Supporting Information

Kyratsous et al. 10.1073/pnas.1701806114

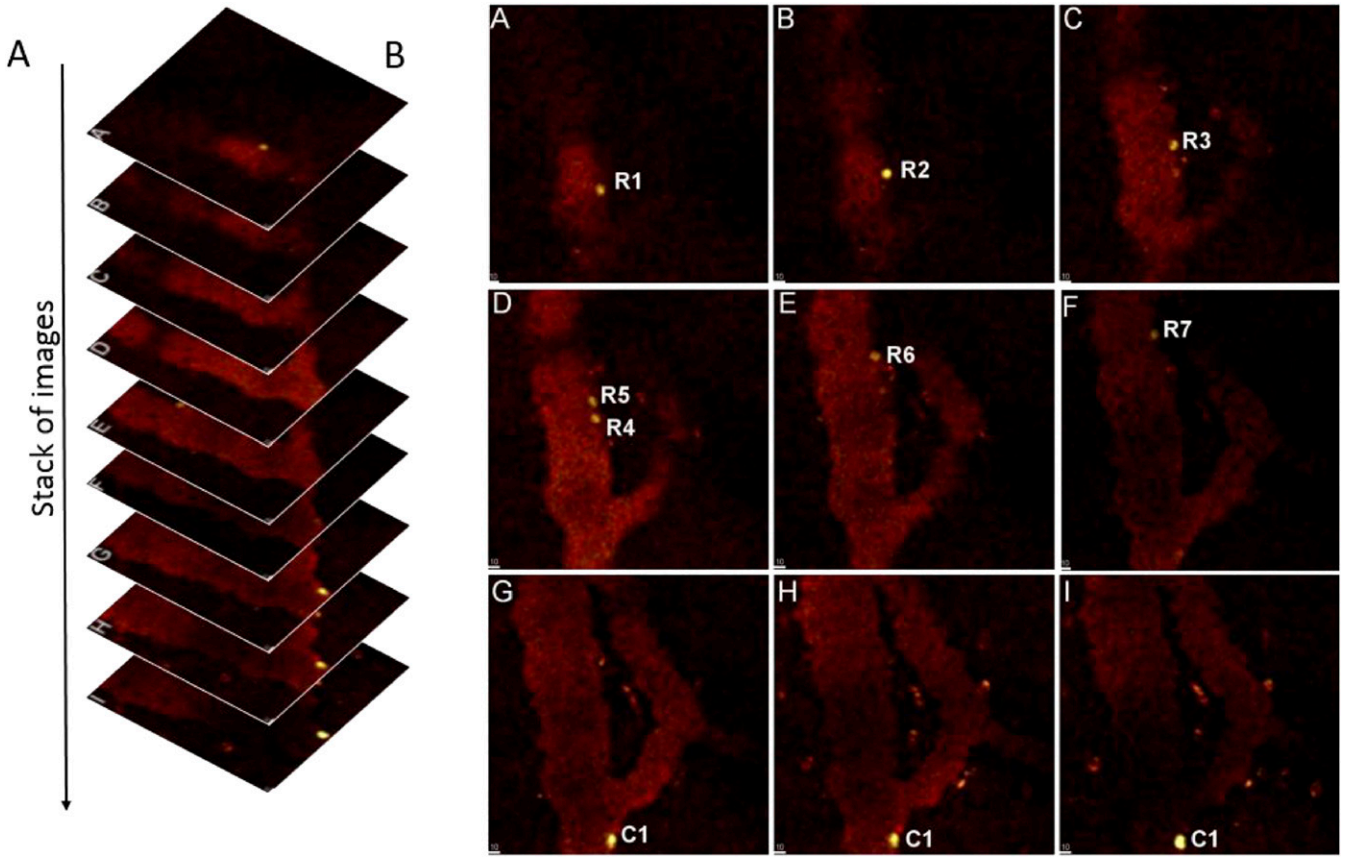


Fig. S1. Detection of rolling and crawling cells by two-photon microscopy. Scheme to detect rolling and crawling cells. (*A*) Scanning of z dimension acquires a series of images. (*B*) Typical cellular size is 10 μm. Because the z interval is around 3–4 μm, one cell can be detected multiple times. The motility of rolling T cells is very high; therefore, a rolling cell locates at different *x*–*y* position in subsequent frames because acquisition of a *x*–*y* plane needs ∼1 s in our setup. For example, a rolling cell appears in six z planes (*A*–*F*) in different positions (R1–R7). In images *G*–*I*, a crawling cell appears (C1). T cells (blue/yellow) and blood vessels (red, visualized by i.v. infusion of fluorescent dextran) are shown in fluorescent overlay. (Scale bar: 10 μm.)

