Supplementary

Table S1 Antibody information

Antibody	Cat No.	Manufacturer
ERK1/2	4695	Cell Signaling Technology
p-ERK1/2	4370	Cell Signaling Technology
MEK	4694	Cell Signaling Technology
p-MEK	2338	Cell Signaling Technology
Vimentin	60330-1-Ig	Proteintech
N-cadherin	22018-1-AP	Proteintech
E-cadherin	20874-1-AP	Proteintech
Snail	21350-1-AP	Proteintech
Ki-67	27309-1-AP	Proteintech
β-actin	66009-1-Ig	Proteintech
GAPDH	60004-1-Ig	Proteintech

https://dx.doi.org/10.21037/tgh-24-115

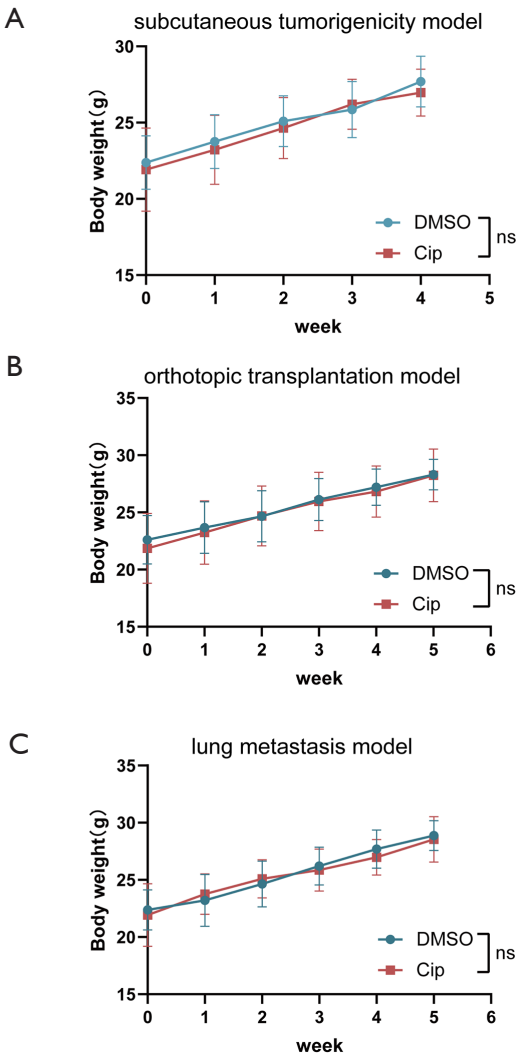

Figure S1 Ciprofol treatment did not affect the growth of mice. (A) The body weight of DMSO- or ciprofol-treated mice for 5 weeks of subcutaneous tumourigenicity model. (B) The body weight of DMSO- or ciprofol-treated mice for 6 weeks of orthotopic transplantation model. (C) The body weight of DMSO- or ciprofol-treated mice for 6 weeks of lung metastasis model. ns, not significant. DMSO, dimethylsulfoxide.

