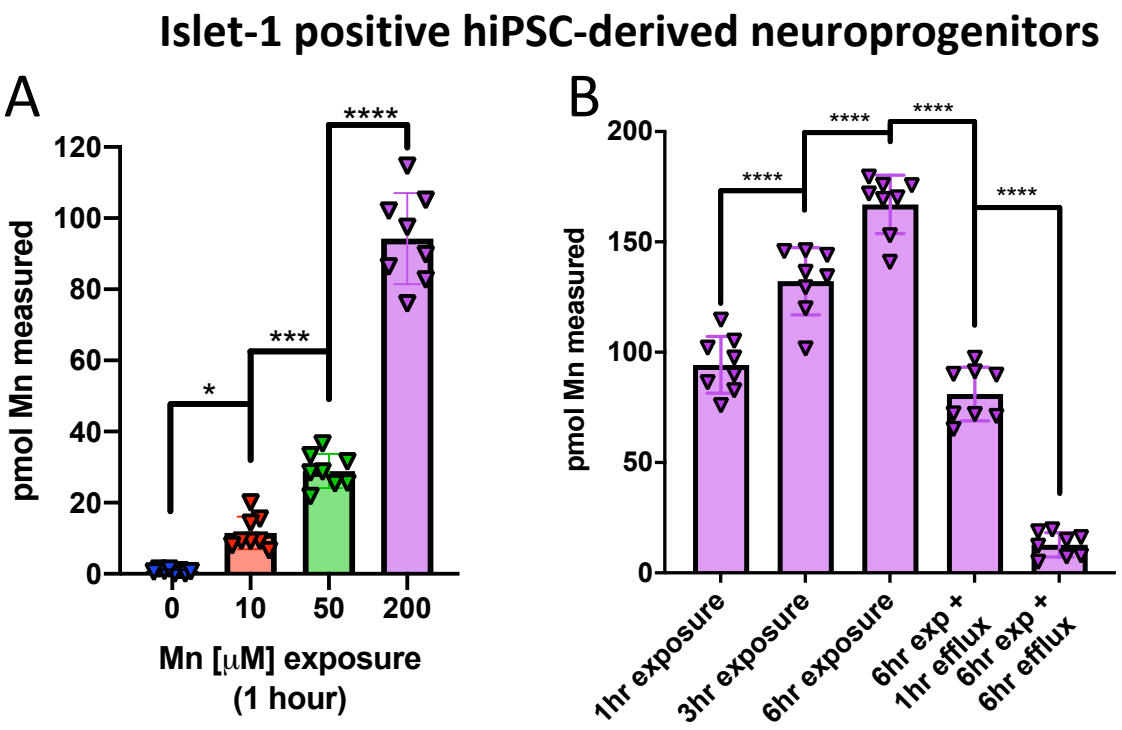
Supporting Figure 1. Efflux screens show other efflux facilitators, but all lack the fast kinetics of MESM.

Cells of the murine striatal neuron lineage, STHdh$^{Q7/Q7}$ (Q7) were pre-exposed to 125μM $MnCl_2$ for 2 hours in HBSS (Hanks Balanced Buffer Solution) before washing away the extracellular Mn and allowing the cells to efflux their intracellular Mn to the extracellular space in PBS (lacking Ca^{2+} and Mg^{2+}) with co-incubation of a small molecule (10 μM). Fluorescent dye (Fura-2; 500nM) was added to the PBS so its fluorescence could be quenched by effluxed Mn. Fluorescence of Fura-2 was measured at 360/535 (Ex/Em) every 15 minutes for 6 hours at 37°C and Mn was calculated based on percent fluorescence quenching. The maximum quantity of Mn effluxed after 6 hours was normalized to 100%. A one-phase exponential decay model of nonlinear regression was used and compared if one curve could adequately fit both data sets (small molecule and vehicle). Least squares regression fitting was used with weighting $1/Y^2$. Green dotted curves of 95% confidence intervals are drawn for every significant curve fit by using an extra sum of squares F-test. ($p < 0.01$). Purple dotted curves of 95% confidence intervals are drawn for fit curves to vehicle and curves not significantly different than vehicle. A (*) is placed in every instance where the calculated $t_{1/2}$ value and 95% confidence intervals are non-overlapping. Of the 22 screened, only 8 small molecules did not have a significant impact on efflux rates. The $t_{1/2}$ values of each small molecule are summarized in a table at the bottom.

