Supplemental Materials and Methods

RNA isolation, reverse transcription, and real time PCR analysis

Total RNA was extracted and purified using TRIzol reagent (Life Technologies), according to manufacturers' protocol. cDNA synthesis was performed with 1 μg of total RNA using iScript cDNA Synthesis Kit (Bio-Rad) and diluted 10-fold. Real time PCR analysis was carried out with SYBR Select Master Mix for CFX (Life Technologies) using the CFX96 Touch Real-Time PCR Detection System (Bio-Rad). Gene specific primer sequences are provided in Supplemental Table S2.

CRISPR/Cas9-mediated knockout of XPC in H9 human ESCs

CRISPR/Cas9 guide RNAs were cloned using the pX330 vector system as previously described (Cong et al. 2013). Human H9 ESCs were transfected with of 3.0 μg hSpCas9-Venus by nucleofection (Lonza; Kit V, Program X-005, 5 x 10^6 Cells). After 48 hours, cells were dissociated with Accutase (Life Technologies) into single-cell suspension and FACS sorted for Venus expression. Sorted cells were grown on inactivated MEF feeders under dilute conditions to obtain individual clones. After 5-7 days, individual clones were isolated and were used for direct cell lysis PCR using DirectPCR (Viagen). Positive clones were further grown in feeder-dependent conditions and knockouts were verified by Sanger sequencing and Western blot analysis. Three independent clones wildtype for XPC were obtained from the procedure in parallel and used as controls. Guide RNA sequences are included in Supplemental Table S2.

Mouse ESC culture

Mouse D3 ESCs were cultured in knockout DMEM (Life Technologies) supplemented with 15% FBS (Hyclone), 2mM GlutaMAX (Life Technologies), non-essential amino acids (Life Technologies), 0.1mM 2-mercaptoethanol (Sigma Aldrich), and 1000 units of LIF (Millipore) on 0.1% gelatin in the absence of feeders.

5mC detection using thin layer chromatography (TLC)

Genomic DNA (2 μg/sample) was digested overnight at 37°C with 100U of MspI (NEB), followed by dephosphorylation with CIP (NEB) for 1 hr. DNA was purified with the QiaQuick Nucleotide Removal Kit (Qiagen). Samples were denatured by heating at 95°C for 10 min, followed by radioactive end labeling with [γ-32P]-ATP (10 μCi, Perkin Elmer) and T4 PNK (NEB) for 2 hr at 37°C. Samples were heat inactivated and treated with 100U S1 Nuclease (Promega) for 3 hr at 37°C. Samples (2 μl) were spotted onto 20 x 20cm PEI cellulose F coated TLC plates (Millipore) and developed with isobutryic acid:water:ammonium hydroxide (66:20:2 v/v/v) until the front reached 1cm from the top. Plates were dried, exposed to a phosphorimager screen (Kodak), and subsequently imaged on the PharosFX Plus (Bio-Rad).

Doubling time determination through CFSE labeling

To determine the doubling time, HDFs were labeled with CFSE-Violet (Life Technologies) at a working concentration of 5.0 μM, according to the manufacturers' protocol. Cells were analyzed for remaining fluorescence on a BD LSRFortessa every day for 1-5 days.

Luciferase Report Assays

U2OS cells were transfected and plated in the same fashion as our single particle tracking experiments. 1.0 μg methylated and mock-treated pGL4.13[Luc2/SV40] (Promega) reporter plasmid was co-transfected with 20ng pRL-CMV (Promega) as an internal control. Cell lysates were prepared 24 and 48 hours after transfection, and luciferase activities were measured using the Dual-Luciferase reporter assay system (Promega), according to the manufacturers' protocol.

References

Cong L, Ran F, Cox D, Lin S, Barretto R, Habib N, Hsu P, Wu X, Jiang W, Marraffini L, et al. 2013. Multiplex Genome Engineering Using CRISPR/Cas Systems. *Science (80-)* **339**: 819–822.