Negative Regulator of MAP Kinase is Increased in Depression and Is Necessary and Sufficient for Expression of Depressive Behavior

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Supplementary Materials

Supplementary Table 1. Case Demographics of matched control subjects.

| Age | Sex/race | Cause of death          | PMI | pH   | Toxicology   |
|-----|----------|-------------------------|-----|------|--------------|
| 37  | M/C      | hemorrhagic pancreatitis| 17  | 6.47 | ND           |
| 43  | M/C      | pulmonary thromboemboli| 23  | 6.49 | propoxyphene |
| 45* | F/AAm    | CVD                     | 9   | 6.86 | ND           |
| 46  | M/AAm    | CVD                     | 19  | 6.95 | ND           |
| 46  | F/C      | homicide                | 24  | 6.32 | ND           |
| 47**| M/C      | pulmonary embolism      | 25  | 6.1  | propoxyphene |
| 49  | F/AAm    | CVD                     | 29  | 6.57 | ND           |
| 50**| F/C      | CVD                     | 27  | 6.74 | ND           |
| 54**| M/AAm    | CVD                     | 19  | 6.52 | ND           |
| 56  | M/C      | hypertrophic cardiomyopathy| 25 | 6.14 | ND           |
| 66  | M/C      | CVD                     | 12  | 7.17 | ND           |
| 67  | F/AAm    | CVD                     | 28  | 6.4  | ND           |
| 69* | M/C      | aortic aneurysm         | 18  | 6.7  | ND           |
| 70  | M/C      | CVD                     | 20  | 6.81 | ND           |
| 77* | M/C      | CVD                     | 24  | 6.56 | ND           |
| 80  | F/C      | CVD                     | 21  | 6.78 | ND           |
| 82  | M/C      | aneurysm                | 16  | 6.72 | ND           |
| 84  | F/C      | CVD                     | 22  | 6.23 | ND           |
| DG Average | 61.1 | 20.5 | 6.61 |
| SD | 16.0 | 5.9  | 0.28 |
| SEM | 4.1  | 1.4  | 0.07 |
| CA1 Average | 58.5 | 21.8 | 6.56 |
| SD | 15.3 | 4.8  | 0.30 |
| SEM | 4.0  | 1.2  | 0.08 |

AAm, African American; C, Caucasian; CVD, cardiovascular disease; F, female; M, male; ND, no psychotropic medication detected, PMI, postmortem interval (hours); *, samples present only in array sets for the dentate gyrus; **, samples present only in array sets for CA1.
## Supplementary Table 2. Case Demographics for subjects with major depressive disorder (MDD).

| Age | Sex/Race | Cause of Death | PMI  | pH   | Toxicology                      | Mediations                      | Axis I Comorbidity               |
|-----|----------|----------------|------|------|---------------------------------|---------------------------------|---------------------------------|
| 30* | M/AAm    | suicide, SIGSW | 18   | 6.91 | ETOH                            | none                            | Hx alcohol abuse                |
| 34**| F/C      | suicide, CO    | 24   | 6.27 | ETOH, CO, alprazolam, valproic acid | none                            |                                |
| 38* | F/C      | suicide, OD    | 12   | 6.41 | diazepam, temazepam             | none                            |                                |
| 40***| F/C      | CVD, accidental OD | 25 | 6.32 | morphine, codeine, hydrocodone  | fluoxetine, temazepam, hydrocodone | sedative, hypnotic and anxiolytic disorder NOS |
| 42**| M/C      | suicide, SIGSW | 20   | 6.8  | ND                              | none                            |                                |
| 43**| M/C      | suicide, hanging | 21   | 6.73 | ND                              | none                            |                                |
| 46  | M/AAm    | homicide       | 17   | 6.26 | ND                              | none                            |                                |
| 47* | M/C      | suicide, SIGSW | 11   | 6.84 | ETOH                            | none                            |                                |
| 48  | M/C      | suicide, SIGSW | 21   | 6.9  | flurazepam, lorazepam           | flurazepam, lorazepam           | Hx alcohol abuse                |
| 54  | M/C      | suicide, CO    | 23   | 6.24 | CO, phenobarbital, phenytoin    | sertraline                      |                                |
| 62  | M/C      | suicide, SIGSW | 20   | 6.47 | ND                              | buspirone, lorazepam            | none                            |
| 63  | F/C      | CVD            | 18   | 6.3  | ND                              | fluoxetine                      | none                            |
| 67  | F/C      | aneurysm       | 17   | 6.98 | ND                              | doxapine                        | agoraphobia, Hx stroke          |
| 68  | M/C      | suicide, CO    | 4    | 6.21 | CO                              | none                            | Parkinson's disease            |
| 73* | M/C      | suicide, SIGSW | 18   | 6.59 | diazepam, codeine               | none                            |                                |
| 74**| M/C      | suicide, hanging | 24   | 6.96 | ND                              | nortriptyline, L-DOPA           | Parkinson's disease            |
| 77* | F/C      | CVD            | 32   | 6.79 | propoxyphene                    | trazodone, thioridazine, venlafaxine, clonazepam | Parkinson's disease            |
| 77* | M/C      | suicide, hanging | 26   | 6.74 | sertraline                      | sertraline                      | none                            |
| 78**| F/C      | suicide, fall from height | 25 | 6.94 | ND                              | none                            | pathological gambling, delusional disorder |
| 82  | M/C      | suicide, CO    | 12   | 6.46 | CO                              | risperidone, sertraline         | benzodiazepine abuse            |
| 87  | F/C      | aortic aneurysm | 24   | 6.56 | ND                              | flurazepam                      | none                            |

