



HAL
open science

Optimal AID expression and efficient immunoglobulin class switch recombination are dependent on the hypoxia-inducible factor

Vincent Heyer, Bernardo Reina San Martin

► **To cite this version:**

Vincent Heyer, Bernardo Reina San Martin. Optimal AID expression and efficient immunoglobulin class switch recombination are dependent on the hypoxia-inducible factor. *European Journal of Immunology*, 2023, 53 (7), 10.1002/eji.202350373 . hal-04219199

HAL Id: hal-04219199

<https://hal.science/hal-04219199>

Submitted on 26 Sep 2023

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

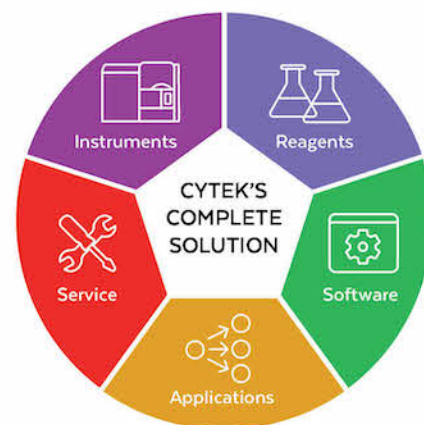
THE BEAUTY OF EXPRESSION

GIVE YOUR CELLS A VOICE

Your cells have a lot to say. Help them express themselves with high-quality single cell data thanks to Cytek's advanced technology and optimized flow cytometry applications.

- **Streamline** your workflow with highly sensitive, flexible instruments that eliminate the need to reconfigure for every assay
- **Accelerate** discovery with pre-optimized, ready-to-use reagent kits integrated with analysis templates
- **Maximize** efficiency with panel design tools and software that are widget-based and seamless
- **Partner** with highly trained service and application specialists that are ready to assist you with training and support

Cytek is committed to providing powerful solutions through scientific innovation. Join the world's most renowned pharmaceutical companies, CRO firms, industrial facilities, and research and academic institutions who have achieved results with our comprehensive approach. **Reveal the Beauty of Cellular Expression.**



CYTEK[®]
TRANSCEND THE CONVENTIONAL
WWW.CYTEKBIO.COM

Short Communication

Optimal AID expression and efficient immunoglobulin class switch recombination are dependent on the hypoxia-inducible factor

Vincent Heyer^{1,2,3,4} and Bernardo Reina-San-Martin^{1,2,3,4} 

¹ Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France

² Institut National de la Santé et de la Recherche Médicale (INSERM), Illkirch, France

³ Centre National de la Recherche Scientifique (CNRS), Illkirch, France

⁴ Université de Strasbourg, Illkirch, France

During immune responses, B cells engaging a cognate antigen are recruited to GCs in secondary lymphoid organs where they will diversify their BCR to generate highly specific and adapted humoral responses. They do so, by inducing the expression of activation-induced cytidine deaminase (AID), which initiates somatic hypermutation (SHM) and class switch recombination (CSR). AID deaminates cytosines in ss DNA, generating U:G mismatches that are processed to induce ds DNA break intermediates during CSR that result in the expression of a different antibody isotype. Interestingly, hypoxia regions have been reported in GCs and suggesting that hypoxia could modulate the humoral response. Furthermore, hypoxia inducible transcription factor (HIF) can bind to the AID promoter and induce AID expression in a non-B-cell setting, suggesting that it might be involved in the transcriptional induction of AID in B cells, hence, regulating SHM and CSR. We, thus, hypothesized that HIF could regulate the efficiency of CSR. Here, we show that the inactivation of both the HIF-1 α and HIF-1 β subunits of the HIF transcription factor in murine CH12 B cells results in defective CSR and that this is due to the suboptimal induction of AID expression.

Keywords: AID · class switch recombination · B cell receptor · HIF-1 α · HIF-1 β



Additional supporting information may be found online in the Supporting Information section at the end of the article.

Introduction

During immune responses, the BCR repertoire is diversified in GC through somatic hypermutation (SHM) and class switch recombination (CSR) [1]. While SHM diversifies the variable region of Ig genes by introducing mutations and modifying antibody affinity for its cognate antigen, CSR provides novel antibody effector functions through a DNA recombination reaction (occurring at the Ig

heavy chain locus) that replaces the isotype expressed (from IgM to IgG, IgE, or IgA). Both of these processes are initiated in GC B cells by the inducible expression of activation-induced cytidine deaminase (AID). AID triggers these molecular events by deaminating cytosines to uracils in DNA, which are then processed by uracil DNA glycosylase and mismatch repair proteins to induce mutations or double stranded DNA breaks [1]. While AID-induced DNA lesions are essential to establish highly specific and adapted humoral responses, AID carries a significant oncogenic potential, which has been implicated at the origin of GC-derived B-cell lymphomas [2].

