Supporting Information

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SI Materials and Methods

Western Blot Analysis. Western blot analysis was performed as previously described (1). The antibodies used in this study were as follows: anti-p53(1C12) (Cell signaling), anti-actin (Sigma), and anti-phospho-Histone H2A.X (Ser139) (Millipore).

RNA Isolation and RT-PCR Analysis. The RT-PCR analysis was performed as previously described (2). Briefly, total RNA was isolated and then subjected to cDNA synthesis using M-MLV reverse transcriptase (Promega). The PCR program used for amplification was (*i*) 94 °C for 5 min, (*ii*) 94 °C for 45 s, (*iii*) 58 °C for 45 s, (*iv*) 72 °C for 1 min, and (*v*) 72 °C for 10 min. From steps *ii*–*iv*, the cycle was repeated 20 times for actin or 28 times for RBM38. The primers for *RBM38* were a forward primer, 5′-GCA CGG CTC ACA GAA GGA-3′, and a reverse primer, 5′-CGA GGA CAG TGA CGG GAC A-3′. The primers for mouse actin were a forward primer, 5′-CCC ATC TAC GAG GGC TAT-3′, and a reverse primer, 5′-AGA AGG AAG GCT GGA AAA-3′. The primers for IL17D were a forward primer, 5′-ACA AGT CTG GAA AGC ATC ACG-3′ and a reverse primer, 5′-GTG GTG GAA GGC GCT GA-3′. The primers for Tnfsf15 were a forward primer, 5′- CCT GCT GCC TGT TGT CAT TT-3′, and a reverse primer, 5′-GCT GTG GTG AAG GCT CAG ATC T-3′. The primers for TLR7 were a forward primer, 5′-AAT ATC CCA GAG GCC CAT GT-3′, and a reverse primer, 5′-TTG GAC CCC AGT AGA ACA GG-3′. The primers for GAPDH were a forward primer, 5′- CCC AGC CTC AAG ATC ATC AGC AAT G -3′, and a reverse primer, 5′- ATG GAC TGT GGT CAT GAG TCC TT-3′. The primers for p16 were a forward primer, 5′- CCC AAC GCC CCG AAC T-3′, and a reverse primer, 5′ CAG AAG AGC TGC TAC GTG AA-3′.

SA-β-Gal Staining. This assay was performed as described previously (3). Briefly, tissues were fixed with 2% (vol/vol) formaldehyde and 0.2% glutaraldehyde for 20 min at room temperature, followed by staining with fresh β-gal staining solution overnight at 37 °C.

Micro-CT Scan. A custom-built micro-CT imaging system was used for the study (4). Mice were anesthetized with isoflurane in an induction chamber [1.5–3% (vol/vol) isoflurane]. The anesthetized mice were placed in the imaging instrument and fitted with a nose cone connected to a vaporizer, to maintain isoflurane (1.0–2.5%) during the procedure.

Histological Analysis. Mouse tissues were fixed in 10% (wt/vol) neutral buffered formalin, routinely processed, and embedded in paraffin blocks. Tissue sections (5 μm) were sectioned and stained with H&E or periodic acid–Schiff (PAS). For immunohistochemistry (IHC) analysis, tissues were stained with B220, CD3, or F4/80 antibody using the Vectastain ABC Elite Kit (Vector Laboratories). Briefly, tissue sections (5 μm) were dewaxed and antigen-retrieved in a citrate buffer (pH 6.0), followed by incubation with a primary antibody overnight at 4 °C and then a secondary antibody for 1 h at room temperature. The slides were visualized by treatment with 3,3′-diaminobenzidine tetrahydrochloride (DAB), and then counterstained with Mayer's hematoxylin.

Irradiation of Mice. At 6–8 wk of age, mice with various genotypes were irradiated with γ rays from a ^{137}Cs source (6.04 gray/min) at a dose of 4 or 8 gray. Mice exposed to 8 gray of γ-irradiation were monitored daily. For mice exposed with 4 gray of γ-irradiation, tissues were collected 4 h postirradiation and subjected to Western blot analysis.

