Loss of signalling via Ga13 in germinal centre B-cell-derived lymphoma

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Germinal centre B-cell-like diffuse large B-cell lymphoma (GCB-DLBCL) is a common malignancy, yet the signalling pathways that are deregulated and the factors leading to its systemic dissemination are poorly defined. Work in mice showed that sphingosine-1-phosphate receptor-2 (S1PR2), a Gu12 and Gu13 coupled receptor, promotes growth regulation and local confinement of germinal centre B cells. Recent deep sequencing studies of GCB-DLBCL have revealed mutations in many genes in this cancer, including in GNA13 (encoding Gu13) and S1PR2 (refs 5–7). Here we show, using in vitro and in vivo assays, that GCB-DLBCL-associated mutations occurring in S1PR2 frequently disrupt the receptor’s A and migration inhibitory functions. Gu13-deficient mouse germinal centre B cells and human GCB-DLBCL cells were unable to suppress pAkt and migration in response to S1P, and Gu13-deficient mice developed germinal centre B-cell-derived lymphoma. Germinal centre B cells, unlike most lymphocytes, are tightly confined in lymphoid organs and do not recirculate. Remarkably, deficiency in Gu13, but not S1PR2, led to germinal centre B-cell dissemination into lymph and blood. GCB-DLBCL cell lines frequently carried mutations in the Gu13 effector ARHGEF1, and Arhgef1 deficiency also led to germinal centre B-cell dissemination. The incomplete phenotype of Gu13- and S1PR2 deficiency led us to discover that P2RY8, an orphan receptor that is mutated in GCB-DLBCL and another germinal centre B-cell-derived malignancy, Burkitt’s lymphoma, also represses germinal centre B-cell growth and promotes confinement via Gu13. These findings identify a Gu13-dependent pathway that exerts dual actions in suppressing growth and blocking dissemination of germinal centre B cells that is frequently disrupted in germinal centre B-cell-derived lymphoma.

We sequenced the S1PR2 coding region in 117 GCB-DLBCL, 31 Burkitt’s lymphoma and 68 activated B-cell-like (ABC)-DLBCL samples. Twelve S1PR2 coding mutations were identified in the GCB-DLBCL samples versus one in each of the Burkitt’s lymphoma and ABC-DLBCL cohorts (Supplementary Tables 1 and 2). The majority of GCB-DLBCL mutations were in conserved transmembrane residues (Fig. 1a) and all were predicted to be structurally damaging. Cell-line transduction experiments showed that five of eight tested mutations disrupted S1PR2 protein expression (Fig. 1b and Extended Data Fig. 1a–c). These same mutations disrupted S1P-mediated inhibition of CXCL12-induced pAkt and migration (Fig. 1c, d and Extended Data Fig. 1d, e). These observations suggested that tumours harbouring single mutant S1PR2 alleles (Extended Data Fig. 2) are often likely to be functionally heterozygous for S1PR2. Using a mixed bone-marrow chimera system in mice, S1pr2 heterozygous B cells showed marked expansion in the germinal centre (GC) relative to the follicular compartment in mesenteric lymph nodes and Peyer’s patches of unimmunized mice (Fig. 1e and Extended Data Fig. 3a, b). Overexpression of WT S1PR2 repressed the outgrowth of S1pr2+/− GC B cells and this was also seen for mutant R329C, whereas the R147C mutation caused the receptor to lose GC growth suppressive activity (Fig. 1f and Extended Data Fig. 3c, d). On the basis of molecular simulation analysis (Supplementary Information and Extended Data Fig. 3e–g) we propose that the R147C S1PR2 mutant cannot attain the active conformation necessary for G-protein recruitment and signalling.

Gu12 and Gα13 often function redundantly in transmitting G-protein-coupled receptor signals. Transcripts for both G-proteins are upregulated in GC B cells, with Gα13 transcripts appearing more abundant (Extended Data Fig. 4a). In accord with recent whole-exome sequencing studies that reported mutations in GNA13 but not GNA12 (refs 5, 6 and 9–11), we found frequent GNA13 coding mutations in GCB-DLBCL and Burkitt’s lymphoma biopsy samples, with a number of biallelic cases (Supplementary Table 2 and Extended Data Fig. 2). Analysis of mixed bone-marrow chimerae revealed that Gα13 deficiency was sufficient to confer a GC B-cell growth advantage in mesenteric lymph nodes and to a lesser extent in Peyer’s patches (Fig. 1g and Extended Data Fig. 4b). Gα13-deficient mesenteric lymph node GC B cells showed increased pAkt relative to WT when incubated ex vivo with CXCL12 and S1P (Fig. 1h). Deficiency in the Gα13 effector, Arhgef1 (p115 Rhogef or Lsc), led to a similar defect in the ability of S1P to repress chemokine induced pAkt (Fig. 1i).

