SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Crystal violet assay

Cells were seeded in 24-well plates and cultured in media supplemented with 10% FBS for 7 days. Media were then removed, plates were briefly washed with PBS and stained with 0.5% crystal violet solution in 20% methanol was added and stained for 5min at 37℃. These cells were washed several times with PBS, solubilized with 1% SDS and absorbance at 600 nm was measured using a microplate reader.

Soft agar assay

Cells were plated into 24-well flat-bottomed plates using a two-layer soft agar system with 1×10^3cells/well in a volume of 400ul/well as described previously. After 15 days of incubation, the colonies were counted and measured. All of experiments were done at least three times using triplicate plates per experimental point.

SUPPLEMENAL FIGURE LEGEND

Supplemental Fig. 1 Knock down of RACK1 increased NSCLC cell lines apoptosis and inhibits cell proliferation and clonogenic growth. A, Apoptotic cells were monitored by Annexin V-PE/PI staining and flow-cytometry analysis. B, PI staining and flow-cytometry analysis of A549 and H23 Si Con or Si RACK1 cells. The sub G1 population represents the apoptotic cells. C, Crystal violet cell growth assay. A549 and H23 Si Con or Si RACK1 cells (2×10^3cells) were plated in 24-well plates. Cells were stained with crystal violet (upper panels) and evaluated at 600 nm on the 7th days after plating. The lower bar graphs depicted the mean±SEM of three experiments, **P<0.01 versus Si Con cells. D, Soft agar assay. The A549 and H23 cells containing either Si Con or Si RACK1 cells (1×10^3cells/well) were grown in soft agar and counted after 15 days. The upper panel shows representative clonogenic growth. The mean number of colonies shown in the lower bar chart was generated from four areas for each group and represents the mean±SEM of triplicate measurements, **P<0.01 versus Si Con cells.

Supplemental Fig. 2 RACK1 activated SHH/GLI transcriptional activity. Luciferase reporter assays showed that Over-expression of RACK1 activated GLIBS luciferase reporter in three different NSCLC cell lines, but did not activate or inhibit TCF-dependent Top Flash reporter activity either without or with addition of Wnt3a conditioned medium. Over-expressed RACK1 has no effect on the Notch1 luciferase reporter activity either without or with over-expression of Notch1IC (mean±SEM; n=3, *P< 0.05, **P< 0.01 versus empty vector-transfected cells (Con)).

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Supplemental Fig. 3 Over-expression of Gli1 reversed the knockdown-RACK1 induced cell apoptosis. FACS assays of Si Con or Si RACK1 6# A549 or H23 cells, Si RACK1 6# A549 or H23 cells transiently transfected with Gli1 expression constructs.

Supplemental Fig. 4 RACK1 interacts with Smo and knock-down RACK1 impairs Smo phosphorylation. A, Knock-down of RACK1 decreased the Ser/Thr phosphorylation of Smo. Proteins from cell extracts were immunoprecipitated with an anti-Smo antibody and immunobloted with an antibody that identifies Ser/Thr phosphorylation. Knock-down of RACK1 diminished the Shh-N induced Smo electrophoretic mobility shift which indicates Smo phosphorylation and Shh-N induced Gli1 accumulation. B, Over-expression of RACK1 promoted the Shh-N induced Smo electrophoretic mobility shift which represents Smo phosphorylation and Gli1 accumulation. C, Exogenous and endogenous co-immunoprecipitation assays demonstrate the interaction between Smo and RACK1. Cell lysates of H23 cells were incubated either with anti-RACK1, anti-Smo, anti-Flag, anti-Myc antibody, IgM or IgG.

Supplemental Fig. 5 NSCLC Cell Lines Lacked Primary Cilium after 72hs of Serum starvation. One normal lung cell line (Bease-2B) and three NSCLC cell lines (H520, A549 and H23) were immunostained with anti-Ac-tublin (Red) which identified primary cilium and anti-γ-tublin (Green) which identified centrosomes, cell nuclei were counterstained with Hochest (blue).

Supplementary Table1: Relationship between levels of expression of Gli1 in NSCLC and clinical and pathological features.

Clinical Characteristics	No. patient	Gli1 negative (n=16)	Gli1 positive (n=47)	Spearman's rho
Gender				
Male	40	11	29	***p***=0.620
Female	23	5	18	
Age				
≤60	29	9	20	***p***=0.350
>60	34	7	27	
Tumor Type				
Squamous carcinoma	16	3	13	***p***=0.440
Adenocarcinoma	41	11	30	
Adenosquamouscarcinoma	6	2	4	
Metastasis				
N0	30	10	20	***p***=0.168
N1&N2	26	5	21	
M1	7	1	6	
Smoke				
No	35	6	29	***p***=0.095
Yes	28	10	18	
Tumor Differentiation				
Well	10	4	6	***p***=0.001**
Moderate	14	8	6	
Poor	39	4	35	
Tumor Size				
<3cm	19	6	13	***p***=0.467
≥3cm	44	10	34	
TNM Stage				
Ⅰ	10	4	6	***p***=0.136
Ⅱ	20	6	14	
Ⅲ	26	5	21	
Ⅳ	7	1	6	

Abbreviations: TNM, tumor node metastasis.