DG Average 61.3  18.2  6.56
SD 16.9  6.8  0.24
SEM 4.4  1.8  0.06
CA1 Average 59.2  19.7  6.54
SD 16.7  5.7  0.27
SEM 4.3  1.5  0.07

AAm, African American; C, Caucasian; CO, carbon monoxide; CVD, cardiovascular disease; ETOH, ethanol; F, female; Hx, history of alcohol abuse but not currently active; M, male; ND, no psychotropic medication detected; OD, drug overdose; PMI, postmortem interval (hours); SIGSW, self-inflicted gunshot wound; 1Psychotropic prescriptions within last month; 2MDD in remission; 3prescriptions for six days prior to death; *, samples present only in array sets for the dentate gyrus; **, samples present only in array sets for CA1.
**Supplementary Table 3.** Microarray-derived expression levels for 23 different dual specificity phosphatase (DUSP) genes in the dentate gyrus (DG) and CA1 hippocampal subregions of MDD subjects’ brains. Note that DUSP1 (MKP-1) is the only DUSP with significant upregulation in both DG and CA1.

| Gene   | Dentate gyrus | CA1          |
|--------|---------------|--------------|
|        | Fold change   | p-value (FDR)| Fold change | p-value (FDR) |
| DUSP1  | 2.27          | 0.038        | 2.42        | 0.004         |
| DUSP2  | **1.72**      | **0.044**    | 1.14        | 0.247         |
| DUSP3  | 1.24          | 0.202        | 1.10        | 0.288         |
| DUSP4  | 0.82          | 0.101        | 0.78        | 0.145         |
| DUSP5  | 1.32          | 0.256        | 0.96        | 0.377         |
| DUSP6  | 1.27          | 0.343        | 1.02        | 0.442         |
| DUSP7  | 0.76          | 0.176        | 0.79        | 0.092         |
| DUSP8  | 0.84          | 0.257        | 1.04        | 0.393         |
| DUSP9  | 1.17          | 0.363        | **1.40**    | **0.003**     |
| DUSP10 | 0.90          | 0.385        | 1.21        | 0.087         |
| DUSP11 | 1.06          | 0.470        | 1.00        | 0.459         |
| DUSP12 | **1.62**      | **0.059**    | **2.52**    | **0.0001**    |
| DUSP13 | na            | na           | 0.89        | 0.208         |
| DUSP14 | 0.88          | 0.393        | 0.97        | 0.427         |
| DUSP15 | 1.19          | 0.333        | 1.11        | 0.340         |
| DUSP16 | 1.03          | 0.499        | 1.22        | 0.196         |
| DUSP18 | 1.00          | 0.553        | 1.15        | 0.187         |
| DUSP19 | **1.25**      | **0.052**    | 0.86        | 0.163         |
| DUSP22 | 1.18          | 0.226        | 1.41        | 0.053         |
| DUSP23 | 1.10          | 0.388        | 0.85        | 0.080         |
| DUSP24 | 0.80          | 0.337        | **0.77**    | **0.022**     |
| DUSP26 | 1.41          | 0.176        | 1.01        | 0.447         |
Supplementary Table 4. Case Demographics for subjects with major depressive disorder (MDD) and matched controls – second cohort of postmortem samples used for in situ hybridization analysis.

