Extended Experimental Procedures

Cells, culturing conditions and viral infections. Primary human small airway epithelial cells (SAECs) were obtained from Lonza, cultured in Small Airway Growth Medium (SAGM) without gentamycin at 3% O_2 as described (Ou et al., 2012; Soria et al., 2010). Primary SAECs were cultured no more than 4 passages. hTERT-SAECs were generated by transducing primary SAECs with a lentivirus expressing hTERT and were cultured to no more than 10 passages. SAECs were cultured to confluence and then maintained for 10 days to promote quiescence prior to infection. A549 cell lines were cultured in DMEM supplemented with 10% heat-inactivated FBS without antibiotics. For infection, A549 cells were seeded at a concentration of 6.4×10^4 cells/cm^2 for 2 hours and infected in 2% FBS DMEM. Infections were carried out at a multiplicity of infection (MOI) of 10 plaque forming units (PFU) per cell for both SAEC and A549. Infections were performed at minimal experimentally determined MOIs to exclude possible artifacts arising from superinfection. Infection media was removed 2 to 4 h.p.i. and replaced with complete growth media.

Drugs. DNA damage was induced by treating cells with 0.5 μg/ml doxorubicin (Sigma) or 30 μg/ml etoposide (Sigma) for 12 hours, unless otherwise indicated. HU inhibits the synthesis of deoxynucleotide precursors and prevents both cellular and viral DNA replication. At low concentrations, aphidicolin inhibits cellular DNA replication by eukaryotic DNA polymerases α and γ, but not viral DNA replication by Adenovirus E2B polymerase (Foster et al., 1982; Kwant and van der Vliet, 1980).

Viruses and viral titer assay. All viruses were grown and titered on 293/E4/pIX complementing cell-lines (O'Shea et al., 2004). To measure total virus yields, cells and culture supernatants were collected and subjected to titering as described previously (O'Shea et al., 2004). Mock infection was performed with a ΔE1 virus (designated AdSyn-CO117), an E1 and E4-ORF3 deleted non-replicating adenovirus expressing GFP under the control of the CMV promoter. The deletion of E4-ORF3 prevents E1A independent expression of E4-ORF3 that could inactivate MRN. Wild-type virus is Ad5. The ΔE1B-55K virus (AdSyn-CO124) was created by mutating the start codon of E1B-55K (ATG to GTG) and I90 of E1B-55K to a stop codon (ATT to TAG). The ΔE4-ORF3 virus (AdSyn-CO118) was created by deleting the coding region of E4-ORF3. The ΔE1B-55K/ΔE4-ORF3 virus (AdSyn-CO140) is the same as ΔE1B-55K except the E4-ORF3 coding region is deleted.

Previous studies have used hybrid Ad2/5 viruses (Shepard and Ornelles, 2004) or viruses that are deleted for multiple E4 genes (E4-ORF1, E4-ORF2, E4-ORF3, E4-ORF3/4, E4-ORF4, E4-ORF6, E4-ORF6/7) (Carson et al., 2009; Carson et al., 2003; Gautam and Bridge, 2013; Lakdawala et al., 2008; Mathew and Bridge, 2007, 2008)., Similar conclusions to the AdSyn series of viruses were obtained were obtained the previously described E1 deleted virus *dl*312 (Jones and Shenk, 1979), wild-type virus WtD (Barker and Berk, 1987), ΔE1B-55K virus *dl*1520/ONYX-015 (Barker and Berk, 1987; Bischoff et al., 1996) and ΔE1B-55K/ΔE4-ORF3 virus *dl*3112 (Shepard and Ornelles, 2003).

