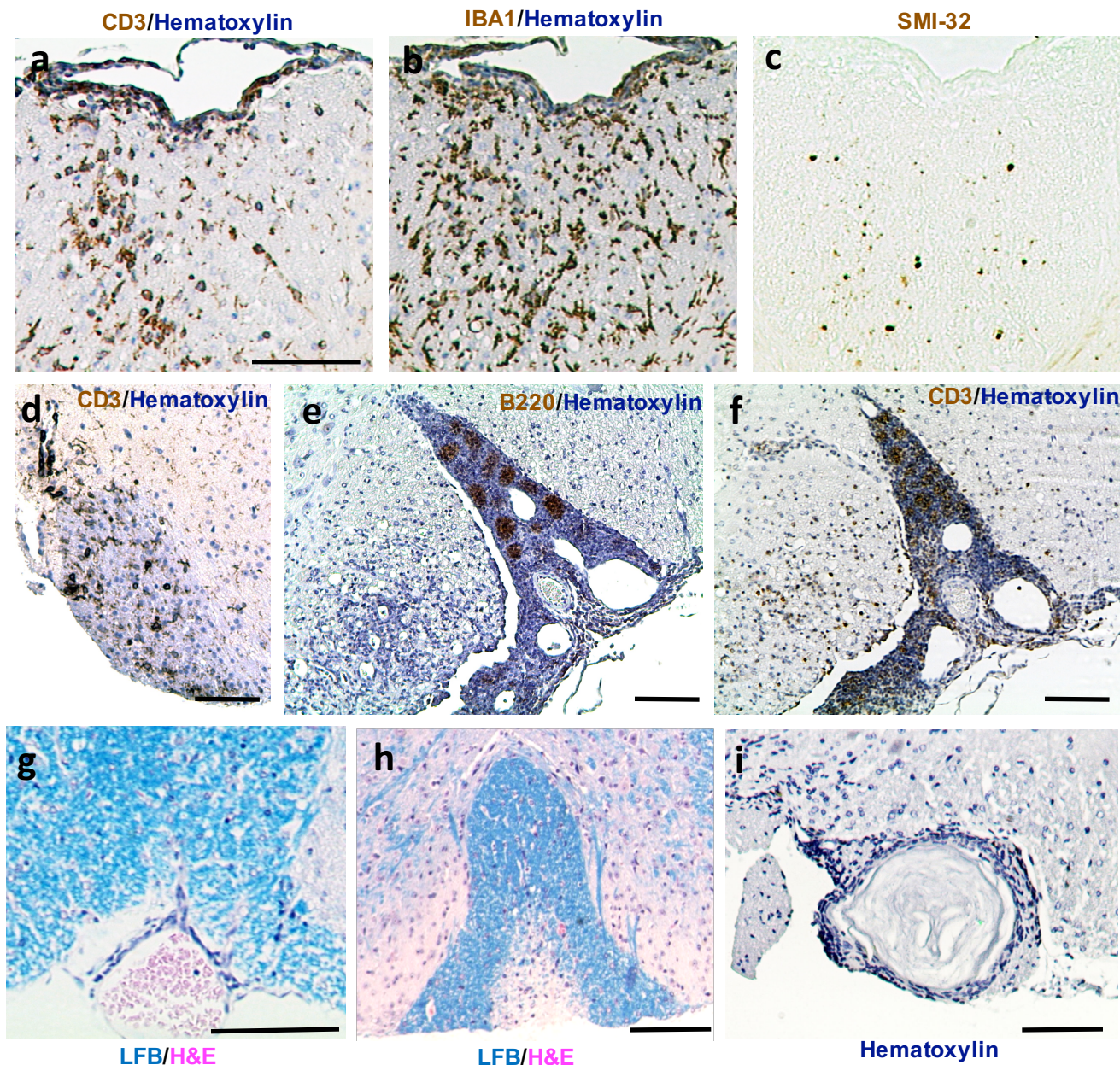
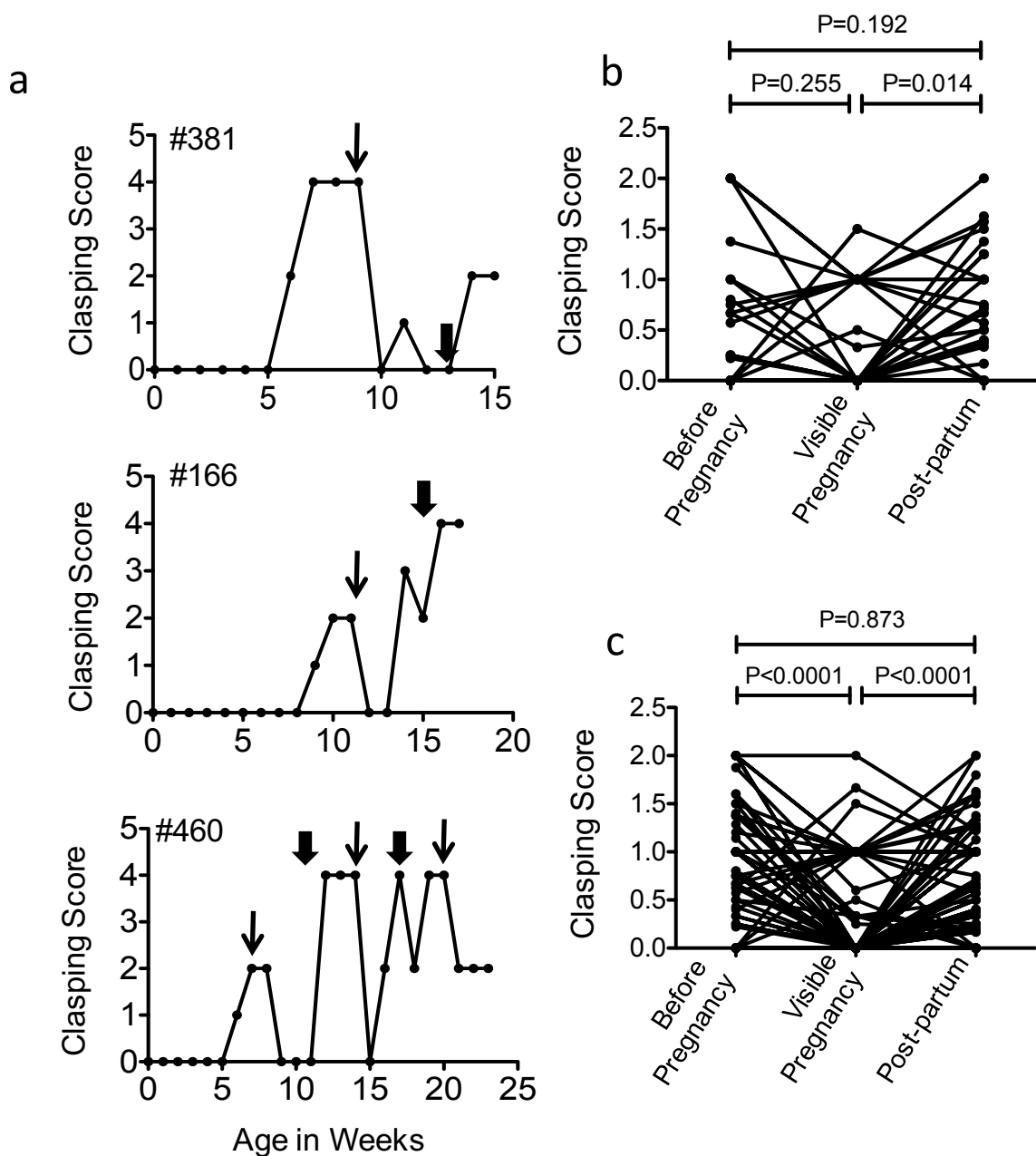


