PROGRESS REPORT
Division of Experimental Animal Immunology

Yoichiro Iwakura, D. Sc.
Members

Faculty Members

Professor

Yoichiro Iwakura, D. Sc.

Assistant Professor

Soo-Hyun Chung, Ph. D.
(until the August 31st, 2022)

Hsi-Hua Chi, Ph. D.
(from the September 1st, 2022)

Postdoctoral Fellows

Xiaoqi Ye, M. D.
Wei Han, Ph. D.
Yulia Makusheva, Ph. D.

Research Assistants

Haiyang Sun

Guest Researchers

Ce Tang, M. D., Ph. D.
(Prof., Sun Yat-sen University)
Shinobu Saijo, M. D.
(Associate Prof., Chiba Univ.)
Kakuta Shigeru, D. V. M., Ph. D.
(Associate Prof., Univ. of Tokyo)
Tomonori Kaifu, Ph. D.
(Junior Associate Prof., Tohoku Medical & Pharmaceutical Univ.)
Masanori Murayama, Ph. D.
(Junior Associate Prof., Kansai Medical Univ.)
Rikio Yabe, Ph. D.
(Tokyo Metropolitan Institute of Medical Science)
Motohiko Kadoki, Ph. D.
(Research Fellow, Massachusetts General Hospital)
Motoko Kotani

Secretaries

Yuko Mikami

Division of Experimental Animal Immunology

Chairman: Yoichiro Iwakura, D.Sc.

1. The Roles of C-type lectin receptors in the development of colitis and colorectal tumors

Xiaoqi Ye[1, 2], Ce Tang[1, 2], Wei Han[1], Yulia Makusheva[1], Haiyang Sun[1], and Yoichiro Iwakura[1]

[1]Division of Experimental Animal Immunology, Research Institute for Biomedical Sciences, Tokyo University of Science, Japan

[2]The First Affiliated Hospital, Sun Yat-sen University, China

C-type lectin receptors (CLRs), containing an extracellular carbohydrate recognition domain and intracellular signal transducing motif such as immunoreceptor tyrosine-based activation motif (ITAM) or immunoreceptor tyrosine-based inhibition motif (ITIM), are one of innate immune receptors mainly expressed on myeloid-lineage cells such as dendritic cells (DCs) and macrophages (Tang et al., *J. Leukoc. Biol*., 2019). Some CLRs play important roles in the host defense against fungal and bacterial infection by recognizing specific carbohydrate components of these pathogens. Recently, these immune receptors have also been reported to be involved in maintaining immune homeostasis of mucosa by recognizing components of microbiota as well as self-components, such as alarmins from dead cells and non-canonical non-carbohydrate ligands. Many glycans, such as mannose structures, Lewis-type antigens and GalNAc, are components of some tumor cells, which are able to be recognized by some CLRs (Tang et al., *J. Leukoc. Biol*., 2019). Therefore, it is important to clarify the potential immunological roles of CLRs in not only fungal infection, but also inflammation, autoimmune disease or even cancer.

Dectin-1, a member of C-type lectin receptor family, recognizes β-glucans on fungal cell-wall and activates anti-fungal immunity of the host. This molecule is highly expressed in gut macrophages and dendritic cells. We have reported that inhibition of Dectin-1 signaling can suppress Dextran Sodium Sulfate (DSS)-induced and naïve T cell-induced intestinal inflammation by increasing intestinal Treg cells through regulation of commensal *Lactobacillus murinus* (Tang et al., *Cell Host Microbe*, 2015). Furthermore, Dectin-1 signaling induces IL-17F in the intestine, leading to the induction of anti-microbial proteins (Kamiya et al., *Mucosal Immunol*., 2018). These antimicrobial proteins suppress the growth of a group of commensal bacteria such as *Clostridium* cluster XIVa and *Lactobacillus*, which can induce Treg cell differentiation in the intestine. Therefore, development of colitis is suppressed in *Il17f*$^{-/-}$ mice due to the expansion of these Treg-inducible bacterial populations (Tang et al., *Nat. Immunol*., 2018).

