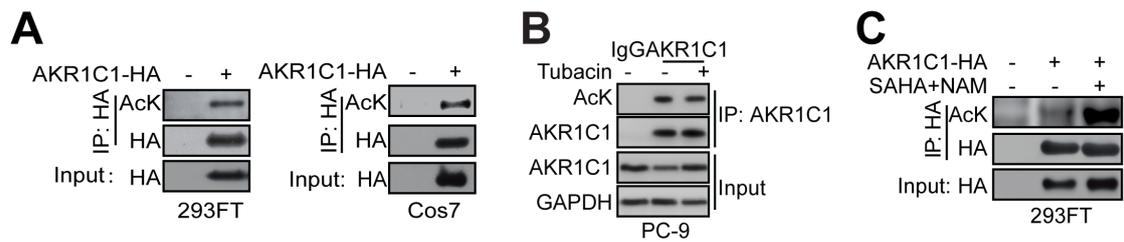
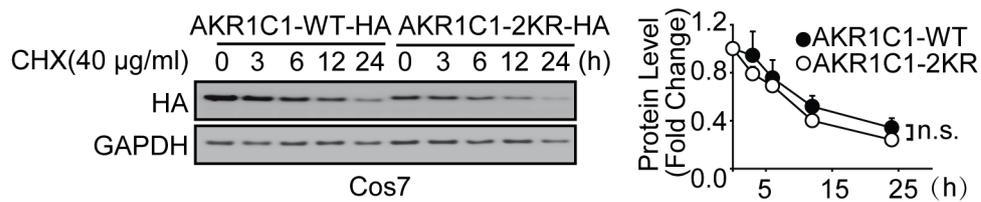


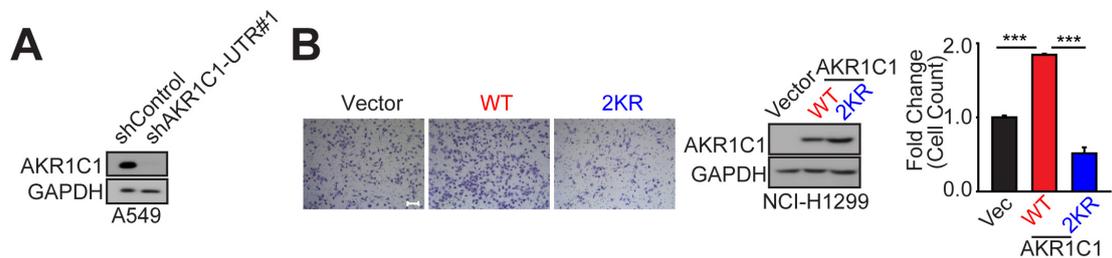
**Supplementary Figures and Legends:**



**Figure S1. AKR1C1 is an acetylated protein.** (A) Exogenous AKR1C1 is acetylated in 293FT cells and Cos7 cells. Acetylation of immunoprecipitated HA-tagged AKR1C1 from 293FT cells and Cos7 cells. (B) Acetylation of immunoprecipitated endogenous AKR1C1 from NSCLC PC-9 cells, with or without the exposure to HDAC6 inhibitor Tubacin. (C) Immunoprecipitated HA-tagged AKR1C1 from 293FT cells was treated with HDAC inhibitors SAHA (4  $\mu$ M) and NAM (5 mM) simultaneously for additional 12 h.