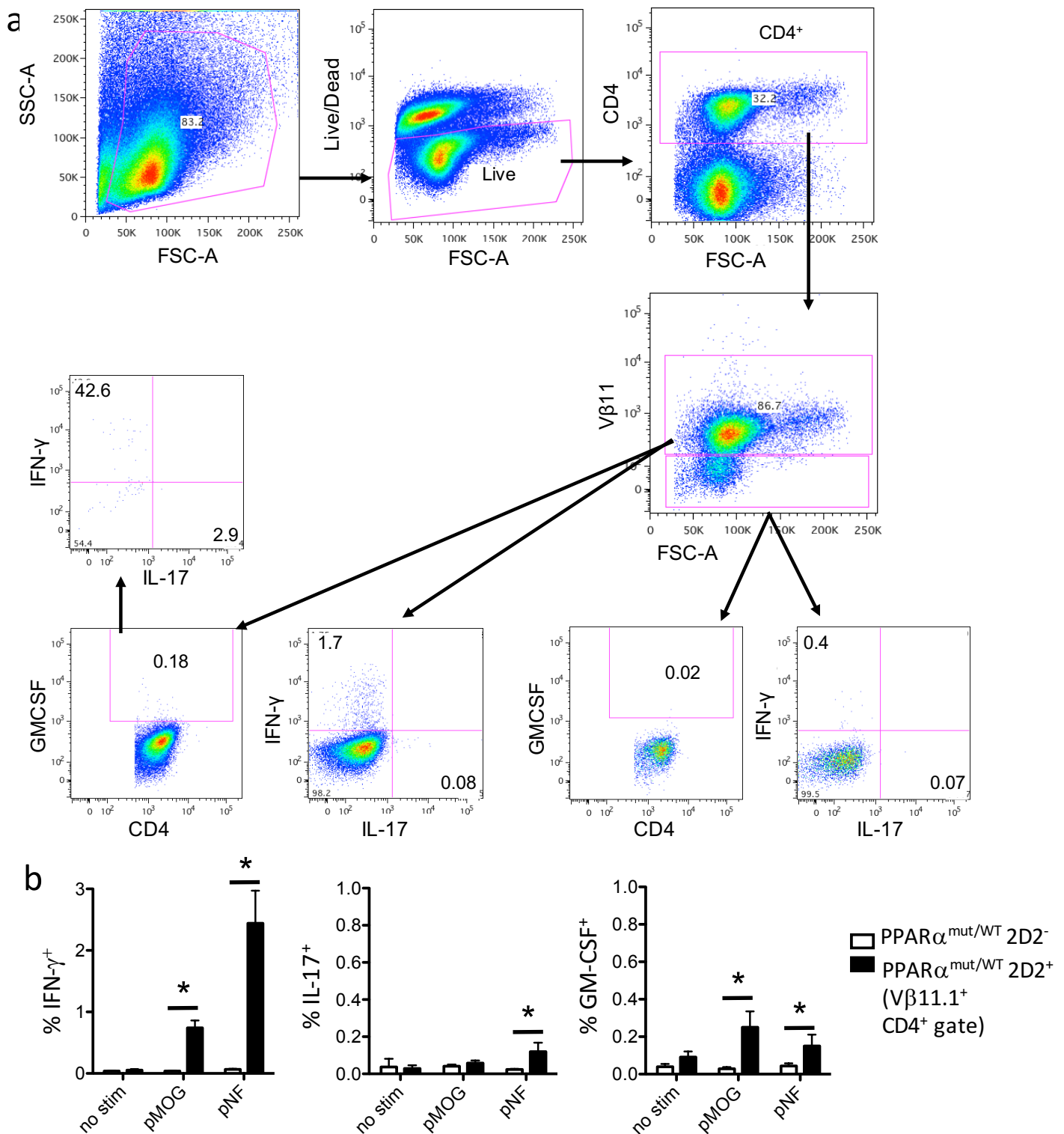
Legend. Occurrence of classic EAE and hindlimb claspings signs in 2D2⁺ mice. (a) Examples of clinical scores in individual 2D2⁺ mice that developed classic EAE signs. 0=no symptoms, 1=limp tail, 2=hindlimb or foot weakness, 3=paralysis of one or both hindlimbs, 4=hindlimb paralysis plus forelimb weakness, 5=moribund or dead. These mice were monitored for clinical signs twice per week, but were provided regular supportive care after the development of symptoms. The x marks when animals were euthanized. (b-d) Representative photos of mice with normal hindlimb splaying upon tail suspension (b), mild claspings in both hindlimbs (c), and severe hindlimb claspings with dystonia (d). (e) The proportion of PPAR $\alpha^{WT/WT}$ 2D2⁺ mice of the various sexes (combined analysis of parental and F2 2D2⁺ colony) that developed classic EAE signs, hindlimb claspings without EAE, or remained asymptomatic during the observation period. These data were collected from N=67 male and N=68 female 2D2⁺ mice.



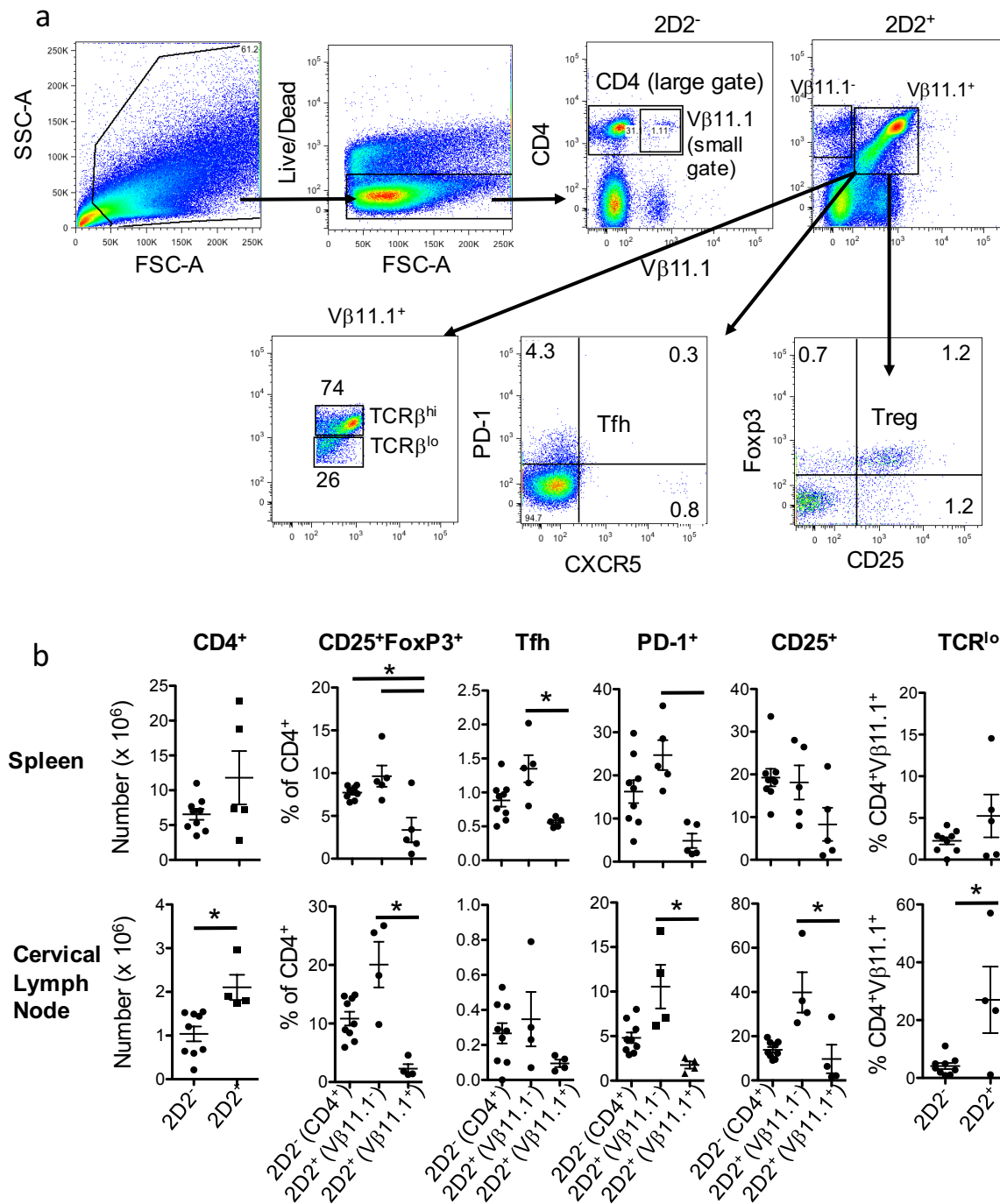
Legend. Other pathological abnormalities in the CNS of $PPAR\alpha^{mut/WT}$ 2D2⁺ mice. (a-c) Area of the posterior cord of a mouse days after the onset of hindlimb claspings stained for anti-CD3 (a), IBA1 (b) and SMI-32 (c). Note that this CD3 antibody weakly labels microglia in the CNS; however T cells can be distinguished from microglia by morphology and dense nuclear staining. (d) A cross-section of the optic tract sampled from a 2D2⁺ mouse stained with anti-CD3. (e, f) One prominent submeningeal inflammatory focus detected in a hindlimb claspings mouse at 1 week post symptom onset stained for the B cell marker B220 (e) and anti-CD3 (f). Note the organized T and B cell zones and the presence of T cells but not B cells in the CNS parenchyma. Submeningeal inflammation was not detected at other levels of the spinal cord in this mouse. (g-i) Examples of pathology seen in female $PPAR\alpha^{mut/WT}$ 2D2⁺ breeding females that had recovered from hindlimb claspings. (g) Example of mild inflammation surrounding a blood vessel in the posterior cord. Other areas of the cord were not inflamed (h) Example of demyelination seen in the posterior spinal cord. Only one other spinal cord section of 10 sampled in this mouse showed a similar pathology. (i) An example of a vacuolar structure seen in hindlimb claspings mice. Scale bar=100 μ m.



