Supplementary methods

Ank1^{E924X} *PCR genotyping*

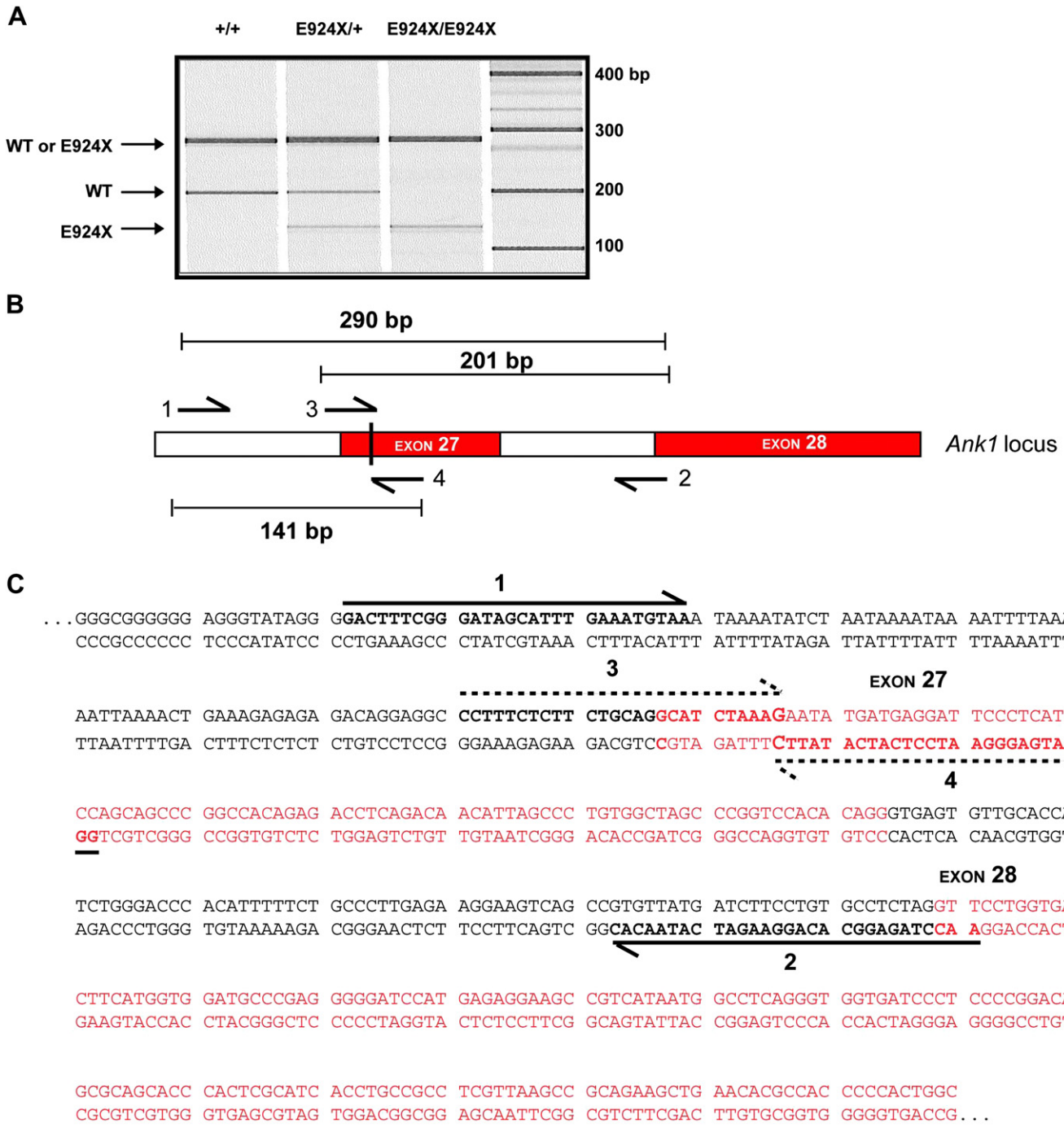
For PCR genotyping, approximately 100 ng genomic DNA was amplified per 25 μL reaction using 1.25 U DNA polymerase in PCR buffer with a final concentration of 0.2 mM dNTP mix and 3 mM $MgCl_2$. Multiplex PCR was performed to genotype WT, *E924X/+*, *E924X/E924X* animals using four primers. For the assay, two outer primers flank the area of the mutation Ank-1 FO (5′-GACTTTCGGGATAG CATTTGAAATGTAA-3′) at 0.3 μM and Ank-1 RO (5′-AACCTAGAGGCACAGGAAGATCATAACAC-3′) at 0.3 μM and two inner primers Ank-1 FI (5′-CCTTTCTC TTCTGCAGGCATCTAGAG-3′) at 0.6 μM amplifies the WT allele (G) and Ank1 RI (5′-GGGAATGAGGGAATCCT CATCATACTA-3′) at 1.2 μM amplifies the mutant allele (T). The PCR reaction produces a 290-bp (control), 202-bp (WT), and 141-bp (*Ank1*^{E924X}) band: WT mice have two bands, 290 bp and 202 bp; *E924X/+* mice have three bands, 290 bp, 202 bp, and 141 bp; and *E924X/E924X* mice have two bands, 290 bp and 141 bp. The PCR cycle profile is as follows: 1 cycle at 94°C for 5 minutes, followed by 35 cycles at 94°C for 30 seconds, 67°C for 30 seconds, and 72°C for 30 seconds and a final extension cycle at 72°C for 10 minutes.

