LETTERS

p53 regulates maternal reproduction through LIF

Wenwei Hu[1]*, Zhaohui Feng[1]*, Angelika K. Teresky[1] & Arnold J. Levine[1,2]

Extensive studies have shown that p53 is important in tumour prevention[1]. However, little is known about its normal physiological function. Here we show that p53 is important in reproduction, in a gender-specific manner. Significant decreases in embryonic implantation, pregnancy rate and litter size were observed in matings with $p53^{-/-}$ female mice but not with $p53^{-/-}$ male mice. The gene encoding leukaemia inhibitory factor (LIF), a cytokine critical for implantation[2], was identified as a p53-regulated gene that functions as the downstream mediator of this effect. p53 can regulate both basal and inducible transcription of LIF. Loss of p53 decreased both the level and function of LIF in uteri. Lower LIF levels were observed in the uteri of $p53^{-/-}$ mice than in those of $p53^{+/+}$ mice, particularly at day 4 of pregnancy, when transiently induced high levels of LIF were crucial for embryonic implantation. This observation probably accounts for the impaired implantation of embryos in $p53^{-/-}$ female mice. Administration of LIF to pregnant $p53^{-/-}$ mice restored maternal reproduction by improving implantation. These results demonstrate a function for p53 in maternal reproduction through the regulation of LIF. Evidence is accumulating that p53 may have a similar function in humans.

p53, the guardian of the genome, regulates the cellular response to various stresses[1]. Most studies have concentrated on *p53* as a tumour suppressor gene, and very little is known about its function in normal cellular processes. Two observations suggest that p53 could have a function in reproduction. First, some alleles of *p53* and its negative regulator, MDM2, are under positive evolutionary selection[3,4]. Second, we observed that crosses of $p53^{+/+}$, $p53^{+/-}$ and $p53^{-/-}$ mice indicated an unusual pattern of maternal effects on pregnancy rate and litter size. In this study, mice of two inbred strains (C57BL/6J and $129SV^{sl}$) with different p53 statuses were bred, and p53 was found to be involved in reproduction in a gender-specific manner. In C57BL/6J mice, pregnancy rate and litter size for $p53^{-/-}$ female mice in breeding pairs with $p53^{+/+}$, $p53^{+/-}$ or $p53^{-/-}$ males were markedly lower, whereas they were unaffected when $p53^{-/-}$ male mice were mated with $p53^{+/+}$ female mice. The decrease was very significant compared with wild-type breeding pairs and was most severe when $p53^{-/-}$ females were mated with $p53^{-/-}$ males, with the embryo genotype of $p53^{-/-}$ (Table 1). A similar, although less severe, phenotype was observed with the $129SV^{sl}$ strain (Table 1), indicating the impact of genetic background. To minimize the effect of genetic background, crosses were performed by mating $p53^{-/-}$ mice from the two different genetic backgrounds (C57BL/6J male and $129SV^{sl}$ female mice). A clear reduction in pregnancy rate and litter size was observed (Table 1), indicating that the defect in reproduction was due to the loss of p53, which could not be compensated for by cross-breeding two different $p53^{-/-}$ inbred strains. Furthermore, the extent of decrease was very close to that in the $129SV^{sl}$ strain (the female breeder) indicating that the severity of the phenotype depended on the genetic background of female mice. These data clearly demonstrate that p53 deficiency in female mice and fetuses results in reduced maternal reproduction, and strongly suggest a crucial function for p53 in maternal reproduction.

As a transcription factor, p53 mainly functions through the transcriptional regulation of its target genes by binding to degenerate DNA-responsive elements (Fig. 1a)[5]. To explore whether p53 regulates maternal reproduction through transcriptional regulation of its target genes, p53 algorithms[6] were employed to search for potential target genes for p53 involved in maternal reproduction. The gene encoding leukaemia inhibitory factor (LIF), a secreted cytokine that is critical for blastocyst implantation[2,7], was identified as a potential p53 target gene with a putative p53-binding element in intron 1 of both mouse and human *LIF* genes (Fig. 1a). Chromatin immunoprecipitation (ChIP) assays were performed in tet-off *p53*-inducible human H1299-WTp53 cells, and in mouse Val5 fibroblasts containing a temperature-sensitive mutant *p53* plasmid. Immunoprecipitation of the chromatin fragments corresponding to the binding elements in both human and mouse *LIF* genes with anti-p53 antibody was observed only when the functional p53 protein was expressed (Fig. 1b). Similar results were obtained in murine embryonic fibroblast (MEF) cells with or without wild-type p53 (Supplementary Fig. 1). These data demonstrate an interaction of p53 with these putative p53-binding elements *in vivo*. Further, pGL2 luciferase reporter plasmids containing these binding elements exhibited a p53-dependent transcriptional activity in *p53*-null human H1299 cells (Fig. 1c), Saos2 and HCT116 $p53^{-/-}$ cells (data not shown).

