FIGURE S1 Representative images of the fluorescent probe C11-BODIPY 581/591. HT1080 cells were treated for 24 h with or without IFNβ (20 ng/mL). The scale bar represents 25 μm.

FIGURE S2 IFNβ treatment induces ferroptosis in tumor cells. **(A)** Representative images by inverted light microscopy show morphology changes in 786-O and HCT116 cells treated with IFNβ (20 ng/mL) for 48 h. Scale bar: 200 μm**. (B)** CCK-8 assay showing the response of 786-O and HCT116 cell lines to IFNβ (20 ng/mL). **(C, D)** Flow cytometric analysis of intracellular Fe^{2+} and lipid ROS levels in 786-O and HCT116 cells treated with or without 20 ng/mL IFNβ for 24 h. n = 6, mean ± SD, statistical significance relative to mock conditions are indicated as * $P < 0.05$ and ** $P < 0.01$.

FIGURE S3 Representative pictures acquired by transmission electron microscopy. HT1080 or 4T1 cells were treated for 24 h with or without IFNβ (20 ng/mL). The scale bar represents 1 μm.

FIGURE S4 IFNβ treatment changes ferroptosis-related gene expression in 4T1 cells. **(A)** Bar graph of functional enrichment analysis by Metascape. **(B)** Venn diagrams showing the differentially expressed genes (DEGs) that overlapped among ferroptosis regulators. **(C)** Heatmap of ferroptosis regulators detected by the RNA-seq. The heatmap shows a fold-change of ferroptosis genes that range from zero to 100 between IFNβ untreated and treated 4T1 cells (n = 3 samples).

FIGURE S5 Activation of the cGAS-STING signaling pathway promotes ferroptosis in heart tissue. **(A)** ELISA analysis showing the IFNβ levels in the serum of cGAMP untreated or treated mice (n = 6). **(B)** RT-qPCR analysis of the mRNA level of IFNB1 in heart tissues of cGAMP untreated or treated mice (n = 6). **(C)** Western blotting analysis showing the total protein levels and phosphorylation status of STAT1 and STAT3 in mice primed with or without cGAMP (n = 3). **(D, E, F)** Levels of intracellular iron, lipid ROS, and GSH in heart tissues of cGAMP untreated or treated mice. **(G)** Relative mRNA levels of ferroptosis regulators expression in heart tissues of cGAMP untreated or

treated mice. n = 6 per group, mean ± SD. Statistical significance relative to mock conditions is indicated as * $P < 0.05$ and ** $P < 0.01$.

FIGURE S6 IFNβ treatment enhances RSL3-induced ferroptosis in 786-O cells. **(A)** Representative images by inverted light microscopy showing morphology changes in 786-O cells treated with IFNβ (20 ng/mL), RSL3 (0.5 μM), or their combination for 48 h. Scale bar, 100 μm. One representative experiment of three independent experiments is shown. **(B)** CCK-8 assay showing the response of 786-O cells to IFNβ or RSL3 or their combination for 48 h. Data indicated as mean ± S.D. (n = 6 replicates). One representative experiment of three independent experiments is shown. **(C)** Flow cytometric analysis of intracellular lipid ROS levels in 786-O cells treated with IFNβ, RSL3, or their combination for 24 h. Data indicated as mean ± S.D. (n = 3 experiments). **(D)** Representative images of western blotting analysis showing the protein levels of STAT1, GPX4 and HMOX1 in 786-O cells treated with IFNβ, RSL3, or their combination for 24 h.

