Supplemental Materials

Molecular Biology of the Cell

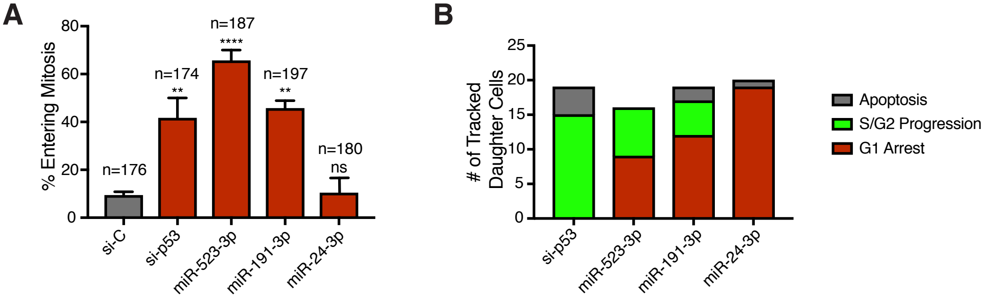
Vittoria et al.

Supplemental Figure 1: **(A)** Percent of tetraploids that enter mitosis, as judged by live-cell imaging, after treatment with the indicated siRNA/miRNA (n=number of cells tracked over three independent experiments, ** $p < 0.01$, **** $p < 0.0001$, ns = non-significant, one-way ANOVA).**(B)** The fates of daughter cells born from tetraploid mitoses when tetraploids were treated with the indicated siRNA/miRNA. **(C)** Table identifying all miRNA hits with an AAGUGC seed motif (red text) and the signaling pathways each miRNA hit modulates. **(D)** Western blot analysis of RPE-1 cells, transfected with the indicated siRNA/miRNA and serum starved for 24 h (left) prior to 1 h restimulation with 5% fetal bovine serum (right). **(E)**Quantification of (D).

Supplemental Figure 2: **(A)** Western blot analysis of RPE-1 cells, transfected with the indicated siRNA/miRNA after treatment with 100 ng/mL doxorubicin for 4 h. miRNAs found to reduce p21 levels more than 40% relative to controls are colored red. **(B)** Quantification of (A). p21 levels are normalized to doxorubicin treated miR-NC cells (n=2, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, two-tailed t test).**(C, D)**Western blot analysis of tetraploid RPE-1 cells treated with the indicated siRNA/miRNA for 48 h (top), with corresponding quantitation (bottom) (n=2, two-tailed t test).**(E)** Relative localization of YAP in representative images of tetraploid RPE-1 cells following transfection with the indicated siRNA/miRNA (bottom). Plot depicts the normalized ratio of YAP immunofluorescence intensity in the nucleus:cytoplasm (N/C) performed in technical duplicate (n >100 cells/condition, two-tailed t test).

Supplemental Figure 3: **(A)**Representative fixed immunofluorescence images of YAP (green) in RPE-1 cells transfected with the indicated siRNA/miRNA for 48 h.

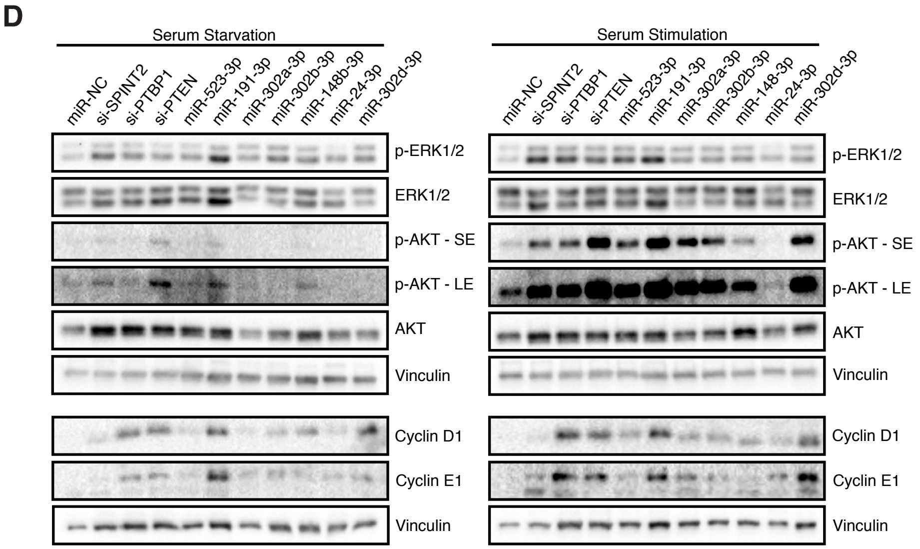
(B) Representative fixed images of YAP in HEK293A cells transfected with the indicated siRNA/miRNA for 48 h(n = 3,>200 cells/condition)**(C)**Quantification of the normalized ratio of YAP immunofluorescence intensity in the nucleus:cytoplasm from (B) (3 independent experiments, n > 200 cells/condition, **** $p \leq 0.0001$, one-way ANOVA). **(D)**Western blot analysis of RPE-1 cells transfected with either negative control or miR-24-3p after 48 h.**(E)**Heat map of top 50 most downregulated genes, according to t-test, in RPE-1 cells overexpressing miR-24-3p with*NF2* highlighted in green. Created using Morpheus matrix visualization software by the Broad Institute (https://software.broadinstitute.org/morpheus/).

