

Electronic supplementary information (ESI)

Zinc intoxication induces ferroptosis in A549 human lung cells

Lauren D. Palmer, Ashley T. Jordan, K. Nichole Maloney, Melissa A. Farrow, Danielle B. Gutierrez, Randi Gant-Branum, William J. Burns, Carrie E. Romer, Tina Tsui, Jamie L. Allen, William N. Beavers, Yuan-Wei Nei, Stacy D. Sherrod, D. Borden Lacy, Jeremy L. Norris, John A. McLean, Richard M. Caprioli, Eric P. Skaar*

*eric.skaar@vumc.org

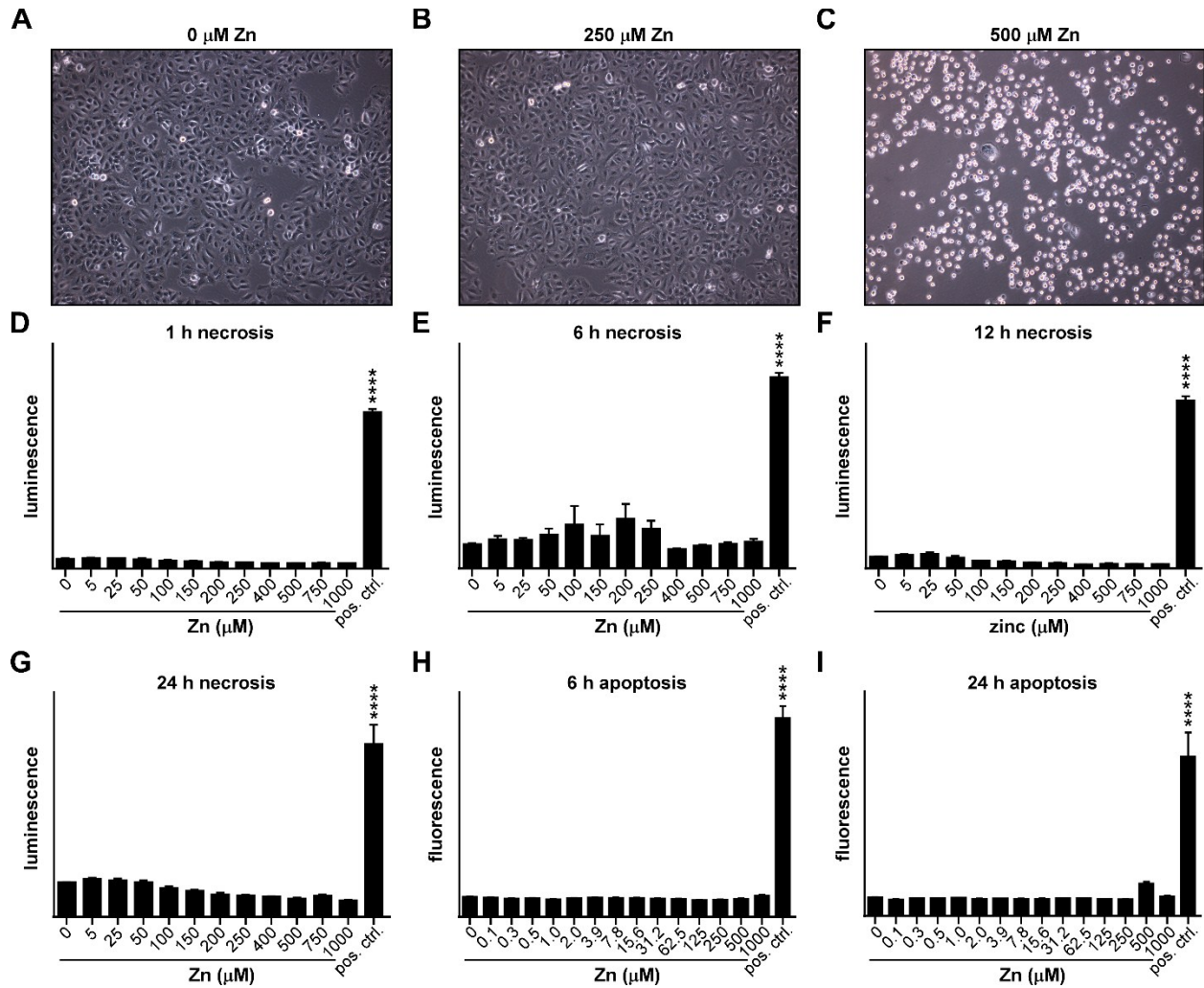


Figure S1: Zinc does not induce apoptosis or necrosis. (A-C) Representative images of A549 cells 24 h after treatment with 0, 250, or 500 µM zinc. (D-G) Necrosis at 1, 6, 12 and 24 h by measurement of lactate dehydrogenase release by CytoTox-Glo. (H-I) Apoptosis at 6 h and 24 h by measurement of caspase-3/7 cleavage by ApoOne. Statistics are by ANOVA with Sidak's multiple comparisons to test significant increase in signal compared to 0 µM zinc.

