Expanded View Figures

Figure EV1. WDR63 suppresses cell migration and invasion.

A, B A549 cells expressing control shRNA, CCDC90B shRNA, NTPCR shRNA, ZNF561 shRNA, EPS8L2 shRNA, or MAST4 shRNA were subjected to wound-healing assay (A). The knockdown efficiency of the indicated proteins was analyzed by real-time RT–PCR (B). The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). **P < 0.01; ***P < 0.001; N.S., no significance; one-way ANOVA. Scale bar: 200 μm.

C A549 cells were infected with lentiviruses expressing control shRNA, WDR63 shRNA#1, WDR63 shRNA#2, PCDH control, or PCDH-Flag-WDR63. Forty-eight hours after infection, cell lysates were analyzed by Western blot. Data shown represent three independent experiments.

D–F H292 cells expressing control shRNA, WDR63 shRNA#1, WDR63 shRNA#2, PCDH control, or PCDH-Flag-WDR63 were subjected to wound-healing (D) and transwell migration (F) assays. Lysates from these cells were also analyzed by Western blot (E). The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). *P < 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA. Scale bar in (D): 200 μm. Scale bar in (F): 100 μm.

G Lysates from A549 cells expressing control shRNA, WDR63 shRNA#1, or WDR63 shRNA#2 were analyzed by Western blot.

H, I Effect of WDR63 knockdown on the migration of H292 cells was analyzed by single-cell tracking with time-lapse microscopy at 10-min intervals for 4 h (I). Cell migration speed, persistence, and mean-squared displacement (MSD) were also analyzed using an Excel macro described by Gorelik and Gautreau. Data shown are mean ± SD. n = 3 independent experiments. **P < 0.01; ***P < 0.001; one-way ANOVA. The successful knockdown of WDR63 in H292 cells was also confirmed by Western blot analysis (H). Scale bar: 100 μm.

J H292 cells expressing control shRNA, WDR63 shRNA#1, WDR63 shRNA#2, PCDH control, or PCDH-Flag-WDR63 were subjected to transwell invasion assay. The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). *P < 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA. Scale bar: 100 μm.

K–N A549 cells were infected with lentiviruses expressing control shRNA, WDR63 shRNA#1, PCDH control, or PCDH-Flag-WDR63. Forty-eight hours after infection, cell lysates were analyzed by Western blot (K). The cell growth curves were measured by counting cell numbers at the indicated time points (L and M). The cell cycle distribution was analyzed by flow cytometry (N). Data shown are mean ± SD; n = 3 independent experiments.

O A549 cells expressing control shRNA, WDR63 shRNA#1, PCDH control, or PCDH-Flag-WDR63 were treated with 1 μg/ml doxorubicin for the indicated periods of time. Cells were then stained with Hoechst 33342, and apoptotic cells were determined by the presence of nuclear fragmentation. Data shown are mean ± SD; n = 3 independent experiments. N.S., no significance; two-tailed Student's t-test.

P, Q A549 cells expressing control shRNA, WDR63 shRNA#1, or WDR63 shRNA#1 plus shRNA-resistant Flag-WDR63 were subjected to wound-healing assay (P). Lysates from these cells were also analyzed by Western blot (Q). The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). ***P < 0.001; one-way ANOVA. Scale bar: 200 μm.

