AKR1C1 Activates STAT3 to Promote the Metastasis of Non-Small Cell Lung Cancer

Supplementary Materials and methods:

Wound-healing assay

PC-9 cells were plated into 24-well plate at a confluence of 95% with complete culture medium. After starving overnight, the cell monolayer was scrapped with a sterilized 200 μL pipette tip. Cells were washed twice, then cultured for indicated times with fresh culture medium. After that, the motility ability of PC-9 cells was photographed using microscope.

Supplementary Figure Legends:

Supplemental Figure 1. ***AKR1C1 has the potential to promote metastasis.*** **(A)** GEO dataset (GDS2865 and GDS3091) analyses of AKR1C1 in metastatic samples [26, 27]. **(B)** The transcript expression of AKR1C1 was highly upregulated in NSCLC clinical patients from 6 datasets [28]. Gene: AKR1C1; Analysis Type: Cancer vs. Normal Analysis; Data Type: mRNA; Sample Type: Clinical Specimen.

Supplemental Figure 2. ***AKR1C1 expression correlates the invasion of NSCLC cell lines.*** **(A)** The AKR1C1 expression among several cell lines (six NSCLC cell lines and a normal lung epithelial cell line) was evaluated by western blot analyses. **(B)** The invasive ability of NCI-H1299 (AKR1C1 low expression) and NCI-H460 (AKR1C1 high expression). **(C-D)** AKR1C1 depletion decreased the invasion of A549 cells. **(E)** AKR1C1 overexpression increased the migration of NCI-H1299 cells. **(F)** AKR1C1 deletion impeded the motility of PC-9 as indicated by wound-healing assay. Statistical significance was determined by a two-tailed, Student's t-test. ** $P < 0.01$.

Supplemental Figure 3. ***The siRNA sequences are specifically targeting AKR1C1.*** **(A)** qRT-PCR analyses were conducted by using high-specific primers to amplify AKR1C1, AKR1C2 and AKR1C3. Statistical significance was determined by a two-tailed, Student's t-test. ** $P < 0.01$.

Supplemental Figure 4. ***The pro-metastatic effects of AKR1C1-stable-overexpression in vitro.*** **(A-B)** The stable overexpression efficiency (A, left) or knockdown efficiency (B, left) were confirmed by western blot. A (middle) and B (middle), SRB assays were used to assess the ability of cellular proliferation. A (right) and B (right), representative photographs were presented for transwell assays. **(C)** AKR1C1 increased inducible STAT3

phosphorylation. Cells were starving overnight, then introduced with IL-6 (20 ng/mL) for 30 min.

Supplemental Figure 5. ***The analyses of metastatic nodules from nude mice.*** **(A-B)** The numbers of metastatic nodules from different tissues (A for lung, B for lung plus liver) were counted under a microscope. Statistical significance was determined by a two-tailed, Student's t-test. * $P < 0.05$.

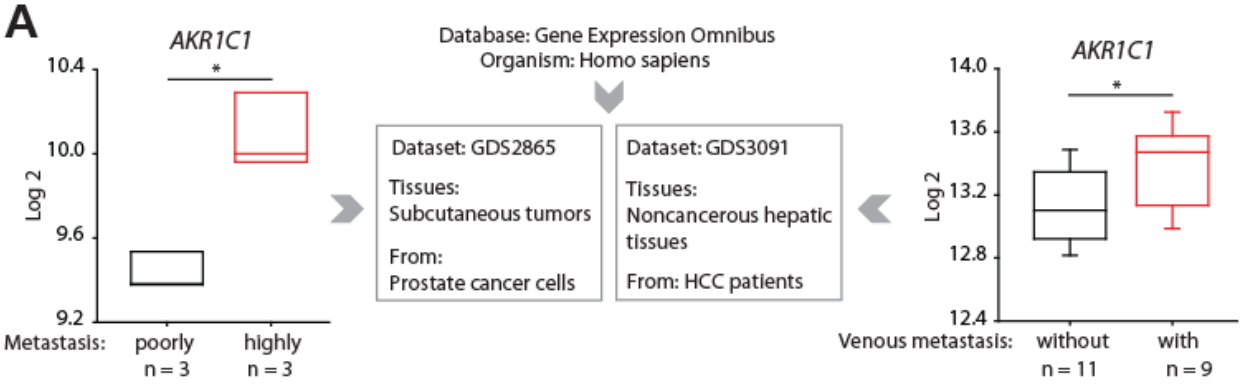
Supplemental Figure 6. ***The microarray analysis of NCI-H460 (siAKR1C1 v.s. NC).*** **(A)** The top 3000 genes were analyzed by functional annotation. **(B)** The proteins of the top 3000 genes were classified. **(C)** Overlay analyses of transcription factors (A) and phosphoproteins (B).

