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**Protective role of fermented whey and pumpkin extract against AFB₁ and
OTA toxicity in Jurkat T-cells**

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Abstract

The beneficial effect of fermented whey and pumpkin extract rich in carotenoids was evaluated in Jurkat cells against aflatoxin B₁ (AFB₁) and ochratoxin A (OTA) cytotoxicity through a proteomic approach. The functional ingredients were added into mycotoxin contaminated bread formulation, which digested *in vitro* in order to simulate human intestinal absorption. Cell cultures were exposed during 7 days to these mycotoxins dissolved in: a) 0.1% organic solvent (DMSO), b) in intestinal digest of bread with pumpkin individually (PID) and c) in intestinal digest of bread with pumpkin mixed with whey fermented (PID+WF). Extracted proteins were subjected to reduction and alkylation and subsequently a tryptic digestion in order to be analyzed by liquid chromatography coupled with quadrupole time of flight (Q-TOF). A total of 496 unique proteins at 1% false discovery rate was detected for DMSO conditions, 352 for PID and 316 for PID+WF using Spectrum Mill software (Agilent). Later, differentially expressed proteins were statistically evaluated through Mass Professional Profiler software (Agilent). Results obtained show the identification of proteins involved in several metabolic pathways, mainly in gluconeogenesis, antioxidant activity and nucleosome assembly. Furthermore, histones' expression implicated in the metabolic pathway of nucleosome assembly (H2A, H2B, H2C, H3 and H4) was increased when exposing cells to functional ingredients, as well as repression of cyclin A2, a profile associated to limiting the growth of carcinogenic cells.

Keywords: aflatoxin B₁, ochratoxin A, proteomics, Jurkat cells, LC/Q-TOF

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3 **1. Introduction**
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6 Aflatoxin B₁ (AFB₁) is the most potent natural carcinogenic toxin which contaminate crops and
7 a wide variety of food materials, such as cereals, nuts, milk, spices, vegetables, fruits, oils and
8 meat (Kowalska *et al.*, 2017). They are polyketide compounds synthesized by secondary
9 metabolic pathway in *Aspergillus* and they have been demonstrated to be carcinogenic,
10 teratogenic and mutagenic in nature (Smith, 2020). AFB₁ is absorbed in the small intestine and
11 metabolized firstly in the liver to release reactive intermediate metabolites but its
12 carcinogenicity has been associated also to remote organs such as kidney, pancreas, bladder,
13 bone, viscera and central nervous system (Benkerroum, 2020). Another widespread mycotoxin,
14 ochratoxin A (OTA), contaminates a great variety of foodstuffs, such as grapes, coffee, cocoa,
15 and infant food. It is a pentaketide derived from dihydrocoumarins family produced by fungi
16 of *Penicillium* and *Aspergillus* genera and has been shown to be nephrotoxic, hepatotoxic,
17 teratogenic and immunotoxic to humans and animals (Tao *et al.*, 2018). As regard its
18 mechanism of action, it is rapidly absorbed in the proximal tubule of the kidney but slowly
19 eliminated and excreted leading to potential accumulation in the body (Zhu *et al.*, 2017) Due
20 to their high toxicity, the Panel on Contaminants in the Food Chain has reported a scientific
21 opinion on the human health risks related to the presence of AFs and OTA in food and estimated
22 its dietary exposure of the European Union population (EFSA, 2020).
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26 Cereal and cereal-based products are classified as one of the leading sources of energy and
27 minerals as well as vitamins for human and animal (Khanegan *et al.*, 2019). However, these
28 products can be contaminated by several mycotoxin species in the field after harvest and
29 storage, or as a consequence of grain infection by fungi. Food processing and innovative
30 technologies can prevent mycotoxins formation and promote its degradation but nonetheless,
31 they cannot eliminate the risk (Gavahian *et al.*, 2020). In order to reduce mycotoxin's
32 absorption and toxicity in humans and animals, the use of functional ingredients has been
33 studied. For instance, the protective effect of dietary antioxidant with high content of
34 carotenoids against mycotoxins induced oxidative stress has been observed at mitochondrial
35 transcriptional level after AFs and OTA exposure in ECV304 cells (Alonso-Garrido *et al.*,
36 2021; Alonso-Garrido *et al.*, 2020). Moreover, it has been shown that they can prevent the onset
37 of cardiovascular diseases by blocking the formation of low-density lipoproteins, improve the
38 communication of GAP-type intercellular junctions, protect cells from mycotoxin-induced
39 toxicity (Juan-Garcia *et al.*, 2019) and limit abnormal cell growth, reducing the risk of certain
40 types of cancers.
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44 Several bioactive compounds, such as probiotic bacteria, are involved in different functions
45 contributing to human health by acting as antioxidant, anti-inflammatory, and immune
46 modulating mediators (Chugh & Kamal-Eldin, 2020). Among them, milk fermented whey
47 (WF) is a functional food by-product formed in milk coagulation step of cheese manufacturing
48 process, which is able to inhibit mycotoxigenic strains growth in food products (Izzo *et al.*,
49 2020a, Izzo *et al.*, 2020b, Dopazo *et al.*, 2021). Recently, Escrivá *et al.*, (2021) evaluated the
50 bioaccessibility and bioavailability of bioactive compounds from milk whey and yellow
51 mustard flour- both fermented with *Lactobacillus plantarum* and non-fermented and evidenced
52 these ingredients may have promising applications in food industry as natural preservatives.
53 Moreover, it has been observed that the addition of milk whey ingredient in contaminated bread
54 formulation, increases its shelf life and antioxidant activity (Luz *et al.*, 2021).
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For this purpose, the aim of the present work was to evaluate the *in vitro* effect of gastrointestinal digest of bread prepared with fermented whey and pumpkin extract on AFB₁- and OTA- induced cytotoxicity in Jurkat cells through a proteomic approach.

2. Material and methods

2.1 Reagents

The standards of Standard solution stock (purity: 99%) of AFB₁ and OTA and phosphate buffer saline (PBS) were obtained from Sigma–Aldrich (St. Louis, MO, USA). All the stock solutions (1000 mg/l) were prepared by dissolving 1 mg of mycotoxin in 1 ml of pure methanol. These stock solutions were diluted with methanol in order to obtain the appropriate multi-compounds working standard solutions. All the standards were kept at –20 °C.

DL-Dithiothreitol (DTT), ≥ 99.0%, Trizma® hydrochloride (Tris-HCl) for proteins extraction, ≥99.0% and Trypsin for digestion, were purchased from Sigma Aldrich (St. Louis, MO, USA). Iodoacetamide (IAA), 98% was acquired from ACROS Organics™, Thermo Fisher Scientific (New Jersey, USA). Thiourea (TU), 99% for lysis buffer preparation was obtained from Thermo Fisher Scientific (Kandel, Germany) and Urea (U), 99% was acquired from F.E.R.O.S.A (Barcelona, Spain). Deionized water (resistivity < 18 MV cm) was obtained using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2 Cell culture and treatments exposure

Jurkat T cells were grown in a humidified incubator with 5% CO₂ and 95% air atmosphere at constant humidity (37°C), using RPMI-glutamax medium supplemented with 100 U/mL penicillin, 100 mg/mL streptomycin and 10% inactivated FBS. Cells were seeded at a density of 5x10⁵ cells/mL in 6-well plates and subsequently exposed to the different treatments. Firstly, cells were treated with AFB₁ and OTA 100 nM in 0.1% DMSO and the solvent concentration as control during 7 days. Secondly, cells were exposed to intestinal digests of two types of bread prepared with lyophilized pumpkin and milk whey fermented (1:10), single or in combination with mycotoxins during 7 days. Carotenoid's content and profile of intestinal digests are reported in Table 1. All the experimental conditions performed were: a) AFB₁ and OTA, individually or in combination, at 100 nM in 0.1% DMSO for 7 days; b) intestinal digest of pumpkin bread (PID), AFB₁ and OTA, individually or in combination; c) PID mixed with fermented whey (WF), AFB₁ and OTA, individually or in combination (Table 2).

Table 1. Concentration (µg/mL) and profile of carotenoids identified from gastrointestinal digests of pumpkin (PID) and PID with fermented whey (WF).

Bread	Carotenoids concentration (µg/mL)					
	β-Carotene	Lutein	Anteraxanthin	Violaxanthin	Zeaxanthin	β-Cryptoxanthin
PID	0.7364	0.0181	n.d.	1.3309	0.0264	1.0241
PID+AFB₁	0.7091	0.0050	0.0001	0.9779	0.0067	0.6988
PID+OTA	0.5394	0.0036	n.d.	0.4978	0.0053	0.3627
PID+AFB₁+OTA	0.2742	0.0036	0.0002	0.6654	0.0019	0.5470
PID+WF	1.8636	0.0180	n.d.	3.3676	n.d.	2.9880
PID+WF+AFB₁	2.5152	0.0122	0.0054	3.0368	0.0054	2.3976
PID+WF+OTA	4.000	0.0120	n.d.	3.3971	n.d.	2.3614

PID+WF+AFB₁+OTA	2.2879	0.0086	0.0580	2.7426	0.0580	1.8554
n.d (not detected)						

Table 2. AFB₁ and OTA Concentrations of each condition employed: A) mycotoxin standard in organic solvent (DMSO), B) intestinal digest of bread with pumpkin (PID), C) intestinal digest of bread with pumpkin and whey fermented (PID+WF)

Treatment		Mycotoxin concentration [nM]	
		AFB ₁	OTA
A	DMSO 0.1 %	-	-
	DMSO 0.1 %+AFB ₁	-	100
	DMSO 0.1 %+OTA	-	-
	DMSO 0.1 %+OTA+AFB ₁	100	100
<i>Pumpkin bread intestinal digest (PID)</i>			
B	PID	-	-
	PID +AFB ₁	137	-
	PID+OTA	-	777
	PID+AFB ₁ +OTA	191	704
<i>Fermented whey-Pumpkin bread intestinal digest (PID)</i>			
C	PID+WF	-	-
	PID+WF+AFB ₁	-	1968
	PID+WF+OTA	-	1037
	PID+WF+AFB ₁ +OTA	187	837

2.3 Protein extraction

Cell’s pellet was washed by deionized water to neutral pH, then dried and resolved in lysis buffer (8 M U/2 M TU/50 mM Tris-HCl). Afterwards, was homogenized by vortexing and sonified 3 times using a methanol: H₂O mixture until protein solubilization (USC 1200D ultrasonicator, VWR, International bvba, Leuven, Belgium). Samples were centrifuged for 30 min at 4 °C and 13000 g and the supernatant was collected in order to quantify protein concentration using a NeoDot UV/Vis Nano Spectrophotometer (Quimigen, Madrid, Spain).

