SUPPLEMENTARY INFORMATION

EMBO-MM

***Igf2* pathway dependency of the *Trp53* developmental and tumour phenotypes**

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Supporting Tables

Parental genotype and strain	Cross	Time-point	WT		*Igf2*$^{+m/-p}$		Total p value χ^2
			♂	♀	♂	♀	
F1=B6 WT ♀ x 129 *Igf2*$^{+m/-p}$ ♂		P0	64 (46) 6.81	46 (46) 0.00	39 (46) 1.14	36 (46) 2.27	185 p<0.05 10.22
		P3.5	56 (39) 7.68	43 (39) 0.47	33 (39) 0.85	23 (39) 6.40	155 p<0.01 15.40
129 WT ♀ x F1 *Igf2*$^{+m/-p}$ ♂	N2	P0	45 (48) 0.16	48 (48) 0.00	54 (48) 0.82	44 (48) 0.29	191 p=NS 1.27
		P3.5	45 (45) 0.00	46 (45) 0.05	49 (45) 0.46	38 (45) 0.95	178 p=NS 1.46
F2=B6 WT ♀ x F1 *Igf2*$^{+m/-p}$ ♂	N2	P0	86 (75) 1.61	90 (75) 3.00	71 (75) 0.21	53 (75) 6.45	300 p<0.05 11.28
		P3.5	79 (55) 16.37	85 (55) 10.47	35 (55) 7.27	21 (55) 21.02	220 p<0.001 55.13
F3=B6 WT ♀ x F2 *Igf2*$^{+m/-p}$ ♂	N3	P0	54 (45) 1.80	56 (45) 2.69	48 (45) 0.20	22 (45) 11.76	180 p<0.001 16.44
		P3.5	50 (31) 12.47	55 (31) 19.68	10 (31) 13.78	7 (31) 18.11	122 p<0.001 64.03
F4=B6 WT ♀ x F3 *Igf2*$^{+m/-p}$ ♂	N4	P0	18 (18) 0.00	22 (18) 1.02	20 (18) 0.29	11 (18) 2.57	71 p=NS 3.87
		P3.5	14 (10) 1.6	18 (10) 6.4	5 (10) 2.5	3 (10) 4.9	40 p<0.01 15.4
F5=B6 WT ♀ x F4 *Igf2*$^{+m/-p}$ ♂	N5	P0	11 (10) 0.05	12 (10) 0.30	10 (10) 0.00	8 (10) 0.49	41 p=NS 0.85
		P3.5	11 (6) 4.17	12 (6) 6.00	1 (6) 4.17	0 (6) 6.00	24 p<0.001 20.33
F6=B6 WT ♀ x F5 *Igf2*$^{-m/+p}$ ♂ (†)	N6	P0	12 (10) 0.40	11 (10) 0.10	9 (10) 0.10	8 (10) 0.40	40 p=NS 1.00
		P3.5	11 (6) 4.17	11 (6) 4.17	1 (6) 4.17	1 (6) 4.17	24 p<0.001 16.67

† - F5 *Igf2*$^{-m/+p}$ ♂ is $^{-m/+p}$ not $^{+m/-p}$ as in all previous stages of breeding

Table S1.
Failure to backcross *Igf2*$^{+m/-p}$ (*Igf2*$^{Tm1Rob-pat/+}$) targeted disruption on C57BL/6J background.
B6 WT females were crossed with 129 *Igf2*$^{+m/-p}$ males and the Mendelian segregation determined at P0 and P3.5. At generations N2 and N3, there was a significant deviation from the expected Mendelian segregation at P0. At P3.5, there was a significant deviation in the expected number of progeny at all generations, with no female and one male *Igf2*$^{+m/-p}$ surviving to P3.5 at the 5th generation. B6 WT females mated with F5 *Igf2*$^{-m/+p}$ males resulted in a normal Mendelian ratio at P0, but almost complete deficit of F6 *Igf2*$^{+m/-p}$ progeny by P3.5. In contrast, 129 WT females crossed with F1 *Igf2*$^{+m/-p}$ males were born at the expected Mendelian distribution at P0 and P3.5. Upper value – observed number. Middle value – expected number. Lower number – χ^2 value. Far right hand column, middle value – p value. All 3 df.

Parental genotype	Strain	WT	*Igf2*$^{+/-}$	*p53*$^{+/-}$	*Igf2*$^{+/-}$, *p53*$^{+/-}$	*p53*$^{-/-}$	*Igf2*$^{-/-}$, *p53*$^{+/-}$	Total p value χ^2 value
Igf2$^{+m/-p}$ ♀ x *p53*$^{+/-}$ ♂	129	46 (40.25) 0.821	37 (40.25) 0.262	38 (40.25) 0.126	40 (40.25) 0.001	-	-	161 p=NS 1.211
p53$^{+/-}$ ♀ x *Igf2*$^{+m/-p}$ ♂	129	10 (11.25) 0.139	12 (11.25) 0.05	12 (11.25) 0.05	11 (11.25) 0.006	-	-	45 p=NS 0.244
Igf2$^{+m/-p}$ x *p53*$^{+/-}$ (†)	129B6F1	14 (17.25) 0.612	15 (17.25) 0.293	19 (17.25) 0.178	21 (17.25) 0.815	-	-	69 p=NS 1.900
Igf2$^{+m/-p}$, *p53*$^{+/-}$ ♀ x *p53*$^{+/-}$ ♂	129B6F2	9 (5.50) 2.227	4 (5.50) 0.409	12 (11.00) 0.091	7 (11.00) 1.454	10 (5.50) 3.682	2 (5.50) 2.227	44 p=NS 10.091

† - 129B6F1 *Igf2*$^{+m/-p}$ x *p53*$^{+/-}$ could be either *Igf2*$^{+m/-p}$ ♀ x *p53*$^{+/-}$ ♂ or *p53*$^{+/-}$ ♀ x *Igf2*$^{+m/-p}$ ♂

Table S2.
Normal Mendelian segregation of progeny from breeding *Igf2* and *p53* heterozygous males and females by post-natal day 10.
On a 129J strain, the progeny from *Igf2*$^{+m/-p}$ females x *p53*$^{+/-}$ males and *p53*$^{+/-}$ females x *Igf2*$^{+m/-p}$ males conformed to the expected Mendelian ratios. The progeny from a 129B6F1 cross also conformed to the expected Mendelian frequencies (3df). Whilst *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ female x *p53*$^{+/-}$ male progeny conformed to the expected Mendelian segregation, although there was a trend towards significance (n=44, p=NS, χ^2 = 10.091) and it may be that the low numbers were confounding the analysis. Upper value – observed, middle value – expected, lower value - χ^2 value. Except right-hand column where middle value – p value.

