**SUPPLEMENTARY MATERIAL**

## List of Supplementary Tables

**Supplementary Table 1.** Patient demographics and clinical characteristics for cerebrospinal fluid samples, presented in Figs 1 and 2.

**Supplementary Table 2.** List of lipids and their catalog numbers, used in the Mini Array experiments shown in Fig. 2.

**Supplementary Table 3.** Total phospholipid levels in control and MS brain samples (nmol/mg protein), as assessed by shotgun lipidomics. The lipid classes identified are shown.

**Supplementary Table 4.** Content of individual lipid species levels in control and MS brain samples, as assessed by single-reaction monitoring.

**Supplementary Table 5.** Total content of palmitic acid in each individual phospholipid class in age-matched MS and normal brain samples, as assessed by single reaction monitoring.

**Supplementary Table 6.** The effect of phospholipase inhibitors on azPC-mediated inhibition of MBP-specific T cell proliferation. Summary of data presented in Fig. S14.

## List of Supplementary Figures

**Fig. S1.** CSF levels of IgG and IgM are elevated in MS.

**Fig. S2.** PGPC, but not sphingomyelin, ameliorates established EAE.

**Fig. S3.** PGPC treatment of mice with EAE lowers T-cell expression of early-activation marker, proliferation, and IFN-γ and TNF production in response to encephalitogenic antigen.

**Fig. S4.** DGPC binds to the PVDF membrane used in the lipid arrays.

**Fig. S5.** Levels of antibodies against PGPC, POPS, azPC-ester, and azPC are higher in RRMS than in OND CSF.

**Fig. S6.** PGPC is present in human brain extract.

**Fig. S7.** azPC is present in human brain extract.

**Fig. S8.** azPC ester is present in human brain extract.

**Fig. S9**. Oxidized phospholipids suppress proliferation of autoreactive T cells.

**Fig S10.** PGPC, azPC ester, azPC, and POPS suppress $PLP_{139-151}$-induced cytokine production and proliferation of splenocytes.

**Fig. S11.** POPS, PGPC, azPC ester, or azPC decrease T-cell proliferation independently of CD1d.

**Fig. S12.** Oxidized lipids suppress proliferation and induce apoptosis of macrophages.

**Fig. S13**. Oxidized lipids have differential effects on proliferation and apoptosis of naive B cells.

**Fig. S14.** Inhibitors of $sPLA_2$ reverse the inhibitory effect of azPC- on $MBP_{Ac1-11}$-induced T-cell proliferation.

## Supplementary Figure Legends

**Fig S1.** Cerebrospinal fluid levels of IgG and IgM are elevated in MS. Levels of total IgG (**A**) or total IgM (**B**) in cerebrospinal fluid (CSF) from patients with relapsing remitting MS (RRMS) or other (non-inflammatory) neurological disease (OND). *$P \geq 0.05$ by Student's *t*-test. (**C**) Comparison of levels of total IgG in CSF from patients with RRMS, OND, or secondary progressive MS (SPMS), or from healthy controls (HC). *$P > 0.05$ by one-way ANOVA.

**Fig S2.** PGPC, but not sphingomyelin, ameliorates established EAE. (**A**) EAE severity in $PLP_{139-151}$-immunized mice administered PGPC or sphingomyelin during the immunization. Six micrograms of PGPC or sphingomyelin were administered during the immunization of mice with $PLP_{139-151}$, and on days 4 and 7 after the immunization. During the immunization, lipids were mixed with the $PLP_{139-151}$-CFA emulsion and injected subcutaneously. On days 4 and 7, lipids were solubilized in 0.05% Tween-20 in PBS and injected into the intraperitoneal cavity. Each point represents the mean + s.e.m. $^{+}P < 0.05$ by Mann-Whitney test comparing vehicle-treated ($n = 5$) and PGPC-treated ($n = 5$) mice; $*P < 0.05$ by Mann-Whitney test comparing vehicle-treated ($n = 5$) and sphingomyelin-treated ($n = 5$) mice. (**B**) EAE severity in $PLP_{139-151}$-immunized mice administered PGPC, sphingomyelin, or vehicle at the onset of disease. Upon developing clinical signs of EAE, mice were intravenously administered 100 µg of PGPC or sphingomyelin , or vehicle alone for a total of five intravenous injections. Each point represents the mean + s.e.m. $^{+}P < 0.05$ by Mann-Whitney test comparing vehicle-treated ($n = 10$) and PGPC-treated ($n = 9$) mice; $*P < 0.05$ by Mann-Whitney test comparing vehicle-treated ($n = 10$) and sphingomyelin-treated ($n = 9$) mice.

