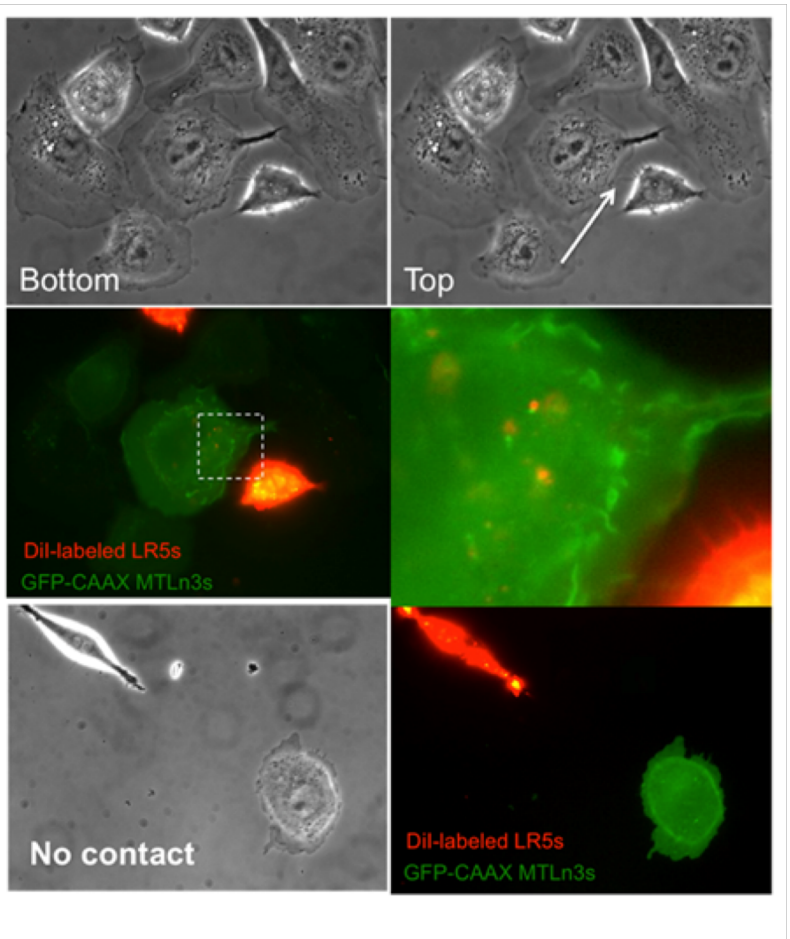

Figure S1: TNT interaction with macrophages mediates the transfer of material. RAW/LR5 macrophages are labeled with DiI (red) prior to co-culture with GFP-CAAX MTLn3 tumor cells. Images show DiI-labeled material transferred to GFP-CAAX tumor cell connected with a macrophage through a TNT (arrow). No transfer is observed in tumor cells not connected to a macrophage (lower panels). Quantitation of material transfer was done using flow cytometry assay, see Figure 2C.