*P<0.05 and**P<0.01 are set for significant and highly significant difference, respectively, by Spearman's rho test.

Grade of Tumor differentiation, tumor type, and metastasis are determined by the pathologists.

Tumor stages are classified according to the TNM classification of the American Joint Committee on Cancer and the International Union.

Tumor size is measured by the surgeons.

Figure S1

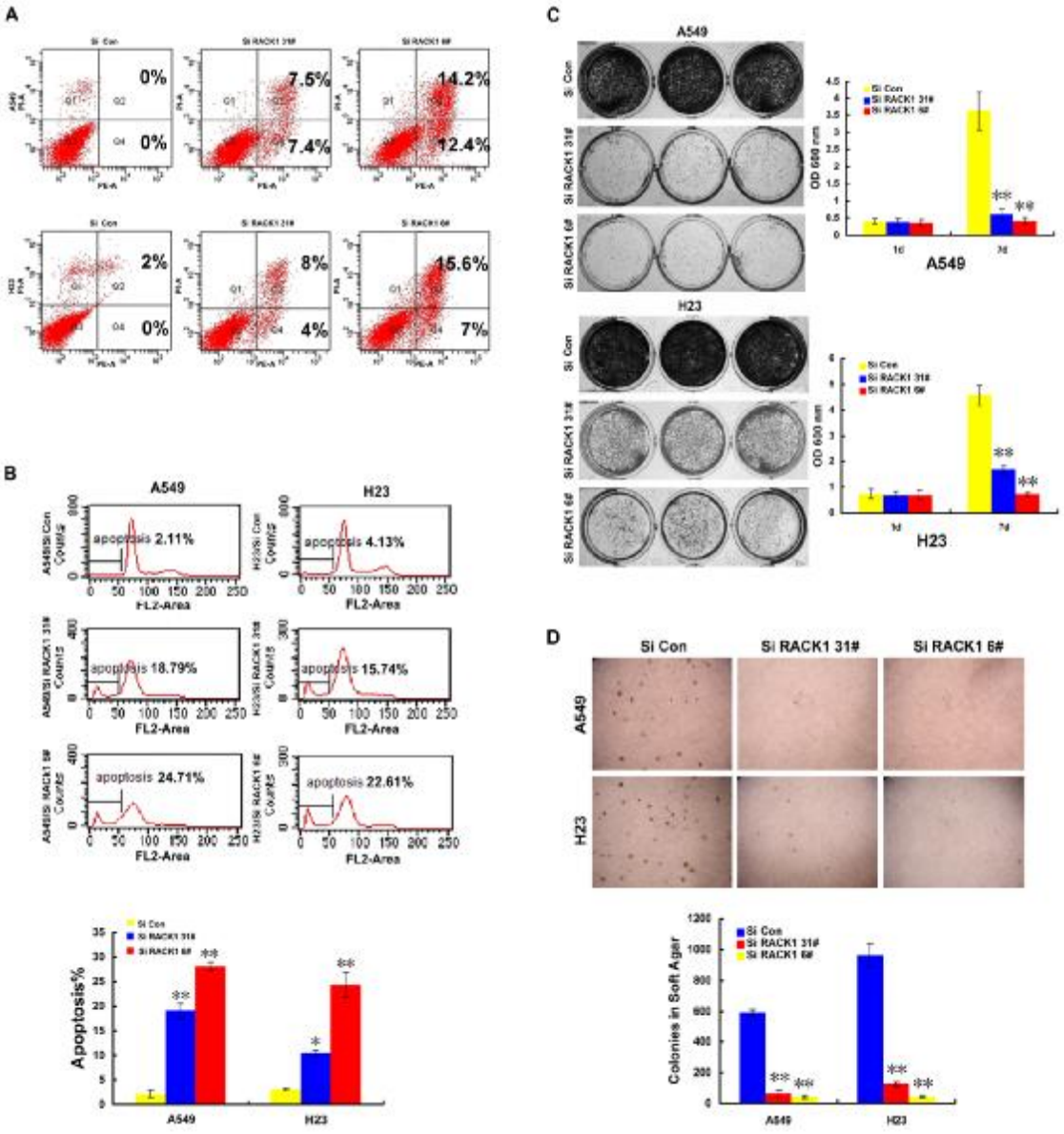
A
0%
0%
7.5%
7.4%
14.2%
12.4%
2%
0%
8%
4%
15.6%
7%
B
A549
H23
apoptosis 2.11%
apoptosis 4.13%
apoptosis 18.79%
apoptosis 15.74%
apoptosis 24.71%
apoptosis 22.61%
FL2-Area
Apoptosis%
C
A549
H23
Si Con
Si RACK1 31#
Si RACK1 6#
OD 690 nm
D
Si Con
Si RACK1 31#
Si RACK1 6#
A549
H23
Colonies in Soft Agar

