Supporting Information

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SI Materials and Methods

Antibodies. The following antibodies were used for Western blot analysis and immunostaining: IKKα (IMG-136A), from IMGENEX; NRF2 (sc-722), p53 (sc-6243), p21 (sc-6246), NOX2 (sc-5827), KEAP1 (sc-15246), Lamin B (sc-6216), CC10 (sc-9772), and SP-C (sc-13979), from Santa Cruz Biotechnology; p65 (8242) and p50 (12540), from Cell Signaling Technology; ubiquitin (MMS-257P) from Covance; β-actin (A-5441) from Sigma-Aldrich; and MDM2 (ab16895), NQO1(ab34173), p53 (ab26), α-tubulin (ab4074) and 8-OHdG (ab26842), from Abcam.

SA-β-Gal Staining. Here 20-μm sections were cut from optimal cutting temperature compound (OTC) -embedded frozen tissues and then fixed in 1% formaldehyde for 1 min. Sections were incubated at 37 °C overnight in 5-bromo-4-chloro-3-indolyl-β-D-galactosidase (X-gal) solution (0.5 mg/mL X-gal, 40 mM citric acid/sodium phosphate buffer, 5 mM potassium ferrocyanide, 5 mM potassium ferricyanide, 2 mM $MgCl_2$, and 150 mM NaCl, pH 5.5) (1). Sections were rinsed with PBS and counterstained with Neutral Red. For cultured cells, cells were washed with PBS and fixed in 1× Fixative Solution (included in Senescence β-Galactosidase Staining Kit 9860; Cell Signaling Technology) for 5 min. The cells were then incubated at 37 °C overnight with the same staining solution. Three fields were selected, and positive cells were counted.

Measurement of ROS. Here 20-μm sections were cut from OCT-embedded frozen tissues and incubated with 10 μM DCF-DA (Molecular Probes) at 37 °C for 0.5∼1 h. The fluorescent signal was detected by fluorescence microscopy, and the intensity was analyzed using ImageJ software. For cultured cells, cells were washed with PBS and incubated with 10 μM DCF-DA at 37 °C for 30 min. The fluorescent signal was detected by a fluorescence microscope or analyzed on a LSR Fortessa flow cytometer (BD Biosciences).

Treatment with NAC and Apocynin. NAC (A7250; Sigma-Aldrich) was administered in the drinking water (10 g/L). Apocynin (acetovanillone, W508454; Sigma-Aldrich) was administered in the drinking water (0.25 g/L) or injected i.p. (100 mg/kg) three times a week. NAC and apocynin supplementation did not affect body weight or food and water intake. For apocynin treatment, both control and apocynin drinking water contained 1.5% ethanol.

Establishment of Kras-CL and $Kras^{IKK\alpha L}$ Cell Lines. Fresh lung tumors from $Kras^{G12D}$ mice were minced and enzymatically digested with trypsin for 1 h at 37 °C. Cells were further disaggregated by pipetting and serial filtration. Single-cell suspensions were placed on culture dishes in RPMI medium 1640 supplemented with 10% serum and 2 mM L-glutamine; these cells were then designated Kras-CL cells. Kras-CL cells were injected intratracheally into C57BL/6 mice. At 3 mo after injection, the lung tumors from these mice were used to establish another cell line, named $Kras^{IKK\alpha L}$, in vitro. Cultured cells were transfected with siRNA (SR417771 for mouse IKKα, SR417549 for mouse NOX2, SR407874 for mouse NQO1, SR427248 for mouse NRF2, and SR30004 for nontargeting siRNA; OriGene) using transfection reagents (Lipofectamine RNAiMAX; Invitrogen). Reintroduction of IKKα, IKKα-KA, or IKKα-ΔLZ was performed in Kras-CL cells using Lipofectamine 2000 (Invitrogen). The truncated IKKα-ΔLZ (Δaa 411–531) expression vector was generated by PCR and subcloned in to the pcDNA vector. The generated vector was verified by sequencing. To compare tumorigenic capabilities, WT mice were injected intratracheally with 5×10^6 of Kras-CL or $Kras^{IKK\alpha L}$ cells and then euthanized at 1.5 mo after injection. In athymic nude mice, cells (5×10^6) were injected s.c. into both flanks and euthanized at 2–3 wk after injection. Tumor volume (mm^3) was calculated with the following formula: (long-axis × short-$axis^2$)/2.

Mutation Analysis for IKKα. Total RNA was isolated from cell lines or tumor tissues, and cDNAs from these samples were synthesized. The PCR fragments were generated with primers (mouse *Ikkα* C-terminal region: 5′-CCCCTCCAGTATCAGCATGG-3′ and 5′-GGCACAAAAGTCCAACCCCT-3′). For *Ikkα* mutation analysis, the PCR products were subcloned into a TOPO TA vector (Invitrogen) and sequenced. The sequences were compared with those at the National Center for Biotechnology Information.

Detection of LOH of *Ikkα* Gene in Mouse Lung Tumors. Paraffin-embedded lung tissues from $Kras^{G12D};Ikk\alpha^{+/-}$ mice were cut into 20-μm-thick sections. To extract genomic DNA, lung tumor areas from lung tissue sections were trimmed, deparaffinized using xylene/ethanol, and then incubated in lysis buffer (50 mM Tris·HCl, 10 mM EDTA, 1% Tween 20, and 200 μg/mL fresh proteinase K) as described previously (2). To determine LOH of the *Ikkα* gene, genomic DNA was amplified by PCR with the following primers included: *Ikkα* WT allele, 5′-CAGGTCATCAGACTTGGTATCAGGC-3′ and 5′-CCAAAGCTCCAATAATCCAGAGTGG-3′; *Ikkα* knockout (KO) allele, 5′-CAACATTAAATGTGAGCGAG-3′ and 5′-GGAACATGGGAGTATTTGG-3′ (12).

Intratracheal Injection of Ad.Cre-GFP. $Kras^{G12D}$ mice with a C57BL/6 background were crossed with $Ikk\alpha^{KA/KA}$, $Ikk\alpha^{f/f}$, and $Ikk\alpha^{+/-}$ mice with a C57BL/6 background. At age 6–8 wk, the mice were intratracheally treated with 2.5×10^7 PFU of Ad.Cre-GFP (3). The Ad.Cre was generated by the Viral Technology and Molecular Detection Groups, Protein Expression Laboratory, Frederick National Laboratory for Cancer Research.