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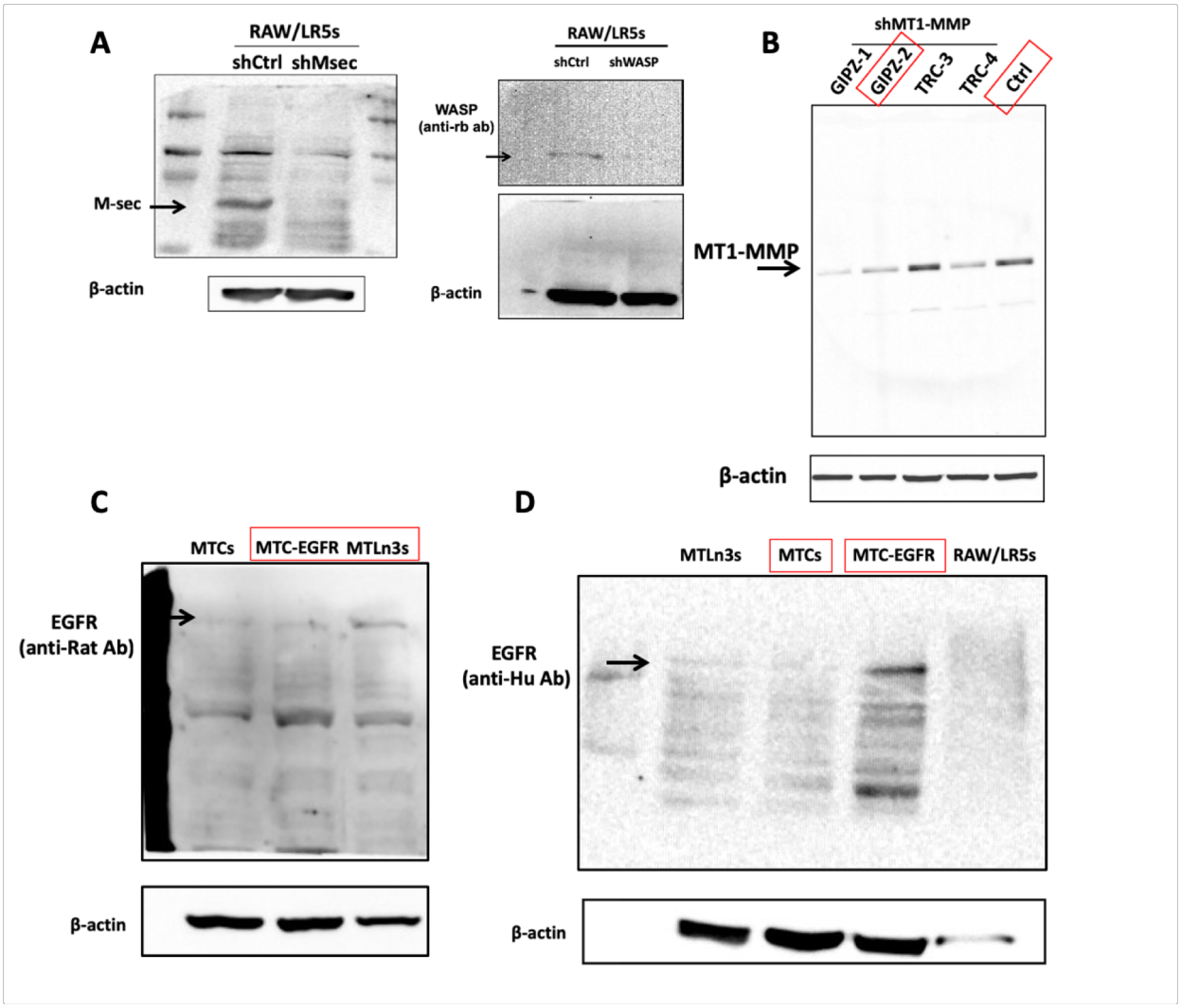
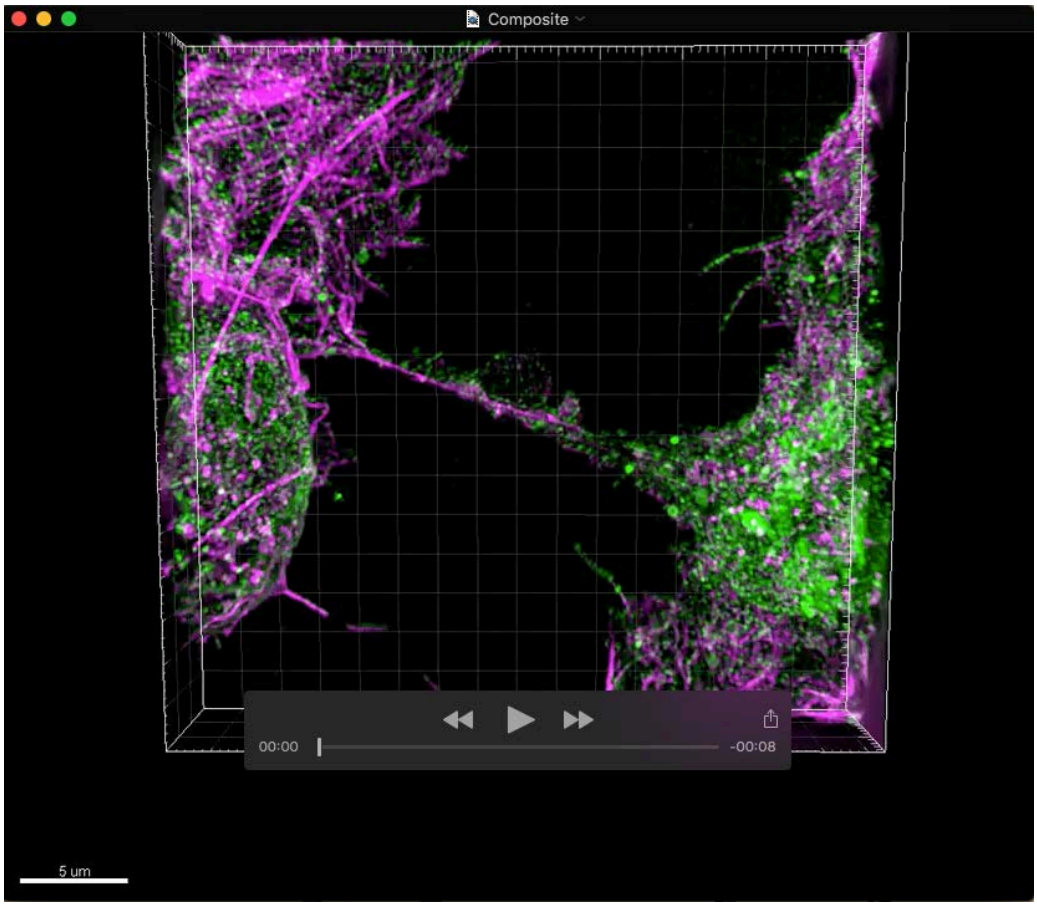
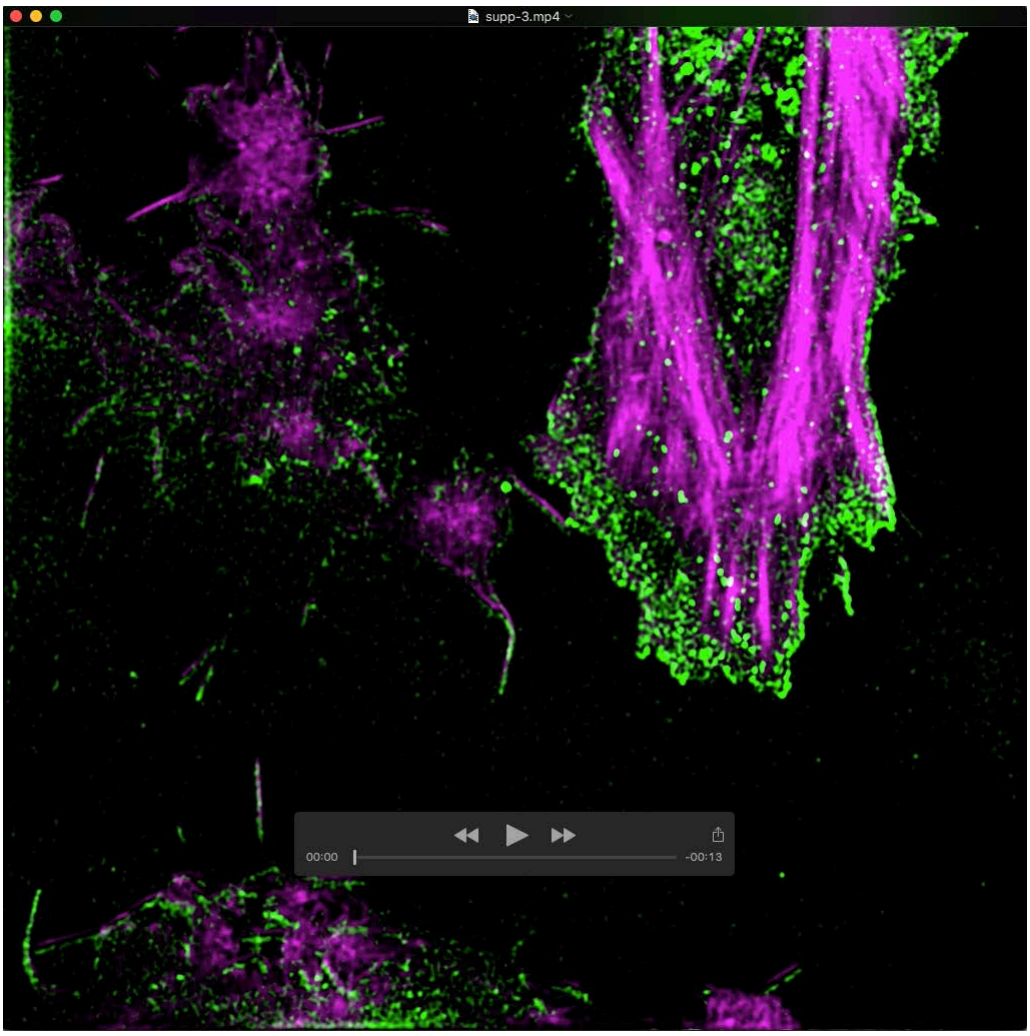


