Supporting Information

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SI Methods

Live-Cell Imaging. MT-2 cells were transfected with pCMVHTdX-NCYFP (NC-YFP; gift from David Derse, Frederick, MD) or p8-mCherry-expressing vectors. Jurkat T cells were prelabeled as described earlier, except that 10 μM CellTracker Violet BMQC 415nm (Invitrogen) was used. Cells were mixed 30 min before imaging in a four-well chambered cover glasses (chambered borosilicate coverglass; Lab-Tek) precoated with fibronectin. Samples were imaged on an SP5-X confocal microscope (Leica Microsystems) using a 63× NA 1.4 objective. Images were collected every 10 to 32 s for 5 to 10 min. Optimal conditions (37 °C, 5% CO_2) were maintained by using a stage-mounted environmental chamber. A 3D volume was constructed from sequential z-sections of cells assembled into a 3D volume in Imaris software (version 6.3.1; Bitplane). All collected images for analyses were deconvolved with Huygens Essential software (version 3.4; Scientific Volume Imaging).

Segmentation and Quantification of Cellular Conduits. The length and curvature of conduits were determined with the Fiji software Simple Neurite Tracer (SNT) plug-in (http://pacific.mpi-cbg.de/wiki/index.php), which automatically generates a trace over the conduits between two selected points, then exported to Excel by the Summarize SNT (SSNT) plug-in. The number of conduits per cell was manually counted and the investigators were blinded to the nature of the samples. The tracings were saved as a compressed XML file (default output option of SNT). The output from SNT consists of the pixel coordinates and the length (in pixels) of each trace. However, to calculate the radius of curvature (r) of each trace (segmented nanotube), an in-house FIJI plugin, SSNT, was used. Briefly, the SSNT plugin operates as follows: first, it accepts the compressed XML file as input and parses it to extract the pixel coordinate vectors (x and y) and the length of each trace. Then, it solves for a_0 and b_0 (center coordinates of the osculating circle) (1) using least mean-square approximation of the following equation:

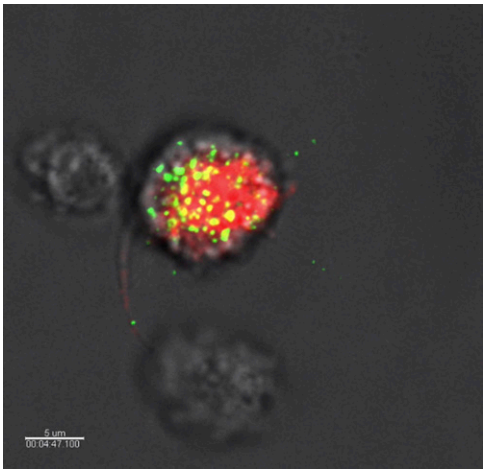
$$a_0X + b_0Y = \frac{\left(X^2 - \mu\left(X^2\right) + Y^2 - \mu\left(Y^2\right)\right)}{2}, \qquad \textbf{[S1]}$$

where $X = x - \mu(x)$ and $Y = y - \mu(y)$ and $\mu(\cdot)$ denotes the arithmetic mean of a vector. Last, the radius of curvature, r, of a trace was calculated using the following equation:

$$r = \frac{1}{\sqrt{\mu\left(X^2\right) + \mu\left(Y^2\right) + a_0^2 + b_0^2}}. \qquad \textbf{[S2]}$$

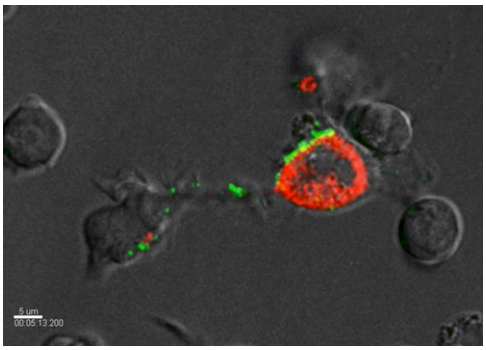
The "solve" function in the JAMA (Java Matrix Package; http://math.nist.gov/javanumerics/jama) library was used for the least mean-square approximation.

1. Trott M (2004) The Mathematica GuideBook for Graphics (Springer-Verlag, New York).



Movie S1. Images were collected every 10 to 32 s for 5 to 10 min. A 3D volume movie was constructed from sequential z-sections of a cells assembled into a 3D volume in Imaris software. This animation shows the elongation of a conduit toward an uninfected cell, transferring YFP-GAG, and retracting of the conduit.

Movie S1



Movie S2. Movie created as described in Movie S1 displays the growth of a conduit after contact with an uninfected cell and movement of YFP-GAG and branching of the conduit.

Movie S2