**iScience, Volume *24***

## Supplemental Information

# NLRC5 promotes transcription of *BTN3A1-3* genes and Vγ9Vδ2 T cell-mediated killing

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## Supplemental Information

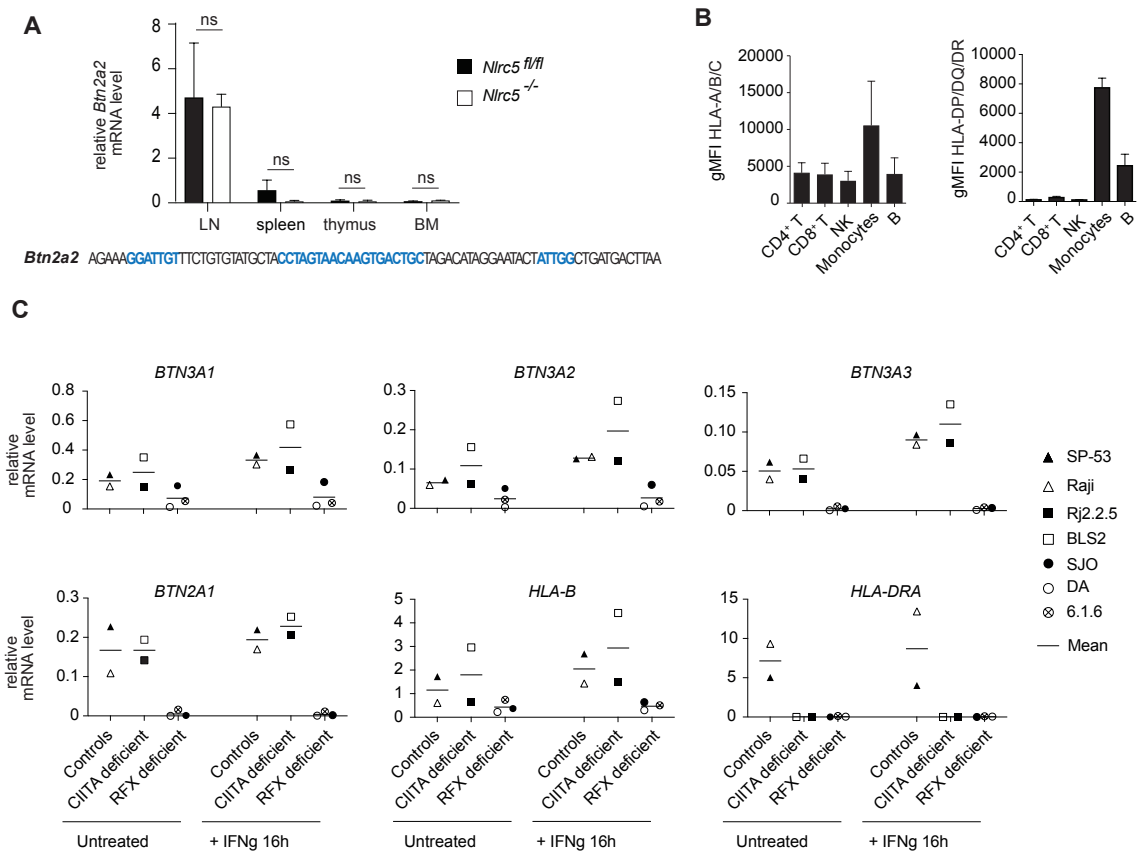


**Figure S1. *BTN3A1, A2* and *A3* mRNA is not decreased in CIITA-deficient cell lines (related to Figure 1).** (**A**) Murine *Btn2a2* promoter sequence and mRNA levels (relative to *Polr2a* mRNA) as assessed by quantitative RT-PCR (qRT-PCR) in lymph node (LN), spleen, thymus, and bone marrow (BM) of *Nlrc5*$^{fl/fl}$ and *Nlrc5*$^{-/-}$ mice. (**B**) Geometric mean fluorescence intensity (gMFI) of HLA-A/B/C and HLA-DR/DQ/DP as measured by flow cytometry in the indicated blood-derived cell subsets. (**C**) *BTN3A1, A2* and *A3, BTN2A1, HLA-B*, and *HLA-DRA* mRNA levels (relative to *POLR2A* mRNA) were measured by qRT-PCR in B cell-derived cell lines not expressing CIITA (Rj2.2.5; black square, BLS-2; white square), RFX5 (SJO; black circle), or RFXAP (DA; white circle, 6.1.6; white circle with cross) or controls (SP-53; black triangle, Raji; white triangle) treated or not with IFNγ for 16 hours. Results depict the mean ± SEM of n=3 mice per genotype and are representative of two independent experiments (**A**), the mean ± SEM of n=3 individual donors (**B**), or the mean of n=2 or n=3 cell lines, each represented as the average of n=3 independent measurements (**C**). (**A**) Statistical differences between genotypes were calculated using unpaired Student's t-test, two-tailed, unequal variance; ns: non significant.

