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https://doi.org/10.1038/s41586-018-0540-1

T cells in patients with narcolepsy target self-antigens of hypocretin neurons

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Narcolepsy is a chronic sleep disorder caused by the loss of neurons that produce hypocretin. The close association with *HLA-DQB1*06:02*, evidence for immune dysregulation and increased incidence upon influenza vaccination together suggest that this disorder has an autoimmune origin. However, there is little evidence of autoreactive lymphocytes in patients with narcolepsy. Here we used sensitive cellular screens and detected hypocretin-specific CD4$^+$ T cells in all 19 patients that we tested; T cells specific for tribbles homologue 2—another self-antigen of hypocretin neurons—were found in 8 out of 13 patients. Autoreactive CD4$^+$ T cells were polyclonal, targeted multiple epitopes, were restricted primarily by HLA-DR and did not cross-react with influenza antigens. Hypocretin-specific CD8$^+$ T cells were also detected in the blood and cerebrospinal fluid of several patients with narcolepsy. Autoreactive clonotypes were serially detected in the blood of the same—and even of different—patients, but not in healthy control individuals. These findings solidify the autoimmune aetiology of narcolepsy and provide a basis for rapid diagnosis and treatment of this disease.

Narcolepsy is a rare, life-long neurological disorder that affects about 0.05% of the general population and presents with excessive daytime sleepiness, cataplexy, hypnagogic hallucinations and sleep paralysis[1,2]. Idiopathic sporadic narcolepsy, which represents over 98% of cases, is due to a selective loss of a small number of hypocretin (HCRT) neurons in the lateral hypothalamus[3,4]. The strong genetic association with *HLA-DQB1*06:02*[5,6], the evidence for immune dysregulation[7,8] and the increased disease incidence upon influenza vaccination[9,10] suggest the possibility that the loss of HCRT neurons reflects the contribution of cellular and humoral immunological responses that manifest in genetically predisposed individuals upon triggering by environmental factors[11–13]. However, so far the unequivocal demonstration of specific autoreactive T lymphocytes in narcolepsy is absent.

HCRT-specific memory CD4$^+$ T cells in narcolepsy

To investigate the autoimmune basis of narcolepsy, we obtained peripheral blood samples from a total of 16 patients with HCRT deficiency (levels of HCRT in cerebrospinal fluid (CSF) < 110 pg ml^{-1}) and clinical diagnosis of narcolepsy with cataplexy (narcolepsy type 1, NT1), of whom 14 carried the disease-associated *HLA-DQB1*06:02* allele (Extended Data Table 1). We also obtained blood samples from three patients who lacked the *HLA-DQB1*06:02* allele, and who had a clinical diagnosis of narcolepsy with mild or no cataplexy and intermediate or normal levels of HCRT in the CSF (narcolepsy type 2, NT2). As controls, we obtained samples from 13 healthy donors who carried the *HLA-DQB1*06:02* allele. Patients were 16–53 years of age (median 32) and had different disease durations (range 1–36 years, median 7 years).

Given the likely low frequency of circulating autoreactive T cells[13,14], we used two different approaches to interrogate the T cell repertoire of patients with narcolepsy. First, memory CD45RA$^-$CD4$^+$ T cells were isolated by cell sorting to high purity (>98%), labelled with carboxyfluorescein succinimidyl ester (CFSE), and then stimulated with autologous monocytes that were either untreated or pulsed with a pool of 15-mer peptides that spanned the entire sequence of the 131-amino-acid precursor molecule (prepro-hypocretin (hereafter defined as HCRT)) that gives rise—through proteolytic processing—to HCRT-1 and HCRT-2 neuropeptides[15]. Out of nine patients with NT1 who were analysed with this method, only one showed a clear response to HCRT on day 7 (patient P8), as demonstrated by the CFSE^{low} profile and the selective upregulation of the activation markers ICOS and CD25 (Fig. 1a). In other cases, few CFSE^{low}-proliferating T cells were detected in both unstimulated and HCRT-stimulated cultures, but only in the latter did a fraction of the cells express ICOS and CD25 (see patient P22, Fig. 1b). Of note, ICOS$^+$CD25$^+$ cells within CFSE^{low}-proliferating cells could be detected in six out of nine patients with NT1 (66%), but in none of six healthy controls (Fig. 1c, d).

As an alternative and more sensitive approach to identify autoreactive T cells, we used the T cell library method that we have previously found to be suitable for detecting rare antigen-specific and autoreactive T cells[16,17]. Memory CD45RA$^-$CD4$^+$ T cells from 15 patients with NT1 and 3 patients with NT2 were initially expanded polyclonally, and then screened for their capacity to proliferate in response to autologous B cells pulsed with a HCRT peptide pool. With the exception of one patient (P24), all patients with NT1 or NT2 showed a clear—and often strong—proliferative response to HCRT, whereas there were only a few proliferating lines in 3 out of 12 healthy controls (Fig. 2a–c). The magnitude of the proliferative response of positive T cell lines of patients with NT1 or NT2 varied from 2.0×10^3 to 76.4×10^3 and was significantly higher compared to the proliferative response of the few positive T cell lines of controls (Fig. 2d). On the basis of these results, we estimated that the frequency of HCRT-reactive T cells in patients with NT1 or NT2 ranged from <1 to 89.7 (21.4 ± 26.4 (mean $\pm$ s.d.), 10.5 (median)) or from 7.9 to 70.9 (36.1 ± 32 (mean $\pm$ s.d.), 29.5 (median)), respectively, in 10^6 memory CD4$^+$ T cells, which was significantly higher

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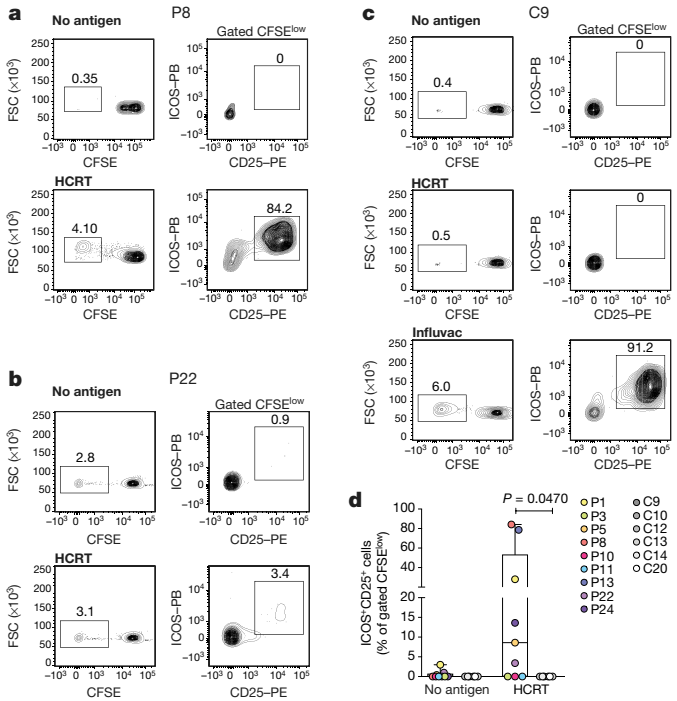

Fig. 1 | Ex vivo stimulation of memory CD4$^+$ T cells from patients with narcolepsy and healthy controls. a–c, Memory CD4$^+$ T cells from the blood of patients with narcolepsy and controls were labelled with CFSE and stimulated with autologous monocytes in the presence or absence of the HCRT peptide pool. On day seven, cells were collected and stained with anti-CD25–phycoerythrin (PE) and anti-ICOS–Pacific Blue (PB) monoclonal antibodies. CFSE profiles and dot plots of CD25 and ICOS expression of gated proliferating CFSE^{low} cells from two representative patients with NT1 (P8 (**a**) and P22 (**b**)) and one control (C9 (**c**)) are shown. Data from the stimulation of T cells from the control C9 with the influenza vaccine Influvac, used as positive control, are also shown. **d**, Pooled data from the indicated patients with NT1 ($n = 9$ biologically independent samples, coloured dots) and controls ($n = 6$ biologically independent samples, white and grey dots) are shown as the percentage of CD25$^+$ICOS$^+$ cells of gated proliferating CFSE^{low} cells in the absence (no antigen) or presence of the HCRT peptide pool. There were no CD25$^+$ICOS$^+$ cells in the pool of CFSE^{high} non-proliferating cells. Each dot represents a donor and boxes are quartile values, whiskers represent the highest and lowest values, and lines represent the median values. Data were analysed using two-tailed Mann–Whitney *U*-test. FSC, forward scatter.

than the frequency in controls (range <1–5.2 (1.8 ± 1.6 (mean ± s.d.), 1.0 (median)) in 10^6 memory CD4$^+$ T cells) (Fig. 2e). Of note, high frequencies of HCRT-reactive T cells were found in patients P10, P5 and P13—who were analysed 27, 6 and 2 years, respectively, after the diagnosis of NT1—and in patient P4, who was analysed 5 years after the diagnosis of NT2. Parallel screening of the same T cell libraries with seasonal influenza A antigens showed that the proliferative response and the frequency of specific memory CD4$^+$ T cells were comparable in patients and controls (Extended Data Fig. 1).

Collectively, the results obtained using ex vivo antigenic stimulation and the sensitive T cell library screening method demonstrate that HCRT-specific memory CD4$^+$ T cells are present in the blood of all of the patients with narcolepsy who had HCRT deficiency, even years after disease onset. HCRT-specific memory CD4$^+$ T cells are also present in the patients with narcolepsy who do not carry the *HLA-DQB1*06:02* allele, and in patients with narcolepsy without HCRT deficiency.

T cells against antigens of HCRT neurons

We took advantage of the possibility of interrogating the T cell libraries with multiple antigens to investigate whether patients with narcolepsy would react to other proteins expressed by HCRT neurons. Memory CD4$^+$ T cell libraries from patients and controls were re-screened for reactivity against tribbles homologue 2 (TRIB2), an intracellular protein produced by HCRT neurons and other cell types that is targeted by antibodies in a fraction of patients with narcolepsy[18–20]. In 8 out of 13 patients, but also in 8 out of 12 controls, we could identify several T cell lines that proliferated in response to autologous B cells pulsed with a pool of overlapping peptides spanning the TRIB2 sequence (Fig. 2f, g). Although the frequency of TRIB2-reactive memory CD4$^+$ T cells was comparable, the magnitude of the proliferative response was significantly higher in patients with narcolepsy compared to controls (Fig. 2h, i). We were also able to establish T cell libraries of memory CD8$^+$ T cells from 13 patients and 9 controls. Although many CD8$^+$ T cell lines from both groups responded comparably to human cytomegalovirus and Epstein–Barr virus, 26 CD8$^+$ T cell lines from patients—but only 2 from controls—responded to HCRT (Extended Data Fig. 2). Therefore, memory CD4$^+$ and rare memory CD8$^+$ T cells in patients with narcolepsy can target HCRT and other self-antigens expressed by HCRT neurons.

Characterization of autoreactive T cells

To further characterize the autoreactive T cells present in narcolepsy, we isolated 184 HCRT-specific CD4$^+$ T cell clones from 9 patients and 30 TRIB2-specific CD4$^+$ T cell clones from 3 patients (Supplementary Table 1), from positive cultures. Sequencing the T cell receptor β-chain variable region (*TRBV*) of all clones identified 64 HCRT-reactive and 15 TRIB2-reactive unique clonotypes. In individual patients, the response could include multiple clonotypes with no bias towards a particular Vβ family (Fig. 3a). When analysed for cytokine production, a panel of HCRT-reactive and TRIB2-reactive T cell clones produced IFNγ and granulocyte–macrophage colony-stimulating factor (GM-CSF) in response to antigenic stimulation (Extended Data Fig. 3a), which indicates that the memory cells are primarily of the T helper 1 (T_H1) type. HCRT- and TRIB2-autoreactive clones expressed high levels of *TBX21* and *STAT4* mRNAs as well as mRNAs encoding T_H1-associated pro-inflammatory cytokines, chemokines and chemokine receptors (Extended Data Fig. 3b).

The specificity of 57 unique HCRT-specific T cell clones and of 15 unique TRIB2-reactive T cell clones was mapped to distinct peptides that span the whole sequence of the two proteins (Fig. 3b, c). Specifically, 22 or 7 HCRT-specific T cell clones recognized peptides in HCRT-2 (amino acids 70–97) or HCRT-1 (amino acids 34–66), respectively, and 17 clones recognized peptides in the signal sequence (amino acids 1–33) or in the C-terminal region (amino acids 98–131) of HCRT[15]. This demonstrates that autoreactive CD4$^+$ T cells target several epitopes that encompass multiple sites of HCRT-1 and HCRT-2 as well as regions that are not found in the mature proteins, with a dominance that varies among patients.

Collectively, these findings show that the response to HCRT and TRIB2 is polyclonal, polarized towards GM-CSF-producing T_H1 cells and directed against multiple epitopes in individual patients.

No T cell cross-reactivity with influenza antigens

A 6–9-fold increase in the risk of narcolepsy was reported in northern Europe after the 2009–2010 campaign of vaccination against pandemic H1N1 influenza[9,10], raising the possibility that the disease might be mediated by cross-reactive T cells or antibodies[21]. However, none of the HCRT- or TRIB2-specific T cell clones proliferated in response to influenza vaccine containing A/California/7/2009 H1N1 or to CA09 H1 haemagglutinin (Extended Data Fig. 4a, b). These findings do not support the notion of a molecular mimicry between epitopes on influenza antigens and HCRT, at least in this group of patients with spontaneous narcolepsy that is not associated with vaccination or infection. They are also consistent with the results of a previous study that failed to identify an immune signature induced by vaccination in its patient cohort[7].