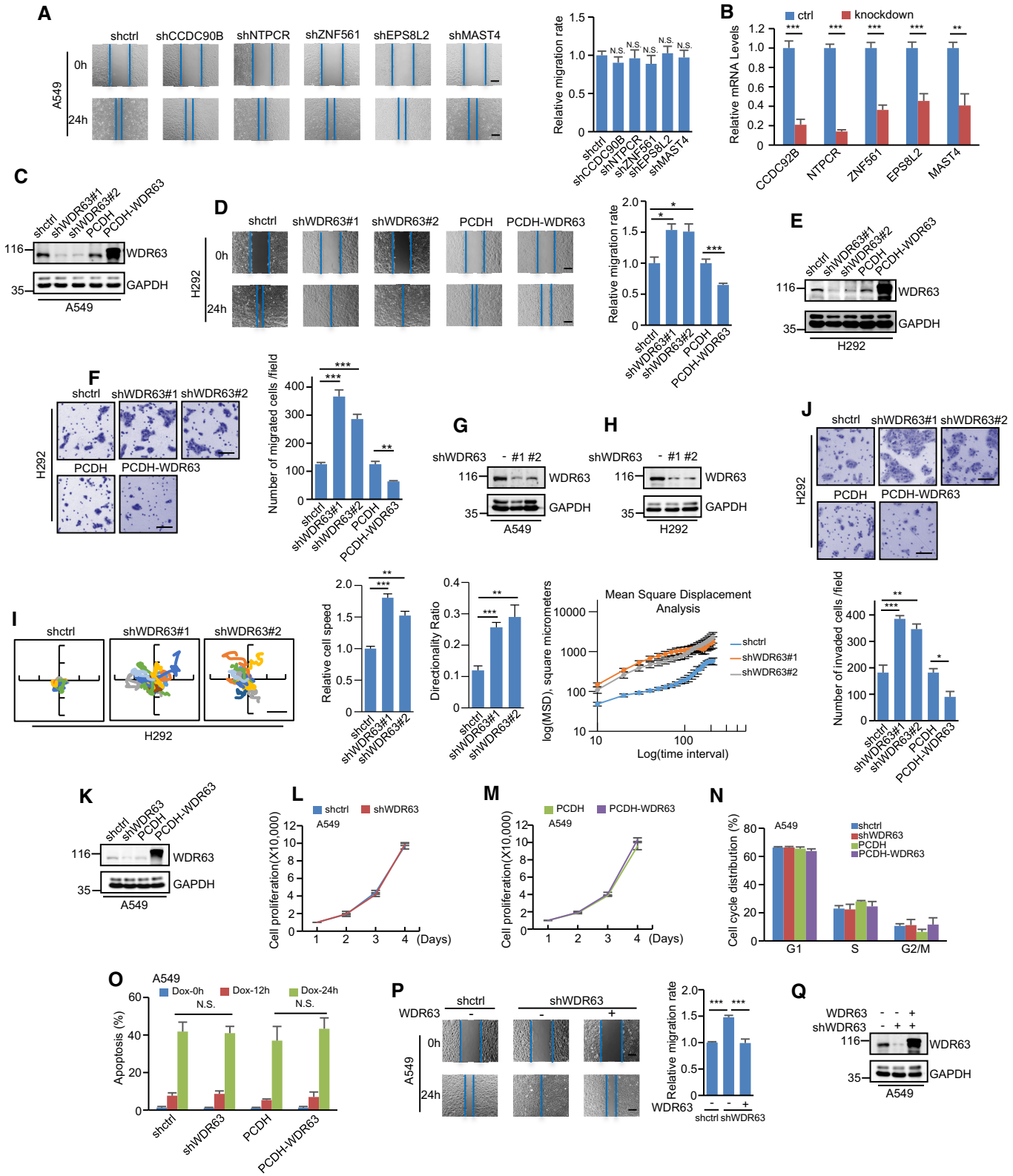
A
shctrl
shCCDC90B
shNTPCR
shZNF561
shEPS8L2
shMAST4
0h
24h
A549
Relative migration rate
N.S.
B
ctrl
knockdown
Relative mRNA Levels
CCDC92B
NTPCR
ZNF561
EPS8L2
MAST4
C
shctrl
shWDR63#1
shWDR63#2
PCDH
PCDH-WDR63
116
WDR63
35
GAPDH
A549
D
H292
Relative migration rate
E
H292
F
Number of migrated cells /field
G
shWDR63
- #1 #2
A549
H
H292
I
Relative cell speed
Directionality Ratio
Mean Square Displacement Analysis
log(MSD), square micrometers
Log(time interval)
J
Number of invaded cells /field
K
A549
L
Cell proliferation(X10,000)
(Days)
M
PCDH
PCDH-WDR63
N
Cell cycle distribution (%)
G1
S
G2/M
O
Dox-0h
Dox-12h
Dox-24h
N.S.
Apoptosis (%)
P
WDR63
Q

Figure EV1.

Figure EV2. WDR63 is upregulated by p53.

