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Deoxynivalenol inhibits the expression by goblet cells of intestinal mucins through a PKR and MAP kinase-dependent repression of the resistin-like molecule β .

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Abbreviations: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT); Deoxynivalenol (DON); Fetal Calf Serum (FCS); Fluorescein isothiocyanate (FITC); Glyceraldehyde 3-phosphate dehydrogenase (GAPDH); Horseradish peroxidase (HRP); Inflammatory Bowel Disease (IBD); Intestinal Epithelial Cell (IEC); Lactate Dehydrogenase (LDH); Mitogen Activated Protein kinase (MAP kinase); Mucin (MUC); Nuclear Factor-kappa B (NF κ B); Paraformaldehyde (PFA); Protein Kinase R (PKR); Pyrrolidine Dithiocarbamate (PDTc); Resistin-like molecule beta (RELM- β); Tight Junction (TJ); Transmission electron microscopy (TEM); Wheat Germ Agglutinin (WGA).

Keywords: Deoxynivalenol; Goblet cell; Mucin; Mucus; Mycotoxin **Abstract**

Scope: The food-associated mycotoxin deoxynivalenol (DON) is known to affect intestinal functions. However, its effect on intestinal mucus is poorly characterized.

Methods and results: We analyzed the effects of DON on human goblet cells (HT29-16E cells) and porcine intestinal explants. Results showed that subtoxic doses of DON (as low as 1 μ M) decreased mucin production. qPCR analysis demonstrated that this inhibition was due to a specific decrease in the level of mRNA encoding for the intestinal membrane-associated (MUC1) and the secreted mucins (MUC2, MUC3). Mechanistic studies demonstrated that DON effect relied on the activation of the protein kinase R and the MAP kinase p38 ultimately leading to the inhibition of the expression of resistin-like molecule beta (RELM- β), a known positive regulator of mucin expression.

Conclusion: Taken together, our results show that at low doses found in food and feed, DON is able to affect the expression and production of mucins by human and animal goblet cells. Due to the important role of mucins in the barrier function and in the interaction of commensal bacteria with the host, such effect could explain the observed modifications in the microbial diversity and the increased susceptibility to enteric infection following exposure to DON.

1. Introduction

Daily, the intestine has to face a challenge: authorizing the passage of nutrients, water and ions and in the mean time limiting the crossing of noxious molecules and micro-organisms. Whereas specific membrane transporters allow the absorption of benefic compounds, the tight junctions (TJs) and the mucus layer are responsible for the barrier function of the gut [1, 2]. In addition to form a physical barrier against mechanical, chemical (including toxins) and physical aggressions, the intestinal mucus contributes to the establishment of the symbiosis between the host and the commensal bacteria forming the microbiota providing them board and lodging. In turn, commensal bacteria have positive impacts on host immunity and nutrition and have an important role in the detoxification of ingested xenobiotics, including mycotoxins [3, 4]. Particular IEC, called goblet cells, are responsible for the production of the intestinal mucus [5]. The major components of the mucus are called mucins. Based on its major roles in the intestinal barrier function and in the symbiosis with the microbiota, perturbations of the composition and / or secretion of mucus are associated with diseases in animals and humans [5, 6].

Mycotoxins are deleterious fungal secondary metabolites present in food / feed able to affect animal and human health [7, 8]. The intestine is the first organ exposed and affected by mycotoxins following their ingestion [9, 10]. These observations lead us, since 2010, to propose that some food-associated mycotoxins could play a role as environmental factors in human IBD [10]. Among the various mycotoxins suspected to play a role in intestinal perturbations, deoxynivalenol (DON) has attracted most attention [10]. DON is part of a family of mycotoxins called trichothecenes that are small sesquiterpenoids with an epoxide group at position 12-13 allowing their binding to ribosomes causing the so called ribosome stress response characterized by the activation of various protein kinases that leads to alterations of the intestinal, immune and brain cell functions [11, 13-19]. Data suggest that DON may affect the intestinal production of mucus. Gajecki's group was the first in 2008 to describe an alteration of the intestinal production of mucus after oral exposure of pigs to a mixture of DON, T-2 toxin and zearalenone [20]. Later on, other *in vivo* studies demonstrated that DON alone is able to decrease the number of mucin-positive goblet cells in animals, questions remaining particularly about the mechanism(s) involved in such effect [21, 22].

In the present paper, we used human goblet cells and porcine intestinal explants, to investigate the effect of DON on the expression of the major membrane and secreted mucins found in human gut and delineated the underlying mechanism involved in such effect.

2. Materials and Methods

Cell culture.

HT29-16E cells (passage number 20 to 35, generous gift from Prof Christian Laboisse, [23]) were routinely grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10 % fetal calf serum (FCS), 1 % L-glutamine and 1% antibiotics (all from Invitrogen, France) and maintained in a 5 % CO₂ incubator at 37 °C. For studying DON effects, HT29-16E cells were seeded at an initial density of 250,000

cells per cm² onto various supports and let to differentiate for 10 to 14 days, preliminary experiments showing that expression of mucins was optimal at that time.

Animals and preparation of jejunal explants.

Six castrated male piglets, acquired just after weaning, were used to prepare intestinal explants. All experiments were conducted under the approval of the Pharmacology-Toxicology Ethics Committee n° 86 Toulouse Midi-Pyrenees (TOXCOM/0017/IO PP, January 18th 2013). Jejunum were rapidly excised, stripped and biopsy punches were laid on sponges and incubated in presence of DON at 39 °C, under a CO₂ controlled atmosphere with orbital shaking [16, 24].

Mycotoxin treatment.

DON (from Romer Labs, France) stock solutions was prepared in anhydrous ethanol and stored at -20 °C. Serial dilutions of DON were prepared in anhydrous ethanol allowing the addition of similar volume of vehicle in all experiments. Cells were treated with the indicated concentrations of DON or equivalent volume of ethanol (1 % final, volume/volume) in DMEM without FCS and without antibiotics. In some experiments, inhibitors of signal transduction pathways (all from Tocris, France) were added 30 min prior DON treatment.

Measurement of the cell viability.

Cell viability was evaluated using lactate dehydrogenase (LDH) and MTT assays as previously described [15, 25]

Mucin labeling and microscopic observations.

HT29-16E cells seeded onto semi-permeable inserts (ThinCert™, 1 cm², 3 μm pore size) were treated or not with DON for various time. Cells were then washed with cold PBS and fixed with 4 % PFA during 60 min at room temperature. Inserts were cut and embedded in Tissue Freezing Medium (Electron Microscopy Sciences, France). Frozen cell sections (5 μm thickness) were obtained using cryostat CM3050 (Leica, Heerbrugg, Switzerland). Cell sections were then labeled for mucins using wheat germ agglutinin (WGA) conjugated to FITC (Sigma Aldrich, France). Briefly, sections were saturated 3 h at room temperature in 5 % PBS-BSA, 0.3 % Triton-X100 and then incubated over night at 4 °C with FITC-conjugated WGA (1:200 dilution in 0.5% PBS-BSA, 0.1% Triton-X100). Finally, sections were observed using similar settings with an epifluorescence microscope (Leitz DMRB microscope (Leica) equipped with a Leica DFC 450C camera). Quantitative analysis of mucin staining was performed using Image J software. Transmission electron microscopy (TEM) was also used to study the impact of DON on cell morphology and ultrastructure of HT29-16E cells. Inserts were fixed with 2.5 % glutaraldehyde in PBS buffer at 4 °C for 1 h. After three washes with PBS, cells were postfixed for 1 h with 2 % osmium tetroxide, washed three times, and dehydrated in an ethanol graded series (70 - 90 - 100 %). Samples were embedded in Epon 812 and perpendicular thin sections (80 nm thickness) were obtained using Reichert Ultracut S (Leica, Switzerland). Sections were stained with 4 % uranyl acetate and 2 % lead citrate. Sections were viewed in a Technai electron microscope operated at 120 kV.

Quantification of mucins and RELM β mRNA.

Total mRNA from porcine intestinal explants or HT29-16E cells grown onto 12 well-plates were extracted and quantified as previously described [16, 26]. Primers used for mRNA quantification were selected using Universal Probe Library Assay Design Center (Roche Applied Science) when available or designed. Their sequences are reported in Table 1 and Table 2. Target mRNA levels were normalized to house-keeping genes, i.e. GAPDH and cyclophilin A, ribosomal protein L32 or β -2-microglobulin, for HT29-16E cells and porcine explants, respectively. The relative quantification of target mRNA levels was performed using the comparative $\Delta\Delta C_t$ method [27].

Quantification of mucin protein expression.

Expression of mucin protein by HT29-16E cells was also studied using WGA conjugated to horseradish peroxidase (HRP) (Sigma Aldrich, France). Briefly, HT29-16E cells seeded onto 96-well plate and treated or not with DON were fixed with 4 % PFA during 30 min at room temperature. To avoid the loss of secreted mucins, 4-times concentrated PFA was directly added to the culture media without washing the cells. After fixation, cells were washed three times with PBS. Cells were saturated 1 h at room temperature in PBS supplemented with 2 % BSA and 0.1 % Triton-X100. HRP-conjugated WGA (1:10,000 dilution) was then added to the wells for 1 h at room temperature. Cells were washed six times with PBS and HRP substrate (Fast OPD from Sigma-Aldrich, France) was added. After 30 min of color development, reaction was stopped with concentrated H₂SO₄ and the optical density was finally measured at 490 nm.

Statistical analysis.

All experiments were conducted at least in triplicate. Two-way ANOVA analyses and t-test were used to address the significant differences between mean values with significance set at $p < 0.05$ (GraphPad ® Prism5 software).

3. Results

DON decreases the labeling of mucins in human goblet cells without affecting the cell viability, integrity or morphology.

Fluorescent WGA staining was used to study the impact of DON on the expression of mucins by HT29-16E cells. Dose-dependent study after 48 h exposure showed that DON inhibited WGA staining at doses superior or equal to 1 μ M (with 68 +/- 17; 84 +/- 12 and 97 +/- 2 % of inhibition at 1, 10 and 100 μ M, respectively) with a half-inhibition at 0.68 μ M (Figure 1 and 3A). Time-dependent experiments conducted at 10 μ M of DON (Figures 2 and 3B) demonstrated that the inhibition of the WGA labeling was statistically significant only after 12 h of exposure (with 47 +/- 18; 49 +/- 22 and 72 +/- 11 % of inhibition at 12, 24 and 48 h, respectively). Results of fluorescent microscopy were confirmed with HRP-conjugated WGA in a 96-well plate assay, giving a half-inhibition of WGA binding at 1 μ M of toxin after 48 h exposure (Figure 4). Since DON is known to cause cell toxicity, we wondered if the observed decrease in WGA labeling was associated with a decrease in cell viability. Evaluation of the cell viability of HT29-16E cells by the LDH assay demonstrated that 48 h exposure to DON caused a dose-dependent increase in the release of intracellular

LDH with a statistically significant effect only after exposure to doses superior or equal to 25 μM (14 \pm 2 % release, $p < 0.02$) and a half effect around 70 μM (Figure 5A). Similar results were obtained with the MTT assay, showing that cell viability was only significantly affected after exposure for 48 h to doses superior or equal to 25 μM (39 \pm 12 % decrease in cell viability) and a half-inhibition around 50 μM (Figure 5B). These results demonstrate that the decrease in mucin staining is not primary associated to a decrease in cell viability, although cell toxicity may contribute to the large decrease in mucin found at 100 μM of DON. TEM was performed to evaluate the impact of DON on HT29-16E morphology (Figure 6). Dose-dependent experiments showed that exposure of human goblet cells for 48 h to increasing concentrations of DON did not significantly alter their general morphology, even at 100 μM . The number of secretory granules was decreased at 100 μM of DON, their abundance being not affected at doses of DON inferior or equal to 10 μM . Interestingly, their electron density was modified suggesting a change in their composition at these doses (Figure 6), leading us to evaluate the effect of DON on mucin expression.

DON decreases the expression of intestinal mucins.

