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Xpc の C 末端領域はマウス胚性幹細胞における *Oct3/4* 転写活性に不必要である

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伊藤　俊介

The C-terminal region *of Xpc* is dispensable for the transcriptional activity of Oct3/4 in mouse embryonic stem cells

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Abstract

The transcription factor Oct3/4 is essential to maintain pluripotency in mouse embryonic

stem (ES) cells. It was reported that the Xpc DNA repair complex is involved in this

process. Here we examined the role of Xpc on the transcriptional activation of the target

genes by Oct3/4 using the inducible knockout strategy. We found that the removal of the

C-terminal region of Xpc, including the interaction sites with Rad23 and Cetn2, showed

faint impact on the gene expression profile of ES cells and the functional *Xpc-ΔC* ES cell

lines retained proper gene expression profile as well as pluripotency to contribute

chimeric embryos. These data indicated that the C-terminal region of Xpc is dispensable

for the transcriptional activity of Oct3/4 in mouse ES cells.

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Key words:　Xpc, Oct3/4 , Pluripotency, Embryonic Stem Cells, Transcriptional Activity, Development

Highlight

The C-terminal region of *Xpc* is dispensable for the maintenance of pluripotency.

The C-terminal region of *Xpc* is dispensable for the transcriptional activity of Oct3/4.

The C-terminal region of *Xpc* is essential for its DNA repair activity.

Introduction

Tissue-specific transcription factors (TFs) bind to the specific sites of the genome in a sequence-specific manner and control the transcription of the target genes positively and negatively. For the activation of transcription, TFs cooperate with coactivators to recruit the general transcription machinery to the promoter, resulting in loading RNA polymerase II (polII) to initiate transcription. As one of the coactivators, the mediator complex (Mediator) has a pivotal role to bridge the TFs binding to their target sites and the general transcriptional machinery [1, 2]. Recent finding indicated that Mediator forms a large complex with multiple TFs bound to a large genomic region to direct the specific gene expression, designated as a super enhancer [3]. The system composed of these factors direct tissue-specific expression of the genes from the genome in cooperation with the epigenetic regulation.

In mouse ES cells, several key TFs have been identified to determine the specific gene expression pattern coupled with pluripotency [4]. The POU family transcription factor Oct3/4 (encoded by *Pou5f1*) is a pivotal TF since it binds to several pluripotency-asscoiated genes to activate their transcription [5] and its elimination causes

the cease of self-renewal and the loss of pluripotency [6]. For the activation of the target

genes, Oct3/4 mainly cooperates with the Sry-related HMG-box transcription factor Sox2

[7]. Oct3/4 directly interacts with Sox2 and these TFs in the heterodimer recognize the

specific sequences adjoining each other without space or with a space of a few base pairs

on the target DNA [8]. Mediator works to transduce the binding of Oct3/4-Sox2 complex

to the distal binding site for the recruitment of polII at the promoter [9]. The requirement

of Mediator to keep the Oct3/4-dependent gene expression pattern was demonstrated by

the knock-down of the component of *Mediator* [9]. It was reported that the DNA repair

complex containing Xpc also act as a coactivator of Oct3/4 [10]. The Xpc complex

consists of Xpc, Rad23B and Cetn2 and mediates the nucleotide excision repair [11]. In

mouse ES cells, the complex directly interacts with Oct3/4 to mediate the transcriptional

activation of the target genes such as *Nanog* [10]. It was demonstrated that the

simultaneous knock-down of *Xpc*, *Rad23b* and *Cetn2* compromised self-renewal of ES

cells with down-regulation of the target genes of Oct3/4 [10]. However, it was also

demonstrated that the *Xpc* knockout mice develop to term [12] and the *Xpc*-null ES cells

can be established from the *Xpc*-null blastocysts [13], which sounds controversial to the

function of the Xpc complex proposed in ES cells.

It has sometimes been observed that the knock-down of a specific gene expression by siRNA or shRNA gives different effects from the knockout of the same gene by the gene-targeting via homologous recombination. For example, several reports demonstrated that the knock-down of *Rex1* (encoded by *Zfp42*) causes differentiation of ES cells [14] although we reported that the *Rex1*-null ES cells continue self-renewal with keeping the ability to contribute chimeric embryos after blastocyst injection [15]. One explanation for this could be the off-target effect of the knock-down strategy. However, it might also be due to the different kinetics of the ablation of the gene function. The expression of the target gene is rapidly eliminated by knock-down. In contrast, the establishment of knockout ES cells from knockout embryos takes long time (several weeks) that may allow adaptation to the loss of the specific gene function. The similar phenomenon could occur in the sequential gene targeting in vitro to generate the knockout ES cells, which was the case of our *Rex1*-null ES cells.

To overcome the controversy between the knock-down and knock-out strategies, inducible knockout of the gene in ES cells provides an ideal solution since it

realizes both the rapid kinetics of the ablation of the specific gene function as in knock-downs and the specificity of the functional ablation as in knock-outs. Here we applied this strategy to reveal the function of *Xpc* in ES cells. As a result, we found that the C-terminal region of the Xpc protein containing all known functional domains is dispensable for the maintenance of pluripotency as well as the activation of the target genes of Oct3/4.