Table 1 | Reproductive data from C57BL/6J and $129SV^{sl}$ mice with different *p53* genotypes

Strain		Genotype of *p53*		Litter size	Pregnancy rate (%)	Number of breeding pairs
Male	Female	Male	Female			
C57BL/6J	C57BL/6J	+/+	+/+	6.71 ± 0.43	100	14
		−/−	+/+	6.41 ± 0.61	100	11
		+/−	+/−	4.79 ± 0.61	79	19
		−/−	+/−	4.93 ± 0.21	89	89
		+/+	−/−	2.18 ± 0.83*	36†	11
		+/−	−/−	1.42 ± 0.55*	33†	12
		−/−	−/−	0.69 ± 0.16*	27†	69
$129SV^{sl}$	$129SV^{sl}$	+/+	+/+	5.41 ± 0.35	95	22
		−/−	+/+	5.58 ± 0.61	100	6
		+/−	+/−	5.21 ± 0.64	100	8
		−/−	+/−	4.8 ± 0.18	84	32
		−/−	−/−	2.35 ± 0.24‡	58§	90
C57BL/6J	$129SV^{sl}$	+/+	+/+	5.64 ± 0.58	100	11
		−/−	−/−	2.62 ± 0.84	63	8

Eight-week-old mice with different *p53* genotypes were set up for mating as indicated and were housed together for at least 6 weeks. Pregnancy rate was calculated as the ratio of the number of females with confirmed pregnancy to the number of females who served as breeders. Where shown, errors are s.e.m.
* $P < 0.001$ versus wild type, *t*-test.
† $P < 0.001$ versus wild type, χ^2 test.
‡ $P < 0.01$ versus wild type, *t*-test.
§ $P < 0.01$ versus wild type, χ^2 test.

[1]Cancer Institute of New Jersey, University of Medicine and Dentistry of New Jersey, New Brunswick, New Jersey 08903, USA. [2]The Simons Center for Systems Biology, The Institute for Advanced Study, Princeton, New Jersey 08540, USA.
*These authors contributed equally to this work.

Regulation of *LIF* expression by p53 was explored in Val5 and Vm10 cells, both containing a temperature-sensitive mutant *p53* plasmid. Activation of p53 at 32 °C increased LIF levels significantly in both cells (up to sevenfold at the messenger RNA level and 14-fold at the protein level) and culture supernatant (up to 16-fold at the protein level) (Fig. 1d). This induction was p53 dependent; there was no increase in 10(1) cells, their *p53*-null parental cells. This p53-dependent induction was also observed in human H1299-WTp53 cells (data not shown). Irradiation with γ-rays resulted in a large induction of *Lif* transcription in spleen (more than 70-fold) and thymus (more than 12-fold), two radiosensitive tissues, in wild-type mice but not in $p53^{-/-}$ mice (Fig. 1e). A clear p53-dependent induction of *Lif* was also observed in uterine tissues under conditions of both whole-body γ-irradiation and *in vitro* irradiation of cultured uterine tissues (Fig. 1e). This induction showed tissue specificity; it was also observed in liver, skin and small intestine but not in kidney, heart or muscle tissues (data not shown). For the sake of comparison, the induction of a panel of known p53-target genes by γ-irradiation in different tissues is listed in Supplementary Fig. 2. Furthermore, endogenous p53, under no apparent stress conditions, had a significant impact on the basal transcription levels of *Lif*. In two pairs of isogenic cell lines (MEF $p53^{+/+}$ and $p53^{-/-}$, and HCT116 $p53^{+/+}$ and $p53^{-/-}$), the basal *Lif* mRNA levels were significantly (twofold to threefold) higher in $p53^{+/+}$ cells than in $p53^{-/-}$ cells (Fig. 1f). These data show that *LIF* is a previously unidentified target of p53, which regulates both basal and inducible transcription of *LIF*.

