

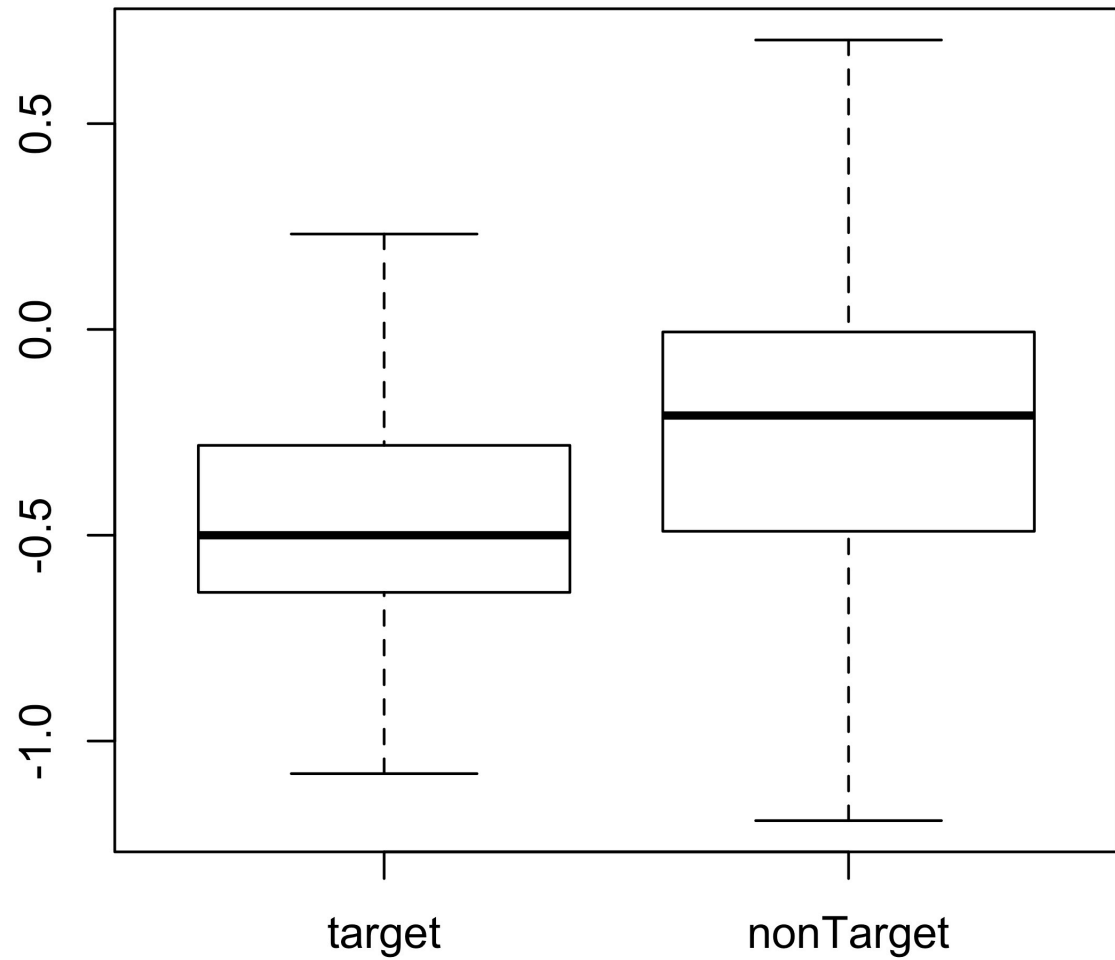
-- Ref 5.18 – KD in MYC --

Referee Comment	13. MYC is known to have profound effects on gene networks. Have the authors considered comparing the results from their MCF7 knockdown experiment to existing data from similar MYC knockdowns to validate the behavior of the network?
Author Response	dc & jz to do the comparison new arrays Search for other MYC KD data
Excerpt From Revised Manuscript	

