Supplemental information: Manganese causes neurotoxic iron accumulation via translational repression of Amyloid Precursor Protein (APP) and H-Ferritin

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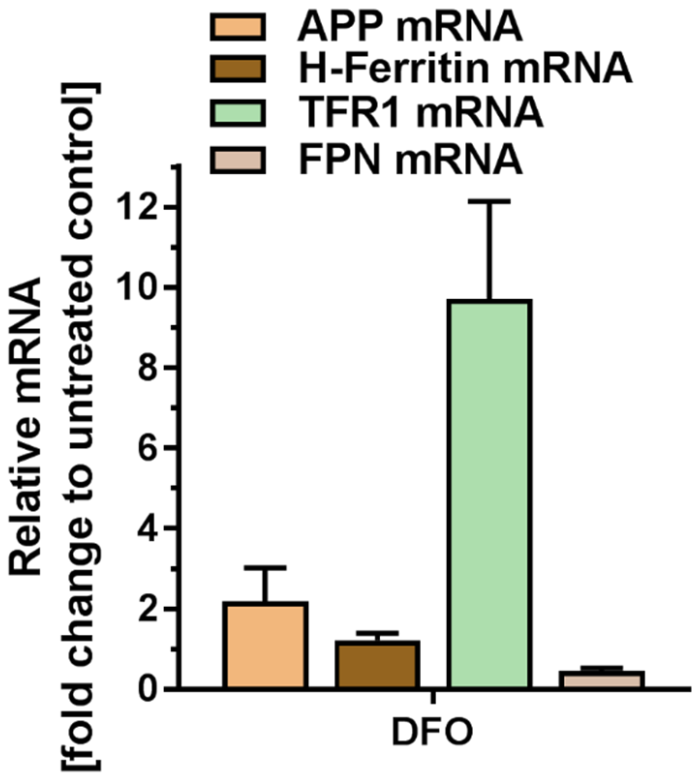
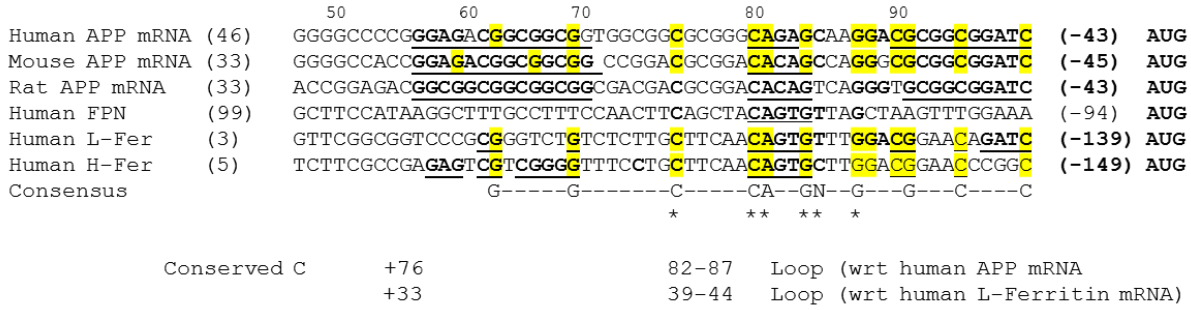
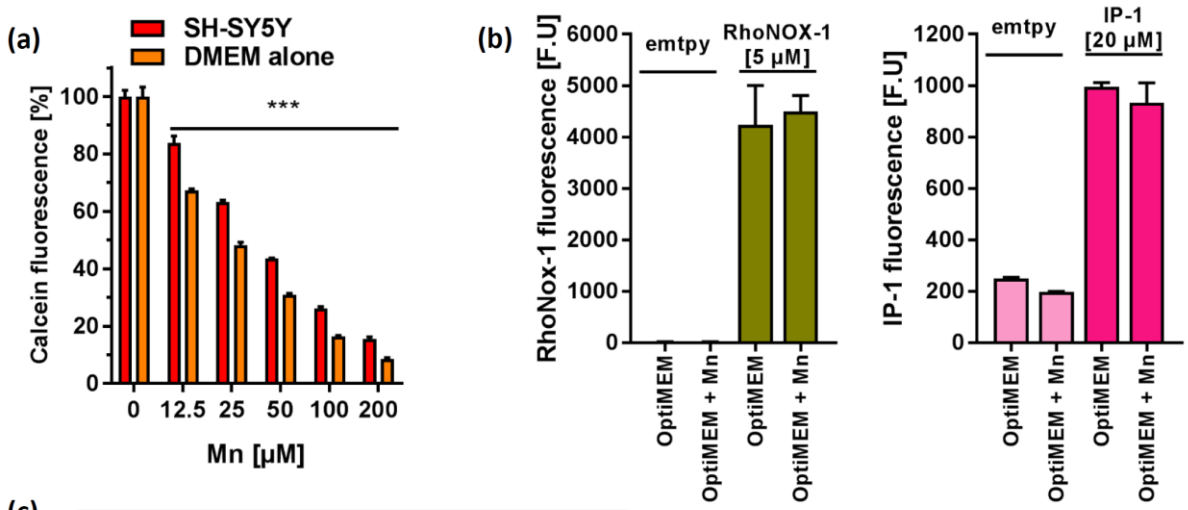


