1 **SHORT COMMUNICATION**

2

3 # Identification of two novel host proteins interacting with *Toxoplasma gondii*
4 # 14-3-3 protein by yeast two-hybrid system

5

6 **Fa-Cai Li[1,*], Qing Liu[1,2], Hany M. Elsheikha[3], Wen-Bin Yang[1,4], Jun-Ling Hou[1], and**
7 **Xing-Quan Zhu[1]**

8

9 (✉) Fa-Cai Li

10 lifacai@caas.cn

11

12 [1] State Key Laboratory of Veterinary Etiological Biology, Key Laboratory of Veterinary
13 Parasitology of Gansu Province, Lanzhou Veterinary Research Institute, Chinese Academy of
14 Agricultural Sciences, Lanzhou, China

15 [2] College of Animal Science and Veterinary Medicine, Shanxi Agricultural University,
16 Taigu, China

17 [3] Faculty of Medicine and Health Sciences, School of Veterinary Medicine and Science,
18 University of Nottingham, Sutton Bonington Campus, Loughborough, LE12 5RD, UK

19 [4] College of Veterinary Medicine, Northwest A&F University, Yangling, China

20

21

22

23

24

25 **Abstract** *Toxoplasma gondii* deploys many effector proteins in order to hijack and
26 manipulate host cell signalling pathways, allowing parasite colonization, subversion of
27 immune responses and disease progression. *T. gondii* effector protein 14-3-3 (Tg14-3-3)
28 promotes parasite dissemination inside the body, by enhancing the migratory ability of
29 infected microglia and dendritic cells. Understanding both the mechanism of action, and the
30 host targets of Tg14-3-3 effector is important because of their importance to the parasite's
31 virulence. The aim of the present study was to explore the function of Tg14-3-3 by utilizing
32 the yeast two hybrid system (Y2HS) to identify novel Tg14-3-3 interactors/substrates in host
33 cells. A human cDNA library was screened using Tg14-3-3 as the bait. Tg14-3-3 (RH strain,
34 Type I) was cloned into the pGBKT7 vector and expressed in the Y2HGold yeast strain. The
35 bait protein expression was validated by Western blotting analysis, auto-activation and
36 toxicity investigation compared with control (Y2HGold yeast strain transformed with empty
37 pGBKT7 vector). Two positive Tg14-3-3 interactors identified by this screening,
38 hCG1821272 and eIF5B (Eukaryotic translation initiation factor 5B), were isolated and
39 characterized. This approach made it possible to gain a better understanding of the function of
40 Tg14-3-3 in regulating host proteins involved in key cellular processes, such as translational
41 initiation and cell migration.

42

43 **Keywords** *Toxoplasma gondii* · Tg14-3-3 · host-pathogen interaction · protein-protein
44 interaction · yeast two-hybrid system

45
46
47
48
49
50
51
52

53 ## Introduction

54

55 It is estimated that more than one-third of the human population are chronically infected with
56 *Toxoplasma gondii* (Montoya and Liesenfeld 2004). This parasite is highly adapted to survive
57 in a variety of microenvironments. On reaching and penetrating the brain the parasite forms a
58 lifelong infection, encysting in a latent state (bradyzoite) for decades from which it is
59 periodically activated via lysis to a fulminant infection (Elsheikha 2008). Global healthcare
60 challenges from this parasite are increasing due to a paucity of human vaccines; while
61 currently available drugs are often toxic and have limited clinical efficacy (Katlama et al.
62 1996), especially against the latent phase of disease.

63 Obligatory intracellular apicomplexan protozoan parasites, such as *T. gondii* cause
64 disease by reiterating their lytic cycle of, host cell invasion, parasite replication, and parasite
65 egress. These parasites are rapidly motile within the body, and succeed in invading and
66 colonizing the host cells through the spatiotemporal deployment of a diverse range of effector
67 proteins (secretome) released by unique secretory organelles (micronemes and rhoptries). This
68 secretome is required for host cell invasion, and plays critical roles in hijacking cellular
69 processes and reprogramming the host cell signaling pathways to enable pathogen survival
70 and proliferation. For example, proteins in the rhoptry neck (RONs) are initially secreted into
71 the host cell membrane, where they help mediate the formation of a moving junction
72 composed of RON2, RON4 and RON5 together with the micronemal protein AMA1. Kinase
73 proteins in the bulb of the rhoptry (ROPs) are then released into the host cell cytosol to
74 perturb various host cell processes (Saffer et al. 1992; Saeij et al. 2006; Taylor et al. 2006;
75 Sibley et al. 2009), where they are directed to the host cell nucleus (e.g., ROP16 and protein
76 phosphatase 2C (PP2C-hn)) or to the surface of the parasitophorous vacuole (PV) (e.g.,
77 ROP2, ROP18 and ROP5).

