Supplementary Materials for

Complete protection by a single-dose skin patch–delivered SARS-CoV-2 spike vaccine

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Published 29 October 2021, Sci. Adv. 7, eabj8065 (2021)
DOI: 10.1126/sciadv.abj8065

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Figs. S1 to S6
Fig. S1. *In vitro* characterisation of SARS-CoV-2 spike. (A) Coomassie-stained SDS-PAGE of purified SARS-CoV-2 S HexaPro with or without DTT. (B) Analytical size-exclusion chromatography of purified HexaPro spike on a Superdex 200 Increase 10/300 GL column, including size markers. (C) ELISA of purified HexaPro spike with a panel of SARS-CoV-2 spike-specific monoclonal antibodies. Data represents mean of n=2 technical replicates and error bars represent SD. (D) Negative-stain TEM of purified HexaPro spike with representative 2D class averages shown in (E) with a box size of 500Å.
Fig. S2. Stability of dried spike. (A) Schematic of the process of excipient screening. SARS-CoV-2 spike was dried in various excipients and reconstituted (B) immediately or stored at 4 °C for (C) 1 or (D) 7 days prior to reconstitution. Recovered protein was analysed via ELISA with the conformation dependent S-specific mAb S309. Percent recovery is relative to a liquid control prepared fresh on the day of each assay.
**Fig. S3. Domain-specific IgG titers.** Serum from mice immunized with SARS-CoV-2 spike was assessed by ELISA against (A) the receptor binding domain (RBD) or (B) N-terminal domain (NTD) of the spike protein. Data representative of geometric mean with error bars representing geometric SD. *P* values indicate results of one-way ANOVA with Tukey’s multiple comparison post-hoc test.
**Fig. S4. Neutralization of SARS-CoV-2 variants.** Serum from mice immunized with SARS-CoV-2 spike via intradermal (i.d.) delivery or HD-MAP delivery, with or without QS21 as an adjuvant, was assessed for neutralization against SARS-CoV-2 variants (A) containing the 614G mutation in the spike protein, (B) a Beta variant (lineage B.1.351) and (C) an Alpha variant (lineage B.1.1.7). Data representative of geometric mean with error bars representing geometric SD. P values indicate results of one-way ANOVA with Tukey’s multiple comparison *post-hoc* test.
Fig. S5. SARS-CoV-2 variant neutralization by K18-hACE2 mouse serum. Serum from K18-hACE2 mice immunized with 1 or 2 doses of HD-MAP delivered spike was analysed for neutralization by PRNT against SARS-CoV-2 variants (A) 614G, (B) Alpha (from the B.1.1.7 lineage) and (C) Beta (from the B.1.351 lineage). Data represents geometric mean and error bars represent geometric SD.
Fig. S6. Histopathology of lungs and brains from K18-hACE2 challenged with SARS-CoV-2. (A) Lungs and (B) brain tissue from naïve or vaccinated K18-hACE2 mice challenged with SARS-CoV-2 were collected on day 6 post-infection for hematoxylin and eosin staining. Representative images are shown. Arrowheads indicate leukocyte infiltration (lungs) or perivascular cuffing (brain).