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The explosively expanding COVID-19 pandemic urges the development of safe, efficacious and fast-acting vaccines. Several vaccine platforms are leveraged for a rapid emergency response¹. We describe the discovery of a live virus-vectored SARS-CoV-2 vaccine candidate using the yellow fever 17D (YF17D) vaccine as vector to express a non-cleavable prefusion form of the SARS-CoV-2 Spike antigen. We assess vaccine safety, immunogenicity and efficacy in several animal models. Vaccine candidate YF-S0 has an outstanding safety profile and induces high levels of SARS-CoV-2 neutralizing antibodies in hamsters, mice and cynomolgus macaques and concomitantly a protective immunity against YFV. Humoral immunity is complemented by a favourable Th1 cell-mediated immune response as profiled in mice. In a stringent hamster model² as well as in non-human primates, YF-S0 prevents infection with SARS-CoV-2. Moreover, in hamsters, a single dose confers protection from lung disease in most vaccinated animals within 10 days. Taken together, the quality of immune responses triggered and the rapid kinetics by which protective immunity can be mounted already after a single dose warrant further development this potent SARS-CoV-2 vaccine candidate.

Vaccine design and rationale

Protective immunity against SARS-CoV-2 and other coronaviruses is believed to depend on neutralizing antibodies (nAbs) targeting the viral Spike (S) protein. In particular, nAbs specific for the N-terminal S1 domain containing the ACE2 receptor binding domain have been shown to prevent viral infection in several animal models³.

The YF17D vaccine is known to rapidly induce broad multi-functional innate, humoral and cell-mediated immune (CMI) responses that may result in life-long protection following a single vaccine dose in nearly all vaccinees⁴. These favorable characteristics translate also to vectored vaccines based on the YF17D backbone⁵. YF17D is used as vector in two licensed human vaccines, generated by swapping genes encoding the

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YF17D surface antigens for those of the Japanese encephalitis (Imojev®) or dengue viruses (Dengvaxia®).

YF17D is a small (+)-ss RNA live-attenuated virus with limited vector capacity, tolerating some insertion of foreign antigens in the viral polyprotein⁶. Such insertions are constrained by (i) the topology and post-translational processing of the YF17D polyprotein; and, (ii) the need to express the antigen of interest in an immunogenic, likely natively folded form, to yield a potent recombinant vaccine.

With the use of an advanced reverse genetics system, a panel of YF17D-based COVID-19 vaccine candidates (YF-S) was designed expressing the S protein of SARS-CoV-2 either in its native cleavable S1/2, or non-cleavable S0 version, or its S1 subdomain as in-frame fusion within the YF17D-E/NS1 intergenic region (YF-S1/2, YF-S0 and YF-S1) (Figure 1a, Extended Data Fig. 1a). As outlined below, variant YF-S0 was finally selected as lead vaccine candidate based on its superior immunogenicity, efficacy and favorable safety profile.

Live attenuated viruses can be rescued by plasmid transfection into BHK-21 cells which are an established substrate for the production of biologicals⁷ and suitable for vaccine production at industrial scale (Extended Data Fig. 2) when following ICH guidelines⁸. Infectious progeny from each recombinant construct showed a unique small plaque phenotype as compared to parental YF17D (Extended Data Fig. 1b), in line with some replicative trade-off posed by the inserted foreign sequences. S or S1 and YF17D antigens were visualized intracellularly in YF-S infected cells by staining with SARS-CoV-2 Spike and YF17D-specific antibodies (Extended Data Fig. 1c). Proper expression and N-glycosylation⁹ of S or S1 was further confirmed by immunoblotting, with or without prior PNGase F treatment (Extended Data Fig. 1d). The full-length S1/2 and S0 antigens containing the original S2 subunit (stalk and cytoplasmic domains) of S may be expected to (i) form spontaneously trimers^{10–12} and (ii) to be intracellularly retained (reinforced by C-terminal fusion to an extra transmembrane domain known to function as ER retention signal) (Extended Data Fig. 1a).

In line with a smaller plaque phenotype, intracranial (i.c.) inoculation in suckling mice¹³ confirmed the attenuation of the YF-S variants (Extended Data Fig. 3a, b). Mouse pups inoculated with 100 plaque forming units (PFU) of parental YF17D stopped growing and succumbed to infection within seven days (median day of euthanasia; MDE), whereas pups inoculated with the YF-S variants continued to grow with MDE of 10, 12 and 17.5 days for YF-S1, YF-S1/2 and YF-S0, respectively. Thus, in particular YF-S0 has a markedly reduced neurovirulence. Importantly, akin classical YF17D potency testing¹³, some mortality confirms the viability and replication competence of the YF-S variants *in vivo*.

Likewise, YF-S0 is also highly attenuated in AG129 mice, that are highly susceptible to neuro-invasive YF17D infection^{14,15}. Whereas i.p. inoculation with 1 PFU of YF17D was uniformly lethal (MDE 16 days), a 10,000 higher inoculum resulted in only 1/12 animals in disease (Extended Data Fig. 3c).

In humans, YF17D itself has an outstanding safety profile^{4,16,17}. However, in very rare cases YF17D vaccination may result in viscerotropic disease^{16,17}. These singular events are poorly understood⁴, yet likely linked to functional immune-deficiencies, including deficiency in antiviral interferon responses¹⁸. Type I and III interferon deficient *STAT2*^{-/-} hamsters² are particularly susceptible to YF17D infection. When inoculated with 10⁴ PFU of YF17D, 12/14 of *STAT2*^{-/-} hamsters rapidly succumbed (MDE 7 days; <15% survival), presenting with severe viscerotropic (hemorrhages in the liver) and neurotropic disease (paralysis) (Extended Data Fig. 3d). By contrast, >90% of *STAT2*^{-/-} hamster (12/13) inoculated with the same dose of YF-S0 survived, without obvious viscerotropic disease and only in 1/13 some signs of neurological involvement¹⁸. As expected, in wild-type hamsters¹⁸ (Extended Data Fig. 3e, f), alike *Irfnar*^{-/-} mice^{19,20} (Extended Data Fig. 3g), YF-S0 was very well tolerated at all doses tested. Viremia as hallmark of viscerotropic disease in humans⁴ was observed in most YF17D vaccinated animals, often prolonged

over several days, whereas inoculation with YF-S0 resulted only in 1/6 hamsters at a single timepoint in any detectable vaccine virus RNA, providing further evidence for YF-S0 being (much) less viscerotropic than YF17D (Extended Data Fig. 3e).

In summary, we generated a set of recombinant replication-competent YF17D variants (YF-S) expressing different SARS-CoV-2 S antigens that are remarkably less neurovirulent, neurotropic and viscerotropic than YF17D.

Full protection in hamsters

Vaccine potency was assessed in a stringent hamster challenge model². Animals were vaccinated at day 0 with a rather low dose^{13,21} of 10³ PFU (i.p. route^{18,22}) of the different constructs or YF17D and sham as negative controls, and boosted after 7 days (Figure 1b). At day 21, all hamsters vaccinated with YF-S1/2 and YF-S0 had seroconverted to high levels of S-specific IgG and virus nAbs (Figure 1c, d; Extended Data Fig. 4a) with log₁₀ geometric mean titers (GMT) respectively for YF-S1/2 of 3.2 (95% CI, 2.9–3.5) for IgG and 1.4 (95% CI, 1.1–1.9) for nAbs; and YF-S0 of 3.5 (95% CI, 3.3–3.8) for IgG and 2.2 (95% CI, 1.9–2.6) for nAbs, with rapid seroconversion kinetics (Figure 1e). Thus in case of YF-S0, nAb levels exceeded those reported for hamsters after experimental SARS-CoV-2 infection (by 2.5 to 7.5-fold)^{23–25}. By contrast, only 1/12 YF-S1 vaccinated hamsters seroconverted and only to low nAbs. An adequate humoral immune responses apparently depends on full-length S antigen.

After 23 or 28 days, hamsters were challenged intranasally with 2 × 10⁵ PFU of SARS-CoV-2. Four days post-infection, high viral loads were detected in lungs of sham-vaccinated controls and animals vaccinated with YF17D as matched placebo (Figure 1f, g). Infection was characterized by a severe lung pathology with multifocal necrotizing bronchiolitis, leukocyte infiltration and edema, resembling findings in patients with severe COVID-19 bronchopneumonia (Figure 1i, j; Extended Data Fig. 5a). By contrast, hamsters vaccinated with YF-S0 were protected against this aggressive challenge (Figure 1f, g), with a median reduction of 5 log₁₀ (IQR, 4.5–5.4) in viral RNA loads (Figure 1f), and of 5.3 log₁₀ (IQR, 3.9–6.3) for infectious virus in the lungs as compared to sham (Figure 1g). Moreover, infectious virus was no longer detectable in 10 of 12 hamsters, and viral RNA reduced to non-quantifiable levels, whereas RNA measured in the two remaining animals equally likely represented residues of inoculum as observed in non-human primate models^{26–28}. Vaccination with YF-S0 also efficiently prevented systemic viral dissemination. In most animals, no or only very low levels of viral RNA were detectable in spleen, liver, kidney and heart four days after infection (Extended Data Fig. 4b). Similarly, a slightly different dose and schedule used for vaccination resulted in all vaccinated hamsters in respectively a 6 log₁₀ (IQR, 4.6–6.6) and 5.7 log₁₀ (IQR, 5.7–6.6) reduction of viral RNA and infectious virus titers as compared to sham (Extended Data Fig. 4f–i). Finally, vaccination with YF-S0 may induce saturating levels of nAbs, since in at least 4/12 YF-S0 vaccinated hamsters no anamnestic nAb response (<2x increase) was observed following challenge (Figure 1h; Extended Data Fig. 4c–e), alike original YF17D vaccination^{29,30}. By contrast, in hamsters vaccinated with the second-best vaccine candidate YF-S1/2, nAb levels further increased following SARS-CoV-2 infection (in 11/12 animals) approaching a plateau only after challenge.

The lungs of YF-S0 vaccinated animals remained normal, or near to normal with no more signs of bronchopneumonia, including (i) a reduction or lack of detectable lung pathology by histological inspection (Figure 1i, Extended Data Fig. 5a); and, (ii) a significant improvement of individual lung scores (Figure 1j, Extended Data Fig. 5b–d) and marked reduction in non-aerated lung volume (Extended Data Fig. 5e) derived by micro-CT of the chest relative to sham. In addition, immunization with YF-S0 resulted in an almost complete, in most cases full, normalization of cytokines, e.g., IL-6, IL-10, or IFN-γ, linked to disease exacerbation in COVID-19 (Figure 1k; Extended Data Fig. 5f). Even most

sensitive markers of infection, such as the induction of antiviral Type III interferons (IFN- λ), or of IFN-stimulated genes such as MX2 and IP-10 showed no elevation in YF-S0 vaccinated animals and remained similar to non-infected healthy controls.

Overall, YF-S0 expressing a non-cleavable S outcompeted construct YF-S1/2 expressing the cleavable version of S; arguing for the stabilized form of S, likely presenting its trimeric prefusion conformation^{11,12,31}, serving as relevant protective antigen. Moreover, in line with its failure to induce nAbs (Figure 1c), construct YF-S1 expressing solely the hACE2 receptor-binding S1 domain (Extended Data Fig. 1d) did not confer any protection (Figure 1f, g, i–h).

Th1 polarization of immunity in mice

Without tools for hamsters, humoral and CMI responses elicited by the different YF-S constructs were studied in parallel in mice. Since YF17D does not readily replicate in wild-type mice^{32,33}, *Ifnar*^{-/-} mice susceptible to vaccination with YF17D were employed^{20,33,34}.

Mice were vaccinated with 400 PFU (of either YF-S variants, YF17D or sham) at day 0 and boosted 7 days later. S-specific IgG was detectable as early as 7 days after first immunization (Figure 2c). At day 21 all YF-S1/2 and YF-S0 vaccinated mice had seroconverted to high levels of S-specific IgG and nAbs with log₁₀ GMT of 3.5 (95% CI, 3.1–3.9) for IgG and 2.2 (95% CI, 1.7–2.7) for nAbs in the case of YF-S1/2, or 4.0 (95% CI, 3.7–4.2) for IgG and 3.0 (95% CI, 2.8–3.1) for nAbs for YF-S0 (Figure 2a, b); with an excess of IgG2b/c over IgG1 indicating a dominant pro-inflammatory and hence antiviral (Th1) polarization of the immune response (Figure 2d) as desirable for protection against SARS-CoV-2^{35–37}. Alike in hamsters, YF-S1 failed to induce SARS-CoV-2 nAbs (Figure 2a, b). However, high potentially protective YF immune responses (Extended Data Fig. 8a–d, g; Extended Data Fig. 9) were conjointly induced by all constructs confirming a consistent immunization.

