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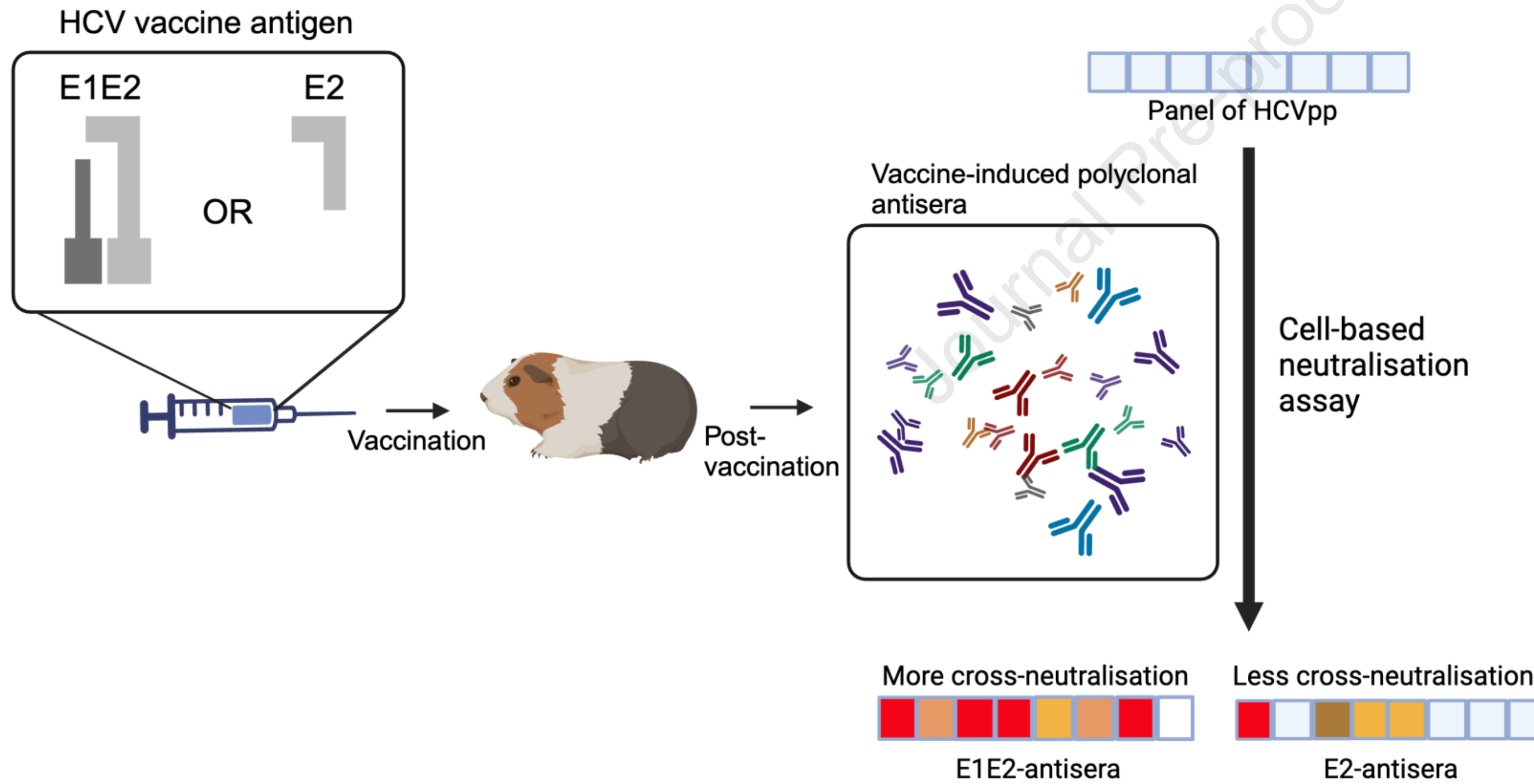
Graphical Abstract



1 **Recombinant H77C gpE1/gpE2 heterodimer elicits superior HCV cross-neutralisation than**
2 **H77C gpE2 alone**
3

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25
26
27 Short title: Comparing immunogenicity of H77C E1E2 and E2
28
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1 **Conflict of Interest Statement**
2 Authors (JK, HTL, ML, DH, AL, KC, MW, JJ, JKK, DLT, MH, JL) own stock in Aurora Vaccines
3 Inc., which is developing a HCV vaccine for clinical and commercial use.
4
5 **Authors' Contributions**
6 JK, DLT, MH, JL conceived and designed the experiments. JK, HTL, ML, DH, AL, KC, MW, JJ,
7 EAT, RAU, JL performed the experiments. JK, HTL, JKK, MH, JL analyzed the data. EAT, RAU,
8 JKB, JRB, JB, ML, SF contributed reagents/materials/analysis tools. JK, MH, JL wrote the paper.
9
10 **Financial Support**
11 This work has been supported by the Canadian Institutes of Health Research (awarded to MH) and
12 Alberta Innovates Health Solutions.
13
14 **Data Availability Statement**
15 The numerical source data for all applicable graphs is provided in the excel file named
16 "Rawdata-Kunduetal2024".

