SUPPLEMENTAL MATERIAL

Kang et al., http://www.jem.org/cgi/content/full/jem.20101295/DC1

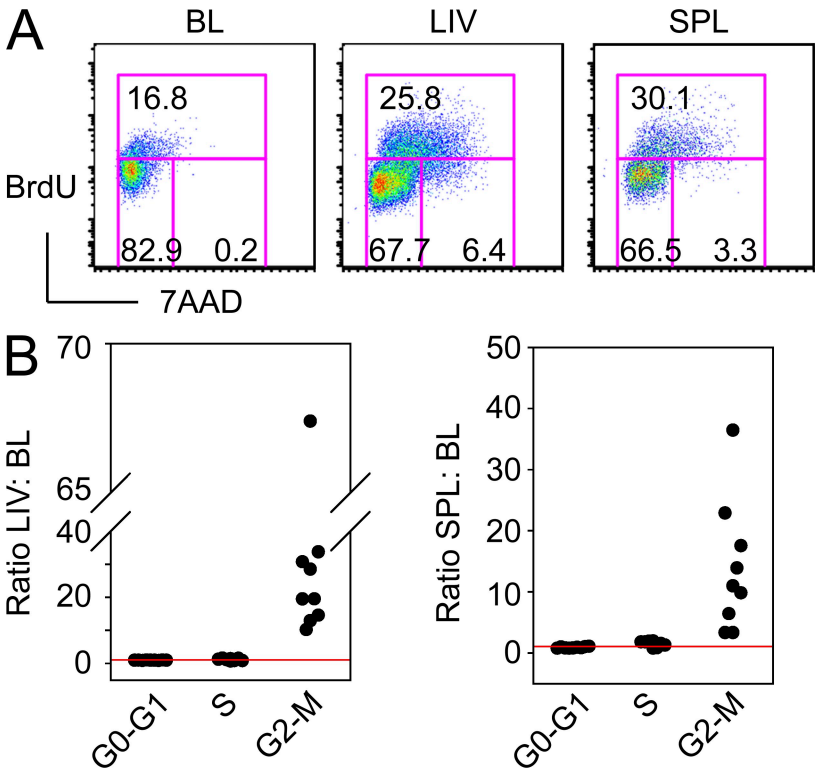


Figure S1. Virus-specific CTLs circulate in cell cycle after VSV infection. To determine whether virus-specific CTLs continue cell cycle during migration in response to another viral pathogen, the blood (BL), liver (LIV), and spleen (SPL) of Ly5.1$^+$ mice seeded with Ly5.2$^+$ OT-I cells were harvested 15 min after i.p. BrdU administration on day 5 after infection with VSV-OVA. (A) Representative flow cytometric plots of BrdU versus 7AAD are shown for Ly5.2$^+$ CD8$^+$ T cells (OT-I cells). (B) The ratio Ly5.2$^+$ OT-I cells found in G0-G1, S, or G2-M stages of cell cycle in the LIV or SPL relative to the blood was calculated for individual mice ($n = 9$). Data were compiled from three independent experiments. The red line is set at a ratio of 1 and indicates no difference relative to the BL. The ratio of cells in G2-M compared with G0-G1 or S was significantly different in all tissues examined ($P < 0.05$, one-way ANOVA).

