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# Supplementary Materials for

## Manganese activates NLRP3 inflammasome signaling and propagates exosomal release of ASC in microglial cells

Souvarish Sarkar, Dharmin Rokad, Emir Malovic, Jie Luo, Dilshan S. Harischandra, Huajun Jin, Vellareddy Anantharam, Xuemei Huang, Mechelle Lewis, Arthi Kanthasamy, Anumantha G. Kanthasamy*

*Corresponding author. Email: akanthas@iastate.edu

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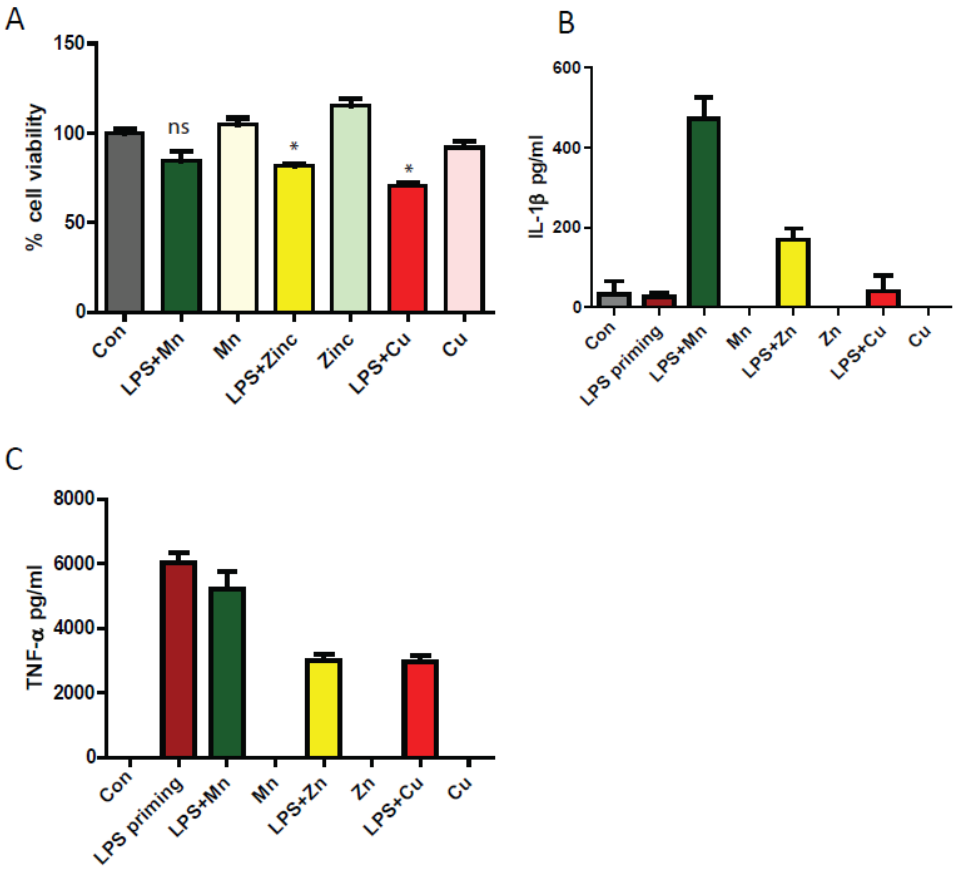


**Fig. S1. Mn specifically induced inflammasome activation in primary microglial culture.** (**A**) MTS assay analysis of cell viability in primary microglial cells treated as indicated. Data are means ± SEM from 4 independent experiments. (**B** and **C**) Luminex assay of IL-1β (B) and ELISA analysis of TNF-α production (C) by primary microglial cells treated as indicated. Data are means ± SEM from 4 independent experiments. *P<0.05 by ANOVA with Tukey post hoc analysis.

