

## Supplementary material

# Discovering the Protective Effects of Quercetin on Aflatoxin B1-Induced Toxicity in Bovine Foetal Hepatocyte-Derived Cells (BFH12)

**Marianna Pauletto <sup>1†</sup>, Mery Giantin <sup>1†</sup>, Roberta Tolosi <sup>1</sup>, Irene Bassan <sup>1‡</sup>, Anisa Bardhi <sup>2</sup>, Andrea Barbarossa <sup>2</sup>, Ludovica Montanucci <sup>3</sup>, Anna Zaghini <sup>2</sup> and Mauro Dacasto <sup>1</sup>**

<sup>1</sup> Department of Comparative Biomedicine and Food Science, University of Padua, Viale dell'Università 16, I-35020 Legnaro, Italy; mery.giantin@unipd.it (M.G.); roberta.tolosi@unipd.it (R.T.); irene.bassan2@gmail.com (I.B.); mauro.dacasto@unipd.it (M.D.)

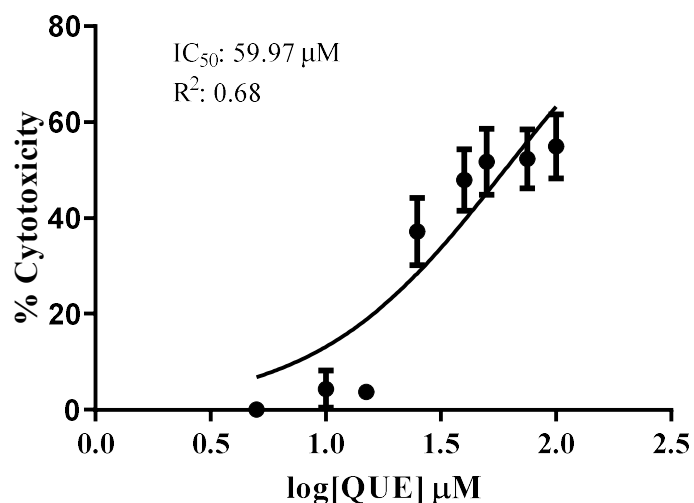
<sup>2</sup> Department of Veterinary Medical Sciences, Alma Mater Studiorum—University of Bologna, Via Tolara di Sopra 50, Ozzano dell'Emilia, I-40064 Bologna, Italy; anisa.bardhi@unibo.it (A.B.); andrea.barbarossa@unibo.it (A.B.); anna.zaghini@unibo.it (A.Z.)

<sup>3</sup> Genomic Medicine Institute, Lerner Research Institute, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, USA; montanl@ccf.org

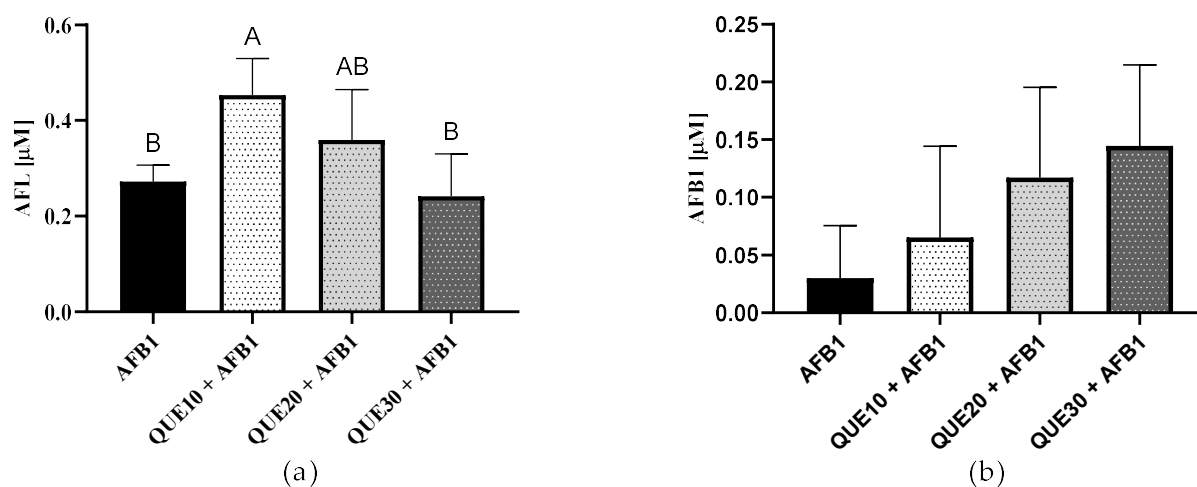
\* Correspondence: marianna.pauletto@unipd.it; Tel.: +39-049-827-2935

† These authors contributed equally to this work.

