Regulation of age-associated B cells by IRF5 in systemic autoimmunity

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Age-associated B cells (ABCs) are a subset of B cells dependent on the transcription factor T-bet that accumulate prematurely in autoimmune settings. The pathways that regulate ABCs in autoimmunity are largely unknown. SWAP-70 and DEF6 (also known as IBP or SLAT) are the only two members of the SWEF family, a unique family of Rho GTPase–regulatory proteins that control both cytoskeletal dynamics and the activity of the transcription factor IRF4. Notably, DEF6 is a newly identified human risk variant for systemic lupus erythematosus. Here we found that the lupus syndrome that developed in SWEF-deficient mice was accompanied by the accumulation of ABCs that produced autoantibodies after stimulation. ABCs from SWEF-deficient mice exhibited a distinctive transcriptome and a unique chromatin landscape characterized by enrichment for motifs bound by transcription factors of the IRF and AP-1 families and the transcription factor T-bet. Enhanced ABC formation in SWEF-deficient mice was controlled by the cytokine IL-21 and IRF5, whose variants are strongly associated with lupus. The lack of SWEF proteins led to dysregulated activity of IRF5 in response to stimulation with IL-21. These studies thus elucidate a previously unknown signaling pathway that controls ABCs in autoimmunity.

Aberrant humoral responses have a key role in the pathogenesis of systemic lupus erythematosus (SLE). While the expansion of germinal center (GC) B cell and plasma cell (PC) populations has long been associated with SLE, additional B cell subsets might also contribute to this disease. Studies of aging mice have identified a B cell subset, age-associated B cells (ABCs), that exhibits a unique phenotype and ‘preferentially’ expands in mice. In addition to expressing classical B cell markers, ABCs also express the myeloid markers CD11c and CD11b. The formation of ABCs is promoted by the cytokines IFN-γ and IL-21 and by engagement of the receptors TLR7 and TLR9. While ABCs exhibit somatic hypermutation, their relationship with GC B cells and PCs is not yet understood. ABCs increase in abundance prematurely in mouse lupus and produce antibodies directed against chromatin. ABC-like B cells (which include IgD –CD27– and CD21lo B cells) have been detected in human autoimmune disorders, including SLE. ABCs express T-bet and depend on this transcription factor for their generation; thus, they are also known as CD11c+T-bet+ B cells. The molecular pathways that promote the expansion and pathogenicity of the ABC population in autoimmunity remain largely unknown.

Several interferon-regulatory factors (IRFs) have been linked to autoimmunity. Among the IRFs, IRF4 has a fundamental role in T cells and B cells, including inducing the production of IL-21, class switching and differentiation into PCs. The multifaceted role of IRF4 has been ascribed to its ability to act together with multiple transactivators, such as members of the AP-1 family (BATF and Jun) and the Ets transcription factor PU.1. Genetic studies have also demonstrated strong associations between variants of IRF5 and human autoimmune disorders, particularly SLE. Furthermore, IRF5 deficiency ameliorates mouse lupus in several models. IRF5 is expressed in myeloid cells and regulates the polarization of macrophages into the classically activated, inflammatory M1 phenotype and the production of IFN-α and of proinflammatory cytokines. Estrogen can modulate the abundance of IRF5 in B cells, in which IRF5 regulates class switching to immunoglobulin G2a (IgG2a) and/or IgG2c and expression of the transcription factor Blimp1. A search for proteins that interact with IRF4 led to the isolation of the protein DEF6 (also known as IBP or SLAT). DEF6 exhibits substantial homology to only one other protein, SWAP-70. SWAP-70 and DEF6 constitute the SWEF family, a unique family of Rho GTPase–regulatory proteins that control both cytoskeletal dynamics and IRF4 activity. Notably, the DEF6 locus has been identified as a genetic risk factor for human SLE. The SWEF proteins have an important immunoregulatory role, and the simultaneous lack of SWAP-70 and DEF6 in C57BL/6 mice (double-knockout (DKO) mice) leads to the spontaneous development of lupus, which, like human SLE, ‘preferentially’ affects females. Autoimmunity in DKO mice is associated with dysregulation of T cells and B cells, increased production of IL-21 and enhanced formation of GC B cells and PCs.

Since ABCs accumulate in autoimmune mice, we investigated this B cell subset in DKO mice. DKO mice exhibited IL-21-dependent expansion of a proliferating ABC population with proinflammatory abilities. DKO ABCs produced autoantibodies and displayed a transcriptome distinct from that of wild-type ABCs, marked by increased transcription of immunoglobulin-encoding genes and diminished expression of molecules in a subset of myeloid-related programs. DKO ABCs exhibited a unique chromatin landscape that showed enrichment for open chromatin regions containing binding motifs for IRF, AP-1–BATF.
and T-bet. In the absence of the SWEF proteins, stimulation of B cells with IL-21 led to dysregulated IRF5 activity and the generation of ABCs. Furthermore, the population expansion of ABCs and development of lupus in female DKO mice was controlled by IRF5. Thus, IRF5 was a regulator of ABCs in these autoimmune settings.

**Results**

**Spontaneous expansion of the ABC population in DKO mice.** The spontaneous development of autoimmunity in female DKO mice led us to investigate whether ABCs accumulated prematurely in DKO mice. The frequency and number of splenic B cells expressing CD11c and CD11b were much greater in female DKO mice than

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**Fig. 1** | Spontaneous expansion of the ABC population in DKO mice. **a,** Flow cytometry of B220+ or B220+CD19+ cells (above plots) from the spleen of female wild-type (WT) and DKO mice over 23 weeks of age (above plots), analyzing the expression of CD11c and CD11b (left), and frequency (middle) and number (right) of CD11c+CD11b+ cells in mice as at left. Numbers adjacent to outlined areas (left) indicate percent CD11c+CD11b+ cells. Data are representative of eight independent experiments (B220+ cells; n = 12 wild-type mice and n = 16 DKO mice) or three independent experiments (B220+CD19+ cells; n = 3 wild-type mice and n = 6 DKO mice). **P = 0.0014 and ***P < 0.0001 (two-tailed Student’s t-test). **b,** Flow cytometry of B220+CD19+ cells from the spleen of female wild-type, DKO, Def6–/– and Swap70–/– mice 18–24 weeks of age (above plots), as in a. Data are representative of three independent experiments (n = 3 wild-type mice, n = 4 Swap70–/– mice, n = 5 Def6–/– mice and n = 5 DKO mice). NS, not significant (P ≥ 0.05); *P = 0.0403 and **P < 0.0001 (one-way analysis of variance (ANOVA) followed by Bonferroni’s multiple-comparisons test). **c,** Expression of various markers (below plots) on B220+CD19+CD11c+CD11b+ or B220+CD19+CD11c–CD11b– cells (key) from the spleen of female DKO mice over 18 weeks of age. Data are representative of seven independent experiments with n = 11 mice (T-bet, CD21 and CD23); four independent experiments with n = 6 mice (CD86 and major histocompatibility complex class II (MHCII)); three independent experiments with n = 5 mice (IgD, IgM, CD43 and CD93); or two independent experiments with n = 4 mice (CD5). **d,** ELISA of IgG antibodies to dsDNA (IgG2c), nRNP and cardiolipin in the supernatants of sorted ABCs (B220+CD19+CD11c+CD11b+) and FoB cells (B220+CD19+CD11c–CD11b–) left unstimulated (−) or stimulated for 7 days in vitro with imiquimod (1 μg/ml) (+imiq) (key); results are presented as optical density at 450 nm (OD450). Data are from one experiment representative of four independent experiments with n = 4 cultures (mean ± s.e.m. of technical replicates). ***P = 0.0004 (anti-dsDNA IgG2c). **P < 0.0001 (anti-nRNP IgG) or **P = 0.0007 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). Each symbol (a,b,d) represents an individual mouse (a,b) or replicate (d); small horizontal lines (a,b) indicate the mean.
IL-21 regulates the generation of DKO ABCs in vitro and in vivo. **a.** Flow cytometry (left) analyzing the expression of T-bet and CD11c by CD23⁺ B cells purified from female wild-type and DKO mice 8–10 weeks of age (left margin) stimulated for 3 d with anti-IgM (5 μg/ml) and anti-CD40 (5 μg/ml) alone (−) or together with IL-21 (50 ng/ml) or imiquimod (1 μg/ml) (above plots), and frequency of ABCs (B220⁺ B cells purified from female wild-type and DKO mice 8–10 weeks of age (left margin) stimulated for 3 d with anti-IgM (5 μg/ml) or mouse (experiments with 6 wild-type mice, n = wild-type, DKO and 5 wild-type mice, n = cells. Data are representative of four independent experiments with n = cultures. Data are representative of four independent experiments with n = 5 wild-type mice, n = 6 DKO mice and n = 8 IL21⁻/⁻ DKO mice. ***P < 0.0002 and ****P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). **b.** Flow cytometry of B220⁺ cells from the spleen of female wild-type, DKO and IL21⁻/⁻ DKO mice over 24 weeks of age (above plots), analyzing the expression of CD11c and CD11b (left), and frequency (middle) and number (right) of ABCs (B220⁺CD11c⁺CD11b⁻) cells in mice as at left. Numbers adjacent to outlined areas (left) indicate percent CD11c⁻CD11b⁺ cells. Data are representative of four independent experiments with n = 5 wild-type mice, n = 6 DKO mice and n = 8 IL21⁻/⁻ DKO mice. ***P < 0.0002 and ****P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). **c.** Flow cytometry of B220⁺ cells from the spleen of female wild-type, DKO and Sap⁻/⁻ DKO mice over 24 weeks of age (above plots), as in **b.** Data are representative of four independent experiments with n = 4 wild-type mice, n = 4 DKO mice and n = 7 Sap⁻/⁻ DKO mice. ***P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). **d.** ELISA of IgG antibodies to dsDNA in the serum of wild-type, DKO, IL21⁻/⁻ DKO, and Sap⁻/⁻ DKO mice (horizontal axis). Data are representative of four independent experiments with n = 6 wild-type mice, n = 8 DKO mice, n = 8 IL21⁻/⁻ DKO mice and n = 6 Sap⁻/⁻ DKO mice. ****P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). Each symbol represents an individual culture (a) or mouse (b–d); small horizontal lines indicate the mean.

