Supplementary Information

Autocrine Fibronectin Inhibits Breast Cancer Metastasis

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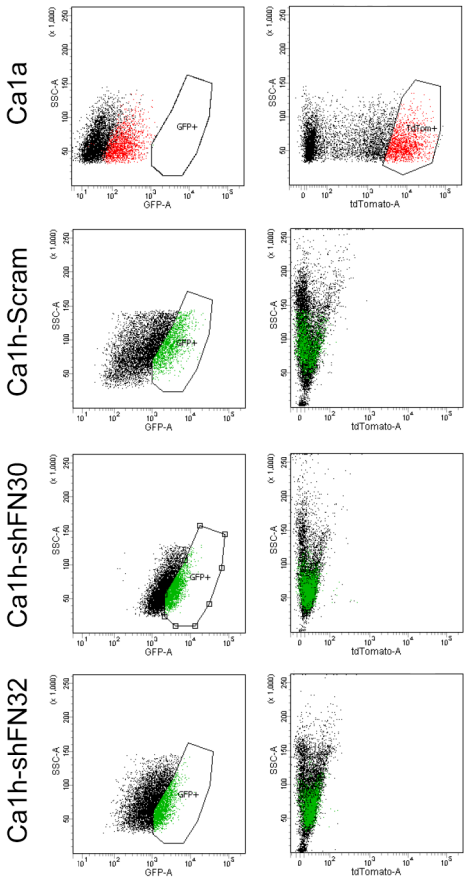
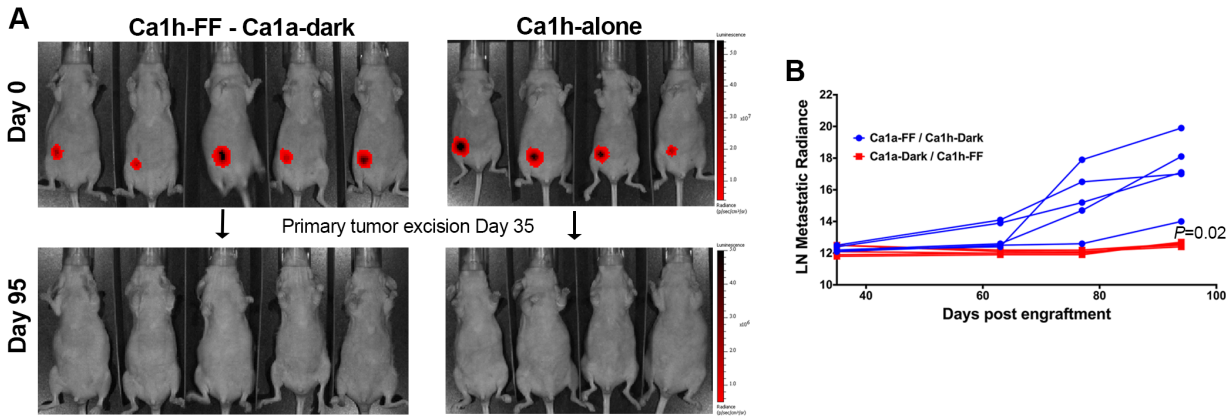
Running Title: Autocrine Fibronectin Limits Metastasis

Supplementary Figure 1.



