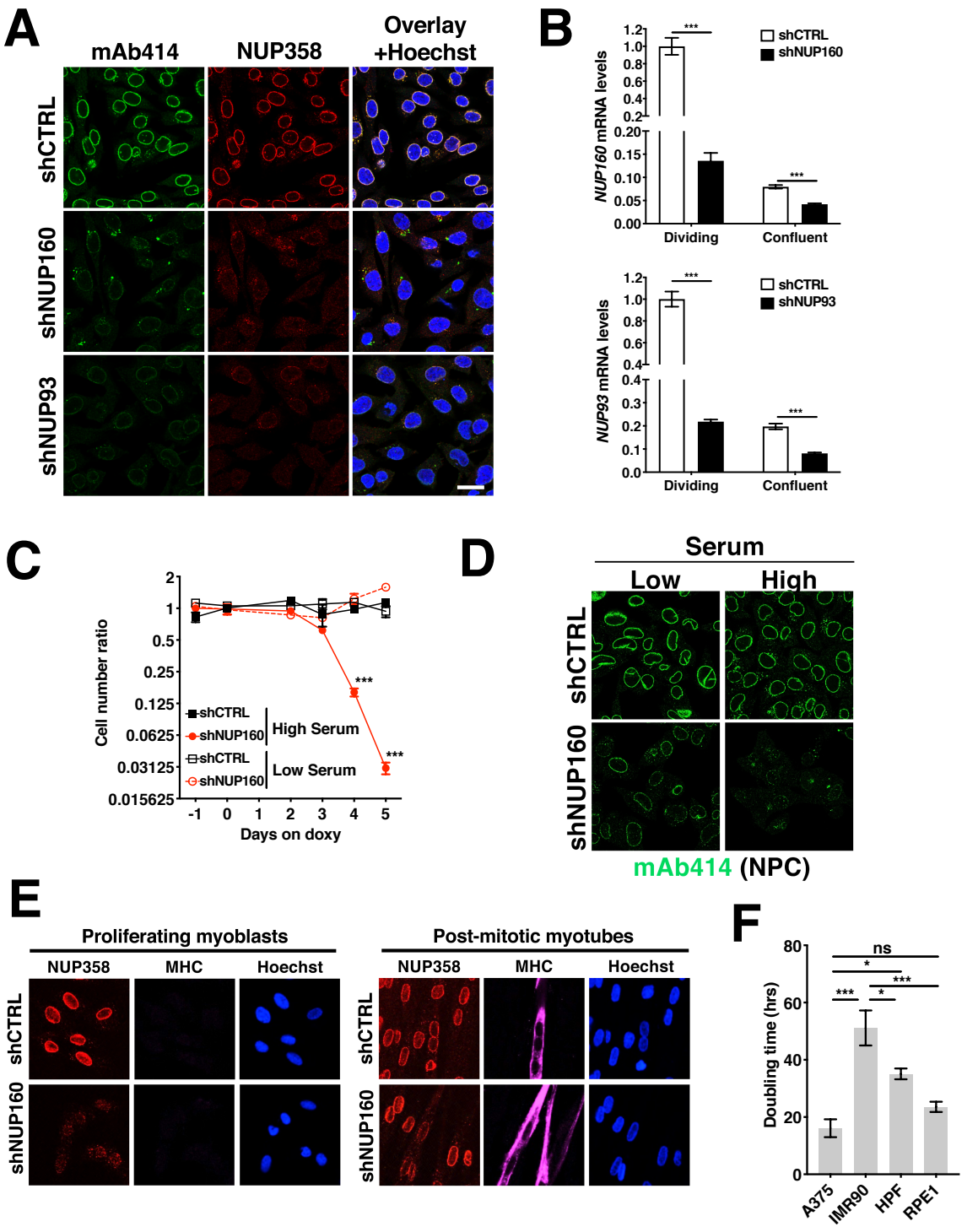
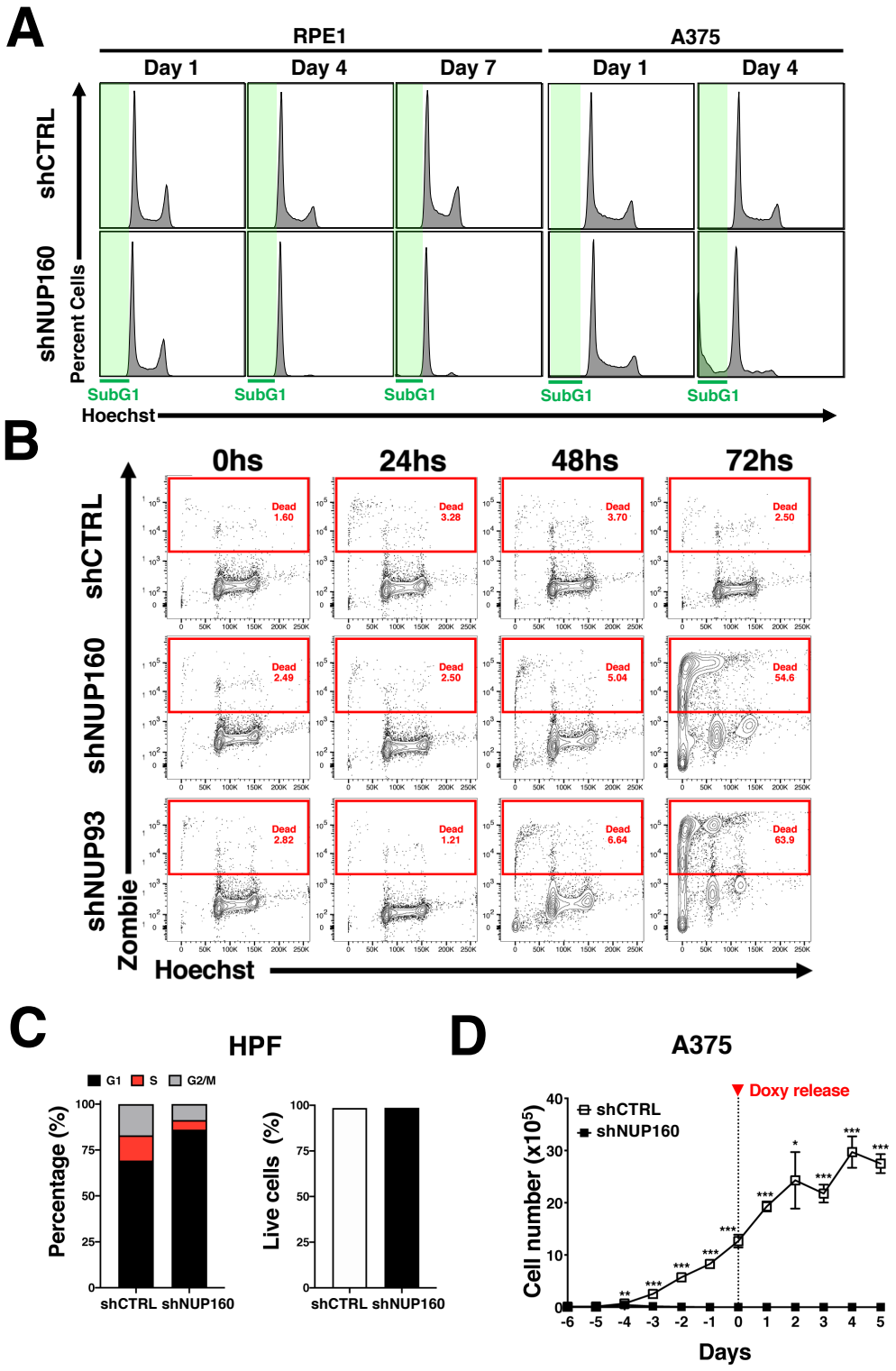