To determine whether loss of Gα13 in B cells could promote lymphomagenesis, we allowed a cohort of Gα13-deficient mice to age. At 1 year, 10 out of 18 Gα13-deficient mice showed a greater than tenfold expansion of GC B cells compared with littermate controls (Fig. 1j, k), and at least five of the outgrowths appeared clonal (Extended Data Fig. 4c). Three of the Gα13-deficient animals showed massive mesenteric lymphadenopathy (Fig. 1l and data not shown), with evidence in one case (number 307) of spleen and Peyer’s patch involvement (Fig. 1l and Extended Data Fig. 4e–i). Immunophenotyping of the Gα13-deficient tumours confirmed they were of GC origin (Extended Data Fig. 4f).
Figure 1 | Lymphoma-associated S1PR2 mutations are functionally disruptive and loss of Gna13 is sufficient to promote GC B-cell survival and lymphomagenesis. a, Schematic of S1PR2 with mutated residues highlighted. Circles denote mutated residues conserved in S1PR2 across species, filled circles denote those conserved across type A G-protein-coupled receptors, squares denote residues not conserved across species, and the asterisk is the position of truncating frameshift mutation. b, Western blot of Flag expression in WEHI231 cells transduced with Flag-tagged WT or mutant S1PR2 or empty vector. One experiment representative of three independent biological replicates is shown. The gap in the gel image marks the position of one lane that was not relevant to this experiment and was removed for clarity. c, WEHI231 cells transduced as in b were stimulated with CXCL12 (100 ng ml\(^{-1}\)) in the presence or absence of S1P (1 nM) for 5 min and analysed for phosphorylation of Akt (pAkt S473) by intracellular fluorescence-activated cell sorting (FACS). Mean fluorescence intensity of pAkt in samples treated with both CXCL12 and S1P relative to CXCL12 alone is shown. Data are pooled from four independent experiments. d, Transwell migration of cells transduced as in b, in response to CXCL12 (100 ng ml\(^{-1}\)) in the presence or absence of S1P (1 nM). The relative migration of transduced cells to CXCL12 in the presence versus absence of S1P is shown. Data are pooled from eight independent experiments. e, Percentages of CD45.2 follicular B cells (FoB) and GC B cells from mesenteric lymph nodes of mixed bone-marrow chimaeras generated with ∼70% WT CD45.1 cells and ∼30% S1pr2 WT (n = 9), heterozygous (n = 28) or knockout (n = 19) CD45.2 bone marrow, assessed by FACS. Gating scheme is shown in Extended Data Fig. 3a. Data are pooled from four independent experiments. f, Fold change in frequency of Thy1.1 reporter+ cells in GC relative to follicular B cells of Peyer’s patches from chimaeras reconstituted with S1pr2+/− bone marrow transduced with retrovirus expressing either WT (n = 17) or mutant S1PR2 (R147C, n = 8; R329C, n = 6). Gating scheme is shown in Extended Data Fig. 3c. Data are pooled from three independent experiments. g, Percentages of CD45.2+ follicular and GC B cells from mesenteric lymph nodes of mixed bone-marrow chimaeras generated with ∼40% Gna13 WT (ff+/f+) (n = 12) or KO (ff mbbcre−/−) (n = 17) CD45.2 cells and ∼60% WT CD45.1 cells. Data are pooled from four independent experiments. h, i, Intracellular FACS for pAkt in GC B cells from mesenteric lymph node of Gna13 (h) or Arhgef1 (i) mixed bone-marrow chimaeras that were stimulated ex vivo with or without CXCL12 (300 ng ml\(^{-1}\)) in the presence or absence of S1P (10 nM) for 10 min. Data are mean ± s.e.m. from one experiment with three biological replicates for each treatment and are representative of four experiments (Gna13) or three experiments (Arhgef1). j, FACS analysis of mesenteric lymph node of 1-year-old Gna13 WT or Gna13 KO (number 307). Percentage of total cells that are GC B cells is indicated. k, GC B-cell number from mesenteric lymph node of Gna13 WT and heterozygous (n = 20) or KO (n = 18) animals aged to 12–16 months. I, Gross appearance of mesenteric lymph node and spleen from Gna13 WT control and two Gna13 KO animals. Arrow in number 307 denotes splenic nodule (see also Extended Data Fig. 4c–e). Scale bar, 1 cm. *P < 0.05, **P < 0.01, ***P < 0.001, unpaired two-tailed Student’s t-test.

