Harmine stimulates proliferation of human neural progenitors

Vanja Dakic [1,2] , **Renata de Moraes Maciel** [1] , **Hannah Drummond** [1,2] , **Juliana M Nascimento** [1,3] , **Pablo Trindade** [1] , **Stevens K Rehen** Corresp. [1,2]

[1] IDOR, D'Or Institute for Research and Education, Rio de Janeiro, RJ, Brazil

[2] Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil

[3] Department of Biochemistry and Tissue Biology / Institute of Biology, State University of Campinas (UNICAMP), Campinas, SP, Brazil

Corresponding Author: Stevens K Rehen
Email address: srehen@lance-ufrj.org

Harmine is the β-carboline alkaloid with the highest concentration in the psychotropic plant decoction Ayahuasca. In rodents, classical antidepressants reverse the symptoms of depression by stimulating neuronal proliferation. It has been shown that Ayahuasca presents antidepressant effects in patients with depressive disorder. In the present study, we investigated the effects of harmine in cell cultures containing human neural progenitor cells (hNPCs, 97% nestin-positive) derived from pluripotent stem cells. After 4 days of treatment, the pool of proliferating hNPCs increased by 71.5%. Harmine has been reported as a potent inhibitor of the dual specificity tyrosine-phosphorylation-regulated kinase (DYRK1A), which regulates cell proliferation and brain development. We tested the effect of analogs of harmine, an inhibitor of DYRK1A (INDY), and an irreversible selective inhibitor of monoamine oxidase (MAO) but not DYRK1A (pargyline). INDY but not pargyline induced proliferation of hNPCs similarly to harmine, suggesting that inhibition of DYRK1A is a possible mechanism to explain harmine effects upon the proliferation of hNPCs. Our findings show that harmine enhances proliferation of hNPCs and suggest that inhibition of DYRK1A may explain its effects upon proliferation *in vitro* and antidepressant effects *in vivo*.

1   **Title: Harmine stimulates proliferation of human neural progenitors**

2   Authors: Vanja Dakic[1,2], Renata de Moraes Maciel[1], Hannah Drummond[1,2],

3   Juliana M. Nascimento[1,3], Pablo Trindade[1], Stevens K. Rehen[1,2,*]

4   [1]D'Or Institute for Research and Education (IDOR), Rio de Janeiro, RJ, Brazil

5   [2]Institute of Biomedical Sciences, Federal University of Rio de Janeiro, Rio de

6   Janeiro, RJ, Brazil

7   [3]Department of Biochemistry and Tissue Biology, Institute of Biology, State

8   University of Campinas, Campinas, SP, Brazil

9   *Corresponding author: S.K.R. (srehen@lance-ufrj.org)

11   **Abstract**

12   Harmine is the β-carboline alkaloid with the highest concentration in the

13   psychotropic plant decoction Ayahuasca. In rodents, classical antidepressants

14   reverse the symptoms of depression by stimulating neuronal proliferation. It has

15   been shown that Ayahuasca presents antidepressant effects in patients with

16   depressive disorder. In the present study, we investigated the effects of harmine

17   in cell cultures containing human neural progenitor cells (hNPCs, 97% nestin-

18   positive) derived from pluripotent stem cells. After 4 days of treatment, the pool of

19   proliferating hNPCs increased by 71.5%. Harmine has been reported as a potent

20   inhibitor of the dual specificity tyrosine-phosphorylation-regulated kinase

21   (DYRK1A), which regulates cell proliferation and brain development. We tested

22   the effect of analogs of harmine, an inhibitor of DYRK1A (INDY), and an

23   irreversible selective inhibitor of monoamine oxidase (MAO) but not DYRK1A

24   (pargyline). INDY but not pargyline induced proliferation of hNPCs similarly to

25   harmine, suggesting that inhibition of DYRK1A is a possible mechanism to

26   explain harmine effects upon the proliferation of hNPCs. Our findings show that

27   harmine enhances proliferation of hNPCs and suggest that inhibition of DYRK1A

28   may explain its effects upon proliferation *in vitro* and antidepressant effects *in*

29   *vivo*.

30

31   **Introduction**

32 Throughout life, specific regions in the human adult brain continuously generate
33 neural cells from a pool of neural progenitor cells (hNPCs). Many physiological
34 and pathological events are able to control neurogenesis by modulating
35 proliferation, differentiation, maturation and integration of new-born neurons into
36 the existing circuitry (Zhao et al. 2008). This balance can be disrupted by chronic
37 stress (Egeland et al. 2015), depression (Mahar et al. 2014), aging (DeCarolis et
38 al. 2015), and neurodegenerative diseases (Winner & Winkler 2015).
39 Classical antidepressants can reverse or block stress-induced hippocampal
40 atrophy in rodents, mostly by stimulating neuronal proliferation (Malberg et al.
41 2000)). Fluoxetine, one of the most used selective serotonin reuptake inhibitors,
42 induces proliferation of rat hypothalamic (Chen et al. 2007; Sachs & Caron 2015;
43 Sousa-Ferreira et al. 2014)) and hippocampal neural progenitors *in vitro* and *in*
44 *vivo* (Chen et al. 2007; Sachs & Caron 2015). Unfortunately, treatment with
45 classic antidepressants leads to full remission in only 50% of patients (Nestler et
46 al. 2002), causes side effects and the time required for achieving therapeutic
47 response is usually measured in weeks. Thus, the demand for novel
48 psychopharmacological agents able to revert depression remains significant.
49 Beta-carbolines, a large group of indole alkaloids are widely distributed in plants.
50 Two members of this group, harmine and harmaline, have been found in human
51 plasma after ingestion of Ayahuasca (Callaway et al. 1996), a psychotropic
52 beverage traditionally used in the Amazonian region of South America as part of
53 local religious ceremonies (Labate & Feeney 2012).
54 Evaluation of the effects of a single dose of Ayahuasca in six volunteers with a
55 current depressive episode suggested that this plant decoction has fast-acting
56 anxiolytic and antidepressant effects (Osorio Fde et al. 2015). Moreover, the use
57 of harmine in rodents leads to the reduction of symptoms associated with
58 depression (Farzin & Mansouri 2006) and re-establishment of normal levels of
59 hippocampal brain-derived neurotrophic factor (BDNF) (Fortunato et al. 2009).
60 Apart of these initial studies, there are no data available regarding the
61 neurogenic effects of harmine in humans. Here we examine the effects of
62 harmine on the proliferation of human neural progenitor cells derived from

63 pluripotent stem cells. We show that harmine increased the pool of neural
64 progenitor cells and that inhibition of DYRK1 is the possible mechanism involved
65 in those proliferative effects.

