A Newly Generated DOCK8-expressing T Follicular Helper Cell Type, aiCD4 T Cell, Causes Systemic Lupus Erythematosus: Self-Organized Criticality Theory

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SUMMARY

Systemic lupus erythematosus (SLE) is a prototypical autoimmune disease of unknown cause. We show here that a novel T follicular helper cell type expressing the guanine nucleotide exchange factor DOCK8 on the cell surface causes SLE. These cells, which we have designated autoantibody-inducing CD4 T (*ai*CD4 T) cells, are generated after resuscitation from anergy following strong TCR stimulation by antigen. When mice normally not prone to autoimmune disease were repeatedly immunized with an antigen such as OVA, they generated DOCK8⁺ CD4 T cells. These DOCK8⁺ CD4 T cells, *in vivo* and also upon transfer to naïve mice, induced a variety of autoantibodies and lesions characteristic of SLE. TCR repertoire analyses showed that a substantial number of novel TCR repertoires were generated in the DOCK8⁺ CD4 T cells, which induced novel autoantibodies upon transfer to naïve mice. DOCK8⁺ CD4 T cells are localized in splenic red pulp, the space immunoreactive against a variety of antigens, and specifically increased in the peripheral blood of SLE patients in association with disease activity. Anti-DOCK8 antibody treatment ameliorated the lesions induced by DOCK8⁺ CD4 T cells and in lupus model (NZB x W) F1 mice. Thus, when CD4 T cells are overstimulated by an external disturbance, i.e., repeatedly stimulated with antigen, to levels that surpass the system's self-organized criticality, these cells express DOCK8 on the cell surface and

60 acquire autoreactivity *via* TCR re-revision at the periphery. These DOCK8⁺ CD4 T cells
61 subsequently induce a variety of autoantibodies and SLE.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a classical systemic autoimmune disease characterized by fever, organ pathologies such as erythematous skin rash or lupus nephritis, and the generation of a variety of autoantibodies including anti-double stranded DNA (anti-dsDNA) Ab. Although SLE has long been suspected to be caused by some type of autoimmune responses, the details of its etiology have remained elusive. Interferon α (IFN α) has been proposed to be a principal driver of SLE (1-16), however, it has also been reported that IFN α can sometimes be protective against SLE (17,18), IFN α treatment itself does not induce anti-Sm Ab, which is considered diagnostic of SLE (1,16,19,20), and monoclonal anti-IFN α Ab therapy is only modestly effective in treating SLE (21-23).

In our studies investigating the etiology of SLE, we found that we could reproducibly induce autoimmune disease in mice normally not prone to such diseases by repeated stimulation with an antigen. This repeated stimulation leads to the generation of a novel type of CD4 T cell, which we designate *ai*CD4 T cell (autoantibody-inducing CD4 T cell), capable of directing the production of a variety of autoantibodies. We have proposed that the induction of *ai*CD4 T cells is due to overstimulation of CD4 T cells to levels that exceed the immune system's "self-organized criticality" (24). The *ai*CD4 T cells thus

generated subsequently overstimulate CD8⁺ T cells, driving them to become cytotoxic T lymphocytes (CTL) *via* antigen cross-presentation (24,25), and this ultimately results in the development of SLE. Thus, SLE is induced not by an autoimmune response, but by the expansion of *ai*CD4 T cells having *de novo* autoreactivity. The key player in this pathogenesis is the *ai*CD4 T cell, and in the present study, we have tried to clarify the nature of these cells. The contribution of *ai*CD4 T cells to the pathogenesis of SLE was investigated by cell transfer studies in mice and by quantification of *ai*CD4 T cells in the peripheral blood and tissue of patients diagnosed with SLE. We also investigated a potential therapy for SLE using an antibody directed against these cells.

Identification of DOCK8⁺ CD4 T cell as *ai*CD4 T cell that induces SLE

To isolate and characterize *ai*CD4 T cells, we first induced the *ai*CD4 T cells in BALB/c mice by 12x repeated intraperitoneal (i.p.) immunization with OVA without adjuvants. Next, various subsets of splenic CD4 T cells were taken and transferred to naïve recipient mice. For each subset we assessed the generation of rheumatoid factor (RF) and anti-dsDNA Ab in the recipient mice and further found that these autoantibodies could be induced by transferring the CD45RB^{lo} CD122^{lo} PD-1⁺ CD4 T cell subset (Extended Data Fig. 1a). The membrane and cytosolic fractions were extracted from these cells, and

subjected to electrophoresis and silver staining. Protein bands differentially expressed were then excised and analyzed by in-gel digestion followed by mass spectrometry. We found that the guanine nucleotide exchange factor DOCK8 was uniquely expressed in the membrane fraction of this T cell subset (Fig. 1a). In mice repeatedly immunized with OVA, the number of DOCK8⁺ CD4 T cells gradually increased in the spleen up to the final 12x immunization (Fig. 1b). We then isolated DOCK8⁺ CD4 T cells from these OVA-immunized mice and transferred them to naïve recipient mice. The recipient mice generated RF and anti-dsDNA Ab and developed renal disease (Extended Data Figs. 2a, 2b). To more closely examine the role of the transferred DOCK8⁺ CD4 T cells in the development of renal disease, we immunized BALB/c mice 8x with OVA. At this time point, nephritis-inducing CTLs had yet to mature. We then treated naïve mice with anti-CD4 Ab to deplete their CD4 T cells and transferred into them, the DOCK8⁺ CD4 T cells isolated from the spleens of the BALB/c mice immunized 12x with OVA. The recipients developed autoantibodies and renal disease characteristic of WHO class IV and V (26) advanced disease (Figs. 1c, 1d). This indicates that DOCK8⁺ CD4 T cells are required for the induction of autoantibodies and renal disease in this mouse model. In addition to kidney disease similar to SLE, transfer of DOCK8⁺ CD4 T cells into naïve mice also induced dermatitis often accompanied by dermal perineuritis in 4 out of 5 recipient mice,

but none in mice receiving control DOCK8⁻ CD4 T cells (Fig. 1e, 1f; $p=0.0476$ by Fisher's exact test). Panniculitis in the dermis and skin epidermal liquefaction degeneration, classical lesion of SLE, were observed in 2 of 5 DOCK8⁺ CD4 T recipients, and none in the control mice (Fig. 1e). Lung interstitial pneumonitis was seen in 4 of 5 DOCK8⁺ CD4 T recipients, and 1 of 5 control mice (Fig. 1f, 1g). Pericholangitis with liver cell necrosis was seen in 2 of 5 DOCK8⁺ CD4 T recipients, and none in the control (Fig. 1f, 1g). Diffuse thyroiditis was seen in 2 of 5 DOCK8⁺ CD4 T recipients, and none in the control. Splenic perivascular fibrosis with amyloid-like deposition and classical onion skin lesion pathognomonic of SLE (27) were seen in all 5 of 5 DOCK8⁺ CD4 T recipients, and none in the control (Fig. 1f, 1g; $p=0.0079$ by Fisher's exact test). Lung interstitial pneumonitis seen in the DOCK8⁺ CD4 T recipients was often accompanied by angiitis (Fig. 1g).

DOCK8⁺ CD4 T cell is a novel T follicular helper cell, located in splenic red pulp

DOCK8⁺ CD4 T cells were labelled with Au-tagged anti-DOCK8 Ab and examined by immunoelectron microscopy. These cells were observed to be relatively large lymphocytes with abundant endoplasmic reticulum and mitochondria (Fig. 2a). DOCK8⁺ CD4 T cells were CD44⁺, CD62L⁻, ICOS⁺ PD-1⁺ CXCR5⁺, CD3⁺, and Thy1.2⁺, indicative of activated T follicular helper cells (28,29) (Fig. 2b; Extended Data Fig.3, 4), in particular, the type of long-lived germinal center (GC) effector T follicular helper cells (30,31) which

also express FR4 and GL7 (Fig.2b). These cells also expressed Ly6C, LFA1, CCR4, CD123, CD23 (32,33) (Fig.2b; Extended Data Fig. 3, 4a; Supplementary Table 1). The DOCK8⁺ CD4 T cells produced higher amounts of IFN γ , IL-4, IL-6, IL-17, IL-21, and IL-22 (Fig. 2c), whereas IL-12 or IFN α was undetectable. The CD25⁺ Foxp3⁺ population were not increased by 12x stimulation with OVA (Extended data Fig. 4b).

In the patients with active SLE, DOCK8⁺ CD4 T cells were mainly located in splenic sinus, red pulp, in a disease-specific fashion (Fig. 2d). This is, likely because they lack CCR7, and also express increased CD147 and LFA1 (Fig. 2b; Extended Data Fig. 3), which should prevent T cells entry from the vasculature into T zones and anchor them against blood flow (34). Splenic sinus, red pulp, is a suitable location to encounter circulating antigens and nurse the autoreactive B cells that derive from bone marrow or shuttle between the marginal and follicular zones by expressing CD138, undergoing class-switch recombination and readily differentiating into plasma cells (35-37). In the spleen of 12x OVA-immunized mice, indeed, activated B cells with CD138 markers classified as plasma cells and follicular B cells were significantly increased (Extended Data Fig. 5). In the red pulp, DOCK8⁺ CD4 T cells could also meet the red pulp macrophages which are highly efficient at cross-presenting antigens to T cells and maturing CTLs (38) and thus important for the induction of lupus tissue injuries (24,25).

Circulating DOCK8⁺ CD4 T cells increase in association with SLE disease activity

Since T follicular helper cells are measurable at the periphery and their frequency reflects *in vivo* dynamics (39), we tried to measure DOCK8⁺ CD4 T cells in the peripheral blood of SLE patients. Higher numbers of circulating DOCK8⁺ CD4 T cells were found in the peripheral blood of patients, and these numbers correlated with higher SLEDAI disease activity scores (40) (Fig. 2e). This indicates that surface expression of DOCK8 on CD4 T cells was associated with high activation levels of CD4 T cells and SLE disease activity. It was noted here that although DOCK8⁺ CD4 T cells were significantly decreased by conventional therapy, a substantial number of patients remained with slightly increased DOCK8⁺ CD4 T cell numbers compared with disease controls. This would be compatible with the therapeutic sequelae of SLE in which the patients require tapered but continued therapy with prednisolone or immunosuppressive agents for several years, suggesting that DOCK8⁺ CD4 T cells, once activated, did not disappear quickly in response to conventional therapy.

DOCK8⁺ CD4 T cells induce autoimmunity, characteristic of SLE

In the DOCK8⁺ CD4 T cells, genes encoding components of V(D)J recombinase complex, recombination-activating genes 1 and 2 (RAG1/2), terminal deoxynucleotidyl transferase (TdT) and surrogate TCR α chain (pT α), were up-regulated (Fig. 3a). TCR basal signaling

molecules, known to suppress RAG expression (41-43), such as CD3 ζ , ZAP70, LAT, SLP-76, PLC γ 1, ERK, Akt, and NFAT1/2 and their nuclear translocation were down-regulated (Fig. 3b), and the transcription factor GATA3 essential for RAG assembly (44-46) was up-regulated (Fig. 2b).

