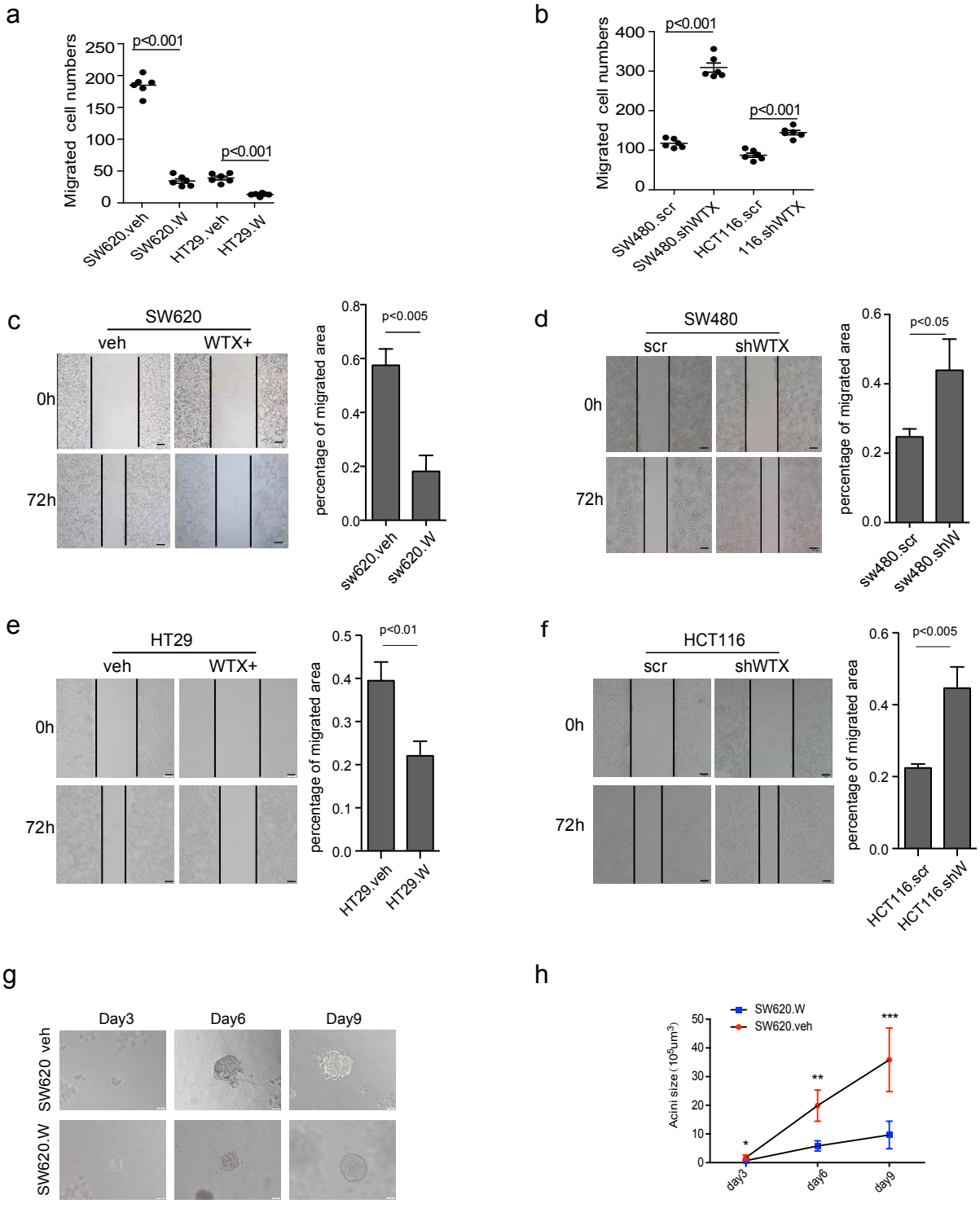
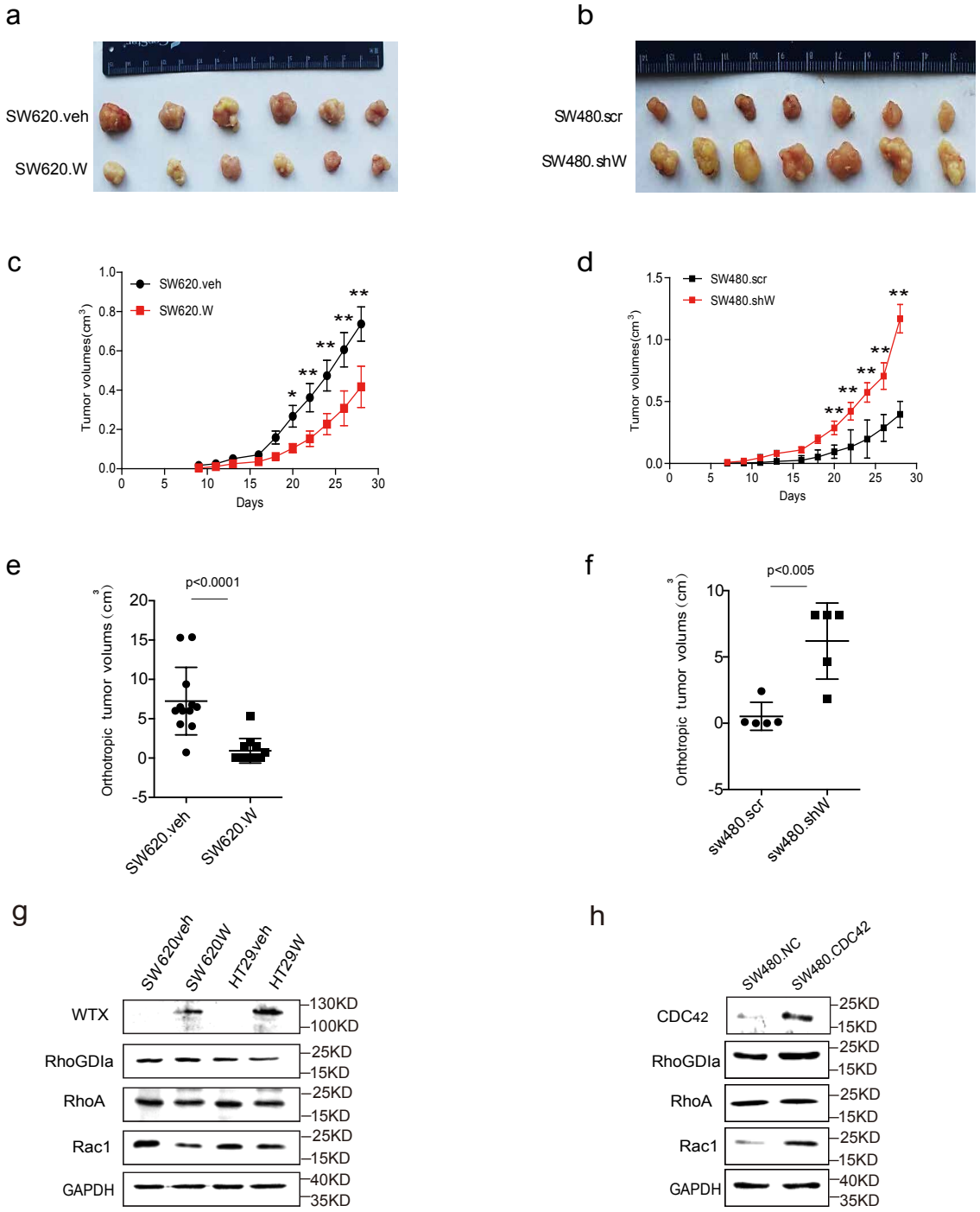
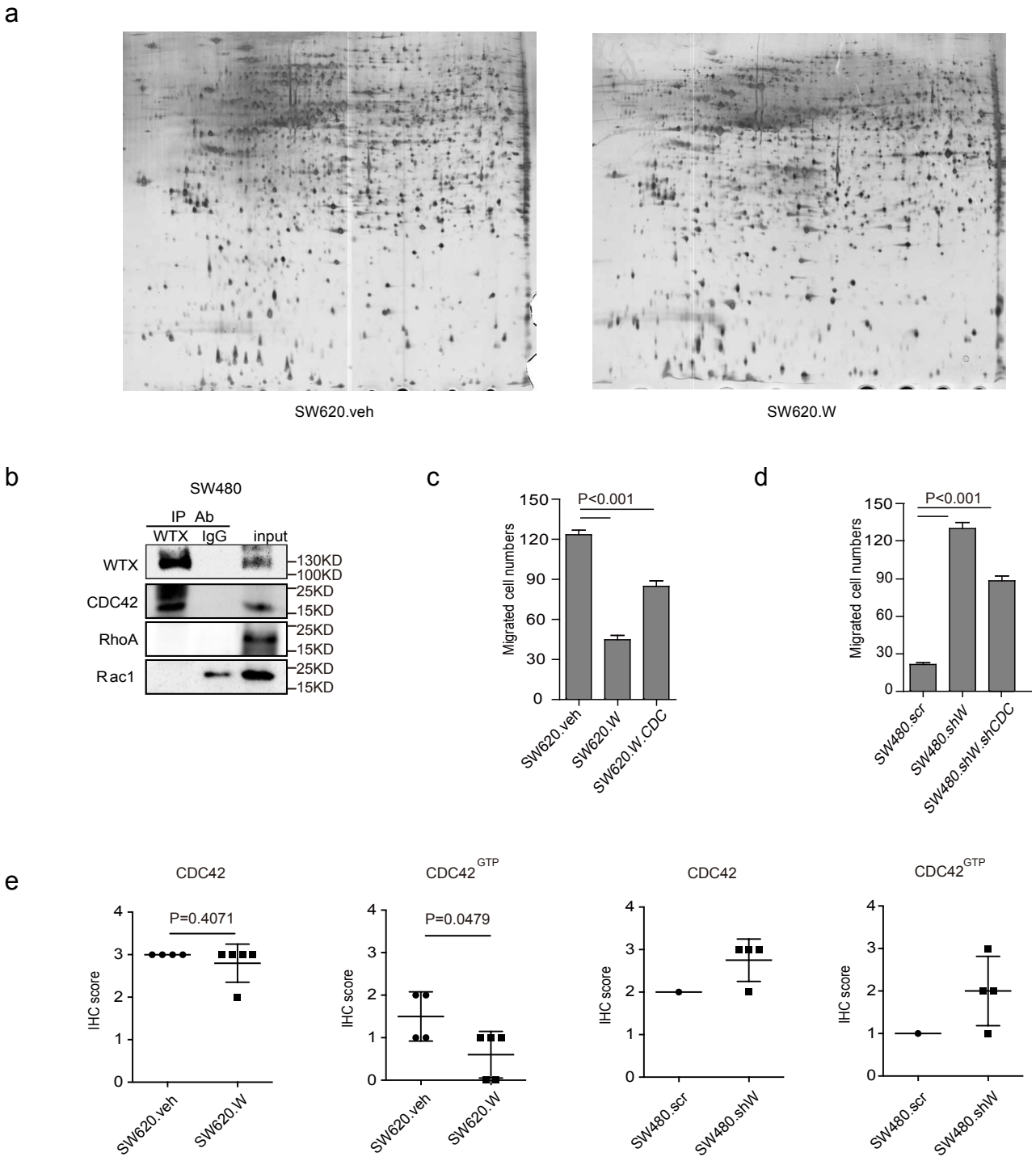


