SUPPLEMENTARY MATERIALS AND METHODS

Human patients and samples

Celiac disease was diagnosed in pediatric patients according to the ESPGHAN (European Society of Pediatric Gastroenterology Hematology and Nutrition) criteria in force at the time of recruitment, including anti-gliadin (AGA), anti-endomysium (EMA) and anti-transglutaminase antibody (TGA) determinations. A confirmatory small bowel biopsy and analyses were performed after informed consent was obtained from all subjects or their parents. All newly diagnosed adult CD patients had elevated TGA titers and displayed characteristic small intestinal histopathologic abnormalities, including villous atrophy, crypt hyperplasia and intraepithelial lymphocytosis. The samples were obtained after informed consent and using a protocol approved by the institutional review board of Columbia University. The study was approved by the Basque Country Clinical Research Ethics Board (CEIC-E ref. PI2019133) and analyses were performed after informed consent was obtained from all subjects or their parents. All experiments were performed in accordance with relevant guidelines and regulations. Biopsy specimens from the distal duodenum of each patient were obtained during routine diagnosis endoscopy. None of the patients suffered from any other concomitant immunological disease. None of the controls showed small intestinal inflammation or any other immunological disease at the time of the biopsy. None was taking any kind of medication. Details on human samples used is included in Supplementary Table 3.

Cell lines and treatments

Intestinal HCT116 (#91091005) cell line was purchased from Sigma-Aldrich (Poole, UK). Murine intestinal cell line C26 was kindly provided by Dr Beatriz Arteta. Both cell lines were cultured in DMEM (Lonza, Basel, Switzerland, #12-604F) supplemented with 10 % FBS (Millipore, Burlington, MA, USA #S0115), 100 units/ml penicillin and 100 µg/ml streptomycin (Lonza, #17-602E). Jurkat (#88042803) cell line was purchased from

Sigma-Aldrich (Poole, UK) and cultured in RPMI (Lonza, #12-115F) with 10 % FBS, 100 units/ml penicillin and 100 µg/ml streptomycin.

HCT116 cells were treated with actinomycin D (Sigma-Aldrich, #A9415) at a final concentration of 5 µg/ml for 3 h and 6 h for mRNA stability assay. XPO1 inhibitor leptomycin B (LMB; Apollo Scientific, Cheshire, UK, #BIL2101) and NFkB inhibitor BAY-11-7082 (Sigma Aldrich #B5556-10MG) were used at a final concentration of 6 ng/ml and 10 uM for 24 h and 48 h respectively.

Mouse models and treatments

Specific pathogen free mice transgenic for HLA-DQ2 (human haplotype DR3-DQ2) with mouse $CD4^+$ cells exclusively, originally generated at the University of Melbourne[24], were bred at McMaster University's Central Animal Facility on a gluten-free diet (Teklad, Harlan Laboratories, Indianapolis, IN) for at least 2 generations. Local animal ethics approval was obtained. One group of mice was gavaged orally using 25µg cholera toxin (CT) (Sigma Aldrich) and 1mg PTG once a week, for 3 weeks. Controls received CT alone. At sacrifice, duodenal sections were processed for RNA extraction.

C57BL/6 mice, purchased from Janvier Labs, France, were used as wild type controls. Briefly, breeders were maintained on GFD (Altromin #C1074) for at least 4 weeks, and experiments were performed using their progeny. At 4 weeks of age, mice were orally sensitized using CT and PTG (500 µg PTG + 25µg CT) once a week for 3 weeks (Enzo Life Sciences, #BML-G117-000). Control mice received CT alone. Control and PTG-treated mice were housed in different cages to avoid PTG exposure through feces in control mice. After the last gavage the mice were sacrificed, and sections of the duodenum processed for RNA and protein extraction. The Animal Experimentation Ethics Committee of the University of Barcelona (CEEA-UB) approved all the procedures with mice. Permits for procedure were obtained from the Government of Catalonia, according to European Directive 2010/63/EU.

Ythdf1 KO mice were received from Dr. He's lab[47] sacrificed at 4 weeks of age. Tissue sections of the duodenum were processed for RNA and protein extraction.

Bioinformatic packages

MeT-DB V2.0 m^{6}A database[25] was used for assessing the existence of m^{6}A peaks in the 5'UTR of *XPO1*. MeT-DB V2.0 records predicted transcriptome-wide m^{6}A peaks and single-base m^{6}A sites from a significantly expanded collection of Methylated RNA Immunoprecipitation Sequencing (MeRIP-Seq) samples. It provides a genome browser to help visualize the m^{6}A sites from different studies. The user can search by genes or genomic location to visualize the relationship of m^{6}A sites from different studies.

