

## **Online Supplementary Material**

*Supplementary Movie 1:* Scanning behavior of CD4+ T cells in the subarachnoid space of naïve mice.

*Supplementary Movie 2:* CD4+ T cells interact with MHC class II+ APCs in the subarachnoid space in the early phases of EAE.

*Supplementary Movie 3:* Behavior of MHC class II positive APCs in the subarachnoid space in the early phases of EAE.

*Supplementary Movie 4:* Migratory behavior of CD4+ T cells in the subarachnoid space during different stages of EAE.

## **Supplementary Movies**

Leptomeningeal explants were prepared from naïve mice or mice immunized with MOG35-55 and harvested on day 10-11 pi (before onset of signs), day 14-15 pi (peak of EAE) or day 17-18 pi (after peak of clinical EAE). Samples were stained with anti-CD4 (green) and -MHC class II (red) and imaged using time-lapse confocal laser-scanning microscopy. In some movies, colors were changed (from red or green to white or blue) during processing to enhance contrast and clarity.

Total imaging times and scanning intervals are described in each movie.

Sequences of z-stacks were transformed into three-dimensional movies and analyzed using LSM 3D analysis software.