Next, we evaluated the effect of DON on the expression of MUC1, MUC2 and MUC3 mRNA. Results showed that exposure for 48 h of HT29-16E cells to increasing doses of DON caused a dose-dependent decrease in MUC1, MUC2 and MUC3 mRNA (Figure 7). MUC2 mRNA were the most affected by DON with 41 \pm 19 and 84 \pm 10 % of inhibition at 0.1 and 1 μM of DON, respectively. MUC1 and MUC3 mRNA were less affected with no significant effect at 0.1 μM and 27 \pm 8 and 64 \pm 9 % of inhibition at 1 μM of DON for MUC1 and MUC3, respectively. Accordingly, results showed that, after 48 h exposure, the IC_{50} (i.e. the dose causing half-inhibition) of DON on mucin expression were 7.2, 0.32 and 0.84 μM for MUC1, MUC2 and MUC3 mRNA, respectively. Time-dependent studies conducted with 10 μM of DON demonstrated that although a weak increase in MUC2 mRNA occurred after short time exposures, DON decreased mucin expression (Figure 8). As for dose-dependent experiments, MUC2 mRNA were the most affected compared to MUC1 and MUC3 mRNA, half-inhibition being observed at 22, 11 and 15 h for MUC1, MUC2 and MUC3 mRNA, respectively. *Ex vivo* experiments were conducted to confirm the results obtained with the HT29-16E cell model. Intestinal explants from pigs were exposed for 8 or 12 h to 10 μM of DON. The choice of using 10 μM of DON in *ex vivo* experiments was based mainly on the fact that such concentration is reached in the intestine of pigs exposed naturally to DON-contaminated cereals and does not affect cell viability of a porcine intestinal cell line (IPEC-1) up to 48 h after treatment [28]. As observed with HT29-16E cells, we found that DON decreased the expression of porcine MUC1, MUC2 and MUC3 mRNA (Figure 9), DON affecting equally the percent expression of the three mRNA (41 \pm 19, 36 \pm 4 and 38 \pm 35 and 70 \pm 8, 77 \pm 9 and 72 \pm 19 for MUC1, MUC2 and MUC3 after 8 or 12 h exposure, respectively).

DON decreases the expression of RELM β prior to affect mucin expression.

We studied the dose-dependent effect of DON on RELM β expression after a 48 h exposure of HT29-16E cells (Figure 10A). Although an increase in RELM β was observed at 0.1 μ M of DON (82 \pm 27 % of increase), DON inhibited its expression (i.e. 84 \pm 3 % of inhibition at 1 μ M) with an IC₅₀ of 0.51 μ M. Similarly, although we found that short exposure increased RELM β expression (41 \pm 18 % of increase after 30 min), time-dependent study (Figure 10B) conducted at 10 μ M of DON showed that inhibition of RELM β expression was very rapid, significant decrease being observed after a 3 h exposure (43 \pm 14 % of inhibition). Importantly, *ex vivo* experiments conducted on porcine explants confirmed that DON inhibited RELM β expression (i.e. 62 \pm 28 % and 92 \pm 7 % of inhibition after 8 and 12 h exposure, respectively) (Figure 11).

Inhibition of the expression of mucins and RELM β by DON relies on the activation of PKR and the MAP kinase p38.

Finally, we studied the implication of various signal transduction pathways in the effect of DON on mucin and RELM β expression. HT29-16E cells were pre-incubated for 30 min with inhibitors prior to DON treatment as explained in the Materials and Methods section. First, we looked at the effect of inhibitors on DON-mediated suppression of mucins mRNA (Figure 12). We found that inhibitor of the NF κ B pathway (PDTC, 100 μ M) or of the MAP kinase ERK1/2 (PD 98059, 50 μ M) did not reverse DON effect on mucin mRNA (Figure 12). Oppositely, inhibitors of the protein kinase R (PKR) (adenine, 2 mM) or of the MAP kinase p38 (SB 203580, 20 μ M) were able to prevent, at least partially, the effect of DON on the expression of MUC1,2 and 3 mRNA. Results obtained by mRNA analysis were confirmed using HRP-conjugated WGA, demonstrating that only p38 and PKR inhibitors prevented DON effect on WGA labeling (Figure 13). qPCR analysis showed that p38 and PKR inhibitors, but not NF κ B or ERK1/2 inhibitors, prevented also the effect of DON on the expression of RELM β mRNA (Figure 14).

4. Discussion

Animal studies demonstrated that DON decreases the number of mucin-positive goblet cells [20-22]. However the mechanism responsible for such inhibition was uncharacterized. Recently, the effect of DON on the expression of colonic cancer-associated mucins MUC5AC and 5B was reported using HT29-MTX cells [29]. Although very instructive, this pioneer study did not evaluate the effect of DON on the expression of the normal intestinal mucins and did not identify the mechanism(s) involved in DON effect. To study the effect of DON on intestinal mucus, we decided to use the HT29-16E cells that, like normal goblet cells, express mainly MUC1, 2 and 3 [23, 30]. Our results demonstrated that DON inhibits the staining of goblet cells by WGA. TEM and cell viability assays showed that the absence of labeling was not associated to major histological changes or toxicity. Our *in vitro* results thus mimicked *in vivo* observations showing a decrease in the number of mucin-positive goblet cells. Secretory granules were still present even after exposure to 100 μ M of toxin, suggesting a specific depletion of mucins from the granules. The inhibition of mucin

expression by DON was confirmed by qPCR analysis. Our results demonstrated that DON inhibits the expression by human goblet cells of MUC1-3 mRNA. Importantly, *ex vivo* experiments performed on intestinal explants from pigs confirmed *in vitro* data, demonstrating the high predictive value of the HT29-16E cells as model of intestinal goblet cells. Based on the critical role played by the resistin-like molecule beta (RELM β) on mucin regulation [5, 31], we wondered if DON could affect its expression. Both *in vitro* and *ex vivo* models confirmed that DON inhibits RELM β expression suggesting the implication of RELM β down-regulation in DON effect.

The modulation of the expression of RELM β and mucin mRNA was observed at sub-toxic doses of DON demonstrating that such effect is independent of general cytotoxicity but rather depends on subtle alterations of signal pathways and gene expression in goblet cells. DON is known to activate various signal pathways. The initial binding of DON to ribosome leads to the activation of PKR that in turn causes the activation of the MAP kinases p38 and ERK1/2, eventually inducing the activation of the NF κ B pathway [11-13, 19]. The involvement of these pathways in DON signaling and DON effects is well characterized and differs depending of the cell type and function affected. For example, the effect of DON on intestinal tight junctions depends on p38 and ERK1/2 [16]. DON effect on the secretion of interleukin 8 by IEC relies on the activation of PKR, p38 and NF κ B [26] whereas its effect on the invasion of enterocytes by *Salmonella* depends on ERK1/2 [32]. The use of specific inhibitors allowed us to demonstrate that the effect of DON on the expression of RELM β and mucins only relies on the activation of PKR and the MAP kinase p38. This is of major importance. Indeed, although studies have clearly demonstrated that RELM β activates the MAP kinases ERK1/2 and p38 [33-35], at present there is no published data describing a regulation of the expression of RELM β by PKR / p38. We are thus the first to demonstrate that MAP kinases, at least p38, could exert a negative feedback on RELM β expression. Questions remain and ongoing experiments tend to identify the mechanism involved in this feedback and its implication in health and disease.

Determination of mycotoxin exposure in Germany by LC-MS/MS multibiomarker approach has shown that german adults may be exposed daily to up to 0.52 μ g of DON per kg of body weight [36], higher exposure being reported by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (up to 2.4 μ g/kg of BW per day). Masked forms of DON potentially further increase the daily exposure of humans to DON [37, 38]. Due to the pivotal role played by the mucus in the gut homeostasis, a depletion of mucins caused by DON at realistic concentrations (i.e. 1 μ M) may explain many gut dysfunctions observed after exposure of animals to this mycotoxin, such as their increased susceptibility to infection and the observed alteration of their microbiota diversity [13, 17, 39, 40]. Importantly, in addition to affect gut immunity and homeostasis, it is possible that such depletion of intestinal mucus caused by DON may have indirect nutritional consequences in humans and animals and potentially increase the intestinal absorption and bioavailability of xenobiotics and toxins, including DON itself.

Author contributions: F.G; A.P; C.N; O.P; P.E; E.D; J.P and M.M performed experiments involving human cells. P.P and I.O performed experiments involving porcine intestinal explants. C.N performed microscopy experiments. I.P and M.M supervised porcine and human experiments, respectively. M.M wrote the paper.

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5. References

- [1] Scaldaferri, F., Pizzoferrato, M., Gerardi, V., Lopetuso, L., Gasbarrini, A., The gut barrier: new acquisitions and therapeutic approaches. *J. Clin. Gastroenterol.* 2012, 46 Suppl, S12-17.
- [2] Brandtzaeg, P., Gate-keeper function of the intestinal epithelium. *Benef. Microbes.* 2013, 4, 67-82.
- [3] Juge, N., Microbial adhesins to gastrointestinal mucus. *Trends Microbiol.* 2012, 20, 30-39.
- [4] Bergstrom, K.-S., Xia, L., Mucin-type O-glycans and their roles in intestinal homeostasis. *Glycobiology.* 2013, 23, 1026-1037.
- [5] Kim, Y.-S., Ho, S.-B., Intestinal goblet cells and mucins in health and disease: recent insights and progress. *Curr. Gastroenterol. Rep.* 2010, 12, 319-330.
- [6] Johansson, M.-E., Sjövall, H., Hansson, G.-C., The gastrointestinal mucus system in health and disease. *Nat. Rev. Gastroenterol. Hepatol.* 2013, 10, 352-361.
- [7] Stoev, S.-D., Food safety and increasing hazard of mycotoxin occurrence in foods and feeds. *Crit. Rev. Food Sci. Nutr.* 2013, 53, 887-901.
- [8] Wu, F., Groopman, J.-D., Pestka, J.-J., Public health impacts of foodborne mycotoxins. *Annu. Rev. Food Sci. Technol.* 2014, 5, 351-372.
- [9] Bouhet, S., Oswald, I.-P., The effects of mycotoxins, fungal food contaminants, on the intestinal epithelial cell-derived innate immune response. *Vet. Immunol. Immunopathol.* 2005, 108, 199-209.
- [10] Maresca, M., Fantini, J., Some food-associated mycotoxins as potential risk factors in humans predisposed to chronic intestinal inflammatory diseases. *Toxicon.* 2010, 56, 282-294.

- [11] Pestka, J.-J., Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. *Arch. Toxicol.* 2010, *84*, 663-679.
- [12] Arunachalam, C., Doohan, F.-M., Trichothecene toxicity in eukaryotes: cellular and molecular mechanisms in plants and animals. *Toxicol. Lett.* 2013, *217*, 149-158.
- [13] Maresca, M., From the gut to the brain: journey and pathophysiological effects of the food-associated trichothecene mycotoxin deoxynivalenol. *Toxins* (Basel). 2013, *5*, 784-820.
- [14] Accensi, F., Pinton, P., Callu, P., Abella-Bourges, N., *et al.*, Ingestion of low doses of deoxynivalenol does not affect hematological, biochemical, or immune responses of piglets. *J. Anim. Sci.* 2006, *84*, 1935-1942.
- [15] Razafimanjato, H., Benzaria, A., Taïeb, N., Guo, X.-J., *et al.*, The ribotoxin deoxynivalenol affects the viability and functions of glial cells. *Glia.* 2011, *59*, 1672-1683.
- [16] Pinton, P., Tsybulskyy, D., Lucioli, J., Laffitte, J., *et al.*, Toxicity of deoxynivalenol and its acetylated derivatives on the intestine: differential effects on morphology, barrier function, tight junction proteins, and mitogen-activated protein kinases. *Toxicol. Sci.* 2012, *130*, 180-190.
- [17] Antonissen, G., Martel, A., Pasmans, F., Ducatelle, R., *et al.*, The impact of Fusarium mycotoxins on human and animal host susceptibility to infectious diseases. *Toxins* (Basel). 2014, *6*, 430-452.
- [18] Ghareeb, K., Awad, W.-A., Böhm, J., Zebeli, Q., Impacts of the feed contaminant deoxynivalenol on the intestine of monogastric animals: poultry and swine. *J. Appl. Toxicol.* 2014, doi: 10.1002/jat.3083.
- [19] Pinton, P., Oswald, I.-P., Effect of deoxynivalenol and other Type B trichothecenes on the intestine: a review. *Toxins* (Basel). 2014, *6*, 1615-1643.
- [20] Obremski, K., Zielonka, L., Gajecka, M., Jakimiuk, E., *et al.*, Histological estimation of the small intestine wall after administration of feed containing deoxynivalenol, T-2 toxin and zearalenone in the pig. *Pol. J. Vet. Sci.* 2008, *11*, 339-345.
- [21] Bracarense, A.-P., Lucioli, J., Grenier, B., Drociunas Pacheco, G., *et al.*, Chronic ingestion of deoxynivalenol and fumonisin, alone or in interaction, induces morphological and immunological changes in the intestine of piglets. *Br. J. Nutr.* 2012, *107*, 1776-1786.
- [22] Basso, K., Gomes, F., Bracarense, A.-P., Deoxynivalenol and fumonisin, alone or in combination, induce changes on intestinal junction complexes and in E-cadherin expression. *Toxins* (Basel). 2013, *5*, 2341-2352.
- [23] Augeron, C., Laboisse, C.L., Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate, *Cancer Res.* 1984, *44*, 3961-3969.
- [24] Kolf-Clauw, M., Castellote, J., Joly, B., Bourges-Abella, N., *et al.*, Development of a pig jejunal explant culture for studying the gastrointestinal toxicity of the mycotoxin deoxynivalenol: histopathological analysis. *Toxicol. In Vitro.* 2009, *23*, 1580-1584.

