

## Dectin-1 intracellular domain determines species-specific ligand spectrum by modulating receptor sensitivity

高野, 智嗣

<https://doi.org/10.15017/1931824>

---

出版情報 : 九州大学, 2017, 博士 (医学), 課程博士  
バージョン :

権利関係 : © 2017 by The American Society for Biochemistry and Molecular Biology, Inc.



# Dectin-1 intracellular domain determines species-specific ligand spectrum by modulating receptor sensitivity

Received for publication, June 13, 2017, and in revised form, August 9, 2017 Published, Papers in Press, August 28, 2017, DOI 10.1074/jbc.M117.800847

Tomotsugu Takano<sup>§¶||</sup>, Chihiro Motozono<sup>¶||\*\*1</sup>, Takashi Imai<sup>‡¶||</sup>, Koh-Hei Sonoda<sup>\*\*</sup>, Yoichi Nakanishi<sup>§</sup>, and Sho Yamasaki<sup>‡¶||##2</sup>

From the <sup>‡</sup>Division of Molecular Immunology, Medical Institute of Bioregulation, <sup>§</sup>Research Institute for Diseases of the Chest, Graduate School of Medical Sciences, and <sup>\*\*</sup>Department of Ocular Pathology and Imaging Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, the <sup>¶</sup>Department of Molecular Immunology, Research Institute for Microbial Diseases, and <sup>||</sup>Department of Molecular Immunology, Immunology Frontier Research Center, Osaka University, Osaka 565-0871, and the <sup>##</sup>Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, Chiba 260-8673, Japan

Edited by Luke O'Neill

C-type lectin receptors (CLRs) comprise a large family of immunoreceptors that recognize polysaccharide ligands exposed on pathogen surfaces and are conserved among mammals. However, interspecies differences in their ligand spectrums are not fully understood. Dectin-1 is a well-characterized CLR that recognizes  $\beta$ -glucan. We report here that seaweed-derived fucan activates cells expressing human Dectin-1 but not mouse Dectin-1. Low-valency  $\beta$ -glucan components within fucan appeared to be responsible for this activation, as the ligand activity was eliminated by  $\beta$ -glucanase treatment. The low-valency  $\beta$ -glucan laminarin also acted as an agonist for human Dectin-1 but not for mouse Dectin-1, whereas the high-valency  $\beta$ -glucan curdlan activated both human and mouse Dectin-1. Reciprocal mutagenesis analysis revealed that the ligand-binding domain of human Dectin-1 does not determine its unique sensitivity to low-valency  $\beta$ -glucan. Rather, we found that its intracellular domain renders human Dectin-1 reactive to low-valency  $\beta$ -glucan ligand. Substitution with two amino acids, Glu<sup>2</sup> and Pro<sup>5</sup>, located in the human Dectin-1 intracellular domain was sufficient to confer sensitivity to low-valency  $\beta$ -glucan in mouse Dectin-1. Conversely, the introduction of mouse-specific amino acids, Lys<sup>2</sup> and Ser<sup>5</sup>, to human Dectin-1 reduced the reactivity to low-valency  $\beta$ -glucan. Indeed, low-valency ligands induced a set of proinflammatory genes in human but not mouse dendritic cells. These results suggest that the intracellular domain, not ligand-binding domain, of Dectin-1 determines the species-specific ligand profile.

This work was supported by Grant-in-Aid 26110009 for Scientific Research on Innovative Areas, Young Scientists (B) Grant 17K15730, Advanced Research and Development Programs for Medical Innovation (AMED-CREST), and Research on Development of New Drugs Grants (16ak0101010h0005 and 17933742) from the Japan Agency for Medical Research and Development (AMED), and Takeda Science Foundation. The authors declare that they have no conflicts of interest with the contents of this article.

This article contains supplemental Figs. S1–S7 and Table S1.

The array data were deposited in the Gene Expression Omnibus (GEO) database under accession number GSE98826.

<sup>1</sup> To whom correspondence may be addressed. Tel.: 81-6-6879-8307; Fax: 81-6-6879-8308; E-mail: motozono@biken.osaka-u.ac.jp.

<sup>2</sup> To whom correspondence may be addressed. Tel.: 81-6-6879-8306; Fax: 81-6-6879-8308; E-mail: yamasaki@biken.osaka-u.ac.jp.

Our bodies are continuously exposed to and infected by various types of pathogens, most of which are directly recognized by pattern recognition receptors such as Toll-like receptors, RIG-I-like receptors, or NOD-like receptors on host cells (1, 2). An additional fourth member of pattern recognition receptors is the emerging C-type lectin receptors (CLRs)<sup>3</sup> that senses pathogens or damaged tissues to trigger innate immune responses (3).

Within this family, Dectin-1 is the first immunoreceptor tyrosine-based activation motif (ITAM)-coupled CLR identified and recognizes  $\beta$ -glucans present in the cell wall of fungi (4–6). Dectin-1 is a type II transmembrane protein expressed by myeloid cells and consists of an extracellular carbohydrate recognition domain (CRD) and a cytoplasmic domain containing an ITAM-like motif (hemITAM). Upon recognition of multivalent  $\beta$ -glucan via its CRD, Dectin-1 multimerizes and is phosphorylated at a tyrosine residue in the hemITAM, providing a binding site for the Syk kinase. The recruited Syk then activates the CARD9-Bcl10-MALT1 and NF- $\kappa$ B pathways to induce inflammatory cytokines, co-stimulatory molecules, and dendritic cell maturation, which promotes Th1 and Th17 responses to orchestrate immunity to pathogens (7, 8). The CRD and hemITAM regions of Dectin-1 are conserved among mammals (5, 9), suggesting the importance of this CLR for promoting acquired immune responses over a wide variety of species. Hence, Dectin-1 agonists hold potential as vaccine adjuvants that may facilitate protective immune responses against pathogens or cancer in mouse models and human patients. Given this potential, it is important to characterize in detail the function of human Dectin-1 in comparison with the evidences accumulated for the more extensively studied mouse Dectin-1 (10, 11).

The ligand potency of  $\beta$ -glucans toward Dectin-1 varies according to their valency. Among several  $\beta$ -glucan-containing polysaccharides, yeast zymosan and bacterial curdlan act as potent agonists, whereas the ligand activity of low valency  $\beta$ -glucans, such as seaweed laminarin, is controversial (12–15).

<sup>3</sup> The abbreviations used are: CLR, C-type lectin receptor; hDectin-1, human Dectin-1; mDectin-1, mouse Dectin-1; rDectin-1, rat Dectin-1; CRD, carbohydrate recognition domain; FcR $\gamma$ , Fc receptor  $\gamma$ -chain; NFAT, nuclear factor of activated T-cells; hemITAM, hem-immunoreceptor tyrosine-based activation motif; hMoDC, human monocyte-derived dendritic cell; mBMDC, mouse bone marrow-derived dendritic cell; PE, phycoerythrin; PBMC, peripheral blood mononuclear cell.

# Intracellular non-ITAM sequence sensitizes human Dectin-1

The inconsistency within studies on the “low-valency” Dectin-1 ligand may be partly due to interspecies difference of Dectin-1. In addition, the function of the cytoplasmic domain, other than its identity as a hemITAM, is not well-characterized.

In this study, we found that low-valency  $\beta$ -glucan can activate cells expressing human Dectin-1, but not mouse Dectin-1. Reciprocal mutagenesis studies revealed that the intracellular domain of human Dectin-1 confers this activity. Furthermore, we found that two intracellular amino acids, which are conserved in primates, play a critical role for enhancing the sensitivity of Dectin-1 independently of the hemITAM.

## Results

### Fucan activates reporter cells expressing hDectin-1 but not mDectin-1

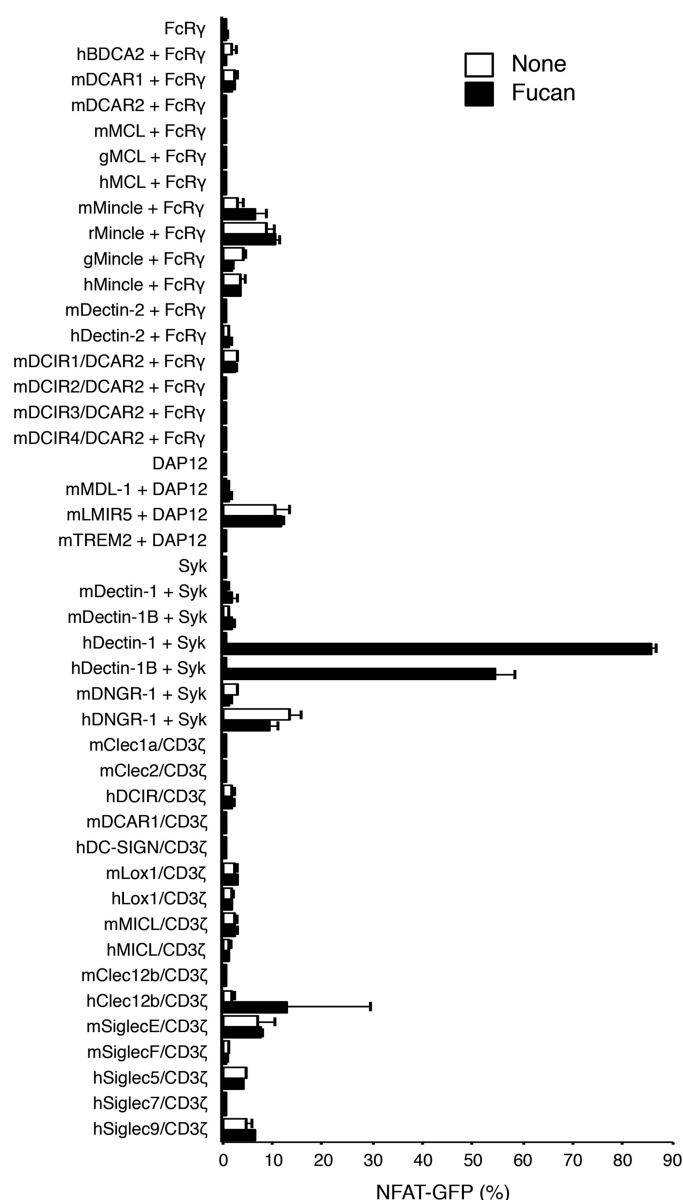
To search for novel CLRs that recognize natural polysaccharides, we employed nuclear factor of activated T-cells (NFAT)-GFP reporter cells expressing various CLRs. After screening several candidates, we found a seaweed-derived, fucose-containing polysaccharide called fucan activated reporter cells expressing human Dectin-1 (hDectin-1) (Fig. 1). Indeed, hDectin-1 induced reporter activity in response to fucan in a dose-dependent manner (Fig. 2A). A hDectin-1 isoform lacking the stalk region (hDectin-1B) similarly responded to fucan (supplemental Fig. S1). However, fucan did not activate cells expressing either forms of mouse Dectin-1 (mDectin-1) (Fig. 2A and supplemental Fig. S1). In contrast, curdlan, a high-molecular weight  $\beta$ -1,3-glucan, comparably activated the reporter for either hDectin-1 or mDectin-1 (Fig. 2B). From these observations, we initially hypothesized that hDectin-1 but not mDectin-1 binds to fucan.

### $\beta$ -Glucan components contained in fucan activate cells expressing hDectin-1

As  $\beta$ -1,3-glucan is a well-established component recognized by Dectin-1 (4), we next examined whether treatment with Westase, a  $\beta$ -1,3- and  $\beta$ -1,6-glucanase, eliminates the activity of fucan. As expected, Westase treatment completely suppressed the ability of curdlan to activate cells via hDectin-1 and mDectin-1 (Fig. 3A, left). Likewise, fucan treated with this enzyme failed to activate cells expressing hDectin-1 (Fig. 3A, right), suggesting that  $\beta$ -glucan-like components included in fucan are responsible for this activity. Size-exclusion separation revealed that the ligand activity was detected in low-molecular weight fractions (supplemental Fig. S2). We therefore characterized the species-specific ligand activity of laminarin, low-molecular weight  $\beta$ -glucan derived from brown seaweed. As with fucan, laminarin activated the reporter cells expressing hDectin-1, but not mDectin-1, and this activity was sensitive to Westase (Fig. 3B). These data suggest that low-valency, soluble  $\beta$ -glucans may selectively activate cells via hDectin-1 but not mDectin-1.

