Immunobiology 220 (2015) 597–604

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Increased production of intestinal immunoglobulins in *Syntenin-1*-deficient mice

Kentaro Tamura[a,b], Masashi Ikutani[c,**], Taketoshi Yoshida[a], Ayumi Tanaka-Hayashi[b], Tsutomu Yanagibashi[c], Ran Inoue[b], Yoshinori Nagai[c], Yuichi Adachi[a], Toshio Miyawaki[a], Kiyoshi Takatsu[c], Hisashi Mori[b,*]

[a] *Department of Pediatrics, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama 930-0152, Japan*
[b] *Department of Molecular Neuroscience, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama 930-0152, Japan*
[c] *Department of Immunobiology and Pharmacological Genetics, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, Toyama 930-0152, Japan*

ARTICLE INFO

Article history:
Received 19 September 2014
Accepted 8 December 2014
Available online 17 December 2014

Keywords:
Antibodies
B cells
Knockout mice
Mucosal immunity
Syntenin-1

ABSTRACT

Syntenin-1 is an intracellular PDZ protein that binds multiple proteins and regulates protein trafficking, cancer metastasis, exosome production, synaptic formation, and IL-5 signaling. However, the functions of Syntenin-1 have not yet been clearly characterized in detail, especially *in vivo*. In this study, we generated a *Syntenin-1* knock out (KO) mouse strain and analyzed the role(s) of Syntenin-1 in IL-5 signaling, because the direct interaction of Syntenin-1 with the cytoplasmic domain of the IL-5 receptor α subunit and the regulation of IL-5 signaling by Syntenin-1 have been reported. Unexpectedly, the number of IL-5-responding cells was normal and the levels of fecal immunoglobulins were rather higher in the *Syntenin-1* KO mice. We also found that IgA and IgM production of splenic B cells stimulated *in vitro* was increased in *Syntenin-1* KO mice. In addition, we showed that a distribution of intestinal microbial flora was influenced in *Syntenin-1* KO mice. Our data indicate that Syntenin-1 negatively regulates the intestinal immunoglobulin production and has a function to maintain the intestinal homeostasis *in vivo*. The analysis of *Syntenin-1* KO mice may provide novel information on not only mucosal immunity but also other functions of Syntenin-1 such as cancer metastasis and neural development.

Introduction

Syntenin-1 (Syndecan-binding protein, Sdcbp) was first identified as an intracellular scaffold protein interacting with the transmembrane heparan sulfate syndecans (Grootjans et al., 1997). It was also termed melanoma differentiation-associated gene-9 (*MDA*-9), which promoted cancer metastasis by regulating cell adhesion (Boukerche et al., 2005, 2007, 2008; Lin et al., 1998). Subsequently, Syntenin-1 has been reported to act as a multifunctional intracellular adapter protein and to regulate protein trafficking and recycling (Zimmermann et al., 2005), Notch signaling (Estrach et al., 2007), IL-5 signaling (Beekman et al., 2009; Geijsen et al., 2001), T cell chemotaxis (Sala-Valdes et al., 2012), HIV infection (Gordon-Alonso et al., 2012), exosome production (Baietti et al., 2012), and synaptic formation in CNS (Hirbec et al., 2005; Jannatipour et al., 2001; Ohno et al., 2004). Additionally, we previously found that extracellular Syntenin-1 in human colostrum could preferentially induce IgA production from cord blood naive B cells (Sira et al., 2009).

IL-5/IL-5R signaling, one of the pathways interacting with Syntenin-1, was reported to maintain mouse B-1 B cells and promote secretion of mucosal IgA (Moon et al., 2004). IL-5 also promotes eosinophil differentiation in humans and mice (Hiroi et al., 1999; Kopf et al., 1996; Moon et al., 2004; Yoshida et al., 1996). Structurally, IL-5R consists of two distinct subunits, an

Abbreviations: BAC, bacterial artificial chromosome; bp, base pair; CNS, central nervous system; ES, embryonic stem; IL-5Rα, interleukin-5 receptor α subunit; KO, knock out; L-LP, large intestinal lamina propria; LP, lamina propria; PC, peritoneal cavity; PDZ, PSD-95/Discs large/zO-1; PEC, peritoneal exudate cells; PP, Peyer's patch; rRNA, ribosomal RNA; sIgA, surface-IgA; S-LP, small intestinal lamina propria; WT, wild type.

* Corresponding author at: Department of Molecular Neuroscience, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0152, Japan. Tel.: +81 76 434 7230; fax: +81 76 434 5015.
** Corresponding author at: Department of Immunobiology and Pharmacological Genetics, Graduate School of Medicine and Pharmaceutical Sciences, University of Toyama, 2630 Sugitani, Toyama 930-0152, Japan. Tel.: +81 76 434 7673; fax: +81 76 434 5009.
E-mail addresses: mikutani@med.u-toyama.ac.jp (M. Ikutani), hmori@med.u-toyama.ac.jp (H. Mori).

http://dx.doi.org/10.1016/j.imbio.2014.12.003

IL-5R-specific α subunit (IL-5Rα) and a common β subunit for the GM-CSF receptor family (Mita et al., 1989). Syntenin-1 was reported to associate with the cytoplasmic tail of IL-5Rα through the PDZ (PSD-95/Discs large/zO-1) domain and to interact directly with the transcription factor Sox4 (Beekman et al., 2009, 2012; Geijsen et al., 2001). Interestingly, Sox4 promotes B cell development, as determined by analysis of *Sox4* KO mice (Schilham et al., 1996; Sun et al., 2013).

Although these various functions of Syntenin-1 *in vitro* have been well reported including the relationship with IL-5R, little is known about the distribution and role of this protein *in vivo*. To clarify the physiological role(s) of Syntenin-1 *in vivo*, we generated a *Syntenin-1* KO mouse strain by gene targeting in this study. We found that *Syntenin-1* KO mice showed no obvious signs of diseases under specific pathogen-free conditions and the Syntenin-1 was widely expressed, particularly in immunologically related organs and CNS. In addition, we focused on the relationship between Syntenin-1 and IL-5 signaling in gut-associated tissues and found that Syntenin-1 was not essential for the maintenance of IL-5-responding cells, and rather negatively regulated immunoglobulin production in the intestine.

Materials and methods

Generation of Syntenin-1 KO mice

Animal care and experimental protocols were approved by the Animal Experiment Committee of the University of Toyama (Authorization No. A2012-MED-35) and were carried out in accordance with the Guidelines for the Care and Use of Laboratory Animal of the University of Toyama.