Methods

Cell culture

EB5 ES cells (derived from male E14tg2a ES cells) were cultured on a gelatin-coated dish in GMEM supplemented with 10% FCS, 1x sodium pyruvate, 1x non-essential amino acids, 10^{-4}M of 2-mercaptoethanol and 1000 U/ml of LIF.

Plasmid construction

For the generation of *Xpc* KO vector, genomic DNA fragments for the 5' and 3' homology arms (Chr:6, 91503806-91500760 and 91497700-91496532 in GRCm38) as well as the floxed region containing exon 9-10 (Chr:6, 91500759-91497701 in GRCm38) were

amplified from EB5 genomic DNA using the primer pairs Xpc S1: Xpc AS1, Xpc S2:

Xpc AS2 and Xpc S3: Xpc AS3 shown in Suplementary Table 1. The 5' and 3'arms were

digested with ClaI+AvrII and AvrII+NotI, respectively, and inserted into ClaI-NotI of

pBR-blue II., resulting in pBR-Xpc-5'+3'. The floxed region was digested by AvrII and

inserted into NheI of ploxP-BNA, resulting in ploxP-Xpc. Then the NotI fragment of

ploxP-Xpc was inserted into EcoRI of pBR-Xpc-5'+3' using the adaptor, resulting in

pBR-Xpc-5'+flox+3'. Finally, the Frt-SAIRESneopA-PGKpactkpA-Frt cassette was

introduced into the EcoRI site of pBR-Xpc-5'+flox+3', resulting in pXpc1-floxKO. The

PiggyBac vectors pPBCAG-MerCreMer-IH and pPBCAG-Egfp-IZ were constructed

based on pGG131 [22].

Generation of inducible *Xpc*-ΔC knockout ES cells

10^7 EB5 ES cells were electroporated with 100 μg of linealized *Xpc1* KO vector DNA at

800 V and 3 μF in a 0.4-cm cuvette using a Gene Pulser (Bio-Rad), followed by culture

with 320 μg/ml of G418 and 1.0 μg/ml of puromycin for 8 days. The resulting stem cell

colonies were picked up, expanded and genotyped by PCR using the primers Xpc S4:

IRES AS and Xpc loxP 1S: Xpc AS4. The correctly targeted clones ($Xpc^{flFRT/+}$) were

seeded in a well of 48-well plate at 10^4 cells per well, and transfected with 1 μg of circular

pCAG-FLPe-IP plasmid using Lipofectoamine 2000 (Invitrogen) followed by the culture

for 3 days. Then these transfected cells were replated and cultured with 1 μM of

Gancyclovir for 8 days. The resulting stem cell colonies were picked up, expanded and

genotyped by PCR using the primers Xpc loxP 2S: Xpc loxP3AS. The clone in which the

SAIRESneopA-PGKpactkpA cassette flanked by Frt were correctly removed ($Xpc^{fl/+}$)

were expanded to 10^7 cells and electroporated with 100 μg of linealized $Xpc1$ KO vector

DNA again. The second round targeting was done as the 1st round, resulting in the

establishment of the ES cells in which both Xpc alleles were modified for insertion of the

loxP sites ($Xpc^{fl/fl}$). The $Xpc^{fl/fl}$ ES cells were seeded on a well of a 48-well plate at 10^4

cells per well, and transfected with 0.25 μg of circular pPB-CAG-MerCreMer-IH, 0.25

μg of circular pPB-CAG-Egfp-IZ, and 0.5 μg of circular pCAG-PiggyBac transposase

(PBase) plasmid using Lipofectoamine 2000 followed by the culture for 3 days. Then

these transfected cells were replated and cultured with 200 μg/ml of Hygromycin B and

20 μg/ml of Zeocin for 8 days. The resulting stem cell colonies were picked up, expanded

and assessed for the expression of Egfp by fluorescent microscopic analysis as well as the

function of MerCreMer by PCR genotyping of the Tx-treated cells using the primers Xpc

S4: Xpc AS4. Finally, *Xpc$^{fl/fl}$:MCM:Egfp* and its descendant *Xpc$^{\Delta/\Delta}$* ES cells were

analyzed in this study.

Production of chimeric embryos

Dissociated ES cells were injected into a C57BL6 blastocyst by microinjection, which

was then transferred into the uterus of a pseudopregnant female ICR mouse. Embryos

were collected at 13.5 dpc to evaluate chimera contribution ability of ES cells by

analyzing with fluorescence microscopy. All animal experiments confirmed to our

Guidelines for the Care and Use of Laboratory animals and were approved by the

Institutional Committee for Laboratory Animal Experimentation (RIKEN Kobe

Institute).

