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# The foodborne contaminant deoxynivalenol exacerbates DNA damage caused by a broad spectrum of genotoxic agents

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## 35 INTRODUCTION

36 Humans are exposed to a broad spectrum of food-contaminants, including mycotoxins  
37 (Payros *et al.*, 2021a). These toxins produced by fungi are the most common naturally  
38 occurring food contaminants and global surveys have estimated that they contaminate up to  
39 70% of world crop production (Streit *et al.*, 2013; Eskola *et al.*, 2020). Mycotoxins can persist  
40 during food processing and are thus found in the consumer's meals (Sugita-Konishi *et al.*,  
41 2006).

42 Deoxynivalenol (DON) is a widespread mycotoxin in food. A recent survey by the  
43 European Food Safety Authority (EFSA) reported that almost 50% of cereals are  
44 contaminated by this toxin; the highest levels being measured in wheat, maize, and oat grains  
45 (Knutsen *et al.*, 2017). Recent assessments using urinary levels as a biomarker, revealed that  
46 around 80% of individuals are exposed to DON (Turner *et al.*, 2008; De Santis *et al.*, 2019).  
47 Based on its toxicity, a tolerable daily intake (TDI) of 1 µg DON/kg body weight/day has  
48 been defined by JEFCA and EFSA (JEFCA, 2011; Knutsen *et al.*, 2017). However, this TDI  
49 can be exceeded in some population groups, especially in children (Knutsen *et al.*, 2017; Vin  
50 *et al.*, 2020).

51 Acute exposure to DON is associated with vomiting and bloody diarrhea (Ruan *et al.*,  
52 2020) while chronic exposure decreases food consumption, induces neuro-endocrine changes,  
53 and alters immune functions (Maresca, 2013; Pinton *et al.*, 2015; Robert *et al.*, 2017; Terziolo  
54 *et al.*, 2018). Upon ingestion of contaminated food, intestinal epithelial cells are the first  
55 target of DON (Maresca, 2013; Pinton and Oswald, 2014; Graziani *et al.*, 2015). Its toxicity  
56 arises from its capacity to bind and inhibit the peptidyl transferase center in the 60S subunit of  
57 the ribosome (Garreau de Loubresse *et al.*, 2014; Pierron *et al.*, 2016a). This results in the  
58 inactivation of protein synthesis and a “ribotoxic stress response”, which leads to the  
59 activation of MAP kinases and their downstream pathways including inflammatory response  
60 and oxidative stress (Pestka, 2008; Mishra *et al.*, 2014; Lucioli *et al.*, 2013; Da Silva,  
61 Bracarense and Oswald, 2018; Payros *et al.*, 2016). DON alters intestinal epithelium  
62 morphology, impairs the barrier function and nutrient absorption (Ghareeb *et al.*, 2014;  
63 Pierron *et al.*, 2016b; Pinton *et al.*, 2009), modifies intestinal microbiota (Waché *et al.*, 2009),  
64 triggers intestinal inflammation (Maresca *et al.*, 2008; García *et al.*, 2018; Pestka, 2010) and  
65 increases susceptibility to intestinal inflammatory diseases (Payros *et al.*, 2020, 2021b).

66 DON is not genotoxic and is not classified as carcinogenic by the International  
67 Agency for Research on Cancer (IARC) (International Agency for Research on Cancer,  
68 1993). However, we previously showed that DON exacerbates DNA damage, characterized

69 by the phosphorylation of the histone H2AX ( $\gamma$ H2AX) induced by colibactin, an *Escherichia*  
70 *coli* genotoxin produced mainly in the intestine, and suspected of being involved in colorectal  
71 cancer (Nougayrede, 2006; Payros *et al.*, 2017; Pleguezuelos-Manzano *et al.*, 2020; Lopez,  
72 Bleich and Arthur, 2021). The aim of the present study was to investigate if realistic doses of  
73 DON exacerbate the genotoxicity caused by different DNA damaging agents, and if  
74 exacerbation is linked to its ribotoxicity.

75

## 76 **METHODS**

77 **Toxins and reagents.** DON, etoposide, cisplatin, anisomycin and cycloheximide were  
78 purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France), and phleomycin (closely  
79 related to bleomycin) from Invivogen (Toulouse, France). DOM-1, a kind gift from G.  
80 Schatzmayr and D. Moll, was obtained by transforming crystalline DON (Romer Labs, Tulln,  
81 Austria) as previously described (Pierron *et al.*, 2016a). Stock solutions were stored at -20 °C;  
82 etoposide (5 mM), phleomycin (13.78 mM), DON (5 mM), DOM-1 (5 mM) and anisomycin  
83 (75  $\mu$ M) were dissolved in DMSO; cisplatin (1.5 mM) and cycloheximide (18 mM) were  
84 dissolved in water.