- [25] Razafimanjato, H., Garmy, N., Guo, X.-J., Varini, K., *et al.*, The food-associated fungal neurotoxin ochratoxin A inhibits the absorption of glutamate by astrocytes through a decrease in cell surface expression of the excitatory amino-acid transporters GLAST and GLT-1. *Neurotoxicology*. 2010, 31, 475-484.
- [26] Maresca, M., Yahy, N., Younès-Sakr, L., Boyron, M., *et al.*, Both direct and indirect effects account for the pro-inflammatory activity of enteropathogenic mycotoxins on the human intestinal epithelium: stimulation of interleukin-8 secretion, potentiation of interleukin-1 β effect and increase in the transepithelial passage of commensal bacteria. *Toxicol. Appl. Pharmacol.* 2008, 228, 84-92.
- [27] Livak, K.-J., Schmittgen, T.-D., Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001, 25, 402-408.
- [28] Cano, P.-M., Seeboth, J., Meurens, F., Cognie, J., *et al.*, Deoxynivalenol as a new factor in the persistence of intestinal inflammatory diseases: an emerging hypothesis through possible modulation of Th17-mediated response. *PLoS One*. 2013, 8, e53647.
- [29] Wan, L.-Y., Allen, K.-J., Turner, P.-C., El-Nezami, H., Modulation of mucin mRNA (MUC5AC and MUC5B) expression and protein production and secretion in Caco-2/HT29-MTX co-cultures following exposure to individual and combined Fusarium mycotoxins. *Toxicol. Sci.* 2014, 139, 83-98.
- [30] Maoret, J.-J., Font, J., Augeron, C., Codogno, P., *et al.*, A mucus-secreting human colonic cancer cell line. Purification and partial characterization of the secreted mucins. *Biochem. J.* 1989, 258, 793-799.
- [31] Krimi, R.-B., Kotelevets, L., Dubuquoy, L., Plaisancié, P. *et al.*, Resistin-like molecule beta regulates intestinal mucous secretion and curtails TNBS-induced colitis in mice. *Inflamm. Bowel Dis.* 2008, 14, 931-941.
- [32] Vandenbroucke, V., Croubels, S., Verbrugghe, E., Boyen, F., *et al.*, The mycotoxin deoxynivalenol promotes uptake of Salmonella Typhimurium in porcine macrophages, associated with ERK1/2 induced cytoskeleton reorganization. *Vet. Res.* 2009, 40, 64.
- [33] Angelini, D.-J., Su, Q., Yamaji-Kegan, K., Fan, C., *et al.*, Resistin-like molecule-beta in scleroderma-associated pulmonary hypertension. *Am. J. Respir. Cell. Mol. Biol.* 2009, 41, 553-561.
- [34] Hsu, W.-Y., Chao, Y.-W., Tsai, Y.-L., Lien, C.-C., *et al.*, Resistin induces monocyte-endothelial cell adhesion by increasing ICAM-1 and VCAM-1 expression in endothelial cells via p38MAPK-dependent pathway. *J. Cell. Physiol.* 2011, 226, 2181-2188.
- [35] Fang, C., Meng, Q., Wu, H., Eid, G. *et al.*, Resistin-like molecule- β is a human airway remodelling mediator. *Eur. Respir. J.* 2012, 39, 458-466.
- [36] Gerding, J., Cramer, B., Humpf, H.,U., Determination of mycotoxin exposure in Germany using an LC-MS/MS multibiomarker approach. *Mol. Nutr. Food Res.* 2014, 58, 2358-2368.
- [37] Berthiller, F., Crews, C., Dall'Asta, C., Saeger, S.,D., *et al.*, Masked mycotoxins: a review. *Mol. Nutr. Food Res.* 2013, 57, 165-186.

[38] Rychlik, M., Humpf, H.,U., Marko, D., Dänicke, S., *et al.*, Proposal of a comprehensive definition of modified and other forms of mycotoxins including "masked" mycotoxins. *Mycotoxin Res.* 2014, 30, 197-205.

[39] Waché, Y.-J., Valat, C., Postollec, G., Bougeard, S., *et al.*, Impact of deoxynivalenol on the intestinal microflora of pigs. *Int. J. Mol. Sci.* 2009, 10, 1-17.

[40] Saint-Cyr, M.-J., Perrin-Guyomard, A., Houée, P., Rolland, J.-G., Laurentie, M., Evaluation of an oral subchronic exposure of deoxynivalenol on the composition of human gut microbiota in a model of human microbiota-associated rats. *PLoS One.* 2013, 8, e80578.

Table 1: Sequences of primers used in this study for human mRNA analysis.

Gene	Accession	Description	Amplicon	Primers	Length	Tm	%GC
GAPDH	NM_002046.3	Glyceraldehyde-3-phosphate dehydrogenase	119 nt	gagtccactggcgtcttcac	20	60	60
				ttcacacccatgacgaacat	20	59	45
MUC1	ENST00000368395.1	Mucin 1 Precursor	95 nt	cgtagccccctatgagaaggtt	21	59	52
				ccctacaagttggcagaagtg	21	59	52
MUC2	NM_002457.2	Mucin 2, oligomeric mucus/gel-forming	90 nt	gctgctatgtcaggacacc	20	60	60
				gggaggagttggtacacacg	20	60	60
MUC3	ENST00000319509.7	Intestinal mucin-3B	102 nt	gtggagatcctgtccctgag	20	59	60
				cacctgctcactactcgctctc	21	60	57
RELM	AF323084.1	Resistin-like molecule beta	85 nt	tgctagtgtcaaaagccaagg	21	60	48
				accacagccatagccacaa	19	60	53

Table 2: Sequences of primers used in this study for porcine mRNA analysis.

Gene	Accession	Description	Amplicon	Primers	Length	Tm	%GC
MUC1	AY243508.1	epithelial mucin	123 nt	gcattacaaaacctccagttacct	24	58	36
				cccagaagcccgtctctttt	20	59	55
MUC2	XM_003122394.1	small intestinal mucin	66 nt	gcagcctgtgcaggaa	17	58	65
				tgatcatatacacagtgccttctg	24	59	46
MUC3	ENST00000319509.7	Human Intestinal mucin-3B	107 nt	gtggagatcctgtccctgag	20	56	60
				cacctgctcactactcgctctc	21	56	57
RETNLB	NM_001103210.1	resistin like beta	74 nt	caaaaactcaggcaaaagtcttc	24	58	42
				cacaggcatagccacaagca	20	59	55
Cyclophilin	NM_214353	peptidylprolyl isomerase A	92 nt	cccaccgtcttctcgacat	20	62	55
				tctgtgtctttggaactttgtct	24	68	41
B2M	NM_213978	beta-2-microglobulin	162 nt	ttctacctctggtccacactga	23	68	48
				tcatccaaccagatgca	18	54	50
RPL 32	NM_001001636	ribosomal protein L32	92 nt	agttcatccggcaccagtca	20	62	55
				gaaccttctccgcaccctgt	20	64	60

Figure legend

Figure 1: DON dose-dependently decreases the labeling of human goblet cells by fluorescent lectin.

HT29-16E cells were treated with increasing doses of DON for 48 h. Cells were then fixed and stained with FITC-conjugated WGA, as explained in Materials and Methods. Conditions were: control cells (**A, B**), DON 0.1 μM (**C, D**), DON 1 μM (**E, F**), DON 10 μM (**G, H**), DON 100 μM (**I, J**). **A, C, E, G, I**: WGA staining (green signal), **B, D, F, H, J**: Nomarski observations.

Figure 2: DON time-dependently decreases the labeling of human goblet cells by fluorescent lectin.

HT29-16E were treated with 10 μM of DON for 6, 12, 24 or 48 h. Cells were then fixed and stained with FITC-conjugated WGA, as explained in Materials and Methods. Conditions were: control cells (**A, B**), 6 h (**C, D**), 12 h (**E, F**), 24 h (**G, H**), 48 h (**I, J**). **A, C, E, G, I**: WGA staining (green signal), **B, D, F, H, J**: Nomarski observations.

Figure 3: Quantification of the effect of DON on the labeling of human goblet cells by fluorescent lectin.

Intensities of the WGA labeling shown in Figures 1 and 2 were measured using Image J, as explained in Materials and Methods. **A**: Dose-dependent study, **B**: Time-dependent study. Results were expressed as percentage of the control staining (means \pm SD ($n = 8$)). Two-way ANOVA analysis was used to address the significant differences between mean values with * $p < 0.05$ and ** $p < 0.01$.

Figure 4: Quantification of the effect of DON on the expression of mucins by human goblet cells using HRP-conjugated lectin.

HT29-16E cells were seeded onto 96-well plate. After differentiation, cells were treated with increasing doses of DON for 48 h before labeling with HRP-conjugated WGA, as explained in Materials and Methods. Results were expressed as means \pm SD ($n = 3$). Two-way ANOVA analysis was used to address the significant differences between mean values with * $p < 0.05$ and ** $p < 0.01$.

Figure 5: Evaluation of the toxicity of DON on human goblet cells using the LDH and MTT assays.

HT29-16E cells were exposed for 48 h to increasing doses of DON and the cell viability and integrity were measured by the LDH (**A**) and the MTT (**B**) assays, as explained in Materials and Methods. Results were expressed as means \pm SD ($n = 3$). Two-way ANOVA analysis was used to address the significant differences between mean values with * p at least < 0.05 .

Figure 6: TEM observation of human goblet cells exposed to DON.

HT29-16E cells were treated with increasing doses of DON for 48 h. TEM observation was then performed as explained in Materials and Methods. Conditions were: control cells (**A**), DON 1 μM (**B**), DON 10 μM (**C**), DON 100 μM (**D**). Goblet cells exposed or not to DON showed apical microvilli (MV) and tight junctions (arrows). Secretory granules (G) were observed in all conditions, their electron density being different in DON-exposed cells compared to control.

Figure 7: DON dose-dependently inhibits the expression of mucins mRNA in human goblet cells.

HT29-16E cells were treated with increasing doses of DON for 48 h. At the end of the incubation, human MUC1 (A), MUC2 (B) and MUC3 (C) mRNA were extracted and quantified, as explained in Materials and Methods. Mucin mRNA were normalized using GAPDH mRNA. Results were expressed as fold increase in target mRNA production compared to untreated cells (means +/- SD, n = 3). Two-way ANOVA analysis was used to address the significant differences between mean values with * $p < 0.05$ and ** $p < 0.01$.

Figure 8: DON time-dependently inhibits the expression of mucins mRNA in human goblet cells.

HT29-16E cells were treated with 10 μ M of DON for various times. At the end of the incubation, human MUC1 (A), MUC2 (B) and MUC3 (C) mRNA were extracted and quantified, as explained in Materials and Methods. Mucin mRNA were normalized using GAPDH mRNA. Results were expressed as fold increase in target mRNA production compared to untreated cells (means +/- SD, n = 3). Two-way ANOVA analysis was used to address the significant differences between mean values with * $p < 0.05$ and ** $p < 0.01$ and *** $p < 0.001$.

Figure 9: DON time-dependently inhibits the expression of mucins mRNA in intestinal explants from pigs.

Explants were treated with 10 μ M of DON for 8 or 12 h. At the end of the incubation, porcine MUC1 (A), MUC2 (B) and MUC3 (C) mRNA were extracted and quantified, as explained in Materials and Methods. Mucin mRNA were normalized using porcine cyclophilin A, ribosomal protein L32 and β -2microglobulin mRNA. Results were expressed as fold increase in target mRNA production compared to untreated cells (means +/- SD, n = 6). Two-way ANOVA analysis was used to address the significant differences between mean values with * $p < 0.05$ and ** $p < 0.01$ and *** $p < 0.001$.

Figure 10: DON inhibits the expression of RELM β mRNA in human goblet cells.

Dose- (A) and time-dependent (B) effect of DON on RELM β mRNA expression was studied, as explained in Materials and Methods. Human RELM β mRNA levels were normalized using GAPDH mRNA. Results were expressed as fold increase in target mRNA production compared to untreated cells (means +/- SD, n = 3). Two-way ANOVA analysis was used to address the significant differences between mean values with * $p < 0.05$ and ** $p < 0.01$ and *** $p < 0.001$.

Figure 11: DON inhibits the expression of RELM β mRNA in intestinal explants from pigs.

Explants were treated with 10 μ M of DON for 8 or 12 h, as explained in Materials and Methods. At the end of the incubation, porcine RELM β mRNA levels were measured and normalized using porcine cyclophilin A, ribosomal protein L32 and β -2microglobulin mRNA. Results were expressed as fold increase in target mRNA production compared to untreated cells (means +/- SD, n = 6). Two-way ANOVA analysis was used to address the significant differences between mean values with * $p < 0.05$.

Figure 12: PKR and p38 inhibitors prevent the effect of DON on mucin mRNA expression.

HT29-16E cells were pre-treated with inhibitors of the MAP kinases p38 (SB 203580 (**SB**) at 20 μ M) or ERK1/2 (PD 98059 (**PD**) at 50 μ M), with NF- κ B inhibitor (pyrrolidine dithiocarbamate (**PDTC**) at 100 μ M) or with PKR inhibitor (adenine (**Ad**) at 2 mM). HT29-16E cells were then treated with 10 μ M of DON for 48 h. At the end of the incubation, human MUC1 (**A**), MUC2 (**B**) and MUC3 (**C**) mRNA were extracted and quantified, as explained in Materials and Methods. Mucin mRNA were normalized using GAPDH mRNA. Results were expressed as fold increase in target mRNA production compared to untreated cells (means \pm SD, n = 3). t-test analysis was used to address the significant differences between mean values and bars without a common letter differ by at least $p < 0.05$.