78 Protein secretion is key to parasite survival, promoting virulence and allowing *T. gondii*
79 to detect and respond to its host environment. In eukaryotic cells, the 14-3-3 family of
80 proteins are critical regulators of key proteins involved in various physiological processes,
81 such as apoptosis (Nomura et al. 2003), cell migration (Kobayashi et al. 2011) and
82 cytoskeleton remodeling (Sluchanko and Gusev 2010). These 14-3-3 proteins have been
83 identified in some apicomplexan protozoans, including *Eimeria tenella*, *Neospora caninum*,
84 *Plasmodium falciparum* and *T. gondii* (Siles-Lucas Mdel and Gottstein 2003). *Toxoplasma*
85 *gondii* effector protein 14-3-3 (known as Tg14-3-3) can induce hyper-migration of parasitized
86 microglia and dendritic cells (Weidner et al. 2016), suggesting that Tg14-3-3 may play a role
87 in the parasite's dissemination to the target host tissues. Results of 14-3-3 gene deletion
88 studies in mammalian and yeast cells (Cognetti et al. 2002; Freeman and Morrison 2011),
89 suggest that knockout of Tg14-3-3 gene produces non-viable *T. gondii* (Weidner et al. 2016).
90 Tg14-3-3 has been considered an important vaccine candidate (Meng et al. 2012). Despite its
91 importance in parasite dissemination within the body (Weidner et al. 2016), the host proteins
92 that interact with Tg14-3-3 to regulate this phenomenon remain unknown.
93 In order to better understand the roles of Tg14-3-3 in molecular host interactions, and
94 define *T. gondii* effectors and molecular mechanisms involved in *T. gondii* pathogenesis, the
95 objective of the present study was to identify the novel host partners interacting with
96 Tg14-3-3 using the yeast two hybrid system (Y2HS).
97

98 Materials and methods

99

100 Parasites culture

101

102 Type I *T. gondii* RH strain was maintained *in vitro* by serial passage on human foreskin
103 fibroblast (HFF) monolayers at 37°C in 5% CO_2. HFF cells were grown in Dulbecco's
104 modified Eagle's medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum
105 (FBS, Gibco, USA), and 1% antibiotics (penicillin-streptomycin solution). When about 90%
106 of infected HFF cells were lysed, the parasites and cells were harvested and centrifuged. The
107 mixture was washed with phosphate buffered saline (PBS) and passed through 25-gauge
108 syringe needles. To reducing the cell debris, the tachyzoites were filtered through 3 μm
109 membrane filters (Millipore, USA), and stored at –80 ℃.

110

111 **Construction of the bait vector**

112

113 Total RNA was isolated from *T. gondii* tachyzoites using TRIzol (Invitrogen, USA) following
114 the manufacturer's instructions. RNA yields and integrity were evaluated by
115 spectrophotometry (NanoDrop Technologies, USA) and gel electrophoresis, respectively.
116 Total RNA was reverse-transcribed into cDNA using the PrimeScript Ⅱ 1st Strand cDNA
117 Synthesis Kit (Takara, Japan), according to the manufacturer's instruction. To construct the
118 Tg14-3-3 bait vector, the Tg14-3-3 gene encoding a 266 residue peptide (from 58 aa to 323
119 aa) (Fig. 1A) was amplified from *T. gondii* cDNA (Fig. 1B) using the specific primers
120 14-3-3-EcoR Ⅰ -1F (5′-GAATTCATGGCGGAGGAAATCAAG-3′) and 14-3-3-BamH Ⅰ -2R
121 (5′-GGATCCTTACTGATCAGCTTGTTCTG-3′). The amplified 14-3-3 fragment (~801 bp)
122 was cloned into pMD19-T (Takara, Japan), and digested with the restriction enzymes *Eco*R Ⅰ
123 and *Bam*H Ⅰ (Fig. 1B). Finally, the purified fragment was ligated into pGBKT7 (designated as
124 pGBKT7-Tg14-3-3), and verified by restriction digestion and sequencing.

125

126 **Expression of the bait protein in yeast**

127

128 To investigate Tg14-3-3 expression in the Y2HGold yeast strain, the pGBKT7-Tg14-3-3 was
129 transformed into the yeast by the lithium acetate method following the manufacture's
130 instruction in Yeast Transformation System 2 Kit (Takara, Japan). The transformants were
131 spread and incubated on plates containing Minimal SD Agar Base (Clontech, USA),
132 supplemented with -Trp DO supplement (Clontech, USA) (SD/-Trp) for 3-5 days. One clone
133 was chosen from the SD/-Trp plate and inoculated into yeast culture medium until the OD600
134 reached 0.5. The proteins were extracted from the cell pellet using the TCA method detailed
135 in the Clontech yeast protocol handbook (http://www.clontech.com). The extracted proteins
136 were separated using SDS-PAGE and detected by Western immunoblotting using mouse
137 anti-Myc primary antibody (CST, USA) and goat anti-mouse IgG-HRP secondary antibody
138 (Santa Cruza Technologies, USA). The blot was observed with the ChemiDoc™ MP Imaging
139 System (Bio-Rad, USA) using the ECL chemiluminescent substrate (Millipore, USA).