85

86 **Cell treatments.** Non-transformed rat intestinal epithelial cells (IEC-6, ATCC CRL-1592)  
87 were cultured in complete DMEM GlutaMAX™ medium (Fisher Scientific) supplemented  
88 with 10% fetal calf serum, 1% non-essential amino acids (Fisher Scientific) and 0.1 U/mL  
89 bovine insulin (Sigma-Aldrich), at 37 °C with 5% CO<sub>2</sub>. Human colon adenocarcinoma cells  
90 (HT-29) were cultured in complete McCoy's 5a Modified medium (Fisher Scientific)  
91 supplemented with 10% fetal calf serum and 1% non-essential amino acids (Fisher Scientific),  
92 at 37 °C with 5% CO<sub>2</sub>. Cells were seeded in black 96-well plates (Greiner bio-one, Les Ulis,  
93 France) or Labtech (Fisher Scientific) and grown for 24-48 h to reach ~ 80% confluence  
94 before treatment. Cells were washed three times with warm HBSS before treatment. Cells  
95 were incubated for 4 h at 37 °C with 5% CO<sub>2</sub> in DMEM HEPES medium (Fisher Scientific)  
96 containing different concentrations of genotoxins (1 to 5  $\mu$ M etoposide, 15 to 25  $\mu$ M cisplatin,  
97 or 1 to 5  $\mu$ M for phleomycin) and ribotoxins. Control cells were treated with DMSO vehicle  
98 (Sigma-Aldrich). After treatment, cells were washed three times with cold PBS and fixed with  
99 4% formaldehyde (Fisher Scientific) for 20 min at room temperature before In-Cell Western  
100 or immunofluorescence assays.

101

102 **Immunofluorescence staining.** After fixation, cells were permeabilized for 15 min with PBS  
103 0.25% Triton X-100 and blocked for one hour in blocking solution (PBS 5% normal goat  
104 serum 0.01% Tween 20). Cells were incubated with monoclonal primary antibody anti  
105  $\gamma$ H2AX diluted 1:500 (mouse monoclonal clone JBW301, Millipore, Burlington, USA) and  
106 anti S33-pRPA32 diluted 1:500 (rabbit polyclonal, Bethyl, Montgomery, USA) in blocking  
107 solution, for 3.5 h at room temperature. Following washing in PBS 0.05% Triton X-100, cells  
108 were incubated for 2 h in the dark at room temperature with anti-mouse AlexaFluor 488 and  
109 anti-rabbit AlexaFluor 568 (Invitrogen, Waltham) diluted 1:1000. After three washes with  
110 PBS, Labtech were mounted using Fluoroshield containing DAPI (Sigma-Aldrich) and  
111 examined with a Zeiss LSM 710 confocal microscope.

112

113 **Quantification of DNA damage by In-Cell Western analysis.** Quantification of  
114  $\gamma$ H2AX by In-Cell Western analysis was performed as previously described (Martin *et al.*,  
115 2013; Theumer *et al.*, 2018). Briefly, the fixed cells were permeabilized with 0.2% Triton X-  
116 100 and incubated in Maxblock (Active Motif) before immuno-staining with rabbit  
117 monoclonal anti- $\gamma$ H2AX diluted 1:200 (20E3; Cell Signaling, Saint-Quentin en Yvelines,  
118 France) followed by near-infrared-fluorescent secondary antibody diluted 1:500 (IRDye  
119 800CW; Rockland) and staining of DNA with RedDot2 diluted 1:1000 (Biotium, Interchim,  
120 Montluçon, France). The DNA and  $\gamma$ H2AX signals were measured at 680 and 800 nm with an  
121 Odyssey infrared imaging scanner (LI-COR Science Tec, Les Ulis, France). The genotoxic  
122 index was calculated by dividing the  $\gamma$ H2AX fluorescence by the corresponding DNA  
123 fluorescence and normalized with the average fluorescence in untreated control cells (Tronnet  
124 and Oswald, 2018). All the data from three biological replicates are presented.