Detection of Cre-Mediated Recombination. Genomic DNA was prepared from tissues or cells. PCR analysis was performed with the *Kras* primers, including 5′-GTCTTTCCCCAGCACAGTGC-3′, 5′-CTCTTGCCTACGCCACCAGCTC-3′, and 5′-AGCTAGCCACCATGGCTTGAGTAAGTCTGCA-3′, to amplify the WT *Kras* (∼622 bp) and recombined $Kras^{G12D}$ allele (∼650 bp). Primer information was obtained from the laboratory of Tyler Jacks (https://jacks-lab.mit.edu/protocols/genotyping/kras_cond). For genotyping of the Cre-deleted *Ikkα* allele, PCR primer sequences were 5′-CTTTGCCATCATCTCTCCGGTTTGTAA-3′ and 5′-CAATAGGATAATCACTAAGCACAGT-3′ (1.2 kb, WT allele; 460 bp, Cre-deleted *Ikkα* allele).

IHC, IF Staining, and Lung Tumor Burden. The Histology and Tissue Core Facility at the Frederick National Laboratory for Cancer Research routinely prepared paraffin sections of mouse organs and performed hematoxylin and eosin (H&E) staining and IHC staining for Ki67 and K5. To assess lung tumor burden, lung tumor areas were quantified by ImageJ software in H&E-stained lung sections and displayed in a bar graph as percentage occupied of total lung area. IF staining for SP-C, CC10, and 8-OHdG was performed on paraffin sections as described previously (4). In brief, paraffin sections were incubated with antibodies against SP-C, CC10, and 8-OHdG overnight at 4 °C, then washed and

stained with the fluorescence-conjugated secondary antibodies for 1 h at room temperature. Finally, the sections were mounted with DAPI (Invitrogen) mounting medium. IF staining was visualized and the cells were photographed under a fluorescence microscope (Nikon Eclipse E800).

Cell Culture and siRNA Knockdown of Gene Expression. Human lung ADC cell line A549 was maintained in RPMI medium 1640 supplemented with 10% serum and 2 mM L-glutamine. Cultured cells were transfected with siRNA (20 nM; SR300826 for human IKKα; SR300136 for human AhR; SR30004 for nontargeting siRNA; OriGene) using transfection reagents (Lipofectamine RNAiMAX; Invitrogen).

Western Blot Assay and IP. Cell lysates or protein extracts from tissues were applied to an acrylamide gel, and the levels of protein expression were measured by Western blot analysis with specific antibodies, as described previously (4). In brief, a lysate (10–50 μg) was dissolved in a protein gel-loading buffer (Bio-Rad), heated for 5 min at 95 °C, and then resolved using 7–12% SDS/PAGE. The separated proteins were transferred to a PVDF membrane (Immobilon-P; EMD Millipore), blocked in 5% nonfat milk, and analyzed using the indicated primary and secondary antibodies. Proteins were detected using a chemiluminescence reagent (PerkinElmer). For IP, the cell lysate (200 μg) was incubated overnight with an anti-AhR or an anti-IKKα antibody and 30 μL of protein A/G beads. The pellet was then washed four times in a lysis buffer. The immunoprecipitates were subjected to SDS/PAGE for Western blot analysis with the indicated antibodies.

Preparation of Nuclear Protein. The cells were washed twice with cold PBS and were collected by spinning at 600 × *g* for 5 min. Then 200–500 μL of cytoplasmic extract buffer (10 mM KCl, 10 mM Hepes pH 7.9, 3 mM $MgCl_2$, and 1.0% Nonidet P-40) was added to the cell pellets. The pellets were gently mixed, kept on ice for 5 min, and then centrifuged at 800 × *g* for 5 min. The supernatants were collected as the cytoplasmic extract. The pellets were washed once with 500 μL of cytoplasmic extract buffer, suspended in 100–200 μL of nuclear extract buffer (400 mM KCl, 10 mM Hepes pH 7.9, 3 mM $MgCl_2$, and 1.0% Nonidet P-40), gently mixed on ice for 5 min, and centrifuged at 10,000 × *g* for 5 min. The supernatants were collected as the nuclear extract.

RT-PCR and Quantitative Real-Time PCR. Total RNA was isolated from the lung tissues or cells using Trizol reagent (Invitrogen). The cDNA was synthesized with the Tectro cDNA synthesis kit (Bioline). For conventional PCR, primers included the following: human Nox2, 5′-GCTGTTCAATGCTTGTGGCT-3′ and 5′-TCTCCTCATCATGGTGCACA-3′; human Gapdh, 5′-CCACCCATGGCAAATTCCATGGCA-3′ and 5′-TCTAGACGGCAGGTCAGGTCCACC-3′; mouse Nox2, 5′- CAGGAGTTCCAAGATGCCTG-3′ and 5′-GATTGGCCTGAGATTCATCC-3′; and mouse Gapdh, 5′-GCAGTGGCAAAGTGGAGATT-3′ and 5′-AGAAGGGGCGGAGATGATGA-3′. For quantitative PCR, genes of interest were subsequently examined using TaqMan Universal PCR Master Mix and an ABI Prism 7300 Detection System (Applied Biosystems), according to the manufacturer's instructions. PCR primers included Nox2 (Cybb; PPM-32951A-200), Nrf2 (Nfe2l2; PPM24614A-200), Nqo1 (PPM03466F-200), Gpx2 (PPM04403B-200), and Gapdh (PPM02946E-200) for mouse, and Nox2 (PPH10407A-200), Nrf2 (PPH0607A-200), Nqo1 (PPH01546C-200), and Gapdh (PPH72843A-200) for human, all from SABioscience. Gene expression was normalized to the level of the *Gapdh* housekeeping gene. Data were analyzed by the ΔΔCt method and expressed as fold change in mRNA expression relative to control values.

