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1 # Gene expression is associated with virulence in murine macrophages
2 # infected with *Leptospira* spp

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4 Erivelto Corrêa de Araújo Junior[1], Leandro Encarnação Garcia[1], Matheus Janeck Araújo[1],
5 Itamar Souza Oliveira-Junior[2], Daniel Robert Arnold[1], Flavia Lombardi Lopes[1], Márcia
6 Marinho[1]*
7
8 [1] Department of Production and Animal Health, São Paulo State University (Unesp),
9 School of Veterinary Medicine, Araçatuba, SP, Brazil.
10 [2] Department of Surgery, Discipline of Anesthesia, Pain and Intensive Medicine, Federal
11 University of São Paulo, São Paulo, Brazil.
12
13 ***Corresponding author**
14 Email: marcia.marinho@unesp.br (MM)

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15

16 ## Abstract

17

18 *Leptospira* genus contains species that affect human health with varying degrees of
19 pathogenicity. In this context, we aimed to evaluate the differences in modulation of host
20 gene expression by strains of *Leptospira* with varied virulence degrees. Our data showed
21 a high number of differentilly expressed transcripts in murine macrophages following 6h
22 of infection with both virulent and culture-attenuated *L. interrogans* and to a lesser
23 degree, with the saprophyte strain *L. biflexa*. That suggests that certain genes are
24 modulated by *Leptospira* infection independent of their degree of virulence, whether
25 others are virulence and species associated. Pathway analysis indicated that Apoptosis,
26 ATM Signaling and Cell Cycle: G2/M DNA Damage Checkpoint Regulation were
27 exclusively regulated following infection with the virulent strain. Results demonstrated
28 that species and virulence play a role during host response to *Leptosppira* spp in murine
29 macrophages.

30 **Keywords:** Leptospirosis; macrophages; immune response; microarray

31 ## Author summary

32 Leptospirosis is an infectious disease that is transmitted from animals to humans. It is a
33 re-emerging neglected zoonosis that is found in a range of environments worldwide, most
34 notably tropical regions prone to flooding. This bacteria is found in soil and water and are
35 eliminated in the urine by rats, their natural host reservoir. Through skin contacts with the
36 bacteria people or animals can get infected however the infection process is still poorly
37 understood, such as the fact that different strains can cause different severity of illness. In
38 this study, we aimed to evaluate the differences in modulation of host gene expression by
39 strains of *Leptospira* varying in virulence. After transcriptomic analysis, the results

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40 showed a high number of differentially expressed genes after 6h of infection by virulent
41 and attenuated *L. interrogans*, and to a lesser extent with *L. biflexa* saprophytic lineage.
42 This suggests that RNAs are modulated after infection by *Leptospira* in macrophages, in
43 a species and virulence related manner. It is hoped that the data produced will contribute
44 to further our understanding on the pathogenesis of leptospirosis.

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54 # Introduction

55 Leptospirosis is a zoonotic bacterial disease that occurs in different
56 epidemiological conditions [1]. The genus *Leptospira* encompasses pathogenic and
57 saprophytic species that differ in their ability to survive and colonize different
58 environments and hosts [2]. *Leptospira* species are classified into three groups according
59 to their pathogenic potential: virulent pathogenic, intermediate, and saprophytes [3].
60 Leptospirosis occurs mainly in vulnerable populations, including urban and rural dwellers
61 [4] of tropical and subtropical developing countries [5-7]. It is a major public health
62 problem, with a recent estimate of 1 million cases per year, and a mortality rate of 5 to
63 10% [4,8-9].

64 Leptospires are capable of infecting humans and many domestic and wild animals,
65 survive and thrive in host tissues, escaping from the host's natural defense mechanisms.
66 Transmission is based on direct or indirect contact with the urine of carriers (mainly
67 rodents); the disease varied from sub-clinical to most serious cases, progressing to renal
68 failure and pulmonary hemorrhage [10-1].

69 Host-specific immune response against pathogenic leptospires are poorly
70 understood, particularly regarding susceptibility resistance to infection. For decades,
71 adaptive humoral immunity was considered as the sole player in leptospirosis, but in
72 recent years some progress has been achieved in the fields of innate and adaptive
73 immunity [11-14].

74 Murray and coworkers [15] identified a number of virulence factors, including the
75 presence of lipopolysaccharides (LPS), heme oxygenase [16], Loa22 lipoprotein [17] and
76 other proteins related to macrophage interaction with *Leptospira*.

77 Phagocytosis is one of the main mechanisms to eliminate invading microbial
78 pathogens at the early stages of infection in individuals without acquired immunity

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79 against the infecting agent, but pathogenic *Leptospira* can escape from complement
80 attack and phagocytosis after infection [18-20]. Pathogenic *Leptospira* is also able to
81 survive and replicate in human macrophages, but it is killed in murine macrophages [21].
82 LPSs of pathogenic *Leptospira* activate human macrophages only through the Toll-like
83 receptor 2 (TLR2) while murine macrophages are activated through TLR2 and TLR4 [13-
84 22]. Vernon Pauillac and Merian [23] have shown that mononuclear macrophages of
85 peripheral blood of hamster infected with a virulent variant of *Leptospira interrogans*
86 secrete proinflammatory cytokines (TNF-α) with a Th1 (IL-12) profile in the first hour,
87 predominating until the fourth day after infection, whereas a Th2 profile appears after 24
88 hours of infection. In the early course of infection, leptospires have to survive and spread
89 in the bloodstream before causing damage to target organs [25].
90 In this study, we applied microarray technology to comparatively analyze early
91 change in murine macrophages genes expression in response to *Leptospira* spp. with
92 varied virulence, and to identify signaling pathways that play a role in an *in vitro* model
93 of macrophageal infection.
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103 # Results

104 ## Data deposition

105 Microarray raw data files are available in Gene Expression Omnibus (GEO) and
106 are accessible through GEO series number GSE105141 [26].

107 ## Gene expression profile via microarray analysis

108 Our data analysis found 892 genes in cells infected with saprophyte, attenuated
109 and virulent leptospirosis compared to control. According to Fig 1, pathogenic leptospires
110 modulates 892 genes (422 up and 470 down-regulated), attenuated leptospires modulates
111 848 genes (400 upregulated and 448 downregulated) and saprophyte 299 genes (128
112 upregulated and 171 downregulated) in a filter criterion of fold change ±2 and false
113 discovery rate (FDR)<0.05 (Fig. 1). Through treatment comparison by Venn diagram, we
114 identify common and specific genes (Fig. 2). A total of 274 genes were common to all
115 infected cells, despite of strains, when compared to control. Virulent and culture-
116 attenuated infected groups groups shared 512 genes in common, while eight genes were
117 shared between virulent and saprophyte groups and only one gene between attenuated
118 and saprophyte infected cells (S1. Table). Average singals (log2) of samples were
119 hierarchically clustered using Pearson correlation and complete-linkage and it was
120 observed again a clustering of samples based on species and virulence, with the virulent
121 and culture-attenuated strains clustering closer together, followed bt the saprophyte strain
122 (Fig. 3).

123

124 **Figure 1. Differentially expressed genes after 6 hours of infection in murine**
125 **macrophages J774A.1 with saprophytic, culture-attenuated and virulent strains of**

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126 ***Leptospira* spp.** The colored bars show the up-regulated (red) or down-regulated (green)
127 genes and grey a total of genes. (n = 3 / assay, FDR-adjusted p<0.05, fold change ± 2).
128
129 **Figure 2. Venn diagram for differentially expressed genes of modulated**
130 **macrophages at 6 hours of infection with different strains of *Leptospira* spp.** Total
131 number of canonical pathways (n=3/treatment; FDR<0.05, fold change ± 2) in the
132 contrasts Infected (Saprophyte; Attenuated and Virulent) vs. Non-infected Control.
133
134 **Figure 3. Heatmap of differentially expressed genes shows the average signal by**
135 **macrophages at 6 hours of infection in different strains of *Leptospira* spp.** The red
136 color indicate increased expression, green color indicates the decreased expression as
137 compared to control (n = 3/treatment; p-value < 0.01; FDR < 0.05; linear fold change ±
138 2).
139 In Table 1 we depict the top 9 DEGs in response to infection. These genes are
140 present in several pathways and biological processes involved in acute inflammatory
141 response.