Correspondence: Dr. Bernardo Reina-San-Martin
e-mail: reinab@igbmc.fr

Given the strong oncogenic potential of AID, its expression and activity need to be tightly regulated. At the transcriptional level, AID expression is only induced in activated B cells and results from a tight balance between the activity of transcription factors (Batf, Hoxc4, etc.) and transcriptional repressors [3]. Interestingly, it has been previously shown that during immune responses, regions of hypoxia are observed within GCs and that the response to hypoxia modulates the humoral response [4, 5]. Nevertheless, little is known about the physiological mechanisms for regulating these processes within the GC microenvironment, and it is possible that oxygen tension and the physiological response to hypoxia may play a key role in regulating the humoral response within GCs [5–7].

The cellular response to hypoxia is mediated by the hypoxia inducible factor complex (HIF), formed by the HIF-1 α and HIF-1 β subunits. In normoxia, the HIF-1 α protein is unstable, it fixes the tumor suppressor factor von Hippel-Lindau (pVHL), an E3 ubiquitin ligase that ubiquitinates it and triggers its degradation through the proteasome. Hypoxia then triggers the stabilization of HIF-1 α , its association with HIF-1 β , and the nuclear translocation of the HIF complex, where it associates with other co-factors, such as p300/CBP, to bind to hypoxia response elements (HREs; RCGTG) and induce the transactivation of hypoxia-sensitive genes [5]. In addition, the HIF signaling pathway can also be activated in an oxygen-independent manner through the activation of the BCR, TLRs, or cytokine receptors, leading to the stabilization of the HIF-1 α protein in B cells [5].

It has been shown that HIF-1 α is capable of binding to the HREs in the *Aicda* promoter (located at -16 and -1620) inducing AID's mRNA production in a non-B-cell setting [8]. We, therefore, hypothesized that the HIF complex could be implicated in regulating the transcriptional activation of the *Aicda* gene in B cells. Here, we explore the role of HIF-1 α and HIF-1 β in the process of CSR in CH12 B cells.

Results and discussion

Hif1 α is required for optimal CSR in CH12 cells

To determine whether HIF-1 α plays a role in CSR, we inactivated the *Hif1 α* gene in CH12 cells (Supporting Information Fig. S1A), a murine B-cell line, that when cultured in vitro in the presence of TGF- β , IL4, and CD40 ligand is capable of undergoing CSR from IgM to IgA very efficiently [9]. The absence of HIF-1 α was verified by Western blot (Fig. 1A). To determine whether CSR is affected by inactivation of *Hif1 α* , WT and *Hif1 α* ^{-/-} cells were induced to undergo CSR. After 72 h, the efficiency of CSR was evaluated by flow cytometry (Fig. 1B, C and Supporting Information Fig. S1D). While the parental cell line displayed 60% of IgA-expressing cells, this percentage was reduced by threefold in *Hif1 α* ^{-/-} CH12 cells (Fig. 1B,C). As CSR is dependent on proliferation, we induced CSR in CFSE-labeled cells and found that all genotypes diluted the CFSE dye at the same extent (Supporting Information Fig. S1C). To confirm that the defective CSR phenotype observed is

due to the lack of *Hif1 α* , we transduced *Hif1 α* ^{-/-} cells with a retrovirus expressing HIF-1 α . We find that re-expression of HIF-1 α is able to rescue significantly the percentage of IgA-expressing cells (Fig. 1B,C). We conclude that *Hif1 α* is required for optimal CSR in CH12 cells, independently of proliferation defects.

Induction of the AID mRNA is compromised in Hif1 α ^{-/-} cells

To determine whether AID expression is defective in the absence of *Hif1 α* , we quantified the level of AID protein in mRNA by RT-qPCR (Fig. 1D) and Western blot (Fig. 1E). We found that induction of the AID mRNA is compromised in *Hif1 α* ^{-/-} cells (Fig. 1D) and that this results in delayed AID expression at the protein level (Fig. 1E) and a concomitant defect in CSR (Fig. 1B,C). Interestingly, we can observe that the re-expression of HIF-1 α can rescue the levels of AID mRNA (Fig. 1D) and protein (Fig. 1E).

Hif1 β is required for optimal AID expression and CSR

The nuclear translocation of HIF-1 α is dependent on HIF-1 β . Thus, in the absence of HIF-1 β , we should observe a similar phenotype. To test this hypothesis, we inactivated the *Hif1 β* gene in CH12 cells (Supporting Information Fig. S1B). Absence of HIF-1 β was verified by Western blot (Fig. 2A). To determine whether CSR is affected by inactivation of *Hif1 β* , WT and *Hif1 β* ^{-/-} cells were induced to undergo CSR (Fig. 2B, C and Supporting Information Fig. S1D). While the parental cell line displayed 55% of IgA-expressing cells, this percentage was significantly reduced in *Hif1 β* ^{-/-} cells (Fig. 2B and C). As *Hif1 α* ^{-/-} cells, *Hif1 β* ^{-/-} cells proliferated at the same level as WT cells (Supporting Information Fig. S1C). To confirm that the defective CSR phenotype observed is due to the lack of HIF-1 β , we transduced *Hif1 β* ^{-/-} cells with a retrovirus expressing HIF-1 β . We find that re-expression of HIF-1 β is able to rescue the CSR defect (Fig. 2B, C). Phenocopying *Hif1 α* -deficiency, *Hif1 β* ^{-/-} cells displayed delayed AID mRNA expression and protein levels (Fig. 2D, E). We conclude that *Hif1 β* is required for optimal AID expression and CSR in CH12 cells, independently of proliferation defects.