Figure S1

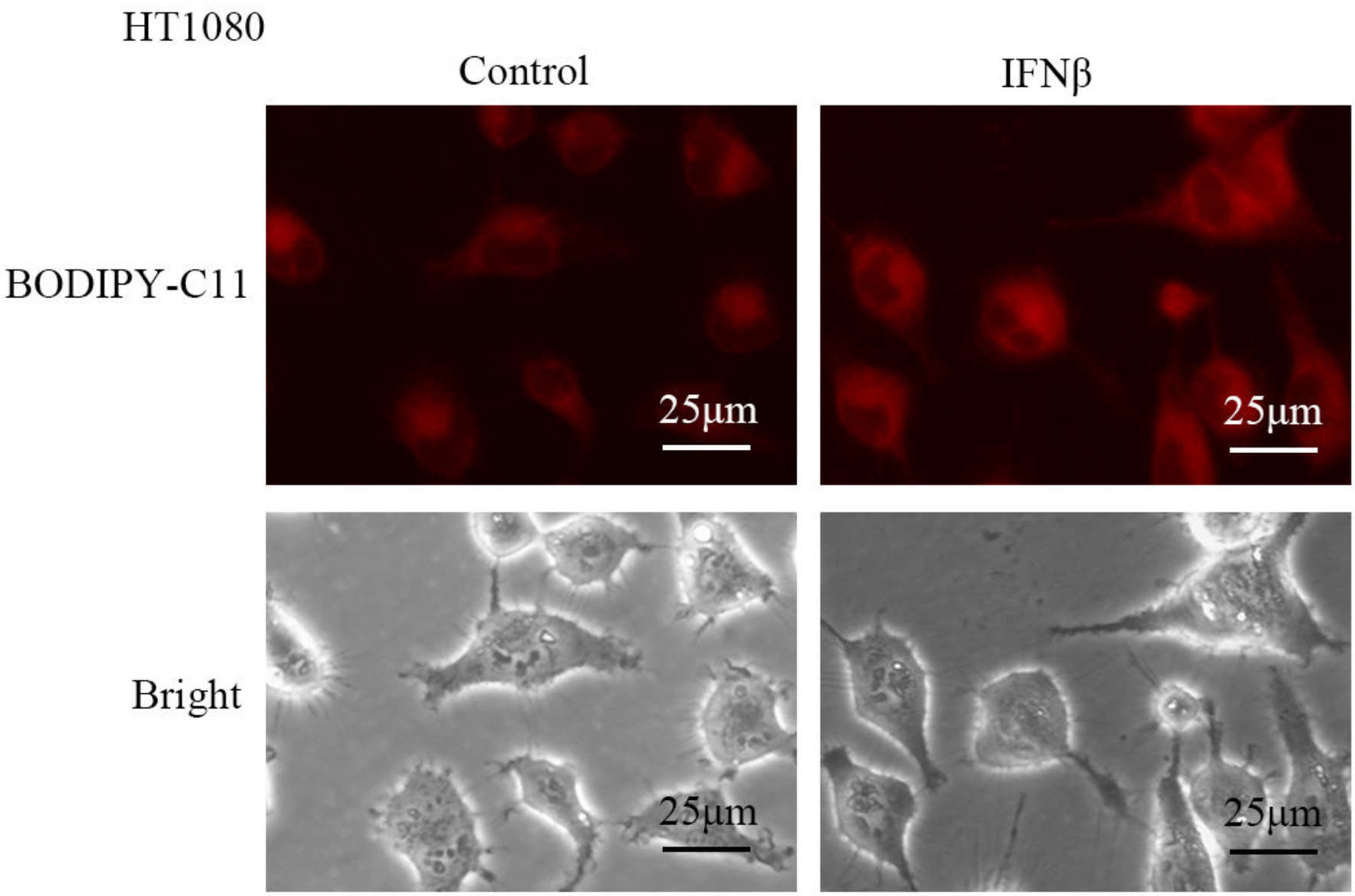


Figure S2

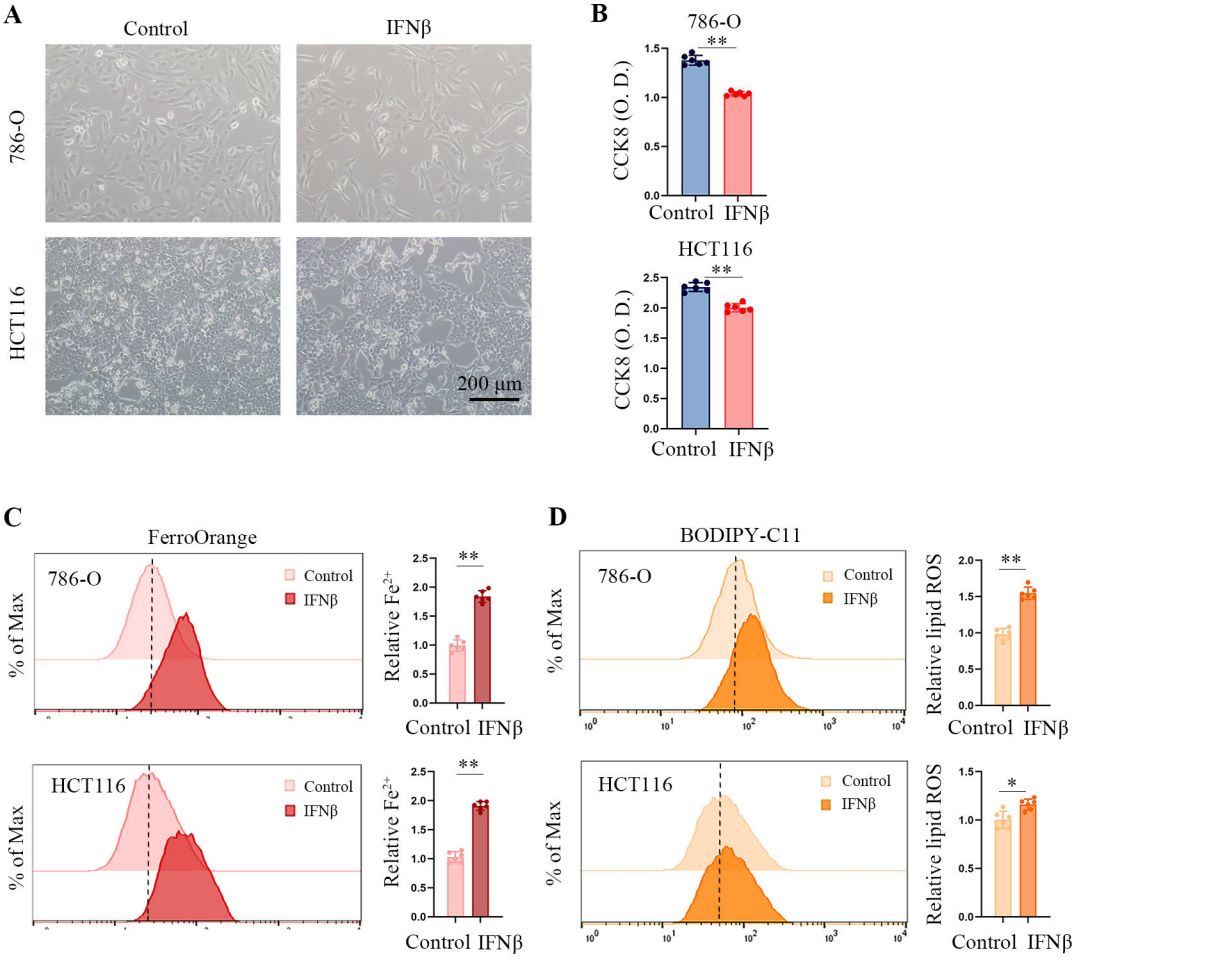

A
Control
IFNβ
786-O
HCT116
200 μm
B
786-O
CCK8 (O. D.)
Control IFNβ
HCT116
CCK8 (O. D.)
Control IFNβ
C
FerroOrange
786-O
Control
IFNβ
% of Max
Relative Fe2+
Control IFNβ
HCT116
Control
IFNβ
% of Max
Relative Fe2+
Control IFNβ
D
BODIPY-C11
786-O
Control
IFNβ
% of Max
Relative lipid ROS
Control IFNβ
HCT116
Control
IFNβ
% of Max
Relative lipid ROS
Control IFNβ

