

Th17 cells require the DNA repair sensor xeroderma pigmentosum complementation Group C to control oxidative DNA damage in a murine model

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T helper 17 cells play essential roles in mucosal immunity and autoimmunity, yet the mechanisms that protect these cells from oxidative DNA damage remain poorly defined. Here we show, in a murine model, that the nucleotide excision repair sensor Xeroderma Pigmentosum Complementation Group C preserves genomic stability and metabolic fitness during T helper 17 cell differentiation. Loss of this factor reduces interleukin 17 production and increases mitochondrial reactive oxygen species and oxidative DNA damage, resulting in altered metabolic programs. Mechanistically, Xeroderma Pigmentosum Complementation Group C interacts with the base excision repair enzyme 8-oxoguanine DNA glycosylase, and its absence enhances oxidative lesion incision activity, indicating defective coordination between DNA repair pathways. Restoring antioxidant capacity rescues cytokine production and limits DNA damage in deficient cells. Together, these findings identify Xeroderma Pigmentosum Complementation Group C as a key coordinator of DNA repair and redox control required for T helper 17 cell function in inflammatory settings.

T helper 17 (Th17) cells are a subset of T helper cells characterized by the production of IL-17A, IL-17F and IL-22 cytokines. They are important for a protective immunity against bacterial and fungal infections at mucosal surfaces. Accordingly, dysregulated Th17 cell responses are involved in the pathogenesis of several inflammatory or autoimmune

diseases, such as inflammatory bowel disease, psoriasis and multiple sclerosis¹. The development of Th17 cells relies on the presence of cytokines such as transforming growth factor- β (TGF- β) and IL-6. TGF- β triggers the activation of SMAD2/3 transcription factors, while IL-6 signals facilitate STAT3 phosphorylation. The concerted action of

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SMAD2/3 and STAT3, along with other transcription factors activated by TCR signaling, promotes the expression of ROR γ t and facilitates Th17 cell differentiation. IL-21 and IL-23 can also transmit signals through STAT3, playing a crucial role in the differentiation of both murine and human Th17 cells^{2–6}.

Endogenous factors threaten genome stability and alter T cell differentiation, which impact on autoimmunity and cancer development^{7–9}. Reactive oxygen species (ROS) have been considered harmful byproducts from metabolism, which damage cellular proteins, lipids and nucleic acids¹⁰. Activated T cells can induce respiratory bursts both through direct contact with phagocytes and via the release of cytokines¹¹. T cell receptor (TCR) ligation triggers the activation of NOX-2, leading to the production of hydrogen peroxide (H₂O₂) that activates GATA-3 and STAT6, while inhibiting STAT3, therefore promoting Th2 and counteracting Th17 cells differentiation¹². In Th17 cells the absence of calcium signaling by STIM1 increases ROS production, which results in DNA damage and cell death¹³. On the other hand, ROS scavenger N-acetylcysteine boosts Th17 cell differentiation¹⁴. Despite being susceptible to increased amounts of ROS in the absence of calcium signaling, WT Th17 cells display less accumulation of ROS, DNA damage and cell death when compared to Th0 and Th1 cells¹⁵, which suggest that Th17 cells own mechanisms to protect against oxidative DNA damage.

The repair of oxidative-induced DNA damage occurs through sophisticated pathways that include the base excision repair (BER) and the nucleotide excision repair (NER). DNA glycosylases, such as 8-oxoguanine DNA glycosylase (OGG1) and S-phase-activated Nei-like (NEIL) family members, recognize and excise oxidized or damaged bases. Following this, Apurinic/apyrimidinic (AP) endonucleases, like APE1, cleave the DNA strand at the apurinic or apyrimidinic sites, creating a nick. DNA polymerases, notably DNA polymerase β , fill in the gap with newly synthesized DNA, and ligases, including DNA ligase III, seal the nick by forming phosphodiester bonds¹⁶. The NER pathway is initiated by the Xeroderma Pigmentosum Complementation Group C (XPC) protein, which is crucial for the recognition of bulk DNA lesions, such as those caused by UV radiation. While XPC plays a key role in damage recognition, it indirectly contributes to the repair of oxidative DNA damage by facilitating the removal of structurally significant lesions, thereby ensuring the initiation of a precise and efficient repair process^{17–20}. Interestingly, Th17 cells upregulate genes associated with the NER DNA repair pathway in the colon during intestinal inflammation²¹.

Beyond its canonical role, increasing evidence supports a direct involvement of NER in repairing oxidative DNA damage. Early studies demonstrated that XPC and other NER proteins can modulate BER enzyme activity¹⁸ showed that, in vitro, XPC physically interacts with and enhances OGG1 glycosylase activity, thereby facilitating the removal of 8-oxo-7,8-dihydroguanine (8-oxoG) lesions in mammalian cells. Consistent with this, live-cell imaging revealed that both XPC and Cockayne syndrome protein B (CSB) rapidly accumulate at sites of oxidative DNA damage (notably 8-oxoG), independent of downstream NER factors²², suggesting a non-canonical, early recognition or scaffolding role for these proteins in the oxidative damage response. Moreover, recent evidence has demonstrated that NER directly removes certain helix-distorting oxidative lesions, particularly 5',8-cyclopurine-2'-deoxynucleosides (cdA and cdG), which cannot be processed by BER glycosylases and are repaired exclusively by NER²³. However, it is unknown whether Th17 cells use the NER DNA repair as a mechanism to control DNA damage. Therefore, we hypothesized that Th17 cells use NER factors to control oxidative DNA damage and genomic instability contributing to an efficient differentiation and function.

Here, we show that Th17 cells upregulate nucleotide-excision repair genes, including *Xpc*, in a BATF-dependent manner and exhibit lower oxidative DNA damage than non-polarized Th0 cells. Genetic

ablation of *Xpc* compromises Th17 differentiation, elevates reactive oxygen species and DNA strand breaks, and drives metabolic reprogramming characterized by reduced glycolytic capacity and mitochondrial dysfunction with increased dependence on oxidative phosphorylation but decreased respiratory efficiency. At the molecular level, XPC physically associates with the base-excision DNA glycosylase OGG1, and, in the absence of XPC, OGG1 incision activity is increased, consistent with a compensatory response to the higher burden of oxidized bases. These findings identify XPC as a coordinator of redox balance and DNA repair pathways in Th17 cells, supporting genome maintenance and the transcriptional program required for effective Th17 function.

Results

XPC is required for Th17 cells differentiation

Initially, we investigated whether lack of XPC affects the differentiation of CD4⁺ and CD8⁺ T cells under steady-state conditions. We collected draining lymph nodes and spleens from 6–8 week-old WT and XPC^{−/−} mice. Flow cytometry analysis revealed no significant difference in the frequency of CD4⁺ and CD8⁺ T cells between WT and XPC^{−/−} mice in the lymph nodes and spleens (Fig. 1a, b). Moreover, the frequency of naive (CD62L⁺CD44[−]) and memory (CD62L[−]CD44⁺) subsets of both CD4⁺ and CD8⁺ T cells was comparable between the two groups (Fig. 1c, d).

We next examined the differentiation of specific T cell subsets, including Th1, Th2, Th17, Treg, and Tc1 cells. UMAP plots confirmed that the overall distribution of these subsets was similar in both WT and XPC^{−/−} mice in the draining lymph nodes and spleen (Fig. 1e). Heatmaps comparing Z-scores of gene expression also confirmed no significant differences in the differentiation of these subsets (Fig. 1f, g).

To further investigate how XPC deficiency affects CD4⁺ T-cell subset differentiation, we analyzed intracellular cytokine expression and transcription factor profiles in CD3⁺CD4⁺ cells isolated from draining lymph nodes of WT and XPC^{−/−} mice. We observed that IFN- γ -producing Th1 and IL-4-producing Th2 cells were comparable between genotypes, whereas IL-17A⁺ Th17 cells were reduced in XPC^{−/−} mice while in IL-10-producing CD4⁺ T cells were increased (Fig. 1h–k). At the transcription-factor level, ROR γ t⁺ CD4⁺ Th17 cells resemble the cytokine defect with a reduction in XPC^{−/−} mice, while Foxp3⁺ Treg were expanded, suggesting a shift toward a regulatory phenotype with selective impairment of the Th17 program (Fig. 1l, m).

To further investigate whether XPC deficiency affects in vitro T helper cell differentiation, we isolated CD4⁺CD62L^{high} T cells from the lymph nodes and spleens of both WT and XPC^{−/−} mice and cultured them under Th1, Th2, Treg, and Th17 cell polarizing conditions for five days. Flow cytometry analysis showed that the deficiency of XPC did not affect the differentiation of Th1, Th2 and Treg cells (Supplementary Fig. 1A, B, D). However, the in vitro differentiation of Th17 cells was significantly impaired in the absence of XPC, as the frequency of IL-17A-producing cells was notably lower compared to WT mice (Supplementary Fig. 1C). This reduction in Th17 differentiation was associated with diminished mRNA expression of *Rorc*, *Stat3*, and *Il17a*, as shown by qPCR analysis (Supplementary Figs. 1E–G).

In summary, while XPC deficiency does not impact the overall differentiation of CD4⁺ or CD8⁺ T cells, nor the development of Th1 or Th2 cells, it significantly impairs the generation of IL-17A-producing Th17 cells. Interestingly, XPC deficiency leads to an increase in FOXP3⁺ Treg cells. These results suggest a critical role for XPC in the balance between Treg and Th17 populations under steady-state conditions.

Th17 cells accumulate less DNA damage with enhanced XPC expression

Next, we investigated the role of XPC in the DNA damage response across different T cell subsets and explored potential regulatory mechanisms controlling its expression. We first measured the levels of γ H2AX, a marker of DNA damage, across various T helper (Th) cell

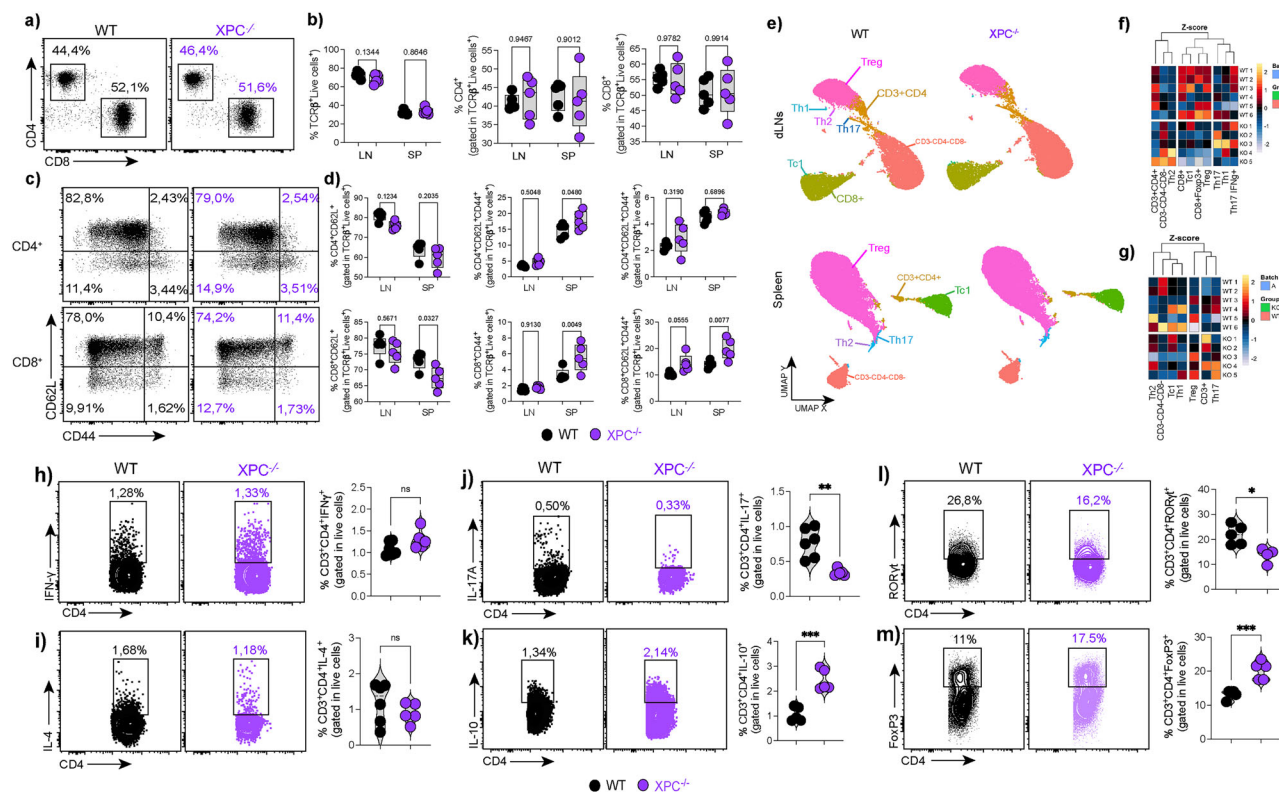


Fig. 1 | XPC deficiency alters CD4⁺ T-cell subset differentiation. **a–b** Flow-cytometric analysis of total CD4⁺ and CD8⁺ T-cell frequencies in draining lymph nodes (LN) and spleen (SP) from WT and XPC^{-/-} mice under steady-state conditions ($n = 5$ per group). Data are shown as box plots, in which the center line represents the median, the bounds of the box indicate the 25th and 75th percentiles, and the whiskers denote minimum and maximum values. Individual dots represent independent biological replicates. Statistical significance was assessed using a two-sided unpaired Student's *t*-test, with exact *P* values indicated. **c–d** Frequencies of naïve (CD62L⁺CD44⁻) and memory (CD62L⁻CD44⁺) subsets within CD4⁺ and CD8⁺ T cells from LN and SP of WT and XPC^{-/-} mice ($n = 5$ per group). Box plots are defined as in (a–b). Statistical analysis was performed using a two-sided unpaired Student's *t*-test, with exact *P* values reported. **e** UMAP projection of flow cytometry data from total CD3⁺ T cells showing comparable overall distribution of major subsets (Th1, Th2, Th17, Treg, Tc1, CD8⁺) between

