**Supplementary Material**

Supplementary Figure 1. Study design for CTCL patients and controls.

- 61 patients with CTCL
  - Age average: 45 years
  - Homogeneous gender.
- 12 Control Skin Samples
  - Age average: 45 years
  - Homogeneous gender.
- 13 patients with Atopic Dermatitis (AD)
  - Age average: 45 years
  - Homogeneous gender.
- 10 patients with Psoriasis
  - Age average: 46 years
  - Homogeneous gender.

**Patients included**

- First cohort
  - 14 patients in plaque stage
  - 12 Control Skin Samples

**Analyses**

- Patients used for CIBAs and intracellular cytokine staining experiments.
- Second cohort
  - 23 patients in plaque stage
  - 12 Control Skin Samples
  - 12 patients with AD
  - 10 patients with Psoriasis

- Evaluation of the expression of the molecules in plaque stage.
- Third cohort
  - 23 patients of second cohort
  - 16 patients in plaque stage
  - 8 patients in tumor stage

- Comparison of the expression of the molecules in plaque and tumor stage.
Supplementary Figure 2. Skin lesions of early stage Cutaneous T Cell Lymphoma patients contain a mixed Th1/Th2 cytokine profile.

Cytokines were quantified using Flow Cytomix Multiplex in the supernatants of dermis and epidermis cultures of skin from CTCL plaques (n=23) and CS (n=10). (A) Quantification of IL-2. (B) Correlation between time since disease onset (years) and levels of IL-2. (C) Quantification of IFN-γ. (D) Correlation between time since disease onset (years) and IFN-γ expression. (E) Quantification of IL-4, IL-5, e IL-13. (F) Expression of T-bet and GATA-3 in CD3+ CD4+ T cells in CS (black bars) and the skin of CTCL patients (grey bars) measured by flow cytometry. Bars show mean fluorescence intensity (MFI) in CD3+ CD4+ T cells from CTCL plaques relative to the average in CS. The mean of data was obtained, and statistical significance was calculated using Mann-Whitney’s t-test. Error bars = SEM. *P<0.05; **P<0.005; ***P< 0.001.
Supplementary Figure 3. Skin lesions of early-stage Cutaneous T Cell Lymphoma patients contain large atypical CD4+ IL-4+ T cell in the epidermis.

Intracellular cytokines staining in CD3+ CD4+ cells by flow cytometry. (A) IFN-γ and IL-4 expression in CD4+ cells from CTCL plaque (n=14). (B) Percentage of IFNγ+ CD4+ and IL-4+ CD4+ cells in the dermis and epidermis cultures of CTCL plaques (n=14) and CS (n=6). (C) Histograms of T-bet and GATA-3 in skin T cells. Total cells were recovered after 72 h of culture and left unstimulated (empty histograms) or stimulated with PMA/Iono for 6 hours (filled histograms), in CTCL plaques (red) and CS (blue) (n=5). The mean of data was obtained, and statistical significance was calculated using Mann-Whitney’s t-test. Error bars = SEM. *P<0.05; **P< 0.01.
Supplementary Figure 4. TOX and IL4 are not overexpressed in psoriasis.

Immunofluorescence of TOX (AF-594) and IL-4 (AF-488) in skin lesions derived from psoriasis patients, a benign inflammatory disease. Quantification of the total expression of TOX and IL-4 in CTCL plaques (n=23) and psoriasis (n=10). Arrows indicate coexpression of markers in the different staining. Error bars = SEM. ****P<0.0001.
Supplementary Figure 5. IFNγ is expressed by TOX+ T cells in CTCL plaques.

(A) Representative immunofluorescence microphotographs of the TOX (AF-594) and IFNγ (AF-488) expression in CTCL plaques. (B) IFNγ (AF-594) and (D) IL-13 (AF-488) expression. Quantification of the total expression of (C) IFNγ and (E) IL-13 in the microphotographs of CTCL plaques (n=23) and CS (n=12). Error bars = SEM. ***P<0.001; ****P<0.0001. Scale bar= 20 nm.
Supplementary Figure 6. IL-4 is mainly produced by TOX+CD4+ T cells associated with clonality in skin lesions of CTCL patients in early stages.

