Video Legends

Video 1. CD40L-treated DC1 express TNT-like protrusions that establish multiple connections between neighboring cells. DC1 were treated with rhCD40L for 20 h prior to staining for cell surface MHC class I (green) and nuclei (blue), followed by confocal imaging. The rotating volume view video displaying 12.5 frames per second (FPS) was generated from a representative Z plane reconstruction image, which highlights numerous TNT-like extensions that formed simultaneous intercellular connections on or above the substratum. Related to Figure 1.

Video 2. CD40L-treated DC2 typically display membrane ruffling, but fail to form TNTs. PGE_2-polarized DC2 were stained for cell surface MHC class I (green) and nuclei (blue), and confocal images collected 20 h post-addition of rhCD40L. A rotating volume view video (12.5 FPS) was generated from a representative Z plane reconstruction image, emphasizing the rounded cell morphology, typical membrane ruffling, and absence of TNT-like membrane extensions. Related to Figure 1.

Video 3. Live-cell imaging captures the dynamic CD40L-induced reticulation process in DC1. DC1 were hyper-stimulated with rhCD40L for 4 h prior to high resolution time-lapse DIC imaging for a duration of 3.6 hours to capture the reticulation process, whereby DC1 membrane extensions formed a dynamic and increasingly complex network between proximal and remote cells. The video is comprised of 36 total frames, which were collected every 6 min and displayed at 5.1 FPS. Related to Figure 4.

Video 4. TNTs support direct intercellular vesicle transfer between CD40L-activated DC1. Mature DC1 were hyper-stimulated with rhCD40L for a duration of 8 h, and subsequently analyzed using live-cell DIC microscopy. A representative 2.5 h time-lapse video reveals multiple endogenous vesicles exiting one cell body into a TNT-like membrane bridge formed between 2 adjacent cells, followed by vesicles trafficking through the tube, and finally entering the connected cell body. The video is composed of 75 total frames collected every 2 min and displayed at 7.5 FPS. Related to Figure 4.

Video 5. Exogenous beads representing Ag rapidly traverse TNTs of donor DC1. Donor DC1 (blue) containing fluorescently labeled 40 nm latex beads (green) were co-cultured with recipient DC1 (red) for 20 h in the presence of rhCD40L prior to live-cell confocal imaging. The video captures the interaction between donor and recipient DC membrane extensions and multiple beads rapidly traversing a dynamic TNT-like structure (blue) expressed by a donor DC1 over a duration of 28 min. Video is comprised of 46 total frames collected every 37 s and displayed at 11.5 FPS. Related to Figure 5.

Video 6. Bacterial pathogens can traffic between DC1 along CD40L-induced TNTs. Labeled DC1 monolayers (blue) stimulated with rhCD40L for 10 h were subsequently exposed to EGFP-expressing *Escherichia coli* for 2 h prior to microscopy. Time-lapse confocal resonant scanning methods were used to capture the bi-directional trafficking of individual bacterium between interconnected DC1 along a membrane bridge (arrows) for a duration of 34 min. Video is made up of 103 total frames collected every 20 s and shown at 14.7 FPS. Related to Figure 6.