TITLE AND AUTHORS

Fanca and *p53* genes cooperate to suppress oral SCC in transgenic mice

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ABSTRACT

Fanconi anemia (FA) patients frequently develop oral squamous cell carcinoma (OSCC). This cancer in FA is diagnosed within the first 3-4 decades of life, and patients respond poorly to current treatments, mainly radiotherapy and chemotherapy. In addition, FA patients develop oral lesions very often that might precede malignant transformation. Research and translation of new chemopreventive and therapeutic strategies has been unsuccessful in part because disease models are scarce or do not fully reproduce the disease. Here we report that *Fanca* gene knockout mice (*Fanca*$^{-/-}$) frequently display pre-malignant lesions in the oral cavity. Moreover, when these animals were crossed with animals having conditional deletion of *p53* gene in oral mucosa (*K14cre*;*Trp53*$^{F2\text{-}10/F2\text{-}10}$) spontaneously develop OSCC with high penetrance and a median latency of less than ten months. Tumors are well differentiated and express markers of squamous differentiation, such as K5 and K10 keratins. In conclusion, *Fanca* and *p53* genes cooperate to suppress oral cancer in mice, and *Fanca*$^{-/-}$*;K14cre;Trp53*$^{F2\text{-}10/F2\text{-}10}$ constitute the first animal model of spontaneous OSCC in FA.

INTRODUCTION

Fanconi anemia (FA) is a heritable syndrome with predisposition to congenital abnormalities, bone marrow failure (BMF), and cancer. In FA patients, pathogenic aberrations have been described in 23 different Fanconi (FANC) genes, giving rise to Fanconi or Fanconi-like clinical phenotype. Most FA children have BMF that can be successfully treated with bone marrow transplantation (BMT). BMT has also been used to treat blood malignancies such as myelodysplastic syndrome (MDS) and leukemia in FA. BMT has significantly improved life expectancy in FA individuals so that there are now more adults living with FA than children diagnosed with this syndrome. Unfortunately, a consequence of this achievement has been the identification of solid tumor predisposition as the most important health challenge in FA adults. Patients have an extraordinarily high lifetime risk of squamous cell carcinoma (SCC) of the head and neck, esophagus, vulva, or anus. This risk is increased by prior BMT, together with a continuing risk of acute myeloid leukemia [1]. Synchronic and metachronic tumors, especially in the oral cavity, are distressingly common in FA [2]. Moreover, many patients with FA, especially BMT recipients, develop multiple visible oral lesions. Frequently, the first clinical manifestation of FA is the diagnosis of SCC in young individuals. Besides SCC, other malignant tumors have also been described in FA, although less frequently: lymphoma, liver cancer, brain tumors, and embryonal tumors such as Wilms tumor and neuroblastoma. However, the risk of these type of tumors is much lower than SCC.

The inability to repair DNA interstrand cross-links (ICLs) is a hallmark feature of FA. FA proteins function to repair ICLs in a common cellular pathway, known as the FA pathway or FA/BRCA pathway. A defective FA pathway leads to the accumulation of genomic aberrations and the emergence of precancerous cells that eventually might progress into invasive carcinomas. However, the etiology of SCC in mucosal epithelia in FA is not well understood. Known drivers of head and neck SCC (HNSCC) are smoking and alcohol consumption, but they are less commonly reported in FA than in the general population. Human papillomavirus (HPV) infection is another driver of HNSCC. However, some reports suggest that HPV may be a major contributor to HNSCC development in patients with FA [3], whereas other studies dispute these results [4]. HNSCC in FA is difficult to treat, as patients cannot tolerate current therapies, including ionizing radiation or DNA cross-linking, platinum-based chemotherapies [5]. In addition, most tumors are detected at advanced stages. Altogether, these events have made cancer as the major reason of early mortality in adults with FA. Therefore, the discovery of non-genotoxic and efficient treatment opportunities in FA patients is of utmost importance.

New therapies against FA HNSCC could be discovered, and preclinically tested, in adequate disease models, such as cellular and animal models. Tremendous efforts have been made to generate, and analyze, whether genetically engineered mouse models (GEMMs) with deleterious mutations in FANC genes could model disease features of FA patients. Thus, lines of mice bearing mutations in *Fanca*, *Fancc*, *Fancd1*, *Fancd2*, *Fance*, *Fancf*, *Fancg*, *Fanci*, *Fancl*, *Fancm*, *Fancn*, *Fanco*, or *Fancp* have been produced [6-8]. To date, all FA mouse models display reduced fertility and cells derived from them are sensitive to ICLs. Embryonic and perinatal lethality has been observed in most FA models, which could be influenced by the mouse genetic background. Some models display other minor developmental abnormalities, such as microphtalmia in *Fanca* [9], *Fanc* [10], *Fancd2* [11], *Fanci* [7], and *Fancp* [12]. The life-threatening anemia that FA patients suffer is not modelled in FA mice, although some defects are present, such as lower blood cellularity in Fancp [12] or reduced proliferation in hypomorphic *Fancd1* [13]. Equally, the high incidence of BMF, leukemia and SCC, accompanied with early mortality in adults with FA, is not well recapitulated in FA mice. Long-term survival has been reported in *Fancc*-, *Fancd2*-, *Fancf*-, *Fancm*- and *Fancp*-deficient mice. However, increased incidence of various solid tumors exists in *Fanca*-, *Fancf*-, *Fancd2*- and *Fancm*-mice. Strikingly, none of them develop SCC in the oral cavity, esophagus or anogenital tissues. These findings demonstrate that the loss of a single Fanc gene is not sufficient condition to model early cancer appearance or SCC development in GEMMs. Double-mutant mice have been investigated to uncover genetic interactions with other cellular pathways conducting to oncogenesis. Cooperation in tumor suppression clearly exists between the FA pathway and p53. Accelerated tumorigenesis is present in double *Fancc-/- p53-/-* [14] and *Fancd2-/- p53-/-* [15], although

these mice do not show increased SCC formation in oral and anogenital tissues, thus precluding their use as animal models of SCC in the appropriate tissue of origin. Fancd2-/- animals, when crossed with K14creE6 or K14creE6E7, and upon treatment with oral carcinogen 4-NQO, developed HNSCC [16,17]. In these animals, no tumor formation were reported in the absence of 4-NQO.

