[F] Supplemental Text and Figures

Supplemental Fig. 1: Parenchymal inflammation and distribution of *T. gondii*

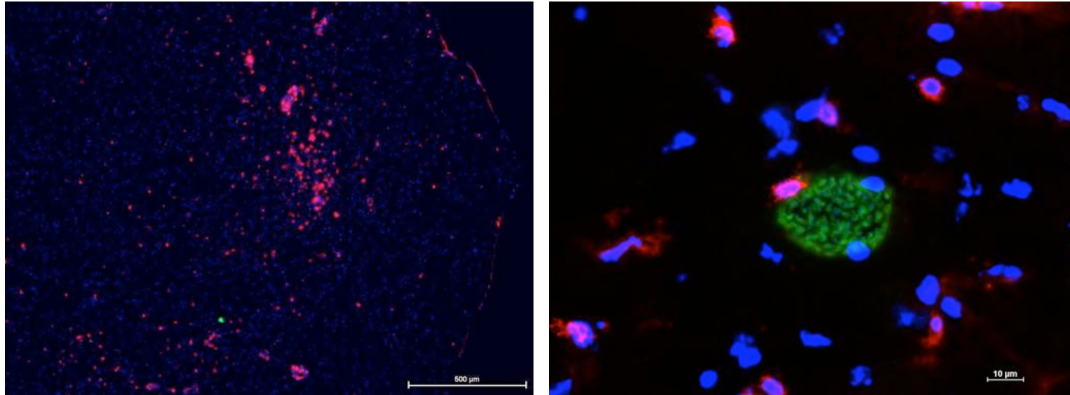

Figure S1 Parenchymal inflammation and d*istribution of T. gondii in the brain.* Chronically infected brains were flash frozen and stained Toxoplasma (green), CD8 (red) and nuclei (DAPI, blue). Scale bar indicates 500μm and 10μm respectively.

Supplemental Fig. 2: peripheral GFP activation

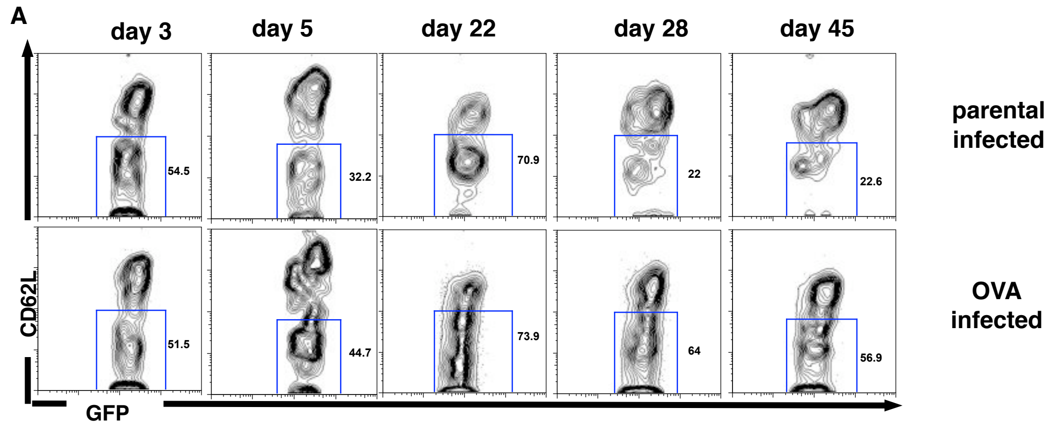

Figure S2 *Peripheral activation of OTI^{GFP} cells.* C57BL/6 mice were infected with either parental control parasites or parasites secreting OVA protein and 3-4 weeks following infection OTI^{GFP} cells were transferred. Splenic cells were analyzed for downregulation of CD62L at various time points following infection. Numbers represent percentage of total GFP cells that have are CD62L low.

