

**Chemistry & Biology, Volume 22**

**Supplemental Information**

**Peroxide-Dependent MGL Sulfenylation Regulates**

**2-AG-Mediated Endocannabinoid Signaling**

**in Brain Neurons**

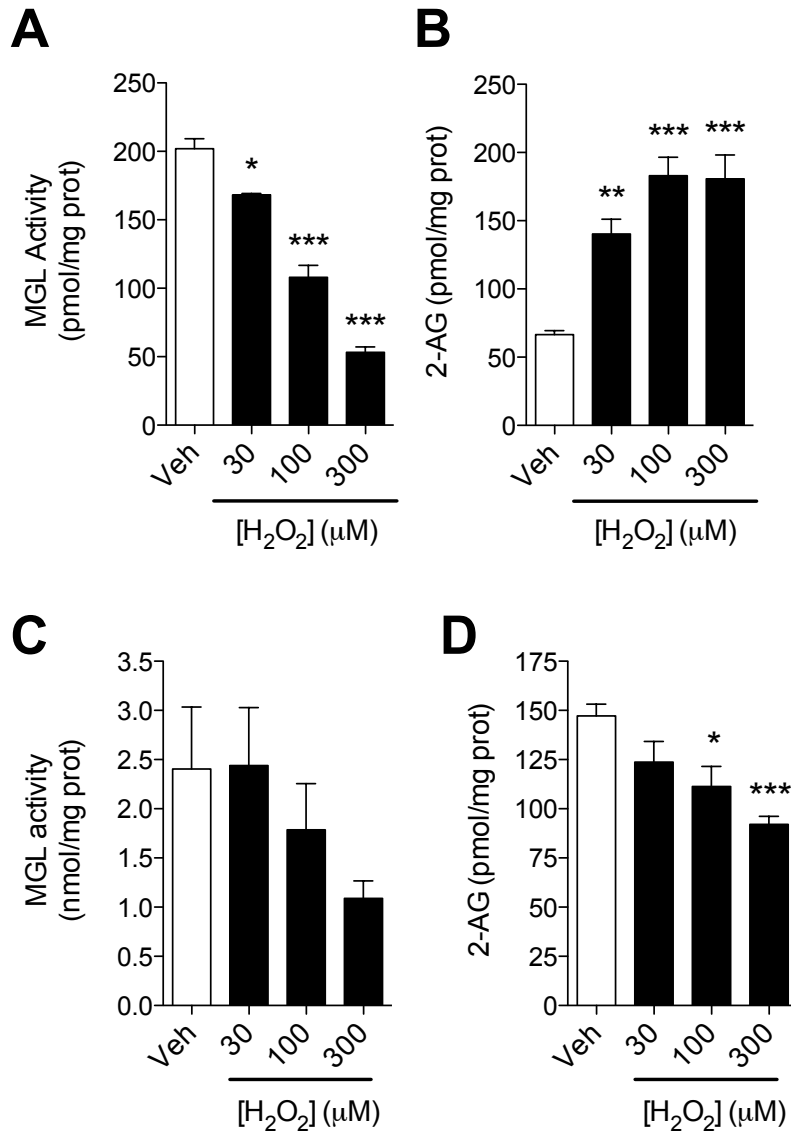
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## **SUPPLEMENTAL INFORMATION**

