SUPPLEMENTAL MATERIALS AND METHODS

Animals. All procedures and experiments involving animals were approved by the Institution Animal Use and Care Committee (IACUC) of the Mayo Clinic. *Usp24* mutant mice were derived from the XB614 embryonic stem cell clone generated by Bay Genomics. Cells were injected into blastocysts and chimeric mice were bred to C57BL/6 mice. Mice were genotyped by PCR using standard procedures. More details may be found in the supplemental *Materials and Methods*. DMBA tumor susceptibility studies were performed as in [37]. We used the Bay Genomics XB614 embryonic stem cell clone (129P2/OlaHsd) to generate chimeric mice on a C57BL/6 background. The exact location of the genetrap in the XB614 clone was not known, but was localized to the intron between exon 37 and 38 (numbering as per NCBI37/mm9) by Bay Genomics (Figure 3A). The insertion site was identified by PCR using a reverse primer anchored in the 5' region of the genetrap vector and a series of forward primers walking from the 3' sequence of exon 37 and continuing 3' into the intron. Sanger sequencing of the products obtained revealed the insertion site. The genetrap is inserted approximately 3819 nucleotides 3' to exon 37.

Immunoreagents. Antibodies used include USP24 (C-terminus; Bethyl, Montgomery, TX, A300-938A), α-tubulin (Cell Signaling), CRMP2 (Cell Signaling #9393), CRMP2B (Cell Signaling #35672), β-galactosidase (Abcam, Cambridge, MA), β-actin (Sigma-Aldrich, St. Louis, MO), histone H3 phospho-Ser10 (Upstate, Lake Placid, NY), anti-centromere antibody (Antibodies Inc., Davis, CA, USA, #15-235. A polyclonal anti-serum against was raised in Rabbits (Covance, Princeton, NJ) against a 97 amino acid region (406-503; VTKLIEDSTLSKSVKNAIDTDRLLDWLVENSVLSIALEGNIDQAQYCDRIKGIIELLGSKL SLDELTKIWKIQSGQSSTVIENIHTIIAAAAVKFNA) from murine USP24. The protein fragment was cloned using PCR into the PET28b vector and soluble HIS-tagged protein was purified from *E. coli* BL21 using Ni-NTA beads using standard procedures. The resulting anti-serum, referred to as the N-terminal antibody, was used to detect the USP24-β-Geo fusion where indicated.

Proteomics. Usp24 wild type and homozygous mutant MEFs (n = four independent lines each) were cultured in multiple 15 cm dishes and were incubated for 6-8 hours with nocodazole (100ng/ml final) prior to isolation of mitotic cells by mitotic shake-off. Frozen cell pellets were solubilized 1% SDS/20mM Tris pH 8, and protein content determined by BCA assay. 75 μg of protein plus 100mM DTT was boiled for 3 min, and diluted in 2D rehydration buffer (8M urea, 4% CHAPS, 40mM DTT, 1% Pharmalyte, 0.25% 3-10 IPG buffer). 18 cm pH 3-10NL IPG strips were rehydrated with sample for 11h, followed by isoelectric focusing for 30000 Vh (~12 h) The focused strips were equilibrated sequentially in 1% DTT, then 2.5% IAA (iodoacetamide) in SDS-PAGE buffer. Strips were mounted on top of 8-14% large format (18x20x0.1cm) Tris glycine HCl gels. BenchMark protein standard was loaded on a paper wick and placed next to the IPG strip for each gel, and both were sealed in place with 0.75% melted agarose in SDS-

PAGE running buffer. The gels were electrophoresed together in a Protean Plus Dodeca Cell for 6h at 4C at 200V, then fixed and stained with SyproRuby fluorescent stain. High resolution images for 2D analysis were obtained using the Molecular Imager FX system (Bio-Rad, Hercules CA). Images were analyzed using REDFIN software (Ludesi, Sweden) and spots with a significant and 2-fold change in volume ($p < 0.025$, ANOVA) were excised from silver-stained gels.