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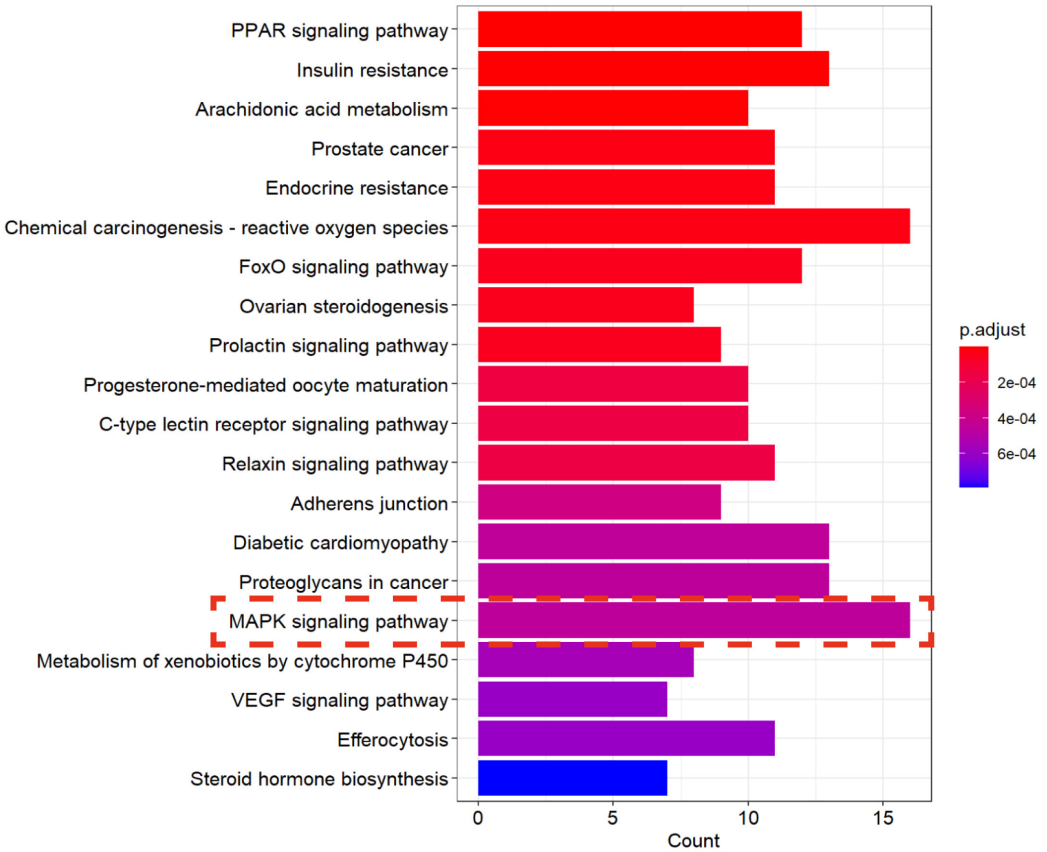

Figure S2 The Raf-MEK-ERK signalling pathway is the downstream target of ciprofol. Pathway enrichment analysis of ciprofol, based on structural predictions by PharmMapper and molecular docking. The red box highlights the MAPK signaling pathway. MAPK, mitogen-activated protein kinase; PPAR, peroxisome proliferator-activated receptor; VEGF, mitogen-activated protein kinase.