125

126 **Statistical analyses.** P-values were calculated using one-way analysis of variance  
127 (ANOVA) followed by multiple comparisons with Bonferroni's multiple-comparison correction  
128 using GraphPad Prism 7.0. For In-Cell Western analyses, the data are expressed as mean  $\pm$   
129 SEM.

130

## 131 **RESULTS**

### 132 **DON exacerbates the genotoxicity caused by etoposide, cisplatin and phleomycin**

133 To assess whether DON modifies the toxicity of a variety of genotoxins, cultured cells  
134 were treated with DON combined with one of the three DNA-damaging compounds with  
135 different modes of action: etoposide (ETP), a topoisomerase inhibitor, which causes DNA

136 double strand breaks (Hande, 1998); cisplatin (CPT), which causes DNA adducts and  
137 crosslinks (Dasari and Tchounwou, 2014); phleomycin (PHM), which causes oxidation of  
138 bases and single strand breaks (Chen and Stubbe, 2005). More precisely, non-transformed rat  
139 intestinal epithelial IEC-6 cells were treated for 4 h with 3  $\mu$ M ETP, 20  $\mu$ M CPT or 3  $\mu$ M  
140 PHM, alone or combined with 10  $\mu$ M DON or 10  $\mu$ M DMSO used as control (vehicle). DNA  
141 damage was visualized using immunofluorescence confocal microscopy of phosphorylated  
142 H2AX (called  $\gamma$ H2AX), a robust and quantitative DNA damage marker (Rogakou *et al.*,  
143 1998). Control cells and cells treated only with DON exhibited low basal levels of  $\gamma$ H2AX in  
144 nuclei (Figure 1). Treatment with the different genotoxins led to an increase in  $\gamma$ H2AX  
145 staining whereas cells treated with both DON and the genotoxins exhibited exacerbated  
146  $\gamma$ H2AX signals (Figure 1). Increased  $\gamma$ H2AX staining was also observed in human colon  
147 cancer HT-29 cells treated with DON and the genotoxins (Figure S1).

148 To confirm that treatment with DON increased DNA damage caused by the  
149 genotoxins, a second DNA damage marker, phosphorylated RPA32 (pRPA32), which is  
150 phosphorylated in response to genotoxic stress (Dueva and Iliakis, 2020), was examined.  
151 Cotreatment with DON and the genotoxins also increased the levels of pRPA32 compared to  
152 the genotoxins alone (Figure S2). In conclusion, DON exacerbates the DNA damage caused  
153 by a variety of DNA-damaging compounds with different modes of action.

154

### 155 **DON induces dose-dependent exacerbation of the DNA damage caused by a variety of** 156 **genotoxins**

157 To quantify the exacerbation of DNA damage caused by the mycotoxin, IEC-6 cells  
158 were treated with varying doses of the three genotoxins together with 10  $\mu$ M DON, then  
159  $\gamma$ H2AX levels in the cell population were measured by In-Cell Western assay. DNA damage  
160 increased when the dose of etoposide was increased from 1  $\mu$ M to 5  $\mu$ M. Additionally, for  
161 each dose of etoposide, cotreatment with 10  $\mu$ M DON markedly exacerbated DNA damage.  
162 Similarly, DNA damage caused by cisplatin and phleomycin increased with an increase in the  
163 dose of genotoxin and were significantly exacerbated at a dose of 10  $\mu$ M DON (Figure 2).

164 To assess whether DON at realistic doses could exacerbate the DNA damage caused  
165 by the different genotoxins, the cells were treated with single doses of DNA-damaging  
166 compounds combined with DON at doses ranging from 0.3  $\mu$ M to 10  $\mu$ M. DNA damage  
167 exacerbation increased with an increase in the dose of DON from 1  $\mu$ M DON for cisplatin and  
168 phleomycin, and from 3  $\mu$ M DON for etoposide (Figure 3, Figure S3). DON exacerbation of  
169 DNA damage was not associated with cell death assessed by DNA staining and quantification

170 (Table S1), consistent with the normal morphology of DAPI-stained nuclei in the cells treated  
171 with both DON and the genotoxins (Figure 1). Taken together, these results show that DON  
172 exacerbates DNA damage caused by different genotoxins at a dose as low as 3  $\mu$ M.

