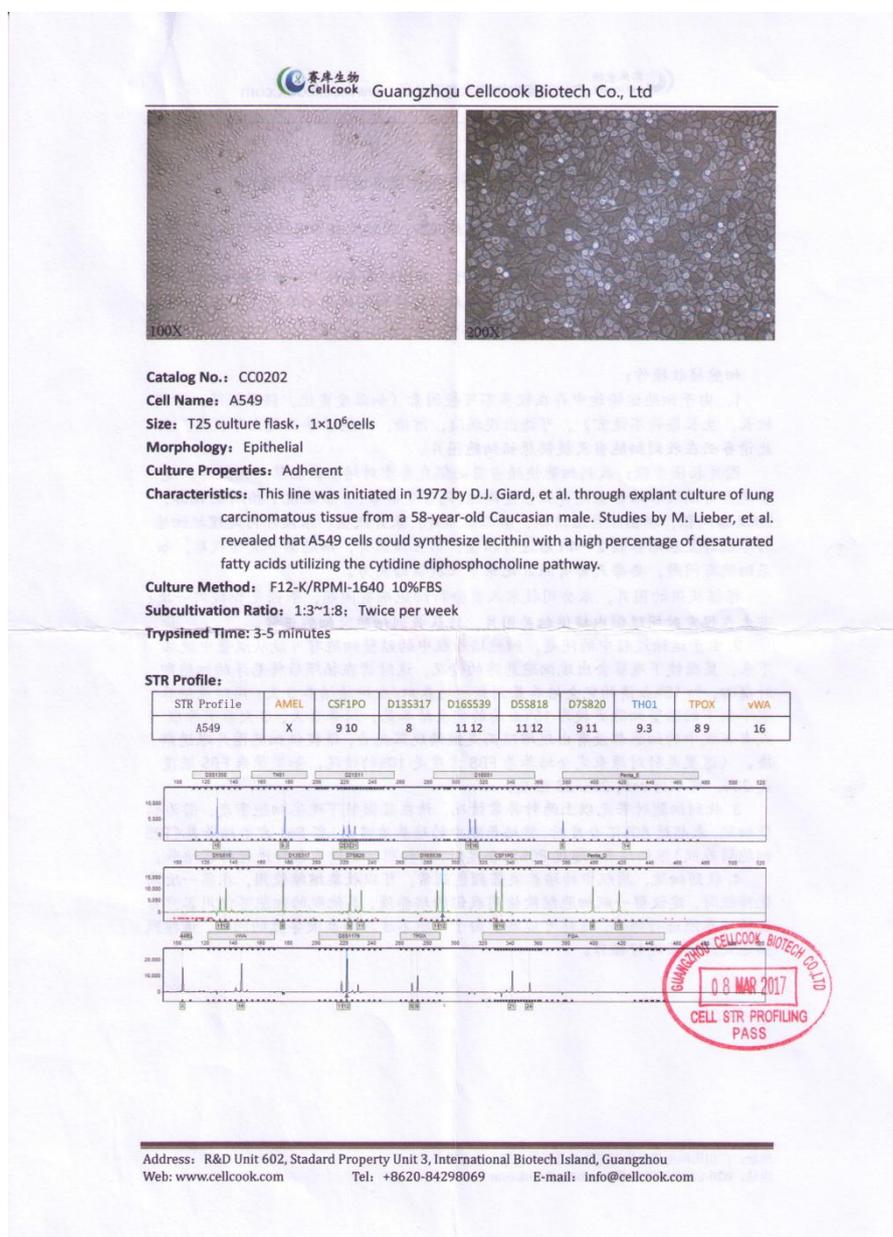
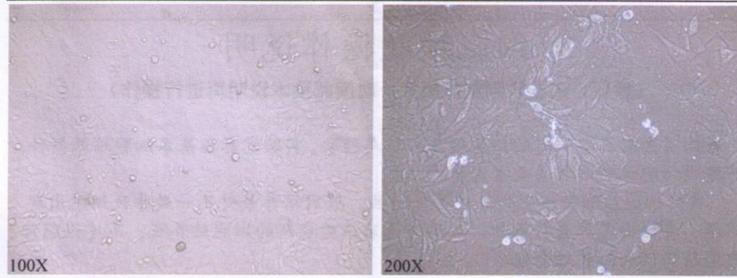


# Trichosanthin enhances sensitivity of non-small cell lung cancer (NSCLC) TRAIL-resistance cells



**Supplemental Figure S1. The authentication of the A549 cell line.** PCR was implemented with STR Multi-amplification KIT (PowerPlex™ 16HS System). No loci had tri-alleles or tetra-alleles. Contamination of other human cells was not detected. 100% matched cell line A549 were found in both ATCC and DSMZ data banks. The cell STR profiling passed on 2017/3/8.



