SUPPLEMENTARY INFORMATION

Material and methods

Determination of 4-hydroxynonenal (HNE)

The amount of lipid peroxidation was determined by measuring the level of 4-hydroxynonenal (HNE) using the OxiSelect™ HNE adduct ELISA kit (Cell Biolabs, Inc., San Diego, CA, U.S.A.) according to the manufacturer's instructions. 100 μL of prefrontal cortical homogenate at a protein concentration of 10 μg/mL was incubated in 96-well protein binding plates at 4 °C overnight. After protein adsorption, HNE adducts in each well were labeled with HNE antibody, followed by HRP-conjugated secondary antibody. Colorimetric development was then performed with substrate solution. Absorbance was recorded at 450 nm using a microplate reader (Molecular Devices Inc., Sunnyvale, CA, U.S.A.), and an amount of HNE adduct in each sample was calculated from the standard curve for HNE-BSA (Shin et al. 2014).

Western blot analysis

Western blotting analysis was performed as previously described (Shin et al., 2005). Proteins (20 μg/lane) were separated by 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membranes, The resulting blot was blocked in phosphate-buffered saline (PBS) containing 3 % skim milk for 30 min. Each blot was incubated overnight at 4 °C with the primary antibody against β-actin (1:300,000; Sigma-Aldrich), IL-1β (1:500; R&D Systems), IL-6 (1:1000; Abcam), IFN-γ (1:5000, Bio-Rad), or TNF-α (1:1000, R&D Systems). After washing in PBS, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary anti-rabbit

immunoglobulin (IgG; 1:5000; Thermo Scientific, Rockford, IL, U.S.A.), anti-goat IgG (1:1000, Sigma-Aldrich), or anti-mouse IgG (1:5000; Sigma-Aldrich) for 2 h. Subsequent visualization was performed using an enhanced chemiluminescence system (GenDEPOT, Houston, TX, U.S.A.). The relative intensities of the bands were quantified by PhotoCapt MW (version 10.01 for Windows) (Vilber Lourmat, Marne la Vallée, France).

Forced swimming test

A forced swimming test was performed as previously described (Noda et al., 1995). Briefly, mice were placed individually in polyethylene cylinders (20 cm high and 15 cm in diameter), containing water (at a temperature of 22 °C and depth of 15 cm) for 6 min. An automated video-tracking system (Noldus Information Technology, Wageningen, The Netherlands) was employed for recording. Immobility time was measured during the final 4 min of the 6-min test period by observers that were blind to the treatment conditions. A mouse was considered to be immobile if it was floating motionlessly or making only movements that were necessary to keep its head above water.

Supplementary Results

Effects of clozapine on PCP-induced alterations in cytosolic and mitochondrial lipid peroxidation in the prefrontal cortex (PFC) of wild-type (WT) and p47^{phox} knockout (KO) mice

As shown in supplementary Figure S1, repeated PCP treatment significantly increased cytosolic and mitochondrial lipid peroxidation (as evaluated by 4-hydroxynonenal; HNE) in the PFC of WT mice (cytosol, $P < 0.05$ vs. saline; mitochondria, $P < 0.01$ vs. saline). Clozapine significantly alleviated mitochondrial lipid peroxidation induced by PCP ($P < 0.05$

vs. WT treated with saline/PCP). Genetic depletion of p47^{phox} significantly attenuated against PCP-induced cytosolic and mitochondrial lipid peroxidation (cytosol, $P < 0.05$ vs. WT treated with saline/PCP; mitochondria, $P < 0.01$ vs WT treated with saline/PCP).

PCP-induced increases in pro-inflammatory cytokine expressions (i.e., IFN-γ, IL-1β, IL-6, TNF-α) in the prefrontal cortex of wild type mice

We investigated whether PCP treatment affects the expression of pro-inflammatory cytokines (i.e., IFN-γ, IL-1β, IL-6, TNF-α) in the PFC. As shown in supplementary Figure S2, the maximal induction of pro-inflammatory cytokines (IFN-γ, $P < 0.01$ vs. Saline; IL-1β, $P < 0.01$ vs. Saline; IL-6, $P < 0.01$ vs. Saline; TNF-α, $P < 0.01$ vs. Saline) were consistently observed 1 d after the final treatment with PCP. In addition, the significant inductions of IFN-γ and IL-1β remain elevated 4 d later (IFN-γ and IL-1β; $P < 0.05$ vs. Saline, respectively). The initial induction of TNF-α was observed 4 h post-PCP. The changes in IL-6 and TNF-α returned to near control level 4 d later (supplementary Figure S2).

