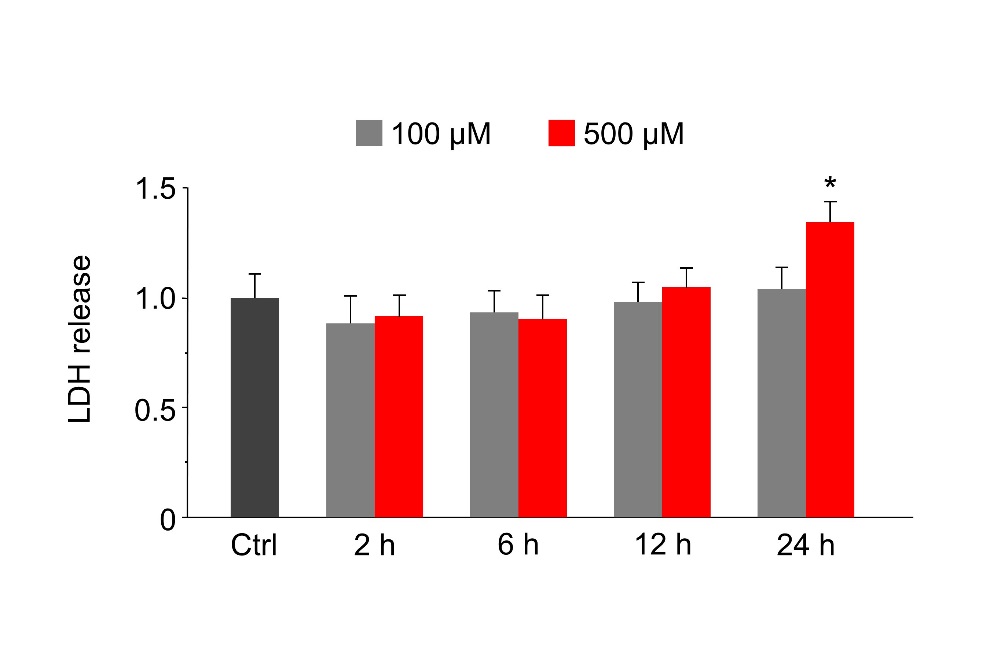
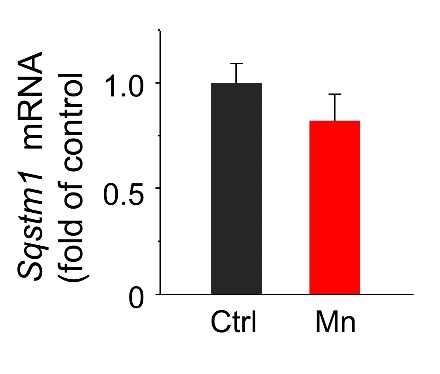
**Supplementary Figures**

**Figure S1**

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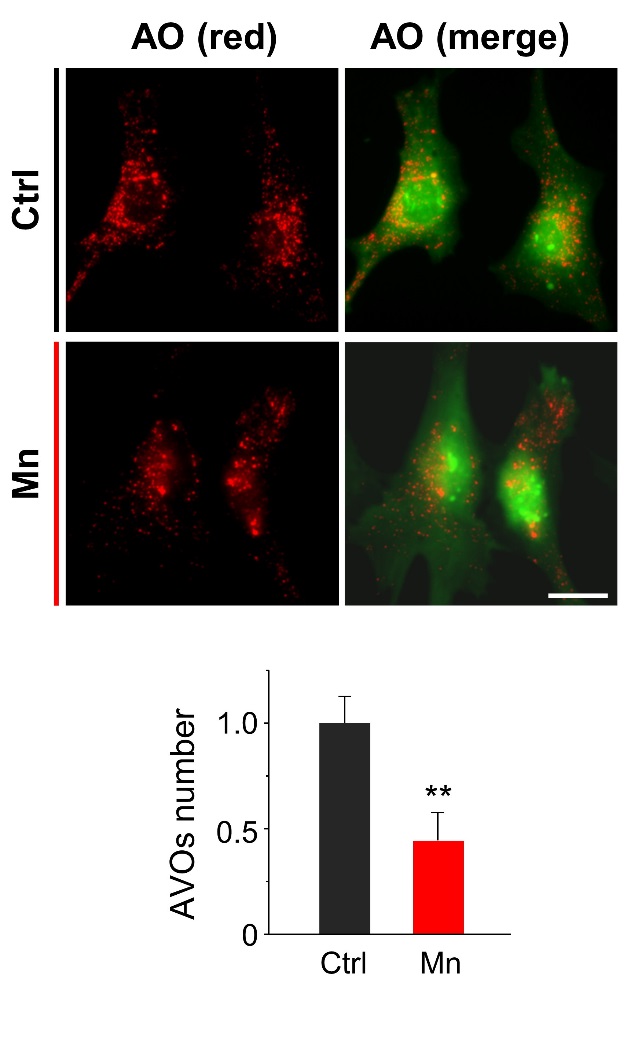
**Figure S1.** Effects of Mn on cell viability assessed by LDH release in primary astrocytes. Primary astrocytes were exposed to 100 μM or 500 μM Mn for the times indicated. Values are normalized to control. n=3. Values reflect means ± SD. *\*p*<0.05.