2.4 Protein denaturation, alkylation, enrichment and digestion

Firstly, samples were standardized to 1 mg/mL and subsequently reduced with DTT (200mM, pH 7.8) for 1 hour at 60 °C. Therefore, IAA 200 mM (pH 7.8) was added for alkylation of cysteine residues and incubated for 30 minutes at 37 °C. Protein digestion was initiated with addition of trypsin (1:40) and incubated once again for 16 hours. Digestion was stopped with addition of 5% acetic acid down to pH 5. Samples were dried for 2 hours in a vacuum concentrator (lyophilizer Freezone 2.5 freeze dryer Benchtop, Labconco) at a -40 °C temperature and vacuum pressure of 0.080 mBar. After the lyophilization, peptides were eluted in 0.1 % acetic acid: acetonitrile (98:2 v/v) to a final concentration of 100 µg/µL.

2.5 Q-TOF mass spectrometry and data analysis

Samples were analyzed using a LC system coupled with quadrupole time of flight (Q-TOF) in duplicate per biological sample. A C18 bioZen™ 2.6 µm, 120 Å, 50 x 2.1 mm (Phenomenex, California, USA) was used to achieve chromatographic separate peptides separation. The injection volume was 10 µL and the column temperature was maintained at 45°C. Total run time was 40 min, at a flow rate of 0.4 mL/min. The eluent consisted of two different phases: A (H₂O containing 0.1% formic acid) and B (ACN containing 0.1% formic acid). The gradient elution was applied with an initial 3% of phase B for 1 min, which went up to 20% in 21 min. Afterwards, the gradient increased to 95% over 3 min and subsequently decreased at 3% in the last 10 min.

As regard Q-TOF-MS conditions, drying gas flow (N₂) was 13.0 L/min; nebulizer pressure, 35 psi; gas drying temperature 325 °C; capillary voltage 4000 V; nozzle voltage 500 V; fragmentor voltage, 175 V; skimmer voltage 65 V and octopole RF peak, 750 V. Positive ionization mode was employed by using Dual AJS ESI interface and positive ions were acquired in the range of 100–3000 m/z for MS scans, and 50–3000 m/z for auto MS/MS scans, at a scan rate of 8 scans/s for MS and 3 scans/s for MS/MS, respectively. MS/MS automatic acquisition mode was carried out using a ramped collision energy with charge state preference of 2, 3, >3 and slope 3.1, 3.6 and 3.6, respectively. Two reference masses of 121.0509 and 922.0098 m/z were used as internal mass correction. Agilent MassHunter Workstation software B.08.00 (Agilent Technologies) was used as instrument controller and data acquisition.

2.6 Bioinformatics and statistical analysis

Spectra were processed using the Spectrum Mill MS Proteomics Workbench Package Rev B.06.00.201 software (Agilent Technologies), which is capable of analyzing data from high quality spectra reducing false positive number and using the Uniprot catalog to identify matched proteins and peptides. MS/MS parameters of spectra were searched and validated according to Cimbalò *et al.*, (2021). Identified proteins were exported to the Mass Profiler Professional (MPP) 15.0 version software (Agilent Technologies) to perform statistical data analysis. This analysis was carried out based on the total spectra intensity of the proteins which were considered as entities in MPP. The baseline of the spectra was adjusted to the mean across all samples. The entities were then filtered based on their frequency of occurrence across at least all replicates of one group. Contrasts for each condition between the experimental mycotoxin dose front the control were analyzed using unpaired t-test with Benjamin-Hochberg adjustment. Results were considered significant with a fold change ≥ 0.7 and p-values cut-offs < 0.05 . Finally, biological processes, molecular functions and metabolic pathways related to these proteins were identified in a meaningful way by using a database for integrated protein annotation, visualization and discovery (DAVID) (Huang *et al.*, 2009a; Huang *et al.*, 2009b).

3. Results

3.1 Identification and quantification of proteins

The analysis of Jurkat cells exposed to different conditions of AFB₁ and OTA, individually and mixed, and their controls (DMSO 0.1%, PID, PID+WF) was performed by gel-free shotgun proteomic approach. A total of 496 unique proteins at 1% false discovery rate was detected for 0.1% DMSO treatment, 352 for the PID treatment and 316 for PID+WF. Subsequently, differentially expressed proteins were identified by a comparison of any treatment and the

control groups (fold change ≥ 0.7 and a p-value ≤ 0.05), as determined by a t-test (See supplementary material for detailed results). From 154 of differentially expressed proteins in DMSO treatment, 74 have shown higher and 80 lower expression levels when compared to the control (Table S1). After PID treatment, 64 were found upregulated and 18 downregulated compared to the control (Table S2). Finally, from 85 proteins identified after PID + WF treatment, 58 displayed higher and 27 lower expressions with respect to the control (Table S3).

3.2 Differentially expressed proteins in Jurkat cells after AFB₁ exposure

After 0.1% DMSO + AFB₁ (100 nM) exposure, gluconeogenesis was as the biological process and metabolic pathway showing more changes in protein expression, identifying glycerol-3-phosphate dehydrogenase (GPDH), glucose-6-phosphate isomerase (GPI), phosphoglycerate kinase (PGK) and phosphoglycerate mutase (PGM) as major proteins, in all cases with lower expression levels compared to the control. Moreover, polyadenylated RNA binding resulted the main molecular function, in which 17 proteins were altered. Among them, ATP-dependent RNA helicase and histone H4 were slightly upregulated (1.17 - 4 LogFC) whereas RNA polymerase II, peroxiredoxin significantly repressed (LogFC= -4) (Table S4).

When cells were exposed to PID + AFB₁, among the overexpressed proteins, there were 4 proteins involved in nucleosome assembly, 2 of mucosal innate immune response and 2 of natural killer (NK) cell-mediated cytotoxicity. The most significant protein involved in these processes is the antioxidant protein peroxiredoxin 1 (PRDX1). Furthermore, analysing the molecular functions, 7 proteins involved in heterodimerization and protein kinase binding were altered, particularly ATP-dependent RNA helicase, PRDX1, histone H2A, H2B, H4 were significantly overexpressed. As regard the metabolic pathways, 6 proteins involved in telomere stress-induced senescence and senescence-associated phenotype were found. The main proteins involved in this metabolic pathway are histones of the H2A, H2B, H3, H4, I, C family, all with higher expression levels compared to the control and cyclin A2, which was down-regulated.

Finally, upon exposure of cells with PID + WF + AFB₁, the stimulation of biological processes such as mitochondrial tRNA methylation, gene silencing by RNA and nucleosome assembly was observed, in addition to the activation of certain molecular functions such as polyadenylated RNA binding and protein kinase binding. Senescence-associated phenotype, transcriptional regulation by small RNA and DNA methylation were the main metabolic pathways activated after treatment.

3.3 Differentially expressed proteins in Jurkat cells after OTA exposure

Proteins expressed after exposure to OTA (100 nM) in presence of DMSO (Table 6) exert their biological processes as translational regulators (whose main proteins are the eukaryotic translation initiation factor 4A1 and the ribosomal proteins S28, S30 and SA), cotranslational proteins, or as part of gluconeogenesis. In terms of their molecular functions, the binding to polyadenylated RNA and the contribution to ribosome structure stand out. The major proteins were mitochondrial ATP synthase, eukaryotic translation elongation factor 2 and 4A, and nucleolin. When PID was added together with OTA, the expressed proteins turn out to be significantly involved in the combined processes of nucleosome assembly, cell division and regulation of serine/threonine kinase activity, and in the molecular functions of protein heterodimerization or histone binding. Finally, in the PID + WF + OTA condition, proteins involved in nucleosome assembly, superoxide radical scavenging and hydrogen peroxide

catalysis are expressed. The molecular functions are related to antioxidant capacity and thioredoxin peroxidase activity (Table S5)

3.4 Differentially expressed proteins in Jurkat cells after AFB₁ + OTA exposure

Biological processes, molecular functions and metabolic pathways in which differentially expressed proteins were involved after exposure with 0.1% DMSO, PID and PID + WF in combination with AFB₁ and OTA are shown below in Table S6.

When cells were exposed to AFB₁ and OTA standards at 100 nM (0.1% DMSO), activation of biological processes related to gluconeogenesis or translational regulation was observed. In addition, 15 proteins in polyadenylated RNA binding and 13 in ATP were stimulated. The addition of pumpkin highlights 4 proteins involved in biological processes related to nucleosome assembly, in the defense response to Gram-positive bacteria 2 in the regulation of ubiquitination. The most characteristic molecular functions were histone binding and protein heterodimerization activity. Finally, when pumpkin and WF were added in combination, 3 proteins of nucleosome assembly and 2 of innate immune defense of the mucosa were altered in its expression. On the other hand, the most significant molecular functions were heterodimerization activity of 4 proteins, binding to DNA of 5 proteins and pyridoxal phosphate binding of 2 proteins.

As for metabolic pathways, the most significant one observed after exposure with 0.1% DMSO turned out to be glycolysis and gluconeogenesis, while adding PID with or without WF, 5 proteins related to secretory phenotype associated with senescence and 4 to DNA methylation were identified.

5. Discussion

Bioactive compounds are substances which naturally occur in human diet and have been identified as having a preventive role against specific diseases caused by food contaminants. This study combined the beneficial effect of pumpkin and fermented whey contained in intestinal digest of bread against AFB₁ and OTA cytotoxicity in Jurkat cells, by focusing on its proteomics profile.