Effects of LY294002, a PI3K inhibitor, on the clozapine-mediated pharmacological activity in response to PCP-induced immobility time in wild-type (WT) and p47^{phox} knockout (KO) mice

Forced swimming test has been widely employed to test antidepressant effect with good reliability and predictive validity, although its construct and face validity is questionable (Petit-Demouliere et al., 2005). In addition, it has been shown that several factors associated with depression, such as stress, social isolation, sleep deprivation and systemic inflammation, could change the basal immobility time in forced swimming test (Bogdanova et al., 2013; Brenes Saenz et al., 2006; Brenes et al., 2008; Lopez-Rodriguez et al., 2004; Tonelli et al., 2008), thus this test has been successfully used to examine depression-like behaviors in gene

mutant mice (Campos et al., 2014; Kaster et al., 2012; Yankelevitch-Yahav et al., 2015) or in rodents withdrawn from repeated treatment with psychostimulants (Horiuchi et al., 2013; Iijima et al., 2013), including PCP (Noda et al., 1995; Noda et al., 2000; Tran et al., 2017).

As shown in supplementary Figure S3, repeated PCP treatments significantly increased "immobility time" in the forced swimming test ($P < 0.05$ vs. saline). Either clozapine or LY294002 significantly attenuated the increase in immobility time 6 d post-PCP in WT mice ($P < 0.05$ vs WT treated with saline/PCP, respectively). LY294002 did not exhibit ant additional effects on clozapine-mediated attenuation against PCP insult. Genetic depletion of $p47^{phox}$ attenuated ($P < 0.05$ vs. WT-treated with saline/PCP) against the increase in immobility time 6 d after the final PCP in mice. Clozapine with or without LY294002 did not alter the attenuation mediated by $p47^{phox}$ knockout in mice.

Effects of clozapine on PHOX activity, ROS formation, protein carbonyl level, HNE level, SOD activity, and GPx activity in the cytosolic and mitochondrial fraction of the prefrontal cortex 6 d after the final PCP treatment in the WT and $p47^{phox}$ knockout mice.

As shown in supplementary Figure S4 –S6, we examined whether clozapine alters changes in PHOX, ROS, protein carbonyl, HNE levels, and SOD and GPx activities 6 d after the final PCP treatment. Neither PCP nor clozapine significantly changed these parameters in WT and $p47^{phox}$ knockout mice, indicating that all the values were returned to saline (control) level 6 d post-PCP.

Effects of intra-mitochondrial Ca^{2+}, mitochondrial membrane potential, mitochondrial complex I, mitochondrial complex II activities 6 d after the final PCP treatment of the prefrontal cortex in the WT and $p47^{phox}$ knockout mice.

As shown in supplementary S7-S8, we examined whether clozapine alters changes in

mitochondrial dysfunction 6 d after the final PCP treatment. Neither PCP nor clozapine significantly changed these parameters in WT and p47^{phox} knockout mice, indicating that PCP-induced mitochondrial parameters initially altered, then they already returned to saline (control) level 6 d post-PCP.