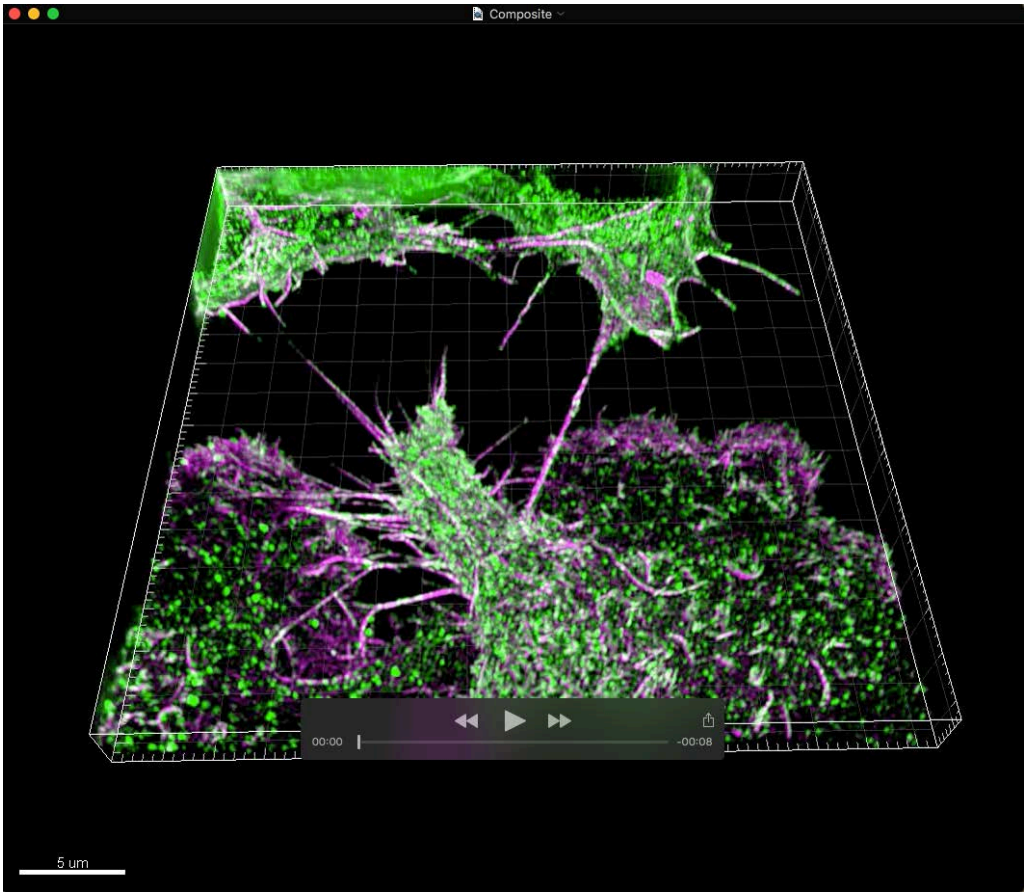
Figure S2: Full western blots. Full western blot used for Figure 2C for protein expression of **(A.)** M-Sec in Figure 2C, **(B.)** MT1-MMP in Figure 4 and **(C.)** EGFR in Figure 5.