A bacterial artificial chromosome (BAC) genomic clone (RP24-301N) originating from the DNA of C57BL/6 mice and containing *Syntenin-1* was obtained from BACPAC Resource Center CHORI (Oakland, CA). A counter-selection BAC modification kit (Gene Bridges, Dresden, Germany) and a MultiSite Gateway Three-Fragment Vector Construction kit (Invitrogen, Carlsbad, CA) were modified for the targeting vector construction. The nucleotide sequence of the mouse genome was obtained from the National Center for Biotechnology Information (NCBI Map Viewer, *Mus musculus* Build 37.1) and the initiation site of translation in *Syntenin-1* (the A of ATG) refers to position +1 and the proceeding residues are indicated by negative numbers in this report. The 5′ arm of ∼5 kbp (base pair) (Nos. −4769 to −334) and 3′ arm of ∼5 kbp (Nos. +322 to +4947) were subcloned into the pDONR P4-P1R and pDONER P2R-P3 vectors, respectively, using the counter-selection BAC modification kit. The 655-bp *Syntenin-1* (Nos. −333 to +321) gene fragment containing exon 2, part of intron 1, and part of intron 2 was amplified by PCR and subcloned between two loxP sequences of a modified pDONR 221 vector containing a pgk-Neo cassette flanked by two FRT sites. To construct the targeting vector, these three plasmids were directionally subcloned into pDEST R4-R3 containing the diphtheria toxin gene (MC1-DTA) by MultiSite Gateway LR recombination reaction. The targeting vector linearized with *Not*I was electroporated into the embryonic stem (ES) cell line RENKA derived from the C57BL/6N strain (Fukaya et al., 2006) as previously described (Miya et al., 2008). After the selection with G418, recombinant ES clone was identified by Southern blot analysis using the 5′ probe (Nos. −5412 to −4923) on *Spe*I-digested genomic DNA, the 3′ probe (+6740 to +7093) on *Apa*I-digested genomic DNA, and the Neo probe (Miya et al., 2008) on *Apa*I-digested genomic DNA. The obtained recombinant ES clone was transfected with the pCre-Pac plasmid (Taniguchi et al., 1998) and pCAGGS-FLP plasmid (Gene Bridges, Dresden, Germany) by electroporation to delete exon 2 and the pgk-neo cassette, respectively. The PCR amplified fragments were verified using the DNA sequencer ABI PRISM 3100 (Perkin-Elmer, Foster City, CA).

The obtained clone was injected into eight-cell stage embryos of the mouse strain ICR. The embryos were cultured to the blastocyst stage and transferred to the pseudopregnant ICR mouse uterus. The resulting male chimeric mice were crossed with female C57BL/6 mice to establish the mutant mouse line. The *Syntenin-1* KO mice were further genotyped by PCR using the following primers; 5′ forward, 5′-TGACCCTGGTTTAGCTGAGGA-3′; 5′ reverse, 5′-TCTGTTCCCACAGCTACCCAA-3′; and 3′ reverse, 5′-GCTCACAACCGTCTAACTCCAAC-3′ (Fig. 1A).

Western blotting

At the age of 6 weeks, wild type (WT) and *Syntenin-1* KO mice were deeply anesthetized with pentobarbital sodium (100 mg/kg body weight, intraperitoneal injection) and then perfused transcardially with ice-cold PBS. Tissues were quickly removed and homogenized in Mammalian Tissue Extraction Reagent (Pierce, Rockford, IL) with Protease Inhibitor (Nacalai, Kyoto, Japan). The homogenate was centrifuged at 14,500 rpm for 15 min to remove large debris. The protein concentration was determined using a BCA Protein Assay kit (Pierce) and the protein samples were diluted at 1:1 in a sample buffer (50 mM Tris–HCl, pH 8.2, 2% SDS, 10% glycerol, 6% 2-mercaptoethanol, and 0.01% bromophenol blue). After denaturation by heating at 95 °C for 5 min, 30 μg of proteins were subjected to SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (Perkin-Elmer). After blocking with 5% skim milk in Tris buffered saline containing 0.1% Tween-20 for 1 h, the membranes were incubated with rabbit polyclonal anti-Syntenin-1 antibody (1:1000, Abcam, Cambridge, UK) or mouse monoclonal anti-β-actin antibody (1:10,000, Sigma–Aldrich, St. Louis, MO) overnight at 4 °C, then with HRP-conjugated goat anti-rabbit IgG (1:25,000, Bio-Rad, Richmond, CA) or goat anti-mouse IgG (1:25,000, Bio-Rad) for 1 h. Protein bands were detected using the ECL chemiluminescence detection system (GE Healthcare, Buckinghamshire, UK).

Antibodies and reagents for flow cytometry

Antibodies used for flow cytometry were anti-mouse B220 (RA3-6B2), CD3ε (145-2C11), CD5 (53–7.3), CD19 (1D3), CD23 (B3B4), and CD45 (30-F11) antibodies purchased from eBioscience (San Jose, CA) and anti-mouse CD21/35 (7G6), surface-IgA (sIgA) (C10-3), and Siglec-F (E50-2440) antibodies purchased from BD Biosciences (San Diego, CA). FcγRs were blocked with anti-mouse FcγR (2.4G2). Flow cytometry was performed using a FACSCanto II (BD Biosciences). Dead cells were gated out by 7-aminoactinomycin D staining (BD Biosciences). FlowJo (Tree Star, Ashland, OR) was used for analysis.

Preparation of lamina propria cells

To obtain lamina propria (LP) cells, the small and large intestines were harvested, and Peyer's patchs (PPs) and cecal patches were removed. The intestines were then opened longitudinally, washed twice with 40 ml of Ca^{2+}- and Mg^{2+}-free HBSS (Sigma–Aldrich) supplemented with 5% FCS, 1 mM DTT, and 5 mM EDTA and then incubated at 37 °C for 40 min with shaking at 150 rpm. Tissues were minced and incubated with RPMI 1640 (Invitrogen) supplemented with 5% FCS. To the small intestine tissues, 1 mg/ml collagenase type I

