Supplementary Materials: The Inhibition of Wnt Restrain *KRAS^{G12V}*-Driven Metastasis in Non-Small-Cell Lung Cancer

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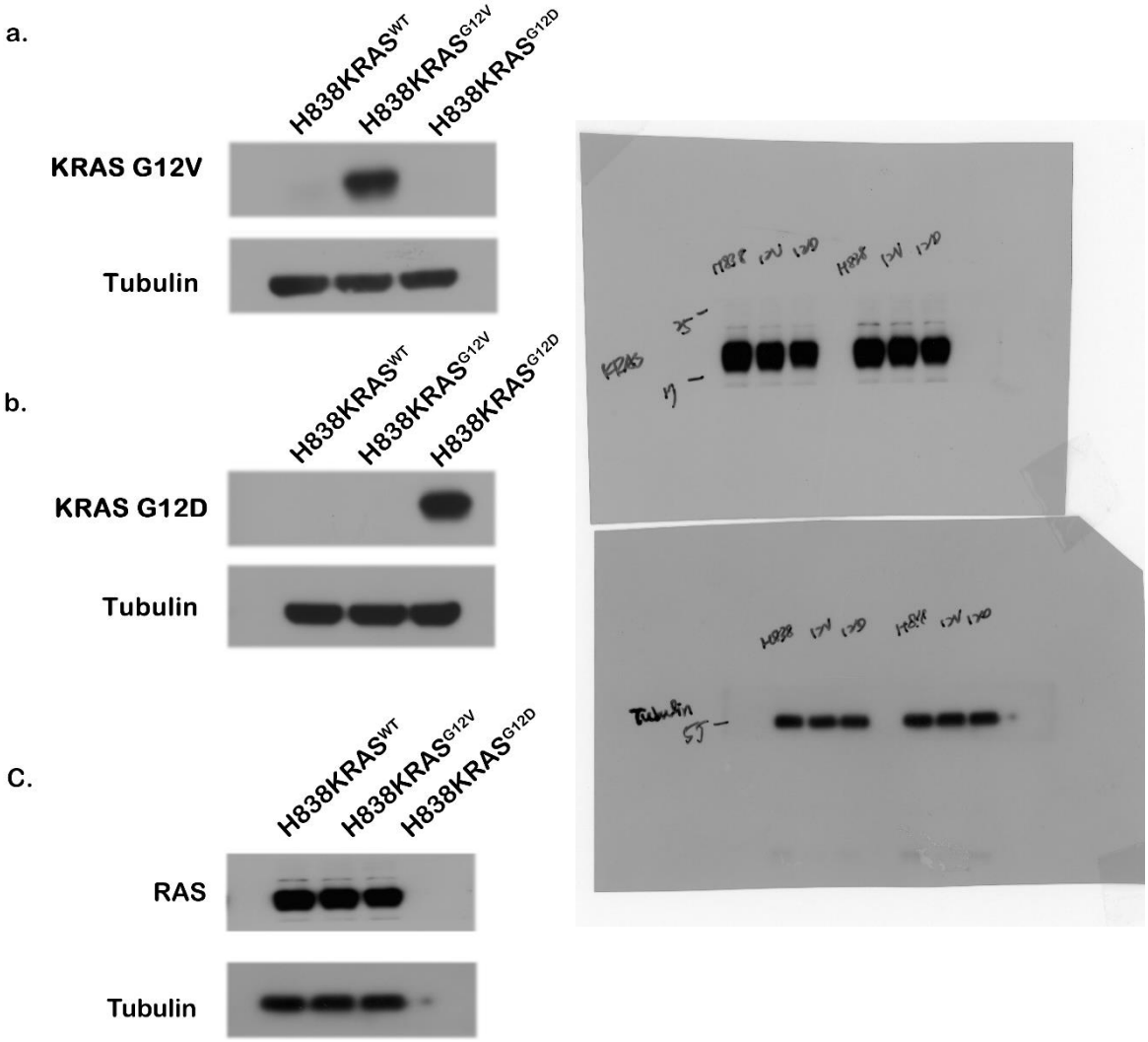


Figure S1. The mutation status of KRAS in H838 KRAS isogenic cells. (**a**). The expression of KRAS ^{G12V} in H838KRAS ^{G12V} cells. (**b**)The expression of KRAS^{G12D} in H838KRAS ^{G12D} cells. (**c**)The western blotting of RAS expression in H838 isogenic cells.