Figure 13: PKR and p38 inhibitors prevent the effect of DON on WGA labeling.

HT29-16E cells seeded onto 96-well plate were pre-treated with inhibitors of the MAP kinases p38 (SB 203580 (**SB**) at 20 μ M) or ERK1/2 (PD 98059 (**PD**) at 50 μ M), with NF- κ B inhibitor (pyrrolidine dithiocarbamate (**PDTC**) at 100 μ M) or with PKR inhibitor (adenine (**Ad**) at 2 mM). HT29-16E cells were then treated with 10 μ M of DON for 48 h. At the end of the incubation, HRP-conjugated labeling was performed as explained in Materials and Methods. Results were expressed as means \pm SD (n = 3). t-test analysis was used to address the significant differences between mean values and bars without a common letter differ by at least $p < 0.05$.

Figure 14: PKR and p38 inhibitors prevent the effect of DON on RELM β mRNA expression.

HT29-16E cells were pre-treated with inhibitors of the MAP kinases p38 (SB 203580 (**SB**) at 20 μ M) or ERK1/2 (PD 98059 (**PD**) at 50 μ M), with NF- κ B inhibitor (pyrrolidine dithiocarbamate (**PDTC**) at 100 μ M) or with PKR inhibitor (adenine (**Ad**) at 2 mM). HT29-16E cells were then treated with 10 μ M of DON for 6 h. At the end of the incubation, RELM β mRNA expression was studied, as explained in Materials and Methods. Human RELM β mRNA levels were normalized using GAPDH mRNA. Results were expressed as fold increase in target mRNA production compared to untreated cells (means \pm SD, n = 3). t-test analysis was used to address the significant differences between mean values and bars without a common letter differ by at least $p < 0.05$.

