Methylglyoxal modification of TrkB promotes synaptic plasticity and enhances resilience to chronic stress

Ziyin Wu[1,2,3]†, Yingxue Fu[2,3]†, Yinfeng Yang[3,4]†, Chao Huang[2,3]†, Chunli Zheng[1,3]†, Zihu Guo[1,3]†, Zihao Yang[6], Xuetong Chen[2,3], Jinglin Zhu[1,3], Jinghui Wang[4], Xiaogang Li[1,3], Liyang Chen[2,3], Weiwei Zhou[3,5], Yangyang Chen[2,3], Jiangmei Wang[2,3], Yang Yang[3,5], Meng Jiang[1,3], Sushing Chen[7], Aiping Lu[8], Jianlin Liu[1], Yan Li[4], Shiguo Sun[6], Zhenzhong Wang[3], Wei Xiao[3]*, Yonghua Wang[1,3]*

[1]Lab of Systems Pharmacology, College of Life Sciences, Northwest University, Xi'an, Shaanxi 710069, China.

[2]Center of Bioinformatics, College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China.

[3]State Key Laboratory of New-tech for Chinese Medicine Pharmaceutical Process, Lianyungang, Jiangsu 222001, China.

[4]Key Laboratory of Industrial Ecology and Environmental Engineering (MOE), Department of Materials Sciences and Chemical Engineering, Dalian University of Technology, Dalian, Liaoning 116024, China.

[5]Pharmacology department, School of Pharmacy, Shihezi University, Shihezi 832002, China.

[6]Shaanxi Key Laboratory of Natural Products & Chemical Biology, College of Chemistry & Pharmacy, Northwest A&F University, Yangling, Shaanxi 712100, China.

[7]Systems Biology Laboratory, Department of Computer Information Science and Engineering, University of Florida, Gainesville, Florida, United States of America.

[8]School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong.

†These authors contributed equally to this work.

*Correspondence should be addressed to Yonghua Wang (yhwang@nwu.edu.cn) and Wei Xiao (xw_kanion@163.com).

Abstract

The endogenous metabolite methylglyoxal (MGO) has recently emerged as a potential mediator of psychiatric disorders, such as anxiety and depression, but its precise mechanism of action remains poorly understood. Here, we find that MGO concentrations are decreased in the prefrontal cortex and hippocampus in rats subjected to chronic stress, and low-dose MGO treatment remarkably enhances resilience to stress and alleviates depression-like symptoms. This effect is achieved by MGO's promotion on the synaptic plasticity in prefrontal cortex and hippocampus. Both *in vitro* and *in vivo* experiments show that MGO provokes the dimerization and autophosphorylation of TrkB and the subsequent activation of downstream Akt/CREB signaling, which leads to a rapid and sustained expression of brain-derived neurotrophic factor (BDNF). We further demonstrate that MGO directly binds to the extracellular domain of TrkB, but not its intracellular domain. In addition, we also identify a natural product luteolin and its derivative lutD as potent inhibitors of Glyoxalase 1 and validate their antidepressant effects in chronic stress rat models. The antidepressant role of endogenous MGO provides a new basis for the understanding and therapeutic intervention design for stress-associated mental disorders.

Introduction

Major depression and anxiety disorders are highly prevalent mental illness characterized by profound disturbances in emotional regulation of affected individuals [1]. Chronic stress is a trigger for depression, and defects in the normal adaptivity to chronic stress can increase the risk of developing depression and anxiety disorder [2]. The ability to cope with stressful situations, resilience, depending on the development of adequate behavioral and psychological adaptations to chronic stress, plays an essential role in modulating the development of and recovery from depression [3,4]. In view of the association between stress resilience and depression, identification of the neural substrates underlying stress resilience is critical for the development of therapeutic strategies for stress-related psychiatric diseases [5,6].

Methylglyoxal (MGO) is an endogenous metabolite mainly generated in the glycolysis process [7]. It is a highly reactive dicarbonyl aldehyde, which can react with protein arginine and/or lysine residues to form advanced glycation end-products (AGEs), and cause non-enzymatic, post-translational modifications (PTMs) of proteins [8,9]. High concentrations of MGO exert cytotoxic effects via evoking the production of reactive oxygen species [10], and have been implicated in pathologies of several diseases including diabetes, aging and neurodegenerative diseases [11-13]. Nevertheless, under normal conditions, MGO can be efficiently detoxified by the glyoxalase system, mainly through Glyoxalase 1 (GLO1), an enzyme catalyzing the conversion of acyclic α-oxoaldehydes to corresponding α-hydroxyacids [14]. Recently, MGO and GLO1 has been identified associated with anxiety- and depression-like behaviors in mice [15,16]. This suggests that MGO may play a potential role in the pathophysiology of depression. However, the cellular function of MGO at physiological concentration and its precise mechanism of action in depression still remain poorly understood.

In this study, we hypothesize that MGO could promote resilience to chronic stress. To test this, we constructed different stress models, and found that MGO levels are remarkably decreased in the prefrontal cortex (PFC), hippocampus (HC) and also plasma in rats subjected to chronic stress, whereas low-dose MGO treatment remarkably enhances resilience to stress and alleviates depression-like symptoms. This effect is achieved by MGO's direct binding to the extracellular domain (ECD) of TrkB to stimulate its dimerization and autophosphorylation. This enables MGO to switch on the TrkB signaling pathways, which leads to a rapid and sustained expression of BDNF. MGO also effectively promotes the hippocampal neurogenesis in CMS rats. In addition, the modulation on MGO concentration by knockdown of GLO1 also exhibits antidepressant-like effects. The antidepressant role of endogenous MGO thus provides a new basis for the design of therapeutic interventions for major depressive disorder.

Results

MGO increases resilience to chronic stress and restores neurotransmitter levels.

To investigate whether chronic stress leads to changes in MGO concentrations in the brain, we constructed a chronic mild stress (CMS) rat model. The CMS model mimics several psychopathological dimensions of depression [17], and adult SD rats that were exposed to the CMS regime exhibited obviously decreased sucrose preference and increased immobility time in the forced swim test (FST) (**Fig. 1E and 1F**). Notably, the concentrations of MGO were lower in the PFC and HC, two critical regions for stress response [18,19], of CMS rats when compared with control rats (**Fig. 1A**), suggesting an MGO deficiency in the brains of rats after exposure to CMS. Accordingly, the plasma MGO levels also dropped remarkedly in CMS rats compared to vehicle (**Fig. 1A**). To determine whether reduced concentrations of MGO in CMS rats are associated with GLO1 activity, we employed quantitative real-time reverse transcription PCR (qRT-PCR) and immunoblot analysis. We found that both GLO1 transcription and expression are upregulated in the PFC and HC of CMS rats (**Fig. 1B** and **1C**). Together, these results suggest that reduced MGO levels in the brain underlie chronic stress-induced depressive behaviors.

To test whether MGO could induce an antidepressant-like effect, we employed the FST and elevated plus-maze (EPM) test. The treatment of low-dose MGO (5 mM/kg per day) for 3 weeks significantly reduced the immobility time of CMS rats in the FST (**Fig. 1E**) and robustly increased their time in the open arms of the EPM (**Fig. 1D** and **Supplementary Fig. S1B**), indicating that MGO could have antidepressant-like effects. Of note, we observed that a 7-d treatment with MGO was sufficient to remarkably increase the sucrose preference in rats with depressive behaviors (**Fig. 1F**). In contrast, a 3-week treatment with the typical antidepressant fluoxetine was necessary to increase the sucrose preference of CMS rats to the baseline levels, and a 1-week treatment with fluoxetine was ineffective. These results suggest that MGO may be a faster-acting antidepressant (**Fig. 1F**). Consistent with its antidepressant activity, MGO also greatly increased the levels of monoamine neurotransmitters such as serotonin and dopamine in both PFC and HC regions of CMS rats (**Fig. 1G**).

To further investigate whether MGO also promotes stress resilience in mice with depressive-like behavior, we employed another widely used stress model: the chronic social defeat stress (CSDS) paradigm (**Supplementary Fig. S1D**). Adult C57BL/6J mice that were subjected to CSDS were separated into susceptible and resilient subpopulations (**Supplementary Fig. S1E**). A physiological concentration of MGO (10 µM, lateral intracerebroventricular (i.c.v.) infusion) decreased the total duration of immobility in adult C57BL6/J mice. Notably, a preinfusion of aminoguanidine, a scavenger of free MGO, blocked MGO's antidepressant-like effects (**Fig. 1H** and **1I**). We then considered whether MGO could reverse CSDS-induced social avoidance, which is a model of stress-induced psychopathology in humans [20,21]. After a 10-d CSDS protocol, the mice that were treated with vehicle exhibited an approximately 70% reduction in time spent in the interaction zone. In contrast, a 7-d treatment with MGO reversed the defeat-related behaviors in mice after CSDS (**Fig. 1H**), suggesting that MGO promotes resilience to chronic stress in adult mice.

MGO promotes the synaptic plasticity in PFC and HC of CMS rats.

To characterize the neurobiological mechanisms that underlie the antidepressant-like effects of MGO, we performed RNA-seq transcriptome profiling of the PFC and HC regions of three groups of rats, i.e., normal control rats and CMS rats treated with either saline or 5 mM/kg MGO for 3 weeks. Differential expression analysis showed that 289 genes in PFC and 278 genes in HC were specifically up- or downregulated in the MGO treatment group compared to the saline group (**Fig. 2A**), of which 63 genes are overlapped between these two regions (**Fig. 2A**). Notably, we found that MGO reversed the expression levels of 25.5% and 47.3% of the misregulated genes in PFC and HC of CMS rats to normal state. Among the top dysregulated genes were those encoding signaling molecules that are essential for differentiation and maintenance of adult neural stem cells in hippocampus, such as adenomatous polyposis coli (*Apc*) and BTG anti-proliferation factor 1 (*Btg1*); and important synaptic proteins such as the synaptotagmin (SYT) family, *Syt2*, *Syt11*, *Syt13,* and the disintegrin and metalloproteinase domain-containing protein 22 (*Adam22*) involved in neurogenesis (**Fig. 2A**). Gene ontology (GO) analysis of these differentially expressed genes revealed an enrichment of neuron-specific GO cellular component terms in both PFC and HC regions, including axon, dendritic spine, synapse, and also biological process terms such as calmodulin binding and calcium-mediated signaling, which are crucial for neurotransmitter release and neuro projection (**Fig. 2B**). As disruption of synapse plasticity is a major phenomenon in depression patients [22], these results suggest that MGO's antidepressant effect is associated with the increased synaptic plasticity in PFC and HC of CMS rats.

To validate the RNA-seq results, we focused on the genes associated with synaptic plasticity and neurogenesis, using qRT-PCR and immunofluorescence analyses (**Fig. 2D** and **Supplementary Fig. 1F**). The results showed that MGO's regulation on the expression of these genes is in accordance with RNA-seq results. Of note, gamma-aminobutyric acid type A receptor related genes (*Gabra1*, *Gabarapl2*) in HC, which were previously implicated in anxious and depression [15,16] were upregulated by MGO compared to CMS group. 5-hydroxytryptamine receptor 2A (*Htr2a*), mutations of which are associated with response to the antidepressant citalopram in MDD patients [23], is downregulated by MGO in the PFC region. In addition, MGO also significantly up-regulated the mRNA expression levels of *Bdnf* and *Adam22* that are involved in the positive regulation of neurogenesis (**Fig. 2D**). To test whether MGO modulates hippocampal neurogenesis *in vivo*, we carried out immunofluorescence analysis on the HC slices of CMS rats treated with MGO. The density of both BrdU+ new born neurons and BrdU+/NeuN+ neurons was decreased in the hippocampal dentate gyrus (DG) of CMS rats (**Supplementary Fig. 1F**). Whereas ip administration of MGO significantly increased the proportion of these cells (**Supplementary Fig. 1F**), indicating that MGO is capable of promoting hippocampal neurogenesis in CMS rats. Together these data suggest that MGO promotes the synaptic plasticity in PFC and hippocampal neurogenesis in CMS rats, and thus presents antidepressant-like effects.