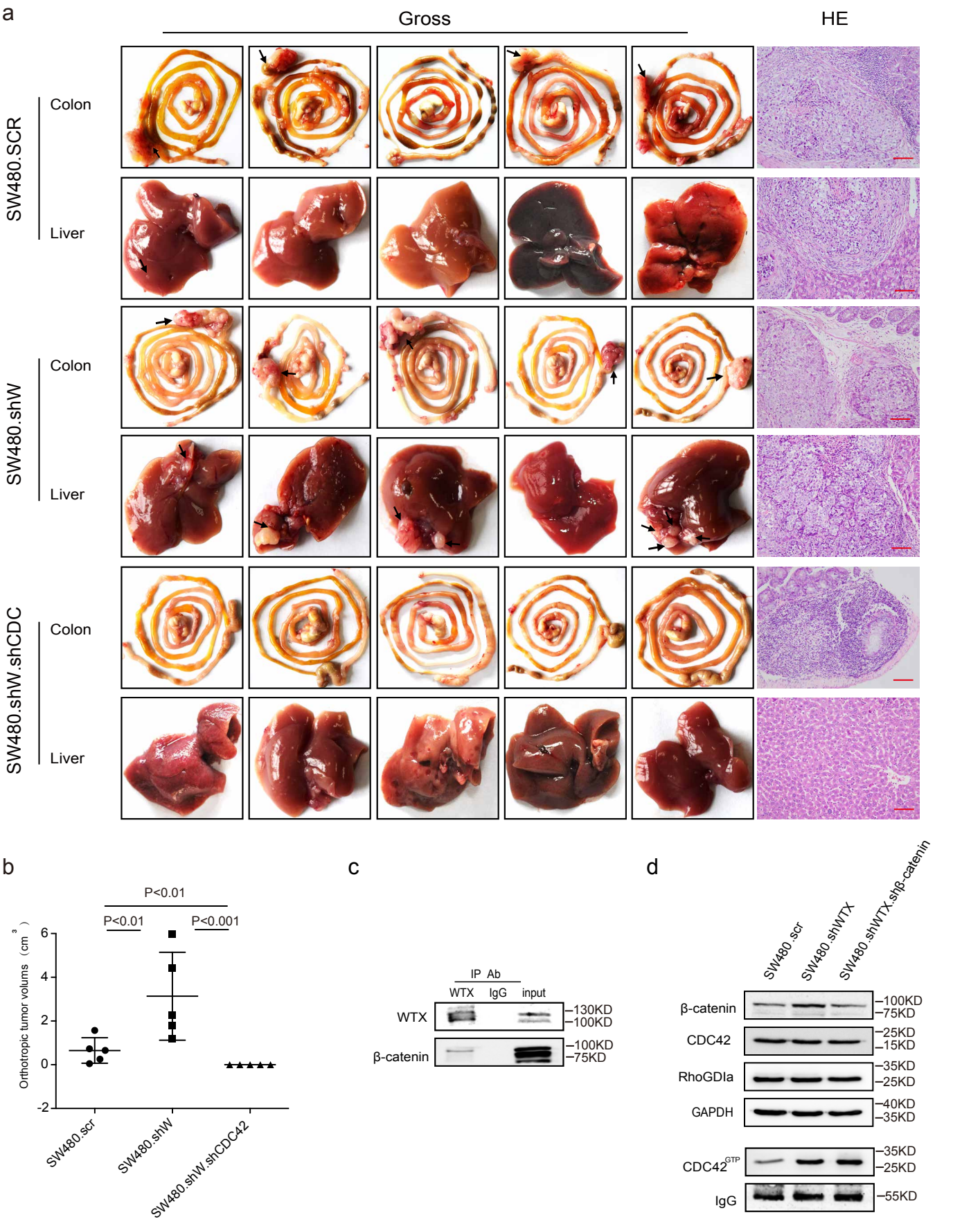

**Supplementary Figure 1. WTX inhibits in vitro cell migration and tumor proliferation.**
**a-b**, Quantification of migrated cells in the indicated cell lines. The migrated cell numbers are counted from three independent experiments respectively. **c-f**, Wound healing assay analyzes the indicated cells. Scale bars, 100 μm. **g-h**, 3D tumor growth observation **(g)** and growth curve **(h)** of the control and SW620.W cells.Scale bars, 50 μm. ***$p$<0.001, **$p$<0.01, *$p$<0.05. Mean±SEM, Two-tailed Student's t-test, 3 biological replicates, with 3 technical replicates each.




