

Identification of Parthenolide Dimers as Activators of Pyruvate Kinase M2 in Xenograft of Glioblastoma Multiforme in vivo

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SUPPORTING INFORMATION

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1. Figures S1-S3

To explore the possibility of dimers PTL (compound **5**) covalently binding on PKM2, we performed the LC-MS/MS analysis on the PKM2 with treatment of **5**. The details are as follows:

1) expressed and purified recombinant PKM2 (rPKM2 in *E.coli*. 2) incubated the rPKM2 protein with or without compound **5** at 37 °C , 2 h. 3) After the incubation, the rPKM2 protein was separated by SDS-PAGE (Figure S1) and the target lane (approximate 60 kDa) was cut and digested with trypsin and then analyzed by LC-MS/MS (ThermoFisher Orbitrap Fusion Lumos). With analysis by Protome Discoverer 2.0 software, we found the mass of the Cys424-containing peptide CCSGAIIVLTK was measured as 1734.87 Da in the presence of **5** (Figure S2). Thus, we speculated the dimers of PTL (compound **5**) might bind to PKM2 through the sulfhydryl group of C424. The speculation is a preliminary study for compound **5** binding to PKM2, and more studies need to be devoted.

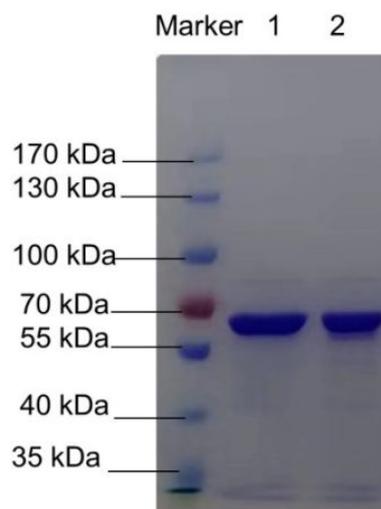


Figure S1. The in-gel digestion samples. Marker (PageRuler Prestained Protein ladder, 26616, Thermo Scientific); 1, rPKM2 with DMSO; 2, rPKM2 with compound **5** treated.

Sequence: CCSGAIIVLTK, C2-(5) (626.27216 Da)

Charge: +2, Monoisotopic m/z: 867.43835 Da (+3.58 mmu/+4.12 ppm), MH+: 1733.86943

Da, RT: 109.10 min,

Identified with: Sequest HT (v1.3); XCorr:3.74, Ions matched by search engine: 0/0

Fragment match tolerance used for search: 0.02 Da

Fragments used for search: b; b-H₂O; y; y-H₂O; y-NH₃

Protein references (1): - pyruvate kinase PKM isoform a [Homo sapiens]

| b⁺ | b²⁺ | Seq. | y⁺ | y²⁺ | #2 |
|----------------------|-----------------------|-------------|----------------------|-----------------------|-----------|
| 104.01647 | 52.51187 | C | | | 11 |
| 833.29782 | 417.15255 | C-GHY-2 | 1630.85309 | 815.93019 | 10 |
| 920.32985 | 460.66856 | S | 901.57174 | 451.28951 | 9 |
| 977.35132 | 489.17930 | G | 814.53971 | 407.77349 | 8 |
| 1048.38844 | 524.69786 | A | 757.51824 | 379.26276 | 7 |
| 1161.47251 | 581.23989 | I | 686.48112 | 343.74420 | 6 |
| 1274.55658 | 637.78193 | I | 573.39705 | 287.20216 | 5 |
| 1373.62500 | 687.31614 | V | 460.31298 | 230.66013 | 4 |
| 1486.70907 | 743.85817 | L | 361.24456 | 181.12592 | 3 |
| 1587.75675 | 794.38201 | T | 248.16049 | 124.58388 | 2 |
| | | K | 147.11281 | 74.06004 | 1 |

