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## More than two decades of Apc modeling in rodents

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### Abstract

Mutation of tumor suppressor gene Adenomatous polyposis coli (*APC*) is an initiating step in most colon cancers. This review summarizes Apc models in mice and rats, with particular concentration on those most recently developed, phenotypic variation among different models, and genotype/ phenotype correlations.

### Keywords

Adenomatous polyposis coli; APC; Apc mouse models; Apc rat models; Apc models; Apc rodent models; FAP models; Apc alleles; Conditional Apc mutations; Intestinal polyposis; LOH / Apc mouse model; Intestinal polyposis in mice; Apc associated tumors; Apc and development

### Adenomatous polyposis coli (APC)

*Adenomatous polyposis coli (APC)* is a critical tumor suppressor gene in the colon. Humans with germline *APC* mutation develop hundreds to thousands of colon tumors in their first few decades of life, a condition referred to as familial adenomatous polyposis (FAP). These tumors are pre-cancerous, and prophylactic colon removal is recommended to avoid progression to invasive carcinoma that otherwise would occur in FAP patients by age 39, on average [1–4]. Notably, *APC* mutation is also an early if not the first step in the development of more than 80% of all sporadic colorectal cancers [5, 6]. In both inherited and sporadic colorectal cancer, *APC* mutations result in premature truncation of the large (2843 amino acid) APC protein, eliminating roughly half to three-quarters of the C-terminal portion [7, 8]. APC interacts with multiple proteins and participates in diverse cellular processes including proliferation, differentiation, apoptosis, adhesion, and migration. One of the first reported APC functions is as a Wnt-signal antagonist. In this capacity, APC forms a complex with GSK3 $\beta$ , axin, and other proteins to mediate phosphorylation and eventual proteasomal destruction of the oncoprotein  $\beta$ -catenin [7, 9]. Animal models have been generated to study APC functions in development and tumorigenesis including *Drosophila*, *C.elegans*, zebrafish, mouse, rat and pig.

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## Apc mouse models

With similar physiological and pathological processes to humans, [10] mice are particularly well-suited to study Apc functions in intestinal homeostasis, tumor suppression, and vertebrate development [11]. All characterized motifs in human APC are conserved in murine Apc and the proteins are 87.9% identical and 91.9% similar at the amino acid level [12]. Furthermore, intestinal tumors from Apc mutant mice displayed expression signatures similar to that in tumors from humans with germline *APC* mutations [13]. Apc mouse models can be divided into two broad categories: mice with a germline *Apc* mutation that results in protein truncation, alteration, or reduced expression in all tissues and mice with conditional *Apc* alterations only in a specific tissue at a particular stage of development. Figure 1 shows the structural domains of Apc and germline mutations in mice. Here, we compare and contrast the published phenotypes of the 43 different Apc mouse models described to date, with particular emphasis on studies from the past several years.

### Apc<sup>Min/+</sup>

The Multiple intestinal neoplasia (Min) mouse was identified in an ethylnitrosourea (ENU) mutagenesis screen and has a nonsense mutation that results in truncation of Apc at codon 851 [14, 15]. Since its first description in 1990, the Apc<sup>Min</sup> model has been used extensively to study Apc functions in suppression of intestinal tumorigenesis and to investigate tumor prevention strategies. Mice homozygous for *Apc*<sup>Min</sup> die early in embryonic development. In the C57Bl/6 background adult Apc<sup>Min/+</sup> mice live for ~120 days and display both intestinal (100% penetrant) and extra-intestinal phenotypes [12, 16–18]. Apc<sup>Min/+</sup> mice typically develop between 20–100 polyps in their gastrointestinal tract [17]. Differences in diet, flora, genetic background, and genetic modifiers can result in even greater variability in the polyp multiplicity [19–21]. The vast majority of Apc<sup>Min/+</sup> polyps are in the small intestine, with a few developing in the colon and even fewer in the stomach [16, 17]. Histologically, most tumors in Apc<sup>Min/+</sup> mice are benign adenomas: polypoidal, sessile, or papillary in nature, with limited dysplasia and atypia. Although these polyps can reach 8 mm in diameter, malignant changes are not typically seen. However, in older Apc<sup>Min/+</sup> mice, polyps express molecular markers of invasiveness seen in malignant tumors, [21] and areas with limited invasion and carcinoma *in situ* have been observed [17]. Furthermore, Apc<sup>Min/+</sup> mice in certain genetic backgrounds live longer and develop fewer polyps than do mice in the B6 background, but show malignant changes and local metastasis to lymph nodes [22]. Likely the short life span of Apc<sup>Min/+</sup> mice limits the accumulation of other genetic mutations in intestinal tumors that are required for progression to invasive carcinoma [22].

In both Apc<sup>Min/+</sup> mice and FAP patients, mutation of the wild-type *Apc* allele (or ‘second hit’) is required for adenoma formation [23]. Genetic and environmental factors that raise mutation rate increase polyp multiplicity in Apc<sup>Min/+</sup> mice [24–27]. However, the nature and predicted mechanism of this second mutation is different in Apc<sup>Min/+</sup> mice and in human FAP patients. In humans with FAP, the somatic *APC* mutation is frequently an independent protein-truncating point mutation. LOH can also occur, presumably by mitotic recombination and particularly in adenomas from the distal bowel when the germline mutation truncates APC at or near codon 1309 [28]. In contrast, most of adenomas from Apc<sup>Min</sup> mice show LOH at the *Apc* locus and the most recent evidence supports loss of the wild-type *Apc* allele with locus diploidy as the mechanism of this LOH [23, 29]. Whether this LOH is the result of somatic recombination or loss and duplication of the entire chromosome 18 (the location of *Apc*) is still not completely resolved. Evidence for somatic recombination as the underlying mechanism of this LOH came from analysis of Apc<sup>Min/+</sup> mice with a Robertsonian translocation, fusing acrocentric chromosomes 7 and 18 (*Rb9* fusion). When placed in trans, cis, or in homozygous distribution with the *Apc*<sup>Min</sup> allele, the

*Rb9* chromosome was associated with reduced tumor burden in  $Apc^{Min/+}$  mice [29]. Because mitotic recombination is compromised in *Rb9* mice, it was concluded that somatic recombination is the mechanism of LOH required for polyp formation. However, there is a chance that the *Rb9* chromosome also affects chromosomal segregation and thus, complete loss and duplication of chromosome 18 in adenomas from  $Apc^{Min/+}$  mice without the *Rb9* chromosome is still a possibility.

