Supplemental Information contains:

1. Figure S1A, B. Related to Figure 1
2. Figure S2A-H. Related to Figure 2
3. Figure S3A-E. Related to Figure 3
4. Legends to Video 1, 2. Related to Figure 3
5. Figure S4A, B. Related to Figure 4
6. Figure S5A-C. Related to Figure 5
7. Figure S6. Related to Figure 6
8. Schematic of nuclear to lysosome DNA clearance process
9. Supplemental experimental procedures

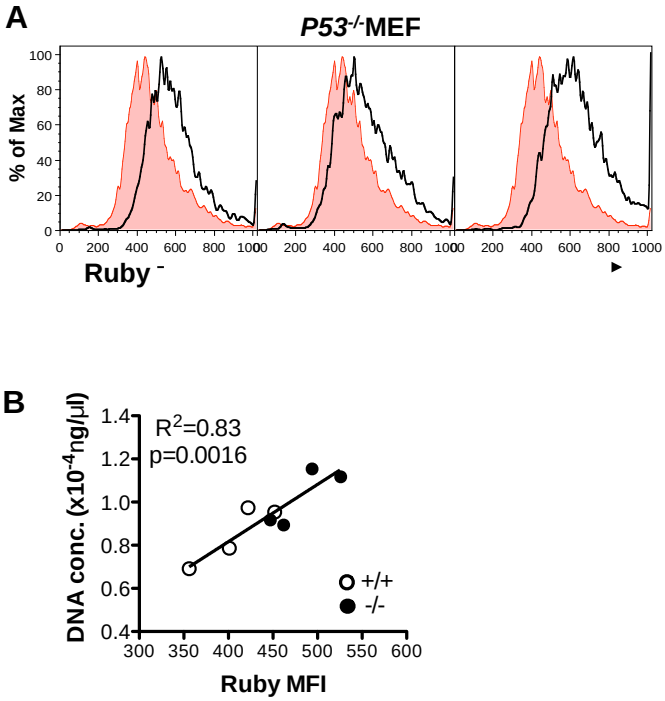

Figure S1. Related to Figure 1

(A) Ruby stain showing DNA content of *p53*$^{-/-}$MEFs with *Dnase2a* silenced by lentiviral-transduced shRNAs (open histogram), non-target shGFP acts as control (closed histogram). Results of 3 different *Dnase2a* shRNA constructs are shown.

(B) Correlation of DNA concentration of purified genomic DNA from *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ MLFs (per cell) and Ruby stain by flow cytometry.