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1 **Abstract**
2
3 **Background & Aims:** An optimal HCV vaccine requires the induction of antibodies that neutralise
4 the infectivity of many heterogenous viral isolates. In this study, we have focused on determining
5 the optimal recombinant envelope glycoprotein component to elicit cross-neutralising antibodies
6 against global HCV genotypes. We compared the immunoreactivity and antigenicity of the
7 recombinant HCV genotype 1a strain H77C envelope glycoprotein heterodimer gpE1/gpE2 with
8 that of recombinant gpE2 alone derived from an infectious molecular clone (H77C).
9 **Methods:** Characterization of the envelope glycoproteins was accomplished by determining their
10 ability to bind to a panel of broadly cross-neutralising monoclonal antibodies (bNAbs).
11 Immunogenicity was determined by testing the ability of vaccine antisera to neutralise the
12 infectivity *in vitro* of a panel of pseudotyped HCV particles in which gpE1/gpE2 derived from
13 representative isolates of the major global HCV genotypes were displayed.
14 **Results:** gpE1/gpE2 binds to more diverse bNabs than gpE2 alone and elicits a broader profile of
15 cross-neutralising antibodies in animals, especially against more heterologous, non-1a genotypes.
16 While not all heterologous HCV strains can be potently inhibited *in vitro* by gpE1/gpE2 antisera
17 derived from a single HCV strain, the breadth of heterologous cross-neutralisation is shown to be
18 substantial.
19 **Conclusions:** Our work supports the inclusion of gpE1/gpE2 in an HCV vaccine in order to
20 maximise the cross-neutralisation of heterogenous HCV isolates. Our data also offers future
21 directions in formulating a cocktail of gpE1/gpE2 antigens from a small selection of HCV
22 genotypes to further enhance cross-neutralisation of global HCV strains and hopefully, achieving
23 global protection.
24
25
26 **Impact and implications:**
27 An HCV vaccine is urgently required to prevent the high global incidence of HCV infection and
28 disease. Since HCV is a highly heterogeneous virus, it is desirable for a vaccine to elicit antibodies
29 that neutralise the infectivity of most global strains. To this end, we have compared the
30 immunoreactivity and antigenicity of recombinant H77C E1E2 heterodimer with that of H77C E2
31 alone and show that the former exhibits more cross-neutralising epitopes and demonstrates a
32 broader cross-neutralisation profile *in vitro*. In addition, our data suggests a way to further broaden
33 cross-neutralisation using a combination of E1E2 antigens derived from a few different HCV
34 clades. Our work provides encouragement for the development of an effective global HCV
35 vaccine.
36
37
38 **Introduction**
39 Despite the availability of curative HCV antivirals, the incidence of HCV infection is rising in
40 many countries of the world due to the increased frequency of injecting drug use and the inhibitory
41 cost of HCV antivirals. In comparison, an HCV vaccine delivered to people-who-inject-drugs
42 (PWIDs) would be highly cost-saving to healthcare budgets around the world and at present,
43 remains the main hope in achieving the WHO goal of eliminating HCV infection as a major
44 infectious disease [1]. There is now persuasive cumulative evidence for the protective role of HCV
45 neutralising antibodies [2–9] and HCV-specific T helper and cytotoxic lymphocyte responses [10–13]. In
46 the only reliable, fully immunocompetent HCV chimpanzee model, the recombinant E1/E2

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heterodimeric envelope glycoprotein antigen remains the only prophylactic vaccine candidate demonstrating statistically significant efficacy against the development of persistent viremia and associated disease [3]. The vaccine was comprised of the two recombinant envelope glycoproteins E1 and E2 derived from a genotype 1a strain HCV1, that fold into a native heterodimer inside the endoplasmic reticulum of transfected mammalian cells [2,14]. Further, an HCV vaccine candidate that elicits just cellular immune responses against HCV, without the production of any virus-neutralising antibodies, failed to demonstrate any efficacy against the persistent carrier state [15], leading the field to now focus on candidates that induce cross-neutralising antibodies that are a well-established correlate of protection for all viral vaccines approved for human use so far.

HCV is extremely heterogeneous with 8 major genotypes identified phylogenetically around the world with each genotype comprising numerous subtypes [16]. HCV genotypes 1 through 6 predominate [17,18]. Of the two envelope glycoproteins encoded by the HCV genome, E2 is known to be the main target for virus-neutralising antibodies. As a result, many groups are developing vaccines using E2 alone [19,20]. Our work has focused historically and currently on the use of the E1E2 heterodimer because of its efficacy to reduce the rate of chronicity in the chimpanzee model, and importantly, it was more immunogenic than E2 alone in chimpanzees and in phase 1 clinical trials [3]. Large-scale production of the E1E2 heterodimer has been a challenge because it's intracellular location in transfected mammalian cells leads to cellular toxicity, low yield, and difficulties with large-scale purification from the complex cellular milieu. However, we have now solved these issues by expressing E1E2 using specially designed conditions in large bioreactors. We also improved purification by utilizing a Fc fusion precursor that allows mature E1E2 to be purified on a large-scale using Protein A-based affinity columns followed by a protease cleavage to remove the Fc [21]. The work reported herein aims to address the important question of the breadth of cross-neutralisation afforded by E1E2 and E2 alone and to understand the underlying mechanisms. We now report data showing that E1E2 contains more diverse cross-neutralising epitopes than E2 alone leading to a superiority of E1E2 in its ability to cross-neutralise the infectivity of many heterogenous, global HCV genotypes and subtypes. Furthermore, our work offers a road-map to producing a cocktail of E1E2 antigens derived from different genotypes to further enhance cross-neutralisation and hopefully, the production of a potent global vaccine.