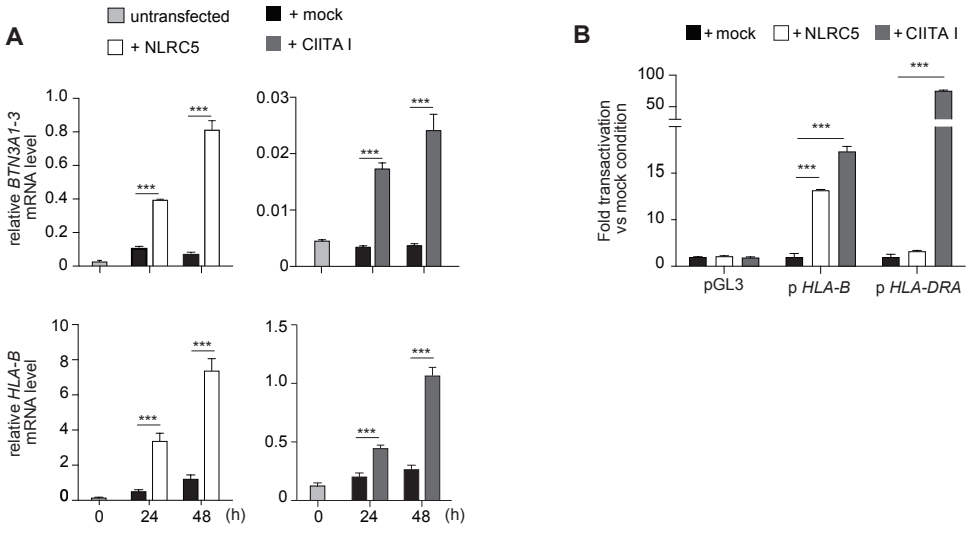


**Figure S2. Overexpressed CIITA transactivates MHC class II and class I promoters (related to Figure 2).** (**A**) *BTN3A1-3* and *HLA-B* mRNA levels (relative to *POLR2A* mRNA) were measured by qRT-PCR at basal (t=0 h, untransfected) and 24 h and 48 h following transfection of plasmids encoding the indicated NLR proteins or an empty vector (mock) in HEK293T. Results are depicted as mean ± SD (n=3 technical replicates) and are representative of at least 2 independent experiments. Statistical differences were determined by two-way ANOVA followed by comparison of the experimental conditions to the corresponding mock transfection, and were corrected for multiple testing using the Holm-Sidak method. (**B**) Luciferase reporter assays were performed in HEK293T cells co-transfected with the parental pGL3 backbone, *HLA-DRA*, or *HLA-B* promoter constructs (reporters), and a vector coding for NLRC5, CIITA I, or an empty (mock) vector. Data are expressed as fold transactivation to the mock condition. Results represent mean ± SD of n=3 technical replicates and are representative of at least three independent experiments. Statistical differences were determined by performing a two-way ANOVA followed by comparison of the experimental conditions to the corresponding mock transfection and were corrected for multiple testing using the Dunnett method. *** $p<0.001$. Only statistically significant differences are illustrated.