Dectin-1 is involved in the development of tumors. Dectin-1 recognizes a N-glycan structure on tumor cells and suppresses tumor growth by activating NK cells (Chiba et al., *Elife*, 2014). However, Dectin-1 can also promote pancreatic cancer growth by recognizing galectin-9 on tumor cells and suppressing M1 macrophage-mediated T cell antitumor immunity (Daley et al., *Nat. Med*., 2017). We found that the development of colorectal tumors was suppressed in Dectin-1 KO mice by using the *APC*^{Min}-DSS- and azoxymethane (AOM)+DSS-induced colorectal tumor models.

Blocking Dectin-1 suppresses prostaglandin E2 production in myeloid-derived suppressor cells and enhances IL-22 binding protein expression, which further prevents colorectal tumors development (Tang et al., *Nat. Commun*., 2023).

Dendritic cell immunoreceptor (DCIR, *Clec4a2*), a CLR family member, is predominantly expressed in dendritic cells (DCs) and macrophages. DCIR has an ITIM in the cytoplasmic region and negatively controls multiple signaling pathways by recruiting the SH2 domain–containing protein tyrosine phosphatases SHP-1 and SHP-2. We found that asialo-biantennary N-glycan(s) (NA2) is a functional ligand of DCIR (Kaifu et al., *J. Exp. Med*., 2021). Previously, we reported that aged *Clec4a2*$^{-/-}$ mice spontaneously develop autoimmune sialadenitis and enthesitis, an inflammation at sites of attachment of ligaments, tendons, and joint capsules to bones, and are highly susceptible to collagen-induced arthritis due to overexpansion of DCs (Fujikado et al., *Nat. Med*., 2008). Th1 cell differentiation is enhanced in *Clec4a2*$^{-/-}$ mice and these mice spontaneously developed ankylosing change of joints due to over production of IFN-γ (Maruhashi et al., *J. Immunol*., 2015). Moreover, experimental autoimmune encephalomyelitis (EAE), a mouse model for multiple sclerosis, is exacerbated in DCIR-deficient mice associated with severe demyelination of the spinal cords (Seno et al., *Exp Anim*., 2015). These observations suggest that DCIR is critically important for the homeostasis of the immune system. Recently, we reported that DCIR deficiency suppresses colitis and colorectal tumors by enhancing granulocyte-macrophage colony-stimulating factor (GM-CSF) production. Blocking the ligand of DCIR by anti-NA2 antibody also ameliorates colitis and colorectal tumors efficiently (Sun et al., *Cell Rep*., 2022). These observations suggested that DCIR is a potential target for the treatment of these diseases.

Clec1A is a relatively poorly characterized member of CLRs and is expressed by endothelial cells and DCs. This receptor is structurally similar