Supplementary References

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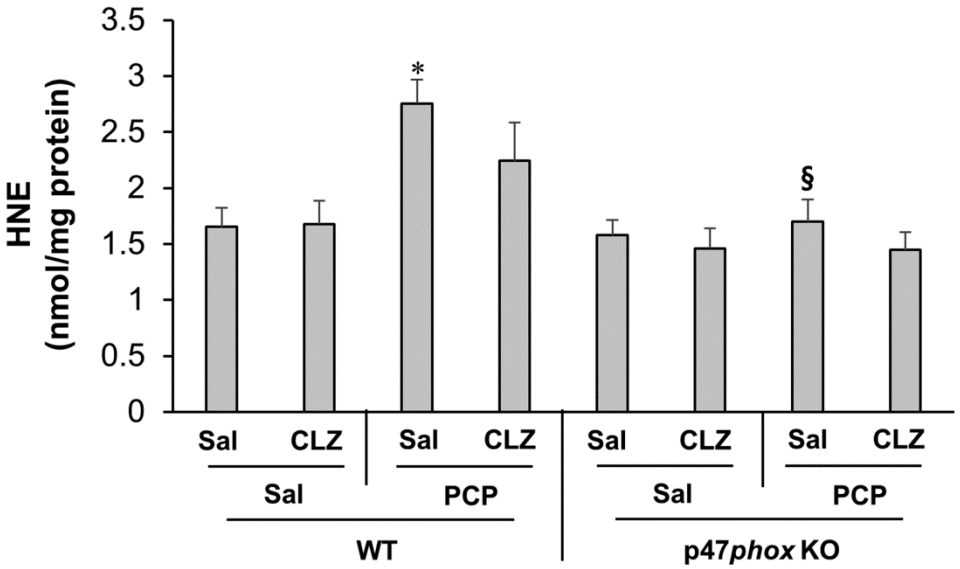
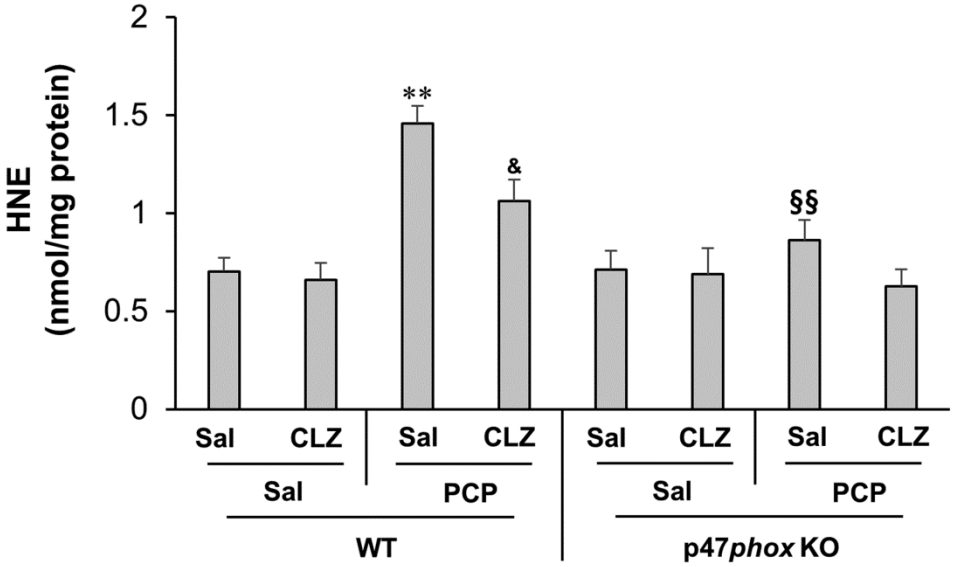
Supplementary Figure Legends

Supplementary Figure S1. Effects of clozapine (CLZ, 10 mg/kg/day, p.o.) on lipid peroxidation (as evaluated by 4-hydroxynonenal; HNE) 1 d after the final PCP treatment in the prefrontal cortex of wild type (WT) and $p47^{phox}$ knockout (KO) mice. **a.** Changes in cytosolic HNE. **b.** Changes in mitochondrial HNE. Each value is the mean ± SEM of six mice. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. corresponding saline. $^{\&}P < 0.05$ vs. corresponding saline with PCP. $^{\S}P < 0.05$, $^{\S\S}P < 0.01$ vs. corresponding WT.