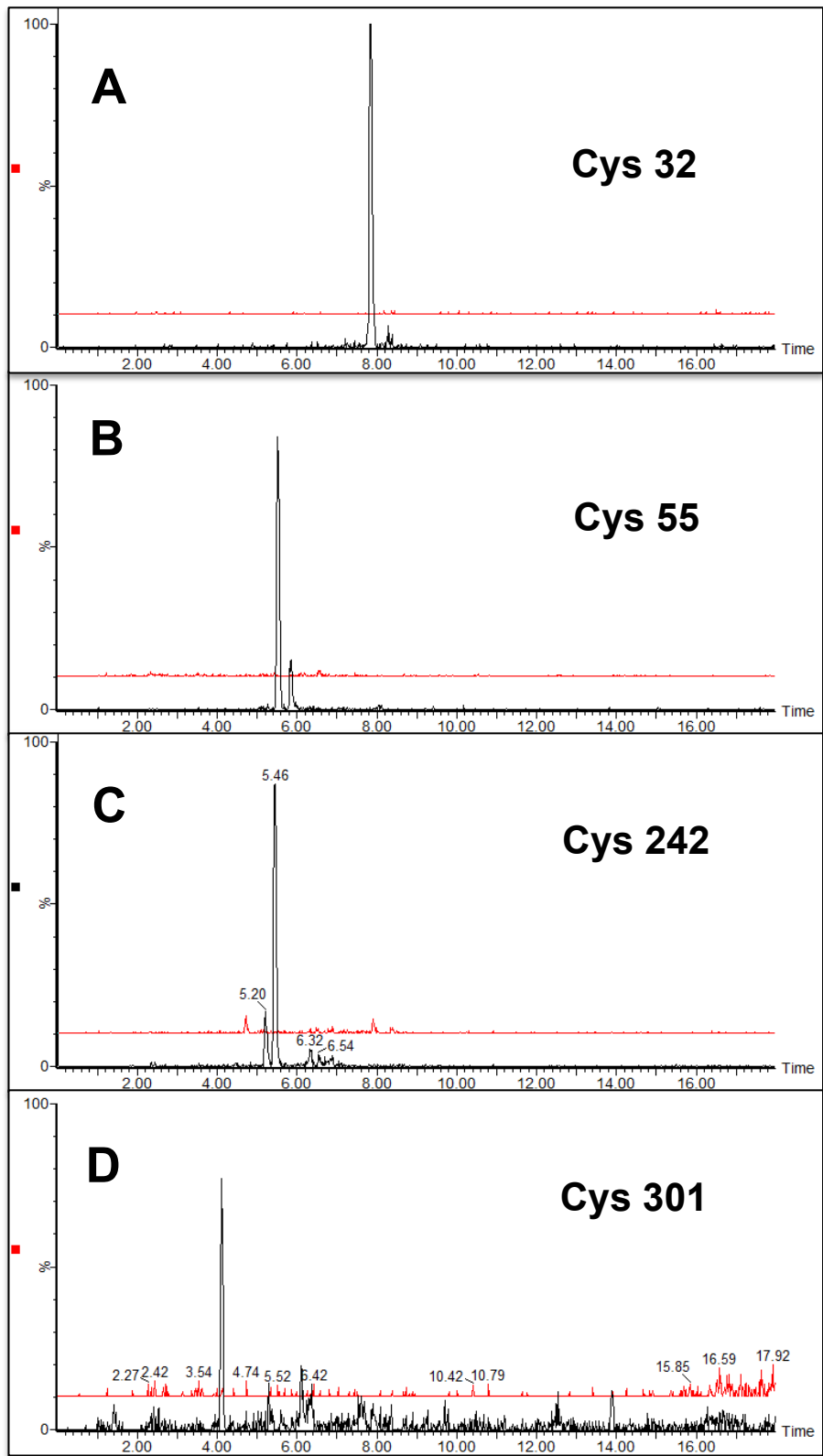
Figure S1-S6

Supplemental Figure Legends

