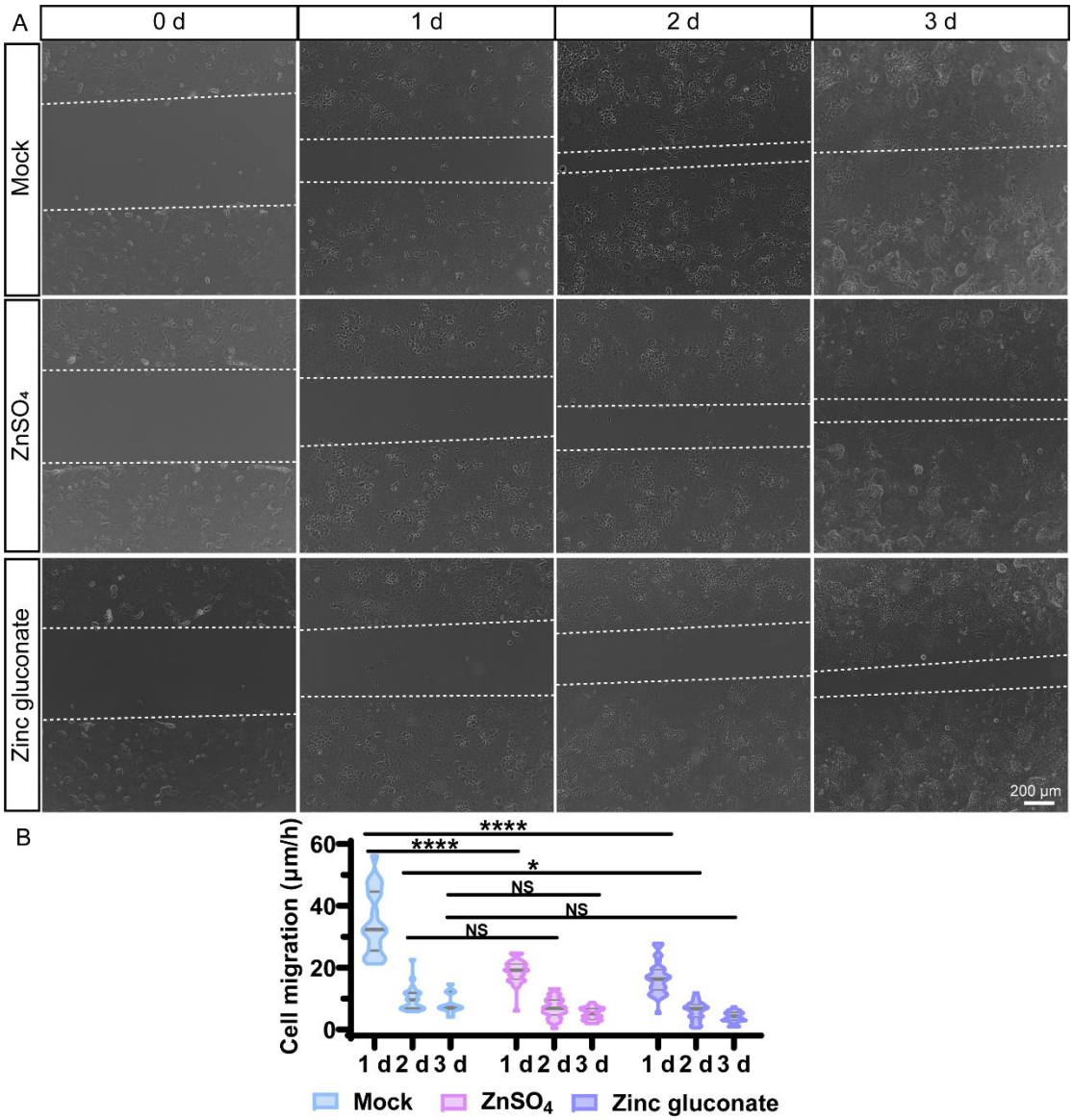
Supplementary Materials

Zinc ions inhibit migrasome biogenesis and impair platelet function by targeting cholesterol and sphingomyelin

