Supporting Information for

Inhibition of major histocompatibility complex-I antigen presentation by sarbecovirus ORF7a proteins

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Other supporting materials for this manuscript include the following:

Dataset S1
**Figure S1 No effect of SARS-CoV-2 proteins on cell surface levels of Tetherin.**

Human 293T cells transduced with pSCRPSY-based lentiviral vector expressing individual SARS-CoV-2 viral proteins, at an MOI of 0.5 and cell surface Tetherin-HA measured with anti-HA followed and flow cytometry. Cells transduced (TagRFP+ population) with a viral protein expression vector (solid line) and empty lentiviral vector (dotted line) were gated and compared. Representative of two experiments.
Figure S2 Redistribution of β2M in SARS-CoV-2 ORF7a-expressing cells.
Immunofluorescent staining of A549 cells with anti-β2M (green) and anti-ORF7a following transduction with empty SCRPSY (vector) or ORF7a-expressing SCRPSY (ORF7a). A single optical section following deconvolution microscopy is shown.
Figure S3 Distribution of MHC-I in bat SARSr-CoV ORF7a-expressing cells. Immunofluorescent staining of A549 cells with anti-HLA-A (green) and anti-ORF7a (red) following transduction with SCRPSY (vector) or SCRPSY expressing ORF7a from the indicated SARSr-CoV. A single optical section following deconvolution microscopy is shown.
Figure S4 Distribution of MHC-I in SARS-CoV-2 or SARS-CoV ORF7a-expressing cells. Immunofluorescent staining of A549 cells with anti-HLA-A (green) and anti-ORF7a (red) following transduction with SCRPSY (vector) or ORF7a-expressing SCRPSY. A single optical section following deconvolution microscopy is shown.
Figure S5 Lack of MHC-1 downregulation by ORF7a in NIH3T3/CycT1 cells.
NIH3T3 cells engineered to express humanized CycT1 to enable efficient expression by the SCRPSY vector were transduced with pSCRPSY-based lentiviral vector expressing SARS-CoV-2, or SARS-CoV ORF7a at an MOI of 0.5. Cells were then stained with isotype IgG (dotted line) or an anti-mouse MHC-I (H-2Ld) antibody (clone 30-5-7S) (solid line) followed by flow cytometry. Cells transduced (TagRFP+ population) with the ORF7a expression vectors or the empty lentiviral vector were gated and compared. Representative of three experiments.

Figure S6 Western blot quantification of co-immunoprecipitated ORF7a and MHC-I
(A) Western blot quantification of ORF7a in immunoprecipitates (IP) as indicated in Figure 4A. The mean and range of values from two independent experiments are plotted. P-values are calculated using the t-test with Welch’s correction.
(B) Western blot quantification of HLA-A in immunoprecipitates (IP) as indicated in Figure 4B. The mean and range of values from two independent experiments are plotted. P-values are calculated using the t-test with Welch’s correction.
Figure S7 Quantification of Endo-H sensitive MHC-I in ORF7a-expressing cells

Cell lysates of A549 cells transduced with SCRPSY expressing no ORF7a (vector) or the ORF7a proteins from sarbecoviruses as shown in Figure 5A were subjected to Western blot quantification, from which the percentage of Endo-H sensitive MHC-I was calculated. The mean and range of values from two independent experiments are plotted. P-values are calculated using the t-test with Welch's correction.
Figure S8 No evidence of effects on CD44 or CDCP1 trafficking and maturation by ORF7a.

Western blot analysis of cell lysates of A549 cells transduced with SCRPSY expressing no ORF7a (vector) or the ORF7a proteins from SARS-CoV-2, SARS-CoV (or mutants thereof) at an MOI of 0.5. Cell lysates were untreated or treated with endoglycosidase H, as indicated prior to analysis.
Figure S9 Western blot quantification of disruption of the MHC-I peptide loading complex assembly by ORF7a

(A) The level of CRT, ERp57, Tap1, TAP2, Tapasin in immunoprecipitates (IP) from 293T cells transduced with SCRPSY expressing no ORF7a (vector) or the SARS-CoV-2 ORF7a protein as indicated in Figure 5B was measured by Western blot quantification and normalized by the corresponding control (vector). The mean and range of values from two independent experiments are plotted. P-values are calculated using the t-test with Welch’s correction.