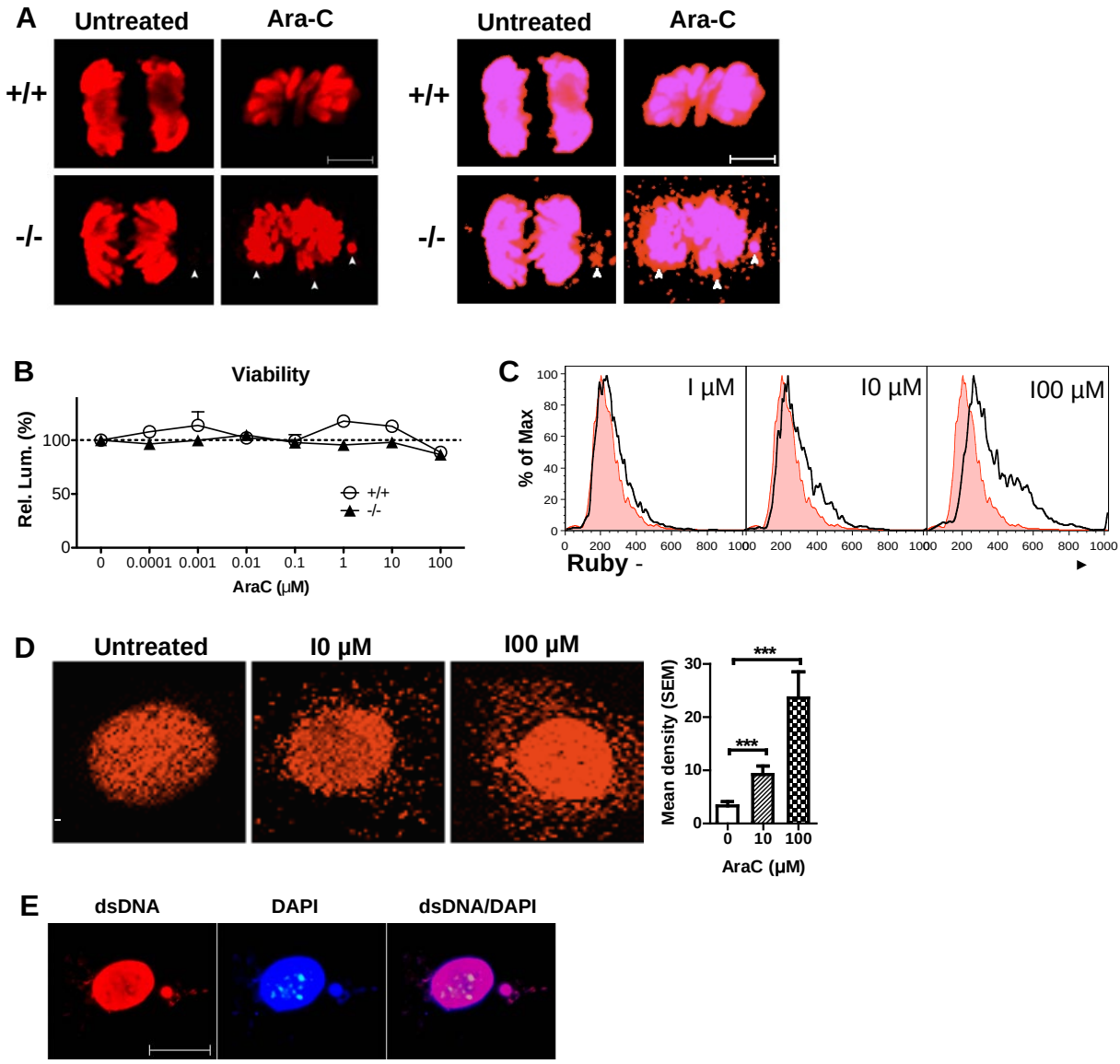

Figure S2. Related to Figure 2

(A) Immunostaining of anti-dsDNA antibodies in dividing wild-type and *Dnase2a*$^{-/-}$ cells, scale bar 10 μm. Left panel, normal intensity with arrow-head highlighting extra-nuclear DNA; right panel, image with enhanced brightness for visualization.

(B) Viability of *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ MLFs examined by CellTitre-glo luminescent assay after different doses of Ara-C treatment for 24 h.

(C) DNA content by Ruby stain of *p53*-deficient MEFs treated with Ara-C as indicated; untreated (closed histogram), treated (open).

(D) The same cells examined by anti-dsDNA immunostaining and its quantitation as mean intensity; scale bar 10 μm.

(E) Anti-dsDNA staining matched with DAPI stain in acetone-fixed Ara-C treated MEFs; scale bar, 10 μm.