MGO activates TrkB signaling pathways and induces the expression of BDNF in PFC and HC of CMS rats.

The neurotrophic factor BDNF and its receptor TrkB play an important role in regulating synaptic plasticity and hippocampal neurogenesis [24,25]. We thus examined whether MGO's antidepressant effects is associated with the BDNF/TrkB signaling pathways in the brains of CMS rats. We found that the phosphorylation levels of two TrkB phosphorylation sites (Y515 and Y706) were significantly upregulated

157   in MGO treatment group compared to the saline group. Accordingly, we also observed increased levels
158   of downstream p-Akt, p-ERK1/2 and p-CREB in both HC and PFC areas of MGO-treated CMS rats (**Fig.
159   3A** and **Supplementary Fig. S2A**), which are important signals that regulate the neuronal survival and
160   local axon growth [26]. More importantly, MGO significantly increased the expression and release of BDNF
161   in both of these brain areas (**Fig. 3A** and **Supplementary Fig. S2A**). To further validate MGO's
162   activation effects on TrkB signaling pathways *in vivo*, we adopted a single acute treatment of MGO (50
163   mM/kg) to CMS rats. The results demonstrated that MGO activates the TrkB signaling pathways in a
164   time-dependent manner and maintains a persistent activation state in 9 h in both PFC and HC regions
165   (**Fig. 3A and Supplementary Fig. S2B**). Together, these results demonstrate that MGO activates the
166   BDNF/TrkB signaling pathways in both PFC and HC areas of CMS rats.

167   We then examined the concentration dependence and time course of MGO-mediated activation of
168   TrkB signaling pathways in cultured PC12 cell lines *in vitro*. Incubation of MGO with PC12 cells for 1 h
169   produced a concentration-dependent activation of the TrkB signaling pathway (**Supplementary Fig.
170   S3A**). Consistently, MGO also increased the expression of BDNF mRNA in a dose-dependent manner
171   (**Supplementary Fig. S3D**). Furthermore, incubation of MGO at 250 µM for 15 or 30 min both robustly
172   increased the levels of p-TrkB, p-Akt, p-ERK1/2 and p-CREB (**Supplementary Fig. S3B**), which
173   demonstrates that MGO provokes the activation of BDNF/TrkB signaling in a fast way. Importantly, the
174   nuclear entry of p-CREB was also significantly promoted by 250 µM MGO (**Fig. 4C**) and the mRNA
175   levels of BDNF robustly increased at 3 h, peaked at 12 h and was detectable until 24 h, which is
176   consistent to its changes at the protein level (**Supplementary Fig. S3C and S3D**). Besides, the
177   phosphorylation of TrkB and the downstream p-ERK1/2 and p-CREB stimulated by 250 µM MGO can
178   last up to 12 h (**Supplementary Fig. S3C**). This long-lasting BDNF/TrkB signaling induced by MGO may
179   enhance the synaptic transmission in adult hippocampus, and thus lead to antidepressant-like effects
180   [27,28].

181   To investigate whether modulation of endogenous MGO levels could also regulate TrkB signaling
182   pathways, two independent, non-overlapping short hairpin RNA (shRNA) lentiviral constructs were
183   designed to knock down GLO1 in PC12 cells (**Supplementary Table S1**). The levels of GLO1 protein
184   and its mRNA were significantly down-regulated, along with a significant increase in MGO concentrations
185   in both of the shRNA groups (**Figs. 3C, 3D** and **Supplementary Figs. S2C**). We then tested whether this
186   knockdown impacts the signaling transduction of BDNF/TrkB pathway. The results show that the
187   expression levels of BDNF protein and its mRNA were both obviously enhanced (**Figs. 3*C* and 3D**).
188   Correspondingly, silencing of GLO1 increased the levels of p-TrkB, p-Akt, p-ERK1/2 and p-CREB (**Fig.
189   3E**), indicating the activation of Akt and ERK signaling pathways. Additionally, the pharmacological
190   inhibition of GLO1 by 10 µM S-p-bromobenzylglutathione cyclopentyl diester (BBGC) in WT PC12 cells
191   also resulted in significantly increased MGO levels and enhanced TrkB signaling (**Supplementary Figs.
192   S3E and 3F***). Reciprocally, the overexpression of GLO1 in PC12 cells significantly increased both the
193   protein and mRNA levels of GLO1, along with decreased MGO levels (**Supplementary Figs. S2C, S2D
194   and *S2E***). And this led to a reduction of p-TrkB, p-Akt, p-ERK1/2 and p-CREB levels, as well as the
195   protein and mRNA levels of BDNF (**Supplementary Figs. S2D*, S2*E and *S2*F**), indicating that GLO1
196   overexpression causes functional inhibition of BDNF/TrkB signaling. To further demonstrate that
197   modulation of endogenous MGO could have antidepressant-like effects *in vivo*, we utilized the adeno-

198 associated viral (AAV) vector-mediated knockdown of GLO1 in the HC of CMS rats (**Fig. 3F** and **G**). The
199 results showed that GLO1 knockdown CMS rats exhibited a significantly decreased immobility time in
200 FST (**Figs. 3H**) and increased entries into open arms in EPM compared to CMS rats with the control
201 vector (**Figs. 3I** and **Supplementary Figs. S2F**). Taken together, these results indicate that both
202 exogenous and endogenous MGO can active the TrkB signaling pathway and induce the expression of
203 BDNF.

204

205 **MGO directly binds to the extracellular domain of TrkB and provokes its dimerization and**
206 **autophosphorylation.**

207   Next, we explored the molecular mechanisms underlying the fast and sustained activation effect of
208 MGO on TrkB signaling pathways. To exclude the activation effects due to the accumulation of BDNF, we
209 added the BDNF antibody to the cell cultures and found that it only partly abrogated MGO-induced
210 phosphorylation of Akt, ERK1/2 and CREB in the first 9 h (**Fig. 4A** and **Supplementary Fig. S4A**). This
211 result suggests that the apparent signaling activation effect caused by MGO does not completely
212 attribute to BDNF. Further, we utilized K252a, a potent selective inhibitor for Trk receptors [29], to
213 investigate whether MGO's effect is associated with the kinase activity of TrkB. K252a potently inhibited
214 the phosphorylation of TrkB, Akt and ERK1/2 that was stimulated by BDNF or 7,8-dihydroxyflavone (7,8-
215 DHF), a known small molecule agonist of TrkB [30]. And the levels of p-TrkB, p-Akt and p-ERK1/2 induced
216 by 250 μM exogenous MGO or 10 μM BBGC were also significantly decreased by this inhibitor (**Fig. 4B**
217 and **Supplementary Fig. S4B**). Additionally, K252a also decreased the p-CREB levels and its nuclear
218 entry induced by MGO (**Figs. 4C** and **4D**). Given that MGO is previously reported as a competitive partial
219 agonist of GABA$_A$R [15] and activation of GABA$_A$R is often related to TrkB signaling [31,32], we further
220 determined whether MGO's activation effect on TrkB signaling is associated with GABA$_A$R. The results
221 show that MGO actives TrkB signaling regardless of the presence of a selective GABA$_A$R antagonist, (-)-
222 bicuculline methochloride (**Supplementary Fig. S4C**), suggesting that MGO directly actives TrkB
223 phosphorylation independent of GABA$_A$R.

224   To examine how MGO regulates the kinase activity of TrkB, we tested whether MGO induces the
225 homodimerization and phosphorylation of TrkB. The pull-down assays showed that treatment of 250 μM
226 MGO or 100 ng/mL BDNF both significantly provoked the homodimerization and autophosphorylation of
227 TrkB (**Fig. 4E**), which indicates that MGO physiologically mimics the functions of BDNF. The biolayer
228 interferometry (BLI) assay showed that MGO directly binds to the extracellular domain (ECD, 32-429aa)
229 of TrkB with a dissociation constant (K_d) of 5.31 μM (**Fig. 4F**). However, MGO does not interact with the

230 intracellular domain (ICD, 454-821aa) of TrkB (**Fig. 4F**). Under physiological condition, MGO binds and

231 modifies arginine, lysine, and cysteine residues in proteins [33]. To explore the modification sites of MGO
232 on the extracellular domain of TrkB, we analyzed the amino acid sequence of the binding regions
233 between TrkB and BDNF. The binding regions in TrkB receptor include amino acid residues 103–181 that
234 contain the third leucine-rich motif and the CC-2 domain and the Ig2 domain (342–394) [34]. We found a
235 significant enrichment of arginine (R) and lysine (K) in the third leucine-rich motif, whereas no arginine (R)
236 or lysine (K) in the Ig2 domain (**Fig. 4G**), suggesting MGO binds to the arginine or lysine residues within

237 the outer binging domain between BDNF and TrkB. Taken in sum, MGO selectively binds to the ECD of
238 TrkB and functions as its agonist, and thus induces a fast and sustained activation of BDNF/TrkB
239 signaling.

240

241

242 **GLO1 is associated with TrkB signaling in MDD patients and serves as a potential drug target for**
243 **depression.**

244 To test whether in MDD patients GLO1 is associated with TrkB signaling, we performed a differential
245 coexpression analysis on a gene expression profiling of brain samples from 34 MDD patients and 55
246 normal individuals [35]. The differentially coexpressed genes (DCGs) of six brain areas, i.e., the
247 dorsolateral PFC (DLPFC), HC, were obtained using an improved weighted correlation network analysis
248 (WGCNA) approach [36] (see Methods, **Supplementary Table S2**). Pathway enrichment analysis of the
249 six DCG sets against KEGG database identified the 'neurotrophin signaling pathway' as one of the most
250 significantly enriched pathways in the DLPFC, AnCg, HC and NAcc areas (**Supplementary Fig. S5B**).
251 Then, by employing the gene set enrichment analysis (GSEA) algorithm, GLO1 was found significantly
252 correlated with the BDNF/TrkB signaling pathway in the DLPFC, HC areas of these samples (**Fig. 5A**
253 **and Supplementary Fig. S5C**), further validating that MGO levels may be involved in regulating the
254 BDNF/TrkB signaling in the DLPFC, HC areas of MDD patients.

255 The antidepressant effects of MGO also makes GLO1 a potential therapeutic target for depression.
256 By application of two in house systems pharmacology-based drug targeting tools, i.e., WES [37] and
257 TCMSP [38], a flavone molecule, luteolin, was screened out as a candidate compound that may target
258 GLO1 (**Supplementary Fig. 6A**). To validate the interaction between luteolin and GLO1, we employed
259 both in vitro fluorescence spectroscopy and in vivo cellular thermal shift assay (CETSA) experiments [39].
260 The fluorescence spectroscopy results showed a K_d value of 47.17 μM for luteolin binding to GLO1 (**Fig.**
261 **5B**). And the *in vivo* CETSA also validates their binding in intact cells (**Fig. 5C**) with the EC_{50}
262 concentration of 45.23 μM for luteolin at which half-maximal thermal stabilization of GLO1 in PC12 cells
263 was observed (**Supplementary Fig. S6E**).

