Published in final edited form as:
Gastroenterology. 2011 May ; 140(5): 1547–55.e10. doi:10.1053/j.gastro.2011.01.048.

The Nuclear Pore Complex Protein Elys Is Required for Genome Stability in Mouse Intestinal Epithelial Progenitor Cells

Nan Gao[1], **Gangarao Davuluri**[2], **Weilong Gong**[2], **Christoph Seiler**[2], **Emma E. Furth**[3], **Klaus Kaestner**[1], and **Michael Pack**[2,4,^]

[1]Department of Genetics, University of Pennsylvania School of Medicine

[2]Department of Medicine, University of Pennsylvania School of Medicine

[3]Department of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine

[4]Department of Cell and Developmental Biology, University of Pennsylvania School of Medicine

Abstract

Background & Aims—Elys is a conserved protein that directs nuclear pore complex (NPC) assembly in mammalian cell lines and developing worms and zebrafish. Related studies in these systems indicate a role for Elys in DNA replication and repair. Intestinal epithelial progenitors of zebrafish *elys* mutants undergo apoptosis early in development. However, it is not known whether loss of Elys has similar effect in the mammalian intestine or whether the NPC and DNA repair defects each contribute to the overall phenotype.

Methods—We developed mice in which a conditional *Elys* allele was inactivated in the developing intestinal epithelium and during pre-implantation development. Phenotypes of conditional mutant mice were determined using immunohistochemical analysis for nuclear pore proteins, electron microscopy, and immunoblot analysis of DNA replication and repair proteins.

Results—Conditional inactivation of the *Elys* locus in the developing mouse intestinal epithelium led to a reversible delay in growth in juvenile mice that was associated with epithelial architecture distortion and crypt cell apoptosis. The phenotype was reduced in adult mutant mice, which were otherwise indistinguishable from wild-type mice. All mice had activated DNA damage responses but no evidence of NPC assembly defects.

Conclusions—In mice, Elys maintains genome stability in intestinal epithelial progenitor cells, independent of its role in NPC assembly in zebrafish.

^Corresponding Authors: Michael Pack, M.D., Department of Medicine, University of Pennsylvania School of Medicine; mpack@mail.med.upenn.edu. Klaus Kaestner, Ph.D., Department of Genetics, University of Pennsylvania School of Medicine; kaestner@mail.med.upenn.edu.
•These authors contributed equally to this work

Author contributions: Study concept and design (NG, GD, WG, CS, KHK, MP); Analysis and interpretation of data (NG, GD, WG, EEF, KHK, MP); Drafting of the manuscript (MP); Critical revision of the manuscript for important intellectual content (NG, GD, KHK, MP).

No conflicts of interests exist for any authors

Keywords

DNA repair; Nuclear pore complex; Elys; Intestinal epithelium; Progenitor cell

The principal role of the nuclear pore complex (NPC) is to regulate transport of macromolecules between the nucleus and cytoplasm. During dissolution of the nuclear envelope in mitosis, the approximately 30 proteins that make up the NPC disassemble into membrane-bound oligomeric subunits. When the nuclear envelope reforms at the end of mitosis, the NPC subcomplexes reassemble to form functional pores. In vitro studies conducted using mammalian cells and *Xenopus* nuclear extracts have suggested that NPC assembly is initiated by Elys, the vertebrate ortholog of *mel-28*, a gene that is required for nuclear integrity in worms[2–7]. Recent work indicates that Elys initiates NPC assembly by first binding AT-rich chromatin sites, then recruiting the Nup107–160 subcomplex, followed by the pore integral membrane proteins POM121 and NDC1[8]. In addition to interacting with nucleoporins, Elys also has been shown to bind kinetochores and proteins of the Mcm2/7 complex, suggesting a role for Elys in cytokinesis and DNA replication[6, 7].

Recently, we reported that Elys is required for NPC assembly in developing zebrafish larvae[2]. Zebrafish *elys*$^{flo\text{-}ti262}$ mutants, hereafter referred to as *flo,* survive to larval stages most likely as a result of maternally derived Elys protein. In *flo* larvae, NPC assembly is disrupted in retinal and intestinal epithelial progenitor cells. The NPC defect within these cells causes development arrest and apoptotic cell death. A non-complementing zebrafish *elys* allele was also reported to disrupt NPC assembly and was associated with epithelial cell apoptosis in the developing intestine[9]. We have shown that apoptosis in *flo i*ntestinal progenitors occurs independently of p53 and is potentiated by DNA replication inhibitors, such as hydroxyurea and UV-irradiation[2]. This finding suggested a role for Elys in the repair of endogenous replication errors, possibly through its previously reported interaction with the Mcm2/7 complex[6]. Supporting this notion, levels of chromatin-bound Mcm2 were reduced in Elys intestinal epithelial progenitors[2]. We speculated that this led to a reduction in the number of dormant origins competent to rescue spontaneously stalled replication forks, as occurs when worms and mammalian cells are partially depleted of Mcm proteins[10, 11]. These findings also raised the possibility that Elys's role in DNA repair is independent of its role in NPC assembly.

Previous studies have shown that Elys is required for the survival of inner mass cells during pre-implantation stages of mammalian development[12]. The precise cause of inner cell mass death in these mutants was not determined, thus, whether Elys is required for NPC assembly during mammalian development is not known. To examine the function of Elys in the mammalian intestine, we engineered a conditional *Elys* loxP allele that facilitated the derivation of intestine-specific *Elys* knockout mice. Strikingly, *Elys* disruption in the developing intestinal epithelium caused widespread apoptosis of crypt progenitor cells that led to growth delay of juvenile mice, but it had no effect on NPC assembly. Progenitor cell apoptosis was partially compensated in adult conditional mutants and this was sufficient to reverse the growth delay phenotype. Persistent apoptosis in adult *Elys* deficient crypts was accompanied by activation of the DNA damage response pathway, thus indicating that Elys is constitutively required for genome maintenance in intestinal progenitors.

All together, these findings indicate that in mammals Elys functions to maintain genome stability in a stage- and tissue-specific manner independent of its previously described role in NPC assembly. Our findings suggest a role for NPC proteins in DNA repair in mammalian cells, as suggested by previous studies in yeast and other fungi[13–16].

Materials and Methods

Animals

All animals were handled in strict accordance with good animal practice as defined by the relevant national and/or local animal welfare bodies, and all animal work was approved by the appropriate animal welfare committee (IACUC).

