1 Supplemental material



3 Fig. S1. GAS5 was the lncRNA with significantly changed expression identified by RNA-seq analysis 4 of liver samples. (A) We first downloaded 2 human NAFLD-related microarray datasets, GSE48452 5 (containing 14 healthy control and 32 NAFLD tissue samples) and GSE107231 (containing 5 normal liver 6 and 5 NAFLD biopsy tissue samples), from Gene Expression Omnibus (GEO) and analysed the 7 downregulated lncRNAs in both datasets. Here, we reconfirmed the top 10 decreased lncRNAs by RT-qPCR 8 in our collected clinical specimens. (B) GAS5 expression was also decreased in the livers of NAFLD mice 9 as determined by lncRNA-seq analysis in our own database. The data are presented as the mean \pm S.D. of 10 three independent experiments. A p value of < 0.05 was considered statistically significant; and *p< 0.05, 11 **p < 0.01, ***p < 0.001 assessed via a two-tailed t test for examining the significance of differences between 12 two groups; ns: not significant. 13



15 Fig. S2. The expression of siRNAs, plasmids, and adenoviruses for knockdown or overexpression of 16 GAS5, miR-28a-5p, and MARCH7 in AML12 cells, HepG2 cells and mouse livers. In this study, AML12 17 and HepG2 cells were transfected with miRNAs, plasmids, or lncRNAs for 12 h. In addition, mice were 18 injected every 10 days with adenoviruses to decrease or increase the hepatic expression of GAS5, miR-28a-19 5p, and MARCH7. After 3 weeks of injection, the mice were prepared for further analysis. Herein, the 20 expression of GAS5, miR-28a-5p, and MARCH7 in vitro and in vivo was measured using RT-qPCR analysis. 21 (A) The expression of miR-28a-5p in AML12 and HepG2 cells. (B) The expression of MARCH7 in AML12 22 and HepG2 cells. (C) GAS5 expression in AML12 and HepG2 cells. (D) The expression of miR-28a-5p in 23 the livers of C57BL/6 mice, HFD-fed mice, and Ob/Ob mice after 3 weeks of the first injection. (E) The 24 expression of MARCH7 in the livers of C57BL/6 mice, HFD-fed mice, and Ob/Ob mice. (F) The expression 25 of GAS5 in the livers of C57BL/6 mice, HFD-fed mice, and Ob/Ob mice. (G) IHC staining of MARCH7 26 levels in liver of mice after 3 weeks of injection. (H) Immunofluorescence detecting of GAS5 and miR-28a-27 5p in liver of mice after 3 weeks of injection. (I) Additionally, the binding sites between GAS5 and miR-28a-28 5p were predicted by sequence alignment, and there were two conserved binding sites between GAS5 and 29 miR-28a-5p (yellow background). The data are presented as the mean ± S.D. of three independent

- 30 experiments. Two-tailed Student's *t* test was used to determine the significance of differences between two 31 groups (A-F). A *p* value of < 0.05 was considered statistically significant; and ${}^{*}p < 0.05$, ${}^{**}p < 0.01$, ${}^{***}p <$
- 32 0.001; ${}^{ns}p > 0.05$, ns: not significant.
- 33



35 Fig. S3. miR-28a-5p knockdown inhibits lipid deposition and inflammation in AML12 and HepG2 cells

- 36 (A-C). Overexpression of miR-28a-5p significantly blocks GAS5 overexpression-induced inhibition of
- 37 lipid accumulation in AML12 and HepG2 cells (D-F).