Legend. Twenty-eight $PPAR\alpha^{mut/WT}$ $2D2^+$ female breeders were assessed 1-2/week for signs of hindlimb clasping (score 0=no signs, score 1=hindlimb clasping, score 2=hindlimb clasping plus weak feet) and pregnancy (presence of distended abdomen), and for the presence of offspring in the cage. Mice were followed for a total of 65 pregnancies (1-6 pregnancies per mouse). (a) Shows the mean clasping score per week plotted against mouse age for three individual mice. Thin arrows indicate times when mice were first observed to have a distended abdomen, while thicker arrows indicate times when mice were first observed to have litters. (b-c) Mean clasping scores for $PPAR\alpha^{mut/WT}$ $2D2^+$ female breeders for the month before visible pregnancy, the period of visible pregnancy, and for the post-partum period. (b) shows the scores of mice for the first pregnancy. (c) shows the mean scores for all 65 pregnancies. In the case of multiple pregnancies, if a mouse became pregnant in the post-partum period, the clasping score assigned for the post-partum period of the first pregnancy and the “before pregnancy” period of the second pregnancy were equivalent and were calculated as the mean of scores collected after birth and before the sign of second pregnancy.

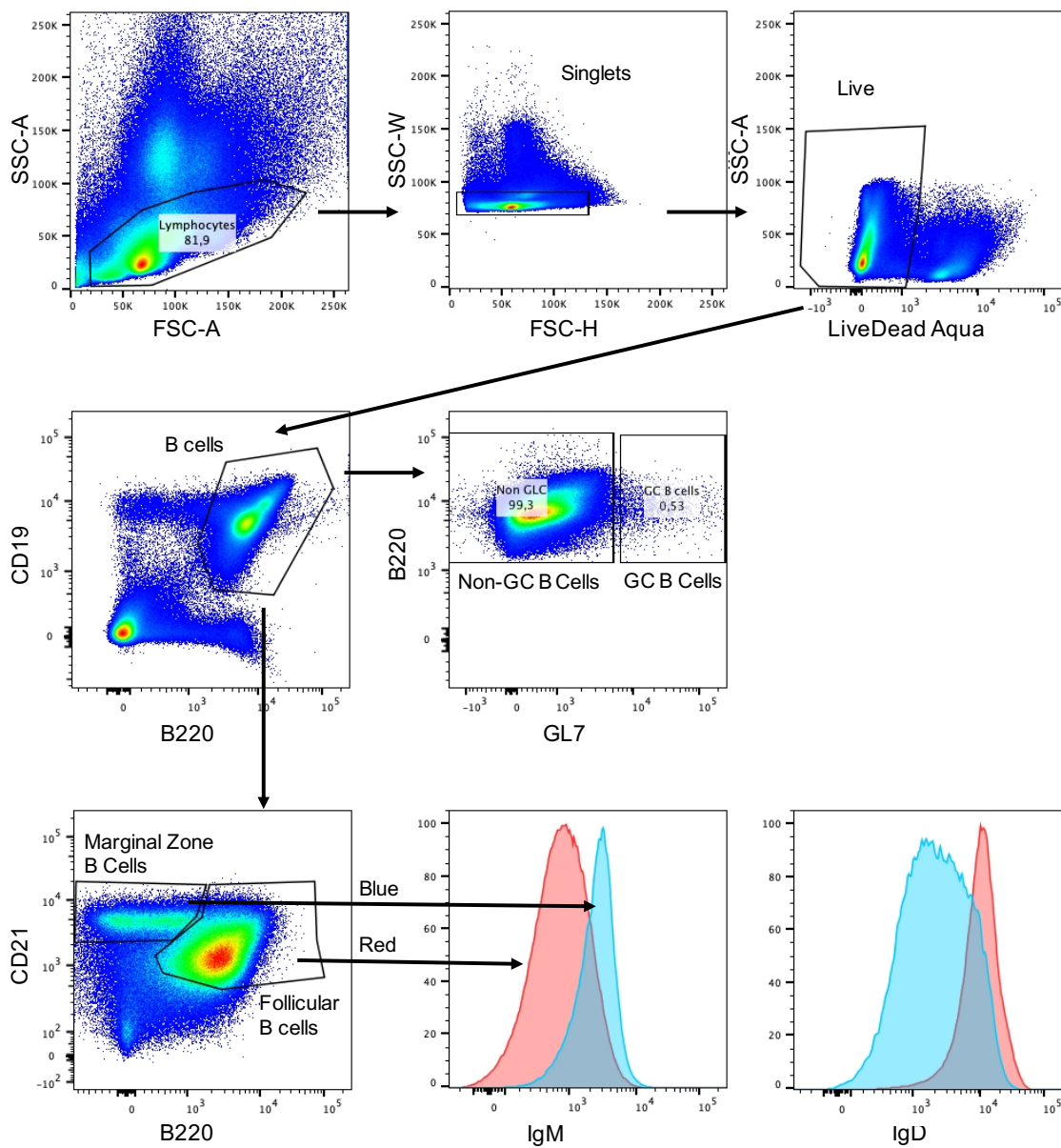


Legend. Antigen-specific Th cytokine responses in PPAR $\alpha^{mut/WT}$ 2D2⁺ and PPAR $\alpha^{mut/WT}$ 2D2⁻ mice. Five PPAR $\alpha^{mut/WT}$ 2D2⁺ and 9 age-matched PPAR $\alpha^{mut/WT}$ 2D2⁻ female mice (aged 7-9 months) were killed and spleens were dissociated into a single cell suspension. Cells were cultured in Complete RPMI media together with MOG p35-55, neurofilament medium (NF-M) peptide or no stimulus for 20 h with GolgiStop added in the last 5 h of culture. Cells were then collected and stained with CD4 and V β 11.1 antibodies, fixed, and intracellular staining for IFN- γ , IL-17, and GM-CSF was performed. (a) Gating strategy for flow cytometry. (b) Percentage of CD4⁺ T cells (in PPAR $\alpha^{mut/WT}$ 2D2⁻) or CD4⁺V β 11.1⁺ cells (in PPAR $\alpha^{mut/WT}$ 2D2⁺) mice that were positive for the indicated cytokines. Note in the gating strategy that the CD4⁺V β 11.1⁺ population in the PPAR $\alpha^{mut/WT}$ 2D2⁻ mice also had low cytokine production. The GM-CSF⁺ cells were IFN- γ , not IL-17-producers. * indicates a difference between the PPAR $\alpha^{mut/WT}$ 2D2⁻ and PPAR $\alpha^{mut/WT}$ 2D2⁺ group by two-tailed Mann-Whitney test ($P \leq 0.05$).



