

*Supporting information for*

**Raman fingerprint provides deep insights into alternations  
in *Ganoderma lingzhi* triterpenes-induced molecular events  
in cells**

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## SUPPLEMENTARYDATA

**Table S1**

Detailed HPLC condition settings for chemical analysis of the *G. lingzhi* triterpenes.

<b>Column:</b> Supersil ODS2 (250×4.6 mm, 5 μm)		
<b>Flowing Phase:</b> (A) Acetonitrile and (B) 0.1% acetic acid in ultrapure water (v/v)		
<b>Detection Wavelength:</b> 254 nm		<b>Column Temperature:</b> 40°C
<b>Detector:</b> UV	<b>Detection Time:</b> 80 min	<b>Flow Rate:</b> 1.0 mL/min
<b>Condition 1</b>	0-5min	40.5% A
	5-45min	40.5%-44% A
	45-70min	44%-94.75% A
	70-80min	94.75% A
	80-85min	94.75%-40.5% A
	85-95min	40.5% A
<b>Condition 2</b>	0-5min	32% A
	5-45min	32%-36% A
	45-70min	36%-94% A
	70-80min	94% A
	80-85min	94%-32% A
	85-95min	32% A
<b>Condition 3</b>	0-5min	34% A
	5-45min	34%-36% A
	45-70min	36%-94% A
	70-78min	94% A
	78-83min	94%-34% A
	83-93min	34% A

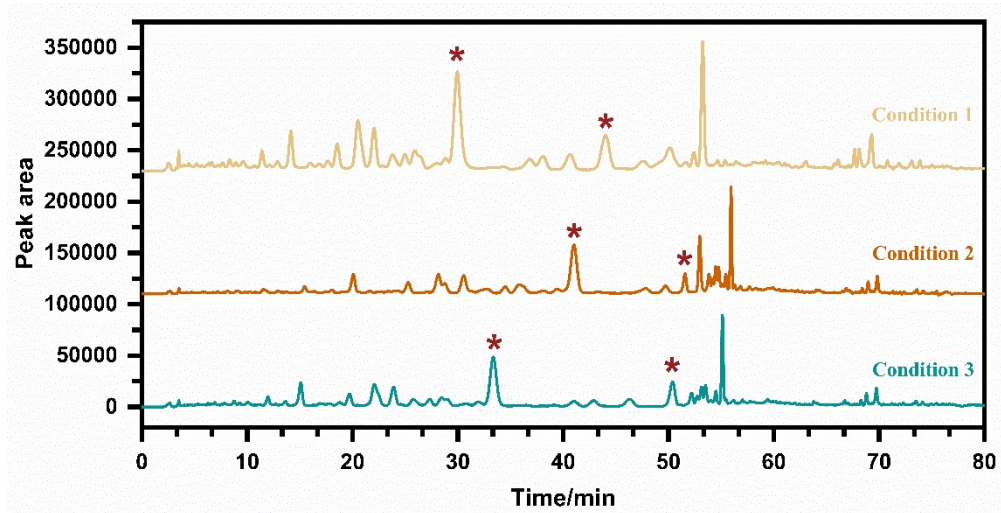


Fig. S1. HPLC analysis of the *G. lingzhi* triterpenes under different conditions.