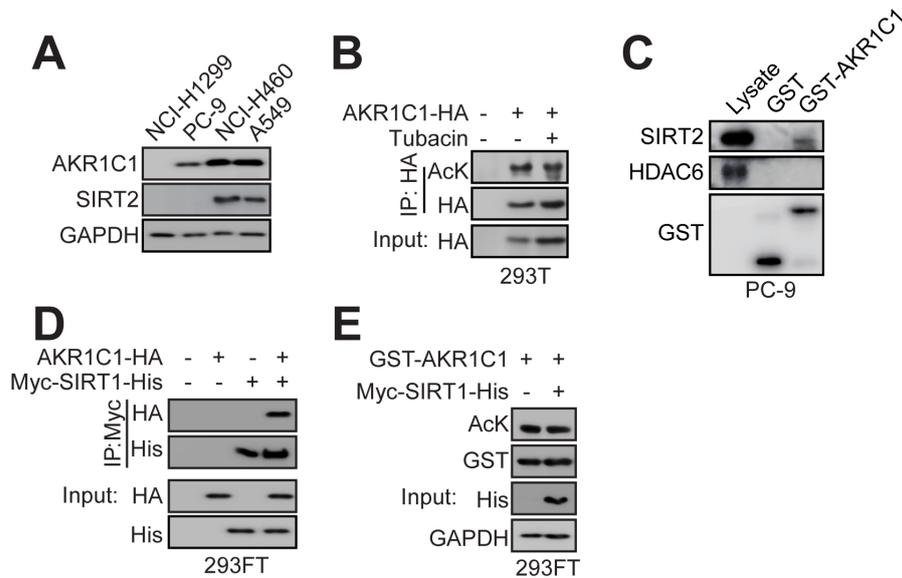


**Figure S2. Acetylation imposes little effects on AKR1C1 protein stability.** AKR1C1 WT and 2KR mutant exhibited equivalent protein half-life (ANOVA analysis was used).

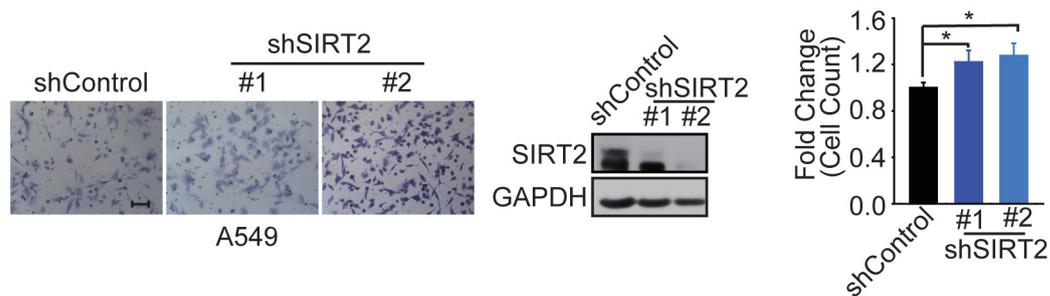


**Figure S3. Acetylation of AKR1C1 is crucial for its pro-metastatic ability of NSCLC cells.** (A) Western Blotting analysis showing the knockdown efficiency of AKR1C1 targeting the untranslated region 1 (UTR#1). (B) Different metastasis phenotypes of NCI-H1299 cells which

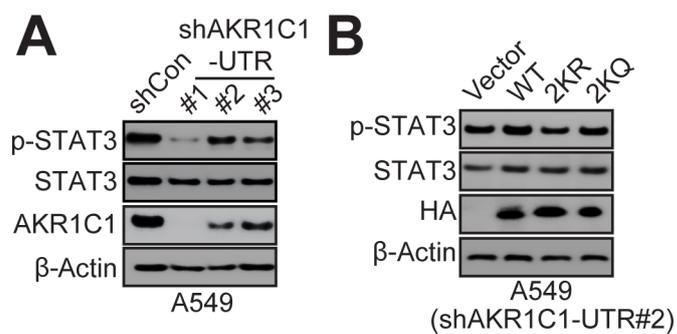
were stably expressing Vector, AKR1C1-WT and AKR1C1-2KR. Western Blotting analysis showing the stable transfected efficiency of AKR1C1s. Scale Bar, 200  $\mu$ m; Statistical significance was determined by Student's t-test ( $n = 3$ ; \*\*\*:  $p < 0.001$ ).