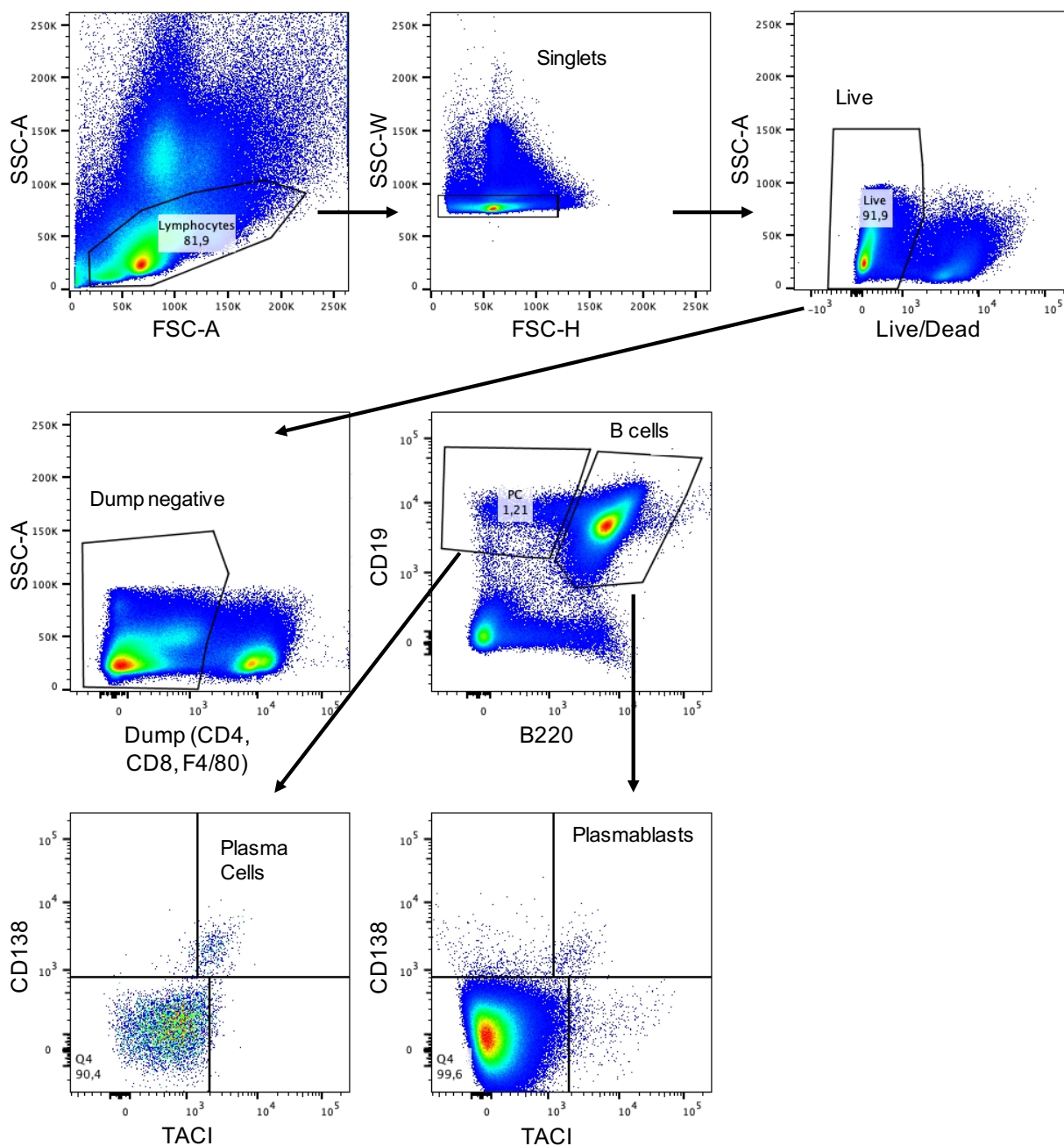
Legend. Characterization of the T helper cell compartment in $PPAR\alpha^{mut/WT}$ $2D2^+$ and $PPAR\alpha^{mut/WT}$ $2D2^-$ mice. 6 $PPAR\alpha^{mut/WT}$ $2D2^+$ ($2D2^+$) and 9 age-matched $PPAR\alpha^{mut/WT}$ $2D2^-$ female ($2D2^-$ mice) (aged 7-9 months) were killed and the cervical lymph nodes and spleens were isolated and dissociated into a single cell suspension. 1 mouse in the $2D2^+$ group was excluded due to lymphoma development. Cells were stained with antibodies specific for CD4, CD25, PD-1 and TCR $V\beta 11.1$ (marker of the TCR transgenic cells). Intranuclear staining for FoxP3 was performed and expression of the indicated markers was evaluated by flow cytometry. (a) Gating strategy that was used to subset total CD4⁺ T cells, $V\beta 11.1^+$ (transgenic) and $V\beta 11.1^-$ (non-transgenic) cells and representative staining for $V\beta 11.1$, PD-1 and CXCR5, and CD25 and FoxP3 in one TCR transgenic mouse. (b) shows number of CD4⁺ T cells and the frequency of CD4⁺ T cells that had expression for the indicated markers. Expression of markers was compared between total CD4⁺ T cells in the $2D2^-$ mice and the $V\beta 11.1^+$ and $V\beta 11.1^-$ populations in the $2D2^+$ mice. For assessment of TCR expression, the % TCR^{lo} cells were compared between $2D2^-$ and $2D2^+$ groups. Data were analyzed by two-tailed Mann-Whitney test (2-groups) or Kruskal Wallis test followed by Dunn's multiple comparison test (3 groups). * indicates a difference between the indicated groups ($P \leq 0.05$).

Fig. S6

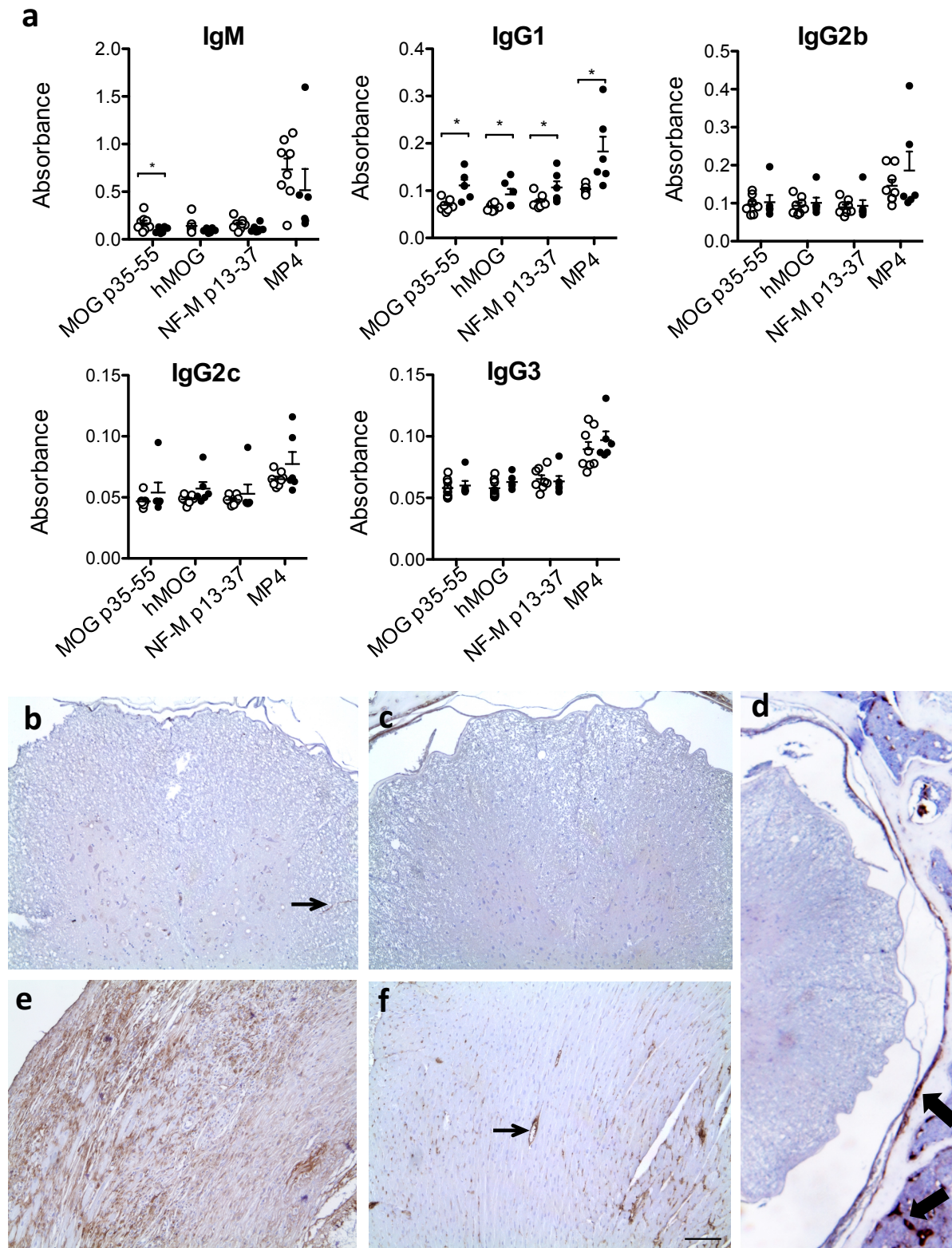


Legend. Gating strategy for identification of germinal center (GC) B cells, marginal zone B cells, and follicular B cells.

