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Supplementary Information for

The CysLT$_2$R receptor mediates leukotriene C$_4$ driven acute and chronic itch

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Other supplementary materials for this manuscript include the following:

Movie S1

Materials and Methods

Mice

All animal experiments were approved by the Harvard Medical School Institutional Animal Care and Use Committee (IACUC). Mice are housed in a specific-pathogen-free animal facility at Harvard Medical School. C57BL/6J, *Trpv1*$^{-/-}$ and *Kit*$^{Wsh/Wsh}$ mice were purchased from Jackson Laboratories and bred in house. *Cysltr2*$^{-/-}$ and *Cysltr1*$^{-/-}$ mice were originally housed at BWH and transferred to Harvard Medical School for subsequent breeding and experiments. For the MC903 model, the AEW model, alloknesis and cheek injections of 48/80, CQ and histamine, balanced numbers of male and female mice were used. For cheek injections of CysLTs, *Alternaria* and IL31, male mice were used. Age-matched, 6- to 14-week-old mice were used for all experiments unless otherwise specified.

BM Chimera Generation

For BM isolation tibia, femur and hips of both sides of either WT (C57BL/6) or *Cysltr2*$^{-/-}$ mice 8-12 weeks of age were dissected and cleaned from all soft tissue. Bones were crushed with mortar and pestle in 25 ml ice cold sterile RPMI/10% FBS. After flushing the bones with the media by pipetting up and down 10-20 times with a 25 ml serological pipette, cell suspension was filtered through a 70µm cell strainer. BM cells were centrifuged at 1300rpm for 5 min and resuspended in 4 ml Red blood cell lysis buffer (Sigma Aldrich) and kept 1 min on ice before diluting in 50 ml ice cold RPMI/ 10% FBS. BM cells were centrifuged 1300rpm for 5 min and resuspended in ice cold HBSS. Recipient animals (WT or *Cysltr2*$^{-/-}$) 6-12 weeks of age underwent lethal irradiation on the day of the transplantation with a total of 9.5-10 Gy. 3-5 h after lethal irradiation, mice were anesthetized with isoflurane for retrobulbar injection of 3 x 10^6 BM donor cells in 100 µl HBSS. After 6-8 weeks mice were ready for behavioral experiments, BM chimera studies were conducted at Mass General Hospital in Dr. Carrie Sokol's laboratory and recordings were performed blinded in a normal light environment.

Mouse RNAScope *in situ* hybridization analysis

DRGs were dissected from mice, embedded in Optimal Cutting Temperature (OCT, Sakura Finetek, Cat# 4583) and stored at -80°C until processing. Cryosections of 16 µm were cut onto Superfrost Plus slides (Thermo Fisher). Multi-labeling ISH was performed using the RNAscope technology (ACD) according to the manufacturer's instructions. Probes against mouse *Cysltr2*, *Trpv1*, *Nppb*, *Mrgpra3*, *Mrgprd*, *Hrh1*, *Tubb3* and *Scn10a* in conjunction with the RNAscope multiplex fluorescent development kit were used. Images were collected on an Olympus FV1000 single point scanning confocal scanhead with 4x multi-alkali PMTs mounted on an IX81 inverted stand equipped with a Prior ProScan II Motorized stage. Images were collected using either an UPlan S Apo 10x/0.40 Air DIC or an UPlan X Apo 20x/0.80 Air DIC objective. Images were captured using FV10-ASW acquisition software for Acquisition.

Human DRG RNAScope analysis

Tissue preparation

Procurement procedures for all human tissue were approved by the Institutional Review Boards at the University of Texas at Dallas and samples were de-identified prior to use in the study. Human lumbar dorsal root ganglions were frozen on dry ice and stored at -80°C. Donor information is provided in **Table 1**. Collection of tissues from organ donors was done within 2 hours of cross-clamp. The human DRGs were progressively embedded in OCT in a cryomold. Tissues were

sectioned with a cryostat at 20 µm onto SuperFrost Plus charged slides. Sections were swiftly thawed in order to adhere to the slide but were immediately returned to the -20°C cryostat chamber until completion of sectioning. The slides were then immediately utilized for histology.

RNAscope in situ hybridization

RNAscope *in situ* hybridization multiplex version 1 was performed following instructions from Advanced Cell Diagnostics (ACD) using a 50:1:1 dilution of *CYSLTR2* (ACD Cat # 543481; Channel 1), *NPPB* (ACD Cat # 448511-C2; Channel 2), *TRPV1* (ACD Cat # 415381-C3; Channel 3) probes. Reagent AMP-4 ALT C (Channel 1 = Atto 550, Channel 2 = Atto 647, Channel 3 = Alexa 488) was used for all experiments. Slides were cover-slipped with Prolong Gold Antifade mounting medium.

Tissue Quality Check

RNA quality was checked in human DRGs using a positive control probe cocktail (ACD) which contains probes for high, medium and low-expressing mRNAs that are present in all cells (ubiquitin C; DNA-directed RNA polymerase II subunit RPB1; Peptidyl-prolyl cis-trans isomerase B). To generate experimental data, DRGs showing signal for all 3 positive control probes were used. A negative control probe was used to check for non-specific/background label targetting the bacterial DapB gene (ACD).

Image Analysis

DRG sections were imaged at 20X magnification on an Olympus FV3000 confocal microscope. 3 20X images were acquired of each human DRG section, and 3 sections were imaged per human donor. The acquisition parameters were set based on guidelines for the FV3000 provided by Olympus. In particular, the gain was kept at the default setting 1, HV ≤ 600, offset = 4, and laser power ≤ 20%. The raw image files were pseudocolored, brightened and contrasted in Olympus CellSens software (v1.18), and then analyzed manually one cell at a time for expression of each gene target. Cell diameters were measured using the polyline tool. Total neuron counts were acquired by counting all neurons that were clearly outlined by DAPI (satellite cell) signal and all of the probe-labeled neurons and contained lipofuscin in the overlay image.

Signal that auto-fluoresced in all 3 channels and/or large globular structures (appear white in the overlay images) were considered to be background lipofuscin and was not analyzed. Aside from adjusting brightness/contrast, no digital image processing was performed to subtract background. While optimization with automated imaging analysis tools wa sattempted for our purposes, these tools were designed to work with fresh, low background rodent tissues, not human samples taken from older organ donors. As such, we implemented a manual approach in our imaging analysis in which we used our own judgement of the negative/positive controls and target images to assess mRNA label. Images were not analyzed in a blinded fashion.