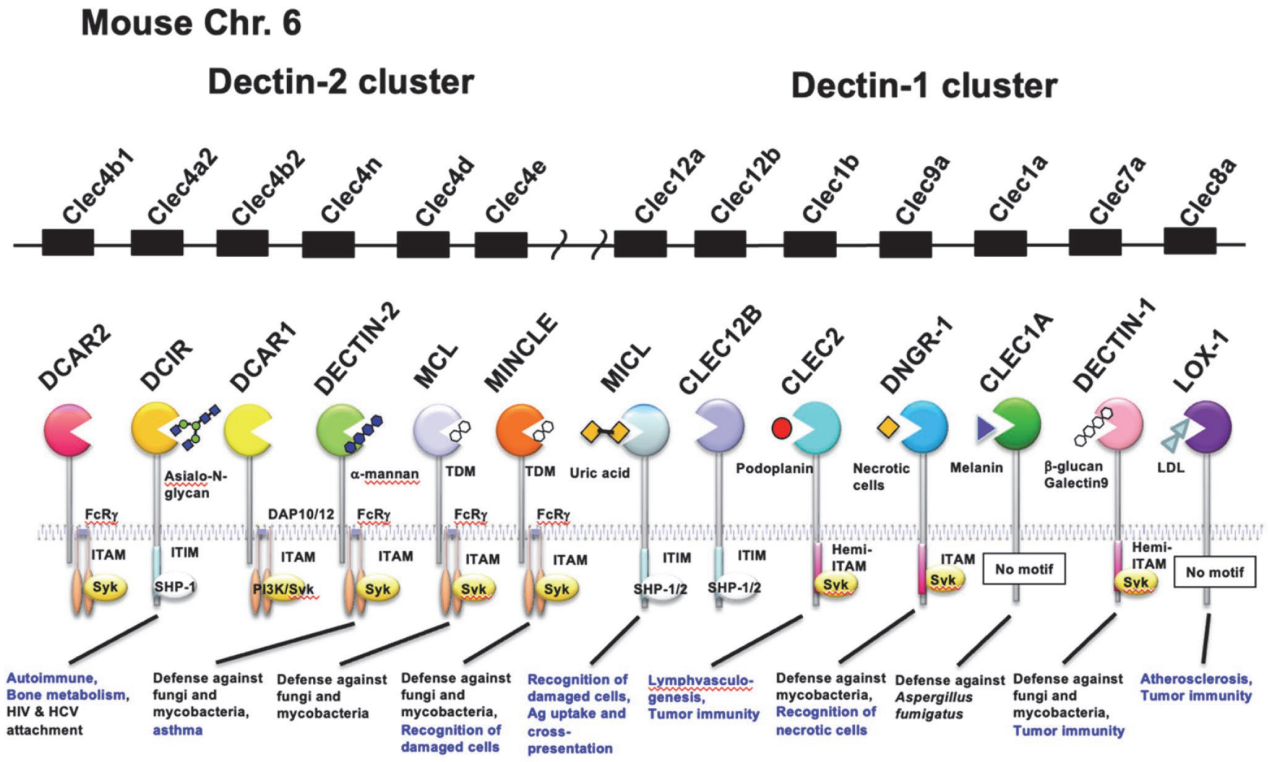

Figure 1. Mouse myeloid C-type lectins

to Dectin-1, although it does not contain any ITAMs or ITIMs. Clec1A plays an important role in host defense against pathological fungi, particularly *Aspergillus fumigatus*, which contains a ligand for Clec1A. We found that *Clec1a*$^{-/-}$ mice are resistant to EAE model. Capacity of antigen presentation in *Clec1a*$^{-/-}$ dendritic cells was impaired, and expression of inflammatory cytokines was greatly decreased in *Clec1a*$^{-/-}$ mice (Makusheva et al., *Exp. Anim*., 2022). Now, we are analyzing the roles of this CLR in AOM+DSS model and DSS-induced colitis model.

We are also analyzing the roles of Clec12B, another CLR member, in the intestinal immune system using AOM+DSS model and DSS-induced colitis model.

2. Blocking Dectin-1 prevents colorectal tumorigenesis by suppressing prostaglandin E2 production in myeloid-derived suppressor cells and enhancing IL-22 binding protein expression

Ce Tang[1, 2, *], Haiyang Sun[1, 2], Motohiko Kadoki[1], Wei Han[1], Xiaoqi Ye[1, 2], Yulia Makusheva[1], Jianping Deng[2], Bingbing Feng[2], Ding Qiu[2], Ying Tan[2], Xinying Wang[2], Zehao Guo[2], Chanyan Huang[2], Sui Peng[2], Minhu Chen[2], Yoshiyuki Adachi[3], Naohito Ohno[3], Sergio Trombetta[4], and Yoichiro Iwakura[1, *]

[3]Laboratory for Immunopharmacology of Microbial Products, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Japan

[4]Boehringer-Ingelheim, Ridgefield, CT, USA

Dectin-1 (gene *Clec7a*), a receptor for β-glucans, plays important roles in the host defense against fungi and immune homeostasis of

