Supplementary Information:

Materials and Methods:

Immunoprecipitation & Western Blots

Cells were harvested and the total cell extract was prepared by incubating the cell pellet for 1h with 2 volumes of NETT 250 buffer (20 mM Tris-HCl [pH 8.0], 0.1 mM EDTA, 250 mM NaCl, 0.5% TritonX-100). After incubation, the lysate was centrifuged at 13,000 rpm for 10 min. Immunoprecipitations were carried out with 1 mg of total protein extract. Lysates were first precleared with 10μl of protein A-sepharose beads (50% slurry) for 1h. The supernatant was then incubated with 1μg of E2F4 antibody followed by 1h incubation with protein A-sepharose beads with gentle rocking. The beads were washed three times with lysis buffer and bound proteins were eluted with gel loading dye, boiled at 100°C for 5 minutes and separated on 8.5% sodium dodecyl sulfate-polyacrylamide gel. For western blots, extracts (150 to 200 μg for large gels or 75-100μg for mini gels) were mixed with gel loading dye, separated on a 8.5% or 10% sodium dodecyl sulfate-polyacrylamide gel and blotted to nitrocellulose. The blots were probed with antibodies to XPC (a generous gift from Dr. Wani; The Ohio State University) and, cdk2 (Santa Cruz), p19ARF (ab80; abcam), E2F4 (C20; Santa Cruz) and DP1 (1DP06; Labvision).

Semiquantitative PCR & Quantitative Real Time reverse transcription-PCR (RT-PCR)

Total RNA were extracted from the DKO (p53-/- MDM2-/-) and TKO (p53-/- MDM2-/- ARF-/-) cells using TRIZOL reagent (Invitrogen). 1.5 micrograms of the total RNA was

treated with DNase I using RQ1 RNase-free DNase I (Invitrogen). 1.5 micrograms of the DNAse treated RNA was then reverse transcribed using an iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's protocol. PCR was performed on 1 μl cDNA products using the Qiagen Taq PCR Core kit for XPB, XPA, XPD, p52 (one of the subunits of TFIIH), ERCC1, XPF and XPG. GAPDH was used as an internal control to normalize the amounts of cDNA. The products were separated on a 1.0% agarose gel by electrophoresis. Quantification of gene expression was obtained with Image J. Primer sequences and PCR conditions are available upon request. Quantitative Real Time PCR amplification was conducted in triplicate using the following primers: mouse XPC forward 5' CAAAAAGCAAGGTGGTGGACC 3' and mouse XPC reverse 5' TTCATCATCCTCCGCAGGTATG 3' annealing temperature 56°C and mouse cyclophilin, 5′ GGCAAATGCTGGACCAAACAC 3′ and 5′ TTCCTGGACCCAAAACGCTC 3′ (annealing temperature, 57.5°C). Each PCR mix consisted of the following: 0.05 μg of cDNA, a 100 nM concentration of each primer, and 1 X iQ SYBR green supermix (Bio-Rad) in a 25 μl reaction mix. Real-time PCR was performed using the MyiQ single color real time PCR detection system (Bio Rad). Melting curve analysis was performed for every reaction to ensure the amplification of one product. To create a standard curve for relative quantification, cDNA obtained from DKO MEFs was used as a standard control, diluted in water (1X, 0.2X, and 0.04X), and subjected to real-time quantitative PCR in triplicate. The dilution value (starting quantity) of the standard was plotted against the threshold cycle number at which fluorescence first increased above the background by the use of MyiQ software (Bio-rad). The expression of the indicated gene in each sample was evaluated with this standard curve. The levels of

XPC mRNA were normalized against the levels of cyclophilin mRNA which was used as an internal control. The change in the level of XPC mRNA was calculated by dividing the normalized values of XPC in TKO MEFs by the normalized values of XPC in DKO MEFs.

Luciferase Assay

For luciferase reporter gene analyses, 73 nt duplex DNA containing -51 to +7 sequences in the mouse XPC promoter containing the E2F site was generated with KpnI and XhoI overhangs for cloning into the pGL3-basic vector (Promega). Another fragment with altered E2F consensus sequence was used as the mutant promoter-reporter construct. For transfection, $1.5x10^5$ NIH-3T3 cells were seeded in triplicate into 24-well culture plates. After 12 hours of serum starvation, cells were transfected with 0.2 μg of CMV empty vector or 0.2 μg of either wild-type or mutant XPC promoter luciferase reporter with the indicated doses or CMV ARF expression construct by using the Lipofectamine 2000 reagent (Invitrogen). Firefly and *Renilla* luciferase activities were measured twenty-six hours after transfection by using the Dual-Luciferase Reporter Assay System kit (Promega) according to the manufacturer's instructions. *Renilla* luciferase activity from a cotransfected pRL-SV40 control vector (1ng/well) was used for normalization. Luciferase was also conducted with a 1.5kB fragment of the XPC promoter.

