

Supplemental Information:

Supplemental Figures:

Supplemental Figure S1: A Cre-versible strategy for comparing wild type and knockout tumors in a single animal, related to Figure 1

(A): Southern blotting strategy to identify targeted $MK2^{CV}$ ES-cells. SpeI-digested ES-cell DNA was examined using either probe A (left) or probe B (right), positions of SpeI-restriction sites and probes are shown in Figure 1A. The 16.3kb band indicates the presence of the wild type $MK2$ allele (+), whereas the 11kb and 7.2kb bands are diagnostic for the Cre-versible allele (CV), respectively.

(B): PCR from cDNA of $MK2^{CV/CV}$ MEFs infected with adenoviral Cre-recombinase (Adeno-Cre), amplified with 5' primer A binding in exon 1, and 3' primer B binding in exon 3. The $MK2^{+CV}$ fragment yields a 317bp PCR product, the $MK2^{CV}$ fragment missing exon 2 a 177bp PCR product. Both PCR products were clones into pZERO Blunt PCR Cloning Vector (Invitrogen) and sequenced.

(C) Alignment of cDNA and corresponding amino acid (AA) sequences from $MK2^{+CV}$ and $MK2^{CV}$ PCR products spanning exon 1 to exon 3 as described in Figure S1B. In the $MK2^{CV}$ orientation, inversion of exon 2 disrupts the splice donor and splice acceptor sites, resulting in a skip from exon 1 directly to exon 3, leading to a frame shift and a STOP-codon early in exon 3.

Supplemental Figure S2: MK2-expressing and MK2-deficient tumors develop in a murine autochthonous model of Non-Small Cell Lung Cancer, related to Figure 2

(A): Genetically engineered mouse lines for the model of NSCLC. $Kras^{LSL-G12D/+}$ and $p53^{flox/flox}$ mice were previously described in (Jackson et al., 2005; Jackson et al., 2001): The endogenous *Kras* locus is targeted to insert a transcriptional inhibitory element ("STOP") flanked by *LoxP* sites into intron 0. In addition, the *Kras* allele contains an activating mutation of codon 12 to mutate Gly (G) to Asp (D). The $p53^{flox}$ allele encodes the wild-type p53 protein; *LoxP* sites flank exons 2 to 10. Exons for *Kras* and *p53*: white boxes, *LoxP* sites: black triangles.

(B): NSCLC tumors and normal lung tissue express MK2 in a MK2 wild type background. Tumors from a $MK2^{+/+};Kras^{LSL-G12D/+};p53^{+/+}$ mouse at the experimental endpoint were

stained with haematoxylin and eosin (H&E, left) or by IHC for MK2 (brown staining, middle). Right: zoom into the region with three MK2+ tumors. Scale bar = 50µm

(C): Loss of MK2 expression in lung tumors by adenoviral Cre-recombinase mediated inversion of exon 2 in vivo. Tumors from a $MK2^{CV/CV};Kras^{LSL-G12D/+};p53^{+/+}$ mouse at the experimental endpoint were stained as described in Figure S2B. Right: zoom into the region between two tumors: MK2+ tumor: brown staining, MK2- tumor: blue counterstain only.

(D): Loss of MK2 has no influence on lung tumor initiation: Quantification of $MK2^{+/+}$ and $MK2^{CV/CV}$ tumors in a $Kras^{G12D/+}; p53^{\Delta/\Delta}$ background at 6 weeks or in a $K-ras^{G12D/+};p53^{+/+}$ background at 9 weeks after tumor induction, shown as percentage of total lung area. n = 4 to 5 mice / strain, error bars represent SEM.

Supplemental Figure S4: Loss of MK2 results in increased chemo-sensitivity in p53-deficient tumor cells *in vitro*, related to Figure 4

(A): Proliferation in murine NSCLC cell lines in response to cisplatin treatment: Ratio of doubling times between MK2+ and MK2- isogenic cell lines and B. (B) Proliferation in human NSCLC cell lines in response to cisplatin treatment: Ratio of doubling times between H1299 cells stably expressing a control hairpin (MK2+) or hairpin against MK2 (MK2-) and A549 cells with and without siRNA against MK2 in combination with siRNA against 53 or control.