T cell antigen recognition and MHC restriction

The notable effect of the *HLA-DQB1*06:02* haplotype on the risk of developing narcolepsy (98% versus 25% frequency in European

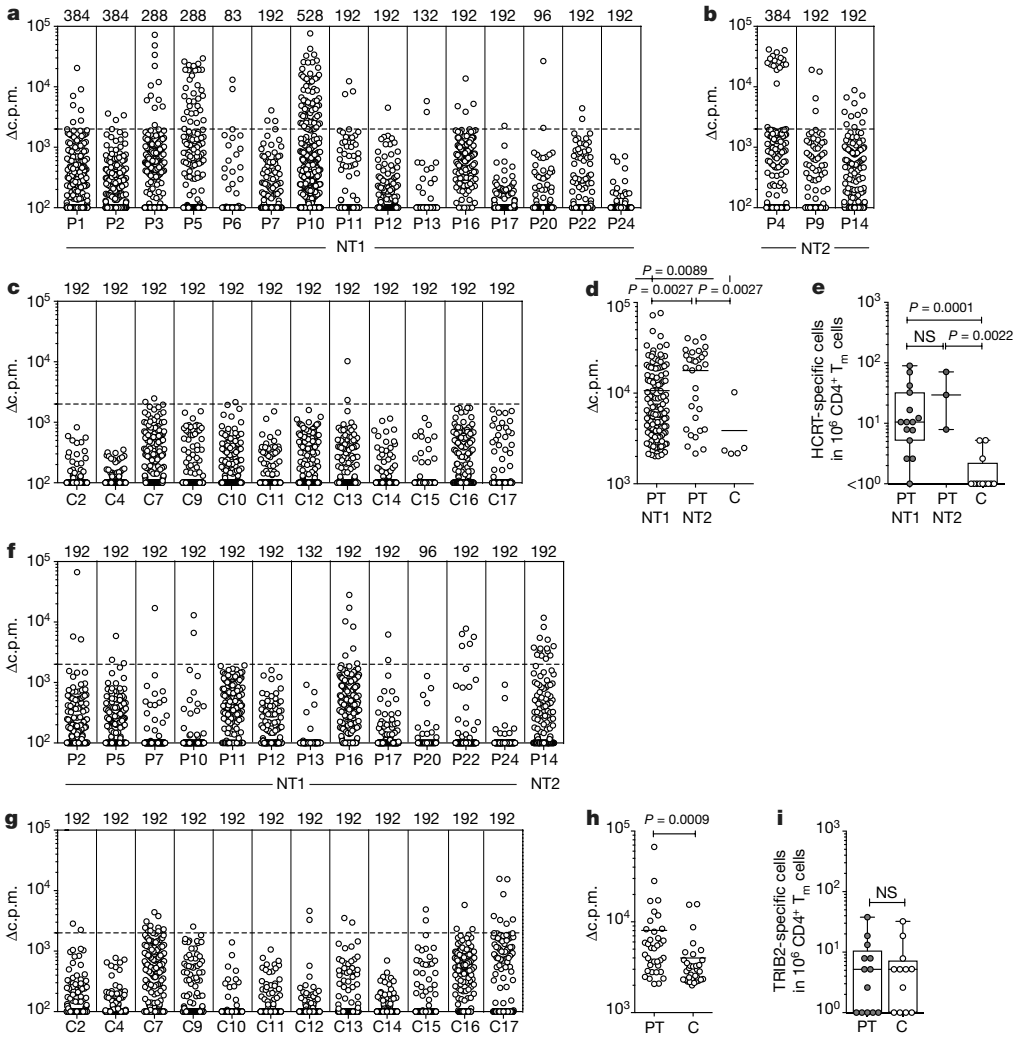

Fig. 2 | Autoreactive memory CD4$^+$ T cells in patients with narcolepsy as detected using the T cell library method. **a**–**c**, **f**, **g**, Memory CD4$^+$ T cell library screening for HCRT (**a**–**c**) or TRIB2 (**f**, **g**). Memory CD45RA$^-$CD4$^+$ T cells from the blood of patients with NT1 or NT2 (patients labelled with P + patient number) and healthy controls (controls labelled with C + control number) were sorted and polyclonally expanded in multiple wells, each containing 2,000 cells. The number of wells ranged from 83 to 528, depending on the number of cells isolated, and is indicated on top of the graphs in **a**–**c** and in **f**, **g**. The individual T cell lines (each represented by a single dot) were screened against pools of overlapping peptides spanning the HCRT (**a**–**c**) or TRIB2 (**f**, **g**) sequence, presented by irradiated autologous B cells. Proliferation was assessed on day 4 after a 16-h pulse with [^{3}H]thymidine. Data are expressed as counts per min (c.p.m.), after subtraction of background proliferation (Δc.p.m.). The background T cell proliferation (mean ± s.d.) in the absence of antigen in patients was 1,551 ± 2,330 c.p.m. (median 859) and in controls was 958 ± 1,121 c.p.m. (median 582). Positive cultures were defined as Δc.p.m. ≥ 2,000 (horizontal dotted line) and stimulation index (SI) ≥ 3. T cell lines with Δc.p.m. ≥ 2,000 and SI < 3 owing to 'autologous mixed lymphocyte reaction'—which were detected in both patients and controls—were removed. **d**, **h**, The Δc.p.m. values of HCRT-reactive lines (**d**; NT1, $n = 140$; NT2, $n = 31$; control, $n = 5$, biologically independent samples) and TRIB2-reactive lines (**h**; patients, $n = 37$; controls, $n = 32$, biologically independent samples) from patients (PT) or controls (C) are shown. **e**, **i**, The frequencies of HCRT-specific cells (**e**) and TRIB2-specific cells (**i**) per million memory CD4$^+$ T (T_m) cells in patients with narcolepsy and controls are shown (**e**, NT1, $n = 15$; NT2, $n = 3$; control, $n = 12$, biologically independent samples; **i**, patients, $n = 13$; controls, $n = 9$, biologically independent samples), calculated using the Poisson distribution. Dots represent frequency of each donor, boxes are quartile values, whiskers represent the highest and lowest values, and lines represent median values. Data were analysed using two-tailed Mann–Whitney *U*-test. NS, not significant (P values > 0.05).

ancestry cases and controls, respectively)[5,6] and the association of the disease with variants in the T cell receptor-α (*TRA*) and cathepsin H (*CTSH*) genes[22,23] suggest the importance of antigen processing and presentation to MHC-class-II-restricted T cells in the pathophysiology of narcolepsy. To investigate the basis for the association between narcolepsy and *HLA-DQB1*06:02*, we determined whether any of the clones isolated were HLA-DQ-restricted. The inhibition of peptide-induced T cell proliferation by blocking antibodies revealed that the majority of autoreactive T cell clones were HLA-DR-restricted, although a few were HLA-DQ- or HLA-DP-restricted (Fig. 3d, e and Extended Data Table 2). We then considered the possibility that the autoreactive clones might recognize HCRT peptides in association with HLA-DRB1*15:01 or HLA-DRB5*01:01 molecules, the genes for which are in linkage disequilibrium with *HLA-DQB1*06:02* and were expressed in all *HLA-DQB1*06:02*-positive patients (Extended Data Table 1). A re-assessment of nine HLA-DR-restricted T cell clones demonstrated that four clones proliferated in response to a HCRT peptide pool presented by autologous B cells and to a HCRT peptide pool presented by an HLA-DRB1*15:01-expressing B cell line (Extended Data Fig. 4c).

To investigate the mode of antigen presentation of neuronal antigens to autoreactive T cell clones, we compared the proliferative response elicited by peptides versus soluble proteins (human TRIB2 or a mixture of human HCRT-1 and HCRT-2), which require processing by antigen-presenting cells (Fig. 3f). Only 1 out of 34 HCRT-specific CD4$^+$ T cell clones and 6 out of 15 TRIB2-specific CD4$^+$ T cell clones proliferated in

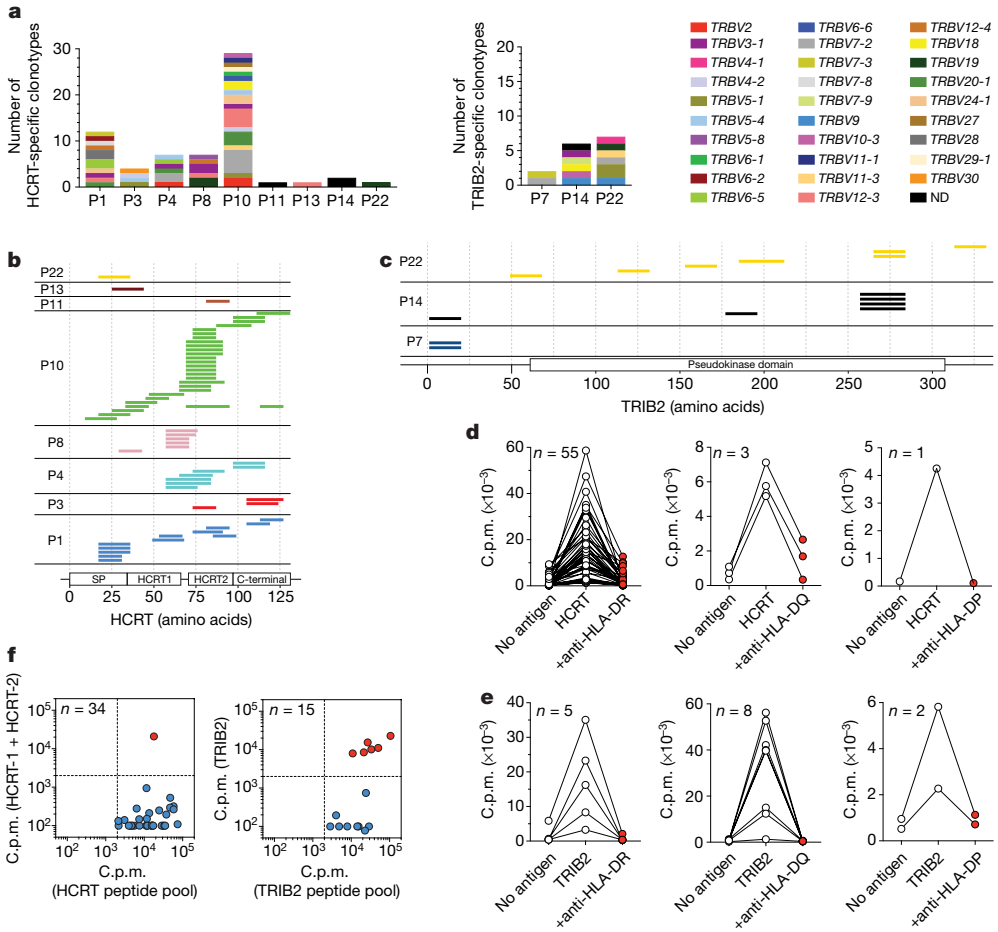

Fig. 3 | Characterization of autoreactive CD4$^+$ T cell clones from patients with narcolepsy. a, TCR Vβ gene repertoire of HCRT-specific (left) or TRIB2-specific (right) CD4$^+$ T cell clones isolated from the indicated patients with narcolepsy. The *y* axis indicates the number of autoreactive clonotypes (that is, the number of T cell clones carrying different TCR Vβ and complementarity-determining region 3 (CDR3) sequences). ND, not determined. **b**, **c**, Epitope mapping of HCRT-specific (**b**, $n = 57$) or TRIB2-specific (**c**, $n = 15$) CD4$^+$ T cell clones from patients with narcolepsy. Epitopes were identified by screening the autoreactive CD4$^+$ T cell clones against overlapping peptides that span the entire HCRT or TRIB2 protein length. Each line represents the sequence recognized by a unique clonotype and each colour indicates a patient. **d**, **e**, MHC restriction of autoreactive CD4$^+$ T cell clones from patients with narcolepsy. HCRT-specific (**d**, $n = 59$) and TRIB2-specific (**e**, $n = 15$) CD4$^+$ T cell clones were stimulated with autologous B cells untreated (no antigen) or pulsed with the indicated antigens in the absence or presence of MHC-class-II blocking antibodies (+ anti-HLA-DR, + anti-HLA-DQ and + anti-HLA-DP). Proliferation was measured on day 3 after a 16-h pulse with [^{3}H]thymidine, and is expressed as c.p.m. Each clone was tested separately with the three antibodies and HLA restriction was determined when inhibition was >80% (red dots). **f**, Type-A and type-B autoreactive CD4$^+$ T cell clones from patients with narcolepsy. HCRT-specific ($n = 34$) and TRIB2-specific ($n = 15$) CD4$^+$ T cell clones were stimulated with autologous B cells in the presence of antigen provided as an HCRT or TRIB2 peptide pool (*x* axis) or a mix of HCRT-1 and HCRT-2 or TRIB2 (*y* axis) protein. The HCRT-specific clones used in this assay recognized epitopes present in HCRT-1 or HCRT-2. Proliferation was measured on day 3 after a 16-h pulse with [^{3}H]thymidine and expressed as c.p.m. Type-A and type-B clonotypes are shown in red and blue, respectively.

response to both forms of antigen (defined as type-A T cell clones), and all of the remaining clones proliferated only in response to peptides (defined as type-B T cell clones)[24]. Comparable findings were obtained using monocytes and B cells (data not shown), which indicates that the failure to generate the correct T cell epitope is shared across different types of antigen-presenting cells. Collectively, these findings suggest that the differential recognition of proteins and peptides may be due to different complex conformations generated by peptides binding to MHC-class-II molecules in late endosomal compartments or to cell-surface or recycling MHC-class-II molecules[24,25].