XPC Lentivirus production & Stable cell line generation

Mouse XPC cDNA obtained from Open Biosciences was cloned into the pLVx-Puro lentiviral expression vector (Clontech) using the PCR Infusion cloning kit (Clontech) (a

generous gift from the Nakamura Lab; University of Illinois at Chicago). Lentivirus was generated according to the manufacturer's instructions. TKO MEFs were infected with 2 ml of virus three times in 4 h intervals. The cells were supplemented with polybrene at a final concentration of 8μg/ml. Cells were allowed to recover for one day. Selection with puromycin (4μg/ml) was conducted for 3 days. Generation of a stable cell line was confirmed by western blot.

Legend to Supplementary Figures:

Supplementary figure 1: Reintroduction of ARF in ARF-/- MEFs corrects the repair deficient phenotype

ARF-/- MEFs were infected with control or ARF-expressing adenovirus. Sixteen hours following infection, the cells were subjected to UV irradiation and UDS assay (Smith et al, 2000). UDS was quantified by counting the number of 3H-thymidine grains/nucleus in 30 nuclei. Autoradiographs of representative nucleus are shown on the top panel, and quantification along with means ± SEM are in the bottom panel. WT AdControl and ARFnull AdARF $p = 0.75$ and therefore the difference is not statistically significant. ARF null AdControl vs. ARFnull AdARF $p < 0.0001$

Supplemental figure 2: XPC mRNA and Protein Level +/- UV

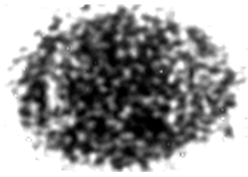
WT MEFs, ARF -/- MEFs, p53-/- MDM2-/- (DKO) MEFs and p53-/- MDM2-/- ARF-/- (TKO) MEFs were UV irradiated and collected 7 hours post irradiation for RNA and protein preparation along with a no UV control for each genotype. RNA and protein plus and minus UV were assayed for XPC by semiquantitative RT-PCR and western blot (A).

The level of ARF is substantially higher in DKO cells than in primary WT MEFs (shown here P5 WT MEFs) (B). (C) Western blot assay confirming knockdown of ARF in DKO MEFs. (D) Western blot assay for XPC in TKO MEFS infected with control or XPC expression virus.

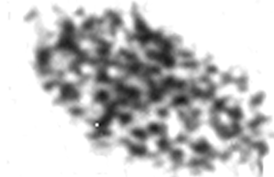
Supplementary Figure 3: **Reintroduction of XPC in ARF null MEFs restores NER**

TKO MEFs were infected with control (pLVx) or XPC-expressing lentivirus (pLVx-XPC) were subjected to UDS assay. Autoradiographs of representative nucleus are shown on the top panel, and quantification from 30 nuclei is shown in the lower left panel. DKO + pLVx and TKO + pLVx-XPC $p = 0.311$ and therefore is not statistically significant. TKO + pLVx vs. TKO + pLVx-XPC $p < 0.0001$.

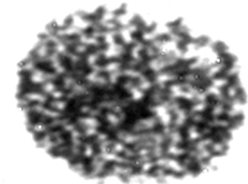
Dominguez-Brauer Supplementary Figure 1



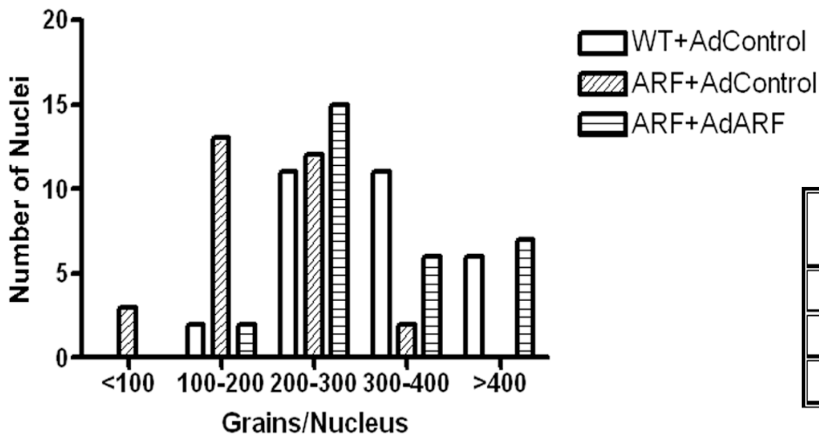
WT (AdControl)