38 On the one hand (A-C), AML12 and HepG2 cells were incubated with FAs for 12 h after 12 h of transfection 39 with the indicated anti-miR-NC and anti-miR-28a-5p constructs. (A) Oil Red O staining; Scale bar, 50 µM. 40 (B) ELISA of TG content. (C) RT–qPCR analysis of inflammation-related genes. On the other hand (D-F), 41 we found that overexpression of miR-28a-5p blocks GAS5-controlled lipid deposition and inflammation in 42 AML12 and HepG2 cells. (D) Oil Red O staining; Scale bar, 50 µM. (E) ELISA of TG content. (F) RT-43 qPCR analysis of inflammation-related genes. All indicated data are shown as the means \pm S.D.s. A p value of < 0.05 was considered statistically significant; and *p<0.05, **p<0.01, ***p<0.001 assessed via a ANOVA 44 45 with the Bonferroni post hoc test for comparisons among more than two groups (B, C, E, F); ns: not 46 significant.

47



Fig. S4. Overexpression of miR-28a-5p significantly reduces GAS5 overexpression-mediated
suppression of hepatic steatosis, inflammation, and insulin resistance in HFD-fed mice.

51 Mice were fed a ND or HFD for 12 weeks. Then, they were injected with adenoviruses every 10 days, *and* 52 these mice were continuously fed a ND or HFD. After 3 weeks of the first injection, the mice were used for 53 further analysis. (A) Food intake was measured every day during ND or HFD feeding (n=5/group). (B) The

- 54 body weight, liver weight, and LW/BW ratios of C57BL/6 and HFD-fed mice after 3 weeks of injection of
- adenoviruses (n=5/group). (C) TG, NEFA, and TC levels were measured using ELISA in the livers of
- 56 C57BL/6 and HFD-fed mice (n=5/group). (D) Oil Red O staining of liver sections from adenovirus-injected
- 57 C57BL/6 and HFD-fed mice (n=5/group); Scale bar, 50 μM. (E) The mRNA levels of inflammation-related
- 58 genes analysed by RT–qPCR (n=5/group). (F) Fasting blood glucose levels of C57BL/6 and HFD-fed mice
- 59 (n=5/group). (G, H) Fasting insulin levels and HOMA-IR scores of adenovirus-injected C57BL/6 and HFD-
- 60 fed mice (n=5/group). (I-L) GTT and ITT of C57BL/6 and HFD-fed mice after 3 weeks of the first adenovirus
- 61 injection and the areas under the curve (AUCs) for the GTT and ITT (N=5/group). The data are presented as
- 62 the mean \pm S.D. of three independent experiments. A *p* value of < 0.05 was considered statistically significant;
- and p < 0.05, p < 0.01, p < 0.001 assessed via a ANOVA with the Bonferroni post hoc test for comparisons
- 64 among more than two groups (A-L); ns: not significant.
- 65



67 Fig. S5. Heterologous overexpression of GAS5 significantly suppresses lipid deposition and 68 inflammation in AML12 and HepG2 cells. Because there are two conserved binding sites between 69 GAS5/miR-28a-5p between humans and mice, we hypothesized that the functions of the GAS5/miR-28a-5p 70 axis were also conserved in the liver between these two species. (A) After overexpression of human GAS5 71 in AML12 normal mouse hepatocytes for 12 h and in the livers of mice for one week, we examined the miR-72 28a-5p levels. (B) the Biotinylated GAS5 pull-down assay showed that human GAS5 interacted with mouse 73 miR-28a-5p in AML12 cells and mouse liver tissues. (C, D, E) After overexpression of human GAS5 in 74 AML12 cells treated with FAs for 24 h and the livers of C57BL/6 mice, we examined the lipid content and

- 75 inflammation; Scale bar, 50 μM. (F) After overexpression of mouse GAS5 in HepG2 human liver cancer
- cells for 12 hours, we determined the miR-28a-5p levels. (G) After overexpression of mouse GAS5 in human
- 177 liver cancer HepG2 cells, we confirmed that mouse GAS5 bound to human miR-28a-5p in HepG2 cells. (H,
- 78 I, J) After overexpression of mouse GAS5 inhibited lipid accumulation and inflammation in HepG2 cells,
- 79 we evaluated the lipid content and inflammation; Scale bar, 50 μ M. The data are presented as the mean \pm
- 80 S.D. of three independent experiments. A p value of < 0.05 was considered statistically significant; and
- 81 p < 0.05, p < 0.01, p < 0.001 assessed via a two-tailed *t* test for examining the significance of differences
- 82 between two groups (A, B, F, G) or ANOVA with the Bonferroni post hoc test for comparisons among more
- 83 than two groups (D, E, I, J); ns: not significant.
- 84