Figure 1

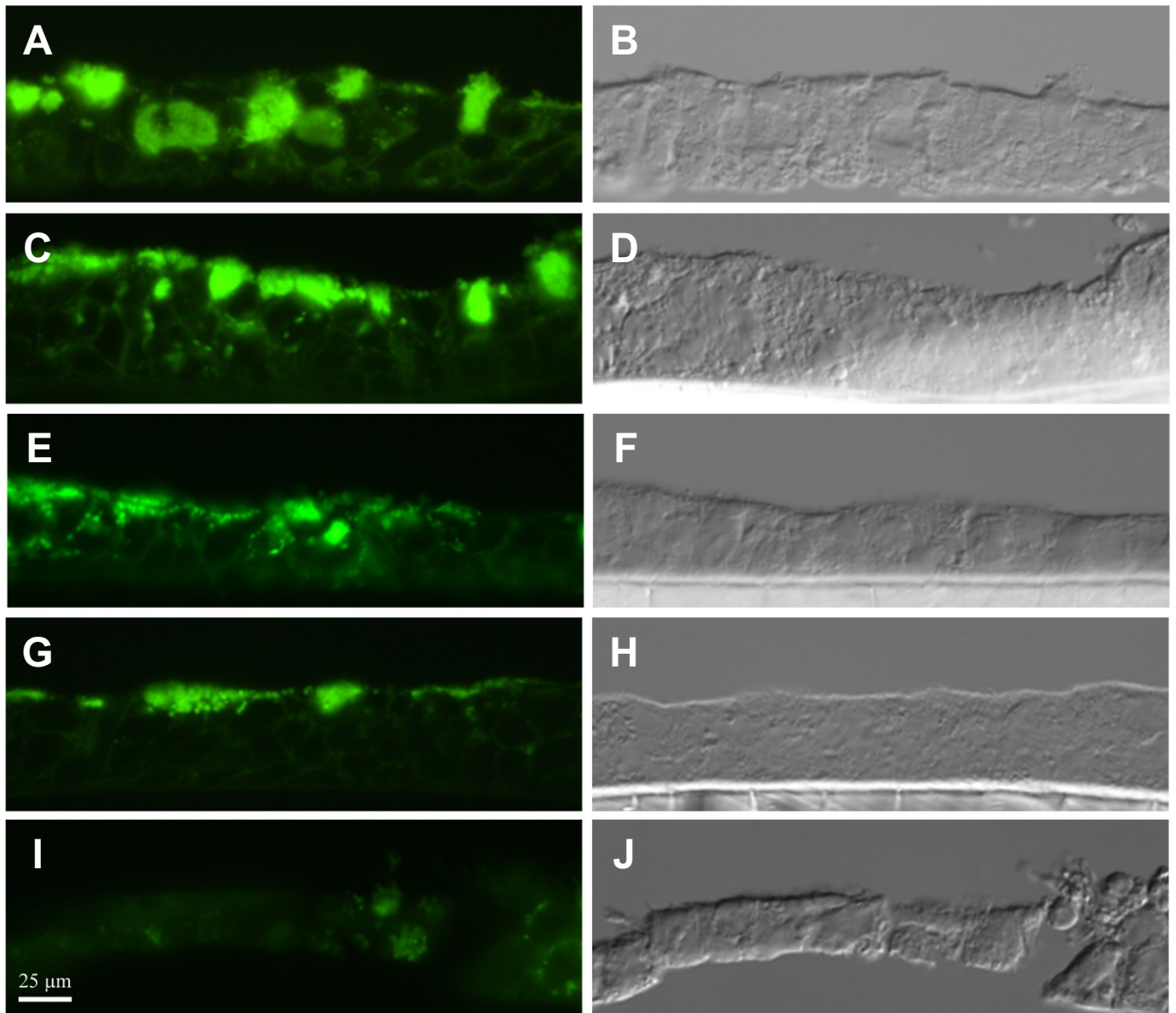


Figure 2

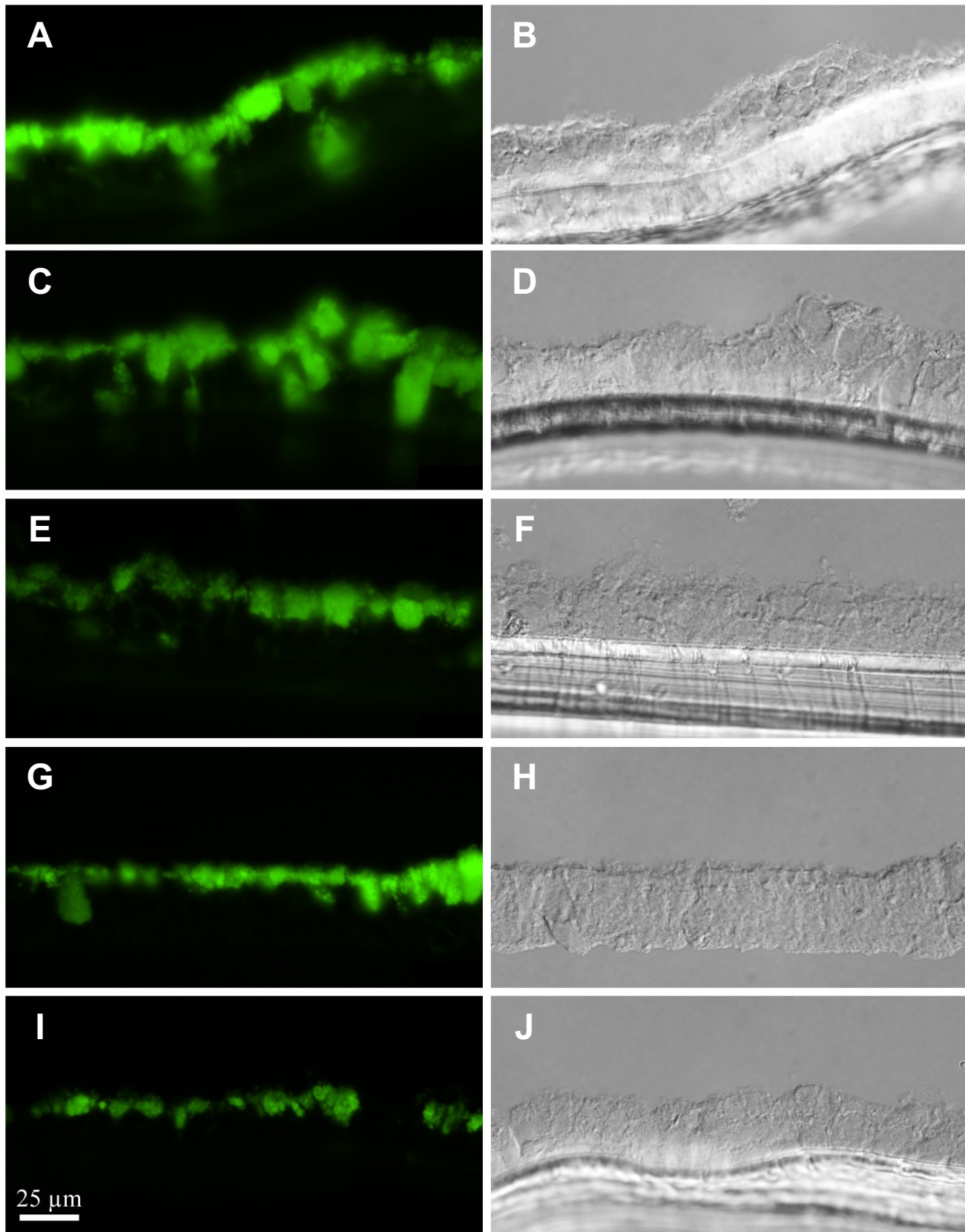


Fig 3

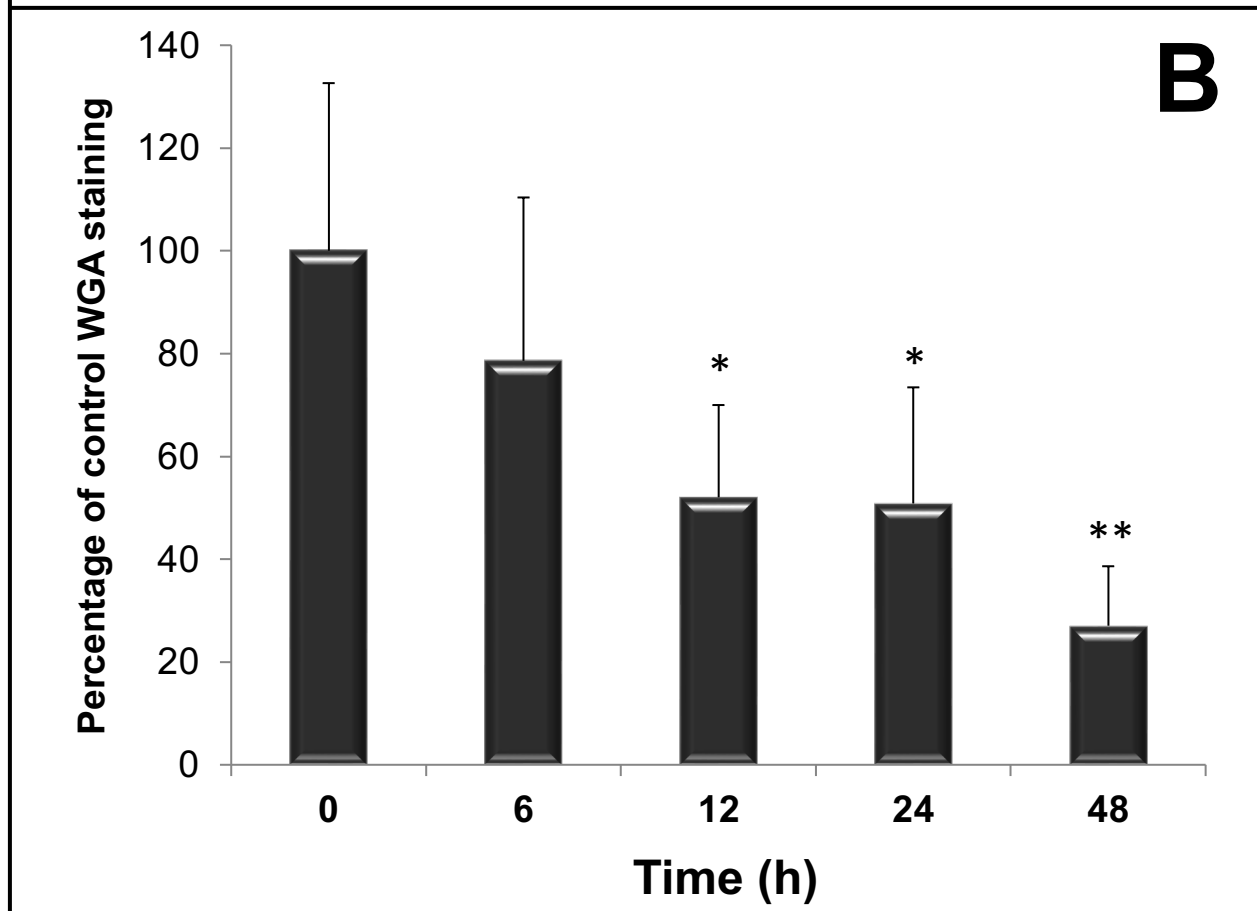
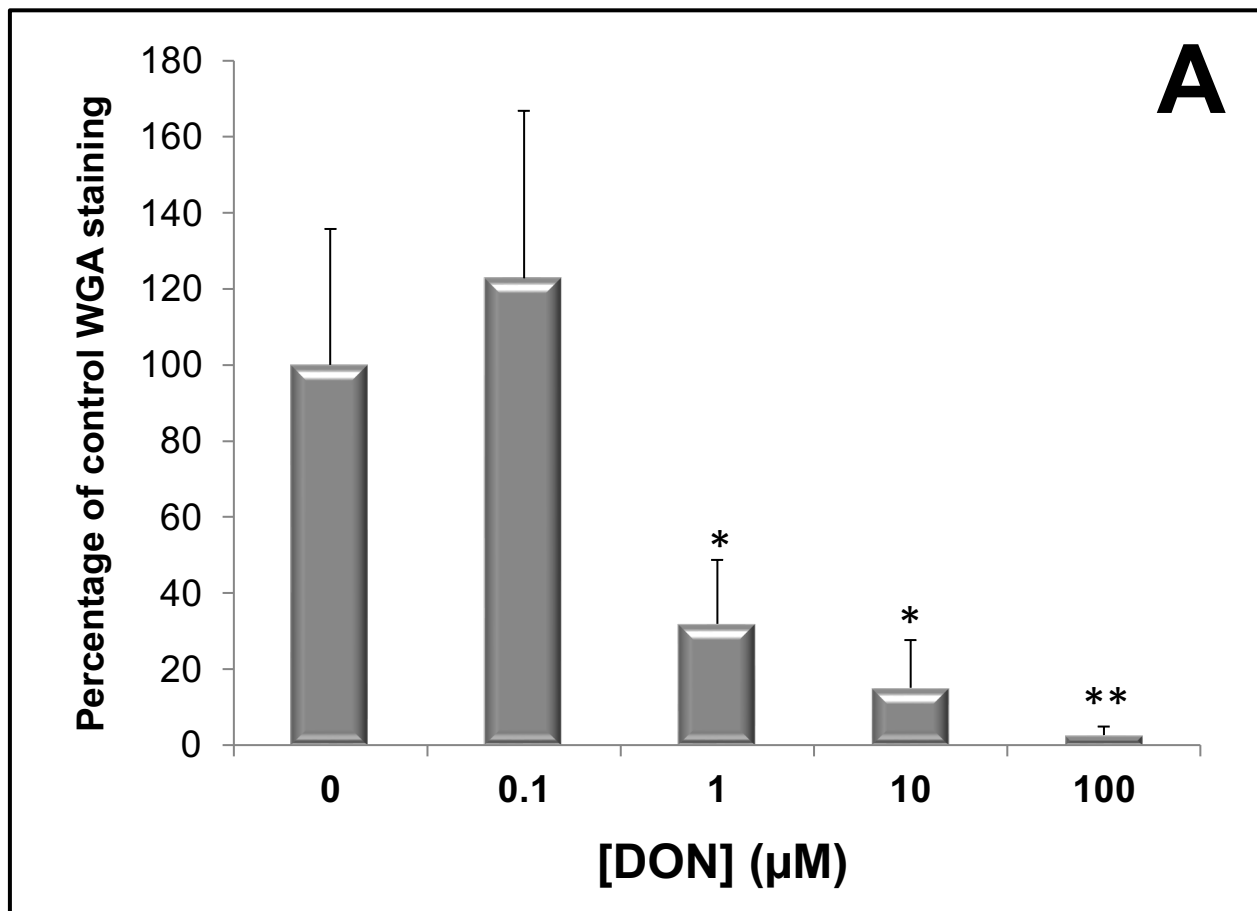


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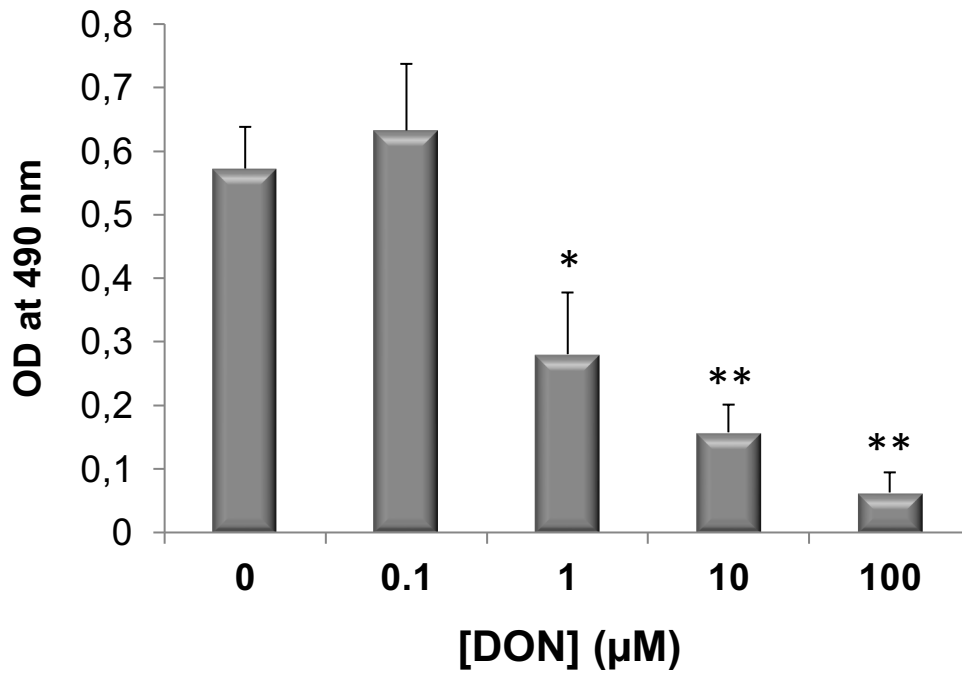


Fig 5

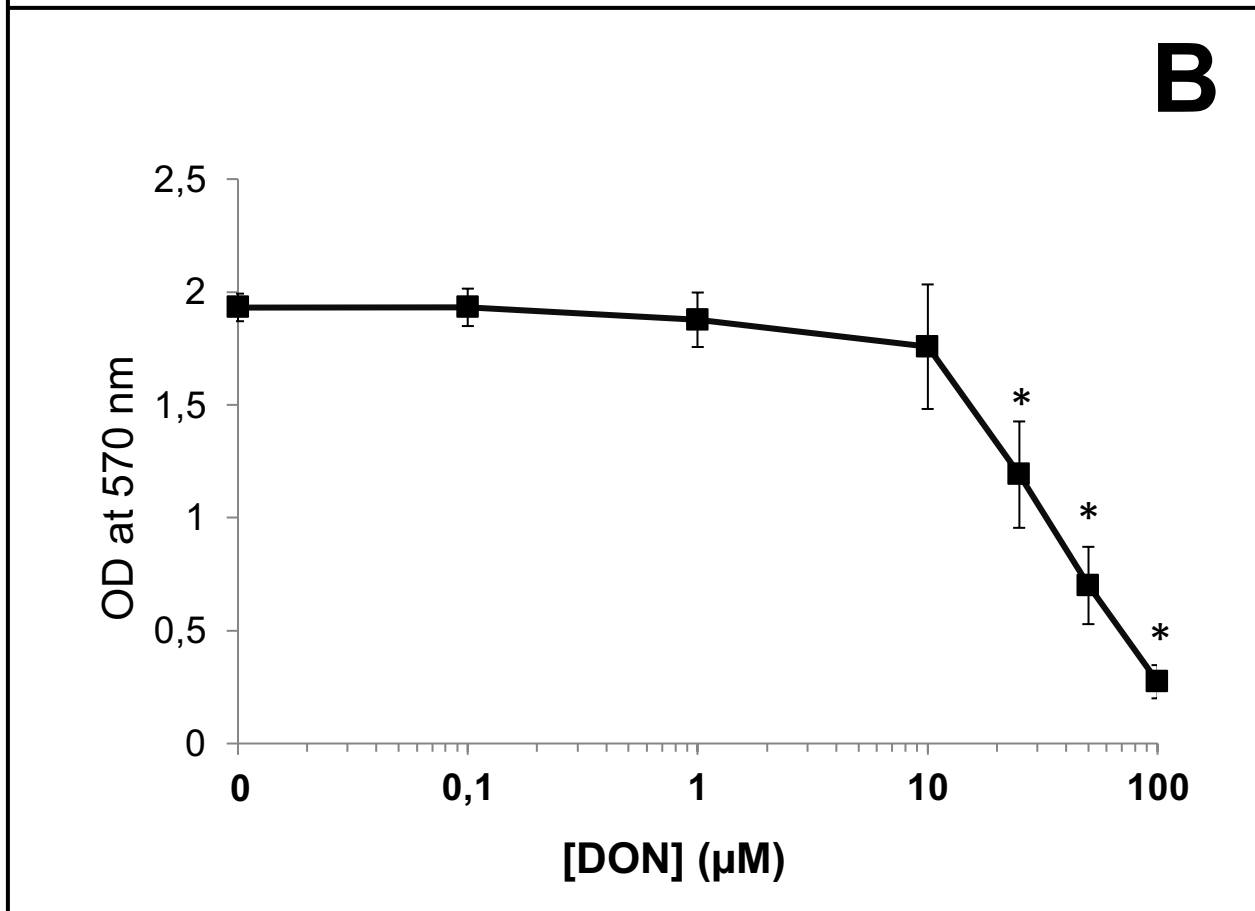
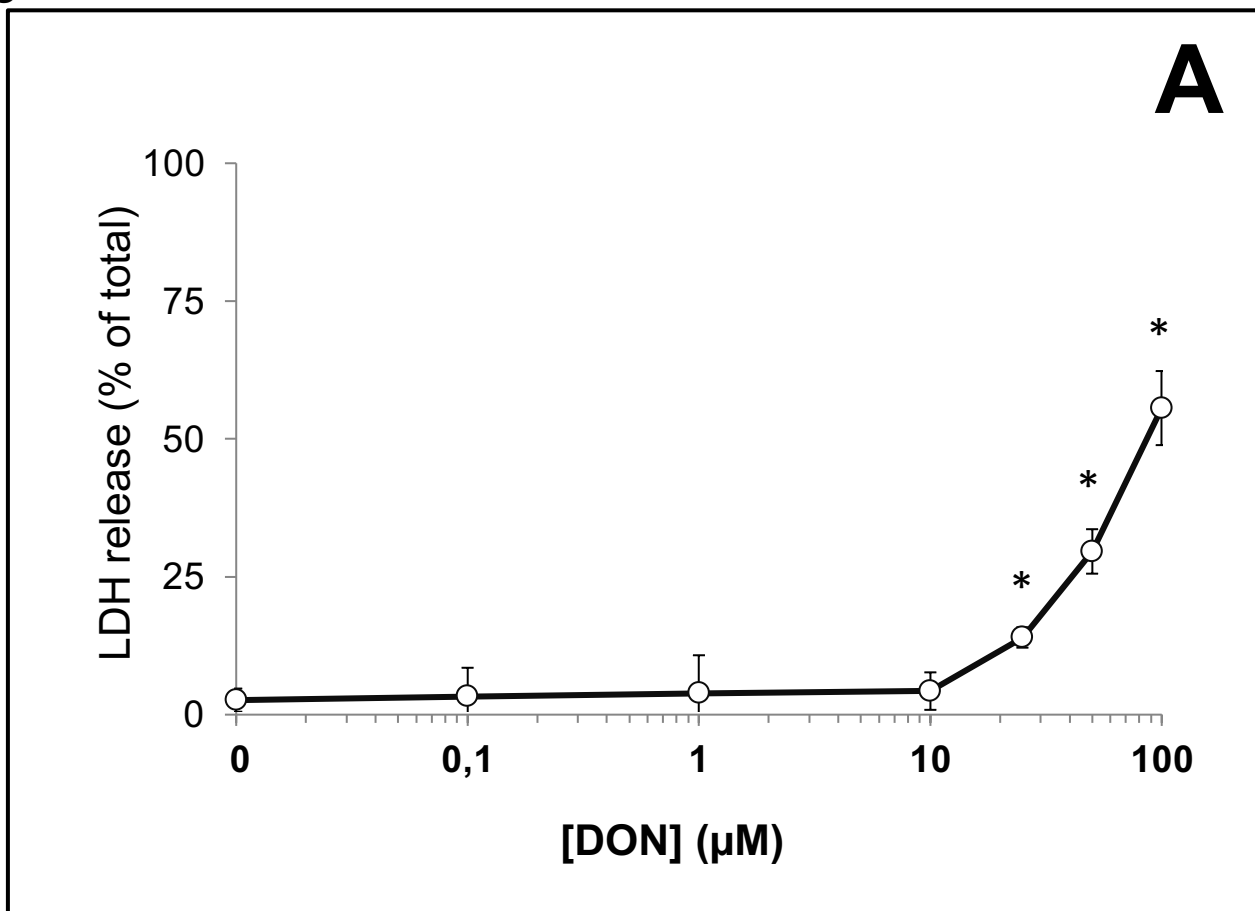