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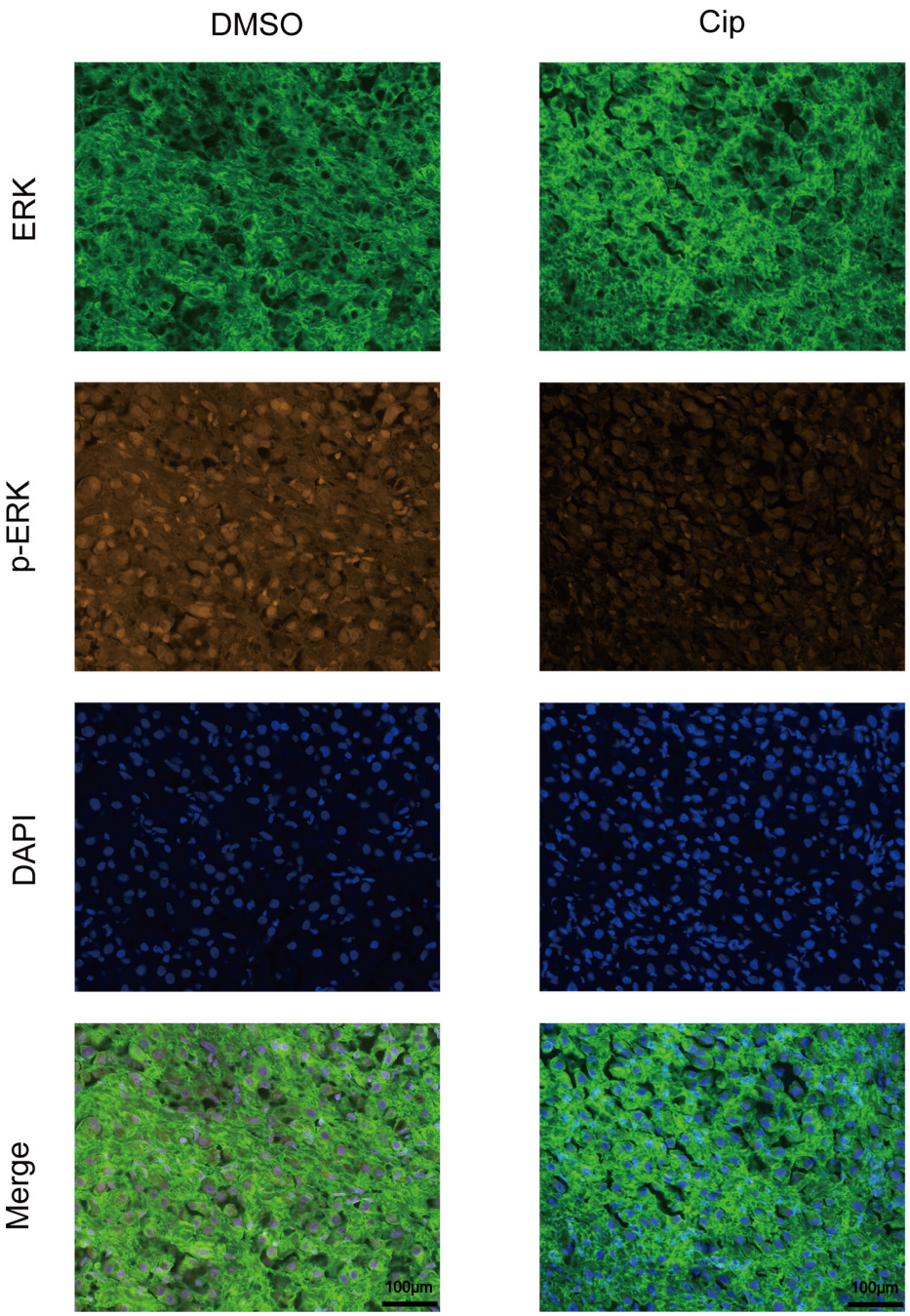

Figure S3 Ciprofol inhibits the expression level of p-ERK. Multiplex IHC assays showed the expression levels of ERK and p-ERK under DMSO- and ciprofol-treatment in subcutaneous tumours of nude mice (n=6) (scale bars, 100 μm). DMSO, dimethylsulfoxide; IHC, immunohistochemistry.