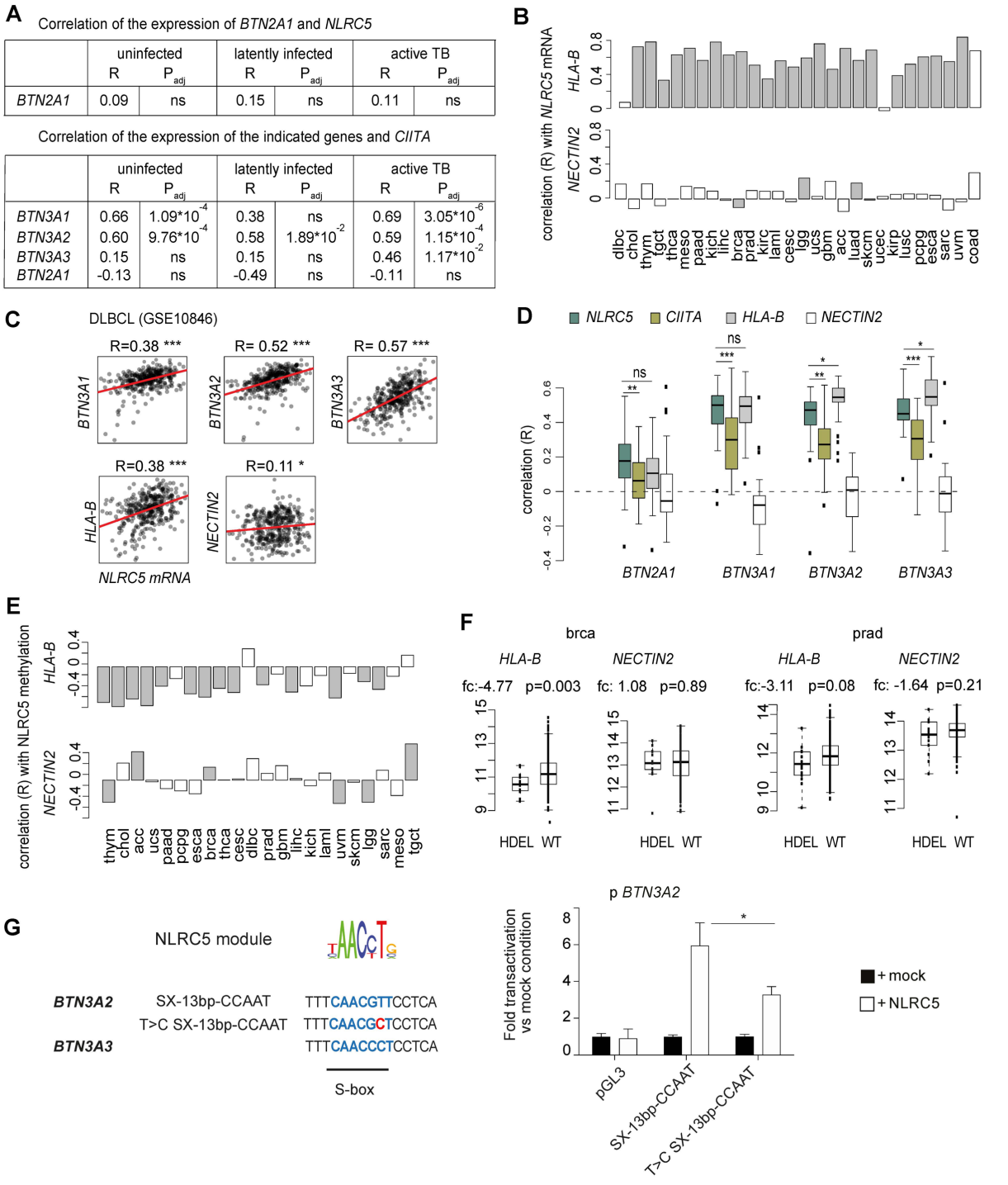


**Figure S3. *NLRC5* mRNA correlates with *HLA-B* transcript abundance (related to Figure 6).** (**A**) Pairwise correlation of *NLRC5* and *BTN2A1* and of *CIITA* and *BTN3A1*, *BTN3A2*, *BTN3A3*, *BTN2A1* expression were analyzed using transcriptome datasets of Gambia *M. tuberculosis (TB)* cohort study (GSE28623). The samples are divided in the groups 'uninfected', 'latently infected', and 'active TB'. The table displays the Spearman's correlation coefficient (R), and Bonferroni adjusted p-values (P adj) for the 3 groups. (**B, D-F**) Data from The Cancer Genome Atlas (TCGA) provisional dataset collections were analyzed after adjustment for CD45 expression. (**B**) Spearman's correlation coefficient (R) for *NLRC5* and *HLA-B* or *NECTIN2* mRNA expression across cancer

types (**C**). Data from GSE10846 lymphoma dataset were analyzed after adjustment for CD45 expression. Scatterplots for *NLRC5* and *BTN3A1*, *BTN3A2*, *BTN3A3*, *HLA-B*, or *NECTIN2* mRNA expression are shown. Spearman's correlation coefficients (R) are indicated and significance was determined using the Bonferroni method. (**D**) Box plot depicting Spearman's correlation coefficient (R) distribution for the indicated *BTN* genes and *NLRC5*, *CIITA*, *HLA-B*, or *NECTIN2* mRNA expression across all cancers. Two group comparisons were performed using unpaired t-tests, two-tailed, unequal variance. (**E**) Spearman's correlation coefficient for *NLRC5* promoter methylation and *HLA-B* or *NECTIN2* mRNA expression across cancer types. (**F**) *HLA-B* and *NECTIN2* mRNA abundance is plotted according to *NLRC5* copy number status. fc: fold change of expression in HDEL (n=14 and n=13 for brca and prad, respectively) over WT group (n=272 and n=361 for brca and prad, respectively). HDEL: homozygous deletion; WT: wild type. Two group comparisons were performed using unpaired t-tests, two-tailed, unequal variance, and p-values are indicated. (**G**) Luciferase reporter assays were performed in HEK293T cells co-transfected with the parental pGL3 backbone or the indicated *BTN3A2* promoter constructs, and a vector coding for NLRC5 or an empty (mock) vector. In the "T>C SX-13bp-CCAAT" construct, the T position was mutated into a C (in red) to resemble the S-box sequence found in the *BTN3A3* promoter. Data are expressed as fold transactivation as compared to the mock condition. Results represent mean ± SD of n=4 technical replicates and are representative of 2 independent experiments. Statistical differences were determined by performing a two-way ANOVA followed by comparison of the SX-13bp-CCAAT to the T>C SX-13bp-CCAAT condition and were corrected for multiple testing using the Holm-Sidak method. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, ns: not significant. (**B, E**) Grey bars indicate significant correlation ($p<0.05$ after Bonferroni correction), white bars non-significant ones.