140

141 **Evaluation of toxicity and auto-activation**

142

143 It was important to examine if the fusion protein had initiated the transcription of the reporter
144 gene by itself. The yeast transformants described above were plated on minimal synthetically
145 defined (SD) medium without tryptophan (SD/-Trp), SD/-Trp medium supplemented with
146 X-α-Gal (SD/-Trp/X-α-Gal) or SD/-Trp/X-α-Gal supplemented with Aureobasidin A (AbA,
147 Clontech, USA) (SD/-Trp/X-α-Gal/AbA) until colonies appeared (~3-5 days). A comparison
148 with the colonies transformed with pGBKT7 empty vector, made it easy to distinguish if the
149 bait plasmid had induced toxicity and auto-activation. Absence of toxicity of the bait plasmid
150 was indicated by the comparable size and appearance of the colonies to those of the pGBKT7
151 control group. Lack of auto-activation of the bait protein was indicated by white colonies on

152 SD/-Trp and absence of blue colonies on the SD/-Trp/X-α-Gal plates and absence of colony
153 growth on the SD/-Trp/X-α-Gal/AbA plates. The yeast two-hybrid screen was performed only
154 when the bait protein showed no toxicity nor auto-activation.
155
156 **Yeast Two-Hybrid System (Y2HS) screening**
157
158 To screen the host proteins that interact with the Tg14-3-3 protein, the Y2HGold transformed
159 with bait vector was mated with the Universal Human cDNA library (Clontech, USA) at 30
160 ℃ for 24 h. The mated cells were then checked, and spread on the 150 mm double dropout
161 medium SD/-Leu/-Trp, supplemented with X-α-Gal and Aureobasidin A
162 (DDO/X-α-Gal/AbA) to be incubated for 3-5 days. The blue colonies (potential positives)
163 were picked from DDO/X-α-Gal/AbA agar plates and re-streaked onto quadruple dropout
164 medium SD/-Ade/-His/-Leu/-Trp, supplemented with X-α-Gal and Aureobasidin A
165 (QDO/X-α-Gal/AbA). To eliminate the duplicate clones, yeast colony PCR was performed
166 and the products were analyzed by electrophoresis. The prey plasmids were extracted from
167 potential positives by Easy Yeast Plasmid Isolation Kit (Clontech, USA) and transformed into
168 *E. coli* DH5α competent cells, followed by selecting on LB/Amp plates. In order to confirm
169 any positive interaction, a point-to-point yeast mating was applied to exclude false positive
170 hits. Briefly, each extracted prey plasmid was transformed into the Y187 strain, which was
171 mated with the Y2HGold transformed with pGBKT7-Tg14-3-3. Mated yeast groups were
172 then spread onto the QDO/X-α-Gal/AbA plates to test for interactions. True positive hits were
173 indicated by blue colonies under these conditions.
174
175 **Analysis of the positive preys**
176

177 When the interaction was confirmed as positive, the insert sequence of the prey plasmid was
178 sequenced by the T7 sequencing primer. We ensured that the open reading frame was fused in
179 frame to the GAL4 transcriptional activation domain. The insert sequence of the positive
180 preys was subjected to BLAST search (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to identify the
181 corresponding human genes, followed by Gene Ontology (GO) analysis.

182

183 Results and discussion

184

185 In this study, the pGBKT7-Tg14-3-3 bait vector containing the conserved 14-3-3 region
186 (66 aa-308 aa) was constructed (Fig. 1) and transformed into yeast strain Y2HGold. In order
187 to detect the Tg14-3-3 protein expression, total proteins of Y2HGold cells were extracted and
188 examined by immunoblotting assay using the Myc-tag (9B11) mouse mAb (Cell Signaling).
189 The relative molecular weight of the Tg14-3-3 fusion protein was determined to be 56 kD
190 (Fig. 2A, lane 1), and as a control, the GAL4 DNA-binding domain protein, which was
191 extracted as above was approximately 21 kD (Fig. 2A, lane 2). Then we investigated whether
192 the bait had autonomously activated (autoactivated) the reporter genes in yeast stain Y2HGold
193 in the absence of a prey protein. The pGBKT7-Tg14-3-3 had no toxicity to yeast, when
194 compared with the colony size and shape of the pGBKT7 control group (Fig. 2B).

