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Prenatal exposure to paraquat and nanoscaled TiO₂ aerosols alters the gene expression of the developing brain

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1

2 Abstract

3 Nanopesticides are innovative pesticides involving engineered nanomaterials in their
4 formulation to increase the efficiency of plant protection products, while mitigating their
5 environmental impact. Despite the predicted growth of the nanopesticide use, no data is
6 available on their inhalation toxicity and the potential cocktail effects between their
7 components. In particular, the neurodevelopmental toxicity caused by prenatal exposures
8 might have long lasting consequences. In the present study, we repeatedly exposed gestating
9 mice in a whole-body exposure chamber to three aerosols, involving the paraquat herbicide,
10 nanoscaled titanium dioxide particles (nTiO₂), or a mixture of both. Particle number
11 concentrations and total mass concentrations were followed to enable a metrological follow-
12 up of the exposure sessions. Based on the aerosols characteristics, the alveolar deposited dose

13 in mice was then estimated. RNA-seq was used to highlight dysregulations in the striatum of
14 pups in response to the *in utero* exposure. Modifications in gene expression were identified at
15 post-natal day 14, which might reflect neurodevelopmental alterations in this key brain area.
16 The data suggest an alteration in the mitochondrial function following paraquat exposure,
17 which is reminiscent of the pathological process leading to Parkinson disease. Markers of
18 different cell lineages were dysregulated, showing effects, which were not limited to
19 dopaminergic neurons. Exposure to the nTiO₂ aerosol modulated the regulation of cytokines
20 and neurotransmitters pathways, perhaps reflecting a minor neuroinflammation. No synergy
21 was found between paraquat and nTiO₂. Instead, the neurodevelopmental effects were
22 surprisingly lower than the one measured for each substance separately.

23

24

25 **Introduction**

26 In response to the growing worldwide demand for food, nanotechnology applications in
27 agriculture elicit a particular interest, taking the form of innovative nanofertilizers and
28 nanopesticides (NPe). This term mostly refers to either pesticides with nano-sized active
29 substances or mixtures in which engineered nanomaterials (ENM) are mixed with
30 conventional active ingredients to control their transport, release, or degradation (Sun et al.,
31 2019). ENMs are defined as intentionally produced products with any external dimension or
32 an internal surface structure at the nanoscale, i.e., in the 1 - 100 nm size range
33 (ISO/TS 80004-1:2015). Overall, nanopesticides may represent important technological
34 innovations to address the sanitary and environmental drawbacks of pesticides, as they could
35 decrease the required tonnages of active substances. More than 1000 patents linked with these
36 technologies have already been granted in the past years (Kah et al., 2019) and a rapid
37 increase of NPe use is expected (Gilbertson et al., 2020).

38 Several authors already emphasized the knowledge gap surrounding the sanitary impact of
39 these emerging products (Kah et al., 2018; Sun et al., 2019; Agathokleous et al., 2020) and
40 health agencies highlighted the necessity to assess the toxicological consequences of a
41 deliberate release of ENM in the environment (USDA, 2015). ENMs can cross the placenta
42 (Bongaerts et al., 2020; Guillard et al., 2020) and the brain-blood barrier (Bencsik et al., 2018;
43 Cena and Jativa, 2018; Boyes and van Thriel, 2020) and might thus alter fetal brain
44 development. Subcutaneous administration of nTiO₂ into gestating mice notably increases
45 dopamine levels in the cortex and striatum of the offspring (Takahashi et al., 2010; Umezawa
46 et al., 2012). Prenatal exposure to nTiO₂ also has long term consequences in rats, enhancing
47 depressive-like behaviors at adult stage (Cui et al., 2014).

48 ENMs contained in nanopesticides might also exert an adverse effect by potentiating the
49 toxicity of pesticides. Although the current regulation enforces to assess the toxicity of both

50 ENMs and pesticides, the novel properties arising from their combination is not carefully
51 addressed in current test guidelines (Kookana et al., 2014). However, ENMs can modify the
52 absorption, biodistribution and reactivity of associated chemicals (Naasz et al., 2018).
53 Synergistic hepatotoxicity of a mixture of paraquat (PQ) and SiO₂ nanoparticles has also been
54 reported in mice (Nishimori et al., 2009).

55 We address here the possible occurrence of synergistic effects induced by the prenatal
56 exposure to aerosols involving a mixture of an active substance and an ENM. As a proof of
57 principle, we associated PQ with TiO₂ P25 (nTiO₂), which is both an ENM suitable for
58 agricultural applications (Wang et al., 2016) and a reference toxicological nanomaterial
59 extensively studied in the literature (Shi et al., 2013; Zhang et al., 2015). We prioritized the
60 developmental period because of its high sensitivity to environmental factors (Barouki et al.,
61 2012) and the neurodevelopment because any adverse effect at this stage could have
62 irreversible consequences.

63 PQ was selected for its known neurotoxicity. Exposures of gestating mice (20 mg/kg/day)
64 provokes irreversible neurobehavioral and cognitive deficits in the offspring (Ait-Bali et al.,
65 2016). Human occupational exposure to PQ is also suspected to increase the occurrence of
66 Parkinson Disease (Kamel et al., 2007; Tanner et al., 2011; Wang et al., 2011; Moisan et al.,
67 2015; Brouwer et al., 2017) . Although the association of PQ and P25 nTiO₂ has not been
68 reported in the fields, it might be suitable for agriculture, as the nTiO₂ photocatalytic activity
69 can degrade PQ under UV illumination (Florencio et al., 2004; Nicosia et al., 2021). Their
70 association could reduce the half-life of PQ in soils, which otherwise can reach several years
71 (Sartori and Vidrio, 2018).

72 We chose to expose mice by inhalation, as it is a major route of occupational exposure to
73 pesticides. Depending on the spraying conditions and the volatility of the pesticide
74 formulation, 10-75% of the applied tonnages are unintentionally disseminated in the air

75 (Pimentel, 2014). As a result, low concentrations of pesticide residues can be detected in air
76 samples of both rural and urban sites (Schummer et al., 2010; Coscollo et al., 2013). In this
77 study, we used an ad hoc aerosol exposure chamber for rodents, which was previously
78 characterized using a metrological protocol, to generate PQ, nTiO₂ and PQ + nTiO₂ aerosols
79 (Hamdaoui et al., 2021).

80 **Material and methods**

81 *Animals and experimental design*

82 Experimentations were conducted according to the European regulation concerning the
83 protection of animals dedicated to scientific purposes (European Directive 2010/63/EU). The
84 protocol was approved by the local Ethics Committee (CECCAPP) and the French Ministry
85 for Research and Higher Education (CECCAPP_ENS_2019_015). Two-month old
86 primiparous C57Bl6/J mice (gestation day 4, GD4, purchased from Charles Rivers) were
87 randomly assigned to housing cages (2 females/cage) and housed in standard environmental
88 conditions (humidity of 55 ± 10%; temperature 22 ± 2°C; 12/12 h light/dark cycle) at the
89 Plateau de Biologie Expérimentale de la Souris (SFR BioSciences Gerland - Lyon Sud,
90 France Accreditation N° D54-547-10). Animals had free access to water and a supplemented
91 diet for pregnant mice (Safe 105 rodent pellets).

92 Groups of gestating mice were exposed to aerosols of either PQ (0.1 mg/m³), nTiO₂ (10
93 mg/m³) or PQ + nTiO₂ (10 mg/m³). These target concentrations were chosen to match the
94 values of occupational exposures reported in the literature, in nTiO₂ production sites (5
95 mg/m³; particle size in the range of 15 – 710.5 nm) (Lee et al., 2011) and in PQ treated fields
96 (0.12 mg/m³) (Morshed et al., 2010). One additional control group was kept in the same
97 conditions during the duration of the exposure sessions, but without aerosol exposure. Each
98 group of exposure was composed of 12 gestating mice exposed at the same time. Gestating
99 mice were repeatedly exposed from GD6 to GD18 for 1.5 hour, 6 days/ week, for a total of 11

100 sessions of exposure, including an individual body weight follow-up. Male and female pups
101 received a lethal intraperitoneal injection (6 mL/kg) at post-natal day 14 (PND14) of a
102 mixture of Ketamine (33 mg/mL) and Xylazine (6.7 mg/mL). Blood was collected on ice in
103 heparin tubes, then centrifuged (15 min at 2,500 g) and the supernatants were stored at -80°C .
104 Organs were weighted and sampled, then stored at -80°C for later analysis. The striatum was
105 dissected and snap frozen in liquid nitrogen.

106 *Aerosol exposures*

107 The aerosols and the exposure chamber used in this study have been previously characterized
108 (Hamdaoui et al., 2021). Briefly, the aerosol exposure chamber was composed of a rodent
109 cage (Tecniplast GR900, internal volume of 19.8 L), used as a whole-body exposure system,
110 coupled with a nebulizer (model 3076, TSI Inc.) to produce aerosols from colloidal
111 suspensions. It was operated to generate three different aerosols i.e., nTiO₂ alone, PQ alone
112 and nTiO₂ mixed with PQ (PQ + nTiO₂). Aerosols were produced from daily prepared
113 colloidal suspensions with PQ dichloride hydrate (Sigma Aldrich reference 856177) in
114 ultrapure water (MilliQ, Millipore, 18.2 M Ω .cm resistivity) and/or bulk powders of nTiO₂
115 (Sigma Aldrich reference 718467). All colloidal suspensions were constantly stirred during the
116 nebulization process. The generated nTiO₂ aerosols were composed of nanostructured
117 agglomerates and aggregates of nTiO₂ nano-objects. The stability of PQ concentration in
118 suspension with nTiO₂ was verified, to ensure that our experimental conditions did not cause
119 its photocatalytic degradation during aerosol generations. The metrological characterization of
120 this experimental set-up was previously described in terms of particle number size
121 distribution, mass and number concentrations, spatial and temporal stability (Hamdaoui et al.,
122 2021). The proper functioning of the device and the stability of aerosols were monitored
123 during each animal exposures in real-time, by using a condensation particle counter (model
124 3007, TSI Inc.) to measure the particle number concentrations inside the exposure chamber.

125 In addition, the mass concentrations of aerosols were also monitored by aerosol filtration
126 using a 47 mm filter-holder (Pallflex® filters, EmfabTMTX40HI20-205 WW type) followed
127 by gravimetric measurements. The chamber average concentrations did not deviate from the
128 mean by more than $\pm 20\%$, in accordance to the test guidelines for inhalation toxicology
129 (OECD, TG 412).

130 *Gestating mice deposited particle mass estimation*

131 The deposition fractions of aerosols in gestating mice and human lungs were calculated using
132 the Multiple Path Particle Dosimetry Model (MPPD version 3.04). Several estimations were
133 necessary to determine the relation between mice model and human exposure by using the
134 alveolar surface as weighting factor (Jarabek et al., 2005). The characteristics of aerosol
135 previously described (Hamdaoui et al., 2021) were used to estimate the alveolar deposition
136 fraction for each aerosol. According to the recommended exposure limit of the National
137 Institute for Occupational Safety and Health (NIOSH) for ultrafine TiO_2 and PQ, $300 \mu\text{g}/\text{m}^3$
138 was used for nTiO_2 and $100 \mu\text{g}/\text{m}^3$ for PQ for the calculation in human. The target
139 concentrations used in this study (10 mg for nTiO_2 and $100 \mu\text{g}/\text{m}^3$ for PQ) were chosen for
140 mice, with a minute ventilation of $22.2 \text{ mL}/\text{min}$ (Tankersley et al., 1994) and an alveolar
141 surface of 0.05 m^2 (Stone et al., 1992). A scenario of occupational exposure was chosen,
142 involving a woman working for 8 months during pregnancy, with a nasal minute ventilation
143 of $19 \text{ L}/\text{min}$, corresponding to workers achieving light duties (Cassee and de Winter-Sorkina,
144 2003) and a human alveolar surface of 102 m^2 (Stone et al., 1992). We assumed that this
145 worker would be exposed 8 h per day, 5 days per week, 20 days per month using the
146 Yeh/Schum symmetric lung model without clearance.

147 *Plasmatic markers of inflammation*

148 Systemic inflammation is known to play an important role in neurologic and
149 neurodevelopmental impairments and inflammation is typically measured by quantifying

150 circulating cytokines. Pups plasma were thawed and pooled by litters ($n \geq 5$ /litter). Fifty
151 microliters were assayed using an ELISA multiplexed kit (MCYTOMAG-70K-26, Bio-
152 Techne) to measure the concentrations of 8 cytokines (MCP-1, Cxcl1, IL-1 beta, IL-4, IL-6,
153 IL-17, TNF-alpha) and the matrix metalloproteinase MMP12 on a Luminex platform
154 (Luminex MAGPIX, ThermoFisher). These markers were selected for their implication in
155 neurodevelopment (MCP-1, IL-1 beta, IL-6 and TNF-alpha) (Nist and Pickler, 2019), their
156 role as pro-inflammatory factors (IL-4, IL-10, IL-17 and Cxcl1), or in extracellular matrix
157 remodeling (MMP12) following tissues insults. The gene *Mmp12* has already been described
158 as an early marker of the lung insults in response to low concentrations of PQ in mice (Tomita
159 et al., 2007).

160 *RT-qPCR*

161 Total RNA was extracted using RNeasy Mini kit (Qiagen) from frozen striatum ($n \geq 8$ /group).
162 On column DNase digestion was used to remove contaminating DNA. RNA concentrations
163 were measured with a Nanodrop spectrophotometer (ThermoScientific) and 1 μ g of each
164 RNA sample was reverse transcribed in murine leukemia virus reverse transcriptase
165 (Promega) and random DNA hexamer primers. Quantitative PCR was performed according to
166 a standard protocol, using the Biorad iQ SYBRGreen kit and the Biorad CFX96 thermocycler.
167 For each pair of primers (Table S1) a standard curve was established to verify PCR efficiency
168 (90%–110%) and quantification was performed using the $2^{-\Delta\Delta C_t}$ method (Livak and
169 Schmittgen, 2001). We verified that the *Gapdh* and *Rplp0* housekeeping genes which were
170 used as internal controls do not vary significantly in our samples according to RNA-seq
171 (Figure S1). Differences among experimental groups were analyzed with the Kruskal-Wallis
172 test followed by Dunn's post hoc test to compare mRNA relative mean levels using Prism
173 (GraphPad 8.3 Software, Inc, San Diego CA).

174 *RNA-seq analysis*

175 Striatum RNA was extracted using the Macherey-Nagel NucleoSpin RNA II kit. cDNA
176 libraries were prepared using the total RNA SENSE kit (Lexogen, Vienna Austria) and
177 analyzed on an Nextseq500 sequencer (Illumina). Raw data of single-end sequencing
178 (GSE169579) were converted to count tables using htseq-count (Galaxy Version
179 0.6.1galaxy3) (Anders et al., 2015). Differential gene expression analysis was performed
180 between exposure and control groups with Deseq2 (Galaxy Version 2.1.8.3, one-factor,
181 parametric fit type, automatic outlier replacement and filtering, p-adjusted values <0.05;
182 minimum read number >10) (Love et al., 2015). RNASeq data are accessible through GEO
183 Series accession number: GSE143933
184 (www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE169579#).

185 *Gene Set Enrichment Analysis (GSEA)*

186 Gene Set Enrichment Analysis (<https://www.gsea-msigdb.org/gsea/index.jsp>) was performed
187 as previously described (Mooney and Wilmot, 2015) Statistical significance was tested by
188 empirical permutation followed by multiple hypotheses correction using Kolmogorov-
189 Smirnov statistics (Subramanian et al., 2005; Tamayo et al., 2016). Several gene set databases
190 were used: hallmark, C2 curated (KEGG, REACTOME) and C5 ontology (biological process)
191 (<https://www.gsea-msigdb.org/gsea/msigdb>). A p-value cut-off of $p < 0.01$ and an FDR <0.05
192 were used to select the significant gene sets, and only a limited number was selected to avoid
193 pathway redundancy. Gene sets were filtered by size, for too specific sets (<15 genes) or too
194 general sets (>300 genes) to compare the control group versus each exposed group, using
195 1000 random permutations of the gene sets for resampling. A network analysis was performed
196 to highlight dysregulated pathways using the Cytoscape software (version 3.8.2) (Cline et al.,
197 2007) with the Enrichment Map plug-in (Merico et al., 2010). The selected gene sets with a
198 False Discovery Ratio <0.05 (FDR), were represented with edges linking gene sets showing
199 an overlap >30% and then clustered in function of their common biological functions.

