Prediction of antiviral drugs against African Swine Fever Viruses based on protein-protein interaction analysis

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The African swine fever virus (ASFV) has severely influenced the swine industry of the world. Unfortunately, there is currently no effective antiviral drug or vaccine against the virus. Identification of new anti-ASFV drugs is urgently needed. Here, an up-to-date set of protein-protein interactions between ASFV and swine were curated by integration of protein-protein interactions from multiple sources. Thirty-eight swine proteins were observed to interact with ASFVs and were defined as ASFV-interacting swine proteins. The ASFV-interacting swine proteins were found to play a central role in the swine protein-protein interaction network, with significant larger degree, betweenness and smaller shortest path length than other swine proteins. Some of ASFV-interacting swine proteins also interacted with several other viruses and could be taken as potential targets of drugs for broad-spectrum effect, such as HSP90AB1. Finally, the antiviral drugs which targeted ASFV-interacting swine proteins and ASFV proteins were predicted. Several drugs with either broad-spectrum effect or high specificity on ASFV-interacting swine proteins were identified, such as Polaprezinc and Geldanamycin. Structural modeling and molecular dynamics simulation showed that Geldanamycin could bind with swine HSP90AB1 stably. This work could not only deepen our understanding towards the ASFV-swine interactions, but also help for the development of effective antiviral drugs against the ASFVs.

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2 protein interaction analysis

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Abstract

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17 Unfortunately, there is currently no effective antiviral drug or vaccine against the virus.

18 Identification of new anti-ASFV drugs is urgently needed. Here, an up-to-date set of protein-

19 protein interactions between ASFV and swine were curated by integration of protein-protein

20 interactions from multiple sources. Thirty-eight swine proteins were observed to interact with

21 ASFVs and were defined as ASFV-interacting swine proteins. The ASFV-interacting swine

22 proteins were found to play a central role in the swine protein-protein interaction network, with

23   significant larger degree, betweenness and smaller shortest path length than other swine proteins.

24   Some of ASFV-interacting swine proteins also interacted with several other viruses and could be

25   taken as potential targets of drugs for broad-spectrum effect, such as HSP90AB1. Finally, the

26   antiviral drugs which targeted ASFV-interacting swine proteins and ASFV proteins were

27   predicted. Several drugs with either broad-spectrum effect or high specificity on ASFV-

28   interacting swine proteins were identified, such as Polaprezinc and Geldanamycin. Structural

29   modeling and molecular dynamics simulation showed that Geldanamycin could bind with swine

30   HSP90AB1 stably. This work could not only deepen our understanding towards the ASFV-swine

31   interactions, but also help for the development of effective antiviral drugs against the ASFVs.

32   **1 Introduction**

33   African swine fever virus (ASFV), the causative agent of African swine fever (ASF), is an

34   enveloped and double-stranded DNA virus with genome size ranging from 170 kbp to 194 kbp

35   (Alonso et al. 2018). ASFV mainly infect suids and soft ticks (Sánchez-Cordón et al. 2018). In

36   swine populations, the virus can cause 100% mortality and severely influence the swine industry

37   (Revilla et al. 2018). The ASFV has caused ASF outbreaks in more than fifty countries in Africa,

38   Europe, Asia, and South America until now (Costard et al. 2013; Malogolovkin et al. 2012).

39   The latest reports showed that the virus has caused outbreaks in all provinces of mainland China

40   (Zhou et al. 2018). How to effectively control the virus is still a great challenge for the globe

41   (Wang et al. 2018).

42   Vaccine and antiviral drugs are believed to be the best tool for prevention and control of viral

43   infection and spread (Monto 2006). Unfortunately, all the attempts to develop effective vaccines

44   against ASFVs had failed (Sánchez et al. 2019). In the absence of vaccines against ASFVs, the

45   antiviral drugs could not only improve host survival, but also help control the epidemic area

46   (Zakaryan & Revilla 2016). Therefore, it is in great need to develop effective antiviral drugs

47   against ASFVs. Several studies have identified multiple compounds which could inhibit ASFV

48   infections. They could be classified into two groups (Arabyan et al. 2019). The first group of

49   antiviral drugs has identified targets and known mechanisms, and includes five kinds of drugs: i)

50   nucleoside analogues, such as iododeoxyuridine (Gil-Fernández et al. 1979), (S)-HPMPA (Gil-

51   Fernández et al. 1987), and Rigid amphipathic fusion inhibitors (Hakobyan et al. 2018); ii)

52   interferons (IFNs) and small peptides, such as IFN-alpha (Paez et al. 1990), IFN-gamma

53   (Esparza et al. 1988) and small peptide inhibitors which could disrupt the interaction between

54   cytoplasmic dynein and viral p54 protein (Hernáez et al. 2010); iii) plant-derived compounds,

55   such as genistein (Arabyan et al. 2018) and genkwanin (Hakobyan et al. 2019). The former

56   interferes with viral type II topoisomerase and the latter disrupts the virus movement along

57   microtubules. iv) antibiotics, such as Rifampicin (Dardiri et al. 1971) and fluoroquinolone

58   (Freitas et al. 2016; Mottola et al. 2013). They inhibit viral DNA-dependent RNA polymerase

59   and type II topoisomerases, respectively. v) small interfering RNA and CRISPR/Cas9. For

60   example, some siRNAs (Keita et al. 2010) which targeted viral genes such as A151R and B646L

61   could significantly reduce the virus titer and RNA transcripts. The other group of antiviral drugs

62   have unknown targets and unknown mechanism, such as Apigenin (Hakobyan et al. 2016),

63   resveratrol (Galindo et al. 2011) and oxyresveratrol (Galindo et al. 2011). However, all the

64   antiviral drugs mentioned above have not been taken forward for commercial production. More

65   candidate drugs are needed for further development.

66   Although most antiviral drugs target the viral proteins, in recent years several studies have

67   attempted to develop antiviral drugs which targeted the host proteins (Mak et al. 2019; Yang et al.

68   2019). Compared to the drugs which target viral proteins, the drugs targeting host proteins have

69   more targets in the host cell since the number of host proteins is much larger than that of viral

70   proteins. Besides, they may be more mutant-insensitive since the host proteins evolve much

71   slower than viral proteins (Luo et al. 2015; Rozenblatt-Rosen et al. 2012). With the rapid

72   development of high-throughput assays, a large amount of protein-protein interactions between

73   virus and host has been accumulated. Analysis of these protein-protein interactions in the

74   perspective of network can help identify host proteins of importance for viral infection, which

75   could be taken as potential targets for antiviral research (Uetz et al. 2006). For example, Han et

76   al. predicted several antiviral drugs against human enterovirus 71 by systematic identification

77   and analysis of protein-protein interactions between the virus and the host, suggesting the

78   important role of protein-protein interaction analysis on developing antiviral drugs targeting host

79   proteins (Han et al. 2017).