TCR repertoire analyses showed that the diversity of TCR gene usage was restricted and skewed in the direction of novel TCR repertoires, even though the stimulation was a single antigen (OVA) (Fig. 3c; Extended Data Fig. 6, 7; Supplementary Table 2). The heatmap representation of this repertoire reveals that this novel TCR gene usage, as characterized by a combination of novel *TRAV* and *TRAJ* gene variations, was restricted to the DOCK8⁺ CD4 T cells (Fig. 3c). A diversity score calculation (47,48), which included the inverted Simpson index, showed that the weighted arithmetic mean of the proportional abundances of TCR repertoires in the DOCK8⁺ CD4 T cells was substantially restricted; 1.22~5.95% of the DOCK8⁻ CD4 T cells (Extended Data Table 1). Unique TCR repertoires, unique sequence reads (USRs) having the combination of *TRAV* and *TRAJ*, and amino acid sequences of the CDR3 that had no identity with other sequence reads, were dominantly found in the DOCK8⁺ CD4 T cells compared to DOCK8⁻ CD4 T cells from the same 12x OVA-immunized mice, or compared to CD4 T cells from control mice mock-immunized with PBS (Extended Data Table 2;

Supplementary Table 2). A variety of autoantibodies were induced when these DOCK8⁺ CD4 T cells from 12x OVA-immunized mice were transferred into naïve mice (Fig. 1c, 3d). These findings together indicate that the DOCK8⁺ CD4 T cells had undergone TCR re-revision at the periphery and were able to induce novel antibodies, including autoantibodies.

TCR gene chromatin accessibility

*ai*CD4 T cells were generated following heavily repeated TCR stimulation with antigen, which released them from their anergic state, irrespective of the antigen used or genetic background, by the help of NKT cells (Extended Data Fig. 8; ref. 24). Thus, even repeated challenge by Staphylococcus enterotoxin B (SEB), which cross-links TCR and MHC, could induce *ai*CD4 T cells (Extended Data Fig.8; ref. 24). Likely due to these strong stimulation, DOCK8 re-localized from its normal site in the cytoplasm to the cell membrane (49-51) and the nuclear lamina (52,53) (Fig. 2a). Further, the progenitor, transforming or reprogramming factors responsible for RAG modulation and chromosomal accessibility (54-65) such as *KLF4*, *KLF5*, *Zeb2*, *EBF1*, *PAX5*, *PAX6*, *Bcl11a* and its target *Tcf4* were up-regulated (Fig. 3e; Supplementary Table 1). Nuclear DOCK8 (65), by binding and activating cdc42 (52,53), can activate nuclear-WASP and up-regulate H3K4 trimethyltransferase (52,53) and thus may disrupt the association of

TCR genes with nuclear lamina *via* actin movement, thereby enhance the chromatin accessibility to transcription factor E2-2 (*Tcf4*) (Fig. 3e). PU.1 (*Spi.1*), which is normally undetectable in mature T cells, was also expressed, and the genes up-regulated by PU.1 such as *Bcl11a*, *Lmo2*, *Mef2c*, *Syk*, *Lyn* and *Cd300a*, but not *Bcl11b*, were up-regulated (54,56,57) (Fig. 3e). Cell surface expression of DOCK8 should also strengthen immunological synapse formation (50,51,65-67) together with LFA1 or HkRP3, WIP, WASP and PtdIns-3-kinase (Fig. 2b, 3e; Extended Fig. 4; Supplementary Table 1).

We performed chromatin-immunoprecipitation sequencing (ChIP-seq) to map histone acetylation (AcH3) and H3K4 methylation (H3K4me3) of the *TCRB* and *TCRA* genes in the *ai*CD4 T cells generated by 8x stimulation with SEB. H3K4me3 was found to be enriched at the *TCRA* J α 45, J α 35, J α 24, J α 12, J α 6 and E α gene segments and *TCRB* 5'D β 1, 5'D β 2, 3'D β 2, and E β gene segments of both control PBS-treated and experimental 8x SEB-stimulated V β 8⁺ *ai*CD4 T cells. This result is in accordance with the previous reports showing that the TCR gene recombination center is an open structure (68-72) (Extended Data Fig. 9). However, in the 8x SEB-stimulated *ai*CD4 T cells, H3K4me3 was also found in the 5' proximal V α 1, V α 16, V α 17, V α 21, TEA, V β 8.3, V β 6, and V β 18 gene segments. These findings are in line with the result of TCR repertoire analysis in the 12x OVA-stimulated DOCK8⁺ *ai*CD4 T cells that 5' proximal V α genes

were dominantly expressed (Extended Data Table 2). To confirm that V(D)J recombination took place in *ai*CD4 T cells, we performed ligation-mediated PCR (LM-PCR) to detect blunt-end DNA fragments harboring a rearranged coding *Jα* region flanked by recombination signal sequences (RSS). We identified the rearranged intermediates corresponding to TCRα joining region 12 (TCRAJ12) in the splenocytes of 8x SEB-stimulated mice (Extended Data Fig. 9). Expression of the *Vα* and *Jα* genes distal from TEA in autoimmunity were compatible with the general rules showing the stepwise and coordinated proximal-to-distal progressions of *Vα* and *Jα* usage on individual *TCRA* alleles in CD4⁺ CD8⁺ thymocytes (73). Thus, while we were unable to show directly the contribution of transcription factor E2-2 or DOCK8 to TCR gene transactivation, the results showed that some novel TCR chromatin regions, particularly distal *Vα* and *J* genes, were accessible in *ai*CD4 T cells.

Treatment of SLE in mice by anti-DOCK8 antibody

We treated 12x OVA-immunized mice with anti-DOCK8 Ab 24 hours before the 6x, 8x, 10x and 12x immunization with OVA, and the mice were studied 9 days after final, 12x, immunization. Anti-DOCK8 Ab-treated mice developed lower titers of RF and anti-Sm and anti-dsDNA Abs compared to control isotype Ig-treated mice (Fig. 4a). Renal disease was significantly ameliorated and almost cured as assessed by histology (Fig. 4b). Lesions

of the skin (Fig. 1e panel E; Fig. 1f), lungs and thyroid were also significantly ameliorated by anti-DOCK8 Ab treatment (Fig. 1f), and the thymic cortex was significantly atrophied.

We also treated female (NZBxNZW) F1 mice, a classical lupus model (74), with anti-DOCK8 Ab every 15 to 24 weeks. Autoantibodies, RF, anti-Sm Ab and anti-dsDNA Ab, and proteinuria were significantly decreased in the DOCK8 Ab-treated mice compared to controls (Fig. 4c), suggesting that generation of disease in this lupus-prone mouse model was mechanistically similar to that generated in antigen-stimulated BALB/c and C57BL/6 mice.

Relationship to interferon α (IFN α) as a cause of SLE

We also observed that IFN response genes were up-regulated in DOCK8⁺ CD4 T cells (Extended Data Fig. 10; Supplementary Table 1). Recent studies have shown that activation of enhancer-like promoter are preferentially associated with the expression of housekeeping or stress-response genes by default, including IFN response genes (75,76). Upon chronic infection, T cells shift towards a T follicular helper phenotype and up-regulate IFN signature genes (77,78). Tcf4, which was up-regulated in DOCK8⁺ CD4 T cells, is a transcription factor specific for plasmacytoid dendritic cells (pDC) that promotes expression of the pDC-enriched genes such as *SpiB*, *Irf8* and *Zeb2* (79-81) (Fig.3e; Extended Data Fig. 10; Supplementary Table 1). Microarray analyses show that

DOCK8⁺ CD4 T cells share some characteristics of pDC (Fig. 3e), however, pDC numbers per se were not increased in 12x OVA-immunized mice (Extended Data Fig. 10). Serum IFN α was also not increased in the sera of 12x OVA- or 8x SEB-stimulated mice (data not shown), nor in most of the SLE patients studied (Fig. 2e). Serum IFN α was slightly higher in 3 treated patients with SLE (indicated by ○ in Fig. 2e), whereas their serum levels were still below the levels detectable in active SLE patients (7), as well as in the mice in which we could induce SLE by the several-fold up-regulation of IFN α (16). Two out of 3 patients with SLEDAI score of 0 and 3.5 (Fig. 2e) had been treated with conventional therapy and potentially could have had higher IFN α levels at an earlier time, which might have induced SLE. Overall however, serum IFN α was not increased in the patients studied here, even though the disease mechanism described here should enhance IFN α generation. Thus, while IFN α may induce SLE (16) in at least some patients, IFN α appears in most cases to exert a disease-modifying, but not causative, role in manifestations such as erythematous skin rash, neuropsychiatric disorders or renal disease (7,16).

CONCLUSIONS

These results show that when CD4 T cells are overstimulated by an external disturbance, i.e., repeated stimulation with antigen, to levels that surpass the system's self-organized criticality, these CD4 T cells express DOCK8 on the cell surface and acquire autoreactivity *via* TCR re-revision at the periphery. These DOCK8⁺ CD4 T cells subsequently induce a variety of autoantibodies and SLE. This represents a novel mechanism of autoantibody development and SLE pathogenesis.

Living organisms are constantly exposed to a variety of pathogens including non-fatal viruses that can invade the host if they circumvent host's CTL defense (82), as exemplified by the recent re-emergence of measles infection in young adults. These repeated rounds of infection, however, may be unapparent at the time of re-infection if CTL memory has lapsed; prodromal signs are indeed minute in SLE. Such antigens, if presented in combination with the appropriate host's HLA, may maximally stimulate the host's TCR. The particular antigens that cause SLE may differ depending upon the host's HLA, but they must be recognized by and must stimulate TCR maximally to levels beyond its self-organized criticality. Upon encounter with peripherally expressed antigens such as mouse mammary tumor virus (Mtv-8) or influenza haemagglutinin (HA), the CD4 T cells reactive against them are deleted or anergized (83,84). However, further repeated stimulation causes a few cells to be recovered from anergy, proliferate, and pass through

294 TCR re-revision at the periphery, acquiring a T follicular helper-like phenotype
295 (77,78,83,84). Since these cells no longer react against the same antigens and reside in a
296 restricted space, splenic GC, immune response will cease (83). However, if further
297 stimulated by an ‘immunogenic’ viral form of influenza HA, the once-angergized T cells
298 will begin to proliferate (84), which is the TCR stimulus that surpass its self-organized
299 criticality. SLE-inducing DOCK8⁺ CD4 T cells are generated in this fashion. This newly-
300 generated DOCK8-expressing T follicular helper cells reside in the splenic red pulp, the
301 location immune reactive against a variety of antigens. Therefore, repeated stimulation
302 with ‘immunogenic’ antigen, either exogenous or endogenous, induces *ai*CD4 T cells and
303 SLE, and the latter of which is consistent with the molecular mimicry theory. Further,
304 since SLE is unique in that a large variety of autoantibodies, sometimes exceeding 100
305 types, are generated in an individual (85,86), it seems rational that SLE and
306 autoantibodies result from TCR re-revision at the periphery rather than reactivation of
307 forbidden clones.

