

Rare phytocannabinoids exert anti-inflammatory effects on human keratinocytes via the endocannabinoid system and MAPK signalling pathway

Daniel Tortolani^{1,2#}, Camilla Di Meo^{3#}, Sara Standoli³, Francesca Ciaramellano¹, Salam Kadhim⁴, Eric Hsu⁴, Cinzia Rapino^{1*} and Mauro Maccarrone^{2,5*}

¹*Department of Veterinary Medicine, University of Teramo, Teramo, 64100, Italy*

²*European Center for Brain Research (CERC)/Santa Lucia Foundation IRCCS, Rome, 00143, Italy*

³*Department of Bioscience and Technology for Food Agriculture and Environment, University of Teramo, Teramo, 64100, Italy*

⁴*InMed Pharmaceuticals Inc., Vancouver BC, V6C 1B4, Canada*

⁵*Department of Biotechnological and Applied Clinical Sciences, University of L'Aquila, 67100 L'Aquila, Italy*

[#]*Equally first authors.*

^{*}*Correspondence: crapino@unite.it (C.R.); mauro.maccarrone@univaq.it (M.M.)*

Supplementary materials

Dose-response curves of tested interleukins and representative images of phospho-kinase arrays

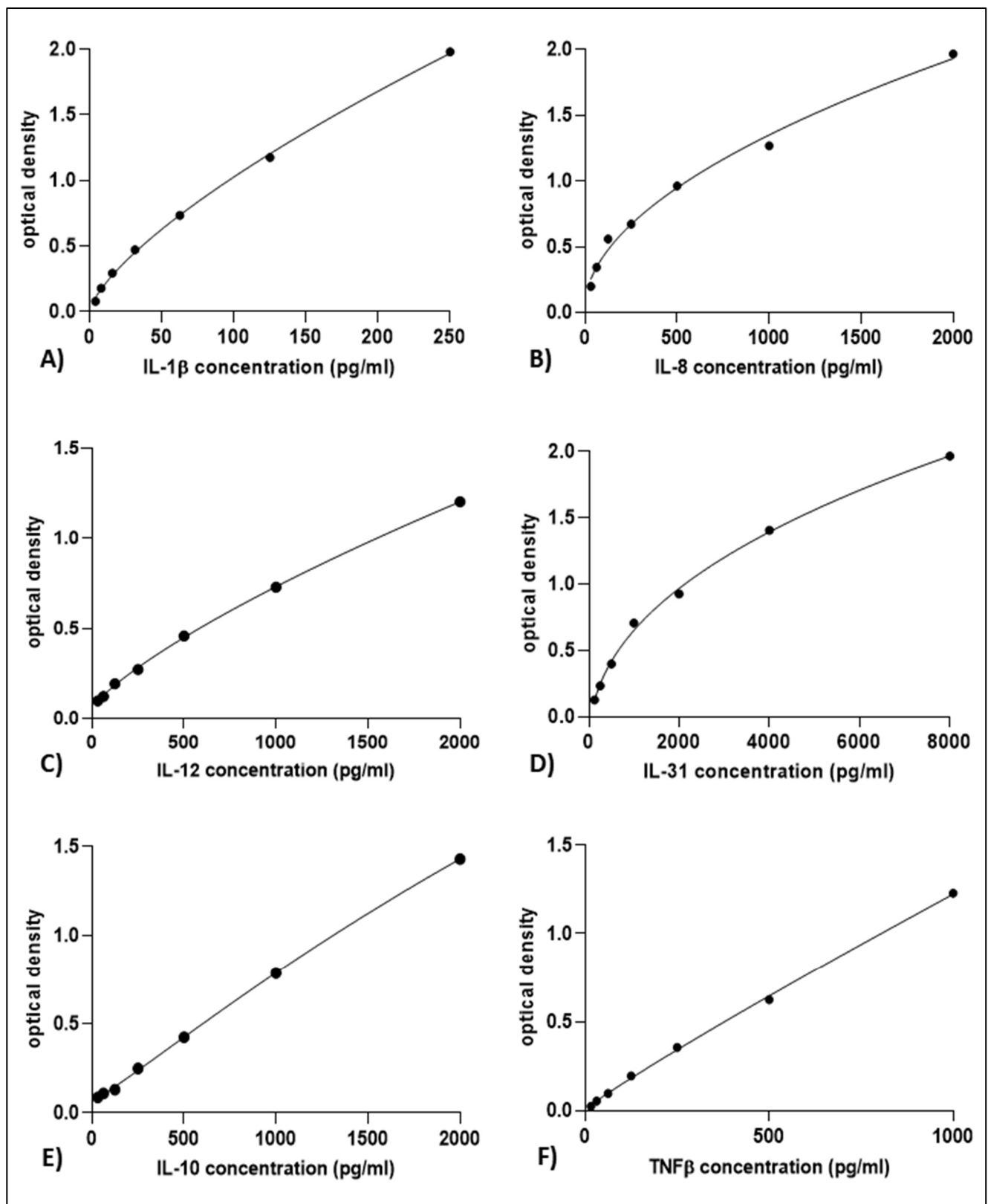


Figure S1. Dose-response curves of tested interleukins (pg/ml). A) IL-1 β ; B) IL-8; C) IL-12; D) IL-31; E) IL-10; and F) TNF β .

