

1 **Immunogenicity and protective potency of Norovirus GII.17**

2 **virus-like particle-based vaccine**

3 Wei Chen¹, Tao Kang¹, Rongliang Yuan², Yuyang Zhang¹, Siqi Xin¹, Congwen

4 Shao^{2*}, Shenrong Jing^{1*}

5 1 Medical School, Kunming University of Science and Technology, Kunming, China;

6 2 Taizhou SCIVAC biotechnology Corporation Limited, Taizhou, China;

7 *Correspondence authors: Congwen Shao and Shenrong Jing, E-mail:

8 jingshenrong@163.com. Address: Medical School, Kunming University of Science

9 and Technology, No. 727, Southern Jingming Road, Chenggong District, Kunming,

10 Yunnan, China 650500. Telephone number/Fax: +86 871 65920776.

11 Wei Chen and Tao Kang are equally contribution to the paper.

12

13 **Abstract** Noroviruses (NoVs) are a major cause of acute viral gastroenteritis in
14 adults and children worldwide. Lacking of cell culture system and animals models
15 that must be considered the virus like particles (VLPs) used as an effective vaccine
16 development. In the present study, we investigated the expression of the major capsid
17 protein (VP1) of Genogroup II, genotype 17 (GII.17) NoV using recombinant
18 baculovirus system in insect cells and saliva binding blockade assay to detect their
19 protective potency. Our results showed that GII.17 VLPs could be successfully
20 generated in sf9 insect cells and electron microscopic revealed that GII.17 VLPs was
21 visualized as spherical particles of -35nm in diameter. Immunized mouse with
22 purified VLPs produced GII.17 specific sera and could efficiently block GII.17 VLPs
23 binding to saliva histo-blood group antigens (HBGAs). Together, these results
24 suggested that GII.17 VLPs represent a promising vaccine candidate against NoV
25 GII.17 infection and strongly support further preclinical and clinical studies.

26 **Keywords:** Norovirus GII.17 (NoV GII.17), virus like particles (VLPs), vaccine, sf9
27 cells;

28

29 **Introduction**

30 Human noroviruses (NoVs) infections are considered a significant public health
31 problem, which cause more than 50% of non-bacterial gastroenteritis characterized by
32 fever, vomiting and diarrhea emerging frequently, especially in adults and children [1,
33 2]. NoVs divided into 5 genogroups designated GI through to GV and subdivided into
34 9 and 22 genotypes which was first linked to human disease in 1972 [3, 4]. From the
35 mid-1990s, Genogroup II, genotype 4 (GII.4) NoV have been predominantly in
36 outbreaks and with every two or three years emergence of new variants [5]. However,
37 since the emergence of the novel Genogroup II, genotype 17 (GII.17) Kawasaki_2014
38 strain, a new pandemic strain has predominant in the world [6]. The novel GII.17 not
39 only caused NoVs outbreaks but also spread sporadically with cases reported in China
40 (Shanghai, Taiwan) and USA during the winter of 2014_2015 [7-9]. From 2015, NoV
41 GII.17 continued to circulate in many countries in South America, Europe and Asia
42 [10-12]. These epidemiological surveys indicate that NoV GII.17 is becoming an
43 important etiological agent of gastroenteritis.

44 NoVs belongs to the family of Caliciviridae and are nonenveloped,
45 single-stranded, positive-sense ribonucleic acid (RNA) viruses. The genomes are
46 7.4-7.7 kb in size and have typically organized into three open reading frames (ORFs).
47 The ORF1 encodes nonstructural proteins, such as the RNA dependent RNA
48 polymerase (RdRp), while the structural proteins, such as the major and minor capsid
49 protein (VP1 and VP2), are encoded by ORF2 and ORF3, respectively [13]. VP1 is a
50 60 KDa protein that can be divided into a shell domain (S domain) and a protruding
51 domain (P domain). The S domain forms a scaffold surrounding the viral RNA and
52 responsible for the shell structure of the capsid, whereas the P domain contains
53 neutralizing epitopes and binds to histo-blood group antigens (HBGAs) receptors,

54 responsible for the genetically variable, builds the viral spikes and facilitates cell
55 attachment [14-16]. VP2, a minor capsid protein, exhibiting multiple functions, which
56 is observed to stabilize and promote expression of VP1 [17].

57 The newly outbreak NoV GII.17 strain has been sweeping all over the world.
58 However, no vaccines or specific treatments are available. Due to a lack of a
59 permissive cell culture system and available an animal model, the main capsid protein
60 VP1 formed the virus like particles (VLPs) has been assembled and used as NoVs
61 vaccine candidates in preclinical and clinical studies [18]. VLPs have shown high
62 immunogenicity, safety and promising results as human enteroviruses vaccines, such
63 as enterovirus 71 (EV71), coxsackievirus A16 (CA16), and enterovirus D68 (EV-D68)
64 [19-21].

65 In the present study, we constructed GII.17 VP1 and expressed using
66 recombinant baculovirus expression system in sf9 cells which lead to formation of
67 VLPs that are morphologically and antigenically similar to true virions. Our results
68 showed that GII.17 VLPs could be readily produced in the baculovirus/insect cell
69 system and these VLPs could induce potent neutralizing antibody responses and
70 provided effective protection.

71

72 **Materials and Methods**

73 **Construction of baculovirus expression vectors**

74 The full-length capsid protein VP1 coding sequence (Genbank number,
75 KT992785) was optimized based on insect cells expression and synthesized by
76 Sangon Biotech (Shanghai, China) with flanked by *Bam*HI and *Xba* I sites at its 5' and
77 3' ends, respectively. The optimized gene cloned into pFastBac-Dual-EGFP vector
78 (our laboratory construction previous), named pFastBac-Dual-EGFP-VP1 (GII.17).