264 To explore the binding mode of luteolin with GLO1, we performed molecular docking coupled with
265 molecular dynamic (MD) simulations. Three residues, E99, Q33 and F62 were identified as crucial
266 residues for the binding, as that in GLO1 active pocket they formed hydrogen bonds and an edge-to-face
267 aromatic interaction with luteolin (**Supplementary Fig. S6C**). Regarding luteolin, its carbonyl, resorcinol
268 and pyrocatechol substituents form H-bonds and hydrophobic-interactions with GLO1, demonstrating the
269 key roles of these groups in luteolin's binding within the active site of GLO1 (**Supplementary Fig. S6C**).
270 More importantly, we found that the hydroxyl group of the resorcinol is freely buried into the positively
271 charged mouth, a big hydrophobic tunnel constituted by residues F62, F67, L69 and T101
272 (**Supplementary Fig. S6C** and **S6D**). To further investigate whether this hydrophobic cavity is beneficial
273 for the binding, we modified the hydroxyl group of the resorcinol into a bulkier group, i.e., 1, 2-
274 dimethoxyethane, and obtained a new derivative, named as lutD (**Supplementary Fig. S6A** and **S7**).
275 Intriguingly, fluorescence spectroscopy analysis presents a 1.5-fold increase of the association constant

276   (K_a) (from 2.12×10^4 L·mol^{-1} to 3.22×10^4 L·mol^{-1}) for lutD compared with luteolin (**Supplementary Fig.**
277   **S6E**), indicating that the bulky groups in resorcinol contribute to a more potent GLO1 binding. Actually,
278   MD simulations on lutD and GLO1 shows that this mouth zone is empty and thus provides sufficient
279   space for bulky substituents (**Supplementary Figs. S6C and S6D**). Besides, the hydrophobic interaction
280   between the methoxyethane group of lutD and F67, L160 (**Supplementary Fig. S6D**) also strengthens
281   the interactions between lutD and GLO1. All these results demonstrate that luteolin is a potent binder of
282   GLO1, in which H-bonds and hydrophobic interactions are important for the binding.

283   We next evaluated whether luteolin exhibits antidepressant effects on CMS rats. The results show
284   that ip administration of 10 mg/kg luteolin per day for 3 weeks markedly reduced the immobility time of
285   rats in FST and increased their number of entries in the open arms in EPM (**Fig. 5D** and **Supplementary**
286   **Fig. S6F**). We also found that chronic luteolin treatment increased the plasma MGO levels and the
287   concentrations of neurotransmitters in HC and PFC areas of rats (**Figs. 5E** and **5F**). These results
288   demonstrate that luteolin exerts antidepressant effects on CMS rats by targeting GLO1. Besides, similar
289   to MGO, luteolin treatment also promoted hippocampal neurogenesis as indicated by obviously
290   increased number of BrdU+ or BrdU+/NeuN+ cells in the hippocampal DG of CMS rats (**Supplementary**
291   **Fig. S6G**). Additionally, single dose treatment of 20 mg/kg luteolin potently activated the BDNF/TrkB
292   signaling in a time-dependent manner in the HC of CMS rats (**Fig. 5G**). These data reveal that through
293   targeting GLO1, luteolin increases MGO concentrations in CMS rats and subsequently triggers the
294   BDNF/TrkB signaling pathway, resulting in desirable antidepressant effects.

295

Discussion

297   In this study, we demonstrated that MGO functions as an endogenous agonist of TrkB. We
298   discovered that MGO directly and selectively binds to the ECD of TrkB and stimulates its dimerization
299   and autophosphorylation. Analysis of amino residues of the binding region between BDNF and TrkB
300   revealed that there is a significant enrichment of arginine and lysine in the third leucine rich motif,
301   suggesting potential MGO modification sites on TrkB. Both in vitro and in vivo experiments showed that
302   MGO triggers a rapid and sustained activation of TrkB-mediated Akt/CREB signaling, which rapidly
303   increases the expression of BDNF, thus being beneficial to MDD. Clinically, increasing the BDNF level in
304   brain is of particular therapeutic interests for depression [40,41]. However, current clinical trials using
305   recombinant BDNF in patients always turn out disappointing due to series of reasons like poor delivery,
306   short half-life, and potential side effects [42]. Our finding that MGO at normal concentrations acts as a
307   switch on activating the BDNF-feedback loop provides a new feasible way to increase the BDNF levels
308   for MDD patients.

309   Importantly, we found that MGO levels in the PFC and HC are significantly decreased in the CMS
310   rats, whereas treatment of low-dose MGO (5 mM/kg) per day for 3 weeks reverses the MGO levels back
311   to normal concentrations and exhibits desirable antidepressant effects. MGO also promotes the synaptic
312   plasticity and neurogenesis of PFC and HC neurons. Besides, we found that chronic administration of
313   MGO does not cause any weight gains in rats (**Supplementary Fig. S1C**), avoiding the major side
314   effects produced by current clinically-used tricyclic antidepressants and monoamine oxidase inhibitors

315   [43,44]. Except for depression, some other psychiatric and neurological disorders such as the epilepsy and
316   Parkinson's disease also possess several common pathological features, like synaptic density reduction
317   and neuron atrophy [45,46]. Thus, manipulating endogenous MGO concentrations in brain provides a new
318   alternative way for treating both MDD and these diseases with more favorable tolerability and efficacy.

319   The present study also highlights the potential of GLO1 as a depression target (**Fig. 6**). And based on
320   this, luteolin was screened out as a GLO1 inhibitor and its binding affinity as well as the interaction
321   features with GLO1 were deeply explored. As a natural flavonoid, luteolin exists in many plants such as
322   *Apium graveolens*, *Petroselium crispum*, and *Capsicum annuum*, with antioxidant, anticancer, memory-
323   improving, and anxiolytic activities [47]. In the present study, chronic administration of luteolin for 3 weeks
324   significantly improves the depression-like behaviors in CMS rats. Luteolin also increased the number of
325   new-born neurons in the HC of CMS rats (**Supplementary Fig. S6G**), which provides a reasonable
326   explanation of why luteolin has strong neuroprotective effects both *in vitro* and *in vivo* [47,48]. Importantly,
327   long-term treatment of luteolin does not exhibit any apparent toxicity in rats (10 mg/kg, i.p. for 3 weeks),
328   which corresponds well with the intraperitoneal LD50 of luteolin in rats (411 mg/kg) [49]. In conclusion, our
329   work not only presents a novel mechanism for MGO's effect on promoting stress resilience, but also
330   identifies a natural product luteolin as a promising antidepressant.

331

Materials and methods

Animals

334   Adult male C57BL/6J mice (7–9 week old, 22–24 g) and adult Sprague-Dawley (SD) male rats (180 -
335   200 g) were purchased from Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Three
336   to four mice were housed in a plastic cage (300 × 170 × 120 mm) at 24 ± 1 °C. Both mice and rats were
337   group-housed with standard laboratory bedding and conditions (12-h light/dark cycle, 22 ± 1°C, ad
338   libitum access to food and water) for 1 week prior to the experiments. The experimental procedures
339   performed in this study were in line with the Guidelines of NIH for the Use of Laboratory Animals as well
340   as approved by the Northwest University Animal Care and Use Committees.

341

Drugs

343   The following drugs were used in this study, including methylglyoxal solution (Sigma-Aldrich, M0252), S-
344   p-bromobenzylglutathione cyclopentyl diester (Sigma-Aldrich, cat. no. SML1306), K252a (Abcam, cat. no.
345   ab120419), luteolin (TCI chemicals, TT2682), 7,8-dihydroxyflavone (7,8-DHF) (Abcam, cat. no.
346   ab120996), and recombinant human BDNF protein (R&D Systems, cat. no. 248-BD-025).

347

CMS procedure

349   To induce the physical, behavioral, biochemical, and physiological phenotypes of depression, all rats
350   were subjected to a schedule of mild psychosocial stressors for 8 weeks (**Supplementary Fig. S1A**).

351   The stressors included alterations of the bedding (sawdust change, removal or damp of sawdust,
352   substitution of sawdust with 21°C water, rat, or cat feces for 1-3 h), cage-tilting (45°,1-3 h) or shaking (5-
353   20 min), cage exchange (rats were positioned in the empty cage of another male for 2-6 h) , predator
354   sounds (5-20 min), and alterations of the length and time of light/dark cycle. To increase stress intensity,
355   stressors were combined after the first week of the CMS regimen [50]. Physical changes were assessed
356   weekly by measuring the body weight of the animal. Additionally, in order to evaluate the neurogenesis

357   by immunocytochemistry, rats were ip injected with BrdU (2 × 150 mg/kg) at the day before treatments.

358   All rats were randomly assigned to three experimental groups, i.e., non-stress control + saline (SAL);
359   stress (CMS) + SAL; stress (CMS) + drugs (5 mM/kg/day of MGO or 10 mg/kg/day of luteolin). To avoid
360   the possible bias induced by the behavioral tests, the rats in each group were divided into two sets (n =
361   10 per set). The first set provided tissue samples used for morphological analysis, western blotting and
362   qRT-PCR analyses, while the second one was adopted for behavioral assessments. During the last 3
363   weeks of the CMS protocol, rats were injected ip daily with drugs.

364

365   **Behavioral assessment**

366   At the end of the CMS protocol, the behavioral tests EPM and FST were conducted. Two days were
367   given for rats between exposures to different behavioral assessments and all behavioral testing
368   experiments were carried out during the daily light phase (09:00 am – 05:00 pm).

369   *Elevated plus-maze test* Rats were placed in the center of a standard EPM apparatus (two open and
370   two closed 33 cm × 5 cm arms) for examining the anxiety-like behaviors of rats. The exploratory activity
371   was measured over a 5-min period. Then, the percentage of time spent in the open arms, an index of
372   anxiety-like behavior, the number of entries in the closed arms, an indicator of locomotion, were
373   determined.

374   *Forced swim test* In brief, SD rats were placed in a glass cylinder filled with water (23°C; 30 cm deep)
375   and a 5-min swim test session was video-recorded. Employing an automated video tracking system, the
376   time spent immobile during the last 4 minutes of the test and the latency to immobility were scored. An
377   increase in immobility time indicates a higher degree of depressive state. All tests were analyzed by
378   TopScan (CleverSys, Inc., CSI) system.

379   *Sucrose preference test.* The sucrose preference test was conducted based on previously described
380   procedures [21]. Briefly, the mice or rats were habituated to a 1% sucrose solution for 2 days before 2
381   additional days of choice testing. The position of the water and sucrose bottles was switched every 12 h
382   to ensure that the mice or rats did not develop a side preference. After this period, the mice or rats were
383   deprived of food and water for 24 h, and exposed to two bottles filled with either 1% sucrose or water for
384   12 h in the dark. Total consumption of each fluid was measured and sucrose preference was calculated
385   as a percentage (amount of sucrose consumed (bottle A) × 100 / total volume consumed (bottles A + B)).
386   To avoid the bias related to body weight, this variable was calculated as an amount of consumed
387   sucrose in mg per gram body weight × 100.

388

CSDS procedure and the social interaction test

390 The CSDS protocol was conducted as previously described [20]. Briefly, a singly C57BL/6J mouse was
391 exposed to a different aggressive CD1 mouse for 5 min each day for a total of 10 days. Following 5 min
392 of contact, the intruder mouse was housed across a perforated plastic divider that allowed visual,
393 olfactory and auditory contacts, but prevented physical interaction from the aggressor CD1 mouse, for
394 the remainder of the 24-h period. Non-defeated control mice were housed opposite to another C57BL/6
395 mouse and the controls were changed daily. Following the 10-day CSDS protocol, the avoidance
396 behaviors were tested to separate the susceptible and unsusceptible subpopulations, the interaction
397 ratio was then calculated as 100 × (time spent in the interaction zone with an "Target")/(time spent in the
398 interaction zone with "No Target") and an interaction ratio of 100 was set as a cutoff. The mice with
399 scores <100 were considered susceptible, and those with scores ≥100 were considered resilient. On day
400 13, each mouse was implanted with an osmotic minipumps (Alzet, model 1007D; 0.5µL/h, 7 d) with a
401 reservoir containing either vehicle (aCSF) or MGO (10 µM) in the right lateral ventricle. To block MGO-
402 induced effect, rats were pre-treated with AG (1 g/L) via drinking water for 48 h (on day 11) before
403 pumps implantation. On day 20, test mice (defeated mice and non-defeated control mice) were subjected
404 to the social interaction test. The approach-avoidance behavior of a test mouse to a CD1 mouse was
405 recorded with a video tracking system. The social interaction test consisted of two 150 s sessions. In the
406 first phase, 'no target' session, the mouse could explore freely in a square-shaped open-field arena (43.5
407 × 43.5 cm) possessing a wire-mesh cage (10 × 6.5 cm). For the second 'target' session, the mouse was
408 reintroduced into this arena with an unfamiliar CD1 mouse placed into the wire mesh cage. The wire
409 mesh cage allowed visual, olfactory and auditory interactions between the test mouse and the target
410 CD1 mouse but prevented direct physical contact. After each trial, the apparatus was cleaned with a
411 solution of 70% ethanol in water to remove olfactory cues and all the behavioral tests were conducted in
412 a dark room. The duration of time that the mice spent in the interaction zone and corner zone was
413 obtained using TopScan (CleverSys, Inc., CSI) system.