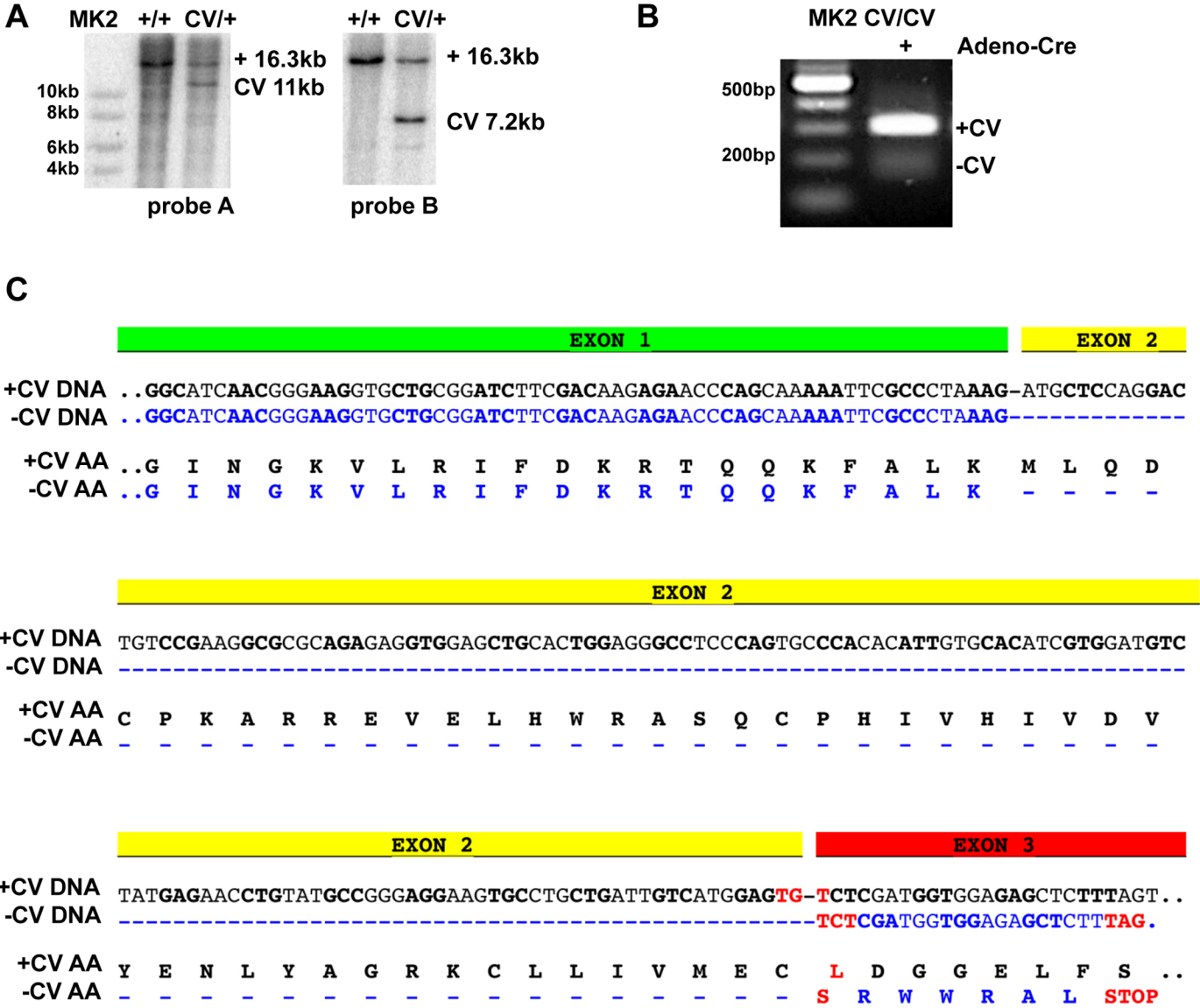
(A), (B): n = 3 independent experiments, ****: $p < 0.0001$, error bars = SEM.

(C), (D): Growth curves for murine NSCLC cell lines A (B) and B (C) in response to cisplatin treatment (Cis), one example for untreated cells (vehicle) is shown.