An elaborate experiment that assessed polyp formation in mice with  $Apc^{Min}$  and mutant *Atp5a1* alleles (both genes are found on chromosome 18) implicated loss and duplication of the entire chromosome 18 as the mechanism of LOH in polyps from  $Apc^{Min/+}$  mice [30]. Homozygous mutation of *Atp5a1* is lethal to cells. Fewer polyps were observed in mice with both mutant *Atp5a1* and  $Apc^{Min}$  alleles are on the same chromosome than in mice with trans-distributed mutant *Atp5a1* and  $Apc^{Min}$  alleles. Furthermore, when compared to polyps from  $Apc^{Min/+}$  mice harboring a wild-type *Atp5a1* allele, the distribution and histopathological characteristics of polyps were different in mice with *Apc* and *Atp5a1* mutations in cis distribution, but not in trans. When mutations in *Apc* and *Atp5a1* are in cis, complete loss of chromosome 18 with or without subsequent duplication results in nonviability since homozygosity for mutant *Atp5a1* is lethal. In contrast, there would not be this selection against loss and reduplication of the whole chromosome if mutated *Apc* and *Atp5a1* are in trans. Since polyps formed in the latter situation were indistinguishable from those that developed in  $Apc^{Min/+}$  mice with wild-type *Atp5a1* alleles, it was concluded that loss and duplication of chromosome 18 is the primary mechanism of LOH in polyps from  $Apc^{Min/+}$  mice [30]. One potential caveat for consideration is that, because chromosome 18 is acrocentric, a single somatic recombination proximal to the *Apc* locus would be difficult to distinguish from complete loss and reduplication of the whole chromosome [29].

In addition to intestinal tumors,  $Apc^{Min/+}$  mice develop mammary tumors, but at a much lower penetrance (5%) and at a relatively older age ( $16 \pm 3.5$  weeks) [18]. Histologically, mammary tumors are usually invasive in nature, with areas of adenocarcinoma and adenoacanthoma, the latter of which have not been reported in humans [18].  $Apc^{Min/+}$  mice treated with the mutagen ENU, exposed to X-rays, or with a mutation in the DNA repair gene *Myh*, have increased mammary tumor incidence but the same tumor morphology, indicating that *Apc* mutation alone is not sufficient for mammary tumorigenesis [26, 31]. In mice with wild-type *Apc*, mammary expression of stabilized  $\beta$ -catenin results in tumor development, consistent with a role for *Apc* in inhibiting mammary tumorigenesis via antagonizing the Wnt signaling pathway [18, 32, 33]. Although humans with FAP also have an increased risk of tumors outside the gastrointestinal tract, including desmoid tumors, mandibular osteomas, and retinal dysplasias, they do not show increased susceptibility to breast cancer [34]. However, *APC* mutation or promoter methylation has been detected in up to 70% of human breast cancers surveyed [35–37]. Moreover, *APC* methylation status was significantly associated with decreased APC protein level and reduced disease-free survival in patients with invasive ductal carcinoma of the breast, supporting a role for APC in suppression of mammary neoplasia [38].

Although intestinal polyposis is the dominant feature of  $Apc^{Min/+}$  mice, these mice also show alterations in other tissues [17, 39]. There is no evidence that LOH is necessary for these extra-intestinal phenotypes of  $Apc^{Min/+}$  mice, which suggests that *Apc* haplo-insufficiency is the underlying mechanism. Anemia was the first such phenotype to be described in  $Apc^{Min/+}$  mice and was used to predict intestinal polyposis before the establishment of  $Apc^{Min}$  genotyping [17]. Although the exact pathogenesis is not completely understood, anemia in  $Apc^{Min/+}$  mice is microcytic-hypochromic, consistent with chronic blood loss from intestinal lesions as the underlying cause [17]. Old  $Apc^{Min/+}$  mice also develop large spleens with enhanced splenic hematopoiesis, therefore, larger spleens might

be an extra-medullary compensatory response to anemia. However, because larger spleens and anemia are not always correlated in  $Apc^{Min/+}$  mice, a different mechanism for large spleen development might be at play [39]. Old  $Apc^{Min/+}$  mice can also develop myelodysplastic disease, with increased formation of myeloid, granulocytic, and erythroid colonies in the spleen [40, 41]. Other hematological changes seen in  $Apc^{Min/+}$  mice include rapid thymus regression, depletion of splenic natural killer (NK) cells, and loss of B-lymphocyte progenitors in spleen and bone marrow [42]. In  $Apc^{Min/+}$  mice the bone marrow micro-environment appears disrupted and gradual loss of the quiescent hematopoietic stem cells occurs [41].

Gonadal changes in  $Apc^{Min/+}$  mice include increased numbers of degenerated and undeveloped ovarian follicles, and under-developed testicular seminiferous tubules, the cause of which is not known [39]. However, conditional truncating *Apc* mutation in testicular Sertoli cells results in premature germ-cell loss and the absence of both Sertoli cell apical extensions and the blood-testis barrier. These changes were not recapitulated by activating mutations in  $\beta$ -catenin, consistent with a Wnt-independent *Apc* function [43]. Disruption and involution of mammary glandular structures has been reported in pregnant  $Apc^{Min/+}$  mice, along with altered proliferation, increased apoptosis, and interrupted epithelial integrity and polarization of mammary epithelial cells. Because these alterations occur in the absence of changes in Wnt target transcript levels and nuclear localization of  $\beta$ -catenin, these mammary gland phenotypes appear to be Wnt-independent [44]. Finally, at 15 weeks,  $Apc^{Min/+}$  mice display a change in their serum lipid profile called dyslipidemia, with increased serum levels of triacylglycerol, cholesterol, and free fatty acids. The exact cause of this dyslipidemia is unknown, but hyperlipidemia has been correlated with the activity and level of the lipid regulatory nuclear receptors PPAR  $\alpha$ ,  $\beta$  and  $\gamma$  [45–47]. Treatment of  $Apc^{Min/+}$  mice with the nonsteroidal anti-inflammatory agent indomethacin decreases polyp number and also improves dyslipidemia in  $Apc^{Min/+}$  mice [48]. These extra intestinal phenotypes indicate that *Apc* functions not only in intestinal epithelial cells, but also in development and maintenance of other tissues.

In addition to its use as a model of FAP,  $Apc^{Min/+}$  mice have been used extensively as a tumor susceptibility model to test the effect of environmental factors, mutations in other genes, and drugs on intestinal tumorigenesis. Such studies have increased our understanding of both intestinal tumorigenesis and cancer biology in general and are summarized in several excellent reviews [11, 19, 49, 50].

Phenotypic variation in  $Apc^{Min/+}$  mice of different genetic backgrounds allowed elucidation of modifier genes that can enhance or attenuate intestinal polyposis, called *Modifiers of Min (Mom)* [19, 50]. Some Mom genes are found on chromosome 18. Some modifiers are single genes, others are thought to represent contiguous genes, and some remain less well-defined [50–54]. The modifiers appear to function as recessive, dominant, or semi-dominant loci. Some modifier genes, such as Mom-1 (*Pla2g2a*), work in a cell-non-autonomous manner [55, 56]. Others, like Mom-2 (*Atp5a1*), appear to inhibit loss of the wild-type *Apc* allele [30]. A detailed discussion of the mechanisms of these modifiers can be found elsewhere [50].