Figure S2

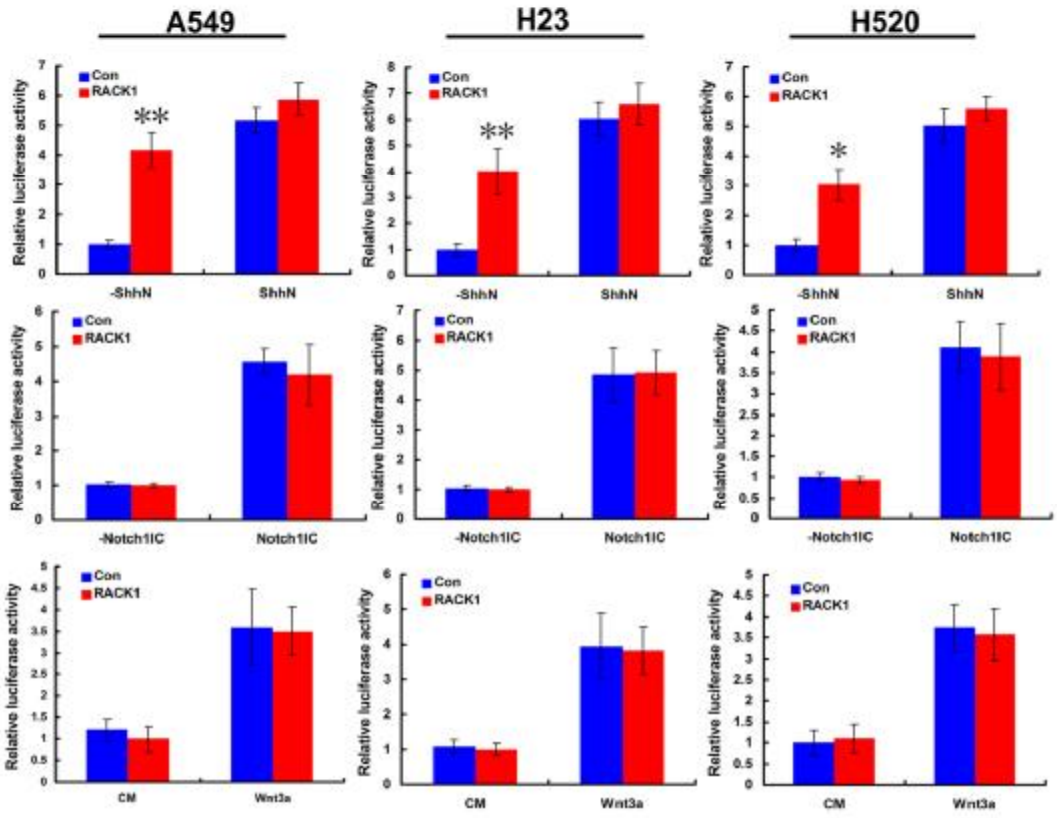
A549
H23
H520
Con
RACK1
Relative luciferase activity
**
**
*
-ShhN
ShhN
-Notch1IC
Notch1IC
CM
Wnt3a

Figure S3

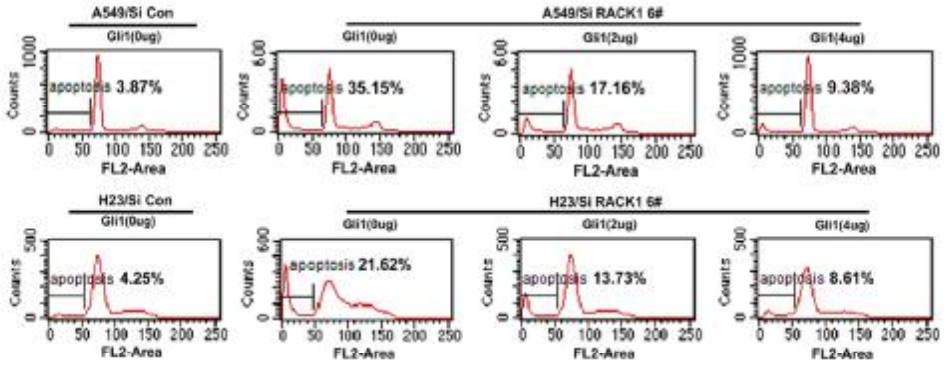
A549/Si Con
Gli1(0ug)
apoptosis 3.87%
A549/Si RACK1 6#
Gli1(0ug)
apoptosis 35.15%
Gli1(2ug)
apoptosis 17.16%
Gli1(4ug)
apoptosis 9.38%
H23/Si Con
Gli1(0ug)
apoptosis 4.25%
H23/Si RACK1 6#
Gli1(0ug)
apoptosis 21.62%
Gli1(2ug)
apoptosis 13.73%
Gli1(4ug)
apoptosis 8.61%
Counts
FL2-Area