200

201 **Results**202 *Experimental design*

203 We exposed 3 groups of gestating mice from the inbred C57Bl6/J strain, which has a well-
204 defined genetic background, to aerosols containing low concentration of either PQ (0.1
205 mg/m³), nTiO₂ (10 mg/m³) or PQ + nTiO₂ (10 mg/m³). These concentrations were selected for
206 their relevance to human exposure (see methods for estimations). Aerosols exposure sessions
207 were started at GD5, before the beginning of neurulation, to ensure a continuous exposure
208 during the entire neurodevelopment. The last session was at GD18, i.e., 1.5 day before birth,
209 to limit maternal stress during the perinatal period.

210 Pups were kept with their mother until PND14, a stage at which the main neurodevelopmental
211 processes are completed. We then used an unbiased transcriptome analysis of gene expression
212 of the post-natal striatum to highlight possible dysregulations caused by *in utero* exposures,
213 and a possible cocktail effect. Following a previous study (Gollamudi et al., 2012), we
214 selected this brain area for several reasons. It first represents a relatively homogenous and
215 well delimited brain area, where 95% of the neurons are GABAergic medium spiny
216 neurons, known to be highly sensitive to moderate neurodevelopmental alterations (Richard et
217 al., 2020). In addition, striatum neurons receive dopaminergic innervation from *substantia*
218 *nigra*, one of the main sites of the PQ neurotoxicity. Thus, we expected that the striatum gene
219 expression could represent a sensitive endpoint to early neurodevelopmental insults.

220 *Aerosol exposures and dosimetry assessment*

221 The particle number concentrations assessed in real-time within the exposure chamber were
222 stable over the duration of exposures for the three aerosols (Figure 1) and the results of
223 particle number and mass concentrations are reported in Table 1. The average number

224 concentrations did not deviate from the mean by more than 9% and the mass concentrations
225 matched the desired target concentrations with a maximum relative standard deviation of 8%.

226 The alveolar deposition fraction in gestating mice and human beings were calculated using the
227 MPPD model to assess the alveolar deposited mass in each species (Table S2). For nTiO₂ and
228 PQ, they were respectively 384 µg/m² and 229 µg/m² in human beings, and 298 µg/m² and 9
229 µg/m² in mice. Therefore, mice exposures during gestation resulted in an alveolar particle
230 deposition below what a pregnant worker could theoretically be exposed to, if working during
231 eight months at levels of exposure corresponding to the occupational exposure limit. The
232 deposition fraction of the aerosol of PQ + nTiO₂ was estimated in a similar manner. Due to
233 similar aerosol characteristics in terms of size distribution, no significant difference was found
234 in its alveolar deposition in comparison with nTiO₂ alone in human being or mice. The
235 mixture resulted in interactions between PQ and nTiO₂ particles in the aerosol phase, but the
236 MMPD modelling cannot deconvolute the deposited mass attributable to each substance
237 separately. Therefore, we were not able to decipher the deposition fraction in each substance
238 (PQ versus nTiO₂) in the aerosol of PQ + nTiO₂.

239 *General health endpoints of exposed females and litters*

240 The treated mice did not display any obvious sign of discomfort. Statistical analysis did not
241 evidence any significant effect of aerosol exposure on gestating mice weight gain, offspring
242 survival, and litter body weights at different post-natal stages (Supplementary data, Figure
243 S2). In addition, prenatal aerosol exposures did not have any incidence on the characteristics
244 of litters (number, sex ratio) or organ weight at PND14 (Table S3). Only two markers were
245 detected in the plasma of pups (MMP12 and Cxcl1) (Figure 2). Cxcl1 chemokine was found
246 to be slightly but significantly increased in the PQ group of exposure in comparison to the
247 control group, which is an indication of a slight systemic inflammation in this group.

248 *Assessing neurodevelopmental toxicity by transcriptome analysis*

249 RNA was extracted from the striatum of pups at PND14 (Table S4). We first used RT-qPCR
250 to address the possible accidental presence of neighboring neural tissues in the striatum
251 samples. The expression of *Drd2* and *Adora2a*, whose expression is restricted to striatum, and
252 *Fezf1* which is selectively expressed in the neighboring cortex, was homogeneous. Sixteen
253 striatum samples (4/group, 50% males and 50% females) coming from sixteen different litters
254 were selected for RNA-seq analysis. Deseq2 was then used to identify differentially expressed
255 genes (DEGs) in each group of exposure in comparison to the control group. The Principal
256 Component Analysis (PCA) of the total normalized counts (Figure 3A) highlighted different
257 profiles for the different groups. The first principal component, explaining 37 % of the
258 variation mainly reflected the groups exposure. The second principal component, explaining
259 26 % of the variation, mainly reflected the distinction between females and males. However,
260 intragroup comparison between males and females did not evidence different response to
261 aerosol exposures. Because of a hypothalamic contamination during dissection, one outlier
262 coming from the PQ group was detected thanks to Deseq2 and excluded from the analysis.

263 Both PQ and nTiO₂ aerosols had a clear influence on gene expression. The number of
264 differentially expressed genes (DEGs) was 2038 for PQ, 1249 for nTiO₂, and only 287 for PQ
265 + nTiO₂. Up-regulation was more frequent than down-regulation (Figure 3B) as up-regulated
266 genes represented 59%, 89% and 85% of the total number of DEGs for PQ, nTiO₂ and PQ +
267 nTiO₂, and the fold-changes exceeded 1.5 for 120, 61 and 36 DEGs respectively. The
268 overlapping DEGs between the three groups of exposure reached 170 (Figure 3C), suggesting
269 that some similarities between the mode of action of PQ and nTiO₂. A smaller subset of 18
270 DEGs were upregulated in the three groups of exposure, with a fold-change superior to 1.5. In
271 agreement with the previous global analysis, the combined exposure to PQ + nTiO₂ had
272 globally a lesser influence on these 18 genes (Figure 3D).

273 RT-qPCR was used to confirm some of the RNA-seq observations on a larger number of
274 animals for 8 genes selected for their known role in neurodevelopment (Figure 4). These
275 genes were markers of neuronal differentiation (*Nefl*, *Nefh*) (Hogberg et al., 2009) reactive
276 astrogliosis resulting from neurological insults (*Gfap*) (Eng and Ghirnikar, 1994) myelination
277 (*Fa2h*, *Mobp*) (Royland et al., 2008; Potter et al., 2011) GABAergic neurons maturation
278 (*Kcnc2*) (Fukumoto et al., 2018) and microglial activation (*Chga*) (Ciesielski-Treska and
279 Aunis, 2000). *Bdnf* encodes a neurotrophin, which is essential for the neuron survival, growth
280 and differentiation, whose modification has been associated with mental disorders (Ikegame
281 et al., 2013). The expression of *Nefl* and *Nefh*, which encode filament proteins expressed
282 respectively during early and late neuronal maturation, were both found dysregulated in the
283 PQ group, while only *Nefl* was found dysregulated in the PQ + nTiO₂ group. Interestingly, the
284 expression of the *Chga* gene colocalizes with activated microglia in neurodegenerative areas
285 (Ciesielski-Treska et al., 1998), was overexpressed in all exposure groups with the same
286 amplitude. Moreover, *Kcnc2* which is highly expressed in the medium spiny neurons of
287 striatum was clearly upregulated in the PQ and PQ + nTiO₂ groups. The expressions of *Gfap*
288 and myelin-forming markers (*Fa2h* and *Mobp*) were upregulated solely in the PQ group.
289 Overall, the dysregulation of these genes, with the possible exception of *Bdnf*, was more
290 pronounced in the PQ group, supporting the observations coming from RNA-seq data.

291 *Interpretation of transcriptome changes - Gene Set Enrichment Analysis*

292 Gene Set Enrichment Analysis (GSEA) can identify differential expression of gene sets, when
293 the expression difference between genes is minimal and cannot be evidence by classical
294 methods on a single gene basis. GSEA was used to interpret the broad but often modest
295 changes in gene expression revealed by RNA-seq, to identify relevant biological pathways.
296 Overall, 77 gene sets were found dysregulated in the striatum of at least one group of mice
297 (Supplementary Tables S5 to S7).

298 PQ exposure resulted in the up-regulation of 7 gene sets involved in gliogenesis and axon
299 ensheathment, and 4 gene sets associated with neuron projection and axon guidance (Figure
300 5A). This suggested an alteration in the timing of differentiation for both oligodendrocytes
301 and the main neuronal cell type, the medium spiny neurons. Among the five identified
302 pathways which were down-regulated, two were likely to reflect a possible direct effect and
303 persisting of PQ in the post-natal brain, following fetal contamination, as they are known to
304 be involved in the etiology of the PD. These are related to redox homeostasis (1 gene set) and
305 mitochondrial electron transport (8 genes sets), which may be linked to PQ oxidant properties.
306 The subset of genes accounting for the enrichment of this pathway, the leading-edge genes
307 according to GSEA results, was analyzed using ClueGO (version 2.5.7) (Bindea et al.,
308 2009) from the Cytoscape package in order to visualize the deregulated pathways in a
309 functionally grouped gene network. A focus presenting the 53 leading edge genes of
310 Parkinson disease gene set (KEGG, total of 110 genes) is presented in Figure 5B, showing
311 that 32 of them were specifically related to the oxidative phosphorylation pathway. In
312 addition, the gene set associated with the cell redox homeostasis (total of 52 genes)
313 encompassed 24 leading edge genes. Within this set, 18 genes were related to the
314 oxidoreductase activity, involving for example the Glutathione reductase (*Gsr*), which is
315 necessary to maintain high levels of reduced glutathione in the cytosol (Yang et al., 2006).

316 The consequences of gestational exposure to nTiO₂ were also highlighted (Figure 5A), with
317 only one down-regulated pathway (centromere complex assembly, 2 gene sets), and 4 up-
318 regulated pathways. These pathways were related to gliogenesis, axon ensheathment and
319 guidance (5 gene sets). This is an indication that nTiO₂ also may have adverse effects on
320 oligodendrocytes differentiation and neuronal maturation. The identification of the ion
321 homeostasis pathway (5 gene sets) also suggested an alteration of the neuronal
322 electrophysiology. The appearance of the pathway “Chemokines production and B-Cell

323 proliferation” pointed out a potential low grade neuroinflammation. Finally, nTiO₂ exposure
324 also altered the “regulation of neurotransmitters” pathway (11 gene sets).

325 The gestational exposure to the combination of PQ + nTiO₂ had a visible influence on only 3
326 pathways (Figure 5A) which is consistent with its decreased influence on the gene expression.
327 These pathways are: “regulation of neurotransmitters” (12 gene sets), “neurons and dendrites
328 extension” (2 gene sets) and “voltage potassium channels” (4 gene sets). As the regulation of
329 neurotransmitters was commonly found upregulated only in the nTiO₂ and PQ + nTiO₂
330 groups, the dysregulation of genes involved in neurotransmitters release is probably caused by
331 nTiO₂, independently of PQ. The first 50 leading edge genes accounting for the enrichment of
332 this pathway were represented using ClueGO (Figure 5B). These common genes accounting
333 for the enrichment of this pathway and their associated fold-changes were represented on a
334 radar plot for the nTiO₂ and PQ + nTiO₂ groups, showing a similar influence of both
335 exposures (Figure S4). This demonstrated that PQ + nTiO₂ did not have an additive effect on
336 this pathway, which is dysregulated by both nTiO₂ and the mixture. Interestingly, two genes
337 related to the glutamate neurotransmission were highlighted: the Glutamate receptor (*Grina3*)
338 and the Metabotropic glutamate receptor 4 (*Grm4*). α -synuclein was also found in the leading
339 edge set of this pathway and different trends were observed, as a slight decrease was found
340 after exposure to PQ (Fold-change=0.80, adjusted p-value=0.01) and nTiO₂ (Fold-
341 change=0.82, adjusted p-value=0.02), but not to PQ + nTiO₂.

342

343 **Discussion**

344 *Effect of maternal exposure to aerosols*

345 We present novel evidence showing that prenatal exposures to aerosols of PQ and/or nTiO₂ at
346 low concentrations, which are theoretically considered safe, have a significant effect on the

347 gene expression in the developing brain . The calculation of the deposition fractions for each
348 aerosol using dosimetry modeling allowed an interspecies extrapolation. We could identify
349 some consequences of the *in utero* exposure by analyzing the gene expression in the post-
350 natal striatum. In this context, transcriptome analysis proved to be an unbiased, powerful and
351 sensitive method to detect neurodevelopmental alterations.

352 *Paraquat neurodevelopmental toxicity*

353 Despite a very low deposition of PQ in maternal lungs, clear changes in gene expression were
354 observed in the striatum of pups after birth, irrespective of the gender, which reflect
355 alterations in different cell lineages. The observed changes suggest moderate defects in
356 neuronal maturation and oligodendrocytes differentiation. They are also indicative of
357 inflammation, with astrogliosis and microglial cells activation. Therefore, the influence of PQ
358 in the developing striatum is not limited to dopaminergic neurons and we propose that it
359 combines direct and indirect effects on neural cells. Based on previous toxicological
360 knowledge and our observations, PQ may directly alter the mitochondrial function and the
361 redox status in neurons, during fetal and early post-natal life, as it does in adult brain to
362 induce Parkinson disease (Jomova et al., 2010). Some of the highlighted changes in gene
363 expression are possibly involved in this process: Glutathione reductase (encoded by the *Gsr*
364 gene) is necessary to maintain high levels of reduced glutathione in the cytosol (Yang et al.,
365 2006), Selenoprotein (product of the *Selenot* gene) protects dopaminergic neurons against
366 oxidative stress in mouse models of Parkinson disease (Boukhar et al., 2016), Parkin RBR
367 E3 ubiquitin protein ligase (*Prkn*), and α -synuclein (*Snca*) maintain the dopamine neuronal
368 homeostasis, whose dysfunction is related to the onset of Parkinson disease (Kuroda et al.,
369 2012; Oczkowska et al., 2013). These initial mitochondrial alterations may indirectly
370 influence the maturation of the neuronal networks, the post-natal myelination process, and
371 eventually favor neuroinflammation. Taken together, these observations suggest that PQ alters

372 mitochondrial functions in the striatum of exposed pups in a way that is reminiscent of the
373 pathological process leading to Parkinson disease.

374 This interpretation is consistent with an analysis of the striatum transcriptome performed
375 after acute exposure of adult mice to PQ (Gollamudi et al., 2012). This also caused
376 dysregulations of the signaling pathways related to Parkinson disease. Exposure of gestating
377 mice to a high dose of PQ via the oral route (Ait-Bali et al., 2016) also induced various
378 neurological and behavioral alterations in the offspring. In a recent study, adult C57Bl/6J
379 mice were exposed to PQ aerosols (Anderson et al., 2021), leading to a significant PQ burden
380 in different brain regions, including the striatum, associated with a male-specific deficit in
381 olfaction, which is part of the prodromal features of Parkinson disease.