The CSR defect in Hif1 α - and Hif1 β -deficient B cells is due to defective AID expression

If HIF-1 α and HIF-1 β are involved in mediating optimal levels of AID mRNA production, then the CSR defect observed in *Hif1 α* ^{-/-} and *Hif1 β* ^{-/-} cells should be rescued by overexpressing AID. To test this hypothesis, we transduced *Hif1 α* ^{-/-} and *Hif1 β* ^{-/-} cells with a retrovirus expressing AID (Fig. 3A,C) and cultured them for 3 days in the presence of TGF- β , IL4, and CD40 ligand to induce CSR. We find that overexpressing AID in *Hif1 α* ^{-/-} or *Hif1 β* ^{-/-} cells is able to rescue CSR (Fig. 3B,D). We conclude that the CSR

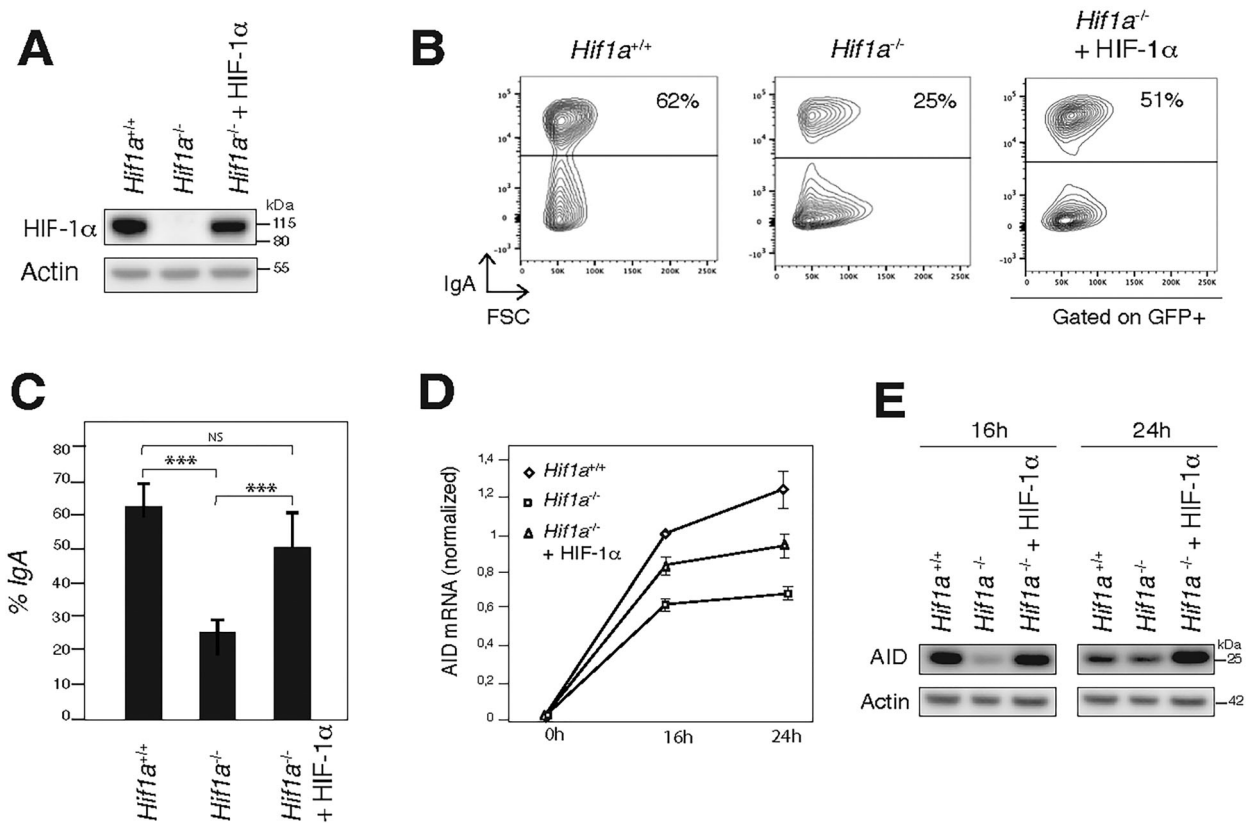


Figure 1. The hypoxia-inducible factor (HIF-1 α) is required for optimal AID expression and CSR. (A) Western blot for HIF-1 α , and β -Actin in WT, *Hif1 α ^{-/-}*, and *Hif1 α ^{-/-}* CH12 cells re-expressing HIF-1 α . Molecular weights (kDa) are indicated. (B) Flow cytometry analysis of IgA expression in WT, *Hif1 α ^{-/-}*, and *Hif1 α ^{-/-}* CH12 cells re-expressing HIF-1 α . Representative plots are shown. (C) Average percentage of IgA⁺ cells from five independent experiments in WT, *Hif1 α ^{-/-}*, and *Hif1 α ^{-/-}* CH12 cells re-expressing HIF-1 α . *p*-Value (two-tailed Student's *t*-test; ****p* < 0.001, NS *p* > 0,05); *p*-values are *Hif1 α ^{+/+}* versus *Hif1 α ^{-/-}* = 5.05×10^{-6} , *p*-values are *Hif1 α ^{-/-}* versus *Hif1 α ^{-/-}* + HIF-1 α = 0.00066, *p*-values are *Hif1 α ^{+/+}* versus *Hif1 α ^{-/-}* + HIF-1 α = 0.05264. (D) Histogram showing the *Aicda* transcript levels in WT, *Hif1 α ^{-/-}* and *Hif1 α ^{-/-}* CH12 cells re-expressing HIF-1 α as determined by RT-qPCR. The mean values of technical triplicates from three independent experiments were normalized to HPRT transcripts ($\Delta\Delta$ CT method) and are shown relative to WT cells, which were set to one. (E) Western blot analysis for AID and β -Actin in WT, *Hif1 α ^{-/-}*, and *Hif1 α ^{-/-}* CH12 cells re-expressing HIF-1 α . Molecular weights (kDa) are indicated. Uncropped Western blot images for Fig. 1A and E are shown in Supporting Information Fig. S2.

defect observed in *Hif1 α ^{-/-}* and *Hif1 β ^{-/-}* cells is related to suboptimal expression of AID.