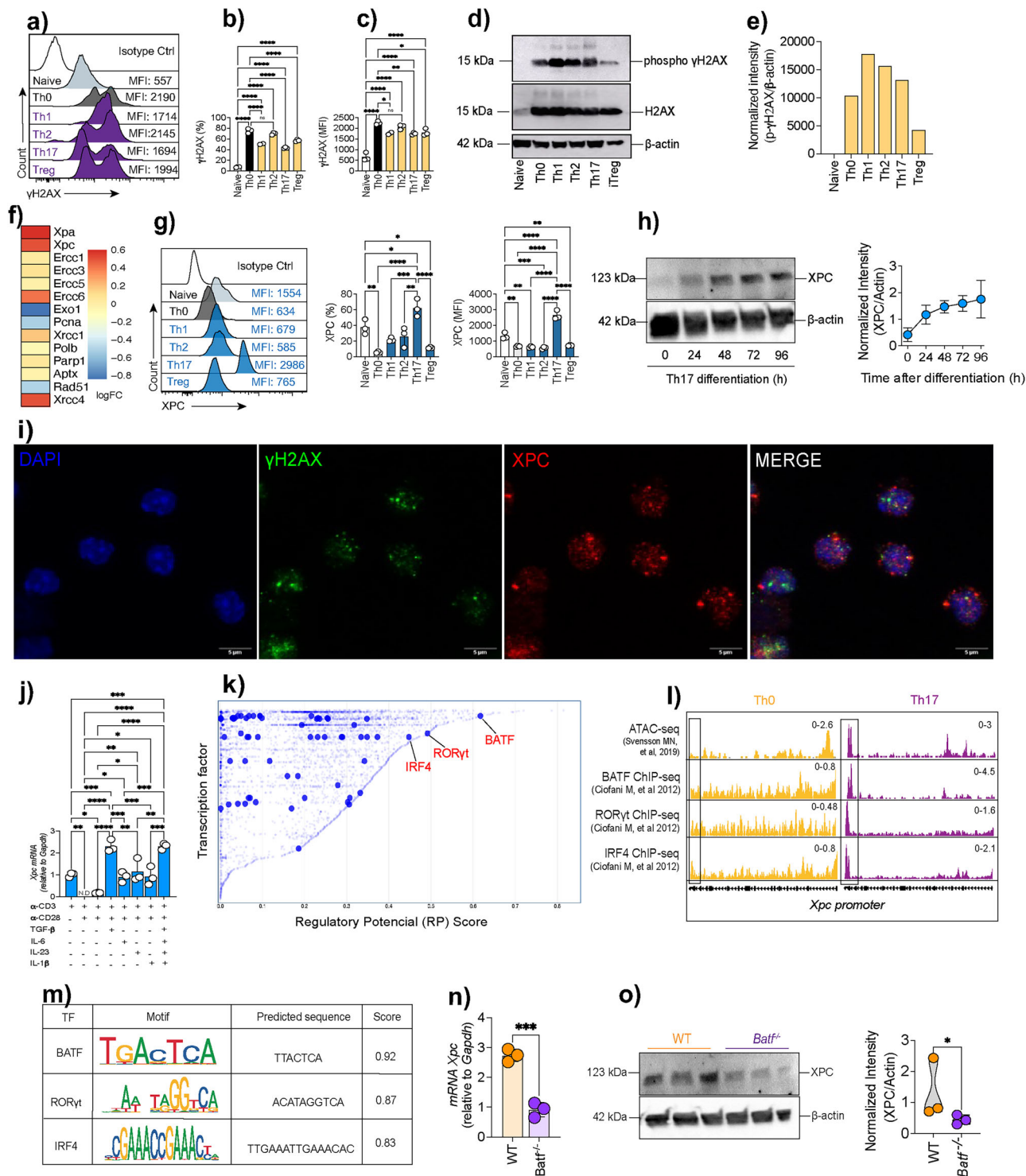
WT and XPC^{-/-} mice in LN and spleen. **f–g** Heatmaps of Z-score-normalized gene expression profiles illustrating similar lineage-specific transcriptional signatures across the two genotypes. Z-scores were calculated per gene across samples. Hierarchical clustering was performed using Euclidean distance and complete linkage. **h–m** Representative flow cytometry plots and quantification of intracellular cytokine and transcription factor staining in CD4⁺ T cells, showing Th1 (IFN- γ), Th2 (IL-4), Th17 (IL-17A, ROR γ t), Treg (Foxp3), and IL-10⁺CD4⁺ populations in WT and XPC^{-/-} mice. Quantitative data are presented as mean $\pm$ SEM, with individual points representing independent biological replicates (WT, $n = 6$; XPC^{-/-}, $n = 5$). Statistical significance was determined using a two-sided unpaired Student's *t*-test. Exact *P* values are shown in the graphs; (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) indicate statistically significant differences. LN, lymph node; SP, spleen. WT samples are shown in black and XPC^{-/-} samples in purple. Data are representative of at least three independent experiments.

subsets and Treg cells using flow cytometry. As shown in Fig. 2a, the naïve T cells displayed the lowest levels of γ H2AX, indicating minimal DNA damage in these resting cells. In contrast, Th0 and Th2 cells showed significantly elevated γ H2AX expression, reflecting higher levels of DNA damage or an active DNA damage response in these subsets. Interestingly, Th1, Th17, and Treg cells exhibited lower γ H2AX levels compared to Th0 cells, though their level was still higher than that of naïve T cells (Fig. 2a–c). Western blot analysis confirmed the presence of γ H2AX across the subsets, further supporting differential activation of the DNA damage response among polarized CD4⁺ T cells (Fig. 2d, e).

We then examined the expression of XPC and other DNA repair genes across Th17 cells compared to non-polarized Th0 cells. Heatmap analysis of log fold changes (*logFC*) showed that Th17 cells had increased expression of several DNA repair genes, including *Xpc*, *Xpa*, *Erccl*, *Erc3*, *Erc5*, *Erc6*, *Xcrcl*, *Polb*, *Parp1*, *Aptx* and *Xcc4* when compared to Th0 cells (Fig. 2f). Consistently, flow cytometry revealed higher XPC abundance in Th17 cells relative to other subsets, and a time-course Western blot corroborated a progressive induction of XPC from 24 h to 96 h of Th17 differentiation (Fig. 2g, h). Immunofluorescence imaging further revealed nuclear co-

localization of XPC with γ H2AX foci, confirming that XPC accumulates at sites of DNA damage in Th17 cells (Fig. 2i).

We next examined the upstream signals and transcriptional regulators that control *Xpc* mRNA expression in Th17 cells. We found that *Xpc* mRNA was induced by anti-CD3 alone or in combination with anti-CD28 and TGF- β , whereas CD28 co-stimulation or individual IL-6, IL-23, or IL-1 β did not increase *Xpc* mRNA. Interestingly, maximal induction occurred only under the full Th17-polarizing condition (anti-CD3 + CD28 + TGF- β + IL-6 + IL-23 + IL-1 β) (Fig. 2j). Next, we used regulatory potential (RP) scores, which rank transcription factors based on their capacity of regulating specific genes. This analysis highlighted several key transcription factors, including BATF, ROR γ t, and IRF4, that exhibited high RP scores and are known to be critical for Th17 differentiation (Fig. 2k). Chromatin immunoprecipitation followed by sequencing (ChIP-seq) reanalyzed from the dataset generated by Ciofani et al., (2012) confirmed the binding of BATF, ROR γ t, and IRF4 to the *Xpc* promoter specifically in Th17 cells, with BATF showing particularly strong enrichment (Fig. 2l). This indicates that these transcription factors likely play a central role in driving *Xpc* expression during Th17 differentiation. Notably, the accessibility of the *Xpc* promoter (as indicated by



ATAC-seq data derived from the dataset generated by Svensson et al., 2019) was significantly higher in Th17 cells compared to non-polarized Th0 cells (Fig. 2l). Motif analysis shows a high affinity binding site of BATF on the *Xpc* promoter. This was supported by the motif scores, which showed BATF as having the highest predicted binding sequence among the transcription factors analyzed, followed by RORγt and IRF4 (Fig. 2m). These results suggest that BATF, directly regulates *Xpc* transcription in Th17 cells. To validate the role of BATF in controlling XPC expression, we examined XPC levels in BATF-deficient (Batf^{-/-}) Th17 cells. qPCR analysis shows a decreased expression of *Xpc* mRNA in Batf^{-/-} Th17 cells (Fig. 2n), which goes in line with western blot analysis showing a

reduction in XPC protein levels in Batf^{-/-} Th17 cells compared to WT controls (Fig. 2o). This suggests that BATF is a critical transcriptional regulator of XPC expression during Th17 differentiation, ensuring that sufficient DNA repair capacity is available to support the proper development of these cells.

The deficiency of XPC impairs the effector program of Th17 cells
Next, we investigate if the impaired IL-17A production by XPC-deficient Th17 cells was related to changes in their effector function. First, we performed transcriptomic analysis by bulk RNA-seq and we found a downregulation of lineage-defining transcription factors (*Rorc*, *Batf*, *Runx1*, *Maf*, *Prdm1*) together with Th17-associated

Fig. 2 | XPC expression and DNA damage response during in vitro T cell differentiation. **a–c** Flow cytometric analysis of γ H2AX in naïve, Th0, Th1, Th2, Th17 and Treg cells showing representative histograms, mean fluorescence intensity (MFI) and quantification across subsets ($n = 3$ technical replicates). Data are presented as mean $\pm$ SEM and analyzed using a two-sided one-way ANOVA followed by Tukey's multiple-comparisons post hoc test; exact P values are indicated in the graphs. **d–e** Immunoblot analysis of γ H2AX in polarized Th subsets, showing a representative blot from a single experiment with densitometric quantification of normalized band intensities (γ H2AX/ β -actin) derived from the same experiment. Data are presented as mean $\pm$ SEM and analyzed using a two-sided one-way ANOVA with Tukey's post hoc test; exact P values are indicated. **f** Heatmap of DNA repair gene expression in Th17 cells displayed as Z-score-normalized values calculated per gene across samples. **g** Flow cytometric quantification of XPC across Th subsets ($n = 3$ technical replicates); box plots show the median, 25th–75th percentiles, and

whiskers indicating minimum and maximum values; two-sided one-way ANOVA with Tukey's post hoc test, exact P values shown. **h** Immunoblot of XPC during Th17 differentiation (0–96 h), representative of a single experiment with densitometry normalized to β -actin from the same experiment; two-sided one-way ANOVA with Tukey's post hoc test, exact P values indicated. **i** Confocal images of Th17 cells stained for γ H2AX (green), XPC (red) and DAPI (blue). Scale bar, 5 μ m. **j–m** qPCR of cytokine-induced *Xpc* ($n = 3$ technical replicates; two-sided one-way ANOVA with Tukey's post hoc test, exact P values shown), regulatory potential scores, ATAC-seq/ChIP-seq tracks and predicted motifs at the *Xpc* promoter. **n–o** qPCR and immunoblot analyses of XPC in WT vs *Batf*^{-/-} Th17 cells ($n = 3$ technical replicates; two-sided unpaired Student's t -test, exact P values shown). Colors indicate cell subsets or genotypes as labeled in each panel. Data are representative of at least three independent experiments unless otherwise indicated. Statistical significance is indicated as follows (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