**Supplementary Figure 2. WTX inhibits *in vivo* tumor proliferation.**
**a-b**, Gross observation of the subcutaneous xenograft tumors of the indicated cell lines.Error bars represent mean ± SEM. Two-sided unpaired t-test. **c-d**, The tumor growth curves of the indicated subcutaneous xenograft tumors. ***$p$<0.001, **$p$<0.01, *$p$<0.05, mean±SEM, Two-tailed Student's t-test, 3 biological replicates, with 3 technical replicates each. **e-f**, Tumor volumes quantify to the control and indicated CRC orthotopic tumors, match to Figure 1n~1o. ***$p$<0.0001, mean±SEM, Two-tailed Student's t-test. **g**, IB analysis of WTX, RhoGDIa, RhoA, and Rac1 expression in indicated WTX overexpression and control cell lines. **h**, IB analysis of CDC42, RhoGDIa, RhoA, and Rac1 expression in indicated CDC42 overexpression and control cell lines.



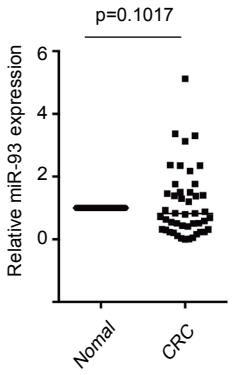

**Supplementary Figure 3. WTX downstream proteins screen and CDC42 activity analysis.**
**a,**2-DGE analyzes the protein patterns of SW620.veh and SW620.W cell lines. **b**, CO-IP analyzes the interaction of WTX and Small GTPases family members. **c-d**, Quantification of transwell experiments of indicated cells. ***p*<0.001, mean±SEM, Two-tailed Student's t-test, 3 biological replicates, with 3 technical replicates each. Two-sided unpaired t-test. **e**, Statistic analysis of IHC staining scores of CDC42 and $CDC42^{GTP}$ expression in CRC orthotopic tumors. **p*<0.05, mean±SEM, Two-tailed Student's t-test.




**Supplementary Figure 4. Establishing orthotropic xenograft CRC models to verify the effect of CDC42 in WTX regulated pathway.**

**a,** The gross and HE of orthotropic xenograft CRC models of SW480.NC, SW480.shW and SW480.shW.shCDC. **b**, Statistical analysis of orthotropic xenograft CRC models of SW480.NC, SW480.shW and SW480.shW.shCDC. **p*<0.05, mean±SEM, *n*=5, Two-tailed Student's t-test. **c**, CO-IP analyzes WTX binding with β-catenin in indicated cells. **d**, IB analyzes CDC42,CDC42$^{GTP}$, and RhoGDIa expression changings in comparing WTX knockdown, WTX and β-catenin double- knockdown cells.

a

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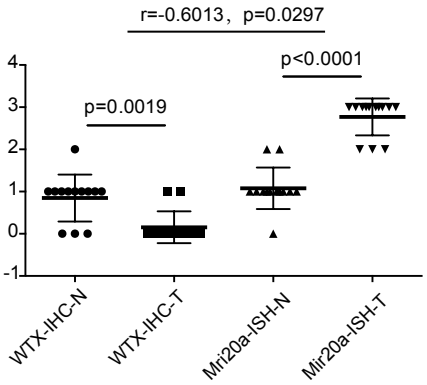


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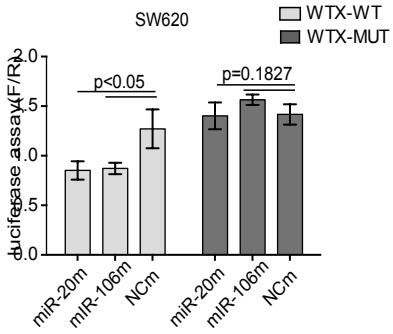


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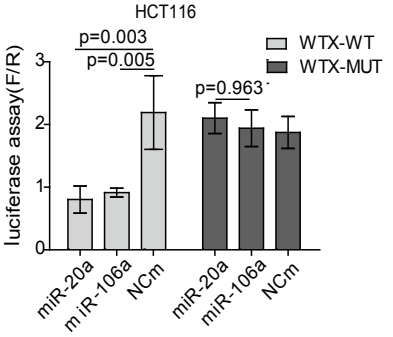


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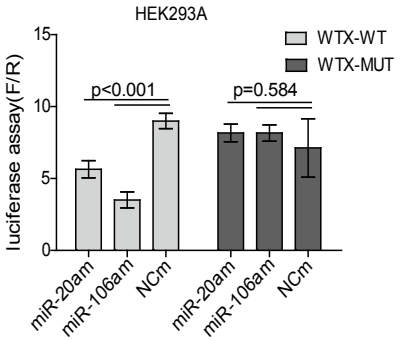


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The blast of WTX-3'UTR sequencing of pGL3-control-WTX-3'UTR plasmid with WTX gene .

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The blast of WTX-3'UTR-Mut sequencing of pGL3-mutant-WTX-3'UTR plasmid with WTX gene .

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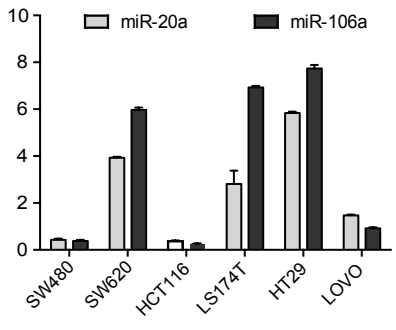
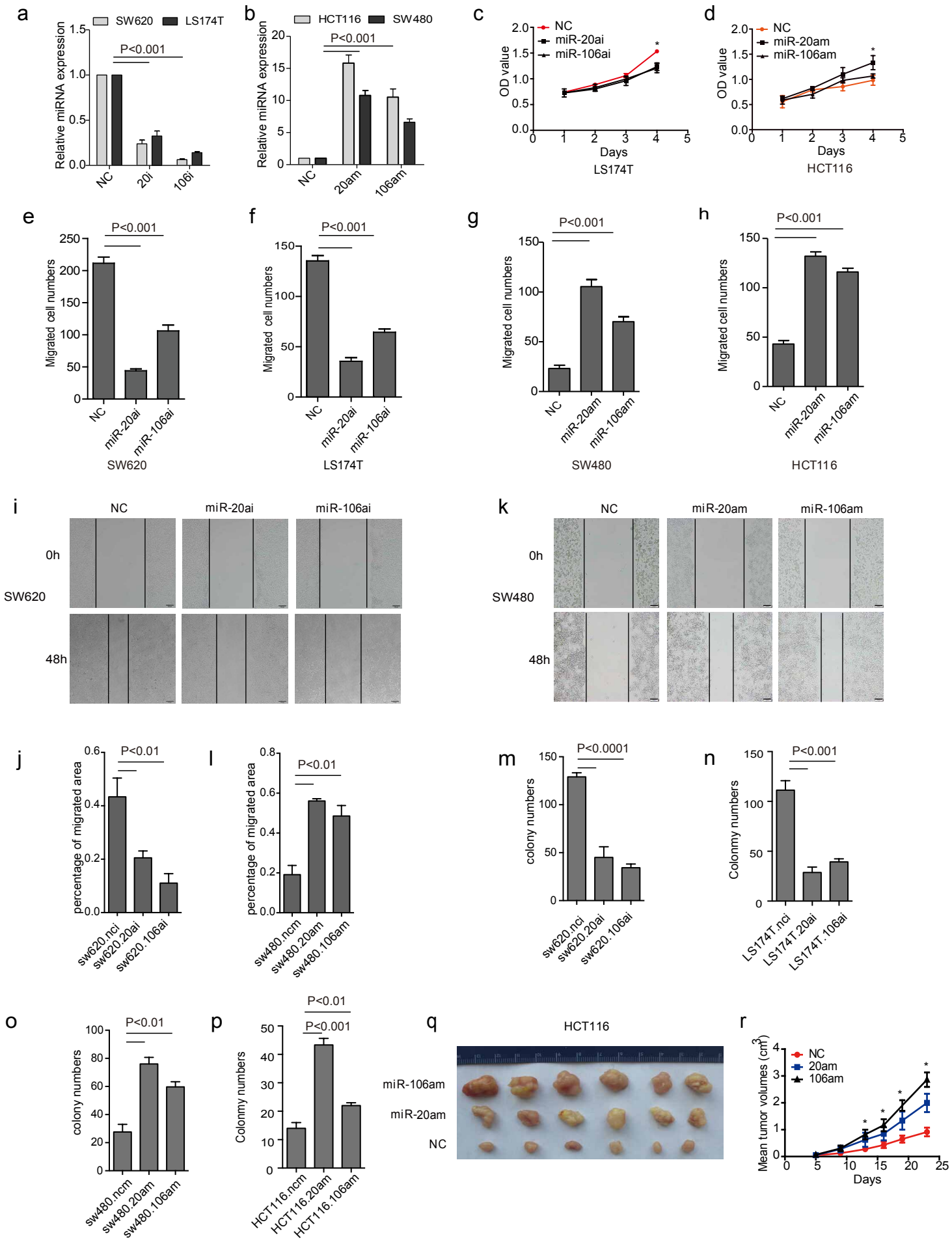