414

Quantification of MGO levels

416 Analysis of the levels of MGO in the plasma (Hycult Biotech, HIT503) and cell lysis (Cell Biolabs, STA-
417 811) was carried out using specific enzyme-linked immunosorbent assays (ELISA) following the
418 manufacturer's instructions. Plasma was collected from aortaventralis and separated into Eppendorf
419 tubes that contained EDTA to inhibit coagulation effect. The mixed blood and EDTA were then inversed
420 and placed on ice for 20 min. After the inversion, the mixture blood sample was centrifuged for 15 min at
421 1500×g at 4°C. The supernatant was then collected and transferred to a fresh polypropylene tube for
422 analysis. To detect MGO-adducts in cell lysate, 50 µg of total protein from fresh tissues or cell cultures
423 were prepared using a Qproteome Mammalian Protein Prep Kit (Qiagen, Germany) in accordance with
424 the manufacturer's protocol.

425

Cell culture and transfection

PC12 and HEK293 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS), 1% (v/v) penicillin and streptomycin at 37°C under an atmosphere containing 5% CO_2. To examine the relationship between GLO1 and TrkB signaling, we modulated the expression levels of GLO1 by lentiviral (LV)-mediated overexpression or shRNA knockdown in PC12 cells. The pLV-CMV-MCS-3flag-EF1-zsgreen-puro vector and the pLV-U6-MCS-zsgreen-puro vector for GLO1 overexpression and GLO1 knockdown were generated and obtained from Sangon Biotech (Shanghai) Co., Ltd.. The sequences of the non-targeting shRNA or GLO1-targeting shRNAs were listed in **Supplementary Table S1**. At 24 h before transfection, approximately 5.0×10^5 cells were seeded in 6-well cell culture dishes. On the next day, they were infected with recombinant lentivirus in the presence of polybrene (5 μg/mL). The medium was replaced 12 h post-transfection with DMEM supplemented with 10% FSB, and cells were allowed to recover for additional 5 days. After transfection, cells were harvested, lysed and then employed for further western blotting analysis. To examine the homodimerization of TrkB induced by MGO, HEK293 cells were co-transfected with 3×Flag-tagged TrkB and 6×His-tagged TrkB. After transfection, these cells were then incubated with either PBS, 250 μM MGO or 100 ng/mL BDNF for 1 h for pull-down assay by Dynabeads His-Tag Pulldown (Invitrogen, cat. no. 10104D) according to the manufacturer's instructions. For *in vitro* mutant studies, HEK293 cells were transfected with human 3×Flag-tagged GLO1 wild type and mutants (Q33E , E99A , F62A). Using Anti-DYKDDDDK Affinity Resin (Invitogen, cat. no. A36803), the Flag-tagged GLO1 wild type and mutants were purified for subsequent experiments.

Quantitative reverse transcription-PCR

Total RNA was prepared from fresh tissues or cell cultures using the RNeasy mini kit (Qiagen, Germany) according to the manufacturer's protocol. cDNAs were synthesized from 100 ng of total RNA using PrimeScript™ RT reagent Kit with gDNA Eraser (Takara, cat. no. RR047). Subsequently, qRT-PCR was performed employing the StepOnePlus Real-Time PCR System (Applied Biosystems) with SYBR Premix Ex Taq II (Takara. cat. no. RR820). Using Primer 3 software, specific gene primers (Sangon Biotech) used for qRT-PCR assays were designed and their sequences are listed in **Supplementary Table S3**. The expression levels of these genes were normalized to that of beta-actin and the fold changes in gene expression were computed by setting the gene expression levels in control samples as one.

Western blot analysis

The expression levels of related proteins were examined by western blot experiments. A total of 30 μg protein samples were firstly separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto poly vinylidene fluoride (PVDF) membranes. After blockade with 5% skimmed milk in TBST at room temperature for 1 h, the membranes were probed with specific primary antibodies to p-TrkB Y706 (Abcam, cat. no. ab197072, 1:1000), TrkB (Abcam, cat. no.

463 ab18987, 1:1000), p-Akt S473 (Cell Signaling, cat. no. 4058, 1:1000), Akt (Cell Signaling, cat. no. 92715,

464 1:1000), p-ERK1/2 T202/Y204 (Cell Signaling, cat. no. 9101, 1:1000), ERK1/2 (Cell Signaling, cat. no.

465 4695, 1:1000), p-CREB S133 (Abcam, cat. no. ab32096, 1:5000), CREB (Abcam, cat. no. ab178322,

466 1:500), BDNF (Abcam, cat. no. ab108319, 1:5000), GLO1 (Abcam, cat. no. ab96032, 1:2000), GAPDH

467 (Cell Signaling, cat. no. 5174, 1:1000), Anti-DDDDK tag (Abcam, cat. no. ab49763, 1:1000), Anti-6X His

468 tag (Abcam, cat. no. ab18184, 1:1000) and then incubated with species-specific peroxidase-conjugated

469 secondary antibodies (antibody to rabbit, Abcam, cat. no., ab6721, 1:20000; antibody to mouse, Abcam,

470 cat. no., ab6789, 1:10000). Using Immun-Star™ WesternC™ Chemiluminescence Kit (Bio-Rad, cat. no.

471 1705070), the protein bands were detected and the optical densities of the bands were quantified by

472 Image-Pro Plus v6 (Media Cybernetics, Silver Spring, MD).

473

Immunocytochemistry

474

475 Cultured PC12 cells were pretreated with either vehicle or 100 nM k252a for 30 min and then treated

476 with 250 µM MGO for 1 h. Subsequently, the cells were fixed in 4% formaldehyde for 10 min and

477 incubated with primary antibodies overnight at 4°C, followed by incubation with the secondary antibodies.

478 The following antibodies are used: p-CREB S133 (Abcam, cat. no. ab32096, 1:250), CREB (Abcam, cat.

479 no. ab178322, 1:500), Goat Anti-Mouse IgG H&L (Alexa Fluor® 488) (Abcam, cat. no. ab150117,

480 1:1000), Goat Anti-Rabbit IgG H&L (Alexa Fluor® 594) (Abcam, cat. no. ab150080, 1:1000). Images

481 were obtained using a DMI 4000B microscope (Leica, Germany) with DFC310 FX camera (Leica,

482 Germany) and Leica application suite, version 4.2.0 (Leica Microsystems).

483

Immunohistochemistry

484

485 Animals were deeply anaesthetized with sodium pentobarbital (50 mg/kg, ip). Brains were then removed

486 and stored at -80°C. Coronal cryosections (20 µm) of the entire hippocampus were prepared with a

487 cryostat (Leica, Wetzlar, Germany). After section, the slides were immediately immersed into 4%

488 paraformaldehyde for 15 min at 4°C. For BrdU labeling, sections were denatured by treatment with 1N

489 HCl for 10 min at 4°C followed by 2N HCl for 20 min at 37°C and then neutralized in 0.1 M borate buffer

490 (pH 8.5) for 10 min. After washed with 0.3% Triton X-100 in PBS, the sections were then incubated with

491 blocking solution containing 10% donkey serum (Jackson ImmunoResearch, cat. no. 017000121) in PBS

492 for 60 min. Afterward, the sections were incubated in the primary antibodies (anti-BrdU, Abcam, cat. no.

493 ab1893, 1:100; anti-NeuN, Abcam, cat. no. ab177487, 1:500) for 16 h at 4°C. Finally, sections were

494 washed three times with PBS and then incubated with the corresponding Alexa-labeled secondary

495 antibodies (donkey anti-rabbit, Abcam, cat. no. ab150061, 1:500; donkey anti-sheep, Abcam, cat. no.

496 ab150180, 1:500) for 1 h at room temperature. After staining, slides were photographed using Leica DM

497 4000B microscope (Leica, Germany) fitted with a digital camera DFC450 C (Leica, Germany), and Leica

498 application suite, version 4.2.0 software (Leica Microsystems) was used for image analysis. For

499 quantification of BrdU-labeled cells, a modified unbiased stereology protocol was used as described

500 previously [51]. Briefly, we collected 5 rats for each group. One in six series of sections of each brain were

501 counted in the dentate gyrus throughout the hippocampus. Results were multiplied by ten to obtain the
502 total number of BrdU-positive cells per dentate gyrus. The average of these values for the individual
503 brain was used for statistical analysis.

504

505 **Quantification of neurotransmitters and their metabolites by LC-MS/MS**

506 To determine the effects of drugs on the depression rats, we measured the levels of the
507 neurotransmitters in HC and PFC areas of rats through a rapid-resolution liquid chromatography–mass
508 spectrometry (LC-MS)/MS analysis. The tissues sample preparation for the LC-MS/MS analysis were
509 obtained from a liquid-solid extraction approach. Using a Waters ACQUITY UPLC system (Waters Corp.,
510 Milford, MA, USA) coupled to a QTRAP 5500 mass spectrometer (Applied Biosystems, Foster City, CA,
511 USA), all analyses were performed. The chromatographic separation was carried out on Acquity UPLC
512 HSS T3 column (2.1 mm × 100 mm, 1.8 μm) and BEH Amide column (2.1 mm × 100 mm, 1.7 μm), and
513 the mass spectrometric measurements were carried out on a QTRAP 5500 quadrupole time-of-flight
514 mass spectrometer equipped with an electrospray ionization source (ESI) operating in the positive and
515 negative ionization modes, which were used for detection (Agilent Technologies). For both platforms,
516 results were denoted as data matrices, in which integrated peak areas were used for determining the
517 metabolites in all the samples.

518

519 **RNA-seq**

520 On Week 7, the hippocampi and PFC of the rat brains were dissected from one normal rat and two CMS
521 rats treated with either saline or 100 mM/kg MGO. Total RNA was extracted and purified from ~30⎵mg
522 tissue using the RNeasy mini kit (Qiagen, Germany) according to the manufacturer's protocol. RNA-seq
523 was performed at Genminix Informatics Ltd.,Co.(Shanghai, China) using Illumina HiSeq ×10 sequencing
524 platform. The original sequencing data have been uploaded to the GEO database (GSE119482).

525

526 **Identification and function enrichment analysis of differentially expressed genes**

527 Quality control of raw sequences from each sample was investigated using FastQC (v0.11.5). Reads
528 were then mapped to the Ensembl rat genome sequence (Rnor_6.0) using HISAT2 (v2.0.4) [52] and
529 assembled into transcripts by StringTie (v1.2.4) [53] using a UCSC rn6 GTF (general transfer format) file.
530 FPKM (fragment per kilobase per million mapped reads) values were used to estimate the transcript
531 abundance. The ballgown [54] R package was employed to detect differentially expressed genes (DEGs)
532 between normal, CMS and MGO-treated CMS rats. The Gene Ontology (GO) and KEGG pathway
533 enrichment analyses of DEGs were performed using the DAVID bioinformatics resources 6.8 [55].

