ING1 and ING2: Multi-Faceted Tumor Suppressor Genes

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Abstract

ING1 (Inhibitor of Growth 1) was identified and characterized as a "candidate" tumor suppressor gene in 1996. Subsequently four more genes, also characterized as "candidate" tumor suppressor genes, were identified by homology search: *ING2, ING3, ING4* and *ING5.* The ING proteins are characterized by a high homology in their C-terminal domain which contains a Nuclear Localization Sequence (NLS) and a Plant HomeoDomain (PHD) which has a high affinity to Histone 3 tri-methylated on lysine 4 (H3K4Me3). The ING proteins have been involved in the control of cell growth, senescence, apoptosis, chromatin remodelling and DNA repair. Within the *ING* family, *ING1* and *ING2* form a subgroup since they are evolutionarily and functionally close. In Yeast, only one gene, *Pho23,* is related to *ING1* and *ING2* and possesses also a PHD. Recently, the ING1 and ING2 tumor suppressor status has been fully established since several studies have described the loss of ING1 and ING2 protein expression in human tumors and both ING1 and ING2 knockout mice were reported to have spontaneously developed tumors, B-cell lymphomas and soft tissue sarcomas respectively. In this review we will describe for the first time what is known about *ING1* and *ING2* genes, proteins, their regulations in both human and mice, and their status in human tumors. Furthermore, we explore the current knowledge about identified functions involving ING1 and ING2 in tumor suppression pathways especially in the control of cell cycle and in genome stability.

Introduction

A tumor suppressor gene (TSG) encodes for a protein which blocks tumor development. These genes negatively regulate cell proliferation and/or contribute to the maintenance of genome stability. Their loss contributes to malignant transformation. They are divided in two classes. Type I TSGs or "caretakers" are directly involved in the maintenance of DNA integrity through DNA repair pathways (e.g.: BRCA1/2, MSH2, etc.); whereas, type II TSGs or "gatekeepers" control cell cycle, senescence, apoptosis, autophagy and suppress angiogenesis and cell invasion (e.g.: p53, pRb, etc.) [1,2].

In 1996, Karl Riabowol's group identified *ING1* (Inhibitor of Growth 1) from an *in vivo* screen which aimed at isolating new candidate TSG [3]. Following ING1 discovery, four genes having a high homology to *ING1* were identified as candidate TSG and were named *ING2-ING5* [4-7]. Subsequently, three *ING* genes were identified in *Saccharomyces cerevisiae: Yng1*, *Yng2* and *Pho23* and two in *Saccharomyces pombe*: *Png1* and *Png2* [8]. Yeast ING proteins share a significant identity of 50 to 60% with the C-terminal region of human ING1 [8]. Since then, ING proteins were also identified in many other species including frog, fish, mosquito, fruit fly, worm, xenopus and plant. The ING proteins are characterized by their C-terminal region which is highly conserved from human to plant and contains a Plant HomeoDomain (PHD) [9-11]. In addition, the C-terminus contains a Nuclear Localisation Signal (NLS) which targets INGs to the nucleus [9]. ING proteins possess also a strongly conserved region that has not yet been described in any other protein and thus has been named the Novel Conserved Region (NCR). The N-terminal domain is unique to each ING [9]. Thus, ING proteins are highly conserved during evolution which suggests their involvement in important biological processes [9,12]. Accordingly, functional studies have characterised ING proteins as "candidate" tumor suppressor proteins that are involved in many processes like cell growth, apoptosis, senescence, migration and DNA repair. Furthermore, many studies have shown that these genes are lost or misregulated in several tumour types [13]. Within the *ING* family, *ING1* and *ING2* form a subgroup since they are evolutionarily and functionally close [9]. Recently, both ING1 and ING2 knockout mice were reported to spontaneously develop tumors with a high frequency [14-17].

In this review we will detail for the first time what is known about ING1 and ING2 gene, protein, their regulations in both human and mice, and their status in human tumors. Furthermore, we discuss the involvement of ING1 and ING2 functions in tumor suppression especially in the control of cell cycle and genome stability.