Materials and Methods

Cell culture and antibodies. Human embryonic kidney 293T (HEK 293T) cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Gibco, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Sigma Aldrich, St Louis, MO, USA), 100 units/ml penicillin and 100 µg/ml streptomycin (Invitrogen, Carlsbad, CA, USA). Human hepatoma Huh7.5 cells were propagated in DMEM (Gibco, Grand Island, NY, USA) containing 10% heat-inactivated FBS (Omega Scientific, Tarzana, CA,USA), 0.1 mM nonessential amino acids (NEAA) (Invitrogen, Carlsbad, CA, USA), and penicillin (100 units/ml) and streptomycin (100 µg/ml) (PenStrep; Invitrogen, Carlsbad, CA, USA) in an incubator supplemented with 5% carbon dioxide (CO_2) at 37°C. CHO cells stably expressing recombinant gpE1/gpE2 (amino acids 192 to 746) or gpE2 construct (amino acids 384 to 661) derived from the genotype 1a H77C infectious clone (GenBank accession number AF009606) [22]were propagated in Iscove's modified Dulbecco's medium (Thermo Fisher Scientific, Waltham, MA, USA) containing 10% heat-inactivated fetal bovine serum (FBS) (Thermo Fisher Scientific, Waltham, MA, USA), 0.1 mM

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1 sodium hypoxanthine – 0.016 mM thymidine (HT supplement; Thermo Fisher Scientific,
2 Waltham, MA, USA), 0.002 mM methotrexate, 100 U/ml penicillin, and 100 µg/ml streptomycin
3 (PenStrep; Invitrogen, Carlsbad, CA, USA). Anti-HCV MAbs (H-111, HC33.4, HC84.26, AR3A,
4 HC-1AM, AR4A and AR5A) were previously described from various co-authors of this study [23–
5 27].
6
7 **Expression and purification of recombinant glycoproteins E1E2 and E2.** E1E2 or E2 were
8 purified from cell pellets expressed as Fc-tagged proteins. For Fc tagged E1E2, a tPA leader was
9 inserted upstream of E1 (amino acids 192-383), followed by a duplication of amino acids 384 to
10 385 (ET), a human IgG1 Fc tag (227 amino acid residues), a human rhinovirus protease 3C
11 (HRV3C) sequence (LEVLFQGP) and the coding sequence for E2 (amino acids 384-746).
12 For Fc-tagged E2, we inserted a tPA leader sequence upstream of E2 (amino acids 384-661).
13 Additionally, at the carboxyl-terminus of E2, we inserted a HRV3C sequence, followed by a
14 human IgG1 Fc tag.
15 Purification of Fc-tagged glycoproteins was as previously described [21]. Briefly, the cell extract
16 was applied to Protein G Sepharose 4 Fast Flow (GE Healthcare, Piscataway, NJ) and then
17 washed. Subsequently, the resin was digested with His6-GST-HRV3C protease (Thermo Fisher
18 Scientific) overnight at 4°C. The digested material was applied to Glutathione Sepharose 4B (GE
19 Healthcare) to remove the protease and the flowthrough was then passed through a
20 hydroxyapatite column (HAP) and subsequently concentrated [21]. The final antigens (E1E2 and
21 E2) reached at least 90% purity (Supplementary figure 1).
22
23 **ELISA**. Microtiter plates (Thermo Scientific CAT # 439454) were coated with recombinant E1E2
24 or E2 antigens (50 ng/well) in phosphate-buffered saline (PBS, 1X) overnight at 4°C. Plates were
25 washed with PBS containing 0.2% Tween 20 (PBST, 1X) and blocked for 1 h in 5% bovine serum
26 albumin (Sigma-Aldrich, St. Louis, MO) in PBST. Mab was added for 1 h and detected by an
27 alkaline phosphatase-conjugated anti-human secondary antibody (1:10,000; Jackson Immuno
28 Research, West Grove, PA) and KPL peroxidase substrate (SeraCare Life Sciences, Milford, MA).
29 The absorbance at 450 nm was read using an Enspire plate reader (Perkin-Elmer, Waltham, MA,
30 USA)
31
32 **Immunization of animals and collection of serum samples.** Hartley guinea pigs (Medimabs,
33 Montreal, QC, Canada), 5 to 7 weeks old, were cared for in accordance with Canadian Council on
34 Animal Care guidelines. Experimental methods were reviewed and approved by the University of
35 Alberta Health Sciences Animal Welfare Committee (AUP00000392). Purified E1E2 or E2 antigen
36 (4 µg) were mixed at a 1:1 equi-volume ratio with 75 µg alum and 7.5 µg monophosphoryl lipid
37 A (MPLA) (Vaccigrade; InvivoGen, San Diego, CA, USA). The final antigenic preparation (100
38 µl) of E1E2 or E2 was administered via intramuscular injection to each guinea pig on days 0, 30
39 and 90. Pre-vaccination blood samples were collected at day 0, and post-vaccination blood samples
40 (terminal bleeds) were obtained 14 days after the final immunization. After clotting, whole blood
41 samples were centrifuged at 5,000 X *g* for 15 min, and sera were collected, and heat inactivated at
42 56°C for 30 min. Serum samples were stored in aliquots at -80°C until use.
43
44 **Production of HCVpp.** Plasmids encoding a panel of various HCV glycoprotein E1E2 were
45 described previously [28–30]. HCVpp were generated by co-transfection of HEK 293T cells with two
46 plasmids encoding HCV glycoprotein gpE1/gpE2, pNL4.3.Luc.R-E containing the luciferase

