**LETTER**

Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system

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Germinal centres (GCs) promote humoral immunity and vaccine efficacy. In GCs, antigen-activated B cells proliferate, express high-affinity antibodies, promote antibody class switching, and yield B cell memory1,2. Whereas the cytokine milieu has long been known to regulate effector functions that include the choice of immunoglobulin class3,4, both cell-autonomous5 and extrinsic6,7 metabolic programming have emerged as modulators of T-cell-mediated immunity8. Here we show in mice that GC light zones are hypoxic, and that low oxygen tension (pO2) alters B cell physiology and function. In addition to reduced proliferation and increased B cell death, low pO2 impairs antibody class switching to the pro-inflammatory IgG2c antibody isotype by limiting the expression of activation-induced cytosine deaminase (AID). Hypoxia induces HIF transcription factors by restricting the activity of prolyl hydroxyl dioxygenase enzymes, which hydroxylate HIF-1α and HIF-2α to destabilize HIF by binding the von Hippel–Landau tumour suppressor protein (pVHL)9. B-cell-specific deletion of pVHL leads to constitutive HIF stabilization, decreases antigen-specific GC B cells and undermines the generation of high-affinity IgG, switching to IgG2c, early memory B cells, and recall antibody responses. HIF induction can reprogram metabolic and growth factor gene expression. Sustained hypoxia or HIF induction by pVHL deficiency inhibits mTOR complex 1 (mTORC1) activity in B lymphoblasts, and mTORC1-haploinsufficient B cells have reduced clonal expansion, AID expression, and capacities to yield IgG2c and high-affinity antibodies. Thus, the normal physiology of GCs involves regional variegation of hypoxia, and HIF-dependent oxygen sensing regulates vital functions of B cells. We propose that the restriction of oxygen in lymphoid organs, which can be altered in pathophysiological states, modulates humoral immunity.

The micro-anatomy of secondary lymphoid organs and rapid proliferation of activated lymphocytes in them9 prompted testing for hypoxia. Using flow cytometry, HIF levels were found to be increased in GC-phenotype B (GCB) cells compared to other B cells in the spleens of immunized mice (Fig. 1a; Extended Data Fig. 1a). Immunofluorescent microscopy revealed that HIF was most increased in GCs (Fig. 1b; Extended Data Fig. 1b). HIF is induced under low oxygen. However, HIF-1α and HIF-2α subunits can be stabilized at normoxic pO2 (ref. 10), so we used chemical probes to mark hypoxic cells in vivo. Spleen, lymph nodes and Peyer's patches were analysed after injection of pimonidazole signals only partially filled GCs, which are subdivided into light and dark zones between which B cells cycle iteratively to promote high-affinity antibodies. EF5 labelling predominantly overlapped a follicular dendritic cell marker (CD35) restricted to the light zone (Fig. 1f). B lymphoblasts proliferate rapidly in the dark zone, whereas cell cycling decreases in the light zone1. The most EF5-positive GCB cells had entered S-phase at lower rates (percentage BrdU+) (Fig. 1g, h) and more frequently activated an executioner caspase (Fig. 1i). Thus, activated B cells experience hypoxia in GCs, predominantly in their light zones. Notably, the more hypoxic GCB cells proliferated less and had increased apoptotic signalling.

To test the effect of hypoxia on antibody class switching, activated B cells cultured in hypoxia (pO2 of 1%) were compared to controls cultured at atmospheric (~21%) or venous (5%) pO2, using conditions that promote IgG1 or the pro-inflammatory isotype IgG2c (Fig. 2a; Extended Data Fig. 2). Hypoxia restricted B cell population growth (Fig. 2a, b), with increased caspase-3 activation and lower BrdU incorporation (Extended Data Fig. 2a, b). Thus, O2 sufficiency promoted B cell proliferation by both improving survival and increasing cell cycling. These effects were paralleled by an altered balance in cell metabolism, as hypoxia promoted a higher glycolytic rate (Extended Data Fig. 2c) in activated B cells. Conversely, in vitro inhibition of prollyl hydroxyl dioxygenase (PHD) reduced O2 consumption, and gene expression profiling of fresh ex vivo B cells showed major differences between the non-GC and GC subsets (Extended Data Fig. 2d, e, respectively). Moreover, IgG2c B cell frequencies were reduced at 1% pO2 (Fig. 2a, Extended Data Fig. 2f). The enteric immune system is a site of physiological hypoxia12; notably, hypoxia did not decrease the frequency of IgA+ B cells in IgA-promoting conditions (Fig. 2a, Extended Data Fig. 2f). Switching requires multiple B cell divisions13. When fluoroescent partitioning was analysed along with switching to IgG2c, hypoxia reduced switching by B cells at the same division number (Fig. 2b). Thus, hypoxia at levels of the GC light zone altered antibody class switching by a direct influence on class choice in addition to reducing proliferation and reprogramming B cell metabolism and survival.

Class switch recombination is executed by AID, which is encoded by the Aicda gene1,4. In IgG switch conditions, Aicda mRNA and AID protein were reduced by hypoxia (Fig. 2c, d; Extended Data Fig. 2g). By contrast, AID was not reduced by hypoxia in IgA switch conditions (Fig. 2d). Switch recombinase is directed to the immunoglobulin heavy chain regions by transcription factors that create accessibility marked by germ-line transcripts (GLTs)14,15. Hypoxia decreased induction of the transcription factor T-bet and the T-bet-dependent Iγ-2c GLT14,15 (Fig. 2e, f), whereas Rora mRNA and the Ic GLT were not reduced in B cells at reduced pO2 (Fig. 2e, f). The PHD inhibitor dimethylxalylglycine (DMOG) reduced proliferation and increased apoptosis of B cells

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Hypoxia in GC light zones. a, Flow cytometry of HIF-1α in GC B cells (GL7+B220+ gate) from sheep red blood cell (SRBC)-immunized mice, and in the GL7−B220− gate, compared to controls (lgG1 instead of primary anti-HIF-1α antibody; Extended Data Fig. 1a, HIF1αΔ/Δ). B cells stained with anti-HIF-1α, b, Left, immunofluorescent staining of HIF-1α or controls (lgG1; as in a) in GCs (GL7+ IgD−) and surrounding follicles (IgD+ GL7−) (n = 12 GCs, 4 spleens in 2 experiments). Scale bar in b, c, f, 100 μm. Right, HIF-1α signals quantified within GCs compared to the GL7− follicular cells (Extended Data Fig. 1b, HIF1αΔ/Δ). B cells stained with anti-HIF-1α, AU, arbitrary units. c–e, GC hypoxia. Adducts, IgD and GL7 were stained after immunized mice were injected with EF5, pimonidazole (hypoxyprobe) or PBS (Veh.). d, Anti-pimonidazole staining of spleen sections (representative of 24 GCs in 9 sections from 3 independent experiments, quantified in Extended Data Fig. 1c). d, Quantified EF5 signals (Extended Data Fig. 1d) within GCs compared to the GL7− follicles, as in b (n = 19 GCs from n = 5 mice each condition, PBS and EF5; 3 independent experiments). e, A representative flow cytometry result (n = 3 experiments) with anti-EF5 staining of spleen cells after intratval injection with EF5 or PBS, as in c and d, gated as in a, f. Hypoxia maps mostly to the light zone. Spleen sections as in e, stained for CD35, GL7 and EF5, and anti-EF5 signals in CD35+ and CD35− regions, quantified as in d. g, Flow cytometric measurements of S-phase (BrdU+) GC B cells that were either hypoxic (EF5+) or not (EF5−), from mice as in e after BrdU injection. b, BrdU incorporation (n = 7 samples in two independent experiments). h, Fractions of cleaved (activated) caspase 3-positive (CC3+) GC B cells, gated as in g. All data are mean ± s.e.m.