**Fig. S3.** PGPC treatment of mice with EAE suppresses T-cell activation. (**A**) Expression of CD69 (an early marker of activation) on $CD4^{+}$ and $CD8^{+}$ propidium iodide-negative (i.e. live) lymphocytes isolated from PGPC- or vehicle-treated EAE mice and cultured for 4 days with 10 µg/ml of $PLP_{139-151}$. The ratio of $CD69^{+}CD4^{+}$:$CD69^{-}CD4^{+}$ is 14.2%:39.6% for lymph node cells from vehicle-treated EAE mice, and 7.96%:55.2% for

cells from PGPC-treated EAE mice. $CD69^+CD4^+$ cells and $CD69^+CD8^+$ cells are boxed in red, and percentages of cells in each quadrant are displayed. (**B**) Proliferation and (**C**) cytokine production of splenocytes isolated from the vehicle- or PGPC-treated EAE mice in panel A. Splenocytes from PGPC-treated mice (gray bars) secreted lower levels of IFN-γ and TNF in response to $PLP_{139-151}$. $^*P < 0.05$, respectively, by Student's *t*-test comparing $PLP_{139-151}$-stimulated cells from PGPC-treated mice with $PLP_{139-151}$-stimulated cells from vehicle-treated mice.

**Fig. S4.** DGPC binds to the PVDF membrane used in the lipid arrays. Luxol fast blue staining of 1-Palmitoyl-2-Azelaoyl-sn-Glycero-3-Phosphocholine (azPC), 1,2-Dipropionoyl-sn-Glycero-3-Phosphocholine (DGPC), L-α-phosphatidylserine (PS), and Cardiolipin (CL) attached to the PVDF membrane used in the lipid antigen arrays in Figs. 1A and 2A, C.

**Fig. S5.** Levels of antibodies against PGPC, POPS, azPC-ester, and azPC are higher in RRMS than in OND cerebrospinal fluid. ELISA analysis of autoantibodies to (**A**) PGPC; (**B**) POPS; (**C**) azPC ester; and (**D**) azPC in cerebrospinal fluid from patients with relapsing remitting MS (RRMS) or other neurological disease (OND). $^*P < 0.05$ by Student's *t*-test.

**Fig. S6.** PGPC is present in human brain. LC-HRMS detection of PGPC in human brain sample obtained at autopsy. (**A**) Accurate mass agreement with calculated mass, (**B**) retention time on the column in comparison to authentic standards, and (**C**) MS/MS fragmentation patterns in comparison to authentic standards.

**Fig. S7.** azPC is present in human brain. LC-HRMS detection of azPC in human brain sample obtained at autopsy. (**A**) Accurate mass agreement with calculated mass, (**B**) retention time on the column in comparison to authentic standards, and (**C**) MS/MS fragmentation patterns in comparison to authentic standards.

**Fig. S8.** azPC ester is present in human brain. LC-HRMS detection of azPC ester in human brain sample obtained at autopsy. (**A**) Accurate mass agreement with calculated mass, (**B**) retention time on the column in comparison to authentic standards, and (**C**) MS/MS fragmentation patterns in comparison to authentic standards.