Western blot

Western blot was performed with anti-Xpc (BETHYL, A301-122A) and anti-Cdk2

(Santa Cruz, sc-163) for the total cell lysate of *Xpc$^{fl/fl}$:MCM:Egfp* ES cells cultured with

or without Tx for 4 days, and *Xpc$^{\Delta/\Delta}$* ES cells.

Real-time PCR analysis

First strand DNA was synthesized from 500 ng of total RNA prepared by QuickGene

RNA cultured cell HC kit (KURABO) in 20 µl of the reaction mixture containing

oligo-dT primers using a ReverTra Ace first strand synthesis kit (TOYOBO). Real-time

PCR was performed with THUNDERBIRD SYBR qPCR Mix (TOYOBO) using

CFX384 Real-Time System (Bio-Rad). Sequence of primer pairs are listed in

Supplementary Table 1 (Xpc QS & QAS, Oct3/4 QS & QAS, Sox2 QS & QAS, Nanog

QS & QAS and Xpc primer pairs for different exons).

UV-sensitivity assay

Xpc inducible knockout ES cells cultured with or without Tx for 4 days, *Xpc*-null ES cells

and EB5 ES cells were dissociated and seeded on a 6-well plate (1000 cells/well). After 5

hours, cells were exposed to different doses of UV (254 nm, Toshiba GL15 lamp)

followed by the culture for 6 days. The colonies were fixed and stained by Leischman

staining solution, and counted to assess the colony-forming ability. All experiments were

performed in triplicate.

Microarray analysis

DNA microarray analyses were performed using a SurePrint G3 Mouse GE Microarray

8x60K (Agilent Technologies). Microarray results were analyzed using NIA Array

Analysis Software. Complete array data will be available on the GEO (NCBI) website.

Results

Generation of inducible knockout ES cells for the C-terminal region of *Xpc*

Xpc (*xeroderma pigmentosum, complementation group C*) consists of 16 exons and

encodes the protein containing 930 amino acids. Xpc protein carries the region conserved

in its yeast orthologue Rad4, which is encoded by exons 3-15. We designed the inducible

knockout vector to remove the exons 9-10 by the Cre-*lox* system, which deletes the

largest exon (exon 9) in the conserved region and causes a frame shit in the downstream

exons (Figure 1-a and 3C). The targeting vector containing 2.8kb of 5' arm and 1.2 kb of

3' arm flanking the *loxP* sites as well as the *SA-IRES-neo-pA:PGK-pacΔtk-pA* cassette

flanked by the *Frt* sites was linealized by ClaI at the end of the 5' arm and introduced into

EB5 ES cells (Figure 1-a). After the selection with G418 and puromycin for 8 days, the

colonies were picked, expanded and genotyped by PCR for the homologous

recombination events at the 5' and 3' arms (Figure 1-b). Among the 48 clones screened,

31 clones were correctly targeted at the 5' ends and 9 of 31 possessed the integration of

the 3' *loxP* site distal from the *Frt* cassette. The remained ones were the products of the

homologous recombination with the 5' arm and with the genomic region upstream of the

3' *loxP* site. One targeted clone was selected and transfected with the expression vector of

FLPe recombinase [16] followed by the selection with gancyclovir, resulting in the

isolation of the clones in which the *Frt* cassette was removed (Figure 1-c). One clone was

chosen for the second round targeting followed by the removal of the *Frt* cassette. As a

result, we obtained ES cells in which both *Xpc* alleles were modified to carry the *loxP*

sites at intron 7 and 10, designated as $Xpc^{fl/fl}$ ES cells (Figure 1-d). Then the expression

vectors for a hormone-inducible Cre recombinase (*MerCreMer*) [17] and *Egfp* were

introduced into $Xpc^{fl/fl}$ ES cells using the Piggy-bac transposon system, resulting in the

establishment of the inducible knockout ES cells for the C-terminal region of *Xpc* (Figure

1-e).

Inducible deletion of the C-terminal region of *Xpc* has no impact on self-renewal of

ES cells

The deletion of exons 9 and 10 is expected to remove the coding sequence for 345 amino acids (between 326 to 670 aa) and to cause the frame shift to the remained downstrem conding sequence, resulting in the deletion of the C-terminal region. We tested the effect of the Cre recombinase activition by 4-hydroxy tamoxifen (Tx) on removing the genomic region flanked by the *loxP* sites. We confirmed the deletion of the exons 9 and 10 and the down-regulation of wild-type *Xpc* transcripts at 1 day after the addition of Tx (Figure 2A and data not shown). At 4 days after the addition of Tx, the morphology of the cells cultured with Tx was indistinguishable from that without Tx (Figure 2B) despite the severe down-regulation of the wild-type Xpc protein observed by western blot with the antibody recognizing the C-terminal region (Figure 2F). There was no immediate effect of the elimination of wild-type Xpc on the expression of pluripotency markers (Figure 2E). To evaluate the impact of the loss of wild-type Xpc on self-renewal of ES cells, the inducible knockout ES cells cultured with or without Tx for 2 days were dissociated and replated at a clonal density, and the numbers of the stem cell colonies were counted based on their morphology (Figure 2C). As a result, there was no change in the number of the stem cell colonies (Figure 2D), indicating that there was no immediate effect of the

elimination of wild-type Xpc on self-renewal of ES cells. When the stem cell colonies

from the ES cells treated with Tx were isolated and genotyped, 91% of the clones were

homozygous for the deletion between the *loxP* sites (data not shown), confirming the

efficient elimination of the wild-type Xpc function by the treatment with Tx. These data

indicated that the inducible knockout system worked efficiently and the elimination of the

C-terminal region of *Xpc* with rapid kinetics had no effect on self-renewal of ES cells.