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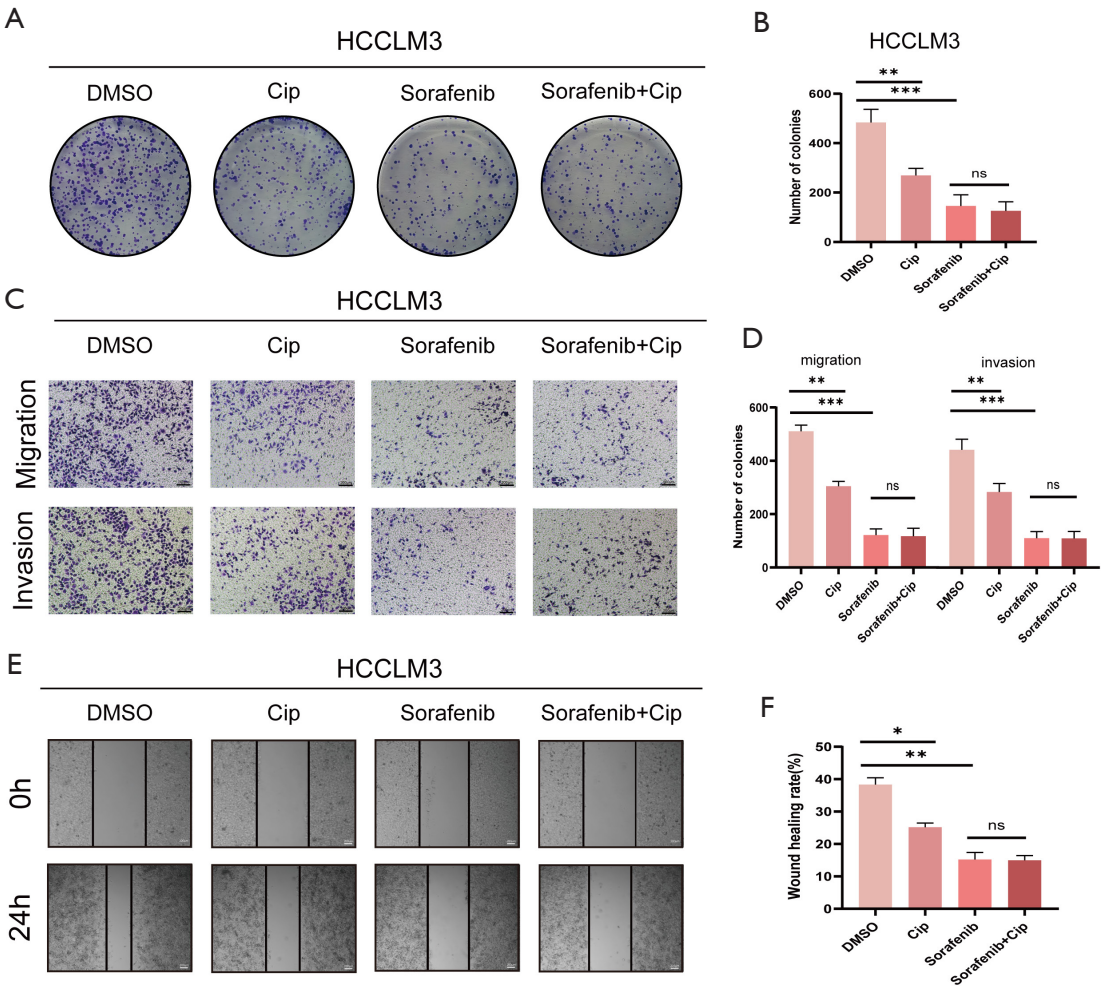

Figure S4 The anti-tumour effect of ciprofol is reversed by sorafenib in HCCLM3. (A,B) Colony formation assay was used to assess the proliferation capacity of HCCLM3 after with different treatments. Cells were treated with ciprofol (10 μM), sorafenib (10 nm) or their combination for 14 days. The clones were fixed, stained by violate crystal. (C,D) The migration and invasion abilities of HCCLM3 in each treatment group were assessed using the transwell assay (scale bars, 200 μm). Cells were treated with ciprofol (10 μM), sorafenib (10 nm) or their combination for 48 hours. The upper chambers were fixed and then stained with violate crystal. (E,F) Wound healing assay showed the migration ability of HCC cells (scale bars, 200 μm). Cells were treated with ciprofol (10 μM), sorafenib (10 nm) or their combination for 24 hours. *, P<0.05; **, P<0.01; ***, P<0.001; ns, not significant. DMSO, dimethylsulfoxide.

