Physapubescin selectively induces apoptosis in VHL-null renal cell carcinoma cells through down-regulation of HIF-2a and inhibits tumor growth

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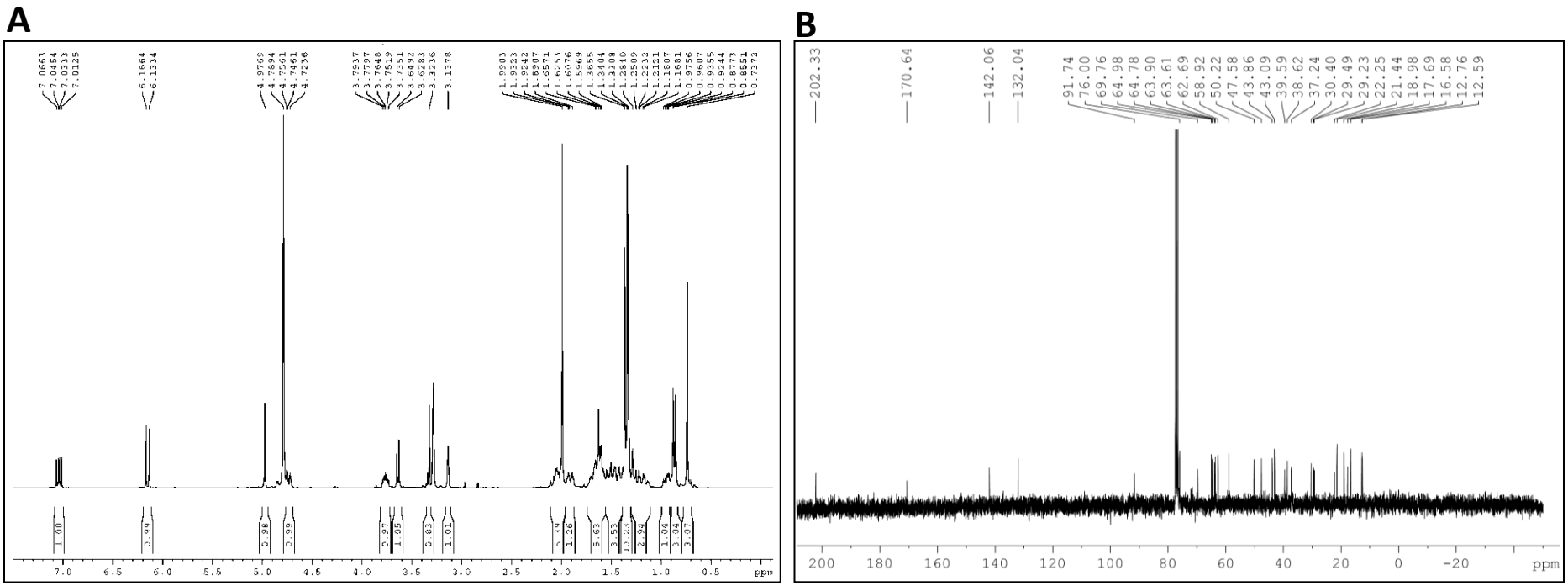
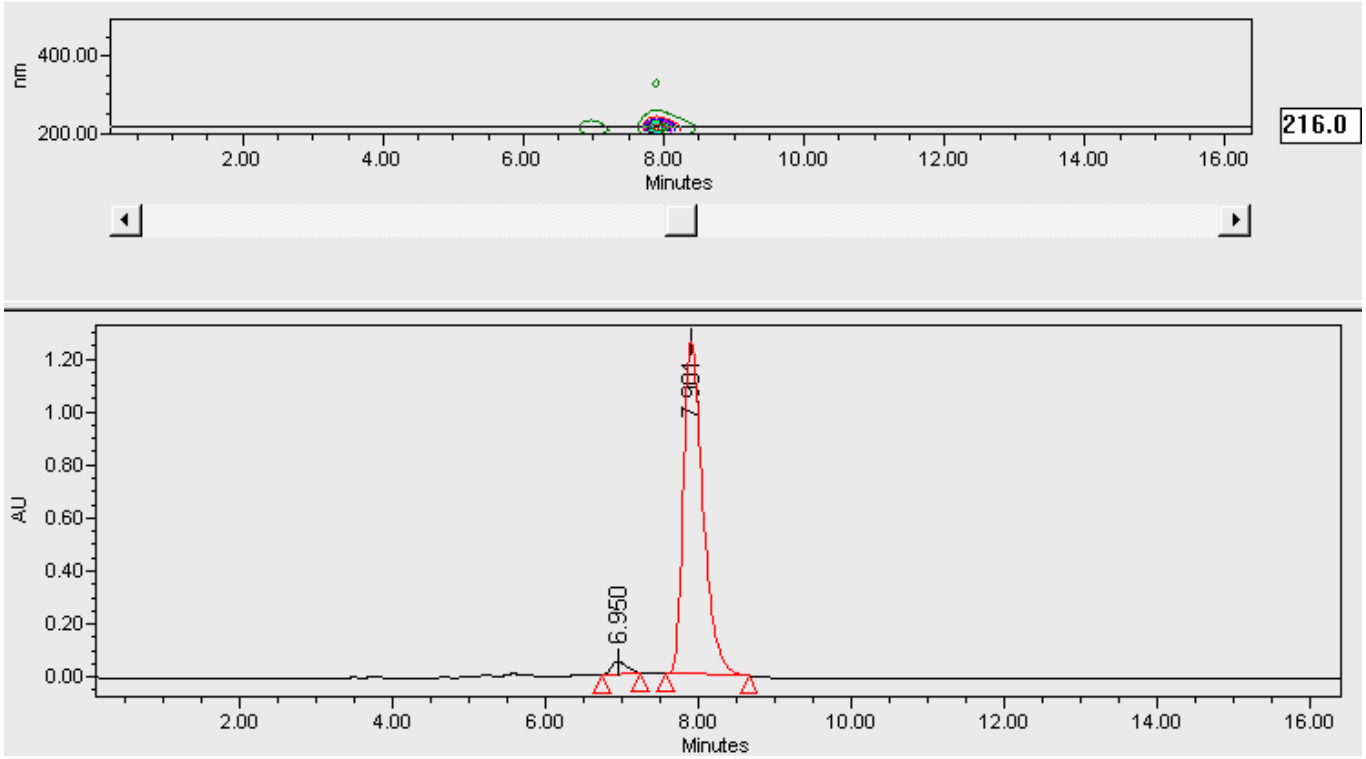
Chen et al. supplementary Fig. 1