LIF has a crucial function in blastocyst implantation[2,7,8]. Transiently increased uterine *LIF* expression coincides with onset of implantation[9]. Similarly to our observation in $p53^{-/-}$ mice, $Lif^{-/-}$ mice have a defect in maternal reproduction as a result of the failure of blastocyst implantation[2]. To investigate the impact of loss of p53 on LIF levels and function during pregnancy, uterine *Lif* expression levels were measured in pregnant mice with different p53 statuses. Simultaneously with increased oestrogen levels, the *Lif* expression levels increased significantly (fivefold to eightfold) in the $p53^{+/+}$ uterus at day 4 (Fig. 2a), the exact onset time for implantation, although no significant increase in the p53 levels (activation) was observed (Supplementary Fig. 3). This transient induction of *Lif* showed tissue specificity; it was not observed in spleen or thymus, where the highest induction by p53 activation after γ-irradiation was observed. The uterine *Lif* expression levels were significantly lower in $p53^{-/-}$ mice than in $p53^{+/+}$ mice, both non-pregnant (day 0) and at day 4 of pregnancy (Fig. 2b). *Lif* is an oestrogen-responsive gene[7] and it has been suggested that oestrogen is involved in the induction of *Lif* expression at day 4 of pregnancy[7]. The impact of the p53 status on *LIF* regulation by oestrogen was examined in a pair of isogenic oestrogen-responsive cell lines: MCF7 ($p53^{+/+}$) and MCF7-p53siRNA (stably expressing a *p53* short interfering RNA (siRNA)). The basal transcription levels of *LIF* in MCF7-p53siRNA were one-third of those in MCF7 cells (Fig. 2c). The *LIF* expression levels increased significantly in both cell lines after treatment with oestrogen. However, significantly lower *LIF* levels were still observed in MCF7-p53siRNA cells (more than threefold to fourfold lower). As a control, there were no significant differences in the levels of *pS2*, an oestrogen-responsive gene not regulated by p53, between these two cell lines, either before or after treatment with oestrogen (data not shown). These results are similar to the observation (Fig. 2b) of different uterine *Lif* levels in day 4 pregnant mice of various p53 statuses, indicating that p53 regulates *LIF* expression in coordination with oestrogen during implantation, which may involve an interaction between p53, oestrogen and oestrogen receptor. Along with the decreased uterine expression of *Lif*, we observed impaired implantation in the $p53^{-/-}$ mice. Examining early embryo implantation sites at day 5 of pregnancy by Chicago blue dye staining demonstrated a significantly smaller number of implantation sites in $p53^{-/-}$ mice than in $p53^{+/+}$ mice (Fig. 2d, e). A significant number of unimplanted blastocysts were recovered from $p53^{-/-}$ mice at day 5 of pregnancy by uterine flushing, whereas