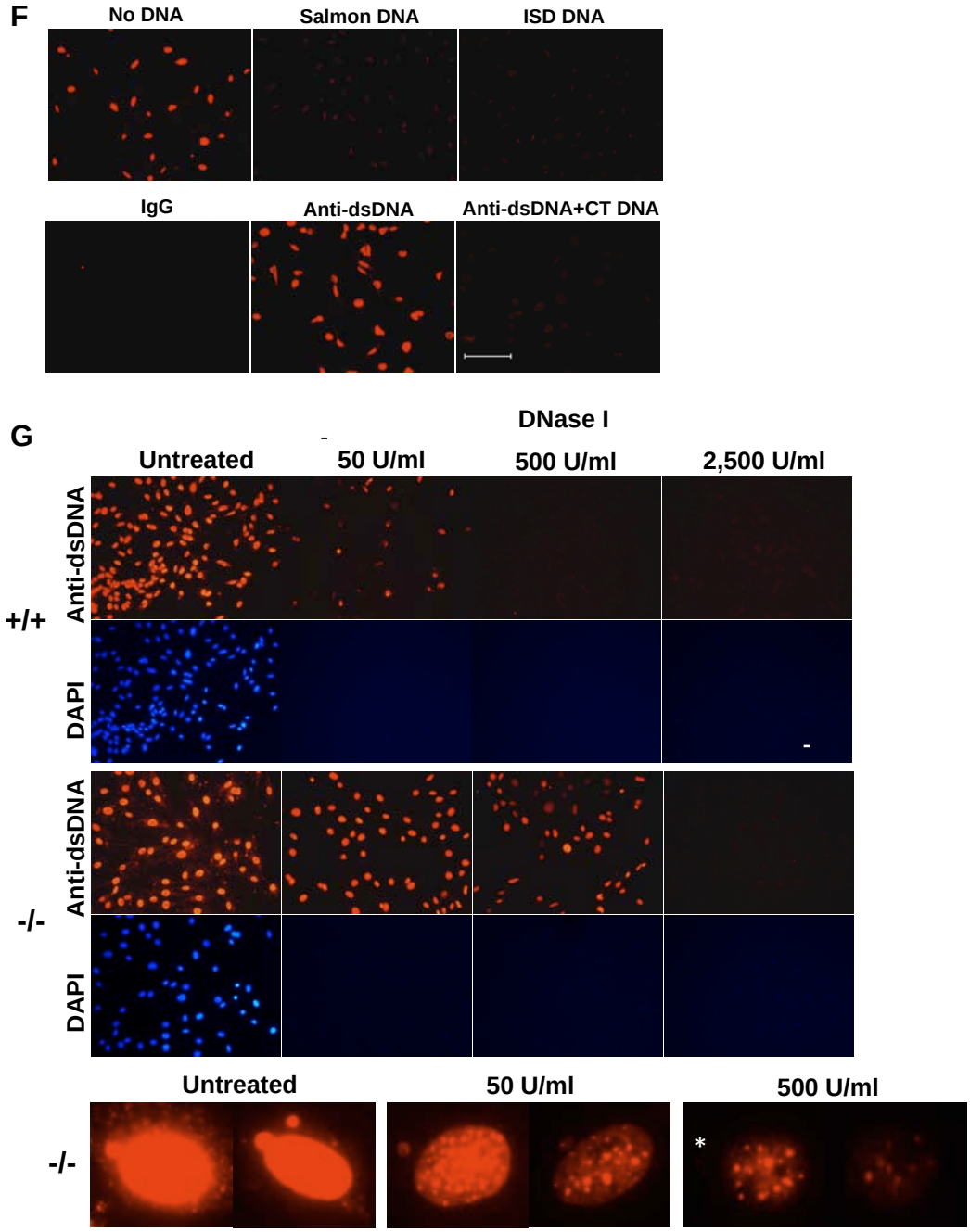

Figure S2 continued. Related to Figure 2

(F) Upper panel, *Dnase2a* KO MLFs treated with Ara-C were stained with anti-dsDNA antibody (red) in the presence of competing DNA indicated. Lower panel, Ara-C treated Dnase2a KD MEFs stained with isotype control, anti-dsDNA antibody with or without DNA. Salmon DNA (10 µg/ml), ISD (5 µg/ml) or calf thymus DNA (10 µg/ml), was incubated with primary antibody for 15 min at room temperature before overnight incubation at 4°C. Scale bar, 50 µm.

(G) *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ MLFs were fixed and permeabilized (0.1% Triton), then treated for 15 min with DNase I at 3 concentrations. DAPI staining (blue) was eliminated at the lowest concentration of DNase I while anti-dsDNA staining (red) was eliminated at higher concentrations. Cells were intact even at the highest concentration of DNase I. Scale bar, 50 µm. Bottom panel shows disappearance of extra-nuclear and nuclear DNA in *Dnase2a*$^{-/-}$ MLFs at 60X. Asterisk marks a faintly visible extra-nuclear DNA piece.