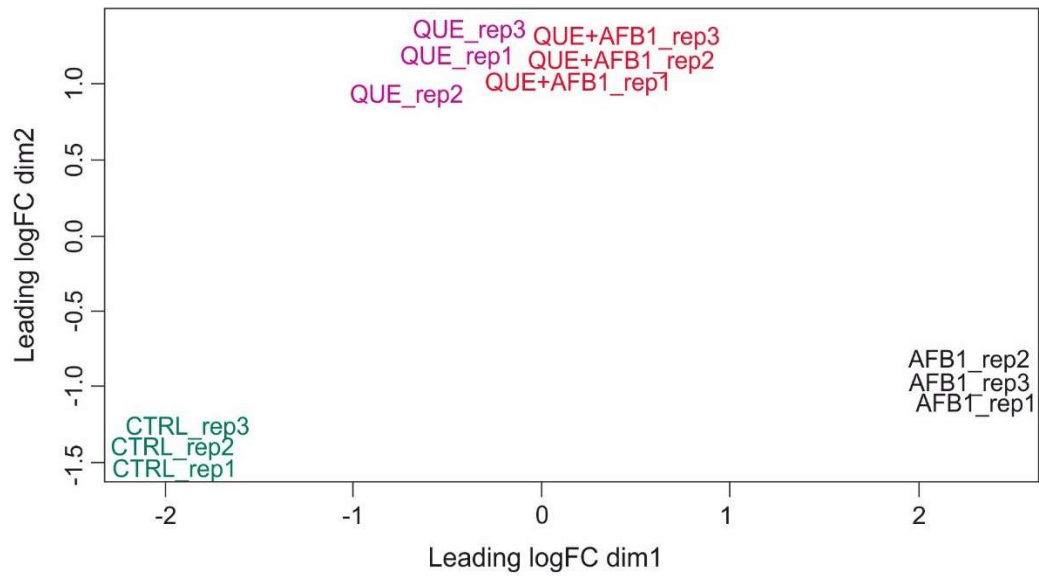
‡ Current address: Chelab srl—Mérieux NutriSciences Italia, Via Fratta 25, I-31023 Resana, Italy.



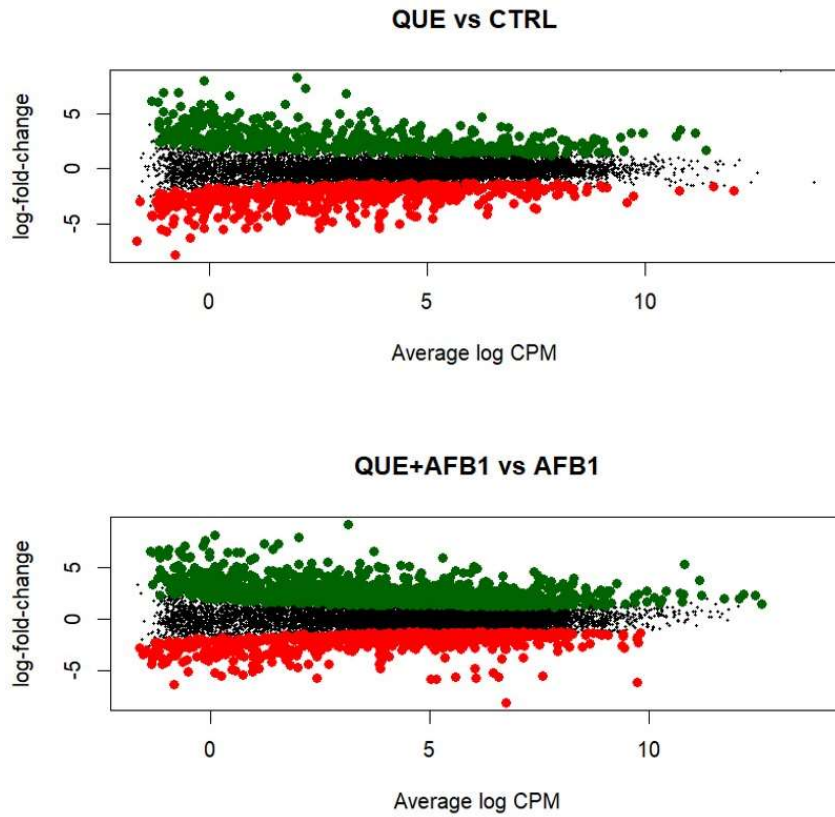
**Figure S1. Cytotoxicity.** Quercetin dose–response curve in BFH12 cells (64 h), based on three independent cell culture experiments, each one run in sextuplicate. Data are expressed in mean cytotoxicity rate  $\pm$  standard error of the mean (SEM).  $IC_{50}$  and  $R^2$  are also reported. QUE=quercetin