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142 **Table 1. Top modulated transcripts in murine macrophages following 6h of**
143 **infection with saprophyte, culture-attenuated and virulent strains of *Leptospira* spp.**

Regulation	Gene Symbol	FDR-adjusted p-value	FC (Sap vs. CT)	FC (Att vs. CT)	FC (Vir vs. CT)
Up	Il1b	0,000037	202,85	253,12	259,03
	Il1a	0,000004	42,58	120	127,28
	Saa3	0,000003	58,32	113,49	95,44
	Il6	0,000005	17,92	90,05	93,09
	Ccl5	0,000008	15,72	56,83	56,56
	Ptgs2	0,000011	38,21	52,72	56,33
	Nos2	0,000008	6,83	48,29	51,82
	Cxcl10	0,000035	8	45,54	51,25
	Ifit1	0,000217	6,16	33,6	37,61
Down	Rasgrp3	0,000059	-6,97	-9,16	-7,8
	Ighm	0,000045	-4,88	-8,22	-7,91
	Hal	0,000022	-4,52	-6,78	-7,01
	Cxcr4	0,000133	-5,65	-5,93	-6,11
	Klhl24	0,000053	-4,81	-5,77	-5,76
	Il18rap	0,000074	-3,7	-5,55	-5,31
	Nrcam	0,000032	-3,37	-5,43	-5,05
	Il1rl1	0,000028	-3,24	-5,35	-4,53
	Ankrd44	0,000476	-2,68	-5,3	-4,67

144 (FC=fold change; SAP= saprophyte; Att=attenuated; Vir=virulent)
145

146 ## Analysis of signaling pathways

147 For functional enrichment of the differentially expressed genes obtained for each
148 treatment, the Ingenuity Pathway Analysis (IPA) software was employed, Core Analysis
149 was performed to identify relevant biological pathways to all 3 strains using the -log BH
150 p-value > 1.3 (equivalent to a p-value <0.01).
151

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152 **Specific pathways modulated by the virulent strain**

153 Several pathways were identified as regulated by the virulent strain, however the
154 Apoptosis pathway, ATM signaling and Cell Cycle: G2 / M DNA Damage Checkpoint
155 Regulation, were exclusively expressed and affected by treatment with the virulent strain
156 (Fig. 4). In the apoptosis pathway, the major up-regulated genes were FAS, IKBKE,
157 NFKB1, NFKBIA, NFKBIB, NFKNID, NFKBIE, TNF, TNFRSF1B; downregulated
158 transcripts were BCL2, CAPN2 and PARP1 (Fig. 5A). In the ATM signaling pathway,
159 the upregulated transcript genes were CDKN1A, GADD45G, MDM2, NFKBIA and
160 TLK2; downregulated transcripts were BRCA1, CBX5, CDK2, CHEK1, CHEK2,
161 FANCD2, MDC1 and TOPBP1 (Fig. 5B). The upregulated genes of the Cell Cycle: G2 /
162 M DNA Damage Checkpoint Regulation pathway were CDKN1A and MDM2;
163 downregulated transcripts were BRCA1, CHEK1, CHEK2, PKMYT1 and WEE1 (Fig.
164 5C).

165 **Figure 4. Venn diagram for pathways of modulated macrophages at 6 hours of**
166 **infection with different strains of *Leptospira* spp.** Total number of canonical pathways
167 (n=3/treatment; FDR<0.05, fold change ± 2) in the contrasts Infected (Saprophyte;
168 Attenuated and Virulent) vs. Non-infected Control.

169

170 **Figure 5. Canonical signaling pathway obtained by the IPA (Ingenuity Pathway**
171 **Analysis program.** Red and green indicate upregulated and down-regulated genes,
172 respectively, compared to control group, and belongs to datasets of DEGs virulent vs.
173 control assays. Color intensity corresponds to the degree of up or downregulation (fold-
174 change). White represents the known genes of the pathway without identification in the
175 transcriptomic analysis. **Panel A)** Canonical signaling pathway of Apoptosis of *in vitro*
176 macrophages. **Panel B)** Canonical signaling of ATM of *in vitro* macrophages. **Panel C)**

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177 Canonical signaling pathway of Cell Cycle: G2 / M DNA Damage Checkpoint
178 Regulation of *in vitro* macrophages.

179 **Validation of microarray data by qRT-PCR**

180 Infection with 10^7 of virulent and attenuated (*L. interrogans* serovar Copenhageni)
181 and saprophytic (*L. biflexa* serovar Patoc), induced significant increase of TNF-α
182 expression in murine macrophages (p <0.0001) compared to control. Regarding
183 expression of IL-1β and NOS2, a similar expression profile was observed between
184 Control and Saprophy, which differed from the profile found in the Attenuated and
185 Virulent samples. The comparative analysis of the expressed values for IL-1β and NOS2
186 were statistically different between the assays, compared to the attenuated and virulent
187 strains, differing when compared to the control groups and infection with the saprophytic
188 strain. Differently from the observed TNF expression results, there was significant
189 difference across all assays (Fig. 6A-C).

190

191 **Figure 6. qRT-PCR of mRNA expression levels in infected macrophages with**
192 **different strains of *Leptospira* compared to non-infected controls. Panel A)** Relative
193 expression of TNF-α in saprophyte, culture-attenuated and virulent compared to control.
194 **Panel B)** Relative expression of IL-1β in saprophyte, culture-attenuated and virulent
195 compared to control. **Panel C)** Relative expression of NOS2 in saprophyte, culture-
196 attenuated and virulent compared to control (p <0.05). Different superscript letters differs
197 significantly (p <0.05).

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198 **Discussion**

199 In this study, we took an *in vitro* approach to analyze the trancriptomic profiles of
200 macrophages in response to saprophytic, culture-attenuated and virulent samples of
201 *Leptospira* spp, to gain a better understanding of the disease's molecular mechanisms and
202 pathways.

203 TREM-1 signaling was the most significant pathway modulated by all threes
204 strains. TREMs are a family of recently discovered receptors of the immunoglobulin
205 superfamily, expressed on various cells of the myeloid lineage, which play important
206 roles in innate immune responses, such as activating inflammatory responses and
207 contributing to septic shock response in microbial-mediated infections [30-31]. Targeted
208 activation of TREM-1 in our study appears to be a first line inflammatory response to the
209 genus, regardless of virulence.

210 Other canonical pathways related to the innate immune system were a common
211 response to all strains, the acute phase response signaling pathway, iNOS, IL-6, IL-1,
212 TNFR1/TNFR2, MIF-regulation of innate immunity and HMGB-1 signaling. Further, all
213 three strains are proposed to negatively regulate the antioxidant action of vitamin C
214 pathway, suggesting that *Leptospira* spp. infection could contribute to oxidative stress
215 associated production of reactive oxygen species (ROS). ROS-mediated intracellular
216 oxidation is prevented by an antioxidant system, which includes low molecular weight
217 antioxidants, such as vitamin C. This pathway is involved in cell process of survival,
218 growth, proliferation and death [32].

219 In the culture-attenuated and virulent samples, Toll-like receptors, Interferon and
220 inflammasome signaling pathways were significantly represented. Innate immune
221 response is initiated by recognizing pathogens through pattern receptors as TLRs.
222 Activation of these receptors is characterized by the massive production release of

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223 proinflammatory mediators, such as cytokines, chemokines and interferons [33]. Our
224 microarray results to virulent strain identified high gene expression for TNF-α, IL-1β, IL-
225 6 and iNOS. Similarly of Iskandar et al [34], verified that the presence of IL-6, IL-8 and
226 IL-1β in serum from human patients with leptospirosis associated with severity of
227 disease. In fact, Schulte et al. [35] concluded that increased TNF-α, IL-1β and IL-6 can
228 activate the coagulation system in endotoxemic models. The high concentration of IL-6 is
229 an indicator of septic shock and correlates with the severity of leptospirosis [36].