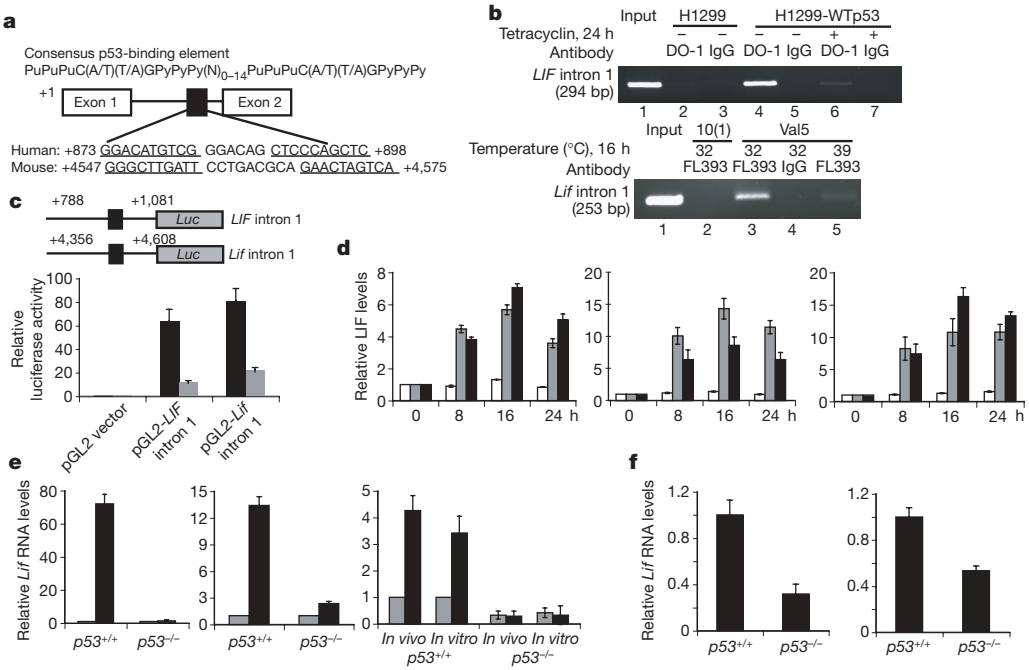
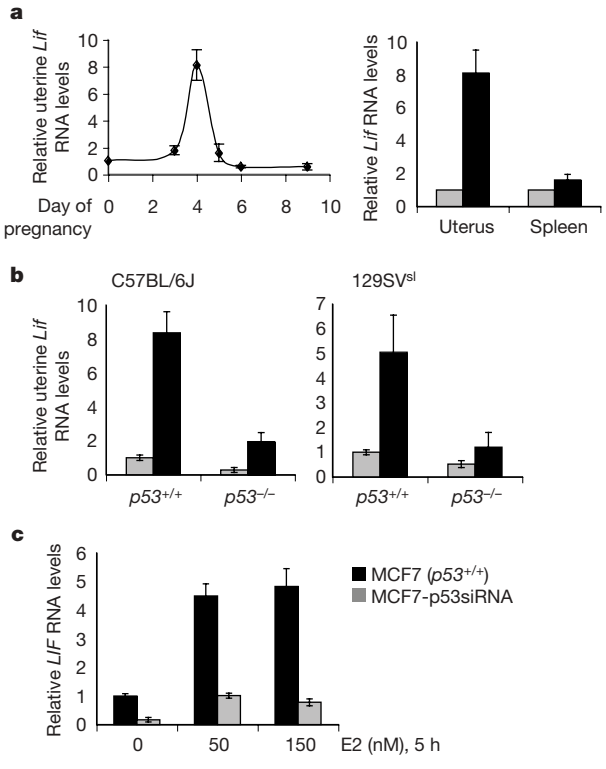
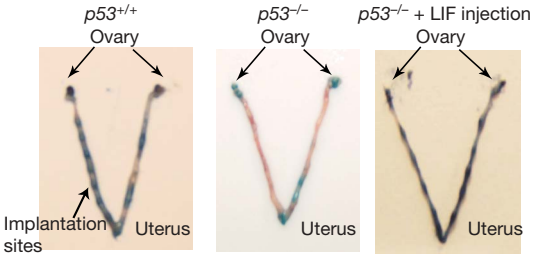


Figure 1 | p53 regulates the expression of *LIF* genes. **a**, Putative p53-consensus binding elements in *LIF* genes. Pu, purine; Py, pyrimidine. **b**, p53 binds to the p53-consensus binding elements in the *LIF* genes (human, *LIF*; mouse, *Lif*) as determined by ChIP assay. bp, base pairs. **c**, p53 transactivates the p53-consensus binding elements in the *LIF* genes as determined by luciferase (*Luc*) reporter assay. Black bars, WTp53; grey bars, mutant p53. **d**, p53 activation increases LIF expression and secretion. Left, RNA; centre, protein (cell lysate); right, protein (culture supernatant). White bars, 10(1) cells; grey bars, Val5 cells; black bars, Vm10 cells. **e**, p53 activation increases *Lif* expression in mice. C57BL/6J mice or uterine tissues from C57BL/6J mice were γ-irradiated (5 Gy) and tissues were harvested 6 h after irradiation ($n = 3$). Left, spleen; centre, thymus; right, uterus. Grey bars, control; black bars, irradiated. **f**, p53 regulates the basal transcription levels of *Lif*. Left, MEF cells; right, HCT116 cells. Results are means and s.e.m.



d

Genotype of *p53*		LIF injection	Implantation sites	Recovered blastocysts	*n*
Male	Female				
+/+	+/+	–	8.4 ± 0.5	0	5
–/–	–/–	–	2.7 ± 0.8	3.2 ± 0.6	6
–/–	–/–	+	7 ± 0.8	0.6 ± 0.6	3



e

Genotype of *p53*		Implanted embryos	*n*
Male	Female		
+/+	+/+	8.9 ± 0.6	7
–/–	–/–	1.4 ± 0.6	8

