ROS

RESEARCH ARTICLE

SUPPLEMENTAL MATERIAL

Manganese-Containing Thiocarbamates Cause Free Radical Production and Caspase-Independent Cell Death following Mitochondrial Dysfunction in Neural Cells

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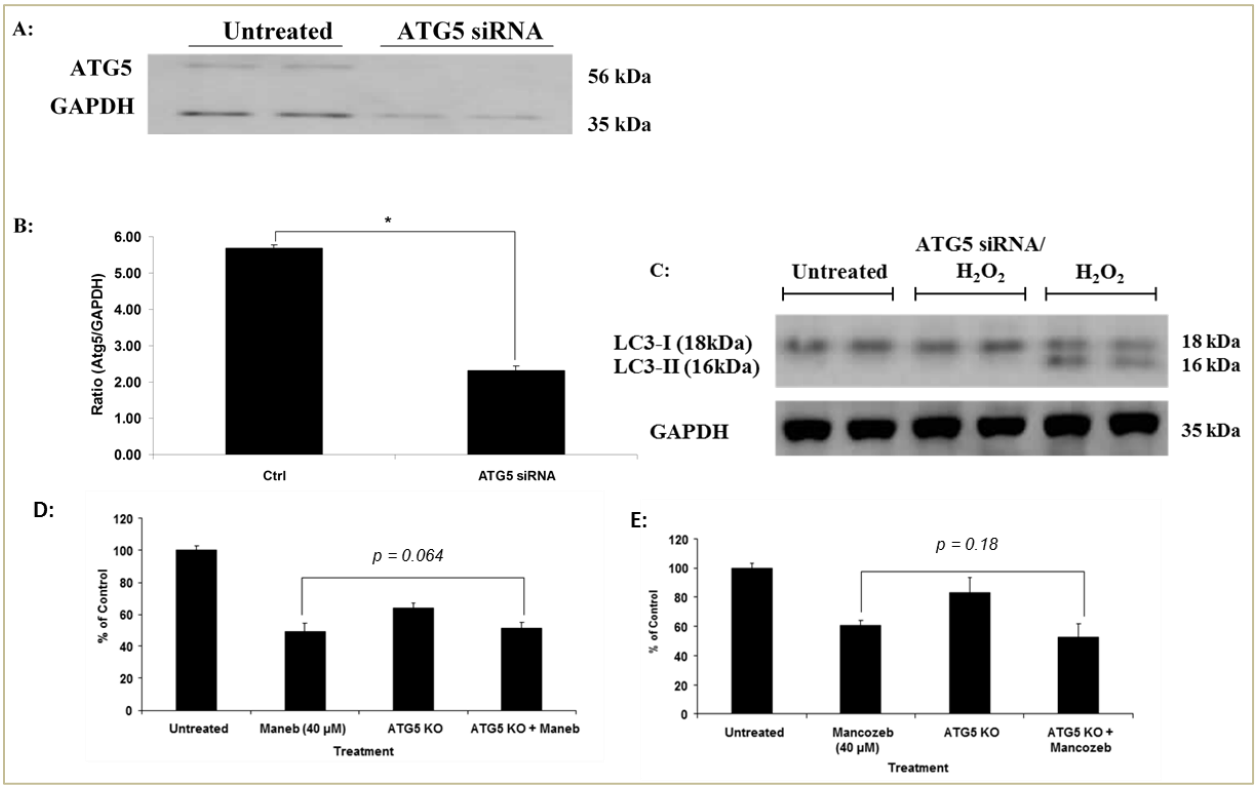
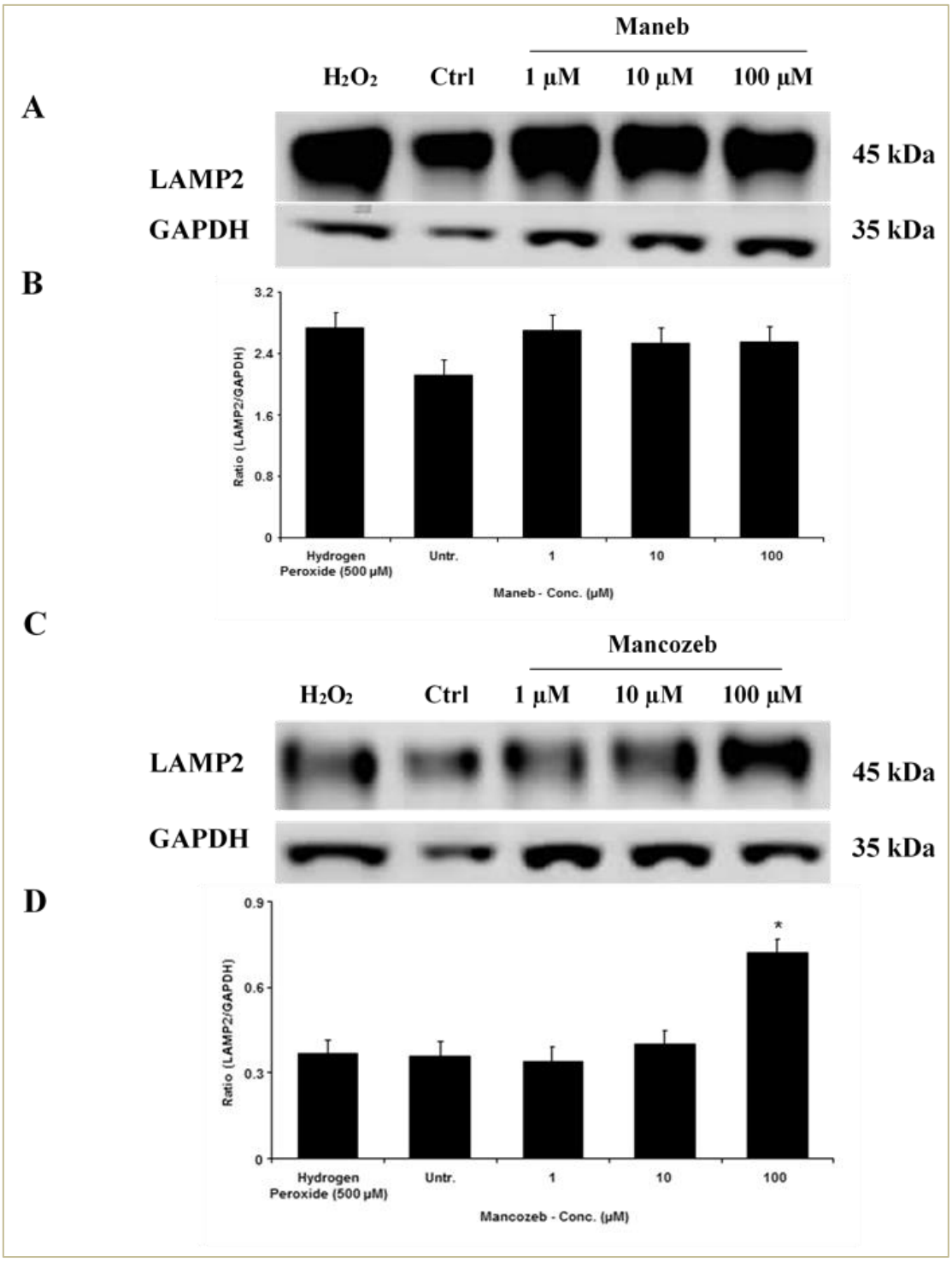
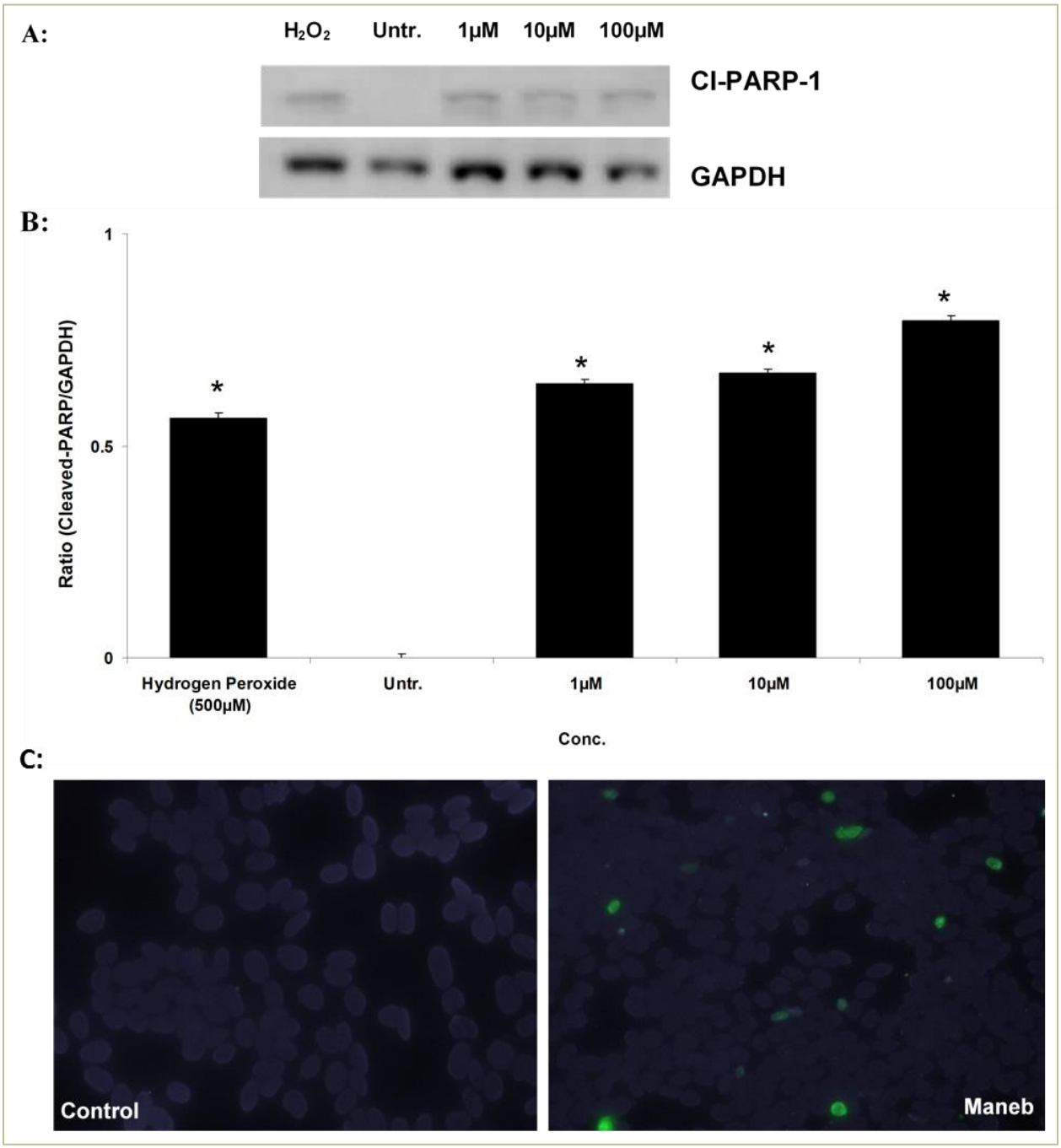