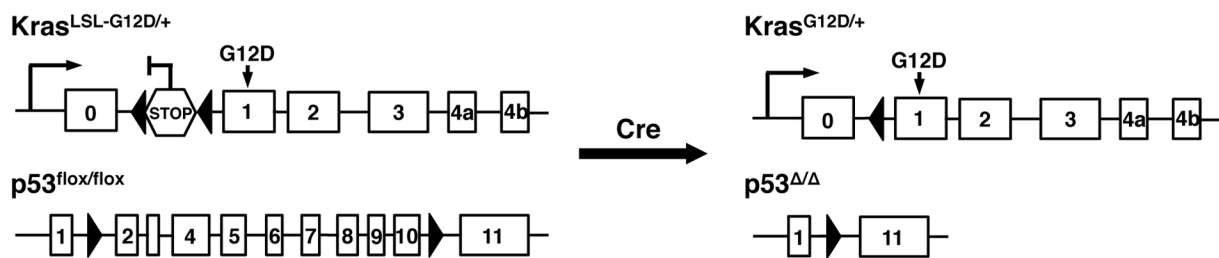
(E) - (G): Growth curves for human NSCLC cell lines H1299 (E), A549 with wild type p53 levels (F) and A549 cells with knock down of p53 (G) in response to cisplatin treatment (Cis), one example for untreated cells (vehicle) is shown.

Supplemental Figure S5: Lack of correlation between mRNA expression levels of MK2 or Chk1 with p53 in human lung adenocarcinoma, related to Figure 5

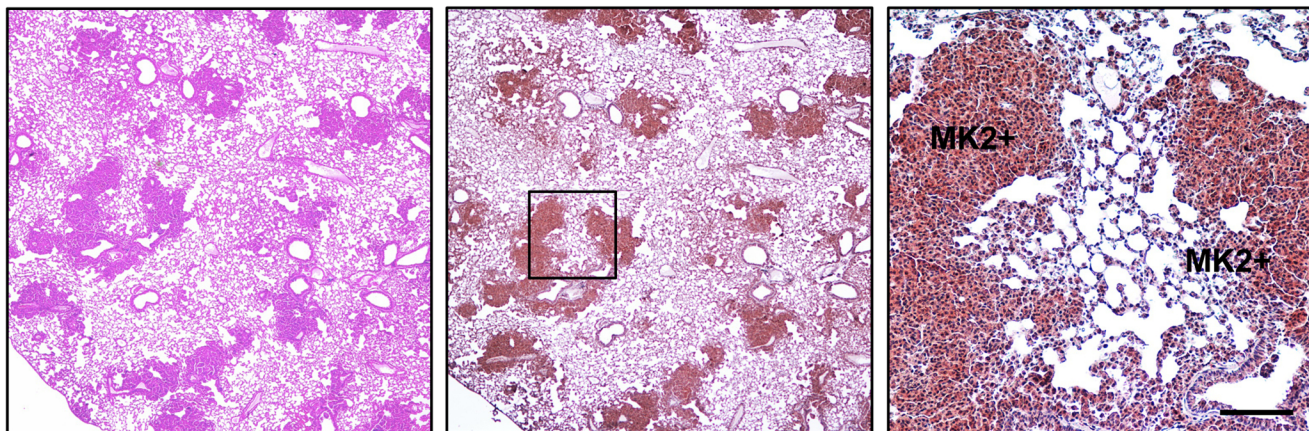
TCGA expression data for *MAPKAPK2* (MK2), *CHEK1* (Chk1) and *TP53* (p53) mRNA levels from 129 human lung adenocarcinoma samples (<http://www.cbioportal.org>, NCI).
r = Pearson's correlation coefficient