### 173 **De-epoxy-deoxynivalenol (DOM-1) does not exacerbate the DNA damage caused by the** 174 **different genotoxins**

175 To probe the mechanism by which DON exacerbates the DNA damage, we  
176 investigated if ribosome inhibition was implicated. Cells were treated with de-epoxy-  
177 deoxynivalenol (DOM-1), a detoxified form of DON that binds to but does not inhibit the  
178 ribosome (Pierron *et al.*, 2016a). In contrast to cells treated with the genotoxins and DON,  
179  $\gamma$ H2AX levels were similar in cells treated with the genotoxins alone or combined with 10  
180  $\mu$ M DOM-1 (Figure 4). DOM-1 and/or the genotoxins did not result in cell death (Table S1).  
181 Thus, non-ribotoxic DOM-1 does not exacerbate the DNA damage caused by different  
182 genotoxic compounds.

183

### 184 **The ribosome inhibitors anisomycin and cycloheximide exacerbate the DNA damage** 185 **caused by the different genotoxins**

186 To confirm the role of ribosome inhibition in exacerbating DNA damage, the capacity  
187 of two ribosome inhibitors with distinct targets to exacerbate DNA damage was tested. Cells  
188 were treated with anisomycin, which, like DON, inhibits the A site of the ribosome, or with  
189 cycloheximide, which binds to the E site and interferes in the translocation step of protein  
190 synthesis (Schneider-Poetsch *et al.*, 2010). Both inhibitors significantly increased the DNA  
191 damage in the cells cotreated with etoposide, cisplatin or phleomycin, in a similar way to  
192 DON without causing cell death (Figure 5, Table S1). Altogether, these results indicate that  
193 the ribotoxicity induced by DON does exacerbate DNA damage.

194

## 195 **DISCUSSION**

196 Given its intrinsic toxicity and prevalence, DON is a major concern for food safety  
197 (Knutsen *et al.*, 2017; Payros *et al.*, 2016). The toxicity of DON is well documented, but little  
198 is known about its interactions with other toxins (Alassane-Kpembi *et al.*, 2017; Luo *et al.*,  
199 2019). We recently observed that DON increases genotoxicity induced by colibactin, a  
200 bacterial toxin that causes peculiar DNA-interstrand crosslink lesions (Payros *et al.*, 2017;  
201 Bossuet-Greif *et al.*, 2018; Xue *et al.*, 2019). The aim of the present study was to determine  
202 whether this phenotype extends to other genotoxins with other modes of action, and, if so, to  
203 investigate the mechanism involved. We observed that DON also exacerbates the genotoxicity

204 of three well-known drugs: etoposide, phleomycin and cisplatin, that respectively induce  
205 DNA double strand breaks, single strand breaks, and adducts (Smart *et al.*, 2008; Povirk,  
206 1996; Siddik, 2003). Thus, although DON is not inherently genotoxic, it exacerbates DNA  
207 damage caused by a broad spectrum of genotoxic agents.

208 The exacerbation of DNA damage caused by DON has been linked to its ribotoxicity.  
209 Indeed, the non-ribotoxic DON derivative DOM-1 (Pierron *et al.*, 2016a) did not exacerbate  
210 genotoxicity. Conversely, ribotoxins with modes of action similar to or distinct from that of  
211 DON (anisomycin and cycloheximide respectively) reproduced the DNA damage  
212 exacerbating phenotype, suggesting that the ribosome inhibitor mode of action is not critical  
213 for DNA damage exacerbation. The mechanism by which DON and other ribotoxins  
214 aggravate the genotoxicity of various genotoxins remains to be identified but several  
215 hypotheses can be proposed. First, ribosomes play an important role in genome preservation  
216 through ribosomal proteins that have a direct role in DNA repair (Mao-De and Jing, 2006). In  
217 addition, upon genotoxic stress, the cell reprograms mRNA translation to quickly synthesize  
218 proteins involved in the stress response (Kabilan *et al.*, 2020; Spriggs, Bushell and Willis,  
219 2010). Thus, DON interference with ribosome function could increase DNA damage by  
220 disturbing DNA damage response. Second, ribotoxins such as DON and anisomycin trigger  
221 the “ribotoxic stress response” with the recruitment of its main mediator, the protein kinase R  
222 (PKR) (Zhou *et al.*, 2014). Activated PKR has been reported to interact functionally with  
223 DNA repair proteins, to repress the repair response, and to sensitize the cells to DNA  
224 damaging agents (Bennett *et al.*, 2006; Zhang *et al.*, 2004). Third, DON and ribotoxins have  
225 been reported to cause upregulation of inflammatory cytokines such as interleukins or IFN $\gamma$ ,  
226 and expression of transcription factors such as NF- $\kappa$ B (Pestka, 2010; Cano *et al.*, 2013; Luo *et al.*,  
227 2021). It is known that inflammation negatively regulates the DNA repair machinery  
228 (Jaiswal *et al.*, 2000). For example, some studies reported that over-expression of NF- $\kappa$ B  
229 triggers the shutdown of tumor suppressor p53 activity, which plays an important role in DNA  
230 repair systems (Hudson *et al.*, 1999; Gudkov, Gurova and Komarova, 2011). Thus, ribotoxin-  
231 induced inflammatory response could sensitize the cells to DNA damage by influencing cell  
232 response to DNA damage. Additional studies are needed to explore these hypotheses.