Statistical Analysis. Fisher's exact test was used for comparison between tumor penetrance from different genotypes. The log-rank test was used to determine the differences in survival of different genotypes. A P value of 0.05 was considered significant.

1. Dohn M, Zhang S, Chen X (2001) p63alpha and DeltaNp63alpha can induce cell cycle arrest and apoptosis and differentially regulate p53 target genes. *Oncogene* 20(25): 3193–3205.
2. Zhang J, Chen X (2007) DeltaNp73 modulates nerve growth factor-mediated neuronal differentiation through repression of TrkA. *Mol Cell Biol* 27(10):3868–3880.
3. Zhang J, et al. (2011) Translational repression of p53 by RNPC1, a p53 target overexpressed in lymphomas. *Genes Dev* 25(14):1528–1543.
4. Liang H, et al. (2007) A microPET/CT system for in vivo small animal imaging. *Phys Med Biol* 52(13):3881–3894.

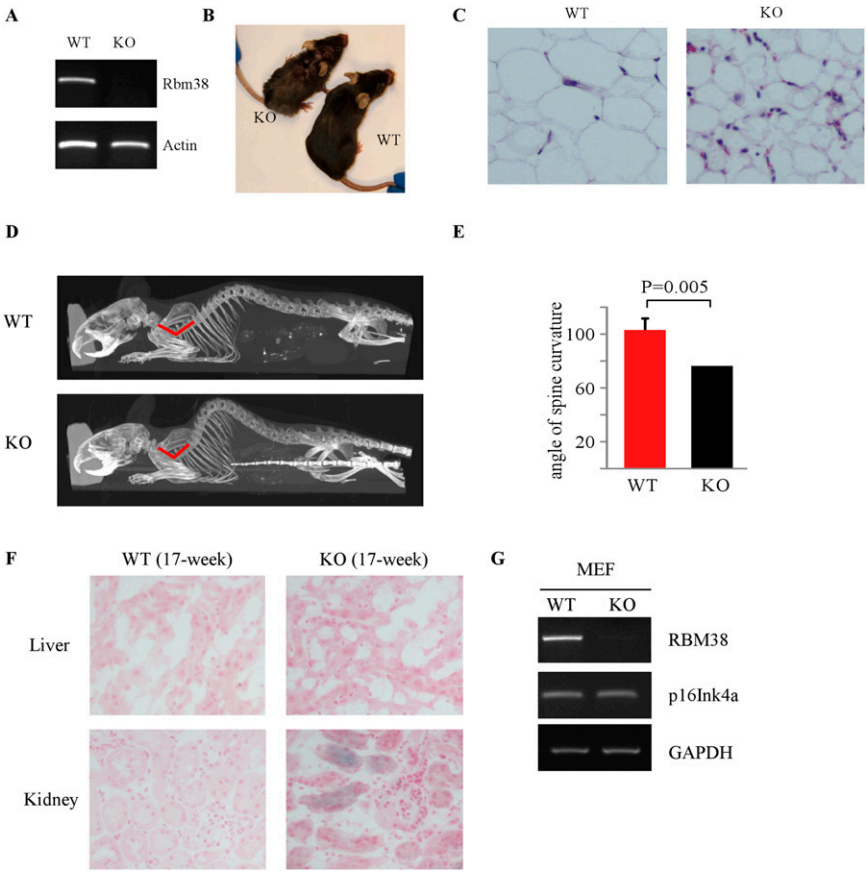

Fig. S1. (*A*) Total RNAs were isolated from WT and *Rbm38*$^{-/-}$ MEFs, and the level of Rbm38 and actin mRNA was determined by RT-PCR analysis. (*B*) Representative photographs of 18-mo-old WT and *Rbm38*$^{-/-}$ mice. (*C*) Representative images of hematoxylin and eosin-stained sections of gonadal adipose tissue from 18-mo-old WT and *Rbm38*$^{-/-}$ mice. (*D*) Representative Micro-CT images from age- and sex-matched WT and *Rbm38*$^{-/-}$ mice. *Rbm38*$^{-/-}$ mouse displays a pronounced lordokyphosis (curvature of the spine) phenotype. (*E*) The spinal angle as indicated in *E* was measured from WT ($n = 3$) and *Rbm38*$^{-/-}$ mice ($n = 4$). Bars represent mean ± SD. (*F*) SA-β-gal staining was performed with fresh-frozen liver and kidney tissues from young (17-wk-old) WT and *Rbm38*$^{-/-}$ mice, followed by counterstaining with nuclear fast red. (*G*) The level of Rbm38, p16Ink4a, and GAPDH transcript was measured in WT and *Rbm38*$^{-/-}$ MEFs.