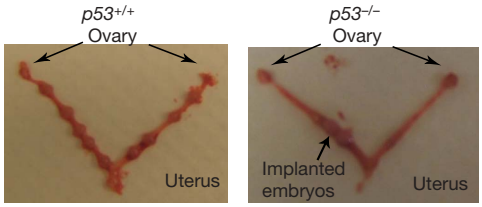


Figure 2 | p53 regulates *LIF* expression in mouse uterus during implantation. **a**, Transiently increased uterine *Lif* expression coincides with the onset of implantation in $p53^{+/+}$ C57BL/6J mice ($n = 4$). Grey bars, day 0; black bars, day 4. **b**, Lower uterine *Lif* expression in $p53^{-/-}$ mice, especially at the implantation stage ($n = 4$). Grey bars, day 0; black bars, day 4. **c**, The impact of p53 on regulation of *LIF* by oestrogen. **d**, **e**, Impaired implantation in $p53^{-/-}$ C57BL/6J mice and its restoration by injection of LIF. **d**, Early implantation examined at day 5 of pregnancy. LIF was injected at day 4 of pregnancy. **e**, Uteri were collected at day 7 of pregnancy and the number of implanted embryos was counted. Results are means and s.e.m.

none was recovered from $p53^{+/+}$ mice. A significantly smaller number of implanted embryos was consistently observed in $p53^{-/-}$ mice by day 7 of pregnancy (Fig. 2d, e). $p53^{-/-}$ mice have normal uterine morphology and ovarian oestrogen levels, and seem to have normal ovulation and fertilization functions (Supplementary Fig. 4), which were unlikely to be causes for the observed low uterine LIF levels and the small number of implanted embryos in $p53^{-/-}$ mice. Taken together, these data demonstrate impaired implantation in $p53^{-/-}$ mice.

Implantation can be restored in $Lif^{-/-}$ mice by the administration of exogenous LIF at day 4 after pregnancy[7]. To investigate whether low uterine LIF levels contributed to the reduced reproduction in $p53^{-/-}$ female mice, LIF was administered (by intraperitoneal injection) to the pregnant $p53^{-/-}$ mice at day 4. LIF injection did indeed improve maternal reproduction significantly in C57BL/6J $p53^{-/-}$ female mice, with 100% fertility and a significantly increased litter size (Table 2); 70% of the mice born were alive and normal. The rest of the mice had developmental abnormalities, mainly defects in the neural tube, which were predominantly associated with females, which is consistent with previous reports[10]. For the 129SV^{sl} strain, only those $p53^{-/-}$ female mice that failed to become pregnant after mating with $p53^{-/-}$ males for at least 1 month were employed for LIF injection. Injection of LIF significantly increased the pregnancy rate (from 0% to 100%) and litter size (Table 2). This effect was p53 dependent; LIF injection did not change the pregnancy rate or litter size in wild-type mice (Table 2). Injection of LIF seemed to act by improving implantation in $p53^{-/-}$ mice; it significantly increased the number of implantation sites and decreased the number of unimplanted blastocysts recovered at day 5 of pregnancy in $p53^{-/-}$ females (Fig. 2d, e). These results demonstrate that *p53* loss decreases the levels and function of uterine LIF, and administration of LIF to pregnant $p53^{-/-}$ mice restores maternal reproduction by improving implantation.

Table 2 | LIF injection increases pregnancy rate and litter size in $p53^{-/-}$ female mice

Strain and genotype	LIF injection	Litter size	Pregnancy rate (%)	Number of breeding pairs
C57BL/6J, $p53^{-/-} \times p53^{-/-}$	–	0.69 ± 0.16	27	69
	+	5.2 ± 0.57*	100†	10
129SV^{sl}, $p53^{-/-} \times p53^{-/-}$	–	2.35 ± 0.24	58	90
	+	3.8 ± 0.32*	100†	5
C57BL/6J, $p53^{+/+} \times p53^{+/+}$	–	6.71 ± 0.43	100	14
	+	7.4 ± 0.81	100	5

Recombinant LIF (5 μg) was administered to female mice twice, with 5 h between, by intraperitoneal injection at day 4 of pregnancy (the day on which a plug was observed was designated as day 1). For the 129SV^{sl} strain, only those $p53^{-/-}$ female mice that failed to get pregnant after mating with $p53^{-/-}$ male mice for at least 4 weeks were used for LIF injection. Where shown, errors are s.e.m.
* $P < 0.001$ versus untreated mice, *t*-test.
† $P < 0.001$ versus untreated mice, χ^2 test.