Parental genotype	Strain	WT	$H19^{+m/-p}$ (*)	$H19^{-m/-p}$	$p53^{+/-}$	$H19^{-m/+p}$, $p53^{+/-}$(*)	$H19^{-m/-p}$, $p53^{+/-}$	$p53^{-/-}$	$H19^{-m/+p}$, $p53^{-/-}$(*)	$H19^{-m/-p}$, $p53^{-/-}$	Total p value χ^2 value
$H19^{-m/+p}$ x $p53^{+/-}$ (†)	129	16 (15.50) 0.016	15 (15.50) 0.016	-	19 (15.50) 0.790	12 (15.50) 0.790	-	-	-	-	62 p=NS 1.613
	B6	17 (18.25) 0.086	13 (18.25) 1.510	-	17 (18.25) 0.086	26 (18.25) 3.291	-	-	-	-	73 p=NS 4.973
	B6129F1	3 (4.75) 0.645	4 (4.75) 0.118	-	5 (4.75) 0.013	7 (4.75) 1.066	-	-	-	-	19 p=NS 1.842
$H19^{-m/+p}$, $p53^{+/-}$ ♀ x $p53^{+/-}$ ♂	129	2 (4.25) 1.191	4 (4.25) 0.015	-	12 (8.50) 1.441	8 (8.50) 0.029	-	5 (4.25) 0.132	3 (4.25) 0.368		34 p=NS 3.176
	B6	6 (5. 50) 0.045	6 (5. 50) 0.045	-	9 (11.00) 0.364	15 (11.00) 1.454	-	4 (5. 50) 0.409	4 (5. 50) 0.409	-	44 p=NS 2.727
$H19^{-m/+p}$, $p53^{+/-}$ ♀ x $H19^{-m/+p}$,$p53^{+/-}$ ♂	129	2 (2.94) 0.299	7 (5.88) 0.215	6 (2.94) 3.192	5 (5.88) 0.130	14 (11.75) 0.431	4 (5.88) 0.598	5 (2.94) 1.448	4 (5.88) 0.598	0 (2.94) 2.94	47 p=NS 9.851
	B6	6 (6.56) 0.048	17 (13.16) 1.144	5 (6.56) 0.372	15 (13.16) 0.268	34 (26.25) 2.288	10 (13.16) 0.744	5 (6.56) 0.372	7 (13.16) 2.858	6 (6.56) 0.048	105 p=NS 8.143
	B6129F2	2 (2.94) 0.299	3 (5.88) 1.407	5 (2.94) 1.448	6 (5.88) 0.003	12 (11.75) 0.005	4 (5.88) 0.598	5 (2.94) 1.448	7 (5.88) 0.215	3 (2.94) 0.001	47 p=NS 5.426

† $H19^{+/-}$ x $p53^{+/-}$ could be either $H19^{-m/+p}$ ♀ x $p53^{+/-}$ ♂ or $p53^{+/-}$ ♀ x $H19^{-m/+p}$ ♂

- $H19^{-m/+p/-}$ progeny from the $H19^{-m/+p}$, $p53^{+/-}$ ♀ x $p53^{+/-}$ ♂ are $H19^{-m/+p}$. Progeny from $H19^{-m/+p}$ x $p53^{+/-}$ and $H19^{+m/-p}$, $p53^{+/-}$ ♀ x $H19^{+/-}$, $p53^{+/-}$ ♂ are a combination of $H19^{-m/+p}$ and $H19^{+m/-p}$.

Table S3.
Bi-allelic *Igf2* expression following maternal allele deletion $H19^{-m/+p}$ (∆13kb) did not alter the embryonic survival of progeny either alone or combined with $p53^{+/-}$ mice.
On 129, B6 and B6129F1 strains, the progeny from $H19^{-m/+p}$ x $p53^{+/-}$ breeding conformed to the expected Mendelian ratios. The progeny from $H19^{-m/+p}$, $p53^{+/-}$ females x $p53^{+/-}$ males conformed to the expected Mendelian frequencies on pure 129 and B6 mouse strains. There was no significant difference in the Mendelian distribution of progeny from $H19^{-m/+p}$, $p53^{+/-}$ females x $H19^{-m/+p}$, $p53^{+/-}$ males, irrespective of mouse strain. Upper– observed, middle– expected, lower- χ^2 value.

Tumor Type	Genotype	Intact non targeted allele	Mutant Non-targeted allele	Type of mutation	Equivalent human amino acid
Lymphoma	*Igf2*$^{+m/-p}$	1	0		
	Igf2$^{+m/+p}$ /*H19*$^{+m/+p}$	5	2	Point mutations	R196R, V203L, E204E, N239S, S241S T211I, E221K, C222R, 228N
	H19$^{-m/+p}$	2	0		
Carcinoma	*Igf2*$^{+m/-p}$	1	1	Point mutations	Y163F, H168H, M169I, T170L, R181W
	Igf2$^{+m/+p}$ /*H19*$^{+m/+p}$	1	1	Point mutations	Y163F, H168H, M169I, T170L, R181W
	H19$^{-m/+p}$	4	0		
Sarcoma	*Igf2*$^{+m/-p}$	1	0		
	Igf2$^{+m/+p}$ /*H19*$^{+m/+p}$	0	0		
	H19$^{-m/+p}$	1	1	Splicing error	Exon 5-6 boundary

Table S4.
Mutations of the intact allele in *p53*$^{+/-}$ tumors by *Igf2* and *H19* genotype and tumor type. Two of five lymphomas and one of two carcinomas WT for *Igf2* had mutation of the intact (non-targeted) *p53* allele. One of two carcinomas that were null for *Igf2* (*Igf2*$^{+m/-p}$) and one of two sarcomas with bi-allelic *Igf2* expression had a mutation in the intact *p53* allele.