80   Several studies have investigated the protein-protein interactions between ASFV and swine.

81   Some important interactions were listed as follows: the viral DP71L protein interacts specifically

82   with and activates protein phosphatase 1 (PP1) (Rivera et al. 2007); the viral A224L interacts

83   with the proteolytic fragment of caspase-3 and inhibits the activity of this protease during ASFV

84   infection (Nogal et al. 2001); the viral p54 binds to the light chain of cytoplasmic dynein (LC8)

85   to hijack the microtubule motor complex during ASFV infection (Alonso et al. 2001); the viral

86   A238L binds to the catalytic subunit of calcineurin and inhibits NFAT-regulated gene

87   transcription in vivo (Miskin et al. 1998); the viral p30 may down-regulate the mRNA translation

88   of host cells by interacting with hnRNP-K (Hernaez et al. 2008); the viral Ep152R interacts with

89   BAG6 to block the immune response during viral infection (Borca et al. 2016); the viral A179L

90   protein was able to interact with the main core Bcl-2 proapoptotic proteins Bax and Bak, and

91   may play an important role during productive ASFV infection (Brun et al. 1996; Galindo et al.

92   2008). This study firstly curated a set of protein-protein interactions between ASFV and swine

93   proteins by integration of protein-protein interactions from public databases and literatures; then,

94   the swine proteins related to ASFV infection were identified; their roles in swine protein-protein

95   interaction network and in interacting with other viruses, and their functions were further

96   investigated; finally, the candidate antiviral drugs targeting these swine proteins and ASFV

97   proteins were predicted. This work could not only deepen our understanding towards the ASFV-

98   swine interactions, but also help for the development of effective antiviral drugs against the

99   ASFVs.

100

2 Materials & Methods

2.1 Protein-protein interactions between ASFV and swine proteins

The protein-protein interactions between ASFV and swine proteins were compiled from three sources (Table S1). First of all, 24 protein-protein interactions with median confidence (scores greater than 0.4) between ASFV and swine, were obtained from the database of Viruses.STRING (Cook et al. 2018) on January 8, 2019.

Secondly, 20 protein-protein interactions between ASFV and swine were obtained from the literature. This was achieved by firstly searching the PubMed database by the key word "ASFV" in the title or abstract on December 29, 2018, which resulted in 630 abstracts. Then, each abstract was manually screened based on whether it contained protein-protein interactions between ASFV and swine, and 117 abstracts were retained. Finally, the full texts of the manuscripts corresponding to these abstracts were read carefully and 20 extra protein-protein interactions between ASFV and swine were compiled from these papers.

Thirdly, 3 protein-protein interactions between ASFV and swine proteins were inferred based on sequence homology. This was conducted by firstly collecting viral proteins (except the ASFV) which interacted with swine proteins based on the database of Viruses.STRING. Then, 159 ASFV proteins encoded by BA71V, which were downloaded from NCBI RefSeq database (Pruitt et al. 2005), were blast (Altschul et al. 1990) against these viral proteins. The hits with e-value smaller than 0.001, coverage greater than 40%, and sequence identity greater than 30%, were considered as homologs of ASFV proteins. The swine proteins which interacted with the hits were predicted to interact with the ASFV proteins. The obtained 3 protein-protein interactions overlapped with those from Viruses.STRING (Table S1).

2.2 Swine protein-protein interaction network

All the swine protein-protein interactions were downloaded from STRING database (Szklarczyk et al. 2016) on January 8, 2019. Only the protein-protein interactions with a median confidence

126 (score greater than 0.4) were kept. Besides, the redundant protein-protein interactions were

127 removed. Finally, a protein-protein interaction network which consisted of 731,174 non-

128 redundant protein-protein interactions between 18,683 swine proteins was obtained for further

129 analysis.

2.3 Network analysis and visualization

131 The igraph package (version 1.2.2) (Csardi & Nepusz 2006) in R was used to analyze the

132 topology of the protein-protein interaction network. The degree and betweenness of proteins in

133 the protein-protein interaction network were calculated with the functions of *degree()* and

134 *betweenness()*, respectively. The shortest path length between two proteins in the protein-protein

135 interaction network was calculated with the function of *shortest.paths()*.

136 The network was visualized with the help of Cytoscape (version 3.7.1) (Su et al. 2014).

2.4 Functional enrichment analysis

138 The Gene Ontology (GO) terms and KEGG pathway enrichment analysis for the ASFV-

139 interacting swine proteins or the ASFV infection-associated swine proteins were conducted with

140 functions of *enrichGO()* and *enrichKEGG()* in the package "clusterProfiler" (version 3.6.0) (Yu

141 et al. 2012) in R (version 3.4.2). All the GO terms and KEGG pathways with adjusted p-values

142 smaller than 0.01 were considered as significant enrichment (Table S2).

2.5 Prediction of candidate drugs targeting ASFV and swine proteins

144 Candidate drugs were predicted with the help of DrugBank (version 5.1.2) (Wishart et al. 2018).

145 The protein sequence of each ASFV protein encoded by BA71V, and that of each ASFV-

146 Interacting swine Protein was queried against DrugBank for similar targets with the default

147 parameters. The drugs targeting the best hit were considered to be candidate drugs for the query

148 protein. The properties of drugs, such as the type and group of drug, and ATC code, were also

149 obtained from DrugBank (Table S3).

2.6 Modeling the interaction between Geldanamycin and swine HSP90AB1

151   The 3D structure of swine HSP90AB1 was predicted using homology modeling method (Eswar

152   et al. 2006). The modelling template was the crystal structure of the Geldanamycin-binding

153   domain of a heat shock protein 90-alpha (HSP90AA1) from Homo sapiens (PDB code: 1YET).

154   Sequence alignment showed that the identity between swine HSP90AB1 and human HSP90AA1

155   was 92.3% in the Geldanamycin-binding domain (208 residues). Besides, only amino acid

156   substitutions but no gaps were observed in the alignment. The highly similar and gap-free

157   alignment indicated that the predicted structure is reliable. In addition, 1YET is the complex

158   structure of Geldanamycin and HSP90AA1, which allowed us to transfer the binding

159   conformation of Geldanamycin from 1YET to the predicted structure of swine HSP90AB1. To

160   validate the binding conformation between Geldanamycin and swine HSP90AB1, molecular

161   dynamics (MD) simulation was performed for 10ns using GROMACS (Abraham et al. 2015).