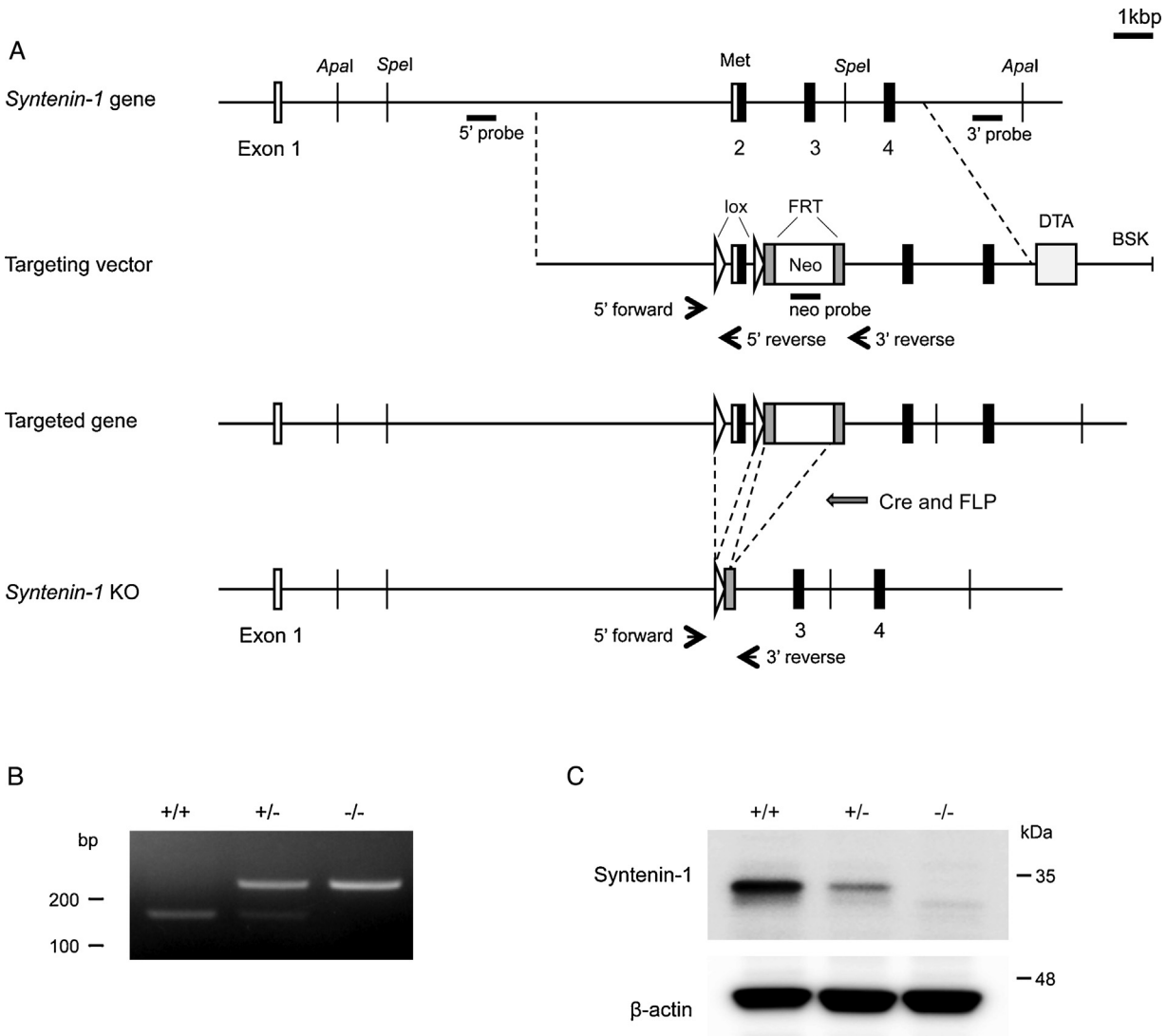

Fig. 1. Generation of *Syntenin-1* KO mice with C57BL/6 genetic background. (A) Schematic representations of *Syntenin-1* gene, targeting vector, targeted gene, and *Syntenin-1* KO gene. The coding and noncoding regions of *Syntenin-1* exons are indicated by closed and open boxes, respectively. Met in exon 2 is the initiation site of translation in *Syntenin-1*. Inserted lox, FRT, and neomycin resistance gene (Neo) are shown. The relevant restriction enzyme sites (*Apa*I and *Spe*I), the location of probes used (5′probe, neo probe, and 3′probe), and the PCR primers (5′forward, 5′reverse, and 3′reverse) are indicated. DTA, diphtheria toxin fragment A; BSK, pBluescript. (B) PCR analysis of genomic DNA from *Syntenin-1*$^{+/+}$ (+/+), *Syntenin-1*$^{+/-}$ (+/−), and *Syntenin-1*$^{-/-}$ (−/−) mice. The 5′forward, 5′reverse, and 3′reverse primers were mixed at a molar ratio of 2:1:1. The positions of DNA size markers are indicated on the left side. (C) Expression of syntenin-1 protein in spleen. Spleen homogenates from *Syntenin-1*$^{+/+}$ (+/+), *Syntenin-1*$^{+/-}$ (+/−), and *Syntenin-1*$^{-/-}$ (−/−) mice were separated by SDS-PAGE and immunoblotted with anti-Syntenin-1 antibody (upper) and anti-β-actin antibody (lower). The positions of protein size markers are indicated on the right side.

(Sigma–Aldrich) was added, and 2 mg/ml collagenase was added to the large intestine tissues. The tissues were then incubated with 100 ng/ml DNase I (Roche Diagnostics, Indianapolis, IN) at 37 °C for 40 min with stirring. Collected cells were placed on the boundary between 40/75% concentrations of Percoll (GE Healthcare, Piscataway, NJ) solution and centrifuged at 1800 rpm at 20 °C for 20 min. After centrifugation, the collected cells were washed and used as LP lymphocytes.

ELISA

Freshly collected fecal samples were weighed, dissolved in PBS (0.1 g/ml), and centrifuged at 15,000 rpm for 5 min. The supernatants were used as fecal extract. The levels of each immunoglobulin isotype in fecal extract and serum were determined by sandwich ELISA using antibodies specific for each murine immunoglobulin isotype (Southern Biotech, Birmingham, AL) according to a protocol.

Splenic B cell purification and cell culture

For collection of resting B cells, single cell suspensions prepared from the spleen isolated from WT and *Syntenin-1* KO mice were purified by magnetic-activated cell sorting (MACS) negative selection using biotin-conjugated anti-mouse CD43 antibody (S7, BD Bioscience) and streptavidin MicroBeads (Miltenyi Biotec, Auburn, CA). The purified resting B cells were cultured at a concentration of 2×10^5 cells/well in RPMI 1640 with 10% FCS, 50 μM 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin. 1 μg/ml LPS (Sigma–Aldrich), 1 ng/ml TGF-β (R and D Systems, Minneapolis, MN), and/or 5 ng/ml IL-5 (R and D Systems) were added to the culture to induce IgA production. 0.1 or 1 μg/ml LPS (Sigma–Aldrich) were used to induce IgM production. 1 μg/ml anti-CD40 antibody (R and D Systems) and 50 ng/ml IL-4 (R and D Systems) were added to induce IgG1 production. The concentration of IgA, IgM and IgG1 in the supernatants was measured by ELISA on day 7, day 5 and day 5, respectively.

DNA extraction from fecal samples and real-time PCR

Fecal samples were collected for 24 h from individually housed *Syntenin-1* KO and WT littermates (6 weeks of age) and stored at −20 °C until analysis. DNA extraction was performed on the fecal samples using DNA stool mini kit (Qiagen, Venlo, The Netherlands). Quantitative real-time PCR assays of 16S ribosomal RNA (rRNA) gene were performed by Cosmobio Co. (Sapporo, Japan) using the method of Matsuki et al. (2002, 2004). Three targets were analyzed: all bacteria, phylum *Firmicutes* and *Bacteroidetes*. The relative ratio of each phylum to all bacteria was measured.

Statistical analysis

All values are represented as mean ± SD. The statistical significance of difference between WT and *Syntenin-1* KO mice was determined by two-tailed Student's t test. Values of $p < 0.05$ indicate a statistically significant difference.

Results

Generation of Syntenin-1 KO mouse strain

To disrupt the *Syntenin-1* locus in ES cells derived from the C57BL/6 mouse strain, we constructed a targeting vector to introduce the loxP sequence into intron 1 and another loxP and the Neo cassette flanked with FRT sequences into intron 2 (Fig. 1A). We obtained an ES cell clone in which the expected homologous recombination occurred at the *Syntenin-1* locus, as detected by Southern blot analysis. The ES cells were treated with Cre and FLP recombinases transiently to delete exon 2 containing the initiation site of translation of *Syntenin-1* and the Neo cassette, respectively. Chimeric mice derived from this clone were mated with C57BL/6 mice to establish the mutant mouse line. The gene deletion in the mutant mice was confirmed by Southern blot (data not shown) and PCR analyses (Fig. 1B). Syntenin-1 protein expression was examined using the homogenate of spleen by Western blot analysis. The rabbit anti-Syntenin-1 antibody recognized a protein detected as a band of 32 kDa corresponding to Syntenin-1 in the WT mice, whereas the band showed decreased intensity in the case of heterozygous mutant mice, and not detected in homozygous mutant mice (Fig. 1C). These findings indicate that *Syntenin-1* was successfully disrupted in the mutant mice.

Mice lacking Syntenin-1 were born at the expected Mendelian ratio (Supplementary Table 1). The *Syntenin-1* KO mice thrived and reproduced as well as their WT littermates and showed no obvious signs of diseases under specific-pathogen free conditions during the first 1 year of life.