79 Then the pFASTBac-Dual-EGFP-VP1 (GII.17) vector purified plasmid DNA
80 transform into DH10Bac™ *E.coli* for transposition into the bacmid. At last, the
81 correct recombinant bacmid DNA transfect into sf9 insect cells to produce
82 recombinant baculovirus.

83 **Production and purification of NoV GII.17 VLPs**

84 After transfection about 96h (the green fluorescence reaches the most), the
85 budded virus released into the medium and collected the medium which was the P1
86 viral stock. Amplified baculoviral stock until P5 viral stock and collected cells.

87 Cells were washed and lysed in phosphate buffer solution (PBS) with ultrasound.
88 The lysates supplemented with 0.5 mol/L NaCl and 10% (W/V) PEG 8000 mixed for
89 6h, and then centrifuged at 10,000 rpm for 30 min. The resultant precipitates were
90 loaded onto a 20% sucrose cushion, and subjected to 10%-50% sucrose gradients for
91 ultracentrifuged at 40,000 rpm for 6h. After ultracentrifuged, fractions were taken
92 from top to bottom and then analyzed contained VLPs by SDS-PAGE. The uninfected
93 sf9 cells were subjected to the same treatments as the negative control. The final
94 purified proteins were dialysis with physiological saline at 4°C overnight and used
95 for immunization experiments.

96 **Electron microscopy**

97 Purified GII.17 VLPs was negatively stained with 0.5% aqueous uranyl acetate,
98 and observed by transmission electron microscopy with Tecnai G2 Spirit at 120V
99 (Thermo, USA).

100 **Mouse immunization**

101 Mice were purchased from Kunming Medical University (Yunnan, China). All
102 animal studies were approved by the Institutional Animal Care and Use Committee at
103 the Kunming University of Science and Technology.

104 GII.17 VLPs or the negative control antigen were adsorbed to the aluminum
105 hydroxide adjuvant with 1:1 by vortexing to a final volume 100 μ L containing 50 μ g
106 of GII.17 VLPs for each injection. Groups of 6 female BALB/c mice (6-8weeks old)
107 were intraperitoneally (i.p.) injected with antigen mixtures at weeks 0 and 4. Blood
108 samples were collected from each immunized mouse after the final immunization, and
109 putted at 37°C for 2h and 4°C for overnight.

110 **Serum antibody measurement assay**

111 NoV GII.17 specific antibodies in immunized mouse sera were detected by
112 indirect ELISA assay. 96-well plates were coated with 0.1 μ g (100 μ L)/well of GII.17
113 VLPs at 4°C for overnight, followed by blocking with 5% milk diluted in PBS
114 containing 0.05% Tween-20 (PBS-T) at 37°C for 1h; then incubated at 37°C for 2 h
115 with 100 μ L/well of serum samples that individual serum samples were diluted in
116 series in PBS-T. At last, incubation with goat anti-mouse IgG HPR (Abcam, Ab6789,
117 UK) at 37°C for 1h. Between each step, the plates were washed three times with
118 PBS-T. The absorbance was measured at 450 nm using a microplate reader (Thermo,
119 USA).

120 **Western blot assay**

121 Mouse sera collected were used to determine their reactivates on VP1 protein. In
122 brief, the VLPs proteins separated by SDS-PAGE were transferred to PVDF
123 membrane and detected using mouse anti-GII.17 VLPs serum at a dilution of 1:20000
124 in PBS-T and then HRP conjugated goat anti-rabbit IgG polyclonal antibody was
125 added at 1:10000 in enzyme buffer. The membrane was developed after another three
126 times wash using PBS-T with DAB.

127 **GII.17 VLPs-HBGAs binding assay in vitro**

128 For the in vitro VLPs-HBGA binding assay, the eighty-four saliva samples

129 collected from blood type A, B, AB and O individuals were used. The protocol for the
130 GII.17 VLPs saliva HBGAs binding assay was conducted based on previously [22,
131 23]. Briefly, human saliva with known ABO antigens was boiled for 10 min and
132 diluted at 1:1000 in PBS, added into 96-well plates (100 μ L/well) and incubated at 4°C
133 for overnight, followed by blocking with 5% milk diluted in PBS-T at 37°C for 1h.
134 GII.17 VLP at 0.1 μ g (100 μ L)/well was incubated at 37°C for 2 h, followed serum
135 samples that were diluted 1:1000 in PBS-T at 37°C for 2 h with 100 μ L /well. At last,
136 incubation with goat anti-mouse IgG HPR (Abcam, Ab6789, UK) at 37°C for 1h.
137 Between each step, the plates were washed three times with PBS-T. The absorbance
138 was measured at 450 nm using a microplate reader. At the same time, we used PBS-T
139 without sera as a negative control.

140 **GII.17 VLPs- HBGAs binding blockade assay**

141 The blocking activity of sera against GII.17 VLPs was determined by in vitro
142 VLPs-HBGAs binding blockade assay. The protocol was performed basically the
143 same as in vitro VLPs-HBGA binding assay except for serum dilution and antibody.
144 Since GII.17 VLPs exhibited the strongest binding to blood type AB salivary HBGAs,
145 a blood type A saliva sample was selected for the blockade assay. All reagents, sera
146 dilutions and reaction times were optimized and conducted saliva-VLPs blocking
147 assay using mouse sera. The 96-well microplates added to purified VLPs containing
148 VLPs mouse serum at 37°C for 1 h, and rabbit anti-GII.17-P domain monoclonal
149 antibodies (Taizhou SCIVAC Bio-Tech CO., Ltd) diluted was added. Wells added
150 with VLPs containing without VLPs mouse serum was selected as control. The
151 blocking index was calculated in % as (mean OD without VLP sera-mean OD with
152 VLP sera)/mean OD without VLP sera \times 100% [24].

