

## Supplementary Materials for

### **Isovalerylsiramycin I suppresses non-small cell lung carcinoma growth through ROS-mediated inhibition of PI3K/AKT signaling pathway**

Zeyu Liu, Moli Huang, Yue Hong, Shaoyang Wang, Yongle Xu, Cheng Zhong, Jingyuan Zhang, Zhengping Zhuang\*, Shan Shan\*, Tao Ren\*

\*Corresponding author. Email: [liuyuanrentao@sjtu.edu.cn](mailto:liuyuanrentao@sjtu.edu.cn) (Tao Ren), [shanshan\\_shcn@126.com](mailto:shanshan_shcn@126.com) (Shan Shan), [zpzhua@hotmail.com](mailto:zpzhua@hotmail.com) (Zhengping Zhuang)

#### **The PDF file includes:**

Fig. S1. ISP-I possessed a vigorous anti-NSCLC effect in vivo.

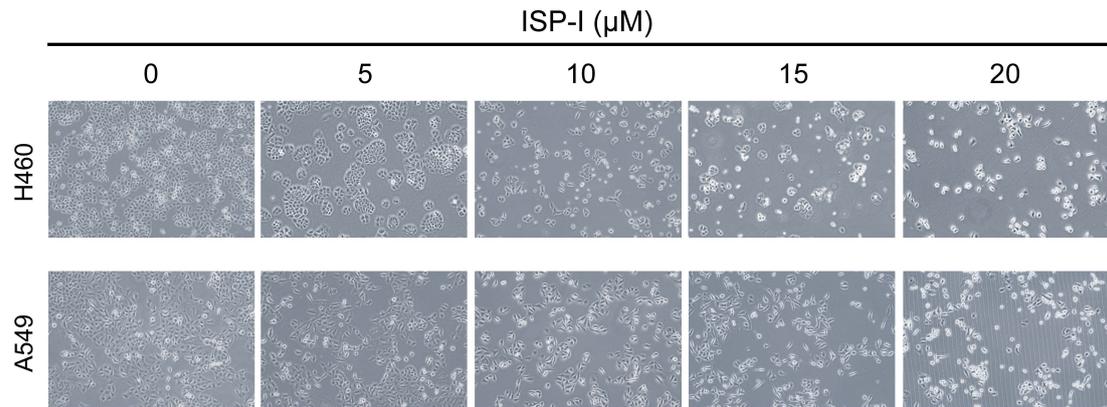
Fig. S2. ISP-I exhibited a favorable tumor inhibition effect in orthotopic lung tumor model.

Fig. S3. Safety assessment of the more proliferating organs after ISP-I treatment.

Table S1. Antibodies used in this study.