Implantation failure is the most frequent cause of lack of human pregnancy after *in vitro* fertilization and embryo transfer[11]. Sufficient uterine LIF protein is an essential condition for implantation, and low LIF levels have been reported in infertile women[12]. Considering the strict regulation of *LIF* by p53, the modulation of p53 function by single nucleotide polymorphisms (SNPs) in the *p53* pathway[4,13] may affect implantation. A recent study reported that the *p53* codon 72 polymorphism (an Arg to Pro change) is associated with recurrent implantation failure in humans, but its mechanism is unclear[11]. Because the proline allele of codon 72 seems to encode a p53 protein with a weaker activity than that from the arginine allele[4], our results suggest a possible mechanism that mediates the impact of this SNP. These studies may also help to explain the positive selection during human evolution of some alleles in the *MDM2* (SNP309)[3] and *p53* genes[4].

This study clearly demonstrates a new function for p53 in the regulation of maternal reproduction, in which *LIF*, a previously unidentified p53 target gene, functions as the downstream mediator of this p53 effect in mice (Supplementary Fig. 5).

METHODS SUMMARY

Wild-type and $p53^{-/-}$ mice were used for breeding assays, and reproduction parameters were measured. Early implantation sites were examined by the Chicago blue dye-staining method at day 5 of pregnancy, and the number of implanted embryos was also counted at day 7 of pregnancy. ChIP assays were performed with an Upstate ChIP assay kit. For reporter assays, putative p53-binding elements were cloned into the promoter region of a pGL2 luciferase reporter plasmid (Promega). The reporter plasmids were transfected into *p53*-null H1299, Soas2 and HCT116 $p53^{-/-}$ cells along with either pRC-wild-type-p53 or pRC-mutant-p53 p53 expression plasmid. The luciferase activities were determined 24 h after transfection. The mRNA levels of different genes were measured by Taqman quantitative real-time PCR. The LIF protein levels were measured by ELISA assay (R & D).

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

Received 24 July; accepted 10 October 2007.

1. Vogelstein, B., Lane, D. & Levine, A. J. Surfing the p53 network. *Nature* **408,** 307–310 (2000).
2. Stewart, C. L. *et al.* Blastocyst implantation depends on maternal expression of leukaemia inhibitory factor. *Nature* **359,** 76–79 (1992).
3. Atwal, G. S. *et al.* Haplotype structure and selection of the *MDM2* oncogene in humans. *Proc. Natl Acad. Sci. USA* **104,** 4524–4529 (2007).
4. Murphy, M. E. Polymorphic variants in the p53 pathway. *Cell Death Differ.* **13,** 916–920 (2006).
5. el-Deiry, W. S., Kern, S. E., Pietenpol, J. A., Kinzler, K. W. & Vogelstein, B. Definition of a consensus binding site for p53. *Nature Genet.* **1,** 45–49 (1992).
6. Hoh, J. *et al.* The p53MH algorithm and its application in detecting p53-responsive genes. *Proc. Natl Acad. Sci. USA* **99,** 8467–8472 (2002).
7. Chen, J. R. *et al.* Leukemia inhibitory factor can substitute for nidatory estrogen and is essential to inducing a receptive uterus for implantation but is not essential for subsequent embryogenesis. *Endocrinology* **141,** 4365–4372 (2000).
8. Song, H., Lim, H., Das, S. K., Paria, B. C. & Dey, S. K. Dysregulation of EGF family of growth factors and COX-2 in the uterus during the preattachment and attachment reactions of the blastocyst with the luminal epithelium correlates with implantation failure in LIF-deficient mice. *Mol. Endocrinol.* **14,** 1147–1161 (2000).
9. Cullinan, E. B. *et al.* Leukemia inhibitory factor (LIF) and LIF receptor expression in human endometrium suggests a potential autocrine/paracrine function in regulating embryo implantation. *Proc. Natl Acad. Sci. USA* **93,** 3115–3120 (1996).
10. Armstrong, J. F., Kaufman, M. H., Harrison, D. J. & Clarke, A. R. High-frequency developmental abnormalities in p53-deficient mice. *Curr. Biol.* **5,** 931–936 (1995).
11. Kay, C., Jeyendran, R. S. & Coulam, C. B. p53 tumour suppressor gene polymorphism is associated with recurrent implantation failure. *Reprod. Biomed. Online* **13,** 492–496 (2006).
12. Hambartsoumian, E. Endometrial leukemia inhibitory factor (LIF) as a possible cause of unexplained infertility and multiple failures of implantation. *Am. J. Reprod. Immunol.* **39,** 137–143 (1998).
13. Bond, G. L. *et al.* A single nucleotide polymorphism in the *MDM2* promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. *Cell* **119,** 591–602 (2004).