Supplemental Fig. 3: peripheral GFP activation by OVA immunization

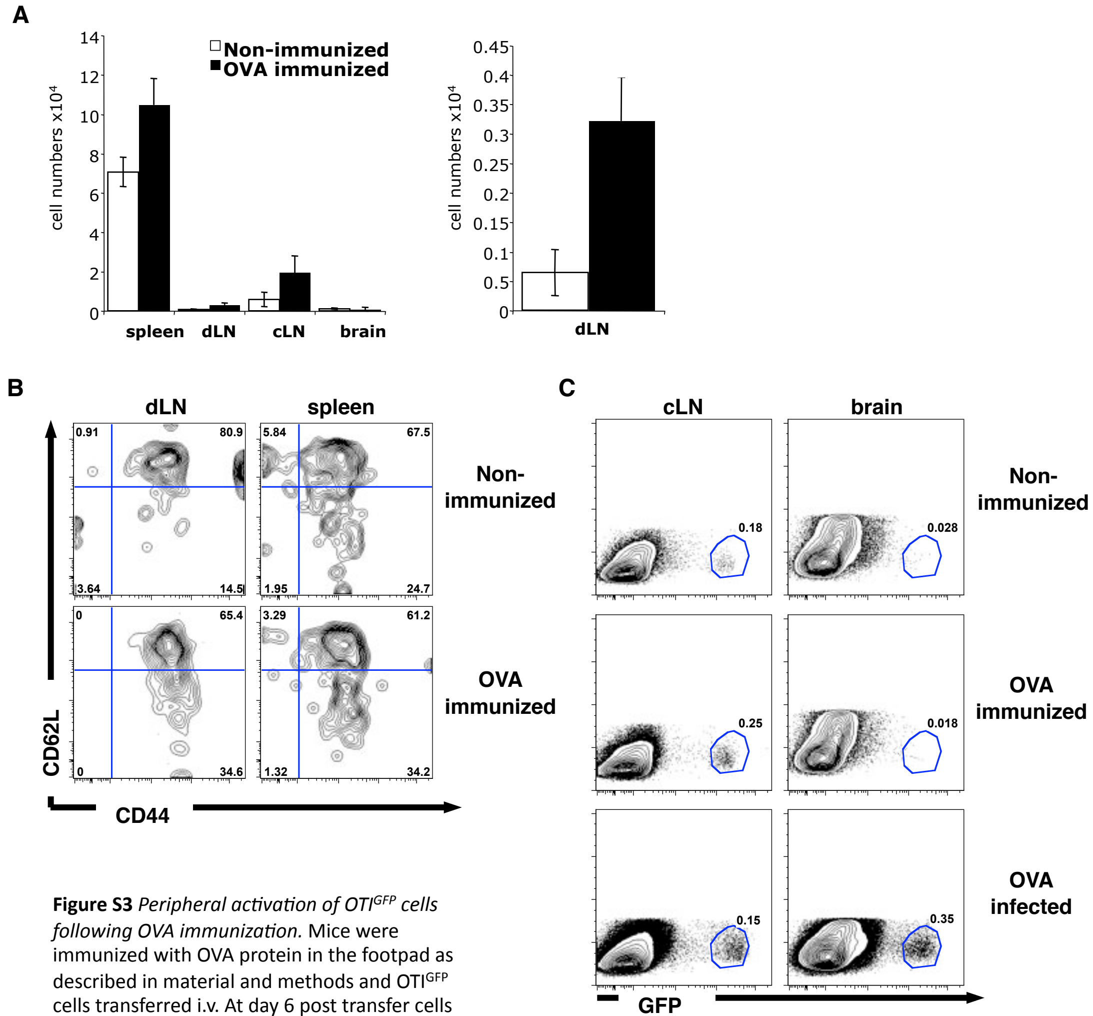

Figure S3 *Peripheral activation of OTI^{GFP} cells following OVA immunization.* Mice were immunized with OVA protein in the footpad as described in material and methods and OTI^{GFP} cells transferred i.v. At day 6 post transfer cells were harvested form spleen, draining LN (inguinal) non-draining LNs (cervical) and brain and OTIGFP cells analyzed for activation and migration to the brain. **A)** total number of cells in non-immunized (open bars) and immunized mice (closed bars). **B)** activation status of OTIGFP cells as measured by CD44 expression and downregulation of CD62L numbers in quadrants represent percentage of total GFP cells. **C)** Number of GFP cells in the cervical LN and brain of non-immunized, immunized and OVA parasite infected mice. Numbers represent percentage of total live cells that are GFP^{+}.