Derivation of mouse *Elys* conditional allele and intestine-specific *Elys* knockouts

Details of these methods are presented as supplemental data. Briefly, a targeting vector containing loxP sites flanking exon 3 of the *Elys* gene was derived through BAC recombineering of a genomic DNA fragment spanning exons 2–6 of the *Elys* locus. A FRT-flanked neomycin resistance gene was recombined into intron3. Targeted ES cell clones were identified by Southern blot analysis. The FRT-flanked neomycin resistance gene was removed by crossing $Elys^{loxP/+}$mice to *Flp1* mice from Jackson Laboratory[17]. The resulting $Elys^{loxP/+}$ mice were crossed with Villin-Cre mice[18] to derive $Elys^{loxP/loxP}$*; Villin-Cre* mice.

RNA isolation, RT-PCR and sequencing analysis

Total RNA was extracted from mouse tissues using DNeasy blood and tissue kit (Qiange). For RT-PCR, 1 μg of total RNA was reverse-transcribed with SuperScript III reverse-transcriptase (Invitrogen Life technologies) using random hexamer primers. 1 μl of first strand cDNA was used in a 50- μl PCR reaction with pfuTurbo DNA polymerase (Stratagene). RT-PCR products amplified by primer sets flanking two loxP sites were purified for direct sequencing (QIAquick, Qiagen).

Chromatin Isolation and Western analyses

Nuclear extracts of intestine were obtained from wild type and *Elys* conditional mutants using standard protocols that are presented as supplemental data. Chromatin immunoprecipitation was performed using a rabbit anti-Histone H2 antibody (Upstate Biotechnology or Sigma Aldrich). Half of the recovered samples were run on a 10% SDS-PAGE gel for western analysis. One tenth of the sample volume was run as a control for immunoprecipitation (input).

Immunohistochemical and transmission EM analysis

The immunohistochemical and EM procedures have been previously described[19]. Primary antibodies used in this study include rabbit polyclonal anti-γH2AX (Cell Signaling, Boston, MA), mouse anti-mAb414 (Covance, Berkeley, CA), and rabbit anti-Nup133 (Abcam, Cambridge, MA). TUNEL staining was performed following the protocol accompanying the Apoptosis Detection Kit (Millipore). For TUNEL quantification, crypts containing at least one TUNEL-positive cell were counted for 5–8 independent intestinal stretches of each animal. At least 2–4 animals of different stages for each genotype were analyzed.

Results

Derivation of the conditional *Elys* allele

The zebrafish $elys^{flo\text{-}ti262c}$ allele encodes a premature stop codon that is predicted to truncate 1209 of the 2519 amino acids in the Elys protein[2]. An independently derived non-complementing *elys* allele encodes a nonsense codon at position 461[9]. To generate a conditional *Elys* allele comparable to $elys^{flo\text{-}ti262c}$, we introduced loxP sites into introns 2 and 3 of the mouse *Elys* locus[20] using a targeting vector engineered through BAC recombineering (Figure 1A, B). Accurate targeting of the *Elys* locus in mouse embryonic stem cells was confirmed by Southern blot analysis and PCR of genomic DNA (Figure 1C,

D). After germline transmission of the targeted allele, the FRT-flanked neomycin resistance gene was removed by crossing *Elys*$^{loxP/+}$mice to *Flp1* deleter mice[17]. Viable and fertile *Elys*$^{loxP/loxP}$ mice with no detectable phenotype were recovered at the expected Mendelian frequency from intercrosses of the *Elys2*$^{loxP/+}$ mice, confirming that the *Elys*^{loxP} allele encodes a functional protein before Cre-mediated deletion.

Mouse embryos homozygous for an *Elys* null allele die before implantation[12]. The conditional *Elys* allele is predicted to generate a null allele following Cre-mediated recombination because of a reading frame shift caused by deletion of exon 3 (Figure 1E, F and Supplemental Figure 1). Consistent with this prediction, cDNA sequence analyses revealed multiple in frame stop codons in mRNA derived from the recombined *Elys* allele (Supplemental Figure 1, 2). To determine whether the frame shift indeed led to an *Elys* null allele *in vivo*, we employed *E2A-Cre* mice to inactivate *Elys*$^{loxP/loxP}$ at the two-cell stage[21]. Of over twenty genotyped offspring, no *Elys*$^{loxP/loxP}$; *E2A-Cre* progeny were recovered from the cross between *Elys*$^{loxP/+}$; *E2A-Cre* and *Elys*$^{loxP/loxP}$ mice. This provided further evidence that removal of exon 3 resulted in a complete loss-of-function *Elys* allele.

Inactivation of *Elys* in the developing intestinal epithelium causes a reversible growth delay phenotype

To examine the role of Elys in the intestine, we derived *Elys*$^{loxP/loxP}$;*Villin-Cre* mice. The *Villin* promoter used in this study drives Cre expression within crypt and villus epithelial cells of the small intestine starting at around embryonic day 14[18]. Viable newborn *Elys*$^{loxP/loxP}$;*Villin-Cre* mice were recovered at the expected Mendelian ratios. Although the newborn mutants and P6 were indistinguishable from wild type littermates, P12–P28 mutants were considerably smaller than their siblings (Figure 2A and Supplemental Figure 3). Western blot analyses showed only a small amount of Elys protein remaining in intestines of P7 and P12 mutants (Figure 2A and data not shown), presumably representing Elys in proliferating smooth muscle and other non-epithelial cells. Remarkably, at P60 the mutants were no longer distinguishable in size from wild type littermates (data not shown). Western blot for Elys at this stage using an antibody directed against a carboxy-terminus epitope showed no detectable Elys protein in the mutant intestine (Figure 2A), because of Elys disruption in the epithelium and, presumably, very low levels of Elys in non-proliferating smooth muscle and other stromal cells. Thus, conditional inactivation of *Elys* within the intestinal epithelium causes a transient growth phenotype in juvenile mice.

Inactivation of *Elys* in the developing intestinal epithelium disrupts progenitor cell survival

To further characterize the Elys-deficient intestinal phenotype, we performed histological analyses of juvenile and adult *Elys* mutants. Severe disruption of small intestinal epithelial architecture with crypt distortion and crypt cell nuclear condensation typical of apoptosis (confirmed by TUNEL assay and anti-Caspase-3 immunostains) were present in P7 and P15 mutant mice (Figure 2A, D; Supplemental Figure 4). The most severe histological changes were detected in the jejunum but TUNEL positive cells were seen in crypts in all small intestinal segments. Crypt architecture distortion was also seen in duodenal and ileal crypts (Supplemental Figure 4). Interestingly, the mutant villi associated with apoptotic crypts were often shorter than in wild type animals but the villus epithelium appeared normal. Many mutant crypts were associated with normal length villi, thus indicating that a subset of progenitor cells differentiated normally (data not shown).