Clonotypic analysis of blood and CSF T cells

Having obtained the TCR sequences of well-characterized autoreactive T cell clones, we next determined whether the same clonotypes could be found in a different sample from the same patient, or in different patients and in control donors. To do so, we performed high-throughput TCR Vβ sequencing of 0.5×10^6 memory CD4$^+$ T cells directly isolated ex vivo from blood of 18 patients (13 *HLA-DQB1*06:02*$^+$ and 5 *HLA-DQB1*06:02*$^-$) and 13 *HLA-DQB1*06:02*$^+$ controls (Supplementary Table 2) and ranked the clonotypes according to their frequency (Fig. 4a and Extended Data Fig. 5a, b). In P10, eight TCR Vβ clonotypes corresponded to those of HCRT-specific T cell clones from the same patient, and two of these were among the most-frequent circulating clonotypes. In P22, three TCR Vβ clonotypes corresponded to those of TRIB2-specific clones, and in P3, P4, P8, P13 and P14 one or two clonotypes corresponded to sequences of autoreactive clones isolated from the same patients. Four TCR Vβ clonotypes associated with HCRT- or TRIB2-autoreactive T cell clones were found also in other patients (marked by an asterisk in Fig. 4a). Of note, none of the autoreactive TCR Vβ clonotypes was found in memory CD4$^+$ T cells isolated from the blood of healthy control donors (Extended Data Fig. 5b). Overall, the TCR Vβ sequencing approach confirmed the presence of expanded autoreactive CD4$^+$ T cell clones in the blood of patients with narcolepsy, and suggested that some autoreactive T cells may share a public TCR Vβ clonotype.

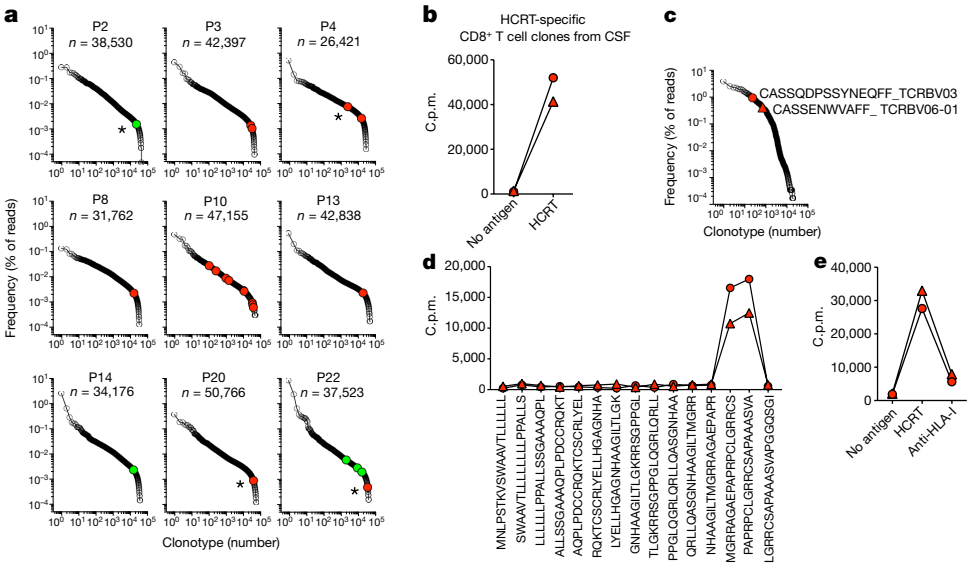

Fig. 4 | Autoreactive CD4$^+$ and CD8$^+$ T cell clonotypes in blood and CSF of patients with narcolepsy. a, TCR Vβ CDR3 sequences of autoreactive CD4$^+$ T cell clones can be found in the blood of the same or of different patients with narcolepsy. TCR Vβ sequencing was performed on memory CD4$^+$ T cells ex vivo, after sorting from peripheral blood of patients with narcolepsy. The frequency distribution of all TCR Vβ clonotypes is shown (*n* indicates total number of clonotypes). Coloured circles indicate TCR Vβ clonotypes identical to those found in HCRT-specific (red) and TRIB2-specific (green) CD4$^+$ T cell clones isolated from the same patient. Asterisk indicates TCR Vβ clonotypes found in autoreactive CD4$^+$ T cell clones isolated from a different patient. TCR Vβ sequencing was performed also on samples from patients P1, P5, P7, P9, P11, P12, P16, P17 and P24 and from 13 healthy controls (see Extended Data Fig. 5). In these samples, no sequences of autoreactive T cell clones were found. **b**, Two HCRT-specific CD8$^+$ T cell clones (red circle, clone 1; red triangle, clone 2) were isolated from the CSF of a patient with NT2 with recent disease onset (P14). The proliferation measured after a 16-h pulse with [^{3}H]thymidine is expressed as c.p.m. **c**, Frequency of the identified HCRT-specific CD8$^+$ T cell clones (red circle and triangle) in CSF. TCR Vβ sequencing was performed on CD8$^+$ T cells sorted from in vitro-expanded CSF T cells. **d**, Epitope mapping of the identified HCRT-specific CD8$^+$ T cell clones from the CSF. Epitopes were identified by screening the CD8$^+$ T cell clones against overlapping peptides that span the entire length of HCRT. **e**, MHC restriction of the HCRT-specific CD8$^+$ T cell clones was evaluated by measuring their proliferation against HCRT peptide pool alone or in combination with MHC-class-I blocking antibody.

We obtained a sample of CSF from 7 of our patients and—after polyclonal expansion—intrathecal CD4$^+$ T cells were analysed by TCR Vβ sequencing (Supplementary Table 2), which led to the identification of 500–3,000 clonotypes in different samples. A comparison of CSF and peripheral blood showed that several clonotypes could be detected in both samples, including some that were highly represented in blood (Extended Data Fig. 6a). However, none of the autoreactive clonotypes identified in peripheral blood was also found in the CSF, possibly owing to the low frequency of the clones or to the low number of T cells analysed. We also performed TCR Vβ sequencing of CD8$^+$ T cells from CSF and peripheral blood (Supplementary Table 2) and found that several clonotypes were shared between the two compartments (Extended Data Fig. 6b). Finally, we searched autoreactive T cell clones in the CSF. This approach led to the isolation of two HCRT-specific CD8$^+$ T cell clones that carried different TCRs from one patient with NT2 with recent disease onset (P14). These clones recognized an epitope within the HCRT (in the region of amino acids 97–124) and were MHC-class-I-restricted, as shown by antibody inhibition experiments (Fig. 4b–e). The presence of HCRT-reactive CD8$^+$ T cells in patients with normal levels of HCRT in CSF and a lack of definite cataplexy may indicate an ongoing destruction, and would be consistent with the notion that a loss of more than 80% of HCRT neurons is necessary for the development of full-blown narcolepsy with cataplexy[4].

Discussion

The findings of this study demonstrate the existence, in patients with narcolepsy, of autoreactive memory CD4$^+$ and—in some cases—CD8$^+$ T cells that target self-antigens expressed by neurons that produce HCRT. The overall low frequency of autoreactive T cells may be due to the temporal gap between the onset of symptoms, the diagnosis of narcolepsy and the immunological analysis[26]. Autoreactive CD4$^+$ and CD8$^+$ T cells were also found in the few cases of NT2 that we analysed; NT2 represents a less severe condition that, in some cases, can progress to full-blown NT1 with cataplexy and HCRT deficiency[27–29]. In this context, it is of note that patient P4—who, at the time blood was drawn, had been diagnosed with NT2—recently developed cataplexy, thus meeting the clinical criteria for NT1. The previous finding of relatively high levels of CD4$^+$ and CD8$^+$ T cells against HCRT in this patient would be consistent with an autoimmune attack that has not (yet) led to a complete loss of neurons that produce HCRT. Future research should test for the presence of autoreactive T cells in larger populations of patients with narcolepsy, including patients with incomplete and evolving clinical manifestations as well as patients with familial and post-infectious (or post-vaccination) narcolepsy, and in populations of patients with other forms of central hypersomnolence disorders.

The finding of autoreactive CD4$^+$ and CD8$^+$ T cells in narcolepsy raises questions as to their possible pathogenic role. CD8$^+$ T cells have the potential to directly kill HCRT neurons, as demonstrated in transgenic mice that express haemagglutinin in HCRT neurons, in which the transfer of cytotoxic haemagglutinin-specific CD8$^+$ T cells led to selective neuronal destruction, sleep attack and cataplexy[12]. Of note, an extensive hypothalamic CD8$^+$ T cell infiltrate was reported in a patient with concomitant Ma2 antibody encephalitis, four months after the onset of symptoms of narcolepsy[11]. By contrast, autoreactive CD4$^+$ T cells may have an indirect effect that promotes the generation of pathogenic CD8$^+$ T cells or autoantibodies, as hypothalamic neurons do not express constitutively MHC-class-II molecules. By producing high levels of IFNγ and GM-CSF, autoreactive CD4$^+$ T cells may also promote local inflammation and loss of integrity of the blood–brain barrier, triggering the influx of effector inflammatory cells and pathogenic antibodies[30].

Most of the T cell clones that we isolated recognized exogenous peptides but not processed protein antigens, suggesting that self-

antigens released by dying neurons may be processed by extracellular proteases into peptides that bind to surface MHC-class-II molecules. While it is still possible that the epitopes may be generated by endogenous processing in HCRT neurons, which may be induced to express MHC-class-II molecules by IFNγ or other cytokines, it appears that in most cases professional antigen-presenting cells are unable to generate these epitopes. These findings are reminiscent of a previous report of type-B T cell clones isolated from mice with spontaneous diabetes[31] and support the notion that extracellular processing and unconventional presentation may be a common mechanism to trigger autoreactive T cells that have escaped from central tolerance[24,25].

Our results do not support a molecular mimicry between HCRT and TRIB2 antigens and influenza virus, and raise questions as to the role of HLA-DQB1*06:02 in antigen presentation. The finding that only a few clones were restricted by HLA-DQ—whereas most were restricted by HLA-DR (including HLA-DRB1*15:01, the gene for which is in linkage disequilibrium with *HLA-DQB1*06:02*)—suggests that the HLA-DR-restricted response may result from an epitope-spreading phenomenon or from a compartmentalization of T cells (blood versus tissue) with different restriction, as previously reported in the case of type 1 diabetes[32–34].

Finally, our results may have major implications in the management of narcolepsy and may indicate new options for an earlier, more reliable and less invasive diagnosis of narcolepsy and its borderland, which currently represents a major clinical challenge. Therapeutically, our findings pave the way for systematic studies of the use of immunomodulatory interventions in narcolepsy, which in small studies have occasionally—but not invariably—been shown to have a positive effect on the evolution of the disease[35].

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of data availability and associated accession codes are available at https://doi.org/10.1038/s41586-018-0540-1.

Received: 5 February 2018; Accepted: 10 August 2018;
Published online: 19 September 2018

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Acknowledgements We thank all patients and their families for their participation in the study. We thank A. Sette and C. Lindestam Arlehamn (La Jolla Institute for Allergy and Immunology) for providing the human cytomegalovirus and Epstein–Barr virus peptide pools, G. Nepom and W. Kwok (University of Washington) and R. Martin and M. Sospedra (University Hospital Zurich) for providing DR2a- and DR2b-transfected B cell lines, the Servizio Tipizzazione of the Policlinico San Matteo, University of Pavia, for HLA typing, H. Hidalgo for logistical support and L. Sallusto for discussions and support. This work was supported by the European Research Council grant (no. 323183, PREDICT, to F.S.) and the Swiss National Science Foundation grants (no. 149475 and no. CRSII3_154483 to F.S.). F.S. and the Institute for Research in Biomedicine are supported by the Helmut Horten Foundation.

Reviewer information *Nature* thanks B. Kornum, E. Unanue and the other anonymous reviewer(s) for their contribution to the peer review of this work.

Author contributions D.L. performed experiments with assistance from E.A., F.M., A.C. and S.J. U.K., J.M., F.Z., R.K., M.M. and C.L.B. recruited participants, performed clinical evaluation and collected biological samples. D.J. performed cell sorting. M.F. performed bioinformatics analysis. M.T. supervised HLA typing. F.S. and C.L.B. conceived and supervised the project. F.S., D.L., C.L.B., U.K., M.T., B.B. and A.L. wrote the manuscript. All authors provided input and reviewed the manuscript.

Competing interests The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41586-018-0540-1.
Supplementary information is available for this paper at https://doi.org/10.1038/s41586-018-0540-1.
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METHODS

No statistical methods were used to predetermine sample size. The experiments were not randomized and investigators were not blinded to allocation during experiments and outcome assessment.

Study subjects. The study included 16 patients with NT1 and 3 patients with NT2, recruited from University Hospital of Bern, Hospital of Lugano (EOC) and Clinic Barmelweid, and 13 healthy donors obtained from University Hospital of Bern (of whom one was a first-degree family member of a patient with NT1) and the Swiss Blood Donation Center of Lugano.

