Pontin is essential for murine hematopoietic stem cell survival

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Online Supplementary Design and Methods

Genotyping of mice and embryos

Genotyping of adult mice and post-implantation embryos was carried out by PCR of genomic DNA using a mix of the following primers for detection of *Ruvbl1* wild-type allele (Primer 1+Primer 2, 300bp product), and *Ruvbl1* KO allele (Primer 1+Primer 3, 372bp product); we performed 30 cycles of amplification (1 min at 95°C, 15 s at 56°C, 30 s at 72°C) using an Eppendorf Thermal Cycler.

Primer 1:	5'-GCT GTC CTA GAA CTC AAT ACG-3'
Primer 2:	5'-AGG ACC TTT GCC ACA GAC AAG-3'
Primer 3:	5'-AAA TGC AGT GCA GTC ACT CG-3'
Primer 4:	5'-GAC TTC CAG CCA ATG CAC-3'

To PCR genotype E2,5 and E3,5 embryos, uteri of plugged females were flushed to collect embryos at a specific stage of development. Single embryos were lysed in 10 μL of lysis buffer (50 mM KCl, 10 mM Tris-HCl pH8.5, 0.1% Triton X-100, 4 mg/mL Proteinase K) overnight at 55°C and heated for 10 min at 95°C. We used 1-2 μL of the reaction for the first round of PCR with the same primer combination. A second round of nested PCR was performed using 2 μL of primary PCR using Primer 4 instead of Primer1 (Primer 4+Primer 2, 280bp product for *Ruvbl1* wild-type allele, Primer 4+Primer 3, 350bp product, *Ruvbl1* KO allele).

Extracts of mouse cells and tissues

Dissected and snap frozen mouse tissues were lysed and homogenized in cold lysis buffer (50 mM NaCl, 20 mM Tris-HCl pH 7.5, 1% Triton X-100, 0.1% SDS, 0.5% sodium deoxycholate). Ten micrograms of protein extract were separated by SDS-PAGE and proteins were transferred and blotted onto Hybond-P (Amersham Biosciences).

Flow cytometry

List of antibodies used in FACS experiments.

Conjugate	Antigen	Clone	Conc.	Dilution	Provider
PE	CD45.1	A20	0.5 mg/mL	1/100	eBioscience
Biotin	CD45.1	A20	0.5 mg/mL	1/50	eBioscience
Biotin	CD45.2	104	0.5 mg/mL	1/50	eBioscience
APC-Alexa-750	CD45.2	104	0.5 mg/mL	1/25	eBioscience
APC	CD150	TC15-12F12.2	0.2 mg/mL	1/100	BioLegend
PE-Cy 7	Sca-1	D7	0.2 mg/mL	1/100	eBioscience
FITC	Mac-1	M1/70	0.2 mg/mL	1/400	eBioscience
PE	B220	RA3-6B2	0.2 mg/mL	1/200	eBioscience
PE	CD48	HM48-1	0.2 mg/mL	1/25	eBioscience
TriColor	Rat IgG		0.2 mg/mL	1/200	CalTag
eFluor605NC	Strept-avidin			1/50	eBioscience

Lineage cocktail

BM cells were Fc-blocked (CD16/CD32, clone 2.4G2, eBioscience) and incubated with purified rat antibodies against the following lineage markers: CD4 (GK1.5), CD5 (53.7.3), CD8 (53-6.7), B220 (RA3-6B2), Mac-1 (M1/70), Gr-1 (RB6-8C5) and TER119 (Ter-119), followed by staining with TriColor-conjugated goat-anti-rat antibody. This was followed by either of the following HSC stains.

