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Supplemental information

Newcastle-disease-virus-induced ferroptosis through nutrient deprivation and ferritinophagy in tumor cells

Xianjin Kan, Yuncong Yin, Cuiping Song, Lei Tan, Xusheng Qiu, Ying Liao, Weiwei Liu, Songshu Meng, Yingjie Sun, and Chan Ding

Supplemental information

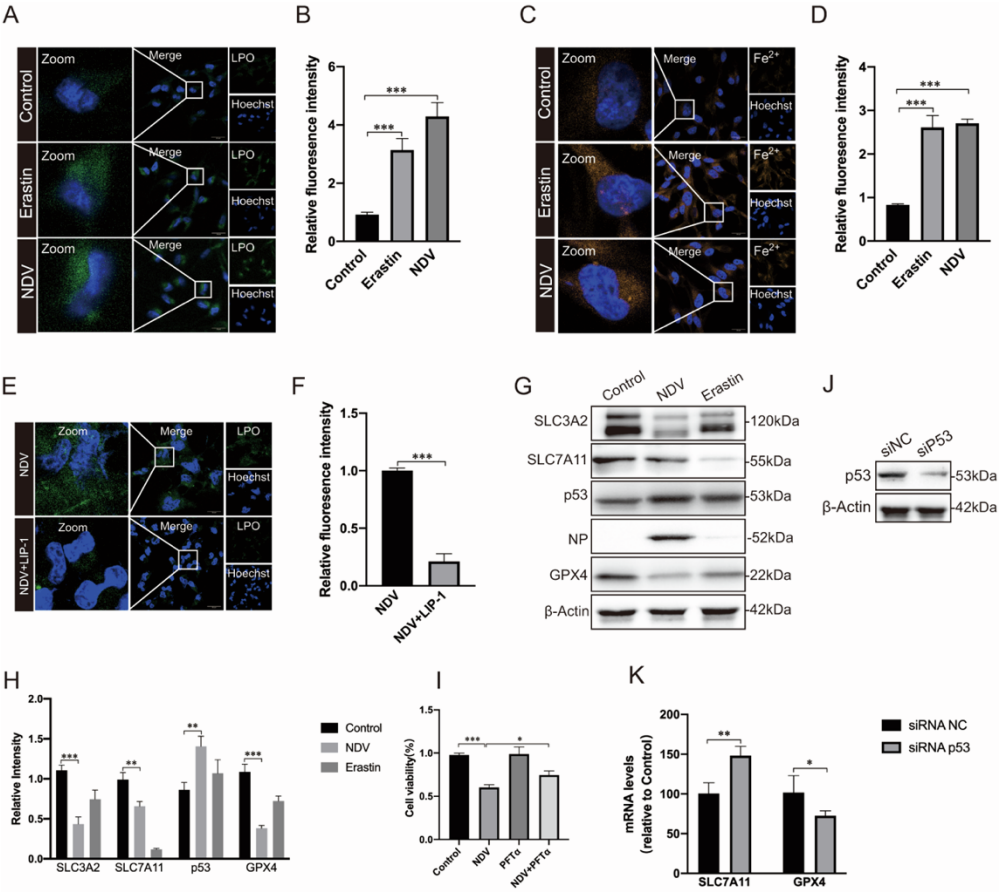
Table S1. qPCR primers for mRNA validation (Related to STAR METHODS)

Genes	Primer Sequence 5’ to 3’
P53-qPCR-F	CGATCCCGGGCAATACAACT
P53-qPCR-R	TGTGCTGGATGCATCTCTCG
GPX4-qPCR-F	CAGTGAGGCAAGACCGAAGT
GPX4-qPCR-R	CCGAACTGGTTACACGGGAA
FTH1-qPCR-F	CTGCGCCCTTCTGGAAAATG
FTH1-qPCR-R	GCAACCCCAGGATTTCAGGA
SLC7A11-qPCR-F	ACAGGGATTGGCTTCGTCAT
SLC7A11-qPCR-R	GGCAGATTGCCAAGATCTCAA
SLC3A2-qPCR-F	ACAGAGTCCTCGGACCTTCA
SLC3A2-qPCR-R	CCGTAGCTGAAAACAGGGGT
β-actin-qPCR-F	GTGGATCAGCAAGCAGGAGT
β-actin-qPCR-R	ATCCTGAGTCAAGCGCCAAA

F: Forward; R: Reverse

Table S2. qPCR primers for siRNA validation (Related to STAR METHODS)

Genes	Primer Sequence 5’ to 3’
siRNA-NCOA4	GGGCUGAACAGCAAAUUAATT
siRNA-p53	CCCGGACGAUAUUGAACAATT
siRNA-ATG5	GCUAUAUCAGGAUGAGAUATT
siRNA-NC	UUCUCCGAACGUGUCACGUTT



S1 Fig. NDV (ZJ1 strain) induces ferroptosis via the p53–SLC7A11–GPX4 signaling pathway. Related to Figure 1, Figure 2, Figure 3.

(A, B) Intracellular LPO in U251 cells treated with or without erastin and NDV (DJ1 strain) for 24 h was determined with the fluorescent probe Liperfluo (Green). Scale bars = 20 μm.

(C, D) Analysis of intercellular ferrous iron levels in U251 cells after erastin and NDV (ZJ-1 strain) treatment for 24 h using the fluorescent probe FerroOrange (Orange). Scale bars = 20 μm.

(E, F) Analysis of intracellular LPO in NDV-infected U251 cells treated with or without LIP-1. Scale bars = 20 μm.