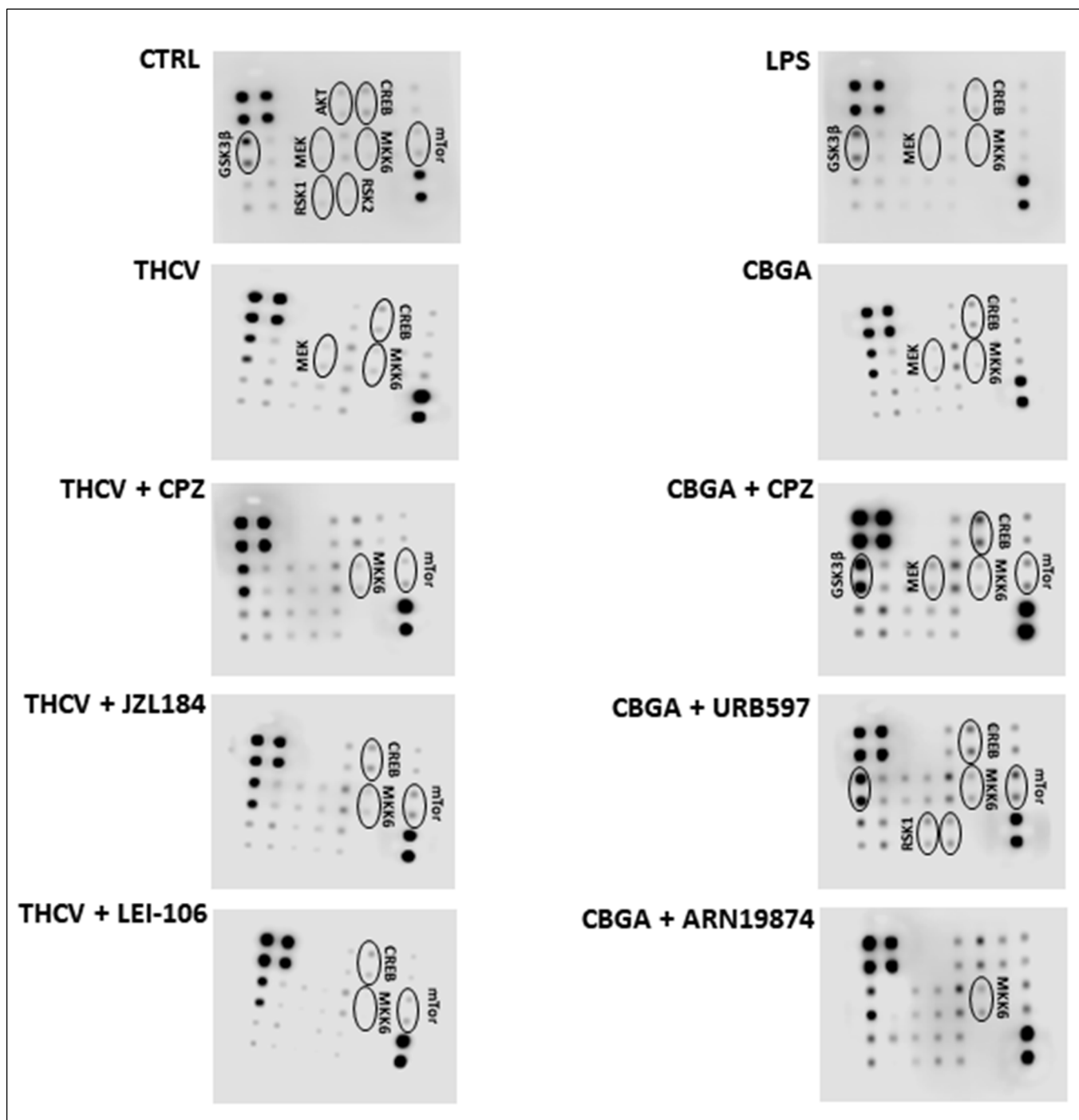


Figure S2. Representative images of phospho-kinase arrays for each treatment, captured by C-DiGit blot scanner. Each membrane detects the following 17 MAPKs: serine/threonine kinase 1 (AKT); cyclic adenosine monophosphate (cAMP) response element-binding protein (CREB); Glycogen synthase kinase 3 α (GSK3 α) and β (GSK3 β); c-Jun N-terminal kinase (JNK); extracellular signal-regulated kinase (ERK1); mitogen-activated protein kinase (MEK1); Mitogen-activated protein kinase kinase 3 (MKK3) and 6 (MKK6); mitogen- and stress-activated protein kinase 2 (MSK2); Heat shock protein 27 (HSP27); mammalian target of rapamycin (mTor); p38 mitogen-activated protein kinase (p38); tumour suppressor protein (p53); p70 ribosomal S6 kinase (P70S6k), Ribosomal S6 kinase 1 (RSK1) and 2 (RSK2) (each spotted in duplicate). The pairs of dots in the upper left corner and the two dots in the lower right corner are positive controls. Black circles denote the 8 proteins (AKT, CREB, GSK3 β , MEK, MKK6, mTor, RSK1 and RSK2) with significant variations under different experimental conditions.

Table S1. Representative membrane with distribution of antibodies used for MAPK array. POS = Positive Control Spot, used for normalization. NEG = Negative Control Spot, used to measure the baseline.

Each antibody is spotted in duplicate vertically		A	B	C	D	E	F	G	H
	1	POS	POS	NEG	NEG	Akt (P-S473)	CREB (P-S133)	ERK1 (P-T202/Y204) ERK2 (P-Y185/Y187)	GSK3a (P-S21)
	2								
	3	GSK3b (P-S9)	HSP27 (P-S82)	JNK (P-T183)	MEK (P-S217/221)	MKK3 (P-S189)	MKK6 (P-S207)	MSK2 (P-S360)	mTOR (P-S2448)
	4								
	5	p38 (P-T180/Y182)	P53 (P-S15)	P70S6K (P-T421/S424)	RSK1 (P-S380)	RSK2 (P-S386)	NEG	NEG	POS
	6								