ING1 and ING2 genes and proteins: structure and regulations

Human *ING1* and *ING2* are located close to chromosome telomere

Human *ING1* and *ING2* are located on two different chromosomes. Human *ING1* has been mapped at chromosome 13q34 and human *ING2* at chromosome 4q35.1. Interestingly, both are located close to the telomeric region [4,6,9] (Fig.1, S1). The amino acid sequence of human ING2 displays 70% homology with ING1 sequence [4] (Fig.2). The subtelomeric location of human *ING1* and *ING2* genes and their high homology suggest that *ING1* and *ING2* could result from the duplication of these regions from a common ancestor [9]. In contrast, mouse *ING1* and *ING2* are located on the same chromosome. *ING1* has been mapped on chromosome 8 A1.1 and *ING2* on chromosome 8 B2 [18] (Fig.S1). Phylogenetic tree analysis has revealed that human and mouse *ING1* from one part and human and mouse *ING2* from another part belong to the same group of related sequences [9] (Fig.S2).

ING1 structure and transcriptional regulation

Human *ING1* is made of four exons, exon 1a, 1b, 1c, and 2, resulting in five transcribed isoforms (ING1a, ING1b, ING1c, and ING1d) (Fig1, S1). They are the result of different promoters and alternative splicing. *ING1* is ubiquitously expressed in tissues. ING1b is the most abundant form among ING1 isoforms (Fig1) (Fig.1 represents a scheme of ING1b isoform and of ING2a and ING2b. ING2 proteins are more detailed than ING1 proteins because the ING1 isoforms have already been published [13] and because ING2b has never been included in a scheme describing ING2 proteins since its identification. A detailed scheme of ING1 isoforms is represented on Fig.S1).

However, their expression can vary greatly e.g. human ING1a mRNA expression is highly expressed in testis; whereas, human ING1b mRNA is highly expressed in thymus, spleen and brain and human ING1c mRNA is highly expressed in thymus and human ING1d is highly expressed in lung, liver, kidney, thymus and small intestine [19]. In contrast, mouse *ING1* consists of four exons, exon 1a, 1b, 1c and 2, resulting in three transcribed isoforms (ING1a, ING1b and ING1c) (Fig.S1) [18].

In addition, human *ING1* transcriptional regulation remains unknown but need to be explored since ING1 mRNA expression is frequently lost in human tumors (see thereafter). The expression of murine *ING1* has been reported to be independent of p53 [20].

ING2 structure and transcriptional regulation

ING2 is made up of three exons, exon 1a, 1b and 2, resulting in two transcribed isoforms: ING2a and ING2b [4,6,21] (Fig.1). They are the result of an alternative splicing between exons 1a and 1b. Both *ING2a* and *ING2b* mRNA are ubiquitously expressed. However, the level of ING2b mRNA transcript expression is much lower. Furthermore, whilst ING2b mRNA expression has been shown at the RNA level, no protein has ever been detected [21,19]. By which mechanism *ING2b* expression may be regulated at the RNA level remains unknown. *ING2* mouse gene is also comprised of three exons giving two transcribed isoforms by alternative splicing: ING2a and ING2b [21] (Fig.S1). Human and mouse ING2a cDNAs share 90% identity whilst human and mouse ING2b cDNAs share 75% identity (Fig. S2A).

ING2 promoter displays regulatory elements that control its transcription. *ING2a* promoter possesses two p53 binding sites and indeed p53 activation by Nutlin-3 has been shown to repress *ING2a* transcription [22]. *ING2a* expression is also regulated by NF-κB as its promoter possesses one NF-κB binding site. Thus, in colon cancer, *ING2a* expression is enhanced through NF-κB activation [23]. So far, no transcriptional regulatory element has been established for *ING2b*. However, a putative Heat Shock transcription Factor 1 and 2 binding site, a C-Rel binding site, a SP1 binding site, five Myeloid Zinc Finger 1 binding sites and a p300 binding site are predicted on *ING2b* promoter [21]. Complementary studies about *ING2* transcriptional regulation would allow a better understanding of ING2 functions since misregulation of these transcription factors could alter *ING2* expression. Interestingly, through still unknown mechanisms, downregulation of *ING2a* results in changes in ING2b mRNA expression. Conversely, downregulation of *ING2b* also triggers changes in ING2a mRNA expression [21]. This raises the possibility of interplay between ING2a and ING2b mRNA expression. Consequently, the analysis of the expression of both ING2a and ING2b mRNAs would be of interest to better characterize the status of *ING2* in human tumors.