Fig. 2 | IL-21 regulates the generation of DKO ABCs in vitro and in vivo. a. Flow cytometry (left) analyzing the expression of T-bet and CD11c by CD23⁺ B cells purified from female wild-type and DKO mice 8–10 weeks of age (left margin) stimulated for 3 d with anti-IgM (5 μg/ml) and anti-CD40 (5 μg/ml) alone (−) or together with IL-21 (50 ng/ml) or imiquimod (1 μg/ml) (above plots), and frequency of ABCs (B220⁺T-bet⁺CD11c⁻ cells) (right) in cultures as at left. Numbers in quadrants (left) indicate percent cells in each. Data are representative of five independent experiments with n = 5 cultures. ****P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). b. Flow cytometry of B220⁺ cells from the spleen of female wild-type, DKO and IL21⁻/⁻ DKO mice over 24 weeks of age (above plots), analyzing the expression of CD11c and CD11b (left), and frequency (middle) and number (right) of ABCs (B220⁺CD11c⁻CD11b⁻) cells in mice as at left. Numbers adjacent to outlined areas (left) indicate percent CD11c⁻CD11b⁺ cells. Data are representative of four independent experiments with n = 5 wild-type mice, n = 6 DKO mice and n = 8 IL21⁻/⁻ DKO mice. ***P < 0.0002 and ****P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). c. Flow cytometry of B220⁺ cells from the spleen of female wild-type, DKO and Sap⁻/⁻ DKO mice over 24 weeks of age (above plots), as in b. Data are representative of four independent experiments with n = 4 wild-type mice, n = 4 DKO mice and n = 7 Sap⁻/⁻ DKO mice. ***P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). d. ELISA of IgG antibodies to dsDNA in the serum of wild-type, DKO, IL21⁻/⁻ DKO, and Sap⁻/⁻ DKO mice (horizontal axis). Data are representative of four independent experiments with n = 6 wild-type mice, n = 8 DKO mice, n = 8 IL21⁻/⁻ DKO mice and n = 6 Sap⁻/⁻ DKO mice. ****P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). Each symbol represents an individual culture (a) or mouse (b–d); small horizontal lines indicate the mean.
Fig. 3 | DKO ABCs exhibit a distinctive transcriptome. a, RNA-seq analysis of RNA from FoB cells (B220−CD19−CD11c−CD11b−CD23+) sorted by flow cytometry from female wild-type mice (n = 2) and DKO mice (n = 3) and from ABCs (B220−CD19−CD11c−CD11b+) sorted by flow cytometry from female DKO mice (n = 3) (below plots), all over 20 weeks of age, presented as z-scores of expression (key), showing hierarchical clustering (left margin and top) of log-transformed expression (counts per million) of genes expressed differentially by these cells (specificity and upregulation (†) or downregulation (‡), right margin). Data are from two (WT) or three (DKO) experiments. b, Gene expression in FoB cells, presented as a volcano plot showing genes expressed differentially (change in expression of over twofold) in cells from wild-type mice (n = 2) relative to their expression in cells from DKO mice (n = 3), plotted against FDR-corrected P value (P < 0.01; dashed horizontal line indicates cutoff of P = 0.01), assessing genes belonging to selected GSEA Hallmark pathways (key). Data are from two (WT) or three (DKO) experiments. c, Proliferation of B220−CD11c−T-bet− cells in the spleen of female wild-type and DKO mice (key) (left) or of B220−CD11c−T-bet− cells and B220−CD11c−T-bet− (ABCs) in the spleen of female DKO mice (key) (right), all over 23 weeks of age, assessed by Ki67 staining and flow cytometry. Data are representative four independent experiments with 5 mice per group (left) or five mice per group (right). d, Gene expression in FoB cells and ABCs (B220−CD19−CD11c−CD11b+) from female DKO mice (n = 3) over 20 weeks of age, presented as a volcano plot showing genes expressed differentially (change in expression of over twofold) in FoB cells relative to their expression in ABCs, plotted against FDR-corrected P value (as in b), assessing genes belonging to selected GSEA Hallmark pathways (key). Data are from three experiments. e, RNA-seq analysis of RNA from FoB cells and ABCs sorted by flow cytometry (cell markers as in a) from female wild-type mice (n = 2) and DKO mice (n = 3) (below plots), presented as z-scores of expression (key), showing hierarchical clustering (left margin and top) of log-transformed expression (as in a) for genes in the GO_inflammatory_response gene-ontology gene set of the Molecular Signatures Database; Pearson’s correlation was used as distance metric between genes. Data are from of two (WT) or three (DKO) experiments. f, qPCR analysis of the expression of Ccl5, Ifng and Cxcl10 mRNA in sorted FoB cells from female wild-type and DKO mice and in ABCs from female DKO mice (horizontal axis; all cell markers as in a); results were normalized relative to those of the control Ppia mRNA and are presented in arbitrary units (AU). Each symbol represents an individual replicate. Data are from one experiment representative of two (Ccl5) or three (Ifng and Cxcl10) independent experiments with n = 2 mice per genotype (Ccl5) or n = 3 mice per genotype (Ifng and Cxcl10) (mean ± s.e.m. of technical replicates). *P = 0.0242 (Ifng; wild-type FoB versus DKO ABC) or 0.0282 (Ifng; DKO FoB versus ABC DKO); **P = 0.0068 (wild-type FoB versus DKO ABC) or 0.0072 (DKO FoB cell versus DKO ABC); and ***P = 0.0002 (Ccl5) (one-way ANOVA followed by Bonferroni’s multiple-comparisons test).
not shown). Stimulated DKO ABCs also produced antibodies to nuclear ribonuclear protein (nRNP) and IgG antibodies to cardiolipin (Fig. 1d). ABCs might thus directly contribute to lupus in DKO mice by producing autoantibodies.

IL-21 regulates the generation of DKO ABCs in vitro and in vivo. The generation of mouse ABCs can be promoted by IL-21 and TLRs. We thus directly investigated the ability of these signals to drive ABC formation in vitro from the B cells of 8- to 10-week-old wild-type and DKO mice. The addition of IL-21 resulted in a significantly greater population of CD11c+T-bet+ ABCs in cultures of DKO B cells than in those of wild-type B cells, but the addition of imiquimod did not (Fig. 2a). Similar results were obtained with CD11c and CD11b as markers (Supplementary Fig. 2a). As reported, the stimulation of wild-type and DKO B cells with either IL-4 or IFN-γ alone did not generate CD11c+T-bet+ B cells, and the addition of IL-4 inhibited the IL-21-mediated formation of these cells in both wild-type cultures and DKO cultures (Supplementary Fig. 2b). DKO B cells therefore exhibited an increased ability to generate ABCs in vitro after stimulation with IL-21.