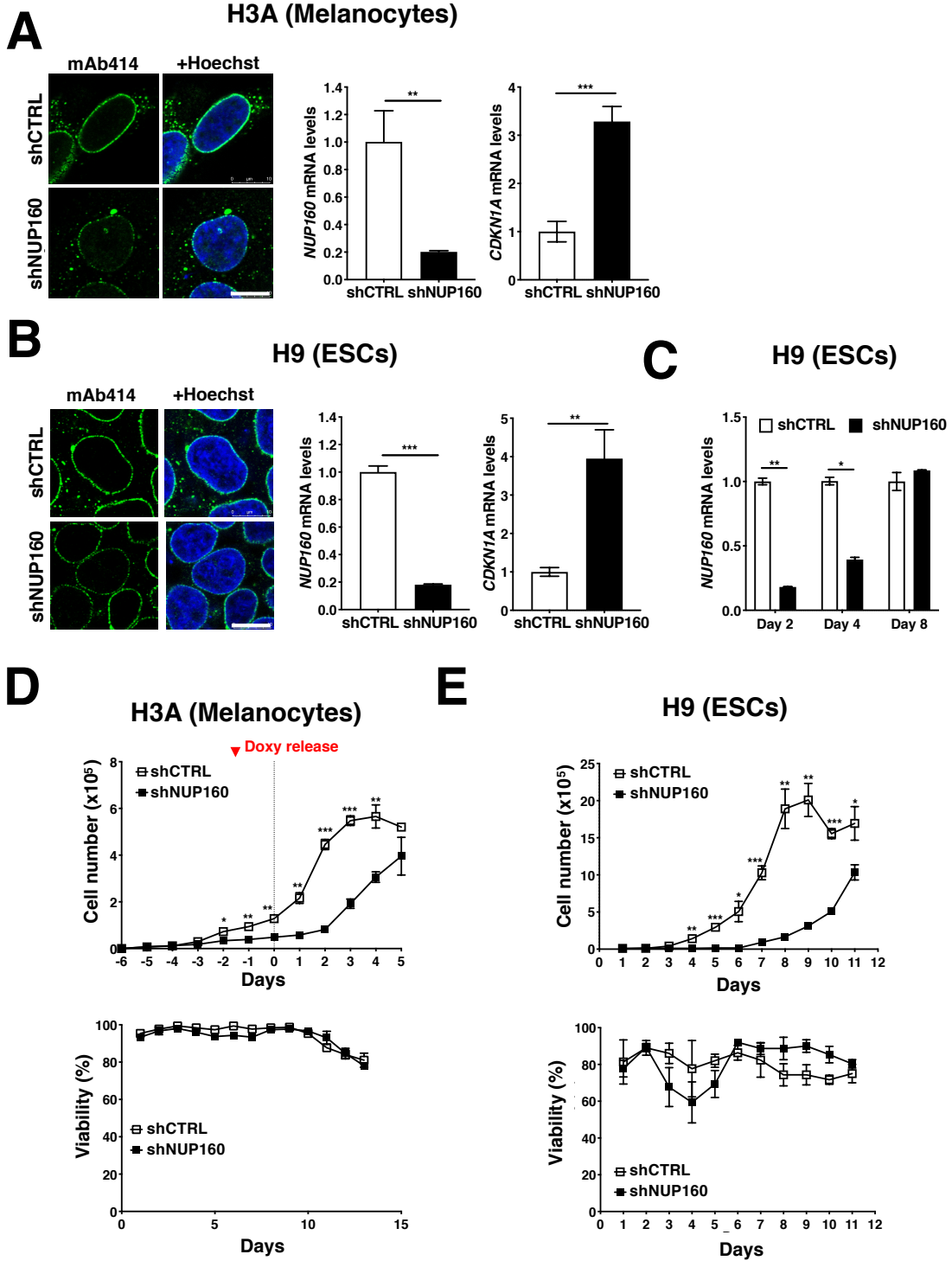
Supplementary Figure 1. NUP160 depletion reduces NPC numbers only in dividing cells. A, Immunofluorescence analysis of mAb414 and NUP358 in A375 cells after 72 hours of Control, *NUP160*, or *NUP93* shRNA induction. **B**, qPCR analysis of NUP160 and NUP93 mRNA levels normalized to GAPDH in proliferating and confluent A375 cells treated with Control, *NUP160*, or *NUP93* shRNAs for 3 or 6 days respectively. As previously reported, the expression of core nucleoporins is strongly downregulated in

non-proliferating cells (10). **C**, Cell count measurements of A375 cells grown in high or low serum after Control or *NUP160* shRNA induction. Cell counts were done by triplicate and normalized to Control shRNA cells. Counts for cells grown in low serum are between 5,000-10,000, while counts for cells grown in high serum vary between 10,000 and 2 million. Graph is representative of 3 independent experiments. **D**, Immunofluorescence analysis of NPCs in A375 cells grown in high or low serum treated for 5 days with Control or *NUP160* shRNAs. **E**, Immunofluorescence analysis of NUP358 and Myosin Heavy Chain (MHC) proteins in primary human myoblasts and differentiated myotubes expressing Control or *NUP160* shRNAs for 3 or 10 days respectively. **F**, Cell doubling time analysis of A375, IMR90, HPF, and RPE1 cells while in log phase growth. 6-20 doubling times were calculated from cell count data. Data are mean ± s.d. ns $P > 0.05$, * $P \leq 0.05$, *** $P \leq 0.001$ by multiple unpaired Student's t tests with Holm-Sidak method to correct for multiple comparisons (A, B) or one-way analysis of variance (ANOVA, E).



