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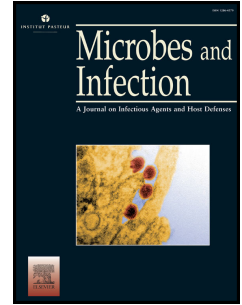
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Mice Humanized for MHC and hACE2 with High Permissiveness to SARS-CoV-2 Omicron Replication

Running title: MHC-humanized COVID-19 preclinical model

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Conflict of Interests

PC is the founder and CSO of TheraVectys. FLC, PA, BV, IF, FM, AN, KN and FA are employees of TheraVectys. LM has a consultancy activity for TheraVectys. Other authors declare no competing interests.

Keywords

Transgenesis / COVID-19 animal model / SARS-CoV-2 / Omicron variant / HLA

Abstract

Human Angiotensin-Converting Enzyme 2 (hACE2) is the major receptor enabling host cell invasion by SARS-CoV-2 via interaction with Spike. The murine ACE2 does not interact efficiently with SARS-CoV-2 Spike and therefore the laboratory mouse strains are not permissive to SARS-CoV-2 replication. Here, we generated new *hACE2* transgenic mice, which harbor the *hACE2* gene under the human keratin 18 promoter, in “HHD-DR1” background. HHD-DR1 mice are fully devoid of murine Major Histocompatibility Complex (MHC) molecules of class-I and -II and express only MHC molecules from Human Leukocyte Antigen (HLA) HLA 02.01, DRA01.01, DRB1.01.01 alleles, widely expressed in human populations. We selected three transgenic strains, with various *hACE2* mRNA expression levels and distinctive profiles of lung and/or brain permissiveness to SARS-CoV-2 replication. These new *hACE2* transgenic strains display high permissiveness to the replication of SARS-CoV-2 Omicron sub-variants, while the previously available B6.K18-ACE2^{2Pr1mn/JAX} mice have been reported to be poorly susceptible to infection with Omicron. As a first application, one of these MHC- and ACE2-humanized strains was successfully used to show the efficacy of a lentiviral-based COVID-19 vaccine.

1. Introduction

With the persistence of the coronavirus disease (COVID)-19 pandemic, the research for second-generation vaccines and drugs remains an important public health issue worldwide and therefore new research tools are still indispensable. Human Angiotensin-Converting Enzyme 2 (hACE2) is the major receptor enabling host cell invasion by SARS-CoV-2 via interaction with Spike glycoprotein [1]. The murine ACE2 ortholog does not interact properly with Spike and therefore the conventional laboratory mouse strains are not permissive to SARS-CoV-2 replication. C57BL/6 mice transgenic for *hACE2* (B6.K18-ACE2^{2PrImn/JAX}) [2] are available at JAX Laboratories. We also generated a new C57BL/6 transgenic strain carrying *hACE2* gene under the human keratin 18 (K18) promoter, namely “B6.K18-hACE2^{IP-THV}”. Compared to B6.K18-ACE2^{2PrImn/JAX} mice, the B6.K18-hACE2^{IP-THV} strain has distinctive characteristics, including higher *hACE2* mRNA expression and strong permissiveness to viral replication in the brain, in addition to the lung [3, 4]. In the present study, we generated three new K18-hACE2 transgenic mice in the “HHD-DR1” genetic background. HHD-DR1 mice are fully devoid of murine Major Histocompatibility Complex (MHC) molecules of class-I (MHC-I) and -II (MHC-II) and express only MHC-I and -II molecules from Human Leukocyte Antigen (HLA) alleles, i.e., HLA 02.01, DRA01.01, DRB1.01.01, widely expressed in human populations, notably in Caucasian, Asian and African populations. More precisely, HLA 02.01 is expressed by 50% of Caucasian, 50% of Asian and 35% of African population, while DR1 is expressed by 20% of Caucasian, 10% of Asian and 8% of African populations. Therefore, in the HHD-DR1 *hACE2* transgenic mice, antigen presentation and cognate interactions between MHC-I and -II molecules and repertoire of T-Cell Receptors for antigen (TCR) reproduce key molecular aspects of antigen presentation in humans.

HHD-DR1 mice are triple transgenic for HLA 02.01, DRA01.01, DRB1.01.01 and quintuple knocked-out for murine β 2-microglobulin (β 2m), H-2D^b, I-A α ^b, I-A β ^b and I-E β ^b. Now homozygote for all these loci, HHD-DR1 mice resulted from: (i) an initial crossing of H2D^b KO mice with mice *Cre Lox*-deleted of a complete 80 kb-long genomic region, encompassing the genes encoding I-A α ^b, I-A β ^b, I-E β ^b MHC-II molecules [5], to select mice having linked the MHC deleted region and the H-2D^b KO locus by crossing over, (ii) a subsequent crossing to the first generation HHD mice, transgenic for a fusion of HLA A2.01 and human β 2m single chain and double KO for murine β 2m and H-2D^b [6], followed by (iii) a final crossing to another HHD strain, double transgenic for HLA DRA01.01, DRB1.01.01 mice [7, 8]. Therefore, any possibility of epitope presentation by the H-2D^b heavy chain alone [9-11] or by a putative HLA DRA.01.01 - I-E β ^b hybrid MHC-II molecule is fully discarded in HHD-DR1 mice [12, 13].

The HHD-DR1 *hACE2* transgenic strains generated in the present study are: (i) permissive to SARS-CoV-2 replication, either in the lung or in the lung and brain, (ii) express high amounts of *hACE2* mRNA, comparable to those detected in the B6.K18-hACE2^{IP-THV} strain [3], and (iii) have marked permissiveness to SARS-CoV-2 Omicron replication, in addition to previous SARS-CoV-2 variants. This last feature also

1 strongly distinguishes HHD-DR1 *hACE2* mice from the B6.K18-ACE2^{2PrImn/JAX} mice [2], which have been
2 reported to have scarce permissiveness to SARS-CoV-2 Omicron replication [14]. As a first application, in
3 one of the established MHC- and ACE2-humanized strains, we demonstrated the strong efficacy of a
4 lentiviral vector-based COVID-19 vaccine candidate, the mode of action of which is largely dependent on
5 T-cell immunity [3, 4, 15, 16].

6

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2. Materials and Methods

2.1. Mice

HHD-DR1 adult males and 3-week-old female mice were purchased from Charles River (Les Oncins, Saint Germain- Nuelles, France) and housed in ventilated cages under specific pathogen-free conditions at the Institut Pasteur animal facilities. B6CBAF1 females were purchased from Janvier Labs (Janvier Labs, Le Genest-Saint-Isle). B6.K18-hACE2^{IP-THV} mice [3, 4] were bred and housed in animal facilities of Institut Pasteur. All procedures were performed in accordance with the European and French guidelines (Directive 86/609/CEE and Decree 87-848 of 19 October 1987) subsequent to approval by the Institut Pasteur Safety, Animal Care and Use Committee, protocol agreement delivered by local ethical committee (CETEA #DAP20007, CETEA #DAP200058) and Ministry of High Education and Research APAFIS#31068-2021041613059523 v1, APAFIS#24627-2020031117362508 v1, APAFIS#28755-2020122110238379 v1.

2.2. *hACE-2* transgenesis in HHD-DR1 mice

The pK18-*hACE2* plasmid, purchased from Addgene (Watertown, MA), was double digested with HpaI and XbaI enzymes, and the 6.8 kb DNA fragment generated was purified with Qiaex II gel extraction kit (Qiagen). Female HHD-DR1 mice were injected with pregnant mare serum gonadotropin and human chorionic gonadotropin with a 46h interval, and then mated with HHD-DR1 males. The fertilized one-cell embryos were collected from the oviducts. Then, 2 ng/μl of the purified transgene were microinjected into the pronuclei of HHD-DR1 zygotes according to standard protocols [17]. The microinjected eggs were then transferred into pseudo-pregnant B6CBAF1 foster mothers (Janvier Labs).

