SUPPLEMENTAL MATERIALS

Supplemental Methods and Materials:

Antibodies and Reagents: Mouse monoclonal antibody to pan-cytokeratin (clone AE1/E3) was purchased from Thermo Scientific (MA1-82041). Mouse monoclonal Antibody to CD45RA (LCA) was obtained from EMD Millipore (05-1413, clone MEM 56). Rabbit polyclonal antibody against RPL5 (NBP1-31413) was obtained from Novus Biological. Mouse monoclonal antibody to GAPDH (ab8245) and anti-RPS24 rabbit polyclonal were purchased from Abcam (ab102986). Rabbit monoclonal antibody to vimentin was purchased from Cell Signaling Technology (5741P), and rabbit polyclonal anti-S100 antibody from Dako was a generous gift from Dr. Volney Sheen (Beth Israel Deaconess Medical Center, Boston, MA). Anti-p53 rabbit polyclonal antibody was purchased from Santa Cruz Biotechnology (clone FL-393, SC-6243). DAB peroxidase substrate was obtained from Vector labs (SK-4100). T-PER tissue protein extraction reagent purchased from Thermo Scientific (78510). HRP-conjugated goat anti-rabbit (170-6515) and anti-mouse (170-6516) antibodies were purchased from Bio-Rad Laboratory. SuperSignal West Femto (34095) and SuperSignal West Pico (34078) chemiluminescent substrates were purchased from Thermo Scientific. NuPAGE Novex 4-12% Bis-Tris precast gels were purchased from Life Technology (NP0335BOX). Anti-β-actin mouse monoclonal antibody was obtained from Sigma-Aldrich (A5441).

Blood Analysis: Blood was collected from the tail vain in microtainer tubes with EDTA (BD Biosciences, Cat# VT365973) and complete blood count was analyzed using HEMAVET 950FS in Boston Children's Hospital mouse facility. The results are average of nine mice per group per experiment.

Methylcellul**o**se colony forming cell assays: Bone marrow cells from 4-6 week old mice were suspended at 1×10^5 cells/mL in Methocult M3334 (StemCell Technologies). Erythroid colony forming unit (CFU-E) colonies were scored 2 to 3 days after plating. For burst forming units (BFU-E) and granulocyte-macrophage

colony-forming units (CFU-GM) assays, bone marrow cells were plated at 2×10^4 cells/mL in Methocult GF M3434 (StemCell Technologies), and colonies were scored 10 to 12 days after plating. Cells were plated in duplicates, and each experiment was performed on a minimum of three *Rpl5+/-*, three *Rps24+/-* and three WT mice.

Real-Time PCR: RNA from bone marrow cells of *Rpl5* +/-, *Rps24* +/-, and wild-type mice was isolated using RNeasy kit from Qiagen (Cat# 74104). One µg of RNA was used to synthesize first-strand cDNA using oligo$(dT)_{20}$ primers supplied with the SuperScript™ III First-Strand Synthesis System from Invitrogen Corporation (Cat# 18080-051). Real-Time PCR was performed using SYBR Green PCR master mix from AB Applied Biosystems(cat# 4309155), and DNA amplification was carried out using 7500 Fast Real-time PCR System (AB Applied Biosystems) and primers were *Gapdh*-F (5'CCAGCCTCGTCCC GTAGAC3'), *Gapdh*-R (5'CCCTTGACTGTGCCGTTG3'), *Rps24*-F (5' ACCGTCTGCTTCAGAGGAAA3'), *Rps24*-R (5'TCCCGAATT TCTGTCTTTGG3'), *Rpl5*-F (5'GCTCGAAAACGATTGGTGAT 3'), *Rpl5*-R (5' GCATATGCTGCACAGACGAT3'). The ΔC_t of the sample was calculated by subtracting the C_t of sample from C_t of housekeeping gene. All reactions were carried out in triplicates.