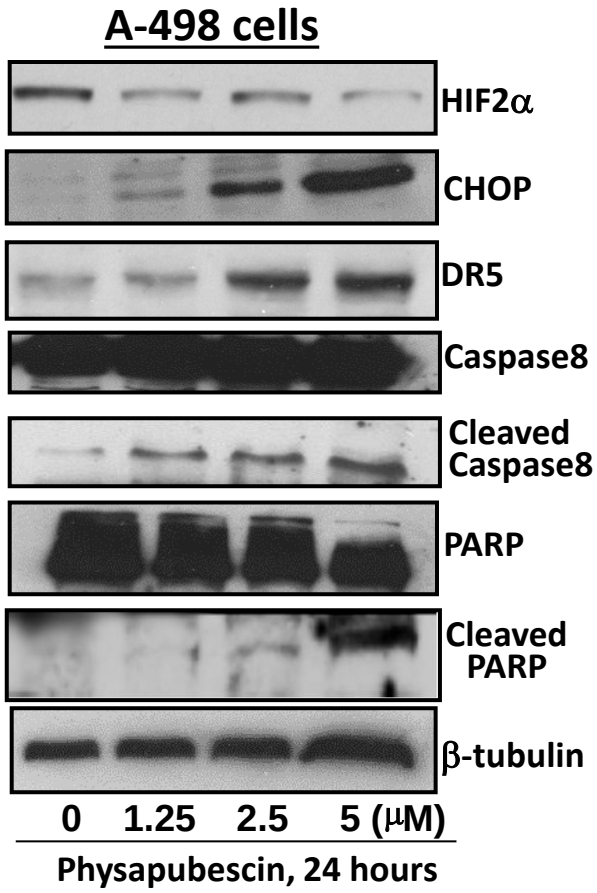
Supplementary Fig.1 Chemical characterization of physapubescin from *Physalis pubescens L.*. A, ^{1}H NMR spectrum (300 MHz, in CD_3OD) of physapubescin. **B,** ^{13}C NMR spectrum (75 MHz, in $CDCl_3$) of physapubescin.