A A549 cells expressing control shRNA, WDR63 shRNA#1, or WDR63 shRNA#2 (each also expressing luciferase) were injected via tail vein into nude mice (n = 6 for each group). Six weeks after injection, tumor formation in lungs was monitored by bioluminescence imaging. Data shown are mean ± SD; *P < 0.05; one-way ANOVA. Scale bar: 1 cm.

B WDR63 expression levels were analyzed in TCGA lung adenocarcinoma (LUAD) and their normal tissues. Data shown are mean ± SD; P-value was calculated by two-tailed Student's t-test.

C WDR63 expression levels were analyzed in TCGA lung squamous cell carcinoma (LUSC) and their normal tissues. Data shown are mean ± SD; P-value was calculated by two-tailed Student's t-test.

D WDR63 expression levels were analyzed in TCGA LUAD with different stages. Data shown are mean ± SD; P-value was calculated by one-way ANOVA.

E WDR63 expression levels were analyzed in TCGA LUSC with different stages. Data shown are mean ± SD; P-value was calculated by one-way ANOVA.

F A549 cells expressing control or p53 shRNA were treated with etoposide (50 μM) for the indicated periods of time. WDR63 mRNA and protein levels were then examined. Data shown are mean ± SD; n = 3 independent experiments. ***P < 0.001; two-tailed Student's t-test.

G A549 cells expressing control or p53 shRNA were treated with Nutlin (10 μM) for the indicated periods of time, followed by Western blot analysis. Data shown represent three independent experiments.

H HCT116 $p53^{+/+}$ and HCT116 $p53^{-/-}$ cells were treated with Nutlin (10 μM) for the indicated periods of time, followed by Western blot analysis. Data shown represent three independent experiments.

I WDR63 expression levels were analyzed in TCGA LUAD harboring wild-type or mutant *TP53* gene using TCGA database. Data shown are mean ± SD; P-value was calculated by two-tailed Student's t-test.

J WDR63 expression levels were analyzed in TCGA LUSC harboring wild-type or mutant *TP53* gene using TCGA database. Data shown are mean ± SD; P-value was calculated by two-tailed Student's t-test.

K A549 cells expressing either control shRNA or p53 shRNA were transfected with the indicated reporter constructs plus Renilla luciferase plasmid. Twenty-four hours later, reporter activity was measured. Data shown are mean ± SD; n = 3 independent experiments. **P < 0.01; ***P < 0.001; two-tailed Student's t-test.