To test the conservation of the Gna13-signalling pathway in human GC B cells, we performed gene rescue experiments in GCB-DLCLB cell lines. Sequencing of S1PR2, GNA13 and ARHGEF1 in a panel of GCB-DLCLB cell lines identified several with deleterious mutations in these genes (Supplementary Table 3 and Extended Data Fig. 5a). The mutations in GNA13 matched those previously described and were associated with reduced protein levels. ARHGEF1 mutations have not previously been reported, probably because the large size (∼24 kilobases) of this 27-exon gene and its multiple splice variants and low transcript abundance make sequence analysis difficult. Remarkably, 10 out of 20 cell lines with analysable ARHGEF1 sequence showed mutations in this gene, several of which resulted in premature stop codons (Supplementary Table 3 and Extended Data Fig. 5a). Using retroviral transduction to restore gene expression, we established that loss of S1PR2, Gna13 and ARHGEF1 were each sufficient to disrupt S1P-mediated suppression of pAKT and, in the case of cell lines that were migratory, to disrupt S1P-mediated inhibition of migration (see Supplementary Information and Extended Data Fig. 5).

The mechanisms by which malignant GC B cells can exit the GC niche and lymphoid organ to spread among multiple lymph nodes or to systemic sites such as bone marrow have not been defined. Consistent with a lack of migration inhibition by S1P (Fig. 2a), mice lacking Gna13 in B cells showed marked disruption of GC architecture in mesenteric lymph nodes (Fig. 2b and Extended Data Fig. 6a). In a mixed transfer system, Gna13-deficient GC B cells were excluded from the interior of otherwise WT GCs (Extended Data Fig. 6b). Remarkably, Gna13-deficient GC B cells were readily detected in lymph and to a lesser extent in blood while WT GC B cells were absent from circulation (Fig. 2c). In mixed bone-marrow chimaeras, Gna13-deficient GC B cells were again detectable in the lymph, indicating that Gna13 was needed intrinsically in GC B cells to inhibit egress (Fig. 2d). Analysis of Arhgef1-deficient mice and chimaeras revealed a similar disruption of mesenteric lymph node GC architecture (Fig. 2b and Extended Data Fig. 6c) and GC B-cell appearance in lymph and blood (Fig. 2e, f). In contrast, S1PR2-deficient GC B cells were not significantly higher in lymph relative to littermate controls (Fig. 2g). Analysis of mice expressing constitutively active myristoylated Akt (myrAkt) or overexpressing BCL2 in B cells established that increased GC B-cell survival was not sufficient to lead to dissemination (Supplementary Information and Extended Data Fig. 7).

GNA13 mutations and BCL2 rearrangements and potentially activating mutations frequently occur together in GCB-DLCLB Cell, GCB cells
in mice with combined Gna13 deficiency and BCL2 overexpression showed enhanced evo survival (Fig. 3a), increased numbers (Fig. 3b), wider dispersal throughout the follicle and interfollicular regions in mesenteric lymph nodes (Extended Data Fig. 7f) and twofold increased frequencies of GL7+CD95+ B cells in lymph nodes (Fig. 3c). A representative FACs plot for GC B cells in lymph nodes is shown in c with the percentage of total cells that are GC B cells indicated. Data are shown as GC B-cell frequency among total cells in lymph and as cells per millilitre in blood. Data in c–g are pooled from between 3 and 13 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001, unpaired two-tailed Student’s t-test.

To examine requirements for GC B-cell persistence after arriving at a distant site, we bypassed the egress step and intravenously transferred mesenteric lymph node cells to congenically distinct recipients. Transferred WT GC B cells were essentially undetectable in recipient spleen and bone marrow after 6 hours (Fig. 3d, e) and Gna13 deficiency alone was insufficient to cause a significant increase in their number (Fig. 3e). BCL2 overexpression alone caused an elevation in GC B-cell frequency in recipient spleens but not bone marrow (Fig. 3e). Loss of Gna13 combined with BCL2 overexpression led to greater accumulation of transferred GC B cells in spleen and now led to an increase in their frequency in bone marrow (Fig. 3e). This combinatorial effect probably reflects an ability of Gna13 deficiency and BCL2 overexpression to cooperate in promoting survival of GC B cells outside the GC niche (Fig. 3a). To determine whether GC B cells could seed distant lymph node sites after entry into lymphatics, we transferred mesenteric lymph node cells intraperitoneally. Small numbers of Gna13-deficient, but not WT, GC B cells were detectable in the draining parathympic lymph nodes after 6 hours (Fig. 3f).