To determine the identity of the apoptotic crypt cells we performed double immunostains for the DNA damage marker γH2AX and the Paneth cell marker lysozyme and the enteroendocrine cell marker Chromogranin-A. These stainings showed that the apoptotic crypt cells were not Paneth cells or enteroendocrine cells (Supplemental Figure 5). This

finding strongly suggests that the dying cells were transit amplifying progenitor cells, although we cannot exclude the possibility that some crypt stem cells are also affected by Elys deficiency. Altered crypt architecture in the juvenile mutants is also consistent with a regenerative response to compensate for progenitor cell death. This regenerative response, coupled with the relative sparing of the villus epithelium and the absence of overt symptoms of malabsorption in the juvenile *Elys* mutants, suggests that their growth delay phenotype arose in part from energy expenditure associated with progenitor cell injury.

In contrast to the histology at juvenile stages, at P60 mutant intestinal histology was indistinguishable from wild type (Figure 2E). However, TUNEL staining at this stage detected persistent crypt apoptosis (Figure 2A, C; two strongly affected mutant crypts are shown), although the absolute number of apoptotic cells was reduced compared with P7 mutants (data not shown). Analysis of mutant intestines at early embryonic and perinatal stages revealed significant apoptosis in cells located within the intervillus epithelial pockets (Figure 2B, and Supplemental Figure 6). These data show that conditional inactivation of *Elys* in developing intestinal epithelia generates a progenitor cell phenotype. In contrast to the requirement of Elys in crypt progenitor cells, Elys is dispensable for the development and survival of the post-mitotic villus epithelial cells.

NPC assembly occurs normally in Elys-deficient in crypt progenitor cells and villus epithelial cells

To determine whether progenitor cell apoptosis in *Elys* mutants was associated with defective NPC assembly, we performed immunostainings using an antibody directed against the FG-Nup nucleoporins[1]. In wild type intestinal epithelia, FG-Nups were detected in a perinuclear distribution in both villus and crypt cells (Figure 3A, B). The distribution of the immunoreactive FG-Nups was less symmetrical than in a cultured Caco2 intestinal cell line (Supplemental Figure 7). We speculate that this arose from tissue processing or sectioning. The distribution of FG-Nups in Elys-deficient intestinal epithelial cells at all stages examined was indistinguishable from control intestinal cells (Figure 3A, B). Cytoplasmic membrane bound nucleoporins (annulate lamellae) indicative of defective NPC assembly were not detected in the Elys-deficient intestines, whereas they are abundant in zebrafish *flo* mutants and Elys-deficient mammalian cells[2, 4, 7, 9]. Western blot analyses of nuclear and cytoplasmic fractions showed only minimal levels of cytoplasmic FG-nup nucleoporins in mutant cells (Figure 3D). Transmission electron micrographs further revealed abundant and morphologically identical NPCs in both crypt and villus epithelial cells of Elys-deficient intestines (Figure 3C, Supplemental Figure 8). All together, these data demonstrate that NPC assembly occurred normally in Elys-deficient intestinal epithelial cells.

Because zebrafish and *C. elegans* germline *elys* mutants have a pronounced NPC phenotype, we considered the possibility that NPC assembly in the conditional *Elys* mutants could be accounted for by a residually active truncated Elys protein that was not detected in Western blots. As noted previously, mRNA derived from the recombined allele was shown to contain multiple in frame nonsense codons (Supplemental Figure 1 and 2). These lie upstream of the nuclear localization signals and the AT-hook domain that is required for Elys interaction with chromatin[6, 8]. Thus, even if this truncated protein was stable following translation, it is not likely that it would retain activity. In addition, expression in zebrafish larvae of a cDNA corresponding to this truncated protein did not cause apoptosis in the intestinal epithelium (Supplemental Figure 9). We also considered the possibility that residual Elys function in the conditional mutants might arise from a truncated Elys protein whose translation was initiated at an in-frame ATG codon immediately downstream of exon 2. This was deemed unlikely however, because lower molecular weight Elys isoforms were not detected in Western blots of whole intestinal extracts or in FG-Nup immunoprecipitation experiments (Figure 3E).

To examine whether Elys deficiency led to a qualitative NPC defect arising from altered nucleoporin stoichiometry, we measured levels of FG-Nups and two other nucleoporins that interact with Elys, Nup107 and Nup133[4, 6]. Elys mutants had normal levels of FG-Nups detected by the Mab414 antibody, normal levels of Nup107, but reduced levels of Nup133 (Figure 3F). However, this reduction in Nup133 is unlikely to account for the Elys mutant phenotype because a comparable reduction in Nup133 levels in ES cells has no obvious effect on ES cell proliferation or the development of Nup133 heterozygous mice[22].

Activation of the DNA damage response in Elys conditional mutants

Normal NPC assembly in conditional Elys mutants suggested that crypt cell apoptosis could be caused by a primary defect in DNA repair. Intestinal progenitor cell apoptosis in zebrafish *flo* mutant larvae is associated with G1 arrest, increased *p53* expression, and activation of the Chk2 checkpoint protein[2]. For this reason, we examined levels of these and other cell cycle regulatory proteins in conditional *Elys* mutants. Western blot analyses of intestinal extracts revealed increased levels of the DNA damage marker γH2AX in *Elys* mutants compared with wild type mice at all stages examined (Figure 4A). Immunohistochemistry showed that γH2AX was present in crypt progenitor cells but not in the post-mitotic villus epithelium (Figure 4C), and γH2AX-positive cells colocalized with TUNEL-positive crypt cells (Supplemental Figure 10; thus arguing against false positive TUNEL staining). Together, this suggests that DNA damage associated with DNA replication triggered the progenitor cell apoptotic response. Levels of p53 also were uniformly elevated in *Elys* mutants, even at P60, when the only detectable intestinal phenotype was crypt cell apoptosis (Figure 4B). Surprisingly, the levels of two checkpoint proteins, phospho-Chk1 and phospho-Chk2, were comparable in Elys mutant and wild type mice (Figure 4D). In contrast, the levels of phosphorylated cyclin dependent kinase, pCdc2 (Tyr15), a DNA damage responsive marker of both S-phase and G2/M phase arrest[23–27], were consistently elevated in the intestinal extracts from *Elys* mutants (Figure 4E). Phosphorylation of Cdc2 Tyr15 has been shown to be a downstream event of activated ATM and ATR checkpoint kinases in response to DNA damage[25, 27]. Consistent with this, activated ATM was detected in intestinal extracts of E16.5 mutants, P7 mutants that have a pronounced intestinal phenotype, and in P60 mutants whose only phenotype was crypt cell apoptosis (Figure 4F).