The lentiviral vector used for the alternative transgenesis method has been previously described [3]. Briefly, the human K18 promoter (GenBank: AF179904.1 nucleotide 90–2,579) was PCR amplified from A549 cells [18, 19]. The K18 promoter is completed in 3' with a modified "i6x7" intron from human K18 gene (GenBank: AF179904.1 nucleotide 2,988–3,740) which was synthesized by Genscript. As a result, the K18 IP-ThV lentiviral pFLAP plasmid includes the human K18 promoter and the i6x7 intron at 5', and the transgene is also flanked with the wildtype WPRE element at 3' (Fig. S2). To facilitate the construction, a ClaI restriction site was introduced between the promoter and the intron. The resulted segment was inserted into a pFLAP lentiviral plasmid between the MluI and BamHI sites. In the case of K18-hACE2 pFLAP plasmid, *hACE2* cDNA was introduced between the BamHI and XhoI sites by restriction/ligation. A high titer ($\approx 10^9$ TU/ml) of integrative lentiviral K18-hACE2 IP-ThV construct was used for transgenesis by means of subzonal microinjection under the pellucida of fertilized HHD-DR1 eggs, followed by transplantation of the microinjected eggs into pseudo-pregnant B6CBAF1 females. Lentiviral vector allows particularly efficient transfer of the transgene into the nuclei of the fertilized eggs [20].

The progeny was studied for integration of *hACE2* gene by using *hACE2*-forward: 5'-TCC TAA CCA GCC CCC TGT T-3' and *hACE2*-reverse: 5'-TGA CAA TGC CAA CCA CTA TCA CT-3' primers in

1 PCR, applied on genomic DNA prepared from toe clipping, as described previously [3]. PCR-identified
2 *hACE2*⁺ founders were backcrossed to HHD-DR1 mice in order to preserve the state of homozygosity for
3 HLA 02.01, DR_α01.01, DR_β01.01 humanized loci and for quintuple KO for β2m, H-2D^b, I-A_α^b, I-A_β^b and
4 I-E_α^b murine loci.

5 It is noteworthy that the female founder F0#9 gave birth to only one *hACE2*⁺ male, in the progeny of
6 which only females were *hACE2*⁺, indicating that the *hACE2* transgene is on the X chromosome. Therefore,
7 in the resulted strain, the *hACE2* transgene insertion is not lethal since males are in good state. The transition
8 to homozygosity will be easy as it can be determined by a simple progeny test with which a *hACE2*⁺ male
9 is "pseudo homozygous", and if crossed with heterozygote females, will give rise to 50% of homozygote
10 females. In addition, whether male or female, the founder F0#9 descendance will express only one
11 functional *hACE2* allele. In fact, in females this will result from methylation of the *hACE2* allele on the
12 second X chromosome.

13 2.3. SARS-CoV-2 inoculation and measurement of viral RNA contents

14 The *hACE2*⁺ offspring were inoculated i.n., with 0.3×10^5 Median Tissue Culture Infectious Dose (TCID)₅₀
15 – contained in 20 µl – of the Delta/2021/I7.2 200 [21], Omicron BA.1 [22] or Omicron BA.5 SARS-CoV-
16 2 clinical isolate. The Delta/2021/I7.2 200 is a clinical isolate of the B.1.617.2 Delta variant, originated
17 from an individual with COVID-19 who had returned to France from India in 2021 [21]. The SARS-CoV-
18 2 Omicron Pango lineage BA.1 has been isolated in Belgium from a traveler returning from Egypt in 2022
19 [22]. The SARS-CoV-2 Omicron BA.5 (hCoV-19/France/BRE-IPP34319/2022 strain) has been isolated
20 by the National Reference Centre for Respiratory Viruses (Institut Pasteur) from a human sample provided
21 by Laboratoire Alliance Anabio, (Melesse, France). For i.n. inoculation, mice were anesthetized by i.p.
22 injection of Ketamine (Imalgene, 80 mg/kg) and Xylazine (Rompun, 5 mg/kg). Animals were then housed
23 in an isolator in BioSafety Level 3 animal facilities of Institut Pasteur. Viral RNA contents were determined
24 in the organs by quantification of total or sub-genomic RNA from ECoV-2 by quantitative real-time (qRT)-
25 PCR. The Esg RT-PCR measures only active viral replication [15, 23-25]. In the context of vaccine efficacy
26 assays, we previously compared head-to-head the viral content quantification by plaque-forming unit (PFU)
27 and Esg qRT-PCR [15]. We observed that, in vaccinated animals, the PFU approach widely underestimated
28 the amounts of cultivable SARS-CoV-2 viral particles because of the high activity of anti-Spike
29 neutralizing antibodies present in the organ homogenates which are incubated with the Vero cells in vitro
30 in the PFU assay. Based on this fact and also the lack of additional information provided by the PFU
31 method, we opted for the Esg qRT-PCR approach.

32 2.4. qRT-PCR for Inflammation study

33 The qRT-PCR quantification of inflammatory mediators in the lungs and brain was performed as
34 detailed elsewhere [15] on total RNA extracted by TRIzol reagent (Invitrogen) and stored at -80°C. The

1 RNA quality was first assessed using a Bioanalyzer 2100 (Agilent Technologies). The RNA Integrity
2 Number (RIN) was 7.5 — 10.0. RNA samples were quantitated using a NanoDrop Spectrophotometer
3 (Thermo Scientific NanoDrop). One μg of each RNA was used per qRT-PCR sample in 96 wells plate.

4 2.5. ELISPOT assay and T-cell epitope mapping

5 Splenocytes from individual mice were homogenized and filtered through 70 μm -pore filters and
6 centrifuged at 300g for 10 min. Cells were treated with Red Blood Cell Lysing Buffer (Sigma) and
7 resuspended at 2.5×10^7 cells/mL in complete α -MEM medium containing 10% heat-inactivated FBS, 100
8 U/mL penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin, 1×10^{-4} M non-essential amino-acids, 10 mM Hepes, 1 mM
9 sodium pyruvate and 5×10^{-5} M of β -mercapto-ethanol. For each mouse, 100 μl of cell suspension ($2.5 \times$
10 10^5 splenocyte/well) were stimulated overnight ($\sim 16\text{h}$) with 100 μl of SARS-CoV-2 matrix peptide pools
11 (2 $\mu\text{g}/\text{ml}$ of each peptide, Mimotopes, UK), SARS-CoV-2 individual peptides (2 $\mu\text{g}/\text{ml}$) or blank control
12 (complete medium with dimethylsulfoxide) onto sterile nitrocellulose MultiScreenIP 96-well plates
13 (Millipore, Bedford, MA) coated with capture antibodies against mouse IFN- γ . Panels of 253 15-mer
14 peptides spanning the prototype Spike were organized into 32 pools. The overlapping peptide pools were
15 assigned to a 2D matrix in which each peptide was represented in 2 different peptide pools. In this way, the
16 intersection of two positive pools identifies a potentially positive peptide [26]. Fifteen-mer candidate
17 peptides at the intersections of the positive matrix-pools were tested individually for confirmation. The
18 results were expressed as IFN- γ spot forming units (SFU) per 10^6 splenocytes and were determined using
19 on an ELISpot analyzer (ImmunoSpot[®], CTL, Germany). To quantify antigen-specific responses, mean
20 spots of the control wells were subtracted from the positive wells.

21 2.6. Intracellular cytokine staining

22 Splenocytes were plated at 4×10^6 cells/well in 24-well plates and co-cultured during 6h in the presence
23 of 10 $\mu\text{g}/\text{ml}$ of appropriate peptide, 1 $\mu\text{g}/\text{ml}$ of anti-CD28 (clone 37.51) and 1 $\mu\text{g}/\text{ml}$ of anti-CD49d (clone
24 9C10-MFR4.B) mAbs (BD Biosciences). During the last 3h of incubation, a mixture of Golgi Plug and
25 Golgi Stop (BD Biosciences) were added. Cells were then collected, washed and stained for 25 min at 4°C
26 with a mixture of Near IR Live/Dead (Invitrogen), Fc γ II/III receptor blocking anti-CD16/CD32 (clone
27 2.4G2), PerCP-Cy5.5-anti-CD3 ϵ (clone 145-2C11), PE-Cy7-anti-CD4 (clone RM4-5) and BV711-anti-
28 CD8 (clone 53-6.7) mAbs (BD Biosciences or eBioscience). Cells were washed in FACS buffer,
29 permeabilized in Cytotfix/Cytoperm kit (BD Bioscience), washed twice with PermWash 1X buffer and
30 incubated with a mixture of BV421-anti-IL-2 (clone JES6-5H4), FITC-anti-TNF- α (MP6-XT22), and
31 APC-anti-IFN- γ (clone XMG1.2) mAbs (BD Biosciences), during 25 min at 4°C. Cells were then washed
32 in PermWash and then in FACS buffer and fixed with Cytotfix (BD Biosciences) overnight at 4°C. Samples

1 were acquired in an Attune NxT cytometer system (Invitrogen) and data were analyzed using FlowJo
2 software (Treestar, OR, USA).

