Supplementary experimental procedures

Senescence associated β-Galactosidase saining

For senescence associated histochemical β-Galactosidase activity staining a commercial kit (#9860, Cell Signaling, Danvers, MA) was used according to the manufacturer's protocol. Freshly harvested whole adrenals were fixed and then incubated in the supplied fixing solution and staining solution (pH6). Tissues were then processed and sectioned using standard procedures (paraffin embedding).

Loss of heterozygosity (LOH) analysis

Tumor tissue was macroscopically isolated from surrounding normal tissue. Tumor and liver DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). Then 500µg of DNA were used in a genotyping reaction for 25 cycles. Amplification products were separated on a 1.5% agarose gel and stained with ethidium bromide. Pictures were taken using a gel documentation system (BioRad, Hercules, CA). To quantify the amount of amplification density was estimated using the Quantity One software (BioRad, Hercules, CA). Background values were subtracted and the ratio for normal liver DNA from heterozygous animals was set as 1 and the ratio for experimental samples were calculated. Complete LOH was arbitrarily defined as ratios <0.33, partial LOH,

reflecting tumor heterogeneity or a significant amount of wt allele containing stroma as 0.33-0.66 and no LOH as values >0.66. For statistical analysis the Mantel-Haenszel Chi-square test of no correlation was used.

Supplementary Figures

Supplementary Figure 1:

(A) A *Acd*$^{+/+}$ *p53*$^{+/+}$ mouse is shown next to a *Acd*$^{acd/acd}$ *p53*$^{-/-}$ mouse. *Acd*$^{acd/acd}$ *p53*$^{-/-}$ mice only differ from their *Acd*$^{+/+}$ *p53*$^{+/+}$ littermates in size.

(B) Hyperpigmentation of the ano-genital region in *Acd*$^{acd/acd}$ *p53*$^{+/+}$ mice. Specifically the genital region is more pigmented than in *Acd*$^{acd/acd}$ *p53*$^{-/-}$ and *Acd*$^{+/+}$ *p53*$^{+/+}$ mice.

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Supplementary Figure 2:

(A) Freshly harvested adrenal glands were stained for senescence-associated β-Galactosidase activity at pH6. Adrenal cortices, like other tissues (e.g. sebaceous glands in the skin) display some background activity. In adrenal cortices from *Acd*$^{acd/acd}$ *p53*$^{+/+}$ animals there were some cells observed with a clearly positive staining. These cells were not observed in *Acd*$^{+/+}$ *p53*$^{+/+}$ adrenal cortices and less were observed in *Acd*$^{acd/acd}$ *p53*$^{-/-}$ adrenal cortices (scale bar 200µm).

Supplementary Figure 3:

(A) Typical macroscopic examples of tumors that developed in *Acd*$^{acd/acd}$ *p53*$^{-/-}$ mice are shown (lymphoma, rhabdomyosarcoma, osteosarcoma and hemangiosarcoma). As described for *p53*$^{-/-}$ mice, most tumors were sarcomas and lymphomas.

Supplementary Figure 4:

(A) Appearance of hydronephrosis at autopsy of *Acd*$^{acd/acd}$ *p53*$^{+/+}$ mice. Hydronephrosis was observed either uni- or bilaterally. Often the remnants of normal kidney tissue appeared atrophic. There is also a pigmented paraaortal lymph node visible. (Black arrow, hydronephrotic cystic kidney, blue arrow hyperpigmented lymphnode, scale bar 500µm).

(B) Microscopically a cystic appearance of the kidney was observed, shown with remnants of normal kidney tissue containing some glomeruli (blue arrow).

Supplementary Figure 5:

(A) *Acd*$^{acd/acd}$ *p53*$^{+/-}$ tumor tissues were genotyped for the *p53*$^{-}$ (*mut*) and *p53*$^{+}$ (*wt*) allele. At least 11 out of 16 tumors showed a loss of the *wt* allele, which could be the result of genomic instability in *Acd*$^{acd/acd}$ tumors.

(B) Genotyping of *Acd*$^{+/+}$ *p53*$^{+/-}$ tumors revealed loss of the *wt* allele in at most 4 out of 14 tumors.

(C) Band intensity was quantified and normalized to normal liver tissue of the same genotype. Partial loss of *wt* allele may represent genetic tumor heterogeneity with subpopulations that had lost the *wt* allele and others that retained the *wt* allele or represent the content of normal stroma that retained the parental genotype. The group of *Acd*$^{acd/acd}$ *p53*$^{+/-}$ tumors had significantly more samples with loss of *wt* allele than the control group (*Acd*$^{+/+}$ *p53*$^{+/-}$) (p=0.0003, Mantel-Haenszel Chi-square test of no correlation).