66

67 **Material and methods**

68 **Chemicals**

69 Harmine (286044), INDY (SML1011), and pargyline hydrochloride (P8013) were
70 purchased from Sigma-Aldrich and diluted in DMSO. Subsequent dilutions were
71 made in aqueous solution. Click-it EdU kit and BOBO™-3 were purchased from
72 Thermo Fisher Scientific. All controls received an amount of vehicle equivalent to
73 drug treatment conditions and no significant difference was observed between
74 controls with (DMSO) or without vehicle.

75

76 **Human pluripotent stem cells**

77 Human embryonic stem cells (Fraga et al. 2011) were cultured under feeder-free
78 culture conditions on Matrigel (BD Biosciences)-coated dishes (Corning) in
79 Essential 8™ Medium (Thermo Fisher Scientific). Passaging was performed
80 enzymatically using Accutase (Millipore) by splitting colonies in clumps every 4-5
81 days and re-plating on Matrigel-coated dishes, having their medium changed
82 every day. All cells were maintained at 37°C in humidified air with 5% CO_2.

83

84 **Human neural progenitor cells**

85 To induce embryonic stem cells to direct neural differentiation, we performed an
86 adaptation of Baharvand and co-workers protocol (Baharvand et al. 2007;
87 Paulsen Bda et al. 2012). Briefly, 70% confluent BR1 culture was differentiated to
88 the neural lineage in defined adherent culture by retinoic acid and basic fibroblast
89 growth factor (bFGF) within 18 days of culture. On the 18th day neural tube-like
90 structures were collected and replated on dishes coated with 10 μg/mL of Poly-L-
91 ornithine and 2.5 μg/mL of laminin (Thermo Fisher Scientific). A population of
92 hNPCs that migrated from neural tube-like structures was tested for expression
93 of neuronal markers and expanded. Expansion was done in N2B27 medium

94   supplemented with 25 ng/mL bFGF and 20 ng/mL EGF (Thermo Fisher
95   Scientific). N2B27 medium consisted of DMEM/F-12 supplemented with 1X N2,
96   1X B-27, 1% penicillin/streptomycin (Thermo Fisher Scientific). Cells were
97   incubated at 37 ºC and 5% CO_2. Medium was replaced every other day. hNPCs
98   were expanded for no more than 5 passages.
99

100 **High Content Screening**
101 Cell proliferation, cell death and DNA damage experiments were performed in a
102 High Content Screening (HCS) format. hNPCs (1,500 cells/per well) were plated
103 on a multiwell 384 µClear plate (Greiner Bio-One) coated with 100 µg/mL Poly-L-
104 ornithine and 10 µg/mL laminin (Thermo Fisher Scientific). After 24h, cells were
105 treated for 4 days in quintuplicate (five wells per condition) with harmine, INDY
106 and pargyline in N2B27 medium supplemented with bFGF and EGF. On day 4
107 cells were labelled with 10 µM EdU for 2h (cell proliferation) or BOBO™-3 (cell
108 death) for 30 min prior to fixation or image acquisition, respectively.
109

110 **High Content Analysis**
111 All images were acquired on Operetta high-content imaging system (Perkin
112 Elmer). For proliferation, incorporated EdU was detected with Alexa Fluor 488
113 using Click-iT EdU kit following manufacturer's instruction. Immunocytochemistry
114 for Ki-67 was performed after EdU AF488 labelling. Total number of cells was
115 calculated by nuclei stained with DAPI. S phase was determined by percentage
116 of total cells labelled with EdU. Whereas dividing cells in all phases of cell cycle,
117 exempting G0, were measured by Ki-67 positive cells as percentage of total
118 cells. Images were acquired with a 10x objective with high numerical aperture
119 (NA).
120 For cell death analysis, cells were labelled with a fluorophore dye cocktail,
121 containing the cell-permeant nuclear dye Hoechst and the cell-impermeant
122 nuclear dye BOBO™-3 in fresh N2B27 medium for 30 min at 37 ºC and 5% CO_2.
123 After incubation, the dye cocktail was replaced for new medium and live cell
124 imaging was performed using temperature and CO_2 control option (TCO) of

125   Operetta, set to 37°C and 5% CO_2 at 10x magnification. For DNA damage
126   analysis, immunocytochemistry was performed on fixed cells after 4 days of
127   treatment using H2AX antibody, and images were acquired at 10x magnification.
128   All quantification analysis were normalised to the number of cells in the well
129   segmented by nucleus dyes. H_2O_2 was used as positive control for both cell
130   death and DNA damage.
131   All analyses sequences were designated by combining segmentation steps with
132   morphological and fluorescence based object characterizations using the image
133   analysis software Harmony 3.5.1 (Perkin Elmer).
134
135   **Immunocytochemistry**
136   hNPCs were fixed in formaldehyde 3.7% for 15 min at RT and permeabilized in
137   0.2% Triton X-100 for 15 min Primary antibodies were incubated overnight in 2%
138   BSA at 4°C, following 40 min of 2% BSA blockage. After washing with PBS,
139   secondary antibodies were incubated for 1h at RT in the dark. Cells were washed
140   3 times with PBS and nuclei were stained with DAPI. Coverslips were mounted
141   on slides using Aqua-Poly Mount (Polysciences) whereas cells on the 384 well
142   plates were covered with glycerol and sealed with AlumaSeal CS (Excel
143   Scientific) for image acquisition in confocal microscopy (Leica) and Operetta
144   (Perkin Elmer), respectively. Primary antibodies used: mouse anti-MAP2 (Sigma-
145   Aldrich), mouse anti-Ki-67 (BD Biosciences), rabbit anti-PAX6 (Santa Cruz
146   Biotechnology), rabbit anti-GFAP (Dako), rabbit anti-γ-H2AX (Cell Signaling
147   Technology), rabbit anti-FOXG1 (Abcam), rabbit anti-DYRK1A (Sigma-Aldrich),
148   rabbit anti-SOX2 (Millipore), mouse anti-β-tubulin III (Millipore), mouse anti-nestin
149   (Millipore), rabbit anti-TBR2 (Millipore). Secondary antibodies used: goat anti-
150   mouse Alexa Fluor 594 and goat anti-rabbit Alexa Fluor 488 (Thermo Fisher
151   Scientific). Data are expressed as relative protein expression in comparison with
152   basal protein expression in control with vehicle (DMSO).
153
154   **Statistical analysis**