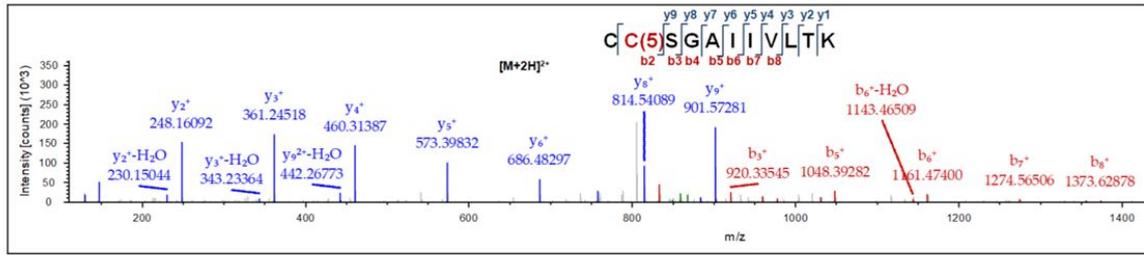


Figure S2 The mass data of LC-MS/MS assay

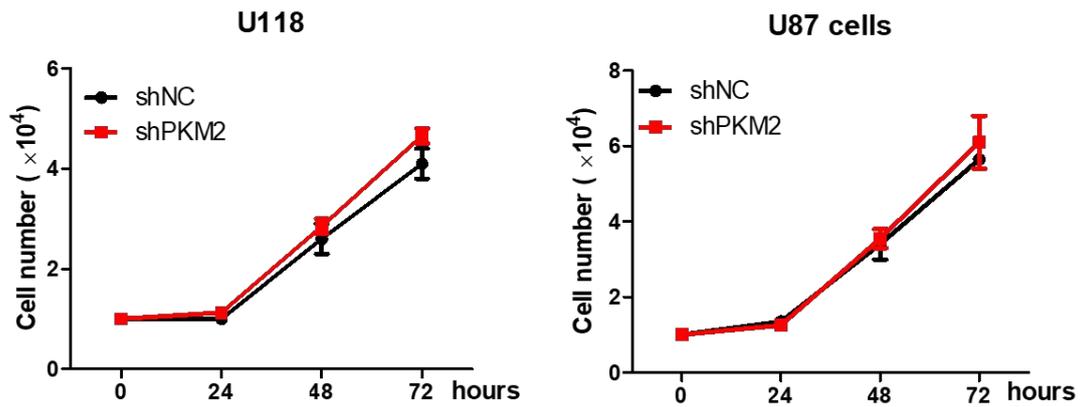
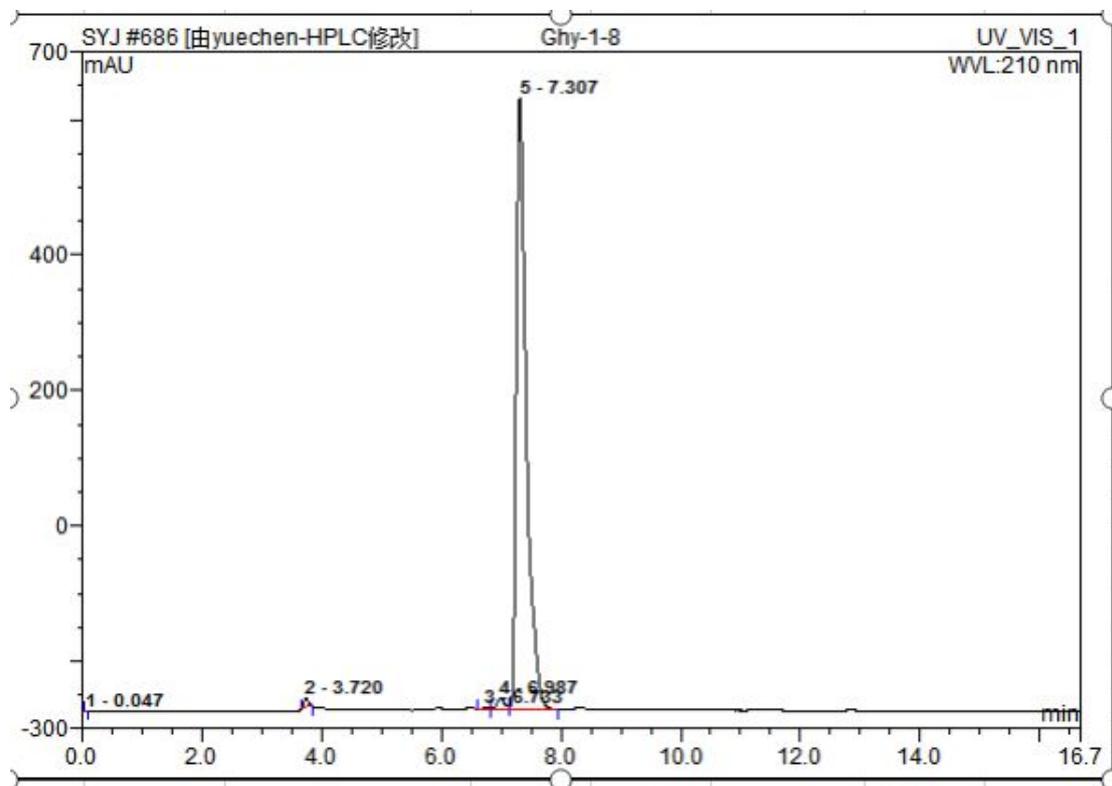


Figure S3. The growth kinetics of U118 and U87 cells after PKM2 knock down.