(A) Immunohistochemistry (IHC) of the expression of TCRVβ22 in Pautrier microabscesses. (B) Correlation between the percentage of TCRVβ22 and the percentage of cells that express both TOX and TCRVβ22 (n=23) from (B). (C) Representative immunofluorescence (IF) images of TOX (AF-594), TCRVβ22 (AF-488) and IL-4 (AF-647) in plaques of CTCL. (D) Representative IF depicting GATA3 (AF-594) expression in CTCL plaques and CS. Quantification of the total expression of GATA3 in CTCL plaques (n= 23), AD (n=12) and CS (n= 12). (E) Representative IF of NLRP3 (AF-488) in psoriasis and CS. Quantification of the total expression of NLRP3 and the percentage of cells with nuclear NLRP3 in psoriasis (n=10) and CTCL plaques (n=23). Error bars = SEM. ***P<0.001, ****P<0.0001. Scale bar= 20 nm.
Supplementary Figure 7. HTB-176 cell line characterization.

(A) Representative dot plot showing IL-4 production, IL-4⁺ cells are shown in blue. (B) Representative histogram of IL-4 in HTB-176 cells. (C) Quantification of the expression of IL4 and (D) IL-13 in the supernatant of HTB-176 cultures (n=3) from (B). (E) Representative immunofluorescence microphotographs of TOX (AF-488), NLRP3 (AF-594), Karyopherin α2 (AF-594), IFNγ (AF-488), TCRVβ22 (AF-488), IL-13 (AF-594) and GATA3 (AF-594). (F) NLRP3 expression was evaluated by Western blot after NLRP3 silencing with specific
siRNA. Graph showing WB analysis and densitometry. Error bars = SEM. Scale bar, 20 nm. NS, non-stimulated; FMO, fluorescence minus one, siNLRP3, siRNA targeting NLRP3; siScr, siRNA scrambled control; MFI, median fluorescence intensity.

Supplementary Figure 8. Expression of NLRP3 and IL-4 are correlative in T cell lines.

(A) The Jurkat cell line was transfected with siNLRP3 and siScr using Lipofectamine (n=3). Quantification of the total expression of NLRP3, IL-4, IL-13 and IFNγ from immunofluorescence microphotographs. (B) Representative Immunofluorescence of NLRP3
(AF-488) and IL-4 (AF-594) in HTB-176 cells treated with phytohemagglutinin (PHA). Quantification of the total expression of NLRP3 and IL-4 after treatment (n=3). Error bars = SEM. *P<0.05; **P<0.01. Scale bar = 20 nm.

Supplementary Figure 9. Nuclear localization of NLRP3 increases by inhibiting the assembly of the inflammasome. (A) Evaluation of the expression of NLRP3 by Western blot (WB) in the cytoplasmic and nuclear fractions of cell extracts of the HTB-176 cell line treated or not with the inhibitor MCC950 + PMA. (B) Quantification of the relative density of NLRP3 cytoplasmic and nuclear fractions in each condition. (C) Evaluation of the expression of TOX by WB in extracts corresponding to the cytoplasm and nuclear fractions. (D) Quantification of the relative density of TOX in each subcellular fraction. (E) Evaluation of the expression of β-actin by WB in extracts corresponding to the cytoplasm and nuclear fractions. (F) Quantification of the relative density of β-actin in each subcellular fraction.
Supplementary Figure 10. Inflammasome inhibition increases NLRP3 binding to the il-4 promoter in HTB-176 cells.

ChIP assays were performed using an NRLP3 specific antibody. The binding of NRLP3 to the NRLP3-1 site in the il-4 promoter region of HBT-176 cells was estimated in basal or inflammasome inhibiting conditions.
Supplementary Figure 11. In tumor stage of CTCL there is an increase of IL-13 and IFNγ is decreased.

Representative microphotographs of the IL-13 (AF-488) expression in CTCL plaque and tumor stage lesions. Quantification of the total expression of (B) IL-13 in the microphotographs of CTCL plaques (n=27) and tumors (n=8). (C) Representative microphotographs of the IFNγ (AF-594) expression in CTCL plaque and tumor stage lesions. Quantification of the total expression of (D) IFNγ in the microphotographs of CTCL plaques (n=27) and tumors (n=8). Error bars = SEM. *P<0.05; **P<0.01. Scale bar= 20 nm.
Supplementary Figure 12. IL-4 promotes TOX expression and proliferation.

(A) Flow cytometric analysis of the cells treated with IL-4. Histogram and quantification of the MFI of TOX and Ki67 expression (n=4). (B) HTB-176 cells stimulated with PHA and treated with anti-IL-4. Microphotographs depicting the expression of Ki67 (AF-488) and TOX (AF-594). Quantification of the total expression of (C) TOX and (D) Ki67 in the
Supplementary Figure 13. Increased IL-13 and decreased IFNγ associated with CTCL progression.

