mixed-grade tumour (top), and deconvoluted z-stack images (bottom) of dashed regions of an adenoma area (left) and adenocarcinoma area (right) are shown. **d**, Proliferation assay in adenocarcinoma-derived cell lines. Knockdown with retrovirally expressed shRNAs to *Trp53* or *Cdkn2a* (Arf-specific transcript), or control cells 48-hours post *Trp53* restoration. Percentage of $BrdU^+$ cells is indicated. **e**, Adenocarcinoma cell lysates from **d** were subjected to immunoblot analysis for p53, p21, Arf, and Hsp90 (loading control).

Supplementary Figure 1 | Genetically engineered mouse lines used in this study. **a,** $KRas^{LA2}$ mice were previously described[14]. Briefly, a tandem duplication of the genomic region including and surrounding exon 2 was generated at the endogenous *Kras* locus. Spontaneous recombination in cis (as shown), or in trans with unequal sister chromatid exchange, results in expression of an activated $KRas^{G12D}$ allele. **b,** $Trp53^{LSL}$ and $Rosa26^{CreERT2}$ mice have been previously described[6]. The endogenous p53 locus was targeted to insert a transcriptional and translational inhibitory element ('STOP') that is flanked by loxP recombinase sites within the first intron. This allele is functionally equivalent to a null allele until Cre-mediated removal of the 'STOP' element. The Cre recombinase is fused to a fragment of the estrogen receptor and is therefore dependent on 4-hydroxytamoxifen for activity. The Cre-ER fusion was targeted to the ubiquitously expressed *Rosa26* locus.

Supplementary Figure 2 | *Trp53* is efficiently restored after tamoxifen administration. a, Low power view of tumour sections highlights similar tumour burden in control and *Trp53*-restored mice. DNA was purified from individual tumours (circled in white) that were harvested by laser capture microscopy. Normal lung tissue (yellow circle) was also harvested for comparison. **b,** *Trp53* is efficiently restored after tamoxifen-mediated activation of $CreER^{T2}$ protein. Normal lung (lanes 1 and 2) and tumour (lanes 3-6) DNA was subjected to allele-specific PCR to detect recombined *loxSTOPLox* sequences. Control DNA from tail biopsies of unrelated mice are shown for comparison on the right. Restored *Trp53* ($Trp53^L$) is only seen in tumours harvested from $Rosa26^{CreERT2}$ mice (Lanes 3 & 6). $Rosa26^{+/+}$ mice do not recombine the *Trp53*

locus after tamoxifen administration (Lanes 4 & 5). Control DNA from *Trp53*$^{LSL/L}$ (lane i) *Trp53*$^{LSL/+}$ (lane ii) and *Trp53*$^{LSL/LSL}$ mice (iii and iv) are included for reference.

Supplementary Figure 3 | Histological survey of *Kras*$^{LA2/+}$*; Trp53*$^{+/+}$ and *Kras*$^{LA2/+}$*;Trp53*$^{LSL/LSL}$ animals at four and ten-week time points. a, Histological sections from four-week old mice showing low grade adenomas. Most cells and nuclei are uniformly shaped. A *Kras*$^{LA2/+}$*;Trp53*$^{LSL/LSL}$ tumour (right) shows multiple cells with pleomorphic nuclei (arrowheads). **b**, Tumours from ten week old animals showing an adenoma in the *Kras*$^{LA2/+}$*; Trp53*$^{+/+}$ (left) and an adenocarcinoma in the *Kras*$^{LA2/+}$*;Trp53*$^{LSL/LSL}$ tumour (right). The adenocarcinoma shows papillary morphology and frequent pleomorphic nuclei (arrows).

Supplementary Figure 4 | *Trp53* restoration leads to reduced tumour growth. Three-dimensional tomograms were analyzed to generate volumes for individual tumours at 8-, 10-, 11-, and 12-week time points. Individual tumour volumes from control (left) and restored (right) mice are plotted.

Supplementary Figure 5 | p53 is detectable in carcinoma areas after *Trp53* restoration by immunohistochemistry. Tumour sections were hybridized with antibodies to p53. Specific labeling of tumour sections was variable; however, when p53 was detected, it was always in tumour areas that had carcinoma features.