| Control | Age | Sex/Race | Cause of Death | PMI | pH  | Toxicology | Medications  
|---------|-----|----------|----------------|-----|-----|------------|--------------|
|         | 54  | M/C      | Gastric Bypass Complications | 26.3 | 6.5 | Morphine | None |
|         | 32  | M/C      | Blunt trauma | 25  | 6.9 | ND | None |
|         | 44  | F/AAm    | CVD + Diabetes | 13.75 | 6.3 | ND | None |
|         | 51  | F/AAm    | CVD | 22  | 6.3 | ND | None |
|         | 44  | F/AAm    | CVD | 32  | 6.7 | ND | None |
|         | 50  | F/AAm    | CVD | 32.3 | 6.6 | ND | None |
|         | 65  | F/C      | CVD | 22  | 6.6 | ND | None |
| Average | 48.6|          |               | 24.8 | 6.5 |          |            |
| SD      | 10.2|          |               | 6.4  | 0.2 |          |            |
| SEM     | 3.9 |          |               | 2.4  | 0.1 |          |            |

| MDD | Age | Sex/Race | Cause of Death | PMI | pH  | Toxicology | Medications  
|-----|-----|----------|----------------|-----|-----|------------|--------------|
|     | 62  | M/AAm   | CVD | 22  | 6.1 | CLEAN | None |
|     | 37  | M/AAm   | SIGSW chest | 31.5 | 6.7 | ETOH | None |
|     | 65  | M/C     | SIGSW chest | 30  | 6.2 | Codeine | Unknown |
|     | 44  | F/C     | SIGSW head | 16  | 6.7 | ND | Bupropion, Effexor |
|     | 50  | F/C     | CVD | 28  | 6.5 | ND | Imipramine |
|     | 61  | F/C     | SIGSW head | 37.45 | 6.7 | ND | None |
|     | 38  | F/C     | OD Unknown substance | 24  | 6.5 | ND | Lorazepam, Citalopram |
| Average | 51.0|          |               | 27.0 | 6.5 |          |            |
| SD     | 11.8|          |               | 7.0  | 0.2 |          |            |
| SEM    | 4.5 |          |               | 2.6  | 0.1 |          |            |

AAm, African American; C, Caucasian; CVD, cardiovascular disease; ETOH, ethanol; F, female; M, male; ND, no psychotropic medication detected; OD, drug overdose; PMI, postmortem interval (hours); SIGSW, self-inflicted gunshot wound; ¹Psychotropic prescriptions within last month; ²MDD in partial remission.
**Supplementary Table 5.** *Gene promoter analysis.* Table illustrating CREB conserved promoter elements in MAPK pathway-related genes.

| Gene | CRE Site     | Homology |
|------|--------------|-----------|
| RAF1 | TGACcTCA     | 7 of 8    |
| MEK1 | TGAaGTCA     | 7 of 8    |
| MEK 2| TGACcTCA     | 7 of 8    |
| ERK1 | TGAaGTCA     | 7 of 8    |
| ERK2 | TGACcTCA     | 7 of 8    |
| MSK1 | TGACcTCA     | 7 of 8    |
| RSK  | little homology |          |
| CREB | TGAaGTCA     | 7 of 8    |
| CREBL1| TGAaGTCA   | 7 of 8    |
| CBP  | TGACcTCA     | 7 of 8    |
| VEGFa| TCAaGTCA     | 7 of 8    |
| VEGFc| TGACcTCA     | 7 of 8    |
| BDNF | TcACGTaA      | 6 of 8    |
| VGF  | TGACGTCA     | 8 of 8    |
| NPY  | TGACaTtA     | 6 of 8    |
**Supplementary Table 6. Daily schedule for the chronic unpredictable stress (CUS) paradigm in both rats (a) and mice (b).** Shown are the types, duration and a total number (#) of times that each stressor was applied during the entire timecourse.

**a**

| Type of Stressor and Duration | Occurrence During 35 Days |
|------------------------------|----------------------------|
| Cold 4C 1h                    | D: 2,8,14,16,26,33; (6)    |
| Swim Stress 18C 10 min        | D: 5,10,17,22,27,34; (6)   |
| Cage Rotation 1h              | D: 1,3,13,20,28,30,32; (7) |
| Isolation Overnight           | D: 4,6,13,18,21,31,33,35; (8) |
| Food/Water Deprivation        | D: 6,10,17,22,25,30; (6)   |
| Overnight                     |                            |
| Light ON Overnight            | D: 1,8,15,27,32,34; (6)    |
| Light OFF Overnight           | D: 2,11,18,23,28,31,35; (7) |
| Odor Overnight                | D: 7,12,20,24; (4)         |
| Stroboscope Overnight         | D: 7,11,19,26,29; (5)      |
| Wet Bedding Overnight         | D: 4,9,15,21,25,29; (6)    |
| Crowding Overnight            | D: 5,12,14,19,23; (5)      |
| Tilt Cage 45 deg. Overnight   | D: 3,9,16,24; (4)          |