Strain	Genotype	Abbreviation (heterozygote)	Generation	Developmental phenotype	Reference
129S2/J	*Igf2*$^{Tm1Rob\text{-}pat/+}$	*Igf2*$^{+/-}$	>20	Paternal K.O. – 60% size of WT Maternal K.O. – same size as WT	Dechiara, 1990
C57BL/6J	*Igf2*$^{Tm1Rob\text{-}pat/+}$	*Igf2*$^{+/-}$	4th	Lethal beyond 4th backcross to generation	Unpublished
129S2/J	*Trp53*$^{Tm1Brd/+}$	*p53*$^{+/-}$ *p53*$^{-/-}$	>20	Unchanged Female specific exencephaly	Clarke, 1993
C57BL/6J	*Trp53*$^{Tm1Brd/+}$	*p53*$^{+/-}$	10-20	Unchanged	Unpublished
C57BL/6J	*H19*$^{Tm1Tilg/+}$	*H19*$^{+/-}$ (*H19*$^{+/\Delta 13kb}$)	>20	Paternal K.O. – same size as WT Maternal K.O. – 128% size of WT	Leighton, 1995
129S2/J	*H19*$^{Tm1Tilg/+}$	*H19*$^{+/-}$	5-9	Ongoing analysis	Unpublished
C57BL/6J	*Igf2*^{loxP}	*Igf2*$^{+/loxP}$ – intact *Igf2*$^{+/\Delta}$ – deleted (deleted region exons 4-6)	>10	Intact allele – unchanged Deleted allele – not quantified	Miguel Constancia Unpublished
B6.FVBN	Trp53^{Tm1Brn}	*p53*$^{+/loxP}$ – intact *p53*$^{+/\Delta}$ – deleted (deleted region exons 2-10)	>5	Intact allele – unchanged Deleted allele – unchanged	Jonkers, 2001
C57BL/6J	*Gt(ROSA)26 CreER*^{T} *Sor*$^{Tm1(cre/Esr1) Nat}$	R26Cre$^{+/-}$	>10	Unchanged	Badea, 2003

Table S5.
Mouse genetic models; strain, genotype and developmental phenotype.
+ denotes WT allele, - denotes allele with targeted mutation (germ-line knock-out), *H19*$^{-m/+p}$ had disruption of *Igf2/H19* imprinting control region and *H19* gene. fl denotes loxP flanked alleles, Δ denotes conditional alleles that have undergone recombination.

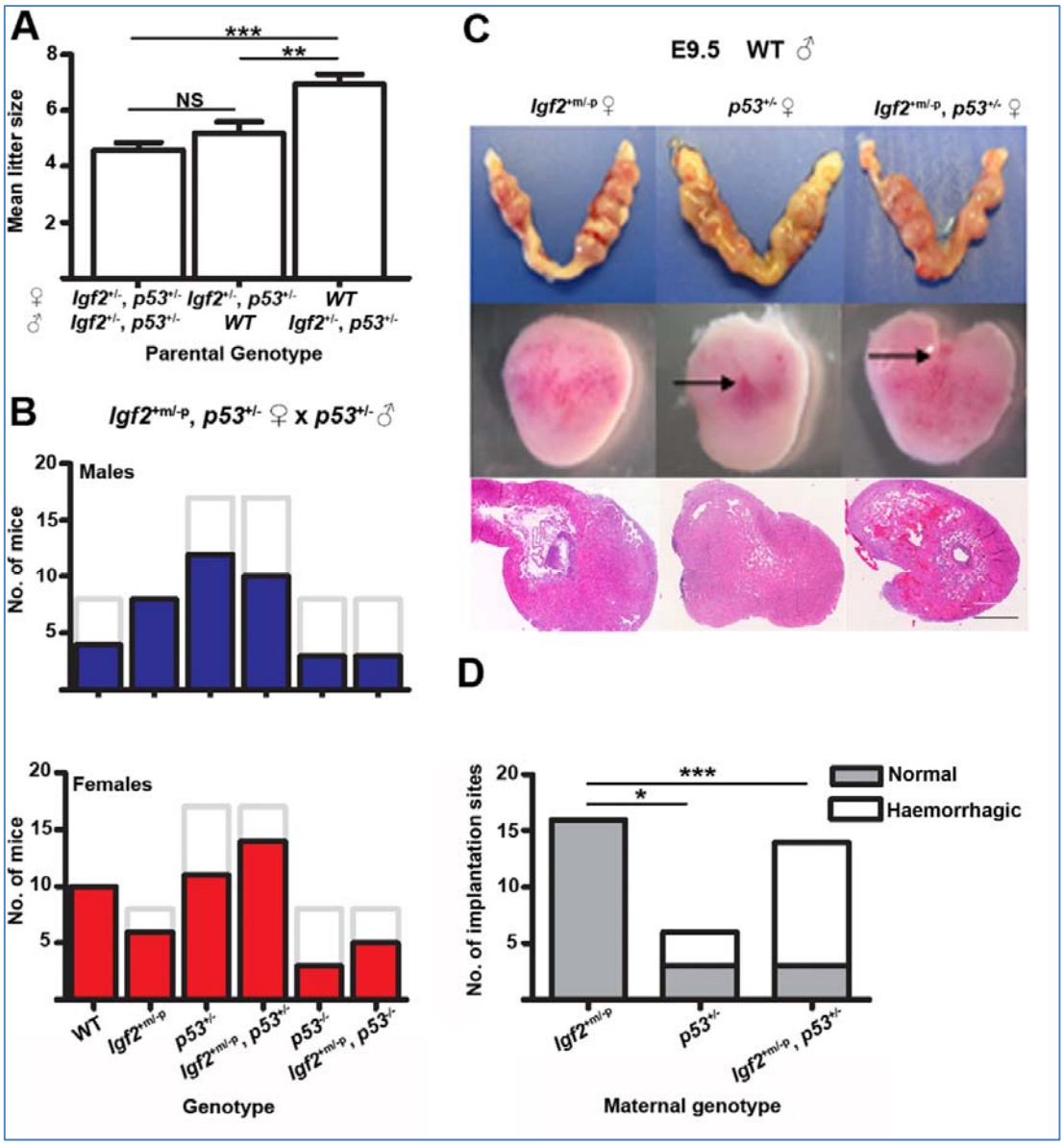


Figure S1. Reduced litter size derived from *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ females was associated with decidual haemorrhage.