## Mouse models expressing truncated *Apc* protein longer than $Apc^{Min}$

APC is a large multi-domain protein that has been implicated in many cellular activities in addition to its role in down-regulating Wnt signaling. APC domains involved in targeting  $\beta$ -catenin for degradation are in the middle region of APC. Interaction of C-terminal APC regions with DNA and with microtubules has been proposed to contribute to tumor suppression [57, 58]. Disruption of the interaction between APC and microtubules affects

spindle formation and mitosis in colon cancer cell lines and in intestinal epithelial cells in  $Apc^{Min/+}$  mice [59]. In addition,  $Apc^{Min/Min}$  embryonic stem (ES) cells show chromosomal instability (CIN) [60]. These observations led to the proposal that loss of the C-terminal one third of APC would promote intestinal tumorigenesis [60–62]. To test this hypothesis, three mouse models with truncations of the C-terminal third of APC have been generated;  $Apc^{1638N}$ ,  $Apc^{1638T}$ , and  $Apc^{1572T}$ . The  $Apc^{1638N}$  mouse was generated by insertion of a neomycin-resistance gene at *Apc* codon 1660.  $Apc^{1638N/1638N}$  mice die as embryos.  $Apc^{1638N/+}$  mice develop intestinal polyps, but very few (less than 10) compared to the number in  $Apc^{Min/+}$  mice, and with a different distribution (gastric and colonic). Intestinal tumors in  $Apc^{1638N/+}$  mice are also invasive, with distant metastasis in the liver detected in one mouse. Because  $Apc^{1638N/+}$  mice live longer than  $Apc^{Min/+}$  mice, the invasive phenotype could reflect tumor progression over time. Intestinal tumorigenesis is enhanced in  $Apc^{1638N/+}$  mice with mutations in other tumor suppressor genes [63–67]. It was initially suggested that LOH in tumors from  $Apc^{1638N}$  mice resulted from loss of the entire chromosome 18 [68]. However, more recent evidence indicates that the *Apc*<sup>+</sup> allele appears to be maintained in most polyps from  $Apc^{1638N/+}$  mice, consistent with inactivation or silencing of the wild-type *Apc* allele [69]. One hundred percent of  $Apc^{1638N/+}$  mice develop desmoid tumors and cutaneous cysts [70]. In humans, desmoid tumors occur in FAP patients [71] and also in patients with an attenuated form of FAP (AFAP) resulting from germ-line mutations in the 3' portion of *APC* [72]. AFAP patients develop only a few polyps, mainly in the duodenum [72–74]. Since only full-length and not truncated Apc protein is detected in these mice using Western blot, *Apc*<sup>1638N</sup> may be considered an essentially null allele [75]. The antibiotic selection cassette used to generate the  $Apc^{1638N}$  mice was inserted in reverse orientation and it is thought that production of an antisense *Apc* transcript might lead to inhibition of the truncated Apc translation. Alternatively, the mild intestinal polyposis phenotype may indicate that the truncated Apc protein is not completely absent, but rather its level is below detection limits due to reduced expression or increased instability of the truncated protein. Possibly, this truncated Apc protein with multiple remaining  $\beta$ -catenin binding and degradation domains can suppress intestinal tumorigenesis in these mice.

In contrast to  $Apc^{1638N/+}$  mice, truncated Apc is expressed in  $Apc^{1638T}$  mice, which have the antibiotic-resistance gene (hygromycin) inserted in the same position as in  $Apc^{1638N}$  mice, but in the sense orientation [76]. Unexpectedly,  $Apc^{1638T/1638T}$  mice are viable and do not develop intestinal or extra-intestinal tumors. Instead, these mice display post-natal growth retardation and nipple-associated cutaneous cysts, and lack preputial glands. The molecular basis for these phenotypes has not been determined, but they indicate a role for the C-terminal region of Apc in development [76].  $Apc^{1638T/1638T}$  mice also show larger thyroid follicles, accumulation of thyroglobulin in the endoplasmic reticulum and less response to exogenous thyroid stimulating hormone relative to wildtype mice [77]. It is puzzling that  $Apc^{1638N/1638T}$  and  $Apc^{Min/1638T}$  mice are not viable. The  $Apc^{1638T}$  protein retains all 15 a.a. repeats, 1 of 3 SAMP motifs, and 3 of 7 20-a.a. repeats.  $Apc^{1638T/1638T}$  embryonic stem (ES) cells have almost no change in Wnt signaling level while Wnt signaling is upregulated in  $Apc^{1638T/1638N}$  ES cells [76]. Perhaps the remaining functions of the truncated *Apc*<sup>1638T</sup> allele are dose-dependent, and thus, the *Apc*<sup>1638T</sup> allele is haplo-insufficient for  $\beta$ -catenin regulation.

More recently, the  $Apc^{1572T}$  mouse model was generated by deleting the remaining SAMP repeat in the  $Apc^{1638T}$  mouse [78]. Although the truncated Apc protein in  $Apc^{1572T/+}$  mice is only 66 amino acids shorter than Apc from  $Apc^{1638T}$  mice, the phenotypes of these two mouse models could not be more different. Unlike *Apc*<sup>1638T</sup>, *Apc*<sup>1572T</sup> germ-line homozygosity is incompatible with viability. One remarkable feature of  $Apc^{1572T/+}$  mice on a B6 background is that they develop no intestinal tumors, but instead develop invasive mammary tumors that can even metastasize to the lungs. While mammary tumor

morphology is similar in both  $Apc^{1572T/+}$  and  $Apc^{Min/+}$  mice, the incidence of mammary tumors is much higher in  $Apc^{1572T/+}$  mice; 100% in virgin females and 30% in males compared to only 5% in  $Apc^{Min/+}$  females.  $\beta$ -catenin activity, as assessed using a “TOPFLASH” reporter assay, is higher in  $Apc^{1572T/1572T}$  ES cells than in wild-type or  $Apc^{1638T/1638T}$  ES cells, but lower than in  $Apc^{1638N/1638N}$  ES cells.  $Apc^{1572T/+}$  mice do develop intestinal polyps if they also have a  $Smad4^{Sad}$  allele, which results in defective TGF- $\beta$  signaling [78]. Because the TGF- $\beta$  pathway inhibits Wnt signaling [79], the authors propose that development of a mammary tumor requires a low level of Wnt signaling which is provided by the  $Apc^{1572T}$  allele. Higher Wnt signaling resulting from reduced TGF $\beta$  signal, or from a second mutant  $Apc$  allele, promotes intestinal polyp formation. Although this model might explain the development of intestinal polyps in mice heterozygous for both  $Apc^{1572T}$  and  $Smad4^{Sad}$  [80], it does not explain the high penetrance of mammary tumors in these mice, given the low penetrance of mammary tumors in other models with higher Wnt signaling.