We have shown that CSR is defective in *Hif1 α* - and *Hif1 β* -deficient B cells. We observe that in these cells there is a delayed expression of the AID mRNA and protein that is visible after 16 h (Fig. 1D,E and 2D,E) and that lasts through the peak of AID expression (24 h). This, per se, explains the CSR defect observed (Fig. 1B,C and 2B,C) and allows us to conclude that deficiency in the HIF transcription factor results in suboptimal levels of AID expression and, hence, defective CSR. This conclusion is supported by the fact that overexpression of AID bypasses the CSR defect observed in a *Hif1 α* - or *Hif1 β* -deficient background (Fig. 3B,D).

While it is clearly established that hypoxia and the HIF1 transcription factor play a fundamental role in B-cell biology [5–7], it is difficult to pinpoint their precise role in shaping the humoral response at the GC reaction in vivo. This might be due, in part, to the pleiotropic effect of HIF1 in controlling intrinsic B-cell responses to hypoxia such as metabolism, cell survival, signaling, or AID expression [5, 7, 10]. In addition, hypoxia can also

influence the function of T-follicular helper cells and/or follicular DCs, thus, having a potential impact on B-cell selection and affinity maturation. Furthermore, there are some apparent contradictions concerning hypoxia in GCs and antibody diversification through CSR and SHM. For example, it has been shown that CSR occurs at the pre-GC stage outside of GCs [11] and that the region in which hypoxia is more pronounced is in the light zone [6] and not in the dark zone, where AID expression is predominant [12]. It is possible that hypoxia triggers the transactivation of the *Aicda* mRNA in the light zone and that its expression, at the protein level, is maximal once B cells reach the dark zone. Furthermore, as the secondary lymphoid organs are in general in a hypoxic environment relative to other tissues [7], this per se may poise follicular and/or GC B cells to enforce optimal AID expression.

Supporting the role of the HIF transcription factor in controlling AID expression, it has been shown that the inactivation of pVHL leads to a strong accumulation of the HIF-1 α protein, and consequently to increased protein levels of the members of the APOBEC3 cytidine deaminase (A3B) family, which includes AID