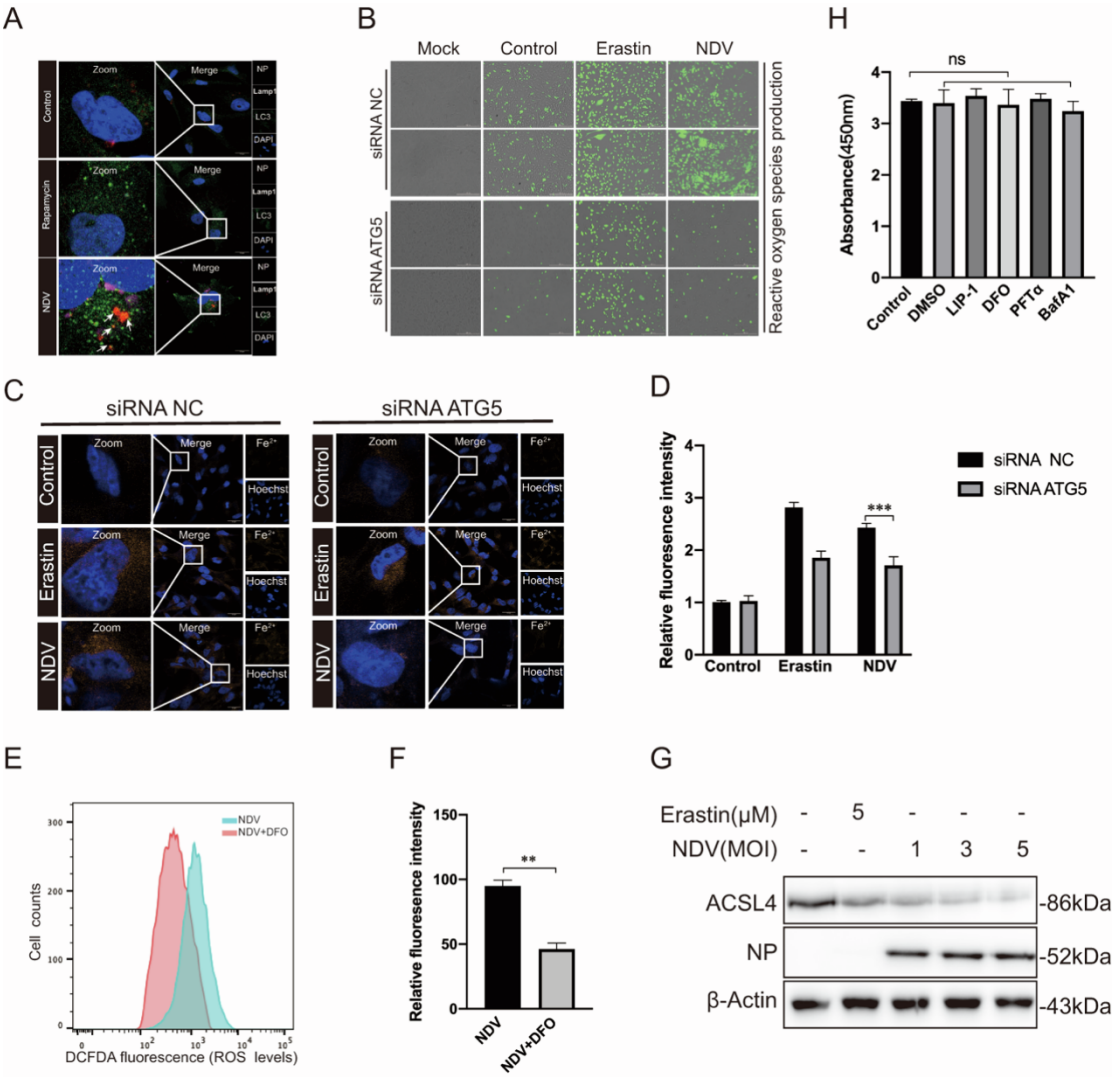
(G, H) Western blotting analyses of the levels of p53, SLC7A11, SLC3A2, GPX4, and NP in U251 cells after ZJ1 strain infection, β-actin was used as the loading control.

(I) Cell death was examined with an LDH assay 24 h after pretreatment with or without PFTα.

(J) P53 expression levels in U251 cells transfected with siRNA-control or siRNA-mediated p53 stable knockdown was determined with western blotting.

(K) mRNA levels of SLC7A11and GPX4 in U251 cells with p53 knockdown. Cells treated with NDV (MOI = 1) for 24h.

Significance was analyzed using a two-tailed Student's t-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Data were expressed as mean ± SEM (n = 3 in each group).



S2 Fig. Ferroptosis induced by NDV depends on ferritinophagy. Related to Figure 4.

(A) Fluorescence microscopy was used to assess the ability of NDV to induce the formation of autolysosomes. Staining with Lamp1 (red), and LC3 (green) in U251cells. Scale bars = 20 μm.
(B) Intracellular ROS production in Atg5-knockdown U251 cells treated with or without erastin and NDV was determined with the fluorescent probe Liperfluo (Green). Scale bars = 200 μm.
(C, D) Expression level of ferrous iron was observed with fluorescence microscopy in Atg5-knockdown cells. Erastin was used as a ferroptosis inducer. Scale bars = 20 μm.
(E, F) Analysis of intracellular ROS levels in U251 cells treated with DFO, using DCFDA staining and flow cytometry.
(G) Western blotting analysis of the expression levels of ACSL4 in NDV-infected U251cells.
(H) Cellular toxicity analysis of LIP-1, DFO, Baf-A1, and PFTα in U251 cells.
Significance was analyzed using a two-tailed Student's t-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Data were expressed as mean ± SEM (n = 3 in each group).