To assess SARS-CoV-2-specific CMI responses (pivotal role for shaping and longevity of vaccine-induced immunity as well as pathogenesis of COVID-19^{38,39}), recall responses in splenocytes from vaccinated mice were analyzed. In general, vaccination with any of the YF-S variants resulted in marked S-specific T-cell responses with a favorable Th1-polarization as detected by IFN- γ ELISpot (Figure 2e), further supported by an upregulation of T-bet (*TBX21*), in particular in cells isolated from YF-S0 vaccinated mice. This CMI profile was balanced by a concomitant elevation of GATA-3 levels (*GATA3*; driving Th2), but no marked overexpression of ROR γ t (*RORC*; Th17) or FoxP3 (*FOXP3*; Treg) (Figure 2f). Intriguingly, in stark contrast to its failure to induce nAbs (Figure 2a), or to protect (Figure 1), YF-S1 vaccinated animals had a greater number of S-specific splenocytes than those vaccinated with YF-S1/2 or YF-S0 (Figure 2e). Thus, even a vigorous CMI may not be sufficient for vaccine efficacy. In-depth profiling of the T-cell compartment by flow cytometry confirmed the presence of S-specific IFN- γ and TNF- α expressing CD8⁺ T-lymphocytes, and of IFN- γ expressing CD4⁺ and $\gamma\delta$ T-lymphocytes (Figure 2g; Extended Data Fig. 6a). A specific elevation of other markers such as IL-4 (Th2 polarization), IL-17A (Th17), or FoxP3 (regulatory T-cells) was not observed, supported by t-SNE analysis of the respective T-cell populations in YF-S1/2 and YF-S0 vaccinated mice (Figure 2h; Extended Data Fig. 6b). Our analyses further revealed, firstly, a similar composition of either CD4⁺ cell sets, comprising an equally balanced mixture of Th1 (IFN- γ ⁺ and/or TNF- α ⁺) and Th2 (IL-4⁺) cells, and possibly a slight raise in Th17 cells for YF-S0. Secondly, for YF-S1/2 and YF-S0 CD8⁺ T-lymphocyte populations were dominated by IFN- γ or TNF- α expressing cells, in line with their transcriptional profiles (Figure 2f). Of note, though similar in numbers, both vaccines YF-S0 and YF-S1/2 showed a distinguished (non-overlapping) profile regarding their CD8⁺ T-lymphocyte populations, with YF-S0 inducing stronger IFN- γ expression (Figure 2h, i; Extended Data Fig. 6b). In summary, YF-S0 induces a vigorous and balanced CMI response with a favorable Th1 polarization, dominated by SARS-CoV-2 specific CD8⁺

T-cells expressing high levels of IFN- γ when encountering SARS-CoV-2 S antigen.

High levels of nAb in non-human primates

Six cynomolgus macaques were vaccinated each with 10⁵ PFU of YF-S0 (similar to a human dose for YF17D^{13,21}, or YF17D-based recombinant vaccines^{40,41}) via the subcutaneous (s.c.) route^{40,41} using a schedule as before. Six animals received recombinant YF17D expressing an irrelevant control antigen as matched placebo. No adverse signs or symptoms were observed. Animals were bled weekly and assessed for seroconversion to nAb. At day 14 and 21, all NHP vaccinated with YF-S0 had seroconverted to consistently high levels of virus nAbs (Figure 3a) with GMT 2.6 (95% CI, 2.4–2.8) and 2.5 (95% CI, 2.3–2.7) respectively; reaching, if not exceeding, those reported for other vaccine candidates^{12,27,28,42–46} [range 0.3 Log₁₀ to 2.6 Log₁₀, and correlating with protection as confirmed by a reduction in SARS-CoV-2 RNA levels in YF-S0 vaccinated animals upon challenge (Figure 3b). Seroconversion occurred rapidly: already at day 7 (following a single dose) 2/6 NHP receiving YF-S0 had SARS-CoV-2 nAbs. In addition, YF-S0 induced protective levels of YFV nAbs^{4,29} (Extended Data Fig. 8e, f).

Rapid protection after single-dose

Vaccination of hamsters using a single-dose of YF-S0 induced high levels of nAbs and bAbs (Figure 4a, b) in a dose- and time-dependent manner (for mice see Extended Data Fig. 7a–d). This single-dose regimen resulted in protection against SARS-CoV-2 challenge, as demonstrated by absence of infectious virus in the lungs in 8/8 animals (Figure 4d) and a marked reduction in lung pathology scores (Figure 4e; Extended Data Fig. 7e). Viral RNA at quantifiable levels was present in only 1/8 animals (Figure 4c). Protective immunity was mounted rapidly. Already 10 days after vaccination, 5/8 animals receiving 10⁴ PFU of YF-S0 were protected against challenge (Figure 4c–e; Extended Data Fig. 7e). Notably, persistence of nAbs and bAbs during long-term follow-up hints towards considerable longevity of immunity induced by single-dose vaccination (Extended Data Fig. 7f, g).

Discussion

Vaccines against SARS-CoV-2 need to be safe and result rapidly, ideally after one single dose, in long-lasting protective immunity. We report encouraging results of YF17D-vectored SARS-CoV-2 vaccine candidate YF-S0, inducing robust immune responses in hamsters, mice and NHP. Since SARS-CoV-2 replicates massively in the lungs of infected hamsters resulting in major lung pathology^{2,23–25}, we selected this model to assess vaccine efficacy. YF-S0 resulted in protection against stringent SARS-CoV-2 challenge, comparable, if not more vigorous, to other vaccine candidates in NHP models^{12,27,28,42–46}. At least in some of the YF-S0 vaccinated animals (4/12) no anamnestic response in nAb levels following SARS-CoV-2 challenge was observed, suggestive that sterilizing immunity similar to that conferred by YF17D vaccination^{29,30} can be achieved. In animals challenged three weeks after single 10⁴ PFU dose vaccination, no infectious virus was detected in the lungs. Considering the severity of the model, it is remarkable, that also in several animals that were challenged already 10 days after vaccination no infectious virus was recovered. Reduction of viral replication mitigated lung pathology with normalization of biomarkers, such as IL-6, associated with infection and disease (Figure 1 and Extended Data Fig. 5). Vaccination of macaques with a relatively low s.c. dose of YF-S0 led to rapid seroconversion to high nAb titers (Figure 3). It is tempting to speculate that such an encouraging potency may translate into a simple one-shot dosing regimen for clinical use in humans.

Moreover, YF-S0 has a markedly improved safety profile over YFV17D in several models (Extended Data Fig. 3); and is well tolerated

in hamsters (Extended Data Fig. 3) and NHP. Notably, updated WHO recommendations endorse general use of YF17D in all people aged 9 months or older living in areas at risk⁴⁷, including elderly and persons with underlying medical conditions^{16,47}. Our data hence suggest that YF-S0 might also be safe in those persons most vulnerable to COVID-19. CMI studied in mice further revealed that YF-S0 favors a Th1 response, which is relevant since a skewed Th2 polarization may cause an induction and dysregulation of alternatively activated wound-healing monocytes/macrophages^{35–37} resulting in an overshooting inflammatory response (cytokine storm) leading to acute lung injury^{38,48}. No indication of such a disease enhancement, neither of antibody-dependent enhancement (ADE)⁴⁹ via Fcγ receptor-mediated mechanisms⁵⁰ was observed in any of our models.

In conclusion, YF-S0 confers vigorous protective immunity against SARS-CoV-2 infection. Remarkably, this immunity can be achieved within 10 days following a single dose vaccination. In light of the threat that SARS-CoV-2 will remain endemic with spikes of re-infection, as a recurring plague, vaccines with this profile may be ideally suited for population-wide immunization programs.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at <https://doi.org/10.1038/s41586-020-3035-9>.