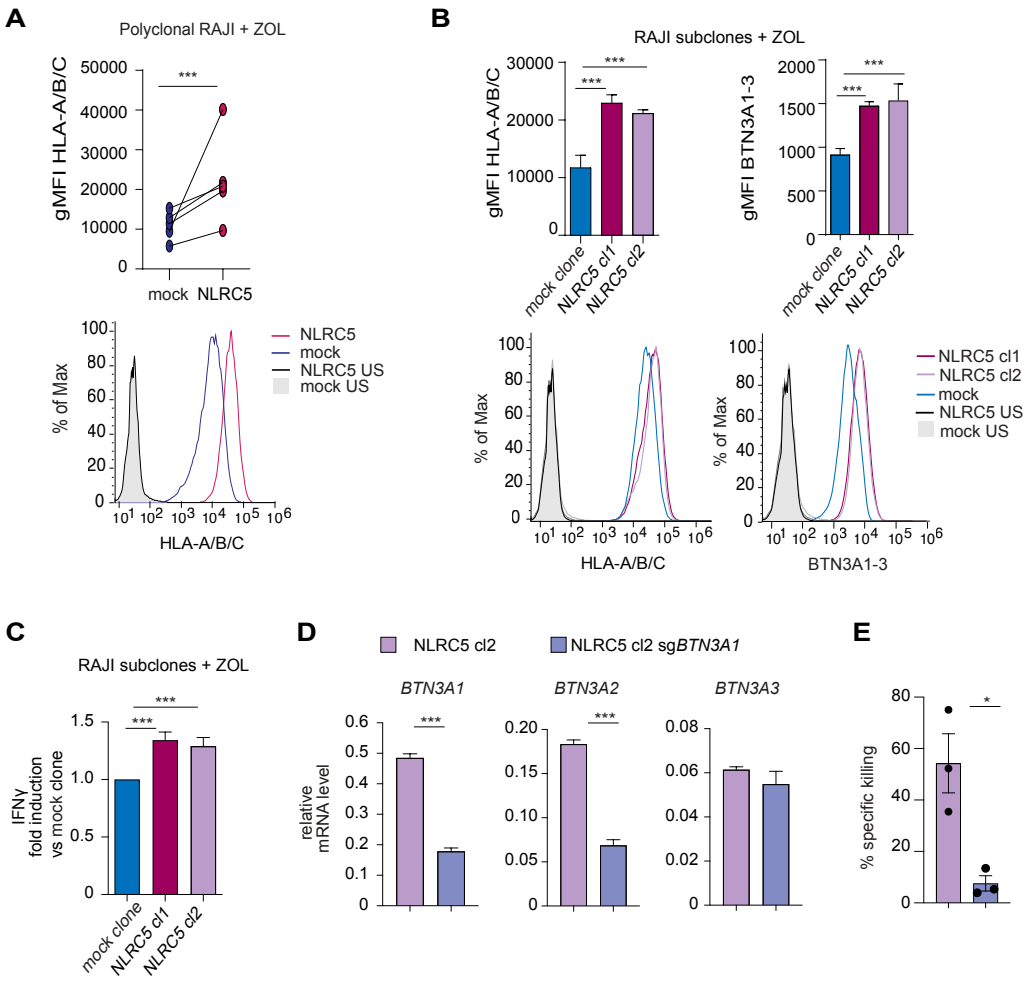


**Figure S4. NLRC5 overexpression increases BTN3A1-3 and HLA-A/B/C expression in Raji cells (related to Figure 7).** (**A**) Polyclonal Raji cells were transduced with NLRC5 or empty vector (mock) and treated with zoledronate (ZOL) for 24 h. HLA-A/B/C surface expression was analyzed by flow cytometry. Graphs depict a quantification of HLA-A/B/C geometric MFI (gMFI; top) and histogram overlays (bottom) show HLA-A/B/C expression for unstained (US; black, grey), mock- (blue) and NLRC5-transduced polyclonal Raji cells (pink). Results represent 5 independent measurements. (**B**) Subclones were generated from Raji cells transduced with NLRC5 or empty vector (mock) and treated with ZOL for 24 h before BTN3A1-3 and HLA-A/B/C surface expression was analyzed by flow cytometry. Graphs depict a quantification of BTN3A1-3 and HLA-A/B/C gMFI (top) and histogram overlays (bottom) show BTN3A1-3 and HLA-A/B/C expression for unstained (US; black, grey), mock- (blue) and NLRC5-transduced Raji subclones (red, violet). (**C**) IFNγ production by Vγ9Vδ2 T cells was measured after 48 h of co-culture with the mock - or the two NLRC5-transduced subclones at an effector-to-target ratio of 10:1 in the presence of ZOL. Results are depicted as IFNγ fold induction in presence of NLRC5-transduced as compared to the mock-transduced subclones. (**D**, **E**) *BTN3A1* was targeted by CRISPR/Cas9 in the NLRC5-transduced Raji subclone 2 (NLRC5 cl2 sg*BTN3A1*). NLRC5 cl2 and NLRC5 cl2 sg*BTN3A1* were assessed for *BTN3A1, A2,* and *A3* mRNA levels (relative to *POLR2A* mRNA) by qRT-PCR (**D**) and for their susceptibility to Vγ9Vδ2 T cell-mediated killing after 24 h of co-culture at an effector-to-target ratio of 10:1 in the presence of ZOL (**E**). Results are depicted as mean ± SEM of n=3 independent measurements (**B**) as mean ± SEM of n=4 healthy donors (**C**), as mean ± SD of n=3 technical replicates (**D**), or as mean ± SEM of n=3 healthy donors (**E**). Results are representative of at least 2 independent experiments (**A-E**). Statistical differences between the condition with and without NLRC5 overexpression were calculated using paired Student's t-test, two-tailed, unequal variance (**A**), by one-way ANOVA followed by comparison of the experimental conditions to the corresponding mock condition and were corrected for multiple testing using the Dunnett method (**B, C**), or by unpaired (**D**) or paired (**E**) Student's t-test, two-tailed, unequal variance. *p<0.05, ***p<0.001. Only statistically significant differences are illustrated.