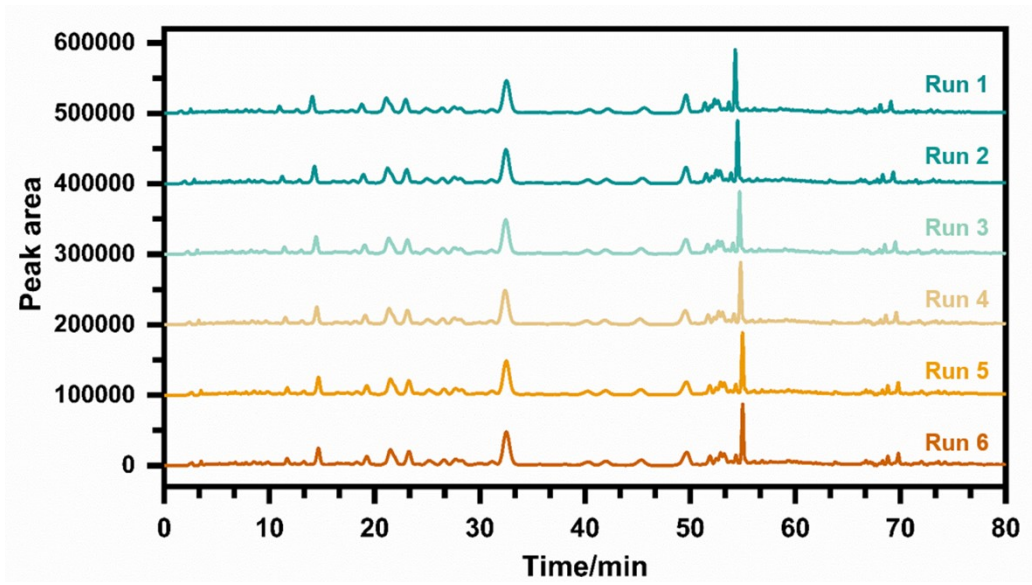
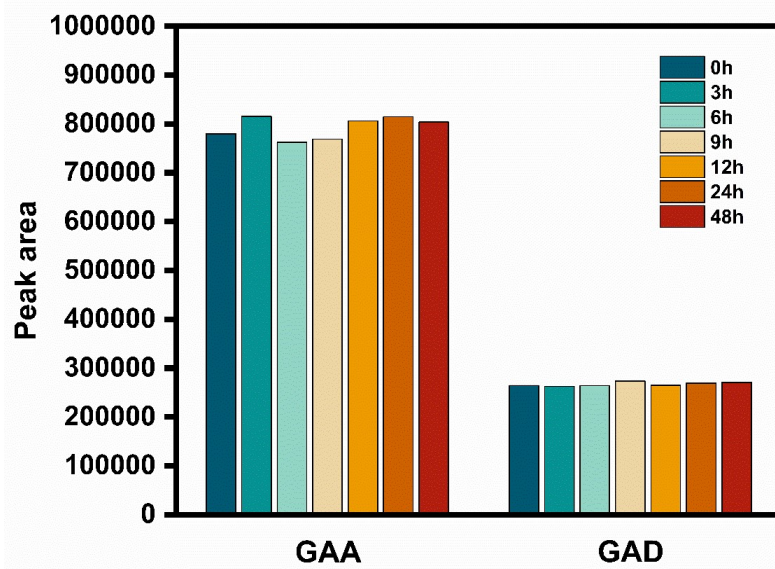


Fig. S2. The precision validation of the *G. lingzhi* triterpenes (n = 6) on the same day.



**Fig. S3.** Stability validation of the *G. lingzhi* triterpenes at different times, including 0h, 3h, 6h, 9h, 12h, 24h and 48 h.

**Table S2**

The reproducibility validation of the GAA in three independent sample solutions.

<b>Sample</b>	<b>Peak area</b>	<b>Content (µg/mL)</b>	<b>Content of Sample (mg/mL)</b>	<b>Sample Weight (mg)</b>	<b>Average contents (mg/g)</b>	<b>RSD%</b>	<b>Content (mg/g)</b>
<b>NO.1</b>	252048	30.14	0.625	141.09	0.69	3.55	0.70
	254120	30.39	0.625	141.09			
	255223	30.52	0.625	141.09			
<b>NO.2</b>	282286	33.77	0.685	137.64	0.68	3.55	0.70
	284482	34.03	0.685	137.64			
	287167	34.35	0.685	137.64			
<b>NO.3</b>	289774	34.66	0.660	139.45	0.73	3.55	0.70
	283986	33.97	0.660	139.45			
	290006	34.69	0.660	139.45			

**Table S3**

The reproducibility validation of the GAD in three independent sample solutions.

<b>Sample</b>	<b>Peak area</b>	<b>Content (µg/mL)</b>	<b>Content of Sample (mg/mL)</b>	<b>Sample weight (mg)</b>	<b>Average contents (mg/g)</b>	<b>RSD%</b>	<b>Content (mg/g)</b>
<b>NO.1</b>	88056	10.22	0.625	141.09	0.22		
	80790	9.39	0.625	141.09			
	81537	9.47	0.625	141.09			
<b>NO.2</b>	98653	11.44	0.685	137.64	0.23	3.99	0.22
	99192	11.51	0.685	137.64			
	98141	11.39	0.685	137.64			
<b>NO.3</b>	88220	10.24	0.660	139.45	0.21		
	85099	9.88	0.660	139.45			
	86648	10.06	0.660	139.45			