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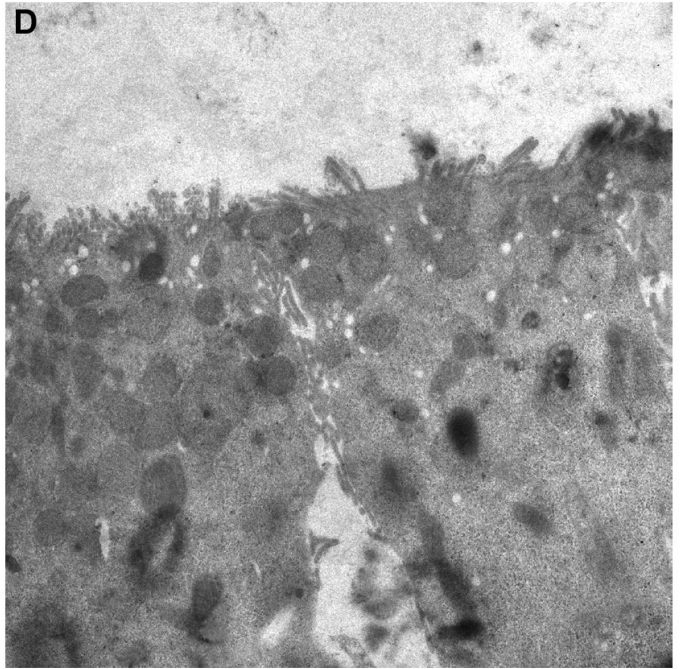
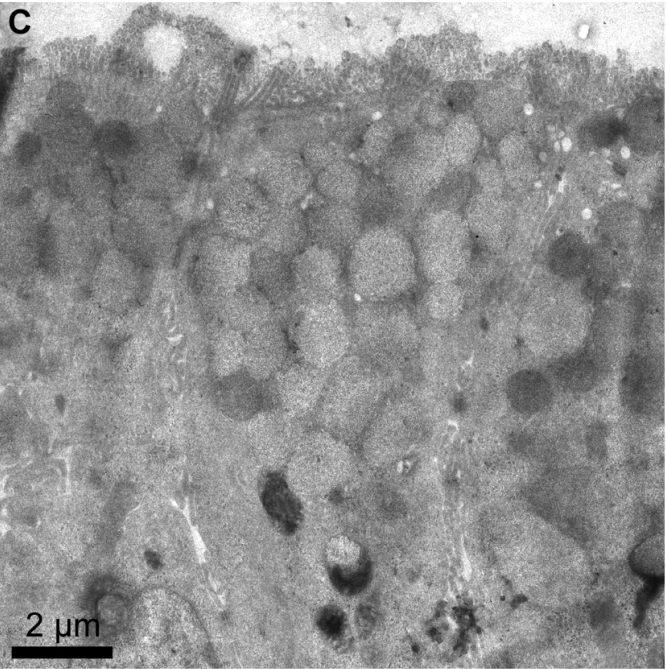
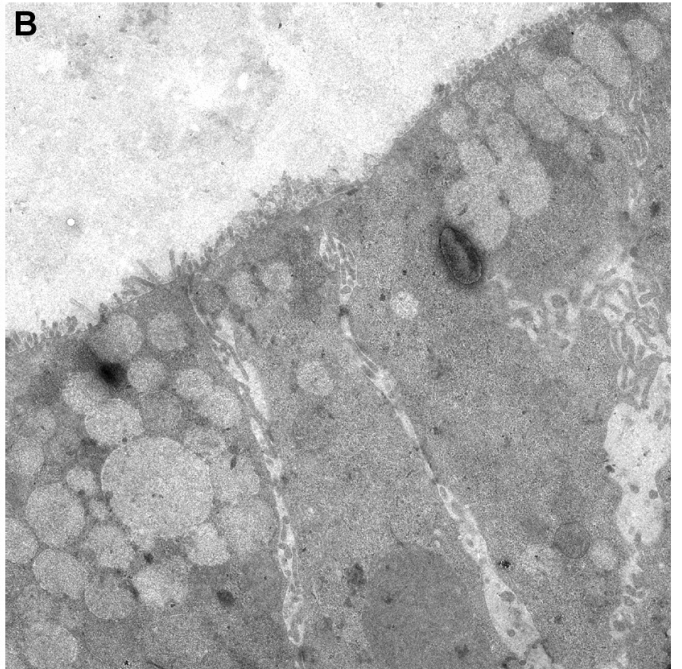
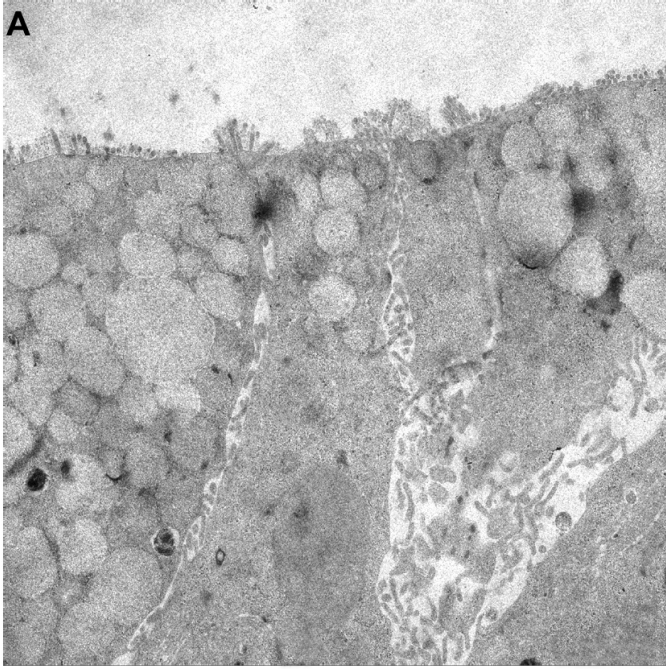