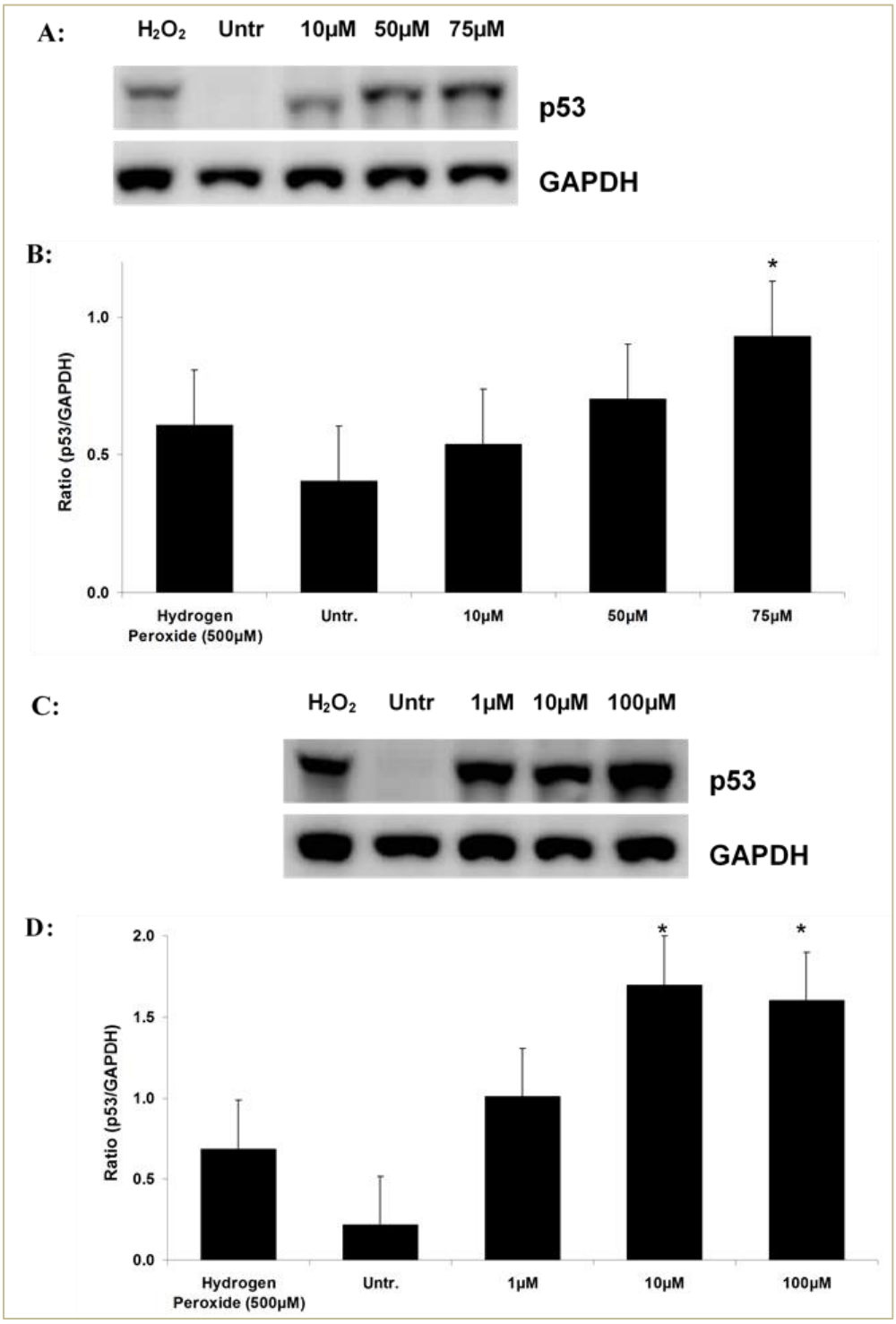
SUPPLEMENTAL FIGURE 1. ATG5 knockdown does not alter response to maneb or mancozeb in SH-SY5Y cells. (A) and (B) ATG5 protein levels were measured using Western blotting and ATG5/GAPDH ratio showed a decrease of ~40% in ATG5 protein levels in siRNA-transfected cells after 72 h. (C) LC3 (16 kDa) bands were only visible in H_2O_2 (500 μM)-treated cells (positive control) and failed to be observed in siRNA-treated cells indicating a positive functional effect. Alamar blue reduction assay showed that ATG5 siRNA knockdown was unable to attenuate toxicity by (D) maneb or (E) mancozeb (mean ± SD, n = 3; *, $p < 0.05$).