230 In regards to signaling pathways regulated by mRNAs modulated specifically
231 following infection with virulent *L. interrogans*, Apoptosis signaling was positively
232 regulated by infection whereas ATM signaling and Cell Cycle: G2/M DNA Damage
233 Checkpoint Regulation, responsible for cell cycle, DNA repair and apoptosis, were
234 negatively modulated. Following DNA damage, cells must detect breaks and transiently
235 block the cell cycle progression allowing time for repair [37]. Jin *et al* 35 concluded that
236 the pathogenic *Leptospira* caused apoptosis between 3-6 hours after infection.
237 Our data corrobarates their finding that virulent *Leptospira* could modulated apoptosis,
238 with just 6 hours of infection, through inhibition of pathways responsible for DNA repair
239 and cell cycle control, as well as by inhibition the BCL-2 (anti-apoptotic gene) in turn
240 leading to DNA damage and degradation. In fact, a previous study from our group has
241 shown that BCL2 is a potentially down-regulated by mmu-mir-7667-3p following
242 infection with *L. interrogans*, suggesting that cell survival could be compromissed after
243 macrophages infection by the spirochete [38].

244 Leptospiral infection in macrophages induces a dependent p53/p21 cell cycle
245 arrest [39]. We verified that the p53 target pathway signaling is regulated after virulent
246 infection by modulation of mRNAs in murine monocyte-machrophages. Homotetrameric
247 transcription factor p53 is reported to directly regulate 500 target genes, thereby

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248 controlling a broad range of cellular processes including cell cycle arrest, cell senescence,
249 DNA repair, metabolic adaptation and cell death [40].

250 Further results from our study support the idea of cellular apoptosis Caspase-3 and
251 8 were elevated in all three infected macrophages, regardless of the pathogenicity
252 notwithstanding, a more pronounced upregulation was induced by the with virulent and
253 attenuated inoculum of *Leptospira*. Whether macrophage apoptosis induction by
254 *Leptospira* is form of evasion mechanism or a host defense response to infection,
255 preventing the spread of infection [jin 35] is still up for debate.

256 Cytokines represent a group of proteins that promote communication between
257 cells, and their activation is through differentiation, receptor expression and cell-mediated
258 immunity [41]. This suggests that the virulence factors, expressed or not during the
259 process of infection of *in vitro* macrophages can guide cell response. In other words,
260 virulent, culture-attenuated and non-pathogenic samples of *Leptospira* should be able to
261 activate the murine macrophages, and the gene expression elicited as a result of infection,
262 is dependent on strain virulence samples. Our results revealed a quantitative and
263 qualitative association of gene expression with the virulence strains, with the virulent *L.*
264 *interrogans* upregulating genes related to acute infection and cellular autophagy, unlike
265 the culture-attenuated and saprophytic strains.

266 A comprehensive overview of gene expression patterns after infection by virulent,
267 culture-attenuated and saprophytic *Leptospira* spp. strains revealed that inflammation and
268 immune response, cytokine signaling, DNA repair, cell movement, death and cell
269 survival were significantly activated following 6 hours of infection. Results demonstrated
270 a group of genes is responsive to antigens present in the genus *Leptospira*, regardless of
271 virulence, whereas species and virulence-specific gene expression was also elicited in the
272 infected macrophages.

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273 # Methods

274 ## Leptospiral Strains

275 Samples of virulent strain *L. interrogans* sorovar Copenhageni (FIOCRUZ L1-
276 130), attenuated strain *L. interrogans* sorovar Copenhageni M20 and saprophyte strain
277 *L.biflexa* sorovar Patoc (FIOCRUZ - Patoc I) that we used in this study were donated by
278 the Laboratory of Bacterial Zoonosis, Department of Preventive Veterinary Medicine and
279 Animal Health of School of Veterinary Medicine and Animal Science, University of São
280 Paulo (FMVZ/USP). All strains were incubated at 30ºC in Fletcher semi-solid culture
281 medium.

282 ## Macrophage Culture

283 Murine monocyte-macrophage cells (*Mus musculus* monocyte-macrophage cell
284 line J774A.1), provided by the Paul Ehrlich cell bank (Rio de Janeiro, Brazil), was used
285 as described [26]. Cells were maintained at 37º C, 5% CO2 in RPMI-1640 media (Sigma,
286 USA) supplemented with 10% heat-inactivated fetal bovine serum (Gibco, USA), 100
287 ug/mL streptomycin (Sigma Chemical Co St.Louis, MO), 0.03% L-glutamine solution
288 (Sigma) and 100 UI/mL of penicillin, in 6-well cell culture plates (3cm/well) until
289 confluency [35].

290 ## Infection of Macrophages

291 After the formation of confluent monolayer cells, they were washed three times
292 in sterile phosphate buffer solution (pH 7.2) for removal of antibiotics and non-adherent
293 cells. Bacteria were harvested by centrifugation and resuspended in RPMI-1640 media
294 (Sigma). Cells were then infected (100:1 bacteria:cell) with *L. interrogans* L1-130
295 (virulent strain), *L. interrogans* M20 (culture-attenuated strain), *L. biflexa* Patoc I
296 (saprophyte strain), as previously described [40]. Non-infected groups and non-infected
297 macrophages were used as controls. All infected cells, in biological triplicates, were

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298 carried in fresh RPMI-1640, devoid of antibiotics, for 6h at 37º C, 5% CO_2. Rate of
299 infection did not differ between strains. At the end of the 6-hour period of infection, RNA
300 extraction was immediately performed.

301 **RNA extraction and Quantification**

302 Total RNA (n=3/experimental group) was extracted from macrophages with
303 RNeasy Mini Kit (Qiagen, USA) according to manufacturer's instructions. RNA samples
304 were immediately stored at -80ºC. The quantification was performed using a NanoDrop
305 (ND-2000 spectrophotometer, Thermo Scientific, Wilmington, DE, USA) and the
306 samples quality was assessed using capillary electrophoresis (Bioanalyzer 2100 Agilent,
307 Santa Clara, CA, USA). All samples used for microarray analysis had a RIN of 10.

308 **Transcriptome Array and Quality control**

309 A WT PLUS Reagent Kit was used to prepare the RNA samples for whole
310 transcriptome expression analysis with Mouse Genome 2.1 ST Arrays Strip Affymetrix
311 (Santa Clara, CA, USA), according to the manufacturer's protocols. Briefly, 100 ng of
312 control RNA sample (Hela cells) was prepared to contain spiked in Poly-A RNA controls
313 (lys, phe, thr and dap) absent in eukaryotic cells and mixed together with RNA samples to
314 generate cDNA. After the amplification process, final cDNA was purified, quantified,
315 fragmented and then labeled for hybridization to the strips, for 20h at 48ºC in the
316 hybridization oven. Finally, strips were processed using the GeneAtlas Hybridization,
317 Wash, Stain Kit for WT Array Strips (Affymetrix) and scanned using the GeneAtlas®
318 System (Affymetrix) generating the raw cell files. Raw intensity values in the cell files
319 were background corrected, log2 transformed and then quantile normalized by the
320 software Expression Console (Affymetrix) using the Robust Multi-array Average (RMA)
321 algorithm.

322

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323 **Identification of differentially expressed genes and functional enrichment**

324 In order to identify differentially expressed genes, we utilized the software
325 Transcriptome Analysis Console (Affymetrix), where statistical analysis was performed
326 by one-way ANOVA (fold change ± 2, FDR corrected $p<0.05$). For the purpose of
327 functional enrichment of the expression profiles obtained for each treatment, we used the
328 Ingenuity Pathway Analysis (IPA) software (Qiagen).

329 **Validation of transcriptome results by qRT-PCR**

330 For the validation of gene expression of selected genes in infected macrophages
331 (saprophyte, culture-attenuated and virulent strains) and non-infected control
332 macrophages, RNA samples were reverse transcribed (1µg of total RNA/sample) using
333 the Moloney Murine Leukemia Virus (MML-V) enzyme (Life Technologies) and Oligo-
334 dT Primers. All primers were designed to span at least one intron, to avoid repeat regions
335 and similarities to other non-specific genomic regions. Mouse genome sequence,
336 available on the University of California, Santa Cruz (UCSC) Genome Browser, was
337 employed for primer design, using the Primer3 program [41]. PCR was performed using a
338 Stratagene QPCR Systems Mx3005P (Agilent Technologies, Santa Clara, CA, USA)
339 using the QuantiTect SYBR Green PCR kit (Qiagen). Expression levels were determined
340 using standard curves for all genes at each individual run, and the expression of the
341 candidate gene is presented as a ratio to an unregulated endogenous control (*β*-actin).
342 The primers used for qPCR validation are listed in Table 2.

