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Attenuation of Allergic Contact Dermatitis Through the Endocannabinoid System

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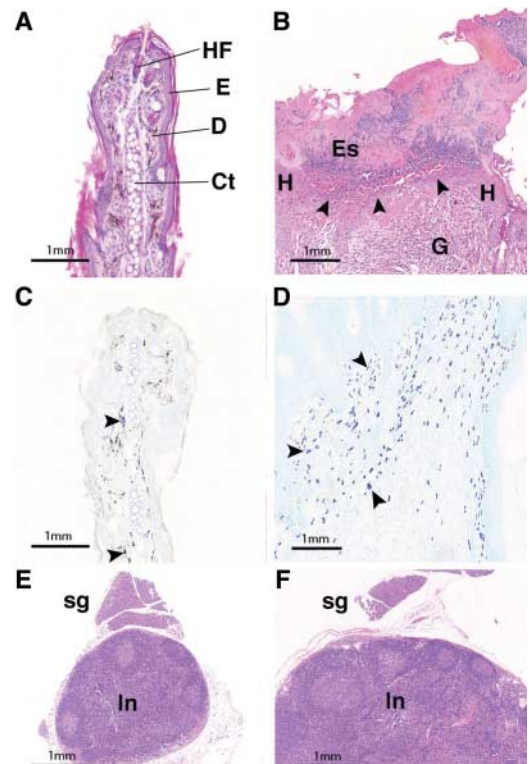
Allergic contact dermatitis affects about 5% of men and 11% of women in industrialized countries and is one of the leading causes for occupational diseases. In an animal model for cutaneous contact hypersensitivity, we show that mice lacking both known cannabinoid receptors display exacerbated allergic inflammation. In contrast, fatty acid amide hydrolase-deficient mice, which have increased levels of the endocannabinoid anandamide, displayed reduced allergic responses in the skin. Cannabinoid receptor antagonists exacerbated allergic inflammation, whereas receptor agonists attenuated inflammation. These results demonstrate a protective role of the endocannabinoid system in contact allergy in the skin and suggest a target for therapeutic intervention.

Allergic contact dermatitis is characterized by a loss of immunological tolerance toward small allergenic molecules (haptens). The allergen or hapten first penetrates the epidermis and is taken up by skin dendritic cells, which migrate to the draining lymph nodes and present haptenated peptide or major histocompatibility complexes (MHC) to naïve antigen-specific T lymphocytes. Upon repeated allergen contact, an inflammatory response is elicited by the recruitment of antigen-specific effector T cells to the skin and the subsequent production of inflammatory cytokines and chemokines (1). Cannabinoid receptors CB1 and CB2 are heterotrimeric GTP-binding protein (G protein)-coupled receptors, which are activated by the cannabinoid Δ^9 -tetrahydrocannabinol (Δ^9 -THC), as well as by endocannabinoids such as arachidonylethanolamide (anandamide or AEA) or 2-arachidonoylglycerol (2-AG) (2). Both cannabinoid receptors are present on keratinocytes in the epidermis, which also express enzymes involved in the synthesis and degradation of endocannabinoids (3, 4).

Although nickel-containing ear tags are generally well tolerated by individuals in our mouse

colony (5), we observed that mice lacking both cannabinoid CB1 and CB2 receptors [CB1 receptor-deficient ($Cnr1^{-/-}$) and CB2 receptor-deficient ($Cnr2^{-/-}$) mice] (6, 7) frequently scratch their ear tags, leading to severe ulcerations in the head and neck regions (Fig. 1, A and B, and fig. S1). Because localized necrotic lesions initially developed around the ear tag, we first considered that impaired wound healing might be the potential primary cause for the skin lesions.

Fig. 1. Histological views of $Cnr1^{-/-}/Cnr2^{-/-}$ mouse ears. Clips with high nickel content cause chronic ear ulceration in $Cnr1^{-/-}/Cnr2^{-/-}$ mice (fig. S1). (A) Hematoxylin and eosin (H&E)-stained sections of ear tissue from healthy $Cnr1^{-/-}/Cnr2^{-/-}$ mice with normal size and histology. Ct, cartilage; D, dermis; E, epidermis; HF, hair follicle. (B) H&E-stained sections of ulcerated ear tissue from $Cnr1^{-/-}/Cnr2^{-/-}$ mice carrying nickel ear tags. Arrowheads point to keratinocytes on the re-epithelized body of the wound. The eschar (Es), the strong hyperproliferative epithelium (H), and the granulation tissue (G) are indicated. (C) Toluidine blue-stained sections of ear tissue from healthy $Cnr1^{-/-}/Cnr2^{-/-}$ mice showing few metachromatic mast cells (arrowheads). (D) Toluidine blue-stained sections of ulcerated ear tissue from $Cnr1^{-/-}/Cnr2^{-/-}$ mice carrying nickel tags with intense infiltration of mast cells (arrowheads). (E) H&E-stained sections of lymph nodes from healthy $Cnr1^{-/-}/Cnr2^{-/-}$ mice. In, lymph node; sg, salivary gland. (F) H&E-stained sections of lymph nodes from $Cnr1^{-/-}/Cnr2^{-/-}$ mice carrying nickel ear tags with reactive enlargement and lymphadenitis of preauricular lymph nodes. The preauricular salivary glands located next to the lymph nodes are the same size in $Cnr1^{-/-}/Cnr2^{-/-}$ mice without (E) and with (F) ear ulceration and serve as an internal standard.



However, experiments to test the wound healing ability revealed no measurable difference between wild-type (WT) and $Cnr1^{-/-}/Cnr2^{-/-}$ double-knockout (DKO) mice (5, 8) (fig. S2). We also noted that skin ulcerations appeared to be particularly prominent in mice with tags containing high nickel content (65 to 70%). In these cases, skin ulcerations were observed in 88 out of 304 $Cnr1^{-/-}/Cnr2^{-/-}$ DKO mice (29%), but were not observed in single $Cnr1^{-/-}$ or $Cnr2^{-/-}$ knockouts or in any other mouse strain. Furthermore, no ulcerations were noted in mice with pure brass tags (fig. S1). Subsequent histopathological analyses of ulcerated ear tissue revealed intense infiltration of mast cells in close proximity to the ulcers (Fig. 1, C and D), suggesting that an allergic reaction might be involved in the pathology seen in $Cnr1^{-/-}/Cnr2^{-/-}$ DKO mice. Consistently, the regional preauricular and submental lymph nodes (Fig. 1, E and F) were swollen, reaching a diameter of 51 ± 2.4 mm in comparison with 20.5 ± 1.9 mm in healthy $Cnr1^{-/-}/Cnr2^{-/-}$ mice, as a result of mixed follicular and interfollicular lymphatic hyperplasia.

We therefore considered the possibility that an increase in allergic responses might exist in knockout (KO) mice. To test this hypothesis, we evaluated cutaneous contact hypersensitivity (CHS) in $Cnr1^{-/-}/Cnr2^{-/-}$ animals using the obligate contact allergen 2,4-dinitrofluorobenzene (DNFB), which generates a specific cutaneous T cell-mediated allergic response upon repeated allergen contact (9). A marked increase in allergic

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responses was apparent in $Cnr1^{-/-}/Cnr2^{-/-}$ mice as compared with WT controls (Fig. 2A). The difference was particularly prominent 48 hours after the second and the third DNFB challenge. Infiltration of the skin with Gr-1⁺ granulocytes was increased in the ears of DNFB-exposed $Cnr1^{-/-}/Cnr2^{-/-}$ mice, as compared with WT animals (fig. S3), and correlated with a higher myeloperoxidase activity, which is indicative of enhanced neutrophil recruitment (10, 11). Furthermore, we found an increased number of MHC II⁺ antigen-presenting cells in $Cnr1^{-/-}/Cnr2^{-/-}$ mice. Upon examination of mice with a single deletion of either CB1 or CB2 receptors, we observed a similarly pronounced increase of allergic ear swelling (Fig. 2B), suggesting a nonredundant role for each cannabinoid receptor in the allergic response to DNFB. This finding would also suggest that the

response to the milder stimulus originating from the nickel ear tag was only detectable in mice lacking both receptors.