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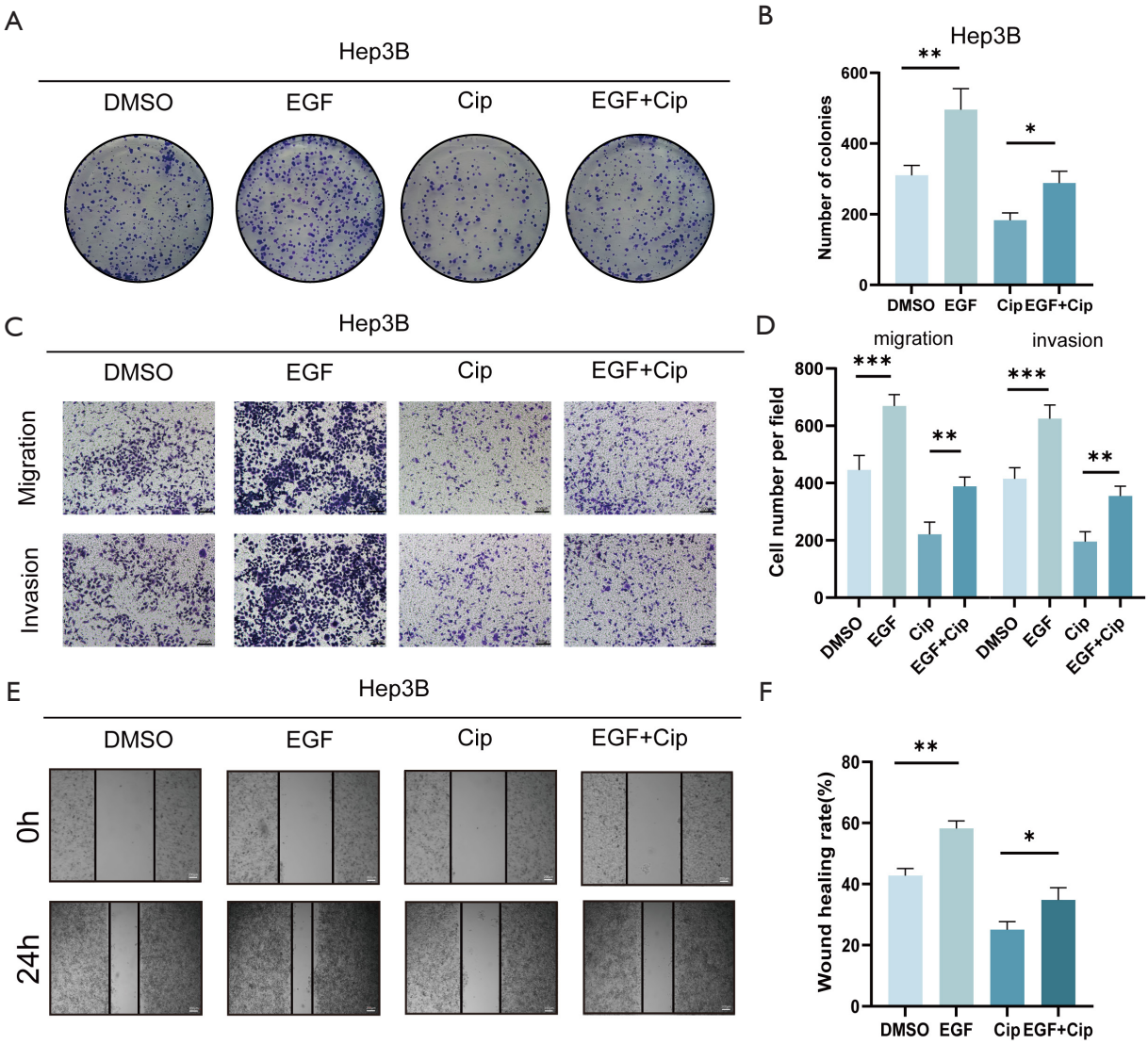

Figure S5 EGF reversed ciprofol-treated inhibition of the malignant progression of HCC cells. (A,B) Colony formation assay was used to assess the proliferation capacity of HCC cells after with different treatments. Cells were treated with ciprofol (10 μM), EGF (50 ng/mL) or their combination for 14 days. The clones were fixed, stained by violate crystal. (C,D) The migration and invasion abilities of HCC cells in each treatment group were assessed using the transwell assay (scale bars, 200 μm). Cells were treated with ciprofol (10 μM), EGF (50 ng/mL) or their combination for 48 hours. The upper chambers were fixed and then stained with violate crystal. (E,F) Wound healing assay showed the migration ability of HCC cells (scale bars, 200 μm). Cells were treated with ciprofol (10 μM), EGF (50 ng/mL) or their combination for 24 hours. *, P<0.05; **, P<0.01; ***, P<0.001. DMSO, dimethylsulfoxide.

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