A. Reduced litter size were derived from *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ females (note *Igf2*$^{+m/-p}$ denoted *Igf2*$^{+/-}$ for clarity). Significantly fewer progeny were obtained from mating of *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ females with *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ males (***p< 0.0001, 1-way ANOVA, mean of 64 litters) and *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ females with *WT* males (**$p < 0.01$, mean of 25 litters) than from *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ males with *WT* females (mean of 45 litters). **B.** Male (top, solid blue bars, total = 40) and female (bottom, solid red bars, total = 49) progeny from *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ females mated with *p53*$^{+/-}$ males segregated according to the expected Mendelian distribution (gray, unfilled bars). The reduction of male offspring was independent of genotype and appeared significant ($P < 0.05$, χ^2-test) compared to the expected numbers derived from all previous breeding from 129 mice. **C.** Uterine horns (upper panels) were removed from pregnant females at E9.5 and representative implantation sites shown (middle panels). In *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ mothers, the maternal deciduas appeared abnormal, that was also evident in a small proportion of *p53*$^{+/-}$ mothers (arrows). Implantation sites from *Igf2*$^{+m/-p}$ mothers appeared normal. (Lower panel) Haematoxylin and eosin sections showed blood-filled implantation sites in *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ mothers (right). Scale bar – 1mm. **D.** *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ females had significantly more haemorrhagic implantation sites (11/14) compared to *p53*$^{+/-}$ females (3/6) and *Igf2*$^{+m/-p}$ females (0/16). (*$P < 0.05$, *** $P <$ 0.0001, Fisher's exact test).

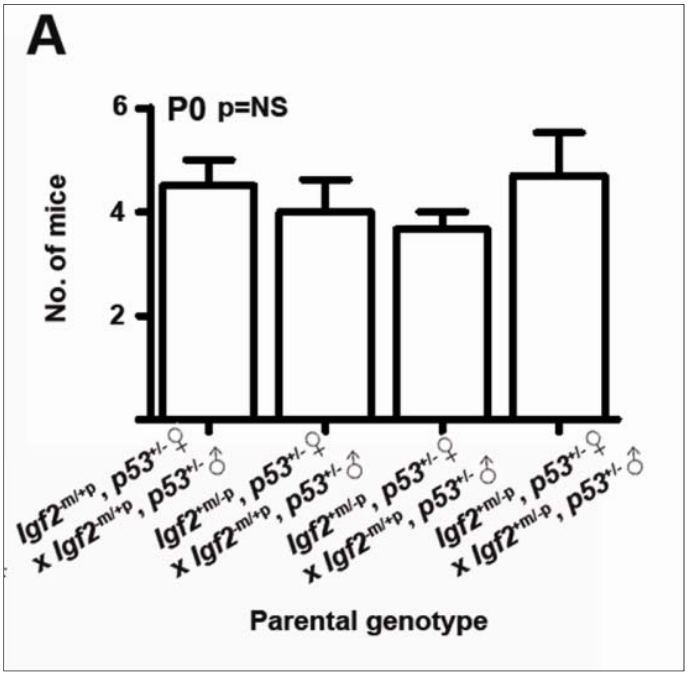


Figure S2.
The reduction in mean litter size from the 129 *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ inter-cross was irrespective of the parental *Igf2* allelic dose.
There was no significant difference (p=0.826, one-way ANOVA) in the mean litter sizes between *Igf2*$^{-m/+p}$, *p53*$^{+/-}$ females mated with *Igf2*$^{-m/+p}$, *p53*$^{+/-}$ males (n=4 litters, mean litter size = 4.500 ± 0.500), *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ females mated with *Igf2*$^{-m/+p}$, *p53*$^{+/-}$ males (n=7 litters, mean litter size = 4.000 ± 0.617), *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ females mated with *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ males (n=3 litters, mean litter size = 3.667 ± 0.333) and *Igf2*$^{-m/+p}$, *p53*$^{+/-}$ females mated with *Igf2*$^{+m/-p}$, *p53*$^{+/-}$ males (n=10 litters, mean litter size = 4.700 ± 0.817).