In this case, recovery of Gna13-deficient GC B cells was not enhanced by the BCL2 transgene. Bone marrow involvement occurs in a fraction of GCB-DLBC patients and is a predictor of worse disease. In some year-old Gna13-deficient mice showing mesenteric lymph node tumours, GC B cells could be detected in the bone marrow (Fig. 3g, h). Moreover, in aged BCL2-tg Gna13 knockout (KO) but not BCL2-tg Gna13 WT mice, GC B cells were frequently found in the bone marrow (Fig. 3i).
growth. Remarkably, human P2RY8 led to a suppressive effect on GC B-cell growth in mouse Peyer’s patches and mesenteric lymph nodes, similar to the effect of S1PR2 overexpression (Fig. 4d and Extended Data Fig. 8c). This suppression required P2RY8 coupling to Gα13 as it was not seen if the cells lacked Gna13 (Fig. 4e and Extended Data Fig. 8d). In short-term transfers, P2RY8-transduced B cells localized in the centre of the follicle immediately around and often within GCs while vector-transduced cells were dispersed throughout the follicle (Fig. 4f, Extended Data Fig. 8e, f and Supplementary Information). In the absence of Gα13, P2RY8 was unable to direct B cells to the follicle centre (Fig. 4g and Extended Data Fig. 8g). Importantly, a control Gα13–coupled G-protein–coupled receptor, Thxa2r, could not suppress GC B-cell growth or confine cells to the GC niche (Extended Data Fig. 9 and Supplementary Information). These observations lead us to suggest that P2RY8 in humans acts to suppress GC B-cell growth and promote B-cell positioning in a GC location via Gα13–dependent pathways.

GC B cells are normally tightly regulated in their growth and strictly confined to the GC, and they lack the ability to exit into circulation or to survive outside the GC niche. Each of these processes breaks down in the GC B-cell–derived malignancies, GCB-DLBCL and Burkitt’s lymphoma. We provide evidence that disruption of Gα13 signalling, via mutations in GNA13, ARHGEF1, S1PR2 or P2RY8, contributes to this breakdown. GNA13 is mutated in 15–33% of GCB-DLBCL and ~15% of Burkitt’s lymphoma.5–7,9–11 (Supplementary Table 2 and Extended Data Fig. 2). This is similar to the frequency of mutations in the histone methyltransferases EZH2 and MLL2, deletions of PTEN and amplifications of mir17-92, genetic alterations that have been highlighted for their role in oncogenesis in GCB-DLBCL.15–21 Our data support a model (Extended Data Fig. 10 and Supplementary Information) where deleterious mutations in Gα13 and its effector, ARHGEF1, are sufficient to deregulate AKT signalling and to cause loss of confinement, allowing egress of GC B cells into circulation; survival of the disseminating cells at distant sites such as bone marrow depends on co-operating mutations affecting additional genes, such as BCL212,22. S1PR2 and P2RY8 mutations are also suggested to deregulate AKT signalling and growth but may lead to less dissemination due to overlapping roles in promoting confinement. Potentially inactivating mutations of RHOA, a direct target of ARHGEF1,23, have been reported in Burkitt’s lymphoma.24 The mechanism by which RHOA inhibits AKT activation is not yet defined but might involve activation of PTEN or inhibition of RAC25–27. We suggest that small molecules that inhibit AKT may replace the missing repressive effects of RHO on growth or survival in cells that harbour defects in the S1PR2/P2RY8–Gα13–ARHGEF1–RHO pathway. Development of active RHO-mimetics may represent a novel therapeutic approach that addresses both lymphoma cell survival and disease dissemination.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Author Contributions J.R.M. designed and performed experiments, interpreted the results and wrote the manuscript. R.S. performed sequencing of cell lines and primary samples, and analysed data. J.A.G. designed experimental procedures used in the manuscript. W.X. analysed sequence data. A.B.L and N.V. performed computer modelling of S1PR2. S.E.B. performed western blots of S1PR2. J.A. performed mouse genotyping and cared for the mouse colony. Y.X. performed quantitative PCR. A.R., G.O., R.R.T., J.R.C., D.D.W. and W.C.C. supplied lymphoma patient samples or lines, and reviewed pathological and clinical data. J.G.C. designed experiments, supervised research and wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to J.G.C. (jason.cyster@ucsf.edu) or L.M.S (lstaudt@mail.nih.gov).
METHODS