Materials and Methods

Mice

BALB/c, B10.D2, C57BL/6, and NZB/W F1 female mice were obtained from Japan SLC, Inc., Hamamatsu, Japan. CD1d knockout (KO) mice of BALB/c background (C.129S2-Cd1^{tm1Gru}/J) were purchased from Jackson Laboratory (Bar Harbor, ME). All animal experiments were conducted in accordance with the guidelines of the Kyushu University Institution Review Board (Permission number A29-174-1) and the Review Board for Experimental Studies of the Institute for Rheumatic Diseases (Permission number A2017-001).

Patients

Human blood samples were studied in accordance with the guidelines of the Kyushu University Institution Review Board for Human Genome/ gene research (Protocol 711-00, approved on March 21, 2017).

Reagents

OVA (grade V) was purchased from Sigma, St.Louis, MO. Staphylococcus enterotoxin B (SEB) and SEA were purchased from Toxin Technologies, Sarasota, FL. Alpha-galactosylceramide (α GalCer) was a gift of Pharmaceutical Research Laboratories, Kirin Brewery Co., Ltd., Gunma, Japan. Carboxyfluorescein diacetate succinimidyl ester

326 (CFSE; C34570, Cell Trace, CFSE Cell Proliferation kit), Protein G magnetic beads
 327 (#100-03D), and normal rabbit IgG (10500C) were purchased from Invitrogen, Thermo
 328 Fisher Scientific, Shibaura, Tokyo, Japan. The following were purchased from Biolegend,
 329 San Diego, CA: anti-B220-PE (RA3-6B2), anti-BST2-FITC (927), anti-CD3-PE/Cy7
 330 (145-2C11), anti-CD4 Ab (GK1.5), anti-human CD4-PE Cy7 Ab (#300511), anti-mouse
 331 CD4-FITC and CD4-PerCP/Cy5.5 Abs (RM4-5), anti-CD5-FITC (53-7.3), anti-CD21-
 332 FITC (7E9), anti-CD23-PE (B3B4), anti-CD23-Alexa Fluor 647 Ab (B3B4), anti-CD25-
 333 PE Ab (PC61), anti-CD27-FITC Ab (LG.3A10), anti-CD43 activation-associated
 334 glycoform-APC (1B11), anti-CD44-FITC Ab (IM7), anti-CD62L-APC Ab (MEL-14),
 335 anti-CD80-FITC (16-10A1), anti-CD83-FITC (Michel-19), anti-CD86-PE (GL-1), anti-
 336 human CD122-APC Ab (#339007), anti-CD123-PE Ab (5B11), anti-CD138-PE (281-2),
 337 anti-CCR4-PE/Cy7 Ab (2G12), anti-CD147-FITC (OX114), anti-CXCR5-FITC
 338 (L138D7), anti-CXCR5-APC Abs (L138D7), anti-DC9-PE/Cy7 (6D5), anti-FR4-FITC
 339 (12A5), anti-GL7-FITC Ab (GL7), anti-GL7-PE (GL7), anti-ICOS-FITC Ab (C398.4A),
 340 anti-rabbit IgG-PE Ab (#406421), anti-rabbit IgG-FITC (Poly4064), IgG-PE Ab
 341 (Poly4064), anti-LFA1-APC (H155-78), anti-IgM-FITC (RMM-1), anti-PD-1-APC Ab
 342 (29F.1A12), anti-SiglecH-PE (551), anti-T-bet-Alexa Fluor 647 Ab (4B10), anti-Thy1.2-
 343 APC (30-H12), and anti-TCR β -FITC (H57-597). Also purchased from Biolegend were

344 ELISA kits for IFN γ , IL-2, IL-4, IL-6, IL-10, IL17 and IL-23. Rabbit anti-DOCK8 Ab
345 (11622-1-AP) was from Proteintech, Co. Ltd., Rosemont, IL. Anti-Bcl6-PE (mGI191E),
346 anti-human CD45RB-Alexa Fluor 488 Ab (53-9458), anti-Foxp3-APC Ab (FJK-16s),
347 anti-GATA3-Alexa Fluor 488 Ab (TWAJ) and anti-ROR γ t-APC Ab (B2D), and ELISA
348 assay kits for TGF β , TNF α , IL-21, and IL-22 were from eBioscience, San Diego, CA.
349 Anti-CD11c-APC (HL3), anti CD28 mAb (37.51), anti-Ly6C-FITC Ab (AL-21), anti-
350 TLR7-PE (A94B10), and anti-V β 8 TCR-PE (F23.1) were from BD Pharmingen, San
351 Diego, CA. Peanut agglutinin (PNA)-fluorescein (FL-1071), alkaline phosphatase avidin
352 D (A-2100) and Animal-free Blocker were purchased from Vector Laboratories
353 (Burlingame, CA). HRP-anti-rabbit IgG Ab and nonimmunized rabbit IgG were
354 purchased from Cappel Laboratories, Inc., Cochranville, PA. Anti-acetyl Histone H3 Ab
355 (acetylated H3K9, K14; 06-599) and anti-trimethyl Histone H3 Ab (Lys4; 07-473) were
356 purchased from Upstate Biotechnology Inc., Lake Placid, NY. Anti-Histone H3 (trimethyl
357 K4) Ab (ChIP grade; ab8580) and rabbit anti-IFN γ (ab96575) Ab were purchased from
358 Abcam, Cambridge, UK. Dynabeads Protein G (DB10003) was from VERITAS, Tokyo,
359 Japan. RNase A (19101) was purchased from Qiagen, Hilden, Germany. Proteinase K
360 (9033) was from TaKaRa Bio Inc., Kusatsu, Japan. Anti-rabbit Novolink (RE7112) was
361 purchased from Leica Camera Frankfurt, GmbH, Germany. Fast red (415261) was

purchased from Nichirei Bioscience, Tokyo, Japan. Histogreen (E109) was from LINARIS Biologische Produkte GmbH, Dossenheim, Germany. T4 DNA ligase was purchased from Roche Diagnostics, Basel, Switzerland.

Animal Studies

BALB/c female mice (8 weeks old) or C57BL/6 mice (8 weeks old) were immunized with 500µg OVA or PBS by means of i.p. injection every 5days for a total of 12injections.

Mice were analyzed 9 days after the final immunization. Mice were also immunized with

25µg SEB, 25µg SEA or PBS by means of i.p. injection every 5days for a total of 8

injections, and analyzed thereafter. For adoptive cell transfer, CD4 T cells were isolated

from mouse spleens to >90% purity using Ab-coated MACS beads (mouse CD4 T cell

isolation kit; Miltenyi Biotec., Germany). They were then incubated with anti-DOCK8

Ab followed by anti-rabbit IgG-coated MACS beads (Miltenyi Biotec.), and the isolated

cells (2.5×10^7 /mouse) were transferred into naïve BALB/c mice by means of i.v. injection.

The recipient mice received a single i.p. injection of 500µg OVA 24h after cell transfer,

and the sera, urine and the organ of recipients were studied 2 weeks afterwards. For

adoptive cell transfer into previously-immunized mice, BALB/c mice were first

immunized 8x with OVA, and 24hr after the final immunization, mice were administered

200µg anti-CD4 Ab (GK1.5) to deplete CD4 T cells. Four days later, DOCK8⁺ CD4 T

cells from the mice immunized 12x with OVA were transferred into the CD4 T-depleted mice. The recipient mice received a single i.p. injection of 500µg OVA 24h after cell transfer and were analyzed 2 weeks later.

For the SEB immunization study, BALB/c or CD1d KO female mice (8-15 weeks old) were immunized with 25µg SEB, 25µg SEA, or 5µg αGC by means of i.p. injection every 5days for a total of 2 or 8 injections. Mice were bled 3 hr after 2nd and 8th immunization, and serum IL-2 and autoantibodies were measured by ELISA. To evaluate T cell division, cells (1×10^7 /ml) were incubated with 10µM CFSE in PBS for 10min and an equal volume of FCS was added to quench the reaction. After washing, CFSE-labeled cells (1×10^5 /200µl/well) were stimulated with 2µg/ml of plate-bound anti-CD28 Ab for 72hr, and the number of mitotic events was measured by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA).

For therapeutic Ab treatment, BALB/c mice immunized with OVA were treated with anti-DOCK8 Ab (100µg) or control rabbit IgG (100µg) 24 hr before each of the 6x, 8x, 10x and 12x OVA immunizations, and analyzed 2 weeks later. The (NZBxNZW) F1 female mice (8 weeks old) were treated either with anti-DOCK8 Ab (100µg) or control rabbit IgG (100µg) every 15 to 24 weeks, and blood and urine samples were analyzed weekly.

Mass spectrometry analysis

398 Cytosolic and membrane proteins were extracted from CD45RB^{hi} CD122^{hi} CD4 T cells,
399 CD45RB^{lo} CD122^{lo} PD-1⁻ CD4 T cells and CD45RB^{lo} CD122^{lo} PD-1⁺ CD4 T cells that
400 had been harvested from spleens of mice immunized 12x with OVA, as well as control
401 CD4 T cells derived from the spleen of mice treated 12x with PBS. One hundred million
402 cells were reacted with Extraction Buffer I (EMD Bioscience, Inc.) containing 1%
403 protease inhibitor cocktail for 10 min at 4 °C, and the supernatants containing cytosolic
404 proteins were taken. The pellets were reacted with Extraction Buffer II (EMD Bioscience,
405 Inc.) containing 1% protease inhibitor cocktail for 30 min at 4 °C, and subjected to
406 centrifugation at 5,500 x g for 10 min, and the supernatants were taken as the membrane
407 protein fraction. Ten µg total protein per sample was fractionated by electrophoresis in a
408 10% polyacrylamide gel, and the gel was stained with silver nitrate. Representative
409 portions in the gel were cut out and digested with trypsin. Digested peptides were eluted
410 with 0.1% formic acid and subjected to LC-MS/MS analysis, performed on a Q-ToF 2
411 quadrupole/time-of-flight hybrid mass spectrometer (Waters Co, Milford, MA) interfaced
412 with a CapLC capillary reverse-phase LC system. The eluted peptides were sprayed
413 directly into the mass spectrometer. MS/MS data were acquired by MassLynx software
414 (Micromass) and converted into a single text file (containing the observed precursor
415 peptide *m/z*, the fragment ion *m/z* and intensity value) by ProteinLynx software

(Micromass). The file was analyzed using the Matrix Science Mascot MS/MS Ion Search software (<http://www.matrixscience.com>) to search and assign the obtained peptides to the NCBI non-redundant database. Proteins (10µg each) were again subjected to electrophoresis in 10% polyacrylamide gel, transferred to an Immobilon-P membrane (Milipore, Bedford, MA), and stained sequentially with specific Ab and labelled 2ndary Ab.

