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► To cite this version:

Rima Souki, Jérémy Amosse, Valentine Genet, Morgane Le Gall, Benjamin Saintpierre, et al.. Small RNA-sequencing reveals the involvement of microRNA-132 in benzo[a]pyrene-induced toxicity in primary human blood cells. *Environmental Pollution*, 2023, 328, pp.121653. 10.1016/j.envpol.2023.121653 . hal-04099863

HAL Id: hal-04099863

<https://hal.science/hal-04099863>

Submitted on 23 May 2023

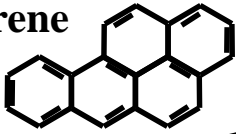
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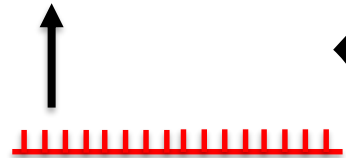


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Polycyclic Aromatic Hydrocarbons

Benzo[*a*]pyrene

CYP1A1/1B1



miR-132

APOPTOSIS

primary human
PBMC

Exposure to polycyclic aromatic hydrocarbons, like benzo[*a*]pyrene, induces miRNA expression changes associated with biological processes relevant to apoptosis in human primary PBMCs.

1 **Small RNA-sequencing reveals the involvement of microRNA-132 in benzo[*a*]pyrene-**
2 **induced toxicity in primary human blood cells**

3
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20
21 Abbreviations: AhR, Aryl Hydrocarbon Receptor; AhRR, AhR Repressor; anti-miR-CTR, miR
22 inhibitor control; anti-miR-132, miR-132 inhibitor; B[*a*]P, benzo[*a*]pyrene ; CYPs,
23 cytochromes P-450; CTP-I, industrial Coal Tar Pitch; CTP-S, synthetic Coal Tar Pitch; DMSO,
24 dimethylsulfoxide; FDR, False Discovery Rate ; IPA, Ingenuity Pathway Analysis; miRNAs,
25 microRNAs; PAHs, Polycyclic Aromatic Hydrocarbons; PBMCs, Peripheral Blood

26 Mononuclear Cells; PYR, pyrene; RNA-seq, RNA sequencing; TIPARP, TCDD Inducible

27 Poly(ADP-Ribose) Polymerase; UTR, untranslated region; Z-VAD, N-benzyloxycarbonyl-

28 Val-Ala-Asp(O-Me) fluoromethyl ketone

29

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30 **Abstract**

31 Polycyclic aromatic hydrocarbons (PAHs) are widely distributed environmental contaminants,
32 triggering deleterious effects such as carcinogenicity and immunosuppression, and peripheral
33 blood mononuclear cells (PBMCs) are among the main cell types targeted by these pollutants.
34 In the present study, we sought to identify the expression profiles and function of miRNAs,
35 gene regulators involved in major cellular processes recently linked to environmental
36 pollutants, in PBMC-exposed to the prototypical PAH, benzo[*a*]pyrene (B[*a*]P). Using small
37 RNA deep sequencing, we identified several B[*a*]P-responsive miRNAs. Bioinformatics
38 analyses showed that their predicted targets could modulate biological processes relevant to
39 cell death and survival. Further studies of the most highly induced miRNA, miR-132, showed
40 that its up-regulation by B[*a*]P was time- and dose-dependent and required aryl hydrocarbon
41 receptor (AhR) activation. By evaluating the role of miR-132 in B[*a*]P-induced cell death, we
42 propose a mechanism linking B[*a*]P-induced miR-132 expression and cytochromes P-450
43 (CYPs) 1A1 and 1B1 mRNA levels, which could contribute to the apoptotic response of
44 PBMCs. Altogether, this study increases our understanding of the roles of miRNAs induced by
45 B[*a*]P and provides the basis for further investigations into the mechanisms of gene expression
46 regulation by PAHs.

47

48

49 **Keywords:** Polycyclic Aromatic Hydrocarbons, Peripheral Blood Mononuclear Cells,
50 microRNAs, Benzo[*a*]pyrene, miR-132-3p, Aryl Hydrocarbon Receptor, Cytochromes P-450
51 1A1 and 1B1

52

53

54 **1. Introduction**

55 Polycyclic aromatic hydrocarbons (PAHs) are a major class of environmental contaminants
56 formed by the incomplete combustion of organic materials, and distillation of coal and
57 petroleum. Human are commonly exposed to these pollutants present in large amounts in food,
58 air pollution, cigarette smoke, and some occupational atmospheres (Mallah et al., 2022). Human
59 exposure to these pollutants has been correlated to various pathologies such as cancer,
60 immunosuppression, and inflammation contributing to cardiovascular and pulmonary diseases
61 (Holme et al., 2019; Stading et al., 2021; Yu et al., 2022). While several particulate PAHs are
62 classified as probable or possible carcinogens by the International Agency for Research on
63 Cancer, benzo[*a*]pyrene (B[*a*]P) is the only PAH classified as a known human carcinogen
64 (International Agency for Research on Cancer, 2010), and the most widely used model
65 compound for studying the effects of PAHs. Most of the B[*a*]P-related toxic effects have been
66 linked to the activation of the aryl hydrocarbon receptor (AhR) and its subsequent binding to
67 specific xenobiotic responsive elements within the promoter of responsive genes such as
68 cytochromes P450 (CYPs) 1A1, 1A2 and 1B1 (Fujii-Kuriyama & Mimura, 2005). We and
69 others showed that activation of the AhR signalling pathway by B[*a*]P produces systemic
70 changes in gene expression contributing in a major way to its toxic effects, especially
71 carcinogenesis in several organs such as lung, liver and lymphoid tissue (Hockley et al., 2006;
72 Sparfel et al., 2010; Liamin et al., 2017, 2018).

73 MicroRNAs (miRNAs) are short (20 to 23 nucleotides long) non-coding RNAs that
74 regulate gene expression at the post-transcriptional level. Typically, they bind to the 3'-UTR
75 (untranslated region) of target mRNAs, leading to suppression of translation and/or mRNA
76 degradation (Bartel, 2009). Through multiple transcript targets, miRNAs have been shown to
77 regulate many aspects of biology such as stress responses, cell death, or carcinogenesis
78 (Gulyaeva & Kushlinskiy, 2016). More recent investigations have examined the relationship

79 between environmental contaminants and miRNA expression (Tumolo et al., 2022). Growing
80 evidence linking miRNAs to pollutants, associated with their regulatory role in gene expression,
81 suggests that miRNAs can serve not only as potential biomarkers of exposure but also
82 contribute to the mechanisms of diseases caused by environmental contaminants. Indeed,
83 miRNA alterations after PAH exposure in humans identified patterns that could be responsible
84 for various health outcomes (Deng et al., 2019; Ruiz-Vera et al., 2019).

85 Peripheral blood mononuclear cells (PBMCs), an easily obtainable blood cell fraction
86 mainly composed of lymphocytes and monocytes, appear to be a major cell type targeted by
87 PAHs. PBMCs are present at the entry points of these contaminants, and circulate permanently
88 through the body, thus acting as the first line of defense. In earlier studies, we have shown that
89 exposure to PAHs inhibits the differentiation of human monocytes into immunocompetent cells
90 such as macrophages (van Grevenynghe et al., 2004). Such an exposure also targets human T
91 lymphocytes, thereby altering their functional properties such as cytokine secretion in current
92 smokers (Prigent et al., 2014) or producing specific B[a]P-derived DNA damage associated
93 with an increasing frequency of mutations (Liamin et al., 2017). Overall, these PAH-induced
94 alterations may participate in the immunotoxic, pro-inflammatory or carcinogenic-related
95 effects; this underlines the importance of studying PBMCs to analyse the effect of
96 environmental pollutants to human health. Whether these effects are associated with an
97 alteration of miRNA expression in PBMCs has not been previously investigated. The present
98 study used high-throughput small RNA sequencing (RNA-seq) technology to characterize
99 miRNA changes caused by B[a]P exposure in human PBMCs. Using bioinformatics tools, we
100 also investigated the possible role of the differentially expressed miRNAs by predicting their
101 downstream target genes and analysed the major regulated signalling pathways and biological
102 functions. Further analyses showed that B[a]P increased, in a dose- and time-dependent
103 manner, the expression of miR-132-3p; that this required AhR activation and that the regulation

104 of CYP1A1/CYP1B1 balance by miR-132 could be a potential modulator of B[a]P's
105 cytotoxicity in PBMCs.

106

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107 **2. Materials and methods**

108 **2.1. Chemicals and reagents**

109 B[a]P, pyrene (PYR), dimethylsulfoxide (DMSO), Hoechst 33342, CH-223191, and N-
110 benzyloxycarbonyl-Val-Ala-Asp(O-Me) fluoromethyl ketone (Z-VAD) were obtained from
111 Merck (Darmstadt, Germany). Lipofectamine and SYTOX[®]Green were purchased from
112 Thermo Fisher Scientific (Courtaboeuf, France). PAH complex mixtures were extracted from
113 the industrial products coal tar pitch (CTP-I), and the synthetic coal tar pitch mixture (CTP-S)
114 was identically reconstituted using PAH standards as previously reported (Bourgart et al.,
115 2019); PAH profiles in CTP-I are indicated in Supplementary materials and methods (Table
116 S1). The applied dose was adjusted to 10 nM and 2 μ M of B[a]P for CTP-I and CTP-S.

117 **2.2. Cell culture and treatment**

118 PBMCs were isolated from buffy coats collected from human blood donor who provided
119 written informed consent for the research protocol according to the regulation for blood
120 transfusion of the French blood organization Etablissement Français du Sang, Rennes (France),
121 AC-2019-3853 by Ficoll (Eurobio, Les Ulis, France) gradient centrifugation (Liamin et al.,
122 2017, 2018). They were then cultivated at 37°C with 5% CO₂ in RPMI medium (Eurobio)
123 supplemented with 20 IU/mL penicillin, 20 μ g/mL streptomycin, and 10% decompemented
124 fetal calf serum (Lonza, Bale, Swiss) and exposed to PAHs, stocked in DMSO. The final
125 concentration of DMSO in the culture medium was always < 0.2% v/v and control cultures
126 received the same dose of DMSO as treated cultures.