Activation of ATM in *Elys* conditional mutants could arise from unresolved DNA replication errors arising during S-phase, or possibly from spindle defects arising during M-phase, as Elys binds kinetochore-associated proteins, and mitosis delay was detected following Elys knockdown via RNAi, although not in zebrafish *flo* mutants[2, 7]. To distinguish these possibilities, we compared levels of the M-phase marker phosphohistone 3 in wild type and *Elys* mutants using immunohistochemistry. These studies showed similar numbers of phosphohistone 3 (pH3) crypt cells in P7 wild type mice and *Elys* mutants (327 pH3 positive cells in 127 wild type crypts vs. 342 pH3 positive cells in 119 mutant crypts; n= 150 crypts counted for both wild type and mutant), thus arguing against mitotic spindle defects as activators of ATM signaling.

Transient reduction in chromatin bound Mcm proteins in *Elys* conditional mutants

Previously, we proposed that apoptosis of intestinal epithelial cell in zebrafish *flo* mutants was caused by DNA damage arising from unrepaired DNA replication errors[2]. We attributed this phenotype to a reduction in the levels of chromatin bound Mcm2, a component of the DNA replication helicase that was reported to bind Elys[6]. We speculated that this led to a reduction in the number of dormant replication origins that become activated under conditions of replication stress[10, 11, 28–29]. Previously, interaction of Elys with Mcm proteins was only directly demonstrated in an in vitro system with an abbreviated cell cycle (*Xenopus*

oocyte extracts). Here, using immunoprecipitation experiments we confirmed that Elys interacts with chromatin bound Mcm2 and Mcm4 proteins in the adult mouse intestine (Figure 5A, B). Chromatin precipitation by a Histone-2 antibody followed by Western blot analyses of total intestinal extracts revealed normal chromatin loading of Mcm2 and Mcm4 proteins in P7 mutants (Figure 4C), with a 20% reduction of chromatin bound Mcm2 in P15 mutants, as revealed by quantification of Western blot band intensity (Figure 5C). Levels of chromatin bound Mcm2 and Mcm4 were unchanged in P60 mutants (Figure 5D).

Discussion

In this study we confirm that the nuclear pore associated protein Elys is required for survival of intestinal crypt progenitor cells but not NPC assembly in these cells. These findings were unexpected given recent studies showing that NPC assembly is disrupted by either Elys RNAi knockdown in *in vitro* systems[4, 6, 7,] or zygotic *elys* mutations in zebrafish and worms[2, 3, 5, 9]. Non-concordance of the *in vitro* knockdown and mouse conditional *Elys* mutant phenotypes we present here is not without precedent, however, as NPC assembly occurs normally in mouse embryos carrying mutations of *Nup133*, a gene previously shown to be required for normal NPC assembly in cultured cells[22]. The discordant zebrafish *flo* and mouse conditional *Elys* mutant NPC phenotypes were unexpected but are perhaps best explained by a stage-specific role for Elys during intestinal development that occurs before the *Villin-Cre* transgene is first activated (embryonic day 14). Indeed, the idea that Elys and other nuclear pore associated proteins might play a critical role at selected developmental stages in specific tissues is supported by the embryonic expression pattern of genes such as *Nup133* and *Nup50*[22, 30], and the varied phenotypes arising from inactivating mutations of these and other nucleoporins[22, 31–35].

A recent study reported that Elys is required for post-mitotic NPC assembly but is not required for NPC growth that occurs during interphase in synchronized cultured mammalian cells[36]. This finding raises the interesting possibility that NPCs present in conditional *Elys* mutants could have formed during interphase rather than when the nuclear envelope reforms at the end of mitosis. Arguing against this idea, however, we did not detect a reduction in the number of NPC complexes present in *Elys* deficient cells predicted by this model (based on nucleoporin levels, immunofluorescence and ultrastructural studies). Further studies will be required to determine whether only post-mitotic NPC assembly is altered by in vivo Elys deficiency.

In contrast to the stage-specific role of Elys in NPC assembly, our findings show that Elys is constitutively required for survival of intestinal epithelial progenitor cells. Based on the closely related phenotype of zebrafish *elys*^{flo} mutants, the role of Elys in DNA replication in *Xenopus*[6], and conserved binding of Elys to the Mcm2/7 DNA replication helicase[6] (Figure 5), we argue that Elys is required for either preventing or repairing DNA damage that normally arises during DNA replication. The requirement for Elys is most pronounced during later stages of embryonic development and in perinatal and juvenile stages. By contrast, Elys deficiency is well tolerated in adults, although crypt apoptosis and biochemical evidence of DNA repair pathway activation were both evident at this stage. The less severe adult Elys conditional phenotype could arise from direct compensation of Elys, or through improved efficiency of DNA replication or DNA repair pathways that do not involve Elys. Alternatively, the types of DNA replication errors occurring in neo-natal and juvenile crypt progenitor cells might differ from those that occur in adult progenitor cells, and repair of these errors may not require Elys. Improved progenitor cell DNA repair capability in adult *Elys* mutants, as evidenced by restoration of normal crypt architecture and reduced crypt cell apoptosis is the most likely explanation for the recovery of growth delay

manifest in juvenile mutants. We speculate that the adult mutants expend less energy maintaining their intestinal epithelium than juvenile mutants.

How Elys functions in DNA repair remains an open question at this time. It is uncertain whether the modest and transient reduction in Mcm levels in the juvenile conditional mouse mutants supports a role for Elys in the maintenance of dormant replication origins, as proposed for zebrafish *flo* mutants. It is conceivable, however, that the levels of these Mcm proteins are more significantly reduced in a subpopulation of the progenitor pool, and that this was not detected in the Western blot analysis of extracts derived from whole intestine. Indeed, at a comparable phenotypic stage the *flo* intestine is comprised solely of progenitor cells[2, 37]. Alternatively, the conditional *Elys* mutation could impart a qualitative defect on Mcm2/7 function. Besides disrupting Mcm2/7 complex function at dormant replication origins, Elys deficiency could interfere with DNA repair by altering the function of Nup107, a nucleoporin that binds Elys and whose yeast ortholog, Nup84, functions in DNA repair at the NPC[13]. As Nup107 levels were unchanged by conditional Elys mutation, this theoretical defect must arise from a qualitative effect on Nup107 function. Finally, Elys deficiency could have an indirect effect on DNA repair at the NPC. Involvement of the NPC in DNA repair mechanisms appears to be a generalized phenomenon in yeast, as it plays a role in the maintenance of eroded telomeres and the resolution of DNA double strand breaks occurring in subtelomeric regions[14, 15]. Yeast lack a clear Elys ortholog[8], however Elys has been detected at the NPC of worms, zebrafish and mammals. The findings presented in this study lead us to speculate that proteins associated with the NPC, such as Elys, play a role in DNA repair in vertebrates.