- WHO. Draft landscape of COVID-19 candidate vaccines, <<https://www.who.int/who-documents-detail/draft-landscape-of-covid-19-candidate-vaccines>> (retrieved November 4, 2020).
- Boudewijns, R. et al. STAT2 signaling as double-edged sword restricting viral dissemination but driving severe pneumonia in SARS-CoV-2 infected hamsters. *bioRxiv*, 2020.2004.2023.056838, <https://doi.org/10.1101/2020.04.23.056838> (2020).
- Cao, Y. et al. Potent neutralizing antibodies against SARS-CoV-2 identified by high-throughput single-cell sequencing of convalescent patients' B cells. *Cell*, <https://doi.org/10.1016/j.cell.2020.05.025> (2020).
- Barrett, A. D. & Teuwen, D. E. Yellow fever vaccine - how does it work and why do rare cases of serious adverse events take place? *Curr Opin Immunol* **21**, 308–313, <https://doi.org/10.1016/j.coi.2009.05.018> (2009).
- Draper, S. J. & Heeney, J. L. Viruses as vaccine vectors for infectious diseases and cancer. *Nat Rev Microbiol* **8**, 62–73, <https://doi.org/10.1038/nrmicro2240> (2010).
- Bonaldo, M. C., Sequeira, P. C. & Galler, R. The yellow fever 17D virus as a platform for new live attenuated vaccines. *Hum Vaccin Immunother* **10**, 1256–1265, <https://doi.org/10.4161/hv.28117> (2014).
- Afonja, O. et al. Baby hamster kidney cell-derived recombinant factor VIII: a quarter century of learning and clinical experience. *Expert Rev Hematol* **9**, 1151–1164, <https://doi.org/10.1080/17474086.2017.1259559> (2016).
- International Council for Harmonisation of technical requirements for pharmaceuticals for human use (ICH). *Quality guidelines for pharmaceutical quality based on Good Manufacturing Practice (GMP): Quality of Biotechnological Products (Q5A–Q5E)*, <<https://www.ich.org/page/quality-guidelines>> (retrieved November 4, 2020).
- Walls, A. C. et al. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* **181**, 281–292.e286, <https://doi.org/10.1016/j.cell.2020.02.058> (2020).
- Du, L. et al. The spike protein of SARS-CoV-2 a target for vaccine and therapeutic development. *Nat Rev Microbiol* **7**, 226–236, <https://doi.org/10.1038/nrmicro2090> (2009).
- Wrapp, D. et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. *Science* **367**, 1260–1263, <https://doi.org/10.1126/science.abb2507> (2020).
- Mercado, N. B. et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. *Nature*, <https://doi.org/10.1038/s41586-020-2607-z> (2020).
- Ferguson, M., Shin, J., Knezevic, I., Minor, P. & Barrett, A. WHO Working Group on technical specifications for manufacture and evaluation of yellow fever vaccines, Geneva, Switzerland, 13–14 May 2009. *Vaccine* **28**, 8236–8245, <https://doi.org/10.1016/j.vaccine.2010.10.070> (2010).
- Kum, D. B. et al. Limited evolution of the yellow fever virus 17D in a mouse infection model. *Emerg Microbes Infect* **8**, 1734–1746, <https://doi.org/10.1080/22221751.2019.1694394> (2019).
- Mishra, N. et al. A chimeric Japanese encephalitis vaccine protects against lethal yellow fever virus infection without inducing neutralizing antibodies. *mBio* **11**, <https://doi.org/10.1128/mBio.02494-19> (2020).
- Thomas, R. E., Lorenzetti, D. L., Spragins, W., Jackson, D. & Williamson, T. The safety of yellow fever vaccine 17D or 17DD in children, pregnant women, HIV+ individuals, and older persons: systematic review. *The American journal of tropical medicine and hygiene* **86**, 359–372, <https://doi.org/10.4269/ajtmh.2012.11-0525> (2012).
- Rafferty, E., Duclos, P., Yactayo, S. & Schuster, M. Risk of yellow fever vaccine-associated viscerotropic disease among the elderly: A systematic review. *Vaccine* **31**, 5798–5805, <https://doi.org/10.1016/j.vaccine.2013.09.030> (2013).
- Mateo, R. I. et al. Yellow fever 17-D vaccine is neurotropic and produces encephalitis in immunosuppressed hamsters. *Am J Trop Med Hyg* **77**, 919–924 (2007).
- Erickson, A. K. & Pfeiffer, J. K. Spectrum of disease outcomes in mice infected with YFV-17D. *J Gen Virol* **96**, 1328–1339, <https://doi.org/10.1099/vir.0.000075> (2015).
- Watson, A. M., Lam, L. K., Klimstra, W. B. & Ryman, K. D. The 17D-204 vaccine strain-induced protection against virulent yellow fever virus is mediated by humoral immunity and CD4+ but not CD8+ T cells. *PLoS Pathog* **12**, e1005786, <https://doi.org/10.1371/journal.ppat.1005786> (2016).
- Martins, R. M. et al. 17DD yellow fever vaccine: a double blind, randomized clinical trial of immunogenicity and safety on a dose-response study. *Human vaccines & immunotherapeutics* **9**, 879–888, <https://doi.org/10.4161/hv.22982> (2013).
- Tesh, R. B., Travassos da Rosa, A. P., Guzman, H., Araujo, T. P. & Xiao, S. Y. Immunization with heterologous flaviviruses protective against fatal West Nile encephalitis. *Emerg Infect Dis* **8**, 245–251, <https://doi.org/10.3201/eid0803.010238> (2002).
- Chan, J. F.-W. et al. Simulation of the clinical and pathological manifestations of Coronavirus Disease 2019 (COVID-19) in golden Syrian hamster model: implications for disease pathogenesis and transmissibility. *Clin Infect Dis*, <https://doi.org/10.1093/cid/ciaa325> (2020).
- Sia, S. F. et al. Pathogenesis and transmission of SARS-CoV-2 in golden hamsters. *Nature*, <https://doi.org/10.1038/s41586-020-2342-5> (2020).
- Imai, M. et al. Syrian hamsters as a small animal model for SARS-CoV-2 infection and countermeasure development. *Proc Natl Acad Sci U S A*, 202009799, <https://doi.org/10.1073/pnas.2009799117> (2020).
- Rockx, B. et al. Comparative pathogenesis of COVID-19, MERS, and SARS in a nonhuman primate model. *Science* **368**, 1012–1015, <https://doi.org/10.1126/science.abb7314> (2020).
- van Doremalen, N. et al. ChAdOx1 nCoV-19 vaccine prevents SARS-CoV-2 pneumonia in rhesus macaques. *Nature*, <https://doi.org/10.1038/s41586-020-2608-y> (2020).
- Yu, J. et al. DNA vaccine protection against SARS-CoV-2 in rhesus macaques. *Science*, <https://doi.org/10.1126/science.abc6284> (2020).
- Staples, J. E., Barrett, A. D. T., Wilder-Smith, A. & Hombach, J. Review of data and knowledge gaps regarding yellow fever vaccine-induced immunity and duration of protection. *npj Vaccines* **5**, 54, <https://doi.org/10.1038/s41541-020-0205-6> (2020).
- Casey, R. M. et al. Immunogenicity of Fractional-Dose Vaccine during a Yellow Fever Outbreak — Final Report. *New England Journal of Medicine* **381**, 444–454, <https://doi.org/10.1056/NEJMoa1710430> (2018).
- Barnes, C. O. et al. Structures of human antibodies bound to SARS-CoV-2 Spike reveal common epitopes and recurrent features of antibodies. *Cell* **182**, 828–842.e816, <https://doi.org/10.1016/j.cell.2020.06.025> (2020).
- Meier, K. C., Gardner, C. L., Khoretonenko, M. V., Klimstra, W. B. & Ryman, K. D. A mouse model for studying viscerotropic disease caused by yellow fever virus infection. *PLoS Pathog* **5**, e1000614, <https://doi.org/10.1371/journal.ppat.1000614> (2009).
- Erickson, A. K. & Pfeiffer, J. K. Dynamic viral dissemination in mice infected with yellow fever virus strain 17D. *J Virol* **87**, 12392–12397, <https://doi.org/10.1128/jvi.02149-13> (2013).
- Kum, D. B. et al. A yellow fever–Zika chimeric virus vaccine candidate protects against Zika infection and congenital malformations in mice. *npj Vaccines* **3**, 56, <https://doi.org/10.1038/s41541-018-0092-2> (2018).
- Channappanavar, R. et al. Dysregulated type I interferon and inflammatory monocyte-macrophage responses cause lethal pneumonia in SARS-CoV-infected mice. *Cell Host Microbe* **19**, 181–193, <https://doi.org/10.1016/j.chom.2016.01.007> (2016).
- Channappanavar, R. & Perlman, S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin Immunopathol* **39**, 529–539, <https://doi.org/10.1007/s00281-017-0629-x> (2017).
- Page, C. et al. Induction of alternatively activated macrophages enhances pathogenesis during severe acute respiratory syndrome coronavirus infection. *J Virol* **86**, 13334–13349, <https://doi.org/10.1128/jvi.01689-12> (2012).
- Grifoni, A. et al. Targets of T cell responses to SARS-CoV-2 coronavirus in humans with COVID-19 disease and unexposed individuals. *Cell* **181**, 1489–1501.e1415, <https://doi.org/10.1016/j.cell.2020.05.015> (2020).
- Ni, L. et al. Detection of SARS-CoV-2-Specific Humoral and Cellular Immunity in COVID-19 Convalescent Individuals. *Immunity* **52**, 971–977.e973, <https://doi.org/10.1016/j.immuni.2020.04.023> (2020).
- Appiahgari, M. B. & Vratil, S. IMOJEV(®): a Yellow fever virus-based novel Japanese encephalitis vaccine. *Expert Rev Vaccines* **9**, 1371–1384, <https://doi.org/10.1586/erv.10.139> (2010).
- Guy, B. et al. A recombinant live attenuated tetravalent vaccine for the prevention of dengue. *Expert review of vaccines* **16**, 1–13, <https://doi.org/10.1080/14760584.2017.1335201> (2017).
- Gao, Q. et al. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* **369**, 77–81, <https://doi.org/10.1126/science.abc1932> (2020).
- Wang, H. et al. Development of an Inactivated Vaccine Candidate, BBIBP-CorV, with Potent Protection against SARS-CoV-2. *Cell* **182**, 713–721.e719, <https://doi.org/10.1016/j.cell.2020.06.008> (2020).
- Yang, J. et al. A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. *Nature*, <https://doi.org/10.1038/s41586-020-2599-8> (2020).
- Feng, L. et al. An adenovirus-vectored COVID-19 vaccine confers protection from SARS-CoV-2 challenge in rhesus macaques. *Nat Commun* **11**, 4207, <https://doi.org/10.1038/s41467-020-18077-5> (2020).
- Erasmus, J. H. et al. An Alphavirus-derived replicon RNA vaccine induces SARS-CoV-2 neutralizing antibody and T cell responses in mice and nonhuman primates. *Sci Transl Med* **12**, <https://doi.org/10.1126/scitranslmed.abc9396> (2020).
- WHO. International Travel and Health. Yellow fever, <<https://www.who.int/ith/vaccines/yf/en/>> (retrieved November 4, 2020).

48. Lambert, P. H. *et al.* Consensus summary report for CEPI/BC March 12-13, 2020 meeting: Assessment of risk of disease enhancement with COVID-19 vaccines. *Vaccine* **38**, 4783-4791, <https://doi.org/10.1016/j.vaccine.2020.05.064> (2020).
49. Liu, L. *et al.* Anti-spike IgG causes severe acute lung injury by skewing macrophage responses during acute SARS-CoV infection. *JCI Insight* **4**, <https://doi.org/10.1172/jci.insight.123158> (2019).

50. Smatti, M. K., Al Thani, A. A. & Yassine, H. M. Viral-induced enhanced disease illness. *Front Microbiol* **9**, 2991-2991, <https://doi.org/10.3389/fmicb.2018.02991> (2018).

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ACCELERATED ARTICLE PREVIEW

Fig. 1 | Immunogenicity and protective efficacy in hamsters. **a**, Schematic representation of YF17D-based vaccine candidates (see also Extended Data Fig. 1). **b**, Syrian hamsters were immunized twice i.p. with 10^3 PFU of each vaccine constructs and inoculated with 2×10^5 TCID₅₀ SARS-CoV-2 ($n=12$ from two independent experiments for all groups). **c–d**, Neutralizing antibodies (nAb) (**c**) and total binding IgG (bAb; sera of three animals were pooled) (**d**) on day 21 post-vaccination. **e**, Seroconversion rates at indicated days. **f–g**, Viral loads in hamster lungs four days after infection quantified by RT-qPCR (**f**) and virus titration (**g**). **h**, Box plot showing nAbs prior and four days after challenge; center line represents the median, the lower and upper hinges represent the first and third quartiles, and the whiskers represent the minimum and maximum. **i**,

Representative H&E images of sham- or YF-S0-vaccinated hamster lungs after challenge. Peri-vascular edema (blue arrow); peri-bronchial inflammation (red arrows); peri-vascular inflammation (green arrow); bronchopneumonia (circle), apoptotic bodies in bronchial wall (red arrowhead). Black scalebar: 100 μ m. **j**, Spider-web plot showing histopathological score for lung damage normalized to sham (grey). **k**, Heat-map showing normalized RNA levels of antiviral and pro-inflammatory cytokine genes in lungs after challenge as determined by RT-qPCR relative to non-treated and non-infected controls ($n=4$). LLOQ - lower limit of quantification; LLOD - lower limit of detection. Data are medians $\pm$ IQR. Two-tailed uncorrected Kruskal-Wallis test (**c–g**), or a two-tailed Wilcoxon matched-pairs rank test (**h**) was applied (ns = Not-Significant).

Fig. 2 | Humoral and cell-mediated immune responses in mice. *Ifnar*^{-/-} mice were vaccinated twice at day 0 and 7 i.p. with 400 PFU of each construct. **a-b**, nAbs (a) and bAbs (b) on day 21 post-vaccination. **c**, Seroconversion rates at indicated days. **d**, Ratios of IgG2b or IgG2c over IgG1 compared to a theoretical Th1/Th2 response. Panels a-d: animals vaccinated with YF-S1/2 (n=11), YF-S0 (n=11), YF-S1 (n=13), sham (n=9) and YF17D (n=9) from three independent experiments. For bAb quantification (b) and IgG subtyping (d) serum minipools from two to three animals were used. **e**, Number of IFN-γ-secreting cells after SARS-CoV-2 Spike peptide pool stimulation. Animals vaccinated with YF-S1/2 (n=11), YF-S0 (n=10), YF-S1 (n=7), sham (n=9) and YF17D (n=5). YF-S1 and YF17D from two, YF-S1/2, YF-S0 and sham from three independent experiments. **f**, Normalized mRNA expression levels of TBX21, GATA3, RORC, FOXP3 quantified by RT-qPCR in Spike peptide-stimulated splenocytes. Data are expressed as

fold-change over median of uninfected controls (n=5 YF-S1/2, YF-S0 and YF-S1, n=7 sham and n=3 uninfected control from a single experiment). **g**, Percentage of IFN-γ and TNF-α expressing CD8, and IFN-γ expressing CD4 and γδ T-cells after Spike peptide stimulation (animals vaccinated with YF-S1/2 (n=11), YF-S0 (n=10), YF-S1 (n=5), YF17D (n=5) and sham (n=8); YF-S1 and YF17D from a single experiment; YF-S1/2, YF-S0 and sham from three independent experiments). **h-i**, t-distributed Stochastic Neighbor Embedding analysis of spike-specific CD8 T-cells positive for at least one intracellular marker (IFN-γ, TNF-α, IL-4) after Spike peptide stimulation (n=6 for YF-S1/2 and YF-S0). **i**, Heatmap of IFN-γ expression density of spike-specific CD8 T-cells after YF-S1/2 and YF-S0 vaccination. Scale bar represents IFN-γ expressing density (blue – low, red – high expression). Data in panels b-k are medians ± IQR. Two-tailed uncorrected Kruskal-Wallis test (b-c, f-k) or a One-Sample T-test (e) was applied (ns = Not-Significant).

Fig. 3 | Immunogenicity and protective efficacy in cynomolgus macaques.

Twelve cynomolgus macaques (*Macaca fascicularis*) were immunized twice at day 0 and 7 s.c. with 10^5 PFU of YF-S0 ($n=6$) or matched placebo ($n=6$). On day 21 post-vaccination, all NHP were challenged with 1.5×10^4 TCID₅₀ SARS-CoV-2. **a**, nAbs on indicated days after first vaccination. Data are medians $\pm$ IQR. **b**, Virus RNA loads in throat swabs at indicated time points quantified by RT-qPCR.