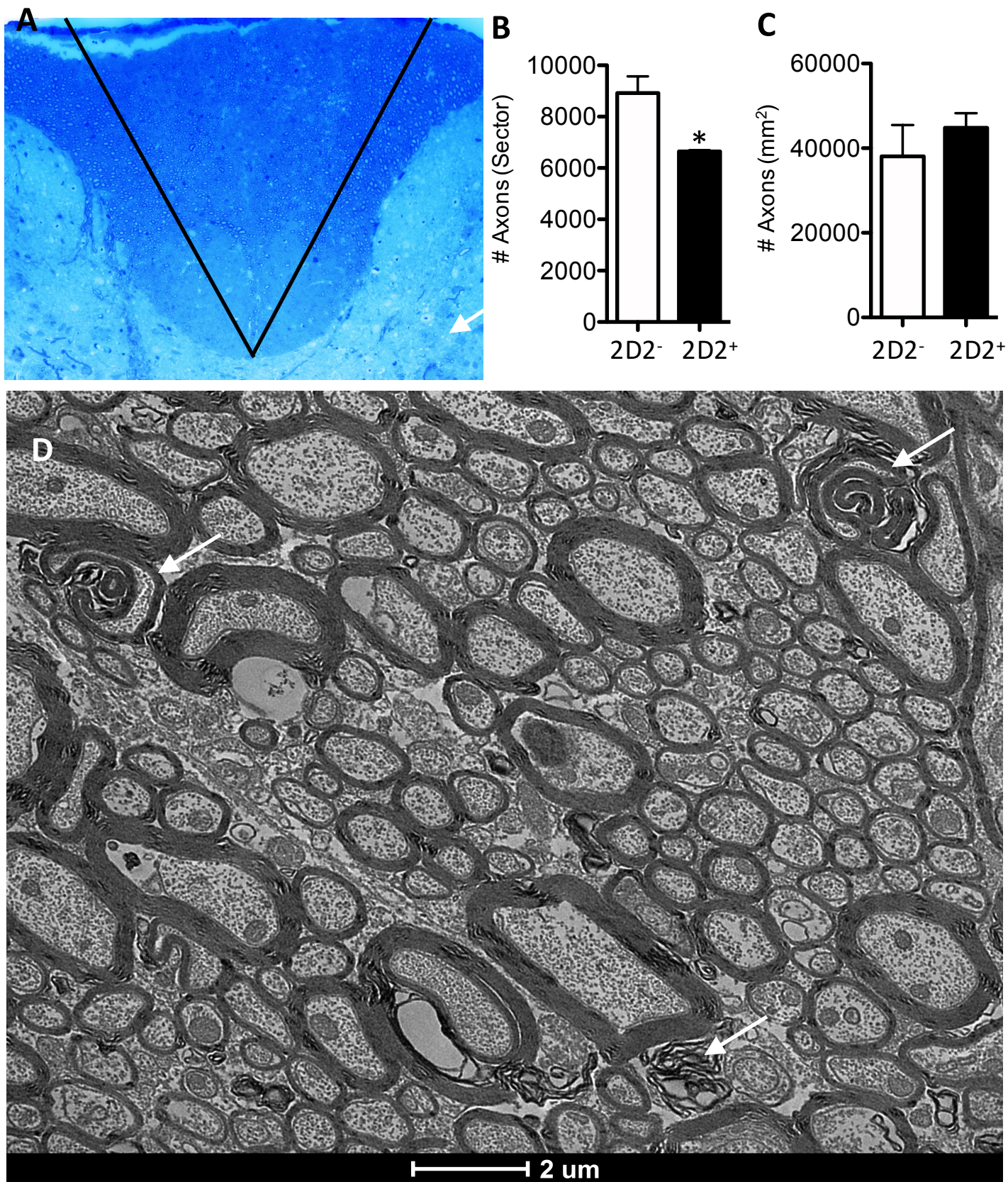
Fig. S7



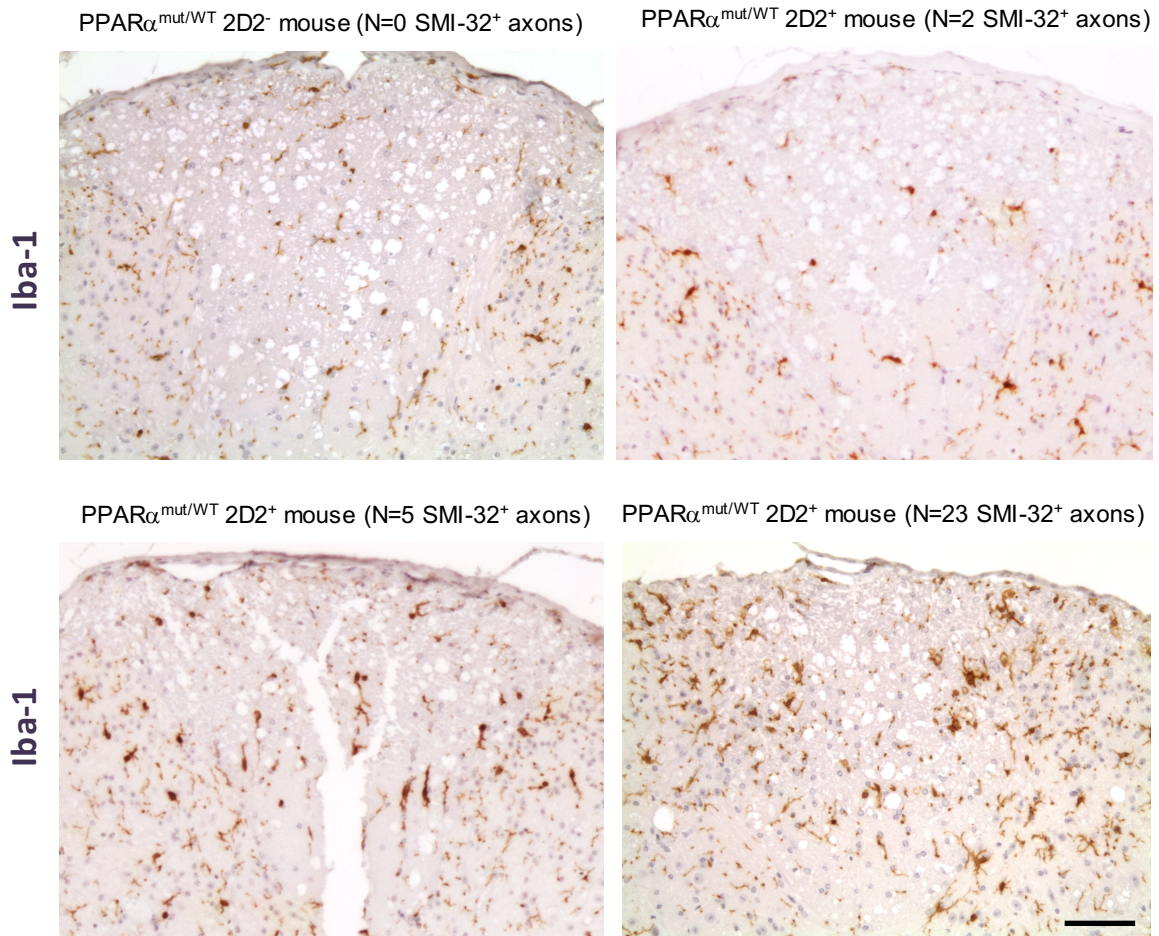
Legend. Gating strategy for characterization of plasma cells and plasmablasts.



Legend. Autoantibody production and CNS deposition of Ig in $PPAR\alpha^{mut/WT}$ 2D2⁻ and $PPAR\alpha^{mut/WT}$ 2D2⁺ mice. Serum levels of IgM, IgG1, IgG2b, IgG2c, and IgG3 reactive against MOG p35-55, whole human MOG (hMOG), neurofilament medium (13-37 amino acids) and MP4 (a MBP/PLP fusion protein) were measured by sandwich ELISA. Shown are the absorbance measures (OD) determined for each assay. (b-d) show representative images of the $PPAR\alpha^{mut/WT}$ 2D2⁻ (b) and $PPAR\alpha^{mut/WT}$ 2D2⁺ (c, d) spinal cord stained with anti-mouse IgG. Only blood vessels stained with Ig in the spinal cord (small arrow in a). These results are representative of staining in N=10 mice/group. As a positive control (arrows in d), we detected staining in the bone marrow and outside the meninges in $PPAR\alpha^{mut/WT}$ 2D2⁺ mice. (e, f) shows positive and negative controls for staining: hearts that had been transplanted into MHC Class II-mismatched mice and rejected via antibody-mediated mechanisms (e) or transplanted into syngeneic recipients (f). (e) shows massive IgG deposition whereas the negative control showed Ig deposition in blood vessels (thin arrow in f). Scale bar=100 μ m.



Legend. Female $PPAR\alpha^{mut/WT}$ 2D2⁺ females (aged, 9, 11, and 11 months) were fixed and processed for TEM. Thin sections were prepared and stained with toluidine blue. A, shows a representative area of the dorsal spinal cord in a $PPAR\alpha^{mut/WT}$ 2D2⁺ mouse and a 55 degree sector that was sampled. B, Number of axons counted in this sector. C, Number of axons per mm² sampled. D, shows an example of the pathology seen at higher frequency in of $PPAR\alpha^{mut/WT}$ 2D2⁺ than age-matched $PPAR\alpha^{mut/WT}$ 2D2⁻ mice. Thin arrows show examples of degenerated axons with collapsed myelin sheaths.