(B) Western blot quantification of CRT (left panel) and ERp57 (right panel) in immunoprecipitates (IP) as indicated in Figure 5C. The mean and range of values from two independent experiments are plotted. P-values are calculated using the t-test with Welch’s correction.
| Target Antigen | Target species | Host species | Clone | Company   | Catalog #   |
|----------------|----------------|--------------|-------|-----------|-------------|
| SARS-CoV / SARS-CoV-2 ORF7a | Mouse | 3C9 | GeneTex | GTX632602 |
| ORF7a | Rabbit | polyclonal | Bioworld | NCP0011 |
| HLA-A | Human | Rabbit | EP1395Y | abcam | ab52922 |
| HLA-A, B, C | Human | Mouse | EMR8-5 | abcam | ab70328 |
| CD44 | Human | Rabbit | polyclonal | abcam | ab243894 |
| CD51 | Human | Rabbit | polyclonal | ThermoFisher | PA5-17245 |
| Calreticulin | Human | Rabbit | polyclonal | abcam | ab2097 |
| ERp57 | Human | Rabbit | polyclonal | abcam | ab10287 |
| TAP1 | Human | Rabbit | polyclonal | ThermoFisher Proteintech | 11114-1-AP |
| TAP2 | Human | Rabbit | polyclonal | abcam | ab180611 |
| TAP2 | Human | Rabbit | polyclonal | ThermoFisher Invitrogen | PA5-37414 |
| Tapasin | Human | Rabbit | polyclonal | ThermoFisher Invitrogen | PA5-42731 |
| Tapasin | Human | Mouse | TO-3 | Santa Cruz | sc-80647 |
| Tubulin | Human | Mouse | DM1A | Sigma | T9026 |

| Target Antigen | Target species | Host species | Clone | Company   | Catalog #   |
|----------------|----------------|--------------|-------|-----------|-------------|
| HLA-A, B, C | Human | Mouse | W6/32 | AF488 | Biolegend | 311413 |
| HLA-A, B, C | Human | Mouse | W6/32 | AF647 | Biolegend | 311414 |
| HLA-A2 | Human | Mouse | BB7.2 | APC | Biolegend | 343308 |
| HLA-A | Human | Rat | YTH 862.2 | abcam | Ab00200-8.1 |
| H-2L<sup>d</sup> | Mouse | Mouse | 30-5-7S | AF488 | Cedarlane | CL9011AF4 |
| HA epitope tag | Mouse | 16B12 | AF488 | Biolegend | 901509 |
| HA epitope tag | Mouse | 16B12 | APC | Biolegend | 901524 |

| Target Antigen | Target species | Host species | Clone | Company   | Catalog #   |
|----------------|----------------|--------------|-------|-----------|-------------|
| SARS-CoV / SARS-CoV-2 ORF7a | Mouse | 3C9 | GeneTex | GTX632602 |
| HLA-A, B, C | Human | Mouse | W6/32 | Biolegend | 311402 |
| HLA-A, B, C | Human | Mouse | 5C5B7 | Proteintech | 60013-1-lg |
| HLA-B | Human | Rabbit | polyclonal | ThermoFisher Invitrogen | PA5-35345 |

| Target Antigen | Target species | Host species | Clone | Company   | Catalog #   |
|----------------|----------------|--------------|-------|-----------|-------------|
| SARS-CoV / SARS-CoV-2 ORF7a | Rabbit | | Bioworld | NCP0011 |
| HLA-A, B, C | Human | Mouse | W6/32 | Sigma | H1650-100TST |
| HLA-A | Human | Rabbit | EP1395Y | Abcam | ab52922 |
| B2M | Human | Mouse | B2M/961 | Novus | NBP2-44471 |
| IgG H+L | Rabbit | Goat | Novus | Nucleotide sequences of codon-optimized SARS-CoV-2 ORFs |
|---------|--------|------|-------|------------------------------------------------------|
| IgG H+L | Mouse  | Goat | Novus | NBP1-72739JF646                                      |
| IgG H+L | Mouse  | Goat | Thermo| A11029                                              |