**Supplementary Figure 1. Construction of *Rnf138*-knockdown (*Rnf138*-KD) C2C12 cell lines.**
(A) Western blot analysis of RNF138 protein levels in stable *Rnf138*-KD C2C12 myoblast cell lines. Cells were infected with lentivirus-mediated shRNA targeting *Rnf138* (shRnf138 #1, #2, and #3) or a scrambled control (SCR).

(B) Quantification of *Rnf138* mRNA levels in stable *Rnf138*-KD and SCR C2C12 cell lines. Relative mRNA expression of *Rnf138* was measured by qPCR. Data are normalized to the values in the SCR and presented as the mean ± SEM and statistical significance was determined using Student's *t*-test, comparing each *Rnf138*-KD group to the SCR group (***$p < 0.001$).

**Supplementary Figure 2. Clustering analysis of differentially expressed genes (DEGs) following *Rnf138*- knockdown (*Rnf138*-KD) in proliferating C2C12 myoblasts.**
(A) Venn diagram showing the overlap and unique gene expression profiles between *Rnf138*-KD and control (SCR) proliferating C2C12 myoblasts. Numbers indicate unique and shared genes between the two groups.

(B) Volcano plot illustrating the distribution of DEGs between *Rnf138*-KD and control groups. DEGs with statistical significance (adjusted p-value < 0.05) and log2(Fold Change) > 1 are highlighted in green (downregulated) and red (upregulated).

(C) Heatmap of DEGs identified between RNF138 knockdown and control groups, showing hierarchical clustering of samples and genes. Red represents upregulated genes, and green represents downregulated genes.

(D) Gene ontology (GO) biological process (BP) enrichment analysis of significant DEGs, highlighting the top 10 enriched terms.

(E-H) Heatmaps showing the expression levels of key genes within specific GO categories: (E) "muscle organ development," (F) "muscle cell development," (G) "muscle-skeletal system development," and (H) "muscle cell differentiation."

**Supplementary Figure 3. GO analysis RNF138 interactome based on IP-MS**.

Co-immunoprecipitation followed by mass spectrometry (Co-IP/MS) was performed in HEK293 cells overexpressing GFP-RNF138, with GFP as a control. The identified RNF138 interactome was analyzed through Gene Ontology (GO) enrichment. GO terms were categorized into biological processes (A), molecular functions (B), and cellular components

(C), and significant enrichments were determined based on -log10(p-value) and percentage of proteins.

**Supplementary Figure 4. *Ctnnb1*-knockdown (*Ctnnb1*-KD) impairs C2C12 myoblasts differentiation**.

(A) Western blot analysis of β-catenin protein levels in C2C12 cells transfected with scrambled control (SCR) or shCtnnb1 constructs (#1, #2, #3).

(B) Relative mRNA levels of *Ctnnb1* in C2C12 cells were assessed by qRT-PCR. Data are normalized to the values in the SCR and presented as mean ± SEM and statistical significance was determined using Student's *t*-test, comparing each shCtnnb1 group to the SCR group (*$p < 0.05$, **$p < 0.01$).

(C) Western blot analysis of MyHC, RNF138, and β-catenin levels in *Ctnnb1*-KD (shCtnnb1#1 and shCtnnb1#3) and SCR cells after 5 days of differentiation (DMd5).

(D) Representative immunofluorescence images of MyHC (red) and nuclei (DAPI, blue) in differentiated C2C12 myoblasts (DMd5) with SCR or *Ctnnb1*-KD (shCtnnb1#1 and shCtnnb1#3). Scale bar: 100 μm.

(E) Quantification of MyHC-positive cells (%) from (D). Data are presented as mean ± SEM from three independent fields per group. Statistical significance was determined using Student's *t*-test, comparing each *Rnf138*-KD group to the SCR group (***$p < 0.001$).

(F) Relative mRNA expression levels of myogenic markers *Myod1*, *Myogenin* (*Myog*), *Myomaker*, and *Myh2b* in SCR and *Ctnnb1*-KD cells after 2 days of differentiation (DMd2). Data are normalized to the values in the SCR group and presented as mean ± SEM from three independent experiments. Statistical analysis was performed using Student's *t*-test, comparing each *Ctnnb1*-KD group to the SCR group (***$p < 0.001$).

(G) Relative mRNA levels of *Myod1*, *Myog*, *Myomaker*, and *Myh2b* in C2C12 myoblasts treated with DMSO (control) or XAV939 (Wnt/β-catenin inhibitor) after 2 days of differentiation (DMd2). Data are normalized to the values in the SCR group and presented as mean ± SEM from three independent experiments. Statistical analysis was performed using Student's *t*-test, comparing the XAV929 group to the DMSO group (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

**Supplementary Figure 5. β-catenin is positively correlated with RNF138**.

(A) Western blot analysis of β-catenin levels in A549 cells transduced with scrambled control (SCR) or RNF138 knockdown constructs (shRNF138 #1 and #2).

(B) Time-course analysis of β-catenin protein levels in A549 cells overexpressing GFP-RNF138 for 24, 36, and 48 hours.

