Supporting Information

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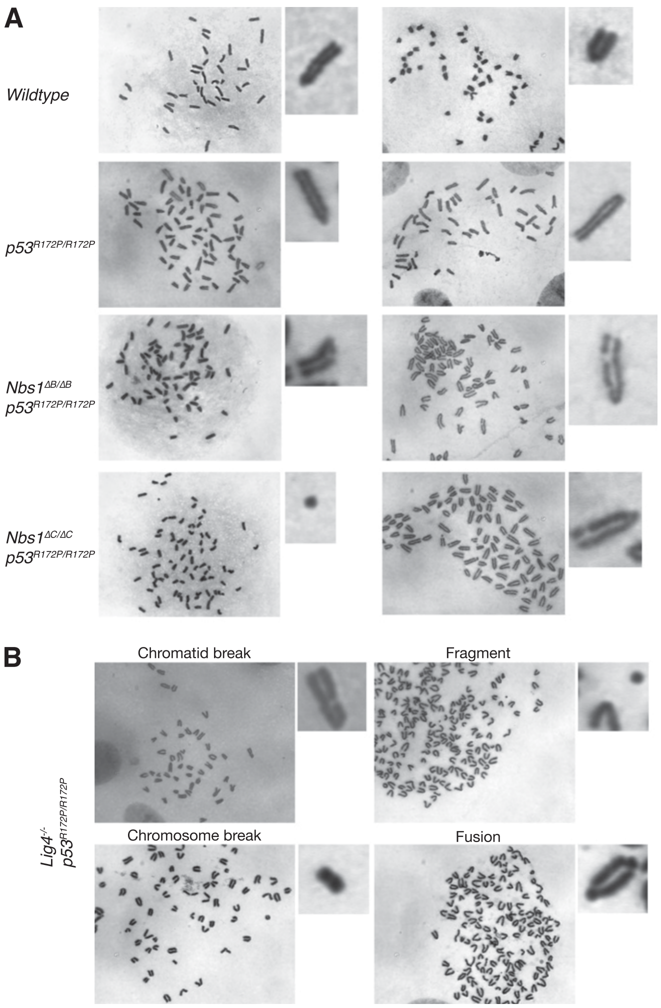

Fig. S1. (*A*) Examples of metaphase spreads used for analysis of chromosomal instability in Fig. 1*E*. All samples are from early-passage ear fibroblast cultures from the indicated genotypes. Examples of a chromosome fragment (*Left*) and a chromatid break (*Right*) are shown at higher magnification for *Nbs1*$^{\Delta C/\Delta C}$ *p53*$^{R172P/R172P}$, and two chromatid breaks are shown for *Nbs1*$^{\Delta B/\Delta B}$ *p53*$^{R172P/R172P}$. Magnified normal chromosomes are shown for wild type and *p53*$^{R172P/R172P}$ genotypes. (*B*) Examples of *Lig4*$^{-/-}$ *p53*$^{R172P/R172P}$ metaphase spreads used for analysis in Fig. 3*E*.

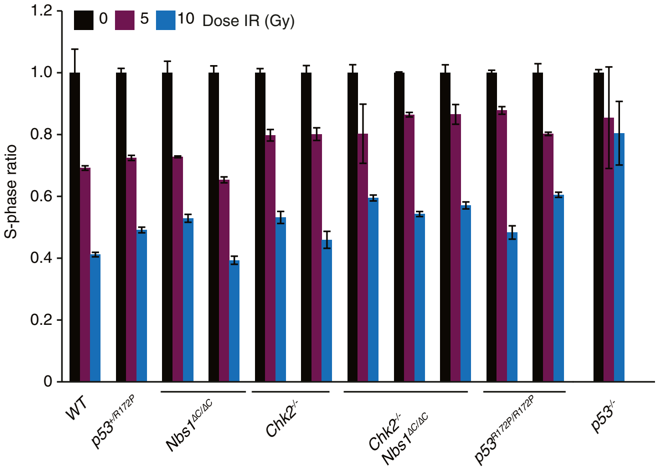

Fig. S2. Individual culture results for analysis of the G1/S checkpoint. S-phase ratios (%BrdU+ in ionizing radiation or mock treated samples/average %BrdU+ in mock treated samples) of triplicate samples are plotted with SD. Genotypes and ionizing radiation dose are indicated. Averaged results of each genotype are shown in Fig. 1.