162   The RMSDs (root mean square deviation) and binding energies of the complex between

163   Geldanamycin and swine HSP90AB1 were calculated.

164

165   3 Results

166   3.1 Interactions between ASFV and swine proteins

167   We firstly attempted to collect the interactions between ASFV and swine proteins as more as

168   possible. In total, we obtained 44 protein-protein interactions between them (Figure 1A),

169   including 24 protein-protein interactions from the database of Viruses.STRING, 20 protein-

170   protein interactions from the literature and 3 protein-protein interactions inferred from protein-

171   protein interactions between other viruses and swine based on sequence homology (details in

172   MATERIALS AND METHODS). 16 ASFV proteins were involved in the protein-protein

173   interactions. Half of ASFV proteins interacted with only one swine protein. For the remaining

174   half of ASFV proteins, the DNA-directed DNA polymerase (DPOL) interacted with 13 swine

175   proteins, while the A179L and A238L both interacted with four swine proteins. Thirty-eight

176   swine proteins were involved in the protein-protein interactions between ASFV and swine,

177   which were defined as ASFV-interacting swine proteins. All of them only interacted with one

178   ASFV protein except the proteins of DNAJA3, FBXO2 and SNAPIN.

179   **3.2 Construction of the ASFV-swine protein interaction network and topological analysis**

180   To investigate the role of ASFV-interacting swine proteins in the swine, a swine protein-protein

181   interaction network was constructed from the STRING database, which contained 731,174 non-

182   redundant protein-protein interactions between 18,683 swine proteins. 35 (94%) ASFV-

183   interacting swine proteins were found to interact with 4184 other swine proteins. The latter 4184

184   swine proteins were defined as ASFV infection-associated swine proteins. The ASFV-interacting

185   swine proteins together with ASFV infection-associated swine proteins formed a protein

186   interaction network with 9305 non-redundant interactions (Figure 2A), including 68 interactions

187   between ASFV-interacting swine proteins.

188   To investigate the centrality of ASFV-interacting and ASFV infection-associated swine proteins

189   in the swine protein-protein interaction network, we calculated the degree and betweenness

190   centrality, and the average shortest path length of each protein in the swine protein-protein

191   interaction network (Figure 2B-D). The node (protein) degree was defined as the number of

192   connections the node has to other nodes in the network; the node betweenness was defined as the

193   number of shortest paths that pass through the node; the average shortest path length of a node

194   was defined as the average length of all shortest paths from the node to other nodes. All of them

195   measure the importance of a node in the network. The median degree and betweenness centrality,

196   and the median shortest path length of all proteins in the swine protein-protein interaction

197   network were 32, $10^{3.5}$ and 59.2 , respectively, whereas these values for the ASFV-interacting

198   swine proteins were 144, $10^{4.6}$ and 58.7, respectively, and they were 120, $10^{4.2}$ and 58.8,

199   respectively, for the ASFV infection-associated swine proteins (Figure 2B). The ASFV-

200   interacting swine proteins and ASFV infection-associated swine proteins were observed to have

201   significant larger degrees and betweenness, and smaller shortest path length than all swine

202   proteins, with p-values much smaller than 0.001 in the two-sided Wilcoxon rank-sum test. This

203   suggested that the ASFV-interacting swine proteins and ASFV infection-associated swine

204   proteins played a central role in the swine protein-protein interaction network.

205   **3.3 Functional analysis of ASFV-interacting swine proteins and ASFV infection-associated**

206   **swine proteins**

207   Since the ASFV-interacting swine proteins and ASFV infection-associated swine proteins were

208   observed to play a central role in swine protein-protein interaction network, we next investigated

209   their functions. Functional enrichment analysis was conducted on the ASFV-interacting swine

210   proteins and ASFV infection-associated swine proteins (Table S2). Only a few GO terms in the

211   domain of Molecular Function were enriched. Interestingly, nearly 50 KEGG pathways were

212   enriched in the ASFV-interacting swine proteins (Table S2). The pathways of Necroptosis and

213   Alcoholism were two of the most enriched pathways, both of which included more than 20% of

214   all ASFV-interacting swine proteins. Besides, three pathways related to virus infection, such as

215   "Herpes simplex virus 1 infection", were also enriched.

216   We further conducted the functional enrichment analysis on the ASFV infection-associated

217   swine proteins. Figure 3 (A-D) showed the top ten GO terms in three domains of GO and KEGG

218   pathways enriched in the ASFV infection-associated swine proteins. In the domain of Biological

219   Process, six of top ten enriched GO terms were related to cell death or apoptotic process; in the

220   domain of Cellular Component, the ASFV infection-associated swine proteins were enriched in

221   the nuclear and cytoskeleton; in the domain of Molecular Function, the ASFV infection-

222   associated swine proteins were enriched in the GO terms of binding and enzyme activity. For the

223   KEGG pathways, "Herpes simplex virus 1 infection" was most enriched. Besides, several

224   signaling pathways were also enriched, such as "PI3K−Akt signaling pathway" and "MAPK

225   signaling pathway".

226   **3.4 The role of ASFV-interacting swine proteins in the protein-protein interactions between**

227   **swine and other viruses**

228   We then investigated the role of ASFV-interacting swine proteins in the protein-protein

229   interactions between swine and other viruses. All the protein-protein interactions between

230   ASFV-interacting swine proteins and other viral proteins which were public available in the

231   Viruses.STRING database were obtained. As was shown in Figure 4A, 48 protein-protein

232   interactions were obtained and shaped a network, which included 15 proteins from 11 other

233   viruses (nodes in square) and 16 ASFV-interacting swine proteins (nodes in ellipse). 13 ASFV-

234   interacting swine proteins were observed to interact with more than one other virus. Besides,

235   they also interacted with several hundreds of proteins in the swine protein-protein interaction

236   network (Figure 4B). For example, the heat shock protein 90s, including HSP90AB1,

237   HSP90AA1 and HSP90B1, could interact with proteins from other five viruses, and interact with

238   more than 1,500 swine proteins, suggesting their central roles in both the virus-swine protein-

239   protein interaction network and swine protein-protein interaction network.