Although epithelial homeostasis is restored in the intestine of adult *Elys* conditional mutants, we still detected biochemical evidence of DNA damage in Elys deficient progenitor cells at this stage. Because of this, we speculate that these cells are not fully competent to respond to regenerative challenges or oncogenic stress. This aspect of the *Elys* conditional mutant phenotype, which could be revealed through genetic interaction with tumor suppressor gene mutations or mouse inflammatory disease models, may have direct clinical relevance. Indeed, mutations in other NPC proteins have recently been causally linked to human disease[38].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Kristin Lorent for assembling figures and editing the manuscript and Karrie Brondell for animal care.

Funding sources: NIH 1K01DK085194-01 (N.G.), NIH R01-DK053839 (K.H.K.), NIH R01-DK61142, R21-CA120491 (M.P.) and core facilities provided by P30-DK50306

Abbreviations

Elys	embryonic large molecule derived from yolk sac
NPC	Nuclear pore complex
flo	*flotte lotte*
Mcm	minichromosome maintenance
Chk	Checkpoint protein

Nup Nucleoporin

References

1. Suntharalingam M, Wente SR. Peering through the pore: nuclear pore complex structure, assembly, and function. Dev Cell. 2003; 4:775–789. [PubMed: 12791264]
2. Davuluri G, Gong W, Yusuff S, et al. Mutation of the zebrafish nucleoporin elys sensitizes tissue progenitors to replication stress. PLoS Genet. 2008; 4:e1000240. [PubMed: 18974873]
3. Fernandez AG, Piano F. Network analysis of C. elegans early embryogenesis identifies MEL-28 as a central coordinator of chromatin maintenance and nuclear envelope function. Curr Biol. 2006; 16:1757–1763. [PubMed: 16950115]
4. Franz C, Walczak R, Yavuz S, et al. MEL-28/ELYS is required for the recruitment of nucleoporins to chromatin and postmitotic nuclear pore complex assembly. EMBO Rep. 2007; 8:165–172. [PubMed: 17235358]
5. Galy V, Askjaer P, Franz C, et al. MEL-28, a novel nuclear envelope and kinetochore protein essential for zygotic nuclear envelope assembly in C. elegans. Curr Biol. 2006; 16:1748–1756. [PubMed: 16950114]
6. Gillespie PJ, Khoudoli GA, Stewart G, et al. ELYS/MEL-28 chromatin association coordinates nuclear pore complex assembly and replication licensing. Curr Biol. 2007; 17:1657–1662. [PubMed: 17825564]
7. Rasala BA, Orjalo AV, Shen Z, et al. ELYS is a dual nucleoporin/kinetochore protein required for nuclear pore assembly and proper cell division. Proc Natl Acad Sci U S A. 2006; 103:17801–17806. [PubMed: 17098863]
8. Rasala BA, Ramos C, Harel A, et al. Capture of AT-rich chromatin by ELYS recruits POM121 and NDC1 to initiate nuclear pore assembly. Mol Biol Cell. 2008; 19:3982–3996. [PubMed: 18596237]
9. de Jong-Curtain TA, Parslow AC, Trotter AJ, et al. Abnormal nuclear pore formation triggers apoptosis in the intestinal epithelium of elys-deficient zebrafish. Gastroenterology. 2009; 136:902–911. [PubMed: 19073184]
10. Ibarra A, Schwob E, Méndez J. Excess MCM proteins protect human cells from replicative stress by licensing backup origins of replication. Proc Natl Acad Sci U S A. 2008; 105:8956–8961. [PubMed: 18579778]
11. Woodward AM, Göhler T, Luciani MG, et al. Excess Mcm2–7 license dormant origins of replication that can be used under conditions of replicative stress. J Cell Biol. 2006; 173:673–683. [PubMed: 16754955]
12. Okita K, Kiyonari H, Nobuhisa I, et al. Targeted disruption of the mouse ELYS gene results in embryonic death at peri-implantation development. Genes Cells. 2004; 9:1083–1091. [PubMed: 15507119]
13. Nagai S, Dubrana K, Tsai-Pflugfelder M, et al. Functional targeting of DNA damage to a nuclear pore-associated SUMO-dependent ubiquitin ligase. Science. 2008; 322:597–602. [PubMed: 18948542]
14. Khadaroo B, Teixeira MT, Luciano P, et al. The DNA damage response at eroded telomeres and tethering to the nuclear pore complex. Nat Cell Biol. 2009; 11:980–987. [PubMed: 19597487]
15. Therizols P, Fairhead C, Cabal GG, et al. Telomere tethering at the nuclear periphery is essential for efficient DNA double strand break repair in subtelomeric region. J Cell Biol. 2006; 172:189–199. [PubMed: 16418532]
16. De Souza CP, Hashmi SB, Horn KP, Osmani SA. A point mutation in the Aspergillus nidulans sonBNup98 nuclear pore complex gene causes conditional DNA damage sensitivity. Genetics. 2006; 174:1881–93. [PubMed: 17028324]
17. Rodríguez CI, Buchholz F, Galloway J, et al. High-efficiency deleter mice show that FLPe is an alternative to Cre-loxP. Nat Genet. 2000; 25:139–140. [PubMed: 10835623]
18. Madison BB, Dunbar L, Qiao XT, et al. Cis elements of the villin gene control expression in restricted domains of the vertical (crypt) and horizontal (duodenum, cecum) axes of the intestine. J Biol Chem. 2002; 277:33275–33283. [PubMed: 12065599]