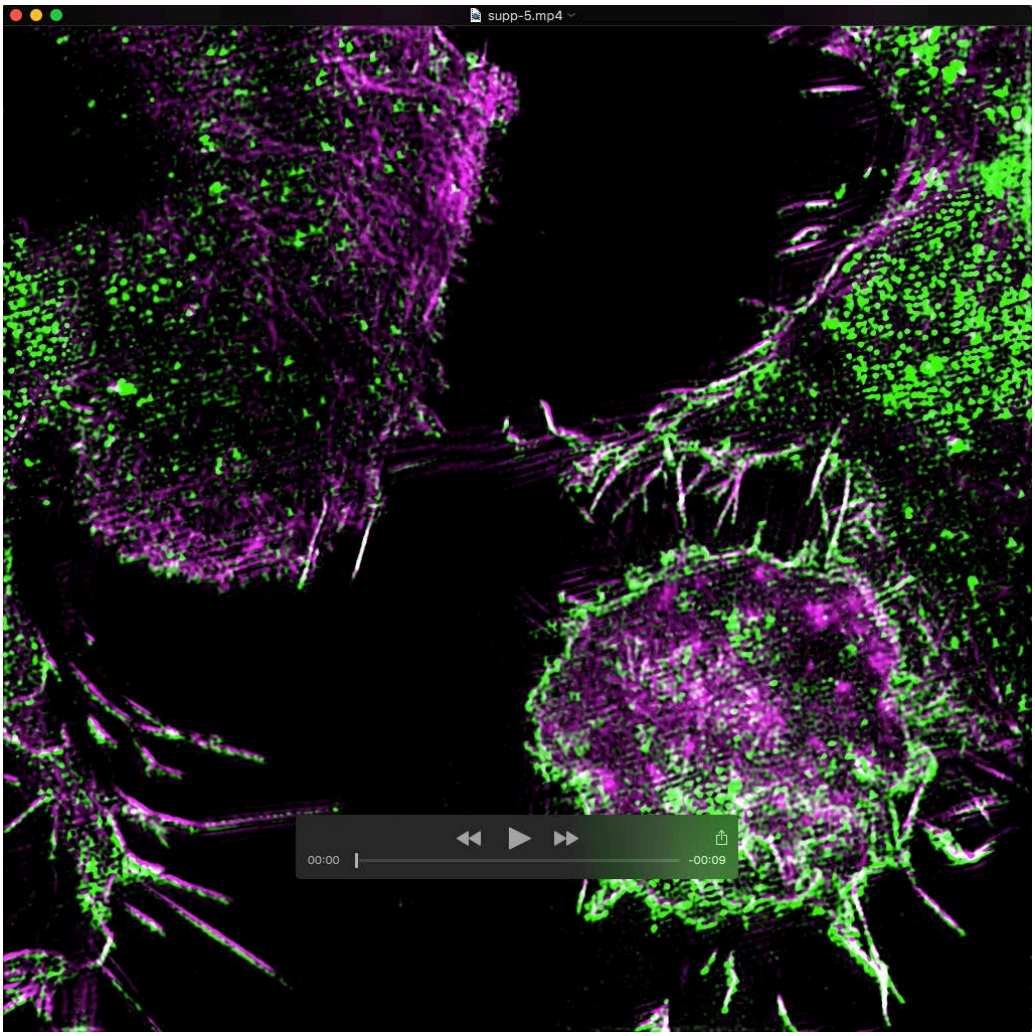
Movie 1: 3D reconstruction of Figure 1A showing TNT connecting RAW/LR5 macrophages with MTLn3 tumor cells. Cells are stained with WGA (green) to label the membrane, and F-actin (magenta). Images were acquired using a Nikon Structured Illumination N-SIM system on an inverted Nikon ECLIPSE Ti-E equipped with a 100× 1.49 NA objective. 3D reconstruction was generated using Imaris software calibrated and maintained by the Analytical Imaging Facility at Albert Einstein College of Medicine.