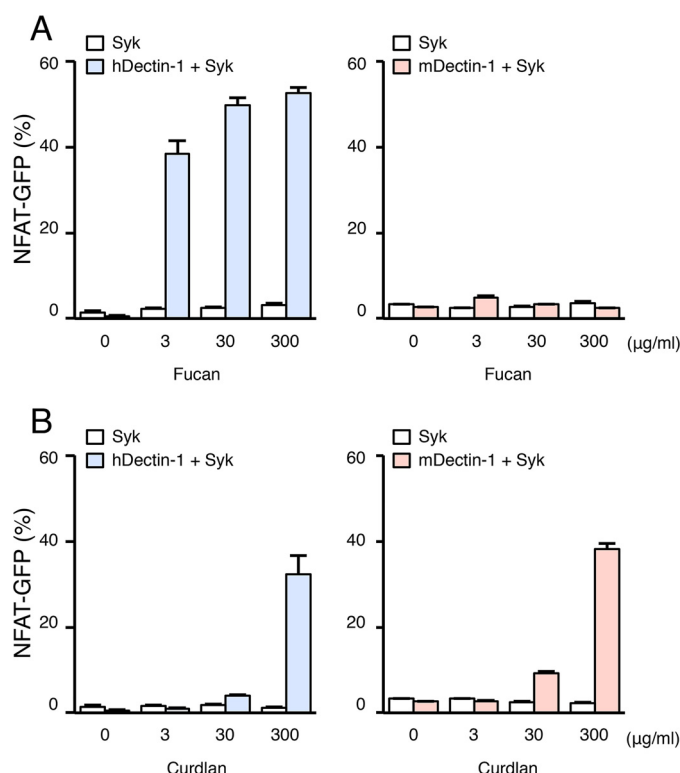
### Non-CRD region of hDectin-1 confers reactivity to low-valency $\beta$ -glucan

As the CRD of Dectin-1 mediates direct ligand binding (16), we initially suspected that the slightly different amino acid



**Figure 1. hDectin-1-expressing cells are selectively activated by fucan.** 2B4 NFAT-GFP cells expressing the indicated receptors and signaling components were left untreated (None) or stimulated with 30  $\mu$ g/ml of fucan (Fucan). m, mouse; r, rat; g, guinea pig; h, human. The detail of receptor constructs and co-transfected genes are described under “Experimental procedures.” The expression of GFP was analyzed by flow cytometry. All data are presented as the mean  $\pm$  S.D., and representative results from two independent experiments with similar results are shown.

sequences within the CRDs comparing hDectin-1 and mDectin-1 might determine the reactivity to soluble  $\beta$ -glucan. To address this possibility, we generated a chimeric mDectin-1 protein harboring the CRD from hDectin-1 (mDectin-1<sup>hCRD</sup> chimera) (Fig. 4A). Contrary to our initial assumption, the reporter cells expressing the mDectin-1<sup>hCRD</sup> chimera were not activated by laminarin similar to mDectin-1-bearing cells (Fig. 4B, mD1<sup>hCRD</sup>), although they showed substantial activity upon stimulation with high-valency curdlan (Fig. 4C). These results suggest that the direct ligand-binding domain, CRD, is not responsible for determining laminarin sensitivity to hDectin-1. We therefore created a hDectin-1 chimera harboring the CRD



**Figure 2. Fucan activates 2B4 NFAT-GFP reporter cells expressing hDectin-1 but not mDectin-1.** A and B, 2B4 NFAT-GFP reporter cells transduced without (Syk) or with hDectin-1 or mDectin-1 were stimulated with the indicated concentration of fucan (A) or curdlan (B) for 18 h and expression of GFP analyzed by flow cytometry. All data are presented as the mean  $\pm$  S.D., and representative results from three independent experiments with similar results are shown.

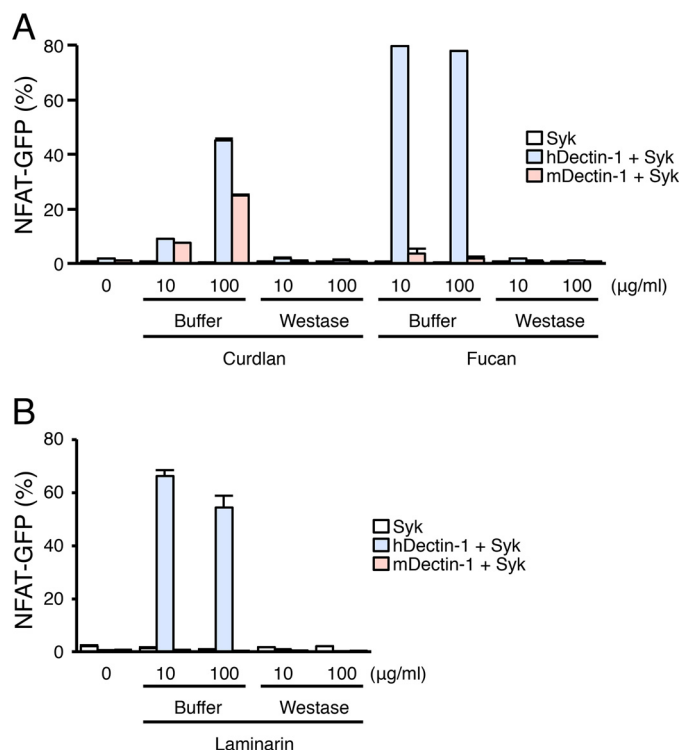
from mDectin-1 (hDectin-1<sup>mCRD</sup>) and found that the combination of mouse CRD and human non-CRD acted as a functional activating receptor for laminarin (Fig. 4B, hD1<sup>mCRD</sup>), suggesting that mouse CRD is capable of recognizing low-valency  $\beta$ -glucan. Indeed, fluorescence-labeled laminarin did bind to mDectin-1 as well as hDectin-1 (supplemental Fig. S3). Furthermore, soluble Ig-fusion protein derived from mDectin-1 and hDectin-1 showed comparable binding to laminarin (Fig. 4D). Taken together, these findings suggest that the non-CRD region of hDectin-1 confers the sensitivity to laminarin.

### Opposite effect of low-valency $\beta$ -glucan on mDectin-1 and hDectin-1

Given the observation that mDectin-1 binds low-valency  $\beta$ -glucan but is unable to deliver activating signaling, we next examined whether these ligands act as antagonists for mDectin-1. To assess this, we added a graded amount of fucan or laminarin to mDectin-1 cells in the presence of suboptimal concentrations of a high-valency ligand zymosan. Indeed, both ligands suppressed mDectin-1-induced reporter activation (Fig. 5A), in sharp contrast to their agonistic effects on hDectin-1 (Fig. 5B). These results indicate that low-valency  $\beta$ -glucan acts oppositely on mDectin-1 and hDectin-1.

### Cytoplasmic N-terminal region of hDectin-1 determines its sensitivity to low-valency $\beta$ -glucan

As the stalk region of hDectin-1 is dispensable for the reactivity to fucan and laminarin (supplemental Fig. S1A), we next



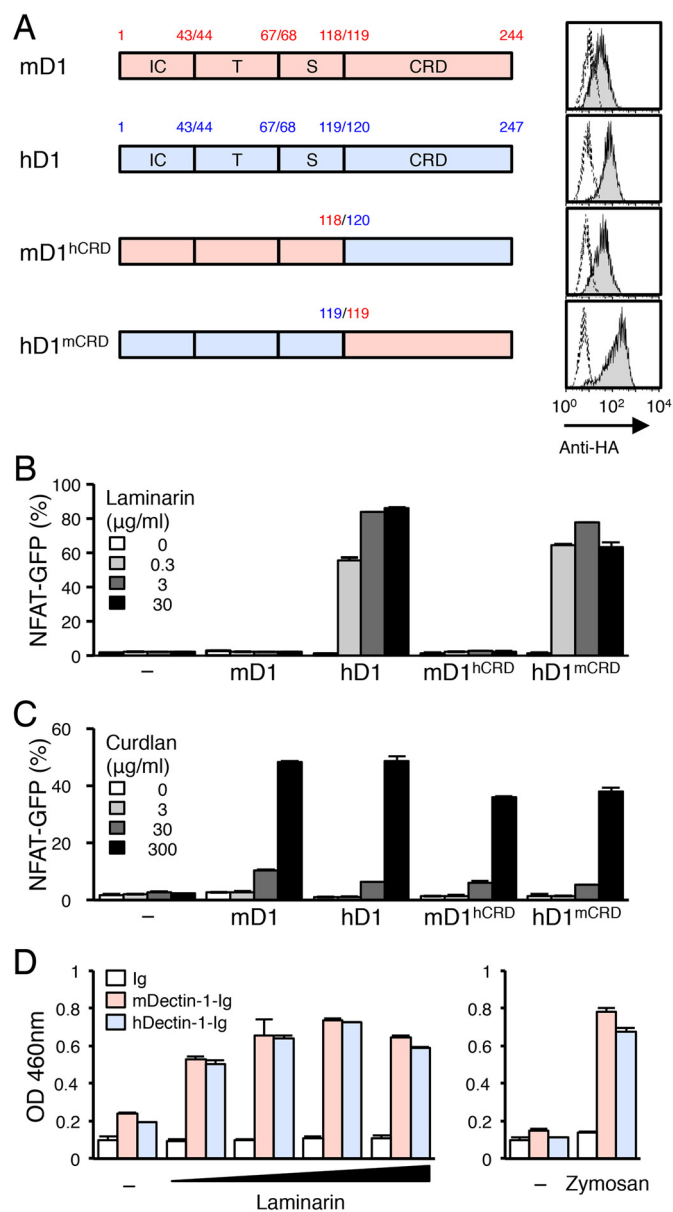
**Figure 3. Westase treatment eliminates the hDectin-1 activity to fucan as well as laminarin.** A and B, 2B4 NFAT-GFP reporter cells were left unstimulated or stimulated with curdlan, fucan (A), or laminarin (B) at the indicated concentrations. Ligands were left untreated (Buffer) or treated with Westase (Westase). GFP expression was analyzed by flow cytometry. All data are presented as the mean  $\pm$  S.D., and representative results from two independent experiments with similar results are shown.

focused on the cytoplasmic domain of hDectin-1. To this end, we generated a series of chimeric mDectin-1 proteins in which each region of the cytoplasmic domain was replaced with the corresponding region of hDectin-1 (Fig. 6A). In contrast to full-length (WT) mDectin-1, mDectin-1 possessing 30 amino acids of the human N-terminal region (mDectin-1<sup>hN30</sup>) normally responded to laminarin (Fig. 6, B and C). Further chimeric analysis revealed that as little as a 10-amino acid sequence at the N terminus of hDectin-1 (mDectin-1<sup>hN10</sup>) was sufficient to confer reactivity to low-valency  $\beta$ -glucan to mDectin-1 (Fig. 6B).