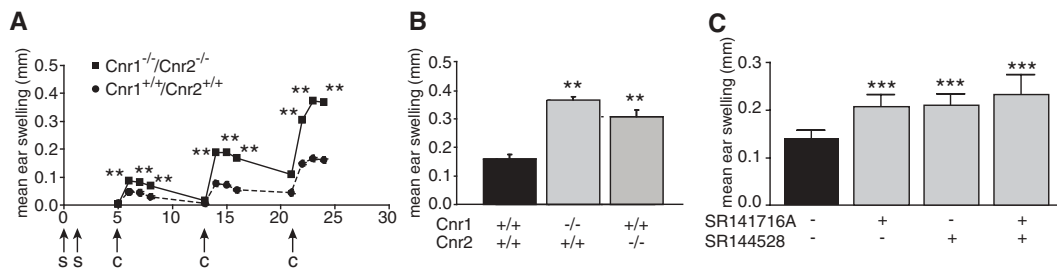
In order to obtain independent evidence for a role of the CB1 and CB2 receptors in the regulation of CHS, we examined WT mice after administration of the CB1 receptor antagonist SR141716A (Rimonabant) and the CB2 receptor antagonist SR144528, respectively. After the induction of CHS, animals received three injections of the corresponding antagonist 30 min before and after each challenge. In these cases, ear swelling was significantly increased in treated mice as compared with control mice (Fig. 2C), further supporting the role of both receptors in CHS. In contrast, however, acute oral or topical administration of the CB2 receptor antagonists appeared to ameliorate inflammatory skin responses (12, 13),

thus suggesting that CB2 antagonism may be initially beneficial but detrimental upon chronic blockade.

To explore this issue more carefully, we investigated the synthesis of endogenous cannabinoids and the expression of cannabinoid receptors during experimental contact allergy studies. After DNFB treatment, the levels of 2-AG increased in the DNFB-treated ears of WT controls and to an even larger extent in the ears of $Cnr1^{-/-}/Cnr2^{-/-}$ mice (Fig. 3A), whereas AEA production was strongly induced in both genotypes. Endocannabinoids may have been overproduced in KO animals as a result of the lack of communication between receptors and ligands (14) or as a further consequence of the exacerbated allergic response. Keratinocytes are known to express CB1 receptors (4), although the expression of CB2 on these

Fig. 2. Contact allergy in cannabinoid receptor-deficient mice.

(A) $Cnr1^{-/-}/Cnr2^{-/-}$ and $Cnr1^{+/+}/Cnr2^{+/+}$ mice were sensitized (indicated by "s") with DNFB on the shaved abdomen. On day 5, mice were challenged (indicated by "c") with DNFB on the right ear. A second and third challenge was performed on day 13 and 21. Shown is the mean ear swelling over time $\pm$ SEM



of a representative experiment with nine mice per group. Statistical significance was evaluated with the Wilcoxon-Mann-Whitney two-sample test (** $P < 0.01$). This experiment was independently repeated four times with similar results. (B) $Cnr1^{-/-}/Cnr2^{+/+}$, $Cnr1^{+/+}/Cnr2^{-/-}$, and $Cnr1^{+/+}/Cnr2^{+/+}$ mice were sensitized as described in (A). Ear swelling 48 hours after the third challenge of a representative experiment with eight mice per group is shown. Similar results were

obtained in four independent experiments with a total of 23 mice (** $P < 0.01$). (C) Contact allergic response in C57BL/6 mice after treatment with the indicated CB receptor antagonists. Ear swelling 48 hours after the second challenge of a representative experiment with 10 mice per group is shown. Similar results were obtained in four independent experiments with a total of 25 mice (*** $P < 0.001$). Error bars in (B) and (C) indicate SEM.

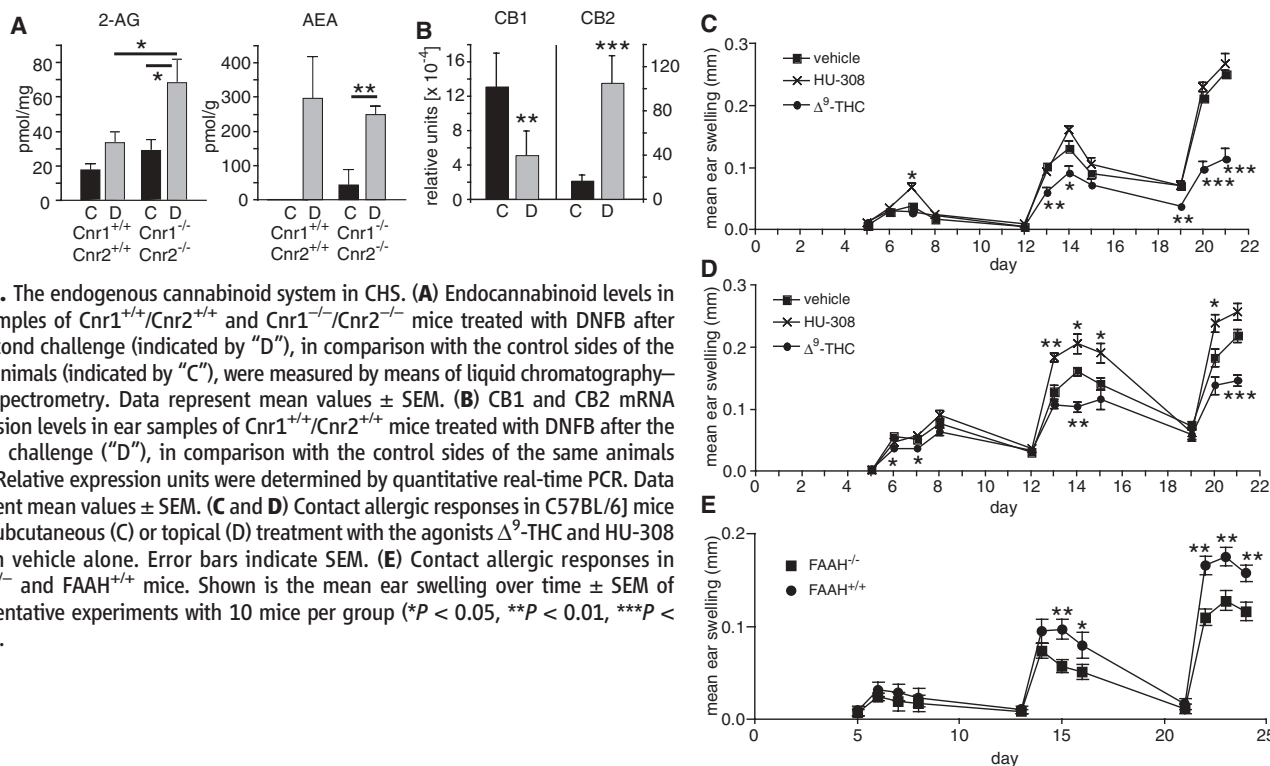


Fig. 3. The endogenous cannabinoid system in CHS. (A) Endocannabinoid levels in ear samples of $Cnr1^{+/+}/Cnr2^{+/+}$ and $Cnr1^{-/-}/Cnr2^{-/-}$ mice treated with DNFB after the second challenge (indicated by "D"), in comparison with the control sides of the same animals (indicated by "C"), were measured by means of liquid chromatography-mass spectrometry. Data represent mean values $\pm$ SEM. (B) CB1 and CB2 mRNA expression levels in ear samples of $Cnr1^{+/+}/Cnr2^{+/+}$ mice treated with DNFB after the second challenge ("D"), in comparison with the control sides of the same animals ("C"). Relative expression units were determined by quantitative real-time PCR. Data represent mean values $\pm$ SEM. (C and D) Contact allergic responses in C57BL/6 mice after subcutaneous (C) or topical (D) treatment with the agonists Δ^9 -THC and HU-308 or with vehicle alone. Error bars indicate SEM. (E) Contact allergic responses in $FAAH^{-/-}$ and $FAAH^{+/+}$ mice. Shown is the mean ear swelling over time $\pm$ SEM of representative experiments with 10 mice per group (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

cells has been debated (3, 12, 15). We could identify both receptor mRNAs in human HaCat keratinocytes (fig. S4). CB1 receptor mRNA was down-regulated in HaCat cells that had been stimulated for 24 hours with polyinosinic-polycytidylic acid and also in DNFB-exposed ears, whereas CB2 mRNA and protein expression was up-regulated in both cases (Fig. 3B and fig. S4). Taken together, these findings indicate that DNFB-induced CHS caused an activation of the endocannabinoid system in the skin and imbalanced expression of both receptors.

