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1 **Induction of DNA damages upon Marek's disease virus infection: implication in viral**
2 **replication and pathogenesis**

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4 **Running title:** DNA damage upon MDV infection

5

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19

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21 **ABSTRACT**

22 Marek's disease virus (MDV) is a highly contagious alphaherpesvirus that infects chickens
23 and causes a deadly neoplastic disease. We previously demonstrated that MDV infection
24 arrests cells in S-phase and that the tegument protein VP22 plays a major role in this process.
25 In addition, expression of VP22 induces double strand breaks (DSB) in the cellular DNA,
26 suggesting that DNA damage and the associated cellular response might be favorable for the
27 MDV lifecycle. Here, we addressed the role of DNA damage in MDV replication and
28 pathogenesis. We demonstrated that MDV induces DSB during lytic infection *in vitro* and in
29 the PBMCs of infected animals. Intriguingly, we did not observe DNA damage in latently
30 infected MDV-induced lymphoblastoid cells, while MDV reactivation resulted in the onset of
31 DNA lesions, suggesting that DNA damage and/or the resulting DNA damage response might
32 be required for efficient MDV replication and reactivation. In addition, reactivation was
33 significantly enhanced by the induction of DNA damage using a number of chemicals.
34 Finally, we used recombinant viruses to show that VP22 is required for the induction of DNA
35 damage *in vivo* and that this likely contributes to viral oncogenesis.
36

37 **IMPORTANCE**

38 Marek's Disease Virus is an oncogenic alphaherpesvirus that causes fatal T-cell lymphomas
39 in chickens. MDV causes substantial losses in poultry industry and is also used as a small-
40 animal model for virus-induced tumor formation. DNA damage is not only implicated in
41 tumor development but also aids in the life cycle of several viruses, however its role in MDV
42 replication, latency and reactivation remains elusive. Here, we demonstrated that MDV
43 induces DNA lesions during lytic replication *in vitro* and *in vivo*. DNA damage was not
44 observed in latently infected cells, however is reinitiated during reactivation. Reactivation
45 was significantly enhanced by the induction of DNA damage. Recombinant viruses that
46 lacked the ability to induced DNA damage were defective in the induction of tumors,
47 suggesting that DNA damage might also contribute to cellular transformation processes
48 leading to MDV-lymphomagenesis.

49

50 **Keywords:** Herpesvirus, Marek's disease virus, DNA damage, oncogenesis, viral replication,

51 VP22

52

53 INTRODUCTION

54 Marek's disease (MD) is an oncogenic lymphoproliferative disease caused by the Marek's
55 disease virus (MDV), also referred as to *Gallid herpesvirus 2*. MDV is a member of the
56 *Alphaherpesvirinae* subfamily, mostly due to its genomic organization. However, MDV
57 shows similarities with gammaherpesviruses considering its lymphotropic nature and
58 oncogenic properties (1). Infection of susceptible chickens with very virulent MDV strains
59 induces a rapid onset of tumors within 3-4 weeks and a high mortality. Intriguingly, MDV
60 and the human herpesvirus 6 (HHV-6) integrate their genome into the telomeres of latently
61 infected cells (2-5), allowing the long-life persistence of the virus in the host. Therefore,
62 MDV is used to assess herpesvirus integration as well as virus-induced lymphomagenesis (6).
63 The MDV life cycle is complex and can be broken down into four phases (7, 8): (i) an early
64 cytolitic phase, corresponding to the replication of MDV in B- and T-lymphocytes during the
65 first week of infection; (ii) the establishment of a latent infection in CD4+ T-lymphocytes
66 between 7-10 days post-infection (dpi) during which MDV is thought to integrate its genome
67 into host telomeres ; (iii) the reactivation of the virus from latently infected cells, which is
68 accompanied by its late replication and continuous shedding of the virus from the feather
69 follicle epithelium; and (iv) the tumorigenic phase characterized by transformation of CD4+
70 T-lymphocytes and the development of T-cell lymphoma.

71 Several viral oncogenes have been identified such as the latent oncoprotein Meq and the viral
72 telomerase RNA subunit, vTR (9-15), however the exact mechanism leading to lymphoma
73 development remains poorly understood. We recently demonstrated that during lytic
74 replication MDV triggers cell proliferation and subsequently delays the cell cycle in S-phase
75 (16). In addition, we showed that the tegument protein VP22 is able to induce the S-phase
76 arrest in the absence of other viral proteins. This blockade is associated with a massive onset
77 of double strand breaks (DSBs). The VP22 tegument protein is encoded by the UL49 viral

78 gene and is part of the MDV virion. VP22 is involved in cell-to-cell spread of MDV and is
79 essential for MDV replication (17). Beyond its role in MDV replication, VP22 potentially
80 contributes to the establishment of latency and/or transformation by its ability to interact with
81 DNA/histones, to interfere with the cell cycle progression, and to mediate DNA damage.
82 Moreover, it was shown that a recombinant MDV expressing a VP22 with a C-terminal GFP-
83 tag is highly attenuated *in vivo*, suggesting that VP22 plays a role in MDV-induced
84 tumorigenesis (18). In addition, we have previously observed that such a modification of the
85 VP22 C-terminus abolishes its ability to modulate the cell cycle and to induce DNA damage
86 upon overexpression of the protein in proliferating cells (16). Of note, the fusion of GFP to
87 the N-terminus of VP22 did not affect these properties *in vitro* and only mildly attenuates the
88 virus *in vivo* (16, 19).

89 Chromosomal aberrations and modulation of DNA damage response (DDR) are commonly
90 encountered during viral infections and are important for the viral life cycle as reviewed
91 previously (20-26). This has been particularly evidenced in herpesvirus infections for which
92 the ATM (Ataxia Telangiectasia-mutated) and ATR (ATM and Rad3 related) DNA damage
93 pathways proteins play a beneficial role for viral replication (27-31). Effectors of the DDR
94 and DNA repair pathways also facilitate virus maintenance and the establishment of latency
95 (31-33). Moreover, in the case of oncogenic viruses such as the Epstein Barr virus (EBV) and
96 the Kaposi sarcoma-associated herpesvirus (KSHV), the deregulation of these pathways and
97 the induction of DNA damage are of particular importance since genomic instability promotes
98 the establishment of neoplastic processes (34-40). DNA damage has been previously observed
99 in the blood of chickens diagnosed with MD infected with uncharacterized field viral strains
100 (41), however it remained unclear if this damage occurs in lymphocytes and if this is also the
101 case during early infection.

102 In the present study, we aimed to elucidate the kinetics of DNA damage in MDV infection to
103 determine the role of DNA damage on the MDV life cycle. We demonstrated that DNA
104 breaks accumulate in lytically infected cells *in vivo* and *in vitro*, but not in latently infected
105 cells. We also showed that DNA damage and/or DDR are actively induced upon MDV lytic
106 replication and reactivation from the latent stage. We demonstrated *in vivo* that VP22 is
107 required for the induction of DNA damage using recombinant viruses. Also, we observed that
108 a recombinant virus that lacked the ability to induced DNA damage was defective in the
109 induction of tumors, suggesting that DNA damage induction might participate to the
110 oncogenicity of MDV.

111

112 MATERIALS AND METHODS

113 Cells and viruses

114 Chicken embryo skin cells were prepared from 12 day-old specific pathogen-free (SPF) White
115 Leghorn (LD1) embryos and maintained in culture as previously described (42). The MDCC-
116 3867K cell line was derived from a renal lymphoma induced upon infection of chicken with
117 the highly pathogenic recombinant vRB-1B 47EGFP virus encoding the UL47 gene fused to
118 the enhanced green fluorescent protein (EGFP) (43). 3867K cells were cultured in RPMI 1640
119 supplemented with 2 mM glutamine, 1% pyruvate, 1% non-essential amino acids, 1%
120 glucose, 10% tryptose phosphate broth and 10% fetal bovine serum (FBS) and maintained at
121 41 °C in a 5% CO₂ atmosphere. RECC-CU91 T-cells, a reticuloendotheliosis virus (REV)-
122 transformed chicken T-cell line, were cultured in RPMI 1640 supplemented with 1%
123 pyruvate, 1% non-essential amino acids, 10% fetal bovine serum (FBS) and
124 penicillin/streptomycin, and maintained at 41°C in a 5% CO₂ atmosphere (44).

125 To visualized virus infected cells, EGFP was fused to the 5' end of the UL49 gene in the
126 avirulent BAC20, resulting in recEGFPVP22 (45). Very virulent, spread competent vRB-1B

127 virus was reconstituted from the infectious bacterial artificial chromosome (BAC) of RB-1B
128 as described previously (46). In addition, recombinant RB-1B were previously generated with
129 EGFP fuses to the 5' and 3' end of VP22, termed vRB-1B EGFP22 and vRB-1B 22EGFP
130 respectively (18, 19). All recombinant viruses were reconstituted, propagated and titrated as
131 described previously (45).

132 Infections of RECC-CU91 T-cells were performed by co-cultivation with infected CESC
133 (47). One million CESC were infected with 3×10^4 PFU of RB-1B_TK-GFP that expresses
134 GFP under the control of the early HSV-1 TK promoter for 3-4 days in 6-well plates.
135 Subsequently, 10^6 RECC-CU91 T-cells were added to the highly infected CESC monolayer
136 for 16 hours at 41°C. RECC-CU91 cells were carefully removed at day 1, 2 and 3 post-
137 infection for further analysis.