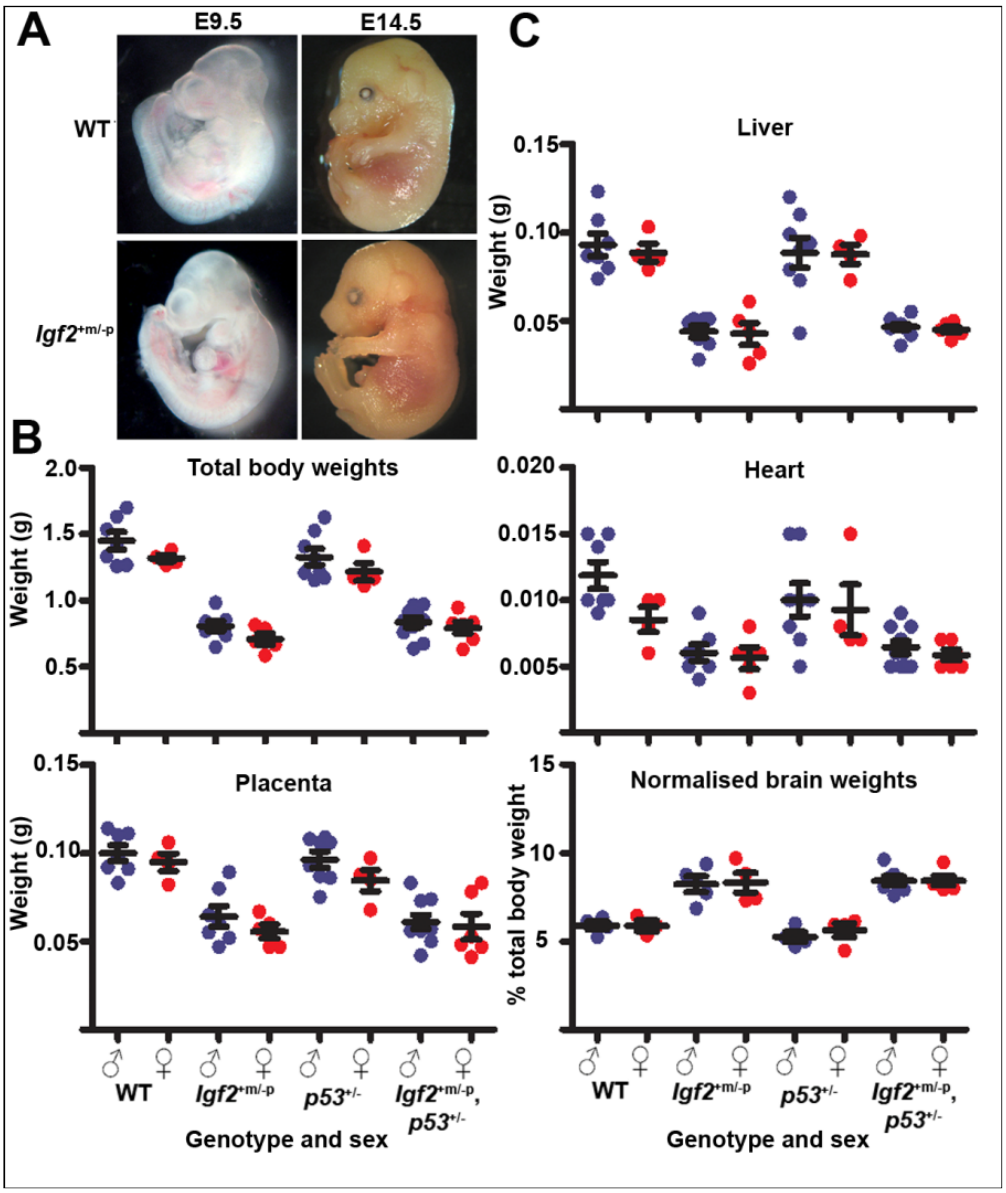


Figure S3.
Normal gross morphology but reduced growth in 129 *Igf2*$^{+m/-p}$ female embryos.
A. 129 *Igf2*$^{+m/-p}$ females did not exhibit gross morphological abnormalities during development. Upper panel – WT embryo at E9.5 (n=7 examined) and E14.5-15.5 (n=1 examined). Lower panel – *Igf2*$^{+m/-p}$ females at E9.5 (n=4 examined) and E14.5-15.5 (n=1 examined). **B. & C.** Reduced embryonic growth of mice that lack the paternal allele of *Igf2* was independent of p53 and embryo sex (blue-males, red-females). Significant weight differences were matched in the embryo, placenta, liver, heart and brains (including brains weight normalised to body weight) of mice with intact *Igf2* paternal allele (WT and *p53*$^{+/-}$) compared to mice functionally null for *Igf2* (*Igf2*$^{+m/-p}$ and *Igf2*$^{+m/-p}$, *p53*$^{+/-}$, p<0.0001, 1-way ANOVA, Tukey's multiple comparison).

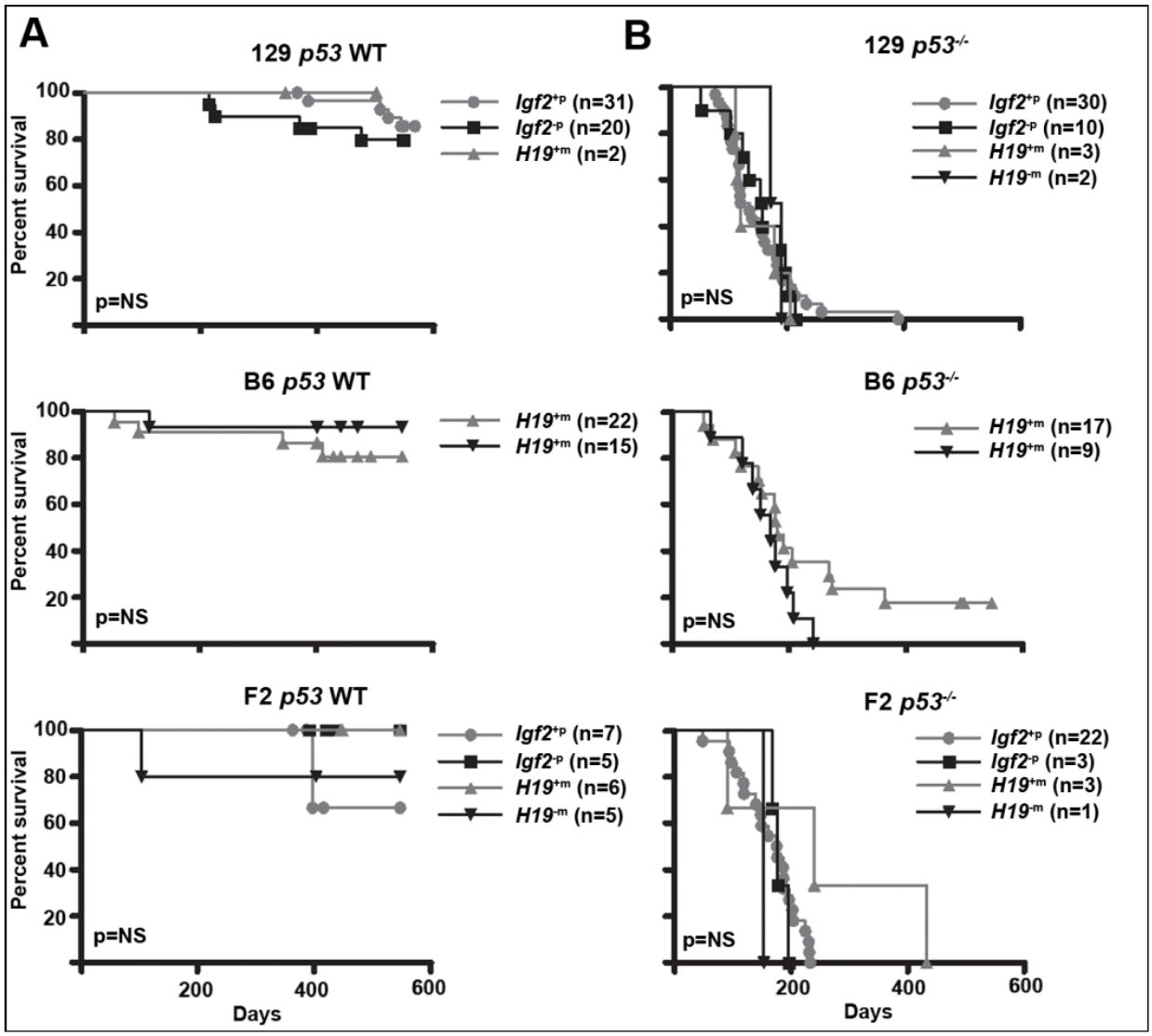
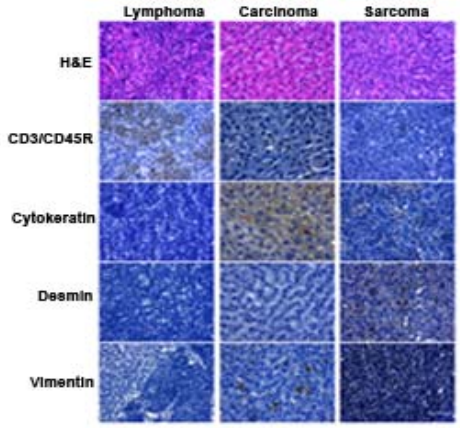