| Α | AM | AML12 | | | ည္ HepG2 ည | | | | |
|--|----------------------------|--|------------------------------|---|---|------------------------------------|------------------------------------|--------------------------------------|--|
| | | NC | RP3 | NLR | | NC | RP3 | NLR | |
| | -NC | p+si- | INL | p+si-] | -NC | p+si-] | -NL | p+si- | |
| | VC+s | 8a-5 | VC+8 | 8a-5 | VC+s | 8a-5 | VC+s | 8a-5 | |
| | √-Jir | niR-2 | uiR-N | niR-2 | uR-♪ | uR-2 | uiR-N | iR-2 | |
| Tubulin-o | x — | - | - | - | - | - | - | | |
| GSDMD-N | 1 | - | | | | - | | | |
| Cleaved Caspase- matured IL-1 | 1 β | = | | | | = | | | |
| F | | | | | | | | | |
| Binding sites of miR-28a-5p 3'UTR WT 3'UTR Mutant | miR-2 3'G 5'A 5'A | 28a-5p AGUU AGGA AGGA | on M JAU(AUG/ AUG/ | IARC CUGA AUCU AU <mark>G</mark> L | H7 3" CACI IGUG I <mark>CUC</mark> | UTR JCGA AAC. AAC. | (Mou AGG/ AUG AUG | ISE) AA5' UU3' UU3' | |
| miR-28a-5p 3'UTR WT 3'UTR Mutant | 3'G 5'A 5'A | AGUU ACUC ACUC | JAUC BAAA BAAA | | CACI UUG. UUC. | UCG/ AGCU A <mark>CC/</mark> | \GG/ JGG/ \GG/ | AA5' AC3' AC3' | |
| Binding sites of miR-28a-5p 3'UTR WT 3'UTR Mutant | miR-3 3'0 5'4 5'4 | 28a-5p 3AGU NUGG2 NUGG2 | on M UAU AUG AUG | IARC CUGA AUCI AU <mark>G</mark> I | H7 3' ACAC JGUG J <mark>CUC</mark> | UTR UCG AAC AAC | (Hu r AGG AUA AUA | nan) AA5' AG3' AG3' | |
| miR-28a-5p 3'UTR WT 3'UTR Mutant | 3 °€ 5°€ | GAUG | 5640 CUAU | gug <i>i</i> J <mark>AG</mark> U | NCAC I <mark>CUC</mark> | V66 UGG | 068 UCAI | ðð§ [;] UG3' | |
| miR-28a-5p 3'UTR WT | 3'C 5'C | GAGUU GUCAA | JAU AUAI | CUGA JGGC | CAC CGAA | UCG. ACC | AGG CCG | AA5' UC3' | |
| 3'UTR Mutant | 5′0 | iU <mark>G</mark> AU | JUU | UGGG | CGAA | | CCG | UC3' | |
| miR-28a-5p | 3'(| BAGU | JAU | CUGA | ACAC | UCG. | AGG | AA5' | |
| 3'UTR Mutant | 5' <i>F</i> 5' A | | ACC/ | ACCU AC <mark>G</mark> U | GUG. | Αυυ Αυπ | UGA. UGA. | AC3' AC3' | |
| miR-28a-5p | 3, (| AGII | | CUG | | UCG | AGG | ΔΔ 5' | |
| 3'UTR WT | 5'C | GUU | JGA | GACU | JGUU | GGU | UUU | AA3' | |
| 3'UTR Mutant | 5'0 | GUU | JGA | CAGU | J <mark>C</mark> UU | GGU | UUU | AA3' | |
| miR-28a-5p 3'UTR WT 3'UTR WT | 3'C 5'U 5' | JAGUI | UAU CAA/ | CUGA AUUC | ACAC UAC AC | UCG. CACI CACI | AGG JCCI UCCI | AA5' JU3' UU3' | |
| 3'UTR Mutant | 5' | | | 0110 | | G | UGC | AU3' | |
| 3'UTR WT | 3'C 5'T | јАGUU ЛЛЛЛ | JAU UUU | UUGA UACI | IGUG | UCG. | AGG GUA | AA5' GU3' | |
| 3'UTR Mutant | 5't | ກບບບ | UUU | UA <mark>G</mark> I | JCUC | CCU | GUA | GU3' | |
| miR-28a-5p | 3'0 | GAGU | JAU | CUG/ | CAC | UCG. | AGG | AA5' | |
| 3'UTR WT 3'UTR WT | 5'U 5'A | | AUAU AUAA | JUGA AUAA | ACAU AAU | UAG AGA | UAA CAU | UA3' CU3' | |
| 3'UTR Mutant | 5' 27 C | | | | | 1100 | | | |
| 3'UTR WT | 5C | JAGUU | AUA | GACA | AUCU | CAA | UCA | аа5 [°] CU3 [°] | |
| 3'UTR Mutant | 5't | JAAA | JUA | CAGA | UCU | CAA | UCA | CU3' | |
| miR-28a-5p | 3'(| GAGU | JAU | CUG/ | ACAC | UCG. | AGG | AA5' | |
| 3'UTR WT | 5'C | CUCA/ | \UC/ | ACUA | UAC | AAA | AUC | UC3' | |
| 3'UTR Mutant | 5′ <mark>(</mark> | JUGA | JUC | ACUA | | AAA | AUC | UC3' | |
| 3'UTR WT 3'UTR Mutant | 5'(5'(5'(| GUCA/ GUCA/ GU <mark>GA</mark> I | JAU AUA JUU | AAAA AAAA | ACAC AGAG AGAG | ACU ACU | AGG ACA ACA | AA5' CA3' CA3' | |
| | | | | | | | | | |