RNAfold web server available at Vienna RNA web services[26] was used to predict the secondary structure of the allele specific *XPO1* 5'UTRs. The RNAfold web server predicts secondary structures of single stranded RNA sequences up to 7,500 nt.

M^{6}A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP) data of HCT116 cells was downloaded from GEO repository (GSE128699). Sequence runs SRR8767363 and SRR8767364, corresponding to the m^{6}A mapping (mCLIP) results of wild-type HCT116 cells deposited in GEO (https://www.ncbi.nlm.nih.gov/geo) experiments GSM3682895 and GSM3682896, respectively, were obtained from the Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra) and reads were aligned against the *XPO1* 5'UTR sequence using NCBI Nucleotide Blast (https://blast.ncbi.nlm.nih.gov). Alignments were downloaded as plain text files, and the number of total matches and mismatches in each position were counted using MS Excel.

DNA, RNA and protein extraction

For human biopsies NucleoSpin TriPrep kit (Macherey-Nagel, Düren, Germany, #740966.50) was used following manufacturer instructions. Protein pellets were resuspended in RIPA buffer (150 mM NaCl, 1.0 % NP-40, 0.5 % NaDeoxycholate, 0.1 % SDS, 50 mM TrisHCl, 1 mM EDTA) with 1x protease inhibitor cocktail (PI) (Roche, Basel, Switzerland, #11836153001). For mice samples Direct-zol RNA miniprep kit (Zymo research, Irvine, USA, #R2053) was used for RNA extraction. Proteins were lysed in RIPA buffer with 2x PI. For HCT116, C26 and Jurkat cell lines RNA extraction was

performed using NucleoSpin RNA Kit (Macherey Nagel, #740984.50) and cells were lysed in RIPA buffer.

Epithelial and Immune subcellular fractions from human intestinal biopsies were obtained following the previously described protocol[46]. Briefly, cells were mechanically separated from fresh biopsies by rotation-agitation in 10 ml of RPMI medium supplemented with 2% FBS, 1% DTT and 5 nM EDTA for 1 hour. The lamina propria and debris were removed by filtration through 30 µm pre-separation filters (cat. no. 130-041-407) and dead cell removal kit (cat. no. 130-090-101) was used to prepare a viable single-cell homogenous suspension. Live cells were labelled with CD45 magnetic microbeads (cat. no. 130-045-801) in the presence of FcR blocking reagent (cat. no. 130-059-901) to increase the specificity of antibody labelling. The antibody-labelled cell-suspension was applied to a magnetic separation column (cat. no. 130-042-201) and unlabelled CD45- cells (mainly the epithelial CD326+ fraction) were collected with the flow-through, while CD45+ cells were recovered after removing the column from the magnet.

Gene expression analyses

500-1000 ng of RNA were used for the retrotranscription reaction using iScript cDNA Synthesis Kit (BioRad, CA, USA, #1708890). Expression values were determined by q-PCR using Taqman Gene Expression Assays (Thermofisher, Waltham, MA) or by q-PCR using Sybr Green (iTaq SYBR Green Supermix, Bio-Rad, #1725124) and specific primers. *HPRT* gene was used as endogenous control both in human and murine samples. Reactions were run in a BioRad CFX384 and melting curves were analyzed to ensure the amplification of a single product. All qPCR measurements were performed in duplicates and expression levels were analyzed using the $2^{-\Delta\Delta Ct}$ method. All Taqman Gene expression Assays and primers are listed in Table1.

IL8 expression in complete human intestinal biopsies as well as in epithelial and immune fractions from non-celiac controls and active disease patients was analyzed using a previously performed RNAseq from our group[46].

SNP genotyping

Genotyping of the SNP rs3087898 was performed in DNA samples of human biopsies using a custom rhAmp SNP Assay (IDT, Newark, NJ, USA) following the manufacturer's instructions.

