Supplemental Materials

Molecular Biology of the Cell

Menko et al.

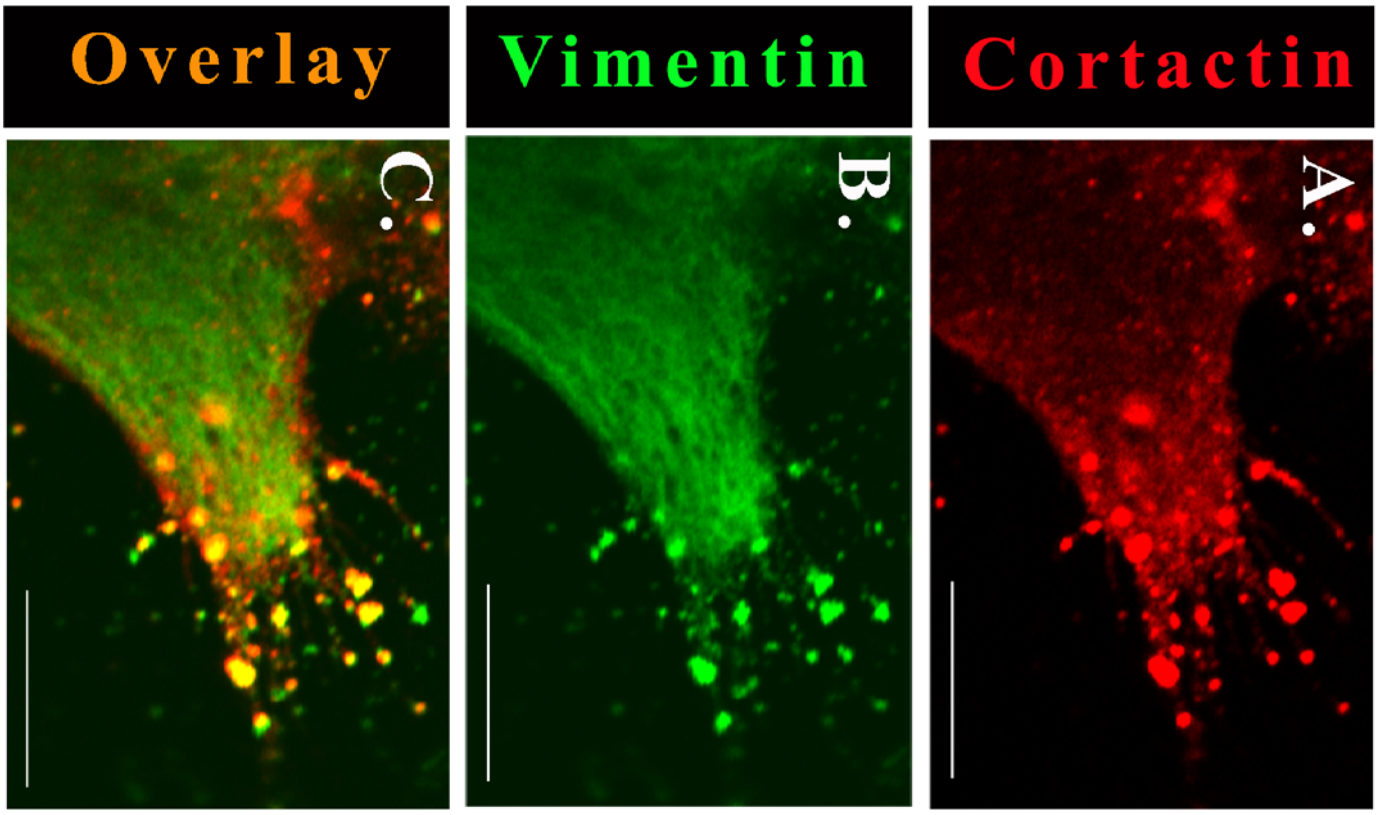
Supplemental Figure 1. Controls for immunofluorescence staining. Ex vivo explants were co-labeled with the following combinations of secondary antibodies: rhodamine anti-mouse (A) and fluorescein anti-rabbit (B) or fluorescein anti-mouse (C) and rhodamine anti-rabbit (D). Results revealed that incubation with secondary antibodies did not result in non-specific staining. Mag. Bars = 20 μm.