(A) Representative Immunofluorescence images of the expression of IL-13 (AF-594) and IFNγ (AF-488) in biopsies from representative slow progressor and fast progressor CTCL patients. Quantification of the total expression of (B) IL-13 and IFNγ in the microphotographs of representative slow progressor and fast progressor CTCL patients. Error bars = SEM. **P<0.01, ***P<0.001. Scale bar= 20 nm.
Supplementary video 1. Reconstruction of Z-stack image from a CTCL plaque stage biopsy. Reconstruction of Z-stack images of the expression of NLRP3 (AF-488) and Karyopherin α2 (AF-594) in CTCL plaque biopsy.

Supplementary video 2. Reconstruction of Z-stack image from an Atopic Dermatitis biopsy. Reconstruction of Z-stack images of the expression of NLRP3 (AF-488) and Karyopherin α2 (AF-594) in AD biopsy.

Supplementary video 3. Reconstruction of Z-stack image from HTB-176 cells. Reconstruction of Z-stacks image of the expression of NLRP3 (AF-488) in the HTB-176 cells nonstimulated with MCC950 inhibitor.

Supplementary video 4. Reconstruction of Z-stack image from HTB-176 cells. Reconstruction of Z-stacks image of the expression of NLRP3 (AF-488) in the HTB-176 cells after treatment with MCC950 inhibitor plus PMA.
**Supplementary Table 1.** Demographic and clinical data of CTCL patients with tissue paraffin-embedded

|   | Personal Data | Stage | Time of disease evolution (years) | Affected Body Surface Area (%) | TNM | WHO-EORTC |
|---|---------------|-------|----------------------------------|---------------------------------|-----|------------|
| 1 | Masculine     | 47    | Plaque                           | 8                              | 39.5| IB         |
| 2 | Feminine      | 63    | Plaque                           | 2.5                            | 78  | IB         |
| 3 | Masculine     | 57    | Plaque                           | 7                              | 40  | IB         |
| 4 | Feminine      | 9     | Plaque                           | NA                             | 37  | IB         |
| 5 | Feminine      | 28    | Plaque                           | 1                              | 19.75| IB        |
| 6 | Masculine     | 41    | Plaque                           | 5                              | 50  | IB         |
| 7 | Masculine     | 20    | Plaque                           | 8                              | 24.5| IB         |
| 8 | Feminine      | 29    | Plaque                           | 10                             | 39  | IB         |
| 9 | Masculine     | 37    | Plaque                           | 0.25                           | 59  | IB         |
|   |               |       |                                  |                                 |     | Folliculotropic MF |
| 10| Feminine      | 23    | Plaque                           | 5                              | 26  | IB         |
| 11| Feminine      | 72    | Plaque                           | 10                             | 54.5| IB         |
| 12| Feminine      | 9     | Plaque                           | 9                              | 21  | IB         |
| 13| Feminine      | 29    | Plaque                           | 7                              | 35.5| IB         |
| 14| Masculine     | 41    | Plaque                           | 1                              | 68  | IB         |
|   | Gender    | Age | Plaque Type | Value1 | Value2 | IB | MF     |
|---|-----------|-----|-------------|--------|--------|----|--------|
| 15| Masculine | 25  | Plaque      | 2      | 79     | IB | MF     |
| 16| Feminine  | 64  | Plaque      | 30     | 76     | IB | MF     |
| 17| Masculine | 40  | Plaque      | 8      | 73     | IB | MF     |
| 18| Masculine | 59  | Plaque      | 19     | 33     | IB | MF     |
| 19| Feminine  | 65  | Plaque      | 60     | 25     | IB | MF     |
| 20| Feminine  | 58  | Plaque      | 0.25   | 25.75  | IB | MF     |
| 21| Masculine | 43  | Plaque      | 0.67   | 39     | IB | Folliculotropic MF |
| 22| Feminine  | 61  | Plaque      | 7      | 80     | IIA| MF     |
| 23| Feminine  | 60  | Plaque      | 11     | 70     | IIA| MF     |
| 24| Feminine  | 14  | Plaque      | 0.13   | 59     | IB | MF     |
| 25| Masculine | 25  | Plaque      | 10     | 75     | IB | MF     |
| 26| Masculine | 19  | Plaque      | 1      | 20     | IB | MF     |
| 27| Masculine | 37  | Plaque      | NA     | 3.5    | IA | MF     |
| 28| Masculine | 35  | Plaque      | 2      | 13.5   | IB | MF     |
| 29| Masculine | 49  | Plaque      | 20     | 45     | IB | MF     |
| 30| Feminine  | 38  | Plaque      | 12     | 28     | IB | MF     |
| 31| Feminine  | 18  | Plaque      | 15     | 60.25  | IB | MF     |
| 32| Masculine | 40  | Plaque      | 6      | 40     | IB | MF     |
| 33| Feminine  | 48  | Plaque      | 20     | 81     | IIA| MF     |
|   | Gender   | Age   | Tumor Type  | Stage | Subtype     |
|---|----------|-------|-------------|-------|-------------|
| 34 | Masculine| 34    | Plaque      | 10    | 50          | IB | MF |
| 35 | Masculine| 30    | Plaque      | 2     | 22.5        | IB | MF |
| 36 | Masculine| 39    | Plaque      | 5     | 60          | IIA| MF |
| 37 | Masculine| 57    | Plaque      | 2     | 1           | IA | MF |
| 38 | Feminine | 59    | Plaque      | 5     | 72          | IB | MF |
| 39 | Feminine | 39    | Plaque      | 5     | 60          | IIA| MF |
| 40 | Masculine| 57    | Plaque and tumor | 39 | 40 | IIB | Folliculotropic MF |
| 41 | Masculine| 48    | Tumor       | 1     | 5           | IIB| MF |
| 42 | Feminine | 65    | Tumor       | 3     | 100         | IVB| SS |
| 43 | Feminine | 73    | Tumor       | 4     | 80          | III| MF |
| 44 | Masculine| 42    | Tumor       | 2     | 20          | IIB| MF |
| 45 | Feminine | 60    | Tumor       | 11    | 70          | IIB| MF |
| 46 | Masculine| 39    | Tumor       | 7     | 60          | IIB| MF |
| 47 | Masculine| 41    | Tumor       | 5     | 50          | IIB| Folliculotropic MF |