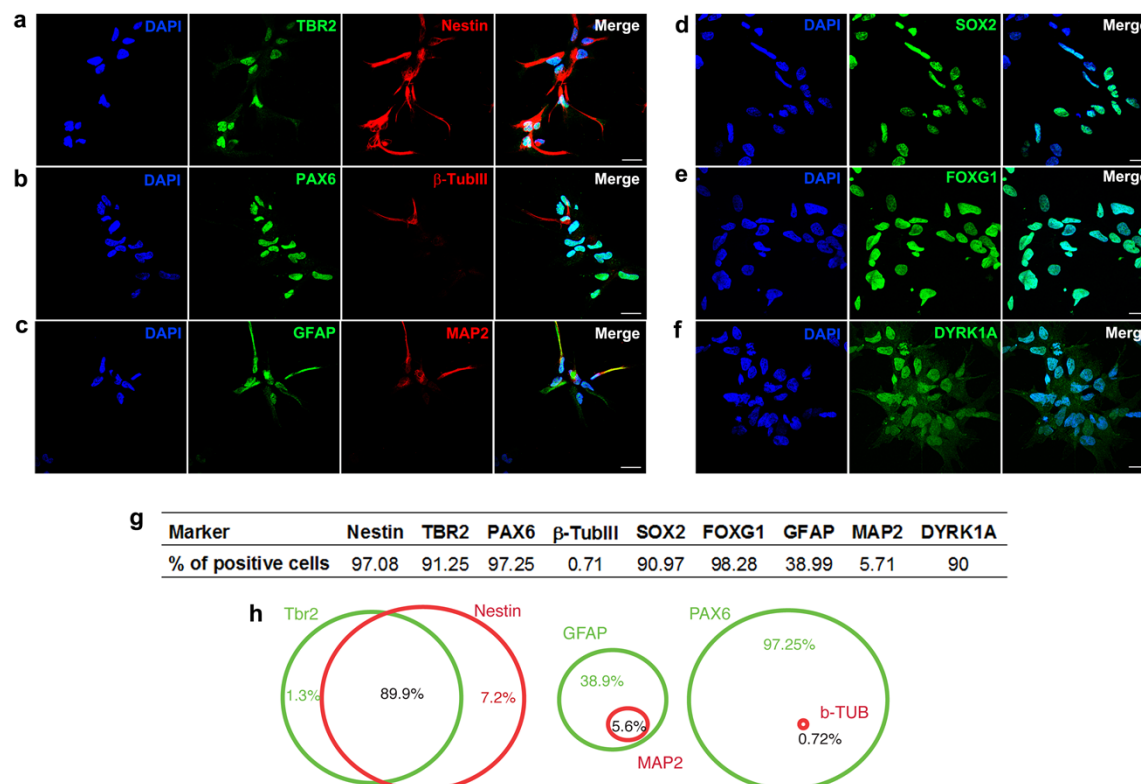
155 All data are expressed as mean ± sem. Results were accepted as statistically
156 significant at $p < 0.05$, as determined using one-way ANOVA with Tukey's
157 multiple comparison test. A minimum of 10,000 hNPCs was counted per
158 condition/per experiment.

159

160 **Results**

161 We generated hNPCs from human embryonic stem cells using a protocol that
162 recapitulates the early steps of nervous system development (Baharvand et al.
163 2007). Characterization of specific cell markers was done on day 0
164 (Supplementary Fig. 1). More than 90% of the hNPCs express characteristic
165 markers of neural progenitors, such as SOX2 (sex determining region Y-box 2),
166 nestin (intermediate filament protein; neuroectodermal stem cell marker), PAX6
167 (paired box 6), FOXG1 (forkhead box G1; transcriptional repressor important for
168 development of the brain and telencephalon), TBR2 (transcription factor Eomes;
169 key regulator of neurogenesis in the subventricular zone) (Fig. 1a, b, d, e, g).
170 Additionally, differentiated cells expressing β-Tubulin III (a class III member of the
171 β tubulin protein family primarily expressed in neurons), GFAP (glial fibrillary
172 acidic protein, marker of astrocytes), or MAP2 (marker of neuronal soma and
173 dendrites) are also observed (Fig. 1b, c, g). Ninety percent of cells express the
174 dual specificity tyrosine-phosphorylation-regulated kinase 1A (DYRK1A) (Fig. 1f,
175 g).

