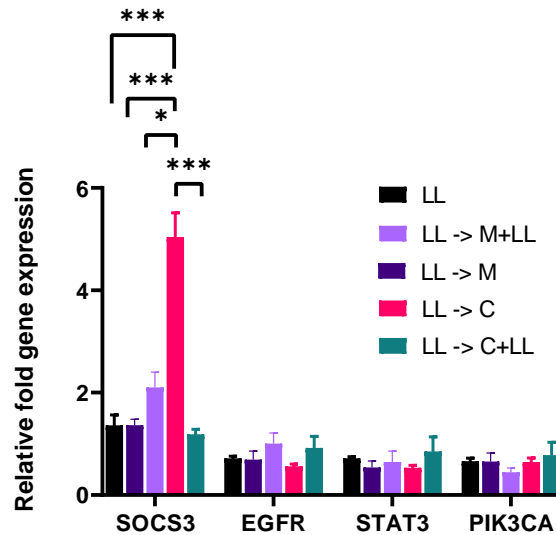
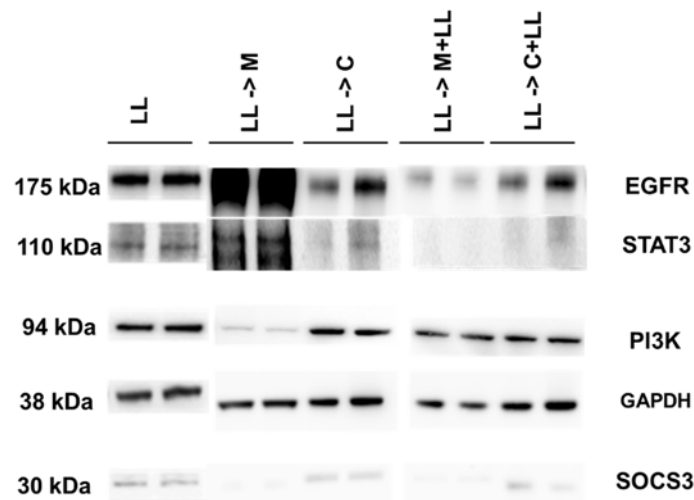


## ML218 HCl is more efficient than Capsaicin in Bacterial Antigen induced Cal 27 oral cancer cell proliferation



**Figure S1.** Expression of tumour suppressor and proliferation-related proteins by RT-qPCR analysis in Cal 27. All the gene expressions are relative to Cal 27 cells without bacterial antigen stimulation and ML218 HCl or capsaicin treatment. M: ML218 HCl, C: capsaicin, LL = LPS + LTA. Letter before the arrow represents pre-stimulation, e.g., LL-> M is LL pre-stimulation and then treatment with ML218 HCl. \*  $p \leq 0.05$ , \*\*\*  $p \leq 0.001$  is statistically significant.  $n = 9$  biological replicates. Error bars represent standard error of the mean.



**Figure S2.** Western blot image of different tumour suppressor and proliferation related proteins in Cal 27. Cal 27 cells were stimulated with LPS + LTA (LL) for 72 h, and then treated with ML218 HCl or capsaicin for 24 h in the absence (LL -> M / LL -> C) or presence (LL -> M+LL / LL -> C+LL) of oral bacterial antigens. M = ML218 HCl, C = capsaicin, LL = LPS + LTA. Letter before arrow represents pre-stimulation e.g., LL -> M is LL pre-stimulation and then treatment with ML218 HCl.

Table S1: Detail of materials and reagents used in the study.