Table S1. Statistical analysis of overall cohort survival (Mantel–Cox log rank test)

Genotype	$p53^{R172P/R172P}$	$Nbs1^{\Delta C/\Delta C}$ $p53^{R172P/R172P}$	$Lig4^{-/-}$ $p53^{R172P/R172P}$
$Chk2^{-/-}$ $Nbs1^{\Delta C/\Delta C}$	<0.0001		
$Nbs1^{\Delta C/\Delta C}$ $p53^{R172P/R172P}$	0.0002		
$Nbs1^{\Delta B/\Delta B}$ $p53^{R172P/R172P}$	<0.0001	0.4876	
$Lig4^{+/-}$ $p53^{R172P/R172P}$	0.1190		<0.0001
$Lig4^{-/-}$ $p53^{R172P/R172P}$	<0.0001		
$Lig4^{-/-}$ $Chk2^{-/-}$	<0.0001		0.2122

Analysis of overall cohort survival and comparison of indicated genotypes using the Mantel–Cox log rank test. Note that cohort survival of $p53^{R172P/R172P}$ mice is significantly affected by the homozygosity of either $Nbs1^{\Delta C}$ or $Nbs1^{\Delta B}$.

Table S2. Statistical analysis of overall cohort survival (6× proportional hazards model, compared with $p53^{R172P/R172P}$)

Genotype	HR	CI lower	CI upper	*P* value
$Nbs1^{\Delta C/\Delta C}$ $p53^{R172P/R172P}$	2.09	−1.02	4.45	5.17e-02
$Nbs1^{\Delta B/\Delta B}$ $p53^{R172P/R172P}$	4.31	1.92	9.66	3.52e-04
$Lig4^{+/-}$ $p53^{R172P/R172P}$	1.09	−1.86	2.23	8.04e-01
$Lig4^{-/-}$ $p53^{R172P/R172P}$	4.40	2.10	9.20	2.83e-05
$Lig4^{-/-}$ $Chk2^{-/-}$	5.48	2.40	12.48	5.52e-05

CI, confidence interval; HR, hazard ratio.

Table S3. Median survival of indicated cohorts

Genotype	Median survival (months)
$p53^{R172P/R172P}$	13.0
$Nbs1^{\Delta C/\Delta C}$ $p53^{R172P/R172P}$	9.2
$Nbs1^{\Delta B/\Delta B}$ $p53^{R172P/R172P}$	7.9
$Lig4^{+/-}$ $p53^{R172P/R172P}$	12.0
$Lig4^{-/-}$ $p53^{R172P/R172P}$	6.6
$Lig4^{-/-}$ $Chk2^{-/-}$	5.2

Table S4. Numbers and percentage of tumor-free or tumor-bearing animals in each cohort

Genotype	No. tumor-free	% tumor-free	No. with tumor	% with tumor	Total no.
$p53^{R172P/R172P}$	2	15.4	11	84.6	13
$Nbs1^{\Delta C/\Delta C}$ $p53^{R172P/R172P}$	5	26.3	14	73.7	19
$Nbs1^{\Delta B/\Delta B}$ $p53^{R172P/R172P}$	2	12.5	14	87.5	16
$Lig4^{+/-}$ $p53^{R172P/R172P}$	0	0	21	100	21
$Lig4^{-/-}$ $p53^{R172P/R172P}$	16	57.1	12	42.9	28
$Lig4^{-/-}$ $Chk2^{-/-}$	14	93.3	1	6.7	15

Raw data for tumor incidence graphed in Figs. 1 and 3.

Table S5. Statistical significance of tumor incidence (Fisher's exact test)

Genotype	$p53^{R172P/R172P}$	$Nbs1^{\Delta C/\Delta C}$ $p53^{R172P/R172P}$	$Lig4^{-/-}$ $p53^{R172P/R172P}$
$Nbs1^{\Delta C/\Delta C}$ $p53^{R172P/R172P}$	0.6707		
$Nbs1^{\Delta B/\Delta B}$ $p53^{R172P/R172P}$	1.0	0.4150	
$Lig4^{+/-}$ $p53^{R172P/R172P}$	0.1390		<0.0001
$Lig4^{-/-}$ $p53^{R172P/R172P}$	0.0181		
$Lig4^{-/-}$ $Chk2^{-/-}$	<0.0001		0.0166

Table S6. Statistical significance of tumor incidence (6× proportional hazards model, compared with $p53^{R172P/R172P}$)

Genotype	HR	CI low	CI high	*P* value
$Nbs1^{\Delta C/\Delta C}$ $p53^{R172P/R172P}$	1.89	−1.24	4.40	1.4e-01
$Nbs1^{\Delta B/\Delta B}$ $p53^{R172P/R172P}$	4.49	1.87	10.82	7.0e-04
$Lig4^{+/-}$ $p53^{R172P/R172P}$	1.31	−1.62	2.80	4.7e-01
$Lig4^{-/-}$ $p53^{R172P/R172P}$	2.26	−1.09	5.56	7.5e-02
$Lig4^{-/-}$ $Chk2^{-/-}$	−2.35	−18.93	3.43	3.7e-01