**Supplementary Figure 5. Screening out miRNAs which regulated WTX expression in CRC.**
**a,** The qRT-PCR analyzes miR-93 expression in colorectal cancer and adjacent normal colorectal mucosa tissues. $p$=0.1017. **b**, Statistics analyzes the correlations of the expressions of miR-20a and WTX. **$p$<0.01, n=13. **c-e**, Luciferase activity assay verify the binding of miR-20a/106a with WTX 3'-UTR in SW620(*$p$<0.05), CT116(**$p$<0.01), and HEK293A(***$p$<0.001) cells. **f**, Sequence alignment of pGL3-control-WTX-3'UTR plasmid luciferase vector. **g**, Sequence alignment of pGL3-mutant-WTX-3'UTR plasmid luciferase vector. **h**, The qRT-PCR analyzes miRNA-20a/106a expression level in CRC cell lines.Mean±SEM, Two-tailed Student's t-test, 3 biological replicates, with 3 technical replicates each.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SW620
LS174T
Relative miRNA expression
P<0.001
NC
20i
106i
b
HCT116
SW480
Relative miRNA expression
P<0.001
NC
20am
106am
c
NC
miR-20ai
miR-106ai
OD value
Days
LS174T
d
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miR-20am
miR-106am
OD value
Days
HCT116
e
P<0.001
Migrated cell numbers
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miR-20ai
miR-106ai
SW620
f
P<0.001
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miR-106ai
LS174T
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miR-106am
SW480
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miR-106ai
0h
SW620
48h
k
NC
miR-20am
miR-106am
0h
SW480
48h
j
P<0.01
percentage of migrated area
sw620.nci
sw620.20ai
sw620.106ai
l
P<0.01
percentage of migrated area
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sw480.106am
m
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Colonmy numbers
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o
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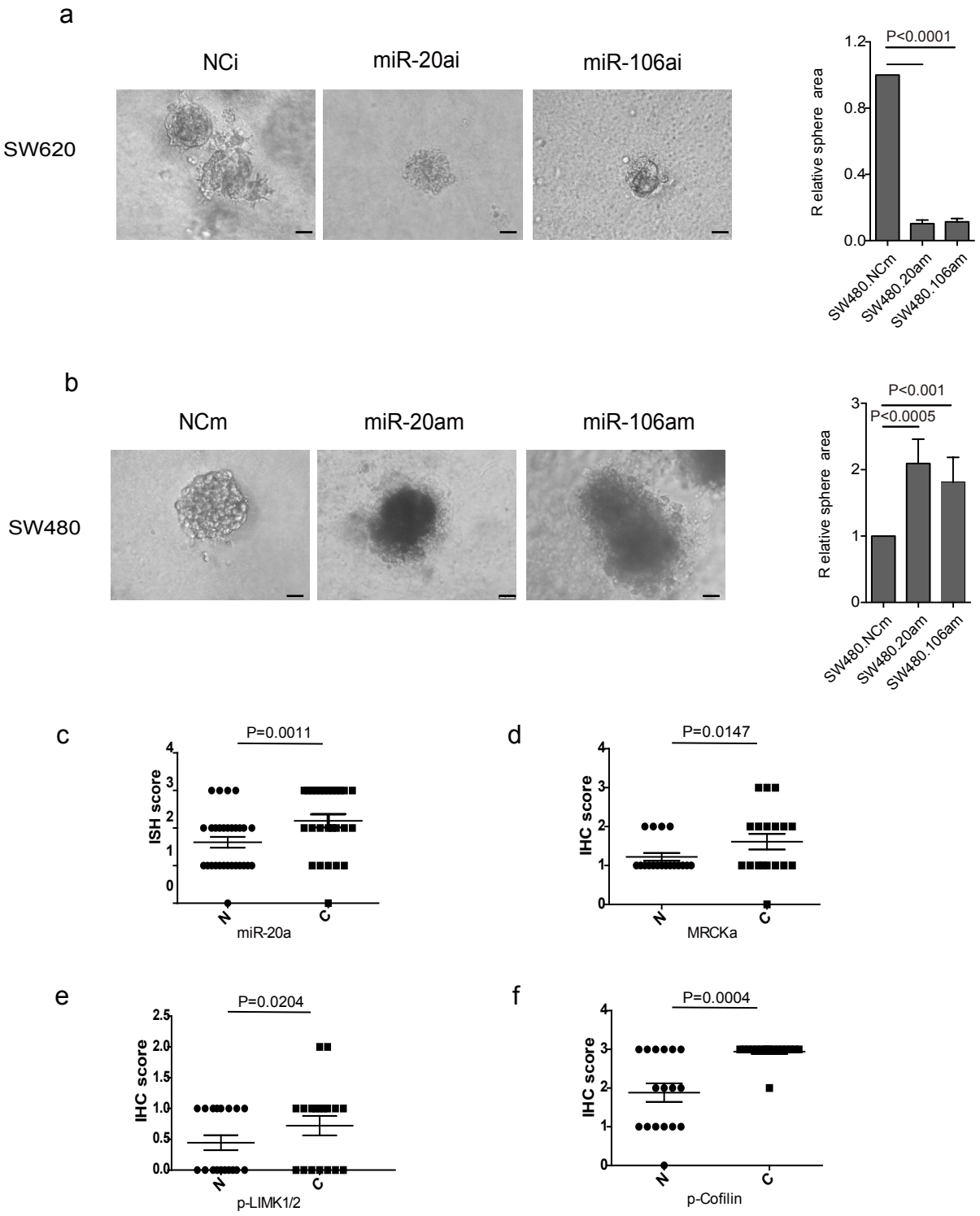
p
P<0.01
P<0.001
Colonmy numbers
HCT116.ncm
HCT116.20am
HCT116.106am
q
HCT116
miR-106am
miR-20am
NC
r
NC
20am
106am
Mean tumor volumes (cm3)
Days

**Supplementary Figure 6. MiR-20a/106a inhibits WTX expression and promotes CRC cell proliferation and migration.**

**a-b,** The qRT-PCR analyzes miRNA-20a/106a expression in the indicated cells. ***$p$<0.001,. **c-d**, CCK8 assays analyze cell proliferation in the indicated cells. *$p$<0.05. **e-h**, Quantifications of cell migration of the indicated cell lines. ***$p$<0.001. **i-l**, Wound healing assays and quantifications analyze the cell migrations of the indicated cells. **$p$<0.01. **m-p**, Quantifications analyze the colony formations of the indicated cell lines. ***$p$<0.001, **$p$<0.01. **q**, Gross observation of the subcutaneous xenograft tumor models of the indicated cell lines. **r**, Growth curves of the subcutaneous xenograft tumor models of the control and miR-20a/106a overexpression HCT116 cells. *$p$<0.05, mean±SEM, n=6 mice. Two-tailed Student's t-test. 3 biological replicates, with 3 technical replicates for the in vitro experiments.