One patient with NT1 (P10) also suffered from multiple sclerosis. All participants provided written informed consent for participation in the study. The study was approved by the Ethical committees of Bern (no. 054/15), Lugano (no. 2909) and Barmelweid (no. 205-116). Human primary cell protocols were approved by the Federal Office of Public Health (no. A000197/2 to F.S.). All healthy donors and 14 out of 19 patients were *HLA-DQB1*06:02*-positive. All patients included in the study were HLA-typed at the Policlinico San Matteo, University of Pavia (Extended Data Table 3).

Peptides and antigens. Peptides were synthesized as crude material on a small scale (1 mg) by A and A (San Diego). Peptides used in the study included 15-mers overlapping by 11 (30 peptides) or 20-mers overlapping by 12 (15 peptides), covering the entire length of the 131-amino-acid long precursor HCRT, comprising both HCRT-1 and HCRT-2 neuropeptides as well as peptides of the signal sequence and of the C-terminal sequence; 20-mers overlapping by 12 (42 peptides) covering the entire length of TRIB2 protein; 15-mers overlapping by 10 (112 peptides) covering the haemagglutinin (HA) sequence from A/California/07/2009 (H1N1); human cytomegalovirus (CMV) and Epstein–Barr virus (EBV) HLA-class I peptides (122 peptides, 46 EBV and 76 CMV), provided by A. Sette and C. Lindestam Arlehamn (La Jolla Institute for Allergy and Immunology). The sequences of HCRT and TRIB2 peptides are reported in Supplementary Table 3. Peptides were combined into unique pools per antigen. HCRT-1 (sequence: XPLPDCCRQKTCSCRLYELLHGAGNHAAGILTL, modifications: X = pyroglutamic acid, disulfide bridge between 6–12, 7–14, Leu33 = C-terminal amide) and HCRT-2 (sequence: RSGPPGLQGRLQRLLQASGNHAAGILTM, modifications: Met28 = C-terminal amide) neuropeptides were purchased from R&D Systems, rhTRIB2 from Sino Biological and seasonal influenza virus vaccine (Influvac) from Mylan.

Cell purification and sorting. Peripheral blood mononuclear cells were isolated with Ficoll-Paque Plus (GE Healthcare). Monocytes, B cells and total $CD4^+$ T cells were enriched by positive selection using CD14, CD19 and CD4-coated microbeads, respectively (Miltenyi Biotec). $CD19^+$ B cells, memory $CD4^+$ and $CD8^+$ total cells were further sorted to over 98% purity on a FACSAria III (BD) excluding $CD45RA^+$, $CD25^{bright}$, $CD8^+$ or $CD4^+$, $CD14^+$ and $CD56^+$ cells. The following fluorochrome-labelled mouse monoclonal antibodies were used for staining: CD4–PE–Texas Red (clone S3.5) and CD45RA–QD655 (clone MEM-56) from Thermo Fisher Scientific, CD8–APC (clone B9.11), CD14–PE–Cy5 (clone RMO52) and CD56–PE–Cy5 (clone N901) from Beckman Coulter, CD19–FITC (clone HIB19) and CD25–PE (clone M-A251) from BD Biosciences, CCR7–BV421 (clone G043H7) from BioLegend. Cells were stained on ice for 15–20 min and sorted with FACSAria III (BD Biosciences). CSF samples (1–2 ml) were collected by lumbar puncture. Within few hours of sampling, CSF was spun down and the pellet of cells was stimulated polyclonally with 1 μg/ml PHA (Remel) in the presence of irradiated (45 Gy) allogeneic feeder cells (1×10^5 per well) and IL-2 (500 IU/ml) in a 96-well plate format. On day 15, expanded T cells were stained with CD3–PE (UCHT1), CD4–PE–Texas Red (clone S3.5), CD8–APC (clone B9.11), CD19–PE–Cy7 (SJ25C1) and CD56–PE–Cy5 (clone N901) antibodies, and $CD3^+$ $CD4^+CD8^-CD19^-CD56^-$ or $CD3^+CD8^+CD4^-CD19^-CD56^-$ T cells were sorted on a FACSAria III (BD Biosciences). The small number of CSF T cells in patients with narcolepsy limits this experimental approach and may only be informative in a small subgroup of patients with recent disease onset.

Ex vivo T cell stimulation. T cells were cultured in RPMI 1640 medium supplemented with 2 mM glutamine, 1% (v/v) nonessential amino acids, 1% (v/v) sodium pyruvate, penicillin (50 U/ml), streptomycin (50 μg/ml) (all from Invitrogen) and 5% heat-inactivated human serum (Swiss Red Cross). Sorted memory $CD4^+$ T cells or expanded and sorted $CD4^+$ and $CD8^+$ CSF T cells were labelled with CFSE and cultured at a ratio of 2:1 with irradiated autologous monocytes untreated or pulsed for 2 h with peptide pools covering the entire sequence of HCRT or TRIB2 (3 μg/ml per peptide). After 7 days, cells were stained with antibodies to CD25–PE (clone M-A251) and ICOS–Pacific Blue (clone C398.4A) from Biolegend. The list of samples analysed ex vivo is reported in Extended Data Table 4.

T cell library. T cells were cultured in RPMI 1640 medium supplemented with 2 mM glutamine, 1% (v/v) nonessential amino acids, 1% (v/v) sodium pyruvate, penicillin (50 U/ml), streptomycin (50 μg/ml) (all from Invitrogen) and 5% human serum (Swiss Red Cross). Sorted memory $CD4^+$ or $CD8^+$ T cells from blood were polyclonally stimulated with 1 μg/ml PHA (Remel) in the presence of irradiated (45 Gy) allogeneic feeder cells (5.0×10^4 per well) and IL-2 (500 IU/ml) in a 96-well plate format (1,000-2,000 cells per well) and T cell lines were expanded as previously described[16]. Autologous B cells to be used as antigen-presenting cells were obtained by expansion with CD40L according to an established protocol[36]. Library screening was performed at day 20–25 by culturing extensively washed T cells ($\sim 2.5 \times 10^5$ cells per well) with irradiated autologous B cells (2.5×10^4 cells per well), untreated or pulsed with different antigens, including HCRT peptide pool (3 μg/ml per peptide), TRIB2 peptide pool (3 μg/ml per peptide), CMV + EBV HLA-class I peptide pool (0.5 μg/ml per peptide), or influenza virus vaccine (5 μg/ml). Proliferation was measured on day 4 after 16-h incubation with 1 μCi/ml [methyl-^{3}H]thymidine (Perkin Elmer). Precursor frequencies were calculated based on numbers of negative wells, assuming a Poisson distribution, and are expressed per million cells. Stringent criteria were used to score positive T cell lines based on a cut-off value of (i) a Δ value $\geq 2 \times 10^3$ (c.p.m. with antigen and APC – c.p.m. with APC only) and (ii) a stimulation index ≥ 3 (c.p.m. with antigen and APC/c.p.m. with APC only). This threshold was chosen based upon previous observations made across multiple negative and positive samples assessed by the T cell library technique and with a variety of donors and antigens[16,37,38]. Note that the high sensitivity of the library method can explain differences with the ex vivo analysis, especially in the case of low-frequency, low-affinity responses such as those against self-antigens. The list of samples analysed with the T cell library method is reported in Extended Data Table 4.

Isolation of autoreactive T cell clones. To isolate autoreactive T cell clones, $CFSE^{low}CD25^+ICOS^+$ T cells from ex vivo cultures or library T cell lines were sorted and cloned by limiting dilution, as previously described[39]. The list of samples that were cloned is reported in Extended Data Table 4. T cell clones were analysed by stimulation with irradiated autologous B cells untreated or pulsed for 2 h with HCRT or TRIB2 peptide pool (3 μg/ml per peptide) or, in some experiments, with soluble HCRT-1 and HCRT-2 (10 μg/ml each) or rhTRIB2 proteins (10 μg/ml). To determine MHC restriction, the assay was performed in the absence or presence of blocking anti-MHC-class-II antibody (anti-HLA-DR, clone L243; anti-HLA-DQ, clone SPVL3; anti-HLA-DP, clone B7/21) or blocking anti-pan-MHC-class-I antibody (clone W6/32). Some autoreactive T cell clones were also tested using different types of antigen-presenting cells, MHC-class-II-negative type-II bare lymphocyte syndrome (BLS II) B cells transfected with *DRB1*15:01* (DR2b: *DRB1*15:01*, *DRA*01:01*) or *DRB5*01:01* (DR2a: *DRB5*01:01*, DRA*01:01) (provided by G. Nepom and W. Kwok, University of Washington). In these experiments, autoreactive T cell clones were stimulated with irradiated autologous B cells or with irradiated DR2a- and DR2b-transfected B cell lines, pulsed or not with prepro-HCRT peptide pool (3 μg/ml per peptide). Excess of antigen was eliminated by washing 3 times after 4-h pulse of antigen-presenting cells. T cell proliferation was measured on day 3 after 16-h incubation with 1 μCi/ml [methyl-^{3}H]thymidine (Perkin Elmer). In the cross-reactivity experiments with influenza virus antigens, autoreactive T cell clones were stimulated with irradiated autologous B cells after 2–3-h pulse with HA peptide pool (3 μg/ml per peptide) or influenza virus vaccine (5 μg/ml). Epitope mapping experiments were performed by stimulation of autoreactive T cell clones with irradiated autologous B cells after 2-h pulse with single 15-mer or 20-mer overlapping peptides (3 μg/ml per peptide) covering the whole HCRT or TRIB2 protein length. In all experiments proliferation was measured on day 3 after 16-h incubation with 1 μCi/ml [methyl-^{3}H]thymidine (Perkin Elmer). Cytokine concentrations in the 48-h culture supernatants were assessed by Luminex bead-based assay (Thermo Fisher Scientific) according to the manufacturer's instructions.

Gene expression analysis. Autoreactive T cell clones were stimulated for 2 h in vitro with plate-bound anti-CD3 (clone TR66; 1 μg/ml) and anti-CD28 (BD Pharmingen; 1 μg/ml) monoclonal antibodies. RNA was extracted with E.Z.N.A. Total RNA kit I (Omega Biotek) following the manufacturer's instructions. Treatment with DNase enzyme (Omega Biotek) was performed during the RNA extraction procedure. RNA expression levels for a total of 594 genes were analysed by NanoString technology using nCounter Immunology Panel (Human_V2) (https://www.nanostring.com/products/gene-expression-panels/ncounter-immunology-panels) according to the manufacturer's instructions. In brief, 8 μl of the hybridization mix, including the Reporter CodeSet, was distributed in 12 tubes containing 25 ng total RNA in 5 μl ddH_2O from each sample (6 HCRT-specific and 6 TRIB2-specific $CD4^+$ T cell clones). Subsequently, 2 μl of the Capture ProbeSet was added and, after mixing samples, the hybridization reaction was run at 65 °C for 20 h. After the incubation, samples were loaded on the Nanostring cartridge lanes following the manufacturer's instructions. The assay was run on nCounter SPRINT Profiler device. Data were normalized by the nSolver Analysis software.

Amplification of TCR Vβ and HLA genes. Total cDNA from individual T cell clones was obtained from 10^3–10^4 cells per reaction. Reaction was carried out using HPLC-purified oligo dT(25) primers (Microsynth) and Maxima H Minus reverse transcriptase (Thermo Fisher Scientific), in a reaction mix containing 0.2% Triton, dNTPs, Ribolock RNase inhibitor (Thermo Fisher Scientific). Reactions

were run with the following conditions: 50 °C × 60 min, 55 °C × 5 min. Five microlitres cDNA was added to a PCR mix (final volume 25 μl) containing Q5 Hot Start High-Fidelity DNA Polymerase (New England Biolabs). Sequences were amplified using TCR Vβ-specific forward primer pool, as previously described[40], and Rev84 reverse primer pairing to C1–C2 β-chain constant region with the following conditions: 98 °C × 1 min; (98 °C × 10 s ; 55 °C × 20 s; 72 °C × 40 s) × 35 cycles; 72 °C × 2 min. Sequence amplification was assessed through agarose gel electrophoresis; successfully amplified fragments were sequenced by Sanger method using Rev64 primer, and TCR sequence annotation was carried out by using IMGT/V-QUEST algorithm. Forward primers. TBV-A: TCAGGT GTGATYCAATTTC; TBV-B: AGGTGTGATCCAATTTCG; TBV-C: TGTGTCC TGGTACCAACAG; TBV-D: GTATCGACAAGACCCAGG; TBV-E: GTATCG ACAAGACCYGGG; TBV-F: CTCACCTGAATGCCCCAA; TBV-G: ATGTTYT GGTAYCGTCAG; TBV-H: CCTTTACTGGTACCDGCAGA; TBV-I: ACAGAG ATGGGACAAGAAG; TBV-J: GCCATGTACTGGTAYMGA; TBV-K: CCCCAT CTCTAATCACTTATAC; TBV-L: ACATCAAACCCCAACCTATAC; TBV-M: AC CAGCAGAAGTCAAGTCA; TBV-N: TGTSTACTGGTACCARCAG; TBV-O: GGGAAGGACAGAAAGCAAAA; TBV-P: TTACTCAGTTCCCCAGCC; TBV-Q: AGATGCAGCCCAATGAAA; TBV-R: ACAGATGGGAAACGACAA; TBV-S: GTATCRACAAGAYCCAGGA. Reverse primers. TRBC-rev84: TGTGGCCT TTTGGGTGTGG; TRBC-rev64: AGATCTCTGCTTCTGATGGC. HLA genotype of *DQB1*, *DRB1* and *DRB5* loci was determined on extracted genomic DNA (prepared using the QIAamp DNA Micro Kit, Qiagen) by PCR amplification of exon 2 using allele-specific primers on genomic DNA, followed by Sanger sequencing of the PCR products in both the forward and reverse directions. Primers targeting the intron 3 of *DRB1* were used as internal positive control of the PCR reaction. PCR for *DQB1*06:02* (mix 1, forward: CCCGCAGAGGATTTCGTGTT, reverse: AACTCCGCCCGGGTCCC, 218 bp product; mix 2, forward: CGTGCGTCTTGTGACCAGAT, reverse: AACTCCGCC-CGGGTCCC, 156 bp product) and *DRB1*15:01* (forward: ACGTTTCCTGTGG-CAGCCTAA, reverse: TGCACTGTGAAGCTCTCCACAA, 262 bp product) was performed as follows: 98 °C for 1 min; (98 °C for 10 s; 72 °C for 40 s) × 36 cycles; 72 °C for 2 min. PCR for *DRB5*01:01* (forward: CTTGCAGCAGGATAAGTAT-GAG, reverse: CTGTG-AAGCTCTCACCAACC, 251 bp product) and *DRB1* intron 3 (forward: TGCCAAGTG-GAGCACCCAA, reverse: GCATCTTGCTCTGTGCAGAT, 782 bp product) was performed as follow: 98 °C for 1 min; (98 °C for 10 s; 63 °C for 20 s; 72 °C for 30 s) × 36 cycles; 72 °C for 2 min. All reactions were carried out using Q5 Hot Start High-Fidelity DNA Polymerase (New England Biolabs), with 100 ng template gDNA in 25 μl volume.

