

Quantification of E-cadherin Expression in H1299 NSD3 Knockdown Cells

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Objective

Having observed downregulation of E-cadherin protein levels in response to NSD3 overexpression in H1299 cells (exp021), I was next interested in testing the consequences of NSD3 knockdown. To do so, I transfected H1299 cells with siRNA targeting NSD3, both short and long isoforms, and assayed E-cadherin expression by western blotting.

Experimental Details

Knockdown of NSD3 in H1299 cells and western blotting was performed in triplicate as described in exp019. A detailed protocol for cell lysis and western blotting can be found on protocol.io (<https://dx.doi.org/10.17504/protocols.io.pxndpme>). Quantification of fluorescence signal was determined using Licor software - Image Studio Lite v5.2. The code below was used to plot western blot quantification data and test significance.

siRNA Used

Target	Supplier - Cat#	Final Concentration
Non-targeting Control	Sigma - SIC001	5 nM
NSD3	Ambion - 4392420-s29725	5 nM

Antibodies Used

Target	Supplier - Cat#	Dilution
NSD3 (RabPoly)	ProteinTech - 11345-1-AP	1:1000
E-cadherin (RabPoly)	abcam - ab53033	1:1000
Actin (MouseMon)	abcam - ab3280	1:5000
Histone H3 (RabPoly)	abcam - ab70550	1:5000