343 Table 2. The primers used for qPCR validation.

GENE	***Forward***	***Reverse***
IL-1β	TCATTGTGGCTGTGGAGAAG	AGCTCATATGGGTCCGACAG
NOS2	GACGAGACGGATAGGCAGAG	CACATGCAAGGAAGGGAACT
TNF-α	CGAGTGACAAGCCTGTAGCC	CGAGTGACAAGCCTGTAGCC

344

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345 **Statistical analysis**

346 Differential expression of each gene was determined by one-way ANOVA with
347 two criteria, a fold change of ±2 comparing all infected groups to the non-infected control
348 and a Benjamini-Hochberg (BH) corrected p-value (FDR)<0.05. For pathways
349 enrichment analysis on the Ingenuity Pathway Analysis (IPA) software (Qiagen),
350 multiple testing was also (BH corrected (p<0.05). Real-time PCR data were analyzed
351 using least-squares analysis of variance and the general linear model procedures of SAS
352 (SAS Institute, Cary, NC, USA; p <0.01). Comparison of means was done using
353 Duncan's multiple range test.

354 **Ethics Statement**

355 The present study was approved by the Research Ethics Committee of São Paulo
356 State University (FMVA- UNESP), under the protocol number 2015-00895. No animal
357 experimentation was performed in the experiments described herein.

358

359 ## Acknowledgments

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361 providing the *Leptospira* spp., and to Cilene Vudovix Táparo for her valuable assistance
362 during the experiments.

363

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473 ## Supporting information

474 **S1 Table. DEGs (gene symbol) modulated by macrophages at 6h of infection by**
475 **different strains of *Leptospira* spp.**

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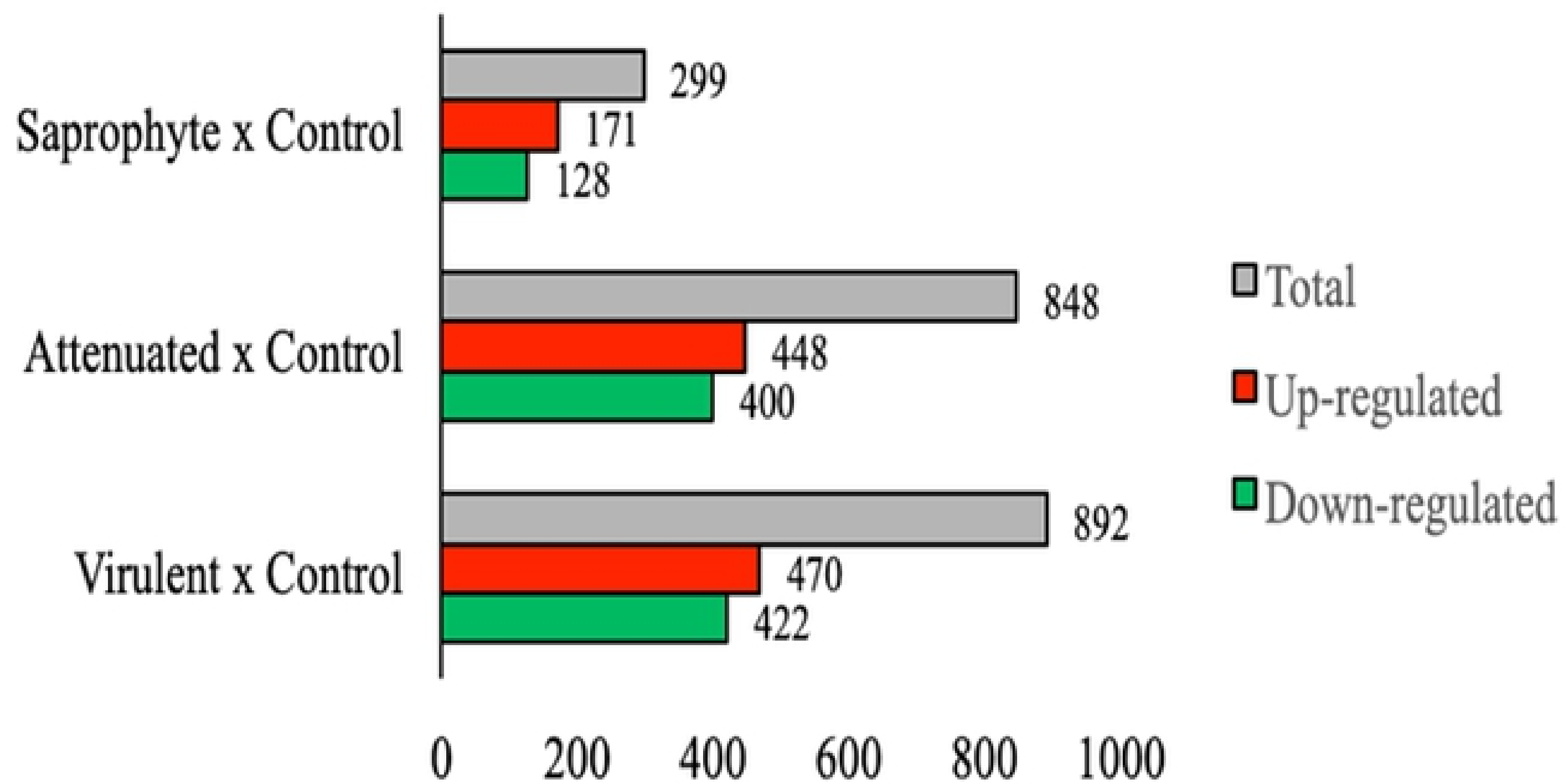