153 **Statistical analysis of data**

154 Statistical analyses were performed using Graphpad Prism v5 software. Antibody
155 titers or OD values between groups were compared by Student's two-tailed t-test.

156

157 **Results**

158 **Expression and characterization of NoV GII.17 VLPs in sf9 cells**

159 In order to confirm the expression of GII.17 VP1 protein in sf9 cells, EGFP and
160 VP1 genes were together cloned into pFastBac-Dual vector under the control of pH
161 and p10 promoters, respectively (Fig.1A). The recombinant baculovirus was using
162 Bac-to-Bac baculovirus expression system and then infected insect cells. When the
163 green fluorescence reaches the most under the fluorescence microscope, we collected
164 cells. Sucrose gradient ultracentrifugation to purification the VP1 revealed by
165 SDS-PAGE and the results which corresponds to the full-length VP1 protein of
166 -58KDa (Fig.1B). The purity and integrity of VLPs were showed by transmission
167 electron microscopy (Fig.1C). It should be noted that smaller, larger and full VLPs
168 (about 35 nm) were observed.

169 **Specificity of the antibody response following immunization**

170 To determine the immunogenicity, GII.17 VLPs was used to i.p. immunize
171 BALB/c mice three at four weeks. Another group of mice was injected with PBS as a
172 control. Serum samples were collected from two weeks after the final immunization
173 and subjected to ELISA analysis for antibody measurement. All sera from VLPs
174 immunized mice exhibited high binding activities towards GII.17 VLPs, whereas the
175 control group did not exhibited significant reactivity (Fig. 2A). As showed in Western
176 blot analysis (Fig. 2B) can confirmed the expression of VP1 protein. These results
177 indicate that immunization with the VLPs but not the control antigen could induce
178 GII.17 VLPs specific antibody responses in mice.

179 **Assembled VLPs bind to salivary HBGAs**

180 An in vitro VLP-HBGA binding assay was used to characterize the binding
181 profiles of GII.17 capsid protein assembled VLPs. As shown in Fig. 3, VLPs bound to
182 blood type A, B and AB salivary HBGAs, respectively, while weak binding to type O
183 salivary HBGA.

184 **The capacity of VLPs immunized mouse sera to block GII.17 VLPs saliva** 185 **binding**

186 As we known, the protective function of anti-NoV serum antibodies correlates
187 with their capacity to block NoV VLPs binding to HBGAs, therefore, the serum
188 antibody-blocking assay was conducted to test the NoV neutralization [25-27]. We
189 collected the saliva HBGAs, including A, B and O antigens and has therefore been
190 used in the blockade assay. We used rabbit sera against GII.17 P domain as antibody
191 in our study. Saliva sample collected from a blood type AB individual was used in the
192 in vitro VLPs-HBGA binding blockade assay which represented the highest binding
193 signal. As was shown in Fig. 4, the blocking indices were higher for antisera from
194 blood type AB. Addition of serum against GII.17 VLPs blocked the binding of
195 GII.17-VP1 capsid protein assembled VLPs to salivary HBGAs.

196

197 **Discussion**

198 NoVs are the major cause of acute non-bacterial gastroenteritis all over the world
199 especially in children. As rapid evolution of NoV GII.4 with new strain replacing
200 previous strain every two or three years, the newly outbreak NoV GII.17 strain has
201 been sweeping all over the world. Thus, it is highly desirable to formulate an effective
202 and rapid NoV GII.17 vaccine to achieve. In our study, we represented that the fist
203 insect cell expressed GII.17 VLPs is promising vaccine.

204 The NoVs capsid protein is divided into two domains: S domain, forming the
205 core of the virus-like particle, and the P domain, mainly involved in receptor binding
206 [14]. Comparison of avidity of IgG from mice immunized from VLPs and P particles,
207 the results suggested that significant higher avidity for VLPs immunized mice and
208 also showed immunization route and adjuvants have no effect on the IgG avidity [28].
209 Some of NoV VLPs candidates have shown efficacy in human trials [26, 29]. As we
210 known, the VP1 which can self-assemble into VLPs when expressed in sf9 cells using
211 recombinant baculovirus expression system [30]. Therefore, in our research, GII.17
212 VLPs has been regarded as the most promising vaccine candidates and were
213 intraperitoneally (i.p.) injected with antigen mixture with aluminum hydroxide
214 adjuvant.

215 Due to the lack of an animal model available for human NoVs infection,
216 challenge study cannot be conducted to assess the efficacy of immunization. In
217 recently, B cells as a cellular target of human NoVs and could be development of an
218 in vitro infection model [31]. Prior to that, as we known, the HBGAs as important
219 receptors for NoVs infection and the HBGAs-VLPs blocking assay has been used as a
220 surrogate method for evaluation of NoVs neutralization in vitro.

221 Among the GII.17 VP1, the P region is further divided into the P1 and the P2
222 subregion. The P2 subregion is located at the outermost layer of the entire capsid,
223 which is important for the binding of NoVs and antibody recognition [32]. In
224 *Escherichia coli*, expression of P domain can lead to formation of P-particle, a 12- or
225 24 dimers, which contained all the elements required for receptor binding and had
226 been possessed the immunogenic in animal studies [33, 34]. So to characterize the
227 binding specificity of GII.17 VP1 capsid protein assembled VLPs, we used rabbit sera
228 against GII.17 P domain in our study.