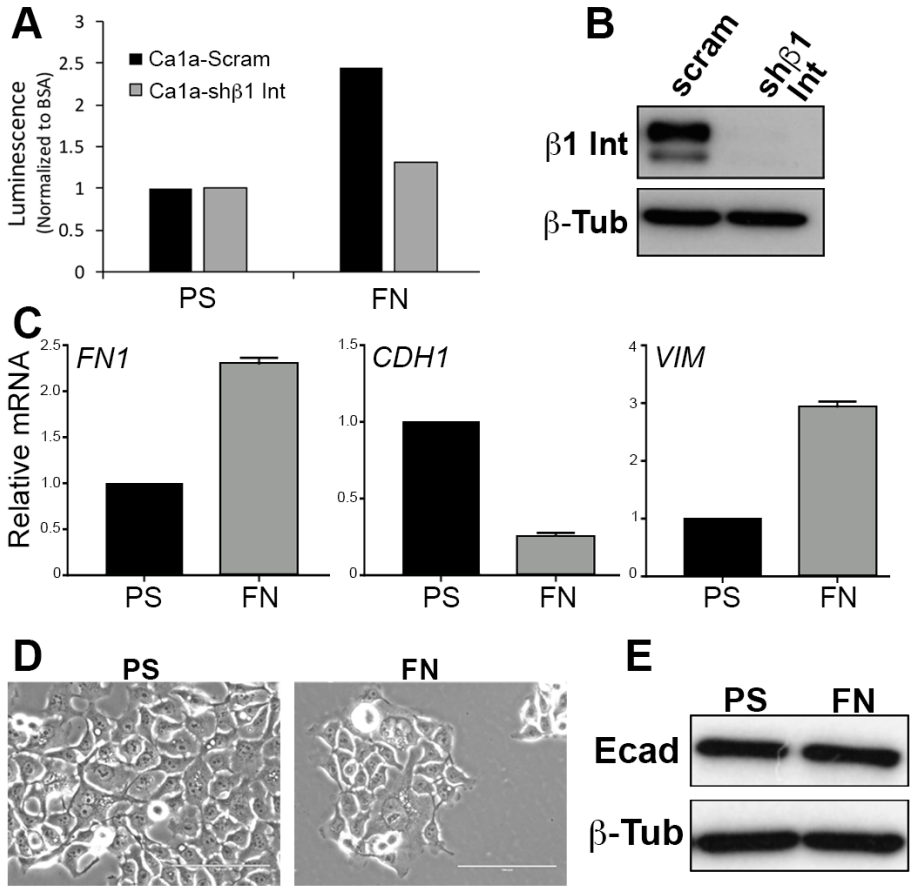
Supplementary Figure 1. Differential fluorescent labeling of epithelial and mesenchymal cells. Ca1a cells stably expressing a d-Tomato encoding vector were isolated from the indicated gate. Similarly, control (scram) and FN-depleted (shFN30, shFN32) Ca1h cells expressing a eGFP encoding vector were isolated from the indicated gates in the GFP channel.

Supplementary Figure 2.



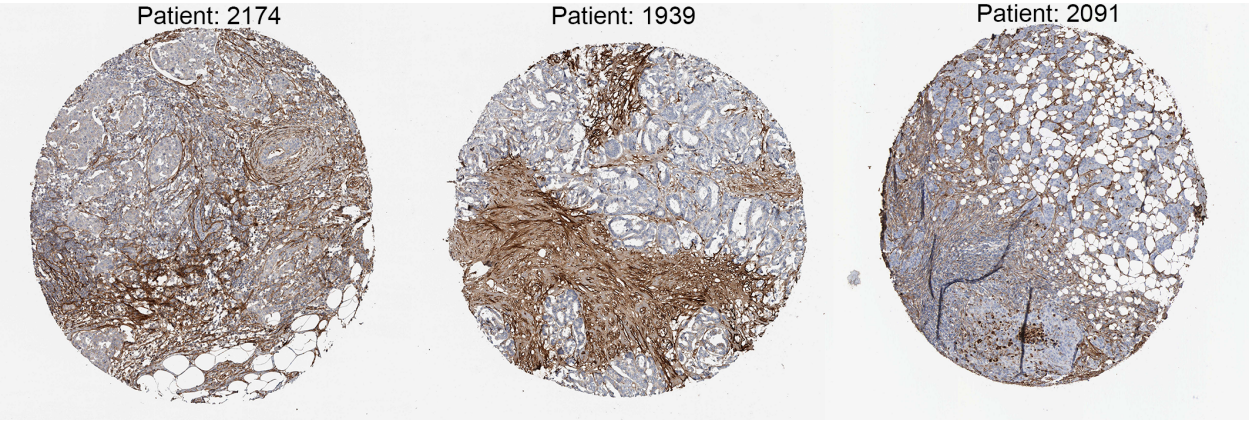
Supplementary Figure 2. Ca1h cells are not metastatic. (A) Firefly luciferase expressing Ca1h cells were engrafted onto the mammary fat pad. Bioluminescence was imaged immediately after engraftment (Day 0) and 95 days later (Day 95). Primary tumors were removed 35 days post engraftment. (B) Metastatic bioluminescent radiance was quantified at the indicated time points in mice bearing the indicated heterogeneous tumors. Data are expressed as the natural log (LN) of the metastatic radiance values for each individual animal resulting in the indicated *P* value.