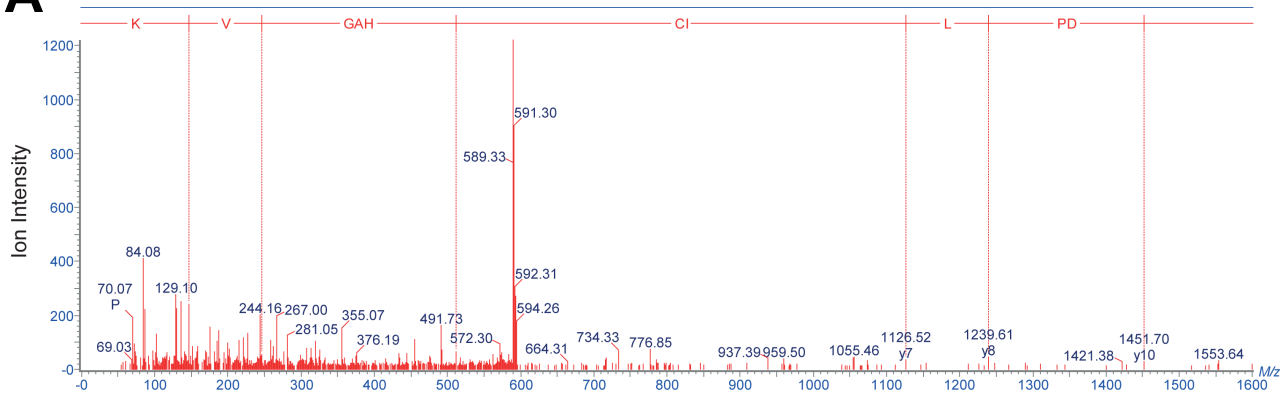
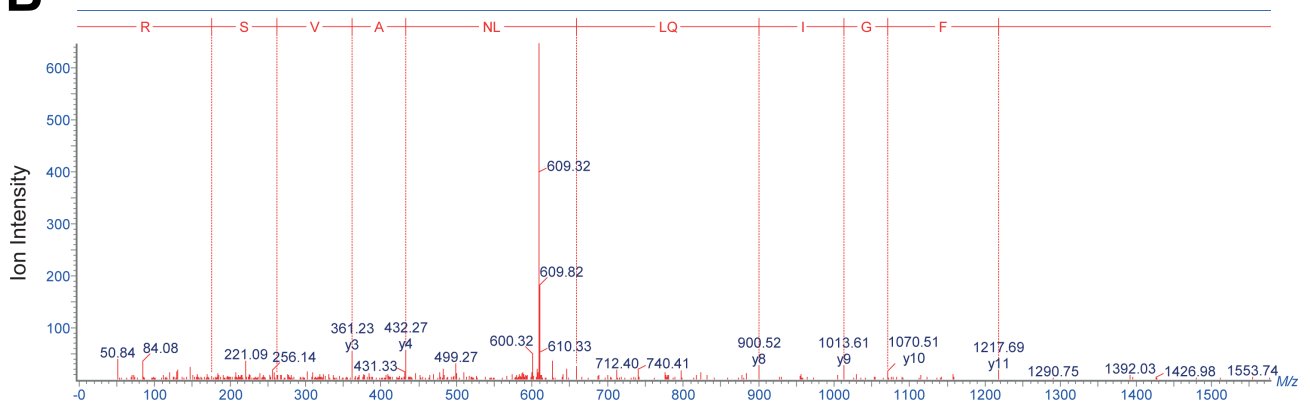
Table S1