**Figure S2**



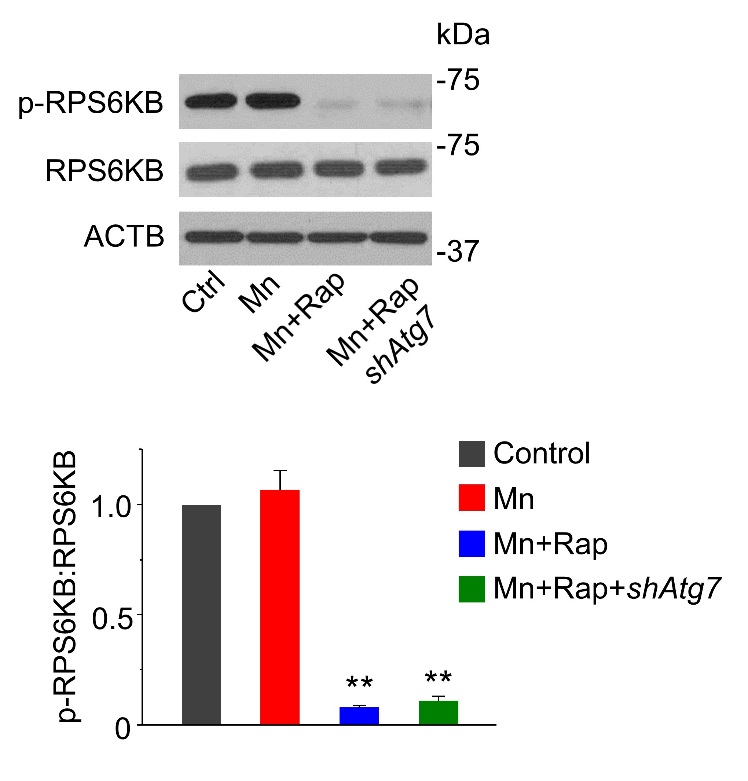
**Figure S2.** Mn exposure does not significantly alter *Sqstm1* mRNA expression. Primary astrocytes were exposed to 100 μM Mn for 24 h. The *Sqstm1* mRNA expression levels were determined by real-time qPCR. No significant difference is observed between control and Mn-treated astrocytes. n=4. Values reflect means ± SD.