Xpc-ΔC ES cells retain pluripotency

We successfully expanded the ES cell clones homozygous for the deletion between the

loxP sites. These *Xpc*-ΔC ES cells were morphologically normal (Figure 2B) and

proliferated as fast as the wild-type ES cells. They kept the expression of the

pluripotency-associated genes *Oct3/4, Sox2* and *Nanog* but lacked the wild-type Xpc

protein detectable by the antibody that recognizes the epitope at C-terminal (Figure 2E

and F). When these ES cells were injected into the blastocysts followed by the transfer

into the uteri of pseudo-pregnant mother mice, we obtained chimeric embryos at 13.5

days with high contribution of *Xpc*-ΔC ES cells (Figure 2G). These data indicated that the

Xpc-ΔC ES cells retain proper pluripotency.

Proper deletion event occurs in the *Xpc* inducible-knockout ES cells

It was reported that the Xpc protein possesses three functional sites at the C-terminal region; the Rad23B interaction site (position 488-727 aa), the single strand DNA binding site (position 599-735 aa), Centrin2 binding site (position 840-859 aa) and the TFIIH interaction site (position 809-930 aa) [18, 19]. To confirm the proper elimination of these sites in our inducible C-terminal knockout ES cells, we examined the transcripts produced by the knockout allele lacking exons 9 and 10. QPCR analyses of *Xpc* transcripts in *Xpc*^fl/fl^ ES cells cultured with or without Tx, and the *Xpc*-ΔC ES cells revealed that the truncated transcripts lacking exon 9 and 10 are expressed by the knockout allele at comparable levels to the wild-type transcripts expressing in *Xpc*^fl/fl^ ES cells cultured without Tx, which can be detected by the primer pairs set in the remained exons; exons 2-4, 5-6, 12-13 and 14-16 (Figure 3A). The QPCR with the primers specifically detect the mutant form of transcripts (8/11 S and AS in Figure 3C), in which the splicing event between exon 8 and 11 occured, revealed that the proper deletion event

was mediated by the Cre-*loxP* system in Tx-dependent manner with low background

(Figure 3A). The RT-PCR with the primers in exon 7 and 12 showed that the majority of

the truncated transcripts were produced by the splicing between exon 8 and 11 based on

the size of the PCR products that fit to the expected size (Figure 3B). When these PCR

products were sequenced, we found the precise splicing event between exon 8 and 11

(data not shown). The splicing between exon 8 and 11 causes frame shift and premature

termination of translation that resulted in production of the truncated protein of 326 aa

followed by 26 aa frame-shift product (Figure 3C). These data suggested that the

modified *Xpc-ΔC* allele in our inducible C-terminal knockout ES cells might produce the

short truncated form of Xpc lacking the C-terminal region including all known functional

domains.

Knockout of *Xpc* causes hypersensitivity to DNA damage

It was reported that the loss of *Xpc* function resulted in increased sensitivity to DNA

damage since *Xpc* is required to mediate the DNA excision repair [13]. To confirm the

functional abolishment of *Xpc* in our inducible C-terminal knockout ES cells, we tested

their sensitivity to ultraviolet (UV) light before and after the excision of the floxed exons.

Xpc inducible C-terminal knockout ES cells cultured with or without Tx for 4 days,

Xpc-ΔC ES cells and wild-type ES cells were seeded at a clonal density. After 5 hours,

these ES cells were exposed to different doses of UV followed by the culture for 6 days to

form colonies. The stem cell colony formation ability of the inducible C-terminal

knockout ES cells treated with Tx and the *Xpc*-ΔC ES cells were severely affected by

low-doses of UV (Figure 3A and B), indicating their hypersensitivity to UV-induced

DNA damage. These data confirmed the proper loss of function of *Xpc* for DNA repair in

our inducible C-terminal knockout ES cells.

Gene expression profile in inducible and constitutive *Xpc*-ΔC ES cells

Since the function of the Xpc complex as a coactivator of Oct3/4 was suggested, the gene

expression profiles were quantitatively examined by the microarray analyses in the *Xpc*

inducible C-terminal knockout ES cells cultured with or without Tx for 4 days and the

Xpc-ΔC ES cells. A pair-wise comparison of the inducible C-terminal knockout ES cells

treated with or without Tx (false discovery rate (FDR)<0.05, gene expression difference

> 1.5-fold) showed no genes whose expression levels was significantly different (Figure 4A). Since the probe for Xpc was set at the exon 1, the expression level of *Xpc* was not significantly altered by the inducible C-terminal knockout. The similar comparison of the inducible C-terminal knockout ES cells without Tx and the *Xpc-ΔC* ES cells showed only 136 differentially-expressed genes (Figure 4B), indicating that the loss of the C-terminal region of Xpc has a faint impact on the global gene expression pattern in ES cells.