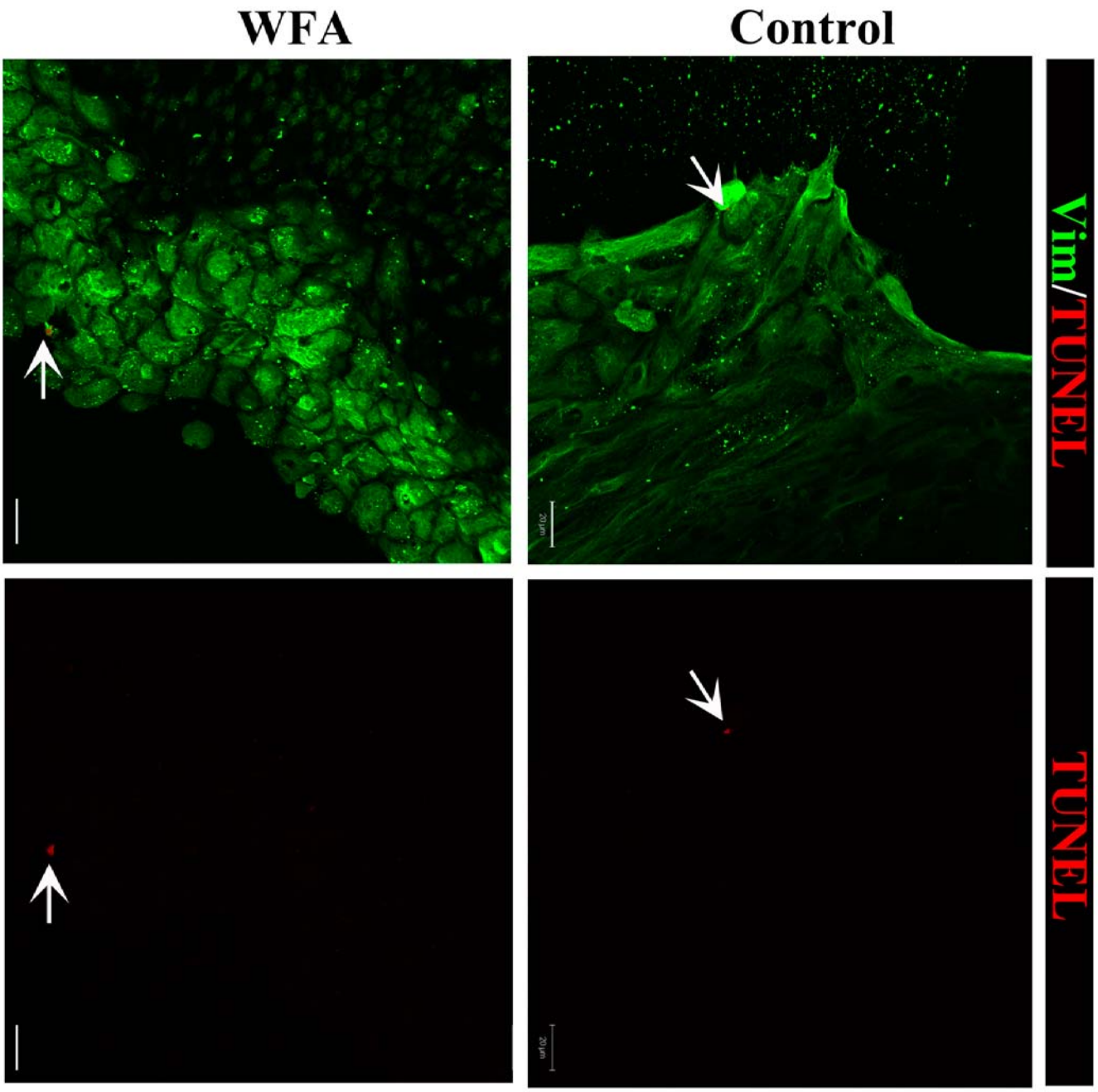
Supplemental Figure 2. Vimentin rich protrusions are enriched for the lamellipodia marker cortactin. Ex vivo explants were co-immunolabeled for cortactin (A, red) and vimentin (B, green) and overlaid in (C). Confocal imaging focused at the wound edge revealed that vimentin rich protrusions were enriched for cortactin at the tips of the lamellipodia. Mag. Bar = 10μm Secondary antibody controls were performed which demonstrated specificity of antibody staining (see Suppl. Fig. 1).