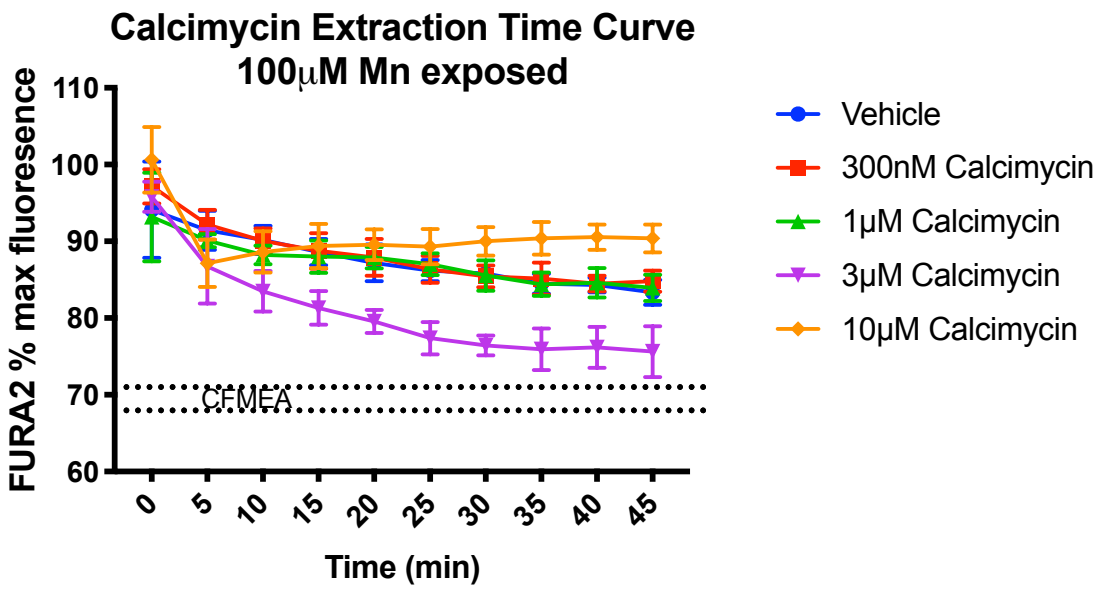


Supporting Figure 2. Higher than optimal concentrations of MESM interfere with Mn quantification.

