

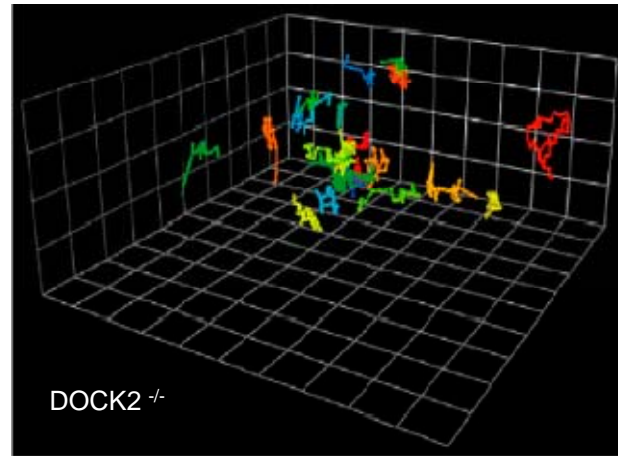
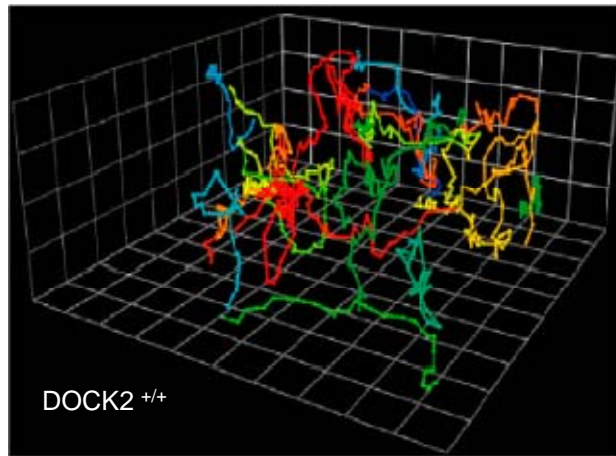


## Application Note

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# Three dimensional tracking of T-Cells



**Figure: Paracortical T cell migration in the absence of DOCK2.** Fluorescently labeled T cells were adoptively transferred into wild-type recipients, and their migratory behavior inside lymph nodes was visualized using MP-IVM. Cell motility was analyzed using **Volocity** software. Three dimensional tracks of control T cells (DOCK2<sup>+/+</sup>) and DOCK2<sup>-/-</sup> T cells over 30 min. Control cells vigorously migrated in a random walk pattern. In contrast, most DOCK2<sup>-/-</sup> T cells oscillated around the initial tracking spot, unable to initiate steady movement in any direction. Each colored line represents a single T cell track. The grid length of each square corresponds to 20.8  $\mu\text{m}$ .

An essential feature of the adaptive immune system is the continuous recirculation of lymphocyte through the body in search of foreign antigen. Lymphocytes are constantly moving from the blood into secondary lymph organs (SLOs), such as peripheral lymph nodes (PLNs) and spleen, where they screen for antigen presenting cells (APCs). Recirculation is completed by migration of lymphocytes back to the blood stream via lymphatic vessels. Their long life span and constant migration make lymphocytes one of the most motile mammalian cell types. Dr Jens Stein and colleagues are investigating this lymphocyte migration *in vivo* with a focus on DOCK2, an intracellular protein which is a key regulator of cell motility via the small GTPase Rac.

The researchers used recently established multiphoton intravital microscopy (MP-IVM) that allows visualization of the dynamics of lymphocyte motility within their microenvironment of primary and secondary lymphoid tissues. Image stacks of CMTMR-labeled control lymphocytes (DOCK2<sup>+/+</sup>, red) and CFSE-labeled DOCK2-deficient lymphocytes (DOCK2<sup>-/-</sup>, green) were taken in intervals over 30 min. Sequences of image stacks were transformed into volume rendered four dimensional movies (<http://www.jem.org/cgi/content/full/jem.20061780/DC1/5>) using **Volocity Visualization**. Semi-automated tracking of cell motility in three dimensions was performed in **Volocity Quantitation** (see **figure**).

These experiments have provided novel information about intracellular signaling factors required for efficient lymphocyte motility, and uncovered a central role for the Rac guanine exchange factor DOCK2. DOCK2-deficient lymphocytes show markedly reduced cell motility and a delayed egress from PLNs. In combination with impaired T cell receptor signaling, it was shown that the deletion of DOCK2 enables long term survival in a murine model of cardiac allograft transplants.

In summary, the findings suggest DOCK2 is an interesting therapeutic target to simultaneously alter various processes controlling lymphocyte trafficking and function while not completely shutting down the immune system.