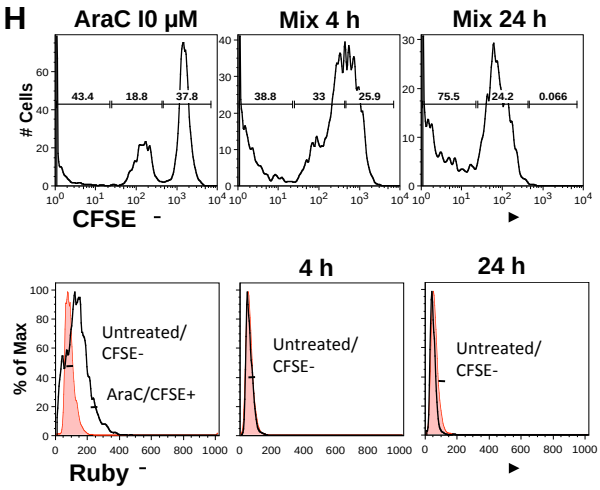

Figure S2 continued. Related to Figure 2

(H) Upper panel: Ara-C treated cells were CFSE labeled (left). Ara-C treated CFSE+ cells were mixed with untreated CFSE- cells for 4h (middle) or 24 h (right). Lower panel: Ara-C/ CFSE+ and untreated/CFSE- cells were stained for DNA content with Ruby before mixing (left). Ara-C treated CFSE+ cells were mixed with untreated CFSE- cells for 4h (middle) or 24 h (right); only CFSE- cells are shown. DNA levels (by Ruby) did not increase in CFSE- cells, suggesting there is no significant uptake of DNA from Ara-C/CFSE+ cellular material.

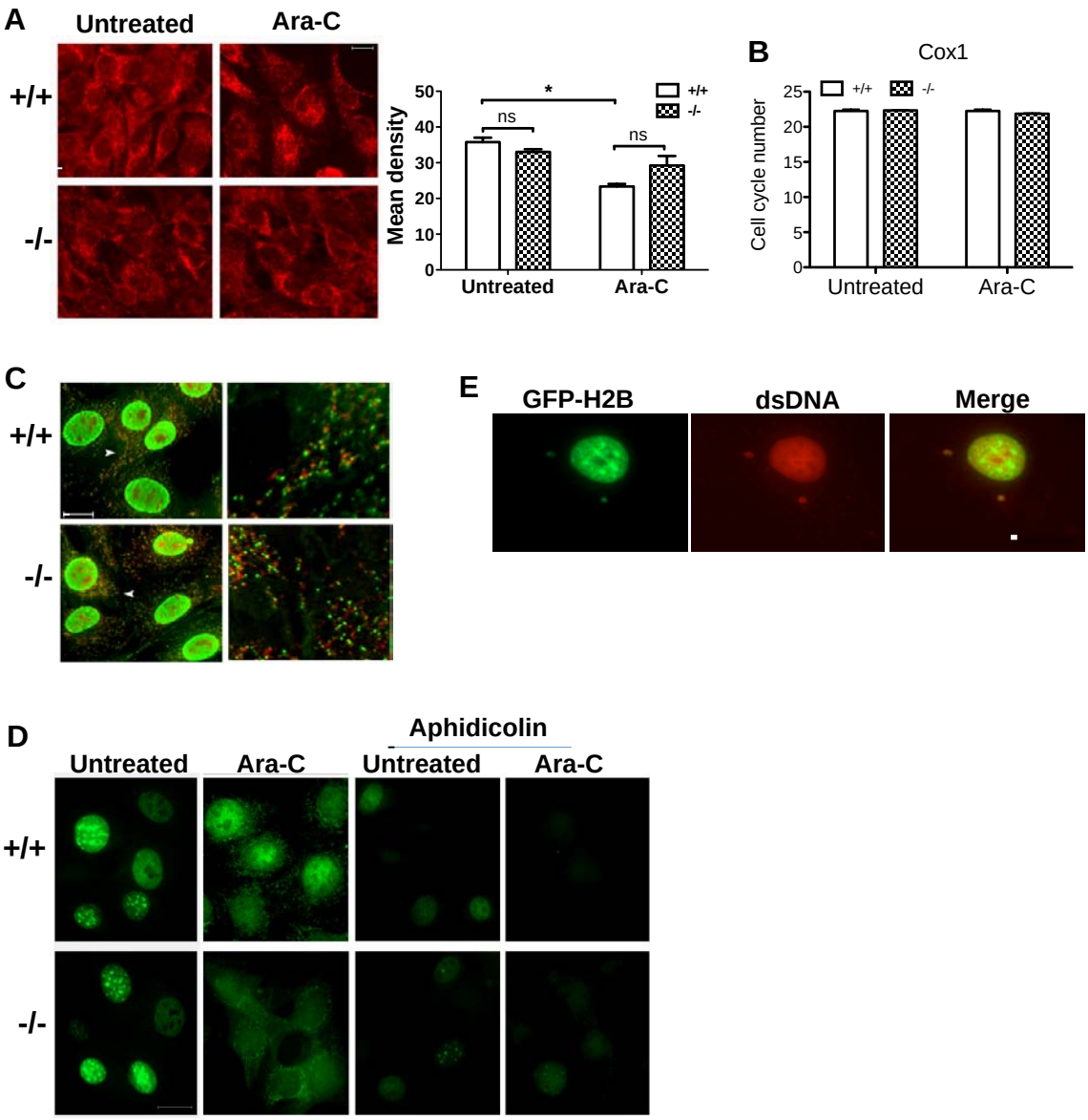

Figure 3. Related to Figure 3

(A) Staining of Mitotracker in *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ MLFs with quantitation; scale bar, 20 μm.