### Glu<sup>2</sup> and Pro<sup>5</sup> of hDectin-1 confer sensitivity to low-valency $\beta$ -glucan to mDectin-1

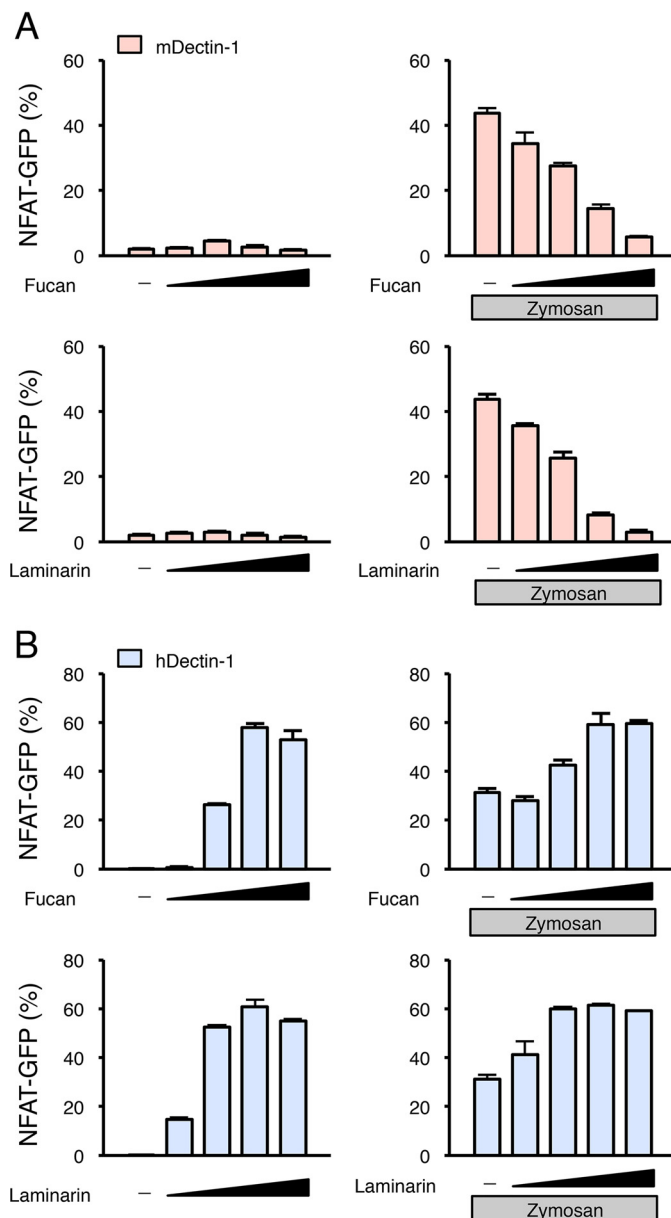
Within the 10-amino acid N-terminal sequence, hDectin-1 differs from mDectin-1 in four residues: Glu<sup>2</sup>, Pro<sup>5</sup>, Asp<sup>6</sup>, and Leu<sup>7</sup> (Fig. 7A). We therefore individually substituted these 4 amino acids of mDectin-1 with the corresponding human-specific residues (Fig. 7B). Mutants carrying the combination of K2E and S5P substitutions selectively responded to laminarin (Fig. 7C, mD1<sup>K2E/S5P/H6D/I7L</sup>, mD1<sup>K2E/S5P/I7L</sup>, mD1<sup>K2E/S5P/H6D</sup>, and mD1<sup>K2E/S5P</sup>). Conversely, the introduction of mouse-specific Lys<sup>2</sup> and Ser<sup>5</sup> substitutions into hDectin-1 (hDectin-1<sup>E2K/P5S</sup>) resulted in the reduction of its reporter activity against laminarin (supplemental Fig. S4). Taken together, these results suggest that two amino acids derived from mDectin-1, Lys<sup>2</sup> and Ser<sup>5</sup>, are critical for “desensitizing” Dectin-1 to low-valency  $\beta$ -glucan. The importance of these 2 amino acids are also supported by Dectin-1 derived from another species, rat. Although

# Intracellular non-ITAM sequence sensitizes human Dectin-1



**Figure 4. Non-CRD region of hDectin-1 confers mDectin-1 with ligand activity for laminarin.** *A*, schematic structure of mDectin-1 (mD1), hDectin-1 (hD1), and mouse/human chimeric proteins (mD1<sup>hCRD</sup> and hD1<sup>mCRD</sup>). Red and blue boxes represent the domain of mDectin-1 and hDectin-1, respectively, and the numbers indicate amino acid residues. Cells expressing the indicated constructs were left unstained (open area) or stained with anti-HA-PE antibody (gray area) and analyzed with flow cytometry. IC, intracellular domain; T, stalk domain; S, transmembrane domain. *B* and *C*, 2B4 reporter cells expressing Syk alone (–) or together with WT or chimeric Dectin-1 proteins (hD1 or mD1) were stimulated with laminarin (*B*) or curdlan (*C*) for 18 h. Induction of GFP was analyzed by flow cytometry. Data are presented as the mean  $\pm$  S.D. White, non-stimulated; light gray, 0.3  $\mu$ g/ml of laminarin or 3  $\mu$ g/ml of curdlan; dark gray, 3  $\mu$ g/ml of laminarin or 30  $\mu$ g/ml of curdlan; black, 30  $\mu$ g/ml of laminarin or 300  $\mu$ g/ml of curdlan. *D*, binding of mDectin-1-Ig and hDectin-1-Ig to laminarin. 50  $\mu$ l of laminarin (0.03, 0.1, 0.3, or 1 mg/ml) or buffer only (–) was coated (left). 3  $\mu$ g/well of zymosan in isopropyl alcohol or isopropyl alcohol only (–) was coated (right). hlgG1-Fc (Ig) was used as a control. Data are presented as the mean  $\pm$  S.D. All data are representative results from two independent experiments with similar results.

rat Dectin-1 (rDectin-1) shares high homology with mDectin-1, it uniquely lacks the N-terminal 9-amino acid sequence that contains Lys<sup>2</sup> and Ser<sup>5</sup> in mouse (Fig. 8, *A* and *B*, and supplemental Fig. S7). In the reporter cell assay, rDectin-1 was

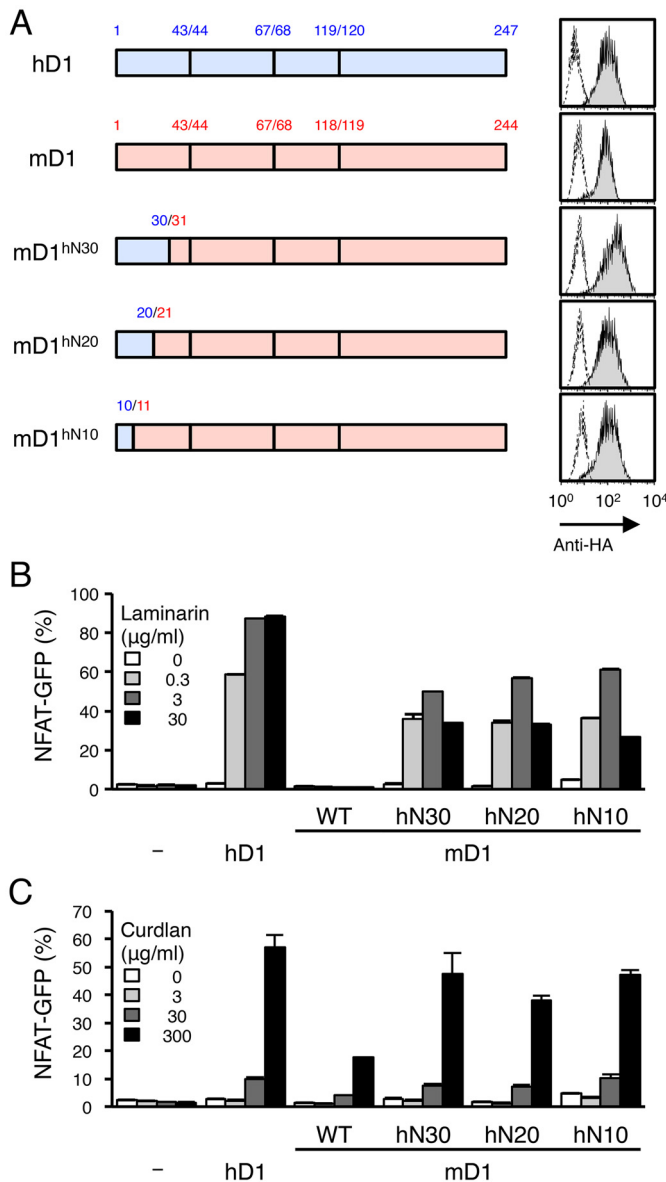


**Figure 5. Agonistic versus antagonistic effect of low-valency  $\beta$ -glucan on hDectin-1 versus mDectin-1, respectively.** *A* and *B*, 2B4 NFAT-GFP reporter cells expressing mDectin-1 (*A*) or hDectin-1 (*B*) were stimulated with laminarin or fucan (0.3, 3, 30, or 300  $\mu$ g/ml) in the presence of a suboptimal concentration of zymosan (3  $\mu$ g/ml) for 18 h. GFP expression was analyzed by flow cytometry. All data are presented as the mean  $\pm$  S.D., and representative results from two independent experiments with similar results are shown.

found to respond well to laminarin (Fig. 8, *C* and *D*), supporting our idea that inactivation of Lys<sup>2</sup> and Ser<sup>5</sup> may sensitize Dectin-1 to low-valency  $\beta$ -glucan.

## Low-valency Dectin-1 ligands activate human DCs but not murine DCs

To confirm the observed hDectin-1-specific phenomenon using primary cells, we finally compared the reactivity of human and murine myeloid cells to low-valency Dectin-1 ligand in a non-biased manner by stimulating human monocyte-derived dendritic cells (hMoDCs) and mouse bone marrow-derived dendritic cells (mBMDCs) with laminarin.



**Figure 6. The cytoplasmic region of hDectin-1 determines its sensitivity to laminarin.** A, schematic structures and surface expression of WT and mutant Dectin-1 proteins. Red and blue boxes represent the regions derived from mDectin-1 and hDectin-1, respectively, and the numbers indicate the amino acid residues. Cells were left unstained (open area) or stained with anti-HA-PE antibody (gray area) and analyzed with flow cytometry. B and C, 2B4 reporter cells expressing Syk alone (–) or together with WT or chimeric Dectin-1 proteins (hD1 or mD1) were stimulated with laminarin (B) or curdlan (C) at the indicated concentrations for 18 h. Induction of GFP was analyzed by flow cytometry. Data are presented as the mean  $\pm$  S.D. All data are representative results from four independent experiments with similar results.

Laminarin potently activated hMoDCs to induce a set of inflammatory genes including *IL1B*, *IL1A*, and *CLEC4E*, which are reportedly induced by CLR-mediated signaling (17, 18). In contrast, the majority of mouse orthologues of genes up-regulated in hMoDCs did not show substantial induction in mBMDs (Fig. 9). Note that mBMDs constitutively expressed Dectin-1 (see GSE98814 and GSE98825), and responded normally to other stimuli, such as LPS (supplemental Fig. S5). Collectively, these data support the idea that hDectin-1, but not mDectin-1, is an activating receptor for low-valency  $\beta$ -glucan.