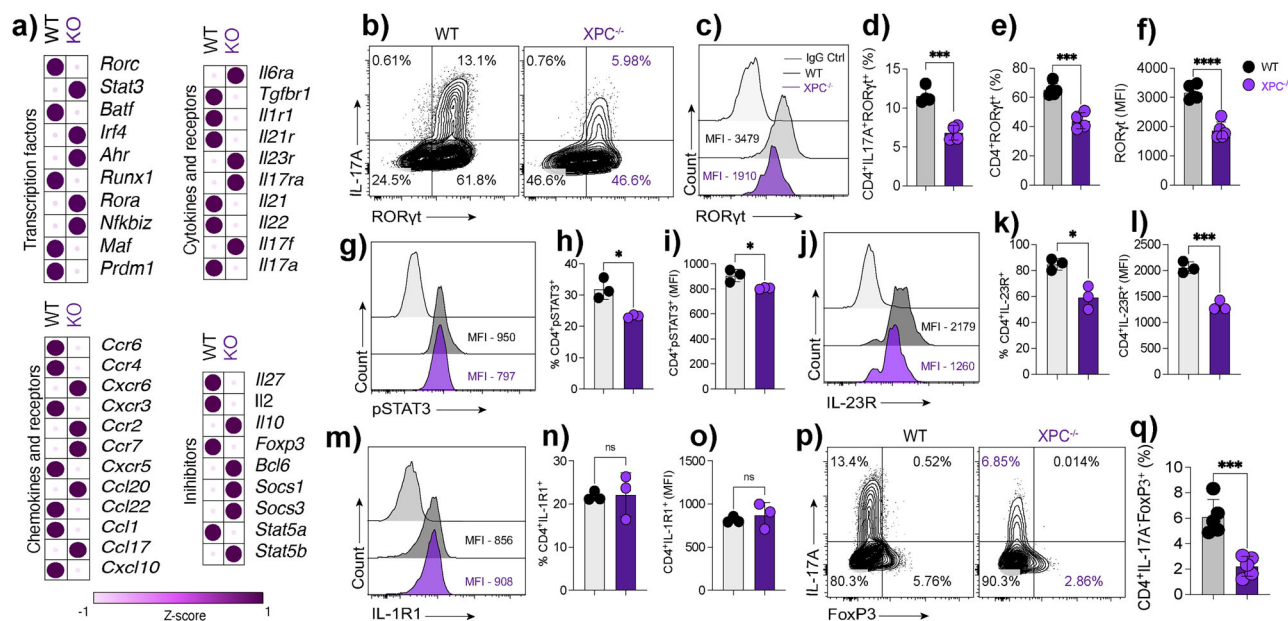


Fig. 3 | Impaired differentiation and altered expression of key signaling molecules in XPC-deficient Th17 cells. **a** Heatmap from bulk RNA-seq of WT and XPC^{-/-} Th17 cells showing genes related to transcriptional regulation, cytokine signaling, and chemokine responses, displayed as Z-score-normalized values calculated per gene across samples. **b–c** Flow cytometry contour plots and histograms showing IL-17A and ROR γ t expression in CD4⁺ T cells from WT and XPC^{-/-} mice, with percentages of IL-17A⁺ROR γ t⁺ and IL-17A⁻ROR γ t⁺ populations and mean fluorescence intensity (MFI) values indicated ($n = 5$ technical replicates). **d–f** Quantification of CD4⁺IL-17A⁺ROR γ t⁺ cells, CD4⁺ROR γ t⁺ cells, and ROR γ t expression (MFI) in WT and XPC^{-/-} mice ($n = 5$ technical replicates). Data are presented as mean $\pm$ SEM and analyzed using a two-sided unpaired Student's t -test, with exact P values indicated. **g–i** Flow cytometric analysis and quantification of phosphorylated STAT3 (pSTAT3) expression in CD4⁺ T cells from WT and XPC^{-/-} mice, shown as percentage of pSTAT3⁺ cells and MFI ($n = 5$ technical replicates). Data are presented as mean $\pm$ SEM and analyzed using a two-sided unpaired Student's t -test,

with exact P values shown. **j–l** Flow cytometry histograms and quantification of IL-23R expression in CD4⁺ T cells from WT and XPC^{-/-} mice, shown as percentage of IL-23R⁺ cells and MFI ($n = 5$ technical replicates). Data are presented as mean $\pm$ SEM and analyzed using a two-sided unpaired Student's t -test, with exact P values indicated. **(m–o)** Flow cytometry histograms and quantification of IL-1R1 expression in CD4⁺ T cells from WT and XPC^{-/-} mice, shown as percentage of IL-1R1⁺ cells and MFI ($n = 5$ technical replicates). Data are presented as mean $\pm$ SEM and analyzed using a two-sided unpaired Student's t -test, with exact P values shown. **p–q** Flow cytometry contour plots and quantification of CD4⁺IL-17A⁺FoxP3⁺ T cells in WT and XPC^{-/-} mice ($n = 5$ technical replicates), presented as mean $\pm$ SEM and analyzed using a two-sided unpaired Student's t -test with exact P values indicated. WT samples are shown in black and XPC^{-/-} samples in purple. Data are representative of at least three independent experiments. Statistical significance is indicated as follows (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

cytokines and receptors (*Tgfb1*, *Il1r1*, *Il21r*, *Il21*, *Il22*, *Il17a*) and chemokine pathways (*Ccr6*, *Ccr4*, *Cx3cr1*, *Cxcr5*, *Ccl22*, *Ccl1*, *Cxcl10*) in XPC-deficient Th17 cells. Conversely, genes linked to regulatory and inhibitory signaling including *Il10*, *Bcl6*, *Socs1*, *Socs3*, and *Stat5b* were upregulated in the absence of XPC (Fig. 3a). The differentiation and function of Th17 cells rely on the activity of ROR γ t, a transcription factor responsible for the expression of lineage signature genes such as *Il17a* and *Il23r*^{4–3}. We observed that the reduction of IL-17A production by XPC-deficient Th17 cells was associated with a significantly decreased expression of ROR γ t (Fig. 3a–f). In addition, these findings go along with a decreased STAT3 phosphorylation,

and a decline in the expression of IL-23R but not IL1R1 in the Th17 cells that developed in the absence of XPC (Fig. 3g–o).

The differentiation of Th17 cell and Tregs share a common signaling pathway involving TGF- β . In this context, low concentrations of TGF- β , in conjunction with IL-6 and IL-21, promote the differentiation of Th17 cells, whereas high concentrations inhibit Th17 cells differentiation and instead promote the expression of FOXP3, leading to Treg differentiation. We investigated if the decreased expression of ROR γ t in XPC-deficient Th17 cells could be related to an increase in FoxP3 expression. Surprisingly, we found that even the expression of FoxP3 was downregulated in XPC^{-/-} Th17 cells (Fig. 3p, q).

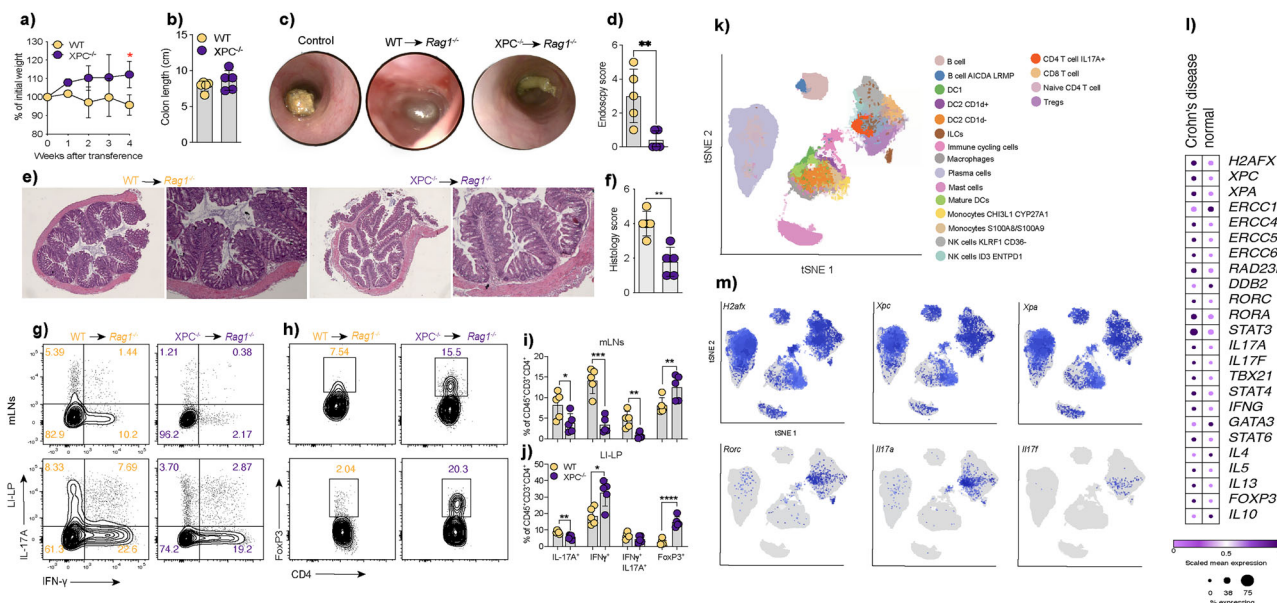


Fig. 4 | Induction of colitis by CD4⁺ T cell transfer from WT and XPC^{-/-} mice into Rag1^{-/-} mice, and single-cell RNA-seq analysis of CD45⁺ cells from Crohn's disease patients. **a** Body weight change, expressed as percentage of initial weight, in Rag1^{-/-} mice receiving CD4⁺CD45RB^{high} T cells from WT or XPC^{-/-} donors, monitored weekly for 4 weeks after transfer ($n = 5$ per group). Data are presented as mean $\pm$ SEM and analyzed using a two-sided two-way ANOVA with repeated measures followed by Tukey's multiple-comparisons post hoc test. **b** Colon length measured at the experimental endpoint in Rag1^{-/-} mice receiving WT or XPC^{-/-} CD4⁺ T cells ($n = 5$ per group). Data are shown as mean $\pm$ SEM and analyzed using a two-sided unpaired Student's *t*-test. **c–f** Representative colonoscopy images, hematoxylin and eosin–stained histological sections, and quantification of endoscopy and histology scores in Rag1^{-/-} mice receiving WT or XPC^{-/-} CD4⁺ T cells ($n = 5$ per group). Quantitative data are shown as individual data points with mean $\pm$ SEM and analyzed using two-sided unpaired Student's *t*-tests with exact *P* values indicated. **g–h** Flow cytometry contour plots showing IFN-γ, IL-17A, and

FoxP3 expression in CD4⁺ T cells isolated from mesenteric lymph nodes (mLNs) and large-intestine lamina propria (LI-LP) of Rag1^{-/-} mice receiving WT or XPC^{-/-} CD4⁺ T cells. **i–j** Quantification of CD4⁺CD45⁺ T cell subsets (IL-17A⁺, IFN-γ⁺, and FoxP3⁺) from mLNs and LI-LP ($n = 5$ per group). Data are presented as mean $\pm$ SEM and analyzed using two-sided unpaired Student's *t*-tests with exact *P* values indicated. **k–m** t-SNE analysis of immune cell populations from colonic tissue of Crohn's disease patients, heatmap showing scaled expression of DNA repair genes, transcription factors, and cytokines, and t-SNE plots displaying expression of selected genes (H2AFX, XPC, XPA, RORC, IL17A). Single-cell RNA-seq data were obtained from the Broad Institute Single Cell Portal (SCP1884) and originally published by Kong et al. (2023). WT samples are shown in yellow and XPC^{-/-} samples in purple. Data are representative of at least three independent experiments unless otherwise indicated. Statistical significance is indicated as follows (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

CD4⁺ T cells lacking XPC exhibit an inability to differentiate into IL-17A⁺/IFN-γ⁺ producing cells and are incapable of inducing T cell-induced colitis in Rag1^{-/-} mice

To investigate the impact of XPC absence on the function and differentiation of CD4⁺ T cells in vivo, we sorted CD4⁺CD45RB^{high} naive T cells from both WT and XPC^{-/-} mice and transferred them into Rag1^{-/-} mice. Subsequently, we monitored the development of colitis. Conversely to Rag1^{-/-} mice receiving WT CD4⁺ T cells, those that received XPC^{-/-} CD4⁺ T cells did not exhibit weight loss or colon shortening and displayed reduced colon inflammation. Endoscopy analysis revealed fewer signs of granularity, fibrin, and diarrhetic stool in Rag1^{-/-} mice that received XPC-deficient CD4⁺ T cells (Fig. 4a–f).