Figure 2 | Hypoxia regulates B cell survival, proliferation and class switching. a, O2 modulates the spectrum of antibody isotypes. Surface IgG1, IgG2c and IgA on B220+ gated cells, measured by flow cytometry after activation of purified B cells culture at P50, of 21% (normoxia), 5% or 1% (hypoxia) using conditions promoting IgG1, IgG2c or IgA. Flow cytometry data from one representative experiment (top) along with bar graphs showing aggregate results of cell numbers and switch efficiencies (bottom) (n = 4 for 5%, n = 7 for 1% and 21% P50). LPS, lipopolysaccharide. b, Flow cytometry of surface IgG2c (right) on B cells gated by division number (left) after activation of CellTrace Violet (CTV)-stained B cells and culture with IFNγ as in a. Inset numbers (bold font) denote the percentage of switched B cells at indicated division numbers in this analysis; mean ± s.e.m.) values from the independent replicate experiments (n = 3) are italicized. Shaded overlay: CTV fluorescence of undivided cells cultured only in BAFF. c, d, AID regulated by oxygen sufficiency. c, Aicda mRNA was quantified in B cells activated and cultured as in a, or in the presence or absence of PHD inhibitor DMOG. d, Relative AID expression, measured as GFP fluorescence in AID–GFP transgenic B cells stained with CTV, activated and cultured in the conditions of a and b. Representative GFP fluorescence versus divisions for B220+ cells quantified from four independent replicate analyses are in Extended Data Fig. 2g. e, Hypoxia and PHD inhibition reduce T-bet and Iγc GLT induction, but not Rora or Ie, Iγc GLT (e) and Tbx21 (f) mRNA measured after B cell cultures in IgG2c conditions; IgO GLT (e) and Rora (f) mRNA in B cells cultured for IgA switching (n = 3–4 experiments). BLD, below limit of detection. Data are mean ± s.e.m.

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Figure 3 | B cell-intrinsic role of pVHL in antibody response qualities. a–c, In adoptive transfer experiments (schematic diagram, Extended Data Fig. 5a), B cells purified from tamoxifen-treated mice were transferred into recipients after mixing with CD4+ T cells (polyclonal.OVA-specific OT-II cells = 4:1). Recipients were analysed after primary immunization, or, for memory responses (Extended Data Fig. 5d), after the primary and a recall immunization. a, b, VHL reduction causes HIF-dependent alterations in antibody responses. a, Primary NP-specific IgG2c antibody response in Rapgα−/− recipients of wild-type (WT) or Vhl−/−,Epas1−/−Cre (Vhl cKO) B cells from tamoxifen-treated donor mice (n = 5 recipients of each genotype, distributed evenly between two independent replicates). Other antibody isotypes are in Extended Data Fig. 5. b, High-affinity (NP20) all-affinity (NP20) anti-NP antibodies of the indicated isotypes in sera from immunized recipients, measured by ELISA. Each dot represents one mouse (n = 9 of each genotype, distributed evenly among 3 independent experiments). Horizontal lines denote the mean. WT, wild type; Vhl cKO, pVHL-depleted conditional knockout (VhlΔ/Δ); Vhl−/−,HIF-1α−/−. c, HIF-dependent reduction of antigen-specific B cell populations. Flow cytometry results scoring NP-binding GCB cells (B220+ GL7+ IgD−) and early memory (B220+ CD38+ IgM− IgD−) phenotypes. One representative result from the same mice and experiments as shown in b (Extended Data Fig. 6c, d), d, VHL B cells promote Aicda and Tbx21 expression. Wild-type and Vhl−/− B cells were activated, cultured and analysed as in Fig. 2. e, B cells transduced with MIT, MIG, MIT-T-bet or pMX-GFP-AID retrovectors were cultured with BAFF and LPS ± IFNγ in the presence or absence of DMOG, EM, empty retrovector. Frequencies of surface IgG2c events among B220+ cells analysed 4 days after transduction, with flow data from one experiment in Extended Data Fig. 4e (n = 3 independent experiments). Data are mean ± s.e.m.

Figure 4 | mTORC1 activity in B cells regulates antibody qualities but is attenuated by hypoxia. a, Immunoblots of lysates prepared from activated B cells cultured overnight at 21% or 1% O2, before (−) and after (+) re-stimulation with anti-IgM. b, Immunoblots of B cell extracts as in a, using conditionally pVHL-depleted cells with either normal (Vhl cKO) or deficient (Vhl−/−,H2 cKO) HIF expression. c, d, Raport promotes generation of high-affinity antibodies and switch to IgG. IgG2a (donor B-cell-derived) allotype anti-NP antibodies were measured after immunization of IgHb allotype mice that had received wild-type or Raptor−/− B cell transfers. ELISA results for all-affinity anti-NP IgG in primary response sera from recipient mice (n = 9 WT, n = 8 Raptor−/−), captured on NPb (c), and high-affinity antibodies (IgM, 1:100; IgG1, 1:50) captured on NPb (d). IgG2c was undetectable, as in e, f, mTORC1 promotes AID expression. GFP in B220+ gated cells by flow cytometry after B cells were cultured for 4 days with LPS, BAFF and IL-4 or IFNγ as indicated. n values for f in e, f, n = 3, 4 replicates, respectively.

pVHL-depleted B cells (Fig. 3d, Extended Data Fig. 4c). To test the impact of decreased AID and T-bet, we forced expression of these proteins in activated B cells. T-bet did not increase the frequency of IgG2c-positive B cells during PHD inhibition, although it bypassed the need for IFNγ with control B cells (Fig. 3e, Extended Data Fig. 4e). By contrast, forcing AID expression normalized switching in these assays (Fig. 3e, Extended Data Fig. 4e). We conclude that the PHD/HIF/VHL axis regulates the qualities of antibody responses, with modulation of AID levels as a major mechanism for hypoxic influence on the Ig class preferences.