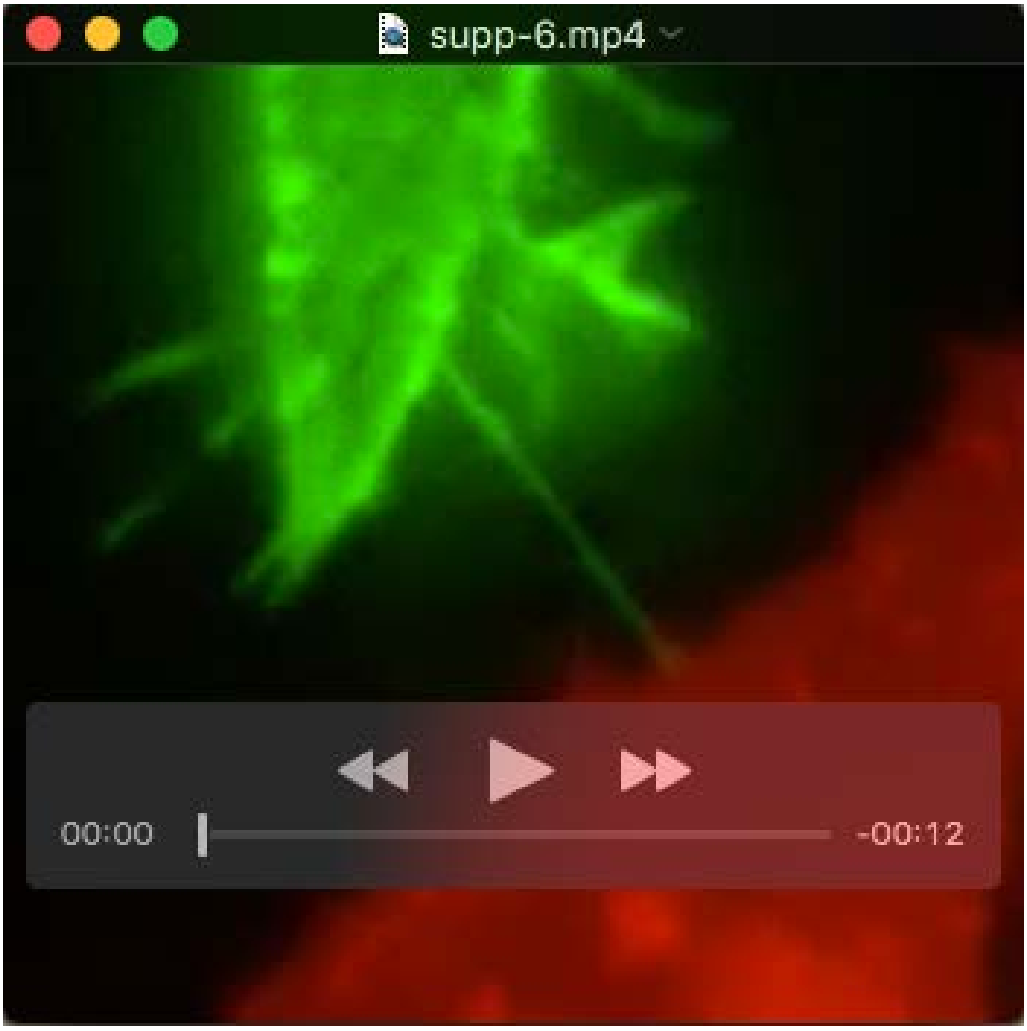
Movie 2: 3D reconstruction showing the Z-stack images with step size = 0.12 µm. Z-stack shows the bottom plane and the upper plane of the image showing the presence of the TNTs connecting a RAW/LR5 macrophage with MTLn3 tumor cells. Cells were fixed and stained for WGA (green) and F-actin (magenta). homotypic TNTs (indicated by white arrows) are often observed between two macrophages or two tumor cells. Images were acquired using a Nikon Structured Illumination N-SIM system on an inverted Nikon ECLIPSE Ti-E equipped with a 100× 1.49 NA objective. Analysis performed with ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016).



