Supplementary Information

Supplementary materials and methods

siRNA transfection

Human siRNA oligos were synthesized from Sangon Biotech in Shanghai. The following sequence was used in the knockdown experiments: siMLKL: 5′-CAAAACUUCUGUAACUCA-3′, siRIPK3: 5′-CCAGCACUCUCGUAAUGAUTT-3′ and siNC (5′-UUCUCCGAACGUGUCACGUTT-3′. The cells were transfected with the siRNA oligos using Lipofectamine2000 according to the manufacturer's instructions. The efficiency of RIPK3 or MLKL siRNA transfection was confirmed by western blot analysis at 24 h after transfection.

Supplementary Figure
Figure S1. Effect of NSA and siRIPK3 and siMLKL on PmPOD-induced HCT116 and HT29 cell death. (A) Dose-dependent (in μM) of necrosulfonamide (NSA) in HCT116 and HT29 cells treated with PmPOD for 48 h. Cell viability was determined by MTT assay. (B-C) Western blots of RIPK3, MLKL in HCT116 transfected with siRIPK3, siMLKL or siNC. (D) Cell viability assay in HT29 and HCT116 cells treated with PmPOD after transfected with siRIPK3 and siNC for 48h. (E) Cell viability assay in HT29 and HCT116 cells treated with PmPOD after transfected with siMLKL and siNC for 48h. Each graphical representation indicates the mean ± S.D. of at least three independent experiments. *P<0.05. **P<0.01.