Syntenin-1 protein was widely expressed, especially in immunologically related organs and CNS

To evaluate the detailed expression pattern of the Syntenin-1 protein *in vivo*, equal amounts of the protein from various organs of WT mice were examined by Western blotting (Fig. 2). The Syntenin-1 protein was widely expressed in mouse tissues, and relatively high expression levels were detected in the spleen, thymus, and brain. The Syntenin-1 expression levels in the liver and kidney were lower than those in the other tissues. We used lysates of human embryonic kidney (HEK) 293 cells transfected with a human *Syntenin-1* expression plasmid as a positive control. The Syntenin-1 protein was not detected in any organs of *Syntenin-1* KO mice (Fig. 2 and data not shown), suggesting the specificity of the primary antibody used against Syntenin-1.

The number of IL-5-responding cells was not affected in Syntenin-1-deficient mice

IL-5 signaling is a key regulator for the maintenance of B-1 B cells, IgA production, and eosinophils in mice (Hiroi et al., 1999; Moon et al., 2004; Tominaga et al., 1991). To clarify whether Syntenin-1 is involved in the development and maintenance of the IL-5-responding cells, we examined the number of lymphocytes and eosinophils in the peritoneal cavity (PC), mesenteric lymph node (MLN), PP, and small and large intestinal lamina propria (S-LP and L-LP, respectively). The proportions of $B220^+$ $sIgA^-$, $B220^+sIgA^+$, and $B220^-sIgA^+$ B cells in the S-LP and L-LP and $B220^+sIgA^-$ and $B220^+sIgA^+$ B cells in PP and MLN, as well as $CD3\varepsilon^+$ T cells, were normal in *Syntenin-1* KO mice (Supplementary Fig. 1A). Siglec-F^+ eosinophils in the S-LP and L-LP of *Syntenin-1* KO mice also normally developed (Supplementary Fig. 1B). B-1a and B-1b cells (characterized as $CD19^+CD21^-CD23^-CD5^+$ and $CD19^+CD21^-CD23^-CD5^-$, respectively) and conventional B-2 cells in PC seemed to be not affected by Syntenin-1 deficiency (Supplementary Fig. 1C). The number of the analyzed immune cells showed no significant differences between *Syntenin-1* KO and WT mice (Table 1). These results suggest that Syntenin-1 little affected on the development of lymphocytes and eosinophils in the steady state.

Production of fecal immunoglobulins were increased in Syntenin-1-deficient mice

Given that IL-5 is a key cytokine for IgA production, isotype-specific ELISA was performed to determine levels of IgA together with those of IgG1 and IgM in the fecal extract and serum in *Syntenin-1* KO and WT mice. Although the levels of immunoglobulins in the serum did not change, IgA, IgG1 and IgM levels in the fecal extract increased significantly in *Syntenin-1* KO mice (Fig. 3Aand B). These results showed that Syntenin-1 negatively regulates immunoglobulin production in the intestine.

In vitro analysis of immunoglobulins production

As intestinal immunoglobulin production was increased in *Syntenin-1* KO mice, we next examined the ability of B cells to secrete immunoglobulins in WT and *Syntenin-1* KO mice *in vitro*. We purified splenic resting B cells by magnetic cell isolation and cultured them in the presence of LPS, TGF-β, and IL-5 to induce IgA secretion; LPS to induce IgM secretion; IL-4 and anti-CD40 antibody to induce IgG1 secretion. IgA production was significantly higher in B cells derived from *Syntenin-1*-deficient mice than in B cells derived from WT mice (Fig. 4A). IgM production of *Syntenin-1* KO B cells stimulated with only LPS was also increased (Fig. 4B). IgG1 production had no significant difference between these mouse strains (Fig. 4C). These results support the idea that Syntenin-1 negatively regulates immunoglobulin production in the intestine and suggest that such an enhanced immunoglobulin production occurs in a B cell-intrinsic manner.

Distribution of intestinal microbiota was influenced in Syntenin-1-deficient mice

Mucosal immunity including intestinal secretory immunoglobulins is closely related to microbiota to maintain intestinal homeostasis (Strugnell and Wijburg, 2010). The obtained results in *Syntenin-1* KO mice raise a question whether a distribution of intestinal microbial flora could be influenced. We next examined the percentages of total 16S rRNA gene of the phylum *Firmicutes* and *Bacteroidetes*, which consist mostly of mouse intestinal microbiota. The percentage of *Firmicutes* in the stool of *Syntenin-1* KO

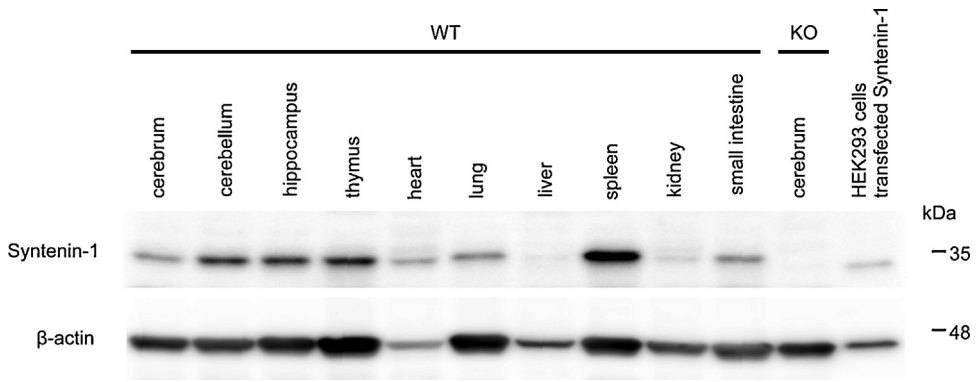

Fig. 2. Expression pattern of syntenin-1 protein. Western blot analysis of protein extracts from cerebrum, cerebellum, hippocampus, thymus, heart, lung, liver, spleen, kidney, and small intestine in WT mice and cerebrum in *Syntenin-1* KO mice using anti-Syntenin-1 antibody (upper panels) and anti-β-actin antibody (lower panels). Equal amounts (30 μg) of protein measured by BCA protein assay were loaded to all lanes. Lysate of human embryonic kidney (HEK) 293 cells (5 μg) transfected with the human *Syntenin-1* expression plasmid was used as a positive control. The positions of protein size markers are indicated on the right side. Data are representative of at least three independent experiments.

mice was significantly higher than WT mice, although the percentage of *Bacteroidetes* and the ratio of *Firmicutes* to *Bacteroidetes* were not significantly different (Fig. 5).

Discussion

We have generated a *Syntenin-1* KO mouse strain to clarify the function(s) of Syntenin-1 *in vivo*. Although Syntenin-1 was widely expressed in mouse organs, homozygous *Syntenin-1*-deficient mice showed no obvious signs of diseases. We found that the levels of fecal immunoglobulins in *Syntenin-1* KO mice were higher than those in WT mice. We also showed that the levels of IgA and IgM secretion from LPS-stimulated splenic B cells were significantly higher in *Syntenin-1* KO mice, therefore the mechanism underlying enhanced fecal immunoglobulins production in *Syntenin-1* KO mice is likely B cell-intrinsic. Additionally, we indicated a possibility that the intestinal microbiota was influenced in *Syntenin-1* KO mice. Taken together, our results imply that Syntenin-1 has a function to maintain the intestinal homeostasis *in vivo*.