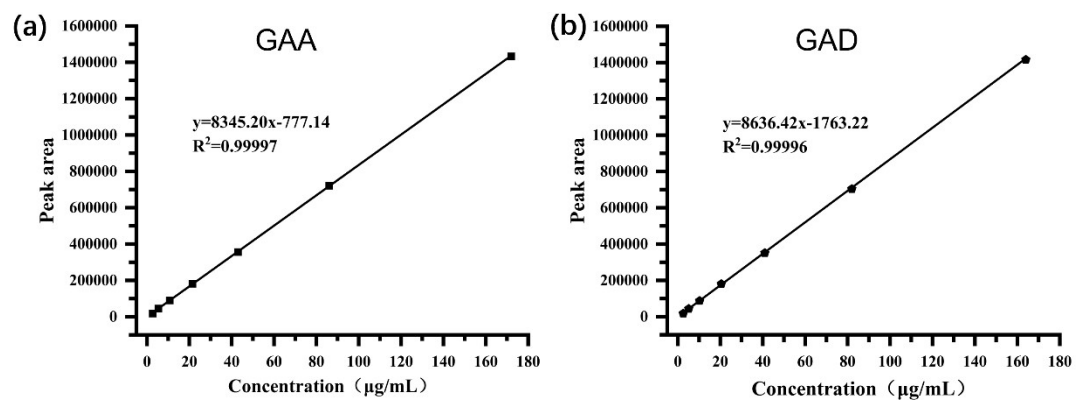


Fig. S4. Liner fitting curves of (a) GAA and (b) GAD standard solutions, respectively.



**Table S4**

The lower limit of detection (LLOD) of two quality control markers.

	<b>Peak area of GAA</b>	<b>S/N</b>	<b>Peak area of GAD</b>	<b>S/N</b>
1	1369	3.82	3582	3.98
2	1377	3.66	3789	3.80
3	1440	3.97	3931	3.73
4	1452	3.03	3873	3.59
5	1365	3.95	3958	3.56
6	1325	3.80	3847	3.80
Mean	1388	3.71	3830	3.74
RSD%	3.50		3.54	

**Table S5**

The lower limit of quantification (LLOQ) of two quality control markers.

	<b>Peak area of GAA</b>	<b>S/N</b>	<b>Peak area of GAD</b>	<b>S/N</b>
1	7871	10.65	11158	10.28
2	7374	10.90	10179	10.55
3	7403	10.41	10606	10.59
4	7276	10.94	10158	10.68
5	7622	10.52	10283	10.41
6	7895	10.52	10715	10.38
Mean	7573.5	10.66	10516.5	10.48
RSD%	3.50		3.70	

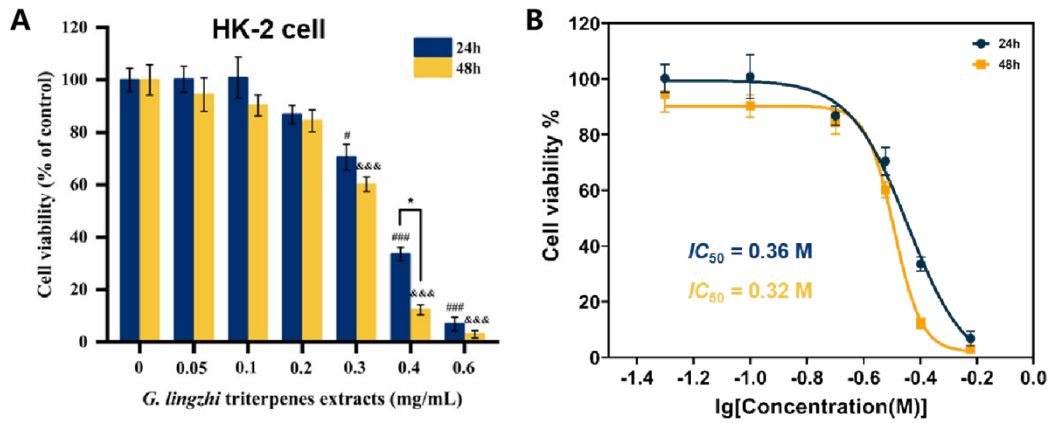
**Table S6**

The recovery validation of the two quality control markers.

