1 **Title:**

2 **Microbiota-stimulated Interleukin-22 regulates brain neurons and protects**

3 **against stress-induced anxiety**

4

5 **Authors:**

6 Anoj Ilanges[1,5], Mengyu Xia[2,5], Junmei Lu[2,5], Lei Chen[3,5], Rani Shiao[1], Changchun Wang[2],

7 Zheyu Jin[2], Ru Feng[3], Qingqing Qi[2], Han Yi[2], Jixi Li[2], Marc Schneeberger[1,4], Boxun Lu[2], Jeffrey

8 M. Friedman[1,6], Xiaofei Yu[2]*

9 **Affiliations:**

10 [1] Laboratory of Molecular Genetics, Howard Hughes Medical Institute, The Rockefeller

11 University; New York, NY 10065, USA.

12 [2] State Key Laboratory of Genetic Engineering, School of Life Sciences, Fudan University;

13 Shanghai, Shanghai 200438, China.

14 [3] Shanghai Institute of Immunology, Department of Immunology and Microbiology, and the

15 Ministry of Education Key Laboratory of Cell Death and Differentiation, Shanghai Jiao Tong

16 University School of Medicine; Shanghai, Shanghai 20025, China.

17 [4] Current address: Department of Cellular and Molecular Physiology, Yale University School

18 of Medicine, New Haven, Connecticut 06510, USA.

19 [5] These authors contributed equally to this work.

20 [6] Senior author.

21

22 * Correspondence: xiaofei_yu@fudan.edu.cn (XY).

23

1 **Summary**:

2 Psychological stress and its sequelae are a major public health problem. While the immune

3 system has been implicated in the development of stress-related disorders, how the immune

4 signals modulate neural responses to stress is poorly understood. Contrary to our expectations,

5 we found that the immune cytokine Interleukin (IL)-22 is the key mediator of an immune-to-

6 brain pathway that diminishes, rather than amplifies, stress-induced anxiety. We showed that

7 stress induced T_H17 differentiation and IL-22 production in the intestine following barrier

8 dysfunction and microbiota stimulation. IL-22 then directly signaled to septal neurons in the

9 brain to mitigate anxiety-like behavior. Accordingly, mice treated with exogenous IL-22 showed

10 resilience to chronic stress-induced anxiety disorders. Our study thus reveals a previously-

11 unappreciated immune-to-brain axis that defends against psychological stress, suggesting a

12 potential intervention strategy for stress-related mental diseases.

13

14 **Keywords:**

15 Stress, anxiety disorders, IL-22, gut-brain axis, T_H17 cells.

16

Introduction

Animals thrive in constantly changing environments by sensing and mounting coordinated responses to stress. The responses are delicately orchestrated across the nervous system and peripheral organs, which are collectively termed allostasis (McEwen et al., 2015). The dysregulation of this process has been shown to contribute to the development of a broad spectrum of psychological diseases such as anxiety disorders and depression (McEwen *et al.*, 2015). However, allostatic responses to stress have been primarily studied from the perspective of the brain (Calhoon and Tye, 2015; McEwen *et al.*, 2015), with less known about other organ systems involved.

The immune system, which predominantly monitors and protects the internal integrity of the body, has recently been implicated in allostatic responses to stress. On the one hand, numerous studies suggest that the immune system promotes the development of stress-related psychological diseases, such as anxiety disorders and depression (Glaser and Kiecolt-Glaser, 2005; Haykin and Rolls, 2021; Huh and Veiga-Fernandes, 2020; Menard et al., 2017; Pape et al., 2019; Salvador et al., 2021). For example, inflammation and immune dysregulation have been observed in patients with stress-related diseases. T lymphocytes, an important arm of the immune system, have also been reported to induce anxiety-like behavior by producing metabolic or cytokine signals, particularly when pathologically activated (Alves de Lima et al., 2020; Choi et al., 2016; Fan et al., 2019; Miyajima et al., 2017). On the other hand, fewer studies have also indicated that lymphocytes, including $CD4^+$ T cells, could confer stress resilience and ameliorate stress-induced psychological disorders (Brachman et al., 2015; Cohen et al., 2006; Rattazzi et al., 2013; Scheinert et al., 2016), but the underlying mechanisms remain elusive. Therefore, it is likely that the immune system is key to the balanced allostatic response to stress (Medzhitov,

1   2021), which may also exacerbate psychological disorders if dysregulated. Given the immune

2   sensing of resident microbiota that has been shown to influence host behavior (Li et al., 2022;

3   Morais et al., 2021; Needham et al., 2022; Veiga-Fernandes and Mucida, 2016), the immune

4   system may integrate microbial danger signals to instruct the host response to stress. Therefore, it

5   is critical to understand how the immune system responds to stress and how the immune

6   response integrates with neural pathways during stress, which are yet to be elucidated.

7

8   **Results**

9   ***IL-22 is sufficient and necessary to suppress anxiety in response to stress***

10   To investigate whether and how the immune system participates in the allostatic response to

11   stress, we studied stress-induced anxiety in mice lacking the T cell receptor β subunit ($Tcrb^{-/-}$)

12   (Mombaerts et al., 1992). $Tcrb^{-/-}$ mice have targeted deficiency in αβ T cells and develop an

13   imbalanced immune system but are generally healthy when maintained in SPF facilities. We

14   reasoned that $Tcrb^{-/-}$ mice could represent an immune imbalance state that might alter immune-

15   to-brain signaling while minimizing potential confounding effects of physical illness. We

16   assessed allostatic responses to stress by testing for anxiety-like behavior using the classical open

17   field test (OFT; more time spent in the center of the arena is indicative of reduced anxiety) and

18   elevated plus maze (EPM; more time spent in the open arms of the arena is indicative of reduced

19   anxiety) (Calhoon and Tye, 2015). Importantly, at the resting state, wild-type (WT) and $Tcrb^{-/-}$

20   mice spent similar amounts of time exploring the center of the OFT arena (Figure 1A and 1B)

21   and the open arms of the EPM maze (Figure S1A and S1B). As an internal control, wild-type

22   (WT) and $Tcrb^{-/-}$ mice traveled similar distances (Figure 1A and 1B), indicating comparable

23   locomotor activities. Thus, $Tcrb^{-/-}$ mice exhibited comparable anxiety-like behavior as WT mice

1   at baseline, making them an ideal discovery model to study immune-brain communications in

2   response to stress.

3   We then examined stress-induced anxiety by subjecting both groups to repeated mild

4   stress, which consists of a daily 30-minute period of restraint for 3 consecutive days. This

5   paradigm introduces stress but is not sufficient to induce mood disorders such as anxiety

6   disorders and depression that usually require 2 hours of restraint per day for 2-3 weeks

7   (Buynitsky and Mostofsky, 2009). The time frame of our treatment paradigm is also long enough

8   to allow early immune responses to occur. Compared to similarly stressed WT mice, $Tcrb^{-/-}$

9   mice spent more time exploring the center of the OFT arena and the open arms of the EPM maze,

10   while traveling similar distances. Therefore, $Tcrb^{-/-}$ mice showed reduced anxiety compared to

11   WT mice after mild stress exposure (Figure 1A, 1B, S1A and S1B).

12   We next tested whether this difference could be attributed to T cells by transplanting WT

13   T cells into $Tcrb^{-/-}$ ($Tcrb^{-/-}+T$) mice (Figure 1C, S1C and S1D). We found that introducing

14   normal T cells increased the anxiety-like behavior of $Tcrb^{-/-}$ mice after stress, equivalent to that

15   observed in WT mice (Figure 1C, 1D, S1E and S1F). Given that αβ T cells are critical for

16   maintaining immune balance, these data collectively suggested that a T cell-regulated immune

17   signal modulates anxiety-like behavior in response to psychological stress.

18   To identify the putative signal(s), we profiled peripheral cytokines, the primary immune

19   signals mediating communications between the immune system and other organ systems such as

20   the brain (Glaser and Kiecolt-Glaser, 2005; Salvador *et al.*, 2021), in stressed WT, $Tcrb^{-/-}$ and

21     $Tcrb^{-/-}+T$ mice (Figure 1E). Coupled with behavior data, we found that there was a significant

22   inverse correlation between IL-22 levels and anxiety-like behavior: IL-22 concentrations were

23   significantly higher in $Tcrb^{-/-}$ mice than in WT mice, with restoration to WT levels in $Tcrb^{-/-}+T$

1   mice (Figure 1E). No clear correlation was detected for all other cytokines examined, including

2   IFN-γ and IL-17A which have been previously shown to directly signal to CNS neurons to

3   modify social and anxiety-like behavior, respectively (Alves de Lima *et al.*, 2020; Choi *et al.*,

4   2016; Filiano et al., 2016; Reed et al., 2020).

5   To test whether IL-22 was sufficient to exert an anxiolytic effect, we intraperitoneally

6   (i.p.) injected WT mice with recombinant IL-22 and subjected them to mild restraint stress

7   (Figure 1F). IL-22 injection significantly reduced stress-induced anxiety-like behavior,

8   recapitulating the phenotype of $Tcrb^{-/-}$ mice with higher IL-22. We then examined whether IL-22

9   was necessary for maintaining proper anxiety in response to stress. While IL-22-deficient (IL-22^{-}

10   $^{/-}$) and IL-$22^{+/-}$ control mice showed no difference in anxiety at baseline, IL-$22^{-/-}$ mice expressed

11   increased anxiety after stress (Figure 1G). Together, our data showed that IL-22 is both

12   necessary and sufficient to regulate anxiety in response to stress. We next set out to define its site

13   of action.