**Catalog No.:** CC0206

**Cell Name:** NCI-H1975

**Size:** T25 culture flask,  $1 \times 10^6$  cells

**Morphology:** Epithelial

**Culture Properties:** Adherent

**Characteristics:** The line was established in July 1988. The tissue donor was a non-smoker.

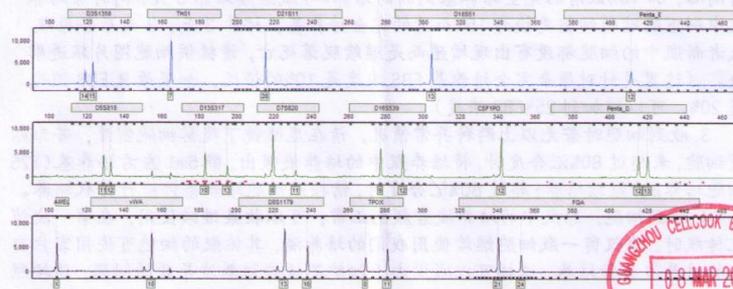
**Culture Method:** RPMI-1640 10%FBS

**Subcultivation Ratio:** 1:3~1:6; Twice per week

**Trypsined Time:** 3-5 minutes

**STR Profile:**

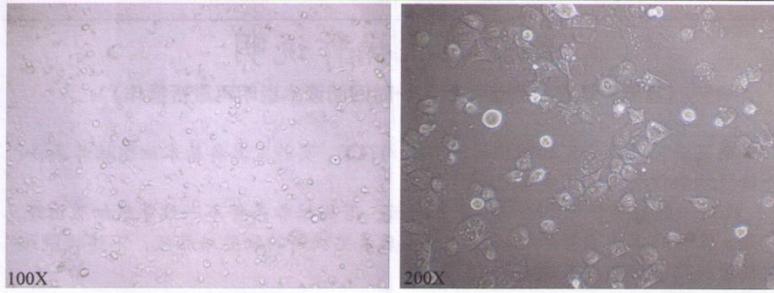
STR Profile	AMEL	CSF1PO	D13S317	D16S539	D5S818	D7S820	TH01	TPOX	VWA
NCI-H1975	X	12	10, 13	9, 12	11, 12	8, 11	7	8, 11	18



**Supplemental Figure S2. The authentication of the H1975 cell line.** PCR was implemented with STR Multi-amplification KIT (PowerPlex™ 16HS System). No loci had tri-alleles or tetra-alleles. Contamination of other human cells was not detected. 100% matched cell line H1975 were found in both ATCC and DSMZ data banks. The cell STR profiling passed on 2017/3/8.



Guangzhou Cellcook Biotech Co., Ltd



**Catalog No.:** CC0203

**Cell Name:** NCI-H1299

**Size:** T25 culture flask,  $1 \times 10^6$  cells

**Morphology:** Epithelial

**Culture Properties:** Adherent

**Characteristics:** The cell line was established from a lymph node metastasis of the lung from a patient who had received prior radiation therapy. The cells have a homozygous partial deletion of the p53 protein, and lack expression of p53 protein. They reported to be able to synthesize the peptide neuromedin B (NMB) at 0.1 pmol/mg protein, but not the gastrin releasing peptide (GRP).