TCR Vβ deep sequencing. Ex vivo-sorted total memory $CD4^+$ or $CD8^+$ T cells and in vitro-expanded and sorted $CD4^+$ or $CD8^+$ T cells from CSF ($2.5–5 \times 10^5$ cells) were analysed by deep sequencing. In brief, cells were centrifuged and washed in PBS, and genomic DNA was extracted from the pellet using QIAamp DNA Micro Kit (Qiagen), according to manufacturer's instructions. Genomic DNA quantity and purity were assessed through spectrophotometric analysis. Sequencing of TCR Vβ CDR3 was performed by Adaptive Biotechnologies using the ImmunoSEQ assay (http://www.immunoseq.com). In brief, following multiplex PCR reaction designed to target any CDR3 Vβ fragments; amplicons were sequenced using the Illumina HiSeq platform. Raw data consisting of all retrieved sequences of 87 nucleotides or corresponding amino acid sequences and containing the CDR3 region were exported and further processed. The assay was performed at survey level (detection sensitivity, 1 cell in 40,000). A clonotype was defined as a unique combination of a CDR3 amino acid sequence and its related V gene. Data processing was done using the productive frequency and the diversity metrics provided by ImmunoSEQ Analyzer V.3.0 (http://www.immunoseq.com). Antigen-specific clonotypes in each donor's repertoire were defined according to identical amino acid sequence in the Vβ CDR3 and identical V gene usage. The samples analysed are listed in Supplementary Table 2 and the TCR sequences are available as a .txt file in Supplementary Information.

Code availability. The Java scripts used to search for antigen-specific clonotypes in donor's repertoire are available at: https://bitbucket.org/mathildefog/narcolepsy. This code is distributed as open source under the terms of the GNU Free Documentation License.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this paper.

Data availability

The data presented in this manuscript are included in the paper and its Supplementary Information. TCR sequences from samples listed in Supplementary Table 2 are available as a .txt file. The sequences have also been deposited in the ImmunoAccess database (http://clients.adaptivebiotech.com/pub/Latorre-2018-nature; https://www.doi.org/10.21417/B73H0P).

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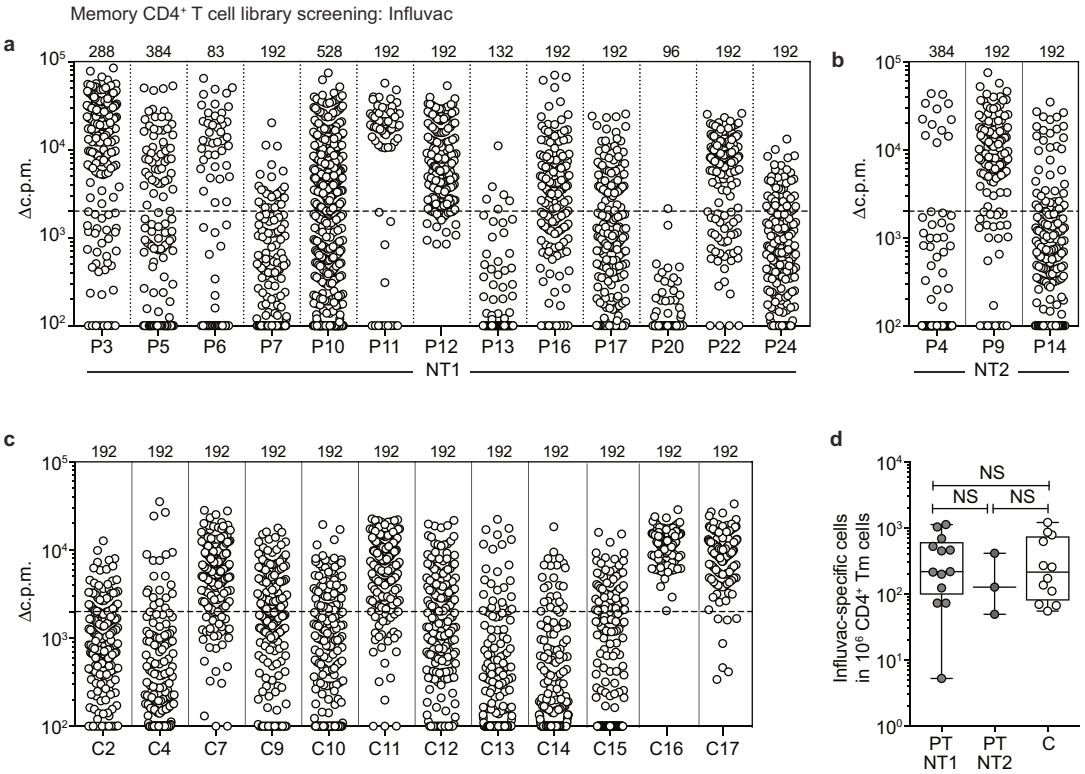
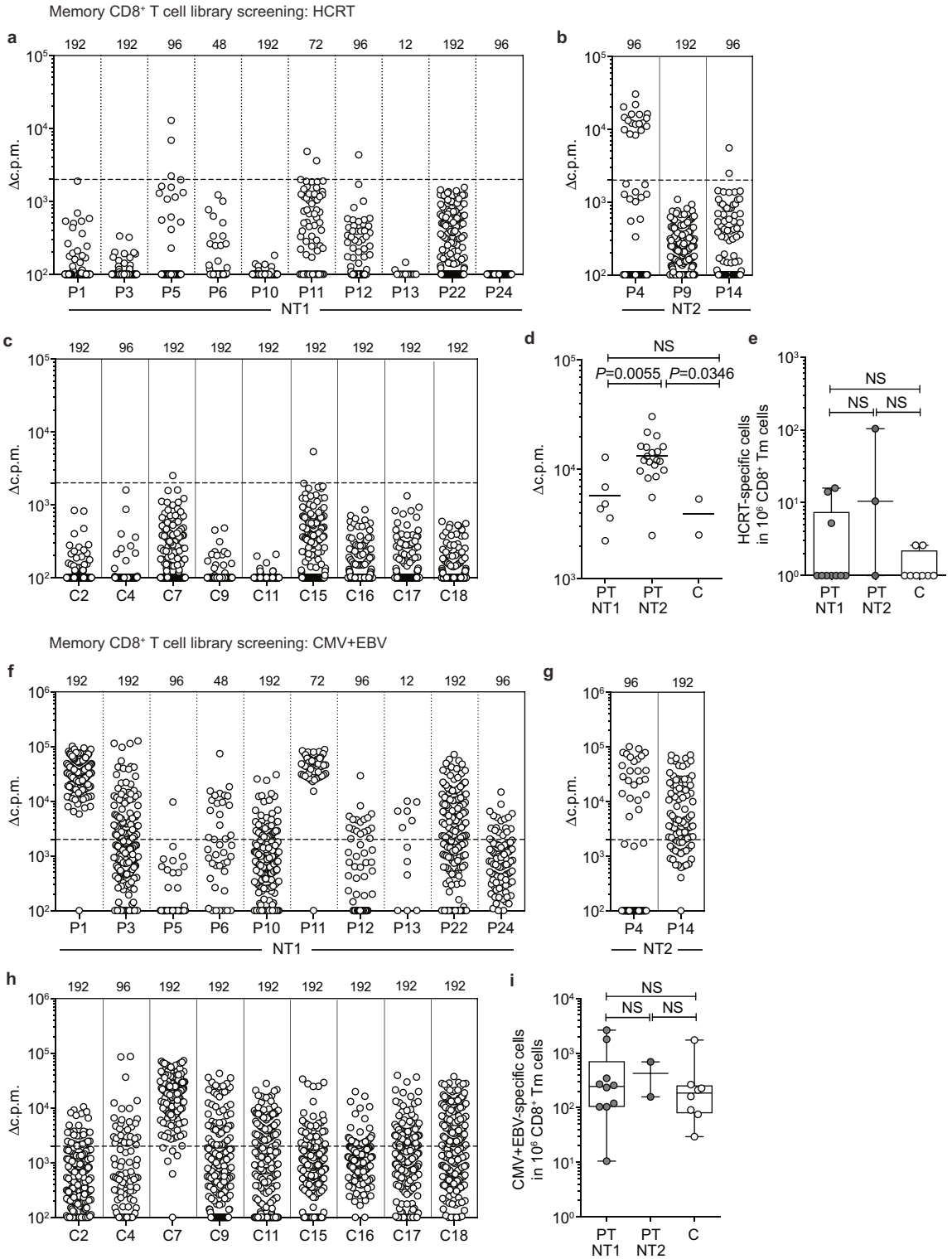
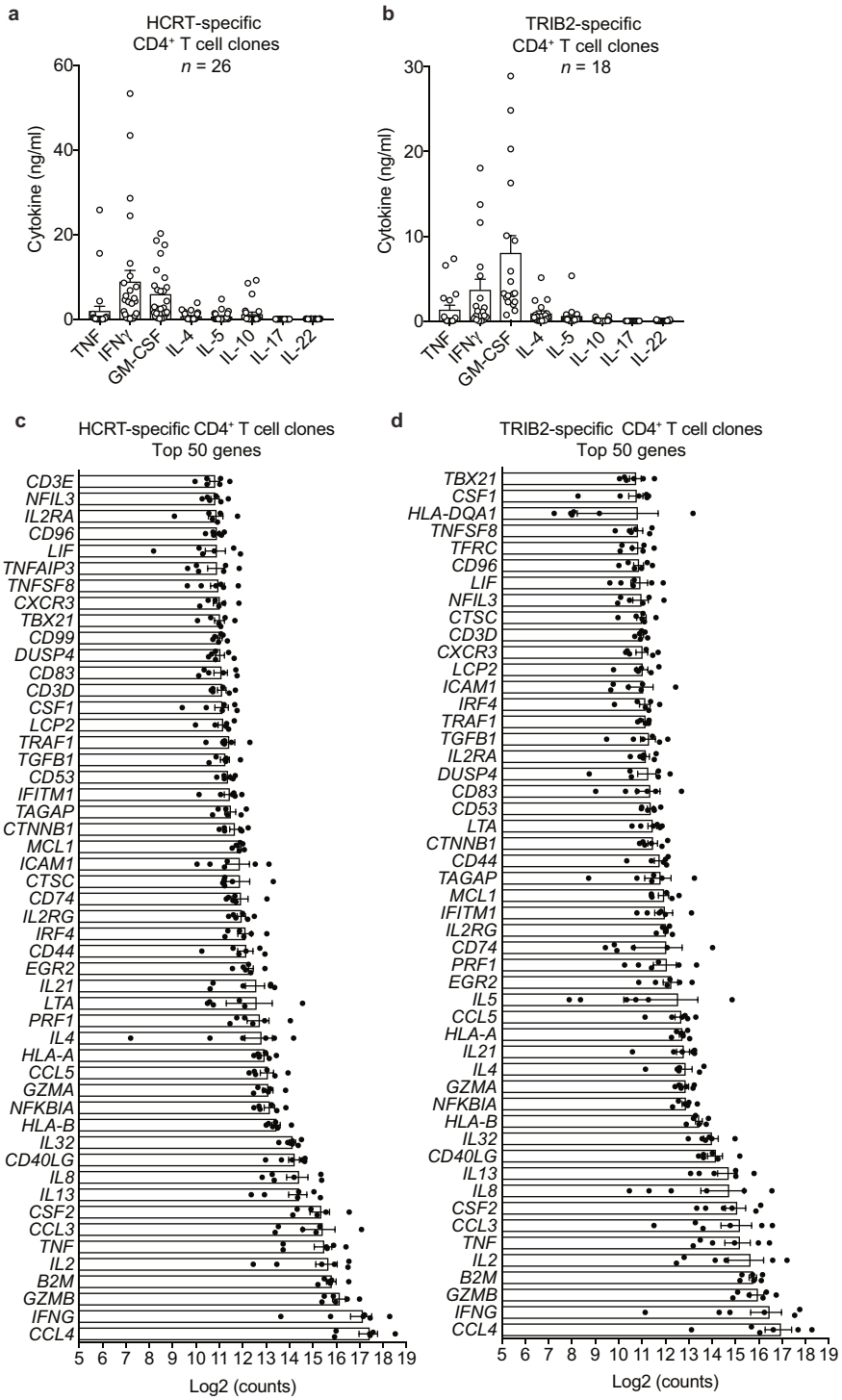