Human samples and sequencing. All clinical samples were studied with informed consent according to an institutional review board protocol approved by the National Cancer Institute. Genomic DNA for the single exon coding region of S1PR2 and complementary DNA (cDNA) for Gna13 or ARHGEF1 was amplified by PCR. PCR products were bidirectionally sequenced using an ABI 3730 Genetic Analyzer (Applied Biosystems). Sequence electropherograms were manually reviewed. ARHGEF1 encodes multiple splice variants with up to 28 coding exons per splice variant. We were unable to sequence the open reading frame of ARHGEF1 from cDNA in some cell lines in our panel, probably because of splice variation or insufficient transcript. In some cell lines, regions containing coding exons for ARHGEF1 were amplified from genomic DNA. Primers used for amplification and sequencing are shown in Supplementary Table 4. The following NCBI (RefSeq) accession numbers were used to report mutations: ARHGEF1, NM_004706 and NP_004697; Gna13, NM_006572 and NP_006563; S1PR2, NM_004220 and NP_004221.

Mice and bone marrow chimeras. Adult C57BL6/Ly5.2 (CD45.1+) mice at least 7 weeks of age were from the National Cancer Institute. S1pr2-/- mice were backcrossed for at least six generations to C57BL6/J (Ly5.2). S1pr2+/- mice were backcrossed to C57BL6/J (Ly5.1) and used for at least six generations for all experiments, except in the ageing cohort of mice to backcrossed to B6/J for at least six generations.

Lymph was collected from the cisterna chyli via fine glass micropipette as previously described3. To generate activated B cells from spleen, mesenteric lymph nodes or lymph node GC B cells, mesenteric lymph node was harvested in RPMI-1640 medium containing 5% (v/v) FBS, antibiotics (penicillin (50 IU ml-1) and streptomycin (50 μg ml-1)), and was filtered through a 70 μm filter. The remaining lymphocytes were incubated in serum-free media containing 0.5% (w/v) fatty-acid-free BSA (migration media; EMD Biosciences). Sequence electropherograms were manually reviewed. Products were bidirectionally sequenced using an ABI 3730 Genetic Analyzer (Applied Biosystems). For quantitative PCR analysis of gene expression in GC B cells, Ptprc (encoding CD45) was used as a control since its expression was unchanged between follicular and GC B cells by microarray (http://www.immgen.org/ and unpublished data), RNA sequencing analysis (unpublished data) and by surface staining. In contrast, Gapdh and Hprt values were considered significant when less than 0.05.

To study the expression of S1pr2 in bone marrow, mesenteric lymph nodes and spleen, we used total mesenteric lymph node cells that were RBC lysed twice or transduced with retrovirus containing vector, WT or mutant S1pr2, P2RY8, Tbxa2r, GNA13 or ARHGEF1.

RETROVIRAL CONSTRUCTIONS AND TRANSIENT TRANSFESSIONS

To generate activated B cells from spleen, mesenteric lymph nodes or lymph node GC B cells, mesenteric lymph node was harvested in RPMI-1640 medium containing 5% (v/v) FBS, antibiotics (penicillin (50 IU ml-1) and streptomycin (50 μg ml-1)), and was filtered through a 70 μm filter. The remaining lymphocytes were incubated in serum-free media containing 0.5% (w/v) fatty-acid-free BSA (migration media; EMD Biosciences). Sequence electropherograms were manually reviewed. Products were bidirectionally sequenced using an ABI 3730 Genetic Analyzer (Applied Biosystems). For quantitative PCR analysis of gene expression in GC B cells, Ptprc (encoding CD45) was used as a control since its expression was unchanged between follicular and GC B cells by microarray (http://www.immgen.org/ and unpublished data), RNA sequencing analysis (unpublished data) and by surface staining. In contrast, Gapdh and Hprt values were considered significant when less than 0.05.

Retroviral constructs and transductions. S1PR2, P2RY8, GNA13, ARHGEF1 retroviral constructs were made by inserting the human open reading frame into the MSCV-2.2 retroviral vector following an internal ribosome entry site (IRES) and Thy1.1 or green fluorescent protein (GFP) as an expression marker. The mouse genome was not blinded from the investigator and mice were not randomized. Mice were housed in a specific pathogen-free environment in the Laboratory Animal Research Center at the University of California, San Francisco, and all animal procedures were approved by the Institutional Animal Care and Use Committee.

Western blotting. WEHI231 cells transduced with vector, WT or mutant human S1PR2 were washed twice in migration media and incubated at 37 °C for 30 min, washed once in cold PBS and lysed in 0.5% Brij 35, 0.5% NP40, 150mM NaCl, 10 mM Tris-HCl, pH 7.4 with protease inhibitor cocktail (Roche) on ice. Lysates were centrifuged and supernatants were mixed with loading buffer and reducing agent and incubated at room temperature of 21–23 °C for 30 min.