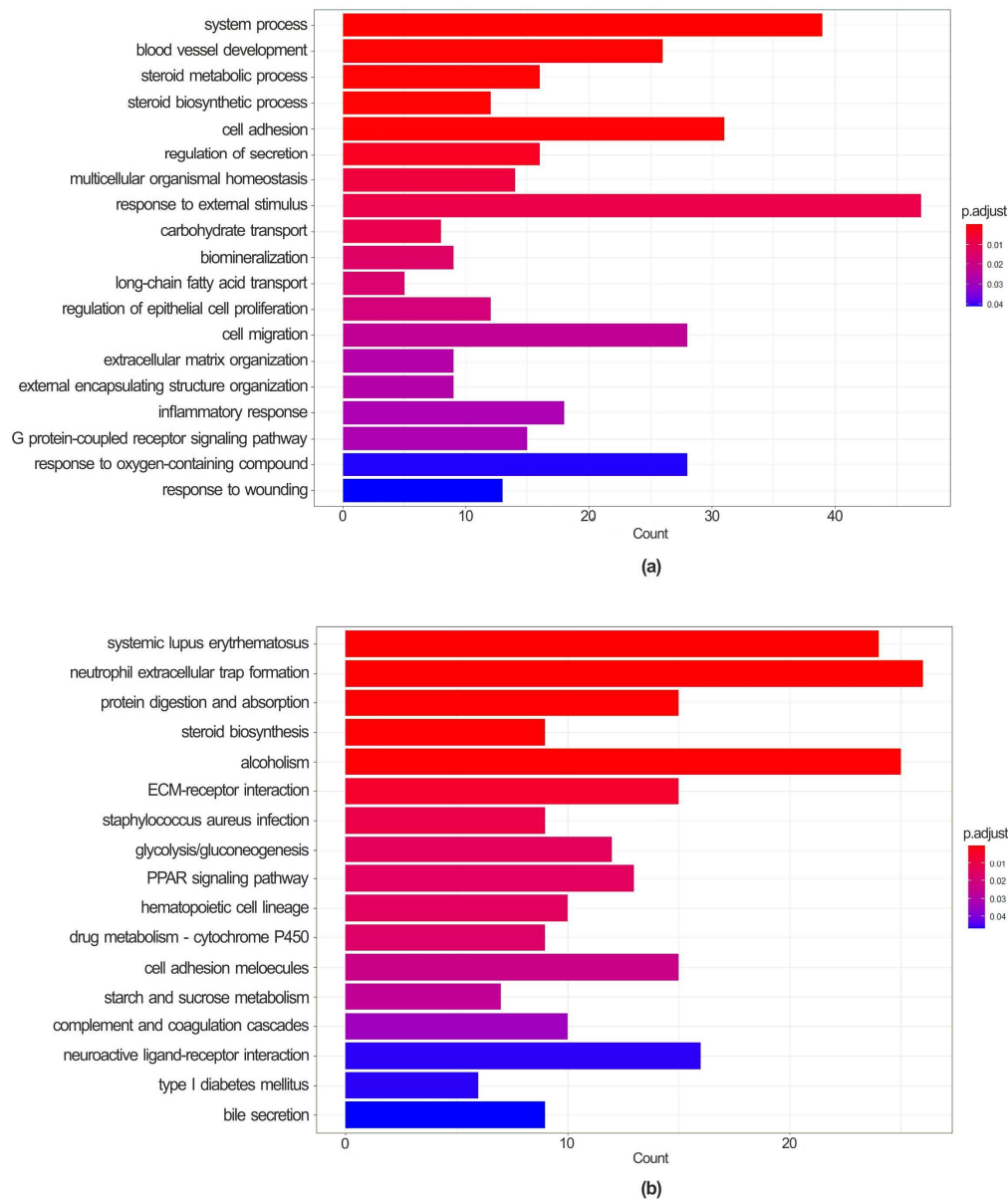
**Figure S2. Effects of QUE in AFB1 biotransformation.** Bars represent the amount ( $\mu M$ ) of either AFL detected in the cellular medium (a) or AFB1 detected in the cellular pellet (b) after 48 h of exposure to 3.6  $\mu M$  AFB1 alone or in combination with QUE increasing concentrations (10, 20, and 30  $\mu M$ ). Data are expressed as mean concentrations  $\pm$  standard deviations of four independent cell culture experiments. Different letters above error bars indicate significant differences ( $p \leq 0.05$ ) among groups (Tukey's post-hoc test). Graphs were obtained by means of GraphPad prism software. AFB1=aflatoxin B1; QUE=quercetin.