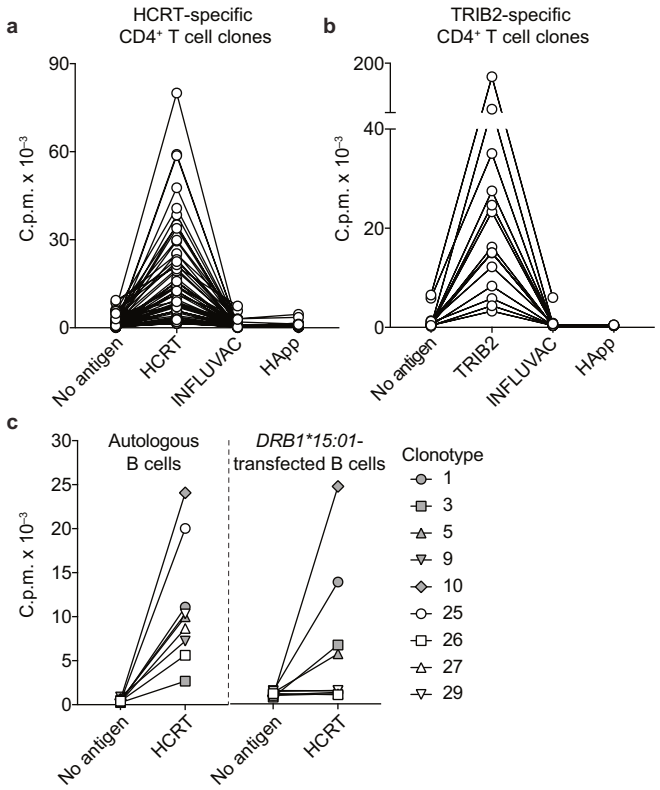
Extended Data Fig. 1 | Screening of memory CD4$^+$ T cell library from patients with NT1 or NT2 and healthy controls. a–c, Memory CD4$^+$ T cell screening for Influvac. Memory CD4$^+$ T cell libraries from patients with NT1 or NT2 and control donors shown in Fig. 2 were also screened for their capacity to proliferate in response to the influenza vaccine Influvac, used as a positive control. On day 4, proliferation was measured after a 16-h pulse with [^{3}H]thymidine. The number of tested T cell lines per donor is indicated on top of the graphs and the proliferation of individual T cell lines (each line is represented by a single dot) is shown as Δc.p.m. value. Positive lines were defined as Δc.p.m. ≥ 2,000 (horizontal dotted line) and SI ≥ 3. **d**, The frequency of Influvac-specific memory CD4$^+$ T cells in patients with narcolepsy and controls (NT1, $n = 13$; NT2, $n = 3$; control, $n = 12$, biologically independent samples) is shown. Dots represent frequency in each donor, boxes are quartile values, whiskers represent the highest and lowest values, and lines represent the median values. Results are presented as precursor frequency per million memory CD4$^+$ T cells. Data were analysed using two-tailed Mann–Whitney *U*-test. NS, not significant (*P* values > 0.05).



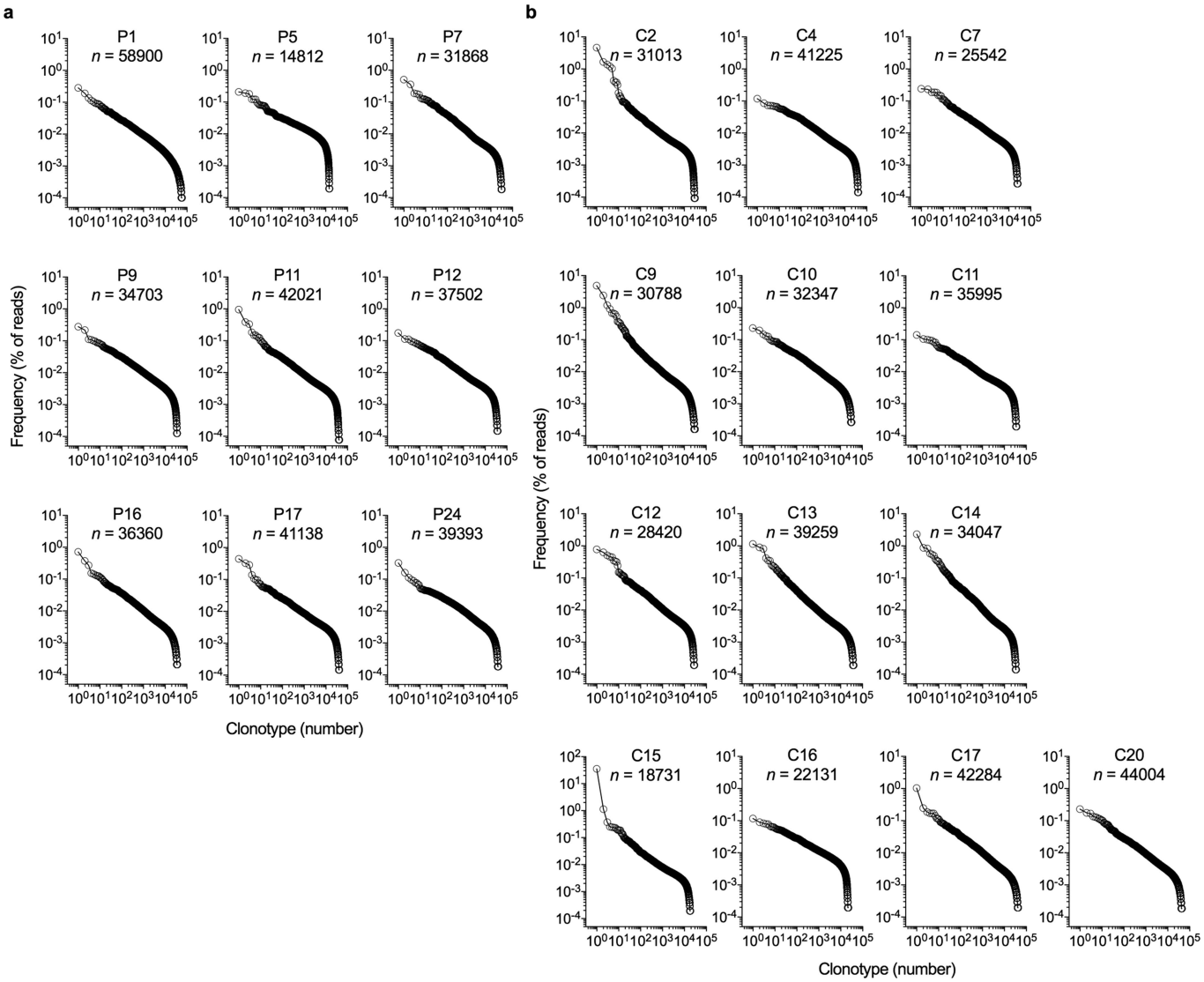
Extended Data Fig. 2 | Autoreactive memory CD8$^+$ T cells in patients with NT1 or NT2 as detected using the T cell library method. **a**–**c**, **f**–**h**, Memory CD8$^+$ T cells were polyclonally expanded and screened for their capacity to proliferate in response to HCRT peptide pool (**a**–**c**) or CMV + EBV peptide pool (**f**–**h**), used as positive control, in the presence of irradiated autologous B cells. On day 4, proliferation was measured after a 16-h pulse with [^{3}H]thymidine. The number of tested T cell lines per donor is indicated on top of the graphs and the proliferation of individual T cell lines (each represented by a single dot) is shown as Δc.p.m. value. Positive responses were defined as Δc.p.m. ≥ 2,000 (horizontal dotted line) and SI ≥ 3. **d**, The Δc.p.m. values of HCRT-positive T cell lines (NT1, $n = 6$; NT2, $n = 20$; control, $n = 2$, biologically independent samples) from patients (PT) and controls (C) are shown. **e**, **i**, The frequency of HCRT-specific (**e**) and CMV + EBV-specific (**i**) cells per million memory CD8$^+$ T cells in patients with narcolepsy and controls is shown (**e**, NT1, $n = 10$; NT2, $n = 3$; control, $n = 9$, biologically independent samples; **i**, NT1, $n = 10$; NT2, $n = 2$; control, $n = 9$, biologically independent samples). Dots represent frequency in each donor, boxes are quartile values, whiskers represent the highest and lowest values, and lines represent the median values. Results are presented as precursor frequency per million memory CD8$^+$ T cells. Data were analysed using two-tailed Mann–Whitney *U*-test. NS, not significant (P values > 0.05).



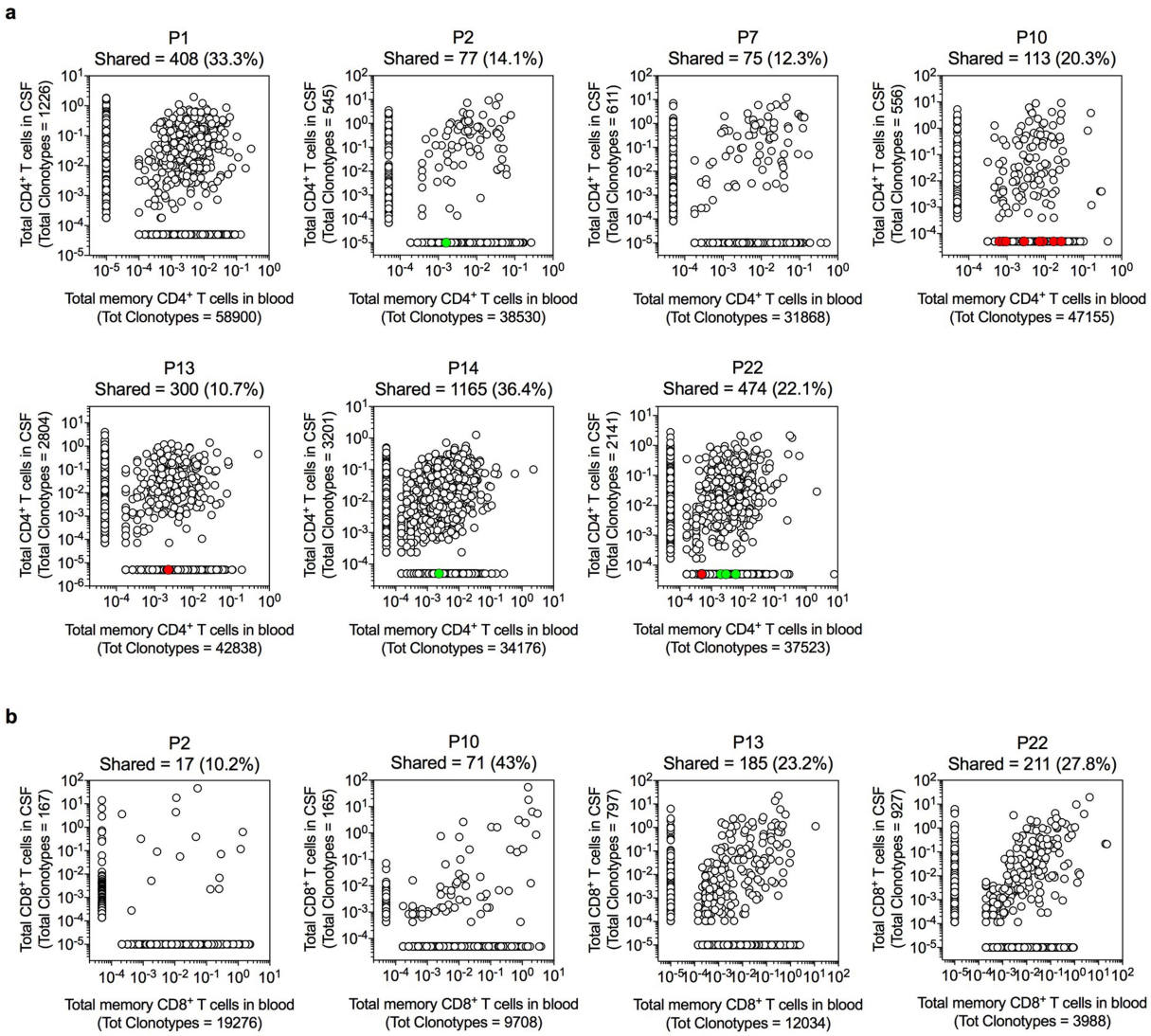
Extended Data Fig. 3 | Cytokine production and gene expression by autoreactive CD4$^+$ T cell clones from patients with narcolepsy. **a**, **b**, HCRT-specific (**a**, $n = 26$ biologically independent samples) and TRIB2-specific (**b**, $n = 18$ biologically independent samples) CD4$^+$ T cell clones derived from patients with narcolepsy were stimulated with HCRT peptide pool (**a**) or TRIB2 peptide pool (**b**) presented by irradiated autologous B cells. Cytokines released in the 48-h culture supernatants were quantified by bead-based multiplex assay. Data represent the mean + s.e.m. **c**, **d**, mRNA expression levels for 579 genes in HCRT-specific (**c**, $n = 6$ biologically independent samples; $n = 3$ from P1 and $n = 3$ from P8) and TRIB2-specific (**d**, $n = 6$ biologically independent samples; $n = 4$ from P22 and $n = 2$ from P14) CD4$^+$ T cell clones were measured using NanoString technology. The top 50 expressed genes are shown. Data represent the mean ± s.e.m.