Immunobloting Assay: Tumor and normal skin tissues from *RPL5*+/- and *PPS24*+/- mice and skin tissue from wild-type mouse were homogenized in T-PER lysis buffer (containing phosphatase and protease inhibitors and 250 mM NaCl) and incubated on ice for 20 minutes followed by centrifugation for 20 minutes at 4°C at 12000 rpm. Protein concentration was measured using BCA Protein Assay kit from Thermo Scientific (23225). The cell lysates were either used immediately for immunoassay experiments or stored at -80°C. Fifteen microgram of total cell extract was subjected to SDS PAGE using NuPAGE Novex electrophoresis system. After transfer of proteins to PVDF membrane, membrane was blotted with either anti-p53 antibody (1:100 in TBS with 0.1%

Tween-20) or anti-β-actin antibody (1:1000 in TBS with 0.1% Tween-20 and 5% dry milk) over night at 4°C. Bands were visualized using either SuperSignal West Femto or SuperSignal West Pico chemiluminescent. For better comparison of the expression levels, the intensity of the expression of β-actin in wild-type tissue was considered 1 and the ratio of the intensity of other actin was compared to the wild-type and set to 1. The intensity of the p53 and actin bands for each tissue was measured using ImageJ and normalized to actin for each sample. Then, fold change was compared with wild-type.
For detection of RPS24 and RPL5 proteins, bone marrow cells were isolated from Rps24+/-, RPl5+/-, or wild-type mice. Proteins were extracted, as described above. Twenty microgram of total protein was subjected to SDS-PAGE, and membranes were blotted with anti-RPS24 (1:100 in TBS with 0.1% Tween-20), anti-RPL5 (1:1000 in 5% milk in TBS with 0.1% Tween-20), or anti-GAPDH (1:3000 in 5% milk in TBS with 0.1% Tween-20) antibodies over-night at 4°C. Bands were visualized, as described above.

Immunohistochemistry Assay: Frozen sections were prepared from skin and tumor tissues isolated from *RPL5*+/- and *PPS24*+/- mice. The sections were air-dried, fixed in cold acetone for 10 minutes at -20C, and permeabilized with 0.5% Triton-X100 in PBS for 5 minutes at room temperature. After three washes with PBS, the sections were incubated in 3% H_2O_2 for 5 minutes and washed in PBS. After blocking in 3% BSA in PBS, the sections were incubated with primary antibodies in blocking buffer overnight at 4ºC. Following incubation with HRP-conjugated secondary antibodies (1:500 dilutions in blocking buffer), the color was developed with DAB substrate. After a brief wash in dH_2O, counterstaining was performed with hematoxylin, followed by dehydration in 100% ethanol, 95% ethanol, and xylene. The sections were mounted and examined under a Nikon Eclipse SO7 microscope and processed using Nikon Software.

Hematoxylin and Eosin Staining: Frozen sections were incubated in absolute methanol for 30 seconds and rinsed in water, followed by incubation in

hematoxylin for 60 seconds. After rinsing in distilled water and ammonia water, they were incubated in 1% eosin (20 seconds), 95% ethanol (20 seconds), 100% ethanol (3 times each for 20 seconds), and xylenes (20 seconds), respectively. The sections were analyzed under a Nikon Eclipse SO7 microscope and processed using Nikon Software.

Supplemental Figure Legends:

Supplemental Figure 1. Analysis of Hematopoietic Colony Formation in *Rpl5* +/-, *Rps24* +/-, and Wild-type Mice. The data represents three individual mice for each type of colony formation. Bone marrow cells from each mouse were plated in duplicates. (**A**) CFU-E, (**B**) BFU-E, (**C**) CFU-GM