233 Exacerbation of genotoxicity was observed from a dose as low as 1  $\mu$ M of DON. This  
234 result is biologically pertinent given the concentrations of DON to which consumers are  
235 exposed. Indeed, DON concentrations of 0.16-2  $\mu$ g/mL (0.5-7  $\mu$ M) can be considered as  
236 realistic in the human gut (Sergent *et al.*, 2006; Maresca, 2013). The lower concentration  
237 corresponds to prolonged daily intake by consumers and the higher one corresponds to the



238 level that can be reached after consumption of heavily contaminated food (Knutsen *et al.*,  
239 2017; Vin *et al.*, 2020; Alassane-Kpembi *et al.*, 2013). Consequently, the level of DON to  
240 which humans are exposed could potentiate the genotoxicity of foodborne genotoxins.  
241 Humans are exposed to many naturally occurring dietary genotoxins, or that are produced  
242 during food processing (Sakita *et al.*, 2017; Goldman and Shields, 2003). These genotoxins  
243 can cause different forms of DNA damage such as single or double strand breaks and adducts  
244 (Barnes *et al.*, 2018). For example, a daily intake of 26 ng/kg body weight of heterocyclic  
245 amines, which are formed during cooking and cause DNA strand breaks and adducts, has  
246 been described in the US population (Layton *et al.*, 1995). Similarly, European are exposed to  
247 2 µg/kg body weight of the DNA strand break-inducing heavy metal cadmium (European  
248 Food Safety Authority, 2012).

249         Given the high prevalence of DON in human food, one can assume that intestinal  
250 epithelial cells are co-exposed to this mycotoxin together with dietary genotoxins. The present  
251 study demonstrates that realistic doses of DON exacerbate DNA damage induced by various  
252 type of genotoxic drugs (etoposide, cisplatin and phleomycin) and preliminary results in our  
253 laboratory indicate that DON aggravated DNA damage in intestinal cells exposed to dietary  
254 genotoxins such as pesticide or alcohol-derived compounds. DNA damage is pivotal in  
255 cancer, because it can lead to gene mutations, chromosomal instability and ultimately, cell  
256 transformation and neoplasia (Jackson and Bartek, 2009). We therefore suggest that DON  
257 could enhance the carcinogenic potential of intestinal mutagens. In 1993, IARC concluded  
258 that DON cannot be classified with respect to its carcinogenicity for humans (International  
259 Agency for Research on Cancer, 1993; Claeys *et al.*, 2020). However, a preliminary report on  
260 a large-scale epidemiological study including half a million participants from 10 European  
261 countries followed for 15 years points to an association between the risk of proximal colon  
262 cancer and long-term exposure to DON (Huybrechts *et al.*, 2019). Further studies are needed  
263 to examine whether exposure to DON (and other ribosome inhibitors) could promote cancer  
264 by exacerbating the genotoxicity of endogenous and dietary mutagens.

265

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274

#### 275 **FIGURE LEGENDS:**

276 **Figure 1: DON exacerbates phosphorylation of H2AX caused by etoposide, cisplatin and**  
277 **phleomycin.** Non-transformed rat intestinal epithelial IEC-6 cells were treated for 4 hours  
278 with 10  $\mu$ M DON or 10  $\mu$ M DMSO vehicle combined with 3  $\mu$ M ETP, 20  $\mu$ M CPT or 3  $\mu$ M  
279 PHM, and  $\gamma$ H2AX was examined by immunofluorescence. Representative photos. Scale bar =  
280 20  $\mu$ m.