127 **2.3. RNA isolation**

128 Parallel isolation of small and large RNA from PBMCs was performed using the NucleoSpin
129 RNAs kit (Macherey-Nagel, Düren, Germany) with minor changes, and were quantified as
130 detailed in Supplementary materials and methods.

131 **2.4. RT-qPCR assays**

132 The reaction is performed on total RNA (1 µg), reverse-transcribed into cDNA using the RT
133 Applied Biosystems kit (Thermo Fischer Scientific). For qPCR assays, gene-specific primers
134 from Eurogentec (Seraing, Belgium) were used as previously described (Liamin et al., 2018).

135 The small RNA fraction (5 ng) was reverse-transcribed using an adapter-based TaqMan
136 Advanced miRNA cDNA Synthesis kit (Thermo Fischer Scientific) with a preamplification
137 step according to the manufacturer's protocol. For qPCR assays, predesigned probe-based
138 TaqMan Advanced miRNA Assays and TaqMan Fast Advanced Master Mix (Thermo Fisher
139 Scientific) were used. For qPCR validation, we spiked the samples with an exogenous miRNA
140 (miR-39) for normalization before RNA extraction, and used it as the reference miRNA to
141 control technical variability as recommended (Hu et al., 2020).

142 **2.5. Western blotting**

143 Total cellular protein extracts were prepared by lysis of PBMCs in a buffer containing 50 mM
144 Tris-HCl, pH 8, 5 mM EDTA, 150 mM NaCl, 1% Nonidet-40, 0.5% sodium desoxycholate, 1
145 mM sodium fluoride, 2 mM dithiothreitol, 1 mM sodium orthovanadate and 1 mM
146 phenylmethanesulphonyl fluoride, supplemented with a cocktail protease inhibitor (Roche
147 Diagnostics, Meylan, France).

148 Protein separation was performed on a polyacrylamide gel followed by electrophoresis
149 transfer onto nitrocellulose membranes (Merck Millipore, Burlington, MA, USA). After
150 blocking, membranes were incubated with a mouse monoclonal antibody to human Dicer
151 (ab14601) (Abcam, Cambridge, UK) or with a rabbit anti-human PUMA antibody (Cell
152 Signaling Technology, Danvers, MA, United States). A peroxidase-conjugated antibody was
153 next used as secondary antibody and immunolabelled proteins were visualized by
154 chemoluminescence using a gel imaging system (Bio-Rad) equipped with a CCD camera.
155 Protein amounts were quantified by densitometry using Image Lab™ software (Bio-Rad) and

156 normalized using HSC70 (Santa Cruz Biotechnology (Heidelberg, Germany) as a loading
157 control.

158 **2.6. Small RNA-seq**

159 **2.6.1. Library preparation and small RNA-seq**

160 To construct the libraries, 12 independent PBMC cultures each grown in untreated (DMSO)
161 and 2 μ M B[a]P-treated conditions for 48 h, were combined in 3 equal pools (with 4 separate
162 blood donors *per* pool) for each condition. Due to the biological variability between donors
163 (Sparfel et al., 2010; Liamin et al., 2018), we pooled the 12 cultures in 3 equal pools, either
164 with or without B[a]P exposure, prior to isolate 5-15 ng of the small RNA fraction and
165 processed using Qiaseq miRNA library prep kit (Qiagen, Hilden, Germany) according to the
166 manufacturer's instructions and as detailed in Supplementary materials and methods.
167 Sequences obtained in the study have been deposited in GenBank with accession numbers
168 GSE222837. After filtering low-quality reads and adaptor sequences, a total of $3251955 \pm$
169 468873 and 2604815 ± 227816 miRNA reads were obtained for the untreated (DMSO) and
170 B[a]P-treated PBMCs, respectively (Tables S3 and S4).

171 **2.6.2. Small RNA-seq data processing and mapping**

172 Fastq files were aligned using the QIAGEN pipeline (QIAseq® miRNA Primary
173 Quantification). The number of differentially expressed miRNAs between the two conditions
174 were determined with the DESeq2 R package (DESeq2_1.26.0). The standard DESeq2
175 normalization method (DESeq2's median of ratios with the DESeq function) was used, with a
176 pre-filter of reads and genes (reads uniquely mapped on the genome, or up to 10 different loci
177 with a count adjustment, and genes with at least 10 reads in at least 3 different samples).
178 Following the package recommendations, the Wald test was used with the contrast function and
179 the Benjamini-Hochberg False Discovery Rate (FDR) control procedure to identify
180 differentially expressed genes.

181 **2.7. Functional analysis**

182 Lists of miRNAs significantly induced or repressed after B[a]P exposure were analysed
183 through the use of Ingenuity Pathway Analysis (IPA) software (QIAGEN Inc.,
184 <https://www.qiagenbioinformatics.com/products/ingenuity-pathway-analysis> v. 73620684).
185 Significantly over-represented functions were identified with a right-tailed Fisher's Exact Test
186 that calculates an overlap *p-value* determining the probability that each term associated with
187 our list of differential miRNAs was due to chance alone.

188 **2.8. miRNA transfection**

189 To study the functions of miR-132, PBMCs were transfected with 8.5×10^{-9} M miR-132
190 inhibitor (anti-miR-132) or miR inhibitor control (anti-miR-CTR) (Thermo Fisher Scientific)
191 using lipofectamine. Twelve hours later, PBMCs were exposed to 2 μ M B[a]P for 48 h.

192 **2.9. Cell death evaluation**

193 PBMCs were tested for both apoptotic and necrotic cell death, as previously reported
194 (van Meteren et al., 2019). Apoptotic cells were identified by chromatin condensation and
195 morphological changes of the nucleus using the blue-fluorescence chromatin dye Hoechst
196 33342; in parallel, necrotic cells were evidenced by the SYTOX[®]Green nucleic acid stain, only
197 permeant to cells with compromised plasma membranes. Briefly, untreated (DMSO) and PAH-
198 treated PBMCs were stained at 37 °C with 10 μ g/ml Hoechst 33342 and 125 μ M
199 SYTOX[®]Green for 15 minutes. Apoptotic and necrotic cells were then manually counted using
200 a fluorescence microscope (Axio Scope A1, ZEISS, Marly le Roi, France); a total of 300 nuclei
201 were examined for each condition (magnification x40).

202 **2.10. Statistical analysis**

203 Data are expressed as mean \pm SEM from at least 3 independent experiments. Statistical
204 significance was assessed using GraphPad InStat (GraphPad Software, INC., La Jolla, CA,

205 USA) by paired Student's t-test, or analysis of variance followed by Dunnett or Student-

206 Newman-Keuls post hoc tests. A *p-value* ≤ 0.05 was considered significant.

207

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208 3. Results

209 3.1. Identification of B[a]P-regulated miRNAs in human PBMCs using high throughput 210 small RNA-seq

211 We isolated primary human PBMCs from healthy blood donors and treated them with vehicle
212 (DMSO) or with the prototypical PAH, B[a]P, at previously and commonly chosen
213 concentrations for *in vitro* toxicity studies of 10 nM and 2 μ M (Liamin et al., 2017) for 48 h.
214 The expression of four well-known AhR-target genes (*CYP1A1*, *CYP1B1*, AhR Repressor
215 (*AhRR*), and TCDD Inducible Poly(ADP-Ribose) Polymerase (*TIPARP*)) was analysed in
216 PBMCs by RT-qPCR. As expected (Hockley et al., 2006; Sparfel et al., 2010), a 48-h exposure
217 to 2 μ M B[a]P was found to up-regulate the mRNA expression of these AhR-target genes, with
218 statistically significant induction of *CYP1A1* and *CYP1B1* while no significant difference was
219 observed in PBMCs treated with 10 nM B[a]P (Figure 1A). We then investigated the effect of
220 2 μ M B[a]P on the expression of genes and proteins involved in the miRNA processing
221 machinery. As shown in Figure 1B, *DROSHA*, *DCRG8*, *XPO5*, *DICER* and *AGO2* mRNA
222 expression was not affected. Consistent with the unaltered mRNA levels, protein levels for
223 *DICER* were not significantly increased in the presence of B[a]P (Figure 1C). Taken together,
224 these data indicate that primary human PBMCs fully responded to a 2 μ M B[a]P 48 h exposure,
225 and possess components of the miRNA machinery unaltered by such exposure, allowing an
226 analysis of global changes in miRNA profiles in these conditions.

227 To identify differentially expressed miRNAs in response to B[a]P exposure, we performed deep
228 sequencing of small RNAs extracted from PBMCs from 12 independent donors cultured in the
229 presence of 2 μ M B[a]P or DMSO for 48 h. As shown in Figure 2A, B[a]P treatment did not
230 significantly alter the proportion of miRNA reads which accounted for approximately 28% and
231 25% of the total number of reads for DMSO and B[a]P-treated samples, respectively. Overall,
232 we detected 765 mature known miRNAs expressed with a base mean of reads > 10 in at least

233 one of the two conditions (Table S5), with 436 (57%) shared by the two conditions as shown
234 by the Venn diagram (Bardou et al., 2014) (Figure 2B). By using the principal component
235 analysis on the top 500 miRNA reads, we revealed distinct clusters between untreated (DMSO)
236 and B[a]P-treated PBMCs (Figure S1). After filtering with an adjusted p -value ≤ 0.05 and a
237 $|\log_2\text{FoldChange}| \geq 0.4$ or ≤ -0.4 , 285 miRNAs showed differential expression after B[a]P
238 treatment compared with the DMSO control with 65 up-regulated and 220 down-regulated
239 miRNAs (Figure 2C, Table S6). Using as a cutoff miRNAs with more than ≥ 100 read counts
240 in at least one of the 6 conditions, 15 miRNAs whose expression was altered upon B[a]P
241 treatment were detected, including 4 up-regulated miRNAs (miR-132-3p, miR-1246, miR-
242 3069, and miR-320c) and 11 down-regulated miRNAs (miR-221-3p, miR-23b-3p, miR-454-3p,
243 miR-7-5p, miR-30c-5p, miR-142-3p, miR-144-5p, miR-301a-3p, miR-4515, miR-1909-5p,
244 miR-6815-5p) (Figure 2E). The heatmap (Figure 2D) indicated that these differentially-
245 regulated miRNAs were well clustered into 2 classes defining distinct expression patterns
246 between untreated (DMSO) and B[a]P-treated PBMCs.