229 HBGAs have been proposed as receptors for NoVs. Due to the NoVs different
230 genotypes, such as GI.3 and GII.2 did not bind to synthetic or salivary HBGAs,
231 whereas GII.4 could bind to salivary HBGAs [35, 36]. In our study, VLPs from capsid
232 proteins of epidemic NoV GII.17 exhibit broader HBGAs recognition except blood
233 type O individual, but lacking of the reasons to explain. Maybe the same as another
234 study reported that the recombinant MxV (GII.3) VLPs demonstrated binding to
235 salivary HBGAs from a blood type A individual [37].

236 At the same time, we used human saliva to evaluate in the blockade assay. Our
237 results indicate that rabbit serum against GII.17 VLPs blocked the binding of capsid
238 protein assembled VLPs to salivary HBGAs from a blood type AB individual and
239 blocking rate up to 60%, whereas the anti-PBS did not have such effects. The
240 discovery of new receptors or factors that promote the binding of GII.17 VLPs to
241 HBGAs is a key for following vaccine development and vaccine efficacy evaluation.
242 These data also suggest important information about the presence of other factors
243 involved in the binding of GII.17 VLPs to HBGAs.

244

245 **Conclusion**

246 Considering the facts of prevalence GII.17, the NoVs vaccine should be urgently
247 developed. Our results provided that the GII.17 VLPs induced antibodies should be
248 protective against in vivo NoV GII.17 infection. Therefore, we identified GII.17
249 VLPs as a viable candidate. NoVs vaccine represents a step forward in developing
250 broad-spectrum, multivalent vaccines against acute viral gastroenteritis.

251

252 **Acknowledgments**

253 This research is supported by grants from the National Natural Science

254 Foundation of China (No. 81860357) and Yunnan Provincial Department of
255 Education (2017ZZX138).

256

257 **Author Contributions**

258 Conceptualization: Congwen Shao, Shenrong Jing

259 Data curation: Rongliang Yuan, Yuyang Zhang, Siqi Xin

260 Methodology: Wei Chen, Tao Kang, Yuyang Zhang

261 Project administration: Congwen Shao, Shenrong Jing

262 Supervision: Shenrong Jing

263 Writing-original draft: Wei chen

264 Writing-review&editing: Shenrong Jing

265

266 **References**

267 1. Ahmed SM, Hall AJ, Robinson AE, Verhoef L, Premkumar P, Parashar UD, et al.
268 Global prevalence of norovirus in cases of gastroenteritis: a systematic review
269 and meta-analysis. *Lancet Infect Dis.* 2014;14(8):725-30. doi:
270 10.1016/S1473-3099(14)70767-4. PubMed PMID: 24981041.

271 2. Kaplan JE, Feldman R, Campbell DS, Lookabaugh C, Gary GW. The frequency
272 of a Norwalk-like pattern of illness in outbreaks of acute gastroenteritis. *Am J*
273 *Public Health.* 1982;72(12):1329-32. PubMed PMID: 6291414; PubMed Central
274 PMCID: PMCPMC1650540.

275 3. Kroneman A, Vega E, Vennema H, Vinje J, White PA, Hansman G, et al.
276 Proposal for a unified norovirus nomenclature and genotyping. *Arch Virol.*
277 2013;158(10):2059-68. doi: 10.1007/s00705-013-1708-5. PubMed PMID:
278 23615870; PubMed Central PMCID: PMCPMC5570552.

279 4. Zheng DP, Ando T, Fankhauser RL, Beard RS, Glass RI, Monroe SS. Norovirus
280 classification and proposed strain nomenclature. *Virology.* 2006;346(2):312-23.

- 281 doi: 10.1016/j.virol.2005.11.015. PubMed PMID: 16343580.
- 282 5. Ramani S, Atmar RL, Estes MK. Epidemiology of human noroviruses and
283 updates on vaccine development. *Curr Opin Gastroenterol.* 2014;30(1):25-33.
284 doi: 10.1097/MOG.000000000000022. PubMed PMID: 24232370; PubMed
285 Central PMCID: PMCPMC3955997.
- 286 6. Emergence of a novel GII.17 norovirus – End of the GII.4 era? *Euro*
287 *surveillance : bulletin Europeen sur les maladies transmissibles = European*
288 *communicable disease bulletin.* 2015;20(26).
- 289 7. Chen H, Qian F, Xu J, Chan M, Shen Z, Zai S, et al. A novel norovirus GII.17
290 lineage contributed to adult gastroenteritis in Shanghai, China, during the winter
291 of 2014-2015. *Emerg Microbes Infect.* 2015;4(11):e67. PubMed PMID:
292 26975060; PubMed Central PMCID: PMCPMC4661427.
- 293 8. Lee CC, Feng Y, Chen SY, Tsai CN, Lai MW, Chiu CH. Emerging norovirus
294 GII.17 in Taiwan. *Clin Infect Dis.* 2015;61(11):1762-4. doi: 10.1093/cid/civ647.
295 PubMed PMID: 26306886.
- 296 9. Parra GI, Green KY. Genome of Emerging Norovirus GII.17, United States,
297 2014. *Emerg Infect Dis.* 2015;21(8):1477-9. doi: 10.3201/eid2108.150652.
298 PubMed PMID: 26196235; PubMed Central PMCID: PMCPMC4517714.
- 299 10. Andrade JSR, Fumian TM, Leite JPG, Assis MR, Bello G, Mir D, et al. Detection
300 and molecular characterization of emergent GII.P17/GII.17 Norovirus in Brazil,
301 2015. *Infect Genet Evol.* 2017;51:28-32. doi: 10.1016/j.meegid.2017.03.011.
302 PubMed PMID: 28300648.
- 303 11. Khamrin P, Kumthip K, Yodmeeklin A, Supadej K, Ukarapol N, Thongprachum
304 A, et al. Molecular characterization of norovirus GII.17 detected in healthy adult,
305 intussusception patient, and acute gastroenteritis children in Thailand. *Infect*
306 *Genet Evol.* 2016;44:330-3. doi: 10.1016/j.meegid.2016.07.031. PubMed PMID:
307 27469026.
- 308 12. Huang XY, Su J, Lu QC, Li SZ, Zhao JY, Li ML, et al. A large outbreak of acute
309 gastroenteritis caused by the human norovirus GII.17 strain at a university in
310 Henan Province, China. *Infect Dis Poverty.* 2017;6(1):6. doi:

- 311 10.1186/s40249-017-0236-z. PubMed PMID: 28143569; PubMed Central
312 PMCID: PMC5286658.
- 313 13. Glass RI, Parashar UD, Estes MK. Norovirus gastroenteritis. *N Engl J Med.*
314 2009;361(18):1776-85. doi: 10.1056/NEJMra0804575. PubMed PMID:
315 19864676; PubMed Central PMCID: PMC3880795.
- 316 14. Prasad BV, Hardy ME, Dokland T, Bella J, Rossmann MG, Estes MK. X-ray
317 crystallographic structure of the Norwalk virus capsid. *Science.*
318 1999;286(5438):287-90. PubMed PMID: 10514371.
- 319 15. Debbink K, Donaldson EF, Lindesmith LC, Baric RS. Genetic mapping of a
320 highly variable norovirus GII.4 blockade epitope: potential role in escape from
321 human herd immunity. *J Virol.* 2012;86(2):1214-26. doi: 10.1128/JVI.06189-11.
322 PubMed PMID: 22090110; PubMed Central PMCID: PMC3255819.
- 323 16. Tan M, Jiang X. The p domain of norovirus capsid protein forms a subviral
324 particle that binds to histo-blood group antigen receptors. *J Virol.*
325 2005;79(22):14017-30. doi: 10.1128/JVI.79.22.14017-14030.2005. PubMed
326 PMID: 16254337; PubMed Central PMCID: PMC1280206.
- 327 17. Lin Y, Fengling L, Lianzhu W, Yuxiu Z, Yanhua J. Function of VP2 protein in
328 the stability of the secondary structure of virus-like particles of genogroup II
329 norovirus at different pH levels: function of VP2 protein in the stability of NoV
330 VLPs. *J Microbiol.* 2014;52(11):970-5. doi: 10.1007/s12275-014-4323-6.
331 PubMed PMID: 25277406.
- 332 18. Lucero Y, Vidal R, O'Ryan GM. Norovirus vaccines under development.
333 *Vaccine.* 2017. doi: 10.1016/j.vaccine.2017.06.043. PubMed PMID: 28668568.
- 334 19. Dai W, Zhang C, Zhang X, Xiong P, Liu Q, Gong S, et al. A virus-like particle
335 vaccine confers protection against enterovirus D68 lethal challenge in mice.
336 *Vaccine.* 2018;36(5):653-9. doi: 10.1016/j.vaccine.2017.12.057. PubMed PMID:
337 29295756.
- 338 20. Zhang C, Liu Q, Ku Z, Hu Y, Ye X, Zhang Y, et al. Coxsackievirus A16-like
339 particles produced in *Pichia pastoris* elicit high-titer neutralizing antibodies and
340 confer protection against lethal viral challenge in mice. *Antiviral Res.*

- 341 2016;129:47-51. doi: 10.1016/j.antiviral.2016.02.011. PubMed PMID: 26902108.
- 342 21. Zhang C, Ku Z, Liu Q, Wang X, Chen T, Ye X, et al. High-yield production of
343 recombinant virus-like particles of enterovirus 71 in *Pichia pastoris* and their
344 protective efficacy against oral viral challenge in mice. *Vaccine*.
345 2015;33(20):2335-41. doi: 10.1016/j.vaccine.2015.03.034. PubMed PMID:
346 25820068.
- 347 22. Marionneau S, Airaud F, Bovin NV, Le Pendu J, Ruvoen-Clouet N. Influence of
348 the combined ABO, FUT2, and FUT3 polymorphism on susceptibility to
349 Norwalk virus attachment. *J Infect Dis*. 2005;192(6):1071-7. doi:
350 10.1086/432546. PubMed PMID: 16107962.
- 351 23. Huang P, Farkas T, Marionneau S, Zhong W, Ruvoen-Clouet N, Morrow AL, et
352 al. Noroviruses bind to human ABO, Lewis, and secretor histo-blood group
353 antigens: identification of 4 distinct strain-specific patterns. *J Infect Dis*.
354 2003;188(1):19-31. doi: 10.1086/375742. PubMed PMID: 12825167.
- 355 24. Huo Y, Wang W, Ling T, Wan X, Ding L, Shen S, et al. Chimeric VLPs with
356 GII.3 P2 domain in a backbone of GII.4 VP1 confers novel HBGA binding
357 ability. *Virus Res*. 2016;224:1-5. doi: 10.1016/j.virusres.2016.08.006. PubMed
358 PMID: 27521750.
- 359 25. Reeck A, Kavanagh O, Estes MK, Opekun AR, Gilger MA, Graham DY, et al.
360 Serological correlate of protection against norovirus-induced gastroenteritis. *J*
361 *Infect Dis*. 2010;202(8):1212-8. doi: 10.1086/656364. PubMed PMID: 20815703;
362 PubMed Central PMCID: PMCPMC2945238.
- 363 26. Atmar RL, Bernstein DI, Harro CD, Al-Ibrahim MS, Chen WH, Ferreira J, et al.
364 Norovirus vaccine against experimental human Norwalk Virus illness. *N Engl J*
365 *Med*. 2011;365(23):2178-87. doi: 10.1056/NEJMoa1101245. PubMed PMID:
366 22150036; PubMed Central PMCID: PMCPMC3761795.
- 367 27. Tan M, Jiang X. Norovirus-host interaction: multi-selections by human
368 histo-blood group antigens. *Trends Microbiol*. 2011;19(8):382-8. doi:
369 10.1016/j.tim.2011.05.007. PubMed PMID: 21705222; PubMed Central PMCID:
370 PMCPMC3149758.