Donor #	DRG	Sex	Age	Surgery/Cause of Death
1	Lumbar	Female	34	Opioid overdose
2	Lumbar	Male	29	Head trauma
3	Lumbar	Female	44	Cardiac arrest

Table 1. Human DRG tissue Information

Behavioral analysis

For all behavior experiments, experimenters were blinded to experimental groups and/or genotypes. Recording of behaviors were performed with an experimental setup that enables to record the mice in the dark in an experimenter-free environment: infrared Behavioral Observation Box (iBOB, Crimson Scientific) unless otherwise specified. There are two major chambers separated into 4 quadrants each in iBOB, one for habituation and the other for recordings/measurements, both outfitted with infrared LEDs. In the recording chamber, mice are recorded from high resolution video camera from below the glass chamber **(Supplemental video 1)**. The actual behavioral analysis is performed post-recording by scoring of these videos by blinded observers.

Acute itch / pain behavior

Cheeks of the mice were shaved at least 2 days prior to the experiment. Mice were then habituated in the iBOB chamber for 30-90 minutes. They were then injected in the cheek intradermally with a volume of 20µl with Leukotriene C_4 (Cayman Chemical, 20210), Leukotriene D_4 (Cayman Chemical, 20310), N-methyl Leukotriene C_4 (Cayman chemical, 13390), histamine (Sigma, H7125), chloroquine (Sigma, C6628), compound 48/80 (Sigma, C2313), interleukin-31 (Sigma, SRP3209) or *Alternaria alternata* extract (Greer laboratories). HC-030031 (Sigma, H4415) was dissolved in methylcellulose 0.5% and sonicated (Sonic Dismembrator 550, Fisher Scientific).

Mice were then were placed back in the iBOB chamber in the dark and recorded with an infrared camera situated below the mice for 30-90 minutes depending on the experiments (see legends). Itch was scored either as total number of hindpaw-scratching bouts or total time spent scratching, with 'one scratching bout' defined as the action whereby the mouse raises its hind leg to scratch, and then lowers it back. For total time spent scratching, blinded observers used a timer to start and stop the timing whenever the mice showed active hindpaw scratching during the total 30-minute period. As described in (1), pain was scored in cheek injections experiments by counting the numbers of forepaw wipings.

Bout duration quantification

Duration of individual bouts was measured, and bouts were then classified in 3 categories according to their length: <0.3s, 0.3s-1s and 1s. Bout durations was reported as the averaged total number of bouts per mouse or the averaged percentage of bouts per mouse.

Touch-induced itch (alloknesis)

For touch-induced itch, the napes of neck of the mice were shaved 2 days prior to the experiment. Mice were habituated to the chambers for an hour, then they were injected intradermally in the back with a volume of 50 µl. The area was mechanically stimulated using a 0.07g Von Frey filament for 1 second 3 times in a row, with this sequence repeated 3 times, and the scratching responses was recorded out of a total of 9.

MC903 model of chronic itch

Mice were habituated to the iBOB chambers prior to the start of the experiment. Mice were anesthetized with isoflurane; if required, ear thickness was recorded as the average of 3 measurements were taken using a digital thickness gage (Mitutoyo). Then 20 µl of either vehicle (ethanol 95%) or 2 nmol of MC903 (Tocris, 2700) were applied to the front and the back of the ear daily for 12 days. On days where the scratching behavior was assessed, the recordings were performed before the daily application.

Dry-skin model (acetone-ether-water)

Dry-skin-evoked itch behaviors assessment was carried out as previously described (2). Briefly, the mouse back was shaved and treated twice daily with a piece of cotton immersed in either a 1:1 mixture of acetone and ether or water for 15s, directly followed

in both cases the application of a piece of cotton immersed in water for 30s for a duration of 5 days.

Histology

Mice were euthanized by CO_2 inhalation and skin samples were dissected and post-fixed in PBS/4% paraformaldehyde solution (PFA, Sigma, Cat# P6148) at 4°C before being embedded in paraffin, sectioned, and stained using Hematoxylin and Eosin (H&E) or Toluidine Blue by the Harvard Medical School Rodent Histopathology Core. Stained sections were imaged by light microscopy on an Eclipse Ti-S/L100 inverted microscope (Nikon), and images collected by NIS-Elements AR software.

Cysteinyl leukotriene detection

Whole ears from mice were collected and CysLTs generation was measured in acetone-precipitated homogenates by a commercially available enzyme immunoassay (ELISA) according to the manufacturer's protocol (Cayman) based on competition between CysLTs and CysLT-acetylcholinesterase conjugate for a limited amount of CysLT ELISA Monoclonal Antibody. The detection lower limit was 60 pg/ml, and the following reported reactivity are leukotriene C_4 (100%), leukotriene D_4 (100%), leukotriene E_4 (79%), 5,6-DiHETE (3.7%), leukotriene B_4 (1.3%), 5(S)-HETE (0.04%), and arachidonic acid (<0.01%).

Mass spectrometry

Samples preparations

Samples were cut in 1-3 mm squares and transferred to screw-top vials with garnet pieces in 1 ml methanol and 100 ul of internal standard solution (D4-d5 199nM in 2:1 Mobile phase A:mobile phase B). Samples were homogenized in a tissueLyser LT (Qiagen, 20min at 50Hz) before incubation for 10 min in an ultrasound bath. After centrifugation, the supernatants were dried down under nitrogen flow and reconstituted in 100ul of 2:1 Mobile phase A:mobile phase B. A standard curve was prepared by a eight level serial dilution (1/5, starting at 1uM) for all compounds (Cayman), in the same solution as the samples (2:1 A:B).

LCMS

Samples were analyzed on an ultimate 3000 LC coupled with a Q-exactive plus mass spectrometer (ThermoFisher), with a method based on (3, 4). 5 ul of samples were injected on a Kinetex C18 column (2.6u, 150x2.1 mm, Phenomenex), maintained at 30°C. The LC mobile phases were A: 0.5% acetic acid, 0.07% ammonium hydroxide in water with a final pH of 5.5 and B: Acetonitrile:Methanol 65:35. The gradient was as follow: starting at 25% B, to 85% B in 12 min, then to 100% B in 1 min, followed by 7 min at 100%, and 5 min re-equilibration at 25% B. The flow rate was maintained at 0.2 mL/min. The mass spec was set to positive polarity, with a resolution of 70000 and scanning mz 300 to 700. Compounds were quantified by integrating the area under the peaks of the compounds (extracted accurate mass at 5ppm, retention time confirmed by the authentic standards) and using the ratio with the IS to calculate the absolute concentration, using the calibration curve.