Supplemental Figure 3. Repair cells piled at the wound edge remained viable in the presence of WFA. To determine if WFA affected the viability of repair cells piled at the wound edge which could impact wound closure, control and WFA treated ex vivo wounded explants were fixed 1D post-injury and analyzed by TUNEL assay (red) and immunostained for vimentin (green). Little TUNEL staining (red, arrow) was noted in the repair cells at the wound edge in both control and WFA treated ex vivo explants. Mag. Bars = 20 μm.

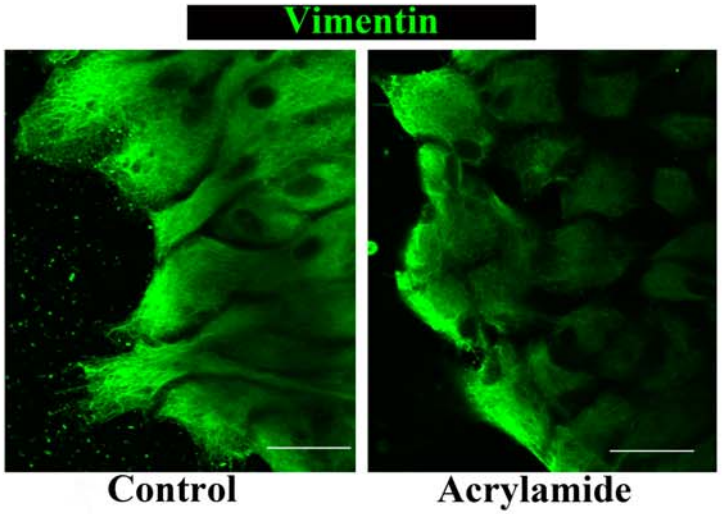
Supplemental Figure 4. Disruption of vimentin function with acrylamide interferes with wound closure. In the presence of Acrylamide, a known vimentin disrupter, vimentin (green) organization was altered, and no longer formed an organized intermediate filament network as seen in cells at the wound edge in control treated explants (A). Similar to WFA, treatment with Acrylamide slowed wound closure in a dose-dependent manner (B). Wound closure for control wounded explants compared to wounded explants treated with 2mM Acrylamide shown in phase contrast images (C). (A) Mag. Bar = 20μm. Phase images Mag. Bar = 500μm. Secondary antibody controls were performed which demonstrated specificity of antibody staining (see Suppl. Fig. 1).