ING2 expression is regulated by tissues-dependent mechanisms since *ING2a* is highly expressed in the skeletal muscle, lung, thymus and the in pancreas [19,21]. Interestingly,

ING2 has been involved in muscle differentiation by regulating *myogenin* transcription [24]. Moreover, both *ING2a* and *ING2b* are especially highly expressed in human and mouse testis [17,21]. Indeed it has been shown that ING2 plays a role in male fertility [17]. It suggests that ING2 may also play a role in the physiology of the lung, thymus and pancreas.

ING1 and ING2 proteins structure

Human ING1a and ING1b are the most abundant ING1 isoforms. No information has currently been published regarding functions of the others. Human ING1a and ING1b have respectively a molecular weight of 47 kDa and 33 kDa (Fig1, 2, S1). Amino acid sequence alignment of human ING1 versus mouse ING1 isoforms has revealed a high similarity between mouse ING1b and human ING1b since they share 89% identity (Fig.S2). Mouse ING1b has a molecular weight of 37kDa and possesses the same domains than human ING1b. The alternative splicing between mouse ING1a, ING1b and ING1c results in the absence of the PCNA-interacting protein (PIP) and Partial Bromo Domain (PBD) domains on ING1a and ING1c. Consequently, ING1a and ING1c have a predicted molecular weight of 31 kDa [18] (FigS1, 2). Thus, there is no equivalent to human ING1a in mice. Anyway, human and mouse ING1b have a high homology. Consequently, mouse models are pertinent for ING1b *in vivo* studies (Fig.S1, S2).

Human ING2a has a molecular weight of 33 kDa whilst ING2b has a predicted molecular weight of 28 kDa. However, ING2b protein has never been experimentally detected [4,6,21]. The alternative splicing between human ING2a and ING2b results in the absence of the Leucine Zipper Like (LZL) domain on ING2b (Fig.1C). Human and mouse ING2a proteins are very close as they differ by only ten amino acids distributed across the length of the protein and are made of the same protein domains (Fig.S2B). Moreover, mouse ING2a has the same molecular weight as human ING2a. On the other hand, mouse ING2b is smaller than human ING2b and has a predicted molecular weight of 20,3 kDa since mouse ING2b is also truncated of some part of its NCR domain [21] (Fig.1, S1). Overall, human and mouse ING2a and ING2b have a high homology. Consequently, mouse models are pertinent for ING2 *in vivo* studies (Fig.S1, S2).

ING1 and ING2 C-terminal domain contain a PHD with a zinc binding motif described as a domain having a high affinity for the histone 3 tri-methylated on lysine 4 (H3K4Me3) [9,10,25-30] (Fig.1, S1). ING1 and ING2 also have a PolyBasic Region (PBR)

adjacent to the PHD [9] (Fig.2). As its closest paralog to ING1, ING2 also contains a amino acids motif in its C-terminal part. The REASP amino acids motif has been described as a 14-3-3 binding motif containing a phosphoserine [31]. ING1, phosphorylated on its serine 199, interacts with the 14-3-3η protein through its REASP motif to be shuttled from the nucleus to the cytoplasm [9,32]. In the cytoplasm, ING1 is dephosphorylated and interacts with karyopherines α2 and β1 through this region to allow its import to the nucleus [33]. One can hypothesize that ING2 is also phosphorylated on its serine located inside the REASP motif and that ING2 may also interacts with the 14-3-3η and with karyopherines α2 and β1 through its REASP motif [9] (Fig.2). ING1 and ING2 C-terminal domains also contain a NLS which possesses three Nucleolar Targeting Sequences (NTS), to be targeted to the nucleolus [21,34] (Fig.1, 2). ING1 and ING2 proteins are characterized by a NCR in their N-terminal part [9] (Fig.1, S1). NCR domain has been suggested to be necessary for the binding between ING1 and histone acetyltransferase (HAT) or histone deacetylase (HDAC) [28]. This domain was also subsequently named Lamin Interaction Domain (LID) because ING1, as well as ING2, ING3 and ING4, coprecipitates with Lamin A through this domain. It has been shown that the binding of ING1 to Lamin A through the ING1 LID regulates ING1 protein level and ING1 nuclear localisation [35]. It is thus possible that the ING2 LID could have a similar function.