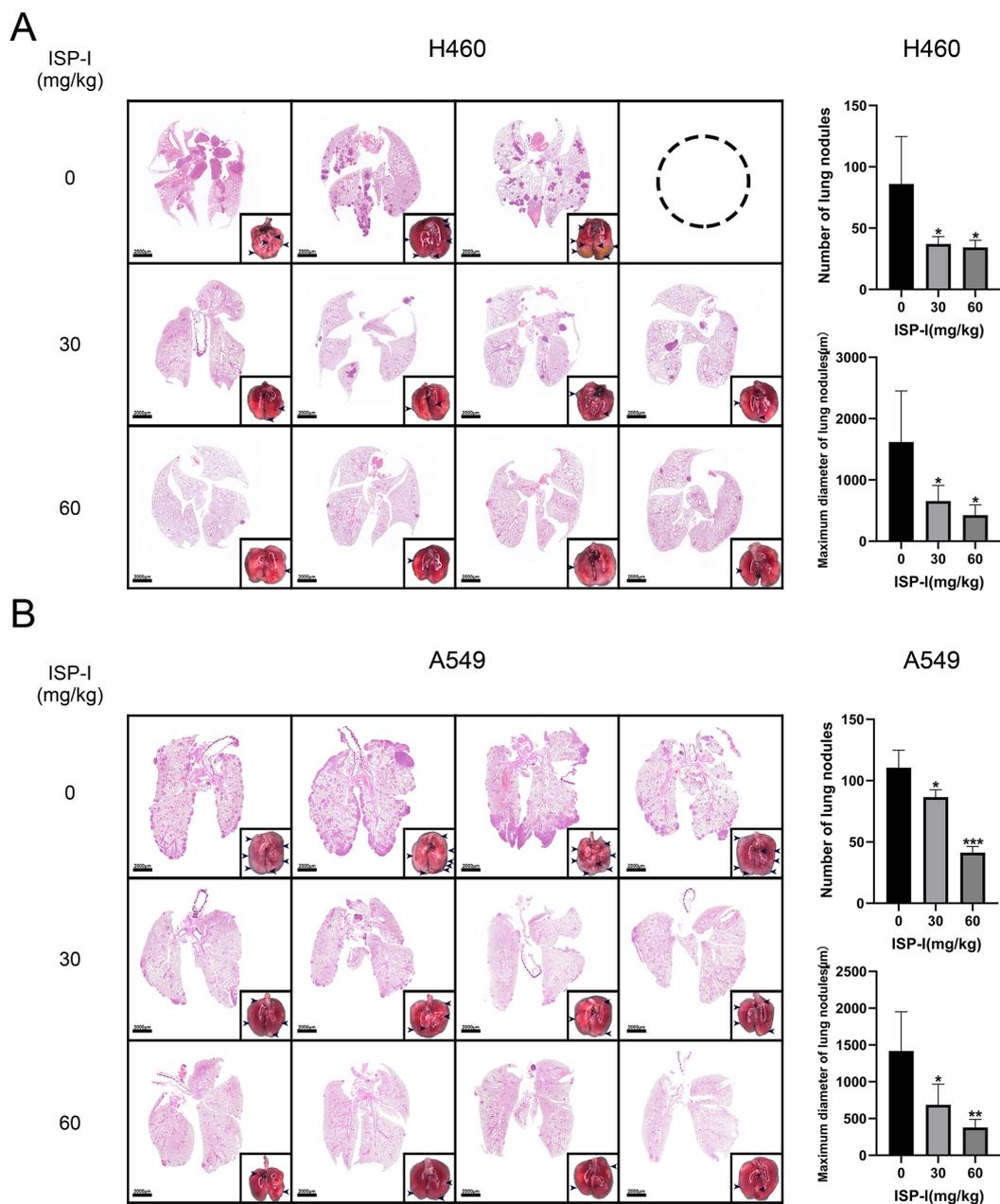
Table S2. Primers' sequences used for qRT-PCR.

**Fig. S1**



**Fig. S1. ISP-I possessed a vigorous anti-NSCLC effect in vivo.** Human NSCLC cell lines (H460 and A549) were treated with different doses of ISP-I or DMSO (control) for 48 h. A Nikon Eclipse Ts2 inverted microscope (100× magnification) was used to observe and image cell morphology and numbers.