Immunofluorescence. Cells were fixed in 4% paraformaldehyde/$PBS^{-/-}$ for 30 minutes at room temperature, permeabilized in 0.5% TritonX-100/$PBS^{-/-}$, and stained as described previously (O'Shea et al., 2004; O'Shea et al., 2005). ΔE1 virus infected cells were identified through GFP

fluorescence. ΔE1B-55K/ΔE4-ORF3 infected cells were identified using E2A co-staining. For MRE11 and NBS1 staining in SAECs, cells were pre-permeabilized (20 mM HEPES-KOH pH 7.9, 50 mM NaCl, 3 mM $MgCl_2$, 300 mM sucrose, 0.5% TritonX-100) for 5 minutes at room temperature prior to fixing. For NBS1 and MDC1 staining in A549 cells, cells were fixed directly in ice-cold methanol for 15 minutes. Primary antibodies are described in Table S1. Alexa 488-, 555- and 633-conjugated secondary antibodies (Molecular Probes) were used for detection of primary antibodies. Hoechst-33342 was used to counter-stain DNA, and nucleus borders were outlined using Adobe Photoshop. Images were acquired with a Zeiss LSM780 imaging system with a 63× objective. For analyzing E2A viral replication centers in SAECs, nascent viral replication centers in A549 cells, and mitotic bodies in A549 cells, a maximum intensity projection of 40 optical z-stacks across 16 μm was generated. All other images are single z-planes. Imaris 7.5 (Bitplane) was used to segment and render the 3D surface of E2A- and Phospho-ATM and to generate the movie.

Immunoblot. Cells were directly harvested in SDS-PAGE loading buffer and sonicated using a Misonix S-4000 cup horn bath sonicator. Cells were counted from an identical well at the time of harvest. Lysate from an equal number of cells was loaded from each sample for SDS-PAGE and immunoblot analysis. β-ACTIN was used as a loading control. Primary antibodies are listed in Table S1. Anti-mouse, anti-rat, and anti-rabbit secondary antibodies conjugated to either IRDye800 (Rockland) or Alexa Fluor 680 (Molecular Probes) fluorophores were used to detect primary antibody staining on a LI-COR-Odyssey scanner. Quantification was performed using the LI-COR Odyssey 3.0 software with the user defined background normalization method. Signals were analyzed as integrated intensities of the bands of interest. For assessing

phosphorylation, Phospho-protein levels were divided by total-protein levels and normalized to the "t = 0" sample. For MRE11 and RAD50 knockdown, MRE11 and RAD50 protein levels were divided by β-ACTIN levels and normalized to the control siRNA treated sample.

Q-PCR analysis of viral genomes. Total DNA was extracted using the QiaAMP DNA Micro kit (Qiagen) following the manufacturer's protocol. Taqman probes for quantifying Adenovirus DNA were as described previously (Johnson et al., 2002). Q-PCR was performed with 2.5 ng of total DNA in 5 µl reactions using the Applied Biosystems 7900HT device (Life Technologies), as described previously (O'Shea et al., 2004). Reactions were performed in triplicate or quadruplicate. Viral DNA was quantified relative to 18S rDNA to obtain a ΔC_t for each sample (Livak and Schmittgen, 2001). The standard deviation for target N, is calculated by: $2^{-\Delta Ct}$ with ΔC_t+s and ΔC_t-s, where s is the standard deviation of the ΔC_t value. For absolute quantitation, cells were trypsinized and submitted to nuclear fractionation prior to DNA purification. Q-PCR was performed using a standard curve of Ad5 DNA to determine absolute virus genome copy numbers using the SDS 2.3 software (Life Technologies).

Chromatin immunoprecipitation. A549 cells were infected as described above and harvested at 12 h.p.i. Plates were incubated with 20 mM HEPES-KOH pH 7.9, 50 mM NaCl, 3 mM $MgCl_2$, 300 mM sucrose, 0.5% TritonX-100 for 5 minutes at room temperature prior to fixing. Samples were processed as described previously for ChIP (Soria et al., 2010). Briefly, cells were fixed with 1% formaldehyde for 10 minutes and stopped with 0.125 M glycine, lysed in 50 mM Tris pH 8.0, 10 mM EDTA pH 8.0, 1% SDS and sonicated to shear genomic DNA. Lysates from 1×10^6 cells were diluted 1:10 in 20 mM Tris pH 8.0, 2 mM EDTA pH 8.0, 150 mM NaCl, 1%

