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
H3K36me2 (MouseMono)	Active Motif - 61019	1:5000
Histone H3 (RabPoly)	abcam - ab70550	1:5000

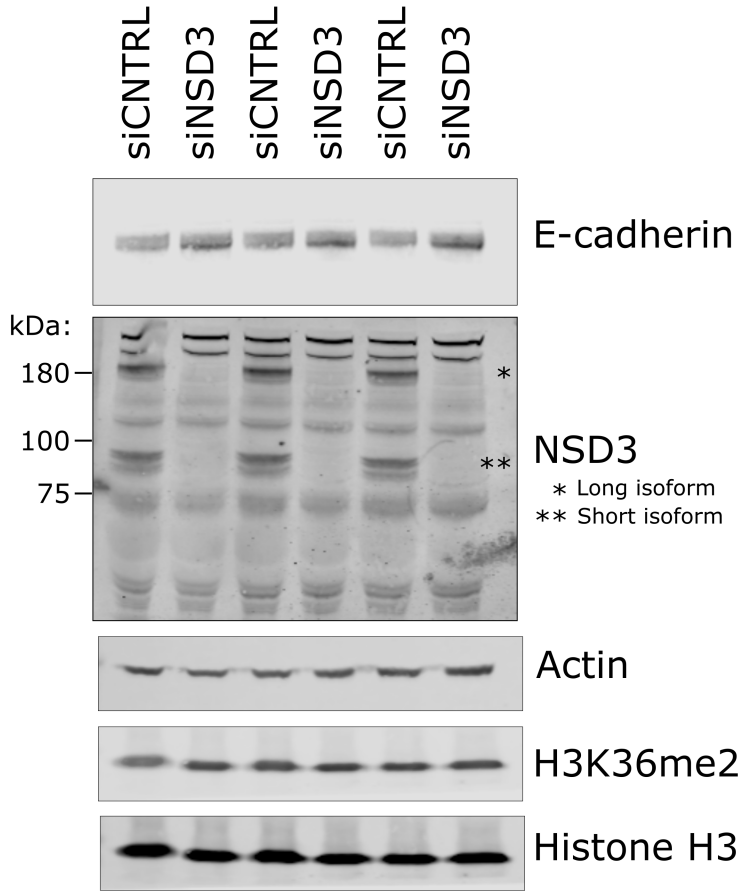


Figure 1: Western blot of of NSD3 Knockdown in H1299 Cells

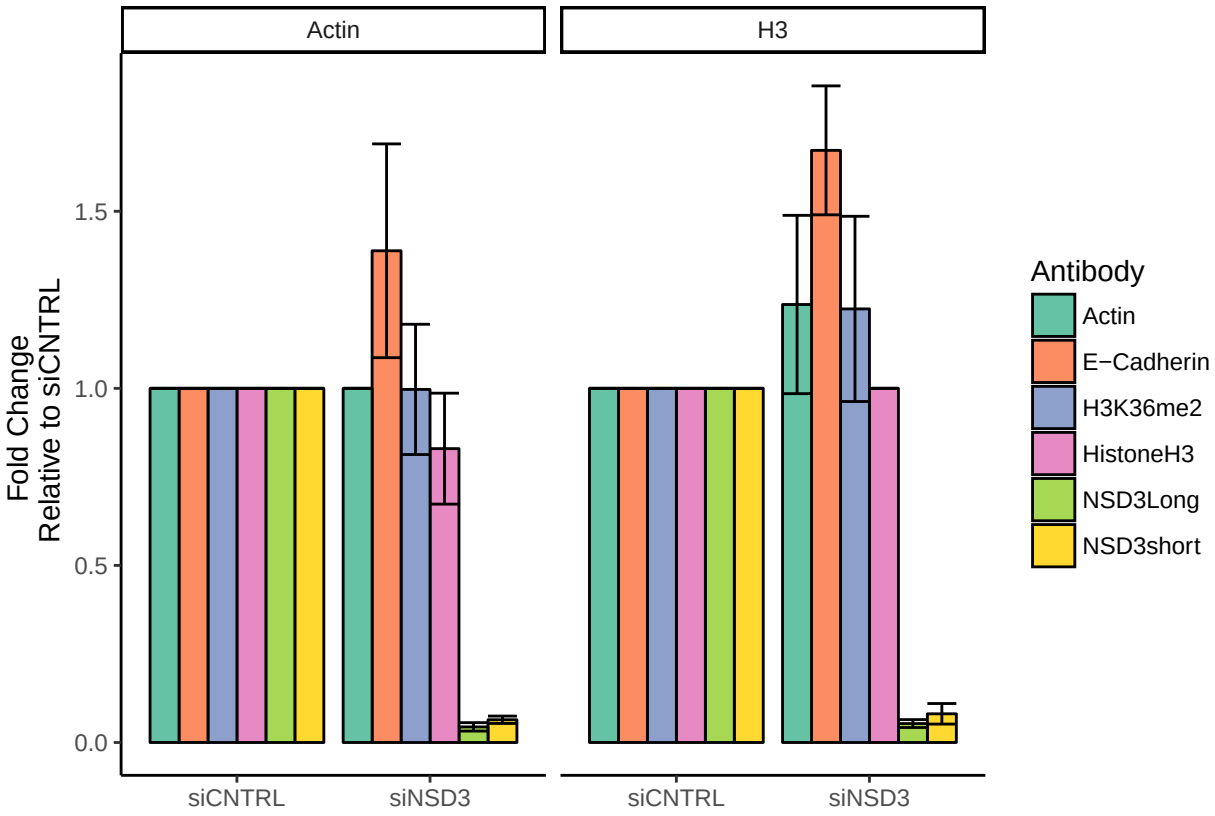
R Script

```
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      siCNTRL 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
```

```r
# 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(ymax = FoldChange + StdDev,
                    ymin = 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