Supplementary Figure 6 | Apoptosis is not apparent after *Trp53* restoration. a, Photomicrographs of H&E, p21, and cleaved caspase 3 (CC3) stained sections shows robust induction of p21 at early time points and decreased signal frequency with time. CC3 stain was negative for all time points examined. Scale bars=10μm. **b**, TUNEL staining after *Trp53* restoration is negligible. Background signals in *Control* and *Restored* samples emanate from autofluorescent red blood cells. Apoptotic cells are identified by positive staining (green) as in the *Trp53*$^{-/-}$*;Rb1*$^{-/-}$ small cell lung tumor section shown (+Control).

Supplementary Figure 7 | Histological evaluation of micro-dissected tumours determines tumour stage and evidence of *Trp53* activation. Carcinomas with high p-Erk, adenomas with low p-Erk, and *Trp53* restored tumours with low to moderate p-Erk are shown. H&E photomicrographs depict a high-grade papillary carcinoma, a dense adenoma, and a *Trp53*-restored tumour that shows less dense tumour cell packing and the appearance of fissures between populations of lower grade tumour cells that are filled with foam macrophages (arrows in inset). Scale bars=100μm

Supplementary Figure 8 | Expression of *Cdkn2a* is elevated in carcinomas relative to adenomas. a, Expression levels derived from microarray are plotted in log base 2 scale and show that *Cdkn2a* levels are increased an average of 2.44-fold relative to adenomas. Significance determined by two-tailed t-test. **b**, Histological analysis from Fig. 3 with an added serial section (iv) stained with Arf antibodies. Arf expression is seen in areas where high p-Erk (iii), high p-Mek (ii), and adenocarcinomas features (i) are localized in serial sections. **c**, Deconvoluted image of an adenocarcinoma hybridized with antibodies to Arf (green) and p-Erk (red). Nuclei are counterstained with Hoechst (blue).

Supplementary Figure 9 | *Trp53* restoration leads to similar phenotypes and gene expression in carcinomas and cell lines derived from carcinomas. a, Cells were treated with 4-hydroxytamoxifen to restore *Trp53*; and after 24, 48, or 72 hours, cell cultures were pulsed with BrdU for two hours. Cells were harvested, fixed, then stained with BrdU specific antibodies. Flow cytometry analysis highlights the cytostatic effect of *Trp53* restoration as the percentage of BrdU positive cells decreases with time after *Trp53* restoration. **b**, Unlike lymphoma cell lines derived from $Trp53^{LSL/LSL};Rosa26^{CreERT2}$ mice, adenocarcinoma-derived cell lines do not undergo apoptosis after *Trp53* restoration. Cells were treated with 4-hydroxytamoxifen to restore *Trp53,* and after 24 hours proteins were harvested and subjected to immunoblot analysis. While induction of p53 is observed in both cell types, the apoptotic marker cleaved caspase 3 was only detected in lymphoma cells. **c**, RT-PCR analysis for expression of *Trp53* mRNA in cells shown in Fig. 4. *Trp53* mRNA is significantly expressed after 4-hydroxytamoxifen in

control and shArf expressing cells but is reduced in p53 shRNA-expressing cells. Average expression relative to GAPH and standard deviation is plotted on the log scale. **d**, Differential gene expression analysis was performed on three independent carcinoma-derived cell lines after *Trp53* restoration. Differentially expressed genes from cell lines and tumours (Fig. 4) were compared and expressed as a Venn diagram. A significant proportion of genes (18% of cell line genes and 15% of tumour genes) were common to both tumours and cell lines (blue stripes). **e**, Gene ontology (GO) analysis was performed to determine the similarity of gene expression programs that are significantly enriched after *Trp53* restoration in tumours and cell lines. The genes that are common between tumours and cell lines (blue stripes), unique to tumours (blue), or unique to cell lines (orange) were used to determine the most significantly enriched GO terms associated with each class. The differentially expressed genes that were common between cell lines and tumours fueled the discovery of top-most significant GO terms. This suggests that the relevant gene expression programs in the two systems are highly similar.

Supplementry References

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