## Transparent Methods

### *Mice*

Sex- and age-matched 6- to 12-week-old *Nlrc5*$^{fl/fl}$ and *Nlrc5*$^{-/-}$ (Staehli et al., 2012) mice on a C57BL/6 (H2$^{b}$) background were housed at the animal facility of the University of Lausanne. All animal experimental protocols were approved by the Veterinary office regulations of the State of Vaud, Switzerland, and all methods were performed in accordance with the Swiss guidelines and regulations.

### *Cell lines and transfections*

HEK293T cells were cultured using DMEM high glucose supplemented, whereas the B-cell lines SP-53, Raji, Rj2.2.5, BLS-2, SJO, DA, and 6.1.6 (previously described in (Ludigs et al., 2015; Tarantelli et al., 2018)) were cultured in RPMI 1640 medium (Life Technologies) containing at least 10 mM HEPES. Culture media were all supplemented with GlutaMAX (2 mM), sodium pyruvate (1 mM), 10% fetal calf serum (FCS), 100 U/ml penicillin and 100 µg/ml streptomycin. For HEK293T cells, 0.1 mM MEM Non-essential Amino Acids (NEAA) were added in some experiments. Cells were incubated at 37°C in 5% $CO_2$. For transfection, HEK293T cells were subconfluently seeded and transfected the following day using PEI reagent (1.5:1 up to 3:1 PEI: DNA ratio) and harvested at the indicated time point for analysis.

### *Quantitative RT-PCR and sample preparation*

To assess expression in human immune tissues, cDNA included in the "Human immune system MTC$^{TM}$ panel" (TAKARA) were used (pool from at least 9 individuals). Peripheral blood mononuclear cells (PBMC) were isolated by density centrifugation over a Ficoll-Hypaque

gradient (LymphoPrep) from blood of healthy human donors. Total PBMCs were surface stained with $CD4^+$ T, $CD8^+$ T, NK, monocyte and B cell markers, and sorted by flow cytometry using FACSAria III sorter (BD Biosciences) as $CD4^+CD3^+$, $CD8^+CD3^+$, $CD56^+CD3^-$, $CD14^+$, and $CD19^+$ cells, respectively. Immune organs were collected and processed from *Nlrc5*$^{fl/fl}$ and *Nlrc5*$^{-/-}$ mice. RNA from cell lines, PBMC subsets, and murine lymphoid tissues was extracted using TRIzol® reagent (Ambion, Life Technologies) according to manufacturer's instructions. Annealing with random primers (Life Technologies) was performed at 70 °C for 5 min, followed by retrotranscription to cDNA with M-MLV RT, RNase H(–) point mutant (Promega), in presence of buffer, nucleotides (Roche Diagnostics), and RNasin® Plus RNase Inhibitor (Promega). Reaction was incubated at 40 °C for 10 min, 45 °C for 50 min and 70 °C for 15 min. cDNAs were diluted and quantitative PCR was performed using the LightCycler 480 SYBR Green I Master (Roche Diagnostics) on a LightCycler 480 machine (Roche Diagnostics). Expression was determined relative to *POLR2A*. Data were analyzed, and transcript abundance (gene/POLR2A) and s.d. were calculated using the LightCycler 480 software. Primers (Fwd; Rev) used were as follows: *Polr2a: (*5'-CCGGATGAATTGAAGCGGATGT-3'; 5'-CCTGCCGTGGATCCATTAGTCC-3'); *Btn2a2:* (5'-TAGGGGTCTCTCCACACAGC-3'; 5'-TATGACCAGGCAACCATGAA-3'); *POLR2A: (*5'-CGCACCATCAAGAGAGTCCAGTTC-3'; 5'-GTATTTGATGCCACCCTCCGTCA-3'); *BTN3A1-3: (*5'-ATGAAAAAGCCCTGGTGGAG-3'; 5'-TGTATTTGGGGTTGGGGGTA-3'); *HLA-B: (*5'-CTACCCTGCGGAGATCA-3'; 5'-ACAGCCAGGCCAGCAACA-3'); *HLA-DRA :* (5'-GCCAACCTGGAAATCATGACA-3'; 5'-AGGGCTGTTTGTGAGCACA-3'); *BTN2A1* : (5'-TGCTCGGCCAGAAGAAAGAA–3'; 5'-CCACAATGATAGGCAGGGC-3'); *BTN3A1:* (5'-CTTCAGCTGCTCATGCCTCA-3'; 5'-CAGATCAGCGTCTTCACCCA-3'); *BTN3A2* : (5'-