Triton X-100 and immunoprecipitated with 5 µl of anti-MRE11, anti-γH2AX, or anti-Phospho-SQ/TQ motif antibodies (Cell Signaling) or 5 µg of an anti-H3 (Abcam), anti-ATM (Novus), or anti-MDC1 (Genetex) antibodies. 5 µg of an anti-GST antibody (Santa Cruz) was used as a control for specificity. Immuno-complexes were isolated using Protein G Dynabeads (Life Technologies). Crosslinking was reversed by incubation at 65^{o}C overnight. DNA was purified using the QiaAMP DNA Micro kit (Qiagen). For Real-time Quantitative PCR, ChIP DNA samples were analyzed in quadruplicate using the Power SYBR green master mix (Life Technologies) using the Applied Biosystems 7900HT device. Primers for Q-PCR were designed against the Ad5 genome, Ad5 Primer Set 1 (5'-GTTACTCATAGCGCGTAATATTTGTCTAG-3' and 5'-CCCGGAACGCGGAAA-3'), which amplifies base pairs 323-424 and Ad5 Primer Set 2, (5'-CGGAAGTGACGATTTGAGGAA-3' and 5'-AAACCGCACGCGAACCTA-3'), which amplifies base pairs 35,689-35,759. Q-PCR Primers for cellular Alu genome sequences (5'-ACGAGGTCAGGAGATCGAGA-3' and 5'-CTCAGCCTCCCAAGTAGCTG-3') were as described (Zheng et al., 2014). A 10-fold dilution series of input DNA was used to determine the efficiency of the PCR for each primer set. Each primer set demonstrated linear amplification with an R^2 value of at least 0.9 across 15 C_t values. ChIP DNA samples were normalized relative to their respective input DNAs using the ΔC_t method to obtain percent input values or normalized relative to the ΔE1 sample using the $\Delta\Delta C_t$ method (Livak and Schmittgen, 2001) to obtain values 'relative to ΔE1'. Standard deviations from ΔC_t or $\Delta\Delta C_t$ values were incorporated into the statistical range as described above and referred to as 'standard deviation' in the legends.

Cell cycle analysis. For DNA content analysis, quiescent SAECs were infected with the indicated viruses and fixed in 70% EtOH/1.5 mM glycine, pH 2.8. Cells were treated with

RNaseA overnight and stained with 0.5 μg/ml propidium iodide immediately before flow cytometry using an LSR II flow cytometer (BD Biosciences). Data was analyzed using FlowJo. For analyzing the effects of HU and aphidicolin on cellular DNA replication, cells were fixed, stained with Hoechst-33342, and DNA content quantified using the ImageXpress Micro high content screening device (Molecular Devices). Data was analyzed by ImageJ (NIH) to generate integrated density values from at least 30,000 nuclei. Cell cycle phase distribution was analyzed in Microsoft Excel.

siRNA. Stealth siRNAs were purchased from Life Technologies: *RAD50* (5'-GGAAGCCCAGUUAACAUCUUCAAAG-3' and 5'-GGACCAUUCAGUGAAAGACAGAUUA-3') and *MRE11* (5'-ACAUGUUGGUUUGCUGCGUAUUAAA-3' and 5'-UCAUGGAGGAUAUUGUUCUAGCUAA -3') in addition to corresponding non-silencing control siRNAs (Cat# 12935-200). siRNAs were transfected using PepMute Plus (Signagen) at a concentration of 20 nM, unless otherwise indicated. ATM knockdown was performed using a lentivirus vector, pLentiX2 (Campeau et al., 2009), encoding the following sequence (5'-GGATTTGCGTATTACTCAG-3') reported previously (Ariumi et al., 2005). Quiescent SAECs were infected with high titer lentivirus preparations. Adenovirus infection experiments were performed after 10 days.