To further evaluate the importance of IL-21 in the expansion of the DKO ABC population, we assessed female DKO mice lacking Il21 (Il21−/− DKO mice). The accumulation of ABCs was completely abolished in these mice, in contrast to the presence of
Fig. 5 | Wild-type and DKO ABCs exhibit distinct transcriptional and chromatin profiles. a, RNA-seq analysis of RNA from ABCs (B220⁺CD19⁺CD11c⁺CD11b⁺) sorted by flow cytometry from female wild-type and DKO mice (n = 2 per group) (below plots), all over 33 weeks of age, presented as z-scores of expression (key), showing hierarchical clustering (left margin of right plot) of log-transformed expression (counts per million) of genes expressed differentially by these cells. b, Gene expression summary statistics for all differentially expressed genes (n = 713), immunoglobulin-encoding genes (n = 34) and the macrophage signature of the ARCHS4 RNA-seq database (http://amp.pharm.mssm.edu/archs4/index.html) (above plots) in wild-type and DKO ABCs (horizontal axis), presented as a violin plot showing median expression (horizontal line), first and third quartiles (vertical box bounds), value spread (central vertical line bound at 1.5 interquartile range) and outliers (black circles) with the kernel density estimate of expression values distribution. Pairwise comparisons (approximation of the Wilcoxon-Mann-Whitney test): all differentially expressed genes, P < 2.2 × 10⁻¹⁰⁵; Z = 16.65, 95% confidence interval, [1.952, 2.415]; immunoglobulin-encoding genes, P = 0.00044, Z = -3.44, 95% CI, [-2.4170, -0.8235]; and ARCHS4 macrophages signature, P < 2.2 × 10⁻¹⁰⁵, Z = 11.52, 95% CI, [2.148, 2.9175]. c, qPCR analysis of various genes in ABCs (as in a) sorted from female wild-type and DKO mice (n = 2 per group); results were normalized to those of Fplia mRNA. Each symbol represents an individual replicate. *P = 0.00273 (Lifr) or 0.0254 (Axl); **P = 0.0081 (Nfil3) or 0.0054 (Merk); and ***P = 0.0007 (Maf) or 0.0003 (Jun) (two-tailed Student’s t-test). d, Normalized ATAC-seq tag density distributions for 4-kb window centered at the summit of wild-type ABC-specific peaks (top left; n = 27,483) or DKO ABC-specific peaks (top right; n = 1583), and average distribution of ATAC-seq normalized tag densities (bottom) (Kolmogorov-Smirnov test). e, De novo motif-enrichment analysis in wild-type ABC-specific and DKO ABC-specific ATAC-seq peaks; binomial distribution was used for motif scores (confidence level, 95%). (f) Functionally enriched gene ontology categories (left margin) for wild-type-specific and DKO-specific peaks of ATAC-seq. Data are from two independent experiments (a,b) or one experiment representative of two independent experiments with similar results (c–f, mean ± s.e.m. of technical replicates).
such cells in age-matched female DKO mice (Fig. 2b), *Il21*−/− DKO mice also failed to accumulate follicular helper T cells (Tfh), GC B cells or PCs and did not produce autoantibodies to dsDNA (Fig. 2d and Supplementary Fig. 2c–f). In addition to being produced by Tfh cells, IL-21 can also be produced by innate sources. To determine whether direct T cell–B cell contacts were necessary for the population expansion of DKO ABCs in vivo, we assessed their presence in DKO mice lacking Sh2d1a, which encodes the adaptor SAP (‘signaling lymphocytic-activation molecule–associated protein’; called ‘Sap’ here: Sap−/− DKO mice), which mediates sustained T cell–B cell interactions. The absence of SAP in DKO mice strongly inhibited the accumulation of ABCs, Tfh cells, GC B cells and PCs and autoantibody production (Fig. 2c,d and Supplementary Fig. 2c–f). Thus, the aberrant population expansion of DKO ABCs was dependent on IL-21 and cognate T cell–B cell interactions.

**SWEF proteins regulate the proliferation and proinflammatory ability of ABCs.** To gain insight into the mechanisms by which the SWEF proteins regulate ABCs, we next sorted B cells on the basis of their expression of CD11c and CD11b and employed RNA-based next-generation sequencing (RNA-seq) to compare the transcriptomes of wild-type FoB cells, DKO FoB cells and DKO ABCs. A total of 3,049 genes were expressed differentially by the three different populations (a change in expression of onefold (log value); false-discovery rate (FDR), <0.01) (Fig. 3a). A set of genes were either upregulated (cluster 2) or downregulated (cluster 1) in DKO B cells regardless of their expression of CD11c and CD11b (Fig. 3a), which suggested that the lack of SWEF proteins altered the expression of these genes in B cells independently of their differentiation state. On the basis of gene-set–enrichment analysis (GSEA) (Fig. 3b and Supplementary Fig. 3a), we concluded that the lack of SWEF proteins affected the control of B cell proliferation, potentially via...
the E2F family of transcription factors and regulators of the G2-M checkpoint. Analysis of staining with the proliferation marker Ki67 revealed that CD11c T-bet DKO B cells included a smaller population of highly proliferative cells than did their wild-type counterparts (Fig. 3c). DKO ABCs proliferated even more robustly than did CD11c T-bet DKO B cells (Fig. 3c). No differences in apoptosis were observed (Supplementary Fig. 3b). In vitro experiments demonstrated that DKO ABCs proliferated to a greater extent than did wild-type ABCs after stimulation with IL-21 (Supplementary Fig. 3c) while exhibiting similar survival (Supplementary Fig. 3d,e).

Thus, SWEF proteins regulated the proliferation of B cells and had an important role in restraining ABC proliferation in response to IL-21.