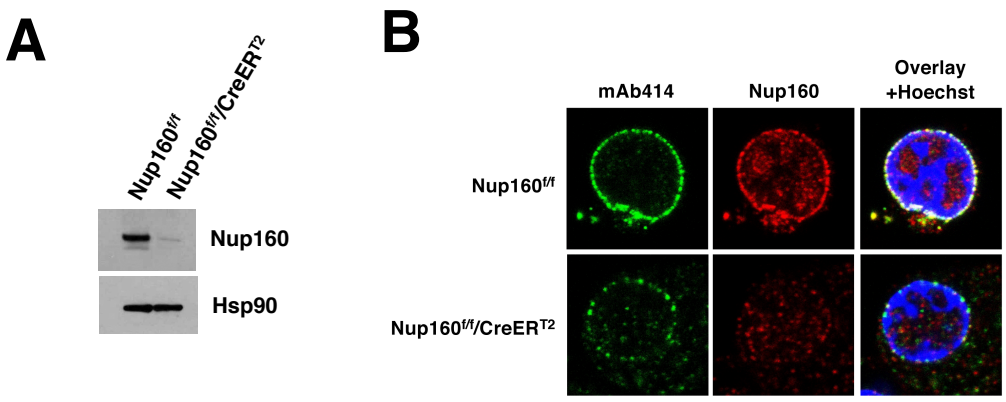
Supplementary Figure 2. A375 cells undergo cell death after NUP160 knockdown. **A**, Flow cytometry-based cell cycle analysis of RPE1 and A375 cells at the indicated times of doxycycline induction of Control or *NUP160* shRNAs. **B**, Flow cytometry analysis of

Hoechst and Zombie viability dye in A375 cells expressing Control, NUP160, or NUP93 shRNAs for the indicated times. Dead cells are indicated by the red rectangle. **C**, Flow cytometry analysis of Hoechst cell cycle phases and Zombie negative (live) dye stain quantifications in HPF cells after 7 days of treatment with Control or *NUP160* shRNAs. **D**, A375 cells expressing inducible or Control or *NUP160* shRNAs were treated with doxycycline (doxy) for 6 days. Cell were then incubated with doxycycline-free media (T= 0) to shut down shRNA expression. The number of viable cells before and after doxy release was counted over time by trypan blue exclusion. Data are mean ± s.d. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ by multiple unpaired Student's t tests with Holm-Sidak method to correct for multiple comparisons (D). Unless otherwise stated, experiments are representative of a minimum of 3 independent repeats.

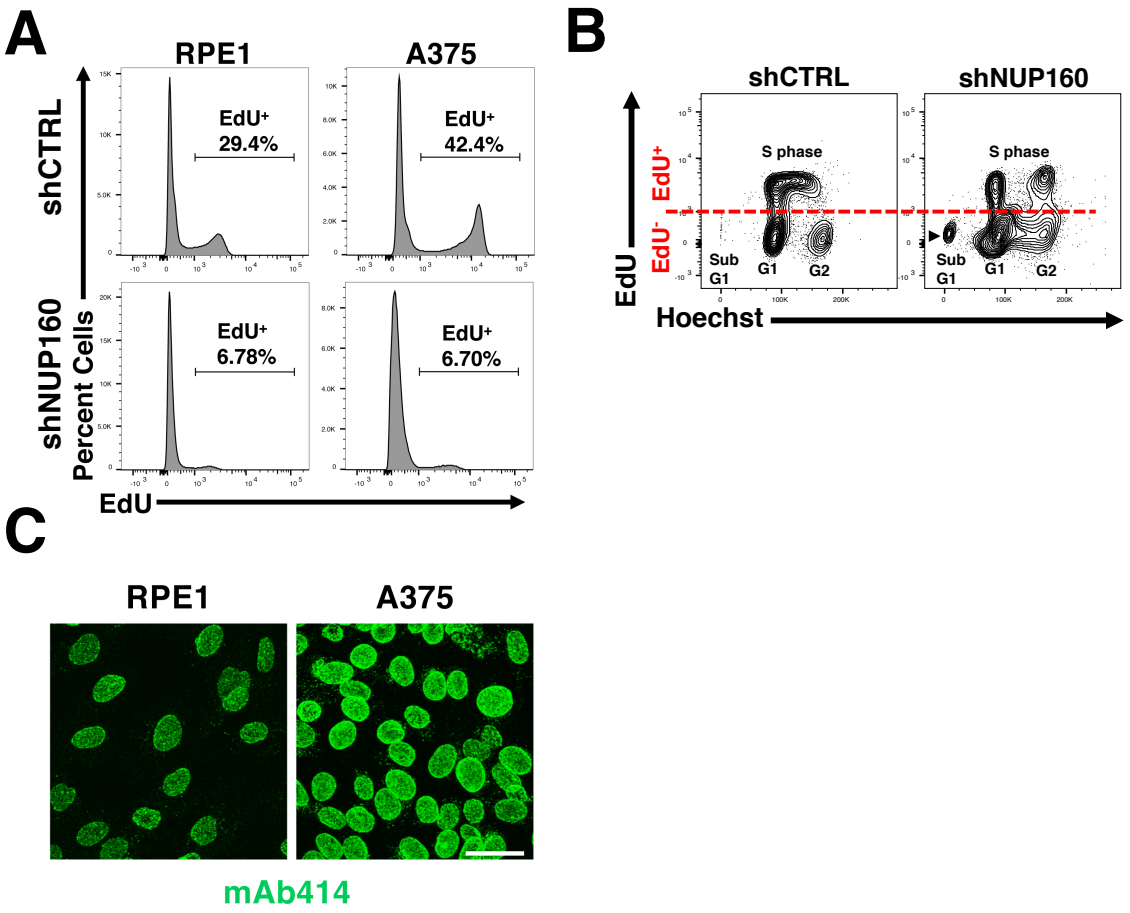


Supplementary Figure 3. H3A melanocytes and H9 ESCs cells undergo reversible cell cycle arrest after *NUP160* knockdown. A, Immunofluorescence (left) analysis of NPCs (mAb414) and qPCR analysis of *NUP160* and *CDKN1A* mRNA levels in H3A melanocytes after 72 hours of treatment with Control or *NUP160* shRNAs. Representative of 2 independent experiments. **B**, Immunofluorescence (left) analysis of NPCs (mAb414)