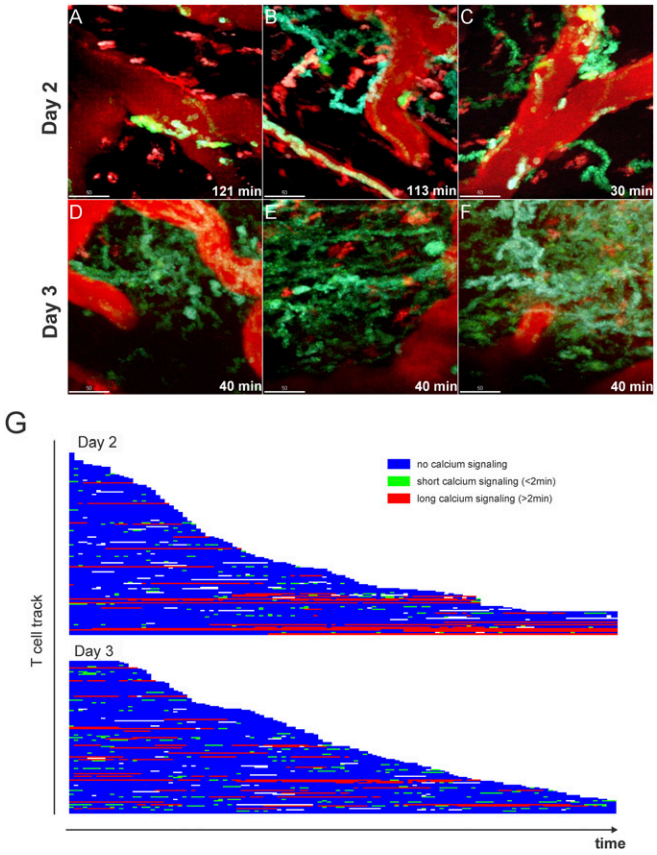


Fig. S2. Leptomeningeal scanning of encephalitogenic T cells on day 2 and day 3. Time projections from six representative movies are shown for $T_{MBP\text{-}Twitch1}$ cells (green) and blood vessels (red, visualized by i.v. infusion of fluorescent dextran) on day 2 (*A*–*C*) and day 3 p.t. (*D*–*F*). Inserted numbers indicate the duration of image acquisition. (*G*) Calcium history plots of $T_{MBP\text{-}Twitch1}$ cells on days 2 and 3 p.t. Each horizontal line represents a single T-cell track. In the calcium history plots, a blue color indicates low calcium levels, a green color indicates high calcium levels shorter than 2 min, and a red color indicated high calcium levels longer than 2 min.