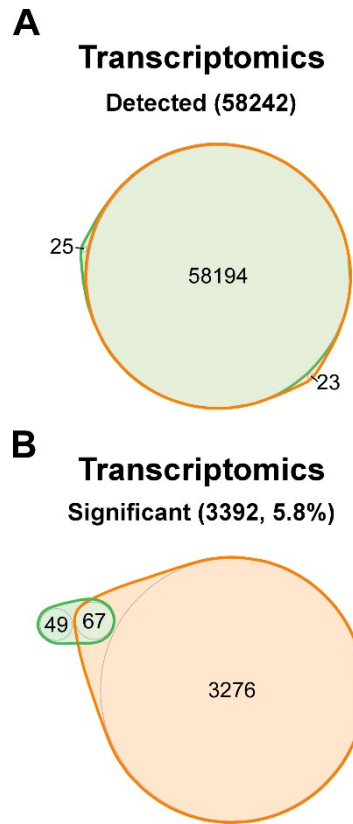


Figure S2: Transcriptomics data over time. (A) Detected transcripts are displayed across time.

(B) Significantly changed transcripts are displayed across time.

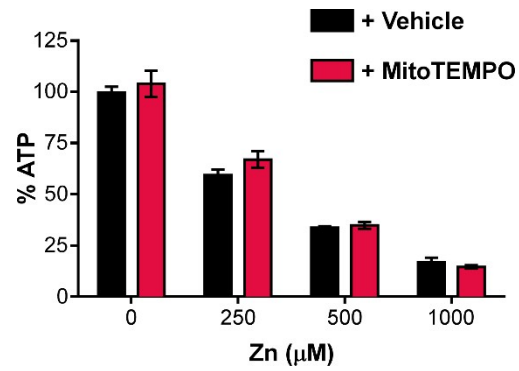


Figure S3: Addition of MitoTEMPO does not rescue viability of A549 cells intoxicated with zinc. A549 cells were treated with Zn (0-1000 μM) and MitoTEMPO (100 μM) overnight and viability was assessed. No significant difference between vehicle and MitoTEMPO treated conditions by two-way ANOVA with Sidak's multiple comparisons.

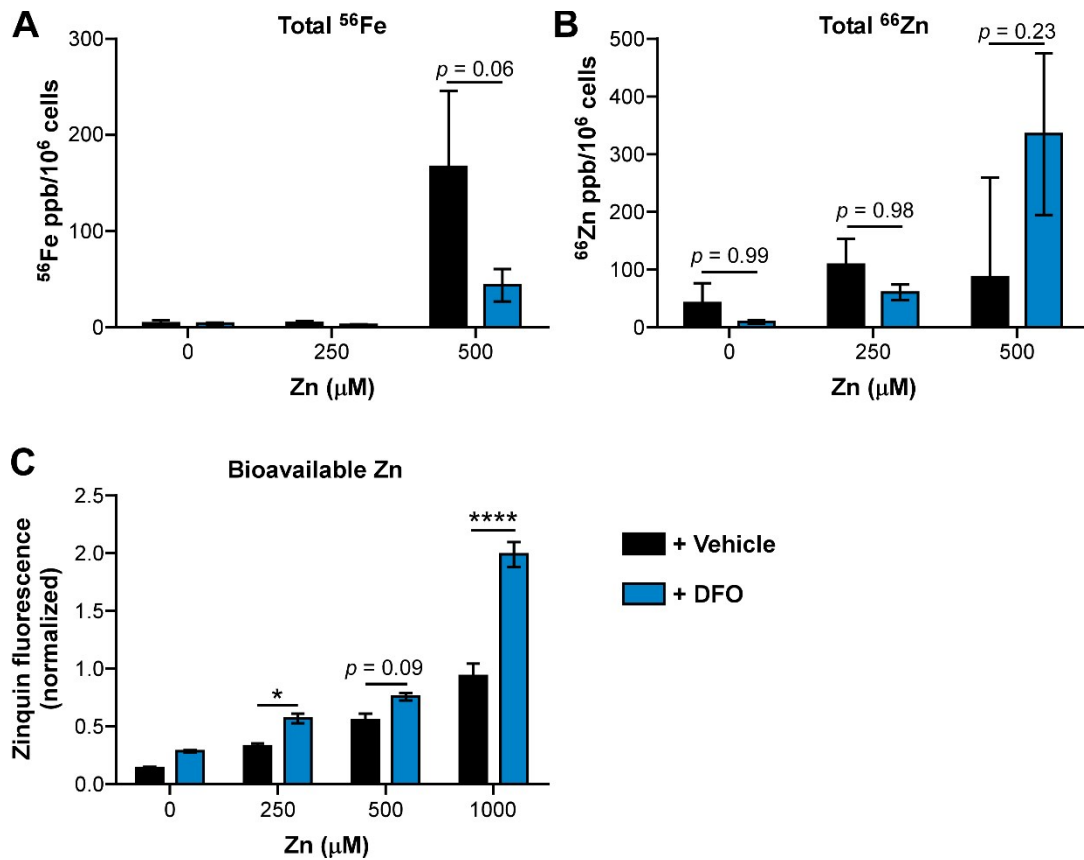


Figure S4: Desferoxamine treatment does not decrease total or bioavailable zinc. A549 cells were pre-treated with ddH₂O (vehicle) or desferoxamine (DFO; 100 μM) 30 min prior to Zn intoxication. (A-B) ^{56}Fe and ^{66}Zn were measured by inductively coupled mass spectrometry. (C) Bioavailable Zn was measured by Zinquin fluorescence normalized to SytoOrange 82 fluorescence. Statistics indicated are two-way ANOVA with Sidak's multiple comparisons.