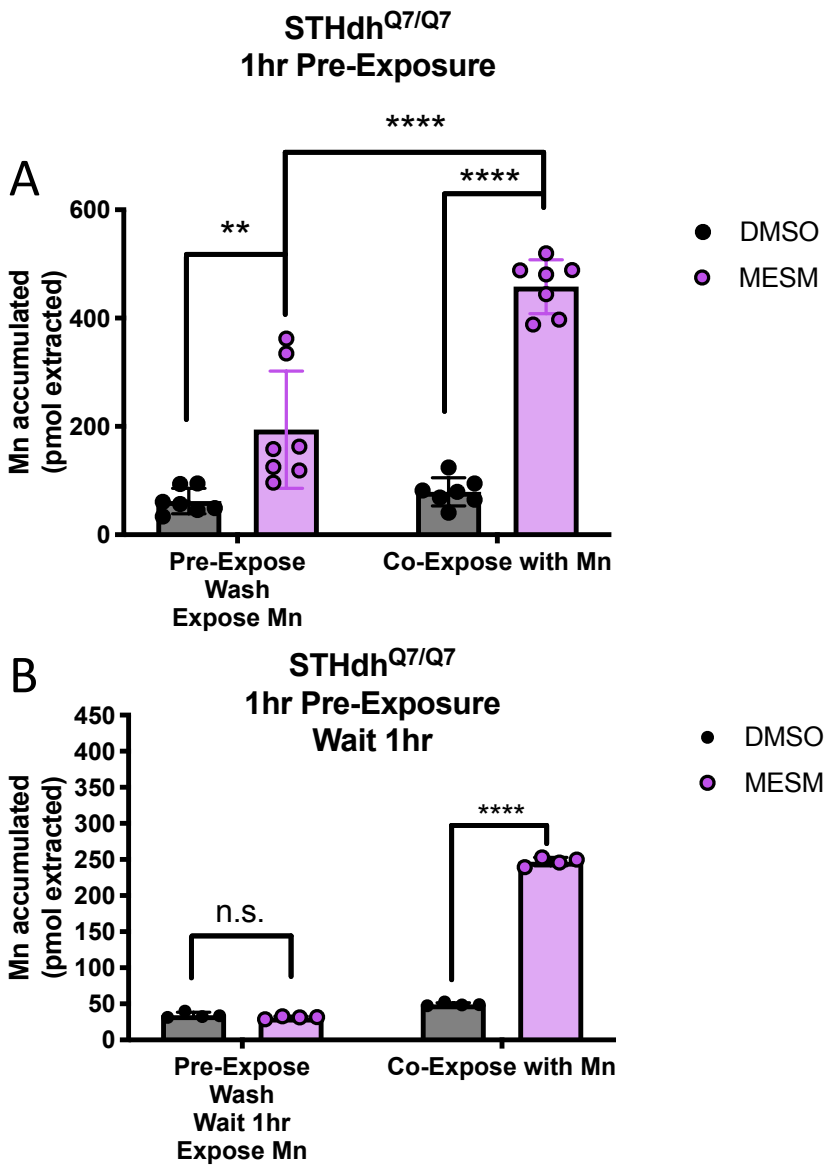
Various concentrations of Mn were added to empty wells (no cells) in PBS (without Ca^{2+} and Mg^{2+}) in the presence of Fura-2 (0.5μM), Fura-2 with 10μM MESM, or Fura-2 with an equivalent DMSO vehicle. The plates were read at 360nm/535nm Ex/Em as earlier described. N=3 of individual replicates performed on different days, with four technical replicate wells for each condition each day. Error bars shown represent standard deviation of the three independent replicate averages. A Two-Way ANOVA yielded a significant main effect for Mn concentration ($p < 0.0001$), and a main effect for MESM concentration ($p < 0.0001$). Sidak's multiple comparisons tests found significant differences between 30 μM MESM and Vehicle Fura-2 % fluorescence at 0.1, 0.3, and 1.0 μM Mn concentrations. (*, $p < 0.05$; **, $p < 0.01$; *** $p < 0.001$). These concentrations are 10-fold higher than required for Mn extraction, but the parabolic effect seen in this MESM concentration curve suggests a competition for Mn binding between MESM and Fura-2.