Fig1

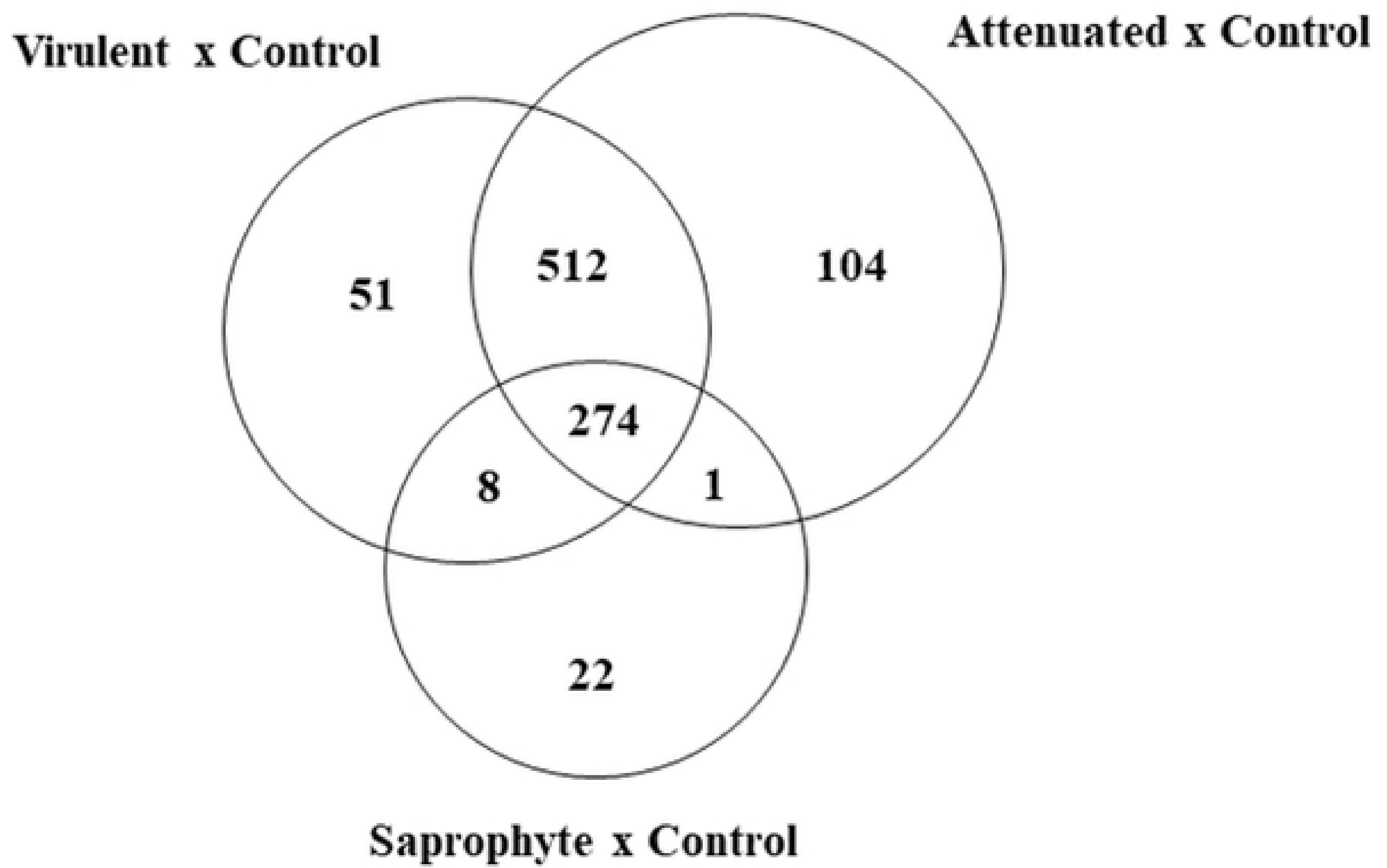
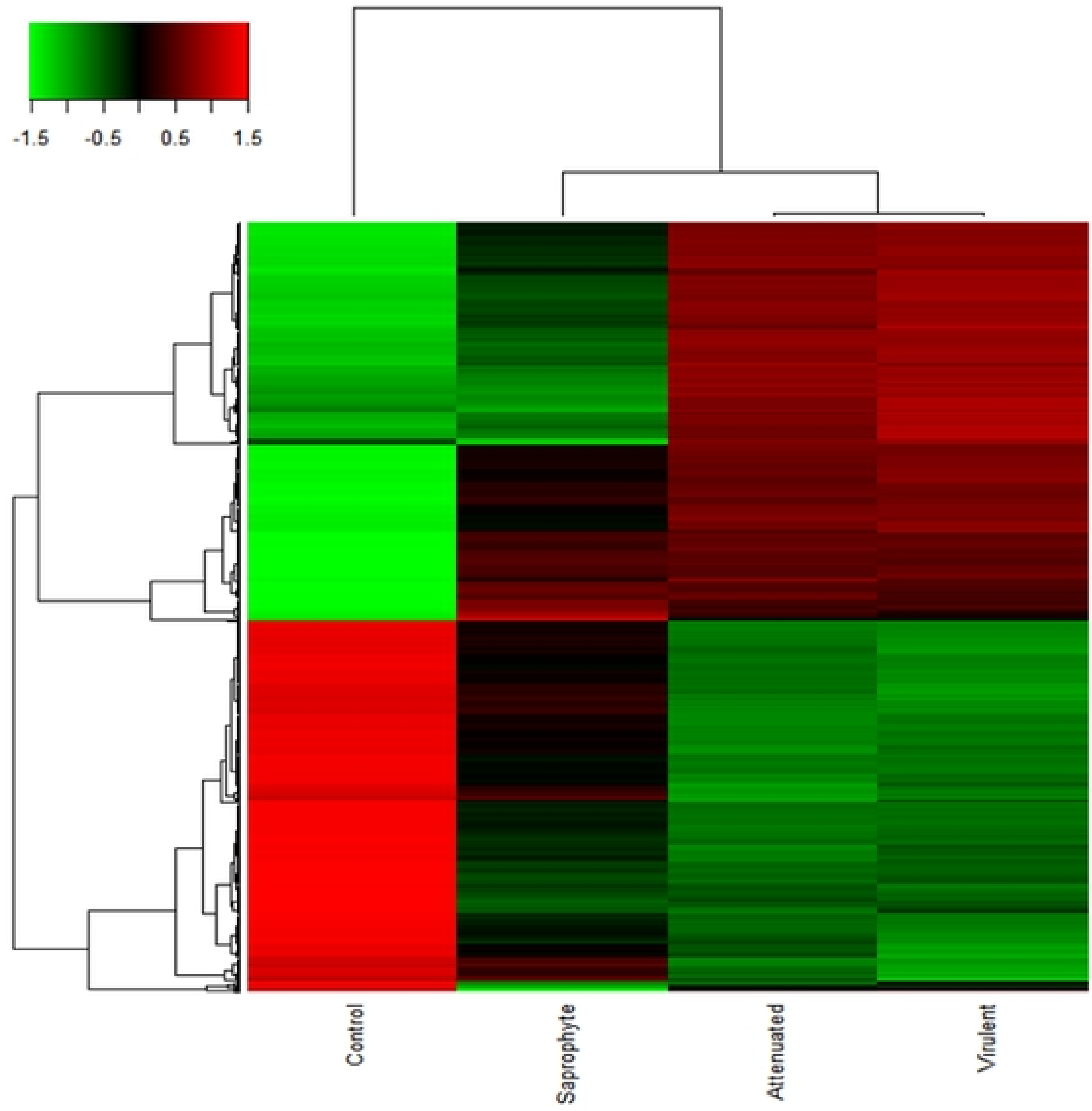


Fig2



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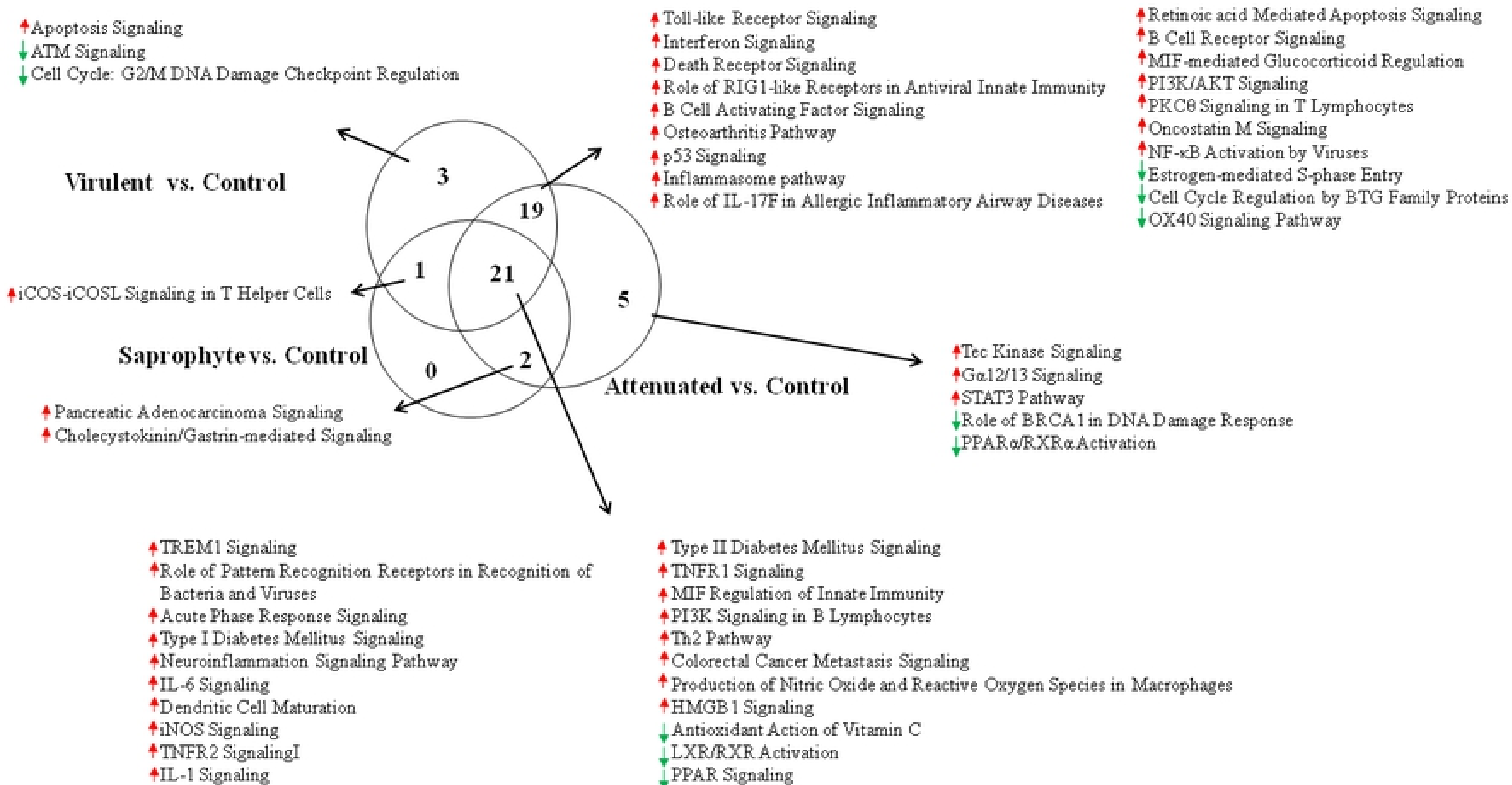