Fig. S6. Interfering with NLRP3 expression blocks miR-28a-5p-mediated activation of pyroptosis. (A)
Interfering with NLRP3 expression blocks miR-28a-5p-mediated activation of pyroptosis, as evidenced by

88 Western blot analysis of pyroptosis-associated proteins after 24 h of FA treatment in anti-miR-NC-, si-NC,

- 89 anti-miR-28a-5p, or si-NLRP3-transfected AML12 and HepG2 cells. (B) The hepatocytes were transfected
- 90 with miR-NC, miR-28a-5p, anti-miR-NC, anti-miR-28a-5p for 24 h, and they were collected to determine
- 91 NLRP3 mRNA expressions evidenced by RT-qPCR. (C) The hepatocytes were transfected with miR-NC,
- 92 miR-28a-5p, and co-transfected with NLRP3 3'UTR for 24 h. Then, they were prepared for luciferase
- 93 reporter assay. (**D**) miR-28a-5p significantly inhibited CHX-induced degradation of NLRP3 protein. They
- 94 were incubated with 30 μM CHX along with miR-NC or miR-28a-5p transfection for 0 h, 3 h, 6 h. (E) The
- 95 potential binding sites of miR-28a-5p in the MARCH7 3'UTR (mouse or human) were examined by sequence
- alignment. The data are presented as the mean \pm S.D. of three independent experiments. A *p* value of < 0.05
- 97 was considered statistically significant; and *p < 0.05, **p < 0.01, ***p < 0.001 assessed via a two-tailed *t* test for
- 98 examining the significance of differences between two groups (B, C) or ANOVA with the Bonferroni post
- 99 hoc test for comparisons among more than two groups (D); ns: not significant.
- 100