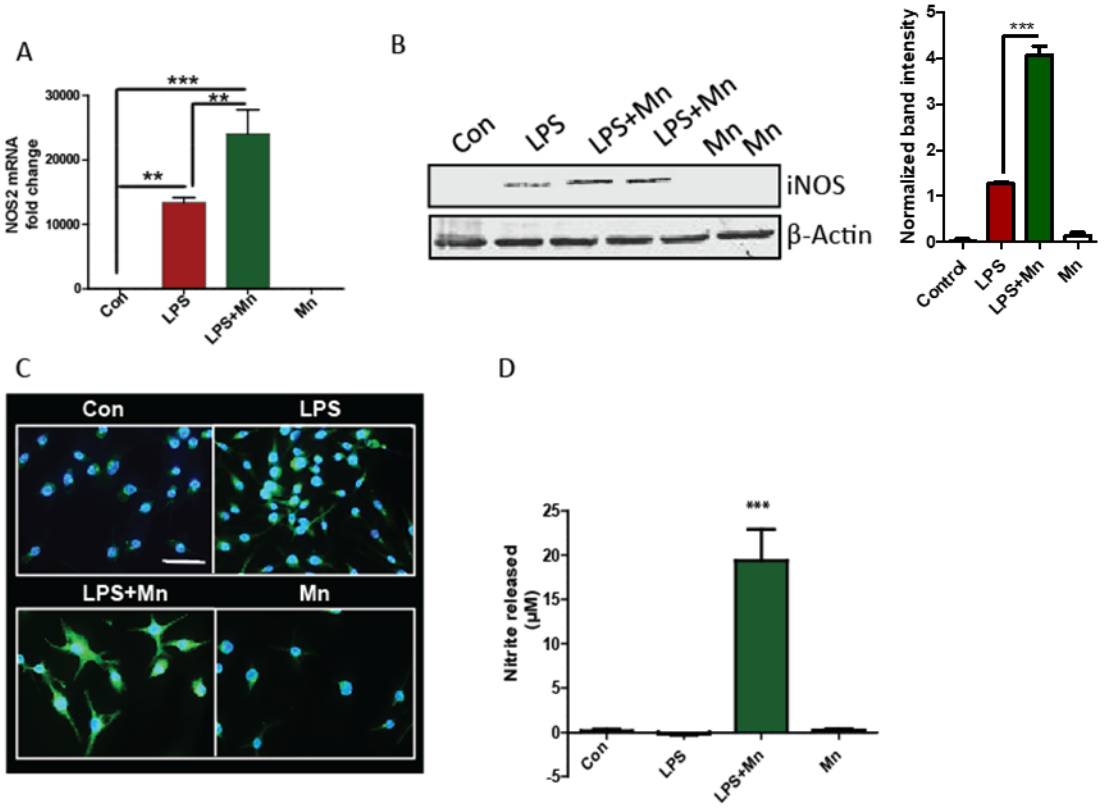


**Fig. S2. Mn enhanced LPS-induced nitrite generation.** (**A**) *Nos2* expression was analyzed by qRT-PCR in microglial cells. Data are means ± SEM pooled from 3 independent experiments. (**B** and **C**) Western blot (B) and immunofluorescence microscopy (C) analysis of iNOS abundance in microglial cells. Blots and images are representative of 3 independent experiments. Quantified band intensity values (right panel of B) are means ± SEM of all experiments. (**D**) Nitrite release was analyzed by the Griess colorimetric assay in microglial cells. Data are means ± SEM pooled from 4 independent experiments. **P<0.01 and ***P<0.001 by ANOVA with Tukey post hoc analysis.