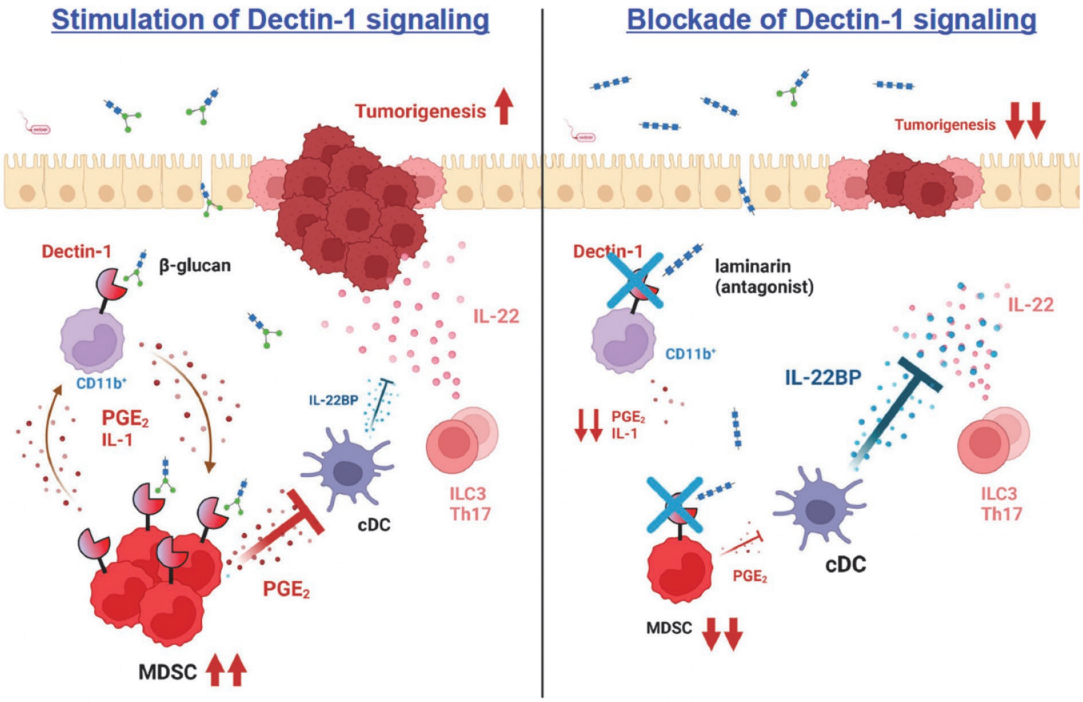

Figure 2. β-glucans activate Dectin-1 to induce PGE2 and IL-1β production in CD11b^{+} cells in colonic tumor microenvironment. PGE2 and IL-1β promote CD11b^{+} MDSC differentiation and proliferation. β-glucans activate MDSCs to produce PGE2, that suppresses production of IL-22BP. Thus, excess Dectin-1 signaling aggravates colorectal tumor development by enhancing PGE2 production and suppressing IL-22BP expression.

the intestine. Although this molecule is also suggested to be involved in the regulation of tumorigenesis, the role in intestinal tumor development remains to be elucidated. In this study, we found that azoxymethane-dextran-sodium-sulfate-induced and *Apc*^{Min}-induced intestinal tumorigenesis are greatly suppressed in *Clec7a*$^{-/-}$ mice independently from commensal microbiota. Dectin-1 was preferentially expressed on myeloid-derived suppressor cells (MDSCs). In the *Clec7a*$^{-/-}$ mouse colon, the proportion of MDSCs and MDSC-derived prostaglandin E_2 (PGE_2) levels were reduced, while the expression of IL-22 binding protein (IL-22BP; gene *Il22ra2*) was up-regulated. Dectin-1 signaling induced PGE_2-synthesizing enzymes and PGE_2 suppressed *Il22ra2* expression *in vitro* and *in vivo*. Administration of short chain β-glucan laminarin, an antagonist of Dectin-1, suppressed the development of mouse colorectal tumors. Furthermore, in patients with colorectal cancer (CRC), the expression of *CLEC7A* was also observed in MDSCs and correlated with the death rate and tumor severity. Dectin-1 signaling upregulated PGE_2-synthesizing enzyme expression and PGE_2 suppressed *IL22RA2* expression in human CRC-infiltrating cells. These observations indicate a pivotal role of the Dectin-1-PGE2-IL-22BP axis in regulating intestinal tumorigenesis, suggesting Dectin-1 as a potential target for CRC therapy.