B cell activation, class switch recombination, and development into antibody-secreting cells are effected by receptors that stimulate mTOR. Hypoxia and HIF-1 have been shown to either inhibit or enhance mTORC1 activity in tumour and endothelial cells. In hypoxic and DMOG-treated B cells, BCR engagement elicited less phosphorylation of proteins downstream from mTORC1 (Fig. 4a, Extended Data Fig. 7a). Depletion of pVHL also reduced BCR-stimulated mTORC1 by a HIF-dependent mechanism (Fig. 4b). Thus, hypoxia restrained mTORC1 in normal B cells. In vitro experiments suggest that HIF-mediated limitation of increased amino acid transport contributes to this effect. B cell activation increased leucine uptake and expression of transporters used for nutrient uptake; HIF stabilization impaired this induction (Extended Data Fig. 7b–e). Moreover, adequate supplies of leucine were crucial, and partially sufficient, for BCR re-activation of
mTORC1 in B lymphoblasts (Extended Data Fig. 7e). HIP deletion did not completely restore either the antibody response or amino acid uptake to normal in pVHL-deficient B cells. However, two additional mechanisms previously shown to suppress mTORC1 were evoked in hypoxic B cells in vitro—steady-state ATP pools were halved, accompanied by increased AMPK activity, and expression of the Redd1 (also known as Ddit4) gene was increased (Extended Data Fig. 8a–c).

Disruption of mTOR function by means that impair both mTORC2 and mTORC1 altered the balance between class-switched and IgM antibody against specific antigen19,20. By contrast, HIP stabilization only partially inhibited mTORC1 and spared mTORC2 (Extended Data Fig. 8d, e). Accordingly, we tested whether partially reduced mTORC1 activity effects high-affinity antibody production, proliferation, AID levels, or biases of Ig class switching using disruption of Rptor, which encodes a protein essential for mTORC1 (ref. 21). Ruptor haploinsufficiency in B cells reduced mTORC1 activity (Extended Data Fig. 9a), and yielded results of in vitro switching and humoral responses in vivo (Fig. 4c, d, Extended Data Fig. 9) similar to those obtained with hypoxia and the PDI/HIF/VHL axis. IgG2c reductions were more substantial than those of IgM or IgG1 (Fig. 4c), and NP-specific GCB cells and IgG2c anti-NP-antibody-secreting cells (Extended Data Fig. 9b–d) were reduced. Partial mTORC1 loss reduced switching to IgG2c (Extended Data Fig. 10a) and suppressed high-affinity IgG1 antibody production (Fig. 4d). IgG1 switch conditions promoted higher expression of a tracking allele, green fluorescent protein (GFP)-tagged AID, which was partially reduced by Ruptor hemizygosity (Fig. 4e), whereas IgG2c conditions led to less AID in control cells and a greater reduction in Ruptor +/−/− B cells. Moreover, Ruptor haploinsufficiency led to reduced T-bet expression, and decreased mRNA levels of both Tbx21 and Aicda in activated B cells (Extended Data Fig. 10b, c). Pharmacological inhibition of mTOR with rapamycin substantially reduced AID levels18,20 (Fig. 4f) and switching to IgG2c, an effect mitigated by forced AID and T-bet expression (Extended Data Fig. 9a), substantially reduced AID levels19,20 (Fig. 4f) and switching to IgG2c, an isotype has particular functions in anti-microbial immunity.

Limiting pathology from unchecked inflammation in normal intestinal epithelial cells limits local inflammation12,27,28, providing now recognized in a wide range of inflammatory settings in which the susceptibility to respiratory infection 23. Hypoxia has also hypoxaemic lung disease exhibit lower serum IgG levels and heightened region with the spectrum of Fc receptors on cells22. Many patients with IgG2c reductions were substantially reduced AID levels (Extended Data Fig. 10a) and suppressed high-affinity IgG1 antibody production (Fig. 4d). IgG1 switch conditions promoted higher expression of a tracking allele, green fluorescent protein (GFP)-tagged AID, which was partially reduced by Ruptor hemizygosity (Fig. 4e), whereas IgG2c conditions led to less AID in control cells and a greater reduction in Ruptor +/−/− B cells. Moreover, Ruptor haploinsufficiency led to reduced T-bet expression, and decreased mRNA levels of both Tbx21 and Aicda in activated B cells (Extended Data Fig. 10b, c). Pharmacological inhibition of mTOR with rapamycin substantially reduced AID levels18,20 (Fig. 4f) and switching to IgG2c, an effect mitigated by forced AID and T-bet expression (Extended Data Fig. 10d–f). Overall, localized hypoxia and HIF induction are effect mitigated by forced AID and T-bet expression (Extended Data Fig. 9a), substantially reduced AID levels19,20 (Fig. 4f) and switching to IgG2c, an isotype has particular functions in anti-microbial immunity.

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**Author Information** The results of RNA-seq have been deposited in the NCBI Gene Expression Omnibus (GEO) database under accession code GSE77113. 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 M.R.B. (mark.boothby@vanderbilt.edu).

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Mice and B cell transfer models. Mice (C57BL/6 mice, CD45.1 congenic, Ig G1 allotype-disparate (IgFβ), Rag2, AID-GFP Tg, pVH, conditional knockout (Vhβαν; ERβ2-Cre)25, pVH; HIF-1α; HIF-2α; triple conditional knockout (Vhβαα; HIF-1αα; HIF-2αα; Epsαααα; ERβ2-Cre) or, and raptor conditional knockout (Rptorfl/fl; ERT2-Cre)) were housed in ventilated micro-isolators under specified pathogen-free conditions in a Vanderbilt University mouse facility and used at 6–8 weeks of age following approved protocols. Healthy mice of useful genotype were randomly selected for the experiments, without preference to size or gender, or other potential confounding factors. All figures are based on data generated in independent biological replicates, typically conducted weeks or months apart in time and involving different cages of donor and recipient mice, and always with parallel handling and manipulation of the mice and cells of samples to be compared. For adoptive transfer experiments, B cells (from 1–2 donor mice of each genotype) were purified by depleting T cells using biotinylated anti-Thy1.2 antibody followed by streptavidin-conjugated microbeads (Imag; BD Biosciences). Pooled wild-type CD4+ T cells and OT-II CD4+ T cells (4 × 10^6 and 1 × 10^6 cells per recipient, respectively, typically from two donor mice of each background) were purified by positive selection with L3T4 anti-CD4 microbeads and, in adoptive transfers into Rag2 or Ig G1 allotype-disparate (IgFβ) mice, mixed with pools of wild-type, Vhβαα; Vhβαα; HIF-1αα; HIF-2αα; Epsαααα, or Rptorfl/fl B cells (5 × 10^6 cells per recipient) and injected intravenously (i.v.) into Rag2 or Ig Fβ recipients. Recipient mice of similar ages (6–8 weeks) were randomly selected for the experiments, without preference to size or gender. Experiments using the conditional Vh alleles (Vhβααα) were designed to avoid distortions rapidly imposed by systemic pVHL loss (for example, extra-medullary haematopoiesis36). Those using Rptorf/fl drove excision with the same Rosa26;ERβ2-Cre allele and with tamoxifen-initiated Cre activity so as to be more directly comparable to the Vh experiments and because of distortions of B cell development observed even in the contexts of nearby genes with mβ1-Cre (dilution at outset of B lymphoid ontogeny) (A.L.R. and M.R.R., unpublished observations).

