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**The N-terminal domain of spike glycoprotein mediates SARS-CoV-2 infection
by associating with L-SIGN and DC-SIGN**

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24 **The widespread occurrence of SARS-CoV-2 has had a profound effect on society and a**
25 **vaccine is currently being developed. Angiotensin-converting enzyme 2 (ACE2) is the**
26 **primary host cell receptor that interacts with the receptor-binding domain (RBD) of the**
27 **SARS-CoV-2 spike protein. Although pneumonia is the main symptom in severe cases of**
28 **SARS-CoV-2 infection, the expression levels of ACE2 in the lung is low, suggesting the**
29 **presence of another receptor for the spike protein. In order to identify the additional**
30 **receptors for the spike protein, we screened a receptor for the SARS-CoV-2 spike protein**
31 **from the lung cDNA library. We cloned L-SIGN as a specific receptor for the N-terminal**
32 **domain (NTD) of the SARS-CoV-2 spike protein. The RBD of the spike protein did not bind**
33 **to L-SIGN. In addition, not only L-SIGN but also DC-SIGN, a closely related C-type lectin**
34 **receptor to L-SIGN, bound to the NTD of the SARS-CoV-2 spike protein. Importantly, cells**
35 **expressing L-SIGN and DC-SIGN were both infected by SARS-CoV-2. Furthermore, L-**
36 **SIGN and DC-SIGN induced membrane fusion by associating with the SARS-CoV-2 spike**
37 **protein. Serum antibodies from infected patients and a patient-derived monoclonal antibody**
38 **against NTD inhibited SARS-CoV-2 infection of L-SIGN or DC-SIGN expressing cells. Our**
39 **results highlight the important role of NTD in SARS-CoV-2 dissemination through L-SIGN**
40 **and DC-SIGN and the significance of having anti-NTD neutralizing antibodies in antibody-**
41 **based therapeutics.**

42

43 **Introduction**

44 Coronavirus disease 2019 (COVID-19) is caused by the novel human coronavirus, a severe acute
45 respiratory syndrome coronavirus 2 (SARS-CoV-2)^{1,2}. SARS-CoV-2 is an enveloped virus and
46 requires specific cellular receptors to infect cells in a manner similar to that observed in other
47 enveloped viruses such as the influenza virus and herpesviruses³⁻⁷. The spike glycoprotein of
48 SARS-CoV-2 associates with ACE2 on host cells and mediates membrane fusion with the host
49 cell membrane during infection^{3,4}. The spike glycoprotein is organized as a homotrimer and each
50 chain is composed of two main segments, the S1 and S2 subunits. The S1 subunit is responsible
51 for receptor binding, whereas the S2 subunit mediates membrane fusion^{8,9}. Within the S1 subunit
52 are two domains known as the N-terminal domain (NTD) and receptor-binding domain (RBD).
53 The RBD is recognized as the main domain that interacts with the ACE2 receptor on the host cells
54 and antibodies against RBD block infection⁹. Although the function of surface-exposed NTD that
55 is structurally coupled with the RBD of the neighboring chain still remains unclear, certain human
56 anti-NTD monoclonal antibodies neutralize SARS-CoV-2 infection¹⁰. This suggests that NTD as
57 well as RBD play a pivotal role in SARS-CoV-2 infection.

58 Pneumonia is the primary complication associated with SARS-CoV-2 infection in severe
59 cases¹¹. Although ACE2 is highly expressed in the intestine and kidney, the expression of ACE2
60 in the lung is limited, and relatively low levels of ACE2 expression are observed only in type-II
61 alveolar cells¹². Therefore, it has remained unclear how the low levels of ACE2 in the lung are
62 involved in pneumonia caused by SARS-CoV-2 infection. In addition, a single-cell RNA
63 sequencing study of bronchoalveolar-lavage samples revealed that viral RNA could be detected in
64 cell populations that did not express ACE2¹³. Taken together, this evidence indicates that ACE2
65 expression alone cannot explain the tropism of SARS-CoV-2 infection, and alternative entry

66 receptors might be involved in SARS-CoV-2 infection of ACE2-low or deficient cells. Besides,
67 SARS-CoV-2 is also detected in multiple non-pulmonary organs such as the intestine, liver, kidney,
68 and blood vessels of COVID-19 patients¹⁴. Based on these observations, we sought to identify a
69 potential alternative receptor for the SARS-CoV-2 spike protein using a lung cDNA library and
70 discuss the mechanisms of SARS-CoV-2 dissemination within the host.

71

72 **Results**

73

74 **Lung cDNA library screening identified L-SIGN as a receptor for NTD of the SARS-CoV-2** 75 **spike protein (SCoV2-NTD).**

76 In order to identify a receptor in the lung for the SCoV2-spike protein, we used expression cloning
77 with a lung retrovirus cDNA library. Cells transfected with a lung cDNA library were stained with
78 SCoV2-NTD human IgG-Fc fusion protein (SCoV2-NTD-Fc) as well as RBD-Fc fusion protein
79 (SCoV2-RBD-Fc) and the stained cells were enriched using a cell sorter (Fig. 1a). After two rounds
80 of enrichment, 83.7% of the cells were stained with SCoV2-NTD-Fc (Fig. 1b) while we could not
81 obtain cells stained with SCoV2-RBD-Fc even after three rounds of enrichment. We obtained
82 single-cell clones that were stained with SCoV2-NTD-Fc and amplified the genes derived from
83 the library using PCR. Notably, L-SIGN (CD299) was identified from all the clones that were
84 stained with SCoV2-NTD-Fc (Fig. 1c). Following this observation, the cell clones were stained
85 with antibodies against L-SIGN (Fig. 1d). These data suggested that L-SIGN is a major molecule
86 that interacts with SCoV2-NTD in the lung. Although L-SIGN is mostly expressed in the liver, the
87 lung has the second-highest expression of L-SIGN according to the RNA-seq tissue data obtained
88 from the Genotype-Tissue Expression (GTE) project (Suppl. Fig. 1).

89
90 **NTD of the spike facilitates the binding of SCoV2 to L-SIGN and DC-SIGN via unique**
91 **glycans**

92 L-SIGN shares a high amino acid sequence homology and functional similarity with DC-SIGN
93 that is also present in the lung (Suppl. Fig. 1). On the other hand, CD147 has been reported to be
94 involved in SARS-CoV-2 infection¹⁵. Accordingly, we transfected L-SIGN, DC-SIGN, ACE2,
95 and CD147 into HEK293T cells and evaluated the binding of NTD- or RBD-Fc fusion proteins
96 (Fig. 2a). We observed that the NTD-Fc bound to both surface-expressed L-SIGN and DC-SIGN
97 transfectants but not to ACE2 and CD147 transfectants. The RBD-Fc fusion protein bound to
98 ACE2 but not L-SIGN, DC-SIGN, or CD147 transfectants. This indicates that the binding of NTD-
99 Fc to L-SIGN or DC-SIGN is mediated by NTD and not the Fc portion. Anti-DC-SIGN mAb
100 blocked the binding of NTD-Fc to DC-SIGN transfectants but not to L-SIGN transfectants (Fig.
101 2b). L-SIGN and DC-SIGN have been shown to bind to intercellular adhesion molecule 3 (ICAM-
102 3) through sugar chain recognition. In addition, mannan is known to be a ligand of both L-SIGN
103 and DC-SIGN. When DC- or L-SIGN transfectants were preincubated with mannan, the binding
104 of NTD-Fc to DC-SIGN and L-SIGN was drastically reduced (Fig. 2b). These data indicate that
105 unique glycans on NTD of SARS-CoV-2 are involved in the interaction with L-SIGN and DC-
106 SIGN.