HNSCCs from FA patients frequently display typical alterations found in HPV-negative HNSCC tumors from the general population, such as mutation in the *p53* gene [18,19]. Here, we have also detected mutations in p53 in all six SCCs from the oral cavity of three FA patients. In order to develop a mouse model of FA oral SCC, we decided to cross *Fanca*$^{-/-}$ animals with deficiency in *Fanca* gene with K14*cre*;*Trp53*$^{F2\text{-}10/F2\text{-}10}$ mice lacking p53 in stratified epithelia (such as oral mucosa). Previously, we have shown that K14*cre*;*Trp53*$^{F2\text{-}10/F2\text{-}10}$ animals spontaneously develop skin SCC, demonstrating a predominant tumor suppressor role of p53 in mouse epidermis [20]. Furthermore, K14*cre*;*Trp53*$^{F2\text{-}10/F2\text{-}10}$ mice display oral cavity tumors when combined with constitutive Akt activity [21] or IKKβ overexpression [22]. We hypothesize that combined loss of *Fanca* and *p53* genes in the oral cavity might produce spontaneous oral tumors. Here we describe the phenotype of double *Fanca*$^{-/-}$; K14*cre*;*Trp53*$^{F2\text{-}10/F2\text{-}10}$ mice and we demonstrate that loss of both *Fanca* and *p53* genes in oral mucosa phenocopies human FA HNSCC.

MATERIALS AND METHODS

Patients. Oral squamous cell carcinoma (OSCC) from Fanconi anemia patients were analyzed. The study was conducted in accordance with the precepts established in the Declaration of Helsinki, Good Clinical Practice guidelines, and all applicable regulatory requirements. The ethics committee of Hospital 12 Octubre approved the study protocol.

Tumor sequencing from FA patients. Deep-sequencing was performed from FFPE tumor blocks. Briefly, the cancerous tissue on 4–5 slides (4 µm sections) per patient was dissected out. These were then deparaffinated using deparaffinisation solution and areas containing >30% tumor cells as determined by an expert pathologist were macrodissected. DNA was extracted and purified with . DNA concentration was quantified using Qubit™ ds DNA High-Sensitive Assay kit (Thermo Fisher Scientific, Waltham, MA, USA) on the Qubit fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). All library preparation was performed manually for Oncomine Comprehensive Assay v3 (OCAv3) (Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions MAN0015885. Multiplex PCR amplification was conducted using a DNA concentration of approximately 20 ng as input. For sequencing, prepared libraries were loaded according to manufacturer's instructions (Ion 550™—Chef, MAN0017275) onto Ion 550™ Chips (Thermo Fisher Scientific, Waltham, MA, USA) and prepared using the Ion Chef™ System. Sequencing was performed using the Ion S5™ XL Sequencer (Thermo Fisher Scientific, Waltham, MA, USA). The data was mapped to the human genome assembly 19, embedded as the standard reference genome in the Ion Reporter™ Software (v. 5.14) (Thermo Fisher Scientific, Waltham, MA, USA). We used the Ion Reporter™ Software for initial automated analysis. Oncomine Comprehensive v3—w4.0—DNA—Single Sample as analysis workflow. Additionally, coverage analysis reports from the Ion Reporter™ Software providing measurements of mapped reads, mean depth, uniformity and alignment over a target region were used as quality assessment of the sequencing reactions.

Mice. *Fanca*$^{-/-}$ animals were maintained in FVB/NJ background, and obtained from the laboratory of Dr. Paula Rio (Biomedical Innovation Unit, CIEMAT, Madrid, Spain) [23]. *Trp53*$^{F2\text{-}10/F2\text{-}10}$ and K14*cre* mice were maintained in a mixed FVB/NJxC57BL6 background. Animals were genotyped by PCR using specific primers as previously described [20,23,24]. All animals showing tumors, or significant morbidity, over a period of 24 months were sacrificed for necropsy. Histopathological analysis was systematically performed on the oral cavity, including lips, palate, tongue and oral mucosa. Overt tumors existing in other organs were also sampled for histopathology. All mice husbandry and experimental procedures were performed according to European and Spanish regulations and were approved by the local Animal Ethical Committee and competent authority (code PROEX006/18).

Histopathology and Immunohistochemistry. Mouse tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections (5 microns) were stained with H&E, or processed for immunostaining. Sections were incubated with primary antibodies and thereafter with biotinylated secondary antibodies (Jackson Immunoresearch Laboratory, Ely, UK). Primary antibodies used are: anti-Keratin 5 polyclonal antibody, clone Poly19055 (BioLegend); anti-Keratin 10 antibody, lone Poly19054 (BioLegend). Immunoreactivity was revealed using the ABC-peroxidase system and the DAB substrate kit (Vector Laboratories; Burlingame, CA, USA), and the sections were counterstained with hematoxylin. Anti-Cytokeratin 5 antibody was purchased to Santa Cruz (sc-32721). The control experiments without the primary antibody gave no signal.

Survival Curves. Tumor-free survival curves were obtained with Prism software (Graphpad Software, Inc., San Diego, CA, www.graphpad.com). Statistical significance of survival between genotypes was calculated with the log-rank test yielding a p-value.

RESULTS

Oral squamous carcinomas from Fanconi anemia patients display mutations in *p53*. We performed deep-sequencing of DNA from cancerous areas of six FFPE blocks from three FA patients, which were diagnosed with squamous cell carcinoma in the oral cavity and treated with surgery. Patient clinical and sample information is shown in Table 1. Genes included in the sequencing panel genes are frequently mutated in solid tumors (Supplementary Table 1). Interestingly, mutations in *p53* previously reported to be oncogenic drivers were detected in all six tumor samples analyzed (Table 2 and Supplementary Table 2). In addition, we also found the germline mutations in the *FANCA* gene that we previously diagnosed in all three FA patients [25] (Table 2). These results are in line with the high frequency of *FANCA* mutations in FA patients in Spain and worldwide, as well as the frequent detection of *p53* alterations in SCC from FA patients [18,19].