14

15   ***The septal area of the brain is an important interfacing point for IL-22 to suppress anxiety***

16   Stress activates multiple brain areas to elicit anxiety (Calhoon and Tye, 2015), so we

17   hypothesized that IL-22 might modulate these stress-activated neural pathways. Since $Tcrb^{-/-}$

18   mice are intrinsically high in IL-22, we mapped brain-wide differential neural activities between

19   WT and $Tcrb^{-/-}$ mice by examining the expression of the immediate-early gene cFOS with

20   whole-mount brain clearing and immunostaining (iDISCO) (Renier et al., 2014). We performed

21   a time-course analysis to fully capture neural activities in response to stress, which consists of 4

22   time points: baseline (Resting), two hours after the start of restraint stress (2h), one day after

23   stress (1-day), and two hours after the OFT performed one day after stress (1-day OFT) (Figure

1   2A). WT and *Tcrb*^{-/-} mice showed similar neuronal activation profiles at baseline (Figure S2A

2   and S2B), in line with comparable anxiety-like behavior at the resting state. In contrast, WT and

3   *Tcrb*^{-/-} mice showed substantial differences in neuronal activation after restraint stress (2h, 1-day,

4   1-day OFT) (Figure S2A), in brain regions involved in sensation and emotion processing such as

5   primary somatosensory areas (SSp), cortical amygdalar area (COA), bed nuclei of the stria

6   terminalis (BST) and lateral septal complex (LSX) at one or more post-stress timepoints (Figure

7   S2B).

8   To pinpoint the specific brain region(s) responsible for attenuated anxiety in *Tcrb*^{-/-} mice

9   after stress, we focused on those regions that showed no difference at baseline (Resting) between

10   WT and *Tcrb*^{-/-} mice but showed consistent differences at all three post-stress timepoints (2h, 1-

11   day, and 1-day OFT). By summarizing the post-stress *Tcrb*^{-/-}-to-WT differences into aggregate z

12   scores, we found that the medial septal nucleus (MS), anterodorsal preoptic nucleus (ADP),

13   ventral medial nucleus of the thalamus (VM), and lateral septal nucleus (LSX) showed most

14   decreased neural activation while the tuberomammillary nucleus (TM) and spinal nucleus of the

15   trigeminal (caudal part) (SPVC) showed most increased neural activation in stressed *Tcrb*^{-/-} mice

16   compared to WT mice (Figure 2B). MS and LSX were of particular interest as they are

17   subregions of the septal area that is involved in the anxiety regulation (Calhoon and Tye, 2015).

18   Significant reduction of neural activation in *Tcrb*^{-/-} mice was detected in MS and LSX at both

19   mapped pixel and region levels after stress with no difference at baseline (Figure 2B, 2C and 2D).

20   The whole septal area expressed 17.1%, 64.6%, and 32.6% fewer activated neurons in *Tcrb*^{-/-}

21   mice than in WT mice at 2h, 1-day, and 1-day OFT, respectively (Figure S2C). Together, these

22   data demonstrated reduced neural activation in the septal area of *Tcrb*^{-/-} mice in response to stress

23   (2h) that persisted to the next day (1-day) and after exposure to OFT (1-day OFT).

1   To test whether stress-activated septal neurons were sufficient to drive anxiety-like

2   behavior, we selectively labeled stress-activated septal neurons with the neuron-activating

3   hM3D(Gq) DREADD receptor (Figure 2E and 2F). This was achieved by stereotactic injection

4   of an AAV encoding a Cre-dependent hM3D(Gq) into the septal area of TRAP2 ($Fos^{2A-iCreERT2}$)

5   mice (DeNardo et al., 2019), followed by administration of 4-hydroxytamoxifen (4-OHT) under

6   resting and stressed conditions, which enabled hM3D(Gq) expression and labeling of active

7   neurons under either condition. Reactivation of septal neurons labeled at the resting condition

8   with clozapine N-oxide (CNO) did not affect anxiety in mice (Figure 2G). In contrast,

9   reactivation of septal neurons labeled under the stressed condition significantly increased

10   anxiety-like behavior. Thus, stress-activated septal neurons were sufficient to drive anxiety-like

11   behavior, indicating that reduced septal neuron activation by stress in $Tcrb^{-/-}$ mice may underlie

12   their decreased anxiety-like behavior.

13   To delineate the mechanisms of reduced septal neuron activation by stress in $Tcrb^{-/-}$ mice,

14   we performed RNA-seq analysis of septal tissues from stressed WT and $Tcrb^{-/-}$ mice. Analysis of

15   the expression of cell type-specific markers failed to reveal any significant differences in the

16   overall cellular composition of the septal area (Figure S2D). Nevertheless, Gene Set Enrichment

17   Analysis (GSEA) revealed that a 206-gene stress-induced signature derived from our previous

18   work (Azevedo et al., 2020) was significantly reduced in $Tcrb^{-/-}$ mice compared to *WT* mice

19   (Figure S2E), in line with reduced neuronal activity in stressed $Tcrb^{-/-}$ mice. Consistent with the

20   immune imbalance in $Tcrb^{-/-}$ mice, several immune signaling pathways were highly enriched in

21   $Tcrb^{-/-}$ mice, with the most significant alterations in the JAK-STAT pathway which mediates the

22   signaling of numerous immune cytokines including IL-22 (Figure 2H and S2F). Indeed, a recent

23   report has confirmed that IL-22 activates the JAK-STAT pathway in an immortalized mouse

1 hippocampal cell line (Lee et al., 2022). Because the septal area is anatomically adjacent to the

2 recently characterized plexus vascular barrier (GVB) that is permissive to large molecules

3 (Carloni et al., 2021), our data raised the possibility that IL-22 might directly signal to neurons in

4 the septal area to modulate anxiety-like behavior.

5   To test this hypothesis, we implanted cannulas locally into the septal area and assayed

6 anxiety-like behavior after injecting saline or IL-22 (Figure 2I). Similar to systemic

7 administration (Figure 1F), local injection of IL-22 into the septum significantly decreased

8 anxiety-like behavior in mice, suggesting that septal IL-22 signaling is sufficient to suppress

9 anxiety. In contrast, local injection of IFN-γ, another cytokine signaling through JAK-STAT

10 failed to induce an effect on anxiety (Figure 2I). Together, our data suggest that the septal area

11 regulates anxiety in response to stress and could be a direct target of IL-22.

12

13 *IL-22 suppresses septal neuron activation*

14 We next sought to identify the cellular target of IL-22 in the septal area. IL-22 signals through a

15 dimeric receptor consisting of a selective IL22RA1 subunit and a shared IL10RB subunit

16 (Ouyang and O'Garra, 2019). We examined *Il22ra1* expression in the septum by single-molecule

17 RNA *in situ* hybridization (RNAscope) and found that it was largely co-expressed with the pan-

18 neuronal marker *Rbfox3* (encoding NeuN) (Figure 3A). To further characterize IL-22 receptor-

19 expressing neurons in the septum, we purified NeuN$^+$ nuclei by fluorescence-activated cell

20 sorting (FACS) and performed single-nuclei RNA-sequencing (snRNA-Seq) (Figure S3A and

21 S3B). Neurons in the septum show distinct gene expression profiles and cluster separately from

22 neurons of the adjacent cortical/hippocampal and striatal areas (Figure S3C). A total of 6735

23 septal neurons were further partitioned into 17 clusters, with each cluster defining a distinct cell

type. Fourteen clusters express *vGat*(*Slc32a1*)/*Gad1*/*Gad2* and are inhibitory and three clusters

express *vGlut2*(*Slc17a6*)/*vGlut1*(*Slc17a7)* and are excitatory (Figure 3B, 3C, S3D and S3E).

Though snRNA-seq is limited by the low amount of mRNA in the nucleus (Slyper et al., 2020)

and may thus underestimate gene expression, we successfully detected the expression of both IL-

22 receptor subunits, *Il22ra1* and *Il10rb,* in septal neurons (Figure 3D and S3E). Simultaneous

expression of both receptor subunits was also confirmed at the individual nucleus level (Figure

3E). 79.5% of IL-22 receptor-expressing neurons are GABAergic, including clusters in1, in3, in5,

in6, in8, in10, in14, and 20.5% are glutamatergic including cluster ex2 (Figure 3E). Therefore,

our data demonstrated that the IL-22 receptor apparatus is expressed by septal neurons.

The expression of IL-22 receptor subunits in septal neurons suggested the possibility for

neurons to respond directly to IL-22. To test this hypothesis, we performed whole-cell patch-

clamp recordings on acutely prepared septal slices in the presence or absence of IL-22 (Figure

3F). IL-22 significantly decreased the resting potential of septal neurons, and concomitantly

reduced firing frequencies and increased peak amplitudes of action potentials (Figure 3F and 3G).

IL-22 treatment also led to an increase of inhibitory tonic currents, with larger than 5 pA inward

currents detected in 66.7% of examined neurons (Figure 3H and 3I). These data showed direct

suppressive effects of IL-22 on septal neurons. Interestingly, a recent report showed that an

immortalized mouse hippocampal cell line could also respond to IL-22 (Lee *et al.*, 2022).

