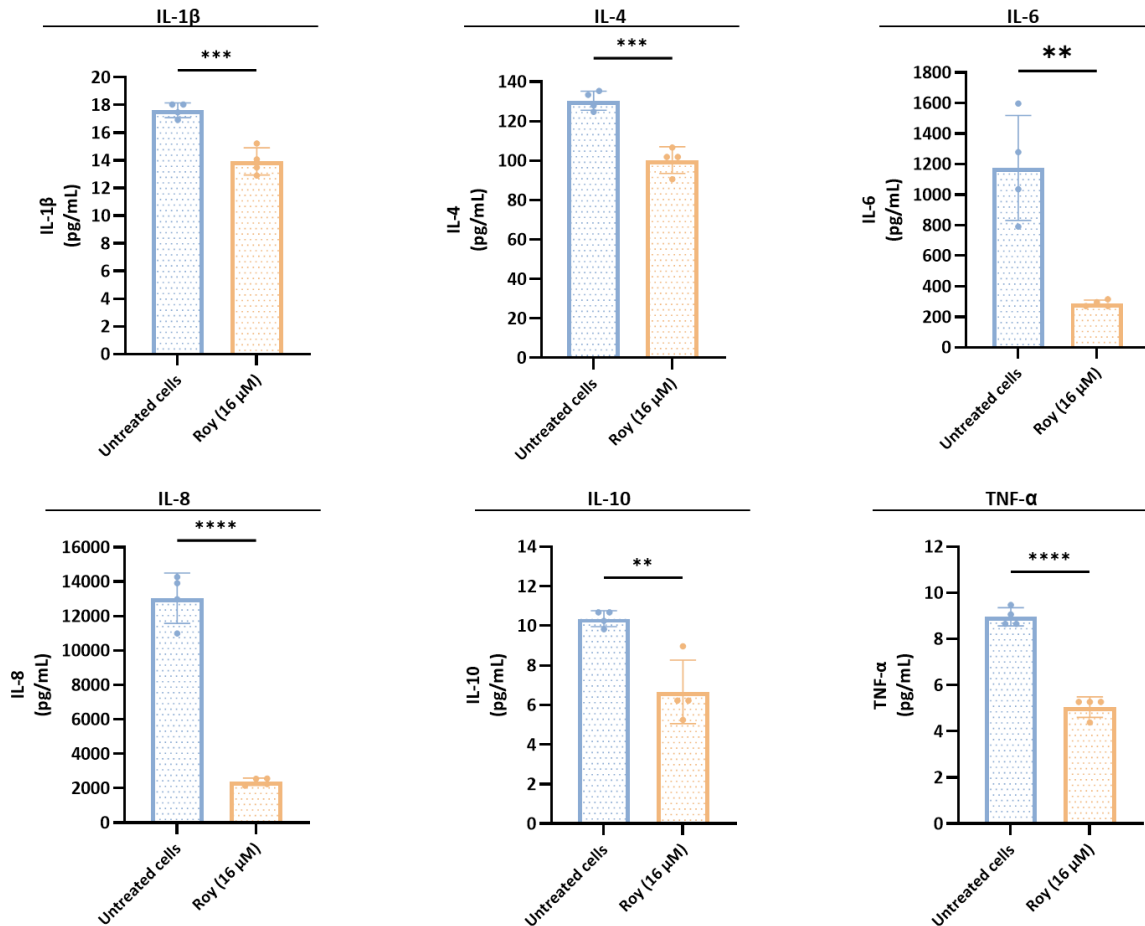


Supplementary Material

1 Supplementary Figures and Tables

1.1 Supplementary Figure 1



Supplementary Figure 1. Assessment of cytokines levels released by glioblastoma cells after treatment with Roy. U87 cells were treated with 16 μ M of Roy and incubated for 48 h. After incubation, the secretome of treated and untreated cells was collected and analyzed by multiplexed Luminex® immunoassay. The expression of IL-1 β , IL-4, IL-6, IL-8, IL-10, and TNF- α was estimated from the standard curve using a fifth-order polynomial equation and expressed as pg/mL. Asterisks (** p <0.01, *** p <0.001, and **** p <0.0001) represent the values that significantly differ from the control (untreated cells). Data are presented as mean \pm SD and it is representative of at least four independent experiments.

1.2 Supplementary Table 1

Supplementary Table 1. Sequence of the primers and respective amplicon size, for each optimized reaction, in quantitative real-time PCR (qRT-PCR). Design and specificity of primers were performed using the Primer Blast tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast/>).

Gene	Amplicon length (bp)	Forward (5'-3')	Reverse (5'-3')
<i>VEGFA</i>	81	TGCAGATTATGCGGATCAAACC	TGCATTACATTTGTTGTGCTGTAG
<i>STAT3</i>	176	ATCACGCCTTCTACAGACTGC	CATCCTGGAGATTCTCTACCACT
<i>STAT5A</i>	80	CGACGGGACCTTCTTGTTG	GTTCCGGGGAGTCAAACCTCC
<i>STAT5B</i>	130	GAACACCCGCAATGATTACAGT	ACGGTCTGACCTCTTAATTCGT
<i>JAK2</i>	130	TCTGGGGAGTATGTTGCAGAA	AGACATGGTTGGGTGGATACC
<i>IL6</i>	149	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTTCAGGTTG
<i>CDK4</i>	128	AGCCGAAACGATCAAGGAT	GCTTGACTGTTCCACCACTTG
<i>GAPDH</i>	131	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA

1.3 Supplementary Table 2

Supplementary Table 2. Optimized conditions for each qRT-PCR reaction. **NOTE:** Primer concentration optimization was performed using calibration curves generated from cell lines exhibiting upregulation of the target genes, based on data from the Human Protein Atlas (<https://www.proteinatlas.org/>). Reaction efficiency for each assay was determined from the standard curves calculated by the equipment.

Gene	Primer concentration (nM)	Melting Temperature (°C)	Efficiency (%)	Cell line	Enzyme / qPCR equipment
<i>VEGFA</i>	200	60	84.11	U87	Xpert Fast SYBR MDM2 Green Mastermix 2X with ROX / QuantStudio® 3 RealTime PCR Systems
<i>STAT3</i>	300	60	85.00	HEL	
<i>STAT5A</i>	200	60	94.98	K562	
<i>STAT5B</i>	200	60	85.77	MOLT-4	
<i>JAK2</i>	200	60	102.88	HEL	
<i>IL6</i>	200	60	99.88	U87	
<i>CDK4</i>	100	60	108.00	THP1	
<i>GAPDH</i>	150	60	92.15	NB4	