Structurally, Syntenin-1 is a 32-kDa protein with two PDZ domains (Das et al., 2012). The PDZ domains can bind to short amino acid sequences at the C-terminal end of the transmembrane or intracellular proteins (Chimura et al., 2011). Through the PDZ domains, Syntenin-1 is capable to bind to various proteins, such as syndecans, the tyrosin kinase Src, IL-5Rα, CD63, Delta1, and adhesion molecules, for synaptic formation in the CNS (Boukerche et al., 2008; Estrach et al., 2007; Geijsen et al., 2001; Grootjans et al., 1997; Hirbec et al., 2005; Jannatipour et al., 2001; Ohno et al., 2004; Pols and Klumperman, 2009). In general, several PDZ proteins are reported to act as a negative regulator of various signals and transcription factors (Alewine et al., 2006; Gupta et al., 2012; Stephenson et al., 2007). In fact, Chen et al. reported that Syntenin specifically interacted with TNF receptor associated factor 6 (TRAF6) and played inhibitory role in TLR4-mediated NF-kB activation signaling pathway (Chen et al., 2008). Therefore, Syntenin-1 could potentially inhibit the signaling pathway of immunoglobulin production.

Although the interacting partners of Syntenin-1 were well reported, little has been known about the distribution of the Syntenin-1 protein *in vivo*. Jeon et al. analyzed the expression pattern of the Syntenin protein in mouse embryos by immunohistochemistry (Jeon et al., 2013). They reported that the Syntenin protein was detected temporally during an early developmental period, and that Syntenin may play a prominent role in cell proliferation and differentiation in normal mouse development. However, in this study we found that the Syntenin-1 protein was widely expressed in adult mouse organs, especially in the spleen, thymus, and brain. Our expression data are consistent with various reports which describe the functions of Syntenin-1 in the immune system and CNS (Gordon-Alonso et al., 2012; Jannatipour et al., 2001; Koroll

Table 1
Numbers of sIgA$^+$ cells, T cells, eosinophils, and B-1a, B-1b, and B-2 cells in gut-associated lymphoid tissues and peritoneal cavity.

Syntenin-1		S-LP	L-LP	MLN	PP	PC
	B220$^+$sIgA$^-$	1.4 ± 0.3	3.0 ± 0.9	11.1 ± 3.2	8.3 ± 2.0	
	B220$^-$sIgA$^+$	19.6 ± 8.2	1.1 ± 0.4			
	B220$^+$sIgA$^+$	0.1 ± 0.1	0.1 ± 0.1	1.0 ± 0.3	1.0 ± 0.4	
	CD3ε$^+$T cell	5.4 ± 3.3	1.3 ± 0.6	22.4 ± 9.5	2.3 ± 0.5	
−/−	Eosinophil	2.0 ± 1.8	0.2 ± 0.1			
	B-1a					2.9 ± 1.5
	B-1b					0.5 ± 0.2
	B-2					5.8 ± 1.1
	B220$^+$sIgA$^-$	1.7 ± 1.1	2.6 ± 1.0	11.1 ± 7.1	10.5 ± 3.3	
	B220$^-$sIgA$^+$	19.6 ± 10.3	1.1 ± 0.4			
	B220$^+$sIgA$^+$	0.1 ± 0.1	0.1 ± 0.0	1.1 ± 0.4	1.3 ± 0.5	
+/+	CD3ε$^+$T cell	5.2 ± 1.7	1.4 ± 0.7	20.7 ± 13.2	2.7 ± 1.2	
	Eosinophil	1.8 ± 1.3	0.3 ± 0.2			
	B-1a					4.7 ± 1.5
	B-1b					0.9 ± 0.4
	B-2					6.0 ± 3.0

The results indicate the mean cell numbers ± SD ($\times 10^5$) calculated on the basis of Supplementary Fig. 1 ($n = 5$ for each group). There were not significantly different between *Syntenin-1* KO mice and WT mice. S-LP, small intestinal lamina propria; L-LP, large intestinal lamina propria; MLN, mesenteric lymph node; PP, Peyer's patch; PC, peritoneal cavity; sIgA, surface-IgA.

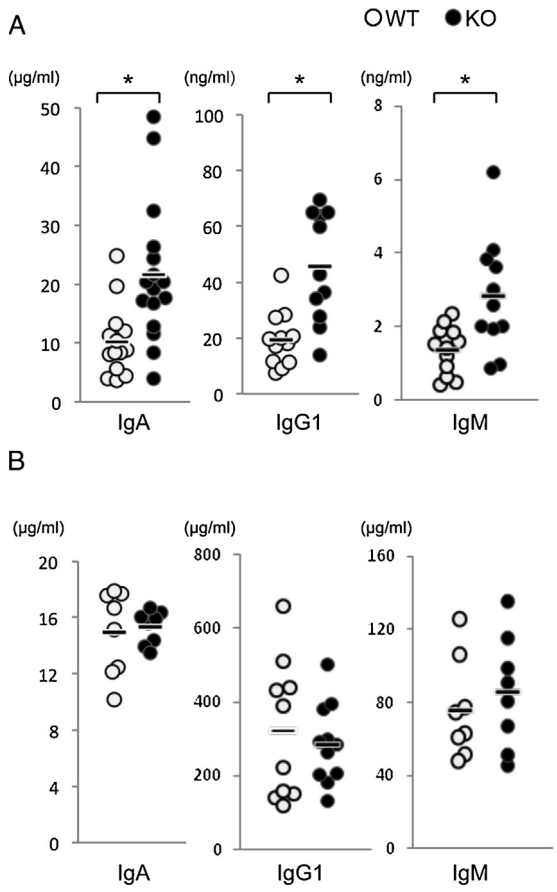

Fig. 3. Immunoglobulin levels in the fecal extract and serum of *Syntenin-1* KO mice. The concentrations of IgA, IgG1, and IgM in fecal extracts (A) and serum (B) in WT (○) and *Syntenin-1* KO (●) mice were determined by isotype-specific ELISA. Each spot represents an individual mouse (6–8 weeks old). The mean levels of immunoglobulins are presented as bars. Asterisks indicate statistically significant differences (*$p < 0.05$), as calculated by two-tailed Student's *t* test.