We next sought to determine if alterations of IL-22 signaling in septal neurons could

affect anxiety-like behavior. Because our snRNA-seq data indicated most IL-22 receptor-

expressing neurons were GABAergic inhibitory neurons, we crossed *Il22ra1^flox/flox^* mice with

vGat-Cre mice to delete IL22RA1 from GABAergic neurons (*Il22ra1^ΔvGat^*) (Figure S3F). We

found that *Il22ra1^ΔvGat^* mice displayed increased anxiety compared to control mice, indicating

1   that IL-22 signaling in GABAergic neurons is critical for maintaining proper anxiety at baseline.

2   We next ablated the IL-22 receptor specifically in septal neurons by stereotactic injection of a

3   *Syn-Cre-GFP* AAV into the septum of *Il22ra1^{flox/flox}* mice (Figure 3J). Compared to control-

4   injected mice (*Syn-GFP* AAV), *Cre*-injected *Il22ra1*-deleted mice showed increased anxiety,

5   suggesting IL-22 signaling in septal neurons mediated an anxiolytic effect. In aggregate, these

6   data showed that IL-22 reduces anxiety by suppressing the activity of septal neurons that express

7   its receptor.

8

9   **IL-22 is induced by stress via an IL-1β-T$_H$17 pathway**

10   To understand the physiological role of IL-22 in response to stress, we investigated the dynamics

11   of IL-22 during stress. IL-22 is known to maintain host barrier integrity against microbial insults

12   and the intestine is a major source of IL-22, where abundant microbes reside (Ahlfors et al., 2014;

13   Keir et al., 2020; Shaler et al., 2021). Since psychological stress increases intestinal permeability

14   (termed "leaky gut"), exposing host tissues to luminal contents, such as microbiota (Shaler *et al.*,

15   2021; Soderholm and Perdue, 2001), we reasoned that IL-22 could be induced by stress in the

16   gut. Supporting our hypothesis, we observed increased gut permeability to orally gavaged

17   Dextran-FITC and microbial exposure in mice treated with our repeated mild stress paradigm

18   (Figure 4A). Accordingly, IL-22 increased significantly in the blood after stress (Figure 4B),

19   consistent with a recent report (Shaler *et al.*, 2021). We then quantified major IL-22-producing

20   cells (T$_H$17, ILC3, and $\gamma\delta$ T cells) in the gut (Ahlfors *et al.*, 2014). While no difference was

21   observed in ILC3 cells and $\gamma\delta$ T cells before and after stress, IL-22-producing T$_H$17 cells showed

22   significant increases in the frequency and absolute number after stress (Figure 4C, S4A and S4B).

23   Thus, stress induces T$_H$17 cells that produce IL-22 in the gut.

1   To identify mechanisms by which stress induced T_H17, we profiled the fecal microbiota

2   between resting and stressed mice by 16S sequencing but failed to detect significant changes in

3   the microbiota composition (bacteria and fungi) (Figure S4C and S4D), suggesting that the IL-22

4   and T_H17 induced by repeated mild stress were unlikely due to microbial dysbiosis. We then

5   isolated naïve $CD4^+$ T cells from the spleens of stressed mice and examined their T_H17

6   differentiation potential *in vitro* (Figure 4D). Naïve T cells from stressed mice were more prone

7   to develop into T_H17 cells than those from resting mice, consistent with increased T_H17 cells in

8   stressed mice. Since splenic T cells are exposed to circulating factors, we tested the effects of

9   plasma from resting and stressed mice on T_H17 cell differentiation. We found that plasma from

10   stressed mice promoted naïve T cells from resting mice to develop into T_H17 cells (Figure 4E).

11   Because T_H17 development is critically regulated by innate immune signals, we reasoned that

12   stress-provoked gut leakage could lead to the production of T_H17-skewing factors in the intestine

13   and the blood.

14   To this end, we performed transcriptome analysis on the intestine tissues of resting and

15   stressed mice. In agreement with stress-induced gut leakage, anti-microbial immune defense

16   factors were significantly upregulated in the intestine after stress, such as S100, defensin and

17   immunoglobin genes (Figure 4F, 4G and S4C). Importantly, the expression of IL-1β, a T_H17-

18   promoting cytokine downstream of microbial-sensing pathways, increased significantly in the

19   intestine after stress (Figure 4F and 4H). No changes were detected for other known T_H17-

20   skewing factors including TGF-β1, serum amyloid As (SAAs), IL-6 and IL-23 in our setting

21   (Figure 4F and S4C). Furthermore, the circulating IL-1β levels were also elevated after stress. To

22   test whether IL-1β could account for the T_H17-promoting effects of stressed mouse plasma, we

23   antagonized IL-1β with its competitive inhibitor, IL1RA (Figure 4I). The addition of IL1RA to

1   stressed mouse plasma blunted T_H17 differentiation *in vitro* in a dose-dependent manner (Figure

2   4I). Taken together, our data suggested that stress promotes T_H17 differentiation and IL-22

3   production through microbiota-stimulated IL-1β in the gut, thereby initiating a gut-brain pathway

4   that protects mice from stress-induced anxiety disorders.

5

6   ***IL-22 intervention reduces chronic stress-induced anxiety disorders in mice***

7   Excessive chronic stress is a major etiological inducer of psychological diseases such as anxiety

8   disorders and depression, which lack efficacious treatment and pose a huge challenge to modern

9   societies. Thus, the IL-22-mediated immune-to-brain axis we identified here presents a

10   promising therapeutic target for stress-induced psychological diseases. As our data thus far has

11   shown an anxiolytic effect of IL-22 under a mild stress condition, we wanted to test whether IL-

12   22 would confer protection against excessive chronic stress.

13   To this end, we subjected mice to an intense chronic stress paradigm (2-hour daily

14   restraint for 21 consecutive days) known to induce mood disorders and intervened with daily

15   injection of recombinant IL-22 (Figure 5A). In agreement with our previous data, IL-22

16   treatment led to a significant reduction (~50%) of septal neuron activation (cFOS[+]) by stress

17   (Figure 5A). While chronic stress led to anxiety disorders, IL-22 treatment significantly reduced

18   the disease severity, suggesting IL-22 is also anxiolytic during chronic stress (Figure 5B).

19   Furthermore, it has been reported that the antidepressants citalopram and mirtazapine can induce

20   IL-22 from patient leukocytes *in vitro* (Munzer et al., 2013), suggesting that IL-22 might

21   contribute to the anti-depressive benefits of these drugs. We then subjected IL-22-treated mice

22   to the forced swimming test and tail suspension test, which are widely used to evaluate the

23   therapeutic effects of antidepressants. We found that IL-22 treatment significantly reduced the

1   immobility time of mice in both assays, suggesting that IL-22 ameliorated the development of

2   depression induced by chronic stress (Figure 5C and 5D).

3   Finally, because endogenous IL-22 can be modulated by diet-derived compounds

4   (Gutierrez-Vazquez and Quintana, 2018), we tested whether the IL-22-inducing compound 6-

5   formylindolo[3,2-b]carbazole (FICZ), a tryptophan derivative related to compounds enriched in

6   cruciferous vegetables, could also increase stress resilience. As expected, treatment with FICZ

7   during chronic stress significantly increased circulating IL-22 levels (Figure S5A). Similar to IL-

8   22 treatment, FICZ treatment also reduced the development of anxiety disorders and depression

9   induced by chronic stress (Figure 5E, 5F and 5G). Together, our data suggested that increasing

10   IL-22 levels can protect animals against the accrued psychopathological effects of excessive

11   chronic stress. Therefore, IL-22 intervention may provide a putative therapeutic strategy for

12   stress-induced psychological diseases.

13

14   **Discussion**

15   In summary, our work has revealed the cytokine IL-22 as the key mediator of an immune-to-

16   brain axis defending against psychological stress. IL-22, and the T_H17 cells that produce it, were

17   induced by stress, following stress-provoked gut leakage and microbiota-stimulated IL-

18   1β production. While IL-22 is well known to maintain gut barrier integrity, we have shown that

19   it directly suppressed septal neurons that would otherwise drive anxiety-like behavior. We

20   further demonstrated that IL-22 was both necessary and sufficient to reduce anxiety in mice in

21   response to stress. In addition, manipulation of the IL-22 pathway by exogenous IL-22 or a

22   dietary compound ameliorated anxiety disorders and depression elicited by excessive chronic

23   stress in mice. Indeed, the antidepressants citalopram and mirtazapine have been shown to

1   induce IL-22 from human leukocytes *in vitro* (Munzer *et al.*, 2013), suggesting that IL-22 might

2   contribute to the therapeutic effects of these drugs. Therefore, our study suggests that the

3   microbiota-stimulated IL-22 is critical for balanced allostatic responses to stress and represents

4   an exciting opportunity to treat stress-related psychological diseases that have been increasingly

5   prevalent in modern societies.

6   It has been a long-standing question regarding the physiological role of the immune

7   system in the stress response. Mounting evidence suggests that pathological immune activation

8   can lead to stress-related psychological diseases such as anxiety disorders and depression in

9   animal models (Choi *et al.*, 2016; Fan *et al.*, 2019; Miyajima *et al.*, 2017) and immune

10   alterations have been observed in human patients with these diseases (Glaser and Kiecolt-Glaser,

11   2005; Menard *et al.*, 2017). However, this may lie within the pathogenic extreme of the immune

12   response spectrum and reflect the cumulative cost of immune activation during stress (Medzhitov,

13   2021). With our mild stress model of which the duration and intensity can be tolerated by WT

14   mice, we observed initial immune activation and IL-1β expression in the gut following stress-

15   provoked barrier dysfunction, which is known to increase microbiota exposure to the immune

16   system (Shaler *et al.*, 2021). IL-1β then induced T_H17 differentiation and IL-22 production,

17   which restored behavioral homeostasis in mice by suppressing septal neuron activation and

18   anxiety. Therefore, the immune system senses microbiota danger signals during stress to regulate

19   host neuronal and behavioral responses, thereby integrating environmental information to

20   instruct host adaptation. Together, our study uncovers a previously-unappreciated defense role of

21   the immune system against psychological stress. Note that T cells, the adaptive immune cells that

22   take days to differentiate and function, might be optimally positioned to respond to chronic stress

23   and defend against chronic stress-elicited psychological diseases. Further study of the immune-

1   to-brain axis may yield new insights into how the immune system senses and responds to

2   stressful encounters to ensure animal survival in its natural living environments.