Figure S4

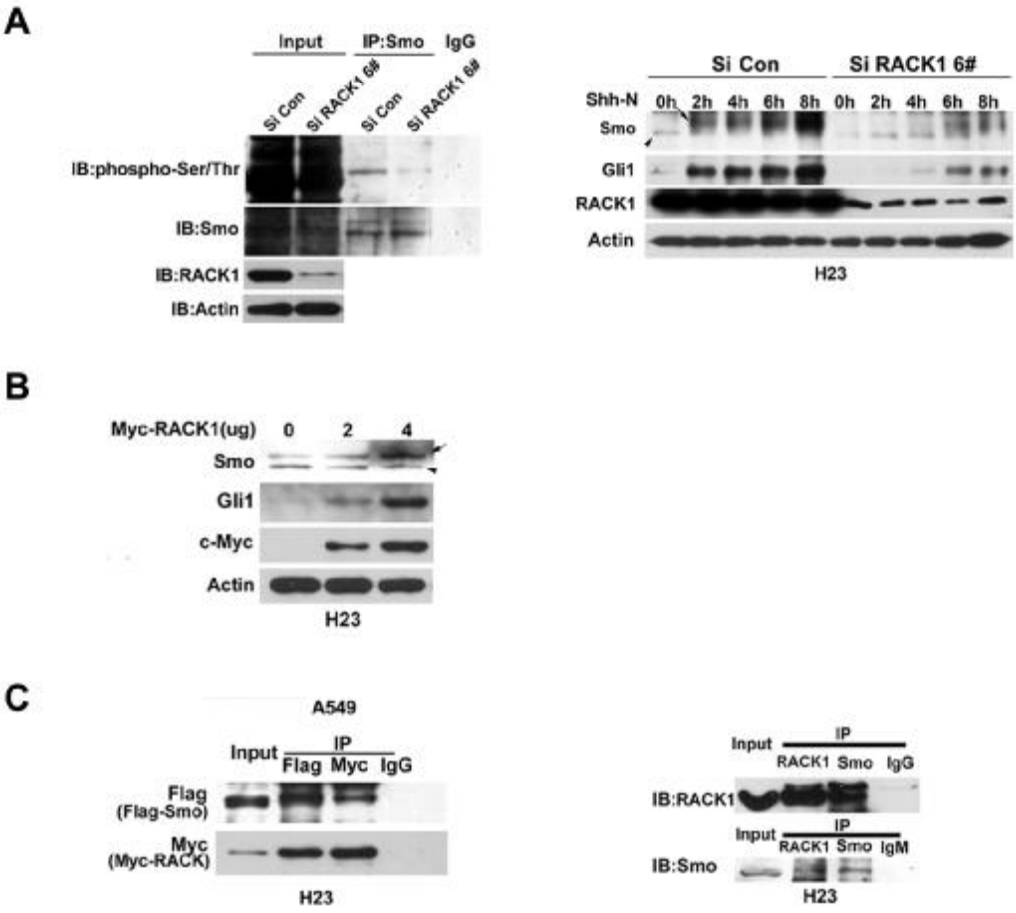
A
Input
IP:Smo
IgG
Si Con
Si RACK1 6#
Si Con
Si RACK1 6#
IB:phospho-Ser/Thr
IB:Smo
IB:RACK1
IB:Actin
Si Con
Si RACK1 6#
Shh-N
0h 2h 4h 6h 8h 0h 2h 4h 6h 8h
Smo
Gli1
RACK1
Actin
H23
B
Myc-RACK1(ug) 0 2 4
Smo
Gli1
c-Myc
Actin
H23
C
A549
Input
IP
Flag Myc IgG
Flag
(Flag-Smo)
Myc
(Myc-RACK)
H23
Input
IP
RACK1 Smo IgG
IB:RACK1
Input
IP
RACK1 Smo IgM
IB:Smo
H23

Figure S5