Supplemental information

A molecularly engineered, broad-spectrum anti-coronavirus lectin inhibits SARS-CoV-2 and MERS-CoV infection in vivo

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Figure S1. The cytotoxicity of H84T-BanLec in different cell lines. The cytotoxicity of H84T-BanLec in VeroE6, Huh7, BSC-1, HFL, and Calu-3 cells was evaluated using the CellTiterGlo® luminescent cell viability assay according to manufacturer’s instructions. H84T-BanLec did not display any obvious cytotoxicity in any of these cell lines even at the concentration of 10µM (ie: 10,000nM). Related to Figure 1.
Figure S2. Antiviral activity of H84T-BanLec against other human-pathogenic coronaviruses. The antiviral activity of H84T-BanLec against SARS-CoV by A, plaque reduction assay and B, (left) CPE inhibition assay; and B, (right) against HCoV-229E by CPE inhibition assay. Related to Figure 1.
Figure S3. Antiviral activity of H84T-BanLec against emerging SARS-CoV-2 variants. The antiviral activity of H84T-BanLec against SARS-CoV-2: A, B.1.1.7 (Alpha, with N501Y), B, B.1.617.2 (Delta), C, P.3 (Theta, with N501Y, E484K, and 141 to 143 deletion), and D, B.1.1.529 (Omicron) variants were evaluated by (left) viral load reduction and (right) plaque reduction assays in VeroE6-TMPRSS2 cells. Data are mean ± s.d., n = 3 biological replicates. One-way ANOVA. ***P<0.001, **P<0.01, *P<0.05. Related to Figure 1.
Figure S4. Intranasal delivery of H84T-BanLec. BALB/c mice were administered AF647-tagged H84T-BanLec intranasally. Mice were visualized by in vivo fluorescence imaging over 24 hours, and the fluorescence signal in the nasal area was quantified. Related to Figures 2 and 3.
**Figure S5.** H84T-BanLec targets SARS-CoV-2 entry. Time-of-drug-addition assay indicated that H84T-BanLec interfered with SARS-CoV-2 entry. Student’s t-test. **P**<0.01. Related to Figures 1 to 3.
Figure S6. Detection of SARS-CoV-2 entry by flow cytometry. A, Representative histograms of mock- or SARS-CoV-2-infected cells (MOI = 1.0) permeabilized and then quantified by fluorescein isothiocyanate (FITC) staining after anti-SARS-CoV-2 nucleocapsid protein antibody and then anti-Alexa Flour 488 conjugated secondary antibody were added. The median fluorescence intensity (MFI) of each group is shown. B, SARS-CoV-2 nucleocapsid protein-positive cells were quantified after H84T-BanLec or PBS treatment. The results were shown as mean ± SD of the PBS control and in triplicate. One-way ANOVA. ***P<0.001, *P<0.05. Related to Figures 1 to 3.
Figure S7. Co-localization of H84T-BanLec with SARS-CoV-2 spike protein in the autopsied lung section of a deceased COVID-19 patient. Top, Multiplex immunohistochemical analysis of SARS-CoV-2-infected autopsy lung section (SARS-CoV-2 nucleocapsid protein, yellow; SARS-CoV-2 spike protein, red; and H84T-BanLec, orange). Total original magnification 200×. Sections were counterstained with DAPI to visualize nuclei (blue) and scanned using the Polaris Vectra multiplex spectral scanner. Bottom, The digital metadata were uploaded to FIJI for co-localization. The mean pixel intensity for each fluorochrome (0-256) at a particular centroid was plotted and the number of co-localizations calculated. The Pearson correlation calculation ranged from -1 to +1 and indicated inverse correlation (-1) through no correlation (0) to positive correlation (+1). The values calculated (0.6 and 0.58) for spike protein vs nucleocapsid protein and spike protein versus H84T-BanLec respectively represented high degrees of co-localization. Related to Figures 1 to 3.