195 To identify putative host proteins that specifically interact with Tg14-3-3, we screened a
196 human cDNA library using the Y2HS. Over 50 colonies were grown on the plates, six of
197 which turned blue and the inserts were amplified by prey primers (T7 primer and 3′AD primer;
198 Fig. 3A, P1-P6). Finally, zygotes containing the prey vector (Fig. 3A, P3 or P4) and
199 pGBKT7-Tg14-3-3 appeared blue on the QDO/X-α-Gal/AbA (Fig. 3B, 1 or 2), but zygotes
200 harboring the prey vector (P3 or P4) and pGBKT7 did not (Fig. 3B, C1 or C2). These results
201 indicate that the two prey vectors (P3 and P4) specifically interacted with the

202 pGBKT7-Tg14-3-3. To determine the host gene identity, BLAST analysis showed that these
203 two inserts (Fig. 3A, P3 and P4) shared high similarity (99-100%) to the human gene
204 hCG1821272 (EAW77836.1) and to the *Homo sapiens* eukaryotic translation initiation factor
205 5B (HseIF5B; BC032639.1), respectively. Gene Ontology (GO) analysis showed that
206 hCG1821272 as a hypothetical, non-annotated protein, whereas HseIF5B is mainly involved
207 in translational initiation.

208 Eukaryotic initiation factor 5 subunit B (eIF5B) is the eukaryotic ortholog of the bacterial
209 IF2 initiation factor and is one of two essential GTPases required for ribosomal subunit
210 joining during translation initiation (Acker et al. 2006; Aitken and Lorsch et al. 2012; Kuhle
211 and Ficner 2014; Nag et al. 2016). Relevant to the present study is the ability of eIF5B to
212 enhance the proliferation and metastasis of hepatocellular carcinoma cells via upregulation of
213 ASAP1, a protein involved in regulation of membrane trafficking and cytoskeleton
214 remodeling (Wang et al. 2016). It is likely that the enhanced migratory ability of *T.*
215 *gondii*-infected immune cells is mediated through Tg14-3-3-eIF5B interaction. 14-3-3
216 proteins are known to act by binding to partner proteins, often leading to altered subcellular
217 localization of the binding partner proteins (Muslin and Xing 2000). Additional experiments
218 measuring Tg14-3-3 influence specifically on eIF5B localization may fully characterize the
219 outcome of this interaction in the host. Further investigation into the consequences on
220 hyper-migration of host infected cells should be pursued in order to determine whether the
221 identified interaction represents the most crucial host target by Tg14-3-3 or if other targets are
222 more pertinent to virulence.

223 The Y2HS has been successfully applied to study protein-protein interactions in *T. gondii*
224 (Ahn et al. 2006; Kim et al. 2008; Cheng et al. 2012; Wang et al. 2014) and has provided new
225 insight into the interaction between important parasite proteins and host signaling pathways.
226 However, due to the limitations of this method (i.e. the necessity for the bait-prey interaction

227 to occur in the cell nucleus for the reporter gene to be activated, the presence of negative
228 positives and the lack of the ability to screen components *in situ*), and the use of the truncated
229 peptide rather than the full-length of Tg14-3-3, it is likely that the spectrum of host interactors
230 with Tg14-3-3 has not been fully defined. It could be argued that fewer positives were
231 detected because Tg14-3-3 is localized within the parasitophorous vacuolar space (Assossou
232 et al. 2003; Assossou et al. 2004; Weidner et al. 2016). However, Tg14-3-3 protein has
233 already produced a positive interaction with two host proteins. It is also possible that
234 Tg14-3-3 may have a *GRA14*-like unique topology in the parasitophorous vacuole membrane
235 (PVM), with the C terminus facing the host cytoplasm and N terminus facing the
236 parasitophorous vacuolar lumen (Rome et al. 2008). Recently developed technologies, such as
237 proximity-dependent biotin identification (BioID), which uses a promiscuous bacterial biotin
238 ligase to detect protein-protein associations and proximate proteins in living cells, and to label
239 proteins in subcellular compartments in *T. gondii* (Chen et al. 2015; Nadipuram et al. 2016),
240 may circumvent some of the limitations associated with the Y2HS to investigate how *T.*
241 *gondii* effector proteins, localized to non-nuclear compartments, can regulate cellular
242 processes inside the parasitized host cells.
243 In conclusion, the present study identified two new host interacting proteins, eIF5B and
244 hCG1821272, that are involved in many important biological processes. These findings enrich
245 the previously discovered array of interactions that occur between *T. gondii* effectors and host
246 proteins. The identified interaction between Tg14-3-3 and the two host proteins merits further
247 investigations to fully characterize the regulatory mechanisms by which Tg14-3-3 regulates
248 its target host proteins. Such studies will unmask urgently needed novel therapeutic targets.
249