Supplementary Figure 3.



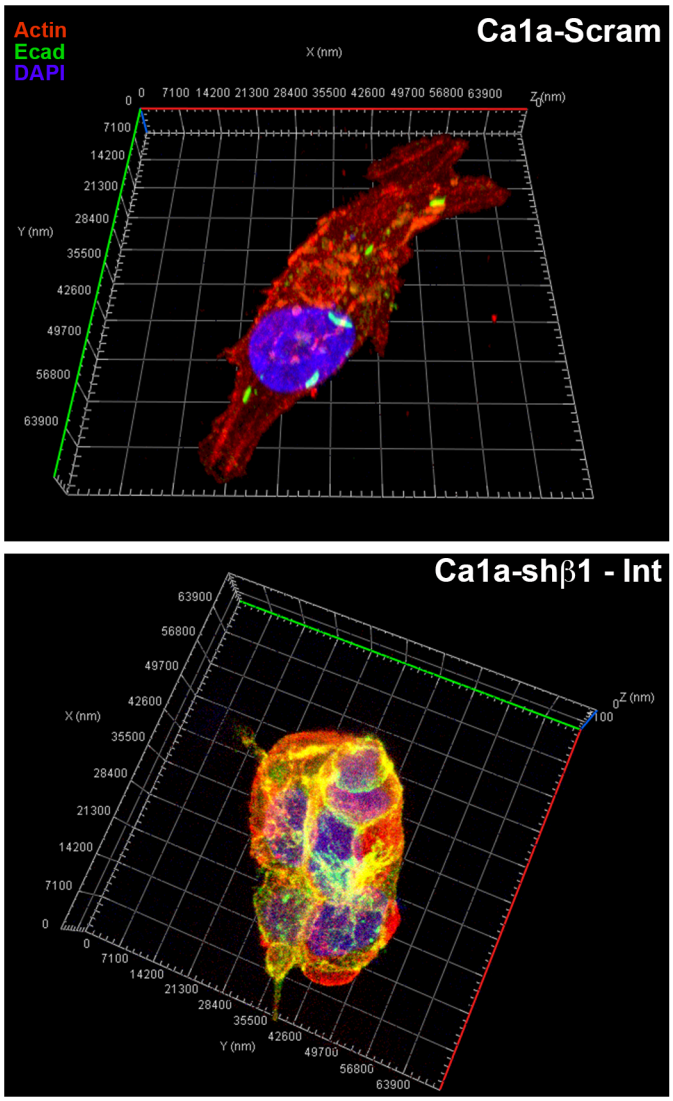
Supplementary Figure 3. Fibronectin coated polystyrene induces a partial epithelial-mesenchymal transition. (A) Control (scram) and β1 integrin depleted (shβ1 Int) Ca1a cells were cultured on BSA or FN coated polystyrene for one hour, washed with PBS and cell-derived bioluminescence was measured. (B) Depletion of β1 integrin (β1 Int) was confirmed using immunoblot analysis. (C) Ca1a cells were cultured on FN coated (FN) or uncoated polystyrene (PS) 2D surfaces for 6 passages, and mRNA transcripts for *FN1*, *CDH1* and *VIM* were quantified by RT-PCR. (D) Phase contrast images demonstrating the epithelial morphology of Ca1a cells cultured on FN-coated (FN) and uncoated polystyrene (PS). (E) Immunoblot analyses for Ecad from Ca1a cells cultured as described in panel D. Expression of β-tubulin served as a loading control. All data are representative of at least three independent analyses.

Supplementary Figure 4.