Immunohistochemical analysis. Cryosections 7 μm in thickness from mesenteric lymph nodes and spleen were cut and processed as described. Tumour immunophenotyping was performed using goat polyclonal IRF4 antibody (Santa Cruz, sc-6059) or biotinylated mouse anti-CD138 (clone 281-2; BD Biosciences). For Bcl-6 staining, cryosections were fixed with 4% FPA for 10 min and stained with rabbit polyclonal B6d antibody (Santa Cruz, sc-3688). Images were captured with a Zeiss AxioObserver Z1 inverted microscope.

Statistical analysis. Prism software (GraphPad) was used for all statistical analysis. Data were analysed with a two-sample unpaired (paired, where indicated) Student’s t-test. P values were considered significant when less than 0.05.
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Extended Data Figure 1 | Lymphoma-associated mutations result in loss of expression and function of S1PR2. a–c, Surface expression of Flag (a) quantitative PCR of human S1PR2 (b) or Thy1.1 reporter expression (c) in mouse WEHI231 B lymphoma cells transduced as described in Fig. 1b. Shown in a are histograms of transduced cells (Thy1.1⁺) in blue and untransduced cells (Thy1.1⁻) in grey. Five of eight S1PR2 mutations showed loss of protein expression despite strong transcript and reporter expression. Loss of expression in these five mutants was probably a result of degradation of improperly folded proteins in the endoplasmic reticulum. d, Representative FACS plots of transwell migration of WEHI231 cells transduced with vector, WT or R147C mutant S1PR2 to the indicated stimuli or the input sample. Numbers indicate percentage of cells positive for the Thy1.1 reporter. e, WEHI231 cells stimulated as in Fig. 1d were analysed for phosphorylation of Akt (pAkt S473) by western blot or by intracellular FACS. Data in a and c are representative of four independent experiments. Data in b are from one experiment. Data in d and e are representative of three independent experiments.
Extended Data Figure 2 | Frequency of mutations in **GNA13**, **S1PR2** and **P2RY8** in aggressive lymphoma. a, b, Summary of overall mutation frequencies (a) and allelic frequencies (b) of non-synonymous coding mutations in S1PR2, GNA13 and P2RY8 in GCB-DLBCL, Burkitt’s lymphoma or ABC-DLBCL cases shown in Supplementary Table 2. Unmutated indicates no coding region mutations in the genes shown. Since the sequencing was performed on genomic DNA, the data may underestimate the frequency of biallelic cases as some disruptive mutations may occur in non-coding regulatory elements.
Extended Data Figure 3 | S1PR2 heterozygosity confers a survival advantage to GC B cells and R147C S1PR2 fails to function.  

**a, b**, Flow cytometry of follicular and GC B cells from mesenteric lymph node and Peyer’s patches of mixed bone-marrow chimaeras generated as in Fig. 1e. Gating strategy for follicular B cells and GCB in mesenteric lymph node is shown in a and percentages of CD45.2+ cells in follicular and GC B cells from Peyer’s patches are shown in b. Data in b are pooled from four independent experiments.  

**c, d**, Gating strategy of Thy1.1 reporter expression in follicular and GC B cells from Peyer’s patches (c) or fold change in Thy1.1+ cells in GC relative to follicular B cells of mesenteric lymph node (d) of retrovirally transduced bone-marrow chimaeras as described in Fig. 1f. Data in d are pooled from three independent experiments. *P < 0.05, **P < 0.001, unpaired two-tailed Student’s t-test.***

There was increased variability in mesenteric lymph node relative to Peyer’s patches when WT S1PR2 was transduced into S1PR21/2 bone marrow. Nine of 17 animals reconstituted with WT S1PR2 showed a reduction in expression of Thy1.1 in mesenteric lymph node GC relative to follicular B cells, whereas in six of eight animals reconstituted with R147C S1PR2 there was increased reporter expression.  

**e**, The hydrogen bond formed between Y141 in ICL2 and D130 on transmembrane helix 3 (TM3) has been observed only in the active state of β2-adrenergic receptor (shown in pink) and not in the inactive state (shown in cyan).  

**f**, Population distribution of the conformational states showing the predicted hydrogen bond network between R147 (TM4), Y140 (ICL2) and E129 (TM3) of the WT (solid lines) and R147C mutant (dashed lines) of S1PR2.  