107 The SCoV spike protein has a high sequence similarity (approximately 76%) to the SCoV2
108 spike protein. However, SCoV-NTD-Fc fusion protein did not bind to DC-SIGN or L-SIGN
109 transfectants unlike the SCoV2-NTD-Fc fusion protein (Fig. 2c). Similarly, DC-SIGN-Fc and L-
110 SIGN-Fc fusion proteins bound to flag-tagged SCoV2 spike transfectants, but bound weakly to
111 SCoV spike transfectants although both transfectants were well stained with anti-flag mAb (Fig.

112 2d). Likewise, DC-SIGN-Fc and L-SIGN-Fc fusion proteins did not bind to the human
113 coronaviruses, OC43 and HKU1 (Suppl. Fig. 2). These results also suggested that the NTD of the
114 SCoV2 spike protein is specifically modified by unique glycans that mediate the binding of NTD
115 to L-SIGN and DC-SIGN.

116 There are seven and eight N-glycosylation sites located on the NTD of SCoV and SCoV2,
117 respectively (Fig. 3a). The protein sequence alignment of both NTDs revealed that there are two
118 unique glycosylation sites, i.e., N74 and N149, on the SCoV2-NTD (Fig. 3a). Interestingly, both
119 glycosylation sites are in the most variable region within the NTD. To identify which glycosylation
120 sites interact with DC-SIGN, we substituted SCoV2-NTD glycosylation site Asn (N) with Gln (Q)
121 and analyzed the binding to DC-SIGN (Fig. 3b, c). The mutation at N149Q diminished the NTD
122 binding to DC-SIGN, while N74Q and N282Q mutation resulted in a substantial reduction of the
123 NTD binding to DC-SIGN. These data suggest that unique glycans on N149 of NTD are
124 responsible for the interaction with SIGNs.

125

126 **L-SIGN and DC-SIGN mediate SCoV2 infection by inducing membrane fusion**

127 It has been reported that L-SIGN and DC-SIGN are involved in infection by several viruses such
128 as HIV, HCV, and SARS-CoV. To determine whether L-SIGN and DC-SIGN are involved in
129 SCoV2 infection, we used SCoV2 spike-pseudotyped vesicular stomatitis virus (SCoV2-PV)
130 carrying a luciferase reporter gene to infect HEK293T cells stably transfected with L-SIGN or DC-
131 SIGN and analyzed the outcome (Fig. 4a, Suppl. Fig. 2). L-SIGN and DC-SIGN transfectants were
132 considerably infected with SCoV2-PV. Furthermore, bona fide SCoV2 also infected L-SIGN and
133 DC-SIGN transfectants. This indicated that DC-SIGN and L-SIGN serve as receptors for SCoV2
134 infection.

135 Being an enveloped virus, membrane fusion between the viral envelope of SCoV2 and the
136 host cell membrane is a critical step in viral infection. To understand the function of L-SIGN and
137 DC-SIGN in membrane fusion during SCoV2 infection, we used a cell-cell fusion assay using a
138 T7 promoter-based luciferase reporter system^{7,16}. In this system, we cocultured effector cells
139 (transfected with SCoV2 spike and T7 polymerase) with target cells (transfected with DC-SIGN
140 or L-SIGN with luciferase gene regulated by a T7 promoter) and the luciferase gene is expressed
141 only when cell-cell fusion between effector cells and target cells is induced. Interestingly, cell-cell
142 fusion activity occurred when effector cells were cocultured with target cells expressing L-SIGN
143 or DC-SIGN (Fig. 4c). Additionally, when effector cells expressing SCoV2 spike protein and red
144 fluorescence protein were cocultured with target cells expressing DC-SIGN and green
145 fluorescence protein, fused cells expressing both red and green fluorescence proteins were detected
146 (Fig. 4d). These data suggest that L-SIGN or DC-SIGN mediate membrane fusion during SCoV2
147 infection of SIGN-expressing cells.

148

149 **moDCs facilitate SCoV-2 dissemination in trans via DC-SIGN.**

150 We then sought to establish the function of endogenously expressed L-SIGN or DC-SIGN. Since
151 L-SIGN has been reported to be expressed in type II alveolar cells, L-SIGN is likely involved in
152 the SARS-CoV-2 infection of lungs. However, we could not find any cell lines expressing
153 endogenous L-SIGN. On the other hand, DC-SIGN is expressed on monocyte-derived DCs
154 (moDCs). For that reason, we isolated CD14⁺ cells from human PBMC and differentiated them
155 into DCs in the presence of cytokines, IL-4 and GM-CSF. The moDCs expressed DC-SIGN, low
156 CD14, high MHC-II, and CD74 (Fig. 5a). As observed with DC-SIGN transfectants (Fig. 2),
157 SCoV2-NTD-Fc fusion protein bound to moDCs (Fig. 5b). Anti-DC-SIGN monoclonal antibodies

158 and mannan prevented the binding of NTD-Fc to moDCs (Fig. 5c). These results indicated that
159 DC-SIGN was expressed on primary cells associated with the NTD of the SCoV2 spike protein.
160 However, moDCs were not infected with SCoV2-PV, but with the VSV-G PV (Fig. 5d). Recently,
161 CD74 induced by CIITA has been reported to inhibit the membrane fusion of SCoV2¹⁷. The
162 considerable expression of CD74 by moDCs (Fig. 5a) may confer resistance to SCoV2 infection.
163 Therefore, there is likely that L-SIGN or DC-SIGN expressed in CD74 negative cells could play
164 a critical role in SARS-CoV-2 infection.

165 NTD-Fc has been observed to bind well to moDCs; therefore, SCoV2 virions bound to DC-
166 SIGN on moDCs may be infectious to other cells. We incubated moDCs with SCoV2-PV followed
167 by extensive washing. Thereafter, moDCs incubated with SCoV2-PV were cocultured with Vero
168 cells. Significant infection was observed in Vero cells and the infection was completely blocked
169 by anti-DC-SIGN antibodies or mannan (Fig. 5e). Due to the general circulation of DCs in the
170 body, SCoV2 bound to DCs via DC-SIGN may be involved in the spread of SCoV2 throughout
171 the body.

172

173 **Neutralization of SIGN-mediated SCoV2 infection by antibodies**

174 Antibodies are of importance in host defense against SARS-CoV-2 infection. The function of
175 antibodies in SIGN-mediated SARS-CoV-2 infection was evaluated. 4A8 and 4A2 are COVID-19
176 patient-derived anti-NTD mAbs¹⁰ and C144 is an anti-RBD mAb¹⁸ (Suppl. Fig. 3). Both 4A8 and
177 C144 have been reported to neutralize SCoV2 infection. Surprisingly, anti-RBD C144 mAbs as
178 well as anti-NTD 4A8 blocked SCoV2-PV infection of HEK-L-SIGN and HEK-DC-SIGN. In
179 contrast, the 4A2 mAb did not block infection (Fig. 6a, b). These data indicate that RBD as well
180 as NTD are involved in SCoV2 infection of SIGN-expressing cells. The effect of antibodies

181 derived from SCoV2-infected patients was investigated; their sera contain antibodies against NTD
182 and RBD (Suppl. Fig. 4). SCoV2-PV infection of DC-SIGN transfectants was hindered by the sera
183 from two SCoV2-infected patients but not from healthy control serum (Fig. 6c). On the other hand,
184 the sera could not neutralize VSV-G PV infection. (Fig. 6d). This shows that SIGN-mediated
185 infection is counteracted by serum antibodies against spike proteins similar to ACE2-mediated
186 infection. In addition, our data imply that antibodies against NTD as well as RBD are useful in
187 blocking SCoV2 infection^{19,20}.