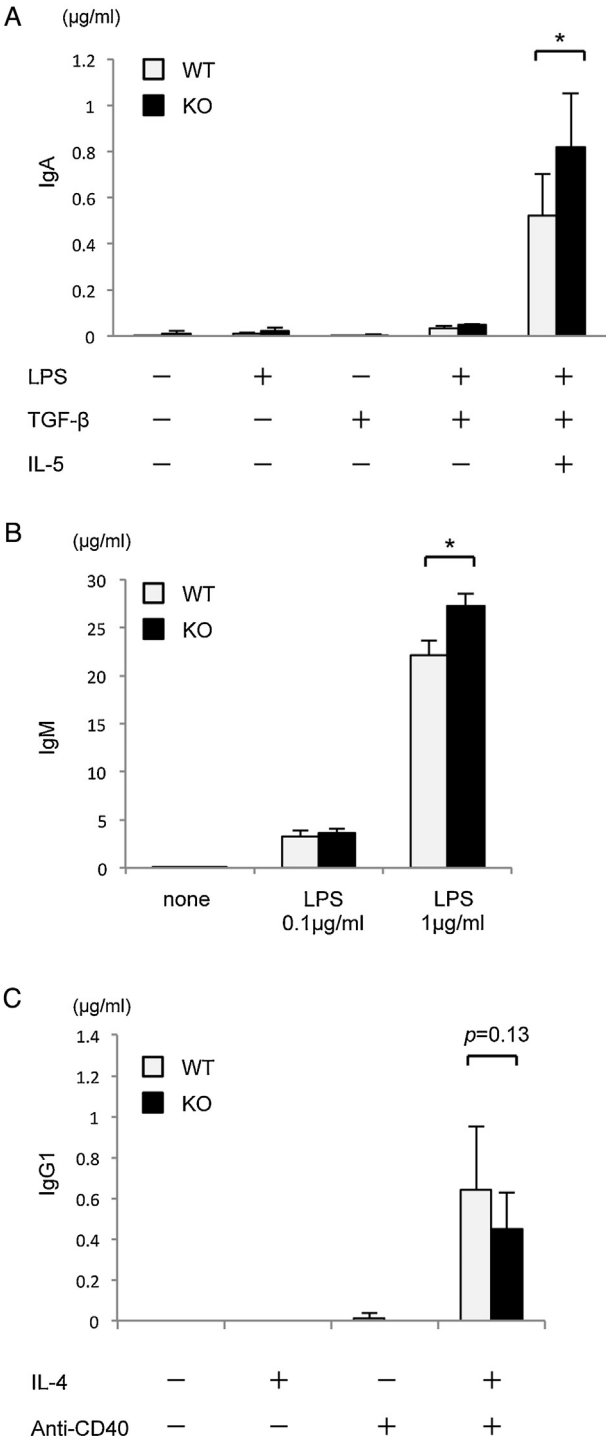

Fig. 4. Increased levels of IgA and IgM secretion from B cells obtained from *Syntenin-1* KO mice *in vitro*. Purified splenic resting B cells from WT (□) and *Syntenin-1* KO (■) mice were cultured with LPS, TGF-β and/or IL-5 for 7 days to induce IgA secretion (A), LPS for 5 days to induce IgM secretion (B), and IL-4 and anti-CD40 antibody for 5 days to induce IgG1 secretion (C). The levels of immunoglobulins in the supernatants were measured by ELISA. Data are shown as mean ± SD and are representative of two or three independent experiments performed in triplicate. Asterisks indicate statistically significant difference (*$p < 0.05$), as calculated by two-tailed Student's *t* test.

et al., 2001; Sala-Valdes et al., 2012) and imply that Syntenin-1 may play important roles in these organs.

Despite the broad expression of the Syntenin-1 protein in all the organs analyzed, the homozygous deletion of *Syntenin-1* was not lethal, and the *Syntenin-1* KO mice showed no obvious signs of diseases under specific pathogen-free conditions. It is possible that some redundant proteins may provide compensatory signals to maintain the homeostasis *in vivo*. In mice and humans, Syntenin has two isoforms, Syntenin-1 and Syntenin-2. Syntenin-2 shares 61% identity in amino acid sequence with Syntenin-1 in mice. Although little is known about the functions of Syntenin-2, it is possible that the presence of Syntenin-2 could compensate for the functions of Syntenin-1 in *Syntenin-1* KO mice. Dual deletion of those *Syntenin* genes might reveal their roles in immunity.

In this study, we focused on the relationship between Syntenin-1 and IL-5 signaling. IL-5 is one of the key regulators in mucosal immunity in mice, especially the development of B-1 B cells and IgA-producing cells and the production of intestinal IgA (Hiroi et al., 1999; Moon et al., 2004; Tominaga et al., 1991). Because Syntenin-1 is reported to interact with IL-5Rα *in vitro* and may affect IL-5 signaling (Geijsen et al., 2001), we investigated the role(s) of Syntenin-1 in gut-associated lymphoid organs. However, the lymphocyte populations in gut-associated lymphoid tissues were not significantly different between *Syntenin-1* KO mice and WT mice, and we found that the levels of immunoglobulins in fecal extract were higher in *Syntenin-1* KO mice. These results suggest that Syntenin-1 plays only minor roles in the IL-5/IL-5R pathway in the mucosal immune system and rather negatively regulates immunoglobulin production in the intestine.

We showed that the IgA and IgM production from stimulated spleen B cells increased in *Syntenin-1* KO B cells. Under physiological conditions, intestinal B cells differentiate into plasma cells via

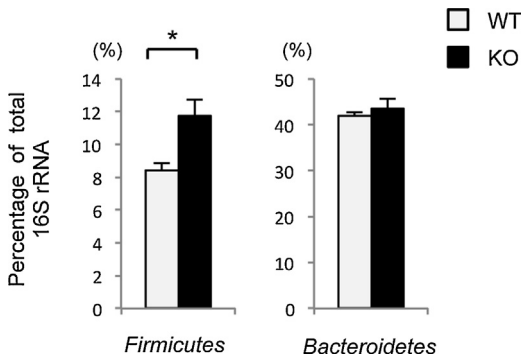

Fig. 5. Percentage of total 16S rRNA gene of phylum *Firmicutes* and *Bacteroidetes* in stool from individually housed *Syntenin-1* KO (■) and WT (□) lettermate mice ($n = 5$). Quantitative real-time PCR assays of 16S ribosomal RNA (rRNA) gene were performed by using total bacterial primers and phylum-specific primers. The relative ratio of each phylum to total bacteria was measured. Data are shown as mean ± SD and an asterisk indicates statistically significant differences ($^*p < 0.05$), as calculated by two-tailed Student's *t* test.

T cell-dependent and T cell-independent pathways, and secreted immunoglobulins at LP are then transported to the lumen by immunoglobulin receptors (Strugnell and Wijburg, 2010; Horton and Vidarsson, 2013). We showed the lymphocyte populations including intestinal B cells and $B220^{-}IgA^{+}$ cells (which represent plasma cells) were not significantly different and IgA secreting cells in LP were not also different by using ELISPOT assays (data not shown). Although the precise mechanisms were not delineated in this study, our *in vitro* results indicate that such an enhanced immunoglobulin production in the intestine is caused by excessive immunoglobulin production of individual *Syntenin-1*-deficient plasma cells.

Mucosal IgA plays crucial roles in host defense and maintenance of normal gut microbiota (Fagarasan et al., 2002; Horton and Vidarsson, 2013; Strugnell and Wijburg, 2010; Suzuki et al., 2004). Additionally, recent studies have shown that mucosal IgM and IgG can also provide humoral protection from various pathogens (Horton and Vidarsson, 2013; Saeland et al., 2003; Stapleton et al., 2011). On the other hand, mucosal IgG or IgM production is excessively increased in patients with an inflammatory bowel disease such as ulcerative colitis and Crohn's disease (Helgeland et al., 1992; Macpherson et al., 1996; Thoree et al., 2002). In this manner, the relationship between host immunity and intestinal microbiota is essential to maintain homeostatic balance in the gut. In this study, we showed that a distribution of intestinal microbiota in *Syntenin-1* KO mice could be influenced at the phylum level. Although the detailed analysis at the genus level was not examined in this study, it is possible that Syntenin-1 is involved in the maintenance of normal intestinal microbiota and mucosal immune balance by regulating immunoglobulin production.

Previously, we demonstrated that Syntenin-1 exists in the human colostrum and could induce IgA production from naive B cells (Sira et al., 2009). In the present study, we found the negative regulation of Syntenin-1 in IgA production. These incompatible functions of Syntenin-1 may be due to differences among species or effector sites. As our present data might be influenced by the function of extracellular Syntenin-1 in milk, we analyzed littermate offspring from heterozygous breeding pairs to exclude such differences in components of milk in this study. Further studies are necessary to clarify the role and mechanisms of actions of Syntenin-1 in the colostrum *in vivo*.