The N-terminus is unique to each ING protein. ING1b N-terminus possesses a PIP domain. Among the ING1 proteins this domain is only restricted to ING1b isoform. ING1b PIP domain has been shown to interact with PCNA [34] (Fig.1, 2, S1). A PBD is also present close to the PIP on ING1b [36,37]. As mentioned previously, ING2a N-terminus also contains a LZL motif. This motif contains leucine residues spanning every seven amino acids and forming a hydrophobic patch. This LZL could present a leucine zipper coiled-coil conformation. Such conformation could allow the interaction with other leucine zipper–containing proteins [9]. Indeed, ING4 also contains a LZL domain and its homodimerisation has recently been demonstrated by cristallography [38,39]. Subsequently, putative homodimers have also been predicted for the others INGs, including ING2 [38]. Nevertheless, this hypothesis remains to be tested for ING2.

So far, only a few studies have investigated ING1 and ING2 protein regulation. Nonetheless, in response to genotoxic stress, human ING1b has been described to be phosphorylated on its serine 126 by the kinases Cdk1 or Chk1. This phosphorylation promote its protein stability [40]. In addition, another study has reported that ING1b can be degraded through the 20S proteasome complex. The NAD(P)H quinone oxidoreductase has been described to inhibit this degradation when ING1b is phosphorylated on its serine 126 [41].

Moreover, human ING2a protein level has been shown to be regulated by ubiquitylation. A member of the E6AP carboxyl terminus ubiquitin ligases (HECT) family named SMURF1 (Smad Ubiquitination Regulating Factor 1) interacts with ING2, inducing ING2 polyubiquitylation and its proteasomal degradation. ING2a interacts with SMURF1 through its central region corresponding to the NCR and the NLS domains while its PHD is the domain targeted by ubiquitinylation [42]. This degradation could play a more important role in the pancreas and testis where both ING2a and SMURF1 are highly expressed [19,21,43,44]. Moreover, *SMURF1* expression is amplified in pancreatic cancer [44]. Thus, it could be interesting to analyze whether ING2a protein expression is reduced in pancreatic cancer because of an enhancement of its degradation by an increased SMURF1 ubiquitinylation.

ING1 and ING2 status in human tumors

Most studies have described that ING1 and ING2 expression is decreased or lost in human tumors which argues for a role for ING1 and ING2 as a tumor suppressor gene [13].

A high frequency of loss of heterozygosity (LOH) of the *ING1* chromosomal region 13q34 has been found in head and neck carcinomas and in esophageal squamous cell carcinomas [45-47]. Expression of *ING1* is frequently lost in breast cancer tumors [13,48,49]. Most of the time, ING1 expression is lost at the RNA level. ING1 mRNA status has been studied using primers targeting exon E2 corresponding to all ING1 isoforms or targeting exon E1a corresponding to ING1b mRNA (Fig.1, S1). Consequently, there is currently no data available regarding ING1a, ING1c or ING1d status in human tumors [13]. Less frequently, some mutations have been found on *ING1* gene. These mutations occur in the PHD [13,50]. They thus could decrease ING1 binding to H3K4Me3 [29] (see thereafter). Moreover, in breast cancer and neuroblastoma, ING1 loss has been associated with more differentiated tumors suggesting that ING1 loss is associated with poor prognosis [13,51,52]. In addition, in oral carcinomas, ING1 loss results in a shift of the protein localization from the nucleus to the cytoplasm resulting in the loss of its nuclear functions [13,53].

In the case of ING2, a high frequency of LOH of the *ING2* chromosomal region 4q32-35.1 has been found in basal cell carcinomas, in head and neck squamous cell carcinomas and in hepatocellular carcinomas [54-56]. ING2 LOH is associated with advanced tumor stage in head and neck squamous cell carcinomas [54]. Strikingly, no report has shown the presence of

ING2 mutation in human tumors. But, several studies have described that ING2 is lost at the RNA level. Indeed, ING2 RNA expression is reduced in breast and ovarian cancers, in hepatocellular carcinomas and in non-small cell lung carcinomas [19,45,53,57]. In melanoma, loss of ING2 nuclear expression has been reported but it remains to be investigated if this is due to a loss of ING2 mRNA expression [58]. In addition, no shift of the protein localization has been reported for ING2 but it remains possible since it has been described for ING1 and for other INGs [13,53].