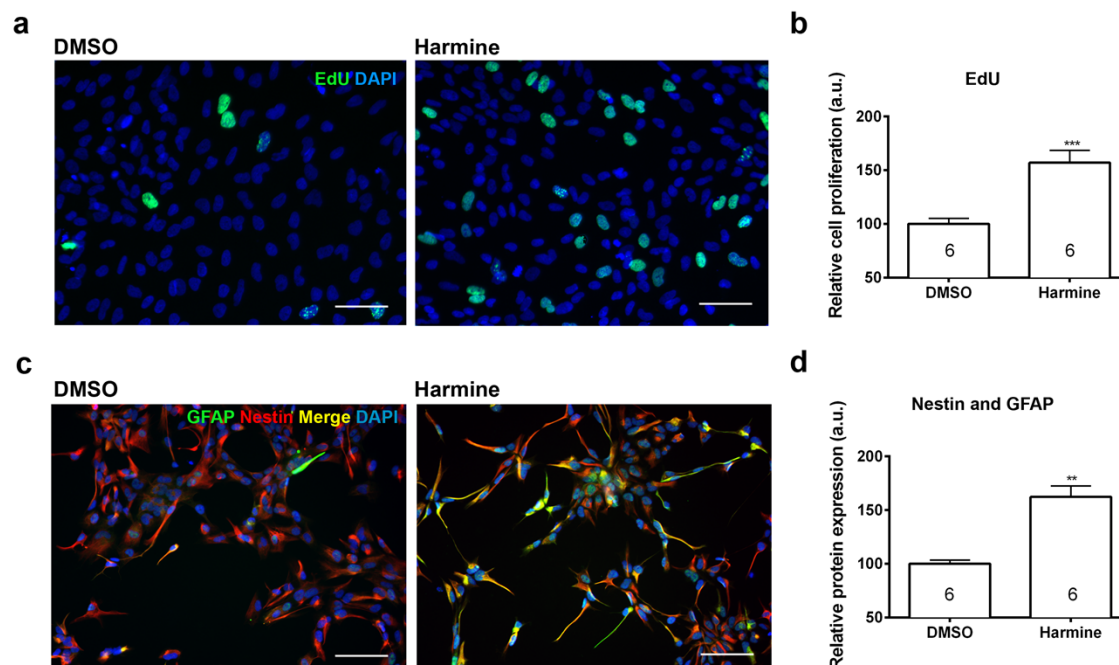
176

Marker	Nestin	TBR2	PAX6	β-TubIII	SOX2	FOXG1	GFAP	MAP2	DYRK1A
% of positive cells	97.08	91.25	97.25	0.71	90.97	98.28	38.99	5.71	90

177
Figure 1: Characterization of neural progenitor cells derived from human embryonic stem cells. Representative images showing hNPCs stained for (**a**) TBR2 and Nestin, (**b**) PAX6 and β-Tubulin III, (**c**) GFAP and MAP2, (**d**) SOX2, (**e**) FOXG1, (**f**) DYRK1A. (**g**) Quantification of cell markers. (**h**) Venn diagram showing percentage of double stained cells. A minimum of 10,000 hNPCs was counted per marker. Scale bar: 25 µm.

184

Harmine increases proliferation of human neural progenitors: a possible role of DYRK1

Pharmacokinetic studies have shown a high variability in the bioavailability of ayahuasca alkaloids in the human plasma: 36.4-222.3 ng/mL (Callaway et al. 1996), 0.5-5 ng/mL (Yritia et al. 2002). On the other hand, *in vitro* studies have been using a wide spectrum of harmine concentrations, ranging from 333 nM to 22.5 µM (Gockler et al. 2009; Hammerle et al. 2011; Martinez de Lagran et al. 2012; Mazur-Kolecka et al. 2012; Wang et al. 2015). In order to examine its effects upon proliferation, we incubated human neural cells with harmine ranging from 0.1 to 22.5 µM for 96h (Supplementary Figs. 1 and 2). The 7.5 µM concentration of harmine was the most effective on proliferation of hNPCs (Supplementary Fig. 2), increasing the pool of proliferating cells by 71.5% (Fig.

197 2a, b). No evidence of cell death or DNA damage in response to harmine as
198 measured by BOBO-3 (Fig. 3a, b) and H2AX labelling (Fig. 3c, d) was observed.
199 Treatment with harmine increased by 64.4% the specific pool of neural
200 progenitors, which actively participates in adult neurogenesis (Nestin and GFAP
201 labelled, Fig 2c, d) (Fukuda et al. 2003). The number of cells positive for SOX2,
202 MAP2 and FOXG1 was not altered (Supplementary Fig. 3).

203
204 **Figure 2: Quantification of the proliferation and differentiation of hNPCs**
205 **after treatment with harmine.** (**a**) Representative images of EdU staining. (**b**)
206 Cell proliferation (S phase) relative to DMSO control, measured by EdU
207 incorporation. (**c**) Representative images of GFAP and Nestin staining. (**d**)
208 Expression of Nestin and GFAP proteins relative to DMSO control. A minimum of
209 10,000 hNPCs was counted per condition/per experiment. Data were analysed
210 by one-way ANOVA with Tukey's multiple comparison test, ** $p<0.001$,
211 ***<0.0001. Values represent mean ± sem. The number inside the bar represents
212 the number of experiments in each group. Scale bar: 100 μm.
213