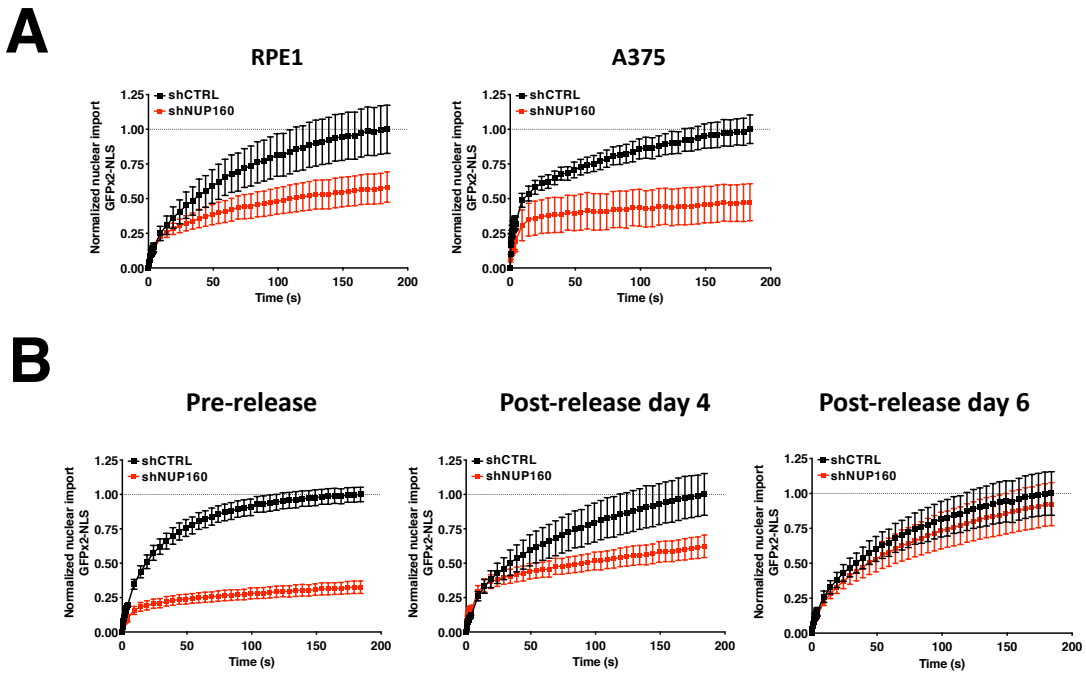
and qPCR analysis of *NUP160* and *CDKN1A* mRNA levels in H9 ESCs after 72 hours of treatment with Control or *NUP160* siRNAs. Representative of 3 independent experiments. **C**, H9 ESCs were transfected with Control or NUP160 siRNAs and NUP160 mRNA levels were quantified over time by qPCR. Representative of 2 independent experiments. **D**, H3A cells expressing inducible or Control or *NUP160* shRNAs were treated with doxycycline (doxy) for 6 days. Cell were then incubated with doxycycline-free media (T= 0) to shut down shRNA expression. The number of viable cells before and after doxy release was counted over time by trypan blue exclusion (top graph). Percent of viable cells is shown in bottom graph. **E,** H9 cells were transfected with Control or *NUP160* siRNAs and the number of viable cells was counted over time by trypan blue exclusion (top graph). Percent of viable cells is shown in bottom graph. Data are mean ± s.d. * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ by multiple unpaired Student's t tests with Holm-Sidak method to correct for multiple comparisons (E, D). Unless otherwise stated, experiments are representative of a minimum of 3 independent repeats.



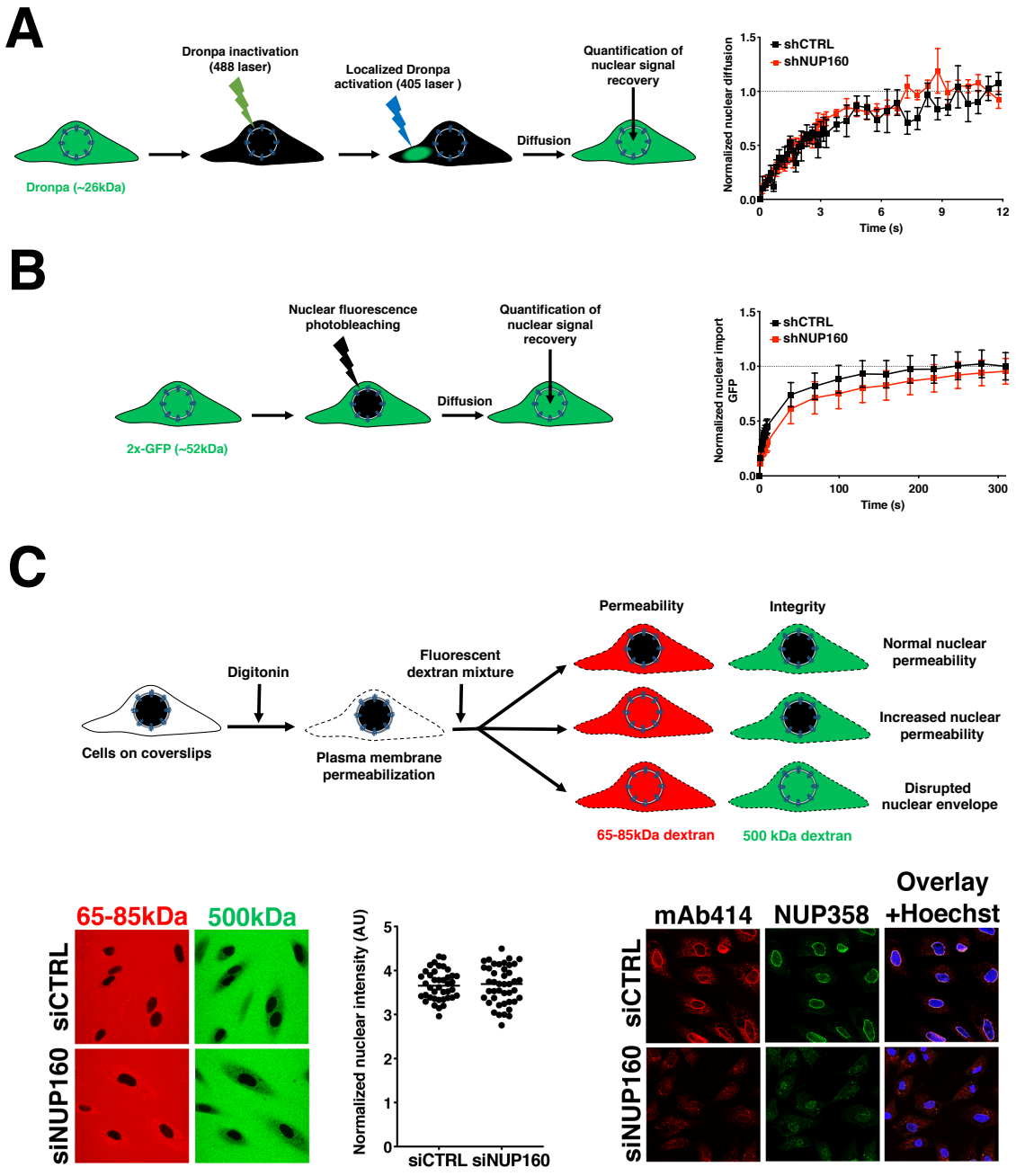
Supplementary Figure 4. NUP160 knockout in primary cells. Hematopoietic progenitors were isolated from control (*Nup160*$^{f/f}$) or *Nup160* inducible knockout mice (*Nup160*$^{f/f}$/CreER^{T2}) and treated with tamoxifen. Nup160 protein levels were analyzed 72 hours after treatment by western blot analyses (**A**) or immunofluorescence (**B**). Hsp90 was used as loading control and the mAb414 antibody was used to stain NPCs.