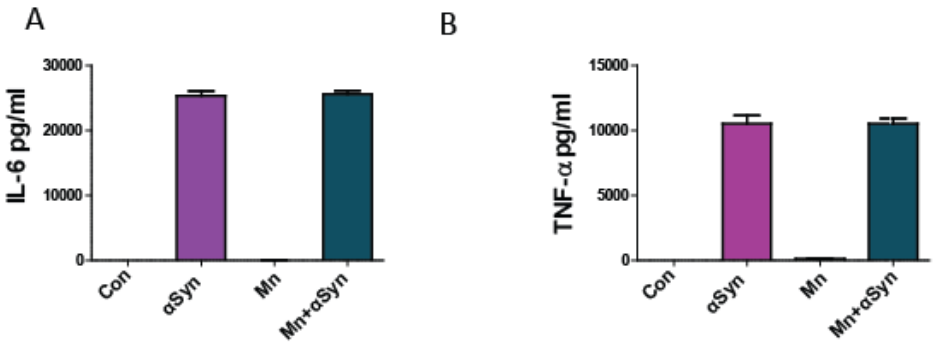


**Fig. S3. Mn exposure did not inhibit αSyn$_{Agg}$-induced proinflammatory cytokine production.** (**A** and **B**) Luminex assay analysis of IL-6 (A) and TNF-α (B) production by microglial cells treated as indicated. Data are means ± SEM pooled from 4 independent experiments.

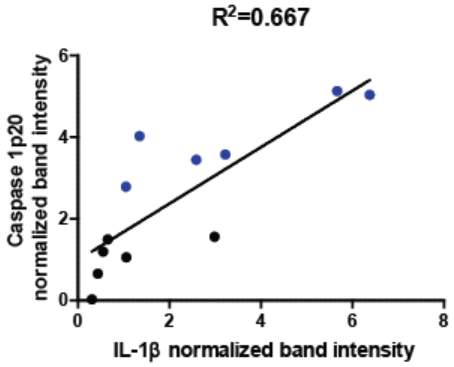


**Fig. S4. Caspase-1-p20–induced cleavage of pro–IL-1β.** Correlation of band intensity values from Western blot analysis of Caspase-1-p20 and IL-1β abundance in lysates from microglial cells of control (black) or $Mn^{2+}$-treated (blue) animals (Fig. 2D). Data are means from 6 independent experiments.

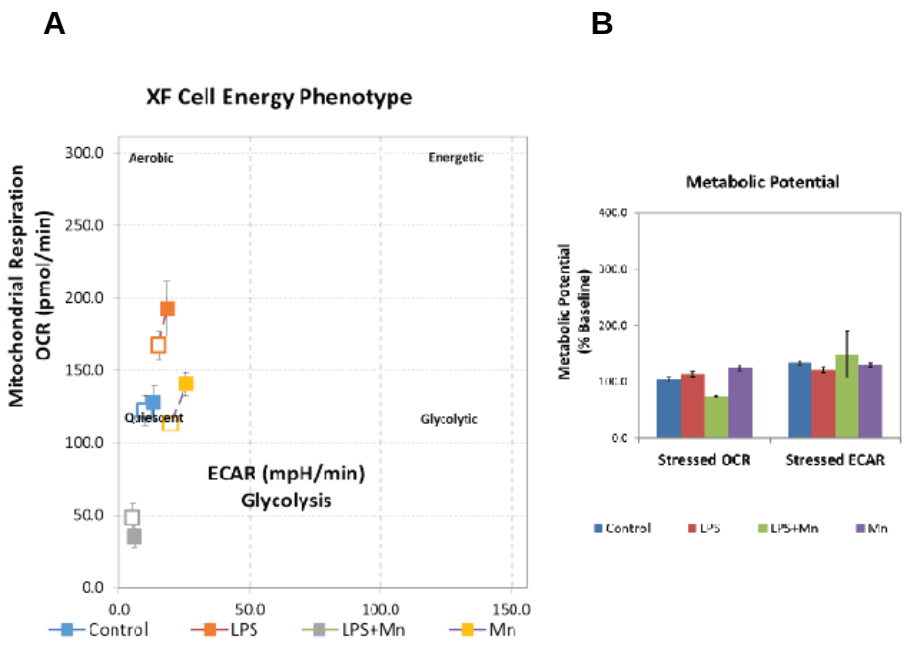


**Fig. S5. Mn exposure inhibited microglial mitochondrial respiration.** (**A** and **B**) Seahorse MitoStress analysis of the metabolic phenotype (A) and the metabolic potential (B) of microglial cells treated as indicated. Data are means ± SEM pooled from 5 independent experiments.

