Supporting Information

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SI Methods and Materials

Animals. Male CD45.2+ C57BL/6 mice and CD45.1+ C57BL/6 mice (20–30 g; The Jackson Laboratory, Stock #000664/#002014) were obtained at 7–8 wk of age for social defeat experiments and allowed 1 wk of acclimation to housing facilities before the start of the experiments. BM transplant hosts (CD45.2+ C57BL/6 and CD45.1+ C57BL/6) were obtained at 3 wk of age and allowed 1 wk of acclimation before irradiation. IL-$6^{-/-}$ mice (B6.129S2-$Il6^{tm1kopf}$/J) on a CD45.2+ C57BL/6 background were bred at the Mount Sinai School of Medicine from stock obtained from a commercial vendor (The Jackson Laboratory, stock #002650) and used at 7–8 wk old. Male CD-1 mice (35–45 g; Charles River Laboratories, stock #482) used as aggressors were sexually experienced retired breeders at least 4 mo of age. Aggressors were singly housed at all times other than during the social defeats. All other animals were group housed before social defeat and singly housed following social defeat. All studies were performed using male mice. All animals were maintained on a 12 h light/dark cycle with ad libitum access to food and water. Mouse procedures were performed in accordance with the guidelines published in the NIH Guide for Care and Use of Laboratory Animals, and all protocols were approved by the Mt. Sinai School of Medicine Institutional Animal Care and Use Committee.

Repeated Social Defeat Stress. Repeated social defeat stress (RSDS) was performed as previously described (1). CD-1 mice were screened for aggressive behavioral responses before the start of social defeat experiments based on previously described criteria (1). At least 24 h before defeat, CD-1 aggressor mice were housed within a hamster cage (26.7 width × 48.3 depth × 15.2 height cm; Allentown Inc.) on one side of a clear perforated Plexiglas divider (0.6 × 45.7 × 15.2 cm; Nationwide Plastics). Experimental mice were exposed to a novel CD-1 aggressor mouse for 10 min daily over 10 consecutive days. After the physical contact, experimental mice were removed and placed on the opposite side of the clear perforated partition within the aggressor's home cage to allow for sensory contact during the subsequent 24-h period. Experimental mice were then relocated to a new cage each day and defeated by a novel CD-1 aggressor to prevent stress habituation. Control mice were housed two mice per cage, on opposite sides of a perforated divider, rotated daily in a manner similar to the defeat group, but never exposed to aggressive CD-1 mice. Twenty-four hours following the final social defeat stress, experimental mice were singly housed. Animals were visually inspected during the course of RSDS. Any animals with wounds larger than 1 cm were removed from the study. In a subset, animals' physical appearance was scored 24 h after the last defeat as a series of points. Animals received 1 point for disheveled fur, 1 point for tail bites, and 1 point for nonvisible back bites (determined by touch).

Subthreshold Defeat Stress. To measure increased susceptibility to stress, we adapted a subthreshold variation on the repeated social defeat protocol as previously described (1, 2). CD45.2+ C57BL/6 mice were subjected to a novel CD-1 aggressor for three consecutive 5-min defeat bouts, with a 15-min intertrial interval between exposures. Twenty-four hours later, mice were assessed for social avoidance behavior. Under control conditions, this protocol does not result in social avoidance behavior but will reveal differences when animals have shifted to a stress-susceptible phenotype.

Social Avoidance Testing (Social Interaction Test). Social interaction testing was performed as previously described (1, 2). All social interaction testing was performed under red-light conditions. Mice were placed in a novel interaction, open-field arena custom-crafted in white Plexiglas (42 × 42 × 42 cm; Nationwide Plastics) with a small wire animal cage placed at one end (10 cm × 6.5 cm × 42 cm; Nationwide Plastics). Animal movements were tracked automatically (Ethovision 3.0; Noldus Information Technology) for 2.5 min in the absence of a novel CD-1 mouse. This phase was used to calculate baseline exploratory behavior. A novel CD-1 mouse was placed in the small wire animal cage, and exploratory behavior by the experimental mouse was recorded for 2.5 min. Social interaction behavior was calculated as the ratio of the time spent in an interaction zone near the novel animal divided by the time spent in the same area near the empty cage. Animals with a ratio of above 1 spent more time near the novel animal than the empty cage and were classified as resilient. Animals with a ratio below 1 spent less time near the empty cage than the novel animal and were classified as susceptible.

Imiprimine Treatment. Male CD45.2+ C57BL/6 mice were exposed to 10 d of RSDS or were housed under control conditions. Animals went through social interaction testing 24 h after the last defeat. Subjects were classified as susceptible, resilient, or control; single housed; and treated for 34 d with Imipramine (20 mg/kg i.p.) or saline vehicle. On day 35 subjects were tested for social interaction, 15–20 min after injection of Imipramine or vehicle.

IL-6 Antibody Study. Male CD45.2+ C57BL/6 mice were given daily injections (i.p.) of mouse anti-IL6 monoclonal antibody (mAb) (R&D Systems, Clone MP5-20F3), rat IgG_1 isotype control (R&D Systems, Clone 43414), or saline vehicle. Antibodies were given at a dose of 4 μg per mouse per day in 0.2 mL of saline vehicle. Antibodies/saline were injected 5 min prior to RSDS. No antibody was given before social interaction testing.

Witness Defeat. Male CD 45.2+ C57BL/6 mice (witness experiment) and male CD45.1+ C57BL/6 mice that received BM transplants from IL-$6^{-/-}$ or WT high–IL-6 releasing controls [IL-$6^{-/-}$ bone marrow transplant (BMT) witness study] watched a CD45.2+C57BL/6 mouse go thorough 10-min daily bouts of RSDS. Witness mice were placed on one side of a perforated Plexiglas divider, and the aggressor and defeat mouse were paired together. Immediately after witnessing the defeat bout, the witness mouse was removed and housed on the separate side of a perforated Plexiglas divider from a novel aggressor. At no point did the witness mouse have physical contact with any aggressor. This was repeated with novel aggressors every day for 10 d. After defeat, all animals were single housed. As previously reported, witness stress takes 30 d to incubate, resulting in social avoidance behavior (3). IL-$6^{-/-}$ and WT BMT mice were tested for social interaction 30 d after witnessing their last bout of RSDS.

Subthreshold Witness Defeat. We used a modified version of the witness defeat. Male CD45.2+ C57BL/6 mice that received BM transplants from susceptible or control CD45.1+ C57BL/6 mice witnessed a CD45.2+ C57BL/6 mouse go through RSDS as described above. Two weeks following the last defeat, mice were given a social interaction test. Two weeks following witnessing RSDS mice normally do not demonstrate social avoidance behavior.