382 The amplitude of the observed changes is modest but does not rule out unforeseen latter
383 consequences, as early alterations can exacerbate the sensitivity to additional stressors at adult
384 stage (Barouki et al., 2012). Previous analysis also showed that early exposure to PQ + Zn in
385 rats affects the oxidative stress, inflammation, cell death, dopamine metabolism and storage
386 regulating machineries (Mitra et al., 2020). Therefore, re-exposure of adult rats caused more
387 pronounced changes in all the observed endpoints of toxicity, compared with rat exposed only
388 during adulthood, thereby showing that early insults emphasized the toxicity of secondary
389 exposures. Similar conclusions were drawn for the early exposure to PQ combined with
390 maneb (Colle et al., 2020).

391 *nTiO₂ neurodevelopmental toxicity*

392 The neurotoxicity of nTiO₂ was previously addressed at higher concentrations than those used
393 here. Subcutaneous injection of large quantities of nTiO₂ to gestating mice induces a
394 mitochondrial respiration dysfunction in the pups brain (Hathaway et al., 2017) altering the
395 gene expression in the post-natal striatum of pups (Umezawa et al., 2012) and having long
396 term consequences on their cognitive functions (Engler-Chiurazzi et al., 2016). Prenatal

397 exposure to nTiO₂ aerosols also results in maternal and fetal microvascular dysfunction
398 (Stapleton et al., 2015) and affects the production of neurotransmitters (Shimizu et al., 2009).
399 Our results confirm that gestational exposure to nTiO₂ has adverse effect on striatum
400 maturation, at a concentration, which is much lower than those used in previous studies.
401 Changes in gene expression suggest a modulation of neuronal maturation, neurotransmitter
402 production, post-natal myelination, and possibly a low-grade neuro-inflammation. The
403 convergence of our conclusions with previous studies suggests that the observed changes are
404 neither transient nor adaptative but rather reflects moderate adverse effects on the brain
405 maturation. The possibility that exposure to aerosols containing realistic concentrations of
406 nTiO₂ could compromise the neurodevelopment should thus be a matter of concern when
407 considering their extensive use.

408 *Interaction between nTiO₂ and PQ exposure.*

409 We did not observe a synergistic nor an additive effect when combining PQ and nTiO₂.
410 Instead, their association in aerosol phase seemed to lessen the effect of each substance.
411 RNA-seq and RT-qPCR showed that the number of differentially expressed genes and the
412 amplitude of the changes in gene expression were globally lower compared to pups exposed
413 to either PQ or nTiO₂ solely. Moreover, unlike the PQ group, the concentration of the Cxcl1
414 inflammation marker was not increased in the pups plasma. The reduction of the mixture
415 effect is not due to the PQ degradation by nTiO₂, as verified previously (Hamdaoui et al.,
416 2021) but might reflect interactions between PQ and nTiO₂ particles. The aerosols of nTiO₂
417 and PQ + nTiO₂ presented very similar particle size distributions, and the mixture displayed
418 number concentrations that did not correspond to the sum of the reported concentrations for
419 PQ and nTiO₂ taken separately. These elements suggest that mixing these substances prior to
420 the aerosol generation does not produce two distinct populations of particles, but rather a
421 composite population of particles associating PQ and nTiO₂. As PQ can be adsorbed on nTiO₂

422 in suspension (Vohra and Tanaka, 2003; Florencio et al., 2004; Phuinthiang and
423 Kajitvichyanukul, 2019) these interactions are likely to occur during the nebulization process
424 and in the aerosol phase. We hypothesize that this sorption changes the deposition fraction,
425 biodistribution and particle toxicokinetics in a way which remains to be addressed. While PQ
426 is suspected to cross the placental barrier (Ingebrigtsen et al., 1984) sorption to nTiO₂ might
427 prevent this translocation. It has been shown previously that nTiO₂ caused a retention of the
428 Cd²⁺ ions, thereby decreasing their bioavailability and preventing ecotoxicological effects.
429 Eventually, nTiO₂ might have the same effect on the PQ toxic forms (PQ²⁺/PQ⁺), by
430 decreasing its bioavailability and therefore its toxicity. *Limitations of the present study*

431 We generated ultrafine PQ particles that do not strictly correspond to the primary size of
432 sprayed pesticide particles in agriculture, which are rather coarse particles (>10 µm) mostly
433 considered as not inhalable (Chester and Ward, 1984). Nevertheless, according to its long
434 environmental persistence and the wind erosion of soil particles containing PQ, occupational
435 or residential populations can be exposed to inhalable PQ particles. This is in line with a size
436 range of 0.1 - 1 µm measured for airborne residue of some pesticides (Coscollo et al., 2013).
437 Secondary exposure to pesticide residues therefore encompass particles that may be deposited
438 in the airways after their atmospheric transport.

439 We studied the consequences of gestational exposures to PQ and nTiO₂ aerosols at a single
440 time-point in the striatum, which does not allow to address the reversibility of the observed
441 changes. Also, in the absence of complementary histological, biochemical and behavioral
442 analysis, the interpretation of these changes remains hypothetical. Finally, further work would
443 be needed to clarify the mechanisms by which the combination of PQ and nTiO₂ seems to
444 lower their inhalation effects, and whether this conclusion would also apply to other cocktails
445 of substances potentially suitable for nanopesticide formulation.

446 Conclusions

447 This study demonstrates the pertinence to use an aerosol exposure chamber and RNA-seq
448 analysis to better evaluate the unknown effects of low and repeated exposures to
449 nanopesticide aerosols on neurodevelopment. Considering the growing demand for these
450 innovative products and the lack of precise guidelines surrounding their health risk
451 assessment, these results should reinforce awareness about their future sanitary impact. Taken
452 together, these data also suggest that the risk associated with the exposure of aerosols
453 containing PQ or nTiO₂ during critical stages, like pregnancy and early development, may
454 have been previously underestimated.

455

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464

465 **Authors contribution.**

466 QH conceived the aerosol exposure device, performed the experiments, the data analysis, the
467 tissue collection and wrote the manuscript, YZ performed data analysis, SR, DA, RG, SM and
468 KG participated to mouse breeding and tissue collection. FGL conceived the aerosol exposure
469 device. AB conceived and supervised the experiments, FF supervised the project and wrote
470 the manuscript. All authors reviewed and edited the manuscript.

471

472 Figure legends

473 *Figure 1. Real time measurement of aerosol particle number concentration in the chamber*
474 *during animal exposures.*

475 Data are presented as means \pm standard deviation (SD) for each aerosol.

476 *Figure 2. Plasmatic Markers of inflammation in pups (PND14) exposed in utero.*

477 Two out of 8 assessed cytokines were detectable by ELISA. No changes were detected in
478 MMP12 expression in any of the exposure groups. The Cxcl1 expression is similar between
479 control and pups exposed to nTiO₂ alone or associated to PQ alone. PQ exposures caused a
480 significant increase in Cxcl1 in comparison to the control group (n \geq 5/litter, * p-value <0.05),
481 thereby suggesting a low grade inflammation. Comparisons between groups were performed
482 with the Kruskal-Wallis test (nonparametric test), followed by multiple mean comparisons
483 with Dunn's test. Data presented as means \pm SEM.

484 *Figure 3. Transcriptome analysis of pups striatum.* (A) PCA of the gene expression in 15
485 pups. (B) Volcano plots summarizing the comparisons between the groups of exposure versus
486 the control group. (C) Venn diagram showing the overlap between DEGs for the 3 exposed
487 groups (adjusted p-value <0.05; no threshold on fold-change), a main part of genes is
488 associated with the PQ exposure only, nTiO₂ shared 68% of its DEGs with PQ, PQ + nTiO₂
489 shared 81% with PQ and 65% with nTiO₂. (E) Fold-changes of the 18 DEGs up-regulated in
490 the 3 groups with a fold-change >1.5; note that the response to the PQ + nTiO₂ is always
491 equal or lower than the response to the single substances.

492 *Figure 4. RT-qPCR analysis of gene expression in pups striatum at PND14.*

493 Comparisons between groups (N \geq 8 for each group, 50% females and 50% males coming
494 from at least 4 different litters) were performed with the Kruskal-Wallis test, followed by

495 multiple mean comparisons with Dunn's test. * $p < 0.05$ ** $p < 0.01$. Data presented as means \pm
496 SEM.

497

498 *Figure 5. Gene Set Enrichment Analysis of striatum gene expression profiles.*

499 (A) Three MSigDB genes collections were analyzed: Gene Ontology (GO) Biological Process
500 ontology and two Canonical Pathways gene sets derived from the KEGG pathway database
501 and from the Reactome pathway database. Each node represents a gene set significantly
502 enriched in comparison with controls. The gene sets corresponding to each node, can be found
503 in supplementary data (Figure S3). Nodes are clustered in pathways based on their shared
504 genes and their common biological functions. Edges are filtered with an overlap cutoff: the
505 minimum edge between two nodes depict that 35% of one gene set is common with the other
506 (B) ClueGO analysis of the leading-edge gene set associated with KEGG Parkinson disease in
507 the PQ group, represented to highlight the main biological function associated with those,
508 notably the oxidative phosphorylation. (C) ClueGO analysis of the leading-edge gene set
509 associated with the GO regulation of neurotransmitter levels in the nTiO₂ and PQ+nTiO₂
510 groups.

511

512 **References**

513 Agathokleous, E., Feng, Z.Z., Iavicoli, I., Calabrese, E.J., 2020. Nano-pesticides: A great
514 challenge for biodiversity? The need for a broader perspective. *Nano Today* 30, 100808-
515 100810.

516 Ait-Bali, Y., Ba-M'hamed, S., Bennis, M., 2016. Prenatal Paraquat exposure induces
517 neurobehavioral and cognitive changes in mice offspring. *Environ Toxicol Pharmacol* 48, 53-
518 62.

519 Anders, S., Pyl, P.T., Huber, W., 2015. HTSeq--a Python framework to work with high-
520 throughput sequencing data. *Bioinformatics* 31, 166-169.

521 Anderson, T., Merrill, A.K., Eckard, M.L., Marvin, E., Conrad, K., Welle, K., Oberdorster,
522 G., Sobolewski, M., Cory-Slechta, D.A., 2021. Paraquat Inhalation, a Translationally
523 Relevant Route of Exposure: Disposition to the Brain and Male-Specific Olfactory
524 Impairment in Mice. *Toxicol Sci* 180, 175-185.

525 Barouki, R., Gluckman, P.D., Grandjean, P., Hanson, M., Heindel, J.J., 2012.
526 Developmental origins of non-communicable disease: implications for research and public
527 health. *Environ Health* 11, 42.

528 Bencsik, A., Lestaevel, P., Guseva Canu, I., 2018. Nano- and neurotoxicology: An
529 emerging discipline. *Prog Neurobiol* 160, 45-63.

530 Bindea, G., Mlecnik, B., Hackl, H., Charoentong, P., Tosolini, M., Kirilovsky, A.,
531 Fridman, W.H., Pages, F., Trajanoski, Z., Galon, J., 2009. ClueGO: a Cytoscape plug-in to
532 decipher functionally grouped gene ontology and pathway annotation networks.
533 *Bioinformatics* 25, 1091-1093.

534 Bongaerts, E., Nawrot, T.S., Van Pee, T., Ameloot, M., Bove, H., 2020. Translocation of
535 (ultra)fine particles and nanoparticles across the placenta; a systematic review on the evidence
536 of in vitro, ex vivo, and in vivo studies. *Part Fibre Toxicol* 17, 56.

- 537 Boukhzar, L., Hamieh, A., Cartier, D., Tanguy, Y., Alsharif, I., Castex, M., Arabo, A., El
538 Hajji, S., Bonnet, J.J., Errami, M., Falluel-Morel, A., Chagraoui, A., Lihrmann, I., Anouar, Y.,
539 2016. Selenoprotein T Exerts an Essential Oxidoreductase Activity That Protects
540 Dopaminergic Neurons in Mouse Models of Parkinson's Disease. *Antioxid Redox Signal* 24,
541 557-574.
- 542 Boyes, W.K., van Thriel, C., 2020. Neurotoxicology of Nanomaterials. *Chem Res Toxicol*
543 33, 1121-1144.
- 544 Brouwer, M., Huss, A., van der Mark, M., Nijssen, P.C.G., Mulleners, W.M., Sas, A.M.G.,
545 van Laar, T., de Snoo, G.R., Kromhout, H., Vermeulen, R.C.H., 2017. Environmental
546 exposure to pesticides and the risk of Parkinson's disease in the Netherlands. *Environ Int* 107,
547 100-110.
- 548 Cassee, F., de Winter-Sorkina, R., 2003. From concentration to dose: Factors influencing
549 airborne particulate matter deposition in humans and the rat human dose extrapolation.
550 *Toxicological Sciences* 72, 39-39.
- 551 Cena, V., Jativa, P., 2018. Nanoparticle crossing of blood-brain barrier: a road to new
552 therapeutic approaches to central nervous system diseases. *Nanomedicine (Lond)* 13, 1513-
553 1516.
- 554 Chester, G., Ward, R.J., 1984. Occupational exposure and drift hazard during aerial
555 application of paraquat to cotton. *Arch Environ Contam Toxicol* 13, 551-563.
- 556 Ciesielski-Treska, J., Aunis, D., 2000. Chromogranin A induces a neurotoxic phenotype in
557 brain microglial cells. *Adv Exp Med Biol* 482, 291-298.
- 558 Ciesielski-Treska, J., Ulrich, G., Taupenot, L., Chasserot-Golaz, S., Corti, A., Aunis, D.,
559 Bader, M.F., 1998. Chromogranin A induces a neurotoxic phenotype in brain microglial cells.
560 *J Biol Chem* 273, 14339-14346.

561 Cline, M.S., Smoot, M., Cerami, E., Kuchinsky, A., Landys, N., Workman, C., Christmas,
562 R., Avila-Campilo, I., Creech, M., Gross, B., Hanspers, K., Isserlin, R., Kelley, R., Killcoyne,
563 S., Lotia, S., Maere, S., Morris, J., Ono, K., Pavlovic, V., Pico, A.R., Vailaya, A., Wang, P.L.,
564 Adler, A., Conklin, B.R., Hood, L., Kuiper, M., Sander, C., Schmulevich, I., Schwikowski,
565 B., Warner, G.J., Ideker, T., Bader, G.D., 2007. Integration of biological networks and gene
566 expression data using Cytoscape. *Nat Protoc* 2, 2366-2382.

567 Colle, D., Santos, D.B., Naime, A.A., Goncalves, C.L., Ghizoni, H., Hort, M.A., Farina,
568 M., 2020. Early Postnatal Exposure to Paraquat and Maneb in Mice Increases Nigrostriatal
569 Dopaminergic Susceptibility to a Re-challenge with the Same Pesticides at Adulthood:
570 Implications for Parkinson's Disease. *Neurotox Res* 37, 210-226.

571 Coscollo, C., Yahyaoui, A., Colin, P., Robin, C., Martinon, L., Val, S., Baeza-Squiban, A.,
572 Mellouki, A., Yusa, V., 2013. Particle size distributions of currently used pesticides in a rural
573 atmosphere of France. *Atmos Environ* 81, 32-38.

574 Cui, Y., Chen, X., Zhou, Z., Lei, Y., Ma, M., Cao, R., Sun, T., Xu, J., Huo, M., Cao, R.,
575 Wen, C., Che, Y., 2014. Prenatal exposure to nanoparticulate titanium dioxide enhances
576 depressive-like behaviors in adult rats. *Chemosphere* 96, 99-104.

577 Eng, L.F., Ghirnikar, R.S., 1994. GFAP and astrogliosis. *Brain Pathol* 4, 229-237.

578 Engler-Chiurazzi, E.B., Stapleton, P.A., Stalnaker, J.J., Ren, X., Hu, H., Nurkiewicz, T.R.,
579 McBride, C.R., Yi, J., Engels, K., Simpkins, J.W., 2016. Impacts of prenatal nanomaterial
580 exposure on male adult Sprague-Dawley rat behavior and cognition. *J Toxicol Environ Health*
581 A 79, 447-452.