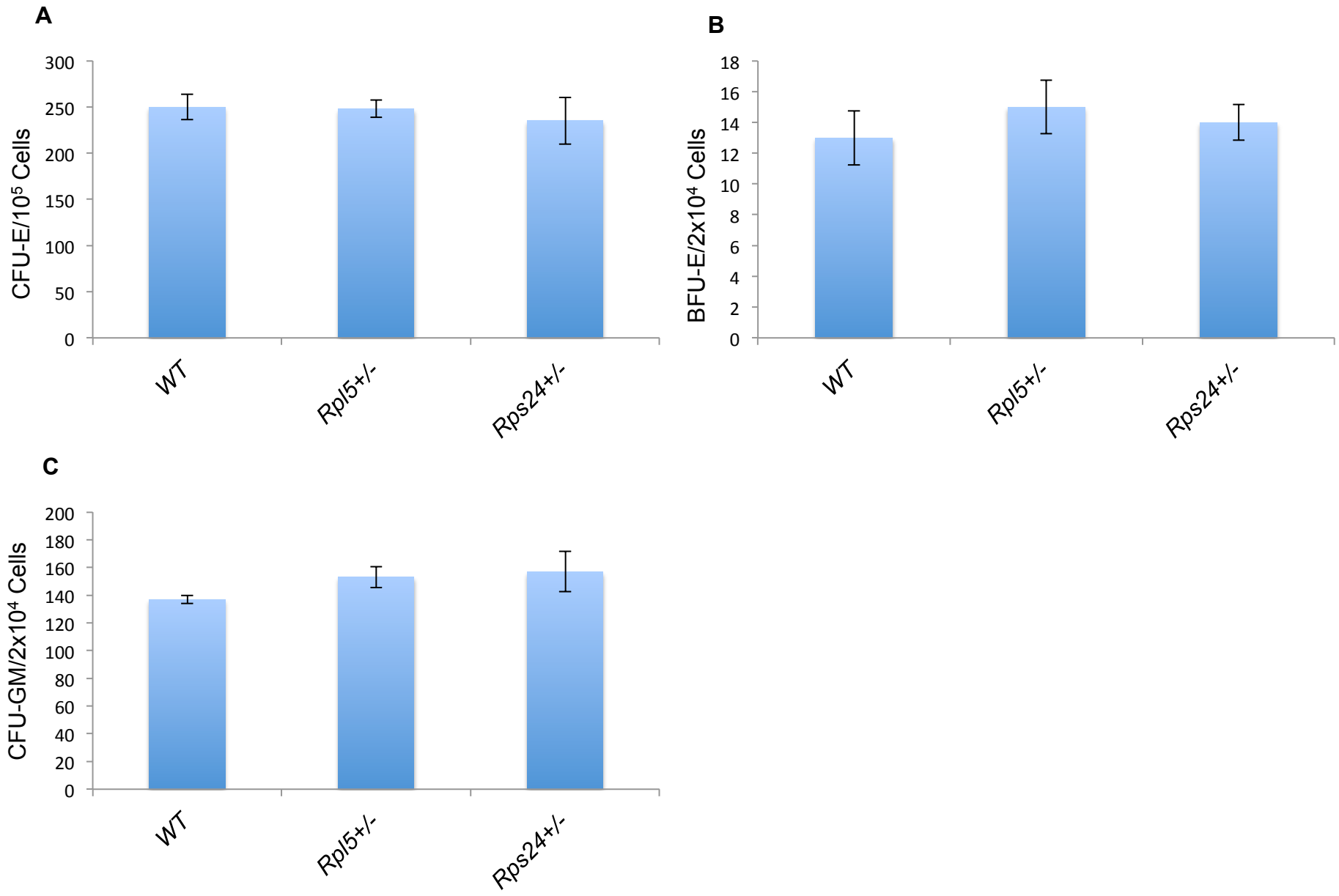
Supplemental Figure 2. Comparison of the Expression Levels of RPL5 and RPS24 Protein in the Bone Marrow Cells of *Rpl5* +/-, *Rps24* +/-, and Wild-Type Mice. Immunoblot assay was performed to compare the expression level of RPL5 (**A**) and RPS24 (**B**) proteins in the bone marrow cells isolated from *Rpl5* +/- and *Rps24* +/- mice with those of wild-type mice. GAPDH was used as loading control.

Supplemental Figure 3. **Detection of Tumors in *Rps24*+/- and *Rpl5*+/- Mice.** Among all monitored ribosomal protein S24 or L5 heterozygous mice, one female *Rps24*+/- mouse developed a very large tumor (2 cm) around upper left ear to head and neck region, at 17 months of age (**A**) and two male *Rpl5*+/- mice developed a single tumor (0.5 cm) in the head region at 22 months of age (**B** and **C**).