Reagents. IFNγ, IL-4 and monoclonal antibody (purified, biotinylated or fluorophore-conjugated) were from BD Pharmingen or Tonbo Biosciences unless otherwise indicated. IL-5 was from Peprotech, TGFβ3 and BAAFF were from R&D Systems. NP-BSA (for capture ELISA), NP-OVA (4–hydroxy-3-nitrophenylacetyl hapten conjugated to ovalbumin, cat No. N-5001-50), and NP-O-succinimide (4–hydroxy-3-nitrophenylacetic acid active ester, or NP-Osu, for NP–allophycocyanin (APC) conjugation; cat No. N-1010) were obtained from Biosearch. SRBCs (sheep red blood cells), n-glucose, and 2–deoxyglucose were from Thermo Fisher Scientific. Tamoxifen, 4–hydroxy-tamoxifen, chicken ovalbumin, all-trans retinoic acid and LPS were from Sigma-Aldrich Chemicals. DMOG (HIF–hypoxiaase inhibitor dimethylxalylglycine, Calbiochem cat no. 400091) and oligomycin were from EMD Millipore. Fluorescent proteins APC and (R)-phycocerythrin (rPE; Prozyme) were used for conjugation reactions with NP–O-succinimide to generate fluor-conjugated NP.

Immunohistochemistry, flow cytometry and detection of hypoxia. C57BL/6 mice were immunized with SRBCs (2 × 10^6 cells per mouse). At 1 week after immunization, mice were injected with EF5 (ref. 31) or pimonidazole HCl for these and other flow cytometric analyses, fluorescence emission data on cell suspensions were collected on BD LSRII or FACS Calibur flow cytometers driven by BD FACS Diva software, then processed using Flow-Jo software (TreeStar).

Immunizations, and measurements of antibody responses. After collection of pre-immune sera, mice were immunized with NP63–ovalbumin (OVA) (100 μg intraperitoneally) in alu (Imject, Thermo Fisher Scientific) as described37. Alternatively, this primary immunogen was mixed with NP75–BSA (high valency, to capture all affinities of antibodies) or NP95 (low valency, to restrict binding to the high-affinity antibody). Specific classes or isotypes were then detected using the series of isotype-specific secondary antibody of the SBA Clonotyping System (Southern Biotech), as described37. Data for antigen-specific antibodies are shown after subtraction of low absorbance (A) values from pre-immune controls analysed together with the immune sera and were separately determined to match values yielded by titration. Antibody-secreting cells were analysed by ELISpot as previously described38 and quantitated using an ImmunoSpot Analyzer (Cellular Technology). Antigen-specific B cells were detected and enumerated using flow cytometry to score B lineage-marked cells binding to fluor (APC or rPE)-conjugated NP, using a dump channel (7–AAD and APC-conjugated monoclonal antibody against IgD, F4/80, Gr1, CD11b, CD11c, CD4, and CD8) to exclude non-specific signal.

Gene expression profiling. Mice were injected with SRBCs and euthanized 10 days after immunization. Single-cell suspensions from spleens were stained with anti-B220 (RA3–6B2) and anti-GL7. B220+ GL7+ and B220+ GL7+ splenocytes were sorted with TRIZol reagent (Ambion). Total RNA was isolated from biological replicates and provided to the Vanderbilt VANTAGE shared resource for library construction and sequencing. Briefly, libraries were constructed from poly-adenylated RNAs and sequenced with an Illumina HiSeq 2500 on an SR-50 run aiming for 30M reads/sample. Reads were aligned to the mm10 mouse transcriptome using TopHat and differential gene expression was determined using Cuffdiff as previously described39. Gene set enrichment analysis (GSEA) was performed using software available from the Broad Institute (http://www.broadinstitute.org/gsea), which tested for enrichment based on hypergeometric distribution with respect to published gene signatures. For hypoxia regulated gene signature, GSEA plots comparing a gene set pre-ranked by log, fold change in gene expression (GL7+ B220− versus GL7− B220+) to a hypoxia signature published previously40 were generated. A significant enrichment was defined as having a false discovery rate (FDR) q ≤ 0.05.

In vitro B cell cultures for class-switched antibody production. Splenic B cells were purified (90–95%) by depleting T cells using biotinylated anti-Thy1.2 monoclonal antibody followed by streptavidin-conjugated microbeads. For IgG1, B cells (0.5 × 10^6 cells ml^−1) were activated with LPS or for (Fab2), anti-IgM (Southern Biotechnological) and anti-CD40 (BD Pharmingen), cultured with IL-4 and IL-7. For IgG2c, B cells (0.5 × 10^6 cells ml^−1) were activated with LPS or anti-IgM and anti-CD40, cultured with BAFF and IFNγ. For IgA, B cells (0.5 × 10^6 cells ml^−1) were activated with LPS (1 μg ml^−1) or anti-IgM and anti-CD40 and cultured with BAFF (10 ng ml^−1), TGFβ (5 ng ml^−1), IL-4 (10 ng ml^−1), IL-5 (10 ng ml^−1), and all-trans retinoic acid (RA) (10 μM). B cells were stained with 10% FBS, penicillin/streptomycin, L-glutamine, 1-glucuronate, and D-marcaptoethanol. To analyse the partitioning of cell division, purified B cells were stained with CellTrace Violet (Thermo Fisher Scientific) according to manufacturer’s instruction or CFDA-SE as described previously41. Cells were cultured (4 days) at P0.7 of 21%, 5% or 1%, after which surface Ig was analysed by flow cytometry. In comparisons of all three oxygen tensions, experiments were performed by dividing one common pool of B cells and using two separate hypoxia chambers maintained at constant PO2 using nitrogen.