***Fanca/p53*^{EPI} and *p53*^{EPI} animals develop spontaneous squamous cell carcinomas in the oral cavity (OSCC).** Mouse of the indicated genotypes were monitored for a period of 24 months, and spontaneous lesions in the oral cavity were analyzed. We found OSCC in K14*cre*;*Trp53*$^{F2-10/F2-10}$ (*p53*^{EPI}) and *Fanca*$^{-/-}$;K14*cre*;*Trp53*$^{F2-10/F2-10}$ (*Fanca/p53*^{EPI}) animals, but not in Control or *Fanca*$^{-/-}$ (*Fanca*) ones (Table 3 and Figure 1A). SCCs were well differentiated, frequently infiltrated, and located in the lips, tongue, and oral mucosa (Figure 1A and Figure 2). Tumors in the lips were frequently located at the mucocutaneous junction, which is a transitional region from oral mucosa to skin (Figure 2). Some animals developed more than one tumor (multicentric SCCs) (Figure 2B). The incidence of SCCs was significantly higher in *Fanca/p53*^{EPI} than in *p53*^{EPI}: 91% *versus* 66% (p-val<0.05, Fisher's exact test). Importantly, double mutant *Fanca/p53*^{EPI} mice developed OSCC much earlier than *p53*^{EPI} as analyzed by a Kaplan-Meier plot (median latencies of 303 *versus* 432 days, respectively) (Fig 1B). Tumors in the tongue and in the oral mucosa were more frequent in double mutant mice (Figure 1A), which are the prevalent locations of HNSCC in FA patients [1,26]. Therefore, Fanca and p53 cooperate to suppress SCC in the oral cavity in transgenic mice. Importantly, *Fanca/p53*^{EPI} animals constitute the first animal model of spontaneous HNSCC in FA.

***p53*^{EPI} and *Fanca/p53*^{EPI} and human squamous oral tumors express epithelial differentiation markers.** SCC formation involves deregulation of normal cell differentiation. Immunodetection of differentiation biomarkers in both human and animal oral SCCs showed expansion of K5-expressing cells from the basal location into suprabasal compartment both in hyperplasic areas and in malignant cells (Fig. 3 and 4). K10, a marker of suprabasal cells in epidermis that is ectopically expressed in human oral epithelial dysplasia and in skin and oral SCCs [27], was also detected in hyperplastic/dysplastic areas and in malignant cells (Fig. 3 and 4). Therefore, the expression pattern of keratin markers corroborates squamous differentiation of the oral carcinomas in FA patients and in *p53*^{EPI} and double *Fanca/p53*^{EPI} animals.

Other malignancies in *p53*^{EPI} and *Fanca/p53*^{EPI} mice. We have previously reported spontaneous skin SCC development in FVB/NJ *p53*^{EPI} mice [20], with aggressive behavior and molecular signatures of poor prognostic human cancer [28]. In addition, we and others reported the development of mammary carcinomas in *p53*^{EPI} mice [29,30]. As expected, we found skin and mammary tumors both in *p53*^{EPI} mice and in double *Fanca/p53*^{EPI} (Supplementary Figure 1). Skin tumors were mainly SCCs, although basal cell carcinomas (BCC), sebaceous glands carcinomas and fibrosarcomas were also diagnosed. Some *p53*^{EPI} and *Fanca/p53*^{EPI} animals were sacrificed as they develop overt thymic lymphomas (Supplementary Figure 1). These findings suggest that *Fanca/p53*^{EPI} mice constitute also a model for FA-associated cancer in locations other than head and neck.

***Fanca* mice display frequent pre-tumoral lesions in the oral cavity.** FA patients display a very high incidence of premalignant, non-invasive lesions in the oral cavity, which are difficult to

prevent, diagnose and treat. We performed a careful inspection on histology samples from oral tissues collected at the time of animal sacrifice, in order to detect possible lesions indicative of cellular transformation. Interestingly, most *Fanca* mice develop aberrant tissue phenotypes in the oral cavity (including atypia, acantolysis, picnosis or vacuolization) associated with hyperplasia or, more importantly, carcinoma *in situ* (CIS), which represents the highest atypia of premalignant epithelial cells (Table 4 and Figure 5). Incidences were significantly higher than in Control animals (p-val<0.05, Fisher's exact test), even though mean age at the analysis was very similar in both genotypes (Control=18 months *versus Fanca*=19.4 months). Lesions were observed in the lips, palate, tongue, oral mucosa and esophagus (Figure 5 and 6), and were also found in *p53*^{EPI} and, mainly, in double *Fanca/p53*^{EPI}. Some animals had more than one (multicentric) pre-tumoral lesion. Globally, the results showed that *Fanca* gene mutation is associated with development of premalignant lesions in the oral mucosa of transgenic mice.

DISCUSSION

Fanconi anemia patients display an exacerbated susceptibility to develop HNSCC early in life. Due to their sensitivity to DNA damaging agents, cancer treatments based on radiotherapy and chemotherapy should be avoided. Poorer survivals after HNSCC development, when compared with non-FA patients, can be explained partially by this suboptimal treatment. Therefore, modeling HNSCC in animals is important to understand the oncogenic mechanisms behind, discover cancer drivers and biomarkers, and perform preclinical tests of new therapies. Here, we are reporting the first mouse model of spontaneous SCC in the oral cavity with genetic depletion of a Fanconi pathway gene.

FA patients display numerous pre-malignant lesions in the oral cavity, which are difficult to diagnose and treat [2]. These lesions may lead carcinoma formation, and new methods to predict their malignant transformation have been recently developed [31]. However, the molecular events that give rise to such lesions are largely unknown. We have found that *Fanca* animals develop hyperplastic and CIS lesions that might resemble patient's pre-malignant changes (Table 4 and Figures 5 and 6). These results represent the first animal model of pre-malignant oral lesions with mutation in a Fanconi pathway gene, whereby new quimiopreventive and therapeutic approaches could be tested.