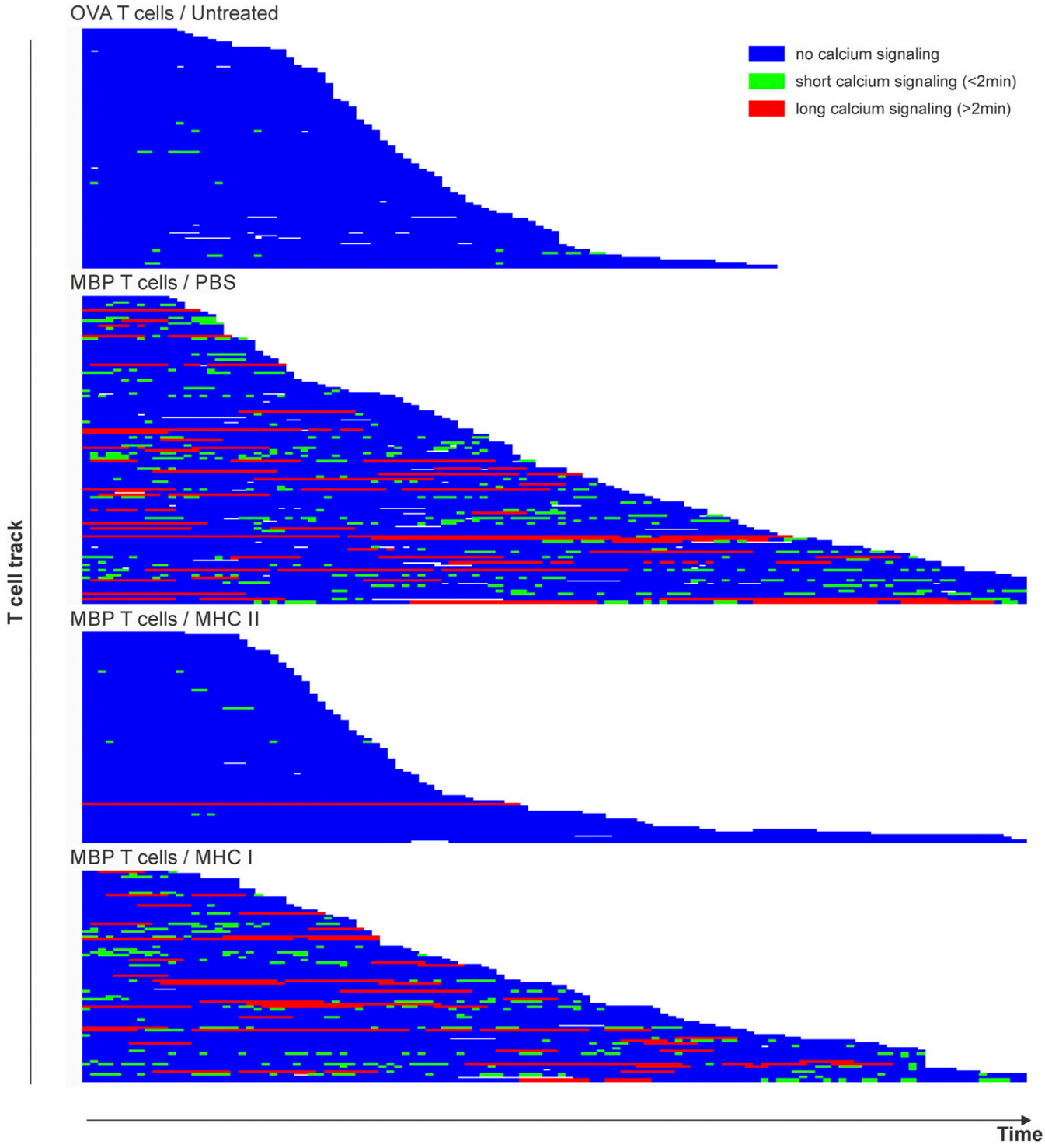


Fig. S3. Calcium signaling after MHC blocking. Calcium history plots of nontreated $T_{OVA\text{-}Twitch1}$ cells and $T_{MBP\text{-}Twitch1}$ cells on day 3 p.t. with or without antibody treatment. Each horizontal line represents one single T-cell track. In the calcium history plots, the blue color indicates low calcium levels, the green color indicates high calcium levels shorter than 2 min, and the red color indicates high calcium levels longer than 2 min.

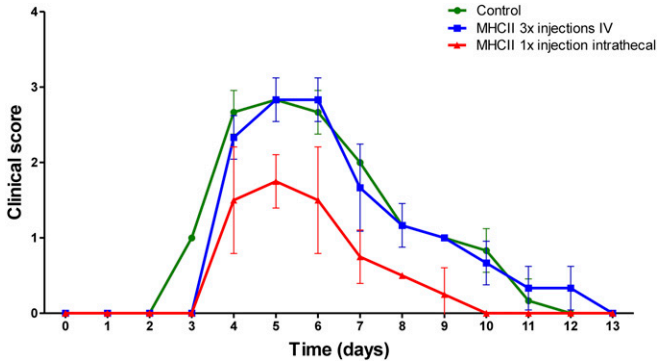


Fig. S4. Effect of anti-MHC class II antibody on clinical EAE. EAE clinical score after injection of anti-MHC class II antibody by i.v. or intrathecal injection. Representative results from at least three experiments are shown.