In addition to the DKO cell–specific clusters 1 and 2, clusters 3 and 5 were uniquely regulated in DKO ABCs and not in FoB cells from either wild-type mice or DKO mice (Fig. 3a,d). As expected, DKO ABCs exhibited higher expression of Tbx21 (which encodes T-bet), Iga (which encodes the integrin subunit αδ (CD11c)) and
Fig. 8 | Monoallelic deletion of *Irf5* abolishes the accumulation of ABCs and lupus development in DKO mice. a. Flow cytometry of cells from the spleen of female wild-type, *Irf5*^fl/fl^ DKO, *Irf5*^−/−^ DKO and *Cd21*^−/Cre^ DKO mice over 20 weeks of age (above plots). Numbers adjacent to outlined areas indicate percent CD11c^−CD11b^+ B cells. Data are representative of ten independent experiments. b. Frequency (left) and number (right) of ABCs in mice as in a (horizontal axis). Data are from ten independent experiments with *n* = 9 wild-type mice, *n* = 10 *Irf5*^fl/fl^ DKO mice, *n* = 10 *Irf5*^−/−^ DKO mice, *n* = 10 *Cd11c*^−/Cre^ DKO mice and *n* = 5 *Cd21*^−/Cre^ DKO mice. ***P* < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). c, d, Microscopy (left) of antinuclear antibodies in the serum (1:200 dilution) of mice as in a (above images), and fluorescence intensity (right) of antinuclear antibodies in such mice (horizontal axis). Data are from ten independent experiments with *n* = 7 wild-type mice, *n* = 9 *Irf5*^fl/fl^ DKO mice, *n* = 9 *Irf5*^−/−^ DKO mice, *n* = 12 *Cd11c*^−/Cre^ *Irf5*^−/−^ DKO mice and *n* = 7 *Cd21*^−/Cre^ *Irf5*^−/−^ DKO mice. *P* = 0.0142, **P = 0.004 and ***P < 0.0001 (Mann-Whitney test). c. ELISA of IgG, IgG1 and IgG2c antibodies to ssDNA in mice as in a (horizontal axis). Data are from ten independent experiments with *n* = 9 (IgG and IgG2c) or 5 (IgG1) wild-type mice; *n* = 9 (IgG and IgG2c) or 11 (IgG1) *Irf5*^fl/fl^ DKO mice; *n* = 6 *Irf5*^−/−^ DKO mice; *n* = 10 (IgG and IgG2c) or 4 (IgG1) *Cd11c*^−/Cre^ *Irf5*^−/−^ DKO mice; and *n* = 5 (IgG and IgG2c) or 9 (IgG1) *Cd21*^−/Cre^ *Irf5*^−/−^ DKO mice. *P* = 0.0206 (IgG) or 0.0280 (IgG1); ***P = 0.003; and ****P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). d. ELISA of IgG antibodies to ssDNA, cardiolipin and nRNP in the serum of mice as in a (horizontal axis). Data are from ten independent experiments with *n* = 6 (ssDNA and nRNP) or 8 (cardiolipin) wild-type mice; *n* = 17 (ssDNA and nRNP) or 19 (cardiolipin) *Irf5*^fl/fl^ DKO mice; *n* = 7 *Irf5*^−/−^ DKO mice; *n* = 6 (ssDNA and nRNP) or 4 (cardiolipin) *Cd11c*^−/Cre^ *Irf5*^−/−^ DKO mice; and *n* = 9 (ssDNA and cardiolipin) or 8 (nRNP) *Cd21*^−/Cre^ *Irf5*^−/−^ DKO mice. *P* < 0.05, **P < 0.01; ***P < 0.001 and ****P < 0.0001 (one-way ANOVA followed by Bonferroni’s multiple-comparisons test). e, f, Microscopy of renal sections from wild-type, DKO and *Irf5*^−/−^ DKO mice (including *Cd11c*^−/Cre^ *Irf5*^−/−^ DKO and *Cd21*^−/Cre^ *Irf5*^−/−^ DKO mice) (above images), assessed with periodic acid-Schiff staining (left), and glomerulonephritis (GN) scores of such mice (right). Scale bar (left), 20 μm. Data are from three independent experiments with *n* = 3 wild-type mice, *n* = 6 DKO mice and *n* = 4 *Irf5*^−/−^ DKO mice. *P* = 0.0275 (wild-type versus DKO) or 0.0307 (DKO versus *Irf5*^−/−^ DKO) (Mann-Whitney test). g. Microscopy of renal sections from mice as in f (above images), assessing deposition of IgG (left), and mean fluorescence intensity (MFI) of IgG in such renal sections (right). Scale bar (left), 30 μm. Data are representative of three independent experiments with five glomeruli per mouse (*n* = 3 wild-type mice, *n* = 6 DKO mice and *n* = 4 *Irf5*^−/−^ DKO mice). **P = 0.0071 (wild-type versus DKO) or 0.0021 (DKO versus *Irf5*^−/−^ DKO). Each symbol (b–g) represents an individual mouse (b–f) or glomerulus (g); small horizontal lines (b–f) indicate the mean.
Igαm (which encodes the integrin subunit αm (CD11b)) than that of FoB cells (Fig. 3d). GSEA indicated that among the sets showing the greatest enrichment (FDR, q < 0.05) in DKO ABCs relative to the number of genes in those sets that were expressed in FoB cells, several gene sets were related to the control of inflammation, chemotaxis, integrin binding and cell adhesion (Fig. 3d,e and Supplementary Fig. 3f). Prominent among the upregulated genes were several genes encoding chemokines (for example, Cxcl9, Cxcl10 and Ccl5), cytokine receptors (for example, Il1r2, Il12rb2 and Il18r1) and cytokines (Fig. 3d and Supplementary Fig. 3f); some of these results were further validated by qPCR in sorted cells (Fig. 3f). Thus, DKO ABCs were endowed with greater proinflammatory ability than that of FoB cells and had unique migratory and adhesive attributes relative to those of FoB cells.

The chromatin landscape of DKO ABCs shows enrichment for IRF and AP-1–BATF motifs. We next employed an assay for transposase-accessible chromatin followed by deep sequencing (ATAC-seq)\(^3\) to investigate the chromatin landscape of DKO ABCs. ATAC-seq signals from DKO ABCs were compared with those of DKO FoB cells sorted from the same mice (Fig. 4a). We identified 3,666 ABC-specific peaks that were found mainly in intergenic and intronic regions and only rarely in promoters (Fig. 4b). Loci with accessibility in ABCs different from that in FoB cells included those encoding proinflammatory cytokines (such as Ifng and Il6) and other key genes (such as the Cxcl10 cluster of genes) (Fig. 4c). ABC-specific peaks were associated with genes that were more transcriptionally active in DKO ABCs than in DKO FoB cells, and pathway analysis showed that many of the differentially expressed ATAC-seq associated genes encoded molecules involved in locomotion and cellular adhesion (Supplementary Fig. 4a–c).

To gain insight into the mechanisms underlying the distinctive chromatin profile of DKO ABCs, we identified the transcription factor–binding motifs over-represented in ABC-specific peaks (Fig. 4d). ABC-specific accessible loci displayed enrichment for binding motifs for AP-1–BATF, IRFs and T-bet (Fig. 4d). The ABC-specific peaks exhibited substantial positional bias in the distribution of binding motifs for IRFs and T-bet that coincided with the peak summit (Fig. 4e). In contrast, FoB cell–specific peaks exhibited enrichment for motifs for a distinct set of transcription factors, including Pou2f2 (Fig. 4d,e). Thus, DKO ABCs exhibited a unique chromatin landscape that, in addition to showing enrichment for T-bet motifs, showed enrichment for IRF and AP-1–BATF motifs and correlated with a distinctive transcriptional profile.

Distinctive transcriptional and epigenomic programs of ABCs from autoimmunity-prone DKO mice. We next investigated whether the transcriptional profiles of ABCs from autoimmunity-prone mice differed from those of the ABCs that slowly accumulated in aging wild-type female mice. ABCs from wild-type and age-matched DKO mice had similar expression of Tbx21 (Supplementary Fig. 5a). A total of 711 genes were expressed differentially by the two populations (change in expression of onefold (log value); FDR < 0.01); among these, 111 genes were upregulated and 600 genes were downregulated in DKO ABCs relative to their expression in wild-type ABCs (Fig. 5a). DKO ABCs expressed several immunoglobulin-encoding gene transcripts more abundantly than did wild-type ABCs but downregulated a subset of myeloid-related transcripts (Fig. 5a,b and Supplementary Table 1). No change in the expression of genes encoding key regulators of PC differentiation, such as Ifr4, Ifr8, Bcl6 or Prdm1 (Supplementary Fig. 5a), was detected in DKO ABCs, which suggested that the differences were not due to the presence of contaminating plasmablasts. However, DKO ABCs exhibited alterations in the expression of genes encoding other transcription factors, including upregulation of Jun and Nfil3 and downregulation of Maf, MafB and Pparg, while their Spi1 expression was similar to that of wild-type ABCs (Fig. 5c and Supplementary Fig. 5a). Differential expression of selected genes, including genes, encoding key regulators of the engulfment of apoptotic cells, such as Mertk and Axl, was further confirmed by qPCR (Fig. 5c). Thus, ABCs from autoimmunity-prone DKO mice were endowed with a greater immunoglobulin-producing capacity than that of wild-type ABCs but downregulated their expression of genes encoding some of the myeloid-related features associated with this B cell subset.

To identify the differences in the chromatin landscapes of wild-type ABCs and DKO ABCs that might accompany the distinct transcriptional profiles noted above, we compared the ATAC-seq signals of sorted ABCs from wild-type and age-matched DKO mice. We identified 27,483 wild-type ABC–specific peaks and 1,583 DKO ABC–specific peaks (Fig. 5d). Most of the wild-type ABC– or DKO ABC–specific peaks were found mainly in intergenic and intronic regions and only rarely in promoters (Supplementary Fig. 5b). DKO ABC–specific accessible loci displayed enrichment for binding motifs for IRF, AP-1–BATF and T-bet (Fig. 5e). In contrast, wild-type ABC–specific peaks were associated with enrichment for binding motifs for the transcription factors Pu.1, Maf and C/EBP (Fig. 5e). These results were consistent with the downregulation of Maf and Mafb observed in DKO ABCs and were reflected in differences in the accessibility of the Maf and Mafb loci detected by ATAC-seq (Supplementary 5c). Gene-ontology categories of genes associated with wild-type cell– or DKO cell–specific peaks indicated that wild-type ABC–specific peaks were positively associated with the transcriptional programs of genes encoding molecules that regulate phagocytosis and other myeloid-related functions, while DKO ABC–specific peaks showed enrichment for genes encoding molecules in processes linked to B cell differentiation, B cell activation, and immunoglobulin regulation (Fig. 5f). These findings supported the idea that the difference between wild-type ABCs and DKO ABCs in chromatin accessibility was functionally important. Thus, the chromatin landscape of ABCs from autoimmunity-prone mice was characterized by dual abnormalities, relative to that of wild-type ABCs, whereby enrichment for IRF and AP-1–BATF motifs was coupled to depletion of Pu.1- and MAF-bound regulatory regions.