Chronic Variable Stress. Male mice (8 wk of age) were exposed to 21-d variable stress. Stressors were applied in the following order: day 1, 100 foot shocks presented over 1 h (0.45 mA); day 2, 1 h of tail suspension; day 3, 1 h of restraint stress in 50-mL conical tubes (Fisher) with drilled breathing holes. Stressors were applied in the above order for a total of 21 d. Twenty-four hours after the last stressor, trunk blood was collected at the time of sacrifice.

Elevated Plus Maze. Mice were acclimated to the testing facility for 1 h before testing. Animals were placed in the elevated plus maze under red-light conditions for 5 min. Each arm of the maze measured 12 × 50 cm. The black Plexiglas cross-shaped maze consisted of two open arms with no walls and two closed arms (40 cm high walls) and was on a pedestal 1 m above floor level. Behavior was tracked using an automated system (Noldus Ethovision; Noldus Interactive Technologies). Behavior was measured as total time in combined open arms and total time in combined closed arms.

Human Participants. Study participants were recruited through an academic outpatient psychiatric clinic. Following informed consent, healthy volunteers or clinical patients underwent a medical and psychiatric evaluation and were required to be medically healthy to participate in the study. Healthy volunteers were free of lifetime psychiatric illness and had no significant medical problems. Clinical patients were diagnosed with MDD based on the Structured Clinical Interview for *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV) and a diagnostic interview with a study clinician. The majority of patients were free of antidepressants or other concomitant psychotropic medication at the time of blood sample collection. Patients with MDD were determined to be treatment-resistant if they had failed to respond to at least two Food and Drug Administration-approved antidepressant medication trials of sufficient dose and duration as determined by the Antidepressant Treatment History Form (4). Depression severity at the time of sample collection was determined using the Quick Inventory of Depressive Symptomatology–Self-Report (QIDS-SR) (5). The study was approved by the Program for the Protection of Human Subjects of the Icahn School of Medicine at Mount Sinai. For full demographics, see Table S1.

Description of Rating Instruments. The Structured Clinical Interview for DSM-IV Axis I Disorders is a semistructured interview for making the major DSM-IV Axis I diagnoses, and was the primary means by which volunteers were diagnosed and screened for mental illness.

QIDS-SR is a 16-item self-rated instrument designed to assess the severity of depressive symptoms present in the past 7 d. The 16 items cover the nine symptom domains of major depression and are rated on a scale of 0–3. Total score ranges from 0 to 27, with ranges of 0–5 (normal), 6–10 (mild), 11–15 (moderate), 16–20 (moderate to severe), and 21+ (severe).

Blood Sample Collection—Human. Eligible participants arrived at a clinical research unit in the morning following an overnight fast. Approximately 10 mL of blood was drawn with the use of a vacutainer from the forearm of the nondominant arm. Blood samples were incubated at room temperature until clotted (maximum 2 h) and then centrifuged at 1,100 × *g* for 15 min at +4 °C, and serum was transferred into aliquots of 0.25 mL and stored in a –80 °C freezer until analyzed.

Blood Sample Collection—Human Cohort 2. A total of 71 participants (50 controls and 21 patients) contributed blood samples. Demographics are listed in Table S2. All patients met the DSM-IV Text Revision criteria for MDD, and clinical tests including administration of the Hamilton Depression Scale (HAMD) assessment were performed by psychiatrists under good clinical practice–compliance to minimize variability. Patients and controls were fasting at sample collection and free of acute and chronic infections, allergies, autoimmune diseases, cancer, or systemic diseases. All subjects gave informed written consent. Clinical investigations were conducted according to the Declaration of Helsinki, and the University of Muenster ethical committee approved the study.

Blood samples were collected from all participants in the Department of Psychiatry, University of Muenster, Germany by venous puncture into S-Monovette 7.5-mL serum tubes (Sarstedt). Serum was prepared according to standard protocols by leaving the samples at room temperature for 2 h to allow coagulation followed by centrifugation at 1,000 × *g* for 5 min at 21 °C to remove the clotted material. The resulting supernatants were stored at –80 °C in Low Binding Eppendorf tubes before analysis. Patients were sampled before and after standard antidepressant treatment following an average of 2.5 ± 1. 3 mo.

Blood Sample Collection—Mice. Submandibular vein bleeds (6) were taken from mice over time to obtain within-subjects information about the time course of cytokine release. To collect plasma, blood was sampled 4 d before RSDS, 20 min after the first defeat, and 48 h after the last defeat in EDTA-lined tubes (Sarstedt). For witness defeat, blood was collected in EDTA-lined tubes (Sarstedt) ∼30 min after social interaction testing. Blood was centrifuged at 956 × *g* for 20 min, and plasma was removed and stored frozen (–20 °C) until analysis (Fig. 1*B*). For serum, whole blood was collected via submandibular bleed in protein lobind tubes (Eppendorf), sat for 1 h at room temperature for clotting, and then was centrifuged for 15 min at 956 × *g*. Serum was collected and stored at –80 °C until analysis. Blood for chimeras was sampled using submandibular bleeds into a solution of PBS and 10 mM EDTA. Blood was stored at room temperature in the dark until same-day processing and analysis.

Single Target Enzyme Linked Immunosorbent Assays. IL-6 levels in plasma, serum, and supernatants from cell cultures were measured in duplicate. Human samples were measured using a Human IL-6 high-sensitivity ELISA with signal amplification (eBioscience). Mean sensitivity was 0.03 pg/mL, the intra-assay variability was 4.9%, and the interassay variability was 6.0%. IL-6 levels from subjects used in the time course and witness study were detected with a commercially available solid phase sandwich ELISA (BD Biosciences). Intra-assay variability ranged from 6.4–6.9%. The mean interassay variability was 4–9.6%, and the mean sensitivity was 3.8 pg/mL. To validate basal concentrations of IL-6 below 3.8 pg/mL, a high-sensitivity ELISA with signal amplification and a mean sensitivity of 0.21 pg/mL (eBioscience) was used. The intra-assay variability was 5.1%, and the interassay variability was 10.02%. Corticosterone (CORT) levels were measured in plasma with a commercially available sandwich ELISA kit (ImmunoDiagnostic Systems). The intra-assay variability for the CORT assay ranged from 3.8–6.6%, and the interassay variability ranged from 7.5–8.6%; mean assay sensitivity was 0.55 ng/mL.