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements We thank S. Christen for critical review of the manuscript, and I. Snyder and the staff of the University of Medicine and Dentistry of New Jersey animal facility for providing animal care. This research was supported by grants from the Breast Cancer Research Foundation and the National Cancer Institute.

Author Contributions W.H., Z.F. and A.J.L. designed the research. W.H., Z.F. and A.K.T. performed the research. W.H., Z.F. and A.J.L. analysed data and wrote the paper.

Author Information Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to A.J.L. (alevine@ias.edu).

METHODS

Mice and cell lines. The C57BL/6J and 129SV^{sl} strain mice were purchased from the Jackson Laboratory. Mice deficient in *p53* (C57BL/6J*129SV) were provided by T. Jacks. The *p53*$^{-/-}$ C57BL/6J and 129SV^{sl} strains were produced by backcrossing ten times to C57BL/6J and 129SV^{sl}, respectively. The wild-type and *p53*$^{-/-}$ MEFs were generated as described previously[14]. Mouse fibroblast Val5 and Vm10 cells, which contain a temperature-sensitive mutant *p53* plasmid (Ala 135 to Val) and express mutant *p53* at 39 °C but wild-type *p53* at 32 °C, were established as described previously[15,16]. HCT116 *p53*$^{+/+}$ and *p53*$^{-/-}$ human colon cancer cells were gifts from B. Vogelstein. Saos2 *p53*-null human osteosarcoma cells and MCF7 (*p53* wild-type) human breast cancer cells were obtained from the American Type Culture Collection (ATCC). The MCF7-p53siRNA cell line stably expressing *p53* siRNA, to knock down p53 protein[17], was a gift from A. Gartel. The human H1299-WTp53 cell line, which contains a *p53* expression plasmid under the control of tetracycline and expresses the wild-type p53 protein on tetracycline withdrawal, was established from the *p53*-null H1299 cell line as described previously[18].

Mouse breeding assay and examination of reproduction parameters. For breeding assays, mating pairs (8-week-old mice) were placed in cages for at least 6 weeks, and female mice were checked every morning for copulation plugs and signs of parturition. The day on which the plug was observed was considered to be day 1 of pregnancy. Pregnancy rate was calculated as the ratio of the number of females with confirmed pregnancy to the number of female mice housed with male mice. The oestrous cycle was evaluated by cytological analysis of daily vaginal smears for 2 weeks. The serum oestrogen levels were measured with an oestradiol enzyme immunoassay kit (Cayman). The number of corpora lutea on each ovary as an indicator of recent ovulation was recorded. Uterine horns were flushed with M2 medium (Sigma) to recover blastocysts[19]. Early implantation sites were examined by the Chicago blue dye-staining method[19] to reveal blue bands at day 5 of pregnancy. The number of implanted embryos was also counted in uteri collected from mice at day 7 of pregnancy.

Injection of LIF. Recombinant mouse LIF (5 μg; Millipore) was administered to female mice twice, with 5 h between, by intraperitoneal injection at day 4 of pregnancy.

Irradiation with γ-rays. Six-week-old C57Bl/6J wild-type and *p53*$^{-/-}$ mice were subjected to 5 Gy of total body irradiation with a ^{137}Cs γ-ray source. Mice were killed 6 h after irradiation, and different tissues were harvested for further experiments. For *in vitro* irradiation, uteri collected from *p53*$^{+/+}$ or *p53*$^{-/-}$ C57Bl/6J mice were irradiated (5 Gy) and cultured in DMEM medium supplemented with 10% fetal bovine serum for 6 h before being harvested. At least three mice were used in each group.