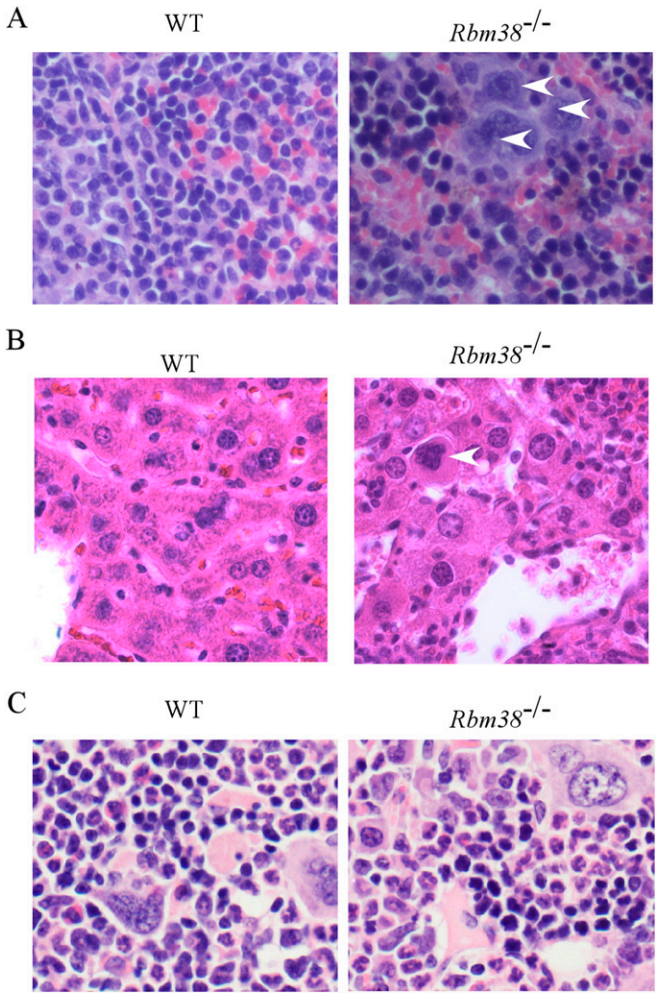

Fig. S2. (*A*) Representative images of hematoxylin and eosin-stained sections of spleens from WT and *Rbm38*$^{-/-}$ mice. The arrows indicate megakaryocytes. (*B*) Representative images of hematoxylin and eosin-stained sections of livers from WT and *Rbm38*$^{-/-}$ mice. The arrow indicates a megakaryocyte. (*C*) Representative images of hematoxylin and eosin-stained sections of bone marrows from WT and *Rbm38*$^{-/-}$ mice.