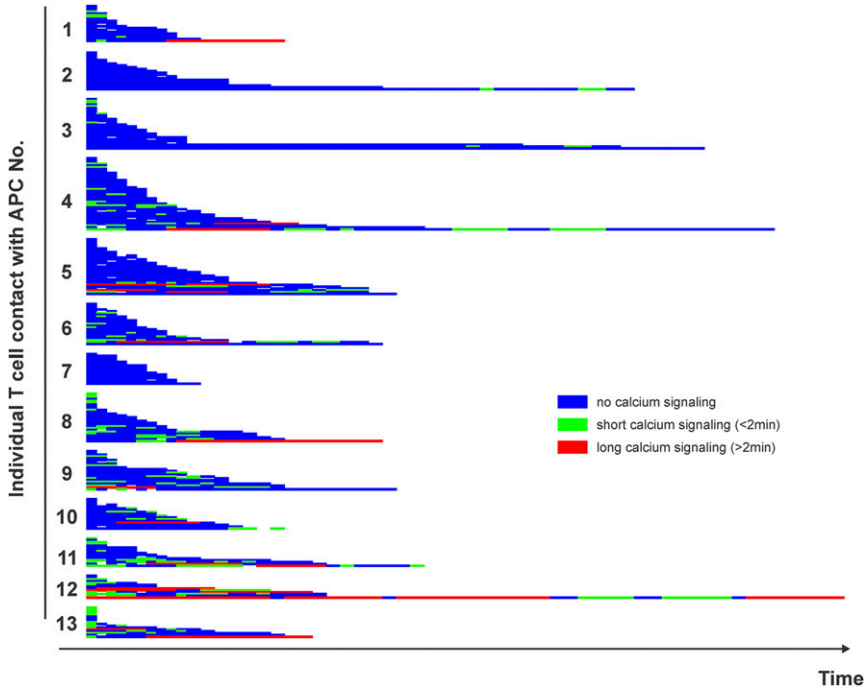


Fig. S5. Calcium history plot during contact with particular APCs. Calcium history plots of the $T_{MBP\text{-}Twitch1}$ cells during contact with APCs are shown. Numbers represent APCs identified in Fig. 6 *A*, *ii*. Each horizontal line represents a single continuous contact. Blue color indicates low calcium levels, green color indicates high calcium levels shorter than 2 min, and red color indicates high calcium levels longer than 2 min.