582 Florencio, M.H., Pires, E., Castro, A.L., Nunes, M.R., Borges, C., Costa, F.M., 2004.
583 Photodegradation of Diquat and Paraquat in aqueous solutions by titanium dioxide: evolution
584 of degradation reactions and characterisation of intermediates. *Chemosphere* 55, 345-355.

- 585 Fukumoto, K., Tamada, K., Toya, T., Nishino, T., Yanagawa, Y., Takumi, T., 2018.
586 Identification of genes regulating GABAergic interneuron maturation. *Neurosci Res* 134, 18-
587 29.
- 588 Gilbertson, L.M., Pourzahedi, L., Laughton, S., Gao, X.Y., Zimmerman, J.B., Theis, T.L.,
589 Westerhoff, P., Lowry, G.V., 2020. Guiding the design space for nanotechnology to advance
590 sustainable crop production. *Nat Nanotechnol* 15, 801-+.
- 591 Gollamudi, S., Johri, A., Calingasan, N.Y., Yang, L., Elemento, O., Beal, M.F., 2012.
592 Concordant signaling pathways produced by pesticide exposure in mice correspond to
593 pathways identified in human Parkinson's disease. *PLoS One* 7, e36191.
- 594 Guillard, A., Gaultier, E., Cartier, C., Devoille, L., Noireaux, J., Chevalier, L., Morin, M.,
595 Grandin, F., Lacroix, M.Z., Comera, C., Cazanave, A., de Place, A., Gayrard, V., Bach, V.,
596 Chardon, K., Bekhti, N., Adel-Patient, K., Vayssiere, C., Fiscaro, P., Feltin, N., de la Farge,
597 F., Picard-Hagen, N., Lamas, B., Houdeau, E., 2020. Basal Ti level in the human placenta and
598 meconium and evidence of a materno-foetal transfer of food-grade TiO(2)nanoparticles in an
599 ex vivo placental perfusion model. *Particle and Fibre Toxicology* 17.
- 600 Hamdaoui, Q., Bencsik, A., Flamant, F., Delcour, S., Macé, T., Vaslin-Reimann, S., Gaie-
601 Levrel, F., 2021. Development and characterization of an aerosol exposure chamber to
602 explore the nanopesticides inhalation effects on rodents *Aerosol and Air Quality Research* in
603 press.
- 604 Hathaway, Q.A., Nichols, C.E., Shepherd, D.L., Stapleton, P.A., McLaughlin, S.L.,
605 Stricker, J.C., Rellick, S.L., Pinti, M.V., Abukabda, A.B., McBride, C.R., Yi, J., Stine, S.M.,
606 Nurkiewicz, T.R., Hollander, J.M., 2017. Maternal-engineered nanomaterial exposure disrupts
607 progeny cardiac function and bioenergetics. *Am J Physiol Heart Circ Physiol* 312, H446-
608 H458.

- 609 Hogberg, H.T., Kinsner-Ovaskainen, A., Hartung, T., Coecke, S., Bal-Price, A.K., 2009.
610 Gene expression as a sensitive endpoint to evaluate cell differentiation and maturation of the
611 developing central nervous system in primary cultures of rat cerebellar granule cells (CGCs)
612 exposed to pesticides. *Toxicol Appl Pharm* 235, 268-286.
- 613 Ikegame, T., Bundo, M., Murata, Y., Kasai, K., Kato, T., Iwamoto, K., 2013. DNA
614 methylation of the BDNF gene and its relevance to psychiatric disorders. *J Hum Genet* 58,
615 434-438.
- 616 Ingebrigtsen, K., Nafstad, I., Andersen, R.A., 1984. Distribution and transplacental transfer
617 of paraquat in rats and guinea-pigs. *Gen Pharmacol* 15, 201-204.
- 618 Jarabek, A.M., Asgharian, B., Miller, F.J., 2005. Dosimetric adjustments for interspecies
619 extrapolation of inhaled poorly soluble particles (PSP). *Inhal Toxicol* 17, 317-334.
- 620 Jomova, K., Vondrakova, D., Lawson, M., Valko, M., 2010. Metals, oxidative stress and
621 neurodegenerative disorders. *Mol Cell Biochem* 345, 91-104.
- 622 Kah, M., Kookana, R.S., Gogos, A., Bucheli, T.D., 2018. A critical evaluation of
623 nanopesticides and nanofertilizers against their conventional analogues. *Nat Nanotechnol* 13,
624 677-684.
- 625 Kah, M., Tufenkji, N., White, J.C., 2019. Nano-enabled strategies to enhance crop
626 nutrition and protection. *Nat Nanotechnol* 14, 532-540.
- 627 Kamel, F., Tanner, C.M., Umbach, D.M., Hoppin, J.A., Alavanja, M.C.R., Blair, A.,
628 Comyns, K., Goldman, S.M., Korell, M., Langston, J.W., Ross, G.W., Sandler, D.P., 2007.
629 Pesticide exposure and self-reported Parkinson's disease in the agricultural health study. *Am J*
630 *Epidemiol* 165, 364-374.
- 631 Kookana, R.S., Boxall, A.B., Reeves, P.T., Ashauer, R., Beulke, S., Chaudhry, Q.,
632 Cornelis, G., Fernandes, T.F., Gan, J., Kah, M., Lynch, I., Ranville, J., Sinclair, C., Spurgeon,

- 633 D., Tiede, K., Van den Brink, P.J., 2014. Nanopesticides: guiding principles for regulatory
634 evaluation of environmental risks. *J Agric Food Chem* 62, 4227-4240.
- 635 Kuroda, Y., Sako, W., Goto, S., Sawada, T., Uchida, D., Izumi, Y., Takahashi, T.,
636 Kagawa, N., Matsumoto, M., Matsumoto, M., Takahashi, R., Kaji, R., Mitsui, T., 2012.
637 Parkin interacts with Klokin1 for mitochondrial import and maintenance of membrane
638 potential. *Hum Mol Genet* 21, 991-1003.
- 639 Lee, J.H., Kwon, M., Ji, J.H., Kang, C.S., Ahn, K.H., Han, J.H., Yu, I.J., 2011. Exposure
640 assessment of workplaces manufacturing nanosized TiO₂ and silver. *Inhal Toxicol* 23, 226-
641 236.
- 642 Livak, J.L., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-
643 time quantitative PCR and the 2-DDCT method. *Methods* 25, 402-408.
- 644 Love, M.I., Anders, S., Kim, V., Huber, W., 2015. RNA-Seq workflow: gene-level
645 exploratory analysis and differential expression. *F1000Res* 4, 1070.
- 646 Merico, D., Isserlin, R., Stueker, O., Emili, A., Bader, G.D., 2010. Enrichment Map: A
647 Network-Based Method for Gene-Set Enrichment Visualization and Interpretation. *Plos One*
648 5.
- 649 Mittra, N., Chauhan, A.K., Singh, G., Patel, D.K., Singh, C., 2020. Postnatal zinc or
650 paraquat administration increases paraquat or zinc-induced loss of dopaminergic neurons:
651 insight into augmented neurodegeneration. *Mol Cell Biochem* 467, 27-43.
- 652 Moisan, F., Spinosi, J., Delabre, L., Gourlet, V., Mazurie, J.L., Benatru, I., Goldberg, M.,
653 Weisskopf, M.G., Imbernon, E., Tzourio, C., Elbaz, A., 2015. Association of Parkinson's
654 Disease and Its Subtypes with Agricultural Pesticide Exposures in Men: A Case-Control
655 Study in France. *Environ Health Persp* 123, 1123-1129.
- 656 Mooney, M.A., Wilmot, B., 2015. Gene set analysis: A step-by-step guide. *Am J Med*
657 *Genet B Neuropsychiatr Genet* 168, 517-527.

- 658 Morshed, M.M., Omar, D., Mohamad, R., Wahed, S., Rahman, M.A., 2010. Airborne
659 Paraquat Measurement and its Exposure to Spray Operators in Treated Field Environment. *Int*
660 *J Agric Biol* 12, 679-684.
- 661 Naasz, S., Altenburger, R., Kuhnel, D., 2018. Environmental mixtures of nanomaterials
662 and chemicals: The Trojan-horse phenomenon and its relevance for ecotoxicity. *Sci Total*
663 *Environ* 635, 1170-1181.
- 664 Nicosia, A., Vento, F., Di Mari, G.M., D'Urso, L., Mineo, P.G., 2021. TiO₂-Based
665 Nanocomposites Thin Film Having Boosted Photocatalytic Activity for Xenobiotics Water
666 Pollution Remediation. *Nanomaterials-Basel* 11.
- 667 Nishimori, H., Kondoh, M., Isoda, K., Tsunoda, S., Tsutsumi, Y., Yagi, K., 2009.
668 Influence of 70 nm silica particles in mice with cisplatin or paraquat-induced toxicity.
669 *Pharmazie* 64, 395-397.
- 670 Nist, M.D., Pickler, R.H., 2019. An Integrative Review of Cytokine/Chemokine Predictors
671 of Neurodevelopment in Preterm Infants. *Biol Res Nurs* 21, 366-376.
- 672 Oczkowska, A., Kozubski, W., Lianeri, M., Dorszewska, J., 2013. Mutations in PRKN and
673 SNCA Genes Important for the Progress of Parkinson's Disease. *Curr Genomics* 14, 502-517.
- 674 Phuinthiang, P., Kajitvichyanukul, P., 2019. Degradation of paraquat from contaminated
675 water using green TiO₂ nanoparticles synthesized from *Coffea arabica* L. in photocatalytic
676 process. *Water Sci Technol* 79, 905-910.
- 677 Pimentel, D., 2014. Pesticides Applied for the Control of Invasive Species in the United
678 States. *Integrated Pest Management: Current Concepts and Ecological Perspective*, 111-123.
- 679 Potter, K.A., Kern, M.J., Fullbright, G., Bielawski, J., Scherer, S.S., Yum, S.W., Li, J.J.,
680 Cheng, H., Han, X., Venkata, J.K., Khan, P.A., Rohrer, B., Hama, H., 2011. Central nervous
681 system dysfunction in a mouse model of FA2H deficiency. *Glia* 59, 1009-1021.

682 Richard, S., Guyot, R., Rey-Millet, M., Prioux, M., Markossian, S., Aubert, D., Flamant,
683 F., 2020. A Pivotal Genetic Program Controlled by Thyroid Hormone during the Maturation
684 of GABAergic Neurons. *iScience* 23, 100899.

685 Royland, J.E., Parker, J.S., Gilbert, M.E., 2008. A genomic analysis of subclinical
686 hypothyroidism in hippocampus and neocortex of the developing rat brain. *J Neuroendocrinol*
687 20, 1319-1338.

688 Sartori, F., Vidrio, E., 2018. Environmental fate and ecotoxicology of paraquat: a
689 California perspective. *Toxicol Environ Chem* 100, 479-517.

690 Schummer, C., Mothiron, E., Appenzeller, B.M.R., Wennig, R., Millet, M., 2010.
691 Gas/particle partitioning of currently used pesticides in the atmosphere of Strasbourg
692 (France). *Air Qual Atmos Hlth* 3, 171-181.

693 Shi, H.B., Magaye, R., Castranova, V., Zhao, J.S., 2013. Titanium dioxide nanoparticles: a
694 review of current toxicological data. *Particle and Fibre Toxicology* 10.

695 Shimizu, M., Tainaka, H., Oba, T., Mizuo, K., Umezawa, M., Takeda, K., 2009. Maternal
696 exposure to nanoparticulate titanium dioxide during the prenatal period alters gene expression
697 related to brain development in the mouse. *Particle and Fibre Toxicology* 6.

698 Stapleton, P.A., McBride, C.R., Yi, J., Nurkiewicz, T.R., 2015. Uterine microvascular
699 sensitivity to nanomaterial inhalation: An in vivo assessment. *Toxicol Appl Pharmacol* 288,
700 420-428.

701 Stone, K.C., Mercer, R.R., Gehr, P., Stockstill, B., Crapo, J.D., 1992. Allometric
702 Relationships of Cell Numbers and Size in the Mammalian Lung. *Am J Resp Cell Mol* 6, 235-
703 243.

704 Subramanian, A., Tamayo, P., Mootha, V.K., Mukherjee, S., Ebert, B.L., Gillette, M.A.,
705 Paulovich, A., Pomeroy, S.L., Golub, T.R., Lander, E.S., Mesirov, J.P., 2005. Gene set

706 enrichment analysis: a knowledge-based approach for interpreting genome-wide expression
707 profiles. *Proc Natl Acad Sci U S A* 102, 15545-15550.

708 Sun, Y., Liang, J., Tang, L., Li, H., Zhu, Y., Jiang, D.N., Song, B., Chen, M., Zeng, G.M.,
709 2019. Nano-pesticides: A great challenge for biodiversity? *Nano Today* 28.

710 Takahashi, Y., Mizuo, K., Shinkai, Y., Oshio, S., Takeda, K., 2010. Prenatal exposure to
711 titanium dioxide nanoparticles increases dopamine levels in the prefrontal cortex and
712 neostriatum of mice. *J Toxicol Sci* 35, 749-756.

713 Tamayo, P., Steinhardt, G., Liberzon, A., Mesirov, J.P., 2016. The limitations of simple
714 gene set enrichment analysis assuming gene independence. *Stat Methods Med Res* 25, 472-
715 487.

716 Tankersley, C.G., Fitzgerald, R.S., Kleeberger, S.R., 1994. Differential Control of
717 Ventilation among Inbred Strains of Mice. *Am J Physiol-Reg I* 267, R1371-R1377.