In conclusion, data collected from  $Apc^{1572T}$  and  $Apc^{1638T}$  mice implicates the C-terminal portion of  $Apc$  in control of mammary tumorigenesis and development. However, these models provide no direct evidence that the  $Apc$  C-terminal region suppresses intestinal tumorigenesis.

### **$Apc^{1309}$ and $Apc^{1322T/+}$ mice**

Although  $Apc^{Min/+}$  mice have been used to model  $APC$  mutation in humans, similarly sized  $APC$  truncations are uncommon in both inherited and sporadic human colon cancers. Mutations in  $APC$  associated with colon cancer typically truncate the C-terminal half of the protein, leaving the first 20-amino acid (20-a.a.) repeat intact in at least one  $APC$  allele [81]. As this 20-a.a. repeat can bind to  $\beta$ -catenin, one would anticipate differences in cells expressing shorter  $Apc$  truncations, such as  $Apc^{Min}$ , and cells with longer  $APC$  (as in human CRC) [82, 83]. The  $Apc^{1309}$  and  $Apc^{1322T}$  mouse models were generated to express truncated  $Apc$  that retains the first 20-a.a. repeat [81, 84, 85]. As with  $Apc^{Min/+}$ , both  $Apc^{1309/+}$  and  $Apc^{1322T/+}$  mice develop polyps mainly in the small intestine, but these polyps are more proximal than those from  $Apc^{Min/+}$  mice. The levels of Wnt target gene transcripts are lower in polyps from  $Apc^{1322T/+}$  mice than in polyps from  $Apc^{Min/+}$  mice, as expected since the  $Apc^{1322T}$  protein includes the first 20-a.a. repeat [86]. However,  $Apc^{1322T/+}$  mice develop more polyps (> 200 polyps by 12 weeks) and have more intestinal stem cells than do  $Apc^{Min/+}$  mice [84]. Together, these results support the “just right” hypothesis that predicts that inclusion of the first 20-a.a. repeat in truncated  $APC$  proteins will result in only slight elevation of Wnt signaling, which is more conducive to intestinal tumor growth than is elevation of Wnt signaling to a higher level [82]. Extra-intestinal phenotypes reported for  $Apc^{1322T/+}$  mice include anemia and large spleens, similar to  $Apc^{Min/+}$  mice [84]. In contrast,  $Apc^{1309/+}$  mice develop far fewer intestinal tumors (~ 35), mainly in the small intestine at the age of 12–14 weeks, and have hyperlipidemia that develops at an even earlier age than in  $Apc^{Min/+}$  mice [85, 87]. Potential explanations for this large discrepancy in polyp number between mouse models that differ in truncated  $APC$  length by only 13 amino acids include the influence of environmental factors, genetic background, and different technologies used to generate these mice. Table 1 summarizes the intestinal phenotype in different  $Apc$  mouse models with truncated  $Apc$  longer than  $Apc^{Min}$ .

### **Mouse models expressing truncated $Apc$ protein shorter than $Apc^{Min}$**

Seven mouse models with mutations upstream to that in  $Apc^{Min}$  have been described.  $Apc^{\Delta 242}$  [88],  $Apc^{\Delta 474}$  [89], and  $Apc^{\Delta 716}$  [90–92] mice have  $Apc$  truncation mutations at codons, 242, 474, and 716, respectively, while  $Apc^{\Delta 580}$  [93],  $Apc^{580D}$  [94], and  $Apc^{\Delta 14}$  [95] mice each have a deletion of exon 14, resulting in a frameshift and a nonsense mutation

at codon 580.  $Apc^{\Delta 15}$  mice have a deletion of the last *Apc* exon and the 3'UTR region and have no detectable expression of the mutant allele [96]. These seven mouse models share many phenotypes with  $Apc^{Min/+}$  mice, including embryonic lethality in the homozygous state, and in heterozygous mice, development of anemia and intestinal polyps predominantly in the small intestine that are indistinguishable at the microscopic level [88–94, 96, 97]. Although polyp number varies between these seven models (Table 2), in most cases, direct comparative studies have not been performed. Mammary tumors have been reported for 14.3% of  $Apc^{\Delta 580}$ , 18.5% of  $Apc^{\Delta 474}$ , and 9% of  $Apc^{\Delta 14}$  mice [89, 93, 97].

Is the variation in polyp number in these mouse models due to the progressive deletion of particular *Apc* domains (see figure 1)? The  $Apc^{\Delta 716}$  protein is 134 a.a. shorter than the  $Apc^{Min}$  protein and lacks an additional portion of the armadillo repeat region. Although it is tempting to speculate that the three-fold increase in polyp number seen in  $Apc^{\Delta 716/+}$  mice compared to  $Apc^{Min/+}$  mice results from interruption of the armadillo repeat region,  $Apc^{\Delta 242/+}$  mice, which have a truncating *Apc* mutation that eliminates the entire armadillo repeat region, develop fewer polyps than  $Apc^{\Delta 716/+}$  mice. Moreover,  $Apc^{\Delta 580/+}$ ,  $Apc^{580D/+}$ ,  $Apc^{\Delta 14/+}$  and  $Apc^{\Delta 474/+}$  mice, which have truncating mutations in the middle of the armadillo repeat region, have reported intestinal polyp numbers similar to that seen in  $Apc^{Min/+}$  mice (Table 2).

## Complete deletion of *Apc*

In human CRC, *APC* mutations are predominantly found in a region referred to as the mutation cluster region (MCR), and result in truncation of the C-terminal half of APC [98]. Complete deletion of APC has been reported in FAP syndrome only rarely [99, 100], leading to the hypothesis that truncated APC protein can enhance tumorigenicity in a dominant-negative manner. A mouse model with complete deletion of all 15 *Apc* exons ( $Apc^{\Delta e1-15}$ ) was generated to test the requirement of truncated APC for tumor formation [101].  $Apc^{\Delta e1-15/+}$  mice develop intestinal polyps of the same distribution and morphology as those seen in  $Apc^{Min/+}$  mice, but with increased frequency. Polyps from  $Apc^{\Delta e1-15/+}$  mice had lower levels of  $Apc^+$  mRNA compared to normal tissue, consistent with a requirement for loss of the wild-type allele for intestinal tumor development, although this was not directly examined.  $Apc^{\Delta e1-15/+}$  mice also develop more severe anemia than  $Apc^{Min/+}$  mice, and one  $Apc^{\Delta e1-15/+}$  mouse developed a mammary tumor. Female  $Apc^{\Delta e1-15/+}$  mice showed more severe phenotypes than did males. Polyps from  $Apc^{\Delta e1-15/+}$  mice had lower mRNA levels of Wnt target genes *Axin2*, *c-Jun*, and  $\beta$ -*catenin* than polyps from  $Apc^{Min/+}$  mice [101]. Although puzzling in terms of the underlying mechanism and pathogenesis, this observation is consistent with the hypothesis that there is a level of Wnt signaling optimal for polyp formation, and signaling in excess of this level inhibits polyposis [101].