**g**, The network of predicted hydrogen bonds mediated by Y140 on ICL2. The hydrogen bond network tightens the interactions between transmembrane helices TM3 and TM4. We hypothesize that this network stabilizes the putative active state conformation of S1PR2. Such a network is broken in the R147C mutant and hence this mutant does not activate the G protein.
Extended Data Figure 4 | Aged Gna13-deficient mice develop GC-derived lymphoma. a, Quantitative PCR analysis of Gna12 and Gna13 transcript abundance in follicular and GC B cells relative to the control gene Ptprc. b, Flow cytometry of follicular and GC B cells from Peyer’s patches of mixed bone-marrow chimaeras as described in Fig. 1g. c, PCR analysis of VHJ558–DJJH, VK–JK and VL–JL rearrangements from indicated tissues of Gna13 KO animals. The space in the gel image marks the position of lanes that were not relevant to this experiment and were removed for clarity. This PCR analysis was done using bulk rather than sorted GC B cells from tumours and thus probably under-reports the number of animals with clonal outgrowths. Samples scored as having clonal outgrowths (and thus probably harbouring tumours) were numbers 307, 377, 418, 1310 and 443. In the case of number 307, the splenic nodule and enlarged Peyer’s patches showed enrichment of the same VHJ558 clonal bands observed in the mesenteric lymph node. d, Gross appearance of small intestine of Gna13 KO number 307 mouse. Box denotes enlarged Peyer’s patches analysed by PCR in c; arrows denote two uninvolved Peyer’s patches. Scale bar, 1 cm. e, Immunohistochemical analysis of splenic nodule from number 307 (see Fig. 1l) for GC marker GL7 (blue) and naive B-cell marker IgD (brown). Scale bar, 500 μm. f, Control or enlarged Gna13 KO mesenteric lymph nodes were stained for the GC B-cell markers GL7 and Bcl6, the plasma cell markers CD138 and IRF4, and the follicular B-cell marker IgD. Scale bar, 200 μm in all samples in f.
Extended Data Figure 5 | Defective regulation of pAkt and cell migration in human GCB-DLBCL cell lines harboring mutations in the S1PR2-signalling pathway. a, Frequency of non-synonymous coding mutations in S1PR2, GNA13 and ARHGEF1 in GCB-DLBCL lines, and the fraction that were mono- or biallelic, summarized from Supplementary Table 3. Unmutated S1PR2
signalling pathway. a, Intracellular FACS for pAkt in human GCB-DLBCL cell lines that are WT or mutant for S1PR2, GNA13 or ARHGEF1 as indicated and which were stimulated with CXCL12 (100 ng ml$^{-1}$) in the presence or absence of S1P (10 nM) for 5 min. pAkt staining of cells treated with wortmannin (200 nM; staining control). b, DOHH2 (GFP*) or Karpas422 (GFP*) transduced with retrovirus expressing the reporter alone (vector) or S1PR2 (10 nM) or wortmannin (200 nM; staining control). g, Intracellular FACS for pAkt in the ARHGEF1 mutant cell line Ly19 transduced with retrovirus expressing reporter alone (vector) or ARHGEF1 that were treated as in b or with the PI3K inhibitor GS-1101 (2 μM; staining control). h, Quantitative PCR analysis of S1PR2 transcript abundance in human GCB-DLBCL cell lines relative to GAPDH. i, Intracellular FACS for pAkt in NUDUL1 cells transduced with retrovirus expressing reporter alone (vector), S1PR2, GNA13 or ARHGEF1, treated as in d. Data in b and d are representative of at least three independent experiments. Pooled data from at least three independent experiments are shown in b, e, f, g and i. Data in b are one experiment representative of two. **$P<0.01$, paired two-tailed Student’s t-test.
Extended Data Figure 6 | Loss of GC B-cell confinement in the absence of Gna13 or Arhgef1. a, Additional examples of mesenteric lymph node sections from Gna13 WT or KO mice stained for GC B cells (GL7, blue) and naive B cells (IgD, brown). In the absence of Gna13, the GC border is indistinct and IgD-positive follicular B cells are interspersed with GL7-positive GC B cells throughout the central region of the follicle. The disruption of mesenteric lymph node GC architecture caused by Gna13 deficiency appears more severe than observed in S1pr2-deficient mice3. b, Mixed B-cell transfer showing exclusion of Gna13-deficient GC B cells from the interior of otherwise WT GCs. Gna13 WT or KO CD45.2+ B cells were mixed with WT CD45.1+ B cells and transferred into MD4 Ig-transgenic CD45.1+ recipients that were then immunized with SRBCs intraperitoneally, and splenic tissue was analysed by immunohistochemistry and FACS after 8 days for CD45.2+ B cells. This transfer approach allows efficient participation of transferred polyclonal B cells in the GC as the Ig-transgenic recipient B cells are hen-egg lysozyme specific and do not respond to SRBCs. Note that CD45.2+ WT B cells are distributed uniformly through the GL7+ GCs (upper panels) whereas the CD45.2+ Gna13 KO B cells are located at the perimeter of the GC or in the surrounding follicle (lower panels). In each case, two example images are shown and the GL7 and CD45.2 stains are of adjacent sections. c, Additional sections of mesenteric lymph nodes from Arhgef1 WT or KO mice, stained for GL7 and IgD. Scale bar, 200 μm in a–c. Data in b are one experiment representative of two.
Extended Data Figure 7 | Augmented GC B-cell survival is not sufficient to promote dissemination of GC B cells. 

