Supplemental Information

Materials and Methods

Antibodies and Reagents

For detection of viral proteins, the following antibodies were used: anti-E1A (sc-430, Santa Cruz Biotechnology), anti-DBP (clone B6-8)[1], anti-E4ORF3 (clone 6A11)[2], anti-E1B55K (clone 2A6)[3], anti-pVII [4], anti-Hexon (sc-58085, Santa Cruz Biotechnology, and 65H6, Abnova), anti-Penton (gift of Dr. Carl Anderson, Brookhaven National Laboratory, Upton, NY). For detection of PML-NB components, anti-PML (H-238, Santa Cruz Biotechnology), anti-Daxx (25C12, Cell Signaling Technology) and anti-Sp100 (AB1380, Chemicon International) antibodies were used. For EMSA antibody supershift reactions, anti-E2F-3 (C-20), anti-E2F-4 (C-20 and C-108), anti-E2F-5 (C-20), anti-Rb (C-15), anti-p107 (C-108 and SD9X), anti-p130 (C-20X) and anti-DP-1 (K-20) were from Santa Cruz Biotechnology and anti-E2F-1 (KH95) was from Lab Vision/NeoMarkerr. For immunoprecipitation and chromatin immunoprecipitation reactions, anti-GABPα (H-180), anti-GABPβ (E-7), anti-Rb (C-15), and anti-p107 (C-108) were from Santa Cruz Biotechnology and anti-HA was from Rockland Immunochemicals. Human IFNα (universal type I interferon 11200-2) and human IFNγ (11500-2) were from PBL Assay Science, dissolved in phosphate buffered saline (PBS) containing 0.1% BSA according to the manufacturer’s instructions. PD0332991 (Palbociclib) was from APExBIO and dissolved in DMSO.

Immunoprecipitation and immunofluorescence assays

HDF-TERT cells were treated with IFNs or left untreated for 24 hr, and immunoprecipitation assays were carried out as described previously [5]. Immunoprecipitations were performed using 2 µg GABPα antibody with protein A-agarose and analyzed by western blot using an antibody against GABPβ. HDF-TERT
cells were seeded on glass coverslips and infected as described above. At appropriate time points, the cells were fixed with ice-cold methanol and processed for immunofluorescence as described [6]. Images were captured and analyzed using a Zeiss Axiovert 200M digital deconvolution microscope with AxioVision 4.8.2 SP3 software.

**Electrophoretic mobility shift assays (EMSA)**

HDF-TERT cells were incubated with IFNs or left untreated for 24 hr, and nuclear extracts were prepared as described previously [7]. EMSA binding reactions were performed in a total volume of 15 µl containing 20 mM HEPES, pH 7.9, 5 mM MgCl₂, 50 mM KCl, 1 mM DTT, and 6% glycerol. 4 µg of each nuclear extract was incubated with 250 ng sonicated salmon sperm DNA, with or without 0.2 µg specific antibody, for 15 min at room temperature. 10 fmol (80,000 cpm) of ³²P-labeled probe was added to each reaction and incubated for an additional 30 min at room temperature. DNA-protein complexes were resolved in a native polyacrylamide gel. The gels were dried and autoradiographed. The E1A-ENH probe, 5'-'GGTTCCATTTTCGCGGGAAAACTGCCGC-3', corresponds to Ad5 nt 271-288.

**Generation of shRNA knockdown cell lines**

For individual depletion of PML, Daxx or Sp100, retroviral vectors expressing shRNAs directed against PML, Daxx and Sp100 (pSIREN-RetroQ-shPML/-shDaxx/-shSp100), as well as negative control pSIREN-RetroQ-shLuci, were used [8,9]. Replication-deficient retroviruses were generated by cotransfection of 293FT cells (Life Technologies) with helper virus and pVSV-G in combination with the respective pSIREN-RetroQ plasmids. Retrovirus-containing medium was repeatedly collected at 2 days, 3 days and 4 days post-transfection. For double and triple depletion of PML-NB components, lentiviral vectors, pLKO-shDP/shPS/shDPS, as well as a scrambled negative control, pLKO-shneg were used [10]. Lentivirus stocks were prepared as
previously described [10]. To deplete endogenous IFI16 protein, scrambled shRNA in pLKO-shneg was replaced with shRNA against IFI16. shRNA target sequences against IFI16 were obtained from the RNAi consortium (TRC) shRNA library, siIFI16-1, GATCATTGCCATAGCAAATT; siIFI16-3, GGAAACTCTGAAGATTGATT. Low passage HDF-TERT cells were transduced with the retrovirus or lentivirus supernatants in the presence of 7.5 µg/ml polybrene (Sigma-Aldrich, Germany) overnight. To increase the transduction efficiency, HDF-TERT cells were reinjected with retroviruses or lentiviruses at day 2 and day 3. Stably-transduced pools of cell populations were selected using 2 µg/ml puromycin. At least two independent pools of cells with efficient knockdown of individual target genes were generated and analyzed.