188

189 **Discussion**

190 SCoV2 is more widespread than its closest human coronavirus, SCov3, and therapeutic
191 interventions are yet to be identified. ACE2 is the primary receptor that interacts with the SCoV2
192 spike glycoprotein (i.e., RBD) and consequently facilitates viral entry³. However, the
193 understanding of the pathogenesis of SCoV2 remains incomplete, including the mechanism of
194 pneumonia, thrombosis, endothelitis, and cytokine storm, which are often associated with severe
195 cases of the disease²¹⁻²³. It is unclear how SCov2 causes pneumonia since the expression of ACE2
196 in the lung is relatively low and limited to type-II alveolar cells and some (<2%) of the respiratory
197 epithelial cells¹². Additionally, the presence of SCoV2 in non-pulmonary organs¹⁴ as well as the
198 detection of viral DNA in non-ACE2 expressing cells¹³ point to an alternative receptor for SCoV2
199 entry and an unknown virus-spreading mechanism within the host. Recently, neuropilin has been
200 reported to be involved in the SCoV2 cell entry by associating with the proteolytically processed
201 spike protein. However, transfection of neuropilin alone to HEK293 cells did not mediate the
202 SCoV2 infection, and the coexpression of ACE2 and TMPRSS2 with neuropilin was required for
203 the SCoV2 infection^{24,25}. On the other hand, L-SIGN, cloned from lung cDNA library as an NTD

204 binding receptor, and DC-SIGN mediated SCoV2 infection in the absence of ACE2 and TMPRSS2.
205 Indeed, L-SIGN is expressed in lung type-II alveolar cells and endothelial cells²⁶. Furthermore,
206 DCs or specialized macrophages expressing DC-SIGN can be found in the lung^{27,28}. Their
207 localization in the lung further strengthens the increasingly likely role of L-SIGN and DC-SIGN
208 as receptors of SCoV2 and may therefore be important in the pathogenesis of pneumonia.

209 The interaction of the SCoV2-NTD with L-SIGN and DC-SIGN is specific and glycan
210 dependent. Despite the NTD of SCoV being a highly glycosylated domain, our analyses indicate
211 that the interaction between NTD and DC-SIGN or L-SIGN is apparently unique to the current
212 SCoV2 because L-SIGN-Fc or DC-SIGN-Fc hardly bound to the spike protein of SCoV as well as
213 human coronavirus OC43 and HKU1. Previous findings have shown that SCoV spike protein
214 interacts with DC-SIGN and L-SIGN albeit with a low binding efficiency^{26,29}. Similarly, we
215 observed a very weak binding of DC- and L-SIGN-Fc to SCoV spike protein, whereas the binding
216 to the SCoV2 spike was stronger. The overall glycosylation profile of the SCoV2 spike protein is
217 quite different from that of SCoV³⁰⁻³² with the N-linked glycan at residue 149 being the major
218 glycan involved in the DC-SIGN and NTD interaction. The N-linked glycosylation site at residue
219 149 of SCoV2-NTD is absent in SCoV-NTD, although other N-linked glycosylation sites are
220 conserved in SCoV-NTD. This may be the reason why L- and DC-SIGN interactions with NTD
221 are more apparent in the current SCoV2. Indeed, both L-SIGN and DC-SIGN recognize a
222 pentasaccharide motif (GlcNAc₂Man₃)³³ that is also present in the glycans at N149 of SCoV2-
223 NTD³⁰.

224 We demonstrated that L-SIGN and DC-SIGN mediate both the pseudotyped and bona fide
225 SARS-CoV-2 infections. Furthermore, both L-SIGN and DC-SIGN induce cell-cell fusion via
226 their association with the spike protein. SCoV2 requires proteolytic cleavage of the spike protein

227 by the host proteases (e.g., TMPRSS2) after binding to an entry receptor³. Besides, the lysosomal
228 protease, cathepsin L was shown to proteolytically activate the SCoV spike protein during viral
229 entry thereby facilitating membrane fusion with the host cell membrane³⁴. Recent research
230 revealed that the thyroglobulin domain (cathepsin L inhibitor) of the CD74 p41 isoform blocks the
231 cathepsin L-mediated viral entry of SCoV2¹⁷. CD74 is an essential molecule for the proper
232 assembling of MHC class II molecules and its expression is regulated by CIITA, a principal
233 transcription factor for MHC class II expression³⁵. Because moDCs express both CD74 and MHC
234 class II molecules favorably, CD74 appears to be responsible for the resistance of moDCs to
235 SCoV2 infection. However, expression of CD74 is largely limited to immune cells such as B cells,
236 monocytes, and dendritic cells, while most cells including endothelial cells do not express CD74.
237 Therefore, L-SIGN or DC-SIGN expressing cells that lack CD74 expression appear to be
238 susceptible to SCoV2 infection.

239 We also show that moDCs facilitate pseudotyped SCoV2 infection in Vero cells in trans.
240 Trans-infection by DCs is well reported in HIV infection³⁶. Due to their migration in the body,
241 DCs may function as transporters of SCoV2 by associating with the virus via DC-SIGN to infect
242 cells expressing ACE2 or L-SIGN, which in turn facilitates virus dissemination in the host. This
243 might explain the presence of viral protein in other organs, including the liver¹⁴, where L-SIGN is
244 highly expressed in human liver sinusoidal endothelial cells that do not express CD74³⁷.
245 Furthermore, infection of the endothelial cells of blood vessels may consequently lead to
246 thrombosis, which is frequently linked to severe disease conditions²². Several human tissues
247 express similar mRNA expression levels of L-SIGN as those of the lung, making them likely
248 targets of SCoV2 infection.

249 The presence of neutralizing antibodies for DC-SIGN-mediated SCoV2 infection in
250 COVID-19 patients implies the relevance of NTD as a therapeutic target besides RBD. Notably,
251 the majority of the isolated mAb from SCoV2 patients did not recognize RBD and those mAbs
252 that neutralize SCoV2 failed to prevent binding of the spike to ACE2¹⁰. In our study, the NTD-
253 specific mAb, 4A8, neutralized SIGN-mediated infection of SCoV2, suggesting the therapeutic
254 potential of anti-NTD antibodies against SIGN-mediated infection. More importantly, anti-RBD
255 mAb also inhibited the SCoV2-PV infection of DC-SIGN and L-SIGN expressing cells. Although
256 HEK293 cells do not express ACE2, certain molecules on HEK293 cells may interact with RBD
257 becoming involved in infection of SIGN-expressing cells. This may suggest that L-SIGN or DC-
258 SIGN is required for the infection of ACE2-low or ACE2-negative cells, where levels are not
259 enough to mediate SCoV2 infection. Further analysis of L-SIGN or DC-SIGN-mediated infection
260 would be important to understand the etiology of SARS-CoV-2-related diseases. Also, targeting
261 SIGN-mediated infection as well as ACE2-mediated infection would be important for effective
262 vaccine development.

263

264 **Methods**

265

266 **Antibodies.** Mouse anti-CD209 mAb (clone 9E9A8), mouse IgG2a isotype control antibody
267 (BioLegend, San Diego, CA, USA), mouse anti-L-SIGN mAb (clone 19F7), mouse anti-MHCII
268 (clone WR18) (Santa Cruz Biotechnology, Dallas, TX, USA), mouse anti-human ACE2 mAb
269 (R&D Systems, Minneapolis, MN, USA), rat anti-Flag mAb (Sigma-Aldrich, St Louis, MI, USA),
270 mouse anti-human CD14-APC mAb (eBioscience, San Diego, CA, USA), Donkey anti-mouse

271 IgG-APC mAb, anti-human IgG-Fc fragment specific-APC mAb, anti-rat IgG-APC mAb (Jackson
272 ImmunoResearch, West Grove, PA, USA).

