

The ARRIVE reporting checklist.

Item	Item No	Detail	Reported on Page Number/Line Number	Reported on Section/Paragraph
Title	1	Ferroptosis is involved in the progression of hepatocellular carcinoma through the circ0097009/miR-1261/SLC7A11 axis	Page1/line2-3	Title/ Paragraph1
Abstract	2	Circ0097009 acts as a competing endogenous RNA to regulate the expression of SLC7A11, a key regulator of cancer cell ferroptosis, by sponging miR-1261 in HCC. Circ0097009 may be used as a diagnostic biomarker for HCC and as a potential target for HCC therapy.	Page2/line43-64	Abstract/ Paragraph1
INTRODUCTION				
Background	3	Primary liver cancer is the 6th most commonly diagnosed cancer and the 4th leading cause of cancer-related death worldwide (1). Circular RNAs (circRNAs), a class of non-coding RNAs, have been found to be widely expressed in mammals (2,3). Revealing the role that circRNAs play in hepatocellular carcinoma(HCC) is critical to gaining an in-depth understanding of the molecular mechanisms underlying HCC progression and finding new biomarkers or therapeutic targets for HCC (4).	Page4/line71-97	Introduction/ Paragraph1-3
Objectives	4	Our findings indicated that circ0097009 might act as a ceRNA to regulate the expression of SLC7A11 by sponging miR-1261, and circ0097009 may be used as a potential therapeutic target in HCC.	Page4-5/line98-110	Introduction/ Paragraph4
METHODS				
Ethical statement	5	This study was approved by the Ethics Committee of Sun Yat-Sen University Cancer Center Health Authority, and was performed according to the ethical	Page12/line357-364	Footnote/ Paragraph2

		standards of the Declaration of Helsinki. Patient consent was obtained before the study commenced. All animal studies were approved and performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Sun Yat-Sen University Cancer Center.		
Study design	6	a. one experimental(si-circ0097009 groups) and one control groups. b. Any steps taken to minimise the effects of subjective bias when allocating animals to treatment (randomisationprocedure). c. The experimental unit (group of animals).	Page5-8/line113-208	Methods/ Paragraph1-11
Experimental procedures	7	Circ0097009 is upregulated in HCC and that the knockdown of circ0097009 inhibits HCC cell growth and invasion in vivo and vitro. Moreover, circ0097009 functions as a ceRNA to regulate SLC7A11 expression by sponging miR-1261. The circ0097009/miR-1261/SLC7A11 axis mediates HCC progression by regulating ferroptosis. The details are shown in the manuscript.	Page5-8/line113-208	Methods/ Paragraph1-11
Experimental animals	8	a. Four-week-old female BALB/c nude mice. Intratumoral injection (40 μ L of si-circ0097009 or negative control siRNA) was administered every 4 days.	Page6/line155-164	Methods/ Paragraph6
Housing and husbandry	9	a. specific pathogen free [SPF]. b. Barrier environment. All animal studies were approved and performed according to the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Sun Yat-Sen University Cancer Center.	Page12/line357-364	Footnote/ Paragraph2
Sample size	10	5 mice per group for tumor formation and 6 mice per group for lung metastases.	Page6/line155-164	Methods/ Paragraph6

Allocating animals to experimental groups	11	a. BALB/c nude mice were randomly divided into two groups. b. Intratumoral injection (40 μ L of si-circ0097009 or negative control siRNA) was administered every 4 days. Cells were intravenously injected into the tail veins of mice.	Page6/line155-164	Methods/ Paragraph6
Experimental outcomes	12	Tumor weights were measured and the number of metastatic lung nodules was counted and verified by evaluation of hematoxylin-eosin (HE)-stained sections under a microscope.	Page6/line155-164	Methods/ Paragraph6
Statistical methods	13	Data are expressed as mean \pm standard error of the mean (SEM). Comparisons among groups were analyzed by one-way ANOVA and the differences between groups were analyzed by two-tailed t tests.	Page8/line204-207	Methods/ Paragraph11
RESULTS				
Numbers analysed	14	5/10 for tumor formation and 6/12 for lung metastases.	Page6/line155-164	Methods/ Paragraph6
Outcomes and estimation	15	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (e.g., 95% confidence interval).	Page8/line204-207	Methods/ Paragraph11
DISCUSSION				
Interpretation/ scientific implications	16	The findings of the present study indicated that circ0097009 is upregulated in HCC and that the knockdown of circ0097009 inhibits HCC cell growth and invasion. Moreover, circ0097009 functions as a ceRNA to regulate SLC7A11 expression by sponging miR-1261. The circ0097009/miR-1261/SLC7A11 axis mediates HCC progression by regulating ferroptosis.	Page12/343-348	Discussion/ Paragraph5
Generalisability/ translation	17	Circ0097009 may be a diagnostic biomarker for HCC and a potential target for HCC therapy.	Page12/347-348	Discussion/ Paragraph5
Funding	18	National Natural Science Foundation of China (No. 81901850, Ning Lyu).	Page12/351-352	Acknowledgments/ Paragraph1

References:

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin* 2018;68:394-424.
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3. Kristensen LS, Andersen MS, Stagsted LVW, et al. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet* 2019;20:675-91.
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