Supplementary Figure 5. Decreasing NPC numbers reduces cell proliferation. A, Flow cytometry analysis of EdU DNA incorporation after a 2-hour EdU pulse in RPE1 and A375 cells treated with Control or *NUP160* shRNAs for 96 hours. **B**, Flow cytometry analysis of Hoechst and EdU incorporation 16 hours after the 2-hour EdU pulse in A375 cells treated with Control or *NUP160* shRNAs for 96 hours . Arrowhead shows SubG1 cells are EdU negative. **C**, Immunofluorescence analysis of NPCs in RPE1 and A375 cells. Representative images show the maximum projection of cells stained with the NPC antibody mAb414. Scale bar, 25 μm. Experiments are representative of 3-5 independent experiments.

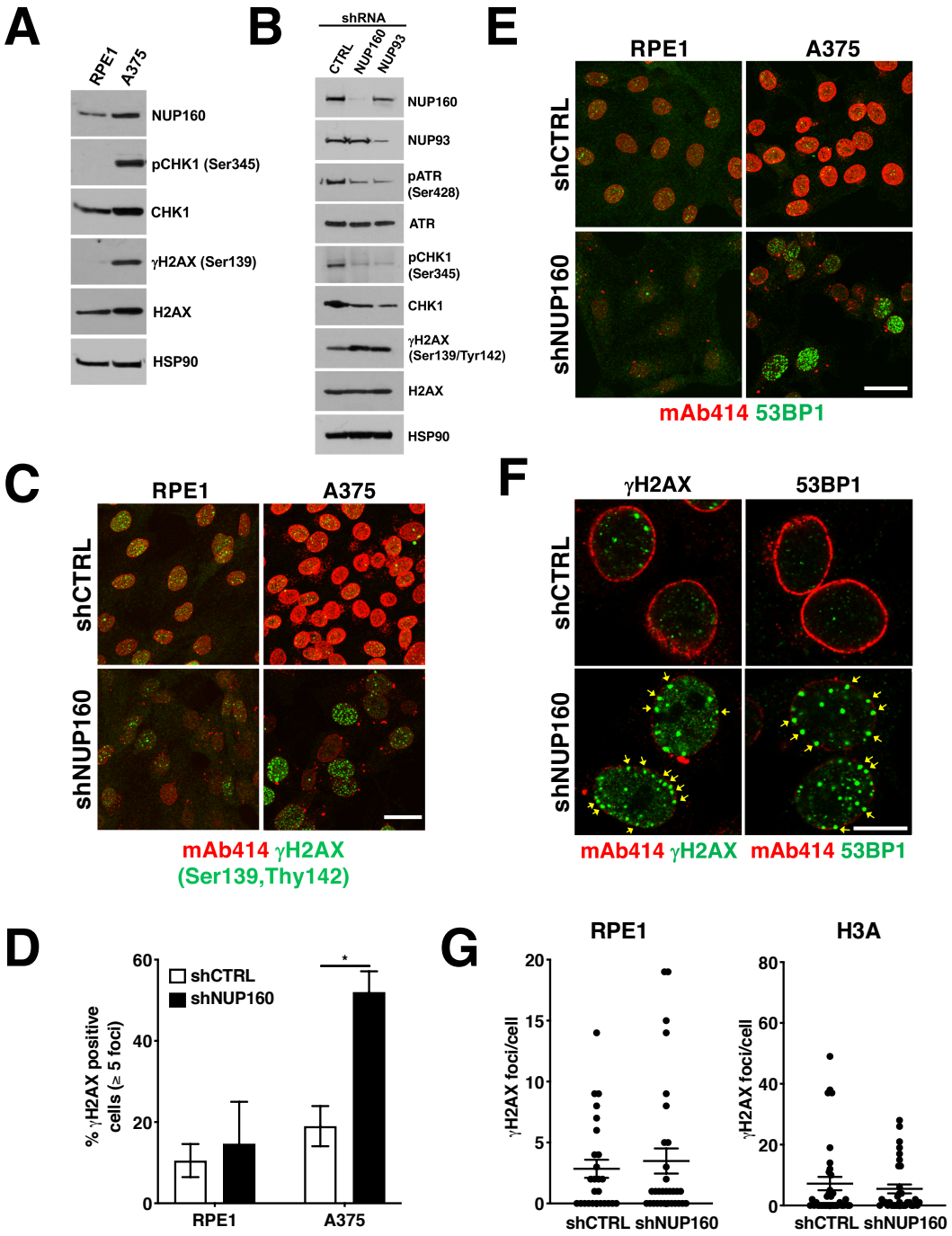


Supplementary Figure 6. Depletion of NUP160 causes a gradual decline in nuclear import efficiency. A, Fluorescence recovery after photobleaching (FRAP) analysis showing nuclear import of the GFPx2-NLS reporter in RPE1 (left) and A375 (right) cells after treatment for 72 hours with Control or *NUP160* shRNAs. The data are expressed as percentage of FRAP relative to maximum recovery of Control (n = 13-14 cells). **B**, FRAP analysis showing nuclear import of the GFPx2-NLS reporter in RPE1 cells after treatment with Control or *NUP160* shRNAs. shRNA was induced by doxycycline for 4 days (left), removed after 6 days and released for 4 days (middle) or 6 days (right). The data are expressed as percentage of FRAP relative to maximum recovery of Control (n = 9-10 cells). Data are mean ± s.e.m. Representative of 3 independent experiments.