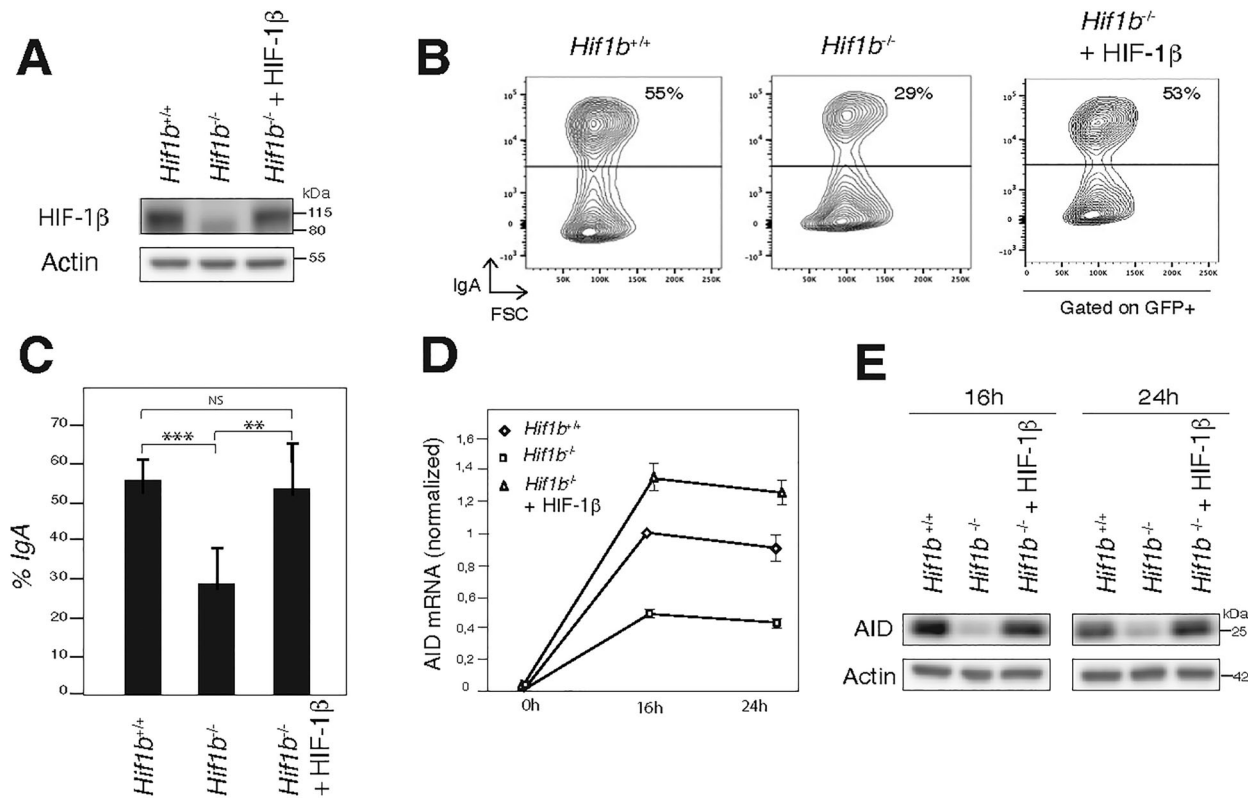


Figure 2. The hypoxia-inducible factor (HIF1- β) is required for optimal AID expression and CSR. (A) Western blot for HIF-1 β and β -Actin in WT, *Hif1b*^{-/-}, and *Hif1b*^{-/-} CH12 cells re-expressing HIF-1 β . Molecular weights (kDa) are indicated. (B) Flow cytometry analysis of IgA expression in WT, *Hif1b*^{-/-}, and *Hif1b*^{-/-} CH12 cells re-expressing HIF-1 β . Representative plots are shown. (C) Average percentage of IgA⁺ cells from five independent experiments in WT, *Hif1b*^{-/-}, and *Hif1b*^{-/-} CH12 cells re-expressing HIF-1 β . *p*-Value (two-tailed Student's *t*-test; ****p* < 0.001, ***p* < 0.01, NS *p* > 0.05); *p*-values are *Hif1a*^{+/+} versus *Hif1a*^{-/-} = 0.0007, *p*-values are *Hif1a*^{-/-} versus *Hif1a*^{-/-} + HIF-1 α = 0.0079, *p*-values are *Hif1a*^{+/+} versus *Hif1a*^{-/-} + HIF-1 α = 0.75732. (D) Histogram showing the *Aicda* transcript levels in WT, *Hif1b*^{-/-}, and *Hif1b*^{-/-} CH12 cells re-expressing HIF-1 β as determined by RT-qPCR. The mean values of technical triplicates from three independent experiments were normalized to HPRT transcripts ($\Delta\Delta$ CT method) and are shown relative to WT cells, which were set to one. (E) Western blot analysis for AID and β -Actin in WT, *Hif1b*^{-/-}, and *Hif1b*^{-/-} CH12 cells re-expressing HIF-1 β . Molecular weights (kDa) are indicated. Uncropped Western blot images for Fig. 2A and E are shown in Supporting Information Fig. S2.

[13]. Thus, we would have expected that inactivation of pVHL would lead to enhanced HIF-dependent transcriptional activity [13], resulting in increased production of the AID mRNA and protein and more efficient CSR and SHM in a hypoxic environment in the GC or in response to cytokine signaling. Nevertheless, a B-cell-specific KO of pVHL, which resulted in constitutive HIF-1 α stabilization [6, 13], surprisingly led to impaired affinity maturation and defective CSR [6]. These results highlight the underlying difficulty of exploring the role of hypoxia and HIF in antibody diversification in vivo.

As the HIF signaling pathway can be activated (independently of oxygen) through cytokine signaling [5, 10], notably through TGF- β , IL4, and CD40, our results highlight the possibility of an oxygen-independent role of the HIF transcription factor in AID's expression and, hence, modulating the efficiency of the humoral response. Nevertheless, the role of HIF in SHM, which requires the generation of a KO mouse model, remains to be determined. In addition, as the efficiency of CSR in the absence of *Hif1a*, is not completely abolished, it is possible that there is a functional compensation by *Hif2a*, whose role in B cells is unclear [5].