Extended Data Fig. 4 | Evaluation of T cell cross-reactivity with influenza virus antigens and MHC restriction of autoreactive T cell clones. a, **b**, HCRT-specific (**a**, $n = 61$ biologically independent samples) and TRIB2-specific (**b**, $n = 14$ biologically independent samples) $CD4^+$ T cell clones from patients with narcolepsy were stimulated in the presence of irradiated autologous B cells pulsed with HCRT peptide pool, TRIB2 peptide pool or influenza vaccine (Influvac) and—for some clones ($n = 29$, HCRT-specific and $n = 9$, TRIB2-specific)—with haemagglutinnin (HA) peptide pool. Each clone represents an individual clonotype. The c.p.m. values indicate the proliferation of autoreactive T cell clones measured after a 16-h pulse with [^{3}H]thymidine are shown. **c**, The proliferative response of HCRT-specific, HLA-DR-restricted $CD4^+$ T cell clones ($n = 9$ biologically independent samples) after stimulation with irradiated autologous B cells or a *DRB1*15:01*-transfected B cell line in absence or presence of HCRT peptide pool, is shown. Proliferation was measured on day 3 after a 16-h pulse with [^{3}H]thymidine.



Extended Data Fig. 5 | Clonotypic analysis of blood memory CD4$^+$ T cells. a, **b**, TCR Vβ sequencing was performed on memory CD4$^+$ T cells ex vivo after sorting from peripheral blood of the indicated patients with narcolepsy (**a**) and healthy controls (**b**). The frequency distribution of all TCR Vβ clonotypes (*n* indicates total number of clonotypes) is shown. In these samples, no sequences of autoreactive T cell clones were found. The number of sequenced clonotypes was comparable in patients and controls; a range of 14,812–58,900 (39,494 ± 10,514 (mean ± s.d.)) and a range of 18,731–44,004 (33,354 ± 7,831 (mean ± s.d.)), respectively. TCR sequencing data are available as Supplementary Information.



Extended Data Fig. 6 | Clonotype sharing of blood and CSF CD4$^+$ and CD8$^+$ T cells from patients with narcolepsy. a, TCR Vβ sequencing was performed on memory CD4$^+$ T cells ex vivo after sorting from blood, and on CD4$^+$ T cells sorted from in vitro-expanded CSF T cells. The comparison of clonotype frequency distribution of CD4$^+$ T cells from CSF (*y* axis) and blood (*x* axis) from the same patient is shown. Coloured circles represent autoreactive clonotypes (red, HCRT-specific; green, TRIB2-specific). **b**, TCR Vβ sequencing was performed on memory CD8$^+$ T cells ex vivo after sorting from blood, and on CD8$^+$ T cells sorted from in vitro-expanded CSF T cells. The comparison of clonotype frequency distribution of CD8$^+$ T cells from CSF (*y* axis) and blood (*x* axis) from the same patient is shown. The total number of clonotypes in each sample is indicated on the axis. Values on top of the plots represent the number and the percentage of shared clonotypes between the two samples, calculated on the total number of clonotypes found in the CSF. TCR sequencing data are available as Supplementary Information.

Extended Data Table 1 | Patients and control donors included in this study

ID	Gender	Age	Disease duration*	Cataplexy	HLA-DQB1*06:02	HLA-DRB1*15:01	HLA-DRB5*01:01	Treatment	CSF HCRT (pg/mL)
Narcolepsy type 1 patients (NT1)									
P1	M	21	3	Yes	+	+	+	Modafinil	64
P2	M	28	12	Yes	+	+	+	Modafinil	0
P3	F	16	4	Yes	+	+	+	Modafinil, Fluoxetin	31
P5	F	18	6	Yes	+	+	+	Methylphenidate	N.A.
P6	M	24	3	Yes	+	+	+	Modafinil, Fluoxetin	<20
P7	M	28	7	Yes	+	+	+	Modafinil	25
P8	M	48	7	Mild	+	+	+	Methylphenidate, Venlafaxine, Agomelatine	N.A.
P10†	F	44	27	Yes	+	+	+	Sodium Oxybate, Venlafaxine, Natalizumab	0
P11	M	32	14	Yes	–	–	–	Venlafaxine	0
P12	M	29	11	Yes	+	+	+	Methylphenidate, Venlafaxine	0
P13	F	21	2	Yes	+	+	+	Modafinil	96
P16	M	53	9	Yes	+	+	+	Modafinil, Bupropion	N.A.
P17	F	52	36	Yes	+	+	+	Ephredine	0
P20	F	46	12	Yes	+	+	+	Modafinil, Fluoxetin	<20
P22	M	25	2	Yes	–	–	–	None	<20
P24	M	35	1	Yes	+	+	+	Modafinil	68
Narcolepsy type 2 patients (NT2)									
P4‡	M	38	5	Mild	–	–	–	Methylphenidate, Venlafaxine	128
P9	F	51	31	No	–	–	–	Modafinil	N.A.
P14	F	43	2	No	–	–	–	Modafinil	395
Control donors									
C2	F	51	–	–	+	+	+	–	–
C4§	F	45	–	–	+	+	+	–	–
C7	N.A.	N.A.	–	–	+	+	+	–	–
C9	N.A.	N.A.	–	–	+	+	+	–	–
C10	N.A.	N.A.	–	–	+	+	+	–	–
C11	N.A.	N.A.	–	–	+	+	+	–	–
C12	N.A.	N.A.	–	–	+	+	+	–	–
C13	N.A.	N.A.	–	–	+	+	+	–	–
C14	N.A.	N.A.	–	–	+	–	–	–	–
C15	N.A.	N.A.	–	–	+	+	+	–	–
C16	N.A.	N.A.	–	–	+	+	+	–	–
C17	N.A.	N.A.	–	–	+	+	+	–	–
C20	N.A.	N.A.	–	–	+	+	+	–	–

N.A., not available.
*Duration in years.
†Co-morbidity, multiple sclerosis.
‡At a subsequent visit, nocturnal sleep of patient P4 deteriorated and cataplexy episodes were more frequent.
§First-degree family member of a patient with NT1.

Extended Data Table 2 | Epitope mapping and HLA restriction of autoreactive CD4$^+$ T cell clones from patients with narcolepsy

Clonotype	PT	Specificity	Epitope (aa)	Restriction
1	P1	HCRT	17-36	HLA-DR
2	P1	HCRT	113-127	HLA-DR
3	P1	HCRT	17-36	HLA-DR
4	P1	HCRT	53-67 / 85-99	HLA-DR
5	P1	HCRT	17-31	HLA-DR
6	P1	HCRT	73-91	HLA-DR
7	P1	HCRT	N.D.	HLA-DR
8	P1	HCRT	49-78	HLA-DQ
9	P1	HCRT	17-36	HLA-DR
10	P1	HCRT	17-31	HLA-DR
11	P1	HCRT	105-119	N.D.
12	P1	HCRT	81-95	HLA-DQ
13	P3	HCRT	105-124	HLA-DR
14	P3	HCRT	73-87	HLA-DR
15	P3	HCRT	105-127	HLA-DP
16	P3	HCRT	N.D.	HLA-DR
17	P4	HCRT	57-76	N.D.
18	P4	HCRT	97-116	HLA-DR
19	P4	HCRT	73-92	HLA-DR
20	P4	HCRT	57-84	HLA-DR
21	P4	HCRT	97-116	HLA-DR
22	P4	HCRT	57-84	HLA-DR
23	P4	HCRT	65-84	HLA-DR
24	P8	HCRT	57-75	HLA-DR
25	P8	HCRT	57-76	HLA-DR
26	P8	HCRT	57-71	HLA-DR
27	P8	HCRT	57-71	HLA-DR
28	P8	HCRT	N.D.	N.D.
29	P8	HCRT	57-71	HLA-DR
30	P8	HCRT	29-43	HLA-DQ
31	P10	HCRT	69-87	HLA-DR
32	P10	HCRT	47-68	HLA-DR
33	P10	HCRT	45-59	HLA-DR
34	P10	HCRT	69-87	HLA-DR
35	P10	HCRT	33-52	HLA-DR
36	P10	HCRT	N.D.	HLA-DR
37	P10	HCRT	87-108	HLA-DR
38	P10	HCRT	25-44	HLA-DR
39	P10	HCRT	97-116	HLA-DR
40	P10	HCRT	69-91	HLA-DR
41	P10	HCRT	9-28	HLA-DR
42	P10	HCRT	65-84	HLA-DR
43	P10	HCRT	65-84	HLA-DR
44	P10	HCRT	69-91	HLA-DR
45	P10	HCRT	69-87	HLA-DR
46	P10	HCRT	69-87	HLA-DR
47	P10	HCRT	33-47/69-95/113-127	HLA-DR
48	P10	HCRT	111-131	HLA-DR
49	P10	HCRT	17-36	HLA-DR
50	P10	HCRT	97-116	HLA-DR
51	P10	HCRT	65-92	HLA-DR
52	P10	HCRT	69-97	N.D.
53	P10	HCRT	73-87	HLA-DR
54	P10	HCRT	69-91	HLA-DR
55	P10	HCRT	73-87	HLA-DR
56	P10	HCRT	69-91	HLA-DR
57	P10	HCRT	69-87	HLA-DR
58	P10	HCRT	73-87	HLA-DR
59	P10	HCRT	N.D.	N.D.
60	P11	HCRT	81-95	HLA-DR
61	P13	HCRT	25-44	HLA-DR
62	P14	HCRT	N.D.	HLA-DR
63	P14	HCRT	N.D.	HLA-DR
64	P22	HCRT	17-36	HLA-DR
65	P7	TRIB2	1-20	HLA-DP
66	P7	TRIB2	1-20	HLA-DP
67	P14	TRIB2	257-284	HLA-DQ
68	P14	TRIB2	177-196	HLA-DQ
69	P14	TRIB2	257-284	HLA-DQ
70	P14	TRIB2	257-284	HLA-DQ
71	P14	TRIB2	257-284	HLA-DQ
72	P14	TRIB2	1-20	HLA-DQ
73	P22	TRIB2	153-172	HLA-DR
74	P22	TRIB2	113-132	HLA-DR
75	P22	TRIB2	185-212	HLA-DQ
76	P22	TRIB2	265-284	HLA-DR
77	P22	TRIB2	49-68	HLA-DR
78	P22	TRIB2	313-332	HLA-DQ
79	P22	TRIB2	265-284	HLA-DR

N.D., not determined.

Extended Data Table 3 | HLA typing of patients with narcolepsy included in this study

ID	HLA-DRB1*	HLA-DRB1*	HLA-DQB1*	HLA-DQB1*	HLA-DQA1*	HLA-DQA1*	HLA-DPB1*	HLA-DPB1*	HLA-A*	HLA-A*	HLA-B*	HLA-B*	HLA-C*	HLA-C*
P1	01:01	15:01	05:01	06:02	01:01	01:02	04:01	13:01	03:01	11:01	07:02	35:01	04:01	07:02
P2	15:01	16:01	05:02	06:02	01:02	01:02	04:01	13:01	01:01	11:01	07:02	55:01	03:03	07:02
P3	11:01	15:01	03:01	06:02	01:02	05:05	04:01	20:01	32:01	68:01	27:07	44:02	07:04	15:02
P5	04:01	15:01	03:01	06:02	01:02	03:03	04:01	04:01	03:01	24:02	07:02	39:01	07:02	12:03
P6	15:01	16:01	05:02	06:02	01:02	01:02	10:01	11:01	01:01	02:01	07:02	55:01	03:03	07:02
P7	15:01	16:01	05:02	06:02	01:02	01:02	03:01	04:01	03:01	31:01	07:02	51:01	07:02	15:02
P8	11:04	15:01	03:01	06:02	01:02	05:05	02:01	04:01	25:01	25:01	18:01	44:05	02:02	12:03
P10	11:04	15:01	03:01	06:02	01:02	05:05	04:01	04:02	24:02	29:01	44:03	51:01	12:03	16:01
P11	01:01	04:07	03:01	05:01	01:01	03:03	02:01	04:01	02:01	11:01	07:02	35:01	04:01	07:02
P12	08:01	15:01	04:02	06:02	01:02	04:01	03:01	04:01	01:01	02:01	07:02	51:01	07:02	14:02
P13	13:02	15:01	06:02	06:04	01:02	01:02	02:01	02:01	01:01	24:02	39:06	58:01	07:02	07:01
P16	13:02	15:01	06:02	06:04	01:02	01:02	04:01	15:01	03:01	24:02	07:02	15:17	07:01	07:02
P17	04:07	15:01	03:01	06:02	03:03	01:02	04:01	04:01	11:01	11:01	35:01	56:01	01:02	04:01
P20	04:07	15:01	03:01	06:02	01:02	03:03	04:01	04:01	02:01	03:01	07:02	44:02	07:02	07:04
P22	01:01	16:01	05:01	05:02	01:01	01:02	04:01	14:01	03:01	24:02	35:01	37:01	04:01	06:02
P24	11:04	15:01	03:01	06:02	05:05	01:02	03:01	04:01	01:01	23:01	07:02	49:01	07:02	07:01
P4	04:01	13:01	03:01	06:03	01:03	03:03	04:01	04:01	11:01	30:02	15:01	44:02	03:04	05:01
P9	03:01	08:01	02:01	04:02	04:01	05:01	02:01	04:01	01:01	03:01	07:02	55:01	03:03	07:02
P14	01:02	04:02	03:02	05:01	01:01	03:01	02:01	04:01	02:01	29:01	44:03	45:01	16:01	16:01