Reagent/kit	Manufacturer	LOT	Catalogue No.	Description
<b>Growth medium and buffers for the cell culture</b>				
Keratinocyte-Serum Free Medium (K-SFM)	Life Technologies	-	1700504	Contains 2.5 µg human recombinant epithelial growth factor (EGF) (0.035 µg/µl), catalogue No. 10450-013, LOT 1969988 and 25 mg bovine pituitary extract (15.1 mg/ml), catalogue No. 13028-014, LOT 2014313
Foetal bovine serum (FBS)	Life Technologies	-	10099141	Endotoxin level: ≤ 5 EU/ml. Haemoglobin level: ≤ 30 mg/dL (levels routinely ≤ 25 mg/dL).
1X phosphate buffered saline (PBS)	Gibco	-	20012-027	1.5 mM KH <sub>2</sub> PO <sub>4</sub> ; 155.2 mM Cl; 2.7 mM Na <sub>2</sub> HPO <sub>4</sub> ·7H <sub>2</sub> O (pH 7.2)
Trypsin-EDTA	Sigma Life Sciences	T4049	SLBZ7364	0.25%, bioreagent 2.5 gm porcine trypsin and 0.2 gm EDTA; 4 Na per litre of Hank's balanced salt solution with phenol red
Dulbeccos Modified Eagles Medium (DMEM)	Gibco	-	11960-044	4,500 mg/L glucose, sodium pyruvate and sodium bicarbonate
Penicillin–streptomycin (P/S) solution	Life Technologies	2068825	15140-148	10,000 units/ml penicillin and 10,000 µg/ml streptomycin

Reagent/kit	Manufacturer	LOT	Catalogue No.	Description
Rosewell Park Memorial Institute (RPMI) 1640 medium	Gibco	1394048	21879-076	Without L-glutamine, and HEPES, with phenol red. Contains biotin, vitamin B12, PABA, vitamin inositol and choline. Used sodium bicarbonate buffer (2.0 g/L), and therefore required 5–10% CO <sub>2</sub> environment to maintain physiological pH
<b>Freezing of the cells</b>				
Tenak Slim tube cryogenic vials	Fisher Scientific	290992017	TE78315	RNAse–DNAse-free, 2-ml cryogenic vials
Dimethyl sulfoxide (DMSO)	Sigma Life Sciences	RNBH8443	D2650	Organic solvent, (CH <sub>3</sub> ) <sub>2</sub> SO
<b>Drugs and bacterial antigens</b>				
LPS 25 mg	Sigma-Aldrich	-	L2630	Lipopolysaccharides from <i>Escherichia coli</i> O111:B4, water soluble
PHA 20 mg	Sigma-Aldrich	37614100	11082132001	Phytohemagglutinin-M (PHA-M) from <i>Phaseolus vulgaris</i> , water soluble
LTA 5 mg	Sigma-Aldrich	049M4155V	L3140	Lipoteichoic acid from <i>Streptococcus pyogenes</i> , water soluble
Capsazepine 5 mg	Sigma-Aldrich	039K4615	C191	N-[2-(4-Chlorophenyl)ethyl]-1,3,4,5-tetrahydro-7,8-dihydroxy-2H-2-benzazepine-2-carbothioamide, polar compound soluble in organic solvent

Reagent/kit	Manufacturer	LOT	Catalogue No.	Description
ML218 HCl (hydrochloride) 10 mg	Tocris	1A/226248	RDS450710	3,5-Dichloro-N- [[[(1 $\alpha$ ,5 $\alpha$ ,6-exo,6 $\alpha$ )- 3-(3,3- dimethylbutyl)-3- azabicyclo [3.1.0] hex-6-yl] methyl]- benzamide hydrochloride, polar compound soluble in organic solvent
Capsaicin 250 mg	Sigma-Aldrich	MKBV4243V	360376	8-Methyl-N- vanillyl-trans-6- nonenamide, polar compound soluble in organic solvent
CID16020046 5 mg	Sigma-Aldrich	093M4730V	SML0805	4-[4,6-Dihydro-4-(3- hydroxyphenyl)-3- (4-methylphenyl)-6- oxopyrrolo[3,4-c] pyrazol-5(1H)-yl]- benzoic acid, polar compound soluble in organic solvent
Calcium chloride	Univar	D3247	127/500 g	CaCl <sub>2</sub> ·2H <sub>2</sub> O supplied as white powder. Water soluble; pH 5% solution at 25°C– 4.5–8.5
<b>Proliferation assay</b>				
RealTime-Glo MT Cell Viability Assay	Promega	0000383765	G9712	10 ×100 reactions
MT Cell Viability Substrate, 1000X	Promega	0000362418	G971A	10 $\mu$ l solution supplied in opaque tubes
NanoLuc Enzyme, 1000X	Promega	0000362418	E499A	0.4 mg/ml
Trypan blue stain 0.4%	Invitrogen	2117644	T10282	Trypan blue 0.4% to identify dead cells via colorimetric detection; supplied as 21 ml vials
Crystal violet acetate	Sigma Aldrich	69H3634	C5042	9(amino-5-imino- 5H-benzo[a] phenoxyazine acetate salt supplied as powder