Next we examined the expression patterns of the putative target genes of Oct3/4 that we identified previously [5]. When the altered expression levels of the 358 Oct3/4 target genes by the Tx treatment in the inducible knockout ES cells were compared to those of the all genes, there was no significant difference (Figure 4C). These data demonstrated that there is no specific effect of the deletion of the C-terminal region of Xpc on the expression of the Oct3/4 target genes.

Discussion

Pluripotency is primarily defined by the specific gene expression pattern directed by a set of transcription factors as evidenced by the ability of the ectopic expression of 4

transcription factors (Oct3/4, Sox2, Klf4 and Myc) to trigger the reprogramming of somatic cells to pluripotent stem cells [20]. Among them, Oct3/4 has a pivotal role and its expression at the optimized level is required for the continuous self-renewal of mouse ES cells [6]. To keep its transcriptional activity within the optimal range, there are multiple co-regulators of Oct3/4 that regulate the transcriptional activity either positively or negatively [21, 22]. The Xpc DNA repair complex was proposed as one of the coactivators of Oct3/4 in mouse ES cells [10], but here, using the inducible C-terminal knockout system, we revealed that the function of the C-terminal region of Xpc including the binding sites for Rad23B and Cetn2 is dispensable for the function of Oct3/4.

To settle down the common controversy between the knockdown and knockout strategies, we applied the inducible knockout using the Cre-*loxP* system. We confirmed the rapid kinetics of the elimination of the floxed exons after the addition of Tx and the functional abolishment of *Xpc* for DNA repair by the increase of UV-sensitivity as reported previously [14]. Moreover, the inducible knockout system allowed the precise pair-wise comparison of the transcriptome of ES cells with or without the C-terminal region of Xpc, which resulted in no significant change. Finally the pluripotency of the

Xpc-ΔC ES cells was confirmed by their ability to contribute to chimeric embryos after blastocyst injection. On the other hand, we failed to rule out the possibility that the knockout allele produce the truncated form of Xpc containing 326 aa of the N-terminal region since the truncated transcripts were expressed at the comparable levels to that of wild-type transcripts found in *Xpc$^{fl/fl}$* ES cells and no antibody was available for the N-terminal region of Xpc to detect the putative truncated protein. However, since the putative product from the C-terminal knockout allele lacks all known functional sites of Xpc including the interaction sites with Rad23B and Cetn2 to form the Xpc/Rad23b/Cetn2 complex [18,19], these data strongly suggested that the Xpc complex formation is dispensable for the maintenance of pluripotency in mouse ES cells. It was proposed that the function of Xpc in the Xpc/Rad23b/Cetn2 complex might be dispensable but there are no experimental evidence supporting this idea [10]. The inducible knockout of *Rad23a/b* and *Cetn2* will provide a clear answer to this argument.

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Competing Financial Interests

The authors declare no competing financial interests.

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Figure legends

Figure 1. Scheme of inducible knockout of *Xpc* in ES cells. The knockout vector of

Xpc carrying the 2 *loxP* sites and the drug-resistant gene cassette flanked by the *Frt* sites

were introduced into ES cells followed by the selection with G418 and puromycin (a) and

the correctly targeted clones were identified by genome PCR (b). The drug-resistant gene

cassette was removed by the transient expression of *FLPe* followed by the selection with

Gancyclovir (c). The steps a~c were repeated for the second round targeting, resulting in

the establishment of the $Xpc^{fl/fl}$ ES cells (d). Then the expression vector for

hormone-inducible form of Cre (MerCreMer) was introduced into the $Xpc^{fl/fl}$ ES cells to

establish the Xpc inducible knockout ES cells, in which the floxed exons 9 and 10 were

removed by the treatment with Tx (e). *SA: En2 splice acceptor, IRES: internal ribosome*

entry site of mouse encephalomyocarditis virus, neo: neomycin phosphotransferase

II, pA: polyA signal, PGKp: mouse phosphoglycelate kinase promoter, pacΔtk: a

fusion gene of puromycin N-acetyltransferase and *herpes simplex virus thymidine kinase,*

CAG: CAG promoter [24]*, MerCreMer: hormone inducible from of Cre* [17]*, hph:*

hygromycin B phosphotransferase.