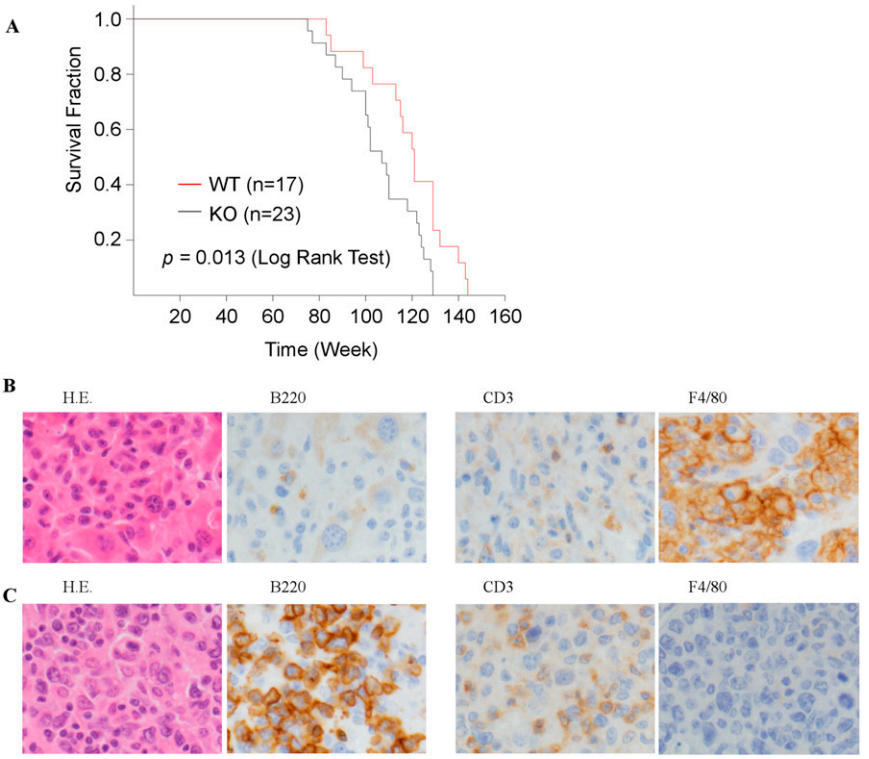

Fig. S3. (*A*) Kaplan–Meyer survival curves of WT ($n = 17$) and *Rbm38*$^{-/-}$ ($n = 23$) mice. The median survival time is 121 wk for WT mice and 107 wk for *Rbm38*$^{-/-}$ mice ($P = 0.013$ by log-rank test). (*B*) Representative hematoxylin and eosin-, B220-, CD3-, and F4/80-stained histiocytic sarcoma in the liver of an *Rbm38*$^{-/-}$ mouse. (*C*) Representative hematoxylin and eosin-, B220-, CD3-, and F4/80-stained lymphoma in the liver of an *Rbm38*$^{-/-}$ mouse.

Table S1. WT mice: Survival time, tumor spectrum, and EMH

ID	Genotype	Survival, wk	Tumor	EMH
1-24-2	WT	83	No	No
2-15-2	WT	140	Lymphoma	Liver
2-19-6	WT	132	No	Spleen
2-19-2	WT	143	No	Spleen
3-11-3	WT	129	No	No
3-28-5	WT	85	No	Spleen
3-9-7	WT	129	No	No
5-12-3	WT	99	Lymphoma	Spleen
7-9-9	WT	116	No	No
8-2-6	WT	120	No	No
10-24-7	WT	130	No	Spleen
10-26-6	WT	129	Histiocytic sarcoma	Liver, spleen
11-10-7	WT	121	No	No
11-7-3	WT	121	No	No
11-29-2	WT	144	No	Spleen
12-2-4	WT	113	No	No
12-20-7	WT	115	No	Spleen

EMH, extramedullary hematopoiesis.