3. Blocking DCIR mitigates colitis and prevents colorectal tumors by enhancing the GM-CSF-STAT5 pathway

Haiyang Sun[1], Ce Tang[1,2], Soo-Hyun Chung[1], Xiaoqi Ye[1], Yulia Makusheva[1], Wei Han[1], Masato Kubo[5], Shigeyuki Shichino[6], Satoshi Ueha[6], Koji Matsushima[6], Kazuho Ikeo[7], Masahide Asano[8], and Yoichiro Iwakura[1]

[5]Division of Molecular Pathology, and

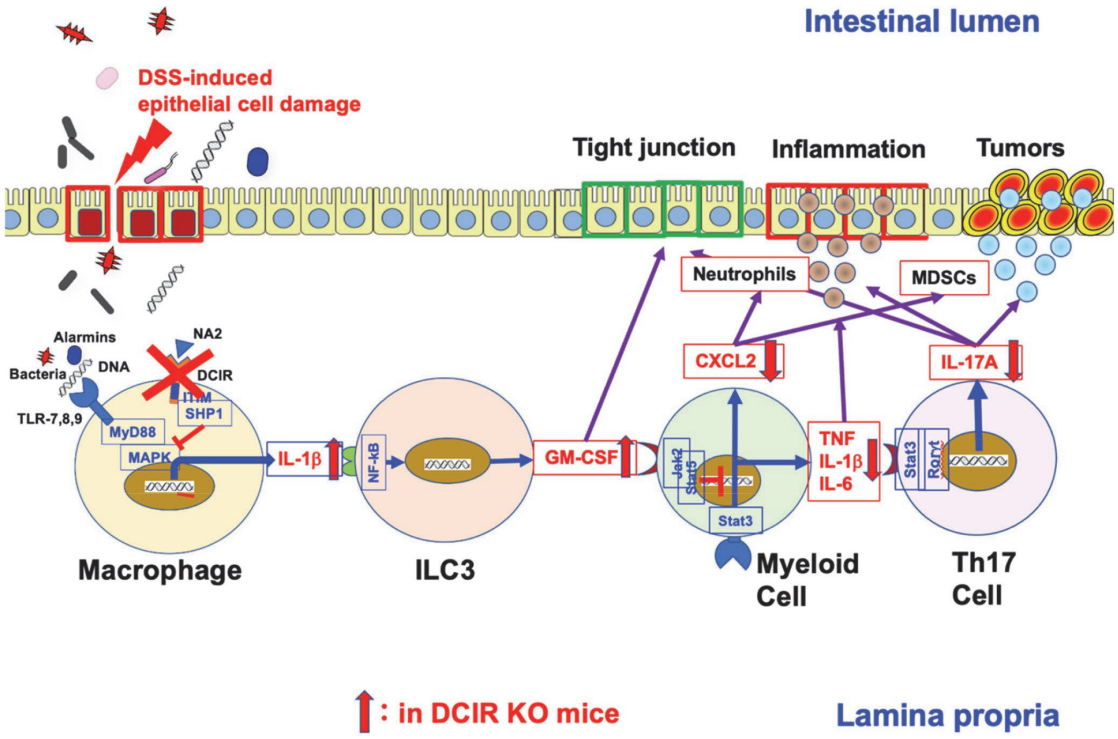

Figure 3. ● DCIR deficiency suppresses colitis and colonic tumor in mice
● Blocking DCIR enhances IL-1β production by macrophages and stimulates ILC3
● ILC3-derived GM-CSF activates STAT5 to suppress intestinal inflammation and tumor

[6]Division of Molecular Regulation of Inflammatory and Immune Diseases, Research Institute for Biomedical Sciences, Tokyo University of Science, Japan
[7]DNA Data Analysis Laboratory, National Institute of Genetics, Japan
[8]Institute of Laboratory Animals, Graduate School of Medicine, Kyoto University, Japan

Dendritic cell immunoreceptor (DCIR; *Clec4a2*), a member of the C-type lectin receptor family, plays important roles in homeostasis of the immune and bone systems. However, the intestinal role of this molecule is unclear. Here, we show that dextran sodium sulfate (DSS)-induced colitis and azoxymethane-DSS-induced intestinal tumors are reduced in *Clec4a2*$^{-/-}$ mice independently of intestinal microbiota. STAT5 phosphorylation and expression of Csf2 and tight junction genes are enhanced, while *Il17a* and *Cxcl2* are suppressed in the *Clec4a2*$^{-/-}$ mouse colon, which exhibits reduced infiltration of neutrophils and myeloid-derived suppressor cells. Granulocyte-macrophage colony-stimulating factor (GM-CSF) administration ameliorates DSS colitis associated with reduced *Il17a* and enhanced tight junction gene expression, whereas anti-GM-CSF exacerbates symptoms. Furthermore, anti-NA2, a ligand for DCIR, ameliorates colitis and prevents colorectal tumors. These observations indicate that blocking DCIR signaling ameliorates colitis and suppresses colonic tumors, suggesting DCIR as a possible target for the treatment of these diseases.

