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# *Supplementary Materials*

# Chrysosplenol D, a Flavonol from *Artemisia annua*, Induces ERK1/2-Mediated Apoptosis in Triple Negative Human Breast Cancer Cells

**Sophia J. Lang [1], Michael Schmiech [1], Susanne Hafner [1], Christian Paetz [2], Katharina Werner [1], Menna El Gaafary [1,3], Christoph Q. Schmidt [1], Tatiana Syrovets [1,*,‡], and Thomas Simmet [1,*,‡]**

[1] Institute of Pharmacology of Natural Products and Clinical Pharmacology, Ulm University, 89081 Ulm, Germany; sophia.lang@uni-ulm.de (S.J.L); michael.schmiech@uni-ulm.de (M.S.); susanne.hafner@uni-ulm.de (S.H.); katharina.werner@uni-ulm.de (K.W.); mennat_elgaafary@yahoo.com (M.E.G); christoph.schmidt@uni-ulm.de (C.Q.S.)

[2] Max Planck Institute for Chemical Ecology, 07745 Jena, Germany; cpaetz@ice.mpg.de[3] Department of Pharmacognosy, College of Pharmacy, Cairo University, Cairo 11562, Egypt

***** Correspondence: tatiana.syrovets@uni-ulm.de (T.S.); thomas.simmet@uni-ulm.de (Th.S.); Tel.: +49-731-500-65604 (T.S.); +49-731-500-65600 (Th.S.)