## Discussion

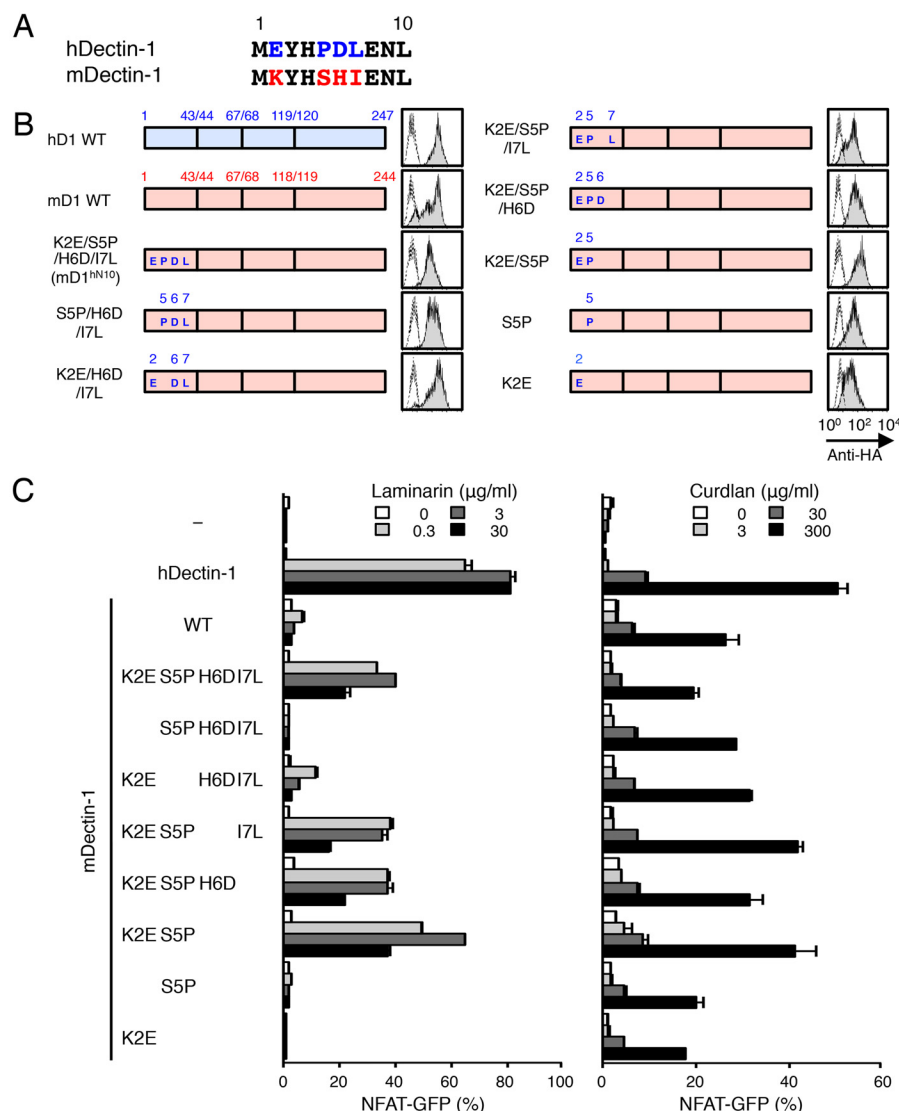
In this study, we report that hDectin-1 responds to low-valency  $\beta$ -glucan and its cytoplasmic region is critical for this function.

Within the cytoplasmic N terminus, we show that Glu<sup>2</sup> of hDectin-1 is one of the critical residues controlling the sensitivity of hDectin-1 to low-valency  $\beta$ -glucan, as a substitution of a mouse-specific residue in this position (hDectin-1<sup>E2K</sup>) impaired this activity. It appears that it is important that the residue at position 2 be a “non-Lys” residue, as substitutions to other residues did not reduce the activity (hDectin-1<sup>E2A</sup> or hDectin-1<sup>E2D</sup>) (supplemental Fig. S6). In line with these observations, Lys residues of mDectin-1 are reported to undergo ubiquitination, which results in the degradation and desensitization of mDectin-1 upon ligand binding (19). Thus, the inactivation of the Lys<sup>2</sup> residue might be one of the reasons that sensitize hDectin-1.

Meanwhile, the precise role of hDectin-1 Pro<sup>5</sup>, which is conserved in primates, is not yet clear. The reciprocal single substitution (mDectin-1<sup>S5P</sup> and hDectin-1<sup>P5S</sup>) had no impact (Fig. 7 and data not shown), whereas mDectin-1 carrying the double mutation (mDectin-1<sup>K2E/S5P</sup>) was active, suggesting that Pro<sup>5</sup> renders hDectin-1 sensitive, albeit it is not sufficient in and of itself. One potential explanation is that mouse Ser<sup>5</sup>, which is conserved in most non-primates mammals (supplemental Fig. S7), may act to reduce the receptor signaling by promoting protein modification as Ser phosphorylation is linked to ubiquitination in several signaling molecules (20–22). Alternatively, Ser<sup>5</sup> may interfere with the function of hemITAM through phosphorylation or other modifications. Indeed, phosphorylation of the Ser residue in the cytoplasmic region of FcR $\gamma$  and Ig $\alpha$  is reported to inhibit tyrosine phosphorylation of their own ITAMs, which then inhibits downstream signaling (23, 24). More detailed analysis is needed to clarify whether Pro<sup>5</sup> (human) cancels the negative function of Ser<sup>5</sup> (mouse), or, alternatively, actively promotes downstream signaling.

From the analysis of the phylogenetic comparisons, one could speculate that “elimination” of Lys<sup>2</sup> and Ser<sup>5</sup> by substitution (human) or truncation (rat) (Fig. 8A and supplemental Fig. S7) might be a common strategy to increase the sensitivity of Dectin-1 to low-valency  $\beta$ -glucan during evolution. In support of this idea, the 5′-UTR of the mRNA for rDectin-1 contains a sequence corresponding to the Ser<sup>5</sup> found in mice (Fig. 8A), suggesting that Ser<sup>5</sup> is preserved as a relic in rDectin-1, which has been inactivated by the introduction of a downstream start codon, as speculated in other immune receptors (25).

Despite the different sensitivities to low-valency  $\beta$ -glucan, the various Dectin-1 mutants used in this study retained substantial reactivities to curdlan. This suggests that Dectin-1, like other ITAM-coupled receptors such as the TCR and BCR, has the capacity to sense the quality of ligand. Recently, another CLR, mMincle and hMincle, recognize different ligands and induce distinct responses (26, 27). It is tempting to speculate that the CLR family members might have modulated their sensitivities and ligand spectrums during evolution to adapt to their environment, an idea that warrants further investigation.



**Figure 7. Glu<sup>2</sup> and Pro<sup>5</sup> of hDectin-1 confer sensitivity to laminarin to mDectin-1.** *A*, sequence alignment of 10 amino acids at the N terminus of hDectin-1 and mDectin-1. Blue and red letters indicate amino acids differences between hDectin-1 and mDectin-1, respectively. *B*, schematic structure and surface expression of WT and mutant Dectin-1 proteins with the indicated amino acid substitutions. Numbers indicate amino acid residues. The “human-type” amino acids introduced are shown in blue. Cells were left unstained (open area) or stained with anti-HA-PE antibody (gray area) and analyzed with flow cytometry. *C*, each reporter cell line expressing Syk alone (–) or together with WT or mutant Dectin-1 proteins was stimulated with laminarin or curdlan at the indicated concentrations for 18 h. Induction of GFP was analyzed by flow cytometry. Data are presented as the mean ± S.D. All data are representative results from two independent experiments with similar results.

Thus, the role of Dectin-1 in fungal infection characterized by use of gene-deficient mice (10, 11) may not fully reflect the function of human orthologue. These findings demonstrate that the therapeutic approaches targeted to Dectin-1 requires re-evaluation of its functions *in vivo* by generating models expressing hDectin-1 in future studies.

## Experimental procedures

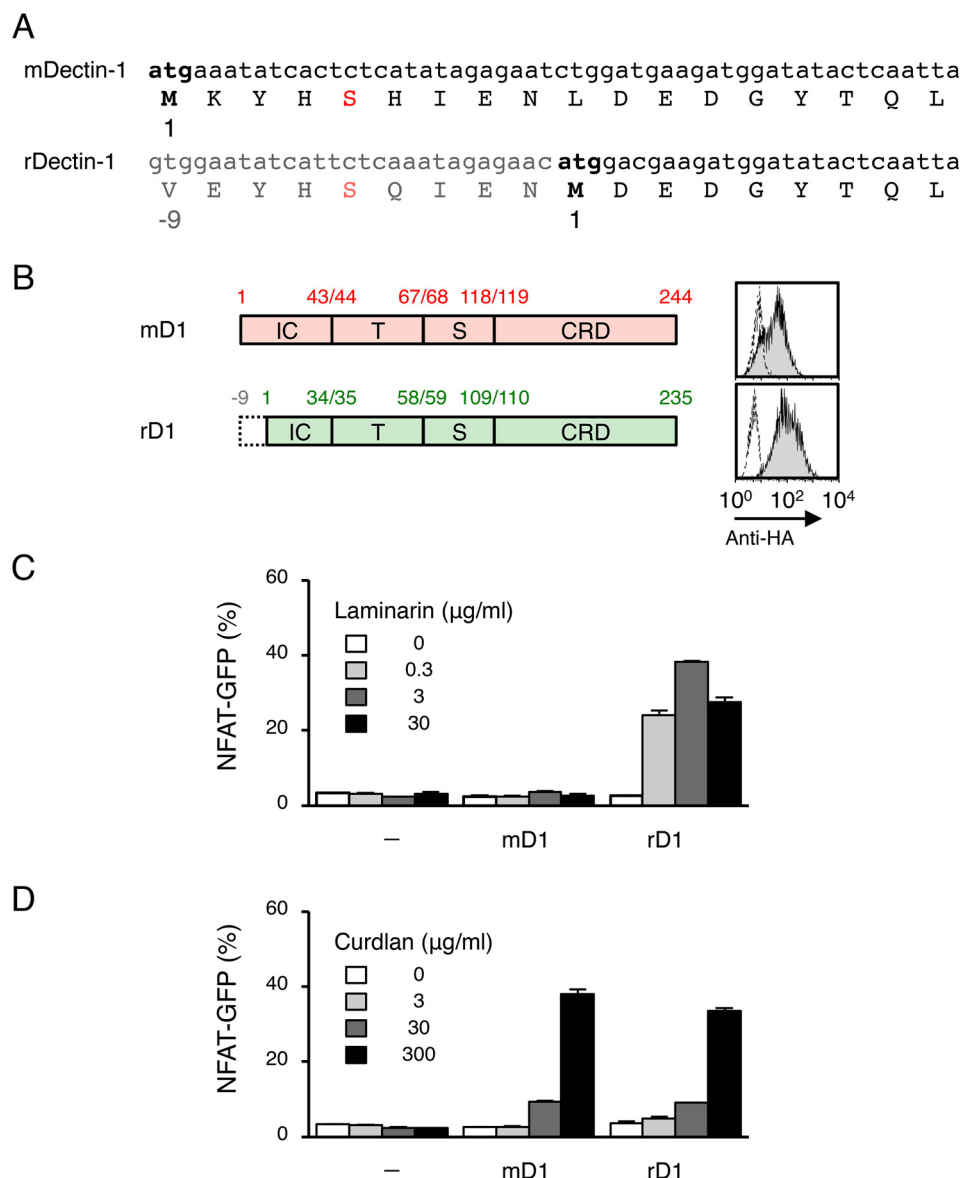
### Reagents and antibodies

Laminarin and curdlan were purchased from InvivoGen. Zymosan (Z4250) and LPS (L4516) were purchased from Sigma. Westase (9095) was purchased from TaKaRa. Fucan from *Cladosiphon novae-caledoniae* Kylin was kindly provided by Daiichi Sangyo and the supernatant was collected after centrifugation at 20,000 × *g* and used as a stimulant. Phycoerythrin

(PE)-conjugated anti-HA Ab (clone 16B12) was purchased from Abcam.

### Cells

2B4-NFAT-GFP reporter cells expressing various CLRs were prepared as previously described (26, 28). For Fcγ- or DAP12-coupled receptors, reporter cells were co-transfected with Fcγ or DAP12, respectively. hemITAM-harbored receptors were co-expressed with Syk. Receptors that are not coupled to ITAM signaling were expressed as chimeric receptors by fusing to CD3ζ. mBMDs were prepared as previously described (29). Briefly, BM cells from a WT C57BL/6J mouse were suspended in RPMI1640 medium supplemented with 10% FBS, antibiotics, and β-mercaptoethanol at a density of 5 × 10<sup>5</sup> cells/ml in the presence of culture supernatant of MGM-5 (provided by Dr. S.