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1 reporter and env-defective HIV proviral genome (National Institute of Health AIDS Reagent
2 Program), and pAdvantage (Promega, Madison, WI) using Lipofectamine 2000 (Thermo Fisher
3 Scientific) according to the manufacturer's protocol. Twenty-four hours post-transfection, the
4 medium was replaced by DMEM containing 3% FBS. At 48 h and 72 hours post-transfection, the
5 cell culture supernatants containing HCVpp were harvested by passing through 0.45 µm filters and
6 stored at -80°C for future use.
7
8 **Neutralisation assays.** For neutralisation assays, $1x10^4$ human hepatoma (Huh7.5) cells were
9 plated onto poly-lysine-coated 96-well plates 1 day prior to infection. HCVpp was pre-mixed with
10 heat-inactivated sera from immunised guinea pigs at 1: 100 dilutions for 1 h at 37°C, followed by
11 addition to Huh7.5 cells. At 5 h post-infection, the immune sera-virus inoculum was replaced with
12 fresh culture medium and was incubated for an additional 72 hours at 37 °C. Pre-immune sera
13 (Pre) were used as control. For E1E2 antisera, each dot represents pre-immunization serum from
14 each animal whereas for E2 antisera, each dot in pre-immunization sera indicated technical
15 replicates. Luciferase activity was measured according to manufacturer's protocol (Promega Inc,
16 Madison, WI). Briefly, cells were lysed using Cell Lysis Buffer, followed by addition of substrate
17 buffer for 5 minutes. The luminescence (RLU) was measured using the EnSpire 2300 multilabel
18 reader (Perkin-Elmer). The percent neutralisation was calculated [29]: % Neutralisation = [1- (RLU
19 $_{\text{post-vaccinated}}$/ RLU $_{\text{Pre-vaccinated sera}}$)] X 100%.
20
21 **Statistical Analysis.** Statistical analysis (paired Student's t test) was done using GraphPad Prism
22 9 software. P-values less than (<) 0.05 were considered statistically significant.
23
24
25 **Results**
26
27 Intracellular H77 E1E2 (aa 192-746) and intracellular E2 (aa 384-661) were each expressed and
28 purified from CHO cell pellets to greater than 90% purity. Each glycoprotein was then used to coat
29 EIA wells and the relative immunoreactivity determined using seven bNabs which broadly
30 neutralise the *in vitro* infectivity of many heterogeneous HCV genotypes (Table 1). Figure 1 shows
31 that E1E2 strongly bound all Mabs tested whereas E2 only bound four strongly. Of the three bNabs
32 showing specificity for the E1E2 heterodimer, two (AR4A and AR5A) have been described as
33 recognizing conformational epitopes formed by the E1 and E2 interaction, while H-111 recognizes
34 E1 and not E2 (Table 1). As expected, H-111 binds only to the E1E2 heterodimer (Figure 1). The
35 remaining four bNabs that bind equally well to E1E2 and E2 (AR3A, HC-33.4, H84.26, HC-1AM)
36 have been reported previously to bind to different epitopes within E2 (Table 1).
37
38 Next, we assayed the ability of the guinea pig antisera to neutralise the *in vitro* infectivity of HCV
39 pseudoparticles (HCVpp) derived from the same 1a strain as the vaccine antigens (H77C
40 sequence). Figure 2A shows that guinea pigs immunized with E1E2 elicited high titers of
41 neutralising antibodies against homologous H77C HCVpp although there was not a statistically
42 significant difference as compared with E2 alone antisera (Figure 2B).
43 In terms of cross-neutralising antibodies against heterologous HCVpp's, we observed that the
44 cross-neutralising profile of E1E2 and E2 antisera against HCVpp's derived from 8 different
45 genotype 1a strains (the same basic genotype from which the vaccines were derived) were similar,
46 although E1E2 tended to elicit stronger and broader cross-neutralising antibodies (Figure 3A).

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1 Interrogations of a representative panel of non-genotype 1a HCVpp's revealed a greater
2 differentiation of these two antigens. E1E2 elicited significant neutralising antibodies against 3/4
3 different genotype 1b HCVpp's, whereas E2 did not significantly neutralise any 1b HCVpp (Figure
4 3B). Of four more genetically distinct HCVpp's derived from HCV genotypes 2, 3, 4, and 5, E1E2
5 antisera significantly inhibited the infectivity of all four, whereas E2 only significantly neutralised
6 one derived from genotype 5, albeit less potently than E1E2 antisera (Figure 3B). Figure 3C shows
7 a relative heat map of these data further illustrating this differentiation of E1E2 and E2 antisera.
8 To compare the breadth of cross-neutralisation, we have tallied the number of isolates that each
9 antiserum can neutralise by over 50%, as described by Osburn *et. al.* [7]. E1E2 antisera exhibited a
10 broader neutralisation breadth than E2 antisera (Figure 3D). Finally, we interrogated the cross-
11 neutralising activity of E1E2 antisera against a wider selection (addition of 14 more)
12 heterogeneous HCVpp's (Figure 4A). The relative heat map (combined with data in Figure 3C,
13 total of 30) is shown (Figure 4B). It revealed that 7/9 different genotype 1a HCVpp's were partially
14 but significantly neutralised by a 1:100 dilution of guinea pig antisera. 3/5 genotype 1b HCVpp's
15 were also significantly and partially neutralised, whereas 1/5, 3/5, 3/4, and 2/2 HCVpp's were
16 significantly neutralised from genotypes 2, 3, 4 and 5, respectively, using 1:100 dilutions of E1E2
17 antisera. This demonstrates the ability of the E1E2 antigen derived from a single HCV strain to
18 prime broad cross-neutralising antibodies against the very heterogeneous human Hepacivirus
19 genus.
20
21 **Conclusions & Discussion**
22
23 Our previous findings from vaccinating small animals, chimpanzees and humans have shown that
24 neutralising antibodies elicited by E1E2 derived from a single HCV 1a strain (HCV1) are *not*
25 restricted to the homologous strain used [31–35]. The current study extends upon these findings by
26 showing that E1E2 of another genotype 1a strain (H77) binds numerous discrete, broadly
27 neutralising bNabs (Figure 1) and that E1E2 antisera from immunised guinea pigs exhibits
28 significant cross-neutralising antibodies against a wide variety of HCVpp's derived from a broad
29 spectrum of predominant global genotypes (Figure 4). In addition, the current study shows that
30 H77C E1E2 outperforms H77C E2 alone in terms of binding to various highly cross-neutralising
31 bNabs and in the breadth and titer of elicited cross-neutralising antibodies against highly
32 heterogenous HCV genotypes. Importantly, in both vaccinated chimpanzees and human
33 volunteers, HCV-1 E1E2 has been shown to be substantially more immunogenic than HCV-1 E2
34 alone ([3] & MH, unpublished).
35
36 Neutralising antibodies correlate with protection against heterologous HCV genotypes in chimeric
37 mice harboring human hepatocytes [4], in chimpanzees [2,6], and in various patient cohorts [5,7–9] and
38 adjuvanted E1E2 has been demonstrated to be capable of substantially reducing the chronic carrier
39 state in vaccinated chimpanzees subsequently challenged with homologous and heterologous 1a
40 strains [2,3,14]. No other vaccine candidate has demonstrated such efficacy in the reliable chimpanzee
41 model [36] or in any other animal model. Given that severe clinical manifestations of HCV infection
42 are predominantly associated with viral persistence over many years, our current work provides
43 further encouragement that an effective, broadly neutralising, global vaccine can be produced
44 which, if necessary, can be further enhanced relatively easily by producing an optimal cocktail of
45 E1E2 antigens derived from just a few different genotypes. We are reporting only partial *in vitro*
46 neutralisation of most HCVpp's at a 1:100 dilution of antisera and so it remains to be seen if this

