Supplemental figures

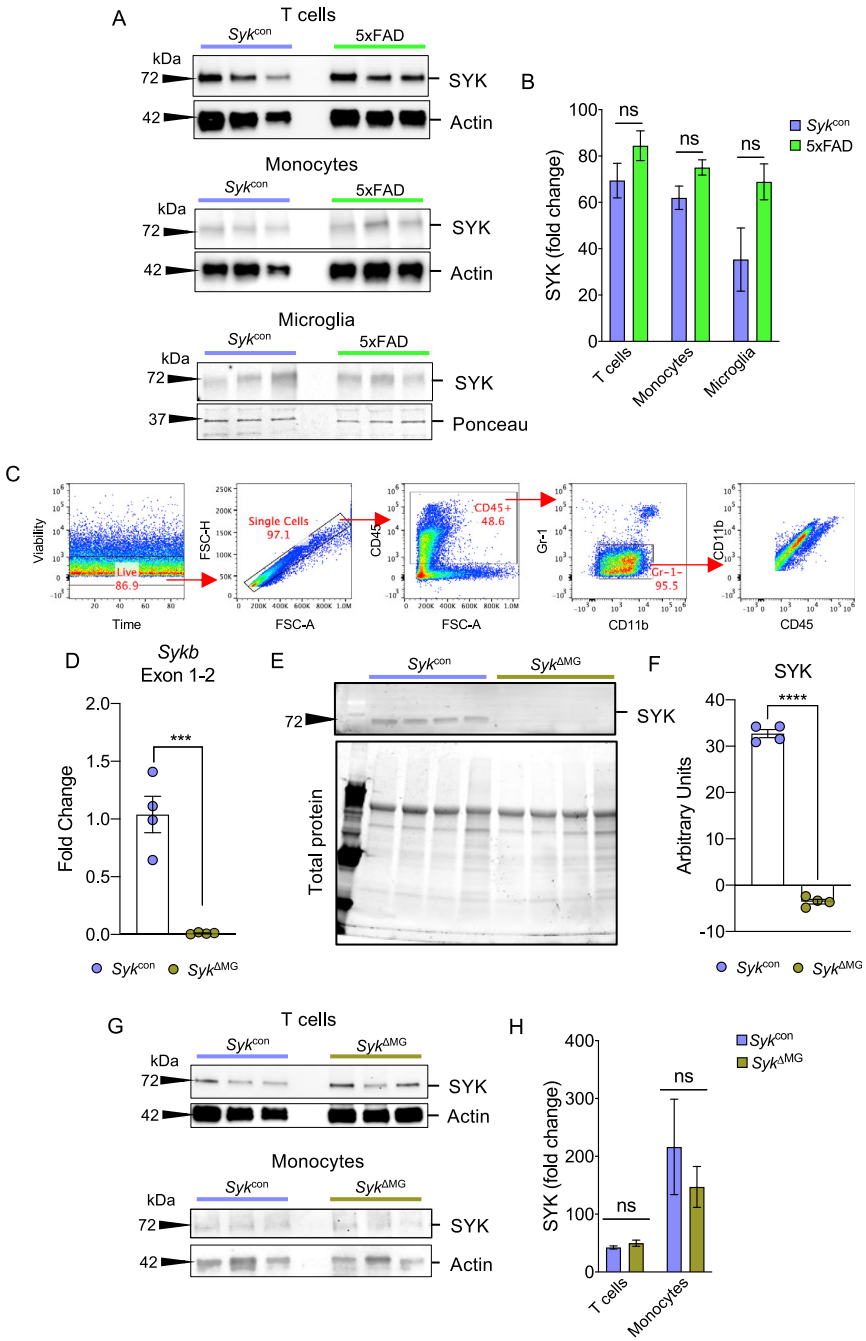

Figure S1. Targeted deletion of SYK in tamoxifen-treated *Syk*$^{fl/fl}$ *Cx3cr1*^{Ert2Cre} mice, related to Figure 1

(A-B) Cre-negative 5xFAD *Syk*$^{fl/fl}$ littermate controls (5xFAD mice) and Cre-negative *Syk*$^{fl/fl}$ littermate controls (*Syk*^{con} mice) received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food for the remainder of the experiment. Spleens and brains were harvested at 5 months of age to evaluate SYK expression in T cells, monocytes, and microglia. T cells, monocytes, and microglia were sorted from single-cell suspensions using respective anti-CD90.2^{+}cells), anti-CD11b^{+}, and anti-CD11b+(microglia) -coated magnetic beads and magnetic column sorting. (A) MACS-sorted splenic T cells and monocytes and MACS-sorted brain microglia were harvested from 5-month-old *Syk*^{con} and 5xFAD mice, and levels of SYK protein (top panel) and Actin or Ponceau-stained protein (bottom panel) were measured by Western blotting. (B) Quantification of intensity of SYK protein bands normalized to total Actin in sorted splenic T cells and monocytes, and intensity of SYK protein bands normalized to Ponceau staining in brain microglia from *Syk*^{con} and 5xFAD mice. (C-F)