Figure 2. Inducible knockout of *Xpc* causes no effect on pluripotency. A. The PCR of

genomic DNA from $Xpc^{fl/fl}$ ES cells cultured with or without Tx for 3 days. The floxed

allele (fl) gives 7.5 kb band and the deleted allele (Δ) gives 4.0 kb band. **B.** The

morphology of the colonies of wild-type EB5, *Xpc^{fl/fl}* ES cells cultured with or without Tx,

and *Xpc*-knockout (*Xpc^{Δ/Δ}*) ES cells cultured 4 days. **C.** The colony staining derived from

Xpc^{fl/fl} ES cells cultured with or without Tx for 2 days. **D.** The colony numbers derived

from *Xpc^{fl/fl}* ES cells cultured with or without Tx for 2 days. Data were obtained from

triplicated samples and shown with standard deviation. **E.** The gene expression pattern of

of wild-type EB5, *Xpc^{fl/fl}* ES cells cultured with or without Tx, and *Xpc*-knockout (*Xpc^{Δ/Δ}*

ES cells cultured 4 days for pluripotency markers and *Xpc*. Q-PCR was performed with

biological triplicates and the relative expression levels to wild-type ES cells (set at 1.0)

were shown with standard deviation. **F.** Western blot analysis of Xpc in *Xpc^{fl/fl}* ES cells

cultured with or without Tx, and *Xpc*-knockout (*Xpc^{Δ/Δ}*) ES cells cultured 4 days. **G.**

Chimeric embryos obtained by the injection of *Xpc^{fl/fl}* and *Xpc^{Δ/Δ}* ES cells carrying the

ubiquitous EGFP expression vector.

Figure 3. Transcripts from the Xpc knockout allele lacking exons 9 and 10. A. QPCR

analyses of the Xpc transcripts expressing in *Xpc^{fl/fl}* ES cells cultured with or without Tx,

and *Xpc*-knockout (*Xpc^{Δ/Δ}*) ES cells. Q-PCR was performed with biological triplicates

and the relative expression levels to $Xpc^{fl/fl}$ ES cells cultured without Tx (set at 1.0) were shown with standard deviation. **B.** The RT-PCR of Xpc transcripts from $Xpc^{fl/fl}$ ES cells cultured with or without Tx and Xpc-knockout ($Xpc^{\Delta/\Delta}$) ES cells. The predicted size of the transcripts from floxed allele (wt) and deleted allele (Δ) were 1399 bp and 364 bp, respectively. **C.** The structure of the transcripts from the deleted allele. The positions of primers 8/11 S and 8/11 AS to detect the truncated transcripts were indicated.

Figure 4. Survival of ES cells after exposure to UV-light. A. Wild-type ES cells (EB5) and Xpc knockout ES cells ($Xpc^{\Delta/\Delta}$) were seeded in 6-well plates (3 of each) and exposed to the UV-light at the indicated doses. The colonies derived from the survived cells were counted after 6 days and the relative survival ratio (no UV-light exposure was set at 100%) were shown from the triplicated samples with standard deviation. **B.** $Xpc^{fl/fl}$ ES cells cultured with or wothout Tx for 4 days were assayed as A.

Figure 5. Gene expression profiles in Xpc inducible knockout ES cells. A. Scatter plot of DNA microarray data from $Xpc^{fl/fl}$ ES cells cultured with or without Tx for 4 days. No significant difference was observed (false discovery rate (FDR)<0.05, gene expression

difference > 1.5-fold). **B.** Scatter plot of DNA microarray data from $Xpc^{fl/fl}$ ES cells

cultured without Tx for 4 days and Xpc-null ES cells ($Xpc^{\Delta/\Delta}$). 136 genes showed

significant difference for their expression levels (false discovery rate (FDR)<0.05, gene

expression difference > 1.5-fold). **C.** Comparison of the relative gene expression levels of

the putative Oct3/4 target genes and the other genes in $Xpc^{fl/fl}$ ES cells cultured with or

without Tx for 4 days. 100 each of target and non-target genes were randomly selected

and their relative expression levels from the DNA microarray data set were compared in

triplicate.

Table 1. Sequences of the primers.

Primer name	Sequence (5' to 3')
Xpc S1	aatatcgataagctcaaaatgggtaggaacctggag
Xpc AS1	attcctaggtgggactcacactgtggaagacaagag
Xpc S2	attcctaggaattcgggactgaactcccattgtcaggcttg
Xpc AS2	attcctaggatatcctacagccttgtattttaacagtcctg
Xpc S3	attgaattcaagtgttcctcgaatctaatgtacacc
Xpc AS3	attgcggccgctcagctagcacctctaacctgtttgag
Xpc S4	tggaaggtgcatgttgtcttagtcagg
Xpc AS4	tctttcaggaaagctcaacactctctg
IRES AS	ccttattccaagcggcttcggccag
Xpc loxP 1S	accatgttaggcacctcgatccataac
Xpc loxP 2S	tccacagtgtgagtcccagaattgaac
Xpc loxP 3AS	aggaacacttgaattgaaccggtagcg
Xpc QS	tcggagcctacttacggaga
Xpc QAS	gcttctccacgacaataccc
Oct3/4 QS	aagccctccctacagcagat
Oct3/4 QAS	ctgggaaaggtgtccctgta
Sox2 QS	gagtggaaactttgtccgaga
Sox2 QAS	gaagcgtgtacttatccttcttcat
Nanog QS	ccaggttccttccttcttcc
Nanog QAS	ggtgagatggctcagtggat
Xpc Ex2 S	gccaaagccactgctaaatc
Xpc Ex4 AS	catgtccagcacaggttcag
Xpc Ex5 S	atacctgcggaggatgatga
Xpc Ex6 AS	agcgaattggaatgatggag
Xpc Ex12 S	gacttgggcctctatggtca
Xpc Ex13 As	tcaggttaggcagggtcatc
Xpc Ex15 S	tgaacaggccatcattgaaa
Xpc E16 AS	tgtgaactggtcccttcctc
Xpc Ex7 S	ctgtcaacgctgacctttca