138 **Pharmacological induction of DNA damages**

139 DNA damage was induced in cells by etoposide (ETP; Sigma-Aldrich), bleomycin
140 (Calbiochem), hydroxyurea (HU; Sigma-Aldrich) and hydrogen peroxide (H_2O_2 ; Sigma-
141 Aldrich) treatments. At 6h post-infection, CESC infected with the recEGFPVP22 were
142 treated by addition in the culture media of the pharmacological agents at appropriate
143 concentrations (0.033, 0.066 or 0.132 μ M of ETP; 0.125 to 1 μ M of bleomycin; 10 to 75 μ M
144 of HU and 12.5 to 100 μ M of H_2O_2). ETP treated infected cells were analyzed at 24, 48, 72,
145 and 96h post-infection and bleomycin, hydroxyurea and hydrogen peroxide treated cells at
146 72h. RECC-CU91 cells were treated at the time of infection with 0.02 μ M or 0.1 μ M ETP for
147 24, 48 and 72h pi. Treatments of 3867K cells were performed for 48h with 0.004 to 0.5 μ M of
148 ETP; 1 to 25 μ M of bleomycin; 0.0625 to 1 mM of HU and 0.1 to 1 mM of H_2O_2 . In all
149 experiments, DMSO (for ETP) or H_2O (for bleomycin, HU and H_2O_2) was used as negative
150 control and added in the culture medium at a volume equivalent to the corresponding to the
151 highest concentration of drug treatments.

152 **Animal experiments**

153 Two *in vivo* experiments were conducted in this study according to the guidelines and
154 regulations as outlined that were approved by the local ethic committee “Comité d’Ethique
155 pour l’Expérimentation Animale de Val de Loire” (CEEA VdL, protocol number 2012-09-3).
156 In the first experiment (Fig. 4), 24 days-old SPF white leghorn chicks (B13/B13 haplotype)
157 were infected intramuscularly (pectoral muscles) with 1,000 pfu of vRB-1B (n=13) or mock
158 infected (n=10) and housed in isolation units. In the second experiment (Fig. 5), chickens
159 were either infected with 1,500 pfu of vRB-1B (n=6), vRB-1B EGFP22 (n=12), vRB-1B
160 22EGFP (n=13) or mock infected (n=10) as described above. Of note, in order to inoculate
161 an equal amount of virus to chickens, vRB-1B EGFP22 and vRB-1B 22EGFP viral inoculum
162 were exclusively constituted of EGFP+ positive sorted CESC (i.e. infected cells). Birds were
163 evaluated daily for symptoms of MD. In the case of clinical evidence of MD, the chickens
164 were euthanized and examined postmortem for the presence of gross MD lesions. At the end
165 of the experiments (35 dpi in experiment #1 and 49 dpi in experiment #2), all surviving birds
166 were euthanized and necropsied. To assess the DNA damage in peripheral blood mononuclear
167 cells and to follow the viral load, blood was collected on 3% sodium citrate from all birds at
168 0, 7, 14, 21, 28 and 35 dpi for experiment #1 and 0, 7, 14, 21, 28, 35 42 and 49 dpi for
169 experiment #2. PBMCs were isolated from 1 ml of whole blood using lymphocytes-separation
170 medium LSM (Eurobio, France) as previously described (19).

171 **DNA extraction and real-time quantitative PCR (qPCR)**

172 To quantify the viral load, DNAs were extracted from 30 µl whole blood (animal experiment
173 #1) or from 2×10^6 isolated cells (PBMCs in the animal experiment #2, CESC and RECC-
174 CU91 cells), using the QIAamp DNA mini kit according to the manufacturer’s instructions
175 (Qiagen). MDV genome copies were quantified by qPCR as previously described (19, 48).

176 The MDV genome was detected using primers and probes against the ICP4 gene and was
177 normalized to 10^6 copies of cellular genome quantified by the detection of the iNOS gene.

178 **RNA extraction and quantitative Reverse Transcription-PCR (qRT-PCR)**

179 RNAs were extracted from 10^6 non-infected and recEGFPVP22 infected CESC, PBMCs
180 isolated from vRB-1B infected chickens and 3867K cells, using the RNeasy Mini kit
181 following the manufacturer's instructions (Qiagen). RNAs were treated with RNase-free
182 RQ1 DNase (Promega, France) and the RNA concentration was measured with a NanoDrop
183 spectrophotometer. One μg of total RNA was reverse transcribed using 100 $\mu\text{g}/\text{mL}$ oligo(dT)
184 primers (Promega) and M-MLV reverse transcriptase (Promega). The expression of the genes
185 of interest was then assessed by qRT-PCR using the Supermix SYBR green (Bio-Rad) as
186 previously described (16). The sequence of the specific primer pairs used for the amplification
187 of the viral and cellular genes are depicted in Table 1. Expression of the chicken
188 glyceraldehyde phosphate dehydrogenase (GAPDH) was used for the normalization and the
189 relative changes in gene expression were determined by the $2(-\Delta\Delta\text{CT})$ method.

190 **Cell sorting and flow cytometry analysis**

191 Sorting of CESC, PBMCs infected with recombinant viruses expressing fluorescent VP22 proteins
192 were performed at 4 dpi. The 3867K cells exhibiting MDV lytic replication were sorted on the
193 basis of the expression of the UL47 protein tagged with EGFP. Mock-infected CESC
194 (negative control) were also sorted to avoid experimental bias linked to sorting. Damaged
195 cells and debris were eliminated on the basis of morphological criteria. EGFP positive and
196 negative cells were sorted using a MoFlo[®] high-speed cell sorter (Beckman Coulter, Fort
197 Collins, CO, USA).

198 The percentage of lytically infected 3867K and RECC-CU91 T cells was determined by
199 cytometry on the basis of the expression of the GFP (associated to the expression of UL47
200 and TK-promoter, respectively). Cell viability was estimated using the fixable viability dye

201 eFluor® 780 (eBioscience) at dilution of 1:1000. Staining was performed for 15 minutes on
202 ice in the dark. Cells were washed twice in PBS before being fixed with paraformaldehyde
203 (PAF) 1%. Cell viability was analyzed with a 780/40 nm band-pass filter.

204 **Alkaline comet assay**

205 Alkaline comet assays were performed from 2×10^5 cells as previously described (16, 49).
206 Two slides for comet assays were prepared for each condition. Comets were observed using
207 the Axiovert 200 M inverted epifluorescence microscope (Zeiss) and images were taken with
208 an Axiocam MRm camera (Zeiss). A minimum of 50 comets was analyzed for each replicate
209 using the CometScore software version 1.5 (TriTek). The olive tail moment parameter (OTM)
210 was calculated on the basis of the tail length and the relative proportion of DNA contained in
211 the tail. Results are presented as the mean (\pm SD) of the OTM calculated for each condition or
212 as a distribution of the comets with respect to their respective OTM value (i.e. the percentage
213 of cells presenting a defined OTM).

214 **Reactive oxygen species (ROS) assay**

215 ROS production was assayed from supernatant (80 μ l) of mock- and recEGFPVP22-infected
216 CESC_s at 24h, 48h, 72h and 96h pi using the ROS-Glo™ H₂O₂ assay following
217 manufacturer's instructions (Promega). Luminescence quantification was performed using a
218 Glomax® Multidetector system luminometer (Promega). Results were recorded as relative
219 luminescent units (RLU). Assays were done in triplicates at each time point. Results obtained
220 from infected-cells were normalized to that of mock-infected conditions and expressed as
221 means (\pm SD).

222 **Nitric oxide (NO) assay**

223 NO produced from infected and mock-infected CESC_s was measured at 24, 48, 72 and 96 hpi
224 by detecting the accumulation of nitrite (NO₂⁻) in the culture media using the Griess reaction
225 (50). Fifty microliters of cell culture supernatant were collected at each time point in a 96-

226 well plate (in triplicates) and incubated for 10 min in the dark with 100 μ l of Griess reagent
227 (mixture (1:1) of 1% sulfanilamide (Sigma-Aldrich) in 1.2 N hydrochloric acid and 0.3% N-
228 1-naphthylethylenediamine dihydrochloride (Sigma-Aldrich)). Absorbance was then
229 measured at 540 nm. Nitrite concentrations were calculated with reference to a calibration
230 curve established using standard solutions ranging from 0 to 200 μ M of sodium nitrite
231 (Sigma-Aldrich) diluted in culture medium. A positive control consisting of supernatant of
232 *E.coli* infected cells was included in the assay as well as cell-free media as negative control.