**b**

| Type of Stressor and Duration | Occurrence During 35 Days |
|------------------------------|----------------------------|
| Cold 4C 1h                    | D: 1,7,14,20,25,33; (6)   |
| Swim Stress 18C 10 min        | D: 4,6,9,23; (4)           |
| Cage Rotation 1h              | D: 2,6,8,10,13,21,27,32; (8) |
| Food/Water Deprivation        | D: 1,9,15,19,27,31; (6)    |
| Overnight                     |                            |
| Light ON Overnight            | D: 4,6,8,11,13,17,18,21,28, 30,34; (11) |
| Light OFF Overnight           | D: 1,3,8,11,14,15,18,22,28; (9) |
| Odor Overnight                | D: 2,4,9,12,23,32; (6)     |
| Stroboscope Overnight         | D: 2,5,10,12,15,16,17,19,22, 26,29; (11) |
| Wet Bedding Overnight         | D: 5,11,14,24,26,31; (6)   |
| Restraint 1h                  | D: 3,7,10,13,18,24,29,33; (8) |
| Tilt Cage 45 deg. Overnight   | D: 3,5,7,12,16,17,20,25,30, 34; (10) |
Supplementary Figure 1.  *Influence of Mkp-1 over-expression on behavior in rodent models of depression.* a) Compared to rAAV-GFP controls, animals infused with rAAV-Mkp-1 displayed an increased immobility during a 15 minute forced swim test (FST). Data are shown as the time (s) spent immobile during each 5 min increment as well as 15 min total. b) Infusions of rAAV-Mkp-1 produced no effect on the locomotor activity. c) Rats infused with rAAV-Mkp-1 also spend less time in the open arm during elevated plus maze (EPM) test and displayed a significant increase in the number of entries into the closed arm. Results for all tests are expressed as mean ± S.E.M. (n = 6-9); *P < 0.05, #P < 0.10 compared to the rAAV-GFP control group (Student’s t-test).
Supplementary Figure 2. Baseline behaviors were evaluated in WT (\(Mkp-1^{+/+}\)) and \(Mkp-1\) null (\(Mkp-1^{-/-}\)) mice. a) Open field test showed no significant differences between the two genotypes in time spent or distance traveled in the outside vs. center zones. No differences were observed in (b) the forced swim test immobility times or (c) consumption of sucrose. d) Similar results for both genotypes were also obtained in the elevated plus maze test, determined by the number of enteries in open or closed arms, as well as time spent in the open arms.
Supplementary Figure 3. Open field test in mice exposed to CUS. *Mkp-1* null mice (*Mkp-1^−/−*) and WT littermates (*Mkp-1^{+/+}*; 11 different stressors; three daily stressors for the first 20 days, followed by two stressors per day for the last 15 days. Both groups performed similarly in the open field test, with no differences in time spent in the center vs outside zones or the overall distance traveled.
Supplementary Materials and Methods

**Human Subjects and Tissue Preparation.** Brain samples were collected at autopsy at the Cuyahoga County Coroner’s Office (Cleveland, OH). Informed written consent was obtained from the legal next-of-kin of all subjects. Tissues from 28 depressed subjects and 25 age-matched psychiatrically healthy control subjects were obtained at autopsy from the Coroner’s Office of Cuyahoga County, Cleveland, Ohio, USA. An ethical protocol approved by the Institutional Review Board of the University Hospitals of Cleveland was used, and informed written consent was obtained from the next-of-kin for all subjects. Blood and urine samples from all subjects were examined by the coroner’s office for psychotropic medications and substances of abuse. Two depressed subjects were co-morbid for Parkinson’s disease and another depressed subject had suffered a stroke. There was no evidence of a neurological disorder in any of the other subjects. Retrospective, informant-based psychiatric assessments were performed for all control and depressed subjects (Supplementary Tables 1–3). A trained interviewer administered the Schedule for Affective Disorders and Schizophrenia: lifetime version (SADS-L) to knowledgeable next-of-kin of 19 of the depressed subjects, as previously described. The Structured Clinical Interview for DSM-IV Psychiatric Disorders (SCID) was administered to next-of-kin of the nine remaining depressed subjects. Axis I psychopathology was assessed and consensus diagnosis was reached in conference using information from the interview and medical records. Responses from the 19 subjects evaluated with the SADS-L were also recorded in the SCID.