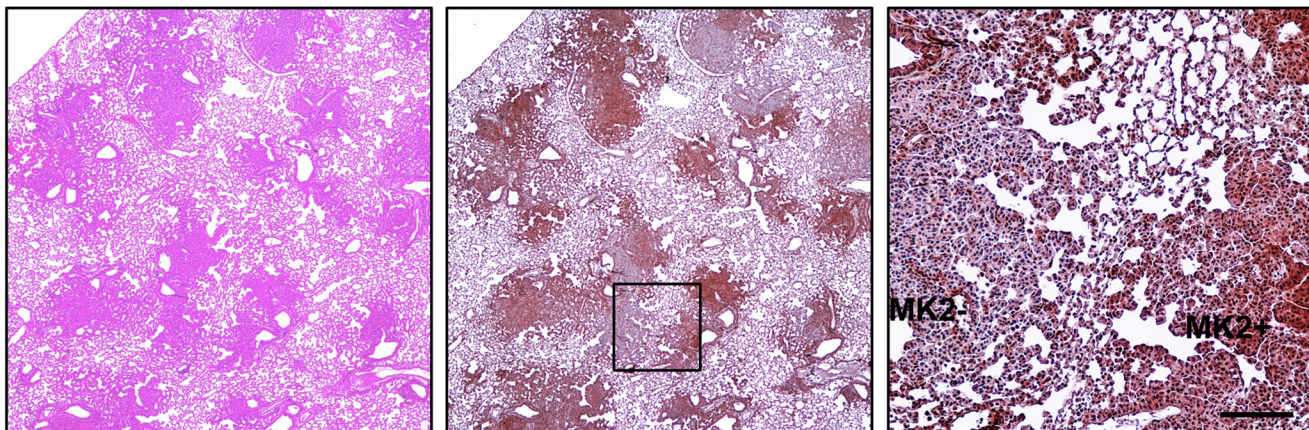
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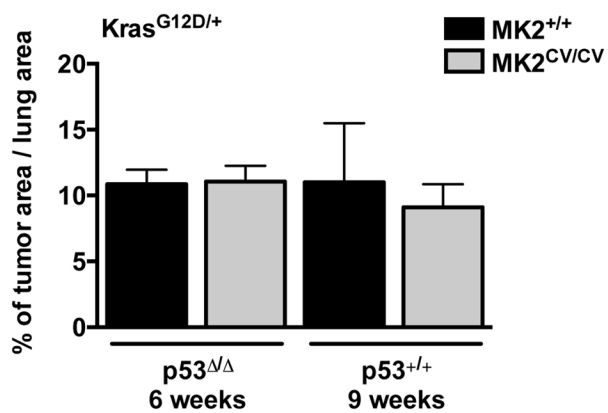
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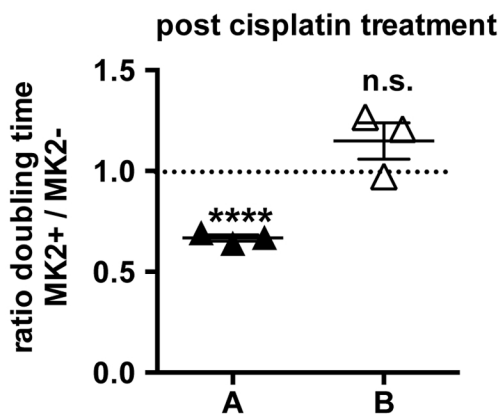