Supplementary Figure 6:

(A) Table of overlapping segments of amplification that were present in more than one sample (*- shown in **Suppl. Fig. 6B and 6C**).

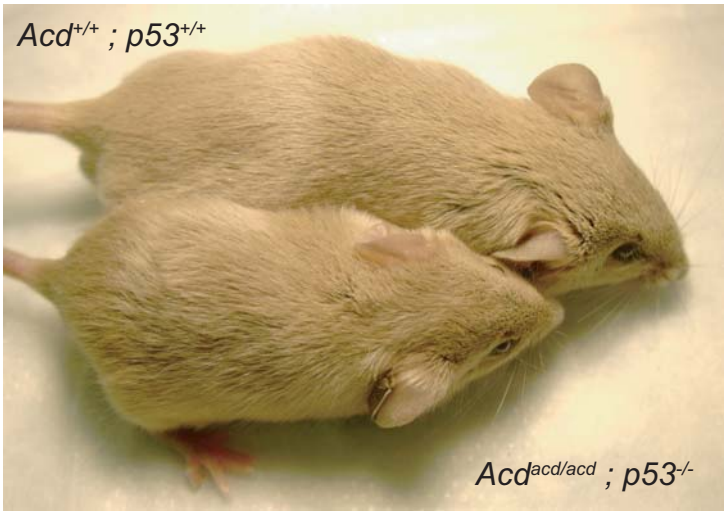
(B) Amplified region of chromosome 6 from two *Acd*$^{acd/acd}$ tumors.

(C) Amplified region of chromosome 11 from two *Acd*$^{acd/acd}$ tumors.

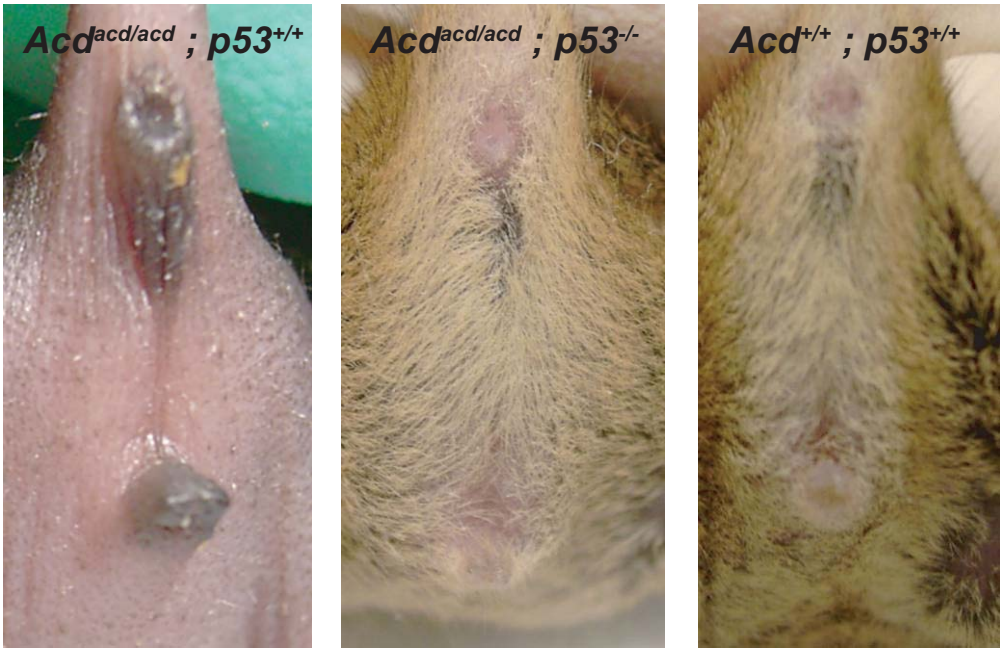
Supplemental Text and Figures

Supplementary Figure 1

A.