247

248 **3.2. Functional analysis and target gene prediction of B[a]P-regulated miRNAs in human** 249 **PBMCs**

250 To explore the over-represented functions in which these 15 B[a]P-regulated miRNAs are
251 involved, we used IPA software. Cellular development, growth/proliferation and
252 death/survival-related functions were 3 of the top 5 functions altered in response to B[a]P
253 treatment (Table 1). The use of the TAM 2.0 tool to perform functional annotations of B[a]P-
254 regulated miRNAs by over-representation analysis also reported an enrichment in miRNAs
255 contributing to cell death and apoptosis (Table S7).

256 Since the functional enrichment analysis of miRNAs is limited by current miRNA
257 functional annotations, we performed a target gene prediction of the 15 differentially-regulated

258 miRNAs using the four analysis online tools, TargetScan, FunRich, miRDIP, and miRWALK.
259 We thus identified 313 common genes (Figure S2A). IPA software was then used to analyse
260 the most significant diseases and disordered biological functions linked to these genes. As
261 shown in Table S8, the most significant disease was cancer, the most significant molecular and
262 cellular function was linked to function and maintenance, and organismal survival appeared as
263 the most significant category in physiological development and system function. Using the
264 online prediction tool WebGestalt, we then performed an identification of enriched categories
265 based on a $FDR \leq 0.05$ for the 313 predicted target genes. Interestingly, the GO biological
266 process analysis showed that these genes are involved in the negative regulation of intrinsic
267 apoptotic signalling pathway to DNA damage, and in the regulation of cellular senescence
268 (Figure S2B). The KEGG analysis also identified autophagy among the five most significant
269 categories selected on *p-value* (Figure S2C). Altogether, these results indicate that the
270 differentially-regulated miRNAs and their predicted genes may participate in regulation of the
271 balance between cell survival and death.

272

273 **3.3. Validation of B[a]P-regulated miRNAs and associated biological pathways in human** 274 **PBMCs by RT-qPCR**

275 We then performed RT-qPCR assays using cultures of independent PBMC samples, in order to
276 confirm the transcriptional changes of miRNAs identified by the RNA-seq analysis. From the
277 list of the 15 most differentially-regulated miRNAs, we selected 8 miRNAs (miR-132-3p, miR-
278 1246, miR-3609, miR-221-3p, miR-454-3p, miR-7-5p, miR-30c-5p, miR-142-3p) that
279 demonstrated the highest reads and depending on TaqMan probes available for RT-qPCR
280 validation. As shown in Figure 3A, all the miRNAs examined show a trend consistent with the
281 miRNA-seq results. We detected levels of up-regulation for miR-132, miR-1246, miR-3609
282 and lower levels for miR-221, miR-454, miR-7, miR-30c, miR-142 in B[a]P-treated PBMCs

283 (Figure 3A). However, results of RNA-seq data were more pronounced than those obtained by
284 RT-qPCR results; indeed, regarding these latter results, the effect of B[a]P was only statistically
285 significant for miR-132 (Figure 3A). Due to its highest change in expression and the robustness
286 of its analysis, miR-132 was selected for further investigations. To further evaluate the impact
287 of B[a]P on miR-132 expression, PBMCs were exposed to B[a]P at increasing concentrations
288 and times. We showed that miR-132 increased with B[a]P concentration with a significant
289 effect from 2 μ M compared to DMSO-controls (Figure 3B). Time-course of its induction in
290 PBMCs exposed to 2 μ M B[a]P showed that miR-132-3p miRNA levels increased over time
291 and became significantly up-regulated at 48 h (Figure 3C).

292 Due to the ontology analysis, and since B[a]P is known to initiate cell death in different
293 cell types (Hardonnière et al., 2017), we evaluated whether B[a]P caused apoptosis, necrosis,
294 and/or autophagy in PBMCs. Using Hoechst 33342 and SYTOX[®]Green co-staining, we first
295 observed that the number of apoptotic PBMCs increased with B[a]P concentrations and was
296 significant at 2 μ M B[a]P after a 48 h-exposure (Figure 4A) with several apoptotic
297 morphological features such as chromatin condensation and DNA fragmentation (magnified
298 images on the right panel, Figure 4A). By contrast, B[a]P treatment did not cause any significant
299 changes in the number of necrotic cells (Figure 4A). Additionally, analysis by RT-qPCR
300 experiments revealed that the expression of several genes involved in the regulation of the
301 apoptotic signalling pathway in response to DNA damage by p53 were increased after treatment
302 with 2 μ M B[a]P for 48 h (Figure 4B). A significant increase was notably found for *DDB2*,
303 which plays a central role in defining the response to DNA damage as well as for the pro-
304 apoptotic genes *BBC3* and *BID* (Figure 4B). In agreement with mRNA expression, protein
305 levels of PUMA encoded by the *BBC3* gene were also significantly increased after treatment
306 with 2 μ M B[a]P for 48 h (Figure 4C). Next, we examined cell autophagy by acridine orange
307 staining and flow cytometry to assess acidic vacuoles, that increase upon autophagy induction

308 (Thomé et al., 2016). We found that the percentage of acridine orange-stained cells was not
309 significantly altered upon B[a]P treatment (Figures S3A and S3B). Furthermore, treatment with
310 2 μ M B[a]P for 48 h did not significantly alter mRNA levels of typical markers of autophagy
311 such as *ATG5*, *ATG7*, *ATG12*, and *BECLIN1* (Figure S3C) or the protein LC3 conversion
312 (LC3B-I to LC3B-II) (Figure S3D) (Mizushima & Yoshimori, 2007). Finally, given the
313 association of oxidative stress with cell death/survival, we used the oxidative-sensitive
314 H2DCFDA probe and flow cytometry to detect generation of reactive oxygen species in
315 PBMCs exposed to B[a]P for 48 h. As shown in Figure S4A, a 48-h treatment with 10 nM or
316 2 μ M B[a]P did not modify the production of reactive oxygen species. However, we observed
317 alterations in the expression of markers linked with the oxidative machinery; we thus found a
318 significantly decreased mRNA expression of *SOD2* mRNA and *HMOX1*/HO-1 mRNA and
319 protein levels after B[a]P treatment with 2 μ M for 48 h (Figures S4B and S4C), therefore
320 suggesting that an early oxidative stress probably could contribute to B[a]P-induced apoptosis
321 as previously described (Huc et al., 2007).

322

323 **3.4. Role of miR-132 in B[a]P-induced cell death in human PBMCs**

324 In the next set of experiments, we first investigated the involvement of the apoptosis induced
325 by B[a]P in miR-132 expression using Z-VAD, an apoptosis inhibitor (Fearnhead et al., 1995;
326 van Meteren et al., 2019). Although Z-VAD prevented the increase in the number of apoptotic
327 cells (Figure 5A, right panel), it did not change the B[a]P-induced miR-132 expression (Figure
328 5A), thus indicating that this was not mediated by apoptosis. We next examined the effects of
329 the potent AhR antagonist CH-223191 (Zhao et al., 2010), inhibiting B[a]P-induced CYP1A1
330 and CYP1B1 mRNA levels in PBMCs (data not shown) on the miR-132 up-regulation.
331 Interestingly, CH-223191 strongly inhibited miR-132-induced expression in B[a]P-treated
332 PBMCs (Figure 5B); it also significantly prevented the induction of apoptosis in B[a]P-treated

333 PBMCs (Figure 5B, right panel). We thereafter analysed the effects of several AhR activators
334 on miR-132. PYR, known as a weak activator of AhR (Boonen et al., 2020), failed to increase
335 miR-132 levels (Figure 5C). By contrast, realistic and synthetic CTPs, adjusted at
336 concentrations of 10 nM and 2 μ M B[a]P and containing 17.3 nM and 3.46 μ M PYR
337 respectively, markedly increased miR-132 expression in PBMCs (Figure 5D). Interestingly,
338 PYR, did not significantly induce apoptosis whereas real and synthetic PAH mixtures
339 significantly increased apoptotic PBMCs (Figures 5C and 5D, right panels). Altogether, these
340 data indicate that B[a]P-induced AhR activation is necessary to increase miR-132 levels and
341 cause apoptosis in PBMCs at a 48 h-exposure.

342 We further analysed the role of miR-132 on B[a]P-induced effects in human PBMCs.
343 Firstly, we explored the effects of miR-132 knockdown on B[a]P-induced apoptosis by
344 transfecting PBMCs with an anti-miR-132 or with an anti-miR-CTR. Transfection and
345 knockdown efficiencies were confirmed by measuring miR-132 expression levels in PBMCs
346 (Figure S5A). The results showed that transfection of the anti-miR-132 significantly mitigated
347 the number of B[a]P-induced apoptotic PBMCs compared to PBMCs transfected with the anti-
348 miR-CTR (Figure 6A). Interestingly, RT-qPCR analysis showed that transfection with the anti-
349 miR-132 in B[a]P-treated PBMCs did not alter the mRNA expression of pro-apoptotic and anti-
350 oxidative genes such as *BBC3* and *Bcl2* (Figure 6B) and of *SOD2* and *HMOX1*,
351 respectively (Figure S5B). Since we observed a strong decrease in expression of AhR target
352 genes, *CYP1A1* and *CYP1B1* associated with the increased miR-132 expression over 48 h
353 (Figure 6C), we explored a potential regulatory role for miR-132 in the AhR signalling pathway
354 and activation of its downstream genes. Interestingly, transfection with the anti-miR-132
355 increased B[a]P-induced, although not significantly, *CYP1A1* mRNA levels, and significantly
356 decreased those of *CYP1B1* (Figure 6D), while it did not modify *AhR* or *ARNT* expression when
357 compared to B[a]P-treated PBMCs transfected with the anti-miR-CTR (Figure 6E). Altogether,

358 these data suggest an effect of B[a]P on the CYP1A1/CYP1B1 balance, which could be
359 associated with its induction of apoptosis in PBMCs.