(B) Expression of mitochondrial protein *Cox1* in *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ MLFs, cytosolic portion of the same number of cells is assessed by qPCR.

(C) Co-staining of dsDNA (green) and Mitotracker (red) in Ara-C treated MLFs; scale bar, 10 μm, enlarged images on right show little co-localization.

(D) *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ MLFs are pulsed with BrdU (15 μM) and treated with aphidicolin and Ara-C (both at 10 μM) for 6 h as indicated, then stained for anti-BrdU (green); scale bar, 20 μm.

(E) Double-staining of GFP-H2B and dsDNA (red) in MLFs, scale bar, 20 μm.

Legends to Video 1 and 2. Related to Figure 3

Time-lapse live imaging of wild-type or *Dnase2a*$^{-/-}$ MLFs is captured by a spinning disc microscope after Ara-C treatment during 0-6 h. Each movie is set for 2 h at 630X, each frame is acquired every 3-5 min. While *Dnase2a*$^{-/-}$ MLFs accumulates more DNA, similar spatiotemporal changes of DNA are also observed in wild-type cells with Ara-C.

Video 1: Formation of nuclear bud in wild-type MLF, 0-2 h.

Video 2: Detachment of nuclear DNA to the cytosol in *Dnase2a*$^{-/-}$ MLF, 4-6 h.

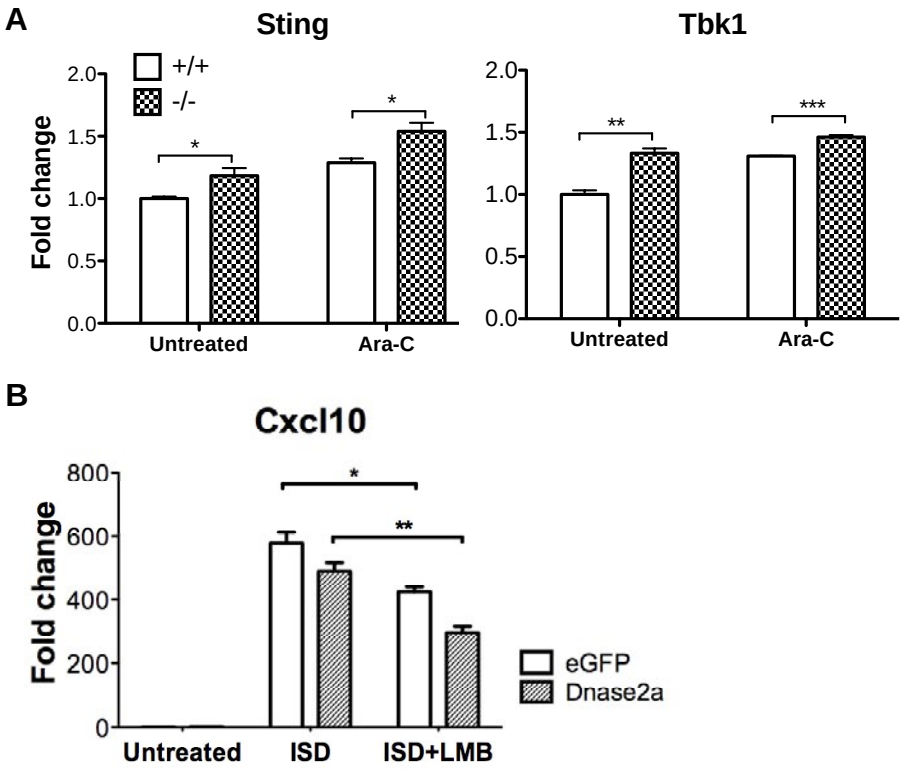

Figure S4. Related to Figure 4

(A) *Sting* and *Tbk1* expression in *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ MLFs, untreated or treated with Ara-C.