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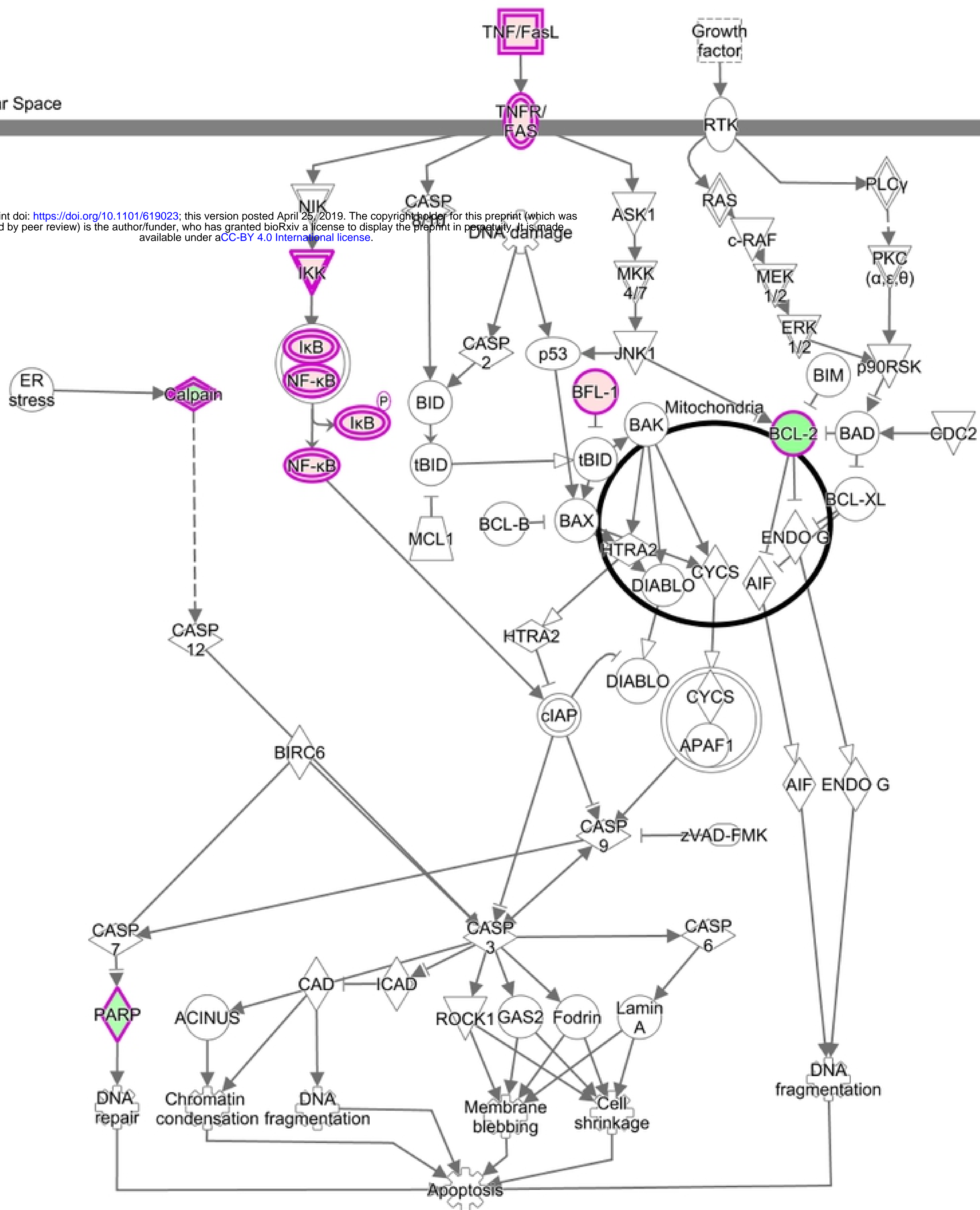