240   **3.5 Drug prediction for treating ASFVs**

241   The wide involvement of ASFV-interacting swine proteins in protein-protein interactions

242   between swine and multiple viruses, and the central role of ASFV-interacting swine proteins in

243   swine protein-protein interaction network, suggested the possibility of their use as broad-

244   spectrum host-dependent antiviral drug targets. Therefore, we attempted to predict drugs

245   targeting the ASFV-interacting swine proteins with the help of DrugBank (Table S3). As was

246   shown in Figure 5, a total of 142 drugs (in ellipse or square) were predicted to target 21 ASFV-

247   interacting swine proteins (pink circles). Most of the drugs were small molecules (colored

248   ellipses); the other drugs were protein or peptide (colored squares). Some ASFV-interacting

249   swine proteins were targeted by multiple drugs, such as the heat shock protein 90 alpha family

250   class A member 1 (HSP90AA1) and tumor necrosis factor (TNF). HSP90AA1 was targeted by

251   more than 30 drugs, most of which were small molecules and were in experimental; while TNF

252   were also targeted by more than 30 drugs, most of which were approved or investigational.

253   We also predicted drugs targeting the ASFV proteins. Twenty-nine small molecules were

254   predicted to target ten ASFV proteins. Among them, both the proteins of F778R and A240L

255 were targeted by eight drugs. However, these ten ASFV proteins were not involved in the

256 protein-protein interactions between ASFV and swine. Interestingly, the Gallium nitrate

257 (DrugBank ID: DB05260), a drug used for treating hyper-calcemia, and Rifabutin (DrugBank ID:

258 DB00615), a antibiotic with potent antimycobacterial properties, were observed to both target the

259 ASFV-interacting swine proteins and ASFV proteins. Both of them (highlighted in red-edge)

260 were approved for use, suggesting their potential use for treating the ASFVs.

261 Some drugs were observed to have strong specificity on the ASFV protein or ASFV-interacting

262 swine proteins, such as the Hydroxyurea (DB01005), Infliximab (DB00065), Adalimumab

263 (DB00051), and so on. Hydroxyurea specifically targeted F778R. It is an antineoplastic agent

264 that inhibits DNA synthesis through the inhibition of ribonucleoside diphosphate reductase. It

265 may be used to inhibit DNA synthesis of the ASFV virus, thus blocking the proliferation of the

266 virus. Infliximab specifically targeted TNF and is primarily related to inflammation control and

267 neurological indications. It may be used to block the necrosis during the ASFV infection.

268 Some drugs were observed to target multiple ASFV-interacting swine proteins, such as

269 Geldanamycin (DB02424), Polaprezinc (DB09221) and Andrographolide (DB05767). For

270 example, Geldanamycin could target the HSP90AA1, HSP90AB1 and HSP90B1, all of which

271 played a central role in the swine protein-protein interaction network and swine-virus protein-

272 protein interaction network (Figure 4B). This suggested that Geldanamycin may have a broad-

273 spectrum effect in disrupting the swine-ASFV protein-protein interaction network, and may

274 inhibit the viral infections effectively. Therefore, we further investigated the mechanism of

275 Geldanamycin by modeling the interactions between Geldanamycin and swine HSP90AB1

276 (Figure 6). The 3D structure of the Geldanamycin-binding domain of swine HSP90AB1 was

277 modeled using the template of human HSP90AA1 (PDB code: 1YET). The binding

278 conformation of Geldanamycin was also predicted by referring the same compound in the

279 template structure (Figure 6A) (see Methods). As the sequence identity between the template and

280 swine HSP90AB1 was as high as 92.8%, particularly in the ligand-binding regions, their binding

281 modes with Geldanamycin in two structures should be highly similar. Molecular dynamics was

282   performed for the complex structure of Geldanamycin and swine HSP90AB1 to investigate their

283   interactions. The result showed that the RMSD (root mean square deviation) of Geldanamycin

284   and swine HSP90AB1 were less than 1.6 Å in the 10 ns simulation (Figure 6B), which indicated

285   the binding conformation was highly stable. The key contributions of binding energy were from

286   ASN51, ASP54, LYS58, ASP93, MET98, ASP102, ASN106, PHE138 and THR184, which was

287   almost identical to the template (Figure S1). In addition, the binding free energy for swine

288   HSP90AB1 and Geldanamycin complex structures was -76.8kcal/mol which was even stronger

289   than -69.1kcal/mol of the template (Figure S2). Hence, Geldanamycin could bind with swine

290   HSP90AB1, similar to that in Homo sapiens.

291

Discussion

293   Vaccines and antiviral-drugs are considered as the most effective tools for fighting against

294   viruses. Unfortunately, nearly all attempts to develop vaccines against ASFVs have failed to

295   induce effective protection (Wang et al. 2018). Therefore, it is necessary to develop antiviral

296   drugs against the virus. Previous studies have found several antiviral drugs which could possibly

297   inhibit ASFV infection in vitro (Arabyan et al. 2019), including nucleoside analogues (Berry &

298   Kinsella 2001), genistein (Arabyan et al. 2018), genkwanin (Hakobyan et al. 2019), Rifampicin

299   (Dardiri et al. 1971), fluoroquinolone (Mottola et al. 2013), sulfated polysaccharides (García-

300   Villalón & Gil-Fernández 1991), lauryl gallate (Hurtado et al. 2008), stilbenes resveratrol

301   (Galindo et al. 2011), oxyresveratrol (Galindo et al. 2011), histone deacetylases enzymes

302   (HDACs) inhibitor (Frouco et al. 2017), small peptide inhibitors (Hernáez et al. 2010), and so on.

303   This study computationally predicted several candidate drugs targeting the ASFV proteins and

304   ASFV-interacting swine proteins, which may be helpful for the development of more effective

305   drugs against the ASFV.