## *Apc* mouse models with interstitial *Apc* mutations

Two mouse models have been recently described in which the engineered mutations result in changes within, rather than truncation of, *Apc* protein:  $Apc^{mNLS}$  and  $Apc^{\Delta SAMP}$  models.

### $Apc^{mNLS}$ model

APC is perhaps best known as a Wnt signal antagonist. In this capacity, APC is a component of a cytoplasmic complex that targets the oncoprotein  $\beta$ -catenin for proteasomal degradation [7]. APC also shuttles between the nucleus and the cytoplasm, aided by at least 2 nuclear localization signals (NLS) and 5 nuclear export signals (NES) [102]. Studies using cultured cells indicate that APC and  $\beta$ -catenin can interact in the nucleus, resulting in transcriptional repression of Wnt target genes and inhibition of cellular proliferation [9, 103]. In addition,

nuclear APC interacts with Topoisomerase II $\alpha$ , a critical enzyme required for DNA replication and a target for traditional cancer chemotherapeutics [104]. APC also has a role in DNA repair and synthesis [105, 106]. To study the role of nuclear APC in tissue homeostasis and tumor suppression, a mouse model was generated in which nuclear import of Apc was compromised via the introduction of inactivating mutations into both NLSs (Apc<sup>mNLS</sup>) [107]. Apc<sup>mNLS/mNLS</sup> mice are viable, with no significant alteration in lifespan. Compared to Apc<sup>+/+</sup> mice, intestinal epithelia from Apc<sup>mNLS/mNLS</sup> mice were more proliferative and showed higher levels of Wnt target gene mRNA. In addition, Apc<sup>Min/+</sup> mice develop more and larger intestinal tumors when they also harbor the Apc<sup>mNLS</sup> allele (Apc<sup>mNLS/Min</sup>). Together, studies using the Apc<sup>mNLS</sup> model support a role for nuclear Apc in inhibition of proliferation, Wnt signaling, and tumorigenesis [107].

### Apc <sup>$\Delta$ SAMP</sup> model

The Apc <sup>$\Delta$ SAMP</sup> mouse, with a deletion of Apc amino acids 1322 to 2005, was generated to delineate the contribution of the Apc C-terminus to tumor suppression [108]. This Apc deletion eliminates all but the first 20-a.a. repeat and all SAMP motifs, but retains the C-terminal region of Apc. Phenotypes of the Apc<sup>1322T/+</sup> and Apc <sup>$\Delta$ SAMP</sup> mice were similar with regard to polyp number, distribution, size, and morphology, severity of dysplasia, differentiated and stem cell populations, and expression of Wnt target genes. Thus, it appears that in the Apc<sup>1322T/+</sup> model, the C-terminal region of Apc is not involved in suppression of intestinal adenoma [108].

### Changing the level of Apc expression

Two Apc mouse models with reduced *Apc* expression were generated by inserting a neomycin cassette into *Apc* intron 13 in either reverse (Apc<sup>NeoR</sup>) or forward orientation (Apc<sup>NeoF</sup>) [109, 110]. The neomycin cassette disrupts an enhancer and reduces the level of full-length Apc expressed from the mutant allele to 20% of normal levels for Apc<sup>NeoR</sup>, and 10% for Apc<sup>NeoF</sup>. Each allele produces an embryonic lethal phenotype in the homozygous state. By the age of 15 months, Apc<sup>NeoR/+</sup> and Apc<sup>NeoF/+</sup> develop intestinal polyps with relatively low incidence (19% and 50%, respectively) and multiplicity (0.26 $\pm$ 5.4 and 1.09 $\pm$ 8.5 polyps per mouse, respectively). The polyps in Apc<sup>NeoR</sup> and Apc<sup>NeoF</sup> mice display loss of the wild-type *Apc* allele and have less  $\beta$ -catenin stability and accumulation of nuclear  $\beta$ -catenin than do polyps from Apc <sup>$\Delta$ 716/+</sup> mice [109, 110]. Thus, in mice, there appears to be a critical threshold level of Apc to support tumor suppression.

### A transgenic mouse expressing truncated Apc

Based in part on the correlation of FAP severity with specific truncating *APC* mutations, and on the ability of truncated APC to bind to full-length APC, it was proposed that particular APC truncations act in a dominant-negative manner [111]. However, a direct test of this hypothesis revealed no increased polyp susceptibility in mice carrying a transgene encoding Apc amino acids 1–716, even though the truncated Apc protein was detected in intestinal cells. It is possible that the proposed dominant -negative activity of truncated Apc could not overcome functional Apc from two wild-type *Apc* alleles that could compensate for any deleterious effect of the truncated allele. To explore this possibility, the transgene for truncated Apc was introduced into Apc <sup>$\Delta$ 716/+</sup> mice [90]. Because intestinal tumor number, distribution, and morphology were the same in Apc <sup>$\Delta$ 716/+</sup> mice with and without the extra truncated Apc transgene, it was concluded that in this mouse model, truncated Apc does not act in a dominant-negative manner [90].



## Conditional *Apc* mouse models

Mouse models with germline *Apc* mutations have been useful to probe many aspects of APC biology, especially in intestinal tumorigenesis. However, most of these models are limited by a short life span, the predominance of intestinal phenotypes, and embryonic lethality in the homozygous state. To study functions of APC at different developmental stages and in organs other than the intestine, investigators have developed mice with conditional *Apc* mutations [112]. A critical component of most conditional systems is CRE recombinase, which induces recombination between two *loxP1* sites, resulting in excision of the DNA between these sites. In conditional *Apc* mouse models, *loxP1* sequences are inserted into introns of the mouse *Apc* gene flanking particular exon(s). In the presence of *Cre*, excision of the *lox*-flanked DNA leads to a frameshift mutation and truncation of *Apc*. Five different conditional *Apc* alleles have been made; *Apc*<sup>580S</sup>, *Apc*<sup>CKO</sup>, *Apc*<sup>Δex14</sup>, *Apc*<sup>15fllox</sup> and *Apc*<sup>lox468</sup> [93–96, 113, 114]. The specificity of these *Apc* mutations is achieved by placing *Cre* under control of a tissue- or developmental stage-specific promoter or an inducible promoter, or by infecting tissues with *Cre*-expressing Adenovirus [115]. Table 3 summarizes different conditional *Apc* mouse models.