Different symbols (squares, triangle, diamond) indicate values for individual animals followed over time with virus RNA loads above LLOQ. Histological examination of the lungs (day 21 post challenge) revealed no evidence of any SARS-CoV-2-induced pathology in either YF-S0 or placebo vaccinated animals. Two-tailed uncorrected Kruskal-Wallis test was applied (ns = Not-Significant).

Fig. 4 | Single shot vaccination in hamsters. Three groups of hamsters were vaccinated once i.p. with sham (white) or YF-S0 at two different doses; 10³ PFU (low, green circles) and 10⁴ PFU (high, green triangles) of YF-S0 at 21 days prior to challenge. A fourth group was vaccinated with the high 10⁴ PFU dose of YF-S0 at 10 days prior to challenge (green squares). Data are from n=8 animals per group from a single experiment; **a-b**, nAbs (a) and bAbs (n=3; sera from two to

three animals were pooled) (b) prior to challenge. **c-d**, Viral loads in lungs four days after infection quantified by RT-qPCR (c) and virus titration (d). **e**, Spider-web plot showing histopathological score for lung damage normalized to sham (white circles). Data are medians $\pm$ IQR. Two-tailed uncorrected Kruskal-Wallis test was applied (ns = Not-Significant).

Methods

Cells and viruses

BHK-21J (baby hamster kidney fibroblasts) cells⁵¹, generously provided by Prof. Peter Bredenbeek (LUMC, The Netherlands), were maintained in Minimum Essential Medium (Gibco), Vero E6 (African green monkey kidney, ATCC CRL-1586) and HEK-293T (human embryonic kidney cells, ATCC CRL-3216) cells were maintained in Dulbecco's Modified Eagle Medium (Gibco). All media were supplemented with 10% fetal bovine serum (Hyclone), 2 mM L-glutamine (Gibco), 1% sodium bicarbonate (Gibco). BSR-T7/5 (T7 RNA polymerase expressing BHK-21)⁵², generously provided by Prof. Ian Goodfellow (University of Cambridge, UK), cells were kept in DMEM supplemented with 0.5 mg/ml geneticin (Gibco). BHK-21J (baby hamster kidney fibroblasts) cells⁵¹ were maintained in Minimum Essential Medium (Gibco), Vero E6 (African green monkey kidney, ATCC CRL-1586) and HEK-293T (human embryonic kidney cells, ATCC CRL-3216) cells were maintained in Dulbecco's Modified Eagle Medium (Gibco). All media were supplemented with 10% fetal bovine serum (Hyclone), 2 mM L-glutamine (Gibco), 1% sodium bicarbonate (Gibco). BSR-T7/5 (T7 RNA polymerase expressing BHK-21)⁵² cells were kept in DMEM supplemented with 0.5 mg/ml geneticin (Gibco).

For all challenge experiments in hamsters, SARS-CoV-2 strain Beta-Cov/Belgium/GHB-03021/2020 (EPI_ISL_407976|2020-02-03) was used from passage P4 grown on Vero E6 cells as described². YF17D (Stamaril®, Sanofi-Pasteur) was passaged twice in Vero E6 cells before use. All cells were regularly monitored for the absence of mycoplasma contamination.

Vaccine design and construction

Different vaccine constructs were generated using an infectious cDNA clone of YF17D (in an inducible BAC expression vector pShuttle-YF17D, patent number WO2014174078 A1)^{34,53,54}. A panel of several SARS-CoV-2 vaccine candidates was engineered by inserting a codon optimized sequence of either the SARS-CoV-2 Spike protein (S) (GenBank: MN908947.3) or variants thereof into the full-length genome of YF17D (GenBank: X03700) as translational in-frame fusion within the YF-E/NS1 intergenic region^{6,55}. The variants generated contained (i) either the S protein sequence from amino acid (aa) 14-1273, expressing S in its cleavable and/or non-cleavable form or version (YF-S1/2 and YF-S0, respectively), or (ii) its subunit-S1 (aa 14-722; YF-S1). To ensure a proper YF topology and correct expression of different S antigens in the YF backbone, transmembrane domains derived from WNV were inserted.

The SARS2-CoV-2 vaccine candidates were cloned by combining the S cDNA (obtained after PCR on overlapping synthetic cDNA fragments; IDT) by a NEB Builder Cloning kit (New England Biolabs) into the pShuttle-YF17D backbone. NEB Builder reaction mixtures were transformed into *E.coli* EPI300 cells (Lucigen) and correct integration of the S protein cDNA was confirmed by Sanger sequencing. Recombinant plasmids were purified by column chromatography (Nucleobond Maxi Kit, Machery-Nagel) after growth overnight, followed by an additional amplification of the BAC vector for six hours by addition of 2 mM L-arabinose as described³⁴.

Infectious vaccine viruses were generated from plasmid constructs by transfection into BHK-21J cells using standard protocols (TransIT-LT1, Mirus Bio). The supernatant was harvested four days post-transfection when most of the cells showed signs of cytopathic effect (CPE). Infectious virus titers (PFU/ml) were determined by a plaque assay on BHK-21J cells as previously described^{15,34}. The presence of inserted sequences in generated vaccine virus stocks was confirmed by RNA extraction and DNase I treatment (Direct-zol RNA kit, Zymo Research) followed by RT-PCR (qScript XLT, Quanta) and Sanger sequencing, and by immunoblotting of extracts of freshly infected cells (see *infra*). YF-S0 vaccine virus stock used for NHP studies was concentrated by tangential flow filtration (Minimate, Pall), and subjected to deep sequencing on a MiSeq platform (Illumina) following an established metagenomics pipeline^{56,57}

or quality control (identity, consistency and genetic homogeneity) and to confirm the absence of adventitious agents as before².

Analysis of genetic stability of YF-S0 vaccine virus

To test the genetic stability of YF-S0 vaccine virus, virus supernatants recovered from transfected BHK-21J cells (P0) were plaque purified once (P1) and serially passaged on BHK-21J cells (P3-P6). Furthermore, the genetic stability of 25 plaque isolates from a second round of plaque purification were analysed after amplification (P4*).

For all passages, fresh BHK-21J cells were infected for 1 hour with a 1:2 dilution of the virus supernatant from the respective previous passage. After infection the cells were washed twice with PBS. Supernatants of the infected cells were routinely harvested 72 hours post infection. The presence of inserted sequences in generated passages was confirmed by RNA extraction and DNase I treatment (Direct-zol RNA kit, Zymo Research) followed by RT-PCR (qScript XLT, Quanta) and Sanger sequencing, and by immunoblotting of freshly infected cells (see *infra*).

Immunofluorescent staining

In vitro antigen expression of different vaccine candidates was verified by immunofluorescent staining as described previously by Kum et al³⁴. Briefly, BHK-21J cells were infected with 100 PFU of the different YF-S vaccine candidates. Infected cells were stained three days post-infection (3dpi). For detection of YF antigens polyclonal mouse anti-YF17D antiserum was used. For detection of SARS-CoV-2 Spike antigen rabbit SARS-CoV Spike S1 antibody (40150-RP01, Sino Biological; 1:250 dilution) and rabbit SARS-CoV Spike primary antibody (40150-T62-COV2, Sino Biological; 1:250 dilution) were used. Secondary antibodies were goat anti-mouse Alexa Fluor-594 and goat anti-rabbit Alexa Fluor-488 (Life Technologies; 1:500 dilution). Cells were counterstained with DAPI (Sigma). All confocal fluorescent images were acquired using the same settings on a Leica TCS SP5 confocal microscope, employing a HCX PL APO 63x (NA 1.2) water immersion objective.

Immunoblot analysis

Infected BHK-21J cells were harvested and washed once with ice-cold phosphate buffered saline, and lysed in radioimmunoprecipitation assay buffer (Thermo Fisher Scientific) containing 1x protease inhibitor and phosphatase inhibitor cocktail (Thermo Fisher Scientific). After centrifugation at 15,000 rpm at 4 °C for 10 minutes, protein concentrations in the cleared lysates were measured using BCA (Thermo Fisher Scientific). Immunoblot analysis was performed by a Simple Western size-based protein assay (Protein Simple) following manufactures instructions. Briefly, after loading of 400 ng of total protein onto each capillary, specific S protein levels were identified using specific primary antibodies (NB100-56578, Novus Biologicals and 40150-T62-CoV2, Sino Biological Inc.; both 1:100 diluted), and HRP conjugated secondary antibody (Protein Simple; 1:100 dilution). Chemiluminescence signals were analyzed using Compass software (Protein Simple). To evaluate the removal of N-linked oligosaccharides from the glycoprotein, protein extracts were treated with PNGase F according to manufactures instructions (New England Biolabs).

Animals

Hamsters and mice. Wild-type Syrian hamsters (*Mesocricetus auratus*) and BALB/c mice and pups were purchased from Janvier Laboratories, Le Genest-Saint-Isle, France. Type I interferon receptor deficient *Ifnar1*^{-/-} mice (Ref. ⁵⁸), type I and II interferon receptor deficient AG129 mice (Ref. ⁵⁹) and *STAT2*^{-/-} hamster were bred in-house. The generation of *STAT2*^{-/-} hamsters (Gene ID: 101830537) has been described before⁶⁰. Functional ablation of type I and III signaling has been confirmed². *STAT2*^{-/-} hamsters have been demonstrated to be particularly susceptible to flavivirus infection⁶¹.

Six- to ten-weeks-old, male and female *Ifnar1*^{-/-} mice, six- to eight-weeks-old, male and female AG129 mice and six- to eight-weeks-old female

wild-type hamsters were used throughout the study. For vaccine safety studies, equal amounts of age-matched male and female wild-type as well as *STAT2*^{-/-} hamsters were used.

Non-human primates (NHP). Twelve outbred mature male cynomolgus macaques (*Macaca fascicularis*) were used in this study. Animals were purpose-bred and housed at the Biomedical Primate Research Centre (BPRC, Rijswijk, The Netherlands). All animals selected for the study were in good physical health with normal baseline biochemical and haematological values.

Animal Experiments

Hamsters and mice. Animals were housed in individually ventilated cages (Sealsafe Plus, Tecniplast), per 5 (mice, cage type GM500) or per 2 (hamsters, cage type GR900), at 21 °C, 55% humidity and 12:12 light/dark cycles. Animals were provided with food and water *ad libitum*, as well as cotton and cardboard play tunnels (mice) or extra bedding material and wooden gnawing blocks (hamsters). This project was approved by the KU Leuven ethical committee (P015-2020), following institutional guidelines approved by the Federation of European Laboratory Animal Science Associations (FELASA). This project was approved by the KU Leuven ethical committee (P015-2020), following institutional guidelines approved by the Federation of European Laboratory Animal Science Associations (FELASA). Animals were euthanized by intraperitoneal administration of 100 µl (mice) or 500 µl (hamsters) Dolethal (200 mg/ml sodium pentobarbital, Vetoquinol SA).

For mice and hamsters, sample sizes were chosen based on the results of pilot experiments. Pivotal studies have been performed in at least two independent biological repeats. In the case of NHP studies, statistical power calculations considered the number of animals required to detect significant induction of immune responses compared to non-vaccinated controls. With groups of $n=6$ a vaccine efficacy >85% can be demonstrated [$\alpha=0.05$, $\beta=0.2$ (power=80%), normal distribution]. Allocation of experimental groups was done randomly. Analysis of animal samples was performed blinded, except for ELISpot (machine-based counting), flow cytometry (gates defined on negative control samples, identical gates for all groups) and survival data (predefined humane end-points).

Immunization and infection of hamsters

Hamsters were intraperitoneally (i.p.) vaccinated with the indicated amount of PFUs of the different vaccine constructs using a prime and boost regimen (at day 0 and 7). As a control, two groups were vaccinated at day 0 and day 7 with either 10^3 PFU of YF17D or with MEM medium containing 2% FBS (sham). All animals were bled at day 21 to analyze serum for binding and neutralizing antibodies against SARS-CoV-2. At the indicated time after vaccination and prior to challenge, hamsters were anesthetized by intraperitoneal injection of a xylazine (16 mg/kg, XYL-M®, V.M.D.), ketamine (40 mg/kg, Nimatek®, EuroVet) and atropine (0.2 mg/kg, Sterop®) solution. Each animal was inoculated intranasally by gently adding 50 µl droplets of virus stock containing 2×10^5 TCID₅₀ of SARS-CoV-2 in both nostrils. Animals were monitored daily for signs of disease (lethargy, heavy breathing or ruffled fur). Four days after challenge, all animals were euthanized to collect end sera and lung tissue in RNA later, MEM or formalin for gene-expression profiling, virus titration or histopathological analysis, respectively. To determine the viremia profile of the vaccine virus a subset of hamsters was vaccinated with 10^4 PFU YF17D or YF-S0 i.p. and bled on days 1, 2, 3, 4, 5, 7, 9, 11, 15 and 29 to measure serum viral load.