NA: Not Available

**Supplementary Table 2.** Demographic and clinical data of CTCL patients without tissue paraffin-embedded
Patients used for CBA and intracellular cytokine staining experiments for whom we do not have available tissue paraffin-embedded

| Gender      | Age | Stage | Time of disease evolution (years) | Affected Body Surface Area (%) | TNM | WHO-EORTC |
|-------------|-----|-------|----------------------------------|--------------------------------|-----|-----------|
| Feminine    | 18  | Plaque| 9                                | 68                             | IB  | MF        |
| Masculine   | 64  | Plaque| 10                               | 38                             | IB  | MF        |
| Masculine   | 38  | Plaque| 0.5                              | 64                             | IB  | MF        |
| Feminine    | 9   | Plaque| 4                                | 20                             | IB  | MF        |
| Feminine    | 59  | Plaque| 19                               | 33                             | IB  | MF        |
| Masculine   | 63  | Plaque| 0.167                            | 85.5                           | IIA | MF        |
| Masculine   | 78  | Plaque| 9                                | 80                             | IIA | MF        |
| Feminine    | 41  | Plaque| 4                                | 93                             | IIA | MF        |
| Masculine   | 43  | Plaque| 0.667                            | 39                             | IB  | MF        |
| Masculine   | 38  | Plaque| 0.5                              | 64                             | IB  | MF        |
| Masculine   | 12  | Plaque| 4                                | 33                             | IB  | MF        |
| Feminine    | 32  | Plaque| 12                               | 55                             | IB  | MF        |
| Feminine    | 54  | Plaque| 26                               | 40                             | IB  | MF        |
| Masculine   | 69  | Plaque| 40                               | 29                             | IB  | MF        |
### Supplementary Table 3. Antibodies used for flow cytometry and immunofluorescence.