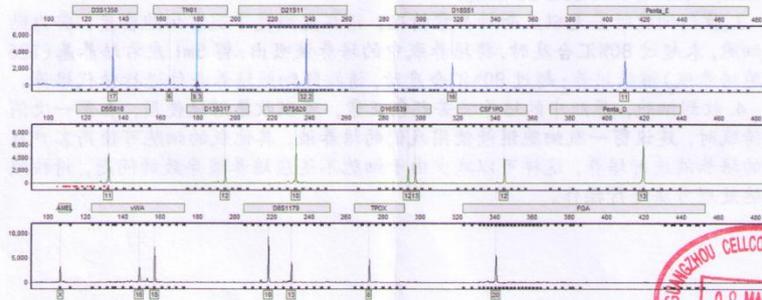
**Culture Method:** RPMI-1640 10%FBS

**Subcultivation Ratio:** 1:3~1:6; Twice per week

**Trypsined Time:** 3-5 minutes

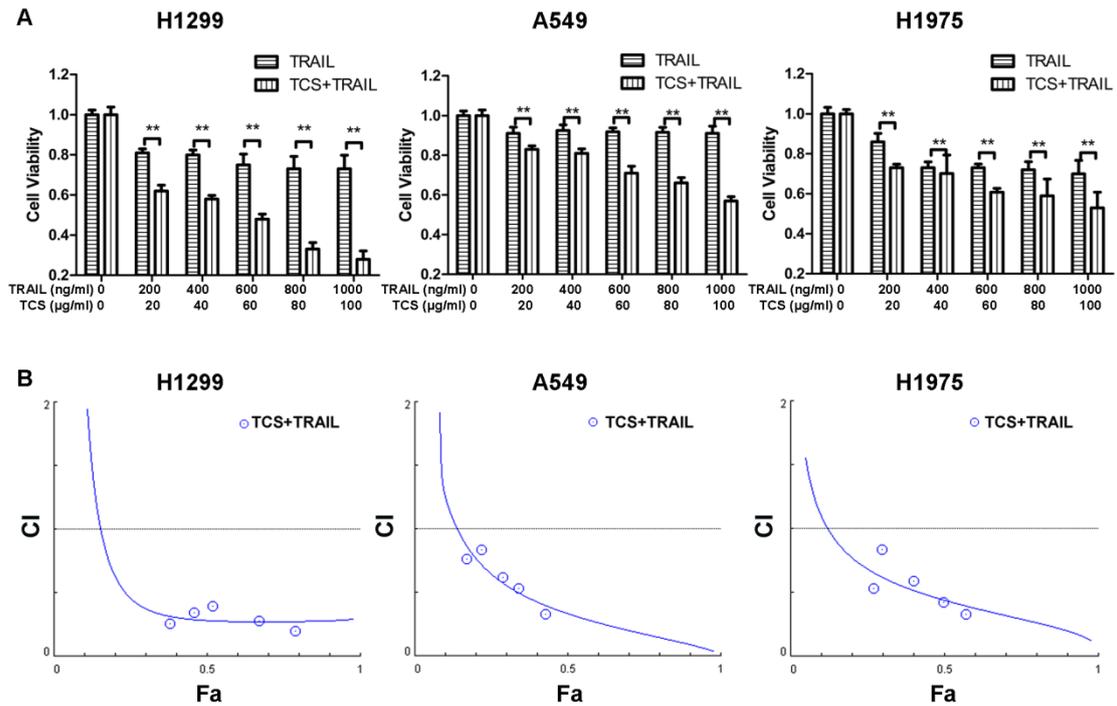
**STR Profile:**

STR Profile	AMEL	CSF1PO	D13S317	D16S539	D5S818	D7S820	TH01	TPOX	vWA
NCI-H1299	X	12	12	12 13	11	10	6 9.3	8	16 17 18

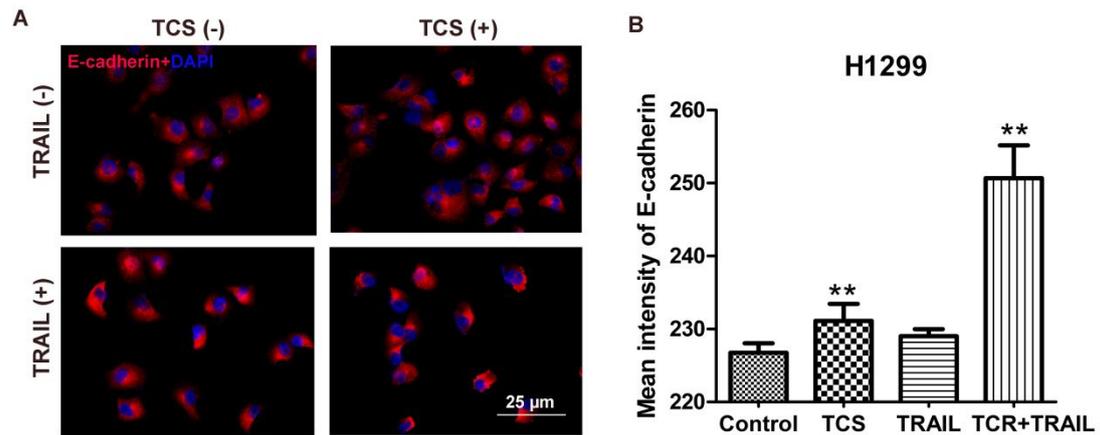


Address: R&D Unit 602, Stadar Property Unit 3, International Biotech Island, Guangzhou  
 Web: www.cellcook.com Tel: +8620-84298069 E-mail: info@cellcook.com