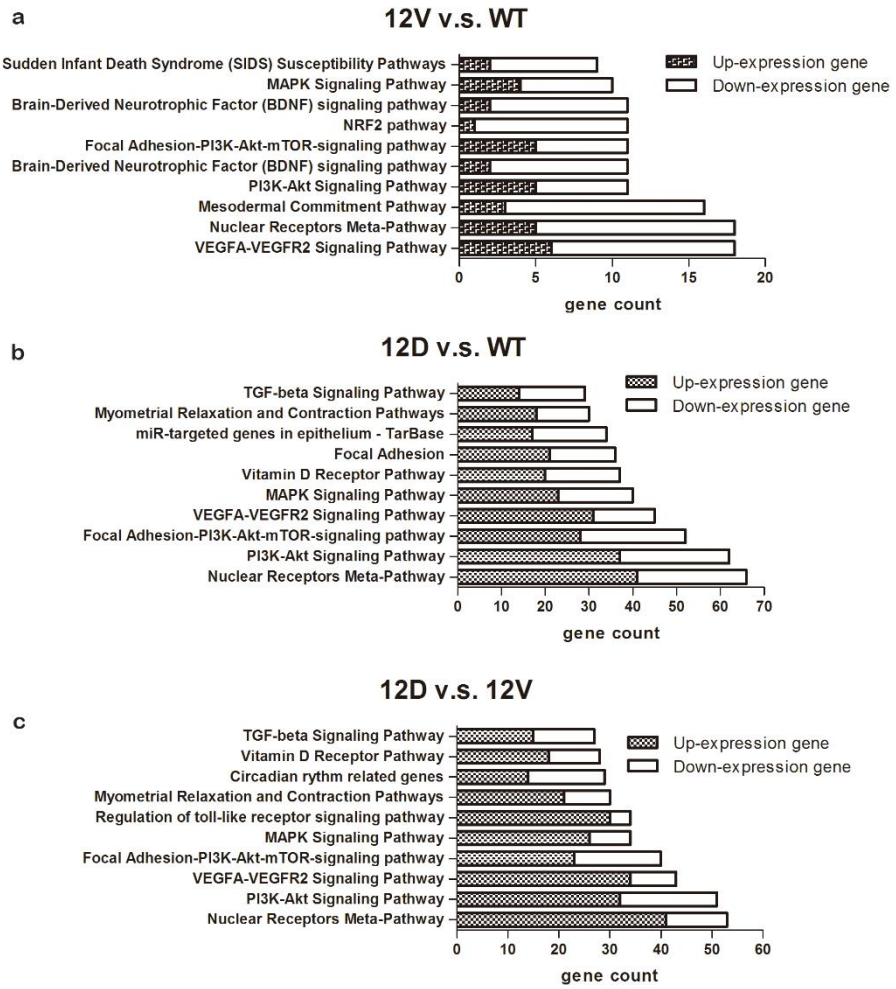


Figure S2. The gene ontology and pathway analysis in H838 KRAS isogenic cells. The top 10 Pathways were predicted to be associated with H838KRAS^{WT}, H838KRAS ^{G12V}, and H838KRAS^{G12D} on *Y* -axis and genes count on *X* -axis.