**‡** These authors contributed equally to this work

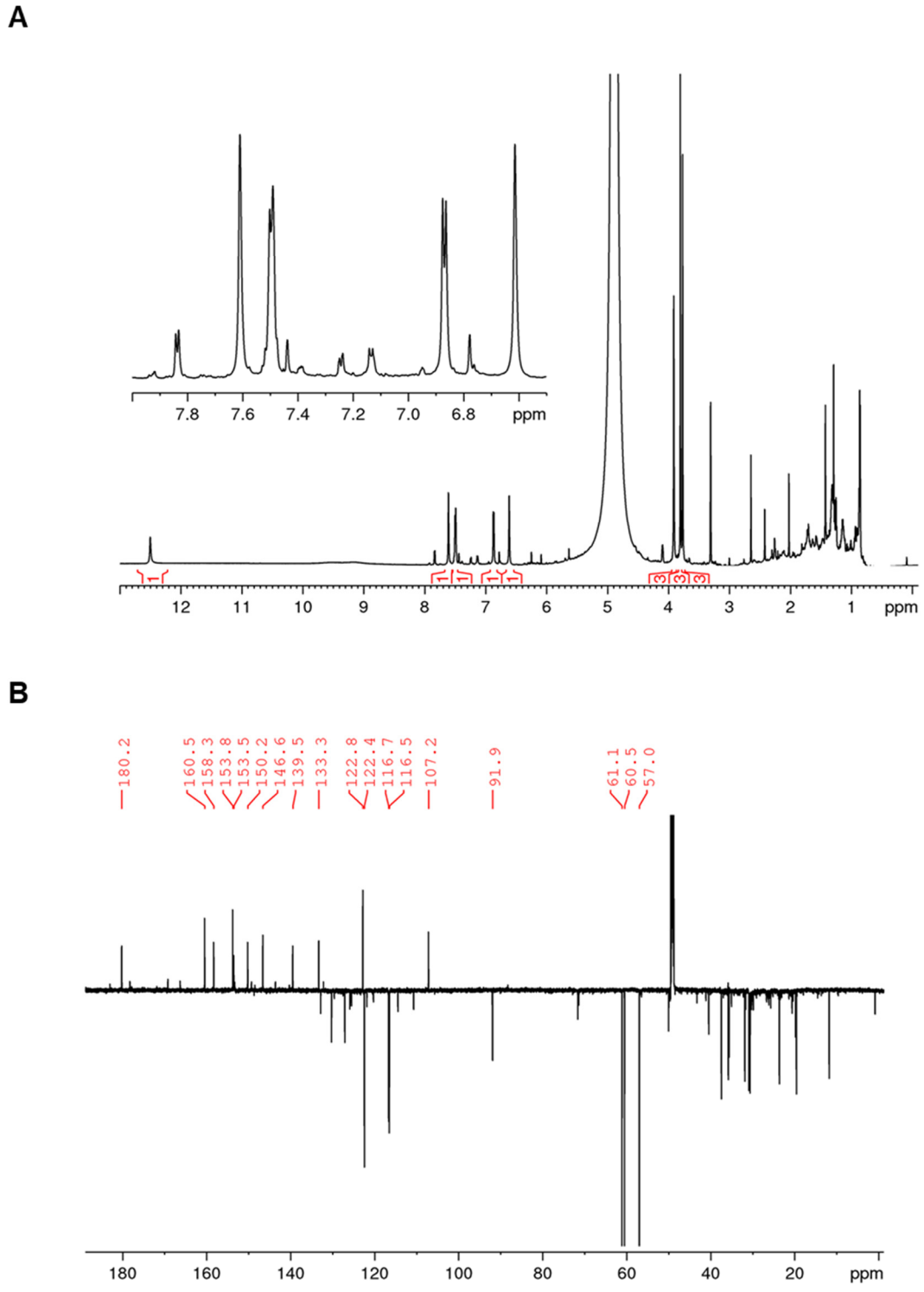


**Figure S1.** One dimensional $^{1}H$ and $^{13}C$ NMR spectra of chrysosplenol D. (**A**) $^{1}H$ NMR spectrum, 700 MHz, in MeOH-d3. (**B**) $^{13}C$ DEPTQ spectrum, 175 MHz, in MeOH-d3.