19. Gao N, White P, Kaestner KH. Establishment of intestinal identity and epithelial-mesenchymal signaling by Cdx2. Dev Cell. 2009; 16:588–599. [PubMed: 19386267]
20. Okita K, Nobuhisa I, Takizawa M, et al. Genomic organization and characterization of the mouse ELYS gene. Biochem Biophys Res Commun. 2003; 305:327–332. [PubMed: 12745078]
21. Lakso M, Pichel JG, Gorman JR, et al. Efficient in vivo manipulation of mouse genomic sequences at the zygote stage. Proc Natl Acad Sci U S A. 1996; 93:5860–5865. [PubMed: 8650183]
22. Lupu F, Alves A, Anderson K, et al. Nuclear pore composition regulates neural stem/progenitor cell differentiation in the mouse embryo. Dev Cell. 2008; 14:831–842. [PubMed: 18539113]
23. Geng L, Zhang X, Zheng S, et al. Artemis links ATM to G2/M checkpoint recovery via regulation of Cdk1-cyclin B. Mol Cell Biol. 2007; 27:2625–2635. [PubMed: 17242184]
24. Nakanishi M, Katsuno Y, Niida H, et al. Chk1-cyclin A/Cdk1 axis regulates origin firing programs in mammals. Chromosome Res. 2010; 18:103–113. [PubMed: 20013152]
25. Satyanarayana A, Hilton MB, Kaldis P. p21 Inhibits Cdk1 in the absence of Cdk2 to maintain the G1/S phase DNA damage checkpoint. Mol Biol Cell. 2008; 19:65–77. [PubMed: 17942597]
26. Takizawa CG, Morgan DO. Control of mitosis by changes in the subcellular location of cyclin-B1-Cdk1 and Cdc25C. Curr Opin Cell Biol. 2000; 12:658–665. [PubMed: 11063929]
27. Tyagi A, Singh RP, Agarwal C, et al. Resveratrol causes Cdc2-tyr15 phosphorylation via ATM/ATR-Chk1/2-Cdc25C pathway as a central mechanism for S phase arrest in human ovarian carcinoma Ovcar-3 cells. Carcinogenesis. 2005; 26:1978–1987. [PubMed: 15975956]
28. Blow JJ, Ge XQ. A model for DNA replication showing how dormant origins safeguard against replication fork failure. EMBO Rep. 2009; 10:406–412. [PubMed: 19218919]
29. Doksani Y, Bermejo R, Fiorani S, et al. Replicon dynamics, dormant origin firing, and terminal fork integrity after double-strand break formation. Cell. 2009; 137:247–258. [PubMed: 19361851]
30. Smitherman M, Lee K, Swanger J, et al. Characterization and targeted disruption of murine Nup50, a p27(Kip1)-interacting component of the nuclear pore complex. Mol Cell Biol. 2000; 20:5631–5642. [PubMed: 10891500]
31. Babu JR, Jeganathan KB, Baker DJ, et al. Rae1 is an essential mitotic checkpoint regulator that cooperates with Bub3 to prevent chromosome missegregation. J Cell Biol. 2003; 160:341–353. [PubMed: 12551952]
32. Faria AM, Levay A, Wang Y, et al. The nucleoporin Nup96 is required for proper expression of interferon-regulated proteins and functions. Immunity. 2006; 24:295–304. [PubMed: 16546098]
33. van Deursen J, Boer J, Kasper, et al. G2 arrest and impaired nucleocytoplasmic transport in mouse embryos lacking the proto-oncogene CAN/Nup214. EMBO J. 1996; 15:5574–5583. [PubMed: 8896451]
34. Wu X, Kasper LH, Mantcheva RT, et al. Disruption of the FG nucleoporin NUP98 causes selective changes in nuclear pore complex stoichiometry and function. Proc Natl Acad Sci U S A. 2001; 98:3191–3196. [PubMed: 11248054]
35. Zhang X, Chen S, Yoo S, et al. Mutation in nuclear pore component NUP155 leads to atrial fibrillation and early sudden cardiac death. Cell. 2008; 135:1017–1027. [PubMed: 19070573]
36. Doucet DM, Talamas JA, Hetzer MW. Cell cycle-dependent differences in nuclear pore complex assembly in metazoa. Cell. 2010; 141:1030–1041. [PubMed: 20550937]
37. Wallace KN, Akhter S, Smith EM, et al. Intestinal growth and differentiation in zebrafish. Mech Dev. 2005; 122:157–73. [PubMed: 15652704]
38. Capelson M, Hetzer MW. The role of nuclear pores in gene regulation, development and disease. EMBO Rep. 2009; 10:697–705. [PubMed: 19543230]

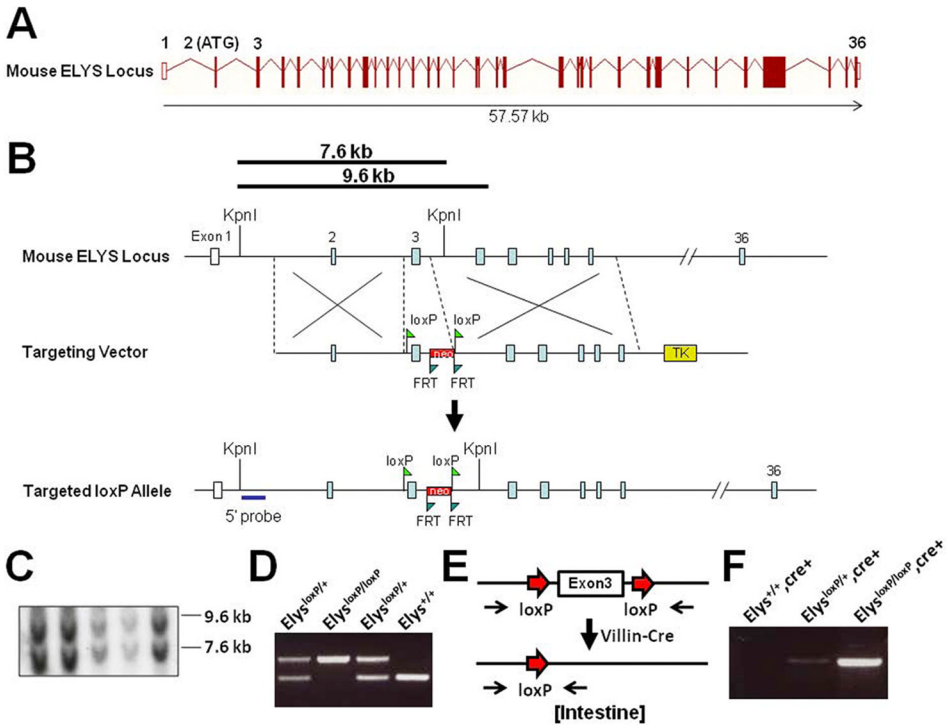

Figure 1.

Derivation of the conditional *Elys* allele. (A) Schematic of the mouse *Elys* locus. The *Elys* gene contains 36 exons. (B) Schematic diagram depicting the targeting strategy of deriving $Elys^{loxP}$ allele. (C) Southern blot confirms correct targeting of *Elys* locus in 5 independent ES cell clones; (targeted allele 9.6kb; wild type allele 7.6kb) (D) Genomic DNA PCR confirms genotypes of $Elys^{loxP/loxP}$, $Elys^{loxP/+}$ and $Elys^{+/+}$ mice. The larger molecular weight band is amplified from the conditional allele. (E, F) Cre-mediated recombination at the *Elys* locus in intestinal cells was confirmed by genomic PCR. The primers flanking the two loxP sites allow amplification of a 400 bp fragment following Cre-mediated recombination.