Because our data indicate that activation of the endocannabinoid system may function to dampen the CHS response, we considered the possibility that administration of cannabinoids such as Δ^9 -THC might attenuate CHS in WT animals. Therefore, after CHS was induced, we injected mice with Δ^9 -THC (5 mg per kilogram of body weight, administered subcutaneously) 30 min before, as well as 24 and 48 hours after, DNFB challenge. Indeed, Δ^9 -THC significantly decreased ear swelling (Fig. 3C) and reduced the recruitment of Gr-1⁺ granulocytes in comparison with swelling and granulocyte recruitment in untreated mice [vehicle: 483 ± 62 Gr-1⁺ cells per high power field (HPF) versus 200 ± 50 Gr-1⁺ cells per HPF in subcutaneous THC treatment]. A similar therapeutic effect of Δ^9 -THC was observed after topical application of 30 μ g of Δ^9 -THC 30 min before, as well as 24 and 48 hours after, DNFB administration (Fig. 3D). In contrast, the CB2-specific agonist HU-308 either showed no efficacy or even increased the allergic response after subcutaneous or topical application (Fig. 3, C and D) (13). It has recently been shown that leukocyte chemotaxis is inhibited by the CB2 receptor inverse agonist *N*-[1(S)-[4-[(4-

methoxy-2-[(4-methoxyphenyl)sulfonyl]phenyl]-sulfonyl]phenyl]ethyl]methanesulfonamide (Sch. 336) and, thus, HU-308 administration to the inflamed ear may have further increased leukocyte infiltration (16).

Because endocannabinoid levels were regulated in DNFB-treated ears and Δ^9 -THC had a beneficial effect on CHS, we asked whether mice having higher endocannabinoid levels would show altered allergic responses after DNFB treatment. Therefore, we analyzed the allergic reaction in mice lacking the enzyme fatty acid amide hydrolase (FAAH), which have augmented anandamide levels (17). As shown in Fig. 3E, we found a significantly decreased allergic response in FAAH KO mice after the second and third DNFB challenge when compared with the response in WT mice. Similar to our results, mice with only peripherally elevated fatty acid amides showed a reduced inflammation in the carrageenan foot-pad model (18). All data indicate that the anti-inflammatory responses were peripheral, rather than central, and that FAAH inhibitors are a potential therapeutic approach for inflammation (and a potentially useful alternative to direct CB1/CB2 agonists).

In order to gain insight into the molecular mechanism that may contribute to the increased magnitude of CHS in cannabinoid receptor-deficient mice, we performed a series of microarray experiments with RNA isolated from DNFB-treated ears of *Cnr1*^{-/-}/*Cnr2*^{-/-} and *Cnr1*^{+/+}/*Cnr2*^{+/+} mice, as well as from the untreated ears of control mice. In all, 830 probe sets were differentially expressed between control and DNFB-treated ears, of which 674 were up-regulated and only 156 were down-regulated. We grouped the probe sets by using gene ontology (GO) annotation

(19, 20) (Fig. 4A). Immune-related probe sets represented the largest group of differentially expressed genes (40 down-regulated and 117 up-regulated probe sets). Notably, large numbers of chemokine C-C and C-X-C motif ligands (21) and receptors were found in the up-regulated group (table S1) and represented 22% of the immune-related probe sets (table S2). Fifty-four genes were differentially expressed in DNFB-treated whole-ear specimens of *Cnr1*^{+/+}/*Cnr2*^{+/+} mice versus those of *Cnr1*^{-/-}/*Cnr2*^{-/-} mice (fig. S5). The only chemokine gene transcript in this group was monocyte chemoattractant protein 2 (MCP-2)/chemokine (C-C motif) ligand 8 (CCL8). Using real-time reverse transcription polymerase chain reaction (RT-PCR) and in situ hybridization, we confirmed the differential expression of MCP-2/CCL8 mRNA in keratinocytes (Fig. 4, B and C). Further in vitro experiments showed dynamic regulation of MCP-2/CCL8 production in activated keratinocytes through cannabinoid agonists (fig. S6). MCP-2/CCL8 is a member of the MCP family, which has been shown to play a major role in the recruitment of macrophages, activated lymphocytes, and mast cells into inflammatory sites (22, 23). Taken together, our results suggest that the endocannabinoid system regulates allergic inflammation through a modulation of the chemokine system.

The observation that the endocannabinoid system is activated in a model of CHS, together with the fact that genetic deletion or pharmacologic blockade of CB receptors enhanced contact allergic inflammation, whereas stimulation of CB receptors reduced such inflammation, strongly suggests that the endocannabinoid system serves to attenuate the inflammatory re-

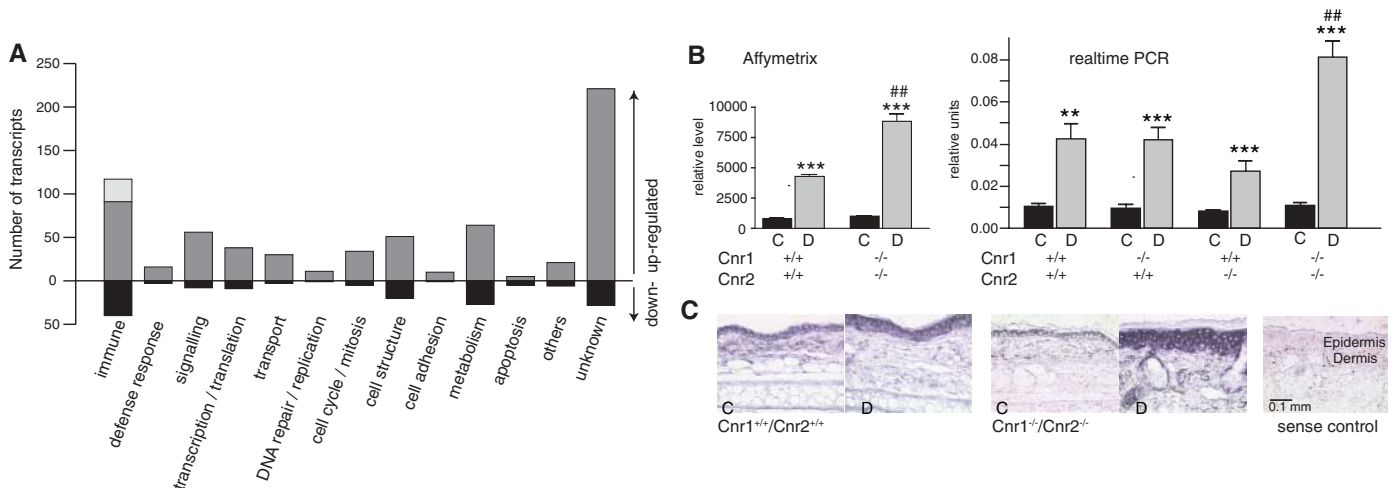


Fig. 4. Gene expression analysis. (A) Microarray analysis of ear samples of *Cnr1*^{+/+}/*Cnr2*^{+/+} and *Cnr1*^{-/-}/*Cnr2*^{-/-} mice treated with DNFB 48 hours after the second challenge ("D") in comparison with the control sides of the same animals ("C") revealed 674 up-regulated and 156 down-regulated probe sets in response to the treatment. Grouping was performed by GO annotation. Chemokine C-C motif and C-X-C motif ligands and receptors in the up-regulated group are presented in light gray in the column of immune-related genes. (B) Transcripts for the chemokine MCP-

2/CCL8 in DNFB-treated ears of *Cnr1*^{+/+}/*Cnr2*^{+/+} and *Cnr1*^{-/-}/*Cnr2*^{-/-} mice resulting from microarray expression study (left panel) and real-time RT-PCR quantification (right panel). Shown are representative results of three independent experiments (***P* < 0.01, ****P* < 0.001). Error bars indicate SEM. (C) In situ hybridization with the use of a digoxigenin-labeled probe for MCP-2/CCL8 in ear tissue of DNFB-treated and control ears from *Cnr1*^{+/+}/*Cnr2*^{+/+} and *Cnr1*^{-/-}/*Cnr2*^{-/-} mice. The sense probe showed no staining.

sponse in cutaneous CHS. The finding that topical application of Δ^9 -THC reduced allergic inflammation points to the promising potential of developing pharmacological treatments (24) with the use of selective CB receptor agonists or FAAH inhibitors.