Multiplex ELISA (MiliplexMAP). A total of 22 cytokine and chemokines were measured in 25 μL of serum collected 20 min after the first encounter with an aggressor. On the day of sample preparation, samples were defrosted on ice and diluted by half with assay buffer. Cytokines and chemokines were analyzed using the commercially available mouse cytokine/chemokine magnetic bead panel (Luminex–MCYTOMAG–70K PMX) on a Luminex 200 multiplex immnuoassay system at the Human Immune Monitoring Core at the Hess Center for Science and Medicine (Icahn School of Medicine at Mt. Sinai). The immunoassay-based technology combines multiplexed ELISAs with a flow cytometery approach. The assays were calibrated using duplicate

8-point standard curves, and raw intensity measurements were converted to absolute protein concentrations using proprietary software. Sample analysis was randomized to avoid any sequential bias. Samples were only included in analysis if there was 80–120% recovery for each data point on the standard curve. Results for each well fell within 20% of the known values for each analyte, and duplicates did not vary more than 20% from each other. Samples within each analyte that did not meet minimum detection values were not included in analysis. Additionally, statistical outlier tests were performed and samples that varied more than twice the SD from the mean were removed.

Multiplexed Serum Profiling (DiscoveryMAP v 1.0) Human Cohort 2. A total of 188 analytes were measured in 200–250 μL serum using the multiplexed immunoassay *Human*MAPv 1.0 platform at a Clinical Laboratory Improvement Amendments (CLIA)-certified laboratory (Rules Based Medicine) (7).

The panel of analytes is involved in various hormonal, immunological, metabolic, and neurotrophic pathways. The immunoassay-based technology combines multiplexed ELISA assays with a flow-through cytometer approach. The assays were calibrated using duplicate 8-point standard curves, and raw intensity measurements were converted to absolute protein concentrations using proprietary software. Machine performance was verified using quality control samples at low, medium, and high levels for each analyte, and sample analysis was randomized to avoid any sequential bias due to the diagnosis, age, or sample collection dates.

Leukocyte Isolations/Immune Challenge. To isolate leukocytes, whole blood was taken via submandibular bleed and stored overnight, in the dark, at room temperature in heparin–Li-coated tubes (Eppendorf North America Biotools). Whole blood (200 μL) was then transferred to a 15-mL conical tube and mixed with 2 mL of complete media (RPMI 1640, 20% horse serum, 10% FBS, 2 mM l-glutamine, 100 units per mL of penicillin, and 100 μg/mL streptomycin). The blood/media mixture was layered over an equal volume of Ficoll-Paque Plus (GE Healthcare). Samples are centrifuged (790 × *g*, 15 min, 25 °C) to form a buffy coat layer. Cells were removed, washed in BEP solution (PBS with 0.5% BSA and 2 mM ETDA), and centrifuged (529 × *g*, 8 min, 25 °C). The supernatant was removed, and cells were resuspended in 200 μL of BEP solution. Cell aliquots were stained with trypan blue, and cells were counted on a hemocytometer. Cells were plated at 1×10^6 cells per well in 1 mL of media or media + 34 μg/mL LPS. Cells were stored for 24 h at 37 °C with 5% CO_2. After 24 h, cells and media were removed from plates, centrifuged (2,348 × *g*, 5 min), and supernatant was removed and stored at –20 °C until IL-6 analysis.

Donor Selection.

Stress-susceptible BM chimera. CD45.1+ C57BL/6 mice (8 wk of age, 20–30 g) were selected as donors based on their in vitro release of IL-6 in response to LPS stimulation before RSDS and their social interaction ratio following 10 d of defeat.

IL-6$^{-/-}$ BM chimera. CD45.2+ C57BL/6 donors were selected based on their in vitro release of IL-6 in response to an LPS challenge. The IL-6$^{-/-}$ donor was selected based on having nondetectable levels of IL-6, and the control donor was selected based on a high release of IL-6 in response to LPS.

Generation of BM Chimeras. As previously described (8), 4-wk-old CD45.2+ C57BL/6 (for stress-susceptible BM chimera) or CD45.1+ C57BL/6 (for IL-6$^{-/-}$ BM chimera) host mice were irradiated with 1,200 rad delivered in two doses of 600 rad delivered 10–11 h apart to ablate the peripheral immune system. During irradiation, animals were given a mixture of ketamine (100 mg per kg body weight) and xylazine (10 mg per kg body weight), and their heads were secured inside of lead shielding (Nuclead). Following the second bout of irradiation, BM hematopoietic progenitor cells from a donor mouse were introduced through a retro-orbital injection. Host mice were treated with antibiotics (sulfatrim) for 3 wk and given a minimum of 5 wk of recovery to allow the new immune cells to mature. The degree of repopulation by donor was determined by measuring the percentage of CD45.1+ cells among total B220+ B cells, Ly6C/G+ CD115– granulocytes, and CD115+ monocytes in the blood 5–8 wk after transplantation via flow cytometry (see methods below).

Cell Suspension Preparations. Nucleated single-cell suspensions were enriched from peripheral blood. All blood cell suspensions were red blood cell (RBC) lysed with 1× RBC Lysis solution (eBioscience). Cell suspensions were made from the brain before analysis by flow cytometry. Briefly, brain tissues were carefully freed from cephalic mesenchyme and meninges, and minced tissues were incubated in 1:10 concentration of FBS to HBSS and Collagenase type IV (0.2 mg/mL, working activity of 770 U/mg) (Sigma) for 1 h and then passed through a 19G syringe to obtain a homogeneous cell suspension. Brain cell suspensions were then resuspended in 37% isotonic Percoll and layered with 70% isotonic Percoll (GE Healthcare) centrifuged at 600 × *g* for 25 min at room temperature. Cells at the interphase were collected and washed before labeling for flow cytometry. Analysis was carried out by flow cytometry, gating on singlet DAPI– (4,6-diamidino-2- phenylindole) CD45+ cells.

Flow Cytometry and Cell Cycle Analysis. Flow cytometry studies were performed using a Fortessa and LSRII (Becton Dickinson) and subsequently analyzed using FlowJo software (Tree Star). Fluorochrome or biotinconjugated mAbs specific for mouse B220 (clone RA3-6B2), MHC class II I-A/I-E (clone M5/114.15.2), CD11b (clone M1/70), CD45 (clone 30F11), CD45.1 (clone A20), CD45.2 (clone 104), CSF-1R (also called CD115) (clone AFS98), Gr-1Ly6C/G (clone RB6-8C5), CD3 (clone 17A2), SIRP1 (clone P84), and Mac-3 (also called CD163) (clone M3/84); the corresponding isotype controls; and the secondary reagents (allophycocyanin, peridinine chlorophyll protein, and phycoerythrin–indotricarbocyanine-conjugated streptavidin) were purchased either from BD Biosciences or eBioscience. Anti-F4/80 (A3-1) mAb was purchased from Serotec.