## *Apc* rat models

A rat model with a germline nonsense mutation at *Apc* codon 1137 (*Apc*<sup>am1137</sup>) was generated to overcome some of the limitations of *Apc* mouse models [134]. Rats homozygous for the *Apc*<sup>am1137</sup> allele die as embryos. *Apc*<sup>am1137/+</sup> rats develop both small intestinal and colonic polyps with 100% penetrance, and are called “PIRC” rats for Polyposis In Rat Colons [134]. The polyps in PIRC rats are adenomas with malignant changes and local invasion seen in old rats. No signs of metastasis have been detected in these rats. As seen in humans with germline *Apc* mutations, the polyps from PIRC rats show β-catenin nuclear translocation in advanced but not in early adenomas. As with *Apc*<sup>Min/+</sup> mice, most intestinal polyps in PIRC rats show LOH. Because chromosome 18, which carries the *Apc* gene in rats, is metacentric, pyrosequencing could be used to demonstrate that LOH in PIRC rats predominantly occurs by means of homologous recombination [134]. The greater width of rat intestines and colons, relative to those of mice, allows for growth of larger intestinal tumors, which facilitates study of tumor progression beyond the early stage. Wider colons and higher colonic tumor multiplicities in rats also supported a longitudinal endoscopic study of tumorigenesis [134]. Male PIRC rats have more polyps than do females [101]. Most *Apc* mouse models do not show a gender bias. However, in *Apc*<sup>Min-FCCC/+</sup> mice, an *Apc*<sup>Min/+</sup> mouse model with a different genetic background, males also develop more colonic polyps than do females [135]. In contrast, female *Apc*<sup>Δe1–15/+</sup> mice display more severe phenotypes than do males [101]. In humans, women appear to be slightly less affected by colon cancer than are men [136]. PIRC rats also show high incidence of jaw tumors, which are the main cause of morbidity in female PIRC rats [134]. This extra-intestinal phenotype has also been described in patients with FAP syndrome [137].

A second *Apc* rat model (Kyoto *Apc* Delta or KAD rat) was developed with a germline nonsense mutation in the *Apc* gene, resulting in deletion of the C-terminal 321 amino acids [138]. This deletion does not appear to affect life expectancy even in homozygous KAD rats, and no spontaneous polyps develop in the KAD rat intestines. However, KAD rats showed enhanced inflammation-mediated colon tumorigenicity, consistent with a Wnt-independent role for the C-terminal domain of *Apc* in tumor suppression.

In summary, rodent models with *Apc* mutations were first generated more than 2 decades ago. Studies of 45 rodent models with germline and conditional *Apc* mutations have led to greater understanding of the role of APC in development, differentiation, and homeostasis of

intestinal epithelial cells. In addition, these models have allowed exploration of the role of APC in intestinal and extra-intestinal development and tumorigenesis. Mouse and rat models with germline *Apc* mutations have permitted experimental testing of different molecular pathways and investigation of genetic and environmental contributions to tumor formation, not only in the gastrointestinal tract but also in other tissues. These models have also facilitated testing different preventive and therapeutic agents in preclinical studies. Continued effort should be made to clarify some of the less understood features of the different *Apc* rodent models. These lingering mysteries include identifying the variables that contribute to differences in extra-intestinal phenotypes, and polyp distribution and number. The full potential of the *Apc* models has not yet been reached; it is expected that they will continue to provide insight into *Apc* and cancer biology for decades to come.

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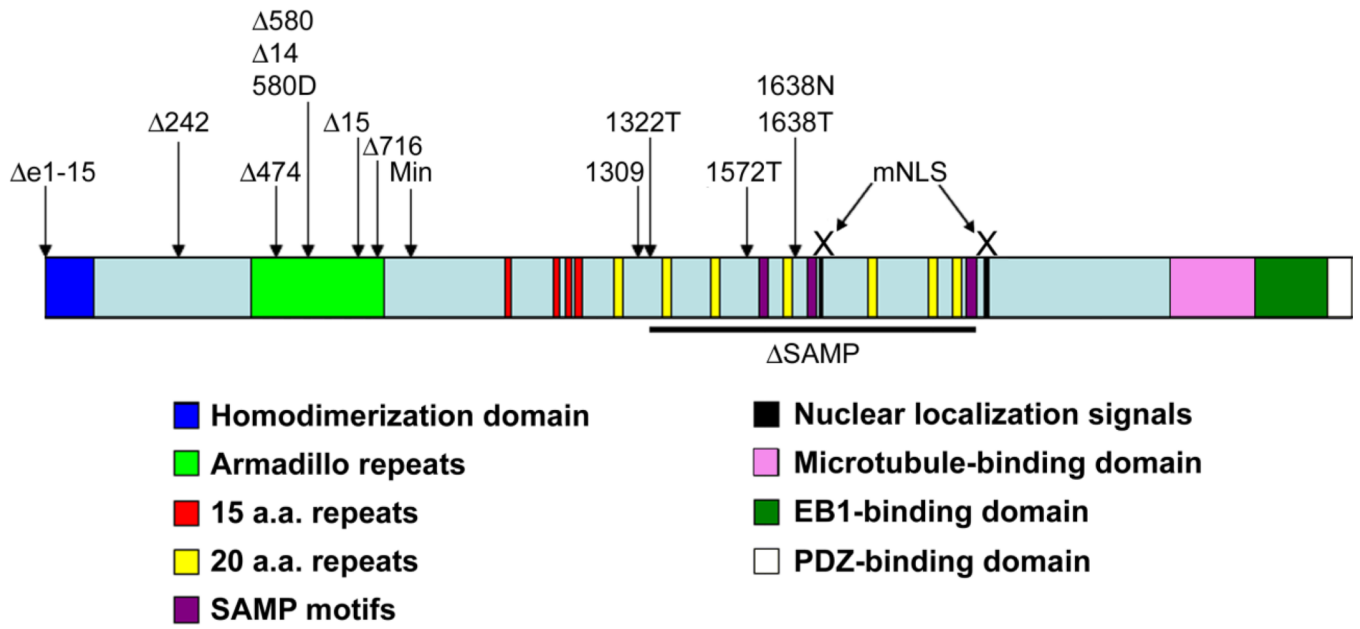
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**Figure 1.**  
 Apc protein structure and different rodent models with germline *Apc* mutations