Supplemental Figure 7. ***Semi-quantitative densitometry analyses of protein levels in Fig. 5A.*** Normalized to corresponding expression of internal standard, the quantitative fold changes were presented as relative optical densities of bands. Statistical significance was determined by a two-tailed, Student's t-test. * $P < 0.05$, ** $P < 0.01$.

Supplementary Figures:

Supplemental Figure 1

A



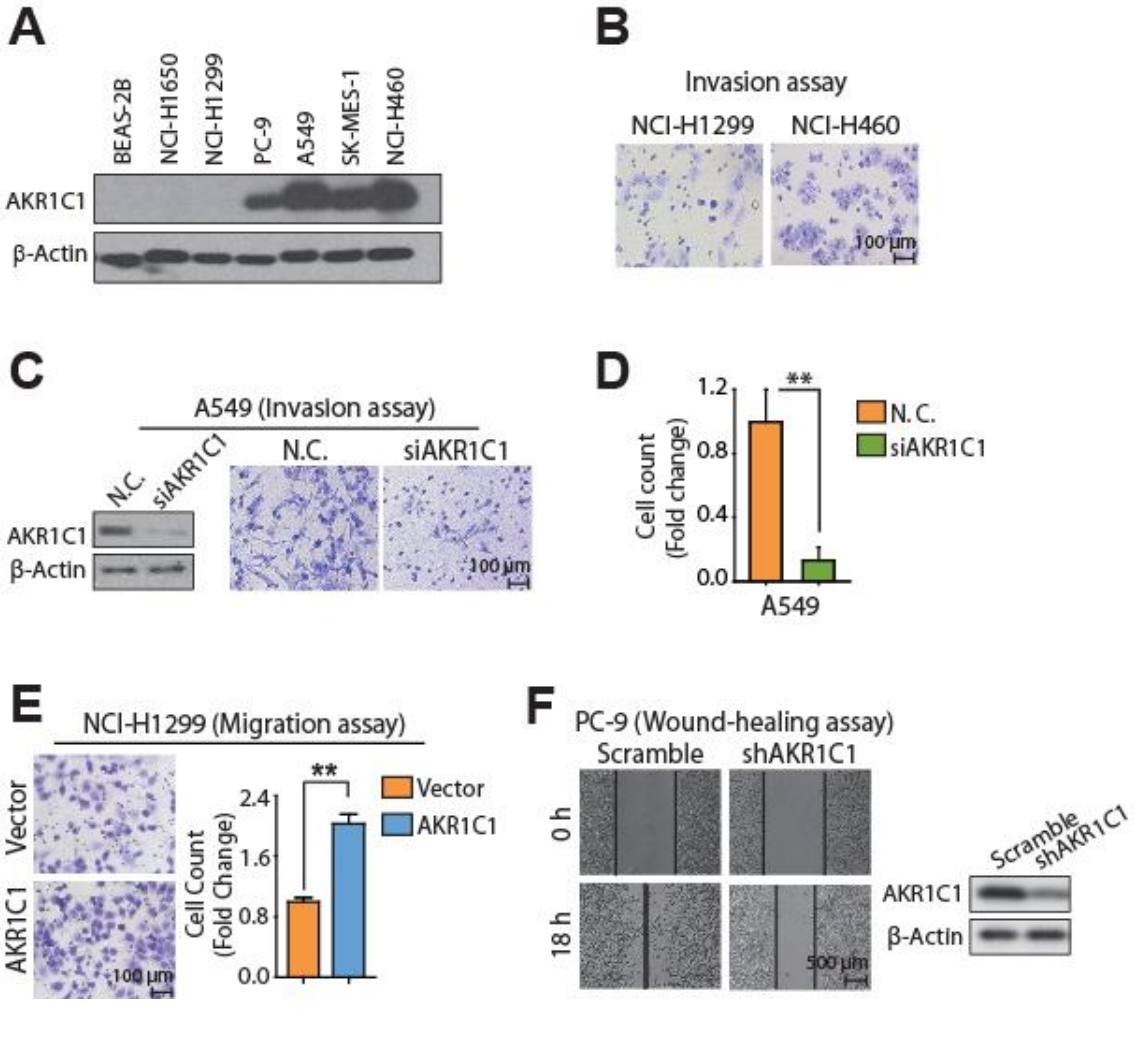
B

Upregulation of *AKR1C1* in NSCLC clinical samples*

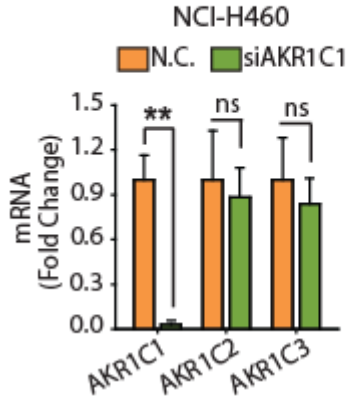
Upregulation of AKR1C1 in lung cancer	Fold change	p-Value	Case number	Database
Squamous Cell Lung Carcinoma vs. Normal	24.361	7.72×10^{-7}		
Large Cell Lung Carcinoma vs. Normal	6.594	0.018	31	Yamagata Lung
Lung Adenocarcinoma vs. Normal	4.954	0.004		
Squamous Cell Lung Carcinoma vs. Normal	5.514	7.40×10^{-9}		
Large Cell Lung Carcinoma vs. Normal	2.753	0.008	6	Hou Lung
Lung Adenocarcinoma vs. Normal	1.359	0.038		
Squamous Cell Lung Carcinoma vs. Normal	7.564	9.78×10^{-4}	10	Wachi Lung
Squamous Cell Lung Carcinoma vs. Normal	5.552	0.001	203	Bhattacharjee Lung
Squamous Cell Lung Carcinoma vs. Normal	9.726	0.002	73	Garber Lung
Squamous Cell Lung Carcinoma vs. Normal	2.231	0.004	93	Talbot Lung