102 Fig. S7. MARCH7 overexpression reversed the miR-28a-5p overexpression-induced enhancement of

- 103 pyroptosis. First, AML12 and HepG2 cells were transfected with overexpression plasmids for 12 h. Then,
- 104 the cells were incubated with FAs for 12 h. The cells were collected for further analysis. (A, B) Western blot
- analysis of the expression of pyroptosis-related molecules in AML12 and HepG2 cells after 24 h of
- 106 transfection with miR-NC, pcDNA, miR-28a-5p, or pcDNA-MARCH7. (C, D) Western blot analysis of the
- 107 expression of pyroptosis-related molecules in the livers of C57BL/6 mice after adenovirus injection for 7
- 108 days. All the data are shown as the means \pm S.D.s. The data are presented as the mean \pm S.D. of three
- 109 independent experiments. (E, F) Western blot analysis of pyroptosis-related gene expression in AML12 and
- 110 HepG2 cells after 24 h transfection with pcDNA, miR-NC, GAS5, and another 12 h of FA treatment. (G, H)
- 111 Western blot analysis of pyroptosis-related gene expression in the livers of HFD-fed mice after 7 days of
- 112 injection with adenoviruses. The data are presented as the mean \pm S.D. of three independent experiments. A
- 113 p value of < 0.05 was considered statistically significant; and *p<0.05, **p<0.01, ***p<0.001 assessed via a
- 114 ANOVA with the Bonferroni post hoc test for comparisons among more than two groups (B, D, F, H); ns:
- 115 not significant.
- 116





118 Fig. S8. Overexpression of MARCH7 attenuates lipid deposition in hepatocytes. AML12 and HepG2 119 cells were transfected with the indicated plasmids and miRNAs for 12 h and incubated with FAs for another 120 12 h. First, (A, D) Oil Red O staining of lipid droplets in hepatocytes; Scale bar, 50 µM. (B, E) ELISA of TG 121 content in AML12 and HepG2 cells. (C, F) RT-qPCR assay of inflammation-related gene expression. 122 Afterwards, we found that MARCH7 overexpression significantly inhibited FA-induced hepatic lipid 123 accumulation and that interfering with MARCH7 expression reversed the miR-28a-5p-mediated suppression 124 of pyroptosis in AML12 and HepG2 cells. The data are presented as the mean \pm S.D. of three independent experiments. A p value of < 0.05 was considered statistically significant; and *p< 0.05, **p< 0.01, ***p< 0.001125

- assessed via a ANOVA with the Bonferroni post hoc test for comparisons among more than two groups (B,
- 127 C, E, F); ns: not significant.
- 128



130 Fig. S9. Knockdown of MARCH7 reverses anti-miR-28a-5p-mediated suppression of hepatic steatosis, 131 inflammation, and insulin resistance in HFD-fed mice. Mice were fed a ND or HFD for 12 weeks. Then, 132 they were injected once a week with the adenoviruses AD-anti-miR-28a-5p, AD-sh-NC, AD-anti-miR-28a-133 5p, and AD-anti-sh-MARCH7, and these mice were continuously fed a ND or HFD. After 3 weeks of the 134 first injection, the mice were used for further experiments. (A) The food intake of ND- and HFD-mice 135 (n=5/group). (B) The body weights, liver weights, and LW/BW ratios of C57BL/6 and HFD-fed mice 136 (n=5/group). (C) TG, NEFA, and TC levels were measured using ELISA in the livers of C57BL/6 and HFD-137 fed mice (n=5/group). (**D**) Oil Red O staining of mouse liver sections (n=5/group); Scale bar, 50 μ M. (**E**) 138 The mRNA levels of inflammation-related genes were measured by RT–qPCR (n=5/group). (F) Fasting blood 139 glucose levels of C57BL/6 and HFD-fed mice (n=5/group). (G, H) Fasting insulin levels and HOMA-IR 140 scores of adenovirus-injected C57BL/6 and HFD-fed mice (n=5/group). (I-L) GTT and ITT of C57BL/6 and 141 HFD-fed mice after 3 weeks of the first adenovirus injection and the areas under the curve (AUCs) for the