References and Notes

1. S. Grabbe, T. Schwarz, *Immunol. Today* **19**, 37 (1998).
2. T. Bisogno, A. Ligresti, V. Di Marzo, *Pharmacol. Biochem. Behav.* **81**, 224 (2005).
3. M. M. Ibrahim *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 3093 (2005).
4. M. Maccarrone *et al.*, *J. Biol. Chem.* **278**, 33896 (2003).
5. Materials and methods are available as supporting material on Science Online.
6. A. Zimmer, A. M. Zimmer, A. G. Hohmann, M. Herkenham, T. I. Bonner, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 5780 (1999).
7. N. E. Buckley *et al.*, *Eur. J. Pharmacol.* **396**, 141 (2000).
8. S. Werner *et al.*, *Science* **266**, 819 (1994).
9. J. Knop, R. Stremmer, C. Neumann, E. De Maeyer, E. Macher, *Nature* **296**, 757 (1982).
10. R. I. Lehrer, J. Hanifin, M. J. Cline, *Nature* **223**, 78 (1969).
11. C. Nathan, *Nat. Rev. Immunol.* **6**, 173 (2006).
12. S. Oka *et al.*, *J. Immunol.* **177**, 8796 (2006).
13. Y. Ueda, N. Miyagawa, T. Matsui, T. Kaya, H. Iwamura, *Eur. J. Pharmacol.* **520**, 164 (2005).
14. V. Di Marzo *et al.*, *J. Neurochem.* **75**, 2434 (2000).
15. S. Stander, M. Schmelz, D. Metz, T. Luger, R. Rukwied, *J. Dermatol. Sci.* **38**, 177 (2005).
16. C. A. Lunn *et al.*, *J. Pharmacol. Exp. Ther.* **316**, 780 (2006).
17. B. F. Cravatt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 9371 (2001).
18. B. F. Cravatt *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **101**, 10821 (2004).
19. J. S. Lee, G. Katari, R. Sachidanandam, *BMC Bioinform.* **6**, 189 (2005).
20. M. Ashburner *et al.*, *Nat. Genet.* **25**, 25 (2000).
21. M. F. Bachmann, M. Kopf, B. J. Marsland, *Nat. Rev. Immunol.* **6**, 159 (2006).
22. A. de Paulis *et al.*, *Int. Arch. Allergy Immunol.* **124**, 146 (2001).
23. D. D. Taub *et al.*, *J. Clin. Invest.* **95**, 1370 (1995).
24. T. W. Klein, *Nat. Rev. Immunol.* **5**, 400 (2005).
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Supporting Online Material

www.sciencemag.org/cgi/content/full/316/5830/1494/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 and S2
References

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Genome-Wide Mapping of in Vivo Protein-DNA Interactions

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In vivo protein-DNA interactions connect each transcription factor with its direct targets to form a gene network scaffold. To map these protein-DNA interactions comprehensively across entire mammalian genomes, we developed a large-scale chromatin immunoprecipitation assay (ChIPSeq) based on direct ultrahigh-throughput DNA sequencing. This sequence census method was then used to map in vivo binding of the neuron-restrictive silencer factor (NRSF; also known as REST, for repressor element-1 silencing transcription factor) to 1946 locations in the human genome. The data display sharp resolution of binding position [± 50 base pairs (bp)], which facilitated our finding motifs and allowed us to identify noncanonical NRSF-binding motifs. These ChIPSeq data also have high sensitivity and specificity [ROC (receiver operator characteristic) area ≥ 0.96] and statistical confidence ($P < 10^{-4}$), properties that were important for inferring new candidate interactions. These include key transcription factors in the gene network that regulates pancreatic islet cell development.

Although much is known about transcription factor binding and action at specific genes, far less is known about the composition and function of entire factor-DNA interactomes, especially for organisms with large genomes. Now that human, mouse, and other large genomes have been sequenced, it is possible, in principle, to measure how any transcription factor is deployed across the entire genome for a given cell type and physiological condition. Such measurements are important for systems-level studies because they provide a global map of candidate gene network input connections. These direct physical interactions between transcription factors or cofactors and the

chromosome can be detected by chromatin immunoprecipitation (ChIP) (1). In ChIP experiments, an immune reagent specific for a DNA binding factor is used to enrich target DNA sites to which the factor was bound in the living cell. The enriched DNA sites are then identified and quantified.

For the gigabase-size genomes of vertebrates, it has been difficult to make ChIP measurements that combine high accuracy, whole-genome completeness, and high binding-site resolution. These data-quality and depth issues dictate whether primary gene network structure can be inferred with reasonable certainty and comprehensiveness, and how effectively the data can be used to discover binding-site motifs by computational methods. For these purposes, statistical robustness, sampling depth across the genome, absolute signal and signal-to-noise ratio must be good enough to detect nearly all in vivo binding locations for a regulator with minimal inclusion of false-positives. A further challenge in genomes large or small is to map factor-binding sites with high positional resolution. In addition to making com-

putational discovery of binding motifs feasible, this dictates the quality of regulatory site annotation relative to other gene anatomy landmarks, such as transcription start sites, enhancers, introns and exons, and conserved noncoding features (2). Finally, if high-quality protein-DNA interactome measurements can be performed routinely and at reasonable cost, it will open the way to detailed studies of interactome dynamics in response to specific signaling stimuli or genetic mutations. To address these issues, we turned to ultrahigh-throughput DNA sequencing to gain sampling power and applied size selection on immuno-enriched DNA to enhance positional resolution.

The ChIPSeq assay shown here differs from other large-scale ChIP methods such as ChIPArray, also called ChIPchip (1); ChIP-SAGE (SACO) (3); or ChIPPet (4) in design, data produced, and cost. The design is simple (Fig. 1A) and, unlike SACO or ChIPPet, it involves no plasmid library construction. Unlike microarray assays, the vast majority of single-copy sites in the genome is accessible for ChIPSeq assay (5), rather than a subset selected to be array features. For example, to sample with similar completeness by an Affymetrix-style microarray design, a nucleotide-by-nucleotide sliding window design of roughly 1 billion features per array would be needed for the nonrepeat portion of the human genome. In addition, ChIPSeq counts sequences and so avoids constraints imposed by array hybridization chemistry, such as base composition constraints related to T_m , the temperature at which 50% of double-stranded DNA or DNA-RNA hybrids is denatured; cross-hybridization; and secondary structure interference. Finally, ChIPSeq is feasible for any sequenced genome, rather than being restricted to species for which whole-genome tiling arrays have been produced.

ChIPSeq illustrates the power of new sequencing platforms, such as those from Solexa/Illumina and 454, to perform sequence census counting assays. The generic task in these applications is to identify and quantify the molecular

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Supporting Online Material for
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Materials and Methods
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Supporting Online Material

1. Materials and methods.

Animals. For pharmacological studies, 8-10 week old female C57BL/6J mice were obtained by Harlan, Germany. Fatty acid amide hydrolase (FAAH^{-/-}) knockout mice (1), CB1-receptor-deficient (Cnr1^{-/-}) (2), CB2-receptor-deficient (Cnr2^{-/-}) (3) and CB1/CB2-receptor-deficient mice (Cnr1^{-/-}/Cnr2^{-/-}) (4) and their wild-type controls were bred at our animal facility. We have been using metallic ear tags for the management of our mouse colonies for more than 10 years and tagged well over 50,000 animals from numerous strains of mice, including many different knockouts. These tags are generally well tolerated. Animal experiments were approved by the Bezirksregierung Köln, Germany, or the local veterinary authorities of Zurich, Switzerland. The institutional and national guidelines for the care and use of laboratory animals were followed.

Wound healing experiment. Ten- to 12-week-old mice were anaesthetized with ketamine/xylazine. Four full-thickness excisional wounds (5 mm diameter) were generated on the shaved back, two on each side of the vertebral column, by excising skin and panniculus carnosus (5). For histological analysis bisected wounds were fixed overnight in 4 % PFA in PBS or in 95 % ethanol/1 % acetic acid, followed by paraffin embedding. Sections (6 µm) from the middle of the 5-day wounds of a) Cnr1^{+/+}/Cnr2^{+/+} and b) Cnr1^{-/-}/Cnr2^{-/-} mice were stained with hematoxylin/eosin and used for morphometric analysis (6, 7). Only littermates of the same sex were used for direct histological comparison.

Contact hypersensitivity. DNFB (1-fluoro-2,4 dinitrobenzene, Merck) was diluted in acetone/olive oil (4:1) immediately before use. Mice without any skin lesions and ear tags were sensitized by painting 50 µl of 0.2% DNFB on the shaved abdomen on two consecutive days. Controls were treated with 50 µl acetone-olive oil. For elicitation of CHS, ears of mice were painted with 10 µl of 0.3% DNFB on day 5. Ear thickness was measured 24 h, 48 h and 72 h after challenge using an engineers micrometer (Oditest, Fa. Kroeplin, Schlüchtern, Germany) and ear swelling was calculated in each mouse as the difference in ear thickness between the unchallenged (left) and the challenged (right) ear (8). Statistical significances were evaluated with the Wilcoxon-Mann-Whitney two-sample test. p values less than 0.05 were considered significant, p values less than 0.01 were considered highly significant.

Drugs. Δ⁹-THC (Sigma) was stored at 4°C until use. SR141716A (Rimonabant) and SR144528 (kindly provided by NIMH) were diluted in DMSO and stored at -20°C until use. Mice were injected s.c. with SR141716A or SR144528 at 3 mg/kg, and with Δ⁹-THC or HU-308 at 5 mg/kg body weight 30 min before challenge, as well as 24 h and 48 h after challenge. Control mice received the appropriate vehicle solution only. Antagonists were dissolved by using one drop Tween 80 in 12% DMSO in saline. Agonists were diluted in ethanol/DMSO/Cremophor/saline (1:1:2:36) for s.c. injections. Topical applications with agonists were performed using 30 µg Δ⁹-THC or 10 µg HU-308 per mouse in a 3% ethanol solution in acetone/olive oil (4:1).

