

Supplementary Materials for
Metformin inhibited GSDME to suppress M2 macrophage pyroptosis
and maintain M2 phenotype to mitigate cisplatin-induced intestinal
inflammation

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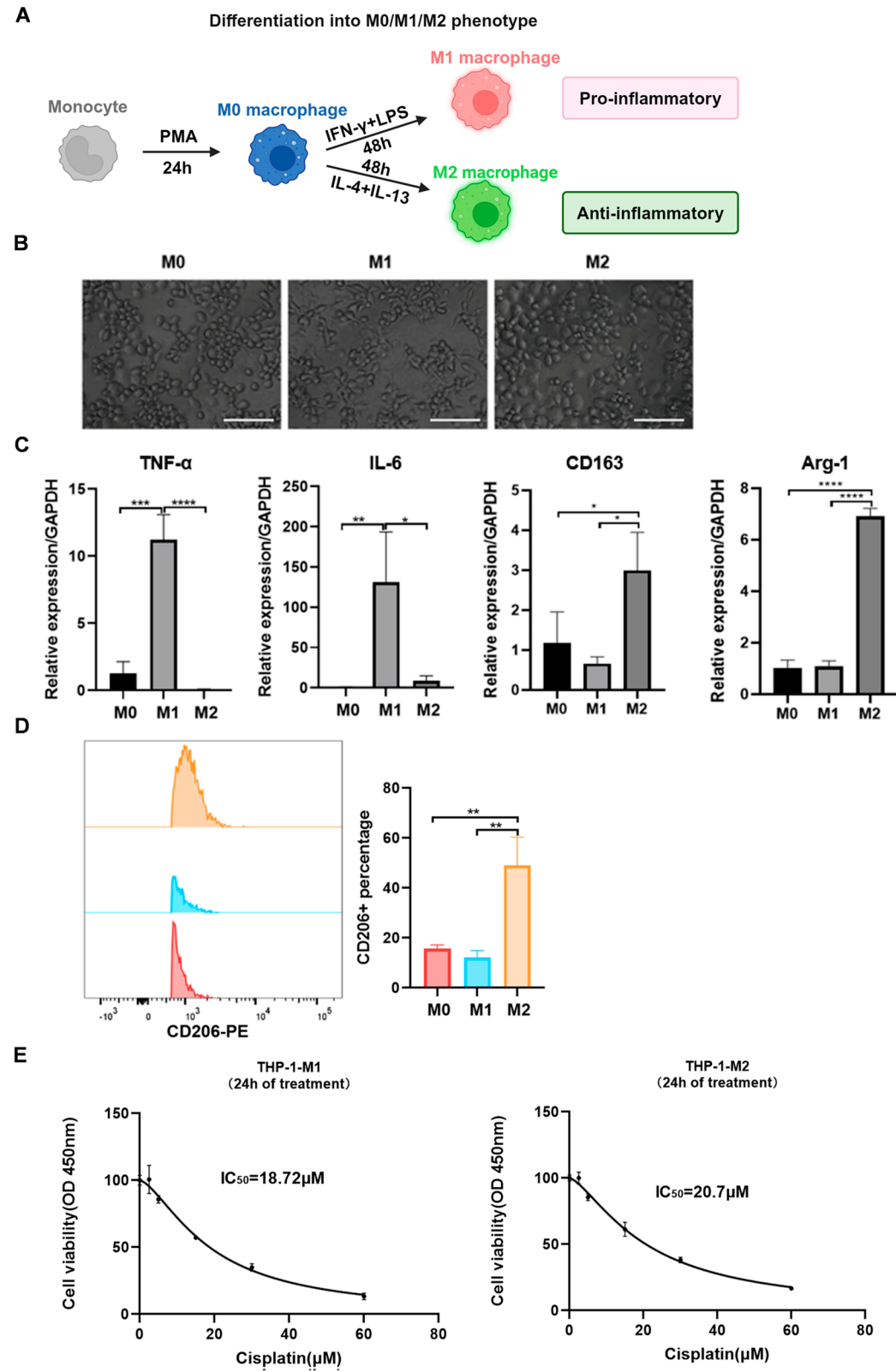
blg@hbu.edu.cn

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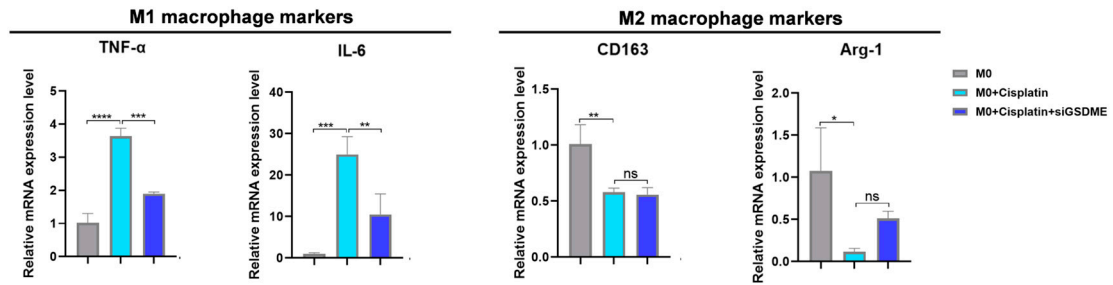
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Figure S1