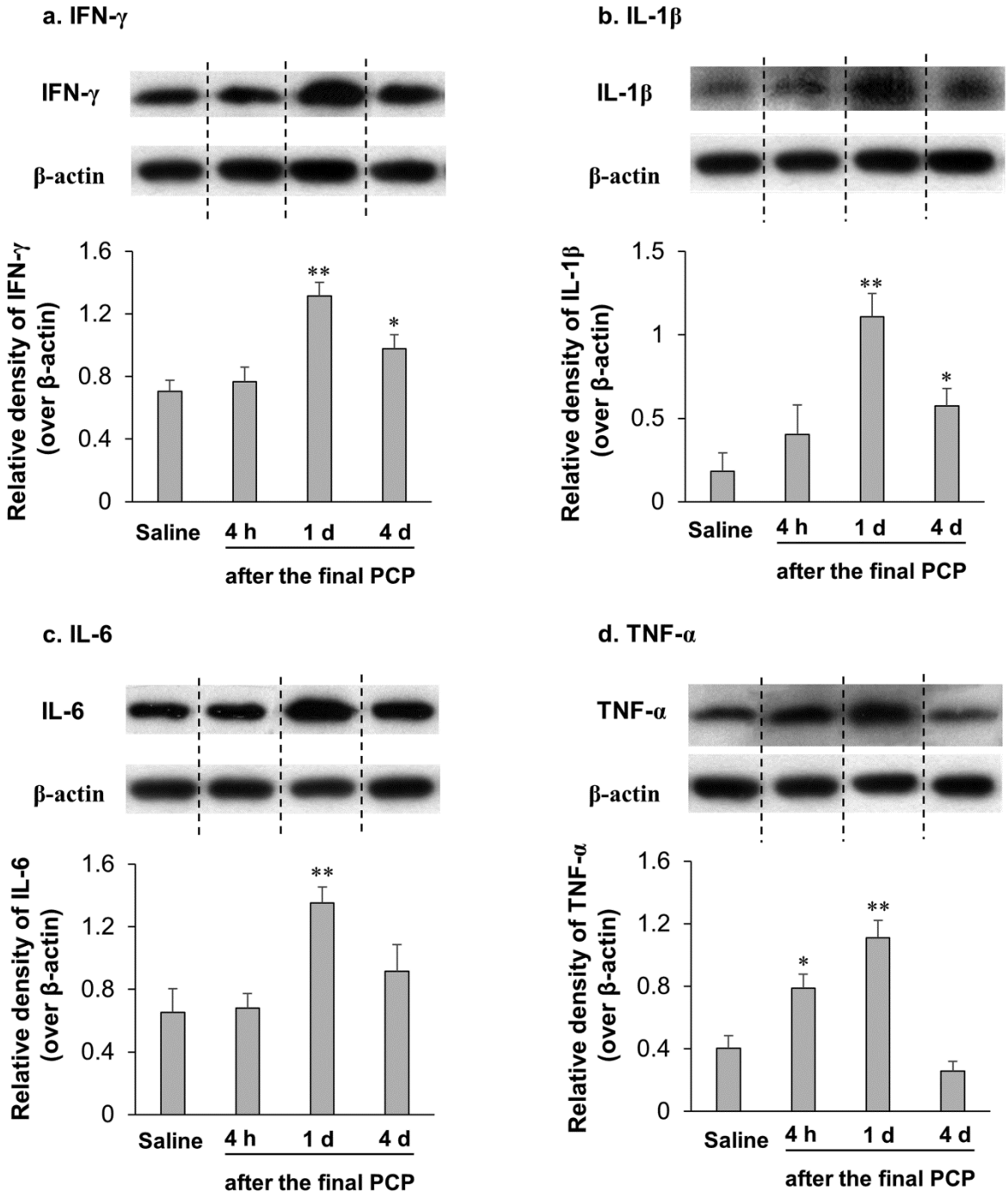
a. Lipid peroxidation – Cytosolic – 1 d post-PCP



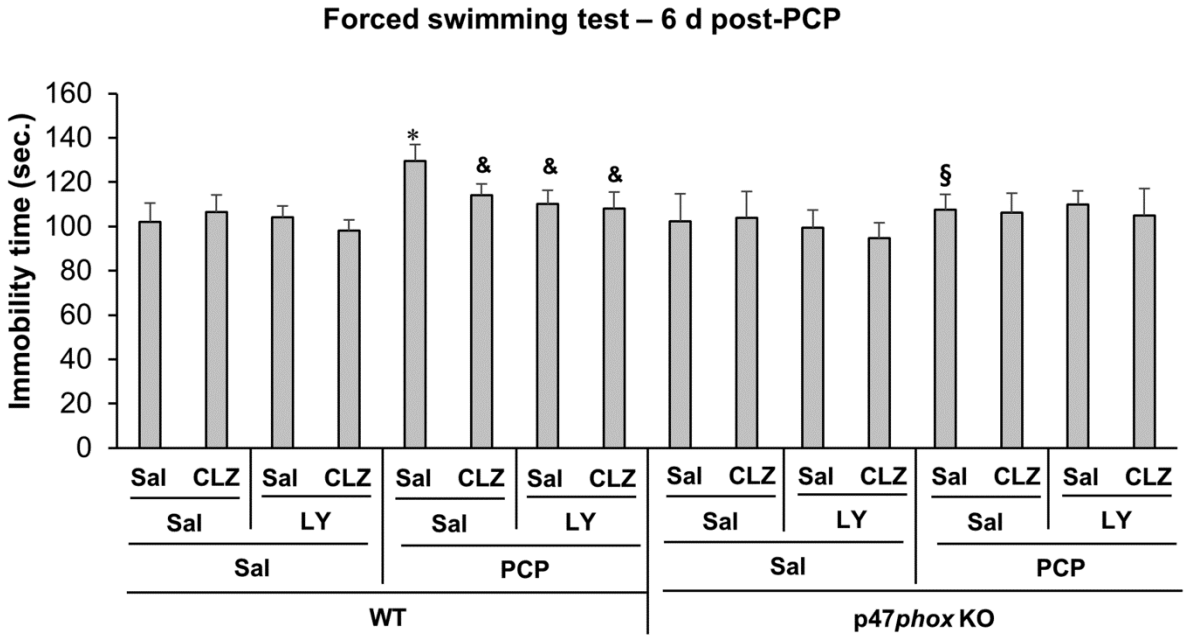
b. Lipid peroxidation – Mitochondrial – 1 d post-PCP