Figure S4.
Survival of *p53* WT or *p53* homozygous null mice to 18 months was independent of *Igf2* allelic dose and mouse strain.
A, Top – There was no significant difference in survival of 129 *p53* WT mice (p=NS, Log Rank test) with loss of *Igf2* (*Igf2*$^{-p}$, n=31) compared to mice mono-allelic for *Igf2* (*Igf2*$^{+p}$, n=20 or *H19*$^{+m}$, n=2). **Middle** – Gain of *Igf2* function through disruption of *H19* (*H19*$^{-m}$, n=15) did not alter survival of B6 *p53* WT mice (p=NS, Log Rank test) compared to mice mono-allelic for *Igf2* (*H19*$^{+m}$, n=22). **Bottom** – Loss of *Igf2* (*Igf2*$^{-p}$, n=5) or gain of *Igf2* (*H19*$^{-m}$, n=5) did not significantly alter survival of F2 hybrid *p53* WT mice compared to mice mono-allelic for *Igf2* (*Igf2*$^{+p}$, n=7 or *H19*$^{+m}$, n=6, p=NS, Log Rank test). **B, Top** – There was no significant difference in survival of 129 *p53*$^{-/-}$ mice with loss of *Igf2* (*Igf2*$^{-p}$, n=31) or gain of *Igf2* (*H19*$^{-m}$, n=2) compared to mice mono-allelic for *Igf2* (*Igf2*$^{+p}$, n=10 or *H19*$^{+m}$, n=3, p=NS, Log Rank test). **Middle** – Gain of *Igf2* (*H19*$^{-m}$, n=15) did not alter survival of B6 *p53*$^{-/-}$ mice (p=NS, Log Rank test) compared to mice mono-allelic for *Igf2* (*H19*$^{+m}$, n=22). **Bottom** – Loss of *Igf2* (*Igf2*$^{-p}$, n=3) or gain of *Igf2* (*H19*$^{-m}$, n=1) did not significantly alter survival of F2 hybrid *p53*$^{-/-}$ mice compared to mice mono-allelic for *Igf2* (*Igf2*$^{+p}$, n=22 or *H19*$^{+m}$, n=3, p=NS, Log Rank test). NB – note low numbers in F2 analysis. **Note abbreviations;** *Igf2*$^{+p}$ = *Igf2*$^{-m/+p}$ and *Igf2*$^{+/+}$**.** *Igf2*$^{-p}$ = *Igf2*$^{+m/-p}$ and *Igf2*$^{-/-}$. *H19*$^{\mathbf{+m}}$ **=** *H19*$^{\mathbf{+m/-p}}$ and *H19*$^{+/+}$. *H19*$^{\mathbf{-m}}$ **=** *H19*$^{\mathbf{-m/+p}}$ and *H19*$^{-/-}$.



Genotype	Tumour type	No. of tumours
Igf2^{+p}, *p53*$^{+/-}$ (129)	Lymphoma	9(4)
	Neuroendocrinoma	1(1)
	Adenocarcinoma (undiff)	1(0)
	Carcinoma (lung)	1(0)
	Carcinoma (GI)	1(1)
	Keraticoma	1(1)
	Leiomyoma	1(0)
	Leiomyosarcoma	1(1)
	Undifferentiated round cell	2(0)
Igf2^{+p}, *p53*$^{+/-}$ (129)	Lymphoma	2(1)
	Squamous cell	2(1)
	Adenocarcinoma (undiff.)	1(1)
	Carcinoma (lung)	1(1)
	Adenoma	1(1)
	Sarcoma	3(3)
H19^{+m}, *p53*$^{+/-}$ (B6)	Lymphoma	10(6)
	Adenocarcinoma (breast)	1(0)
	Keratioacanthoma	1(1)
	Carcinoma (uterine)	1(0)
	Carcinoma (GI)	2(2)
H19^{-m}, *p53*$^{+/-}$ (B6)	Lymphoma	3(1)
	Hepatocellular carcinoma	1(1)
	Carcinoma (GI)	4(3)
	Sarcoma	2(0)

Figure S5.
Representative immunohistochemical labelling and classification of tumours using DAB substrate (brown) and haematoxylin (blue). All images x40 objective. Left column – lymphoma; positive for CD3 (T cell lymphoma), CD45R (B cell lymphoma) or both (mixed). Negative for cytokeratin, desmin and vimentin. Middle column (hepatocellular carcinoma) – positive for cytokeratin, vimentin positive = stromal cells, negative for CD3/CD45R and desmin. Right column (sarcoma) – negative for CD3/CD45R and cytokeratin, positive for desmin and vimentin (stromal cells). **Table** – tumour histio-type assembled by genotype; classified by Histopathologist (FP) blinded to genotype. Number in brackets = males.