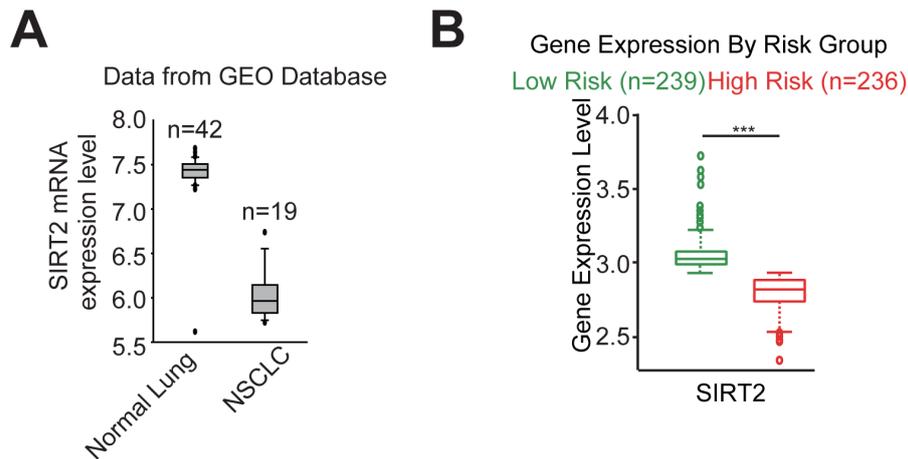
**Figure S4. SIRT1 could not deacetylate AKR1C1.** (A) The AKR1C1 and SIRT2 protein levels were detected in different NSCLC cell lines. (B) HA-tagged AKR1C1 was transfected into 293FT cells for 48 h. Cells were treated with vehicle or Tubacin, and then subjected to analyses of acetylation levels. (C) Recombinant GST-tagged AKR1C1 protein was purified and incubated with whole cell lysate of PC-9 cells, and subjected to GST-pull down. (D) HA-tagged AKR1C1 and His-tagged SIRT1 were transfected into 293FT cells for 48 h. Immunoprecipitation-western blot for analyzing the interaction between AKR1C1 and SIRT1. (E) *In vitro* deacetylation assay was used to evaluate the effect of SIRT1 on AKR1C1, mix recombinant GST-AKR1C1 with purified protein His-tagged SIRT1 in 37°C for 2 h. Western Blot analysis shows the acetylation of GST-AKR1C1 in reaction mixtures.



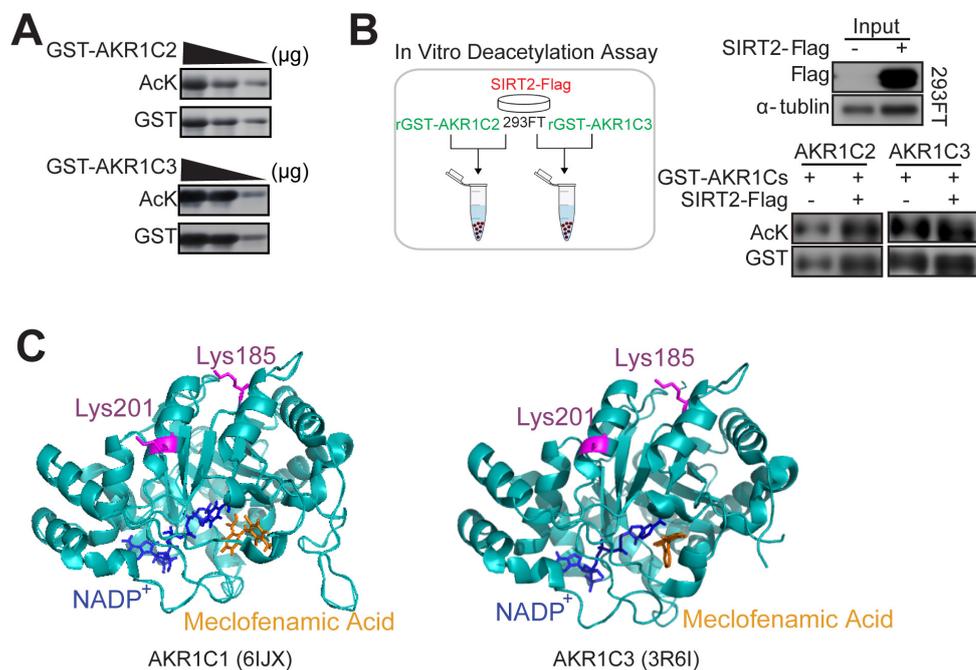
**Figure S5. Depletion of endogenous SIRT2 increases the mobility of NSCLC cells.** SIRT2 in A549 cells was depleted using shRNA (2 different sequences were introduced), and a transwell assay was utilized to examine the metastatic potential. Scale Bar, 100  $\mu$ m. Data were analyzed using two-tailed Student's t-tests. (n = 3; \*:  $p < 0.05$ )