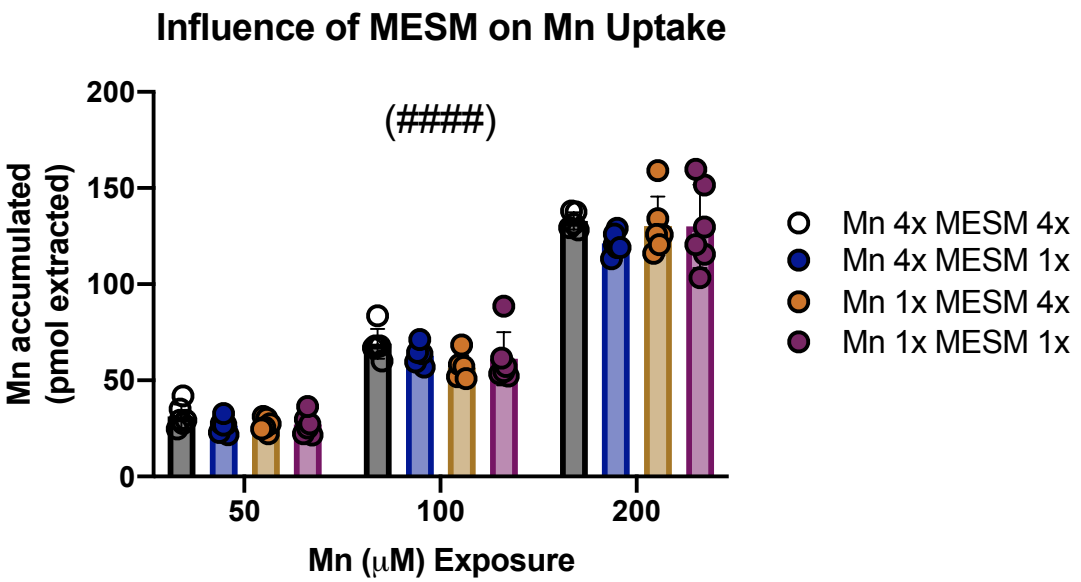
Supporting Figure 3. Manganese extraction by MESMER in hiPSC-derived cells differentiated to day 11 striatal-like lineage, Islet-1-positive, neuroprogenitors, show time- and concentration-dependent effects of Mn exposure.
(A) Islet-1-positive NPCs were exposed to various concentrations (10, 50, 200μM) of Mn in medium for one hour. Two independent differentiations with 4 individual wells each, for a total N=8. Ordinary One-Way ANOVA with Tukey's multiple comparisons test. (*, $p < 0.05$; ***, $p < 0.001$); ****, $p<0.0001$). (**B**) At the highest concentration (200μM), cells were exposed for 1, 3, or 6 hours, combined with efflux conditions, where after Mn exposure, the medium was replaced with medium without Mn for 1 or 6 hours, thus allowing free efflux of Mn from cells, before assaying intracellular Mn with MESMER. MESMER was able to detect increases in intracellular Mn concentrations with increasing exposure times, and subsequent time-dependent decreases in Mn, after allowing Mn efflux from cells. Two independent differentiations with 4 individual wells each, for a total of N=8. Ordinary One-Way ANOVA with Sidak's multiple comparisons. (****, $p<0.0001$).



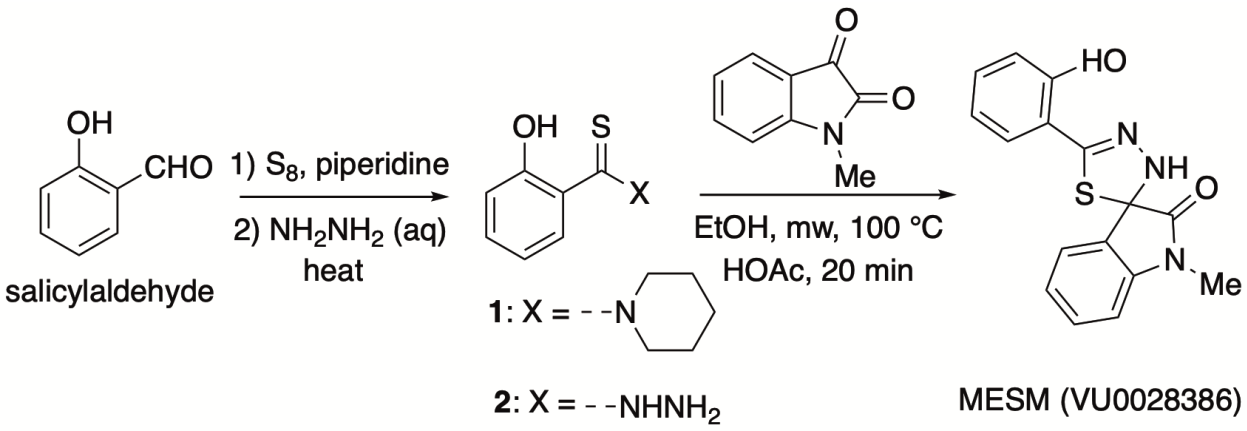
Supporting Figure 4. Calcimycin continuesly extracts Mn over time, but fails to reach the CFMEA range. Murine striatal neuron lineage Q7 cells were exposed to 100μM Mn in HBSS for two hours at 37°C. Cells were then washed five times in PBS (lacking Ca^{2+} and Mg^{2+}) and then exposed to 0.5μM Fura-2 and varying concentrations of calcimycin for 45 minutes. Fluorescence was read every 5 minutes at 360nm/535nm Ex/Em. A separate group of cells had Mn extracted by traditional CFMEA means after Mn exposure, for comparison. CFMEA dotted lines (in black) denote range of one standard deviation from CFMEA average. Each point outside the black dotted lines is significantly different from CFMEA, as determined by a Two-Way ANOVA with Sidak's multiple comparisons. Error bars represent standard deviation of all biological replicates.