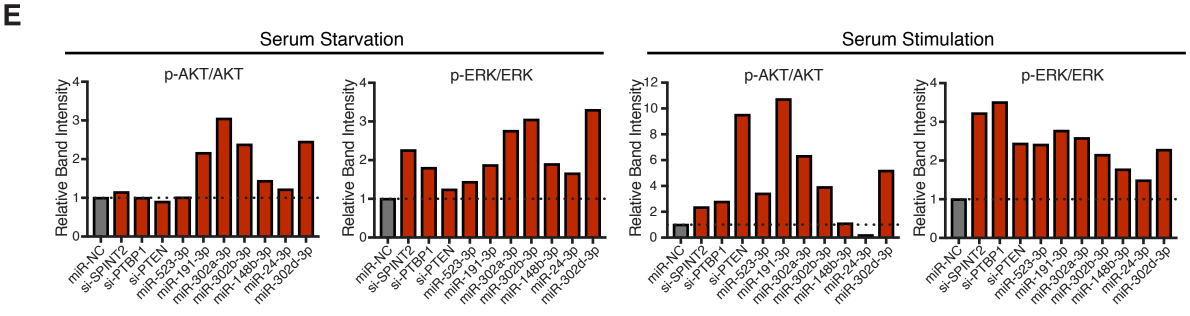


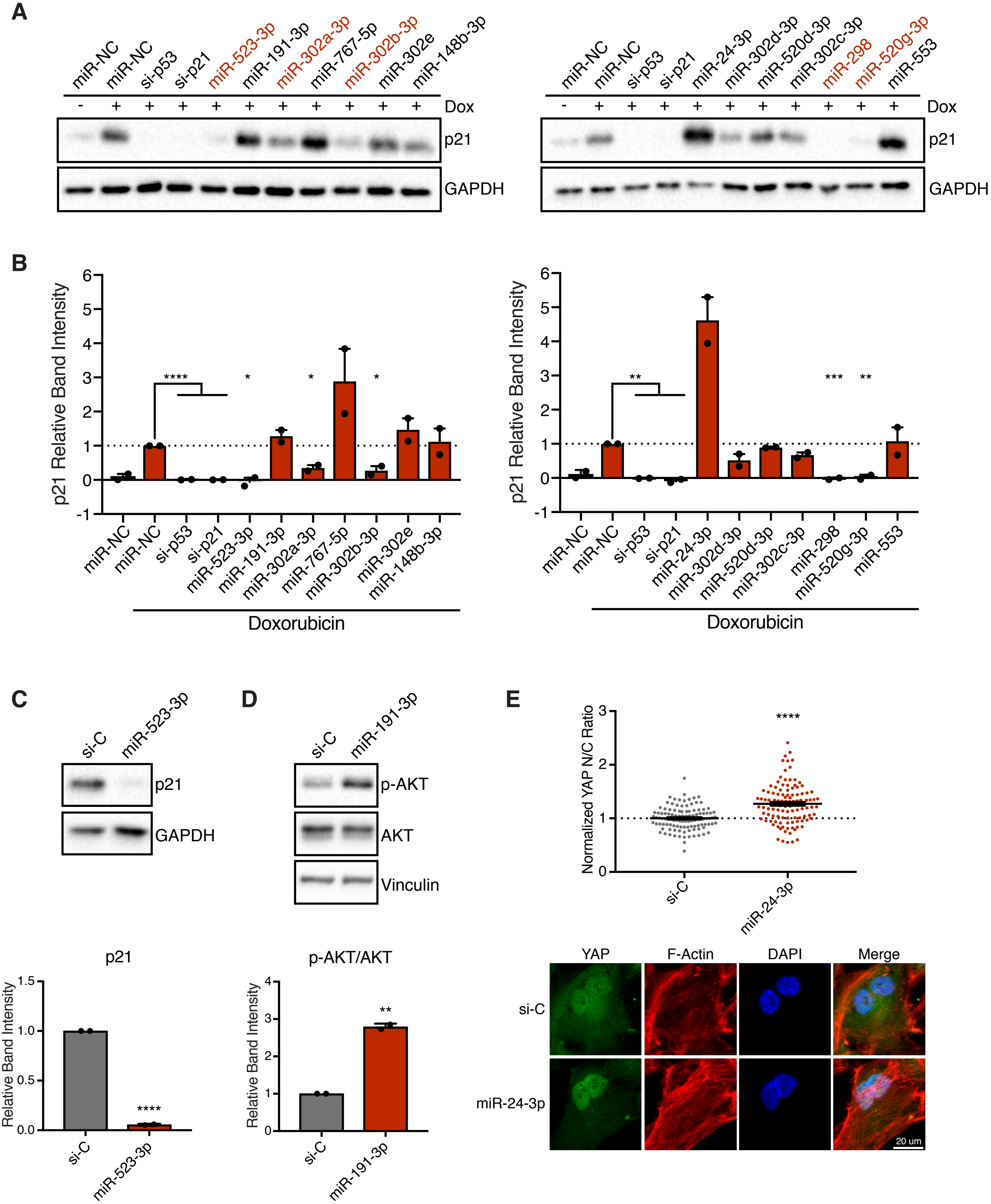
C

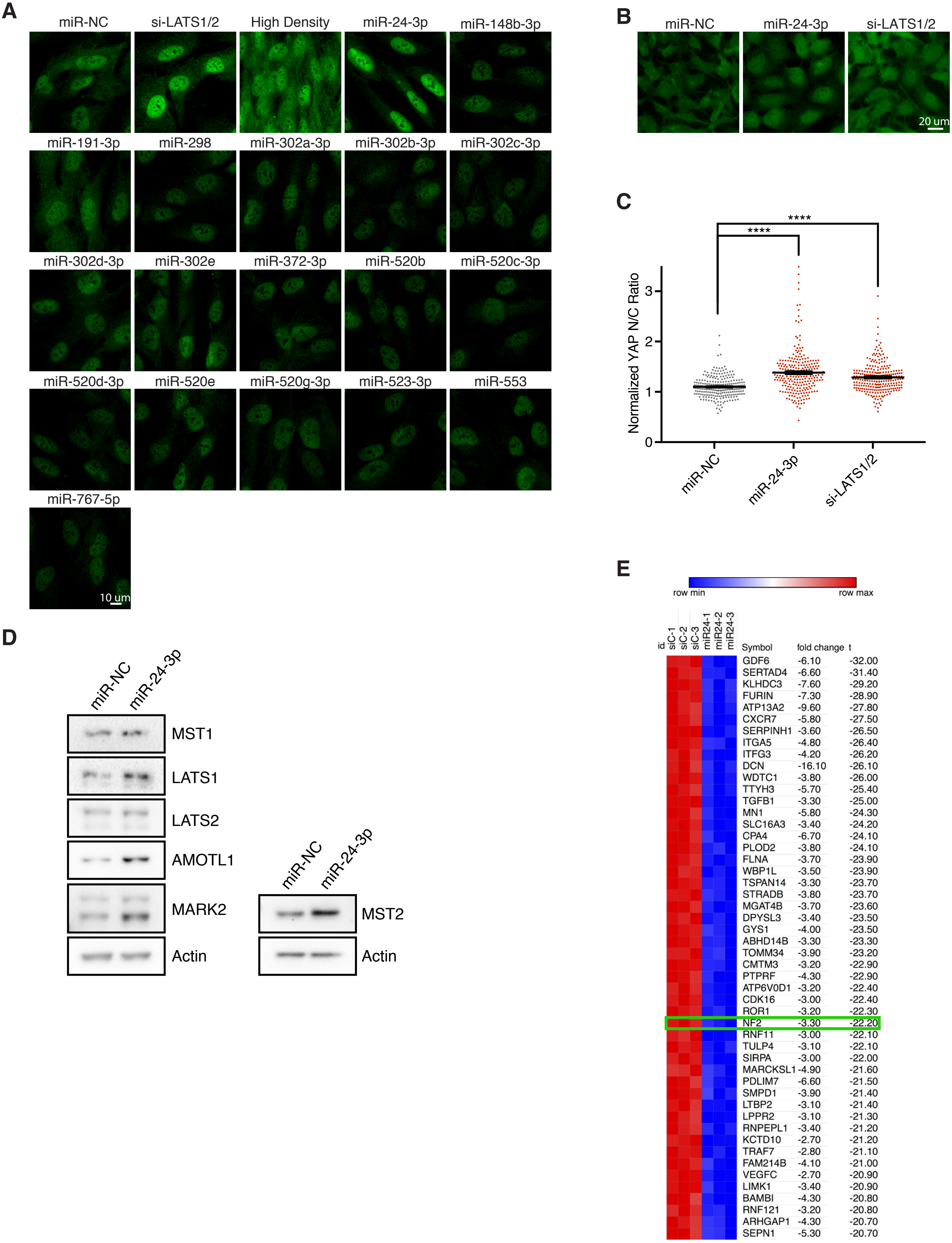
Ambion ID	Human ID	Sequence	Activates Mitogenic	Inactivates p21	Hippo
hsa-miR-523	hsa-miR-523-3p	GAACGCGCUUCCCUAUAGAGGGU	✓	✓	-
hsa-miR-191*	hsa-miR-191-3p	GCUGCGCUUGGAUUUCGUCCCC	✓	-	-
hsa-miR-372	hsa-miR-372-3p	AAAGUGCUGCGACAUUUGAGCGU	NT	NT	NT
mmu-miR-302a	hsa-miR-302a-3p	UAAGUGCUUCCAUGUUUUGGUGA	✓	✓	-
hsa-miR-767-5p	hsa-miR-767-5p	UGCACCAUGGUUGUCUGAGCAUG	NT	-	NT