CAGTACTTGACTCGTGGAGAG-3'; 5'-TCAGGCTGACTTATTGGTATCGG-3'); *BTN3A3 :* (5'-TCGTGGAGAGAAGTCTTTGG-3'; 5'-ACATCCGCAGGTTTGAAGA-3'); *NLRC5 :* (5'-GTGCCTCTGGACCTGGAG-3'; 5'-GAGATTCAGGTTGGCTTTTCC-3'); *CIITA :* (5'-AGCCAAGTCCCTGAAGGATG-3'; 5'-TCTTAAGGTCCCGAACAGCAG-3').

*Flow Cytometry*

For flow cytometry analysis, sorted PBMCs or cell lines were surface stained using antibodies against HLA-A/B/C (W6/32, eBioscience or BioLegend), HLA-DP/DQ/DR (Tu39, BioLegend) and BTN3A1-3/CD277 (BT3.1, BioLegend or Miltenyi Biotec). Data were acquired using a FACS Canto (Becton Dickinson) or a Gallios (Beckman Coulter) flow cytometer and analyzed with FlowJo software (LCC, Becton Dickinson).

*Plasmids and constructs*

NOD1, NOD2, NLRC3 expression plasmid were obtained from the laboratory of the late J. Tschopp. CD72 expression plasmid was a kind gift from M. Thome-Miazza (UNIL, Switzerland). HLA-B170 (referred as p *HLA-B*) luciferase reporter plasmid was kindly provided by P.J. van den Elsen (Leiden University, Netherlands). HLA-DRA luciferase reporter and CIITA I/III expression plasmids were kindly gifted by W. Reith (UNIGE, Switzerland). For overexpression experiments, a NLRC5 encoding plasmid previously described was used (Staehli et al., 2012). For ChIP assays, a plasmid encoding NLRC5 in frame with a N-terminal HA-tag, referred here as wt NLRC5, has been generated. ORF was amplified using the KAPA Hifi PCR kit (KAPA Biosystems) using NLRC5 containing plasmid as template. PCR primers were extended with KpnI or XhoI restriction sites for oriented cloning into the pCMV-HA backbone.

NLRC5 Walker A domain mutant (K234A), named as mt NLRC5, was generated by site directed point mutagenesis using the wt NLRC5 construct as template. The pHRSIN-CS-Luc-IRES-emGFP plasmid was a kind gift from A. Rodriguez (UAM, Spain). The pHRSIN-CS-IRES-mTagBFP2 was generated by removing the luciferase and substituting the emGFP for the mTagBFP2 in the pHRSIN-CS-Luc-IRES-emGFP plasmid using the Gibson assembly cloning method. The lentiviral construction pHRSIN-CS-HA-NLRC5-IRES-mTagBFP2 coding for NLRC5 was generated by Gibson assembly using the pHRSIN-CS-IRES-mTagBFP2 as recipient plasmid and the HA-NLRC5 insert PCR amplified from the wt NLRC5 plasmid. Lentiviral packaging plasmids pCMVDR8.74 and pMD2.G were a gift from D. Trono (EPFL, Switzerland). Luciferase reporter plasmids were created by replacing the MluI-BglII fragment spanning the HLA-DRA SXY region in the pDRAprox plasmid (Krawczyk et al., 2004) with *BTN3A* promoter regions. Inserts were obtained either by PCR amplification using the GoTaq polymerase (Promega) or using annealed purchased DNA oligos (Microsynth AG). The pGL3 plasmid containing the remaining HLA-DRA core promoter (from −60 to +10) in the same reporter plasmid was used as negative control. All generated constructs were verified by sequencing. Sequences of primers used for cloning are available upon request.

*Luciferase reporter assay*

HEK293T cells were seeded into a 96-well plate and transfected the following day using PEI reagent with human NLRC5, or human CIITA I expression vectors, or an empty backbone as control (mock), and the indicated luciferase reporter constructs. The pRLTK (Renilla) luciferase reporter was included for normalization. Cells were harvested between 20 and 30 h post-transfection and cell lysates were analyzed using the Dual-Luciferase® Reporter Assay System

(Promega) following manufacturer's instruction. Bioluminescence was measured using the Enspire™ Alpha2390 Multilabel Reader (PerkinElmer).