**Western blot analysis**

Whole cell extracts were prepared by suspending cell pellets in SDS lysis buffer (50 mM Tris-HCl, pH 6.5, 4% SDS) and boiled for 10 min. The samples were clarified by centrifugation and the protein concentration was determined using a Bicinchoninic Acid (BCA) kit (Pierce). Equal amounts of whole cell extracts were resolved on SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were blocked with Tris-HCl-buffered saline (TBS) buffer containing 3% BSA for 1 hr at room temperature, followed by incubation with primary antibodies (as indicated in the text and figure legends) at 4°C overnight. Membranes were washed with TBS buffer containing 0.1% Tween-20 (TBS-T) and then incubated with IRDye® 800CW-conjugated goat anti-rabbit antibody (926-32211, Li-COR) and IRDye® 680RD-conjugated goat anti-mouse antibody (925-068071, Li-COR) for 1 hr at room temperature. After three wash with TBS-T, images was captured using the ODYSSEY® CLx infrared imaging system (Li-COR). Alternatively, HRP-conjugated secondary antibodies were used in conjunction with ECL western blotting (Millipore Immobilon).

**Cell fractionation**
For the preparation of isolated nuclei for qPCR, cells were washed twice with ice-cold PBS, harvested, resuspended in 1 ml ice-cold isotonic buffer containing NP-40 (10 mM Tris-HCl, pH 7.5, 140 mM NaCl, 1.5 mM MgCl₂, 0.6% NP-40), and incubated on ice for 10 min. Nuclei were pelleted by centrifugation at 2,000 x g for 10 min at 4 °C. Nuclear and cytoplasmic fractions for Western blot analysis were prepared using NE-PER™ nuclear and cytoplasmic extraction kit (Pierce).

**Figure Legends**

**Figure S1. Analysis of the adenovirus life cycle in HDF-TERT cells.** (A) HDF-TERT cells on glass coverslips were infected with *dl309* at 1,000 virus particles/cell and fixed at the indicated time points. Immunofluorescence assay was carried out using antibodies against PML (green) and Ad5 protein VII (red). (B, C) HDF-TERT cells were infected with *dl309* at 25 virus particles/cell. Nuclear DNA and total RNA were purified at various time points. Viral DNA levels were quantified by qPCR and normalized to GAPDH DNA levels. Viral mRNA levels were quantified by RT-qPCR and normalized to GAPDH mRNA levels. (D) HDF-TERT cells were infected with *dl309* at 200 virus particles/cell and harvested at 12, 16, 20 and 24 hr post-infection. Western blots were performed to examine viral early (E4-ORF3) and intermediate (IVa2) protein expression. γ-tubulin was used as a loading control for the samples. (E) HDF-TERT cells were pretreated with IFNs or left untreated, and then infected with *dl309* at 25 virus particles/cell. Cells were harvested at 48, 97 and 144 hr post-infection and viral late protein levels (Penton, Hexon) were analyzed by Western blot. γ-tubulin was used as a loading control for the samples. (F) HDF-TERT cells on glass coverslips were pretreated with IFNα, IFNγ or left untreated for 24 hr, then infected with *dl309* at 1,000 particles/cell. Cells were fixed at 2 hr post-infection. Immunofluorescence assays were carried out using antibodies against PML (green) and Ad5 protein VII (red).
Figure S2. Comparison of E1A expression levels. (A) Whole cell extracts were prepared from HDF-TERT cells (lane 1), HDF-TERT-E1A cells (lane 2), HDF-TERT cells infected with dl309 at 200 virus particles/cell and harvested at 48 hr post-infection (lane 3), and HDF-TERT cells infected with in340-Δ2-CMV-E1A (lane 4) or Δ1-3-CMV-12SHA (lane 5) at 5,000 virus particles/cells and harvested at 24 hr post-infection. E1A and γ-Tubulin levels were analyzed by Western blot. (B) HDF-TERT cells were infected with Ad5-WT, Ad5-mut1 or Ad5-mut2 virus at 25 particles/cell. Cells were harvested at 24 and 48 hr post-infection. E1A mRNA levels were analyzed by RT-qPCR and normalized to GAPDH. (C) HDF-TERT cells were infected with Ad5-WT at 1,000 particles/cell. Cells were harvested at 48 hr post-infection. E1A mRNA levels were analyzed by RT-qPCR and normalized to GAPDH.