hsa-miR-520c-3p	hsa-miR-520c-3p	AAAGUGCUUCCUUUUAGAGGGU	NT	NT	-
hsa-miR-302b	hsa-miR-302b-3p	UAAGUGCUUCCAUGUUUUAGUAG	✓	✓	-
hsa-miR-302e	hsa-miR-302e	UAAGUGCUUCCAUGCUU	NT	-	-
xtr-miR-148b	hsa-miR-148b-3p	UCAGUGCAUCACAGAACUUUGU	-	-	-
bta-miR-24	hsa-miR-24-3p	UGGCUCAGUUCAGCAGGAACAG	-	-	✓
hsa-miR-520b	hsa-miR-520b	AAAGUGCUUCCUUUUAGAGGG	NT	NT	-
hsa-miR-520a-3p	hsa-miR-520a-3p	AAAGUGCUUCCCUUUGGACUGU	NT	NT	NT
hsa-miR-302d	hsa-miR-302d-3p	UAAGUGCUUCCAUGUUUGAGUGU	✓	-	-
hsa-miR-520e	hsa-miR-520e	AAAGUGCUUCCUUUUUGAGGG	NT	NT	-
hsa-miR-520d-3p	hsa-miR-520d-3p	AAAGUGCUUCUCUUUGGUGGGU	NT	-	-
gga-miR-302c	hsa-miR-302c-3p	UAAGUGCUUCCAUGUUUCAGUGG	NT	-	-
hsa-miR-32	hsa-miR-32-5p	UAUUGCACAUUACUAAGUUGCA	NT	NT	NT
hsa-miR-298	hsa-miR-298	AGCAGAAGCAGGGAGGUUCUCCCA	NT	✓	-
rno-miR-874	hsa-miR-874-3p	CUGCCCUGGCCCGAGGGACCGA	NT	NT	NT
mmu-miR-200a*	hsa-miR-200a-5p	CAUCUUACCGGACAGUGCUGGA	NT	NT	NT
hsa-miR-520g	hsa-miR-520g-3p	ACAAAGUGCUUCCCUUUAGAGUGU	NT	✓	-
hsa-miR-553	hsa-miR-553	AAAACGGUGAGAUUUUGUUUU	NT	-	✓
hsa-miR-517b	hsa-miR-517b	UCGUGCAUCCCUUUAGAGUGUU	NT	NT	NT