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Syk$^{fl/fl}$ *Cx3cr1*^{ERT2Cre} (*Syk*$^{\Delta MG}$ mice) and Cre-negative *Syk*$^{fl/fl}$ littermate controls (*Syk*^{con} mice) received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food for the remainder of the experiment. Brains and spinal cords were later harvested to evaluate SYK deletion. Microglia were sorted from single-cell suspensions using anti-CD11b^{+}-coated magnetic beads and magnetic column sorting. (C) Representative flow cytometry gating strategy used to validate purity of MACS-sorted microglia from naive *Syk*^{con} and *Syk*Δ^{MG} combined brain and spinal cord samples. (D) Expression levels of *Sykb* mRNA from MACS-sorted microglia quantified by qPCR. (E) Levels of SYK protein (top panel) and total protein loaded (bottom panel) from MACS-sorted microglia determined by Western blotting and SDS-PAGE with a stain-free gel, respectively. (F) Quantification of intensity of SYK protein bands normalized to intensity of bands from total protein loaded. (G-H) *Syk*^{con} and *Syk*$^{\Delta MG}$ mice received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food for the remainder of the experiment. Spleens were harvested at 5 months of age to evaluate SYK expression in T cells and monocytes. (G) Levels of SYK protein (top panel) and Actin protein (bottom panel) from MACS-sorted splenic T cells and monocytes determined by Western blotting. (H) Quantification of intensity of SYK protein bands normalized to total Actin in sorted splenic T cells and monocytes from *Syk*^{con} and *Syk*$^{\Delta MG}$ mice. Statistical significance between experimental groups was calculated by unpaired Student's *t* test (B, D, F, H). ns = not significant, ***$p < 0.001$, ****$p < 0.0001$. Error bars represent mean ± SEM and each data point represents an individual mouse.

