Electronic Supplementary Material (ESI) for Molecular Omics.
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ITEM TO CHECK	IMPORTANCE	CHECKLIST
EXPERIMENTAL DESIGN		
Definition of experimental and control groups	E	**Experimental groups:** Activated Raji cells treated with maslinic acid (19 μM) and incubated over a time-point period (4h, 8h, 12h, 24h and 48h; h = hours) **Control groups:** Activated Raji cells treated with maslinic acid (19 μM) at 0 hour
Number within each group	E	n = 3
Assay carried out by core lab or investigator's lab?	D	
Acknowledgement of authors' contributions	D	
SAMPLE		
Description	E	Raji cells, derived from human lymphoblastoid cell line were obtained from RIKEN cell bank. Only cultures with more than 90% cell viability as determined by trypan blue exclusion method were used for the antitumour-promoting bioassay.
Volume/mass of sample processed	D	1 x 10^6 cells/mL of Raji cells
Microdissection or macrodissection	E	RPMI1640 media supplemented with EBV-infected serum. The cell cultures were prepared in triplicates and the cells were maintained at 37 ˚C in 5% CO 2 atmosphere.
Processing procedure	E	Upon time-point incubation completion, cells were collected via centrifugation and (nucleic acid) extracted for downstream processing
If frozen - how and how quickly?	E	
If fixed - with what, how quickly?	E	
Sample storage conditions and duration (especially for FFPE samples)	E	Samples prepared fresh prior to nucleic acid extraction
NUCLEIC ACID EXTRACTION		
Procedure and/or instrumentation	E	A total of 1 x 10 6 cells/ml of Raji cells were incubated in 2 ml of RPMI complete growth medium containing 3 mM of SnB, 0.05 μMPMA and 19 μM maslinic acid at 37 ˚C in 5 % CO 2 atmosphere for 48 hours. The cells were harvested periodically at 0, 4, 8, 12, 24 and 48 hours and the samples were processed immediately with Total RNA/Protein extraction kit (Macherey-Nagel, Germany). Eluted RNA and solubilised protein were stored in aliquots at -80 ºC until further use.
Name of kit and details of any modifications	E	Total RNA/Protein extraction kit (Macherey-Nagel, Germany)
Source of additional reagents used	D	
Details of DNase or RNAse treatment	E	RNaseZap® (Thermo Fisher Scientific, Cat.#AM9780)
Contamination assessment (DNA or RNA)	E	
Nucleic acid quantification	E	RNA concentration was determined by measuring the absorbance at 260 nm UV light
Instrument and method	E	Nanodrop® UV-Vis Spectrophotometer (Implen,Germany) and 1.2 % denaturing agarose gel.
Purity (A260/A280)	D	1.9-2.0
Yield	D	
RNA integrity method/instrument	E	RNA integrity as evaluated using 1.2 % denaturing agarose gel.
RIN/RQI or Cq of 3' and 5' transcripts	E	
Electrophoresis traces	D	
Inhibition testing (Cq dilutions, spike or other)	E	
REVERSE TRANSCRIPTION		
Complete reaction conditions	E	The Applause WT-Amp Plus ST System synthesizes microgram quantities of ST-cDNA starting with a total RNA input of at least 50 ng. In approximately seven hours, the system produces sufficient cDNA for labeling and subsequent hybridization to GeneChip Gene 1.0 ST and Exon 1.0 ST Arrays. The size of the majority of the cDNA products produced by the amplification process is between 0.1 and 2.0 kilobases.
Amount of RNA and reaction volume	E	20 ng/μL
Priming oligonucleotide (if using GSP) and concentration	E	RNA was used to generate biotin-labeled cDNA with the Applause WT-Amp ST System (NuGEN, CA) kit
Reverse transcriptase and concentration	E	As prepared by Applause WT-Amp ST System (NuGEN, CA) kit in the form of a mastermix
Temperature and time	E	Program 1 Primer Annealing 65°C – 5 min, hold at 4°C Program 2 First Strand Synthesis 4°C – 1 min, 25°C – 10 min, 42°C – 10 min, 70°C – 15 min, hold at 4°C SECOND STRAND cDNA SYNTHESIS Program 3 Second Strand Synthesis 4°C – 1 min, 25°C – 10 min, 50°C – 30 min, 70°C – 5 min, hold at 4°C POST-SECOND STRAND ENHANCEMENT Program 4 Post-Second Strand Enhancement 4°C – 1 min, 37°C – 15 min, 80°C – 20 min, hold at 4°C SPIA AMPLIFICATION Program 5 SPIA Amplification 4°C – 1 min, 47°C – 90 min, 95°C – 5 min, hold at 4°C POST-SPIA MODIFICATION Program 6 Post-SPIA Modification I 4°C – 1 min, 37°C – 15 min, 95°C – 5 min, hold at 4°C Program 7
Manufacturer of reagents and catalogue numbers	D	Applause WT-Amp ST System (NuGEN, CA) kit
Cqs with and without RT	D*	
Storage conditions of cDNA	D	
qPCR TARGET INFORMATION		
If multiplex, efficiency and LOD of each assay.	E	
Sequence accession number	E	Solaris pre-designed assay AX-004357-00-0200 Solaris Human qPCR Gene Expression Assay, Human XAF1 (54739) AX-007881-00-0100 Solaris Human qPCR Gene Expression Assay, Human CXCL9 (4283) AX-004599-00-0200 Solaris Human qPCR Gene Expression Assay, Human IFI44L (10964) AX-012511-00-0100 Solaris Human qPCR Gene Expression Assay, Human DDX58 (23586) AX009768-00-0200 Solaris Human qPCR Gene Expression Assay, Human OAS2 (4939) AX-004235-00-0100 Solaris Human qPCR Gene Expression Assay, Human ISG15 (9636) AX-004253-00-0100 Solaris Hman qPCR Gene Expression Assay, Human GAPDH (2597)