**Fig. S9.** Oxidized phospholipids suppress proliferation of autoreactive T cells. Proliferation of naive splenocytes stimulated for 48 hours with $MBP_{Ac1-11}$ in the presence of 30 μg/ml of PGPC or lipids related to PGPC: (**A**) other fatty side-chain derivatives, or (**B**) other head-group derivatives. *$P < 0.05$ by Student's *t*-test. Values are the mean ± s.e.m. of triplicates. Results are representative of 2 independent experiments.

**Fig. S10.** PGPC, azPC ester, azPC, and POPS suppress $PLP_{139-151}$-induced cytokine production and proliferation of splenocytes. (**A**) Cytokine production and (**B**) proliferation of splenocytes isolated from $PLP_{139-151}$-immunized SJL mice (10 days after immunization) and re-stimulated *in vitro* with $PLP_{139-151}$ in the presence of 30 μg/ml of lipids for 48 hours. *$P < 0.05$ by Student's *t*-test. Values are the mean ± s.e.m. of triplicates. Results are representative of 2 independent experiments.

**Fig. S11.** POPS, PGPC, azPC ester, and azPC suppress T-cell proliferation independently of CD1d. Proliferation of T cells isolated from splenocytes of wild-type (*wt*) and CD1d-deficient (*Cd1d*$^{-/-}$) mice and stimulated for 48 hours with (**A**) anti-CD3 or (**B**) anti-CD3 and anti-CD28 in the presence of 30 μg/ml of POPS, PGPC, azPC ester, or azPC. Values are the mean + s.e.m. **P* < 0.05 for comparisons made by Student's *t*-test between each lipid treatment and vehicle treatment of *wt* cells.

**Fig. S12.** POPS, PGPC, azPC ester, and azPC suppress proliferation and induce apoptosis of macrophages. (**A**) Proliferation and (**B**) apoptosis (annexinV and 7AAD staining) of RAW 264.7 cells stimulated with 100 ng/ml of lipopolysaccharide (LPS) for 48 hours in the presence of 30 μg/ml of lipid. **P* < 0.05 by Student's *t*-test comparing each lipid treatment to treatment with LPS alone. Values are the mean ± s.e.m. of triplicates. Results are representative of 2 independent experiments.

**Fig. S13.** POPS, PGPC, azPC ester, and azPC have differential effects on proliferation and apoptosis of naive B cells. (**A**) Proliferation and (**B**) apoptosis (annexinV and 7AAD staining) of B cells isolated from spleens of naive mice and stimulated with soluble anti-IgM F(ab')2 fragment antibody (5 μg/ml) and anti-CD40 antibody (5 μg/ml) for 48 hours in the presence of 30 μg/ml of lipid. **P* < 0.05 by Student's *t*-test comparing each lipid treatment to treatment with anti-IgM and anti-CD40 antibodies alone. Values are the mean + s.e.m. of triplicates. Results are representative of 2 independent experiments.

**Fig. S14.** $sPLA_2$ inhibitors reverse azPC-induced inhibition of $MBP_{Ac1-11}$-specific T-cell proliferation (**A**) Inhibitors of PLC (U73122), $cPLA_{2\alpha}$ (EMD525143), or $iPLA_2$ (FKGK11) had no effect on the inhibition of $MBP_{Ac1-11}$-specific T-cell proliferation by azPC. (**B**) Inhibitors of $sPLA_2$ Groups IIA, IID, IIE, V, X (YM 26734), $sPLA_2$ Groups IIA, V (LY 311727), $sPLA_2$ Group V (CAY10590), or $sPLA_2$ Group IIA (EMD525145) partially reversed the inhibition of $MBP_{Ac1-11}$-specific T-cell proliferation by azPC. Naive $MBP_{Ac1-11}$ splenocytes were pre-incubated with each phospholipase inhibitor or vehicle for 40 minutes before being stimulated with 2 μg/ml of $MBP_{Ac1-11}$ for 48 hours in the presence of 30 μg/ml of azPC. Data are representative of two independent experiments. Proliferation response for MBP + azPC is the ratio between the average of triplicate wells for MBP + azPC divided by the average of triplicate wells for MBP.