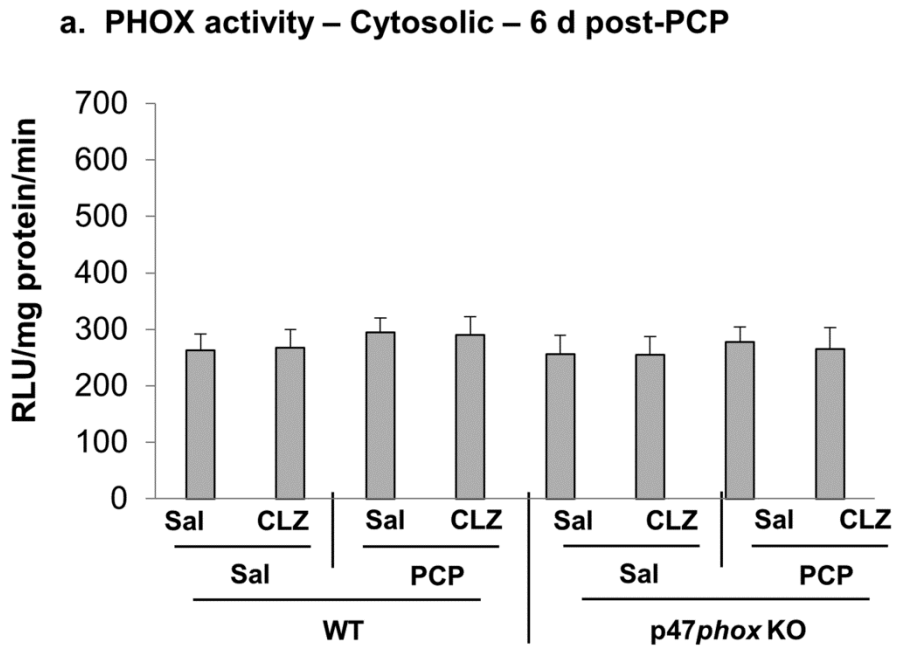
Supplementary Figure S2. Changes in the expression of pro-inflammatory cytokines 4h, 1 d and 4 d after the final PCP treatment in the prefrontal cortex of wild type mice. a. Changes in IFN-γ expression. b. Changes in IL-1β expression. c. Changes in IL-6 expression. d. Changes in TNF-α expression. Each value is the mean ± SEM of six mice. *P < 0.05 vs. corresponding saline. $^{**}P < 0.01$ vs. corresponding saline.

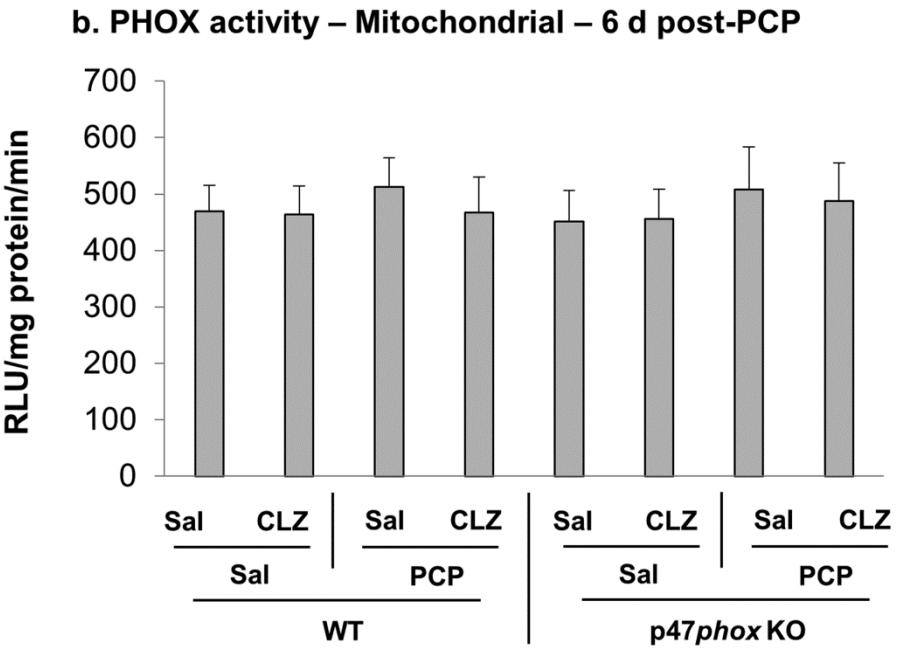


Supplementary Figure S3. Effects of LY 204002 (LY) on the pharmacological activity of clozapine (CLZ, 10 mg/kg/day, p.o.) in response to the immobility time in forced swimming test 6 d after the final PCP treatment in wild type (WT) and p47^{phox} knock out (KO) mice. Each value is the mean ± SEM of ten mice. $^{*}P < 0.05$ vs. corresponding saline. $^{\&}P < 0.05$ vs. corresponding saline with PCP. $^{\S}P < 0.05$ vs. corresponding WT.