K_i-67 Labeling. Animals were deeply anesthetized with a lethal dose of choral hydrate and transcardially perfused with PBS followed by 4% paraformaldehyde in a 0.1-M phosphate buffer (pH 7.3). Brains were postfixed in 4% paraformaldehyde for a minimum of 24 h and then transferred to a PBS/azide (0.1%). Unilateral coronal sections (50 μm) from the entire rostrocaudal extent of the dentate gyrus were cut on a vibratome. Every sixth section was slide mounted 24 h before staining. Sections were prepared via immunohistochemical staining for Ki67, a nuclear protein the presence of which indicates active cell proliferation. For antigen retrieval, sections were placed in boiling citric acid and maintained at 90 °C for 15 min. Slides were then rinsed in PBS and then incubated overnight at room temperature in primary (1:500, Ki67 Rabbit mAb clone SP6, ThermoScientific) blocking solution (3% normal donkey serum, TBS, 0.3% triton). Slides were rinsed and incubated in secondary blocking solution (1:200 AlexaFluor488 Conjugated Affinipure Donkey Anti-Rabbit IgG, cat. no. 711–545-152; Jackson Scientific). Sections were rinsed, counterstained with DAPI, dehydrated, and coverslipped. Slides were coded before quantitative analysis, and cells were counted blind to the experimental conditions. Cells were imaged at 40× on a confocal microscope (Zeiss). Photomicrographs were tiled and density was calculated by tabulating the maxima identified within an area of interest, and dividing the sum by volume, calculated as the product of the mean area of

the regions sampled in an animal, the number of sections evaluated, and the thickness of each section.

Core Body Temperature Measurement. C57BL/6J mice were implanted with IPTT-300 temperature transponders (Biomedic Data Systems) s.c. under aseptic conditions with isofluorane anesthesia. Each mouse was fitted with a new transponder in the superior dorsal/interscapular region. A DAS-502 electronic transponder reader (BMDS) was used to record basal temperature after 10 d of RSDS immediately before the social interaction test.

Statistical Analysis. Differences between two groups were compared using *t* tests. Comparisons of multiple means were analyzed by univariate ANOVAs when appropriate, with Newman–Keuls used for post hoc analysis. Comparisons of multiple factors or repeated measures were analyzed by bivariate ANOVAs with a Bonferroni posttest for post hoc analysis. Percentages of cell populations were analyzed using χ^2 tests. All statistical analyses were performed using Graph Pad Prism 5.0 software (Graphpad Software Inc.). Statistical significance was set at $P < 0.05$.

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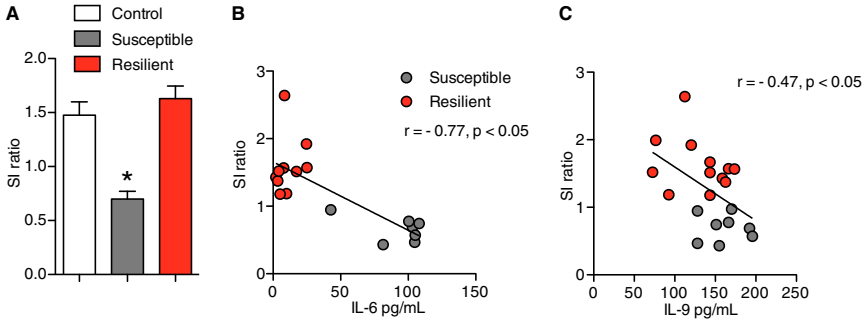

Fig. S1. Behavior of the animals used for the multiplex ELISA study. (*A*) Susceptible mice ($n = 8$) had lower social interaction ratios than control ($n = 10$) or resilient ($n = 12$) mice, indicating social avoidance behavior ($F_{2,29} = 17.48$, $P < 0.0001$). (*B*) SI ratio after 10 d of RSDS negatively correlated with circulating levels of IL-6 20 min after the first encounter with an aggressor ($r = –0.77$, $P < 0.05$). (*C*) Circulating levels of IL-9 20 min after the first encounter with an aggressor negatively correlated with the SI ratio 10 d later ($r = –0.47$, $P < 0.05$). Graphs display mean ± SEM. * denotes a significant effect of phenotype, and circles denote individual animals.