## Supplementary Materials and Methods

**ELISA for measuring levels of antibodies to POPS, PGPC, azPC, and azPC ester.** Lipids dissolved in methanol were added to Corning Costar 3590 enzyme immunoassay plates at 5 nmol/well. The methanol solvent was then evaporated under nitrogen gas. Plates were blocked with BD OptEIA diluent for 3 hours. Standard curves were generated using standardized human serum containing autoantibody against cardiolipin (ImmunoVision). Detection of primary antibodies was achieved using HRP-conjugated goat anti-human IgG antibody (1:10,000).

**Luxol fast blue stain.** Membranes were blocked in 1% fat-free bovine serum albumin overnight, washed in 100% ethanol and 95% ethanol, and then incubated in luxol fast blue solution (NovaUltra Stain Kit) at 56˚C overnight. Membranes were immersed in lithium-carbonate solution and then in 70% ethanol, and finally washed with distilled water.

**EAE induction.** Animal experiments were approved by, and performed in compliance with, the National Institute of Health guidelines of the Institutional Animal Care and Use Committee at Stanford University. To induce EAE in SJL/J mice (Jackson Mice), we immunized 8- to 12-week-old female animals subcutaneously with 100 µg of $PLP_{139-151}$ emulsified in CFA (Difco Laboratories). **Prophylactic administration of lipid**: Three injections of PGPC or sphingomyelin (6 µg/mouse/injection) or vehicle (0.05% Tween-20 in PBS) were delivered on days 0, 4, and 7 after immunization with $PLP_{139-151}$. On day 0 the lipid or vehicle was emulsified together with $PLP_{139-151}$ in CFA and administered by subcutaneous injection. For subsequent time points, lipid or vehicle was injected intraperitoneally as previously described (*1,2*). Clinical disease was monitored daily using the following scoring system: 0, no disease; 1, limp tail; 2, hind limb weakness; 3, hind limb paralysis; 4, hind limb and forelimb paralysis; 5, death. **EAE treatment with lipid (Fig. S2B):** 100 µg of PGPC or sphingomyelin or vehicle (0.5% Tween-20 in PBS) was administered in 0.2 ml intravenously in the tail. Treatment with lipid or vehicle was initiated once the $PLP_{139-151}$-immunized mice developed paralysis (representing clinical EAE) and repeated 3, 6, 12, and 18 days later, for a total of five separate injections.

**Proliferation and cytokine assays.** **PLP-stimulated cells from $PLP_{139-151}$-immunized mice administered PGPC prophylactically:** SJL/J mice were co-injected with PGPC and $PLP_{139-151}$ in CFA and sacrificed 48 days later. Lymph node cells and splenocytes were then harvested and re-stimulated *in vitro* ($2.5 \times 10^6$ cells/ml) with 10 µg/ml of $PLP_{139-151}$ or with media alone. **PLP-stimulated cells from untreated $PLP_{139-151}$-immunized mice:** SJL/J mice were immunized with $PLP_{139-151}$ in CFA and sacrificed 10 days later. Splenocytes were then harvested and re-stimulated *in vitro* (at $5 \times 10^5$ cells/ml) with 10 ug/ml of $PLP_{139-151}$ in the presence of 30 µg/ml of POPS, PGPC, azPC ester, or azPC. **$Cd1d^{-/-}$ T cells:** We harvested splenocytes from *$Cd1d^{-/-}$* mice and their wild-type littermates and used $CD3^+$ T-cell enrichment columns (R&D systems) to isolate T cells. We then stimulated the T cells with plate-bound anti-CD3 antibodies, or plate-bound anti-CD3 plus anti-CD28 antibodies, in the presence of 30 µg/ml of POPS, PGPC, azPC ester, or azPC. **MBP-stimulated cells from $MBP_{Ac1-11}$ transgenic mice:** Splenocytes were harvested from mice possessing a transgene encoding a T-cell receptor specific for $MBP_{Ac1-11}$. $5 \times 10^6$ cells/ml were stimulated *in vitro* with 2 µg/ml of $MBP_{Ac1-11}$ in the presence of various concentrations of palmitic acid (Sigma), other lipids, or 100% Ethanol (vehicle alone). **Anti-CD3/anti-CD28-stimulated purified T cells:** We harvested lymph nodes and spleens from naive C57BL/6 mice and used $CD3^+$ T-cell enrichment columns (R&D systems) to isolate $CD3^+$ T cells. We then stimulated $1 \times 10^6$ cells/ml of T cells with 5 µg/ml of plate-bound anti-CD3 antibodies plus anti-CD28 antibodies in the presence of 30 µg/ml of POPS, PGPC, azPC ester, brain sulfatides, or azPC, 0.5 mM palmitic acid, or 100% ethanol (as the vehicle control). **LPS-stimulated RAW 264.7 mouse macrophage cells:** $1 \times 10^5$ cells/ml of RAW 264.7 cells were stimulated with 100 ng/ml of lipopolysaccharide (LPS) in the presence of 30 µg/ml of