(C) Relative mRNA levels of *CTNNB1* in A549 cells transfected with GFP or GFP-RNF138. Data are normalized to the values in the GFP group and presented as mean ± SEM from three independent experiments. Statistical analysis was performed using Student's *t*-test, comparing the GFP-RNF138 group to the GFP group (ns: not significant).

(D, E) Western blot analysis of β-catenin, RNF138, and γ-H2A.X (Ser139) levels in HEK293T, U2OS, and C2C12 cells treated with arsenate (As) (D) or etoposide (Eto) (E).

**Supplementary Figure 6. GO analysis of DEGs related to the canonical Wnt signaling pathway upon *Rnf138*-knockdown**.

Heatmap of differentially expressed genes (DEGs) related to the canonical Wnt signaling pathway in differentiating (A) and proliferating (B) myoblasts upon *Rnf138*-knockdown.

**Supplementary Figure 7. Correlation analysis of RNF138 and MKRN1 mRNA expression in various human tissues.**

Scatter plots showing the correlation between RNF138 and MKRN1 mRNA expression levels across various human tissues. Data were generated using the online tool GEPIA (http://gepia.cancer-pku.cn/) on November 24, 2024. Pearson correlation coefficients (R) and *p*-values are shown in the corresponding figures. All analyses were conducted entirely by the GEPIA platform without manual intervention.

Table S1. The sequences of primers used in this study.

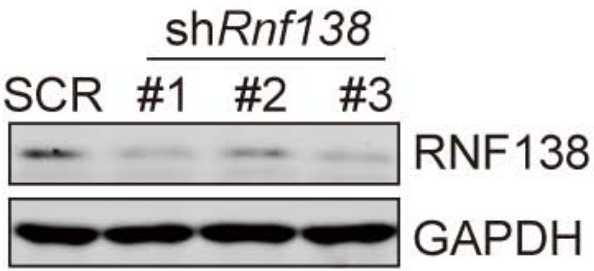
| Name | Sequence (5' ~ 3') |
|---|---|
| **Primers used for generating constructs (Mus musculus)** | |
| Rnf138-F | ATGTCCGAGGAACTTTCGG |
| Rnf138-R | TCACATGTTTACTTGAAAAGACTCTT |
| **Primers used for generating constructs (Homo sapiens)** | |
| RNF138-F | ATGGCCGAGGACCTCTC |
| RNF138-R | TCAGATGTTTACTTGAAAAGATTCTT |
| β-catenin-F | ATGGCTACTCAAGCTGATTTG |
| β-catenin-R | TTACAGGTCAGTATCAAACCAGG |
| APC-Nter-F | CAGTCTGCTGGATTTGGTTCTAGGGTG |
| APC-Nter-R | TTAAACAGATGTCACAAGGTAAGACCC |
| **Primers used to generate deletion mutants of RNF138 for functional domain studies (Homo sapiens)** | |
| RNF138-ΔRING-F | CGAAGATGATTTCTACGGAAATGTGACTAGAAGAGAGAGAGCATGTC |
| RNF138-ΔRING-R | TTCTAGTCACATTTCCGTAGAAATCATCTTCGGTGTAGGACGTGGC |
| RNF138-ΔZNF1-F | GAAGTTTTCTGGTAGCAAGAAGTATCAGGATGAATATGGTGTTTC |