(B) Dnase2a KO MLFs, rescued with Dnase2a or infected with eGFP ORF as a negative control, are transfected with dsDNA (ISD=45 bp dsDNA, 1 µg/ml) complexed with lipofectamine. Levels of *Cxcl10* are measured in the presence or absence of leptomycin (LMB).

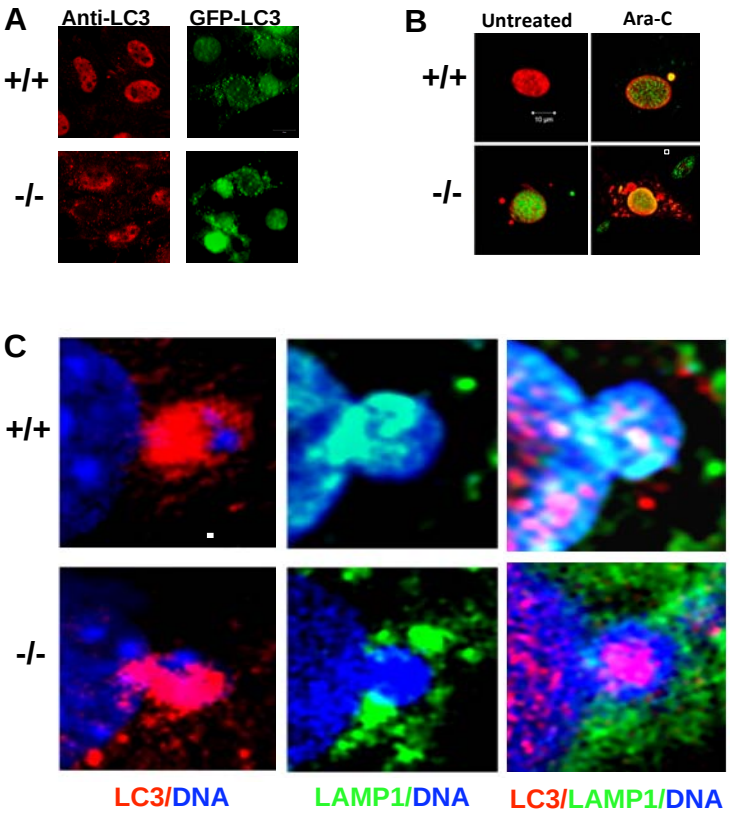

Figure S5. Related to Figure 5

(A) Similar nuclear and cytosolic punctate pattern of anti-LC3 staining (red, left panel) and GFP-LC3 (green, right) in Ara-C treated MLFs; scale bar, 20 µm.

(B) In GFP-LC3-expressing MLFs, GFP-LC3 (green) co-localizes with extra-nuclear dsDNA (red), as single aggregate in *Dnase2a*$^{+/+}$ MLFs and in enlarged image in *Dnase2a*$^{-/-}$ cells.

(C) Three-color confocal imaging of dsDNA (blue), LC3 (red) and LAMP1 (green) in *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ MLFs after Ara-C treatment, showing co-localization of LC3 and LAMP1 with dsDNA in nuclear bud of wild-type, but LC3 does not co-localize with LAMP1 in *Dnase2*$^{-/-}$ cells; scale bar, 2 µm.