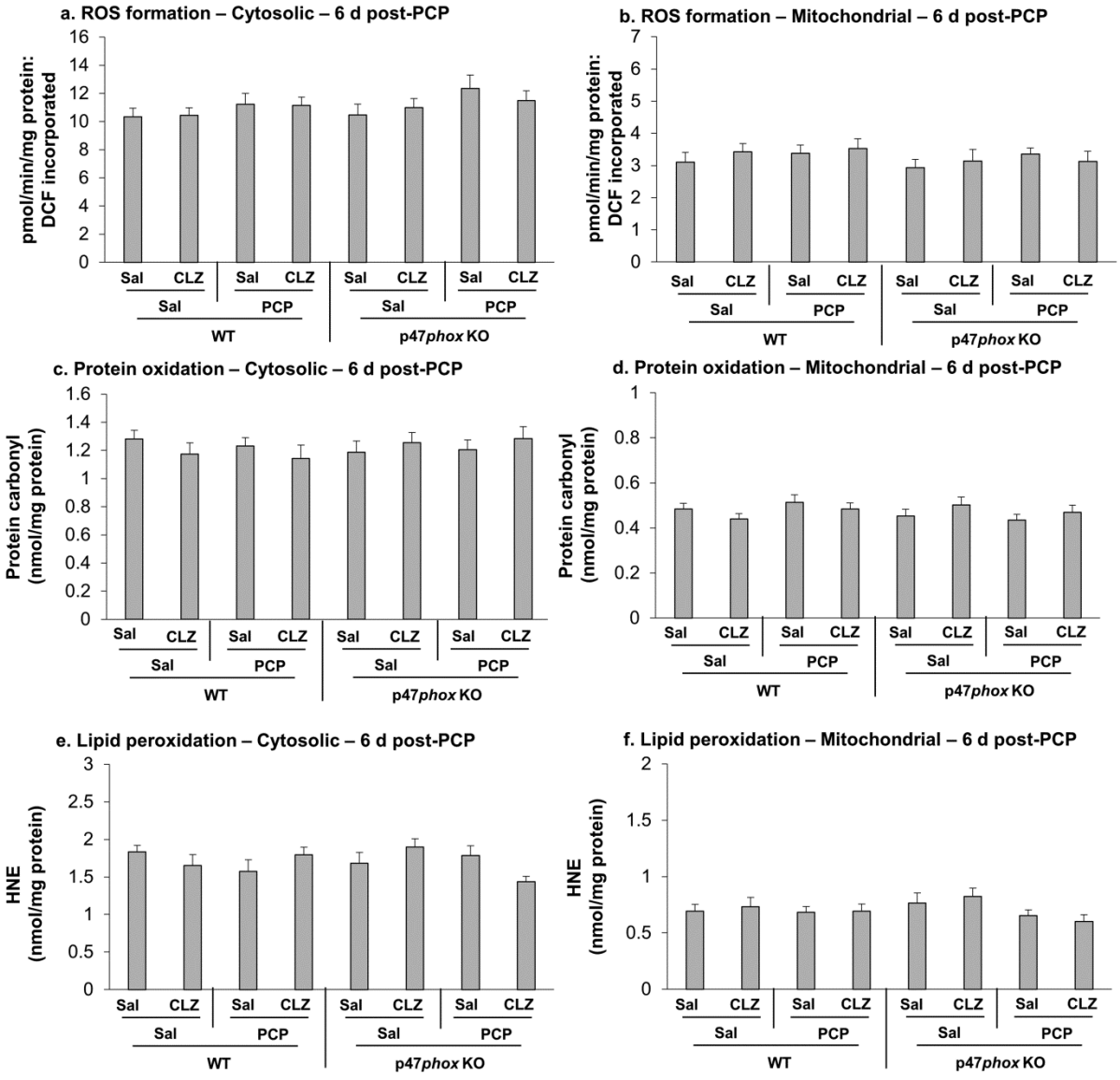


Supplementary Figure S4. Effects of clozapine (CLZ, 10 mg/kg/day, p.o.) on NADPH oxidase (PHOX) activity 6 d after the final PCP treatment in the prefrontal cortex of wild type (WT) and p47^{phox} knockout (KO) mice. **a.** Changes in cytosolic PHOX oxidase activity. **b.** Changes in mitochondrial PHOX oxidase activity. Each value is the mean ± SEM of six mice.