Measurements of RNA and proteins. RNA was isolated using TRIzol reagent (Invitrogen) according to manufacturer’s instructions. After DNA synthesis by reverse transcription, expression of genes was analysed in duplicate samples using SYBR green PCR master-mix (Qigen) by quantitative reverse transcriptase PCR (qRT–PCR). Data are presented as values normalized to wild-type control, and averaged over PCR normalized to levels of internal control (actin). Primer pairs and cycle conditions are freely available on request. Proteins in whole-cell extracts were separated by SDS–PAGE, transferred onto nylon membranes (Millipore), and then incubated with rabbit antibodies against p-S6 (S235/S236), p-70S6K(S371), π-Act (S473), π-Tub (T308), p-ACC (S79), p-AMPK (T172) (Cell Signaling Technologies), or goat anti-actin (Santa Cruz) antibodies followed by the appropriate fluorophore-conjugated, species–specific secondary antibody conjugates (Rockland Immunochemicals, and LI-COR). Proteins were visualized and quantitated by laser excitation and infrared imaging (Odyssey, LI-COR). For measurements of the induction of S6K, S6 and Akt phosphorylation, purified B cells were cultured 2 days in BAFF (10 ng ml^−1) and F(ab)2, anti-IgM (1 μg ml^−1), washed, rested 18h, and then re-stimulated (15 min)
in the presence or absence of F(ab')2 anti-IgM (2.5 μg ml\(^{-1}\)). To test the effect of amino acid supply on S6K and S6 phosphorylation, B lymphoblasts were washed, cultured in complete medium overnight, then rinsed, cultured in amino acid-free RPMI1640 (US Biological) for 1 h, and re-stimulated in the presence or absence of anti-IgM, with readdition of l-leucine (Sigma) or all 20 amino acids. For the induction of p-ACC and p-AMPK, purified B cells were cultured for 2 days in LPS, BAFF and IFNγ.

**Glycolysis and oxygen consumption assays.** Purified B cells were cultured for 2 days at 37 °C at P\(_{O2}\) of 21% (normoxia) or P\(_{O2}\) of 1% (hypoxia) in the presence of BAFF, LPS and IFNγ. To quantify glycolysis, 1 × 10\(^6\) viable cells were washed, pulsed with 1μCi of 5-\([\text{3} \text{H}]\)glucose in 24-well plates (37 °C, 1 h), and returned to their previous oxygen condition. Glycolytic conversion was then quantitated as described\(^{32}\). Oxygen consumption rates were measured using Seahorse assays. Because this instrument cannot be used in a hypoxia chamber, purified B cells (1 × 10\(^6\) cell ml\(^{-1}\)) were activated with 1μg ml\(^{-1}\) LPS and cultured 48 h with 10 ng ml\(^{-1}\) BAFF in complete IMDM medium supplemented as described\(^{32}\) in the presence or absence of 0.5 mM DMOG. After 48 h, cultured B cells were washed twice, resuspended in XF Base Media (Seahorse Bioscience) supplemented with 2 mM l-glutamine, and equal numbers of Trypan Blue-excluding B cells (1.5 × 10\(^5\)) were plated on extracellular flux assay plates (Seahorse Bioscience) coated with CellTak (Corning) according to the manufacturer’s protocol. Before extracellular flux analysis, B cells were rested (25 min at 37 °C, atmospheric CO\(_2\)) in XF Base Media. Oxygen consumption rate (OCR) and the extracellular acidification rate (ECAR) were measured using a XF96 extracellular flux analyser (Seahorse Bioscience) before and after the sequential addition of 10 mM d-glucose, 1μM oligomycin, and 50 mM 2-deoxyglucose.

**Amino acid uptake assay.** Purified B cells were activated and cultured for 2 days with LPS and BAFF. Viable cells were washed and incubated with amino acid uptake buffer (5.4 mM KCl, 140 mM NaCl, 1.8 mM CaCl\(_2\), 0.8 mM MgSO\(_4\), 5 mM d-glucose, 25 mM HEPES, and 25 mM Tris, pH 7.5) for 30 min to deplete intracellular amino acids. Triplicate samples (1 × 10\(^6\) cells per sample) were incubated with 1μCi of l-[3, 4, 5-\([\text{3} \text{H}]\)leucine (American Radiolabelled Chemicals, Inc.) in amino acid uptake buffer for 2 min at room temperature and immediately spun through a layer of bromoiodoacetic acid (200μl) into 8% sucrose/ 20% perchloric acid (25μl). Tubes were frozen in a dry ice/ethanol bath and cut with dog nail clippers to separate the cells from unincorporated \([\text{3} \text{H}]\)leucine. 25μl of 10% Triton X-100 and liquid scintillation cocktail were added and the cell-associated \([\text{3} \text{H}]\) were measured by liquid scintillation counting.

**Statistical analysis.** The primary analyses were conducted on pooled data points from independent samples and replicate experiments (minimum two, generally three, biologically and temporally independent replicate experiments for all data, with multiple independent samples in the case of two biological replicates), using an unpaired two-tailed Student’s t-test with post-test validation of its suitability. Welch’s or Mann–Whitney testing were used instead of the t-test where indicated based on statistical analysis of the distribution of variances in the samples to be compared. Data are displayed as mean ± s.e.m., that is, ‘centre values’ were mean as ‘average’. Results were considered statistically significant when the P value of for the null hypothesis of a comparison was <0.05. Since the extent or direction of difference between samples was unknown, and regulations mandate reducing the number of animals used to the lowest feasible level, no statistical methods were used to determine pre-specified sample sizes. The experiments were not randomized and the investigators were not blinded during the experiments. Corrections for multiple comparisons were not used. Statistical approaches for RNA-seq-related data are outlined in that section.

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Extended Data Figure 1 | See next page for caption.
Extended Data Figure 1 | Landscape of hypoxic cells in follicles and GCs of lymphoid organs. a, b, Controls for anti-HIF-1α antibody staining of GCs and portions of the surrounding splenic follicle, as in Fig. 1a, b, with fluorescent signals at the same intensity settings when analysing samples processed together, using SRBC immunization of wild-type and Hif-deleted mice and either anti-HIF-1α sera or non-immune rabbit IgG (rIgG1), as indicated. Shown are flow cytometry results of intracellular staining performed after exposure of lymphoblasts of the indicated genotypes to 4-hydroxytamoxifen and hypoxia (a), and confocal images (original magnification, ×40) (b), as in Fig. 1a and b, respectively.