Among the biological processes in which identified differentially expressed proteins participate, gluconeogenesis stands out. This process converts non-carbohydrate substrates (such as lactate, amino acids and glycerol) into glucose under fasting conditions and is modulated by external factors such as nutrients, energy, exercise and stress reactions (Zhang *et al.*, 2019). In this work, 5 of the enzymes implicated in gluconeogenesis process, such as GPDH, GPI, PGK and PGM, were commonly downregulated after DMSO treatment along with mycotoxins. In particular, deficiency of GPDH and GPI strongly downregulated after mycotoxins exposure (LogFC = -4), was implicated in hepatic steatosis and nonalcoholic fatty liver (Zheng *et al.*, 2019; Lin *et al.*, 2020). Moreover, the loss of PGK observed also in the present study after AFB₁ (LogFC=-0.1672) and OTA (LogFC=-0.4104) exposure alone, but not in combination, is associated with early prognosis in cancer, since is the first enzyme to produce ATP in the glycolytic pathway (Fu and Yu, 2020). As regard PGM, which catalyzes the reversible transformation of 3-phosphoglycerate to 2-phosphoglycerate, was downregulated after single mycotoxin exposure (LogFC=4) but upregulated with mix (LogFC=0.4309). Based on these latter results, it has been reported that PGM overexpression induced necroptosis in primary cardiomyocytes whereas on the contrary, its deficiency increased mitochondrial DNA

and transcripts levels, normalized mitochondrial respiration and repressed mitochondrial ROS production (Zhu *et al.*, 2021).

Regarding the antioxidant activity, peroxiredoxins and thioredoxins expression changed depending on treatment employed in Jurkat cells. These proteins protect cells from oxidative aggressions produced by mycotoxins and help to maintain their natural homeostasis. In particular, peroxiredoxins catalyze the formation of peroxides species, in order to balance H₂O₂ content at cellular level, since they are essential to regulate signaling and oxidative stress mechanisms (Ye *et al.*, 2019). When cells were exposed to AFB₁ and OTA alone or in combination in 0.1% DMSO, PRDX1 decreased its expression down to 4-fold when compared to the control. On the contrary, when functional ingredients with antioxidant capacity were added, PRDX 1 and PRXD2 were overexpressed up to 4-fold, along with TRX-1 which was probably activated by the synergistic action of the two mycotoxins. According to that, *in vitro* and *in vivo* studies reported higher expression of peroxiredoxin and thioredoxin as a consequence of the increased cells' natural defenses after different mycotoxins exposure, alone or in combination (Brennan *et al.*, 2017). Moreover, higher expression of peroxiredoxins was observed in this work in biological processes related to the mucosal innate immune response, superoxide radical scavenging and H₂O₂ catabolic process.

Nucleosome assembly is a process by which DNA is packaged into nucleosomes by assisting chromatin replication, in order to maintain genome stability. Each nucleosome is composed of an octamer of four core histones which are H2A, H2B, H3 and H4. The expression of histones, which are highly basic proteins that help the assembly of new nucleosomes, facilitate genome replication and conservation of hereditary epigenetic information (Liu *et al.*, 2017; Zhao and Shilatifard, 2019). It has been demonstrated that the dysregulation of certain chromatin-associated proteins act as drivers in certain type of cancer. Low expression of histone variants, for instance, was associated with a higher amount of DNA damage in cervical cancer cell lines, suggesting a correlation with DNA repair mechanism (Li *et al.*, 2017). In this study, when exposing cells to functional ingredients and mycotoxins, the overexpression of 6 histones species, (H1, H2A, H2B, H2C, H3, H4) was observed in all conditions employed. Moreover, histone mutations which are partly incorporated into chromatin such as H1.3 and H3.3, have been identified in cancer cell growth by changing the structural properties of nucleosome (Arimura *et al.*, 2018). Also in this case, the histone variants H1.3 and H3.3 levels were strongly increased (LogFC=4) after exposing cells to OTA and mix. Hence, pumpkin and WF could promote the development of new nucleosomes contributing to genome conservation.

Considering metabolic pathways, senescence-associated secretory phenotype related was the most frequent found in cells treated with intestinal digest. This metabolic pathway sensitizes cells and renders them senescent to prevent genomic stress processes such as DNA damage, telomere shortening and chromatin alteration from inducing the loss of vital cell functions. In addition, it has the ability to stimulate the expression of immune-related proteins and paralyze cell proliferation in the process of cancer formation, including promoting tissue repair and development (Özcan *et al.*, 2016). In all conditions where this metabolic pathway was altered, the expression of cyclin A2 was commonly decreased when compared to the control. This protein, has an essential role in progression of cell cycle phase transitions and its up-regulation has been confirmed in certain types of cancer (Pei *et al.*, 2019). On the contrary, Tu *et al.* (2019) demonstrated that down-regulation of cyclin A2 and cyclin-dependent kinases studied in colon cancer cell lines attenuated cell proliferation, as well as in the present study. Based on these findings, functional ingredients can act as protectors against genomic stress caused by AFB₁

and OTA also in this case, preventing the loss of vital cell functions and paralyzing the growth of carcinogenic cells.

6. Conclusions

Digested intestinal extracts of bread enriched with pumpkin and whey fermented contributed positively to the normal functioning of biological processes involved in cells activity against AFB₁ and OTA toxicity, especially gluconeogenesis, antioxidant activity and nucleosome assembly. Moreover, the overexpression of proteins involved in the metabolic pathway of the secretory phenotype associated with senescence (histones of the H2A, H2B, H3 and H4 families) and repression of cyclin A2 confirms the preventive effect of these functional ingredients.

Conflicts of interest

All authors declare have no conflict of interest (financial or non-financial) in the subject matter or materials discussed in this manuscript.

Author's contribution

A.Cimbalo: visualization, conceptualization, experimental part and writing original draft. **M. Frangiamone:** experimental part and writing original draft. **M. Lozano:** methodology, data curation, statistical analysis. **L. Escrivá:** review & editing. **Vila-Donat:** writing final draft, review & editing. **L. Manyes:** conceptualization, funding acquisition, project administration and supervision, review & editing.

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For Peer Review

Uniprot Accession	Log FC	Regulation	p-value	FC	Abund Diff (Raw)
P51665		4 up	0.01762523	16	2363770
P14060		-4 down	4.6853E-08	-16	-35829
P42765		4 up	0.01608898	1.117064	83200.69
P62857		4 up	0.02464994	16	17365.996
P08865		-4 down	0.00024042	-16	-978723.44
P05386		-4 down	0.03639147	-16	-32373.979
P11230	-0.7069859	down	0.00645515	-1.6323886	-6739.998
B3KTV4		4 up	3.0369E-05	16	63713.008
P06733	0.02551269	up	0.02299051	1.3980635	43221.016
P16157		4 up	0.01205456	16	17921.002
P43490		-4 down	0.00046173	-16	-9263.001
P06576		-4 down	0.03950807	-16	-55135.973
O43681		-4 down	0.0007779	-16	-93276.59
O94761		-4 down	0.00039751	-16	-670794.1
O00148	1.1712914	up	0.00496209	2.2521327	36432.004
H3BP20		-4 down	0.00260806	-16	-469780.06
P31327		-4 down	0.0171401	-1.0086838	-8751.9375
Q70JA7		4 up	0.00097482	16	37814.992
P27708		4 up	0.03688859	16	228981.88
O15318		4 up	0.02094653	16	40409.39
C9J2Y9		-4 down	0.01113175	-1.4843638	-12867.596
P13639	0.07700246	up	0.01007148	1.0548248	4037.5469
P60842		4 up	0.03860403	1.4737688	24729.312
P06744		-4 down	0.03727964	-16	-22418.004
P21695		-4 down	0.04212526	-16	-26285.988
P07900	-0.0393743	up	0.00159574	1.1023188	1402.8457
P52272		4 up	0.02332856	16	13606.009
P04629		-4 down	0.00012432	-16	-21787.002
Q06210	0.14351945	up	0.00063744	1.1045972	9787.055
P62805		4 up	0.00836238	16	64697.87
P36776		-4 down	8.7727E-05	-16	-71597.97
P40925		4 up	0.00020858	16	17023.508
P19338		-4 down	0.00015717	-16	-17774.51
Q06830		-4 down	0.01071603	-16	-28268.014
H0YM31	0.26691148	up	0.032335	1.20323	132700.19
P00558	-0.1672763	down	0.02845348	-1.1334736	-13191.359
P18669		-4 down	6.3169E-05	-16	-152713.95
O75604		4 up	0.00152906	16	294722.1
Q6IN84		4 up	0.00808035	16	29487
Q92979		4 up	0.0007223	16	101617.055
P24928		-4 down	0.00169313	-16	-378016.97
P18754		-4 down	0.00358621	-16	-378016.97
P02768		-4 down	0.04778986	-16	-54050.707
P62304		4 up	0.00382694	16	141332.1
P23246		-4 down	0.04128197	-16	-8414.997
P16949		-4 down	0.00097257	-16	-51514.008
O43252		4 up	0.01608898	16	802287.7