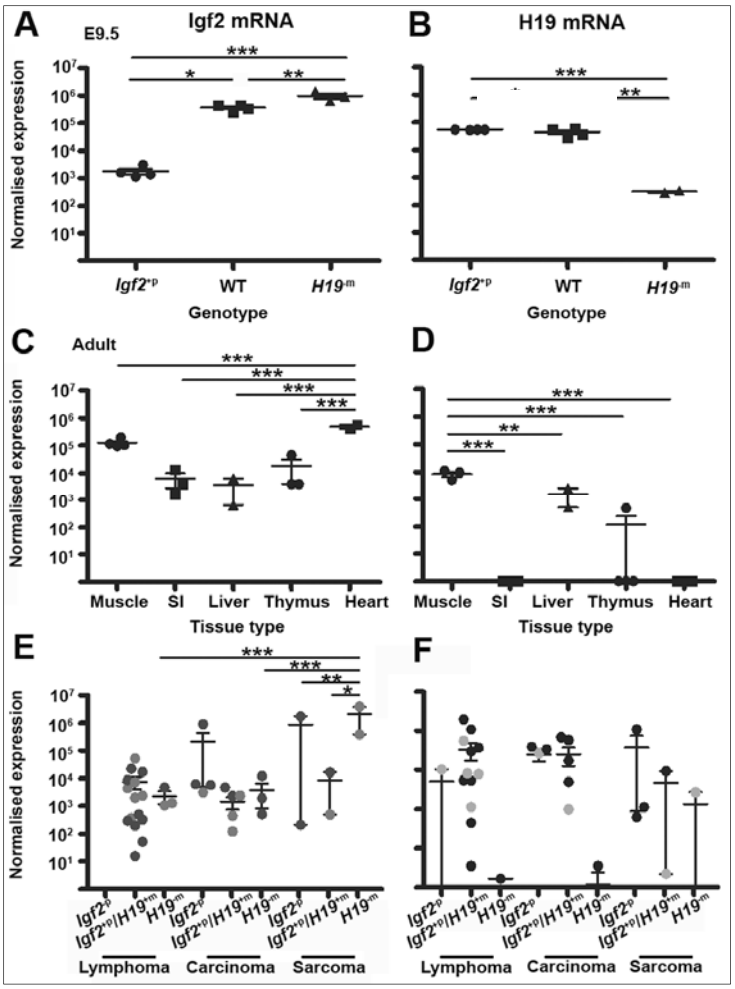


Figure S6.
Sarcomas from *p53*$^{+/-}$ mice with bi-allelic *Igf2* expressed higher *Igf2* mRNA levels comparable to embryonic levels of expression.
Igf2 and *H19* mRNA expression normalised to 100,000 copies of *β-actin*. **A**. Embryos null for *Igf2* paternal allele had a significantly lower *Igf2* mRNA expression (*Igf2*$^{+m/-p}$, n=5) compared to WT (*Igf2*$^{+/+}$, n=4, *p<0.05) and embryos bi-allelic for *Igf2* (*H19*$^{-m/+p}$, n=3, ***p<0.0001). WT embryos had a significantly lower expression compared to embryos bi-allelic for *Igf2*. **B**. There was no significant differences in *H19* mRNA expression between embryos null for *Igf2* (*Igf2*$^{+m/-p}$, n=4) compared to WT (*Igf2*$^{+m/+p}$, n=4). Mice with bi-allelic *Igf2* expression (*H19*$^{-m/+p}$, n=2) had significantly lower *H19* mRNA expression compared to mice null and WT for *Igf2*. **C**. *Igf2* mRNA expression was significantly different in the heart (n=2) compared to muscle (n=4), small intestine (n=3), liver (n=2) and thymus (n=3). There was no significant difference between other tissues. **D**. *H19* mRNA expression was significantly different in the muscle (n=3) compared to small intestine (n=3), liver (n=2), thymus (n=4,) and heart (n=4). There was no significant difference between other tissues. **E.** *Igf2*$^{+p}$ equals *Igf2*$^{-m/+p}$ and *Igf2*$^{+/+}$. *Igf2*$^{-p}$ equals *Igf2*$^{+m/-p}$ and *Igf2*$^{-m/-p}$. *H19*$^{+m}$ = *H19*$^{+m/-p}$ and *H19*$^{+m/+p}$. *H19*$^{-m}$ = *H19*$^{-m/+p}$ and *H19*$^{-m/-p}$. Black circles – male. Gray circles – female. *Igf2* allelic dose did not affect *Igf2* expression in lymphomas (*Igf2*$^{-p}$ n=1, *Igf2*$^{+p}$/*H19*$^{+m}$ n=16, *H19*$^{-m}$ n=3) or carcinomas (*Igf2*$^{-p}$ n=4, *Igf2*$^{+p}$/*H19*$^{+m}$ n=7, *H19*$^{-m}$ n=4). Loss of paternal *Igf2* did not significantly alter *Igf2* mRNA expression in sarcomas (*Igf2*$^{-p}$ n=2) relative to *Igf2*$^{+p}$/*H19*$^{+m}$ (n=2), implicating maternal allele expression (loss of imprinting). There was a significant increase in *Igf2* mRNA expression in sarcomas with bi-allelic *Igf2* expression (n=2) compared to monoallelic *Igf2* tumors (*Igf2*$^{+p}$, p<0.05) and mice null for *Igf2* (*Igf2*$^{-p}$, p<0.001). **F**. There was no significant difference in *H19* mRNA expression dependent on *Igf2* allelic dose in lymphomas (*Igf2*$^{-p}$ n=2, *Igf2*$^{+p}$/*H19*$^{+m}$ n=14, *H19*$^{-m}$ n=1), carcinomas (*Igf2*$^{-p}$ n=4, *Igf2*$^{+p}$/*H19*$^{+m}$ n=6, *H19*$^{-m}$ n=3) or sarcomas (*Igf2*$^{-p}$ n=3, *Igf2*$^{+p}$/*H19*$^{+m}$ n=2, *H19*$^{-m}$ n=2). All 1-way ANOVA, Tukey's multiple comparison; ***p<0.0001, **p<0.001, *p<0.01.

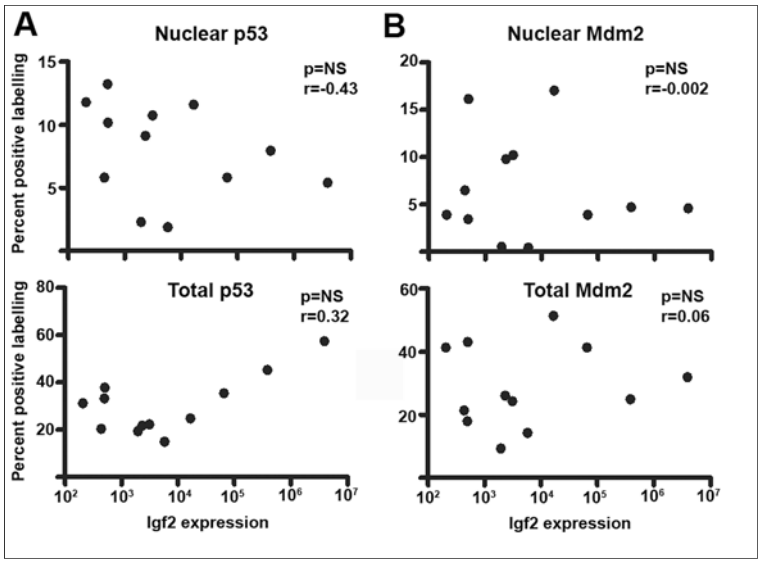


Figure S7.
***Igf2* expression did not correlate with either p53 or Mdm2 protein levels or localisation in solid tumours. A.** There was no correlation between *Igf2* mRNA level and nuclear p53 levels (above, n=13, p=NS, r = -0.43), or total p53 levels (below, n=13, p=NS, r = 0.32), **B.** Nuclear Mdm2 levels (above, n=13, p=NS, r = -0.002) and total Mdm2 levels (below, n=13, p=NS, r = 0.06, Spearman's) also did not correlate with *Igf2* mRNA expression. (NS=not significant).