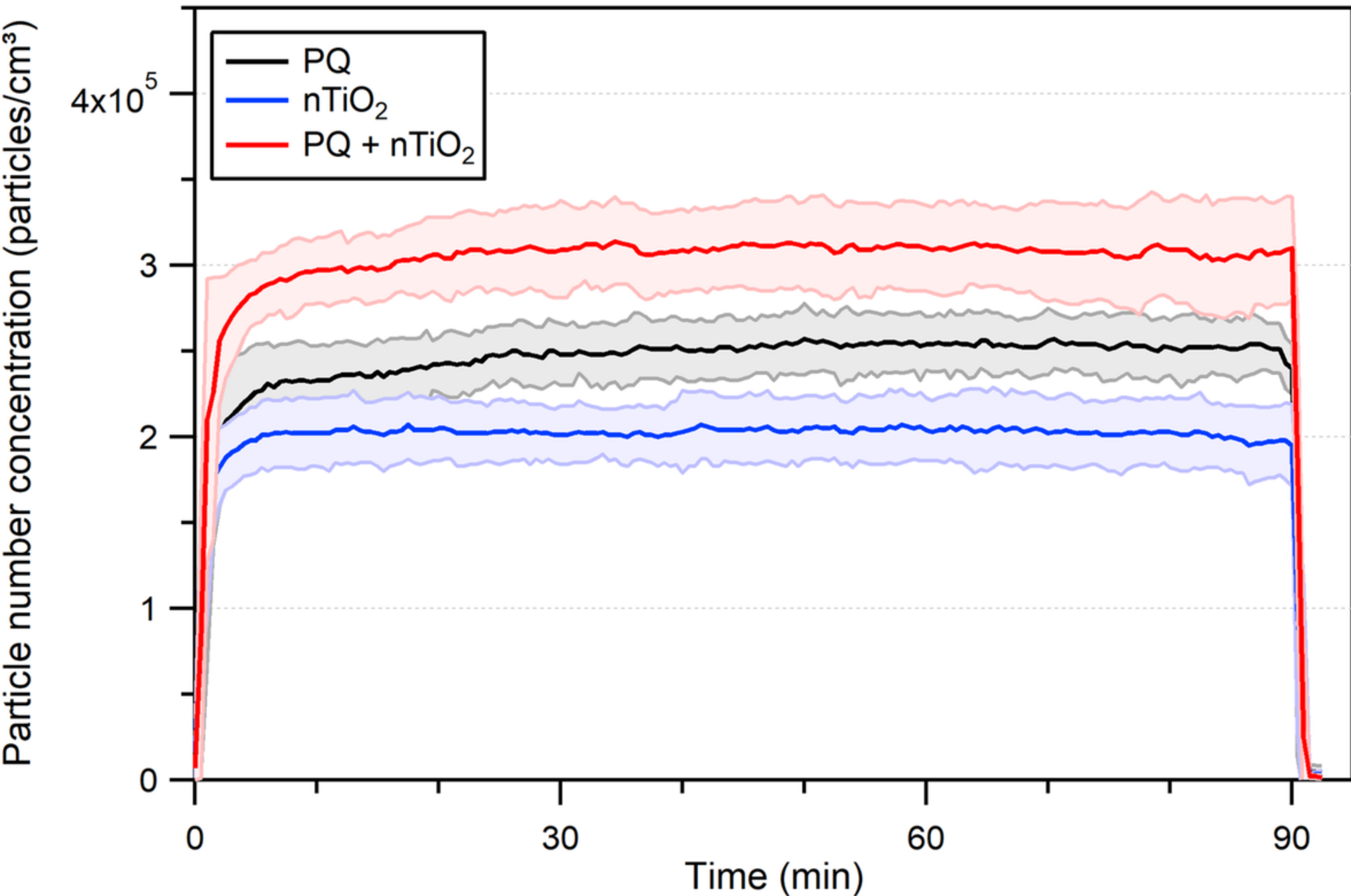
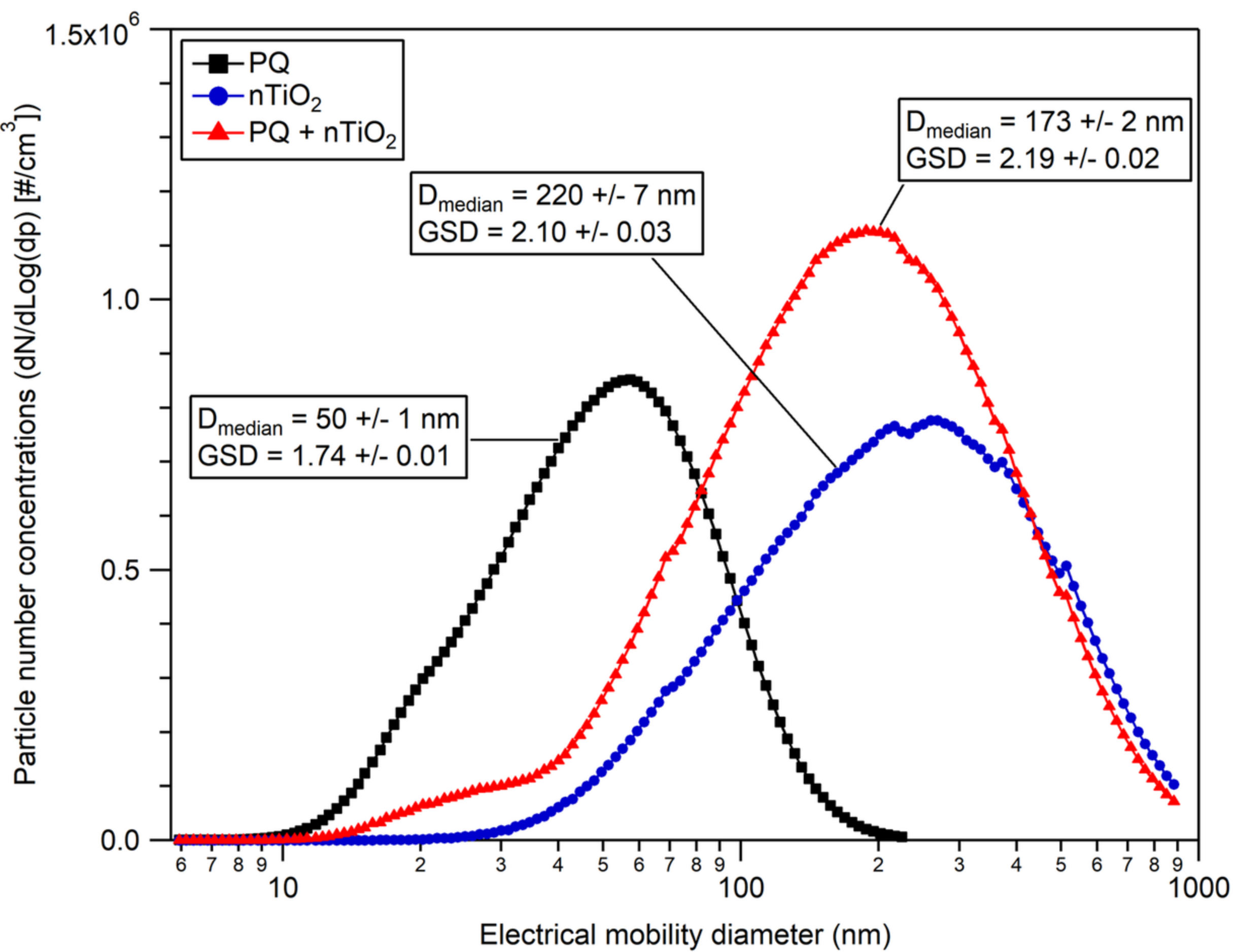
718 Tanner, C.M., Kamel, F., Ross, G.W., Hoppin, J.A., Goldman, S.M., Korell, M., Marras,
719 C., Bhudhikanok, G.S., Kasten, M., Chade, A.R., Comyns, K., Richards, M.B., Meng, C.,
720 Priestley, B., Fernandez, H.H., Cambi, F., Umbach, D.M., Blair, A., Sandler, D.P., Langston,
721 J.W., 2011. Rotenone, Paraquat, and Parkinson's Disease. *Environ Health Persp* 119, 866-872.

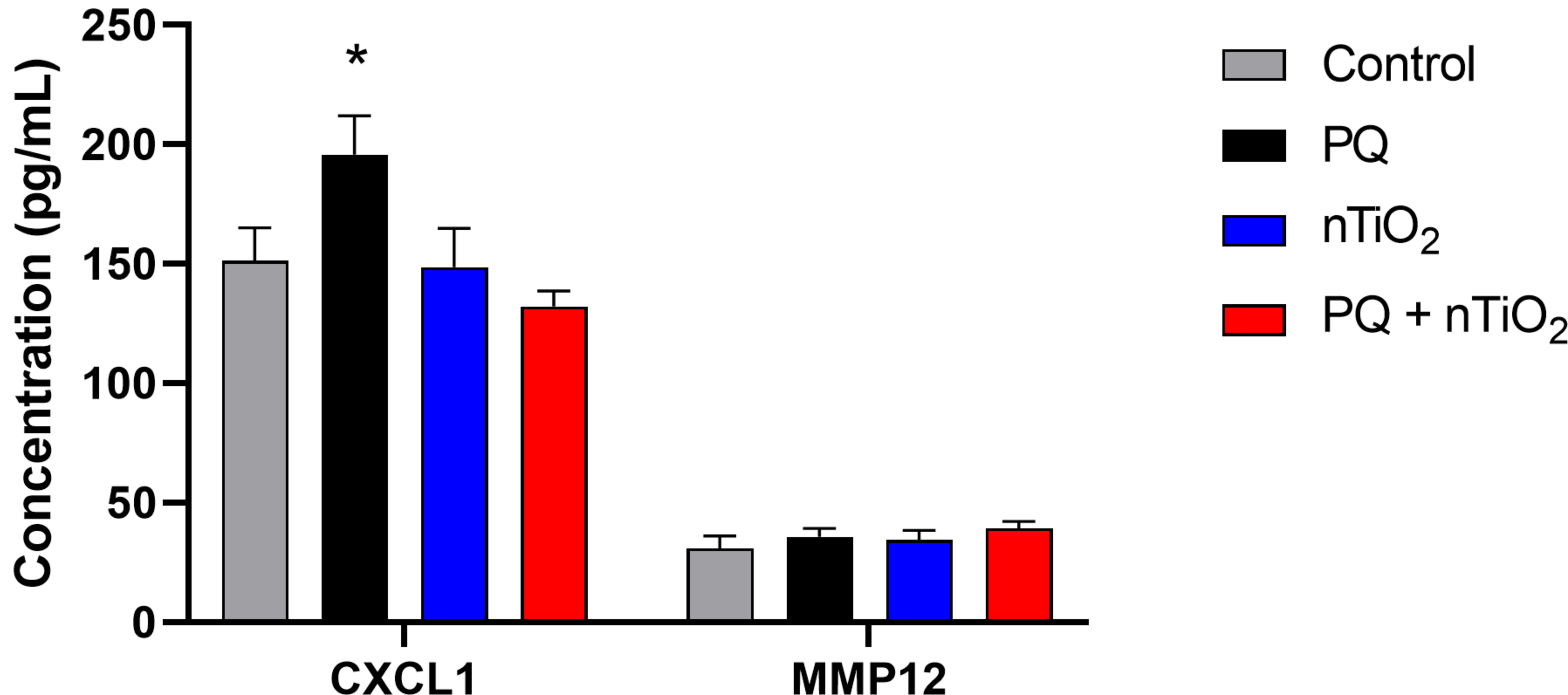
722 Tomita, M., Okuyama, T., Katsuyama, H., Miura, Y., Nishimura, Y., Hidaka, K., Otsuki,
723 T., Ishikawa, T., 2007. Mouse model of paraquat-poisoned lungs and its gene expression
724 profile. *Toxicology* 231, 200-209.