250 **Acknowledgments** This study was supported by the National Natural Science Foundation of
251 China (Grant No. 31230073), the Fundamental Research Funds of Chinese Academy of

252 Agricultural Sciences (Grant No. Y2016JC05) and the Agricultural Science and Technology
253 Innovation Program (ASTIP) (Grant No. CAAS-ASTIP-2016-LVRI-03).
254

255 **References**

256

257 Acker MG, Shin BS, Dever TE, Lorsch JR (2006) Interaction between eukaryotic initiation
258 factors 1A and 5B is required for efficient ribosomal subunit joining. J Biol Chem
259 281:8469-8475
260 Ahn HJ, Kim S, Kim HE, Nam HW (2006) Interactions between secreted GRA proteins and
261 host cell proteins across the paratitophorous vacuolar membrane in the parasitism of
262 *Toxoplasma gondii*. Korean J Parasitol 44:303-312
263 Aitken CE, Lorsch JR (2012) A mechanistic overview of translation initiation in eukaryotes.
264 Nat Struct Mol Biol 19:568-576
265 Assossou O, Besson F, Rouault JP, Persat F, Brisson C, Duret L, Ferrandiz J, Mayençon M,
266 Peyron F, Picot S (2003) Subcellular localization of 14-3-3 proteins in *Toxoplasma gondii*
267 tachyzoites and evidence for a lipid raft-associated form. FEMS Microbiol Lett
268 224:161-168
269 Assossou O, Besson F, Rouault JP, Persat F, Ferrandiz J, Mayençon M, Peyron F, Picot S
270 (2004) Characterization of an excreted/secreted antigen form of 14-3-3 protein in
271 *Toxoplasma gondii* tachyzoites. FEMS Microbiol Lett 234:19-25
272 Chen AL, Kim EW, Toh JY, Vashisht AA, Rashoff AQ, Van C, Huang AS, Moon AS, Bell
273 HN, Bentolila LA, Wohlschlegel JA, Bradley PJ (2015) Novel components of the
274 *Toxoplasma* inner membrane complex revealed by BioID. MBio 6:e02357-14

275 Cheng L, Chen Y, Chen L, Shen Y, Shen J, An R, Luo Q, Du, J (2012) Interactions between
276 the ROP18 kinase and host cell proteins that aid in the parasitism of *Toxoplasma gondii*.
277 Acta Trop 122:255-260
278 Cognetti D, Davis D, Sturtevant J (2002) The *Candida albicans* 14-3-3 gene, BMH1, is
279 essential for growth. Yeast 19:55-67
280 Elsheikha HM (2008) Congenital toxoplasmosis: priorities for further health promotion action.
281 Public Health 122:335-353
282 Freeman AK, Morrison DK (2011) 14-3-3 Proteins: diverse functions in cell proliferation and
283 cancer progression. *Semin.* Cell Dev Biol 22:681-687
284 Katlama C, De Wit S, O'Doherty E, Van Glabeke M, Clumeck N
285 (1996) Pyrimethamine-clindamycin versus pyrimethamine-sulfadiazine as acute and
286 long-term therapy for toxoplasmic encephalitis in patients with aids. Clin Infect
287 Dis 22:268-275
288 Kim JY, Ahn HJ, Ryu KJ, Nam HW (2008) Interaction between parasitophorous vacuolar
289 membrane-associated GRA3 and calcium modulating ligand of host cell endoplasmic
290 reticulum in the parasitism of *Toxoplasma gondii*. Korean J Parasitol 46:209-216
291 Kobayashi H, Ogura Y, Sawada M, Nakayama R, Takano K, Minato Y, Takemoto Y, Tashiro
292 E, Watanabe H, Imoto M (2011) Involvement of 14-3-3 proteins in the second epidermal
293 growth factor-induced wave of Rac1 activation in the process of cell migration. J Biol
294 Chem 286:39259-39268
295 Kuhle B, Ficner R (2014) eIF5B employs a novel domain release mechanism to catalyze
296 ribosomal subunit joining. EMBO J 33:1177-1191
297 Meng M, He S, Zhao G, Bai Y, Zhou H, Cong H, Lu G, Zhao Q, Zhu XQ (2012) Evaluation
298 of protective immune responses induced by DNA vaccines encoding *Toxoplasma gondii*
299 surface antigen 1 (SAG1) and 14-3-3 protein in BALB/c mice. Parasit Vectors 5:273

300 Montoya JG, Liesenfeld O (2004) Toxoplasmosis. Lancet 363:1965-1976

301 Muslin AJ, Xing H (2000) 14-3-3 proteins: regulation of subcellular localization by molecular
302 interference. Cell Signal 12:703-709