IRF5 regulates the IL-21-mediated formation of DKO ABCs. The enrichment for IRF motifs in the chromatin landscape of DKO ABCs suggested that IRFs might contribute to the generation and/or function of ABCs. Since the SWEEF proteins can regulate IRF4 activity\(^3\), we first investigated whether DKO ABCs depended on IRF4. Analysis of Cd11c-Cre Irf4\(^{fl/fl}\) DKO mice (with deletion of lacP-flanked Irf4 alleles by Cre recombinase expressed from the promoter of Igαx (called ‘Cd11c-Cre: Cd11c-Irf4’), previously generated to evaluate dendritic cells\(^3\), revealed that deletion of Irf4 in CD11c-expressing cells did not significantly affect the accumulation of ABCs (Supplementary Fig. 6a) or other parameters of autoimmunity\(^3\), which suggested that DKO ABCs might not require IRF4.

Given the homology among IRF DNA-binding domains, we next pursued the possibility that another IRF might regulate DKO ABCs.
in DKO B cells in an IL-21- and Irf5-dependent manner (Fig. 6f). Thus, the IL-21-driven abnormalities in the generation and function of DKO ABCs were dependent on IRF5.

Since the ATAC-seq had revealed enrichment for IRF-binding sites in ABC-specific peaks located at the Il6 transcription start site (TSS), the Cxcl10 cluster, the Ighg2c region and Jun, we next performed chromatin immunoprecipitation (ChIP) assays to assess the binding of IRF5 to these regulatory regions. DKO B cells exhibited enhanced binding of IRF5 to these sites relative to that of wild-type B cells only after stimulation with IL-21 (Fig. 7a and Supplementary Fig. 7a). IL-21-mediated phosphorylation of the transcription factor STAT3 and translocation of IRF5 to the nucleus were similar in wild-type and DKO B cells (Supplementary Fig. 7b,c). Minimal IRF5 binding was observed in Cd21-Cre Irf5fl/– DKO B cells (Fig. 7a and Supplementary Fig. 7a), in support of the specificity of the findings. To evaluate whether ABC-specific peaks bound by IRF5 could also be targeted by T-bet, we performed ChIP assays with anti-T-bet (Fig. 7b and Supplementary Fig. 7a). DKO B cells exhibited increased binding of T-bet to the ABC-specific regions at the Cxcl10 cluster, the Ighg2c peak and Jun but not to the Il6 TSS or a site in Zeb2 known not to bind T-bet. Notably, deletion of Irf5 in DKO B cells resulted in decreased binding of T-bet to the Cxcl10 cluster, the Ighg2c peak and Jun. Further confirmation that stimulation of DKO B cells with IL-21 led to aberrant ability of IRF5 and T-bet to target the Cxcl10 cluster was obtained by oligonucleotide-precipitation assays (ONP assays). As observed with the ChIP assays, the presence of IRF5 was necessary for T-bet to bind to the Cxcl10 cluster, while no binding of T-bet to the Il6 TSS was detected (Fig. 7c and Supplementary Fig. 7d). Co-transfection of cells with constructs encoding T-bet with IRF5, coupled with a mutational analysis, confirmed that optimal recruitment of T-bet to the Cxcl10 cluster required DNA binding by IRF5 (Fig. 7d and Supplementary Fig. 7e). Together these findings supported a model whereby in the absence of the SWEF proteins, stimulation with IL-21 leads to increased ability of IRF5 to target ABC-specific peaks. Targeting of these regions by IRF5 subsequently enables the recruitment of T-bet to a subset of these sites.

We next investigated the possibility that the SWEF proteins were able to interact with IRF5 and thus restrain its activity. Endogenous IRF5 in B cells was found to interact with both DEF6 and SWAP-70 (Fig. 7e). The association of IRF5 with either DEF6 or SWAP-70 mapped to the C-terminal portion of the SWEF proteins, which contains their IRF-interacting region, and required the IRF-association domain of IRF5 (Supplementary Fig. 7f–h). No interaction of either DEF6 or SWAP-70 with T-bet was detected (Supplementary Fig. 7i). Co-transfection of a construct encoding IRF5 with a construct encoding DEF6 or SWAP-70, followed by an ONP assay, demonstrated that the full-length SWEF proteins interfered with the ability of IRF5 to bind to the Il6 TSS, but mutants unable to interact with IRF5 did not (Fig. 7j). In the course of these studies, we also observed that DEF6 and SWAP-70 were able to form heterodimers (Supplementary Fig. 7j). These results suggested that the interaction of IRF5 with the SWEF proteins was able to regulate IRF5’s activity and thus indirectly altered the recruitment of T-bet to selected target genes.

Monoallelic deletion of Irf5 abolishes the accumulation of ABCs and lupus development in DKO mice. We next evaluated the effect of Irf5 deficiency on the in vivo population expansion of DKO ABCs. Monoallelic deletion of Irf5 significantly decreased the accumulation of ABCs regardless of the markers used to identify these cells (Fig. 8a,b and Supplementary Fig. 8a,b). Further deletion of Irf5 using Cd21-Cre or Cd11c-Cre to target B cells or CD11c+ cells, respectively, did not exert additional effects (Fig. 8a,b and Supplementary Fig. 8a–c). Loss of ABCs was accompanied by a substantial decrease in splenomegaly, TgN cells, GC B cells, PCs and autoantibodies (Fig. 8c,d and Supplementary Fig. 8d–f). The reduction in the titers of antibodies to dsDNA reflected mainly a decrease in IgG2c antibodies rather than IgG1 antibodies (Fig. 8d). The production of autoantibodies to single-stranded DNA (ssDNA), cardiolipin and nRNP was also substantially affected by the loss of Irf5 (Fig. 8e). Furthermore, diminished expression of Irf5 ameliorated several parameters of renal injury in DKO mice, including expansion of the mesangial matrix, the presence of hylane deposits, a decrease in capillary loops and the deposition of immunocomplexes (Fig. 8f,g). Thus, the aberrant population expansion of DKO ABCs in vivo was dependent on IRF5. Furthermore, decreasing the expression of Irf5 corrected several of the abnormalities observed in female DKO mice and considerably ameliorated the spontaneous development of lupus in these mice.

Discussion

The molecular networks that control ABCs in autoimmunity have remained largely unknown. Here we demonstrated that the SWEF proteins limited the generation of ABCs in response to IL-21. These cells exhibited a unique transcriptional profile and chromatin landscape enriched not only for T-bet binding sites but also for IRF and AP-1–BATF motifs. At a mechanistic level, the SWEF proteins inhibited the IL-21-driven formation of ABCs by controlling the accessibility of IRF5 to key targets. These studies thus elucidate a previously unknown pathway for the regulation of ABCs in autoimmunity.

The lack of SWEF proteins results in abnormalities in several key processes, including cell proliferation. This could promote both the premature accumulation of DKO ABCs and their dysregulated differentiation due to the close coupling of cell division and the acquisition of B cell transcriptional and epigenetic programs. The expansion of DKO ABC populations could subsequently fuel autoimmunity via their dual ability to secrete proinflammatory mediators and produce autoantibodies. The deletion of Irf5 in DKO mice, however, could have affected other subsets, such as GC B cells and PCs, that produce autoantibodies and are dysregulated in DKO mice. Thus, it remains to be established whether DKO ABCs contribute directly to autoimmunity or whether their accumulation is secondary to the chronic inflammation of autoimmune conditions.