**Supplementary Figure 7. 3D culture and statistical analysis to verify the function and mechanism of miRNAs.**

**a-b**, 3D culture analyze the invasion ability changings of miR-20ai/106ai**(a)**, miR-20am/106am **(b)** CRC cell lines. **c-f**, Statistic analysis of ISH and IHC staining score of miR-20a**(c)**, MRCKa**(d)**, p-LIMK1/2**(e)**, and p-Cofilin expression**(f)**. ***$p$<0.001, **$p$<0.01, mean±SEM, Two-tailed Student's t-test, 3 biological replicates, with 3 technical replicates each.

**WB#1**

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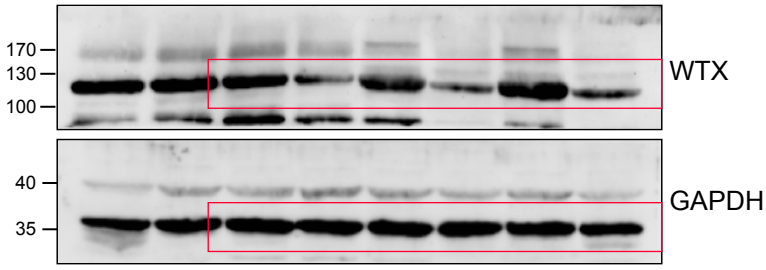


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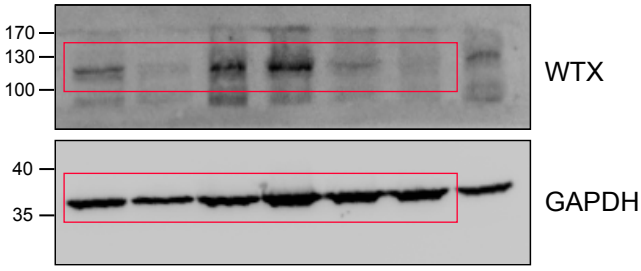


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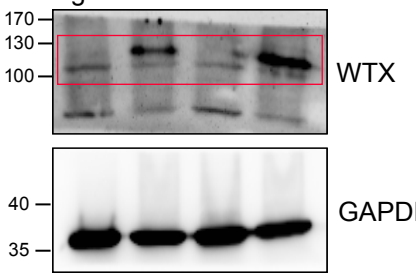


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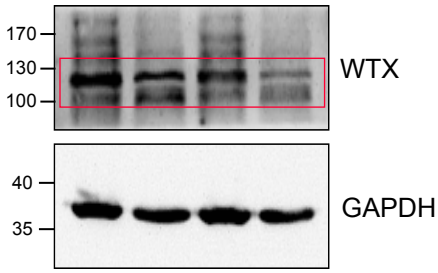


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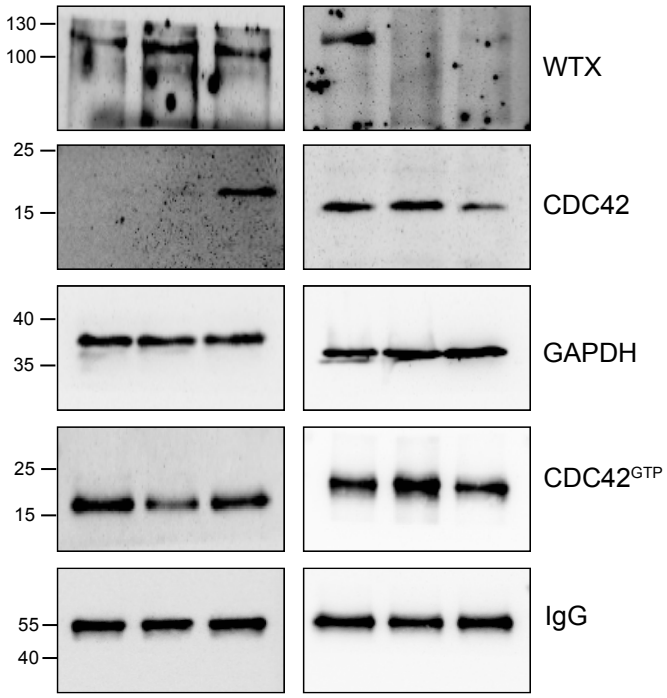


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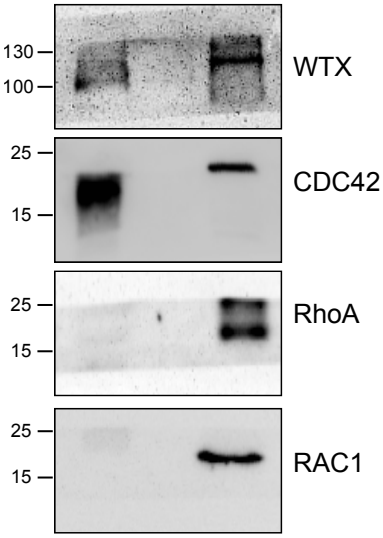


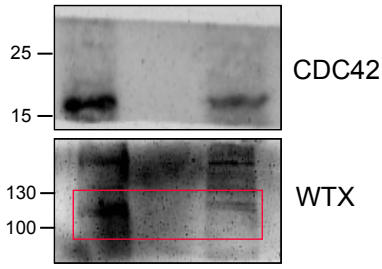


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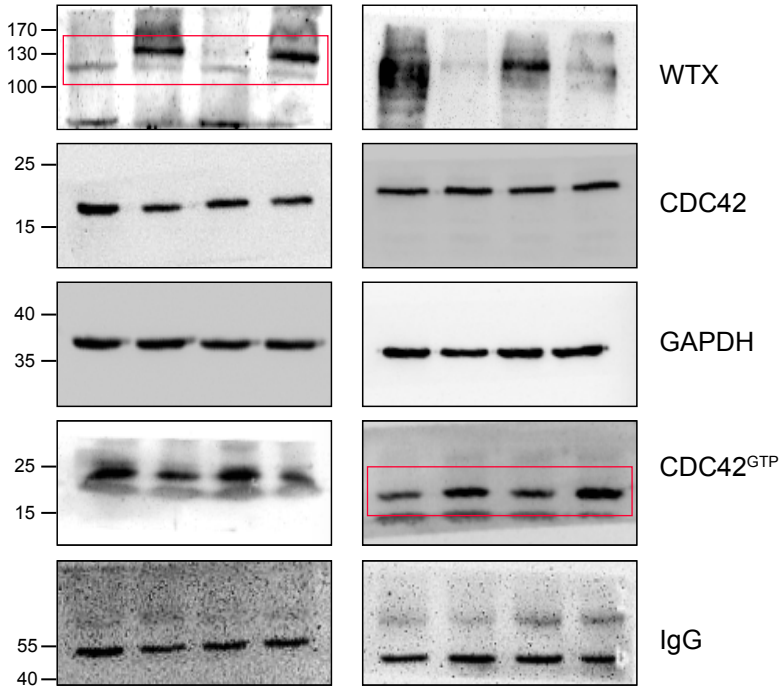