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361 Discussion

362 miRNAs play major roles in the regulation of responses to environmental stresses and pollutant
363 exposures. Alteration of their expression can influence a variety of cellular functions, which
364 justifies investigating their expression profiles to understand toxicological responses of various
365 cell types. Among ubiquitous pollutants, PAHs, especially the prototypical B[a]P, exert various
366 toxic effects, mainly associated with AhR activation and the subsequent induction of CYP1-
367 metabolizing enzymes (Shimada, 2006). We have previously characterized B[a]P-induced
368 alterations and their involvement in immunotoxic, pro-inflammatory or carcinogenic effects
369 using primary human blood cells (Liamin et al., 2017; Prigent et al., 2014; van Grevenynghe et
370 al., 2004). However, the key questions of how miRNA expression is regulated and what the
371 roles of dysregulated miRNAs are in B[a]P-exposed human blood cells, remain to be
372 elucidated. In the present study, primary human PBMCs representing normal non-transformed
373 human cells that express all the miRNA processing machinery (Beer et al., 2014), were used as
374 a model system to assess the effect of B[a]P on the expression of miRNAs.

375 Recent advances in next-generation sequencing technologies have enabled the
376 development of comprehensive and robust approaches such as small RNA-seq to quantify
377 miRNA expression genome-wide. To our knowledge, this is the first study using the high-
378 throughput small RNA-seq method to profile B[a]P-regulated miRNAs, which allowed us to
379 identify them as potential novel modulators of the effects of B[a]P, on human PBMCs. We
380 observed that B[a]P altered the expression of 285 miRNAs, most of them being down-
381 regulated. This down-regulation has previously been described, notably the effects of PAHs
382 and cigarette smoke on miRNA profiles in humans have been reported (De Flora et al., 2012;
383 Deng et al., 2014). By selecting highly expressed miRNAs, we focused on 15 miRNAs whose
384 expression is altered in B[a]P-treated PBMCs. Some of these miRNAs have already been
385 associated with B[a]P treatment such as miR-221 (recently proposed as a genetic marker for

386 bladder cancer for which PAH exposure is a risk factor; Martin-Way et al., 2022), miR-30c
387 regulated by B[a]P exposure (Wu et al., 2019) or miR-142, a PAH-responsive marker in
388 epidemiological studies (Rani, 2021). In the context of realistic environmental exposure to
389 which a human may be exposed, a number of such miRNA signatures have been described, for
390 example, miR-30c and miR-142 for cigarette smoke, and the miR-144 for air pollution by
391 diesel particles (Vrijens et al., 2015). The miR-132 also appeared regulated under human
392 exposure to cigarette smoke (Donate et al., 2021), our use of an occupational mixture of coal
393 tar reinforces this data in a real exposure situation. Interestingly, our *in silico* analysis indicated
394 that these 15 differentially-expressed miRNAs and their predicted targets could be enriched in
395 link with various functions, including well-documented B[a]P-regulated processes and
396 signalling pathways. Indeed, IPA analysis revealed the prominence of biological categories
397 involved in apoptosis and autophagy processes essential to cell death and survival, which we
398 have previously been reported to be associated with B[a]P exposure (Lagadic-Gossmann et al.,
399 2019). Analysis of signalling pathways activated by B[a]P-regulated miRNAs and their targets
400 genes such as *MDM2* and *DDB2* support involvement of PI3K-Akt pathway and DNA damage
401 response. DNA damage-induced Akt activation provides an opportunity for cells to survive and
402 repair DNA damage as we reported (Liamin et al., 2017). The consequence of DNA damage
403 depends on the balance between death and survival signals even if the molecular basis
404 underlying the balance between death and survival remains to be clarified under such our
405 experimental conditions. Our experimental data confirmed that exposure of PBMCs to B[a]P
406 induces cell apoptosis, supporting the hypothesis that miRNAs altered by B[a]P might be
407 implicated in the cellular alterations caused by this pollutant in PBMCs. Subtle regulation of
408 miRNAs has been described as able to significantly inhibit different individual key targets to
409 modify biological responses (Flynt & Lai, 2008). For example, miR-221, known to promote
410 cell growth and protect cell from apoptosis (Liu et al., 2020; Xie et al., 2018), appeared weakly

411 down-regulated in B[a]P-treated PBMCs whereas its pro-apoptotic target *BBC3/PUMA* was
412 up-regulated at both mRNA and protein levels. A down-regulation of miR-142, leading to
413 changes in gene expression associated with an increase in B[a]P-DNA adducts in lung and liver
414 tissues, has also been reported (Halappanavar et al., 2011).

415 In the present study, we focused on miR-132 as it was the only miRNA whose altered
416 expression was validated by RT-qPCR. When we used TaqMan advanced probe-based
417 technology to validate miRNAs on independent PBMC samples, we did not statistically
418 validate by RT-qPCR the changes in expression of the 15 miRNAs identified by RNA-seq.
419 Although the consistency of these two techniques has been described (Yagi et al., 2017),
420 discrepancies have also been reported leading to a low percentage of validation by RT-qPCR
421 (Chettimada et al., 2020; Ogando et al., 2016). This could be due not only to a difference in the
422 specificity between targeted qPCR *versus* whole library amplification of RNA-seq, but also to
423 a difference in the approaches used for data normalization. Indeed, non-variable small RNAs
424 have not been proposed for normalization in qPCR as there is no standard strategy (Prieto-
425 Fernández et al., 2019). Nevertheless, the trends in the qPCR quantification of selected
426 miRNAs was consistent with the RNA-seq results, supporting the validity of the sequencing
427 data.

428 Interestingly, the most induced miRNA, the miR-132, is a highly conserved miRNA
429 belonging to the cluster miR212/132 associated with pleiotropic roles in multiple cell types and
430 different biological processes such as neurological development, inflammation, angiogenesis
431 and cancer (Wanet et al., 2012). miR-132 has also been described as regulated in several
432 immune contexts such as experimental autoimmune encephalomyelitis (Hanieh & Alzahrani,
433 2013), in patients suffering from arthritis (Donate et al., 2021) or multiple sclerosis (Sağır et
434 al., 2021). Regarding its transcriptional regulation, miR-132 appears typically controlled by the
435 cAMP-response element binding transcription factor (Wanet et al., 2012), but in inflammatory

436 contexts, recent evidences demonstrated that AhR activation is implicated in the regulation of
437 miR-132 expression (Hanieh & Alzahrani, 2013; Abdullah et al., 2019; Donate et al., 2021).
438 Our data demonstrated the major contribution of AhR in B[a]P-induced miR-132 expression in
439 PBMCs. Indeed, realistic and synthetic CTPs with B[a]P content enhanced miR-132
440 expression, whereas the weak AhR agonist PYR exhibiting low AhR activity (Boonen et al.,
441 2020) did not significantly alter miR-132 levels. In addition, the AhR antagonist CH-223191
442 fully counteracted the induction of miR-132 expression by B[a]P, whereas the use of pifthrin-
443 α , a p53 inhibitor, also reducing B[a]P metabolism (Sparfel et al., 2006), did not change it (data
444 not shown). Our findings are consistent with the description of an AhR binding consensus motif
445 located in the miR-132 gene, as described by Hanieh et al., (2015). Despite these advances
446 indicating the involvement of AhR in the B[a]P-induced miR-132 expression independently
447 from metabolism and activation of p53 pathway, the transcriptional control of the miR-132
448 expression by AhR remains incompletely elucidated. Although the induction of miR-132 by
449 AhR has been suggested to play an important role in mediating some of the immunomodulatory
450 effects of AhR (Wanet et al., 2012), its exact role in PBMCs awaits further investigation. Since
451 PYR did not induce up-regulation of miR-132 in PBMCs and because CH-223191
452 concomitantly decreased B[a]P-triggered apoptosis and miR-132 up-regulation, we first
453 postulated a role of miR-132 in apoptosis. To ascertain this role, B[a]P-induced miR-132
454 expression was inhibited *via* an anti-miR-132 that mitigated apoptosis in B[a]P-treated PBMCs,
455 in agreement with the proposed role of miR-132 as a tumor suppressor in mantle cell lymphoma
456 (B. Wu et al., 2020). By antagonizing miR-132 in PBMCs, the mRNA expression levels of pro-
457 and anti-apoptotic genes remained unchanged while those of CYP1A1 and CYP1B1 were
458 altered. Then, we hypothesized that miR-132 might modulate the CYP1A1/CYP1B1 balance.
459 Since CYP1A1 is important for detoxication whereas CYP1B1 plays a prominent role in the
460 metabolic activation and genotoxicity of PAHs (Uno et al., 2006), we propose that modulation

461 by miR-132 in favour of CYP1B1 could be correlated to B[a]P-induced apoptosis. However,
462 the mechanism by which B[a]P-induced miR-132 regulates CYP1A1 and CYP1B1 mRNAs
463 remains to be determined. Interestingly, a negative correlation between miR-132 and CYP1A1
464 has already been described (Rieger et al., 2013). Moreover, the description of miR-132 binding
465 sites on the CYP1A1 3'UTR in the miRWALK database suggests a direct regulation of
466 CYP1A1 by miR-132 through transcript degradation. To our knowledge, no studies have so far
467 linked miR-132 to CYP1B1 but an indirect transcriptional regulation mediated by a down-
468 regulation of the SIRT1 deacetylase that regulates CYP is possible (Lee et al., 2010). A role for
469 cAMP also cannot be ruled out since we have previously reported that the B[a]P-activated β 2-
470 adrenoreceptor-dependending signalling pathway is crucial for PAH-mediated up-regulation of
471 CYP1B1 (Lagos et al., 2010; Mayati et al., 2012). We then propose a model in which B[a]P-
472 induced miR-132 mediates an increase of CYP1B1, associated to a reduction of CYP1A1
473 mRNA levels, thus potentiating the apoptotic response of PBMCs. Biological functions
474 mediated by these potential miR-132 targets are worthy of further investigations in the future,
475 especially during exposure to xenobiotics.