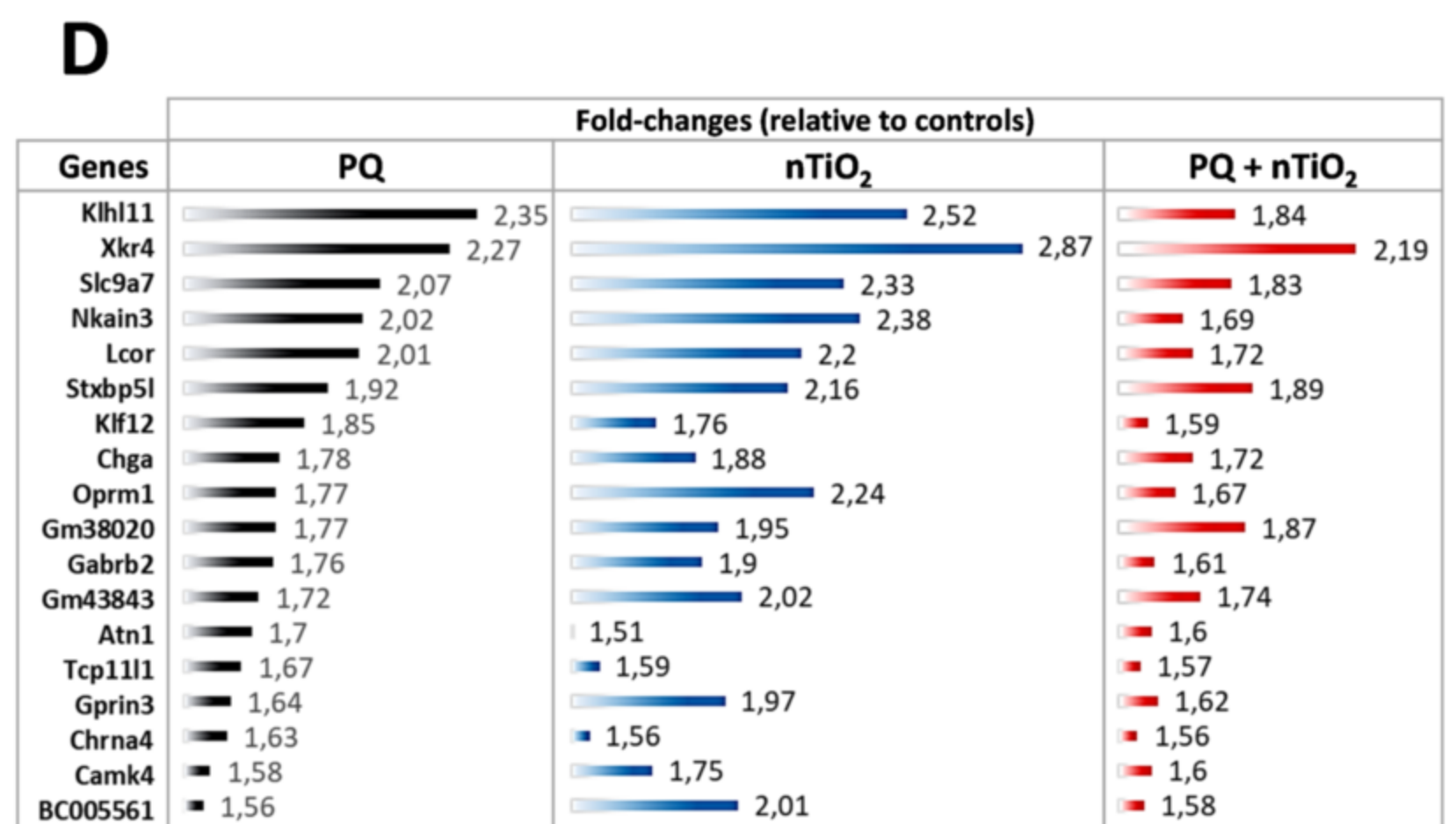
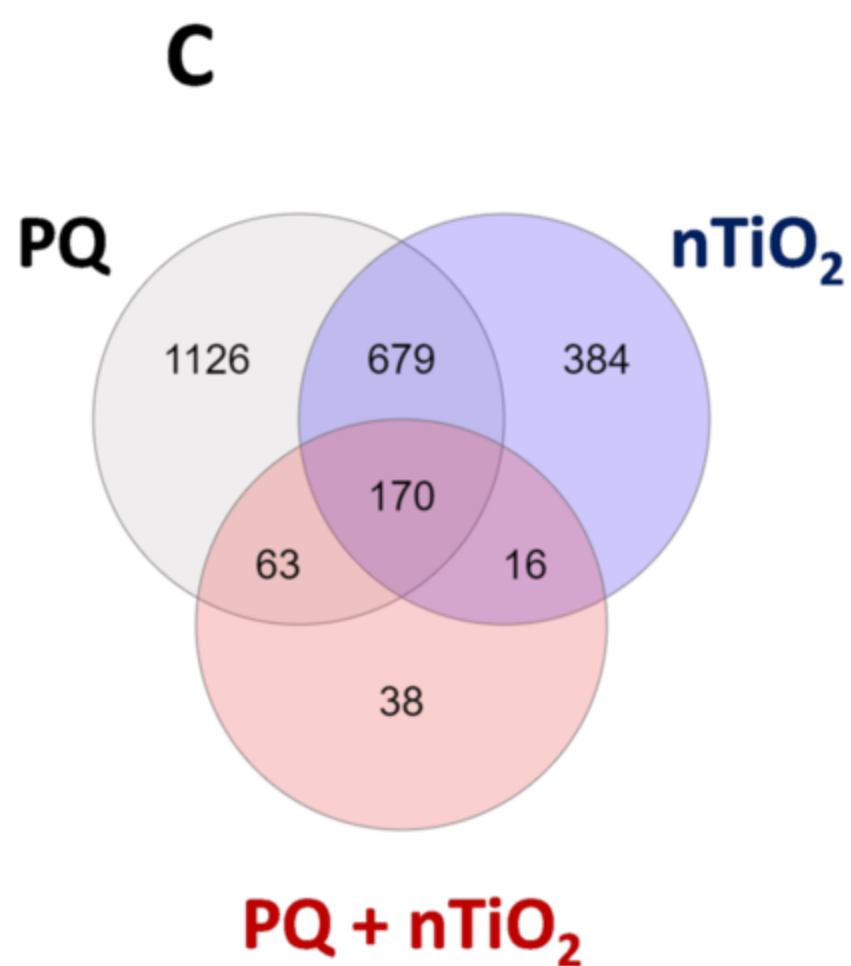
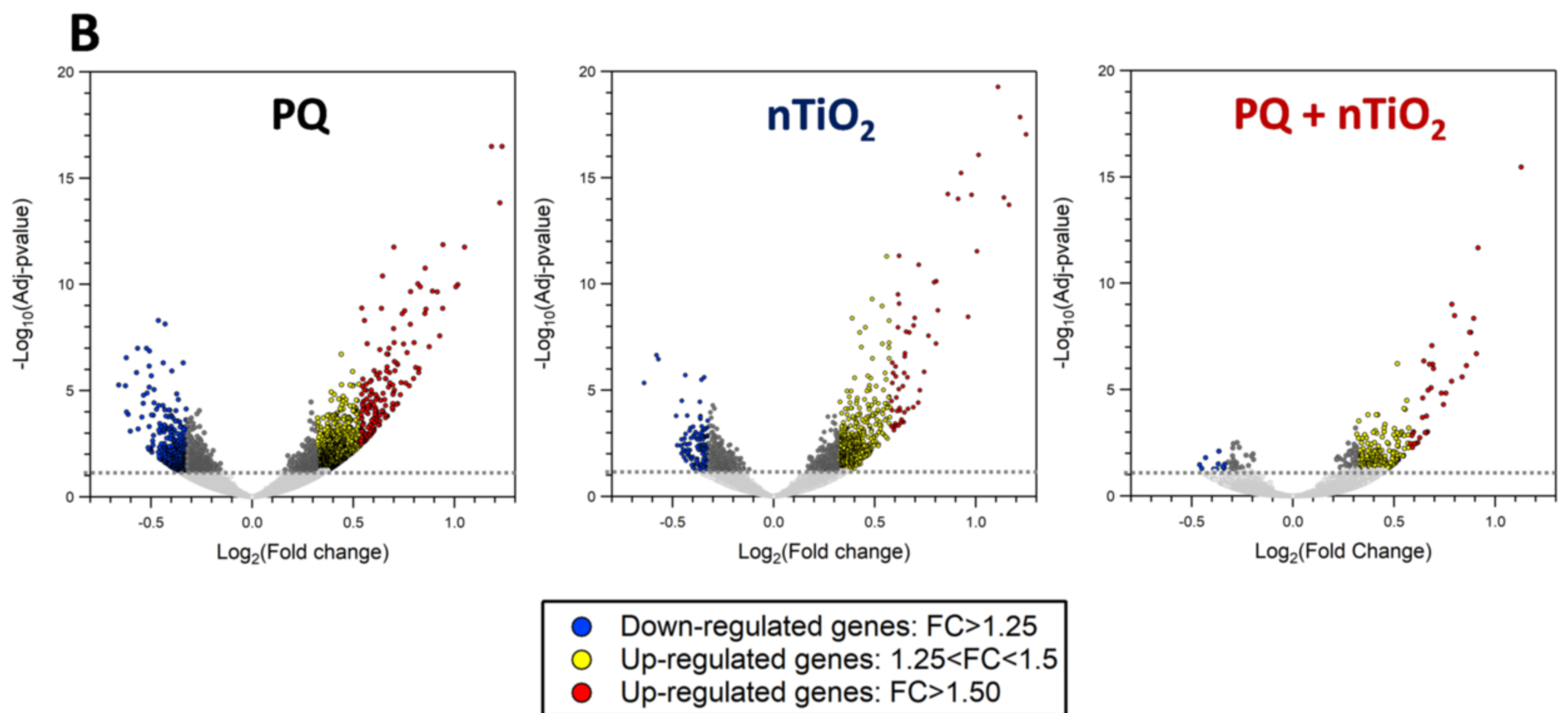
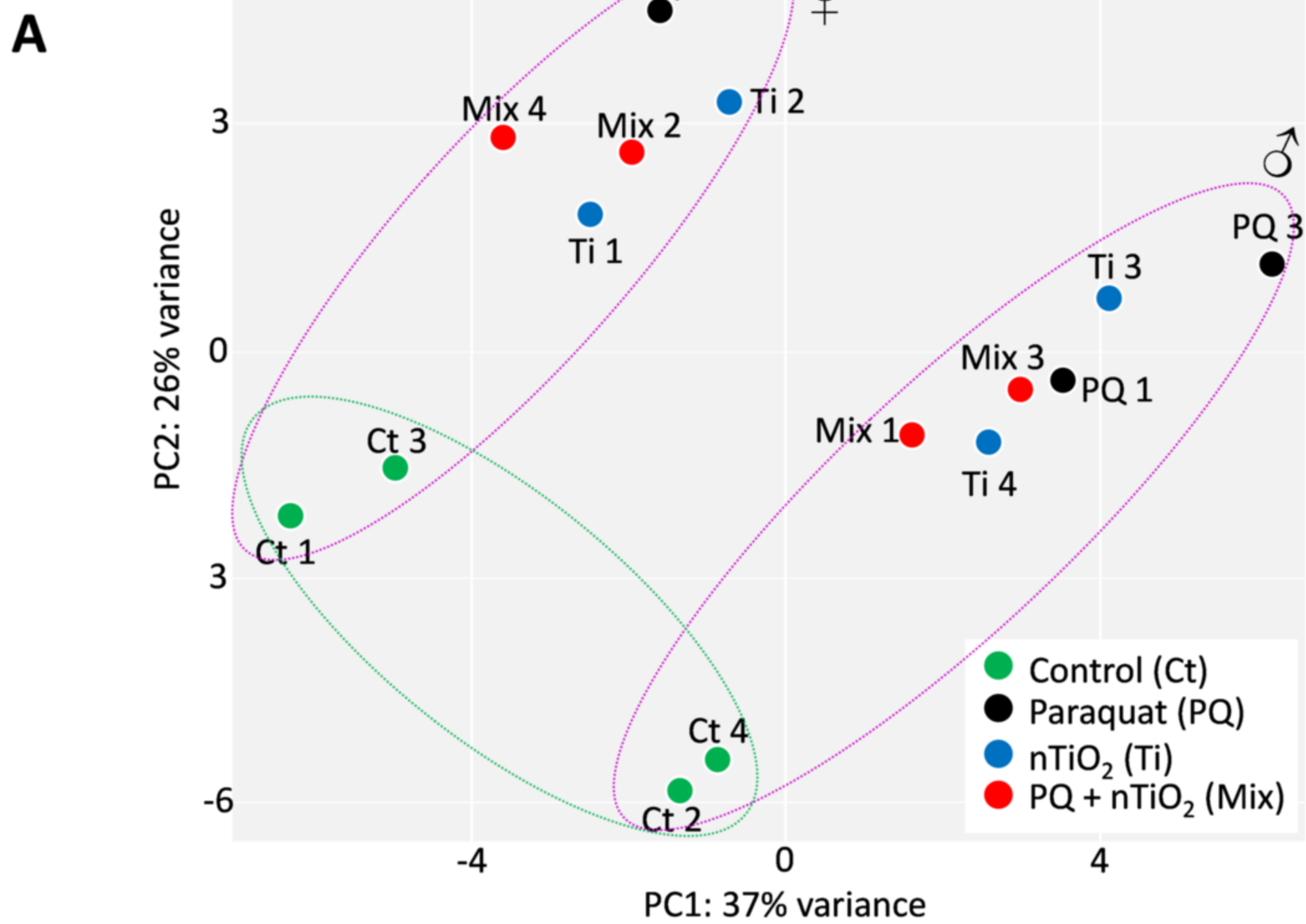
725 Umezawa, M., Tainaka, H., Kawashima, N., Shimizu, M., Takeda, K., 2012. Effect of fetal
726 exposure to titanium dioxide nanoparticle on brain development - brain region information. *J*
727 *Toxicol Sci* 37, 1247-1252.