Subsequently, we assessed cytokine production by WT and XPC-deficient CD4⁺ T cells in the mesenteric lymph nodes (mLNs) and colonic lamina propria (cLP) of Rag1^{-/-} mice four weeks post-T cell transfer. The frequency of TCR-β⁺CD4⁺IL17A⁺ and TCR-β⁺CD4⁺IL17A⁺IFN-γ⁺ producing cells were significantly diminished in mice transferred with XPC-deficient CD4⁺ T cells compared to those receiving WT CD4⁺ T cells (Fig. 4g–j). Furthermore, the frequency of TCR-β⁺CD4⁺FOXP3⁺ T cells was increased in mice transferred with XPC-deficient CD4⁺ T cells compared to Rag1^{-/-} mice receiving WT ones (Fig. 4h–j).

Next, we analyzed single-cell data obtained from Crohn's disease (CD) patients, accessible through the Single Cell portal at the Broad Institute (SCP1884) and obtained from the study of Kong et al.²⁴, in order to understand if Th17 cells from CD patients express genes

related to DNA damage and the NER DNA repair pathway including XPC. In this dataset, CD45⁺ cells were isolated from colon biopsies of both healthy controls and individuals with CD, and then subjected to single-cell sequencing. CD45⁺ cells derived from CD patients displayed heightened expression levels of DNA repair genes, including *XPA*, *XPC*, *ERCC4*, *ERCC6* and *RAD23B*. Th17 cells from CD patients exhibited increased expression of XPC alongside with the classical genes of its signature, *RORC*, *STAT3*, *IL17A* and *IL17F*, suggesting a potential role for XPC in the differentiation of human Th17 cells during the course of CD (Fig. 4k–m).

The deficiency of XPC alters the expression of genes associated with DNA repair pathways in Th17

To unravel the mechanism by which XPC influences Th17 cell differentiation, we next analyze our bulk RNA-seq on both WT and XPC-deficient Th17 cells. Remarkably, our analysis identified 1758 genes that were significantly downregulated and 1447 genes that were upregulated in XPC-deficient Th17 cells (Fig. 5a). Gene ontology analysis unveiled an enrichment of genes associated with various pathways, prominently including DNA repair and DNA replication, which are important for DNA processing and repair (Fig. 5b). Based on the $\log_2$ FC, we rank the top differentially expressed genes (DEGs), and we observed that genes such as *Exo1* involved in the BER DNA repair pathway was significant downregulated, and the gene *Txnip* that encodes the protein Thioredoxin-interacting protein (TXNIP) and is involved in the inhibition of antioxidant response²⁵ was significant

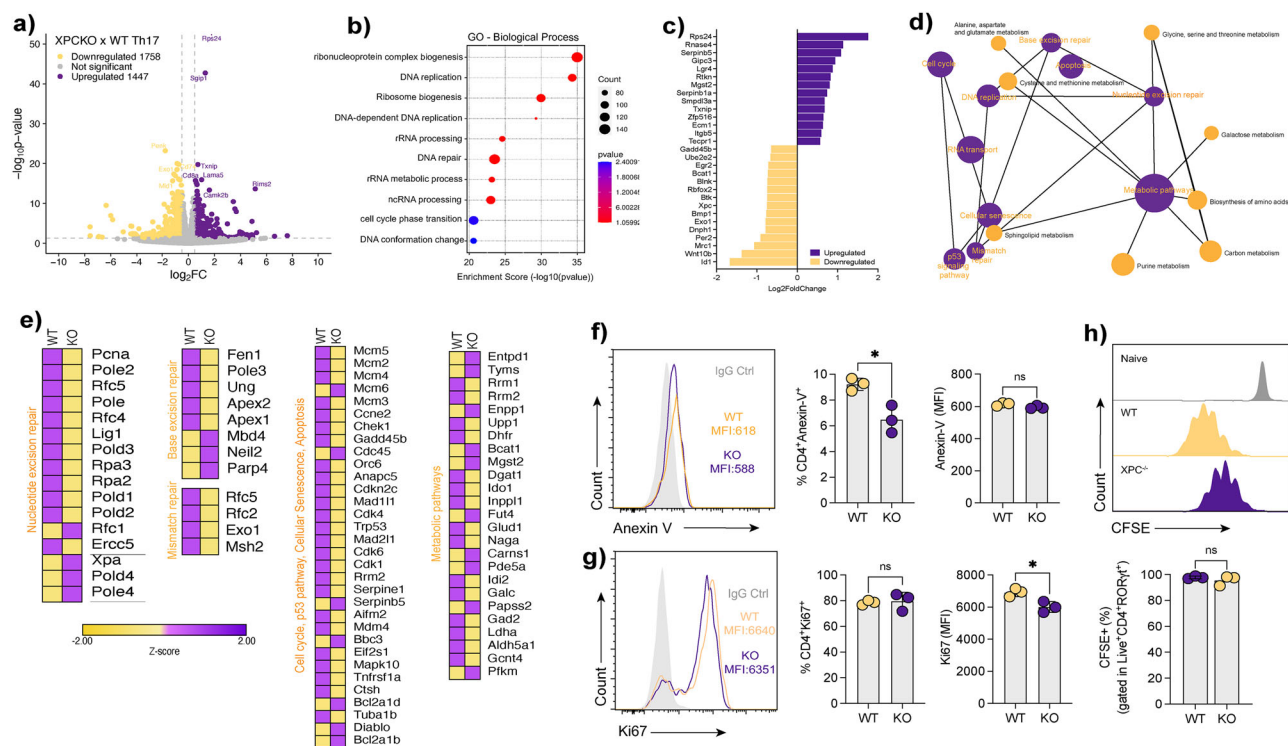


Fig. 5 | XPC deficiency induces coordinated transcriptional changes linking DNA repair, metabolism, and proliferation in Th17 cells. **a** Volcano plot showing differentially expressed genes (DEGs) between XPC^{-/-} and WT Th17 cells. Genes upregulated in XPC^{-/-} cells are highlighted in yellow, and downregulated genes in purple. Differential expression was determined using an adjusted *P* value threshold as described in Methods. **b** Gene Ontology (GO) enrichment analysis of DEGs, showing biological processes enriched in XPC^{-/-} Th17 cells. Dot size represents the number of genes per category, and color indicates adjusted *P* values. **c** Bar plot showing selected differentially expressed genes ranked by log₂ fold change (log₂FC) between XPC^{-/-} and WT Th17 cells. **d** Network map illustrating interactions between metabolic and DNA repair pathways enriched in XPC^{-/-} Th17 cells. Nodes represent pathways, and edges indicate functional associations, highlighting cell cycle, DNA repair, and metabolism. **e** Heatmaps displaying Z-score-normalized expression of genes involved in nucleotide excision repair, base excision repair, mismatch repair, cell cycle, cellular senescence, and metabolic pathways in WT and

XPC^{-/-} Th17 cells. Z-scores were calculated per gene across samples. **f** Flow cytometry histogram and quantification of Annexin V staining in WT and XPC^{-/-} Th17 cells. Data are presented as mean ± SEM with individual technical replicates (n = 3) and analyzed using a two-sided unpaired Student's *t*-test, with exact *P* values indicated. **g** Flow cytometry histogram and quantification of Ki-67 expression in WT and XPC^{-/-} Th17 cells, shown as mean ± SEM (n = 3 technical replicates) and analyzed using a two-sided unpaired Student's *t*-test with exact *P* values indicated. **h** Flow cytometry analysis of CFSE dilution in WT and XPC^{-/-} Th17 cells showing proliferative capacity. Quantification represents the percentage of CFSE⁺ cells gated on live CD4⁺RORγ⁺ Th17 cells. WT samples are shown in yellow and XPC^{-/-} samples in purple. Data are presented as mean ± SEM (n = 3 technical replicates) and analyzed using a two-sided unpaired Student's *t*-test with exact *P* values indicated. Data are representative of at least three independent experiments. Statistical significance is indicated as follows (**p* < 0.05, ***p* < 0.01, ****p* < 0.001).

upregulated in Th17 cells lacking XPC (Fig. 5c). The gene enrichment network illustrated interconnected and enriched genes from pathways such as BER, NER, and mismatch repair, intertwined with pathways related to cell cycle, cellular senescence, and metabolic processes (Fig. 5d).

Upon detailed examination of the altered genes within these pathways, a notable reduction in the expression of several genes associated with the NER pathway was observed, including *Pcna*, *Lig1*, *Pole*, and *Ercc5* (Fig. 5e). Furthermore, downregulation extended to genes linked with cell cycle, p53 pathway, and cellular senescence. Conversely, genes associated with resistance to apoptosis, such as *Bcl2a1d* and *Bcl2a1b*, exhibited upregulation in the absence of XPC (Supplementary Fig. 2A). The absence of XPC in Th17 cells is associated with the downregulation of genes associated with various metabolic pathways, such as *Dgat1* and *Ldha*, while showing an upregulation of genes like *Mgdt2* and *Pd5a*, which are associated with oxidative stress (Fig. 5e) and (Supplementary Fig. 2B–G). Consistent with these findings, flow cytometry showed less Annexin V-positive cells in XPC-deficient Th17 cells. Moreover, Ki-67 expression was reduced despite comparable CFSE dilution between WT and XPC^{-/-} cultures, indicating attenuated cell-cycle activity without detectable differences in cumulative divisions (Fig. 5f–h).

Taken together, these data suggest that XPC sustains transcriptional programs for DNA repair/proliferation and cell-cycle progression in Th17 cells while restraining stress- and oxidative-response pathways.

XPC-deficient Th17 accumulated DNA damage

Given the crucial role of XPC in DNA repair, we hypothesized that its absence might lead to increased DNA damage in Th17 cells, resulting in genomic instability and compromising their differentiation.

In order to understand if Th17 cells accumulates DNA damage in the absence of XPC, we cultured WT and naïve XPC-deficient CD4⁺ T cells under Th17 cells polarizing conditions and measured DNA damage by the expression of the phosphorylated H2AX (γH2AX) and comet assay. Our findings indicated elevated levels of γH2AX and increased DNA damage in XPC-deficient Th17 cells when compared to WT cells (Fig. 6a–c). In addition, alkaline comet assay indicates increased DNA strand breaks as well as increased levels of formamidopyrimidine DNA glycosylase (FPG) sites, which suggest accumulation of oxidized bases in XPC-deficient Th17 cells (Fig. 6d, e).

Following the observation of increased γH2AX in XPC-deficient Th17 cells (Fig. 6a–e), we next investigated how the DNA damage response (DDR) network is transcriptionally modulated in the absence of XPC. Bulk RNA-seq analysis revealed a broad