The silver stained 2D-gel spots were destained with 15mM potassium ferricyanide and 50mM sodium thiosulfate in water until clear, then rinsed with water several times to remove all color. The spots were then reduced with 30 mM DTT/50mM Tris, pH 8.1 at 55ºC for 40 minutes and alkylated with 40mM iodoacetamide at room temperature for 40 minutes in the dark. Proteins were digested in-situ with 25µl (0.005µg/µl) trypsin (Promega Corporation, Madison WI) in 20 mM Tris pH 8.1 / 0.0002% Zwittergent 3-16, at 37ºC overnight followed by peptide extraction with 25µl of 2% trifluoroacetic acid, then 50µl of acetonitrile. The pooled extracts were concentrated to less than 5µl on a SpeedVac spinning concentrator (Savant Instruments, Holbrook NY) and then brought up in 0.15% formic acid/0.05% trifluoroacetic acid for protein identification by nano-flow liquid chromatography electrospray tandem mass spectrometry (nanoLC-ESI-MS/MS) using a Thermo Finnigan LTQ Orbitrap Hybrid Mass Spectrometer (Thermo Electron, Bremen, Germany) coupled to an Eksigent nanoLC-2D HPLC system (Eksigent, Dublin CA). The digest peptide mixture was loaded onto a 250nl OPTI-PAK trap (Optimize Technologies, Oregon City OR) custom packed with Michrom Magic C8 solid phase (Michrom Bioresources, Auburn CA). Chromatography is performed using 0.2% formic acid in both the A solvent (98% water/2% acetonitrile) and B solvent (80% acetonitrile/10% isopropanol/10% water), and a 5% B to 45% B gradient over 60 minutes at 400 nl/min through a Michrom packed tip capillary Magic C18 75µm x 150mm column. The LTQ Orbitrap mass spectrometer experiment was set to perform a FT full scan from 375-1600 m/z with resolution set at 60,000 (at 400m/z), followed by linear ion trap MS/MS scans on the top five ions. Dynamic exclusion was set to 2 and selected ions were placed on an exclusion list for 40 seconds [60]. The lock-mass option was enabled for the FT full scans using the ambient air polydimethylcyclosiloxane (PCM) ion of m/z = 445.120024 or a common phthalate ion m/z = 391.284286 for real time internal calibration [61]. The MS/MS raw data were converted to DTA files using extract_msn.exe from Bioworks 3.2 and correlated to theoretical fragmentation patterns of tryptic peptide sequences from the Swissprot mouse database using Mascotô (Matrix Sciences London, UK) [62]. All searches were conducted with fixed modification of carbamidomethyl-cysteine and variable modifications allowing oxidation of methionines for methionine sulphoxide, and protein N-terminal acetylation. The search is restricted to full trypsin generated peptides allowing for 2 missed cleavages and is left open to all species. Peptide mass search tolerances were set to 10 ppm and fragment mass tolerances were set to ± 0.8 Daltons. All protein identifications were considered when Mascot individual peptide scores were above the 95% percentile for probability and rank number one of all the hits for the respective MS/MS spectra.

Microscopy. Live-cell imaging experiments were performed as previously described in detail [36,57]. A lentiviral construct encoding YFP-tagged H2B (pTSIN-H2B-YFP) was used to visualize chromosomes by fluorescence microscopy [55,58]. Indirect immunofluorescence was performed as described (58, 60). A laser scanning microscope (LSM 880, Zeiss) with Axiovert 100M (Zeiss) with a c-Apochromat ×100 oil immersion or x40 water immersion objective was used to analyze immunostained cells and capture images, using ZEN software (Zeiss). Images were converted into composite images then those were analyzed by using ImageJ (NIH) to generate quantitative measurements. For the CRMP2 staining we developed the following staining that is a slightly modified version of [59] Cells were flash permeabilized for 1 minute in in PHEM buffer (25 mM HEPES, 10 mM EGTA, 60 mM PIPES, 2 mM $MgCl_2$, pH 6.9 with 0.5% Triton X-100), fixed for 5 minutes in 3% PFA (in PBS) then continued the permeabilization for an addition 10 minutes in PHEM. Background was blocked for an hour in 3% milk then cells were incubated in the following antibodies o/n at 4ºC.