ChIP assay. ChIP assays were performed with an Upstate ChIP assay kit in accordance with the instructions of the manufacturer. Human H1299-WTp53 and *p53*-null H1299 cells were cultured in the absence of tetracycline for 24 h, followed by ChIP assay with DO-1 anti-p53 antibody. Mouse Val5 and *p53*-null 10(1) cells were cultured at 32 °C for 16 h followed by ChIP assay with FL393 anti-p53 antibody. The primer sets were designed to encompass the potential p53-binding elements in intron 1 of the human and mouse *LIF* genes. The sequences for the *LIF* genes are as follows: human, 5′-ACCCCGGCCC-CCACCATCTTC-3′ and 5′-ACTCATCCGCTGTCCCTTGTCC-3′; mouse, 5′-TGACCTGGGGGAGAGCAACCTAAC-3′ and 5′-AGCCAACAGCCCCA-GCATCAC-3′.

Construction of reporter plasmids and luciferase activity assay. The TOPO II vector (Invitrogen) was used to clone PCR fragments containing the putative p53-binding elements in intron 1 of human and mouse *LIF* genes by using PCR primers for ChIP assays. The sequence-confirmed clones were subcloned into pGL2 luciferase reporter plasmid (Promega). The pGL2 reporter plasmids containing one copy of each putative p53-binding element were transfected into *p53*-null H1299 cells, Saos2 and HCT116 *p53*$^{-/-}$ cells by using LipofectAMINE 2000 (Invitrogen) along with 1 μg of pRC-wtp53 (wild-type human *p53* expression plasmid) or pRC-273H (mutant human *p53* expression plasmid containing a substitution at R273H) and 0.5 ng of pRL-SV40 plasmid expressing *Renilla* luciferase as an internal control to normalize transfection efficiency. The luciferase activity was measured 24 h after transfection and normalized with the internal standard.

Treatment with oestrogen. For treatment with oestrogen, cells were cultured in Phenol red-free culture medium supplemented with 10% charcoal-stripped fetal bovine serum for 3 days before being treated with various concentrations of 17-β-oestradiol (Sigma) for various durations.

Quantitative real-time PCR. Total RNA was prepared from cells or mouse tissues with the RNeasy kit (Qiagen) and treated with DNase I to remove residual genomic DNA. The complementary DNA was generated with random primers using a TaqMan reverse transcription kit (Applied Biosystems). Real-time PCR was performed in triplicate with TaqMan PCR mixture (Applied Biosystems) in the 7000 ABI sequence detection system. All human and mouse primers were purchased from Applied Biosystems. The expression of genes was normalized to the housekeeping gene encoding β-actin.

Measurement of LIF protein levels. Val5 and Vm10 cells were cultured at 32 °C for various durations before cells and culture supernatants were collected. Cells were lysed in cell extraction buffer for enzyme-linked immunosorbent assay (ELISA; Biosource). Culture supernatants were concentrated with an Amicon Ultra-4 centrifugal filter device (Millipore) after a brief centrifugation to remove any cell debris. The LIF protein levels were measured with a mouse LIF ELISA kit (R & D) in accordance with the manufacturer's instructions.

14. Harvey, D. & Levine, A. J. p53 alteration is a common event in the spontaneous immortalization of primary BALB/c murine embryo fibroblasts. *Genes Dev.* **5,** 2375–2385 (1991).
15. Pochampally, R., Fodera, B., Chen, L., Lu, W. & Chen, J. Activation of an MDM2-specific caspase by p53 in the absence of apoptosis. *J. Biol. Chem.* **274,** 15271–15277 (1999).
16. Wu, X. & Levine, A. J. p53 and E2F-1 cooperate to mediate apoptosis. *Proc. Natl Acad. Sci. USA* **91,** 3602–3606 (1994).
17. Radhakrishnan, S. K., Gierut, J. & Gartel, A. L. Multiple alternate p21 transcripts are regulated by p53 in human cells. *Oncogene* **25,** 1812–1815 (2006).
18. Chen, X., Ko, L. J., Jayaraman, L. & Prives, C. p53 levels, functional domains, and DNA damage determine the extent of the apoptotic response of tumor cells. *Genes Dev.* **10,** 2438–2451 (1996).
19. Nagy, A., Gertsenstein, M., Vintersten, K. & Behringer, R. *Manipulating the Mouse Embryo, a Laboratory Manual* 3rd edn (Cold Spring Harbor Press, New York, 2003).