3

Author contributions:

Conceptualization: XY, JMF

Methodology: AI, JL, LC, MS

Investigation: AI, MX, JL, LC, RS, CW, RF, ZJ, QQ, JL, BL, XY

Visualization: AI, JL, LC, ZJ, XY

Funding acquisition: XY

Supervision: XY

Writing – original draft: XY, AI, JMF

Declaration of interests:
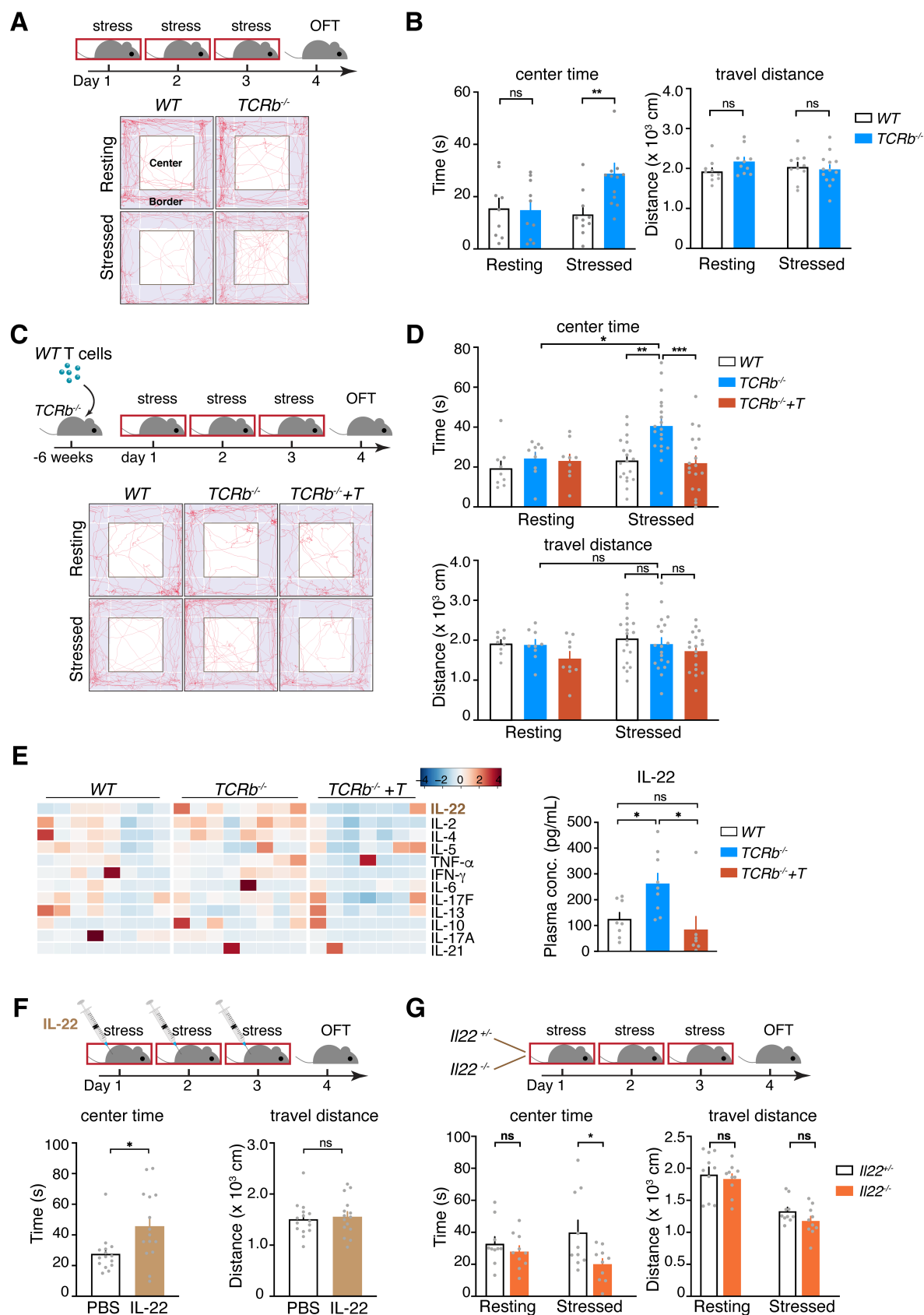
The authors declare no competing interests.

Figure 1

Figure 1. IL-22 reduces mouse anxiety in response to stress.

(**A,B**) Wild-type (WT) and T cell-deficient $Tcrb^{-/-}$ mice were subjected to repeated mild restraint stress (30-minutes restraint/day for 3 days) and examined for anxiety-like behavior with the Open Field Test (OFT). (A) Illustration of the experimental procedure and representative track plots. (B) Quantification of mouse anxiety (center time) and locomotor activity (travel distance, as an internal control). n=9, 10, 10, 13 for WT-resting, $Tcrb^{-/-}$-resting, WT-stressed, and $Tcrb^{-/-}$-stressed, respectively.

(**C,D**) WT, $Tcrb^{-/-}$ and T cell-rescued $Tcrb^{-/-}$ ($Tcrb^{-/-}+T$) mice were stressed and tested for anxiety. (C) Illustration of the experimental procedure and representative track. (D) Quantification of mouse anxiety and locomotor activity. n=9, 9, 9, 19, 19, 18 for WT-resting, $Tcrb^{-/-}$-resting, $Tcrb^{-/-}+T$-resting, WT-stressed, $Tcrb^{-/-}$-stressed and $Tcrb^{-/-}+T$-stressed, respectively.

(**E**) Circulating cytokine levels in WT, $Tcrb^{-/-}$ and $Tcrb^{-/-}+T$ mice (heatmap), with IL-22 concentrations shown in a bar plot. n=8, 8, 7 for WT, $Tcrb^{-/-}$ and $Tcrb^{-/-}+T$ mice, respectively.

(**F**) WT mice were treated with recombinant mouse IL-22 and tested for anxiety-like behavior in response to stress. n=15 for both PBS and IL-22-treatment groups.

(**G**) $Il22^{-/-}$ and $Il22^{+/-}$ control mice were analyzed for anxiety-like behavior. n=10 for both groups.

Data were pooled from or representative of 2-3 independent experiments and shown as Means ± SEMs. Statistical analysis was performed with 2-way ANOVA with Sidak's multiple comparisons (B,D,G), 1-way ANOVA with Tukey's multiple comparisons (E), and Unpaired t-test (F). *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.
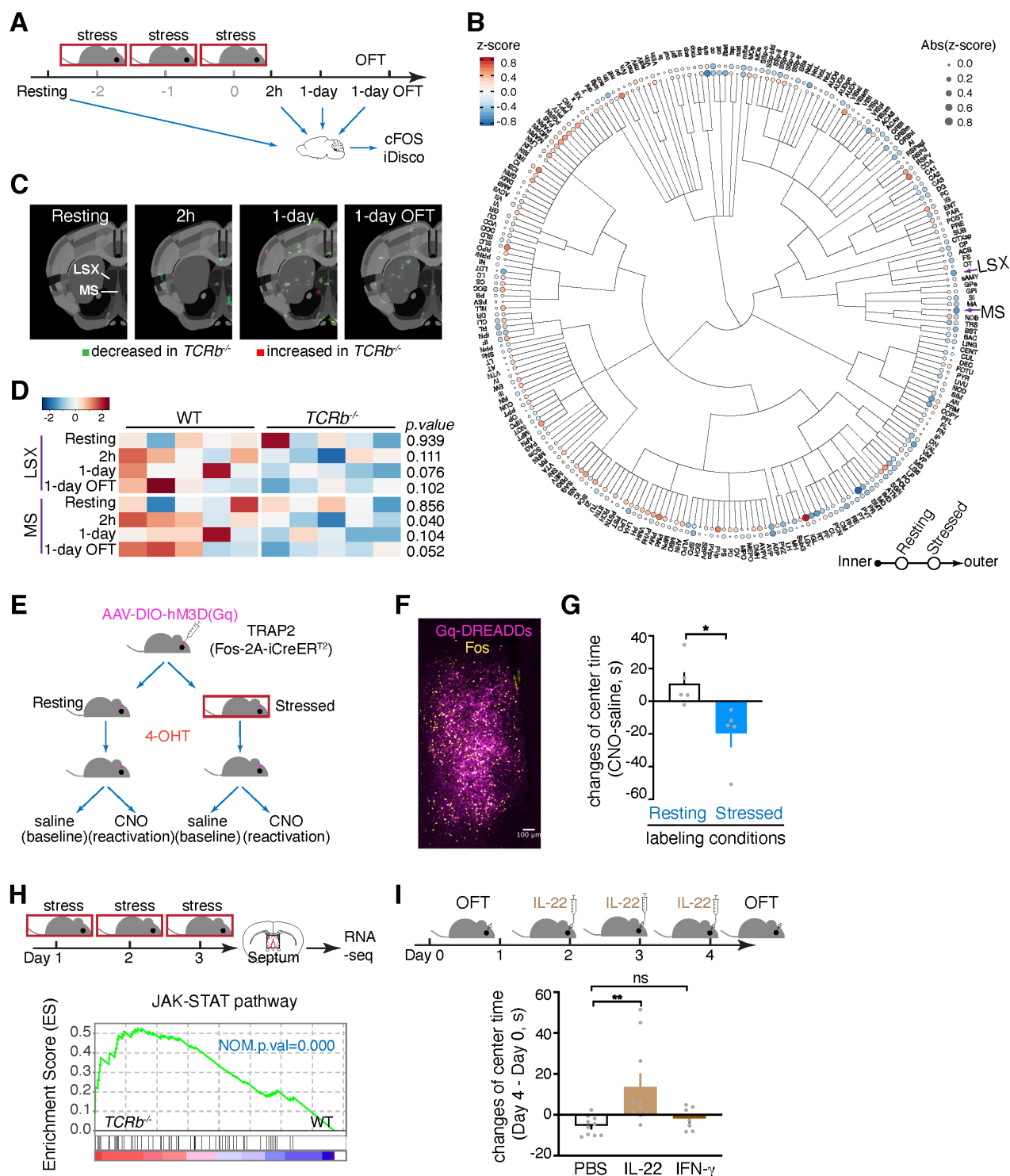
Figure 2