**a**, **b**, Transduced GC B-cell frequency among total cells in mesenteric lymph node (**a**) and lymph (**b**) of mice reconstituted with bone marrow transduced with B-cell-restricted control (vector, *n*= 5) or myr-Akt (*n*= 5) expressing retrovirus. 

**c**, Immunohistochemical analysis of mesenteric lymph node sections from mice in **a**, stained for GL7 and IgD. Scale bar, 100 μm. 

**d**, **e**, BCL2-tg or Gna13 KO GC B-cell frequency among total cells in mesenteric lymph node (**d**) and lymph (**e**) of BCL2-tg:Gna13 KO mixed chimaeras (*n*= 8). 

**f**, Immunohistochemical analysis of mesenteric lymph nodes from BCL2-tg Gna13 WT or BCL2-tg Gna13 KO mice. Scale bar in low-magnification images (left) is 200 μm and in high-magnification images (right) is 100 μm. Data in **a**, **b**, **d** and **e** are pooled from two independent experiments. Data in **c** and **f** are representative of at least three mice of each type.
Extended Data Figure 8 | Human P2RY8 suppresses GC B-cell growth and promotes B-cell confinement to the GC in mice. a, b, P2RY8 mutations arising in GCB-DLBCL and Burkitt’s lymphoma disrupt receptor expression. Flag-tagged versions of six point mutants and the WT receptor were expressed in WEH231 B cells and surface expression examined by Flag flow cytometry (a). The transduction efficiency of each construct was confirmed to be similar based on IRES-Thy1.1 reporter expression (b). c, d, Fold change in Thy1.1 reporter+ GC relative to follicular B cells from mesenteric lymph node of chimaeras described in Fig. 4d, e. e–g, Immunohistochemical analysis of splenic sections from SRBC-immunized mice given Ig-transgenic (e), Gpr183+/− (f) or Gna13 WT or KO (g) B cells transduced as in Fig. 4f, g and assessed 24 h after cell transfer. Data in a and b are representative of three independent experiments. Data in e and g are additional examples of the experiments shown in Fig. 4f, g, respectively. Data in f are representative of four independent experiments. Scale bar, 200 μm in e–g. *P < 0.05, **P < 0.01, unpaired two-tailed Student’s t-test.
Extended Data Figure 9 | P2RY8-dependent suppression of GC B-cell survival and promotion of B-cell confinement to the GC niche is receptor specific. 

**a**, Transwell migration of WEHI231 cells transduced with retrovirus encoding the control Gz13-coupled receptor, Tbxa2r, towards CXCL12 (100 ng ml\(^{-1}\)) in the presence or absence of the thromboxane A2 analogue, U-46619. **b, c**, Fold change in frequency of Thy1.1 reporter \(^{+}\) GC relative to follicular B cells of Peyer’s patches (b) or mesenteric lymph node (c) from bone marrow chimaeras reconstituted with Slpr2 KO bone marrow transduced with empty vector (control) or Tbxa2r. **d**, Immunohistochemical analysis of splenic sections from SRBC-immunized mice given Gpr183\(^{+/+}\) B cells transduced with empty vector, Tbxa2r or P2RY8, and assessed 24 h after cell transfer. Scale bar, 200 \(\mu\)m. Data in **a** and **d** are one experiment representative of two. Data in **b** and **c** are from one experiment (\(n = 4\) in each group). **P < 0.01, unpaired two-tailed Student’s \(t\)-test.
Extended Data Figure 10 | Model relating disruptions in S1PR2/P2RY8–
Gna13–ARHGEF1 migration- and Akt-inhibitory pathway to increases in
GC B-cell survival, dispersal in the follicle, egress into circulation and
dissemination to bone marrow. a, Summary of signalling pathway.
b, Schematic diagram showing GC-containing lymph node follicle, with connection to efferent lymphatic, blood and bone marrow. Suggested distribution of S1P and of putative P2RY8 ligand within lymph node is shown by dots. Comparative migration and survival behaviour of GC B cells with loss (S1PR2, P2RY8, GNA13, ARHGEF1) or gain (BCL2) of function mutations is summarized.