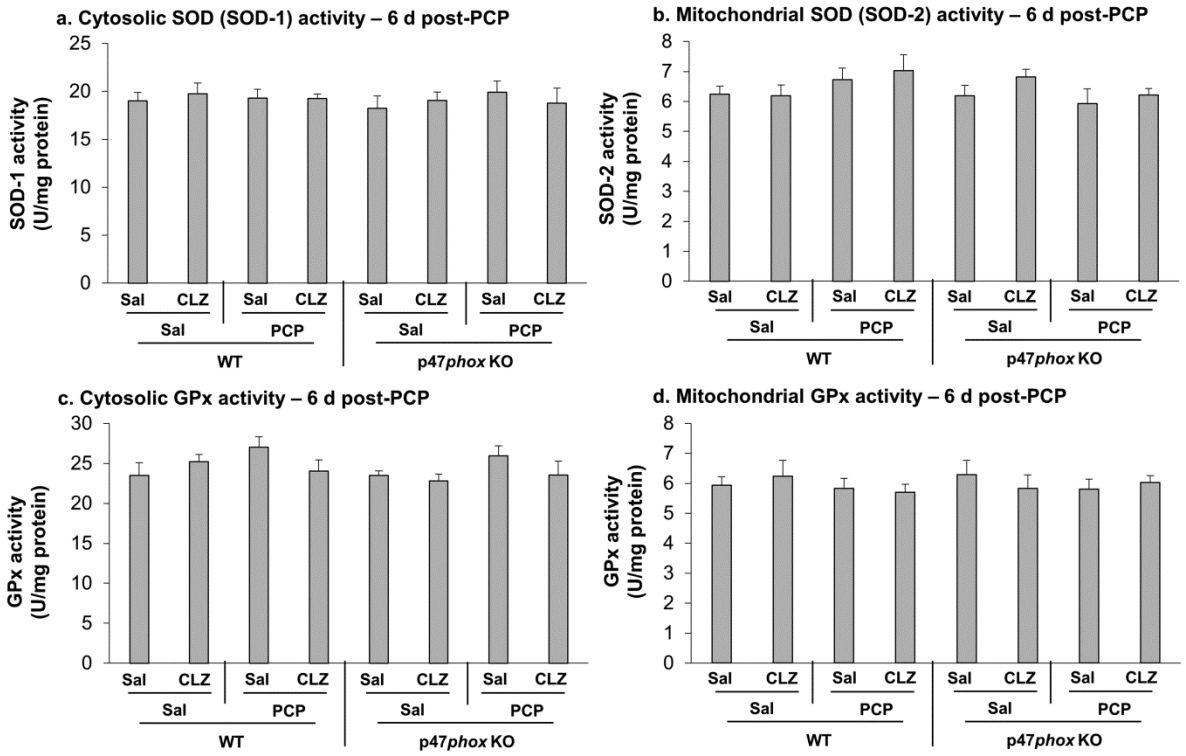




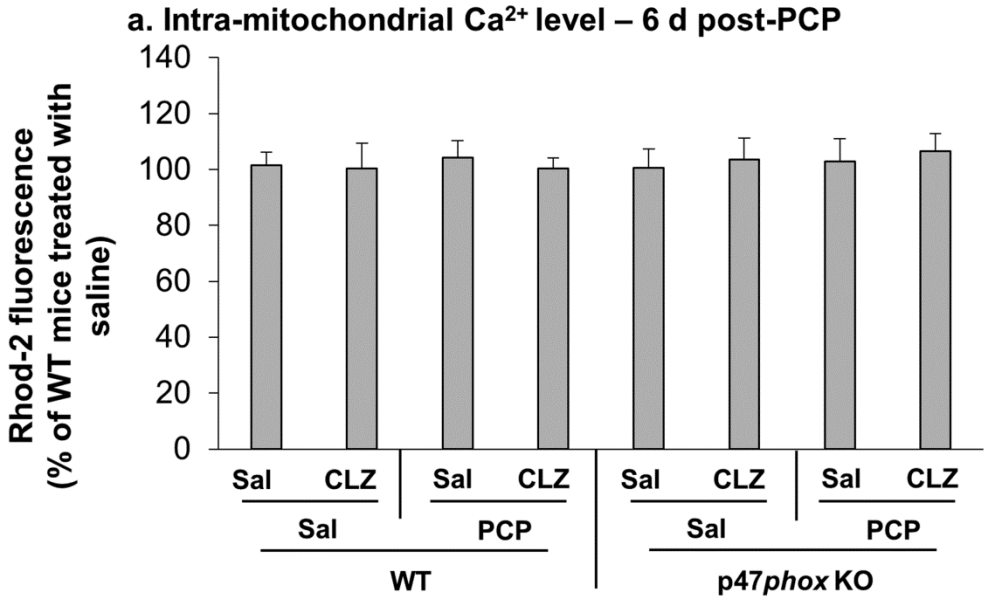
Supplementary Figure S5. Effects of clozapine (CLZ, 10 mg/kg/day, p.o.) on ROS formation, protein carbonyl, and HNE 6 d after the final PCP treatment in the prefrontal cortex of wild type (WT) and p47^{phox} knock out (KO) mice. **a.** Changes in cytosolic ROS formation. **b.** Changes in mitochondrial ROS formation. **c.** Changes in cytosolic protein oxidation (as shown by protein carbonyl). **d.** Changes in mitochondrial protein oxidation (as shown by protein carbonyl). **e.** Changes in cytosolic lipid peroxidation (as shown by HNE). **f.** Changes in mitochondrial lipid peroxidation (as shown by HNE).. Each value is the mean ± SEM of six mice.