Legend. Examples of IBA1 staining seen in the posterior spinal cord of 9 month old PPAR $\alpha^{mut/WT}$ 2D2 $^{+}$ and PPAR $\alpha^{mut/WT}$ 2D2 $^{-}$ females. Five μ m thick paraffin sections from PPAR $\alpha^{mut/WT}$ 2D2 $^{-}$ and PPAR $\alpha^{mut/WT}$ 2D2 $^{+}$ mice (N=5/group) that were adjacent to sections stained for SMI-32 were stained with anti-IBA1. All specimens had been stained in the same staining run. (a) shows an example of IBA1 staining in a PPAR $\alpha^{mut/WT}$ 2D2 $^{-}$ mouse with normal microglia appearance. (b-d) show examples of IBA1 staining in PPAR $\alpha^{mut/WT}$ 2D2 $^{+}$ mice where axon injury was present in the adjacent section. (d) shows the most severe case of microglia activation observed in the PPAR $\alpha^{mut/WT}$ 2D2 $^{+}$ mice. Scale bar=100 μ m.

Supplementary Methods

Genotyping. Weaning aged mice were screened for the presence of the 2D2 TCR transgene by flow cytometry by conducting surface staining for CD4 and the V β 11 TCR. In brief, a drop of blood was collected from the saphenous vein of mice into a FACS tube that contained 15 μ l of 1 x PBS and 5 U/ml of heparin (Sigma). Cells were stained with FITC-CD4 (Clone GK1.1, ThermoFisher) and PE-V β 11 antibody (Clone RR3-15, BD Biosciences) in 20 μ l of FACS buffer (1 x PBS containing 2% fetal calf serum) at room temperature in the dark for 30 min. Red blood cells were lysed via the addition of 2 mL of Ammonium-Chloride-Potassium lysis buffer (0.15 M NH₄Cl, 10 mM KHCO₃, 0.1 mM Na₂EDTA) to each tube. Once the cell suspension became transparent, 2 ml of FACS buffer was added to stop the cell lysis. Cells were centrifuged at 335 x g at 4 °C for 5 min, the supernatant was decanted and cells were resuspended in FACS buffer for analysis. Staining was analyzed using a FACSCaliburTM and CellQuest software (BD Biosciences). Mice positive for the 2D2 transgene were identified as having a large majority of cells (>80%) within the CD4 gate that were positive for V β 11.1. PCR genotyping of PPAR α wildtype or mutant alleles was done using DNA obtained from tail or each punch samples as described previously (Dunn et al., 2007).

Rotarod and Hanging Grip Test. Mice were placed on a rotarod (Model ENV-575M, Med Associates Inc.) at 32 rpm constant speed for a maximum of 60 seconds. The time to fall was recorded using a force platform (Med Associates Inc.). On the test day, mice were given three trials, separated by 10 minutes. The longest time to fall in these three trials is reported. For the hanging grip test, mice were placed on the bottom of a stainless steel open box with smooth sides (31 cm wide x 31 cm long x 28 cm high) that had 1 cm x 1 cm wire grid bottom. Mice were

placed in the box and then the box was slowly inverted and the time to fall was recorded. Three trials were performed for each mouse, each separated by 15 minutes. The average time to fall is presented. Mice were trained on each of these tasks for three trials/day on three consecutive days prior to testing. Tests were conducted in the same room by the same experimenter at the same time of day.

Histological and Immunohistochemical (IHC) Staining of the Brain and Spinal cord. Spinal cord and brain cross-sections were fixed in 10% formalin and embedded in paraffin. For some of the long-term clasping mice, specimens were also decalcified in 3% formic acid in PBS over 5 days prior to embedding in paraffin. For gross analysis of inflammation and demyelination, spinal cord cross-sections (10-12/mouse) and five brain coronal slides were embedded in a single paraffin block. CNS sections (5 μ m thick) were cut and stained with (LFB) followed by hematoxylin and eosin (H&E) (Toronto Centre for Phenogenomics, histology core), mouse Ig (horse anti-mouse Ig, Vector Cat# BT-2001) or were immunostained for anti-CD3 (Dako Cat#556970), anti-neurofilament heavy non-phosphorylated (SMI-32, Cat#801702, Biolegend), IBA1 (Cat# ab178847, Abcam), NeuN (Millipore, Clone A60), or synaptotagmin (Synaptic Systems, Cat#105002). In brief, paraffin sections heated in a 55 degrees celsius oven for 15 min and then were de-waxed with xylene (10 min) and then citrosolve (5 min) and then hydrated in successive ethanol baths (100% x 2, 95% x 2, 70% x 1), and washed in 1 x PBS + 0.05% Tween-20 (PBS-T). Endogenous peroxidase activity was quenched (15 min in 3% hydrogen peroxide in methanol), sections were washed in PBS-T (2 x 5 min) and antigen retrieval was performed in a pressure cooker for 5 minutes in 10 mM trisodium citrate (pH=6.0) containing 1% Tween-20 or 10 mM Tris 1 mM EDTA (in the case of synaptotagmin). For anti-CD3, synaptotagmin, and

IBA1 IHC, sections were washed in PBS-T and blocked for 30 minutes at room temperature in blocking buffer (1 x PBS-T containing 2% bovine serum albumin, and 2% serum of host of secondary antibody, Vector Laboratories, Burlingame, CA, USA) plus avidin (4 drops/ml, Vector Laboratories). Sections were then incubated in the same blocking buffer supplemented instead with biotin (4 drops per ml, Vector Laboratories) that contained anti-CD3 (1:1000), anti-synaptotagmin (1:100), or anti-IBA1 (1:8000) overnight at 4 °C. The following day, sections were washed in PBS-T and probed either with either biotinylated goat anti-rabbit IgG (BA-100) (1:200 diluted in blocking buffer) for anti-CD3 and anti-IBA1 or poly horseradish peroxidase goat anti-mouse/rabbit/rat IgG (1:1 diluted in PBS) (Immunologic). After 30 min at room temperature, slides were washed in PBS-T and were incubated in ABC Elite reagent (Vector Laboratories) for 30 min at room temperature. Sides were washed in PBS-T and developed with 3,3-diaminobenzidine tetrahydrochloride (DAB) (Vector Laboratories). The substrate development was stopped by incubating slides in running tap water. Sections were then counterstained in Mayer's hematoxylin (Electron Microscopy Sciences), dehydrated, cleared with xylene, and mounted with Permount™. Ig staining was conducted by the Pathology Research Program at the University Health Network.

Staining with SMI-32 (1:5000 dilution) and NeuN (1:500 dilution) antibodies was done using the Mouse-on-Mouse (M.O.M.) kit (Vector Laboratories). All steps were the same as described above up until the blocking steps, after which blocking and incubations were performed using reagents and instructions provided in the M.O.M kit. For all IHC procedures, sections were stained with secondary antibody alone as a negative control with the exception for mouse Ig staining where negative control tissues were used. The positive control for Ig staining was a heart that had been rejected by antibody-mediated mechanisms.

Histological Analysis of Inflammation, Perivascular Inflammation, and Meningeal inflammation.

Slides that contained LFB/H&E stained spinal cord cross-sections (5 μm thick, 10-12 sections collected at different levels of the cord) were blinded by an investigator who was not conducting the analysis by covering the slide labels with tape and then coding the slides. Each spinal cord cross-section was divided into four quadrants (ventral, posterior, lateral, lateral). The presence of submeningeal infiltrates or perivascular cuffs was scored as 1 observation. These observations were then summed across sections and expressed as the percentage of total quadrants examined. To determine the inflammation score, a similar analysis was done except that the severity of inflammation in the CNS parenchyma was scored in each quadrant (0=no inflammation, 1=mild inflammation, 2=moderate inflammation, or 3=severe inflammation). The inflammation score for each mouse was determined by summing the scores for all examined sections and then dividing this number by the number of quadrants sampled.

CD3⁺ and SMI-32⁺ Counting in Symptom Onset Mice.

CD3- and SMI-32-stained slides were blinded as described above and then representative thoracic sections for each mouse were identified. T cells (CD3 stained cells that contained a dark hematoxylin-stained nucleus) were counted in ventral, posterior, and lateral quadrants of these sections. The area of the white matter sampled was determined using a microscope (DM-1000) and camera that were interfaced with an image analysis system (Leica Application Suite, Version 4, Leica Microsystems). The total T cell count is expressed per mm^2 white matter tissue sampled. To help determine the geographical distribution of the T cells, we measured the T cell counts for ventral, posterior, and the 2 lateral aspects of each spinal cord section in 1-3 sections per mouse.