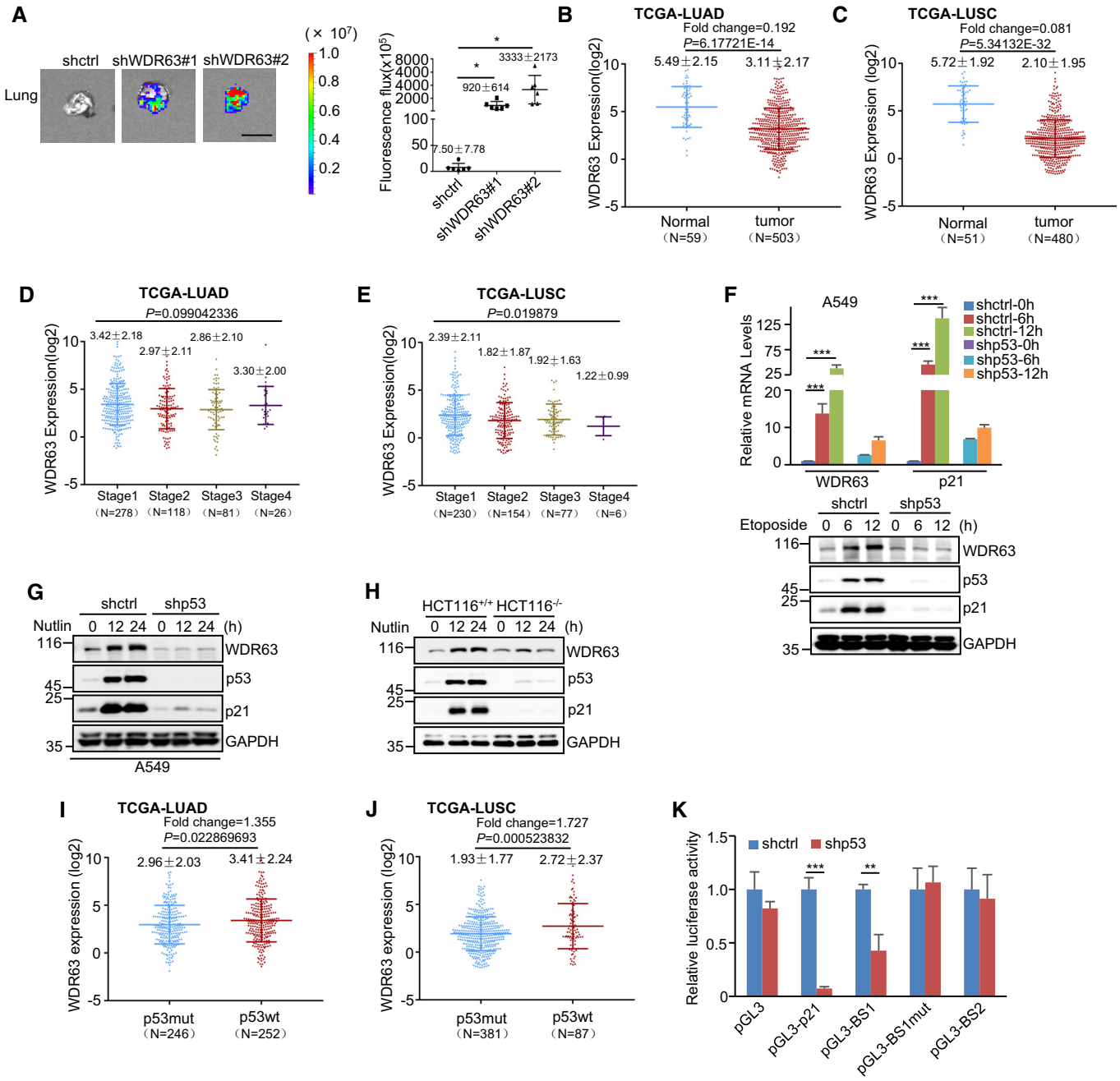
A
shctrl
shWDR63#1
shWDR63#2
Lung
(× 10^7)
1.0
0.8
0.6
0.4
0.2
Fluorescence flux(x10^5)
7.50±7.78
920±614
3333±2173
shctrl
shWDR63#1
shWDR63#2
B
TCGA-LUAD
Fold change=0.192
P=6.17721E-14
5.49±2.15
3.11±2.17
WDR63 Expression(log2)
Normal (N=59)
tumor (N=503)
C
TCGA-LUSC
Fold change=0.081
P=5.34132E-32
5.72±1.92
2.10±1.95
WDR63 Expression (log2)
Normal (N=51)
tumor (N=480)
D
TCGA-LUAD
P=0.099042336
3.42±2.18
2.97±2.11
2.86±2.10
3.30±2.00
WDR63 Expression(log2)
Stage1 (N=278)
Stage2 (N=118)
Stage3 (N=81)
Stage4 (N=26)
E
TCGA-LUSC
P=0.019879
2.39±2.11
1.82±1.87
1.92±1.63
1.22±0.99
WDR63 Expression(log2)
Stage1 (N=230)
Stage2 (N=154)
Stage3 (N=77)
Stage4 (N=6)
F
A549
shctrl-0h
shctrl-6h
shctrl-12h
shp53-0h
shp53-6h
shp53-12h
Relative mRNA Levels
WDR63
p21
shctrl
shp53
Etoposide 0 6 12 0 6 12 (h)
WDR63
p53
p21
GAPDH
G
shctrl
shp53
Nutlin 0 12 24 0 12 24 (h)
WDR63
p53
p21
GAPDH
A549
H
HCT116+/+ HCT116-/-
Nutlin 0 12 24 0 12 24 (h)
WDR63
p53
p21
GAPDH
I
TCGA-LUAD
Fold change=1.355
P=0.022869693
2.96±2.03
3.41±2.24
WDR63 expression (log2)
p53mut (N=246)
p53wt (N=252)
J
TCGA-LUSC
Fold change=1.727
P=0.000523832
1.93±1.77
2.72±2.37
WDR63 expression (log2)
p53mut (N=381)
p53wt (N=87)
K
shctrl
shp53
Relative luciferase activity
pGL3
pGL3-p21
pGL3-BS1
pGL3-BS1mut
pGL3-BS2

Figure EV2.