Immunization and infection of mice

Ifnar1^{-/-} mice were i.p. vaccinated with different vaccine constructs by using a prime and boost of each 4×10^2 PFU (at day 0 and 7). As a control, two groups were vaccinated (at day 0 and 7) with either YF17D or sham. All mice were bled weekly and serum was separated by centrifugation

for indirect immunofluorescence assay (IIFA) and serum neutralization test (SNT). Three weeks post first-vaccination, mice were euthanized, spleens were harvested for ELISpot cytokine detection, transcription factor analysis by RT-qPCR and intracellular cytokine staining (ICS). For yellow fever challenge, mice were inoculated with a lethal dose of YF17D via the intracranial (i.c.) route as described^{14,34}. In brief, three weeks after i.p. vaccination with 4×10^2 PFU of either YF-S0 or YF17D, or sham, animals were anesthetized and inoculated i.c. with 30 µl containing 3×10^3 PFU of YF17D, and then monitored daily for signs of disease and weight change for 4 weeks. Sick mice were euthanized based on morbidity (hind limb paralysis, weakness and ruffled fur), or weight loss of more than 25%.

Vaccine safety testing in suckling mice, AG129 mice and *STAT2*^{-/-} hamsters

To evaluate neurovirulence and neurotropism, BALB/c mice pups and AG129 mice were, respectively, intracranially or i.p. inoculated with the indicated PFU amount of YF17D and YF-S vaccine constructs and monitored daily for morbidity and mortality for 21 days post inoculation. To study viscerotropic disease, *STAT2*^{-/-} hamsters were inoculated for highest exposure via the i.p. route with 10^4 PFU of YF17D or YF-S0 and followed daily for 21 days for well-being. Euthanasia was performed if the following occurred: total weight loss of > 20%; day to day weight loss of > 10%; clear signs of paralysis; signs of distress (laboured breathing, ruffled hair, abnormal/hunched posture, severe agitation or depression).

Immunization and infection challenge of cynomolgus macaques

All housing and animal procedures took place at the BPRC, upon positive advice by the independent ethics committee (DEC-BPRC), under project license AVD5020020209404 issued by the Central Committee for Animal Experiments (CCD), and following approval of the detailed study protocol by the institutional animal welfare body (AWB). All animal handlings were performed within the Department of Animal Science (ASD) according to Dutch law, regularly inspected by the responsible national authority (Nederlandse Voedsel- en Warenautoriteit, NVWA), and the AWB.

Animals were pair-housed with a socially compatible cage-mate and randomly assigned to two groups. Six ($n=6$) cynomolgus macaques vaccinated s.c. in the inner upper limbs using a dose of 10^5 PFU of YF-S0 at days 0 (prime) and 7 (boost). As a control, $n=6$ animals were vaccinated twice with 10^5 PFU of a matched placebo vaccine, consisting of recombinant YF17D with an irrelevant control antigen with no sequence homology to SARS-CoV-2 inserted in the same location (E/NS1 junction).

A temperature monitor was implanted in the abdominal cavity of each animal three weeks before the start of the study (Anapill DSI, 's-Hertogenbosch, The Netherlands) providing continuous real-time measurement of body temperature and activity. Health was checked daily and animals monitored for appetite, general behaviour and stool consistency. Blood was collected for regular assessment of whole blood counts and clinical chemistry with no changes out of normal ranges detected.

On day 21 post vaccination, all NHP were challenged by a combined intranasal-intratracheal inoculation with nominally 1.5×10^4 TCID₅₀ of SARS-CoV-2 (as determined by back-titration on Vero cells) in total volume 5 ml; split over the trachea (4 ml) and nares (0.25 ml each). Resulting virus RNA loads were quantified in throat swabs using RT-qPCR as described with a lower limit of detection (LLOD) of 200 RNA copies/ml (Ref. ⁶²). After a follow-up for 21 days, animals were euthanized for histological analysis of their lungs²⁶.

SARS-CoV-2 and YF RT-qPCR

The presence of infectious SARS-CoV-2 particles in hamster lung homogenates was quantified by RT-qPCR (Ref. ²). Briefly, for quantification of viral RNA levels and gene expression after challenge, RNA

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was extracted from homogenized organs using the NucleoSpin™ Kit Plus (Macherey-Nagel), following the manufacturer's instructions. Reactions were performed using the iTaq™ Universal Probes One-Step RT-qPCR kit (BioRad), with primers and probes (Integrated DNA Technologies) listed in Supplementary Table 1. The relative RNA fold-change was calculated with the $2^{-\Delta\Delta C_t}$ method⁶³ using housekeeping gene β -actin for normalization. For detection of vaccine virus in blood, RNA from 50 μ l of serum was extracted with the NucleoSpin RNA virus kit (Macherey-Nagel, Germany). Primers and probe were derived from the YF nonstructural gene 3 and in Supplementary Table 1. RT-qPCR was performed using the ABI 7500 Fast Real-Time PCR System (Applied Biosystems, Branchburg, NJ). For absolute quantification, standard curves were generated using 5-fold dilutions of a cDNA plasmid template (plasmid pShuttle/YFV-17D^{14,34}) of known concentration. Based on repeated standard curves the lower limit of detection (LLOD) was established at 8900 copies/ml, corresponding to a C_t value of 35. C_t values above 35 were considered below the limit of detection and represented as the square root of the LLOD.

End-point virus titrations

To quantify infectious SARS-CoV-2 particles, endpoint titrations were performed on confluent Vero E6 cells in 96-well plates. Lung tissues were homogenized using bead disruption (Precellys®) in 250 μ l minimal essential medium and centrifuged (10,000 rpm, 5 min, 4 °C) to pellet the cell debris. Viral titers were calculated by the Reed and Muench method⁶⁴ and expressed as 50% tissue culture infectious dose (TCID₅₀) per mg tissue.

Histology

For histological examination, lung tissues (for NHP: after inflation with 10% neutral-buffered formalin) were fixed in 4% paraformaldehyde (hamsters) or 10% neutral-buffered formalin (NHP) and embedded in paraffin. Tissue sections (5 μ m for hamsters; 3 μ m for NHP) were stained with hematoxylin and eosin and analyzed for lung damage by an expert pathologist.

Micro-computed tomography (CT) and image analysis

To monitor the development of lung pathology after SARS-CoV-2 challenge, hamsters were imaged using an X-cube micro-computed tomography (CT) scanner (Molecubes) as described before². Quantification of reconstructed micro-CT data were performed with Data-Viewer and Ctan software (Bruker Belgium). A semi-quantitative scoring of micro-CT data was performed as primary outcome measure and imaging-derived biomarkers (non-aerated lung volume) as secondary measures, as previously described^{2,65-68}.

Detection of total binding IgG and IgG isotyping by indirect immunofluorescent assay (IIFA)

To detect SARS-CoV-2 specific antibodies in hamster and mouse serum, an in-house developed IIFA was used. Using CRISPR/Cas9, a CMV-SARS-CoV-2-Spike-Flag-IRES-mCherry-P2A-BlastiR cassette was stably integrated into the ROSA26 safe harbor locus of HEK293T cells⁶⁹. To determine SARS-CoV-2 Spike binding antibody end titers, 1/2 serial serum dilutions were made in 96-well plates on HEK293T-Spike stable cells and HEK293T wt cells in parallel. Goat-anti-mouse IgG Alexa Fluor 488 (A11001, Life Technologies; 1:250 dilution), goat-anti-mouse IgG1, IgG2b and IgG2c Alexa Fluor 488 (respectively 115-545-205, 115-545-207 and 115-545-208 from Jackson ImmunoResearch; all 1:250 diluted) were used as secondary antibody. After counterstaining with DAPI, fluorescence in the blue channel (excitation at 386 nm) and the green channel (excitation at 485 nm) was measured with a Cell Insight CX5 High Content Screening platform (Thermo Fischer Scientific). Specific SARS2-CoV-2 Spike staining is characterized by cytoplasmic (ER) enrichment in the green channel. To quantify this specific SARS-CoV-2 Spike staining the difference in cytoplasmic vs. nuclear signal for the

HEK293T wt conditions was subtracted from the difference in cytoplasmic vs. nuclear signal for the HEK293T SARS-CoV-2 Spike conditions. All positive values were considered as specific SARS-CoV-2 staining. The IIFA end titer of a sample is defined as the highest dilution that scored positive this way. Because of limited volume of serum, IIFA end titers for most conditions (unless indicated otherwise) were determined on minipools of two to three samples.

SARS-CoV-2 pseudotyped virus and YF17D virus serum neutralization test (SNT)

SARS-CoV-2 VSV pseudotypes were generated as described previously⁷⁰⁻⁷². Briefly, HEK-293T cells were transfected with a pCAGGS-SARS-CoV-2_{Δ18}-Flag expression plasmid encoding SARS-CoV-2 Spike protein carrying a C-terminal 18 amino acids deletion^{73,74}. One day post-transfection, cells were infected with VSVΔG expressing a GFP (green fluorescent protein) reporter gene (MOI 2) for 2h. The medium was changed with medium containing anti-VSV-G antibody (1I, mouse hybridoma supernatant from CRL-2700; ATCC) to neutralize any residual VSV-G virus input⁷⁵. 24h later supernatant containing SARS-CoV-2 VSV pseudotypes was harvested.

To quantify SARS-CoV-2 nAbs, serial dilutions of serum samples were incubated for 1 hour at 37 °C with an equal volume of SARS-CoV-2 pseudotyped VSV particles and inoculated on Vero E6 cells for 18 hours.

Neutralizing titers (SNT₅₀) for YFV were determined with an in-house developed fluorescence-based assay using a mCherry tagged variant of YF17D virus^{34,54}. To that end, serum dilutions were incubated in 96-well plates with the YF17D-mCherry virus for 1h at 37 °C after which serum-virus complexes were transferred for 72 h to BHK-21J cells. The percentage of GFP or mCherry expressing cells was quantified on a Cell Insight CX5/7 High Content Screening platform (Thermo Fischer Scientific) and neutralization IC₅₀ values were determined by fitting the serum neutralization dilution curve that is normalized to a virus (100%) and cell control (0%) in Graphpad Prism (GraphPad Software, Inc.).

SARS-CoV-2 and YF17D plaque reduction neutralization test (PRNT)

Sera were serially diluted with an equal volume of 70 PFU of SARS-CoV-2 or 40 PFU of YF17D before incubation at RT °C for 1h. Serum-virus complexes were added to Vero E6 (SARS-CoV-2) or BHK-21J (YF17D) cell monolayers in 24-well plates and incubated at 37 °C for 1h. Three days later, overlays were removed and stained with 0.5% crystal violet after fixation with 3.7% PFA. Neutralization titers (PRNT₅₀) of the test serum samples were defined as the reciprocal of the highest test serum dilution resulting in a plaque reduction of at least 50%.

Antigens for T cell assays

PepMix™ Yellow Fever (NS4B) (JPT-PM-YF-NS4B) and subpool-1 (158 overlapping 15-mers) of PepMix™ SARS-CoV-2 spike (JPT-PM-WCPV-S-2) were used as recall antigens for ELISpot and ICS. Diluted Vero E6 cell lysate (50 μ g/mL) and a combination of PMA (50 ng/mL) (Sigma-Aldrich) and Ionomycin (250 ng/mL) (Sigma-Aldrich) served as negative and positive control, respectively.

