

"

"

"

---

## **SUPPLEMENTARY INFORMATION LEGENDS**

**Supplementary Figure 1.** Snapshots from a typical movie acquired using TIRFM (256 pixels by 402 pixels, 57 frames/s).

## **SUPPLEMENTARY METHODS**

The supplementary files below are packaged into a single zip file. The files are separated into three folders:

\FRAP\ Three sample FRAP recordings (in Nikon .nd2 file format), Matlab programs, instructions, and auxiliary files.

\Fusion\Epi\ Three sample fusion events cropped from larger movies (10 Hz acquisition rate, far-field epifluorescence mode), sample PointPicker files for analysis of docking and fusion events, Matlab programs, instructions, and auxiliary files. The movies are 16 bit tif stacks that can be opened using e.g. ImageJ.

\Fusion\TIRFM\ Three sample fusion events cropped from larger movies (57 Hz acquisition rate, TIRFM mode), sample SpeckleTrackerJ track files for analysis of docking and fusion events, Matlab programs, instructions, and auxiliary files. The movies are 16 bit tif stacks that can be opened using e.g. ImageJ.

In addition, supplementary figure 1 shows a few annotated snapshots from a movie acquired using TIRFM.