306   In the era of systems biology, a large amount of protein-protein interactions have been

307 accumulated, including the virus-host protein-protein interactions. This study compiled an up-to-

308 date protein-protein interaction network between ASFV and swine. Analysis of the network

309 could help identify possible associations between viral activities and host defense strategies,

310 which may facilitate development of potential therapies by disrupting host-virus interactions

311 (Tan et al. 2007). Several ASFV-interacting swine proteins and lots of ASFV infection-

312 associated swine proteins were identified based on the protein-protein interaction network. They

313 were observed to interact with more proteins or have larger influences on the information flow

314 throughout the swine protein-protein interaction network than other proteins, suggesting their

315 central roles in the swine protein-protein interaction network. Some ASFV-interacting swine

316 proteins were observed to interact with multiple viruses. They could be used as antiviral drug

317 targets. Besides, the predicted drugs targeting these ASFV-interacting swine proteins, such as

318 Polaprezinc and Geldanamycin, may have a broad-spectrum effect against viral infections.

319 Both the ASFV-interacting swine proteins and ASFV infection-associated swine proteins were

320 enriched in the functions of cell death, apoptosis or necroptosis. This suggested that these

321 processes may play an important role in viral infections. Previous studies have shown that ASFV

322 infection induced TNF-alpha production which further induced apoptosis in the infected cell (Del

323 Moral et al. 1999). Actually, ASFV induced apoptosis of infected cells both *in vitro* and *in vivo*

324 (Ramiro-Ibáñez et al. 1996). ASFV has several strategies to regulate apoptosis. It encodes

325 several anti-apoptotic proteins such as A179L and A224L to delay the execution step of the

326 apoptotic pathway. The virus also has the capacity of regulating the unfolded protein response to

327 prevent early apoptosis and ensure viral replication (Galindo et al. 2012). In the late stage of

328 ASFV infection, induction of apoptosis could favour virus spread without the activation of

329 inflammatory responses (Carrascosa et al. 2002). Therefore, the drugs which could inhibit cell

330 death, apoptosis or necroptosis, may be candidates for treatment of ASFVs. For example, the

331 infliximab, which is a TNF blocker and primarily related to inflammation control (Keane et al.

332 2001), may be used to block the necrosis during the ASFV infection.

333   Lots of drugs were predicted to target the ASFV-interacting swine proteins or ASFV proteins.

334   Several strategies could be used to select the candidate drugs. For specificity, the drugs with high

335   specificity on the ASFV-interacting swine proteins or ASFV proteins could be selected, such as

336   the Hydroxyurea and Infliximab; for broad-spectrum effect, the drugs which targeted the ASFV-

337   interacting swine proteins with high degrees in the swine protein-protein interaction network,

338   such as Polaprezinc, or those which targeted multiple ASFV-interacting swine proteins, such as

339   Geldanamycin, could have a large influence on the protein-protein interactions between swine

340   and ASFV. Two drugs, i.e., Gallium nitrate and Rifabutin, were observed to target both the

341   ASFV-interacting swine proteins and ASFV proteins. They could also be used for broad-

342   spectrum inhibitory effect against ASFV infections.

343   Most antiviral drugs target the viral proteins. Drug resistance frequently appears due to rapid

344   mutation of viruses. On the contrary, the drugs targeting the host protein may have the advantage

345   of stable effect since the host proteins generally evolve far slower than viral proteins (He et al.

346   2007; Tavassoli 2011). Besides, some host proteins may interact with multiple viruses, such as

347   HSP90AB1 mentioned above. The drugs targeting them may have broad-spectrum antiviral

348   effect. Bioinformatics analysis of the accumulated protein-protein interactions between virus and

349   host cell can facilitate the identification host proteins which are vital for viral infection. As the

350   accumulation of protein-protein interactions and the rapid development of bioinformatics

351   methods, several antiviral molecules with reduced side effects have been proposed and validated

352   (Sessions et al. 2009). Previous studies have developed antiviral drugs against the host proteins

353   such as the ubiquitin-proteasome system (Barrado-Gil et al. 2017) and HDACs (Frouco et al.

354   2017) to inhibit the replication of ASFVs. This study investigated the prediction of antiviral

355   drugs against ASFV infections. The ASFV-interacting swine proteins could be taken as potential

356   antiviral-drug targets. The candidate drugs identified here may facilitate further development of

357   effective drugs against the virus.

358   There were two limitations to this study. Firstly, the protein-protein interactions between swine

359   and ASFV are far from complete. The ASFV encodes more than 150 proteins (Rodríguez &

360 Salas 2013). Only 16 of them were involved in the protein-protein interactions analyzed here.

361 Much more efforts are needed to generate a comprehensive protein-protein interaction network

362 between swine and ASFV. Fortunately, based on the limited protein-protein interactions between

363 swine and ASFV, several antiviral drugs were predicted and had the potential for further

364 development. Secondly, the drugs predicted here need further experimental validations. Several

365 drugs with high specificity on ASFV-interacting swine proteins, or with broad-spectrum effect,

366 such as Polaprezinc and Geldanamycin, could be prioritized for validation.

367 Conclusions

368 In conclusion, this study curated an up-to-date set of protein-protein interactions between swine

369 and ASFV as far as we know, and identified the ASFV-interacting swine proteins which were

370 vital for viral infection. The ASFV-interacting swine proteins were observed to play a central

371 role in swine protein-protein interaction network, and also took part in interactions between

372 swine and several other viruses. They could be taken as potential antiviral-drug targets. Several

373 drugs were predicted to target the ASFV-interacting swine proteins and ASFV proteins. They

374 could be helpful for further development of effective drugs against the virus.

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381

382 CONFLICT OF INTEREST

383 The authors have declared that no competing interests exist.

384

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523

524

Figure 1

Overview of protein-protein interactions between the ASFV and swine.