To our knowledge, mouse models with deletion on Fanconi genes developing spontaneous oral SCC have not been previously reported. Our results support that loss of *Fanca* gene alone does not promote the development of SCCs, even at prolonged times of observation (up to 24 months of age). Previous works have shown that the specific ablation of p53 gene in stratified epithelia confers high susceptibility to SCC development [20,21,29]. Our data indicate that the simultaneous absence of *p53* and *Fanca* genes cooperate in the SCC development. Furthermore, *Fanca* loss augments incidence and accelerates oral SCC $p53^{EPI}$ in stratified epithelia (Figures 1 and 2). Cooperation between p53 and FA genes has been already described: tumor appearance in full body deletion in *p53* ($p53^{-/-}$ mutant mice) is accelerated when combined with loss of *Brca2*, *Fancd2* or *Fancc* [14,15,24]. Cells from $Fanca^{-/-}$ animals activate p53 protein function as a mechanism to control replication of cells with DNA damage [32,33], which eventually could give rise to neoplastic cells. We propose that SCC development in FA individuals is a sequential process in which DNA instability produced by germline mutations in Fanconi genes might eventually give rise to alterations affecting additional cancer genes. Such double mutant cells might acquire a selective advantage over neighbor cells, and expand to produce transformed clones with differential invasive capabilities. As *p53* is the most frequent type of somatic alteration in SCCs from FA patients (Table 2, Supplementary Table 1 and 2) [18,19], and *Fanca* and *p53* cooperate to suppress SCC in our mouse models (Fig. 1), we suggest that *p53* genomic alterations might constitute early events during carcinogenesis in FA individuals. The finding that *p53* mutation is an early event during HPV negative, non-FA HNSCC is agree with our hypothesis [34].

Wong et al [9] reported tumor formation, mainly lymphomas, in mice with targeted disruption of exons 1 to 6 of the *Fanca* gene, in line with formation of overt lymphomas in the thymus of some of our animals. However, a more systematic analysis should be performed to understand if *Fanca* or $Fanca/p53^{EPI}$ mice have a higher incidence of such tumors when compared with control, normal littermates. Although the incidence and risk of skin SCC development in FA individuals has not been properly addressed, some reports showed a high incidence of BCC and SCC in FA, and at significant younger ages than in the general population [35,36]. As both skin SCC and BCC were observed in double $Fanca/p53^{EPI}$ mice, we propose that these mice represent a model for skin SCC in FA patients. Despite the known high lifetime risk of FA individuals of the development of SCCs of the mucosae (head, neck, esophagus, vulva or anus), further epidemiological studies should be performed to demonstrate that FA patients display also higher risk of development of SCCs/BCC of the skin, in comparison to the general population.

In conclusion, *Fanca* and *p53* genes cooperate to suppress OSCC in mice, and *Fanca/p53*^{EPI} mice is an animal model of OSCC in FA whereby analyses of the molecular mechanisms of the disease, new biomarkers and new therapies is warranted.

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Figure Legends

Figure 1. *Fanca* and *p53* genes cooperate to suppress oral SCC in mice. Mice from the indicated genotypes were long term-monitored (24 months), sacrificed at the observance of tumor/s development or significant morbidity, and analyzed histologically. **A)** Percentages of animals in *p53^{EPI} and Fanca/p53^{EPI}* genotypes having oral SCC, shown by sub-anatomical location. **B)** A Kaplan-Meier plot of the percentage of oral SCC free survival is shown. Note that *Fanca* loss accelerates SCC development in *p53^{EPI}* animals (*Fanca/p53^{EPI}* versus *Fanca*). Statistical significance between curves was determined using log rank test. ***P < 0.001, ****P<0.0001, P > 0.05 (NS).

Figure 2. Histopathology of oral SCC in *p53^{EPI}* and *Fanca/p53^{EPI}* mice. A) Well differentiated SCC at the mucocutaneous junction of the lips in a *p53^{EPI}* mouse. The tumor infiltrates deep into the subcutaneous muscle. Observe the mucosal (left dotted box) and epidermal (right dotted box) epithelia surrounding the carcinoma. **A')** and **A'')** are magnifications of the dotted boxes in **A)**, where mucosal and cutaneous junctions are shown, respectively. **B)** Well differentiated SCCs developing at the oral mucosa and the epidermis enclosing the lips (dotted boxes) in a *Fanca/p53^{EPI}* animal. **B')** and **B'')** are magnifications of the dotted boxes in **B)**, where mucosal and cutaneous (yellow arrow) SCCs are shown.

Figure 3. Oral SCCs in FA patients express K5 and K10 squamous differentiation keratin markers. A) H&E staining of a well differentiated SCC in the retromolar trigone of tumor #FA903-17, with a "horny pearl" or concentric foci of squamous differentiation (asterisk in **A**). K5 and K10 are expressed in malignant cells of the #FA903-17 SCC shown in **A)** (**B** and **D**), and in a tongue SCC (#FA310-18) from another FA patient (**C** and **E**).

Figure 4. Oral SCC in *Fanca/p53^{EPI}* mice express K5 and K10 squamous differentiation keratin markers. A) and **B)** H&E staining of well differentiated SCC in the oral mucosa (A) and tongue (B) of two different *Fanca/p53^{EPI}* animals (#1135 and #9339). Both are well differentiated SCCs since "horny pearls" or concentric foci of squamous differentiation are evident in the tumors (asterisks in **A** and **B**). K5 and K10 expression is still present in hyperplastic and malignant cells of the SCCs shown in **A)** (**C** and **D**) and **B)** (**E** and **F**).

Figure 5. *Fanca* gene prevents the development of hyperplastic and CIS lesions in the oral cavity. Percentages of animals in all genotypes (Control, *Fanca*, *p53^{EPI} and Fanca/p53^{EPI}*) showing hyperplastic **(A)** or CIS **(B)** changes in the oral cavity, shown by sub-anatomical location.

Figure 6. Histology of carcinoma *in-situ* (CIS) in *Fanca* mice. CIS premalignant changes found in the epithelia of the oral cavity of different *Fanca* animals. **A)** Soft palate with a foci of lichenoid inflammation, pseudocarcinomatous basal hyperplasia and severe atypia of the epithelium, characterized by nuclear pyknosis, increase of the nuclear:cytoplasm ratio, and loss of nuclear polarity of keratinocytes (yellow square bracket). **B)** Pseudocarcinomatous basal hyperplasia and severe atypia of the epithelium in the hard palate. **C)** Foci of severe atypia and epithelial hyperplasia associated to lichenoid inflammation in the labial mucosa. **D)** Foci of extreme atypia with epithelium vacuolization of the dorsal tongue filiform papillae (square bracket). A, B, C (200X), C (400X).