Table S2. *Rbm38*$^{-/-}$ mice: Survival time, tumor spectrum, and EMH

ID	Genotype	Survival, wk	Tumor	EMH
1-12-2	*RBM38*$^{-/-}$	100	Lymphoma	Spleen
1-24-5	*RBM38*$^{-/-}$	83	No	No
1-11-3	*RBM38*$^{-/-}$	100	No	No
1-11-14	*RBM38*$^{-/-}$	110	Histiocytic sarcoma	Spleen
2-14-2	*RBM38*$^{-/-}$	109	No	Spleen
2-14-3	*RBM38*$^{-/-}$	101	No	Spleen
2-25-1	*RBM38*$^{-/-}$	107	Lymphoma	Spleen
3-7-2	*RBM38*$^{-/-}$	101	Lymphoma	Spleen
3-7-5	*RBM38*$^{-/-}$	129	Lymphoma	Liver
3-7-4	*RBM38*$^{-/-}$	129	Hepatoma	Spleen
6-15-3	*RBM38*$^{-/-}$	90	No	Spleen, liver
8-2-3	*RBM38*$^{-/-}$	94	No	Spleen
9-30-4	*RBM38*$^{-/-}$	102	Hemangiosarcoma	Spleen, liver
10-26-3	*RBM38*$^{-/-}$	75	No	Spleen
11-3-14	*RBM38*$^{-/-}$	77	No	Spleen
11-10-10	*RBM38*$^{-/-}$	124	Lymphoma and hemangiosarcoma	Spleen, liver
11-16-14	*RBM38*$^{-/-}$	128	No	Spleen, liver
11-16-7	*RBM38*$^{-/-}$	125	Hepatoma and lymphoma	Spleen
12-19-3	*RBM38*$^{-/-}$	123	Lymphoma	Spleen
12-19-8	*RBM38*$^{-/-}$	118	Hepatoma	Spleen, liver
12-19-6	*RBM38*$^{-/-}$	87	No	No
12-24-2	*RBM38*$^{-/-}$	122	No	No
12-28-5	*RBM38*$^{-/-}$	102	Hepatoma and lymphoma	Spleen

EMH, extramedullary hematopoiesis.

Table S3. Tumor spectrum of *p53*$^{+/-}$ and *Rbm38*$^{-/-}$; *p53*$^{+/-}$ mice

Tumors	*p53*$^{+/-}$ (n = 24)	*Rbm38*$^{-/-}$; *p53*$^{+/-}$ (n = 21)
Lymphoma		
Thymic T-cell lymphoma	2	0
Diffuse large B-cell lymphoma	3	2
Unclassified	4	1
Sarcoma		
Osteosarcoma	4	3
Liposarcoma	3	0
Fibrosarcoma	5	0
Angiosarcoma	0	2
Granulocytic sarcoma	0	3
Rhabdomyosarcoma	0	1
Carcinoma		
Squamous cell carcinoma	3	1
Adenocarcinoma	1	1
Hepatocellular carcinoma	1	0
Endometrial carcinoma	0	1
Total tumors	26	15

Table S4. $p53^{+/-}$ mice: Survival time and tumor spectrum

ID	Genotype	Survival, wk	Tumor
15	$p53^{+/-}$	60	Adenocarcinoma and liposarcoma
2-1-7	$p53^{+/-}$	97	Lymphoma
85	$p53^{+/-}$	84	Fibrosarcoma
2-1-4	$p53^{+/-}$	62	Thymic T-cell lymphoma
1-4-4	$p53^{+/-}$	96	DLBL and hepatocellular carcinoma
1-9-15	$p53^{+/-}$	31	Thymic T-cell lymphoma
2-16	$p53^{+/-}$	91	Osteosarcoma
63	$p53^{+/-}$	62	Liposarcoma and osteosarcoma
17	$p53^{+/-}$	61	Fibrosarcoma
61-2	$p53^{+/-}$	68	Lymphoma
24	$p53^{+/-}$	36	Fibrosarcoma
72	$p53^{+/-}$	57	SCC
41	$p53^{+/-}$	33	Lymphoma
1-4-3	$p53^{+/-}$	34	SCC
2-3	$p53^{+/-}$	64	Fibrosarcoma
70	$p53^{+/-}$	68	Osteosarcoma
91	$p53^{+/-}$	74	Lymphoma
68	$p53^{+/-}$	67	SCC
84	$p53^{+/-}$	77	DLBL
2-1-6	$p53^{+/-}$	92	DLBL
53	$p53^{+/-}$	109	Osteosarcoma
18	$p53^{+/-}$	48	Fibrosarcoma
80	$p53^{+/-}$	65	Liposarcoma
19	$p53^{+/-}$	61	No

DLBL, diffuse large B lymphoma; SCC, squamous-cell carcinoma.