273

274 **Cell lines and cell culture.** The following cell lines were used in this study: Vero E6 is an African
275 Green Monkey Kidney cell line, HEK293T cells are a human kidney cell line, HEK293T-DC-
276 SIGN is a stable transfectant of HEK293T expressing DC-SIGN, HEK293T-L-SIGN is a stable
277 transfectant of HEK293T expressing L-SIGN, HEK293T-ACE2 is a stable transfectant of
278 HEK293T expressing ACE2, HEK293T-L-SIGN/TMPRSS2 is a stable transfectant of HEK293T
279 expressing L-SIGN and TMPRSS2, HEK293T-DC-SIGN/TMPRSS2 is a stable transfectant of
280 HEK293T expressing L-SIGN and TMPRSS2. Stable cell lines were prepared using retrovirus
281 transfection produced from Plat-E³⁸. Briefly, Plat-E cells were co-transfected with target gene
282 vectors and an amphotropic envelope vector using polyethylenimine (PEI; Polysciences,
283 Warrington, PA, USA) as described previously³⁹. Culture supernatant containing retroviruses was
284 harvested after 48 hours and premixed with DOTAP liposomal transfection reagent (Roche, Basel,
285 Switzerland) prior to spin transfection at 2400 rpm at 32°C for 2 hours into target cells. The
286 transfected cells were cultured for three days and subsequently sorted using an SH800S cell sorter
287 (Sony, Minato City, Tokyo, Japan). All cell lines were maintained in DMEM (Nacalai Tesque,
288 Kyoto, Japan) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biological
289 Industries, Cromwell, CT, USA), penicillin (100 U/mL), and streptomycin (100 µg/mL) (Nacalai
290 Tesque, Japan) and cultured at 37°C in 5% CO₂.

291

292 **Genes**

293 The followings genes were used in the current study, SARS-CoV-2 spike (amino acid from 1–
294 1273, GenBank accession NC_045512.2), SARS-CoV spike (amino acid from 1–1255, GenBank
295 accession AY278491), human coronavirus HKU1 spike (amino acid from 1–1352, GenBank
296 accession NC_006213), human coronavirus OC43 (amino acid from 1–1353, GenBank accession
297 KY674921) DC-SIGN (GenBank accession NM_021155.4), L-SIGN (gene obtained from lung
298 cDNA library screening corresponding to GenBank accession NM_001144904.2), human ACE2
299 (GenBank accession NM_021804.3), human TMPRSS2 (GenBank accession BC051839.1),
300 human CD147 (GenBank accession NG_007468), human IgG1 Fc segment^{6,40,41}, genes for
301 variable regions of mAbs, C144, 4A2, and 4A8, were retrieved from previous studies^{10,18}.

302

303 **cDNA library screening**

304 A retrovirus cDNA library was constructed based on a previous study⁴². Briefly, cDNA was
305 generated from a human lung poly-A⁺ RNA (cat. 636105, Takara Bio, Kusatsu, Shiga, Japan) using
306 a cDNA library construction kit (Takara Bio, Japan) and cloned into EcoRI and NotI sites of pMXs
307 retrovirus vector. The ligated cDNA was transformed into XL10-gold and the plasmids were
308 purified using a plasmid plus midi kit (Qiagen, Germantown, MD, USA). The complexity of the
309 cDNA library was 2×10^6 . The cDNA library was then transfected into PLAT-E packaging cells
310 to generate a retrovirus cDNA library. Subsequently, the retrovirus cDNA library was transfected
311 into 2B4 mouse T cell hybridoma and sorted for cells binding to the SCoV2-NTD-Fc fusion protein
312 using an SH800S cell sorter (Sony, Japan). Single-cell clones were obtained and the genomic DNA
313 was then isolated using a Wizard SV genomic DNA purification kit (Promega, Madison, WI, USA).
314 The target gene was amplified by PCR using pMXs vector forward
315 (5'GGTGGACCATCCTCTAGA3') and reverse (5'CCCTTTTTCTGGAGACTAAAT3')

316 primers. The identity of the target gene was determined by DNA sequencing. The L-SIGN gene
317 obtained corresponded to GenBank accession NM_001144904.2.

318

319 **Mutagenesis of N-linked glycosylation sites of SCoV2-NTD**

320 Single point SCoV2-NTD-Fc N-linked glycosylation site mutants (N61, 74, 122, 149, 165, 234,
321 282Q) were generated using a QuikChange site-directed mutagenesis kit (Agilent Technologies,
322 Santa Clara, CA, USA) to change the respective asparagine (N, Asn) residue to glutamine (Q, Gln).

323

324 **Recombinant protein production**

325 Recombinant DC-SIGN-Fc, L-SIGN-Fc, SCoV-NTD-Fc (amino acids 14–279), SCoV2-NTD-Fc
326 (amino acids 14–292), SCoV2-RBD-Fc (amino acids 335–587), and SCoV2-NTD-Fc N-
327 glycosylation site mutant fusion proteins were independently expressed as a secreted protein using
328 HEK293T cells. The recombinant mAbs, C144, 4A2, and 4A8 were produced using Expi293 cells.
329 The Fc fusion protein in the culture supernatant was quantified using a protein A beads assay.
330 Essentially, the protein A was coupled to 4 µm aldehyde/sulfate latex beads (Invitrogen, USA)
331 according to the manufacturer's instructions and used to capture the Fc fusion protein in the culture
332 supernatant. The captured Fc fusion proteins were then detected using anti-human IgG-Fc
333 conjugated to APC and measured using flow cytometry. The protein concentration was calculated
334 using a known concentration of IgG-Fc fusion protein as a standard. For recombinant proteins that
335 required purification, the culture supernatant containing the secreted fusion proteins was purified
336 using protein-A conjugated Sepharose beads (Cytiva, Marlborough, MA, USA).

337

338 **Flow cytometry analysis**

339 FACSVerse™ or FACSCalibur flow cytometer (Becton Dickinson, Franklin Lake, NJ, USA) was
340 used for flow cytometry analysis. For the binding study, cells were transiently co-transfected with
341 the plasmid containing the respective receptors and GFP using a PEI transfection reagent³⁹. Forty-
342 eight hours post-infection, cells were then stained with Fc fusion proteins for 30 minutes at 4°C in
343 Hank's buffer containing 0.1% BSA and identified using goat anti-human IgG-Fc-APC Ab prior
344 to flow cytometry analysis. For DC- and L-SIGN blocking experiments, the cells were pre-blocked
345 with 5 µg/mL of anti-CD209 mAb or 50 µg/mL of mannan (Sigma, USA) for 30 minutes before
346 staining with Fc fusion protein. For the staining of the spike protein, DC-SIGN-Fc (5 µg/mL) or
347 L-SIGN-Fc fusion protein (20 µg/mL) premixed with goat anti-human IgG-Fc-APC mAb (3
348 µg/mL) was used. For the analysis of SCoV2-NTD N-linked glycosylation site mutants'
349 interaction with DC-SIGN, 10 µg/mL of each mutant was used. Surface-expressed SCoV2-NTD
350 (amino acids 14–333), SCoV2-RBD (amino acids 335–587), and SCoV2-S2 (amino acids 588–
351 1219) were generated by fusing the respective domains with a transmembrane region derived from
352 PILRα at the C-terminal⁶.