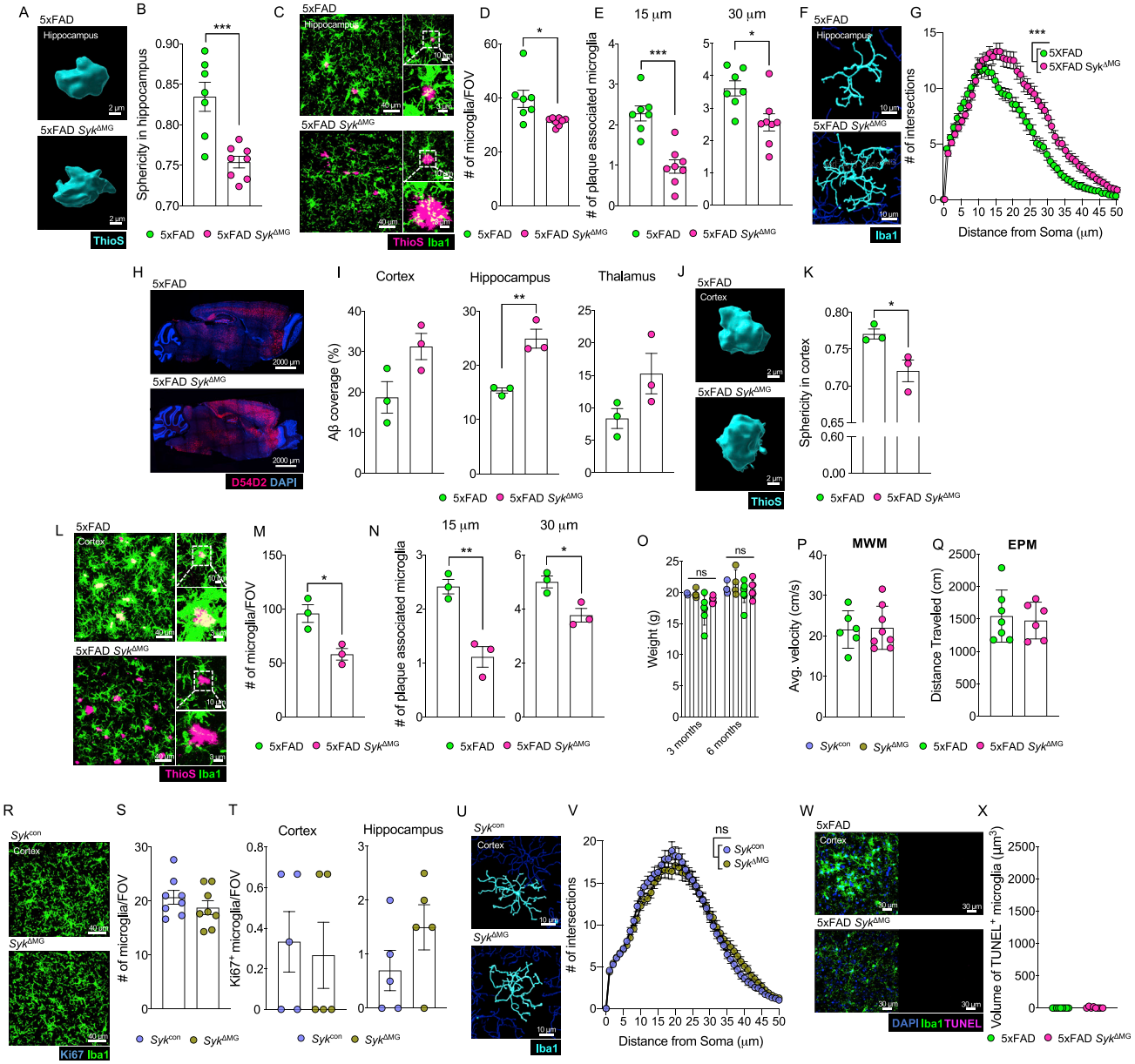

Figure S2. Microglial SYK deletion in the hippocampus and after disease onset significantly alters AD pathology and microgliosis, however, the loss of SYK does not affect microgliosis in the absence of Aβ, related to Figures 1, 2, and 3

(A-G) 5xFAD *Syk*$^{\Delta MG}$ mice and 5xFAD littermate controls received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food for the remainder of the experiment. Brains were then harvested at 5 months of age to evaluate plaque sphericity and microgliosis in the hippocampus. (A-B) Microglial response to plaques measured by (A) the sphericity of ThioS-labeled and Imaris-rendered Aβ plaques in the hippocampus of matched sagittal sections. (B) Quantification of sphericity with 1.00 being the most spherical, combined data from a total of 50–100 plaques from 3 matching brain sections per mouse. (C-E) Microglia were imaged by labeling with Iba1 (green) surrounding Aβ plaques labeled with ThioS (pink) to assess microglial coverage and proximity to plaques. (C) Representative images of Iba1 and ThioS staining in the hippocampus of matching sagittal brain sections. (D) Quantification of microglial numbers in the field of view (FOV) in 40 μm images. (E) Quantification of the number of microglia within a 15 and 30 μm radius surrounding ThioS-labeled Aβ plaques. (F) Representative Imaris rendering of Iba1^{+} microglia in the hippocampus of 5xFAD and 5xFAD *Syk*$^{\Delta MG}$ mice. (G) Sholl analysis quantification from a total of 12 microglia from 3 matching brain sections per mouse (5xFAD n = 7, 5xFAD *Syk*$^{\Delta MG}$ n = 7). (H-N) 5xFAD *Syk*$^{\Delta MG}$ mice and 5xFAD mice received tamoxifen food for 2 weeks starting at 4 months of age and then mice were returned to regular food for the remainder of the experiment. Brains were then harvested at 8 months of age in this delayed deletion model to evaluate amyloid beta (Aβ) load in the brain. (H-I) Immunofluorescence staining of Aβ (D54D2, red; DAPI, blue) was performed on sagittal sections and the percent area covered by Aβ in the cortex, hippocampus, and thalamus was quantified. (J) Sphericity of ThioS-labeled and Imaris-rendered Aβ plaques in the cortex of matched sagittal sections. (K) Quantification of sphericity with 1.00 being the most spherical, combined data from a total of 50–100 plaques from 3 matching brain sections per mouse. (L-N) Microglia were imaged by labeling with Iba1 (green) surrounding Aβ plaques labeled with ThioS (pink) to assess microglial coverage and proximity to plaques. (L) Representative images of Iba1 and ThioS staining in the cortex of matching sagittal brain sections. (M)

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Quantification of microglial numbers in the FOV in 40 μm images. (N) Quantification of the number of microglia within a 15 and 30 μm radius surrounding ThioS-labeled Aβ plaques. (O-X) 5xFAD $Syk^{\Delta MG}$ mice, 5xFAD mice, $Syk^{\Delta MG}$ mice, and Syk^{con} mice received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food for the remainder of the experiment. (O-Q) Mouse body weight was measured at 3 and 6 months of age, while memory and anxiety-related behaviors were evaluated in the Morris water maze (MWM) and elevated plus maze (EPM) at 4 months of age. (O) Mouse body weight recorded in grams (g) across experimental groups. (P) Average velocity in the MWM on day 4 of the acquisition phase of the test. (Q) Distance traveled in the EPM. (R-V) Brains from $Syk^{\Delta MG}$ and Syk^{con} mice were harvested at 5 months of age to evaluate microgliosis in the absence of Aβ. (R) Iba1 (green) and Ki67 (blue) staining was performed on sagittal sections to evaluate $Syk^{\Delta MG}$ and Syk^{con} microglial numbers and proliferation. (S) Quantification of microglial numbers in the FOV averaged from 3 matching cortical sections per mouse. (T) Quantification of $Ki67^{+}$ microglia in the FOV of the cortex and hippocampus of mice averaged from 3 matching brain sections per mouse. (U) Representative Imaris rendering of Iba1+ microglia in the cortex of Syk^{con} and $Syk^{\Delta MG}$ mice. (V) Sholl analysis quantification from a total of 12 microglia from 3 matching brain sections per mouse (Syk^{con} n = 5, $Syk^{\Delta MG}$ n = 5). (W-X) Brains were harvested from 5xFAD $Syk^{\Delta MG}$ and 5xFAD mice at 5 months of age to evaluate microglial apoptosis by TUNEL staining. (W) 5xFAD $Syk^{\Delta MG}$ and 5xFAD microglia labeled with Iba1 (green) and TUNEL (pink) surrounding Aβ plaques. (X) Quantification of TUNEL volume within $Iba1^{+}$ microglia as a measure of apoptosis. Statistical significance between experimental groups was calculated by an unpaired Student's t test (B, D-E, I, K, M−N, P-Q, S-T, X), two-way ANOVA with a Bonferroni post-hoc test (G, V), and one-way ANOVA with multiple comparisons (O). ns = not significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Error bars represent mean ± SEM. Each data point represents an individual mouse (B, D-E, I, K, M-Q, S-T, X).