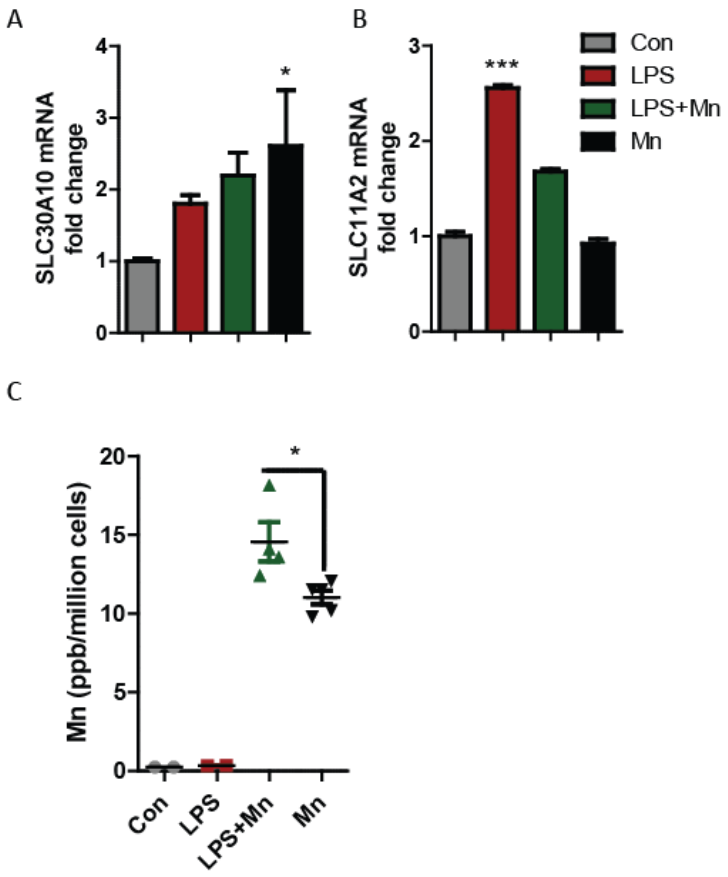


**Fig. S6. LPS increased Mn transporters and Mn uptake.** (**A** and **B**) qRT-PCR analysis of *Slc30a10* (A) and *Slc11a2* (B) mRNA expression in microglial cells treated as indicated. (**C**) Mass spectrometry analysis of Mn abundance in microglial cells treated as indicated. All data are means ± SEM pooled from 3-4 independent experiments. *P<0.05 and ***P<0.001 by ANOVA with Tukey post hoc analysis.

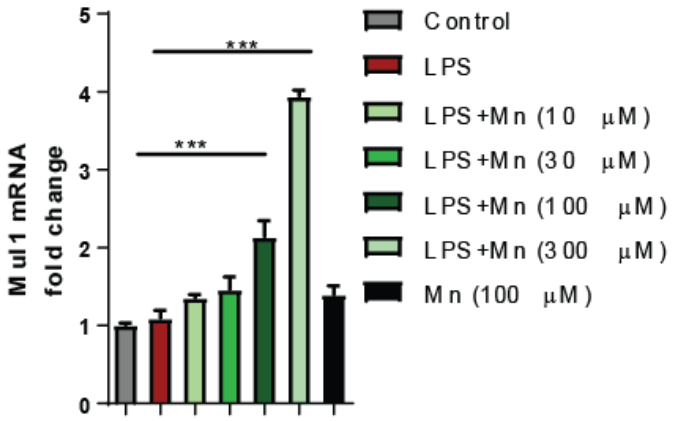


**Fig. S7. Mn increased Mul1 expression in a dose-dependent manner.** qRT-PCR analysis of *Mul1* mRNA expression in microglial cells after Mn treatment at the indicated dose. Data are means ± SEM pooled from 3 independent experiments. ***P<0.001 by ANOVA with Tukey post hoc analysis.