Population doubling analysis. A549 cells were infected and seeded in triplicate at subconfluent densities in 24-well plates. At 6 hours, 12 hours, 1 day, 2 days, and 4 days post infection, cells were trypsinized and counted. Population doublings were calculated according to the following

formula $\log_2$ (number of cells at time of harvest/number of cells at time of seeding). Error bars indicate standard deviations from cell counts across three wells.

Primary antibodies used for ChIP, immunoblot, immunofluorescence, and ELISA.

Antibody	Company	Product Number	Lot	Application
β-ACTIN (Clone AC-15)	Sigma	A5441	10TK480	IB
Adenovirus Type 5 (polyclonal)	Abcam	ab6982-100	158869	IB, ELISA
MRE11 (Clone 12D7)	Genetex	GTX70212	26626	IB
MRE11 (Clone 31H4)	Cell Signaling	4847	1, 2	ChIP
RAD50 (Clone 13B3/2C6)	Genetex	GTX71228	9111	IB
NBS1 (Clone 1D7)	Genetex	GTX70224	26330	IB
NBS1 (polyclonal)	Novus	NB100-143	L-2, L-3	IF
NBS1 phospho-Ser343 (Clone EP178)	Novus	NBP1-44411	YG080801	IB
ATM/ATR phospho-S/TQ substrate motif antibody (polyclonal)	Cell Signaling	2851	3	ChIP
ATM (Clone MAT3-4G10/8)	Sigma	A1106-24ul	010M4826	IB
ATM (polyclonal)	Novus	NB100-104	P-1	ChIP
ATM phospho-Ser1981 (Clone EP1890Y)	Epitomics	2152-1	YH101212D	IB
DNA-PKcs (Clone Ab-2)	Calbiochem	NA57	D00114972	IB
DNA-PKcs phospho-Ser2056 (polyclonal)	Abcam	ab18192-100	696143	IB
CHK2 (Clone 7)	Upstate/Millipore	05-649	28044	IB
CHK2 phospho-Thr68 (polyclonal)	Cell Signaling	2661	7	IB
CHK1 (Clone 2611D5)	Upstate/Millipore	05-965	DAM1447556	IB
CHK1 phospho-Ser345 (Clone 133D3)	Cell Signaling	2348	8	IB
H2AX (Clone 322105)	RnD Systems	MAB2406	YBU0110121	IB
H2AX phospho-Ser139 (Clone 20E3)	Cell Signaling	9718	8	IB, IF, ChIP
KAP1 (Clone 4E1)	Cell Signaling	5868	1	IB
KAP1 phospho-Ser824 (polyclonal)	Cell Signaling	4127	1	IB
RPA32 (Clone 4E4)	Cell Signaling	2208	1	IB
RPA32 phospho-S4/S8 (polyclonal)	Bethyl	A300-245A	A300-245A-2	IB
53BP1 (polyclonal)	Novus	NB 100-304	A4	IF
MDC1 (polyclonal)	Genetex	GTX11170	28485	IF, ChIP
CYCLIN B1 (Clone Ab-3 GNS1)	NeoMarkers	MS-868-P	868P911E	IB

CYCLIN A (Clone Ab-6 6E6)	NeoMarkers	MS-1061-S	1061S10040	IB
Histone H3 phospho-Ser10 (polyclonal)	Upstate/Millipore	06-570	0703053576	IF
Histone H3	Abcam	ab1791	GR66870-1	ChIP
p53 (Clone DO-1)	Santa Cruz	sc-126	G1112	IB
MDM2 (Clone 2A10)	Calbiochem	OP115	D00124127	IB
p21 (Clone CP36, CP74)	Upstate/Millipore	05-345	DAM1787838	IB
GST (Clone B-14)	Santa Cruz	sc-138	L3005, I1009	ChIP
E1B-55K (Clone 2A6)				IB
E2A (Clone B-6)	received from Dr. Arnold Levine's laboratory			IF
E4-ORF3 (Clone 6A11)	Ascention			IB, IF
E1A (Clone m73)	Dr. Edward Harlow			IB

Supplemental References

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