Location of amplicon	D	
Amplicon length	E	
In silico specificity screen (BLAST, etc)	E	BLAST analysis is a critical component of any comprehensive qPCR assay design protocol and BLAST analysis has been integrated into the Solaris design algorithm. The algorithm utilizes genomic transcript and pseudogene databases to identify and eliminate sequences that are more likely to lead to erroneous priming and detection (i.e. off-target effects).
Pseudogenes, retropseudogenes or other homologs?	D	
Sequence alignment	D	
Secondary structure analysis of amplicon	D	
Location of each primer by exon or intron (if applicable)	E	
What splice variants are targeted?	E	
qPCR OLIGONUCLEOTIDES		
Primer sequences	E	OAS2 GTGAACACCATCTGTGACG(F) TACCATCGGAGTTGCCTCTT (R) TGGACGGAAAACAGTCTTA (P) ISG15 ATGCTGGCGGGCAACGAAT(F) GTGATCTGCGCCTTCAG (R) TCCATGTCGGTGTCAGAGC (P) XAF1 GCAGCCTATGACATTCTGAG (F) TAACCACCGGCATTTCTCCTGA (R) TCCTAAATCAACATCAG (P) CXCL9 TTCTGATTGGAGTGCAAGGAAC (F) CAAGGATTGTAGGTGGATAGTC (R) TCAGCACCAACCAAGGG (P) IF144L TTTGGAACTGGACCCCATGA (F) GCTCTAACGCTCTCTCAA (R) TGAGGAAACTGGTGCAAT (P) DDX58 GCGAATCAGATCCCAGTGTATG (F) GCCTGTAACTCTATACCCATGT (R) AAAATACTTTGAAAGAC (P) GAPDH GCCTCAAGATCATCAGCAATG (F) CTTCCACGATACCAAAGTTGTC (R) GCCAAGGTCATCCATGA (P)
RTPrimerDB Identification Number	D	
Probe sequences	D**	
Location and identity of any modifications	E	Not applicable
Manufacturer of oligonucleotides	D	Thermo Scientific, USA
Purification method	D	
qPCR PROTOCOL		
Complete reaction conditions	E	Reaction contains 1μL of cDNA aliquot and 9 μL of Solaris qPCR mastermix. The cycling conditions comprise a 15 minutes enzyme activation step at 95°C, followed by 40 cycles of denaturation step at 95°C for 15 seconds and annealing/extension step at 60°C for 60 seconds. The expression of the selected genes were normalized to a housekeeping gene, GAPDH.
Reaction volume and amount of cDNA/DNA	E	10μL
Primer, (probe), Mg++ and dNTP concentrations	E	800 nM of forward and reverse primers each and 200 nM of gene specific probe
Polymerase identity and concentration	E	Solaris qPCR mastermix (Thermo Scientific, USA)
Buffer/kit identity and manufacturer	E	Solaris qPCR mastermix (Thermo Scientific, USA)
Exact chemical constitution of the buffer	D	
Additives (SYBR Green I, DMSO, etc.)	E	Solaris qPCR mastermix (Thermo Scientific, USA)
Manufacturer of plates/tubes and catalog number	D	
Complete thermocycling parameters	E	15 minutes enzyme activation step at 95°C, followed by 40 cycles of denaturation step at 95°C for 15 seconds and annealing/extension step at 60°C for 60 seconds.
Reaction setup (manual/robotic)	D	
Manufacturer of qPCR instrument	E	RotorGene thermal-cycler (QIAGEN, Germany).
qPCR VALIDATION		
Evidence of optimisation (from gradients)	D	Assay is pre-optimised as per suggested by manufacturer (Solaris pre-designed assays)
Specificity (gel, sequence, melt, or digest)	E	Evaluated from the melt curve of the assay, no template control was included in every run
For SYBR Green I, Cq of the NTC	E	
Standard curves with slope and y-intercept	E	
PCR efficiency calculated from slope	E	
Confidence interval for PCR efficiency or standard error	D	
r2 of standard curve	E	
Linear dynamic range	E	
Cq variation at lower limit	E	
Confidence intervals throughout range	D	
Evidence for limit of detection	E	
If multiplex, efficiency and LOD of each assay.	E	
DATA ANALYSIS		
qPCR analysis program (source, version)	E	Rotor-Gene® Q
Cq method determination	E	
Outlier identification and disposition	E	
Results of NTCs	E	
Justification of number and choice of reference genes	E	
Description of normalisation method	E	
Number and concordance of biological replicates	D	
Number and stage (RT or qPCR) of technical replicates	E	n = 3 technical replicates
Repeatability (intra-assay variation)	E	
Reproducibility (inter-assay variation, %CV)	D	
Power analysis	D	
Statistical methods for result significance	E	
Software (source, version)	E	
Cq or raw data submission using RDML	D	

Table 1. MIQE checklist for authors, reviewers and editors. All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if available. If using primers obtained from RTPrimerDB, information on qPCR target, oligonucleotides, protocols and validation is available from that source.

*: Assessing the absence of DNA using a no RT assay is essential when first extracting RNA. Once the sample has been validated as RDNA-free, inclusion of a no-RT control is desirable, but no longer essential.

**: Disclosure of the probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay vendors provide this information, it cannot be an essential requirement. Use of such assays is advised against.