POPS, PGPC, azPC ester, or azPC. **Anti-IgM F(ab')2 fragment/anti-CD40-stimulated purified B cells:** We harvested spleens from naive C57BL/6 mice and used a B-cell isolation kit (Miltenyi Biotec) to negatively isolate B cells. We then stimulated 5 x $10^5$ cells/ml of B cells with 5 μg/ml of each of soluble anti-IgM F(ab')2 fragment (Jackson ImmunoResearch) antibody and anti-CD40 antibody (eBioscience) in the presence of 30 μg/ml of POPS, PGPC, azPC ester, or azPC. **Phospholipase inhibition:** Splenocytes were harvested from mice possessing a transgene encoding a T-cell receptor specific for $MBP_{Ac1-11}$. 5 x $10^6$ cells/ml were pre-incubated in complete RPMI media for 40 min at 37°C with 5% $CO_2$, in the presence of vehicle (DMSO or 100% ethanol) or a phospholipase inhibitor: U 73122 (500 nM, Tocris Bioscience), 525143 (5 μM, EMD, Calbiochem), FKGK 11 (50 μM, Caymen Chemical), YM 26734 (250 μM, Tocris Bioscience), LY 311727 (500 μM, Tocris Bioscience), CAY10590 (200 μM, Caymen Chemical), 525145 (5μM, EMD, Calbiochem). The splenocytes were then stimulated with 2 μg/ml of $MBP_{Ac1-11}$ in the presence of 30 μg/ml azPC for 48 hours.

All cells (except for RAW 264.7) were cultured in complete RPMI 1640 containing 10% fetal bovine serum supplemented with L-glutamine (2 mM), HEPES (25mM), sodium pyruvate (1 mM), non-essential amino acids (0.1 mM), penicillin (100 U/ml), streptomycin (0.1 mg/ml), and 2-mercaptoethanol (50 μM). RAW 264.7 cells were cultured in DMEM containing high glucose, 10% fetal bovine serum supplemented with L-glutamine (2 mM), sodium pyruvate (1 mM), and penicillin (100 U/ml), streptomycin (0.1 mg/ml).

For assessment of proliferation, 1 μCi of $^{3}$H-thymidine was added to each well for the final 18 hours of culture, and incorporation of radioactivity was quantified using a Betaplate scintillation counter.

Cytokine production by cells from the lymph nodes and spleens of PGPC co-immunized mice was measured after 66 hours of stimulation by using the BD OptEIA™ Mouse IFN-γ ELISA kit (BD Biosciences). Cytokine assays for the anti-CD3/anti-CD28 antibody-stimulated T cells and $PLP_{139-151}$-stimulated splenocytes were performed on culture supernatants after 48 hours of stimulation using the BD OptEIA™ Mouse IL-6, IFN-γ, and TNF alpha ELISA kits (BD Biosciences) and the Mouse IL-17 DuoSet ELISA Development kit (R&D Systems).