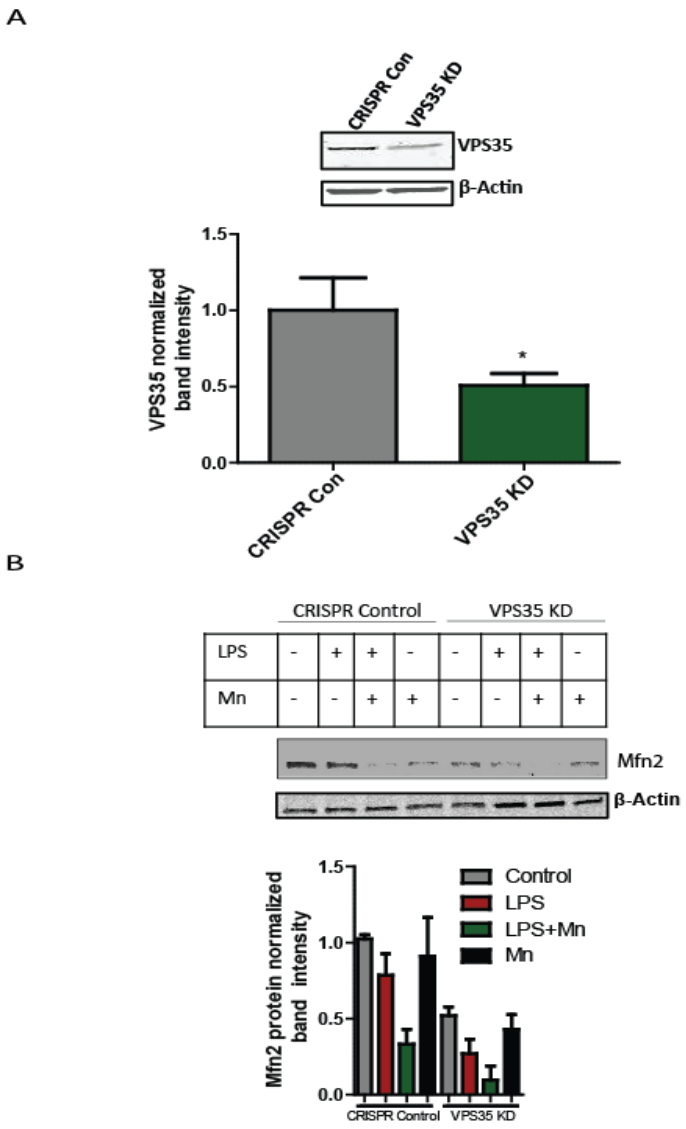


**Fig. S8. CRISPR-Cas9 VPS35 KD results in Mfn2 degradation.** (**A**) Western blot analysis of Mfn2 abundance in lysates of VPS35 knockdown microglial cells. Blots (upper) are representative of 3 independent experiments. Quantified band intensity values (lower) are means ± SEM of all experiments. (**B**) Western blot analysis of Mfn2 abundance in lysates of VPS35 knockdown microglial cells treated as indicated. Blots (upper) are representative of 3 independent experiments. Quantified band intensity values (lower) are means ± SEM of all experiments. *P<0.05 by two-way ANOVA with Tukey post hoc analysis.