The distinctive features of ABCs from autoimmunity-prone mice were highlighted by their unique chromatin landscape, which exhibited enrichment for binding sites for IRFs and AP-1–BATF, in addition to the expected presence of motifs for T-bet. Those results were further supported by studies linking IRF5 to the regulation of ABCs. Given the known interplay between AP-1 and IRF5, AP-1 proteins are also probably involved in the regulation of ABCs. IRF5–AP-1 crosstalk could be further facilitated by a potential feed-forward loop set-up by the IRF5-mediated induction of Jun. Notably, absence of the SWEF proteins resulted in increased binding of T-bet to several ABC-specific peaks, which occurred in an IRF5-dependent manner; this would suggest cooperativity between IRF5 and T-bet for at least some regulatory regions. That was reinforced by the requirement for the DNA-binding domain of IRF5 in the optimal recruitment of T-bet to ABC-specific sites. It remains to be determined whether IRF5 can function as a focused ‘pioneer factor’ for ABCs, as has been shown for IRF1 in T regulatory type 1 cells.

The enrichment for IRF motifs in DKO ABC peaks was mechanistically linked to increased IRF5 activity due to lack of the inhibitory effects of the SWEF proteins. Both DEF6 and SWAP-70 are found in the nucleus, which suggests that they inhibit the activity of nuclear IRF5, a finding supported by our biochemical studies. Given their ability to bind to the IRF-association domain of IRF5, they could also potentially interfere with its crosstalk with AP-1 proteins. Published studies showing that SWAP-70 can be recruited to some but not all IL-4-inducible promoters furthermore suggest...
that the SWEF proteins could be recruited to distinct regulatory regions, depending on the precise composition and/or modifications of SWEF protein–containing complexes. This might enable the inhibitory actions of SWEF proteins to target specifically either IRF4 or IRF5. The inhibitory effects of SWEF proteins might also depend on the relative abundance of IRF5 and IRF4, which could vary depending on the ABC differentiation stage. Indeed, the IRF motifs within ABC-specific peaks could accommodate the binding of other IRFs such as IRF4, which could mark a more terminally differentiated ABC not captured by our analysis here. Furthermore, given the role of IRF4 in GC B cells14,15, we cannot exclude the possibility that IRF4 might be necessary at the earliest stages of ABC generation, which might not have been affected by deletion of IRF4 in CD11c-expressing DKO cells16. Given the complex array of biological responses controlled by DEF6 and SWAP-70, these two SWEF proteins might also restrain ABCs in vivo by acting separately on additional IRF-independent pathways.

ABCs from autoimmunity-prone mice also exhibited a substantial loss of accessible chromatin regions containing PU.1, MAF and C/EBP motifs, relative to the abundance of such regions in wild-type ABCs. Those changes were associated with downregulation of Maf and MafB but not of Sp1, a pattern reminiscent of that employed by IFN-γ to disassemble enhancers that regulate M2-like macrophage programs17–19. This mechanism might be directly responsible for the decreased expression, in DKO ABCs, of Merkt and other genes encoding myeloid-related molecules involved in the engulfment of apoptotic cells, a pathway highly relevant to the pathogenesis of lupus. Given the known repressive role of PU.1 in antibody production and PC differentiation20–22, selective depletion of PU.1-bound peaks might also lessen the PU.1-mediated inhibitory effects that directly contribute to the increased transcription of immunoglobulin-encoding genes in DKO ABCs and endow them with an enhanced ability to undergo PC differentiation after exposure to environmental stimuli. Thus, dysregulated IRF5 activity, coupled with the loss of PU.1-containing repressive complexes, might represent a key mechanism employed by ABCs from autoimmunity-prone mice to bypass critical checkpoints that govern the transition of B cells into antibody-secreting cells.

While the role for IRF5 in TLR7 signaling is well known23, our studies now indicate involvement of IRF5 downstream of IL-21 and thus position IRF5 as a common mediator of two key pathways for the generation of ABCs in autoimmunity. The convergence of these pathways onto IRF5 probably contributes to the dramatic effects of monoallelic deletion of Irf5 on the development of lupus in our model and other models24,25. Such strong gene-dosage effects might be particular relevant for human SLE, in which IRF5 risk variants can affect IRF5 expression26. Associations between SLE and variants of IL21, IL21R, DEF6 and IRF5 have all been identified in genome-wide association studies, which raises the intriguing possibility that improper regulation of this pathway has a key role in the pathogenesis of SLE. Several polymorphisms in DEF6, which is located centromeric to the locus encoding the major histocompatibility complex27, have been reported, and population expansion of ABC-like cells and aberrancies in IL21–IL21R or IRF5 have also been observed in other autoimmune conditions such as rheumatoid arthritis and inflammatory bowel disease28–30. Dysregulation of the ability of the SWEF proteins to restrain IRF5’s activity in response to IL-21 and to properly control ABCs might thus contribute to multiple autoimmune diseases.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at https://doi.org/10.1038/s41590-018-0056-8.

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Author contributions
M.M. designed and performed the experiments, interpreted the experiments and wrote the manuscript; S.G., E.R. and M.S. performed the experiments; Y.C. conducted the RNA-seq bioinformatics analysis; T.P. assisted with the histological analysis; R.J. generated the mice and performed the experiments, interpreted the experiments and wrote the manuscript; and A.B.P. designed and supervised the study, interpreted the experiments, and wrote the manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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**Methods**

**Mice.** Female C57BL/6, Cd21-Cre and Cd11c-Cre mice were obtained from Jackson Laboratory. DEF6-deficient (Def6.−/−) mice were generated by Lexicon Pharmaceuticals by a gene trapping strategy as previously described1. Swap-70-deficient mice (Swap-70.−/−) were generated as previously described1. Swap-70−/− and Def6−/− mice were backcrossed into a C57BL/6 background for >10 generations1. Sup−/− mice were obtained from Taconic and were crossed to DKO mice to obtain Sup−/− DKO mice. I2I−/− mice on mixed strain background were obtained from the Mutant Mouse Regional Resource Centers (Lexicon strain ID 011723-UCD), then were backcrossed into a C57BL/6 background for over ten generations and then crossed with DKO mice to obtain I2I−/− DKO mice. Cd11c-Cre Irf6−/− DKO mice were generated as previously described1. Irf5−/− mice, which can do the Dock2 mutation, were originally obtained from P. Pitha-Rowe (Johns Hopkins University, MD). These mice were further crossed with DKO mice expressing either Cd21-Cre or Irf5-Cre to obtain pIRES2-EGFP biotin expression vector (Clonetech). Various deletion mutants of human Swap-70 were generated by PCR using the appropriate primers. The full-length wild-type human Irf5 expression construct in pcDNA3 was a gift from I. Rogatsky. Full length human Irf5 (variant 5) and its variant deletion mutants were constructed in pXFLAG-CMV-10 expression vector (Sigma) using the Irf5 construct (GenScript) as a PCR template. The expression plasmid for untagged T-bet was generated in pIRES2-EGFP biotin expression vector (Clonetech) using the T-bet expression construct (GenScript) as a PCR template.

**Immunoblot analysis and immunoprecipitation.** Nuclear and cytoplasmic extracts were prepared with NE-PER Nuclear and Cytoplasmic Extraction Reagents (Pierce), as previously described. For expression analysis, cell extracts were analyzed by immunoblot analysis with the following antibodies: anti-STAT3 (clone 9B9, BD Bioscience), antibody to STAT3 phosphorylated at Tyr705, anti-IL6Rα (4D4, Cell Signaling), anti-IRF5 (49B3, Cell Signaling) or anti-ID3 (2025, Cell Signaling). For protein-protein interaction studies, cell extracts were immunoprecipitated with an anti-IRF5 (49B3, Cell Signaling) or anti-HA (31F0, Roche Applied Science). The immunoprecipitates were resolved by 8% SDS-PAGE, transferred to a nitrocellulose membrane and then analyzed by immunoblot with either anti-STAT3 (70–23, Santa Cruz Biotechnology), anti-IRF5 antisera or anti-HA (31F0, Roche Applied Science).

**ChIP assay.** Cd21+ B cells were purified and stimulated in vitro for 48 h. After harvesting, the cells were cross-linked with formaldehyde, and chromatin extracts were prepared using the truChIP Chromatin Immunoprecipitation Reagents ( Covaris) according to manufacturer’s instructions. The DNA–protein complexes were immunoprecipitated with an anti-IRF5 (Abcam, ab21689) or an anti-T-bet (Santa Cruz; sc-21749X) specific antibody or a control antibody. After cross-linking was reversed and proteins were digested, the DNA was purified from the immunoprecipitates as well as from input extracts and then was analyzed by quantitative PCR. The qPCR method and normalized to cyclophilin a (Cyclophilin a).