All depressed subjects met diagnostic criteria for MDD according to the Diagnostic and Statistical Manual of Mental Disorders IV (American Psychiatric Association, 1994) using information collected with either structured diagnostic interview. With the exception of two subjects, one in full remission and another in partial remission, all depressed subjects met criteria for a major depressive episode within the last two weeks of life. The depressed subjects consisted of 12 women and 16 men. The deaths of 20 of the 28 depressed subjects were ruled to be suicide by the coroner. Of the subjects with depression, 12 had a prescription for an antidepressant drug filled in the last month of life, however only sertraline was detected postmortem in one
depressed subject (Supplementary Table 2). The control subjects, consisting of 12 women and 13 men, never met criteria for an Axis I disorder at any time in their lives and were closely matched with the depressed subjects. For the microarray analysis and real-time PCR (qPCR), dentate gyrus (DG) and CA1 samples from fifteen pairs of subjects were matched for age (DG: ± 5.1 yr, CA1: ± 4.9 yr), gender, tissue pH, or postmortem interval (Supplementary Tables 1 and 2). There was an overlap in the brain pairs used for each region, although there were some differences due to availability of tissue samples. For in situ hybridization, a separate cohort of seven pairs of subjects was matched for age, gender, tissue pH and postmortem interval (Supplementary Table 3).

The hippocampal formation was dissected from the right temporal lobe at autopsy, frozen in dry ice-cooled isopentane, and stored at –80 °C. Tissue samples from age matched pairs of control and depressive subjects were coded throughout all histological procedures so that laboratory personnel were not aware of the psychiatric diagnoses assigned to the samples. Coded blocks of tissue from both cohorts were sectioned in an alternating manner to avoid a possible difference in histological treatment of tissue. For the microarray and qPCR studies, frozen tissues were sectioned on a cryostat at 60 µm in thickness. An average of 15 punches (2 mm in diameter, weighing a total of ~ 30 mg per region per subject) was collected from the granule cell layer of the dentate gyrus and the CA1 pyramidal cell layers from between 6 and 27 sections, depending on the profile size of the hippocampus. For in situ hybridization, three to four frozen sections were collected at 20 µm in thickness.

Microarray Analysis. Total RNA from the dentate gyrus granule and CA1 pyramidal cell layer punches was extracted using RNAqueous kit (Ambion, Austin, TX), followed by cleanup with RNeasy MinElute kit (Qiagen, Valencia, CA). Total RNA (RIN > 4.8, A260/280 > 1.9) from MDD and matched control human samples (n = 15) were reverse-transcribed into cDNA and indirectly labeled with highly sensitive fluorescent dendrimers (Genisphere, Hatfield, PA). Oligonucleotide (70mer) human whole genome expression MI Ready microarrays (Microarray, Inc., Huntsville, AL) were used to analyze changes in gene expression. These microarrays contain probes from the HEEBO (Human Exonic
Evidence Based Oligonucleotide) set. In a two-step process, array chips were initially hybridized to cDNA overnight, followed by a series of stringent washes to reduce nonspecific probe binding, and then post-stained with fluorescent Cy3 and Cy5 dendrimers. Following the post-hybridization washes, slides were scanned using a GenePix scanner (Axon Instruments, Union City, CA). Microarray image analysis was performed using GenePix Pro 6.0 (Axon Instruments) software.

Data from all 30 two-channel arrays were analyzed using R/Bioconductor. First, probes with more than three missing values out of 30 (10%) were dropped. Data were normalized using linear-Log normalization to stabilize the variance of low expressing genes, and then spatial plus intensity based LOWESS to remove spatial and intensity related biases, using the MAANOVA library. Array number eight from dentate gyrus set was dropped from further analysis because of a poorly conditioned MA plot. The resulting data matrix was then fit using a linear mixed effects model with array as a random effect and depression status and whether the subject had taken an antidepressant in the last month as fixed effects, followed by 1,000 permutation tests using a high performance computing cluster. P values were then adjusted to control false discovery rate (FDR) at 0.05 using the Q-value package. P values were calculated using distributions generated using permutation methods and standard error estimates were therefore not used in the P value calculations.

Quantitative Real-Time PCR (qPCR). Total RNA (500 ng) extracted from human hippocampal tissue was reverse-transcribed into cDNA in a 20 μL reactions using oligo-dT primers (Genisphere, Hatfield, PA). qPCR was performed utilizing a hot-start SYBR Green method with ABI 7900 instrument (Applied Biosystems, Foster City, CA) set for 40 cycles with the following parameters: 2 s @ 94 °C (denaturation), 30 s @ 60 °C (annealing), and 30 s @ 72 °C (elongation). Utilizing coding DNA sequence obtained from GenBank (National Center for Biotechnology Information), high-melt temperature forward and reverse primers for human MKP-1 and house-keeping genes were designed using Primer3 v. 0.4.0 software (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi; Whitehead Institute for Biomedical Research, Cambridge, MA). Specificity of primer sequences were additionally verified with nucleotide blast
software (BLAST Interface, NCBI). Quantitect SYBR Green PCR kit (Qiagen, Valencia, CA) was used in 16 µL reactions containing 1 µL of cDNA, and 1 µL of 5 pmol µL⁻¹ primer mixture, followed by melt-curve analysis to further verify specificity and well-to-well consistency of specific product generation. MKP-1 gene fold changes in MDD vs. controls were determined by utilizing ΔΔCt (Ct = cycle number at threshold) analytical method that includes normalization against house-keeping genes cyclophilin and GAPDH. Sequences of qPCR primers (forward and reverse) used are as follows: MKP-1, 5'-ccggtggaatctgccctttc-3' and 5'-ggttgagcaattggctgagc-3'; Cyclophilin, 5'-caaactcagatggacaggtgag-3' and 5'-gttgggtttgcgccctgcatttg-3'; GAPDH, 5'-cggaaactgtggcgtatgg-3' and 5'-gccagttagcctttccggt-3'.