activity will translate to broad protection in humans. Protection against HCV infection, as in the case of the approved HBV and HPV vaccines, will likely rely more heavily on the generation of vaccine-mediated HCV-specific B and T cell memory responses rather than maintaining high circulating levels of antibodies and activated T cells required for optimal protection against fast-acting respiratory viruses like SARS-CoV-2, influenza, and RSV viruses [37–41]. The observed priming of broadly cross-neutralising antibodies by E1E2 implies the simultaneous generation of broad memory B and T cells that could be cross-protective, but efficacy trials in human volunteers will be required to definitively address these questions possibly through rapid human challenge trials [42].

As has been common practice in developing effective vaccines against heterogeneous viruses and bacteria in the past, combinations of vaccine antigens derived from different HCV genotypes/strains can be readily produced to enhance the potency of a global HCV vaccine and our data reported in this study offers some pointers on what combinations may be appropriate to this end. Vaccines against HCV and HIV that only elicit cellular immune responses in the absence of neutralising antibodies have proven to be unsuccessful in human efficacy trials [15,36,43,44]. Our data indicates that the use of E1E2 antigens in either recombinant form or encoded using RNA or vectored platforms remains a promising approach for preventing global HCV disease and transmission. Finally, it should be noted that the work reported here compared H77C E1E2 with H77C E2 derived from the common global HCV genotype 1a. Given the large heterogeneity of HCV and its complex cell entry mechanisms, it remains to be seen if the superiority of E1E2 applies to all other HCV genotypes.

Figure legends:

Figure 1. Binding of HCV cross-neutralising human MAbs to purified E1E2 and E2 antigens. Microtiter plates were coated either with purified recombinant E1E2 (red) or E2 (blue) and probed with the 2-fold decreasing concentrations of HCV neutralising human MAbs (starting at 0.5 µg/mL to 10 µg/mL). Bound antibodies were detected by an alkaline phosphatase-conjugated anti-human secondary antibody. The mean optical densities (OD) measured at 450 nm for each Mab tested in two independent experiments are plotted versus Mab concentration (µg/mL). E2-specific antibodies are AR3A, HC33.4, HC84.6 and HC-1AM. E1E2-specific antibodies are AR4A, AR5A. E1-specific antibody is H-111.

Figure 2. Homologous neutralisation activity against H77C HCVpp from E1E2 and E2-immunised guinea pig antisera. (A) Antisera from guinea pigs (G1-G8) either immunised with E1E2 (top) or E2 (bottom) were serially diluted and their abilities to block entry of HCVpp pseudotyped with H77C E1E2 were determined as described. (B) IC_{50} of these antisera were determined using GraphPad Prism software (version 9). The Mean of IC_{50} between antisera from E1E2- and E2-immunised guinea pigs were compared. Unpaired Student's T-test showed P-value >0.05 (non-significant (ns)).

Figure 3. Comparison of the neutralisation activity between antisera from E1E2- and E2-immunized guinea pigs. The infectivity of sera from guinea pigs pre- or post-vaccination with E1E2 and E2 was tested against a panel of eight genotype 1a HCVpp (A) and eight non genotype 1a HCVpp (B) in Huh7.5 cells. Pre- and post-vaccinated sera were diluted at a 1:100 ratio. The