476

477 **Conclusion**

478 Our results contribute to a better understanding of the roles of miRNA, particularly that of miR-
479 132, in the response to B[a]P exposure in human PBMCs, and provide the foundations for
480 further investigations on the regulatory mechanisms of gene expression associated with PAH
481 exposure.

482

483 **Acknowledgments**

484 This work was supported by The French National Research Program for Environmental and
485 Occupational Health of Anses (2019/1/010). Rima Souki had a doctoral fellowship from the

486 French Ministry for Higher Education and Research and Jeremy Amosse was a post-doctoral
487 recipient from Anses. We are also thankful to the faculty of Pharmacy of Rennes, for funding
488 the extended contract of Rima Souki. We thank Dr Catherine Lavau for critical reading of the
489 manuscript and English editing. The authors are also grateful to the flow cytometry platform of
490 Biosit, University of Rennes 1 (France).

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492 **Legends of figures**493 **Fig.1: Primary human PBMCs are fully responsive to 2 μ M B[a]P exposure for 48 h and**494 **express several components of the miRNA machinery.** Primary human PBMCs were treated495 with vehicle (DMSO), with 10 nM or 2 μ M B[a]P for 48 h. (A, B) mRNA expression of AhR-

496 target genes (A) and miRNA machinery genes (B) was measured by RT-qPCR. Data are

497 expressed relative to mRNA expression levels in DMSO-treated PBMCs, arbitrarily set at 1

498 unit and are shown as mean \pm SEM of 8 (A) and 4 (B) independent experiments performed with499 PBMCs from separate blood donors tested in triplicate *per* experiment. (C) Expression of

500 DICER protein was analyzed by Western blotting. HSC70 was used as a loading control. A

501 representative blot is displayed on the right panel. Data are expressed relative to protein

502 expression levels in DMSO-treated PBMCs, arbitrarily set at 1 unit and are shown as mean \pm 503 SEM of 5 independent experiments performed with PBMCs from separate blood donors. *, $p \leq$

504 0.05 when compared with DMSO-treated PBMCs (analysis of variance followed by Dunnett's

505 multirange test) (A).

506

507 **Fig.2: Identification of B[a]P-regulated miRNAs in human PBMCs using high-**508 **throughput small RNA-seq.** Primary human PBMCs were treated with vehicle (DMSO) or 2509 μ M B[a]P for 48 h and small RNA-seq was used to profile miRNA expression. (A) Distribution

510 of percentages of differentially mapped miRNAs. (B) Venn diagram showing numbers of

511 shared and unique miRNAs expressed in PBMCs. miRNAs were identified with a base mean

512 of reads > 10 in at least one of the two conditions. (C) Volcano plot of the differentially513 expressed miRNAs with $\log_2(\text{Fold Change})$ on the x-axis and $-\log_{10}(\text{adjusted } p\text{-value})$ on the514 y-axis. Genes with an adjusted $p\text{-value} \leq 0.05$ and a $\log_2(\text{Fold Change}) \geq 0.4$ or ≤ -0.4 are

515 highlighted in bright red for the up-regulated annotations, representing a total of 65 miRNAs,

516 and bright blue for down-regulated annotations, with a total of 220 miRNAs. (D) Visual

517 heatmap representation of the top 15 miRNAs (<http://www.heatmapper.ca/expression>) showing

518 unsupervised hierarchical clustering performed on rows (miRNAs) and columns (samples)
519 using Spearman rank correlation on z-scored normalized counts. (E) Table indicating
520 differential expression of the top highest expressed 15 miRNAs significantly regulated after
521 B[a]P exposure.

522

523 **Fig.3: Selected B[a]P-regulated miRNAs identified by RNA-seq are validated by RT-**
524 **qPCR in human PBMCs.** Primary human PBMCs were treated with vehicle (DMSO) or 2 μ M
525 B[a]P for 48 h (A), with various increasing B[a]P concentrations for 48 h (B) or with 2 μ M
526 B[a]P for various increasing times (C). miRNA expression was measured by RT-qPCR. Data
527 are expressed relative to miRNA expression levels in DMSO-treated PBMCs (dotted black
528 line), arbitrarily set at 1 unit and are shown as mean \pm SEM of 12 (A), 5 (B) and 6 (C)
529 independent experiments performed with PBMCs from separate blood donors tested in
530 triplicate *per* experiment. *, $p \leq 0.05$ when compared with DMSO-treated PBMCs (paired
531 Student's *t* test (A) and analysis of variance followed by Dunnett's multirange test (B, C)).

532

533 **Fig.4: B[a]P induces apoptosis in human PBMCs.** Primary human PBMCs were treated with
534 vehicle (DMSO), with 10 nM (A) or 2 μ M B[a]P (A, B, C) for 48 h. (A) Apoptosis was
535 identified by microscopic observation (magnification x40) of the number of cells with
536 condensed and/or fragmented chromatin after nuclear staining by Hoechst 33342, and necrosis
537 was estimated by SYTOX[®]Green staining. Representative photographs are displayed on the
538 right panel with digitally magnified images (x5) indicating apoptotic features such as chromatin
539 condensation and DNA fragmentation. Data are expressed as percentages of dead cells and are
540 shown as mean \pm SEM of 5 independent experiments performed with PBMCs from separate
541 blood donors. (B) mRNA expression of genes involved in DNA damage and apoptosis was
542 measured by RT-qPCR. Data are expressed relative to mRNA expression levels in DMSO-

543 treated PBMCs, arbitrarily set at 1 unit and are shown as mean \pm SEM of 8 independent
544 experiments performed with PBMCs from separate blood donors tested in triplicate *per*
545 experiment. (C) Expression of PUMA protein was analyzed by Western blotting. HSC70 was
546 used as a loading control. A representative blot is displayed on the right panel. Data are
547 expressed relative to protein expression levels in DMSO-treated PBMCs, arbitrarily set at 1
548 unit and are shown as mean \pm SEM of 6 independent experiments performed with PBMCs from
549 separate blood donors. *, $p \leq 0.05$ when compared with DMSO-treated PBMCs (analysis of
550 variance followed by Dunnett's multirange test (A) and paired Student's *t* test (B, C)).

551

552 **Fig.5: B[a]P-induced miR-132 expression does not depend on apoptosis but on AhR**
553 **activation in human PBMCs.** miR-132 expression was measured by RT-qPCR in primary
554 human PBMCs pre-treated or not for 1 h with 10 μ M Z-VAD (A) or with 3 μ M CH-223191
555 (B) prior to a B[a]P treatment for 48 h (A, B), with the vehicle (DMSO) or 2 μ M PYR for 48 h
556 (C) or with industrial and synthetic CTP mixtures (CTP-I and CTP-S) (D). (A, B, C, D, left
557 panels) miR-132 expression was measured by RT-qPCR. Data are expressed relative to miRNA
558 expression levels in DMSO-treated PBMCs, arbitrarily set at 1 unit and are shown as mean \pm
559 SEM of 6 (A, B), 8 (C), 7 (D, CTP-I) and 6 (D, CTP-S) independent experiments performed
560 with PBMCs from separate blood donors tested in triplicate *per* experiment. (A, B, C, D, right
561 panels) Apoptosis was identified by microscopic observation of the number of cells with
562 condensed and/or fragmented chromatin after nuclear staining by Hoechst 33342 staining. Data
563 are expressed as percentages of dead cells and are shown as mean \pm SEM of 4 (A), 5 (B), 8 (C)
564 and 6 (D) independent experiments performed with PBMCs from separate blood donors. *, $p \leq$
565 0.05 when compared with B[a]P (A, B)- and DMSO (D)-treated PBMCs (analysis of variance
566 followed by the Student-Newman-Keuls's multirange test (A, B) or by Dunnett's multirange
567 test (D)).

568 **Fig.6: miR-132 inhibition regulates B[a]P-induced CYP1A1 and CYP1B1 mRNA**
569 **expression and attenuates B[a]P-dependent apoptosis in human PBMCs.** Primary human
570 PBMCs were transfected with an anti-miR-132 or an anti-miR control (anti-miR-CTR) (A, B,
571 D, E) prior to a B[a]P-treatment for 48 h (A, B, D, E) or with the vehicle (DMSO) or 2 μ M
572 B[a]P for various increasing times (C). (A) Apoptosis was identified by microscopic
573 observation of the number of cells with condensed and/or fragmented chromatin after nuclear
574 by Hoechst 33342 staining. Data are expressed as percentages of dead cells and are shown as
575 mean \pm SEM of 6 independent experiments performed with PBMCs from separate blood
576 donors. (B, C, D, E) mRNA expression (B, C, D, E) and miR-132 expression (C) was measured
577 by RT-qPCR. Data are expressed relative to expression levels in DMSO-treated PBMCs,
578 arbitrarily set at 1 unit and are shown as mean \pm SEM of 6 independent experiments performed
579 with PBMCs from separate blood donors tested in triplicate *per* experiment. *, $p \leq 0.05$ when
580 compared with B[a]P-transfected PBMCs with an anti-miR-CTR (paired Student's *t* test (A,
581 D)).