353

354 **Cell-cell fusion assay**

355 Effector cells were prepared by transiently transfecting T7 RNA polymerase plasmid, pCAG-T7,
356 and SCoV2 spike plasmid into HEK293T cells. For the target cells, HEK293T cells were
357 transiently co-transfected with T7 promoter-luciferase plasmid, pT7EMCV-Luc, and DC-SIGN in
358 a pcDNA3.4 vector, L-SIGN in a pcDNA3.4 vector, or a pcDNA3.4 empty vector (mock). After
359 two days of transfection, the effector cells (1×10^4 cells) were cocultured with the target cells (1
360 $\times 10^4$ cells) in a 384-well white plate (Greiner Bio-One, Kremsmünster, Austria). The cells were
361 then incubated for 24 hours at 37°C in 5% CO₂ and the luciferase activity was measured using the

362 ONE-Glo™ luciferase assay (Promega, USA) according to the manufacturer's instructions. The
363 signals were recorded using a luminescence plate reader TriStar LB941 (Berthold Technologies,
364 Bad Wilbad, Germany). For the cell-cell fusion assay using fluorescence protein, effector cells
365 were prepared by transiently co-transfecting with SCoV2 spike and red fluorescence protein (pCI-
366 neo-DsREDExp) into HEK293T cells. For target cells, HEK293T cells were transiently co-
367 transfected with DC-SIGN and green fluorescence protein (pMX-GFP). After two days of
368 transfection, the effector and target cells were enriched using a Sony SH800 cell sorter (Sony,
369 Japan) prior to co-culture as described above. Cells were then analyzed using an IX83 Olympus
370 microscope (Olympus, Shinjuku City, Tokyo, Japan).

371

372 **SCoV2 spike-pseudotyped virus**

373 HEK293T cells were transiently transfected with expression plasmids for the SCoV2 spike protein.
374 Twenty-four hours post-transfection, VSV-G-deficient vesicular stomatitis virus (VSV) carrying
375 a luciferase reporter gene complemented in trans with VSV-G protein⁴³ was added and incubated
376 for two hours. Cells were then carefully washed with DMEM media without FBS and incubation
377 continued with DMEM supplemented with FBS at 37°C in 5% CO₂ for 48 hours. The supernatant
378 containing the pseudotyped SCoV2 virions was harvested and aliquoted before storage at -80°C.

379

380 **CD14⁺ cell preparation and moDCs differentiation**

381 Peripheral blood mononuclear cells (PBMCs) were prepared from healthy individuals by
382 centrifugation using Ficoll-Paque™ PLUS (GE Healthcare, Chicago, IL, USA). CD14⁺ cells were
383 isolated by positive selection using anti-CD14 conjugated microbeads (Miltenyi Biotec, Bergisch
384 Gladbach, Germany) according to the manufacturer's instructions. Monocyte-derived dendritic

385 cells (moDCs) were stimulated as previously described⁴⁴. Briefly, 5×10^5 cells/mL of CD14⁺
386 enriched cells were stimulated using recombinant human (rh) GM-CSF and rhIL-4 (BioLegend,
387 USA) at a concentration of each 500 IU/mL in RPMI1640 media supplemented with 10% FCS at
388 37°C in 5% CO₂. The expression of CD209 was detected after two days of stimulation using an
389 anti-CD209 monoclonal antibody.

390

391 **Direct infection assay**

392 For pseudotyped SCoV2 infection, 5×10^3 cells were mixed with the pseudovirus for 24 hours at
393 37°C in 5% CO₂ in a 384-well plate. Luciferase activity was then measured using the ONE-Glo™
394 luciferase assay (Promega, USA) according to the manufacturer's instructions. The signals were
395 recorded using a luminescence plate reader TriStar LB941 (Berthold Technologies, Germany). For
396 neutralization assay, essentially the same protocol was conducted with the exception that the
397 pseudovirus was preincubated with diluted patient sera or anti-NTD, clone 4A8 and 4A2
398 monoclonal antibodies for 30 minutes at room temperature. SARS-CoV-2 convalescent patients'
399 sera were collected from Osaka University Hospital. Patients' sera collection was approved by the
400 IRB (No. 19546). Recombinant SCoV2 virus with a high-affinity NanoBiT (HiBiT) luciferase
401 gene in the N-terminus of the ORF6 gene was produced as previously described⁴⁵. The virus (MOI
402 3) was added to 1.5×10^4 cells for 24 hours at 37°C, 5% CO₂ in a 96-well plate. SCoV2 infected
403 cells were collected and luciferase activity was then measured using a Nano-Glo HiBiT lytic assay
404 system (Promega, USA), according to the manufacturer's instructions. The signals were recorded
405 using a luminometer.

406

407 **Trans-infection assay**

408 moDCs were incubated with pseudotyped SCoV2 at 37°C for 60 minutes. For the blocking
409 experiment, moDCs were preincubated with anti-CD209 antibodies (5 µg/mL), mouse IgG2a
410 antibodies (Isotype control), or mannan (100 µg/mL) before incubation with pseudovirus. After
411 incubation, the cells were washed three times with DMEM and subsequently added to Vero cells
412 at a 1:1 ratio. The cells were incubated at 37°C in 5% CO₂ for 24 hours and luciferase activity was
413 measured using a ONE-Glo™ luciferase assay (Promega, USA) according to the manufacturer's
414 instructions.

415

416 **Sequence, data, and statistical analysis**

417 The amino acid multiple sequence alignment in Fig. 3a was generated using the CLUSTAL Omega
418 multiple sequence alignment tool from NCBI. FlowJo (BD Biosciences, San Jose, CA, USA) was
419 used for analyzing flow cytometry data and Graphpad Prism version 7.0e was used for graph
420 generation and statistical analysis. Asterisks indicate statistical significance.

421

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430

431 **Author contributions**

432 W.T.S., M.Y., S.T., and H.A. conceived experiments. W.T.S., Y.L., N.E.E., and O.C., performed
433 experiments. N. H. collected patients' sera. W.T.S. and H.A. wrote the manuscript. All authors
434 read, edit, and approved the manuscript.