***In vitro* autoantibody and cytokine assay**

Sera were assayed 9days after final priming by ELISA for RF (FUJIFILM Wako Shibayagi Co., Gunma, Japan), anti-dsDNA Ab (Worthington Biochemical Co., Lakewood, NJ), or anti-Sm Ab using Sm antigen (ImmunoVision, Springdale, AR). Isolated DOCK8⁺ CD4 T cell CD4 T cells (1x10⁶/ml) were stimulated with 2 µg/ml anti-CD3 Ab and 5 µg/ml anti-CD28 Ab at 37° C for 24hr, and the culture supernatants were assayed for cytokines by ELISA.

Flow cytometry and cell sorting.

Lymphocyte surface markers of T and B cells of mice and human peripheral blood were assayed using anti-CD4-FITC Ab, anti-CD4-PerCP/Cy5.5 Ab or the respective Abs after blocking non-specific FcR binding with anti-mouse CD16/32 Ab (Biolegend). For DOCK8 staining, lymphocytes were treated with rabbit anti-DOCK8 Ab followed by

staining with anti-rabbit IgG-FITC Ab or anti-rabbit IgG-PE Ab. For intracellular staining, DOCK8-stained cells were fixed and permeabilized in fixation/ permeabilization solution and permeabilization buffer from the Foxp3/ Transcription Factor Staining Buffer Set (eBioscience). They were next stained with the respective Abs against Foxp3, T-bet, GATA3 and ROR γ t. Cells were washed once, resuspended in PBS and analyzed on a FACSCallibur flow cytometer (BD Biosciences, San Jose, CA).

All antibodies have been validated by their suppliers and references can be found on their websites or online validation databases. Dilutions were 1:5,000 for surface staining with PNA-fluorescein, 1:1,000 for surface staining with anti-GL7-PE Ab, 1:200 for surface staining with anti-CD4FITC Ab or anti-CD4-PerCP/Cy5.5 Ab, and 1:100 for the staining with the other Abs.

For cell isolation, CD4 T cells, DOCK8⁺ T cells were isolated from spleens of mice to >90% purity using Ab-coated MACS beads (Miltenyi Biotec, Germany). Cell numbers were determined using AccuCheck Counting Beads (Life Technologies, Invitrogen) according to manufacturer's instructions.

Pathological Studies

Pathological studies were done using 10% formalin-fixed and H-E stained organ specimens. For immunoperoxidase staining of DOCK8⁺ cells, specimens were serially

452 reacted with anti-DOCK8 Ab and HRP-anti-rabbit IgG Ab followed by diaminobenzidine
453 H₂O₂ reaction. Nonimmunized rabbit IgG was used as a control. For immunoelectron
454 microscopic studies, CD4 T cells were isolated from the spleens of 12x OVA-immunized
455 mice to >90% purity using Ab-coated MACS beads, pelleted by centrifugation, washed,
456 and stained directly with Au-conjugated goat anti-DOCK8 Ab RT for 4hr. They were then
457 washed with PBS, fixed in 1% OsO₄ for 2 hr at 4 °C, and then dehydrated in graded
458 alcohol and embedded in Epon 812. Sections 90 nm thick were cut using an LKB
459 ultramicrotome, counter-stained with uranyl acetate and lead, and observed under a
460 Hitachi H-7600 electron microscope for Au-stained cells. Renal Pathology was classified
461 according to SLE human renal disease classification (26). Spleens from patients with SLE
462 and controls obtained at autopsy were fixed in 10% formalin and embedded in paraffin.
463 Paraffin sections of 4µm thickness were deparaffinized by graded xylene and graded
464 alcohol sequentially, treated with 0.01M citric acid in an autoclave at 121°C for 10min,
465 and blocked with Animal-free Blocker for 10min. Specimens were then treated with
466 biotinylated anti-DOCK8 monoclonal Ab diluted at 1:200 and rabbit anti-interferonγ Ab
467 diluted at 1:1,000 at 4°C for 24hr. After blocking endogenous peroxidase with hydrogen
468 peroxide RT for 15min, the specimens were reacted serially with alkaline phosphatase
469 avidin D and anti-rabbit Novolink RT for 30min, followed by Fast red and Histogreen

470 staining RT for 40min and 2min, respectively. After washing, the specimens were air dried,
471 embedded, and observed under microscope.

472 **RT-PCR for recombinae.**

473 Total RNA was reversely transcribed to cDNA and amplified by PCR. The products were
474 fractionated by electrophoresis and transferred to nylon membranes (Roche Diagnostics,
475 Mannheim, Germany). The membranes were hybridized to fluorescein end-labelled
476 probes and visualized by alkaline phosphatase (ALP)-labeled anti-fluorescein antibody
477 and Gene Images CDP-Star chemiluminescence reaction (Amersham Pharmacia Biotech,

478 Piscataway, NJ). The primers and probes were: 5'-

479 CCAAGCTGCAGACATTCTAGCACTC-3' (forward), 5'-

480 CAACATCTGCCTTCACGTCGATCC-3' (reverse) and 5'-

481 AACATGGCTGCCTCCTTGCCGTCTACCCT-3' (probe) for RAG1 (87); 5'-

482 CACATCCACAAGCAGGAAGTACAC-3' (forward), 5'-

483 GGTCAGGGACATCTCCTACTAAG-3' (reverse) and 5'-

484 GCAATCTTCTCTAAAGATTCCTGCTACCT-3' (probe) for RAG2 (87); 5'-

485 GAACAACCTCGAAGAGCCTTCC-3' (forward), 5'-

486 CAAGGGCATCCGTGAATAGTTG-3' (reverse) and 5'-

487 ATTCGGTCACCCACATTGTGGCAGAGAAC-3' (probe) for TdT; 5'-

488 CAACTGGGTCATGCTTCTCC-3' (forward), 5'-TGGCTGTCTGAAGATTCCC-3'
489 (reverse) and 5'-CCGTCTCTGGCTCCACCCATCACACTGCT-3' (probe) for pTα.

490 **Next-generation sequencing of TCR genes**

491 One microgram total RNA from DOCK8⁺ CD4 T cells, DOCK8⁻ CD4 T cells, and CD4
492 T cells were each converted into complementary DNA (cDNA) with Superscript III
493 reverse transcriptase (Invitrogen, Carlsbad, CA). BSL-18E primer containing polyT₁₈ (5'-
494 AAAGCGGCCGCATGCTTTTTTTTTTTTTTTTTTTVN-3') and an *Sph*I site was used
495 for cDNA synthesis. Double stranded (ds)-cDNA was synthesized using *E.coli* DNA
496 polymerase I, *E.coli* DNA ligase and RNase H (Invitrogen). The ds-cDNA was then
497 blunted with T4 DNA polymerase (Invitrogen). P10EA/ P20EA adaptor (5'-
498 GGGAATTCGG-3'/ 5'-TAATACGACTCCGAATTCCC-3') was ligated to the 5' end of
499 the ds-cDNA followed by digestion with *Sph*I restriction enzyme (88). After removing
500 adaptor and primer by MinElute Reaction Cleanup kit (Qiagen), PCR was performed
501 using either specific for TCRα-chain constant region (mCA1; 5'-
502 TCATGTCCAGCACAGTTTTG-3') or TCRβ-chain constant region (mCB1; 5'-
503 AGGATTGTGCCAGAAGGTAG-3') and P20EA adaptor using KAPA HiFi DNA
504 Polymerase (Kapa Biosystems, Woburn, MA). Next, a second PCR was performed using
505 the P20EA and 2nd PCR primers for TCRα (mCA2; 5'-GTTTTTCGGCACATTGATTG-

506 3') or TCR β (mCB2; 5'-TTGTAGGCCTGAGGGTCC-3'). Amplicons were
 507 subsequently prepared by amplifying the second PCR products using P22EA-ST1-R Tag
 508 primers (5'-
 509 GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTAATACGACTCCGAATT
 510 CCC-3') and mCA-ST1-R Tag primer for TCR α (5'-TCGTCGGCAGCGTCAGAT
 511 GTGTATAAGAGACAGGTGGTACACAGCAGGTTCT-3') or mCB-ST1-R Tag
 512 primer for TCR β (5'-
 513 TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTTGGGTGGAGTCACATTT-
 514 3'). After PCR amplification, index (barcode) sequences were added by amplification
 515 with the Nextera XT Index kit v2 setA or setD (illumine, San Diego, CA). The indexed
 516 amplicon products were mixed in equal molar concentrations and quantified by a Qubit
 517 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA). Sequencing was performed
 518 using the Illumina Miseq paired-end platform (2x300bp). All the paired-end reads were
 519 classified by index sequences. All sequence reads were classified by MID Tag sequences.
 520 Artificially added sequences and sequences with low quality scores were removed from
 521 both terminals of sequence reads using software installed on the 454 sequencing system
 522 (88). The remaining sequences were used for assignment of TRAV and TRAJ for TCR α
 523 sequences, and TRBV and TRBJ for TCR β sequences. Sequences were assigned by

determining highest identity in a data set of reference sequences for all TRAV, TRAJ, TRBV and TRBJ genes including pseudogenes and open reading frame (ORF) reference sequences available from the international ImMunoGeneTics information system® (IMGT) database (<http://www.imgt.org>). Data processing, assignment, and data aggregation were automatically performed using repertoire analysis software RG (Repertoire Genesis Inc., Osaka, Japan), which implemented a program for sequence homology searches using BLATN, an automatic aggregation program, a graphics program for gene usage, and CDR3 length distribution (90). Sequence identities at the nucleotide level between query and entry sequences were automatically calculated by carefully optimizing for respective repertoires for parameters that increased sensitivity and accuracy; i.e. E-value threshold, minimum kernel, and high-scoring segment pair (HSP) score. CDR3 sequences were defined as the sequences ranging from a conserved Cysteine at position 104 (Cys104) in the IMGT nomenclature to a conserved Phenylalanine (Phe118) or Tryptophan (Trp118) at position 118, and these, together with the next Glycine (Gly119), were translated into the deduced amino acid sequences (89). A unique sequence read (USR) was defined as a sequence read having no identity with other sequence reads in TRV, TRJ and deduced CDR3 amino acid sequences. The copy number of identical USR was automatically counted by the RG software in each samples and

ranked by copy number. Percentage frequencies of sequence reads within TRAV, TRAJ, TRBV and TRBJ genes to total sequence reads were calculated. To estimate TCR diversity in deep sequencing data, several diversity indices were used, including Shannon-Weaver index H' and inverted Simpson index $1/\lambda$. U, unique read and copy numbers were used for species and individuals in these equations (47,48).