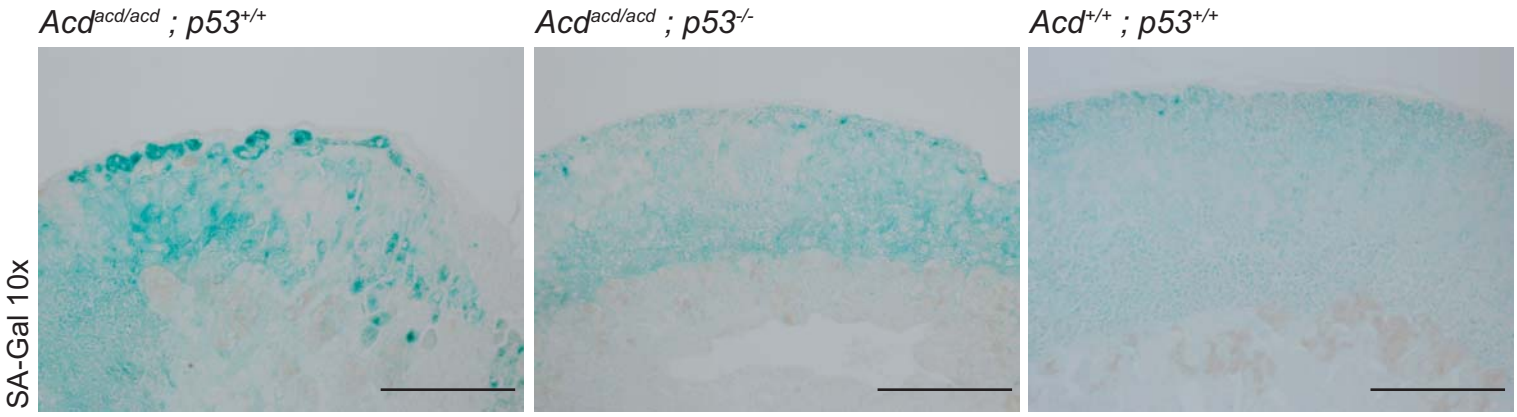


B.



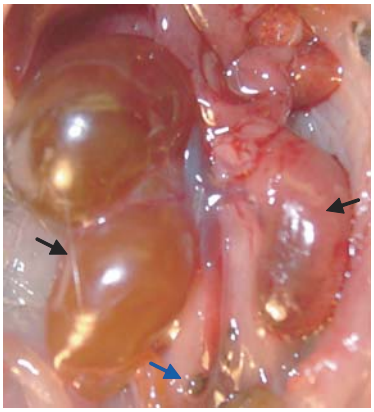
Supplementary Figure 2

A.



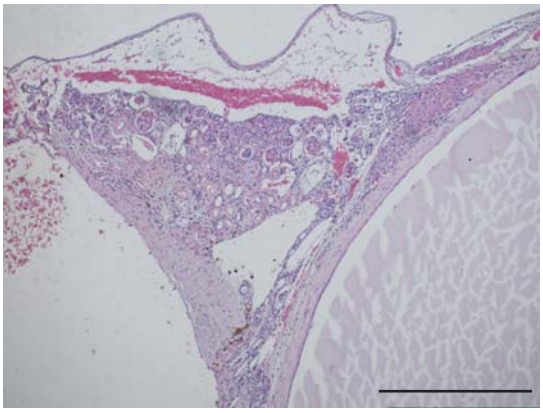
Supplementary Figure 3

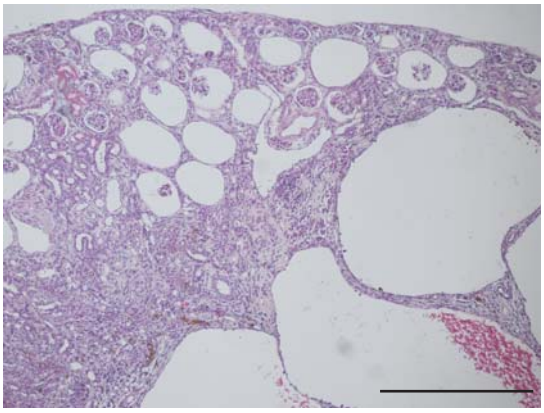
A.



bilateral hydronephrosis
(macroscopic)

B.



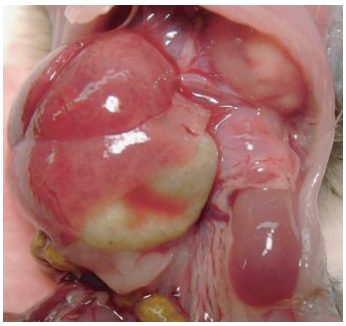


hydronephrosis
(microscopic)

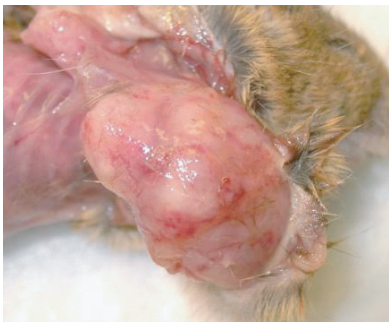
Supplementary Figure 4

A.

lymphoma
(thymus)



rhabdomyosarcoma
(diaphragm)