582

583

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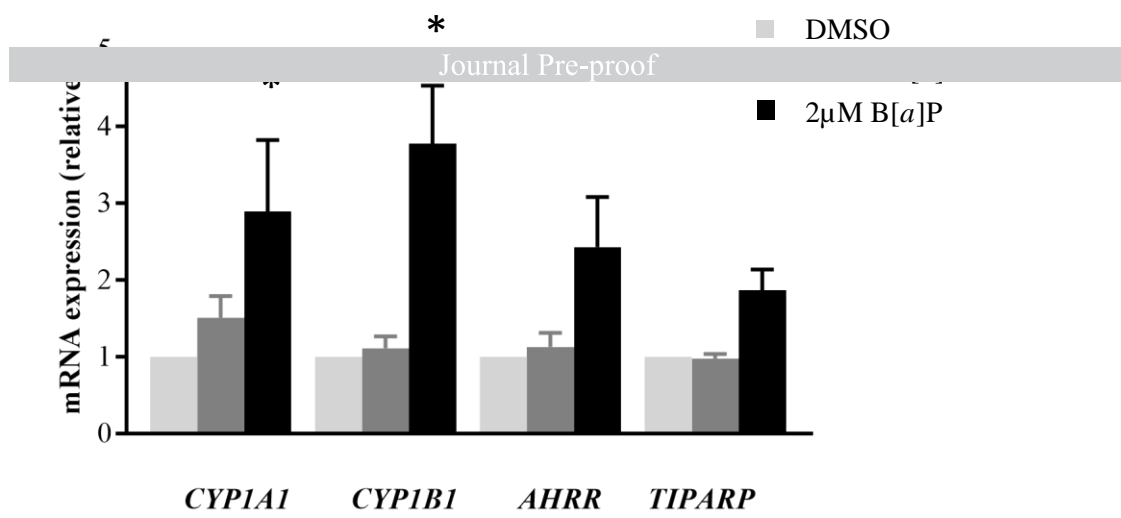
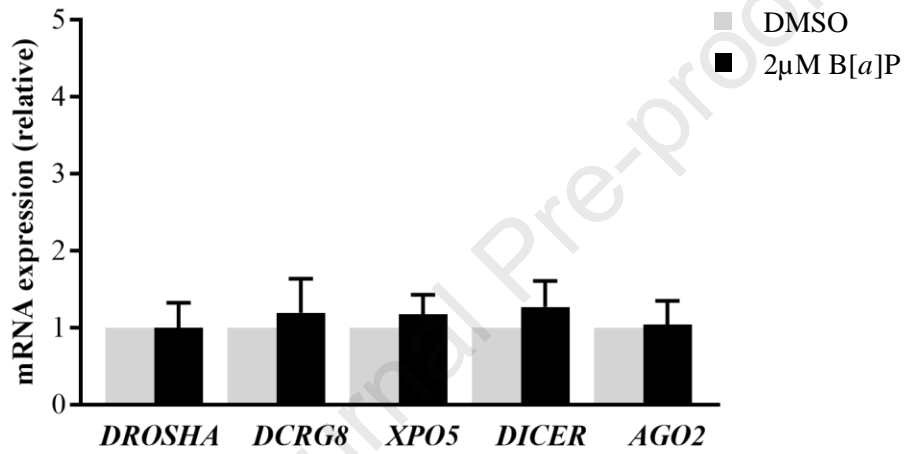
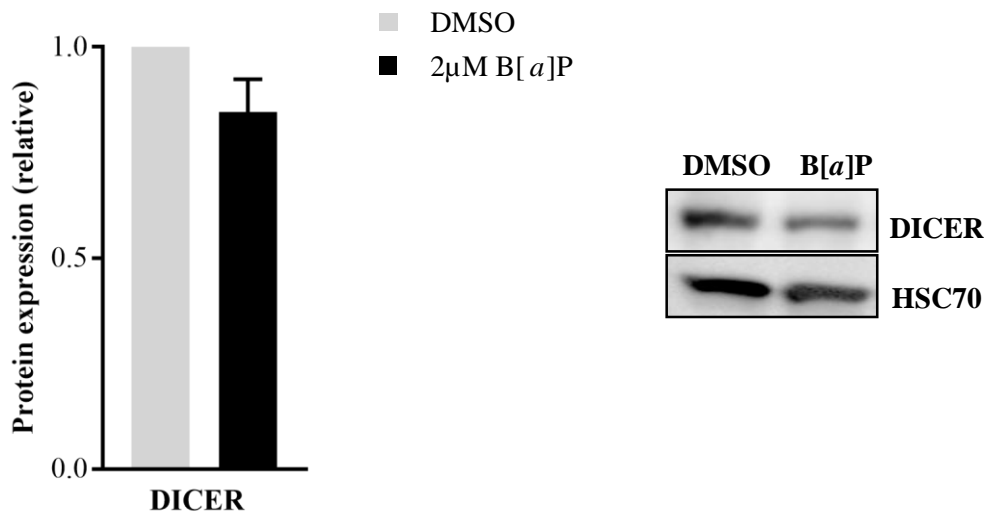
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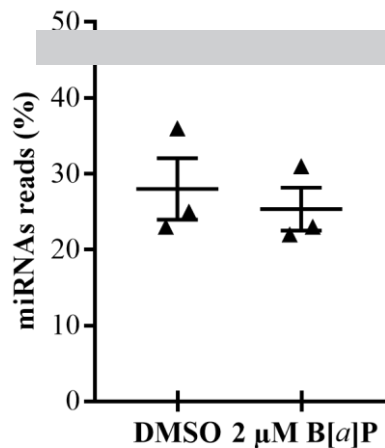
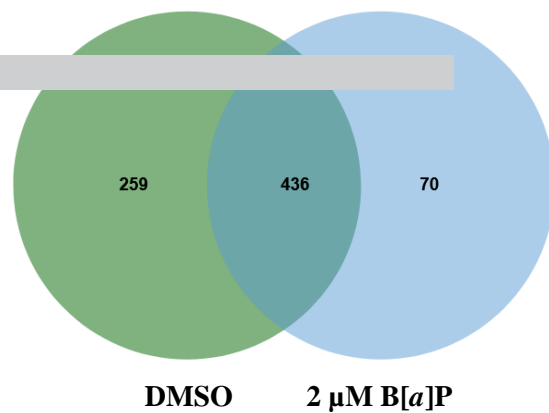
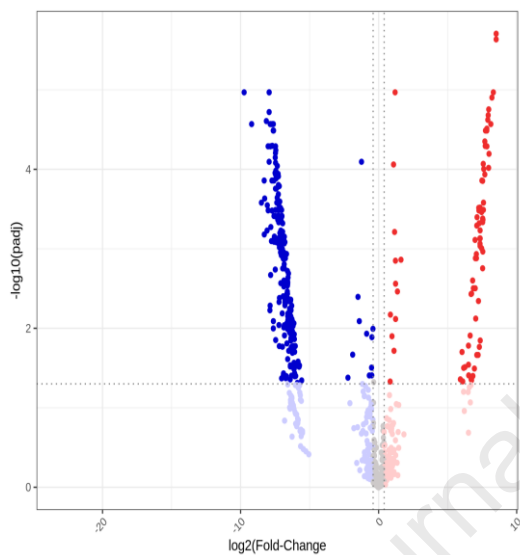
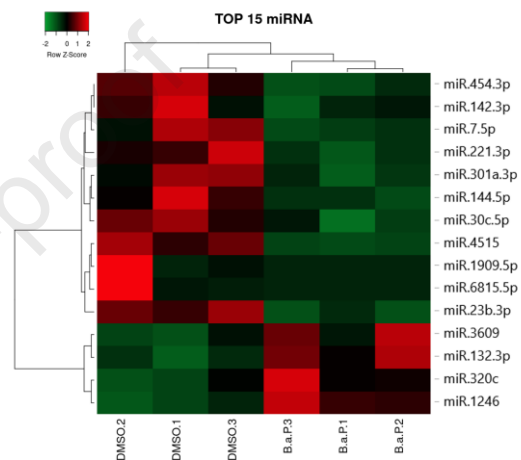
Table 1: Top functions regulated by the 15 differentially-expressed miRNAs in PBMCs after exposure to 2 μ M B[a]P for 48 h.

Molecular and Cellular functions		
Top Functions	<i>p</i>-value^a	miRNAs
Cellular Development	3.98E-02 - 4.83E-06	130a-3p, 132-3p, 144-5p, 221-3p, 23a-3p, 30c-5p, 320b, 7a-5p
Cellular Growth and Proliferation	3.98E-02 - 4.83E-06	130a-3p, 132-3p, 144-5p, 221-3p, 23a-3p, 30c-5p, 320b, 7a-5p
Cell Death and Survival	4.87E-02 - 1.96E-04	130a-3p, 132-3p, 142-3p, 221-3p, 23a-3p, 30c-5p, 320b, 7a-5p
Cell-To-Cell Signaling and Interaction	3.32E-02 - 1.38E-03	142-3p, 221-3p
Cellular Function and Maintenance	4.16E-02 - 1.84E-03	130a-3p, 142-3p, 30c-5p, 320b

^ap-values were calculated using the IPA software.

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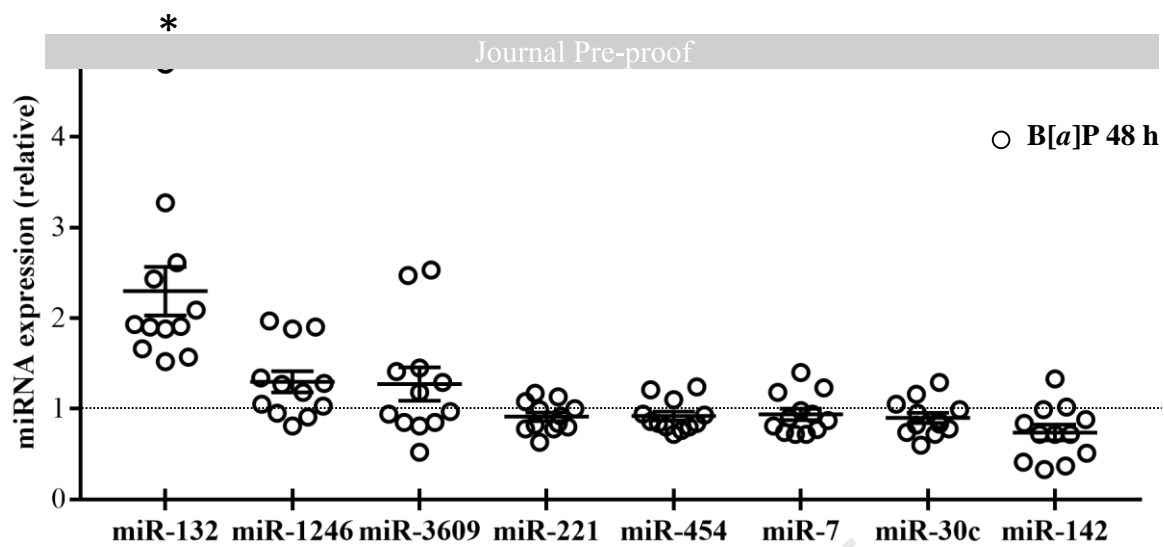
A**B****C****Fig. 1**