- 371 28. Huo Y, Wan X, Ling T, Wu J, Wang Z, Meng S, et al. Prevailing Sydney like
372 Norovirus GII.4 VLPs induce systemic and mucosal immune responses in mice.
373 Mol Immunol. 2015;68(2 Pt A):367-72. doi: 10.1016/j.molimm.2015.09.006.
374 PubMed PMID: 26375574.
- 375 29. Bernstein DI, Atmar RL, Lyon GM, Treanor JJ, Chen WH, Jiang X, et al.
376 Norovirus vaccine against experimental human GII.4 virus illness: a challenge
377 study in healthy adults. J Infect Dis. 2015;211(6):870-8. doi:
378 10.1093/infdis/jiu497. PubMed PMID: 25210140; PubMed Central PMCID:
379 PMC5914500.
- 380 30. Jiang X, Wang M, Graham DY, Estes MK. Expression, self-assembly, and
381 antigenicity of the Norwalk virus capsid protein. J Virol. 1992;66(11):6527-32.
382 PubMed PMID: 1328679; PubMed Central PMCID: PMC240146.
- 383 31. Jones MK, Watanabe M, Zhu S, Graves CL, Keyes LR, Grau KR, et al. Enteric
384 bacteria promote human and mouse norovirus infection of B cells. Science.
385 2014;346(6210):755-9. doi: 10.1126/science.1257147. PubMed PMID:
386 25378626; PubMed Central PMCID: PMC4401463.
- 387 32. Tan M, Huang P, Meller J, Zhong W, Farkas T, Jiang X. Mutations within the P2
388 Domain of Norovirus Capsid Affect Binding to Human Histo-Blood Group
389 Antigens: Evidence for a Binding Pocket. Journal of Virology.
390 2003;77(23):12562.
- 391 33. Fang H, Tan M, Xia M, Wang L, Jiang X. Norovirus P particle efficiently elicits
392 innate, humoral and cellular immunity. PLoS One. 2013;8(4):e63269. doi:
393 10.1371/journal.pone.0063269. PubMed PMID: 23638188; PubMed Central
394 PMCID: PMC3639243.
- 395 34. Tamminen K, Huhti L, Koho T, Lappalainen S, Hytonen VP, Vesikari T, et al. A
396 comparison of immunogenicity of norovirus GII-4 virus-like particles and
397 P-particles. Immunology. 2012;135(1):89-99. doi:
398 10.1111/j.1365-2567.2011.03516.x. PubMed PMID: 22044070; PubMed Central
399 PMCID: PMC3246655.
- 400 35. Huo Y, Wan X, Ling T, Shen S. Biological and immunological characterization

401 of norovirus major capsid proteins from three different genotypes. *Microb*
402 *Pathog.* 2016;90:78-83. doi: 10.1016/j.micpath.2015.11.022. PubMed PMID:
403 26616166.

404 36. Huo Y, Wan X, Ling T, Wu J, Wang Z, Meng S, et al. Prevailing Sydney like
405 Norovirus GII.4 VLPs induce systemic and mucosal immune responses in mice.
406 *Molecular Immunology.* 2015;68(2):367-72.

407 37. Huang P, Farkas T, Zhong W, Tan M, Thornton S, Morrow AL, et al. Norovirus
408 and Histo-Blood Group Antigens: Demonstration of a Wide Spectrum of Strain
409 Specificities and Classification of Two Major Binding Groups among Multiple
410 Binding Patterns. *Journal of Virology.* 2005;79(11):6714-22.