**Figure 8. Rat Dectin-1 activates reporter cells in response to laminarin.** *A*, sequence alignment of the N-terminal amino acids and cDNAs of mDectin-1 and rDectin-1. Ser<sup>5</sup> of mDectin-1 and the untranslated serine in the 5'-UTR of rDectin-1 are shown in red. Methionines coded by start codons are shown in bold. The 5'-UTR of rDectin-1 cDNA and amino acids corresponding to the nucleotide sequence are shown in gray. Numbers indicate the amino acid residues. *B*, schematic structure and surface expression of mDectin-1 and rat Dectin-1. The box surrounded by dotted lines represents the region missing from the rDectin-1 protein and the numbers indicate amino acid residues. Cells were left unstained (open area) or stained with anti-HA-PE antibody (gray area) and analyzed with flow cytometry. *C* and *D*, each reporter cell line expressing mDectin-1 or rDectin-1 was stimulated with laminarin (*C*) or curdlan (*D*) at the indicated concentrations for 18 h. Induction of GFP was analyzed by flow cytometry. Data are presented as the mean  $\pm$  S.D. All data are representative results from two independent experiments with similar results.

Nagata) as a source of GM-CSF containing conditioned medium, and cultured for 7 days at 37 °C. hMoDCs were also generated as previously described (30). Briefly, peripheral blood mononuclear cells (PBMCs) from a healthy donor were isolated by Lymphocyte Separation Solution ( $d = 1.077$ ) (Nacalai Tesque) for gradient centrifugation. Human CD14<sup>+</sup> monocytes were purified from PBMCs using anti-human CD14 MicroBeads (Miltenyi Biotech), and cultured in RPMI1640 supplemented with 10% FBS, non-essential amino acid, antibiotics, 10 ng/ml of human GM-CSF (PeproTech), and 10 ng/ml of human IL-4 (PeproTech) for 7 days at 37 °C. The collection and use of human PBMCs were approved by the institutional

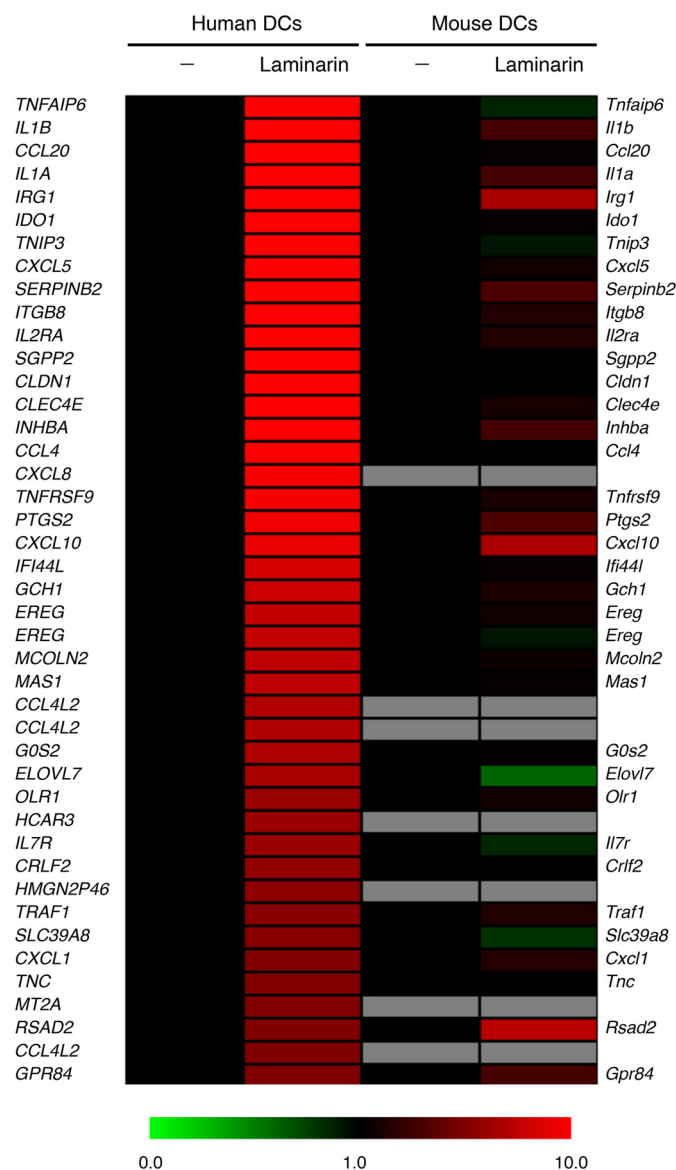
review boards of Research Institute for Microbial Diseases, Osaka University (29-4).

#### In vitro stimulation

The reporter cells were stimulated with curdlan, zymosan, fucan, or laminarin for 18 h at 37 °C. The GFP expression of reporter cells was evaluated by FACS Calibur flow cytometer (BD Biosciences).

#### Westase treatment

Curdlan and fucan were diluted to 1 mg/ml with buffer (McIlvain's disodium phosphate/citric acid buffer, pH 6.0)



**Figure 9. Gene expression profiles of hMoDCs and mBMDCs stimulated with laminarin.** A heat map of the top 40 genes up-regulated in hMoDCs stimulated with laminarin compared with unstimulated hMoDCs (–) is displayed on the left. Genes are listed in the order of sample/reference ratio. A heat map of the mouse orthologues of the human genes is displayed on the right. mBMDCs were left untreated or stimulated with laminarin. Columns of genes whose mouse orthologues are not conserved are displayed as gray.

containing 2 units/ml of Westase,  $\beta$ -1,3- and  $\beta$ -1,6-glucanase, or buffer alone and were incubated for 5 min at 100 °C to inactivate Westase following incubation for 12 h at 37 °C.

### Construction of chimeric Dectin-1 receptors

For mouse/human Dectin-1 chimeras, constructs were generated by overlapping extension PCR. Primers used were listed on supplemental Table S1. The resulting constructs were cloned into pMX-IRES-hCD8 or pMX-puro retroviral vector containing HA tag at the C terminus and delivered into 2B4 NFAT-GFP cells expressing Syk as previously described (28, 29).

### Microarray analysis

$1 \times 10^6$  hMoDCs from a healthy volunteer or  $1 \times 10^6$  mBMDCs from a WT mouse were left untreated or stimulated with 500  $\mu$ g/ml of laminarin for 8 h at 37 °C. Total RNA was isolated by TRIzol (Thermo Scientific). DNA microarray analysis was performed using Human Gene 1.0 ST array (Affimetrix) or Mouse Gene 1.0 ST array (Affimetrix). A Z-score was calculated for each gene between each sample. Genes with both a Z-score exceeding (or equal to) 2 and a sample/reference ratio exceeding (or equal to) 1.5 were defined as up-regulated. The array data were deposited in the Gene Expression Omnibus (accession number GSE98826).

### Dectin-1-Ig fusion protein

Extracellular domains of hDectin-1 (amino acids 68–247) or mDectin-1 (amino acids 98–244) were fused to the N terminus of the human IgG1 Fc region (hIgG1) as described previously (31). Zymosan diluted in isopropyl alcohol was added to 96-well plates followed by evaporation. Laminarin dissolved in  $\text{NaHCO}_3$  buffer was coated on the plates by incubation at 37 °C for 8 h. 100  $\mu$ g/ml of hIgG1-Fc, hDectin-1-Ig, or mDectin-1-Ig diluted in buffer consisting of 20 mM Tris-HCl, 150 mM NaCl, 1 mM  $\text{CaCl}_2$ , and 2 mM  $\text{MgCl}_2$  (pH 7.0) was incubated with plate-coated ligands. Anti-hIgG-HRP was used for the detection of bound protein.

### Real-time PCR

$1 \times 10^6$  hMoDCs from a healthy volunteer or  $1 \times 10^6$  mBMDCs from a WT mouse were stimulated or left untreated for 8 h at 37 °C. Total RNA was prepared using Sepasol RNA I Super G (Nacalai Tesque) and used to generate cDNA templates with ReverTra Ace (TOYOBO). Quantitative PCR was performed by using THUNDERBIRD SYBR qPCR mix (TOYOBO) and ABI PRISM 7000 (Applied Biosystems). Human and mouse  $\beta$ -actin mRNA were used for normalization. All primers for specific target genes are listed in supplemental Table S1.

**Author contributions**—T. T., C. M., and S. Y. designed the research. T. T. and T. I. performed the experiments. K. S. and Y. N. provided materials. T. T., C. M., and S. Y. wrote the manuscript.

**Acknowledgments**—We thank K. Toyonaga for screening, G. Brown for discussions, M. Kurata for technical assistance, and M. Tanaka for animal support.

### References

1. Takeuchi, O., and Akira, S. (2010) Pattern recognition receptors and inflammation. *Cell* **140**, 805–820
2. Brubaker, S. W., Bonham, K. S., Zanoni, I., and Kagan, J. C. (2015) Innate immune pattern recognition: a cell biological perspective. *Annu. Rev. Immunol.* **33**, 257–290
3. Iborra, S., and Sancho, D. (2015) Signalling versatility following self and non-self sensing by myeloid C-type lectin receptors. *Immunobiology* **220**, 175–184
4. Brown, G. D., and Gordon, S. (2001) Immune recognition: a new receptor for  $\beta$ -glucans. *Nature* **413**, 36–37
5. Ariizumi, K., Shen, G. L., Shikano, S., Xu, S., Ritter, R., 3rd, Kumamoto, T., Edelbaum, D., Morita, A., Bergstresser, P. R., and Takashima, A. (2000)