233 **Fluorescence Microscopy**

234 CESC's were grown on glass coverslips and infected with recEGFPVP22. At 4 days pi,
235 infected and non-infected cells were fixed with 4% PFA for 20 min at room temperature (RT)
236 and permeabilized with 0.5% Triton X-100 for 5 min at RT. After blocking with PBS, 0.1%
237 Triton X-100 and 2% Bovine Serum Albumin (BSA), cells were incubated with mouse
238 monoclonal IgG directed against phospho-histone H2AX (Ser139) (Millipore; clone JBW301)
239 at a dilution of 1:500. Goat anti-mouse IgG Alexa-Fluor 594 secondary antibody (Invitrogen)
240 was used at 1:2000. Nuclei were counterstained with Hoechst 33342 (Invitrogen). EGFP
241 fluorescence was directly observed from cells expressing the viral EGFP-tagged VP22
242 protein. Cells were observed under an Axiovert 200 M inverted epifluorescence microscope
243 equipped with a 40 \times PlanNeofluar oil/Dic objective or a 63 \times PlanApochromat oil/DIC and
244 the Apotome imaging system (Zeiss). Images were captured with a CCD AxioCam MRm
245 camera (Zeiss) by using the Axiovision software.

246 **MDV plaque size measurement assay**

247 At 48 hpi, CESC's monolayers infected with the recEGFPVP22 virus and treated with ETP
248 were fixed with 4% PFA. Fluorescence emitted from the viral EGFP-tagged VP22 protein
249 was detected using Axiovert 200 M inverted epifluorescence microscope equipped with a 5 \times
250 Fluor objective. Viral plaques were measured and analyzed as previously described (45).

251 **Statistical analysis**

252 All graphs and statistics were performed using the GraphPad Prism software version 5.02
253 (San Diego, USA). Data are presented as means and standard deviations (\pm SD) or medians.
254 The one-way ANOVA test was used to compare differences in multiple groups and the Mann-
255 Whitney (two-tailed) was used to compare nonparametric variables between two groups.
256 Significant differences were determined using Student's *t*-test. *P* values <0.05 were
257 considered statistically significant as indicated in the figure legends.

258 **RESULTS**

259 **MDV replication induces double strand breaks in the host genome**

260 Until now, it remained unknown if MDV induces DNA damage during virus replication.
261 Therefore, we infected CESC cells with a recombinant virus containing EGFP fused to the N-
262 terminus of VP22 (recEGFPVP22). GFP-positive MDV infected and GFP-negative cells were
263 sorted by flow cytometry 96 hpi and DNA damage was assessed by alkaline comet assay.
264 MDV infection increased the rate of DNA damage by 8.5-fold and 6-fold compared to GFP-
265 negative and mock-infected cells, respectively (Fig. 1A). In addition, up to 30% of the
266 infected cells had an OTM score greater than 10, indicative of highly damaged DNA (Fig.
267 1B). To identify the nature of the DNA damage in infected cells, we monitored the expression
268 and localization of γ -H2AX, a marker classically used to detect double strand breaks (DSBs).
269 Immunofluorescence analyses revealed a significant increase in intensity of γ -H2AX and a
270 typical localization of the protein as foci in the nucleus of CESC cells infected with the
271 recEGFPVP22 virus (Fig. 1C) indicative of the presence of DSBs.

272 **Induction of DNA damage enhances MDV replication**

273 To determine if the induction of DNA damage and/or the subsequent DDR is beneficial for
274 MDV replication, we infected CESC cells with recEGFPVP22 in the presence or absence of
275 potent DSB inducer etoposide (ETP) (51) and monitored MDV replication by qPCR (Fig. 2A

276 and B). MDV replication was significantly increased in the presence of ETP at 96 hpi
277 compared to DMSO treated control cells, and the greatest increase was observed for the
278 highest ETP concentration (Fig. 2A and B). To confirm that the observed effect is indeed due
279 to the induction of DNA damage, we also tested a number of pharmacological agents known
280 to generate single-strand and DSB, replicative stress and/or oxidative stress (bleomycin,
281 hydroxyurea (HU) and H₂O₂). As for ETP treatment, CESC were infected with
282 recEGFPVP22 and treated with these drugs at different concentrations and MDV copy
283 number was assayed at by qPCR (Table 2). The overall effect of the DNA damaging agents
284 tested was more moderate than ETP on MDV infected-cells, although all reagents tended to
285 increase slightly viral replication, underlining that DNA damage enhances MDV replication.
286 In addition, we could demonstrate that ETP increases the percentage of viable GFP-VP22
287 expressing cells in a dose dependent manner when compared to DMSO-treated control cells
288 (Fig. 2C). This increase in infected cells was significant for the highest ETP concentrations.
289 Furthermore, MDV plaques were also significantly larger upon induction of DNA damage
290 (Fig. 2D), indicating that the virus spread more efficiently to surrounding cells. Beyond that,
291 we also assessed the effect of ETP treatment on MDV replication in T cells, the natural target
292 of MDV infection. RECC-CU91 T cell were infected with RB-1B_TK-GFP in presence of
293 ETP and MDV copy number was monitored by qPCR (Fig. 2E). The impact of ETP treatment
294 on MDV replication in T-cells is more mitigated than in CESC, however MDV genome
295 copies slightly increase from one day pi when cells were treated with the highest ETP
296 concentration.
297 Taken together, our data demonstrate that additional induction of DNA damage and/or the
298 subsequent DDRs are beneficial for MDV replication.

299
300

301 **MDV replication induces reactive oxygen species (ROS) and nitric oxide (NO)**
302 **production in CESC**

303 Next we assessed if MDV induced DNA damage is mediated by oxidative stress, a common
304 cause of DSBs (52). We first monitored the level of ROS by measuring the production of
305 hydrogen peroxide (H₂O₂) in the supernatant of MDV or mock-infected cells (Fig. 3A). A
306 significant accumulation of H₂O₂ was detected in the supernatant of infected cells from 48 to
307 96 hpi. Besides ROS, reactive nitrogen species such as nitric oxide (NO) also plays a role in
308 metabolic stress and oxidative DNA damage in cells (53). We measured NO production in the
309 supernatant of infected and mock-infected cells and observed a significant increase of NO at
310 72 hpi (Fig. 3B). NO can be generated by the inducible nitric oxide synthases (iNOS) that is
311 generally activated upon immune response. Intriguingly, NO production coincided with an
312 increase expression of iNOS at 72 hpi (Fig. 3C). Our data suggest that MDV infection is
313 associated with an increased level of reactive oxygen and nitrogen species that could
314 contribute to the DNA damage in infected cells.

315 **DNA damage induction in chicken peripheral blood mononuclear cells upon MDV**
316 **infection**

317 To determine if MDV induces DNA lesions *in vivo*, we infected chickens with the very
318 virulent MDV strain vRB-1B. Blood was collected from all animals at various time points.
319 Viral load was assessed by qPCR on whole blood and DNA damage was determined using the
320 alkaline comet assay on isolated peripheral blood mononuclear cells (PBMCs). In addition,
321 the establishment of viral latency was followed by analyzing the mRNA expression of Meq
322 by qRT-PCR in non-infected and vRB-1B infected PBMCs. Intriguingly, DNA damage was
323 significantly increased (about 3-fold higher) in PBMCs of infected chickens during the lytic
324 phase of the MDV life cycle at 7 dpi (Fig. 4A). In contrast, no increase in DNA lesions was
325 observed in the latent phase of infection after 14 dpi. The increased in DNA damage was

326 associated with a 100-fold increase in viral load in the blood during the lytic phase of
327 infection (Fig. 4B). After day 14, high levels of the major oncogene Meq were observed by
328 qRT-PCR, corresponding to latently infected or MDV transformed cells (Fig. 4C) and are in
329 agreement with previous reports on the establishment of latency (54, 55). Thus, our data
330 shows that MDV early lytic infection is associated with transient DNA damage in PBMCs,
331 while no DNA damage was detected at later stages of infection.

332 **VP22 contributes to the DNA damage upon MDV infection *in vivo*.**

333 We previously demonstrated that overexpression of VP22 arrests the cell cycle and induces
334 DNA damage *in vitro* (16). In addition, we showed that MDV harboring EGFP fused to the C-
335 terminus of VP22 (vRB-1B 22EGFP) is severely attenuated, while fusion to the N-terminus of
336 VP22 (vRB-1B EGFP22) induces only a mild decrease in oncogenicity (18, 19), suggesting
337 that the C-terminal fusion affects VP22 function. Based on this observation, we assess the
338 induction of DNA damage mediated by these recombinant viruses *in vivo*. We infected SPF
339 chickens with either wild-type vRB-1B, vRB-1B 22EGFP or vRB-1B EGFP22 and monitored
340 DNA damage, virus replication and tumor development. Intriguingly, DNA damage at day 7
341 was only observed in PBMCs of birds infected with wild-type vRB-1B and vRB-1B EGFP22,
342 suggesting that the fusion of GFP to the N terminus of VP22 does not affect DNA damage
343 induction (Fig. 5A). In contrast, levels of DNA damage in PBMCs of vRB-1B 22EGFP
344 infected animal was comparable to mock infected chickens, indicating that fusion of GFP to
345 the C-terminus of VP22 disrupts its ability to mediate DNA lesions. To ensure that this effect
346 was not just due to a reduced virus replication, we monitored virus load in PBMCs by qPCR
347 and could demonstrate that virus replication was only mildly reduced at day 7. In contrast, a
348 significant decrease was observed at days 14 and 28 pi between vRB-1B 22EGFP and the
349 parental virus (Fig. 5B). Since DNA damage also plays an important role in cancer
350 development, we also monitored tumor incidence in the infected chickens. As observed

351 previously, tumor formation was severely impaired for vRB-1B 22EGFP (31 %) that cannot
352 induce DNA damage, while tumors were efficiently induced by wild-type vRB-1B (100 %)
353 and vRB-1B EGFP22 (66%). Our data shows that DNA damages is dependent on a functional
354 VP22 and that tumor formation is severely impaired for a virus that cannot induce DNA
355 damage.