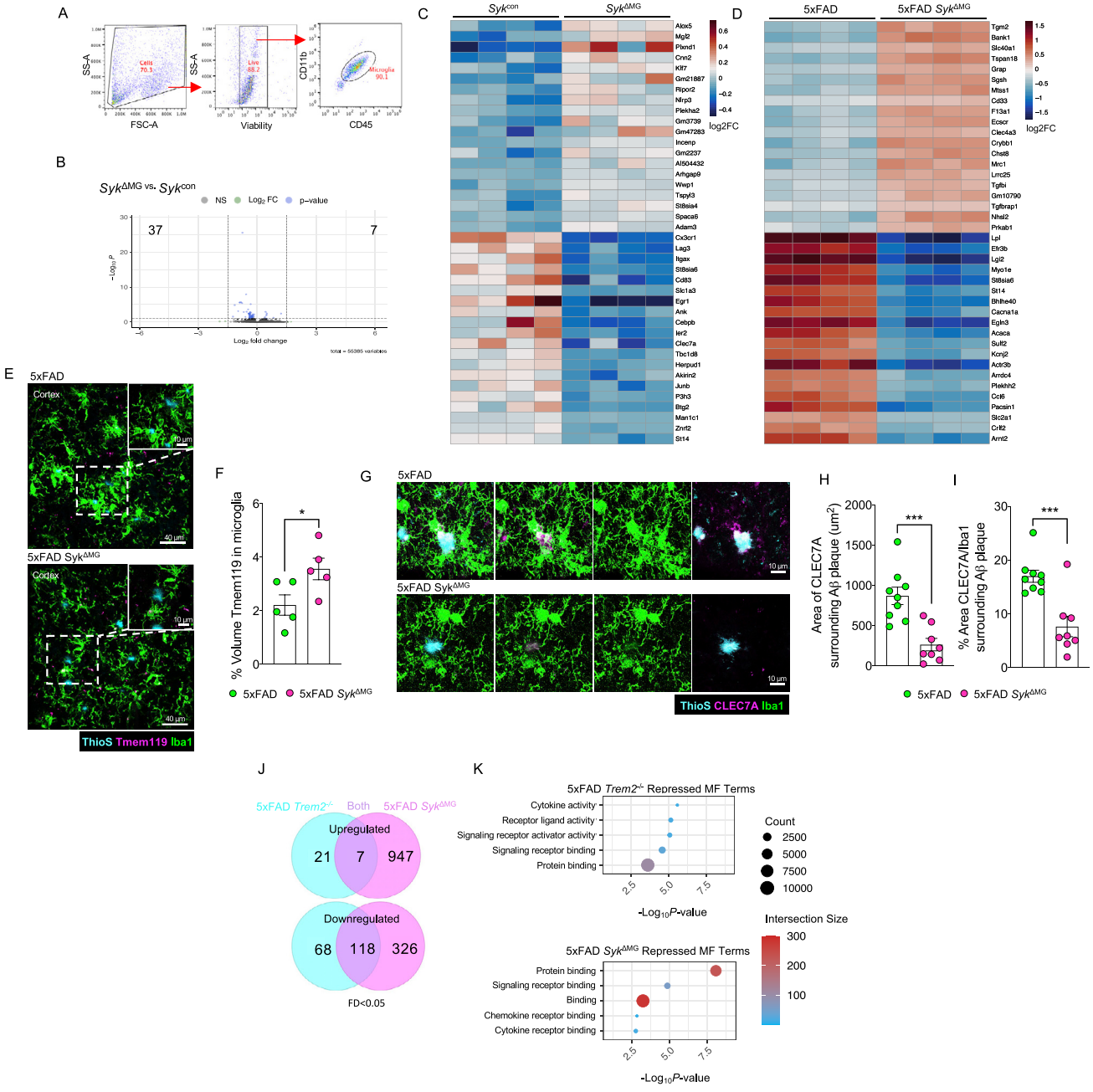

Figure S3. Effects of SYK deficiency on microglial gene expression under steady-state conditions and in response to Aβ pathology, related to Figure 4