Figure S3

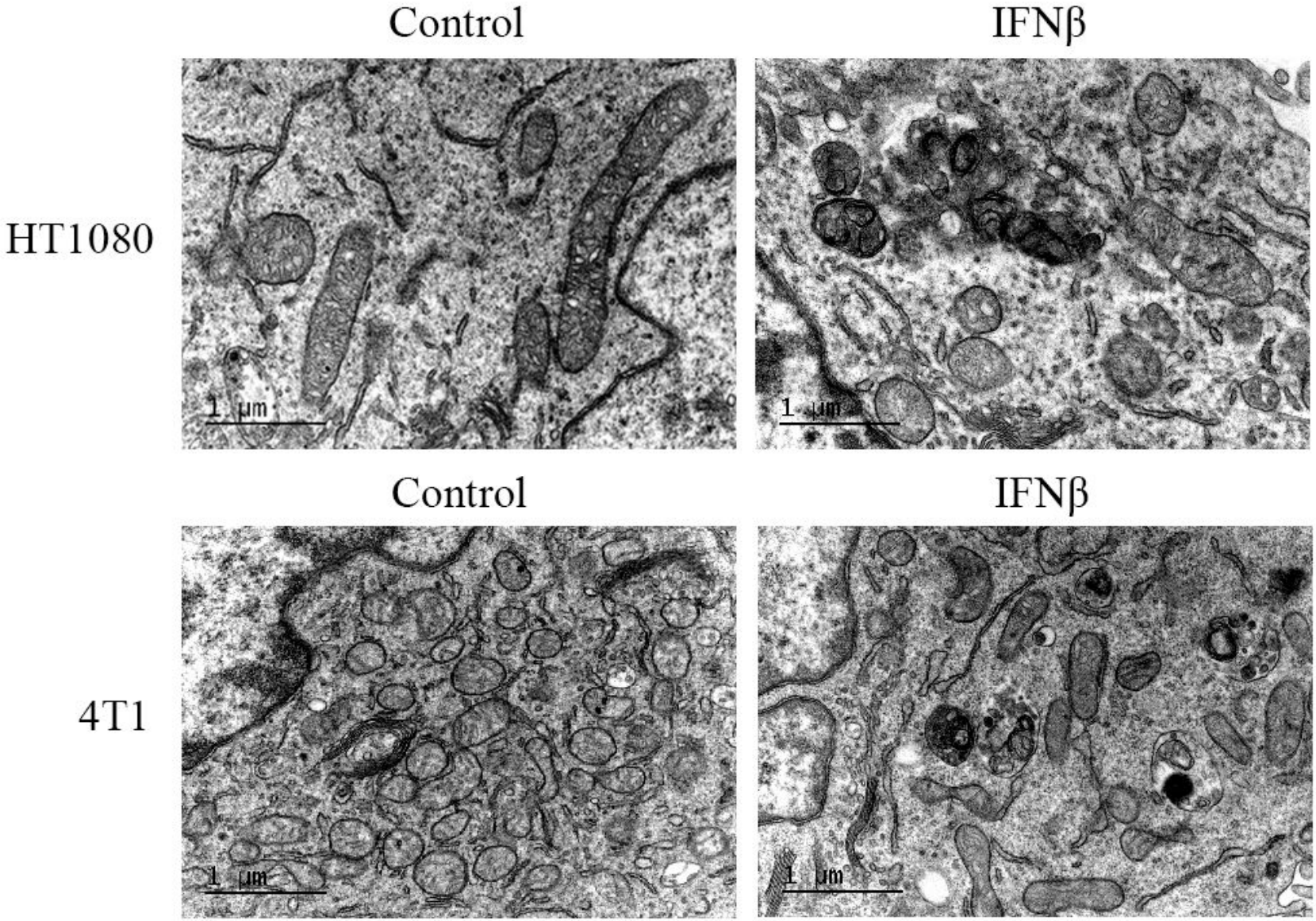
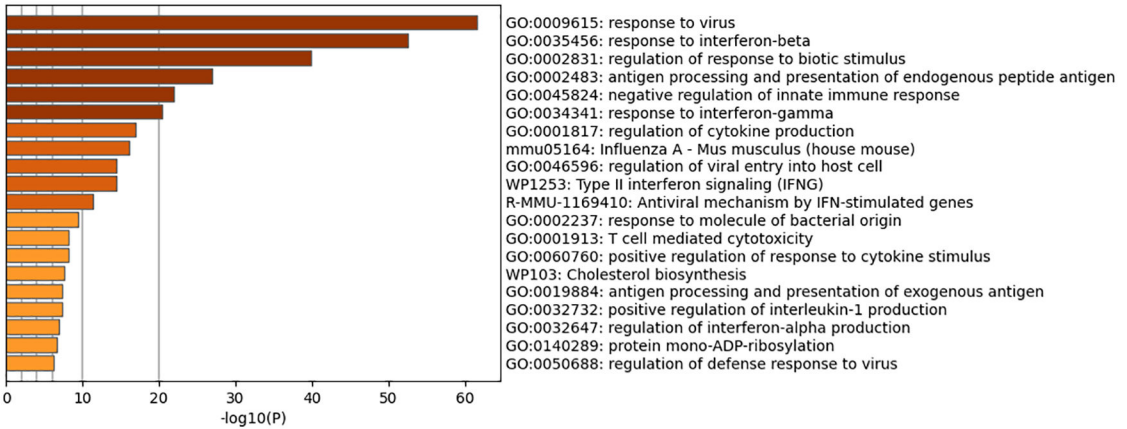