356 **MDV reactivation is accompanied and enhanced by DNA damage**

357 Next, we set to determine if DNA damage is induced upon MDV reactivation. We
358 used a lymphoblastoid cell line that expresses GFP fused to the tegument protein UL47 upon
359 reactivation (3867K) as described previously (43). We sorted EGFP positive and negative
360 cells and assessed DNA damage by comet assays. DNA damage was significantly increased
361 in reactivating (GFP+) cells compared to the latent, GFP negative cells (Fig. 6A), indicating
362 that MDV reactivation in T cells is associated with DNA damage. An increased proportion of
363 reactivating cells also showed high levels of DNA damage (8%, OTM>10), while only
364 minimal damage is seen in latently infected cells (Fig. 6B). Next, we evaluated if DNA
365 damage can also increase reactivation. We induced DNA damage in 3867K with increasing
366 concentration of ETP for 48 hours. Both ICP4 expression and the number of GFP expressing
367 cells was significantly increased in a dose dependent manner (Fig. 6C and D). To confirm that
368 effect of DNA damage on MDV reactivation, we treated 3867K cells with bleomycin, HU and
369 H₂O₂. MDV reactivation was significantly increased in a dose dependent manner for all three
370 DNA damage inducing drugs (Fig. 6E). Taken together, our data demonstrate that DNA
371 damage is induced upon MDV reactivation and that induction of DNA damages seem to be
372 favorable for MDV reactivation.

373 **DISCUSSION**

374 The hallmark of the present study is the observation of an onset of DNA lesions in cells
375 sustaining MDV replication *in vitro* and *in vivo*. This was initially shown *in vitro* in MDV-

376 infected CESCes in which we detected DNA DSBs at 96 hpi. *In vivo*, we demonstrated that
377 MDV early cytolitic replication is associated with an increase in DNA damages in PBMCs of
378 infected chickens early after infection (7 dpi). Moreover, we showed *in vitro* that
379 lymphoblastoid cells (3867K) undergoing MDV replication induced from the spontaneous
380 reactivation of the virus are also affected by DNA damage. Of note, DNA damage during
381 MDV reactivation in the PBMCs of birds infected with the highly virulent RB-1B was not
382 statistically significant different from that of the mock-infected birds at 21 dpi, the time point
383 at which a peak of viral reactivation is expected. This might be due to the fact that only a
384 small number of CD4+ T cells reactivate in the blood and the low sensitivity of the comet
385 assay.

386 Intriguingly, DNA lesions were detected at 7 dpi in the PBMCs of chickens infected with both
387 vRB-1B or vRB-1B EGFP22 virus, but not with the attenuated vRB-1B 22EGFP virus, even
388 though all 3 viruses showed similar robust viral DNA replication as was assessed by qPCR
389 results. This observation may indicate that MDV replication is not sufficient to induce DNA
390 breaks. However, at 14 and 28 dpi the attenuated vRB-1B 22EGFP virus displayed a lower
391 replication rate compared to the wild-type vRB-1B virus. We could assume that this growth
392 defects might be associated with the low rate of DNA lesions occurring during the early
393 replication of the virus (at 7 dpi), and thus that DNA damage might be favorable for MDV
394 replication. These observations also confirmed that VP22 is a major viral determinant
395 associated to DNA damages *in vivo*. The VP22 tegument protein is abundantly expressed
396 during viral lytic infection and essential for MDV replication (17, 42). In a previous study, we
397 also reported that the overexpression of VP22 leads to DSB induction in proliferating cells,
398 and that this activity of VP22 depends on an unmodified C-terminal extremity (16). Herein,
399 we show *in vivo*, in an infectious context, that VP22 is involved in the induction of DNA
400 lesions observed during MDV early cytolitic infection, and that the modification of the C-

401 terminal extremity of the protein subverted the ability of MDV to trigger DNA damage in
402 PBMCs. It should also be noted that the level of DNA lesions detected at 7 dpi in PBMCs
403 from infected birds is somewhat surprising given the low number of circulating infected cells
404 and seems to indicate that non-infected cells might also be subjected to DNA damage. The
405 lesions observed in the non-infected population could be attributed to the inflammatory
406 immune response and/or paracrine signaling molecules emitted from infected cells and
407 responsible of a bystander effect (56-58). Also, despite conflicting reports about the
408 intercellular trafficking property of the VP22 protein, we cannot exclude that VP22 could
409 spread to non-infected surrounding cells and contribute to the generation of DNA lesions in
410 these cells (59-61). The mechanism by which VP22 is involved in the onset of DNA lesions is
411 still unclear. MDV VP22 could have a direct genotoxic activity on DNA since VP22 can
412 interact with DNA and histones (16, 42), or could activate cellular metabolism pathways
413 leading to DNA damage. In support to the latter hypothesis, we showed that MDV infection
414 triggers oxidative stress in CESC. We detected an increase of hydrogen peroxide production
415 from 48 hpi in CESC's infected with MDV. In addition, a higher level of nitrites associated to
416 an increase of iNOS mRNA expression was detected at 72 hpi in the supernatant of MDV-
417 infected CESC's compared to mock-infected cells. Previous studies reported that MDV
418 infection influences the production of NO (41, 48). A correlation was notably established
419 between the virulence of MDV strains and its ability to induce NO, with the most virulent
420 strains inducing the highest level of NO (48). It is believed that NO plays a role in MDV
421 pathogenesis through its involvement in the immune suppression observed early after
422 infection (48). Nevertheless, the role of NO on MDV replication is still not clearly elucidated.
423 NO production was identified as an anti-viral process by inhibiting MDV replication *in vitro*
424 and *in vivo* (62, 63). However, Jarosinski *et al.* demonstrated that despite a high NO response,
425 chickens infected with very virulent MDV strains showed an enhanced cytolytic infection

426 (48). Oxidative stress is a major generator of DNA breaks including DSBs (64). We could
427 thus hypothesize that oxidative stress generated during MDV replication could participate in
428 the generation of DNA lesions, which in turn would facilitate MDV replication and
429 consequently potentiate the virulence of MDV. We have indeed demonstrated that DNA
430 damage seems to favor the replication of the virus, since we have shown that DNA damaging
431 pharmacological agents can promote MDV replication and enhance MDV reactivation from
432 latent infection. Of note, the impact on MDV replication seemed to depend on the drug used
433 and thus probably on the nature of the damages generated and/or the damage response (DDR)
434 associated. Also, the response to different treatments might vary between cells according to
435 their lineage (lymphoid, fibroblastic) and their proliferative potential (primary versus cell
436 lines). Hence, ETP treatment (inducing mainly DSBs) resulted in an increase of MDV
437 replication in CESC_s, while bleomycin, hydroxyurea and H₂O₂ treatments had a weaker effect
438 in CESC_s. One explanation might be the low proliferative rate of CESC_s that may counteract
439 the activity of drugs inducing damage during S phase. ETP had also mild effect on MDV
440 replication in CU-91 T- cell line. Also, CU-91 were initially transformed with REV, we could
441 thus hypothesize that the presence of a replicative retrovirus might disturb the DNA damage
442 responses in these cells and/or have an impact on MDV replication. On the other hand, all
443 treatments induced a significant increase of MDV reactivation in a lymphoid cell line
444 transformed by MDV. We cannot currently specify whether DNA damages only or the
445 induction of the DDR associated with the onset of damage promotes MDV replication. As
446 previously demonstrated for a number of viruses and especially herpesviruses, the DNA
447 damage response (DDR) plays a major role in viral replication (for review see (20, 22-24, 26,
448 65)). Unfortunately, we were not able to characterize more precisely the DDR pathways
449 induced during MDV infection in the present study due to a lack of specific tools cross-
450 reacting with chicken proteins. Nevertheless, we hypothesized that MDV infection induces

451 the activation of a DDR as it was demonstrated for other herpesviruses. We identified at least
452 two processes that could contribute to the DDR pathways activation: (i) the generation of
453 DNA lesions in cellular DNA triggered upon MDV replication, and (ii) the increase of
454 oxidative stress in MDV-infected CESC. Elevated levels of ROS are known to activate DDR
455 pathways, as demonstrated notably upon EBV infection in which the latent protein EBNA1
456 promotes ROS accumulation and consequently ATM-dependent DDR activation (34).
457 Moreover, our previous study demonstrated that MDV induces an S-phase arrest in fibroblasts
458 (16). This constitutive S-phase induction may generate a favorable environment for viral
459 replication but could also lead to replicative stress, a potent mechanism responsible of DDR
460 induction. Although we currently cannot determine the DDR pathways activated by MDV, we
461 can speculate that ATM signaling may be induced in response to the DSBs and to the
462 oxidative stress generated during MDV infection (66, 67). Of note, ATM pathway activation
463 seems to be a common characteristics of herpesviruses infections (as previously reported
464 notably for the human herpesvirus type-1 (HSV-1), the cytomegalovirus (CMV, EBV and
465 KSHV), and in most cases ATM has been demonstrated to be beneficial for viral replication
466 (28, 68-70).