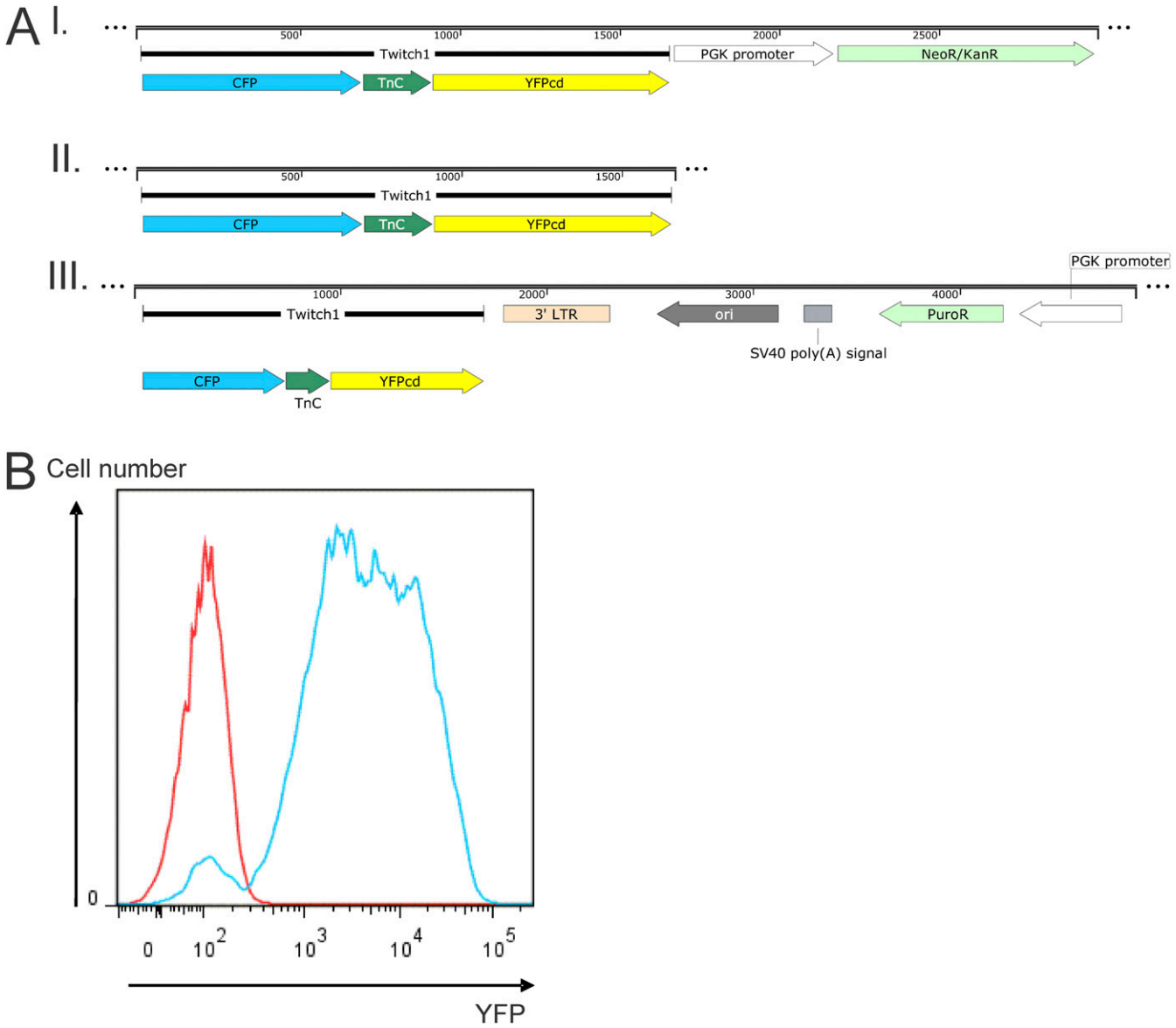


Fig. S6. Improved retroviral vector coding Twitch1. (*A*) Twitch1 retroviral constructs. The elements of each construct are shown here. (*I*) pMSCVneoTwitch1. (*II*) pMSCVΔneoTwitch1. (*III*) PINCOpuroTwitch1. Ori, origin of replication; puro, puromycin resistance gene. (*B*) Representative flow cytometric histogram depicting Twitch1 expression levels in $T_{MBP\text{-}Twitch1}$ (blue) and nonfluorescent MBP-specific T cells (red) at the seventh round of restimulation on day 2.

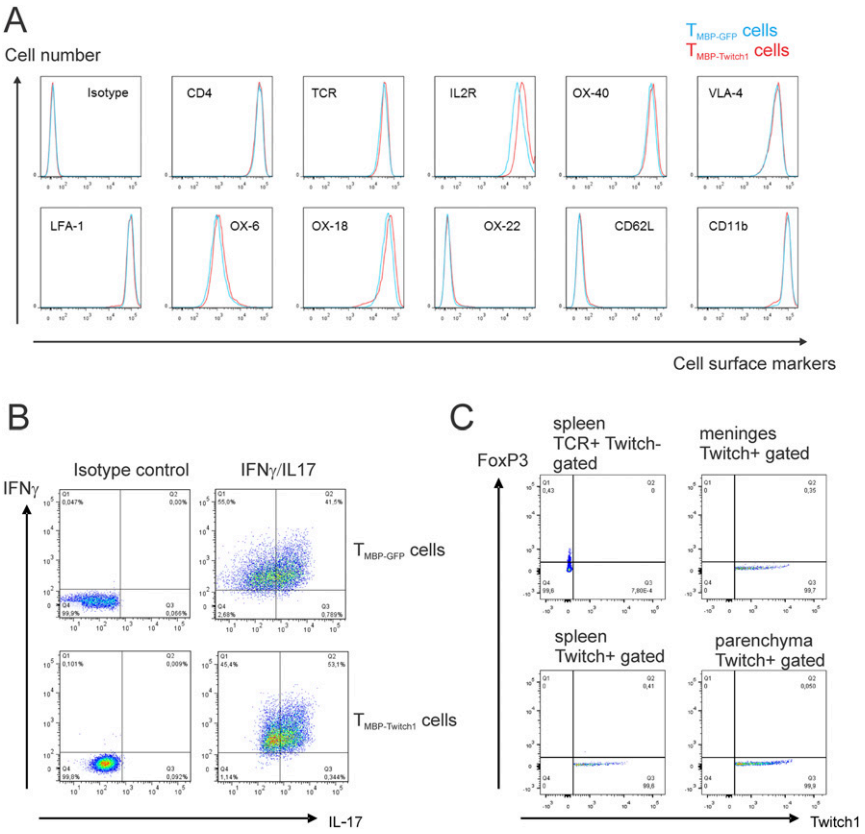


Fig. S7. Cellular phenotype of Twitch1-expressing T cells. (*A*) The phenotype of Twitch1-expressing T cells is compared with GFP-expressing T cells for cell surface markers (*A*) and the production of inflammatory cytokines (*B*). (*A*) The histograms depict the expression of cell surface markers on in vitro-activated GFP- or Twitch1-labeled T cells. $T_{MBP-GFP}$ (blue lines) and $T_{MBP-Twitch1}$ (red lines) cells were stained with specific antibodies as indicated and analyzed by flow cytometry. (*B*) Dot plots of IFNγ/IL-17 intracellular staining. The inserted numbers indicate the proportion of cells in each quadrant. Representative data from three independent experiments per cell line are shown. (*C*) Dot plots of Twitch1 expression and FoxP3 staining in ex vivo isolated T cells. Living cells were further gated as indicated. Representative result from two independent experiments including two rats per experiment is shown.