Supplemental Fig. 4: Splenic cell numbers following anti-VLA-4 treatment

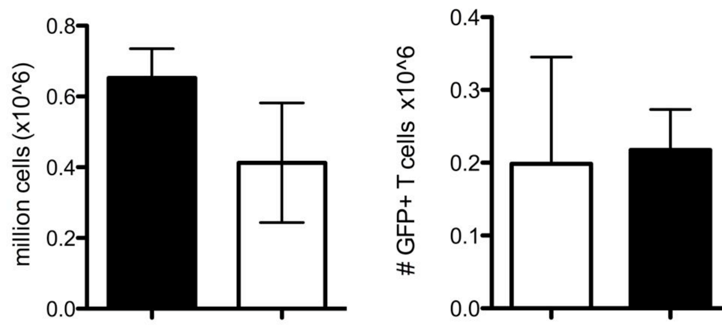

Figure S4 Splenic cell numbers following anti-VLA-4 treatment: Left graph endogenous cells, Right graph GFP numbers. Filled bar = no treatment; open bar = anti-VLA-4 treatment. No significant difference is observed.

Supplemental Fig. 5: Cell division and clustering within the brain

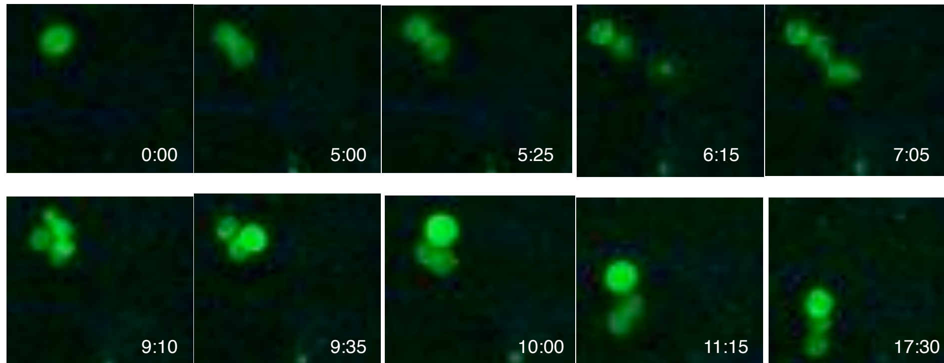

Figure S5 *Cell division and clustering within the brain.* Snapshots of OTI^{GFP} cells dividing and clustering in the brain as imaged by multi-photon microscopy. Time in minutes since the start of imaging is noted.

Supplemental Fig. 6: Transfer of naïve cells leads to a similar pattern of behavior

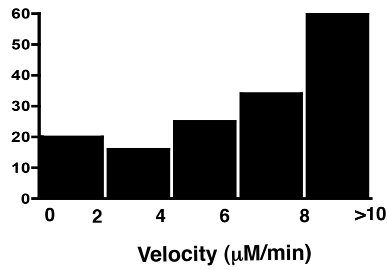

Figure S6 *Transfer of naïve cells leads to a similar pattern of cell behavior.* The number of cells graphed to a range of velocites tracked following transfer of naïve OTI^{GFP} cells followed by infection with Pru^{OVA} and imaged at day21 post transfer

Supplemental Fig. 7: association of SHG with areas of parasite replication

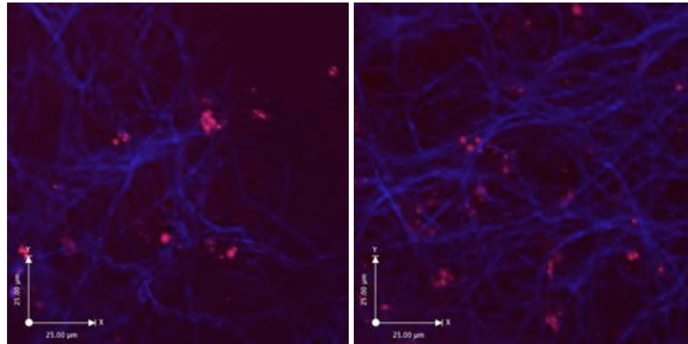

Figure S7 *Second harmonic structures in areas of parasite replication.* Mice were infected with RFP expressing parasites and multi-photon imaging was conducted collecting SHG signal in PMT1 (457-487nm) and RFP signal in PMT 4 (580-652nm). Two examples are provided (scale bar = 25μm) individual parasites can be seen in rosette patterns typical of Toxoplasma.