303 Nadipuram SM, Kim EW, Vashisht AA, Lin AH, Bell HN, Coppens I, Wohlschlegel JA,
304 Bradley PJ (2016) *In vivo* biotinylation of the *Toxoplasma* parasitophorous vacuole
305 reveals novel dense granule proteins important for parasite growth and pathogenesis.
306 MBio 7:e00808-16

307 Nag N, Lin KY, Edmonds KA, Yu J, Nadkarni D, Marintcheva B, Marintchev A (2016)
308 eIF1A/eIF5B interaction network and its functions in translation initiation complex
309 assembly and remodeling. Nucleic Acids Res 44:7441-7456

310 Nomura M, Shimizu S, Sugiyama T, Narita M, Ito T, Matsuda H, Tsujimoto Y (2003) 14-3-3
311 Interacts directly with and negatively regulates pro-apoptotic Bax. J Biol Chem
312 278:2058-2065

313 Rome ME, Beck JR, Turetzky JM, Webster P, Bradley PJ (2008) Intervacuolar transport and
314 unique topology of GRA14, a novel dense granule protein in *Toxoplasma gondii*. Infect
315 Immun 76:4865-4875

316 Saeij JP, Boyle JP, Coller S, Taylor S, Sibley LD, Brooke-Powell ET, Ajioka JW, Boothroyd
317 JC (2006) Polymorphic secreted kinases are key virulence factors in toxoplasmosis.
318 Science 314:1780–1783

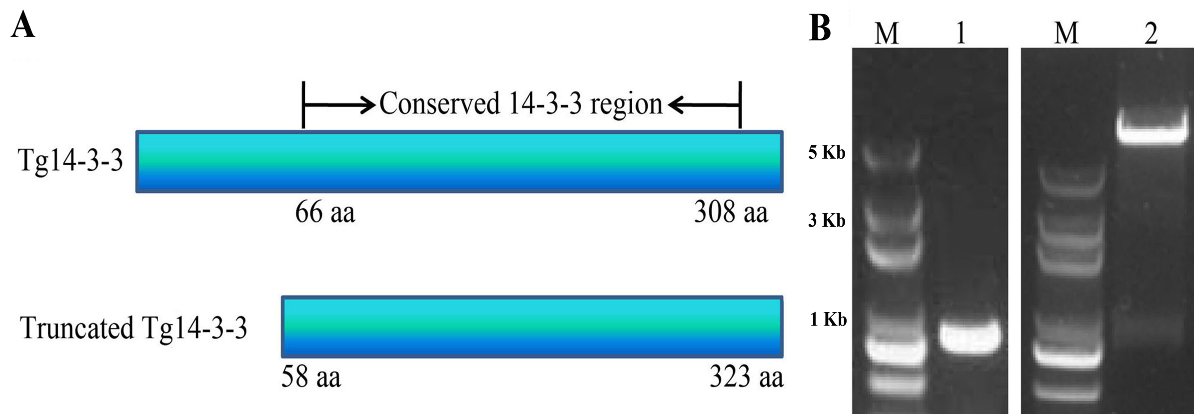
319 Saffer LD, Mercereau-Puijalon O, Dubremetz JF, Schwartzman JD (1992) Localization of a
320 *Toxoplasma gondii* rhoptry protein by immunoelectron microscopy during and after host
321 cell penetration. J Protozool 39:526-530

322 Sibley LD, Qiu W, Fentress S, Taylor SJ, Khan A, Hui R (2009) Forward Genetics in
323 *Toxoplasma gondii* Reveals a Family of Rhoptry Kinases That Mediates Pathogenesis.
324 Eukaryot Cell 8:1085-1093

325 Siles-Lucas Mdel M, Gottstein B (2003) The 14-3-3 protein: a key molecule in parasites as in
326 other organisms. Trends Parasitol 19:575-581
327 Sluchanko NN, Gusev NB (2010) 14-3-3 proteins and regulation of cytoskeleton.
328 Biochemistry (Mosc) 75:1528-1546
329 Taylor S, Barragan A, Su C, Fux B, Fentress SJ, Tang K, Beatty WL, Hajj HE, Jerome M,
330 Behnke MS, White M, Wootton JC, Sibley LD (2006) A Secreted Serine-Threonine
331 kinase determines virulence in the eukaryotic pathogen *Toxoplasma gondii*. Science
332 314:1776-1780
333 Wang Y, Fang R, Yuan Y, Hu M, Zhou Y, Zhao J (2014) Identification of host proteins
334 interacting with the integrin-like A domain of *Toxoplasma gondii* micronemal protein
335 MIC2 by yeast-two-hybrid screening. Parasit Vectors 7:543
336 Wang ZG, Zheng H, Gao W, Han J, Cao JZ, Yang Y, Li S, Gao R, Liu H, Pan ZY, Fu SY, Gu
337 FM, Xing H, Ni JS, Yan HL, Ren H, Zhou WP (2016) eIF5B increases ASAP1 expression
338 to promote HCC proliferation and invasion. Oncotarget 7:62327-62339
339 Weidner JM, Kanatani S, Uchtenhagen H, Varas-Godoy M, Schulte T, Engelberg K, Gubbels
340 MJ, Sun HS, Harrison RE, Achour A, Barragan A (2016) Migratory activation of
341 parasitized dendritic cells by the protozoan *Toxoplasma gondii* 14-3-3 protein. Cell
342 Microbiol 18:1537-1550