**Figure S3**

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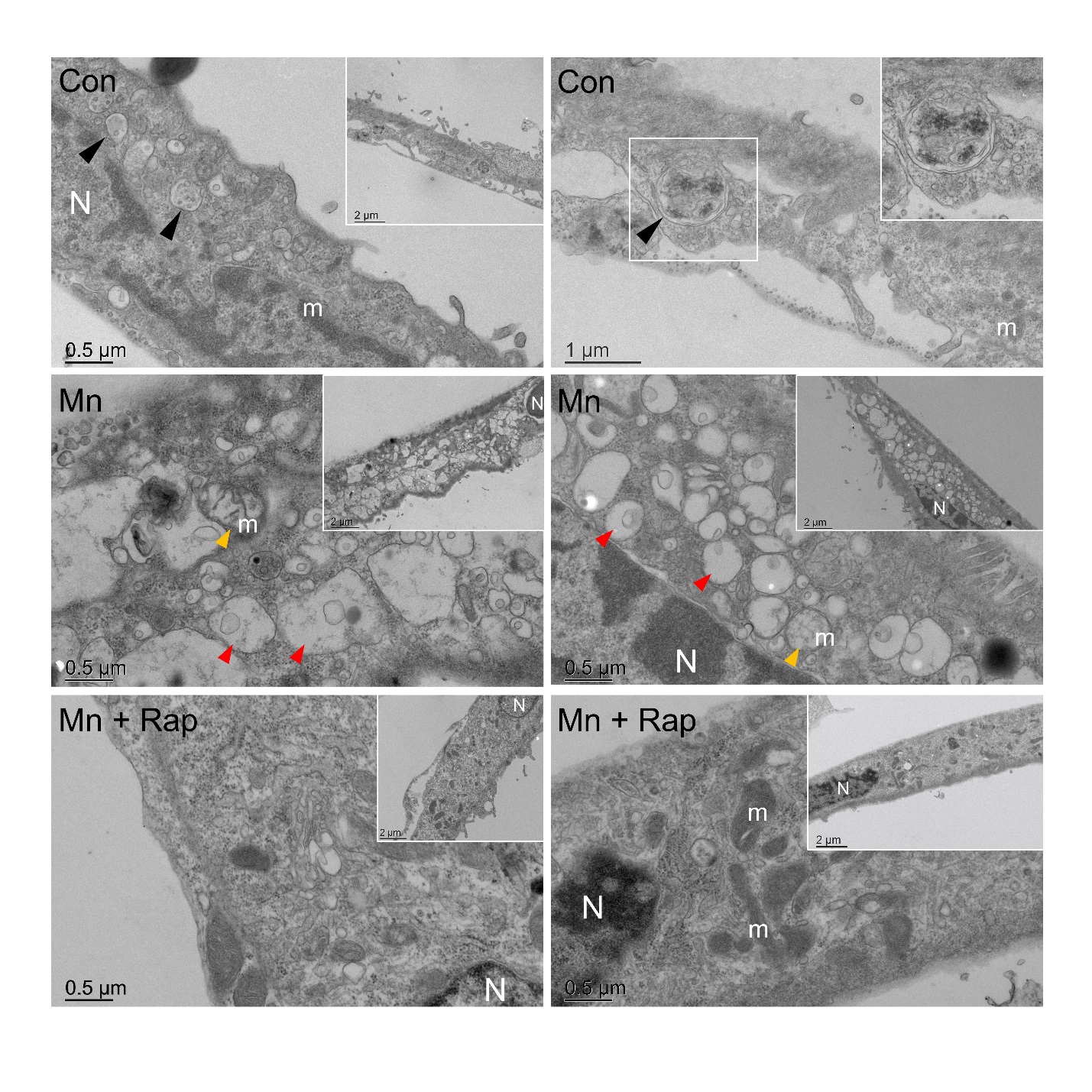
**Figure S3.** Mn reduces the number of acidic vesicular organelles (AVOs) in primary astrocytes. Primary astrocyte cultures were treated with 100 μM Mn for 24 h and stained with acridine orange (AO). Red staining is associated with acidic vesicles. Scale bar: 15 μm. Summary data show average number of red puncta per cell and are normalized to control group. n ≥ 60 astrocytes from 4 independent experiments. Values reflect means ± SD. *\*\*p*<0.01.