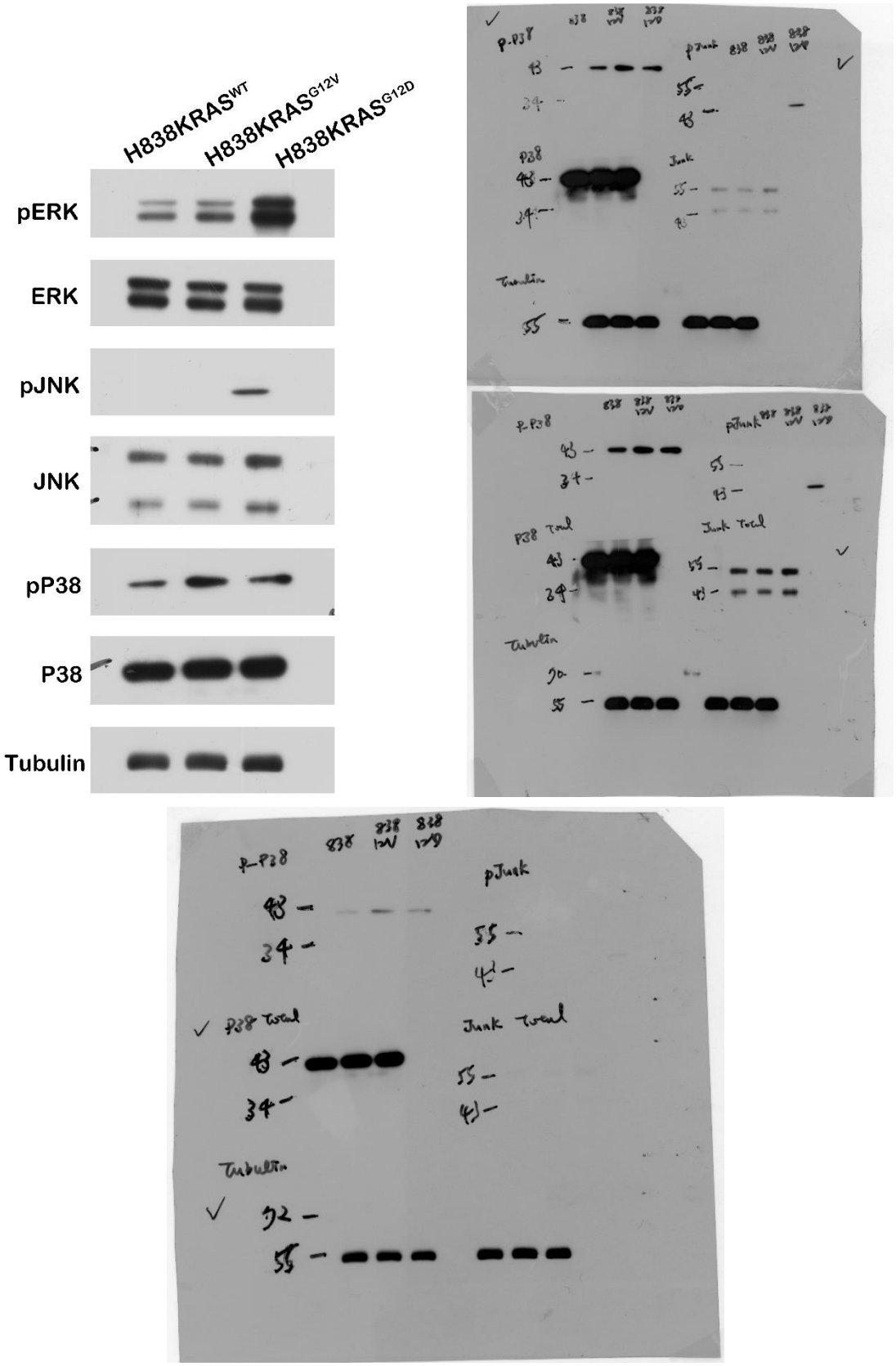


Figure S3. The MAPK pathway in H838 KRAS isogenic cells. Immunoblot blot were probed with ERK (phospho/Total), JNK (phospho/Total) and p38 (phospho/Total) antibody. Tublin was used as an internal control.

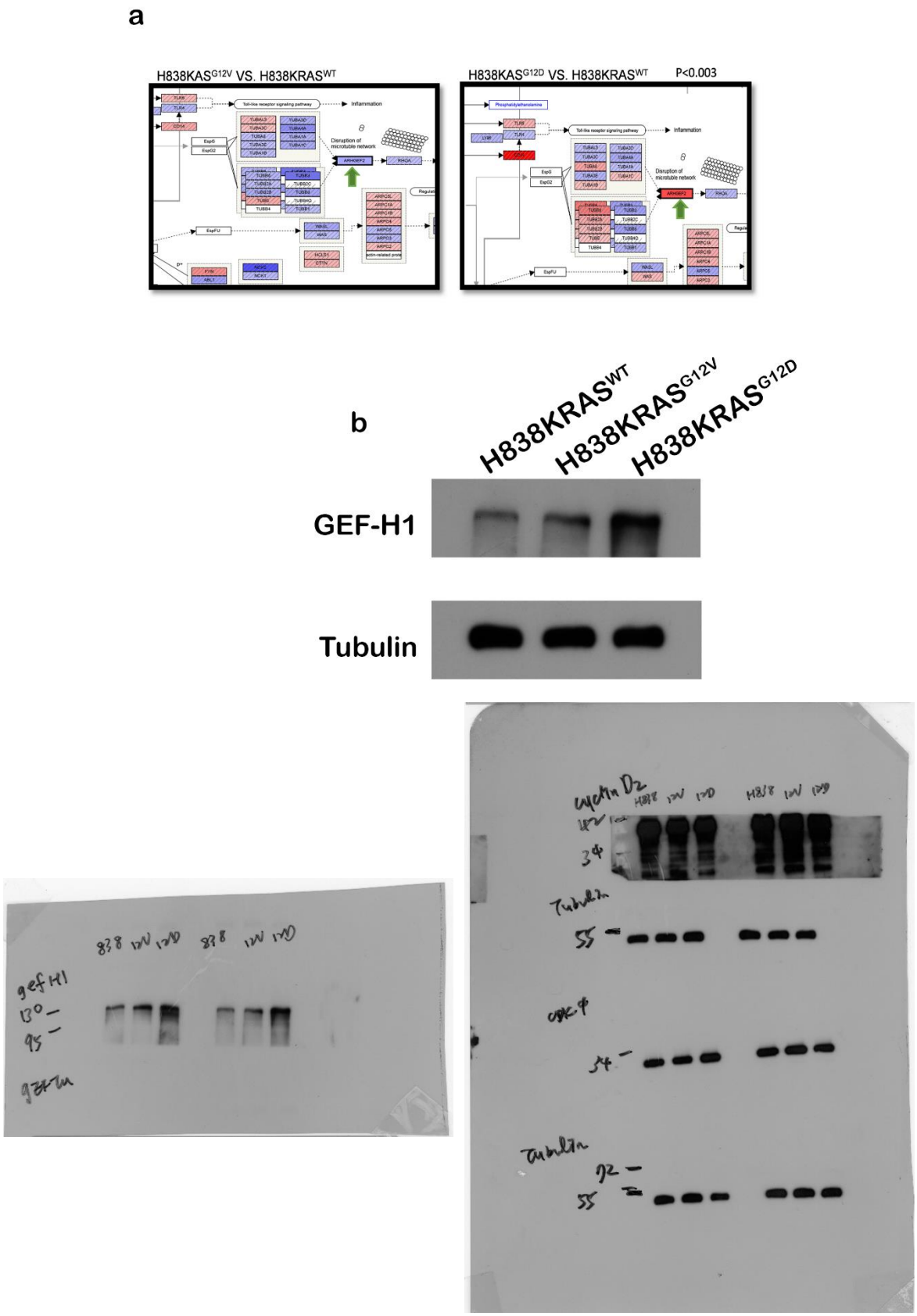


Figure S4. GEF-H1 was increased in H838KRAS^{G12D} cells. (**a**). The analysis of Transcriptome Analysis Console (TAC) of GEF-H1 and GEF-H1 related pathway. (**b**). The immunoblot analysis of GEF-H1 in H838KRAS isogenic cells. Tubulin was used as an internal control.