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1 amount of virus entry was measured by quantifying the HCVpp encoded luciferase activity 48-
2 hour post incubation and the proportion infectivity was normalized with HCVpp incubated without
3 serum. Results were shown from at least two independent experiments in triplicate. Mean
4 proportion of infectivity is denoted by a solid line. Student's t-tests were performed to compare
5 between pre- and post-vaccination. **** indicates p-value < 0.0001; *** indicates p-value < 0.001;
6 **demonstrates p-value < 0.01 and * demonstrates p-value < 0.05. NS indicates no
7 significance. (**C**) The normalized neutralisation activity of post-vaccination antisera was
8 represented in a heat map. Color codes: >75% Neutralisation (red), >50% Neutralisation (orange),
9 >25% Neutralisation (light green), and <25% Neutralisation (white). Patterns of neutralisation
10 from individual guinea pigs immunised with either E1E2 (upper panel) or E2 (lower panel) against
11 a panel of 16 HCVpp's are shown. Another representation of this figure is shown in Supplementary
12 Figure 2 by arranging the HCVpp as tiers of neutralisation resistance as described [29]. **(D)** The
13 breadth of the cross-neutralisation conferred by E1E2 or E2 induced antisera was compared. The
14 breadth of cross-neutralisation is determined by the number of isolates (out of the 16 HCVpp
15 tested) that were neutralised by >50%. The mean of this number between guinea pigs immunised
16 with E1E2 or E2 was compared by student's t-tests. * indicates statistical significance, P-value <
17 0.05
18
19 **Figure 4. The cross-neutralisation profile of antisera from E1E2-immunised guinea pigs.** The
20 neutralisation activity of sera from guinea pigs pre- or post-vaccination with E1E2 was tested
21 against an expanded panel of heterologous HCVpp's in Huh 7.5 cells. Pre- and post-vaccinated
22 sera were diluted at 1:100. The amount of virus entry was measured by quantifying the HCVpp
23 encoded luciferase activity 48 hour post incubation and the proportion infectivity was normalized
24 with HCVpp incubated without serum. The data was calculated from three independent
25 experiments, each performed with triplicate wells. Means of infectivity % are indicated. Student's
26 t-tests were performed to compare between pre- and post-vaccination. **** indicates p-value <
27 0.0001; *** indicates p-value < 0.001; **demonstrates p-value < 0.01 and * demonstrates p-value
28 < 0.05. NS indicates no significance. The normalized neutralisation activities of post-vaccination
29 antisera against 30 HCVpp are represented in a heat map. Same scale was used as indicated in
30 Figure 3.
31
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33 valuable reagents. This work has been supported by the Canadian Institutes of Health Research
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35 MW, JJ, JKK, DLT, MH, JL) own stock in Aurora Vaccines, Inc., which is developing an HCV
36 vaccine for clinical and commercial use. SF's work was supported in part by NIH U19AI159840.

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Monoclonal Antibody	Epitope	Specificity	Capacity to block E1E2 binding to CD81	Neutralising activity	Ref.
AR4A	Conformational Discontinuous E1(201-206), E2(459-487), E2(543-597), E2(652-698)	E1E2	No	1a,1b,2a,3a,4a,5a,6a (HCVcc); 1a,1b,2a,2b,3a,4,5,6 (HCVpp)	[24,45]
AR5A	Conformational Discontinuous E1(201-206), E2(459-486),E2(513-597),(639-692)	E1E2	No	1a,2a,4a,5a,6a (HCVcc); 1a,1b,4,5,6 (HCVpp)	[24,45]
AR3A	Conformational Discontinuous E2(394-424), E2 (437-447), E2(523-540)	E2	Yes	1a,1b,2a,2b,3a,4,5,6 (HCVpp); 1a,1b,2a,3a,4a,5a,6a (HCVcc)	[27]
HC33.4	Linear E2(413,418,420,421)	E2	Yes	1a,2a,3a,4a,5a,6a (HCVcc)	[46]
HC84.26	Conformational Discontinuous E2(418–446); E2(611–616)	E2	Yes	1a,2a,3a,4,5,6 (HCVcc)	[23]
HC-1AM	Conformational E2 (523-540)	E2	Yes	1a,1b,2a,2b,3a (HCVcc)	[47]
H-111	Linear E1(192-205)	E1	N/A	N/A	[26]

2 N/A, not available

3

4 Table 1. HCV cross-neutralising bNabs used in this study.

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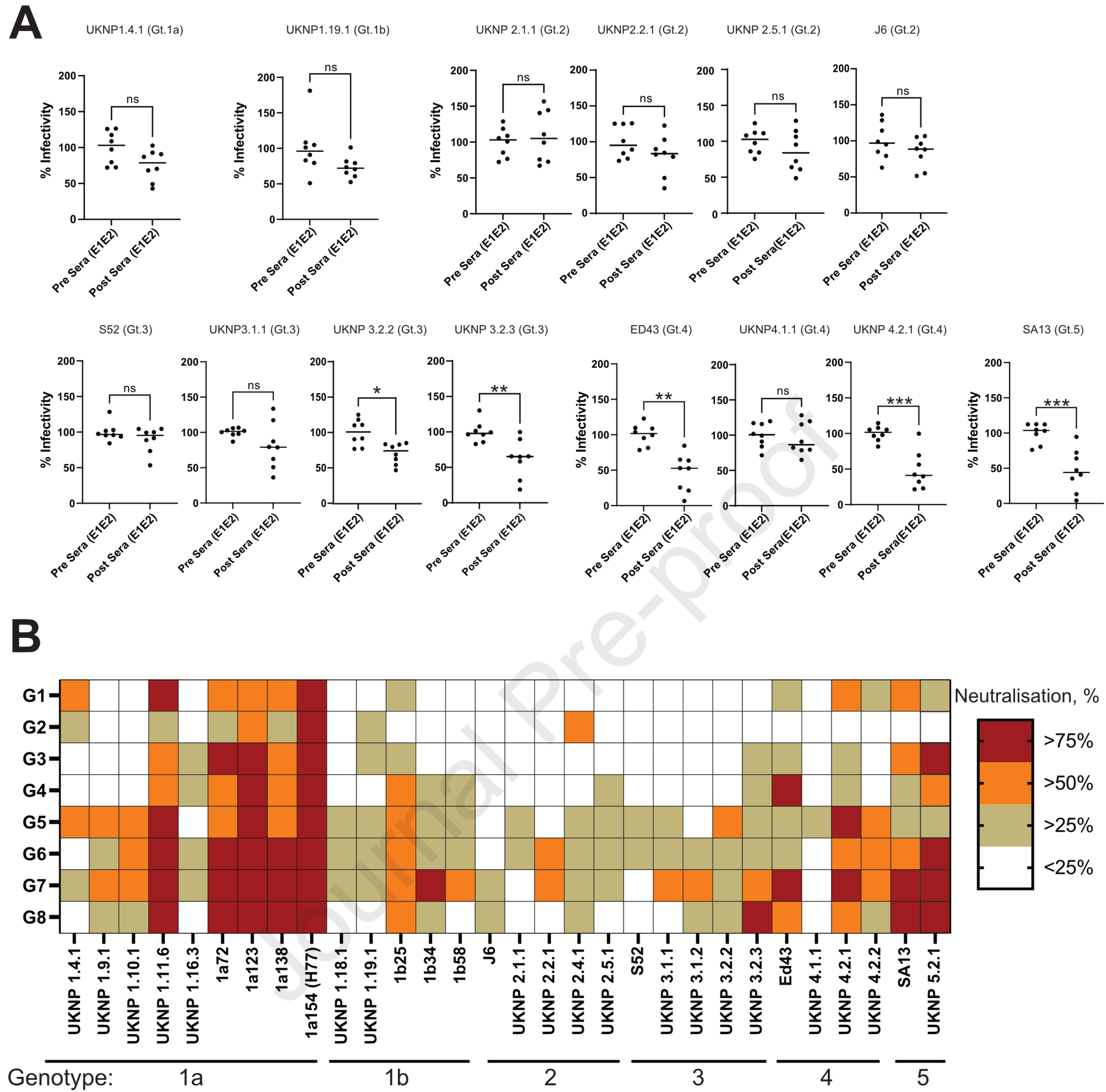