(A) Collected protein-protein interactions between ASFV and swine proteins. AIP, ASFV-interacting swine proteins. (B) All the ASFV proteins involved in protein-protein interactions and the number of interacted swine proteins. PPI, protein-protein interaction.
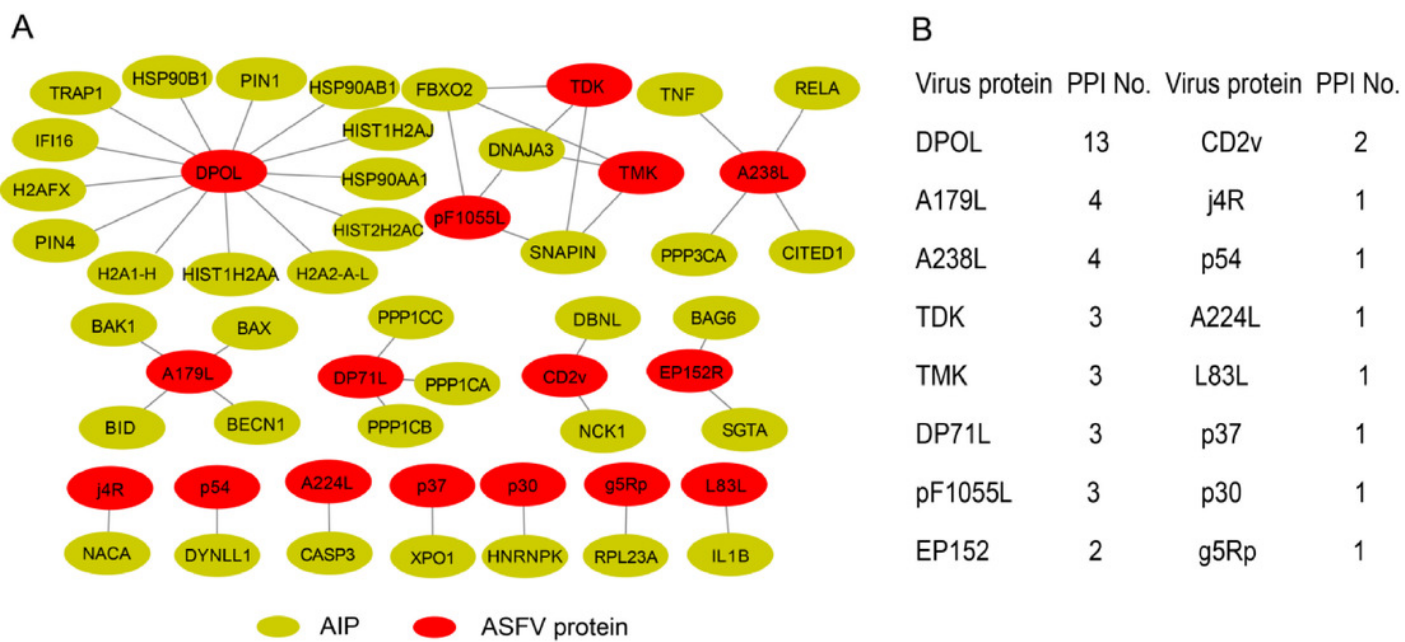
Figure 2

The protein-protein interaction network between ASFV-interacting swine proteins and ASFV infection-associated proteins, and the topological analysis of these proteins.

(A) The protein-protein interaction network between ASFV-interacting swine proteins (AIPs) and ASFV infection-associated proteins (AAPs). (B-D) Distribution of the betweenness centrality, degree and shortest path length for all proteins (ALL), ASFV-interacting swine proteins (AIPs) and ASFV infection-associated swine proteins (AAPs) in the swine protein-protein interaction network.
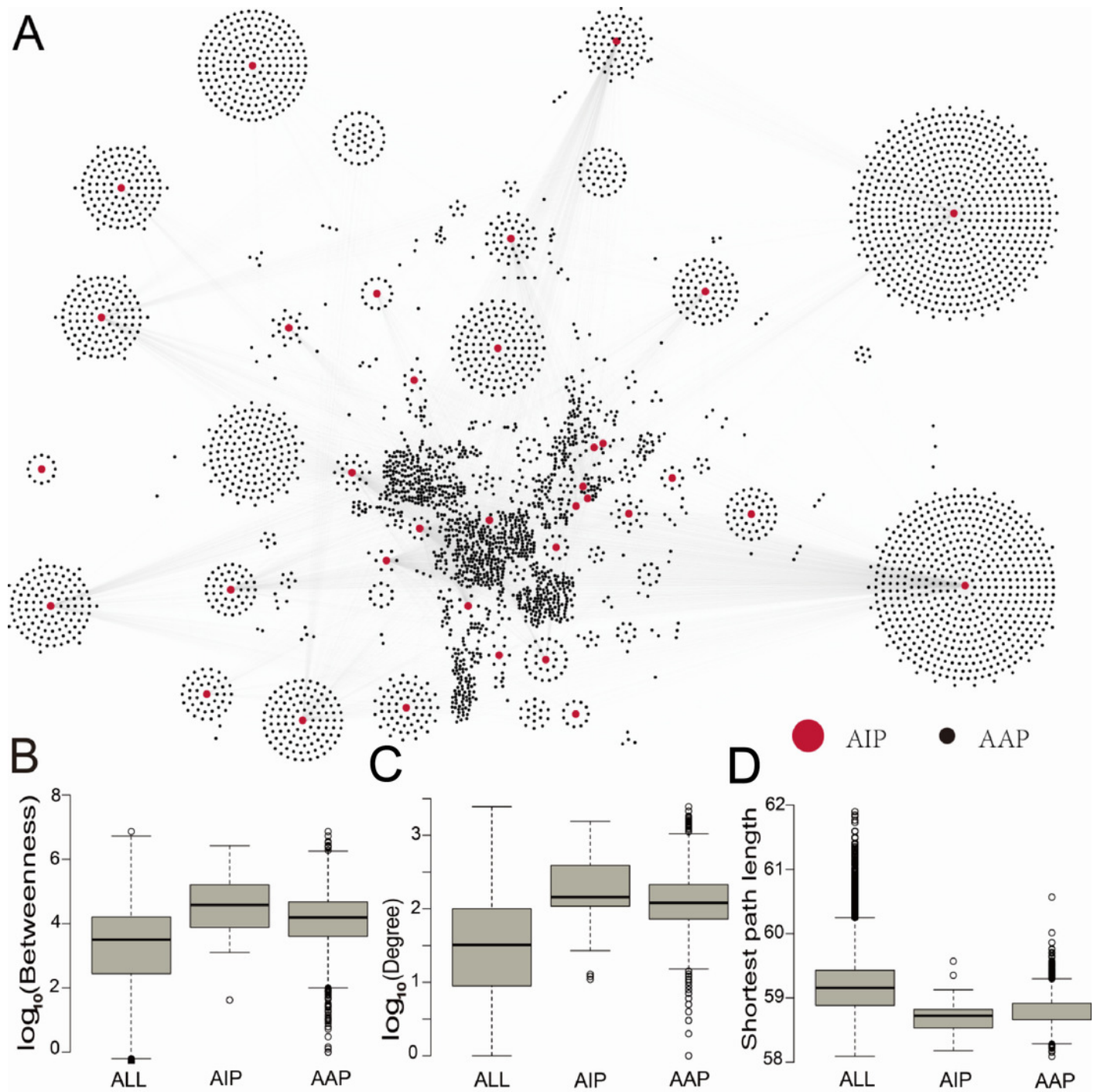
Figure 3

Functional enrichment analysis of AAPs.