- Identification of a novel, dendritic cell-associated molecule, Dectin-1, by subtractive cDNA cloning. *J. Biol. Chem.* **275**, 20157–20167
6. Rogers, N. C., Slack, E. C., Edwards, A. D., Nolte, M. A., Schulz, O., Schweighoffer, E., Williams, D. L., Gordon, S., Tybulewicz, V. L., Brown, G. D., and Reis e Sousa, C. (2005) Syk-dependent cytokine induction by Dectin-1 reveals a novel pattern recognition pathway for C type lectins. *Immunity* **22**, 507–517
7. LeibundGut-Landmann, S., Gross, O., Robinson, M. J., Osorio, F., Slack, E. C., Tsoni, S. V., Schweighoffer, E., Tybulewicz, V., Brown, G. D., Ruland, J., and Reis e Sousa, C. (2007) Syk- and CARD9-dependent coupling of innate immunity to the induction of T helper cells that produce interleukin 17. *Nat. Immunol.* **8**, 630–638
8. Leibundgut-Landmann, S., Osorio, F., Brown, G. D., and Reis e Sousa, C. (2008) Stimulation of dendritic cells via the dectin-1/Syk pathway allows priming of cytotoxic T-cell responses. *Blood* **112**, 4971–4980
9. Yokota, K., Takashima, A., Bergstresser, P. R., and Ariizumi, K. (2001) Identification of a human homologue of the dendritic cell-associated C-type lectin-1, dectin-1. *Gene* **272**, 51–60
10. Taylor, P. R., Tsoni, S. V., Willment, J. A., Dennehy, K. M., Rosas, M., Findon, H., Haynes, K., Steele, C., Botto, M., Gordon, S., and Brown, G. D. (2007) Dectin-1 is required for beta-glucan recognition and control of fungal infection. *Nat. Immunol.* **8**, 31–38
11. Saijo, S., Fujikado, N., Furuta, T., Chung, S. H., Kotaki, H., Seki, K., Sudo, K., Akira, S., Adachi, Y., Ohno, N., Kinjo, T., Nakamura, K., Kawakami, K., and Iwakura, Y. (2007) Dectin-1 is required for host defense against *Pneumocystis carinii* but not against *Candida albicans*. *Nat. Immunol.* **8**, 39–46
12. Gantner, B. N., Simmons, R. M., and Underhill, D. M. (2005) Dectin-1 mediates macrophage recognition of *Candida albicans* yeast but not filaments. *EMBO J.* **24**, 1277–1286
13. Underhill, D. M., Rossmagle, E., Lowell, C. A., and Simmons, R. M. (2005) Dectin-1 activates Syk tyrosine kinase in a dynamic subset of macrophages for reactive oxygen production. *Blood* **106**, 2543–2550
14. Willment, J. A., Gordon, S., and Brown, G. D. (2001) Characterization of the human  $\beta$ -glucan receptor and its alternatively spliced isoforms. *J. Biol. Chem.* **276**, 43818–43823
15. Sahasrabudhe, N. M., Schols, H. A., Faas, M. M., and de Vos, P. (2016) Arabinoxylan activates Dectin-1 and modulates particulate  $\beta$ -glucan-induced Dectin-1 activation. *Mol. Nutr. Food Res.* **60**, 458–467
16. Adachi, Y., Ishii, T., Ikeda, Y., Hoshino, A., Tamura, H., Aketagawa, J., Tanaka, S., and Ohno, N. (2004) Characterization of  $\beta$ -glucan recognition site on C-type lectin, dectin 1. *Infect. Immun.* **72**, 4159–4171
17. Steele, C., Rapaka, R. R., Metz, A., Pop, S. M., Williams, D. L., Gordon, S., Kolls, J. K., and Brown, G. D. (2005) The  $\beta$ -glucan receptor dectin-1 recognizes specific morphologies of *Aspergillus fumigatus*. *PLoS Pathog.* **1**, e42
18. Werninghaus, K., Babiak, A., Gross, O., Hölscher, C., Dietrich, H., Agger, E. M., Mages, J., Mocsai, A., Schoenen, H., Finger, K., Nimmerjahn, F., Brown, G. D., Kirschning, C., Heit, A., Andersen, P., Wagner, H., Ruland, J., and Lang, R. (2009) Adjuvant activity of a synthetic cord factor analogue for subunit *Mycobacterium tuberculosis* vaccination requires FcR $\gamma$ -Syk-Card9-dependent innate immune activation. *J. Exp. Med.* **206**, 89–97
19. Xiao, Y., Tang, J., Guo, H., Zhao, Y., Tang, R., Ouyang, S., Zeng, Q., Rapleye, C. A., Rajaram, M. V., Schlesinger, L. S., Tao, L., Brown, G. D., Langdon, W. Y., Li, B. T., and Zhang, J. (2016) Targeting CBLB as a potential therapeutic approach for disseminated candidiasis. *Nat. Med.* **22**, 906–914
20. Brown, K., Gerstberger, S., Carlson, L., Franzoso, G., and Siebenlist, U. (1995) Control of I $\kappa$ B $\alpha$  proteolysis by site-specific, signal-induced phosphorylation. *Science* **267**, 1485–1488
21. Iwasaki, H., Takeuchi, O., Teraguchi, S., Matsushita, K., Uehata, T., Kuniyoshi, K., Satoh, T., Saitoh, T., Matsushita, M., Standley, D. M., and Akira, S. (2011) The I $\kappa$ B kinase complex regulates the stability of cytokine-encoding mRNA induced by TLR-IL-1R by controlling degradation of regnase-1. *Nat. Immunol.* **12**, 1167–1175
22. Winston, J. T., Strack, P., Beer-Romero, P., Chu, C. Y., Elledge, S. J., and Harper, J. W. (1999) The SCF $\beta$ -TRCP-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in I $\kappa$ B $\alpha$  and  $\beta$ -catenin and stimulates I $\kappa$ B $\alpha$  ubiquitination *in vitro*. *Genes Dev.* **13**, 270–283
23. Shah, S., Gibson, A. W., Ji, C., Darrington, E., Mobley, J., Kojima, K., Edberg, J. C., and Kimberly, R. P. (2017) Regulation of FcR $\gamma$  function by site-specific serine phosphorylation. *J. Leukoc. Biol.* **101**, 421–428
24. Müller, R., Wienands, J., and Reth, M. (2000) The serine and threonine residues in the Ig- $\alpha$  cytoplasmic tail negatively regulate immunoreceptor tyrosine-based activation motif-mediated signal transduction. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 8451–8454
25. Arase, H., and Lanier, L. L. (2004) Specific recognition of virus-infected cells by paired NK receptors. *Rev. Med. Virol.* **14**, 83–93
26. Kiyotake, R., Oh-Hora, M., Ishikawa, E., Miyamoto, T., Ishibashi, T., and Yamasaki, S. (2015) Human mincle binds to cholesterol crystals and triggers innate immune responses. *J. Biol. Chem.* **290**, 25322–25332
27. Hattori, Y., Morita, D., Fujiwara, N., Mori, D., Nakamura, T., Harashima, H., Yamasaki, S., and Sugita, M. (2014) Glycerol monomycolate is a novel ligand for the human, but not mouse macrophage inducible C-type lectin, Mincle. *J. Biol. Chem.* **289**, 15405–15412
28. Ishikawa, E., Ishikawa, T., Morita, Y. S., Toyonaga, K., Yamada, H., Takeuchi, O., Kinoshita, T., Akira, S., Yoshikai, Y., and Yamasaki, S. (2009) Direct recognition of the mycobacterial glycolipid, trehalose dimycolate, by C-type lectin Mincle. *J. Exp. Med.* **206**, 2879–2888
29. Miyake, Y., Toyonaga, K., Mori, D., Kakuta, S., Hoshino, Y., Oyama, A., Yamada, H., Ono, K., Suyama, M., Iwakura, Y., Yoshikai, Y., and Yamasaki, S. (2013) C-type lectin MCL is an FcR $\gamma$ -coupled receptor that mediates the adjuvant activity of mycobacterial cord factor. *Immunity* **38**, 1050–1062
30. Yonekawa, A., Saijo, S., Hoshino, Y., Miyake, Y., Ishikawa, E., Suzukawa, M., Inoue, H., Tanaka, M., Yoneyama, M., Oh-Hora, M., Akashi, K., and Yamasaki, S. (2014) Dectin-2 is a direct receptor for mannose-capped lipoarabinomannan of mycobacteria. *Immunity* **41**, 402–413
31. Mori, D., Shibata, K., and Yamasaki, S. (2017) C-type lectin receptor Dectin-2 binds to an endogenous protein  $\beta$ -glucuronidase on dendritic cells. *PLoS ONE* **12**, e0169562

## Supplemental material for:

Dectin-1 intracellular domain determines species-specific ligand spectrum  
by modulating receptor sensitivity

**Tomotsugu Takano, Chihiro Motozono, Takashi Imai, Koh-Hei Sonoda,  
Yoichi Nakanishi and Sho Yamasaki**

### Supplemental Figures

Fig. S1. Fucan activates cells expressing hDectin-1B but not mDectin-1B

Fig. S2. Low molecular weight components in fucan activate hDectin-1-expressing cells

Fig. S3. Fluorescence-labeled laminarin binds to both hDectin-1 and mDectin-1

Fig. S4. Substitution of Lys2 and Ser5 into hDectin-1 reduces the ligand activity of laminarin

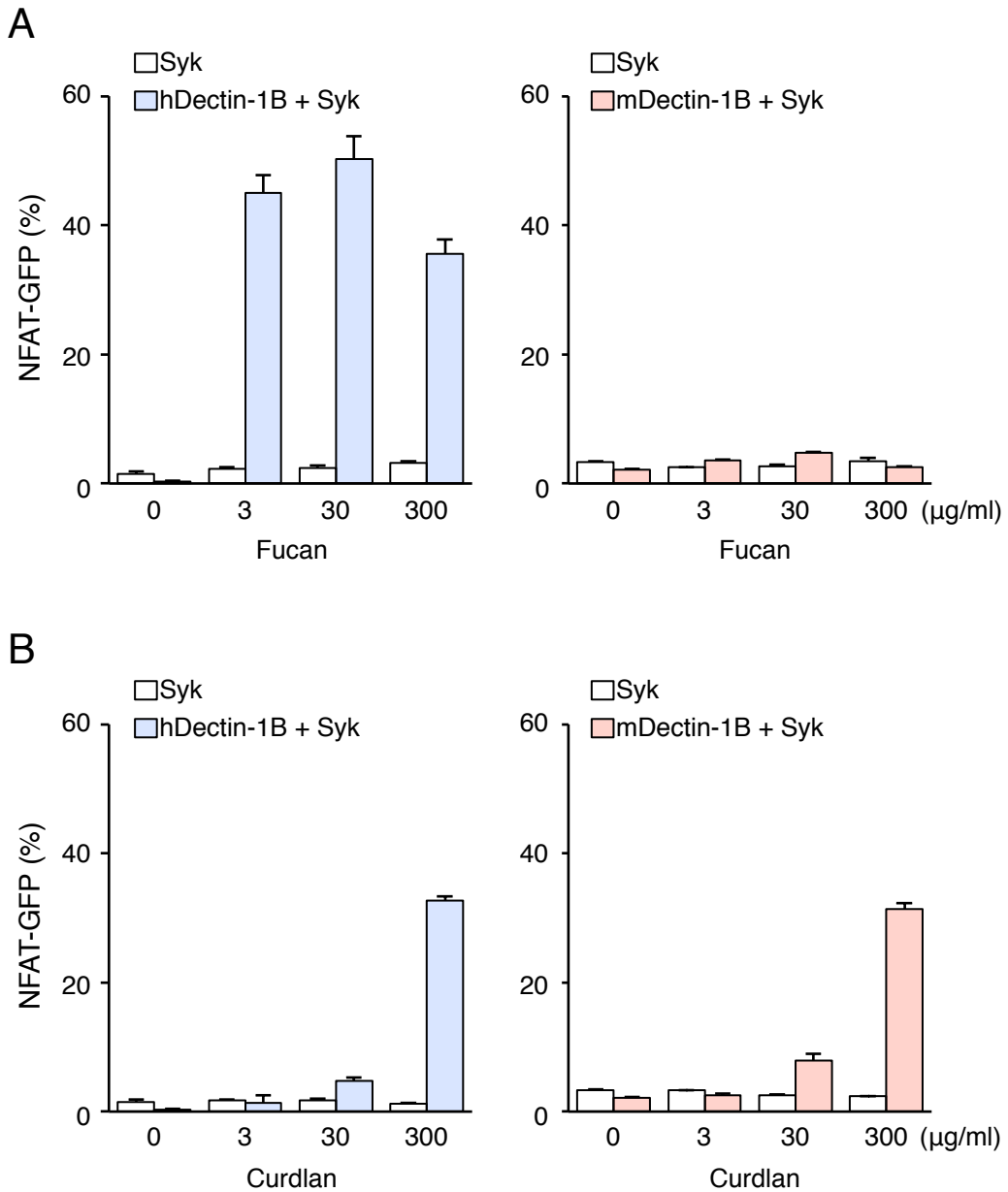
Fig. S5. mBMDCs respond to LPS to induce inflammatory genes