281

282 **Figure 2: Dose-dependent genotoxicity of etoposide, cisplatin and phleomycin and its**  
283 **exacerbation by DON.** Non-transformed rat intestinal epithelial IEC-6 cells were treated for  
284 4

285 hours with 10  $\mu$ M DON or 10  $\mu$ M DMSO vehicle combined with different doses of ETP  
286 (red), CPT (blue) or PHM (green), then H2AX phosphorylation levels were quantified by In-  
287 Cell Western. All the data are expressed as mean  $\pm$  SEM (3 independent experiments).  
288 Statistical analysis was performed using one-way ANOVA with Bonferroni's multiple-  
289 comparison correction. Values that differ significantly from vehicle are indicated by black  
290 asterisks, and values that differ significantly from the genotoxin alone are indicated by red  
291 asterisks. n.s.: not significant, \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ .

292

293 **Figure 3: DON exacerbates genotoxicity caused by etoposide, cisplatin and phleomycin**  
294 **in a dose-dependent manner.** Non-transformed rat intestinal epithelial IEC-6 cells were  
295 treated for 4 hours with the doses of DON shown in the figure, combined with 5  $\mu$ M ETP  
296 (red), 25  $\mu$ M CPT (blue) or 5  $\mu$ M PHM (green), then H2AX phosphorylation levels measured  
297 in three independent experiments were quantified by In-Cell Western analysis. All the data  
298 are expressed as mean  $\pm$  SEM (3 independent experiments). All P-values are calculated using  
299 one-way ANOVA with Bonferroni's multiple-comparison correction. Values that differ  
300 significantly from the vehicle are indicated by black asterisks, and values that differ  
301 significantly from the genotoxin alone are indicated by red asterisks. \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p$   
302  $< 0.0001$ , n.s: not significant.

303

304 **Figure 4: DOM-1 does not exacerbate genotoxicity caused by etoposide, cisplatin and**  
305 **phleomycin.** Non-transformed rat intestinal epithelial IEC-6 cells were treated for 4 hours

306 with 10  $\mu$ M DON or DOM-1 and 5  $\mu$ M ETP, (red), 20  $\mu$ M CPT (blue), or 5  $\mu$ M PHM (green),  
307 then H2AX phosphorylation levels measured in three independent experiments were  
308 quantified by In-Cell Western analysis. All the data are expressed as mean  $\pm$  SEM (3  
309 independent experiments). All Pvalues are calculated using one-way ANOVA with  
310 Bonferroni's multiple-comparison correction. Values that differ significantly from the vehicle  
311 are indicated by black asterisks, and values that differ significantly from the genotoxin alone  
312 are indicated by red asterisks. \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ , n.s: not significant.

313

314 **Figure 5: Cycloheximide and anisomycin exacerbate genotoxicity caused by etoposide,**  
315 **cisplatin and phleomycin.** Non-transformed rat intestinal epithelial IEC-6 cells were treated  
316 for 4 hours with 1  $\mu$ M CHX or 100 pM ANC combined with 5  $\mu$ M ETP (red), 20  $\mu$ M CPT  
317 (blue) or 5  $\mu$ M PHM (green), then H2AX phosphorylation measured in three independent  
318 experiments was quantified by In-Cell Western analysis. All the data are expressed as mean  $\pm$   
319 SEM (3 independent experiments). All P-values are calculated using one-way ANOVA with  
320 Bonferroni's multiplecomparison correction. Values that differ significantly from the vehicle  
321 are indicated by black asterisks, and values that differ significantly from the genotoxin alone  
322 are indicated by red asterisks. \*\*:  $p < 0.01$ , \*\*\*:  $p < 0.001$ , \*\*\*\*:  $p < 0.0001$ , n.s: not  
323 significant.