| | |
|---|---|
| RNF138-ΔZNF1-R | TTCATCCTGATACTTCTTGCTACCAGAAAACTTCCTCATTATATTTTC |
| RNF138-ΔZNF2-F | GTCATCCTACTTTTAAGCTATTTCAGATAGTTCCTGTGACATG |
| RNF138-ΔZNF2-R | CAGGAACTATCTGAAATAGCTTAAAAGTAGGATGACCAGAAGAAC |
| RNF138-ΔZNF3-F | GATAGTTCCTGTGACACAATTTGATTATGGAGAATTTGTGAATC |
| RNF138-ΔZNF3-R | TTCTCCATAATCAAATTGTGTCACAGGAACTATCTGAAATAGG |
| RNF138-ΔUIM-F | GAATCTTCAGCTAGATAACATCTGAGAATTCTGCAGATATC |
| RNF138-ΔUIM-R | CAGAATTCTCAGATGTTATCTAGCTGAAGATTCACAAATTCTC |
| RNF138-ΔNLS-F | GCCTTAGACCTTGAAAATATATACAAATCTTGTAAGAAGTATCAGGATG |
| RNF138-ΔNLS-R | GATACTTCTTACAAGATTTGTATATATTTTCAAGGTCTAAGGCCCG |
| **Primers used for constructing vectors expressing short hairpin RNAs (shRNAs) of various genes (Mus musculus)** | |
| Rnf138_shRNA#1-F | CCGGCTTCTGTCATTCCAAACTTTACTCGAGTAAAGTTTGGAATGACA GAAGTTTTT |
| Rnf138_shRNA#1-R | AATTAAAAACTTCTGTCATTCCAAACTTTACTCGAGTAAAGTTTGGAA TGACAGAAG |
| Rnf138_shRNA#2-F | CCGGCTGCATCTGATAACACAGAAACTCGAGTTTCTGTGTTATCAGAT GCAGTTTTT |
| Rnf138_shRNA#2-R | AATTAAAAACTGCATCTGATAACACAGAAACTCGAGTTTCTGTGTTAT CAGATGCAG |
| Rnf138_shRNA#3-F | CCGGCCCTTATGTCAAGAGTCAAATCTCGAGATTTGACTCTTGACATA AGGGTTTTT |
| Rnf138_shRNA#3-R | AATTAAAAACCCTTATGTCAAGAGTCAAATCTCGAGATTTGACTCTTG ACATAAGGG |
| Ctnnb1_shRNA#1-F | CCGGGCTGATATTGACGGGCAGTATCTCGAGATACTGCCCGTCAATAT CAGCTTTTT |
| Ctnnb1_shRNA#1-R | AATTAAAAAGCTGATATTGACGGGCAGTATCTCGAGATACTGCCCGTC AATATCAGC |
| Ctnnb1_shRNA#2-F | CCGGCACGCAAGAGCAAGTAGCTGATATTCTCGAGAATATCAGCTACT TGCTCTTGCGTGTTTTT |
| Ctnnb1_shRNA#2-R | AATTAAAAACACGCAAGAGCAAGTAGCTGATATTCTCGAGAATATCAG CTACTTGCTCTTGCGTG |
| Ctnnb1_shRNA#3-F | CCGGCCCTCAGATGGTGTCTGCCATTGTACTCGAGTACAATGGCAGAC ACCATCTGAGGGTTTTT |
| Ctnnb1_shRNA#3-R | AATTAAAAACCCTCAGATGGTGTCTGCCATTGTACTCGAGTACAATGG CAGACACCATCTGAGGG |
| **Primers used for constructing vectors expressing short hairpin RNAs (shRNAs) of various genes (Homo sapiens)** | |
| RNF138_shRNA#1-F | CCGGGCTAGATGAAGAAACCCAATACTCGAGTATTGGGTTTCTTCATC TAGCTTTTT |
| RNF138_shRNA#1-R | AATTAAAAAGCTAGATGAAGAAACCCAATACTCGAGTATTGGGTTTCT TCATCTAGC |
| RNF138_shRNA#2-F | CCGGGTCGTGGAAATGTGACTAGAACTCGAGTTCTAGTCACATTTCCA CGACTTTTT |
| RNF138_shRNA#2-R | AATTAAAAAGTCGTGGAAATGTGACTAGAACTCGAGTTCTAGTCACAT TTCCACGAC |
| RNF138_shRNA#3-F | CCGGGCATGAGACATCATTACAAATCTCGAGATTTGTAATGATGTCTCA TGCTTTTT |
| RNF138_shRNA#3-R | AATTAAAAAGCATGAGACATCATTACAAATCTCGAGATTTGTAATGAT GTCTCATGC |
| **Primers used for qRT-PCR (Mus musculus)** | |
| Rnf138-f | TTCTACTGCCCTGTCTGTCA |
| Rnf138-r | CGTTCCGGACATGCTCTTTC |
| Myod1-f | CCACTCCGGGACATAGACTTG |
| Myod1-r | AAAAGCGCAGGTCTGGTGAG |
| Myogenin-f | CTACAGGCCTTGCTCAGCTC |
| Myogenin-r | CACGATGGACGTAAGGGAGT |
| Mrf4-f | CGGCTGGATCAGCAAGAGAA |
| Mrf4-r | CCACGTTTGCTCCTCCTTCC |
| Myomaker-f | ATCGCTACCAAGAGGCGTT |
| Myomaker-r | CACAGCACAGACAAACCAGG |
| Myh2b-f | TGATGCAGGCTGAGATCGAGGAG |
| Myh2b-r | TTGGTGTTGATGAGGCTGGTGTTC |
| IL-6-f | GAACAACGATGATGCACTTGC |
| IL-6-r | CTTCATGTACTCCAGGTAGCTATGGT |
| Fos-f | GTTTCCGGCATCATCTAGGC |

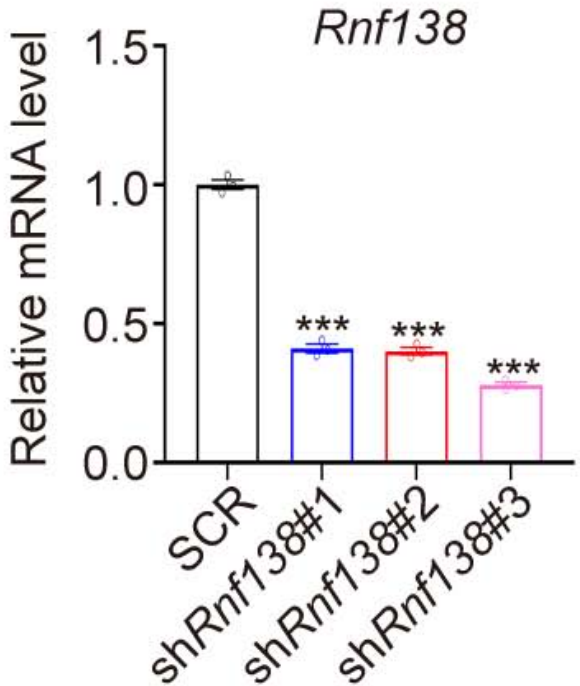
| | |
|---|---|
| Fos-r | GAAGGAGTCAGCTTCAGGGT |
| Sox8-f | CAGAGCTCAGCAAGACCCTA |
| Sox8-r | TCACACTCTTCCTTCGCCTT |
| Notch1-f | ACACCGTGTAAGAATGCTGGA |
| Notch1-r | GCCTGCTGACATGATTTTCCTG |
| Ctnnb1-f | CACAACCTTTCTCACCACCG |
| Ctnnb1-r | TTTCTGCAGTCCACCAGCTA |
| Gapdh-f | GACGTGCCGCCTGGAGA |
| Gapdh-r | GAAGAGTGGGAGTTGCTGTTGAA |