214

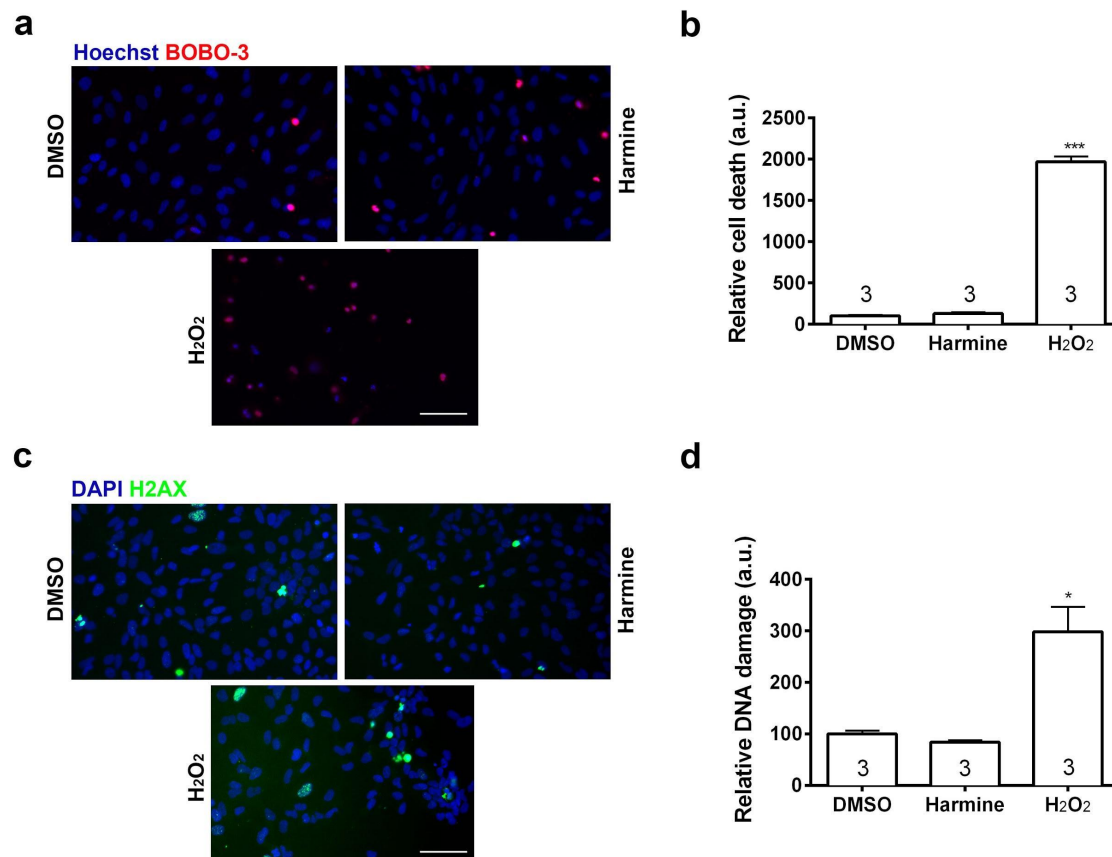



Figure 3: Percentage of cell death and DNA damage in neural cells treated with harmine. (**a**) Representative immunostaining images of BOBO-3 (red) positive cells. Nuclei are stained with Hoechst (blue). (**b**) Cell death relative to DMSO control, measured as the percentage of cells stained with BOBO-3. (**c**) Representative images of cells positive for H2AX (green). Nuclei are stained with DAPI (blue). (**d**) Quantification of H2AX positive cells relative to DMSO control, as percentage of cells stained with H2AX. H_2O_2 was used as positive control for both cell death and DNA damage. A minimum of 10,000 hNPCs was counted per condition/per experiment. Data were analysed by one-way ANOVA with Tukey's multiple comparison test, * $p<0.05$, ***<0.0001. Values represent mean ± sem. The number inside the bar represents the number of experiments in each group. Scale bar: 100 µm.

227

228   Harmine has been shown to be an inhibitor of DYRK1A (Becker & Sippl 2011;

229   Gockler et al. 2009), other DYRK family members (Gockler et al. 2009) and

230   monoamine oxidase (MAO) (Santillo et al. 2014). To verify whether these two

231   targets are involved in the harmine-dependent increase in neural proliferation, we

232   examined the effect of two functional analogs of harmine in human neural cells:

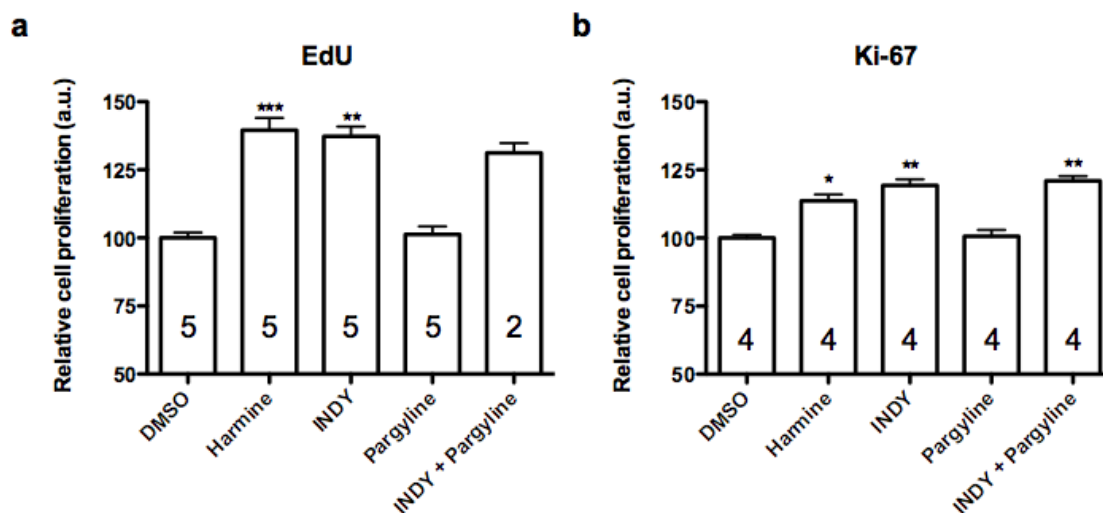
233   INDY (15 µM), an inhibitor of DYRK1A; and pargyline (10 µM), an irreversible

234 selective inhibitor of MAO but not DYRK1A.

235 Harmine and INDY increased proliferation of hNPCs in comparison with control,

236 analysed by two different methods, while pargyline did not induce the same

237 effect. Concomitant inhibition of both DYRK1A and MAOs produced similar effect

238 as harmine or INDY alone (Fig. 4).

239
240 **Figure 4: Quantification of EdU and Ki-67 labelling in human neural**
241 **progenitor cells in response to harmine and its functional analogs.** (**a**) Cell
242 proliferation relative to DMSO control, measured by EdU incorporation. (**b**) Cell
243 proliferation relative to DMSO control, measured by Ki-67. Values represent
244 mean ± sem. A minimum of 10,000 hNPCs was counted per condition/per
245 experiment. Data were analysed by one-way ANOVA with Tukey's multiple
246 comparison test, * $p<0.05$, ** $p<0.001$, ***<0.0001. Values represent mean ±
247 sem. The number inside the bar represents the number of experiments in each
248 group.
249

250 **Discussion**

251 The use of *in vitro* models can potentially clarify mechanisms related to

252 proliferation and differentiation of new-born neurons (Cai & Grabel 2007), which

253 happens massively during embryogenesis but also later in specific brain regions.