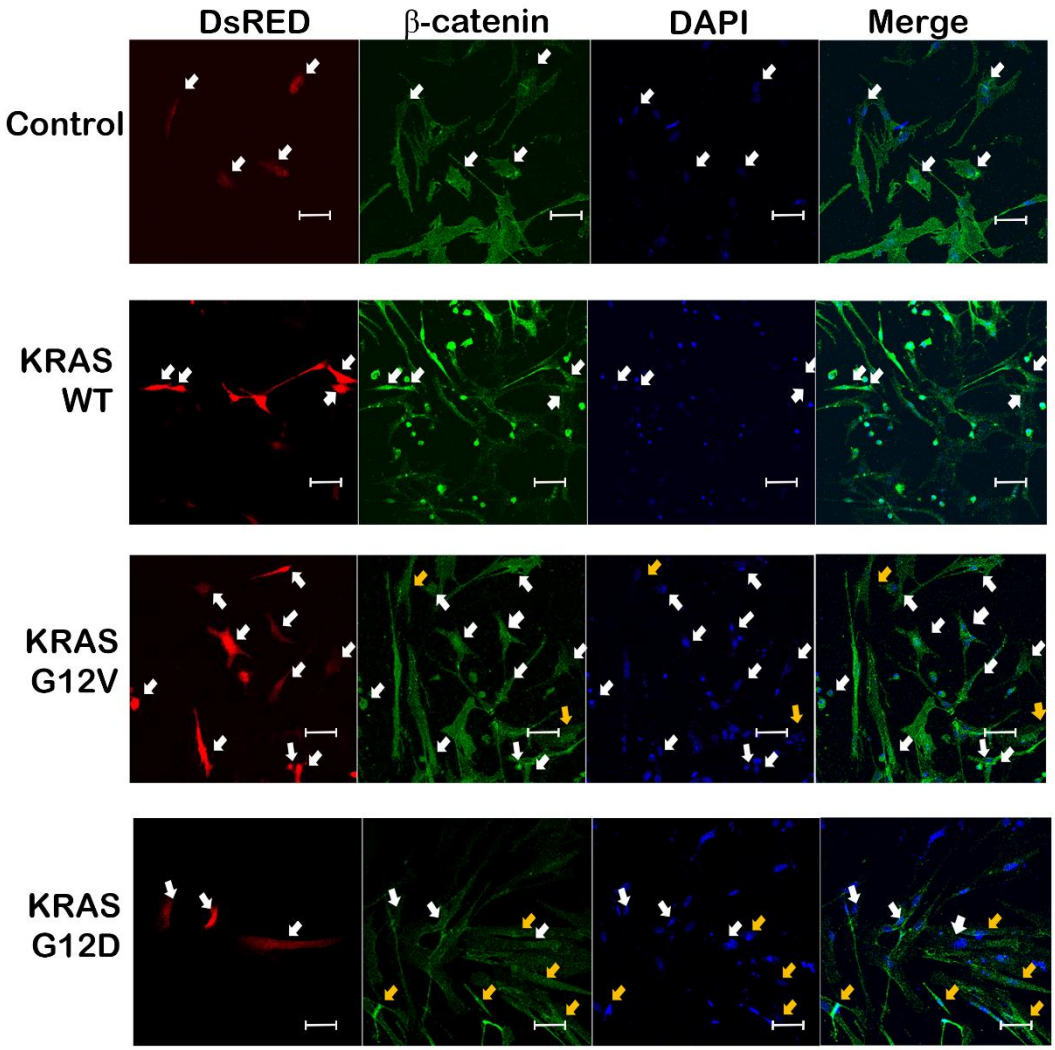
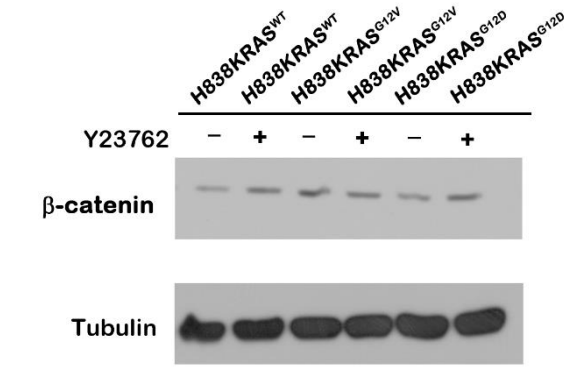


Figure S5. KRAS G12D inactivate the activity of WNT /β-catenin in MRC-5 cells. KRAS G12D did not induce WNT /β-catenin translocation into nucleus. White arrows indicate the plasmid transfected cells. Yellow arrows indicate the non-transfected cells. MRC-5 cells were transfected with DsRed-tagged KRAS WT, KRAS G12V, and KRAS G12D plasmid. IF staining after EGF stimulation. β-catenin is visualized with green fluorescent. Hochest3342 is the blue nuclear stain. Immunoreactivity was captured by confocal microscopy. Scale bar 50 μm.