*Chromatin immunoprecipitation (ChIP)*

HEK293T cells were transiently co-transfected with plasmids encoding for HA-tagged human NLRC5 (wt NLRC5) or human NLRC5 Walker A mutant (mt NLRC5) and human CD72. After 48 h, cells were collected and stained with FITC-labeled α-CD72 (3F3; BioLegend) followed by incubation with α-FITC magnetic beads for MACS enrichment (Miltenyi Biotech). Chromatin was prepared from $CD72^+$ cells as previously described (Masternak et al., 2003). Immunoprecipitation was performed using a ChIP grade anti-HA tag antibody (ab9110, Abcam). Analysis of specific DNA regions was performed by quantitative PCR. The amount of immunoprecipitated DNA was calculated from the standard curves generated with the input chromatin and fold enrichment was determined relative to the mt NLRC5 condition. The promoter of *HOXC8*, which is not a NLRC5 target, is used as negative control. Primers (Fwd; Rev) are listed hereafter: *BTN3A1: (5'-* GGGAGGTAGGGCAGGAATTT -3'; 5'-CACTGAGGAAGGCTGAAATGA-3'); *BTN3A2: (5'-* TGAGAAACATCACCTCTGAGCCA; 5'- CCATGAGAAACAGTAAGAGTCGC-3'); *HLA-B: (5'-* GTGTCGGGTCCTTCTTCCA-3'; 5'- CCAATGGGAGTGGGAAGTG-3'); *HOXC8: (5'-*CTCAGGCTACCAGCAGAACC-3'; 5'-TTGGCGGAGGATTTACAGTC-3').

*Generation of Raji target cells for cytotoxicity assays*

To produce lentiviral particles HEK293T cells were co-transfected with the pCMVDR8.74 and pMD2.G packaging vectors, the pHRSIN-CS-Luc-IRES-emGFP plasmid encoding for the

luciferase, and either the pHRSIN-CS-HA-NLRC5-IRES-mTagBFP2 construct expressing NLRC5 or the empty pHRSIN-CS-IRES-mTagBFP2 as control (mock). After transduction, Raji cells double positive for mBFP2 and luciferase expression were isolated using the MoFlo Astrios cell sorter (Beckman Coulter) and were used for the cytotoxicity assays as polyclonal population. To obtain Raji subclones, single-cell subcloning was performed using limiting dilution of the polyclonal Raji cells. In this context, culture medium was supplemented with 2-β-mercaptoethanol (0.05 mM).

CRISPR/Cas9-mediated gene disruption of BTN3A1 was conducted with a combination of two pre-designed chemically stabilized Alt-R CRISPR-Cas9 crRNAs (crRNA XT; IDT) and the Alt-R CRISPR Cas9 system of Integrated DNA Technologies. For duplex formation, the chemically stabilized crRNAs XT Hs.Cas9.BTN3A1.1.AL (5'-ACCAUCAGAAGUUCCCUCCU-3', IDT) and Hs.Cas9.BTN3A1.1.AP (5'-GAUGUGAAGGGUUACAAGGA-3', IDT) were mixed in equimolar amounts with Alt-R CRISPR-Cas9 tracrRNA (IDT) to a final oligo concentration of 44 μM, heated to 95°C for 5 min and cooled down to RT for gRNA duplex formation (crRNA XT:tracrRNA). Alt-R S.p. Cas9 Nuclease V3 protein (IDT) was used at 36 μM. For ribonucleoprotein (RNP) assembly, the gRNA duplex was mixed with Alt-R S.p. Cas9 Nuclease V3 protein at equal volumes and incubated for 20 min at RT. RNP complexes were then stored on ice prior utilization. Electroporation of Raji cells was conducted with the Neon Transfection System (Invitrogen, Life Technologies, 1 pulse of 1,350 V for 30 ms) and in the presence of Alt-R Cas9 Electroporation Enhancer (10.8 μM; IDT). Cells were then transferred into pre-warmed complete medium and incubated at 37°C and 5% CO2.