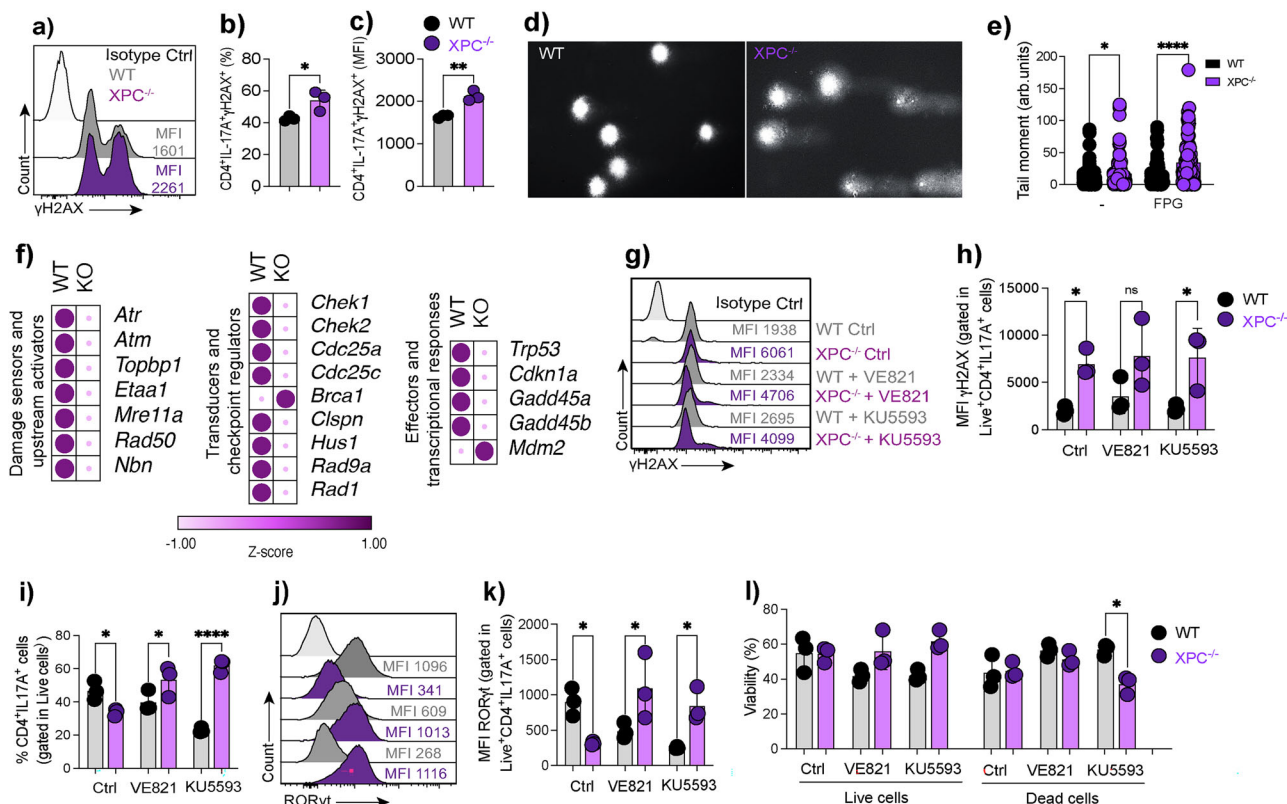


Fig. 6 | Loss of XPC increases DNA damage in Th17 cells. **a** Flow cytometry histogram showing γ H2AX expression in $CD4^+IL-17A^+$ Th17 cells from WT and $XPC^{-/-}$ conditions, with mean fluorescence intensity (MFI) values indicated. **b–c** Quantification of $CD4^+IL-17A^+\gamma$ H2AX $^+$ cells and γ H2AX expression (MFI) in WT and $XPC^{-/-}$ Th17 cells ($n = 3$ technical replicates). Data are presented as mean $\pm$ SEM and analyzed using two-sided unpaired Student's t -tests with exact P values indicated. **d** Representative comet assay images showing DNA damage in $CD4^+$ T cells from WT and $XPC^{-/-}$ conditions. **e** Quantification of tail moment in the comet assay for $CD4^+$ T cells treated with or without formamidopyrimidine DNA glycosylase (FPG) to assess oxidative DNA damage ($n = 5$ technical replicates). Data are shown as mean $\pm$ SEM and analyzed using a two-sided unpaired Student's t -test with exact P values indicated. **f** Heatmap showing Z-score-normalized expression of genes involved in DNA damage sensing and checkpoint signaling in WT and $XPC^{-/-}$ Th17

cells, calculated per gene across samples. **g** Flow cytometry histograms showing γ H2AX expression in WT and $XPC^{-/-}$ Th17 cells following ATR (VE-821) or ATM (KU-55933) inhibition. **h–k** Quantification of γ H2AX and ROR γ t expression (MFI) and frequency of $CD4^+IL-17A^+$ Th17 cells following ATR or ATM inhibition in WT and $XPC^{-/-}$ conditions ($n = 3$ technical replicates). Data are presented as mean $\pm$ SEM and analyzed using two-sided unpaired Student's t -tests with exact P values indicated. **l** Quantification of viable and dead cells following checkpoint inhibition in WT and $XPC^{-/-}$ Th17 cells treated with KU-55933 ($n = 3$ technical replicates). Data are shown as mean $\pm$ SEM and analyzed using a two-sided unpaired Student's t -test with exact P values indicated. WT samples are shown in black and $XPC^{-/-}$ samples in purple. Data are representative of at least three independent experiments unless otherwise indicated. Statistical significance is indicated as follows (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

downregulation of genes involved in DNA-damage sensing and checkpoint activation, including *Atr*, *Atm*, *Tophbp1*, *Etaa1*, *Mre11a*, *Rad50*, and *Nbn*, as well as transducers and cell-cycle regulators such as *Chek1*, *Chek2*, *Cdc25a*, *Clspn*, *Hus1*, *Rad9a*, and *Rad1*. Downstream effector genes associated with p53 signaling and DNA repair, including *Trp53*, *Cdkn1a*, *Gadd45a*, and *Gadd45b*, were also significantly reduced (Fig. 6f).

To determine whether ATR and ATM signaling contribute to the elevated levels of γ H2AX observed in XPC-deficient cells, we pharmacologically inhibited each kinase using VE-821 and KU-55933, respectively. Both treatments led to a further increase in γ H2AX intensity in XPC-deficient Th17 cells, suggesting that in the absence of XPC, ATM/ATR inhibition exacerbates unresolved DNA lesions rather than alleviating them (Fig. 6g, h). Despite this, blockage of ATR or ATM signaling resulted in increased IL-17A and ROR γ t expression in XPC-deficient Th17 cells, partially restoring the Th17 effector phenotype (Fig. 6i, k). This indicates that while ATR and ATM activation restrain Th17 differentiation under conditions of DNA damage, their transient inhibition can relieve this checkpoint-mediated suppression and permit partial reactivation of the lineage program. Cell viability was unaffected by these treatments, with comparable frequencies of live cells across all conditions, although a

modest reduction in cell death was observed following KU-55933 exposure in XPC-deficient cultures (Fig. 6l).

Collectively, these findings demonstrate that loss of XPC leads to the accumulation of oxidative DNA lesions and the downregulation of key DDR components. Under these conditions, ATR/ATM inhibition further enhances DNA-damage accumulation but simultaneously alleviates checkpoint-driven suppression of Th17 differentiation.

XPC deficiency impairs mitochondrial function, increases oxidative stress, and disrupts DNA repair in Th17 cells

Given the marked increase in DNA damage observed in XPC-deficient Th17 cells, we next investigated whether the absence of XPC affects the transcriptional and metabolic programs controlling redox homeostasis. Transcriptomic profiling revealed extensive reprogramming of genes associated with antioxidant defense, energy metabolism, and oxidative stress response. Glycolytic enzymes such as *Hk1*, *Hk2*, *Pfkfb* and *Ldha* were downregulated, whereas genes involved in oxidative phosphorylation, including *Nudfa9*, *Ndufv1*, *Sdh* and *Cox5b* were upregulated. In parallel, genes encoding antioxidant response, such as *Gpx1*, *Prdx1*, *Txn1*, *Txn2*, were decreased, while there was an increase in genes involved in oxidative stress response such as *Nfe2l2*, *Hspa1b*,

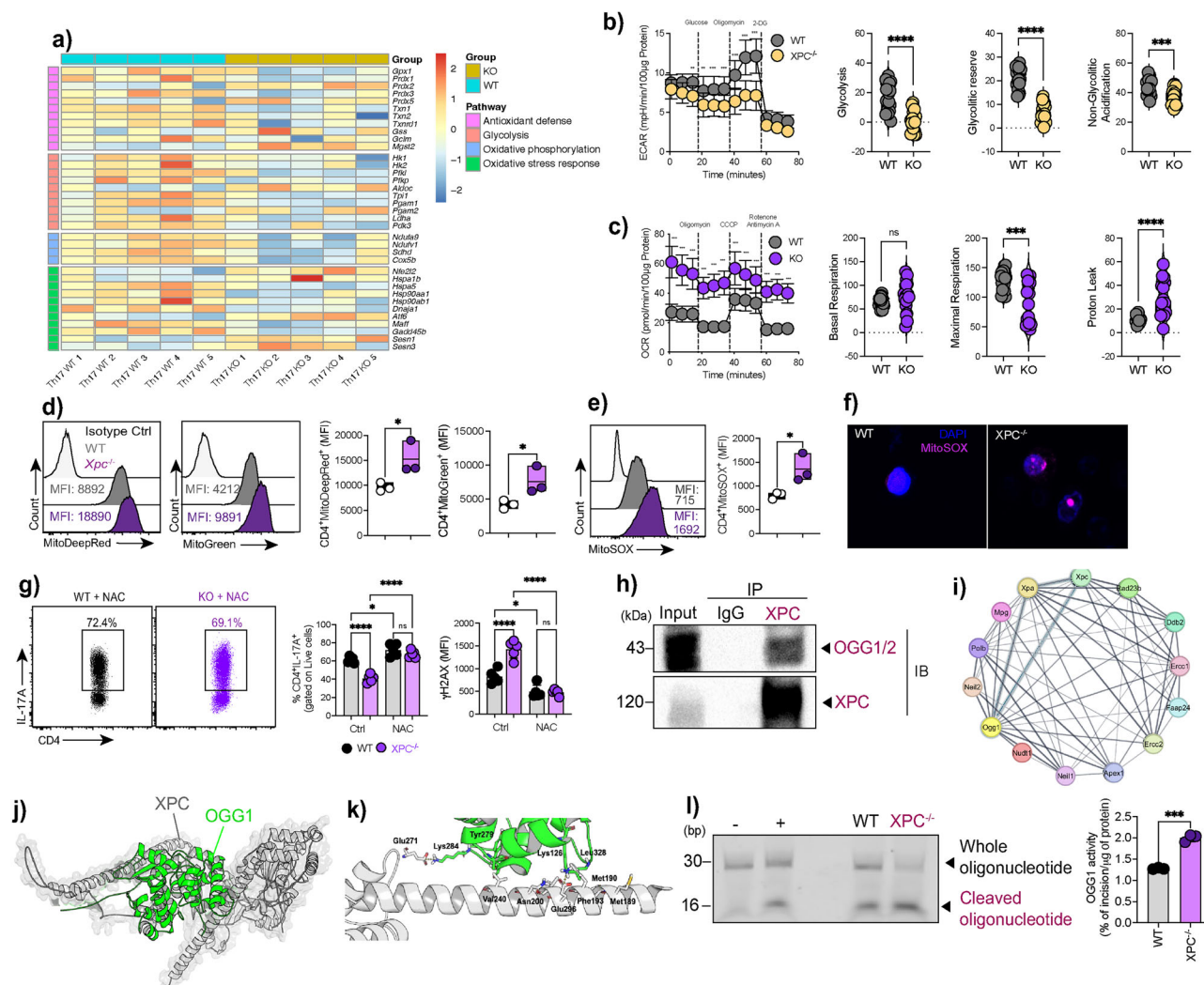


Fig. 7 | Mitochondrial oxidative stress and OGG1-XPC interaction dynamics in Th17 cells. **a** Heatmap showing differentially expressed genes involved in antioxidant defense, glycolysis, oxidative phosphorylation, and oxidative stress responses in WT and XPC^{-/-} Th17 cells, displayed as Z-score-normalized values calculated per gene across samples. **b–c** Seahorse analysis of extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) in WT and XPC^{-/-} Th17 cells, including glycolytic rate, glycolytic reserve, basal respiration, maximal respiration, and proton leak ($n = 10$ technical replicates). Data are presented as mean $\pm$ SEM and analyzed using two-sided unpaired Student's t -tests with exact P values indicated. **d–e** Flow cytometric analysis and quantification of mitochondrial membrane potential (MitoTracker Deep Red), mitochondrial mass (MitoTracker Green), and mitochondrial reactive oxygen species (MitoSOX) in WT and XPC^{-/-} Th17 cells ($n = 3$ technical replicates). Data are shown as mean $\pm$ SEM and analyzed using two-sided unpaired Student's t -tests with exact P values indicated. **f** Representative confocal microscopy images of WT and XPC^{-/-} Th17 cells stained with MitoSOX and DAPI. Scale bar as indicated. **g** Flow cytometric quantification of

IL-17A⁺ CD4⁺ T cells and γ H2AX expression (MFI) following N-acetylcysteine (NAC) treatment in WT and XPC^{-/-} Th17 cultures ($n = 5$ technical replicates). Data are presented as mean $\pm$ SEM and analyzed using a two-sided one-way ANOVA followed by Tukey's multiple-comparisons post hoc test, with exact P values indicated. **h** Co-immunoprecipitation analysis showing interaction between XPC and OGG1 proteins in Th17 cell lysates, with representative immunoblots shown. **i–k** Protein-protein interaction network generated by STRING analysis and molecular docking simulations illustrating predicted functional associations and the interaction interface between XPC and OGG1. **l** Representative OGG1 incision assay using nuclear extracts from WT and XPC^{-/-} Th17 cells, with quantification of cleavage activity expressed as percentage of incision ($n = 3$ technical replicates). Colors indicate cell subsets or genotypes as labeled in each panel. Data are shown as mean $\pm$ SEM and analyzed using a two-sided unpaired Student's t -test with exact P values indicated. Data are representative of at least three independent experiments unless otherwise indicated. Statistical significance is indicated as follows (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Hspa5, *Hsp90aa1*, *Atf6*, *Sesn1*, *Sesn3*, suggesting activation of compensatory oxidative-stress defenses in the absence of XPC (Fig. 7a).