Table 1. Details of FA patients with OSCC

Patient ID	Age at OSCC (years)	Location	HP grading	Tumor ID	BMT	Age at BMT (years)
FA310	35	Tongue	Well differentiated	FA310-14	yes	9
	38	Tongue	Well differentiated	FA310-18a		
	38	Tongue	Well differentiated	FA310-18b		
FA551	33	Palate	Differentiated	FA551-18	no	
FA903	41	Retromolar trigone	Well differentiated	FA903-17	no	

Abreviations: HP, hystopathologic; OSCC: oral squamous cell carcinoma; BMT: bone marrow transplantation.

Table 2. FANCA and p53 mutations found in OSCC from Fanconi anemia patients.

Sample	Locus	Gene	Genotype	AA-change	Type
FA310-14	chr16:89816213	FANCA	C/A	p.Arg1055Leu	SNV
FA310-14	chr16:89807256	FANCA	AGAA/A	p.Phe1263del	INDEL
FA310-14	chr17:7577581	TP53	A/C	p.Tyr234Asp	SNV
FA310-18a	chr16:89816213	FANCA	C/A	p.Arg1055Leu	SNV
FA310-18a	chr16:89807256	FANCA	AGAA/A	p.Phe1263del	INDEL
FA310-18a	chr17:7577570	TP53	C/A	p.Met237Ile	SNV
FA310-18b	chr16:89816213	FANCA	C/A	p.Arg1055Leu	SNV
FA310-18b	chr16:89807256	FANCA	AGAA/A	p.Phe1263del	INDEL
FA310-18b	chr17:7577570	TP53	C/A	p.Met237Ile	SNV
FA551-18	chr16:89805578	FANCA	G/C	p.Ser1377Ter	SNV
FA551-18	chr16:89807256	FANCA	AGAA/A	p.Phe1263del	INDEL
FA551-18	chr17:7578190	TP53	T/C	p.Tyr220Cys	SNV
FA903-17	chr16:89831435	FANCA	G/A	p.Gln881Ter	SNV
FA903-17	chr17:7577120	TP53	C/T	p.Arg273His	SNV

Table 3. Incidences of oral SCC in animal genotypes

Genotype	Group size, *n*	Oral SCC, *n* (%)
Control	12	0 (0)
Fanca	16	0 (0)
p53^{EPI}	12	8 (66)
Fanca/p53^{EPI}	11	10 (91)

NOTE: The difference in OSCC incidence between *p53*^{EPI} and *Fanca/p53*^{EPI} groups was statistically significant (p-val<0.05). Statistical comparisons were performed by using the Fisher's exact test.

Table 4. Incidence of non-invasive lesions in the oral cavity in the animal genotypes

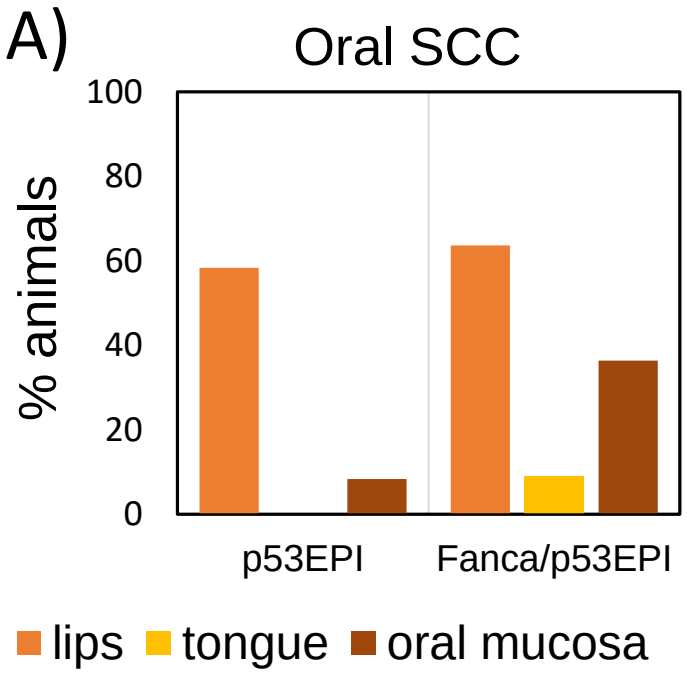
Genotype	Group size, *n*	Lesions in the oral cavity, *n* (%)	
		hyperplasia	carcinoma *in situ* (CIS)
Control	12	2 (17)	0 (0)
Fanca	16	12 (75)	7 (44)
p53^{EPI}	12	7 (44)	5 (42)
Fanca/p53^{EPI}	11	9 (82)	9 (82)

NOTE: The difference in hyperplasia or CIS incidence between *Control* and *Fanca* groups was statistically significant (p-val<0.05). Statistical comparisons were performed by using the Fisher's exact test.

Supplementary Figure Legends

Supplementary Figure 1. *p53*^{EPI} and *Fanca/p53*^{EPI} mice develop overt tumors in organs such as mammary gland (**A** and **B**, adenocarcinomas), skin (**C** and **D**, SCCs; **E**, sebaceous gland carcinoma; and **F**, BCC) and thymus (**H**, lymphoma).