Reagent/kit	Manufacturer	LOT	Catalogue No.	Description
Methanol	Chem-Supply	UN1230	MA004-2.5L-P	Analytical reagent
Cell Countess cell-counting chamber slides	Invitrogen	I31A9 Q422	C10283	For live cell and dead cell percentage via calorimetric detection
<b>Protein estimation</b>				
Pierce BCA Protein Assay kit	Thermo Fisher Scientific	UD2969	23225	Two-component, high-precision, detergent-compatible protein assay for determination of protein concentration
<b>Kinase profiling</b>				
Proteome Profiler Human Phospho-Kinase Array kit	R&D Systems	P236166	ARY003C	8 arrays (4 Part A, and 4 Part B), 1 transparency overlay, 1 8-well multi-dish and reagents
Human Phosphokinase array	R&D Systems	1569148	899190	8 nitrocellulose membranes (4 Part A, 4 Part B) each containing antibodies printed in duplicate
Array Buffer 1	R&D Systems	P173293	895477	21 ml of buffered protein base with preservatives
Array Buffer 2 5X Concentrate	R&D Systems	P200594	895478	21 ml of concentrated buffered protein base with preservatives
Array Buffer 3	R&D Systems	P184800	895008	21 ml of buffered protein base with preservatives
Wash Buffer Concentrate	R&D Systems	P230760	895003	2 vials (21 ml/vial) of a 25-fold concentrated solution of buffered surfactant with preservative
Detection Antibody Cocktail A	R&D Systems	1568310	899188	1 vial of biotinylated antibody cocktail; lyophilised

Reagent/kit	Manufacturer	LOT	Catalogue No.	Description
Detection Antibody Cocktail B	R&D Systems	1568313	899189	1 vial of biotinylated antibody cocktail; lyophilised
Streptavidin-HRP	R&D Systems	1549191	893019	200 µl of streptavidin conjugated to horseradish peroxidase (HRP)
Chemi Reagent 1	R&D Systems	P222081	894287	2.5 ml of stabilised hydrogen peroxide with preservative
Chemi Reagent 2	R&D Systems	P222083	894288	2.5 ml of stabilised luminol with preservative
8-well multi-dish	R&D Systems	-	607591	Clear 8-well rectangular multi-dish
Transparency overlay template	R&D Systems	-	608281	1 transparency overlay template for coordinate reference
<b>Determination of proliferation related gene expression</b>				
Pure Link RNA mini kit	Invitrogen	2137737	12183018A	Total RNA extraction
Trizol reagent	Invitrogen	260702	15596026	Total RNA extraction
Ethyl alcohol	Sigma Aldrich	SHBH7551	E7023-1L	CH <sub>3</sub> CH <sub>2</sub> OH; pure
SSIV VILO Master mix W/ EzDNASE	Invitrogen	00831746	11766050	DNA digestion and Reverse transcription PCR
POWRUP SYBR Master Mix, 5 ml	Invitrogen	00799448	A25742	Real-time PCR Master Mix
Chloroform	BDH AnalaR	19073	VWRC22711.260	CHCl <sub>3</sub> , contains 1% v/v of ethanol as preservative
MicroAmp Fast plate	Applied Biosciences, Life Biosystems	-	4346907	96-well reaction plate (0.1 ml)
Optical adhesive cover	Applied Biosystems, Life Technologies	-	4360954	qPCR-compatible optical adhesive covers
<b>Western blot</b>				