1   **Figure 2. The brain septal area is an important interfacing point for IL-22 to suppress**

2   **anxiety.**

3   (**A-D**) Unbiased time course profiling of neuronal activation in WT and *Tcrb*^{-/-} mice was

4   performed by examining the expression of cFOS in the whole brain with iDISCO whole-mount

5   imaging. (A) Illustration of the experimental design and timepoints examined: baseline (Resting),

6   two hours after the final restraint stress (2h), one day after the final restraint stress (1-day), and

7   after exposure to OFT one day after the final restraint stress (1-day OFT). (B) Differential

8   activation of brain regions by stress in WT and *Tcrb*^{-/-} mice. The inner dots show baseline

9   (Resting) z scores of each brain region and the outer dots show aggregate z scores of post-stress

10   timepoints (2h, 1-day, 1-day OFT). Blue and red indicate negative and positive z scores,

11   respectively, corresponding to decreased and increased neuronal activation in *Tcrb*^{-/-} mice

12   compared to WT mice. The size of dots and intensity of colors reflect the absolute values of z

13   scores. The tree structure shows the hierarchical organization of brain regions. (C) Statistical

14   analysis of neuronal activation in the septal area at the mapped pixel level. Green and red

15   indicate significant decrease ($p<0.01$) and increase ($p<0.01$) in *Tcrb*^{-/-} mice. (D) Heatmap

16   analysis of neuronal activation in the septal area at the region level. n=5 for all groups.

17   (**E-G**) Reactivation of stress-activated septal neurons to examine their sufficiency to drive

18   anxiety-like behavior. (E) Design of the reactivation experiment. Stress-activated septal neurons

19   were labeled with the activating hM3D(Gq) DREADD receptor in TRAP2 mice and reactivated

20   by CNO. (F) Representative images showing labeling of activated (cFOS[+], yellow) septal

21   neurons by hM3D(Gq) (magenta) in stressed mice. (G) Changes of center time of individual

22   mice injected with CNO and saline (baseline) were calculated to reflect the anxiety-driving

23   effects of septal neurons. n=5 for both groups.

1   **(H)** RNA-seq analysis of septal tissues of WT and *Tcrb*^{-/-} mice. GSEA analysis was performed to

2   compare the enrichment of JAK-STAT pathway genes in the septum. n=2 for both groups.

3   **(I)** WT mice were stereotactically injected with IL-22 into the septum and analyzed for anxiety-

4   like behavior. Changes in the center time of individual mice before and after IL-22 injection

5   were calculated and depicted. IFN-γ was injected as a control. n=10, 10, and 7 for PBS, IL-22,

6   and IFN-γ groups, respectively.

7   Data were pooled from 2 independent experiments for (I) and presented as Means ± SEMs.

8   Statistical analysis was performed with ClearMap (B,C,D), Unpaired t-test (G), GSEA (H), and

9   1-way ANOVA with Dunnett's multiple comparisons **(I)**. *P < 0.05; **P < 0.01; ns, not

10   significant.

11
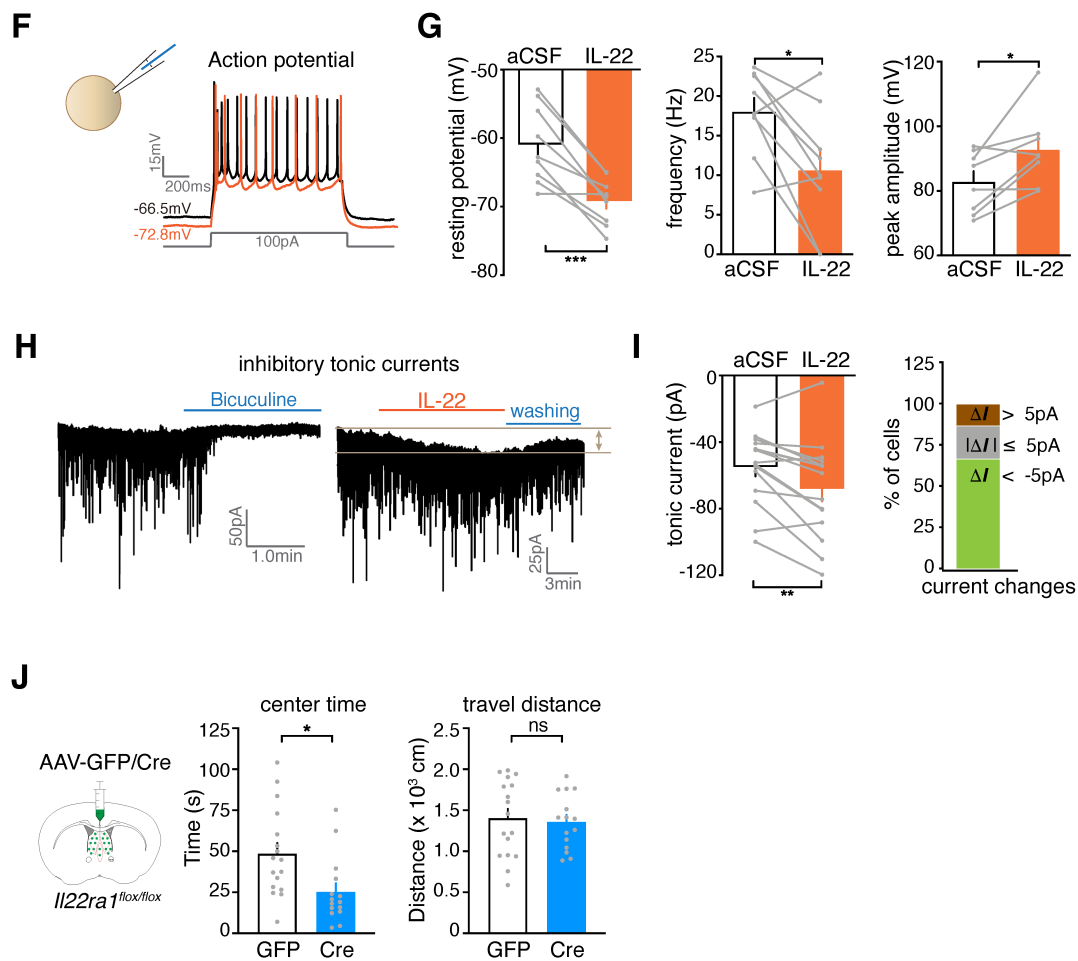
Figure 3

Figure 3 (cont'd)

1 **Figure 3. IL-22 inhibits septal neuron activation and reduces anxiety.**

2 (**A**) Detection of the IL-22 receptor *Il22ra1* expression by RNAscope. Representative staining of

3 DNA (DAPI, blue), *Il22ra1* (green), the pan-neuronal marker *NeuN* (*Rbfox3*, red), and the

4 merged image are shown.

5 (**B-E**) snRNA-seq analysis of septal neurons. (B) UMAP embedding of single-cell profiles (dots)

6 for the septal area, colored by cluster membership. (C) Dotplot showing top marker genes for

7 each septal neuron cluster. (D) Expression of *Il22ra1*, *Il10rb*, vGat (*Slc32a1*), and vGlut2

8 (*Slc17a6*) in septal neurons. (E) Co-expression of *Il22ra1* and *Il10rb* in individual neurons.

9 Ratios of neurons expressing both receptor subunits in each cluster are shown.

10 (**F-I**) Electrophysiological analysis of septal neurons responding to IL-22. (F) Representative

11 action potential recording from septal neurons. (G) Summary of the membrane potential and the

12 frequency and peak amplitude of the action potential of septal neurons treated with buffer (aCSF)

13 or IL-22. (H) Example current trace of mIPSCs when septal neurons were treated with

14 bicuculline (a blocker, left) and IL-22 (right). (I) Quantification of tonic current shift and the

15 proportion of tonic current shift when septal neurons were treated with IL-22.