Fig 7

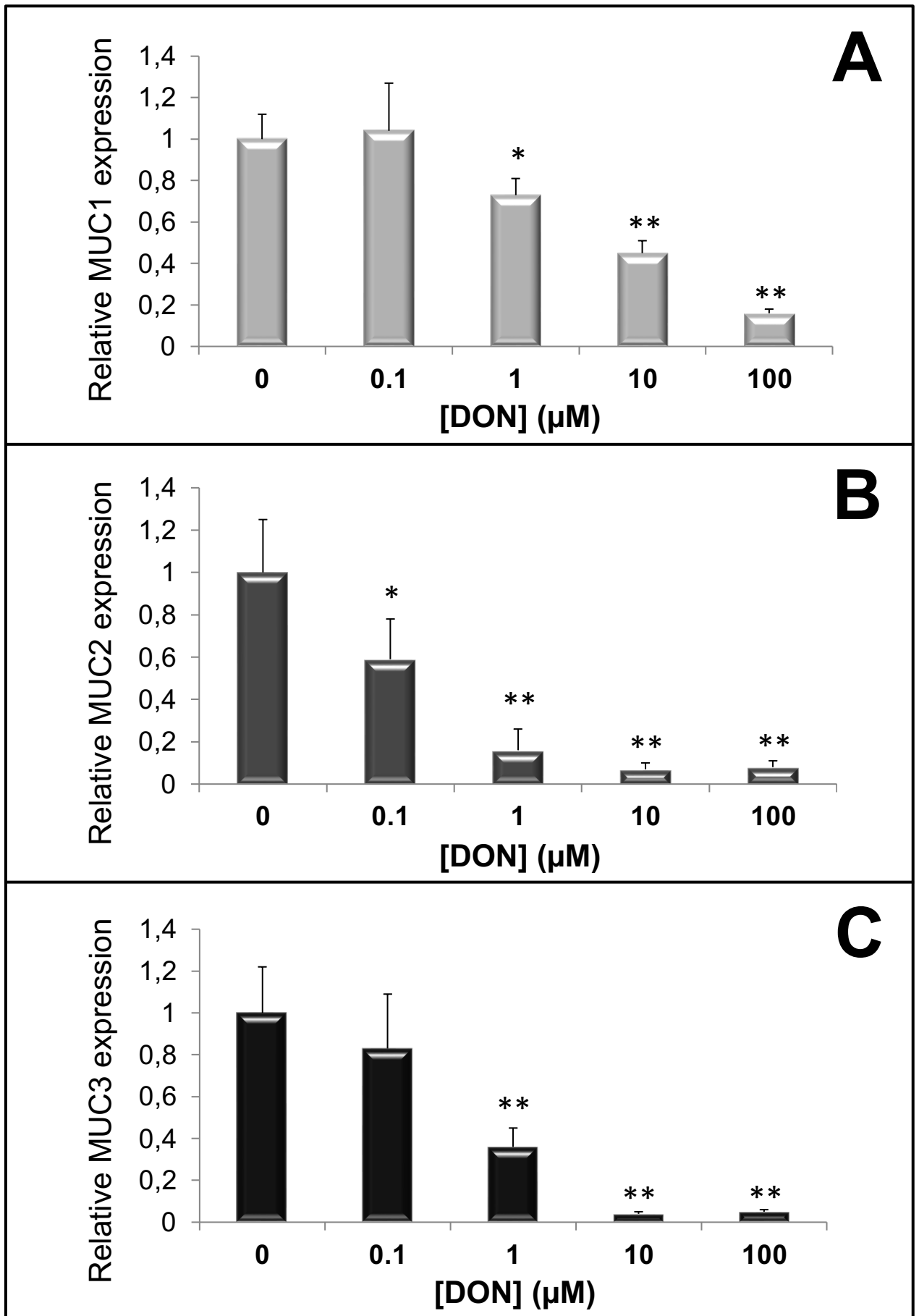


Fig 8

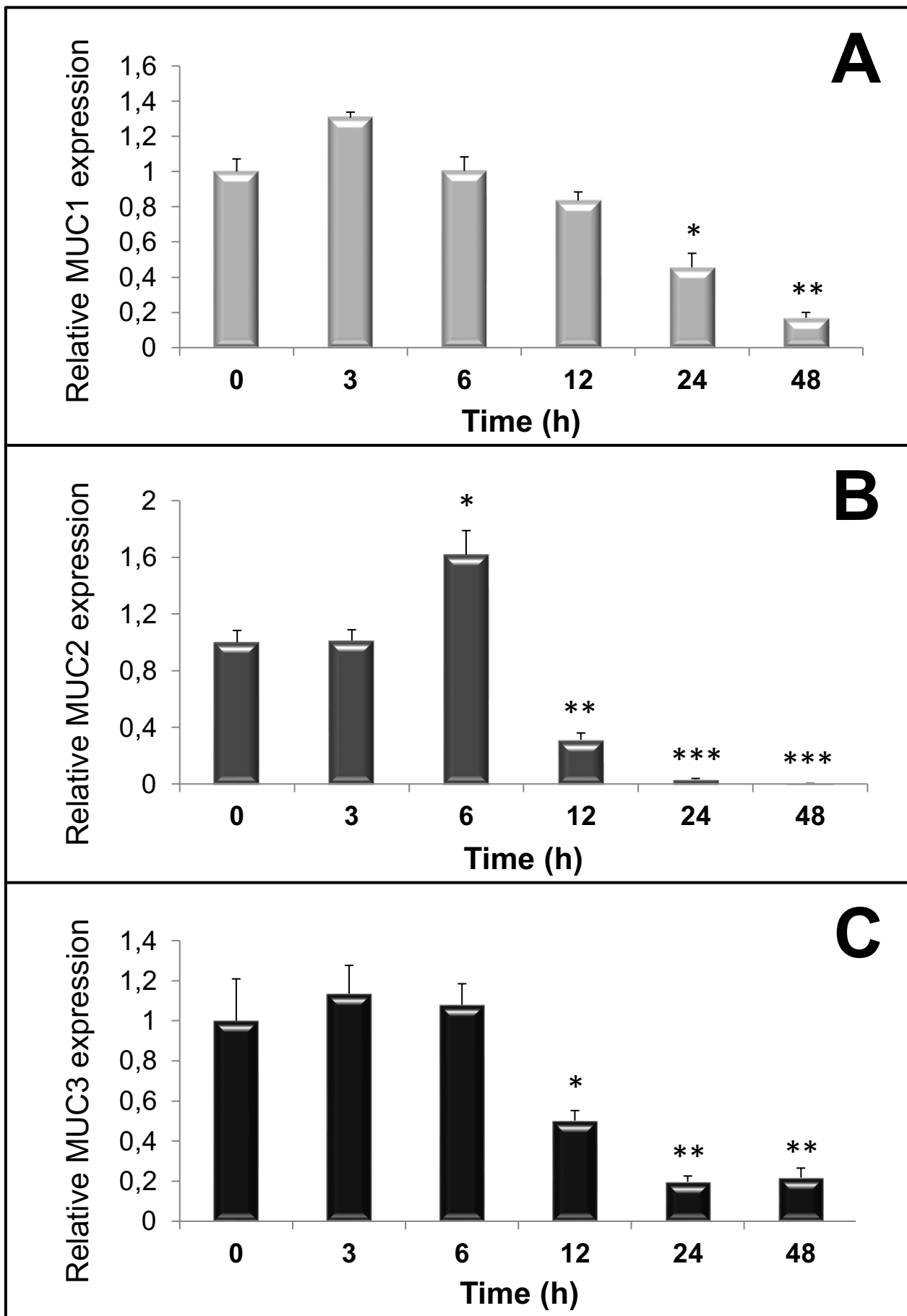


Fig 9

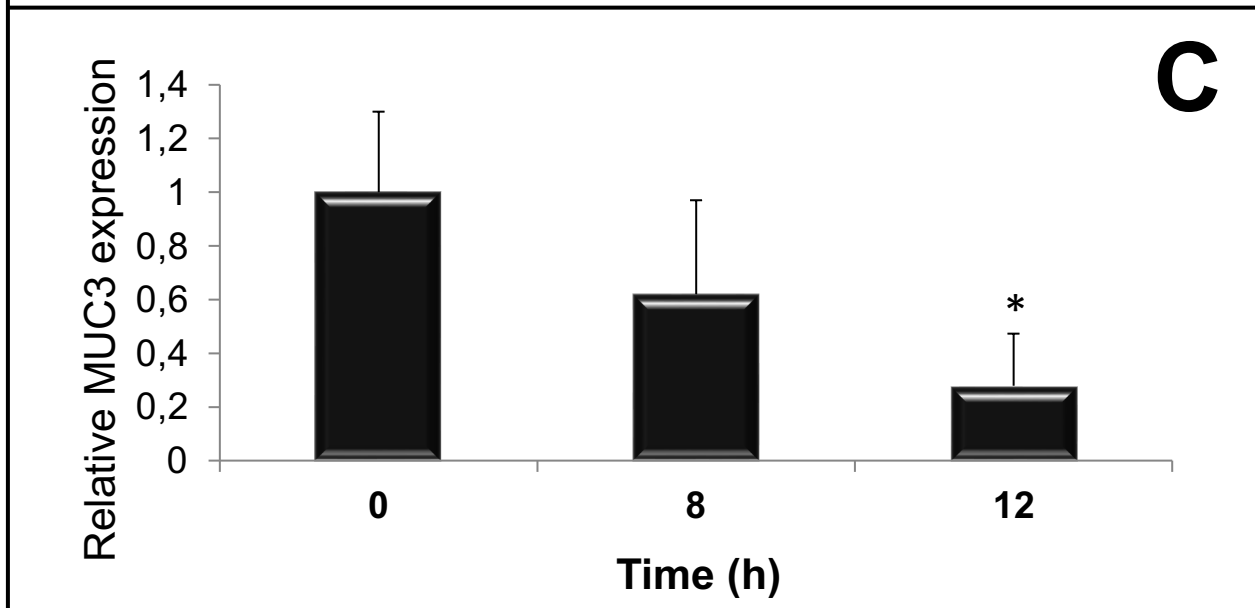
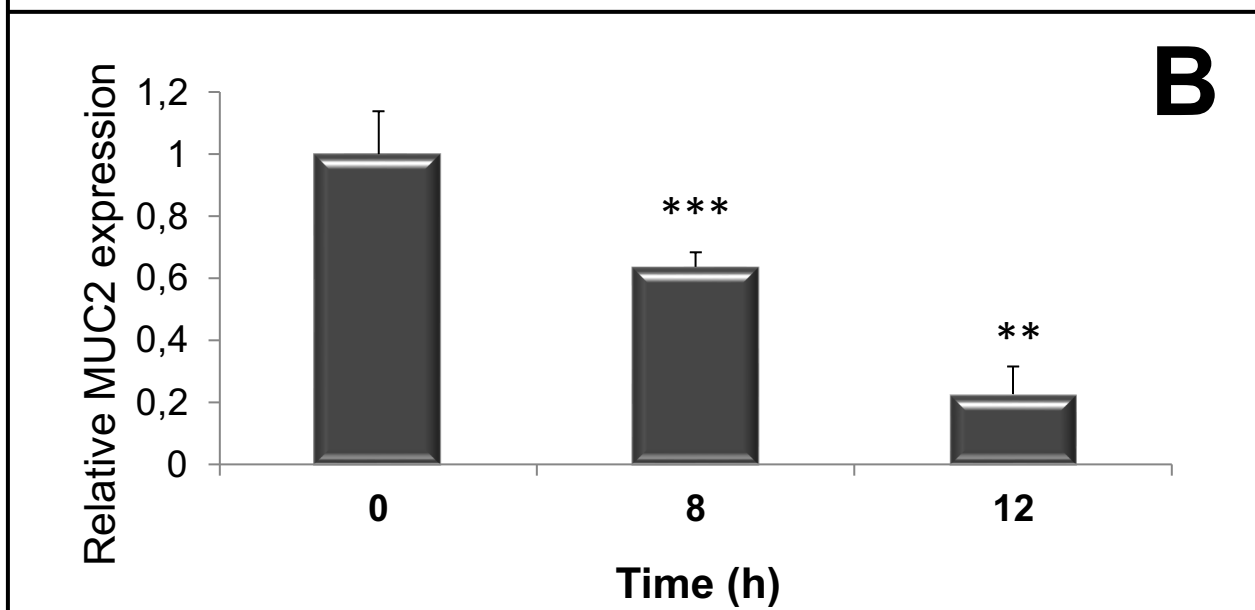
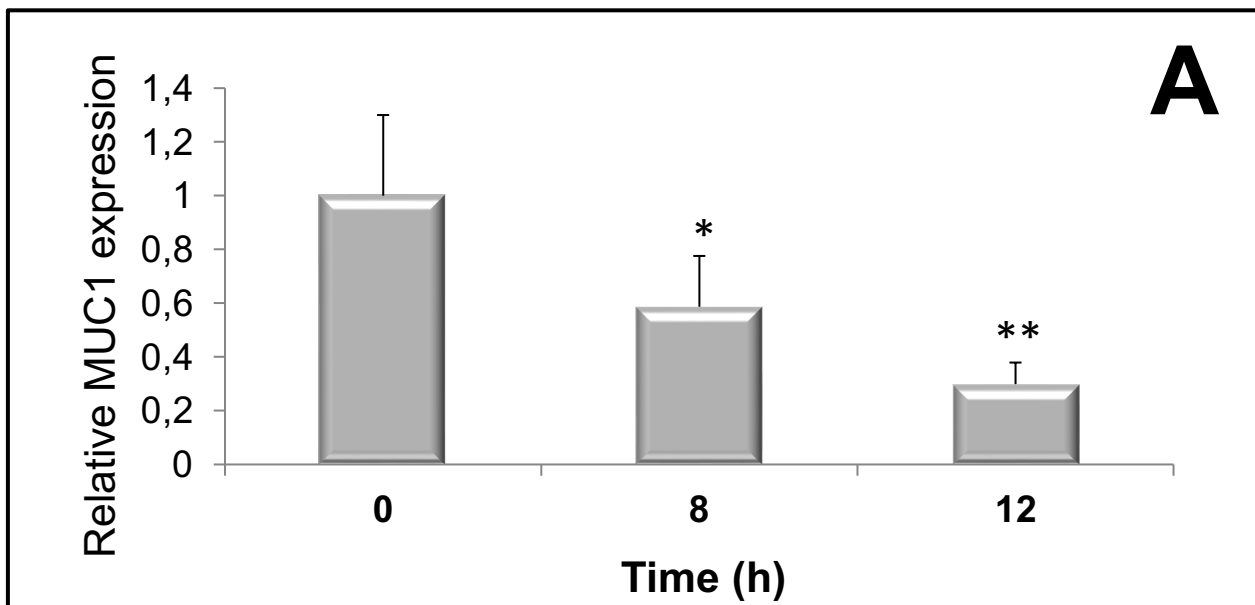


Fig 10

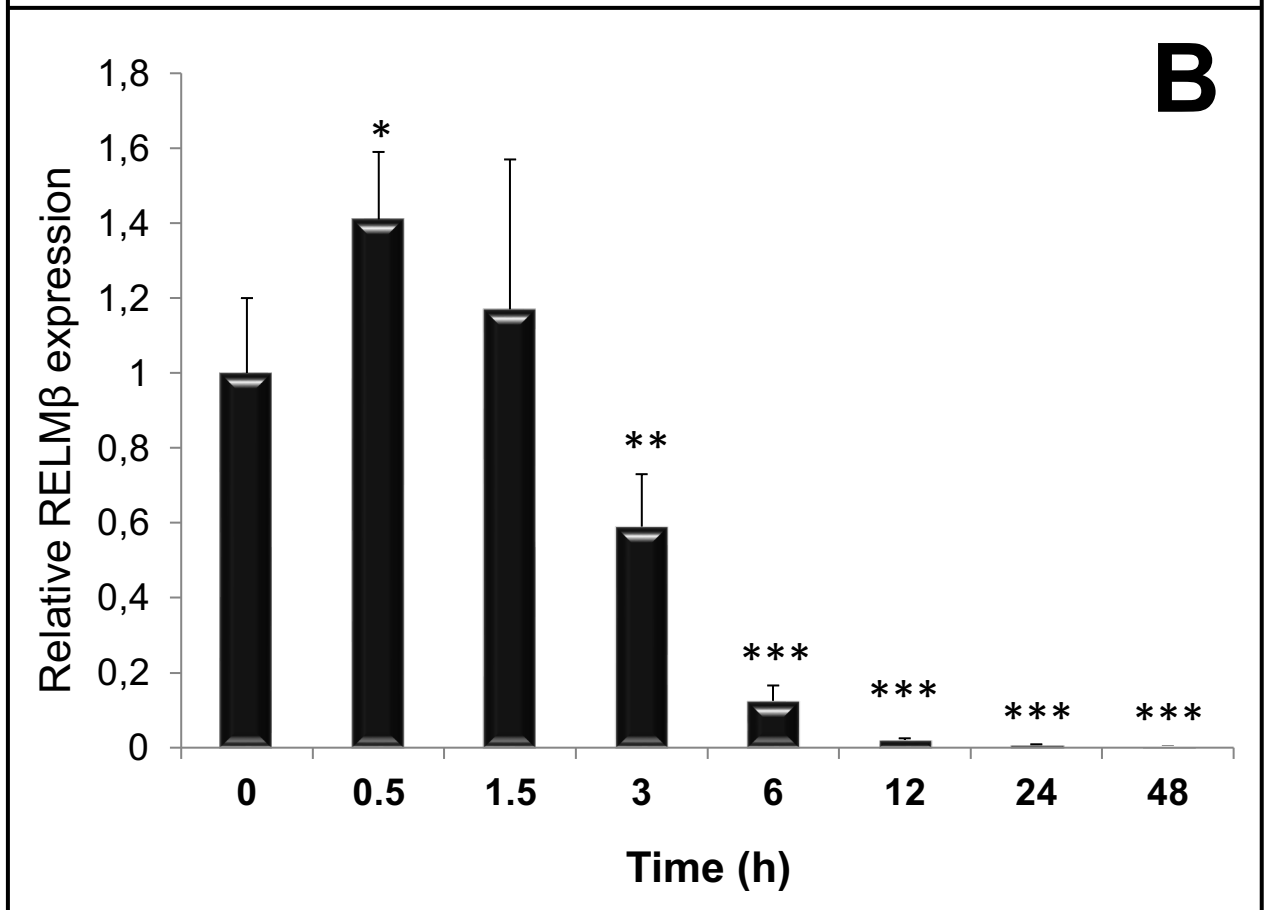
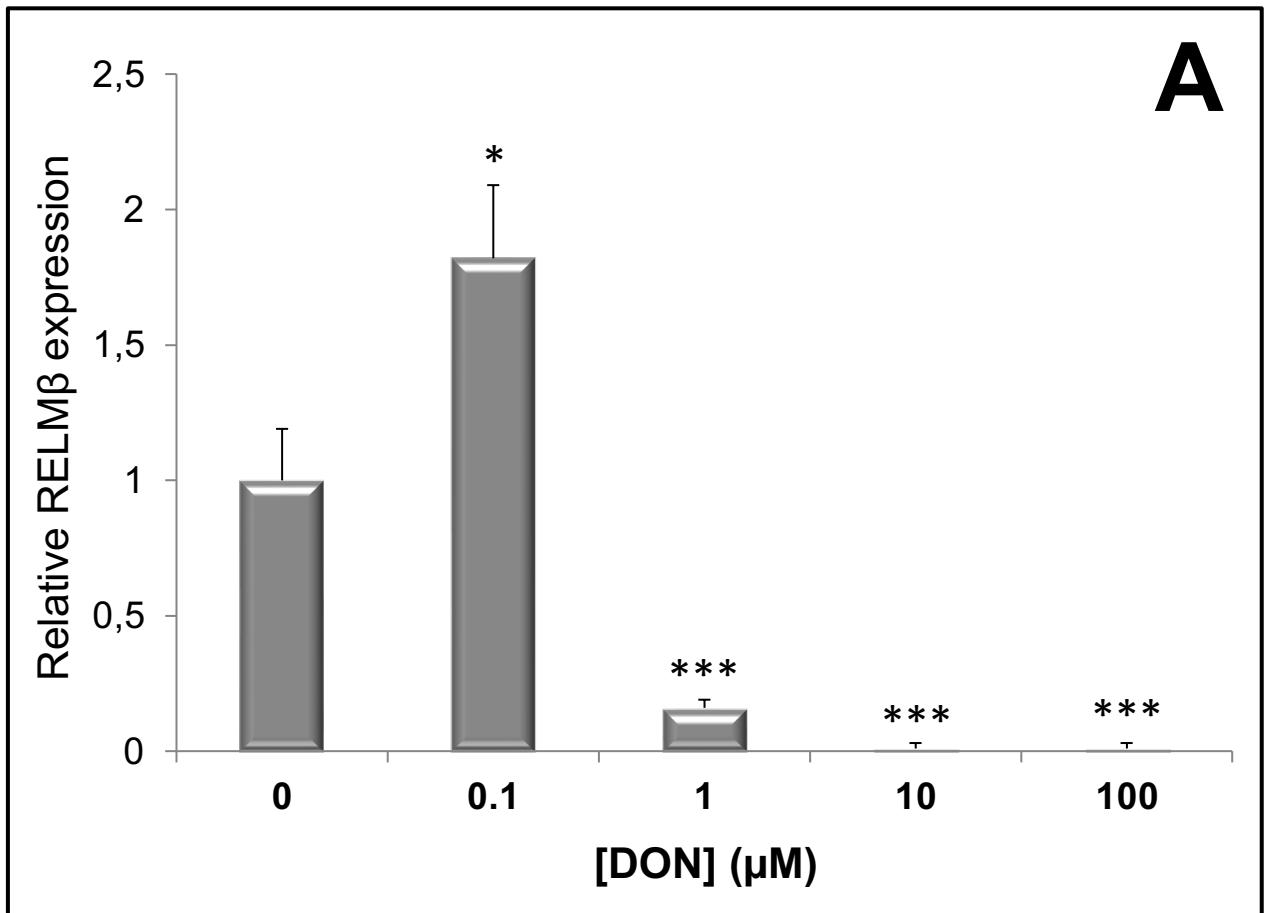