Extended Data Figure 1 | Landscape of hypoxic cells in follicles and GCs of lymphoid organs. c, Quantified data obtained from samples represented in Fig. 1c. Shown are the mean (± s.e.m.) specific fluorescence intensities of hypoxprobe (anti-pimonidazole) staining in GCs (delimited as GL7+ and GL7−IgD+) follicular B cell regions after subtracting background signal (mean fluorescence intensities in these regions after anti-pimonidazole staining of samples from PBS-injected control mice). d, Immunostaining of EF5-modified cells. Shown are confocal microscopic images of spleen sections from SRBC-immunized mice injected with EF5 (left) or PBS (right) 2 h before collection, followed by direct immunofluorescent staining of frozen sections with anti-GL7, anti-IgD and anti-EF5 antibodies, representative of the quantified data presented in Fig. 1d (n = 7 GC from 3 mice in biological replicate analyses). e, Representative images of mesenteric lymph nodes after injections and immunostaining as in Fig. 1c. f, Low magnification (×10; panels are 900 μm × 900 μm) image of anti-pimonidazole immunohistochemistry on spleen sections from SRBC-immunized mice injected with pimonidazole (left) or PBS (right) before collection. Among stained sections for both anti-pimonidazole and EF5, ~75% of GC sections were unequivocally positive (n = 14 sections from 4 spleens in biological replicate analyses). g, Representative images of Peyer’s patches from non-immune, EF5-injected mice processed as in Fig. 1c (n = 6 samples from 3 mice in biological replicate analyses). h, Representative images of spleen sections from unimmunized mice injected with hypoxprobe (left) or PBS (right) 3 h before collection, processed in parallel with sections from immunized mice injected with probe, and imaged by confocal microscopy at the same time and settings as for the sections from immunized mice (for each, n = 4 sections from 2 spleens in independent biological replicates). i, GSEA plots comparing gene set pre-ranked by log2-fold change in relative expression (GL7+/GL7−) in a hypoxia gene signature.

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Extended Data Figure 2 | See next page for caption.
Extended Data Figure 2 | Altered B cell survival, proliferation and metabolism in reduced $P_{O_2}$. a, Increased executioner caspase-3 activation in hypoxic B cells. Left, representative flow histograms of cleaved caspase-3 (CC3) in the B cell gate after activated B cells were cultured in $P_{O_2}$ of 21% (normoxia) and 1% (hypoxia). B cells were stimulated with BAFF, LPS and IFNγ, cultured for 4 days at the indicated oxygen tension and processed for detection of activated caspase-3 using fluorescent-conjugated CC3 antibody. Right, quantitative data for the frequencies of B cells positive for caspase-3 cleavage in three independent replicate experiments (mean ± s.e.m.). b, $O_2$ sufficiency enhances cell cycle rates. As in a, but cells were pulsed with BrdU and frequencies of S-phase during the cultures are displayed in relation to IgG2c switching. Left, a representative result. Right, quantification of the overall B220+ cell populations in three independent replicate experiments. B cells were cultured for 4 days with BAFF, LPS and IFNγ at the indicated oxygen levels, pulsed for 4 h with BrdU, and then stained with anti-IgG2c, -B220 and -BrdU antibodies after fixation, permeabilization and processing. c, d, Pools of purified wild-type B cells were stimulated with BAFF and LPS, divided and cultured for 2 days in $P_{O_2}$ of 21% (normoxia) and 1% (hypoxia). c, Rates of glycolysis were measured after returning to their previous oxygen conditions, using equal numbers of surviving B cells after culture as detailed in the Methods. Glycolysis rates were measured in three independent experiments (mean ± s.e.m.). d, Inhibition of PHD activity decreases cellular respiration of B lymphoblasts. Purified B cells were activated and cultured for 2 days with LPS and BAFF in the presence or absence of DMOG (0.5 mM). The oxygen consumption rate (OCR) was measured with cultured viable B cells ($1.5 \times 10^5$ cells) (see Methods). The OCR was measured from technical triplicates in one experiment representative of three independent replicates with similar results (mean ± s.d.). e, Metabolic gene expression profile of GL7+ GCB cells. Genes showing significant expression changes in GL7+ GCB cells were mined for genes important for the indicated cellular processes. The heat map depicts values for the indicated genes shown as the value derived as log10 of the fragments per kilobase per million (reads) after adding 1 to each value (FPKM + 1). f, Hypoxia limits switch to IgG among B cells activated via BCRs and CD40. As in Fig. 2a, except that the B cell preparations were activated by cross-linking their surface IgM and CD40 without addition of LPS. g, Quantified mean fluorescence intensities for GFP expression in the full set of replicate experiments conducted as in Fig. 2d, presented as mean (± s.e.m.) data for each condition of culture ($P_{O_2}$ of 21, 5 or 1%, with cytokines and retinoic acid for Ig class switch conditions as indicated, and as for Fig. 2a, b).
Extended Data Figure 3 | See next page for caption.
Extended Data Figure 3 | HIF stabilization alters B cell survival, proliferation and class switched antibody level. a, Purified wild-type B cells were activated and cultured for 4 days with LPS and BAFF in the presence or absence of DMOG, after which frequencies of cells with cleaved caspase 3 or BrdU uptake, as indicated, were measured as in Extended Data Fig. 2 (representative result from one experiment among n = 3 independent replicate experiments). b, Purified wild-type B cells were activated and cultured in conditions for switching to IgG1, IgG2c and IgA, as in Fig. 2a, b, but at atmospheric (21%) $P_{O_2}$ in the presence or absence of DMOG. The frequencies of surface IgG1, IgG2c and IgA among B220$^+$-gated cells were measured as in Fig. 2 (see Methods). FACS plots display the surface levels of IgG1, IgG2c and IgA on B220$^+$-gated cells in one experiment representative of three independent replicates. c, HIF inhibition impedes the hypoxia-induced alteration of antibody class switch choices. B cells were activated and cultured for 4 days with BAFF, LPS and the indicated switching conditions as in Fig. 2a (IL-4, IgG1; IFNγ, IgG2c; retinoic acid, TGFβ, IL-4 and -5, IgA) at $P_{O_2}$ of 21% (normoxia) or 1% (hypoxia) in the presence or absence of the HIF inhibitor Bay 87-2243. FACS plots displaying the surface levels of IgG1, IgG2c and IgA on B220$^+$-gated cells in one representative result among three independent experiments are shown. Flow data shown in this figure were acquired on a BD FACScalibur but otherwise analysed as detailed in the Methods.
Extended Data Figure 4 | See next page for caption.
Extended Data Figure 4 | Hypoxia and PHD inhibition repress T-bet induction. a, B cells from wild-type mice were activated and cultured in LPS, BAFF and IL-4 or IFN-γ for 4 days under normoxic and hypoxic conditions (a) or cultured with and without DMOG at P_{O_2} of 21% (b). Shown are results of immunoblots using anti-T-bet antibody along with actin as a loading control. Shown is one representative result from three independent experiments. 

c, HIF-dependent regulation of T-bet expression by pVHL. B cells from wild-type or conditionally deleted Vhl^{Δ/Δ} and Vhl^{Δ/Δ} Hifa^{Δ/Δ} Epas1^{Δ/Δ} (Vhl and V;H1;H2 cKO, respectively) mice were activated and cultured for 4 days in LPS and BAFF in the presence or absence of IFN-γ, as indicated. Results of one representative immunoblot (from three independent experiments) probed for HIF-1α, T-bet and actin are shown. 

