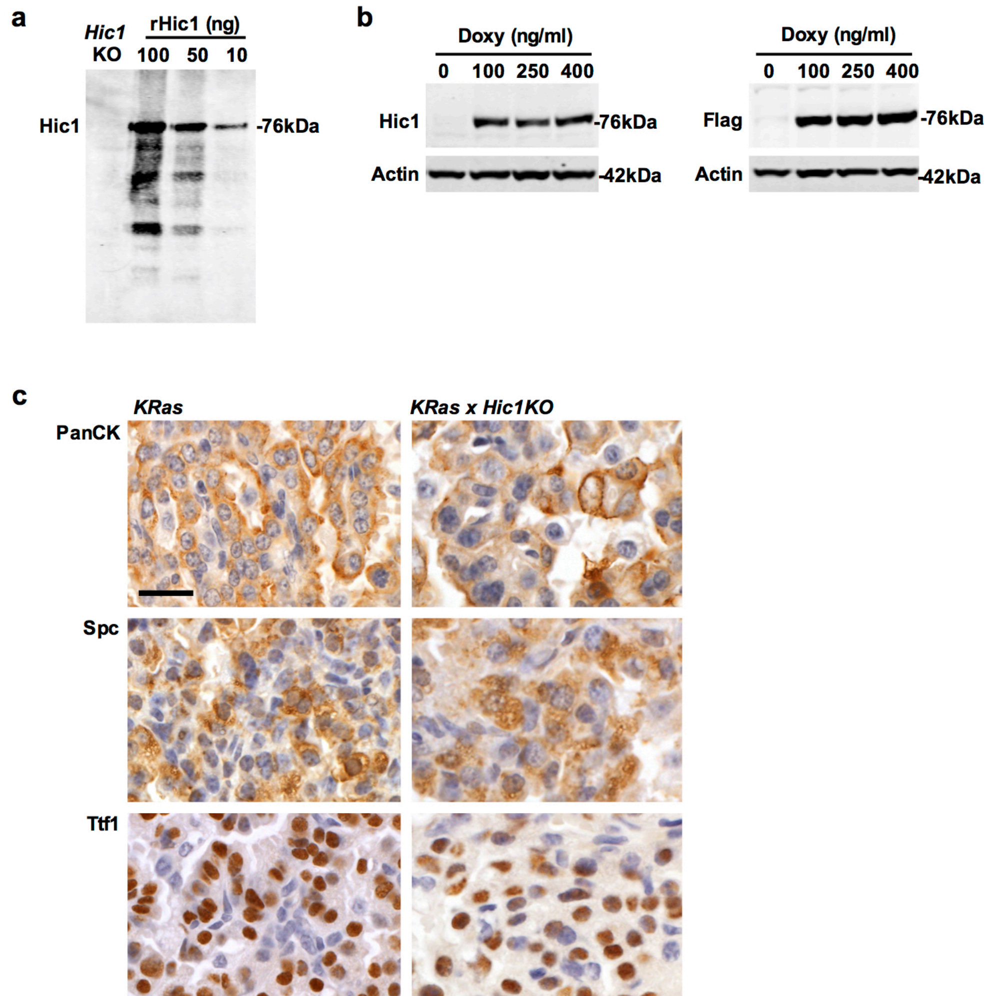

Supplementary Figure S1. (**a**) Western blot analysis of *Hic1*KO MEF cell lysate, or recombinant Hic1 protein probed with a novel Hic1 rabbit polyclonal antibody. **(b)** Western blot analysis of HEK293 cells transiently transfected with a doxycycline-inducible Hic1-Flag expression vector, and then treated with doxycycline (Doxy, Sigma-Aldrich, St Louis, MO, USA #D9891) probed with a novel rabbit polyclonal anti-Hic1 antibody or anti-Flag (Sigma-Aldrich, St Louis, MO, USA F3165). Western blot analysis was performed as described in Figure 1. (**c**) Effects of *Hic1* deletion (*Hic1*KO) on the development of lung tumors induced by the conditional activation of an activating $KRas^{G12D}$ (*KRas*) mutant in the airway epithelium of adult mice. Representative photomicrographs of sections from *KRas* and *KRas* x *Hic1*KO shown in stained with immunoperoxidase (brown) for Pancytokeratin (PanCK, Abcam, Cambridge, MA, USA #ab27988), Surfactant Protein C (Spc, Abcam #ab90716) or Thyroid Transcription Factor 1 (Ttf1, Abcam #ab76013) and countersained with hematoxylin (blue). Scale bar = 100μm. Staining was performed as described.[1]

Supplementary Methods

Microarray Gene Expression

Microarray expression analysis of RNA were performed by the Australia Genome Research Facility (Melbourne, Australia) using the MouseWG-6 v2.0 Expression BeadChip (Illumina), which contains 45,281 probes representing over 30,000 genes. Image processing and probe quantification was performed using the GenomeStudio software package (version 2011.1, Illumina, Inc., San Diego, CA, USA). A common pre-immortal set of WT samples was used as a control for both pre-immortal and immortal gene expression analysis.

Unprocessed probe intensities were exported from GenomeStudio and were background corrected, quantile normalized and $\log_2$ transformed using neqc function (default parameters)[2] from limma's Bioconductor package.[3] Only probes with a detection p value < 0.05 in at least 3 samples were used for further analysis.

Differentially probe expression testing was performed using limma's lmFit and eBayes functions. Orthogonal contrasts were generated to compare different experimental groups ($\log_2$FC 0.5 for the pre-immortal contrast and $\log_2$FC 1 for the immortal set contrasts; false discovery rate (FDR) cut-off P < 0.05 [Benjamini–Hockberg FDR (BH-FDR).[4] Gene ontology analysis was performed on selected differentially expressed probes, aggregated by gene, using DAVID 6.8.[5] A cut-off value of 0.05 (Benjamini) was applied to select significantly differentially enriched ontology categories.

Principal coordinate analysis was performed using ord function from made4 Bioconductor package.[6] Three-dimensional plots and gene expression Heatmaps were created using ComplexHeatmap[7] and the rgl R package.

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Supplementary Tables S1-5.xlsx

Table S1
Differentially expressed genes in preimmortal mouse embryonic fibroblasts (MEFs), wild type (WT) vs *Hic*1KO 48 hours after tamoxifen treatment.

Table S2
Gene ontology analysis of differentially expressed genes in preimmortal MEFs, wild type (WT) vs *Hic*1KO 48 hours after tamoxifen treatment

Table S3
Differentially expressed genes in immortalized MEFs, *EsrCre* vs *Hic1*KO.

Table S4
Differentially expressed genes in immortalized MEFs, *EsrCre* vs *p53*KO.

Table S5
Differentially expressed genes in immortalized MEFs, *Hic1*KO vs *p53*KO.