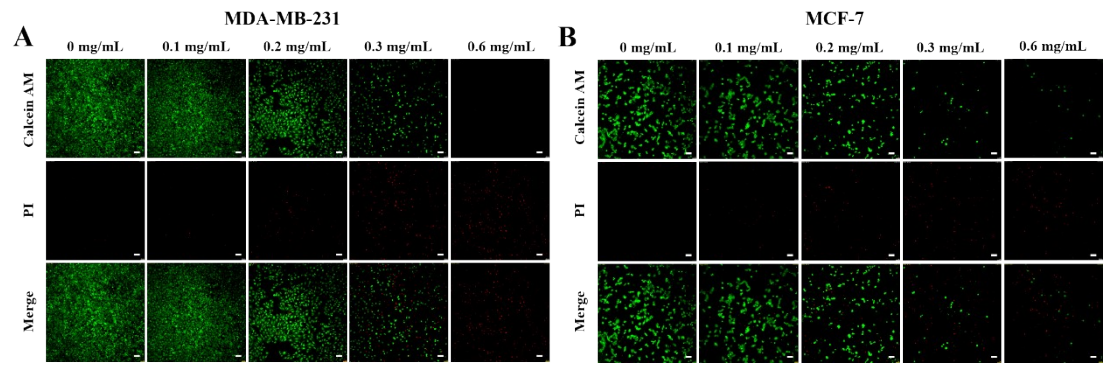
<b>Component</b>	<b>Detectable concentration (µg/mL)</b>	<b>Original concentration (µg/mL)</b>	<b>Added concentration (µg/mL)</b>	<b>Recovery (%)</b>	<b>average recovery (%)</b>	<b>RSD %</b>
<b>GAA</b>	44.83	28.91	15.47	102.87	103.67	0.91
	44.86	28.91	15.47	103.10		
	44.90	28.91	15.47	103.33		
	61.16	28.91	30.94	104.24		
	61.07	28.91	30.94	103.95		
	61.48	28.91	30.94	105.26		
	76.99	28.91	46.41	103.59		
	77.42	28.91	46.41	104.53		
	76.32	28.91	46.41	102.15		
<b>GAD</b>	14.77	9.12	5.84	96.77	96.07	1.10
	14.75	9.12	5.84	96.40		
	14.78	9.12	5.84	96.92		
	20.41	9.12	11.68	96.62		
	20.35	9.12	11.68	96.17		
	20.35	9.12	11.68	96.14		
	25.88	9.12	17.52	95.63		
	26.03	9.12	17.52	96.50		
	25.49	9.12	17.52	93.44		

**Table S7**Detailed quantitative analysis of GAA and GAD in *G. lingzhi* triterpenes.

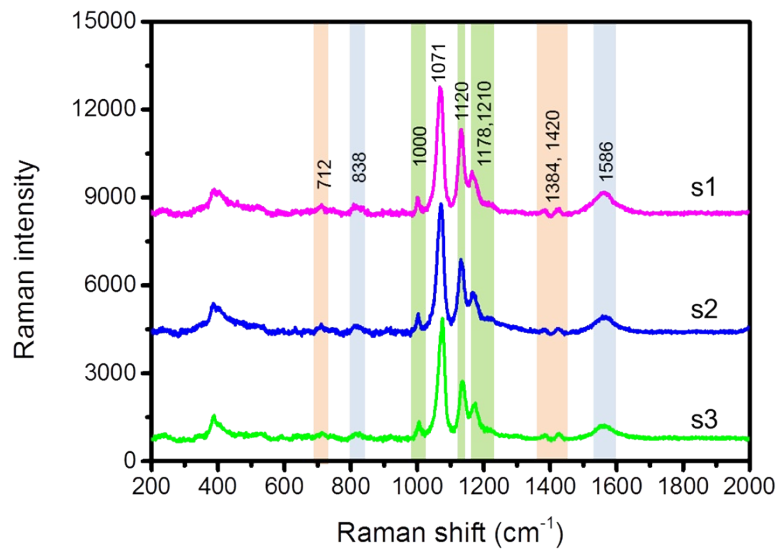
	Precision	Stability	Reproducibility	Accuracy		Content (mg/g)
				Recovery Rate	RSD	
<b>GAA</b>	1.87%	2.80%	3.55%	103.67%	0.91%	0.67
<b>GAD</b>	0.89%	1.54%	3.99%	96.07%	1.10%	0.23



**Fig. S5.** HK-2 cells were treated with *G. lingzhi* triterpenes (0-0.6 mg/mL) for 24 and 48 hours, then subjected to cell counting. (n=5, # represents compared with the 24h control group,  $P<0.05$ ; ### represents compared with the 24h control group,  $P<0.001$ ; &&& represents compared with the 48h control group,  $P<0.001$ ; \* represents the comparison between 24h and 48h at the same concentration,  $P<0.05$ .)



**Fig. S6.** Confocal laser scanning microscopy images of (A) MDA-MB-231 and (B) MCF-7 cells stained by calcein AM (green) and PI (red) after treatment with various concentrations of the *G. lingzhi* triterpenes for 48 hours. Scale bar: 50  $\mu\text{m}$ .



**Fig. S7.** Fingerprinting the contents of MDA-MB-231 cells in three parallel samples (n=3).