(A-D) Top ten enriched terms in the domain of Biological Process, Cellular Component and Molecular Function, and KEGG pathways were shown.
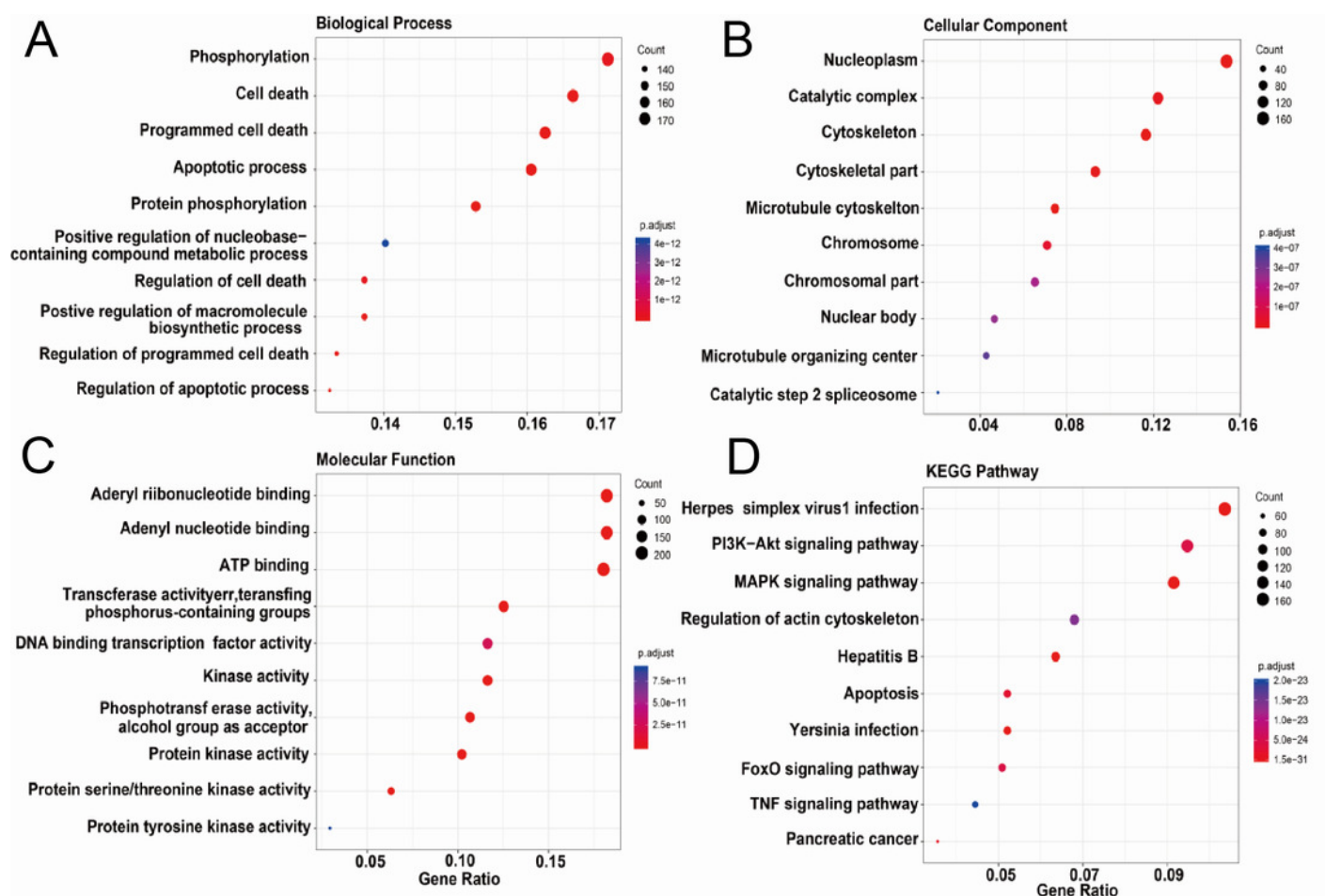
Figure 4

ASFV-interacting swine proteins and their interactions with other viruses.

(A) The protein-protein interaction network between ASFV-interacting swine proteins (AIPs) and other viruses. ASFV-interacting swine proteins were represented as ellipse in gray. Viruses were represented as squares and colored according to the legend in the bottom right. VESE, Vesicular exanthema of swine virus; Swinepox, Swinepox virus; TTSV1a, Torque teno sus virus 1a; TeschoA, Teschovirus A; Nodamura, Nodamura virus; FMDV, Foot-and-mouth disease virus; TTSVk2, Torque teno sus virus k2; FLUCV, Influenza C virus; EMCV, Encephalomyocarditis virus; (B) The number of interacted virus and the degree of ASFV-interacting swine proteins in the swine protein-protein interaction network. HISTH2AC, histone H2A type 2-C; H2A1-H, histone H2A type 1-H; HIST1H2AA, histone H2A type 1-A; HIST1H2AJ, histone H2A type 1-like; H2A2-A-L, histone H2A type 2-A-like; H2AFX, histone H2AX.
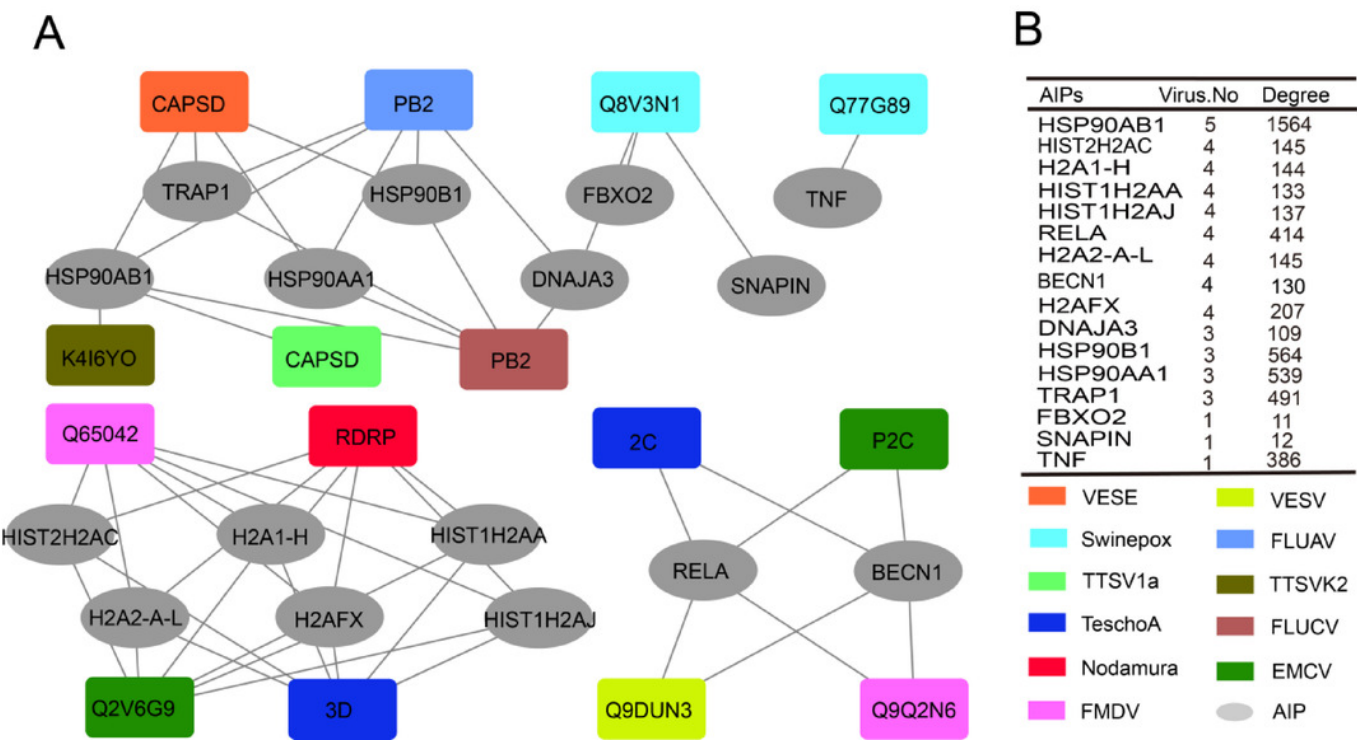
Figure 5