d, HIF superinduction by pVHL depletion in B cells at 1% P_{O_2}. Wild-type and B cells after conditional Vhl^{f/f} deletion were activated, cultured in 1% P_{O_2}, as in Extended Data Fig. 1a, and analysed by flow cytometry after processing together for indirect immunofluorescent staining of intracellular HIF-1α as in Fig. 1a and Extended Data Fig. 1a. Numbers denote the mean fluorescent intensity (MFI) of the B cells of each type. e, Flow cytometric data from one representative experiment as in Fig. 3e, in which B cells were transduced with MIT, MIG, MIT-T-bet or pMx-GFP-AID retrovectors, and cultured with BAFF and LPS ± IFN-γ in the presence or absence of DMOG. The frequencies of surface IgG2c^+ events among B220^+ cells analysed 4 days after transduction are shown, with flow data from one experiment of three independent experiments.
Extended Data Figure 5 | See next page for caption.
Extended Data Figure 5 | VHL regulates antigen-specific antibody production. a, Schematic outline of adoptive transfer experiments. B cells purified from tamoxifen-treated wild-type, Vhl\(^{f/f}\), or Vhl\(^{f/f};Hif1a\(^{f/f}\);Epas1\(^{f/f}\);ERT\(^{2}\)-Cre mice were transferred into recipients after mixing with CD4\(^+\) T cells (polyclonal:OVA-specific OT-II cells = 4:1). Recipients were analysed after primary immunization, or, for memory responses, after the primary and a recall immunization. 
b, As in Fig. 3a, except B cells from wild-type or conditionally deleted Vhl knockout mice were mixed with CD4\(^+\) OT-II TCR transgenic T cells, transferred into Ig C\(_{\alpha}\) allotype-disparate (Ig\(\alpha\)) recipient mice, followed by immunization with NP-OVA and collected 3 weeks after primary immunization. Donor- (b allotype) and recipient- (a allotype) derived NP-specific IgM and IgG1 levels in the sera were analysed by ELISA. The mean (± s.e.m.) absorbance data averaging independent samples (n = 8 WT and n = 7 Vhl cKO) obtained in two separate transfer experiments (measured on the same ELISA plate) are shown. c, d, As in Fig. 3a, wild-type or Vhl\(^{\Delta\Lambda}/\Delta\Lambda\) (Vhl cKO) B cells were mixed with wild-type CD4\(^+\) T cells (polyclonal:OVA-specific OT-II cells = 4:1), and transferred into Rag\(^{\gamma}\) recipients that were then immunized with NP-OVA, and analysed for NP-specific antibody levels 3 weeks after primary immunization (c) or, for memory response, 9 weeks after the primary immunization and 1 week after a recall immunization (d) (n = 5 independent recipients per genotype in two independent experiments) (c). Mean (± s.e.m.) ELISA data for all-affinity IgM anti-NP from the same samples as Fig. 3b are shown. d, Impaired immune memory follows interference with the B cell hypoxia response system. Terminal sera obtained from the recipient mice (Fig. 3a) 1 week after recall immunization were analysed by ELISA for all-affinity anti-NP antibodies of the indicated isotypes at the same time as the primary response samples (as in c and Fig. 3a).
## Extended Data Figure 6 | See next page for caption.
Extended Data Figure 6 | HIF-dependent regulation of antigen-specific B cell population and antibody response by pVHL. a, b, As in Fig. 3, wild-type, Vhl<sup>Δ/Δ</sup> (Vhl cKO), or Vhl<sup>Δ/Δ</sup> Hifa<sup>Δ/Δ</sup> Epas1<sup>Δ/Δ</sup> (V;H1;H2 cKO) B cells were mixed with wild-type CD4<sup>+</sup> T cells (polyclonal:OVA-specific OT-II cells = 4:1), transferred into Rag<sup>δ</sup> recipients that were then immunized with NP-OVA and analysed for NP-specific antibody levels after primary immunization as in Fig. 3b, c. Using the same mice and samples as in Fig. 3b, c, cells in spleen secreting IgG2c anti-NP were quantified by ELISpot and averaged as frequencies of antibody-secreting cells (ASC) in the sample (a). Mean (± s.e.m.) frequencies for all samples (n = 9 each) are shown. b, Anti-NP IgA levels in the sera of the samples used in Fig. 3b were quantified by ELISA. c, d, VHL regulation of antigen-specific GCs and memory B cells is HIF-dependent. As in Fig. 3b, c, wild-type, Vhl cKO or V;H1;H2 cKO B cells were mixed with CD4<sup>+</sup> T cells (polyclonal:OVA-specific OT-II cells = 4:1), transferred into Rag<sup>δ</sup> mice, immunized with NP-SRBC along with NP-OVA, boosted with NP-OVA at 3 weeks after primary immunization, and analysed at 1 week after the boost. Shown are the mean (± s.e.m.) frequencies or numbers of antigen (NP)-binding B cells of GC (IgD<sup>−</sup>GL7<sup>+</sup> ) (c), and early memory (IgD<sup>−</sup>GL7<sup>−</sup>CD38<sup>hi</sup>) phenotypes (d) derived from each donor population and recovered in the recipient mice, as determined by enumeration and flow cytometric phenotyping with fluor-conjugated NP antibody. P values, as indicated in the figure, were derived using Welch’s test for comparisons in a, c and d, where the variances were unequal but followed a normal (Gaussian) distribution.
Extended Data Figure 7 | See next page for caption.
Extended Data Figure 7 | Hypoxia interrupts an activation-induced feed-forward loop in which mTORC1 increases leucine uptake by B cells. **a**, PHD inhibition attenuates mTORC1 activity. Wild-type B cells were activated with anti-IgM and cultured for 2 days in BAFF, rested for 20 h in the presence or absence of DMOG, and then re-stimulated for 20 min with anti-IgM. Shown are immunoblots probed with anti-HIF-1α, anti-p-S6K, anti-p-S6 and anti-S6 antibody along with anti-actin as a loading control. Data are the results from one representative experiment among three independent replicates. **b–f**, Hypoxia and HIF stabilization reduce leucine uptake and mTORC1 activation. **b, c**, Reduced leucine uptake (**b**) and Slc7a5 mRNA encoding the large neutral amino acid transporter LAT1 (**c**) with inhibition of PHD proteins or mTOR. Wild-type cells were analysed after culture in 1% O2 or at PO2 of 21%, in presence of vehicle, DMOG or mTORC1 inhibitor (rapamycin) as indicated. **b**, B cell uptake of leucine, in n = 3 independent experiments. **c**, Relative mRNA level, normalized to actin (n = 3 independent experiments). **d, e**, Activated B cells of the indicated genotypes were assayed for leucine uptake (**d**) and induction of the Slc7a5 gene encoding a large neutral amino acid transporter (**e**). **d**, Leucine uptake by the cultured cells, normalized in each independent experiment (n = 3) to activated wild-type cells. **e**, VHL loss leads to HIF-dependent attenuation of Slc7a5 mRNA levels. Wild-type or conditional knockout B cells of the indicated genotypes were activated and cultured at 21% O2 as in Fig. 3d. qPCR results normalized first to actin for level within a sample, and then to the wild-type control in each independent experiment (n = 3). **f**, Leucine stimulates mTORC1 activity in activated B cells. Activated wild-type B cells, divided and cultured overnight in medium lacking or sufficient for the indicated amino acid, were restimulated and analysed as in Fig. 4a, b. Data are mean ± s.e.m.