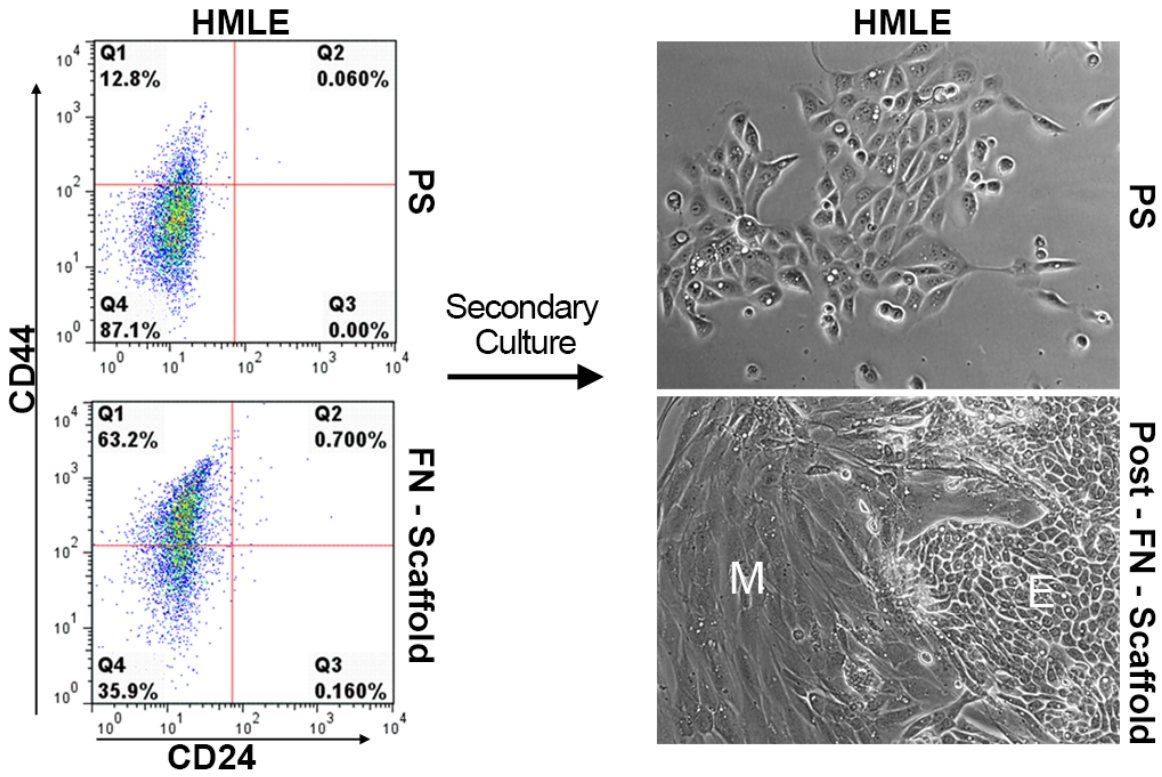
Supplementary Figure 4. Fibronectin is in a fibular form in patient tumors. Biopsies from the indicated patients were stained for FN and counter stained with hematoxylin to visualize nuclei. Images were obtained from the protein atlas (www.proteinatlas.org).

Supplementary Figure 5.



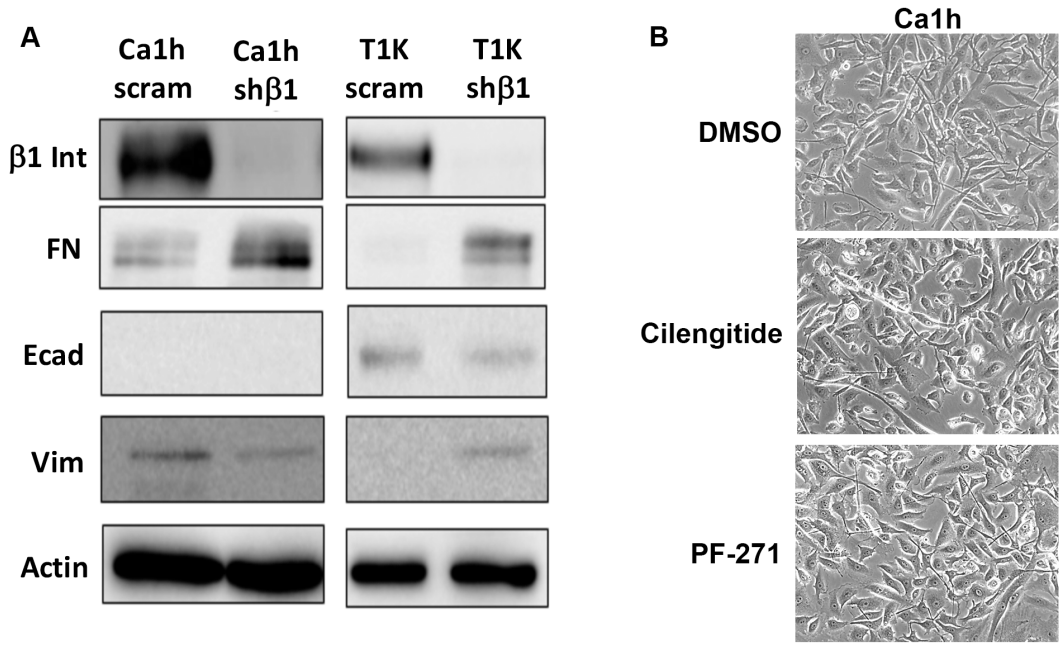
Supplementary Figure 5. Fibrillar fibronectin induction of EMT is dependent on the expression of β1 integrin. 3D reconstructions cells shown in Figure 7B. Control (scram) and β1 integrin depleted (sh β1-Int) Ca1a cells growing on fibrillar FN off of the solid support of our scaffold. Cells were stained with antibodies against Ecad (green), phalloidin (Red) and DAPI (blue).