Given the transcriptional enrichment of mitochondrial and redox pathways, we next examined the metabolic profile of Th17 cells using Seahorse extracellular flux analysis. XPC-deficient Th17 cells exhibited markedly reduced glycolytic capacity, glycolytic reserve, and non-glycolytic acidification compared to WT cells (Fig. 7b), indicating impaired glycolytic flux. Oxygen consumption rate (OCR) analysis showed comparable basal respiration between groups, but a significant decrease in maximal respiratory capacity and a marked increase in proton leak in XPC-deficient cells (Fig. 7c). Together, these

findings demonstrate that loss of XPC leads to mitochondrial dysfunction and reduced bioenergetic efficiency, rather than a metabolic shift toward oxidative phosphorylation.

Flow-cytometry analysis revealed increased MitoSOX fluorescence, confirming higher mitochondrial superoxide levels in XPC-deficient cells. In parallel, staining with MitoTracker Deep Red and MitoTracker Green showed preserved mitochondrial mass but increased mitochondrial membrane potential, indicative of hyperpolarized and metabolically active mitochondria (Fig. 7d, e). Confocal microscopy corroborated these findings, revealing an accumulation of MitoSOX signal in XPC-deficient Th17 cells (Fig. 7f). Moreover, BATF-

deficient Th17 cells exhibited a phenotype resembling that of XPC-deficient Th17 cells, characterized by elevated levels of mitochondrial ROS and increased DNA damage (Supplementary Fig. 3A–D).

To determine whether oxidative stress contributes causally to the Th17 defect in the absence of XPC, we treated the cultures with the antioxidant N-acetylcysteine (NAC). NAC treatment markedly restored IL-17A production (Fig. 7g) and pSTAT3 activation, and ROR γ t expression in XPC-deficient Th17 cells (Supplementary Fig. 4A, B). In addition, NAC decreased γ H2AX levels in both WT and XPC-deficient cells (Fig. 7g), linking oxidative damage to the accumulation of DNA breaks and impaired Th17 differentiation. These results demonstrate that redox imbalance represents a major driver of the differentiation defect observed in the absence of XPC.

Because the comet assay incorporating FPG in Fig. 6d, e indicated an accumulation of oxidized bases in XPC-deficient Th17 cells, we next investigated whether XPC interacts with base-excision repair (BER) enzymes, which are responsible for repairing such lesions. Prior studies have shown a functional association between XPC and OGG1¹⁸, the DNA glycosylase that removes 8-oxo-7,8-dihydroguanine (8-oxoG) and initiates BER of oxidative DNA damage. Co-immunoprecipitation experiments confirmed a physical interaction between XPC and OGG1 in Th17 cells (Fig. 7h). Protein–protein network analysis using STRING further positioned XPC and OGG1 within a BER-associated cluster that includes PARP1, APEX1, AND POLB (Fig. 7i). To elucidate the structural basis of this interaction, protein–protein docking simulations were performed using the ClusPro and HADDOCK 2.4 servers, guided by the interaction partners identified in the network analysis. Two interaction configurations were explored: a scaffolded OGG1–XPA–XPC complex and a direct OGG1–XPC complex. Docking simulations yielded Z-scores of 1.68 and 2.03 for OGG1–XPA and OGG1–XPC, respectively, consistent with moderate-to-high confidence interaction scores predicted by STRING (0.53 for OGG1–XPC). Energetic decomposition analysis using PPCheck indicated that the direct OGG1–XPC complex displays a large buried surface area (>1400 Å²) and a favorable solvation free energy (Δ G = −19.7 kcal/mol), corresponding to a total stabilizing energy of approximately −54 kJ/mol, primarily driven by van der Waals and electrostatic forces (Fig. 7j). Structural mapping revealed that the OGG1–XPC interface is distinct from the canonical β -hairpin DNA-binding domains (BHD1–3) of XPC that mediate lesion recognition. Instead, OGG1 interacts with α -helical regions located in the central domain of XPC through a network of salt bridges and hydrophobic contacts. Salt bridges were observed between Asp81 and Asp82 of OGG1 and Arg851 of XPC, while hydrophobic interactions were formed between Leu2, Ala21, and Val85 of OGG1 and aliphatic residues within XPC's α -helical surface. Additional stabilizing contacts included interactions between Lys126 and Lys284 of OGG1 and Glu196 and Glu271 of XPC, as well as aromatic stacking between Tyr279 of OGG1 and Phe193 of XPC (Fig. 7k).

Finally, OGG1 activity was quantified using a duplex oligonucleotide incision assay in which nuclear extracts from in-vitro-polarized Th17 cells (WT or XPC-deficient) were incubated with a 5'-FITC-labeled 30-mer double-stranded oligonucleotide bearing a single 8-oxoG lesion at position 11. Representative gels show a stronger cleaved-product band in extracts from XPC-deficient Th17 cells compared with WT, and quantification across biological replicates confirms a significant increase in incision activity in XPC-deficient Th17 cells (Fig. 7l). These data indicate that BER is functionally upregulated at the OGG1 step in the absence of XPC.

Together, these results demonstrate that XPC integrates redox control and DNA repair during Th17 differentiation. In the absence of XPC, Th17 cells display reduced glycolytic activity and a compensatory dependence on mitochondrial respiration, which is characterized by elevated ROS generation and oxidative DNA lesions. This heightened oxidative burden, together with the loss of XPC–OGG1 coordination in

base excision repair, uncouples oxidative stress signaling from effective DNA repair, thereby constraining Th17 differentiation.

Discussion

T cells are frequently exposed to harmful factors that can threaten genomic stability. These can lead to alterations in their differentiation and impact autoimmunity²⁶. ROS are commonly regarded as detrimental byproducts of cellular metabolism, causing damage to nucleic acids¹⁰. In autoimmune conditions, T cells exhibit abnormal expression of DNA damage response (DDR) genes and proteins, coupled with oxidative stress. These factors have been linked to changes in the differentiation of cells into immunogenic subsets and/or an elevated rate of apoptosis^{27,28}. The increased DNA damage observed in T cells during autoimmunity could also be associated with a compromised DNA repair²⁹. However, it remains unknown how T cells use the DNA repair machinery to remove DNA damage. Here, we define the DNA repair protein XPC as a positive regulator of Th17 cells differentiation and function by coordinating oxidative DNA damage response. The deficiency of XPC leads to compromised Th17 cell differentiation and function, impacting inflammatory responses in vivo. Additionally, we found that the transcription factor BATF promotes XPC expression in Th17 cells, which suggests a potential functional relationship between BATF and XPC in maintaining DNA repair machinery and genomic integrity in Th17 cells.

XPC plays an essential role in initiating GG-NER³⁰. Individuals with inherited mutations in the XPC and other genes of the XP family, including XP-A, XP-B, XP-D, XP-F, XP-G, XP-V, develop a rare disorder named Xeroderma pigmentosum (XP) characterized by heightened vulnerability to skin cancer, attributed to impaired removal of genotoxic dipyrimidine photoproducts induced by solar UV radiation through GG-NER³¹. Growing evidence suggests that XP patients have defects in their immune system, which could explain the vulnerability to skin cancer and other conditions observed in these patients³². XP patients have reduced numbers of CD4⁺ T cells as well as reduced NK lytic activity³². In addition, T cells from XP patients exhibited markedly reduced levels of the antiapoptotic BCL-2 and CD95 molecule compared to non-XP T cells, resulting in the highest BAX/BCL-2 ratio for XP T-cells suggesting a susceptibility to undergo apoptosis³³. On the other hand, some XP patients are susceptible to urinary infections and also display bilateral pneumonia with bacterial and fungal infection³⁴. In our study, we found that Th17 cells that lack XPC display a compromised expression of the main identity proteins ROR γ t, STAT3 and IL-23R, therefore leading to an impaired function, which suggest that XP patients could be more susceptible to these infecting agents due to an impaired Th17 cell differentiation. Th17 cells play a protective role against skin cancers such as squamous cell carcinoma and melanoma, while Treg cells have a pro-tumor function in various types of cancer. Our findings, which show that XPC-deficient mice have an impaired Th17 response along with an increase in Treg cells, may help explain the higher susceptibility to cancer in XP patients. It is possible that the elevated Treg cells within tumors contribute to tumor growth, suggesting that this imbalance between Th17 and Treg cells could be a factor in the increased risk of carcinogenesis in these patients^{35–38}.

The expression of XPC is regulated by a variety of transcription factors that ensure proper DNA repair and genomic stability in different cell types. In Th17 cells, we identified BATF as key regulator of *Xpc* expression. In this sense, BATF emerged as a critical player, with BATF-deficient Th17 cells exhibiting a similar phenotype to XPC deficiency, characterized by increased mitochondrial ROS, DNA damage, and reduced XPC expression. These findings suggest a potential BATF–XPC pathway that plays a central role in DNA repair and maintaining genomic integrity in Th17 cells. BATF is a well-established transcription factor essential for Th17 cell identity and differentiation. BATF-deficient mice show impaired Th17 differentiation and dysregulation of key cytokine genes. BATF directly binds to conserved regulatory elements within the

IL-17A/F locus, as well as to the promoters of *Il17a*, *Il21*, and *Il22*, further underscoring its importance in Th17 cell function³⁹. Nevertheless, BATF deficiency in Th17 cells is associated with a less enrichment of genes related to DNA repair pathways⁴⁰. Therefore, the direct interaction between XPC and BATF further suggests a potential enhancement of BATF-mediated transcriptional functions facilitated by XPC in Th17 cells.

After identifying a helix-distorting lesion, either XPC or blocked RNA polymerase II signal for NER, involving TFIIH to unwind DNA, XPA stabilization, and incision by XPF/ERCC1. Repair includes coordinated synthesis, further incision by XPG, and nick sealing by DNA ligases^{41–43}. The findings from XPC-deficient Th17 cells reveal a significant impact on DNA repair pathways, particularly the NER pathway. Additionally, downregulation extends to genes related to BER and the mismatch repair pathway, along with those associated with the cell cycle, p53 pathway, and cellular senescence. This comprehensive downregulation could explain the compromised differentiation and function of XPC-deficient Th17 cells due to impaired DNA repair. These results suggest that XPC may have an essential role in preserving genomic stability in Th17 cells by ensuring effective DNA repair.

In Th17 cells, the deficiency of STIM-1 and MINK1 is associated with the accumulation of ROS, DNA damage, cell death and genomic instability^{13,14}. A similar phenotype was observed in XPC-deficient Th17 cells with increased DNA damage, oxidative stress and altered cellular metabolism. ROS, including hydroxyl radicals ($\cdot\text{OH}$), can react with DNA bases. These modifications can disrupt the typical structure of DNA, potentially causing mutations and compromising genomic integrity^{44,45}. When DNA bases undergo oxidation, they can interfere with transcription processes. Oxidized bases may affect the progression of RNA polymerase during transcription, leading to transcriptional errors or even transcriptional arrest^{46,47}. Nevertheless, fibroblasts exhibiting reduced XPC protein expression undergo a shift in the use of oxidative phosphorylation complexes (OXPHOS), associated with increased nuclear DNA damage and mitochondrial generation of H_2O_2 ¹⁷. These results align with our findings that XPC-deficient Th17 cells show increased *Nox-1* expression and metabolic changes, including reduced glycolysis and increased OXPHOS. The altered mitochondrial membrane potential, elevated mitochondrial ROS levels, and reduced glycolytic capacity, together with decreased maximal respiration and increased proton leak, indicate mitochondrial dysfunction and bioenergetic inefficiency rather than a metabolic shift toward oxidative phosphorylation. These alterations, along with the accumulation of DNA damage and oxidized bases, may underlie genome instability and impaired differentiation and function of Th17 cells lacking XPC.

The repair of oxidized bases involves intricate pathways such as BER¹⁶ and NER¹⁹. In line with this, we have identified a physical interaction between XPC and OGG1 in Th17 cells, suggesting a potential link between these proteins. These results are consistent with previous findings that keratinocytes and fibroblasts that lack XPC display a reduction in the repair efficiency of 8-OH-Gua. In addition, using recombinant proteins, the XPC-HR23B complex stimulates the activity of the DNA glycosylase, OGG1¹⁸. Unexpectedly, we observed that OGG1 incision activity was increased in XPC-deficient Th17 cells. This paradoxical effect suggest a compensatory response to the accumulation of oxidative lesions and elevated mitochondrial ROS observed in the absence of XPC (Figs. 6d, e and 7d–g). The sustained oxidative environment and excess of damaged substrates may therefore drive hyperactivation of the BER pathway, reflected by increased OGG1 activity.