Table S5. *RBM38*$^{-/-}$; $p53^{+/-}$ mice: Survival time, tumor spectrum, and other abnormalities

ID	Genotype	Survival, wk	Tumor	Abnormalities in spleen and liver
3-1-1	*RBM38*$^{-/-}$; $p53^{+/-}$	70	Granulocytic sarcoma	Not determined
3-1-8	*RBM38*$^{-/-}$; $p53^{+/-}$	70	Granulocytic sarcoma	Not determined
3-1-4	*RBM38*$^{-/-}$; $p53^{+/-}$	75	Liposarcoma	Not determined
10-23-3	*RBM38*$^{-/-}$; $p53^{+/-}$	64	Angiosarcoma and osteosarcoma	Not determined
10-23-2	*RBM38*$^{-/-}$; $p53^{+/-}$	82	Endometrial carcinoma	Not determined
4-13-5	*RBM38*$^{-/-}$; $p53^{+/-}$	87	Osteosarcoma and DLBL	Not determined
10-4-6	*RBM38*$^{-/-}$; $p53^{+/-}$	64	Adenocarcinoma	Not determined
2-27-2	*RBM38*$^{-/-}$; $p53^{+/-}$	76	Angiosarcoma	Not determined
1-16-4	*RBM38*$^{-/-}$; $p53^{+/-}$	65	Osteosarcoma and lymphoma	Not determined
1-16-5	*RBM38*$^{-/-}$; $p53^{+/-}$	68	Rhabdomyosarcoma	Not determined
10-4-7	*RBM38*$^{-/-}$; $p53^{+/-}$	74	DLBL	Not determined
12-15-2	*RBM38*$^{-/-}$; $p53^{+/-}$	115	SCC and granulocytic sarcoma	Not determined
11-21-4	*RBM38*$^{-/-}$; $p53^{+/-}$	91	No	Splenic follicular hyperplasia
7-11-13	*RBM38*$^{-/-}$; $p53^{+/-}$	52	No	Splenic follicular hyperplasia
11-21-3	*RBM38*$^{-/-}$; $p53^{+/-}$	36	No	Hepatitis
6-13-6	*RBM38*$^{-/-}$; $p53^{+/-}$	60	No	No change
6-13-2	*RBM38*$^{-/-}$; $p53^{+/-}$	69	No	Steatosis and splenic follicular hyperplasia
9-5-3	*RBM38*$^{-/-}$; $p53^{+/-}$	42	No	Steatosis and splenic follicular hyperplasia
10-23-1	*RBM38*$^{-/-}$; $p53^{+/-}$	75	No	Steatosis and splenic follicular hyperplasia
10-25-5	*RBM38*$^{-/-}$; $p53^{+/-}$	101	No	Splenic follicular hyperplasia
12-15-4	*RBM38*$^{-/-}$; $p53^{+/-}$	75	No	Splenic follicular hyperplasia

DLBL, diffused large B lymphoma; SCC, squamous-cell carcinoma.

Table S6. *p53*$^{-/-}$ mice: Survival time and tumor spectrum

ID	Genotype	Survival, wk	Tumor
5	*p53*$^{-/-}$	26	Angiosarcoma, hibernoma, and lymphoma
50	*p53*$^{-/-}$	25	Thymic T-cell lymphoma
9	*p53*$^{-/-}$	21	Thymic T-cell lymphoma
42	*p53*$^{-/-}$	30	Angiosarcoma and osteosarcoma
57	*p53*$^{-/-}$	22	Rhabdomyosarcoma
48	*p53*$^{-/-}$	18	Diffuse large B-cell lymphoma
92	*p53*$^{-/-}$	22	Diffuse large B-cell lymphoma
95	*p53*$^{-/-}$	20	Lymphoma
52	*p53*$^{-/-}$	29	Lymphoma
4	*p53*$^{-/-}$	31	Diffuse large B-cell lymphoma
68	*p53*$^{-/-}$	27	Thymic T-cell lymphoma
25	*p53*$^{-/-}$	34	Histiocytic sarcoma and rhabdomyosarcoma
3	*p53*$^{-/-}$	26	Thymic T-cell lymphoma
8	*p53*$^{-/-}$	23	Thymic T-cell lymphoma and hemangioma
1-1	*p53*$^{-/-}$	22	Thymic T-cell lymphoma and hemangioma
1-2	*p53*$^{-/-}$	25	Thymic T-cell lymphoma and granulocytic sarcoma
2	*p53*$^{-/-}$	22	Lymphoma
76	*p53*$^{-/-}$	20	Lymphoma
85	*p53*$^{-/-}$	22	Thymic T-cell lymphoma and histiocytic sarcoma
36	*p53*$^{-/-}$	24	Thymic T-cell lymphoma
83	*p53*$^{-/-}$	22	Rhabdomyosarcoma and histiocytic sarcoma
9-2	*p53*$^{-/-}$	25	Diffuse large B-cell lymphoma
55	*p53*$^{-/-}$	28	Thymic T-cell lymphoma
19	*p53*$^{-/-}$	29	Thymic T-cell lymphoma