Fig. S6. E2A and E2D do not impair hDectin-1 reactivity to laminarin

Fig. S7. Sequence alignment of Dectin-1 intracellular domain in mammals

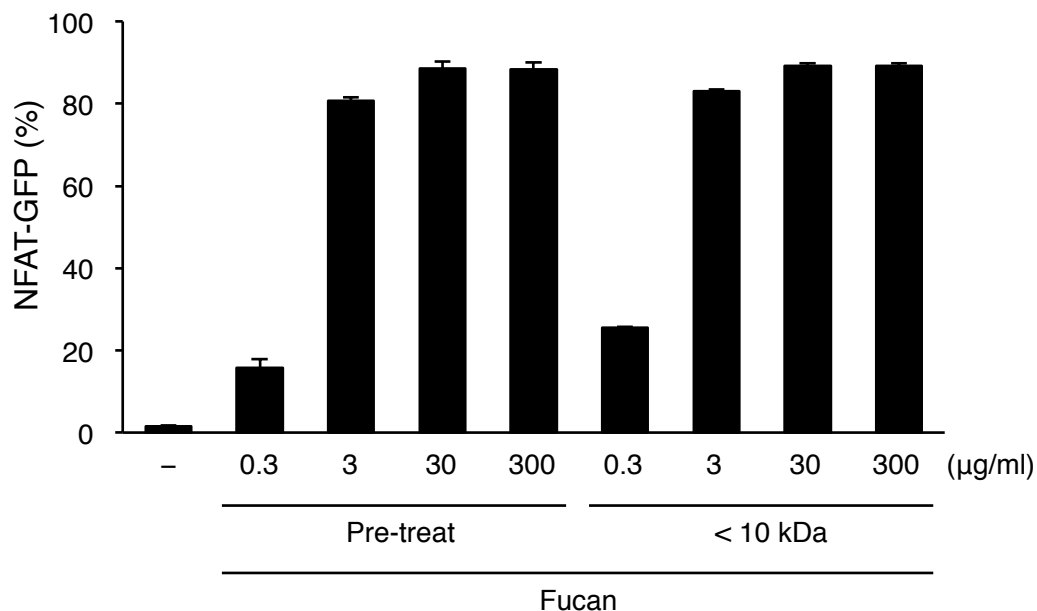
### Supplemental table

Table S1. Primers used in this study



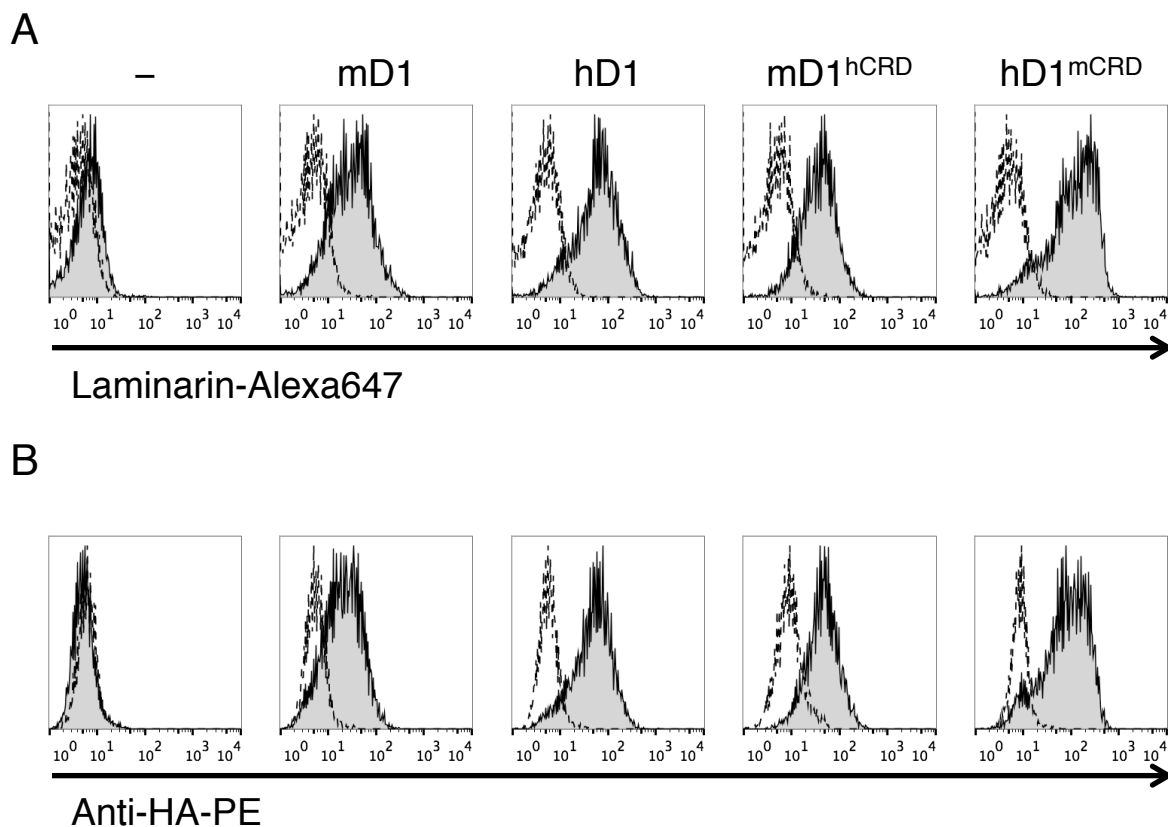
**supplemental Fig. S1. Fucan activates cells expressing hDectin-1B but not mDectin-1B.**

(A and B) 2B4 NFAT-GFP reporter cells transduced without (Syk) or with hDectin-1B or mDectin-1B were stimulated with the indicated concentrations of fucan (A) or curdlan (B) for 18 hr. The expression of GFP was analyzed by flow cytometry. All data are presented as the means  $\pm$  S.D. for duplicate assays, and representative results from three independent experiments with similar results are shown.



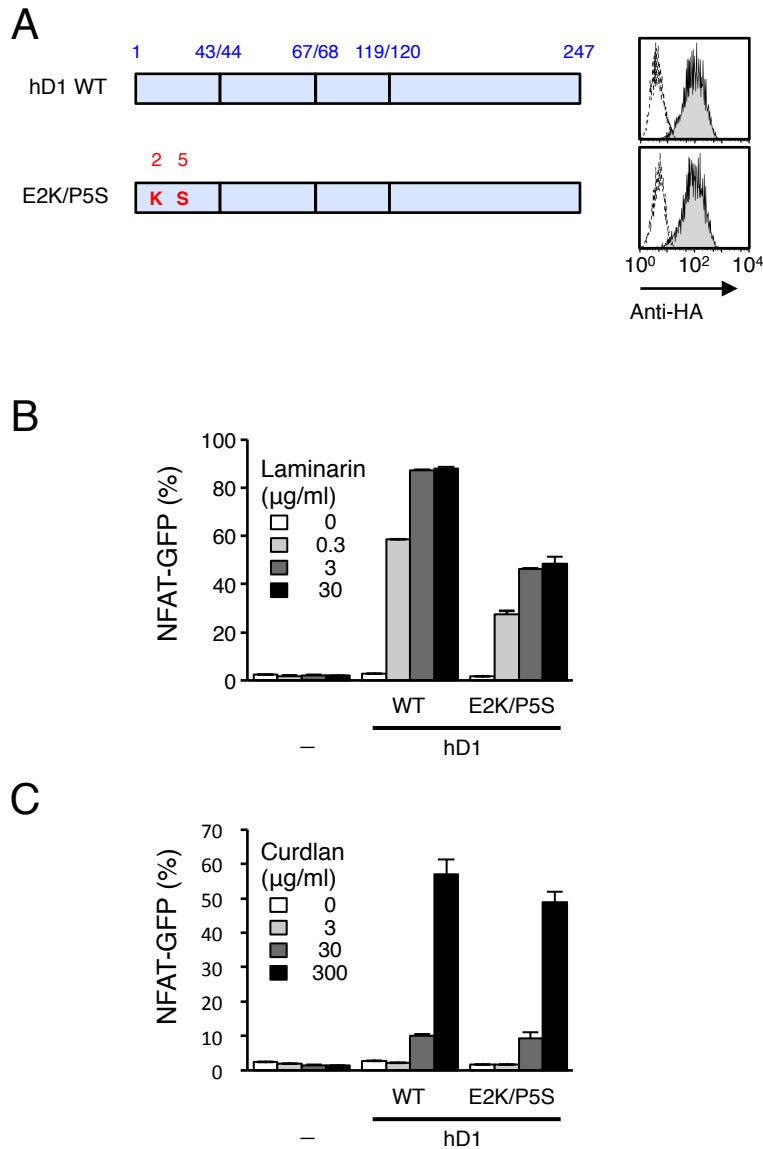
**supplemental Fig. S2. Low molecular weight components in fucan activate hDectin-1-expressing cells.**

Fucan was centrifuged through a ultrafiltration spin column (molecular weight cut-off 10 kDa) at  $4,000 \times g$  for 20 min and wash three times with 10 ml of water. The flow-through fraction with molecular weight below 10 kDa was collected and weighed following lyophilization. 2B4 NFAT-GFP reporter cells expressing hDectin-1 were stimulated with the indicated concentrations of fucan (Pre-treat) or the flow-through fraction of fucan (< 10 kDa) for 18 hr. The expression of GFP was analyzed by flow cytometry. Data are presented as the means  $\pm$  S.D., and representative results from two independent experiments with similar results are shown.



**supplemental Fig. S3. Fluorescence-labeled laminarin binds to both hDectin-1 and mDectin-1.**

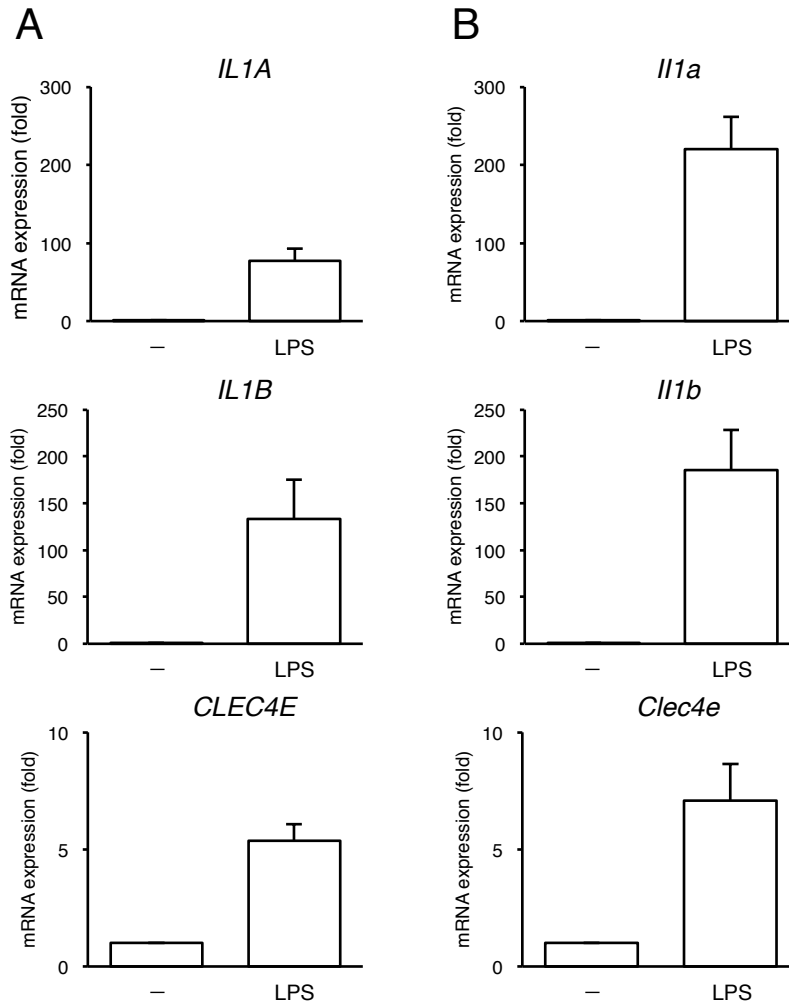
(A and B) Laminarin was oxidized with 0.1 M sodium acetate (pH 5.2) and 5 mM KIO<sub>4</sub> at 4°C for 24 hr. The oxidized laminarin was dried up following dialysis with a dialysis tube (molecular weight cut-off 3.5 kDa) and were dissolved with 0.1 M sodium acetate (pH 5.2) and reacted with 300 µg/ml of Alexa Fluor™ 647 Hydrazide for 20°C for 24 hr. After dialysis to remove the free dye, 1 × 10<sup>5</sup> 2B4 cells expressing Syk alone (–), or together with mDectin-1 (mD1), hDectin1 (hD1), or mouse/human chimeric proteins (mD1<sup>hCRD</sup> and hD1<sup>mCRD</sup>) were stained with 1.5 µg/ml of fluorescence-labeled laminarin (A) or anti-HA-PE antibody (B) and analyzed with flow cytometry. Open histograms represent unstained controls. Data are representative results from two independent experiments with similar results.