In Situ Hybridization Analysis. In situ hybridization procedure was conducted according to standard protocols used in our laboratory. MKP-1 specific antisense cRNA probes were generated by an in vitro transcription reaction using PCR product-derived template and MAXIscript kit T7 polymerase (Ambion, Austin, TX). For generation of the probe template, the following primers (forward and reverse) were used in PCR reaction:

Rat, 5'-cttcccgggagatgctgac-3';
Rat, 5'-CAAGCCCTTCTAATACGACTCCTATAGGGGAGACtgagtaagcaaaggcatgg-3';
Human, 5'-ctgagtactagcctccctgacagc-3';
Human, 5'-CCAAGCCTTCTAATACGACTCCTATAGGGGAGAcaaaccccttcctcagc-3'.

Reverse primer contained a T7-binding promoter (sequence shown in capital letters). MKP-1 specificity of the PCR product was verified by sequencing. Probes were radio-labeled with ³⁵S-rCTP and purified through a NucAway spin column (Ambion, Austin, TX). Frozen cryostat cut brain coronal sections (16 µm), were mounted on Probe-On Plus slides (Fisher Scientific Co., St. Louis, MO) and post-fixed in 4% paraformaldehyde for 10 min. Following a 10 min wash with 50 mM phosphate buffered saline (PBS), slides were incubated in 0.1 M triethanolamine (TEA), pH 8.0, with 0.25% acetic anhydride (v/v) for 10 min with stirring. Slides then washed twice with 2x saline-sodium citrate (SSC) for 2 min and dehydrated briefly with 30%, 50% and
100% ethanol. Two million counts (per slide) of MKP-1 specific probes in hybridization buffer [50% formamide (v/v), 3X SSC, 50 mM NaPO₄, 10 mM (dithiothreitol) DTT, 1X Denhart’s, 0.25 g L⁻¹ tRNA, 10% dextran SO₄] were applied to the sections and allowed to hybridize at 55 °C overnight in a humidifying chamber. The following day, slides were washed in 2X SSC for 10 min and treated with RNase solution (0.5 M NaCl, 10 mM Tris-Cl pH 8.0, 1 mM EDTA pH 8.0, 20 mg mL⁻¹ RNase A, 1 U mL⁻¹ RNase T1) for 30 min at room temperature. Slides were then transferred to 2X SSC for 10 min, followed by 20 min incubations in 0.2X SSC (with 5mM DTT) and 0.1X SSC (with 5mM DTT) at 55 °C. Following a rinse in dH₂O and 100% ethanol, slides were air dried for several hours, before being exposed to Kodak MR autoradiographic film. Images were captured using a computer-controlled digital camera (Cohu, Poway, CA) and imported into Image J (Scion Corp, Frederick, MD) for densitometric analysis. Using the manufacturer's calibration scale, raw densitometry data were converted to nCi of ¹⁴C per gram of tissue, which are linearly related to the tissue levels of the specific mRNA.