435

436 **Competing interests**

437 All authors declare no conflict of interest.

438

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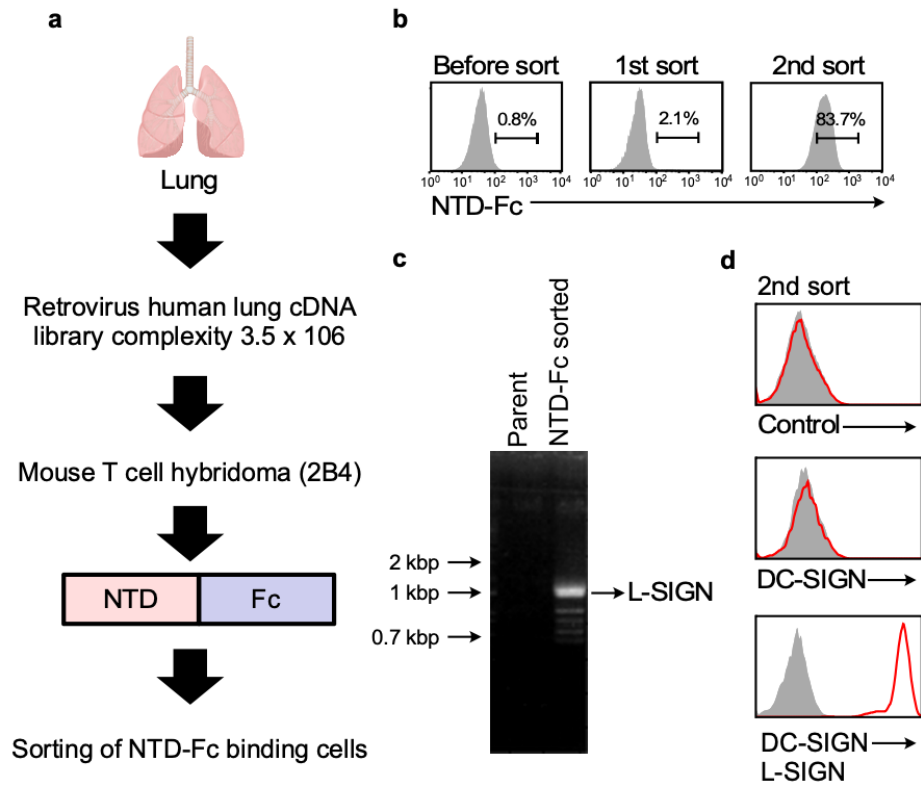
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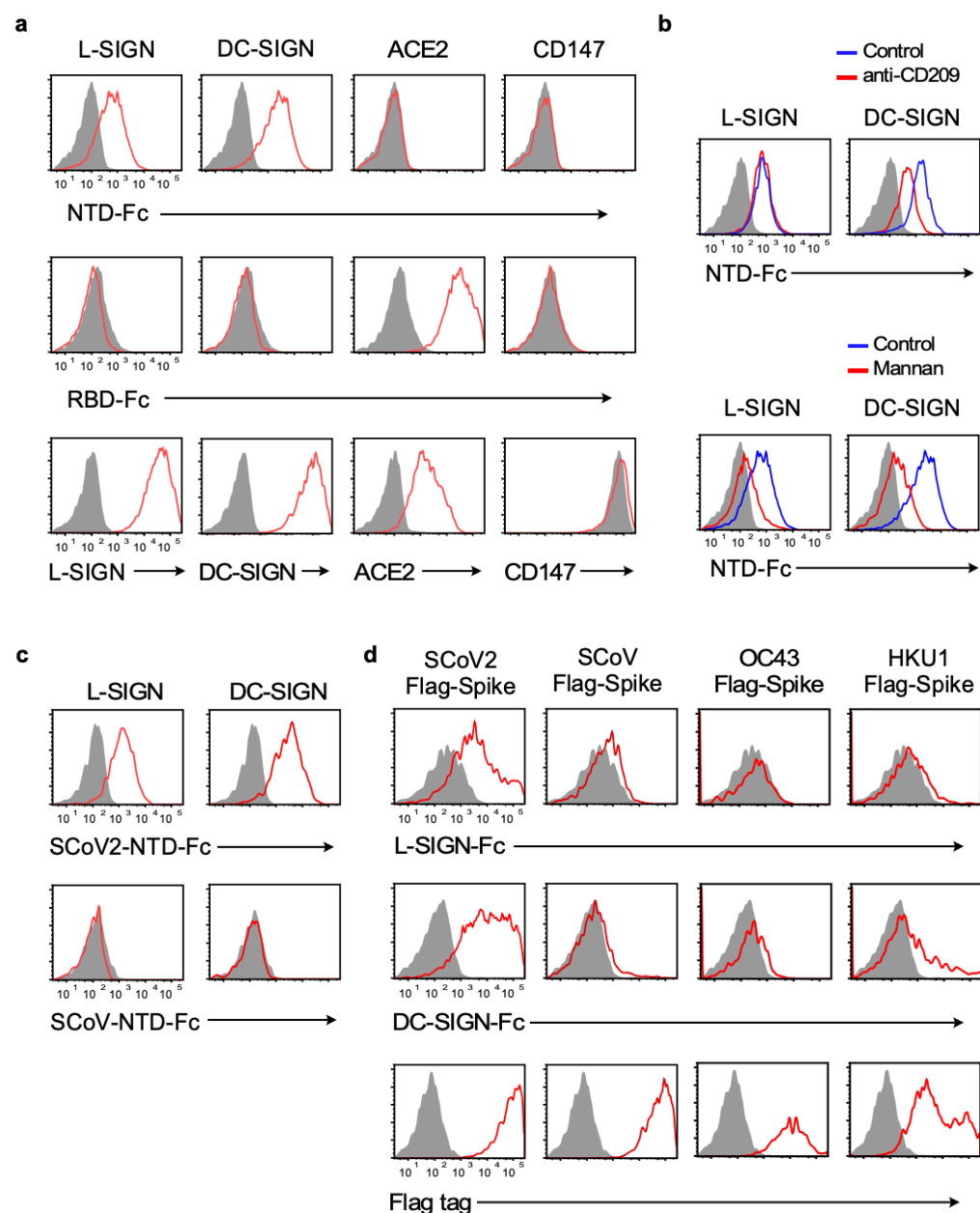
543 **Figures and figure legends**



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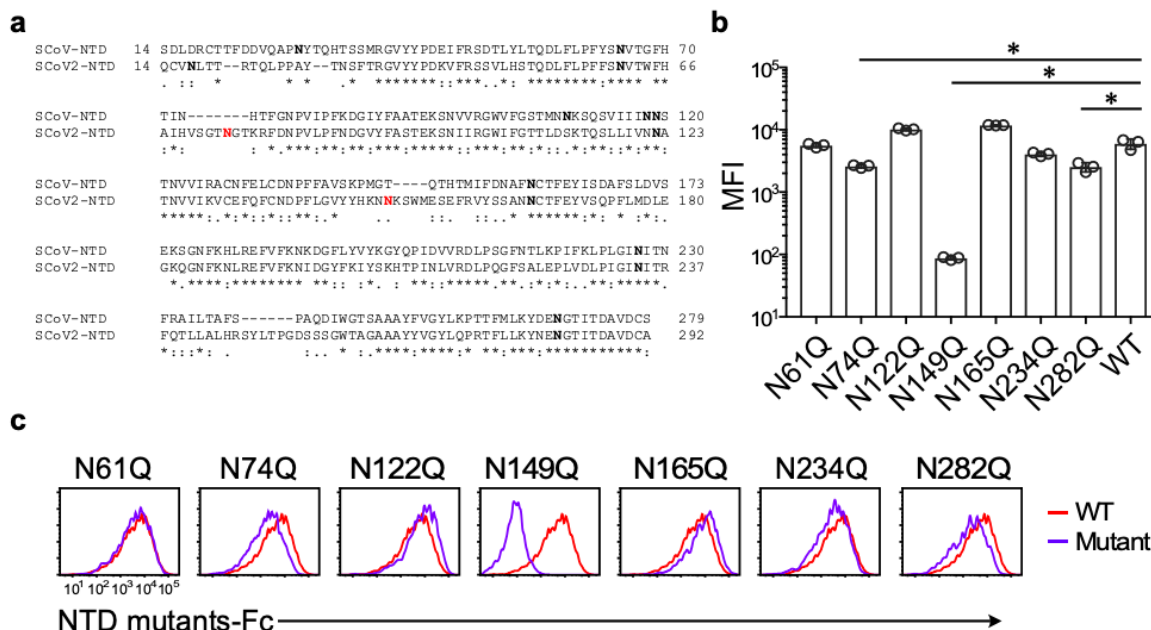
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546 **Fig. 1 Lung cDNA library screening using the NTD-Fc of SCoV2 spike.** **a**, The workflow of
547 lung cDNA library screening. **b**, Cells transfected with retrovirus cDNA library were stained with
548 SCoV2-NTD-Fc. Proportions of cells stained with SCoV2-NTD-Fc are shown. **c**, DNA agarose
549 gel electrophoresis of cDNA library derived genes amplified from a single-cell clone stained with
550 SCoV2-NTD-Fc. The indicated band was identified as L-SIGN. **d**, Single-cell clone stained with
551 SCoV2-NTD-Fc was analyzed by anti-DC-SIGN mAb and anti-DC/L-SIGN mAb (red line) or
552 control (shaded gray).
553



554
 555 **Fig. 2 Cell surface binding analyses of the SCoV2 spike bound to DC-SIGN and L-SIGN**
 556 **using flow cytometry. a,** Cells transfected with ACE2, L-SIGN, DC-SIGN, and CD147 (red line)
 557 or mock (shaded gray) were stained with SCoV2-NTD-Fc and RBD-Fc fusion protein (10 μ g/mL).
 558 Each transfectant was also stained with a specific monoclonal antibody. **b,** Effect of mannin and
 559 anti-CD209 (anti-DC-SIGN) antibody on SCoV2-NTD-Fc binding to DC- and L-SIGN
 560 transfectants or mock (shaded gray). The transfectants were preincubated with mannin (light blue
 561 line) or anti-CD209 antibody (dark blue), followed by the staining with NTD-Fc fusion protein. **c,**
 562 Staining of DC- and L-SIGN transfectants (red line) or mock (shaded gray) with SCoV2-NTD-Fc
 563 and SCoV-NTD-Fc fusion proteins (10 μ g/mL). **d,** Cells transfected with flag-tagged spike
 564 proteins of SCoV2, SCoV, human coronavirus OC43, or HKU1 (red line) or mock (shaded gray)
 565 were stained with DC-, L-SIGN-Fc fusion proteins or anti-Flag-tag antibody. Proportions of the
 566 stained cells are shown. Data are representative of three independent experiments.