While it has been shown that hypoxia leads to contrasting effects on CSR [4, 6, 14], here we demonstrate that inactivation of the HIF transcription factor in murine CH12 B cells (independently of pleiotropic effects on metabolism, cell survival, or oxygen dependence) results in defective CSR and that this is due to the suboptimal induction of AID expression. This has implications for the effectiveness of the humoral response within the GCs, as regions of hypoxia and cytokine stimulation might potentiate AID expression to enforce the diversification of the BCR and to accomplish the establishment of highly specific and adaptive immunity.

Materials and methods

Cell culture

CH12 cells were cultured in RPMI 1640 (Gibco) supplemented with 10 mM HEPES, 10% heat-inactivated Fetal Calf Serum (Bodinco BV), 1 mM Na pyruvate, 100 U/mL penicillin/streptomycin,

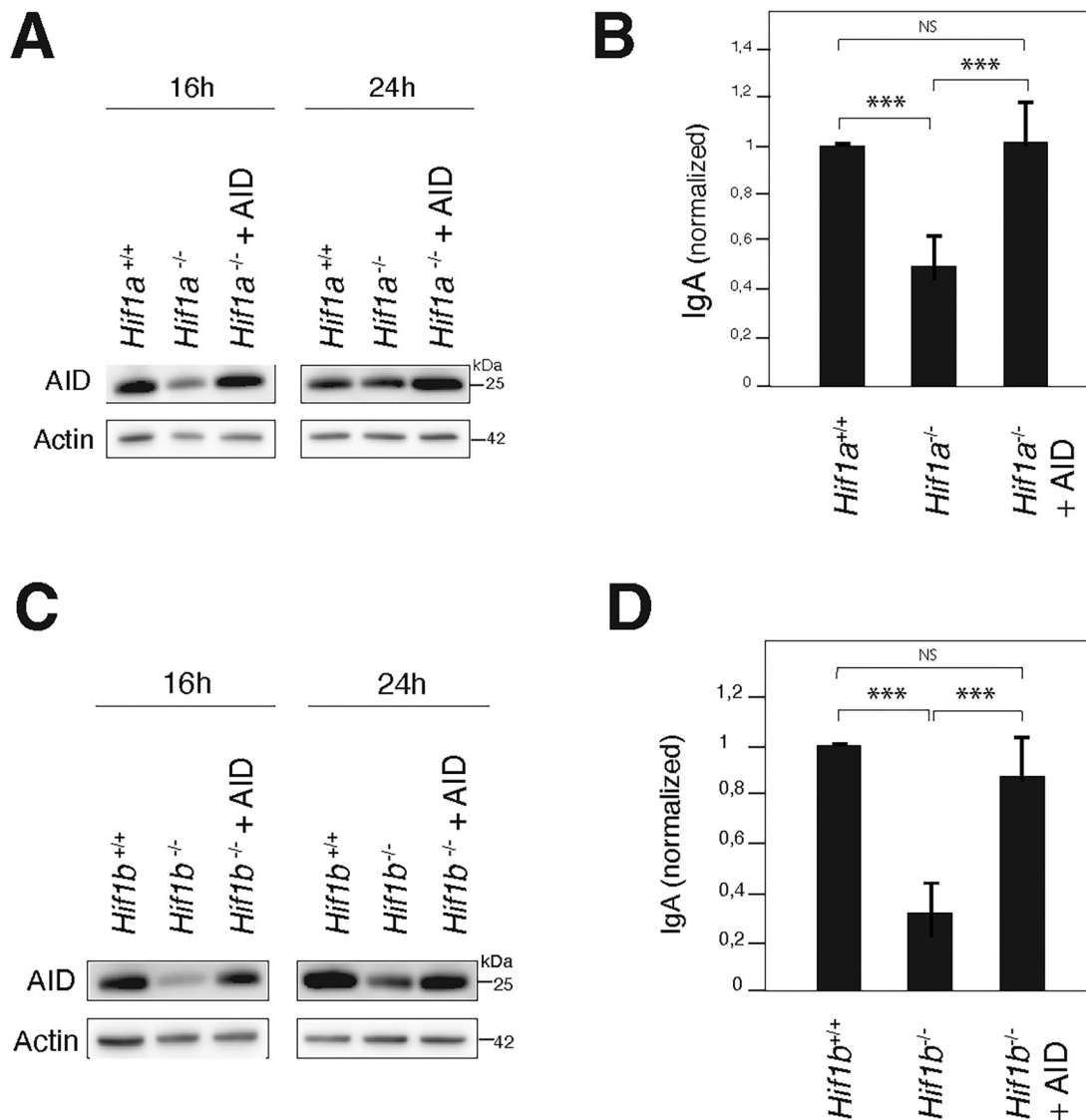


Figure 3. Overexpression of AID rescues the CSR defect in Hif1a- and Hif1b-deficient B cells. Western blot for AID and β -Actin in (A) WT and Hif1a^{-/-} CH12 cells overexpressing AID or not and (C) WT, Hif1b^{-/-}, and Hif1b^{-/-} CH12 cells overexpressing AID or not. Molecular weights (kDa) are indicated. Percentage of IgA-expressing cells in (B) Hif1a^{-/-} or Hif1b^{-/-} (D) CH12 cells re-expressing AID (or not), relative to WT controls. Data are from five independent experiments. *p*-Value (two-tailed Student's *t*-test; ****p* < 0.001, NS *p* > 0.05); *p*-values are Hif1a^{+/+} versus Hif1a^{-/-} = 0.00018; Hif1a^{-/-} versus Hif1a^{-/-} + AID = 0.00079; Hif1a^{+/+} versus Hif1a^{-/-} + AID = 0.65873; Hif1b^{+/+} versus Hif1b^{-/-} = 6.5×10^{-6} ; Hif1b^{-/-} versus Hif1b^{-/-} + AID = 0.00066; Hif1b^{+/+} versus Hif1b^{-/-} + AID = 0.14032. Uncropped Western blot images for Fig. 3A and C are shown in Supporting Information Fig. S2.

and 50 μ M β -mercaptoethanol. HIF-1 α protein was induced with 1% O₂, 35 μ g/mL COCl₂, or 5 mM DMOG, for 6 h. Bosc23 cells were cultured in DMEM (Gibco) supplemented with glucose (4.5g/L), 10% of heat-inactivated fetal calf serum, 1 mM Na pyruvate, 100 U/mL penicillin/streptomycin.

CRISPR/Cas9

CH12 cells were transfected with a plasmid expressing Cas9 high-fidelity (Cas9-HF1) fused to EGFP and co-expressing