Movie 3: 3D reconstruction of Figure 1C showing TNT connecting RAW/LR5 macrophages with MDA-MB-231 tumor cells. Cells are stained with WGA (green) to label the membrane, and F-actin (magenta). Images were acquired using a Nikon Structured Illumination N-SIM system on an inverted Nikon ECLIPSE Ti-E equipped with a 100× 1.49 NA objective. 3D reconstruction was generated using Imaris software calibrated and maintained by the Analytical Imaging Facility at Albert Einstein College of Medicine.



Movie 4: 3D reconstruction showing the Z-stack images with step size = 0.12 µm. Z-stack shows the bottom plane and the upper plane of the image showing the presence of the TNTs connecting a RAW/LR5 macrophage with MDA-MB-231 tumor cells. Cells were fixed and stained for WGA (green) and F-actin (magenta). Images were acquired using a Nikon Structured Illumination N-SIM system on an inverted Nikon ECLIPSE Ti-E equipped with a 100× 1.49 NA objective. Analysis performed with ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016).



Movie 5: Time-lapse imaging of GFP-CAAX RAW/LR5 macrophages (green) in co-culture with mCherry-CAAX MTLn3 tumor cells (red) showing heterotypic TNT formed by a macrophage towards a tumor cell. Duration of original sequence at least 30 mins. Magnification 60X, 2X2 binning. Frame interval: 10 s. Scale bar = 10 μm. Analysis performed with ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016).