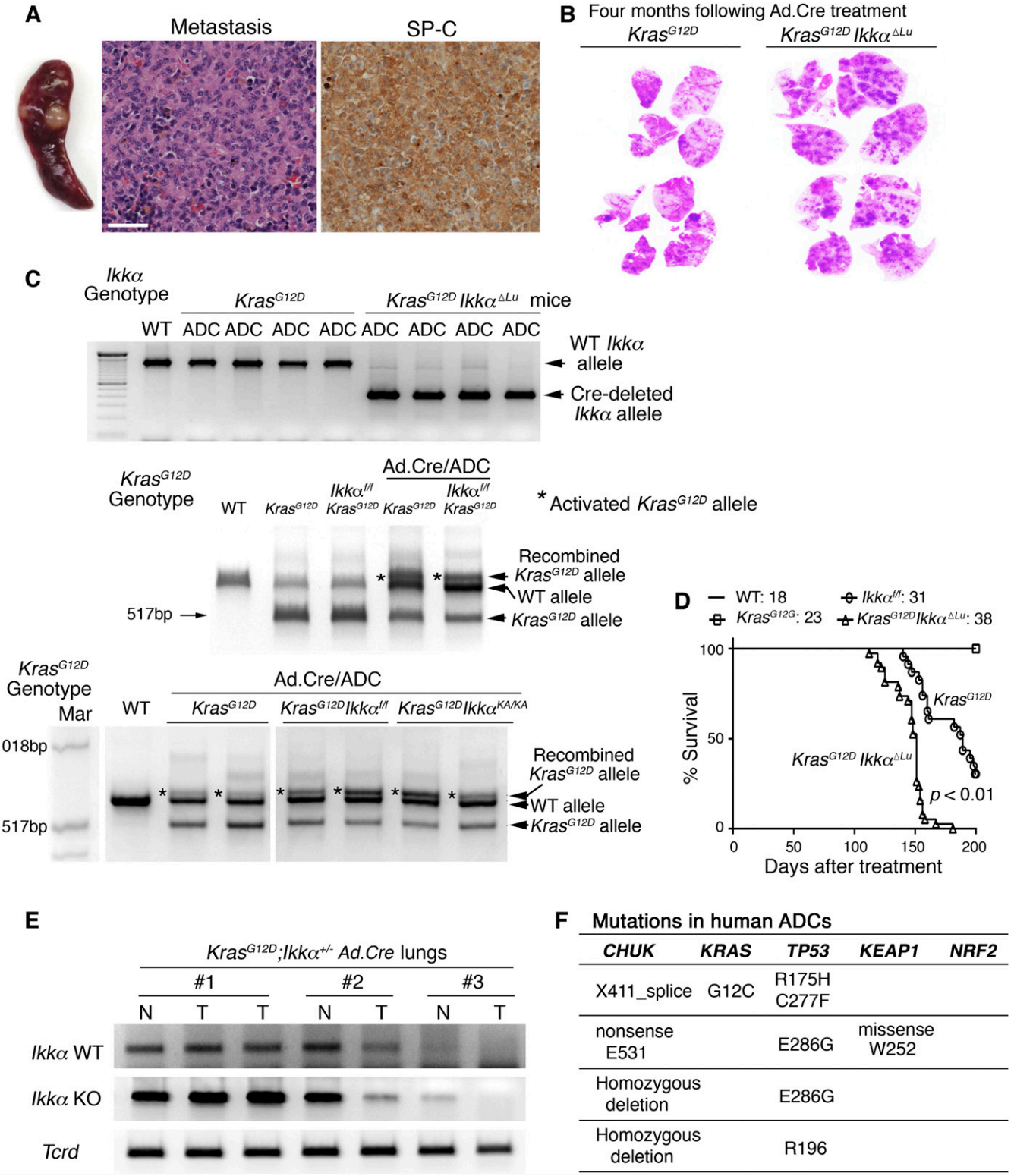
ChIP Assay. The ChIP assay was performed following the instructions of the kit (17-295; EZ-ChIP; EMD Millipore). Cells were cross-linked with 1% formaldehyde and then lysed and sonicated on ice to generate DNA fragments with an average length of 200–800 bp. After preclearing, 1% of each sample was saved as an input fraction. Immunoprecipitation was performed using antibodies against IKKα (M280; Santa Cruz Biotechnology), AhR (H-211; Santa Cruz Biotechnology), NRF2 (C-20; Santa Cruz Biotechnology), trimethyl-H3-K9 (07-442; Upstate Biotechnology), Dnmt3a (ab2850; Abcam), and suv39H1 (05-615; EMD Millipore). DNA was eluted and purified from complexes, followed by PCR amplification of the target promoters using primers of human Nox2: 5′-CAGATTGGTCCCAAACTCCT-3′ and 5′-TTGGCCAATGATGAACCAC-3′; mouse Nox2: 5′-CAATCTTTGTGCCCCATCTT-3′ and 5′-CTGTCACAGAGCCATTTCCA-3′; and mouse Nrf2: 5′-CAGCTGCTAATCTCTAGCAAGG-3′ and 5′-CCAGTGGAAGGAGCAAGAG-3′.

Microarray Analysis. Total RNA was isolated from lung tissues of WT, $Kras^{G12D}$, and $Kras^{G12D}$;$Ikk\alpha^{\Delta Lu}$ mice and from Kras-CL and $Kras^{IKK\alpha L}$ mouse ADC cells using Trizol reagent (Invitrogen). RNA quality was examined on a Bioanalyzer (Agilent) and then analyzed on the Affymetrix GeneChip Mouse Genome 430 2.0 array at the Laboratory of Molecular Technology, Frederick National Laboratory for Cancer Research. Microarray results (accession nos. GSE84159 and GSE84163) have been deposited at the National Center for Biotechnology Information's Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/).

Quantification and Statistical Analysis. Statistical analyses were performed using GraphPad Prism software. Statistical significance was determined by the *t* test, Mantel–Cox log-rank test, and Fisher's exact test, as indicated in the figure legends. $P < 0.05$ was considered to indicate statistical significance, and the error bars represent SD.

Data and Software Availability. The microarray analyses for mouse lung tissues (accession no. GSE84159) and mouse lung ADC cells (accession no. GSE84163) have been deposited at the National Center for Biotechnology Information's Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/).

1. Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O (2009) Protocols to detect senescence-associated beta-galactosidase (SA-beta-gal) activity, a biomarker of senescent cells in culture and in vivo. *Nat Protoc* 4:1798–1806.
2. Liu B, et al. (2006) A critical role for I kappaB kinase alpha in the development of human and mouse squamous cell carcinomas. *Proc Natl Acad Sci USA* 103:17202–17207.
3. DuPage M, Dooley AL, Jacks T (2009) Conditional mouse lung cancer models using adenoviral or lentiviral delivery of Cre recombinase. *Nat Protoc* 4: 1064–1072.
4. Xiao Z, et al. (2013) The pivotal role of IKKα in the development of spontaneous lung squamous cell carcinomas. *Cancer Cell* 23:527–540.



F Mutations in human ADCs

CHUK	*KRAS*	*TP53*	*KEAP1*	*NRF2*
X411_splice	G12C	R175H C277F		
nonsense E531		E286G	missense W252	
Homozygous deletion		E286G		
Homozygous deletion		R196		

Fig. S1. IKKα deletion in ADCs causes and promotes lung ADC. (*A*) Splenic metastasis of $Ikk\alpha^{\Delta Lu}$ lung ADC was stained with H&E and analyzed by IHC for SP-C. (Scale bar: 25 μm.) (*B*) Comparison of ADC spots in H&E-stained histological slides in lungs of $Kras^{G12D}$ and $Kras^{G12D};Ikk\alpha^{\Delta Lu}$ mice at 4 mo after Ad.Cre treatment. (*C*) Genotyping of activated $Kras^{G12D}$ alleles in $Kras^{G12D}$, $Kras^{G12D};Ikk\alpha^{\Delta Lu}$, and $Kras^{G12D};Ikk\alpha^{KA/KA}$ lung ADCs and genotyping of $Ikk\alpha$ ablation in $Kras^{G12D};Ikk\alpha^{\Delta Lu}$ lung ADCs using gene-specific PCR primers. In row 3, there are two splices (spaces) between lane 1 and lane 2 and between lane 4 and lane 5 because these results were obtained from two different gels. (*D*) Survival curves of 38 $Kras^{G12D};Ikk\alpha^{\Delta Lu}$ mice and 23 $Kras^{G12D}$ mice. Data were statistically analyzed using the Mantel–Cox log-rank test. $^{***}P < 0.001$. (*E*) Genotyping of WT *Ikkα* and *Ikkα* KO alleles with PCR. *Tcrd* served as a DNA-loading control. T, ADC; N, adjacent tissue to ADCs. (*F*) *KRAS*, *TP53*, *KEAP1*, and *NRF2* mutations identified in human lung ADCs carrying *CHUK* mutations or deletions (Fig. 1*A*; www.cbioportal.org).

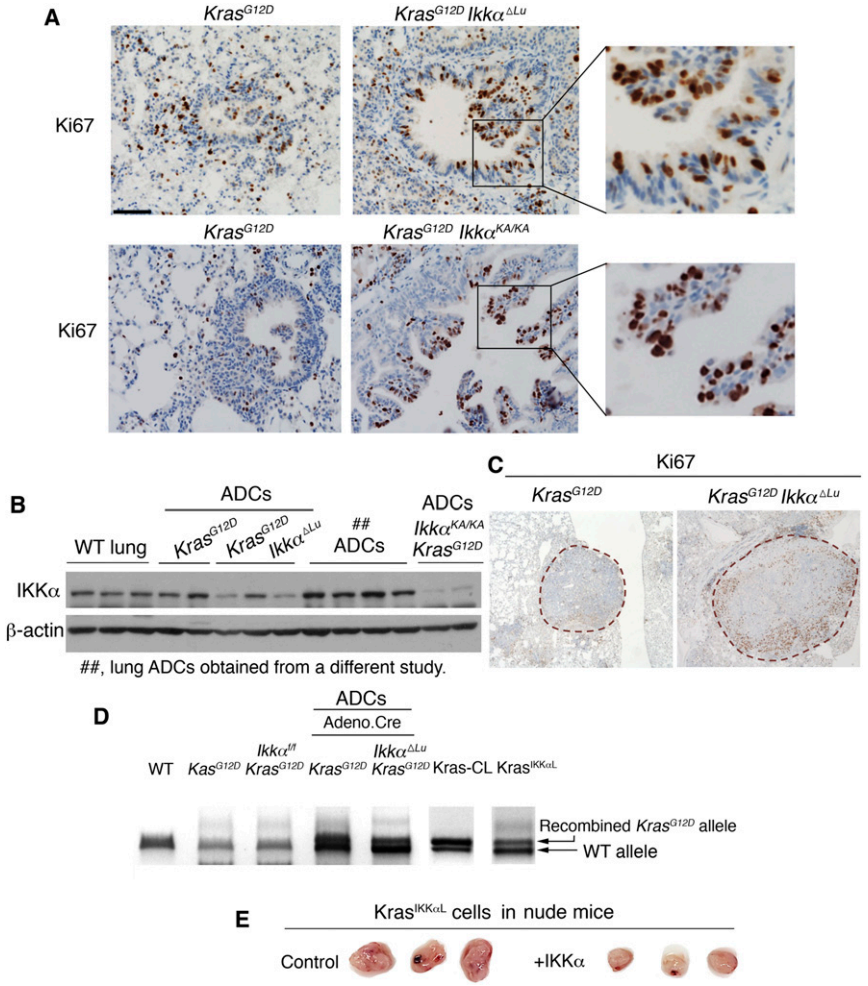


Fig. S2. IKKα deletion promotes cell proliferation but attenuates cell senescence. (*A*) IHC-stained Ki67-positive bronchial epithelial cells (dark brown) in the lungs of $Kras^{G12D}$, $Kras^{G12D}$;$Ikk\alpha^{\Delta Lu}$, and $Kras^{G12D}$;$Ikk\alpha^{KA/KA}$ mice (Fig. 2 *A* and *B*). The boxes on the left are amplified on the right. (Scale bar: 40 μm.) (*B*) IB analysis showing IKKα levels in WT lungs, $Kras^{G12D}$ ADCs, and $Kras^{G12D}$;$Ikk\alpha^{KA/KA}$ ADCs. β-actin served as a protein-loading control. (*C*) IHC-stained Ki67-positive cells in ADCs derived from $Kras^{G12D}$ and $Kras^{G12D}$;$Ikk\alpha^{\Delta Lu}$ mice (n = 3 mice for 10 slides/group). Brown color, positive staining. (*D*) Genotyping recombined $Kras^{G12D}$ allele in Kras-CL and $Kras^{IKK\alpha L}$ ADC cells using specific gene PCR primers and agarose gels. The activated $Kras^{G12D}$ allele genotypes of $Kras^{G12D}$, $Kras^{G12D}$;$Ikk\alpha^{\Delta Lu}$ lung ADCs are used as a positive control. (*E*) Tumors derived from athymic nude mice after the mice received $Kras^{IKK\alpha L}$ cells transfected with control vector or IKKα vector at day 14.