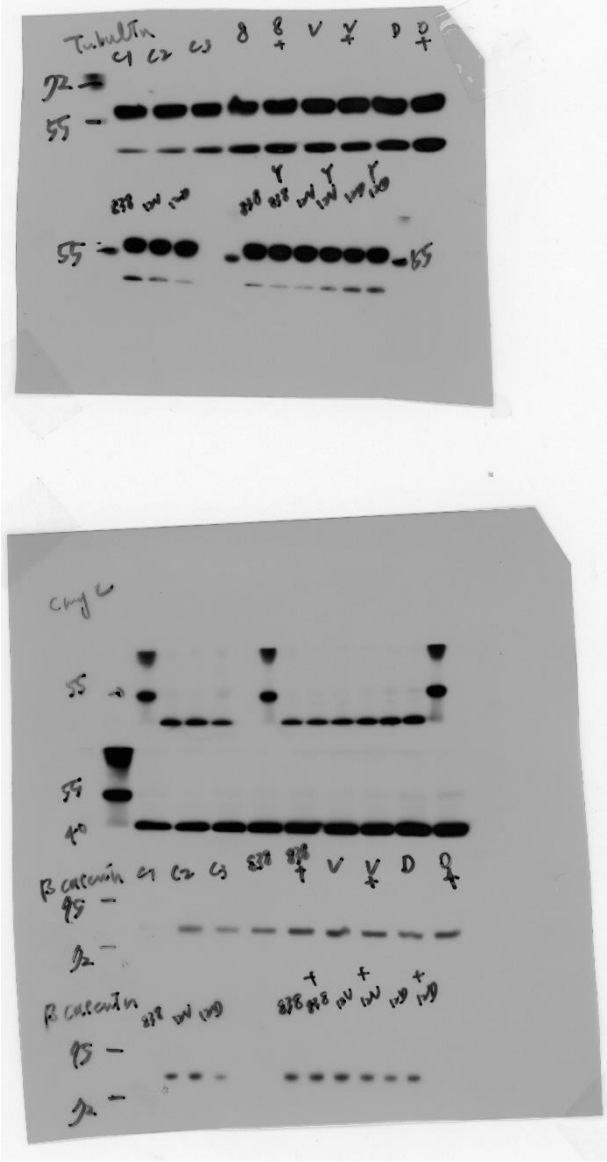


Figure S6. The RhoA suppress the expression of β-catenin in H838 KRAS isogenic cells. The western blotting of β-catenin in H838 KRAS isogenic cells after 30μM Y23762 treatment (RhoA inhibitor).

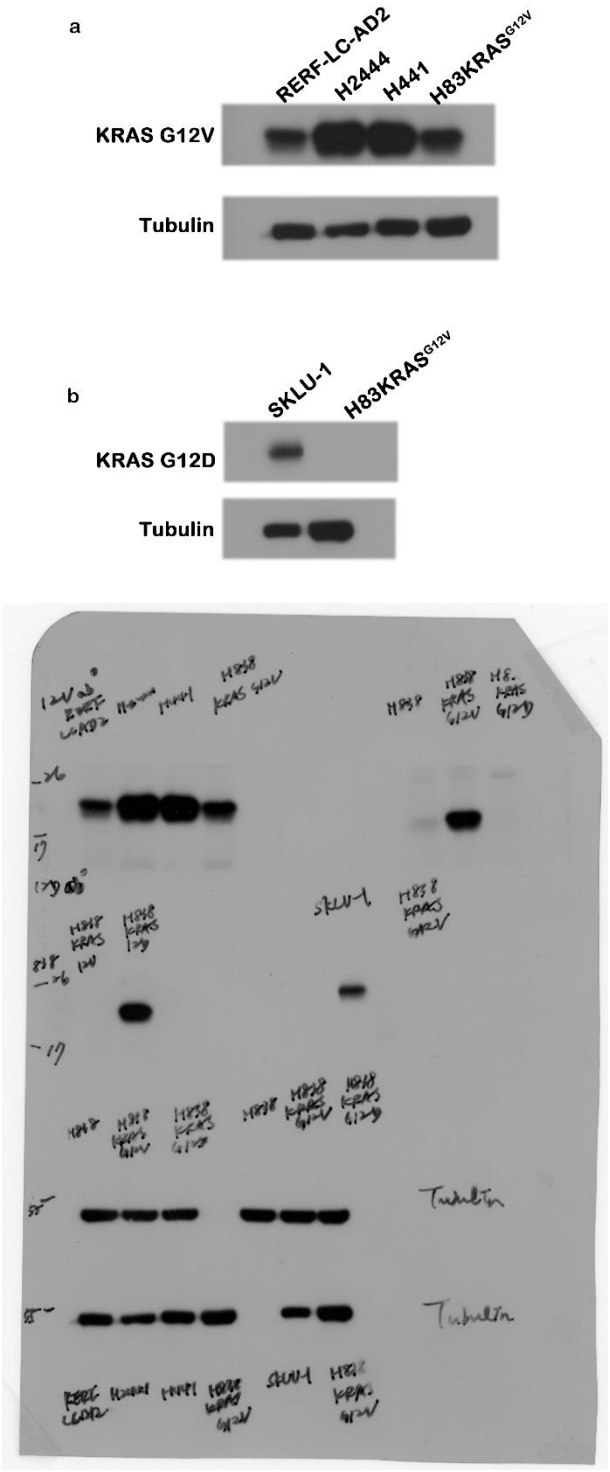


Figure S7. The mutation status of KRAS in KRAS-mutant NSCLC cells. (**a**) The expression of KRAS ^{G12V} in RERF-LC-AD2, H441, H2444, and H838KRAS ^{G12V} cells. H838 KRAS ^{G12V} cells as a positive control. (**b**) The expression of KRAS^{G12D} in SKLU-1 and H838KRAS^{G12V} cells. H838KRAS^{G12V} cells as a negative control.