Figure S4

A



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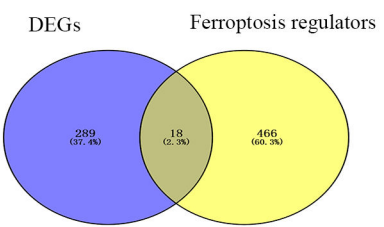

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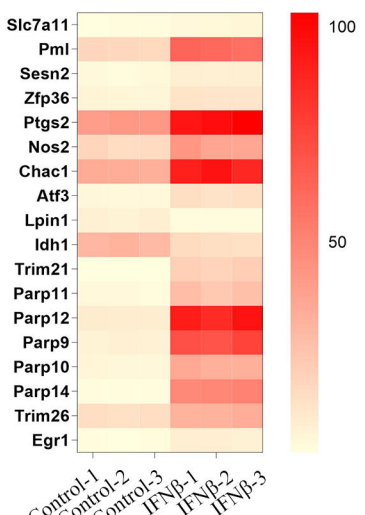

Figure S5

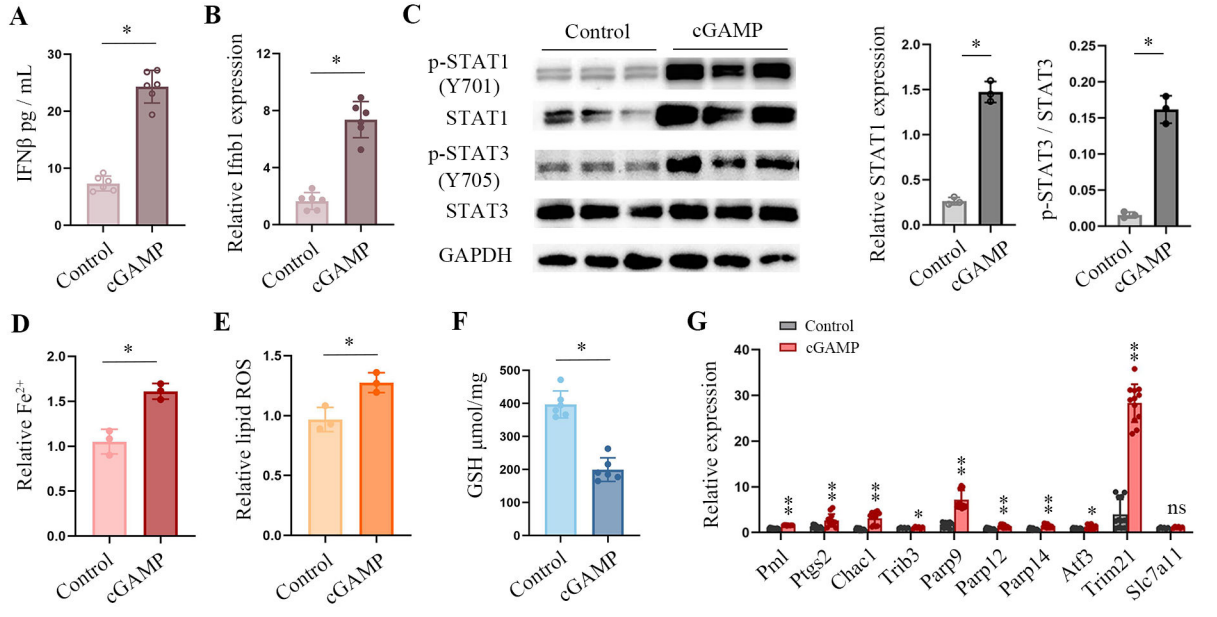
A
IFNβ pg / mL
Control
cGAMP
B
Relative Ifnb1 expression
C
Control
cGAMP
p-STAT1 (Y701)
STAT1
p-STAT3 (Y705)
STAT3
GAPDH
Relative STAT1 expression
p-STAT3 / STAT3
D
Relative Fe2+
E
Relative lipid ROS
F
GSH μmol/mg
G
Relative expression
Pml
Ptgs2
Chac1
Trib3
Parp9
Parp12
Parp14
Atf3
Trim21
Slc7a11
ns

Figure S6

786-O