*: data collected from Oncomine database

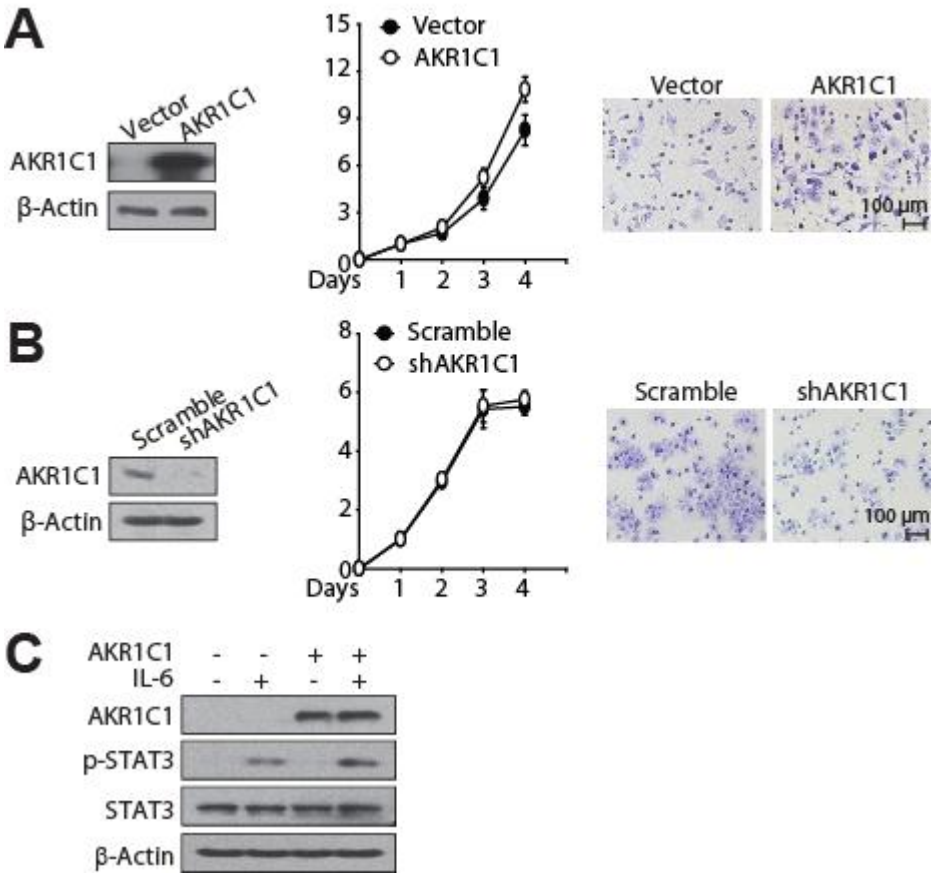
Supplemental Figure 2



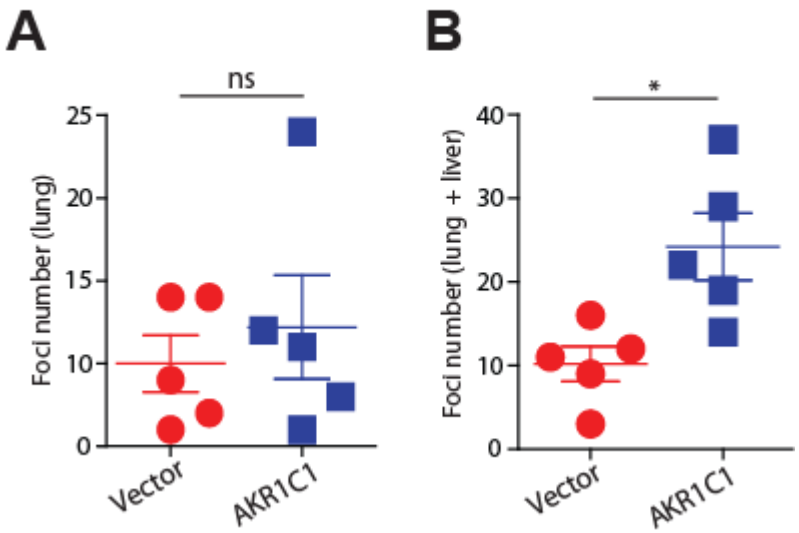
Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6

A

Functional Annotation Clustering

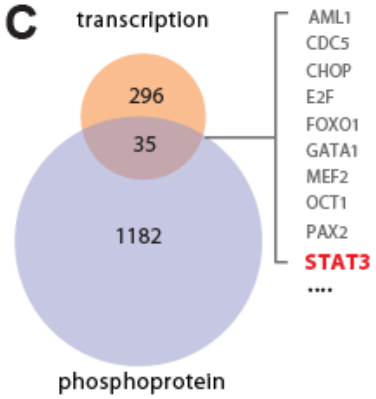
	Term	Count	P_Value
Annotation Cluster 1	nucleus	650	2.1×10^{-12}
	transcription	296	7.3×10^{-4}
Enrichment Score: 4.63	transcription regulation	286	1.9×10^{-3}
	dna-binding	243	1.1×10^{-1}
Annotation Cluster 2	zinc	320	8.3×10^{-5}
	metal-binding	420	1.2×10^{-4}
Enrichment Score: 3.79	zinc-finger	252	4.6×10^{-4}
Annotation Cluster 3	cell cycle	99	9.7×10^{-9}
	mitosis	43	2.4×10^{-5}
Enrichment Score: 5.52	cell division	54	1.1×10^{-4}
Annotation Cluster 4	P-loop	29	2.1×10^{-4}
	nucleotide-binding	25	1.8×10^{-3}
Enrichment Score: 2.56	GTP binding	14	5.4×10^{-2}