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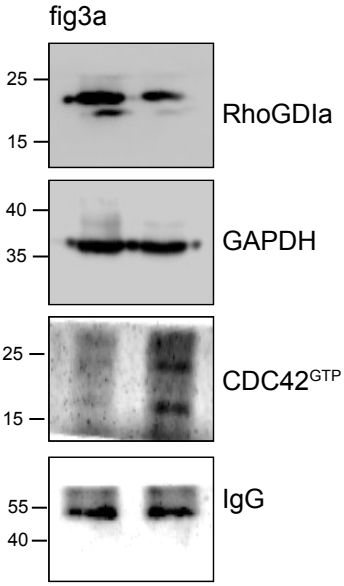

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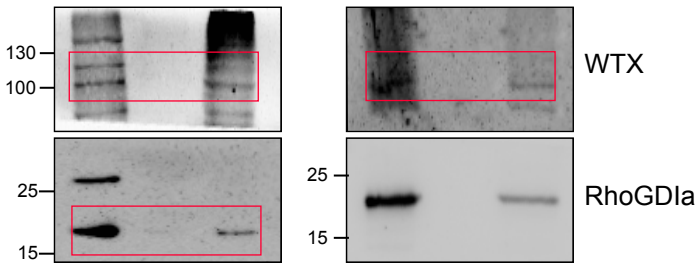


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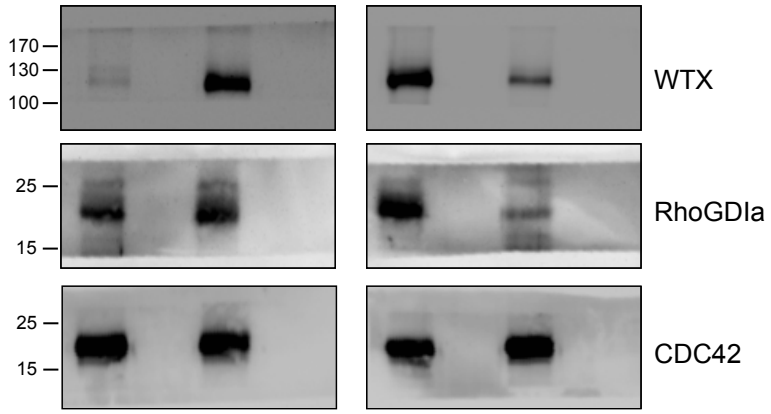


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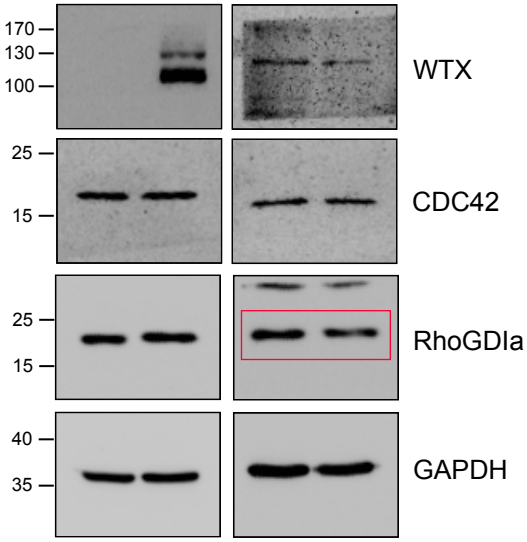


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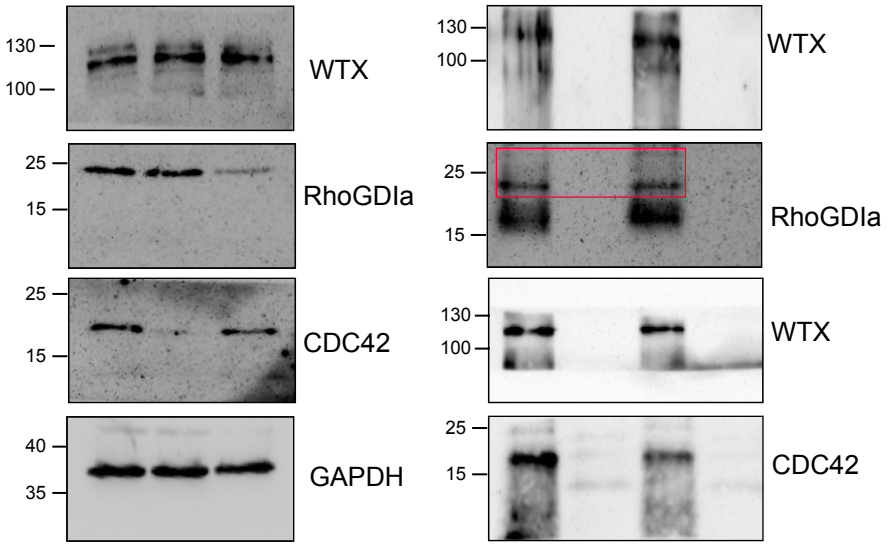


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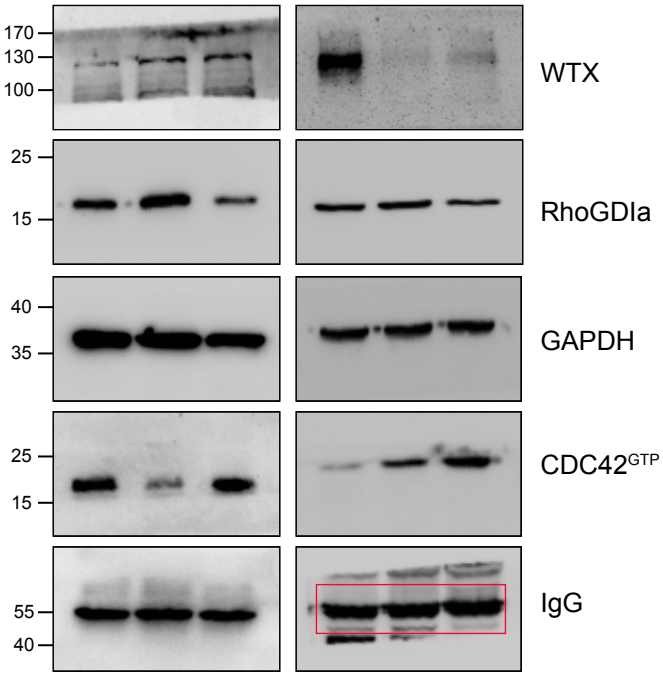


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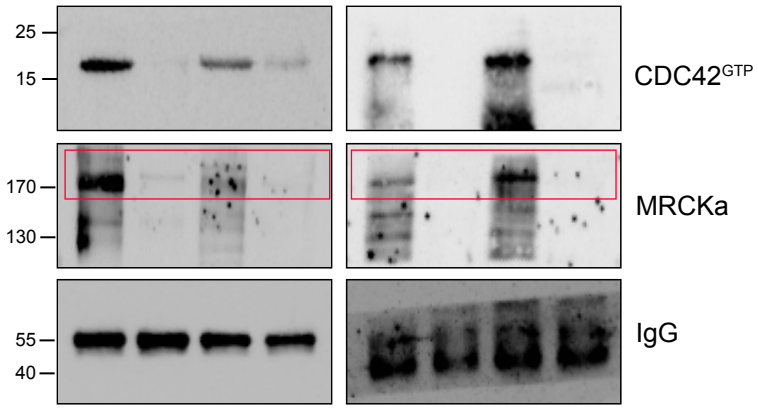