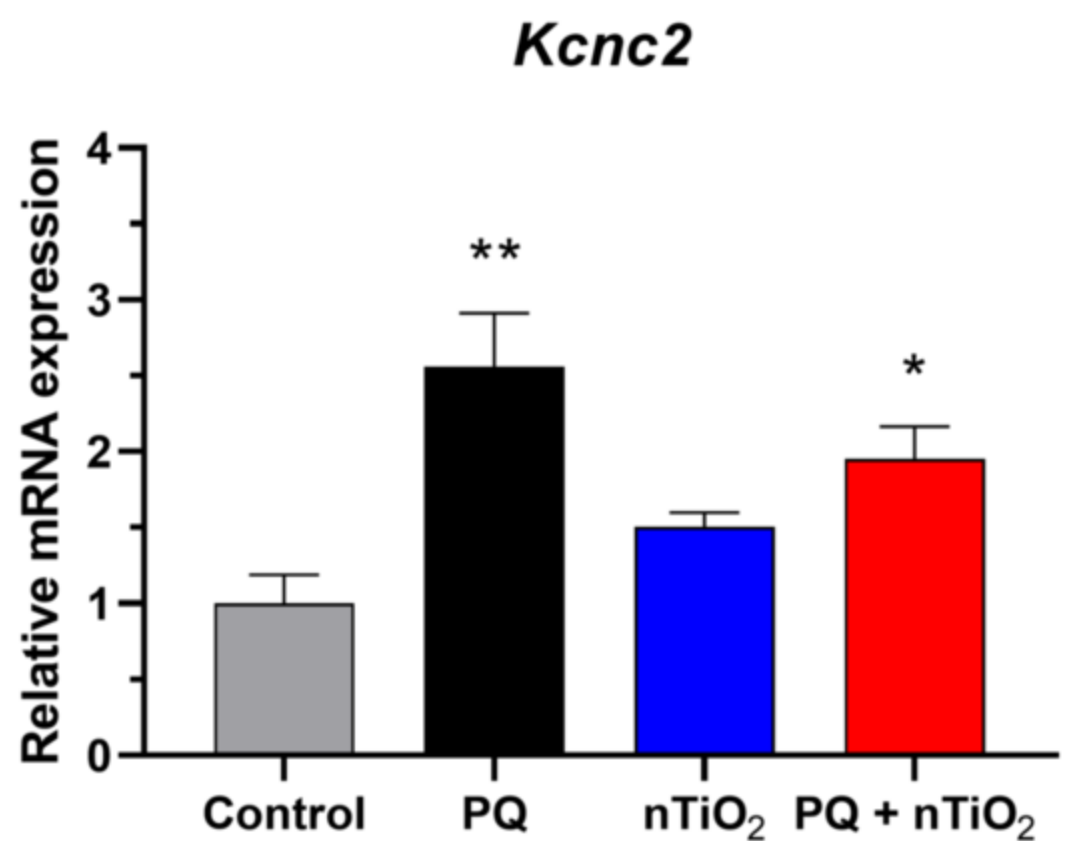
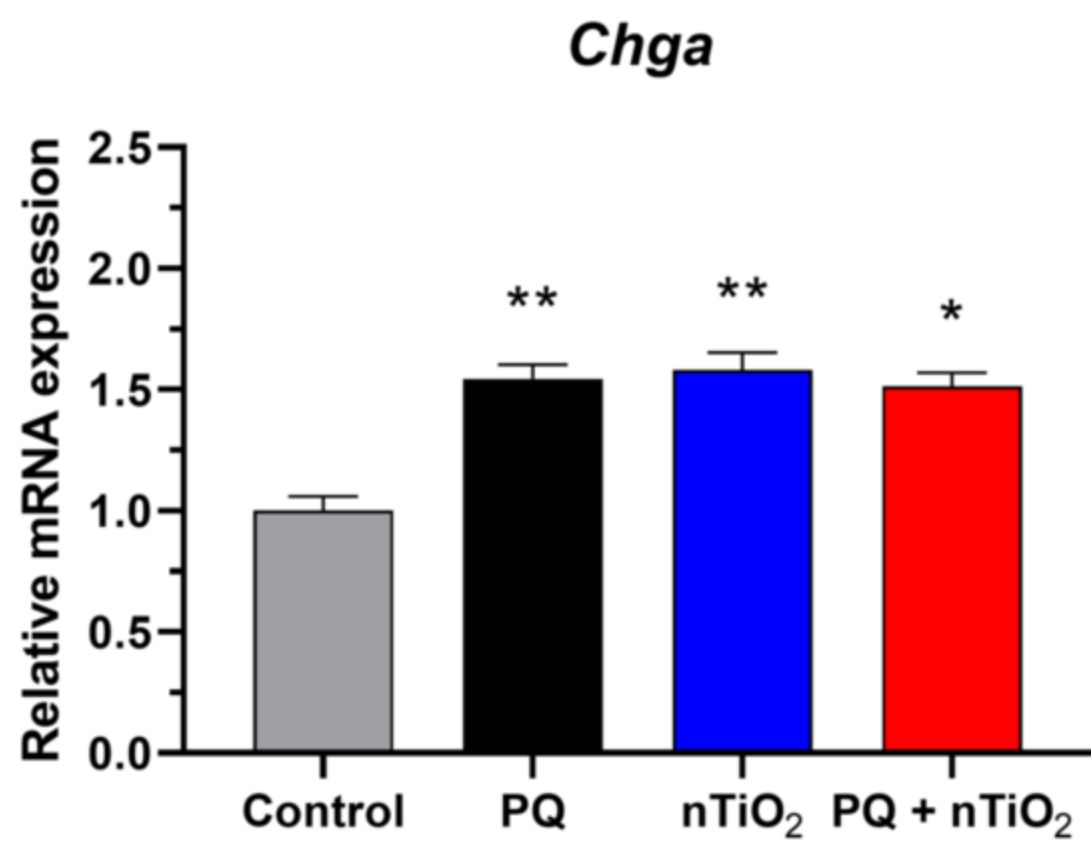
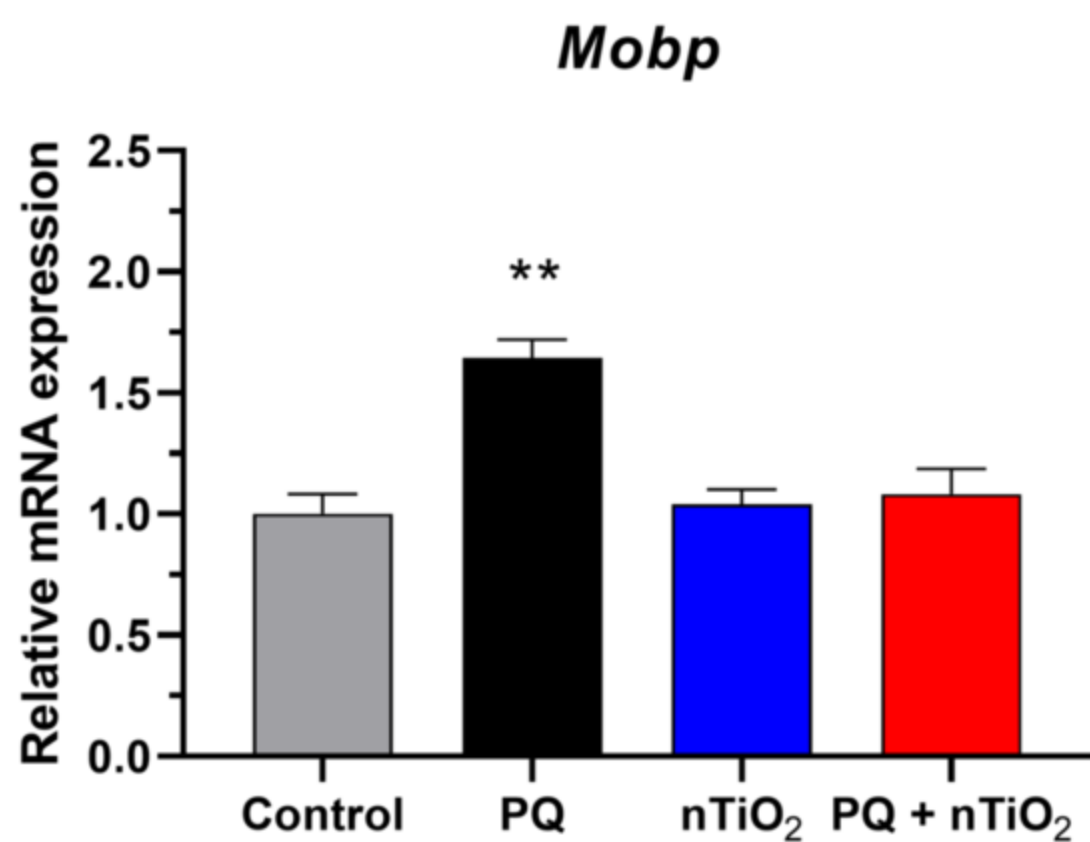
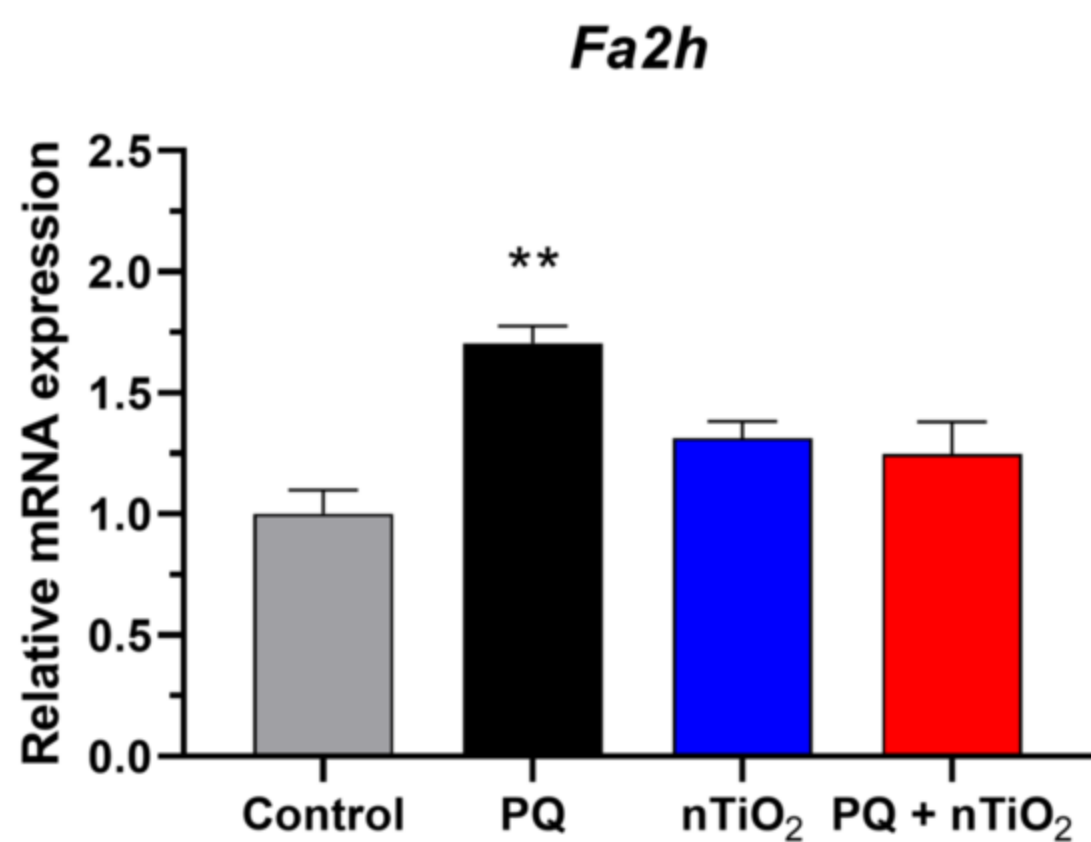
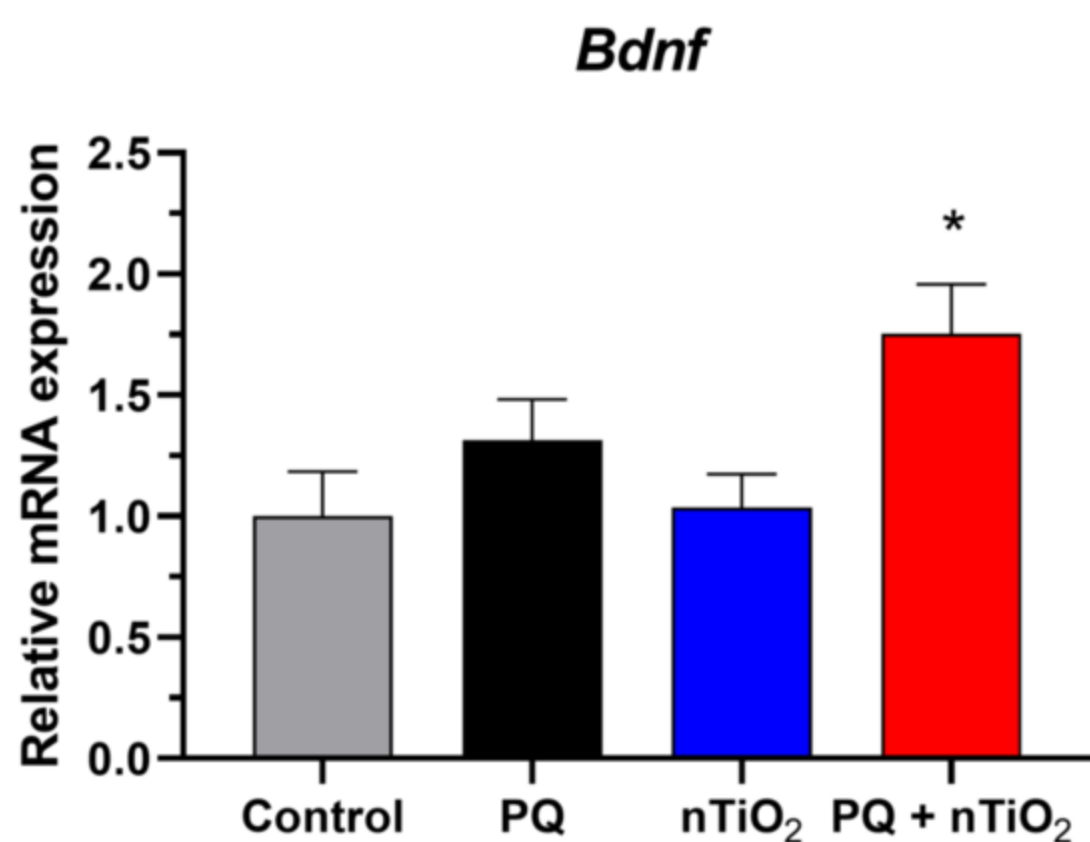
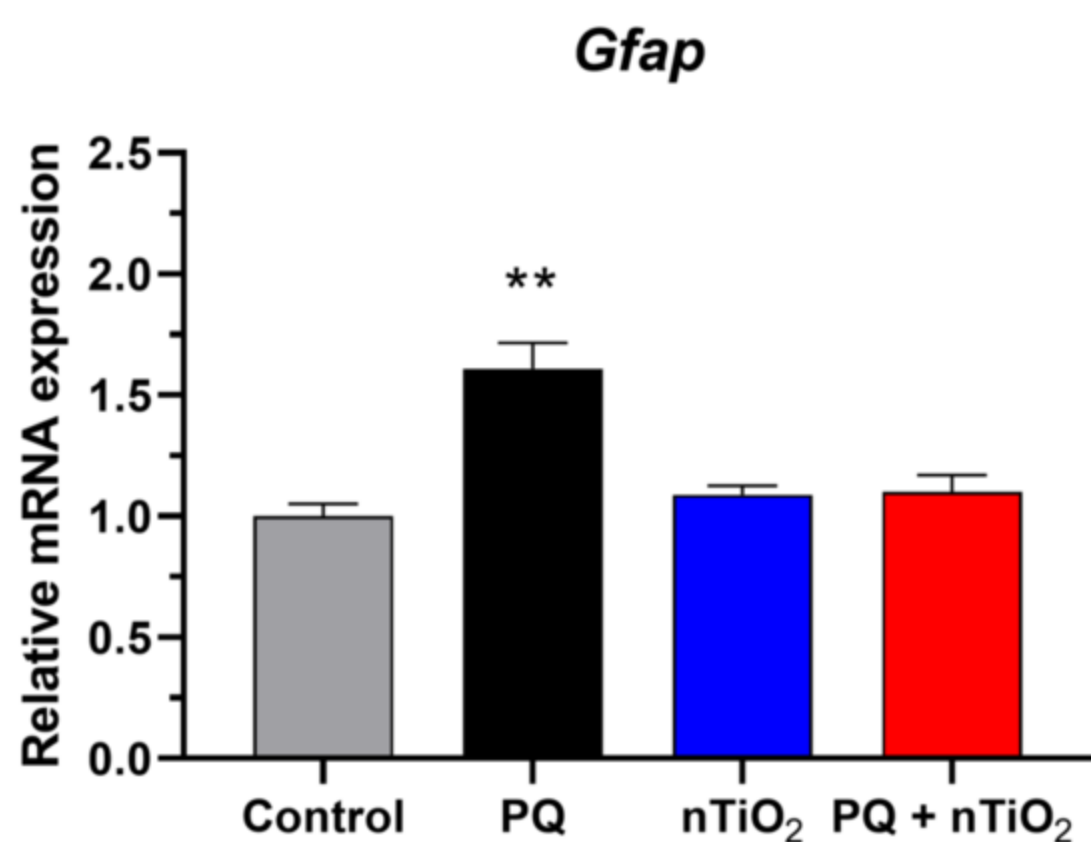
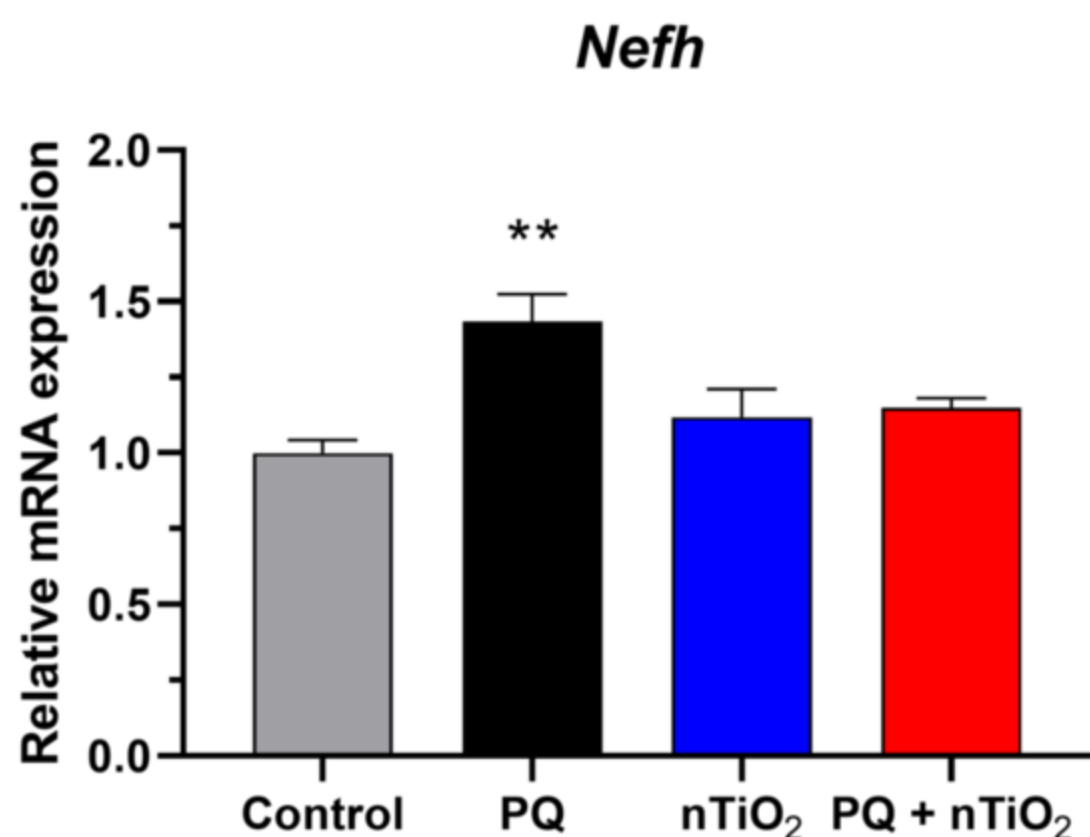
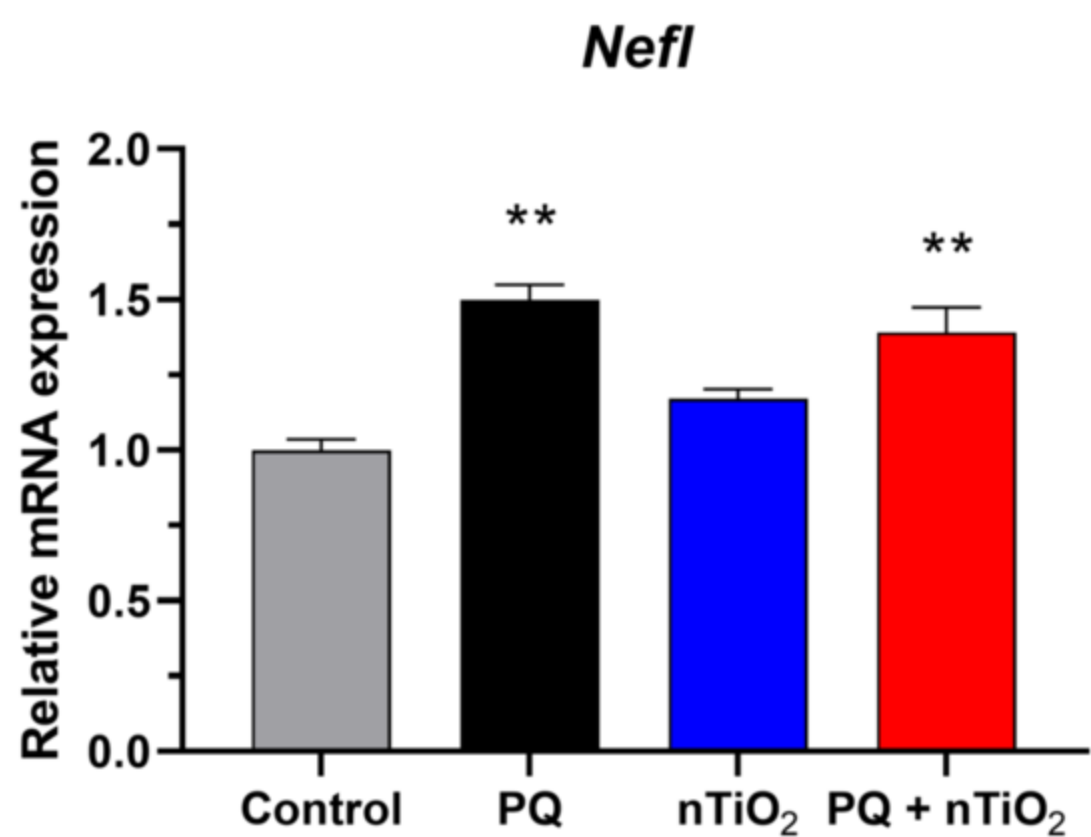
728 Vohra, M.S., Tanaka, K., 2003. Photocatalytic degradation of aqueous pollutants using
729 silica-modified TiO₂. *Water Res* 37, 3992-3996.