4. The role of CTRP-PAQR pathways in renal fibrosis development

Hsi-Hua Chi[1], Masanori A. Murayama[1, 9] and Yoichiro Iwakura[1]
[9]Institute of Biomedical Science, Kansai Medical University

C1q/TNF-related proteins (CTRPs) including CTRP6 are a group of secreted proteins which have a complement C1q-like domain in common, and play versatile roles in lipid metabolism, inflammation, tumor metastasis and bone metabolism. Previously we showed that CTRP6 binds adiponectin receptor 1 (AdipoR1), but not AdipoR2, in vitro. We also showed that AdipoR2

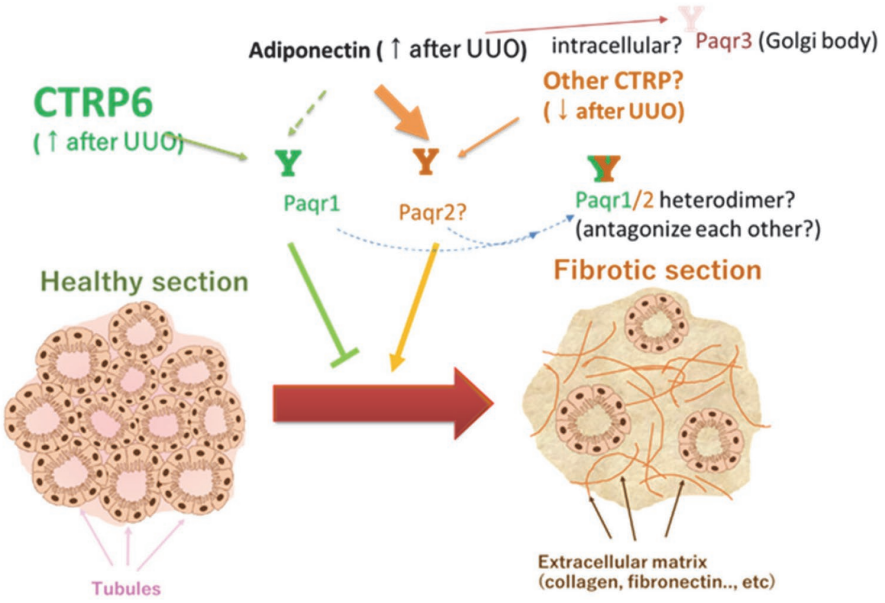

Fig. 4. The role of CTRP-PAQR pathways in renal fibrosis development

is the receptor for CTRP3. Although adiponectin is suggested to play a role in the development of acute kidney injury and renal fibrosis, the results are controversial, leaving the roles of AdipoR1 and AdipoR2 in the development of renal tubulointerstitial fibrosis obscure. Thus, we are now trying to generate *Paqr* KO mice to study the roles of each CTRP and PAQR family member in acute kidney injury and renal fibrosis using various kidney disease models.

Unilateral ureter obstruction (UUO) model is used for the analysis of pathogenic mechanisms of kidney diseases, such as tubular cell injury, interstitial inflammation, and fibrosis during subacute as well as chronic kidney diseases. By using a panel of gene knockout mice, we have found that each PAQR has a distinct role by interacting with a particular CTRP molecule in UUO model. We are now analyzing downstream signaling pathways of each receptor.

We also found that activation of complement system is involved in renal fibrosis progression. Previously, we showed that CTRP6 is a regulator of the complement alternative pathway (Murayama et al., *Nat. Commun*., 2015). Thus, CTRP6 regulates renal fibrosis not only by activating PAQR but also by inhibiting complement alternative pathway.