3 2.7. Vaccine assay

4 Methods used for the measurement of Spike-specific antibody and T-cell responses were described
5 elsewhere [15]. Mice were immunized (i.m. or i.n.) with 1×10^8 TU of LV::S_{Beta-2P}, contained in 50 μ l for
6 i.m. injection and in 20 μ l for i.n injection. Mice were inoculated i.n. with 0.3×10^5 TCID₅₀ of the Delta
7 variant of SARS-CoV-2 clinical isolate and housed in filtered cages in an isolator in biosafety level 3 animal
8 facilities. The organs recovered from the infected animals were manipulated according to the approved
9 standard procedures of these facilities in a Bio-Safety Level 3 laboratory.

10 2.8. Lung histopathology

11 Histopathological study was performed on the left lung lobes, fixed in formalin for 7 days, then
12 transferred in ethanol and embedded in paraffin. Five μ m-thick sections were stained with hematoxylin and
13 eosin. Lesions were scored on images acquired in a double-blinded manner on an Axioscan Z1 Zeiss slide
14 scanner, using the Zen 2 blue edition software.

15 2.9. Statistical analyses

16 Statistical significances were determined by use of two-tailed unpaired t test or one-way Anova. When
17 indicated data were subjected to Pearson correlation coefficient analyses. All statistical analyses were
18 performed by use of Prism GraphPad v9.

19 2.10. Data availability

20 The published article includes all datasets generated and analyzed during this study. All plasmids,
21 lentiviral vectors and mouse strains generated in this study will be available under material transfer
22 agreement for research use. Further information and requests for resources and reagents should be directed
23 to and will be fulfilled by the corresponding author Laleh Majlessi (laleh.majlessi@pasteur.fr).

3. Results

3.1. *hACE-2* transgenesis in HHD-DR1 mice

Two technologically distinct transgenesis methods were performed by: (i) pronuclear DNA micro-injection of a 6.8-kb HpaI I-XbaI fragment from the pK18-hACE2 vector [2] into the fertilized HHD-DR1 eggs, or (ii) sub-zonal micro-injection of the integrative LV::pK18-hACE2 lentiviral vector under the zona pellucida of fertilized HHD-DR1 eggs. With the former approach, the DNA fragment must be directly injected into the pronuclei of the zygotes and usually multiple head-to-tail tandem copies of the transgene are inserted into a unique site of the host genome [27]. With the second technology, the nuclear transfer of the genetic material from the zona pellucida to the nuclei of fertilized HHD-DR1 eggs is assured by cell invasion followed by retro-transcription of the lentiviral recombinant RNA into double stranded proviral DNA and very efficient nuclear import of the transgene into the host nuclei. The strong transduction capacity of lentiviral vector is related to a three-stranded DNA structure, known as a "DNA flap", which is formed following retro-transcription of RNA into double stranded proviral DNA [28]. In net opposition to the first approach, with the lentiviral technology, the transgene is not in tandem and is generally integrated at numerous potential integration sites [29].

The progenies were studied for integration of *hACE2* gene. The *hACE2*⁺ F0#8 male, F0#9 female and F0#11 female founders were identified in the DNA fragment-based transgenesis. The *hACE2*⁺ F30# male and F0#47 male founders were identified in the lentiviral vector-based transgenesis (Table S1). To stabilize the progenies, each founder was backcrossed to HHD-DR1 non-*hACE2* transgenic mice to preserve the homozygous status for: (i) HLA 02.01, DRA01.01 and DRB1.01.01 humanized loci, and (ii) KO genes encoding for murine $\beta 2m$, H-2D^b, I-A _{α} ^b, I-A _{β} ^b and I-E _{α} ^b.

3.2. Permissiveness of the *hACE2*⁺ offspring to SARS-CoV-2 replication

First, the *hACE2*⁺ offspring of the founders generated by the DNA fragment-based transgenesis were evaluated for their permissiveness to SARS-CoV-2 replication. Individuals from the previously described B6.K18-hACE2^{IP-THV} strain [3, 4] were also included as positive controls. Mice were inoculated intranasally (i.n.) with 0.3×10^5 TCID₅₀ of a Delta SARS-CoV-2 clinical isolate [21]. Four days post-inoculation (dpi), viral loads were determined in the lung and brain by E_{CoV-2} (E)- or sub-genomic E (Esg)-specific quantitative real-time (qRT)-PCR. It is well established that the Esg qRT-PCR is an indicator of only active viral replication [24, 25, 30]. We previously established the good correlation between viral contents determined by PFU and Esg qRT-PCR methods in non-immune mice and hamsters. [3, 15]. The Esg qRT-PCR method is also technically easier to perform, especially in brain homogenates, which are very rich in fat material and difficult to culture in PFU assay.

The progeny of the founder F0#8 was not permissive to SARS-CoV-2 replication (Fig. 1A). In contrast, the progeny of the founder F0#9, hereafter named "HHD-DR1.ACE2^{Hu1}", displayed a notable lung – but

1 not brain – permissiveness to SARS-CoV-2 replication. The progeny of the founder F0#11, hereafter named
2 “HHD-DR1.ACE2^{Hu2}”, was not only markedly permissive to SARS-CoV-2 replication in their lung (Fig.
3 1A, top) but also displayed significant Esg RNA contents in their brain (Fig. 1A, bottom). The viral RNA
4 contents in the lungs of HHD-DR1.ACE2^{Hu1} and HHD-DR1.ACE2^{Hu2} mice were statistically comparable
5 to those of the previously described B6.K18-hACE2^{IP-THV} strain. The main characteristics of these strains
6 are recapitulated in the Table S1. Comparative assessment of *hACE2* mRNA transcription showed that the
7 non-permissive F0#8 offspring had detectable but the lowest *hACE2* mRNA expression level in the lung
8 and brain (Fig. 1B). Such transcription levels were obviously not sufficient to allow productive SARS-
9 CoV-2 infection. Analysis of the *hACE2* mRNA transcription levels in both lung and brain established the
10 following hierarchy: B6.K18-hACE2^{IP-THV} = HHD-DR1.ACE2^{Hu2} > HHD-DR1.ACE2^{Hu1} > F0#8 offspring
11 (Fig. 1B).

12 In the lentiviral vector-based transgenesis, the progeny of the founder F0#30 was not permissive to
13 SARS-CoV-2 Delta replication, whereas the progeny of the founder F0#47, hereafter named "HHD-
14 DR1.ACE2^{Hu3}", displayed strong lung and brain permissiveness to SARS-CoV-2 Delta replication (Fig.
15 2A). Comparative assessment of the *hACE2* mRNA transcription levels in the lung and brain (Fig. 2B) of
16 the F0#30 offspring and HHD-DR1.ACE2^{Hu3} mice established a positive correlation between the *hACE2*
17 transgene expression levels and the permissiveness to viral replication. Therefore, based on the transgenesis
18 method, *hACE2* expression levels and permissiveness of the organs to SARS-CoV-2 replication, we defined
19 three distinct humanized HHD-DR1.ACE2^{Hu} strains (Table S1).

20 HHD-DR1.ACE2^{Hu3} mice, which had the highest viral loads notably in the brain, were further
21 characterized for their infection-mediated inflammation after inoculation with SARS-CoV-2 Delta. At 4
22 dpi, as evaluated by qRT-PCR study of 20 pro- or anti-inflammatory analytes, applied to RNA extracted
23 from total lung homogenate, no sizable modification of the inflammatory transcriptome was observed in
24 the lungs of the permissive HHD-DR1.ACE2^{Hu3} mice, compared to the non-permissive F0#30 offspring
25 (Fig. 2C, left). In net contrast, at this time point, the brain of the permissive HHD-DR1.ACE2^{Hu3} mice
26 displayed a marked inflammatory status, compared to the non-permissive F0#30 offspring (Fig. 2C, right).
27 This brain inflammation was characterized by statistically significant upregulation of IFN- α , IFN- γ , TNF-
28 α , IL-1 β , IL-5, IL-6, IL-12p40, CCL2, CCL3, CCL5, CXCL9 and CXCL10 (Fig. 2C, right), in a correlative
29 manner (Fig. 3D). No correlation was observed between IL-2 and IL-4 expression and the former.