Flow cytometry

Ears were mechanically separated and minced, then digested in RPMI (Life Technologies) containing 10% FBS, 150 μg/ml liberase (Roche) and 50 μg/ml DNase at 37 °C for 90 min at 200 r.p.m. The cell mixture was filtered through a 70-μm cell strainer (BD). Cells were

washed 3 times in FACS buffer (PBS, 2% FBS, 0.5% BSA and 0.5 mM EDTA) and treated with Fc Block (Biolegend). Incubations with antibody cocktails were conducted on ice for 30 min, and samples were subjected to two washes and resuspension in PBS with 2% PFA and 1 mM EDTA before flow cytometry. Antibodies used for staining included: anti-CD45-Alexa Fluor 700 (Biolegend), anti-CD3-PerCP-eFluor 710 (eBioscience), anti-IgE-Brilliant Violet 421 (BD Biosciences), anti-CD117-APC (BioLegend), anti-Ly-6G-FITC (Biolengend), anti-Ly-6C-PE-Dazzle 594 (Biolegend), anti-Siglec-F-PE (BD Biosciences), anti-CD11b-Brilliant Violet 605 (Biolengend), anti-GR1-FITC (Biolegend), anti-CD11c-PE (Biolegend),anti-MHCII-FITC (Biolegend) and anti-F4/80-APC (Biolegend). Cells were fixed using BD Cytofix™ Fixation Buffer. Flow cytometry was conducted on an LSRII flow cytometer (BD). Data were collected with BD DIVA software, and files were analyzed with FlowJo (Treestar, version 10.0.8r1). A live-cell stain (eFluor 506, ebioscience) was used to exclude dead cells. Positive staining and gates for each fluorescent marker was defined by comparing full stain sets with fluorescence minus one (FMO) control stain sets.

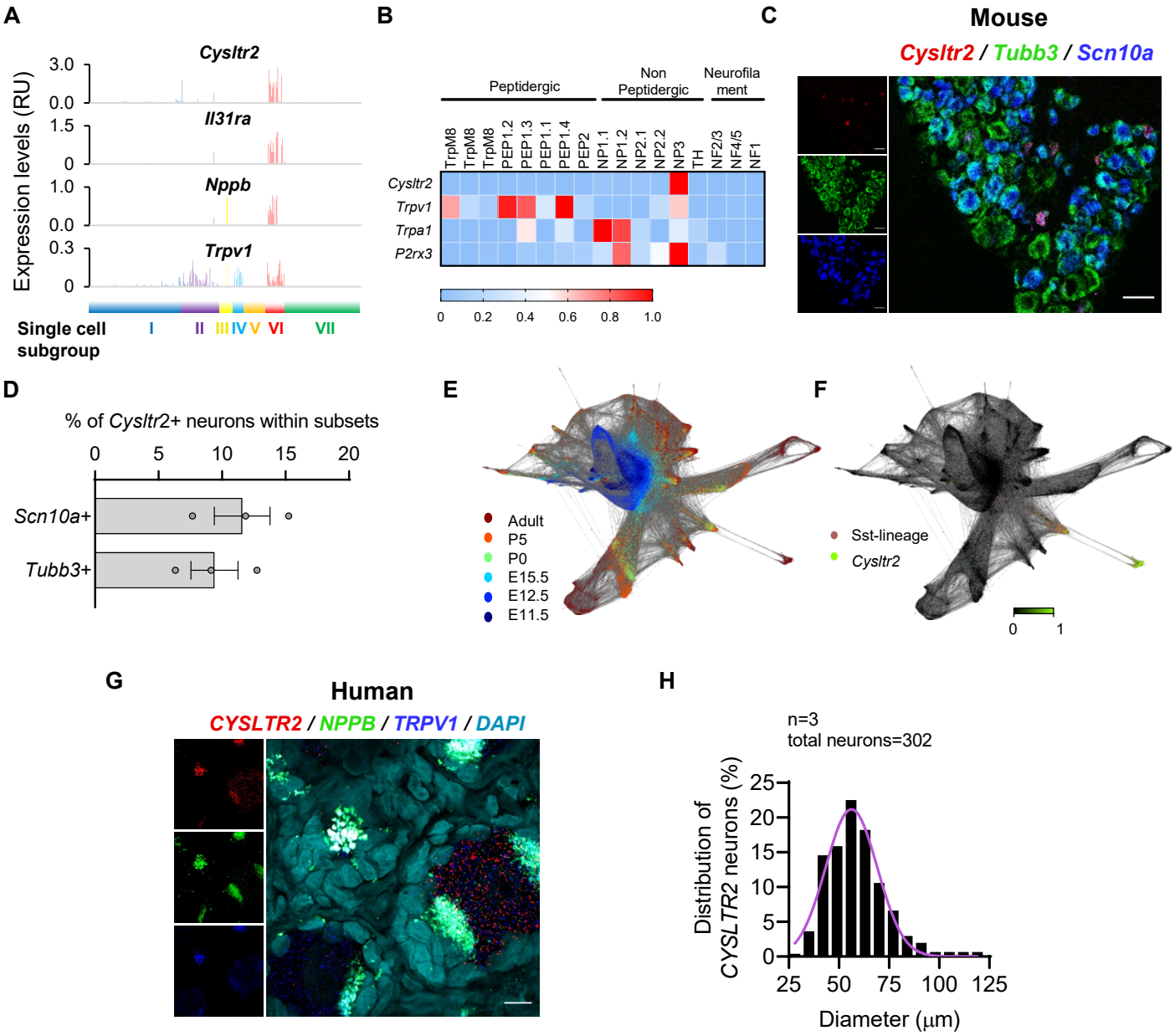

Figure S1. Expression data of *Cysltr2* transcript in mouse and human DRGs

(A) Expression of selected transcripts in mouse DRG neuron populations clustered into functional subsets based on single-cell microarray data. Full dataset and methods are available (5).
(B) Expression of TRP channels transcripts in mouse DRG neuron populations clustered into functional subsets based on single-cell RNA-seq data. Full dataset and methods are available (6).
(C) ISH done with RNAscope in mouse DRG (*Cysltr2* – red, *Tubb3* – green, *Scn10a* - blue). Scale : 50um.
(D) ISH quantification (n= 3 mice).
(E-F) Force-directed layout of DRG sensory neurons from scRNA-seq data overlaid with **(E)** time points and **(F)** SST-lineage neurons (red) and expression of Cysltr2 (green). Full data and methods available in (7).
(G) High magnification image displaying *CYSTLR2* positive neurons. Scale : 10 um.
(H) Histogram with Gaussian distribution displaying the size profile of all *CYSLTR2*-positive neurons in human DRG.
Values represented as mean ± SEM.