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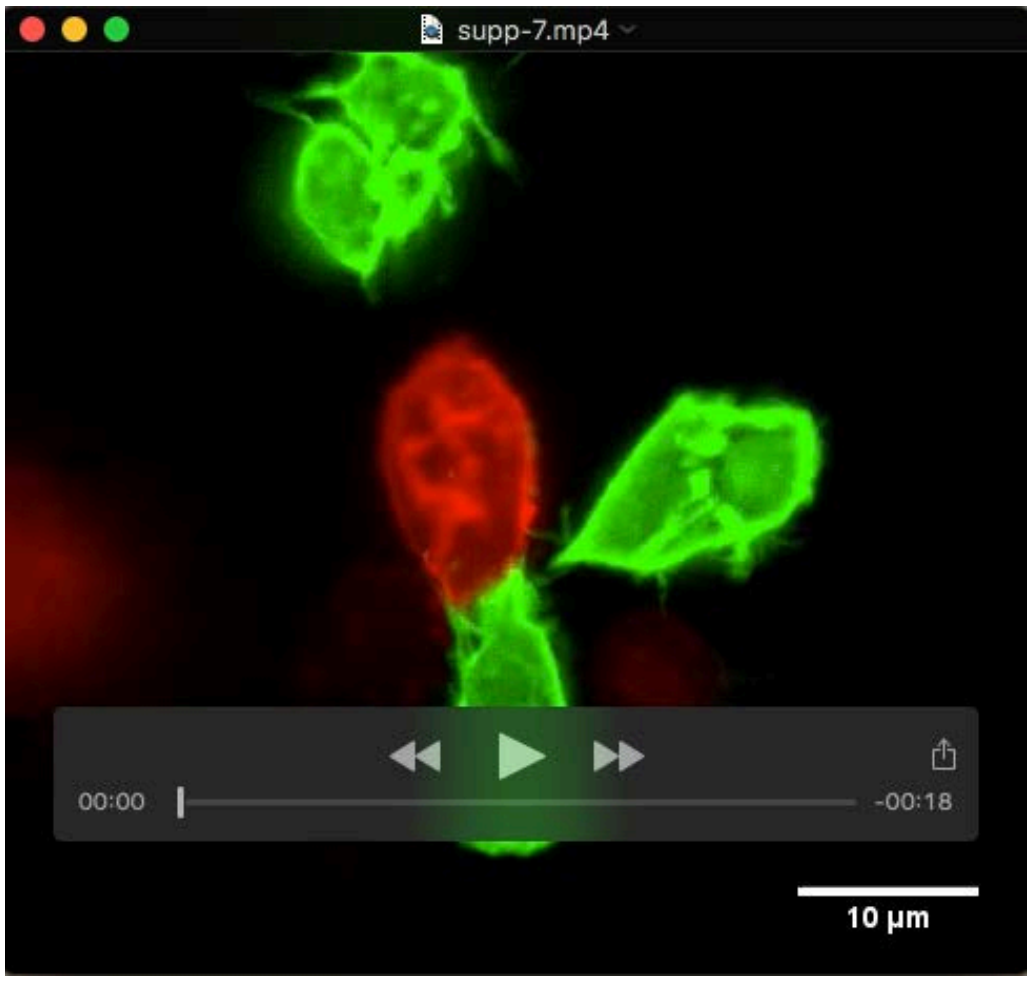
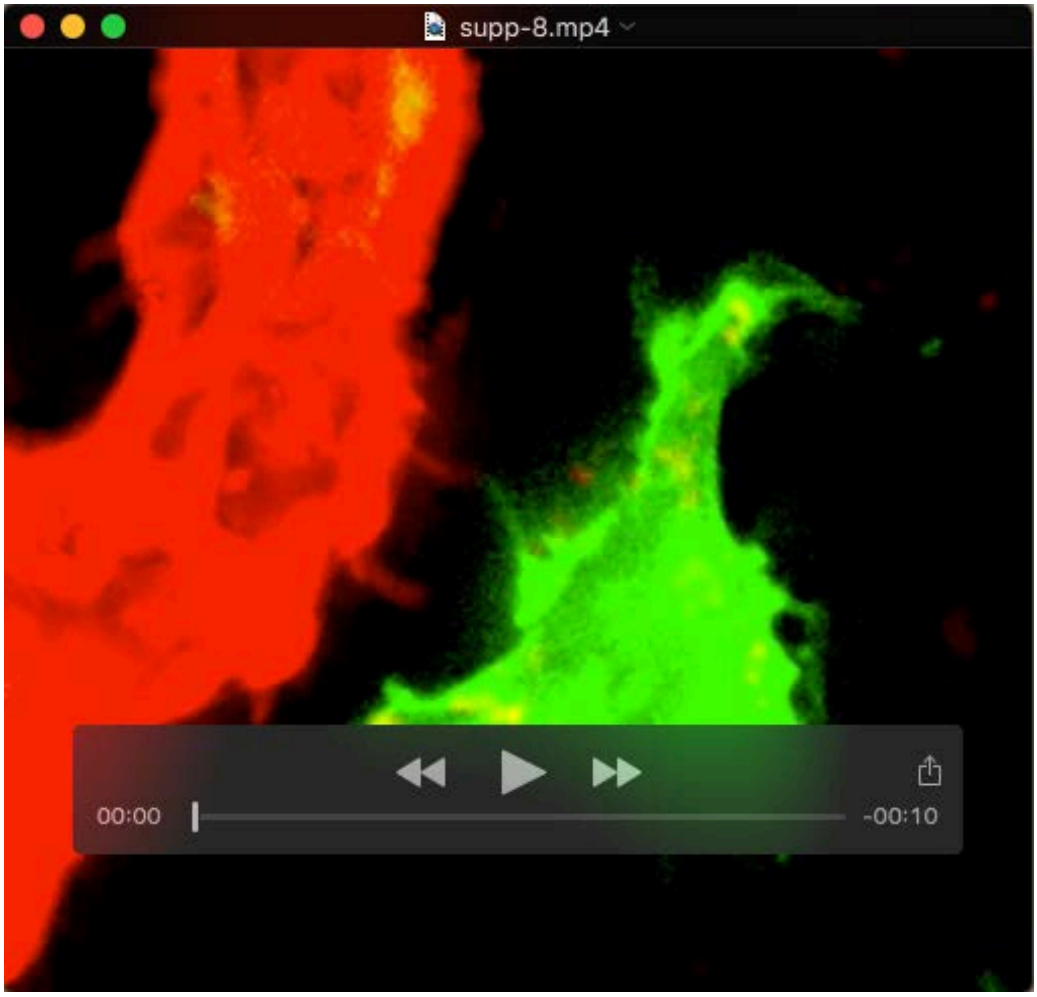


Movie 6: Time-lapse imaging of GFP-CAAX RAW/LR5 macrophages (green) in co-culture with mCherry-CAAX MTLn3 tumor cells (red) showing two examples of TNT-like protrusions initiated from macrophages towards a tumor cells indicated by white arrows. Duration of original sequence at least 30 mins. Magnification 60X, 2X2 binning. Frame interval: 10 s. Scale bar = 10 µm. Analysis performed with ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016).