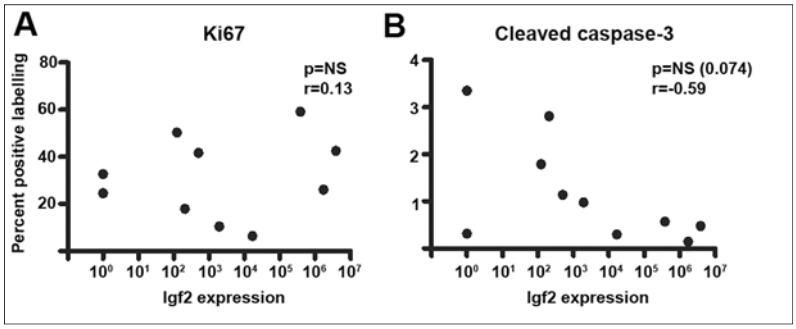


Figure S8.
Proliferation and apoptosis did not correlate with *Igf2* mRNA expression in carcinomas and sarcomas *p53*$^{+/-}$ tumours. A. Mean percentage proliferating cells did not correlate with *Igf2* expression levels (n=10, r = 0.13, p=NS). **B** – Whilst there was no correlation between mean percentage apoptotic cells and *Igf2* expression (n=10, r = -0.59, p=NS) this was approaching significance (p=0.074, Spearman's).

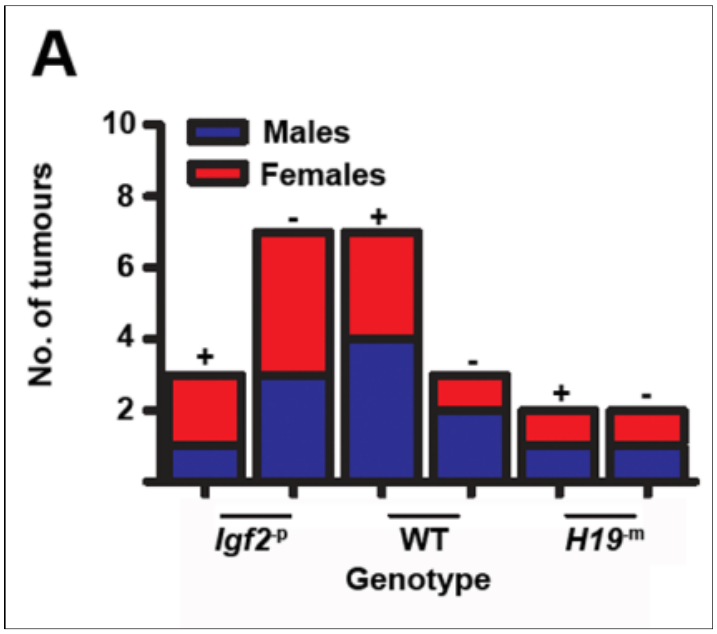


Figure S9.
***Igf2* allelic dose did not alter the frequency of ER-α positive solid tumours in *p53*$^{+/-}$ mice. A.** There was no significant difference in the proportion of ER-α positive *p53*$^{+/-}$ tumours in *Igf2*$^{-p}$ (n=1 male and 2 females) and ER-α negative tumours (n=3 males and 4 females) relative to *Igf2* WT ER-α positive *p53*$^{+/-}$ tumours (n=4 males and 3 females) and ER-α negative tumours (n=2 males and 1 female, p=NS, Fisher's exact). There was no significant difference in the proportion of ER-α positive *p53*$^{+/-}$ tumours with bi-allelic *Igf2* expression *H19*$^{-m}$ (n=1 male and 1 female) and ER-α negative tumours (n=1 male and 1 female) relative to *Igf2* WT *p53*$^{+/-}$ tumours (p=NS, Fisher's exact test).

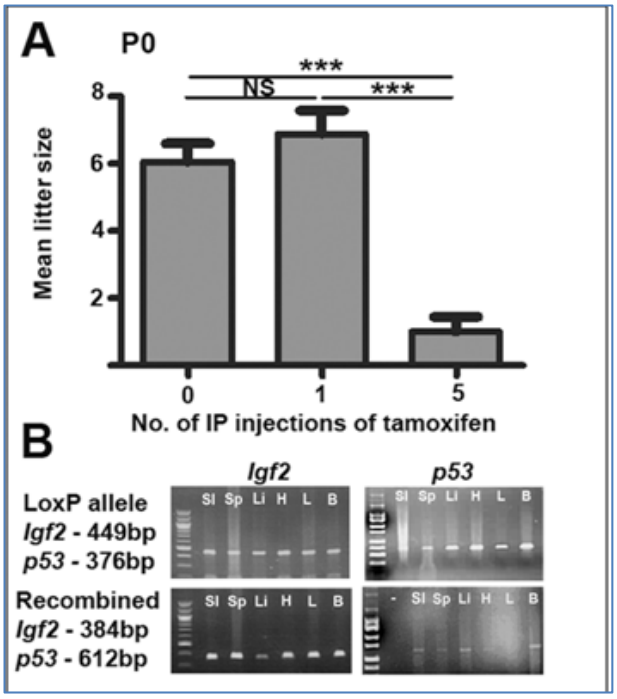


Figure S10.
Mean litter size of conditional mouse breeding following Tamoxifen injection.
A. Mean litter size of conditional mouse breeding was reduced following injections of 1mg Tamoxifen at E10.5. There was a significant deficit in the mean litter size following 5 daily injections (n=10 litters) compared to no injections (n=15 litters. ***p<0.0001) and 1 injection at E10.5 (n=18 litters, ***p<0.0001). **B**. Recombination of the *Igf2*$^{fl(loxP)}$ alleles was induced in all organs following a single 1mg IP injection of Tamoxifen at E10.5, but may not have been 100% efficient. Recombination of the *p53*^{loxP} alleles was induced in all organs but relative levels differed. Above; PCR of intact alleles using F1 and R1 primers. Below; PCR of recombined alleles using F1 and R10 primers. (Sl – small intestine, Sp – spleen, Li – liver, H – heart, L – lung, B – brain). Note: The progeny of *Igf2*$^{fl/fl}$, *p53*$^{fl/fl}$ females mated with *Igf2*$^{+/fl}$, *p53*$^{+/fl}$, R26Cre$^{+/-}$ males were born at the normal Mendelian ratios without Tamoxifen (n=60, p=NS, χ^2 = 6.894, 7df). Male (total = 37) and female (total = 28) progeny from the conditional cross of *Igf2*$^{fl/fl}$ *p53*$^{fl/fl}$ females x *Igf2*$^{fl/+}$, *p53*$^{fl/+}$, R26Cre$^{+/-}$ and injection of 1mg of Tamoxifen at E10.5 lead to normal Mendelian segregation at P10.