- 142 GTT and ITT (N=5/group). The data are presented as the mean ± S.D. of three independent experiments. A
- 143 p value of < 0.05 was considered statistically significant; and *p<0.05, **p<0.01, ***p<0.001 assessed via a
- 144 ANOVA with the Bonferroni post hoc test for comparisons among more than two groups (A-L); ns: not
- 145 significant.
- 146



147

148 Fig. S10. MARCH7 was required for miR-28a-5p-induced inhibition of NLRP3 ubiquitination-

149 mediated degradation to promote pyroptosis. (A) After MARCH7 knockdown for 48 h, we overexpressed 150 miR-28a-5p in AML12 and HepG2 cells for another 48 h. Then, we harvested them for Western blot analysis 151 of pyroptosis and for analysis of NLRP3 ubiquitination after 12 h of incubation with 20 µM MG132. (B, C) 152 After MARCH7 knockdown for 48 h, we overexpressed miR-28a-5p in AML12 and HepG2 cells for another 153 48 h, and they were incubated with FAs for 8 h; Scale bar, 50 μM. In addition, mice were fed a ND or HFD 154 for 8 weeks. Then, they were injected with adenoviruses every 10 days, and these mice were continuously 155 fed a ND or HFD. After the first injection for 3 weeks, the mice were used for Oil Red O staining, TG 156 measurement, and RT-qPCR. (D) After MARCH7 overexpression for 48 h, we overexpressed miR-28a-5p 157 in AML12 and HepG2 cells for another 48 h, and we collected them for analysis of NLRP3 ubiquitination 158 after 12 h of incubation with 20 µM MG132. (E) The purified proteins were prepared by Sangon (Shanghai) 159 and mixed together in cell lysis buffer, and the MARCH7-bound proteins were identified using a GST pull-160 down assay. (F, G) The purified proteins were mixed together in cell lysis buffer following the instructions 161 of an in vitro ubiquitination assay kit (VIVA Bioscience, Amyjet Scientific Inc, Wuhan, China) and an in vitro 162 ubiquitination assay protocol (DOI: 10.21769/BioProtoc.928); the effects of GST-MARCH7 on the levels of 163 HA-NLRP3, Flag-GSDMD-N, and Myc-caspase-1 were examined by western blotting; and the ubiquitination of HA-NLRP3 was analysed. The data are presented as the mean \pm S.D. of three independent 164 experiments. A p value of < 0.05 was considered statistically significant; and *p< 0.05, **p< 0.01, ***p< 0.001165 assessed via a ANOVA with the Bonferroni post hoc test for comparisons among more than two groups (B, 166 167 C); ns: not significant.



169

170 Fig. S11. The MARCH7 mutant fails to suppress hepatic steatosis, inflammation, and insulin resistance 171 in HFD-fed mice. Mice were fed a ND or HFD for 12 weeks. Then, they were injected every 10 days with 172 the adenoviruses AD-vector, AD-MARCH7-WT, and AD-MARCH7-Mut, and these mice were continuously 173 fed a ND or HFD. After 3 weeks of the first injection, the mice were used for analysis. (A) The food intake 174 of ND- and HFD-mice (n=5/group). (B) The body weights, liver weights, and LW/BW ratios of C57BL/6 175 and HFD-fed mice (n=5/group). (C) Hepatic TG, NEFA, and TC levels were measured using ELISA 176 (n=5/group); (D) Oil Red O staining of mouse liver sections (n=5/group); Scale bar, 50 μ M. (E) The mRNA 177 levels of inflammation-related genes analysed by RT–qPCR (n=5/group). (F) Fasting blood glucose levels of 178 C57BL/6 and HFD-fed mice (n=5/group). (G, H) Fasting insulin levels and HOMA-IR scores of C57BL/6 179 and HFD-fed mice (n=5/group). (I-L) GTT and ITT of C57BL/6 and HFD-fed mice and the areas under the 180 curve (AUCs) for the GTT and ITT (N=5/group). The data are presented as the mean \pm S.D. of three independent experiments. A p value of < 0.05 was considered statistically significant; and *p < 0.05, **p < 0.01, 181 182 ***p < 0.001 assessed via a ANOVA with the Bonferroni post hoc test for comparisons among more than two 183 groups (A-L); ns: not significant.



