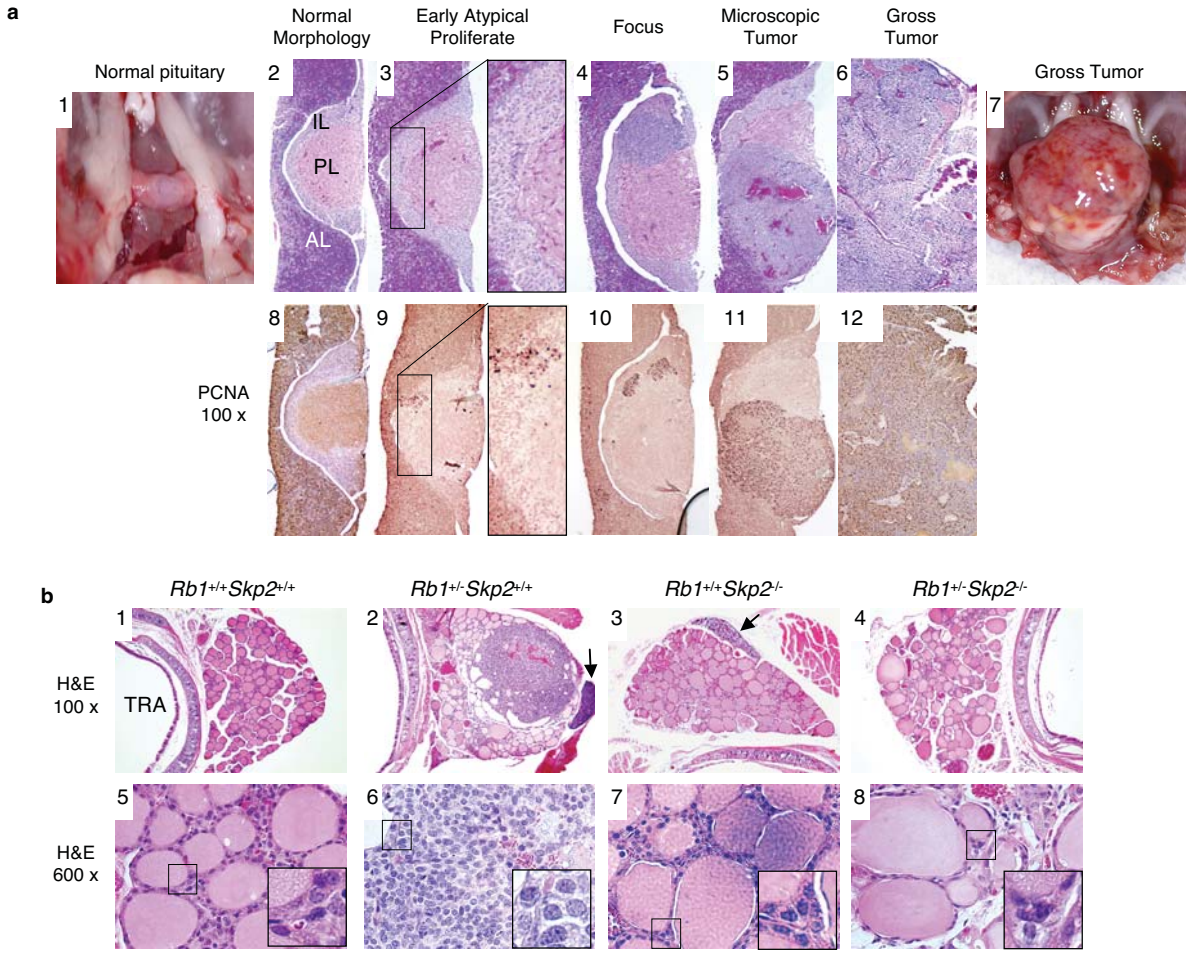
Supplemental Information

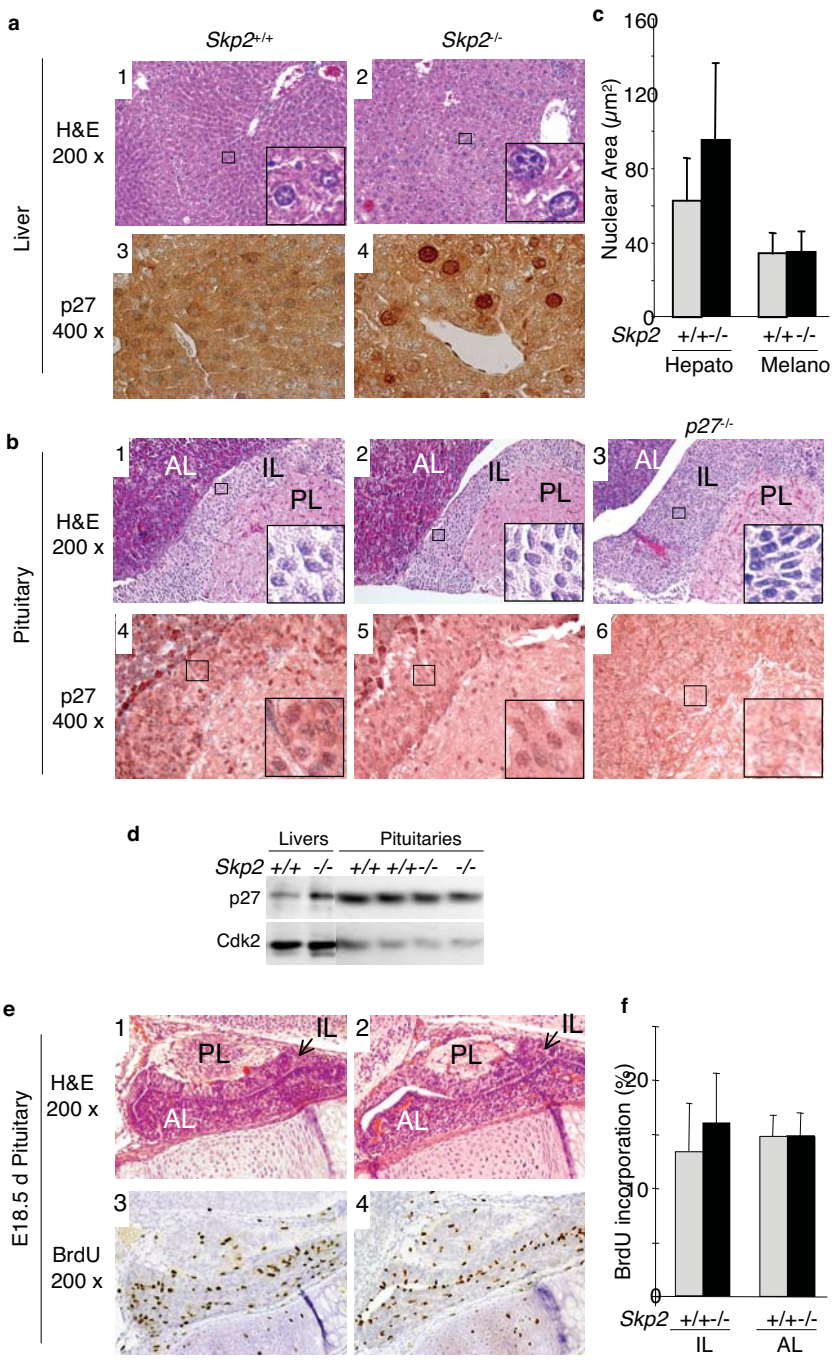
Skp2 is required for survival of aberrantly proliferating *Rb1*-deficient cells and for tumorigenesis in *Rb1*$^{+/-}$ mice

Hongbo Wang[1], Frederick Bauzon[1*], Peng Ji[1*], Xiaoliang Xu[3*], Daqian Sun[1], Joseph Locker[2], Rani S. Sellers[2], Keiko Nakayama[4], Keiich I. Nakayama[5], David Cobrinik[3], and Liang Zhu[1]