467 Finally, a major point of interest of the present study is the potential involvement of the onset
468 of DNA lesions in MDV-induced lymphomagenesis. Many reports have shown that DNA
469 damage and the DDR can contribute to genomic instability in cells and in turn to the
470 development of tumors. Moreover, DSBs are among the most deleterious lesions occurring in
471 cells and if not repaired, accumulation of DSBs could promote cell-death or the loss of
472 genome integrity possibly leading to carcinogenesis (71). Several studies demonstrated the
473 interplay between oncogenic viruses and DNA damage/DDR signaling and its crucial
474 implication in virus mediated-tumorigenesis (for review see (35, 39, 40)). Our data support
475 these findings since we observed a greater rate of DNA damage in PBMCs from chickens

476 infected with virulent MDV viruses (vRB-1B and vRB-1B EGFP22) and to a significantly
477 lesser extent in PBMCs of birds infected with an attenuated recombinant virus (vRB-1B
478 22EGFP). The low oncogenicity of the vRB-1B 22EGFP virus might well be associated with
479 its diminished replication capacity (as observed at day 14 pi). However, in the light of our
480 results, one can also speculate that the viral phenotype is due to the absence of an early onset
481 of DNA lesions in infected leukocytes that otherwise might contribute to MDV induced
482 tumorigenesis. One hypothesis would be that the enhanced MDV replication upon, could
483 result in an increased number of latently infected cells from which transformed T cells
484 originate. DNA damage could also play a direct role in the establishment of viral latency and
485 more precisely in the integration process of the viral genome. We indeed cannot exclude that
486 the DNA lesions observed at an early time point of MDV infection could arise before or
487 concomitantly to MDV latent infection in CD4+ T-lymphocytes and thus could facilitate
488 MDV genome integration either directly or indirectly by triggering DDR and DNA repair
489 pathways, especially homologous recombination, as it was previously suggested for the
490 hepatitis B virus, human Papillomaviruses, Merkel Cell Polyomavirus and EBV (2, 3, 72, 73).
491 However, one should not underestimate the impact of a potential genomic instability
492 originating from DNA damage generated during MDV replication. It is indeed conceivable
493 that, in a particular sequence of events including cell cycle deregulation, reprogramming of
494 gene expression by viral oncogenes (notably Meq) and telomerase activation, the
495 accumulation of DNA lesions upon MDV infection may also contribute to the transformation
496 process ultimately leading to MD lymphoma formation.

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- 693

694 **FIGURE LEGENDS**

695 **Figure 1. Induction of DNA damage in MDV lytically infected cells.** CESC_s were infected
696 with 10⁴ pfu of recEGFPVP22. (A) DNA damage analysis in mock- or recEGFPVP22-
697 infected CESC_s. At 4 dpi, EGFP-positive and -negative cells were sorted by flow cytometry
698 and DNA damage was analyzed from 2x10⁵ cells by alkaline comet assays. Two slides per
699 comet assays were prepared for each condition and analyzed using the CometScore software.
700 Results are presented as the mean of OTM score (\pm SD; ***p<0,001) and representative
701 images of comets are shown as photographs. (B) Frequency distribution of the comets with
702 respect to their OTM value. (C) Expression and localization of γ H2AX in CESC_s infected
703 with recEGFPVP22. At 4 dpi, mock- and recEGFPVP22-infected CESC_s were subjected to
704 immunofluorescence using a mouse anti- γ H2AX monoclonal antibody and an AlexaFluor
705 594-conjugated secondary antibody (red). Nuclei were stained with Hoechst 33342 (blue) and
706 infected cells expressing the EGFP-tagged VP22 were directly visualized by fluorescent
707 microscopy (green).

708 **Figure 2. DNA damage induction enhances MDV replication.** (A-D) CESC_s were infected
709 with recEGFPVP22 and treated at 6 hpi with etoposide (ETP), Bleomycin, hydroxyurea (HU)
710 and H₂O₂ at the indicated concentrations or with DMSO or H₂O (as negative controls). (A-B)
711 At 24, 48, 72, and 96 hpi, DNA was extracted from cells treated with ETP and MDV
712 replication was assessed using qPCR. For each group, the number of MDV genome copies
713 (corresponding to ICP4 copies number) was normalized to 10⁶ cells (estimated by the iNOS
714 copies number). (A) Representative growth curve from a total of 3 independent experiments.
715 Means of qPCR triplicates are indicated (\pm SD). (B) Fold change in MDV copies in ETP-
716 treated cells relative to DMSO-treated cells (**p<0.05). (C) Number of cells lytically infected
717 with MDV upon ETP treatment. The percentage of viable GFP positive infected cells was
718 determined at 96 hpi by FACS. Viable cells were detected using the viability dye eFluor-780.

719 Means are represented as bars (\pm SD; * p <0.05). (D) Effect of ETP on MDV plaques size.
720 Images of fluorescent MDV plaques were taken and plaque sizes measured at 48 hpi. Means
721 are presented as histograms (\pm SD; * p <0.05; *** p <0.001). (E) Impact of ETP induced DNA
722 damage on MDV replication in RECC-CU91 T-cells. (E) RECC-CU91 cells were infected
723 with RB-1B_TK-GFP and treated with 0.02 and 0.1 μ M of ETP or with DMSO (as a negative
724 control). At 24, 48 and 72 hpi, MDV genome copy number was quantified by qPCR and data
725 shown as a fold change (\pm SD) relative to DMSO-treated cells.

726 **Figure 3. MDV replication induces production of ROS and NO.** CESC's were mock-
727 infected or infected with recEGFPVP22. (A) ROS accumulation in supernatant of mock- and
728 recEGFPVP22-infected cells. At 24, 48, 72, and 96 hpi, supernatants of mock- and
729 recEGFPVP22-infected cells were collected, and H₂O₂ accumulation was quantified using the
730 ROS-Glo™ kit (Promega). Results were normalized to RLU values obtained from mock-
731 infected cells and expressed as means (\pm SD). (B) NO production in supernatant of mock- and
732 recEGFPVP22-infected cells. At indicated time points, supernatants of mock- and
733 recEGFPVP22-infected cells were collected, and nitrite accumulation was quantified using
734 the Griess reaction. Absorbance values (at 540 nm) obtained from supernatant of infected
735 cells were reported to these from mock-infected cells and expressed as means (\pm SD). (C)
736 Expression of inducible nitric oxide synthase (iNOS) in MDV infected cells. Total mRNA
737 was isolated from mock- and MDV infected CESC's at the time point indicated and qRT-PCR
738 were performed with iNOS specific primers. Results were normalized to GAPDH expression
739 and expressed as mRNA fold change compared to expression of iNOS in mock-infected cells
740 (\pm SD).

741 **Figure 4. Induction of DNA damage in PBMCs of chickens infected with MDV.** Specific
742 pathogen free (SPF) susceptible white leghorn chicks (B13/B13 haplotype) were inoculated
743 intramuscularly with 1,000 pfu of the very virulent MDV strain vRB-1B. DNA damage onset

744 in PBMCs was assessed in 10 non-infected chickens (circles) and 13 birds infected with vRB-
745 1B (triangles). Blood was collected from all birds at indicated time points. (A) DNA damage
746 analysis in PBMCs isolated from mock- and vRB-1B infected chickens by alkaline comet
747 assays. Two slides per comet assays were prepared for each animal at each time point. A
748 minimum of 50 comets were observed and further analyzed on each replicate slide using the
749 CometScore software. Results are presented as dot plot, each dot representing an animal and
750 the mean of OTM for each group is indicated as a bar (**p<0.001). (B) Viral load estimated
751 after DNA extraction from whole blood and quantification of MDV genome copies using
752 qPCR. For each group, the number of ICP4 copies in the MDV genome was normalized to
753 10⁶ copies of cellular genome estimated by the detection of iNOS copies. The medians copy
754 numbers are indicated as a bar. (C) Meq mRNA expression upon MDV infection. Total RNA
755 was extracted from PBMCs isolated from blood of birds infected with vRB-1B. Quantitative
756 RT-PCRs were performed in order to detect the mRNA expression of Meq. Gene expression
757 was normalized to GAPDH expression and fold changes are presented as box plot (Min/max).

758 **Figure 5. Role of VP22 and DNA damage in MDV-mediated oncogenicity in chickens.**

759 SPF white leghorn chicks were inoculated with 1,500 pfu of vRB-1B, vRB-1B EGFP22, or
760 vRB-1B 22EGFP. Blood was collected from all birds at indicated time points and PBMCs
761 were isolated. (A) DNA damage was quantified from 2x10⁵ PBMCs using the alkaline comet
762 assay. Results are presented as dot plots, each dot representing an animal. For each group, the
763 median of the OTM is indicated as a bar (**p<0.001) (B). MDV viral load was evaluated by
764 qPCR on DNA extracted from PBMCs. The number of MDV copies per 10⁶ cells is presented
765 as a dot plot, each dot representing an animal. For each group, the median is indicated as a bar
766 (*p<0.05; **p<0.005).