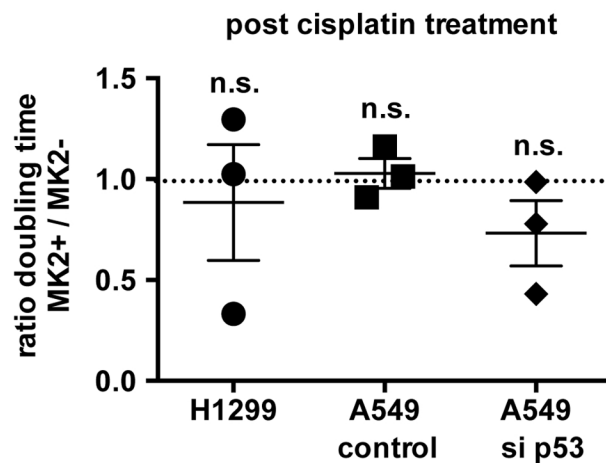
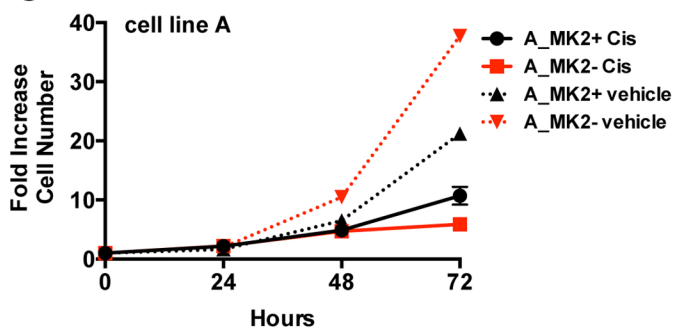
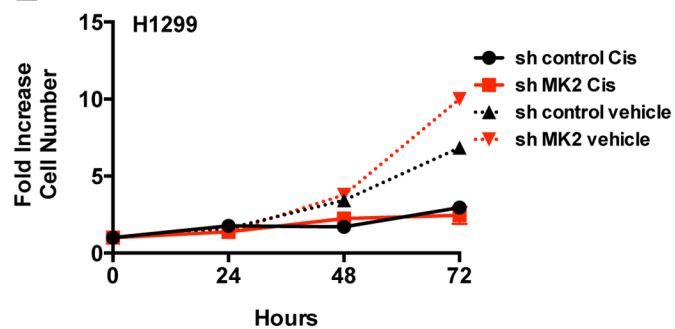
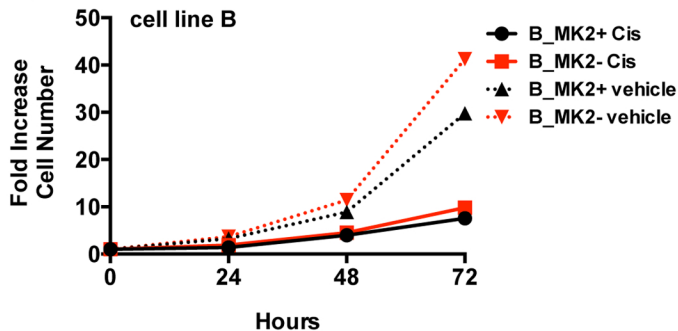
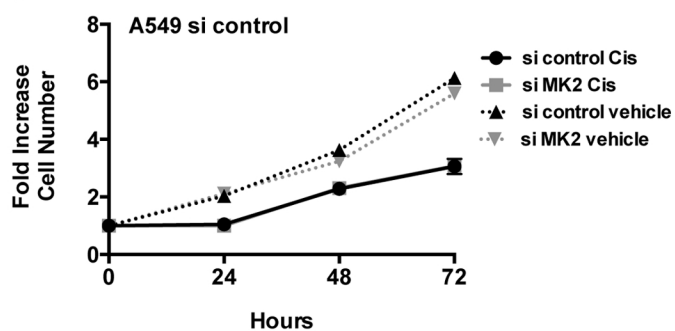
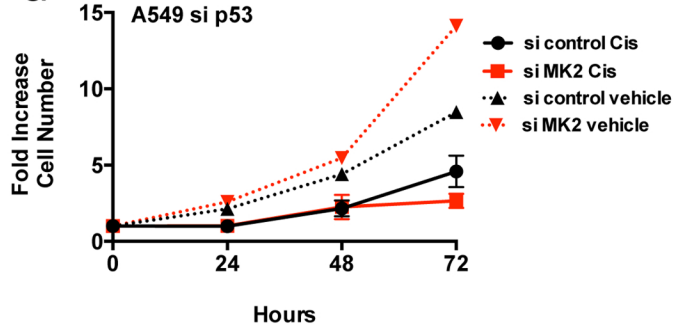