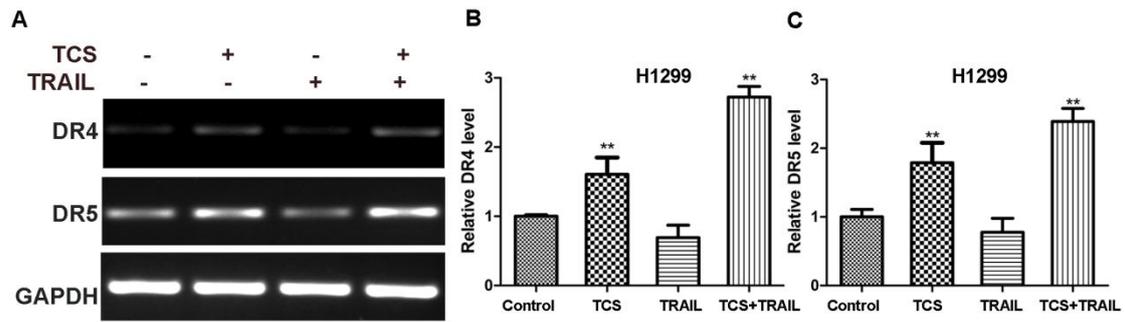
**Supplemental Figure S3. The authentication of the H1299 cell line.** PCR was implemented with STR Multi-amplification KIT (PowerPlex™ 16HS System). No loci had tri-alleles or tetra-alleles. Contamination of other human cells was not detected. 100% matched cell line H1299 were found in both ATCC and DSMZ data banks. The cell STR profiling passed on 2017/3/8.



**Supplemental Figure S4. Effects of TCS and TRAIL on the proliferation of NSCLC cells.** (A) The cells were incubated with different concentrations of TCS and TRAIL at a constant ratio of 1:10. The cell viability of H1299, A549 and H1975 were determined by CCK8. (B) Combination index (CI) values of each drug fraction were calculated using the Chou-Talalay method in H1299, A549 and H1975 cells respectively.



**Supplemental Figure S5. The combination of TCS and TRAIL upregulated E-cadherin expression.** (A) Representative images of immunofluorescence for E-cadherin in H1299 cells treated with or without TCS and TRAIL. The original magnification was 400X. The scale bar: 25  $\mu$ m. (B) The combination of TCS and TRAIL increased the immunofluorescent intensity of E-cadherin. \*\*  $p < 0.01$ .



**Supplemental Figure S6. The combination of TCS and TRAIL upregulated the transcription of DR4 and DR5.** (A) RT-PCR analysis for DR4 and DR5 after treatment with 50 ng/ml TRAIL or / and 40  $\mu$ g/ml TCS for 48 h. GAPDH was used as an internal control. (B-C) The combination of TCS and TRAIL increased the mRNA levels of DR4 and DR5. \*\*  $p < 0.01$ .



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### **To whom it may concern**

The paper "Trichosanthin enhances sensitivity of non-small cell lung cancer (NSCLC) TRAIL-resistant cells" by Chengcheng You, Yingming Sun, Shiyu Zhang, Guiliang Tang, Nannan Zhang, Chunyang Li, Xiaoli Tian, Shijing Ma, Yuan Luo, Wenjie Sun, Feng Wang, Xuefeng Liu, Yan Gong, Yu Xiao, Junhong Zhang, Conghua Xie was edited by Elsevier Language Editing Services.