185

186 Fig. S12. Metformin exerts anti-NAFLD activity by affecting the levels of GAS5, miR-28a-5p,

188 kilogram of body weight for a month accompanied by 12 weeks of ND or HFD feeding. AML12 and HepG2 189 cells were incubated with FAs for 6 h, and they were incubated with both 10 nM metformin and FA for 190 another 6 h. (A) Oil Red O staining of mouse liver sections; Scale bar, 50 μ M. (B) The TG content was 191 measured using ELISA in the livers of 12-week-old ND- or HFD-fed C57BL/6 mice (n=8/group). (C) RT-192 qPCR analysis of inflammatory factors in the livers of mice. (D) Oil Red O staining of hepatocytes (AML12 193 and HepG2); Scale bar, 50 µM. (E) The TG content was measured using ELISA in hepatocytes. (F) RT-194 qPCR analysis of inflammatory genes in hepatocytes. (G) RT-qPCR analysis of the expression of miR-28a-195 5p, MARCH7, and GAS5 in the livers of mice. (H) RT-qPCR analysis of the expression of miR-28a-5p, MARCH7, and GAS5 in hepatocytes. (I) Western blot analysis of MARCH7-mediated pyroptosis genes in 196 197 the livers of mice. (J) Western blot analysis of MARCH7-mediated pyroptosis genes in hepatocytes. The 198 data are presented as the mean \pm S.D. of three independent experiments. The one-way analysis of variance 199 (ANOVA) with the Bonferroni post hoc test was conducted for comparisons among more than two groups

MARCH7, and NLRP3. C57BL/6 mice (N=5) were administered daily with 200 mg metformin per

- 200 (B, C, E-J). A *p* value of < 0.05 was considered statistically significant; and **p* < 0.05, ***p* < 0.01; ****p* < 0.001;
- 201 $^{ns}p > 0.05$, ns: not significant.

202



Fig. S13. Knockdown of GAS5 significantly inhibited the effects of metformin on pyroptosis and hepatic lipid deposition. After 48 h of transfection, AML12 and HepG2 cells were incubated with 10 nM metformin for another 6 h. After one week of injection of AD-GAS5, C57BL/6 mice were administered 200 mg metformin per kilogram of body weight daily for one month. Then, AML12 cells, HepG2 cells, and mouse livers were collected for further experiments. (**A**, **B**) RT–qPCR was used to determine the effects of metformin on miR-28a-5p levels in GAS5-knockdown AML12 cells, HepG2 cells, and mouse livers. (**C**, **D**)

- 210 Western blot analysis of the effects of metformin on pyroptosis in GAS5-knockdown AML12 cells, HepG2
- 211 cells, and mouse livers. (E, F) Oil Red O staining was used to examine the effects of metformin on lipid
- accumulation in GAS5-knockdown AML12 cells, HepG2 cells, and mouse livers; Scale bar, 50 µM. The data
- 213 are presented as the mean ± S.D. of three independent experiments. The one-way analysis of variance
- 214 (ANOVA) with the Bonferroni post hoc test was conducted for comparisons among more than two groups
- 215 (A, B, F). A *p* value of < 0.05 was considered statistically significant; and **p* < 0.05, ***p* < 0.01, ****p* < 0.001;
- 216 $^{ns}p > 0.05$, ns: not significant.