324

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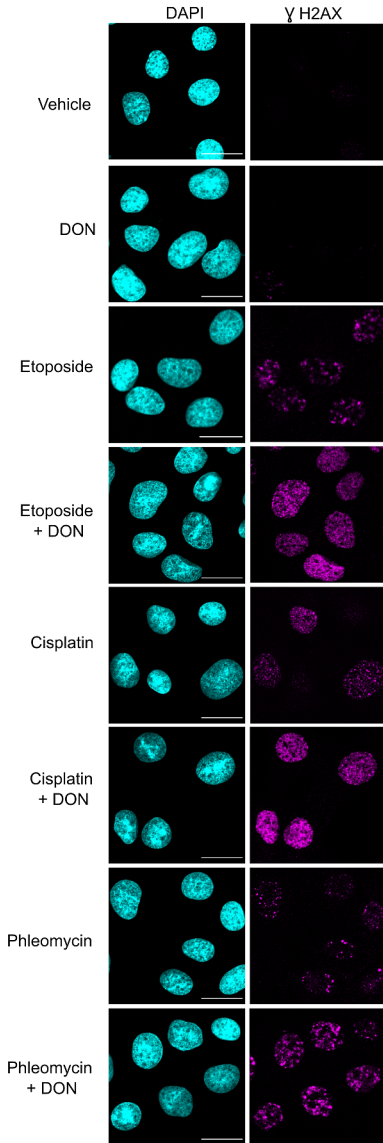
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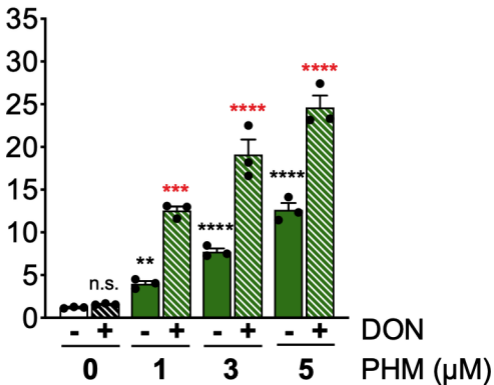
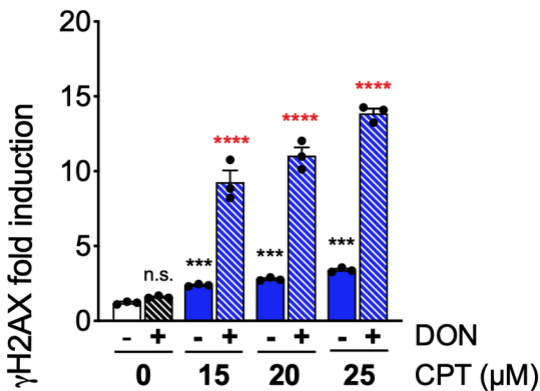
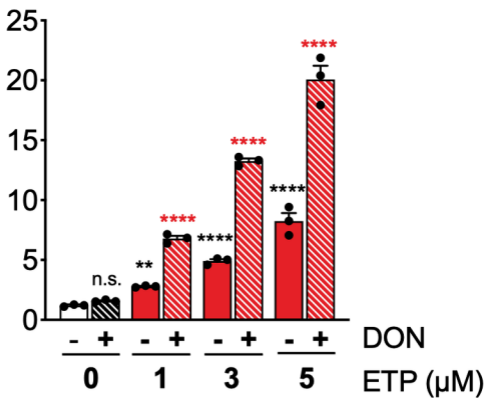
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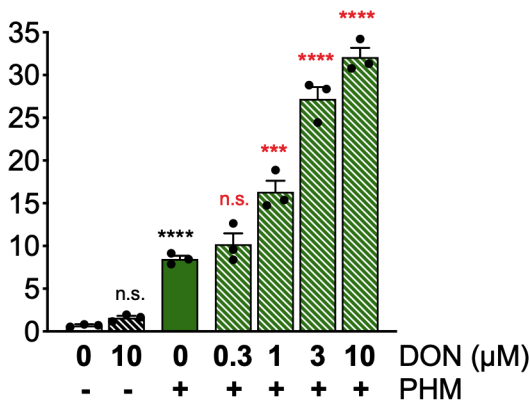
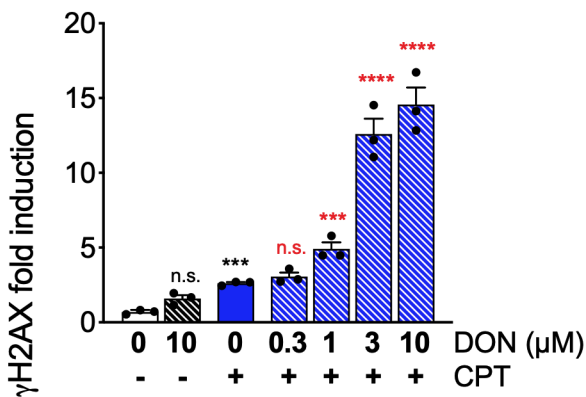
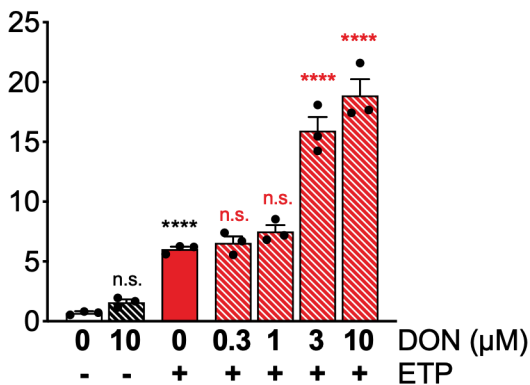
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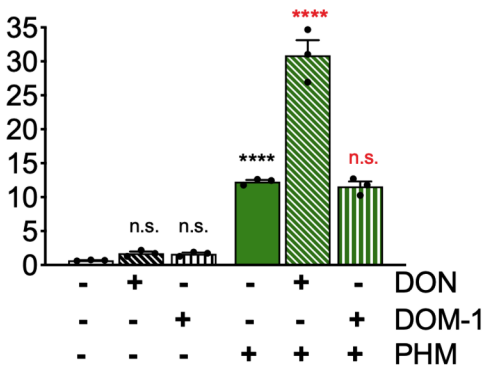
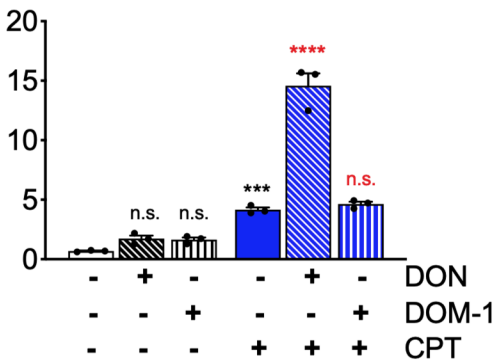
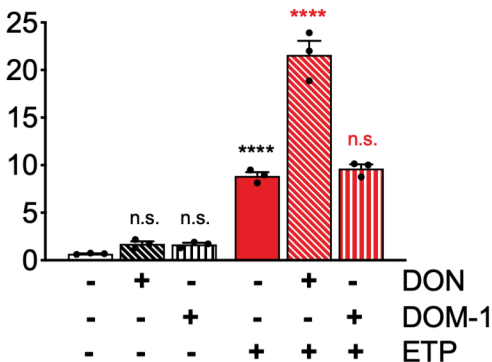
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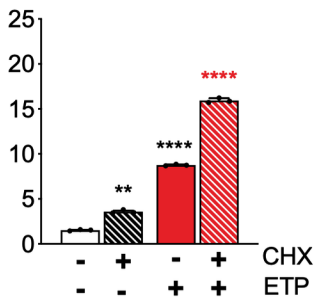