Movie 7: Time-lapse imaging of GFP-CAAX RAW/LR5 (green) macrophages in co-culture with mCherry-CAAX MTLn3 tumor cells (red) showing TNT-like protrusions extending from both cell types where they appear to intertwine and/or interact at the tips of the TNT-like protrusions. Duration of original sequence at least 30 mins. Magnification 60X, 2X2 binning. Frame interval: 10 s. Scale bar = 10 μm. Analysis performed with ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016).

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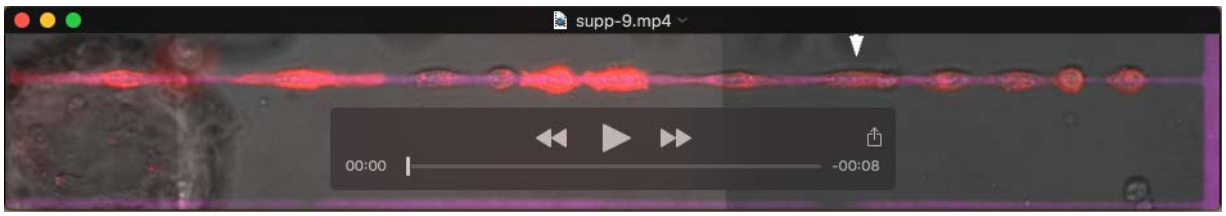
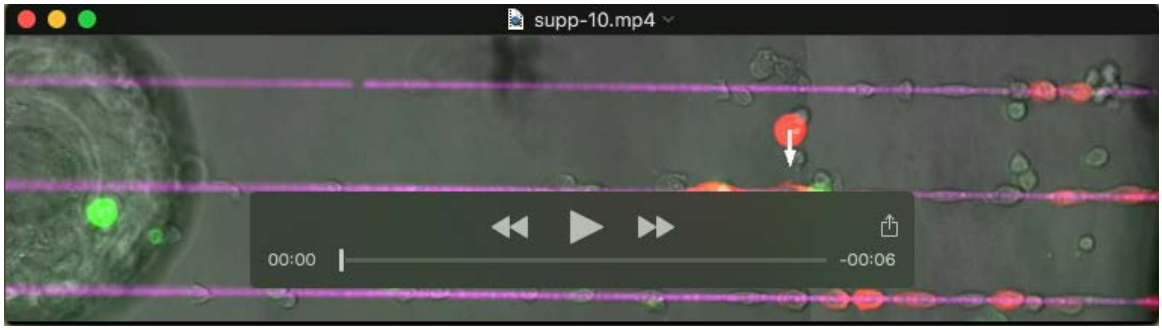


Movie 8: Supplemental Movie S8 Time-lapse live imaging of the 1D assay where mCherry-CAAX MTLn3 tumor cells (red) are plated alone on fibronectin coated strips (magenta). Cells are allowed to migrate towards a HUVEC coated bead (far left). Time lapse images were obtained on the wide-field DeltaVision microscope (Albert Einstein College of Medicine, Bronx, NY). Images were acquired every 10 minutes for up to10 hours. Image analysis was performed using the LOCI plug-in and the “Merge Channels” command in ImageJ ((Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016).



Movie 9: Time-lapse live imaging of the 1D assay where mCherry-CAAX MTLn3 tumor cells (red) are plated on fibronectin coated strips (magenta) in co-culture with Control GFP-CAAX RAW-LR5 macrophages (green). Cells are allowed to migrate towards a HUVEC coated bead (far left). Time lapse images were obtained on the wide-field DeltaVision microscope (Albert Einstein College of Medicine, Bronx, NY). Images were acquired every 10 minutes for up to10 hours. Image analysis was performed using the LOCI plug-in and the “Merge Channels” command in ImageJ ((Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016).

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Movie 10: Time-lapse live imaging of the 1D assay where mCherry-CAAX MTLn3 tumor cells (red) are plated on fibronectin coated strips (magenta) in co-culture with shM-Sec GFP-CAAX RAW-LR5 macrophages (green). Cells are allowed to migrate towards a HUVEC coated bead (far left). Time lapse images were obtained on the wide-field DeltaVision microscope (Albert Einstein College of Medicine, Bronx, NY). Images were acquired every 10 minutes for up to10 hours. Image analysis was performed using the LOCI plug-in and the “Merge Channels” command in ImageJ ((Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2016).