Supplementary Figure 6.



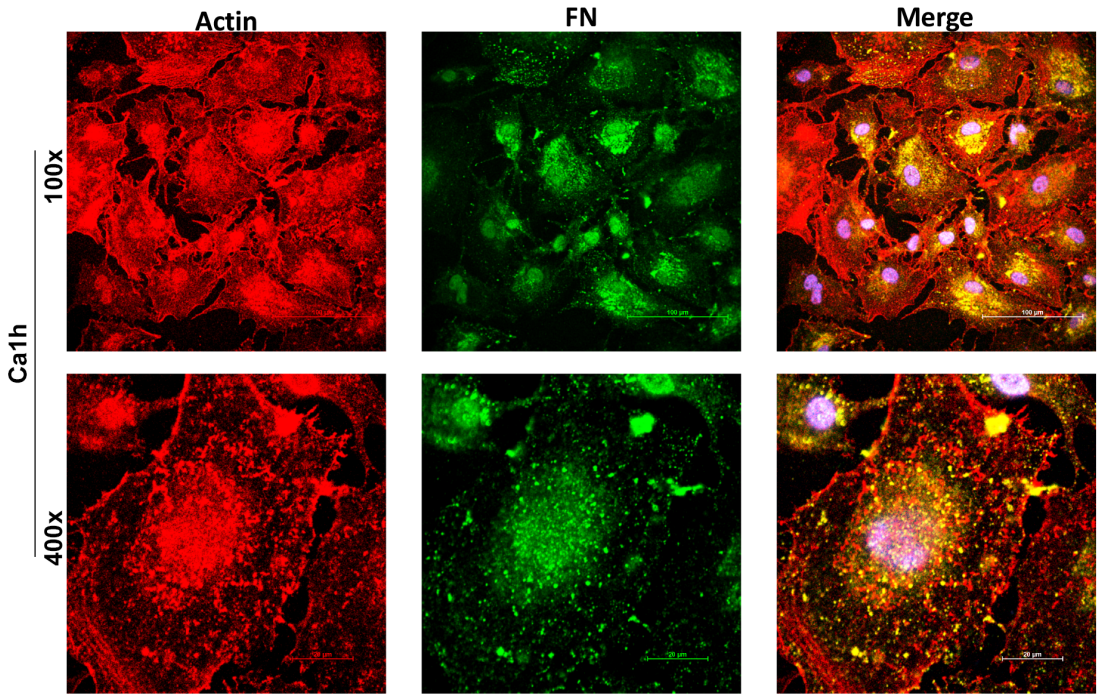
Supplementary Figure 6. Fibrillar fibronectin induces a mesenchymal phenotype. Human mammary epithelial (HMLE) cells were cultured on polystyrene (PS) or FN coated scaffolds for 6 days and these cells were analyzed by flow cytometry for cell surface expression of CD44 and CD24. Subsets of these cells were returned to traditional 2D culture for three days and imaged by phase contrast microscopy to visualize the distinct epithelial (E) and mesenchymal (M) cell populations.

Supplementary Figure 7.