Chen et al. supplementary Fig. 2



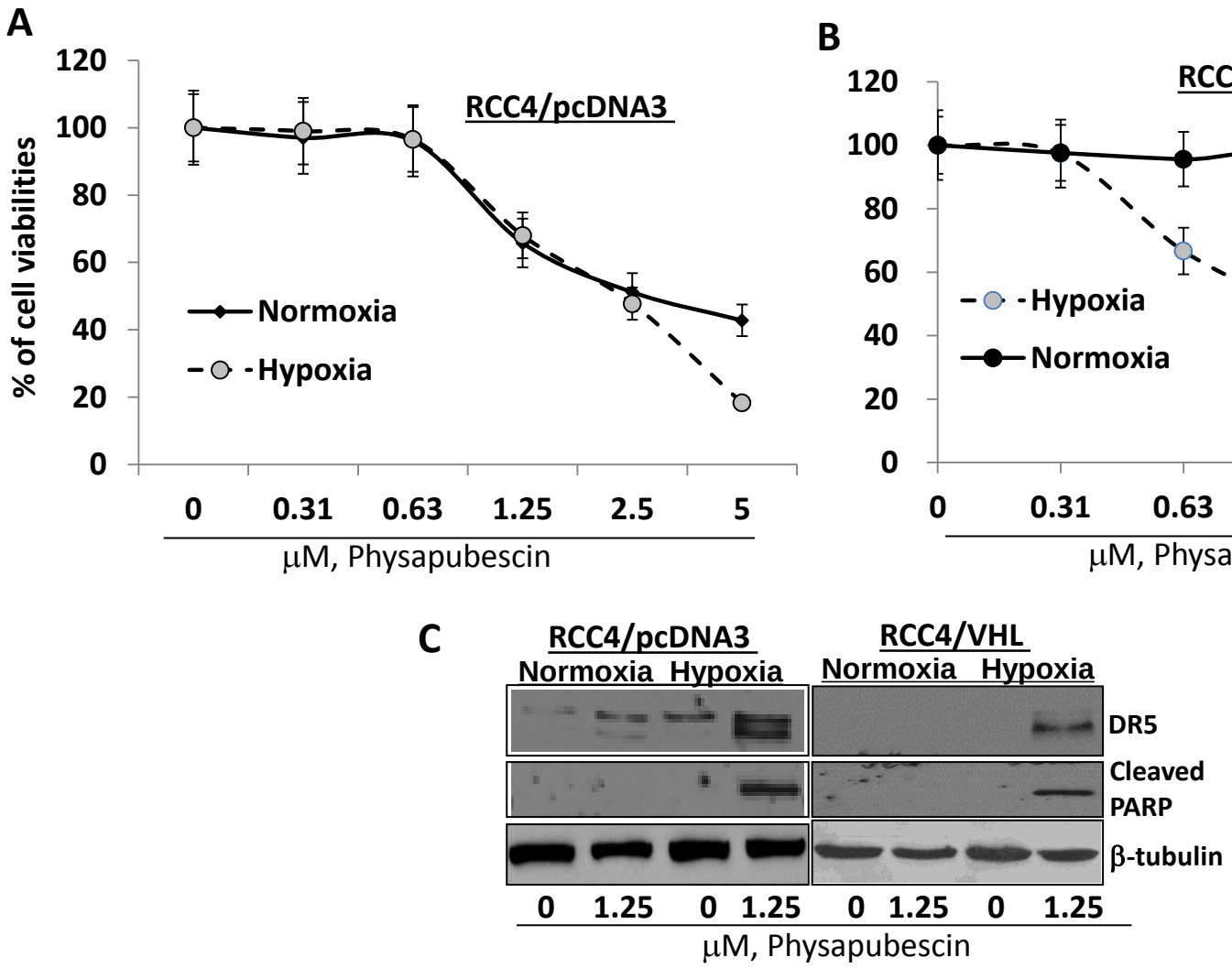
Supplementary Fig. 2 A representative chromatograph of physapubescin analyzed by HPLC [column: Aglient Zorbax SB-C18, 4.6 × 150 mm, 5 µm; solvent phase: methanol−H2O (60:40)].

Chen et al. supplementary Fig. 3



Supplementary Fig.3 Physapubescin decreases the expression of HIF-2α and increased the expression of CHOP and DR5 leading to activation of caspase cascade in VHL null A-498 cells. Protein expression of HIF-2α, CHOP, DR5, cleaved caspase-8 and PARP after indicated treatments hours was detected by Western blotting analysis. β-Tubulin was detected as a loading control. A representative blot was shown from three independent experiments.

Chen et al. supplementary Fig. 4



Supplementary Fig.4 The effects of physapubescin on reducing cell viabilities and modulating expression of related biomarkers are enhanced under hypoxia vs. normoxia conditions. RCC4/pcDNA3 cells were seeded at a density of $5x10^4$ cells/well in six well plates under normoxic (21% O2), hypoxic (1% O2) conditions. After 24 hours of seeding, (A) cells were treated with 0.05% DMSO or physapubescin at the indicated concentrations for 72 hours. Cell densities were measured by MTT assay. Each value represents mean $\pm$ SEM of three samples for each treatment; (B) the protein expression of DR5 and cleaved PARP at indicated treatments for 24 hours was analyzed by Western blotting. β-Tubulin was detected as a loading control. A representative blot was shown from three independent experiments.