Gene Expression Microarray Assay

Total RNA was extracted with the RNeasy Mini Kit (Qiagen, Hilden, Germany), and total RNA was quantified by absorbance at 260 nm using a Nano Drop 2000 spectrophotometer (Thermo Fisher Scientific, MA. USA). RNA samples were quantified by an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE) and the quality was confirmed with the Experion System (Bio-Rad Laboratories, Hercules, CA). The mRNA expression profile was determined using a SurePrint G3 mouse GE Microarray ($8 \times 60K$ v2) (Agilent Technology, Santa Clara, CA), and assay was performed by Cell Innovator Inc. (Fukuoka, Japan). Relative hybridization intensities and background hybridization values were calculated using Agilent Feature Extraction Software (9.5.1.1). Raw signal intensities and Flags of two samples were calculated from hybridization intensities using gProcessedSignal and spot information using gIsSaturated module in Bioconductor software (91). The data was $\log_2$ -transformed and normalized by quantile algorithm with

‘preprocessCore’ library package (90). Probes named ‘P’ flag were set at least in one sample, and Z-scores (92) and ratios (non-log scaled fold-change) from normalized signal intensities of each probe for comparison between control and experiment sample were calculated. The genes for which the Z score and the ratio were above 2.0 and 1.5, respectively, were considered up-regulated. In contrast, the genes for which the Z scores and ratios were below -2.0 and 0.66, respectively, were considered down-regulated. Data Analysis consisted of Flag criteria on GeneSpring Software which classified the results as A: absent, indicating that the feature was not-positive and significant and that the feature was not above background; M: marginal, indicating that the feature was not uniform, saturated and a population outlier; P: present, indicating values other than A or M.

Autoantibody Microarray Assay

Antigens diluted to optimal concentration were spotted in duplicate and random fashion using Microgrid II micro-arrayer onto Nitrocellulose-coated 16-pad FAST™ slides (Whatman Schleicher & Schleicher BioScience, Keene, NH) (93). A 16-section frame was applied to each slide to separate individual assays. After washing with blocking PBS buffer containing 1% bovine serum albumin and Tween, sera diluted 1:100 with blocking buffer were added, and incubated with Cy3-labelled anti-mouse IgG. The arrays were

washed with buffer, and the frames were removed. The slides were immersed in PBS and then spun dry. A Genepix 4000B scanner with 532nm laser wavelength was used to generate Tif images for analysis (94).

Chromatin immunoprecipitation (CHIP) assay

Forty million cells were suspended in 1ml RPMI, to which 0.1ml of 11% formaldehyde was added and the tube rocked RT for 5 min. Next, 0.1ml of 1.5M glycine was added to terminate crosslinking. Cells were pelleted (3K x g, 5 min, 4°C), and dissolved in 1ml FACS solution (PBS containing 2%FBS and 0.05% NaN₃) and washed. After the second wash, cells were suspended in 0.2ml SDS lysis buffer (50mM Tris-HCl, pH 8.0, 10mM EDTA, and 1%SDS) containing 1% Protease inhibitor cocktail on ice for 10 min. Chromosomal DNA was sheared using a Bioruptor UCD-200 on ice for 10 min. After centrifugation at 14K x g, 4°C for 10min, the supernatant was transferred to a 2ml siliconized tube. The chromatin sample was stored by addition of 0.1vol 50% glycerol at –80°C. Fifty µl Dynabeads Protein G were washed twice with Blocking solution (PBS containing 0.5% BSA), dissolved in 0.5ml of the same, and loaded with 5µg of anti-acetyl histone H3 Ab, anti-trimethyl-Histone H3 Ab (Lys4), or normal rabbit IgG. After loading, the pellets were washed twice with Blocking solution. For ChIP analyses, Dynabeads Protein G were diluted with 1.8ml ChIP dilution buffer at 4°C. Chromatin derived from

2 million cells was used for each ChIP experiment with each antibody. The chromatin samples were then added to the rabbit Ig-loaded beads and rocked at 4°C for 6hr. Beads were spun down at 15K x g, 4°C for 2min, and 2ml supernatants were divided into 2 tubes, from which 980µl was transferred to new tubes containing a second batch of rabbit Ig-loaded beads, and rocked 1 hr at 4°C. These were next washed 5x with RIPA buffer (50mM HEPES-KOH, pH7.6, 500mM LiCl, 1mM EDTA, pH8.0, 1% NP-40, and 0.7% sodium deoxycholate) and once with TE buffer (10mM Tris-HCl, pH8.0, 1mM EDTA, and 50mM NaCl). Twenty µl was used as 1% Input. Beads were reacted with 210µl of ChIP direct elution buffer (10mM Tris-HCl, 300mM NaCl, 5mM EDTA, and 0.5% SDS) at 65°C for 30min with rotation. After centrifugation at 15K x g, RT for 1min, 200µl of supernatant was incubated at 65°C. The 2%input chromatin was treated identically. Two hundred µl of the supernatant was mixed with RNase A (final 0.2mg/ml), rotated, spun down and incubated at 37°C for 30min. Subsequently, proteinase K (final 0.2mg/ml) was added, the sample rotated, spun down and incubated at 55°C for 1hr. DNA was then extracted using the QIA quick PCR purification kit, and reacted with TCR locus primers by PCR at 94°C for 10min, followed by PCR amplification at 94°C 30s, 58°C 1min, and 72°C 1min for 35 cycles, and incubation at 72°C for 10min. PCR products (10µl) were applied to a 2% agarose gel. The primer and probe sequences were shown in

Supplementary Table 3. Input chromatin samples were diluted so that IP and input samples would give approximately equal qPCR signals if 2% of the region of interest were present in the IP sample.

Ligation-mediated PCR (LM-PCR)

Genomic DNA (1 µg) was ligated to 20 pmols BW linker, which was made by annealing the oligonucleotides BW-1 and BW-2, in 50µL of reaction buffer containing 66mM Tris-HCl (pH7.5), 5mM MgCl₂, 5mM DTT, 1mM ATP and 2.5 U of T4 DNA ligase (Roche) for 16 hours at 16°C. Ligated DNA was diluted with an equal volume of a buffer containing 10 mM Tris (pH8.3), 50 mM KCl, 0.5% Nonidet P-40 (NP-40) and 0.5% Tween20 and heated for 15 min at 95°C. Ligated DNA (100 ng) was amplified for 12 cycles with the locus-specific primer for first PCR and linker-specific primer BW-1H. For nested LM-PCR reactions, 1µL of the products was then amplified for another 27 cycles with the locus-specific primer for nested PCR and BW-1H. The LM-PCR products were fractionated by electrophoresis and transferred to Hybond N⁺ membranes (GE Healthcare, Buckinghamshire, England). The membranes were hybridized to alkaline phosphatase (ALP)-labeled probes and visualized by Gene Images CDP-Star chemiluminescence reaction (GE Healthcare). The sequences of the linkers and linker-specific primers were;

5'-GCGGTGACCCGGGAGATCTGAATTC-3' for BW-1 linker, 5'-GAATTCAGATC -

633 3' for BW-2 linker and 5'-CCGGGAGATCTGAATTCCAC -3' for BW-1H primer. The
 634 sequences of the locus-specific primers and probes were as follows: 5'-
 635 TTACAAGAACTCCCTCTGCCTCTC-3' for first PCR, 5'-
 636 AAGACACACTGGCTAGAGTTCTGGT-3' for nested PCR and
 637 5'AGATGCCTAGGCTTCTGTAAAGGTGTCACCTGCAGTGGAATTCAG-3'
 638 (probe) for signal end (SE) of J α 2 (J α 2 SE);
 639 5'-AGGAGATGCAAGCAAAGGAGTT-3' for first PCR, 5'-
 640 AGGCAGAATTCGACTTGAGTAAG G-3' for nested PCR and 5'-
 641 ACCTGTGGGTGTTTTTGACTGACTAAGAAACACTGTGGAATTCAG-3' (probe)
 642 for J α 12 SE; 5'-GAACACAGAAGAAGGCCCAATATC-3' for first PCR, 5'-
 643 AAAAGGAGATCAGAGAGGGGTGTT-3' for nested PCR and 5'-
 644 CTGGGGACGCCATTTTGTAGACGTGTTTGTTCACAGTGGAATTCAG-3' (probe)
 645 for J α 24 SE; 5'-GCCTCTGTGGTCTAGTGTTTCTCA-3' for first PCR, 5'-
 646 TGAGAACTTTACCCAGGAGGAAGA-3' for nested PCR and 5'-
 647 CTCTGAGGAAGTTTTTGTGTAGAGTCAGCCACTGTGGAATTCAG-3' (probe)
 648 for J α 35; 5'-GTCAAAGCACCCAGAATAAAGAGG-3' for first PCR, 5'-
 649 CCTGCACTATCACTGACTGTTTCC-3' for nested PCR and 5'-
 650 CCTTACCTTGGTTTATGTAGAGACACAGAACACTGTGGAATTCAG-3' (probe)

651 for J α 40 SE; 5'-CAATGGAGGGAAACAACACTAGC-3' for first PCR, 5'-
652 AGAAGCTAGGGAATCAGGGAGAAT-3' for nested PCR and 5'-
653 GATGCCACGAGTTTTTGCAAAGCCCTTCAGTGCAGTGGAATTCAG-3' (probe)
654 for J α 58 SE.

655 **Anti-DOCK8 monoclonal Ab**

656 Recombinant GST/His-tagged mouse DOCK8 encompassing the DHR1 domain (N-
657 terminal 561-730 amino acids; Uniprot accession number Q8C147), was expressed in *E.*
658 *coli*. After capturing on glutathione sepharose column, His-tagged mouse DOCK8 was
659 eluted by protease-digestion, and fractionated with size-exclusion chromatography. Using
660 this purified antigen, anti-DOCK8 antibodies were generated from non-immune phage
661 antibody libraries developed in KAN Research Institute. Purified His-tagged mouse
662 DOCK8 was immobilized on Dynabeads M280-Tosylactivated (Thermo Fisher Scientific,
663 Waltham, MA), and monoclonal single chain Fvs (scFvs) were selected by 3x repeated
664 reaction with the beads, followed by interaction with His-tagged mouse DOCK8 coated
665 onto MaxiSorp immunotubes (Thermo Fisher Scientific). Monoclonal scFvs picked from
666 the 3rd and 4th rounds of selection were expressed as bacterial supernatants. Positive scFvs
667 were identified by ELISA reactive against His-tagged mouse DOCK8. ELISA-positive
668 scFvs obtained were sequenced and genetically converted to human IgG format. Anti-

DOCK8 mAbs were expressed in the Expi293 system (Thermo Fisher Scientific) and purified by Protein A resin. The anti-DOCK8 IgG mAbs thus obtained were validated by size-exclusion chromatography, ELISA reactive against His-tagged mouse DOCK8₅₆₁₋₇₃₀, and intracellular staining of mouse CD4⁺ T cells by flow-cytometry. Finally, mAb generated were used for staining DOCK8⁺ CD4 T cells from SLE patients and control individuals. DOCK8⁺ CD4 T cells in the peripheral blood were stained with anti-DOCK8 mAb and anti-rabbit IgG, and DOCK8⁺ CD4 T cell fraction within the each patient's CD4 T cells were compared with individual SLE disease activity indices (SLEDAI) (40).