**Table 1**Intestinal phenotypes in mice with truncated Apc longer than that of Apc<sup>Min</sup>

Mouse model	Polyp number	Apc <sup>Min/+</sup> Polyp number *	Notes	Ref
<i>Apc</i> <sup>1638N/+</sup>	<10	-	Malignant changes are detected in old mice	[75]
<i>Apc</i> <sup>1638T/+</sup>	None	-	Viable homozygous mutant mice	[76]
<i>Apc</i> <sup>1572T/+</sup>	None	-		[78]
<i>Apc</i> <sup>1322T/+</sup>	192 (age 10–12 weeks)	154 (age 110–130 days)	Majority of polyps are in the proximal 2/3 of the small intestine	[84, 86]
<i>Apc</i> <sup>1309/+</sup>	36.7±2.7	-		[87]

\* included in the same study

**Table 2**Intestinal phenotypes in mice with truncated Apc shorter than that of Apc<sup>Min</sup>

Mouse model	Polyp number	Apc <sup>Min/+</sup> Polyp number*	Notes	Ref
<i>Apc</i> <sup>Δ716</sup>	256±55	1/3 of those in <i>Apc</i> <sup>Δ716</sup>		[91]
<i>Apc</i> <sup>Δ14</sup>	36±29	34±18	More polyps in distal colon and rectum relative to Apc <sup>Min/+</sup> , number of polyps increases in germ-free environment	[97]
<i>Apc</i> <sup>Δ15/+</sup>	184.7	-	Tumors are mainly in the ileum, no comparative data to Apc <sup>Min</sup> mice	[96]
<i>Apc</i> <sup>Δ580</sup>	120±37	-	No full-length or truncated APC proteins were detected in polyps	[93]
<i>Apc</i> <sup>580D</sup>	??	-		[94]
<i>Apc</i> <sup>Δ474</sup>	123±9.6		No comparative data to Apc <sup>Min</sup> mice	[89]
<i>Apc</i> <sup>Δ242</sup>	177±30	106±28		[88]

\* included in the same study

Table 3

mice with conditional mutations in *Apc*

<i>Apc</i> <sup>580S</sup> allele; Expression of Cre recombinase results in excision of <i>Apc</i> exon 14 and a stop codon at a.a. 580				[94]
Mouse	Organ	Developmental stage	Cre delivery/phenotype	Ref
<i>Apc</i> <sup>580S</sup>	Colon and rectum	Adult	Cre delivered via Adenovirus vector injected in the colon through the anus. Develop colon adenomas in the distal 3 cm of the colon. Malignant transformation seen in old lesions.	[94]
<i>CPC;Apc</i> <i>CDX2P 9.5-NLS-Cre; Apc</i> <sup>+/loxP</sup> )	Distal ileum, cecum, colon and rectum	Day 8.5 embryonic	Cre is expressed using 9.5-Kb DNA fragment from the homeobox gene <i>Cdx2</i> promoter. Mice develop ~10 tumors in ileum, cecum and colon by the age of 300 day. Malignant transformation in 66.1% of mice. Mice show anemia and stunted growth.	[132]
<i>CDX2P9.5-G22Cre; Apc</i> <sup>flox/flox</sup>	Distal ileum, cecum, colon and rectum	Day 8.5 embryonic	Cre is expressed using 9.5-Kb DNA fragment from the homeobox gene <i>Cdx2</i> promoter. There is a string of 22 guanine nucleotides after the ATG initiation codon (out of frame). Restoration of in-frame sequence occurs by mitotic microsatellite instability. Mice die at age of 10–27 days and develop large number of adenomatous polyps in the distal ileum and large intestine.	[133]
<i>AhCre-Apc</i> <sup>fl/fl</sup>	Small intestine, large intestine. Possibly the liver	Adult	Cre expressed using <i>Cyp1A</i> promoter when mice were injected with $\beta$ -naphthoflavone. Upregulation in Wnt signaling. Intestinal cell differentiation, proliferation, migration, and apoptosis disrupted. Mice died 4 days after induction.	[129]
<i>MMTV-Cre-Apc</i> <sup>flox/flox</sup> <i>Pten</i> <sup>flox/flox</sup>	Salivary glands	??	Cre expressed using <i>MMTV</i> promoter. In B6X129 background, <i>MMTV</i> promoter is active in salivary gland and less active in mammary gland. Salivary gland tumors only with <i>Pten</i> deletion.	[117]
<i>Mx1-Cre</i> <sup>+</sup> <i>Apc</i> <sup>fl/fl</sup>	Hematopoietic stem and progenitor cells transplanted into WT mice	Adult	Cre expressed under the type-1 interferon	[126]

<i>Apc</i> <sup>580S</sup> allele; Expression of Cre recombinase results in excision of <i>Apc</i> exon 14 and a stop codon at a.a. 580				[94]
Mouse	Organ	Developmental stage	Cre delivery/phenotype	Ref
			inducible promoter <i>Mx1</i> after injection of polyinosinic-polycytidylic acid (pI-pC). This promoter is active in other tissues including; intestinal epithelium, oseoblasts and kidney. To restrict the expression in only hematopoetic stem cells and progenitor cells, bone marrow cells from <i>Mx1-Cre<sup>+</sup> Apc<sup>fl/fl</sup></i> mice were implanted into WT mice. Induction of Cre results in increased cell cycle entry, apoptosis and exhaustion of hematopoetic and depletion of myeloid progenitor pool	
<i>Ksp-Cre Apc</i> <sup>58S/580S</sup>	Renal tubular epithelial cells and developing genitourinary tract	Embryonic	Cre is expressed using <i>Ksp-cadherin</i> promoter. Neonatal death with signs of renal failure. Rare mice that live to adulthood develop renal cysts, adenoma and elevated blood urea level	[125]
<i>AhCre-Apc</i> <sup>fl/fl</sup>	Kidney	Day 14.5–18.5 embryonic	Cre is expressed using <i>Cyp1A</i> promoter with no $\beta$ -naphthoflavone induction. Renal carcinoma in ~1/4 mice at 6 months, increased incidence with co-existence of p53 mutations	[128]
<i>OC-Cre Apc</i> <sup>fllox/fllox</sup>	Osteoblasts	Starting from embryonic day 17	Cre is expressed using promoter of <i>oseocalcin</i> . Increased bone formation with distorted bone architecture. Reduced survival to time of weaning (10%).	[119]
<i>Apc</i> <sup>CKO</sup> allele; Expression of Cre recombinase results in excision of <i>Apc</i> exon 14 and a stop codon at a.a. 580				[93]
Mouse	Organ	Developmental stage	Cre delivery/phenotype	Ref
<i>Apc</i> <sup>CKO/CKO-LSL-Kras</sup>	Distal colon	Adult	Cre is delivered via adenovirus vector injected in the colon through the anus, resulting in excision of <i>Apc</i> exon 14 and expression of mutant constitutively active <i>Kras</i> . Adenocarcinoma in the distal colon that show spontaneous metastasis to the liver after 24 weeks	[120]