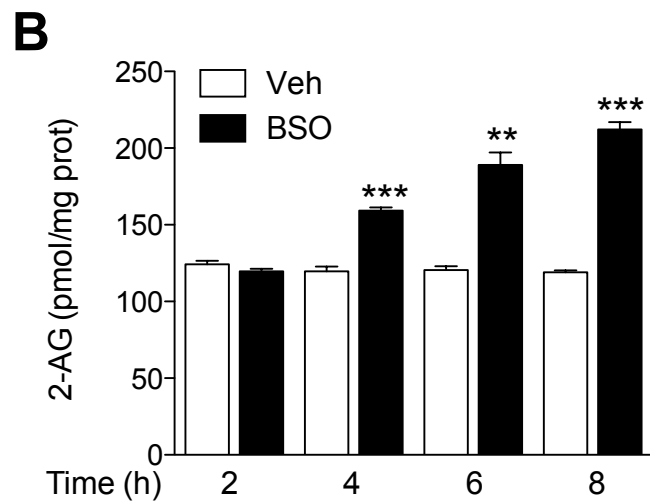
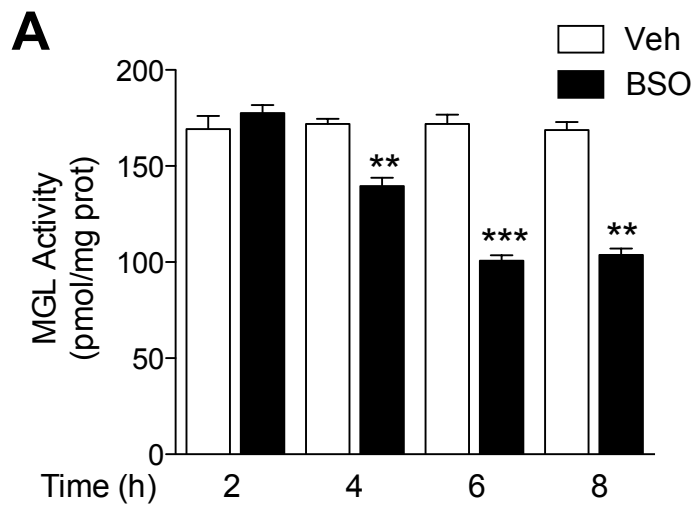


**Figure S1**

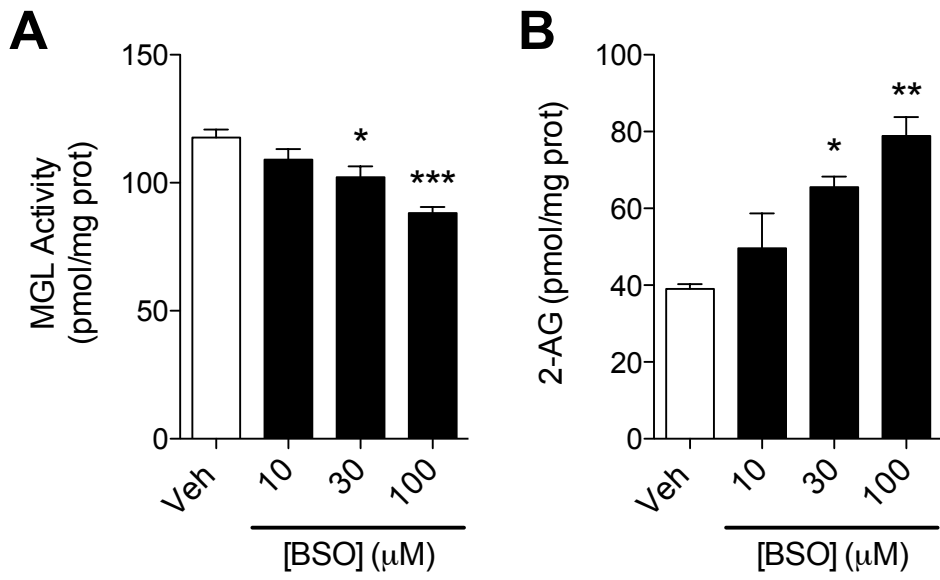


**Figure S2**

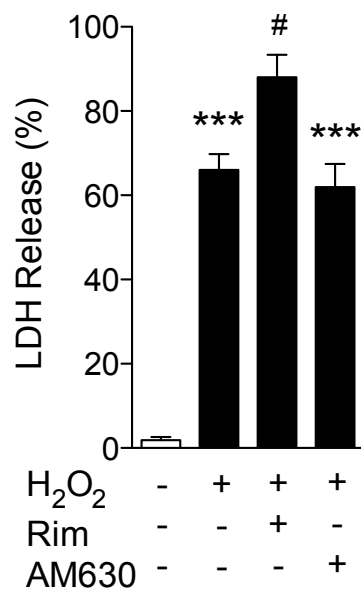
**A****B****Figure S3**



**Figure S4**



**Figure S5**



**Figure S6**



## SUPPLEMENTAL FIGURE LEGENDS

**Figure S1, Related to Figure 1. Hydrogen peroxide inhibits MGL activity and increases 2-AG levels in Neuro-2a cells.** (A-B) Effects of H<sub>2</sub>O<sub>2</sub> (filled bars) or vehicle (open bars) in naïve Neuro-2a cells on the (A) MGL activity and (B) 2-AG levels. (C-D) Effects of H<sub>2</sub>O<sub>2</sub> (filled bars) or vehicle (open bars) in Neuro-2a cells transfected with pEF6-C201/208A MGL plasmids, encoding the double Cysteine mutant form of MGL, on the (C) MGL activity and (D) 2-AG levels.