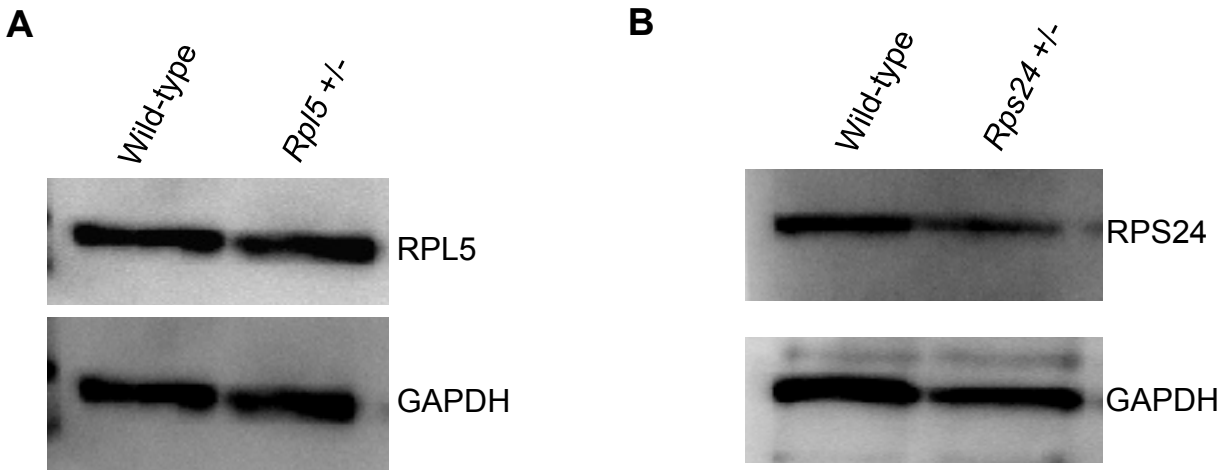
Supplemental Figure 4. Comparison of the Expression Level of p53 Protein in Wild-type, *Rps24*+/-, and *Rpl5*+/- Normal Skin and Tumor Tissues. Immunoblot assay was performed to determine the expression level of p53 protein in sarcoma tissues compared with *Rps24*+/- and *Rpl5*+/- normal skin and

wild-type skin. In this experiment β-actin was used as loading control (**A**). The level of protein expression was compared to wild-type (as described in Supplemental Materials and Methods) (**B**). The results are the average of two independent experiments.