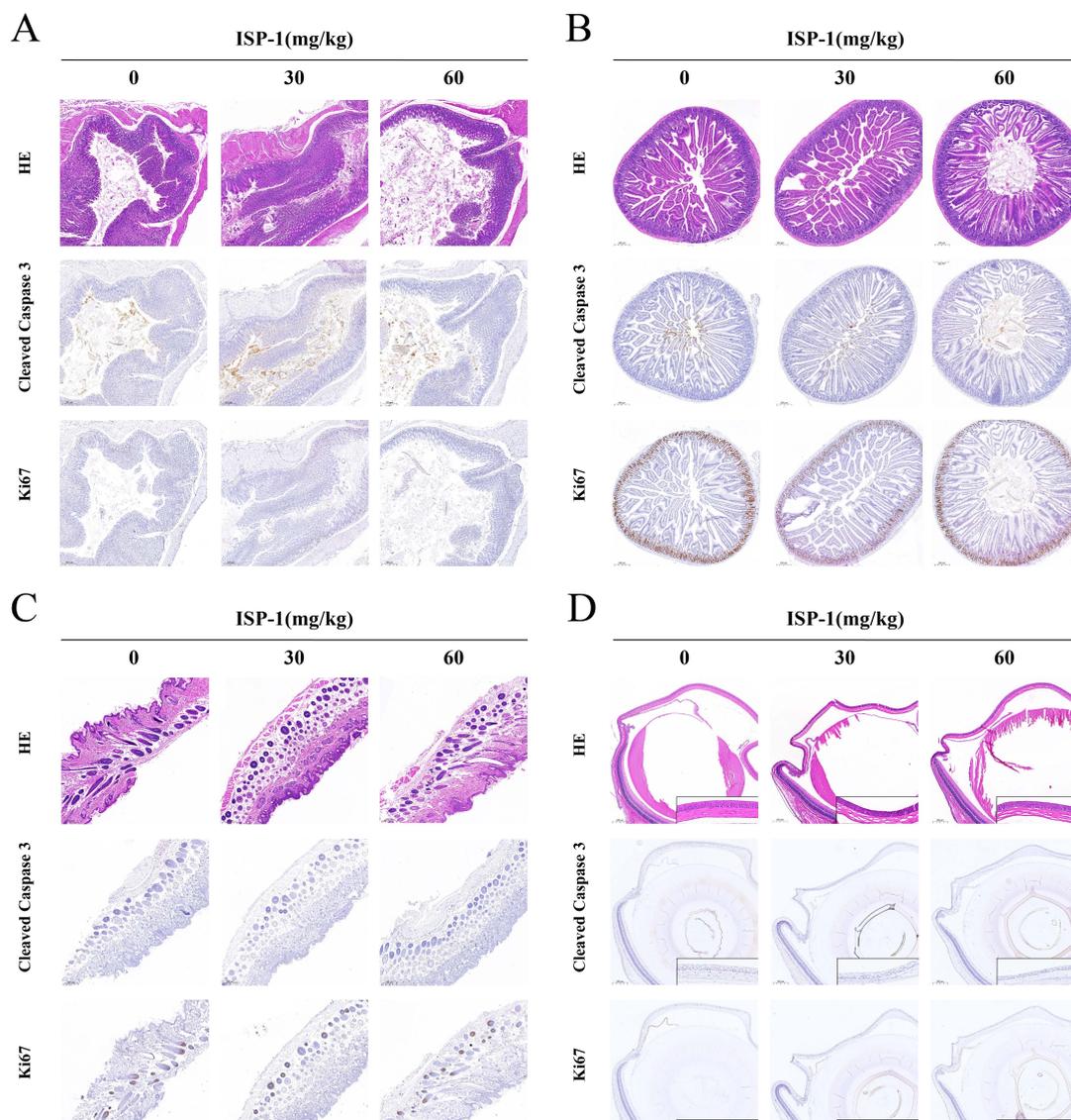
**Fig. S2**



**Fig. S2. ISP-I exhibited a favorable tumor inhibition effect in orthotopic lung tumor model.** ISP-I (30 or 60 mg/kg, experimental groups) or vehicle (water containing PEG, vegetable oil and Tween-80, control group) were administered daily by oral gavage in nude mice with orthotopic lung tumors formed by H460 (**A**) and A549 (**B**). After 18 days, the mouse lungs were

removed for gross observation and HE staining (Left panels). Number and maximum diameter of lung nodules were measured further (Right panels). Data are expressed as mean  $\pm$  standard deviation, n=4 unless one mouse in the H460-control group died during the experiment (black circle).

**Fig. S3**



**Fig. S3. Safety assessment of the more proliferating organs after ISP-I treatment.** After 18 days treatment of vehicle or ISP-I (30 or 60 mg/kg), the more proliferating organs, including stomach (A), small intestine (B), skin (C) and eyes (D), were removed for HE and IHC staining of Ki67 and cleaved Caspase3. No pathological changes on tissue morphology, proliferative activity and apoptosis were found in ISP-I treated groups, compared with control group.