30

31 3.3. Permissiveness of HHD-DR1.ACE2 mice to replication of SARS-CoV-2 Omicron 32 sub-variants

33 The previously available B6.K18-ACE2^{2PrImn/JAX} transgenic mice are much less permissive to Omicron
34 replication than to the other previously emerged SARS-CoV-2 variants [14]. In net contrast, the B6.K18-

1 hACE2^{IP-THV} mice, that we previously generated [3], allow the replication of Omicron as efficiently as other
2 SARS-CoV-2 variants like Delta [4]. Correlatively, B6.K18-hACE2^{IP-THV} mice express significantly higher
3 amounts of *hACE2* mRNA in lungs and brain than B6.K18-ACE2^{2PrImn/JAX} [3].

4 Here, we first evaluated the permissiveness of the three HHD-DR1.ACE2^{Hu1/2/3} lineages to SARS-CoV-
5 2 Omicron BA.1 sub-variant. As determined at 4 dpi, by SARS-CoV-2 E- or Esg-specific qRT-PCR, the
6 lungs of all three HHD-DR1.ACE2^{Hu1/2/3} strains were strongly permissive to the replication of Omicron
7 BA.1 (Fig. 3A). The brain viral RNA content of HHD-DR1.ACE2^{Hu1} was not studied here, as we previously
8 determined the brain unpermissiveness in this strain to SARS-CoV-2 replication (Fig. 1A). In HHD-
9 DR1.ACE2^{Hu2} mice, the brain E viral RNA content was relatively weak and no brain Esg viral RNA was
10 detected (Fig. 3B). In contrast, substantial Omicron BA.1 replication was detected in the brain of HHD-
11 DR1.ACE2^{Hu3} mice which, like the previously described B6.K18-hACE2^{IP-THV} mice, resulted from
12 lentiviral vector-based transgenesis (Fig. 3B).

13 Analysis of *hACE2* mRNA expression levels established the following hierarchy: HHD-DR1.ACE2^{Hu3}
14 = HHD-DR1.ACE2^{Hu2} > HHD-DR1.ACE2^{Hu1} in the lungs (Fig. 3C). In the brain, we observed a tendency
15 to a higher *hACE2* mRNA expression in HHD-DR1.ACE2^{Hu3} compared to HHD-DR1.ACE2^{Hu2}. Although
16 this trend did not reach significance, it was associated with a significantly higher permissiveness of HHD-
17 DR1.ACE2^{Hu3} mice to Omicron BA.1 mice (Fig. 3D).

18 Like the B6.K18-hACE2^{IP-THV} mice, the HHD-DR1.ACE2^{Hu2} and HHD-DR1.ACE2^{Hu3} mice were also
19 strongly permissive to replication of Omicron BA.5 (Fig. 3E). In contrast to the brains of HHD-
20 DR1.ACE2^{Hu2} mice, which were not permissive to replication of Omicron BA.5, the brain of 3 out of 5
21 HHD-DR1.ACE2^{Hu3} mice had high cerebral Omicron BA.5 loads (Fig. 3F).

22 Therefore, permissiveness to the two Omicron sub-variants tested was observed in both lungs and brains
23 of the MHC-humanized mice, reaching levels as high or even higher than in B6.K18-hACE2^{IP-THV} mice.

24 25 3.4. Use of HHD-DR1.ACE2^{Hu1} to evaluate the efficacy of a lentiviral vector-based 26 COVID-19 vaccine candidate

27 To conduct a vaccine efficacy evaluation in one of the HHD-DR1.ACE2^{Hu} strains, we first studied, in
28 HHD-DR1 mice, the immunogenicity of the lentiviral vector-based COVID-19 vaccine candidate, namely
29 "LV::*S_{Beta-2P}*" [3, 4, 15, 16]. LV::*S_{Beta-2P}* encodes the full length sequence of Spike from the SARS-CoV-2
30 Beta variant, stabilized by K^{986P} and V^{987P} substitutions in the S2 domain [4].

31 As we previously established that an intramuscular (i.m.) prime followed by an i.n. boost was the most
32 efficacious scheme to achieve anti-SARS-CoV-2 protection, HHD-DR1 mice were primed (i.m.) with 1 ×
33 10⁸ Transduction Units (TU) of LV::*S_{Beta-2P}* at wk 0 and then boosted (i.n.) with the same amount of this

1 vaccine at wk 8. Control mice received an empty LV vector (Ctrl LV). The LV::S_{Beta-2P}-immunized mice
2 mounted high titers of serum anti-Spike IgG, albeit weaker than those detected in the conventional C57BL/6
3 mice (Fig. S2) (see Discussion).

4 T-cell immunogenicity of LV::S_{Beta-2P} in HHD-DR1 mice was studied by IFN- γ ELISPOT epitope
5 mapping of Spike, based on a 2D-peptide pool matrix [26], using splenocytes (Fig. 4A, B). To do so, panels
6 of 253 15-mers spanning the full-length Spike protein were organized into 32 pools, assigned to a 2D matrix
7 in which each peptide was represented in two distinct pools. In this approach, the intersection of two
8 immunogenic pools predicts presence of a potentially positive epitope. By analyzing 5 individual LV::S_{Beta-2P}-
9 vaccinated HHD-DR1 mice, we identified the positive IV, XII, XVI, XX, XXIII and XXVII peptide
10 pools (Fig. 4A, B). The intersection peptides, as well as S:511-525 (#103, VVLSFELLHAPATVC) and
11 S:976-990 (#196, VLNDILSRLDKVEAE), previously identified in humans by others [31, 32], were then
12 individually tested in IFN- γ ELISPOT (Fig. 4C). This assay clearly confirmed the immune-recognition of
13 S:316-330 (#64, SNFRVQPTESIVRFP), S:511-525 (#103, VVLSFELLHAPATVC), S:536-555 (#108 and
14 #109, NKCVMNFNGLTGTGVLTES), S:816-830 (#164, SFIEDLLFNKVTLAD), and S:976-990 (#196,
15 VLNDILSRLDKVEAE) by T cells from HHD-DR1 vaccinated mice. Interestingly, none of these identified
16 T-cell epitopes overlapped with regions where mutations occurred in the Omicron Spike. This suggests that
17 LV::S_{Beta-2P}-induced T cells will recognize Omicron-infected host cells as efficiently as those infected with
18 the ancestral virus.

19 Intracellular cytokine staining (ICS) and cytometric analysis performed with splenocytes from LV::S_{Beta-2P}-
20 vaccinated HHD-DR1 mice, stimulated in vitro with these identified peptides, showed that: (i) S:511-
21 525 (#103, VVLSFELLHAPATVC) was recognized by CD4⁺ T cells and thus restricted by DRA01.01 +
22 DRB1.01.01 MHC-II molecule (Fig. 4D, E, and Fig. S3). In parallel, S:316-330 (#64,
23 SNFRVQPTESIVRFP), S:511-525 (#103, VVLSFELLHAPATVC) and S:536-555 (#108 and #109,
24 NKCVMNFNGLTGTGVLTES) were recognized by CD8⁺ T cells and thus contain epitopes restricted by
25 HLA 02.01 MHC-I molecule (Fig. 4D, F, and Fig. S3). Note that S:511-525 (#103, VVLSFELLHAPATVC)
26 contains both MHC-I- and -II-restricted epitopes. Other peptides detected positive in the ELISPOT (Fig.
27 4C) were not identifiable by ICS, the most likely because of the weaker sensitivity of ICS compared to
28 ELISPOT. Further analysis of these immunogenic regions by the SYFPEITHI software
29 (<http://www.syfpeithi.de/bin/MHCServer.dll/EpitopePrediction.htm>) also indicated the presence of high-
30 scoring predicted T-cell epitopes in all these identified regions (Table 2S).