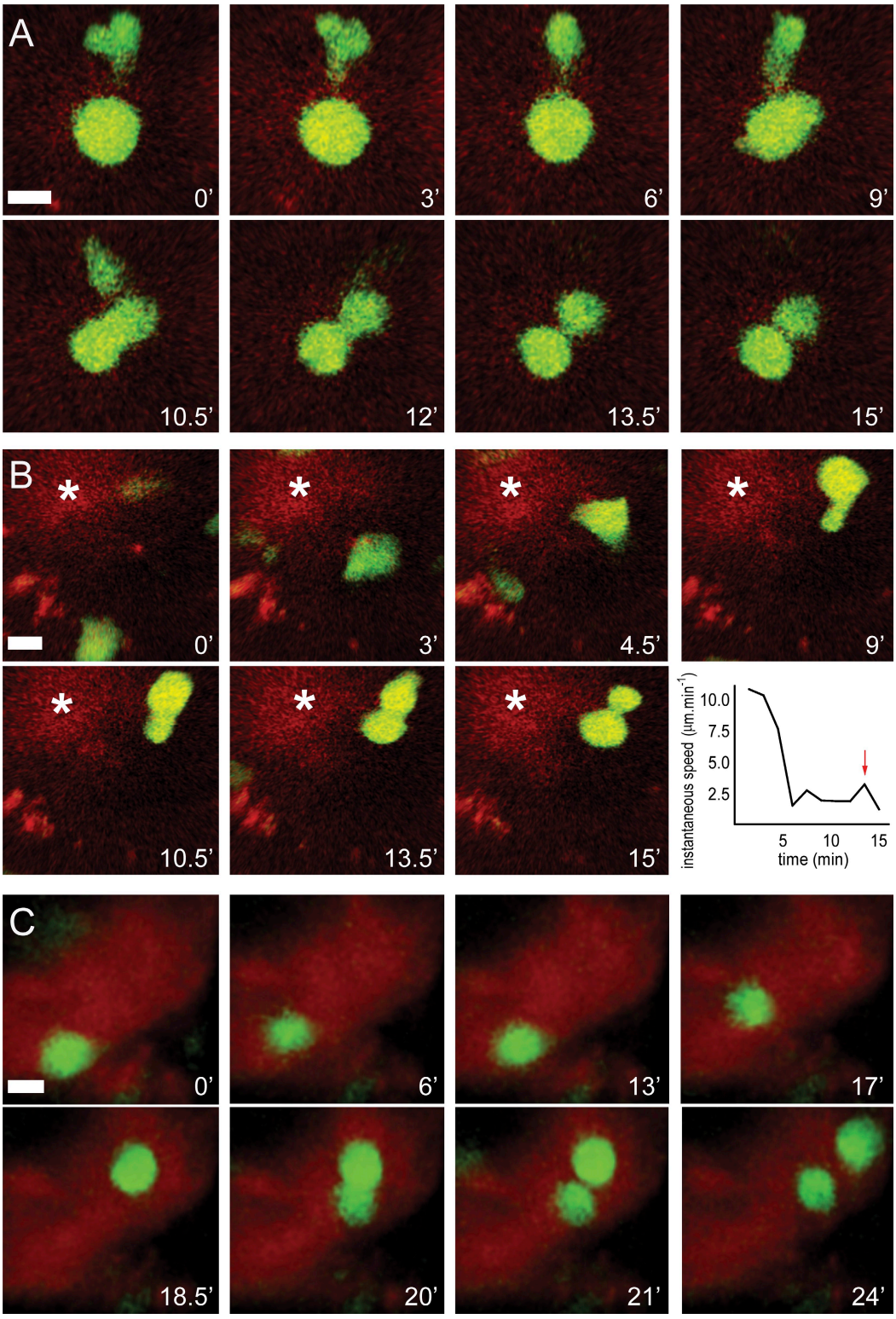


Figure S2. Representative time lapses of CTL undergoing mitosis after arrest in the meninges. (A–C) Representative two-photon time-lapse images demonstrate GFP$^+$ P14 cells (green) dividing in the meninges (A and B) and inside of a meningeal blood vessel (C). The mitochondrial dye rhodamine-2AM (red) was used as a tissue contrast agent to reveal meningeal cells in time lapses (A and B). Meningeal vasculature was visualized in C by injecting quantum dots 10 min before imaging. The graph in B shows the velocity of the P14 CTL over time. The CTL decelerates to <2.0 μm/min at 5 min (indicative of arrest) and divides by 15 min (red arrow). P14 CTL can also divide within meningeal blood vessels as shown in time lapse (C). Bars, 5 μm. Data are representative of six independent experiments (n = 35 mice; see Video 1).

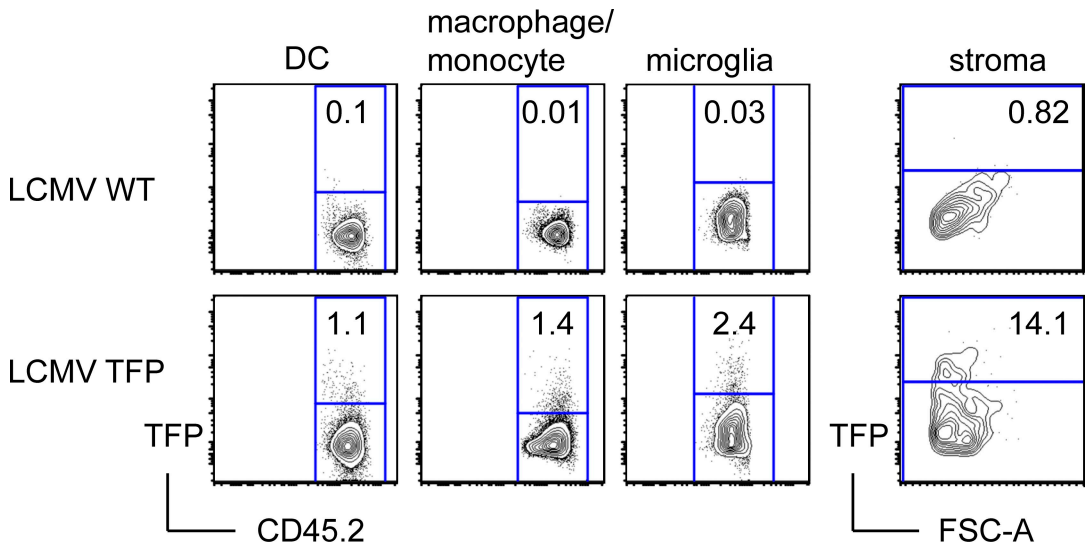
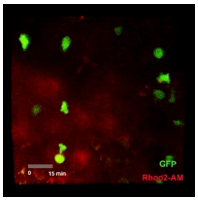
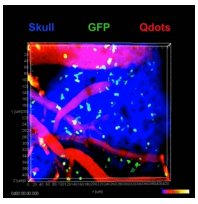