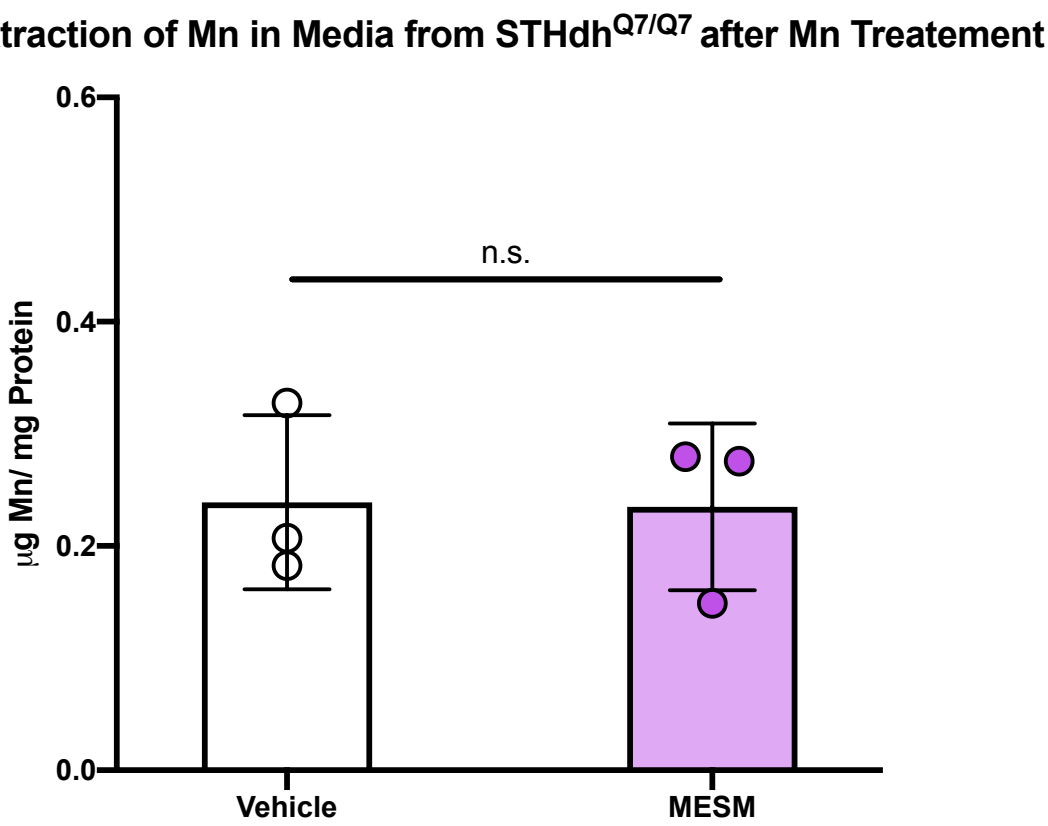
Supporting Figure 5. Cells pre-exposed to MESM, but washed prior to Mn exposure, show a similar trend to cells co-exposed with MESM and Mn.
(**A**) STHdh cells were pre-exposed to 10μM MESM or Vehicle equivalent for one hour before washing off with HBSS and adding 125μM Mn for one hour. These cells were compared to cells co-exposed with 125μM Mn and 10μM MESM or equivalent Vehicle for one hour. Mn accumulation was measured by CFMEA. N=7. A Two Way ANOVA showed a main effect for MESM/DMSO ($p < 0.0001$), a main effect for pre-exposure/co-exposure ($p < 0.0001$), and a significant interaction ($p < 0.0001$). In Sidak's multiple comparisons test, significance is denoted by **($p < 0.01$) or **** ($p < 0.0001$)**.** DMSO controls for each exposure paradigm (black bars) were not statistically significant from each other in Sidak's multiple comparisons test. If a similar experiment is run but the cells are incubated an extra hour in between MESM pre-exposure and Mn exposure (**B**), the effect of MESM on Mn accumulation is lost (n.s.).