This compensatory activation of OGG1 in the absence of XPC likely contributes to the maintenance of cell viability observed in XPC-deficient Th17 cells (Fig. 5f), despite the increased burden of oxidative lesions. However, this response appears to come at the cost of proper lineage specification. Notably, our assays were performed at late stages of Th17 differentiation (96 h), when cells have already undergone

extensive proliferation and metabolic remodeling. It is therefore plausible that defective DNA repair and elevated ROS during early activation phases compromise the transcriptional capacity required for Th17 polarization. Persistent oxidative stress, checkpoint activation, and a reduction in glycolysis coupled with increased dependence on OXPHOS under reduced respiratory efficiency create an environment incompatible with coordinated STAT3–ROR γ t function. In this context, heightened BER activity may preserve genome integrity and survival but fails to reestablish redox and signaling balance, ultimately impairing IL-17A production. Consistent with a primary role for redox imbalance, antioxidant treatment with NAC in XPC-deficient Th17 cells reduced DNA damage and restored IL-17A production together with ROR γ t expression and STAT3 phosphorylation (Fig. 7g). Collectively, our data support a model in which XPC integrates redox homeostasis with DNA repair to sustain both survival and differentiation.

Besides its function as a DNA damage sensor, XPC also functions as a transcription regulator by interacting with E2F1 and promoting binding to its DNA element⁴⁸. The transcription factor E2F1 is a central player involved in cell cycle progression, DNA-damage response, and apoptosis⁴⁹ and its target genes include *Ogg1* and *Mutyh*. In addition, E2F1 is a protective factor against oxidative stress⁵⁰. XPC is required for efficient RNA synthesis, optimal DNA demethylation, and histone posttranslational modifications (H3K4/H3K9 methylation, H3K9/14 acetylation)⁵¹. Thus, the observed decrease in DNA repair and antioxidant response gene expression in Th17 cells lacking XPC suggests that XPC's transcriptional regulatory role plays a crucial part in modulating the cellular response to oxidative damage.

In summary, our study reveals a major role of XPC in maintaining genomic stability and influencing Th17 cell differentiation. Our findings demonstrate the role of XPC in Th17 biology, linking DNA repair, metabolic state, and effector function. Given the importance of Th17 responses in autoimmunity and host defense, modulation of XPC-related pathways may provide a rationale for therapeutic intervention in Th17-driven disorders.

Methods

Ethics statement

All experimental procedures were conducted in accordance with relevant ethical regulations. Animal experiments were approved by the Ethics Committee on Animal Experimentation of the University of São Paulo (CEUA-ICB, protocol 35881860349) and performed in compliance with the principles of the Brazilian College of Animal Experimentation (COBEA). Male wild-type C57BL/6, XPC^{-/-}, and BATF^{-/-} mice aged 6–8 weeks and weighing 20–25 g were used in this study. All efforts were made to minimize animal suffering and to reduce the number of animals used. Human single-cell RNA-seq data from Crohn's disease patients analyzed in this study were obtained from a publicly available dataset deposited in the Broad Institute Single Cell Portal (SCP1884) and were originally published by Kong et al. (2023). The use of these data complies with the ethical approvals, informed consent procedures, and data-sharing policies described in the original publication. No new human samples were collected for this study.

Animals

Male wild-type C57BL/6, XPC^{-/-} and BATF^{-/-} mice aged 6–8 weeks and weighing 20–25 grams were used. All procedures were performed in accordance with the principles proposed by the Brazilian College of Animal Experimentation (COBEA) and approved by the Ethics Committee on Animal Experimentation of the University of São Paulo (CEUA ICB: 35881860349).

In vitro T helper cells polarization

In vitro T helper cells polarization was performed by isolating naïve CD4⁺ T cells from the spleen and lymph nodes of WT and XPC^{-/-} mice using microbeads coated with anti-CD4 (clone L3T4, cat 130-117-043,

Miltenyi Biotec, Bergisch Gladbach, Germany). The cells were then stained with fluorochrome-conjugated anti-CD4, anti-CD44, and anti-CD62L antibodies, and CD4⁺CD44^{low}CD62L^{high} cells were isolated by sorting (FACS Aria III sorter, BD Biosciences). For the differentiation, the CD4 T cells obtained from the sorting were placed in 48-well plates coated with anti-CD3 (2 µg/mL - BD Bioscience Cat. 553058, Clone - 145-2C11) anti-CD28 (1 µg/mL - BD Bioscience Cat. 553294, Clone: 37.51 RUO), and subsequently stimulated with a cocktail of cytokines and neutralizing antibodies. Th17: 2.5 ng/mL of TGF-β (R&D - Cat. 7666MB), 50 ng/mL of IL-6 (PeproTech - Cat. 216-16), 50 ng/mL of IL-23 (R&D, Cat. 1887-ML), 20 ng/mL of IL-1β (PeproTech, Cat. 211-11B) and 1 µg/mL of anti-IFNγ (BD Bioscience, Cat. 554408), anti-IL-4 (BD Bioscience, Cat. 554432, Clone: 11B11) and anti-IL-2 (BD Bioscience, Cat. 554424, Clone: JE56-1A12). Th1: 20 ng/mL of IL-12 (Cat. 212-12, Peprotech) and 1 µg/mL anti-IL-4 (Cat. 554432, BD Bioscience). Th2: 10 ng/mL of IL-4 (Cat. 214-14, Peprotech) and 1 µg/mL of anti-IFNγ (BD Bioscience, Cat. 554408). iTreg: 10 ng/mL TGF-β (R&D - Cat. 7666MB) and 1 µg/mL of anti-IFNγ (BD Bioscience, Cat. 554408).

Flow cytometry (FACS)

Quantitative and phenotypic analysis of cells differentiated in vitro or isolated from lymph nodes, spleen, or intestinal lamina propria was performed by flow cytometry. Briefly, after being isolated, cells were counted and evaluated for viability with trypan blue. Subsequently, the cells were plated in a 96-well plate and stimulated with phorbol myristate acetate (PMA) (50 ng/mL; Sigma Aldrich) and ionomycin (500 ng/mL; Sigma Aldrich), and brefeldin A (transport inhibitor of golgi - 1.5 µL/mL; BD Biosciences), for 4 h in an oven at 37 °C (5% CO₂ and 8% humidity). The number of cells used were 5 × 10⁵/cells per well for experiments derived from in vitro culture of lymphocytes or 2 × 10⁶/cells per well for in vivo experiments. Then, the cells were washed twice with PBS 1x and centrifuged at 450 g for 5 min and incubated for 15 min with Fc block (1:200) to block Fc receptors, and subsequently washed with PBS 1x, centrifuged at 450 g per 5 and stained with cell viability marker (Fixable viability dye; 1:1000; eBioscience) and antibodies against extracellular markers for 10 min at room temperature (Table II). Then, the cells were centrifuged and fixed using the kit's fixative (transcription factor staining buffer set, eBioscience) for 60 min at room temperature, with subsequent centrifugation. The next step was intracellular labeling with antibodies listed. Briefly, the antibodies were diluted in the kit's permeabilizer, and the cells were incubated with permeabilization buffer containing the antibody mix for 60 min at 4 °C. The following antibodies were used: anti-mouse CD4 (BioLegend, Cat#100432, clone GK1.5, 1:1000), anti-mouse CD62L (BioLegend, Cat#104405, clone MEL-14, 1:200), anti-mouse CD45 (BioLegend, Cat#103138, clone 30-F11, 1:300), anti-mouse TCRβ (BioLegend, Cat#109212, clone H57-597, 1:1000), anti-mouse IL-17A (eBioscience, Cat#11-7177, clone eBio17B7, 1:200), anti-mouse IFNγ (eBioscience, Cat#25-7311, clone XMGL2, 1:400), anti-mouse RORγt (BD Biosciences, Cat#562894, clone Q31-378, 1:800), anti-mouse FOXP3 (eBioscience, Cat#17-5773, clone FJK-16s, 1:400), anti-mouse IL-1R1 (BD Biosciences, Cat#557489, clone 35F5, 1:200), anti-mouse IL-23R (BD Biosciences, Cat#562497, clone O78-1208, 1:200), and anti-mouse phospho-γH2AX (Ser139) (BD Biosciences, Cat#613411, clone 2F3, 1:200). After this step, the cells were centrifuged again and resuspended in PBS for acquisition by flow cytometry (FACS Canto II, BD Biosciences). In this step, between 100,000 and 250,000 events were acquired according to the experiment. Sample analyses were performed using the FlowJo X software (BD Biosciences).

Real-time PCR

To assess gene expression in Th17 T cell cultures, cells were harvested and total RNA was extracted using the RNA Extraction Kit (Promega), according to the manufacturer's instructions. RNA

concentration and purity were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific). For cDNA synthesis, 500 ng of total RNA were reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific), following the manufacturer's protocol. Quantitative real-time PCR was performed using SYBR Green Master Mix (Thermo Fisher Scientific), gene-specific primers, and cDNA templates on a StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). Relative gene expression was calculated using the comparative cycle threshold (Ct) method, with normalization to Gapdh expression and fold-change determination using the 2^{-ΔΔCt} method. Primer specificity was confirmed by the absence of amplification in no-template controls and by the presence of a single peak in melting curve analyses, and all primer pairs exhibited amplification efficiencies greater than 90%. The primer sequences used in this study were as follows (5'–3'): Xpc forward GGTATTGTCGTGGA-GAAGCAGTC and reverse CACGGTTAGAGAAGCCTTTCACC; Il17a forward GCTCCAGAAGGCCCTCAG and reverse CTTTCCCTCCGATTGACA; Rorc (Roryt) forward GAGTTTGC-CAAGCGGCTTT and reverse TCCATTGCTCCTGCTTTCAGTACA; Stat3 forward AGGAGTCTACAACGGCAGCCT and reverse GTGGTACACCTCAGTCTCGAA.

RNA-seq analysis

Total RNA extraction from five independent biological replicates of each Th17 WT and KO cells was performed using TRIzol reagent (Life Technologies, Carlsbad, CA, United States) following manufacturer instructions. After, RNA samples were treated with DNase I (1 U/µg of RNA) (Thermo Scientific, Lithuania, EU) at 37 °C for 15 min. The absence of DNA contamination was determined from the A260/A280 ratio using a spectrophotometer Nanodrop ND1000 (Thermo Scientific, United States). RNA integrity was evaluated using an Agilent 2,100 Bioanalyzer and a Pico Agilent RNA 6000 kit (Agilent Technologies, Santa Clara, CA, United States). rRNA depletion was performed using Ribo-Zero Human/rat/mice Ribo-Zero plus rRNA Depletion Kit (Illumina). Library preparation was performed using 1 µg of rRNA-depleted total RNA using Truseq Stranded Total RNA LT Sample Prep Gold kit, without molecular barcodes. Sample preparation followed preparation using the NovaSeq 6000 S4 Reagent Kit (Illumina) according to manufacturer's instructions for RNA sequencing submission. The sequencing was performed with paired-ends (100 bp) using the Illumina NovaSeq 6000 Platform in the Center for Cell-Based Therapy Facility at Ribeirao Preto Medical School Ribeirão Preto. The raw data was deposited at Sequence read archive - SRA under the accession code: PRJNA1160718.

Western blot

To evaluate XPC protein expression, samples were collected in RIPA buffer (Sigma Aldrich), homogenized with protease, centrifuged, and the supernatant collected. Then, protein quantification was performed using the Bicinchoninic acid protein assay (BCA; Sigma Aldrich). For the experiments with samples of CD4 T cells, 30 µg of proteins were used. Sample preparation was performed with 2x Laemmli sample buffer (Bio-rad), and heated for 10 min at 95 °C for protein denaturation. Subsequently, the samples were applied to a 12% gradient polyacrylamide gel (Bio-rad) and, after separation, were transferred to 0.2 µm nitrocellulose membranes (Bio-rad), through the Trans-Blot Turbo transfer system (Bio-rad). Then, the membranes were blocked in TBST (TRIS-HCl 100 mM pH 7.5, NaCl 150 mM, Tween20 0.05%) with 5% milk (Cell Signaling) to reduce nonspecific binding, washed with TBST and incubated with the primary antibodies diluted in 5% TBST BSA (Sigma Aldrich), overnight, at 4 °C under slow agitation. Then, the membranes were washed and secondary antibodies (anti-rabbit XPC Cat. #MA1-23328, Thermo

Scientific) and/or anti-Phospho-Histone H2A.X (Ser139) Rabbit Monoclonal Antibody (Clone: 20E3, Cat. 9718 T, Cell Signaling Technology) were added in TBST with 5% milk (Cell Signaling), for 2 h at room temperature and slow stirring. Subsequently, the membranes were washed for 30 min with TBST and the substrate Lumina (Millipore) was added for chemiluminescence detection, using the ChemiDoc™ XRS equipment (Bio-rad) and analyzed with the Image Lab 3.0 program (Bio-rad). An anti-beta actin antibody (#4970S Cell Signaling) was used as a protein loading control.