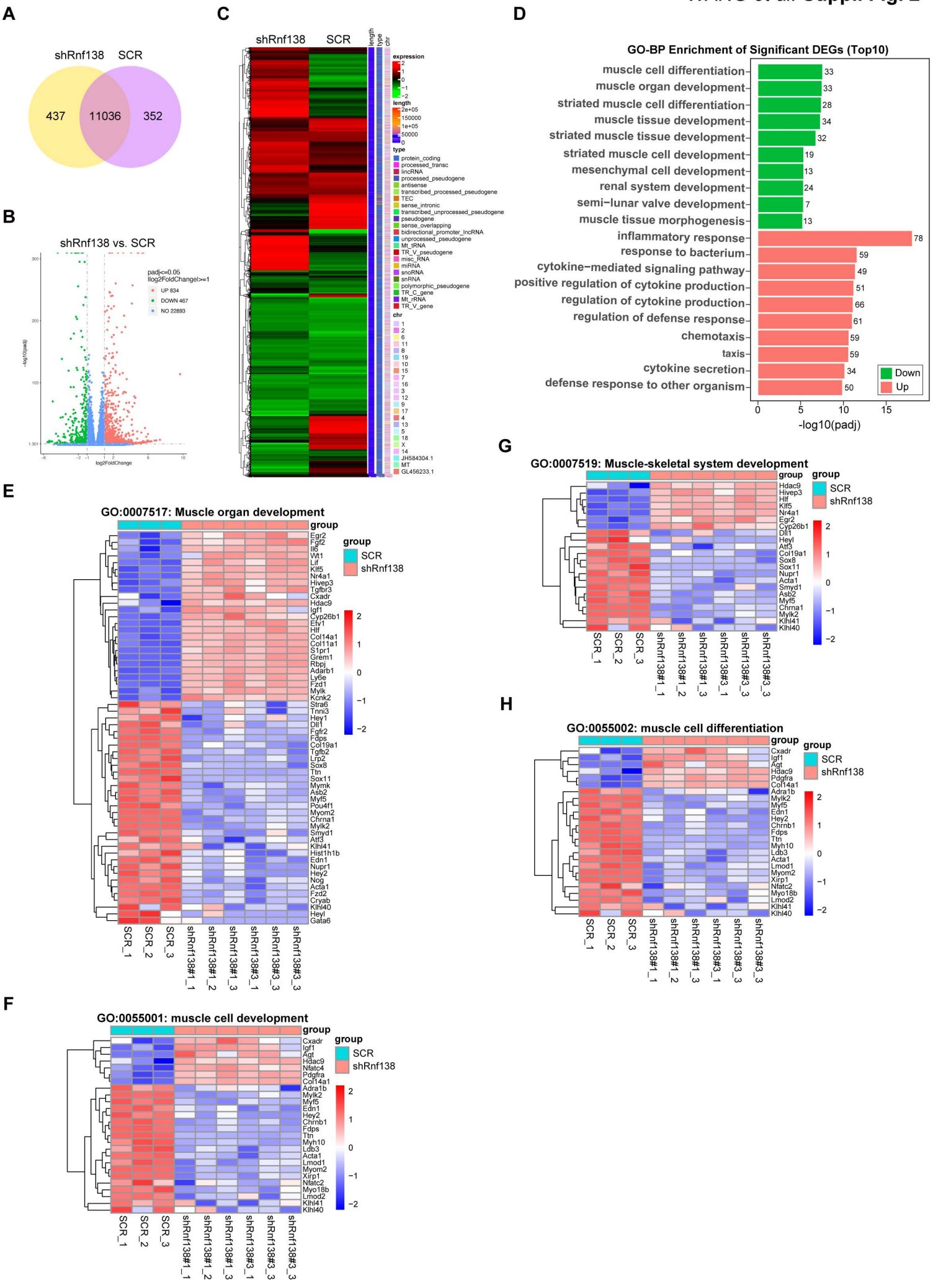
# *WANG et al.* **Suppl. Fig. 1**

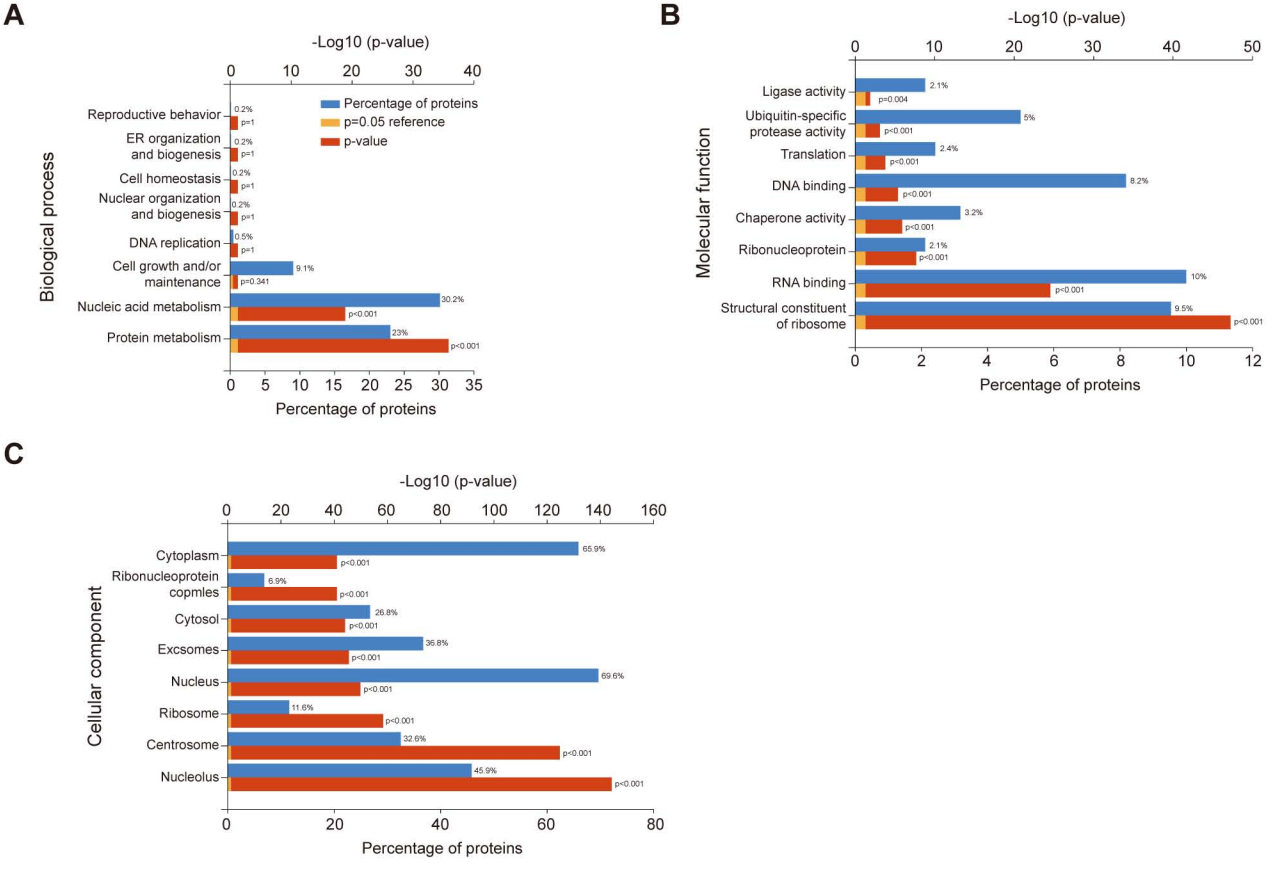
**A**

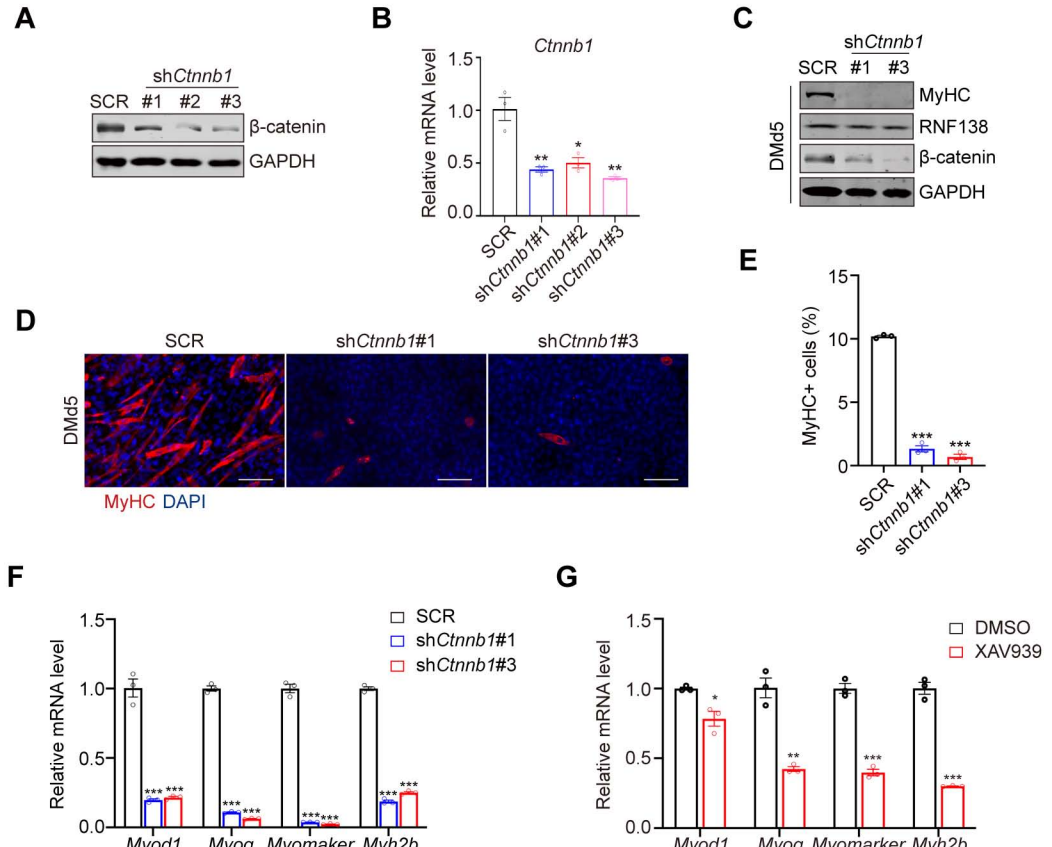


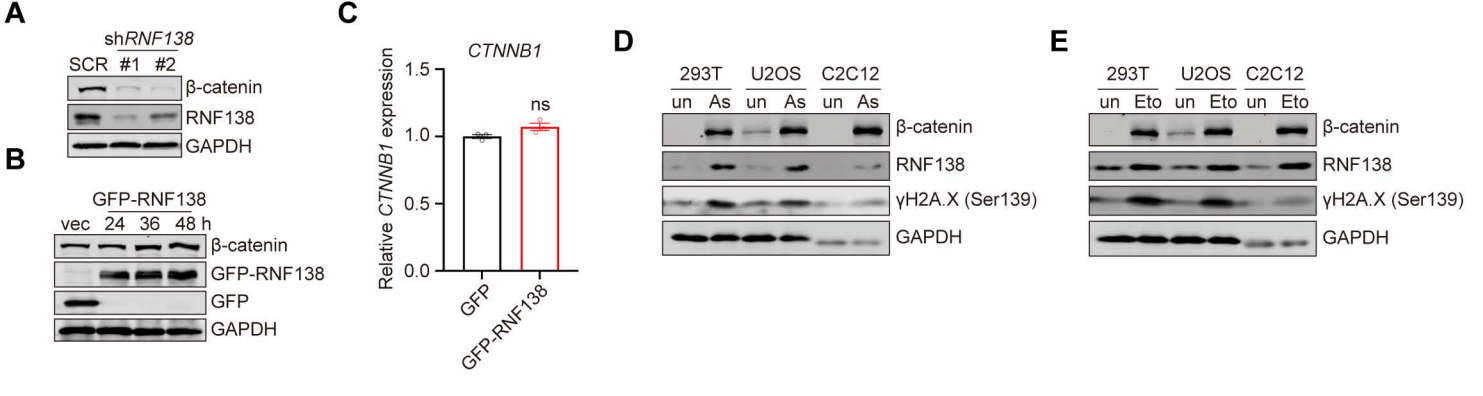

**B**



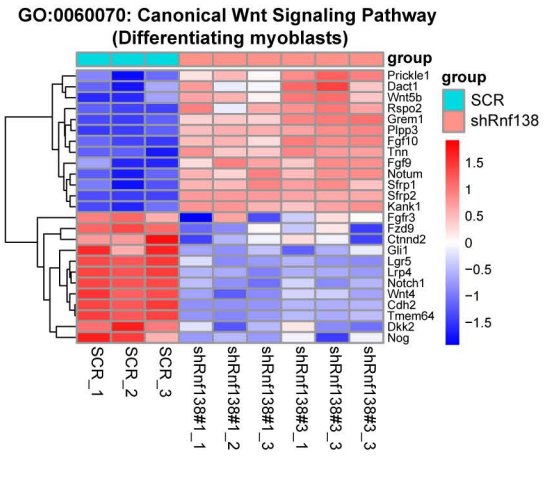

A
shRnf138
SCR
437
11036
352
B
shRnf138 vs. SCR
padj<=0.05
|log2FoldChange|>=1
UP 834
DOWN 467
NO 22893
-log10(padj)
log2FoldChange
C
shRnf138
SCR
length
type
chr
expression
length
type
chr
D
GO-BP Enrichment of Significant DEGs (Top10)
muscle cell differentiation
muscle organ development
striated muscle cell differentiation
muscle tissue development
striated muscle tissue development
striated muscle cell development
mesenchymal cell development
renal system development
semi-lunar valve development
muscle tissue morphogenesis
inflammatory response
response to bacterium
cytokine-mediated signaling pathway
positive regulation of cytokine production
regulation of cytokine production
regulation of defense response
chemotaxis
taxis
cytokine secretion
defense response to other organism
33
33
28
34
32
19
13
24
7
13
78
59
49
51
66
61
59
59
34
50
Down
Up
-log10(padj)
E
GO:0007517: Muscle organ development
group
SCR
shRnf138
SCR_1
SCR_2
SCR_3
shRnf138#1_1
shRnf138#1_2
shRnf138#1_3
shRnf138#3_1
shRnf138#3_3
shRnf138#3_3
F
GO:0055001: muscle cell development
G
GO:0007519: Muscle-skeletal system development
H
GO:0055002: muscle cell differentiation