534

535 **Biolayer interferometry**

536 The kinetics of MGO binding to the extracellular or cytoplasmic domain of recombinant human TrkB

537 protein was assessed using biolayer interferometry with an Octet K2 system (ForteBio). All of the

538 experiments were performed at 30°C under buffer conditions of PBST (0.1% Tween 20), pH 7.4, 8%

539 DMSO. NI-NTA biosensors (FortéBio Inc., Menlo Park, CA) were used to capture TrkB proteins onto the

540 surface of the sensor. After reaching baseline, sensors were moved to association step for 60 s and then

541 dissociated for 60 s. Curves were corrected by a double-referencing technique, using both NI-NTA pins

542 dipped into the experimental wells and a buffer-only reference. After double referencing corrections, the

543 subtracted binding interference data were applied to the calculations of binding constants using the

544 FORTEBIO analysis software (Version: 9.0.0.10) provided with the instrument.

545

Stereotactic injection of adeno-associated virus

547 Adeno-associated viral vectors (AAVs) serotype 2 expressing either mCherry or GLO1 were generated

548 and purified by Sangon Biotech (Shanghai) Co., Ltd. The rats used for the stereotactic injection were

549 weighted from 280 g to 320 g. These rats were deeply anaesthetized with isoflurane gas (3.0%

550 isoflurane for induction and 2.0% for maintenance) and placed into a stereotactic apparatus (RWD Life

551 Science Inc.). Stereotaxic surgery was performed to deliver viruses into the hippocampus of these rats.

552 AAV-GLO1 vector or AAV-mCherry was bilaterally infused using stereotaxic coordinates according to the

553 rat brain atlas [56]. By using a precision Hamilton micro-syringe with a 26 G needle, a total of 2 µL viral

554 solution was bilaterally injected into the hippocampus (AP -3.6 mm, ML ± 2.0 mm, DV 3.4 mm). Viruses

555 were infused at a speed of 0.2 µL/min for 10 min and the needle was left in place for additional 10 min

556 before slowly being withdrawn. The rats were recovered on hot pad (37°C) until waken up and returned

557 to cages, and then they were allowed to recover for 3 weeks before starting the behavioral paradigm.

558

Gene expression data of MDD patients

560 Normalized gene expression data for MDD patients were collected from the GEO database (GSE45642)

561 [35]. GSE45642 includes a total of 670 whole-genome gene expression profiles of six brain areas, i.e.

562 DLPFC, AnCg, HC, AMY, NAcc and CB for 55 normal controls and 34 MDD patients.

563

Differentially coexpressed genes

565 To identify the DCGs in the six brain areas of MDD patients, we applied an I-WGCNA method [57]

566 (**Supplementary Fig. S1*A***). Firstly, to decrease the noise content, we discarded non-informative genes

567 based on their variability and expression levels by using the functions of *variabilityBasedfilter* and

568 *expressionBasedfilter* of DCGL R package [36]. Specially, for each brain area, the genes used for

569 subsequent analysis were obtained by $(S_c(exp) \cap S_c(var)) \cup (S_d(exp) \cap S_d(var))$, where $S_c(exp)$, $S_c(var)$

570 represent the gene sets filtered by the *expressionBasedfilter* and v*ariabilityBasedfilter* functions in control

571 group, whereas $S_d(exp)$, $S_d(var)$ represent those corresponding ones in depression group, respectively.

572 Then, the DCGs in each brain area were identified by using the I-WGCNA approach [36,57]. Briefly

573 speaking, the coexpression gene networks were constructed for both the control and depression groups
574 with the edge weight (a_{ij}) presented by Eq. (1),

$$a_{ij} = |\frac{1 + cor(e_i, e_j)}{2}|^\beta$$

575 (1)

576 where $cor(e_i, e_j)$ is the expression Spearman's correlation coefficient (SCC) between gene i and j in
577 control or depression group. The power value of β is set as 2 in order to emphasize large correlations at
578 the expense of low correlations. For a specific gene i, we defined a WGCNA score (WGCNA$_i$) to evaluate
579 its edge weight difference between the control and depression coexpression networks (Eq. (2)),

$$WGCNA_i = \sqrt{\frac{(|a_{i1} - a'_{i1}| \times w_{i1})^2 + ... + (|a_{i,k} - a'_{i,k}| \times w_{i,k})^2 + ... + (|a_{i,m-1} - a'_{i,m-1}| \times w_{i,m-1})^2}{m-1}}$$

580 (2)

581 where m is the number of genes in a coexpression network, a_i and a'_i are the edge weights between
582 gene i with other genes in the control and depression coexpression networks, respectively. It is worth
583 noting that we introduced a weighted factor ($w_{i,k}$) in the WGCNA score instead of using the original
584 length-normalized Euclidean distance (Eq. (3)),

585

$$w_{i,k} = (cor(e_i, e_k) - cor(e_i, e_k)') \times \max(|cor(e_i, e_k)|, |cor(e_i, e_k)'|)$$

586 (3)

587 where the $cor(e_i, e_k)$ and $cor(e_i, e_k)'$ are expression SCCs of gene i and k in control and depression groups,
588 respectively. $w_{i,k}$ is responsible for distinguishing the importance when two pairs of genes at two groups
589 have the same SCC difference. Finally, the sample permutation is repeated 10, 000 times, with P-value
590 for each gene be estimated from an empirical null distribution. And the Benjamini-Hochberg (BH) method
591 was used to control the false discovery rate. The pathway enrichment analysis of DCGs was performed
592 against KEGG database using the *enrichKEGG* function in *clusterProfiler* R Package [58]. A rank-based
593 enrichment analysis was then carried out to detect the potential genes that regulate these enriched
594 pathways. Specifically, for a candidate gene, we generated a gene list of its coexpression genes which
595 are ranked according to their correlation differences calculated with Eq. (3). The relationship between
596 the candidate gene and the enriched pathway was then determined by the enrichment score (ES)
597 calculated by the GSEA algorithm [59]. The randomization step was repeated 10,000 times, with the
598 empirical *P*-value estimated by calculation of the fraction of permutations in which the random count was
599 higher than or equal to the observed values. In addition, to control the false discovery rate, Benjamini-
600 Hochberg (BH) method was employed.

601

602 **Statistical analyses**

603 All data given in text and figures indicate mean ± s.e.m. For analysis of experiments with two groups, we
604 used the parametric unpaired two-tailed Student's t test. For analysis of experiments with three or more

605   groups, the parametric one-way ANOVA with Dunnett's multiple comparisons test or two-way ANOVA

606   with Sidak's multiple comparisons test were used. Differences were considered significant when P was <

607   0.05. NS = not significant, * P < 0.05, ** P < 0.01, *** P < 0.001.

Supplemental Information

Supplemental Information can be found in the online version of this article.

Acknowledgements

Author Contributions

Z.Y.W., Y.X.F., C.H., W.X. and Y.H.W. conceived the study. Z.Y.W. assisted by Y.X.F., C.H. and C.L.Z. designed and performed majority of the experiments. C.H. and C.L.Z. generated and analyzed the data from GEO. Z.H.G. contributed to data analysis of RNA-seq. Y.X.F. and Z.H.G. oversaw all bioinformatics analyses. J.L.Z. and L.Y.C. contributed to *in vivo* surgeries and behavioral experiments. X.G.L., L.Y.C., W.W.Z., Y.Y.C., J.M.W., Y.Y. and M.J. helped with the Q-RTPCR, western blot and Immunohistochemistry experiments. S.G.S. and Z.H.Y. were responsible for the design and synthesis of lutD, Z.H.Y. also performed fluorescence spectroscopy analysis. X.T.C. screened out candidate compounds of GLO1 inhibitor. J.H.W., X.T.C. and Y.F.Y. performed molecular docking and Molecular dynamics simulation. J.L.L. and A.P.L. provided guidance and advice. Z.Z.W. provided technical assistance. Y.L. provided advice and oversaw a portion of the work. Y.X.F., Y.F.Y., Z.Y.W., C.H., and C.L.Z. wrote the manuscript. All authors discussed the results and commented on the manuscript.

Conflict of interest

The authors declare that they have no conflict of interest.

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759

760 **Figure legends**

761 **Figure 1. MGO increases resilience to chronic stress and restores neurotransmitter levels.** (***A***) MGO concentrations
762 in the HC, PFC and plasma of normal control rats (Ctrl) and CMS rats. MGO levels were measured by MGO-H1 protein
763 adducts using ELISA assays (n = 5 rats per group). (***B***) Measurement of mRNA expression levels of GLO1 and BDNF by
764 qRT-PCR in in the HC and PFC of normal rats (control) and CMS rats (n = rats 5 per group). (***C***) Quantification of GLO1
765 protein levels (percentage of protein to GAPDH) in the HC and PFC of normal rats (control) and CMS rats (n = 5 rats per
766 group). (***D***) Comparison of immobility time in the forced swim test (FST) of normal rats (control) and CMS rats treated with
767 either vehicle or MGO 5 mM/kg intraperitoneal (ip) per day for 3 weeks. MGO treatment significantly decreased the
768 immobility time compared to CMS rats (n = 10 rats per group). (***E***) In the elevated plus-maze (EPM) test, CMS rats
769 exhibited decreased number of entries to open arms, whereas treating with MGO 5 mM/kg ip per day for 3 weeks
770 significantly increased the entry times to open arms (n = 10 rats per group). (***F***) In the sucrose preference test, in week 7,
771 the CMS rats were treated with saline, MGO or fluoxetine (FLU) (n = 10 rats per group). (***G***) LC-MS/MS analysis of the
772 concentrations of monoamines and their metabolites in the PFC and HC homogenates in rats (n = 5 rats per group). 5-HT,
773 serotonin; DA, dopamine; 5-HIAA, 5-hydroxyindoleacetic acid; DOPAC, 3,4-dihydroxyphenylacetic acid; HVA, homovanillic
774 acid. (***H***) Interaction zone times for the control mice and mice treated with saline, MGO or AG+MGO (i.c.v.) in conditions of
775 no target and CD1 target (n = 10 rats per group). (***I***) The sucrose preference test for the mice after 7-d treatment with
776 saline, MGO or AG+MGO (i.p.) (n = 10 rats per group). Data in A, B and C are analyzed using multiplet-test with Holm–
777 Sidak correction. Data in D, E and G are analyzed using one-way ANOVA with Dunnett's multiple comparisons test. Data
778 in F, H and I are analyzed using two-way ANOVA with Sidak's multiple comparisons test. Data are presented as mean
779 ±s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, NS = not significant.

780

781 **Figure 2. MGO promotes the synaptic plasticity in PFC and HC of CMS rats.** (***A***) Scatter plot showing the differentially
782 expressed genes (DEGs) in HC and PFC among the control rats and CMS rats treated with either vehicle or MGO 5
783 mM/kg intraperitoneal (ip) per day for 3 weeks. The horizontal axis represents the $\log_2$(fold change) of genes between
784 CMS+MGO and CMS+SAL groups, and the vertical axis represents the $\log_2$(fold change) of genes between Ctrl+SAL and
785 CMS+SAL groups. The colored dots represent DEGs with ∟$\log_2$(fold change)∟> ∟2 and FDR < 0.05 (red, CMS+MGO vs
786 CMS+SAL; blue, Ctrl+SAL vs CMS+SAL; pink, overlapped genes). (***B***) The enriched GO terms of MGO-induced DEGs in
787 PFC and HC regions of CMS rats. (***C***) Heatmap showing the DEGs in the PFC and HC of different groups of rats. (***D***) qRT-
788 PCR analysis of the DEGS related to synaptic plasticity in the PFC and HC lysates of different group of rats. Data are
789 analyzed using two-way ANOVA with Sidak's multiple comparisons test. Data are presented as mean ± s.e.m., n = 5 rats
790 per group, *P < 0.05, **P < 0.01, ***P < 0.001, NS = not significant.