254 Numerous studies have shown a close relationship between disturbed adult

255 neurogenesis and depression, highlighting the need for more efficacious and

256 faster-acting treatments. Thus, the aim of this study was to investigate the effects

257 of harmine, a compound with potential antidepressant properties, on human

258 neural progenitor cells derived from pluripotent stem cells. Here we show that

259   harmine increases proliferation of hNPCs positive for both nestin and GFAP *in*
260   *vitro*, having inhibition of DYRK1 as the possible mechanism.

261   We describe, in cell cultures containing more than 90% of hNPCs, that treatment
262   with harmine increased proliferation without DNA damage or cell death. These
263   results are consistent with findings published by Hämmerle and others (2011), in
264   which exposure of chick embryos to harmine resulted in a strong increase in
265   BrdU incorporation and number of mitotic cells in the spinal cord.

266   Interestingly, harmine treatment also increased the number of early progenitor
267   cells, stained with both GFAP and Nestin. At first, this result suggests that
268   harmine drives the differentiation of hNPCs into radial glial cells, which are the
269   major source of neuronal and glial progenitors in the developing brain (Gotz &
270   Barde 2005). Similarly, in the adult hippocampus of rodents, these neural
271   precursors are responsible for late neurogenesis and gliogenesis (Zhao et al.
272   2008). In this context, levels of GFAP tend to increase on cells that shifted to a
273   glial fate. On the other hand, despite being GFAP+ cells, at this developmental
274   stage, these progenitors can also shift to a phenotype PSA-NCAM+, which can
275   give rise to neurons (Fukuda et al. 2003). In the light of this evidence, we suggest
276   that harmine could potentiate proliferation of radial glia-like cells
277   (GFAP+/Nestin+) derived from hNPCs, which are capable to generate both
278   neurons and astrocytes. Further experiments should be done in order to confirm
279   the terminal fate of harmine-treated hNPCs.

280   Harmine has been described as an inhibitor of MAO and DYRK1A (Gockler et al.
281   2009; Santillo et al. 2014). MAO inhibition increases serotonergic
282   neurotransmission in the adult brain (Finberg, 2014), which is a key component
283   of the classical antidepressant action. The main outcome of treatment with MAO
284   inhibitors is upregulation of cell proliferation and neurogenesis in the
285   hippocampus (Manev et al. 2001). On the other hand, it has been reported that
286   mice lacking MAO presented reduced proliferation of neural progenitor cells
287   (Cheng et al. 2010). In order to verify if MAO inhibition plays a role in harmine-
288   induced proliferation, we used the specific MAO inhibitor, pargyline. Pargyline did
289   not alter the levels of harmine-induced proliferation. While these results indicate

290 that MAO is not involved in the proliferative effects of harmine, we can not rule
291 out that the lack of effect of pargyline is due to the absence of MAO in neural
292 progenitor cells. In fact, MAO-dependent changes in NPCs proliferation were
293 described late in embryonic development, mediated by serotonin (Cheng et al.
294 2010).

295 On the other hand, treatment with INDY, an inhibitor of DYRK1, induced
296 proliferation similarly to that observed with harmine. These results are consistent
297 with the findings of Wang and collaborators, where harmine-dependent DYRK1A
298 inhibition increases the proliferation of pancreatic beta cells (Zhou et al. 2015).
299 Since both harmine and INDY have multiple targets on DYRK family, including
300 regulators of cell cycle (Becker & Sippl 2011), we should consider other potential
301 players of INDY- and harmine-mediated increase in proliferation.

302 INDY can also inhibit DYRK1A closely related kinases, DYRK1B and DYRK2. In
303 cancer studies DYRK1B and DYRK2 have been described as modulators of
304 proliferation (Adayev et al. 2011; Zhou et al. 2015). In fact, harmine also inhibits
305 DYRK1B and DYRK2, but the efficiency of this inhibition is, respectively, 5- and
306 50-fold lower in comparison to DYRK1A (Adayev et al. 2011; Bain et al. 2007;
307 Gockler et al. 2009). Thus, DYRK1A emerges as the major candidate for
308 mediating the increase in proliferation seen in this study. Also, it was shown that
309 DYRK1A directly phosphorylates p53 and leads to the induction of p53 target
310 genes, attenuating proliferation of rat and human neural progenitor cells (Park et
311 al. 2010). Further studies are needed to reveal other aspects of human cell
312 proliferation stimulated by harmine.

313 It is worth to mention that DYRK1A can also modulate migration (Pons-Espinal et
314 al. 2013) and differentiation (Kurabayashi & Sanada 2013; Yabut et al. 2010) of
315 neural progenitors. Neurogenesis contemplates the interplay of proliferation,
316 migration and differentiation. While our data show that harmine stimulates
317 proliferation of hNPCs, further studies upon migration and differentiation may
318 clarify whether neurogenesis (or gliogenesis) are indeed increased by harmine in
319 hNPCs.

320 Taken together our results suggest that harmine exert proliferative effects in

321 human neural progenitors, particularly in radial glia-like cells (GFAP+/Nestin+),

322 by inhibiting DYRK1A. These findings shed light on the possible mechanisms

323 behind the antidepressant effects of Ayahuasca described in patients.

324

325

326 **Abbreviations**

327

328 hNPCs - human neural progenitors; DYRK - dual-specificity tyrosine
329 phosphorylation-regulated kinase; MAO - monoamine oxidase; GFAP - Glial
330 fibrillary acidic protein; MAP2 - Microtubule-Associated Protein 2;

331

332 **Acknowledgements**
333 This work is part of the PhD thesis of VD. We thank Ismael Gomes, Marcelo
334 Costa and Igor Lima da Silva for technical assistance and Dr. Mauro de Freitas
335 Rebelo for assistance with statistical analysis.