ARF -/- (AdControl)



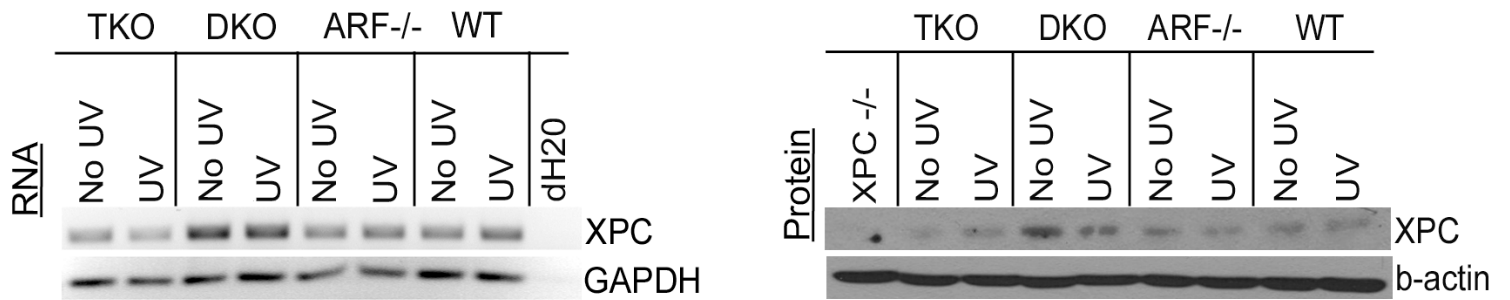
ARF -/- (AdARF)



Genotype	Mean Number of Grains/Nucleus
WT + AdControl	338.5 ± 14.7
ARFnull + AdControl	184.9 ± 11.6
ARFnull + AdARF	330.8 ± 19.4

Dominguez-Brauer Supplementary Figure 2

A



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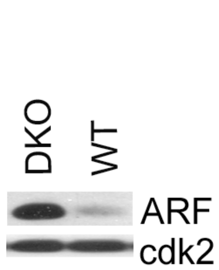

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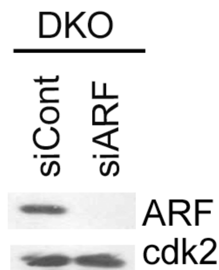
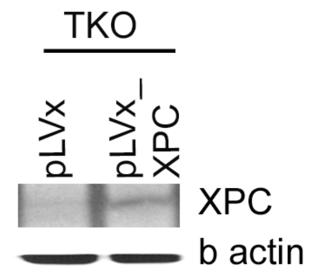


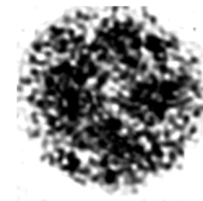
D



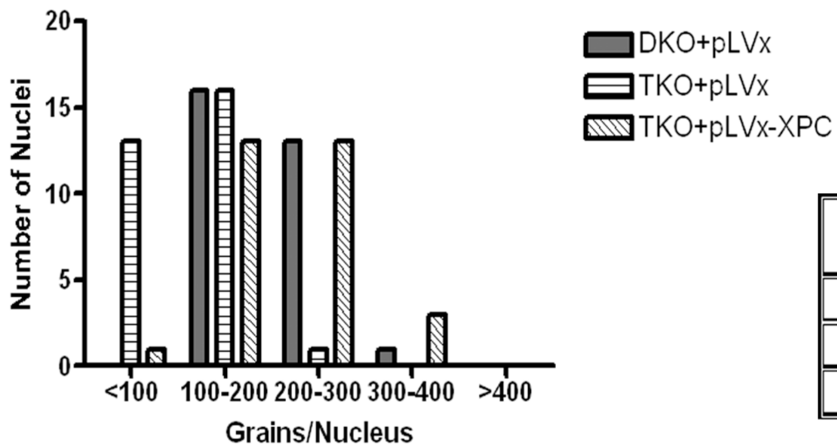
Dominguez-Brauer Supplementary Figure 3

DKO+pLVx

TKO+pLVx



TKO+pLVx-XPC



Genotype	Mean Number of Grains/Nucleus
DKO+pLVx	207.3 ± 8.6
TKO+pLVx	109.5 ± 7.5
TKO+pLVx-XPC	222.7 ± 12.4