Immunohistology. Immunostaining of zinc fixed tissue was performed with primary antibodies for CD3 (1:100, Serotec), B220, MHC-II and Gr-1 (1:25, BD Pharmingen). Slides were incubated with biotinylated rabbit anti-rat IgG and a Streptavidin-AP Fuchsin Chromogen System (Dako Cytomation) was used for colour development. After every step slides were washed in TRIS buffered saline. Counterstaining was performed with hematoxylin. Mast cell staining was performed with 1% alcoholic toluidine-blue.

In situ hybridization. Sections were treated with proteinase K and post-fixed in 4% paraformaldehyde. A 208bp PIQOR™ cDNA fragment (9) for MCP-2/CCL8 (accession no. NM_021443; nt 259-466) was used as template for generation of digoxigenin-labeled hybridization probes. Digoxigenin labeling was performed according to the protocol of the supplier (Roche). Visualisation was performed using alkaline phosphatase-conjugated anti-dig. Fab fragments (Roche) and NBT/BCIP (Sigma) dissolved in AP- buffer (see instructions of the manufacturer).

Myeloperoxidase assay. Ears of Cnr1^{-/-}/Cnr2^{-/-} and Cnr1^{+/+}/Cnr2^{+/+} were snap frozen, placed in HTAB buffer (50mM KH₂PO₄ + 0,5% hexadecyltrimethylammonium pH 6.0) and homogenized. Homogenates were centrifuged and 50 µl supernatant were added to 200 µl MPO reagent. Absorbance was measured at 460 nm. Data were normalized to the value obtained in control groups (10).

Endocannabinoid measurement. Tissues were homogenized with chloroform/methanol/-Tris-HCl 50mM pH=7.4 (1/1/1 by vol) containing d8-AEA and d5-2AG as internal standards. The lipid-containing organic phase was dried down, weighed, and pre-purified by open-bed chromatography on silica gel and analyzed by liquid chromatography (LC)-atmospheric pressure chemical ionization (APCI)-mass spectrometry (MS) (LCAPCI-MS) using a Shimadzu HPLC apparatus (LC-10ADVP) coupled to a Shimadzu (LCMS-2010) quadrupole MS via a Shimadzu APCI interface. MS analyses were carried out in the selected ion-monitoring (SIM) mode as described previously (11). Anandamide and 2-AG quasimolecular ions were quantified by isotope dilution with the above-mentioned deuterated standards and their amounts in pmols normalized per g of wet tissue extracted. Data were statistically evaluated by t-test analysis (*, p<0.05; **, p<0.01; ***, p<0.001).

In vitro MCP-2/CCL8 assay. The human keratinocyte cell line HaCaT were used for all in vitro assays. Cells were cultured in 10% FCS containing DMEM (Invitrogen) in a 5% CO₂ humidified atmosphere at 37°C. One day before stimulation cells were plated in a 24-well plate. MCP-2/CCL8 release was tested after stimulation with IFN γ (100ng/ml) and TNF α (50ng/ml), LPS (100ng/ml), CpG (1nmol/ml) or poly-(I:C) (50µg/ml). For further experiments HaCaT cells were stimulated always with 50µg/ml poly-(I:C) (Sigma) and the cannabinoid agonist HU-210. HU-210 (1µM) was applied in DMEM containing less than 0.001% ethanol/DMSO (1:9). Supernatants were collected after 24 hours and used for MCP-2/CCL8 ELISA detection according to the manufacturer's instructions (RayBio®, ELH-MCP-2-001). In vitro assays were performed at least three times in triplicates.

Microarray experiments. DNFB treated and control ears from three $Cnr1^{-/-}/Cnr2^{-/-}$ and three $Cnr1^{+/+}/Cnr2^{+/+}$ mice were used for the gene expression analysis. Tissue was harvested 48h after the 2nd challenge and immediately snap frozen in liquid nitrogen. Total RNA was isolated using Trizol (Invitrogen) and purified using RNeasy columns (Qiagen). Five μ g of total RNA was converted into double-stranded cDNA using T7-oligo dT coupled primers. Ten μ g of fragmented cRNA samples were hybridized to MG_430 2.0 Affymetrix GeneChips. All samples reported a GAPDH and β -actin average 3' to 5' ratio >0.9 on the GeneChips. All mice reported a comparable number of genes expressed (58.3% in treated and 57.5% in the control groups). Microarray expression values were generated using GC-RMA (Irizarry et al., 2003) using software "ArrayAssist 3.3" (Stratagene). For the treatment effects, which were independent of the genotype, all control (n=6) and DNFB-treated ears (n=6) were statistically analyzed using t-test with $p \leq 0.05$ after Bonferroni correction and a differential expression higher than fourfold (given as an average log 2 ratio; ALR). The genotype effects were obtained by comparison of DNFB-treated ears of $Cnr1^{+/+}/Cnr2^{+/+}$ (n=3) versus $Cnr1^{-/-}/Cnr2^{-/-}$ (n=3) with $p \leq 0.001$ and $ALR \geq 1$. Results for treatment and genotype effects were compared using Venn diagram. Expression data for the 12 samples analyzed in this study are available online through the Gene Expression Omnibus database (<http://www.ncbi.nlm.nih.gov/geo/>) in series entity GSE7694.

RT-PCR. RNA was isolated from HaCaT cells as described above and reverse transcribed using Omniscript (Qiagen) and oligo-dT18 primers. PCR was performed with 300 ng cDNA per reaction with HotStar Taq Polymerase (Qiagen). The PCR program included an annealing temperature of 60°C and 35 repeating steps (for house keeping gene 28 cycles). HaCaT cells were plated in 3 cm cell culture dishes, cells were stimulated 24h with 20 μ g/ml poly-(I:C) or with control medium (C) All results were obtained in three independent experiments. Sequences of primers: hCB1 forward (CTCACAGCCATCGACAGGTAC), hCB1 reverse (GTGCGAGGTCCTTACTCCTC), hCB2 forward (ATCATGTGGGTCCTCTCAGC), hCB2 reverse (GACCTGGTCACTGAGCGTAGTG)

Real-time PCR. RNA isolation from DNFB treated and untreated tissue 48h after the 2nd challenge from 9 mice was performed as described above. Two μ g of RNA were reverse transcribed with Omniscript (Qiagen). Real-time RT-PCR analysis was performed using Applied Biosystems TaqMan® Gene Expression Assays. Data was normalized to β -Actin. The relative quantification of CB1 and CB2 mRNA levels was obtained by using the Δ Ct-method, whereas fold-changes of MCP-2/CCL8 were calculated by the $\Delta\Delta$ Ct-method. (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Assay ID (sequences of primers and reporters are not provided by the company): Mm00607939_s1 with context sequence (TACTGAGCTGCGTTTTACACCCTTT); CB1, Mm00432621_s1 with context sequence (TCCACCGCAAAGATAGTCCCAATGT); CB2, Mm00438286_m1 with context sequence (CGGCTGACAAATGACACCCAGTCTT). The real-time analysis for CCL8 was performed by using an Applied Biosystems Custom TaqMan® Gene Expression Assay with the following primer sequences:

forward primer CTGTCAAGCCAGAGAAGCT,
reverse primer TTTAGTACATGAAAGCAGCAGGTGA,

reporter sequence CTGGAGCCTTATCTGGCC.

2. Supporting figures

Figure S1

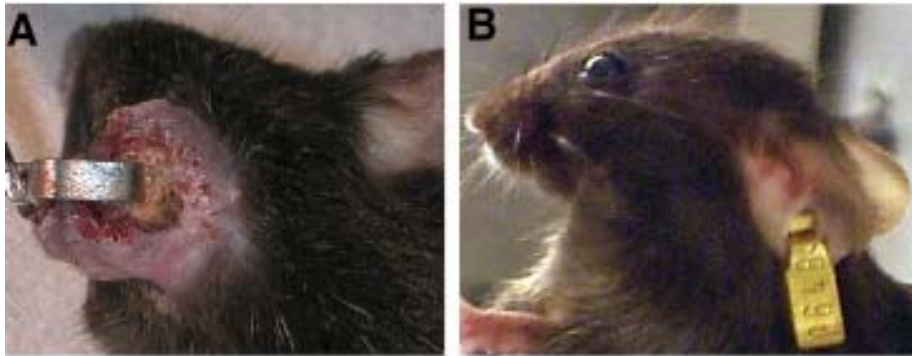


Fig. S1. Macroscopic views of ear-tagged mice. Clips with high nickel content cause chronic ulceration and intense infiltration of mast cells in $Cnr1^{-/-}/Cnr2^{-/-}$ mice (**A**). No ulcerations were observed in ears clipped with pure brass tags (**B**).