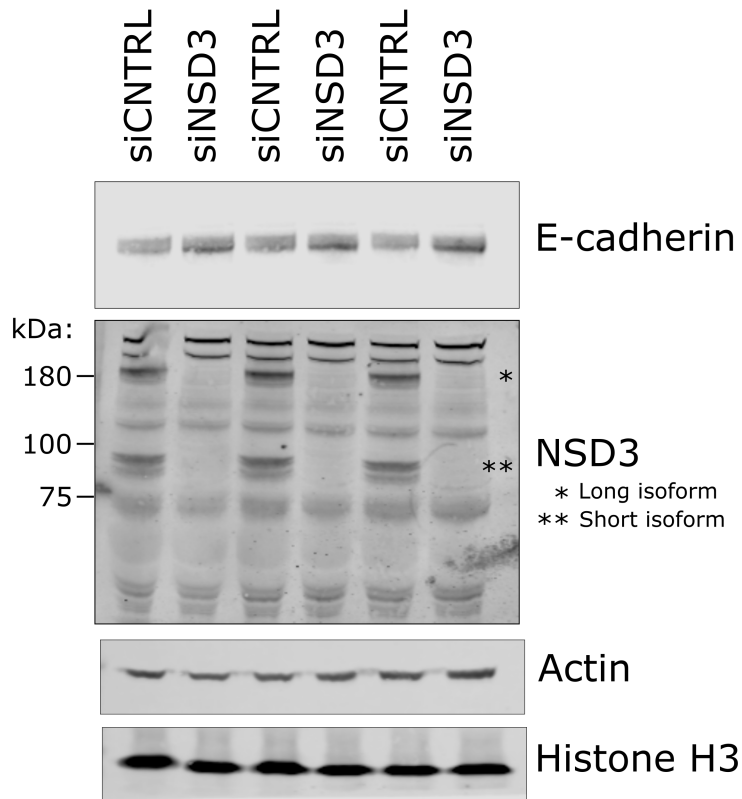


Figure 1: Western blot of of NSD3 Knockdown in H1299 Cells

R Script

```
library(tidyverse)

# Read data into R.
quant <- read_csv(file = "blot_quantifications_26042018.csv")

# Summarise the data for plotting.
summary <- quant %>% select(Sample, Antibody, Norm.H3, Norm.Actin) %>%
  group_by(Antibody, Sample) %>%
  summarise(H3 = mean(Norm.H3),
            Actin = mean(Norm.Actin),
            sd.H3 = sd(Norm.H3),
            sd.Actin = sd(Norm.Actin))

print(summary)

## # A tibble: 12 x 6
## # Groups:   Antibody [?]
##   Antibody Sample      H3 Actin sd.H3 sd.Actin
##   <chr>      <chr>    <dbl> <dbl> <dbl> <dbl>
## 1 Actin     siCTRL  1      1      0      0
## 2 Actin     siNSD3  1.24   1      0.252  0
```

```

## 3 E-Cadherin siCNTRL 1      1      0      0
## 4 E-Cadherin siNSD3  1.67  1.39  0.182  0.302
## 5 H3K36me2  siCNTRL 1      1      0      0
## 6 H3K36me2  siNSD3  1.22  0.997 0.262  0.184
## 7 HistoneH3 siCNTRL 1      1      0      0
## 8 HistoneH3 siNSD3  1      0.830 0      0.157
## 9 NSD3Long  siCNTRL 1      1      0      0
## 10 NSD3Long siNSD3  0.0534 0.0441 0.0111 0.0119
## 11 NSD3short siCNTRL 1      1      0      0
## 12 NSD3short siNSD3  0.0810 0.0642 0.0290 0.0104

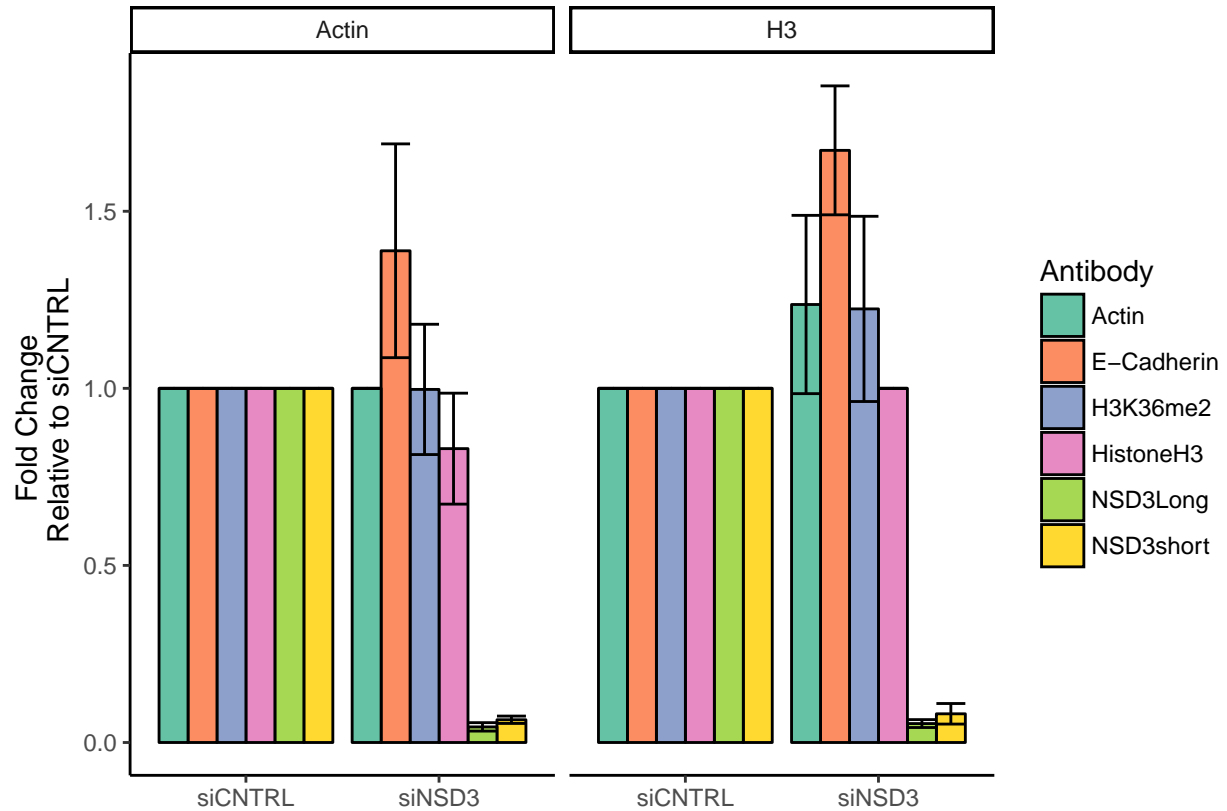
```

```
# Plot results as a bar graph.
```

```

summary %>%
  gather(Normalizer, FoldChange, 3:4) %>%
  mutate(StdDev = case_when(Normalizer == "Actin" ~ sd.Actin,
                           Normalizer == "H3" ~ sd.H3)) %>%
  mutate(Normalizer = str_replace(Normalizer, "mean.Actin", "Normalized to Actin")) %>%
  mutate(Normalizer = str_replace(Normalizer, "mean.H3", "Normalized to H3")) %>%
  select(Antibody, Sample, FoldChange, Normalizer, StdDev) %>%
  ggplot(aes(Sample, FoldChange, fill = Antibody)) +
  geom_bar(stat = "identity", colour = "black",
          position = position_dodge()) +
  geom_errorbar(aes(ymin = FoldChange - StdDev,
                   ymax = FoldChange + StdDev),
               position = position_dodge()) +
  facet_grid(. ~ Normalizer) +
  theme_classic() +
  ylab("Fold Change \n Relative to siCNTRL") +
  xlab("") +
  scale_fill_brewer(palette = "Set2")

```



```
# Prepare Data for T-test Significance Testing.
```

```
siCNTRL <- quant %>%
  filter(Antibody == "E-Cadherin"
         & Sample == "siCNTRL") %>%
  select(Norm.H3, Norm.Actin)

siNSD3 <- quant %>%
  filter(Antibody == "E-Cadherin"
         & Sample == "siNSD3") %>%
  select(Norm.H3, Norm.Actin)
```

```
# T-test Signal Normalized to Histone H3
```

```
t.test(siCNTRL$Norm.H3, siNSD3$Norm.H3)
```

```
##
## Welch Two Sample t-test
##
## data: siCNTRL$Norm.H3 and siNSD3$Norm.H3
## t = -6.3948, df = 2, p-value = 0.02359
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
## -1.1241433 -0.2198574
## sample estimates:
## mean of x mean of y
```

```
##      1.000      1.672
# T-test Signal Normalized to Actin
t.test(siCNTRL$Norm.Actin, siNSD3$Norm.Actin)

##
## Welch Two Sample t-test
##
## data:  siCNTRL$Norm.Actin and siNSD3$Norm.Actin
## t = -2.2288, df = 2, p-value = 0.1556
## alternative hypothesis: true difference in means is not equal to 0
## 95 percent confidence interval:
##  -1.138244  0.361416
## sample estimates:
## mean of x mean of y
##  1.000000  1.388414
```

Observations

With transfection of NSD3-targeting siRNA (Ambion Silencer Select - s29725) we observe significant knockdown of both long and short isoforms of NSD3. When normalizing samples to histone h3 expression, we observe a significant increase in e-cadherin expression, suggesting depletion of NSD3 promotes a more epithelial state.

ExpID-023