🟩 ✓ Modulates Pathway
🟥 - No Appreciable Modulation
NT Not Tested






A
miR-NC
miR-NC
si-p53
si-p21
miR-523-3p
miR-191-3p
miR-302a-3p
miR-767-5p
miR-302b-3p
miR-302e
miR-148b-3p
- + + + + + + + + + +
Dox
p21
GAPDH
miR-NC
miR-NC
si-p53
si-p21
miR-24-3p
miR-302d-3p
miR-520d-3p
miR-302c-3p
miR-298
miR-520g-3p
miR-553
- + + + + + + + + + +
Dox
p21
GAPDH
B
p21 Relative Band Intensity
6
5
4
3
2
1
0
-1
****
*
*
*
miR-NC
miR-NC
si-p53
si-p21
miR-523-3p
miR-191-3p
miR-302a-3p
miR-767-5p
miR-302b-3p
miR-302e
miR-148b-3p
Doxorubicin
p21 Relative Band Intensity
6
5
4
3
2
1
0
-1
**
***
**
miR-NC
miR-NC
si-p53
si-p21
miR-24-3p
miR-302d-3p
miR-520d-3p
miR-302c-3p
miR-298
miR-520g-3p
miR-553
Doxorubicin
C
si-C
miR-523-3p
p21
GAPDH
p21
Relative Band Intensity
1.5
1.0
0.5
0.0
****
si-C
miR-523-3p
D
si-C
miR-191-3p
p-AKT
AKT
Vinculin
p-AKT/AKT
Relative Band Intensity
4
3
2
1
0
**
si-C
miR-191-3p
E
Normalized YAP N/C Ratio
3
2
1
0
****
si-C
miR-24-3p
YAP
F-Actin
DAPI
Merge
si-C
miR-24-3p
20 um



Symbol	fold change	t
GDF6	-6.10	-32.00
SERTAD4	-6.60	-31.40
KLHDC3	-7.60	-29.20
FURIN	-7.30	-28.90
ATP13A2	-9.60	-27.80
CXCR7	-5.80	-27.50
SERPINH1	-3.60	-26.50
ITGA5	-4.80	-26.40
ITFG3	-4.20	-26.20
DCN	-16.10	-26.10
WDTC1	-3.80	-26.00
TTYH3	-5.70	-25.40
TGFB1	-3.30	-25.00
MN1	-5.80	-24.30
SLC16A3	-3.40	-24.20
CPA4	-6.70	-24.10
PLOD2	-3.80	-24.10
FLNA	-3.70	-23.90
WBP1L	-3.50	-23.90
TSPAN14	-3.30	-23.70
STRADB	-3.80	-23.70
MGAT4B	-3.70	-23.60
DPYSL3	-3.40	-23.50
GYS1	-4.00	-23.50
ABHD14B	-3.30	-23.30
TOMM34	-3.90	-23.20
CMTM3	-3.20	-22.90
PTPRF	-4.30	-22.90
ATP6V0D1	-3.20	-22.40
CDK16	-3.00	-22.40
ROR1	-3.20	-22.30
NF2	-3.30	-22.20
RNF11	-3.00	-22.10
TULP4	-3.10	-22.10
SIRPA	-3.00	-22.00
MARCKSL1	-4.90	-21.60
PDLIM7	-6.60	-21.50
SMPD1	-3.90	-21.40
LTBP2	-3.10	-21.40
LPPR2	-3.10	-21.30
RNPEPL1	-3.40	-21.20
KCTD10	-2.70	-21.20
TRAF7	-2.80	-21.10
FAM214B	-4.10	-21.00
VEGFC	-2.70	-20.90
LIMK1	-3.40	-20.90
BAMBI	-4.30	-20.80
RNF121	-3.20	-20.80
ARHGAP1	-4.30	-20.70
SEPN1	-5.30	-20.70