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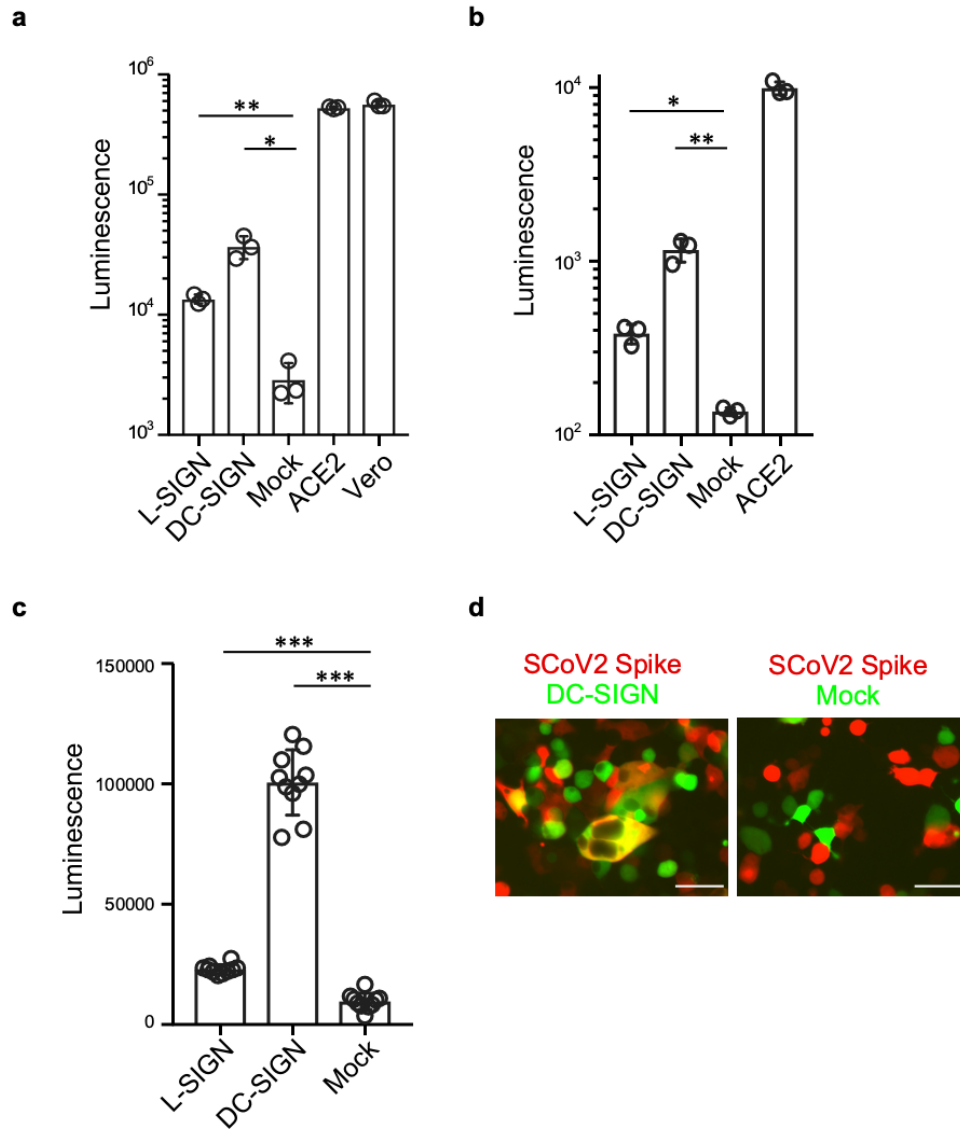
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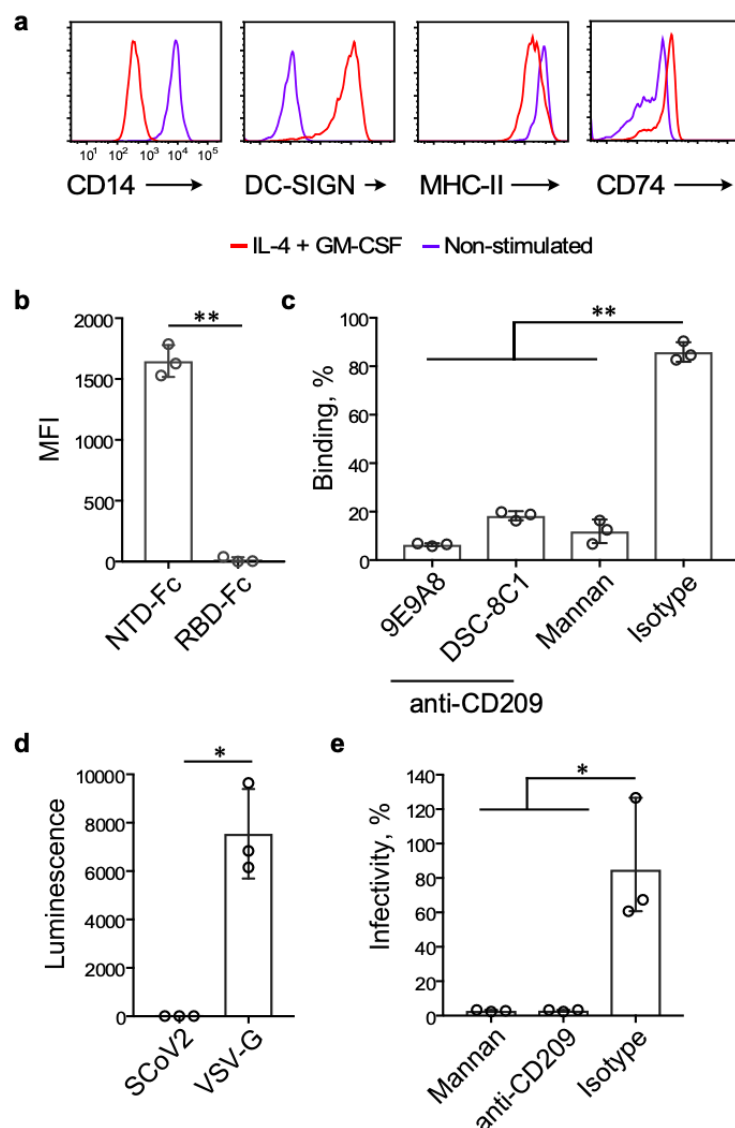
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Fig. 3 Effect of N-linked glycosylation on SCoV2-NTD interaction with DC-SIGN. a, Amino acid sequence alignment of the NTD between SCoV and SCoV2 spike. Bold asparagine (N) residues indicate the N-linked glycan sites³⁰⁻³². Highlighted asparagine residues in red indicate the unique N-glycosylation sites in SCoV2. **b**, DC-SIGN transfectants were stained with NTD-Fc fusion proteins in which N-glycosylation sites were mutated and analyzed by flow cytometry. Mean fluorescence intensities (MFI) of the cells were shown. Asterisks indicate statistical significance derived from unpaired T-test; * $P < 0.0001$. **c**, Representative histogram of DC-SIGN transfectants stained with NTD-Fc mutants is shown. Proportion of cells stained with NTD-Fc mutants were shown. Data are representative of three independent experiments.