[1]Department of Developmental and Molecular Biology, and Medicine, and Pathology[2], The Albert Einstein Comprehensive Cancer Center and Liver Research Center, Albert Einstein College of Medicine, Bronx, NY 10461, USA. [3]Dyson Vision Research Institute, Weill Medical College of Cornell University, New York, NY 10021. [4]Department of Functional Genomics, Division of Developmental Genetics, Tohoku University Graduate School of Medicine, Sendai 980-8575, Japan, [5]Department of Molecular and Cellular Biology, Medical Institute of Bioregulation, Kyushu University, Fukuoka 812-8582, Japan



Supplemental Figure 1. Pituitary IL and thyroid C-cell tumorigenesis in *Rb1*$^{+/-}$ mice. **a**. Progression of pituitary IL tumorigenesis. EAPs were detected with PCNA stain (**a**3,9). **b**. Thyroid C-cell tumorigenesis. Arrows mark the parathyroid glands in **b**2,3. TRA, Trachea.



Supplemental Figure 2. Effects of Skp2 knockout in the pituitary gland intermediate lobe in comparison to the liver. In susceptible tissues such as the liver, Skp2 knockout leads to enlarged nuclei and accumulation of p27 protein (**a**). Unlike the livers in the same animals pituitary glands of 6-month old *Skp2*$^{-/-}$ mice did not contain enlarged nuclei or detectably higher levels of p27 protein in melanotrophs (**b** and **c**). Anti-p27 Western blotting with extracts of liver and pituitary glands of *Skp2*$^{+/+}$ and

Skp2$^{-/-}$ mice confirmed results obtained by anti-p27 IHC (**d**). To directly determine the effects of Skp2 knockout on cell proliferation in pituitary glands, we measured the BrdU incorporation rates in pituitaries of *Skp2*$^{+/+}$ and *Skp2*$^{-/-}$ embryos at 18.5 days of gestation and found them to be similar (**e** and **f**). Thus, Skp2 is not required for normal organogenesis and homeostasis of the pituitary gland including its intermediate lobe. Anterior lobe (AL), intermediate lobe (IL), and posterior lobe (PL), big square inserts are enlarged views of areas marked by small square boxes. Quantification of nuclear sizes (areas) of about 600 cells from three *Skp2*$^{+/+}$ or *Skp2*$^{-/-}$ mice was by Axiovision (Zeiss) image analysis software. "Hepato", hepatocytes; "Melano", melanotrophs of IL. Averages and s.d. are shown. Quantification of BrdU labeling was performed with three embryos each of the indicated genotypes.

a

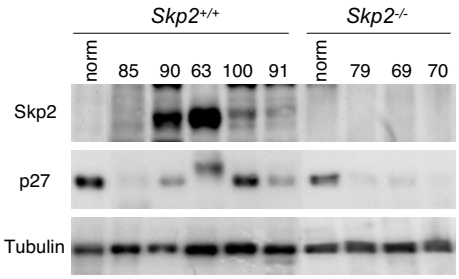
	Sub-mandible Lymphoma sizes (number of mice and % total)			Thoracic Lymphoma sizes (number of mice and % total)			(number of mice and % total)		
	5 mm	3 mm	2 mm	5 mm	3 mm	2 mm	**S**	**H**	**B**
***Skp2*$^{+/+}$ (n=44)**	5 11.4%	14 31.8%	2 4.5%	3 6.82%	7 15.9%	5 11.4%	22 50%	19 43.2%	16 36.4%
***Skp2*$^{-/-}$ (n=35)**	5 14.3%	11 31.4%	6 17.1%	4 11.4%	7 20%	6 17.1%	15 42.9%	16 45.7%	14 40%