336

337 **References**

338

339 Adayev T, Wegiel J, and Hwang YW. 2011. Harmine is an ATP-competitive
340   inhibitor for dual-specificity tyrosine phosphorylation-regulated kinase 1A
341   (Dyrk1A). *Arch Biochem Biophys* 507:212-218. 10.1016/j.abb.2010.12.024
342 Baharvand H, Mehrjardi NZ, Hatami M, Kiani S, Rao M, and Haghighi MM. 2007.
343   Neural differentiation from human embryonic stem cells in a defined
344   adherent culture condition. *Int J Dev Biol* 51:371-378.
345   10.1387/ijdb.72280hb
346 Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H, Klevernic I, Arthur
347   JS, Alessi DR, and Cohen P. 2007. The selectivity of protein kinase
348   inhibitors: a further update. *Biochem J* 408:297-315. 10.1042/BJ20070797
349 Becker W, and Sippl W. 2011. Activation, regulation, and inhibition of DYRK1A.
350   *FEBS J* 278:246-256. 10.1111/j.1742-4658.2010.07956.x
351 Cai C, and Grabel L. 2007. Directing the differentiation of embryonic stem cells to
352   neural stem cells. *Dev Dyn* 236:3255-3266. 10.1002/dvdy.21306
353 Callaway JC, Raymon LP, Hearn WL, McKenna DJ, Grob CS, Brito GS, and
354   Mash DC. 1996. Quantitation of N,N-dimethyltryptamine and harmala
355   alkaloids in human plasma after oral dosing with ayahuasca. *J Anal
356   Toxicol* 20:492-497.
357 Chen SJ, Kao CL, Chang YL, Yen CJ, Shui JW, Chien CS, Chen IL, Tsai TH, Ku
358   HH, and Chiou SH. 2007. Antidepressant administration modulates neural
359   stem cell survival and serotoninergic differentiation through bcl-2. *Curr
360   Neurovasc Res* 4:19-29.
361 Cheng A, Scott AL, Ladenheim B, Chen K, Ouyang X, Lathia JD, Mughal M,
362   Cadet JL, Mattson MP, and Shih JC. 2010. Monoamine oxidases regulate
363   telencephalic neural progenitors in late embryonic and early postnatal

364   development. *J Neurosci* 30:10752-10762. 10.1523/JNEUROSCI.2037-
365   10.2010
366 DeCarolis NA, Kirby ED, Wyss-Coray T, and Palmer TD. 2015. The Role of the
367   Microenvironmental Niche in Declining Stem-Cell Functions Associated
368   with Biological Aging. *Cold Spring Harb Perspect Med* 5.
369   10.1101/cshperspect.a025874
370 Egeland M, Zunszain PA, and Pariante CM. 2015. Molecular mechanisms in the
371   regulation of adult neurogenesis during stress. *Nat Rev Neurosci* 16:189-
372   200. 10.1038/nrn3855
373 Farzin D, and Mansouri N. 2006. Antidepressant-like effect of harmane and other
374   beta-carbolines in the mouse forced swim test. *Eur*
375   *Neuropsychopharmacol* 16:324-328. 10.1016/j.euroneuro.2005.08.005
376 Finberg JP. 2014. Update on the pharmacology of selective inhibitors of MAO-A
377   and MAO-B: Focus on modulation of CNS monoamine neurotransmitter
378   release. Pharmacol Ther. 143(2):133-52.
379   10.1016/j.pharmthera.2014.02.010.
380 Fortunato JJ, Reus GZ, Kirsch TR, Stringari RB, Stertz L, Kapczinski F, Pinto JP,
381   Hallak JE, Zuardi AW, Crippa JA, and Quevedo J. 2009. Acute harmine
382   administration induces antidepressive-like effects and increases BDNF
383   levels in the rat hippocampus. *Prog Neuropsychopharmacol Biol*
384   *Psychiatry* 33:1425-1430. 10.1016/j.pnpbp.2009.07.021
385 Fraga AM, Sukoyan M, Rajan P, Braga DP, Iaconelli A, Jr., Franco JG, Jr.,
386   Borges E, Jr., and Pereira LV. 2011. Establishment of a Brazilian line of
387   human embryonic stem cells in defined medium: implications for cell
388   therapy in an ethnically diverse population. *Cell Transplant* 20:431-440.
389   10.3727/096368910X522261
390 Fukuda S, Kato F, Tozuka Y, Yamaguchi M, Miyamoto Y, and Hisatsune T. 2003.
391   Two distinct subpopulations of nestin-positive cells in adult mouse dentate
392   gyrus. *J Neurosci* 23:9357-9366.
393 Gockler N, Jofre G, Papadopoulos C, Soppa U, Tejedor FJ, and Becker W. 2009.
394   Harmine specifically inhibits protein kinase DYRK1A and interferes with
395   neurite formation. *FEBS J* 276:6324-6337. 10.1111/j.1742-
396   4658.2009.07346.x
397 Gotz M, and Barde YA. 2005. Radial glial cells defined and major intermediates
398   between embryonic stem cells and CNS neurons. *Neuron* 46:369-372.
399   10.1016/j.neuron.2005.04.012
400 Hammerle B, Ulin E, Guimera J, Becker W, Guillemot F, and Tejedor FJ. 2011.
401   Transient expression of Mnb/Dyrk1a couples cell cycle exit and
402   differentiation of neuronal precursors by inducing p27KIP1 expression and
403   suppressing NOTCH signaling. *Development* 138:2543-2554.
404   10.1242/dev.066167
405 Kurabayashi N, and Sanada K. 2013. Increased dosage of DYRK1A and DSCR1
406   delays neuronal differentiation in neocortical progenitor cells. *Genes Dev*
407   27:2708-2721. 10.1101/gad.226381.113