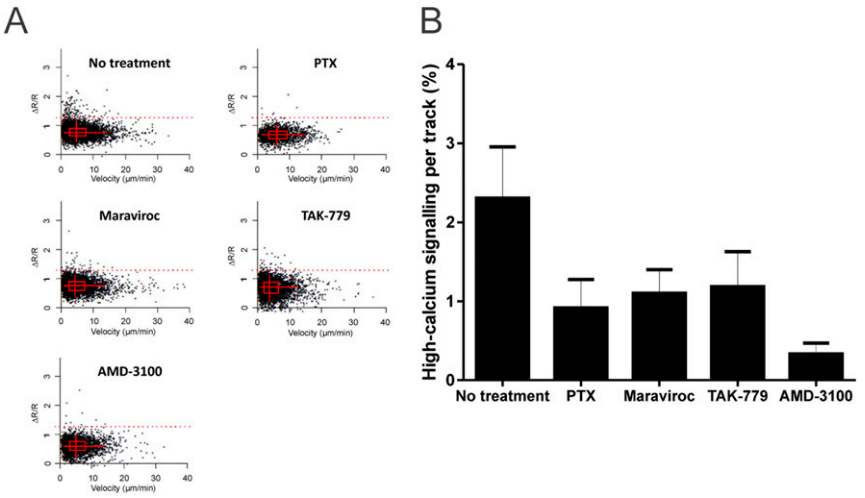
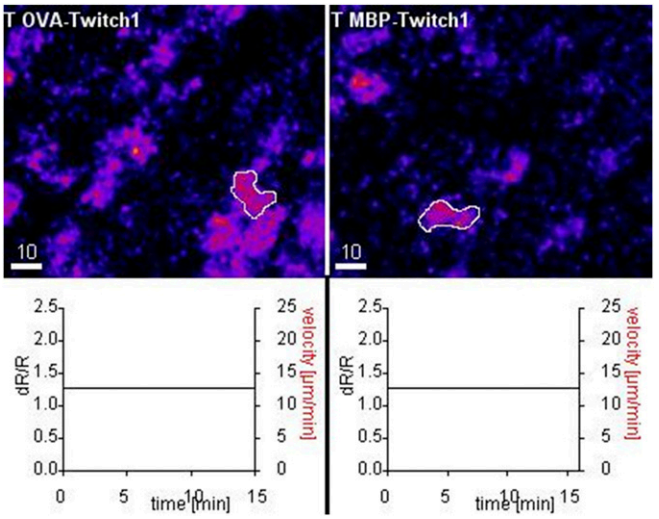
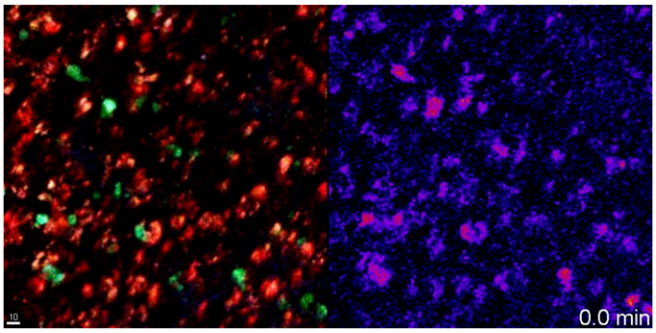


Fig. S8. Contribution of chemokine signaling to short-lived calcium signaling. (*A*) Scatterplots showing the $T_{OVA\text{-}Twitch1}$ velocity versus the calcium-indicator ratio change for each individual time point with or without inhibitor treatment. Mean values for ΔR/R and velocity are indicated along with a 2D box plot. The results are the sum of at least three independent experiments per treatment. (*B*) Percentage of high-calcium signaling per track in the spleen with or without inhibitor treatment.



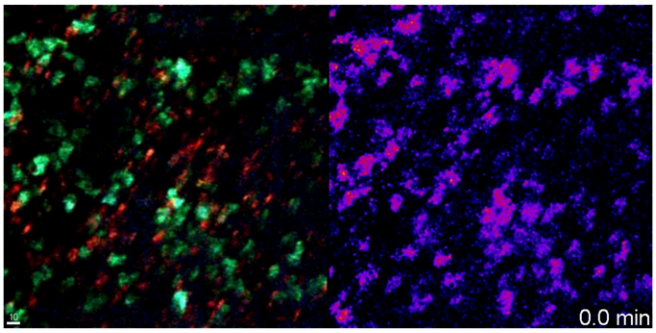
Movie S1. Short calcium signaling in spleen. $T_{OVA\text{-}Twitch1}$ cells (*Left*) and $T_{MBP\text{-}Twitch1}$ cells (*Right*) were imaged in the spleen on day 3 p.t. (*Upper*) Intracellular calcium level by pseudocolor ratio images. (*Lower*) Instantaneous velocity (red lines) and intracellular calcium levels as ΔR/R (black lines) at indicated time point. All movies run 10 frames per second (×250 speed).