S: Splenomegaly

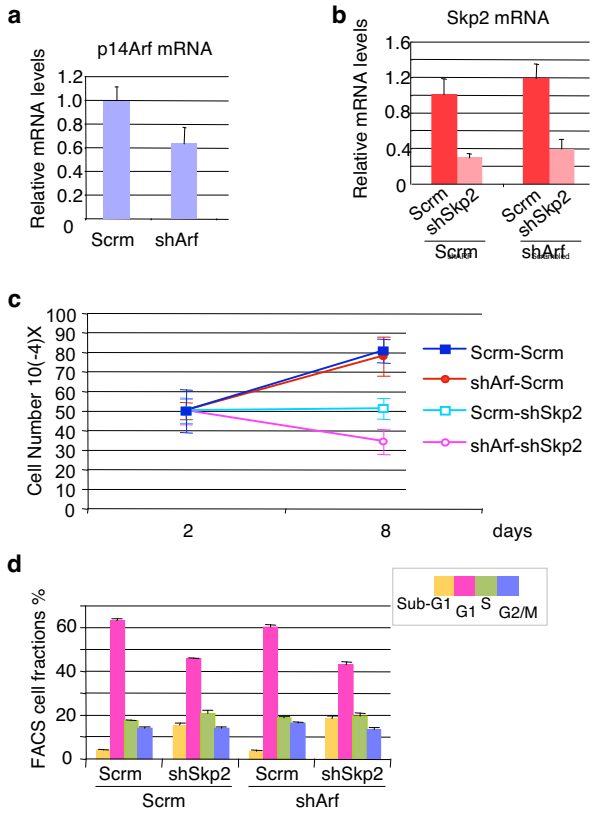
H: Hepatomegaly

B: Both Splenomegaly and Hepatomegaly

b



Supplemental Figure 3. Skp2 inactivation does not protect mice from ENU induced tumorigenesis. (**a**) Lymphomas are the most prominent tumors in ENU-treated mice. Presence of sub-mandible lymphoma and thoracic lymphoma of the indicated sizes in 44 Skp2 WT and 35 littermate Skp2 KO mice is shown; the presence of splenomegaly and hepatomegaly was also determined in these mice. (**b**) Protein expression levels for Skp2 and p27 in representative normal spleen and lymphoma tissues were determined by Western Blot.



Supplemental Figure 4. Effects of p14Arf knockdown and Skp2 knockdown on early passage RB177 retinoblastoma cells. **(a)** RB177 cells were infected with lentiviruses expressing shRNA targeting p14Arf or scrambled shRNA (Scrm), and p14Arf mRNA levels determined by Q-RT-PCR. (**b**) Cells from (**a**) were infected with lentiviruses expressing shRNA targeting Skp2 or Scrm shRNA, and Skp2 mRNA levels determined by Q-RT-PCR on day 4. (**c**) Cell numbers measured on the indicated days for cells treated as indicated. (**d**) Cell cycle profile by FACS, including sub-G1 cell fraction for apoptotic cells. Skp2 knockdown-induced increase in sub-G1 fraction was not diminished by co-knockdown of p14Arf.

Supplemental Table 1. Oligonucleotide primers.

Skp2 KO mice genotyping	
Skp2WT-5'	5- AGAGTGGAAGAACCCAGGCAGGAC-3
Skp2WT-3'	5- CCCGTGGAGGGAAAAAGAGGGACG -3
Skp2MUT-5'	5- GCATCGCCTTCTATCGCCTTCTTG-3
Skp2MUT-3'	5- TTCCCACCCCCACATCCAGTCATT-3
Rb1 lox/lox mice genotyping	
Rb5lox	5-CTCTAGATCCTCTCATTCTTC-3
Rb3lox	5-CCTTGACCATAGCCCAGCAC-3
Rosa26R(YFP) mice genotyping	
YFP1	5- AAGTTCATCTGCACCACCG -3
YFP2	5- TGCTCAGGTAGTGGTTGTCG –3
p27T187A KI mice genotyping	
Y1	5-GAGCAGGTTTGTTGGCAGTCGTACACCTCC-3
H3	5-CCAATATGGCGGTGGAAGGGAGCTGA-3
mSkp2 Q-PCR	
	5-AGCTGCTCCTTGGGATCTTT-3
	5- ACGTCTGGGTGCAGATTTTT-3
mGAPDH Q-PCR	
	5-GGATGATGTTCTGGGCAG-3
	5-GGATGATGTTCTGGGCAG-3