**Supplemental Materials and Methods**

**RNA isolation and real-time PCR**

Total RNA was extracted using TRNzol (TianGen Biotech, Beijing, China) and converted to cDNA using the Maxima First Strand cDNA Synthesis Kit (Thermo Scientific, Pittsburgh, PA). Total RNA purification and reverse transcription procedures were performed according to the manufacturer's protocol. Quantitative PCR was performed with PowerUpTM SYBRTM Green Master Mix (Thermo Fisher Scientific) using an ABI 7500 instrument (Applied Biosystems, Carlsbad, CA). The quantitative data were normalized by internal control (β-actin) and triplicate assays were performed.

Primers and shRNA sequence:

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| --- | --- |
| PYGL-F | 5’CACTTCAGTGGCAGATGTGGTG 3’ |
| PYGL-R | 5’GCAGTGGAAATCTGCTCTGACAG 3’ |
| GLUL-F | 5’CTCCACTGTACGGCGTAGTC 3’ |
| GLUL-R | 5’ TGCTTTCCCCAACACACCAA 3’ |
| GLUD1-F | 5’ CCTGGGCGAAGCGCTGTTGCT 3’ |
| GLUD1-R | 5’ GGGCTGTCCCCGGGCCCA 3’ |
| PDC-E1α-F | 5’ AGAGAATTCGGATCCATGAGGAAGATGCTCGCCGC 3’ |
| PDC-E1α-R | 5’ CTTCCATGGCTCGAGCCCGGGTTAACTGACTGACTTAAACTTGATCCAC 3’ |
| PDC-E1β-F | 5’ AGAGAATTCGGATCCATGGCGGCGGTGTCTGGCTT 3’ |
| PDC-E1β-R | 5’ CTTCCATGGCTCGAGCCCGGGCTAAATATTTAATGTTTTCTTTATTGCAAATATG 3’ |
| PDC-E2-F | 5’ AGAGAATTCGGATCCATGTGGCGCGTCTGTGCGC 3’ |
| PDC-E2-R | 5’ CTTCCATGGCTCGAGCCCGGGTTACAACAACATAGTGATAGGTTTTTCAAG 3’ |
| PDC-E3-F | 5’ AGAGAATTCGGATCCATGCAGAGCTGGAGTCGTGT 3’ |
| PDC-E3-R | 5’ CTTCCATGGCTCGAGCCCGGGAAAGTTGATTGATTTGCCAAATGACG 3’ |
| RIP3-shRNA-#3 | CAGGGTTGGTATAATCATA |
| RIP3-shRNA-#4 | GAGAGACAAGGCATGAACT |
| RIP3-shRNA-#5 | GGCTAAACAAACTGAATCT |
| RIP3-shRNA-#6 | ACTCTCGTAATGATGTCAT |

**Western blot**

Cells were harvested and washed twice with ice-cold PBS, and then lysed in whole-cell extract buffer (25mM Tris–HCl, pH 7.4, 150mM NaCl, 1%NP40, 1mM EDTA, 5% v/v glycerol). Equal amounts of the total proteins from cell preparations and PageRulerTM molecular weight markers (Fermentas life sciences) were resolved by SDS–polyacrylamide gel electrophoresis and electrotransferred to a PVDF membrane. The membranes were blocked and then incubated with specific primary antibodies according to the manufacturer’s recommendations. The primary antibody complexes were then stained with horseradish peroxidase conjugated secondary antibody and developed with the enhanced chemiluminescence detection kit (ECL;Pierce). Visualization was performed using the ChemiDoc XRS system with Image Lab software (Bio-Rad, CA, USA).

**Supplementary figure legends**

Supplementary Figure 1 Cells were treated with different dosages of zVAD.fmk (0,10,20 μM) for 1 h. The protein levels of PARP and cleaved Caspase-3 were detected by western blot assay in (a) HT29 and (b) Raji cells. β-actin was used as a loading control.

Supplementary Figure 2 Cells were pretreated with zVAD.fmk (20 μM) for 1 h, followed by different dosages of TCN (0, 1, 2μM) treatment for 24 h. Cell viability of (a) The mRNA and (b) protein levels of RIPK1in HT29 and Raji cells. HT29 cells were seeded on coverslips overnight and pretreated with zVAD.fmk (20 μM) for 1 h, followed by different dosages of TCN (0, 1μM) treatment for 24 h. (c) The fluorescence of RIPK1 was detected by confocal microscopy and nuclei were stained blue. Quantification of the fluorescent intensity of RIPK1 imaging was shown as bar graphs. (d) The co-localization of RIP3 (green) and RIP1 (red) was determined by fluorescent microscopy, when nuclei were stained blue. Pearson’s correlation coefficient for the colocalization of RIP3 and RIP1 was shown as bar graphs. Scale bar, 25 μm. Data are shown as mean values S.D. of independent, triplicate experiments. The asterisks (\*\*,\*\*\*) indicate significant differences (p< 0.01, p< 0.001,respectively). NS, no significance.

Supplementary Figure 3 The protein levels of RIP3 in HT29 cells transfected with control shRNA or *RIP3* shRNAs (3#, 4#,5# and 6#).