Fig 11

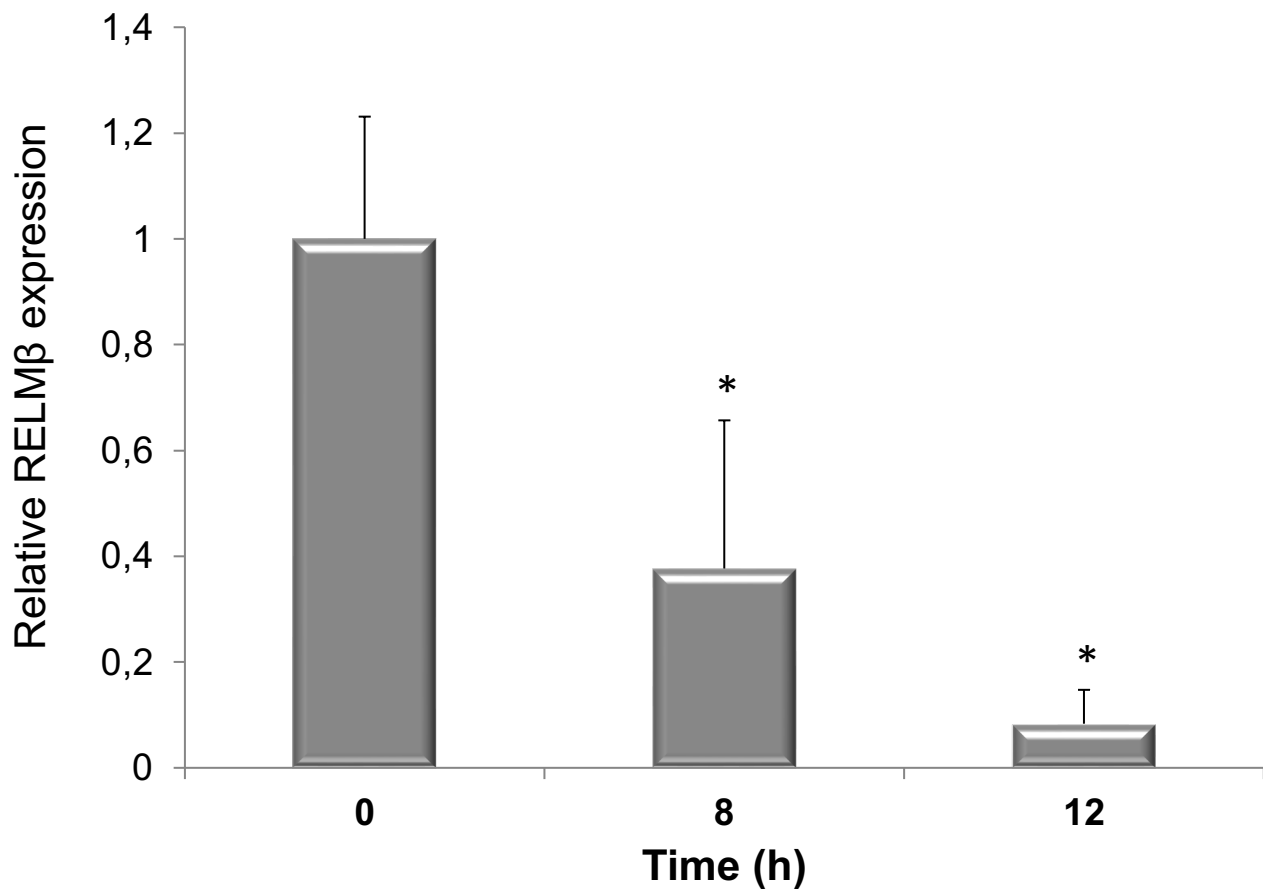


Fig 12

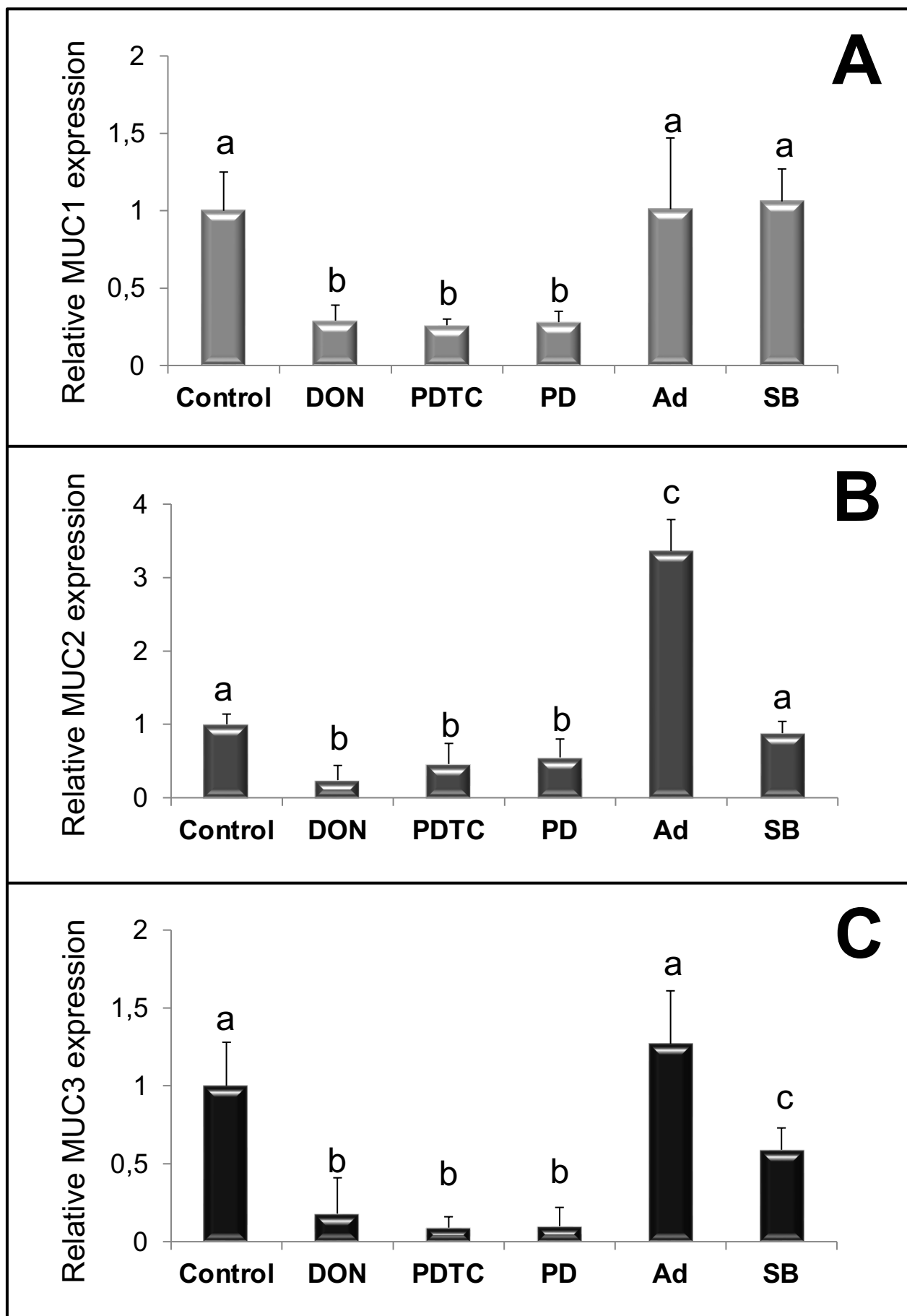


Fig 13

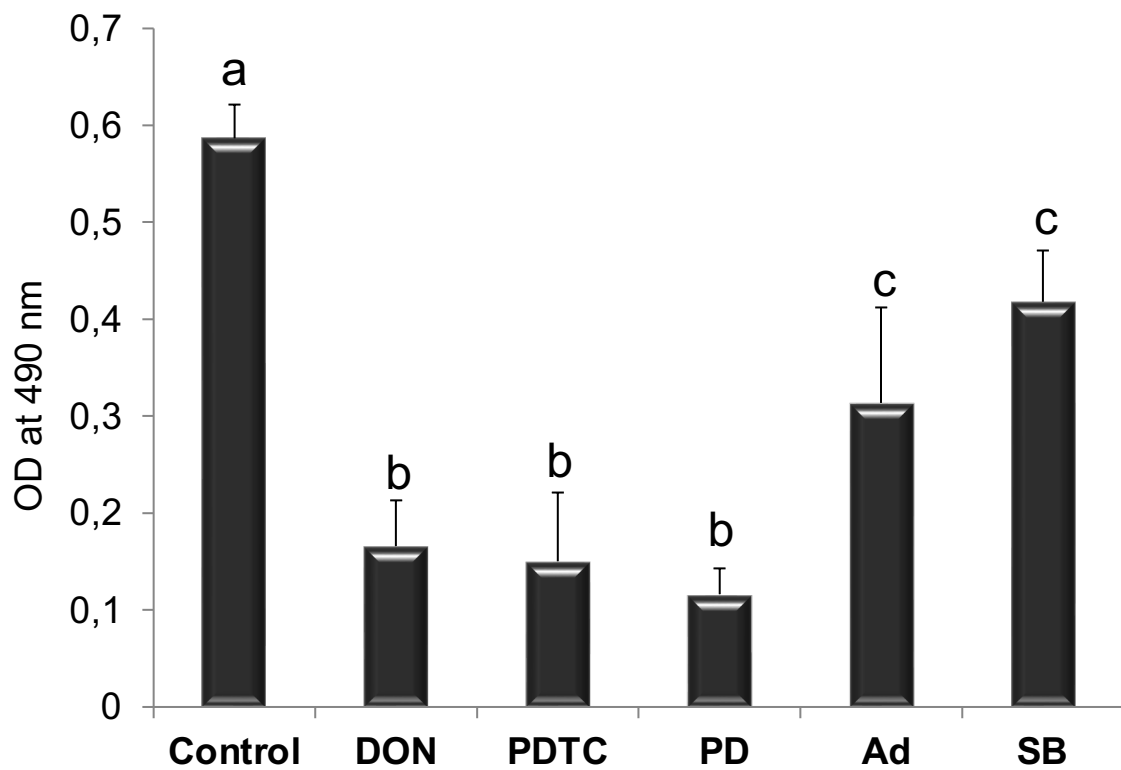


Fig 14