<i>Apc580S</i> allele; Expression of Cre recombinase results in excision of <i>Apc</i> exon 14 and a stop codon at a.a. 580				[94]
Mouse	Organ	Developmental stage	Cre delivery/phenotype	Ref
<i>K14-Cre-Apc<sup>CKO/CKO</sup></i> <i>K14-Cre-Apc<sup>CKO/+</sup></i>	Ectodermal derived tissues including mammary glands	Day 9.5 embryonic	Cre is expressed using <i>Keratin-14</i> promoter in epidermal tissues. Growth retardation, premature death, abnormalities in epidermal derived tissues including: hair follicles, cornea, and teeth. Thymus hypoplasia, squamous metaplasia in the thymus (homozygous), mammary tumors in 76.5% of heterozygous females.	[93, 121]
<i>WAP-Cre-Apc<sup>CKO/CKO</sup></i> <i>WAP-Cre-Apc<sup>CKO/+</sup></i>	Lactating epithelial cells	Lactation	Cre is expressed using <i>Whey Acidic Protein (Wap)</i> promoter. Mammary tumors in nulliparous and multiparous females (less than 20%)	[93]
<i>Ahmr2-Cre-Apc<sup>flox/flox</sup></i>	Uterine stroma (in females) Sertoli cells (in males)	Fetus	Cre is expressed using <i>anti-Mullerian hormone type II receptor</i> promoter in mesenchyme of fetal Mullerian duct. Progressive uterine hyperplasia and endometrial carcinoma. <i>Apc</i> has a cell-non-autonomous role as an endometrial tumor suppressor protein. Large spleens (in females); abnormal spermatogenesis, loss of the apical part of Sertoli cells, disruption of tight junctions, no tumors (in males).	[43, 130]
<i>Pms<sup>2</sup>-Apc<sup>CKO/+</sup></i>		??	Out-of-frame Cre that reverts back in-frame stochastically. Rate of transformation is higher in <i>Apc<sup>1638N/CKO</sup></i> and <i>Apc<sup>Min/CKO</sup></i> mice relative to <i>Apc<sup>CKO/+</sup></i> mice	[118]
<i>Apc<sup>15flox</sup></i> allele; Expression of Cre recombinase results in excision of <i>Apc</i> last exon and 3'UTR region				[96]
Mouse	Organ	Developmental stage	Cre delivery/phenotype	Ref
<i>Fabp1Cre; Apc<sup>15flox/+</sup></i>	Distal small intestine and large intestine	??	Cre is expressed using <i>fatty-acid binding protein-1 (Fabp1)</i> promoter in some cells. Develop adenoma and adenocarcinoma mainly in large intestine	[96]
<i>Ahmr2-Cre-Apc<sup>15flox/15flox</sup></i>	Uterine myometrium	??	Cre is expressed using <i>anti-Mullerian hormone type II receptor</i>	[131]



<i>Apc580S</i> allele; Expression of Cre recombinase results in excision of <i>Apc</i> exon 14 and a stop codon at a.a. 580				
Mouse	Organ	Developmental stage	Cre delivery/phenotype	Ref
			promoter in mesenchyme of fetal Mullerian duct. Myometrial defects, dystocia, reduced number of endometrial glands	
<i>Pgr-Cre-Apc<sup>fllox/fllox</sup></i>	Uterine endometrium& myometrium	??	Cre is expressed using <i>progesterone receptor</i> promoter. Myometrial and endometrial defects, endometriosis interna-like changes	[131]
<i>Math1-Cre-Apc<sup>Fl/Fl</sup></i>	Cerebellum	Day 12.5 embryonic	Cre is expressed using <i>Math-1</i> promoter in Granule cells in the cerebellum. No tumor. Cerebellar cortical hypoplasia, impaired motor coordinator and ataxia	[122]
<i>Col2a1-Cre-Apc<sup>I5lox/I5lox</sup></i>	Mesenchymal cells	Day 9.5 embryonic in sclerotome Day 12.5–16.5 embryonic in chondrogenic and osteogenic cells.	Cre is expressed using <i>Col2a1 (collagen-2a-1)</i> promoter in mesenchymal cells. Embryonic lethal, defective cartilage and bone differentiation	[124]
<i>Apc<sup>I5lox/I5lox</sup> Apc<sup>I5lox/1638N</sup> Apc<sup>I5lox/+</sup> Apc<sup>I5lox/1572T</sup></i>	Hematopoietic stem and progenitor cells	Adult	Cre delivered by a lentiviral vector <i>ex-vivo</i> Different levels of Wnt signaling activation associated with differential effects on hematopoietic stem cell and myeloid and lymphoid differentiation	[123]
<i>Apc<sup>Δex14</sup></i> allele; Expression of Cre recombinase results in excision of <i>Apc</i> exon 14 and a stop codon at a.a. 580				
Mouse	Organ	Developmental stage	Cre delivery/phenotype	Ref
<i>Vil-CreER<sup>T2</sup>-Apc<sup>lox/lox</sup></i>	Small and large intestine	Adult	Cre expressed using <i>Villin</i> promoter when the mice are injected with Tamoxifen. Upregulation of Wnt signaling, increased proliferation and apoptosis, decreased migration, increased number of cells committed to Paneth cell differentiation.	[116]
<i>Apc<sup>Δex14/Δex14</sup></i>	Liver	Adult	Cre driven by a CMV promoter is delivered using Adenovirus injected intravenously. High viral dose causes hepatomegaly, hepatocellular hyperplasia and death. Low viral dose causes hepatocellular carcinoma	[95]

<i>Apc580S</i> allele; Expression of Cre recombinase results in excision of <i>Apc</i> exon 14 and a stop codon at a.a. 580				
Mouse	Organ	Developmental stage	Cre delivery/phenotype	Ref
<i>Ap<sup>lox468</sup></i> allele; Expression of Cre recombinase results in Excision of exons 11 & 12 and a frameshift splicing exons 10–13 and truncating <i>Apc</i> at codon 468.				
Mouse	Organ	Developmental stage	Cre delivery/phenotype	Ref
<i>Ts4Cre-Apc<sup>lox468/+</sup></i>	Colon and distal ileum	??	Cre is expressed using Ts4 promoter. Polyposis in the colon and distal ileum. Lipoteichoic acid-deficient lactobacilli bacteria modulate colonic inflammation and reduce polyp formation.	[114]
<i>LckCre-Apc<sup>lox/lox468</sup></i>	Thymus	Starts at CD4 <sup>-</sup> CD25 <sup>+</sup> double-negative 3 (DN3) stage and complete by DN4 stage of lymphocyte development	Cre is expressed using <i>Lck</i> promoter during the development of thymocytes. Thymic atrophy, reduced T-lymphocyte receptor rearrangement, increasing proliferation of pre-T cells, chromosomal segregation defects, T-cell developmental delay.	[113]

??  
not defined