Table S7. *RBM38*$^{-/-}$; *p53*$^{-/-}$ mice: Survival time and tumor spectrum

ID	Genotype	Survival, wk	Tumor
10-1-2	*RBM38*$^{-/-}$;*p53*$^{-/-}$	19	No
9-5-16	*RBM38*$^{-/-}$;*p53*$^{-/-}$	18	Thymic T-cell lymphoma
7-11-5	*RBM38*$^{-/-}$;*p53*$^{-/-}$	26	Lymphoma
7-6-3	*RBM38*$^{-/-}$;*p53*$^{-/-}$	21	Thymic T-cell lymphoma
7-11-2	*RBM38*$^{-/-}$;*p53*$^{-/-}$	16	Thymic T-cell lymphoma
7-11-1	*RBM38*$^{-/-}$;*p53*$^{-/-}$	27	Thymic T-cell lymphoma
10-1-1	*RBM38*$^{-/-}$;*p53*$^{-/-}$	17	Thymic T-cell lymphoma
7-6-10	*RBM38*$^{-/-}$;*p53*$^{-/-}$	15	Thymic T-cell lymphoma
1-14-4	*RBM38*$^{-/-}$;*p53*$^{-/-}$	29	Diffuse large B-cell lymphoma
4-13-4	*RBM38*$^{-/-}$;*p53*$^{-/-}$	28	Spindle cell sarcoma
7-6-6	*RBM38*$^{-/-}$;*p53*$^{-/-}$	24	Diffuse large B-cell lymphoma
7-11-4	*RBM38*$^{-/-}$;*p53*$^{-/-}$	21	Diffuse large B-cell lymphoma
3-10-10	*RBM38*$^{-/-}$;*p53*$^{-/-}$	19	Lymphoma
12-15-1	*RBM38*$^{-/-}$;*p53*$^{-/-}$	19	Diffuse large B-cell lymphoma
1-14-3	*RBM38*$^{-/-}$;*p53*$^{-/-}$	15	Thymic T-cell lymphoma
10-1-4	*RBM38*$^{-/-}$;*p53*$^{-/-}$	18	Thymic T-cell lymphoma
4-13-1	*RBM38*$^{-/-}$;*p53*$^{-/-}$	21	No
6-21-8	*RBM38*$^{-/-}$;*p53*$^{-/-}$	19	Thymic T-cell lymphoma
5-2-9	*RBM38*$^{-/-}$;*p53*$^{-/-}$	21	Thymic T-cell lymphoma

Table S8. Tumor spectrum of *p53*$^{-/-}$ and *Rbm38*$^{-/-}$; *p53*$^{-/-}$ mice

Tumors	*p53*$^{-/-}$ (n = 24)	*Rbm38*$^{-/-}$; *p53*$^{-/-}$ (n = 19)
Lymphoma		
Thymic T-cell lymphoma	11	10
Diffuse large B-cell lymphoma	4	4
Unclassified	5	2
Sarcoma		
Spindle cell sarcoma	0	1
Rhabdomyosarcoma	3	0
Histiocytic sarcoma	3	0
Angiosarcoma	2	0
Granulocytic sarcoma	1	0
Osteosarcoma	1	0
Hibernoma	1	0
Hemangioma	2	0
Total tumors	33	17
Tumor-free mice	0	2