(A-K) 5xFAD *Syk*$^{\Delta MG}$, 5xFAD, *Syk*$^{\Delta MG}$, and *Syk*^{con} mice received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food for the remainder of the experiment. Brains were later harvested at 5 months of age to evaluate microglial activation. (A-D) Microglia from 5-month-old 5xFAD *Syk*$^{\Delta MG}$, 5xFAD, *Syk*$^{\Delta MG}$, and *Syk*^{con} mice were sorted from single-cell brain suspensions using anti-CD11b^{+}-coated magnetic beads and magnetic column sorting, finally, RNA-Seq was performed. (A) Representative flow cytometry gating strategy used to validate purity of MACS-sorted microglia from 5xFAD *Syk*$^{\Delta MG}$, 5xFAD, *Syk*$^{\Delta MG}$, and *Syk*^{con} brain samples. (B) Volcano plots depicting differentially expressed genes (FDR<0.1) where 37 genes are downregulated and 7 genes are upregulated in *Syk*$^{\Delta MG}$ microglia compared to *Syk*^{con} microglia. (C) Heatmap representation of the 20 most significantly upregulated and downregulated (FDR<0.1) genes between *Syk*$^{\Delta MG}$ and *Syk*^{con} mice. (D) Heatmap representation of the 20 most significantly upregulated and downregulated (FDR<0.1) genes between 5xFAD *Syk*$^{\Delta MG}$ and 5xFAD mice. (E-I) Immunohistochemistry validation of RNA-Seq findings in 5-month-old 5xFAD *Syk*$^{\Delta MG}$ and 5xFAD mice. (E) 5xFAD *Syk*$^{\Delta MG}$ and 5xFAD microglia labeled with Iba1 (green) and Tmem119 (pink) surrounding Aβ plaques labeled with ThioS (blue). (F) Quantification of Tmem119 vol colocalized with Iba1. (G) 5xFAD *Syk*$^{\Delta MG}$ and 5xFAD microglia labeled with Iba1 (green) and CLEC7A (pink) surrounding Aβ plaques labeled with ThioS (blue). (H-I) Quantification of total area of CLEC7A surrounding individual Aβ plaques and percent area of CLEC7A normalized to the area of Iba1^{+} cells per Aβ plaque, respectively. Quantification was determined by averaging the Iba1 and CLEC7A area found surrounding ~30 plaques from 3 matching brain sections per mouse.

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(J-K) Comparison of the transcriptional changes that arise in microglia with the loss of either SYK or TREM2 in 5xFAD mice. 5xFAD *Syk*$^{\Delta MG}$ and 5xFAD brains were harvested at 5 months of age and their microglia were sorted from single-cell brain suspensions using anti-CD11b^{+}-coated magnetic beads and magnetic column sorting to perform RNA-Seq. 8-month-old 5xFAD *Trem2*$^{-/-}$ and littermate 5xFAD microglia were FACS-sorted and analyzed by RNA-Seq in (Griciuc et al., 2019). (J) Venn diagram of significantly upregulated and downregulated genes in 5xFAD *Trem2*$^{-/-}$ and 5xFAD *Syk*$^{\Delta MG}$ microglia compared with their respective littermate 5xFAD controls (FD < 0.05). (K) Molecular function (MF) term enrichment scatterplot highlighting major functions that are repressed in 5xFAD *Trem2*$^{-/-}$ and 5xFAD *Syk*$^{\Delta MG}$ microglia in comparison to littermate 5xFAD microglia. Statistical significance between experimental groups was calculated by unpaired Student's *t* test (F, H-I). *p < 0.05, ***p < 0.001. Error bars represent mean ± SEM and each data point represents an individual mouse.