791

792 **Figure 3. MGO activates TrkB signaling pathways and induces the expression of BDNF in PFC and HC of CMS**
793 **rats.** (***A***) Western blotting analysis of BDNF and other proteins involved in TrkB signaling pathway of PFC lysates of
794 different groups of rats. Compared to CMS rats, MGO-treated rats (5 mM/kg, ip per day for 3 weeks) exhibited increased
795 p-TrkB (Y706), p-Akt (S473), p-ERK1/2 (T202/Y204), and p-CREB (S133) immunoreactivities (n = 4 rats per group). (***B***)
796 Representative western blot of PFC lysates of rats euthanized at various time points after ip administration of single dose
797 MGO (50 mM/kg). MGO exhibits time-dependent activation on TrkB signaling pathways and BDNF expression. (***C***)
798 Immunoblot analysis of protein expressions of GLO1 and BDNF (percentage of protein to GAPDH) in PC12 cells after
799 shRNA-mediated GLO1 knockdown (shA and shB) compared to shCont. Representative western blots are shown on top
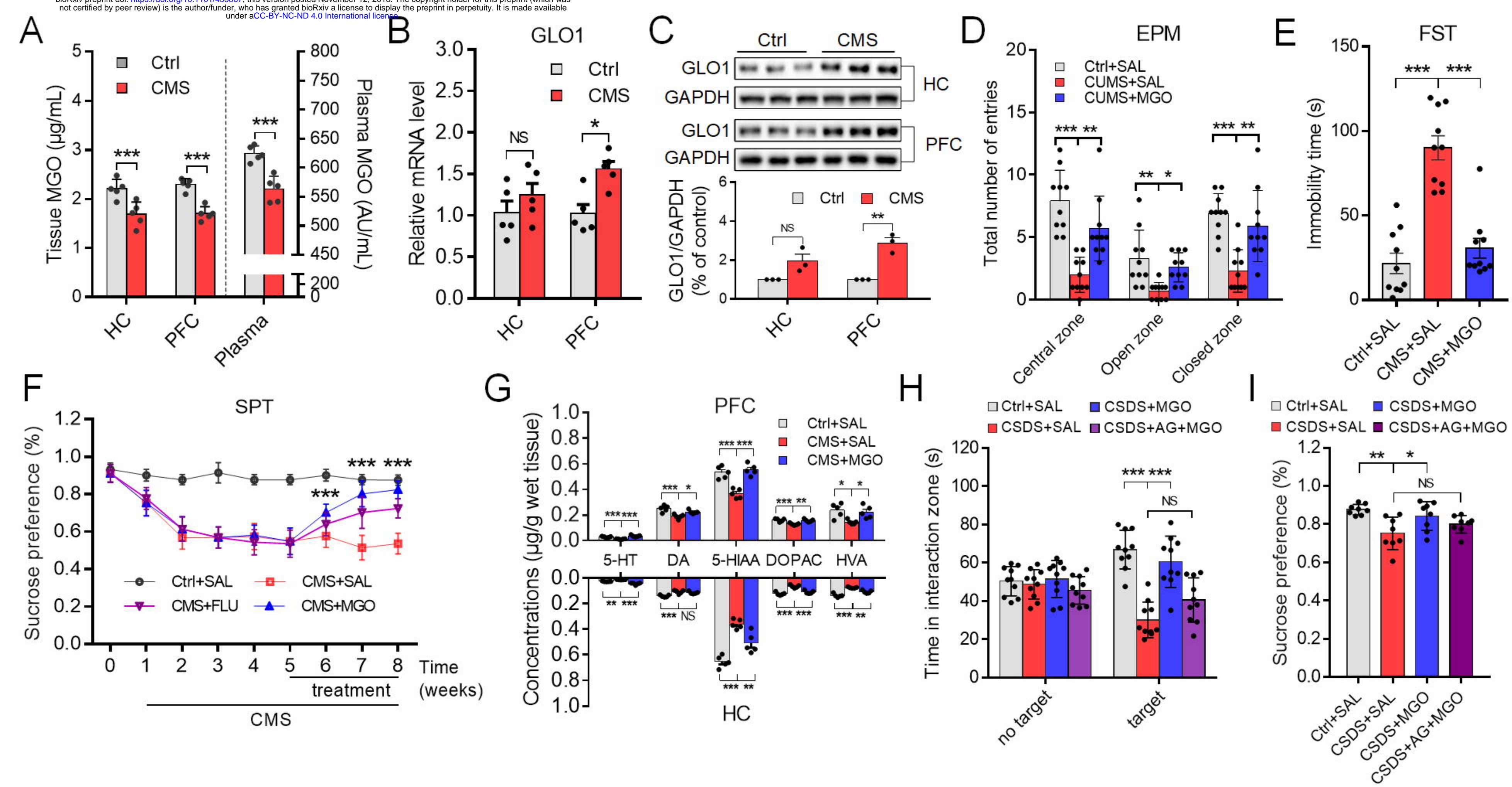
800   of the quantitative plots. shCont, a non-targeting control shRNA; shA and shB are the shRNAs targeting GLO1 (n = 3

801   samples collected independently). (*D*) Measurement of mRNA expression levels of GLO1 and BDNF by qRT-PCR in PC12

802   cells after shRNA-mediated GLO1 knockdown compared to shCont (n = 3 samples collected independently). (*E*)

803   Immunoblot analysis and quantification of protein expression, which highlights the increase of p-TrkB, p-Akt, p-ERK1/2

804   and p-CREB (percentage of phosphorylated protein to total protein) after shRNA-mediated GLO1 knockdown (n = 3

805   samples collected independently). (*F*) AAV injection and CMS experiment procedures for rats. On Week 7, the EPM and

806   FST were conducted. (*G*) The injection site of AAV vector encoding GLO1 shRNA into the dentate gyrus (DG) region of HC

807   (gray box, coronal section at +1.78 mm bregma), with an image of mCherry expression in the DG 3 weeks after injection

808   (scale bar, 400 μm). (*H*) Comparison of immobility time in the forced swim test (FST) of control rats and CMS rats with

809   GLO1-konckdown. GLO1-knockdown rats exhibited decreased immobility time compared to control CMS rats (n = 10 mice

810   per group). (*I*) The elevated plus-maze (EPM) test showing that CMS rats with GLO1-knockdown exhibited significantly

811   increased entry times to open arms compared to control CMS rats (n = 10 mice per group). Data in C, D and E are

812   analyzed using one-way ANOVA with Dunnett's multiple comparisons test. Data in D, E and G are analyzed using one-way

813   ANOVA with Dunnett's multiple comparisons test. Data in H and I are analyzed using two-way ANOVA with Sidak's

814   multiple comparisons test. Data are presented as mean ±s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, NS = not significant.

815

816   **Figure 4. MGO directly binds to the extracellular domain of TrkB and provokes its dimerization and**

817   **autophosphorylation.** (*A*) Western blots of p-Akt (S473), Akt, p-ERK1/2 (T202/Y204), ERK1/2, p-CREB (S133) and

818   CREB are shown. Either 250 μM MGO or additional 20 ng/mL anti-BDNF were preincubated for various periods of time

819   with cultured PC12 cells. (*B*) Preincubating PC12 cells with 10 μM 7,8-DHF, 100 ng/mL BDNF, 250 μM MGO or 10 μM

820   BBGC for 1 h significantly activated the TrkB signaling compared to that with 1% DMSO. While addition of 100 nM K252a

821   markedly reduced the activation effects. (*C*) Treatment with 250 μM MGO for 1 h promoted the entry of p-CREB to nucleus,

822   while the preincubation of PC12 cells with K252a for 30 min blocked this effect. Representative western blots of nucleus

823   CREB and p-CREB (top) and the quantification of ratios of immunoreactivity of p-CREB (S133) to Lamin B1 (bottom) is

824   shown. Data are analyzed using two-way ANOVA with Sidak's multiple comparisons test. Data are presented as mean

825   ±s.e.m., n = 3 samples collected independently, ***P < 0.001, NS = not significant. (*D*) Immunocytochemistry (ICC) assay

826   depicting the total CREB (green) and the phosphorylation levels of CREB (red) in PC12 cells. Treatment with 250 μM

827   MGO for 1 h induced the phosphorylation of CREB, while preincubating the PC12 cells with K252a for 30 min blocked this

828   effect. DAPI (blue) was employed to stain the nuclei. (*E*) Flag-tagged and His-tagged TrkBs were co-transfected into HEK-

829   293 cells. The cells were then treated with either PBS, 250 μM MGO or 100 ng/mL BDNF for 30 min. Subsequently, Flag-

830   tagged TrkB was pulled down and monitored by 6×His-tagged antibody. (*F*) Biolayer interferometry (BLI) data depicting the

831   association and dissociation sensograms at different concentrations of MGO for the interaction analysis of MGO with the

832   extracellular and intracellular domain (ECD and ICD) of TrkB. (*G*) The enrichment of arginine (R) and lysine (K) in the third

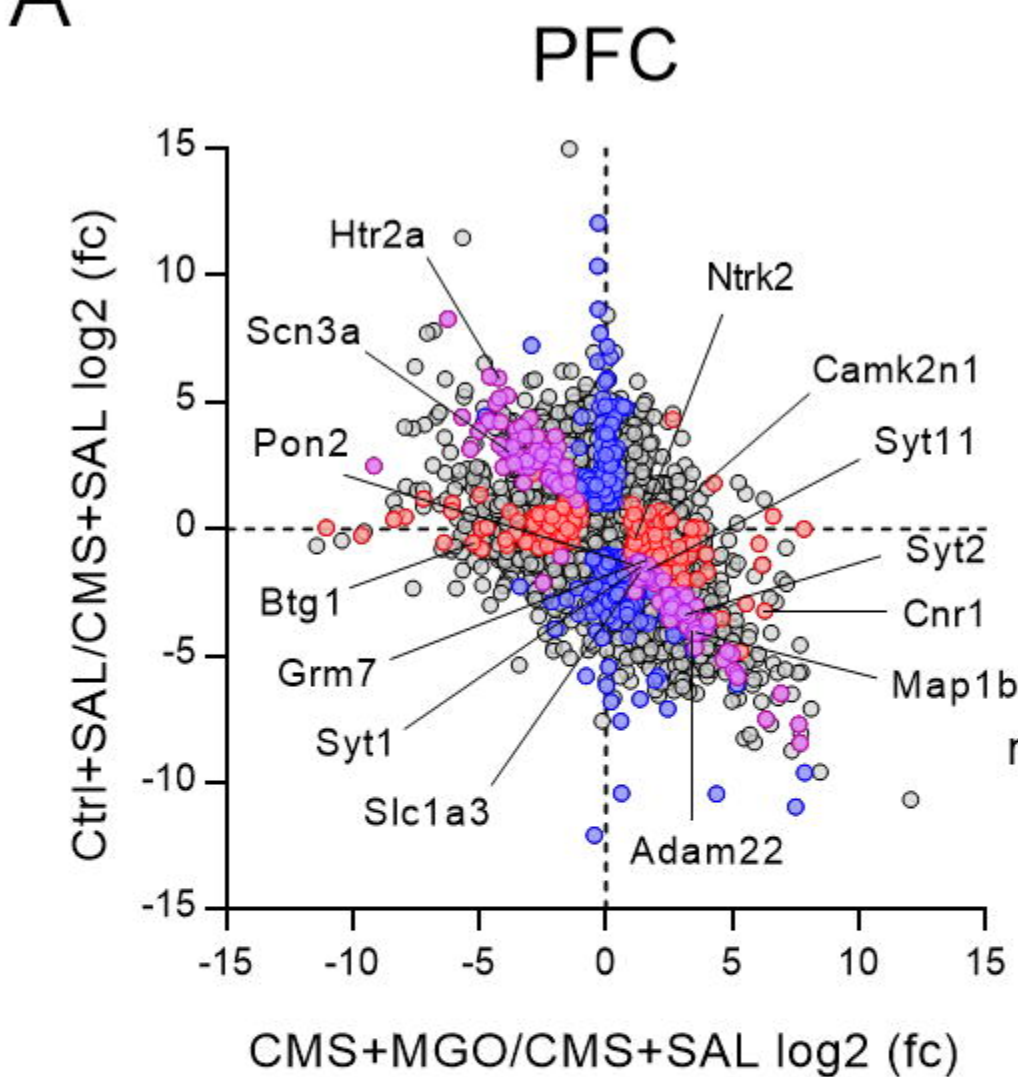833   leucine-rich motif of TrkB from human, rat and mouse.