Supplemental Fig. 8: Development of SHG structure during EAE

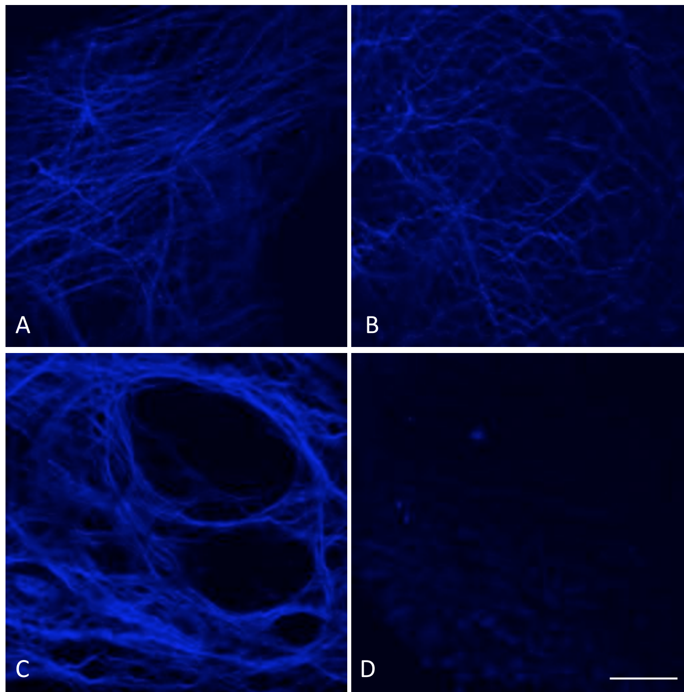

Figure S8 *Development of second harmonic structures during EAE.* Brains and spinal cords were removed from two mice with hind limb paralysis following MOG treatment (94). Imaging was conducted as before on various areas: cortex (**A**); brain stem **(B)**; caudal cervical; rostral lumbar (**C)** and caudal lumbar. There was a correlation between the development of reticular structures as visualized by SHG and inflammation with very dense areas found in the lumbar area. Two naïve mice were also imaged and little or no structure could be detected in either the brain or spinal cord (rostral lumbar: **D**). Images shown are snapshots from z-stacks generated as before and image intensity kept constant between panels. Scale bar = 25μm.

Supplemental Methods: Eight week-old C57Bl/6 mice were subcutaneously immunized with 200 μg Myelin Oligodendroglia Glycoprotein (MOG) peptide 35-55 (custom ordered from CS Bio, Menlo Park, CA) emulsified in 500 μg CFA (Sigma, St. Louis, MO) divided over the four flanks. Intraperitoneal injection of 200 ng pertussis toxin (List Biological laboratories, Campell, CA) diluted into 200 μl of PBS was performed on the day of immunization and 48 hours later. Clinical scoring was undertaken on subsequent days based on standard convention: 0 = no weakness, 1 = limp tail, 2 = mild hind limb paresis, 3 = severe hind limb paresis, 4 = hind limb paralysis, 5 = moribund. Mice used for imaging exhibited persistent paraparesis, with scores of 2 to 3 at chronic time points (>30 days post-immunization). Two-photon imaging was conducted collecting SHG signal in PMT1 (457-487nm) and images of A) cortex, B) brain stem, C) rostral lumbar from EAE mice were generated. D) lumbar region from a naïve mouse. Scale bar indicates 25μm.