A.
Rh anti-Mouse
B.
Fl anti-Rabbit
C.
Fl anti-Mouse
D.
Rh anti-Rabbit

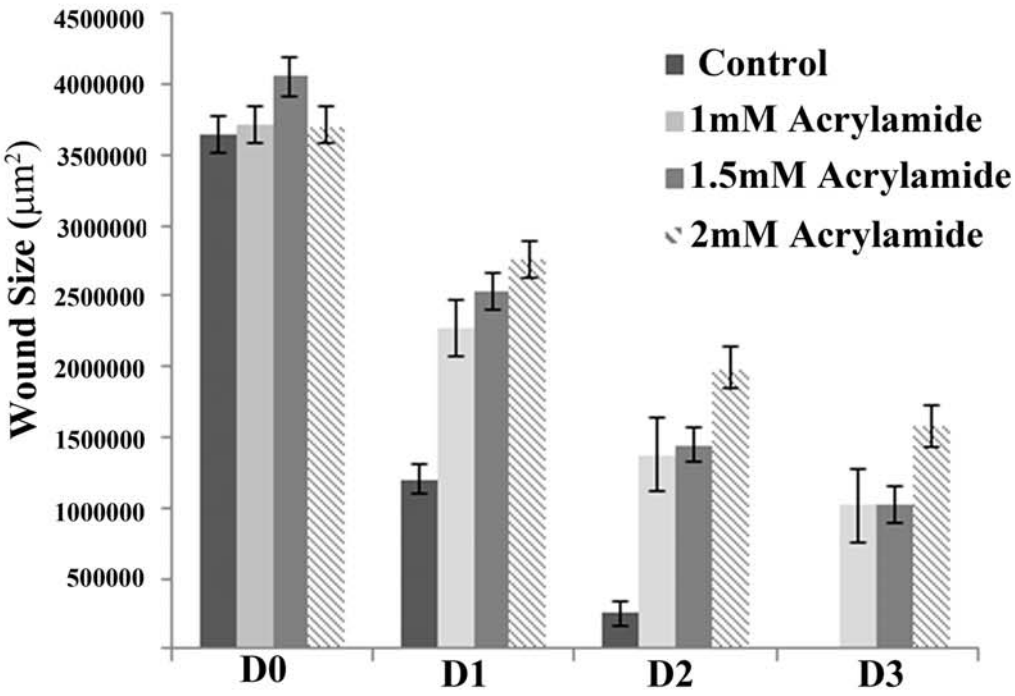

Overlay
Vimentin
Cortactin
C.
B.
A.


WFA
Control
Vim/TUNEL
TUNEL
20 μm
20 μm

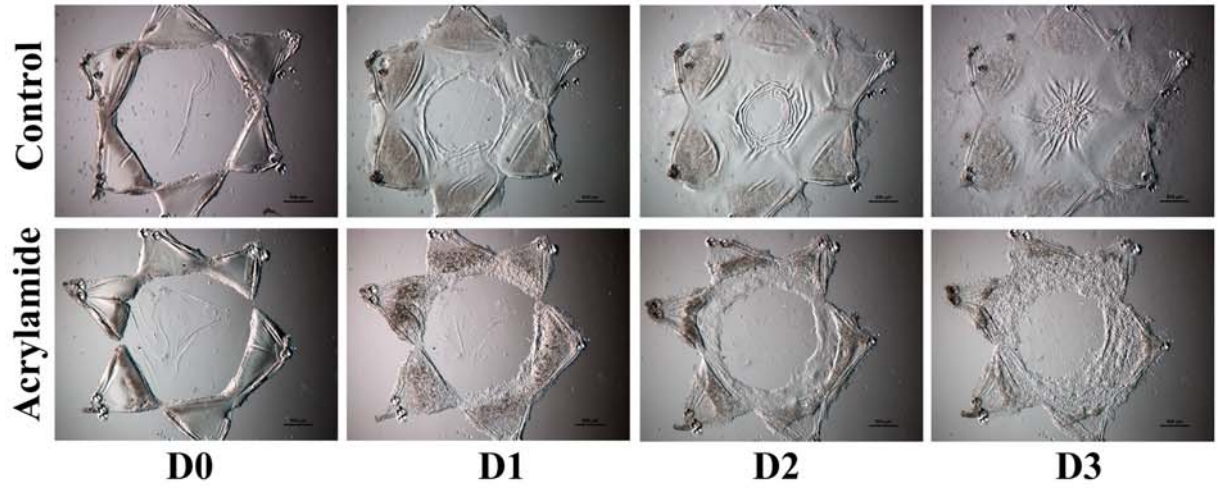
A.


Vimentin
Control
Acrylamide

B.


Wound Size (μm²)
4500000
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
Control
1mM Acrylamide
1.5mM Acrylamide
2mM Acrylamide
D0
D1
D2
D3

C.


Control
Acrylamide
D0
D1
D2
D3