834

835   **Figure 5. GLO1 is associated with TrkB signaling in MDD patients and serves as a potential drug target for**

836   **depression.** (*A*) GSEA analysis between GLO1 coexpression gene signatures and the BDNF/TrkB signaling pathway in

837   HC and DLPFC areas of depression patients. HC, hippocampus; DLPFC, dorsolateral prefrontal cortex. (*B*) Fluorescence

838   spectra analysis of GLO1 in the presence of various concentrations of luteolin. T = 298 K, λex = 280 nm, C_{GLO1} = 5 μM. (*C*)

839   Representative western blot of CETSA in cell lysate for GLO1 targeted by 100 μM luteolin. Data are presented as mean ±

840   s.e.m., n = 3 samples collected independently, *P < 0.05, ***P < 0.001; two-way ANOVA with Sidak's multiple comparisons

841   test between luteolin treatment group and DMSO group. (**D**) Luteolin (10 mg/kg) significantly increased the number of

842   entries of rats in central and open zones during the FST test and decreased immobility time in the EPM test compared to

843   vehicle treatment, indicating an antidepressant-like response (n = 10 rats per group). (**E**) Plasma MGO levels of CMS rats

844   after treated with luteolin for 3, 12, 24 h (n = 5 rats per group). (**F**) Measurements of monoamines and their metabolites

845   levels in the PFC and HC of different groups of rats (n = 10 rats per group). 5-HT, serotonin; DA, dopamine; 5-HIAA, 5-

846   hydroxyindoleacetic acid; DOPAC, 3, 4-dihydroxyphenylacetic acid; HVA, homovanillic acid. (**G**) Time course of TrkB, Akt,

847   ERK1/2 and CREB and their phosphorylation levels as assessed by western blot in the HC of CMS rats after single dose

848   treatment with luteolin (20 mg/kg). Data in D, E and F are analyzed using one-way ANOVA with Dunnett's multiple

849   comparisons test. Data in H and I are analyzed using two-way ANOVA with Sidak's multiple comparisons test. Data are

850   presented as mean ±s.e.m., *P < 0.05, **P < 0.01, ***P < 0.001, NS = not significant.
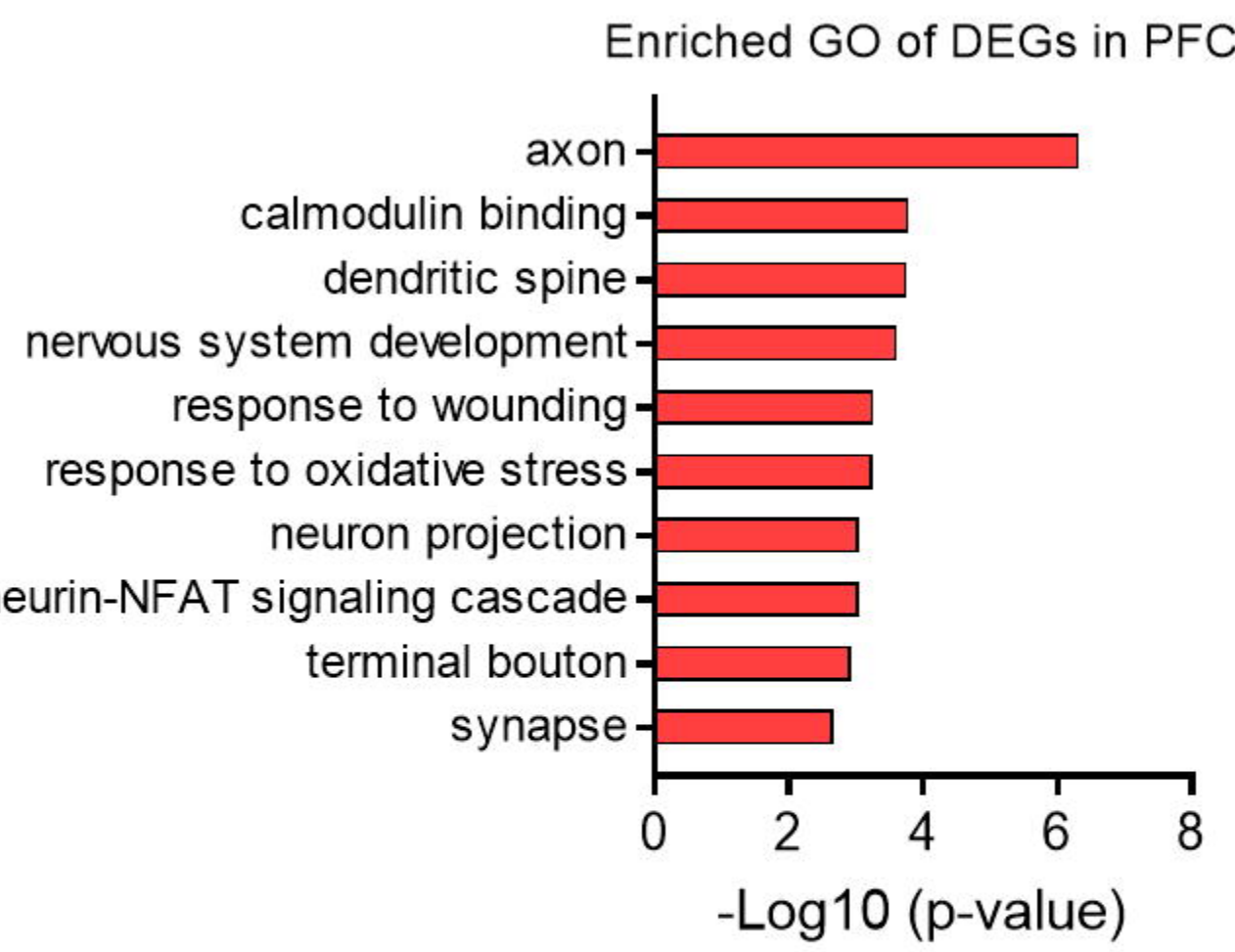
851

852   **Figure 6. The molecular mechanisms underlying the antidepressant effects of MGO.** Under normal conditions, MGO

853   binds to the extracellular domain of TrkB and provokes its dimerization and autophosphorylation, which then activates the

854   downstream Akt and ERK signaling, as well as the phosphorylation of the transcription factor CREB. This effectively

855   induces the expression of BDNF and forms a BDNF-positive feedback loop, which further promotes the synaptic plasticity

856   of nerons. Whereas under depressive state, the concentrations of MGO are significantly decreased partly due to the

857   increased expression levels or enzyme activities of GLO1. This results in inactivation of the BDNF/TrkB signaling. GLO1