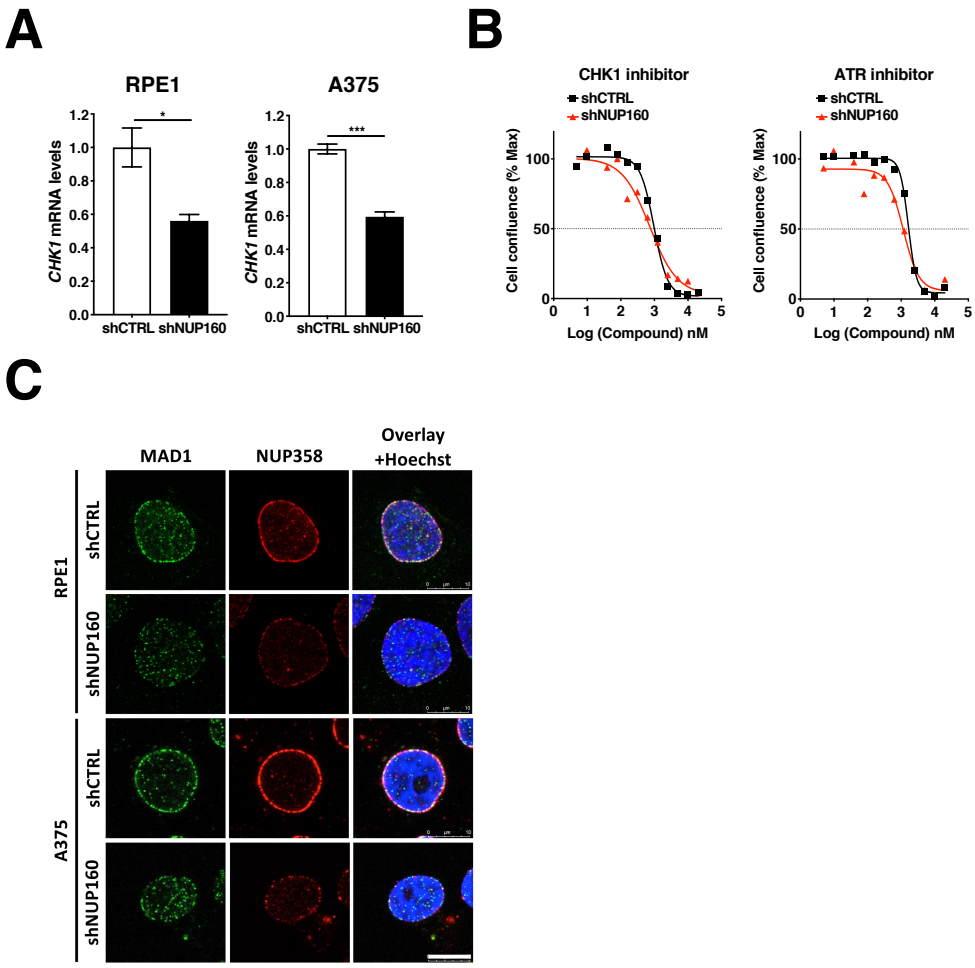
Supplementary Figure 7. Depletion of NUP160 does not cause alterations in nuclear permeability. A, Schematic illustrations of experimental approach to evaluate Dronpa diffusion into the nucleus (left). Normalized nuclear diffusion analysis of activated Dronpa protein in RPE1 cells after treatment for 72 hours with Control or *NUP160* shRNAs (right, n = 6-7 cells) **B**, Schematic illustrations of experimental approach to evaluate 2xGFP diffusion into the nucleus (left). Normalized nuclear diffusion analysis of 2xGFP protein in RPE1 cells after treatment for 72 hours with Control or *NUP160* shRNAs (right, n = 9-11 cells). **C**, Schematic illustrations of experimental approach to evaluate fluorescent dextran

leakage into the nucleus (top). Representative images (bottom left) and quantified analysis (bottom middle) of RPE1 cells were treated for 72 hours with Control or *NUP160* siRNAs, permeabilized, and mixed with fluorescent dextran mixture (n = 40-43 cells). After the nuclear permeability assays cells were fixed and stained with mAb414 and NUP358 to confirm the inhibition of NPC assembly during treatment (bottom right). Data are mean ± s.e.m (A, B) or mean ± s.d. (C). Experiments are representative of 3 independent repeats.

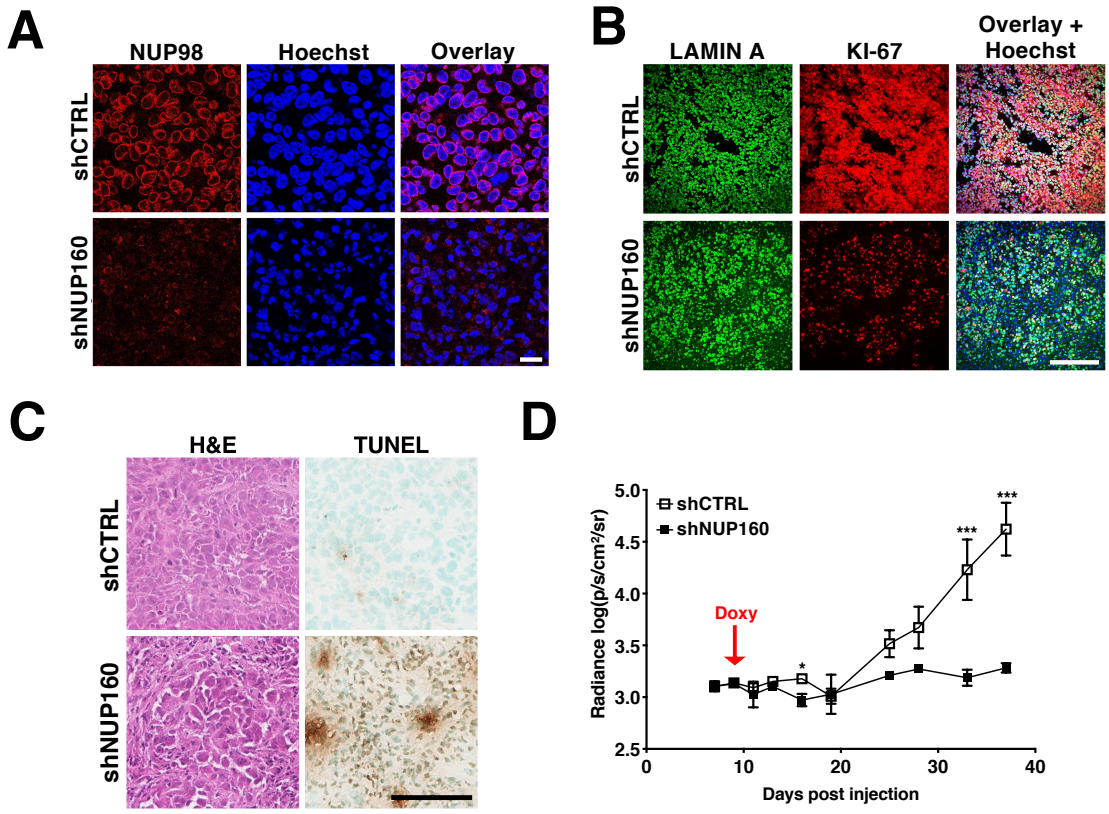


Supplementary Figure 8. Inhibition of NPC formation selectively increases DNA damage in A375 cells. **A**, Western blot analysis of DNA damage response proteins in untreated RPE1 and A375 cells. Representative of 4 independent experiments. **B**, Western blot analysis of DNA damage response proteins in A375 cells after 4 days after Control, *NUP160* or *NUP93* shRNA induction. Representative of 4 independent experiments. **C**, Immunofluorescence analysis of mAb414 and γH2AX (Ser139, Thy142) in RPE1 and A375 cells 96 hours after Control or *NUP160* shRNA induction.