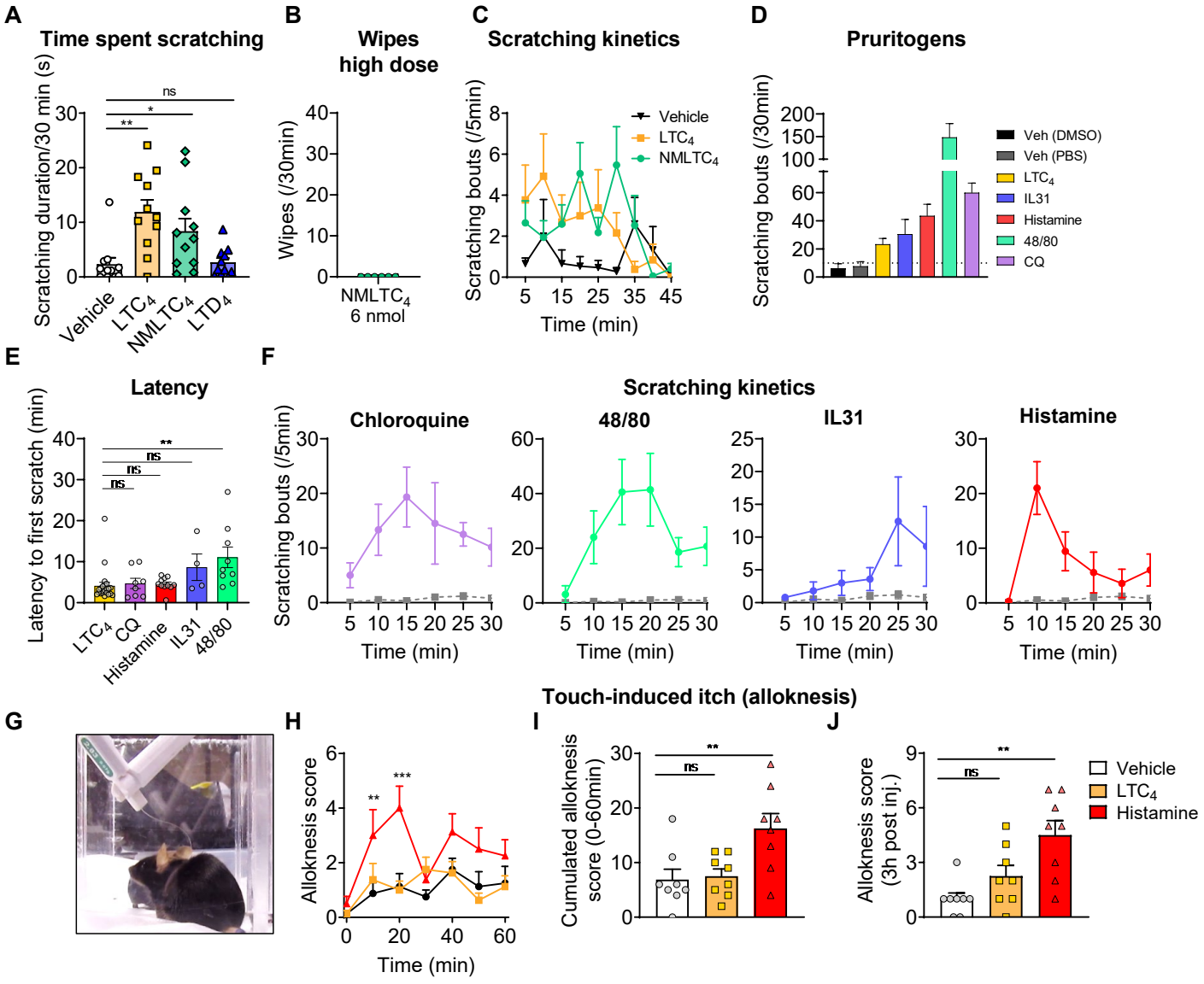

Figure S2. Acute itch caused by CysLTs

(A) Time spent scratching in response to vehicle, LTC_4, $NMLTC_4$ and LTD_4 at 0.6 nmol (n=11).
(B) Wiping responses, indicative of pain, to $NMLTC_4$ at 6 nmol (n=6).
(C) Detailed scratching bouts kinetics over 45 minutes in response to vehicle, LTC_4 (0.6 noml) and $NMLTC_4$ (0.6 nmol) (n=13-17).
(D) Scratching bouts in response to vehicle (PBS), vehicle (DMSO), LTC_4 (0.6 nmol), IL-31 (0.02 nmol), histamine (100 ug), compound 48/80 (100 ug), chloroquine (CQ – 200 ug) (n=5-11).
(E) Latency to first scratching bout after injection with pruritogens.
(F) Scratching bouts kinetics of pruritogens over 30 minutes (n=5-11). Vehicle in dotted lines pooled from several experiments.
(G-J) Touch-induced itch (alloknesis) following the intradermal injection in the back skin of vehicle, histamine (100 ug) or LTC_4 (0.6 nmol) over 60 minutes (n=8).
(G) Picture of mouse stimulated by a Von Frey hair (0.07 g) on the back, scratching responses were recorded out of 9 stimulations per time point.
(H) Time course of the alloknesis score (out of 9) during the first hour post injection. *(* Vehicle vs histamine)*.
(I) Cumulated alloknesis score over the first hour post injection.
(J) Alloknesis score at the three hours timepoint post injection.
*Values represented as mean + SEM. One-way ANOVA with Dunnett's posttest (A, E, I, J), repeated measures two-way ANOVA, Sidak's posttest (H); ns: non-significant, *p<0.05; **p<0.01*