m^{6}A RNA immunoprecipitation

For cell lines, 4 µg of precleared RNA per sample were fragmented with RNA fragmentation buffer (100 mM Tris, 2 mM $MgCl_2$) for 3 min at 95ºC and placed on ice immediately after heating. For epithelial and immune fractions isolated from human intestinal biopsies a pool of 18 human biopsy fractions was used for a total of 2 µg RNA per fraction. 10 % of RNA was kept as input. 1 µg of m^{6}A antibody (Abcam, #ab151230) and control antibody (IgG, Santa Cruz Biotechnologies, Dallas, USA, #sc-2025) were coupled to agarose A beads (GE Healthcare, Chicago, USA) in a rotation wheel for 1 h at 4ºC. After incubation, beads were washed twice in reaction buffer (150 mM NaCl, 10 mM Tris-HCl, 0.1 % NP-40). RNA was added to the antibody-coupled beads and incubated for 3h at 4ºC in a rotating wheel. Subsequently, beads were washed 2X in reaction buffer, 2X in low salt buffer (50 mM NaCl, 10 mM TrisHCl and 0.1 % NP-40) and 2X in high salt buffer (500 mM NaCl, 10 mM TrisHCl and 0.1 % NP-40). After the last wash, beads were resuspended in Lysis buffer and RNA was extracted using the PureLink RNA extraction kit (Invitrogen, Carlsbad, USA, #12183016). In human intestinal HCT116 and human T cell derived Jurkat cell lines *SOCS1* and *HPRT* were used as positive and negative controls respectively. In murine intestinal cell line C26 *Hprt* and *Po* were used as positive and negative controls respectively.

m^{6}A RT-qPCR

RT-qPCR based method was used as previously described[28] for the confirmation of residue specific methylation. (+) primers for the 3 m^{6}A motifs together with a (-) primer, all located in the *XPO1 5'UTR*, were designed. *TUG1* and *HPRT* were used as positive and negative controls respectively. The sequence of the primers used are available upon request.

Plasmid construction

XPO1 5'UTR was amplified from human cDNAs containing T or C allele for rs3087898 SNP and cloned into a modified pIS0 vector (Addgene, #12178) using NcoI restriction sites.

pLKO.1-TRC Cloning vector (Addgene, #10878) was used for the construction of plKO.1-shMETTL3 and plKO.1-shYTHDF1 plasmids. Following Addgene's protocol shRNAs were designed to knock down human *METTL3* (AAGGAACAATCCATTGTTCTCGAGAACAATGGATTGTTCCTT) and *YTHDF1* (AACGGCAGAGTCGAAACAAACTCGAGTTTGTTTCGACTCTGCCGTTC).

For allele-specific overexpression experiments, we generated mammalian expression plasmids containing XPO1 5´UTR sequence followed by the cDNA encoding human full-length XPO1 protein (1071 amino acids). Two different constructs, named *5´UTR-XPO1*T* and *5´UTR-XPO1*C*, were generated, carrying the rs3087898 T or C allele, respectively. To generate these plasmids, a partial *XPO1* cDNA fragment, from nucleotide 187 to the stop codon, was first amplified by PCR using a previously described pEYFP-CRM1 plasmid[48] as template. This PCR product was cloned into the pEYFP-C1 vector (Clontech) as a SalI/BamHI fragment. Next, the EYFP-coding sequence from the resulting plasmid was excised as a NheI/SalI fragment, and replaced by a DNA sequence containing *XPO1* 5´UTR fused to the first 187 nt of *XPO1* cDNA, which was generated using a two-step overlapping PCR approach.

Luciferase Assay

Empty vector pIS0 was used as control. 150000 cells/well were seeded and transfection was performed with X-TremeGENE HP DNA transfection reagent (Sigma-Aldrich, #6366546001) using 250 ng per plasmid during 48 h. Dual-Luciferase Reporter Assay System (Promega, Madison, USA, #E1910) was used following manufacturer's protocol.

Overexpression

For METTL3 overexpression experiments 250 ng plasmid from Addgene (#53739) was used. 150000 cells/well were seeded and transfected using X-TremeGENE HP DNA transfection reagent (Sigma-Aldrich, #6366546001), cells were harvested after 48 h.

For XPO1 overexpression, 1 µg of 5´UTR-XPO1*T or 5´UTR-XPO1*C plasmids were used. 100000 cells/well were seeded and transfection was performed with X-TremeGENE HP DNA transfection reagent (Sigma-Aldrich, #6366546001) for 48 h.

Silencing experiments

Viral particles were produced in HEK293FT cells transfected with 1 µg pLKO.1 shRNA plasmid, 750 ng psPAX2 packaging plasmid (Addgene, #12260) and 250 ng pMD2.G envelope plasmid (Addgene, #12259) using X-TremeGENE HP DNA transfection reagent (Sigma-Aldrich, #6366244001) in DMEM without antibiotics and cells were incubated o/n at 37ºC. Transfection media was replaced with fresh complete DMEM and viral particle containing media was harvested after 24 h and 48 h. Collected media was centrifuged and viral particles were stored in aliquots at -80ºC. HCT116 cells were infected with sh-METTL3, sh-YTHDF1 or pIKO.1 as negative control and selection was performed by puromycin resistance (2 µg/mL).