Representative images from 3 independent experiments show the maximum projection of entire nuclei. **D**, Quantification of cells with ≥5 γH2AX foci from (C) (n = 26-79 cells). **E**, Immunofluorescence analysis of mAb414, γH2AX (Ser139, Thy142), and 53BP1 in A375 cells 4 days after Control or *NUP160* shRNA induction. Representative images from 3 independent experiments show the maximum projection of entire nuclei. **F**, Immunofluorescence analysis of mAb414, γH2AX (Ser139, Thy142), and 53BP1 in A375 cells 4 days after Control or *NUP160* shRNA induction. Representative images from 3 independent experiments show nuclear cross-sections. Arrows indicate γH2AX/53BP1 foci at the nuclear periphery. **G,** RPE1 and H3A cells were treated with doxycycline to express Control or *NUP160* shRNAs for 6 days and released for 4 days to shut down shRNA expression. At the end of the experiment's cells were stained and the number of γH2AX foci/cell was quantified (n = 26-31 cells). Data are mean ± s.e.m. * P ≤ 0.05 by unpaired Student's t tests. Experiments are representative of 3-5 independent repeats.



Supplementary Figure 9. Inhibition of NPC results in multiple cellular alterations. **A**, qPCR analysis of *CHK1* mRNA levels normalized to *GAPDH* in RPE1 and A375 cells 4 days after Control or *NUP160* shRNA induction. Representative of 2 independent experiments, **B**, Cell confluence measurements of A375 cells treated for 72 hours with CHK1 inhibitor (CCT245737) or ATR inhibitor (VE-821) and Control or *NUP160* shRNA as assessed by Celigo cell cytometer. Cell confluency measurements are representative of two independent experiments with eight technical replicates. **C**, Immunofluorescence analysis of MAD1 and NUP358 proteins in RPE1 and A375 cells 72 hours days after Control or *NUP160* shRNA induction. Unless otherwise stated experiments are representative of 3-5 independent experiments.



Supplementary Figure 10. Inhibition of NPC assembly induces cell death and inhibits tumor growth. **A,** Representative images of NUP98 staining in sections from Control or *NUP160* shRNA A375 tumors at the final time point from Figure 7. Scale bar, 25 µm. **B,** Representative images of LAMIN A and KI-67 staining in sections from Control or *NUP160* shRNA A375 tumors at the final time point from Figure 7. Scale bar, 100 µm. **C**, Representative images of Hematoxylin & Eosin (H&E) and Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining of sections from Control or *NUP160* shRNA A375 tumors at the final time point from Figure 7. Scale bar, 100 µm. **D**, Mice were injected with MOLM-13-luciferase cells expressing inducible Control or *NUP160* shRNAs. 9 days after injection Control and *NUP160* shRNAs were induced with doxycycline and in vivo luciferase activity was quantified over time (n = 3-5 mice). Data are mean ± s.e.m. *** P ≤ 0.001 by Two-way analysis of variance (ANOVA) of log normalized values. Images A-C are representative of the staining of 2 independent tumors.

Supplementary Movie. Brightfield live imaging analysis of RPE1 and A375 cells starting immediately after Control or *NUP160* shRNA induction. Frames separated by 20 minutes. Scale bar, 100 μm. Movie is representative of 3 independent experiments.

SUPPLEMENTARY METHODS

Viral transduction

Virus was prepared by the Sanford Burnham Prebys Viral Vectors Core. Cells were transduced with Tripz tetracycline-inducible shRNA lentivirus (Control RHS4743, NUP160 clone V2THS_101965, or NUP93 clone V2THS_259822 from Dharmacon), followed 2 days later by selection with 1.25 µg/ml Puromycin (Gibco). Expression of shRNA was induced with 200 ng/ml doxycycline (Clontech). MOLM-13 cells were also transduced with Firefly Luciferase virus (GenTarget, LVP568) and selected with 10 µg/mL Blasticidin (Gibco). H3A cells were sorted by Fluorescence Activated Cell Sorting (FACS) for shRNA positive cells. Cells were single cell cloned to attain cells with homogeneous expression and different clones were tested to confirmed phenotypes.

siRNA transfection

Cells were transfected with Lipofectamine RNAiMAX Transfection Reagent (Life Technologies) according to manufacturer's protocol with siRNAs (GE Dharmacon) and grown for 3 days before immunofluorescent staining. Primary human myoblasts were grown for 4 days with growth or differentiation media. For the nucleoporin screen, A375 cells were transfected with 10nM siRNA pools (Horizon Discovery SMARTpool siRNA library) for individual nucleoporins in 384-well plates by SBP Functional Genomics Core. Cells were fixed 48 hours later with 4% paraformaldehyde (PFA) and stained with the mAb414 antibody as described below. Imaging was performed at SBP Conrad Prebys Center for Chemical Genomics on an Opera Phenix (PerkinElmer) confocal high content screening system using a 40x 1.1NA water immersion objective. Acquired images were analyzed using Harmony HCS software (PerkinElmer). The main assay parameter was a linear-discriminant analysis (LDA) weighted average of the mAb414 staining intensities of the nuclear envelope and a cytoplasmic ring region. LDA weights were trained using negative (0.1% DMSO-vehicle) and positive (Doxy-treated shNUP160 expressing cells) controls, resulting in a single assay readout that is representative of the NPC inhibition phenotype of decreasing nuclear envelope staining intensity. siRNA catalog numbers: NUP160 # L-029990-01; NUP98 # L-013078-00; NUP205 # L-010646-00; NUP93 # L-020767-01; NUP107 # L-020440-00; NUP155 # L-011967-01; NUP62 # L-012468-00; NUP133 # L-013322-01; NUP85 # L-014478-00; RANBP2 # L-004746-00; Sec13 # L-012351-00; Seh1l # L-013475-01; NUP153 # L-005283-00; NUP43 # L-018906-01; NUP35 # L-018998-01; NUP54 # L-017570-01; AAAS # L-017636-00; NUP214 # L-011980-00; NUP88 # L-017547-01; Elys # L-013961-00; NUP58 # L-013864-01; NUP188 # L-032297-01; NUP50 # L-012369-01; NUP210 # L-017529-01; NUP37 # L-014282-01; CG1 # L-010331-00; RAE1 L-011482-01; Tpr # L-010548-00

