Supplemental Information

Immunogenicity of a new gorilla adenovirus vaccine candidate for COVID-19

Stefania Capone, Angelo Raggioli, Michela Gentile, Simone Battella, Armin Lahm, Andrea Sommella, Alessandra Maria Contino, Richard A. Urbanowicz, Romina Scala, Federica Barra, Adriano Leuzzi, Eleonora Lilli, Giuseppina Miselli, Alessia Noto, Maria Ferraiuolo, Francesco Talotta, Theocharis Tsoleridis, Concetta Castilletti, Giulia Matusali, Francesca Colavita, Daniele Lapa, Silvia Meschi, Maria Capobianchi, Marco Soriani, Antonella Folgori, Jonathan K. Ball, Stefano Colloca, and Alessandra Vitelli
Fig S1. Schematic cartoon of GRAd32 genome backbones and encoded Spike antigens. The full length wild type (S) or the two proline-stabilized (S-2P) Spike gene expression cassette was cloned in E1 in the leftward orientation. The GRAd32b backbone is deleted of E1 and E3 regions, while GRAd32c genome is deleted of E1, E3 and E4 and replaced with the hAd5 E4 orf6
Fig. S2 Restriction analysis of viral genome after 10 passages. Genomic DNA was extracted from GRAd32b-S-2P (A) and GRAd32c-S-2P (B) purified viral particles (V), digested with different restriction enzymes and compared to the pre-adeno plasmid (P). M: 1kb marker, BsrGI+SpeI (lane 2-3) XhoI+SphI (lane 5-6) XmnI (lane 7-8)
Figure S3. Expression of Spike antigens. (A) Western blot analysis of reducing SDS-PAGE of cell lysates from HeLa cells infected with 50 MOI of GRAd32b (lane 1) or GRAd32c (lane 2) backbone vectors encoding Spike 2P, using an anti-HA tag rabbit monoclonal antibody. (B, C) Western blot analyses of reducing SDS-PAGE of cell lysates from HeLa cells not infected (lane 1) or infected with 150 MOI (vp/cell) of GRAd32c vectors encoding Spike 2P (lane 2) or Spike wt (lane 3), using either an anti Spike RBD rabbit polyclonal antibody (B) or an anti-HA tag rabbit monoclonal antibody (C).

Fig. S4 A single GRAd-COV2 administration induces similarly anti Spike IgG1 but more efficiently IgG2a than two administration of a Spike protein formulated in alum adjuvant. A) Spike-binding total IgG, IgG1 and IgG2a titers in sera from mice immunized with 1x10^9 vp of GRAd-COV2 or with two injections 2 weeks apart of 2.5μg Spike protein formulated in alum adjuvant. Sera collected at w4 post first immunization were tested by ELISA on recombinant full length Spike. Data are expressed as endpoint titer. Horizontal lines indicate Geometric Mean.
Fig. S5 Dose-response T cell response in BALB/c mice three weeks after GRAd-COV2 vaccination. BALB/c mice received a single intramuscular injection of between $1 \times 10^9$ to $1 \times 10^5$ vp of GRAd-COV2, and spleens were collected 3 weeks post immunization. Data are expressed as IFN-γ Spot Forming Cells (SFC)/$10^6$ splenocytes. Individual data points represent total Spike response in each animal, obtained by summing reactivity to each of the 2 Spike peptide pools and subtracting 2 times the DMSO background. Red lines represent Geometric mean.
Fig. S6 GRAd32-gag administration induces humoral and cellular responses to gag but not to Spike antigen. A group of 5 BALB/c received intramuscular immunization with $1 \times 10^9$ vp of GRAd32 encoding HIV-1 gag. Sera and splenocytes were isolated 5 weeks post immunization and subjected to ELISA and IFN-γ ELISpot analyses. Black symbols denote IgG endpoint titers measured on recombinant gag p24 or SARS-CoV-2 Spike proteins, and are plotted on the left y axis. Red symbols show T cell response upon stimulation with 15mer peptide pools covering gag or Spike, are expressed as IFN-γ SFC/million splenocytes and are plotted on right y axis. Horizontal lines indicate Geometric mean.