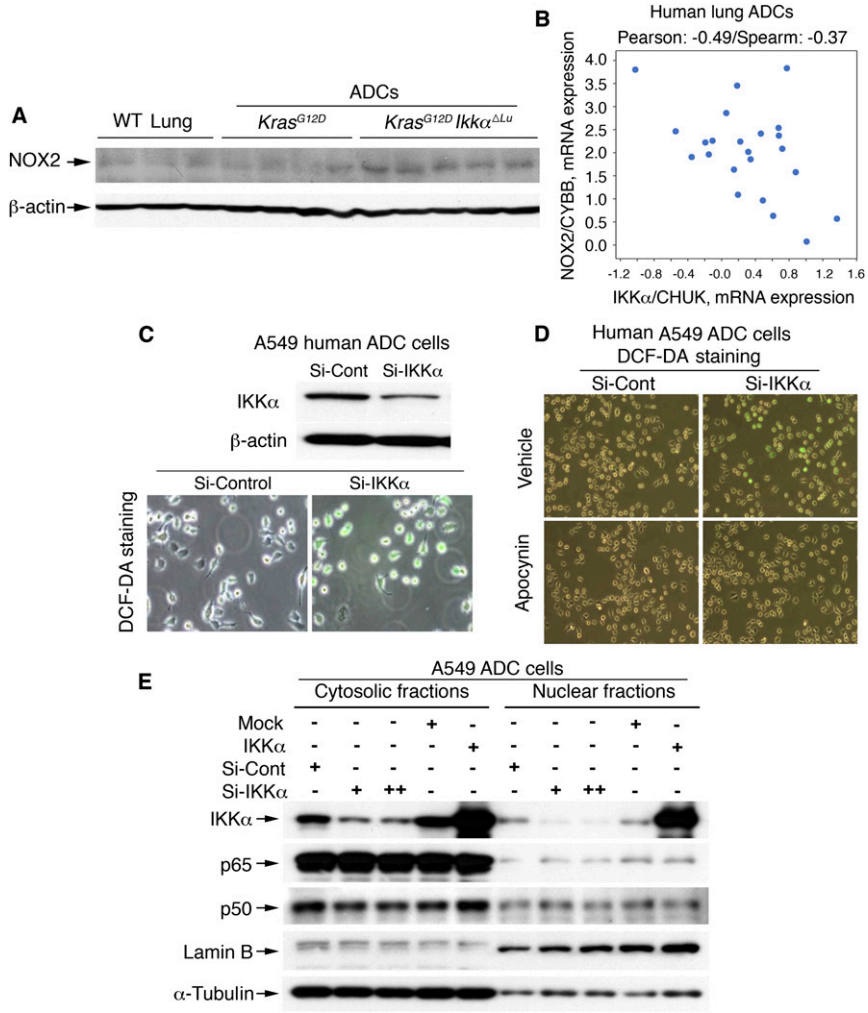


Fig. S3. IKKα deletion increases ROS, and apocynin treatment reduces ROS. (*A*) IB analysis of NOX2 expression in WT mouse lungs and from $Kras^{G12D}$ and $Kras^{G12D}$;$Ikk\alpha^{\Delta Lu}$ lung ADCs. β-actin served as a protein-loading control. (*B*) A negative correlation between NOX2 and IKKα expression in human lung ADC analyzed by microarray (cBioPortal). Blue circle, neither mutated. Pearson correlation, −0.49; moderate correlation from −0.40 to −0.59. (*C*, *Top*) IKKα levels in A549 cells treated with IKKα Si-RNA (Si-IKKα) or control Si-RNA (Si-Cont). β-actin served as a protein-loading control. (*C*, *Bottom*) DCF-DA staining in A549 human ADC cells treated with Si-IKKα or Si-control. (*D*) Treatment with apocynin (100 μM) reduces IKKα down-regulation–induced DCF-DA in human A549 ADC cells. Si-Cont, Si-control. (*E*) Cytosolic and nuclear protein levels of p65 and p50 in A549 cells following reexpression of IKKα or treatment with Si-Cont (control) or Si-IKKα. α-tubulin, a cytosolic protein-loading control; Lamin B, a nuclear protein control.