411

412

413

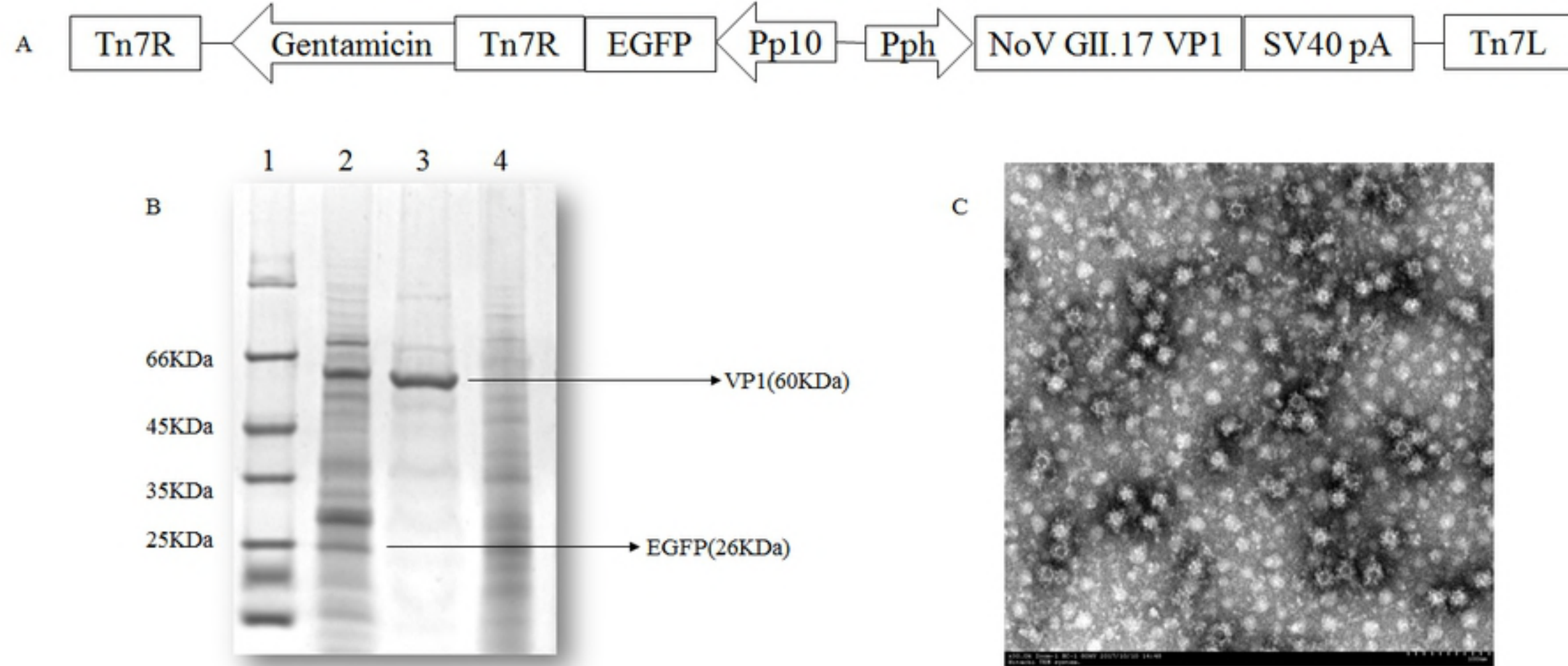


Fig. 1 Co-expression of NoV GII.17 VP1 and EGFP in insect cell

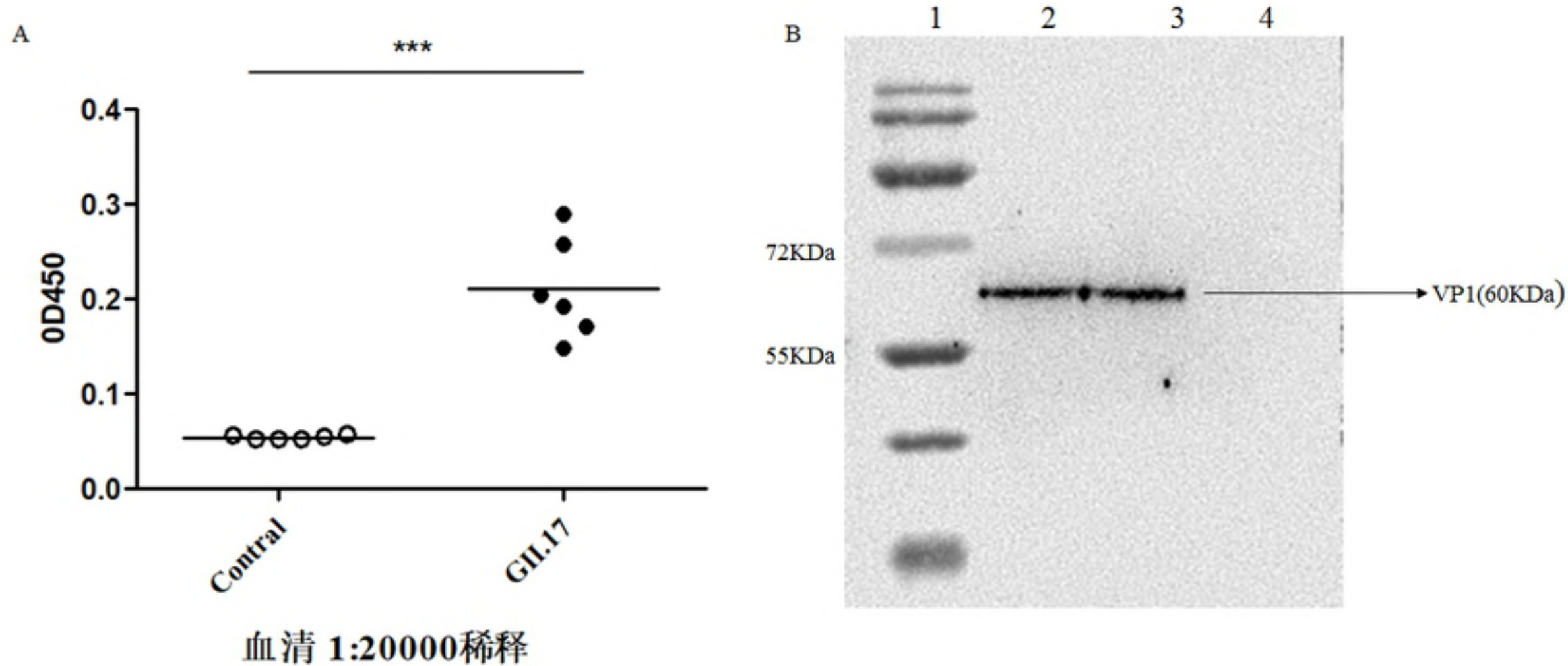


Fig.2 Serum antibody responses in mice after immunization with GII.17 VLPs