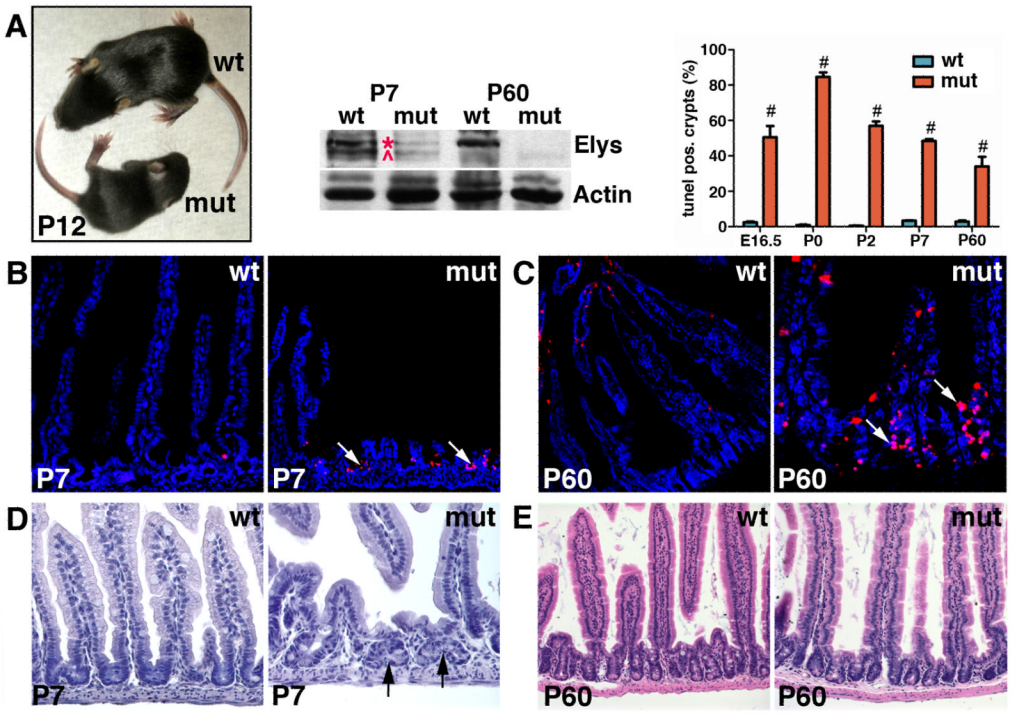

Figure 2.

Intestinal progenitor cell defects in *Elys*$^{loxP/loxP}$;*Villin-Cre* mice. (A) P12 mutant mice (mut) is smaller than its wild type littermate. Western blot analysis shows only a small amount of Elys protein (red *) in intestinal extracts of P7 mutants compared with wild type (wt) littermates, whereas none is detected in the intestine of the P60 mutant. The lower band (^) is non-specific and not consistently present. Bar graph showing the percentage of mutant and wild type intestinal crypts with 1 or more TUNEL positive cells; (# - $p < 0.01$) (B, C) TUNEL staining shows apoptotic cells (red) in mutant and wild type intestines. White arrows point to apoptotic crypt cells in mutants. Panel C shows a severely affected P60 crypt. TUNEL positive cells in P60 wild type intestine are located at the villus tip but not in crypts. (D, E) Histological sections of P7 and P60 Elys-deficient and wild type intestines. Arrows point to abnormal crypts in P7 mutant.

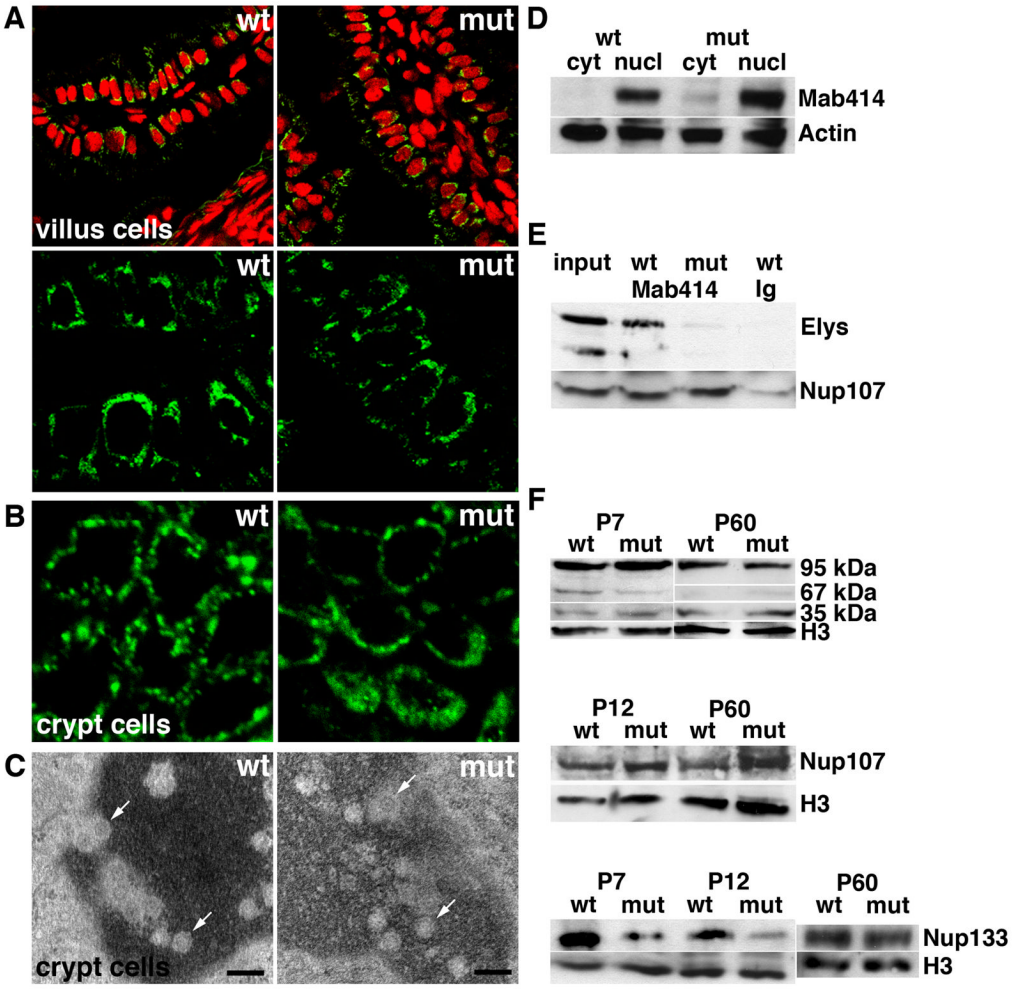

Figure 3.