16 (**J**) *Il22ra1^{flox/flox}* mice were stereotactically injected with a *Syn-Cre-GFP* AAV to locally delete

17 *Il22ra1* in septal neurons and analyzed for anxiety-like behavior. n=17, 15 for GFP (control) and

18 Cre groups, respectively.

19 Data were pooled from 2 independent experiments for (J). Data are presented as Means ± SEMs.

20 Statistical analysis was performed with Paired t-test (G,I) and Unpaired t-test (J). *P < 0.05; **P
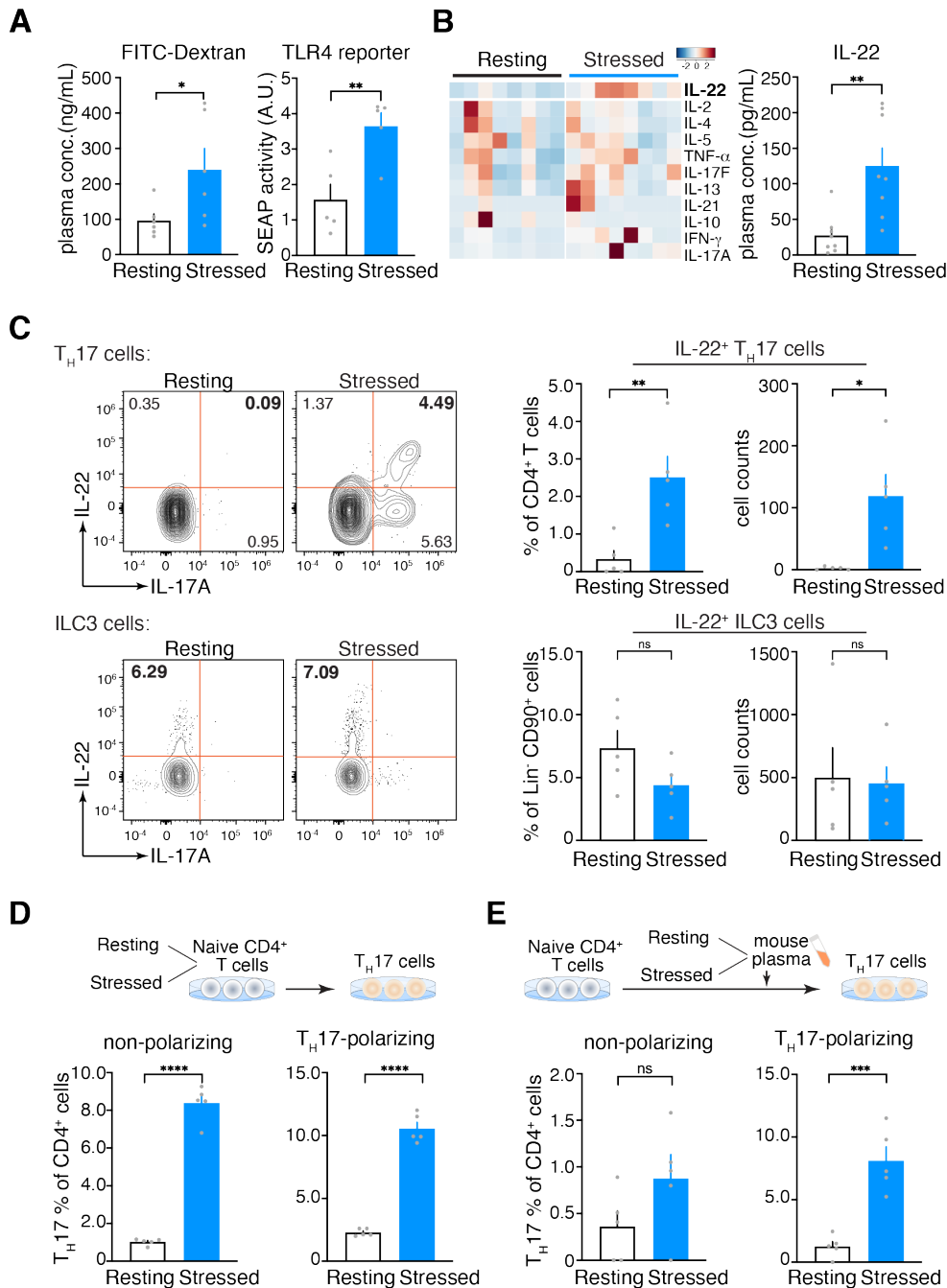
21 < 0.01; ns, not significant.

22

1 # Figure 4

Figure 4 (cont'd)

F

G

H

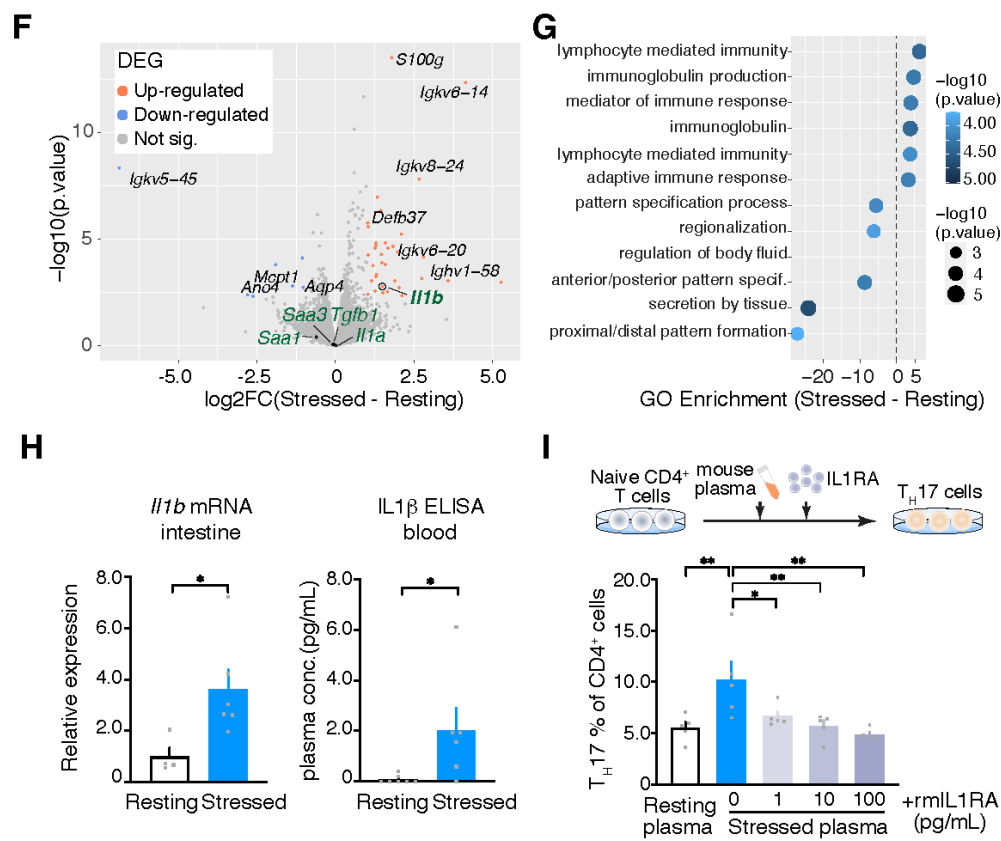*Il1b* mRNA intestine

IL1β ELISA blood

I

1   **Figure 4. IL-22 is induced by stress via an IL-1β-T$_H$17 pathway.**

2   (**A**) Intestinal permeability was determined by oral gavage of FITC-Dextran. n=6 for both groups.

3   Microbial exposure was measured with stimulation of TLR4-SEAP reporter cells with plasma

4   from resting and stressed mice. n=5 for both groups.

5   (**B**) Circulating IL-22 concentrations before and after stress. Cytokines were profiled with the

6   LegendPlex multiplex bead assay. n=8 for both groups.

7   (**C**) Flow cytometry analysis of IL-22-producing T$_H$17 cells in the intestine, showing

8   representative flow plots (left) and quantification summary (right). n=5 for both groups.

9   (**D**) *In vitro* differentiation of splenic naïve CD4$^+$ T cells from resting and stressed mice into

10   T$_H$17 cells in the presence or absence of TH17-polarizing cytokines. n=5 for both groups.

11   (**E**) *In vitro* differentiation of T$_H$17 cells in the plasma of resting and stressed mice. n=5 for both

12   groups.

13   (**F**) Volcano plot showing differentially expressed genes in the intestines of resting and stressed

14   mice. Detected T$_H$17-skewing cytokines were highlighted in green. n=3 for each group.

15   (**G**) Bubble plot showing top enriched GO pathways in the intestines of resting and stressed mice.

16   n=3 for each group.

17   (**H**) IL-1β levels in the intestine (mRNA) and blood (protein) before and after stress. n=4-6 for

18   each group.

19   (**I**) *In vitro* T$_H$17 differentiation in the stressed mouse plasma with increasing concentrations of

20   recombinant mouse IL1RA (rmIL1RA) to blocking of IL-1β. n=4 for all groups.