CFU-C assays

Clonogenic assays were performed as we have described previously [37]. Single-cell suspensions of BM, spleen, and liver were prepared in Iscove's modified Dulbecco's medium (Gibco) with 5% fetal bovine serum. A red blood cell lysis was performed on BM, splenocytes, liver, and PB, using a red blood cell lysis buffer (0.14 M NH_4Cl_2 and 0.017 M Tris). Cell counts and viability were assessed on a ViCell automated cell counter (Beckman-Coulter). For each assay, three aliquots of 2×10^4 (BM) or 1×10^5 (splenocytes, liver, and PB) cells were mixed with the appropriate methylcellulose media, plated, and grown in humidified chambers at 37°C, 5% CO_2. All CFU-C assays were grown in methylcellulose M3434 and CFU-E assays were grown in methylcellulose M3334 (Stemcell Technologies Inc.). CFU-E colonies were counted based on benzidine staining and morphology 2 days after plating. After 8 to 10 days of growth, CFU-Cs were counted based on colony morphology.

Electron microscopy

All reagents and consumables used for scanning electron microscopy were from Canemco Inc. (Canton de Gore, Lakefield, QC, Canada) unless otherwise indicated. One drop of blood, collected as noted, was immediately suspended and mixed in 100 μL 4% paraformaldehyde prepared in 0.1 M sodium cacodylate (0.1 M NaCaco). After 1 minute, an equal volume of a solution containing 4% paraformaldehyde plus 5% gluteraldehyde (in 0.1 M NaCaco) was added and mixed to the blood suspension (final 4% paraformaldehyde, 2.5% gluteraldehyde in 0.1 M NaCaco) and allowed to fix for 20 minutes at room temperature with frequent rotation. The fixed cells were then washed three times in PBS and placed onto dry coverslips precoated with poly-L-lysine (1:10 in distilled water) (Sigma-Aldrich, Oakville, ON, Canada) and allowed to adhere for 15 minutes at room temperature. Coverslips were washed again with PBS and covered with PBS before processing for electron microscopy (within 1 hour). PBS was removed and cells adhered to coverslips were treated with reduced 1% osmium tetroxide (OsO_4) for 1 hour at room temperature, washed with distilled water, treated with 1% thiocarbohydrazide, and further osmicated with 1% aqueous OsO_4 before dehydration in a graded ethanol series (from 30% to 100% ethanol), critical-point dried (Tousimis 815B), mounted on aluminum scanning electron microscopy stubs, and sputter-coated with 4 nm chromium and 8 nm gold (Cressington HR208). Red cells were observed and imaged using a Hitachi S4700 FESEM (Hitachi, Tokyo, Japan) at 2 kV.



Supplementary Figure E1. PCR genotyping strategy to detect the point mutation in exon 27 of the *Ank1*^{E924X} allele. (**A**) Digital photograph of capillary electrophoresis gel showing examples of the banding patterns observed from amplification of WT (+/+), heterozygous (*E924X/+*), and homozygous (*E924X/E924X*) genomic DNA using the primers indicated in (**B**, **C**). The digital photograph was processed in Adobe Illustrator (11.0) and contrast-enhanced for publication (**B**) Schematic of the Ank1 genomic locus adjacent to exon 27 (transcript variant 1) and primer sequences used to detect the *Ank1* E924X and WT alleles. The sizes of expected amplicons for each of the primer pairs are indicated. (**C**) Genomic sequence proximal to the ENU-generated *Ank1*^{E924X} lesion.