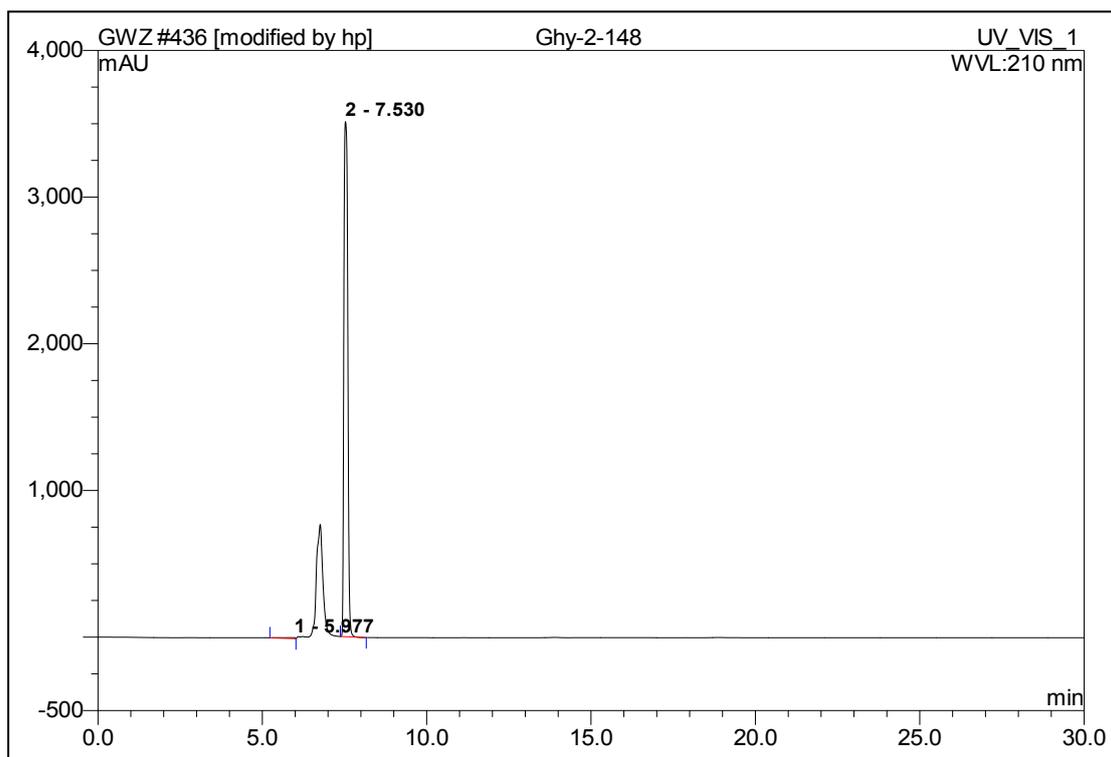
2. HPLC analysis

Compound 5:



| 序号 | 保留时间 min | 峰名称 | 峰高 mAU | 峰面积 mAU*min | 相对峰面积 % | 样品量 n.a. | 类型 |
|-----|-------------|------|-----------|----------------|------------|-------------|------|
| 1 | 0.05 | n.a. | 0.011 | 0.000 | 0.00 | n.a. | BMB |
| 2 | 3.72 | n.a. | 12.997 | 1.248 | 0.69 | n.a. | BMB* |
| 3 | 6.73 | n.a. | 1.370 | 0.187 | 0.10 | n.a. | BM * |
| 4 | 6.99 | n.a. | 16.564 | 2.433 | 1.35 | n.a. | M * |
| 5 | 7.31 | n.a. | 902.379 | 177.026 | 97.86 | n.a. | MB* |
| 总和: | | | 933.322 | 180.895 | 100.00 | 0.000 | |

Compound 16:



| No. | Ret.Time min | Peak Name | Height mAU | Area mAU*min | Rel.Area % | Amount | Type |
|---------------|-----------------|-----------|---------------|-----------------|---------------|--------|------|
| 1 | 5.98 | n.a. | 7.097 | 3.253 | 0.67 | n.a. | BMB |
| 2 | 7.53 | n.a. | 3511.282 | 482.203 | 99.33 | n.a. | BMB* |
| Total: | | | 3518.379 | 485.456 | 100.00 | 0.000 | |

The first peak is fumaric acid.

3. NMR spectra of compounds