Figure 1


A)
Oral SCC
% animals
100
80
60
40
20
0
p53EPI
Fanca/p53EPI
lips
tongue
oral mucosa

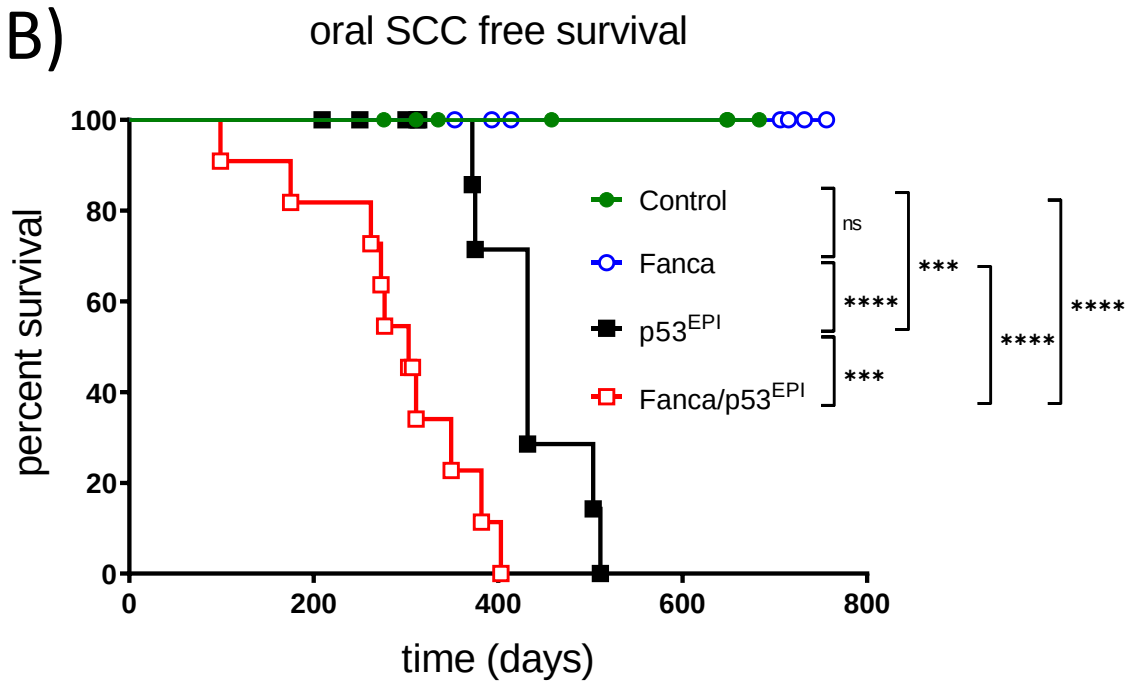

B)
oral SCC free survival
percent survival
100
80
60
40
20
0
0
200
400
600
800
time (days)
Control
Fanca
p53EPI
Fanca/p53EPI
ns
***
****
***
****
****

Figure 2

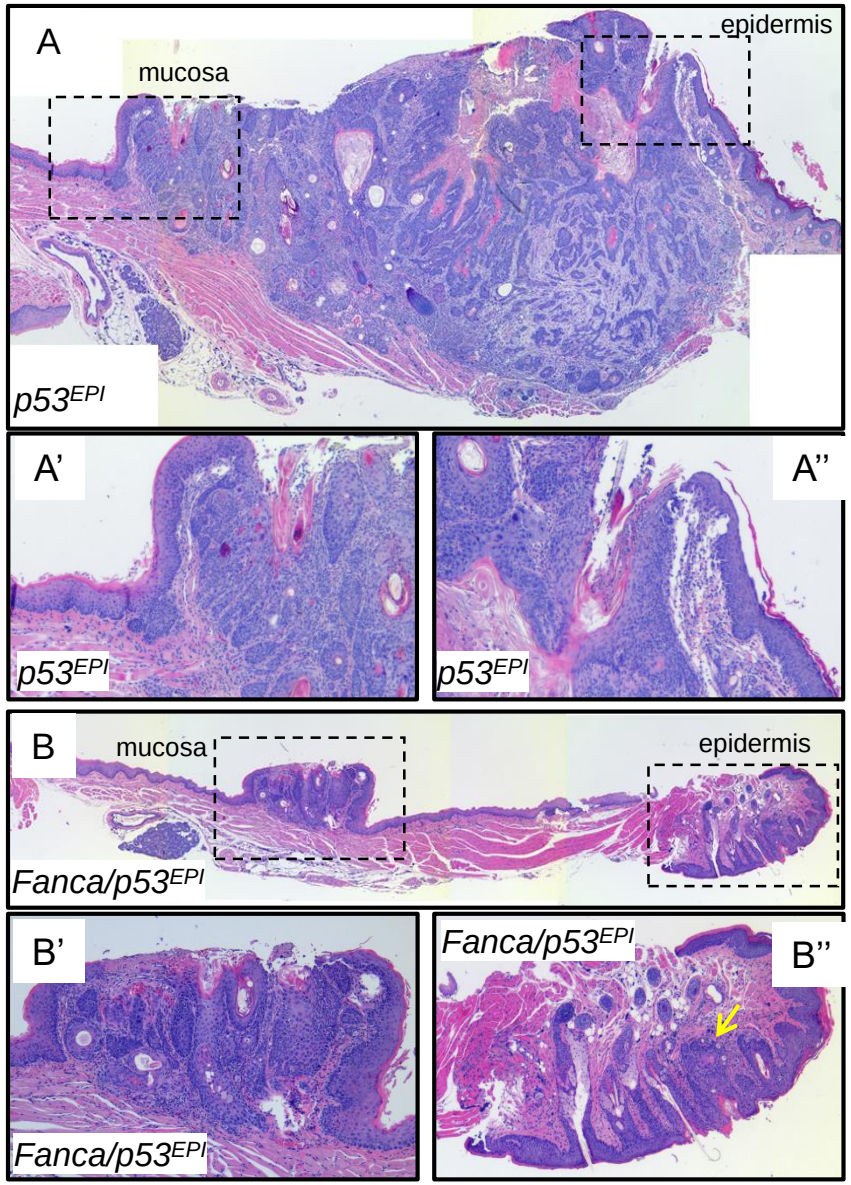

A
mucosa
epidermis
p53EPI
A'
p53EPI
A''
p53EPI
B
mucosa
epidermis
Fanca/p53EPI
B'
Fanca/p53EPI
Fanca/p53EPI
B''