Movie S1



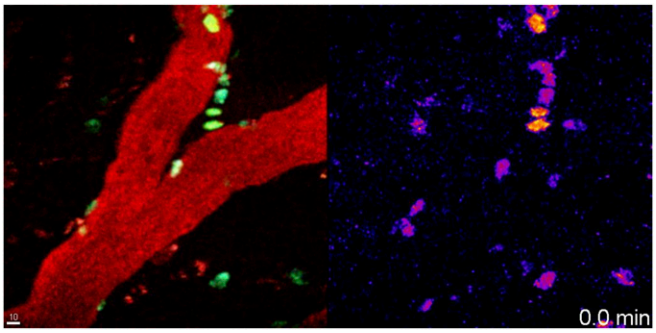
Movie S2. Visualization of calcium signaling in spleen before and after soluble MBP treatment. $T_{MBP\text{-}Twitch1}$ cells were imaged in spleen on day 3 p.t. A fluorescence overlay (*Left*) and a pseudocolor ratio image (*Right*) are depicted. Soluble MBP was injected at indicated time point. Blue/green, $T_{MBP\text{-}Twitch1}$ cells; red, splenic phagocytes visualized by detecting autofluorescence and i.v. infusion of fluorescent dextran at *Left*.

Movie S2



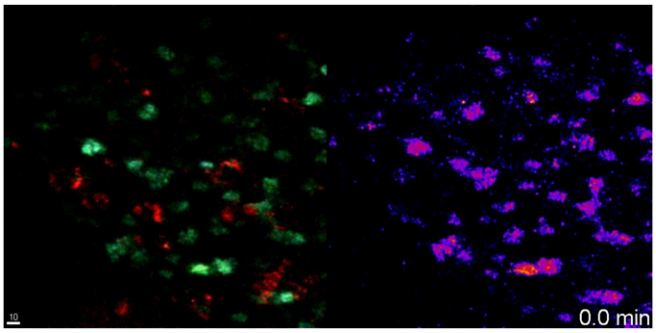
Movie S3. Visualization of calcium signaling in spleen before and after soluble OVA treatment. $T_{OVA\text{-}Twitch1}$ cells were imaged in spleen on day 3 p.t. A fluorescence overlay (*Left*) and a pseudocolor ratio image (*Right*) are depicted. Soluble OVA was injected at indicated time point. Blue/green, $T_{OVA\text{-}Twitch1}$ cells; red, splenic phagocytes visualized by detecting autofluorescence and i.v. infusion of fluorescent dextran at *Left*.

Movie S3



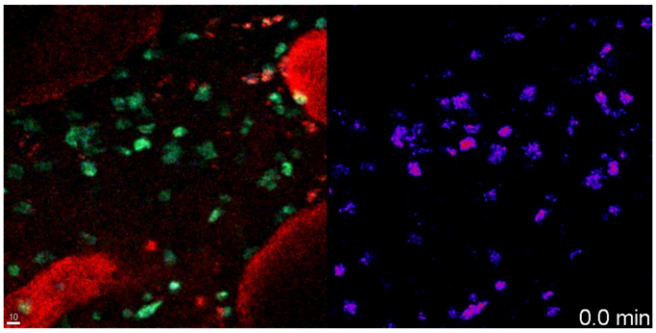
Movie S4. Visualization of calcium signaling of $T_{MBP\text{-}Twitch1}$ cells during the early prodromal phase of infiltration. $T_{MBP\text{-}Twitch1}$ cells were imaged in spinal cord leptomeninges on day 2 p.t. A fluorescence overlay (*Left*) and a pseudocolor ratio image (*Right*) are depicted. Blue/green, $T_{MBP\text{-}Twitch1}$ cells; red, blood vessels and phagocytes visualized by i.v. infusion of fluorescent dextran at *Left*.

