Wound Healing Assays in NSD3short Overexpressing H1299 Cells

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Objective. During epithelial to mesenchymal transitioning (EMT), transcription networks become activated and cytoskeletal rearrangements take place. These events promote migratory capacity and invasiveness. A common assay to measure this phenotype is wound healing. This involves plating cells and culturing until confluent at which point a scratch or wound is made in the culture. This area is imaged over a 24-72 period to monitor the rate of wound closure. Cells with increased migratory capacity will close the wound faster and are indicative of a more mesenchymal state.

1. Experimental Details

1.1 Wound Healing Assay

H1299 cells stably overexpressing NSD3short were plated in triplicate in 96-well plates and incubated overnight. Initially 15000 cells were plated, however the cells were not confluent at the time wound making (Rep1), so I repeated the processes starting with 30 000 cells (Rep2). The next day after plating, wounds were made using the ESSEN Bio science IncuCyte Woundmaker (Cat. No. 4493) following the manufacturer's protocol. Following wound making, cells were washed and fresh media added to remove cell debris. Finally, plates were imaged and analyzed on a IncuCyte Zoom at 0 and 72 (Rep1) or 24 (Rep2) hours.

2. Results & Obeservations

Observations. In the first replicate, in which cells were not fully confluent at the time the wound was made, both NSD3 overexpression lines close the wound over 72hrs, while for the empty vector control a wound still remains. Unfortunately, during the second replicate, plated at a higher density, the wild-type NSD3short overexpressing cells completely died off after being plated. However we can observe from this experiment that the W284A NSD3 mutant again promotes wound healing, indicating PWWP1 function is not required for this activity. These results will be validated by repeating with independently transduced and puro-selected stable lines.