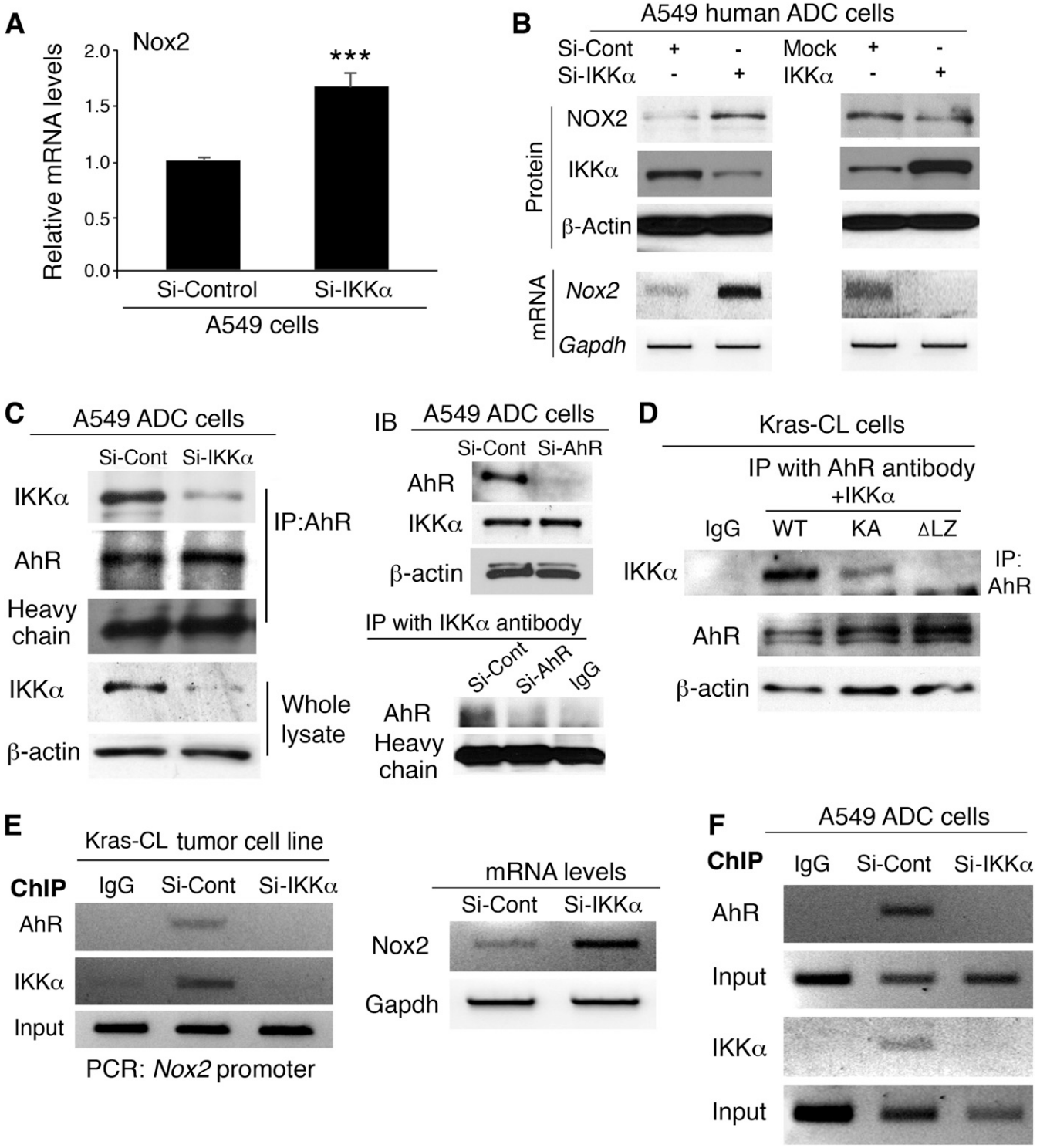


Fig. S4. IKKα deletion up-regulates Nox2 expression. (*A*) Knockdown of IKKα using Si-IKKα increases Nox2 expression in A549 cells ($n = 3$) analyzed by RT-PCR. Data represent mean ± SD (three repeats). ***$P < 0.001$, Student's *t* test. (*B*) Analysis of NOX2 protein and Nox2 mRNA expression in A549 cells treated with Si-IKKα, Si-control, and overexpressed IKKα. Mock served as a control vector; β-actin, as a protein-loading control; Gapdh, as an mRNA-loading control. (*C*) IP experiment showing the interaction between AhR and IKKα. The immunocomplexes were gel- separated and blotted with an anti-AhR or IKKα antibody in A549 ADC cells. β-actin served as a protein-loading control. Si-Cont, control SiRNA; Si-IKKα, SiRNA for IKKα; Si-AhR, SiRNA for AhR. (*D*) IP analysis of the interaction between IKKα and AhR in IKKα-deficient Kras-CL cells overexpressing WT IKKα, IKKα-KA, or IKKα-ΔLZ. An anti-AhR antibody was used for IP. AhR was input; β-actin served as a protein-loading control. (*E*) ChIP analysis for IKKα and AhR binding to *Nox2* promoter in Kras-CL cells with anti-IKKα or anti-AhR antibody and PCR with primers to the *Nox2* promoter. Input, PCR control; Si-IKKα, SiRNA against IKKα mRNA; Si-Cont, control (*Left*); *Gapdh*, an mRNA-loading control (*Right*). (*F*) ChIP analysis performed using anti-AhR or anti-IKKα antibody and PCR with *Nox2* promoter primers in A549 ADC cells. Input, a loading control.