343
344

345
346
347
348
349
350
351
352



353

354

355 **Fig. 1.** Construction of the Tg14-3-3 bait plasmid. (A) Schematic illustration of the full-length

356 Tg14-3-3 and the truncated region of Tg14-3-3 used in the yeast two hybrid screening. (B)

357 Lane1: gel electrophoresis analysis of the region of Tg14-3-3 amplified from *T. gondii* cDNA;

358 Lane 2: the pGBKT7-Tg14-3-3 vector was confirmed by digestion with *Eco*RⅠ and

359 *Bam*HⅠ. Lane M: DL 5000 molecular marker.

360

361

362

363

364

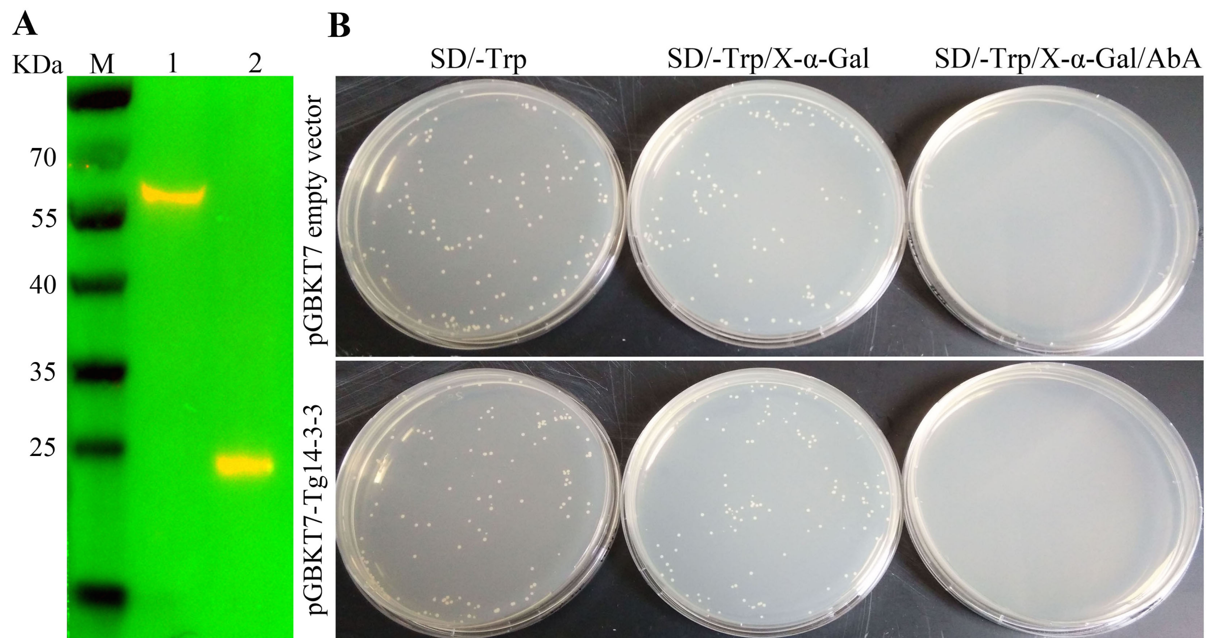
365

366

367

368

369



370

371

372 **Fig. 2.** Expression and autoactivation of Tg14-3-3 in Y2HGold cells. (A) Western blotting
373 detection of the bait vector expression in Y2HGold cells. Lane 1: pGBKT7-Tg14-3-3 vector;
374 Lane 2: pGBKT7 empty vector. (B) Determination of autoactivation and toxicity of the bait
375 vector in Y2HGold cells on different selection plates.

