Supplementary figures
A

TPW  CMV R U5  RRE  cPPT CTS  TRIplex  MCS  ΔU3

TPEW
TPCatalase
TPCuZnSOD
TPMnSOD
TPNOX1

B

TEEshXPC1W  CMV R U5  RRE  cPPT CTS  EF1-α  EGFP  WPRE  H1  shXPC  U5

5'-GGAGGAGAAGGAGAGAATTCAAGAGACCTTTTCCTCCTTCCTTTTT-3'
S+ targeted sequence  A- targeted sequence

5'-GTGGTTTACAGGAAAACGTCAAGAGACCTTTTCCTCCTTCCTTTTT-3'
S+ targeted sequence  A- targeted sequence

5'-ACAATAGCCTTGATTCTCATGTTCAAGAGACATGAGAATCAAGGCTATTGTTTTTT-3'
S+ targeted sequence  A- targeted sequence

5'-GTGGTTCATTGGAACATTTACTTCAAGAGAGTAAATGTTCCAATGAACCACTTTTTT-3'
S+ targeted sequence  A- targeted sequence

5'-ACATGAACTCCTGAATTTCAACTGCATGTTCAAGAGACATGCAGTTGAAATTCAGGCATTTTT-3'
S+ targeted sequence  A- targeted sequence

5'-CCATGAACGAGTTTGAGTACCTTCAAGAGAGGTACTCAAACTCGTTCATGGTTTTT-3'
S+ targeted sequence  A- targeted sequence

5'-ACATGAACTCCTGAATTTCAACTGCATGTTCAAGAGACATGCAGTTGAAATTCAGGCATTTTT-3'
S+ targeted sequence  A- targeted sequence

5'-GCTCCAAGGTGTACGTGAATTCAAGAGATTCACGTACACCTTGGAGCTTTTT-3'
S+ targeted sequence  A- targeted sequence

5'-CCATGAACGAGTTTGAGTACCTTCAAGAGAGGTACTCAAACTCGTTCATGGTTTTT-3'
S+ targeted sequence  A- targeted sequence

5'-GCTCCAAGGTGTACGTGAATTCAAGAGATTCACGTACACCTTGGAGCTTTTT-3'
S+ targeted sequence  A- targeted sequence

C

D

[Graph showing protein expression and activity levels]

HRR-Figure S1
Figure S1. Vector constructs and analysis of the transduction efficiency. (A) Lentiviral vectors constructs used to overexpress EGFP, catalase, CuZnSOD, MnSOD, or NOX1 are shown. Vectors carry an internal cassette for the enhanced green fluorescent protein (EGFP), catalase, CuZnSOD, MnSOD, or NOX1 driven by the promoter of human phosphoglycerate kinase gene (hPGK). ∆U3, R, and U5 are the LTR regions, with a deletion that includes the enhancer and the promoter from U3. CMV is the cytomegalovirus promoter, SD is the major splice donor site, SA is the splice acceptor site, RRE is the rev-response element, cPPT is a nuclear import sequence, and WPRE is a regulatory element of woodchuck hepatitis virus. (B) Lentiviral constructs used to inhibit XPC, NOX1, NOX2, AKT, or red fluorescent protein (RFP) expressions are shown. TEEHshRF PW construct was used as the control shRNA (named shCtrl) plasmid. (C, D) Transduction efficacy was checked by western blotting analysis (C) and also by measuring the specific activity of each antioxidant enzyme in the keratinocytes (D) as described in the Methods section.
Figure S2. XPC downregulation results in change of keratinocyt cell size