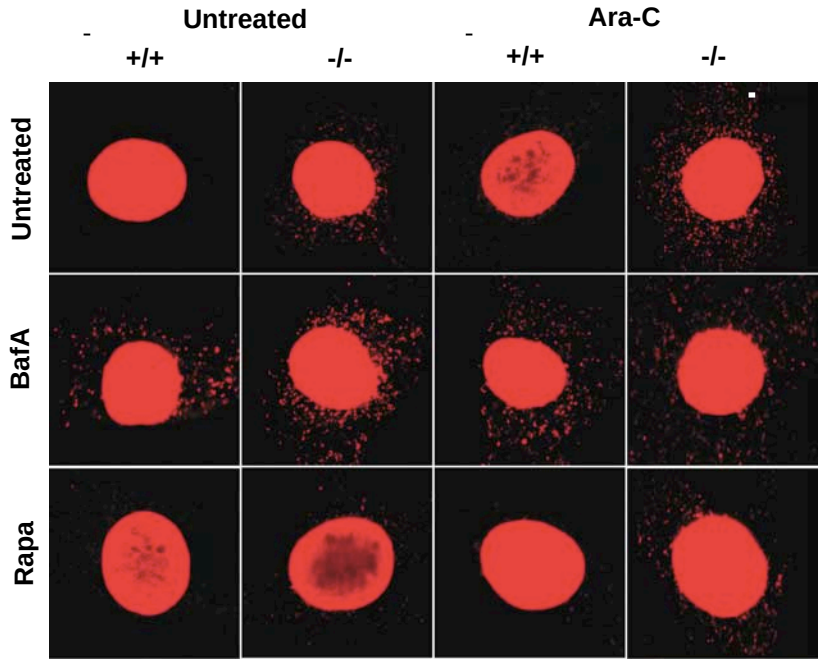

Figure S6. Related to Figure 6
Anti-dsDNA immunostaining in *Dnase2a*$^{+/+}$ and *Dnase2a*$^{-/-}$ MLFs after treatment with bafilomycin (200 nM, 8 h) or rapamycin (100 nM, 1 h) in Ara-C treated or untreated cells; scale bar, 10 µm.

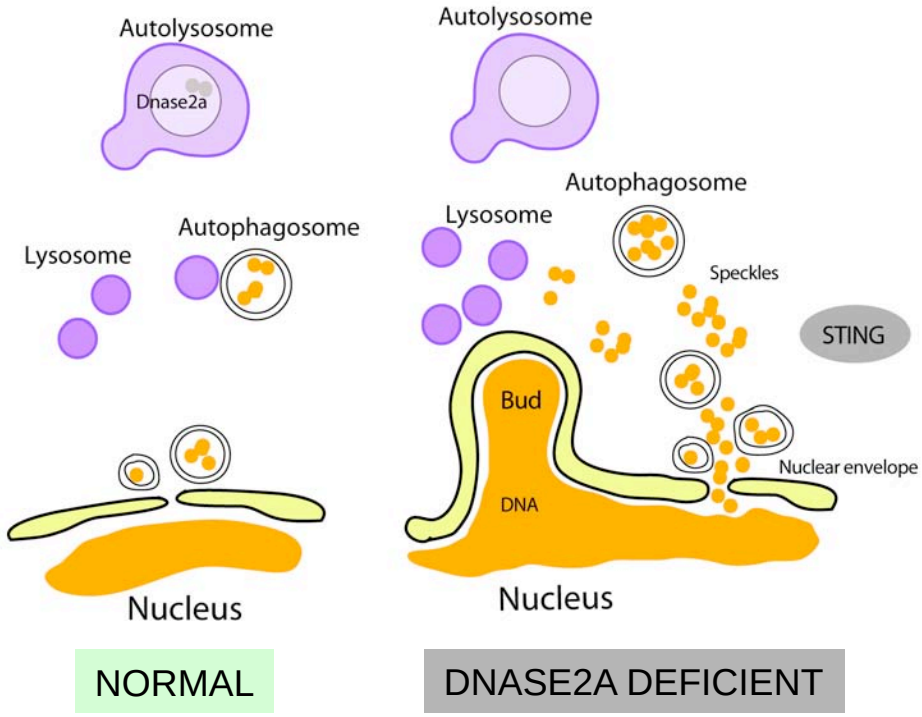

Figure S7
Schematic of the autonomous nuclear-to-lysosome DNA clearance process. Damaged nuclear DNA is exported from the nucleus, enclosed by autophagosomes and targeted to lysosomes to be degraded by Dnase2a. Defects in degradation (*Dnase2a* deficiency) or autophagy (e.g. *Atg5*$^{-/-}$) result in extra-nuclear DNA accumulation that engages a cytosolic *Sting*-dependent pathway to elicit cytokines and chemokines.