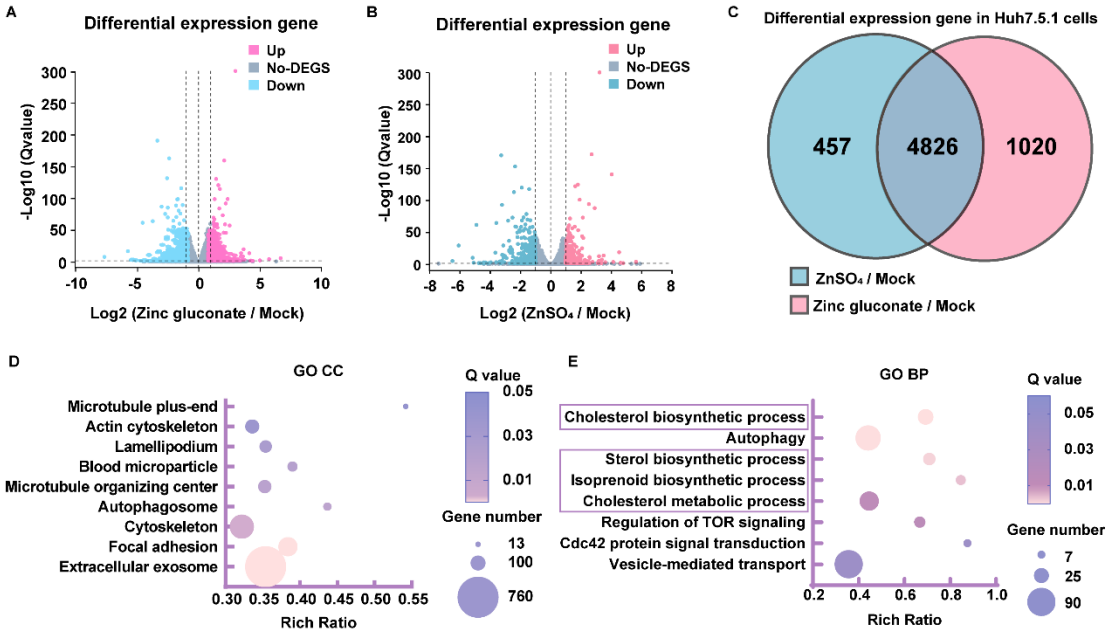
Ang Li[1,2,3], Jingze Liu[3], Shuhao Zhang[3], Jiafeng Zhu[3], Leiliang Zhang[1,3]

[1]Department of Clinical Laboratory Medicine, The First Affiliated Hospital of Shandong First Medical University & Shandong Provincial Qianfoshan Hospital, Jinan 250014, Shandong, China.
[2]School of Pharmaceutical Sciences & Institute of Materia Medica, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117, Shandong, China.
[3]Department of Pathogen Biology, School of Clinical and Basic Medical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117, Shandong, China.

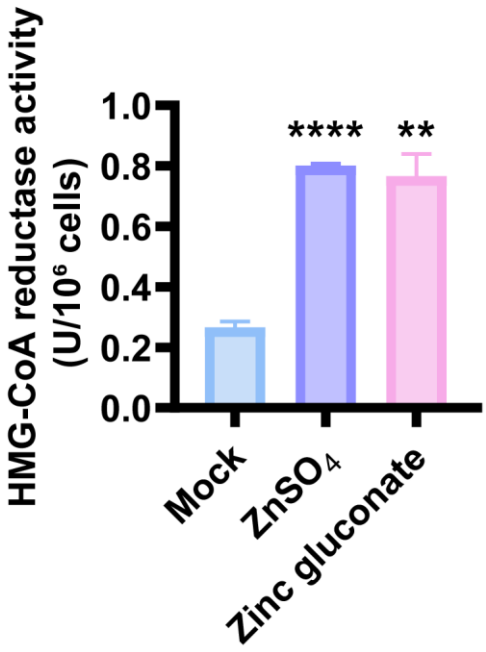
Correspondence to: Dr. Leiliang Zhang, Department of Pathogen Biology, School of Clinical and Basic Medical Sciences, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117, Shandong, China. E-mail: armzhang@hotmail.com



Supplementary Figure 1. $ZnSO_4$ and zinc gluconate inhibit cell migration in Huh7.5.1 cells. (A) Representative images of Huh7.5.1 cells treated with 20 μM $ZnSO_4$, 20 μM zinc gluconate, or left untreated, from 0 h to 3 ds. Scale bar: 200 μm; (B) Quantification of migration distance. n ≥ 11 per group. Data are mean ± SEM from six independent experiments. One-way ANOVA with Tukey-Kramer post hoc test. NS, $P > 0.05$; *$P < 0.05$; ****$P < 0.0001$. ANOVA: Analysis of variance; NS: not significant; SEM: standard error of the mean.



Supplementary Figure 2. RNA sequencing analysis of Huh7.5.1 cells treated with zinc gluconate and ZnSO4. (A) Scatter plot of differentially expressed genes in cells treated with 20 μM zinc gluconate. Upregulated genes, pink; downregulated, blue; (B) Scatter plot of differentially expressed genes in cells treated with 20 μM $ZnSO_4$. Upregulated genes, pink; downregulated, green; (C) Venn diagram comparing gene expression changes between $ZnSO_4$ vs. mock and zinc gluconate vs. mock; (D) GO cellular component (GO CC) enrichment analysis of differentially expressed genes in both models; (E) GO biological process (GO BP) enrichment analysis of differentially expressed genes in both models. BP: Biological process; CC: cellular component; DEGs: differentially expressed genes; GO: Gene Ontology.



Supplementary Figure 3. Zinc ions promote HMGCR activity. Huh7.5.1 cells were treated with $ZnSO_4$ or zinc gluconate for 17 h, and HMGCR activity was measured. Data are mean ± SEM from three independent experiments. One-way ANOVA with Tukey-Kramer post hoc test. $^{**}P < 0.01$; $^{****}P < 0.0001$. ANOVA: Analysis of variance; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; SEM: standard error of the mean.