Predicted drugs targeting the ASFV-interacting swine proteins (AIPs) and ASFV proteins.

The interactions above and below the dotted line referred to those between drugs and ASFV-interacting swine proteins, and those between drugs and ASFV proteins, respectively. The ASFV-interacting swine proteins and ASFV proteins were represented as pink and cyan circles, respectively. Drugs of protein or peptide, and those of small molecule, were represented as squares and ellipses, respectively. Drugs in the stage of approved, investigational and experimental were colored in orange, purple and light green, respectively. Drugs which specifically targeted one ASFV-Interacting swine Protein were highlighted in black-edge. Two drugs which targeted both the ASFV-Interacting swine Protein and ASFV protein were highlighted in red-edge.
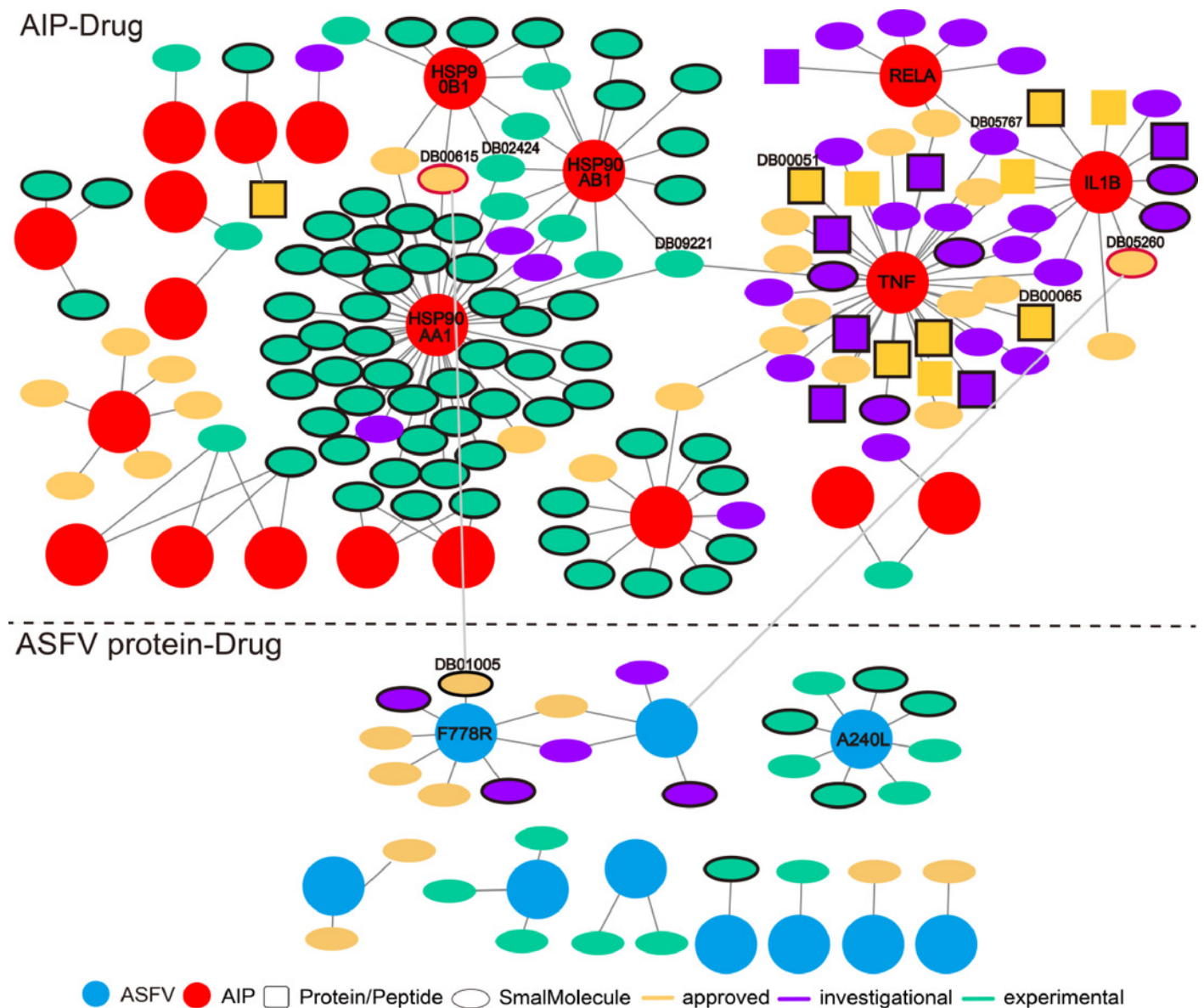
Figure 6

Modelling the interactions between Geldanamycin and swine HSP90AB1.

(A) The predicted 3D structure of the Geldanamycin-binding domain of swine HSP90AB1 and its interaction with Geldanamycin. (B) RMSD of all Cα atoms for the ligand (black) and receptor-ligand complex (red) during MD simulations (10 ns).