Reagent/kit	Manufacturer	LOT	Catalogue No.	Description
Human/Mouse SOCS-3 MAb (Clone 516919), 25 µg	R&D Systems	CCDL0219051	MAB5696	Purified mouse monoclonal IgG
Human/Mouse/Rat STAT3 MAb (Clone 232209), 25 µg	R&D Systems	JXW041912A	MAB1799	Purified mouse monoclonal IgG
Human PI 3-Kinase p110 beta MAb (Clone 269020), 25 µg	R&D Systems	VCK042001A	MAB2686	Purified mouse monoclonal IgG
Anti-G3PDH/GAPDH (T0893), 20 µL	R&D Systems	20481	2275-PC-020	Polyclonal rabbit antibody
Human EGF R/ErbB1 Polyclonal Ab, 25 µg	R&D Systems	AUC1118111	AF231	Affinity purified goat IgG
Novex Sharp pre-stained protein standard	Life Technologies	2115579	LC5800	consists of 12 pre-stained protein bands in molecular weight range 3.5–260 kDa
Clarity Western ECL Substrate	Bio-Rad	102031366 102031363	1705060	Supplied in two parts: peroxide solution and luminol/enhancer solution
Ponceau S solution	Sigma-Aldrich	SLCB3855	P7170-1L	Bioreagent 0.1% (w/v) supplied in 5% acetic acid: $C_{22}H_{12}N_4Na_4O_{13}S_4$
Anti-goat IgG HRP conjugate	R&D Systems	XGD10161011	HAF009	Secondary antibody specific to primary antibody source
Anti-rabbit IgG HRP conjugate	R&D Systems	FIN1819021	HAF008	Secondary antibody specific to primary antibody source
Anti-mouse IgG HRP conjugate	R&D Systems	WVA00919011	HAF018	Secondary antibody specific to primary antibody source

Reagent/kit	Manufacturer	LOT	Catalogue No.	Description
NuPAGE LDS sample buffer (4X)	Invitrogen	1981103	NP0007	Used to prepare protein samples for denaturing gel electrophoresis with Bis-Tris or Tris-Acetate gels. It contains lithium dodecyl sulfate, pH 8.4; contains Coomassie G250 and Phenol Red
NuPAGE 10% bis-tris gel	Invitrogen	19050170	NP0302BOX	1 mm × 12 well
NuPAGE 10% bis-tris gel	Invitrogen	19071070	NP0301BOX	1 mm × 10 well
20X NuPAGE MOPS SDS Running Buffer	Invitrogen	-	NP0001	500 ml contains 50 mM MOPS, 50 mM Tris Base, 0.1% SDS, 1 mM EDTA, pH 7.7
<b>Apoptotic assay</b>				
CellEvent Caspase-3/7 Green Detection Reagent 25 µl	Invitrogen	2119122	C10723	2.0 mM solution in DMSO
<b>TNF α enzyme-linked immunosorbent assay (ELISA)</b>				
Human TNF α ELISA kit	Invitrogen	225688-001	KHC3011	Supplied with all the reagents

Table S2: Primers used in RT-qPCR for proliferation factors.

Gene Name	Accession Number	Sequence (5'-3')	Position	Exon – Exon	PCR product length (bp)
<i>SOCS3</i>	NM_003955.4	GCGCGAAGGCTCCTTTGTG	191	330/331	135
		GGGGGGCTGGTCCCGAATC	336		
<i>STAT3</i>	NM_139276.2	GGACATCAGCGGTAAGACCC	2118	2128/2129	168
		CTCTGGCCGACAATACTTTC	2310		
<i>EGFR</i>	NM_005228.5	AGCTACGGGGTGACTGTTTG	2956	2962/2963	106
		GAACTTTGGGCGACTATCTG	3114		
<i>GAPDH</i>	NM_002046.7	GACAGTCAGCCGCATCTTCT	21	105/106	181
		ACCAAATCCGTTGACTCCGA	112		
<i>PI3KCA</i>	NM_006214.4	TGG GGATGATTTACGGCAAG	2732	2739/2740	129
		TCCACACAGTCACCGATTGA	2861		