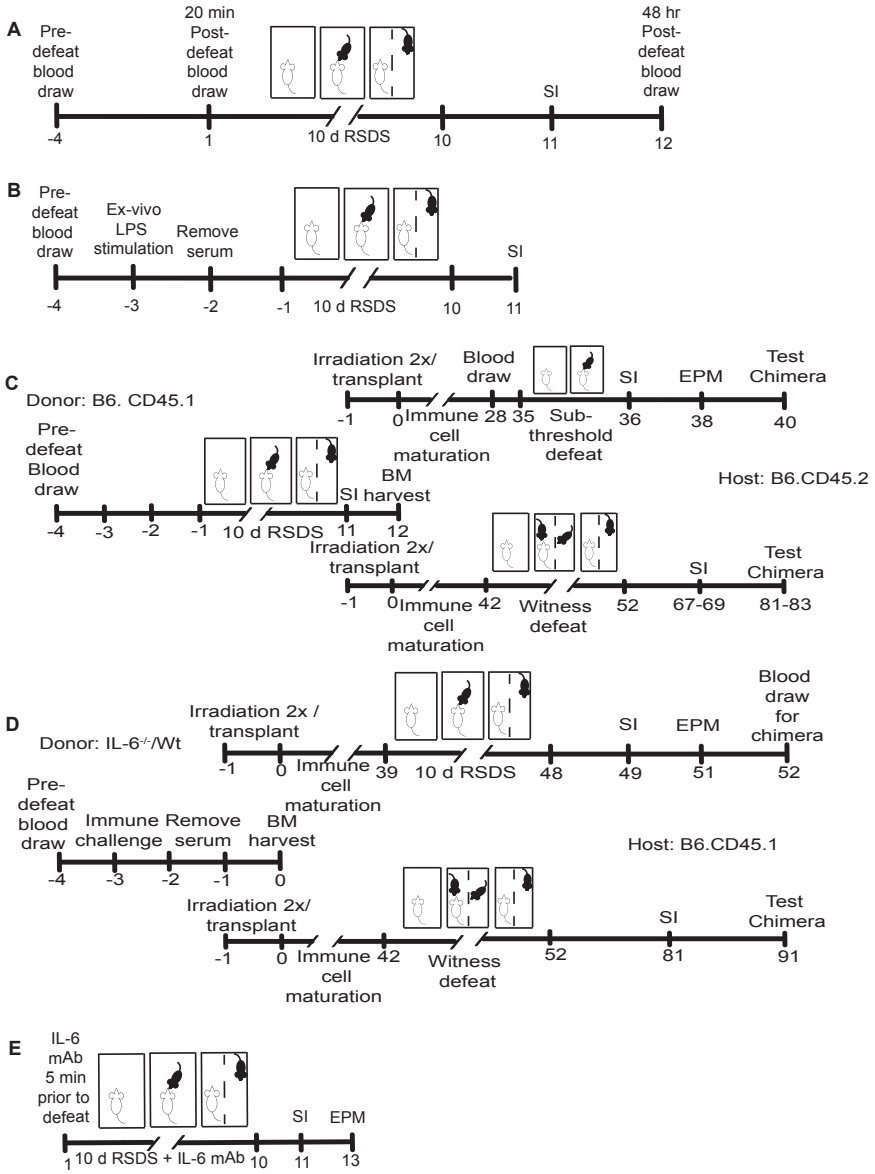

Fig. S2. Schematics of experimental designs. (*A*) Schematic demonstrating time points of blood sampling. (*B*) Schematic of the experimental design used to investigate the predictive power of an ex vivo LPS challenge of leukocytes from animals before RSDS. (*C*) A schematic of the BM transplant procedure in which hematopoietic progenitor cells isolated from CD45.1+ C57BL/6-susceptible ($n = 4$) or control mice ($n = 4$) are injected into head-shielded irradiated host ($n = 58$) CD45.2+ C57BL/6 mice for both subthreshold stress and witness defeat. (*D*) Schematic of BM hematopoietic progenitor cell transplants from IL-$6^{-/-}$ CD45.2+/CD 45.1– mice ($n = 2$) or WT controls ($n = 2$) into host CD45.1+/CD45.2– mice ($n = 31$) for RSDS study and for witness defeat. (*E*) Schematic describing systemic IL-6, IgG mAb, or saline vehicle administration to neutralize IL-6 in the periphery.

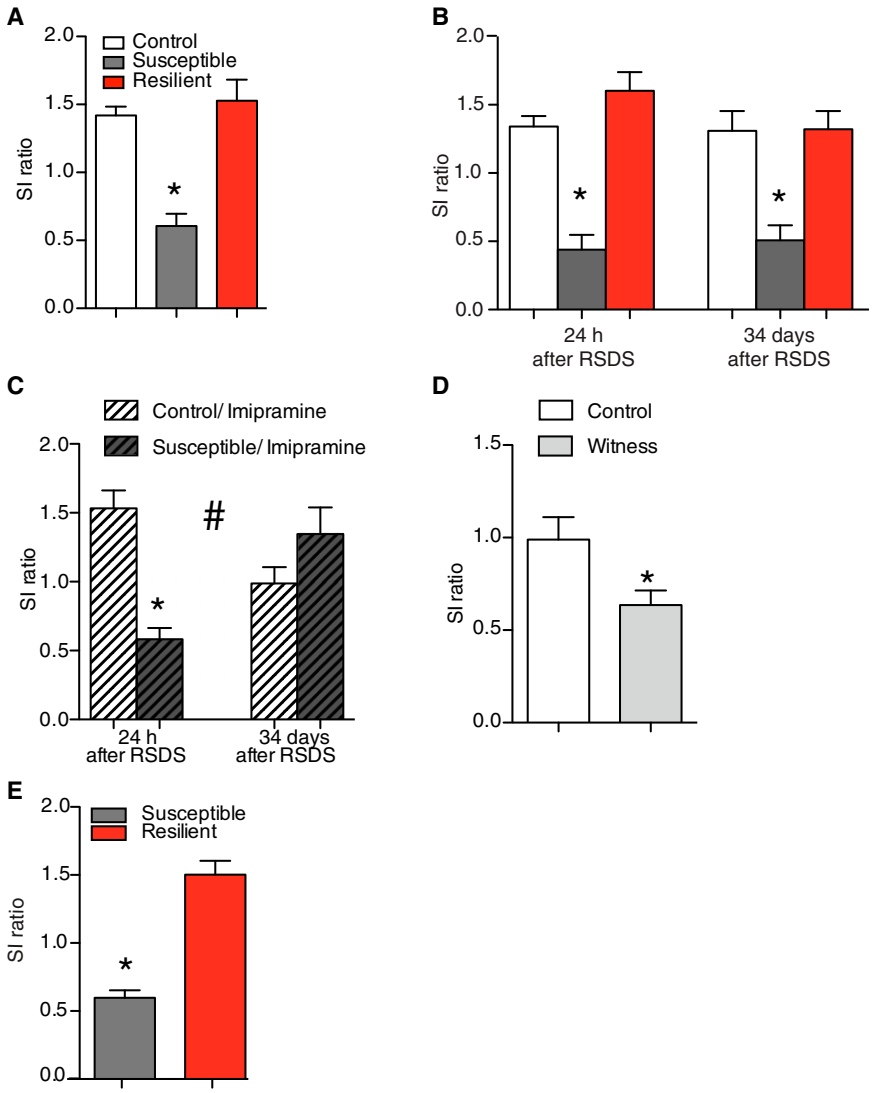

Fig. S3. Social interaction behavior of animals and humans used for cytokine measurements. (*A*) Behavior of animals used for time course study; susceptible mice showed decreased social interaction with a novel aggressor compared with control or resilient mice ($F_{2,19} = 24.77$, $P < 0.001$). (*B*) Social interaction behavior of animals exposed to 10 d of RSDS and tested for social interaction 24 h and 34 d after the last defeat. Susceptible mice ($n = 7$) showed less interaction with a novel aggressor 24 h and 34 d after defeat ($F_{2,23} = 30.89$, $P < 0.0001$) compared with control ($n = 13$) and resilient ($n = 6$) mice. (*C*) Behavior of control ($n = 13$) and susceptible ($n = 11$) animals exposed to 10 d of RSDS and then treated for 35 d with Imipramine; antidepressant treatment rescued social avoidance behavior in susceptible mice, as indicated by a significant interaction ($F_{1,22} = 39.38$, $P < 0.0001$). (*D*) Mice that witnessed defeat spent less time near a novel aggressor than animals that did not witness RSDS ($t_{19} = 2.52$, $P < 0.05$, two tailed). (*E*) Social interaction behavior from animals used for the in vitro IL-6 stimulation study. Resilient animals had higher social interaction scores than susceptible animals when tested 24 h after the last defeat ($t_{36} = 8.05$, $P < 0.001$). Graphs display mean ± SEM. # denotes a significant interaction. * denotes a main effect of phenotype or significant differences between means (*t* test).