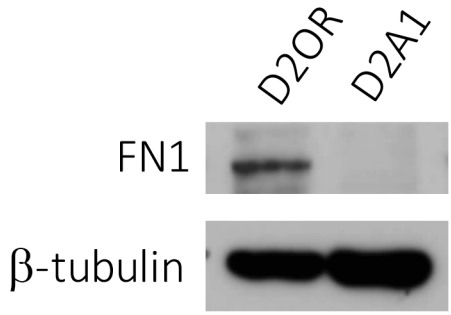
Supplementary Figure 7. Depletion of β1 increases intracellular FN. (A) Ca1h and their isogenic counterpart T1K cells were depleted for expression of β1 integrin via shRNA. Expression of intracellular FN, Ecad and vimentin (Vim) were assessed by immunoblot analyses. Expression of actin and β1 integrin served as loading controls. (B) Ca1h cells were treated for 14 days with the integrin blocking cyclic peptide Cilengitide (1 μM) or the focal adhesion kinase inhibitor PF-562,271 (PF-271; 1 μM). Cells were subsequently imaged by phase contrast microscopy. No changes in cell morphology were observed with these inhibitors.

Supplementary Figure 8.



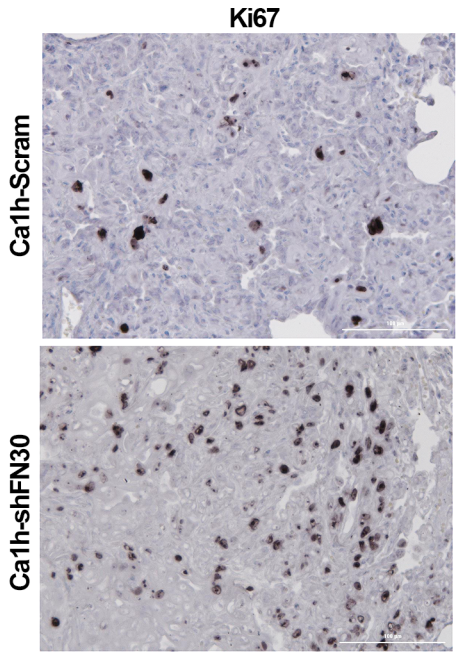
Supplementary Figure 8. Intracellular FN colocalizes with the actin cytoskeleton. Ca1h cells were stained with antibodies directed toward FN and phalloidin to visualize the actin cytoskeleton. These cells were visualized used confocal microscopy. Shown are single, mid-cell plains of focus.

Supplementary Figure 9.



Supplementary Figure 9. Systemically dormant cells express intracellular FN. Whole cell lysates from systemically dormant D2.OR cells and their isogenic but fully metastatic counterpart D2.A1 cells were analyzed for expression of FN. β-tubulin served as a loading control.

Supplementary Figure 10.



Supplementary Figure 10. Depletion of FN increases pulmonary tumor proliferation. The resultant pulmonary tumors formed 35 days after tail vein injection of control (scram) and FN-depleted (shFN30) Ca1h cells were analyzed by IHC staining for the proliferation marker Ki67.

Supplementary Video Legends

Supplementary Video 1 and 2. Ca1a cells (labeled by d-tomato) can migrate and proliferate on FN-fibrils. Our 3D culture scaffolds were coated with FN and Ca1a cells were added. The original position of cells is outlined and cell migration was tracked over the next 10 hours.

Supplementary Video 3. Control Ca1h cells forming fibrillar strains when cultured on our 3D culture scaffold. Video is of images taken every 30 minutes over a 48-hour period.

Supplementary Video 4. Ca1h cells depleted for FN (shFN30) fail to form any fibrillar strains when cultured on our 3D culture scaffold. Video is of images taken every 30 minutes over a 48-hour period.

Supplementary Video 5. Ca1h cells partially depleted for FN (shFN33) demonstrate reduced formation of fibrillar strains when cultured on our 3D culture scaffold. Video is of images taken every 30 minutes over a 48-hour period.

Supplementary Video 6. Ca1a cells (labeled by d-tomato) can migrate off of the solid support scaffold on FN-expressing Ca1h cells. Video is of images taken every 30 minutes over a 36-hour period.

Supplementary Video 7. FN-depleted Ca1h cells (shFN30) cannot facilitate the migration of Ca1a cells (labeled in red) off of the solid support of the scaffold. Video is of images taken every 30 minutes over a 36-hour period.

Supplementary Video 8. FN-depleted Ca1h cells (shFN32) cannot facilitate the migration of Ca1a cells (labeled in red) off of the solid support of the scaffold. Video is of images taken every 30 minutes over a 36-hour period.