SMI-32⁺ axons were counted manually using a microscope and hand counter and were expressed relative to the area of white matter sampled in mm² (determined as above).

Quantification of Synaptotagmin and Luxol Fast Blue Staining

For the quantification of the synaptotagmin positive signal, RGB images of grey matter areas in stained thoracic sections of the spinal cord were acquired at 200 x. For demyelination analysis, RGB images of the thoracic spinal cord stained with LFB (without hematoxylin) and were acquired at 40 x magnification using a Leica Microscope and camera. Analysis of staining was performed using ImageJ 1.15s (National Institutes of Health, USA). For this, RGB images were separated into single color channels (DAB, brown; H&E for blue and purple) using the color deconvolution plugin. Each single color channel was subjected to thresholding followed by Area Fraction measurement of the region of interest. For synaptotagmin, the area fraction of brown staining was determined for the ventral and dorsal horns of the spinal cord. For LFB, area fraction of blue staining was determined within a set traced area of white matter area. The person conducting the analysis was blinded to the identity of the sections.

T cell analysis in the CNS in Aged Mice.

This analysis was performed in the mice for which MR imaging was done. The spinal cord (still in the spinal column) was decalcified and then fixed in formalin. The spinal cord was then cut into 6 pieces, lengthwise, and then these were embedded in cross-section in a single paraffin block. The block was sectioned using a microtome and at least one hundred 5 µm sections were prepared from each block. Every 10/11th thoracic section was stained with anti-CD3 using DAB IHC (as described above) and H&E/LFB to evaluate T cell infiltration and

inflammation/demyelination. Since the decalcification resulted in some microglia being faintly stained with anti-CD3, T cells were defined as round brown cells with no processes that had a dark central hemotoxylin-stained nucleus. These cells were counted and expressed per # thoracic sections sampled.

Axon Counting and Ultrastructure Studies. To conduct a structural analysis of myelinated axons, mice were anesthetized, perfused with PBS followed by 4% PFA. Spinal cords were then harvested and fixed overnight in 2% glutaraldehyde, 4% PFA in 0.1 M Sorenson phosphate buffer. Samples for TEM were post-fixed with 1% osmium tetroxide, dehydrated, and flat embedded in Poly-Bed 812 epoxy resin. Half-micrometer-thick sections of the thoracic spinal cord were cut and stained with toluidine blue. Ultrathin sections (90 nm) were cut from the posterior thoracic cord, stained with uranyl acetate and lead citrate and imaged by TALOS L120C TEM (FEI). For axon counting, TIFF images of scanned slides were imported into ImageScope and all axons were manually counted within a 55 degree sector of the posterior spinal cord using a manual count tool and expressed per mm² area tissue sampled.

Stereological Analysis

The thoracic spinal cord was embedded in cross-section in paraffin and was serially-sectioned (5 μm) (at least 100 sections). After viewing the sections for integrity, a register of 50 serial thoracic sections were selected for analysis. Every seventh section in this register was selected for stereological analysis (7 sections in total). Selected sections were stained with anti-NeuN as described above, slides were blinded with tape, and stereological analysis was performed by

another investigator using a stereology workstation that included a transmitted light microscope (Leica DMRB, Concord, ON, Canada), an integrated motorized stage for automated sampling (Ludl Electronic Products, Hawthorne, NU, USA) and stereology software (StereoInvestigator, MicroBrightField, Williston, VT, USA). For this analysis, the spinal cord grey matter was imaged (20 x objective) and the region-of-interest (ROI) (the grey matter) manually traced using a computer mouse that was interfaced with the software. Dissectors with a frame size of 80 μm x 80 μm were placed over the ROI using the systematic uniform random sampling protocol, sampling 25% of the ROI. For each counting frame, NeuN⁺ nuclei were manually counted using the mouse as the automated staged moved through the section. Only nuclei that were within the frame that did not touch the exclusion lines were counted. On average, 194 ± 8 NeuN⁺ nuclei were counted per slice with an average of 1356 ± 56 NeuN⁺ nuclei counted per brain. The Gundersen coefficient of error was 0.03-0.04 (Gundersen et al., 1999)

Immunophenotyping of Aged mice

Tissue processing and flow cytometry

The cervical lymph nodes and the spleen were dissected from mice (between 7 and 9 months of age) and were dissociated into a single cell suspension by pushing the tissue through a 70 μm sieve into 1 x PBS using the back end of a sterile 3 mL syringe. Cells were centrifuged (300 x g for 10 min), and then red blood cells lysed by incubating the cell pellet with Ammonium-Chloride-Potassium lysis buffer (0.15M NH₄Cl, 10 mM KHCO₃, 0.1 mM Na₂EDTA) for 1 min and 15 s (1 mL/sample). The lysis was stopped by adding 1 x PBS, and then cells were centrifuged and then resuspended in Complete RPMI media (CM) (RPMI 1640, 10% FBS from Hyclone, 1 mM sodium pyruvate, 2 mM L-glutamine, 0.1 mM non-essential amino acids, 100

U/ml penicillin/streptomycin, and 0.5 μ M 2-mercaptoethanol all from Gibco). Cells (8×10^6 /ml) were plated in 96-well flat-bottomed tissue culture plates together with MOG peptide 35-55 (MEVGWYRSPFSRVVHLYRNGK) (GenScript), neurofilament medium peptide 13-37 (RRVTETRSSFVSRVSGSPSSGFRSQS) (GenScript), or no stimulus. Cells were incubated overnight (15 h) at 37 °C and Brefeldin A (BD Biosciences) was added to a final concentration of 0.66 μ l/ml in the last 5 hours of culture. Cells were then centrifuged ($524 \times g$ and 5 min at 4 °C) and washed twice in 1x PBS prior to flow cytometry staining.

All staining steps were conducted at 4 °C in the dark and centrifugations were at $524 \times g$ and 5 min at 4 °C. First, cells (1×10^6 /stain) were blocked with 100 μ l of anti-CD16/CD32 (5 μ g/ml) (ThermoFisher) in FACS buffer. Cells were washed and stained for 30 min in FACS buffer containing specific antibodies for the following antibody panels (see Tables below). The panel to distinguish total B cells, germinal center B cells, marginal zone B cells, and follicular B Cells included Live/dead Fixable Aqua stain (ThermoFisher) and antibodies specific for CD21, CD19, IgM, B220, CD23, IgD, and GL7 (Rojas et al., 2019). The plasma cell/plasmablast panel included Live/dead Fixable Aqua stain and antibodies specific for B220, CD4, CD8, F4/80, CD138, TACI and CD19 (Rojas et al., 2019). The panel to examine regulatory T cells, T follicular, exhausted, and activated CD4⁺ T cells contained Live/dead Fixable Aqua stain and antibodies directed against CD4, CD25, CXCR5, T cell receptor V β 11.1, and PD-1. The T helper cytokine panel contained Live/dead Fixable Aqua stain and antibodies against CD4 and V β 11.1.

To examine intracellular staining of cytokines, cells were fixed for 15 min with 4% PFA in 1 x PBS, washed and then permeabilized in 1 x Perm/Wash BufferTM (BD Bioscience) for 30 min at 4 °C. Cells were then stained for the following intracellular antibodies in 1x Perm/Wash

BufferTM (BD Bioscience): IFN- γ , IL-17, and GM-CSF. Intranuclear staining for FoxP3 was performed using buffers in the FoxP3 staining kit (Thermofisher). Cells from all staining protocols were washed twice with FACS buffer prior to flow cytometry acquisition using LSRII. Data were analyzed using Flowjo (Flowjo LLC). Details of flow antibodies are provided in the tables below.