Supplemental Experimental Procedures

Real-time RT-qPCR

Total RNA was extracted using the RNeasy® Plus Mini Kit (Qiagen) and reverse-transcribed with the High Capacity cDNA Reverse Transcription kit (Applied Biosystems). Quantitative PCR was performed using the Lightcyler® 480 SYBR Master I reagents (Roche) on the Stratagene MX4000 instrument. Levels of mRNA were normalized to *β-actin* or *Gapdh*. Primer pairs used are:
Atg5-F, AGCCAGGTGATGATTCACGG,
Atg5-R, GGCTGGGGGACAATGCTAA;
Atg7-F, GTTCGCCCCCTTTAATAGTGC,
Atg7-R, TGAACTCCAACGTCAAGCGG;
Becn1-F, ATGGAGGGGTCTAAGGCGTC,
Becn1-R, TCCTCTCCTGAGTTAGCCTCT;
Cxcl10-F, CCAAGTGCTGCCGTCATTTTC,
Cxcl10-R, GGCTCGCAGGGATGATTTCAA;
Dnase2a-F, GCTCAGCTGGGGACTCTAC,
Dnase2a-R, GGTCTGGCCGAAGGTTTGA;
Gapdh-F, AGGTCGGTGTGAACGGATTTG,
Gapdh-R, TGTAGACCATGTAGTTGAGGTCA;
Sting-F, GGTCACCGCTCCAAATATGTAG,
Sting-R, CAGTAGTCCAAGTTCGTGCGA;
Tbk1-F, ACTGGTGATCTCTATGCTGTCA,
Tbk1-R, TTCTGGAAGTCCATACGCATTG
β-actin-F, GGCTGTATTCCCCTCCATCG,
β-actin-R, CCAGTTGGTAACAATGCCATGT.

Cell synchronization

Cells were subject to double thymidine block at 2.5 mM. First block was 18 h, with 8 h release, then second block for 12 h to arrest in S phase. Cells were then pulsed with BrdU (10 μM) for indicated times. Alternatively, cells were treated with nocodazole at 100 ng/ml for 16 h.

DNA quantitation by immunofluorescence

Quantification of nuclear and cytosolic DNA was performed using ImageJ. Ten 40X images (~100 cells per image) were analyzed for each phenotype and treatment condition. In each image, a binary image mask was created to define regions of interest (ROI). For nuclear ROI, a median filter was applied to normalize noise and positive signals to a median of 250 data points in each image. An Automatic threshold was then set to include all fluorescence data above background. Using the image calculator, nuclear ROI was subtracted from all fluorescence data to define cytoplasmic ROI. Quantitative fluorescence data was presented using mean (±SEM) of staining intensity.

Tissue histological analysis

Tissues were fixed overnight in 4% PFA at 4°C, then paraffin-embedded or cryo-preserved in Tissue-Tek OCT compound after 30% sucrose dehydration. Joints were decalcified in 20% EDTA in PBS (pH 7.4) for 6 weeks. H&E staining was prepared by standard technique. For immunohistochemistry, sodium citrate was used for antigen retrieval, 3% H_2O_2/methanol (20 min) to reduce endogenous peroxidase, and 1X Biogenex (15 min) for blocking. Primary antibodies incubated overnight were rabbit polyclonal LC3 at 10 mg/ml and γH2AX at 1:50 (Novus Biological, NB100-2331 and NB100-79976), and biotinylated-CD107a at 0.5mg/ml (BioLegend, 121604). Secondary reagents were anti-rabbit antibodies (20 min; Biogenex, HK326-UR) and tertiary was streptavidin horseradish peroxidase (20 min; Biogenex, HK-320-UK). Sections were then incubated in 3, 3-diaminobenzidine tetrahydrochloride (DAB, 0.24% H_2O_2, 5 min), counterstained with hematoxylin, mounted in Permount and imaged with Nikon eclipse ME6000 microscope using Nikon digital camera DXM1200C. Rabbit IgG (Sigma) was negative control.

Electron microscopy and immunogold labeling

Cells were fixed in 4% paraformaldehyde with 0.1% glutaraldehyde (Electron Microscopy Sciences) in 0.1 M sodium cacodylate buffer, pH 7.4, rinsed in PBS, then scraped and pelletted. The pellet was resuspended in 2% agarose and cut to small blocks protected by sucrose (2.3 M). Ultra-thin cryosections were cut on a Leica EM FCS at -80°C and collected on formvar-coated nickel grids. Grids were blocked in 5% normal goat serum and 1% BSA, incubated with primary antibody (mouse-anti-dsDNA, 1:100, Dako diluent, Dako Corp) for 1 h, washed, and then with goat-anti-mouse IgG gold-conjugated secondary antibody (15 nm, Ted Pella) for 1 h. Grids were then washed, floated on tylose and uranyl acetate and dried. They were examined at 80 kV in a JEOL 1011 TEM. Images were acquired using an AMT digital imaging system (Advanced Microscopy Techniques).