**supplemental Fig. S4. Substitution of Lys2 and Ser5 into hDectin-1 reduces the ligand activity of laminarin.**

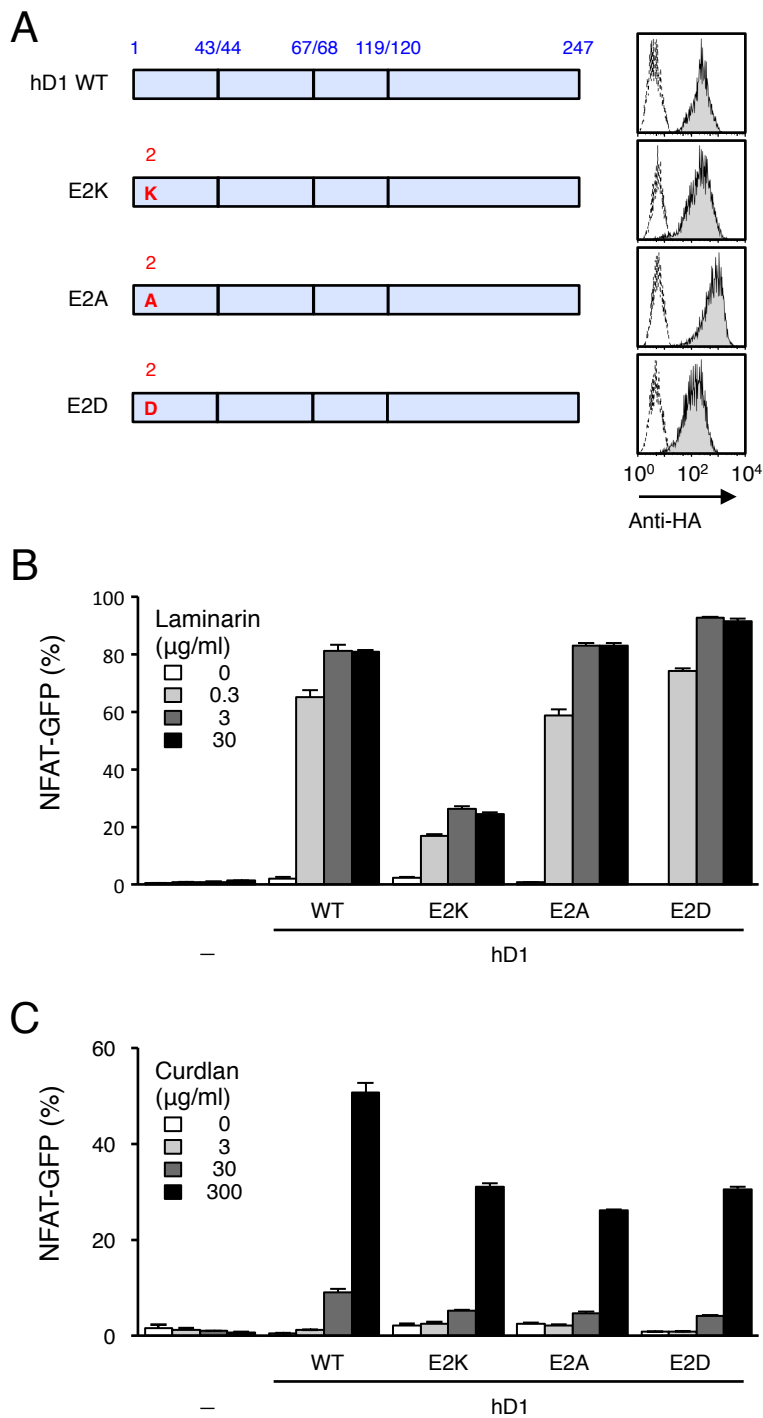
(A) Schematic structure and surface expression of WT hDectin-1 and hDectin-1 protein with the indicated amino acid substitutions. Substituted “mouse-type” amino acids are shown in red. Cells were left unstained (open area) or stained with anti-HA-PE antibody (gray area) and analyzed with flow cytometry. (B and C) Each reporter cell line expressing WT or mutant hDectin-1 protein was stimulated with laminarin (B) or curdlan (C) at the indicated concentrations for 18 hr. Induction of GFP was analyzed by flow cytometry. Data are presented as the means  $\pm$  S.D..

All data are representative results from two independent experiments with similar results.



**supplemental Fig. S5. mBMDCs respond to LPS to induce inflammatory genes.**

(A and B) hMoDCs (A) or mBMDCs (B) were stimulated with 100 ng/ml of LPS or left untreated (-). Gene expression of IL1A (Il1a), IL1B (Il1b) and CLEC4E (Clec4e) was analyzed by real-time PCR. Data are presented as the means  $\pm$  S.D. for triplicate assays.



**supplemental Fig. S6. E2A and E2D do not impair hDectin-1 reactivity to laminarin.**

(A) Schematic structure and surface expression of WT hDectin-1 and hDectin-1 proteins with the indicated substitutions. Substituted “mouse-type” amino acids are shown in red. Cells were left unstained (open area) or stained with anti-HA-PE antibody (gray area) and analyzed with flow cytometry.

(B and C) Each reporter cell line expressing WT or mutant hDectin-1 proteins was stimulated with laminarin (B) or curdlan (C) at the indicated concentrations for 18 hr. Induction of GFP was analyzed by flow cytometry. Data are presented as the means ± S.D..

All data are representative results from two independent experiments with similar results.

	1	10	20	30	40
Mus musculus	MKYHSHIENLDEDGYTQLDFSTQDIHKRPRGSEKGSRAPSSPW				
Rattus norvegicus	-----MDEDGYTQLDFGTRNIHKRPVKSEKGSPAPSSRW				
Homo sapiens	MEYHPDLENLDEDGYTQLHFDSQSNTRIAVVSEKGSAAASPPW				
Macaca mulatta	MEYHPDLENLDEDGYTQLHFDSRSNTRIAVVSEKGSVASPPW				
Pan troglodytes	MEYHPDLENLDEDGYTQLHFDSRSNTRIAVVSEKGSAAASPPW				
Sus scrofa	MEYQSPLENVDEDGYTQLDFSSHNLTRRSVVSEKGPCAASPYW				
Ovis aries	MEYQSSVENLDEDGYTQLDFSSRSITRRSVVSEKGLCAASSRW				
Bos taurus	MEYQSSVENLDEDGYTQLDFSSRNITRRSVVSEKGLCAASSHW				
Canis lupus familiaris	MEYHSGVENLDEDGYTQLNFHSGGITGRPVLKVTCAATSPRW				

**supplemental Fig. S7. Sequence alignment of Dectin-1 intracellular domain in mammals.**

Amino acid sequences of the N-terminal regions of *Mus musculus* (NCBI Reference Sequence: NP\_064392.2), *Rattus norvegicus* (NP\_001166857.1), *Homo sapiens* (NP\_922938), *Macaca mulatta* (NP\_001028115.1), *Pan troglodytes* (XP\_528732.2), *Sus scrofa* (NP\_001130338.1), *Ovis aries* (XP\_012030797.1), *Bos taurus* (NP\_001027022.1) and *Canis lupus familiaris* Dectin-1 (XP\_854143.1) are shown. The amino acids at position 5 in the cytoplasmic domain are shaded.

## Primers used for cloning

Protein	Clone primers: 5' Forward sequence3'	Clone primers: 3' reverse sequence5'
mDectin-1	ttgaattcatgaaatcatcactctcatatagagaatc	ttctcgagcagttccttctcacagatactgtatga
hDectin-1	ttgaattcatggaatcatcctcgatttagaaaatt	actgtcgaccattgaaaacttcttctcac
rDectin-1	gatgaattcatggacgaagatggatatactcaattag	gatctcgagcagttccttctcacagatagtgaatga
mD1K2E/S5P/I7L	tttgaattcatggaatcatcctcatttagaaaaatttg	actgtcgaccattgaaaacttcttctcac
mD1K2E/S5P/H6D	tttgaattcatggaatcatcctcatttagaaaaatttg	actgtcgaccattgaaaacttcttctcac
mD1K2E/H6D/I7L	tttgaattcatggaatcatcctcgatttagaaaaatttg	actgtcgaccattgaaaacttcttctcac
mD1K2E/S5P	tttgaattcatgaaatcatcctcgatttagaaaaatttg	ttctcgagcagttccttctcacagatactgtatga
mD1K2E	tttgaattcatggaatcatcactctcatatagagaatctgg	ttctcgagcagttccttctcacagatactgtatga
mD1S5P	tttgaattcatgaaatcatcaccctcatatagagaatctgg	ttctcgagcagttccttctcacagatactgtatga
hD1E2K/P5S	tttgaattcatggaatcatcaccctcatatagagaatctgg	actgtcgaccattgaaaacttcttctcac
hD1E2K	tttgaattcatgaaatcatcctcgatttagaaaaatttg	actgtcgaccattgaaaacttcttctcac
hD1E2A	tttgaattcatggcatatcatcctcgatttagaaaaatttg	actgtcgaccattgaaaacttcttctcac
hD1E2D	tttgaattcatggactatcatcctcgatttagaaaaatttg	actgtcgaccattgaaaacttcttctcac

## Primers used for overlapping PCR

Protein	Overlap primers: 5' Forward sequence3'	Overlap primers: 3' reverse sequence5'
mD1 <sup>hCRD</sup>	ttctcagccttgcctcctaattggattatatatg	taggaggaagagctgagaaaaacctcctgtagtt
hD1 <sup>mCRD</sup>	ctttccagcccttgccctcctaattggatcatgc	taggaaggcaaggctggaaagaacctctg
mD1 <sup>hN30</sup>	tgctgcattctcaccttgagggccca	tccaaggtgaagatgcagcacacgatcc
mD1 <sup>hN20</sup>	atagctgttgatcagagaaaggaagcc	tctgatccaacagctatcctggattgc
mD1 <sup>hN10</sup>	caattacacttcagcactcaagacatcca	ttgagtgcgaagtgaattgagtatatcc

## Primers used for real-time PCR

Gene	Forward (5'→3')	Reverse (5'→3')
<i>ACTB</i>	aatctggcaccacaccttct	tagcacagcctggatagcaa
<i>IL1A</i>	cgccaatgactcagag	agggcgctcattcagga
<i>IL1B</i>	aagcccttgctgtagtgtg	gaagctgatggcctaataca
<i>CLEC4E</i>	catttcgcattcttcaaacctgtg	attcccagttcaatggacaacaatt

Gene	Forward (5'→3')	Reverse (5'→3')
<i>actb</i>	tggaatcctgtggcat	taaaacgcagctcagt
<i>illa</i>	agcccgtgtgtgtaagga	agaaaatgaggtcgtctcactac
<i>illb</i>	aactgtgaaatgccaccttttgac	gttgatgtgctgctgctgcgagat
<i>clec4e</i>	cagtggcaatgggtggatgatac	agtcccttatggtggcacagtc

**Dectin-1 intracellular domain determines species-specific ligand spectrum by modulating receptor sensitivity**

Tomotsugu Takano, Chihiro Motozono, Takashi Imai, Koh-Hei Sonoda, Yoichi Nakanishi and Sho Yamasaki

*J. Biol. Chem.* 2017, 292:16933-16941.

doi: 10.1074/jbc.M117.800847 originally published online August 28, 2017

---

Access the most updated version of this article at doi: [10.1074/jbc.M117.800847](https://doi.org/10.1074/jbc.M117.800847)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

Supplemental material:

<http://www.jbc.org/content/suppl/2017/08/28/M117.800847.DC1>

This article cites 31 references, 14 of which can be accessed free at <http://www.jbc.org/content/292/41/16933.full.html#ref-list-1>