**Supplementary table 1**: Peptide pools spanning the spike glycoprotein used in T cell analysis

| Peptide pool nomenclature | Amino acid no. | Region | # of peptide pooled |
|---------------------------|----------------|--------|---------------------|
| S1-NTD                    | 1-318          | ![Region Diagram](image) | 77                  |
| RBD                       | 319-685        | ![Region Diagram](image) | 92                  |
| S2                        | 686-1273       | ![Region Diagram](image) | 146                 |
**Supplementary table 2**: Primers used to confirm construction and purity of vaccine stocks

| Locus | Forward primer | Reverse primer |
|-------|----------------|----------------|
| D13L  | 5’-GTGAGTACCTGGATACGAAAT-3’ | 5’-AACCATCTACGTATACGTGTTATAT-3’ |
| A41L  | 5’-TCACATCGTTACGAAATGACGACT-3’ | 5’-CTAGACGAAACCCTCAGACAAAC-3’ |
| Spike | 5’-TATTCAGTTAAGCATACGTAAATTTGTGTAC-3’ | 5’-GTACCAACCACAGAGTAGTAGTACCTTTTGG-3’ |
Supplementary figure 1: SDS-PAGE gel and raw file of immunoblot analysis data to confirm spike protein expression

Left panel: SDS PAGE analysis showing total protein loading prior to transfer for Western blotting.
Right panel: Raw unmanipulated file of Western blot image shown in Figure 1d.
Lane 1: Benchmark ladder
Lane 2: Gangnam ladder
Lane 3: 143B cells infected with SCV-S at an MOI of 1 PFU
Lane 4: 143B cells infected with control SCV at an MOI of 1 PFU
Lane 5: 143B uninfected control
Supplementary figure 2: Illumina read coverage of the SCV-S spike expression cassette in MVS and P10 vaccine stocks.

Viral genomic DNA was purified from SCV-S MVS or P10 (after 10 passages in the MCL, performed in triplicate) vaccine stocks. Nextera XT libraries were prepared and 150 bp paired end Illumina MiSeq sequencing reads were mapped to the SCV-S spike expression cassette and low frequency variant detection was performed using CLC Genomics Workbench 21.0.4. Approximately 324k reads from MVS infected cells mapped to the transgene with an average coverage of ~12k reads (top panel). None of the reads mapped to any reporter/selection genes or genes targeted for deletion in the construction process of SCV-S. Reads from p10 infected cells were combined and ~100k reads mapped to the spike transgene with an average coverage of ~3.8k reads (bottom panel). No variants with frequencies >1% were detected which was consistent with plaque immunostaining and demonstrated the genetic stability of the spike transgene in SCV.
Supplementary figure 3: Spike-specific CTL activity at varying effector: target cell ratios. Groups of C57BL/6J female mice (n = 6) were vaccinated IM with $10^7$ PFU of SCV-S or control vector. At day 7 post-vaccination, splenocytes were pulsed with peptide pools (15AA length with 11mer overlaps) spanning the S1-NTD, RBD or S2 subunit of spike protein and percent specific lysis at effector: target ratios of 100:1, 33:1, 11:1 and 1:1 was assessed by standard $^{51}$Cr release ex vivo CTL assay. Data represented as mean ± SEM from one experiment.
Supplementary figure 4: Gating strategy for spike-specific multifunctional T cells. C57BL/6J female mice (n = 5) were vaccinated IM with $10^7$ PFU of SCV-S. At day 7 post-vaccination, splenocytes were pulsed with peptide pools (15AA length with 11mer overlaps) spanning the S1 subunit of spike protein and viable single CD3+ CD8+ T cells were enumerated for intracellular cytokine staining using the gating strategy shown. The numbers within the gates indicate the percentage of parent population.
**Supplementary figure 5: Magnitude of cytokine production in spike-specific CD8\(^+\) T cell populations.**

C57BL/6J male and female mice (n=8 in total) were vaccinated IM with \(10^7\) PFU of SCV-S. At day 7 post-vaccination, splenocytes were pulsed with peptide pools (15AA length with 11mer overlaps) spanning the S1 and S2 subunits of the spike protein and intracellular cytokine production assessed by flow cytometric methods. The magnitude of IFN-\(\gamma\), TNF, and IL-2 was measured by median fluorescence intensity (MFI) in single, double, and triple cytokine producing CD8\(^+\) T cells specific for the S1 and S2 subunits. Symbols represent individual mice and bars show the mean ± SEM, with data representative of a second independent experiment. Statistical significance was determined using Kruskal-Wallis ANOVA test or Mann-Whitney \(t\)-test, ** \(P < 0.01\); *** \(P < 0.001\); **** \(P < 0.0001\), n/a=not applicable.
Supplementary figure 6: Subclass profiling of spike-specific antibody responses.
C57BL/6J female mice (n = 3; one experiment) were vaccinated with 10^7 PFU of SCV-S or left untreated. At day 21 post-vaccination, subclass profiling of S1- and S2-subunit specific antibody responses was assessed by ELISA to establish Th1 bias.
Supplementary figure 7: Maintenance of spike-specific antibody responses in C57BL/6J mice following single SCV-S vaccination.