**ONP assay.** ONP assays were conducted as previously described. In brief, nuclear extracts were precleared with streptavidin-agarose beads and then incubated with biotinylated double-stranded oligonucleotide containing potential IFN-binding site within the ATAC-seq peak at the Cxcl10 cluster (5′-ATAGTTAAAT GGTTCCTTTA AACCCGATTCG CCTTGAATG TATGCTA AAGTTA-3′) or BtSS (primer 3′-TGTCAGAA GTAG AATG CCAAA GAG AGGAC TACCA CACG-3′ and reverse 5′-GGTTACCTG TACG ACACG AGGAC TACCA CACG-3′) with 100 ng/ml.

**Cytokines and ELISA.** IL-6 and CXCL10 in culture supernatants were measured using the mouse ELISA Max Standard Set (Biolegend) and the Mouse Quantikine ELISA Kit (R&D Systems) respectively.

**Autoantibody ELISA and Ana.** For anti-dsDNA ELISA, plates were coated with 100 μg/ml salmon sperm DNA (Invitrogen AM9680) at 37 °C overnight and blocked in 2% BSA in PBS, at room temperature for 2 h. For anti-cardiolipin ELISA, Immulon 2HB plates (Thermo Fisher) were coated with 75 μg/ml of cardiolipin dissolved in 100% ethanol at 4°C overnight. Serum diluted 1:200 was incubated on coated plates at 25 °C for 2 h. Plates were then incubated for 1 h with horseradish peroxidase–labeled goat anti-mouse IgG (1030-05). IgG1 (1070-05).
were obtained from Alpha Diagnostic International. OD450 was measured on a microplate reader. ANAs were detected on Hep-2 slides (MBL international) at a 1:200 dilution using Alexa Flour 488-conjugated anti-mouse IgG (715-546-151, Jackson ImmunoResearch Laboratories). Fluorescent intensity was semi-quantitated as previously described.

**Histology and immunofluorescence staining.** Tissue specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Tissue sections were stained with periodic acid–Schiff and were analyzed by light microscopy. The nephritis scoring system was adapted from the International Society of Nephrology–Renal Pathology Society classification of human lupus nephritis. At least 40 glomeruli per mouse were evaluated. The final score accounted for morphological pattern (mesangial, capillary and/or membranous) and for the proportion of glomeruli involved. Immunofluorescence analysis of frozen kidney sections was performed by staining with FITC-labeled goat anti-mouse IgG (715-096-151, Jackson ImmunoResearch Laboratories) and specimens were analyzed with a LSM 510 laser scanning confocal microscope (Carl Zeiss, Inc.). Images were captured by Q capture software. Five representative glomeruli per mouse were chosen and mean fluorescence intensity (MFI) was calculated using ImageJ software.

**RNA-Seq analysis.** Total RNA was isolated using RNeasy Plus Mini kit (Qiagen). A SMART-Seq v3 Ultra Low Input RNA Kit (Clontech) followed by preparation of a Nextera library were used to prepare Illumina-compatible sequencing libraries. Quality of all RNA and library preparations were evaluated with BioAnalyser 2100 (Agilent). Sequencing libraries were pair-end sequenced by the Weill Cornell Genomics Core using HiSeq2500 at the depth of ~30–50 million fragments per sample. Sequencing performance was evaluated using FASTQC. 50-bp paired reads were mapped to mouse genome (mm10, build 38.75, 41,128 genes and 87,108 transcripts) with CLC Bio Genomic Workbench 7.5 software (Qiagen). Duplicated reads with more than five copies were discarded. Read count tables were created using unique exon read counts and the differential expression was analyzed using EDGER (Bioconductor). Genes with the expression less than 1 count per million (cpm) in at least three conditions were considered non-expressing and were removed from further analysis. A negative binomial generalized log-linear model was fit to read counts for each gene. A likelihood ratio tests with the null hypothesis that the pairwise contrasts of the coefficients are equal to zero was used to evaluate the significance of differences in expression between analyzed groups. The Benjamini-Hochberg false-discovery rate (FDR) procedure was used to correct for multiple testing. Genes with a FDR-corrected \( P \) value of \( >0.01 \) and a change in expression of less than twofold were filtered out. Genes that passed the filtering were considered to be differentially expressed.

Gene Set Enrichment Analysis (GSEA; [http://www.broad.mit.edu/gsea/index.html](http://www.broad.mit.edu/gsea/index.html)) was performed using the difference of log-transformed count per million (cpm) for contrasted conditions as a ranking metric. The Molecular Signatures Database v 5.2 (Broad Institute) was used as source of gene sets with defined functional relevance. Gene sets ranging between 15 genes and 1000 genes were included into analysis. Nominal \( P \) values were FDR corrected, and gene sets with an FDR of \( <0.05 \) were used to create GSEA enrichment plot. To define the groups of potentially co-regulated genes, we performed unsupervised hierarchical clustering analysis of log-transformed expression values (cpm) in R. The distances between genes were calculated as \( (1 – \text{Pearson correlation}) \). The Euclidean distance was used to determine the distances between samples. Ward.D2 methods was used to perform clustering. The expression values were \( z \)-transformed and visualized using heat maps.

**ATAC-seq, peak calling and annotation.** The nuclei of sorted wild-type and DKO ABCs or DKO FoB cells were prepared by incubation of cells with nuclear preparation buffer (0.30 M sucrose, 10 mM Tris, pH 7.5, 60 mM KCl, 15 mM NaCl, 5 mM MgCl\(_2\), 0.1 mM EGTA, 0.1% NP-40, 0.15 mM spermine, 0.5 mM spermidine and 2 mM 6AA)\(^3\). Libraries were prepared as described previously\(^3\). Paired-end 50-bp sequences were generated from samples on an Illumina HiSeq2500. We used the makeTagDirectory followed by findPeaks command from HOMER version 4.7.2 ([http://homer.salk.edu/homer/](http://homer.salk.edu/homer/)) to identify peaks of ATAC-seq. A false-discovery rate (FDR) threshold of 0.001 was used for all data sets. The following HOMER command was used: cmd = findPeaks <sample tag directory> -style factor or histone –o <output file> -i <input tag directory>. The total number of mapped reads in each sample was normalized to ten million mapped reads. Peak-associated genes were defined based on the closest genes to these genomic regions using ReSeq coordinates of genes. We used the annotatePeaks command from HOMER to calculate ATAC-seq tag densities from different experiments and to create heatmaps of tag densities. Sequencing data were visualized by preparing custom tracks for the UCSC Genome browser.

**Motif enrichment analysis.** De novo transcription factor motif analysis was performed with motif finder program findMotifs.pl from HOMER package, on given ATAC-seq peaks. Peak sequences were compared to random genomic fragments of the same size and normalized G+C content to identify motifs enriched in the targeted sequences.

**Statistics.** \( P \) values were calculated with unpaired two-tailed Student's \( t \)-test for two-group comparisons and by one-way ANOVA followed by Bonferroni's multiple-comparisons test for multi-group comparisons. For statistical analysis of ANA intensity scores, a non-parametric Mann-Whitney test was used. \( P \) values of \( <0.05 \) were considered significant; and the following values were delineated: \( *P<0.05 \), \( **P<0.01 \), \( ***P<0.001 \) and \( ****P<0.0001 \). Statistical analysis was performed with Graphpad Prism 7.

**Life Science Reporting Summary.** Further information on experimental design and reagents is available in the Life Science Reporting Summary.

**Data Availability.** The data that support the findings of this study are available from the corresponding author upon request. The RNA-seq and ATAC-seq sequencing data have been deposited at the GEO database with accession code GSE99480.

**References**

51. Biswas, P. S. et al. Phosphorylation of IRF4 by ROCK2 regulates IL-17 and IL-21 production and the development of autoimmunity in mice. *J. Clin. Invest.* 120, 3290–3295 (2010).

52. Biswas, P. S., Kang, K., Gupta, S., Bhagat, G. & Pernis, A. B. A murine autoimmune model of rheumatoid arthritis and systemic lupus erythematosus associated with deregulated production of IL-17 and IL-21. *Methods Mol. Biol.* 900, 233–251 (2012).