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Author contributions

S.S. conceived and designed the study, identified DOCK8 as *ai*CD4 T marker, performed
EM and pathology studies, interpreted results, and wrote the manuscript. K.T. performed
cell transfer studies, FACS, WB, RT-PCR and cytokine measurements, and prepared
samples for and reconstructed the data of autoantibody microarray and TCR repertoire
next generation assays. Y.M. isolated and fractionated CD4 T cells to
CD45RB^{lo}CD122^{lo}PD-1⁺ fraction. K.U. performed CHIP-seq and LM-PCR studies.
K.Sakurai performed B cell assay. T.Nakashima, H.M., C.S., A.D., Y.F., and M.T.
performed anergy and mechanism of *ai*CD4 T cell induction studies. T.Nakashima and
Y.N. studied NKT cell help for *ai*CD4 T cell induction. M.I. performed OVA-induced
*ai*CD4 T cell in C57BL/6 mice study. Q-Z. L. performed autoantibody microarray assay.
K. M. and Y. M. produced anti-DOCK8 mAb. K.M., T.E., S.T., Y.H. and M.M. performed
human pathology studies. M.M. performed, evaluated blindly, and organized the mouse
and human pathology studies. T. H., T. M., M. O., T. Y., H. K. and S.S. enabled portions

705 of, and K.S. enabled most of patients study under written consent based on Kyushu

706 University Institution Review Board (Protocol 711-00).

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938

Figure Legends

Fig. 1

a, Identification of DOCK8 as a marker of *ai*CD4 T cells.

Membrane and cytosolic fractions of either whole T cells (Lanes 1 and 5), CD45RB^{hi} CD122^{hi} CD4 T cells (Lanes 2 and 6), CD45RB^{lo} CD122^{lo} PD-1⁻ CD4 T cells (Lanes 3 and 7) or CD45RB^{lo} CD122^{lo} PD-1⁺ CD4 T cells (Lanes 4 and 8) from 12x OVA-immunized BALB/c mice, were subjected to 10% polyacrylamide gel electrophoresis, transferred to Immobilon-P membrane, and stained with anti-DOCK8 Ab.

b, Expansion of DOCK8⁺ CD4 T cells after repeated immunization with OVA without an adjuvant.

Flow cytometry analyses of DOCK8⁺ CD4 T cell from splenic CD4 T cells of BALB/c mice after repeated 12x stimulation with PBS, 6x with OVA or 12x with OVA.

c, Transferred DOCK8⁺ CD4 T cells induced autoantibodies in the recipients.

Rheumatoid factor (RF), anti-Sm Ab and anti-dsDNA Ab were quantified in the sera of BALB/c mice that had been pre-immunized 8x with OVA, depleted of CD4 T cells by anti-CD4 Ab and then inoculated with DOCK8⁻ CD4 T cells or DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice. The control group was BALB/c mice pre-immunized 8x with OVA, CD4 T cell-depleted by anti-CD4 Ab, and inoculate with CD4

957 T cells from 12x PBS-immunized BALB/c mice. Anti-dsDNA Ab and anti-Sm Ab titers
 958 were represented by arbitrary unit (AU). Statistical assessment was by Student's t-test.

959 **d**, Glomerulonephritis in the BALB/c mice that had been pre-immunized 8x with OVA,
 960 depleted of CD4 T cells by anti-CD4 Ab and inoculated with DOCK8⁻ CD4 T cells or
 961 DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice.

962 Control group was the BALB/c mice that had been pre-immunized 8x with OVA, depleted
 963 of CD4 T cells by anti-CD4 Ab, and inoculated with CD4 T cells from 12x PBS-
 964 immunized BALB/c mice. Glomerular lesions in mice were classified according to human
 965 WHO classification (26) as follows; class I, normal glomeruli; class II, purely mesangial
 966 disease; class III, focal proliferative glomerulonephritis; class IV, diffuse proliferative
 967 glomerulonephritis; and class V, membranous glomerulonephritis. Statistical assessment
 968 was by Student's t-test. *p<0.05, **p<0.001.

969 **e**, Skin lesion.

970 **A**: Moderate dermatitis with dermal fibrosis, **B**: liquefaction degeneration (arrow) in the
 971 basal cells of epidermis, and **C**: panniculitis in the deep area of the dermis of mice pre-
 972 treated 8x with OVA, depleted of CD4 T cells and inoculated with DOCK8⁺ T cells from
 973 12x OVA-immunized mice. **D**: Discoid lupus-like advanced dermatitis observed in mice
 974 immunized 12x with OVA. **E**: Healed dermatitis with some fibrosis in 12x OVA-

immunized mice treated with anti-DOCK8 Ab 24 hours each before the 6x, 8x, 10x and 12x OVA immunizations with OVA. H & E stain. (bar=30μm; original magnification A,B, x400, C, x300, D,E, x100). *p<0.05, **p<0.001

f, Summary of lesions other than kidney in BALB/c mice 8x pre-immunized with OVA, CD4 T cell-depleted and inoculated with DOCK8⁻ CD4 T cells or DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice. Also shown were results from anti-DOCK8 Ab treatment of 12x OVA-immunized mice: Mice received 100μg anti-DOCK8 Ab 24 hr each before the 6x, 8x, 10x and 12x OVA immunizations with OVA. *Statistical analysis was by Fisher's exact test.

g, Lesion other than kidney or skin including **A**: lung interstitial pneumonitis, **B**: interstitial pneumonitis accompanied by angiitis, **C**: liver pericholangitis, **D**: Onion-skin lesion with amyloid deposition classical to lupus in the spleen, and **F**: thyroiditis, accompanied by giant cells, in the BALB/c mice 8x pre-immunized with OVA, CD4 T cell-depleted, and then inoculated with DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice. **E**: Classical onion-skin-like lesion with amyloid deposits in the spleen, and **G**: perineuritis observed in the dermis of the BALB/c mice immunized 12x with OVA. H & E stain. (bar=30μm; original magnification x200).

Fig. 2

993 Identification of DOCK8⁺ CD4 T cells as long-lived T follicular helper T cells located at
 994 splenic red pulp, which were increased in the peripheral blood of patients with active SLE.
 995 **a, A**, Morphology of DOCK8⁺ CD4 T cell observed by immunoelectron microscopy,
 996 stained with rabbit anti-DOCK8 Ab and Au-tagged ant-rabbit IgG Ab. (bar=1μm;
 997 original magnification x12,000). **B; C**, Nuclear membrane portion of DOCK8⁺ CD4 T
 998 cell, stained likewise. (bar=1μm; original magnification x18,000; x24,000).
 999 **b**, Surface markers of splenic DOCK8⁺ CD4 T cells.
 1000 Flow cytometry for ICOS, CXCR5, and PD-1 expression in splenic DOCK8⁺ CD4 T
 1001 cells taken 9 days after the final immunization of BALB/c mice immunized 12x with
 1002 PBS versus OVA (Left Upper panel). Flow cytometry analysis of ICOS, CXCR5, PD-1,
 1003 GATA3, RORγt, T-bet, Bcl6, Ly6C, IFA1, FR4, and GL7 expression in splenic DOCK8⁻
 1004 CD4 T cells and DOCK8⁺ CD4 T cells isolated from 12x OVA-immunized BALB/c
 1005 mice.
 1006 **c**, Cytokines released in culture supernatants of 1x10⁶/ml DOCK8⁺ or DOCK8⁻ CD4 T
 1007 cells following incubation with 2 μg/ml anti-CD3 and 5 μg/ml anti-CD28 Ab at 37 °C for
 1008 24hr. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.
 1009 **d**, DOCK8⁺ CD4 T cells in the spleen of autopsied SLE patient who died before therapy.
 1010 Tissues were fixed in 10% formalin, deparaffinized by graded xylene and graded alcohol

sequentially, and serially treated with biotinylated anti-DOCK8 monoclonal Ab, alkaline phosphatase avidin D and anti-rabbit Novolink, followed by reaction with Fast Red and Histogreen (Red). CD4 T cells were stained with rabbit anti-IFN γ Ab and anti-rabbit IgG Ab (Blue) serially. S: red pulp sinus. A; central artery. P: penicillar artery. (bar=100 μ m; original magnification x100). **Inset;** Magnified DOCK8⁺ CD4 T cells (Arrows). (bar=20 μ m; original magnification x400).

e, Circulating DOCK8⁺ CD4 T cells in the peripheral blood of patients with SLE and its relationship to the SLE disease activity index (SLEDAI). Data of control patients were depicted as; ■ rheumatoid arthritis, ▲ mixed connective tissue disease, △ polymyositis, ◆ systemic sclerosis, □ microscopic polyangiitis. Also shown were quantification of interferon (IFN) in sera of the same patients with SLE measured by ELISA.

Fig. 3

a. Expression of V(D)J recombinase complex and related molecules in splenic CD4 T cells from 12x PBS-immunized BALB/c mice, and in DOCK8⁻ CD4 T cells and DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice. Two mice were analyzed for each group.

b, Western blot analysis of TCR signal transduction molecules in splenic CD4 T cells from 12x PBS-immunized BALB/c mice, and in DOCK8⁻ CD4 T cells and DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice. Two mice were analyzed for each group. GAPDH, control house keeping glyceraldehyde 3-phosphate dehydrogenase gene.

c, Heatmap visualization of TCR usage of a combination of TRV and TRJ genes in the splenic DOCK8⁻ CD4 T cells and DOCK8⁺ CD4 T cells from 12x OVA-immunized mice. Three mice were analyzed for each group.

d, Autoantibody microarray study. Autoantibodies produced in BALB/c mice pre-immunized 8x with OVA, depleted of CD4 T cells and inoculated with DOCK8⁻ CD4 T cells or DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice. Control was autoantibodies produced in BALB/c mice pre-immunized 8x with OVA, depleted of CD4 T cells and inoculated with CD4 T cells from 12x PBS-immunized BALB/c mice. Two mice were analyzed for each group.

e, A, Western blot analysis of DOCK8 and transcription factors in splenic CD4 T cells from 12x PBS-immunized BALB/c mice, and in DOCK8⁻ CD4 T cells and DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice. Three mice were analyzed for each group.

B, Results of gene expression microarray analysis, abstract from Supplementary Table

1. Ratio of mRNA expression of splenic DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice in comparison to splenic DOCK8⁻ CD4 T cells from 12x OVA-immunized mice, given as a log₁₀ scale, wherein 2, 3, 4, 5 and 6 indicated x10², x10³, x10⁴, x10⁵, and x10⁶, respectively.

Fig. 4.

a, The effect of anti-DOCK8 mAb treatment on autoantibody generation. Autoantibodies, rheumatoid factor (RF), anti-Sm Ab and anti-dsDNA Ab, were analyzed in the sera of 12x PBS-immunized or OVA-immunized mice: Mice received anti-DOCK8 Ab (100μg) or control rabbit IgG (100μg) 24 hr each before the 6x, 8x, 10x and 12x OVA immunizations with OVA. Anti-Sm Ab and anti-dsDNA Ab titers were represented by arbitrary unit (AU).

*Statistical analysis was by Student's t-test; *p<0.05, **p<0.01, ***p<0.005, ****p<0.001.

b, The results of anti-DOCK8 mAb treatment on renal disease.

