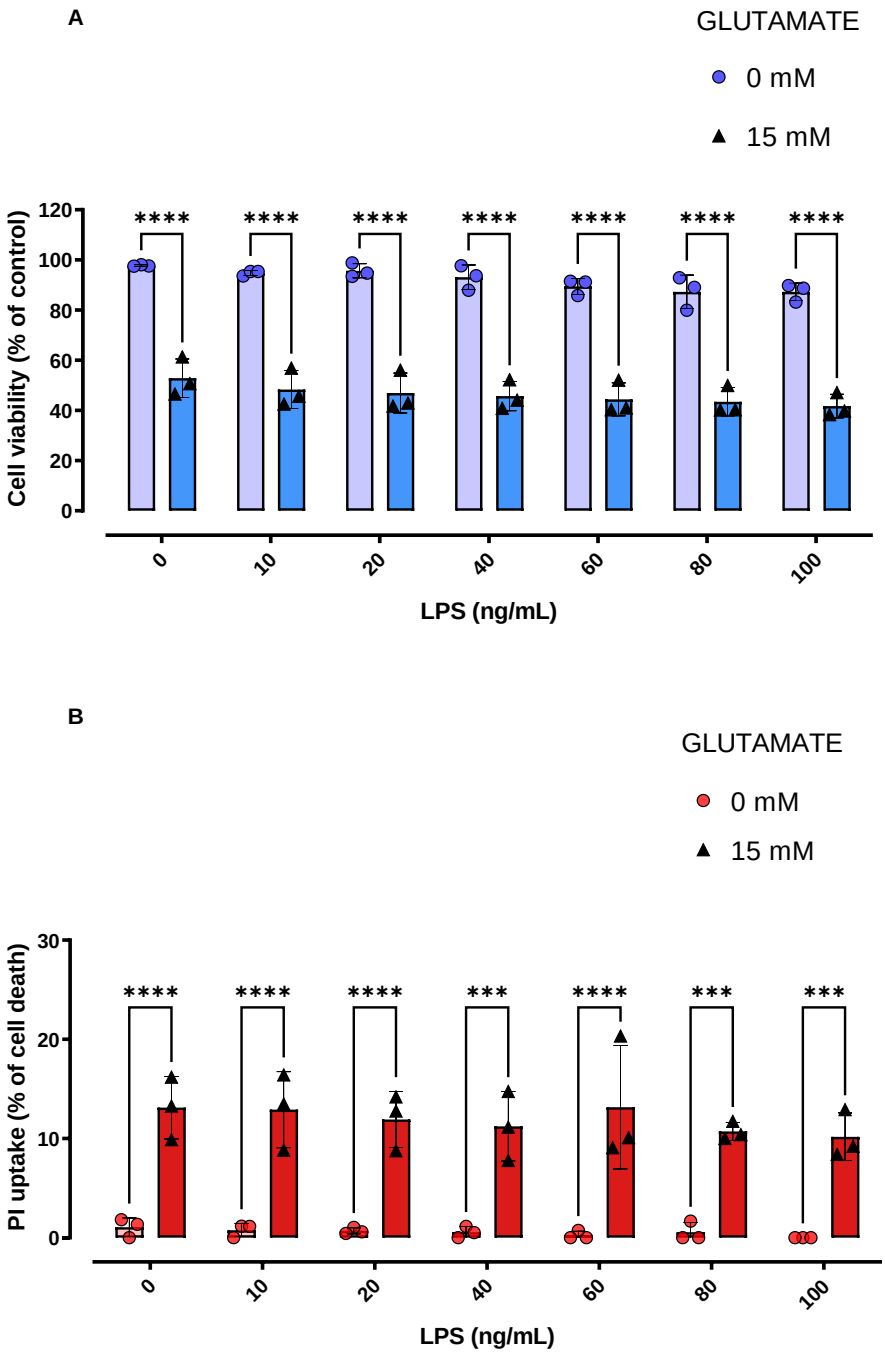
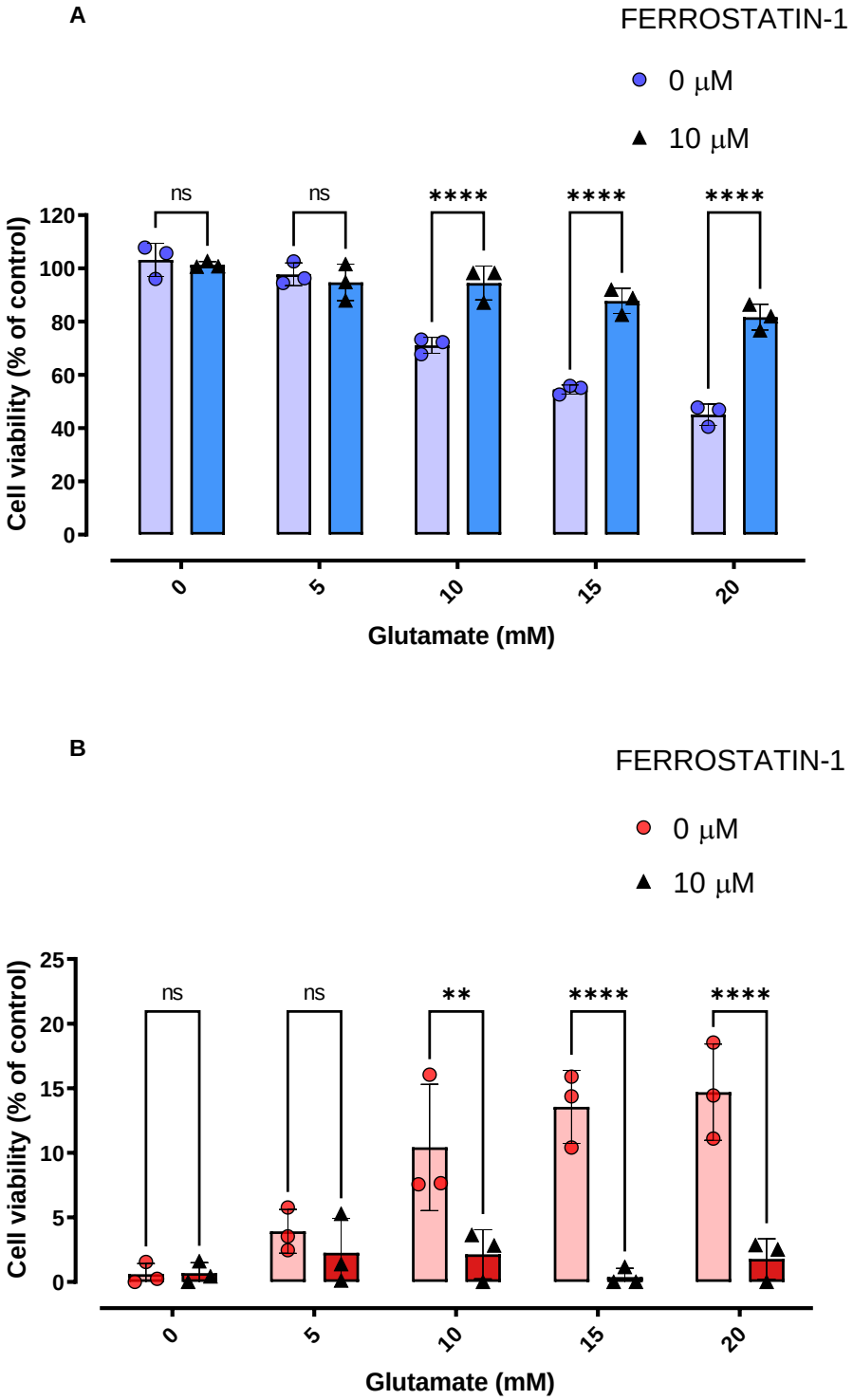
Supplementary Materials