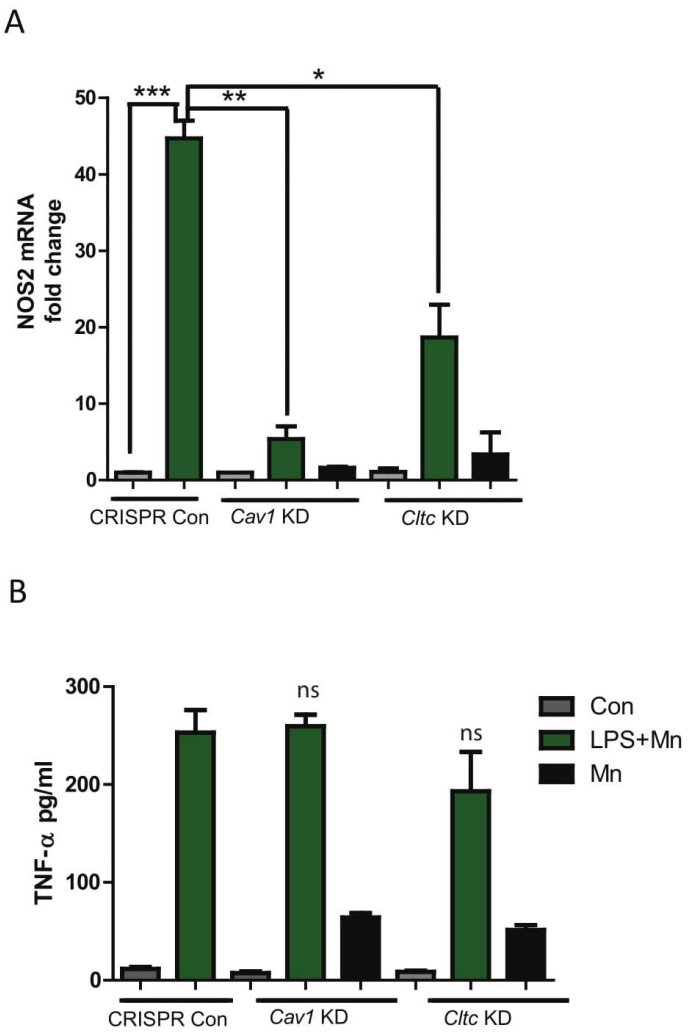


**Fig. S9. Caveolin and clathrin KD reduced Mn-induced Nos2 expression but not TNF-α.** (**A**) qRT-PCR analysis of *Nos2* mRNA expression in caveolin- and clathrin-KD cells after Mn treatment. **B**) Luminex analysis of TNF-α secretion by Mn in a primed microglial cell line. All data are means ± SEM pooled from 8 independent experiments. *P<0.05, **P<0.01, ***P<0.001 via two-way ANOVA with Tukey post hoc analysis.

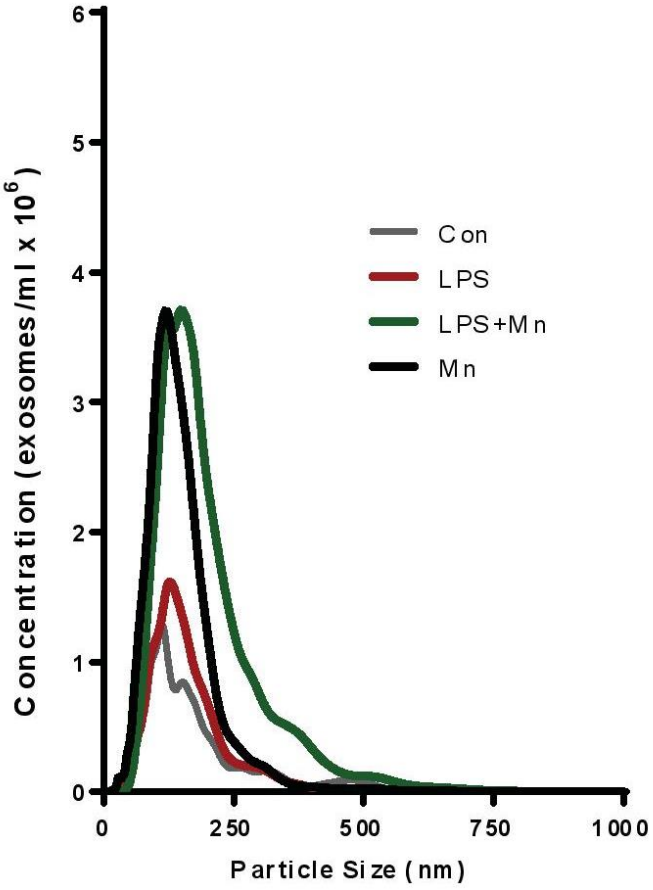


**Fig. S10. Mn exposure did not change the size distribution of the exosomes secreted by microglial cells.** NanoSight analysis of the size distribution of exosomes from microglia exposed to Mn. Data are representative of 3 independent experiments.