Normal NPC assembly in *Elys*$^{loxP/loxP}$;*Villin-Cre* mice. (A, B) Normal perinuclear distribution of FG-Nups (green) detected by the Mab414 antibody in wild type (wt) and mutant (mut) villus epithelial cells (A) and crypt epithelial cells (B). Nuclei were labeled in red by propidium iodide. (C) Morphologically identical nuclear pores (white arrows) are detected in transmission electron micrographs of mutant and wild type crypt epithelial cells. (D) Western blot shows only a very small amount of FG-Nup (95kDa) in the cytoplasmic fraction of intestinal extracts from P12 mutants. (E) Elys is co-immunoprecipitated with FG-Nups from the P12 wild type intestine by the Mab414 antibody. Minimal levels of Elys bound to FG-Nups are detected in the mutant intestine. Levels of Nup107, an FG-Nup serve as loading control. Input refers to intestinal extract prior to immunoprecipitation. (F) Western blot detects comparable levels of three FG-Nups recognized by Mab414 in the wild type and mutant intestine; comparable levels of Nup107 in nuclear extracts from the intestine of P12 and P60 mutant; reduced levels of Nup133 in nuclear extracts from the intestine of conditional of P7 and P12 Elys mutant vs. wild type littermates detected by Western blot. Comparable levels or Nup133 are present in P60 wild type and mutants. H3 – Histone 3 loading control.

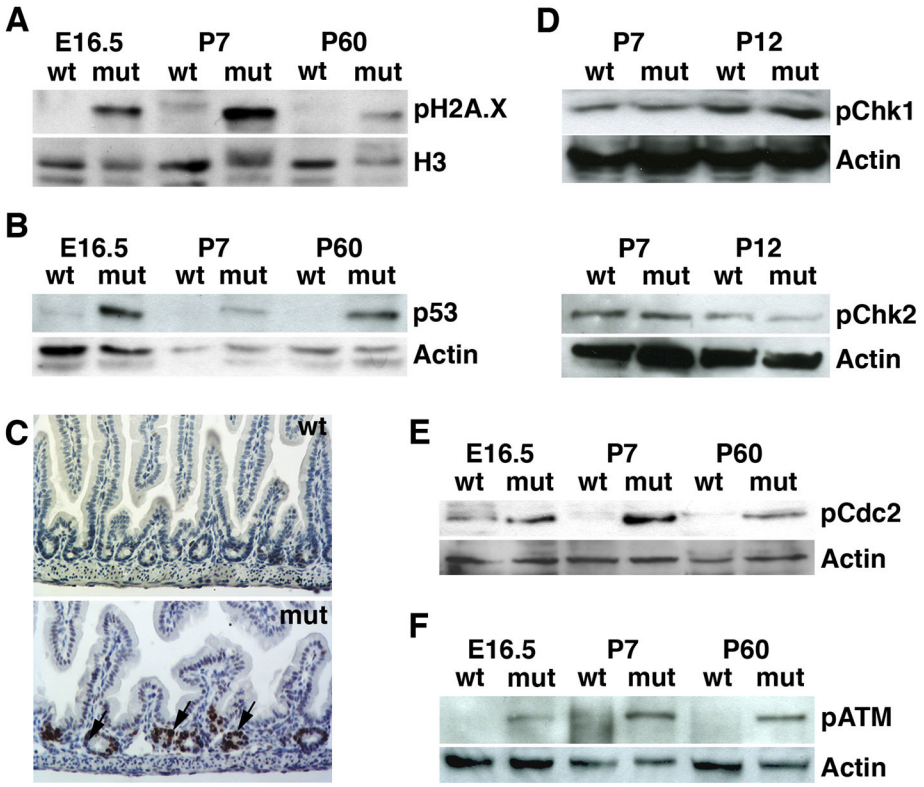

Figure 4.

The DNA damage response is activated in the intestine of *Elys*$^{loxP/loxP}$;*Villin-Cre* mice. (A, B) Western blot detect increased levels of γH2AX and p53 proteins in whole intestinal extracts from E16.5, P7, and P60 *Elys* conditional mutants (mut). (C) Immunohistochemical detection of γH2AX is restricted to intestinal crypts of the P7 conditional *Elys* mutant but is not detected in the wild type intestine. (D) Western blot shows normal levels of phospho-Chk1 (Ser317) and phospho-Chk2 (Ser33/35) in whole intestinal extracts of P7 and P12 conditional *Elys* mutants. (E, F) Western blot shows increased levels of phospho-Cdc2 (Tyr15) and phospho-ATM (Ser1981) in whole intestinal extracts of E16.5, P7, and P60 conditional *Elys* mutants.

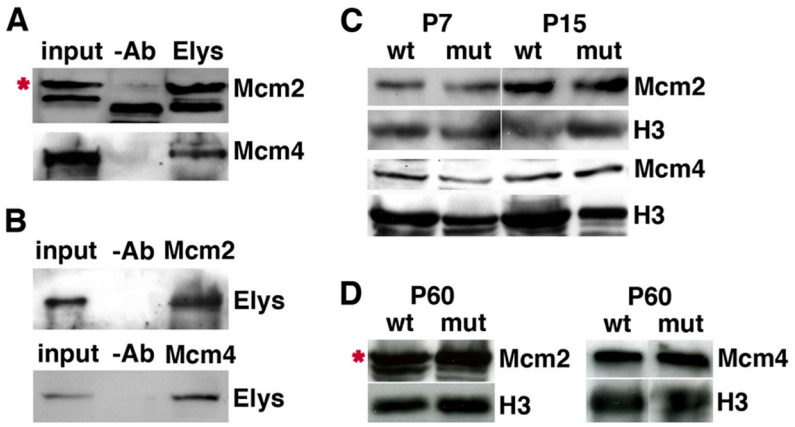

Figure 5.

Elys binds Mcm proteins in mouse intestine. (A, B) Reciprocal immunoprecipitation (IP) experiments show interaction between Mcm2 and Mcm4 and Elys in the adult mouse intestine. (A) Western blot detects Mcm2 and Mcm4 bound to Elys immunoprecipitated from intestinal nuclear extract. Red asterisk indicates band corresponding to full-length Mcm2 protein. Input refers to intestinal extract prior to immunoprecipitation. -Ab refers to mock immunoprecipitation. (B) Western blot detects Elys bound to Mcm2 and Mcm4 immunoprecipitated from intestinal nuclear extract. (C, D) Levels of chromatin bound Mcm2 and Mcm4 in P7, P15 and P60 intestinal extracts from wild type and *Elys* conditional mutants. H3 – Histone 3 loading control.