**Figure S6. Acetylation deficiency impairs the effect of AKR1C1 to transactivated STAT3 in A549 cells with shAKR1C1-UTR#2.** (A) Western Blotting analysis showing the knockdown efficiency of AKR1C1 targeting the untranslated region (UTR) regions. (B) Western Blotting analysis of p-STAT3 and STAT3 protein levels in the wild-type and mutant-type (2KR and 2KQ) AKR1C1 transfected in A549 cells (shAKR1C1-UTR#2).

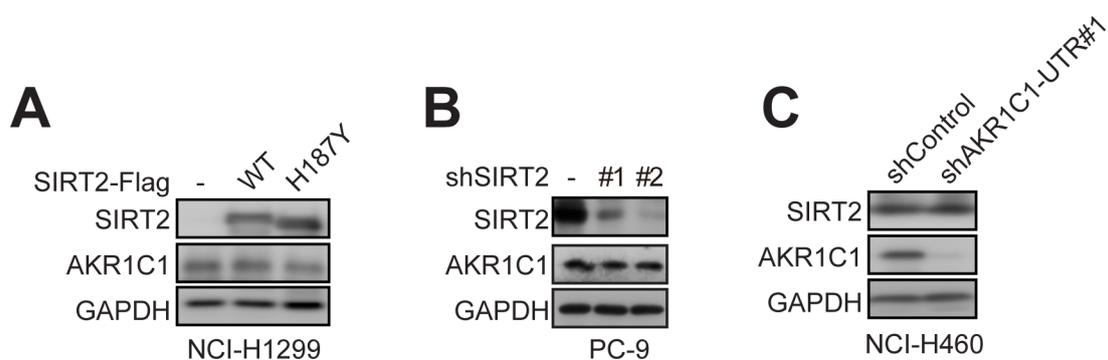


**Figure S7. The inverse correlation of SIRT2 expression levels with NSCLC.** (A) *SIRT2* mRNA levels in normal lung tissues and tumor samples from NSCLC patients (GSE40275). (B) Gene expression levels of *SIRT2* in the low risk (n = 239) and high risk (n = 236) groups of adenocarcinoma of lung cancer patients (TCGA database) (\*\*\*:  $p < 0.001$ ).



**Figure S8. The acetylation of AKR1C2 and AKR1C3 was not regulated by SIRT2.** (A) Purified GST-AKR1C2 and GST-AKR1C3 are acetylated. (B) *In vitro* deacetylation assay was utilized to determine the influence of SIRT2 on GST-AKR1C2/C3 acetylation. (C) The position of lysine 185/201 in the AKR1C1/AKR1C3-NADP<sup>+</sup>-Meclofenamic Acid complexes (RCSB PDB:

AKR1C1/6IJX, AKR1C3/3R6I). The coenzyme NADP<sup>+</sup> and inhibitor Meclofenamic Acid that interact with AKR1C1/C3 are depicted using stick models.



**Figure S9. The protein levels of AKR1C1 and SIRT2 are not influenced by each other. (A)** Exogenous SIRT2 imposed minimal effect on AKR1C1 protein levels. **(B&C)** Knock-down of SIRT2 (B) or AKR1C1 (C) failed to influence the protein levels of AKR1C1 (B) or SIRT2 (C).

**Table S1. Information of plasmid constructs**

Plasmid Constructs	Tag	Plasmid Constructs	Tag
pCMV6-AKR1C1-WT	HA	pEGFP-SIRT2-WT	Flag
pCMV6-AKR1C1-2KR	HA	pEGFP-SIRT2-H187Y	Flag
pCMV6-AKR1C1-2KQ	HA	pEGFP-SIRT1-WT	His/Myc
pCMV6-AKR1C1-WT	No-tag	pGEX-4T1	GST
pCMV6-AKR1C1-2KR	No-tag	pGEX-4T1-AKR1C1-WT	GST
pGEX-4T1-AKR1C1-2KR	GST		