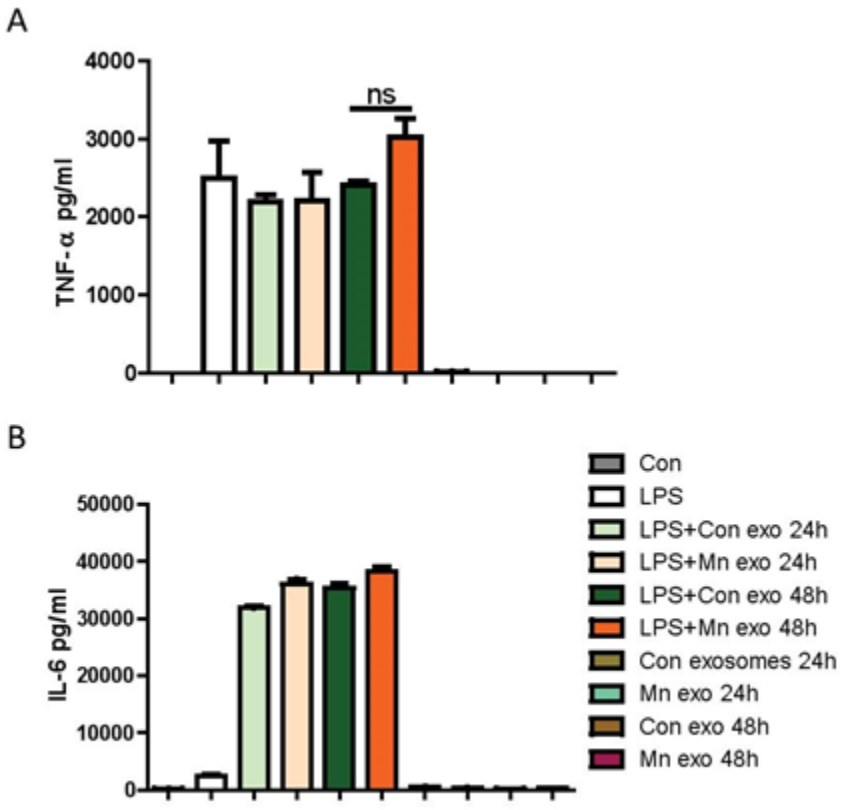


**Fig. S11. Exposure to exosomes isolated from Mn-gavaged animals did not alter release of IL-6 and TNF-α. (A** and **B)** Luminex assay analysis of TNF-α (A) and IL-6 (B) in exosome-treated microglial cells from Mn-gavaged animals. Data are means ± SEM pooled from 8 independent experiments. ns, not significant by Student's t-test.

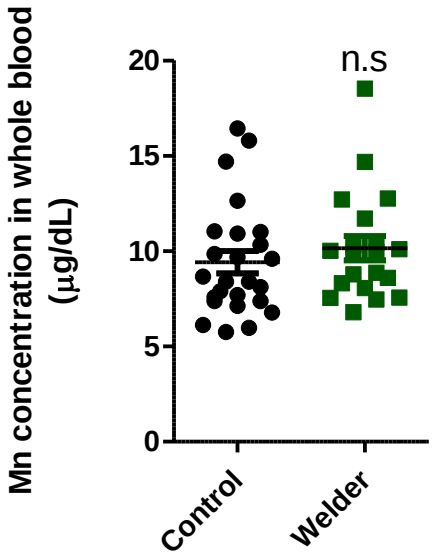


**Fig. S12. Mn concentration in whole blood is unaltered in welders.** ICP-MS analysis of Mn concentration in whole blood from control and welder groups. Data are means ± SEM from 25 control subjects and 16 welders. n.s., not significant by Student's t-test.