Movie S4



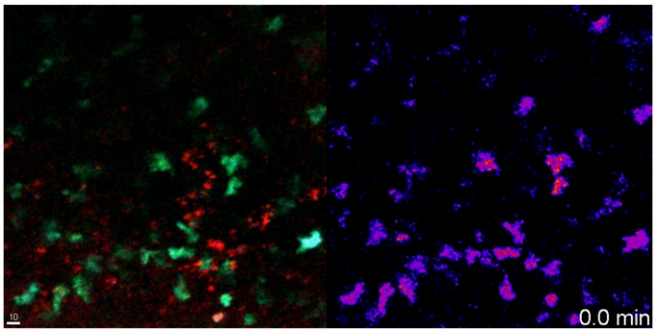
Movie S5. Calcium signaling of $T_{MBP\text{-}Twitch1}$ cells at leptomeninges after onset of EAE. $T_{MBP\text{-}Twitch1}$ cells were imaged in spinal cord leptomeninges on day 3 p.t. A fluorescence overlay (*Left*) and a pseudocolor ratio image (*Right*) are depicted. Blue/green, $T_{MBP\text{-}Twitch}$ cells; red, phagocytes visualized by intrathecal injection of fluorescent dextran at *Left*.

Movie S5



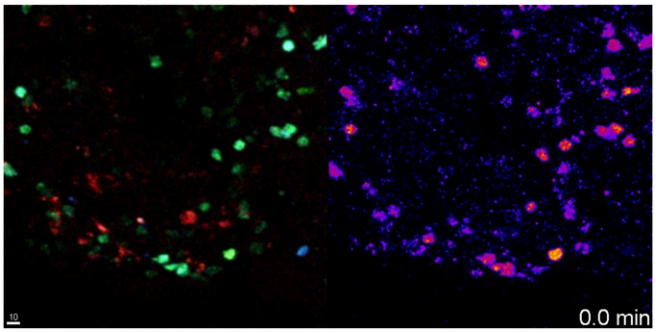
Movie S6. Calcium signaling of $T_{OVA\text{-}Twitch}$ cells at leptomeninges. $T_{OVA\text{-}Twitch1}$ cells were imaged in spinal cord leptomeninges on day 3 p.t. after cotransfer with nonlabeled MBP-specific T cells (not visible). A fluorescence overlay (*Left*) and a pseudocolor ratio image (*Right*) are depicted. Blue/green, $T_{OVA\text{-}Twitch1}$ cells; red, blood vessels and phagocytes visualized by i.v. and intrathecal injection of fluorescent dextran, respectively, at *Left*.

Movie S6



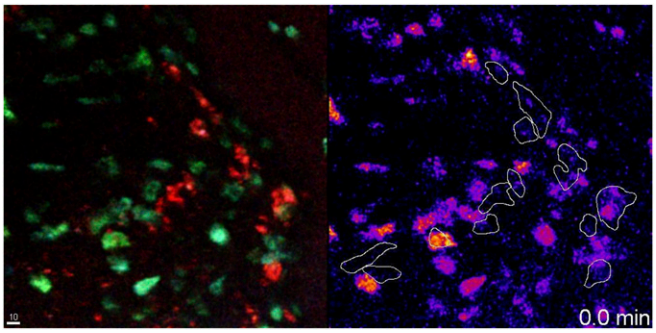
Movie S7. Calcium signaling of $T_{MBP\text{-}Twitch1}$ cells at leptomeninges after intrathecal injection of anti-MHC class II blocking antibody. $T_{MBP\text{-}Twitch1}$ cells were imaged in spinal cord leptomeninges on day 3 p.t. after intrathecal injection of anti-MHC class II blocking antibody. A fluorescence overlay (*Left*) and a pseudocolor ratio image (*Right*) are depicted. Blue/green, $T_{MBP\text{-}Twitch}$ cells; red, phagocytes visualized by intrathecal injection of fluorescent dextran at *Left*.

Movie S7



Movie S8. Calcium signaling of $T_{MBP\text{-}Twitch1}$ cells at leptomeninges after intrathecal injection of anti-MHC class I blocking antibody. $T_{MBP\text{-}Twitch1}$ cells were imaged in spinal cord leptomeninges on day 3 p.t. after intrathecal injection of anti-MHC class I blocking antibody. A fluorescence overlay (*Left*) and a pseudocolor ratio image (*Right*) are depicted. Blue/green, $T_{MBP\text{-}Twitch1}$ cells; red, phagocytes visualized by intrathecal injection of fluorescent dextran at *Left*.

Movie S8



Movie S9. Antigen-presenting capacity of APCs at CNS leptomeninges. $T_{MBP\text{-}Twitch1}$ cells were imaged in spinal cord leptomeninges on day 3 p.t. A fluorescence overlay (*Left*) and a pseudocolor ratio image (*Right*) are depicted. Antigen-presenting cells are indicated with white lines at *Right*. Blue/green, $T_{MBP\text{-}Twitch1}$ cells; red, phagocytes visualized by intrathecal injection of fluorescent dextran at *Left*.

Movie S9