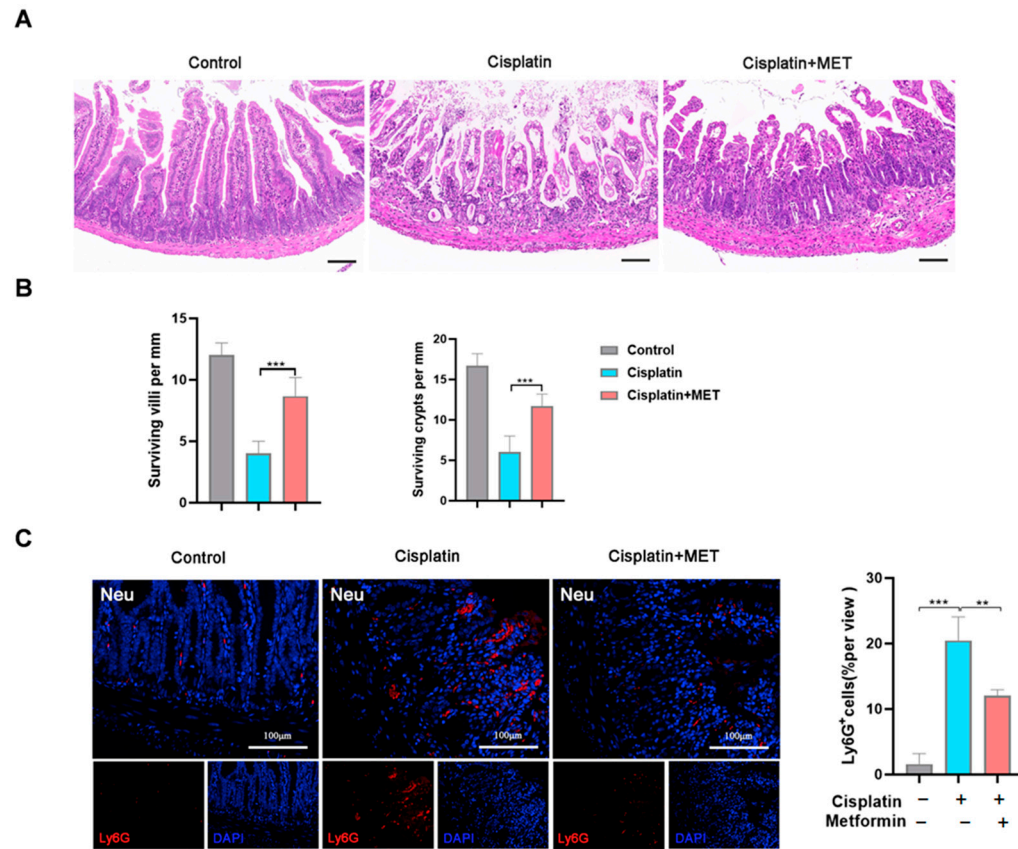
Supplemental Figure S1. The polarization of THP-1 cells into different subtypes of macrophages. **(A)** The schematic of the differentiation of the monocytic cell line THP-1 into M0, M1, and M2 macrophages. **(B)** The morphological change of polarized macrophages by the phase contrast microscope. Scale bar: 100 μ m. **(C)** The mRNA expression levels of the marker genes for M1 (TNF- α , IL-6) and M2 (CD163, Arg-1) in THP-1-derived M0, M1, and M2 macrophages ($n=3$). **(D)** The flow cytometry analysis of CD206⁺ population in different subtypes of macrophages ($n=3$). **(E)** The dose–response curve of M1 macrophages and M2 macrophages exposed to cisplatin ($n=3$). P values were calculated by one-way ANOVA with Dunnett’s test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