Intracellular cytokine staining (ICS) and flow cytometry

Fresh mouse splenocytes were incubated with 1.6 μ g/mL Yellow Fever NS4B peptide mixture; 1.6 μ g/mL Spike peptide subpool-1; PMA (50 ng/mL)/Ionomycin (250 ng/mL) or 50 μ g/mL Vero E6 cell for 18h at 37 °C. After treatment with brefeldin A (Biolegend) for 4h, the splenocytes were stained for viability with Zombie Aqua™ Fixable Viability Kit (Biolegend) and Fc-receptors were blocked by the mouse FcR Blocking Reagent (Miltenyi Biotec) (0.5 μ L/well) for 15 min in the dark at RT. Cells were then stained with extracellular markers BUV395 anti-CD3 (17A2; 1:167 dilution) (BD), BV785 anti-CD4 (GK1.5; 1:100 dilution) (Biolegend),

APC/Cyanine7 anti-CD8 (53-6.7; 1:100 dilution) (Biolegend) and PerCP/Cyanine5.5 anti-TCR γ/δ (GL3; 1:67 dilution) (Biolegend) in Brilliant Stain Buffer (BD) before incubation on ice for 25 min. Cells were washed once with PBS and fixed/permeabilized for 30 min by using the FoxP3 transcription factor buffer kit (Thermo Fisher Scientific) according to the manufacturer's protocol. Finally, cells were stained with following antibodies: PE anti-IL-4 (11B11; 1:50 dilution), APC anti-IFN- γ (XMG1.2; 1:100 dilution), PE/Dazzle™ 594 anti-TNF- α (MP6-XT22; 1:50 dilution), Alexa Fluor® 488 anti-FOXP3 (MF-14; 1:20 dilution), Brilliant Violet 421 anti-IL-17A (TC11-18H10.1; 1:50 dilution) (all from Biolegend) and acquired on a BD LSRFortessa™ X-20 (BD). All measurements were calculated by subtracting from non-stimulated samples (incubated with non-infected Vero E6 cell lysates) from corresponding stimulated samples. The gating strategy employed for ICS analysis is depicted in Fig. S9. The strategy used for comparative expression profiling of vaccine-induced T-cell populations by t-distributed Stochastic Neighbor Embedding (t-SNE) analysis is outlined in Fig. S8. All flow cytometry data were analysed using FlowJo Version 10.6.2 (LLC). T-SNE plot was generated in FlowJo after concatenating spike-specific CD4 and CD8 T cell separately based on gated splenocyte samples.

ELISpot

ELISpot assays for the detection of IFN- γ -secreting mouse splenocytes were performed with mouse IFN- γ kit (ImmunoSpot® MIFNG-1M/5, CTL Europe GmbH). IFN- γ spots were visualized by stepwise addition of a biotinylated detection antibody, a streptavidin-enzyme conjugate and the substrate. Spots were counted using an ImmunoSpot® S6 Universal Reader (CTL Europe GmbH) and normalized by subtracting spots numbers from control samples (incubated with non-infected Vero E6 cell lysates) from the spot numbers of corresponding stimulated samples. Negative values were corrected to zero.

RT-qPCR for transcription factor profile

Spike peptide-stimulated splenocytes were used for RNA extraction by using the NucleoSpin™ Kit Plus kit (Macherey-Nagel). cDNA was generated by using a high-capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific). Real-time PCR was performed using the TaqMan gene expression assay (Applied Biosystems) on an ABI 7500 fast platform. Expression levels of TBX21, GATA3, RORC, FOXP3 (all from Integrated DNA Technologies) were normalized to the expression of GAPDH (IDT). Relative gene expression was assessed by using the $2^{-\Delta\Delta C_q}$ method.

Statistical analysis

GraphPad Prism (GraphPad Software, Inc.) was used for all statistical evaluations. The number of animals and independent experiments that were performed is indicated in the figure legends. Statistical significance was determined using the non-parametric Mann-Whitney U-test and Kruskal-Wallis test if not otherwise stated. Values were considered significantly different at P values of ≤ 0.05 .

Reporting summary

Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

SARS-CoV-2 strain BetaCov/Belgium/GHB-03021/2020 sequence available from GISAID (EPI_ISL_407976|2020-02-03, <https://www.gisaid.org>). Prototypic Wuhan-Hu-1 2019-nCoV sequence is available from GenBank (accession number MN908947.3). Full-length YF17D sequence is available from GenBank (accession number X03700). Source data are provided as supplementary information with this paper. Flow cytometry data supporting the findings in this study are also available from the corresponding author upon reasonable request.

51. Lindenbach, B. & Rice, C. M. Trans-Complementation of yellow fever virus NS1 reveals a role in early RNA replication. *J Virol* **71**, 9608-9617, <https://doi.org/10.1128/JVI.71.12.9608-9617.1997> (1998).
52. Buchholz, U. J., Finke, S. & Conzelmann, K. K. Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter. *J Virol* **73**, 251-259 (1999).
53. Dallmeier, K. & Neyts, J. Simple and inexpensive three-step rapid amplification of cDNA 5' ends using 5' phosphorylated primers. *Anal Biochem* **434**, 1-3, <https://doi.org/10.1016/j.ab.2012.10.031> (2013).
54. Sharma, S. et al. Small-molecule inhibitors of TBK1 serve as an adjuvant for a plasmid-launched live-attenuated yellow fever vaccine. *Hum Vaccin Immunother*, 1-8, <https://doi.org/10.1080/21645515.2020.1765621> (2020).
55. Bredenbeek, P. J. et al. A recombinant Yellow Fever 17D vaccine expressing Lassa virus glycoproteins. *Virology* **345**, 299-304, <https://doi.org/10.1016/j.virol.2005.12.001> (2006).
56. Conceição-Neto, N. et al. Modular approach to customise sample preparation procedures for viral metagenomics: a reproducible protocol for virome analysis. *Sci Rep* **5**, 16532, <https://doi.org/10.1038/srep16532> (2015).
57. Conceição-Neto, N., Yinda, K. C., Van Ranst, M. & Matthijssens, J. NetoVIR: Modular Approach to Customise Sample Preparation Procedures for Viral Metagenomics. *Methods Mol Biol* **1838**, 85-95, https://doi.org/10.1007/978-1-4939-8682-8_7 (2018).
58. Müller, U. et al. Functional role of type I and type II interferons in antiviral defense. *Science* **264**, 1918-1921, <https://doi.org/10.1126/science.8009221> (1994).
59. van den Broek, M. F., Müller, U., Huang, S., Zinkernagel, R. M. & Aguet, M. Immune defence in mice lacking type I and/or type II interferon receptors. *Immunol Rev* **148**, 5-18, <https://doi.org/10.1111/j.1600-065x.1995.tb00090.x> (1995).
60. Fan, Z. et al. Efficient gene targeting in golden Syrian hamsters by the CRISPR/Cas9 system. *PLoS One* **9**, e109755, <https://doi.org/10.1371/journal.pone.0109755> (2014).
61. Siddharthan, V. et al. Zika virus infection of adult and fetal STAT2 knock-out hamsters. *Virology* **507**, 89-95, <https://doi.org/10.1016/j.virol.2017.04.013> (2017).
62. Corman, V. M. et al. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. *Euro Surveill* **25**, 2000045, <https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045> (2020).
63. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25**, 402-408, <https://doi.org/10.1006/meth.2001.1262> (2001).
64. Reed, L. J. & Muench, H. A simple method of estimating fifty per cent endpoints. *Am J Epidemiol* **27**, 493-497, <https://doi.org/10.1093/oxfordjournals.aje.a118408> (1938).
65. Vandeghinste, B. et al. Iterative CT reconstruction using shearlet-based regularization. *IEEE Trans Nucl Sci* **60**, 121, <https://doi.org/10.1117/12.911057> (2012).
66. Vande Velde, G. et al. Longitudinal micro-CT provides biomarkers of lung disease that can be used to assess the effect of therapy in preclinical mouse models, and reveal compensatory changes in lung volume. *Dis Model Mech* **9**, 91-98, <https://doi.org/10.1242/dmm.020321> (2016).
67. Berghen, N. et al. Radiosafe micro-computed tomography for longitudinal evaluation of murine disease models. *Sci Rep* **9**, 17598, <https://doi.org/10.1038/s41598-019-53876-x> (2019).
68. Kaptein, S. J. et al. Antiviral treatment of SARS-CoV-2-infected hamsters reveals a weak effect of favipiravir and a complete lack of effect for hydroxychloroquine. *bioRxiv*, 2020.2006.2019.159053, <https://doi.org/10.1101/2020.06.19.159053> (2020).
69. Geisinger, J. M., Turan, S., Hernandez, S., Spector, L. P. & Calos, M. P. In vivo blunt-end cloning through CRISPR/Cas9-facilitated non-homologous end-joining. *Nucleic Acids Res* **44**, e76, <https://doi.org/10.1093/nar/gkv1542> (2016).
70. Whitt, M. A. Generation of VSV pseudotypes using recombinant Δ G-VSV for studies on virus entry, identification of entry inhibitors, and immune responses to vaccines. *J Virol Methods* **169**, 365-374, <https://doi.org/10.1016/j.jviromet.2010.08.006> (2010).
71. Berger Rentsch, M. & Zimmer, G. A vesicular stomatitis virus replicon-based bioassay for the rapid and sensitive determination of multi-species type I interferon. *PLoS One* **6**, e25858, <https://doi.org/10.1371/journal.pone.0025858> (2011).
72. Hoffmann, M. et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* **181**, 271-280.e278, <https://doi.org/10.1016/j.cell.2020.02.052> (2020).
73. Fukushi, S. et al. Vesicular stomatitis virus pseudotyped with severe acute respiratory syndrome coronavirus spike protein. *J Gen Virol* **86**, 2269-2274, <https://doi.org/10.1099/vir.0.80955-0> (2005).
74. Wang, C. et al. Publisher Correction: A human monoclonal antibody blocking SARS-CoV-2 infection. *Nat Commun* **11**, 2511, <https://doi.org/10.1038/s41467-020-16452-w> (2020).
75. Kleine-Weber, H. et al. Mutations in the Spike protein of Middle East respiratory syndrome coronavirus transmitted in Korea increase resistance to antibody-mediated neutralization. *J Virol* **93**, <https://doi.org/10.1128/jvi.01381-18> (2019).
76. Op De Beeck, A., Rouillé, Y., Caron, M., Duvert, S. & Dubuisson, J. The transmembrane domains of the prM and E proteins of yellow fever virus are endoplasmic reticulum localization signals. *Journal of virology* **78**, 12591-12602, <https://doi.org/10.1128/jvi.78.22.12591-12602.2004> (2004).
77. Bonaldo, M. C. et al. Construction and characterization of recombinant flaviviruses bearing insertions between E and NS1 genes. *Virology Journal* **4**, 115, <https://doi.org/10.1186/1743-422X-4-115> (2007).
78. Barban, V. et al. High stability of yellow fever 17D-204 vaccine: a 12-year retrospective analysis of large-scale production. *Vaccine* **25**, 2941-2950, <https://doi.org/10.1016/j.vaccine.2006.06.082> (2007).

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Competing interests L.S.F., J.N., and K.D. are named as inventors on patent describing the invention and use of coronavirus vaccines. All other authors have nothing to disclose.