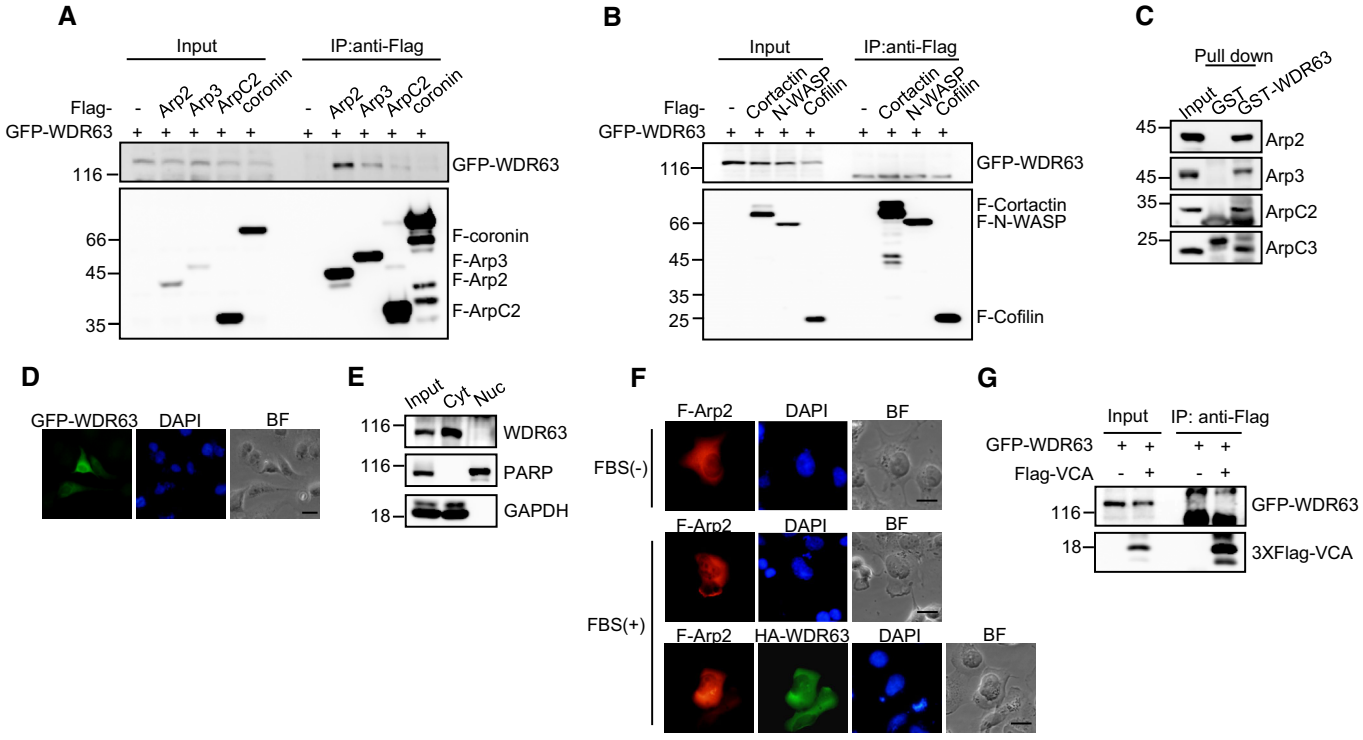

Figure EV3. WDR63 interacts with Arp2/3 and inhibits FBS-stimulated translocation of Arp2 to the leading edge of migrating cells.

A Lysates from HEK293T cells expressing GFP-WDR63 alone or GFP-WDR63 plus Flag-tagged Arp2, Arp3, ArpC2, and coronin 1B as indicated were immunoprecipitated by anti-Flag antibody, followed by Western blot analysis. Data shown represent three independent experiments.