**Figure S4**



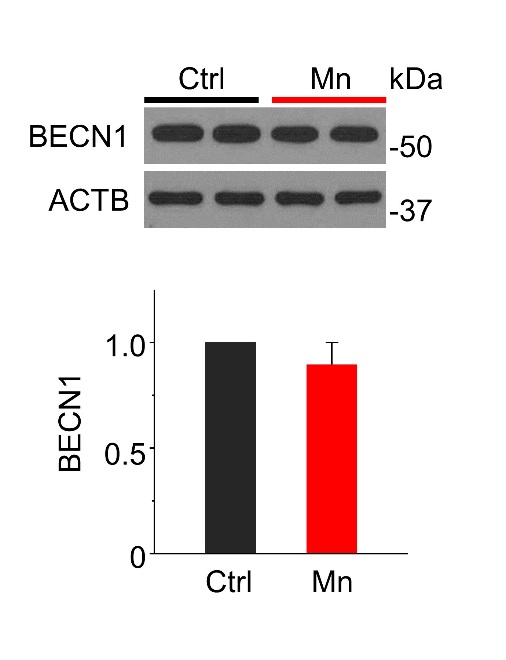
**Figure S4.** Rapamycin inhibits MTOR-RPS6KB signaling pathway in the presence of Mn. Primary astrocytes were transfected with lentivirus carrying non-target shRNA or *Atg7* shRNA. Rapamycin (100 nM) was added 2 h prior to Mn exposure (100 μM, 24 h). Western blots of p-RPS6KB and total RPS6KB. ACTB is used as a loading control. Summary data are normalized to control (n=3). Values reflect means ± SD. *\*\*p*<0.01.

**Figure S5**

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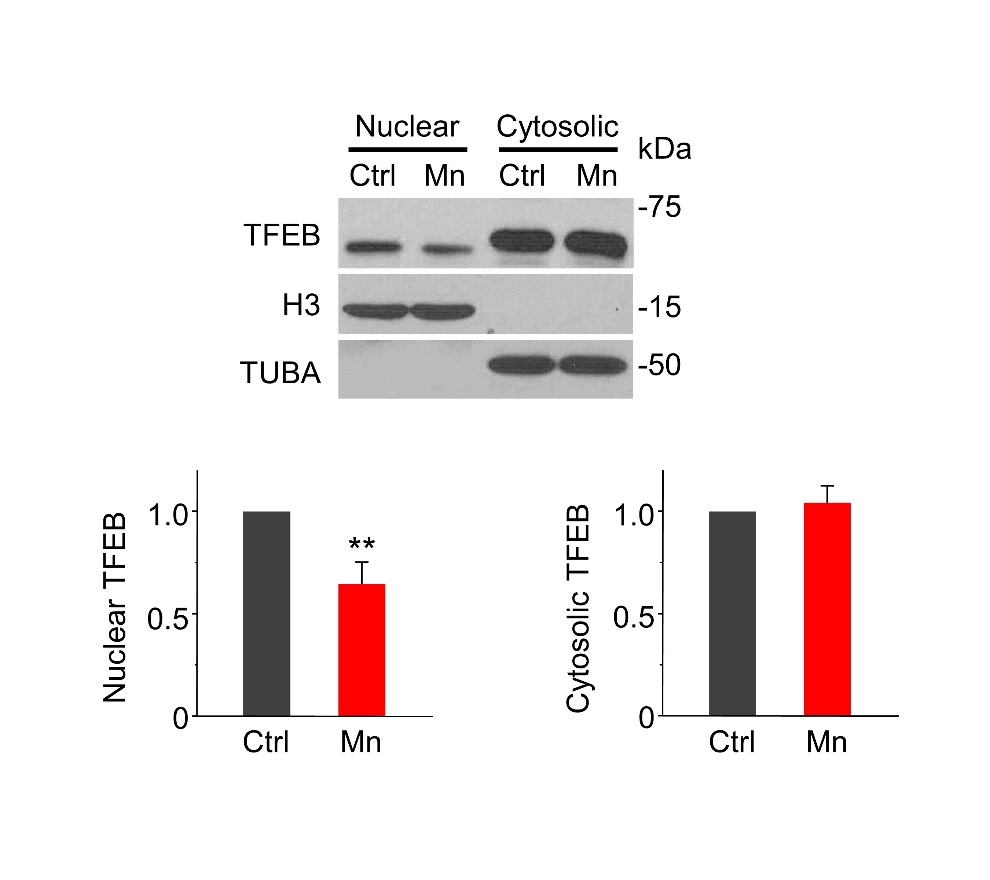
**Figure S5.** Electron micrographs showing rapamycin ameliorates ultrastructural changes in Mn-treated astrocytes. Con: control astrocytes with no Mn exposure; Mn: astrocyte treated with 100 μM Mn for 24 h; Mn+Rap: rapamycin (100 nM) was added 2 h prior to Mn exposure. **N**: nucleus; **m**: mitochondria; yellow arrow : damaged mitochondria; black arrow : autophagic structures; red arrow : unknown vesicles. Scale bar values are indicated in the images.