Inbred C57BL/6J female mice (n = 5) were vaccinated with 10^7 PFU of SCV-S and serum samples collected at times indicated. S1-specific antibody binding was assessed by ELISA (left panel), with endpoint IgG titers reported. Neutralizing titers were determined by cPASS SARS-CoV-2 neutralization antibody detection kit (right panel). Symbols represent individual mice and bars show the mean ± SEM from one analysis of one experiment with results similar to that obtained from 3 other similar experiments. Repeated measures one-way ANOVA with Geisser-Greenhouse correction was used to compare the samples with Fisher’s LSD test used to report multiple comparisons. ***P < 0.001
Supplementary figure 8: Correlation between S1-specific IgG binding titers and SARS-CoV-2 surrogate neutralising activity.
S1-specific IgG binding titers and neutralization activity depicted in Figure 3 (e) were plotted against each other. A positive correlation was established between the two variables by non-parametric Spearman rank correlation test.
Supplementary figure 9: S1-binding antibodies in young and middle-aged mice following prime-boost vaccination

Inbred C57BL/6J female mice young (6-8 weeks) and middle-aged (9-10 months) mice (n=5 per group) were vaccinated with $10^7$ PFU of SCV-S in a prime-boost vaccination strategy and serum samples collected at times indicated (the same cohorts as indicated in Figure 3 (h)). S1-specific antibody binding was assessed by ELISA (left panel) and endpoint IgG titers calculated (right panel). Symbols represent individual mice and bars show the mean ± SEM from one analysis.
Supplementary figure 10: SCV-S induces spike-specific T cell responses in middle-aged mice.

Groups of C57BL/6J middle-aged female mice (9-10 months; n = 5) were vaccinated in a single dose (day 0) or prime-boost strategy (day 0 and 28) with 10^7 PFU SCV-S. Mice vaccinated with control vector on day 0 and 28 were used as controls. At day 120, splenocytes were stimulated with peptide pools (15AA length with 11mer overlaps) spanning the spike protein to examine antigen-specific T cell responses. IFN-γ SFU specific for (a) S1 N-terminal domain region, (c) RBD region and (e) S2 region of the spike protein was quantitated by ELISPOT. Frequency of IFN-γ^+ CD8^+ T cells and absolute numbers of triple cytokine (IFN-γ^+ TNFα^+ IL-2^+) positive cells for (b) S1-NTD region, (d) RBD region and (f) S2 region of the spike protein was enumerated by intracellular cytokine staining and FACS analysis. Graphs illustrate the (g) mean frequency and (h) absolute numbers of RBD-specific single, double, and triple cytokine cells within the cytokine positive CD8^+ T cell compartment. Symbols represent individual mice and bars indicate mean ± SEM from one experiment. Data was log transformed and statistical significance determined using Brown-Forsythe and Welch ANOVA with Dunnett T3 multiple comparison test. * P < 0.05; ** P < 0.01; *** P < 0.001; **** P < 0.0001
Supplementary figure 11: Vector-specific T cell responses increase after boost vaccination.

Groups of C57BL/6J (female; n = 5) were vaccinated with 10⁷ PFU SCV-S following a single dose (day 0) or prime-boost strategy (day 0 and 28). Mice vaccinated with control vector on day 0 and 28 were used as antigen-specific controls. At day 120 post-vaccination, splenocytes were stimulated with the VACV-specific A8 peptide (ITYRFYLI) and resultant A8-specific IFN-γ SFUs quantitated by ELISPOT (left panel). Vector-specific CD3⁺ CD8⁺ T cell responses were assessed by intracellular cytokine staining and FACS analysis, with the frequency of IFN-γ⁺ CD8⁺ T cells (middle panel) and absolute numbers of triple cytokine (IFN-γ⁺ TNF⁺ IL-2⁺) calculated. Symbols represent individual mice and bars show the mean ± SEM from one experiment. Data was log transformed and statistical significance was determined using Brown-Forsythe and Welch ANOVA with Dunnet T3 multiple comparison test. **P < 0.01; ***P < 0.001
Supplementary figure 12: Comparison of effector and central memory CD8 T cell population after single and prime-boost vaccination.

C57BL/6J female mice (n = 5) were vaccinated with 10^7 PFU SCV-S with a single dose (day 0) or prime-boost regimen (day 0 and 28). At day 120, splenocytes were harvested and the central and effector memory populations were identified by FACS analysis using the surface markers CD3, CD8, KLRG1, CD44, CD62L and CD127. Graphs showing absolute numbers of short-lived effector cells (T_{SLE}; CD44^{hi}KLRG1^{hi}CD62L^{lo}), long-lived effector memory cells (T_{EM}; CD44^{hi}KLRG1^{lo}CD62L^{lo}CD127^{hi}), and central memory cells (T_{CM}; CD44^{hi}KLRG1^{lo}CD62L^{hi}CD127^{hi}) in single and prime-boost vaccinated mice are shown. Symbols represent individual mice and bars show the mean ± SEM from one experiment. Data was log transformed and unpaired *t*-test with Welch’s correction was used for statistical analysis. **P < 0.01, ns=not significant
Supplementary figure 13: S2-specific binding titers post-vaccination in young and middle-aged mice.

Groups of young (6-8 weeks old) and middle-aged (9-10 months old) female C57BL/6J mice (n = 5) in one experiment were vaccinated IM with $10^7$ PFU of SCV-S (days 0 and 28) or vector control. S2-specific binding activity in serum samples collected on day 21 (prime) and 50 (boost) were evaluated by ELISA. Symbols represent individual mice and bars show the mean ± SEM from one experiment. Data was log transformed and statistical significance was determined using unpaired t-test with Welch’s correction. **$P < 0.01$
Supplementary figure 14: Comparison of multifunctional T cell population in young and middle-aged mice

Young (6-8 weeks old) and middle-aged (9-10 months old) female C57BL/6J mice (n = 5; one experiment) were vaccinated IM with 10^7 PFU of SCV-S (days 0 and 28). At 9 months post-vaccination, splenocytes were stimulated with peptide pools (15AA length with 11mer overlaps) spanning the spike protein and the proportions of single and polyfunctional T cells in both the young and middle-aged mice specific to S1 N-terminal domain (top panel), RBD (middle panel) and S2 (bottom panel) were enumerated by intracellular cytokine staining and FACS analysis.