Supplementary Table S1: Chemicals and reagents used in the study.

Chemicals/reagents	Supplier	Code
3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT)	Sigma-Aldrich	M2128
Antibiotic-Antimycotic (100X)	Thermo Fisher	15240062
Dimethyl sulfoxide (DMSO)	Sigma-Aldrich	472301; D2650
Dulbecco's modified Eagle's medium (DMEM)	Thermo Fisher	12100046
Fetal bovine serum (FBS)	Thermo Fisher	16140071
Hanks' Balanced Salt solution (HBSS)	Sigma-Aldrich	H8264
L-Glutamic acid	Sigma-Aldrich	G1251
Lipopolysaccharides from *Escherichia coli* O111:B4	Sigma-Aldrich	L2630
N-(1-Naphthyl)ethylenediamine dihydrochloride	Sigma-Aldrich	222488
Propidium iodide (PI)	Sigma-Aldrich	P4170
Sulfanilamide	Sigma-Aldrich	S9251
tert-Butyl hydroperoxide (t-BuOOH)	Sigma-Aldrich	458139
Triton X-100	Sigma-Aldrich	X100
Trypsin-EDTA	Thermo Fisher	15400054



Supplementary Figure S1. LPS did not induce protection against 15 mM glutamate. In order to investigate whether LPS would be able to generate a protective response against glutamate-induced ferroptosis, HT22 cells were exposed for 6 h (item 4.5) to different concentrations of LPS and then to 15 mM glutamate for 18 h. The figure shows significant impairment of metabolic cell viability (A - MTT assay) and increased cell death (B - PI assay) induced by 15 mM glutamate. These results were not significantly altered by pre-exposure to LPS (10-100 ng/mL), i.e., no protective effects against 15 mM glutamate were observed. In B, 100% cell death is represented by cells treated with 0.2% Triton X-100 for 20 minutes. ***$p < 0.001$; ****$p < 0.0001$ compared to control group (untreated cells). n=3.



Supplementary Figure S2. Ferrostatin-1 protected against glutamate-induced cell death. In order to investigate parallel cell death mechanisms that could be co-acting with glutamate-induced ferroptosis, HT22 cells were pre-treated with 10 μM ferrostatin-1 (classic ferroptosis inhibitor) for 30 min and then exposed to 5, 10, 15 and 20 mM glutamate for 24 h. The figure shows significant impairment of metabolic cell viability (A - MTT assay) and increased cell death (B - PI assay) induced by 10, 15 and 20 mM glutamate. These effects were

greatly decreased by pre-treatment with ferrostatin-1, evidencing ferroptosis as the main mechanism of cell death in our paradigms. In B, 100% cell death is represented by cells treated with 0.2% Triton X-100 for 20 minutes. ns: not significant; **$p < 0.01$; ****$p < 0.0001$ compared to control group (untreated cells). n=3.