**Figure S6**

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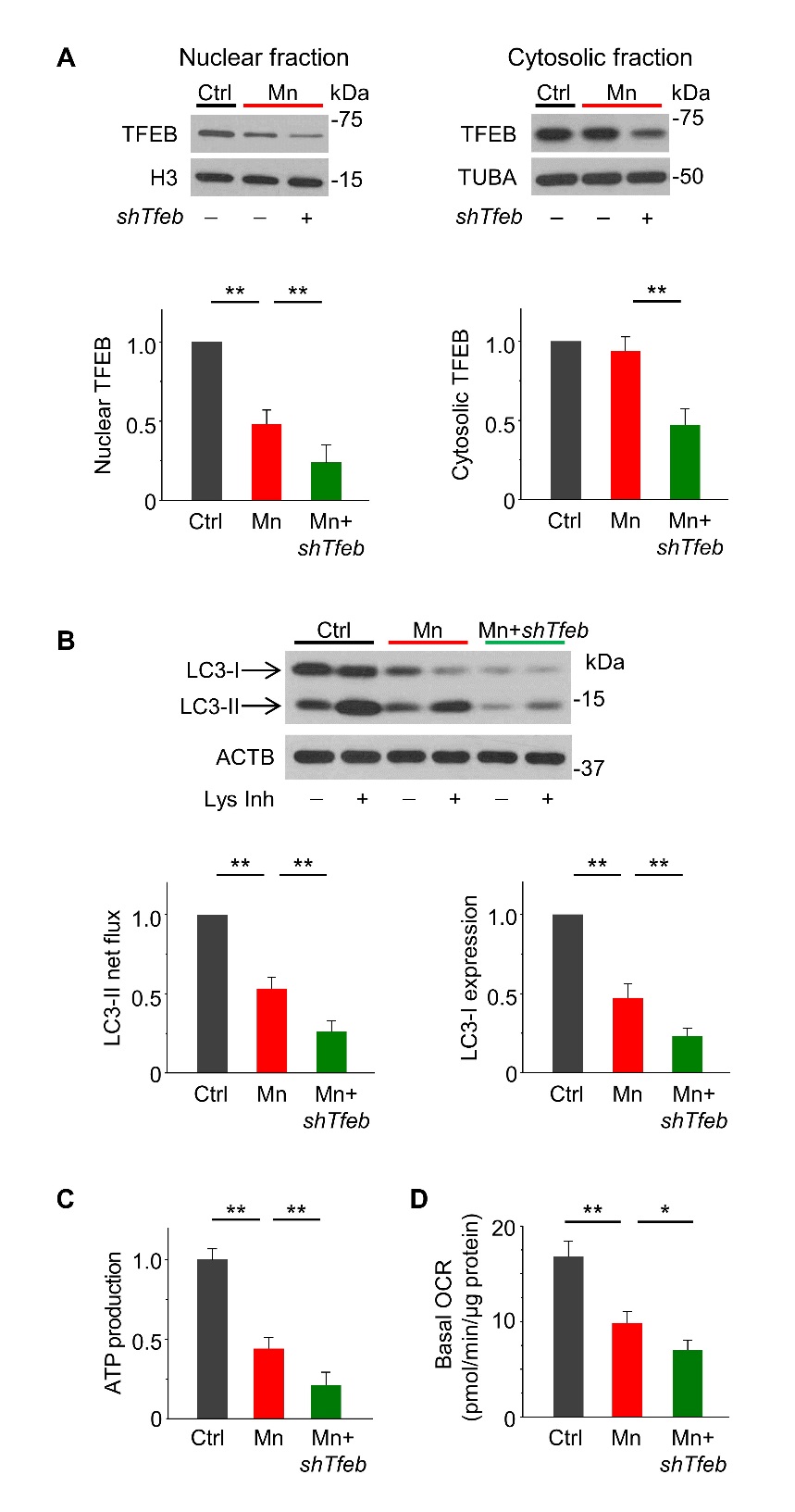
**Figure S6.** Western blots of BECN1 expression in primary astrocytes. Primary astrocytes were exposed to 100 μM Mn for 24 h. ACTB is used as a loading control. Summary data (n=3) are normalized to control. Values reflect means ± SD.