2 gRNAs flanking exon 6 or exon 2 of the *hif1a* or *hif1b* genes, respectively (Hif1a-1: GCATGCCCTGATATACATGG, Hif1a-2: CTCATAGCTAAGATTACAGG, Hif1b-1: AATCCTTCACCCACATGG, Hif1b-2: CTCAGTGGTTAAGAGCACTG). After 24 h of transfection, single CH12 cells expressing Cas9-HF1 (EGFP+) were sorted (FACS Aria Fusion, BD) and cultured for 2 weeks. Clones were genotyped by PCR using specific primers (HIF1a-F: ATGACCAGATTAGTGAAGAAGGAAA-GATAG, HIF1a-R: TGTGTTTTAGTGTTTTAGTTGGTTG, HIF1b-F: GAGTCAATTTTCATGTGGCCTGGATAGT, HIF1b-R: CTGTGAGT-GAGGTCGGATGATAAGTAATAGGTGTG) and sequencing.

Western blot analysis

Whole-cell extracts were prepared using standard methods. Proteins were separated by SDS-PAGE, transferred to a PVDF membrane (Millipore) and blotted using anti HIF-1 α (1/1000; Novus NB100-123), anti-HIF-1 β (1/1000; Novus NB100-110), anti-AID (1/10.000; IGBMC), and anti- β -Actin (1/20.000; Sigma-Aldrich) antibodies.

CSR Assays

CH12 cells were cultured for 72 h in vitro with IL4 (5 μ g/ μ L; Peprotech), TGF- β (1 μ g/ μ L; R&D Systems Europe), and anti-CD40 antibody (200 ng/mL; eBioscience) for 72 h. CSR to IgA was assessed by staining with an anti-IgA-PE antibody (1/500; Southern Biotech). Before analysis, DAPI was added to exclude dead cells. Cells were analyzed by flow cytometry (Celesta BD) using the FlowJo software and following the flow cytometry guidelines. Gating strategy is shown in Supporting Information Fig. S1D.

Retroviral transductions

Cells were transduced with retroviral supernatants obtained by transfecting Bosc23 cells with a retrovirus expressing mHIF-1 α , mHIF-1 β , or mAID and selected with puromycin (1 μ g/mL). After 7 days, cells were submitted to CSR assays.

RT-qPCR

RNA extraction and cDNA synthesis were done using standard techniques. RT-qPCR was performed in triplicate (Roche LightCycler 480) using QuantiTect SYBR green PCR master mix (Qiagen) and specific primers (AID-F: GAAAGTCACGCTG-GAGACCG, AID-R: TCTCATGCCGTCCTTGG, HPRT-F: GTTG-GATACAGCCAGACTTTGTTG, HPRT-R: GATTCAACTGCGCT-CATCTTAGGC).

Acknowledgements: We thank members of the Reina-San-Martin laboratory for discussions. C. Ebel and M. Phillips for assistance with cell sorting. B. Heller for cell culture. This work of the Interdisciplinary Thematic Institute IMCBio, as part of the ITI 2021–2028 program of the University of Strasbourg, CNRS and Inserm, was supported by IdEx Unistra (ANR-10-IDEX-0002), by SFRI-STRA^TUS project (ANR 20-SFRI-0012), and EUR IMCBio (ANR-17-EURE-0023) under the framework of the French Investments for the Future Program and by grants to B.R.S.M. from Fondation ARC, Institut National Contre le Cancer (INCA) and Fondation Recherche Médicale (FRM).