*Expansion of human Vγ9Vδ2 T cells and cytotoxicity assays*

Human Vγ9Vδ2 T cells were expanded from human PBMCs isolated from healthy donors using a Ficoll-Hypaque gradient. Cells were cultured in RPMI 1640 medium + GlutaMAX (Life Technologies) supplemented with 10% fetal calf serum (FCS), 50 U/ml penicillin, 50 µg/ml streptomycin, 10 mM HEPES buffer, 1 mM sodium pyruvate and 1x MEM non-essential amino acid solution. For γδ T cell expansion, PBMCs were stimulated either with 2.5 µM zoledronate and 50 U/ml rIL-2 or with 1 µg/ml concanavalin A and 10 ng/ml rIL-2 and 10 ng/ml rIL-4. For zoledronate expanded Vγ9Vδ2 T cells, fresh rIL-2 was added every second day over a culture period of 21 days. After 7 days, the expanded Vγ9Vδ2 T cells were additionally split at a 1:2 ratio every second day. 14 days after stimulation, the purity of the expanded Vγ9Vδ2 T cells was evaluated by flow cytometry and cells were MACS-enriched if required (TCRγ/δ+ T Cell Isolation Kit, human, Miltenyi Biotech). For bioluminescence-based killing assays, 10,000 luciferase-expressing NLRC5- or mock-transduced Raji cells were co-cultured with either 10,000 (1:1), 50,000 (5:1) or 100,000 (10:1) expanded Vγ9Vδ2 T cells in triplicates for 24 h at 37°C and 5% CO2 in the presence of 10 µM zoledronate. The proportion of living cells was measured as bioluminescence in relative light units (RLU) at an Infinite 200 PRO plate reader (Tecan) in the presence of the substrate D-firefly luciferin potassium salt (37.5 µg/ml, Biosynth). Specific killing was calculated relative to Raji cells treated either with medium alone (spontaneous death) or with 1% Triton X-100 (maximal death) with the following formula: % specific killing = 100 x (average spontaneous death RLU - test RLU) / (average spontaneous death RLU - average maximal death RLU). Quantification of IFNγ production was performed by co-culturing 10,000 NLRC5- or mock-transduced Raji subclone cells with 100,000 Vγ9Vδ2 T cells (10:1) for 48 h in the presence of 10 µM zoledronate, followed by standard enzyme linked immunosorbent assay (ELISA) from Invitrogen, Life Technologies.

*Bioinformatic analyses of Gambia cohort and tumor datasets*

We selected 32 TCGA cancer types from the TCGA provisional data set collections as available from cBioportal (June 2019). Genetic profiles for mRNA expression, methylation and copy number of selected genes were retrieved using the 'cgdsr' R/Bioconductor package. Adjustment for potential confounding factors (here CD45/PTPRC) was performed using the 'removeBatchEffect' function from the limma R/Bioconductor package using CD45 expression as covariate. For the independent diffuse large B-cell lymphoma (DLBCL) cohort, we downloaded gene expression data (GSE10846) from the NCBI GEO database using the 'GEOquery' R package, and corrected the data for CHOP/RCHOP treatment effects and for CD45 expression. Similarly, for the Gambia cohort, we downloaded the gene expression data (GSE28623) from GEO. For GSE10846 DLBCL, we used the 350 samples with ABC or GCB subtype and batch-corrected for chemotherapy. No batch correction or adjustment for CD45 was deemed to be needed. For continuous variables, statistical correlation and significance was assessed using Spearman's correlation. For 2 group comparisons, the t-test (two-sided, unequal variance) was used to assess differences in means. Statistical analysis was performed using R version 3.5.2 and BioConductor 3.8 on Ubuntu/Linux. Cancer types are abbreviated according to the TCGA database (https://gdc.cancer.gov/resources-tcga-users/tcga-code-tables/tcga-study-abbreviations): dlbc: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma; chol: Cholangiocarcinoma; thym: Thymoma; tgct: Testicular Germ Cell Tumors; thca: Thyroid carcinoma; meso: Mesothelioma; paad: Pancreatic adenocarcinoma; kich: Kidney Chromophobe; lihc: Liver hepatocellular carcinoma; brca: Breast invasive carcinoma; prad: Prostate adenocarcinoma; kirc: Kidney renal clear cell carcinoma; laml: Acute Myeloid Leukemia; cesc:

Cervical squamous cell carcinoma and endocervical adenocarcinoma; lgg: Brain Lower Grade Glioma; ucs: Uterine Carcinosarcoma; gbm: Glioblastoma multiforme; acc: Adrenocortical carcinoma; luad: Lung adenocarcinoma; skcm: Skin Cutaneous Melanoma; ucec: Uterine Corpus Endometrial Carcinoma; kirp: Kidney renal papillary cell carcinoma; lusc: Lung squamous cell carcinoma; pcpg: Pheochromocytoma and Paraganglioma; esca: Esophageal carcinoma; sarc: Sarcoma; uvm: Uveal Melanoma; coad: Colon adenocarcinoma.

*Ethics*

Mice were treated in accordance with the Swiss Federal Veterinary Office guidelines. For expression analyses, PBMCs were isolated from blood donations obtained from the Blood Transfusion Center, Switzerland. For experiments using $V\gamma9V\delta2$ T cells, informed consent was obtained from the donors in accordance with the Declaration of Helsinki and Institutional Review Board approval from the University of Freiburg Ethics Committee (412/9). Human cell lines are established cell lines.

*Statistical analysis*

Statistical analyses were performed using either Prism software (GraphPad version 8.2.0) or R version 3.5.2 and BioConductor 3.8 on Ubuntu/Linux. For 2 group comparisons, t-tests (two-tail, unequal variance) were used to assess differences in means. In case of multiple testing, overall effects were determined by ANOVA and *post hoc* comparisons to the control condition were performed using either Dunnett's or Holm-Sidak method. For continuous variables, statistical correlation and significance was assessed using Spearman's correlation, Bonferroni method was

used to adjust for multiple correlations. Differences were considered significant when $P<0.05$ (*), very significant when $P<0.01$ (**) and highly significant when $P<0.001$ (***).