Upper: Proteinuria, assessed 9 d after the final immunization and graded with a score of 0 (<30 mg/dl); 1 (30–99 mg/dl); 2 (100–299 mg/dl); or 3 (300–999 mg/dl).

Lower: Kidney lesions of BALB/c mice immunized 12x with either PBS or OVA, and treated either with anti-DOCK8 Ab (100μg) or control rabbit IgG (100μg) 24 hr each

before the 6x, 8x, 10x and 12x OVA immunizations with OVA. Mouse glomerular lesions were classified according to human WHO classification (26) as follows; class I, normal glomeruli; class II, purely mesangial disease; class III, focal proliferative glomerulonephritis; class IV, diffuse proliferative glomerulonephritis; and class V, membranous glomerulonephritis. Statistical assessment was by Student's t-test; * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$.

c, Results of anti-DOCK8 mAb treatment on (NZBxNZW) F1 female mice. Autoantibodies, anti-dsDNA Ab, anti-Sm Ab and rheumatoid factor (RF), and proteinuria of (NZBxNZW) F1 female mice, treated either with anti-DOCK8 Ab (100 μ g) or control rabbit IgG (100 μ g) every week from 15 weeks until 24 weeks (arrow). Statistical assessment performed by Student's t-test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

Extended Data Table 1

Diversity score calculation. Percentage of sequence reads with TRAV, TRAJ, TRBV and TRBJ genes among total sequence reads were calculated. To estimate TCR diversity in deep sequencing data, diversity indices, Shannon-Weaver index H' and inverted Simpson index $1/\lambda$, were used, with unique read and copy number used for species and individuals in the equations (47,48). Two mice were analyzed for each group.

Extended Data Table 2.

TCR repertoires uniquely expressed in DOCK8⁺CD4⁺ T cells. The TCR repertoires uniquely expressed in DOCK8⁺CD4⁺ T cells from 12x OVA-immunized BALB/c mice, but not in DOCK8⁻CD4⁺ T cells from 12x OVA-immunized BALB/c mice or CD4⁺ T cells from 12x PBS-stimulated mice. Each 2 mice were compared. Two mice were analyzed for each group.

Extended Data Fig. 1

To isolate *ai*CD4 T cells, SLE was induced in the BALB/c mice by 12x repeated intraperitoneal (i.p.) immunization with OVA without adjuvants, and various subsets of splenic CD4 T cells were taken and transferred to naïve recipient mice. For each subset we assessed the generation of rheumatoid factor (RF) and anti-dsDNA Ab in the recipient mice. Rheumatoid factor (RF) and anti-dsDNA antibody (anti-dsDNA Ab) were measured in naïve recipient BALB/c female mice 2weeks after the transfer of either CD4 T cells

from 12x PBS-immunized BALB/c mice, or CD45RB^{lo} CD122^{lo} PD-1⁻ CD4 T cells or CD45RB^{lo} CD122^{lo} PD-1⁺ CD4 T cells from 12x OVA-immunized BALB/c mice. Measurement was by ELISA. Anti-dsDNA Ab titers were represented by arbitrary unit (AU). Statistical assessment performed by Student's t-test; *p<0.05.

Extended Data Fig. 2.

Induction of autoantibodies and renal disease by the transfer of DOCK8⁺ CD4 T cells in naïve recipient mice.

a, Autoantibodies, rheumatoid factor (RF) and anti-dsDNA Ab, measured in naïve recipient mice 2weeks after the transfer of either CD4 T cells from 12x PBS-immunized mice, or DOCK8⁻ CD4 T cells or DOCK8⁺ CD4 T cells from 12x OVA-immunized mice. Anti-dsDNA Ab titers were represented by arbitrary unit (AU). Statistical assessment performed by Student's t-test; *p<0.05, **p<0.01, ***p<0.005.

b, Renal disease. **Left panel**, Proteinuria observed in BALB/c mice that had been immunized x8 with OVA, depleted of CD4 T cells and inoculated either splenic DOCK8⁻ T cells or DOCK8⁺ T cells from 12x OVA-immunized BALB/c mice. Observations were made 2weeks after cell transfer. Proteinuria was assessed 9 d after the final immunization and graded with a score of 0 (<30 mg/dl); 1 (30–99 mg/dl); 2 (100–299 mg/dl); or 3 (300–999 mg/dl). **Middle upper**: Wire loop lesion typical of SLE (indicated by arrow), **middle**

lower: diffuse proliferative WHO type IV lupus nephritis, **right upper:** focal segmented WHO type III nephritis (segmental lesion marked by arrow), and **right lower:** glomerulonephritis (WHO type V) with crescent formation observed in BALB/c mice that had been pre-immunized 8x with OVA, depleted of CD4 T cells and inoculated with DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice. Mouse glomerular lesions were classified according to human WHO classification (26) as follows; class I, normal glomeruli; class II, purely mesangial disease; class III, focal proliferative glomerulonephritis; class IV, diffuse proliferative glomerulonephritis; and class V, membranous glomerulonephritis. H & E stain. (bar=30μm; original magnification x200).

Extended Data Fig. 3.

Flow cytometry analysis of CD3, CD4, CD23, CD27, CD43act (activation-associated glycoform), CD44, CD62L, CD123, CD147, CCR4, CCR7, TLR7, Thy1.2 and TCRβ of DOCK8⁻ or DOCK8⁺ CD4 T cells taken from BALB/c mice 9 days after 12x immunization with OVA.

Extended Data Fig. 4.

a, Flow cytometry analysis of CCR4 and Ly6c, CCR4 and LFA1, and Ly6c and LFA in DOCK8⁻ or DOCK8⁺ CD4 T cells taken from BALB/c mice 9 days after 12x immunization with OVA.

b, Regulatory T cell surface markers. Flow cytometry analysis of DOCK8, CD25, Foxp3 and CD4 expression in the spleens of BALB/c mice immunized 12x with OVA (Upper panel). Flow cytometry analysis of CD25, Foxp3 and CD4 in the spleens of BALB/c mice immunized 12x with OVA compared to those mock-immunized 12x with PBS.

Extended Data Fig. 5.

Flow cytometry analysis of B cells in spleen of 12x OVA-immunized versus 12x PBS-immunized BALB/c mice. Statistical analysis was by Student's t-test; * $p < 0.05$.

Extended Data Fig. 6.

Heatmap visualization of the TCRA usage involving a combination of TRAV and TRAJ genes in splenic DOCK8⁺ CD4 T cells versus DOCK8⁻ CD4 T cells from 12x OVA-immunized BALB/c mice, as well as CD4 T cells from 12x PBS-immunized mice.

Extended Data Fig. 7.

Heatmap visualization of the TCRB usage involving a combination of TRBV and TRBJ genes in splenic DOCK8⁺ CD4 T cells versus DOCK8⁻ CD4 T cells from 12x OVA-immunized BALB/c mice, as well as CD4 T cells from 12x PBS-immunized mice.

Extended Data Fig. 8.

*ai*CD4 T cells are generated thru resuscitation from anergy, irrespective of antigen or genetic background, in the presence of NKT cells.

a, Induction of *ai*CD4 T cells by repeated OVA immunization in mice with a different genetic background, C57BL/6.

Generation of autoantibodies, RF and anti-dsDNA Ab, IFN γ ⁺ CD8⁺ T cells, and proteinuria in C57BL/6 mice after 12x immunization with OVA. Statistical analysis performed by Student's t-test; *p<0.05, ***p<0.005.

b,c, Induction of *ai*CD4 T cells by repeated stimulation with SEB in BALB/c mice.

After 2x stimulation with SEB (25 μ g), SEB-reactive V β 8⁺ CD4 T cells were rendered into anergy; IL-2 production and cell division ceased. Upon further repeated immunization 8x with SEB, once-anergized CD4 T cells began to secrete IL-2 and proliferate. This recovered from anergy was accompanied by autoantibody induction.

Cells did not recover from anergy upon 8x stimulation with SEA (25 μ g), which was incompatible with TCR V β 8, but recovered from anergy upon 8x stimulation with SEA in combination with α -galactosyl ceramide (α GalCer) (25 μ g). The CD4 T cells from CD1d KO mice also did not recover from anergy. Statistical analysis was by *p<0.05, **p<0.01.

Extended Data Fig. 9.

Chromatin-immunoprecipitation sequencing (ChIP-seq) assay and LM-PCR for chromatin accessibility and V(D)J recombination.

a, Histone acetylation (AcH3) and H3K4 methylation (H3K4me3) of the TCRA gene of splenic *ai*CD4 T cells from 8x SEB-stimulated BALB/c mice. Splenic CD4 T cells of 8x SEB-stimulated BALB/c mice which produced amply high titers of autoantibodies were assayed. TEA, TCR α promoter. E α , TCR α enhancer.

b, Histone acetylation (AcH3) and H3K4 methylation (H3K4me3) of the TCRB gene of splenic *ai*CD4 T cells from 8x SEB-stimulated BALB/c mice. E β , TCR β enhancer. MyoD (myoblast determination 1) was used as negative controls.

c, TCR α chain revision in the spleen of mice immunized 8x with SEB was determined by LM-PCR detection of dsDNA breaks at the RSS flanking the TCRAJ12, and its confirmation by sequencing.

Extended Data Fig. 10.

a, Expression of IFN signature genes. Results in gene expression microarray analysis, abstract from Supplementary Table 1. Ratio of mRNA expression of splenic DOCK8⁺ CD4 T cells from 12x OVA-immunized BALB/c mice in comparison to splenic DOCK8⁻ CD4 T cells from 12x OVA-immunized mice, given as a log₁₀ scale, wherein 2, 3, 4, 5 and 6 indicated $\times 10^2$, $\times 10^3$, $\times 10^4$, $\times 10^5$, and $\times 10^6$, respectively.

1184 **b**, Flow cytometry analysis of plasmacytoid dendritic cell (pDC) markers.

1185 BST2 and SiglecH, BST2 and B220, and BST2 and CD11c in DOCK8⁺ versus

1186 DOCK8⁻ CD4 T cells from BALB/c mice 9 days after 12x immunization with OVA.

1187

1188 **Supplementary Table 1.**

1189 Gene expression microarray results. Comparison among splenic DOCK8⁺ CD4 T cells of

1190 x12 OVA-immunized BALB/c mice, the splenic DOCK8⁻ CD4 T cells of x12 OVA-

1191 immunized mice, and the CD4 T cells of x12 PBS-immunized mice.

1192 **Supplementary Table 2.**

1193 Result of next-generation sequencing of TCR genes of splenic DOCK8⁺ CD4 T cells of

1194 x12 OVA-immunized BALB/c mice, the splenic DOCK8⁻ CD4 T cells of x12 OVA-

1195 immunized mice, and the CD4 T cells of x12 PBS-immunized mice.