Conflict of interest: The authors declare no commercial or financial conflict of interest.

Author contributions: Conceptualization, V.H. and B.R.-S.-M.; methodology, V.H. and B.R.-S.-M.; investigation, V.H.; writing-original draft, V.H. and B.R.-S.-M.; writing-review & editing, V.H. and B.R.-S.-M.; supervision, B.R.-S.-M.; funding acquisition, B.R.-S.-M.

Data availability statement: The data that support the findings of this study are available from the corresponding author upon request.

Peer review: The peer review history for this article is available at <https://publons.com/publon/10.1002/eji.202350373>

References

- Feng, Y.Q., Seija, N., Di Noia, J. M. and Martin, A., AID in antibody diversification: there and back again (vol 41, pg 586, 2020). *Trends Immunol.* 2021. 42: 89–89.
- Nussenzweig, A. and M.C. Nussenzweig., Origin of chromosomal translocations in lymphoid cancer. *Cell* 2010. 141: 27–38.
- Tran, T.H., Nakata, M., Suzuki, K., Begum, N. A., Shinkura, R., Fagarasan, S., Honjo, T. et al., B cell-specific and stimulation-responsive enhancers derepress Aicda by overcoming the effects of silencers. *Nat. Immunol.* 2010. 11: 148–U67.
- Abbott, R.K., Thayer, M., Labuda, J., Silva, M., Philbrook, P., Cain, D. W., Kojima, H. et al., Germinal center hypoxia potentiates immunoglobulin class switch recombination. *J. Immunol.* 2016. 197: 4014–4020.
- Zhang, J.W., Wu, X., Ma, J., Long, K., Sun, J., Li, M. and Ge, L., Hypoxia and hypoxia-inducible factor signals regulate the development, metabolism, and function of B cells. *Front. Immunol.* 2022. 13. <https://doi.org/10.3389/fimmu.2022.967576>
- Cho, S.H., Raybuck, A. L., Stengel, K., Wei, M., Beck, T. C., Volanakis, E., Thomas, J. W. et al., Germinal centre hypoxia and regulation of antibody qualities by a hypoxia response system. *Nature* 2016. 537: 234–238.
- Burrows, N. and P.H. Maxwell, Hypoxia and B cells. *Exp. Cell. Res.* 2017. 356: 197–203.
- Liu, Y.Q., Mukhopadhyay, P., Pisano, M. M., Lu, X., Huang, L., Lu, Q. and Dean, D. C., Repression of Zeb1 and hypoxia cause sequential mesenchymal-to-epithelial transition and induction of Aid, Oct4, and Dnmt1, leading to immortalization and multipotential reprogramming of fibroblasts in spheres. *Stem Cells* 2013. 31: 1350–1362.
- Rogier, M., Moritz, J., Robert, I., Lescale, C., Heyer, V., Abello, A., Martin, O. et al., Fam72a enforces error-prone DNA repair during antibody diversification. *Nature* 2021. 600: 329–333.
- Li, L., Feng, C., Qin, J., Li, D., Liu, M., Han, S. and Zheng, B., Regulation of humoral immune response by HIF-1 α -dependent metabolic reprogramming of the germinal center reaction. *Cell. Immunol.* 2021. 367: 104409.
- Roco, J.A., Mesin, L., Binder, S. C., Nefzger, C., Gonzalez-Figueroa, P., Canete, P. F., Ellyard, J. et al., Class-switch recombination occurs infrequently in germinal centers. *Immunity* 2019. 51: 337–350 e7.
- Victoria, G.D. and M.C. Nussenzweig, Germinal centers. *Annu. Rev. Immunol.* 2022. 40: 413–442.

- 13 Scholtes, G.K., Sawyer, A. M., Vaca, C. C., Clerc, I., Roh, M., Song, C. and D'Aquila, R. T., The von Hippel-Lindau Cullin-RING E3 ubiquitin ligase regulates APOBEC3 cytidine deaminases. *Transl. Res.* 2021. 237: 1–15.
- 14 Koers, J., Marsman, C., Steuten, J., Tol, S., Derksen, N. I. L., ten Brinke, A., van Ham, S. M. et al., Oxygen level is a critical regulator of human B cell differentiation and IgG class switch recombination. *Front. Immunol.* 2022. 13: p. 1082154.

Abbreviations: **AID:** activation-induced cytidine deaminase · **SHM:** somatic hypermutation · **CSR:** class switch recombination · **HIF:**

hypoxia inducible transcription factor · **pVHL:** tumor suppressor factor von Hippel-Lindau · **HREs:** hypoxia response elements

Full correspondence: Dr. Bernardo Reina-San-Martin, Institut de Génétique et de Biologie Moléculaire et Cellulaire (IGBMC), Illkirch, France.
e-mail: reinab@igbmc.fr

Received: 12/1/2023

Revised: 31/3/2023

Accepted: 2/5/2023

Accepted article online: 4/5/2023