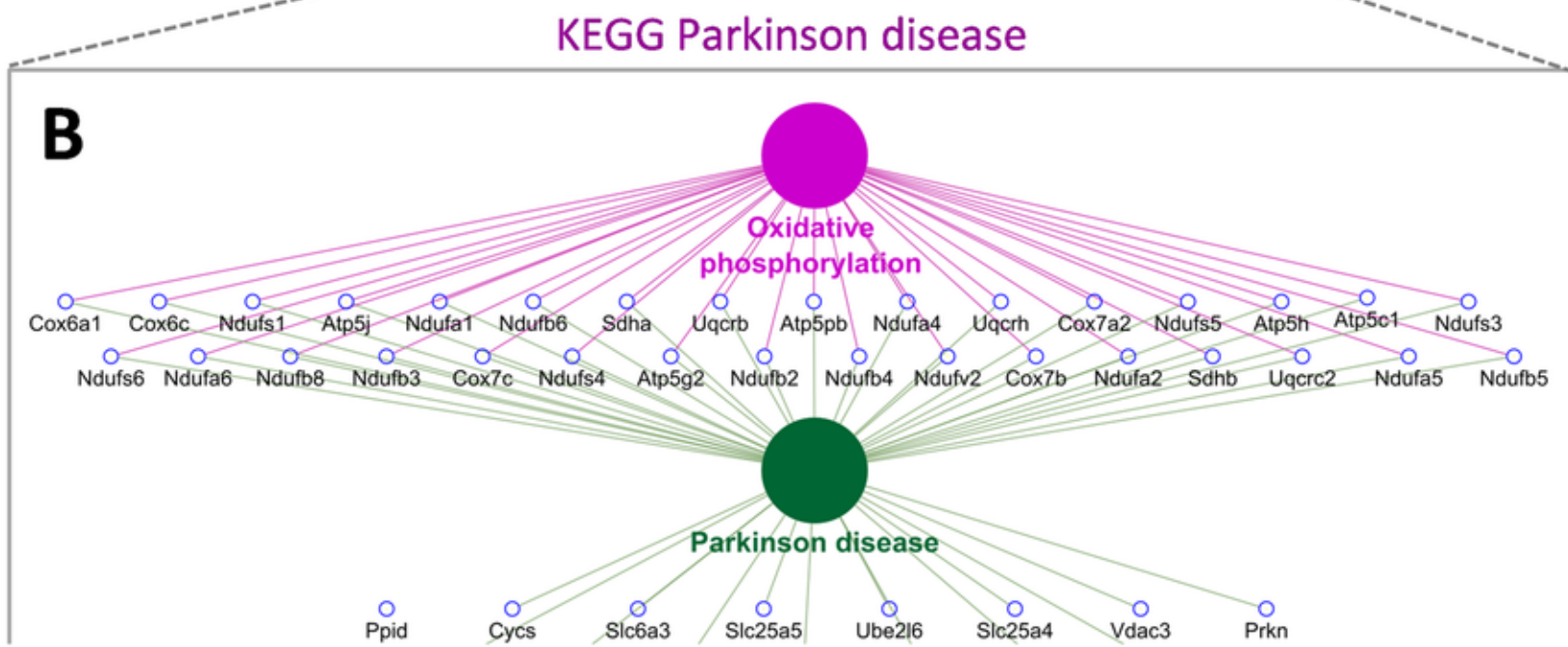
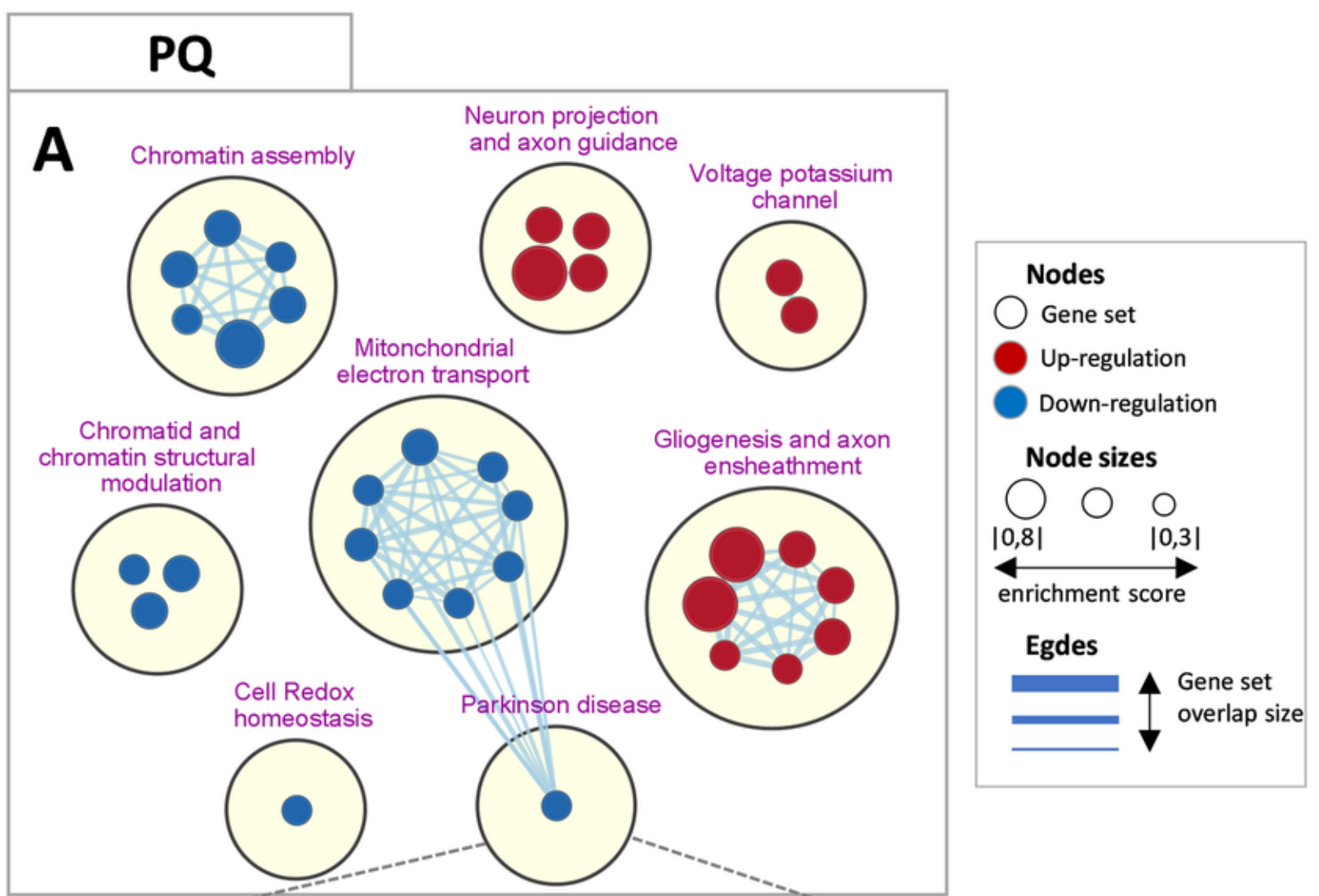
- 730 Wang, A., Costello, S., Cockburn, M., Zhang, X.B., Bronstein, J., Ritz, B., 2011.
731 Parkinson's disease risk from ambient exposure to pesticides. *Eur J Epidemiol* 26, 547-555.
- 732 Wang, Y., Sun, C., Zhao, X., Cui, B., Zeng, Z., Wang, A., Liu, G., Cui, H., 2016. The
733 Application of Nano-TiO₂ Photo Semiconductors in Agriculture. *Nanoscale Res Lett* 11, 529.
- 734 Yang, M.S., Chan, H.W., Yu, L.C., 2006. Glutathione peroxidase and glutathione
735 reductase activities are partially responsible for determining the susceptibility of cells to
736 oxidative stress. *Toxicology* 226, 126-130.
- 737 Zhang, X.C., Li, W., Yang, Z., 2015. Toxicology of nanosized titanium dioxide: an update.
738 *Archives of Toxicology* 89, 2207-2217.
- 739

A**B**



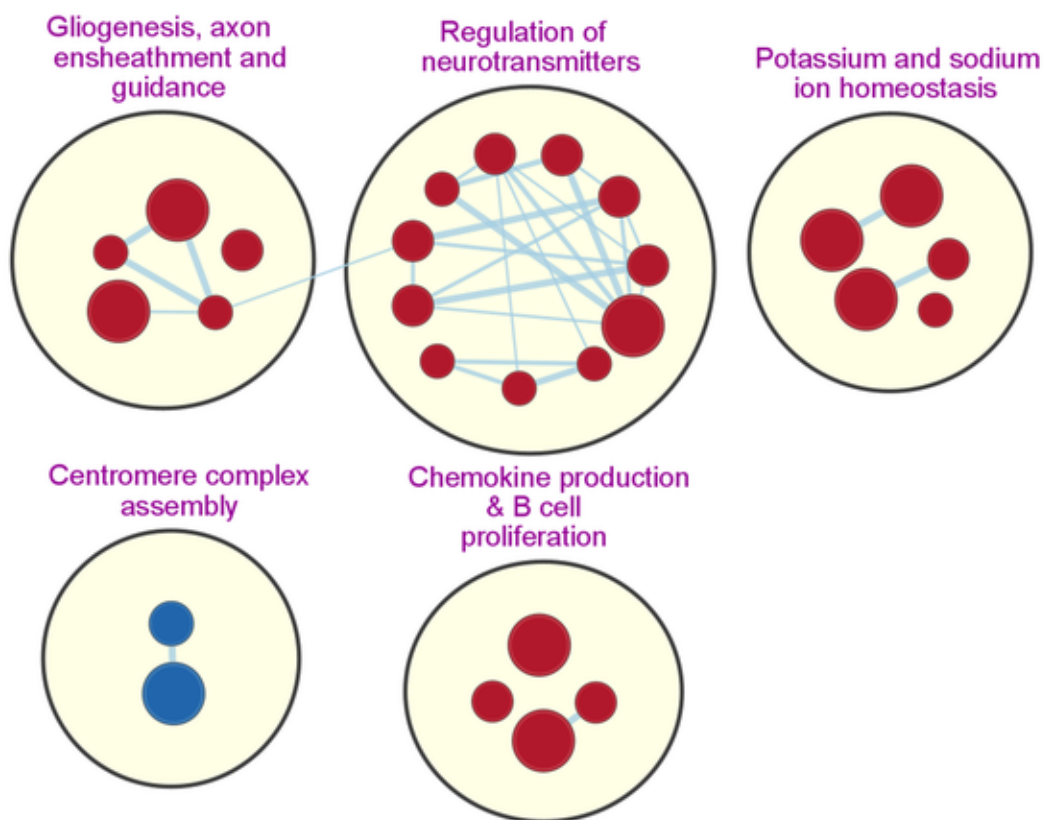




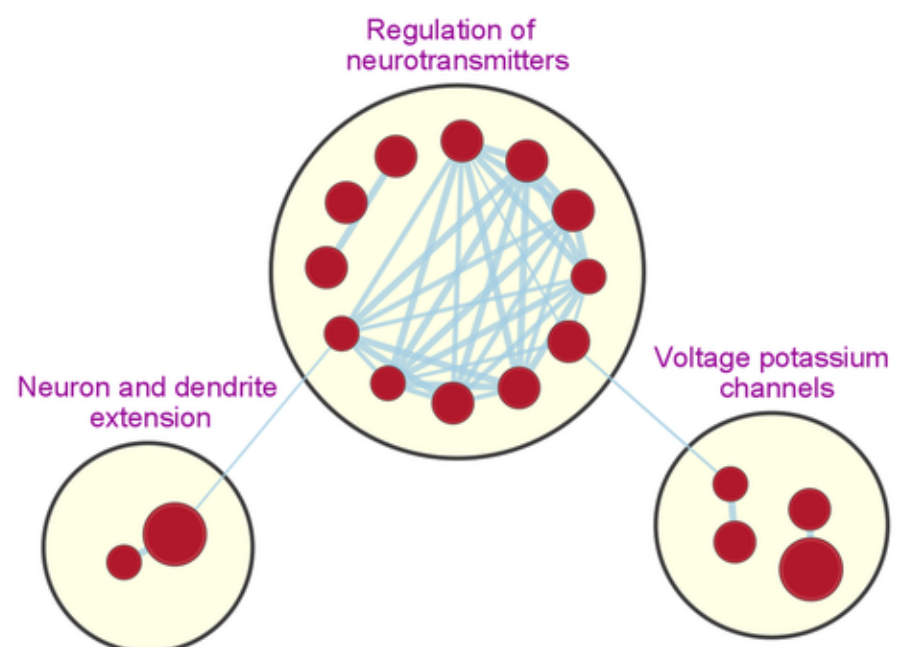


C

nTiO₂



PQ+ nTiO₂



GO regulation of neurotransmitter levels

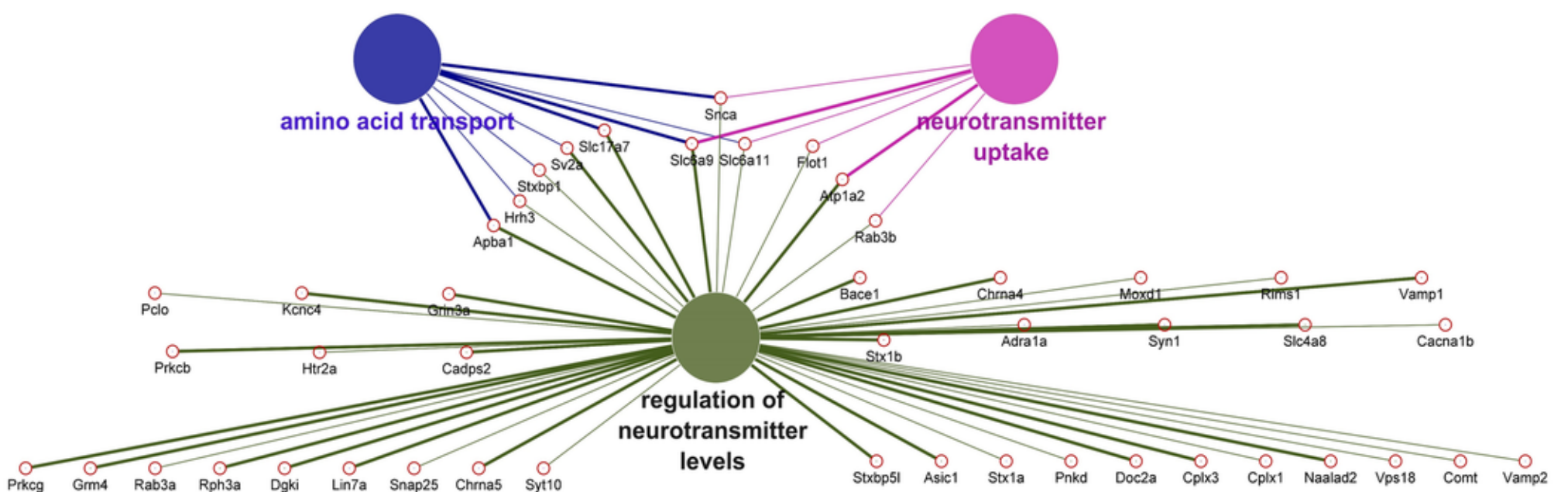


Table 1. Aerosol characteristics

| Aerosol | PQ | nTiO₂ | PQ + nTiO₂ |
|---|----------------------------------|-------------------------|----------------------------------|
| Mass concentration (mg/m ³) | *[98.7 ± 3.2] × 10 ⁻³ | 10.1 ± 0.6 | 9.9 ± 0.8 |
| Number concentration [× 10 ³ particles/cm ³] | 249 ± 17 | 202 ± 17 | 308 ± 27 |
| *Particle median diameter (nm) | 50 ± 1 | 220 ± 7 | 173 ± 2 |
| *Geometric standard deviation | 1.74 ± 0.01 | 2.10 ± 0.03 | 2.29 ± 0.02 |

Means ± SD are shown. * values which were not measured during the animal exposure sessions but during the prior metrological characterization of the exposure device.