B Lysates from HEK293T cells expressing GFP-WDR63 alone or GFP-WDR63 plus Flag-tagged cortactin, N-WASP, and cofilin as indicated were immunoprecipitated by anti-Flag antibody, followed by Western blot analysis. Data shown represent three independent experiments.

C Lysates from A549 cells were incubated with purified GST or GST-WDR63 proteins immobilized on glutathione beads. Input and bead-bound proteins were analyzed by Western blot. Data shown represent three independent experiments.

D The cellular localization of exogenous GFP-WDR63 was determined by immunofluorescence. Data shown represent three independent experiments. Scale bar: 20 μm.

E The localization of endogenous WDR63 in A549 cells was evaluated by nuclear/cytosolic fractionation, followed by Western blot analysis. Data shown represent three independent experiments.

F A549 cells expressing Flag-Arp2 alone or together with HA-WDR63 were serum-starved for 6 h. Cells were then treated with or without 10% FBS for 30 min, followed by immunofluorescence assay. Data shown represent three independent experiments. Scale bar: 20 μm.

G Lysates from HEK293T cells expressing GFP-WDR63 alone or together with Flag-VCA were immunoprecipitated by anti-Flag antibody, followed by Western blot analysis. Data shown represent three independent experiments.

Figure EV4. WDR63 suppresses cell migration and invasion via interacting with the Arp2/3 complex.

A–C H292 cells expressing control shRNA, WDR63 shRNA#1, Arp2 shRNA, or both WDR63 and Arp2 shRNA were subjected to wound-healing (A) and transwell migration (C) assays. Lysates from these cells were also analyzed by Western blot (B). The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). **P < 0.01; ***P < 0.001; N.S., no significance; one-way ANOVA. Scale bar in (A): 200 μm. Scale bar in (C): 100 μm.

D, E A549 (D) and H292 (E) cells expressing control shRNA, WDR63 shRNA#1, or both WDR63 and Arp2 shRNA were analyzed by single-cell tracking with time-lapse microscopy at 10-min intervals for 4 h. Scale bar in (D): 200 μm. Scale bar in (E): 100 μm. Cell migration speed, persistence, and mean-squared displacement (MSD) were also analyzed using an Excel macro described by Gorelik and Gautreau. Data shown are mean ± SD. n = 3 independent experiments. **P < 0.01; ***P < 0.001; one-way ANOVA.

F H292 cells expressing control shRNA, WDR63 shRNA#1, Arp2 shRNA, or both WDR63 and Arp2 shRNA were subjected to transwell invasion assay. The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). *P < 0.05; ***P < 0.001; one-way ANOVA. Scale bar: 100 μm.

G, H A549 cells expressing control shRNA and WDR63 shRNA#1 were treated with or without 100 μM CK666 for 24 h. The cells were then subjected to transwell migration (G) and transwell invasion (H) assays. The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). *P < 0.05; ***P < 0.001; N.S., no significance; one-way ANOVA. Scale bar: 100 μm.

I A549 cells expressing either control shRNA or WDR63 shRNA#1 were infected with lentiviruses expressing Flag-tagged full-length or truncated WDR63 proteins as indicated. Cells were then subjected to wound-healing assay. The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). **P < 0.01; ***P < 0.001; one-way ANOVA. Scale bar: 200 μm.