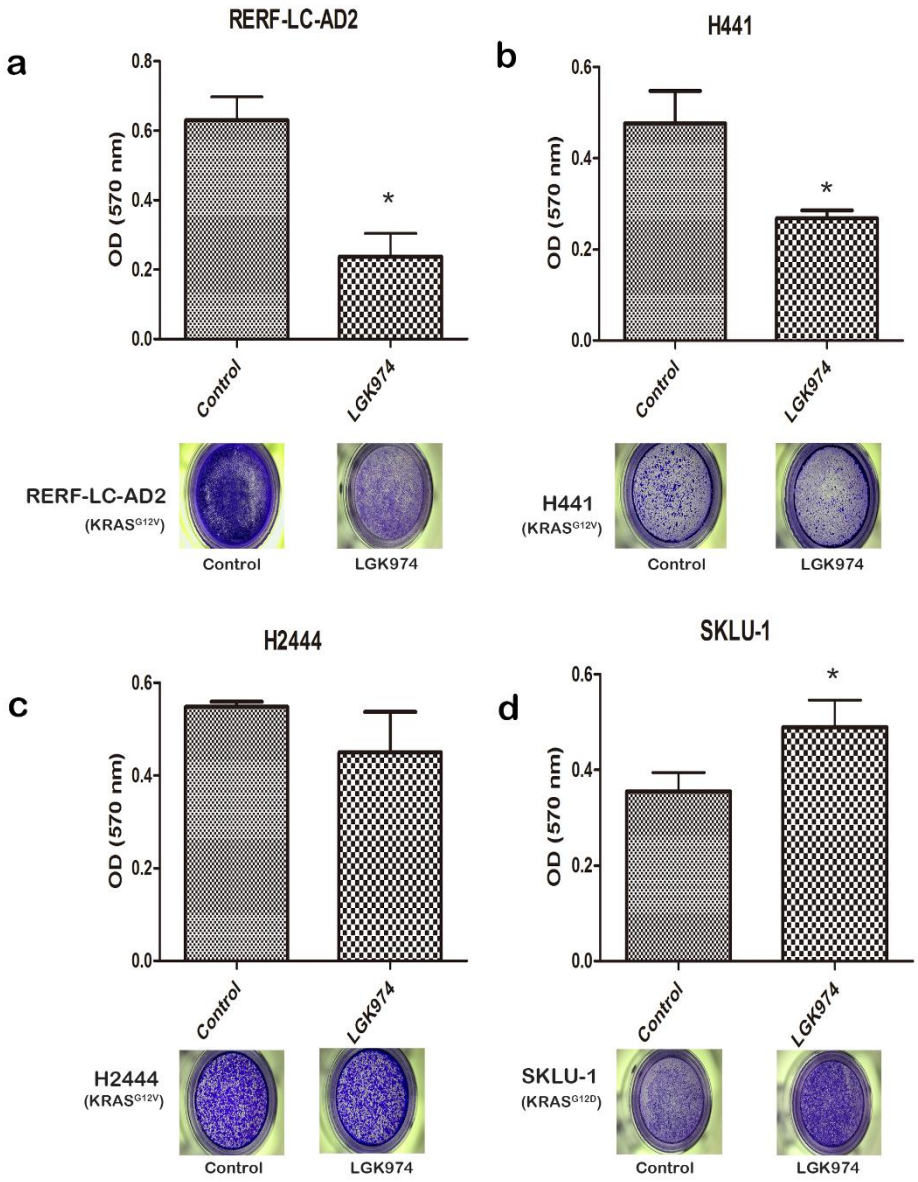


Figure S8. The migration ability of KRAS^{G12V}mutant NSCLC cells were attenuated by LGK974. (**a**). The migration assay of RERF-LC-DA2 after 20nM LGK974 treatment. (**b**). The migration assay of H441 after 20nM LGK974 treatment. (**c**). The migration assay of H2444 after 20nM LGK974 treatment. (**d**). The migration assay of SK-LU-1 after 20nM LGK974 treatment. The values represent the means ± s.d. of three independent assays ($n = 3$, $^*p < 0.05$)