31 Given the emergency of making research tools available to the community in the context of the
32 pandemic, the transgenic mouse colony that most quickly gave us enough age- and sex-matched animals,
33 i.e., HHD-DR1.ACE2^{Hu1} mice, was used to perform a protection experiment. HHD-DR1.ACE2^{Hu1} mice
34 were primed i.m. at wk 0 and boosted i.n. at wk 3 with LV::S_{Beta-2P}, as detailed above [3, 4, 15, 16] or Ctrl
35 LV, and were then challenged i.n. at wk 5 with 0.3×10^5 TCID₅₀ of SARS-CoV-2 Delta variant. LV::S_{Beta-}

1 2P prime-boost vaccination of HHD-DR1.ACE2^{Hu1} mice led to statistically significant protection against
2 SARS-CoV-2 replication in the lungs, as determined at 4 dpi (Fig. 5A, B). At this time point, cytometric
3 analysis of the lung innate immune cell subsets detected significantly lower percentages of CD11b^{int}
4 NKp46⁺ Natural Killer (NK) cells and CD11b⁺ Ly6G⁺ neutrophils among the CD45⁺ cells in the vaccinated
5 HHD-DR1.ACE2^{Hu1} mice, than in the control animals which received Ctrl LV (Fig. 5C, D). NK and
6 neutrophils have been associated with enhanced lung inflammation and poor COVID-19 outcome and their
7 decrease is a biomarker of good prognosis in humans [33, 34]. The reason why the percentages of lung
8 neutrophils in unprotected Ctrl LV-injected mice were reduced compared with unvaccinated, unprotected
9 controls is not known and requires further investigation.

10 Histological examination of SARS-CoV-2-inoculated mice pre-treated with Ctrl LV revealed a mild to
11 moderate inflammation of the lung, with zones of interstitial infiltration sometimes accompanied by
12 alveolar exudates (Fig. 6A). Although the bronchiolar epithelium integrity was preserved, degenerative
13 lesions of epithelium cells could be seen in the inflammatory areas. The histological scores are recapitulated
14 in the heatmap (Fig. 6B). In vaccinated HHD-DR1.ACE2^{Hu1} mice, the alveolo-interstitial syndrome was
15 more limited in size, less severe (minimal to mild), and accompanied by discreet alterations of the
16 bronchiolar epithelium.

17 Therefore, virological, immunological and histopathological criteria showed that HHD-DR1.ACE2^{Hu}
18 mice can be used to evaluate protective potential of COVID-19 vaccine candidates.

19

4. Discussion

We generated three new transgenic mice expressing: (i) hACE2 under the human K18 promoter, and (ii) only human – but not murine – MHC-I and -II molecules from HLA 02.01, DRA01.01, DRB1.01.01 alleles, widely expressed in human populations. The three strains, namely “HHD-DR1.ACE2^{Hu1/2/3}”, are permissive to SARS-CoV-2 replication, yet each displays distinctive characteristics in terms of lung- and/or brain-specific permissiveness to viral replication, and their site-specific *hACE2* mRNA transcription levels. Similar to B6.K18-hACE2^{IP-THV} strain [3], one particularity of the HHD-DR1.ACE2^{Hu1/2/3} strains is their marked permissiveness to the replication of Omicron sub-variant(s). The high *hACE2* mRNA transcription notably in the HHD-DR1.ACE2^{Hu3} generated by lentiviral based transgenesis, is correlated with their permissiveness to SARS-CoV-2 Omicron replication. The reasons why the two independent lentiviral-based transgenes, which gave rise to B6.K18-hACE2^{IP-THV} and HHD-DR1.ACE2^{Hu3} strains, led to high lung and brain transgene expression are not clear. It is plausible that the lentiviral insertion sites in the host chromosome(s) lead to more accessible and more active transcription of the transgene. In addition, the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE), present in the genetic material delivered by the lentiviral vector, enhances the transgene expression and transgenic mRNA export from the host nuclei [35], which is not the case for the transgene inserted by the conventional, DNA fragment-based transgenesis.

Quantitative comparison of *hACE2* transcript levels in *hACE2* transgenic mice with physiological levels of this transcript in humans is very important but also very challenging. The tissue expression pattern and level of *ACE2* mRNA has been established and compared in 31 tissues [36, 37]. Small intestine, testis, kidney, heart, thyroid, and adipose tissue have the highest *ACE2* expression levels, lung, colon, liver, bladder, and adrenal gland have medium *ACE2* expression levels, while blood, spleen, bone marrow, brain, blood vessels, and muscle display the lowest *ACE2* expression levels [37]. These amounts were determined as transcripts per kilobase of exon pattern per million mapped reads, by the RNA-Seq approach [36], and therefore very difficult to compare to the *hACE2* mRNA levels we determined in transgenic mice by qRT-PCR.

Among all SARS-CoV-2 variants of concern, Omicron harbors the highest number, i.e., 37 mutations in its Spike glycoprotein [38-40]. Among these mutations, 15 are located inside the Receptor-Binding Domain (RBD). Inside the Omicron RBD, the 96 amino acid-long Receptor-Binding Motif (RBM) carries 10 mutations, i.e., N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y and Y505H, among which, G446S, E484A, Q493R, G496S, Q498R and Y505H are unique to Omicron and are believed to reinforce by threefold the binding of its Spike to hACE2, compared to the Spike of ancestral or Delta SARS-CoV-2. This strengthened interaction occurs via increased electrostatic and hydrophobic interactions, and formation of ACE2 salt bridge and ACE2 hydrogen bond, improving the potential of Omicron to invade the host cells [38-40]. On the other hand, the Omicron Spike protein is less efficiently

1 cleaved into S1 and S2 subunits, reducing its fusogenic potential and thereby its pathogenicity [41, 42].
2 Other mutations elsewhere in the genome of Omicron may also be responsible its reduced pathogenic
3 potential. Despite the increased affinity of Omicron RBM interaction with hACE2, replication of Omicron
4 variants in the upper and lower respiratory airways of B6.K18-ACE2^{2PrImn/JAX} transgenic mice is 3-to-5
5 log₁₀ lower compared to the earlier SARS-CoV-2 variants [14]. The notable permissiveness of HHD-
6 DR1.ACE2^{Hu1/2/3} strains to Omicron, is probably largely explained by their high expression of *hACE2*
7 transgene transcription levels, because SARS-CoV-2 enters the host cells mainly via hACE2, although
8 alternative routes of entry such as the transmembrane protease serine 2 (TMPRSS2) or the cathepsin-
9 dependent endocytic pathway have been reported [43]. However, these are unlikely to be different in the
10 B6.K18-ACE2^{2PrImn/JAX}, B6.K18-ACE2^{IP-THV} and HHD-DR1.ACE2^{Hu1/2/3} mouse strains, which harbor a
11 common C57BL/6 genetic background.

12 As a first application of these strains, we demonstrated in HHD-DR1.ACE2^{Hu1} mice the efficacy of a
13 lentiviral vector-based COVID-19 vaccine candidate. This vaccine induced anti-Spike serum IgG, CD4⁺
14 and CD8⁺ T cells [3, 4, 15, 16]. In the LV::*S_{Beta-2P}*-immunized HHD-DR1 mice, the titers of anti-Spike IgG
15 were lower than in their LV::*S_{Beta-2P}*-immunized wild type C57BL/6 counterparts. This may be linked to
16 the absence of free murine β 2m in HHD-DR1 mice, which harbor only the human β 2m covalently linked
17 to the HLA 02.01. β 2m contributes to the formation of “neonatal Fc receptor” (FcRn) for IgG [44]. In the
18 absence of association with β 2m, the FcRn heavy chain remains sequestered and unfunctional in endoplasmic
19 reticulum. Increased clearance of IgG in β 2m KO mice has suggested that FcRn protects IgG from
20 degradation, and is thus critical in maintaining IgG levels in the circulation [45]. In a non-mutually-
21 exclusive manner, it is also possible that the size of the T-cell compartment in HHD-DR1 mice is reduced
22 compared to wild-type mice. In this case, the CD4⁺ T-cell helper function may be attenuated, resulting in
23 weaker antibody responses. Spike epitope mapping in LV::*S_{Beta-2P}*-immunized HHD-DR1 mice allowed for
24 the identification of both MHC-I- and II-restricted immunogenic Spike regions for humans. Furthermore,
25 LV::*S_{Beta-2P}*-vaccinated HHD-DR1^{Hu1} mice were protected from a challenge by SARS-CoV-2, as evidenced
26 by virological, immunological and histopathological criteria, validating the HHD-DR1^{Hu} preclinical model
27 for immunogenicity and vaccine evaluation investigations.