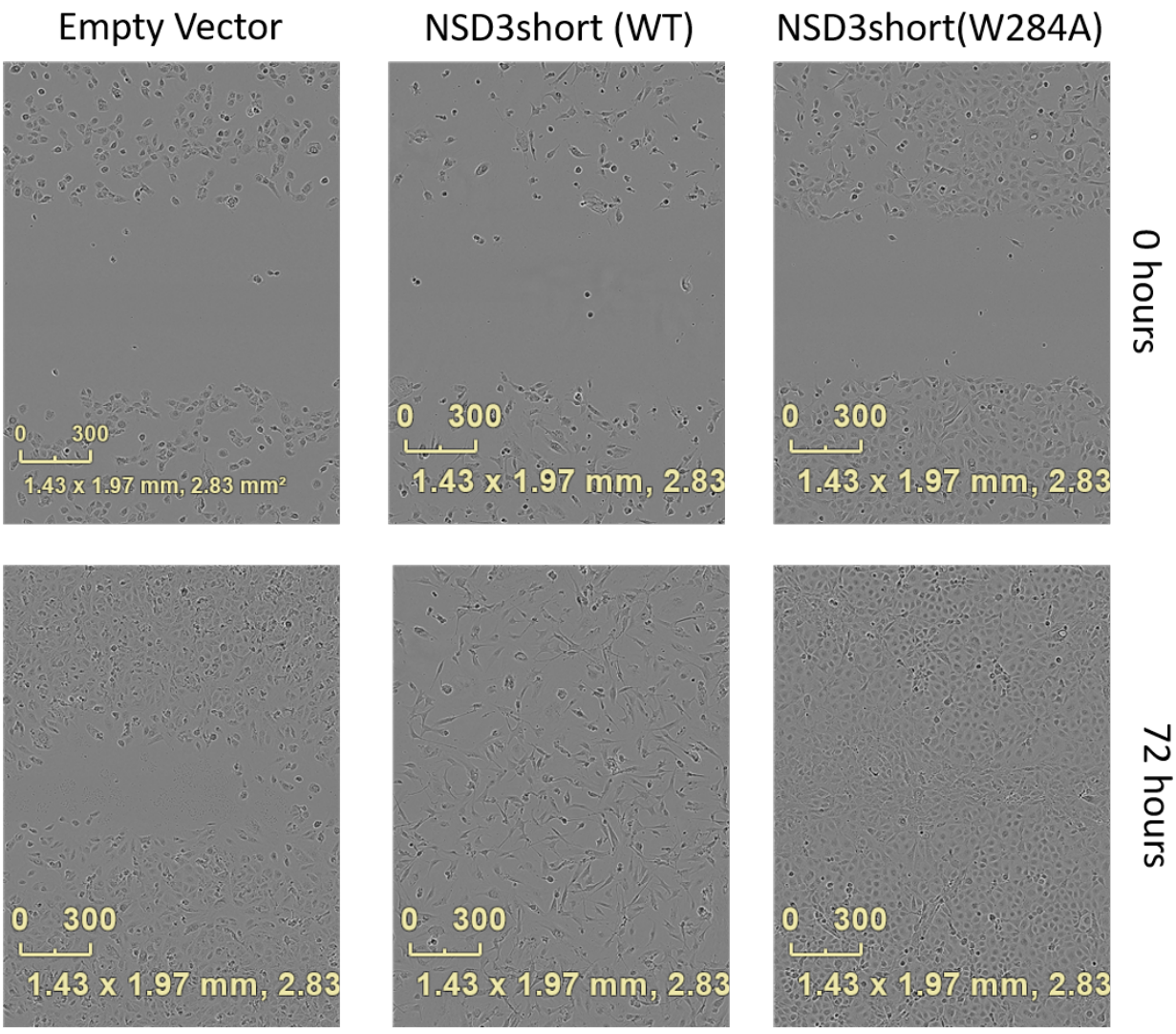

Figure 1: Wound Healing Assay Rep1 - 15 000 cells seeded

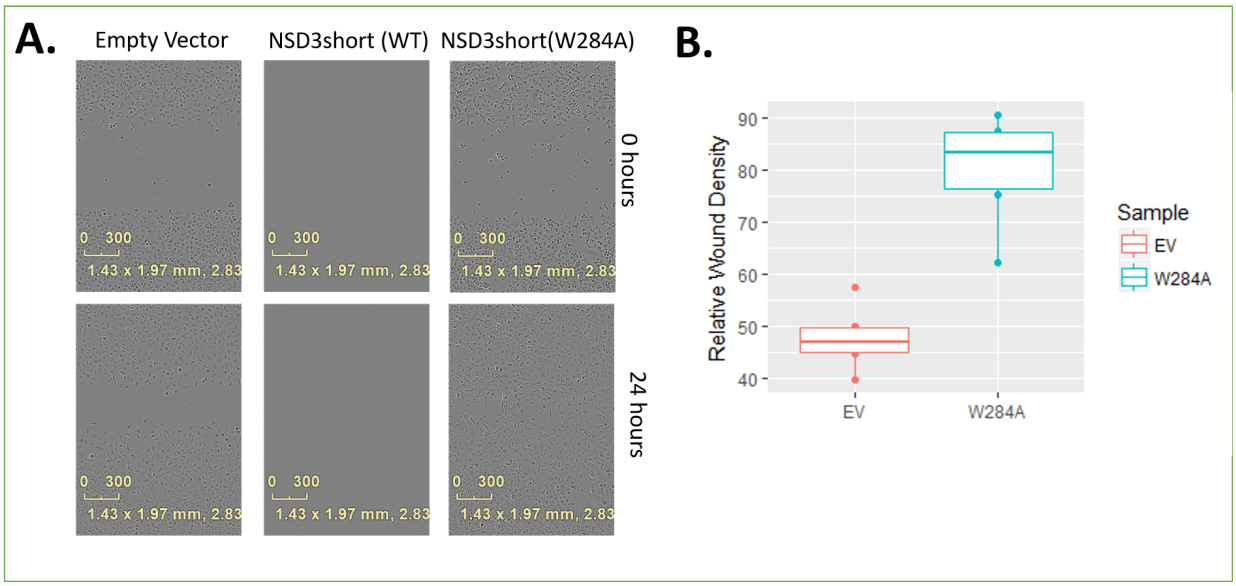

Figure 2: Wound Healing Assay Rep1 - 30 000 cells seeded

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