

staple scanning library of HR2 amino acid sequences 1168-1205 with the structure of the PEG₄-thiocholesterol moiety appended to the C-terminal lysine. WT, unstapled lipopeptide bearing the indicated wild-type HR2 domain sequence. **d–h** The stapled lipopeptide library was tested in infectivity assays using a series of pseudoviruses (PV), including the initial Wuhan-Hu-1 strain (**d**), Omicron variants B.1.1.529.1 (**e**) and B.1.1.529.2 (**f**), and SARS-CoV Urbani (**g**) in ACE2-expressing HEK293T cells, and SARS-CoV-2 Beta strain live virus (**h**) in ACE2-A549 cells at screening doses of 250 nM (**e, f**), 500 nM, (**d, g**) or 4 μ M (**h**). The data are normalized to the percent infected cells treated with vehicle control. Data are mean \pm SEM for assays performed in technical quadruplicate (PV) or triplicate (LV) and then repeated with similar results. The gray shading highlights those lipopeptides that inhibited infectivity to

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