28 HHD-DR1.ACE2^{Hu1/2/3} murine strains provide new pre-clinical research tools for SARS-CoV-2
29 infection and research and development of new COVID-19 drugs or vaccines. These strains will notably
30 pave the way for: (i) identification of SARS-CoV-2-derived epitopes recognized by human T cells, (ii)
31 investigation of T-cell immunogenicity of new-generation COVID-19 vaccines, and (iii) evaluation of
32 protective potential of new-generation COVID-19 vaccine, in small rodent models, in which the MHC-I
33 and -II molecules are from widely expressed HLA alleles in human populations. In addition, since
34 coronaviruses can infect the brain [46-48], the HHD-DR1.ACE2^{Hu2/3} strains provide also valuable pre-

- 1 clinical models for investigation of immune protection of the central nervous system against SARS-CoV-
- 2 2.
- 3

Journal Pre-proof

1

2 **Figure Legends**

3 Fig. 1. Permissiveness of HHD-DR1.ACE2^{Hu1} and HHD-DR1.ACE2^{Hu2} transgenic mice to SARS-CoV-
 4 2 replication. *hACE2*⁺ offspring from the founders #8, #9 (HHD-DR1.ACE2^{Hu1}) or #11 (HHD-
 5 DR1.ACE2^{Hu2}), resulting from a conventional DNA-based transgenesis, or B6.K18-hACE2^{IP-THV} positive
 6 control mice were inoculated i.n. with 0.3×10^5 TCID₅₀/mouse of SARS-CoV-2 Delta variant. **A.** Lung and
 7 brain viral RNA contents were determined by E- (top) or sub-genomic Esg- (bottom) specific qRT-PCR at
 8 4 dpi. Red lines indicate the qRT-PCR detection limits. **B.** Quantification of *hACE-2* mRNA in the lung
 9 and brain of the same mice. Statistical significance was evaluated by one-way Anova (*= $p < 0.05$, **= $p <$
 10 0.01 , ***= $p < 0.001$).

11

12 Fig. 2. Permissiveness of HHD-DR1.ACE2^{Hu3} transgenic mice to SARS-CoV-2 replication and
 13 inflammatory status of their lungs and brain after SARS-CoV-2 inoculation. *hACE2*⁺ offspring from the
 14 founders #30 or #47 (HHD-DR1.ACE2^{Hu3}), resulting from a lentiviral vector-based transgenesis, were
 15 inoculated i.n. with 0.3×10^5 TCID₅₀/mouse of SARS-CoV-2 Delta variant. **A.** Lung and brain viral RNA
 16 contents were determined by E-specific or sub-genomic Esg-specific qRT-PCR at 4 dpi. **B.** Quantification
 17 of *hACE-2* mRNA in the lung and brain of the same mice. **C.** Heatmaps represent log₂ fold change in
 18 cytokine and chemokine mRNA expression in the lungs or brain of the same mice ($n = 4-5$ /group). Data
 19 were normalized versus untreated controls. Statistical significance was evaluated by Mann-Whitney test (ns
 20 = not significant, * = $p < 0.05$, ** = $p < 0.01$). **D.** Pearson correlation coefficient of the analytes studied in
 21 the brain of SARS-CoV-2 Delta-inoculated HHD-DR1.ACE2^{Hu3} mice.

22

23 Fig. 3. Permissiveness of HHD-DR1.ACE2^{Hu1/2/3} mice to replication of SARS-CoV-2 Omicron BA.1 or
 24 BA.5 sub-variants. HHD-DR1.ACE2^{Hu1/2/3} mice or B6.K18-hACE2^{IP-THV} positive control mice were
 25 inoculated i.n. with 0.3×10^5 TCID₅₀/mouse of SARS-CoV-2 Omicron BA.1 or BA.5. **A.** Lung and **B.**
 26 brain viral RNA contents were determined by E-specific (top) or sub-genomic Esg-specific (bottom) qRT-
 27 PCR at 4 dpi with Omicron BA.1. **C-D.** Comparative quantification of *hACE-2* mRNA in the lung and
 28 brain of individual B6.K18-hACE2^{IP-THV} or HHD-DR1.ACE2^{Hu1/2/3} transgenic mice. **E-F.** Lung and brain
 29 viral RNA contents were determined by E-specific (top) or sub-genomic Esg-specific (bottom) qRT-PCR
 30 at 4 dpi with Omicron BA.5. Statistical significance was evaluated by one-way Anova (*= $p < 0.05$, **= $p <$
 31 0.01). ND = Not determined.

32

33 Fig. 4. Spike T-cell epitope mapping in HHD-DR1 mice vaccinated with LV::S_{Beta-2P}. HHD-DR1 mice
 34 were primed (i.m.) at wk 0 and boosted (i.n.) at wk 8 with a control empty lentiviral vector (Ctrl LV) or
 35 LV::S_{Beta-2P}. **A.** Panels of 253 15-mers spanning the Spike protein, were organized into 32 pools (I to
 36 XXXII) containing no more than 16 individual peptides. The peptide pools were assigned to a 2D matrix
 37 system in which each peptide was represented in two different pools. The intersection of two positive pools
 38 identified potentially positive peptides. **B.** Splenocytes from HHD-DR1 mice ($n = 5$) immunized with
 39 LV::S_{Beta-2P} or a Ctrl LV were harvested at wk 2 post boost and were analyzed in an IFN- γ ELISPOT after
 40 stimulation with each of the 32 distinct I to XXXII pools. Heat map representing the number of IFN- γ -
 41 producing cells per million of splenocytes responding to each peptide pool. For each peptide pool, the
 42 background generated by the splenocytes from Ctrl LV-injected mice was subtracted. **C.** Intersection or
 43 previously identified peptides were tested individually in ELISPOT. The threshold of positivity was defined
 44 by a twofold increase in the number of spots upon stimulation with the peptide, compared to the average
 45 number of spots for the same splenocyte sample in the medium alone. **D-F.** The positive peptides identified
 46 were used in CD4 and CD8 surface staining and IFN- γ , IL-2, and TNF- α ICS and cytometric analysis to

1 determine the T-subset specific to the corresponding epitopes. **D.** Gating strategy, **E-F.** IL-2/TNF- α
2 expressing cells within the IFN- γ^+ CD4 $^+$ (**E**) or CD8 $^+$ (**F**) T cells after stimulation with the indicated Spike-
3 derived peptides or a negative control peptide. The numbers in the upper right quadrant of the dot plots
4 indicate the percentage of the IL-2 $^+$ TNF- α^+ IFN- γ^+ population within the CD4 $^+$ (**E**) or CD8 $^+$ (**F**) T cells.
5 See also Fig. S3.

6
7 **Fig. 5.** Use of HHD-DR1.ACE2^{Hu1} mice to evaluate the protective capacity of LV::S_{Beta-2P} vaccine
8 candidate against SARS-CoV-2. **A.** Timeline of vaccination and SARS-CoV-2 challenge. **B.** HHD-
9 DR1.ACE2^{Hu1} mice were untreated or primed (i.m.) at wk 0 and boosted (i.n.) at wk 3 with 1×10^8
10 TU/mouse of LV::S_{Beta-2P} or Ctrl LV, before i.n. challenge at wk 5 with 0.3×10^5 TCID₅₀ of SARS-CoV-2
11 Delta variant. Lung viral Esg-specific RNA contents was evaluated by qRT-PCR at 4 dpi. Red lines indicate
12 the detection limits. **C.** Cytometric detection of neutrophils or NK cells in the lungs of untreated, Ctrl-Lenti-
13 , or LV::S_{Beta-2P}-vaccinated and challenged mice at 4 dpi. **D.** Percentages of neutrophils or NK cells in the
14 lungs of untreated, Ctrl LV-, or LV::S_{Beta-2P}-vaccinated and challenged mice at 4 dpi. Percentages were
15 calculated versus total lung live CD45 $^+$ cells. Statistical significance was evaluated by one-way Anova test
16 (*= $p < 0.05$, **= $p < 0.01$).

17
18 **Fig. 6.** Lung histopathology of unvaccinated or LV::S_{Beta-2P}-vaccinated HHD-DR1.ACE2^{Hu1} mice after
19 inoculation with SARS-CoV-2. Mice are those detailed in the Fig. 5. **A.** Examples of haematoxylin-eosin
20 saffron staining of whole-lung sections (left, scale bar: 500 μ m) and close-up views (right, scale bar: 100
21 μ m), at 4 dpi. Stars mark areas of minimal (bottom) or mild-to-moderate (top) infiltration. In the blow-up
22 panels, red arrows indicate bronchiolar epithelia. While in the bottom blow-up the epithelium is subnormal,
23 in the top panel epithelial degenerative lesions can be seen, e.g., perinuclear clear spaces (green arrow),
24 associated with cells remnants and proteinaceous material in the bronchiolar lumen. **B.** Heatmap
25 representing the histological scores for: (i) inflammation seriousness, (ii) interstitial syndrome, and (iii)
26 alveolar syndrome, (iv) alteration of bronchial epithelium, and (v) percent of abnormal lung zones.