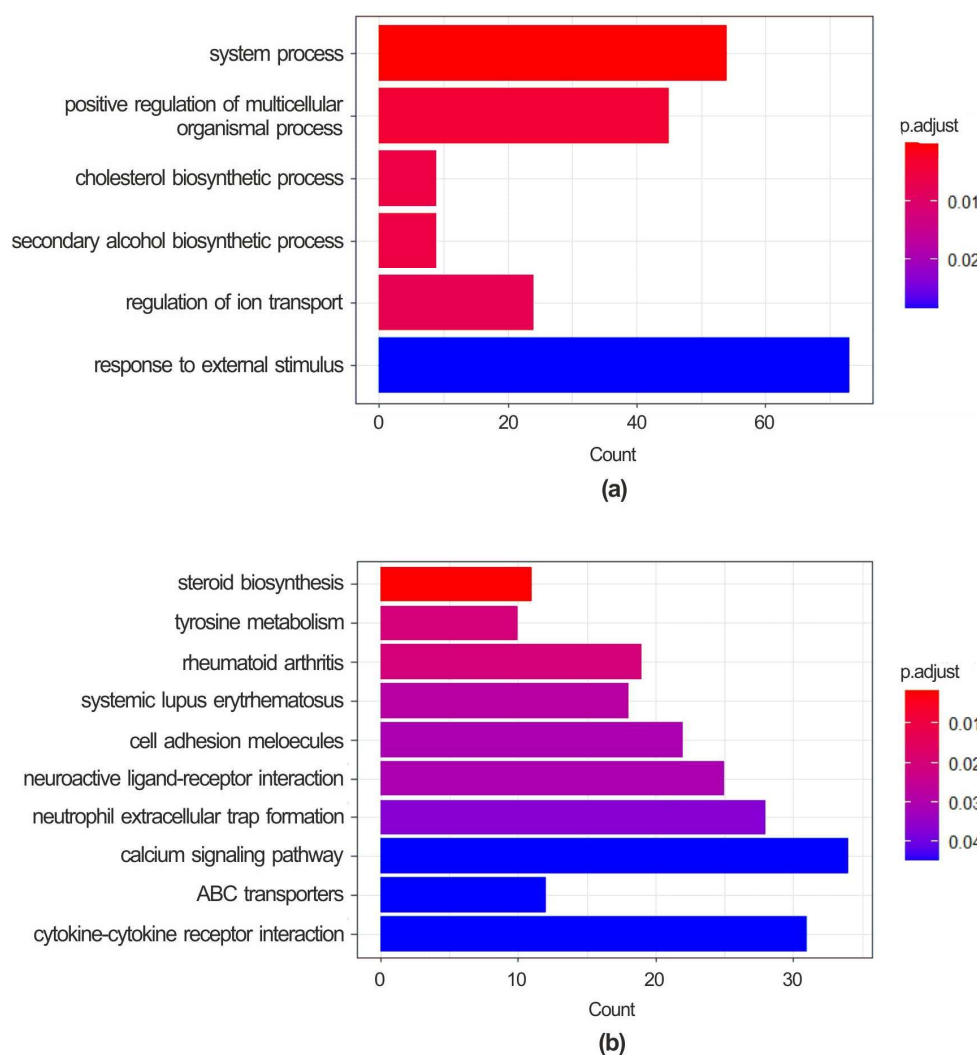
**Figure S3. MDS plot.** The plot shows distances between expression profiles of the twelve RNA-seq libraries evaluated in this study. Biological replicates are represented by rep1, rep2, and rep3 suffix. AFB1=aflatoxin B1; QUE=quercetin.