Supplemental Fig. 1 (corresponding to Fig. 2): RT-PCR analysis of indicated iron responsive target genes (APP, H-Ferritin, TFR1 and FPN) from SH-SY5Y cells incubated for 24 h with 100 µM DFO. All data are normalized to housekeeping gene β-actin and presented as relative fold-induction compared to untreated controls (n=4). N number indicates number of replicates. Data shown represent mean ± SD.



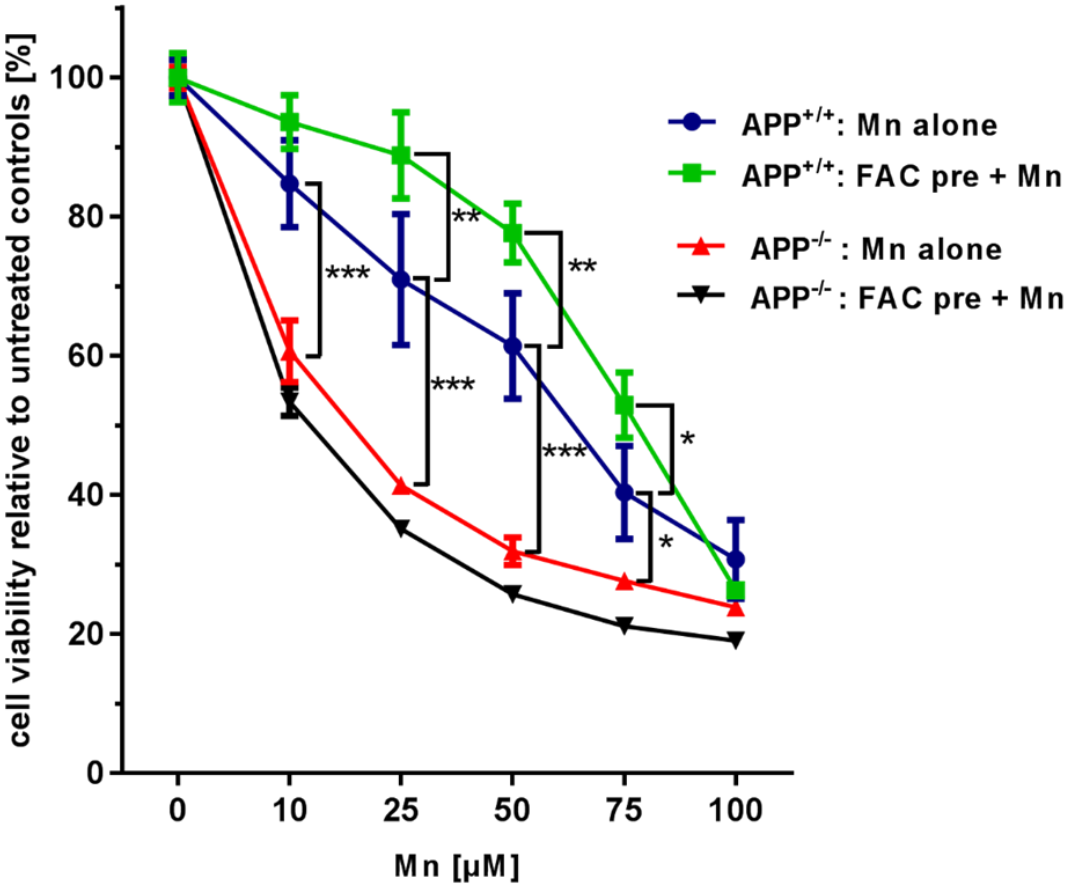
Supplemental Fig. 2 (corresponding to Fig. 3): Bioinformatics alignment of the 5'untranslated regions of APP mRNAs when aligned to the ferritin canonical IREs AGU and AGA tri-loops as a potential IRP 1 binding site (bold lettering).