A**B****C****D****E**

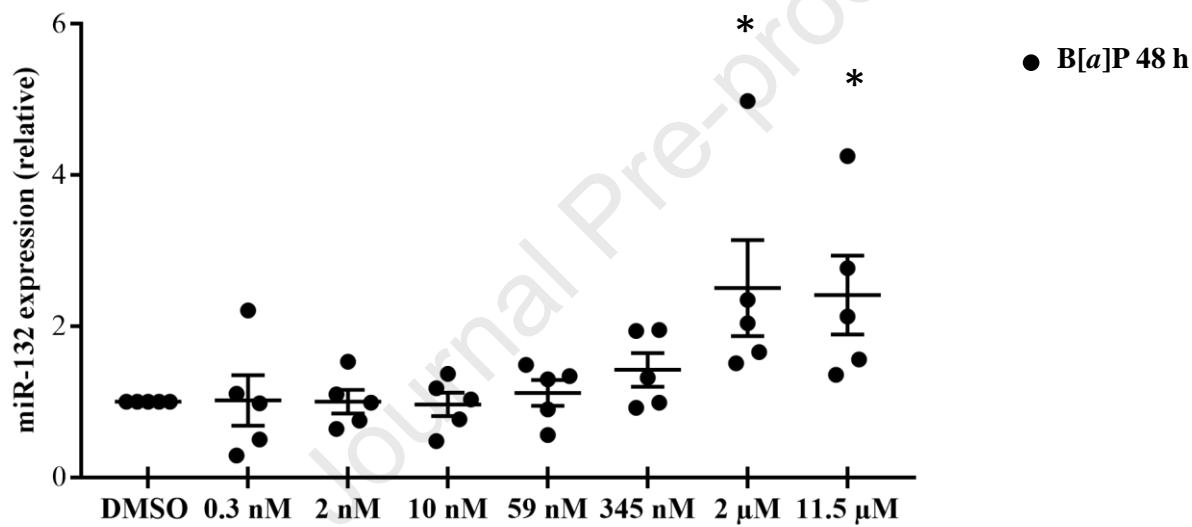
	baseMean	log2FoldChange	<i>p</i> -value	<i>p</i> -value adjusted
hsa-miR-142-3p	48920.68	-0.7	0.01	0.04
hsa-miR-221-3p	7113.01	-0.4	0.02	0.05
hsa-miR-23b-3p	3104.84	-0.4	0.00	0.01
hsa-miR-1246	1708.11	1.2	0.00	0.00
hsa-miR-132-3p	934.27	1.2	0.00	0.00
hsa-miR-454-3p	1536.52	-0.5	0.00	0.01
hsa-miR-30c-5p	1467.31	-0.5	0.01	0.03
hsa-miR-320c	809.59	0.8	0.02	0.05
hsa-miR-7-5p	1121.42	-0.5	0.01	0.04
hsa-miR-3609	412.29	1.1	0.01	0.02
hsa-miR-144-5p	372.74	-0.9	0.00	0.01
hsa-miR-4515	254.03	-1.2	0.00	0.00
hsa-miR-301a-3p	115.06	-1.0	0.02	0.05
hsa-miR-1909-5p	85.36	-9.7	0.00	0.00
hsa-miR-6815-5p	58.60	-9.2	0.00	0.00

Fig. 2

A



B



C

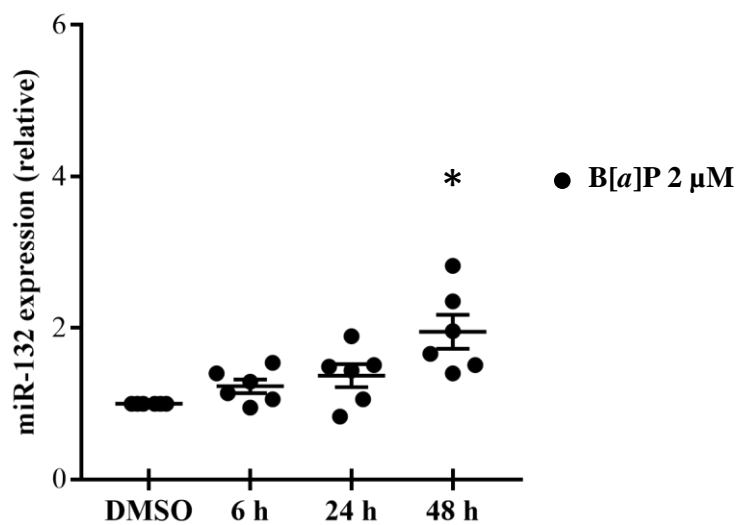


Fig. 3

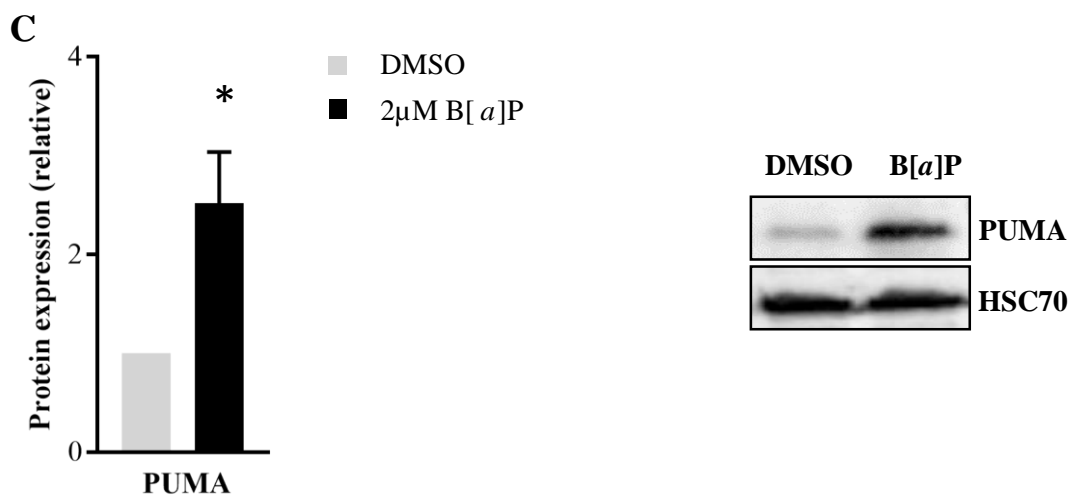
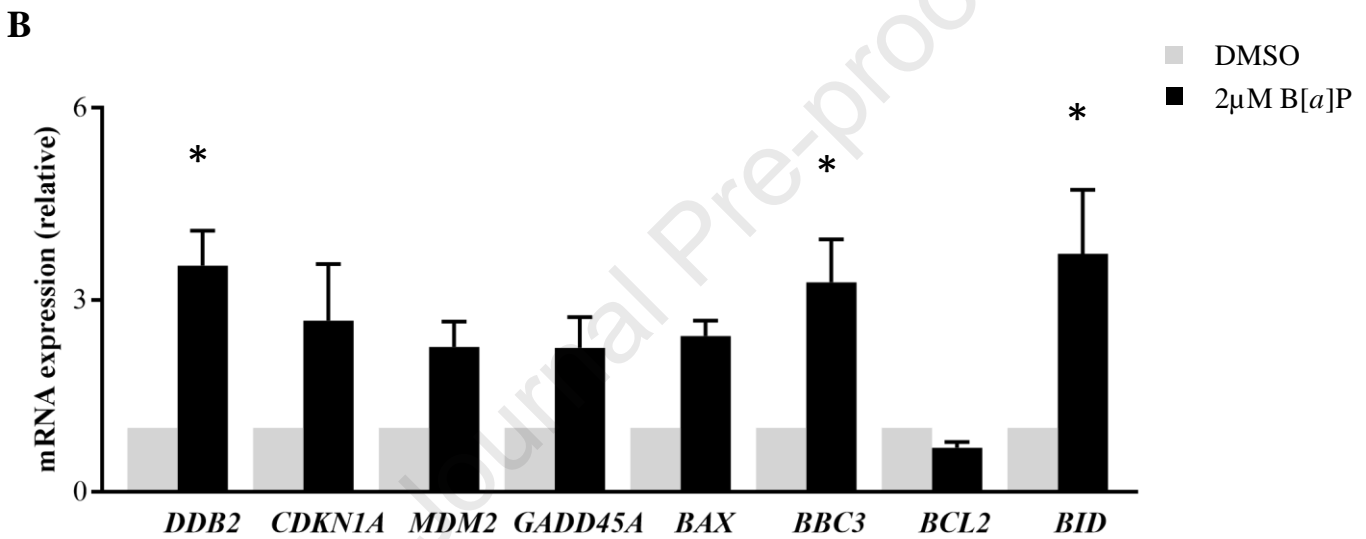
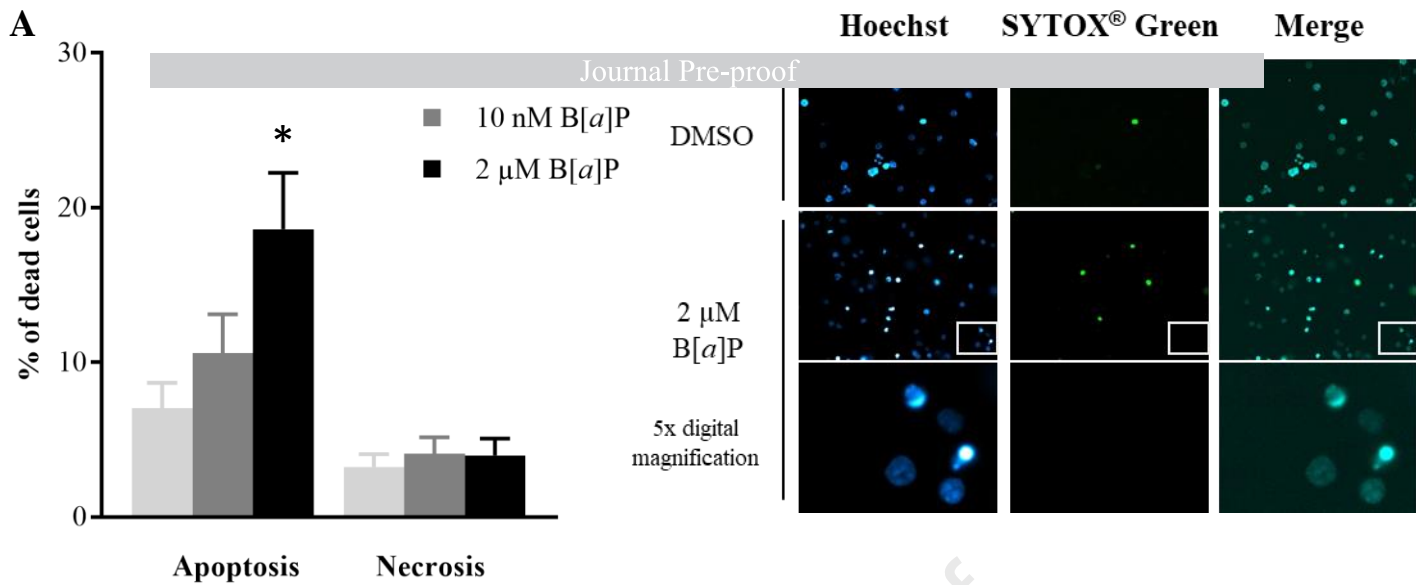