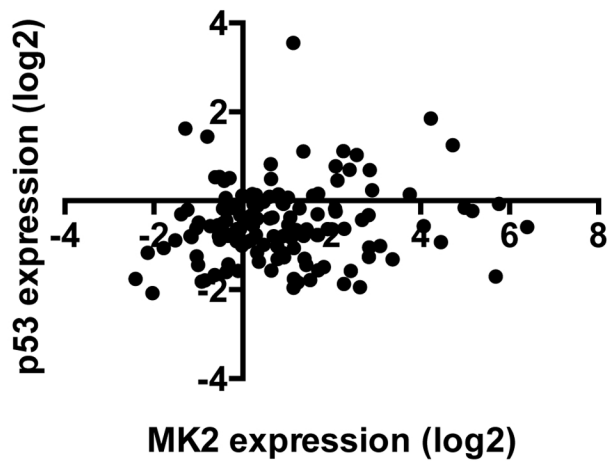
C



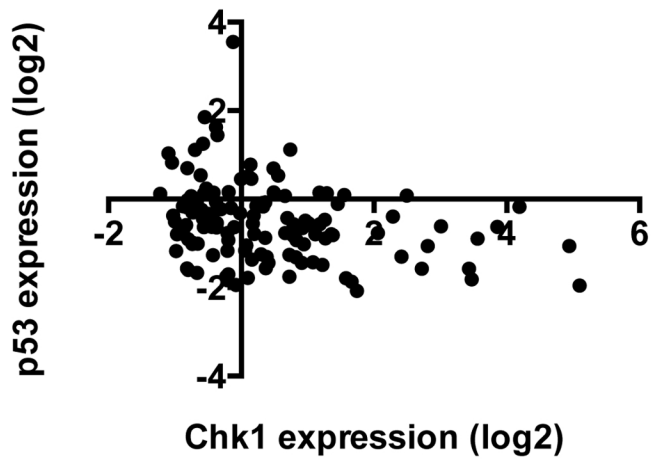
D



A**B****C****E****D****F****G**



$r = 0.137, p = 0.122$



$r = -0.289, p = 0.0009$

Supplemental Experimental Procedures:

Generation of MK2 'Cre-versible' (CV) mice

To generate mice carrying 'Cre-versible' alleles of MK2, we constructed the targeting vector shown in Figure 1A using standard cloning approaches. The targeting vector is based on the pBluescript variant pKS-DTA (generously provided by A. Ventura). The Bac clone RP24-125B12 (Bacpac Resource Center) spanning the entire genomic locus encoding MK2 served as the template for PCR-directed cloning. The final targeting construct consists of 2.7kb of the 5' flanking sequence before exon 2, a *LoxP* site, exon 2, followed by a second *LoxP* site in opposite direction, i.e. in reverse complement sequence as the first *LoxP* site, a *FRT*-Pgk::Neo-*FRT* cassette to select for integration of the targeting construct into ES-cells, and exons 3 to 10. The targeting construct was linearized using *AscI*, which cuts at a unique site in the pBluescript parent backbone of the targeting construct, and electroporated in 129/B6 hybrid ES cells. Transfected ES cell clones were selected by growth in media containing neomycin for 1 week. Proper integration was monitored using Southern blot analysis from *SpeI* genomic DNA-digests to monitor the 5' integration event using a 300bp fragment from the genomic sequence in intron 1-2 that lies outside the targeting construct as probe A (Figure 1A). The probe was generated by PCR amplification followed by radiolabeling with ^{32}P - α -dCTP using random priming. In the wild-type genomic MK2 sequence, the *SpeI* sites in intron 1 and intron 10 gives rise to a 16.3kb band, while correct 5' integration of the targeted allele gives rise to a 11.0kb band due to the presence of a *SpeI* site within the Pgk::Neo cassette (Suppl. Figure S1A). The 3' integration event was monitored by Southern blotting using a 300bp probe B corresponding to the 3' flanking region outside the targeting construct (Figure 1A). The targeted allele gives rise to a 7.2kb band (Suppl. Figure S1A).

One ES cell clone containing the integrated MK2^{CV} allele was injected into host albino FEBN blastocysts and implanted into pseudo-pregnant mice. Chimeric mice were crossed to C57-FLPe mice to eliminate the *FRT*-Pgk::Neo-*FRT* cassette. The offspring was genotyped by PCR using primers P1 and P2 flanking the second *LoxP* sites (Figure 1B).

All mice were maintained on a mixed C57/BL/6J x 129SvJ strain. All mouse studies described in this proposal were approved by the MIT Institutional Committee for Animal Care (CAC), and conducted in compliance with the Animal Welfare Act

Regulations and other federal statutes relating to animals and experiments involving animals and adheres to the principles set forth in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996 (Institutional Animal Welfare Assurance no. A-3125–01).

Immunohistochemical analysis

Immunohistochemistry was performed on formalin-fixed, paraffin-embedded 4µm-sections using the ABC Vectastain kit (Vector Laboratories). Sections were developed with DAB and counterstained with haematoxylin. Haematoxylin and eosin staining (H&E) was performed using standard methods.