LSK CD150

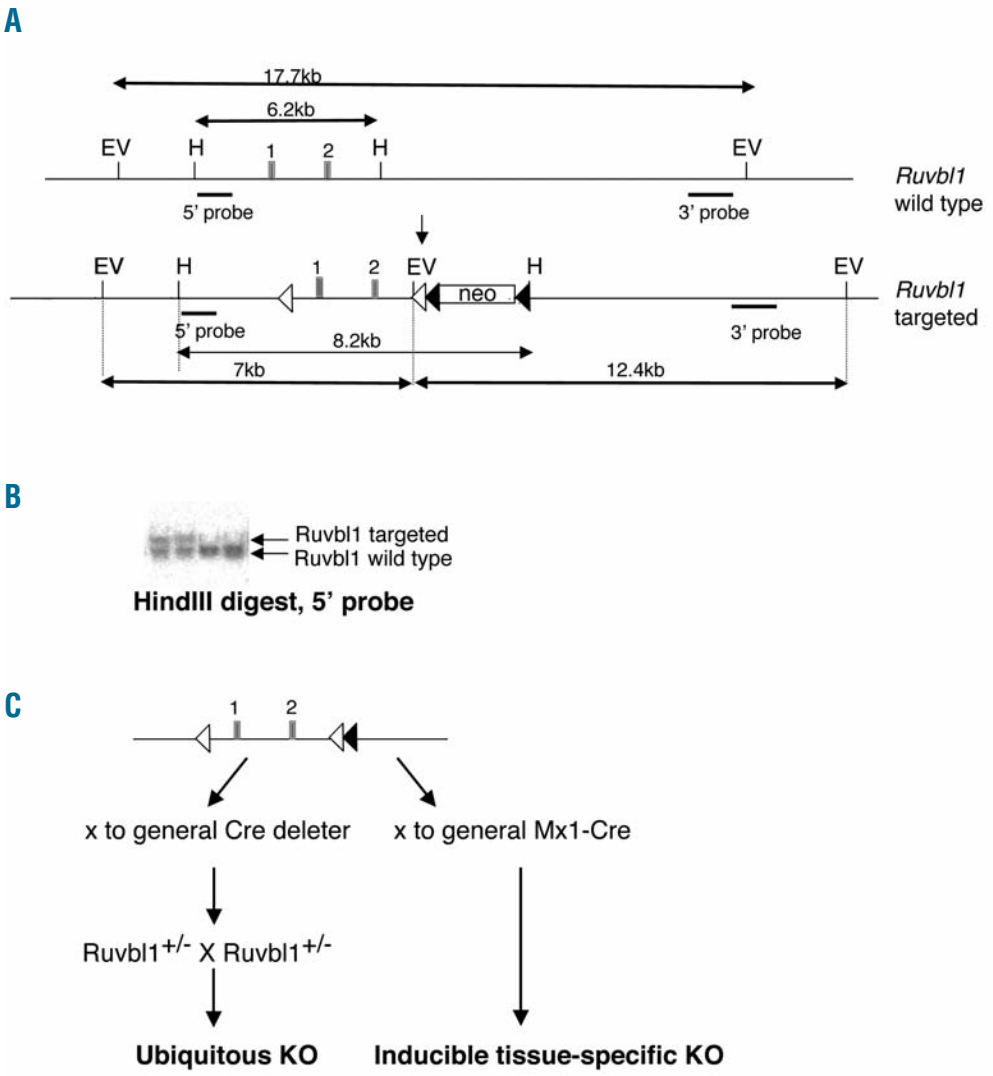
With the following additional antibodies: c-Kit–APC-Alexa780, Sca-1–PECy7, CD150–APC, CD45.1–PE, CD45.2-biotin/Streptavidin- eFluor605NC.

SLAM HSC

Sca-1–PECy7, CD150–APC, CD48-PE, CD45.1–APCAlexa750, CD45.2-biotin/Streptavidin-eFluor605NC.

Apoptosis

Where relevant, to detect apoptotic and dead cells, cells were stained with FITC–AnnexinV according to the manufacturer's instructions (eBioscience); SytoxBlue (Invitrogen) was added 10 min prior to sample analysis.



Online Supplementary Figure S1. Strategy for *Ruvbl1* gene targeting in mice. (A) Schematic representation of wild-type (top) and targeted (bottom) Ruvbl1 locus. Exons are shown in gray boxes, inserted LoxP sited in empty triangles and FRT sites in black triangles. Restriction sites and regions for Southern probe generation used for selection of ES clones are indicated. (B) Southern blot hybridization image of HindIII digestion of the targeted and wild-type *Ruvbl1* loci, 2 representative targeted ES clones along with wild-type ES clones are shown. (C) Scheme of mice breeding to generate mice with *Ruvbl1* deletion in all tissues (ubiquitous KO) and mice with inducible *Ruvbl1* inactivation in hematopoietic system (inducible tissuespecific KO).