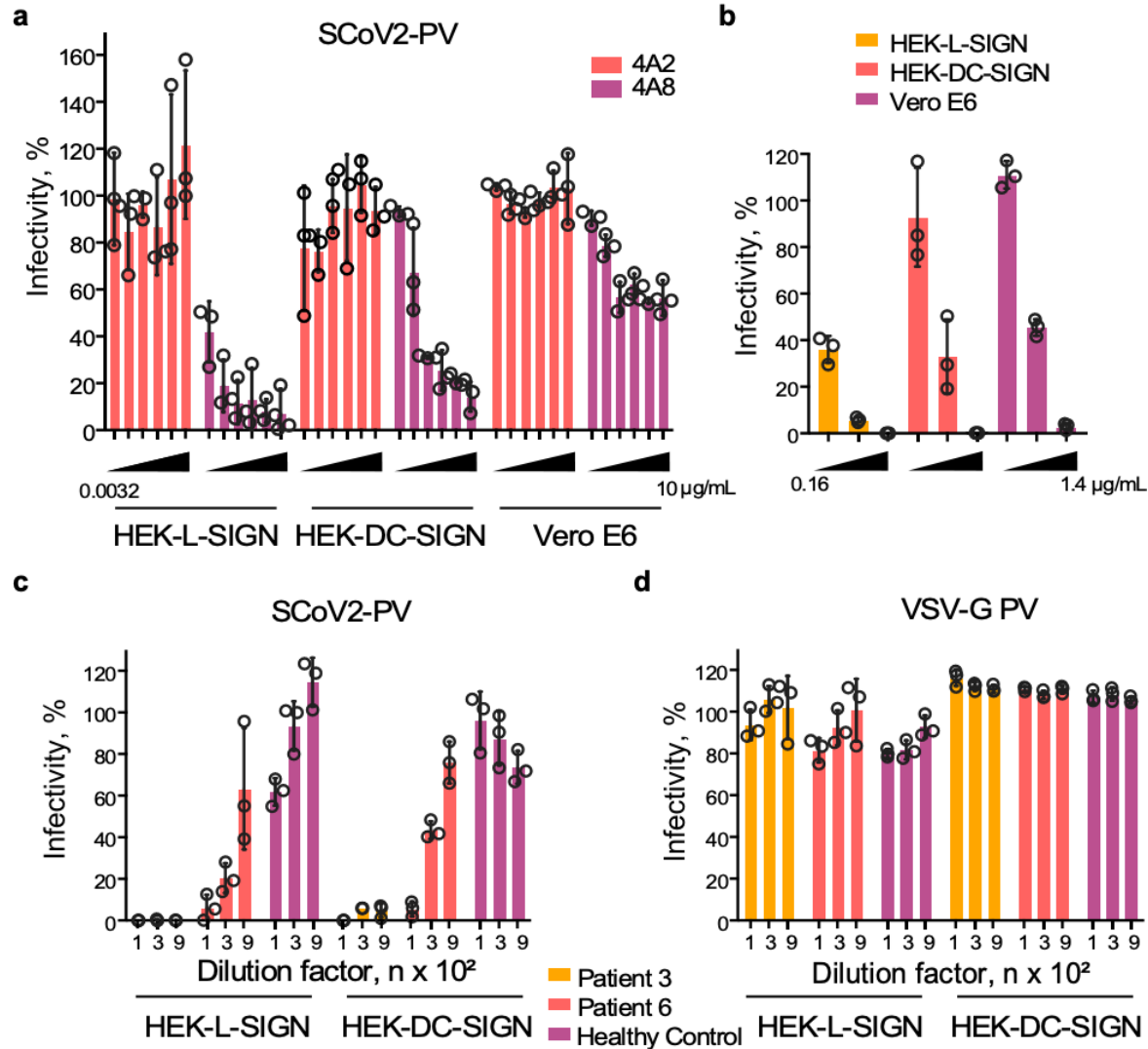
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Fig. 4 DC-SIGN and L-SIGN mediate SCoV2 infection. **a**, SCoV2-PV infection on DC-SIGN, L-SIGN, mock, or ACE2 transfectants and Vero E6 cells. SCoV2-PV carrying a luciferase gene was used for the infection and luciferase activity was measured 24 h later. Asterisks indicate statistical significance derived from unpaired T-test; * $P = 0.0018$; ** $P = 0.0003$ **b**, DC-SIGN or L-SIGN transfectants were infected with recombinant SCoV2 with the NanoBiT luciferase gene. The luciferase activity was measured 24 h later. Asterisks indicate statistical significance derived from unpaired T-test; * $P = 0.001$; ** $P = 0.0006$. **c**, Cell-cell fusion assay of SCoV2 spike transfectants and DC- or L-SIGN transfectants. The effector cells expressing spike and T7 polymerase were cocultured with target cells expressing DC-SIGN, L-SIGN, mock, and T7 promoter-driven luciferase. Luciferase activities were measured after 24 h. Asterisks indicate statistical significance derived from unpaired T-test *** $P < 0.0001$. **d**, Cell-cell fusion assay of SCoV2 spike transfectants (red) and DC-SIGN transfectants (green). Representative images are shown. Scale bar represents 50 μm in length. Data are representative of three independent experiments.



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597

598 **Fig. 5 moDCs facilitate SCoV2 infection in trans through DC-SIGN.** **a**, Phenotype of the
599 CD14⁺ cells from PBMC cultured in the presence (red line) or absence (purple line) of cytokines
600 (IL-4 and GM-CSF, 500 IU/mL each) for three days. **b**, Binding of SCoV2-NTD-Fc and RBD-Fc
601 of SCoV2 spike to moDCs. The amount of Fc-fusion proteins bound to moDCs were presented as
602 mean fluorescence intensity (MFI). Asterisks indicate statistical significance derived from an
603 unpaired T-test; ** $P < 0.0001$. **c**, Blocking of SCoV2-NTD-Fc binding to moDCs by two specific
604 anti-DC-SIGN mAbs, isotype antibody, and mannan. The percentage of binding is calculated
605 based on the MFI value of non-blocking control. Asterisks indicate statistical significance derived
606 from unpaired T-test; ** $P < 0.0001$. **d**, SCoV2-PV and VSV-G PV infection on moDCs. Asterisks
607 indicate statistical significance derived from unpaired T-test; * $P = 0.0021$. **e**, SCoV2-PV infection
608 in trans on Vero E6 cells facilitated by moDCs. moDCs was incubated with SCoV2-PV in the
609 presence or absence of anti-DC-SIGN antibody or mannan, and cocultured with Vero E6 cells after
610 extensive washing. Infectivity was calculated based on the luciferase activity of a non-blocking
611 control. Asterisks indicate statistical significance derived from unpaired T-test; * $P = 0.005$. Data
612 are representative of three independent experiments.



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614

615 **Fig. 6 Neutralizing potency of SCoV2 convalescent patients' sera and anti-NTD antibodies**
 616 **to L-SIGN and DC-SIGN mediated infection.** Neutralization assay of pseudotyped SCoV2
 617 infection on HEK-L-SIGN, HEK-DC-SIGN, and Vero cells by human anti-SCoV2-NTD mAbs (a)
 618 and human anti-SCoV2-RBD (C144) specific mAb (b). SCoV2-PV preincubated with mAbs was
 619 used to infect the cells. Neutralization assay of SCoV2-PV (c) and VSV-G PV (d) infection of L-
 620 SIGN or DC-SIGN transfectants by serum from SCoV2-infected patients or healthy control.
 621 SCoV2-PV or VSV-G PV preincubated with sequentially diluted serum was used to infect L-SIGN
 622 or DC-SIGN transfectants and luciferase activity was measured. The percentage of infectivity was
 623 calculated based on the luminescence value of no serum or mAb control. Data are representative
 624 of three independent experiments.

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