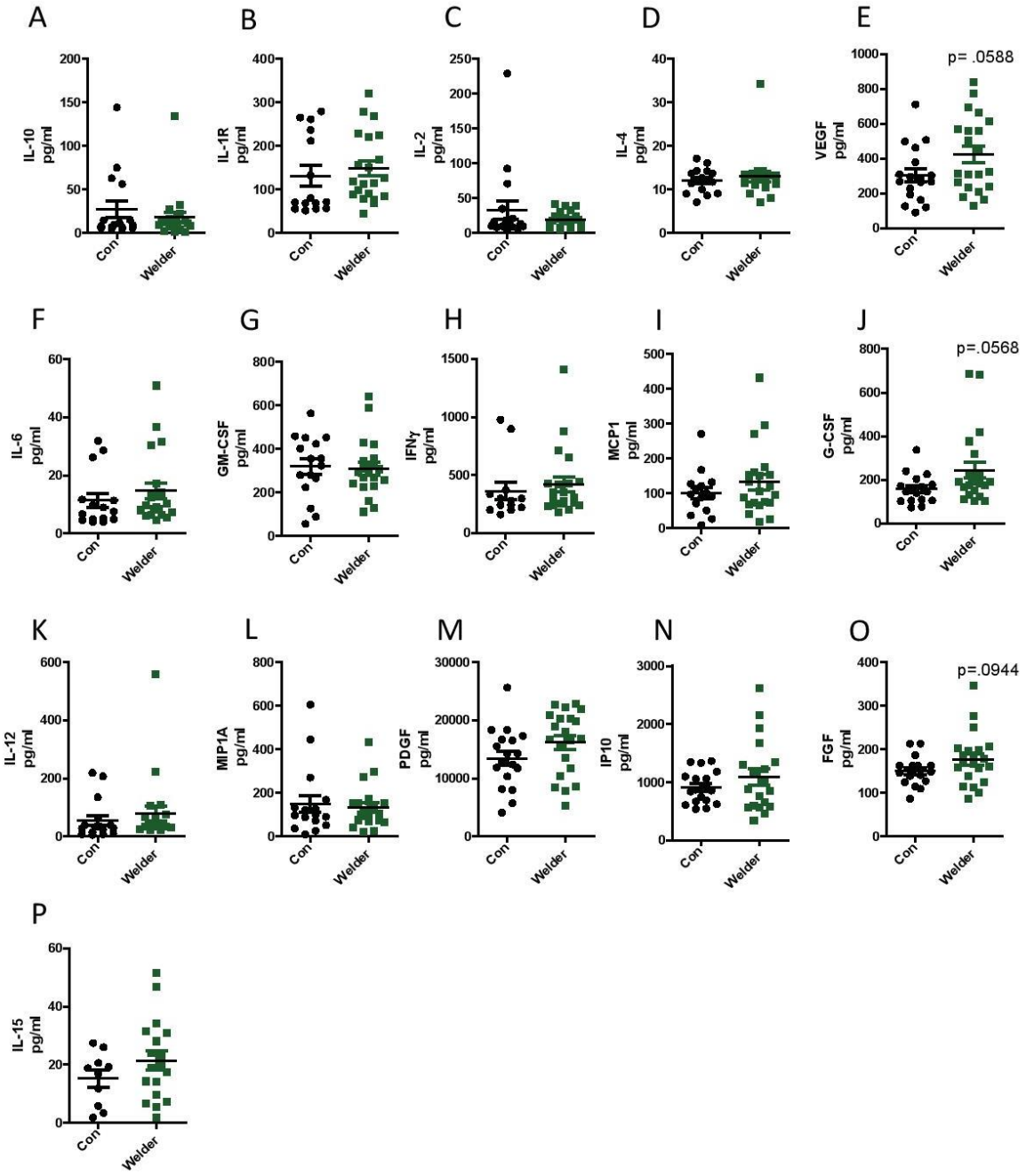


**Fig. S13. Proinflammatory cytokine profile of welder and age-matched control populations.**

(**A** to **P**) Luminex analysis of the indicated cytokines in serum from welders and age-matched controls. Data are means ± SEM from 17 control and 21 welder subjects. P-values were determined by Student's t-test.