**Figure S7**

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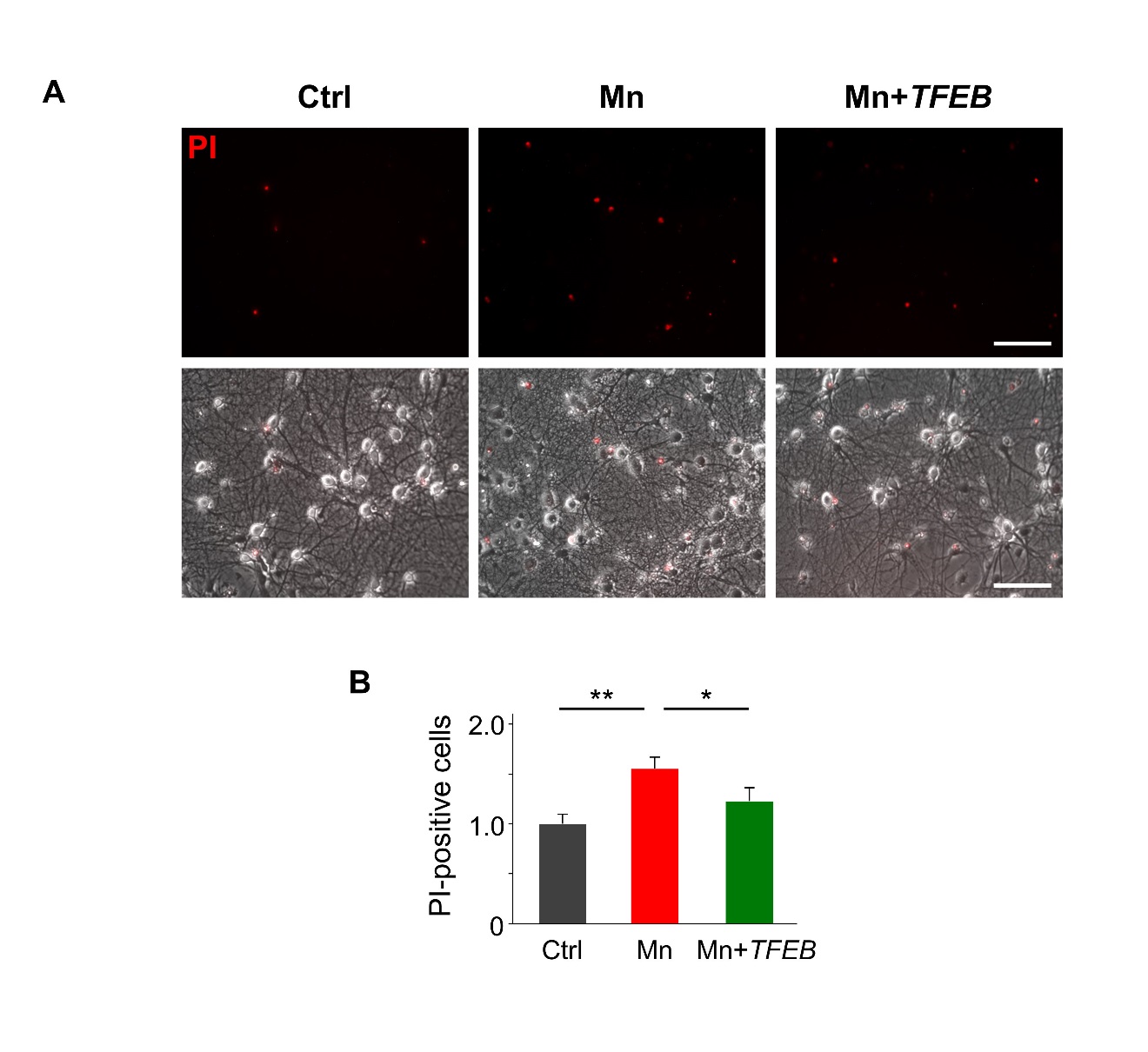
**Figure S7.** Mn decreases TFEB nuclear localization in mouse striatum. Western blots of TFEB in nuclear and cytoplasmic fraction in mice subjected to vehicle or MnCl2∙4H2O (50 mg/kg, s.c., day 0, 3 and 6) injection. Bar graphs show summary data (n=4). Nuclear and cytoplasmic fractions are normalized to histone H3 and TUBA, respectively. Summary data are normalized to control. Values reflect means ± SD. *\*\*p*<0.01.