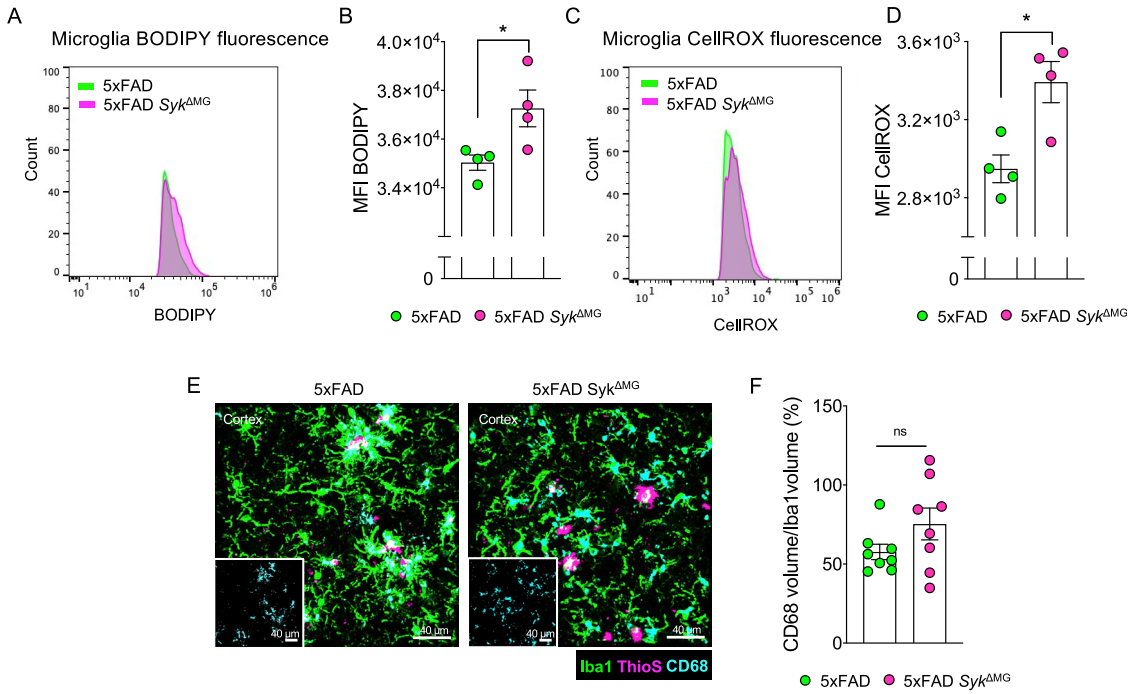

Figure S4. Genetic ablation of SYK in microglia leads to increased levels of microglial lipid droplet accumulation and ROS production in 5xFAD mice, related to Figure 5

(A-D) 5xFAD *Syk*$^{\Delta MG}$ mice and 5xFAD littermate controls received tamoxifen food for 2 weeks starting at 4 months of age and then mice were returned to regular food for the remainder of the experiment. Microglia from 8-month-old 5xFAD *Syk*$^{\Delta MG}$ and 5xFAD mice were sorted from single-cell brain suspensions using flow cytometry. (A) Representative histograms of BODIPY-labeled lipid-droplet accumulation in CD11b^{+}CD45^{int} microglia. (B) Mean Fluorescence Intensity (MFI) quantification of BODIPY in 5xFAD and 5xFAD *Syk*$^{\Delta MG}$ microglia. (C) Representative histograms of CellROX-labeled reactive oxygen species (ROS) in CD11b^{+}CD45^{int} microglia. (D) MFI quantification of CellROX in 5xFAD and 5xFAD *Syk*$^{\Delta MG}$ microglia. (E-F) 5xFAD *Syk*$^{\Delta MG}$ mice and Cre-negative 5xFAD mice received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food for the remainder of the experiment. Brains were later harvested and Iba1 staining was performed at 5 months of age to evaluate microglial activation. (E) 5xFAD *Syk*$^{\Delta MG}$ and 5xFAD microglia labeled with Iba1 (green) and CD68 (blue) surrounding Aβ plaques labeled with ThioS (pink). (F) Volumetric quantification of CD68 normalized to Iba1 volume. Statistical significance between experimental groups was calculated by unpaired Student's *t* test (B, D, F). ns = not significant, *p < 0.05. Error bars represent mean ± SEM and each data point represents an individual mouse.