Extended Data Table 4 | Summary of memory CD4$^+$ T cell screenings

ID	Ex vivo	T cell library	HCRT-T cell reactivity	Isolation of HCRT-reactive clones	TRIB2-T cell reactivity	Isolation of TRIB2-reactive clones
P1	√	√	Yes	Yes	N.D.*	N.D.
P2	N.D.	√	Yes	None	Yes	None
P3	√	√	Yes	Yes	N.D.	N.D.
P5	√	√	Yes	None	Yes	None
P6	N.D.	√	Yes	None	N.D.	N.D.
P7	N.D.	√	Yes	None	Yes	Yes
P8	√	N.D.	Yes	Yes	N.D.	N.D.
P10	√	√	Yes	Yes	Yes	None
P11	√	√	Yes	Yes	None	N.D.
P12	N.D.	√	Yes	None	None	N.D.
P13	√	√	Yes	Yes	None	N.D.
P16	N.D.	√	Yes	None	Yes	None
P17	N.D.	√	Yes	None	Yes	None
P20	N.D.	√	Yes	None	None	N.D.
P22	√	√	Yes	Yes	Yes	Yes
P24	√	√	Yes	None	None	N.D.
P4	N.D.	√	Yes	Yes	N.D.	N.D.
P9	N.D.	√	Yes	None	N.D.	N.D.
P14	N.D.	√	Yes	Yes	Yes	Yes

*N.D., not done.

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☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
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☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	BD FACSDIVA v.6.2 was used for flow cytometry data collection
Data analysis	All the softwares used and their version information, when available, are shown here. Flow-Jo (v10.1) was used for all FACS analyses. IMGT (v3.4.8) was used to analyze TCR Vβ sequences obtained by Sanger method. ImmunoSEQ Analyzer V3.0 (http://www.immunoseq.com) was used for data processing of TCR Vβ CDR3 sequencing performed at Adaptive Biotechnologies. Scripts written in Java by one of the authors (Mathilde Foglierini) were used to define antigen-specific clonotypes in each donor's repertoire according to identical amino acid sequence in the Vβ CDR3 and identical V gene usage. The Java scripts are available at https://bitbucket.org/mathildefog/narcolepsy. This code is distributed open source under the terms of the GNU Free Documentation License. Graphpad Prism (v7) was used to analyze data and create plots. The Microbeta2 Windows Workstation software, version 2.0.0.87, was used to acquire data of T cell proliferation by [3H]-thymidine incorporation on a Beta counter 2 (Perkin Elmer).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Data availability. The data presented in this manuscript are tabulated in the main paper and in the supplementary materials. TCR sequences from samples listed in Supplementary Table 2 are available as .txt file in Supplementary Information. The sequences have been deposited in the immuneACCESS™ database (URL http://clients.adaptivebiotech.com/pub/Latorre-2018-nature, doi:10.21417/B73H0P).

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☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size calculation was performed. Narcolepsy is a rare disease and we included in the study patients enrolled in different centers in Switzerland.
Data exclusions	Data obtained from healthy controls were included only if there was a T cell response to antigens used as positive controls (Flu antigens for CD4+ T cells and CMV+EBV antigens for CD8+ T cells).
Replication	In a few cases it was possible to analyze separate samples from the same patient with comparable results. In the cases of T cell clones, their reactivity against self-antigens was assessed in multiple occasions (in at least 3 separate experiments) with reproducible results.
Randomization	Narcolepsy patients with HCRT deficiency (CSF HCRT levels < 110 pg/mL) and clinical diagnosis of narcolepsy with cataplexy were allocated to NT1 patients group. Narcolepsy patients with intermediate or normal HCRT levels and clinical diagnosis of narcolepsy with mild or without cataplexy were allocated to NT2 patients group. Patients were 16–53 years of age (median 32) and had different disease duration (range 1–36, median 7 years). Healthy controls were obtained from University Hospital of Bern (of which 1 was a 1st degree family member) and the Swiss Blood Donation Center of Lugano and selected for being HLA-DQB1*0602 positive.
Blinding	Blinding of the investigators was not applied for the screening of autoreactive cells in NT versus C groups, because the healthy donors were included later in the study. The investigators were unaware of the allocation of patients to the NT1 and NT2 groups.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☐	☐ ChIP-seq
☐	☒ Flow cytometry
☐	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	There are no restriction in the availability of materials described in the study.

Antibodies

Antibodies used

All the commercial antibodies were used following the manufacturer's instructions.
Supplier name, catalog number and clone name are indicated here and in the Methods section.
CD4-PE-Texas Red, clone S3.5 from ThermoFisher Scientific, cat n MHCD0417, Lot#1710439A, dil 1:800.
CD45RA-QD655, clone MEM-56 from ThermoFisher Scientific, cat n Q10069, Lot#1785496, dil 1:1000.
CD8-APC, clone B9.11 from Beckman Coulter, cat n IM2469, Lot#51, dil 1:30.
CD14-PE-Cy5, clone RMO52 from Beckman Coulter, cat n A07765, Lot#23, dil 1:30.
CD56-PE-Cy5, clone N901 from Beckman Coulter, cat n A07789, Lot#42, dil 1:30.
CD19-FITC, clone HIB19 from BD Bioscience, cat n 555412, Lot#5097663, dil 1:30.
CD25-PE, clone M-A251 from BD Bioscience, cat n 555432, Lot#5314982, dil 1:20.
CCR7-BV421, clone G043H7 from Biolegend, cat n 353207, Lot#B233487, dil 1:80.
CD19-PE-Cy7, clone SJ25C1 from BD Bioscience, cat n 341113, Lot#7048931, dil 1:30.
CD3-PE, clone UCHT1 from Beckman Coulter, cat n A07747, Lot#26, dil 1:30.
ICOS-Pacific Blue clone C398.4A from Biolegend, cat n 313522, Lot#B170453, dil 1:100.
antiHLA-DR, clone L243, ATCC cat n HB55, produced in house from hybridoma cell line, dil 1:20.
anti-HLA-DQ clone SPVL3 (1), produced in house from hybridoma cell line, dil 1:20.
anti-HLA-DP, clone B7/21 (2), produced in house from hybridoma cell line, dil 1:20.
pan anti-MHC class I, clone W6/32, ATCC cat n HB-95, produced in house from hybridoma cell line, dil 1:20

1) Spits H; Keizer G; Borst J; Terhorst C; Hekman A; de Vries JE. Characterization of monoclonal antibodies against cell surface molecules associated with cytotoxic activity of natural and activated killer cells and cloned CTL lines. Hybridoma, 1983, 2(4):423-37.

2) Watson AJ, DeMars R, Trowbridge IS, Bach FH. Detection of a novel human class II HLA antigen. Nature, 1983, 304:358-61.

Validation

Describe the validation of each primary antibody for the species and application, noting any validation statements on the manufacturer's website, relevant citations, antibody profiles in online databases, or data provided in the manuscript.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

MHC-class II-negative Type II bare lymphocyte syndrome (BLS II) cells transfected with DRB1*15:01 (DR2b: DRB1*15:01, DRA*01:01) or DRB5*01:01 (DR2a: DRB5*01:01, DRA*01:01) were used as antigen presenting cells (kindly provided by G. Nepom and W. Kwok, University of Washington, Seattle, USA). B and T cell lines were generated from primary human cells and kept short term in culture. EBV-transformed B lymphoblastoid cell lines were generated and used as APCs. Allogeneic PMBCs were used as feeder cells and were isolated from healthy donors obtained from the Swiss Blood Donation Center of Lugano.

Authentication

BSL II cell lines transfected with DRB1*15:01 or DRB5*01:01 were typed for the presence of DRB1*15:01 or DRB5*01:01 allele and for the absence of the DQB1*06:02 allele by PCR amplification. >99% of the cells expressed HLA-class II molecules on their surface as assessed by flow cytometry.

Mycoplasma contamination

EBV-B lymphoblastoid cell lines are routinely tested for mycoplasma contamination and found to be negative.

Commonly misidentified lines
(See ICLAC register)

No cell lines listed in the ICLAC database have been used in the study.

Human research participants

Policy information about studies involving human research participants

Population characteristics

The study included:
- 16 NT1 with HCRT deficiency (CSF HCRT levels < 110 pg/mL) and clinical diagnosis of narcolepsy with cataplexy (narcolepsy type 1, NT1), of which 14 carried the disease-associated HLA-DQB1*06:02 allele;
- 3 NT2 patients, HLA-DQB1*06:02-negative with a clinical diagnosis of narcolepsy with mild or without cataplexy and intermediate or normal HCRT levels (narcolepsy type 2, NT2);
- 13 healthy donors, HLA-DQB1*06:02-positive

Patients were 16–53 years of age (median 32) and had different disease duration (range 1–36, median 7 years). One NT1 patient (P10) suffered also from multiple sclerosis. All healthy donors and 14 out of 19 patients were HLA-DQB1*06:02 positive. All patients included in the study were HLA-typed at the Policlinico San Matteo, University of Pavia. Patients were recruited from University Hospital of Bern, Hospital of Lugano (EOC) and Clinic Barmelweid and healthy donors were obtained from University Hospital of Bern (of which 1 was a 1st degree family member) and the Swiss Blood Donation Center of Lugano. All participants provided written informed consent for participation in the study.
Additional information on human research participants such as age, gender, disease, HLA typing and HCRT levels in the CSF are provided in Extended Data Table 1 and Extended Data Table 3.

Recruitment

The patients were recruited at the University Hospital of Bern, Hospital of Lugano (EOC) and Clinic Barmelweid. Healthy donors were recruited at the University Hospital of Bern and the Swiss Blood Donation Center of Lugano. All participants provided written informed consent for participation in the study. The study was approved by the Ethical committees of Bern (n. 054/15),

Lugano (n. 2909) and Barmelweid (205-116).

ChIP-seq

Data deposition

☐ Confirm that both raw and final processed data have been deposited in a public database such as GEO.

☐ Confirm that you have deposited or provided access to graph files (e.g. BED files) for the called peaks.

Data access links *May remain private before publication.*	*For "Initial submission" or "Revised version" documents, provide reviewer access links. For your "Final submission" document, provide a link to the deposited data.*
Files in database submission	*Provide a list of all files available in the database submission.*
Genome browser session (e.g. UCSC)	*Provide a link to an anonymized genome browser session for "Initial submission" and "Revised version" documents only, to enable peer review. Write "no longer applicable" for "Final submission" documents.*

Methodology

Replicates	*Describe the experimental replicates, specifying number, type and replicate agreement.*
Sequencing depth	*Describe the sequencing depth for each experiment, providing the total number of reads, uniquely mapped reads, length of reads and whether they were paired- or single-end.*
Antibodies	*Describe the antibodies used for the ChIP-seq experiments; as applicable, provide supplier name, catalog number, clone name, and lot number.*
Peak calling parameters	*Specify the command line program and parameters used for read mapping and peak calling, including the ChIP, control and index files used.*
Data quality	*Describe the methods used to ensure data quality in full detail, including how many peaks are at FDR 5% and above 5-fold enrichment.*
Software	*Describe the software used to collect and analyze the ChIP-seq data. For custom code that has been deposited into a community repository, provide accession details.*

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Memory T cells were enriched from PBMCs of narcolepsy patients and healthy controls by positive selection using microbeads. CD4+ and CD8+ total memory cells were further sorted with BD FACSAria excluding CD8+ or CD4+ cells, and CD45RA+, CD25bright, CD14+ and CD56+ cells.
Instrument	BD FACSAria 335119 BD LSRFortessa 647788
Software	FACS Diva (version 6.2) was used for acquisition of samples and Flow-Jo (version 10.1) was used for all the FACS analyses.
Cell population abundance	Purity of the relevant cell populations (CD4+ and CD8+ memory T cells) was checked after sorting at BD LSRFortessa instrument and found to be >98%.
Gating strategy	CD4+ and CD8+ total memory cells were sorted from PBMC using a BD FACSAria III instrument as CD4+ (or CD8+), CD45RA–, CD14–, CD25–/lo, CD14–, CD56– and CD8– (or CD4–) cells. CD4+ and CD8+ T cells from CSF were sorted from T cell lines expanded in vitro using a BD FACSAria III instrument as CD3+CD4+CD8-–CD19–CD56– or CD3+ CD8+CD4–CD19–CD56– cells.

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*
Volume censoring	*Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings	*Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*
Effect(s) tested	*Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)	*Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.*
Correction	*Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).*

Models & analysis

n/a	Involved in the study
☒	☐ Functional and/or effective connectivity
☒	☐ Graph analysis
☒	☐ Multivariate modeling or predictive analysis