Figure S8. Single molecule force spectroscopy measurements. H84T-BanLec was immobilized onto surfaces and trimeric spike proteins were coupled to AFM tips. **a,** Binding activity and control experiments demonstrating the specificity of the interaction. Binding probability, presented as the percentage of force experiments displaying unbinding events, between H84T-BanLec and the spike of wild-type (WT), N234Q, B.1.617.2, and B.1.1.529 SARS-CoV-2 strains, respectively. Adjacent to the right of each binding activity experiment, control experiments using a surface only functionalized with PEG linker (without H84T, WT spike) or blocking experiments using pre-incubated spike coupled tip in H84T-BanLec solution for 20 min and adding H84T-BanLec (Block) into the measurement solution with N234Q, B.1.617.2 or B.1.1.529); a significant drop in the binding probability was seen in each case. **b,** Determination of kinetic on-rate between H84T-BanLec and the spike of WT, N234Q, B.1.617.2, and B.1.1.529 SARS-CoV-2 strains, respectively. Binding probability was plotted as a function of the interaction time. The solid line was the result of a least-square fit of a mono-exponential decay, assuming pseudo first-order kinetics. Plot of unbinding force versus loading rate for **c,** N234Q spike mutant **d,** B.1.617.2 spike trimer and **e,** B.1.1.529 spike trimer dissociating from H84T-BanLec. A Markov binding model computed the behavior of the double bond (blue) and triple bond (cyan) interactions, using parameters derived from the Bell and Evan’s single-barrier model fit of the single bond (red). Related to Figures 4 and 5.
Figure S9. Surface plasmon resonance (SPR) measurements. H84T-BanLec was injected at the indicated concentrations to surfaces containing immobilized a, full-length trimeric N234Q spike mutant or b, receptor-binding domain to allow for binding (ascending parts), followed by wash out using buffer (descending parts) c, trimeric B.1.627.2 variant spike, and d, trimeric B.1.1.529 variant spike. Data were fitted using the bivalent binding model, consistent with H84T-BanLec being predominantly a dimer. To compare the binding of H84T-BanLec to the N234Q and B.1.627.2 spike proteins to that of the standard spike, see Fig. 4H. Related to Figures 4 and 5.
|                  | H84T-BanLec /Spike trimer | H84T-BanLec /N234Q spike mutant | H84T-BanLec /B.1.617.2 trimer | H84T-BanLec /B.1.1.529 trimer | H84T-BanLec /N234Q spike mutant | H84T-BanLec /B.1.617.2 trimer | H84T-BanLec /B.1.1.529 trimer |
|------------------|---------------------------|---------------------------------|-------------------------------|-------------------------------|---------------------------------|-------------------------------|-------------------------------|
| $k_{\text{off}, 1}$ [1/s] | 0.0457 ± 0.024            | 0.2585 ± 0.0135                 | 0.0476 ± 0.0246               | 0.0314 ± 0.008               | 0.0262 ± 0.007                  | 0.006 ± 0.0027                | 0.0249 ± 0.0031               |
| $k_{\text{on}, 1}$ [1/Ms]  | $9.98 \times 10^4$ ± 5.18 × 10^4 | $2.87 \times 10^5$ ± 0.66 × 10^5 | $1.91 \times 10^6$ ± 0.52 × 10^6 | $3.46 \times 10^6$ ± 0.23 × 10^6 | $2.09 \times 10^6$ ± 0.25 × 10^4 | $2.99 \times 10^4$ ± 0.31 × 10^4 | $4.31 \times 10^4$ ± 0.23 × 10^4 |
| $K_D$ [M]        | $4.6 \times 10^{-7}$ ± 4.37 × 10^{-7} | $9.61 \times 10^{-7}$ ± 2.68 × 10^{-7} | $2.54 \times 10^{-7}$ ± 1.06 × 10^{-7} | $9.17 \times 10^{-7}$ ± 3.16 × 10^{-7} | $1.28 \times 10^{-8}$ ± 0.19 × 10^{-6} | $2.17 \times 10^{-7}$ ± 1.14 × 10^{-7} | $5.76 \times 10^{-7}$ ± 0.41 × 10^{-7} |
| $X_\beta$ [Å]   | 7.83 ± 0.60               | 7.30 ± 0.15                     | 6.53 ± 2.28                   | 6.28 ± 0.32                   |

Table S1. Quantification of parameters obtained with dynamic force spectroscopy (DFS) and surface plasmon resonance (SPR) methods. $k_{\text{on}}$, $k_{\text{off}}$, and $K_D$, are kinetic on-rate, kinetic off-rate, and equilibrium dissociation constant, respectively, of a single bond (DFS) or the first binding step (SPR). $X_\beta$ denotes the distance of the activation barrier from the energy minimum along the pulling axis in DFS experiments. Related to Figures 4 and 5.