Cell lines

MEFs were isolated from E13.5 embryos and used between passages 3 and 5. Murine NSCLC cell lines were generated as described in (Winslow et al., 2011). To generate isogenic $MK2^{+CV/+CV}$ and $MK2^{-CV/-CV}$ clones, single cell clones were generated from murine NSCLC cell lines and infected with Adenovirus expressing Cre-recombinase (Ad5CMVCre, University of Iowa) at 100 MOI in the presence of 8µg/ml polybrene (Millipore). Cells were washed twice after 48h and propagated for second round of single-cell clone generation.

The human NSCLC cell lines H1299 and A549 were obtained from ATCC. MEFs, murine NSCLC cell lines and A549 were cultured in DMEM plus 10% FBS and penicillin/streptomycin, H1299 were grown in RPMI plus 10% FBS and penicillin/streptomycin.

Cleavage of Caspase 3

To measure the induction of cleaved caspase 3 cells were treated with vehicle, 5µM or 10µM cisplatin, harvested after 24h and analyzed by Western Blot.

Primer sequences:

Generation of Southern Blot probes:

Probe A: forward: 5'-GGTTCAGAGGGCTAGAGTCC-3'

reverse: 5'-GCATCCTTCCTCCAGAGGACA-3'

Probe B: forward: 5'-GACCGTTTACTTGTGTTCTCCC-3'

reverse: 5'-GATTTCGACAGTGCTCCAGGTAG-3'

Genotyping:

MK2^{wt}, MK2^{+CV} and MK2^{-CV} alleles:

P1: 5'- GAGCTCTCCACCATCGAGAC-3'

P2: 5'- GCAGACAGCCCACTATGGAT-3'

P3: 5'- GCTGCCTCTCCTTCTGTAGAC-3'

DNA for genotyping was isolated using the Hot Shot Method and Kras^{LSL-G12D}, Kras⁺, p53^{flox} and p53⁺ alleles were genotyped as described in publicly available protocols from the laboratory of Tyler Jacks: http://web.mit.edu/jacks-lab/protocols_table.html

cDNA sequencing:

A (exon 1): 5'-GTTCCCCCAGTTCCACGTCAAG-3'

B (exon 3): 5'- CTAAAGAGCTCTCCACCATCG-3'

Knock down of MK2 and p53

shRNAs were designed using resources available through G. Hannon's laboratory at Cold Spring Harbour Laboratories (<http://katahdin.cshl.org/siRNA/RNAi.cgi?type=shRNA>). Oligonucleotides were cloned into the miR30-based retroviral vector pMLP, a kind gift from M. Hemann (MIT). Retroviral packaging constructs pMDg and pMDg/p were a generous gift from T. Benzing (Cologne). The best oligonucleotide for targeting MK2 (human, NM_032960.2) was 5'-TGCTGTTGACAGTGAGCGAAGCGAAATTGCTTTACTAAATAGTGAAGC CACAGATGTATTTAGTAAAGACAATTTTCGCTCTGCCTACTGCCTCGGA-3', an inactive/scrambled hairpin directed against the human sequence of BAD was used for control knock down. Retroviruses encoding shRNAs were packaged in HEK293T cells using standard procedures. H1299 and A549 cells stably expressing shRNA constructs were generated by retroviral infection (three times for 12h in the presence of 8µg/ml

polybrene), and targeted cells were selected with 10µg/ml puromycin for 5 days.

Silencer select si-RNA duplexes were purchased from Ambion, ID s569 against human MK2, ID s605 against human TP53. A549 cells were transfected with siRNAs using Lipofectamine RNAi-MAX (Invitrogen) according to the manufacturer's instructions. Cells were treated for further experiments after 48h.

Antibodies and chemicals

Antibodies against MK2 and cleaved caspase 3 were obtained from Cell Signaling Technologies, the antibody against p53 (DO-1) was purchased from Santa Cruz Biotechnology and the antibody against p21^{WAF1} (CP74) from Thermo Scientific. Antibodies against β-actin and γ-tubulin, cisplatin (*cis*-Diammineplatinum(II) dichloride) and puromycin were purchased from Sigma –Aldrich.

Supplemental Reference

Winslow, M. M., Dayton, T. L., Verhaak, R. G., Kim-Kiselak, C., Snyder, E. L., Feldser, D. M., Hubbard, D. D., DuPage, M. J., Whittaker, C. A., Hoersch, S., *et al.* (2011). Suppression of lung adenocarcinoma progression by Nkx2-1. *Nature* 473, 101-104.