408 Labate BC, and Feeney K. 2012. Ayahuasca and the process of regulation in
409   Brazil and internationally: implications and challenges. *Int J Drug Policy*
410   23:154-161. 10.1016/j.drugpo.2011.06.006
411 Mahar I, Bambico FR, Mechawar N, and Nobrega JN. 2014. Stress, serotonin,
412   and hippocampal neurogenesis in relation to depression and
413   antidepressant effects. *Neurosci Biobehav Rev* 38:173-192.
414   10.1016/j.neubiorev.2013.11.009
415 Malberg JE, Eisch AJ, Nestler EJ, and Duman RS. 2000. Chronic antidepressant
416   treatment increases neurogenesis in adult rat hippocampus. *J Neurosci*
417   20:9104-9110.
418 Manev H, Uz T, Smalheiser NR, and Manev R. 2001. Antidepressants alter cell
419   proliferation in the adult brain in vivo and in neural cultures in vitro. *Eur J*
420   *Pharmacol* 411:67-70.
421 Martinez de Lagran M, Benavides-Piccione R, Ballesteros-Yanez I, Calvo M,
422   Morales M, Fillat C, Defelipe J, Ramakers GJ, and Dierssen M. 2012.
423   Dyrk1A influences neuronal morphogenesis through regulation of
424   cytoskeletal dynamics in mammalian cortical neurons. *Cereb Cortex*
425   22:2867-2877. 10.1093/cercor/bhr362
426 Mazur-Kolecka B, Golabek A, Kida E, Rabe A, Hwang YW, Adayev T, Wegiel J,
427   Flory M, Kaczmarski W, Marchi E, and Frackowiak J. 2012. Effect of
428   DYRK1A activity inhibition on development of neuronal progenitors
429   isolated from Ts65Dn mice. *J Neurosci Res* 90:999-1010.
430   10.1002/jnr.23007
431 Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, and Monteggia LM. 2002.
432   Neurobiology of depression. *Neuron* 34:13-25.
433 Osorio Fde L, Sanches RF, Macedo LR, Santos RG, Maia-de-Oliveira JP,
434   Wichert-Ana L, Araujo DB, Riba J, Crippa JA, and Hallak JE. 2015.
435   Antidepressant effects of a single dose of ayahuasca in patients with
436   recurrent depression: a preliminary report. *Rev Bras Psiquiatr* 37:13-20.
437   10.1590/1516-4446-2014-1496
438 Park J, Oh Y, Yoo L, Jung MS, Song WJ, Lee SH, Seo H, and Chung KC. 2010.
439   Dyrk1A phosphorylates p53 and inhibits proliferation of embryonic
440   neuronal cells. *J Biol Chem* 285:31895-31906. 10.1074/jbc.M110.147520
441 Paulsen B da S, de Moraes Maciel R, Galina A, Souza da Silveira M, dos Santos
442   Souza C, Drummond H, Nascimento Pozzatto E, Silva H, Jr., Chicaybam
443   L, Massuda R, Setti-Perdigao P, Bonamino M, Belmonte-de-Abreu PS,
444   Castro NG, Brentani H, and Rehen SK. 2012. Altered oxygen metabolism
445   associated to neurogenesis of induced pluripotent stem cells derived from
446   a schizophrenic patient. *Cell Transplant* 21:1547-1559.
447   10.3727/096368911X600957
448 Pons-Espinal M, Martinez de Lagran M, and Dierssen M. 2013. Environmental
449   enrichment rescues DYRK1A activity and hippocampal adult neurogenesis
450   in TgDyrk1A. *Neurobiol Dis* 60:18-31. 10.1016/j.nbd.2013.08.008
451 Sachs BD, and Caron MG. 2015. Chronic fluoxetine increases extra-hippocampal
452   neurogenesis in adult mice. *Int J Neuropsychopharmacol* 18.
453   10.1093/ijnp/pyu029

454 Santillo MF, Liu Y, Ferguson M, Vohra SN, and Wiesenfeld PL. 2014. Inhibition
455   of monoamine oxidase (MAO) by beta-carbolines and their interactions in
456   live neuronal (PC12) and liver (HuH-7 and MH1C1) cells. *Toxicol In Vitro*
457   28:403-410. 10.1016/j.tiv.2013.12.006
458 Sousa-Ferreira L, Aveleira C, Botelho M, Alvaro AR, Pereira de Almeida L, and
459   Cavadas C. 2014. Fluoxetine induces proliferation and inhibits
460   differentiation of hypothalamic neuroprogenitor cells in vitro. *PLoS One*
461   9:e88917. 10.1371/journal.pone.0088917
462 Wang P, Alvarez-Perez JC, Felsenfeld DP, Liu H, Sivendran S, Bender A, Kumar
463   A, Sanchez R, Scott DK, Garcia-Ocana A, and Stewart AF. 2015. A high-
464   throughput chemical screen reveals that harmine-mediated inhibition of
465   DYRK1A increases human pancreatic beta cell replication. *Nat Med*
466   21:383-388. 10.1038/nm.3820
467 Winner B, and Winkler J. 2015. Adult neurogenesis in neurodegenerative
468   diseases. *Cold Spring Harb Perspect Biol* 7:a021287.
469   10.1101/cshperspect.a021287
470 Yabut O, Domogauer J, and D'Arcangelo G. 2010. Dyrk1A overexpression
471   inhibits proliferation and induces premature neuronal differentiation of
472   neural progenitor cells. *J Neurosci* 30:4004-4014.
473   10.1523/JNEUROSCI.4711-09.2010
474 Yritia M, Riba J, Ortuno J, Ramirez A, Castillo A, Alfaro Y, de la Torre R, and
475   Barbanoj MJ. 2002. Determination of N,N-dimethyltryptamine and beta-
476   carboline alkaloids in human plasma following oral administration of
477   Ayahuasca. *J Chromatogr B Analyt Technol Biomed Life Sci* 779:271-281.
478 Zhao C, Deng W, and Gage FH. 2008. Mechanisms and functional implications
479   of adult neurogenesis. *Cell* 132:645-660. 10.1016/j.cell.2008.01.033
480 Zhou N, Yuan S, Wang R, Zhang W, and Chen JJ. 2015. Role of dual specificity
481   tyrosine-phosphorylation-regulated kinase 1B (Dyrk1B) in S-phase entry of
482   HPV E7 expressing cells from quiescence. *Oncotarget* 6:30745-30761.
483   10.18632/oncotarget.5222
484
485