A
-Log10 (p-value)
0 10 20 30 40
Percentage of proteins
p=0.05 reference
p-value
Biological process
Reproductive behavior
ER organization and biogenesis
Cell homeostasis
Nuclear organization and biogenesis
DNA replication
Cell growth and/or maintenance
Nucleic acid metabolism
Protein metabolism
0.2%
p=1
0.2%
p=1
0.2%
p=1
0.2%
p=1
0.5%
p=1
9.1%
p=0.341
30.2%
p<0.001
23%
p<0.001
0 5 10 15 20 25 30 35
Percentage of proteins
B
-Log10 (p-value)
0 10 20 30 40 50
Molecular function
Ligase activity
Ubiquitin-specific protease activity
Translation
DNA binding
Chaperone activity
Ribonucleoprotein
RNA binding
Structural constituent of ribosome
2.1%
p=0.004
5%
p<0.001
2.4%
p<0.001
8.2%
p<0.001
3.2%
p<0.001
2.1%
p<0.001
10%
p<0.001
9.5%
p<0.001
0 2 4 6 8 10 12
Percentage of proteins
C
-Log10 (p-value)
0 20 40 60 80 100 120 140 160
Cellular component
Cytoplasm
Ribonucleoprotein copmles
Cytosol
Excsomes
Nucleus
Ribosome
Centrosome
Nucleolus
65.9%
p<0.001
6.9%
p<0.001
26.8%
p<0.001
36.8%
p<0.001
69.6%
p<0.001
11.6%
p<0.001
32.6%
p<0.001
45.9%
p<0.001
0 20 40 60 80
Percentage of proteins

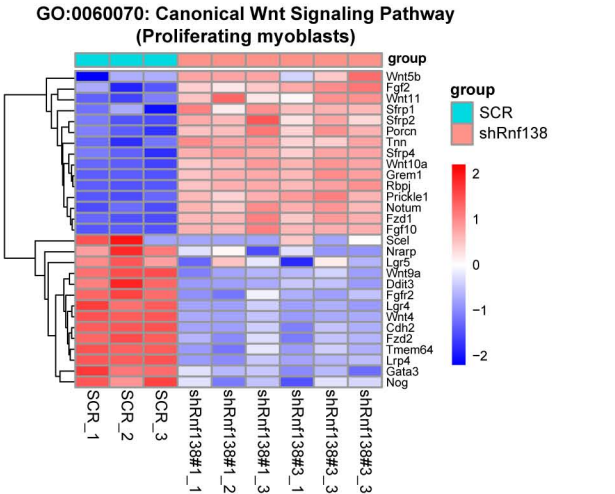

A
shCtnnb1
SCR #1 #2 #3
β-catenin
GAPDH
B
Ctnnb1
Relative mRNA level
1.5
1.0
0.5
0.0
**
*
**
SCR
shCtnnb1#1
shCtnnb1#2
shCtnnb1#3
C
shCtnnb1
SCR #1 #3
DMd5
MyHC
RNF138
β-catenin
GAPDH
D
SCR
shCtnnb1#1
shCtnnb1#3
DMd5
MyHC DAPI
E
MyHC+ cells (%)
15
10
5
0
***
***
SCR
shCtnnb1#1
shCtnnb1#3
F
Relative mRNA level
1.5
1.0
0.5
0.0
SCR
shCtnnb1#1
shCtnnb1#3
***
Myod1
Myog
Myomaker
Myh2b
G
Relative mRNA level
1.5
1.0
0.5
0.0
DMSO
XAV939
*
**
***
***
Myod1
Myog
Myomarker
Myh2b