Xpc Ex8-11 AS	gtatgcacgcaatccctttg
Xpc Ex8-11 S	cagctgtgacaaagggattg
Xpc Ex12 AS	tgaccatagaggcccaagtc

ACCESSION: *Oct3/4(Pou5f1)* NM_013633, *Sox2* NM_011443, *Nanog* NM_028016, *Xpc* NM_009531

Figure 1

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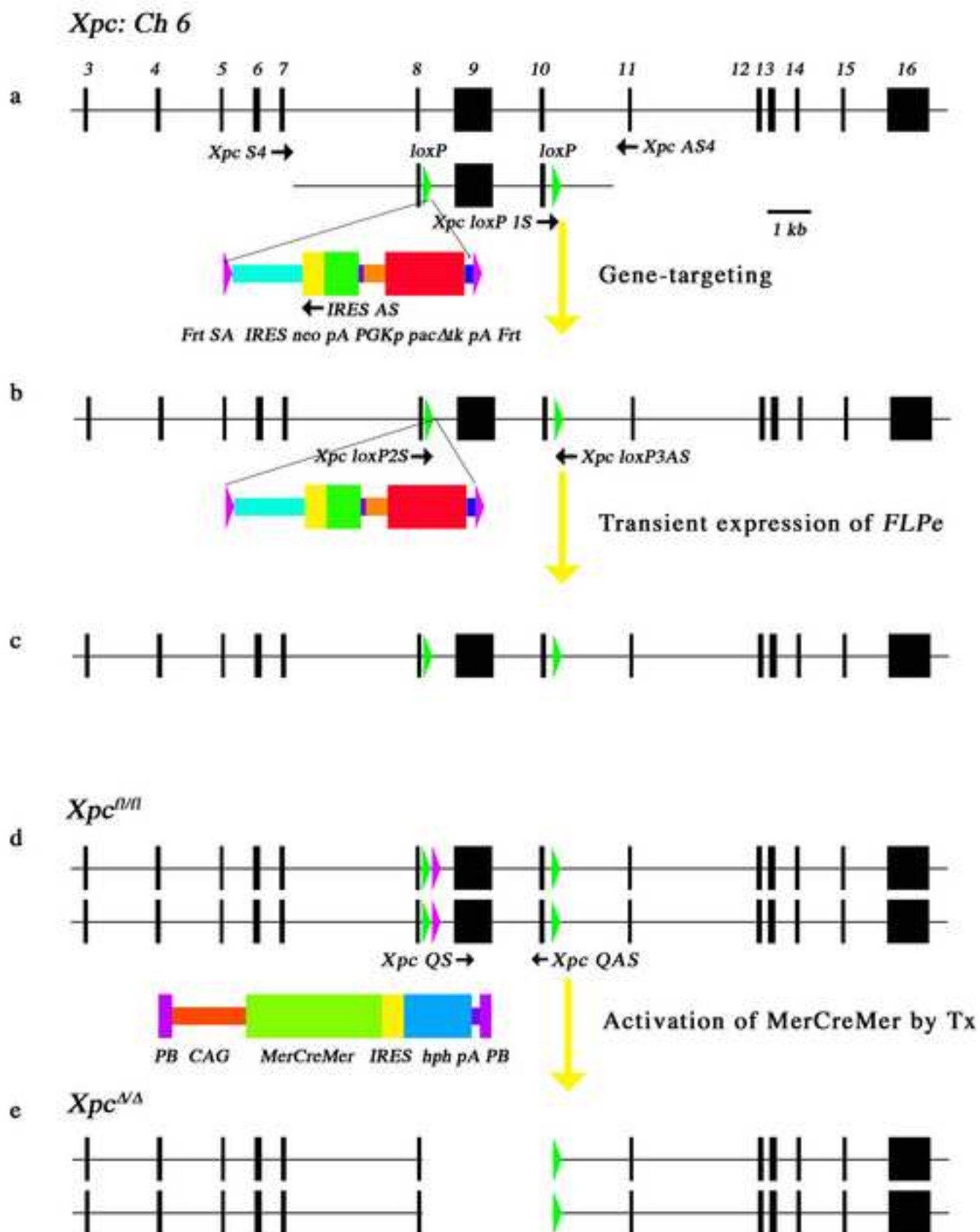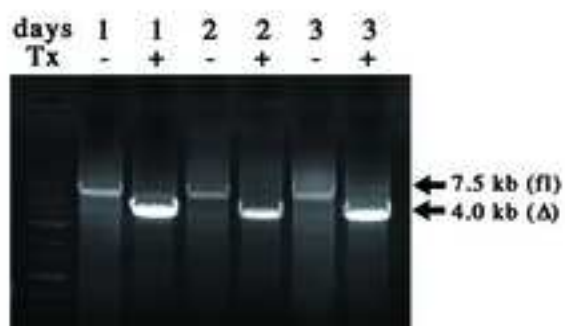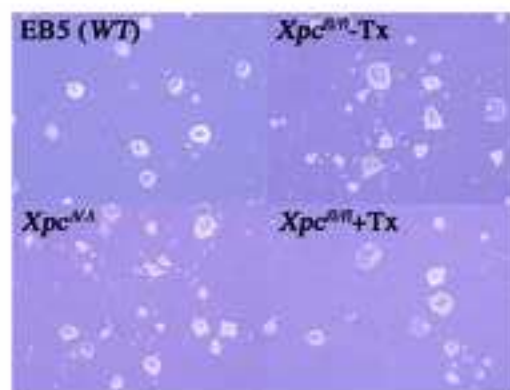