For YTHDF1 silencing in PTG stimulated cells, 30 nM of 2 different siRNAs against YTHDF1 (IDT, # hs.Ri.YTHDF1.13.1 and hs.Ri.YTHDF1.13.2) or negative control siRNA (IDT # 51-01-14-01) were transfected into cells sensitized with PTG and 16 h prior to 350 µg/mL PTG treatment using Lipofectamine RNAimax reagent (Thermo Fisher Scientific).

Dot Blot

300 ng of RNA was crosslinked into a nitrocellulose membrane using UV. Membrane was blocked using 5 % milk in 0.1 % PBST (0.1 % Tween in PBS). Membrane was incubated overnight with a m^6A antibody (1:200) (Abcam, Cambridge, UK, #ab151230) at 4ºC. After washing in 0.1 % PBST, membranes were incubated with a secondary HRP-conjugated anti-rabbit antibody (1:10000) (Santa Cruz Biotechnology, #sc-2357) and the membrane was developed using Clarity Max ECL Substrate (BioRad, #1705062).

PT-Gliadin Stimulations *in vitro* and *ex vivo*

PT-Gliadin was prepared by enzymatic digestion as described previously[49] with minor modifications. Briefly, 2.5 g of gliadin (Sigma-Aldrich, #G3375) was dissolved in 25 ml of 0.2 N HCl and incubated stirring o/n at 37°C with 25 mg of pepsin (Sigma-Aldrich, #6887). pH was adjusted to 7.4 using 1 N NaOH and the resultant dilution was further digested adding 25 mg of trypsin (Sigma-Aldrich, #T9201). The solution was stirred vigorously at 37°C for 5 h, boiled (100°C) for 1 h, centrifuged 10 min at 2000 g, supernatant filtered and aliquots of the resulting solution were kept at -80ºC until used.

100.000 HCT116 cells were plated and incubated o/n at 37ºC. Next day cells were incubated with a low-dose of 30 µg/mL PT-Gliadin (PTG) and incubated for 48 h. Then treatment with PTG at a final concentration of 350 µg/mL was performed and after 24 h supernatants and cells were harvested for further RNA and protein analysis (SF3.A).

For *ex vivo* stimulation experiments, epithelial cells isolated from duodenal fractions from WT and YHDF1 KO mice were divided into two wells and cultured in DMEM + FBS and P/S. PTG stimulation was further performed in freshly isolated cells using 250 µg/mL PTG for 4 h and non-stimulated cells were used as control.

For biopsy stimulation experiments, two biopsy portions, taken from active celiac patients were incubated without any compound or in the presence of 250 µg/ml PTG in 150 µl RPMI-1640 10X medium (Thermofisher, #11875093) at 37°C and 5% CO_2 during 4 h.

Cellular fractionation

For the quantification of RNA amounts in nuclear and cytoplasmic compartments, nuclei were isolated using C1 lysis buffer (1.28 M sucrose, 40 mM Tris-HCl pH 7.5, 20 mM $MgCl_2$, 4 % Triton X-100). The amounts of *XPO1-T/C*, *MALAT1* (nuclear control) and *RPLPO* (cytoplasmic control) mRNAs were measured by q-PCR and compared to the total amount of those RNAs in the whole cell lysate.

For the quantification of protein amounts in nuclear and cytoplasmic compartments, cells were resuspended in NARA buffer (10 mM HEPES pH 7,9, 10 mM KCl, 0.1 mM EDTA) with PI and incubated in ice for 10 minutes. After adding NP-40 to final concentration 0.05 %, lysates were incubated 5 minutes in ice and centrifuged at 400 g for 2 minutes. The supernatant was the cytosolic fraction. Pellet was washed 3 times with NARA buffer and resuspended in NARC buffer (20 mM HEPES, 400 mM NaCl, 1 mM EDTA) + PI, shaken at 4ºC for 30 minutes and centrifuged at 16000 g for 10 minutes. The supernatant was the nuclear extract.