Extended Data Figure 8 | Hypoxia promotes AMPK activity and induction of the mTORC1 inhibitor REDD1 without repressing mTORC2. a, B cells were activated and grown for 2 days in LPS and BAFF at the indicated $pO_2$ and in the presence or absence of IFN-$\gamma$ as indicated. ATP concentrations in equal numbers of cells were then assayed. In each of three replicate experiments with similar results, the [ATP] measured for cells at conventional (21%) $pO_2$ without IFN-$\gamma$ was set as 1, and the mean (± s.e.m.) levels in each sample relative to this reference are shown for three biological replicates. b, Immunoblot results after probing membranes with anti-p-ACC, anti-p-AMPK (T172) and actin are shown for one representative experiment. Numbers indicate the level of signal for cells cultured in hypoxia or DMOG as compared to the reference value of the sample cultured in conventional (21%) $pO_2$, after normalization of each sample relative to this reference value. c, Results of a representative qRT–PCR experiment measuring Redd1 mRNA in wild-type B cells (activated and cultured as in b), with each sample first normalized to Actb mRNA and then to vehicle-treated cells. d, e, Effect of VHL, hypoxia and DMOG on Akt phosphorylation in B cells. d, B cells were activated with anti-IgM and BAFF, cultured for 2 days and rested for 20 h under conditions of hypoxia or normoxia in the presence or absence of DMOG, after which cells were re-stimulated (20 min) with anti-IgM. e, As in d, B cells from wild-type or conditionally deleted Vhl knockout mice were activated with anti-IgM in the presence of BAFF, cultured for 2 days and rested for 20 h, after which cells were re-stimulated (20 min) with anti-IgM. Shown are results of immunoblots probed with antibodies directed against p-Akt (T308), p-Akt (S473), and Akt. Numbers show the quantification of signal relative to B cells that were not restimulated, after adjustment of each sample for loading as determined by total Akt. Data shown are from one representative experiment among three independent replicates.
Extended Data Figure 9 | See next page for caption.
Extended Data Figure 9 | mTORC1 regulates expansion of antigen-specific B cells and antibody class spectrum. a, Results of immunoblots using anti-raptor and anti-p-S6 antibodies, with anti-S6 antibody as a loading control. B cells (wild-type or haploinsufficient for raptor) were activated with F(ab')2 anti-IgM and BAFF, cultured for 2 days and rested for 20h, after which cells were re-stimulated for 20 min with F(ab')2 anti-IgM. Data shown are from one representative experiment among three independent replicates. b, Recipient antibody controls for effect of mTORC1 on class-switched antibody responses. As in Fig. 4c, wild-type or raptor-haploinsufficient B cells (from heterozygous mice that were Rosa26;ERT2-Cre, *Rptor*^f/+^ and converted to *Rptor*^+/Δ^ by tamoxifen injections) were mixed with CD4^+^ OT-II TCR transgenic T cells, transferred into Ig CH allotype-disparate recipient mice, immunized with NP-OVA, and obtained 3 weeks after primary immunization. Donor-derived (b allotype) (in Fig. 4) or recipient-derived (a allotype) NP-specific IgG1 and IgG2c levels in the sera were analysed by ELISA. Absorbance data averaging samples (*n* = 9 WT versus *n* = 8 *Rptor*^+/Δ^) obtained in three separate experiments (measured on the same ELISA plate). c–e, Wild-type or *Rptor*^+/Δ^ B cells were mixed with CD4^+^ T cells (polyclonal:OVA-specific OT-II = 4:1) and transferred into *Rag*^0^ mice and immunized with NP-OVA. Shown are the recoveries of antigen (NP)-binding wild-type versus *Rptor*^+/Δ^ B cells of GC (B220^+^ GL7^+^ IgD^-^) (c) and early memory (B220^+^ CD38^+^ GL7^-^ IgD^-^) (d) phenotypes. e, Generation of antigen-specific IgG2c-secreting cells depends on mTORC1. Mean (± s.e.m.) results of ELISpot assays quantitating NP-binding IgG2c (b allotype) antibody-secreting cells from the experiments in b and Fig. 4c, d, quantified as described in Extended Data Fig. 6a. P values were derived using Welch’s test for comparisons in c–e, in which the variances were unequal but followed a normal (Gaussian) distribution.
Extended Data Figure 10 | See next page for caption.
Extended Data Figure 10 | mTORC1 is rate-limiting for AID expression and switching to IgG2c. **a,** A division-independent mechanism dependent on mTORC1 quantity in B cell switching to IgG2c. Flow cytometric data in the B cell gate, displaying carboxyfluorescein diacetate succinimidyl ester (CFDA-SE) partitioning (fluorescein emission intensities) versus IgG2c, were from one experiment representative of three independent biological replicates. Wild-type or Rptor\(^{+/\Delta}\) B cells were stained with CFSE and cultured with LPS, BAFF and IFN\(\gamma\), and analysed by flow cytometry. **b,** Wild-type or Rptor\(^{+/\Delta}\) B cells were cultured for 2 days with LPS, BAFF and IFN\(\gamma\). mRNA levels of the Aidca (left) and Tbx21 (right) genes were measured in three independent replicate experiments by qRT–PCR normalized to actin in the sample and then to the level in wild-type cells (set as relative level of 1). **c,** Immunoblots probed for raptor, T-bet and actin, as indicated, using B cells as in **b** (representative of \(n = 3\) independent experiments). **d,** mTOR promotes switching to IgG by division-independent mechanisms. As in a, but CFSE-stained wild-type B cells were activated and cultured for 4 days with LPS, BAFF and IFN\(\gamma\) in the presence or absence of rapamycin versus vehicle. **e, f,** mTORC1 regulation of AID level in collaboration with T-bet determines efficient switching to IgG2c. B cells were transduced with MIT, MIG, MIT-T-bet or pMX-GFP-AID retrovectors, and cultured with BAFF and LPS and/or IFN\(\gamma\) in the presence or absence of rapamycin (5 nM). **e,** Representative flow data, from one experiment among three independent replicates, derived as in Extended Data Fig. 4e. **f,** Frequencies of surface IgG2c\(^+\) events among B220\(^+\) cells analysed 4 days after transduction are shown (\(n = 3\) independent experiments). Data are mean ± s.e.m.