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2	Q7Z4L5	-4 down	1.5081E-05	-16	-105384.02
3	Q3MIT2	-4 down	0.03954641	-16	-323822.84
4	Q9Y2Z9	-4 down	0.00554999	-16	-869606.75
5	P26640	4 up	4.0045E-05	16	246660.05
6	P08670	4 up	0.0015823	1.402877	12137.266
7	Q8N823	-4 down	3.0369E-05	-16	-42922.004
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10	P14060	-4 down	0.03727964	-16	-35829
11	P23396	4 up	0.01209612	16	603308
12	A0L900	-0.011734 down	0.00088379	-1.0081666	-5757.1875
13	P62857	4 up	0.00013561	16	18173.951
14	P62861	4 up	0.00260806	16	20401.006
15	P08865	-4 down	3.0369E-05	-16	-978723.44
16	P10809	-4 down	0.00645515	-1.5525551	-34303.098
17	P05388	-4 down	0.00039751	-1.177164	-14759.344
18	P05386	-4 down	0.00043417	-16	-32373.979
19	P11230	-0.1868997 down	0.02861935	-1.1383145	-2113.998
20	P63261	0.5609208 up	0.04778986	1.47926	108359.8
21	B3KTV4	4 up	0.03688859	16	71375.016
22	P43490	-4 down	0.00382694	-16	-9263.001
23	P25705	-0.0193119 up	0.00152906	1.0150217	386.55273
24	P06576	-4 down	0.03688859	-16	-55135.973
25	O43681	-4 down	3.0369E-05	-16	-93276.59
26	O94761	-4 down	0.00152906	-16	-670794.1
27	O00148	1.1577549 up	0.00808035	2.2311003	35820.047
28	H3BP20	-4 down	0.03860403	-16	-469780.06
29	P31327	-4 down	0.00011684	-16	-1016592.1
30	P13639	-0.8750682 down	0.00012719	-1.8340954	-33491.44
31	P60842	4 up	0.00035097	1.9032334	47146.08
32	P06744	-4 down	0.00164067	-16	-22418.004
33	P21695	-4 down	0.00294619	-16	-26285.988
34	O60812	4 up	0.00808035	16	25353.988
35	P52272	4 up	0.00015049	16	8035.9966
36	P04629	-4 down	0.00097482	-16	-21787.002
37	Q06210	-4 down	0.01007148	-16	-93567.96
38	P62805	4 up	0.03470451	16	51665.668
39	P36776	-4 down	2.2966E-05	-16	-71597.97
40	Q8WU39	4 up	0.032335	16	133566.05
41	Q86U38	4 up	0.03727964	16	116149.97
42	P19338	-4 down	0.00046173	-16	-17774.51
43	P62937	4 up	0.0171401	16	76251.97
44	Q06830	-4 down	0.00118826	-16	-28268.014
45	H0YM31	0.11057761 up	0.0010904	1.0796611	52015.188
46	P00558	-0.4104499 down	0.00034294	-1.3291003	-27737.914
47	P18669	-4 down	0.00024296	-16	-152713.95
48	O75604	4 up	0.01850278	16	201553.11
49	P24928	-4 down	0.00097482	-16	-378016.97
50	P18754	-4 down	0.0021484	-16	-378016.97
51	P02768	-4 down	0.00046173	-4.227745	-41266.71
52	P23246	-4 down	0.00018161	-16	-8414.997

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2	P16949	-4 down	0.02845348	-16	-51514.008
3	Q7Z4L5	-4 down	0.00014719	-16	-105384.02
4	O75347	4 up	0.00097257	16	16380.996
5	Q9Y2Z9	-4 down	0.00057609	-16	-869606.75
6	P08670	0.4501762 up	3.0369E-05	1.3662063	11032.516
7	Q8N823	-4 down	5.7991E-05	-16	-42922.004
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10	P14060	0.04549972 up	0.00011244	1.0320406	1148.0195
11	P42765	4 up	0.00133758	1.0826414	58735.5
12	P62857	4 up	0.03770666	16	34289.996
13	P08865	-4 down	0.03688859	-16	-978723.44
14	Q6P1L8	4 up	0.00124926	16	35788.996
15	P05388	4 up	0.00165184	1.5031499	49342.945
16	P05386	-4 down	0.02094653	-16	-32373.979
17	P11230	0.91683966 up	3.0369E-05	1.887975	15448.986
18	B3KTV4	4 up	0.04128197	16	143851.02
19	P0C7T5	4 up	0.00026888	16	75552.04
20	P43490	-4 down	0.00039751	-16	-9263.001
21	P06576	-4 down	6.1595E-06	-16	-55135.973
22	O43681	-4 down	0.03727964	-4.876628	-74150.1
23	O94761	-4 down	8.4713E-05	-16	-670794.1
24	O00148	4 up	0.00169313	2.9803216	57619.34
25	H3BP20	4 up	4.9085E-05	1.1247135	58588.062
26	P31327	-4 down	0.00152906	-16	-1016592.1
27	O75390	-4 down	0.00010496	-16	-33375.355
28	P13639	-4 down	0.00169313	-16	-73643.45
29	P60842	4 up	0.04212526	2.7803922	92931.24
30	P06744	-4 down	9.2337E-05	-16	-22418.004
31	P04406	2.048046 up	0.03727964	4.3335953	937485.75
32	P21695	-4 down	0.00046173	-16	-26285.988
33	P07900	0.3171749 up	4.6853E-08	1.4113647	5640.0166
34	O60812	4 up	0.0171401	16	37612.31
35	P04629	-4 down	0.01850278	-16	-21787.002
36	Q06210	-4 down	0.01608898	-16	-93567.96
37	B2R4S9	4 up	0.00176261	1.9202634	260406.06
38	Q12840	4 up	0.00161005	16	55105
39	O95237	4 up	0.0001574	16	36977.02
40	P36776	0.13183405 up	0.00035097	1.2205746	15792.969
41	Q9H944	4 up	0.02865852	16	130650.07
42	P19338	-4 down	0.04778986	-16	-17774.51
43	Q06830	-4 down	0.00645515	-16	-28268.014
44	HOYM31	4 up	0.0019816	1.1560568	101897.69
45	P00558	0.43131918 up	0.00903664	1.348467	39035.96
46	P18669	0.43094826 up	0.00017951	1.0530009	8094.047
47	P08236	4 up	0.03688859	16	70063.01
48	P12004	4 up	0.00138284	16	46689.992
49	P24928	0.4809122 up	0.00034294	1.3956249	149553.28
50	P18754	0.40566632 up	5.7991E-05	1.3246989	122742
51	P23246	-4 down	0.00759901	-16	-8414.997
52	P16949	-4 down	0.02094653	-16	-51514.008

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2	P51687	4 up	1.5081E-05	16	2551487
3	O60271	4 up	0.00568589	16	417435.7
4	Q7Z4L5	-4 down	0.00152906	-16	-105384.02
5	Q3MIT2	-4 down	3.0369E-05	-16	-323822.84
6	Q9Y2Z9	0.11624337 up	0.00016006	1.0839088	72967.75
7	P26640	4 up	2.2966E-05	16	382686.9
8	P08670	-4 down	4.9085E-05	-16	-30125.512
9	P17017	4 up	0.00018161	16	2534026.5
10	Q8N823	-0.2554002 down	0.00153021	-1.1936656	-6964.0195
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Protein Name

AFB1

26S proteasome regulatory subunit rpn8
 3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type 1
 3-ketoacyl-CoA thiolase
 40S ribosomal protein S28
 40S ribosomal protein SA
 60S acidic ribosomal protein P1
 Acetylcholine receptor subunit beta-type lev-1
 Adenylosuccinate synthetase
 Alpha-enolase
 Ankyrin-1
 ATP phosphoribosyltransferase
 ATP synthase subunit beta
 ATPase ARSA1
 ATP-dependent DNA helicase Q-like 4B
 ATP-dependent RNA helicase DDX39A
 Beta-hexosaminidase
 Carbamoyl-phosphate synthase small chain
 Chondroitin synthase
 Dihydroorotase
 DNA-directed RNA polymerase subunit alpha
 DNA-directed RNA polymerase subunit beta
 Elongation factor 2
 Eukaryotic initiation factor 4A-I
 Glucose-6-phosphate isomerase
 Glycerol-3-phosphate dehydrogenase [NAD(P)+]
 Heat shock protein HSP 90-alpha
 Heterogeneous nuclear ribonucleoprotein M
 High affinity nerve growth factor receptor
 Histidinol-phosphate aminotransferase
 Histone H4, major
 Lon protease homolog, mitochondrial
 Malate dehydrogenase, cytoplasmic
 Nucleolin
 Peroxiredoxin-1
 Phosphoenolpyruvate carboxylase
 Phosphoglycerate kinase 1
 Phosphoglycerate mutase 1
 Probable ubiquitin carboxyl-terminal hydrolase 2
 Ribosomal RNA small subunit methyltransferase A
 Ribosomal RNA small subunit methyltransferase G
 RNA-directed RNA polymerase L
 Segregation and condensation protein A
 Serum albumin
 Small nuclear ribonucleoprotein E
 Splicing factor, proline- and glutamine-rich
 Stathmin
 Sulfate adenylyltransferase

1 Tetratricopeptide repeat protein 21 homolog
 2 tRNA(Ile)-lysidine synthase
 3 Ubiquinone biosynthesis monooxygenase COQ6, mitochondrial
 4 Valine--tRNA ligase
 5 Vimentin
 6 Zinc finger protein 611
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 8 **OTA**
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 10 3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type 1
 11 30S ribosomal protein S3, chloroplastic
 12 3-ketoacyl-CoA thiolase
 13 40S ribosomal protein S28
 14 40S ribosomal protein S30
 15 40S ribosomal protein SA
 16 60 kDa heat shock protein, mitochondrial
 17 60S acidic ribosomal protein P0
 18 60S acidic ribosomal protein P1
 19 Acetylcholine receptor subunit beta-type lev-1
 20 Actin, cytoplasmic 2
 21 Adenylosuccinate synthetase
 22 ATP phosphoribosyltransferase
 23 ATP synthase subunit alpha, mitochondrial
 24 ATP synthase subunit beta
 25 ATPase ARSA1
 26 ATP-dependent DNA helicase Q-like 4B
 27 ATP-dependent RNA helicase DDX39A
 28 Beta-hexosaminidase
 29 Carbamoyl-phosphate synthase small chain
 30 Elongation factor 2
 31 Eukaryotic initiation factor 4A-I
 32 Glucose-6-phosphate isomerase
 33 Glycerol-3-phosphate dehydrogenase [NAD(P)+]
 34 Heterogeneous nuclear ribonucleoprotein C
 35 Heterogeneous nuclear ribonucleoprotein M
 36 High affinity nerve growth factor receptor
 37 Histidinol-phosphate aminotransferase
 38 Histone H4, major
 39 Lon protease homolog, mitochondrial
 40 Marginal zone B- and B1-cell-specific protein
 41 Nucleolar protein 9
 42 Nucleolin
 43 Peptidyl-prolyl cis-trans isomerase A
 44 Peroxiredoxin-1
 45 Phosphoenolpyruvate carboxylase
 46 Phosphoglycerate kinase 1
 47 Phosphoglycerate mutase 1
 48 Probable ubiquitin carboxyl-terminal hydrolase 2
 49 RNA-directed RNA polymerase L
 50 Segregation and condensation protein A
 51 Serum albumin
 52 Splicing factor, proline- and glutamine-rich
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1 Stathmin
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 3 Tetratricopeptide repeat protein 21 homolog
 4 Tubulin-specific chaperone A
 5 Ubiquinone biosynthesis monooxygenase COQ6, mitochondrial
 6 Vimentin
 7
 8 Zinc finger protein 611