At 15 days after transduction cells was photographed with light microscopy. (A) Light micrographs show that XPC\textsuperscript{KD\textsubscript{NHK}} cells are larger than the control cells and that AKT downregulation inhibits this modification. (B) Surface of 100 cells (per condition) was measured. Results are expressed as average cell surface ± SD of three independent experiments. *, p<0.05 for different cells vs shCtrl-transduced cells.
Figure S3. Inhibition of NOX1 expression blocks the effect of XPC downregulation on ROS levels. Intracellular ROS levels were measured in keratinocytes at different days following transduction using CM-H$_2$DCF-DA, the cytoplasmic probe, or MitoSOX, the mitochondrial probe. (A, B) Cytoplasmic (A) and mitochondrial (B) ROS levels were measured in keratinocytes transduced with shCtrl, shXPC, catalase, CuZnSOD, MnSOD, shNOX1, and/or NOX1, and DPI-treated cells by flow cytometry. Data are expressed as the mean ± SD of three independent experiments. †, p<0.05 for different cells vs shCtrl-transduced cells and *, p<0.05 for different cells vs indicated corresponding samples.
Figure S4. NOX1 activation in XPC<sup>KD</sup>NHK triggers metabolic alteration. (A) The relative activity of complex IV of the mitochondrial respiratory chain was assessed. The mRNA levels of COX1 and COX3, ND1 and ND5 (B), HK-2 and PFKFB-3, and GLUT-1 and G6PD (C) were quantified by quantitative real-time RT-PCR at different time intervals following transduction. The results are shown as the average percentage of shCtrl ± SD of six independent experiments. *, p<0.05 for different cells vs shCtrl-transduced cells.
Figure S5. AKT activation in XPC\textsuperscript{KDNHK} triggers metabolic alteration. (A) The protein expression levels of XPC, ND1, COX3, GLUT-1, HK-2, PFKFB-3, G6PD, AKT, phospho-AKT, phospho-GSK-3β were determined by western blot at the indicated days after transduction. β-actin was used as a loading control. (B) The relative activity of complex IV of the mitochondrial respiratory chain was assessed. (C) The mRNA levels of COX1 and COX3, ND1 and ND5 (D). HK-2 and PFKFB-3, and GLUT-1 and G6PD (D) were quantified by quantitative real-time RT-PCR at different time intervals following transduction. The results are shown as the average percentage of shCtrl ± SD of six independent experiments. (E) The mitochondrial network morphology in shCtrl-, shXPC, (shXPC+shAKT)-transduced keratinocytes was determined by microscopy using Mito Tracker. Length of mitochondrial tubules was measured in 50 cells (25 mitochondrial tubules per cell) per condition. Results are expressed as average percentage of mitochondrial tubule size distribution ± SD of three independent experiments. *, p<0.05 for different cells vs shCtrl-transduced cells.
Figure S6. XPC silencing-induced epithelial hyperplasia manifestation blocked by AKT downregulation. Epidermis reconstructed with shAKT- and (shXPC+shAKT)-transduced keratinocytes on days 5 and 15 after transduction. The effect of DNA-PK inhibition on reconstruction of epidermis was assessed by treatment of epidermis with NU7026. Architecture of epidermis was evaluated with H&E staining. Proliferation and differentiation status of epidermis were assessed using immunofluorescence staining of K10, K14, integrin α6, integrin β1, and Ki67. The nuclei were marked with DAPI and are in blue.
Figure S7. The effects of NOX1 on AKT activation in XPC\textsuperscript{KD}NHK. (A) The effects of NOX1 on the protein expression level of AKT, phospho-AKT, phospho-GSK-3β and γH\textsubscript{2}AX were determined by western blot at the indicated days after transduction. β-actin was used as a loading control. (B) Apoptotic cells were analyzed by flow cytometry at indicated time intervals after transduction. Results are expressed as the average percentage of apoptotic and necrotic cells ± SD of three independent experiments. *, p<0.05 for different cells vs shCtrl-transduced cells at the indicated time points.
**Figure S8. XPC protein level in cells from XPC patients.** Total cell lysates from 3 patients (lanes 2, 3, and 5), 2 control samples (lanes 1 and 4), and one control sample transduced with shXPC (lanes 6) were analyzed by western blot and probed with anti-XPC and anti-β-actin antibodies. Anti-β-actin was used to normalize protein levels.
### Supplementary Tables

**Table S1. Primer sequences for quantitative real-time PCR**

<table>
<thead>
<tr>
<th>Target gene</th>
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| ND1         | F: ACGCCATAAAACTCTTTCACCAAG  
R: TAGTAGAAGAGCGATGGTGAGAGCTA |
| ND5         | F: AGTTACAATCGGCATCAACCAA  
R: CCCGGAGCACATAAAATAGTATGG |
| Cox I       | F: CTGCTATAGTGGAGGCGGGA  
R: GGGTGGGAGTATGTTCCCTGC |
| Cox III     | F: CCAATGATGGCGCGATG  
R: CTTTTGAGCAGTAGGTTGTTG |
| GLUT-1      | F: GCTACAACACTGGAGGCACTCAG  
R: GGATCAGCATCTCAAAGGAC |
| HK-1        | F: GCTGGCCGATCAACACCGGT  
R: GCCGTCCGGGGGATGCACA |
| PFKFB-3     | F: GGTGTGCGACGACCTAC  
R: GTACACGATGCGGCTCTG |
| G6PD        | F: ATGATGCAGCCTCCTACCAG  
R: ACAGGGAGGAGATGTGGTTG |
| Ku70/XRCC6  | F: CCGAGATACAGGATCTCCCT  
R: TCCGCAACAGGTCTCTAGC |
| DNA-PKcs    | F: CCTTGACACAGTTCCTGAGGT  
R: TCTCGAGAAGCTGCCCCCTTT |
| Tubulin     | F: GAGTGCATCTCCATCCACGTT  
R: TAGAGCTCCAGCAGGCATT |
Table S2. Primer sequences used to detect mitochondrial deletions

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<thead>
<tr>
<th>Primer name</th>
<th>SEQUENCE</th>
<th>Position</th>
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<tr>
<td>A1*</td>
<td>5'-CTTTTGGCGGTATGCACTTT-3’</td>
<td>404-423</td>
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<td>A2*</td>
<td>5’-GATTATGGATGCGGTTGCTT-3’</td>
<td>4676-4657</td>
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<tr>
<td>B1*</td>
<td>5’-CAACCCTCGCCCATCCTA-3’</td>
<td>491-508</td>
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<tr>
<td>B2*</td>
<td>5’-CCTGCAAAGATGGTAGTAGATGAC-3’</td>
<td>4516-4489</td>
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<td>5’-GCAGATAATATCAATATTTTTCACTG-3’</td>
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* the primer sequences are according to Harbottle and Birch-Machin (Harbottle and Birch-Machin, 2006).
† the primer sequences are according to Berneburg et al (Berneburg et al., 1999).