**Lipidomic analysis of brain samples.** Archived, fresh-frozen, human postmortem samples from MS brain and age-matched healthy brain were analysed by shotgun lipidomics, as previously described (*3*-*5*). MS brain samples are described in the main text. Control brain samples were thoroughly examined to rule out the presence of neurological disease. Samples containing MS lesions were dissected and immediately freeze-clamped in liquid nitrogen, pulverized with a stainless-steel mortar and pestle, and their protein concentrations determined by using a BCA protein assay kit (Pierce). Internal standards were added to each tissue sample to enable normalization according to the protein content and quantification relative to that of a selected internal standard through ion intensity comparison (i.e., ratiometric comparison). Each lipid extract was reconstituted in chloroform/methanol (1:1, v/v) at a volume of 500 μL/mg of protein (calculated on the basis of the original protein content of the sample). The lipid extracts were flushed with nitrogen, capped, and stored at −20 °C until used in electrospray

ionization mass spectrometric analyses (typically within 1 week). A TSQ Quantum Ultra Plus triple-quadrupole mass spectrometer (Thermo Fisher Scientific) equipped with an automated nanospray apparatus (Nanomate HD, Advion Bioscience Ltd.) and Xcalibur system software were used in the study (*6*). Each lipid extract solution was diluted to less than 50 pmol of total lipids/μl with $CHCl_3$/MeOH/isopropanol (1:2:4 by volume) before infusion into the mass spectrometer with the nanomate. Typically, a 1 min period of signal averaging was used for each mass spectrum, and a 2 min period of signal averaging for each tandem mass spectrum.

**LC-HRMS:** Dried Folch extracts of ~100 mg brain were resuspended in 500 microliters of 50:50 DCM:MeOH. The suspensions were sonicated and centrifuged to remove particles. The authentic standards were prepared at 8 – 10 ng / ml in 50:50 DCM:MeOH. We performed chromatography as previously described (*7*). The reversed phase column, which is kept at 50°C, is an Acquity UPLC™ BEH C18 2.1 mm ID × 50 mm length with 1.7 μm particles. The binary solvent system includes A. water (1% 1M $NH_4Ac$, 0.1% HCOOH) and B. acetonitrile/ isopropanol (5:2, 1% 1M $NH_4Ac$, 0.1% HCOOH). The linear gradient starts from 35% B, reaches 100% B in 6 min and remains at this level for the next 7 min. The total run time including a 5 min re-equilibration step is 18 min. The flow rate is 0.200 ml/min, and the volume injected 5 μl. The temperature of autosampler is maintained at 4°C. Mass spectra were collected on an Agilent 6530 Q-TOF. MS1 spectra were collected from m/z 400 – 1000 at a rate of 2 spectra/sec. The gas temperature was 325°C, drying gas was 5L/min, Nebulizer was 20psig, capillary voltage was 3500V, nozzle voltage was 2000V, Sheath gas temperature was 325°C and the

Sheath gas flow was 7.5L/min. For MS/MS spectra, a collision energy of 20V was used with a narrow (1 a.m.u.) isolation window; the scan range was m/z 100 to 700 and collected at a rate of 6 spectra/sec.

**Flow cytometry.** Cells were stained according to standard protocols, run on a FACScan flow cytometer (BD Biosciences), and analysed with CellQuest software (BD Immunocytometry Systems) or with FlowJo software version 6.3.2 (Tree Star, Inc.). The antibody conjugates used were FITC anti-CD4 (clone GK1.5, BD Pharmingen), FITC anti-mouse CD8 (clone 53-6.7, BD Pharmingen), FITC anti-mouse CD3 (clone 145-2C11, eBioscience), PE-anti-rat IgG2A isotype control (BD Pharmingen), and PE-anti-mouse CD69 (clone H1.2F3, eBioscience). 7AAD staining was performed by using the Annexin V-PE Apoptosis Detection Kit I (BD Pharmingen).

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