Publications

1. Lee, S., Byun, S., Lee, C., Park, S. H., Rudra, D., Iwakura, Y., Lee, Y. J., Im, S. H., and Hwang, D. S. Resolving the mutually exclusive immune responses of chitosan with nanomechanics and immunological assay. *Adv. Healthc. Mater*., **11**, e2102667 (2022).
2. Iida, S., Nakanishi, T., Momose, F., Ichishi, M., Mizutani, K., Matsushima, Y., Umaoka, A., Kondo, M., Habe, K., Hirokawa, Y., Watanabe, M., Iwakura, Y., Miyahara, Y., Imai, Y., and Yamanaka, K. IL-17A is the critical cytokine for liver and spleen amyloidosis in inflammatory skin disease. *Int. J. Mol. Sci*., **23**, 5726 (2022).
3. Iwasawa, M. T., Miyachi, H., Wakabayashi, S., Sugihira, T., Aoyama, R., Nakagawa, S., Katayama, Y., Yoneyama, M., Hara, H., Iwakura, Y., Matsumoto, M., Inohara, N., Koguchi-Yoshioka, H., Fujimoto, M., Núñez, G., Matsue, H., Nakamura, Y., and Saijo, S. Epidermal clearance of *Candida albicans* is mediated by IL-17 but independent of fungal innate immune receptors. *Int. Immunol*., **34**, 409-420 (2022).
4. Yamagishi, R., Kamachi, F., Nakamura, M., Yamazaki, S., Kamiya, T., Takasugi, M., Cheng, Y., Nonaka, Y., Yukawa-Muto, Y., Thuy, L. T. T., Harada, Y., Arai, T., Loo, T. M., Yoshimoto, S., Ando, T., Nakajima, M., Taguchi, H., Ishikawa, T., Akiba, H., Miyake, S., Kubo, M., Iwakura, Y., Fukuda, S., Chen, W. Y., Kawada, N., Rudensky, A., Nakae, S., Hara, E., and Ohtani, N. Gasdermin D-mediated release of IL-33 from senescent hepatic stellate cells promotes obesity-associated hepatocellular carcinoma. *Sci. Immunol*., 7, eabl7209 (2022).
5. Sun, H., Tang, C., Chung, S. H., Ye, X.-Q., Makusheva, Y., Han, W., Kubo, M., Shichino, S., Ueha, S., Matsushima, K., Ikeo, K., Asano, M., and Iwakura, Y. Blocking DCIR mitigates colitis and prevents colorectal tumors by enhancing the GM-CSF-STAT5 pathway. *Cell Rep*., **40**, 111158 (2022).
6. Iwayama, T., Iwashita, M., Miyashita, K., Sakashita, H., Matsumoto, S., Tomita, K., Bhongsatiern, P., Kitayama, T., Ikegami, K., Shimbo, T., Tamai, K., Murayama, M. A., Ogawa, S., Iwakura, Y., Yamada, S., Olson, L. E., Takedachi, M., and Murakami, S. Plap-1 lineage tracing and single-cell transcriptomics reveal cellular dynamics in the periodontal ligament. *Development*, **149**, dev201203 (2022).
7. Drummond, R. A., Desai, J. V., Hsu, A. P., Oikonomou, V., Vinh, D. C., Acklin, J. A., Abers, M. S., Walkiewicz, M. A., Anzick, S. L., Swamydas, M., Vautier, S., Natarajan, M., Oler, A. J., Yamanaka D, Mayer-Barber, K.

D., Iwakura, Y., Bianchi, D., Driscoll, B., Hauck, K., Kline. A., Viall, N. S., Zerbe, C. S., Ferré, E. M., Schmitt, M. M., DiMaggio, T., Pittaluga, S., Butman, J. A., Zelazny, A. M., Shea, Y. R., Arias, C. A., Ashbaugh, C., Mahmood, M., Temesgen, Z., Theofiles, A. G., Nigo, M., Moudgal, V., Bloch, K. C., Kelly, S. G., Whitworth, M. S., Rao, G., Whitener, C. J., Mafi, N., Gea-Banacloche, J., Kenyon, L. C., Miller, W. R., Boggian, K., Gilbert, A., Sincock, M., Freeman, A. F., Bennett, J. E., Hasbun, R., Mikelis, C. M., Kwon-Chung, K. J., Belkaid, Y., Brown, G. D., Lim, J. K., Kuhns, D. B., Holland, S. M., and Lionakis, M. S. Human Dectin-1 deficiency impairs macrophage-mediated defense against phaeohyphomycosis. *J. Clin. Invest*., **132**, e159348 (2022).