**Figure S8**

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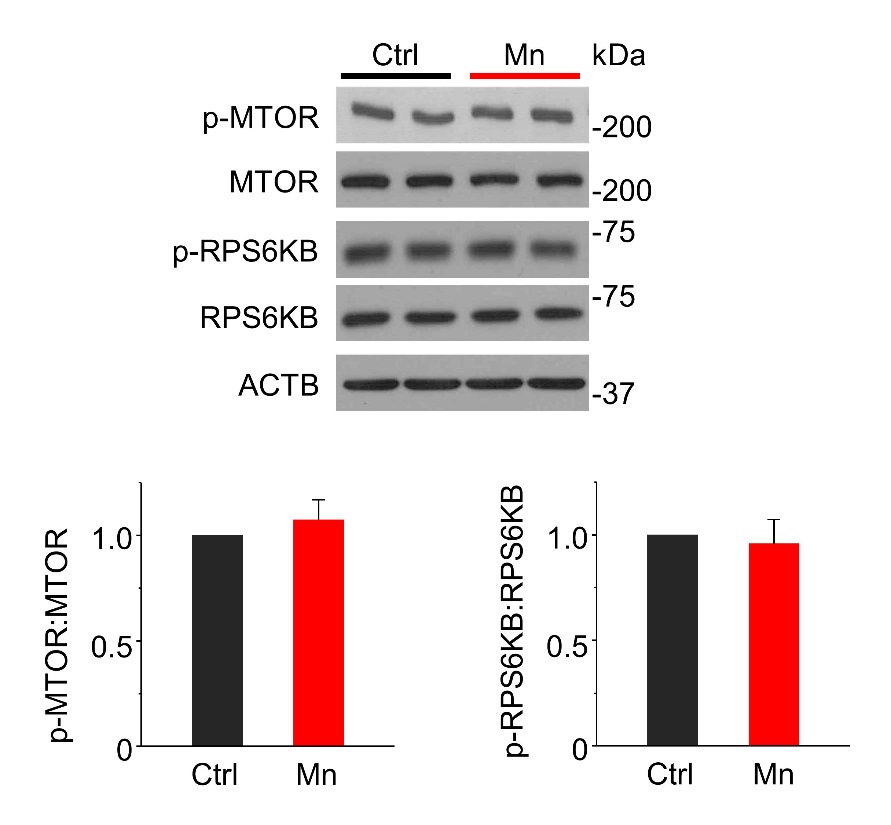
**Figure S8.** TFEB knockdown exacerbates Mn-induced autophagy failure and mitochondrial dysfunction. Primary astrocytes were transfected with lentivirus carrying non-target shRNA or *Tfeb* shRNA and then exposed to 100 μM Mn for 24 h. (**A**) Western blots of TFEB in nuclear and cytoplasmic fractions. Bar graphs show summary data (n=3). Nuclear and cytoplasmic fractions are normalized to histone H3 and TUBA, respectively.(**B**) Western blots of LC3 in the presence and absence of lysosomal inhibitors (Lys Inh, 20 mM NH4Cl and 100 μM leupeptin) for the last 2 h. ACTB is used as a loading control. Bar graphs show LC3-II flux and LC3-I protein levels (n=3). (**C**) Total intracellular ATP (n=3). (**D**) Basal OCR (n=3). Values are normalized to protein content. Values are normalized to control group in (A, B and C). Values reflect means ± SD. *\*p*<0.05, \**\*p*<0.01.