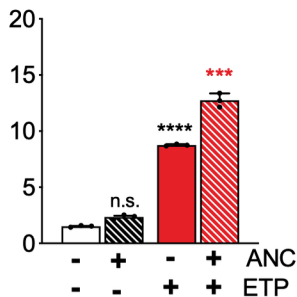




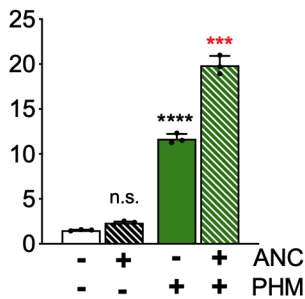
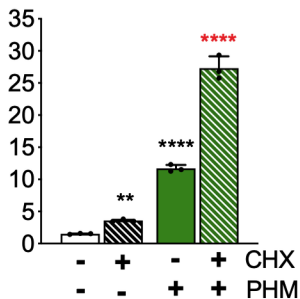
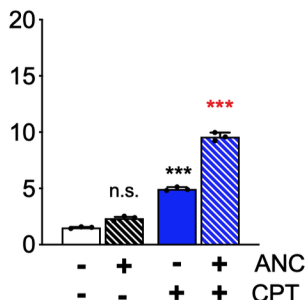
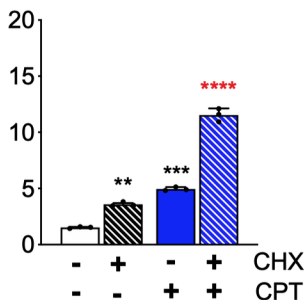
## Cycloheximide (CHX)



## Anisomycin (ANC)



γH2AX fold induction



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2 **GRAPHICAL ABSTRACT**

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