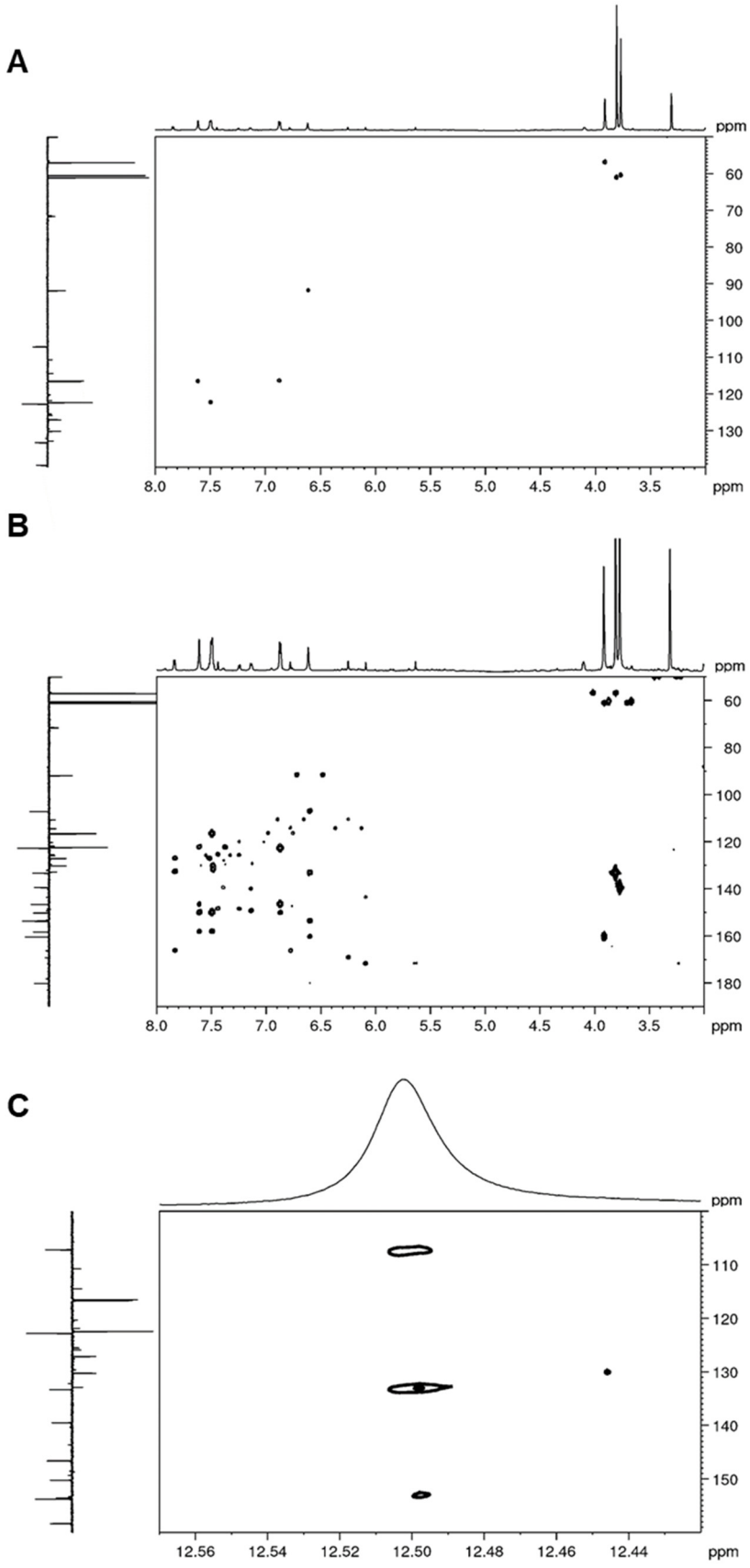


**Figure S2.** Two dimensional $^{1}H$ and $^{13}C$ NMR spectra of chrysosplenol D. (**A**) $^{1}H$-$^{13}C$ HSQC spectrum, 700/175 MHz, in MeOH-d3, level adjusted to main compound. (**B**) $^{1}H$-$^{13}C$. HMBC spectrum, 700/175 MHz, in MeOH-d3, level adjusted to main compound. (**C**) Detail of $^{1}H$-$^{13}C$ HMBC spectrum, 700/175 MHz, in MeOH-d3, long-range $^{1}H$-$^{13}C$ correlations of OH at C-5.

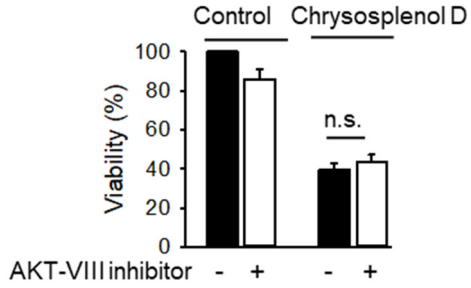


**Figure S3.** Akt activation is dispensable for chrysosplenol D-induced cytotoxicity. MDA-MB-231 cells were treated with an Akt activation inhibitor (AKT VIII inhibitor, Calbiochem, 0.2 μM) for 1 h and then with chrysosplenol D (10 μM) for 48 h. Cell viability was analyzed by using XTT. Data are mean ± SEM, n = 4, n.s. – non-significant.

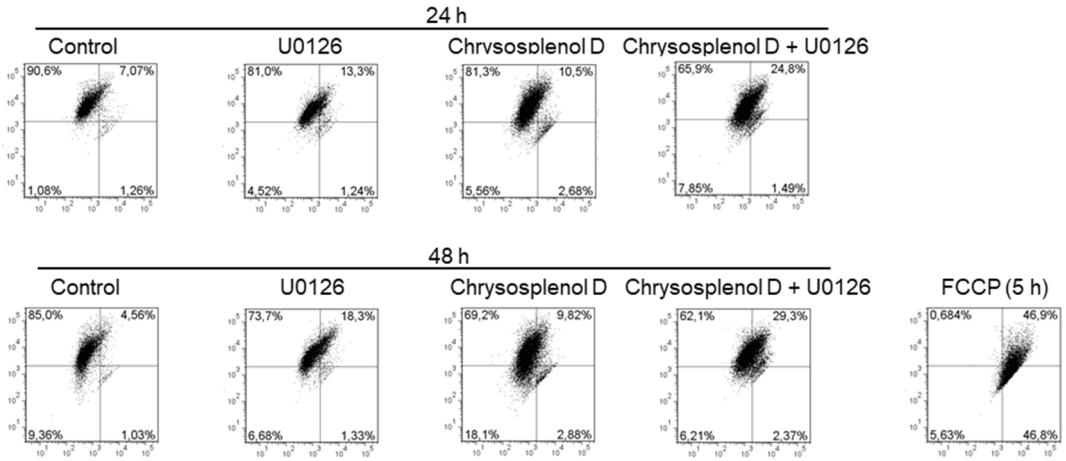


**Figure S4.** The MEK-inhibitor U0126 induces dissipation of the mitochondrial membrane potential ($\Delta\Psi_m$) in breast cancer cells and does not prevent $\Delta\Psi_m$ loss induced by chrysosplenol D. MDA-MB-231 cells were pretreated with the MEK-inhibitor U0126 (5 μM, 1 h) and treated with chrysosplenol D (10 μM) for 24 or 48 h. Loss of the mitochondrial membrane potential was analyzed by using flow cytometry after staining with JC-1. The uncoupling agent FCCP (carbonyl cyanide-4-(trifluoromethoxy)phenylhydrazone, 50 μM, 5 h) was used as positive control. Representative dot plots are shown.