Figure S3. Depletion of PML-NB components does not rescue Ad5 replication in the presence of IFNs. (A) Expression of PML, Daxx and Sp100 in single knockdown cell lines was examined by Western blot. (Left panel) Parental HDF-TERT cells (HDF) were used along with different individual knockdown subclones corresponding to control shRNA (shleer) and shRNAs targeting Daxx, PML and Sp100. (Right panel) Parental HDF-TERT cells (HDF) were used along with pools of cells expressing control shRNA (shneg) and shRNAs targeting Daxx and PML (shDP), PML and Sp100 (shPS), and all three proteins (shDPS). (B) Single-knockdown cell lines (left panel) and double- and triple-knockdown cell pools (right panel) were pretreated with IFNs or left untreated for 24 hr and then infected with dl309 at 1,000 virus particles/cell. Viral DNA replication was quantified by qPCR at 48 hr post-infection and was plotted as mean ± sd, n=4. (C) HDF-TERT cells were infected with dl309 at 1,000 particles/cell. Cells were harvested at 48 hr post-infection. E1A mRNA levels were analyzed by RT-qPCR and normalized to GAPDH.
**Figure S4. Replication of Ad5 in IFI16-depleted cells.** (A) IFI16 proteins levels were examined by Western blot in parental HDF-TERT cells (HDF) along with different individual knockdown subclones corresponding to control shRNA (shneg) and shRNAs targeting IFI16. γ-tubulin was used as a loading control for the samples. (B) shRNA control and IFI16 knockdown cells were treated with IFNs or left untreated for 48 hr. IFI16 protein levels were examined by Western blot. (C) shRNA control and IFI16 knockdown cells were pretreated with IFNs or left untreated and then infected with dl309 at 1,000 virus particles/cell. Viral DNA replication was quantified by qPCR at 48 hr post-infection and was plotted as mean ± sd.

**Figure S5. Analyses of HDF-TERT long-term Ad infections.** (A) Phase contrast images were captured from HDF-TERT cells infected with Ad5-WT or Ad5-mut1, with or without IFNγ, as described in Figure 7 at the indicated days post-infection. (B) E1A expression was analyzed with HDF-TERT cells infected with Ad5-WT or Ad5-mut1 by Western blot at the indicated time points. α-Tubulin was used as a loading control.
Table S1. Oligonucleotides used in qPCR.

| Oligonucleotide | Sequence | Description |
|-----------------|----------|-------------|
| PACK-4          | GCGAAAAATGGCCAAATGTTA | Left-end of Ad5-WT, spanning from the ITR to the E1A enhancer region |
| PACK-5          | TAATGAGGGGGTGGAGTTTG | |
| loxP7           | GTCCGGTTTTCTATGCCAAC | E1A 13S cDNA, does not recognize Ad-CMV and Ad-CMV-12SHA |
| loxP8           | CCGTATTCCTCCCCGATGATAA | |
| E1B-F           | TGTGCCTTTTACTGCTGCTG | E1B cDNA |
| E1B-R           | CACAGCCACGCTTTTCACTA | |
| DBP-F           | CCGTAGTGCGATCCAAAGGT | E2A cDNA |
| DBP-R           | GTCTAGCAAGGCCAAGATCG | |
| E2B-F           | CGCGCGTGAAGTAGCTCTAT | E2B cDNA |
| E2B-R           | CGGTGGAAGATGCTACCCTA | |
| E4ORF6-F        | TACCGGGAGGTTGGAATTA | E4ORF6 cDNA |
| E4ORF6-R        | TCTAAATCCACAGTGCAAA | |
| E1A-ENH-F       | CGCGGGAACCTGAATAAGA | Ad5-WT and dl309-specific, does not recognize in340-Δ2-CMV |
| E1A-ENH-R       | CTTGAGGAACTCACCAGGTA | |
| Ad5-mut1-F      | ATATCTCCCACTGAATAAGA | Ad5-mut1 E1A enhancer-specific |
| E1A-pan-F       | ACTCTTGAGTGCCAGCGAG | |
| Ad5E1A-R        | ACTCTTGAGTGCCAGCGAG | |
| Ad3E1A-R        | TACAGATCGTGCGTACGG | |
| Ad4E1A-R        | AGCGAAGGTGTCTCACAATGG | |
| Ad9E1A-R        | GGGCATCTACCTCCAGATCA | |
| Ad12E1A-R       | CGGCAGACTCCACATCAAG | |
| cGAPDH-F        | ACCCAGAAGACTGTGGATGG | cellular GAPDH cDNA |
| cGAPDH-R        | TTCTAGACGCGGGTCTAGGT | |
| gGAPDH-F        | CCCACACACACACTGCACTTACC | cellular GAPDH genomic sequence |
| gGAPDH-R        | CCTAGTCCAGGGCTTTGATT | |
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