376

377

378

379

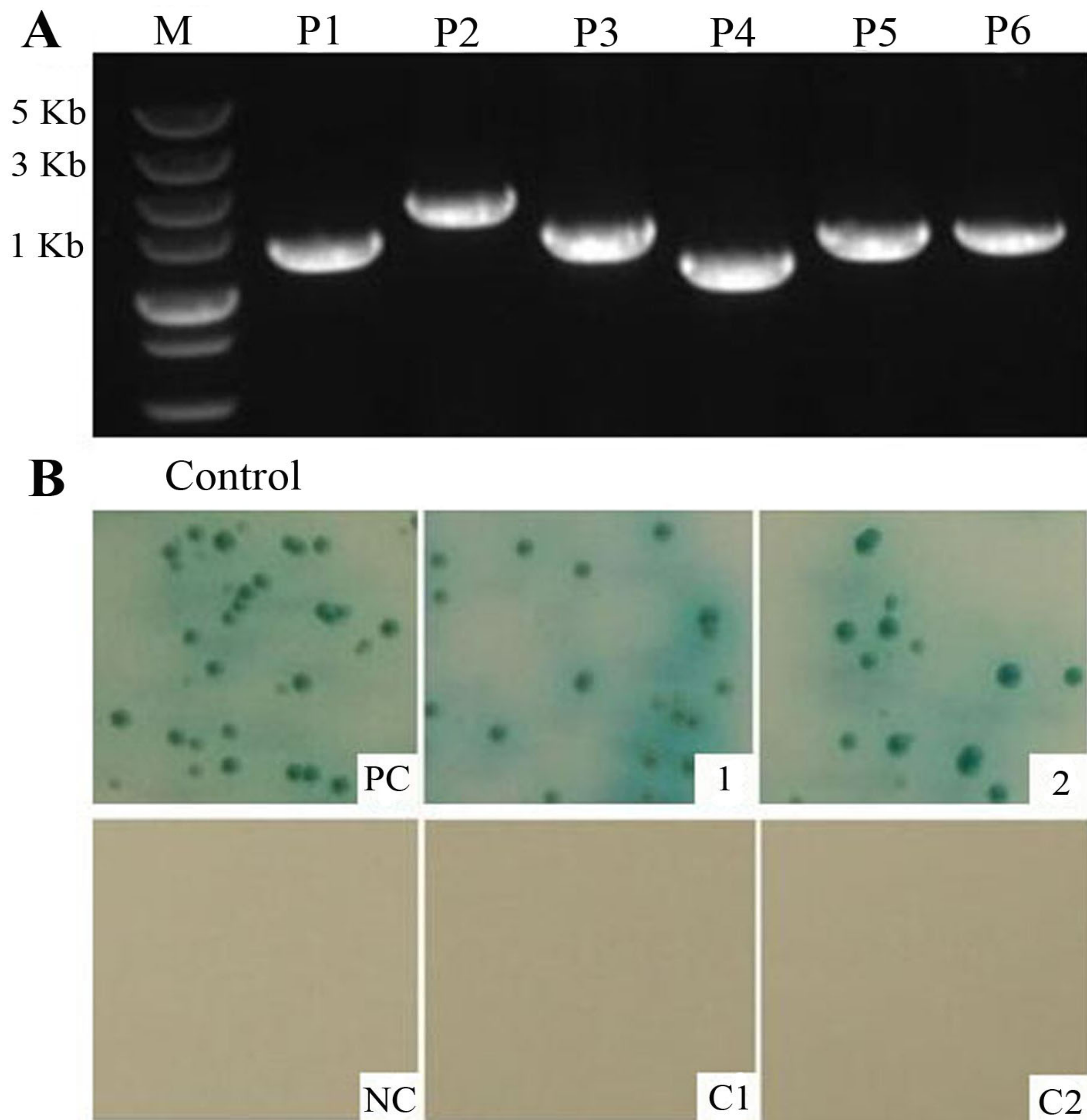
380

381

382

383

384



385

386 **Fig. 3.** Analysis of putative positives. (A) Detecting PCR products amplified from putative
387 positives. Lane P1-P6: PCR products amplified from the putative positives P1-P6 (responding
388 to prey vectors P1-P6); Lane M: DL 5000 marker. (B) Confirmation of putative positives.
389 Y2HGold cells transformed with bait vector were mated with Y187 containing each of prey
390 vectors (P1-P6), respectively. Zygotes were grown on QDO/X-α-Gal/AbA plates. Zygotes
391 containing pGBKT7-53 and pGADT7-T were used as positive controls (Fig. 3B, PC), and
392 zygotes containing pGBKT7 and pGADT7-T were used as negative controls (Fig. 3B, NC).
393 Zygotes containing pGBKT7-Tg14-3-3 and pGADT7-P3/P4 are shown in Fig. 3B, 1/2;
394 zygotes containing pGBKT7 and pGADT7-P3/P4 are shown in Fig. 3B, C1/C2.

395