

NSD3-Short Promotes Migration of A549 Lung Cancer Cells

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Objective

In a previous experiment (exp024), we observed an increase in E-cadherin expression in response to knockdown of the short isoform of NSD3. To determine if this change is functionally relevant, we performed wound healing assays to measure any corresponding alteration in migratory potential of A549 lung epithelial cancer cells.

Experimental Details

1.1 siRNA Transfections

Transfection of A549 cells with NSD3-targeting and control siRNA was performed in quadruplicate following the Life Technologies protocol for RNAiMAX reagent (https://tools.thermofisher.com/content/sfs/manuals/Lipofectamine_RNAiMAX_Reag_protocol.pdf) by reverse transfection of 30 000 cells in 96 well format.

siRNA Used

Target	Supplier - Cat#	Final Concentration
Non-targeting Control	Sigma - SIC001	5 nM
NSD3	Ambion - 4392420-s29725	5 nM
NSD3Long	IDT - CD.Ri.108909.13.1	5 nM
NSD3Short	IDT - CD.Ri.108912.13.4	5 nM

1.2 Wound Healing Assay

A549 cells reverse transfected in 96 well plates were cultured for 48 hours. 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 24 and 48 hours.

Results

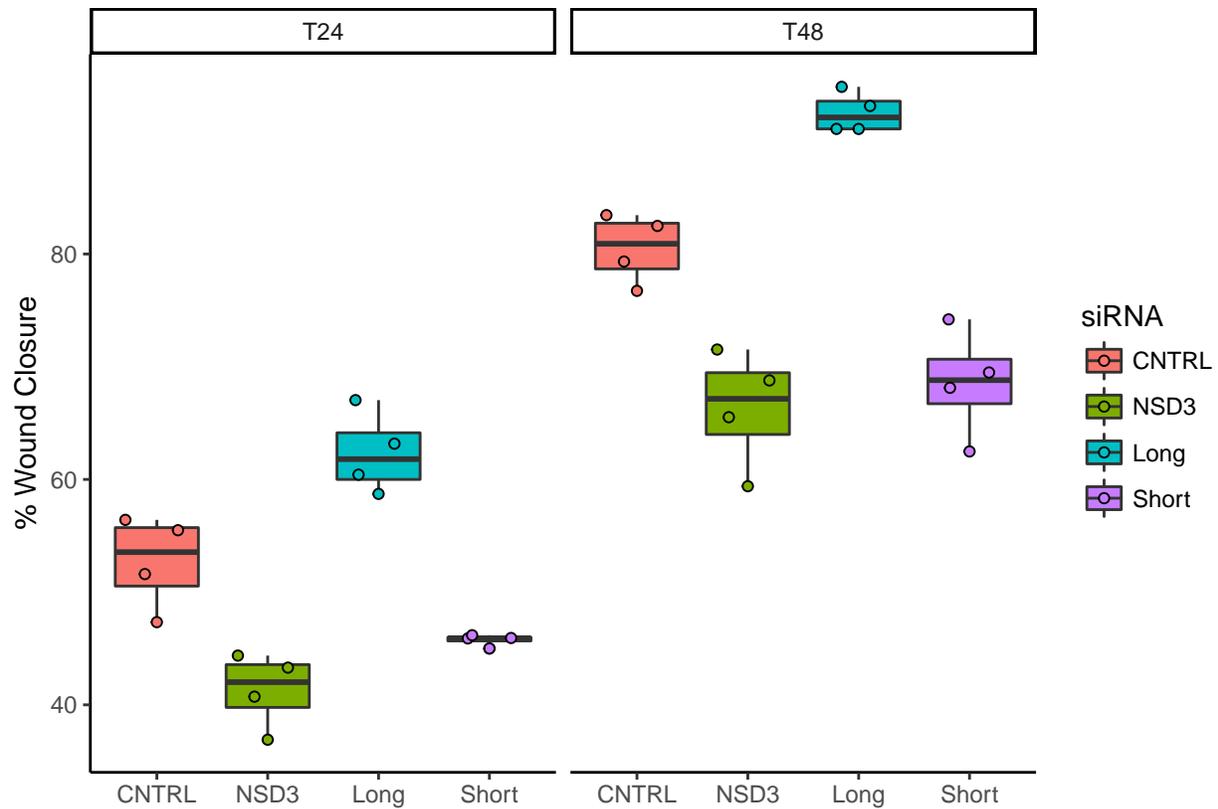
```
require(tidyverse)
require(ggbeeswarm)

read_csv(file= "exp025_WoundHealing.csv") %>%
  mutate(siRNA = factor(siRNA, levels = c("CNTRL", "NSD3", "Long", "Short"))) %>%
  gather(Time.Pt, Closure, 2:4) %>%
  group_by(siRNA, Time.Pt, Cell_Line) %>%
```

```

filter(Cell_Line == "A549") %>%
filter(Time.Pt != "T0" ) %>%
ggplot(aes(siRNA, Closure, fill = siRNA)) +
geom_boxplot() +
geom_quasirandom(pch = 21) +
theme_classic() +
ylab("% Wound Closure") +
xlab("") +
facet_grid(~ Time.Pt)

```



Observations

In agreement with western blotting results showing increased E-cadherin expression in response to NSD3short knockdown, we show here that siRNA targeting the short isoform of NSD3 decreases the migratory ability of a model epithelial lung cell line (A549). Collectively, these results support the hypothesis that NSD3short is a pro-EMT factor.

ExpID-025