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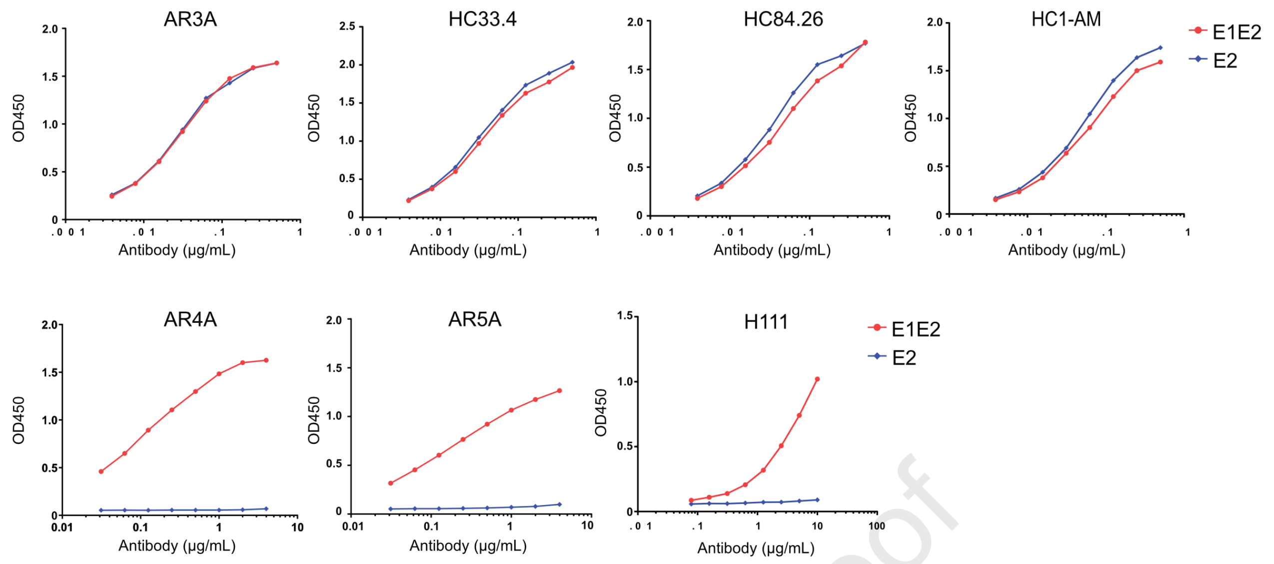

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Figure 1.

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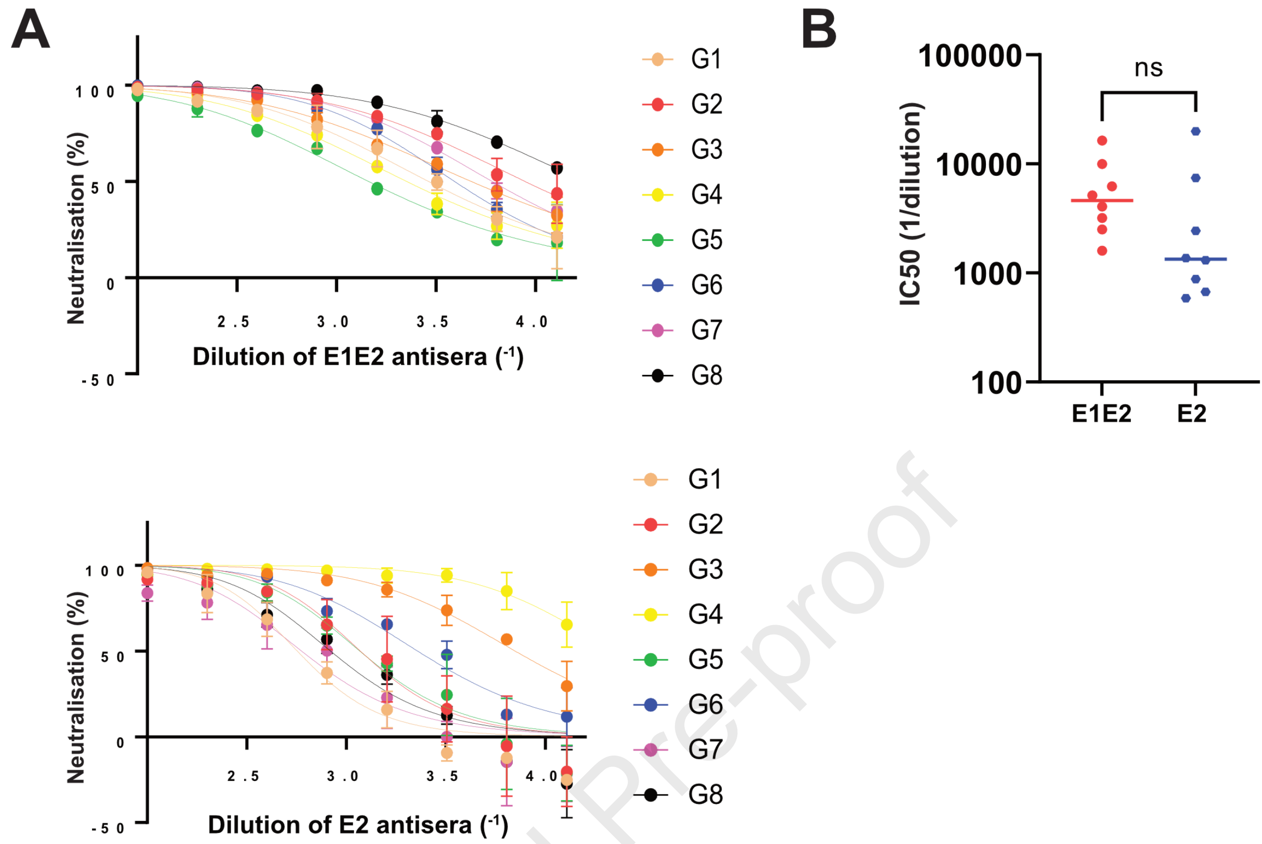