**Western Blot Analysis.** Fresh mouse hippocampal tissue was initially homogenized in lysis buffer (25 mM HEPES, 300 mM NaCl pH 7.4, 2% Triton-100) containing a cocktail of proteinase inhibitors (Roche, Indianapolis, IN) and phosphatase inhibitors (10 mM NaF, 1 mM NaVO₃). Tissue protein concentrations were determined using the BCA protein assay kit (Pierce, Rockford, IL). Protein samples were electrophoretically separated on an SDS-PAGE gel (10% Tris-HCl; Bio-Rad, Hercules, CA) and transferred to polyvinylidene difluoride membranes (0.2 µm pores; Millipore, Bedford, MA). The membranes were blocked with 5% bovine serum albumin (BSA) in Tris-buffered saline solution containing 0.1% Tween 20 (TBST) for 2 h at room temperature (RT). Membranes were then incubated overnight at 4 °C with anti-pErk (detects Erk1/2 MAPKs phosphorylated at Thr²⁰²/Tyr²⁰⁴, 1:1,200; Cell Signaling Technology #9106, Beverly, MA) primary antibodies. All antibodies were diluted in 1% BSA/ TBST buffer solution. After washing in TBST, the blots were incubated with horseradish peroxidase (HRP)-conjugated secondary antibody for 2 h at RT (1:10,000; Vector Laboratories, Burlingame, CA). Following the rinse in TBST, the blots were developed using enhanced chemilluminescence kit (Perkin Elmer, Wellesley, MA) for 1 min and exposed
onto Kodak MR autoradiographic film. To obtain loading controls, the blots were incubated in stripping buffer (25 mM glycine pH 2.0, 2% SDS) for 2 h at 37 °C and reprobed with antibodies recognizing total Erk (1:1,500; Cell Signaling Technology #4695) proteins. Additionally, as a secondary loading control, membranes were also reprobed with antibodies recognizing β-actin (1:1,500; Cell Signaling Technology #4970). Autoradiographic images were captured using a computer-controlled digital camera (Cohu, Poway, CA) and imported into Image J (Scion Corp, Frederick, MD) for quantitative analysis.

**Immunohistochemistry.** Rat brains were initially perfused with cold phosphate buffered saline (PBS), extracted and post-fixed in 4% paraformaldehyde at 4 °C for seven days. Agarose (2%) imbedded brains were cut (60 µm coronal sections) using vibrating blade microtome (Leica VT1000s, Wetzlar, Germany) and stored in PBS at 4°C. Sections were washed in 0.02M potassium phosphate buffered saline (KPBS), incubated in blocking solution [5% normal goat serum (NGS) in KPBS-T (0.02M KPBS, 0.1% Triton-X 100)] for 2 h at room temperature, followed by overnight incubation at 4°C in chicken anti-green fluorescent protein (Gfp) primary antibody (1:200, Aves Labs, Inc. Gfp-1020, Tigard, OR) diluted in 1% NGS/ KPBS-T buffer solution. After washing in KPBS, sections were incubated in AlexaFluor 488 goat anti-chicken secondary antibody (1:200, Invitrogen A-11039, Carlsbad, CA) for 2 h at room temperature. Sections were then washed in KPBS, dried and mounted with Gel/ mount aqueous mounting medium with anti-fading agents (Biomeda Corp., Foster City, CA). Section images were captured using Olympus Fluoview FV1000 confocal microscope (Oympus Corporation, Tokyo, Japan) and Zeiss Axioskop 2 fluorescent microscope and AxioVision 3.1 software (Carl Zeiss Imaging Solutions GmbH, Gottingen, Germany).

**Construction, Preparation and Infusion of Recombinant AAV.** The rat Mkp-1 cDNA was amplified from rat hippocampal cDNA library and subcloned into an AAV2 backbone, containing two CMV promoters to independently drive the expression of target protein (Mkp-1) and EGFP. The same backbone carrying no Mkp-1 cDNA was used as a control (rAAV-control). Recombinant AAV 2/1 pseudo-typed viruses were
generated as previously described \(^5\). Briefly, human embryonic kidney 293 (HEK293) cells were transfected with the AAV cis plasmid, and pDp1 and pDp2 helper plasmids using transfection reagent PerFectin (Genlantis, San Diego, CA). Sixty hours post-transfection cells were harvested and lysed (three freeze-thaw cycles in dry ice/ ethanol bath). Subsequently, viruses were purified using HiTrap heparine HP affinity columns (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). Viral titers were determined in rat cortical primary cultures. All infusion surgeries were performed under aseptic conditions and anesthesia; xylazine \([6 \text{ mg kg}^{-1}, \text{intramuscular (i.m.)}, \text{Lloyd laboratories, Shenandoar, IA)}\) and ketamine \([80 \text{ mg kg}^{-1} \text{i.m., Fort Dodge Animal Health, Overland Park, KS)}\). Virus was delivered into the dorsal hippocampus by bilateral infusions at \(-4.3 \text{ mm (anterior-posterior)}, -3.0 \text{ mm (lateral)}, \) and \(-4.7 \text{ mm (dorsal-ventral)}\) relative to the Bregma \(^6\). Each hippocampal hemisphere was infused with a total of 1.5 \(\mu\text{l}\) of purified virus over a 15 min period followed by 2 min of rest. Behavioral test were performed four weeks after virus infusion.