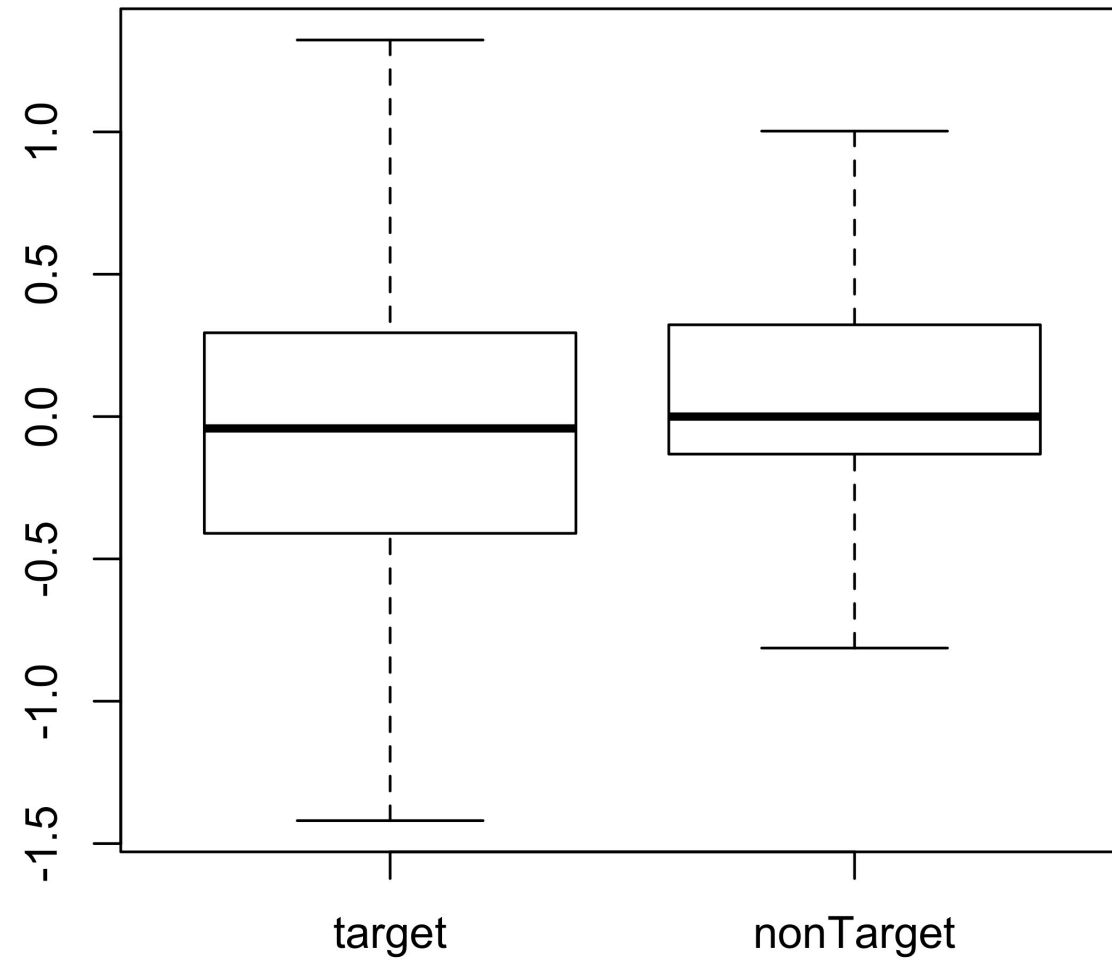
“...data from similar MYC knockdowns” -- Data from GEO is here [accession # 200086504]:
<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE86504>

Summary	<p>MYC is a master regulator of transcription in growing cells. Menin is an enigmatic protein that displays unique ability to either suppress or promote tumorigenesis in a context dependent manner. It's interesting to ask is there any relationship between MYC and menin. Here, we used RNA-seq to study global transcriptomic expression of MYC or MEN1 knockdown HT1080 cells to investigate whether there are any correlations between MYC- and menin- regulated gene expression. Besides, we performed ChIP-seq assays for MYC and Menin binding sequences to address whether Menin and MYC share some common binding sites on chromatin.</p>
Overall design	<p>Human fibrosarcoma HT1080 cells were transfected with MYC shRNAs, MEN1 shRNAs or non-targeting control shRNA followed by RNA extraction and sequenced on Illumina HiSeq 4000 (Homo sapiens). And also we performed MYC ChIP-seq and Menin ChIP-seq to address whether Menin and MYC share some common binding sites on chromatin. RNA Pol II ChIP-seq for NTC and shMEN1 HT1080 cell samples were used to study the effect of Menin on RNA Pol II-mediated elongation.</p>