767

768 **Figure 6. DNA damage during MDV reactivation.**

769 3867K cells undergoing MDV lytic replication were sorted by cytometry on the basis of the
770 expression of the UL47 gene tagged with EGFP. (A) DNA damage analysis in lytically
771 (GFP+) and latently (GFP-) infected cells. The alkaline comet assay was performed on EGFP-
772 positive and -negative sorted cells. Results are presented as the mean of OTM (\pm SD;
773 *** $p < 0.001$) and representative comets images are shown below. (B) Frequency distribution
774 of the comets with respect to their OTM value. (C-E) Effect of DNA damaging
775 pharmacological agents on MDV reactivation. 3867K cells were treated with etoposide
776 (ETP), bleomycin, hydroxyurea (HU) or H₂O₂ at the indicated concentrations for 48 hours.
777 DMSO and H₂O were added to culture media as negative controls. (C) MDV replication was
778 evaluated by quantifying the mRNA expression of the immediate-early gene ICP4 by qRT-
779 PCR. ICP4 expression was normalized to the expression of GAPDH and results are presented
780 as means (\pm SD; ** $p < 0.005$). (D-E) Number of MDV reactivated 3867K cells. The
781 percentage of GFP positive cells (expressing the EGFP-tagged UL47 protein) was determined
782 48 h post-treatment by cytometry specifically in viable cells labeled using the viability dye
783 eFluor-780. Means are represented as bars (\pm SD; * $p < 0.05$). Results are representative of 3
784 independent experiments realized in triplicates.

Target		Sequences	Accession Number
ICP4	For	5'TTTCTAGCAAGGAGCGACGC3'	NC_002229.3
	Rev	5'CTGACTTGCGCTTACGGGAA3'	
Meq	For	5'GTCCCCCTCGATCTTTCTC3'	AY571783.1
	Rev	5'CGTCTGCTTCTGCGTCTTC3'	
iNOS	For	5'TACTGCGTGTCTTTCAACG3'	U46504
	Rev	5'CCCATTCTTCTCCAACCTC3'	
GAPDH	For	5'TGATGATATCAAGAGGGTAGTGAAG3'	K01458
	Rev	5'TCCTTGGATGCCATGTGGACCAT3'	

785 **Table 1. List of primer pairs used for (q)RT-PCR**

786

Treatment	Concentration (μ M)	Mean ICP4/ 10^6 iNOS copies
H ₂ O		(1.46 \pm 0,22) $\times 10^8$
Bleomycin	0.125	(1.33 \pm 0,05) $\times 10^8$
	0.25	(2.25 \pm 0,04) $\times 10^8$
	0.5	(2.16 \pm 0,14) $\times 10^8$
	1	(2.47 \pm 0,33) $\times 10^8$
	10	(2.55 \pm 0,03) $\times 10^8$
Hydroxyurea	25	(2.6 \pm 0,005) $\times 10^8$
	50	(1.76 \pm 0,03) $\times 10^8$
	75	(1.83 \pm 0,25) $\times 10^8$
	12.5	(1.91 \pm 0,009) $\times 10^8$
H ₂ O ₂	25	(1.6 \pm 0,03) $\times 10^8$
	50	(1.38 \pm 0,05) $\times 10^8$
	100	(2.12 \pm 0,23) $\times 10^8$

787 **Table 2. Effect of DNA damage inducers on MDV replication in CESC**

788

Figure 1

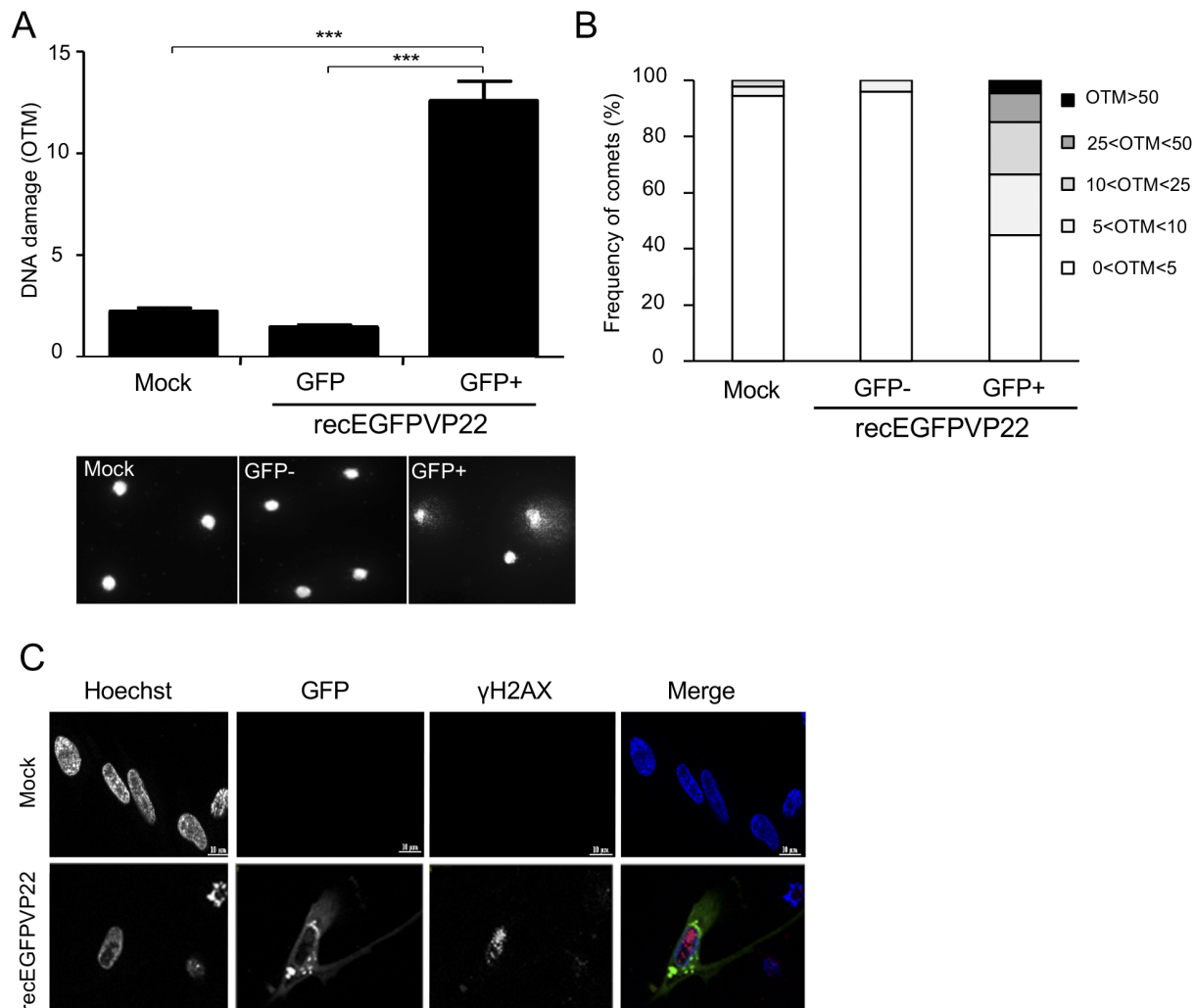


Figure 1. Induction of DNA damage in MDV lytically infected cells. CESC were infected with 10^4 pfu of recEGFPVP22. (A) DNA damage analysis in mock- or recEGFPVP22-infected CESC. At 4 dpi, EGFP-positive and -negative cells were sorted by flow cytometry and DNA damage was analyzed from 2×10^5 cells by alkaline comet assays. Two slides per comet assays were prepared for each condition and analyzed using the CometScore software. Results are presented as the mean of OTM score (\pm SD; *** $p < 0,001$) and representative images of comets are shown as photographs. (B) Frequency distribution of the comets with respect to their OTM value. (C) Expression and localization of γ H2AX in CESC infected with recEGFPVP22. At 4 dpi, mock- and recEGFPVP22-infected CESC were subjected to immunofluorescence using a mouse anti- γ H2AX monoclonal antibody and an AlexaFluor 594-conjugated secondary antibody (red). Nuclei were stained with Hoechst 33342 (blue) and infected cells expressing the EGFP-tagged VP22 were directly visualized by fluorescent microscopy (green).