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Fig5A

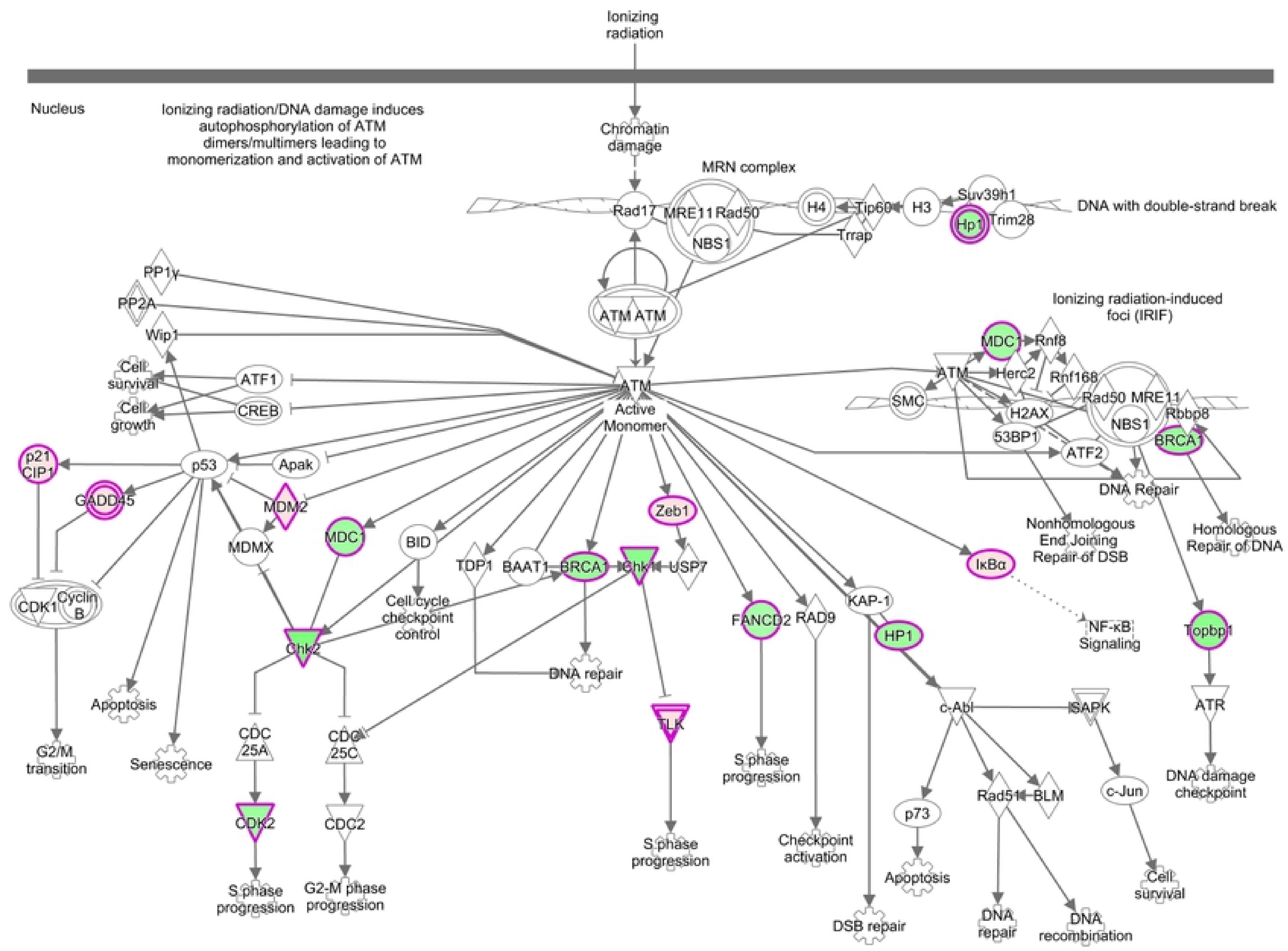

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Fig5B

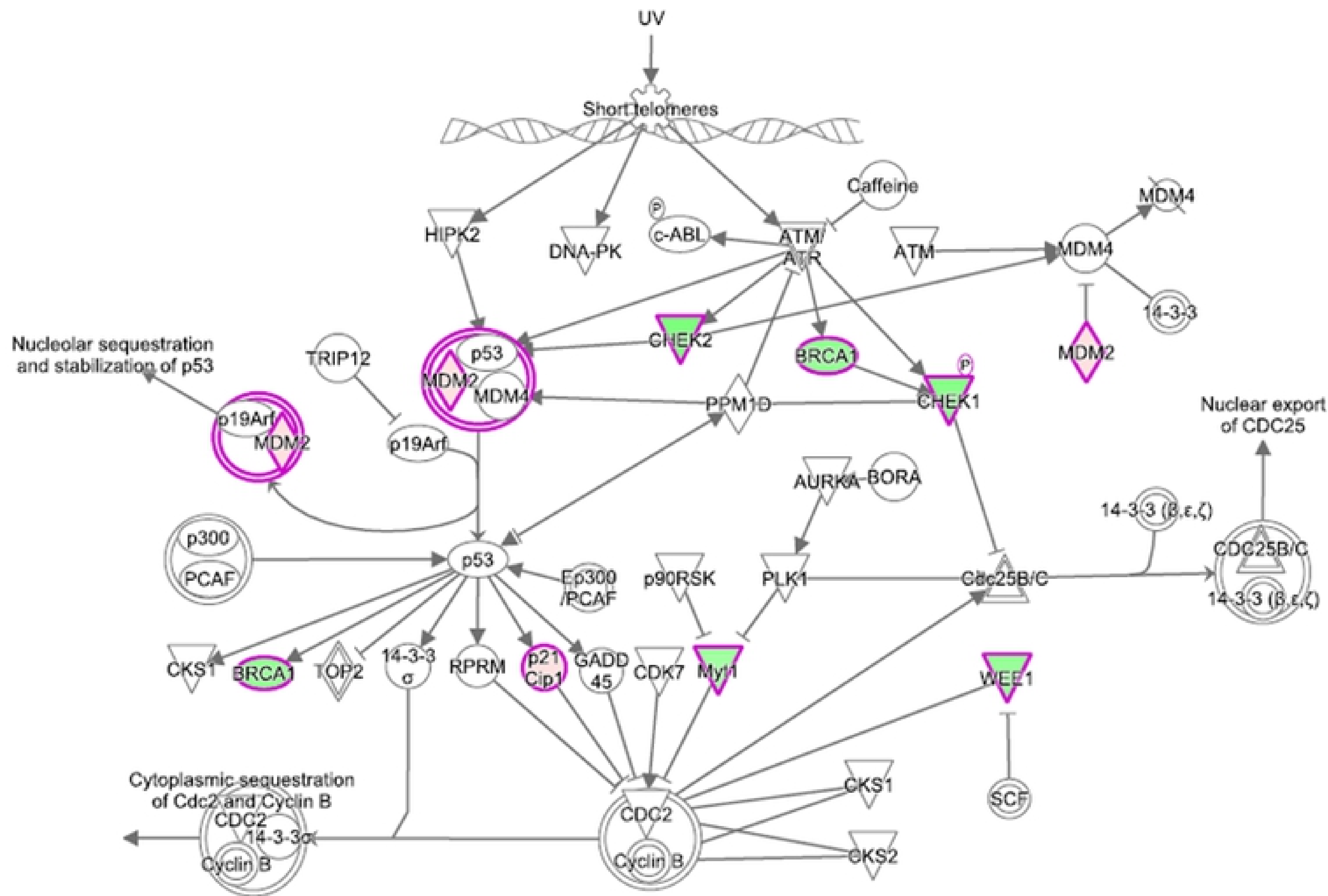