**Table S1. Antibodies used in this study.**

<b>Antibodies</b>	<b>Supplier</b>	<b>Cat no.</b>
cleaved caspase-3(Asp175)	Cell Signaling Technology	#9664
cleaved caspase-8(Asp374)	Cell Signaling Technology	#9496
cleaved caspase-9(Asp330)	Cell Signaling Technology	#52873
p21 Waf1/Cip1	Cell Signaling Technology	#2947
cyclin B1	Cell Signaling Technology	#4138
cdc2	Cell Signaling Technology	#9116
Phospho-cdc2 (Tyr15)	Cell Signaling Technology	#9111
PI3 Kinase p85	Cell Signaling Technology	#4257
Phospho-PI3 Kinase p85(Tyr458)/p55(Tyr199)	Cell Signaling Technology	#4228
Akt	Cell Signaling Technology	#4691
phospho-AKT(Ser473)	Cell Signaling Technology	#4060
mTOR	Cell Signaling Technology	#2983
phospho-mTOR (Ser2448)	Cell Signaling Technology	#5536
FoxO3a	Cell Signaling Technology	#2497
phospho-FoxO3a (Ser253)	Cell Signaling Technology	#9466
FoxO1	Cell Signaling Technology	#2880
phospho-FoxO1 (Ser256)	Cell Signaling Technology	#9461
Bcl-2	Abcam	#ab32124
Bax	Abcam	#ab32503

PUMA	Abcam	#ab9643
GAPDH	Abcam	#ab8245
Ki67	Abcam	#ab16667
HRP-conjugated Goat Anti- Rabbit IgG (H+L)	ABclonal	# AS014
HRP-conjugated Goat Anti- Mouse IgG (H+L)	Abclonal	# AS003

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**Table S2. Primers' sequences used for qRTPCR.**

<b>Gene</b>	<b>Species</b>	<b>Primer sequence</b>
<i>PIK3CB</i>	Human	Forward: GACTTTGCGACAAGACTGCC Reverse: AATCTGAAGCAGCGCCTGAA
<i>AKT1</i>	Human	Forward: CAGGATGTGGACCAACGTGA Reverse: AAGGTGCGTTTCGATGACAGT
<i>MTOR</i>	Human	Forward: TGCCAATGAGAGGAAAGGTG Reverse: CCAATTCGGGTGGCATTTC
<i>EIF4EBP1</i>	Human	Forward: TGTGACCAAAACACCCCCAA Reverse: TGGTAGTGCTCCACACGATG
<i>EIF4</i>	Human	Forward: CAAACCTGCGGCTGATCTC Reverse: TCCCACATAGGCTCAATACCA
<i>FOXO1</i>	Human	Forward: GTGGAGATCGACCCGGACTT Reverse: CCGAGTTGGACTGGCTAAAC
<i>FOXO3</i>	Human	Forward: AGAAGTTCCCCAGCGACTTG Reverse: TCCCCACGTTCAAACCAACA
<i>CDKN1A</i>	Human	Forward: CTGCCCAAGCTCTACCTTCC Reverse: CGAGGCACAAGGGTACAAGA
<i>CDK1</i>	Human	Forward: TGGGGTCAGCTCGTTACTCA Reverse: ATGCTAGGCTTCCTGGTTTC
<i>CCNB1</i>	Human	Forward: GGTCGGGAAGTCACTGGAAA Reverse: AGCATCTTCTTGGGCACACA

<i>BAX</i>	Human	Forward: CGGGTTGTCGCCCTTTTCTA Reverse: GTCCAATGTCCAGCCCATGA
<i>BBC3</i>	Human	Forward: GACCTCAACGCACAGTACGA Reverse: ATGGTGCAGAGAAAGTCCCC
<i>BCL2</i>	Human	Forward: TTCCGCGTGATTGAAGACACC Reverse: ATCTCCCGGTTATCGTACCCT
<i>GADD45A</i>	Human	Forward: AGAGCAGAAGACCGAAAGGATG Reverse: AGGCACAACACCACGTTATC
<i>FADD</i>	Human	Forward: CCTAGACCTCTTCTCCATGCTG Reverse: TCTGAGACTTTGAGCTGACGA
<i>FAS</i>	Human	Forward: ACTGTGACCCTTGCACCAA Reverse: AGACAAAGCCACCCCAAGTT
<i>TNFRSF10A</i>	Human	Forward: CTGTTGTTGCATCGGCTCAG Reverse: CGAAAGTGGACAGCGAGTCT
<i>TNFRSF10B</i>	Human	Forward: TGACCTCCTTTTCTGCTTGCG Reverse: TTCCCCACTGTGCTTTGTACCT

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