Fig. 4

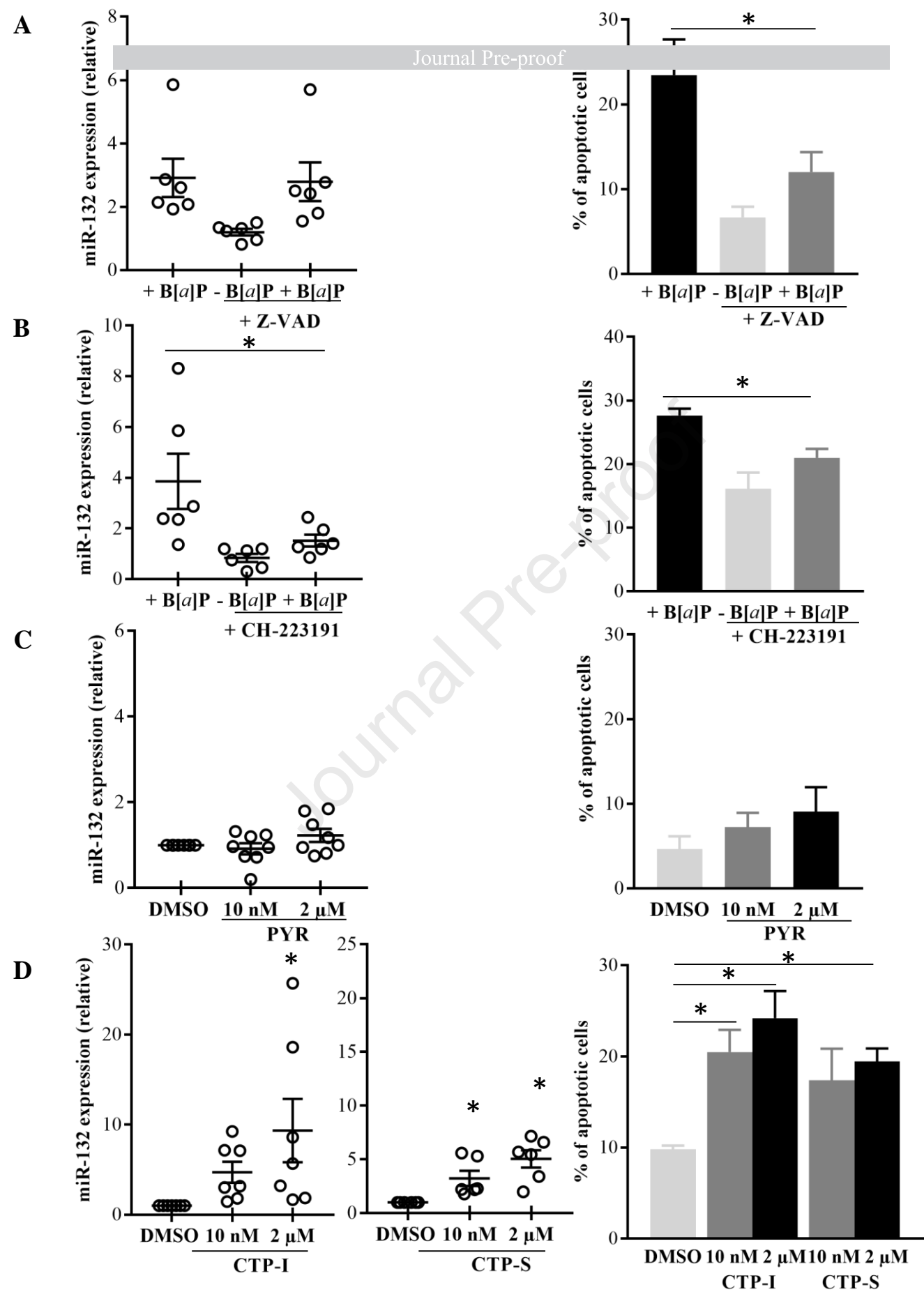


Fig. 5

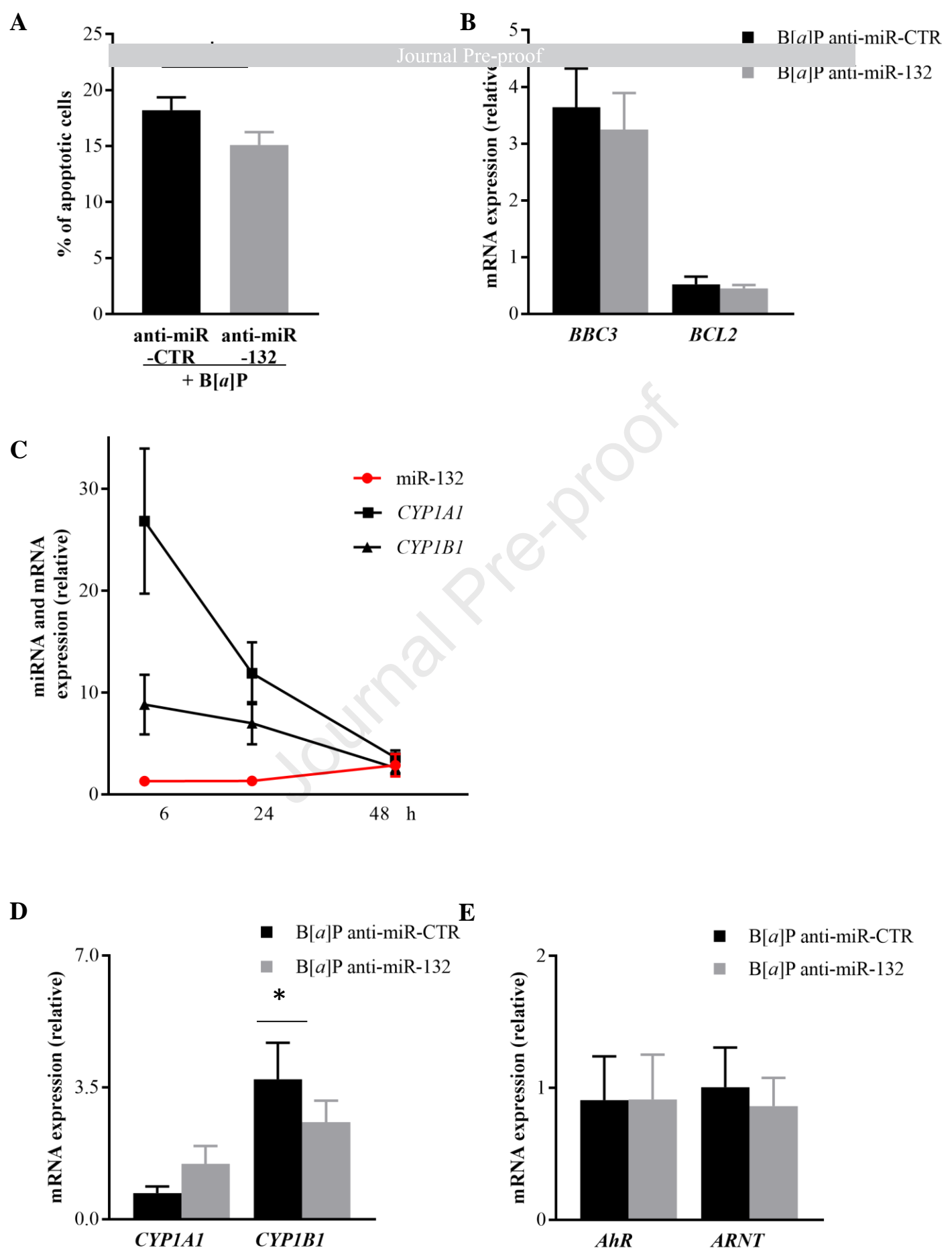


Fig. 6

Highlights

- Small RNA sequencing identifies B[a]P-responsive miRNAs in human PBMCs.
- Predicted targets of B[a]P-regulated miRNAs are related to apoptosis of PBMCs.
- miR-132-3p is the most B[a]P-regulated miRNA in PBMCs.
- B[a]P-induced miR-132 requires AhR activation.
- miR-132 could modulate apoptosis *via* regulation of the CYP1A1/1B1 balance upon B[a]P.

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Project administration: Lydie Sparfel

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Journal Pre-proof