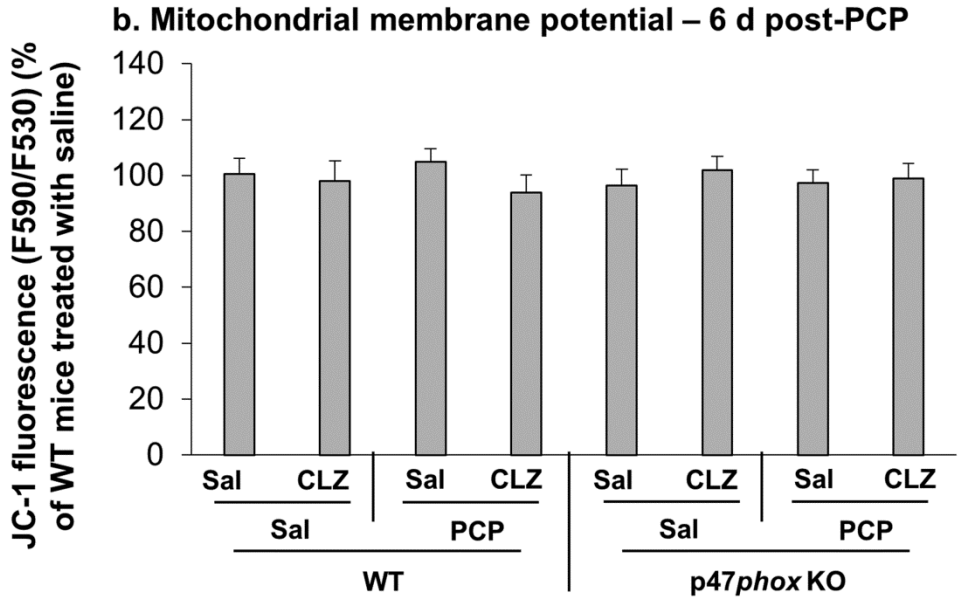


Supplementary Figure S6. Effects of clozapine (CLZ, 10 mg/kg/day, p.o.) on SOD, and GPx activities 6 d after the final PCP treatment in the prefrontal cortex of wild type (WT) and p47^{phox} knock out (KO) mice. **a.** Changes in cytosolic SOD (SOD-1) activity. **b.** Changes in mitochondrial SOD (SOD-2) activity. **c.** Changes in cytosolic GPx activity. **d.** Changes in mitochondrial GPx activity. Each value is the mean ± SEM of six mice.



Supplementary Figure S7. Effects of clozapine (CLZ, 10 mg/kg/day, p.o.) on intra-mitochondrial Ca^{2+} level and mitochondrial membrane potential 6 d after the final PCP treatment in the prefrontal cortex of wild type (WT) and p47^{phox} knock out (KO) mice. **a.** Changes in intra-mitochondrial Ca^{2+} level. **b.** Changes in mitochondrial membrane potential. Each value is the mean ± SEM of six mice.





Supplementary Figure S8. Effects of clozapine (CLZ, 10 mg/kg/day, p.o.) on mitochondrial complex I and II activities 6 d after the final PCP treatment in the prefrontal cortex of wild type (WT) and p47^{phox} knock out (KO) mice. **a.** Changes in mitochondrial complex I activity. **b.** Changes in mitochondrial complex II activity. Each value is the mean ± SEM of six mice.