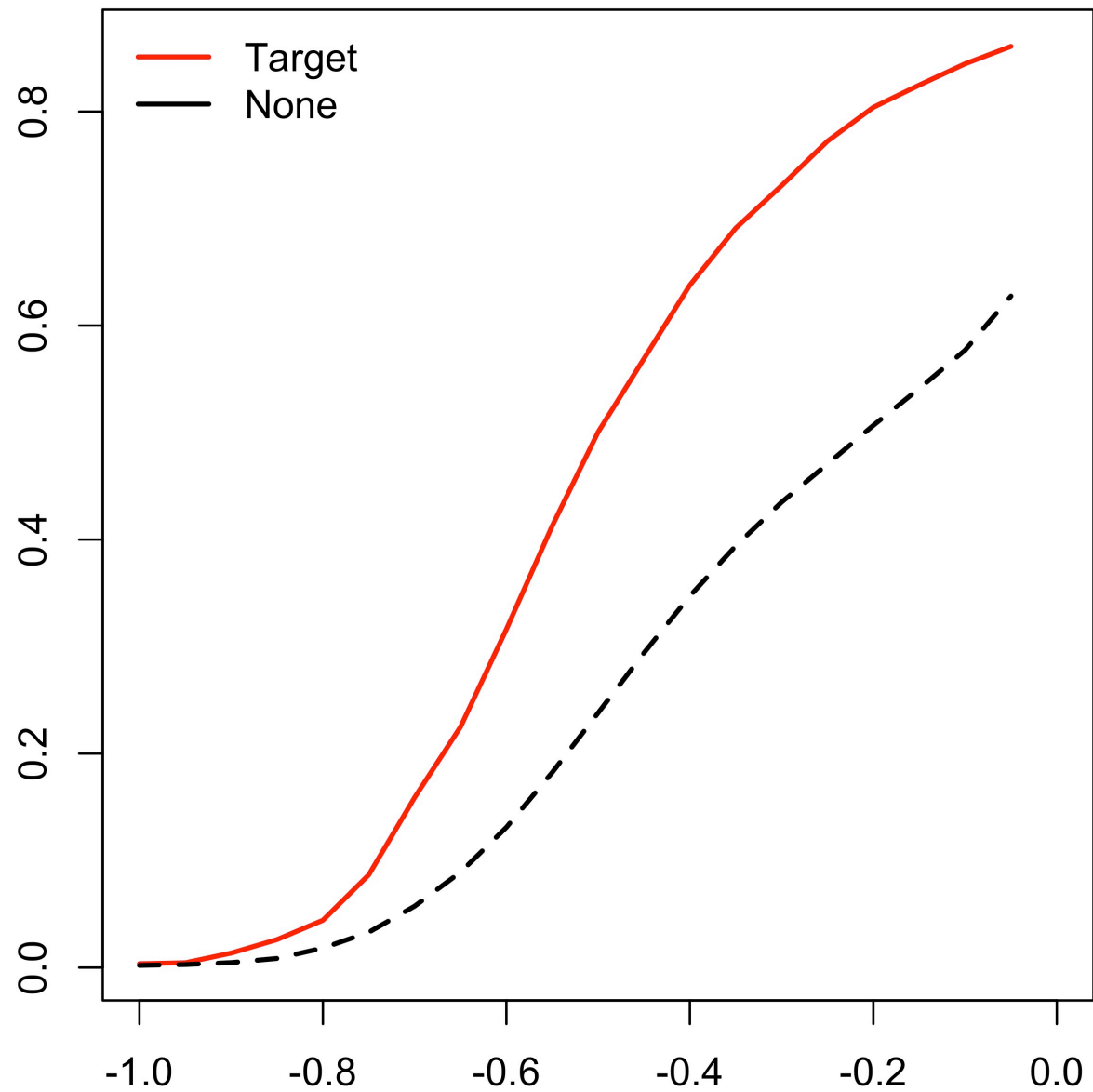
Our original result



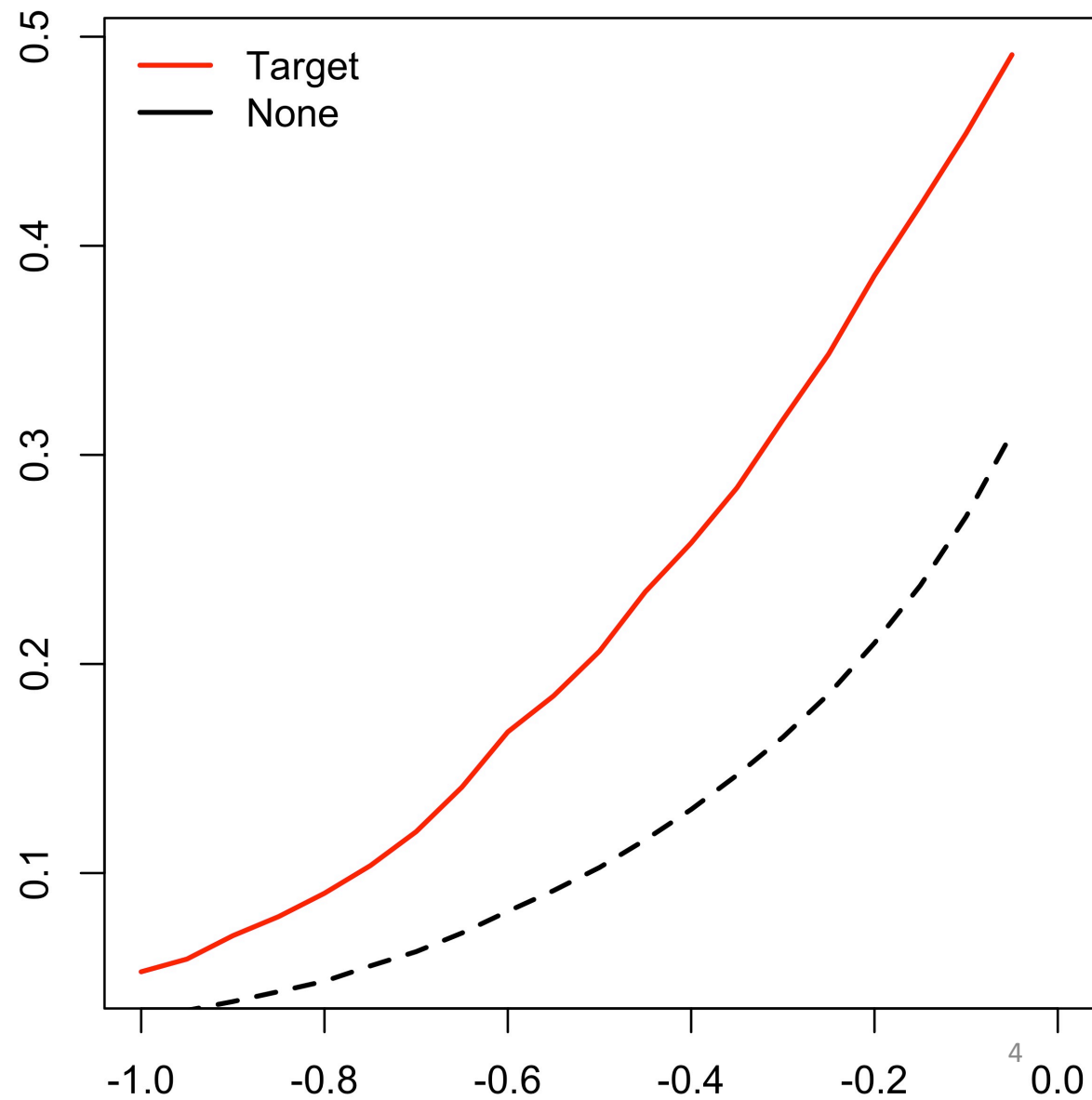
Result using alternative gene expression data from GEO



Our original result



Result using alternative gene expression data from GEO



Our original result

```
> cor.test(data.val$fc, data.val$score)

Pearson's product-moment correlation

data: data.val$fc and data.val$score
t = -56.929, df = 20199, p-value < 2.2e-16
alternative hypothesis: true correlation is not
equal to 0
95 percent confidence interval:
 -0.3836616 -0.3598941
sample estimates:
      cor
-0.3718388
```

Result using alternative gene expression data from GEO

```
> cor.test(data.val$fc, data.val$score)

Pearson's product-moment correlation

data: data.val$fc and data.val$score
t = -1.5299, df = 18573, p-value = 0.126
alternative hypothesis: true correlation is not
equal to 0
95 percent confidence interval:
 -0.025602413  0.003155945
sample estimates:
      cor
-0.01122556
```

Our original result

```
> table(data.val$score>reg.thres)
```

```
FALSE  TRUE  
9094  1107
```

Result using alternative gene expression data from GEO

```
> table(data.val$score>reg.thres)
```

```
FALSE  TRUE  
17590  985
```

Our original result

```
> data.val[data.val$gene=="MYC",]
      gene      score d1.used.FPKM d2.used.FPKM d1.used.gene_short_name d2.used.gene_short_name      fc
11187  MYC 0.7666595      8.45918      5.05832                MYC                MYC -0.6427973
```

Result using alternative gene expression data from GEO

```
> data.val[data.val$gene=="MYC",]
      gene      score d1.used.FPKM d2.used.FPKM d1.used.gene_short_name d2.used.gene_short_name      fc
11187  MYC 0.7666595      47.34291      14.86019                MYC                MYC -1.607895
```

Our original result

```
Wilcoxon rank sum test with continuity correction
```

```
data: x and y
```

```
W = 6630000, p-value < 2.2e-16
```

```
alternative hypothesis: true location shift is not  
equal to 0
```

Result using alternative gene expression data from GEO

```
Wilcoxon rank sum test with continuity correction
```

```
data: x and y
```

```
W = 7390400, p-value = 6.813e-15
```

```
alternative hypothesis: true location shift is not  
equal to 0
```