Figure 3

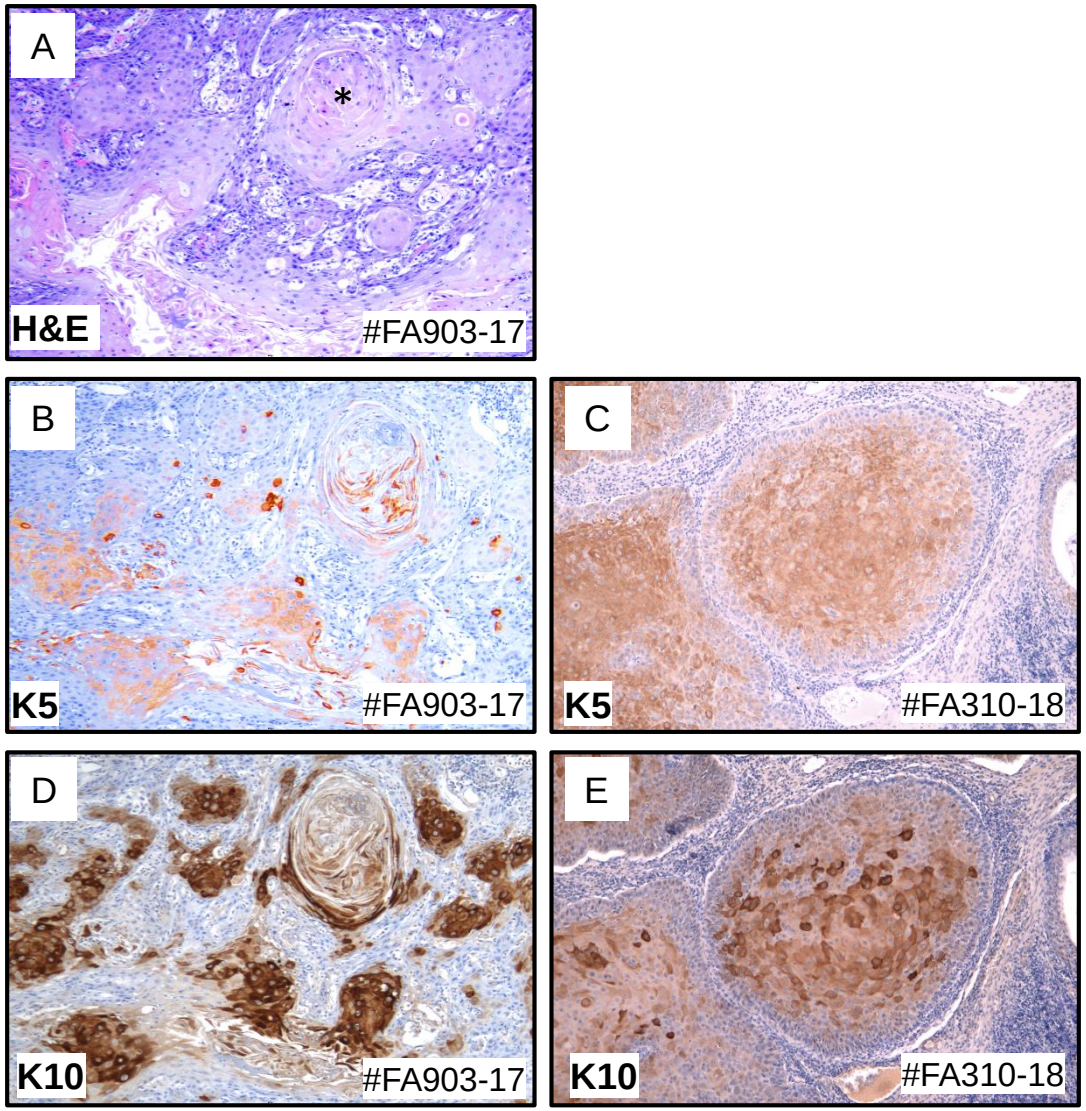

A
*
H&E
#FA903-17
B
K5
#FA903-17
C
K5
#FA310-18
D
K10
#FA903-17
E
K10
#FA310-18

Figure 4

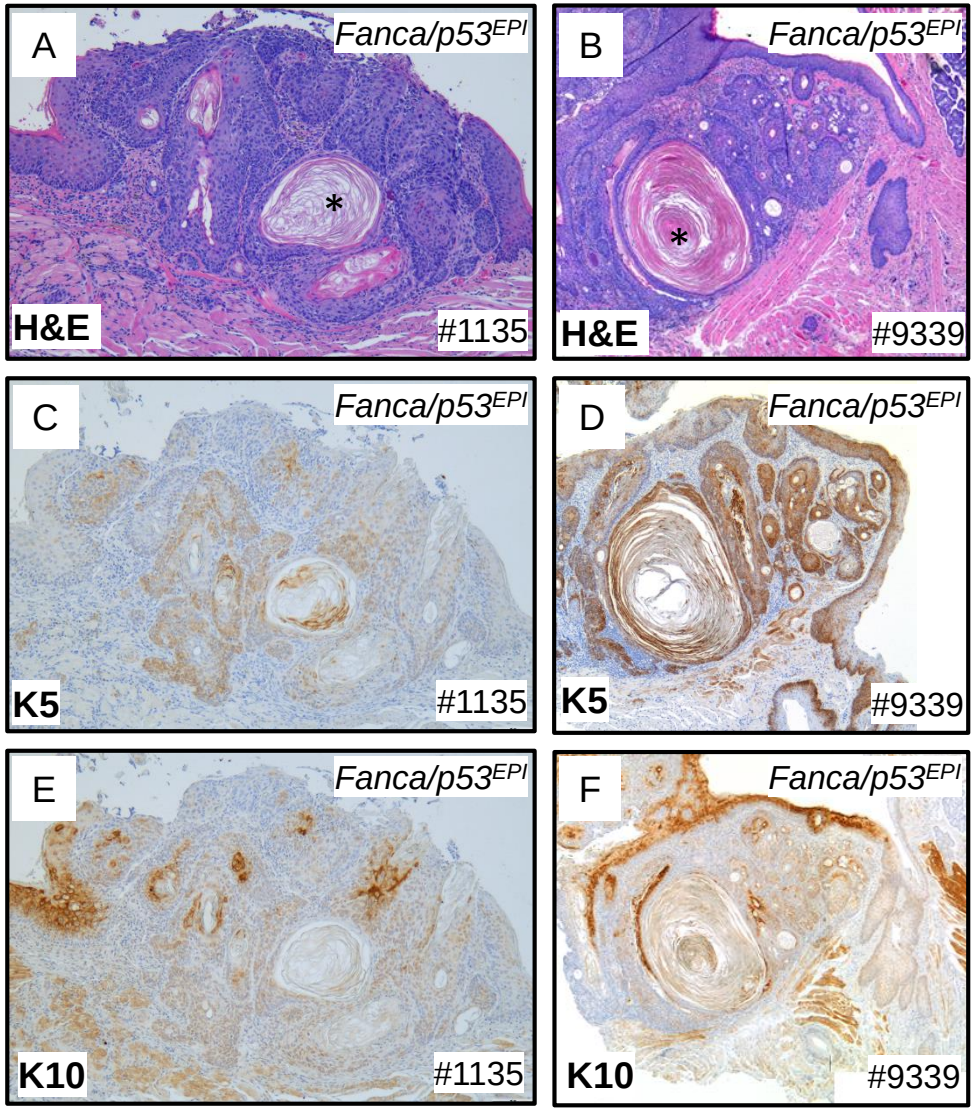

A
Fanca/p53EPI
*
H&E
#1135
B
Fanca/p53EPI
*
H&E
#9339
C
Fanca/p53EPI
K5
#1135
D
Fanca/p53EPI
K5
#9339
E
Fanca/p53EPI
K10
#1135
F
Fanca/p53EPI
K10
#9339

Figure 5

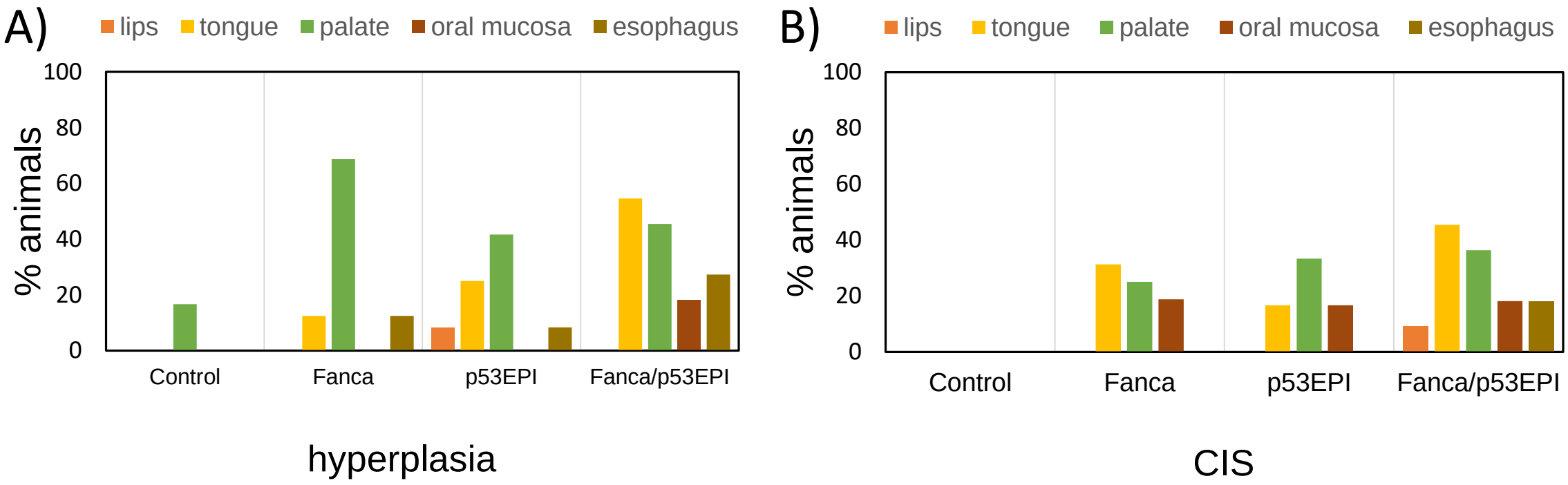