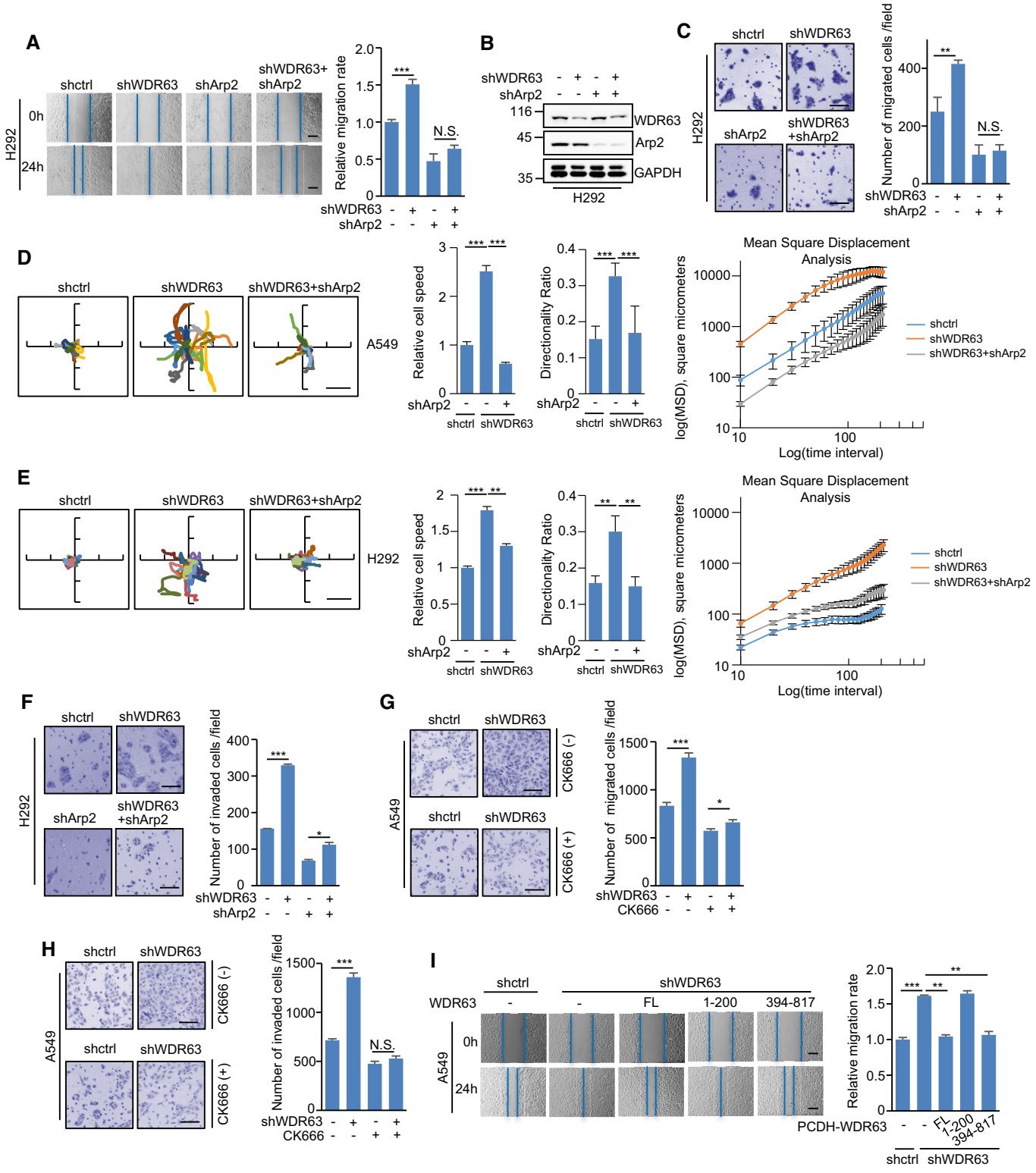

Figure EV4.