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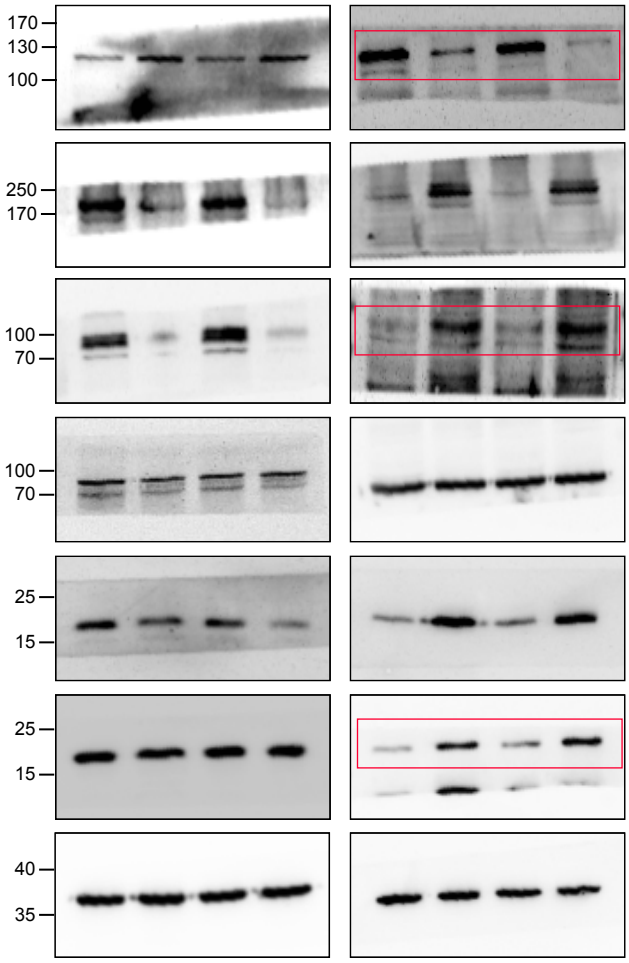


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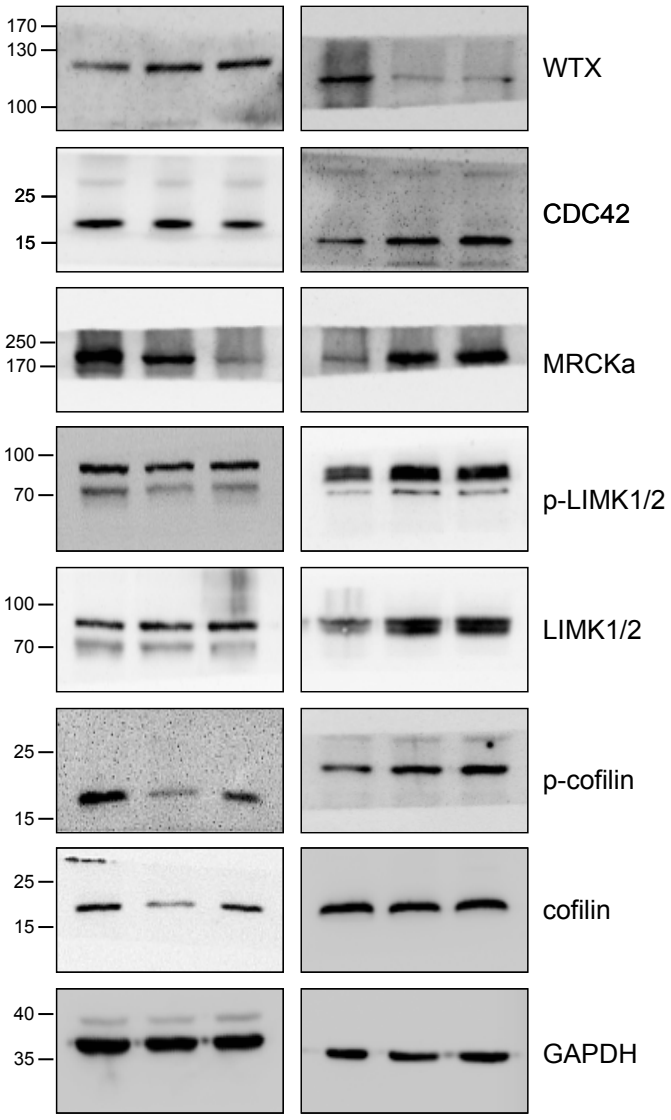


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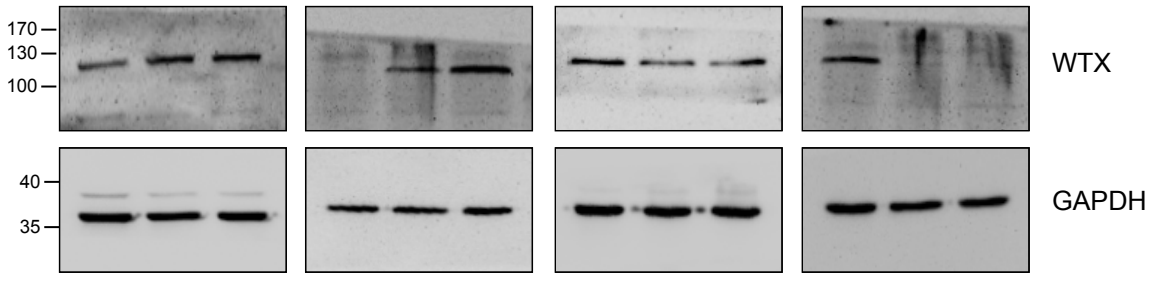


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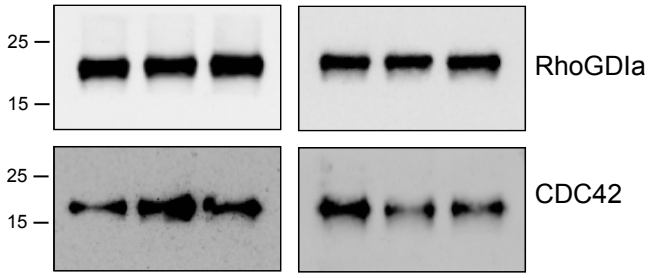


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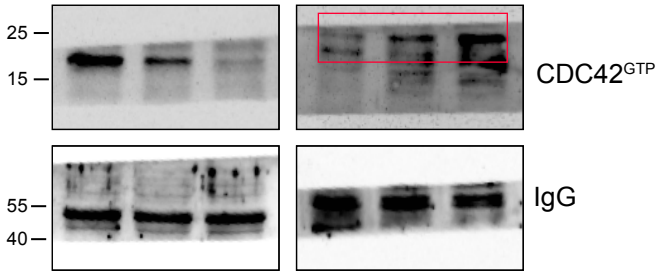

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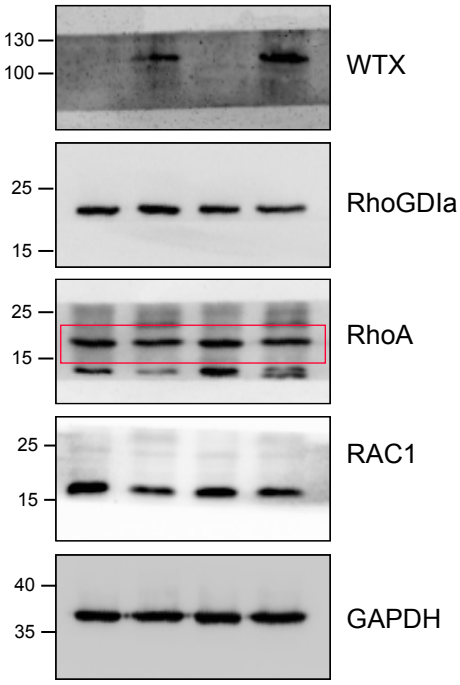


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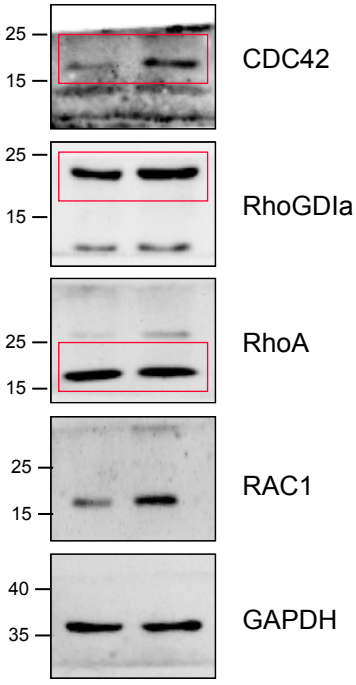