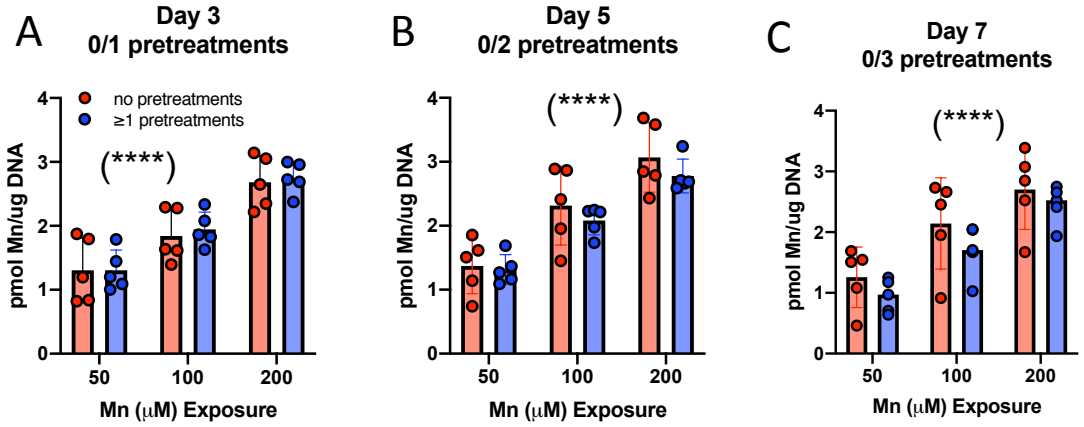
Supporting Figure 6. MESM pre-exposures do not impact future Mn accumulation.
STHdh cells were exposed to Mn (50, 100, or 200μM) once per day for 2 hours in HBSS before being extracted with MESMER, or left without extracting. Afterwards the cells were washed in PBS and returned to media at 37°C. Each day, new cells that were plated at the same time but previously unexposed received Mn. This process occurred up to four times, until at last all cells were extracted by MESMER. A Two-Way ANOVA revealed a significant main effect (####, $p < 0.0001$) for Mn concentration, though no significant main effect for exposure paradigm.



Supporting Figure 7. Schematic of MESM molecule synthesis.



Supporting Figure 8. MESM loses the ability to extract Mn when exposed in DMEM. STHdh cells were exposed to 50µM Mn for 2 hours prior to washing and the Mn-extraction step by 3µM MESM or Vehicle (DMSO). Samples were then prepared for ICP-MS. If MESM was active as an ionophore, one would expect to see a decline from Vehicle to MESM in µg Mn/mg protein, as intracellular Mn would have been extracted from the MESM-treated cells. The two conditions were compared using a two-tailed Student's t-test (not significant; n.s).



Supporting Figure 9. Multiple Mn pretreatments to neuronal cultures do not decrease Mn accumulation. STHdh Q7 cells were exposed to 50μM, 100μM, or 200μM $MnCl_2$ in HBSS at 37 degrees up to four times for 2 hours each before washing and being retuned to DMEM. There were 48 hours in between each pretreatment, so that the ≥1 pretreatment cells at 3 days post plating (**A**) were pre-exposed once before; at day 5 (**B**) pre-exposed twice before; at day 7 (**C**) pre-exposed three times before. "No pretreatment" cells were used as a comparison, which were also plated at Day 0 but never pretreated with Mn. After Mn quantification the number of cells in each condition was approximated with a DNA count using a PicoGreen Assay (see methods) and normalized to microgram DNA. A Two-Way ANOVA analysis (N=5) showed a main effect of Mn concentration at Day 3 (****; $p < 0.0001$), Day 5 (****; $p < 0.0001$) and Day 7 (****; $p < 0.0001$). There was no main effect for pretreatments at Day 3, Day or Day 7. Sidak's multiple comparisons showed no instances of significance at any exposure level for specific days.