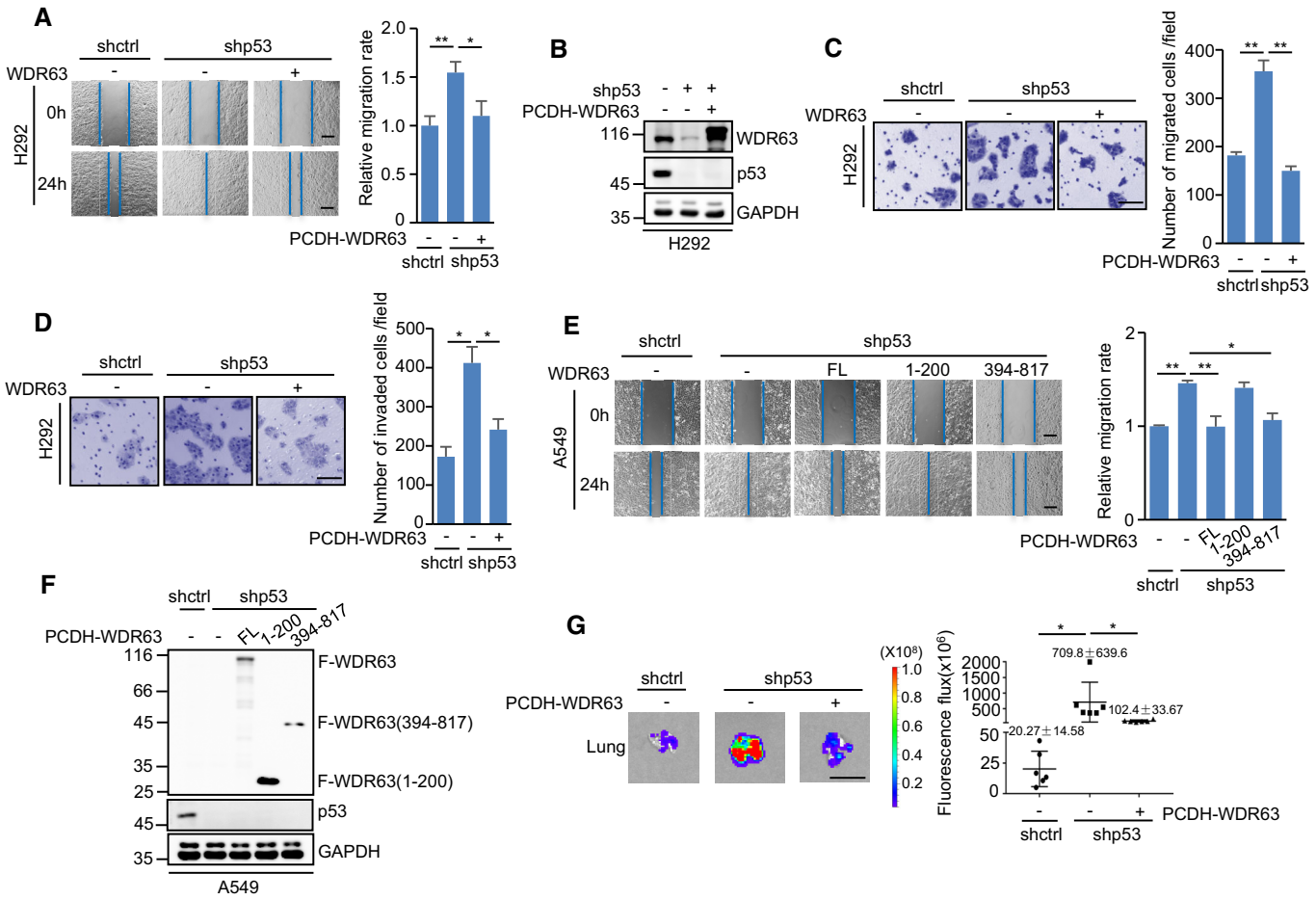

Figure EV5. WDR63 mediates the metastasis-suppressive function of p53.

A–D H292 cells expressing control shRNA, p53 shRNA, or p53 shRNA plus Flag-WDR63 were subjected to wound-healing (A), transwell migration (C), and transwell invasion (D) assays. Lysates from these cells were also analyzed by Western blot (B). The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). *P < 0.05; **P < 0.01; one-way ANOVA. Scale bar in (A): 200 μm. Scale bars in (C and D): 100 μm.

E, F A549 cells expressing either control shRNA or p53 shRNA were infected with lentiviruses expressing Flag-tagged full-length or truncated WDR63 proteins as indicated. Cells were then subjected to wound-healing assay (E). Lysates from these cells were also analyzed by Western blot (F). The shown images are representative of three independent experiments. Data shown are mean ± SD (n = 3). *P < 0.05; **P < 0.01; one-way ANOVA. Scale bar: 200 μm.

G A549 cells expressing control shRNA, p53 shRNA, or p53 shRNA plus Flag-WDR63 (each also expressing luciferase) were injected via tail vein into nude mice (n = 6 for each group). Six weeks after injection, tumor formation in lungs was monitored by bioluminescence imaging. Data shown are mean ± SD; *P < 0.05; one-way ANOVA. Scale bar: 1 cm.