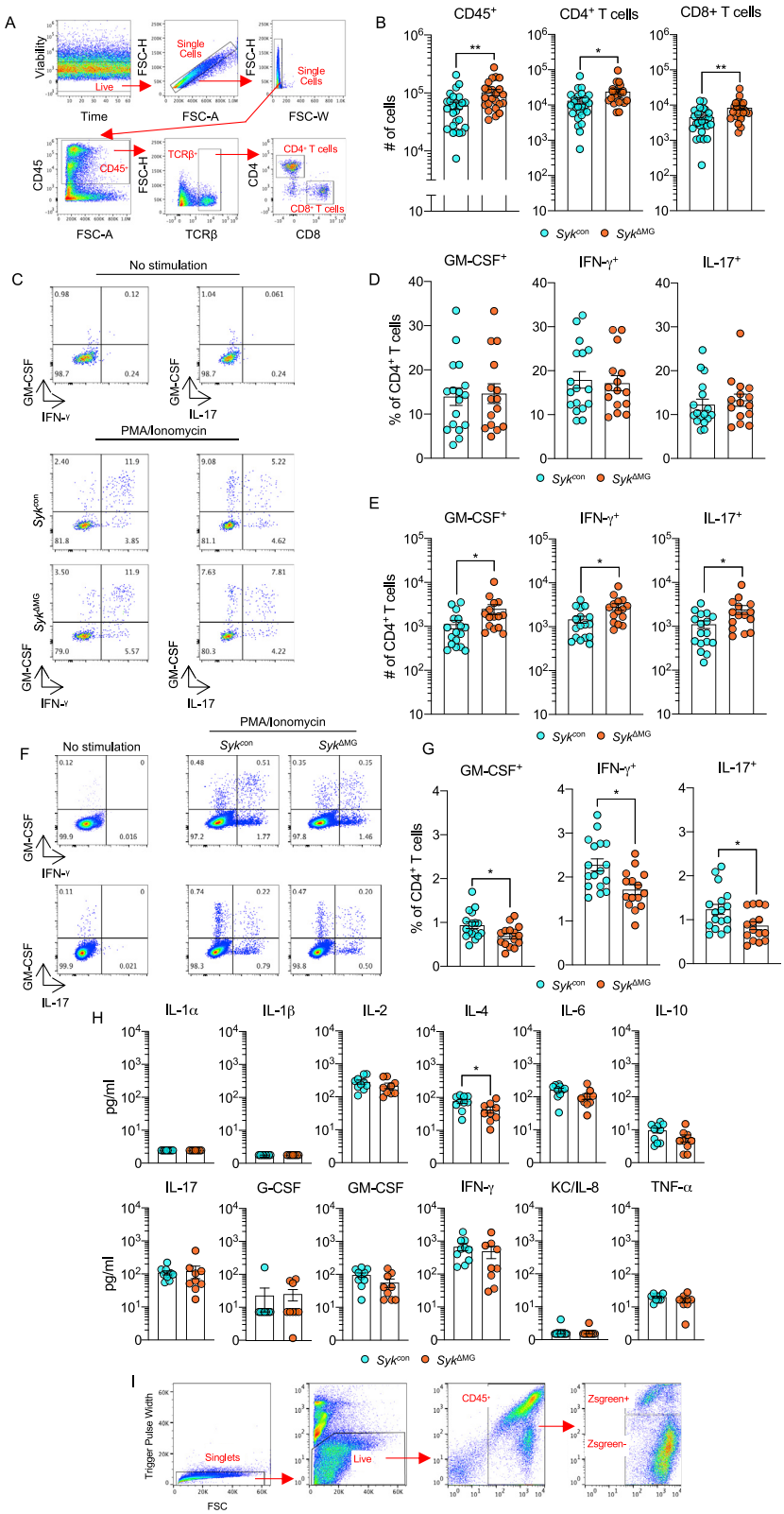

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Figure S5. *Syk*$^{\Delta MG}$ mice have increased numbers of cytokine producing T cells infiltrating the CNS but have modestly reduced peripheral T cell responses during EAE, related to Figure 6

(A-H) *Syk*$^{\Delta MG}$ mice and *Syk*^{con} littermate controls received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food for the remainder of the experiment. Mice were later immunized with MOG + CFA and pertussis toxin at 8–14 weeks of age to induce experimental autoimmune encephalomyelitis (EAE). (A-E) Spinal cords were then harvested from mice during the effector phase of clinical disease (at least 30 days post-immunization) to evaluate immune cell responses by flow cytometry. (A) Representative flow cytometry gating strategy. (B) Quantification of immune cell populations. (C) Representative dot plots of effector cytokine-producing CD4$^+$ T cells after no stimulation or 5 h *ex vivo* stimulation with PMA and ionomycin in the presence of monensin. (D) Quantification of the frequencies of effector cytokine producing CD4$^+$ T cells. (E) Quantification of the total numbers of effector cytokine producing-CD4$^+$ T cells. (F-H) Spleens were harvested from mice during the effector phase of clinical disease (at least 30 days post-immunization) to evaluate immune cell responses by flow cytometry. (F) Representative dot plots of EAE effector-cytokine producing CD4$^+$ T cells from EAE effector phase spleens after no stimulation or 5 h *ex vivo* stimulation with PMA and ionomycin in the presence of monensin. (G) Quantification of effector cytokine production by CD4$^+$ T cells. Data are combined from 2 independent experiments. (H) Quantification of secreted cytokine levels in culture media from *Syk*^{con} and *Syk*Δ^{MG} EAE effector phase splenocytes stimulated *ex vivo* with MOG_{35-55} peptide for 48 h. Levels of indicated analytes were measured by multiplex cytokine assay. (I) Flow cytometry-based sorting of ZsGreen$^+$ microglia from *Syk*$^{\Delta MG-Ai6}$ and *Syk*$^{con-Ai6}$ mice. *Syk*$^{\Delta MG}$ and *Syk*^{con} mice were crossed onto the Ai6-ZsGreen reporter background (denotated as *Syk*$^{\Delta MG-Ai6}$ and *Syk*$^{con-Ai6}$ mice) to isolate microglia in the EAE disease model. Spinal cords were harvested from mice on day 35 post-immunization and single-cell RNA-sequencing was performed on FACS-sorted ZsGreen$^+$ microglia. Statistical significance between experimental groups was calculated by unpaired Student's *t* test (B, D-E, G-H). *$p < 0.05$, **$p < 0.01$. Error bars represent mean ± SEM and each data point represents an individual mouse.