AFB1 + OTA

10 3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type 1
 11 3-ketoacyl-CoA thiolase
 12 40S ribosomal protein S28
 13 40S ribosomal protein SA
 14 50S ribosomal protein L14
 15 60S acidic ribosomal protein P0
 16 60S acidic ribosomal protein P1
 17 Acetylcholine receptor subunit beta-type lev-1
 18 Adenylosuccinate synthetase
 19 Ataxin-1-like
 20 ATP phosphoribosyltransferase
 21 ATP synthase subunit beta
 22 ATPase ARSA1
 23 ATP-dependent DNA helicase Q-like 4B
 24 ATP-dependent RNA helicase DDX39A
 25 Beta-hexosaminidase
 26 Carbamoyl-phosphate synthase small chain
 27 Citrate synthase, mitochondrial
 28 Elongation factor 2
 29 Eukaryotic initiation factor 4A-I
 30 Glucose-6-phosphate isomerase
 31 Glyceraldehyde-3-phosphate dehydrogenase
 32 Glycerol-3-phosphate dehydrogenase [NAD(P)+]
 33 Heat shock protein HSP 90-alpha
 34 Heterogeneous nuclear ribonucleoprotein C
 35 High affinity nerve growth factor receptor
 36 Histidinol-phosphate aminotransferase
 37 Histone H2B 1/2/3/4/6
 38 Kinesin-like protein KIN-5A
 39 Lecithin retinol acyltransferase
 40 Lon protease homolog, mitochondrial
 41 Mediator of RNA polymerase II transcription subunit 20
 42 Nucleolin
 43 Peroxiredoxin-1
 44 Phosphoenolpyruvate carboxylase
 45 Phosphoglycerate kinase 1
 46 Phosphoglycerate mutase 1
 47 Probable alpha-glucuronidase A
 48 Proliferating cell nuclear antigen
 49 RNA-directed RNA polymerase L
 50 Segregation and condensation protein A
 51 Splicing factor, proline- and glutamine-rich
 52 Stathmin

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- 2 Sulfite oxidase, mitochondrial
- 3 Surface protein
- 4 Tetratricopeptide repeat protein 21 homolog
- 5 tRNA(Ile)-lysidine synthase
- 6 Ubiquinone biosynthesis monooxygenase COQ6, mitochondrial
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- 8 Valine--tRNA ligase
- 9 Vimentin
- 10 Zinc finger protein 14
- 11 Zinc finger protein 611
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For Peer Review

Uniprot Accession	Log FC	Regulation	p-value	FC	Abund Diff (Raw)
W1WUJ5		4 up	5.7991E-05	16	64641.016
P61247		4 up	0.0171401	16	266370.97
P10809		4 up	6.1595E-06	16	6258.5
P63261		4 up	9.9027E-05	16	59045.49
P02765		4 up	9.9027E-05	16	21303.998
P51531		4 up	0.00088379	16	476578.75
P20248		-4 down	0.00011244	-16	-26313.996
Q7Z6Z7		4 up	2.2625E-05	16	2588.0005
Q9BTM1		4 up	0.00043417	16	37315.516
P62807		4 up	0.00138284	16	90166.71
P62805		4 up	8.4713E-05	16	28859.996
Q9P2J5		4 up	0.03727964	16	45660
P06748		4 up	0.00067757	16	35518.99
O00746		4 up	0.01762523	16	21397.01
P56715		-4 down	0.01608898	-16	-23534.992
Q06830		4 up	9.816E-06	16	19151.986
Q16822		-4 down	0.00152906	-16	-38463
P18669		4 up	0.00220531	16	27969.988
P07814		4 up	0.00068856	16	19172.988
P14618		4 up	0.0396359	16	23359.008
O95347		-4 down	0.00645515	-16	-26824.99
P29122	3.153655	up	0.02845348	8.899078	265820.12
Q96SZ6	0.69679826	up	0.03860403	1.6209036	8601.997
P07437		4 up	0.00097482	16	30916.004
Q2M389		-4 down	0.00020858	-16	-18504.002
Q6PDB4		4 up	8.0159E-05	16	59715.02
Q9NVS2		4 up	0.03727964	16	24079.986
P20248		-4 down	0.00011244	-16	-26313.996
Q7Z6Z7		4 up	0.00013561	16	20808.002
P16402		4 up	0.032335	16	9819.005
Q16777		-4 down	0.00182297	-16	-15710.998
O60814	1.7780703	up	6.8027E-05	3.4296694	46128.484
P68431		4 up	0.00015049	16	18260.986
P56715	1.1854258	up	0.01035451	2.2743065	29992.035
P32119		4 up	0.01205456	16	16255.999
Q16822		-4 down	0.00088379	-16	-38463
P16104		4 up	0.03470451	16	29893.488
P54687		4 up	0.01608898	16	75862.016
P18754		4 up	0.0171401	16	29831.994
O95347		-4 down	5.7991E-05	-16	-26824.99
P29122		-4 down	0.02845348	-16	-33651.02
Q96SZ6	0.80851555	up	0.03860403	1.7514085	10410.011
Q7LBR1		4 up	0.00138284	16	27091
Q2M389		-4 down	0.00098858	-16	-18504.002
Q9Y2R5		4 up	0.01608898	16	133270.06

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2	P63261	4 up	0.00836238	16	28864.766
3	Q9NZ52	4 up	0.01205456	16	18594.008
4	P02765	4 up	0.0396359	16	27255.002
5	P51531	4 up	0.00152906	16	391180.44
6	Q13206	4 up	9.9027E-05	16	28732
7	Q53ZF3	4 up	0.01762523	16	87888.01
8	P20248	-4 down	0.00496209	-16	-26313.996
9	O75592	4 up	0.00138284	16	104212.02
10	Q7Z6Z7	4 up	0.00067757	16	27109.398
11	Q6IA69	1.1024222 up	0.00067757	2.1358693	105613.07
12	P07900	4 up	0.03860403	16	19330.508
13	Q16777	1.8091373 up	2.2625E-05	3.5043292	39348.016
14	P62807	4 up	0.00152906	16	94729.66
15	O60814	-4 down	0.00159574	-16	-18984.498
16	P84243	4 up	0.0015823	16	35271.203
17	P62805	4 up	4.0045E-05	16	28654.988
18	Q8NAC3	4 up	0.0396359	16	20972.006
19	O14782	4 up	0.02299051	16	27729
20	Q9H3D4	4 up	0.02464994	16	68225.945
21	P56715	-4 down	0.00011684	-16	-23534.992
22	Q06830	4 up	0.00011244	16	23344.512
23	Q16822	-4 down	0.00118826	-16	-38463
24	P14555	4 up	9.9027E-05	16	23545.996
25	P07814	4 up	0.0171401	16	23907.004
26	Q92765	4 up	2.2625E-05	16	135513.89
27	P18754	4 up	0.00645515	16	68357.945
28	O95347	-4 down	0.00088379	-16	-26824.99
29	P29122	-4 down	0.01113175	-16	-33651.02
30	O43396	4 up	0.00159574	16	20452.008
31	Q8N442	4 up	0.032335	16	225024.05
32	Q32P41	4 up	0.01209612	16	68629.98
33	Q96SZ6	1.5497007 up	0.00068856	2.9933202	27615.438
34	Q9H4B7	4 up	0.00020858	16	24578.256
35	P68543	4 up	0.01007148	16	46070.004
36	Q7LBR1	4 up	0.00068856	16	190481
37	P26640	4 up	0.02094653	16	389426
38	Q2M389	-4 down	0.02464994	-16	-18504.002
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Protein Name

AFB1

2-isopropylmalate synthase
 40S ribosomal protein S3a
 60 kDa heat shock protein, mitochondrial
 Actin, cytoplasmic 2
 Alpha-2-HS-glycoprotein
 ATP-dependent helicase fft2
 Cyclin-A2-4
 E3 ubiquitin-protein ligase PUB23
 Histone H2A.J
 Histone H2B type 1
 Histone H4, major
 Leucine--tRNA ligase
 Nucleophosmin
 Nucleoside diphosphate kinase
 Oxygen-regulated protein 1
 Peroxiredoxin-1
 Phosphoenolpyruvate carboxylase
 Phosphoglycerate mutase 1
 Proline--tRNA ligase
 Pyruvate kinase PKM
 Structural maintenance of chromosomes protein 2
 Subtilisin-like protease SBT5.4
 tRNA-2-methylthio-N(6)-dimethylallyl adenosine synthase
 Tubulin beta chain
 WASH complex subunit 4
 Zinc finger protein 880

OTA

30S ribosomal protein S18
 Cyclin-A2-4
 E3 ubiquitin-protein ligase PUB23
 Histone H1.3
 Histone H2A type 2-C
 Histone H2B type 1-K
 Histone H3
 Oxygen-regulated protein 1
 Peroxiredoxin-2
 Phosphoenolpyruvate carboxylase
 Probable histone H2A.6
 Putative aminodeoxychorismate lyase
 Segregation and condensation protein A
 Structural maintenance of chromosomes protein 2
 Subtilisin-like protease SBT5.4
 tRNA-2-methylthio-N(6)-dimethylallyl adenosine synthase
 Vacuolar protein sorting-associated protein 20 homolog 1
 WASH complex subunit 4