Supplemental Fig. 9: Presence of numerous antigen presenting cells in the brains of infected mice

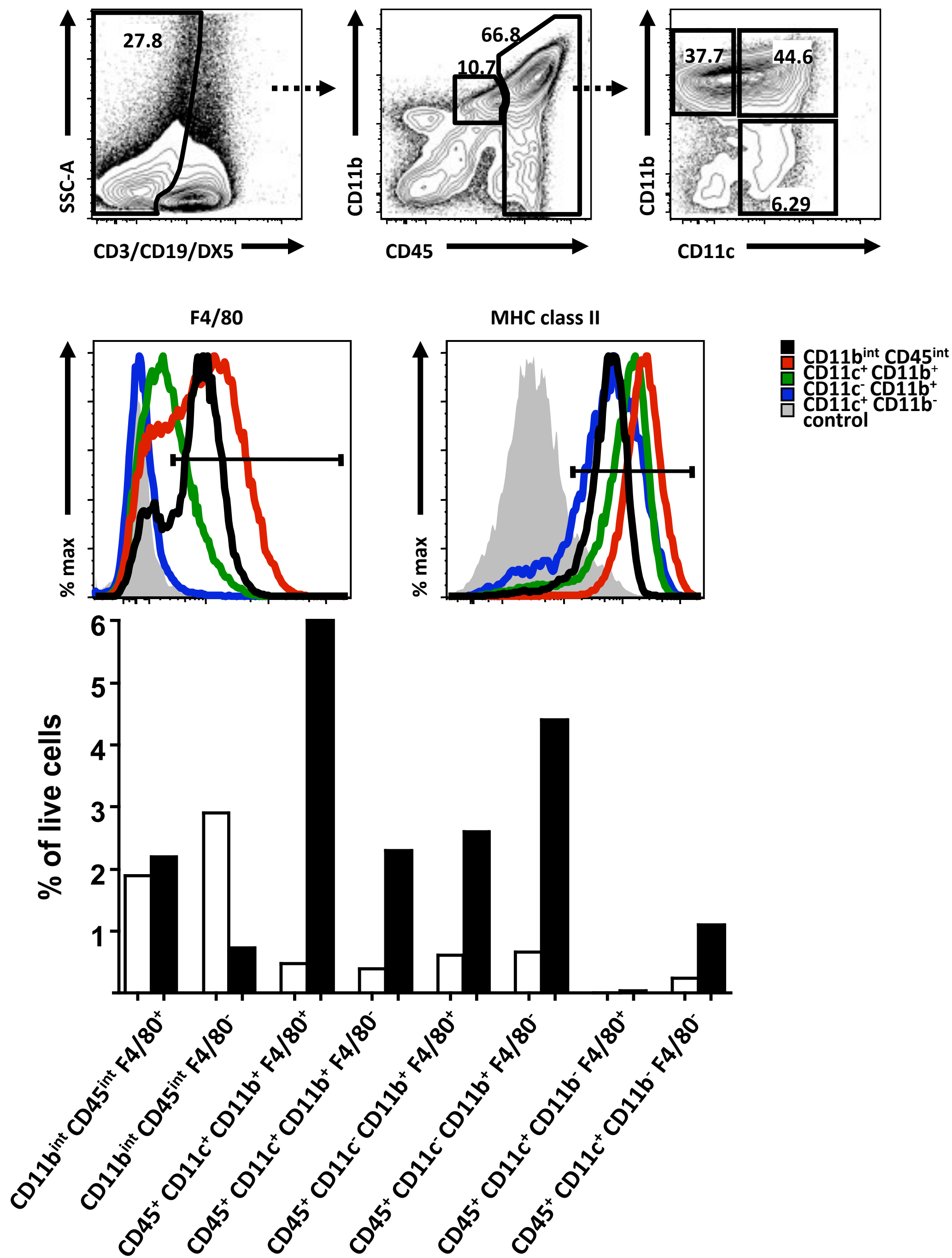

Figure S9: Presence of numerous antigen presenting cells in the brains of infected mice. 3 million BMNC cells prepared for flow cytometry as described in the materials and methods. Cells were then incubated with: FITC CD3/CD19/DX5; PE CD11c; Percep cy5.5; CD8 APC; CD45 APC- Alexa-Fluor 750; CD11b PECy7; F4/80 Pac Blue; MHC II I-A/I-E and gated as detailed above.