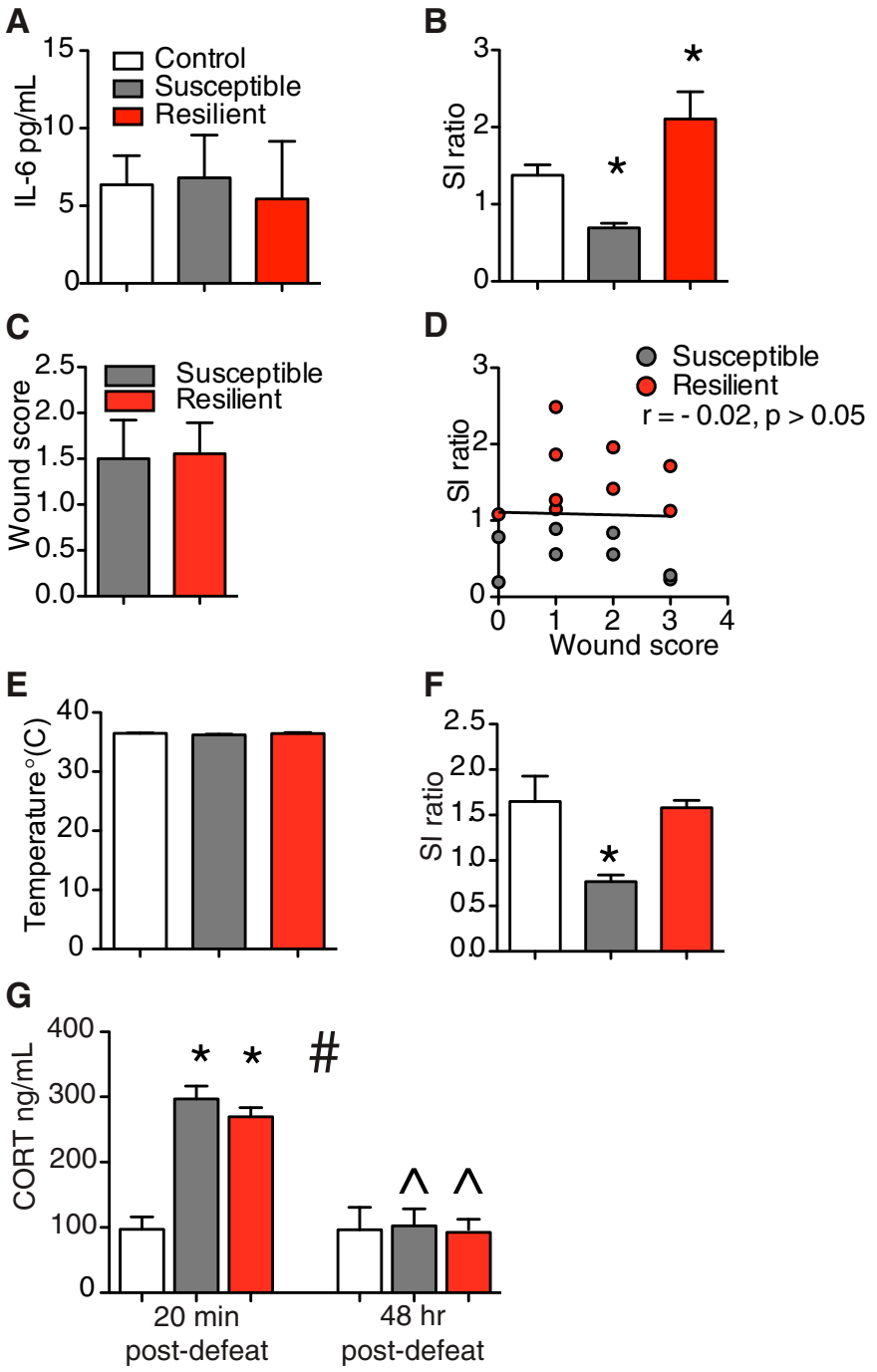

Fig. S4. Physiological profiles of animals exposed to RSDS. (*A*) IL-6 levels were measured 4 d before RSDS in a separate cohort of animals, from plasma using a high-sensitivity (0.21 pg/mL) ELISA (eBiosciences) with signal amplification (control, $n = 8$; susceptible, $n = 8$; resilient, $n = 4$). There were no significant differences in basal levels of IL-6 before social defeat ($F_{2,19} = 0.06$, $P > 0.05$). Plasma levels were below assay sensitivity in 9 of the 29 animals used in the behavioral assays, resulting in their exclusion from the assay data. (*B*) Mice used for the high-sensitivity ELISA were behaviorally divided into control ($n = 10$), susceptible ($n = 11$), or resilient ($n = 8$) based on social interaction scores ($F_{2,28} = 13.30$, $P < 0.001$). (*C*) There were no significant differences in physical appearance between susceptible ($n = 8$) and resilient ($n = 9$) mice ($t_{15} = 0.10$, $P > 0.05$). (*D*) Wound score did not correlate with the social interaction ratio ($r = -0.02$, $P > 0.05$). (*E*) There were no significant differences in body temperature of animals exposed to 10 d of RSDS before the social interaction test ($F_{2,86} = 1.30$, $P > 0.05$). (*F*) Social interaction behavior for mice used to measure circulating levels of corticosterone (CORT). Susceptible mice ($n = 4$) had lower levels of social interaction than control ($n = 6$) or resilient animals ($n = 6$), as indicated by the social interaction ratio ($F_{2,13} = 5.38$, $P < 0.05$). (*G*) CORT was elevated to equal levels in both susceptible and resilient mice compared with controls 20 min after the first defeat, but there were no differences in circulating levels of CORT 48 h after the last defeat, as indicated by a significant interaction of phenotype and time ($F_{2,13} = 8.52$, $P < 0.01$). Graphs display mean ± SEM. # denotes a significant interaction. * denotes significant difference in phenotype or means (*t* test). ^ denotes a main effect of time. Circles denote individual animals (correlations).