Figure 2

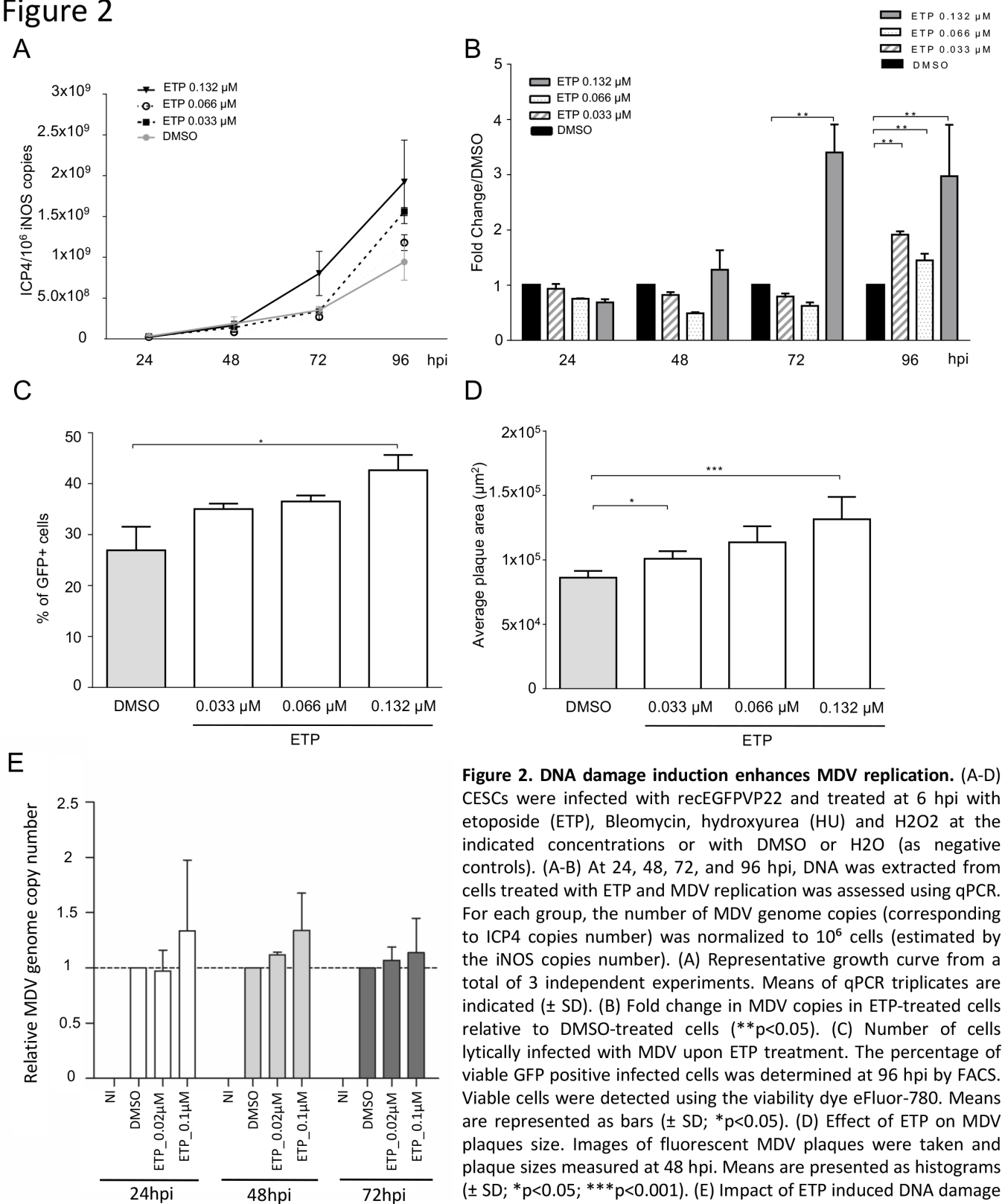


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Figure 3

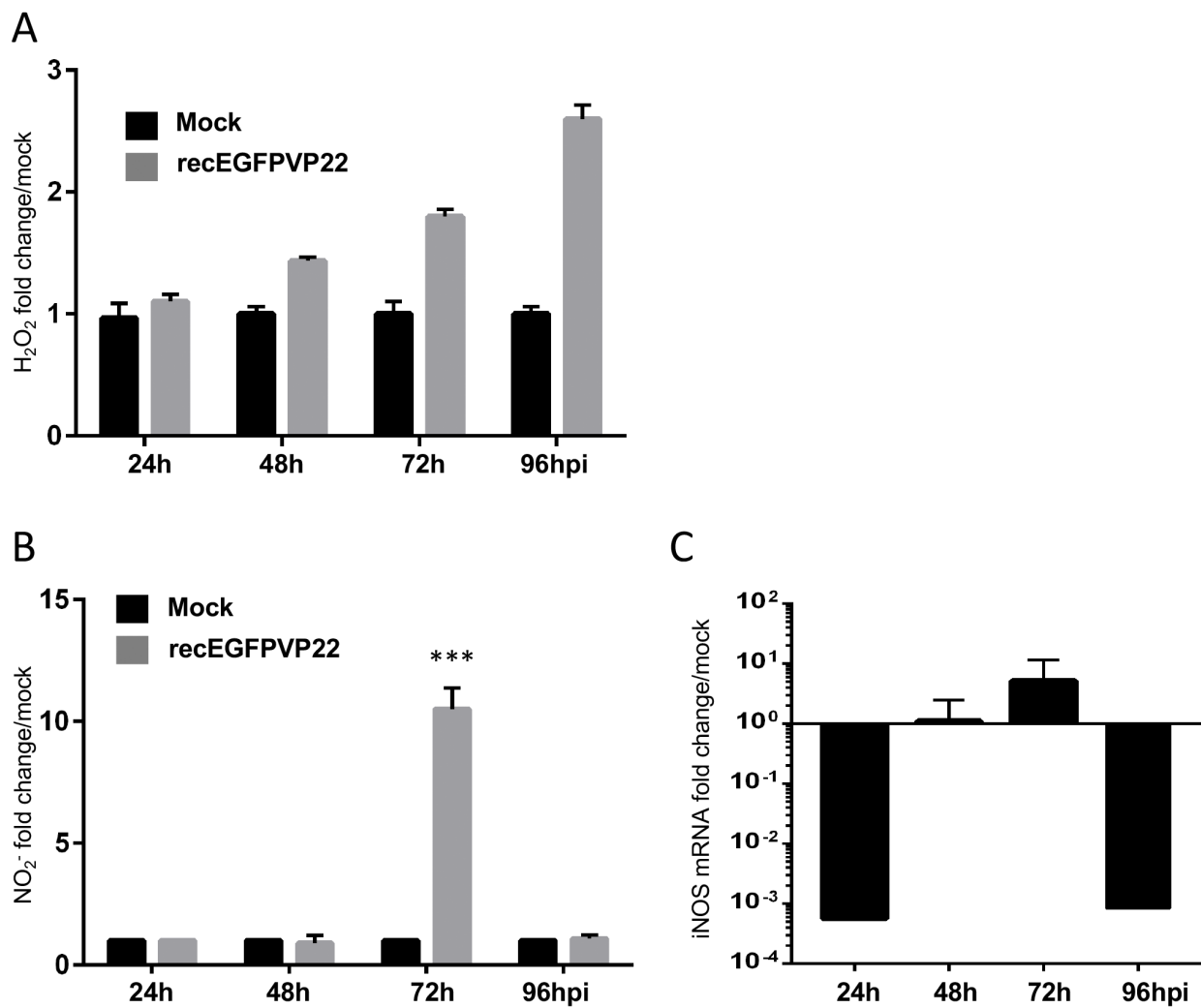


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Figure 4

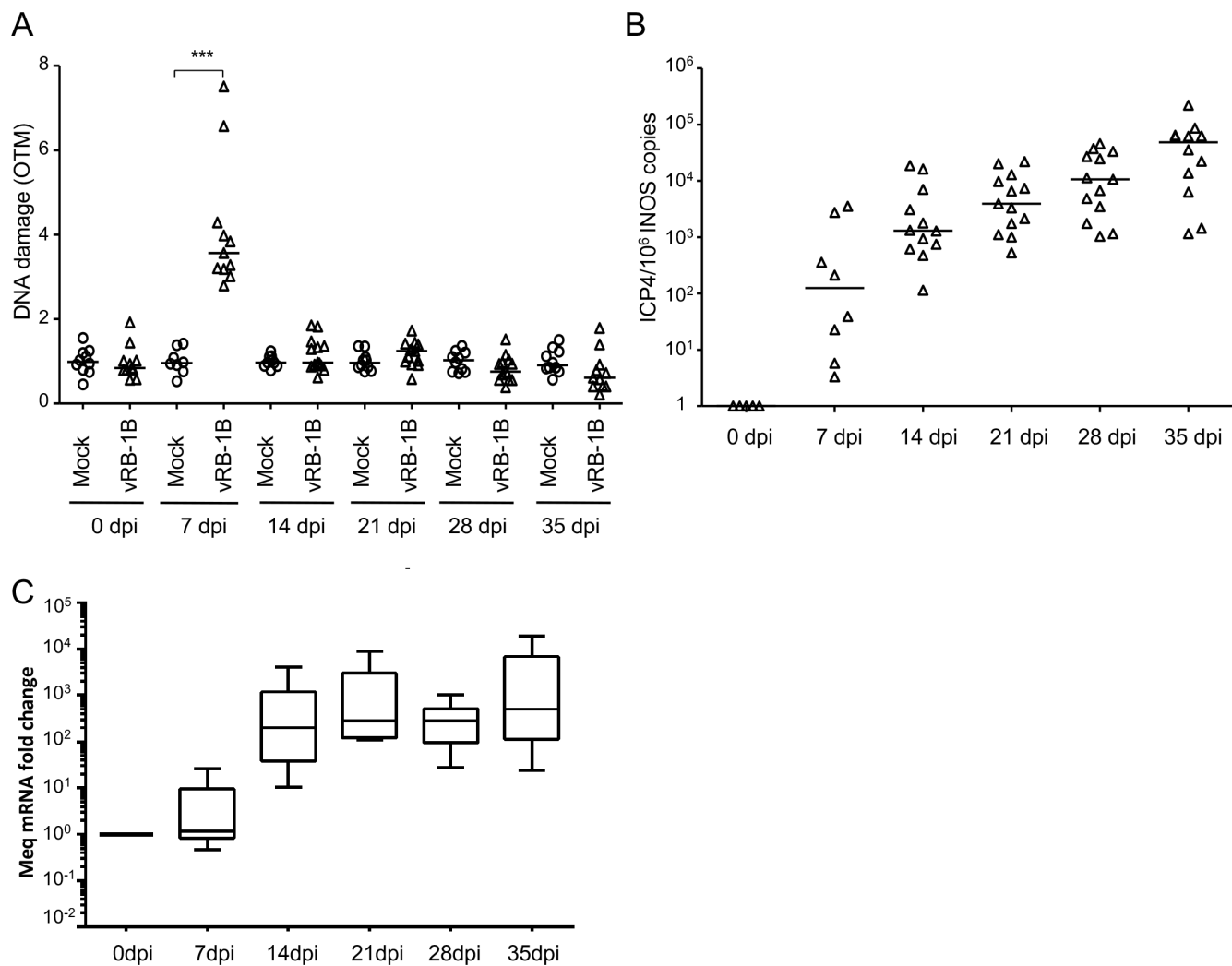
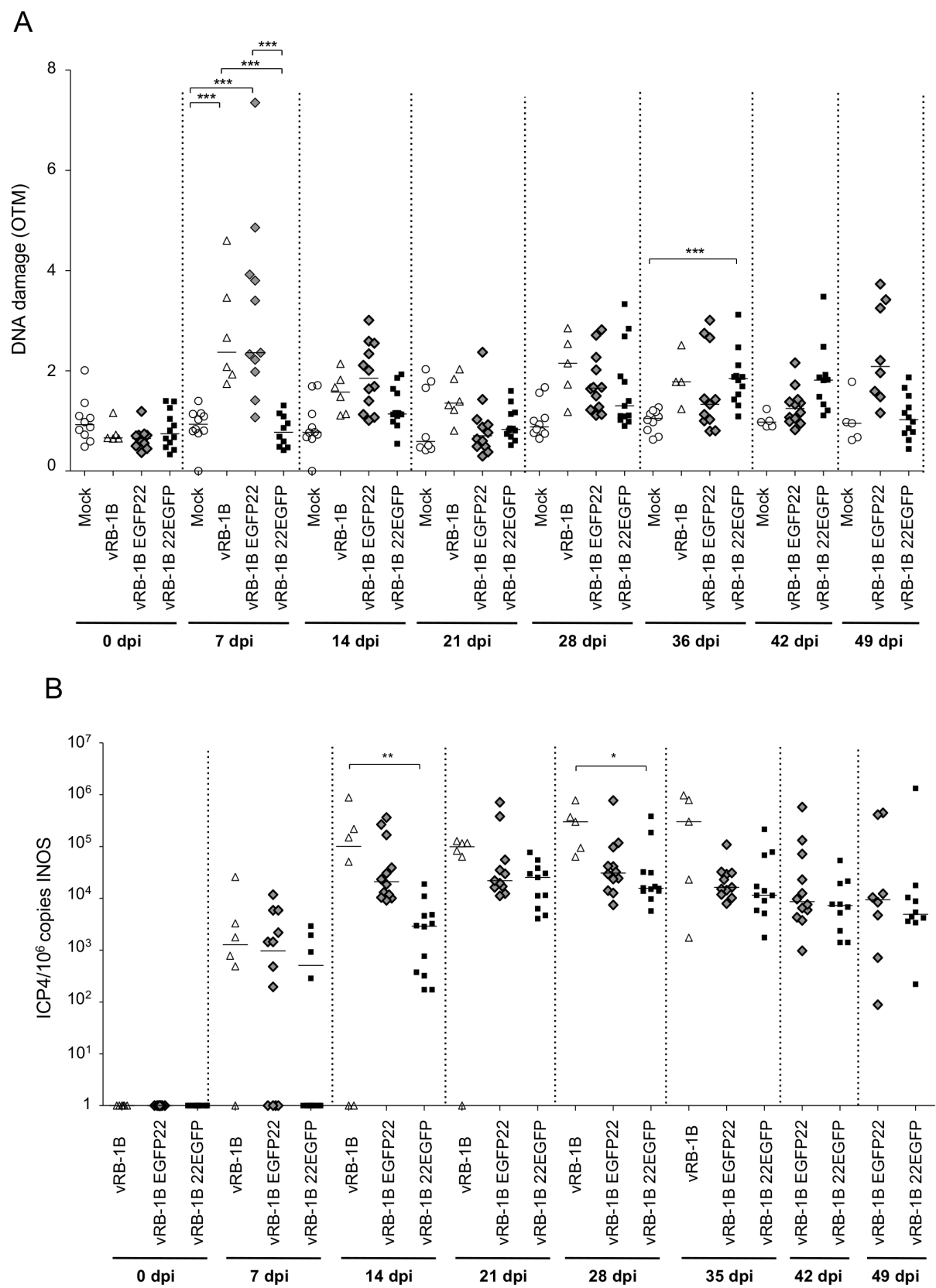


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Figure 5