21   Data are representative of 2-3 independent experiments and shown as Means ± SEMs. Statistical

22   analysis was performed with Unpaired t-test (A-E, H) and 1-way ANOVA with Dunnett's

23   multiple comparisons (I). *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.
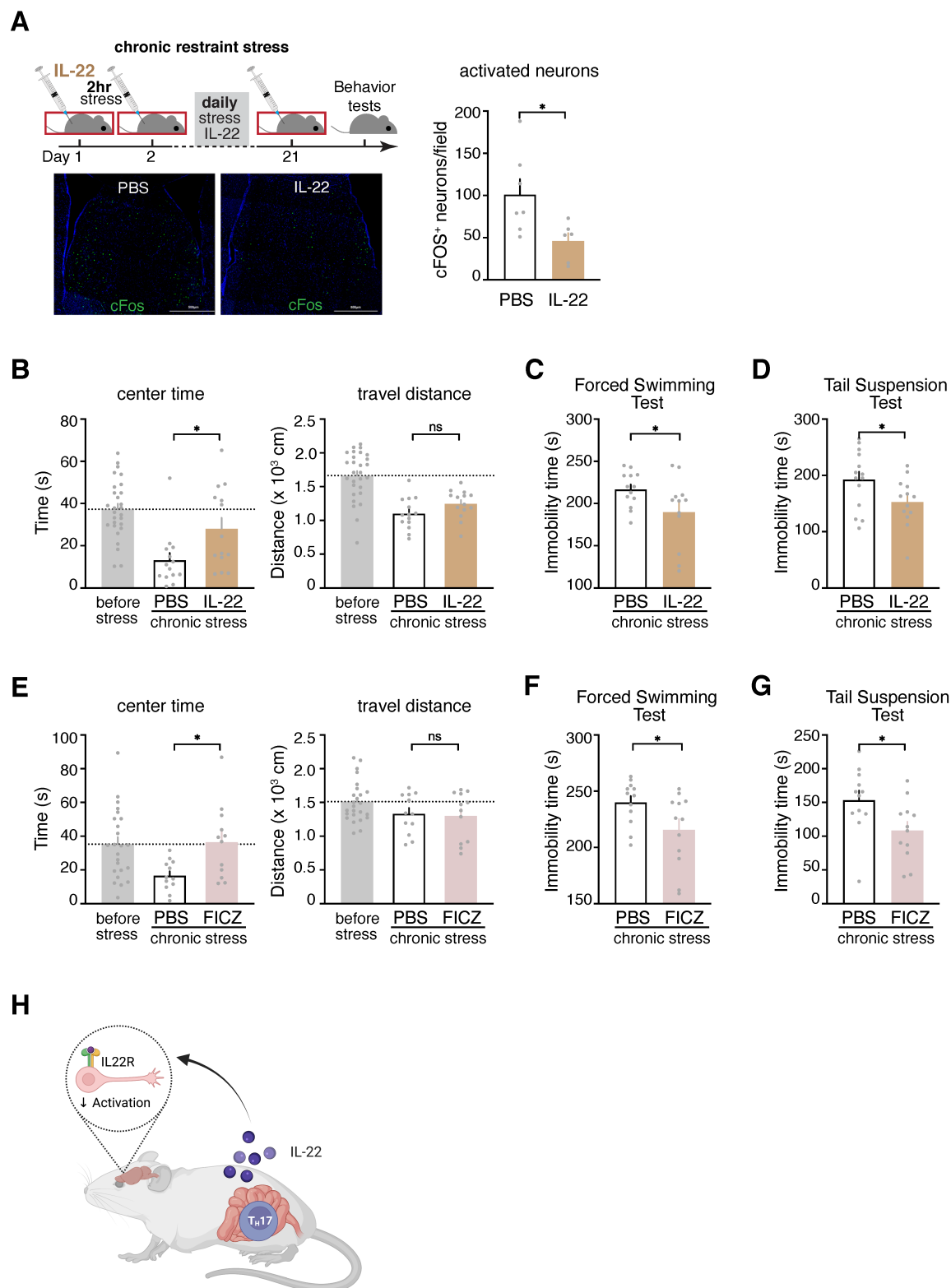
Figure 5

1   **Figure 5. IL-22 protects mice from stress-induced psychological disorders.**

2   (**A-D**) Mice were subjected to excessive chronic stress, treated with IL-22, and analyzed for

3   anxiety disorders and depression. (A) The experimental design and septal neuron activation in

4   treated mice. Representative cFOS staining images (left) and quantification (right) are shown.

5   n=7 and 6 for PBS and IL-22 treatment groups, respectively. (B) Anxiety was assessed with OFT.

6   n=28, 14 and 14 for pre-stress, PBS and IL-22 treatment groups, respectively. (C,D) Depression

7   was assessed with the forced swimming test (C) and tail suspension test (D). More immobility

8   time indicates greater severity of depression in mice. n= 14 and 13 for the PBS and IL-22

9   treatment groups, respectively.

10   (**E-G**) Mice were treated with the IL-22-inducing compound, FICZ, during excessive chronic

11   stress and analyzed for anxiety disorders and depression. (E) Anxiety was examined with OFT.

12   n=24, 12 and 12 for pre-stress, PBS and FICZ treatment groups, respectively. (F,G) Depression

13   was assessed with the forced swimming test (F) and tail suspension test (G). n= 12 for both

14   groups.

15   (**H**) The working model.

16   Data are presented as Means ± SEMs. Statistical analysis was performed with Unpaired t-test

17   (A,C,D,F,G) and 1-way ANOVA with Dunnett's multiple comparisons (B,E). *P < 0.05; ns, not

18   significant.

19

1   **References**

2   Ahlfors, H., Morrison, P.J., Duarte, J.H., Li, Y., Biro, J., Tolaini, M., Di Meglio, P., Potocnik,

3   A.J., and Stockinger, B. (2014). IL-22 fate reporter reveals origin and control of IL-22

4   production in homeostasis and infection. J Immunol *193*, 4602-4613.

5   10.4049/jimmunol.1401244.

6   Alves de Lima, K., Rustenhoven, J., Da Mesquita, S., Wall, M., Salvador, A.F., Smirnov, I.,

7   Martelossi Cebinelli, G., Mamuladze, T., Baker, W., Papadopoulos, Z., et al. (2020).

8   Meningeal gammadelta T cells regulate anxiety-like behavior via IL-17a signaling in

9   neurons. Nat Immunol *21*, 1421-1429. 10.1038/s41590-020-0776-4.

10 Azevedo, E.P., Tan, B., Pomeranz, L.E., Ivan, V., Fetcho, R., Schneeberger, M., Doerig, K.R.,

11   Liston, C., Friedman, J.M., and Stern, S.A. (2020). A limbic circuit selectively links active

12   escape to food suppression. Elife *9*. 10.7554/eLife.58894.

13 Brachman, R.A., Lehmann, M.L., Maric, D., and Herkenham, M. (2015). Lymphocytes from

14   chronically stressed mice confer antidepressant-like effects to naive mice. J Neurosci *35*,

15   1530-1538. 10.1523/JNEUROSCI.2278-14.2015.

16 Buynitsky, T., and Mostofsky, D.I. (2009). Restraint stress in biobehavioral research: Recent

17   developments. Neurosci Biobehav Rev *33*, 1089-1098. 10.1016/j.neubiorev.2009.05.004.

18 Calhoon, G.G., and Tye, K.M. (2015). Resolving the neural circuits of anxiety. Nat Neurosci *18*,

19   1394-1404. 10.1038/nn.4101.

20 Carloni, S., Bertocchi, A., Mancinelli, S., Bellini, M., Erreni, M., Borreca, A., Braga, D.,

21   Giugliano, S., Mozzarelli, A.M., Manganaro, D., et al. (2021). Identification of a choroid

22   plexus vascular barrier closing during intestinal inflammation. Science *374*, 439-448.

23   10.1126/science.abc6108.

1 Choi, G.B., Yim, Y.S., Wong, H., Kim, S., Kim, H., Kim, S.V., Hoeffer, C.A., Littman, D.R.,

2   and Huh, J.R. (2016). The maternal interleukin-17a pathway in mice promotes autism-like

3   phenotypes in offspring. Science *351*, 933-939. 10.1126/science.aad0314.

4 Cohen, H., Ziv, Y., Cardon, M., Kaplan, Z., Matar, M.A., Gidron, Y., Schwartz, M., and Kipnis,

5   J. (2006). Maladaptation to mental stress mitigated by the adaptive immune system via

6   depletion of naturally occurring regulatory CD4+CD25+ cells. J Neurobiol *66*, 552-563.

7   10.1002/neu.20249.

8 DeNardo, L.A., Liu, C.D., Allen, W.E., Adams, E.L., Friedmann, D., Fu, L., Guenthner, C.J.,

9   Tessier-Lavigne, M., and Luo, L. (2019). Temporal evolution of cortical ensembles

10   promoting remote memory retrieval. Nat Neurosci *22*, 460-469. 10.1038/s41593-018-0318-

11   7.

12 Fan, K.Q., Li, Y.Y., Wang, H.L., Mao, X.T., Guo, J.X., Wang, F., Huang, L.J., Li, Y.N., Ma,

13   X.Y., Gao, Z.J., et al. (2019). Stress-Induced Metabolic Disorder in Peripheral CD4(+) T

14   Cells Leads to Anxiety-like Behavior. Cell *179*, 864-879 e819. 10.1016/j.cell.2019.10.001.

15 Filiano, A.J., Xu, Y., Tustison, N.J., Marsh, R.L., Baker, W., Smirnov, I., Overall, C.C., Gadani,

16   S.P., Turner, S.D., Weng, Z., et al. (2016). Unexpected role of interferon-gamma in

17   regulating neuronal connectivity and social behaviour. Nature *535*, 425-429.

18   10.1038/nature18626.

19 Glaser, R., and Kiecolt-Glaser, J.K. (2005). Stress-induced immune dysfunction: implications for

20   health. Nat Rev Immunol *5*, 243-251. 10.1038/nri1571.