**Figure S9**

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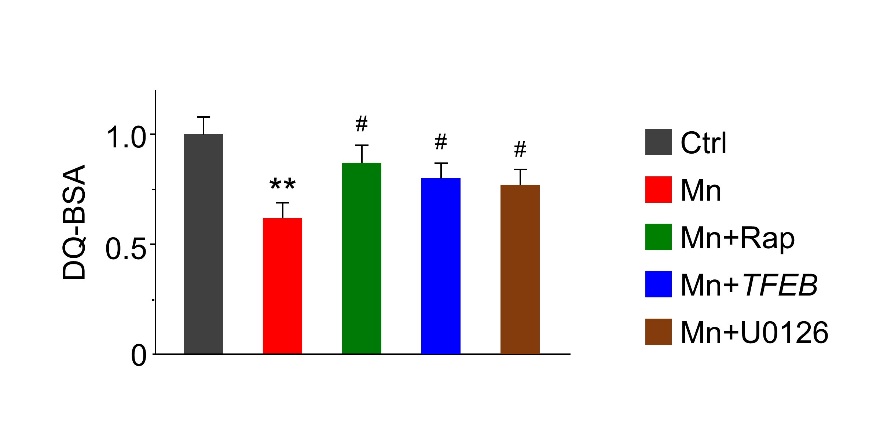
**Figure S9.** TFEB overexpressing astrocytesattenuate Mn-induced neurotoxicity in a neuron-astrocyte co-culture system. Primary astrocytes were plated on transwell inserts and transfected with lentivirus carrying *TFEB* or empty vector. Primary midbrain dopaminergic neurons were plated on 24-well plates and exposed to Mn on day 14. The neuron-astrocyte co-cultures were exposed to 100 μM Mn for 24 h. (**A**) Representative fluorescent and bright-field images showing uptake of PI (red) by primary neurons, an indicator of cell death. Scale bar: 50 μm. (**B**) Summary data of PI-stained neurons. n=5-7 coverslips per treatment group/3 independent experiments. Values are normalized to control and reflect means ± SD. *\*p*<0.05, *\*\*p*<0.01.

**Figure S10**



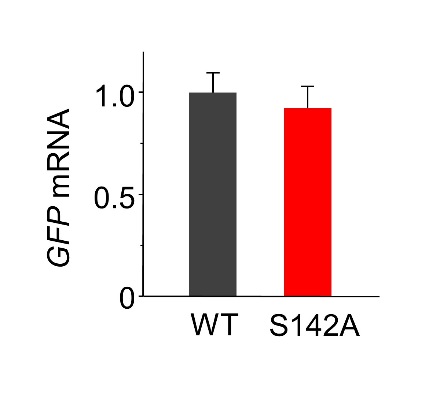
**Figure S10**. Mn exposure does not alter MTOR signaling in primary astrocytes. Primary astrocytes were exposed to 100 μM Mn for 24 h. Western blots of p-MTOR, total MTOR, p-RPS6KB and total RPS6KB. ACTB is used as a loading control. Summary data (n=3) are normalized to control. Values reflect means ± SD.

**Figure S11**



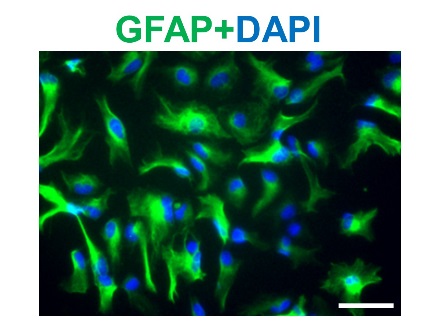
**Figure S11.** Lysosomal proteolytic activity is partially restored by treatment with rapamycin, TFEB overexpression, or U0126 in Mn-exposed astrocytes. Lysosomal proteolytic activity is measured by DQ-BSA fluorescence intensities. Summary data (n=3) are normalized to control. Values reflect means ± SD. *\*\*p*<0.01 vs. control, *#p*<0.05 vs. Mn.

**Figure S12**



**Figure S12.** Quantitative PCR shows equal levels of GFP mRNA (reporter) are expressed in astrocytes transfected with lentivirus expressing wild type (WT) TFEB-GFP or site-mutant TFEBS142A-GFP (n=4). Values reflect means ± SD.

**Figure S13**

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**Figure S13.** Primary astrocyte cultures are >95% positive for the astrocyte marker GFAP. Primary astrocytes were immunostained for GFAP (green) and counterstained with DAPI (blue). Scale bar: 50 μm.