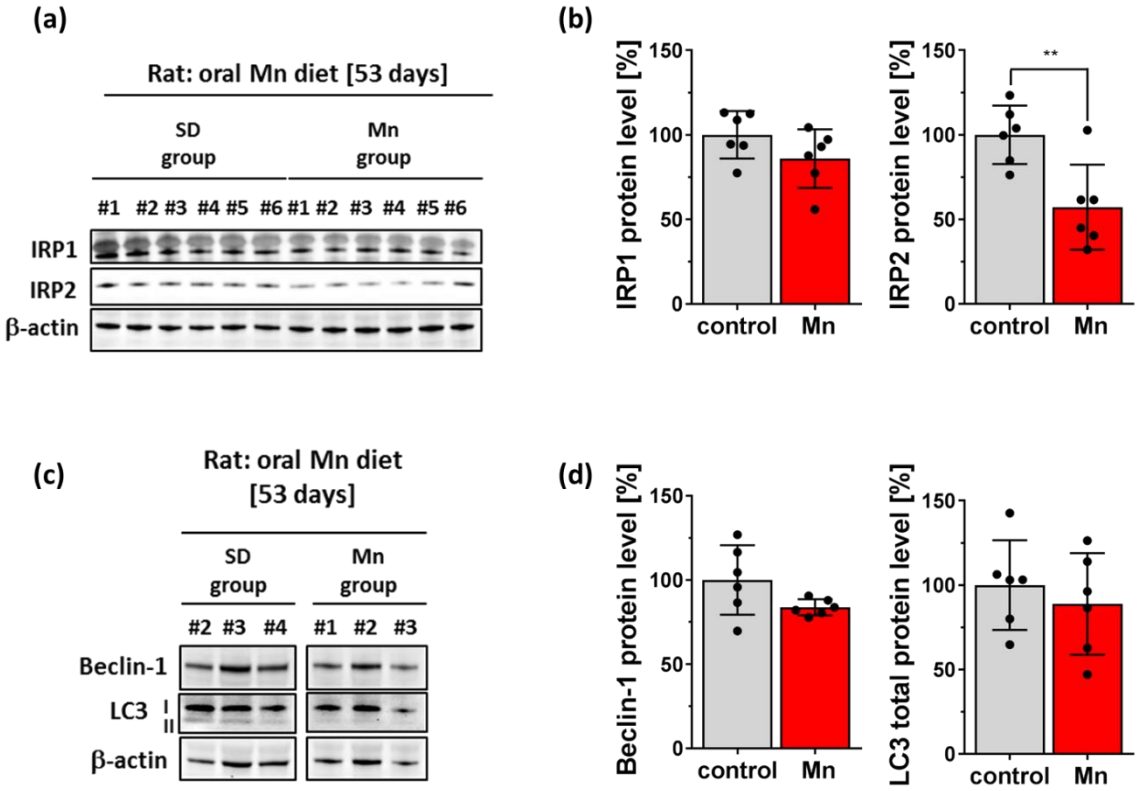
(c)

condition	Fe^{3+} [µg/L]	Fe^{2+} [µg/L]
Control Ex. #1	333.94	84.95
Control Ex. #2	228.76	100.81
Control Ex. #3	237.20	100.86
Mn [100µM] Ex. #1	224.32	170.20
Mn [100µM] Ex. #2	211.92	154.49
Mn [100µM] Ex. #3	224.39	138.75

Supplemental Fig. 3 (corresponding to Fig. 4): (a) SH-SY5Y cells or complete DMEM media alone were incubated with increasing Mn doses for 24 h, loaded with calcein-AM and analyzed for fluorescence. Data are presented as relative fluorescence compared to untreated fluorescence (n=6). **(b)** Cell-free background fluorescence of OptiMEM media ± 100 µM Mn (empty) or OptiMEM media ± 100 µM Mn loaded with either 5 µM RhoNOX-1 (left panel) or 20 µM IP-1 (right panel). Data are presented as absolute fluorescence units (F.U.) (n=3). **(c)** Absolute Fe2+ and Fe3+ values assed by IC-ICP-MS and CE-ICP-MS from SH-SY5Y lysates treated with DMEM media or 100 µM Mn. Corresponding relative Fe^{3+} to total iron and Fe^{2+} to total iron ratio are shown in **Fig. 4b**. Data are presented as mean ± SD. N number indicates number of replicates. Differences were calculated using either two-way ANOVA followed by Bonferroni post-hoc analyses. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).



Supplemental Fig. 4 (corresponding to Fig. 5): Immortalized murine embryonal fibroblasts (MEFs) were isolated from wild-type mice ($APP^{+/+}$) or APP-knockout ($APP^{-/-}$) mice. $APP^{+/+}$ and $APP^{-/-}$ MEFs were either pretreated with 100 µM FAC or media for 24 h and subsequently incubated with increasing doses of Mn for additional 24 h and analyzed for cell viability (n=3). Data are presented as mean ± SD. N number indicates number of replicates. Differences were calculated using two-way ANOVA followed by Bonferroni post-hoc analyses. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).



Supplemental Fig. 5 (corresponding to Fig. 6): Corresponding western blots of IRP1 and IPR2 from whole brains lysates of either Sprague-Dawley rats treated with standard diet (SD group) or Mn (500 mg/kg) for 53 days. β-actin served as loading control. **(b)** Densitometric quantifications were normalized to β-actin (each group: n=6). **(c)** Representative western blots of autophagy marker Beclin-1 and LC3. β-actin served as loading control. **(d)** Densitometric quantifications were normalized to β-actin (each group: n=6)**.** N number indicates number of individual animals. Data are presented as mean ± SD. Differences were calculated using unpaired t-test.