Park et al., http://www.jcb.org/cgi/content/full/jcb.201210099/DC1

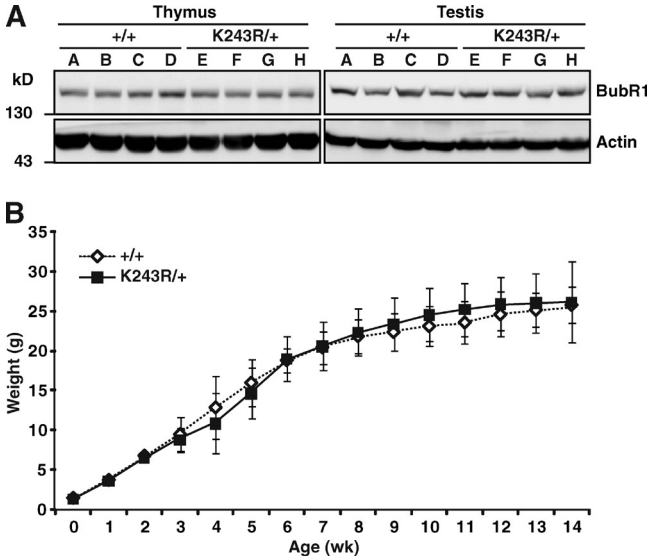


Figure S1. **Analysis of *K243R/+* knock-in mice.** (A) WB analysis of BubR1 protein levels in the thymus and testis. (B) Body weight analysis of WT (n = 50) and *K243R/+* (n = 130). SDs are shown in error bars.