Kind regards,

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**Supplemental Figure S7. The certificate issued by the Elsevier editing service.**

**Supplemental Table S1.** List of primary antibodies used in this study.

<b>Antigen</b>	<b>Species Antibody Raise in</b>	<b>Method</b>	<b>Dilution</b>	<b>Supplier</b>
<b>Ki-67, human</b>	Rabbit, monoclonal	IF	1:200	Abcam, Cat. #ab16667
<b>GAPDH, human</b>	Rabbit, polyclonal	WB	1:5,000	Proteintech, Cat. #10494-1-AP
<b>BCL-2, human</b>	Rabbit, monoclonal	WB	1:2,000	Abclonal, Cat. # A2212
<b>BAX, human</b>	Rabbit, monoclonal	WB	1:1,000	Proteintech, Cat. #50599-2-Ig
<b>PARP, human</b>	Rabbit, monoclonal	WB	1:2,000	CST, Cat. #9532
<b>FADD, human</b>	Rabbit, polyclonal	WB	1:1,000	Proteintech, Cat. #14906-1-AP
<b>Caspase 8, human</b>	Rabbit, polyclonal	WB	1:1,500	Proteintech, Cat. #13423-1-AP
<b>Caspase 3, human</b>	Rabbit, polyclonal	WB	1:1,500	Proteintech, Cat. #19677-1-AP
<b>MMP-2, human</b>	Rabbit, polyclonal	WB	1:1,500	Proteintech, Cat. #10373-2-AP
<b>MMP-9, human</b>	Rabbit, monoclonal	WB	1:1,000	Proteintech, Cat. #10375-2-AP
<b>ICAM-1, human</b>	Rabbit, monoclonal	WB	1:1,500	Proteintech, Cat. #10831-1-AP
<b>E-cadherin, human</b>	Rabbit, monoclonal	WB	1:1,000	CST, Cat. #3195
<b>E-cadherin, human</b>	Rabbit, monoclonal	IF	1:1,00	CST, Cat. #3195
<b>N-cadherin, human</b>	Rabbit, polyclonal	WB	1:1,000	Proteintech, Cat. #22018-1-AP
<b>Vimentin, human</b>	Rabbit, monoclonal	WB	1:1,000	CST, Cat. #5741
<b>CDK2, human</b>	Rabbit, polyclonal	WB	1:1,500	Proteintech, Cat. #10122-1-AP
<b>Cyclin D1, human</b>	Rabbit, monoclonal	WB	1:1,000	CST, Cat. #2978
<b>CCNE1, human</b>	Rabbit, polyclonal	WB	1:1,500	Proteintech, Cat. #11554-1-AP
<b>Cyclin A1 + Cyclin A2, human</b>	Rabbit, monoclonal	WB	1:2,000	Abcam, Cat. #ab185619
<b>P27, human</b>	Rabbit, polyclonal	WB	1:1,200	Proteintech, Cat. #25614-1-AP
<b>ATP1A1, human</b>	Rabbit, polyclonal	WB	1:1,500	Proteintech, Cat. # 14418-1-AP
<b>DR4, human</b>	Rabbit, monoclonal	WB	1:1,000	CST, Cat. # 42533
<b>DR5, human</b>	Rabbit, monoclonal	WB	1:1,000	CST, Cat. # 8074
<b>DR4, human</b>	Rabbit, monoclonal	IF	1:100	CST, Cat. # 42533
<b>DR5, human</b>	Rabbit, monoclonal	IF	1:50	CST, Cat. # 8074
<b>DR4, human</b>	Rabbit, monoclonal	FC	1:50	CST, Cat. # 42533
<b>DR5, human</b>	Rabbit, monoclonal	FC	1:20	Bioss, Cat. # bs-7352R

IF: Immunofluorescence ; FC: Flow cytometry ; WB: Western blot.

**Supplemental Table S2.** List of secondary antibodies and DAPI.

<b>Secondary Detection System Used</b>	<b>Host</b>	<b>Method</b>	<b>Dilution</b>	<b>Supplier</b>
<b>Anti-Mouse-IgG (H + L)-HRP</b>	Goat	WB	1:10,000	Proteintech, Cat. #SA00001-1
<b>Anti-Rabbit-IgG (H + L)-HRP</b>	Goat	WB	1:10,000	Proteintech, Cat. #SA00001-2
<b>Anti-Rabbit-IgG (H + L)-Alexa Fluor 488</b>	Goat	IF	1:500	Proteintech, Cat. #SA00006-3
<b>Hoechst 33342 nucleic acid staining (DAPI)</b>	-	IF	1 µg/ml	Sigma, Cat. #D8417
<b>Anti-Rabbit -IgG(H+L)-Fluorescein (FITC)</b>	Goat	IF, FC	1:100	Proteintech, Cat. #SA00003-2
<b>Anti-Rabbit -IgG(H+L)-Cy3</b>	Goat	IF, FC	1:100	Proteintech, Cat. #SA00009-2

IF: Immunofluorescence; FC: Flow cytometry; WB: Western blot.