AFB1 + OTA

30S ribosomal protein S17

1 Actin, cytoplasmic 2
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3 ADP-ribosylation factor-binding protein GGA3
4 Alpha-2-HS-glycoprotein
5 ATP-dependent helicase fft2
6 ATP-dependent RNA helicase DBP4
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8 Cationic trypsin-3
9 Cyclin-A2-4
10 E3 ubiquitin-protein ligase highwire
11 E3 ubiquitin-protein ligase PUB23
12 Glutamine-dependent NAD(+) synthetase
13 Heat shock protein HSP 90-alpha
14 Histone H2A type 2-C
15 Histone H2B 1/2/3/4/6
16 Histone H2B type 1-K
17 Histone H3.3
18 Histone H4, major
19 Interleukin-17 receptor C
20 Kinesin-II 95 kDa subunit
21 Orofacial cleft 1 candidate gene 1 protein homolog
22 Oxygen-regulated protein 1
23 Peroxiredoxin-1
24 Phosphoenolpyruvate carboxylase
25 Phospholipase A2
26 Proline--tRNA ligase
27 Secreted frizzled-related protein 3
28 Segregation and condensation protein A
29 Structural maintenance of chromosomes protein 2
30 Subtilisin-like protease SBT5.4
31 Thioredoxin-like protein 1
32 Translation factor GUF1 homolog, mitochondrial
33 tRNA (guanine(37)-N1)-methyltransferase
34 tRNA-2-methylthio-N(6)-dimethylallyladenosine synthase
35 Tubulin beta-1 chain
36 UBX domain-containing protein 2
37 Vacuolar protein sorting-associated protein 20 homolog 1
38 Valine--tRNA ligase
39 WASH complex subunit 4
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Uniprot Accession	Log FC	Regulation	p-value	FC	Abund Diff (Raw)
P63261		4 up	0.00153021	16	41100.44
P02765		4 up	0.0001574	16	23204.99
P51530		4 up	0.0001574	16	583165.9
P07477		4 up	0.00011244	16	86163.04
P20248		-4 down	0.03639147	-16	-26456.01
Q96A65		4 up	0.01426697	16	115472.91
P43026		-4 down	0.00169313	-16	-33087.01
P82995		4 up	0.00063744	16	51433.977
Q9BW92		4 up	0.02630597	16	93343.95
Q16777		-4 down	0.00081254	-16	-18183.998
Q9BTM1		4 up	0.00133758	16	35901.504
P62807	1.9681511	up	0.02332856	3.9126663	80493.53
P84243		4 up	0.0019816	16	31275.174
P62805		4 up	0.03688859	16	28572.008
O15047		4 up	0.00030684	16	48272.977
Q8NEV4		-4 down	0.0001574	-16	-44794.02
Q96JN8		4 up	0.03976551	16	740670.44
O00746		4 up	0.00124926	16	20448.008
P56715		-4 down	0.00011244	-16	-29968.992
Q06830		4 up	0.00161005	16	20069.998
P18669		4 up	0.00026888	16	28493.994
Q8TCS8		4 up	0.00252583	16	34647.984
Q32MH5		4 up	0.00300694	16	105377.06
P61619		4 up	0.0004961	16	37226.008
P54687		-4 down	0.04212526	-16	-45917.8
P14618		4 up	0.01382999	16	20877.998
Q7LOY3		4 up	4.9085E-05	16	21948.012
P24928		4 up	0.00153021	16	56392.016
P57059		4 up	0.00011244	16	31272.982
Q96JI7		4 up	0.00025985	16	56431.992
P50991		-4 down	0.03954641	-16	-23909.012
Q32P41		4 up	0.0014282	16	63339.01
Q96SZ6	1.0101323	up	4.9085E-05	2.0747678	18275.357
P07437		4 up	5.7991E-05	16	27385.596
Q9NP79		-4 down	3.0369E-05	-16	-22965.412
Q2M389		-4 down	0.0171401	-16	-23995.734
Q6PDB4		4 up	0.0014282	16	50311.008
Q86VS8		4 up	4.9085E-05	16	17756.998
Q9Y676		4 up	0.00133758	16	24079.986
P63261		4 up	0.02464994	16	47007.49
P02765		4 up	0.0001574	16	22760.996
P51530		4 up	0.00153021	16	314794.88
P07477		4 up	0.0014282	16	63319.504
P20248		-4 down	0.02464994	-16	-26456.01
O75899		4 up	4.9085E-05	16	38738.996
P04406		4 up	3.0369E-05	16	25586.512

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2	P43026	-4 down	0.08128197	-16	-33087.01
3	P82995	4 up	0.03727964	16	17044.51
4	P16402	4 up	0.00568589	16	9819.005
5	Q16777	-4 down	0.01071603	-16	-18183.998
6	Q9BTM1	4 up	0.00554999	16	38452.984
8	P62807	-4 down	9.7727E-05	-16	-27634.686
9	O60814	4 up	0.00159574	16	69645.664
10	B2R4P9	4 up	2.2966E-05	16	26710.13
11	P62805	4 up	0.00023303	16	19118.994
12	Q8NEV4	-4 down	0.01007148	-16	-44794.02
14	O95544	4 up	5.7991E-05	16	31291.988
15	O00746	4 up	3.0369E-05	16	17135.988
16	P56715	0.83677673 up	0.0171401	1.7860553	23558.035
17	Q06830	4 up	0.00067757	16	11657.497
18	P32119	4 up	0.0007779	16	16255.999
19	P18669	4 up	0.00138171	16	31151.002
20	Q5SQT3	4 up	0.0019816	16	29893.488
22	P54687	0.73347753 up	0.00554999	1.6521124	29944.215
23	P18754	4 up	0.00161005	16	29831.994
24	P50991	-4 down	0.02589051	-16	-23909.012
26	Q96SZ6	0.5129424 up	0.01762523	1.4269584	7260.002
27	Q9H4B7	4 up	0.04128197	16	20169.66
28	Q9NP79	0.24134968 up	0.04673324	1.1796356	4125.588
29	Q2M389	-4 down	0.02151751	-16	-23995.734
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32	P20248	-0.688898 down	0.02094653	-1.6120539	-10045.021
33	P62877	-0.1007119 down	0.04778986	-1.0784642	-876.99414
34	P43026	-4 down	0.00046173	-16	-33087.01
35	Q16777	-4 down	0.00645515	-16	-18183.998
36	P62807	-4 down	0.0007223	-16	-27634.686
37	O60814	4 up	0.0004961	16	28314.002
38	P62805	4 up	0.01007148	16	14964
39	Q8NEV4	-4 down	9.2337E-05	-16	-44794.02
40	P56715	-4 down	0.00169313	-16	-29968.992
41	P54687	-4 down	3.0369E-05	-16	-45917.8
42	P02768	0.37525555 up	0.00220531	1.1686254	4578.119
43	P50991	-4 down	0.00097257	-16	-23909.012
44	Q96SZ6	-4 down	0.00759901	-16	-17003.006
45	Q9NP79	0.03508619 up	0.0037571	1.0224924	516.57227
46	Q2M389	-4 down	0.00152906	-16	-23995.734
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Protein Name

AFB1

Actin, cytoplasmic 2
 Alpha-2-HS-glycoprotein
 ATP-dependent helicase fft2
 Cationic trypsin-3
 Cyclin-A2-4
 Exocyst complex component 4
 Growth/differentiation factor 5
 Heat shock protein HSP 90-alpha
 Histidine--tRNA ligase
 Histone H2A type 2-C
 Histone H2A.J
 Histone H2B type 1
 Histone H3.3
 Histone H4, major
 Histone-lysine N-methyltransferase SETD1A
 Myosin-IIIa
 Neuralized-like protein 4
 Nucleoside diphosphate kinase
 Oxygen-regulated protein 1
 Peroxiredoxin-1
 Phosphoglycerate mutase 1
 Polyribonucleotide nucleotidyltransferase
 Protein FAM214A
 Protein transport protein SEC61
 Putative aminodeoxychorismate lyase
 Pyruvate kinase PKM
 Ribonuclease R
 RNA-directed RNA polymerase L
 Serine/threonine-protein kinase cbk1
 Spatacsin
 T-complex protein 1 subunit delta
 tRNA (guanine(37)-N1)-methyltransferase
 tRNA-2-methylthio-N(6)-dimethylallyl adenosine synthase
 Tubulin beta chain
 Vacuolar protein sorting-associated protein 20 homolog 1
 WASH complex subunit 4
 Zinc finger protein 880
 Zygote defective protein 12

OTA

30S ribosomal protein S18
 Actin, cytoplasmic 2
 Alpha-2-HS-glycoprotein
 ATP-dependent helicase fft2
 Cationic trypsin-3
 Cyclin-A2-4
 Gamma-aminobutyric acid type B receptor subunit 2
 Glyceraldehyde-3-phosphate dehydrogenase

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- Growth/differentiation factor 5
- Heat shock protein HSP 90-alpha
- Histone H1.3
- Histone H2A type 2-C
- Histone H2A.J
- Histone H2B type 1
- Histone H2B type 1-K
- Histone H3
- Histone H4, major
- Myosin-IIIa
- NAD kinase
- Nucleoside diphosphate kinase
- Oxygen-regulated protein 1
- Peroxiredoxin-1
- Peroxiredoxin-2
- Phosphoglycerate mutase 1
- Probable histone H2A.6
- Putative aminodeoxychorismate lyase
- Segregation and condensation protein A
- T-complex protein 1 subunit delta
- tRNA-2-methylthio-N(6)-dimethylallyladenosine synthase
- Tubulin beta-1 chain
- Vacuolar protein sorting-associated protein 20 homolog 1
- WASH complex subunit 4

AFB1 + OTA

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- Cyclin-A2-4
- E3 ubiquitin-protein ligase PUB23
- Growth/differentiation factor 5
- Histone H2A type 2-C
- Histone H2B type 1
- Histone H2B type 1-K
- Histone H4
- Myosin-IIIa
- Oxygen-regulated protein 1
- Putative aminodeoxychorismate lyase
- Serum albumin
- T-complex protein 1 subunit delta
- tRNA-2-methylthio-N(6)-dimethylallyladenosine synthase
- Vacuolar protein sorting-associated protein 20 homolog 1
- WASH complex subunit 4