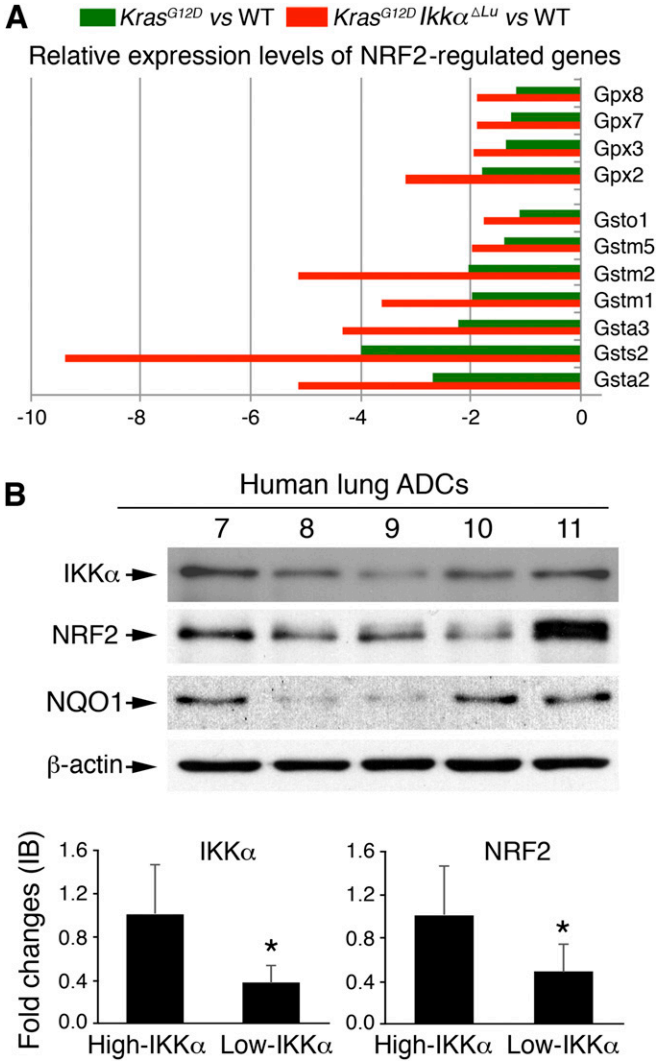


Fig. S5. IKKα ablation reduces expression of NRF2 targets. (*A*) Expression levels of NRF2 target genes in $Kras^{G12D}$;$Ikk\alpha^{\Delta Lu}$ lungs vs. WT (red) and in $Kras^{G12D}$ lungs vs. WT (green), analyzed by gene expression profile array. (*B*, *Top*) IB analysis of IKKα, NRF2, and NQO1 expression in human lung ADCs. β-actin served as a protein-loading control. (*B*, *Bottom*) Statistical analysis of the correlation between IKKα and NRF2 protein levels in 11 human lung ADCs (Fig. 3*E*). (*Left*) These ADCs were divided into two groups: those expressing high levels ($n = 5$) and those expressing low levels ($n = 6$) of IKKα. (*Right*) NRF2 expression was further analyzed in the two groups. $^*P < 0.05$, Student's *t* test.