**Chronic Unpredictable Stress and Behavioral Testing.** Male Sprague-Dawley rats (Charles River, Wilmington, MA) and wild-type \((Mkp-1^+/+)\) and homozygotic \((Mkp-1^{−/−})\) mice (kindly provided by Bristol-Myers-Squibb, bred at Yale University) were housed in groups of 2–4 per cage under a 12 h light/ dark cycle at constant temperature (25 °C) and humidity with *ad libitum* access to food and water (except when indicated). Prior to any treatments or experiments, animals were allowed at least one week of habituation to the housing conditions. All animals were age and weight matched (rats: 250–300 g, mice: 29–33 g) at the time of the first stressor. The maintenance of rat and mouse colonies and all animal treatments and procedures were in accordance with NIH laboratory care standards and approved by the Yale University Care and Use of laboratory animals (YACUC) guidelines.

CUS is a rodent model of depression where animals are exposed to sequence of mild and unpredictable stressors designed to prevent habituation \(^7\). For rat CUS animals were subjected to the sequence of 12 different stressors (two per day for 35 consecutive days) as previously described \(^8,9\) (see Supplementary Table 5). In order to simulate a realistic course of antidepressant intervention, CUS rats were administered
fluoxetine [FLX; 5 mg kg\textsuperscript{-1}, intraperitoneal (i.p.); Eli Lilly, Indianapolis, IN] daily for 21 days with continued CUS \textsuperscript{9}. Active avoidance test (AAT) was administered on day 28, while the sucrose preference test (SPT) was conducted on day 35 of the CUS paradigm. Rats were also tested for locomotor activity on days 15, 28 and 35. For the mouse studies, animals were initially exposed to three stressors per day for the first 20 days, followed by 12 days of two stressors per day. The sucrose (1\%) consumption test (SCT) was administered on days 0 (baseline), 17 and 30. Water consumption was tested on day 20, open field test (OFT) on day 32 and elevated plus maze (EPM) on day 33.

**Behavioral Testing.** During the active avoidance test rats were initially habituated for five min to shuttle boxes (Med Associates), and then subjected to 30 randomized escapable footshocks at an intensity of 0.65 mA \textsuperscript{8}. In shock trial, the gate separating the two halves of the shuttle box opened five sec prior to shock onset and remained open for the duration of the shock. The average inter-trial interval was 60 s (range 20–100 s). The first five trials required one crossing to terminate footshock (FR1) and the remaining 25 trials required two crossings (FR2). Results are expressed as the number of escapes failures, or the number of times that the animal did not terminate the footshock by making the appropriate crosses.

For the sucrose preference test (SPT) rats were first exposed to palatable 1\% or 2\% sucrose solution (Sigma, St Louis, MO) for 48 h on day 33 of CUS. On day 35, SPT was conducted as animals were initially deprived of water for 4 h and then exposed for 1 h to two identical bottles filled with either sucrose solution or water. Total consumption of each fluid was measured and the sucrose preference was defined as the ratio of the volume of sucrose versus water consumed during the 1 h test \textsuperscript{8,9}. Sucrose consumption was used for mice because of difficulty achieving preference in this species. Mice were habituated to 1\% sucrose solution (Sigma) for 48 h prior to testing days 0, 17 and 30. Following overnight fluid deprivation, animals were exposed to sucrose solution for 1 h and the total volume consumed was recorded. Results are expressed in milliliters of sucrose consumed during a 1 h test period.
For novelty suppressed feeding (NSF) animals were initially food deprived over-night (12 h). On the test day animals were placed in an open field box (76.5 cm x 76.5 cm x 40 cm, Plexiglas) with eight pellets of food in the center. The latency to approach the food and take the first bite was recorded (in seconds), with a cutoff maximum set at 15 min. Subsequently, food consumption in the home cage was quantified as a positive control.

The open field test and elevated plus maze were conducted as previously described \(^{10}\) in both non-stressed animals (baseline) as well as at the end of the CUS paradigm on days 32 and 33, respectively. During open field test mice were the placed in the center of a plexiglas box (50 cm x 50 cm x 40.5 cm) in a brightly lit room. During a 10 min session, animals were scored for the time spent and distance traveled in the center of the box (25 cm\(^2\) area) vs. the outside zone (the rest of the box area). Mouse behavior was recorded and subsequently analyzed using EthoVision pro video tracking system and software (Noldus Inc., Leesburg, VA).

For the forced swim test, mice were placed in 19 cm diameter glass cylinder filled to 10 cm with 23–25 °C water and their behavior was videotaped. During analysis of the recordings, immobility was defined as the absence of all movement except motions required to maintain the animal’s head above the water. Results are expressed as time (in seconds) that animals spent immobile during a 10 min session. Each session was further divided into early (0–5 min) and late (6–10 min and 11–15 min) phases.

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