Figure S2

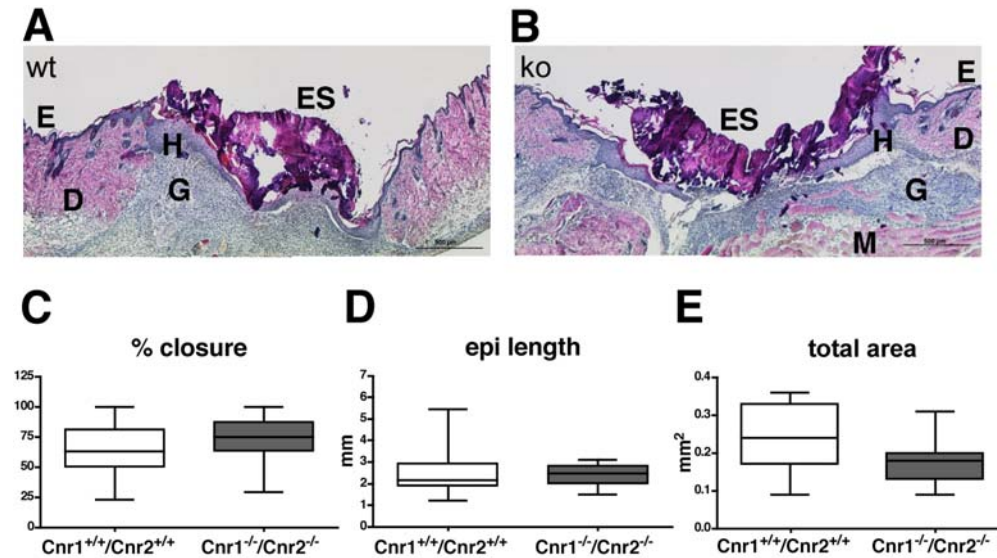


Fig. S2. Wound healing experiment. Ten- to 12-week-old mice were anaesthetized with ketamine/xylazine. Four full-thickness excisional wounds (5 mm diameter) were generated on the shaved back, two on each side of the vertebral column, by excising skin and panniculus carnosus. For histological analysis bisected wounds were fixed overnight in 4 % PFA in PBS or in 95 % ethanol/1 % acetic acid, followed by paraffin embedding. Sections (6 μ m) from the middle of the 5-day wounds of (A) Cnr1^{+/+}/Cnr2^{+/+} and (B) Cnr1^{-/-}/Cnr2^{-/-} mice were stained with hematoxylin/eosin and used for morphometric analysis. Only littermates of the same sex were used for direct histological comparison. Morphometric analysis resulted in no genotypic differences in (C) percentage of closure (D) epithelial length and (E) total area. D: dermis, E: epidermis, G: granulation tissue, H: hyperproliferative wound epidermis, ES: eschar, M: muscle (Panniculus carnosus).

Figure S3

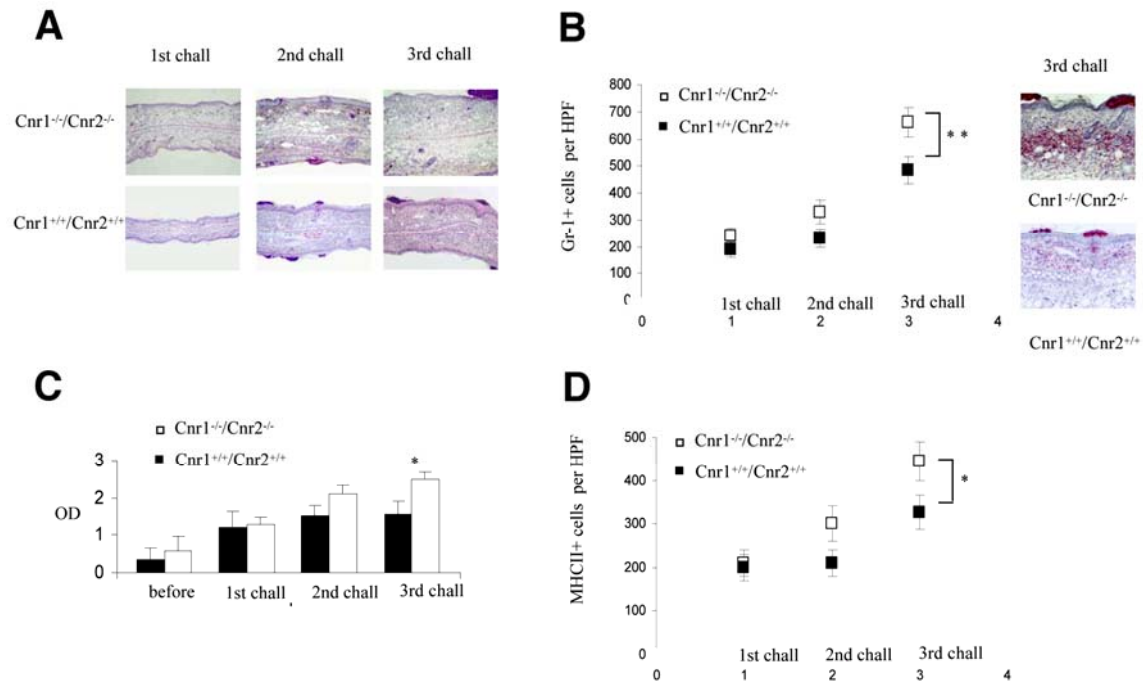


Fig. S3. Histopathology of contact allergic ear inflammation. **(A)** H&E-stained sections of ear tissue from Cnr1^{-/-}/Cnr2^{-/-} double knock-out and wildtype mice 48 h after the 1st, 2nd and 3rd challenge with DNFB are shown (x100 magnification). **(B)** The number of infiltrating Gr-1+ cells per high power field at x200 magnification was counted in immunostained sections of DNFB-treated ears from Cnr1^{-/-}/Cnr2^{-/-} and wildtype mice. Results are shown as mean counts $\pm$ SEM. An example of Gr-1 stained sections taken 48 h after the third challenge is depicted on the right. **(C)** Myeloperoxidase activity activity was determined in ear tissue of DNFB-treated Cnr1^{-/-}/Cnr2^{-/-} and wildtype mice after repeated challenges as an index for neutrophil infiltration into inflamed tissue. ** $p < 0.01$, * $p < 0.05$. **(D)** The number of MHC-II+ antigen-presenting cells 48 h after the 1st, 2nd and 3rd challenge in Cnr1^{-/-}/Cnr2^{-/-} and wildtype mice were counted per per high power field at x200 magnification. Results are shown as mean counts $\pm$ SEM. * $p < 0.05$.