Table S7. Tumor distribution by type: percentage of total number of tumors of the indicated tumor type in each cohort

Tumor type	$p53^{R172P/R172P}$	$Nbs1^{\Delta B/\Delta B}$ $p53^{R172P/R172P}$	$Nbs1^{\Delta C/\Delta C}$ $p53^{R172P/R172P}$	$Lig4^{+/-}$ $p53^{R172P/R172P}$	$Lig4^{-/-}$ $p53^{R172P/R172P}$	$Lig4^{-/-}$ $Chk2^{-/-}$
Total no. of tumors	17	15	23	27	12	1
Hemangiosarcoma	**23.5**	**6.7**	**13.0**	**11.1**	0.0	0.0
Histiocytic sarcoma	**17.6**	0.0	**17.4**	**3.7**	**8.3**	0.0
Lymphoma/leukemia	**23.5**	**46.7**	**26.1**	**22.2**	**25.0**	0.0
Rhabdomyosarcoma	**5.9**	**6.7**	0.0	**3.7**	**8.3**	0.0
Alveolar-bronchiolar adenoma	0.0	0.0	**4.3**	**3.7**	**16.7**	0.0
Alveolar-bronchiolar carcinoma	0.0	0.0	**13.0**	0.0	0.0	0.0
Basal cell carcinoma	0.0	0.0	**4.3**	0.0	0.0	0.0
Lipoma	0.0	0.0	**4.3**	0.0	0.0	0.0
Neuroectodermal tumor	0.0	0.0	**4.3**	**3.7**	0.0	0.0
Sarcoma	0.0	0.0	**4.3**	**11.1**	0.0	0.0
Squamous cell carcinoma	0.0	0.0	**8.7**	0.0	0.0	0.0
Fibrosarcoma	0.0	0.0	0.0	**7.4**	**8.3**	0.0
Adenocarcinoma	0.0	0.0	0.0	**3.7**	**16.7**	0.0
Gonadostromal tumor	0.0	0.0	0.0	0.0	**8.3**	0.0
Granulosa cell tumor	0.0	0.0	0.0	0.0	0.0	**100.0**
Medulloblastoma	0.0	0.0	0.0	**3.7**	0.0	0.0
Cholangiosarcoma	0.0	0.0	0.0	**3.7**	0.0	0.0
Sebaceous adenoma	0.0	0.0	0.0	**3.7**	0.0	0.0
Osteosarcoma	0.0	0.0	0.0	**7.4**	0.0	0.0
Uncharacterized	**29.4**	**40.0**	0.0	**11.1**	**8.3**	0.0

Bold type indicates the percentage of the cohort with the indicated tumor type. The number of tumors is higher than the number of tumor-bearing mice, because some mice had two or three distinct lesions.

Table S8. Breeding schemes and outcomes used to generate double-mutant mice

Genotype	Expected no. of pups	Observed no. of pups	Ratio observed/expected	*P* value*
$Lig4^{-/-}$	10.06[†]	0	0.00	3.07×10^{-5}
$Lig4^{-/-}$ $p53^{R172P/R172P}$	57[‡]	47	0.82	
$Lig4^{-/-}$ $Chk2^{-/-}$	32[§]	19	0.59	
$Lig4^{-/-}$ $Nbs1^{\Delta C/\Delta C}$	12.25[¶]	0	0.00	7.55×10^{-7}
$Lig4^{+/-}$ $p53^{R172P/R172P}$	114[‡]	122	1.07	
$p53^{R172P/R172P}$	57[‡]	59	1.04	
$Lig4^{+/-}$ $Chk2^{-/-}$	64[§]	69	1.08	
$Chk2^{-/-}$	32[§]	40	1.25	
$Nbs1^{\Delta C/\Delta C}$	12.25[¶]	20	1.63	
$Lig4^{+/-}$ $Nbs1^{\Delta C/\Delta C}$	24.5[¶]	29	1.18	

Expected number of pups assumes normal Mendelian inheritance.

**P* values are based on binomial distribution.

[†] One hundred sixty-one pups generated from breeding pairs with a 1 in 16 chance of generating $Lig4^{-/-}$.

[‡] Two hundred twenty-eight pups generated from 10 independent $Lig4^{+/-}$ $p53^{R172P/R172P}$ × $Lig4^{+/-}$ $p53^{R172P/R172P}$ breeding pairs.

[§] One hundred twenty-eight pups generated from seven independent $Lig4^{+/-}$ $Chk2^{-/-}$ × $Lig4^{+/-}$ $Chk2^{-/-}$ breeding pairs.

[¶] Forty-nine pups generated from four independent $Lig4^{+/-}$ $Nbs1^{\Delta C/\Delta C}$ × $Lig4^{+/-}$ $Nbs1^{\Delta C/\Delta C}$ breeding pairs.