8. Chen, X., Hashimoto, D., Ebata, K., Takahashi, S., Shimizu, Y., Shinozaki, R., Hasegawa, Y., Kikuchi, R., Senjo, H., Yoneda, K., Zhang, Z., Harada, S., Hayase, E., Ara, T., Ohigashi, H., Iwakura, Y., Nakamura, K., Ayabe, T., and Teshima, T. Reactive granulopoiesis depends on T-cell production of IL-17A and neutropenia-associated alteration of gut microbiota. *Proc. Natl. Acad. Sci. U. S. A*., **119**, e2211230119 (2022).
9. Huang, Z., You, X., Chen, L., Du, Y., Brodeur, K., Jee, H., Wang, Q., Linder, G., Darbosset, R., Cunin, P., Chang, M. H., Wactor, A., Wauford, B. M., Todd, M. J. C., Wei, K., Li, Y., Levescot, A., Iwakura, Y., Pascual, V., Baldwin, N. E., Quartier, P., Li, T., Gianatasio, M. T., Hasserjian, R. P., Henderson, L. A., Sykes, D. B., Mellins, E. D., Canna, S. W., Charles, J. F., Nigrovic, P. A., and Lee, P. Y. mTORC1 links pathology in experimental models of Still's disease and macrophage activation syndrome. *Nat. Commun*., **13**, 6915 (2022).
10. Ashino, T., Nakamura, Y., Ohtaki, H., Iwakura, Y., and Numazawa, S. Interleukin-6 regulates the expression of hepatic canalicular efflux drug transpoters after cecal ligaiton and puncture-induced sepsis: a comparison with lipopolysaccharide treatment. *Toxicol. Lett*., S0378-4274(22)01808-2 (2022).
11. Nakanishi, T., Iida, S., Maruyama, J., Urushima, H., Ichishi, M., Matsushima, Y., Mizutani, K., Nakayama, Y., Sugioka, K., Nishimura, M., Umaoka, A., Iwakura, Y., Kondo, M., Habe, K., Tsuruta, D., Yamamoto, O., Imai, Y., and Yamanaka, K. Arteriosclerosis derived from cutaneous inflammation is ameliorated by the deletion of IL-17A and IL-17F. *Int. J. Mol. Sci*., **24**, 5434 (2023).
12. Tang, C., Sun, H., Kadoki, M., Han, W., Ye, X.-Q., Makusheva, Y., Deng, J., Feng, B., Qiu, D., Tan, Y., Wang, X., Guo, Z., Huang, C., Peng, S., Chen, M., Adachi, Y., Ohno, N., Trombetta, S., and Iwakura, Y. Blocking Dectin-1 prevents colorectal tumorigenesis by suppressing prostaglandin E2 production in myeloid-derived suppressor cells and enhancing IL-22 binding protein expression. *Nat. Commun*., **14**, 1493 (2023).
13. Ashino, T., Nakamura, Y., Ohtaki, H., Iwakura, Y., and Numazawa, S. Downregulation of the gene expression of Cyp2c29 and Cyp3a11 by cecal ligation and puncture-induced sepsis is associated with interleukin-6. *Int. Immunopharmacol*., **117**, 110039 (2023).
14. Kaifu, T., Maruhashi, T., Chung, S.-H., Shimizu, K., Nakamura, A., and Iwakura, Y. DCIR suppresses osteoclastic proliferation and resorption by downregulating M-CSF and RANKL signaling. *Front. Immunol*., **14**, 1159058 (2023).
15. Shimazu, T., Suzuki, M., Takasaki, R., Besshi, A., Suzuki, Y., and Iwakura, Y. Heat-killed *Levilactobacillus brevis* as a candidate postbiotics through immunostimulation mediated by macrophage-inducible C-type lectin. *Probiotics and Antimicrobial Proteins*, **15**, 774-784 (2023).
16. Fergany, A., Zong, C., Ekuban, F. A., Suzuki, A., Kimura, Y., Ichihara, S., Iwakura, Y., and Ichihara, G. Deletion of IL-1β exacerbates acrylamide-induced neurotoxicity in mice. *Toxicol. Sci*., **195**, 246-256 (2023).