858   inhibitors, such as luteolin, can effectively increase the concentrations of MGO and thus exert antidepressant effects.
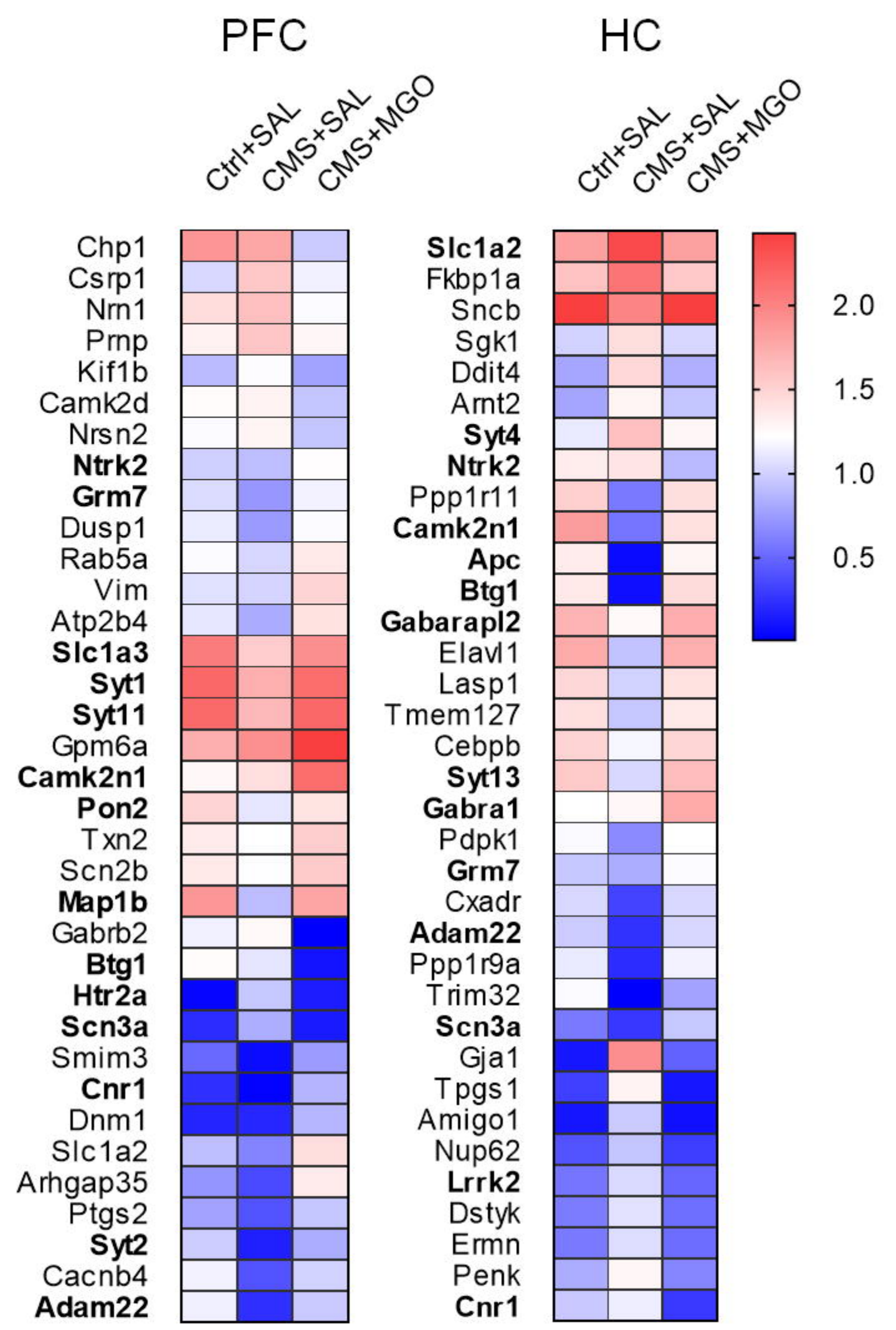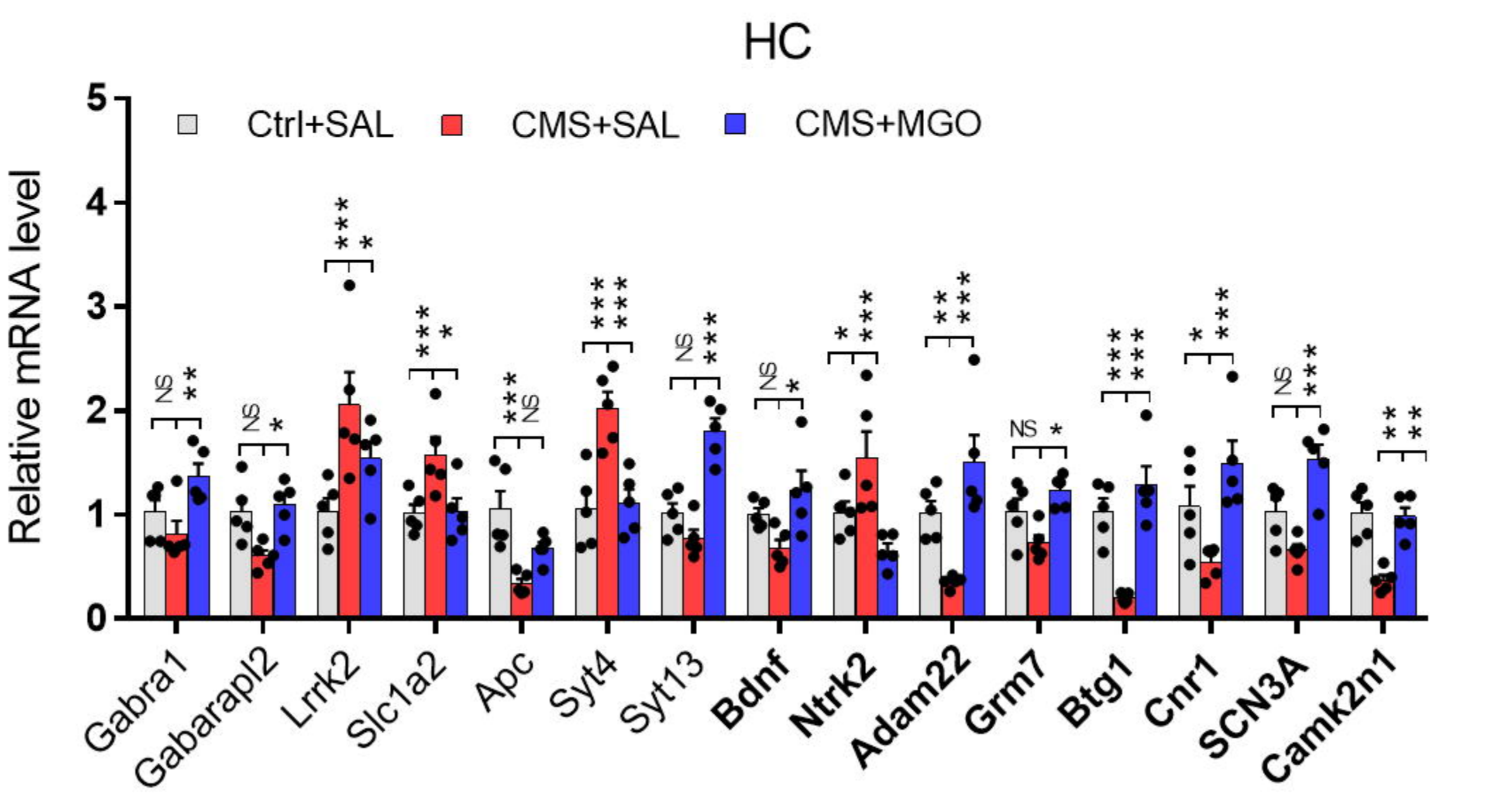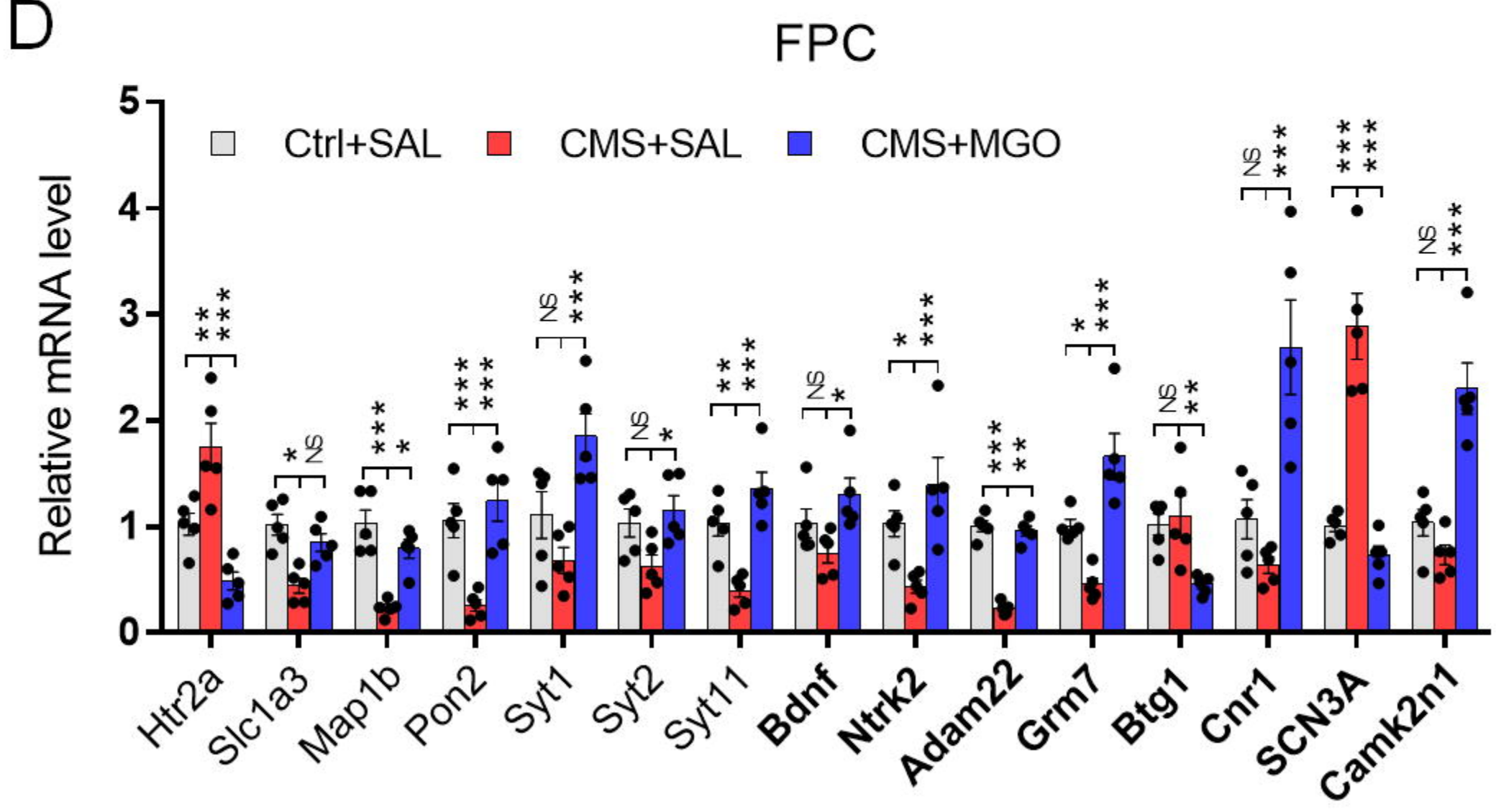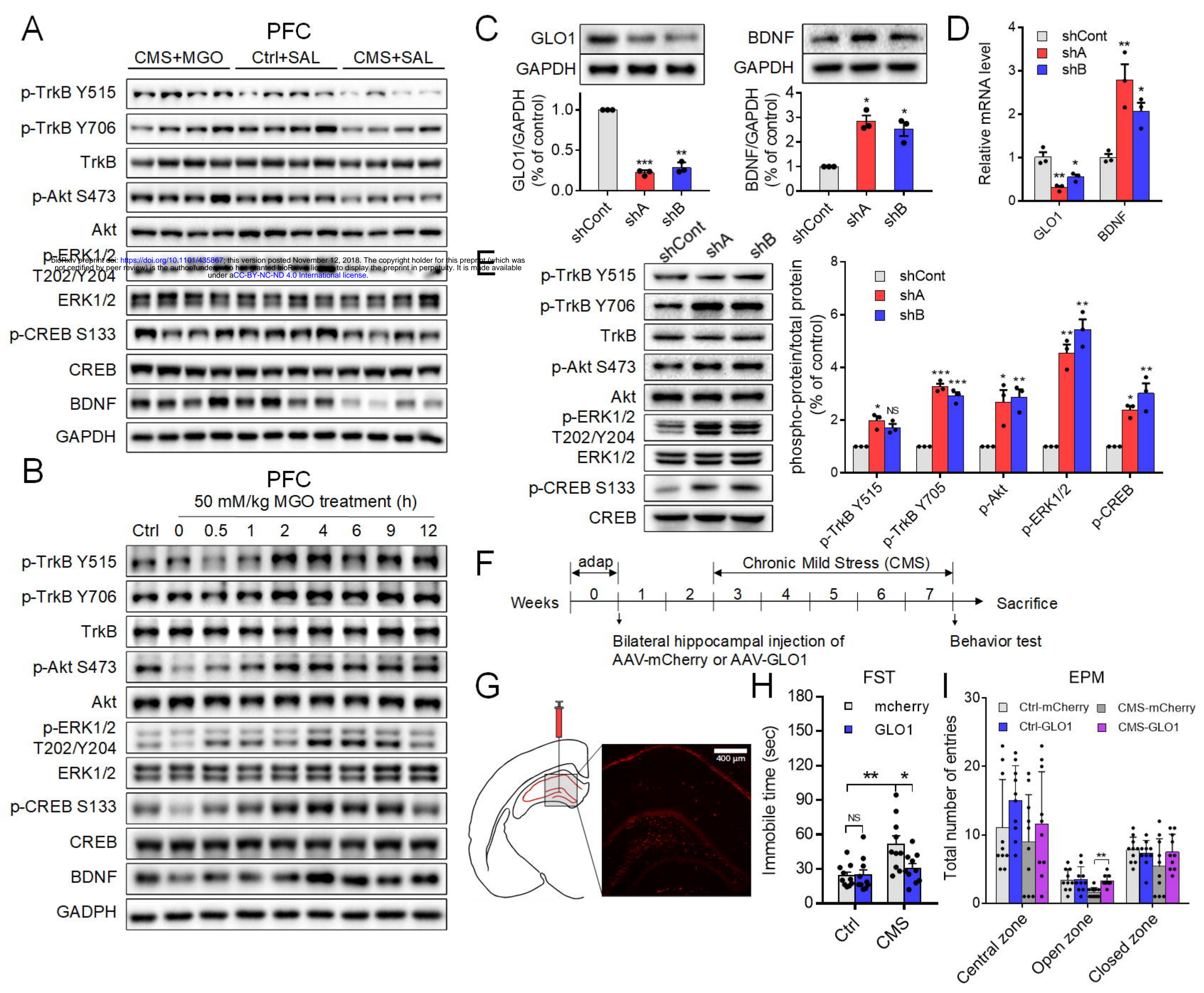
859

A

PFC

CMS+MGO   Ctrl+SAL   CMS+SAL

p-TrkB Y515
p-TrkB Y706
TrkB
p-Akt S473
Akt
p-ERK1/2 T202/Y204
ERK1/2
p-CREB S133
CREB
BDNF
GAPDH

B

PFC

50 mM/kg MGO treatment (h)

Ctrl   0   0.5   1   2   4   6   9   12

p-TrkB Y515
p-TrkB Y706
TrkB
p-Akt S473
Akt
p-ERK1/2 T202/Y204
ERK1/2
p-CREB S133
CREB
BDNF
GADPH

C

GLO1
GAPDH

BDNF
GAPDH

shCont shA shB

D

Relative mRNA level

shCont
shA
shB

GLO1   BDNF

E

shCont shA shB

p-TrkB Y515
p-TrkB Y706
TrkB
p-Akt S473
Akt
p-ERK1/2 T202/Y204
ERK1/2
p-CREB S133
CREB

phospho-protein/total protein (% of control)

shCont
shA
shB

p-TrkB Y515   p-TrkB Y705   p-Akt   p-ERK1/2   p-CREB

F

adap   Chronic Mild Stress (CMS)

Weeks 0   1   2   3   4   5   6   7   Sacrifice

Bilateral hippocampal injection of   Behavior test
AAV-mCherry or AAV-GLO1

G

400 μm

H

FST

Immobile time (sec)

mcherry
GLO1

Ctrl   CMS

I

EPM

Total number of entries

Ctrl-mCherry   CMS-mCherry
Ctrl-GLO1   CMS-GLO1

Central zone   Open zone   Closed zone