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Fig5C

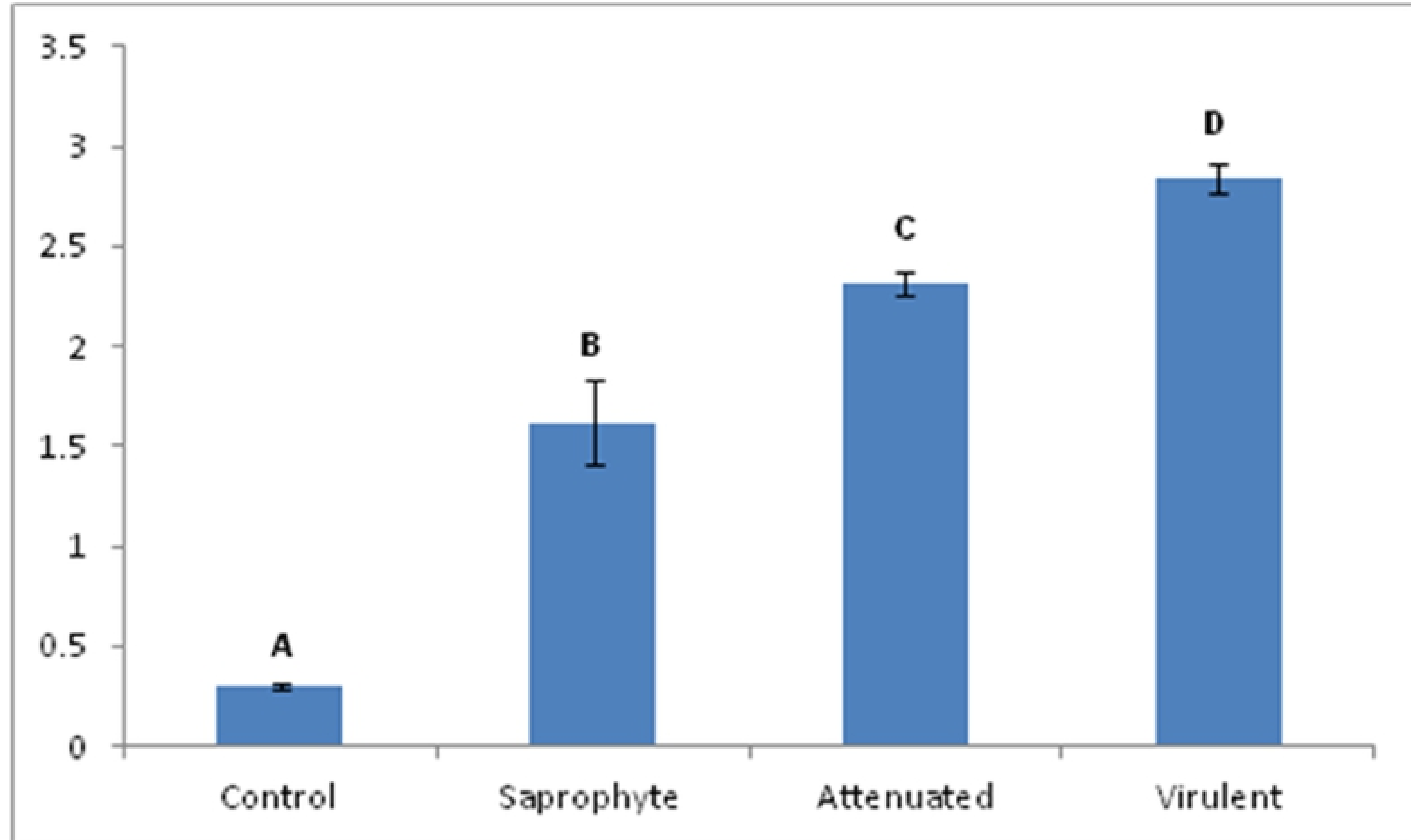

Fig6A

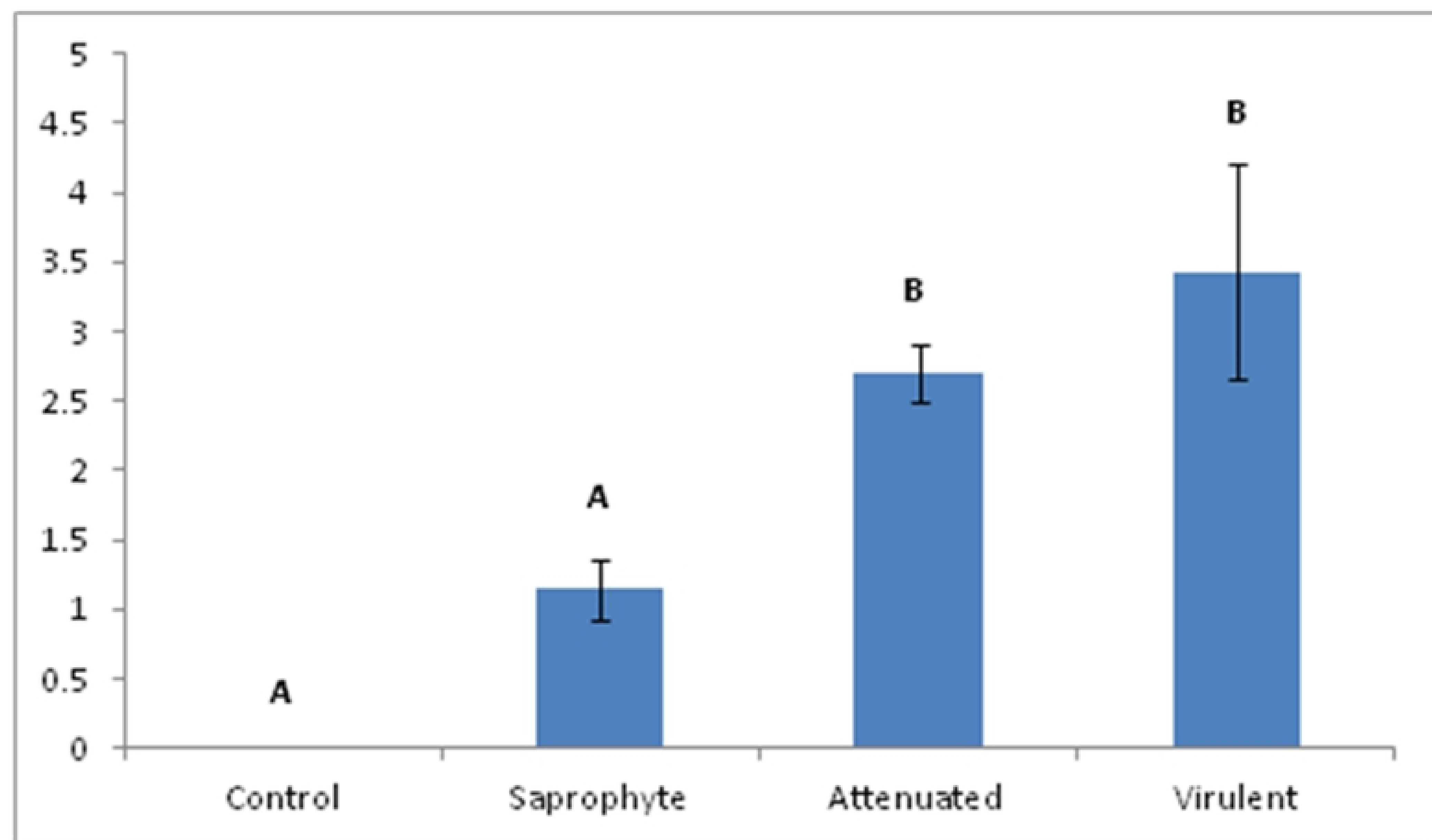

Fig6B

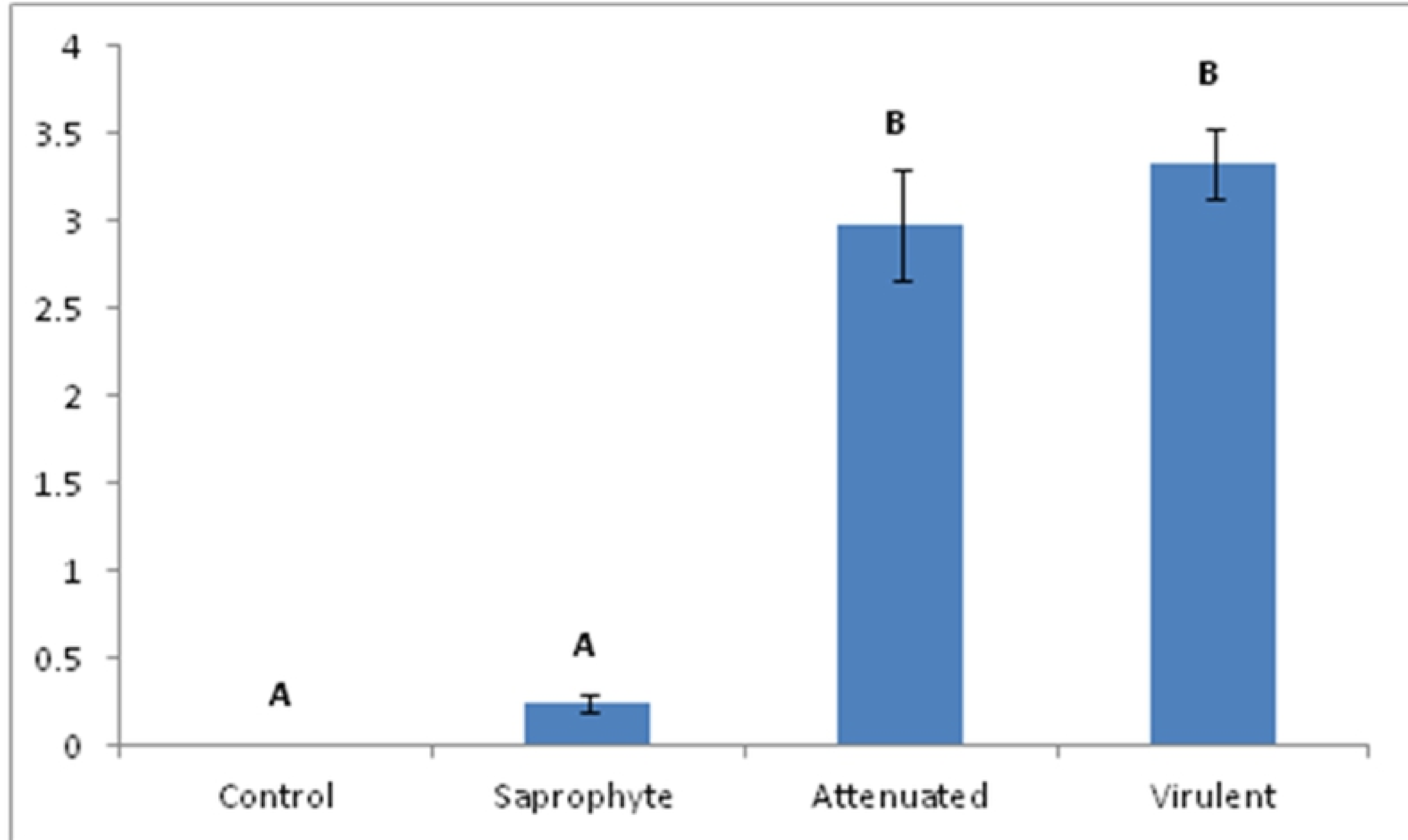

Fig6C