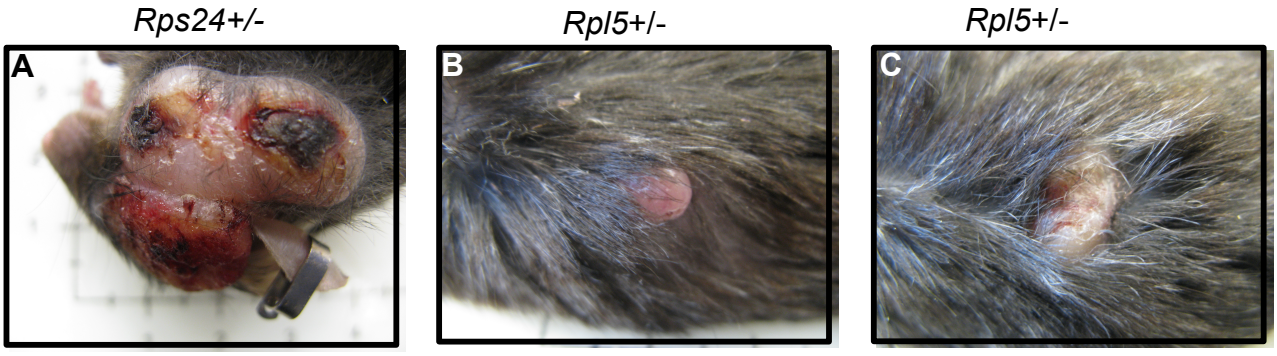
Supplemental Figure 1



Supplemental Figure 2

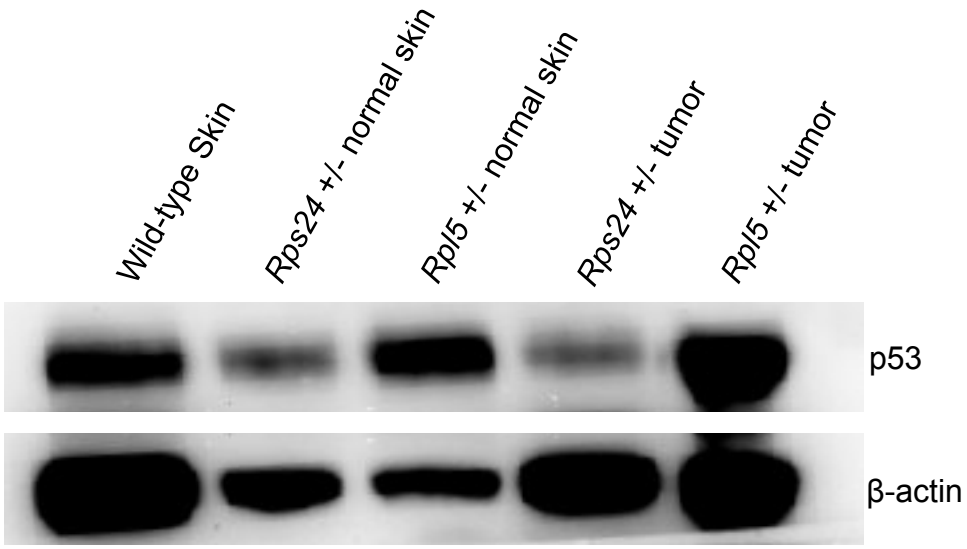


Supplemental Figure 3



Supplemental Figure 4

A



B

Samples	Fold Change
Wild-type	0.93
Rps24+/- normal	0.57
Rpl5+/- normal	1.05
Rps24+/- tumor	0.59
Rpl5+/- tumor	1.4

Supplemental Table 1. Complete Blood Count in Four-week Old *Rpl5+/-*, Rps24+/- and WT Mice

Parameter (Units)	*Rpl5+/-* (Mean Result, n=9)	*Rps24+/-* (Mean Results, n=9)	WT (Mean Results, n=9)
RBC (M/μL)	10.87 ±0.2	10.9±0.2	11.03±0.1
Hb (g/dL)	14.0±0.2	13.2±0.1	13.86±0.3
HTC (%)	48.4±0.6	48.8±1.0	47.67±0.5
MCV (fl)	44.5±0.5	44.7±0.4	43.2±0.5
WBC (K/μL)	9.27±0.8	8.2±1.2	8.14±1.8
NE (K/μL)	1.91±0.4	1.3±0.4	2.13±1.5
LY (K/μL)	7.16±0.6	6.7±1.0	5.78±1.7
MO (K/μL)	0.18±0.1	0.16±0.0	0.2±0.1
EO (K/μL)	0.01±0.0	0.00±0.0	0.03±0.0
BA (K/μL)	0.00±0.0	0.00±0.0	0.07±0.0
PLT (K/μL)	1012±47.1	818.3±116.5	927.4±233.3

RBC – red blood cells; Hb – hemoglobin; HTC – hematocrit; MCV – mean corpuscular volume; WBC – white blood cells; NE – neutrophils; LY – lymphocytes ; MO – monocytes; EO – eosinophils; BA – basophils; PLT – platelets

Supplemental Table 2. qRT-PCR Analysis of *Rpl5* and *Rps24* mRNA Levels in Bone Marrow Cells

A

Expression of *Rpl5* mRNA in Bone Marrow of *Rpl5* +/- and Wild-type Mice

Mice Strain	*Rpl5* Average C_t	*Gapdh* Average C_t	ΔC_t *Rpl5-Gapdh*	*Rpl5* Normalized to *Gapdh* ($2^{-\Delta Ct}$)
Wild-type	20.29±.3	17.08±.1	3.21±.3	.108067(.087778-.133046)
Rpl5 +/-	20.20±.2	16.89±.2	3.31±.2	.100830(.087778-.115824)

Note: C_t- number of quantitative cycles

B

Expression of *Rps24* mRNA in Bone Marrow of *Rps24* +/- and Wild-type Mice

Mice Strain	*Rps24* Average C_t	*Gapdh* Average C_t	ΔC_t *Rps24-Gapdh*	*Rps24* Normalized to *Gapdh* ($2^{-\Delta Ct}$)
Wild-type	18.40±.5	15.28±.4	3.14±.7	.113440(.069830-.184284)
Rps24+/-	18.36±.3	15.81±.2	2.54±.5	.171943(.121582-.243164)

Note: C_t- number of quantitative cycles

Supplemental Table 3. Number of Mice Monitored for Cancer Study

Mouse Genotype	Total Mice	Sarcoma	Dermatitis	Injury	Natural Cause	Continue to Monitor
Rpl5+/-	21	2	8	5	6	0
Rps24+/-	23	1	11	4	4	3
Wild-type	31	0	9	0	3	19