Serum collection and detection of antibodies using ELISA

Mouse blood was collected by cardiac puncture into a 1.5 mL Eppendorf tube and incubated at room temperature for an hour. Samples were then centrifuged at 5,000 x g for 10 minutes to separate the serum from the clot and the serum was collected and frozen at -80 °C until analysis. Antibody levels in the serum of mice were determined using a sandwich ELISA technique as follows. For the following procedure, washing steps consisted of a five times wash with PBS-T. NUNC 96-well ELISA plates were pre-coated overnight at 4 °C with 10 μ g/ml each of the following antigens: whole human MOG (Anaspec), MOG p35-55 (GenScript), neurofilament medium p13-37 (GenScript), and MP4 antigen (obtained from Stefanie Kuerten). The next day, plates were washed and then blocked using the Assay Diluent Buffer (Thermofisher) for 1 hour at room temperature. The plates were washed and then 50 μ L of 1:100 diluted sera (in Assay Diluent Buffer) was added to each well in duplicate. After a 2 h incubation at room temperature, plates were washed and the following secondary antibodies were added to the plate (diluted 1:5000 in Assay diluent): biotinylated-IgG1, biotinylated-IgG2a, biotinylated-IgG2b, HRP-conjugated IgG2c, biotinylated-IgG3 and biotinylated-IgM. All plates (with the exception of those labeled with anti-IgG2c) were washed and then incubated with 50 μ L of streptavidin-HRP (1:250 diluted in Assay Diluent Buffer) (Thermofisher). After a 30 min incubation period, all

plates were washed (7 times with PBS-T) and TMB substrate (ThermoFisher) was added to the plate. Development was stopped with 25 μ L of stop solution (1M H₂SO₄). The plates were read at 450 nm using a plate reader (Victor X3, PerkinElmer) and optical density values were recorded for each well. The raw OD values are presented.

Antibodies used for B cell panels for multicolor flow-cytometry

Antibody	Clone	Company	Conjugation
CD21	7G6	BD Biosciences	FITC
CD19	eBio1D3	ThermoFisher	PE
IgM	RMM-1	Biolegend	PerCPCy5.5
B220	RA3-6B2	Biolegend	BV605
CD23	B3B4	ThermoFisher	PeCy7
IgD	1126-C	ThermoFisher	ef450
GL7	GL-7	ThermoFisher	ef660
MHC	M5/114.14.2	ThermoFisher	APC-ef780
CD4	RM4-5	ThermoFisher	PECy7
CD8	53-6.7	ThermoFisher	PECy7
F4/80	BM8	ThermoFisher	PECy7
CD138	281-2	BioLegend	PerCPCy5.5
TACI	eBio8F10-3	ThermoFisher	APC

Antibodies used for regulatory T cell and intracellular T cell panels for multicolor flow-cytometry

Antibody	Clone	Company	Conjugation
CD4	GK1.5	ThermoFisher	APC-ef780
IFN- γ	XMG1.2	BioLegend	BV421
IL-17	eBio17B7	ThermoFisher	APC
GMCSF	MP1-22E9	ThermoFisher	FITC
V β 11.1	RR3-15	BD Biosciences	PE
PD-1	J43	ThermoFisher	FITC
CXCR5	2G8	BD Biosciences	PE-Cy5
FoxP3	FJK-16s	ThermoFisher	ef450
CD25	PC61.5	ThermoFisher	APC
IFN- γ	XMG1.2	Biolegend	ef450
IL-17	eBio17B7	ThermoFisher	APC
GM-CSF	MP1-22E9	ThermoFisher	FITC

Antibodies used for ELISA

Antibody	Clone	Cat No	Company	Conjugation
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IgG1	Polyclonal	1070-08	Southern Biotech	Biotinylated
IgG2a	Polyclonal	1070-08	Southern Biotech	Biotinylated
IgG2b	Polyclonal	1090-08	Southern Biotech	Biotinylated
IgG3	Polyclonal	1100-08	Southern Biotech	Biotinylated
IgM	Polyclonal	1020-08	Southern Biotech	Biotinylated
IgG2c	Polyclonal	1078-05	Southern Biotech	HRP

References

1. Dunn SE, *et al.* (2007) *J Exp Med* 204(2): 321-330.
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Table S1: B cell subsets in 7-9-month PPAR α ^{mut/WT} 2D2⁺ and PPAR α ^{mut/WT} 2D2⁻ mice

	PPAR α ^{mut/WT} 2D2 ⁻		PPAR α ^{mut/WT} 2D2 ⁺		P value
	Cell Number (10 ⁶)	SEM (10 ⁶)	Cell Number (10 ⁶)	SEM (10 ⁶)	
Cervical Lymph Nodes	4.2	0.7	8.1	1.6	0.042
Spleen	49.9	4.9	37.4	5.4	0.060
B cells	PPAR α ^{mut/WT} 2D2 ⁻		PPAR α ^{mut/WT} 2D2 ⁺		P value
	Frequency (%)	SEM (%)	Frequency (%)	SEM (%)	
Cervical Lymph Nodes					
B cells (CD19 ⁺ B220 ⁺)	34.4	2.8	41.82	7.12	0.364
Germinal Center B cells (CD19 ⁺ B220 ⁺ GL7 ⁺)	1.2	0.2	0.74	0.23	0.364
Follicular B cells (CD19 ⁺ B220 ⁺ GL7 ⁻ CD21 ^{lo} IgM ^{lo} IgD ⁺)	88.6	0.9	94.36	0.97	0.002
Non-follicular B cells (CD19 ⁺ B220 ⁺ GL7 ⁻ CD21 ^{hi} IgM ^{hi} IgD ^{lo})	1.9	0.3	0.76	0.24	0.029
Plasma cells (CD19 ⁺ B220 ⁻ TACI ⁺ CD138 ⁺)	0.3	0.04	0.27	0.05	0.898
Plasmablasts (CD19 ⁺ B220 ⁺ TACI ⁺ CD138 ⁺)	0.01	0.00	0.00	0.00	0.19
Spleen					
B cells (CD19 ⁺ B220 ⁺)	52.7	1.2	50.26	3.40	0.606
Germinal Center B cells (CD19 ⁺ B220 ⁺ GL7 ⁺)	1.6	0.7	1.05	0.26	1
Follicular B cells (CD19 ⁺ B220 ⁺ GL7 ⁻ CD21 ^{lo} IgM ^{lo} IgD ⁺)	68.6	3.1	70.68	3.13	0.699
Marginal Zone B cells (CD19 ⁺ B220 ⁺ GL7 ⁻ CD21 ^{hi} IgM ^{hi} IgD ^{lo})	12.4	1.0	12.24	2.34	1
Plasma cells (CD19 ⁺ B220 ⁻ TACI ⁺ CD138 ⁺)	1.8	0.2	1.096	0.10	0.029
Plasmablasts (CD19 ⁺ B220 ⁺ TACI ⁺ CD138 ⁺)	0.19	0.03	0.09	0.03	0.112
B cells	PPAR α ^{mut/WT} 2D2 ⁻		PPAR α ^{mut/WT} 2D2 ⁺		P value
	Cell Number	SEM	Cell Number	SEM	
Cervical Lymph Nodes					
B cells (CD19 ⁺ B220 ⁺)	1,467,350	309,130	3,585,560	981,154	0.06
Germinal Center B cells (CD19 ⁺ B220 ⁺ GL7 ⁺)	17,863	4,680	23,372	9,773	0.606
Follicular B cells (CD19 ⁺ B220 ⁺ GL7 ⁻ CD21 ^{lo} IgM ^{lo} IgD ⁺)	1,287,262	275,129	3,383,991	941,024	0.06

Non-follicular B cells (CD19 ⁺ B220 ⁺ GL7 ⁻ CD21 ^{hi} IgM ^{hi} IgD ^{lo})	27,920	7,407	23,926	9,544	0.797
Plasma cells	226	103	281	76	0.19
Plasmablasts	149	49	125	40	1
Spleen					
B cells	26,330,166	2,642,927	19,091,400	3,509,231	0.147
Germinal Center B cells (CD19 ⁺ B220 ⁺ GL7 ⁺)	395,806	156,009	218,108	87,889	0.298
Follicular B cells (CD19 ⁺ B220 ⁺ GL7 ⁻ CD21 ^{lo} IgM ^{lo} IgD ⁺)	17,847,169	2,061,700	13,299,100	2,372,489	0.298
Marginal Zone B cells (CD19 ⁺ B220 ⁺ GL7 ⁻ CD21 ^{hi} IgM ^{hi} IgD ^{lo})	3,216,798	418,085	2,283,648	593,163	0.24
Plasma cells (CD19 ⁺ B220 ⁺ TACI ⁺ CD138 ⁺)	62,237	11,939	21,391	5,064	0.012
Plasmablasts (CD19 ⁺ B220 ⁺ TACI ⁺ CD138 ⁺)	52,748	8,363	17,826	5,567	0.019

Values between PPAR α ^{mut/WT} 2D2⁺ and PPAR α ^{mut/WT} 2D2⁻ mice were compared using a two-tailed Mann-Whitney test.

Legend. Supplementary Video 1. This mouse is an example of mild clasping (inward turning in of both feet) upon tail elevation and would be assigned a score of 2 using our scoring system.

Legend. Supplementary Video 2. This mouse is an example of severe clasping and would be assigned a score of 3 using our scoring system. If the mouse also slipped through the cage top while walking, it would be provided a score of 4.