Antibodies

The following primary antibodies were used: mAb414 (BioLegend, #902901); anti-NUP358 (Bethyl Laboratories, #A301-796A); MYH1E (Clone MF20, Developmental Studies Hybridoma Bank) NUP98 (Clone C39A3, Cell Signaling Technology, #13393S); NUP93 (Clone F2, Santa Cruz Biotechnology, #sc-374400); LAMIN A (Sigma Aldrich, L1293); NUP160 (Sigma Aldrich, #SAB4500969); 53BP1 (Cell Signaling Technology, #49375); Phospho-Histone H2A.X (Ser139) (Clone 20E3, Cell Signaling Technologies, #9718); Phospho-Histone H2A.X (Ser139/Tyr142) (Cell Signaling Technology, #5438); Histone H2A.X (Cell Signaling Technology, #7631); Phospho-ATR (Ser428) (Cell Signaling Technology, #2853); ATR (Cell Signaling Technology, #13934); Phospho-

CHK1 (Ser345) (Cell Signaling Technology, #2348); CHK1 (Cell Signaling Technology, #2360); mTOR (Cell Signaling Technology, #2983); Phospho-mTOR (Ser 2448) (Santa Cruz Biotechnology, #sc-293133,); Phospho-S6 Ribosomal Protein (Ser235/236) (Cell Signaling Technology, #4858); HSP90 (R&D Systems, #341320); Mad1 (Clone 9B10, Santa Cruz Biotechnology, #sc-47746); and Ki-67 (Clone 11F6, BioLegend, #151202). The following secondary antibodies from Life Technologies were used: anti-mouse Alexa Fluor 488 (#A21202); anti-mouse Alexa Fluor 546 (#A21570); anti-mouse Alexa Fluor 647 (#A21571); anti-rabbit Alexa Fluor 488 (#A21206); anti-rabbit Alexa Fluor 555 (A31572); and anti-rabbit Alexa Fluor 647 (#A31573). Senescence β-Galactosidase Staining Kit (Cell Signaling Technologies, #9860) was used according to manufacturer's directions. All images were acquired on a Leica SP8 confocal microscope.

RNA sequencing

RNA sequencing was performed as previously described (1). A total of four technical replicates were used. RNA extraction was performed using the RNeasy Plus Micro Kit (QIAGEN). The following steps were performed by the Sanford Burnham Prebys Genomic Core. RNA integrity was confirmed using the Agilent 2100 Bioanalyzer (Agilent Technologies). PolyA RNA was isolated using the NEBNext® Poly(A) mRNA Magnetic Isolation Module and barcoded libraries were made using the NEBNext® Ultra™ Directional RNA Library Prep Kit for Illumina® (NEB, Ipswich MA). Libraries were pooled and single end sequenced (1X75) on the Illumina NextSeq 500 using the High output V2 kit (Illumina Inc., San Diego CA). Read data was processed in BaseSpace (basespace.illumina.com). Reads were aligned to Homo sapiens genome (hg19) using the BaseSpace RNA-Seq Alignment app v1.1 app and Tophat2 aligner (http://tophat.cbcb.umd.edu/) with default settings. Differential transcript expression was determined using the Cufflinks Cuffdiff package (http://cufflinks.cbcb.umd.edu/).

qPCR

Total RNA was extracted from cells using the PureLink RNA Mini kit (Thermo Fisher Scientific), and cDNA was synthesized using the QuantiTect Reverse Transcription Kit (QIAGEN). qPCR was carried out using SYBR Green (ThermoFisher Scientific) or TaqMan (ThermoFisher Scientific). For H3A and H9 cells total RNA was extracted and cDNA was synthesized using TaqMan Fast Advanced Cells-to-CT Kit (Thermo Fisher Scientific) according to manufacturer directions. qPCR data were collected in a CFX384 Real-Time PCR Detection System (Bio-Rad). The following primers were used to detect human transcripts: CHK1 5'-TCTCAGACTTTGGCTTGGCA-3' (forward) and 5'-TCCCATGGCAATTCTCCAGC-3' (reverse); NUP160 5'-CCATGTGCTTCTGGGGGAAT-3' (forward) and 5'-CTGGTCACCCCTGATAGCTG-3' (reverse); CDKN1A (p21): 5'-AGGTGGACCTGGAGACTCTCAG-3' (forward) and 5'-TCCTCTTGGAGAAGATCAGCCG-3' (reverse); GAPDH: 5'-GACAGTCAGCCGCATCTTCT-3' (forward) 5'-ACCAAATCCGTTGACTCCGA-3' (reverse). In addition, the following TaqMan® Gene Expression Assays were used to detect human transcripts: NUP160 (Hs00299567_m1), NUP93 (Hs00206418_m1), and GAPDH (Hs03929097_g1).

Western blotting

For protein extracts, cells were washed with ice-cold PBS containing Protease and Phosphatase inhibitors (Pierce Halt Protease and Phosphatase Inhibitor Cocktail, ThermoFisher Scientific) and harvested with RIPA buffer containing Protease and

Phosphatase inhibitors. Homogenates were incubated on ice for 30 min and passed 5-10 times though a 29 G syringe to shear the DNA. Protein concentration was determined using the Pierce BCA reagent (ThermoFisher Scientific). LDS Sample Buffer premixed with NUPAGE® Sample Reducing Agent (Life Technologies) was added, and samples were incubated for 10 minutes at 70°C. For western blot analysis, 20 or 40 µg of protein were resolved by SDS-PAGE on NUPAGE Novex 3-8% tris-acetate protein gels or Bolt 4-12% Bis-Tris Plus protein gels (Invitrogen) and blotted to nitrocellulose membranes using an iBlot2 Dry Blotting System. Membranes were stained with Ponceau, washed with TBS, and blocked for 1 hour at RT with TBS-0.1% Tween 20 (TBS-T) containing 5% BSA (for phospho-antibodies) or 5% nonfat milk, and incubated with primary antibodies overnight at 4°C. After three washes with TBS-T, the secondary antibody was added and incubated for 1 hour at RT. Membranes were then washed and developed using the Pierce ECL Western Blotting Substrate (ThermoFisher Scientific) or SuperSignal West Pico Plus Chemiluminescent Substrate (ThermoFisher Scientific).

1. Borlido J, Sakuma S, Raices M, Carrette F, Tinoco R, Bradley LM*, et al.* Nuclear pore complex-mediated modulation of TCR signaling is required for naive CD4(+) T cell homeostasis. Nat Immunol **2018**;19(6):594-605 doi 10.1038/s41590-018-0103-5.