Xpc: Ch 6

a

3 4 5 6 7 8 9 10 11 12 13 14 15 16

Xpc S4 →

loxP   *loxP*   ← *Xpc AS4*

Xpc loxP 1S →

1 kb

Gene-targeting

← *IRES AS*

Frt SA IRES neo pA PGKp pacΔtk pA Frt

b

Xpc loxP2S →   ← *Xpc loxP3AS*

Transient expression of *FLPe*

c

d *Xpc^{fl/fl}*

Xpc QS →   ← *Xpc QAS*

PB CAG   MerCreMer IRES hph pA PB

Activation of MerCreMer by Tx

e *Xpc^{Δ/Δ}*

Figure 2

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A

days 1 1 2 2 3 3
Tx - + - + - +

← 7.5 kb (fl)
← 4.0 kb (Δ)

B

EB5 (WT)   Xpc^(fl/fl)-Tx

Xpc^(Δ/Δ)   Xpc^(fl/fl)+Tx

C

D

Number of colonies

+Tx   -Tx

E

■ Xpc^(fl/fl)-Tx
■ Xpc^(fl/fl)+Tx
■ Xpc^(Δ/Δ)
■ Wt

Oct3/4   Sox2   Nanog   Xpc

F

Xpc^(fl/fl)-Tx
Xpc^(fl/fl)+Tx
Xpc^(Δ/Δ)

Xpc

Oct3/4

Cdk2

G

Xpc^(fl/fl)   Xpc^(Δ/Δ)

Figure 3
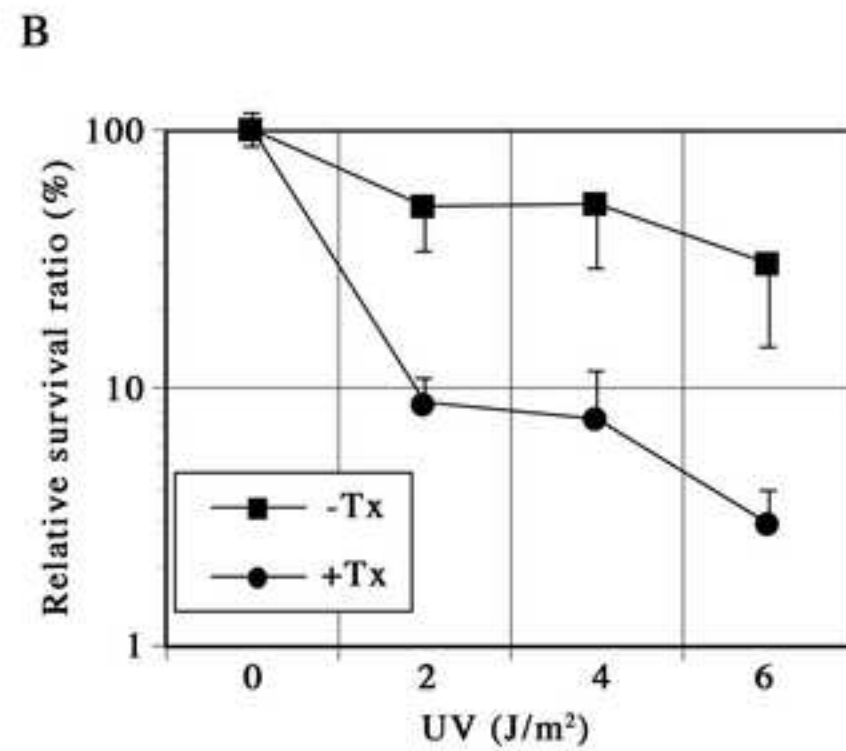
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A

B

C

Figure 4

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A

B

Figure 5

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