Additional information

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Extended Data Fig. 1 | Vaccine design and antigenicity. **a**, SARS-CoV-2 Spike (S1/2, S0 or S1) antigens were inserted into the E/NS1 intergenic region as translational fusion within the YF17D polyprotein (dark grey) inserted in the ER (endoplasmic reticulum; pale blue). To cope with topological constraints of the fold of both SARS-CoV-2 Spike antigens and the polyprotein of the YF17D vector, one extra transmembrane domain^{76,77} (derived from the West Nile virus E-protein; light grey) was added to the C-terminal cytoplasmic domain of the full-length S proteins (S1/2 and S0). Likewise, two transmembrane domains were fused to the ER-resident C-terminus of the S1 subunit in construct YF-S1. Scissors indicate proposed maturation cleavage sites, including the S1/2 furin-cleavage site mutated in YF-S0. **b**, Representative pictures of plaque phenotypes from YF17D and different YF-S vaccine constructs on BHK-21 cells.

c, Confocal immunofluorescent images of BHK-21 cells three days post-infection with different YF-S vaccine constructs staining for SARS-CoV-2 Spike antigen (green) and YF17D (red) (nuclei stained with DAPI, blue; white scalebar: 25 μ m). Similar results were obtained from two independent experiments. **d**, Immunoblot analysis of SARS-CoV-2 Spike (S1/2, S0 and S1) expression after transduction of BHK-21 cells with different YF-S vaccine candidates. Prior to analysis, cell lysates were treated with PNGase F to remove their N-linked oligosaccharides or left untreated (black arrows – glycosylated forms of S; white arrow heads – de-glycosylated proteoforms without N-linked oligosaccharides). Similar results were obtained from two technical repeats from the same experiment. For gel source data, see Supplementary Figure 1.

a BHK passaging schematics

d Spike expression P3-P6 YF-S0

b Primer pairs

c Stability of P3-P6 YF-S0

e Stability of P4* YF-S0

Extended Data Fig. 2 | Genetic stability of YF-S0 during passaging in BHK-21 cells. **a**, Schematic representation of YF-S0 passaging in BHK-21 cells, YF-S0 vaccine virus recovered from transfected BHK-21 cells (P0) was plaque purified once (P1, n=5 plaque isolates), amplified (P2) and serially passaged on BHK-21 cells (P3-P6). In parallel, each amplified plaque isolate (P2, n=5) from the first plaque purification was subjected to a second round of plaque purification (P3*, n=25 plaque isolates) and amplification (P4*). **b**, Schematic representation of tiled RT-PCR amplicons from three different primer pairs used for detection of the inserted SARS-CoV-2 Spike viral RNA sequence present in supernatants of different passages. All data are from a single representative experiment. **c**, RT-PCR fingerprinting performed on the virus supernatant harvested from serial passage 3 (P3) and 6 (P6) of plaque purified YF-S0. **d**, Immunoblot analysis of spike expression by P3 and P6 of YF-S0. **e**, RT-PCR fingerprinting on

amplified plaque isolates from the second round of plaque purification (P4*), 20 individual amplified plaque isolates are shown here (1-20). **c,e**, Control: YF-S0 cDNA (0.5 ng). Ladder: 1 kb DNA ladder. Direct Sanger sequencing confirmed maintenance of full-length S inserts for 25 out of 25 plaques (100%). After two rounds of plaque purification and amplification, only in three isolates a single point mutation was found (two silent mutations and one missense mutation resulting in a S47P amino acid change in the N-terminus of S1); at a low $<10^{-4}$ mutation frequency (i.e. 3 nt changes observed in a total of $25 \times 4196 \text{ nt} = 104900 \text{ nt}$ sequenced; of which $25 \times 3780 \text{ nt} = 94500 \text{ nt}$ were of S transgene sequence). This mutation rate is similar to that of parental YF17D under current vaccine manufacturing conditions^{14,78}. For gel source data, see Supplementary Figure 1.

a Survival and weight Balb/c suckling mice **b** Balb/c suckling mice **c** Survival and weight curve AG129 mice **d** Survival WT and *STAT2*^{-/-} hamsters **e** Viremia WT hamsters **f** Weight WT hamsters **g** Weight *Ifnar*^{-/-} mice

Extended Data Fig. 3 | Attenuation of YF-S vaccine candidates. **a**, Survival and weight curve of suckling Balb/c mice inoculated intracranially (i.c.) with 100 PFU of the different vaccine constructs. Animals inoculated with YF-S1/2 (n=8), YF-S0 (n=8), YF-S1 (n=10), sham (n=10) and YF17D (n=9). **b**, Representative images of Balb/c mice at seven days after i.c. inoculation with sham, 10² PFU of either YF-S0 or YF17D. **c**, Survival and weight curve of AG129 mice (n=8 for each group) after intraperitoneal (i.p.) inoculation with a dose range of YF-S0 (10², 10³ and 10⁴ PFU; green) in comparison to YF17D (1, 10 and 10² PFU black and grey). **d**, Survival curve of WT and *STAT2* knock-out (*STAT2*^{-/-}) hamsters inoculated i.p. with 10⁴ PFU of YF17D or YF-S0. WT hamsters inoculated with

YF17D (n=6) and YF-S0 (n=6); *STAT2*^{-/-} hamsters inoculated with YF17D (n=14) and YF-S0 (n=13). Number of surviving animals at study endpoint indicated (a,c,d). **e-f**, Vaccine virus RNA (viremia) in the serum (e) and weight evolution (f) of WT hamsters after i.p. inoculation with 10⁴ PFU YF17D (n=6) or YF-S0 (n=6). Number of animals that showed viremia on each day post inoculation indicated below (e). **g**, Weight evolution of *Ifnar*^{-/-} mice after i.p. inoculation with 400 PFU each at day 0 and 7 of YF-S0, YF17D and sham. Animals inoculated with YF17D (n=5), YF-S0 (n=5) or sham (n=5). Data in panel d are from two independent experiments, data in other panels are from a single experiment.

a Benchmarking SNT **b Viral RNA levels in organs** **c nAb titers post-challenge** **d nAb titers pre/post-challenge** **e bAb titers post-challenge** **f Vaccination and challenge schedule** **g nAb titers** **h Viral RNA in lungs** **i Infectious virus in lungs** **Extended Data Fig. 4 | Immunogenicity and protective efficacy in hamsters.**

a left, Correlation analysis of nAb titers using SARS-CoV-2 (PRNT) and rVSV-ΔG-spike (SNT) for a panel of seven sera. SNT₅₀ and PRNT₅₀ values were plotted to determine the correlation between the neutralization assays with a Pearson regression coefficient of 0.77 ($P=0.04$). **a right**, nAbs in sera from four convalescent patients as determined by SNT. Hamsters were vaccinated and challenged as depicted in Figure 1b. **b**, Viral RNA in spleen, liver, kidney, heart and ileum of hamsters ($n=6$ for each group from a single experiment) vaccinated with YF-S1/2, YF-S0 or sham, and challenged by infection with SARS-CoV-2. Viral RNA levels were determined by RT-qPCR, normalized against β -actin mRNA levels, and calculated relative to the median of sham-vaccinated animals. **c-e**, nAbs (**c-d**) and bAbs (**e**) in vaccinated hamsters four days after challenge with SARS-CoV-2 ($n=12$ animals per group from two independent

experiments; for bAb quantification sera of three animals were pooled). **d**, Pair-wise comparison of nAbs at day 21 post-immunization (circles), and four days post-challenge (squares). **f**, Syrian hamsters were immunized twice i.p. with 1.5×10^3 PFU each of vaccine construct YF-S0 produced on Vero E6 cells ($n=7$ animals), or sham ($n=3$ animals). At day 23 post-vaccination, animals were inoculated with 2×10^5 TCID₅₀ SARS-CoV-2 and followed up for four days. **g**, nAbs 21 days post-vaccination. **h-i**, Viral loads in lungs of hamsters four days after infection quantified by RT-qPCR (**h**) and virus titration (**i**). Data in panels g-i are from a single experiment. LLOQ - lower limit of quantification; LLOD - lower limit of detection. Data are medians $\pm$ IQR. Two-tailed uncorrected Kruskal-Wallis test (**b,c,e**) or a two-tailed Mann-Whitney test was applied (**g-i**) (ns = Not-Significant).

a Lung pathology sham-vaccinated hamster **b** Histopathology scores of H&E images **c** μ CT images **d** μ -CT-derived lung score **e** μ -CT-derived lung volumes **f** Gene expression levels

Extended Data Fig. 5 | Protection from lung pathology. Hamsters ($n=12$ from two independent experiments for all groups) were vaccinated and challenged as depicted in Figure 1b. **a**, Representative H&E images of a diseased lung (sham-vaccinated and infected). Upper panel: area of bronchopneumonia (circle). Black scalebar: 1 mm. Lower panel: magnification of inflamed tissue showing a mixture of histiocytes (yellow arrows), neutrophils (green arrows) and lymphocytes (blue arrows). Black scalebar: 50 μ m. **b**, Cumulative histopathology score for signs of lung damage in H&E stained lung sections (dotted line – maximum score in sham-vaccinated group). **c**, Representative micro-CT images of sham and YF-S0 vaccinated four days after SARS-CoV-2 infection. Arrows indicate examples of pulmonary infiltrates seen as consolidation of lung parenchyma (cyan). **d-e**, Five transverse cross sections at

different positions in the lung were selected for each animal and scored to quantify lung consolidations (d) or used to quantify the non-aerated lung volume (NALV) (e), as functional biomarker reflecting lung consolidation. **f**, Individual expression profiles for 10 genes in lungs of vaccinated hamsters four days after SARS-CoV-2 infection (as in Figure 1k presented as $\log_{10}$ -fold change relative to uninfected controls ($n=4$)). Levels of individual mRNAs were determined by RT-qPCR and normalized for β -actin mRNA. Changes are reported as values over the median of uninfected controls. Only for IFN- λ , where all control animals had undetectable RNA levels, fold changes were calculated over the lowest detectable value. LLOD – lower limit of detection. Data are medians $\pm$ IQR. Two-tailed uncorrected Kruskal-Wallis test was applied.

a Gating strategy

b t-SNE analysis

Extended Data Fig. 6 | Gating strategy and profiling of CD8 and CD4 T-cells.

a, First, live cells were selected by gating out Zombie Aqua (ZA) positive and low forward scatter (FSC) events. Then, doublets were eliminated in an FSC-H vs. FSC-A plot. T-cells (CD3 positive) were stratified into $\gamma\delta$ T-cells ($\gamma\delta$ TCR⁺), CD4 T-cells ($\gamma\delta$ TCR/CD4⁺) and CD8 T-cells ($\gamma\delta$ TCR/CD8⁺). Boundaries defining positive and negative populations for intracellular markers were set based on non-stimulated control samples. **b**, Full representation of t-distributed Stochastic Neighbor Embedding (t-SNE) analysis of Spike-specific CD4 and CD8

T-cells positive for at least one intracellular marker (IFN- γ , TNF- α , IL-4, or IL17A) from splenocytes of YF-S1/2, YF-S0 and sham vaccinated *ifnar*^{-/-} mice (n=6 per group) after overnight stimulation with SARS-CoV-2 Spike peptide pool (blue – IFN- γ expressing T-cells; red – TNF- α expressing T-cells; green – IL-4 expressing T-cells; yellow – IL17A expressing T-cells). t-SNE plots generated using FlowJo by first concatenating Spike-specific CD8 (upper panels) or CD4 T-cells (lower panels) from all animals.

Extended Data Fig. 7 | Immunogenicity and protective efficacy in mice and hamsters after single dose vaccination. **a**, *ifnar*^{-/-} mice were vaccinated once i.p. with 400 PFU YF-S0 (n=9), sham (n=6) or YF17D (n=6). **b-c**, nAbs (b) and bAbs (c) at day 21 post-vaccination; minipools of sera from two to three animals analyzed for quantification of bAbs. **d**, Spot counts for IFN-γ-secreting cells per 10⁶ splenocytes after stimulation with SARS-CoV-2 Spike peptide pool. **e**, Hamsters (n=8 from a single experiment for all groups) were vaccinated with a single dose of YF-S0 and challenged as outlined in Figure 4. Cumulative

histopathology score for signs of lung damage after SARS-CoV-2 infection in H&E stained lung sections (dotted line – maximum score in sham-vaccinated group). **f-g**, Hamsters (n=6 animals per group from a single experiment) were vaccinated with a single dose of YF-S0 (10⁴ PFU i.p.) and sera were collected at 3, 10 and 12 weeks after vaccination. nAbs (f) and bAbs (g) at the indicated weeks post vaccination. LLOQ - lower limit of quantification; LLOD - lower limit of detection. Data are medians ± IQR. Two-tailed uncorrected Kruskal-Wallis test was applied (ns = Not-Significant).

Extended Data Fig. 8 | YF17D-specific immune responses in hamsters, mice and NHP. **a**, Correlation analysis of nAb titers determined by using YF17D (PRNT) and YF17D-mCherry (SNT) for a panel of 21 antisera (historical samples from previous vaccination studies in mice and hamsters). SNT₅₀ and PRNT₅₀ values were plotted to determine the correlation between both neutralization assays with a Pearson regression coefficient of 0.68 ($P=0.0006$). **b-c**, nAb titers in hamsters ($n=12$ from two independent experiments for all groups) (**b**) and *ifnar*^{-/-} mice (YF-S1/2 ($n=11$), YF-S0 ($n=11$), YF-S1 ($n=8$), sham ($n=9$) and YF17D ($n=8$) from two to three independent experiments) (**c**); sera collected at day 21 post-vaccination in both experiments (two-dose vaccination schedule; Fig. 1 and 2). **d**, *ifnar*^{-/-} mice vaccinated according to a two-dose vaccination schedule (YF-S1/2 ($n=11$), YF-S0 ($n=10$), YF-S1 ($n=7$), sham ($n=9$) and YF17D ($n=8$) from

three independent experiments). Spot counts for IFN γ -secreting cells per 10^6 splenocytes after stimulation with a YF17D NS4B peptide mixture. **e-f**, nAb titers after vaccination in NHP with YF-S0 (**e**) or placebo (**f**) (6 animals per group from a single experiment); sera collected at indicated times post-vaccination (two-dose vaccination schedule; Fig. 3). **g**, *ifnar*^{-/-} mice vaccinated according to a single-dose vaccination schedule (YF-S0 ($n=8$), sham ($n=5$) and YF17D ($n=5$) from two independent experiments). Spot counts for IFN γ -secreting cells per 10^6 splenocytes after stimulation with a YF17D NS4B peptide mixture. LLOQ - lower limit of quantification; LLOD - lower limit of detection. Data are medians $\pm$ IQR. Two-tailed uncorrected Kruskal-Wallis test was applied (ns = Not-Significant).