In summary, in this article we described the generation of *Syntenin-1* KO mice and provided new evidence that Syntenin-1 negatively regulates immunoglobulin production in the intestine. Although Syntenin-1 has been reported to have various functions *in vitro*, additional studies are needed to clarify the mechanisms in detail, especially *in vivo*. Through future studies of *Syntenin-1* KO mice, it will be possible to provide novel evidence of the involvement and functions of Syntenin-1 not only mucosal immunity but also cancer metastasis, protein recycling, exosome formation, and neural network formation.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

This work was supported by a Grant-in Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (Grant number 221S0003). We thank the members of the Departments of Molecular Neuroscience, Immunobiology and Pharmacological Genetics, and Pediatrics of the University of Toyama for their cooperation and for their constructive comments on this study. We also thank Kanako Konno for technical assistance.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.imbio.2014.12.003.

References

Alewine, C., Olsen, O., Wade, J.B., Welling, P.A., 2006. TIP-1 has PDZ scaffold antagonist activity. Mol. Biol. Cell 17, 4200–4211.
Baietti, M.F., Zhang, Z., Mortier, E., Melchior, A., Degeest, G., Geeraerts, A., Ivarsson, Y., Depoortere, F., Coomans, C., Vermeiren, E., Zimmermann, P., David, G., 2012. Syndecan-syntenin-ALIX regulates the biogenesis of exosomes. Nat. Cell Biol. 14, 677–685.
Beekman, J.M., Verhagen, L.P., Geijsen, N., Coffer, P.J., 2009. Regulation of myelopoiesis through syntenin-mediated modulation of IL-5 receptor output. Blood 114, 3917–3927.
Beekman, J.M., Vervoort, S.J., Dekkers, F., van Vessem, M.E., Vendelbosch, S., Brugulat-Panes, A., van Loosdregt, J., Braat, A.K., Coffer, P.J., 2012. Syntenin-mediated regulation of Sox4 proteasomal degradation modulates transcriptional output. Oncogene 31, 2668–2679.
Boukerche, H., Su, Z.Z., Emdad, L., Baril, P., Balme, B., Thomas, L., Randolph, A., Valerie, K., Sarkar, D., Fisher, P.B., 2005. mda-9/Syntenin: a positive regulator of melanoma metastasis. Cancer Res. 65, 10901–10911.
Boukerche, H., Su, Z.Z., Emdad, L., Sarkar, D., Fisher, P.B., 2007. mda-9/Syntenin regulates the metastatic phenotype in human melanoma cells by activating nuclear factor-kappaB. Cancer Res. 67, 1812–1822.
Boukerche, H., Su, Z.Z., Prevot, C., Sarkar, D., Fisher, P.B., 2008. mda-9/Syntenin promotes metastasis in human melanoma cells by activating c-Src. Proc. Natl. Acad. Sci. U. S. A. 105, 15914–15919.
Chen, F., Du, Y., Zhang, Z., Chen, G., Zhang, M., Shu, H.B., Zhai, Z., Chen, D., 2008. Syntenin negatively regulates TRAF6-mediated IL-1R/TLR4 signaling. Cell. Signal 20, 666–674.
Chimura, T., Launey, T., Ito, M., 2011. Evolutionarily conserved bias of amino-acid usage refines the definition of PDZ-binding motif. BMC Genomics 12, 300.
Das, S.K., Bhutia, S.K., Kegelman, T.P., Peachy, L., Oyesanya, R.A., Dasgupta, S., Sokhi, U.K., Azab, B., Dash, R., Quinn, B.A., Kim, K., Barral, P.M., Su, Z.Z., Boukerche, H., Sarkar, D., Fisher, P.B., 2012. MDA-9/syntenin: a positive gatekeeper of melanoma metastasis. Front. Biosci. 17, 1–15.
Estrach, S., Legg, J., Watt, F.M., 2007. Syntenin mediates Delta1-induced cohesiveness of epidermal stem cells in culture. J. Cell Sci. 120, 2944–2952.
Fagarasan, S., Muramatsu, M., Suzuki, K., Nagaoka, H., Hiai, H., Honjo, T., 2002. Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. Science 298, 1424–1427.
Fukaya, M., Tsujita, M., Yamazaki, M., Kushiya, E., Abe, M., Akashi, K., Natsume, R., Kano, M., Kamiya, H., Watanabe, M., Sakimura, K., 2006. Abundant distribution of TARP gamma-8 in synaptic and extrasynaptic surface of hippocampal neurons and its major role in AMPA receptor expression on spines and dendrites. Eur. J. Neurosci. 24, 2177–2190.
Geijsen, N., Uings, I.J., Pals, C., Armstrong, J., McKinnon, M., Raaijmakers, J.A., Lammers, J.W., Koenderman, L., Coffer, P.J., 2001. Cytokine-specific transcriptional regulation through an IL-5Ralpha interacting protein. Science 293, 1136–1138.
Gordon-Alonso, M., Rocha-Perugini, V., Alvarez, S., Moreno-Gonzalo, O., Ursa, A., Lopez-Martin, S., Izquierdo-Useros, N., Martinez-Picado, J., Munoz-Fernandez,