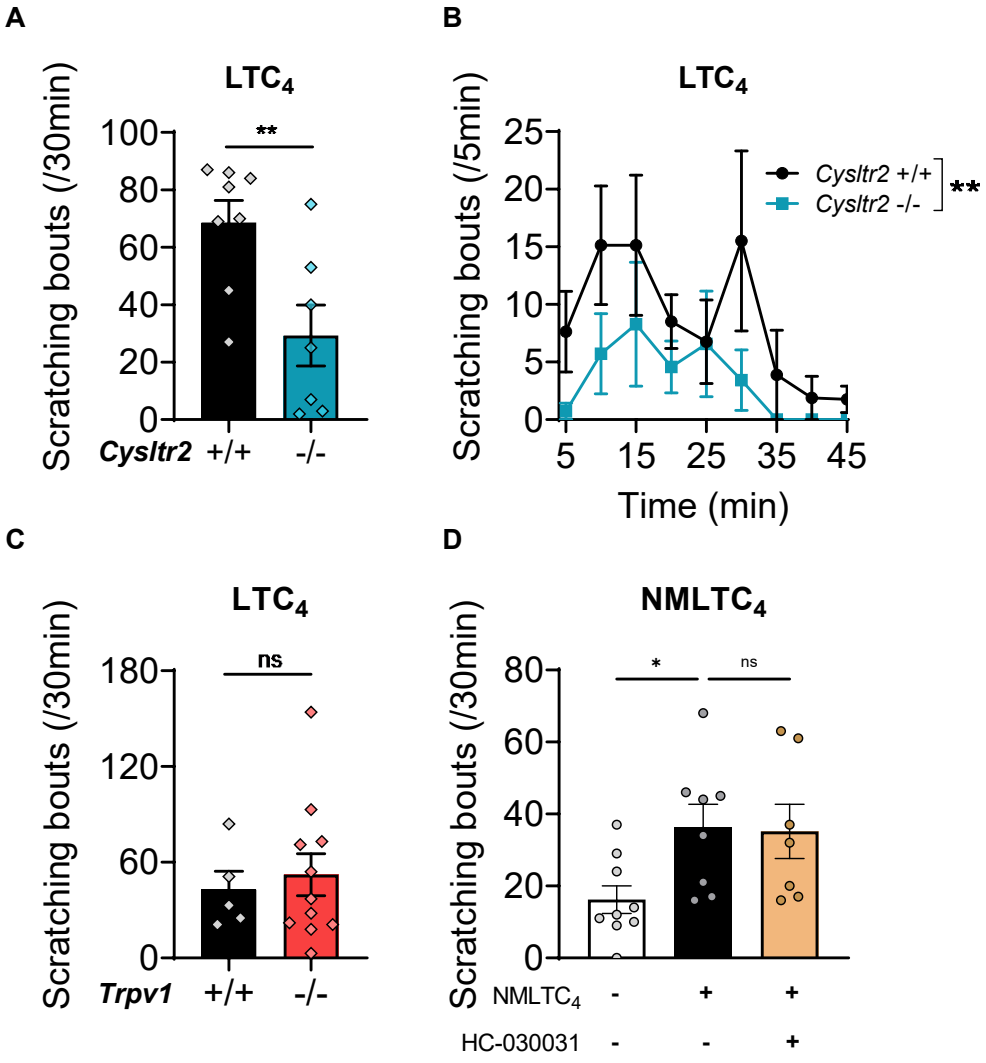

Figure S3. Acute itch caused by LTC_4 is dependent on $CysLT_2R$ receptor

(A-B) Scratching bouts induced by intradermal cheek injection in *Cysltr2*$^{+/+}$ or *Cysltr2*$^{-/-}$ mice of LTC_4 (0.6 nmol) **(B)** and detailed scratching bouts kinetics over 45 minutes **(B)**.
(C) Scratching bouts induced by intradermal cheek injection of LTC_4 (0.6 nmol) in *Trpv1*$^{-/-}$ mice.
(D) Effect of TrpA1 antagonist HC-030031 (100 uM) on scratching when injected intradermally 5 minutes before $NMLTC_4$ (0.6 nmol).
*Values represented as mean ± SEM. Repeated measures two-way ANOVA, Sidak's posttest (B), and unpaired t-test (A, C); one-way ANOVA with Dunnett's post test (D); ns: non-significant, *p<0.05; **p<0.01, ***p<0.001*

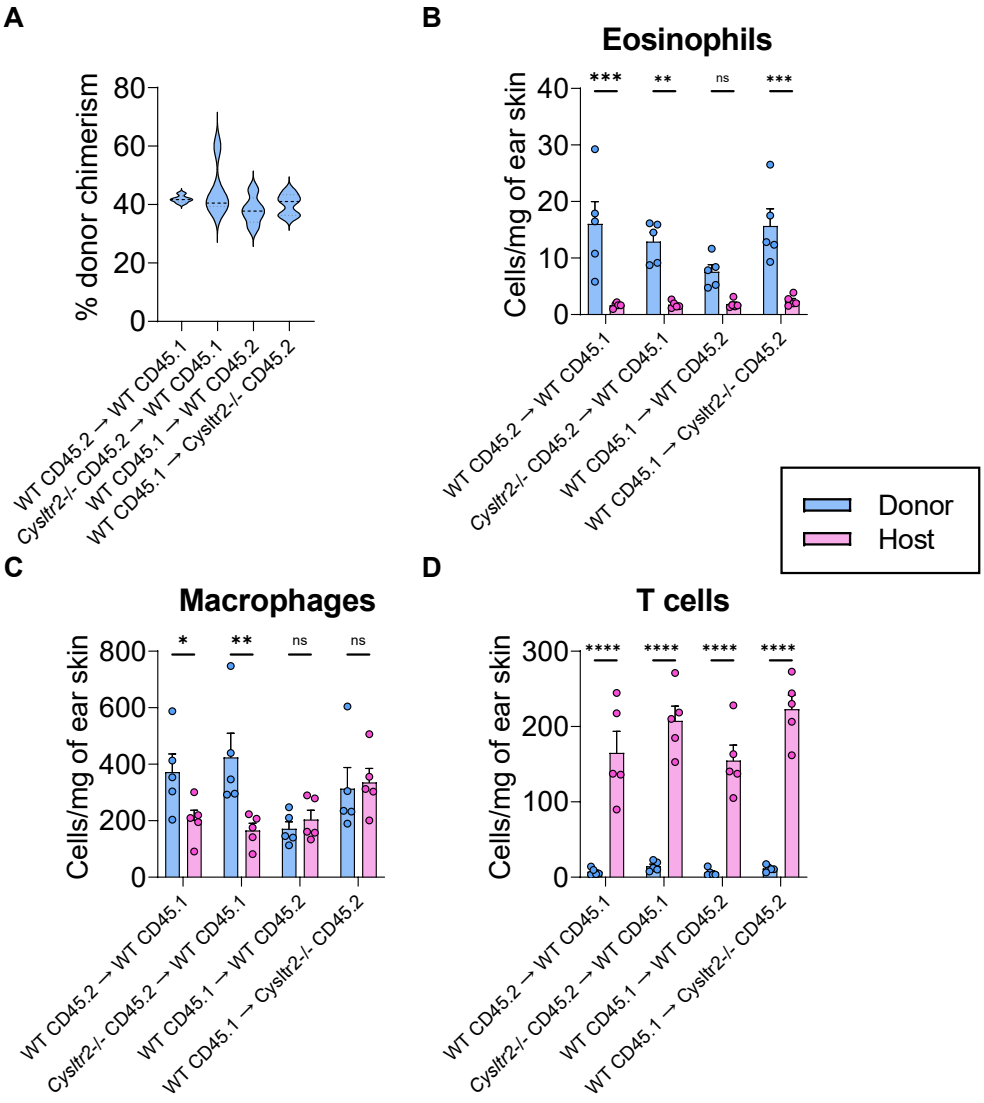

Figure S4. Quantification of donor and dost derived leucocytes in the skin of BM-transplanted mice

(A) Violin plot showing the % of donor derived leucocytes from all leucocytes in the skin of recipient animals measured by flow cytometry (CD45.1/CD45.2 chimerism).
(B-D) Absolute numbers of donor and host derived **(B)** eosinophils (CD45+/Gr1 low/ Siglec F +), **(C)** macrophages (CD45+, CD11b+ F4/80+) and **(D)** T cells (CD45+/CD3+) in 1 mg of skin measured with flow cytometry.
*Values represented as mean + SEM. Repeated measures two-way ANOVA, Sidak's posttest , ns: non-significant, *p<0.05; **p<0.01; ****p<0.0001*