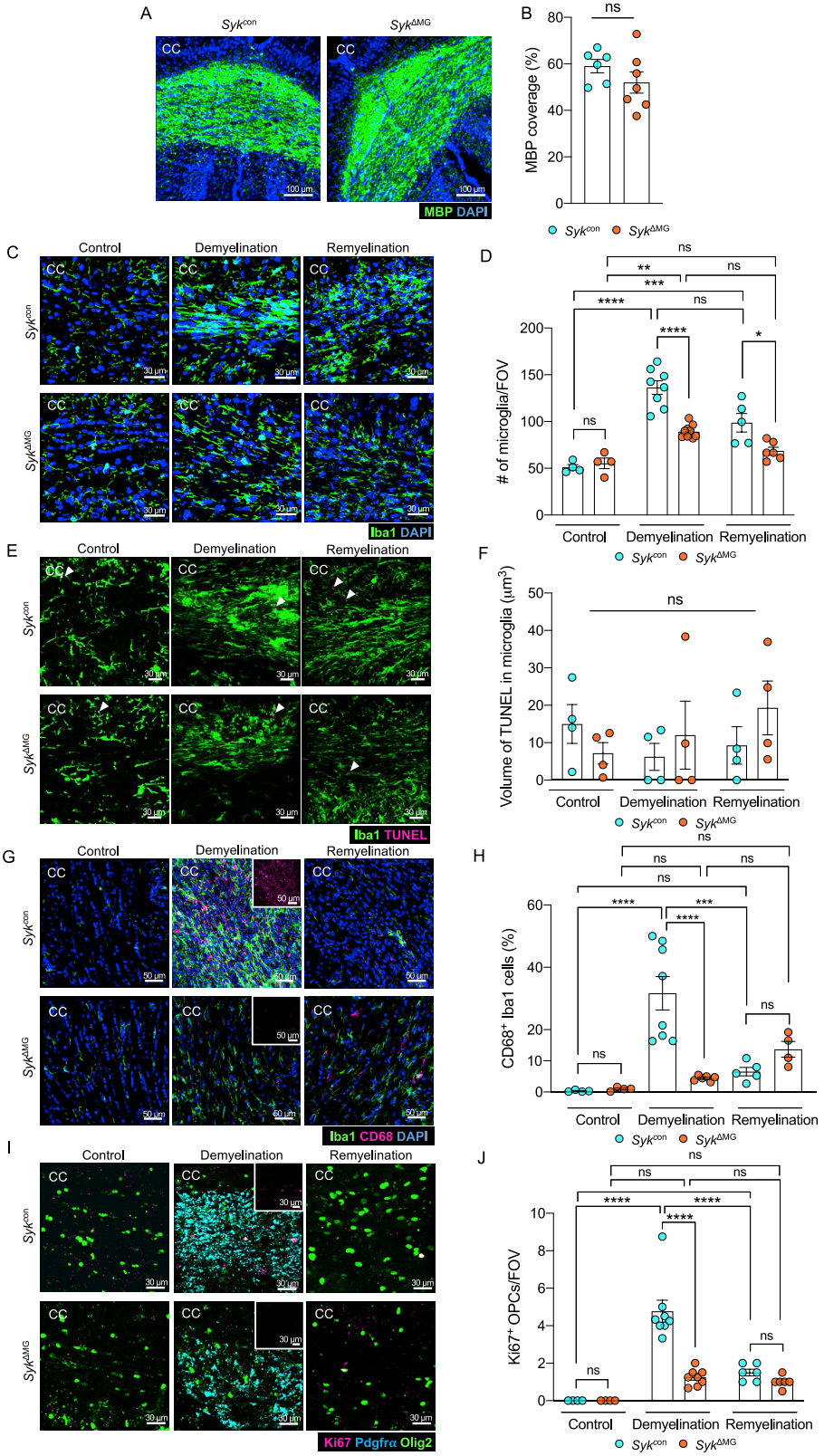

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Figure S6. Disruption of SYK signaling in microglia during demyelinating disease leads to impaired microglial response and reduced OPC proliferation, related to Figure 7

(A-B) $Syk^{\Delta MG}$ mice and Syk^{con} littermate controls received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food for the remainder of the experiment. Mice were harvest at 8 months of age and total myelin levels were evaluated in the corpus callosum. (A) $Syk^{\Delta MG}$ mice and Syk^{con} corpus callosum labeled with myelin basic protein (MBP; green) and DAPI (blue). (B) Quantification of percent area covered by MBP in the corpus callosum at baseline. (C-J) $Syk^{\Delta MG}$ mice and Syk^{con} mice received tamoxifen food for 2 weeks starting at 3 weeks of age and then mice were returned to regular food. Adult (8–12 month old) mice were later fed a diet consisting of 0.3% cuprizone for 5 weeks to induce demyelination. Mice were then either harvested after 5 weeks of cuprizone treatment (demyelination group) or returned to normal chow for one additional week before being harvesting (remyelination group). Control mice were not introduced to the cuprizone diet. (C-D) Representative images and quantification of the number of $Iba1^+$ cells (green) in the corpus callosum. (E-F) Representative images and quantification of microglial apoptosis measured by $TUNEL^+$ volume (pink) in $Iba1^+$ microglia (green) in the corpus callosum. (G) Representative images of $Iba1^+$ microglia (green) and CD68 (pink) expression in the corpus callosum. (H) Volume of CD68 colocalized to the volume of Iba1 in the corpus callosum. (I) Representative images of proliferating $Ki67^+$ (pink) $Pdgfra^+$ $Olig2^+$ (blue; green) oligodendrocyte precursor cells (OPCs) in the corpus callosum. (J) Quantification of $Ki67^+$ OPCs in the corpus callosum. Statistical significance between experimental groups was calculated by unpaired Student's *t* test (B) and one-way ANOVA with multiple comparisons (D, F, H, J). ns = not significant, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$. Data are mean ± SEM and combined from two independent experiments and each data point represents an individual mouse.