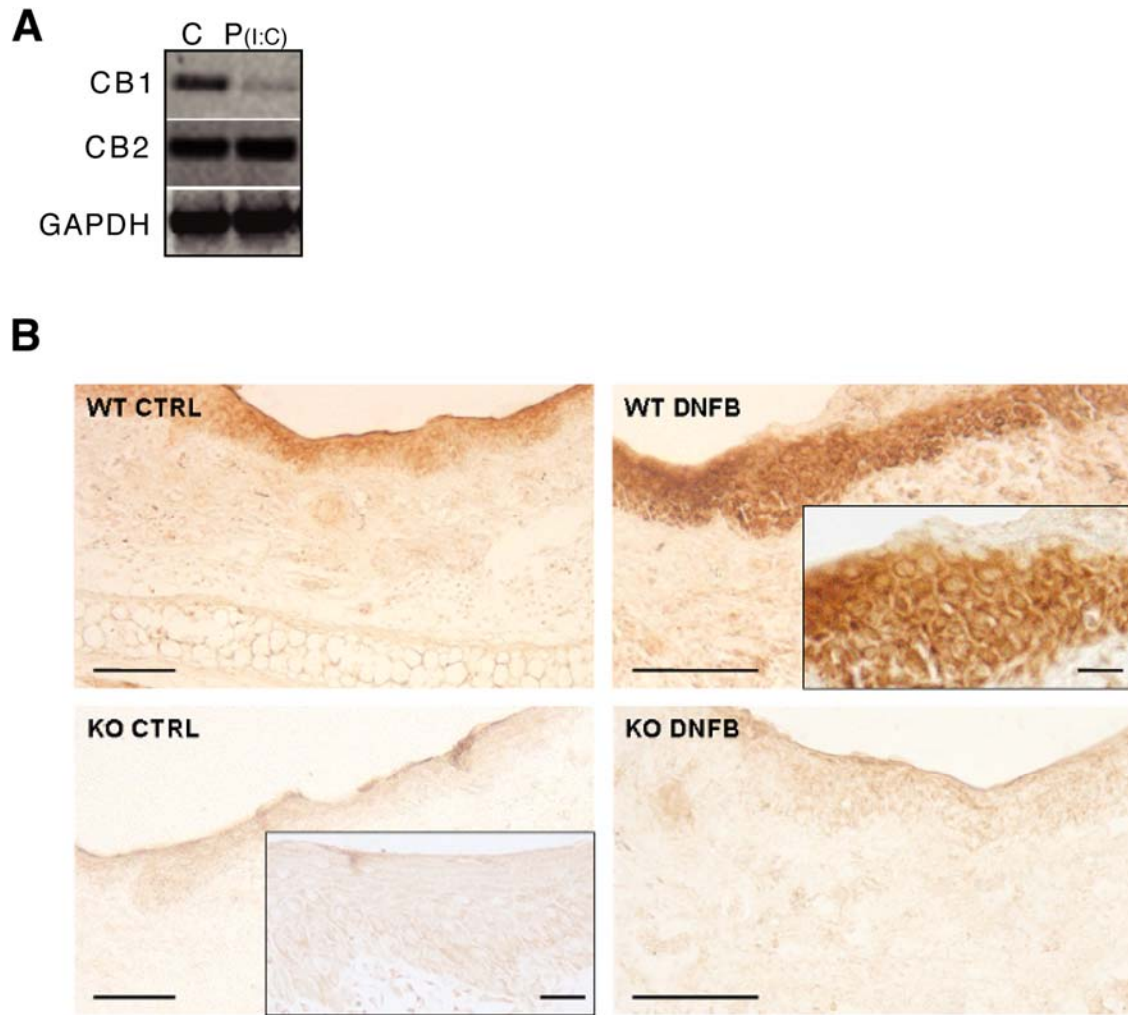
Figure S4

Fig. S4. Expression of CB1 and CB2 receptors in keratinocytes. **(A)** The human keratinocyte cell line (HaCaT) express CB1 and CB2 receptors (left lane, C - control). HaCaT cells stimulated 24 h with 20 $\mu\text{g/ml}$ poly-(I:C) showed a down-regulation of CB1 and no difference in CB2 mRNA expression (right lane, P(I:C)) in three independent experiments. **(B)** Immunohistochemical localization of CB2 in ear samples of mice treated with DNFB and control animals (CTRL). Photomicrographs demonstrating CB2-immunoreactivity in $\text{Cnr1}^{+/+}/\text{Cnr2}^{+/+}$ mainly located to the epidermal layer, most likely keratinocytes (upper left panel). Immunohistochemical staining reveals an increase in CB2-staining in the epidermis after DNFB treatment (upper right panel), as shown also by higher magnification of CB2-positive cells in the boxed area (inset). $\text{Cnr1}^{-/-}/\text{Cnr2}^{-/-}$ mice (see lower left panel) served as controls. Sections shown are representative of observations from two animals in each group and are representative of 3 different experiments. Scale bars correspond to 100 μm and 40 μm in insets.

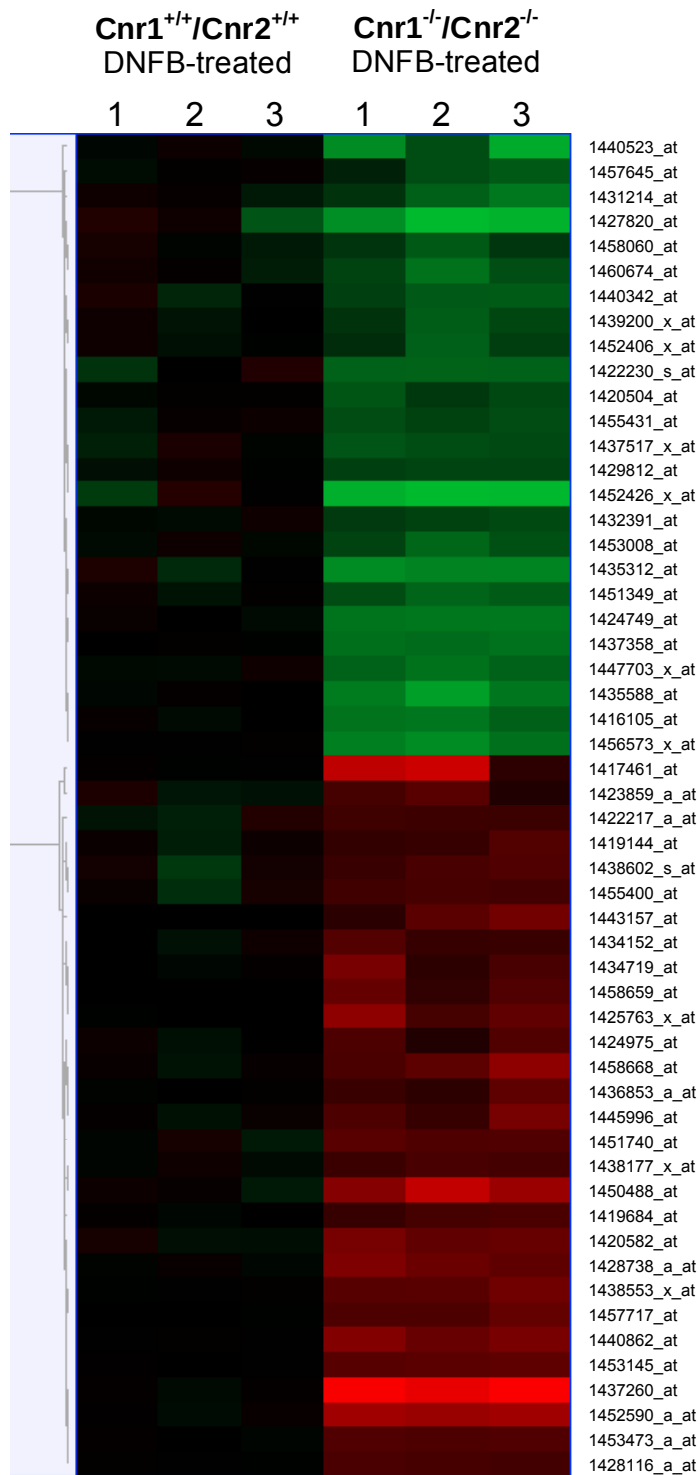
Figure S5

Fig. S5. Transcripts differentially expressed between DNFB-treated *Cnr1*^{+/+}/*Cnr2*^{+/+} and *Cnr1*^{-/-}/*Cnr2*^{-/-} ears as identified by an Affymetrix microarray analysis. Fifty-four probe sets met the inclusion criteria ($p < 0.05$, differential expression ≥ 2). Green color indicates transcripts downregulated in comparison to the baseline (treated *Cnr1*^{+/+}/*Cnr2*^{+/+}), red indicates upregulation.

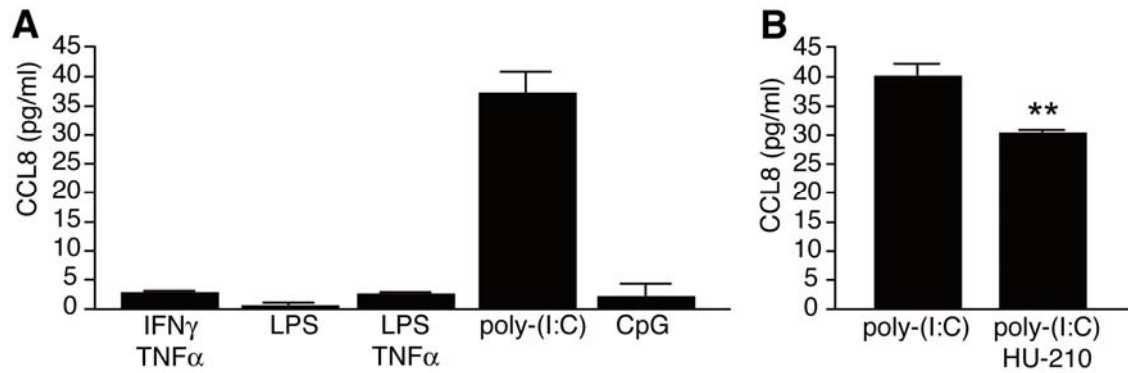
Figure S6

Fig. S6. MCP-2/CCL8 protein production in the spontaneously immortalized human keratinocyte cell line HaCaT. **(A)** HaCaT cells were treated with different stimulants for 24 hours (IFN γ : 100 ng/ml, TNF α : 50 ng/ml), LPS: 100 ng/ml, CpG: 1 nmol/ml or poly-(I:C): 50 μ g/ml). Quantification of MCP-2/CCL8 protein in the supernatant was performed using an ELISA. The toll-like receptor 3 (TLR3) ligand poly-(I:C) stimulated MCP-2/CCL8 protein production efficiently. **(B)** HaCaT cells were co-stimulated with poly-(I:C) and a CB1 and CB2 receptor agonist (1 μ M HU-210). MCP-2/CCL8 chemokine release was reduced by this cannabinoid. Our results demonstrate that the chemokine MCP-2/CCL8 is produced by human keratinocytes and that cannabinoids can modulate its synthesis. HU-210 treatment alone had no effect on MCP-2/CCL8 (data not shown, ** $p < 0.01$).