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Species*Homo sapiens*

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DMSO 0.1%

<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>	
GOTERM_BP_DIRECT	gluconeogenesis	RT	7	13.2
GOTERM_BP_DIRECT	glutamine metabolic process	RT	4	7.5
GOTERM_BP_DIRECT	canonical glycolysis	RT	4	7.5
GOTERM_BP_DIRECT	glycolytic process	RT	4	7.5
GOTERM_BP_DIRECT	mRNA splicing, via spliceosome	RT	5	9.4
<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>	
GOTERM_MF_DIRECT	poly(A) RNA binding	RT	17	32.1
GOTERM_MF_DIRECT	ATP binding	RT	14	26.4
GOTERM_MF_DIRECT	protein binding	RT	38	71.7
GOTERM_MF_DIRECT	DNA-directed RNA polymerase activity	RT	3	5.7
GOTERM_MF_DIRECT	RNA binding	RT	7	13.2
<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>	
REACTOME_PATHWAY	Gluconeogenesis	RT	6	11.3
REACTOME_PATHWAY	Glycolysis	RT	4	7.5
REACTOME_PATHWAY	RNA biogenesis	RT	3	5.7
REACTOME_PATHWAY	Peptide chain elongation	RT	4	7.5
REACTOME_PATHWAY	Processing of Capped Intron-Containing	RT	3	5.7

PID

<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>	
GOTERM_BP_DIRECT	nucleosome assembly	RT	4	12
GOTERM_BP_DIRECT	natural killer cell mediated cytotoxicity	RT	2	8
GOTERM_BP_DIRECT	canonical glycolysis	RT	2	8
GOTERM_BP_DIRECT	regulation of cyclin-dependent protein	RT	2	8
GOTERM_BP_DIRECT	tRNA aminoacylation for protein transl	RT	2	8
<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>	
GOTERM_MF_DIRECT	ATP binding	RT	8	32
GOTERM_MF_DIRECT	protein heterodimerization activity	RT	7	20
GOTERM_MF_DIRECT	poly(A) RNA binding	RT	7	28
GOTERM_MF_DIRECT	histone binding	RT	3	12
GOTERM_MF_DIRECT	protein kinase binding	RT	4	16
<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>	
REACTOME_PATHWAY	DNA Damage/Telomere Stress Induced	RT	6	16
REACTOME_PATHWAY	Deposition of new CENPA-containing n	RT	4	16
REACTOME_PATHWAY	Condensation of Prophase Chromosom	RT	6	16
REACTOME_PATHWAY	Senescence-Associated Secretory Phen	RT	6	16
REACTOME_PATHWAY	DNA methylation	RT	5	12

PID+WF

<u>Category</u>	<u>Term</u>		<u>Count</u>	<u>%</u>
GOTERM_BP_DIRECT	mitochondrial RNA 5'-end processing	RT	2	5.3
GOTERM_BP_DIRECT	protein transport	RT	5	13.2
GOTERM_BP_DIRECT	mitochondrial tRNA methylation	RT	2	5.3
GOTERM_BP_DIRECT	gene silencing by RNA	RT	3	7.9
GOTERM_BP_DIRECT	nucleosome assembly	RT	3	7.9
<u>Category</u>	<u>Term</u>		<u>Count</u>	<u>%</u>
GOTERM_MF_DIRECT	protein heterodimerization activity	RT	5	13.2
GOTERM_MF_DIRECT	poly(A) RNA binding	RT	7	18.4
GOTERM_MF_DIRECT	ATP binding	RT	8	21.1

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2	GOTERM_MF_DIRECT	protein binding	RT	25	65.8	
3	GOTERM_MF_DIRECT	protein kinase binding	RT	4	10.5	
4	Category	Term		Count	%	
5	REACTOME_PATHWAY	Transcriptional regulation by small RNA	RT	6	15.8	
6	REACTOME_PATHWAY	Senescence-Associated Secretory Phen	RT	6	15.8	
7	REACTOME_PATHWAY	RNA Polymerase I Promoter Opening	RT	5	13.2	
8	REACTOME_PATHWAY	DNA methylation	RT	5	13.2	
9	REACTOME_PATHWAY	DNA Damage/Telomere Stress Induced	RT	5	13.2	
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For Peer Review

<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
4.20E-09	1.90E-06	1.90E-06
3.10E-05	7.10E-03	7.00E-03
6.90E-05	1.10E-02	1.10E-02
1.60E-04	1.80E-02	1.80E-02
4.90E-03	3.40E-01	3.40E-01
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
1.10E-07	2.30E-05	2.20E-05
3.80E-04	3.90E-02	3.80E-02
2.20E-03	1.40E-01	1.30E-01
5.90E-03	1.40E-01	1.30E-01
5.90E-03	1.40E-01	1.30E-01
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
3.00E-07	6.60E-05	4.50E-02
4.20E-04	4.50E-02	4.60E-01
7.80E-03	4.60E-01	4.60E-01
8.40E-03	4.60E-01	4.90E-01
1.30E-02	4.90E-01	4.90E-01
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
1.20E-02	1.00E+00	1.00E+00
2.50E-02	1.00E+00	1.00E+00
3.70E-02	1.00E+00	1.00E+00
5.40E-02	1.00E+00	1.00E+00
5.60E-02	1.00E+00	1.00E+00
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
2.90E-03	9.70E-02	9.50E-02
3.30E-03	9.70E-02	9.50E-02
3.30E-03	9.70E-02	9.50E-02
1.20E-02	2.50E-01	2.40E-01
1.40E-02	2.50E-01	2.40E-01
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
3.80E-04	1.60E-02	1.20E-02
5.40E-04	1.60E-02	1.20E-02
5.40E-04	1.60E-02	1.20E-02
1.70E-03	3.70E-02	2.90E-02
8.80E-03	9.10E-02	7.20E-02
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
4.00E-03	8.10E-01	8.10E-01
8.00E-03	8.10E-01	8.10E-01
8.10E-03	8.10E-01	8.10E-01
2.10E-02	1.00E+00	1.00E+00
2.40E-02	1.00E+00	1.00E+00
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
1.50E-02	9.10E-01	9.10E-01
2.70E-02	9.10E-01	9.10E-01
3.10E-02	9.10E-01	9.10E-01

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2	3.60E-02	9.10E-01	9.10E-01
3	4.20E-02	9.10E-01	9.10E-01
4	<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
5	1.10E-05	6.10E-04	5.20E-04
6	1.30E-05	6.10E-04	5.20E-04
7	2.80E-05	6.10E-04	5.20E-04
8	3.20E-05	6.10E-04	5.20E-04
9	3.40E-05	6.10E-04	5.20E-04
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<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>
GOTERM_BP_DIRECT	translational initiation	RT	7 14.6
GOTERM_BP_DIRECT	SRP-dependent cotranslational protei	RT	6 12.5
GOTERM_BP_DIRECT	gluconeogenesis	RT	5 10.4
GOTERM_BP_DIRECT	nuclear-transcribed mRNA catabolic p	RT	6 12.5
GOTERM_BP_DIRECT	rRNA processing	RT	6 12.5
<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>
GOTERM_MF_DIRECT	poly(A) RNA binding	RT	20 41.7
GOTERM_MF_DIRECT	structural constituent of ribosome	RT	6 12.5
GOTERM_MF_DIRECT	ATPase activity	RT	5 10.4
GOTERM_MF_DIRECT	ATP binding	RT	12 25
GOTERM_MF_DIRECT	protein binding	RT	34 70.8
<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>
REACTOME_PATHWAY	Peptide chain elongation	RT	7 14.6
REACTOME_PATHWAY	L13a-mediated translational silencing	RT	7 14.6
REACTOME_PATHWAY	GTP hydrolysis and joining of the 60S	RT	7 14.6
REACTOME_PATHWAY	R-HSA-192823	RT	6 12.5
REACTOME_PATHWAY	Selenocysteine synthesis	RT	6 12.5
REACTOME_PATHWAY	Eukaryotic Translation Termination	RT	6 12.5

PID

<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>
GOTERM_BP_DIRECT	nucleosome assembly	RT	4 22.2
GOTERM_BP_DIRECT	cell division	RT	4 22.2
GOTERM_BP_DIRECT	regulation of cyclin-dependent protei	RT	2 11.1
GOTERM_BP_DIRECT	chromatin silencing	RT	2 11.1
GOTERM_BP_DIRECT	G1/S transition of mitotic cell cycle	RT	2 11.1
<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>
GOTERM_BP_DIRECT	protein heterodimerization activity	RT	5 27.8
GOTERM_BP_DIRECT	chromatin DNA binding	RT	3 16.7
GOTERM_BP_DIRECT	structure-specific DNA binding	RT	3 16.7
GOTERM_BP_DIRECT	histone binding	RT	3 16.7
GOTERM_BP_DIRECT	protein dimerization activity	RT	5 27.8
<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>
REACTOME_PATHWAY	Condensation of Prophase Chromoso	RT	5 27.8
REACTOME_PATHWAY	Senescence-Associated Secretory Phe	RT	5 27.8
REACTOME_PATHWAY	RNA Polymerase I Promoter Opening	RT	4 22.2
REACTOME_PATHWAY	DNA methylation	RT	4 22.2
REACTOME_PATHWAY	DNA Damage/Telomere Stress Induce	RT	4 22.2

PID+WF

<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>
GOTERM_BP_DIRECT	nucleosome assembly	RT	6 18.8
GOTERM_BP_DIRECT	chromatin silencing	RT	3 9.4
GOTERM_BP_DIRECT	negative regulation of gene expressio	RT	3 9.4
GOTERM_BP_DIRECT	removal of superoxide radicals	RT	2 6.2
GOTERM_BP_DIRECT	hydrogen peroxide catabolic process	RT	2 6.2
<u>Category</u>	<u>Term</u>	<u>Count</u>	<u>%</u>
GOTERM_MF_DIRECT	protein heterodimerization activity	RT	7 21.9
GOTERM_MF_DIRECT	thioredoxin peroxidase activity	RT	2 6.2