1196 **Supplementary Table 3.**

1197 Primer and probe sequences used in CHIP assay.

Figures

Figure 1

a, Identification of DOCK8 as a marker of aiCD4 T cells. Membrane and cytosolic fractions of either whole T cells (Lanes 1 and 5), CD45RBhi CD122hi CD4 T cells (Lanes 2 and 6), CD45RBlo CD122lo PD-1- CD4 T cells (Lanes 3 and 7) or CD45RBlo CD122lo PD-1+ CD4 T cells (Lanes 4 and 8) from 12x OVA-immunized BALB/c mice, were subjected to 10% polyacrylamide gel electrophoresis, transferred to Immobilon-P membrane, and stained with anti-DOCK8 Ab. b, Expansion of DOCK8+ CD4 T cells after repeated immunization with OVA without an adjuvant Flow cytometry analyses of DOCK8+ CD4 T cell from splenic CD4 T cells of BALB/c mice after repeated 12x stimulation with PBS, 6x with OVA or 12x with OVA. c, Transferred DOCK8+ CD4 T cells induced autoantibodies in the recipients. Rheumatoid factor (RF), anti-Sm Ab and anti-dsDNA Ab were quantified in the sera of BALB/c mice that had been pre-immunized 8x with OVA, depleted of CD4 T cells by anti-CD4 Ab and then inoculated with DOCK8- CD4 T cells or DOCK8+ CD4 T cells from 12x OVA-immunized BALB/c mice. The control group was BALB/c mice pre-immunized 8x with OVA, CD4 T cell-depleted by anti-CD4 Ab, and inoculate with CD4 T cells from 12x PBS-immunized BALB/c mice. Anti-dsDNA Ab and anti-Sm Ab titers were represented by arbitrary unit (AU). Statistical assessment was by Student's t-test. d, Glomerulonephritis in the BALB/c mice that had been pre-immunized 8x with OVA, depleted of CD4 T cells by anti-CD4 Ab and inoculated with DOCK8- CD4 T cells or DOCK8+ CD4 T cells from 12x OVA-immunized BALB/c mice. Control group was the BALB/c mice that had been pre-immunized 8x with OVA, depleted of CD4 T cells by anti-CD4 Ab, and inoculated with CD4 T cells from 12x PBS-immunized BALB/c mice. Glomerular lesions in mice were classified according to human WHO classification (26) as follows; class I, normal glomeruli; class II, purely mesangial disease; class III, focal proliferative glomerulonephritis; class IV, diffuse proliferative glomerulonephritis; and class V, membranous glomerulonephritis. Statistical assessment was by Student's t-test. *p<0.05, **p<0.001. e, Skin lesion. A: Moderate dermatitis with dermal fibrosis, B: liquefaction degeneration (arrow) in the basal cells of epidermis, and C: panniculitis in the deep area of

the dermis of mice pre-treated 8x with OVA, depleted of CD4 T cells and inoculated with DOCK8+ T cells from 12x OVA-immunized mice. D: Discoid lupus-like advanced dermatitis observed in mice immunized 12x with OVA. E: Healed dermatitis with some fibrosis in 12x OVA-immunized mice treated with anti-DOCK8 Ab 24 hours each before the 6x, 8x, 10x and 12x OVA immunizations with OVA. H & E stain. (bar=30µm; original magnification A,B, x400, C, x300, D,E, x100). *p<0.05, **p<0.001 f, Summary of lesions other than kidney in BALB/c mice 8x pre-immunized with OVA, CD4 T cell-depleted and inoculated with DOCK8- CD4 T cells or DOCK8+ CD4 T cells from 12x OVA-immunized BALB/c mice. Also shown were results from anti-DOCK8 Ab treatment of 12x OVA-immunized mice: Mice received 100µg anti-DOCK8 Ab 24 hr each before the 6x, 8x, 10x and 12x OVA immunizations with OVA. *Statistical analysis was by Fisher's exact test. g, Lesion other than kidney or skin including A: lung interstitial pneumonitis, B: interstitial pneumonitis accompanied by angiitis, C: liver pericholangitis, D: Onion-skin lesion with amyloid deposition classical to lupus in the spleen, and F: thyroiditis, accompanied by giant cells, in the BALB/c mice 8x pre-immunized with OVA, CD4 T cell-depleted, and then inoculated with DOCK8+ CD4 T cells from 12x OVA-immunized BALB/c mice. E: Classical onion-skin-like lesion with amyloid deposits in the spleen, and G: perineuritis observed in the dermis of the BALB/c mice immunized 12x with OVA. H & E stain. (bar=30µm; original magnification x200).

Figure 2

Identification of DOCK8+ CD4 T cells as long-lived T follicular helper T cells located at splenic red pulp, which were increased in the peripheral blood of patients with active SLE. a, A, Morphology of DOCK8+ CD4 T cell observed by immunoelectron microscopy, stained with rabbit anti-DOCK8 Ab and Au-tagged ant-rabbit IgG Ab. (bar=1µm; original magnification x12,000). B; C, Nuclear membrane portion of DOCK8+ CD4 T cell, stained likewise. (bar=1µm; original magnification x18,000; x24,000). b, Surface markers of

splenic DOCK8+ CD4 T cells. Flow cytometry for ICOS, CXCR5, and PD-1 expression in splenic DOCK8+ CD4 T cells taken 9 days after the final immunization of BALB/c mice immunized 12x with PBS versus OVA (Left Upper panel). Flow cytometry analysis of ICOS, CXCR5, PD-1, GATA3, ROR γ t, T-bet, Bcl6, Ly6C, IFA1, FR4, and GL7 expression in splenic DOCK8- CD4 T cells and DOCK8+ CD4 T cells isolated from 12x OVA-immunized BALB/c mice. c, Cytokines released in culture supernatants of 1x10⁶/ml DOCK8+ or DOCK8- CD4 T cells following incubation with 2 μ g/ml anti anti-CD3 and 5 μ g/ml anti anti-CD28 Ab at 37°C for 24hr. *p<0.05, **p<0.01, ***p<0.005, ****p<0.001. d, DOCK8+ CD4 T cells in the spleen of autopsied SLE patient who died before therapy. Tissues were fixed in 10% formalin, deparaffinized by graded xylene and graded alcohol sequentially, and serially treated with biotinylated anti-DOCK8 monoclonal Ab, alkaline phosphatase avidin D and anti-rabbit Novolink, followed by reaction with Fast Red and Histogreen (Red). CD4 T cells were stained with rabbit anti-IFN γ Ab and anti-rabbit IgG Ab (Blue) serially. S: red pulp sinus. A; central artery. P: penicillar artery. (bar=100 μ m; original magnification x100). Inset; Magnified DOCK8+ CD4 T cells (Arrows). (bar=20 μ m; original magnification x400). e, Circulating DOCK8+ CD4 T cells in the peripheral blood of patients with SLE and its relationship to the SLE disease activity index (SLEDAI). Data of control patients were depicted as; $\square$ rheumatoid arthritis, $\square$ mixed connective tissue disease, mixed connective tissue disease, $\square$ polymyositis, polymyositis, $\square$ systemic sclerosis, sclerosis, $\square$ microscopic polyangiitis. Also shown were quantification of interferon (IFN) in sera of the same patients with SLE measured by ELISA.

Figure 3

a. Expression of V(D)J recombinase complex and related molecules in splenic CD4 T cells from 12x PBS-immunized BALB/c mice, and in DOCK8- CD4 T cells and DOCK8+ CD4 T cells from 12x OVA-immunized BALB/c mice. Two mice were analyzed for each group. b, Western blot analysis of TCR signal transduction molecules in splenic CD4 T cells from 12x PBS-immunized BALB/c mice, and in DOCK8- CD4 T cells and DOCK8+ CD4 T cells from 12x OVA-immunized BALB/c mice. Two mice were analyzed for each group. GAPDH, control house keeping glyceraldehyde 3-phosphate dehydrogenase gene. c, Heatmap visualization of TCR usage of a combination of TRV and TRJ genes in the splenic DOCK8- CD4 T cells and DOCK8+ CD4 T cells from 12x OVA-immunized mice. Three mice were analyzed for each group. d, Autoantibody microarray study. Autoantibodies produced in BALB/c mice pre-immunized 8x with OVA, depleted of CD4 T cells and inoculated with DOCK8- CD4 T cells or DOCK8+ CD4 T cells from 12x OVA-immunized BALB/c mice. Control was autoantibodies produced in BALB/c mice pre-immunized 8x with OVA, depleted of CD4 T cells and inoculated with CD4 T cells from 12x PBS-immunized BALB/c mice. Two mice were analyzed for each group. e, A, Western blot analysis of DOCK8 and transcription factors in splenic CD4 T cells from 12x PBS-immunized BALB/c mice, and in DOCK8- CD4 T cells and DOCK8+ CD4 T cells from 12x OVA-immunized BALB/c mice. Three mice were analyzed for each group. B, Results of gene expression microarray analysis, abstract from Supplementary Table 1. Ratio of mRNA expression of splenic DOCK8+ CD4 T cells from 12x OVA-immunized BALB/c mice in comparison to splenic DOCK8- CD4 T cells from 12x OVA-immunized mice, given as a log10 scale, wherein 2, 3, 4, 5 and 6 indicated x102, x103, x104, x105, and x106, respectively.

Figure 4

a, The effect of anti-DOCK8 mAb treatment on autoantibody generation. Autoantibodies, rheumatoid factor (RF), anti-Sm Ab and anti-dsDNA Ab, were analyzed in the sera of 12x PBS-immunized or OVA-immunized mice: Mice received anti-DOCK8 Ab (100µg) or control rabbit IgG (100µg) 24 hr each before

the 6x, 8x, 10x and 12x OVA immunizations with OVA. Anti-Sm Ab and anti-dsDNA Ab titers were represented by arbitrary unit (AU). *Statistical analysis was by Student's t-test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$. b, The results of anti-DOCK8 mAb treatment on renal disease. Upper: Proteinuria, assessed 9 d after the final immunization and graded with a score of 0 (<30 mg/dl); 1 (30–99 mg/dl); 2 (100–299 mg/dl); or 3 (300–999 mg/dl). Lower: Kidney lesions of BALB/c mice immunized 12x with either PBS or OVA, and treated either with anti-DOCK8 Ab (100µg) or control rabbit IgG (100µg) 24 hr each before the 6x, 8x, 10x and 12x OVA immunizations with OVA. Mouse glomerular lesions were classified according to human WHO classification (26) as follows; class I, normal glomeruli; class II, purely mesangial disease; class III, focal proliferative glomerulonephritis; class IV, diffuse proliferative glomerulonephritis; and class V, membranous glomerulonephritis. Statistical assessment was by Student's t-test; * $p < 0.05$, ** $p < 0.005$, *** $p < 0.001$. c, Results of anti-DOCK8 mAb treatment on (NZBxNZW) F1 female mice. Autoantibodies, anti-dsDNA Ab, anti-Sm Ab and rheumatoid factor (RF), and proteinuria of (NZBxNZW) F1 female mice, treated either with anti-DOCK8 Ab (100 µg) or control rabbit IgG (100 µg) every week from 15 weeks until 24 weeks (arrow). Statistical assessment performed by Student's t-test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$, **** $p < 0.001$.

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