3. Supporting tables

Table S1

Upregulated Genes		Probe set	Ctrl	DNFB
Krt2-6b	Mm.196352	1422588_at	-0,55	9,62
2610528A11Rik	Mm.24660	1435639_at	-0,05	9,50
Saa3	Mm.14277	1450826_a_at	0,00	8,18
AW146083		1435761_at	-0,25	8,00
Krt1-16	Mm.19889	1448932_at	-0,49	8,16
Cxcl9	Mm.766	1418652_at	-0,46	7,05
Stfa3	Mm.77709	1419709_at	-0,30	7,97
Il1b	Mm.22150	1449399_a_at	-0,01	7,80
Cxcl2	Mm.4979	1449984_at	0,00	7,72
AV020412		1456539_at	-0,05	7,23
Ms4a4b	Mm.33957	1423467_at	-0,01	6,49
Ms4a4c	Mm.446	1450291_s_at	-0,03	6,41
Plac8	Mm.34609	1451335_at	-0,02	6,21
Spr2f	Mm.10692	1449833_at	0,00	6,95
Chi3l3	Mm.4571	1419764_at	-0,04	6,23
Fcgr3a	Mm.137745	1425225_at	-0,01	6,31
Slfn4	Mm.38192	1427102_at	-0,03	6,66
Al662854		1448025_at	-0,03	6,23
2310002A05Rik	Mm.46390	1456248_at	-0,20	6,92
Ms4a4c	Mm.159648	1420671_x_at	-0,02	6,10
Spr2h	Mm.10693	1422240_s_at	-0,21	6,47
MGI:3524944	Mm.138101	1442339_at	-0,16	7,20
S100a9	Mm.2128	1448756_at	-0,72	6,88
Gzma	Mm.15510	1417898_a_at	-0,01	6,26
Gzmb	Mm.14874	1419060_at	-0,01	6,07
Spr1b	Mm.140151	1422672_at	-0,47	6,33
Al447904	Mm.447	1435330_at	-0,02	5,91
Cxcl10	Mm.877	1418930_at	-0,04	5,75
L38281.1		1427381_at	-0,02	6,54
Ccr1	Mm.57051	1419609_at	-0,01	6,03
S100a8	Mm.21567	1419394_s_at	-0,81	6,33
Krt2-6a	Mm.22629	1422783_a_at	-0,01	6,16
Ccl5	Mm.3370	1418126_at	-0,02	5,84
Spr2a	Mm.6853	1439016_x_at	-0,20	6,20
BE688358		1434372_at	-0,02	5,43
Ccr5	Mm.14302	1424727_at	0,00	5,35
Oasl2	Mm.27162	1453196_a_at	-0,02	5,88
Oas1a	Mm.14301	1424775_at	-0,14	5,91
Gbp2	Mm.24038	1418240_at	-0,05	5,51

Tab. S1. Upregulated probe sets after DNFB-treatment. Shown are the mean log2-transformed expression values for the 40 strongest upregulated transcripts independent of the genotype. Ctrl, control ear; DNFB, DNFB-treated ear.

Table S2.

	Gene	Probeset	wt-ctrl	wt-tr	ko-ctrl	ko-tr
chemokine (C-C motif) ligand 2	Ccl2	1420380_at	0.16	2.85	-0.23	2.54
chemokine (C-C motif) ligand 3	Ccl3	1419561_at	0.01	4.84	-0.01	5.15
chemokine (C-C motif) ligand 5	Ccl5	1418126_at	-0.11	5.72	0.07	5.96
chemokine (C-C motif) ligand 7	Ccl7	1421228_at	0.19	2.64	-0.23	2.75
chemokine (C-C motif) ligand 8	Ccl8	1419684_at	-0.15	2.25	0.13	3.29
chemokine (C-C motif) receptor 1	Ccr1	1419609_at	-0.17	5.98	0.15	6.08
chemokine (C-C motif) receptor 1	Ccr1	1419610_at	0.00	4.18	0.00	3.99
chemokine (C-C motif) receptor 2	Ccr2	1421186_at	-0.04	3.65	0.02	3.50
chemokine (C-C motif) receptor 2	Ccr2	1421187_at	-0.07	3.54	0.06	3.35
chemokine (C-C motif) receptor 2	Ccr2	1421188_at	0.01	2.27	-0.01	1.95
chemokine (C-C motif) receptor 5	Ccr5	1422259_a_at	0.03	4.27	-0.20	4.04
chemokine (C-C motif) receptor 5	Ccr5	1422260_x_at	-0.01	3.04	0.00	3.00
chemokine (C-C motif) receptor 5	Ccr5	1424727_at	0.02	5.47	-0.03	5.23
chemokine (C-C motif) receptor 7	Ccr7	1423466_at	-0.03	1.75	0.03	2.29
chemokine (C-C motif) receptor-like 2	Ccr12	1427736_a_at	-0.05	2.53	0.04	2.91
chemokine (C-X-C motif) ligand 10	Cxcl10	1418930_at	-0.18	5.82	0.09	5.69
chemokine (C-X-C motif) ligand 16	Cxcl16	1418718_at	-0.06	1.94	0.05	2.12
chemokine (C-X-C motif) ligand 16	Cxcl16	1449195_s_at	-0.06	2.15	0.05	2.24
chemokine (C-X-C motif) ligand 2	Cxcl2	1449984_at	0.05	7.55	-0.05	7.89
chemokine (C-X-C motif) ligand 5	Cxcl5	1419728_at	0.01	5.53	-0.01	5.56
chemokine (C-X-C motif) ligand 9	Cxcl9	1418652_at	-1.08	7.19	0.16	6.91
chemokine (C-X-C motif) ligand 9	Cxcl9	1456907_at	-0.15	4.63	0.03	4.50
chemokine (C-X-C motif) receptor 3	Cxcr3	1449925_at	0.01	2.33	-0.02	2.18
chemokine (C-X-C motif) receptor 6	Cxcr6	1422812_at	0.04	2.77	-0.07	2.33
chemokine (C-X-C motif) receptor 6	Cxcr6	1425832_a_at	-0.33	3.27	0.25	3.00

Tab. S2. Chemokine related probesets encoding 16 genes, which were differentially expressed after DNFB-treatment (with $p \leq 0.05$ after Bonferroni correction, differential expression $\geq$ factor 2). Given are the mean log2-transformed expression values ctrl, control ear; tr, DNFB-treated ear; ko, $Cnr1^{-/-}/Cnr2^{-/-}$; wt, $Cnr1^{+/+}/Cnr2^{+/+}$

4. Supporting references

1. B. F. Cravatt *et al.*, *Proc Natl Acad Sci U S A* **98**, 9371 (Jul 31, 2001).
2. A. Zimmer, A. M. Zimmer, A. G. Hohmann, M. Herkenham, T. I. Bonner, *Proc Natl Acad Sci U S A* **96**, 5780 (May 11, 1999).
3. N. E. Buckley *et al.*, *Eur J Pharmacol* **396**, 141 (May 19, 2000).
4. Z. Jarai *et al.*, *Proc Natl Acad Sci U S A* **96**, 14136 (Nov 23, 1999).
5. S. Werner *et al.*, *Science* **266**, 819 (Nov 4, 1994).
6. C. Bamberger *et al.*, *Am J Pathol* **167**, 733 (Sep, 2005).
7. I. S. Thorey *et al.*, *J Biol Chem* **279**, 26674 (Jun 18, 2004).
8. J. Knop, R. Stremmer, C. Neumann, E. De Maeyer, E. Macher, *Nature* **296**, 757 (Apr 22, 1982).
9. S. Tomiuk, K. Hofmann, *Brief Bioinform* **2**, 329 (Dec, 2001).
10. F. Massa *et al.*, *J Clin Invest* **113**, 1202 (Apr, 2004).
11. G. Marsicano *et al.*, *Nature* **418**, 530 (Aug 1, 2002).