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 Bencherit, D., Rémy, S., Le Vern, Y., Vychodil, T., Bertzbach, L. D., Kaufer, B. B., Denesvre, C., Trapp-Fragnet, L. (Auteur de correspondance) (2017). Induction of DNA damages upon Marek's disease virus infection: implication in viral replication and pathogenesis. *Journal of Virology*. 91 (24). 1-36. DOI : 10.1128/JVI.01658-17

Figure 6

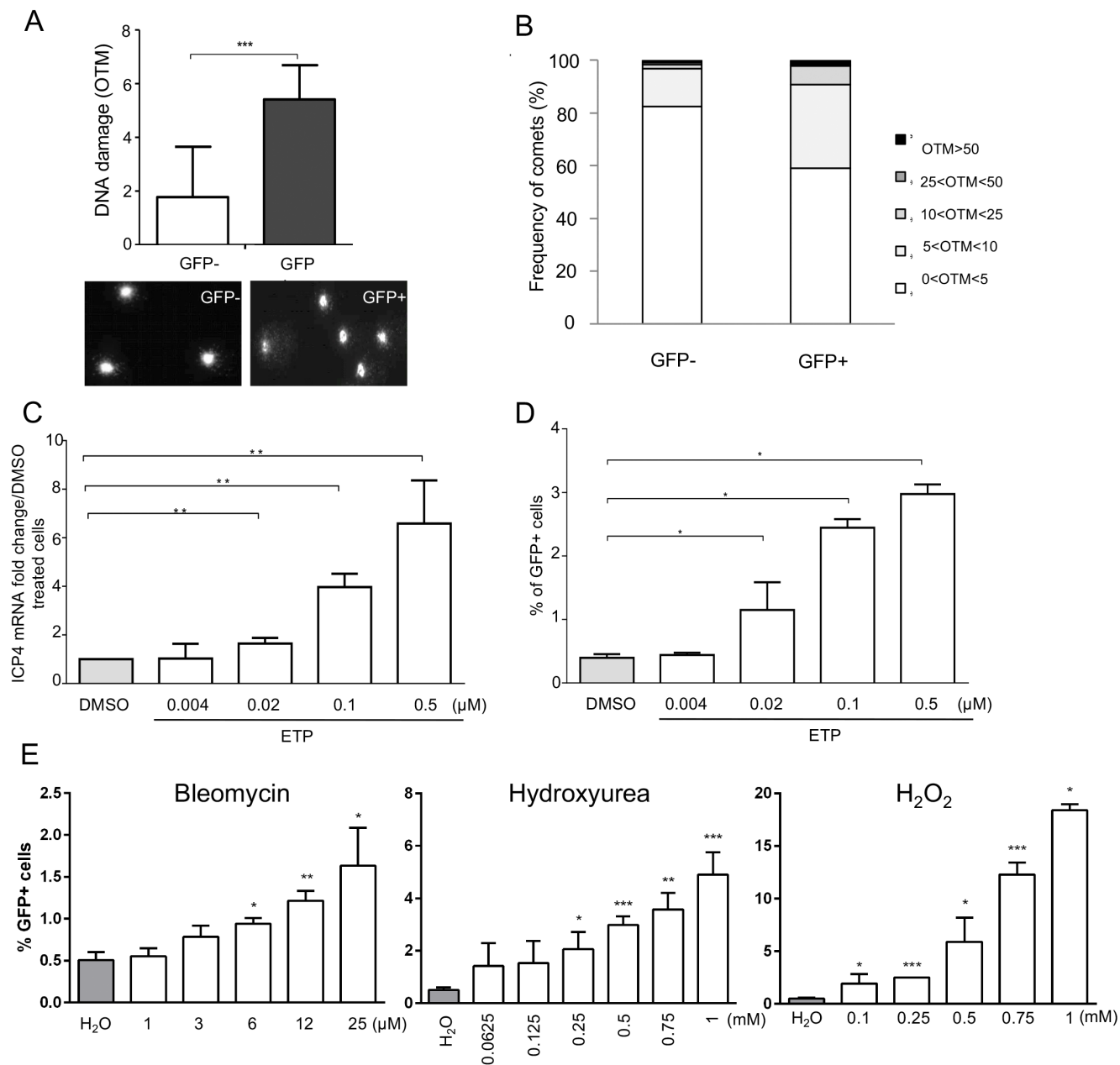


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