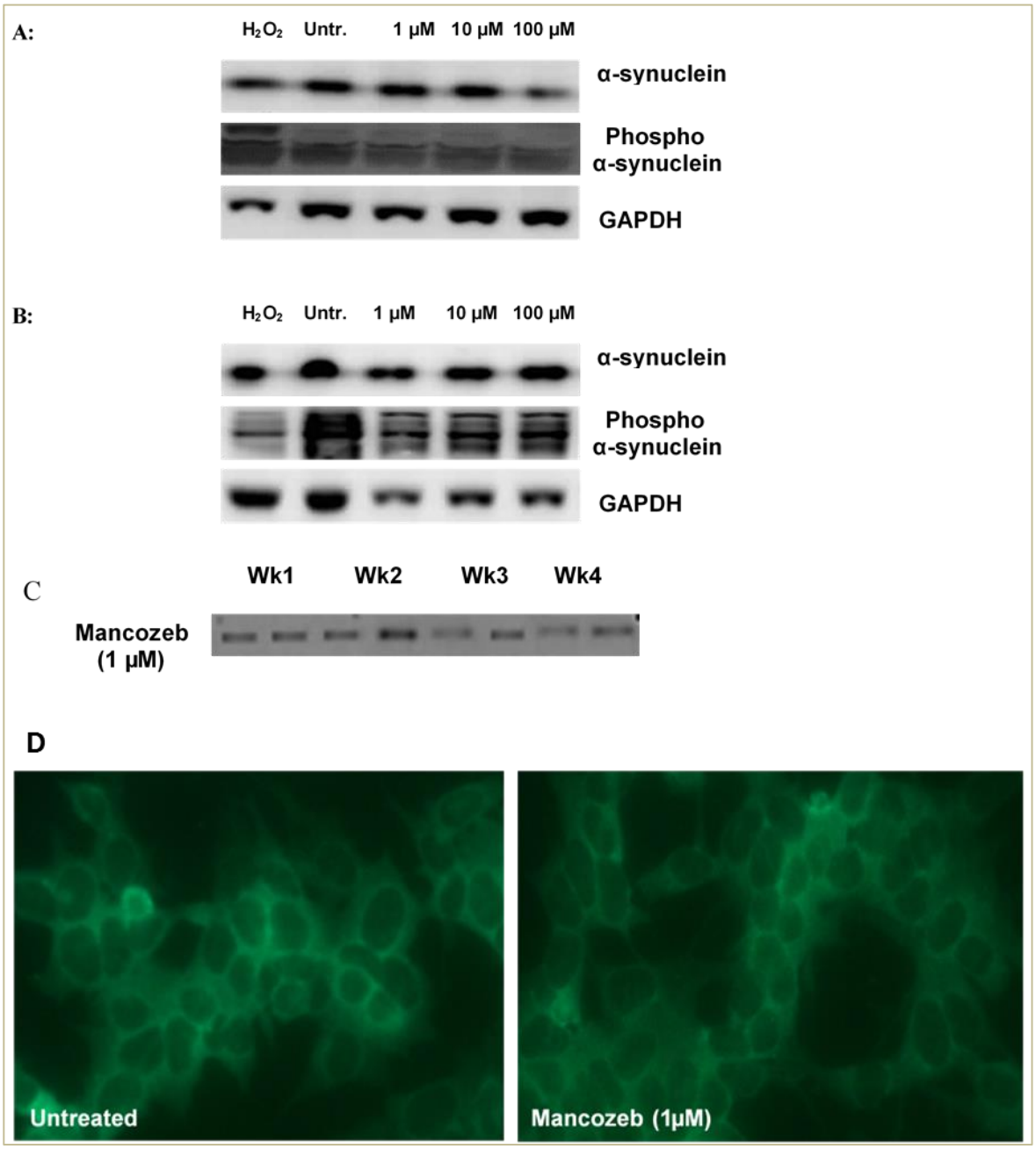
SUPPLEMENTAL FIGURE 2. Toxin-induced changes in LAMP-2 levels. SH-SY5Y cells were treated with different doses of maneb (A and B) or mancozeb (C and D) for 24 h after which cell extracts were probed for LAMP-2 protein (mean ± SD, n = 3; *, $p < 0.05$ compared with untreated control).



SUPPLEMENTAL FIGURE 3. Effect of toxin treatment on the expression of cell death markers. Expression of cleaved-PARP-1 was determined after 24 h treatment. (A, B, and C) Maneb and also mancozeb (not shown) showed significant induction of cleaved PARP only after 24 h exposure to compound with nuclear location of protein (C) (mean ± SD, n = 3; *, $p < 0.05$ compared with untreated control).



SUPPLEMENTAL FIGURE 4. **Toxin-induced changes in p53 levels.** SH-SY5Y cells were treated with (A and B) mancozeb or (C and D) maneb for 24 h after which cell extracts were probed for p53 (mean ± SD, n = 3; *, $p < 0.05$ compared with untreated control).



SUPPLEMENTAL FIGURE 5. Expression of α-synuclein in toxin-treated cells. SH-SY5Y cells were treated with (A) maneb or (B) mancozeb for 24 h after which cell extracts were probed for α-synuclein and phosphorylated α-synuclein. (C) SH-SY5Y cells were additionally grown in medium containing mancozeb, for 4 weeks after which cell extracts were probed for α-synuclein although no change was seen in levels. (D) No change was observed in the distribution of α-synuclein in chronically exposed cells (only mancozeb exposure is shown).