Figure S2



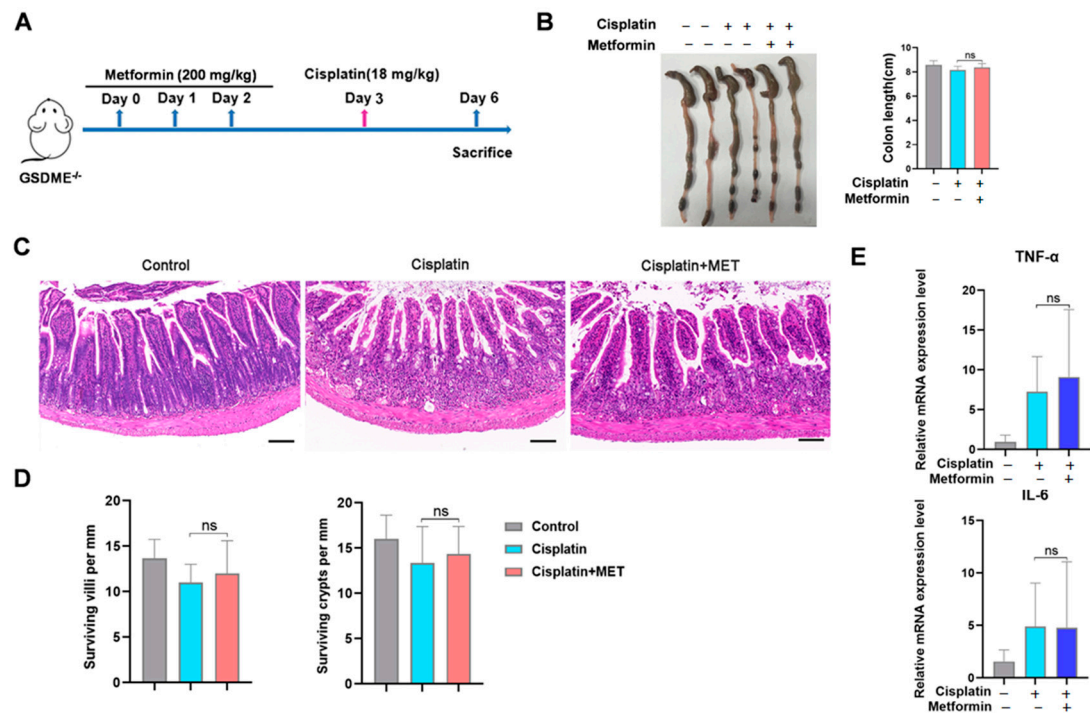
Supplemental Figure S2. The induction of M1- and M2-associated genes in M0 macrophages by cisplatin. The mRNA expression levels of M1 (TNF- α and IL-6) and M2(CD163 and Arg-1) markers were analyzed by qRT-PCR in the control and GSDME-knockdown M0 macrophages upon cisplatin treatment ($n=3$). P values were calculated by one-way ANOVA with Dunnett's test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, and ns (not significant).

Figure S3



Supplemental Figure S3. The *in vivo* effect of Metformin on cisplatin-induced acute intestinal injury. **(A)** The representative images of the H&E staining of the sections of the small intestine derived from the control, cisplatin-treated, and cisplatin/metformin-treated mice are shown ($n=5$). Scale bar: 100 μm . **(B)** The analysis of the loss of crypts and numbers of surviving villi in different mice models ($n=5$). **(C)** The representative images of immunofluorescence staining of the neutrophils (Ly6g⁺) in intestinal tissue ($n=3$); scale bar: 100 μm . P values were calculated by one-way ANOVA with Dunnett's test. ** $P < 0.01$, *** $P < 0.001$.

Figure S4



Supplemental Figure S4. Metformin exerted mitigating effects on cisplatin-induced intestinal inflammation through GSDME. **(A)** The schematic of the *GSDME*^{-/-} mice treated with intraperitoneal injection of metformin and cisplatin at different time points. **(B)** The representative images and the statistical analysis of the colon length of mice from different groups (*n*=6). **(C)** The representative images of H&E staining of the small intestine sections derived from different mice models (*n*=5). Scale bar: 100 μm. **(D)** The analysis of the surviving villi and crypts within the small intestine sections derived from different mice models (*n*=5). **(E)** The mRNA expression of TNF-α, IL-6 in the small intestine of the control, cisplatin-treated, and cisplatin/metformin-treated mice models (*n*=5). P values were calculated by one-way ANOVA with Dunnett's test. ns, not significant.

Supplementary Table S1. Specific primer sequences of qRT-PCR

| Gene name | Sequence |
|-----------------------|--|
| TNF- α (human) | Forward: 5'-TCTCGAACCCCGAGTGACAA-3' Reverse: 5'-TGAAGAGGACCTGGGAGTAG-3' |
| IL-6 (human) | Forward: 5'-CGGGAACGAAAGAGAAGCTCTA-3' Reverse: 5'-CGCTTGTGGAGAAGGAGTTCA-3' |
| CD163 (human) | Forward: 5'-CCAGAAGGAACTTGTAGCCACAG-3' Reverse: 5'-CAGGCACCAAGCGTTTTGAGCT-3' |
| Arg-1 (human) | Forward: 5'-GACAGGGCTCCTTTCAGGAC-3' Reverse: 5'-GCCAAGGTAAAGCCACTGC-3' |
| GAPDH (human) | Forward: 5'-GCAAATTCCATGGCACCGT-3' Reverse: 5'-GCCCCACTTGATTTTGGAGG-3' |
| TNF- α (mouse) | Forward: 5'-CGAGTGACAAGCCTGTAGCCC-3' Reverse: 5'-CTCTTTGAGATCCATGCCGTTG-3' |
| IL-6 (mouse) | Forward: 5'-GCTACCAAACCTGGATATAATCAGGA-3' Reverse: 5'-CCAGGTAGCTATGGTACTCCAGAA-3' |
| GAPDH (mouse) | Forward: 5'-GCAAATTCCATGGCACCGT-3' Reverse: 5'-GCCCCACTTGATTTTGGAGG-3' |

Supplementary Table S2. siRNA sequences

| Gene name | Sequence |
|-----------|-------------------------------|
| GSDME#1 | 5'-GCGGTCCTATTTGATGATGAA-3' |
| GSDME#2 | 5'-GAUGAUGGAGUAUCUGAUCUUTT-3' |