21 Gutierrez-Vazquez, C., and Quintana, F.J. (2018). Regulation of the Immune Response by the

22   Aryl Hydrocarbon Receptor. Immunity *48*, 19-33. 10.1016/j.immuni.2017.12.012.

1 Haykin, H., and Rolls, A. (2021). The neuroimmune response during stress: A physiological

2 perspective. Immunity *54*, 1933-1947. 10.1016/j.immuni.2021.08.023.

3 Huh, J.R., and Veiga-Fernandes, H. (2020). Neuroimmune circuits in inter-organ communication.

4 Nat Rev Immunol *20*, 217-228. 10.1038/s41577-019-0247-z.

5 Keir, M., Yi, Y., Lu, T., and Ghilardi, N. (2020). The role of IL-22 in intestinal health and

6 disease. J Exp Med *217*, e20192195. 10.1084/jem.20192195.

7 Knight, Z.A., Tan, K., Birsoy, K., Schmidt, S., Garrison, J.L., Wysocki, R.W., Emiliano, A.,

8 Ekstrand, M.I., and Friedman, J.M. (2012). Molecular profiling of activated neurons by

9 phosphorylated ribosome capture. Cell *151*, 1126-1137. 10.1016/j.cell.2012.10.039.

10 Krishnaswami, S.R., Grindberg, R.V., Novotny, M., Venepally, P., Lacar, B., Bhutani, K.,

11 Linker, S.B., Pham, S., Erwin, J.A., Miller, J.A., et al. (2016). Using single nuclei for RNA-

12 seq to capture the transcriptome of postmortem neurons. Nat Protoc *11*, 499-524.

13 10.1038/nprot.2016.015.

14 Lee, D., Jo, H., Go, C., Jang, Y., Chu, N., Bae, S., Kang, D., Kim, Y., and Kang, J.S. (2022). The

15 Roles of IL-22 and Its Receptor in the Regulation of Inflammatory Responses in the Brain.

16 Int J Mol Sci *23*. 10.3390/ijms23020757.

17 Li, X.V., Leonardi, I., Putzel, G.G., Semon, A., Fiers, W.D., Kusakabe, T., Lin, W.Y., Gao, I.H.,

18 Doron, I., Gutierrez-Guerrero, A., et al. (2022). Immune regulation by fungal strain

19 diversity in inflammatory bowel disease. Nature. 10.1038/s41586-022-04502-w.

20 McEwen, B.S., Bowles, N.P., Gray, J.D., Hill, M.N., Hunter, R.G., Karatsoreos, I.N., and Nasca,

21 C. (2015). Mechanisms of stress in the brain. Nat Neurosci *18*, 1353-1363. 10.1038/nn.4086.

22 Medzhitov, R. (2021). The spectrum of inflammatory responses. Science *374*, 1070-1075.

23 10.1126/science.abi5200.

1   Menard, C., Pfau, M.L., Hodes, G.E., and Russo, S.J. (2017). Immune and Neuroendocrine

2   Mechanisms of Stress Vulnerability and Resilience. Neuropsychopharmacology *42*, 62-80.

3   10.1038/npp.2016.90.

4   Miyajima, M., Zhang, B., Sugiura, Y., Sonomura, K., Guerrini, M.M., Tsutsui, Y., Maruya, M.,

5   Vogelzang, A., Chamoto, K., Honda, K., et al. (2017). Metabolic shift induced by systemic

6   activation of T cells in PD-1-deficient mice perturbs brain monoamines and emotional

7   behavior. Nat Immunol *18*, 1342-1352. 10.1038/ni.3867.

8   Mombaerts, P., Clarke, A.R., Rudnicki, M.A., Iacomini, J., Itohara, S., Lafaille, J.J., Wang, L.,

9   Ichikawa, Y., Jaenisch, R., Hooper, M.L., and et al. (1992). Mutations in T-cell antigen

10   receptor genes alpha and beta block thymocyte development at different stages. Nature *360*,

11   225-231. 10.1038/360225a0.

12   Morais, L.H., Schreiber, H.L.t., and Mazmanian, S.K. (2021). The gut microbiota-brain axis in

13   behaviour and brain disorders. Nat Rev Microbiol *19*, 241-255. 10.1038/s41579-020-00460-

14   0.

15   Munzer, A., Sack, U., Mergl, R., Schonherr, J., Petersein, C., Bartsch, S., Kirkby, K.C., Bauer,

16   K., and Himmerich, H. (2013). Impact of antidepressants on cytokine production of

17   depressed patients in vitro. Toxins (Basel) *5*, 2227-2240. 10.3390/toxins5112227.

18   Needham, B.D., Funabashi, M., Adame, M.D., Wang, Z., Boktor, J.C., Haney, J., Wu, W.L.,

19   Rabut, C., Ladinsky, M.S., Hwang, S.J., et al. (2022). A gut-derived metabolite alters brain

20   activity and anxiety behaviour in mice. Nature *602*, 647-653. 10.1038/s41586-022-04396-8.

21   Ouyang, W., and O'Garra, A. (2019). IL-10 Family Cytokines IL-10 and IL-22: from Basic

22   Science to Clinical Translation. Immunity *50*, 871-891. 10.1016/j.immuni.2019.03.020.

Pape, K., Tamouza, R., Leboyer, M., and Zipp, F. (2019). Immunoneuropsychiatry - novel perspectives on brain disorders. Nat Rev Neurol *15*, 317-328. 10.1038/s41582-019-0174-4.

Rattazzi, L., Piras, G., Ono, M., Deacon, R., Pariante, C.M., and D'Acquisto, F. (2013). CD4(+) but not CD8(+) T cells revert the impaired emotional behavior of immunocompromised RAG-1-deficient mice. Transl Psychiatry *3*, e280. 10.1038/tp.2013.54.

Reed, M.D., Yim, Y.S., Wimmer, R.D., Kim, H., Ryu, C., Welch, G.M., Andina, M., King, H.O., Waisman, A., Halassa, M.M., et al. (2020). IL-17a promotes sociability in mouse models of neurodevelopmental disorders. Nature *577*, 249-253. 10.1038/s41586-019-1843-6.

Renier, N., Wu, Z., Simon, D.J., Yang, J., Ariel, P., and Tessier-Lavigne, M. (2014). iDISCO: a simple, rapid method to immunolabel large tissue samples for volume imaging. Cell *159*, 896-910. 10.1016/j.cell.2014.10.010.

Salvador, A.F., de Lima, K.A., and Kipnis, J. (2021). Neuromodulation by the immune system: a focus on cytokines. Nat Rev Immunol *21*, 526-541. 10.1038/s41577-021-00508-z.

Sanz, H., Aponte, J.J., Harezlak, J., Dong, Y., Ayestaran, A., Nhabomba, A., Mpina, M., Maurin, O.R., Diez-Padrisa, N., Aguilar, R., et al. (2017). drLumi: An open-source package to manage data, calibrate, and conduct quality control of multiplex bead-based immunoassays data analysis. PLoS One *12*, e0187901. 10.1371/journal.pone.0187901.

Scheinert, R.B., Haeri, M.H., Lehmann, M.L., and Herkenham, M. (2016). Therapeutic effects of stress-programmed lymphocytes transferred to chronically stressed mice. Prog Neuropsychopharmacol Biol Psychiatry *70*, 1-7. 10.1016/j.pnpbp.2016.04.010.

Shaler, C.R., Parco, A.A., Elhenawy, W., Dourka, J., Jury, J., Verdu, E.F., and Coombes, B.K. (2021). Psychological stress impairs IL22-driven protective gut mucosal immunity against colonising pathobionts. Nat Commun *12*, 6664. 10.1038/s41467-021-26992-4.

1 Slyper, M., Porter, C.B.M., Ashenberg, O., Waldman, J., Drokhlyansky, E., Wakiro, I., Smillie,
2   C., Smith-Rosario, G., Wu, J., Dionne, D., et al. (2020). A single-cell and single-nucleus
3   RNA-Seq toolbox for fresh and frozen human tumors. Nat Med *26*, 792-802.
4   10.1038/s41591-020-0844-1.

5 Soderholm, J.D., and Perdue, M.H. (2001). Stress and gastrointestinal tract. II. Stress and
6   intestinal barrier function. Am J Physiol Gastrointest Liver Physiol *280*, G7-G13.
7   10.1152/ajpgi.2001.280.1.G7.

8 Stuart, T., Butler, A., Hoffman, P., Hafemeister, C., Papalexi, E., Mauck, W.M., 3rd, Hao, Y.,
9   Stoeckius, M., Smibert, P., and Satija, R. (2019). Comprehensive Integration of Single-Cell
10   Data. Cell *177*, 1888-1902 e1821. 10.1016/j.cell.2019.05.031.

11 van Dijk, D., Sharma, R., Nainys, J., Yim, K., Kathail, P., Carr, A.J., Burdziak, C., Moon, K.R.,
12   Chaffer, C.L., Pattabiraman, D., et al. (2018). Recovering Gene Interactions from Single-
13   Cell Data Using Data Diffusion. Cell *174*, 716-729 e727. 10.1016/j.cell.2018.05.061.

14 Veiga-Fernandes, H., and Mucida, D. (2016). Neuro-Immune Interactions at Barrier Surfaces.
15   Cell *165*, 801-811. 10.1016/j.cell.2016.04.041.

16 Yu, X., Rollins, D., Ruhn, K.A., Stubblefield, J.J., Green, C.B., Kashiwada, M., Rothman, P.B.,
17   Takahashi, J.S., and Hooper, L.V. (2013). TH17 cell differentiation is regulated by the
18   circadian clock. Science *342*, 727-730. 10.1126/science.1243884.

19

20