Immunofluorescence

Naïve CD4⁺ T cells (CD4⁺CD62L⁺CD44⁻) from WT mice were purified by negative selection, polarized to Th17, and harvested at 96 h. Cells ($2-3 \times 10^5$) were adhered to poly-L-lysine-coated #1.5H coverslips, fixed in 4% PFA (10 min, RT), quenched with 50 mM NH₄Cl, permeabilized with 0.1% Triton X-100 (5 min), and blocked in 3% BSA/5% normal serum (45–60 min). Primary antibodies against XPC and γH2AX were applied overnight (4 °C) in blocking buffer, followed by Alexa Fluor-conjugated secondary antibodies (1 h, RT), DAPI nuclear stain, and mounting in antifade medium. Imaging was performed on a laser-scanning confocal microscope Leica TCS SP8.

Co-Immunoprecipitation Assay

Immunoprecipitation was performed using the Pierce CO-IP Kit (Thermo Scientific) following the manufacturer's protocol. In this experiment, 1×10^7 cells per IP were used. Briefly, control IgG antibodies (Cat. #2729, Cell Signaling) and mouse anti-XPC (Cat. #MA1-23328, Thermo Scientific) were immobilized using AminoLink Plus coupling resin. Equal amounts of Th17 cell lysates were pre-cleaned and subsequently incubated with the antibody-coupled resin overnight at 4 °C. Subsequently, the resin was washed, and the proteins eluted with an elution buffer. Immunoprecipitated samples were analyzed for expression of OGG1/2 protein (Cat. sc-376935, Santa Cruz Biotechnology) and XPC by immunoblotting.

Model of colitis induced by adoptive transfer of CD4 T cells to Rag1^{-/-} mice

CD4⁺CD45RB^{high} T cells (2.5×10^6) were isolated by sorting from lymph node and spleen samples of WT and XPC^{-/-} mice, and subsequently 5×10^5 cells were injected intraperitoneally into Rag1^{-/-} mice. The mice that received the cells had their weight monitored daily, and in week zero and in the fourth week after the transfer they were anesthetized and submitted to a microendoscopy procedure to evaluate inflammation in the colon. After 4 weeks, the animals were euthanized and the mesenteric lymph nodes (LNM), spleen, and colon were collected for flow cytometry and H&E analyses.

Lamina propria cell isolation

Intestines removed from euthanized animals were opened lengthwise, washed with PBS to remove feces, and cut into pieces approximately 1 cm in size. The pieces were then incubated with RPMI medium containing 3% fetal bovine serum, EDTA, and DTT for 20 min at 37 °C with agitation. After incubation, the pieces of intestine were sieved and washed with a medium containing EDTA to separate the intraepithelial lymphocytes. The solution resulting from the washes, which contained the intraepithelial lymphocytes, was discarded. The remaining pieces were then digested with a medium containing liberase (1/250 of a 25 mg/mL solution, Roche, Germany) and DNase I (Sigma-Aldrich, St. Louis, MO, USA) for 25 min at 37 °C with agitation. After digestion, the solution was passed through a sieve (70 μm), centrifuged, resuspended in PBS + 2% FBS, and passed again through a sieve (40 μm). The lamina propria cells obtained were then marked with monoclonal antibodies for analysis of the marking of surface and intracellular molecules, according to the cell population to be analyzed.

Histological analysis

A large intestinal fragment was removed, fixed in metacarn (60% Methanol, 30% Chloroform, and 10% Glacial Acetic Acid) for 24 h at 4 °C and subsequently transferred to a 70% alcohol solution. The material was sent for mounting in paraffin blocks and the prepared slides were stained with H&E (Hematoxylin & Eosin). Images were obtained using a Nikon microscope at 10x, 20x, and 40x. The histology score analysis was performed according to the guidance for scoring colonic inflammation mediated by disturbed immune cell homeostasis published by Erban et al. (2014).

Alkaline comet assay

The alkaline comet assay is a single-cell electrophoresis analysis performed on days 5 after Th17 of differentiation to detect DNA breaks. The assay was conducted using the commercial kit (Trevigen, Gaithersburg, MD, USA) following the manufacturer protocol. However, the method was performed with few alterations to detect DNA oxidized bases. The DNA was digested with formamidopyrimidine DNA glycosylase (Fpg) enzyme that can identify and remove oxidized purines; this principle is often used in the comet assay detecting oxidized bases by quantifying the DNA strand breaks caused by the incubation with the enzyme (Azqueta, 2019). Briefly, 12 μL of cell suspension (1×10^5 cells per mL) was added to 100 μL of low melting point (0.5%) agarose at 37 °C and the solution was pipetted onto pre-coated CometAssay® Kit slides (Trevigen, Gaithersburg, MD, USA). After solidifying the agarose, slides were immersed in a cold lysis solution (CometAssay Lysis Solution, Trevigen, Gaithersburg, MD, USA) for at least 40 min. After lysis, slides were washed and equilibrated in an enzyme buffer (40 mM HEPES, 100 mM KCl, 0.5 mM EDTA, 0.2 mg/mL BSA; pH 8). Each slide was then treated with 50 μL of enzyme buffer with or without (control) the FPG enzyme (0.12 units per gel—New England Bio Labs Inc., Ipswich, MA, EUA) and incubated in a moist box at 37 °C for 40 min. After the treatment, the slides were set at 4 °C for 30 min and then immersed in an alkaline solution (300 mM NaOH and one mM EDTA, pH 13.0) for 20 min, allowing the unfolding of the DNA structure. Later, it was submitted to an electrophoresis process in an alkaline solution (30 mM NaOH and 1 mM EDTA, pH 13.0) at 300 mA and 4 °C for 30 min. Finally, slides were neutralized with dH₂O for 5 min and fixed in 70% ethanol. DNA staining was done using a SYBR™ Green I Nucleic Acid Gel Stain (Invitrogen Molecular Probes, Eugene, Oregon, USA) solution for 5 min. Slides were analyzed under a Zeiss fluorescence microscope (Axiovert 200) with a 510–560 nm filter and a 590 nm barrier. DNA fragmentation parameters were analyzed using the LUCIA Comet Assay™ software (Laboratory Image, Prague, Czech Republic); 100 comets were scored from each point and the tail moment median was considered.

In vitro measurement of OGG1 activity

OGG1 activity was measured using an oligonucleotide incision assay with fluorescent-labeled oligonucleotides containing a site-specific lesion⁵². The oligonucleotide containing 8-oxoguanine (8-oxoG) at position 11 used in this study, with the following sequence 5'-ATA TAC CGC GG 8-oxoG CCG ATC AAG CTT ATT-3', was obtained from Midland Certified Reagent Company, Midland, TX, USA, and was gel-purified and duplexed as described. The reactions contained 40 mM HEPES-KOH pH 7.4; 5 mM EDTA; 1 mM DTT; 75 mM KCl; 10% glycerol; 0.5 μg of poly(dI-dC) and 200 fmoles of oligonucleotide duplex each, and were incubated at 37 °C for 1 h. The reactions were terminated by adding 1 μL of 10 mg/mL Proteinase K in 5% SDS and incubated at 55 °C for 15 min. NaOH was added to a final concentration of 50 mM along with 1:1 vol of Hi-Di Formamide, then the reactions were incubated at 75 °C for 15 min. The amount of protein extract used in the assay, 35 μg of protein, was determined experimentally. The products were resolved under denaturing conditions (20% acrylamide/bis-acrylamide 19:1, 7 M Urea, 1X-tris-borate-EDTA [Tris base 89 mM, Boric acid

89 mM, EDTA 2 mM]), visualized using a Chemidoc (BioRad) and quantified using ImageJ. As a positive control, we used 50 µg of Whole Cell Extract from C57BL/6 mice temporal cortex.

OGG1–XPC interaction analysis by molecular docking

A protein–protein interaction (PPI) network analysis was initially performed to investigate direct or indirect interactions (mediated by interface proteins) between OGG1 and XPC using STRING v12.0 1 (organism: *Mus musculus*; minimum interaction confidence: 0.4), complemented by an extensive literature review. 2–7 Candidate interactors identified through combined STRING scores and literature evidence were selected for structural characterization relevant to the OGG1–XPC axis. Subsequently, 3D structures of OGG1 (UniProt ID: O08760), XPC (UniProt ID: P51612), and XPA (UniProt ID: Q64267), ERRC1 (UniProt ID: P07903), ERRC2 (UniProt ID: O08811), DDB2 (UniProt ID: Q99J79), APEX1 (UniProt ID: P28352), and RAD23B (UniProt ID: P54728) were obtained from AlphaFold. Structural quality of 3D structures was confirmed using MolProbity, examining parameters including Clashscore (steric clashes per 1000 atoms), percentage of poor rotamers, bond and angle outliers, and residues in favored Ramachandran plot regions. The overall quality of 3D structures was summarized by the MolProbity score in Supplementary Table S1. Subsequently, protein–protein docking simulations were performed using the ClusPro web server 8 with default parameters. Docked complexes were filtered by the “balanced” scoring criterion, and the top-ranking models were further evaluated using the PIZSA 9 and PDBePISA 10 web servers. PIZSA assessed interface stability based on Z-scores calculated at a 4 Å cutoff (1.500 indicates a stable interface). PDBePISA was employed to estimate interface area, Δi G, hydrogen bonds, and salt bridges based on solvent-accessible surface area differences between bound and unbound states. These parameters were used to evaluate the docking-derived complexes; thermodynamic stability and structural complementarity. Further interface residues and interaction energies were analyzed using the PPCheck web server. 11 Finally, molecular visualization, structural inspection, and image generation were done using PyMOL (Schrödinger, LLC).

Statistical analysis

Statistical analyses were performed using GraphPad Prism version 10.2.0 (GraphPad Software, San Diego, USA) and R version 4.3.2. Data are presented as mean $\pm$ s.e.m. unless stated otherwise. Normality was assessed using the Shapiro–Wilk test. Comparisons between two groups were made using a two-tailed unpaired Student's *t*-test, while comparisons among multiple groups were performed using one-way ANOVA followed by Tukey's post hoc test. Each data point represents an independent biological replicate, obtained from distinct animals or cell preparations. At least three independent experiments were performed for all analyses. For flow cytometry, qPCR, and Western blot quantifications, *n* values and statistical tests are indicated in the respective figure legends. No statistical methods were used to pre-determine sample size, and data collection and analysis were not randomized or blinded. Effect sizes (Cohen's *d*) were estimated for key comparisons where appropriate.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

The raw RNA-seq data generated in this study have been deposited in the Sequence Read Archive under accession code [PRJNA1160718](https://www.ncbi.nlm.nih.gov/sra/PRJNA1160718). Single-cell RNA-seq data from Crohn's disease patients analyzed in this study are publicly available via the Broad Institute Single Cell Portal under accession [SCTX1884](https://www.broadinstitute.org/single-cell/study/SCTX1884). Publicly available ATAC-seq data from Th0 and Th17 cells used for integrative analyses are available under

accession [GSM3505005](https://www.ncbi.nlm.nih.gov/sra/GSM3505005). Publicly available ChIP-seq datasets include BATF (GSM1004793; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1004793>), RORγt (GSM1004854; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1004854>), and IRF4 (GSM1004830; <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM1004830>), originally published by Ciofani et al. (Cell, 2012) and accessed via the Cistrome DB toolkit. Uncropped and unprocessed scans of all immunoblots and gels, together with the raw numerical data underlying all graphs, are provided as Source Data files accompanying this manuscript. All datasets required to interpret, verify, and extend the findings are publicly available without restriction. Source data are provided with this paper.

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Author contributions

J.A.L., and N.O.S.C. conceived the study. J.A.L. designed and performed the experiments, analyzed data and wrote the manuscript. L.M., E.M., G.S.L.; N.N.B.; G.C.M.G.; P.S.; S.O.; E.S.A.S.; H.R.; I.P.; M.A.; C.P.F.; J.A.O.N.N.; V.Y.Y.; M.C.; B.L.; T.G.; A.G.S.; S.B.; H.W.; T.G.H.; S.S.; B.J.N.; N.C.S.P.; L.M.C.; A.W.; S.M.; C.F.M.M.; N.O.S.C. provided scientific assistance, intellectual support and critically revised the manuscript. N.O.S.C. provided intellectual support in addition to directing and supervising the study. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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