**Figure S4. MD plots.** Plots show the log-fold change and average abundance of each gene in the two comparisons ("QUE vs CTRL" and "QUE+AFB1 vs CTRL"). Genes with fold-changes significantly greater than 2 are highlighted in green (if up-regulated) or red (if down-regulated). CPM = Counts Per Million. AFB1=aflatoxin B1; QUE=quercetin.



**Figure S5. Over-representation analysis: QUE vs CTRL.** Bar plots report the over-represented BP terms (a) and KEGG pathways (b) in the list of DEGs resulted from the pair-wise comparison “QUE vs CTRL”. Count = number of DEGs in each enriched term/pathway. The color gradient reflects the significance level of each term. P-values were adjusted using the Benjamini-Hochberg method.



**Figure S6. Over-representation analysis: QUE+AFB1 vs AFB1.** Bar plots report the over-represented BP terms (a) and KEGG pathways (b) in the list of DEGs resulted from the pair-wise comparison “QUE+AFB1 vs AFB1”. Count = number of DEGs in each enriched term/pathway. The color gradient reflects the significance level of each term. P-values were adjusted using the Benjamini-Hochberg method.

Gene	QUE + AFB1 vs AFB1
CYP1A1	↑*
CYP1B1	↑
CYP3A28	↓
GSTA1	ns
AHR	ns
AHRR	↑ / nd
ARNT	ns
NRF	ns
KEAP1	ns
CAT	ns
GPX1	ns
SOD1	↑ / ns
SOD2	↓
NQO1	↑

**Figure S7. Concordance between qPCR and RNA-seq results.** The figure reports the observed gene expression differences in the comparison QUE + AFB1 vs AFB1. Green and red colours highlight when the two methods agreed or disagreed, respectively. ns: not significant; ne: not detected; ↑: upregulated; ↓: downregulated; \*by means of RNA-seq, this gene was induced with only marginal significance (i.e. FDR = 0.08).

**Table S1. Sequencing and mapping results.** The table reports the RNA-seq libraries sequenced, including for each of them: i) SRA accessions; ii) the number of raw reads obtained; iii) the number of reads after trimming and rRNAs removal; iv) the number of mapped reads (and the percentage of mapped reads).

SRA accession	Sample ID	Raw reads	Reads after trimming and rRNAs removal	Reads mapping (%)
SRR11586297	PCB126_B	22,928,709	22,823,199	22,628,132 (99.15)
SRR11586296	PCB126_C	25,807,296	25,653,873	25,436,649 (99.15)
SRR11586295	PCB126_D	43,005,307	42,793,203	42,432,959 (99.16)
SRR11586294	AFB1_B	20,399,647	20,286,542	20,106,412 (99.11)
SRR11586293	AFB1_C	23,877,815	23,632,276	23,428,800 (99.14)
SRR11586292	AFB1_D	10,774,628	10,714,111	10,614,084 (99.07)
SRR24958754	QUE_B	25,116,232	24,972,157	24,723,237 (99.00)
SRR24958753	QUE_C	22,334,139	22,173,375	21,991,767 (99.18)
SRR24958758	QUE_D	24,530,040	24,401,397	24,193,093 (99.15)
SRR24958757	QUE+AFB1_B	16,266,419	16,154,781	15,981,264 (98.193)
SRR24958756	QUE+AFB1_C	40,451,368	40,169,816	39,850,034 (99.20)
SRR24958755	QUE+AFB1_D	21,003,875	20,887,979	20,701,925 (99.11)

AFB1: aflatoxin B1; ID: identity; PCB: polychlorinated biphenyls; QUE: quercetin