**Figure S2, Related to Figure 2. Hydrogen peroxide sulfenylates C201 and C208.** No other peptides forming DMD adducts were observed in the tryptic digest of rat MGL incubated with 10 μM H<sub>2</sub>O<sub>2</sub> (in the presence of dimedone). (A) Extracted ion current of peptide TPQNV<sup>+</sup>PYQDLPH LVNADGQYLFC<sub>32</sub>R as naïve (black trace, 930.12 m/z, charge state +3) and dimedone adduct form (red trace, 976.71 m/z, charge state +3). (B) Extracted ion current of peptide ALIFVSHGAGEHC<sub>55</sub>GR as naïve (black trace, 518.59 m/z, charge state +3) and dimedone adduct form (red trace, 564.84 m/z, charge state +3). (C) Extracted ion current of peptide LC<sub>242</sub>DSK as naïve (black trace, 564.28 m/z, charge state +1) and dimedone adduct form (red trace, 703.35 m/z, charge state +1). (D) Extracted ion current of peptide C<sub>301</sub>LP as naïve (black trace, 332.13 m/z, charge state +1) and dimedone adduct form (red trace, 471.40 m/z, charge state +1).

**Figure S3, Related to Figure 4. Accurate MS/MS spectra of peptides obtained by tryptic digestion of MGL.** (A) Peptide SEVDLYNSDPLIC\*HAGVK (590.53 m/z, z=4) bearing C201 covalently linked with BP1. (B) Peptide VC\*FGIQLLNAVSR (606.30 m/z, z=3) bearing C208 covalently linked with BP1.

**Figure S4, Related to Figure 5. BSO inhibits MGL activity and increases 2-AG levels in brain neurons.** Time-course of the effects of BSO (100 μM, filled bars) or vehicle (open bars)

on (A) MGL activity and (B) 2-AG levels in rat cortical neurons in primary cultures. \*\*\* $P < 0.001$ , and \*\* $P < 0.01$  compared to vehicle, two-tailed Student's  $t$  test.

**Figure S5, Related to Figure 5. BSO inhibits MGL activity and increases 2-AG levels in Neuro-2a cells.** Effects of BSO (filled bars) or vehicle (open bars) on (A) MGL activity and (B) 2-AG levels in Neuro-2a cells. \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$  compared to vehicle, two-tailed Student's  $t$  test.

**Figure S6, Related to Figure 7. CB<sub>1</sub> receptor blockade enhances the cytotoxic effects of H<sub>2</sub>O<sub>2</sub> in rat brain neurons.** Effects of H<sub>2</sub>O<sub>2</sub> (300  $\mu$ M, filled bars), alone or combined with CB<sub>1</sub> antagonist rimonabant (1  $\mu$ M) or CB<sub>2</sub> antagonist AM630 (1  $\mu$ M), on LDH release from rat cortical neurons in primary cultures. \*\*\* $P < 0.001$  compared to vehicle, and # $P < 0.05$  compared to H<sub>2</sub>O<sub>2</sub> only, two-tailed Student's  $t$  test.

## SUPPLEMENTAL TABLE

**Table S1, Related to Figure 1.** Median inhibitory concentration ( $IC_{50}$ ) values of MGL inhibition by hydrogen peroxide.

MGL	$IC_{50}$ ( $\mu$ M)
Wild-type	8.2
C201A	30.7
C208A	22.7
C201/208A	473.7