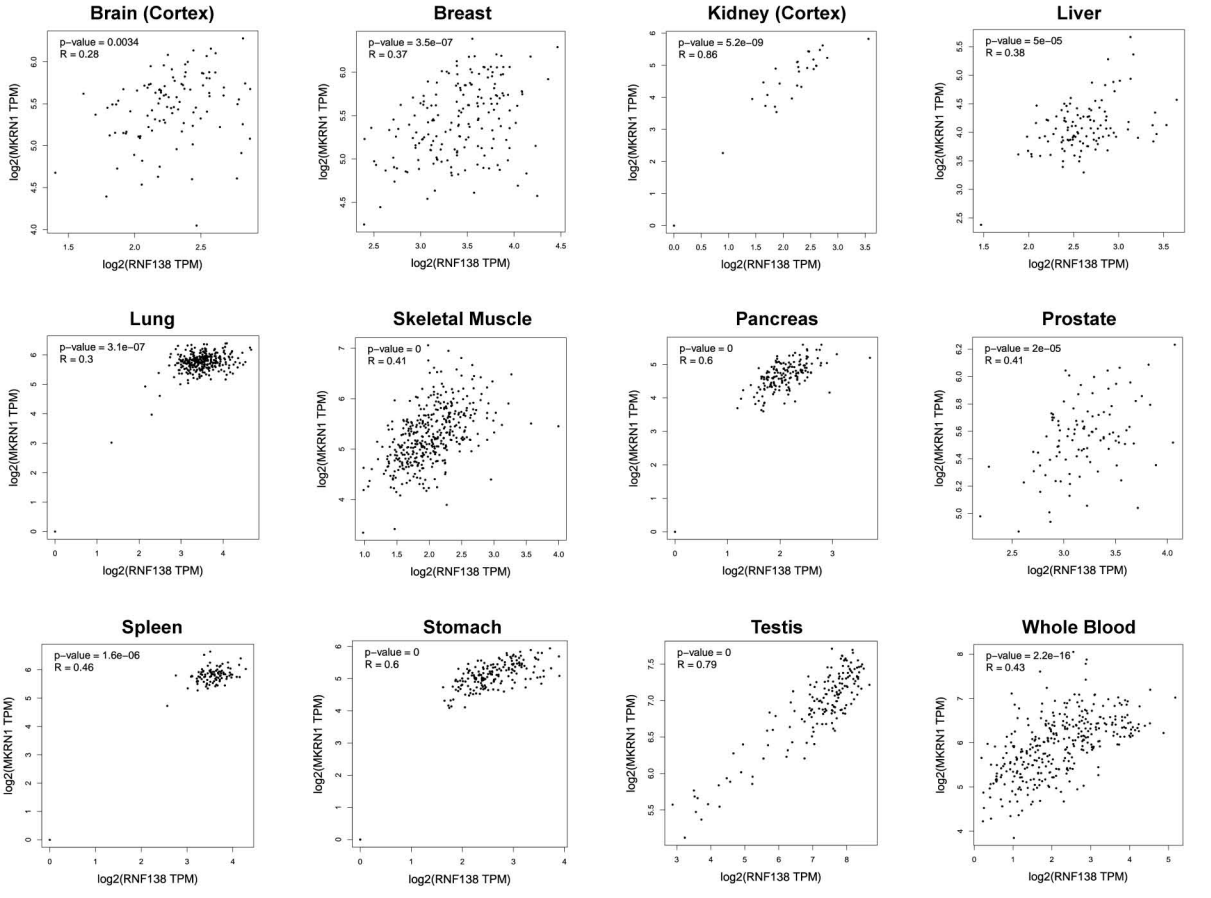

A
shRNF138
SCR #1 #2
β-catenin
RNF138
GAPDH
B
GFP-RNF138
vec 24 36 48 h
β-catenin
GFP-RNF138
GFP
GAPDH
C
CTNNB1
Relative CTNNB1 expression
1.5
1.0
0.5
0.0
ns
GFP
GFP-RNF138
D
293T U2OS C2C12
un As un As un As
β-catenin
RNF138
γH2A.X (Ser139)
GAPDH
E
293T U2OS C2C12
un Eto un Eto un Eto
β-catenin
RNF138
γH2A.X (Ser139)
GAPDH

**A**


GO:0060070: Canonical Wnt Signaling Pathway
(Differentiating myoblasts)
group
Prickle1
Dact1
Wnt5b
Rspo2
Grem1
Plpp3
Fgf10
Tnn
Fgf9
Notum
Sfrp1
Sfrp2
Kank1
Fgfr3
Fzd9
Ctnnd2
Gli1
Lgr5
Lrp4
Notch1
Wnt4
Cdh2
Tmem64
Dkk2
Nog
group
SCR
shRnf138
1.5
1
0.5
0
−0.5
−1
−1.5
SCR_1
SCR_2
SCR_3
shRnf138#1_1
shRnf138#1_2
shRnf138#1_3
shRnf138#3_1
shRnf138#3_3
shRnf138#3_3

**B**


GO:0060070: Canonical Wnt Signaling Pathway
(Proliferating myoblasts)
group
Wnt5b
Fgf2
Wnt11
Sfrp1
Sfrp2
Porcn
Tnn
Sfrp4
Wnt10a
Grem1
Rbpj
Prickle1
Notum
Fzd1
Fgf10
Scel
Nrarp
Lgr5
Wnt9a
Ddit3
Fgfr2
Lgr4
Wnt4
Cdh2
Fzd2
Tmem64
Lrp4
Gata3
Nog
group
SCR
shRnf138
2
1
0
−1
−2
SCR_1
SCR_2
SCR_3
shRnf138#1_1
shRnf138#1_2
shRnf138#1_3
shRnf138#3_1
shRnf138#3_3
shRnf138#3_3


Brain (Cortex)
p−value = 0.0034
R = 0.28
Breast
p−value = 3.5e−07
R = 0.37
Kidney (Cortex)
p−value = 5.2e−09
R = 0.86
Liver
p−value = 5e−05
R = 0.38
Lung
p−value = 3.1e−07
R = 0.3
Skeletal Muscle
p−value = 0
R = 0.41
Pancreas
p−value = 0
R = 0.6
Prostate
p−value = 2e−05
R = 0.41
Spleen
p−value = 1.6e−06
R = 0.46
Stomach
p−value = 0
R = 0.6
Testis
p−value = 0
R = 0.79
Whole Blood
p−value = 2.2e−16
R = 0.43
log2(MKRN1 TPM)
log2(RNF138 TPM)