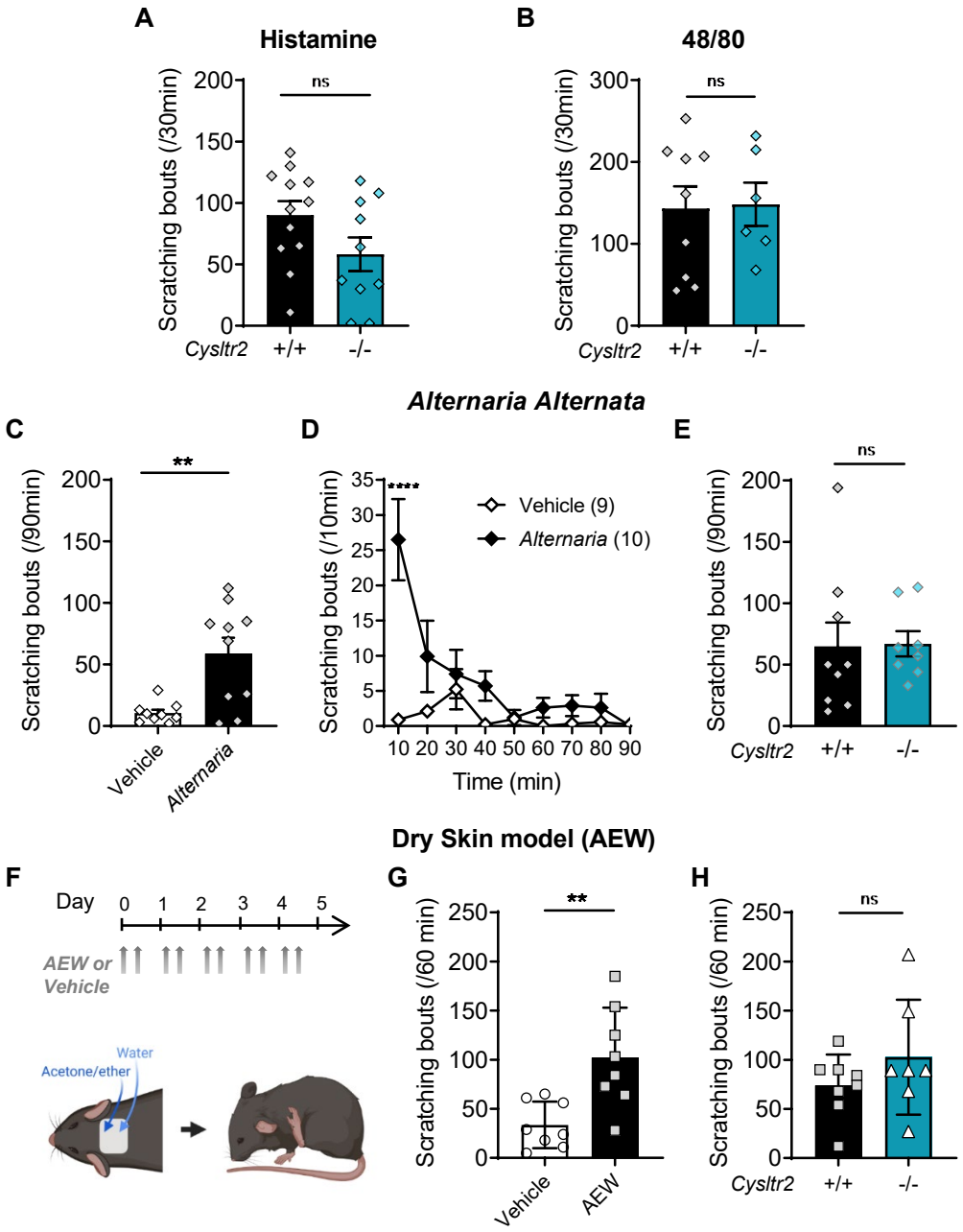

Figure S5. *Cysltr2* $^{-/-}$ mice are not deficient in *Alternaria* or dry skin induced itch

(A-B) Scratching bouts induced by intradermal cheek injection in *Cysltr2*$^{+/+}$ or *Cysltr2*$^{-/-}$ mice of **(A)** histamine (100 ug) and **(B)** compound 48/80 (100 ug).
(C) Scratching bouts induced by intradermal cheek injection of vehicle or *Alternaria alternata* extract (30 ug) in B6 mice.
(D) Detailed scratching bouts kinetics over 90 minutes in response to *Alternaria alternata*.
(E) Scratching bouts induced by intradermal cheek injection in *Cysltr2*$^{+/+}$ or *Cysltr2*$^{-/-}$ mice of *Alternaria alternata*.
(F) Diagram of procedure: twice daily application of vehicle (water) or of a mixture of acetone/ether (AEW) followed by water on the nape of the neck of the mouse for 5 days.
(G) Scratching bouts recorded on day 5 for 60 minutes from B6 mice treated with either vehicle or AEW.
(H) Scratching bouts recorded on day 5 for 60 minutes from *Cysltr2*$^{+/+}$ or *Cysltr2*$^{-/-}$ mice treated with AEW.
*Values represented as mean ± SEM. Unpaired t-test (A-C & E-H) and repeated measures two-way ANOVA, Sidak's posttest (D), ns: non-significant, **p<0.01*