Extended Data Fig. 9 | Protection from lethal YF17D. a, *Ifnar*^{-/-} mice were vaccinated with either a single 400 PFU i.p. dose of YF17D (black, n=7) or YF-S0 (green, n=10), or sham (grey, n=9). After 21 days, mice were challenged by intracranial (i.c.) inoculation with a uniformly lethal dose of 3x10³ PFU of YF17D

and monitored for weight evolution (b) and survival (c). Number of surviving animals at study endpoint (day 15) indicated. Data are from two independent experiments.

Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☐	☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ The statistical test(s) used AND whether they are one- or two-sided
<i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☐	☒ A description of all covariates tested
☐	☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
<i>Give P values as exact values whenever suitable.</i>
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection	Chemiluminescence signals were analyzed using Compass software (Protein Simple, Version 4.0.0, Build ID: 0815). Visualization and quantification of reconstructed micro-CT data was performed with DataViewer and CTan software (Version 1.20.3.0 Bruker micro-CT). Acquisition of flow cytometry data on the BD LSR-Fortessa X-20 was performed with BD FACSDiva software (Version 8.0.2, build 2016 11 22 10 42). High content image collection and analysis performed with CellInsight CX5 High Content Screening platform.
Data analysis	GraphPad Prism Version 8 (GraphPad Software, Inc.) was used for all statistical evaluations. FlowJo Version 10.6.2 (LLC) for analysis of flow cytometry data. High content image collection and analysis performed with CellInsight CX5 High Content Screening platform.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

SARS-CoV-2 strain BetaCov/Belgium/GHB-03021/2020 sequence available from GISAID (EPI_ISL_407976|2020-02-03, <https://www.gisaid.org>). Prototypic Wuhan-Hu-1 2019-nCoV sequence is available from GenBank (accession number MN908947.3). Full-length YF17D sequence is available from GenBank (accession number X03700). Source data are provided as supplementary information with this paper. Flow cytometry data supporting the findings in this study are also available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	(mice and hamsters): Sample sizes were chosen based on the results of pilot experiments. Pivotal studies have been performed in at least two independent biological repeats. (NHPs): Statistical power calculations consider the number of animals required to detect significant induction of immune responses compared to non-vaccinated controls. With groups of n=6 a vaccine efficacy >85% can be demonstrated [$\alpha=0.05, \beta=0.2$ (power=80%), normal distribution]. For continuous variables (challenge virus load) the minimal detectable alternative is $1.8 \times \text{SD}$ for n=6 [$\alpha=0.05, \beta=0.2$ (power=80%), Student t-distribution]. Data from a previous experiment (PMID: 32303590) showed the standard error of the mean was 0.5 log ₁₀ TCID ₅₀ or an SD of $(0.5 \times \sqrt{4}) = 1$ log ₁₀ TCID ₅₀ . This means that we should be able to detect a reduction of 1.8 log ₁₀ TCID ₅₀ on days 1-4.
Data exclusions	No data were excluded from the study.
Replication	Principal data could be successfully replicated in duplicate experiments.
Randomization	Allocation of experimental groups was performed randomly.
Blinding	Data acquisition/analysis of RT-qPCR, CT scans, virus titrations, serology and histology was performed blinded. Not blinded: ELISPOT (machine-based counting, so non-biased) Flow cytometry (boundaries based on negative control samples, so blinding not possible, but based on this, identical gates were used for all groups) Survival data (animals were euthanized based on predefined humane end-points)

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

WES:
SARS Spike Protein Antibody (NB100-56578, Novus Biologicals, 1:100 dilution) (Lot: AB092903C-06)
HRP-conjugated Anti-Rabbit Detection Module (DM-001, ProteinSimple, 1:100 dilution) (Lot: 80636)
rabbit SARS-CoV Spike primary antibody (40150-T62-COV2, Sino Biological, 1:100 dilution) (Lot: HD14MA0908)
Mouse monoclonal anti-GAPDH antibody (Clone 1D4) (NB300-221, Novus Biologicals, 1:2000 dilution) (Lot: HD14MA0908)

Immunofluorescence:
rabbit SARS-CoV Spike S1 antibody (40150-RP01, Sino Biological, 1:250 dilution) (Lot: HC09JL3110-B)
rabbit SARS-CoV Spike primary antibody (40150-T62-COV2, Sino Biological, 1:250 dilution) (Lot: HD14MA0908)
goat anti-rabbit Alexa Fluor-488 (A11034, Life Technologies, 1:500 dilution) (Lot: 2018207)
goat anti-mouse Alexa Fluor-594 (A11005, Life Technologies, 1:500 dilution) (Lot: 1890858)

IIFA for detection of IgG isotypes:

goat-anti-mouse IgG Alexa Fluor 488 (A11001, Life Technologies, 1:250 dilution) (Lot: 2140660 & 2189178)
 goat-anti-mouse IgG1 Alexa Fluor 488 (115-545-205, Jackson ImmunoResearch, 1:250 dilution) (Lot: 143658)
 goat-anti-mouse IgG2b Alexa Fluor 488 (115-545-207, Jackson ImmunoResearch, 1:250 dilution) (Lot: 141849)
 goat-anti-mouse IgG2c Alexa Fluor 488 (115-545-208, Jackson ImmunoResearch, 1:250 dilution) (Lot: 144218)

Flow cytometry:

BUV395 anti-CD3 (17A2) (740268, Becton-Dickinson, 1:167 dilution) (Lot: 0150504)
 BV785 anti-CD4 (GK1.5) (100453, Biolegend, 1:100 dilution) (Lot: B308333)
 APC/Cyanine7 anti-CD8 (53-6.7) (100714, Biolegend, 1:100 dilution) (Lot: B314157)
 PerCP/Cyanine5.5 anti-TCR γ/δ (GL3) (118118, Biolegend, 1:67 dilution) (Lot: B289573)
 PE anti-IL-4 (11B11) (504104, Biolegend, 1:50 dilution) (Lot: B271497)
 APC anti-IFN- γ (XMG1.2) (505810, Biolegend, 1:100 dilution) (Lot: B299238)
 PE/Dazzle™ 594 anti-TNF- α (MP6-XT22) (506346, Biolegend, 1:50 dilution) (Lot: B289898)
 Alexa Fluor® 488 anti-FOXP3 (MF-14) (126406, Biolegend, 1:20 dilution) (Lot: B276008)
 Brilliant Violet 421 anti-IL-17A (TC11-18H10.1) (506926, Biolegend, 1:50 dilution) (Lot: B309658)

Validation

WES:

-SARS Spike Protein Antibody (NB100-56578, Novus Biologicals): antibody was validated by manufacturer for detection of SARS-CoV-2 spike in Western blots. Same used in 'Spike mutation D614G alters SARS-CoV-2 fitness', by Jessica A. Plante et al., Nature 2020 (in press), <https://doi.org/10.1038/s41586-020-2895-3>
 -rabbit SARS-CoV Spike primary antibody (40150-T62-COV2, Sino Biological): antibody was validated by manufacturer for detection of SARS-CoV-2 spike in Western blots. Same used for immunoblotting in 'Development of a multi-antigenic SARS-CoV-2 vaccine using a synthetic poxvirus platform', by F. Chiuppesi, 2020 (preprint), doi: 10.21203/rs.3.rs-40198/v1
 -Mouse monoclonal anti-GAPDH antibody (Clone 1D4) (NB300-221, Novus Biologicals): same used in PMID: 24928958

Immunofluorescence:

-rabbit SARS-CoV Spike S1 antibody (40150-RP01, Sino Biological): same used in PMID: 32795413
 -rabbit SARS-CoV Spike primary antibody (40150-T62-COV2, Sino Biological) same used in PMID: 32379723

Flow cytometry:

-BUV395 anti-CD3 (740268, Becton-Dickinson): same used in PMID: 28399409
 -BV785 anti-CD4 (100453, Biolegend): same used in PMID: 30800128
 -APC/Cyanine7 anti-CD8 (100714, Biolegend): same used in PMID: 30696629
 -PerCP/Cyanine5.5 anti-TCR γ/δ (118118, Biolegend): same used in PMID: 24898474
 -PE anti-IL-4 (504104, Biolegend): same used in PMID: 32402289
 -APC anti-IFN- γ (505810, Biolegend): same used in PMID: 27637330
 -PE/Dazzle™ 594 anti-TNF- α (506346, Biolegend): same clone used in PMID: 31649661
 -Alexa Fluor® 488 anti-FOXP3 (126406, Biolegend): same used in PMID: 28212561
 -Brilliant Violet 421 anti-IL-17A (506926, Biolegend): same clone used in PMID: 17785809

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)

VeroE6 (Peter Bredenbeek, LUMC, the Netherlands; ATCC® CRL-1586™)
 BHK-21J (Peter Bredenbeek, LUMC, the Netherlands; no commercial source, PMID: 9371625)
 HEK293T (Dirk Daelemans, Department of Microbiology, Immunology and Transplantation, Laboratory of Virology and Chemotherapy, Rega Institute, KU Leuven; ATCC® CRL-3216™)

Authentication

No authentication of the cell lines was performed.

Mycoplasma contamination

All cell lines tested negative for mycoplasma contamination.

Commonly misidentified lines
(See [ICLAC](#) register)

None of the commonly misidentified cell lines were used.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Wild-type Syrian hamsters (*Mesocricetus auratus*) and BALB/c mice and pups were purchased from Janvier Laboratories, Le Genest-Saint-Isle, France. Ifnar1^{-/-} and AG129 were bred in-house. Six- to ten-weeks-old female mice and wild-type hamsters were used throughout the study. Animals were housed in individually ventilated cages (Sealsafe Plus, Tecniplast), per 5 (mice, cage type GM500) or per 2 (hamsters, cage type GR900), at 21°C, 55% humidity and 12:12 light/dark cycles. Twelve outbred mature male cynomolgus macaques (*Macaca fascicularis*) were used in this study. Animals were purpose-bred and housed at the Biomedical Primate Research Centre (BPRC, Rijswijk, The Netherlands). All animals selected for the study were in good physical health with normal baseline biochemical and haematological values.

Wild animals	No wild animals were used in the study.
Field-collected samples	No field-collected samples were used in the study.
Ethics oversight	<p>Mice and hamsters: Housing conditions and experimental procedures were approved by the ethical committee of KU Leuven (license P015-2020), following institutional guidelines approved by the Federation of European Laboratory Animal Science Associations (FELASA).</p> <p>NHPs: All housing and animal procedures took place at the BPRC, upon positive advice by the independent ethics committee (DEC-BPRC), under project license AVD5020020209404 issued by the Central Committee for Animal Experiments (CCD), and following approval of the detailed study protocol by the institutional animal welfare body (AWB). All animal handlings were performed within the Department of Animal Science (ASD) according to Dutch law, regularly inspected by the responsible national authority (Nederlandse Voedsel- en Warenautoriteit, NVWA), and the AWB.</p>

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	To generate single-cell suspensions, spleens were pushed through 70-µm cell strainers (BD Biosciences) with syringe plungers. Spleen samples were then incubated with red blood cell lysis buffer (eBioscience) for 8 min at room temperature and washed twice with FACS-B (PBS with 2% FBS and 2mM EDTA).
Instrument	Becton Dickinson LSRFortessa™ X-20
Software	FlowJo Version 10.6.2 (LLC)
Cell population abundance	No cell sorting was performed in this study.
Gating strategy	<p>Gating strategy is provided as supplementary figure (Extended Data Fig 6).</p> <p>Live cells were selected by gating out Zombie Aqua (ZA) positive and low forward scatter (FSC) events. Then, doublets were eliminated in an FSC-H vs. FSC-A plot. T-cells (CD3 positive) were stratified into gδT-cells (gδTCR+), CD4 T-cells (gδTCR-/CD4+) and CD8 T-cells (gδTCR-/CD8+). Boundaries defining positive and negative populations for intracellular markers were set based on non-stimulated control samples.</p>

- ☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.