53. Minnich, M. et al. Multifunctional role of the transcription factor Blimp-1 in coordinating plasma cell differentiation. *Nat. Immunol.* 17, 331–343 (2016).
# Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

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Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study. For final submission: please carefully check your responses for accuracy; you will not be able to make changes later.

## 1. Sample size

Describe how sample size was determined.

The sample size was chosen based on the literature in the field. For in vivo B cells activation, previous studies by Fanzo et al. and Jordan provided the model for observing lymphocyte activation and differentiation. Their results found that approximately 60% of female mice developed lupus-like syndrome in aging Def6-deficient mice compared to none in the comparison group (p<0.005). Using those estimates, it was shown that sample sizes of five (5) achieve 95% power to detect a 60% incidence difference between the comparison groups with a significance level set at 0.05.

## 2. Data exclusions

Describe any data exclusions.

Outliers were identified by the ROUT method (GRAPHPAD) and removed from analysis. In RNA-seq experiments, genes with the expression levels less than 1 count per million (cpm) in at least three conditions were considered non-expressing and removed from further analysis.

## 3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

Each experimental finding was reproducibles. Experimental findings were reproduced at least twice. Most of the findings were reproduced by different laboratory members at different times.

## 4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

No Randomization was used.

## 5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Blinding was used during the ANA evaluation.

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.
6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| Item                                                                 | Required? | Confirmation |
|----------------------------------------------------------------------|-----------|--------------|
| **n/a**                                                             |           |              |
| □ The exact sample size ($n$) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) | ✗         |              |
| □ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly | ✗         |              |
| □ A statement indicating how many times each experiment was replicated | ✗         |              |
| □ The statistical test(s) used and whether they are one- or two-sided |
| Only common tests should be described solely by name; describe more complex techniques in the Methods section. | ✗         |              |
| □ A description of any assumptions or corrections, such as an adjustment for multiple comparisons | ✗         |              |
| □ Test values indicating whether an effect is present |
| Provide confidence intervals or give results of significance tests (e.g. $P$ values) as exact values whenever appropriate and with effect sizes noted. | ✗         |              |
| □ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) | ✗         |              |
| □ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation) | ✗         |              |

See the web collection on statistics for biologists for further resources and guidance.

7. Software

Policy information about availability of computer code

Describe the software used to analyze the data in this study.

Statistics were calculated using Graphpad Prism 7. Softwares used for RNAseq and ATAC-seq analysis are described in the method section. FACS data were collected using FACS DIVA and analyzed using FlowJo v9.9.6. ImageJ 1.48u4 was used for immunoblots and IgG deposition quantification.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

Unique materials are available upon request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Flow cytometry:
CD11c APC(N418, Cat#117310), CD11b PE(M1/70, Cat#101206), CD19 PE(6D5, Cat#115523), B220 PEcy7(RA3-6B2, Cat#103222), T-bet PE(4B10, Cat#644810), CD4 PB(RM4-5, Cat#100531), CD21/CD35 FITC (7E9, Cat#123408), CD23 PE(B3B4, Cat#), CD68APcy7 (GL-1, Cat#105030), MHCII PerCPcy5.5(AF6-120.1, Cat#116416), IgG1 PerCPcy5.5 (RGM1-1, Cat#406612) and IgG2a FITC (RGM2a-62, Cat#407105) were obtained from BioLegend.

Antibodies to CD43 FITC (S7, Cat#553270 ), CD138 APC(281-2, Cat#558626), GL-7 PE (Cat#561530), CXCR5 Biotin (Cat#551960) and Fas (Jo2, Cat#55425G) were obtained from BD. Antibodies to Ki-67 PB (SolA15, Cat#48-5698-80), IgD FITC(11-26, Cat#11-5993-85), IgM PEcy7 (II/41, Cat#25-5790-82), CD93 PEcy7 (AA4.1, Cat#25-5802-81), CD5 PEcy7 (53-7, Cat#25-0051-81), PDCA-1 PerCPeFluor710(eBio927, Cat#46-3172-80), PD1 FITC(J43, 11-9985-85) and Foxp3 APC (FJK-16s, Cat#17-5773-82) were obtained from eBioscience. Immunoblotting:

anti-STAT3 (BD Bioscience, Cat#BD610189), anti-pSTAT3 (Y705, Cell Signaling,Cat#9131s), anti-IRF5 (Cell Signaling, Cat#4950s) or anti-HDAC1 (Cell Signaling, Cat#2062s), anti-HA (3F10; Roche Applied Science) anti–SWAP-70 (Santa Cruz Biotechnology, Inc., Cat# SC81991), All antibodies are commonly available and validated by the manufacturer.

anti-DEF6 antiserum was previously described and validated in "Gupta, S. et al. Molecular cloning of IBP, a SWAP-70 homologous GEF, which is highly expressed in the immune system. Hum Immunol 64, 389-401 (2003)".
10. Eukaryotic cell lines
   a. State the source of each eukaryotic cell line used. 293T cells lines were obtained from ATCC (CRL-3216)
   b. Describe the method of cell line authentication used. 293T cells is a well established cell line, no in house authentication has been done
   c. Report whether the cell lines were tested for mycoplasma contamination. The cell lines were not tested for mycoplasma contamination
   d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use. No commonly misidentified cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
    Provide all relevant details on animals and/or animal-derived materials used in the study. For in vitro studies female 8-10 weeks old mice were used. For in vivo studies female mice >20 weeks old were used. Strains used in the paper included: C57Bl/6, Def6tr/tr, Swap-70–/–, Def6tr/trSwap-70–/– (DKO), Sap–/– DKO, Cd11c-Cre If4fl/fl DKO, If5fl/fl DKO, Cd21-Cre If5fl/– DKO, Cd11c-Cre If5fl/– DKO and If5fl/– DKO.

Policy information about studies involving human research participants

12. Description of human research participants
    Describe the covariate-relevant population characteristics of the human research participants. The study did not involve human participants
Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☐ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
☒ 3. All plots are contour plots with outliers or pseudocolor plots.
☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation. Single cells suspensions from spleens were prepared by filtering on a 40micrometer cell strainer. For sorting, Splenocytes were enriched for B cells by using B220 magnetic beads (Miltenyi) following the kit protocol.

6. Identify the instrument used for data collection. FACS Canto (Becton Dickinson) and for sorting FACS Aria (Becton Dickinson).

7. Describe the software used to collect and analyze the flow cytometry data. Acquisition with BDFACS Diva and analysis with FlowJo 9 (TreeStar) software.

8. Describe the abundance of the relevant cell populations within post-sort fractions. Post-sort FACS FoB>98%, ABC>95%

9. Describe the gating strategy used. FSC/SSC gating was determined according to lymphocytes size. Single Cells were gated using FSC-H/FSC-W and SSC-H/SSC-W. B cells were then gated on B220+ and CD19+. ABC were gated on CD11c+CD11b+ within the B cell gate.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☒
ChIP-seq Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

### Data deposition

1. For all ChIP-seq data:
   - a. Confirm that both raw and final processed data have been deposited in a public database such as GEO.
   - b. Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

2. Provide all relevant data deposition access links.
   *The entry may remain private before publication.*
   - GSE99480

3. Provide a list of all files available in the database submission.
   - GSE99480

4. Provide a link to an anonymized genome browser session (e.g. UCSC), if available.
   - NA

### Methodological details

5. Describe the experimental replicates.
   - n=2 (ATACseq)

6. Describe the sequencing depth for each experiment.
   - PCR conditions: Buenrostro et al., Nature, 2015
   - Mean of total aligned reads: 1.101 x 10^8
   - Mean od uniquely mapped reads: 1.387 x 10^8
   - Length of reads: 50
   - Paired- or single-end: Paired

7. Describe the antibodies used for the ChIP-seq experiments.
   - NA

8. Describe the peak calling parameters.
   - The following HOMER command was used: cmd = findPeaks
   - `<sample tag directory>` -style factor or histone -o `<output file>`
   - `-i <input tag directory>`.

9. Describe the methods used to ensure data quality.
   - A false discovery rate (FDR) threshold of 0.001 was used for all data sets. The total number of mapped reads in each sample was normalized to ten million mapped reads.

10. Describe the software used to collect and analyze the ChIP-seq data.
    - HOMER version 4.7.2 (http://homer.salk.edu/homer/)