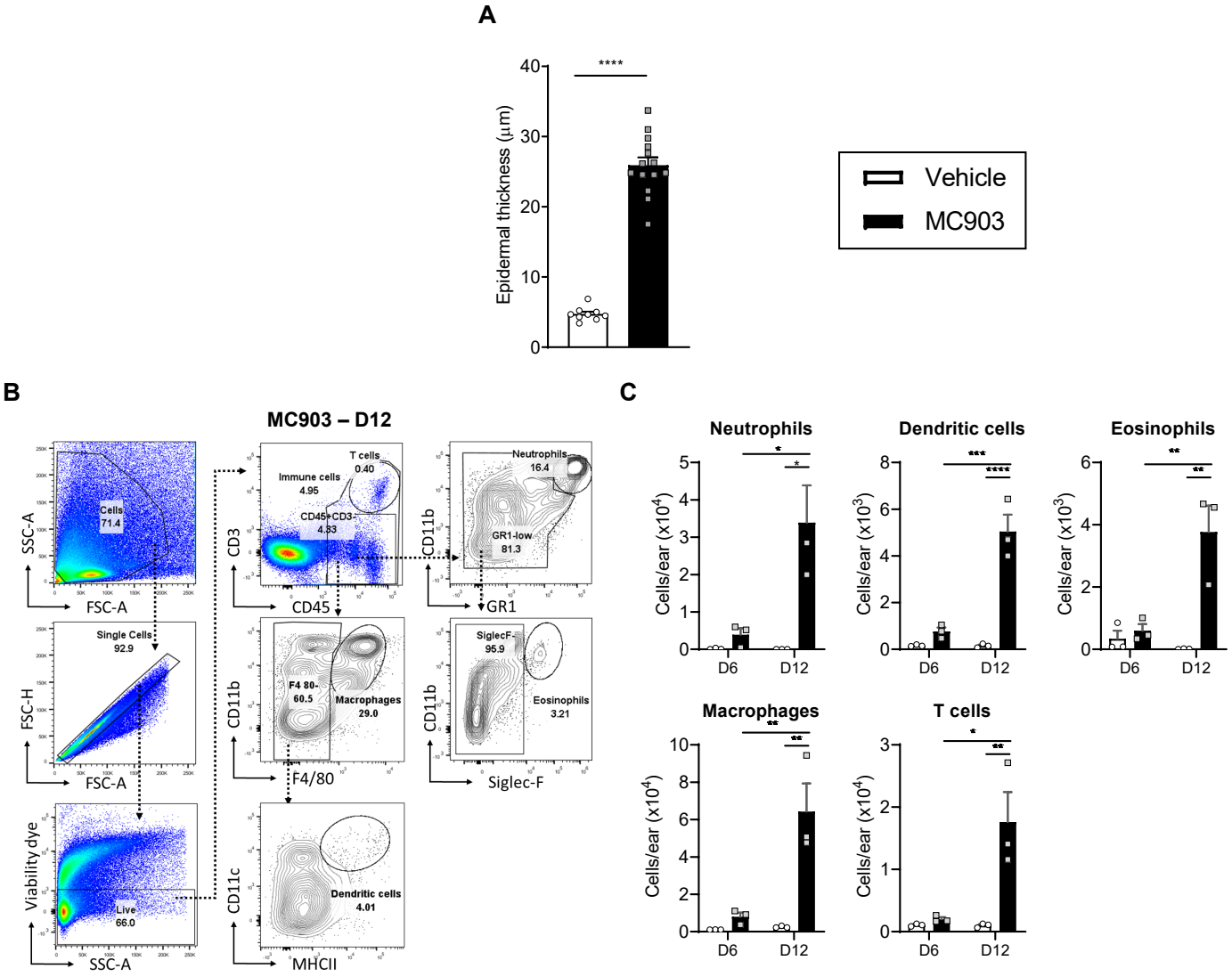

Figure S6. MC903 induces significant immune cell influx and epidermal thickening

(A) Quantification of epidermal thickness at day 12 in mice treated daily with either vehicle or MC903.
(B-C) Characterization of immune cell populations by flow cytometry from ear homogenates collected at day 6 or 12 from mice treated daily with vehicle or MC903 (n=3)
(B) Representative FACS plots of immune cells in ears from MC903-treated mice at day 12.
(C) Quantification of immune cell populations.
*Values represented as mean + SEM. Unpaired t-test (A), two-way ANOVA, Sidak's post test (C), ns: non-significant, *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001*

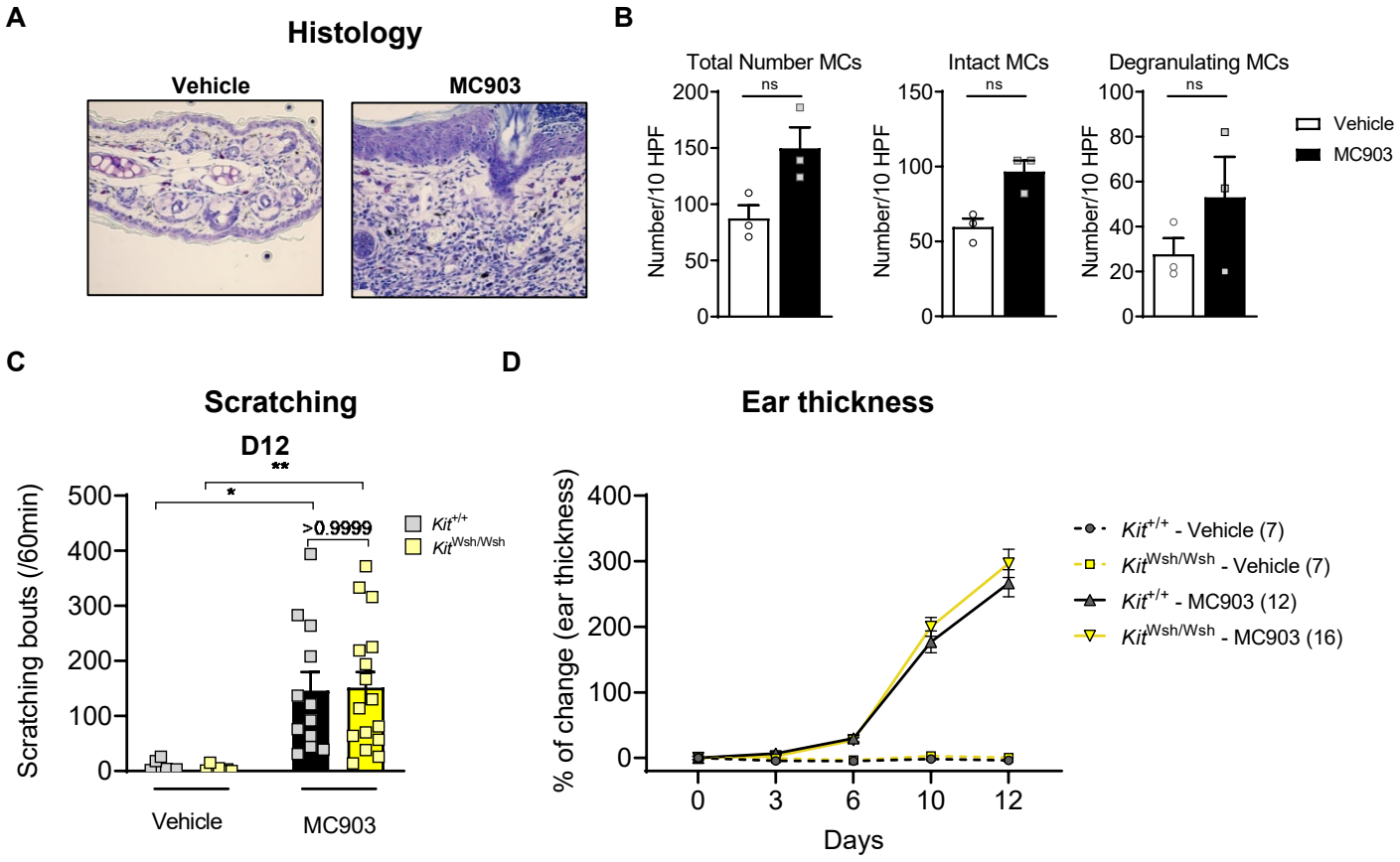

Figure S7. Mast cells are not required for MC903 induced inflammation and itch

(A) Toluidine blue staining in ear section at day 12 after treatment with vehicle or MC903.
(B) Quantification of MC at day 12 (total number, intact MC and degranulating MC) in sections from vehicle and MC903 treated mice.
(C) Scratching bouts recorded on day 12 for 60 minutes in *Kit*$^{+/+}$ or *Kit*$^{Wsh/Wsh}$ mice treated with daily application of vehicle or MC903.
(D) Percentage of ear thickness change following daily application of vehicle or MC903 in *Kit*$^{+/+}$ or *Kit*$^{Wsh/Wsh}$ mice.
Values represented as mean ± SEM. Unpaired t-test (B), two-way ANOVA, Sidak's post test (C), repeated measures two-way ANOVA, Sidak's post test (D), ns*: non-significant, *p<0.05; **p<0.01*