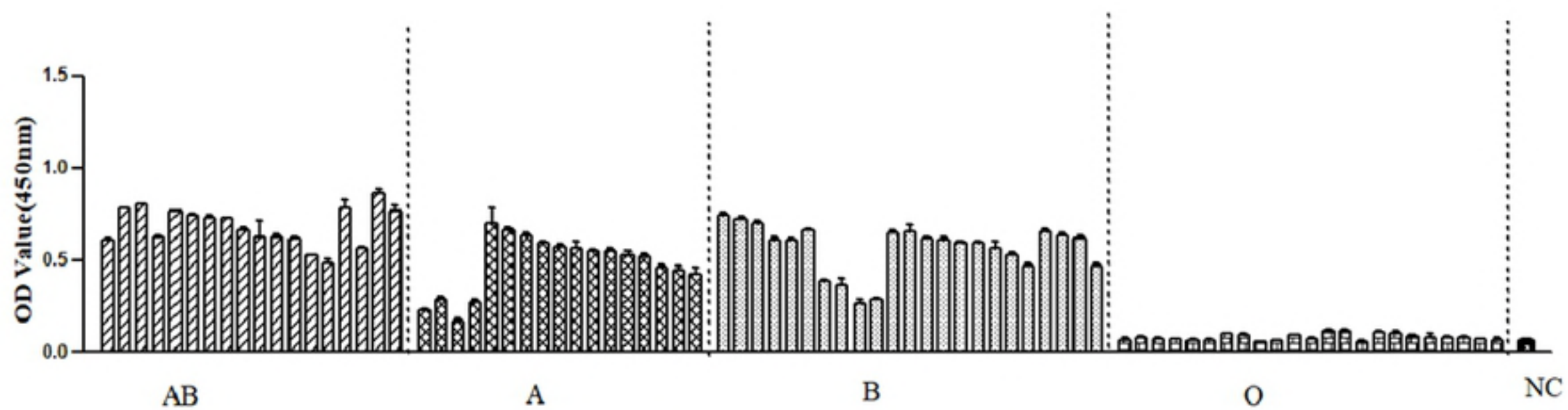


Fig.3 Characterization of binding profiles of GII.17 VLPs to blood type A, B and AB salivary HBGAs, respectively

Figure 3

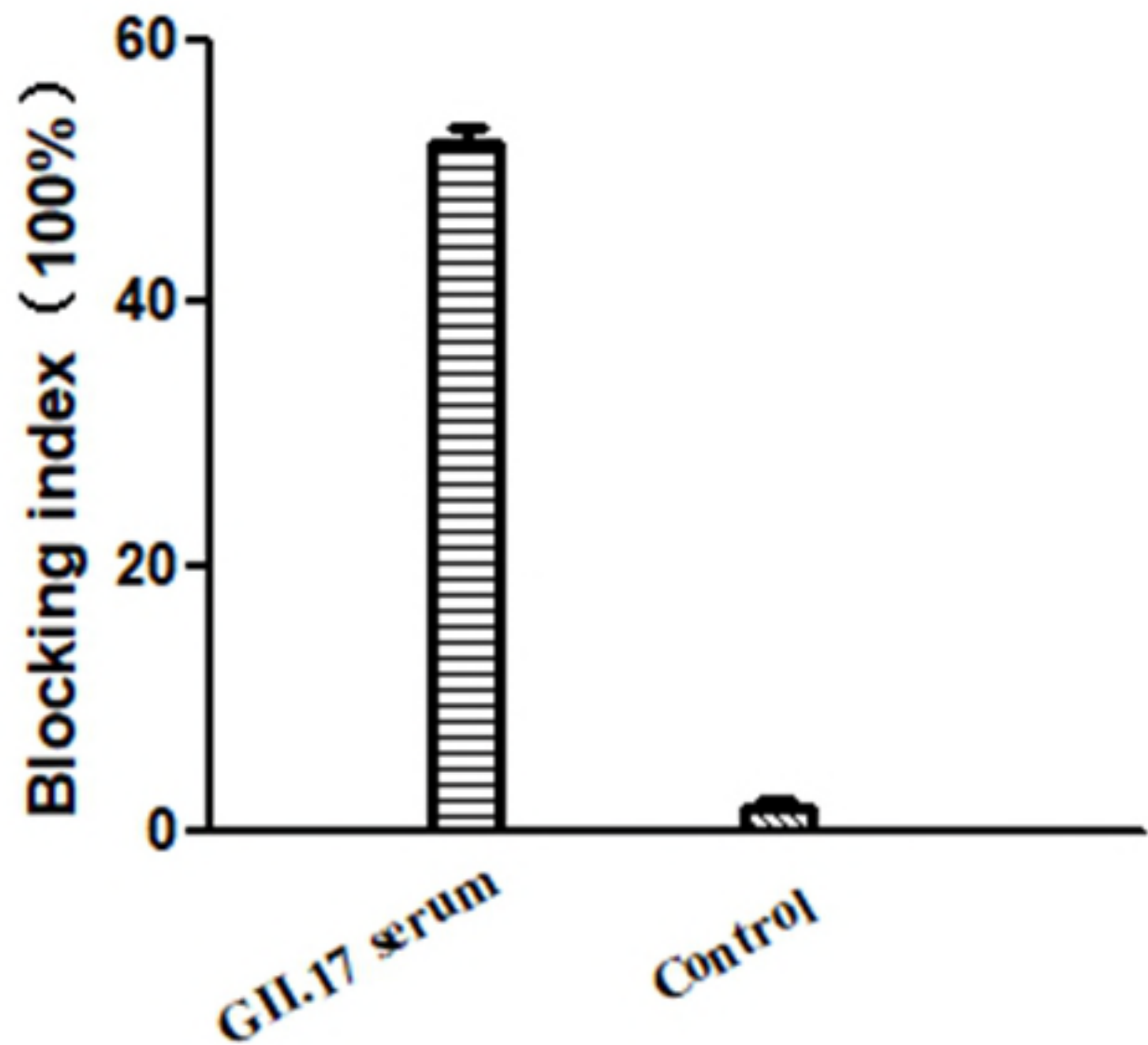


Fig.4 VLP-HBGAs binding blockade assay

Figure 4