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4 Declaration of competing interest

5 PC is the founder and CSO of TheraVectys. FLC, PA, BV, IF, FM, AN, KN and FA are employees of
6 TheraVectys. LM has a consultancy activity for TheraVectys. Other authors declare no competing interests.

8 Author Contributions

9 Funding: MB, PC, Study concept and design: MB, PC, FL, FLV, LM, acquisition of data: FLC, PA, MB,
10 BV, IF, KN, LM, lung histopathology: FG, DH, transgenesis experiments and breeding: SC, DC, YS, FLV,
11 construction and production of lentiviral vector for transgenesis: FM, AN, CB, FA, analysis and
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25

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Figure 1

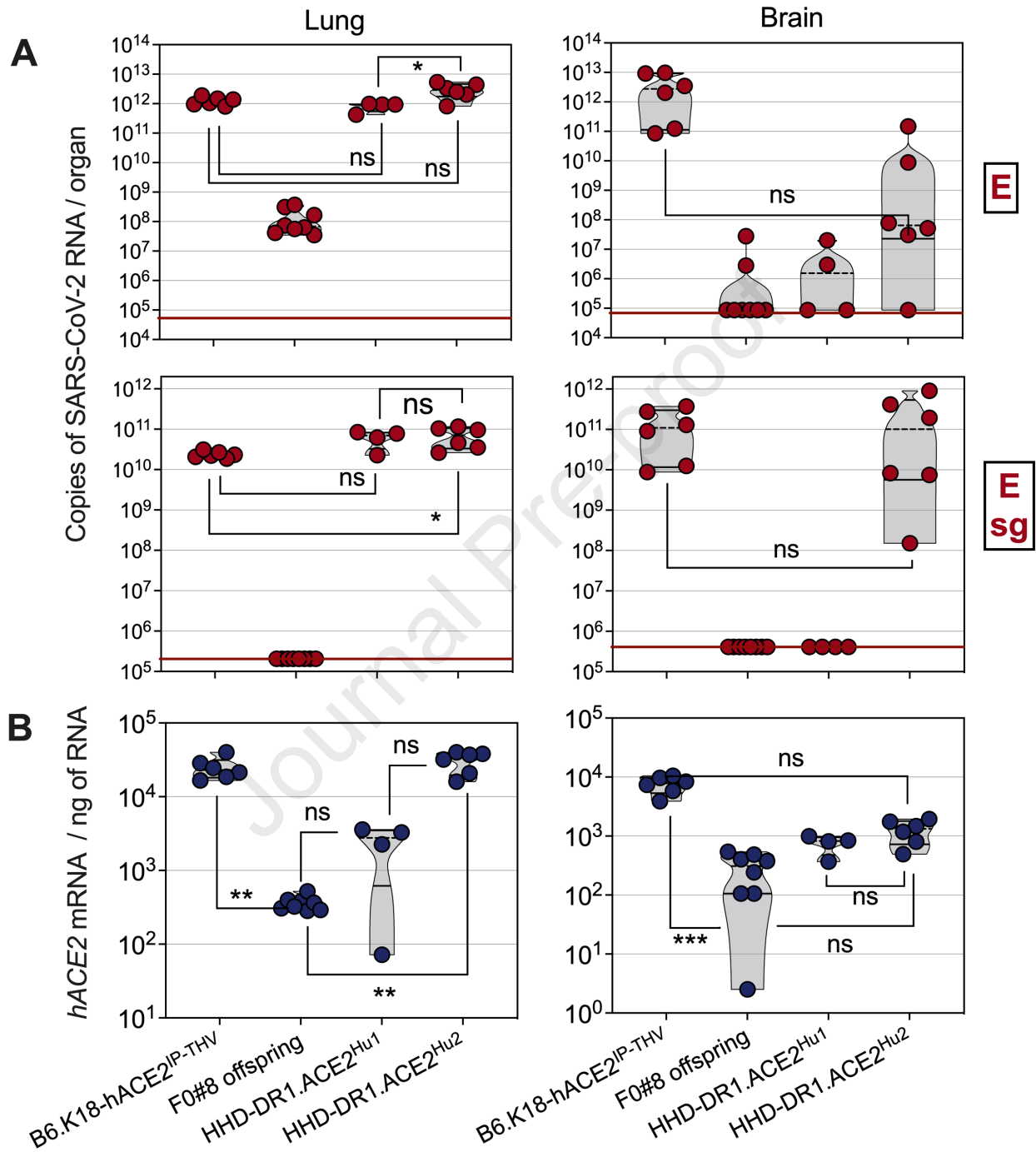


Figure 2

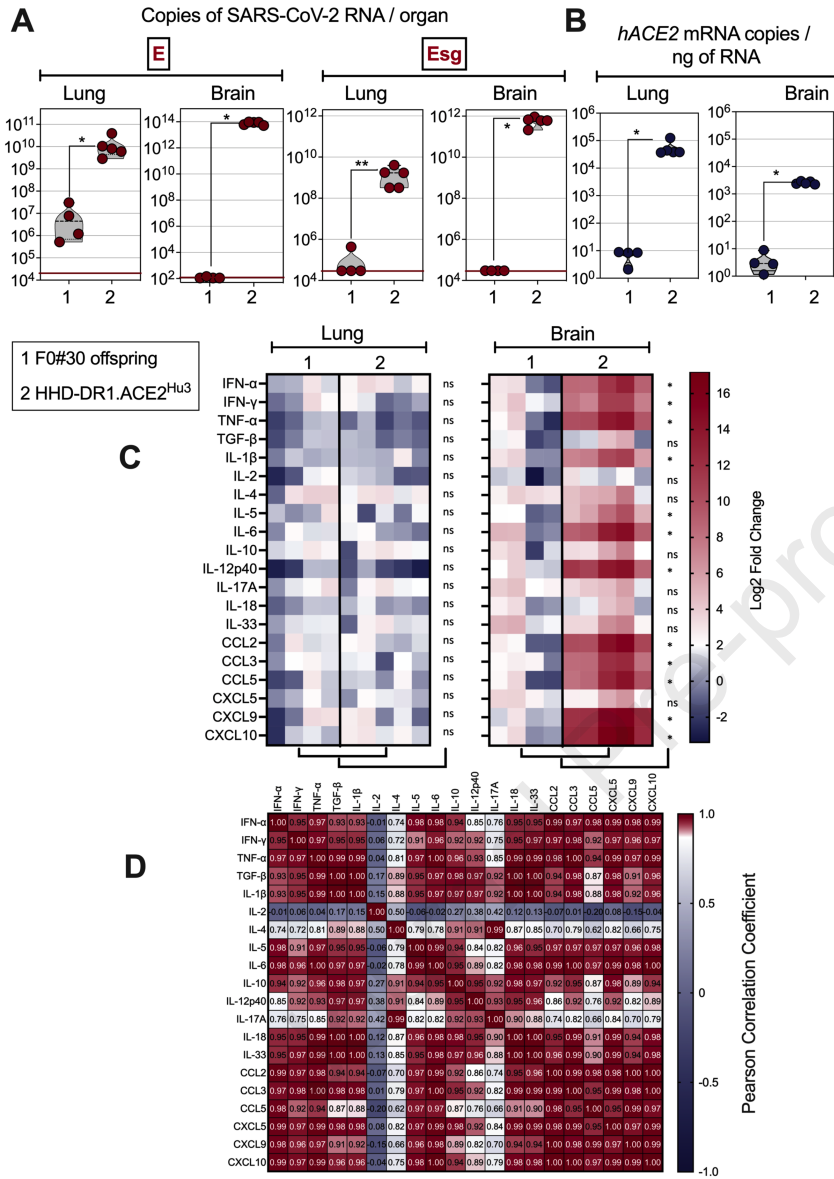
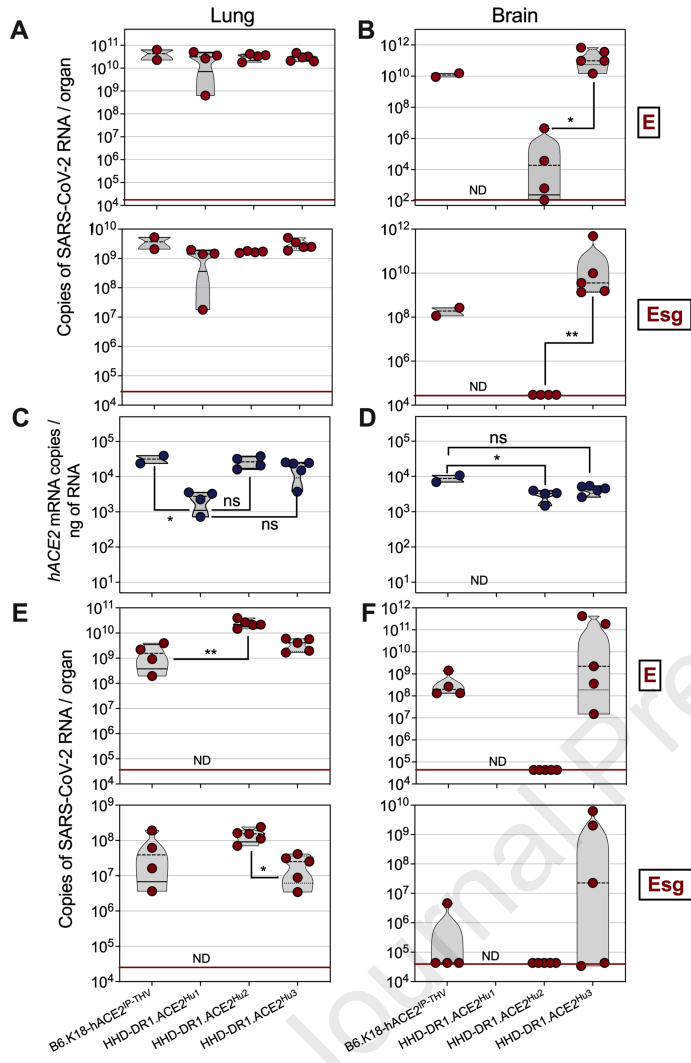