Western Blot

Laemmli buffer (62 mM Tris-HCl, 100 mM dithiothreitol (DTT), 10 % glycerol, 2 % SDS, 0.2 mg/ml bromophenol blue, 5 % 2-mercaptoethanol) was added to the protein extracts in RIPA and were denatured by heat. Proteins were migrated on 10 % SDS-PAGE gels. Following electrophoresis, proteins were transferred onto nitrocellulose membranes using a Transblot-Turbo Transfer System (Biorad) and blocked in 5 % non-fatty milk diluted in TBST (20 mM Tris, 150 mM NaCl and 0.1 % Tween 20) at room temperature for 1 h. The membranes were incubated overnight at 4ºC with primary antibodies diluted 1:1000 or 1:5000 (for ß-ACTIN) in TBST. Immunoreactive bands were revealed using the Clarity Max ECL Substrate (BioRad, #1705062) after incubation with a horseradish peroxidase-conjugated anti-mouse (1:10000 dilution in 2.5 % non-fatty milk) or anti-rabbit (1:10000 dilution in 2.5 % non-fatty milk) secondary antibody for 1 h at room temperature. The immunoreactive bands were detected using a Bio-Rad Molecular Imager ChemiDoc XRS (BioRad) and quantified using the ImageJ software.

The following antibodies were used for Western Blotting: XPO1 (Cell Signalling, Leiden, The Netherlands, #46249S), METTL3 (Abcam, #195352), YTHDF1 (Abcam, #220162), YTHDF1 (Proteintech, Rosemont, USA, #17479-1-AP), actin (Santa Cruz Biotechnologies, #sc47778), tubulin (Sigma-Aldrich, #T9026 and Cell Signalling, #2144S), HSP90 (Cell Signaling; #4874), H3 (Abcam; #ab1791), p50 (Abcam, #ab7971), GAPDH (Novus, St Louis, MI, USA, #ND300-221), GAPDH HRP (Santa Cruz Biotechnologies, #sc-166574 HRP).

RNA immunoprecipitation assay (RIP)

For RIP experiments, HCT116 cells were lysed in RIP buffer (150 mM KCl, 25 mM Tris, 0.5 mM DTT, 0.5 % NP-40, PI), kept on ice for 15 minutes and homogenized using a syringe. Lysates were pre-cleared with protein A-Agarose beads (GE Healthcare, Chicago, USA) for 1 h in a wheel shaker at 4ºC. A-Agarose beads were blocked with 20 % BSA and mixed with pre-cleared lysates and 1 µl of anti-IgG antibody (negative control; Santa Cruz Biotechnologies, #sc-2025) or antibody of interest (METTL3, Abcam or YTHDF1, Abcam). After overnight incubation in a wheel shaker at 4ºC, beads were washed three times with RIP buffer, three times with low salt buffer (50 mM NaCl, 10 mM Tris-HCl, 0.1 % NP-40) and three times with high salt buffer (500 mM NaCl, 10 mM Tris-HCl, 0.1 % NP-40). After the washes, 70 % of beads were resuspended in RNA extraction buffer and 30 % was used for WB. *SOCS1* and *HPRT* were used as positive and negative controls respectively.

ELISA

RNA from HCT116 cells or from intestinal samples of non-celiac controls, active celiac disease patients and celiac disease patients in a gluten free diet were collected for determination of m^6A levels using a commercially available m^6A ELISA kit (Epigentek, NY, USA, # P-9005-96).

Protein extracts from human biopsies or cell culture supernatants were collected for determination of human XPO1 protein levels (Cloud-Clone Corp, Houston, USA, #SEC258Hu) and secreted IL8 chemokine levels were quantified using commercially

available ELISA kit (R&D Systems, Abingdon, UK; #D8000C) following the manufacturer's instructions.

Gel shift assay

Nuclear lysates were prepared from HCT116 cells transfected with an empty vector or overexpressing *XPO1*T* form by incubating them on ice with C1 lysis buffer for 15 min and collecting nuclei by centrifugation for 15 min at 700 g. Nuclei were extracted with EMSA lysis buffer (50 mM KCl, 25 nM HEPES, 125 uM DTT and 0.5 % NP-40). Electrophoretic mobility shift assays were performed by incubating 40 µg of nuclear extracts in 50 µl reaction volume in EMSA binding buffer (50 µg BSA, 20 mM Tris, 50 mM KCl, 0.05 % NP-40, 5 % glycerol, 10 mM b-mercaptoethanol,1 mM EDTA and 0.1 M DTT) with 60 nM of the fluorescently labeled oligos containing the NFkB consensus sequence in the IL8 promoter as described in LASAGNA TFBS web server[50] for 30 min at room temperature. For competition assay 10x of the unlabeled oligos were added to the reaction. Samples were then loaded on a 1 % non-denaturing agarose gel and run at 60 V for 2 h.

Statistical analysis

The data are represented as the mean ± standard error of the mean of at least three biological replicates. Mean comparisons were performed by Student's t-test, Mann Whitney test or ANOVA test. Correlation analyses were done using Pearson correlation. The statistically significance level was set at $p < 0.1$.

Supplementary references

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