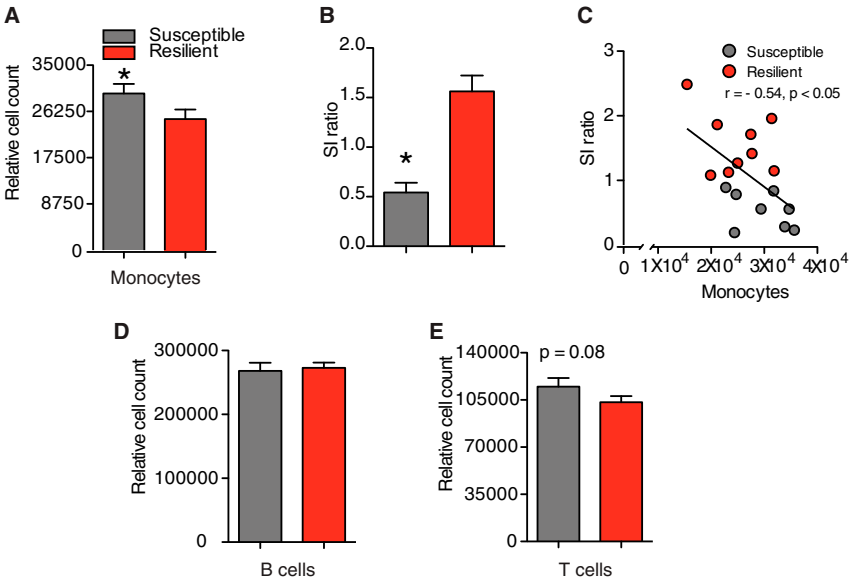

Fig. S5. Populations of leukocytes before RSDS. (*A*) Using flow cytometry in a separate cohort of animals, cell phenotype was examined before exposure to RSDS. There were more circulating monocytes in animals that later displayed a susceptible phenotype ($n = 8$) than animals that displayed resilience ($n = 9$) after RSDS ($t_{15} = 1.85$, $P < 0.05$, one tailed). (*B*) Social interaction scores for animals used to measure cell phenotypes before social defeat. Susceptible mice spent less time interacting with a novel aggressor than resilient mice ($t_{15} = 5.27$, $P < 0.0001$). (*C*) The number of monocytes before RSDS significantly correlated with the social interaction score after RSDS ($r = –0.54$, $P < 0.05$). (*D*) There was no significant difference in B-cell populations before RSDS ($t_{15} = 0.32$, $P > 0.05$, one tailed). (*E*) There was a modest trend for increased T cells before RSDS between animals that went on to demonstrate susceptible or resilient phenotypes 10 d later ($t_{15} = 1.47$, $P = 0.08$, one tailed). Lead shielding protected the brains of mice from irradiation. * denotes significant difference in means (*t* test). Circles denote individual animals (correlations).

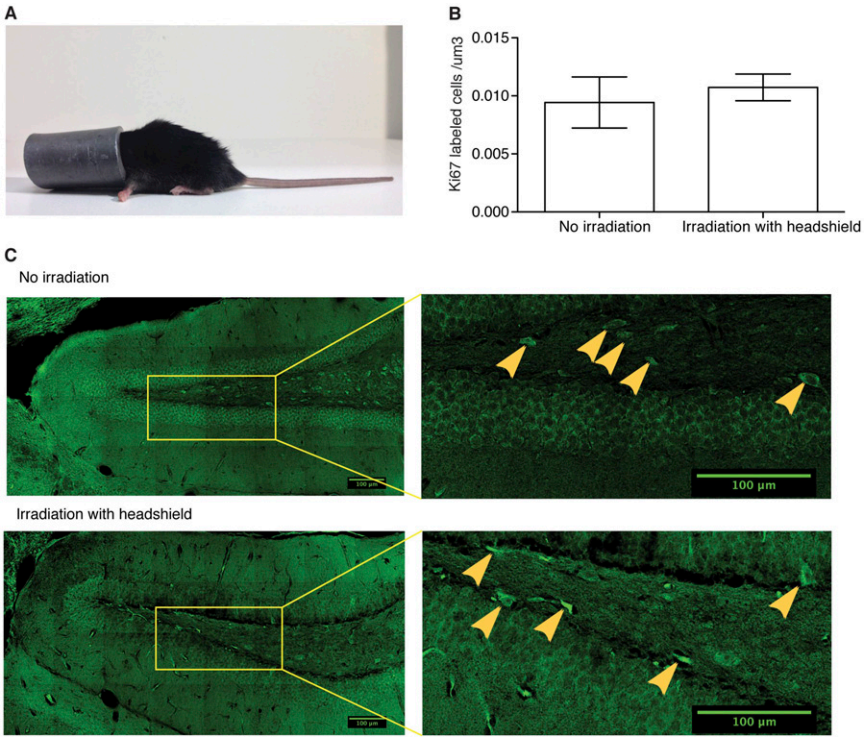

Fig. S6. Protection of brains by lead shielding. (*A*) Photograph of lead shield used to protect the brains of mice undergoing irradiation for BM transplants. (*B*) Mean density of Ki67+ cells in the dentate gyrus in animals exposed to irradiation while wearing protective lead head shields ($n = 4$) or not irradiated ($n = 4$). The mean density of Ki67-labeled cells was not significantly different between groups (P values > 0.05). (*C*) Photomicrographs demonstrating comparable density of Ki67-positive cells within the dentate gyrus among mice receiving no irradiation and mice receiving irradiation with head shielding. Graphs display mean ± SEM. Ki67-labeled cells are indicated by yellow arrows.