M.A., Yanez-Mo, M., Sanchez-Madrid, F., 2012. The PDZ-adaptor protein syntenin-1 regulates HIV-1 entry. Mol. Biol. Cell 23, 2253–2263.
Grootjans, J.J., Zimmermann, P., Reekmans, G., Smets, A., Degeest, G., Durr, J., David, G., 1997. Syntenin, a PDZ protein that binds syndecan cytoplasmic domains. Proc. Natl. Acad. Sci. U. S. A. 94, 13683–13688.
Gupta, S., Braun, A., Morowski, M., Premsler, T., Bender, M., Nagy, Z., Sickmann, A., Hermanns, H.M., Bosl, M., Nieswandt, B., 2012. CLP36 is a negative regulator of glycoprotein VI signaling in platelets. Circ. Res. 111, 1410–1420.
Helgeland, L., Tysk, C., Jarnerot, G., Kett, K., Lindberg, E., Danielsson, D., Andersen, S.N., Brandtzaeg, P., 1992. IgG subclass distribution in serum and rectal mucosa of monozygotic twins with or without inflammatory bowel disease. Gut 33, 1358–1364.
Hirbec, H., Martin, S., Henley, J.M., 2005. Syntenin is involved in the developmental regulation of neuronal membrane architecture. Mol. Cell. Neurosci. 28, 737–746.
Hiroi, T., Yanagita, M., Iijima, H., Iwatani, K., Yoshida, T., Takatsu, K., Kiyono, H., 1999. Deficiency of IL-5 receptor alpha-chain selectively influences the development of the common mucosal immune system independent IgA-producing B-1 cell in mucosa-associated tissues. J. Immunol. 162, 821–828.
Horton, R.E., Vidarsson, G., 2013. Antibodies and their receptors: different potential roles in mucosal defense. Front. Immunol. 4, 200.
Jannatipour, M., Dion, P., Khan, S., Jindal, H., Fan, X., Laganiere, J., Chishti, A.H., Rouleau, G.A., 2001. Schwannomin isoform-1 interacts with syntenin via PDZ domains. J. Biol. Chem. 276, 33093–33100.
Jeon, H.Y., Das, S.K., Dasgupta, S., Emdad, L., Sarkar, D., Kim, S.H., Lee, S.G., Fisher, P.B., 2013. Expression patterns of MDA-9/syntenin during development of the mouse embryo. J. Mol. Histol. 44, 159–166.
Kopf, M., Brombacher, F., Hodgkin, P.D., Ramsay, A.J., Milbourne, E.A., Dai, W.J., Ovington, K.S., Behm, C.A., Kohler, G., Young, I.G., Matthaei, K.I., 1996. IL-5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. Immunity 4, 15–24.
Koroll, M., Rathjen, F.G., Volkmer, H., 2001. The neural cell recognition molecule neurofascin interacts with syntenin-1 but not with syntenin-2, both of which reveal self-associating activity. J. Biol. Chem. 276, 10646–10654.
Lin, J.J., Jiang, H., Fisher, P.B., 1998. Melanoma differentiation associated gene-9, mda-9, is a human gamma interferon responsive gene. Gene 207, 105–110.
Macpherson, A., Khoo, U.Y., Forgacs, I., Philpott-Howard, J., Bjarnason, I., 1996. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. Gut 38, 365–375.
Matsuki, T., Watanabe, K., Fujimoto, J., Kado, Y., Takada, T., Matsumoto, K., Tanaka, R., 2004. Quantitative PCR with 16S rRNA-gene-targeted species-specific primers for analysis of human intestinal bifidobacteria. Appl. Environ. Microbiol. 70, 167–173.
Matsuki, T., Watanabe, K., Fujimoto, J., Miyamoto, Y., Takada, T., Matsumoto, K., Oyaizu, H., Tanaka, R., 2002. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. Appl. Environ. Microbiol. 68, 5445–5451.
Mita, S., Tominaga, A., Hitoshi, Y., Sakamoto, K., Honjo, T., Akagi, M., Kikuchi, Y., Yamaguchi, N., Takatsu, K., 1989. Characterization of high-affinity receptors for interleukin 5 on interleukin 5-dependent cell lines. Proc. Natl. Acad. Sci. U. S. A. 86, 2311–2315.
Miya, K., Inoue, R., Takata, Y., Abe, M., Natsume, R., Sakimura, K., Hongou, K., Miyawaki, T., Mori, H., 2008. Serine racemase is predominantly localized in neurons in mouse brain. J. Comp. Neurol. 510, 641–654.
Moon, B.G., Takaki, S., Miyake, K., Takatsu, K., 2004. The role of IL-5 for mature B-1 cells in homeostatic proliferation, cell survival, and Ig production. J. Immunol. 172, 6020–6029.
Ohno, K., Koroll, M., El Far, O., Scholze, P., Gomeza, J., Betz, H., 2004. The neuronal glycine transporter 2 interacts with the PDZ domain protein syntenin-1. Mol. Cell. Neurosci. 26, 518–529.
Pols, M.S., Klumperman, J., 2009. Trafficking and function of the tetraspanin CD63. Exp. Cell Res. 315, 1584–1592.
Saeland, E., Vidarsson, G., Leusen, J.H., Van Garderen, E., Nahm, M.H., Vile-Weekhout, H., Walraven, V., Stemerding, A.M., Verbeek, J.S., Rijkers, G.T., Kuis, W., Sanders, E.A., Van De Winkel, J.G., 2003. Central role of complement in passive protection by human IgG1 and IgG2 anti-pneumococcal antibodies in mice. J. Immunol. 170, 6158–6164.
Sala-Valdes, M., Gordon-Alonso, M., Tejera, E., Ibanez, A., Cabrero, J.R., Ursa, A., Mittelbrunn, M., Lozano, F., Sanchez-Madrid, F., Yanez-Mo, M., 2012. Association of syntenin-1 with M-RIP polarizes Rac-1 activation during chemotaxis and immune interactions. J. Cell Sci. 125, 1235–1246.
Schilham, M.W., Oosterwegel, M.A., Moerer, P., Ya, J., de Boer, P.A., van de Wetering, M., Verbeek, S., Lamers, W.H., Kruisbeek, A.M., Cumano, A., Clevers, H., 1996. Defects in cardiac outflow tract formation and pro-B-lymphocyte expansion in mice lacking Sox-4. Nature 380, 711–714.
Sira, M.M., Yoshida, T., Takeuchi, M., Kashiwayama, Y., Futatani, T., Kanegane, H., Sasahara, A., Ito, Y., Mizuguchi, M., Imanaka, T., Miyawaki, T., 2009. A novel immunoregulatory protein in human colostrum, syntenin-1, for promoting the development of IgA-producing cells from cord blood B cells. Int. Immunol. 21, 1013–1023.
Stapleton, N.M., Andersen, J.T., Stemerding, A.M., Bjarnarson, S.P., Verheul, R.C., Gerritsen, J., Zhao, Y., Kleijer, M., Sandlie, I., de Haas, M., Jonsdottir, I., van der Schoot, C.E., Vidarsson, G., 2011. Competition for FcRn-mediated transport gives rise to short half-life of human IgG3 and offers therapeutic potential. Nat. Commun. 2, 599.
Stephenson, L.M., Sammut, B., Graham, D.B., Chan-Wang, J., Brim, K.L., Huett, A.S., Miletic, A.V., Kloeppel, T., Landry, A., Xavier, R., Swat, W., 2007. DLGH1 is a negative regulator of T-lymphocyte proliferation. Mol. Cell. Biol. 27, 7574–7581.
Strugnell, R.A., Wijburg, O.L., 2010. The role of secretory antibodies in infection immunity. Nat. Rev. Microbiol. 8, 656–667.
Sun, B., Mallampati, S., Gong, Y., Wang, D., Lefebvre, V., Sun, X., 2013. Sox4 is required for the survival of pro-B cells. J. Immunol. 190, 2080–2089.
Suzuki, K., Meek, B., Doi, Y., Muramatsu, M., Chiba, T., Honjo, T., Fagarasan, S., 2004. Aberrant expansion of segmented filamentous bacteria in IgA-deficient gut. Proc. Natl. Acad. Sci. U. S. A. 101, 1981–1986.
Taniguchi, M., Sanbo, M., Watanabe, S., Naruse, I., Mishina, M., Yagi, T., 1998. Efficient production of Cre-mediated site-directed recombinants through the utilization of the puromycin resistance gene, pac: a transient gene-integration marker for ES cells. Nucleic Acids Res. 26, 679–680.
Thoree, V.C., Golby, S.J., Boursier, L., Hackett, M., Dunn-Walters, D.K., Sanderson, J.D., Spencer, J., 2002. Related IgA1 and IgG producing cells in blood and diseased mucosa in ulcerative colitis. Gut 51, 44–50.
Tominaga, A., Takaki, S., Koyama, N., Katoh, S., Matsumoto, R., Migita, M., Hitoshi, Y., Hosoya, Y., Yamauchi, S., Kanai, Y., et al., 1991. Transgenic mice expressing a B cell growth and differentiation factor gene (interleukin 5) develop eosinophilia and autoantibody production. J. Exp. Med. 173, 429–437.
Yoshida, T., Ikuta, K., Sugaya, H., Maki, K., Takagi, M., Kanazawa, H., Sunaga, S., Kinashi, T., Yoshimura, K., Miyazaki, J., Takaki, S., Takatsu, K., 1996. Defective B-1 cell development and impaired immunity against *Angiostrongylus cantonensis* in IL-5R alpha-deficient mice. Immunity 4, 483–494.
Zimmermann, P., Zhang, Z., Degeest, G., Mortier, E., Leenaerts, I., Coomans, C., Schulz, J., N'Kuli, F., Courtoy, P.J., David, G., 2005. Syndecan recycling is controlled by syntenin-PIP2 interaction and Arf6. Dev. Cell 9, 377–388.