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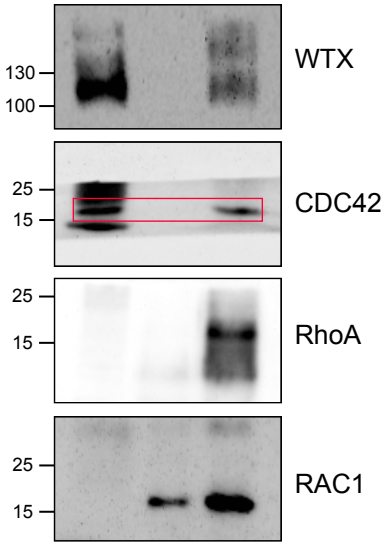
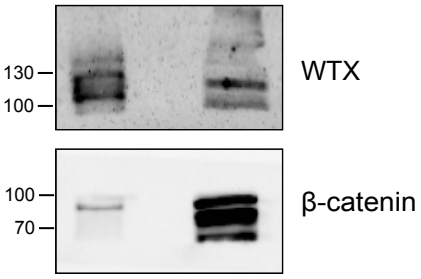
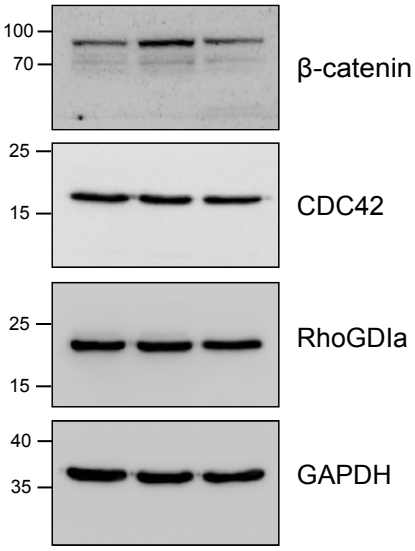
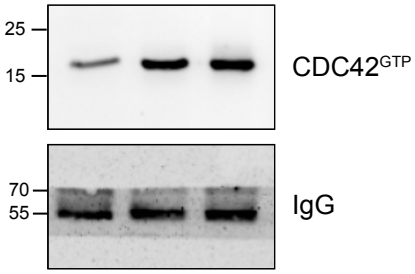



sf4c




sf4d



**Supplementary Table Legends**

Supplementary Table 1: WTX gene modified orthotopic xenograph CRC tumor models formation and liver metastasis analysis.

| | Total mice number | Orthotropic tumor N(%) | High tumor burden N(%) | Liver metastasis N(%) |
|---|---|---|---|---|
| SW620.veh | 11 | 11(100) | 7(63.6) | 7(63.6) |
| SW620.W | 11 | 5(45.5) | 0(0) | 0(0) |
| SW480.scr | 8 | 5(62.5) | 0(0) | 0(0) |
| SW480.shW | 8 | 8(100) | 8(100) | 6(75) |

High tumor burden: The tumor mass is more than 1cm in diameter.

**Supplementary Table 2 : The primers sequences for WTX gene CDS amplification.**

| | | |
|---|---|---|
| WTX | Primer F | 5'-ATCCGGGAATTCGCCACCATGGAGACCCAAAAGGATGAAG-3' |
| | Primer R | 5'-ATCATCGGATCCCGCTTGGCTAGGTTTCCATTCATG-3' |

**Supplementary Table 3: List of primers used in qRT-PCR.**

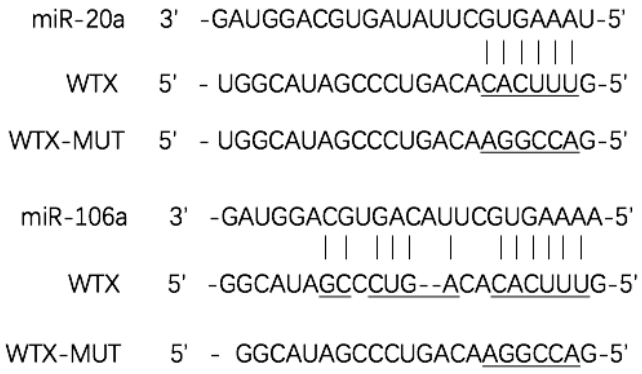
| Primers | Sequence |
|---|---|
| WTX | F, 5’-GACCCAAAAGGATGAAGCT-3’ |
| | R, 5’-CCCCTCCAAAGAAACTAGGC-3’ |
| GAPDH | F, 5’-TGAAGGTCGGAGTCAACGGA-3’ |
| | R, 5’-CCATTGATGACAAGCTTCCCG-3’ |
| WTX 3’UTR | F, 5’-CATCTAGA AACACCACCACTCAAGGG-3’ |

| | |
|---|---|
| | R, 5’-TAAAGGCCGGCCAAGGCTGTCCAAACCATG-3’ |

**Supplementary Table 4: List of shRNAs coding sequences**

| shRNAs | Coding sequence |
|---|---|
| shWTX | TGCCCTATATGAGTTCTAT |
| shCDC42 | AAAGACUCCUUUCUUGCUUGUdTdT |

Supplementary Table 5: miR-20a and miR-106a binding sites on WTX gene predicating.




**Supplementary Table 6: MiR-20a/106a modified orthotopic xenograph CRC tumor formation and liver metastasis analysis.**

| | Total mice number | Orthotropic tumor N(%) | High tumor burden N(%) | Liver metastasis N(%) |
|---|---|---|---|---|
| SW620.NCi | 6 | 6(100) | 6(100) | 4(66.7) |
| SW620-20ai | 6 | 0(0) | 0(0) | 0(0) |
| SW620-106ai | 6 | 0(0) | 0(0) | 0(0) |
| SW480.scr | 5 | 4(80) | 3(60) | 1(20) |
| SW480.shW | 5 | 5(100) | 5(100) | 4(80) |
| SW480.shW.shCDC | 5 | 0(0) | 0(0) | 0(0) |

High tumor burden: The tumor mass is more than 1cm in diameter

**Supplementary Table 7: List of antibodies used in IHC, IF and western blot experiments:**

| Antigen | Catalog number | Source | Application |
|---|---|---|---|
| WTX | 5854s | Cell signaling technology | WB |
| WTX | MAB7374 | R&D systems | WB |
| RhoA | 12441-H07B | Sino Biological Inc. | WB, ELISA, IP |
| RAC1 | sc-95 | Santa Cruz Biotechnology | WB, ELISA, IP, IHC-P |
| CDC42 | A1188 | Abclonal | WB, IHC |
| $CDC42^{GTP}$ | 26905 | BIO-NEWEAST | IP, |
| RhoGDIα | 2977-1 | Eptiomics | WB,IHC,ICC |
| MRCKa | WH0008476M1 | Sigma | WB、IP、IHC-P、IF |
| P-LIMK1/2(T508/505) | ab131341 | Abcam | WB, IHC-p,ICC/IF |
| LIMK1/2 | ab39641 | Abcam | ICC/IF, WB, ELISA |
| Cofilin | ab42824 | Abcam | ICC/IF, WB, IHC-P |
| p-Cofilin(Ser3) | 3313s | Cell signaling technology | WB, IF |
| Ki-67 | 9449 | Cell signaling technology | IHC,IF,F |
| RhoGD1a | ab133248 | Eptiomics | WB, IHC-P, ICC |
| Flag | 66008-2-Ig | ProteinTech | ELISA, WB, IP, IF |
| β-actin | PR-0255 | ZSGB-BIO | WB, ICC/IF, IHC, IP |
| GAPDH | TA336621 | ZSGB-BIO | WB, ICC/IF, IHC, IP |