Figure 3



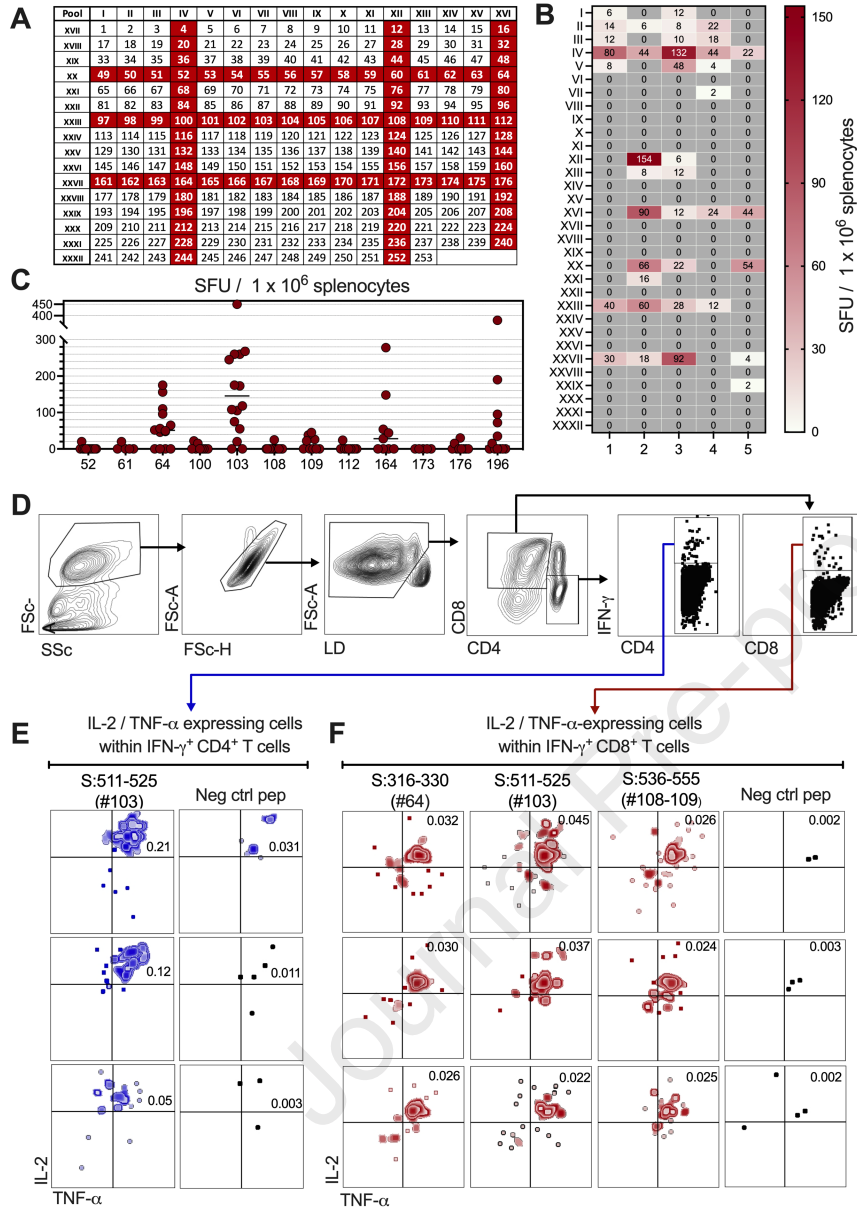


Figure 5

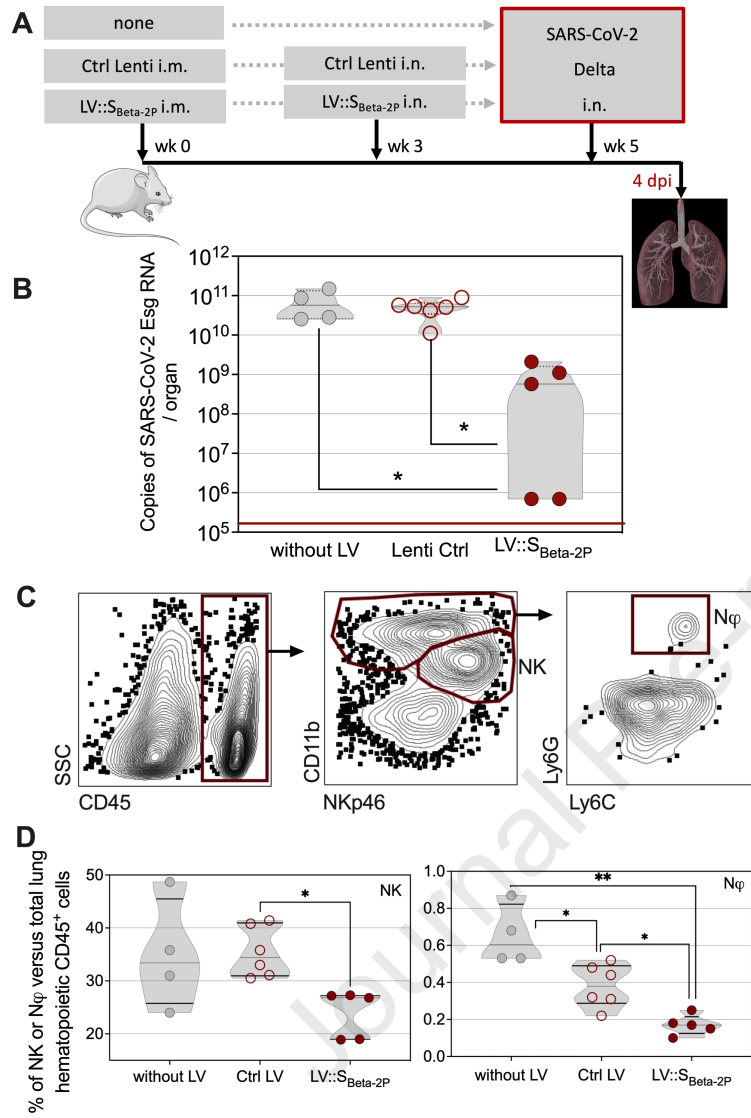


Figure 6