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2	GOTERM_MF_DIRECT	histone binding	RT	3	9.4	
3	GOTERM_MF_DIRECT	DNA binding	RT	8	25	
4	GOTERM_MF_DIRECT	antioxidant activity	RT	2	6.2	
5	Category	Term		Count	%	
6						
7	REACTOME_PATHWAY	Senescence-Associated Secretory Phe	RT	7	21.9	
8	REACTOME_PATHWAY	RNA Polymerase I Promoter Opening	RT	6	18.8	
9	REACTOME_PATHWAY	DNA methylation	RT	6	18.8	
10	REACTOME_PATHWAY	DNA Damage/Telomere Stress Induce	RT	6	18.8	
11	REACTOME_PATHWAY	ctivated PKN1 stimulates transcrip	RT	6	18.8	
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<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
1.70E-06	7.50E-04	8.30E-04
5.10E-06	8.50E-04	8.30E-04
5.60E-06	8.50E-04	1.30E-03
1.60E-05	1.40E-03	1.90E-02
2.60E-04	1.90E-02	3.50E-02
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
1.90E-11	3.40E-09	3.30E-09
3.00E-04	2.70E-02	2.60E-02
1.40E-03	6.80E-02	6.70E-02
1.50E-03	6.80E-02	6.70E-02
2.90E-03	1.00E-01	1.00E-01
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
1.80E-06	3.00E-04	2.80E-04
6.80E-06	3.90E-04	3.60E-04
7.20E-06	3.90E-04	3.60E-04
3.50E-05	1.10E-03	1.00E-03
4.10E-05	1.10E-03	1.00E-03
4.30E-05	1.10E-03	1.00E-03
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
2.20E-04	3.10E-02	3.10E-02
4.90E-03	3.40E-01	3.40E-01
3.90E-02	1.00E+00	1.00E+00
4.50E-02	1.00E+00	1.00E+00
9.80E-02	1.00E+00	1.00E+00
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
7.90E-04	8.60E-02	8.50E-02
4.20E-03	2.30E-01	2.20E-01
7.90E-03	2.80E-01	2.80E-01
1.30E-02	3.50E-01	3.50E-01
2.10E-02	4.50E-01	4.40E-01
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
2.80E-06	1.60E-04	1.00E-04
1.30E-05	3.90E-04	2.60E-04
8.70E-05	9.00E-04	5.90E-04
9.50E-05	9.00E-04	5.90E-04
1.00E-04	9.00E-04	5.90E-04
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
2.00E-06	4.70E-04	4.70E-04
2.90E-03	2.80E-01	2.80E-01
3.60E-03	2.80E-01	2.80E-01
2.10E-02	9.30E-01	9.30E-01
3.50E-02	9.30E-01	9.30E-01
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
1.20E-04	9.80E-03	9.70E-03
8.60E-03	3.60E-01	3.60E-01

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2	1.90E-02	4.30E-01	4.30E-01
3	2.10E-02	4.30E-01	4.30E-01
4	3.40E-02	5.30E-01	5.20E-01
5	<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
6			
7	4.10E-07	1.70E-05	1.40E-05
8	6.60E-07	1.70E-05	1.40E-05
9	7.70E-07	1.70E-05	1.40E-05
10	8.30E-07	1.70E-05	1.40E-05
11	8.90E-07	1.70E-05	1.40E-05
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Category	Term	Count	%
GOTERM_BP_DIRECT	gluconeogenesis	RT	6 11.3
GOTERM_BP_DIRECT	canonical glycolysis	RT	4 7.5
GOTERM_BP_DIRECT	glycolytic process	RT	4 7.5
GOTERM_BP_DIRECT	response to dexamethasone	RT	3 5.7
GOTERM_BP_DIRECT	translational initiation	RT	5 9.4
Category	Term	Count	%
GOTERM_MF_DIRECT	poly(A) RNA binding	RT	15 28.3
GOTERM_MF_DIRECT	ATP binding	RT	13 24.5
GOTERM_MF_DIRECT	structural constituent of ribosome	RT	5 9.4
GOTERM_MF_DIRECT	ATPase activity	RT	4 7.5
GOTERM_MF_DIRECT	large ribosomal subunit rRNA binding	RT	2 3.8
Category	Term	Count	%
REACTOME_PATHWAY	Gluconeogenesis	RT	5 9.4
REACTOME_PATHWAY	Glycolysis	RT	4 7.5
REACTOME_PATHWAY	Peptide chain elongation	RT	5 9.4
REACTOME_PATHWAY	L13a-mediated translational silencing	RT	5 9.4
REACTOME_PATHWAY	GTP hydrolysis and joining of the 60S r	RT	5 9.4

PID

Category	Term	Count	%
GOTERM_BP_DIRECT	nucleosome assembly	RT	4 10.5
GOTERM_BP_DIRECT	defense response to Gram-positive bacteria	RT	3 7.9
GOTERM_BP_DIRECT	cellular protein metabolic process	RT	3 7.9
GOTERM_BP_DIRECT	regulation of protein ubiquitination	RT	2 5.3
GOTERM_BP_DIRECT	cell division	RT	4 10.5
Category	Term	Count	%
GOTERM_MF_DIRECT	histone binding	RT	4 10.5
GOTERM_MF_DIRECT	protein heterodimerization activity	RT	6 15.8
GOTERM_MF_DIRECT	ATP binding	RT	9 23.7
GOTERM_MF_DIRECT	GTP binding	RT	4 10.5
GOTERM_MF_DIRECT	GTPase binding	RT	2 5.3
Category	Term	Count	%
REACTOME_PATHWAY	Condensation of Prophase Chromosome	RT	6 15.8
REACTOME_PATHWAY	Amyloid fiber formation	RT	6 15.8
REACTOME_PATHWAY	Senescence-Associated Secretory Phenotype	RT	6 15.8
REACTOME_PATHWAY	RNA Polymerase I Promoter Opening	RT	5 13.2
REACTOME_PATHWAY	DNA methylation	RT	5 13.2

PID+WF

Category	Term	Count	%
GOTERM_BP_DIRECT	nucleosome assembly	RT	3 20
GOTERM_BP_DIRECT	innate immune response in mucosa	RT	2 13.3
GOTERM_BP_DIRECT	regulation of cyclin-dependent protein	RT	2 13.3
GOTERM_BP_DIRECT	antibacterial humoral response	RT	2 13.3
GOTERM_BP_DIRECT	endosomal transport	RT	2 13.3
Category	Term	Count	%
GOTERM_MF_DIRECT	protein binding	RT	14 93.3
GOTERM_MF_DIRECT	protein heterodimerization activity	RT	4 26.7

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2	GOTERM_MF_DIRECT	pyridoxal phosphate binding	RT	2		13.3
3	GOTERM_MF_DIRECT	protein dimerization activity	RT	4		26.7
4	GOTERM_MF_DIRECT	DNA binding	RT	5		33.3
5	Category	Term		Count	%	
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7	REACTOME_PATHWAY	DNA Damage/Telomere Stress Induced	RT	5		33.3
8	REACTOME_PATHWAY	Senescence-Associated Secretory Ph	RT	5		33.3
9	REACTOME_PATHWAY	Packaging Of Telomere Ends	RT	4		26.7
10	REACTOME_PATHWAY	DNA Polymerase I Promoter Opening	RT	4		26.7
11	REACTOME_PATHWAY	DNA methylation	RT	4		26.7
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For Peer Review

<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
2.10E-07	9.30E-05	9.20E-05
6.50E-05	1.40E-02	1.40E-02
1.50E-04	2.10E-02	2.10E-02
1.90E-04	2.10E-02	2.10E-02
7.90E-04	7.00E-02	6.90E-02
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
4.10E-06	8.50E-04	8.50E-04
1.40E-03	1.40E-01	1.40E-01
4.50E-03	3.10E-01	3.10E-01
1.80E-02	8.50E-01	8.50E-01
2.10E-02	8.50E-01	8.50E-01
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
1.30E-05	2.40E-03	2.30E-03
4.20E-04	3.80E-02	3.80E-02
7.90E-04	4.90E-02	4.80E-02
1.80E-03	7.00E-02	6.80E-02
1.90E-03	7.00E-02	6.80E-02
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
2.30E-03	5.50E-01	5.50E-01
1.50E-02	1.00E+00	1.00E+00
2.80E-02	1.00E+00	1.00E+00
3.50E-02	1.00E+00	1.00E+00
4.10E-02	1.00E+00	1.00E+00
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
2.20E-03	1.70E-01	1.70E-01
2.90E-03	1.70E-01	1.70E-01
1.20E-02	4.70E-01	4.70E-01
4.80E-02	1.00E+00	1.00E+00
5.40E-02	1.00E+00	1.00E+00
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
1.80E-06	2.10E-04	1.80E-04
7.30E-06	4.30E-04	3.50E-04
1.30E-05	5.00E-04	4.10E-04
2.80E-05	5.60E-04	4.60E-04
3.20E-05	5.60E-04	4.60E-04
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
4.30E-03	6.30E-01	6.30E-01
2.10E-02	1.00E+00	1.00E+00
3.20E-02	1.00E+00	1.00E+00
3.60E-02	1.00E+00	1.00E+00
5.40E-02	1.00E+00	1.00E+00
<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
3.00E-03	2.70E-01	2.70E-01
4.80E-03	2.70E-01	2.70E-01

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2	4.30E-02	1.00E+00	1.00E+00
3	5.50E-02	1.00E+00	1.00E+00
4	3.30E-02	7.60E-01	7.60E-01
5	<u>P-Value</u>	<u>Benjamini</u>	<u>FDR</u>
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7	5.20E-07	4.00E-05	3.00E-05
8	4.10E-06	1.60E-04	1.20E-04
9	2.10E-05	4.70E-04	3.40E-04
10	3.70E-05	4.70E-04	3.40E-04
11	4.10E-05	4.70E-04	3.40E-04
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