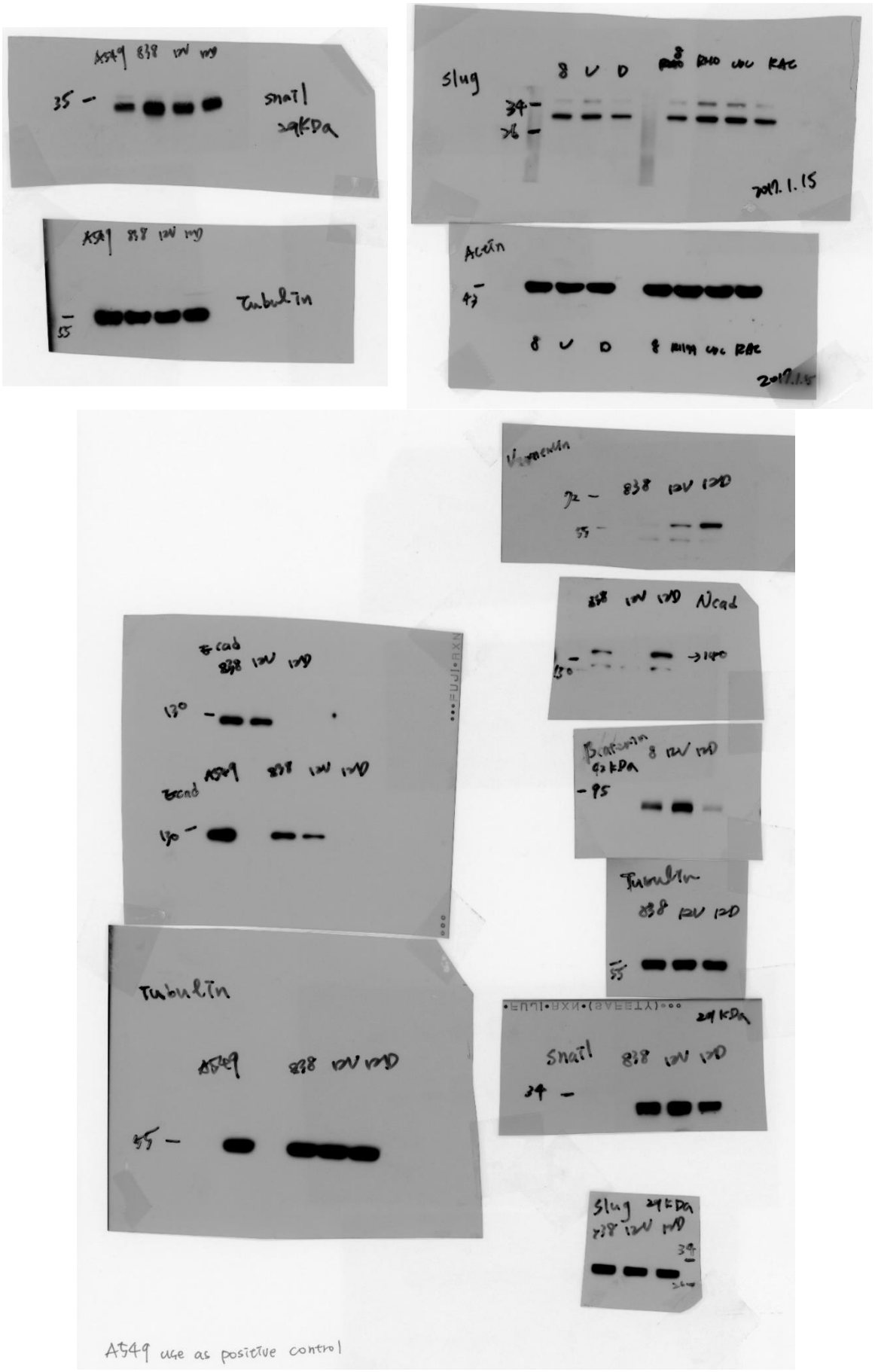
Table S1. Quantitative real-time PCR primers used in this study.

Gene	Product Number
CDH1	Hs01023894_m1
CDH2	Hs00983056_m1
SNAI1	Hs00195591_m1
SNAI2	Hs00161904_m1
VIM	Hs00958111_m1
CTNNB1	Hs00355049_m1
GAPDH	Hs99999905_m1

Table 2. List of antibodies used in western blotting.

Antibody	Name Company

β-catenin (#8480)	Cell Signaling
Vimentin (#5741)	Cell Signaling
Snail (#3879)	Cell Signaling
Slug (#9585)	Cell Signaling
Cyclin D1(GTX108624)	GeneTex
c-Myc (GTX103436)	GeneTex
Tubulin (ab12546)	Abcam
N-cadherin (ab76011)	Abcam
E-cadherin (ab40772)	Abcam
Ras (#3965)	Cell Signaling
Ras G12V mutant (#14412)	Cell Signaling
Ras G12D mutant (#14429)	Cell Signaling
pP38 (#9211)	Cell Signaling
P38 (#8690)	Cell Signaling
pJunk (#9251)	Cell Signaling
Junk (#9252)	Cell Signaling
pERK (#9101)	Cell Signaling
ERK (#9102)	Cell Signaling


A549 838 IW IW
35 –
Snail
29KDa
A549 838 IW IW
Tubulin
55
Slug
8 V D
34 –
26 –
2019. 1. 15
Actin
43 –
8 V D
Vimentin
72 –
838 IW IW
55 –
838 IW IW Ncad
130 –
→140
E-cad
838 IW IW
130 –
Ecad
A549 838 IW IW
130 –
Tubulin
838 IW IW
55 –
Tubulin
A549 838 IW IW
55 –
Snail 838 IW IW
34 –
Slug 29KDa
838 IW IW
34 –
A549 use as positive control