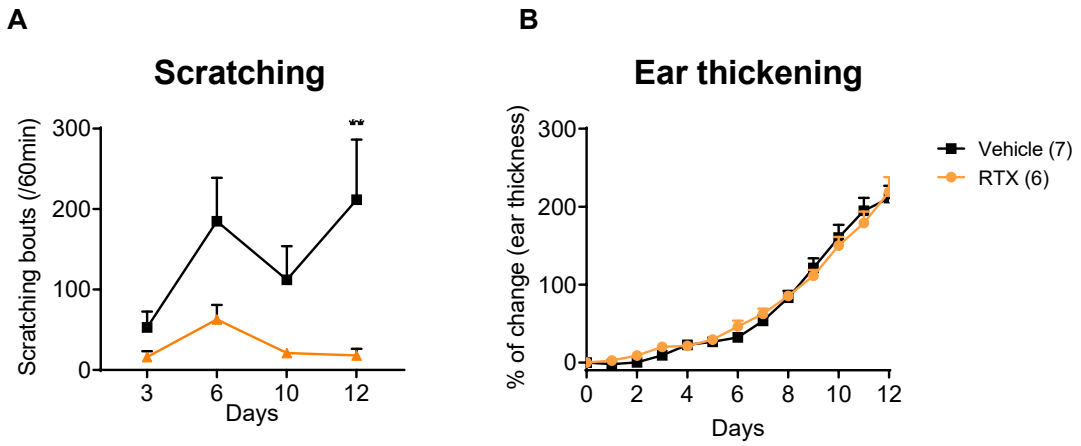

Figure S8. RTX treatment abolishes MC903 induced itch

(A) Scratching bouts recorded on day 3, 6, 10 and 12 for 60 minutes before daily application of MC903 in mice pre-treated with vehicle or RTX.
(B) Percentage of ear thickness change following daily application of MC903 in vehicle or RTX pre-treated mice.
*Values represented as mean + SEM. Repeated measures two-way ANOVA, Sidak's posttest, **p<0.01*

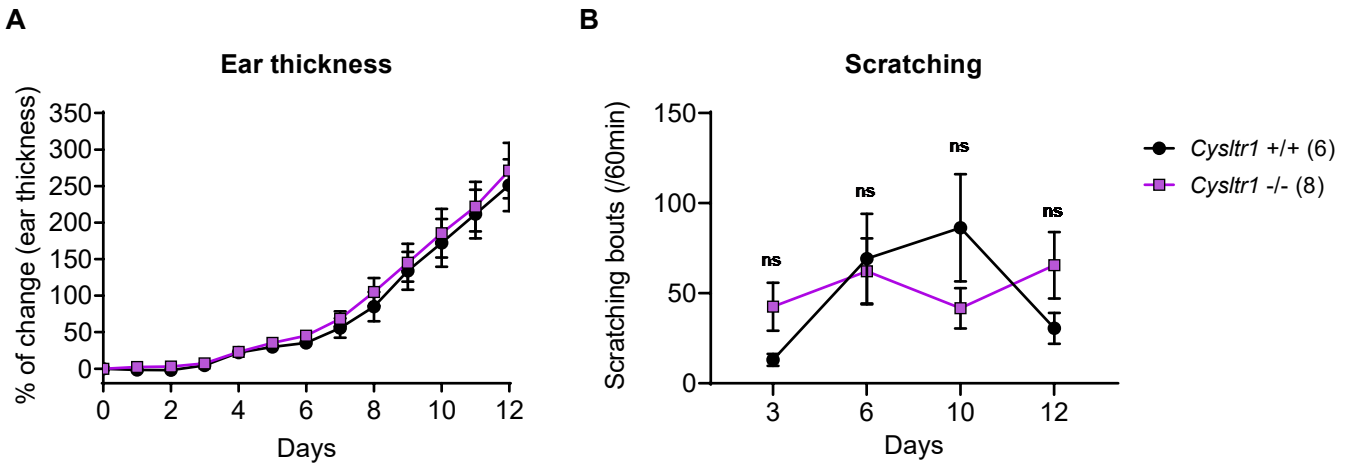

Figure S9. CysLT$_1$R deficiency does not affect MC903 induced itch

(A) Percentage of ear thickness change following daily application of MC903 in *Cysltr1* $^{+/+}$ and *Cysltr1*$^{-/-}$ mice.
(B) Scratching bouts recorded on day 3, 6, 10 and 12 for 60 minutes before daily application of MC903 in *Cysltr1*$^{+/+}$ and *Cysltr1*$^{-/-}$ mice.
Values represented as mean ± SEM. Repeated measures two-way ANOVA, Sidak's posttest, ns: non-significant

Video S1
Example of scratching bouts induced by LTC_4 0.6 nmol injection in left cheek recorded in iBOB

References

1. Shimada SG & LaMotte RH (2008) Behavioral differentiation between itch and pain in mouse. *Pain* 139(3):681-687.
2. Wilson SR*, et al.* (2013) The Ion Channel TRPA1 Is Required for Chronic Itch. *The Journal of Neuroscience* 33(22):9283-9294.
3. Gijon MA, Zarini S, & Murphy RC (2007) Biosynthesis of eicosanoids and transcellular metabolism of leukotrienes in murine bone marrow cells. *Journal of lipid research* 48(3):716-725.
4. Zarini S, Gijón MA, Ransome AE, Murphy RC, & Sala A (2009) Transcellular biosynthesis of cysteinyl leukotrienes in vivo during mouse peritoneal inflammation. *Proceedings of the National Academy of Sciences* 106(20):8296-8301.
5. Chiu IM*, et al.* (2014) Transcriptional profiling at whole population and single cell levels reveals somatosensory neuron molecular diversity. *Elife* 3:[doi: 10.7554/eLife.04660].
6. Zeisel A*, et al.* (2018) Molecular Architecture of the Mouse Nervous System. *Cell* 174(4):999-1014.e1022.
7. Sharma N*, et al.* (2020) The emergence of transcriptional identity in somatosensory neurons. *Nature* 577(7790):392-398.