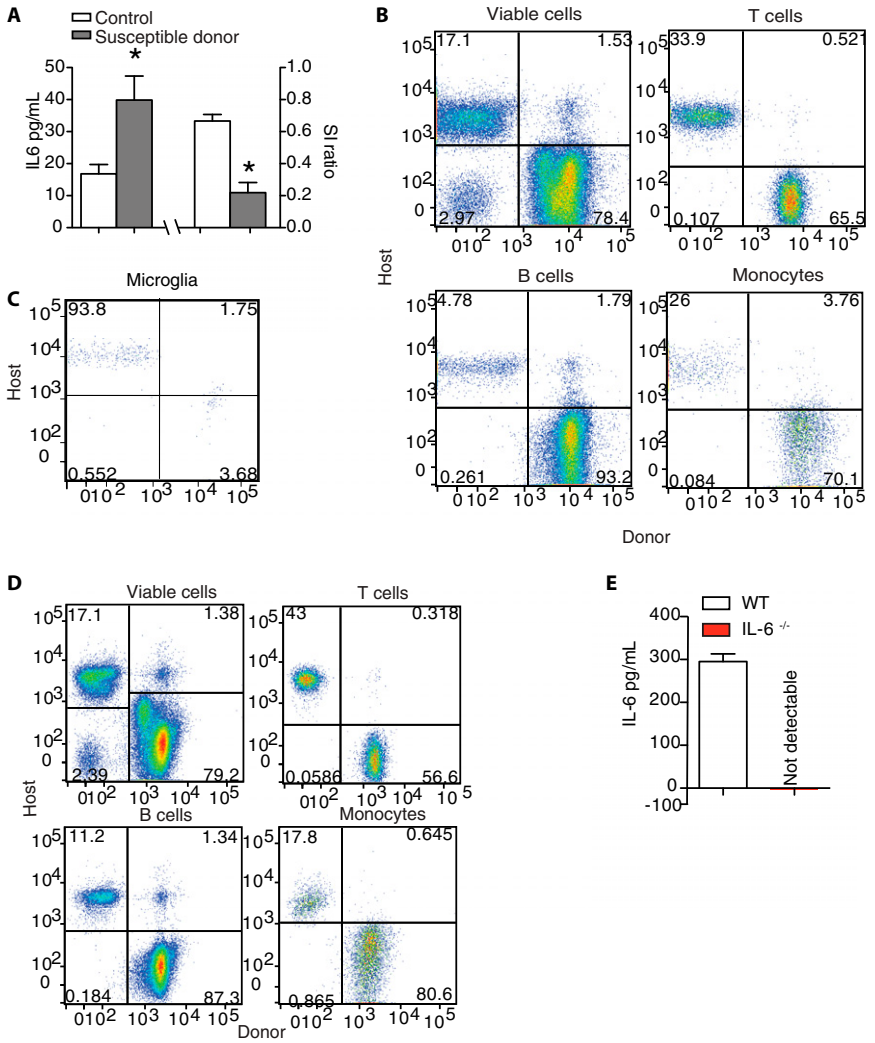

Fig. S7. Donor selection and flow cytometry plots of leukocytes after transplant. (*A*) Blood levels of IL-6 ($t_6 = 2.86$, $P < 0.05$, two tailed) and social avoidance behavior from the donors ($t_6 = 5.92$, $P < 0.05$, two tailed). (*B*) Representative images of flow cytometry gating for viable cells, T cells, B cells, and monocytes in animals labeled CD45.1+/CD 45.2– (donor) or CD45.2+/CD45.1– (host). (*C*) Representative image of flow cytometry gating for microglia collected from the brain of BM chimeras. (*D*) Representative gating of flow cytometry displaying the distribution of viable cells, T cells, B cells, and monocytes from donor and host mice. (*E*) IL-6 levels following LPS challenge of the leukocytes of the donors used for BM transplants. Donors were either high–IL-6 releasing WT ($n = 2$) or IL-$6^{-/-}$ ($n = 2$) mice; IL-6 was not detectable by ELISA in IL-$6^{-/-}$ mice. * denotes significant difference in means (*t* test).

Table S1. Demographics on the human participants used to measure circulating levels of IL-6

Cohort	Control	MDD	MDD/untreated	MDD/antidepressant
Cohort 1 *n*	18	19		
	12 f/6 m	12 f/7 m		
Age	32 ± 2.4	39 ± 2.6		
Comorbid disorders, number of subjects	Obesity, 1 Past cancer, 1 Skin condition, 1	Obesity, 2 Past cancer, 1 Anxiety disorder, 4 Bipolar disorder, 1 Chronic pain, 2 Thyroid condition, 1		
Concomitant psychotropic medication	0	8		
Cohort 2 *n*	50		21	21
	34 f/16 m		14 f/7 m	14 f/7 m
Age	42 ± 3		46 ± 1.5	46 ± 1.5
IL-6	0.2 ± 0.8		1.3 ± 0.3*	1.5 ± 0.3*
HAMD	NA		28 ± 1	7 ± 1*

For cohort 2, Dunnett's test indicated that MDD patients had higher levels of circulating IL-6 than healthy controls and that standard antidepressant treatment did not lower IL-6 levels in the same individuals ($F_{2,89} = 15.80$, $P < 0.0001$). Antidepressant treatment did significantly lower HAMD scores in the same individuals ($t_{20} = 15.58$, $P < 0.0001$). * denotes significant difference.

Table S2. Behavioral data examining anxiety-associated behaviors in susceptible BM chimeras, IL-6$^{-/-}$, IL-6$^{-/-}$ BM chimeras, and IL-6 mAb-treated mice

Group	Time in open arms	Time in closed arms	Test	*P* value
Control donor No stress	21.49 ± 6.2	234.6 ± 10.5	Open arms Interaction $F_{1,25} = 0.17$	NS
Susceptible donor No stress	19.38 ± 4.3	246.0 ± 9.1	Subthreshold defeat $F_{1,25} = 0.76$ Donor $F_{1,25} = 0.70$	NS NS
Control Subthreshold stress	21.85 ± 4.4	223.5 ± 10.8	Closed arms Interaction $F_{1,25} = 0.006$	NS
Susceptible donor stress	21.66 ± 4.4	236.3 ± 5.7	Subthreshold defeat $F_{1,25} = 1.22$ Donor $F_{1,25} = 1.66$	NS
IL-6$^{-/-}$	22.48 ± 4.3	223 ± 6.2	Open arms, t_{18} =1.83	NS
WT	13.12 ± 2.7	235.9 ± 6.5	Closed arms, $t_{18} = 1.4$	NS
IL-6$^{-/-}$ BMT	43.47 ± 4.3	187.6 ± 4.6	Open arms, $t_{12} = 0.43$	NS
WT BMT	46.84 ± 6.1	187.9 ± 5.2	Closed arms, $t_{12} = 0.03$	NS NS
IL-6 mAb	52 ± 4	209.4 ± 4	Open arm mAb, $F_{2,62} = 5.30$	< 0.01
IgG mAb	54.73 ± 5	204.6 ± 6	Closed arm mAb, $F_{2,62} = 0.27$	NS
Saline	35.97 ± 4*	207 ± 4		

Data for susceptible donor BMT were examined using bivariate ANOVA. IL-6$^{-/-}$ vs. WT or IL-6$^{-/-}$ BMT vs. WT BMT data were examined using *t* tests. IL-6 mAb data were analyzed using univariate ANOVA. * denotes a significant main effect of phenotype.