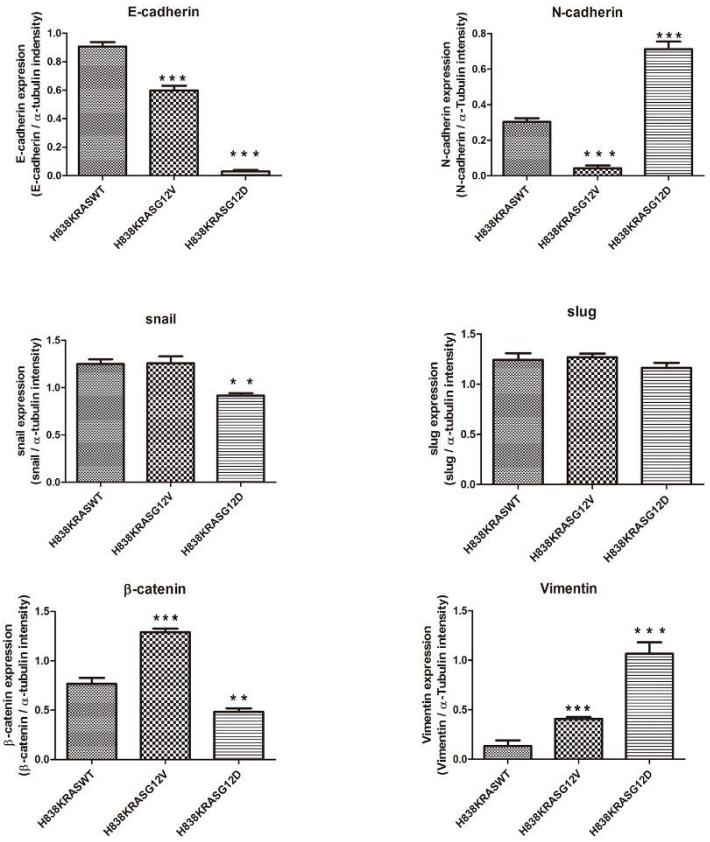


Figure S9. The uncropped blots and molecular weight markers of Figure 5.

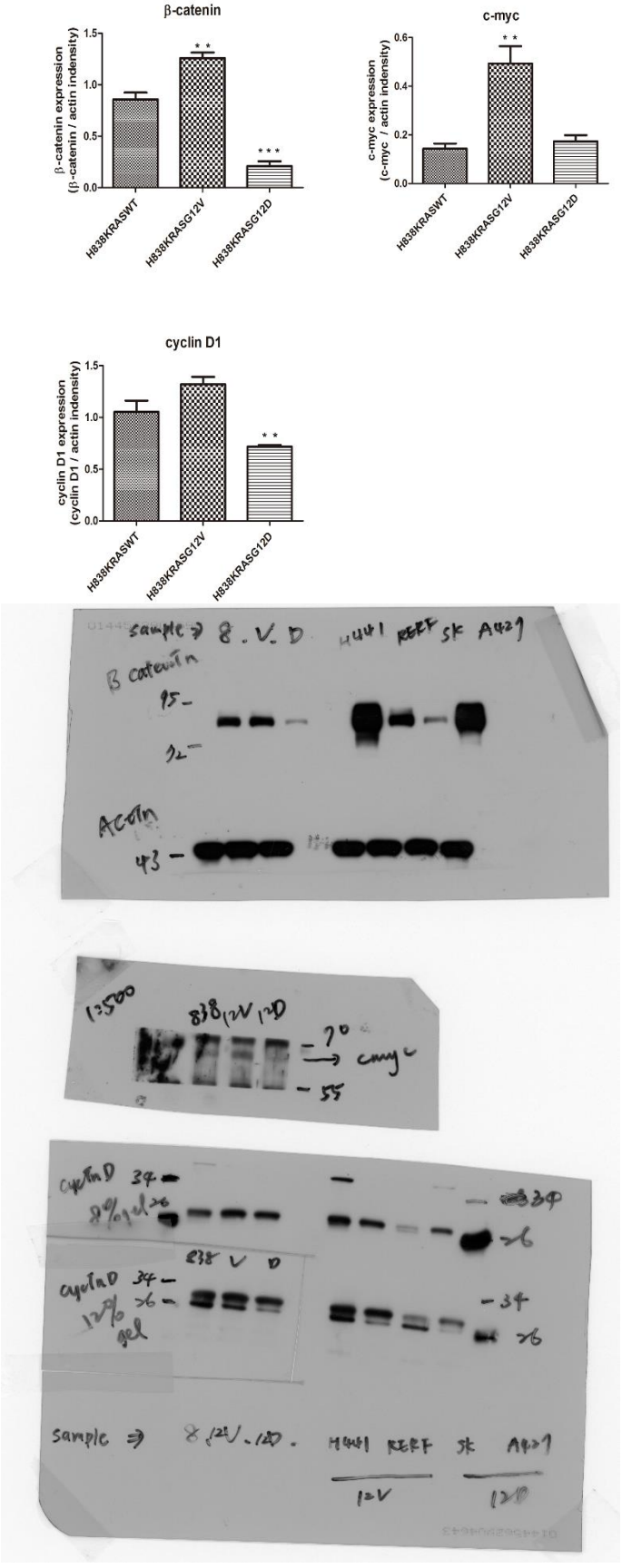


Figure S10. The uncropped blots and molecular weight markers of Figure 6.