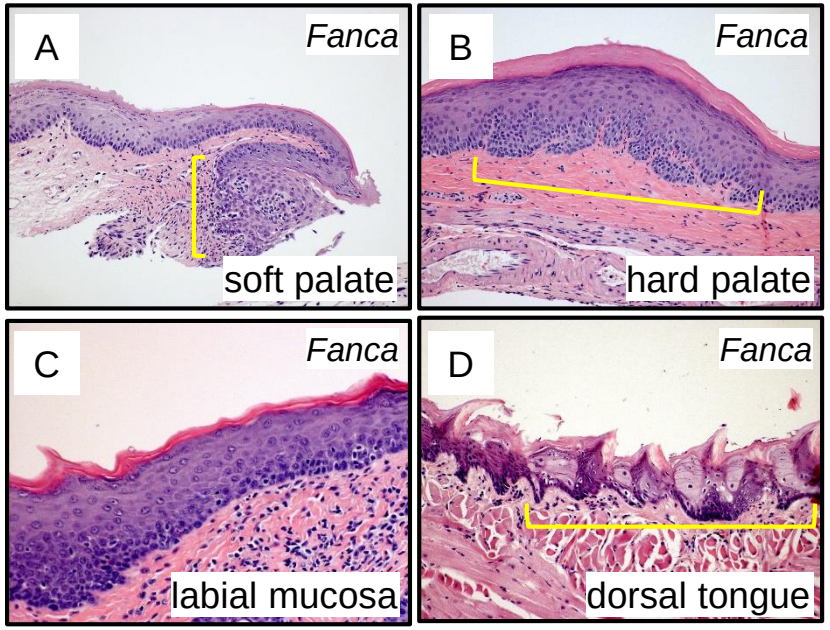

A)
lips
tongue
palate
oral mucosa
esophagus
% animals
100
80
60
40
20
0
Control
Fanca
p53EPI
Fanca/p53EPI
hyperplasia
B)
lips
tongue
palate
oral mucosa
esophagus
% animals
100
80
60
40
20
0
Control
Fanca
p53EPI
Fanca/p53EPI
CIS

Figure 6


A
Fanca
soft palate
B
Fanca
hard palate
C
Fanca
labial mucosa
D
Fanca
dorsal tongue