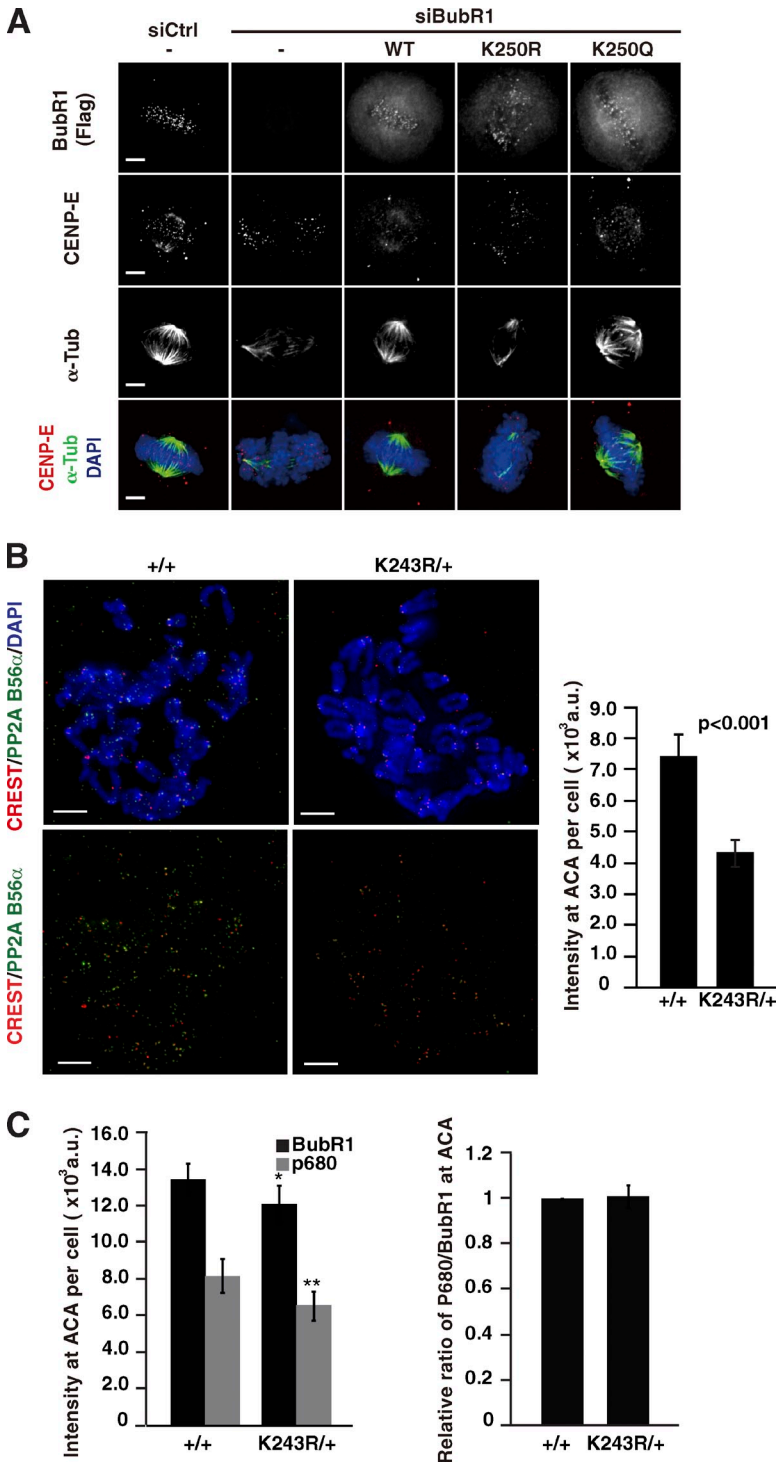


Figure S2. **BubR1 acetylation is involved in proper chromosome congression.** (A) Acetylation-mimetic form of BubR1 (K250Q) is capable of rescuing the defects of KT–MT interaction in BubR1-depleted cells. Hela-FRT-BubR1, -K250R, or -K250Q cells were depleted of endogenous BubR1 by siRNA transfection targeting 3′-UTR. The expression of indicated FLAG-tagged BubR1 constructs was induced by doxycycline treatment. Then they were subjected to cold MT assay after treating cells with MG132 for 1 h. Immunofluorescence assay was performed with anti-BubR1 or anti-FLAG (M2), anti–CENP-E, and anti-tubulin antibodies. Representative metaphase cells are shown (siCtrl, *n* = 11; siBubR1, *n* = 15; siBubR1 + WT, *n* = 10; siBubR1 + K250R, *n* = 15; siBubR1 + K250Q, *n* = 12). Bars, 5 µm. (B) PP2A-B56α recruitment to KTs in WT and *K243R/+* MEFs. Metaphase chromosome spreads were coupled with immunofluorescence assay. Immunofluorescence assay was performed with anti-CREST and anti–PP2A-B56α. The result is from two independent experiments of 27 cells in each setting (mean ± SEM; *n* > 450 KTs). Bars, 5 µm. (C) Relative intensity of p680 BubR1 at KTs of prometaphase cells. MEFs were treated with 200 ng/ml nocodazole and 10 µM Mg132 for 2 h before fixation. Immunofluorescence assay was performed with anti-CREST, anti-pT680 BubR1, and anti-BubR1 antibodies. The result is from two independent experiments of 37 cells in each setting (mean ± SEM; *n* > 350 KTs). Asterisks mark significant p-values calculated by an unpaired *t* test (*, P = 0.32; **, P = 0.18). (right) Relative ratio of p680/BubR1 immunofluorescence intensities are depicted as histograms (SDs are from two independent experiments).

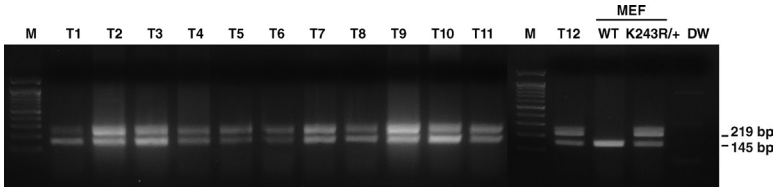
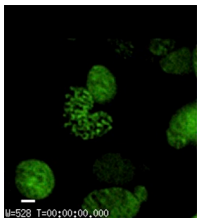
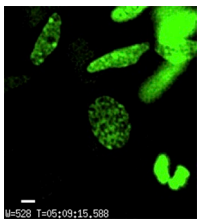


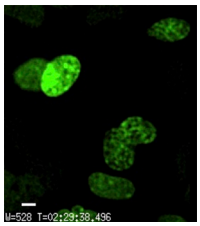
Figure S3. **Detection of WT and K243R allele in primary tumors.** PCR genotyping of *WT-BubR1* and *K243R* from 12 primary tumors obtained from the *K243R/+* mice. Genomic DNA samples from the WT and *K243R/+* MEFs served as the PCR controls.



Video 1. **Live imaging of mitosis in WT MEFs.** MEFs isolated from the mice expressing *GFP-H2B* (i.e., GFP-H2B transgenic mice) were subjected to time-lapse video microscopy using the UPlanFL N 40×/NA 1.30 oil lens on a microscope (DeltaVision Cpre) equipped with a charge-coupled device camera in a CO_2 chamber at 37°C. The images were captured every 5 min using a 20× objective lens. Bar, 5 µm.



Video 2. **Mitosis in *K243R/+; GFP-H2B* MEFs.** The *GFP-H2B* transgenic mice were crossed with *K243R/+* mice, and the MEFs isolated from the resultant *K243R/+; GFP-H2B* mice were subjected to live imaging as in Video 1. The images were taken every 5 min. The chromosomes segregated without forming distinct metaphase plates. Lagging chromosomes are shown. Bar, 5 µm.



Video 3. **Mitosis in *K243R/+; GFP-H2B* MEFs.** The *GFP-H2B* transgenic mice were crossed with *K243R/+* mice, and the MEFs isolated from the resultant *K243R/+; GFP-H2B* mice were subjected to live imaging as in Video 1. The cells exited mitosis without chromosome segregation. The images were captured every 5 min. Bar, 5 µm.