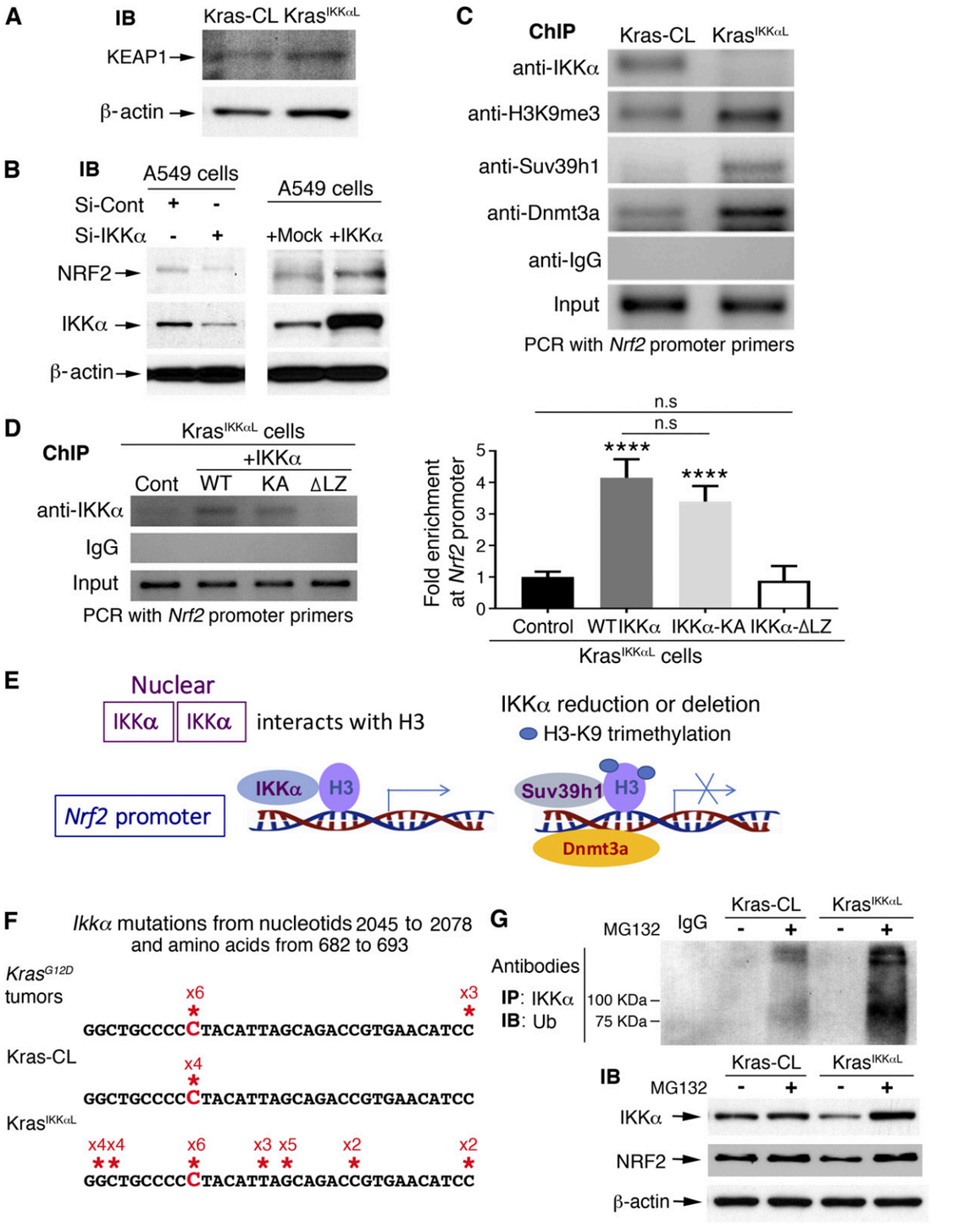


Fig. S6. IKKα regulates NRF2 expression through its promoter activity. (*A*) IB analysis of KEAP1 expression in Kras-CL and Kras$^{IKK\alpha L}$ cells. β-actin served as a protein-loading control. (*B*) IB analysis of IKKα and NRF2 expression following treatment with Si-Cont or Si-IKKα, or after reexpression of IKKα in A549 cells. Mock, control; β-actin, protein-loading control. (*C*) ChIP assays using antibodies against IKKα, trimethyl-H3K9 (H3K9me3), Suv39h1, or Dmnt3a for immunoprecipitation, followed by PCR with *Nrf2* promoter primers. An anti-IgG is used as an antibody control, and the input is used as a loading control. (*D*, *Left*) ChIP analyses for IKKα binding to Nrf2 promoter using an antibody against IKKα for IP and PCR with *Nrf2* promoter primers in Kras$^{IKK\alpha L}$ cells (Cont, control) and Kras$^{IKK\alpha L}$ cells overexpressing WT IKKα, IKKα-KA, or IKKα-ΔLZ. (*D*, *Right*) Data represent mean ± SD (four repeats). ****$P < 0.0001$ compared with control or IKKα-ΔLZ, Student's *t* test. n.s., not significant. (*E*) A working model for *Nrf2* promoter regulation in the presentence or absence of IKKα. H3, histone 3; arrow, gene transcription; cross lines, block of gene transcription. (*F*) *Ikkα* mutations were identified in Kras^{G12D}-initiated lung ADCs, Kras-CL cells, and Kras$^{IKK\alpha L}$ cells by sequencing of IKKα cDNAs. Approximately 80 cDNA clones inserted into a vector from each cell line were sequenced. Red stars, mutations; numbers indicate detection frequencies of mutations. (*G*) IB analysis of IKKα expression and ubiquitination in Kras-CL and Kras$^{IKK\alpha L}$ cells, with or without MG132 treatment to inhibit proteasomal degradation. IP was performed with an IKKα antibody; IB, with a ubiquitin antibody. β-actin served as a protein-loading control.

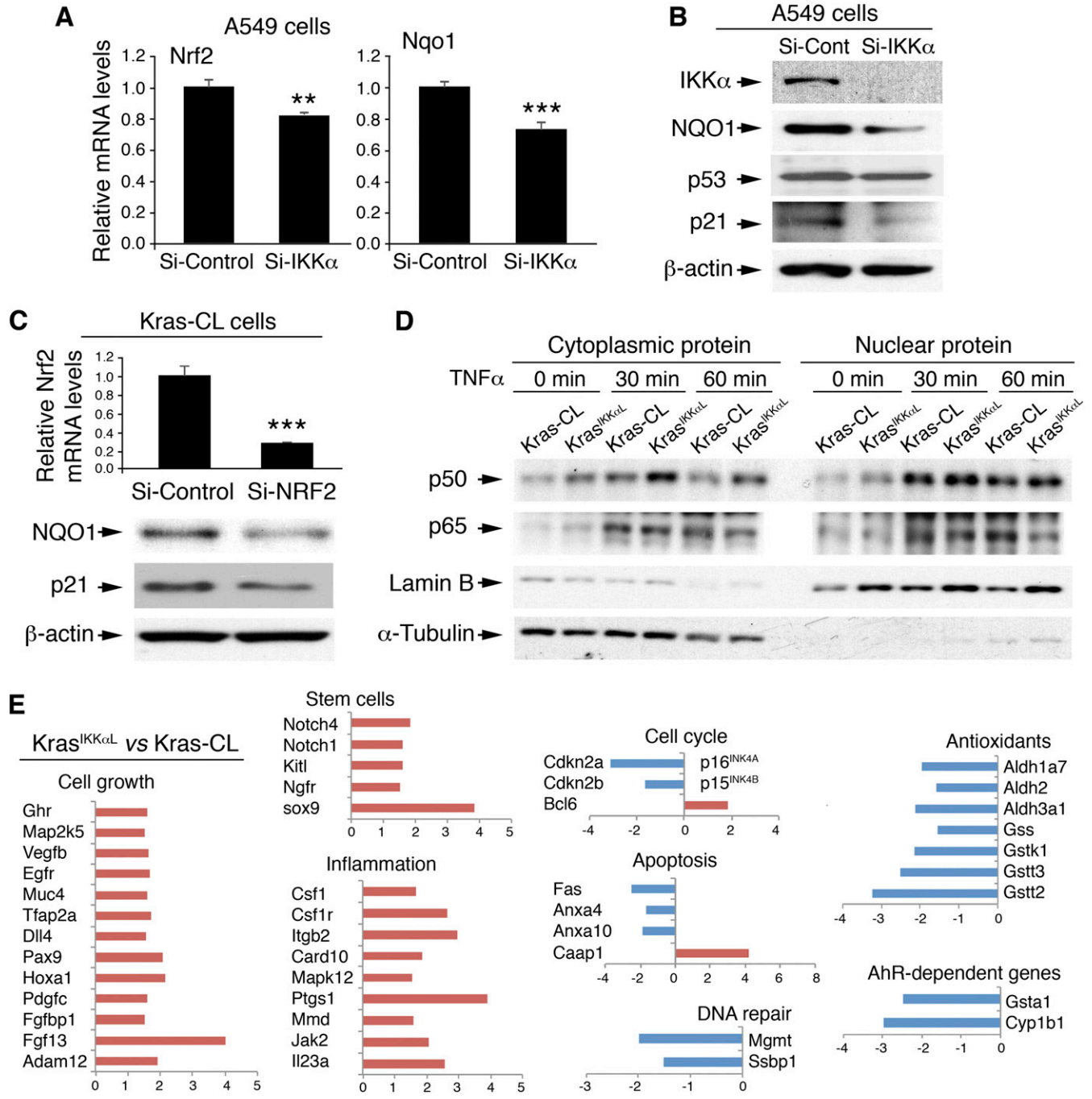


Fig. S7. IKKα up-regulates Nrf2 and Nqo1 expression. (*A*) RT-PCR analysis of Nrf2 and Nqo1 expression in A549 cells treated with Si-Control or Si-IKKα (n = 3/group). Data represent mean ± SD (three repeats). $^{**}P < 0.01$; $^{***}P < 0.001$, Student's t test. (*B*) IB analysis of NQO1, p53, and p21 expression in A549 cells treated with Si-control or Si-IKKα. β-actin served as a protein-loading control. (*C*) Si-RNA NRF2 decreases NRF2, NQO1, and p21 expression in Kras-CL cells. β-actin served as a protein-loading control. (*D*) IB analysis of the expression of cytoplasmic and nuclear p50 and p65 levels in Kras-CL and Kras$^{IKK\alpha L}$ cells following TNFα (10 ng/mL) stimulation. Lamin B served as a nuclear protein-loading control; α-tubulin, as a cytoplasmic protein-loading control. (*E*) Gene expression profile analysis using gene arrays in Kras$^{IKK\alpha L}$ vs. Kras-CL cells. Red bar indicates increased expression; blue bar, decreased expression.