Figure 2.

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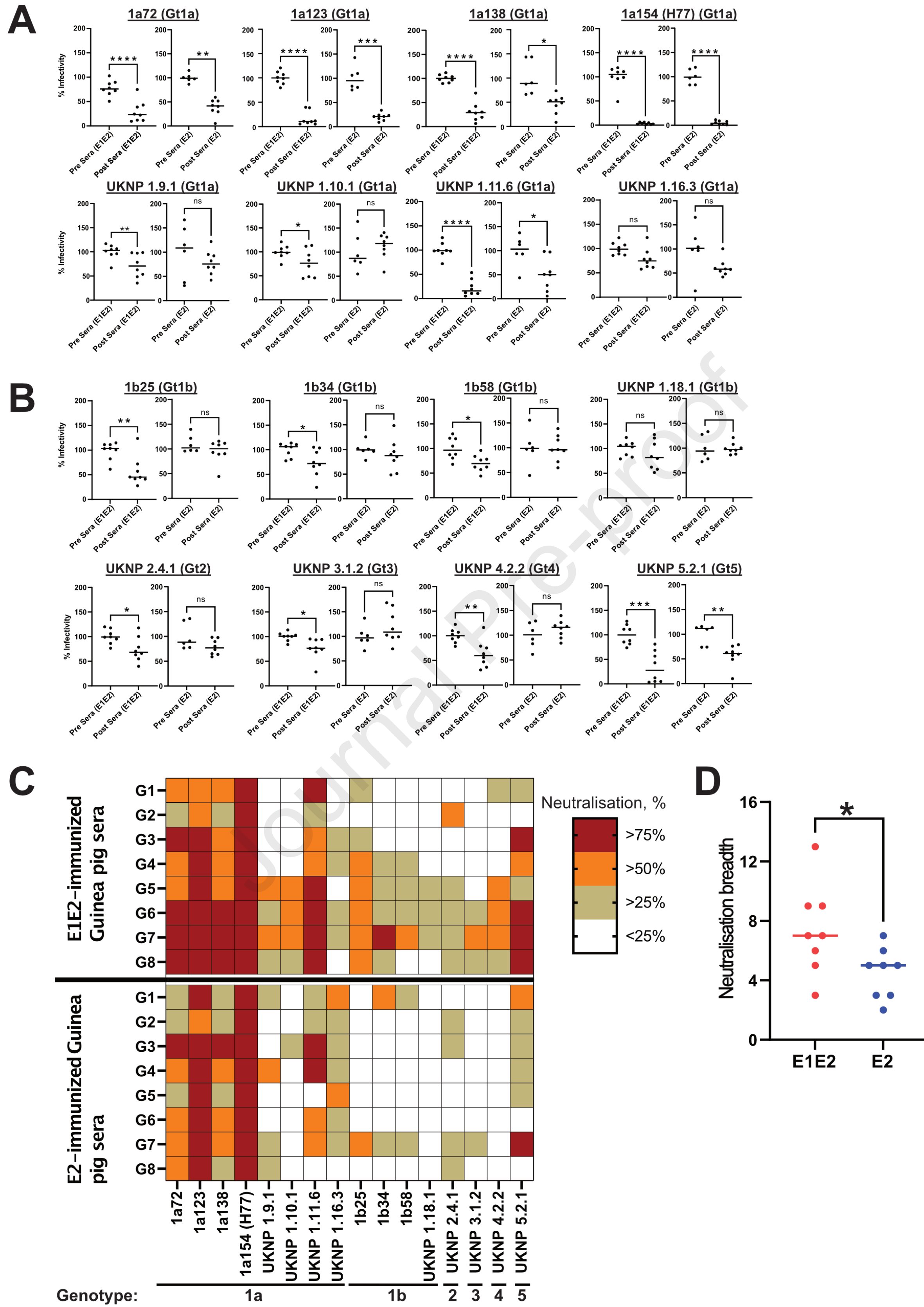

Figure 3.

Highlights:

-Glycoprotein-based HCV vaccines are immunogenic.

-Compared the antigenicities of E1E2 and E2.

-HCV E1E2-based vaccine induced a broader cross-neutralising profile.

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