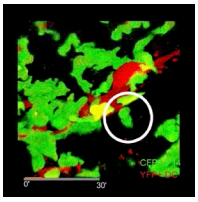
Figure S3. LCMV is detected in both resident and CNS-infiltrating APCs at day 6 after infection. To identify the CNS cell populations that have the potential to present antigen to P14 CTL, we infected mice i.c. with r3LCMV-TFP. WT LCMV Arm was used as a negative control for this experiment. At day 6 after infection, brain mononuclear cells were extracted and analyzed by flow cytometry for expression of TFP. Representative FACS plots of TFP expression in CNS DCs ($CD11c^+$ $CD45.2^{hi}$ $CD11b^+$), macrophage/monocytes ($CD11c^-$ $CD45.2^{hi}$ $CD11b^+$), microglia ($CD45.2^{lo}$ $CD11b^+$), and stromal cells ($CD45.2^-$ $gp38^+$) are shown for mice infected with r3LCMV-TFP or WT LCMV. The data are representative of $n = 12$ animals compiled from three independent experiments.



Video 1. Examples of CTL division in the virally infected meninges. Part 1: a representative time lapse of a 3D reconstruction shows a GFP^+ P14 CTL (green) undergoing mitosis in the meningeal space after a period of arrest in a day 6–infected mouse. The mitochondrial dye rhodamine-2AM (red) was used as a tissue contrast agent. Part 2: another representative time lapse at day 6 after infection shows a P14 CTL migrate adjacent to a density of rhod-2AM signal (presumed to be a cell), decelerate, and then undergo mitosis. This all occurs within 15 min. Part 3: after a period of arrest, a P14 CTL undergoes mitosis inside of a meningeal blood vessel of a day 6–infected mouse. The meningeal blood vessel was visualized by injecting quantum dots (red) i.v.



Video 2. Anatomy and dynamics of CTL division in the virally infected meninges. Part 1: a representative time lapse of a 3D reconstruction shows GFP^+ P14 CTL (green) underneath the thinned skull (blue) and in relation to meningeal vasculature labeled with quantum dots (red). The circle denotes a P14 CTL undergoing mitosis and splitting into two daughter cells during the course of the film. The box represents the region shown in part 2. Part 2: a 3D reconstruction representing one time point from part 1 is animated to reveal the anatomical position of the mitotic CTL. Note that the CTL sits in the meninges beneath the thinned skull and above a meningeal blood vessel. Part 3: a cropped portion of the time lapse from part 1 shows a magnified view of the arrested CTL (white circle) undergoing division.



Video 3. CTLs divide with and without contacting meningeal DCs. Part 1: a representative 30-min time lapse of a 3D reconstruction shows CFP^+ P14 CTL (green) clustering (white arrows) around a CD11c-YFP^+ DC (red) in the meninges at day 6 after infection. The P14 CTL highlighted by the white circle is dividing while engaged in stable contact with the DC. It does not complete the final phase of the cell cycle program (cytokinesis) before detaching. Part 2: a magnified view is shown of the dividing CTL from Part 1. The CTL-DC contact point is noted with an arrow. Part 3: a P14 CTL splits into two daughter cells while contacting (arrow) a meningeal DC. The proximal daughter cell remains attached to the DC whereas the distal daughter moves away. Part 4: in this example, a CTL approaches a DC, makes contact, and then splits into two daughter cells. All of this transpires in 10 min. Part 5: an example is shown of a P14 CTL that undergoes mitosis in the absence of DC contact. Part 6: four P14 CTLs are shown dividing in a single field of view. Only one CTL (pink circle) is in contact with a DC dividing in a single field of view. The other dividing CTLs (blue circles) have no contact with DCs.