B

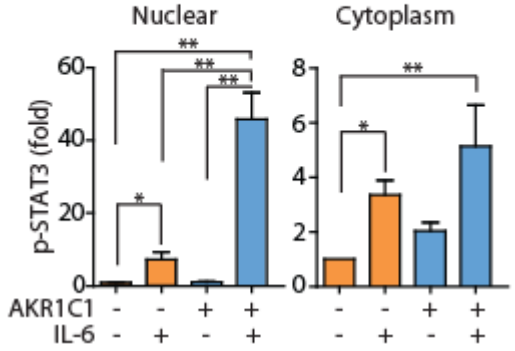
Functional Annotation Chart

Term	Count	P_Value
phosphoprotein	1182	3.8×10^{-44}
alternative splicing	1091	2.9×10^{-18}
nucleus	650	2.1×10^{-12}
acetylation	430	2.2×10^{-12}
cytoplasm	502	6.2×10^{-9}
nucleotide-binding	275	5.0×10^{-8}
coiled coil	298	8.0×10^{-5}
zinc	320	8.3×10^{-5}
metal-binding	420	1.2×10^{-4}
zinc-finger	252	4.6×10^{-4}
transcription	296	7.3×10^{-4}
transcription regulation	286	1.9×10^{-3}

C



Supplemental Figure 7



Supplementary Tables:

Table S1. Human short hairpin RNA target sequences

	Sequence
siAKR1C1	namely, siAKR1C1#1
siAKR1C1#1	5’-AAGCTTTAGAGGCCACCAAAT-3
siAKR1C1#2	5’-ATGTTGACCTCTACCTTATTC-3’
siSTAT3#1	5’-GCAAAGAATCACATGCCACTT-3’
siSTAT3#2	5’-GGCGTCCAGTTCACTACTAAA-3’
siJAK2#1	5’-CCCTGACCCTAAATAATACAT-3’
siJAK2#2	5’-CACAGTTTGAAGAGAGACATT-3’
siEGFR#1	5’-CGCAAAGUGUGUAACGGAAUA-3’
siEGFR#2	5’-GCAAAGUGUGUAACGGAAUAGGUAU-3’
shAKR1C1	5’-CCGGAAGCTTTAGAGGCCACCAAATCTCGAGATTTGGTGGCCTCTAAAGCTTTTTTTG-3’

Table S2. Primers of qRT-PCR

Primer		Sequence
GAPDH	Forward	5’-GTCATCCATGACAACTTTGG-3’
	Reverse	5’-GAGCTTGACAAAGTGGTCGT-3’
Twist	Forward	5’-GGCATCACTATGGACTTTCTCTATT-3’
	Reverse	5’-GGCCAGTTTGATCCCAGTATT-3’
MMP2	Forward	5’-ACAGCAGGTCTCAGCCTCAT-3’
	Reverse	5’-TGAAGCCAAGCGGTCTAAGT-3’
SOX2	Forward	5’-TACAGCATGTCCTACTCGCAG-3’
	Reverse	5’-GAGGAAGAGGTAACCACAGGG-3’
N-cadherin	Forward	5’-ACAGTGGCCACCTACAAAGG-3’
	Reverse	5’-CCGAGATGGGGTTGATAATG-3’
Fibronectin	Forward	5’-TCCAAGCGGAGAGAGT-3’
	Reverse	5’-GTGGGTGTGACCTGAG-3’
CCND1	Forward	5’-GGCGGAGGAGAACAAACAGA-3’
	Reverse	5’-TGTGAGGCGGTAGTAGGACA-3’
MCL1	Forward	5’-GGACAAAACGGGACTGGCTA-3’
	Reverse	5’-CAGCAGCACATTCCTGATGC-3’
ZEB1	Forward	5’-ATGACCTGCCAACAGACCAG-3’
	Reverse	5’-TTGCCCTTCCTTTCCTGTGT-3’
AKR1C1	Forward	5’-ATTTGCCAGCCAGGCTAGTG-3’
	Reverse	5’-AGAATCAATATGGCGGAAGCC-3’
AKR1C2	Forward	5’-CCTAAAAGTAAAGCTCTAGAGGCCGT-3’
	Reverse	5’-GAAAATGAATAAGATAGAGGTCAACATAG-3’
AKR1C3	Forward	5’-GAGAAGTAAAGCTTTGGAGGTCACA-3’
	Reverse	5’-CAACCTGCTCCTCATTATTGTATAAATGA-3’