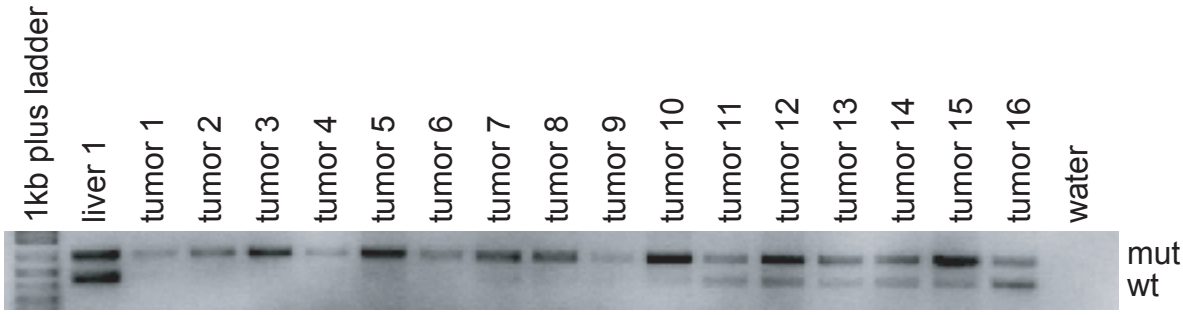


osteosarcoma
(hindlimb)

hemangiosarcoma
(neck)

Supplementary Figure 5

A.



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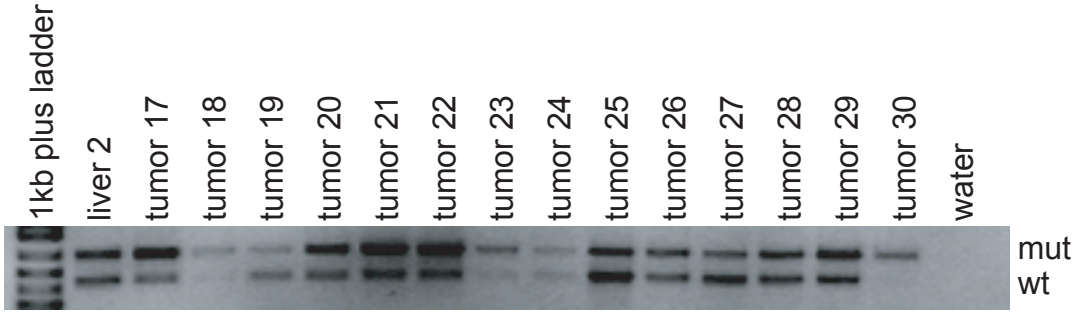


C.

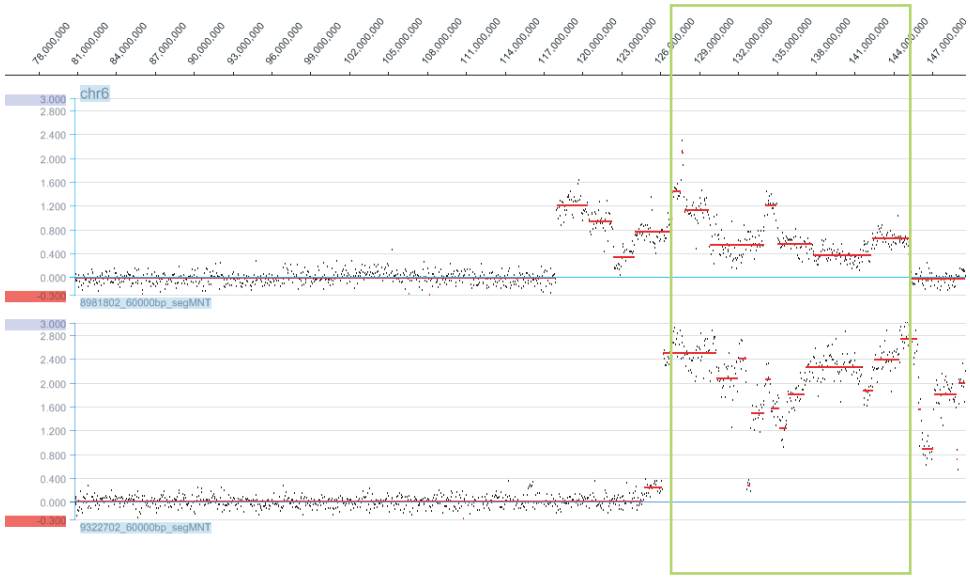
	no LOH	partial LOH	complete LOH	total
Acd$^{acd/acd}$; *p53*$^{+/-}$	1	4	11	16
Acd$^{+/+}$; *p53*$^{+/-}$	10	2	2	14

Supplementary Figure 6

A.

chromosome	region of overlap start	region of overlap stop
1	none	none
3	111,690,000	111,750,000
5	none	none
6*	126,870,000	145,230,000
11*	111,930,000	112,530,000
14	52,290,000	53,130,000
X	none	none

B.



C.