| Antigen | Manufacturer                          | Clone  | Catalog Number | RRID     | Isotype          | Fluorochrome/Use         |
|---------|---------------------------------------|--------|----------------|----------|------------------|--------------------------|
| CD3     | Biolegend                             | OKT3   | 317307         | AB_571912| Mouse IgG2a kappa| Phycoerythrin            |
| CD4     | BD Bioscience                         | RPA-T4 | 560158         | AB_1645478| Mouse IgG1 kappa | APCH7                    |
| IL-4    | Biolegend                             | MP4-25D2 | 500822    | AB_961404| Mouse IgG1 kappa | PerCP-Cy5.5              |
| T-bet   | Biolegend                             | 4B10   | 644805         | AB_1595593| Mouse IgG1 kappa | PerCP-Cy5.5              |
| GATA3   | BD Bioscience                         | L50-823 | 560163   | AB_1645302| IgG1             | Alexa Fluor 488          |
| IFNγ    | BD Bioscience                         | B27    | 557643         | AB_396760| Mouse IgG1 kappa | PECy7                    |
| TOX     | eBioscience                           | TXRX10 | 50-6502-82    | AB_2574265| Rat IgG2a kappa  | eFluor 660               |
| Ki67    | Biolegend                             | Ki67   | 350522         | AB_2563863| Mouse IgG1 kappa | Brilliant Violet 605     |
| IL-4    | Santa Cruz Biotechnologies, TX, USA   | C-19   | sc-1260        | AB_2128970| Goat polyclonal  | IF                       |
| IL-4    | RyD Systen, MI, USA                   | #3007  | MAB304         | AB_2889404| Mouse monoclonal | Neutralization            |
| IL-13   | Abcam, Cambridge, UK                  | Polyclonal | ab106732 | AB_10867235| Rabbit polyclonal | IF                       |
| Protein | Vendor                  | Type       | Catalog Number     | Antibody Code | Species       | Applications |
|---------|-------------------------|------------|--------------------|---------------|---------------|--------------|
| TOX     | Thermo Scientific, MA, USA | Polyclonal | PA5-30328          | AB_2547802    | Rabbit       | IF, WB, IHC  |
| TCRβ22  | Beckman Coulter, CA, USA | IMMU 546   | IM1484             | AB_131022     | Mouse        | IF, FC, IHC  |
| IFNγ    | Biolegend, CA, USA      | MD-1       | 507501             | AB_2122340    | Mouse        | IF           |
| NLRP3   | RyD System, MI, USA     | #768319    | MAB7578            | AB_2889405    | Rat          | IF, WB       |
| Karyopherin α2 | BD Bioscience, CA, USA | C-2        | 610485             | AB_397855     | Mouse        | IF           |
| GATA-3  | Abcam, Cambridge, UK    | Polyclonal | ab106625           | AB_10887935   | Rabbit       | IF           |
| IRF4    | Biolegend, CA, USA      | IRF4.3E4   | 646411             | AB_2728477    | Rat IgG1 kappa | ChIP        |
| Rat IgG (H+L) | Jackson Immunoresearch, PA, USA | Polyclonal | 712-585-153        | AB_2340689    | Donkey     | IF, AF 594   |
| Rabbit IgG (H+L) | Jackson Immunoresearch, PA, USA | Polyclonal | 711-545-152        | AB_2313584    | Donkey     | IF, AF 488   |
| Rabbit IgG (H+L) | Jackson Immunoresearch, PA, USA | Polyclonal | 711-585-152        | AB_2340621    | Donkey     | IF, AF 594   |
| Goat IgG (H+L) | Jackson Immunoresearch, PA, USA | Polyclonal | 705-065-147        | AB_2340397    | Donkey     | IF, AF 647   |
| Mouse IgG (H+L) | Jackson Immunoresearch, PA, USA | Polyclonal | 715-605-151        | AB_2340863    | Donkey     | IF, AF 647   |
## Supplementary Material

| Rat IgG (H+L) | Thermo Scientific, MA, USA | Polyclonal | A-11006 | AB_2534074 | Goat polyclonal | IF, AF 488 |
|--------------|----------------------------|------------|---------|------------|----------------|-----------|
| Rabbit IgG | Leica Biosystems | Polyclonal | DS9800 | AB_2891238 | Goat polyclonal | IHC |
| Bond Polymer Refine Detection | | | | | | |
| Mouse/Rabbit ImmunoDetector Link & AP Label | Bio Science | Polyclonal | BSB-0351 | AB_2891237 | Goat polyclonal | IHC |
| | | | | | | |
| Beta-Actin | GeneTex | Polyclonal | GTX109639 | AB_1949572 | Rabbit polyclonal IgG | WB |
| | | | | | | |
| IRDye 800CW Goat anti-Rat | LI-COR Biosciences | Polyclonal | 926-32219, 926-32211 | AB_1850025, AB_621843 | Goat polyclonal IgG | WB |
| IRDye 800CW Goat anti-Rabbit | LI-COR Biosciences | Polyclonal | 926-32219, 926-32211 | AB_1850025, AB_621843 | Goat polyclonal IgG | WB |

### Supplementary Table 4. Primers used for PCR

| Gene | Forward primer | Reverse Primer |
|------|----------------|----------------|
| NLRP3 (site 1) | ATACCACATGATCTCACGCATA | CACCATGTCGTACAATGGCT |
| NLRP3 (site 3) | CAGGTGCCCTGTAGTCCCAGCT | AGTGCAGTGGCGTGATCTTAGTT |
| IRF4 | GTGACAGAGCAAGATTCCATCT | TGGACACAATAAGGTGCTCATT |
| IgF2 | CAGGCTCCCCAAAATCTA | GGGAACATAGAGAAAGG |

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