Contrary to its status observed in many tumors ING1b mRNA has been once shown to be overexpressed in brain tumor. It has been associated with a mutation within the NLS. Since this mutation was located within a consensus phosphorylation site (serine 81), it has been suggested that ING1b overexpression may be due to a disregulation of a post-translationnal modification [59]. Moreover, in contrast to its status observed in many tumors Kumamoto and coll., have shown in colon cancer that ING2 mRNA expression is upregulated and may enhance tumor invasion by enhancing Matrix Metallo Proteinase 13 expression. NF-κB activation was suggested as a possible mechanism to upregulate ING2a mRNA [23]. However, it remains unclear how and why ING2 is upregulated in colon cancer. These studies raise a new assumption where ING1b or ING2a could have under certain conditions a role as an oncogene. This is not restricted to ING1b or ING2a since other TSGs such as E2F1 which regulates cell death through activation of p53 and p73α pathways have also been described to support tumor cell migration and invasion when it is overexpressed, giving it oncogenic properties [60].

In some cancers, such as in breast and lung cancers, ING1b and ING2a expression decrease could be involved in the tumor initiation and/or progression [13,45,53,49,58] since ING1b and ING2a mRNA loss of expression occurs at an early stage of tumor development. Loss of ING1b or ING2a mRNA could be the result of either misregulation of transcription factors that regulate *ING1* or *ING2* expression or gene inactivation mechanisms such as epigenetic mechanisms including DNA methylation and histone modifications. These losses could also result from misregulation of microRNAs targeting ING1b or ING2a mRNA since in gastric cancer miR-622 have been described to decrease the ING1 mRNA expression by targeting the *ING1* 3'UTR [61].

ING1 and ING2 knockout mice

Development of ING1 and ING2 knockout mice are an interesting *in vivo* model to study the physiological role of ING1 and ING2 since both have been reported to be lost in human tumors. Moreover, human and mouse ING1b or human and mouse ING2a are highly homologous (Fig.S2).

Two models of ING1 knockout mice have been developed. The first one was developed by removing the common exon E2 shared by the three ING1 mouse isoforms (Fig.S1). Thus it results in the absence of the three isoforms (Fig.S1). This model displays an increased incidence of spontaneous tumors with mostly B-cell lymphoma from germinal center origin [14,15]. They also exhibit a decrease in term of survival in response to six daily doses of 2.3 Gy of γ-rays compared to the wild type mice which suggests a role for ING1 in DNA repair [14] (see thereafter). In the second model only mouse ING1b has been inactivated. ING1b-/- mice develop B-cell lymphoma from germinal center origin with a high incidence [14-16]. Strikingly, these ING1b-/- mice have a tumor spectrum slightly different than ING1-/- mice suggesting a role for mouse ING1a and mouse ING1c isoforms by modulating mouse ING1b activity to prevent tumorigenesis. These two models of ING1 knockout mice have demonstrated that ING1 functions are independent of p53. Interestingly, p53-/- ING1b-/- mice develop, with an incidence of 63%, aggressive diffuse large lymphomas (DLBCL) whereas p53-/- mice develop T-cell lymphomas with an incidence of 60%. In addition, expression analysis of *Bax* in the thymus, spleen, liver and brain have been performed in these mouse models in response to ionizing radiations. It appears that for all these tissues, *Bax* expression is highly induced in ING1b-/- mice and slightly less in ING1b -/- p53 -/- mice whereas it is not expressed in p53-/- mice [15], suggesting that ING1 induces *Bax* expression and consequently apoptosis independently of p53. Thus, because of their different tumor spectrum, ING1 has p53-dependent and independent functions. Moreover, it seems that they cooperate to suppress DLBCL [16].

Then, a model of knockout mice targeting both ING2a and ING2b isoforms has been engineered. Saito and coll. have reported that ING2-/- mice develop soft-tissue sarcomas with an incidence of 46%, and most frequently histiocytic sarcomas (28%) [17]. A particular phenotypic trait was observed in ING2-/- male mice. These mice were infertile. They showed deficient spermatogenesis, small testes, seminiferous tubules degeneration, abnormal spermatozoa motility and morphology from the age of eight weeks. Analysis of testes DNA content showed that ING2-/- testis cells failed to complete meiosis II and have an altered meiotic recombination. RNA microarray profiling showed a deregulation of genes specifically expressed in spermatid and spermatozoa suggesting that ING2 could have an effect in

spermatids and spermatozoa differentiation. Moreover, comparisons of public microarray datasets revealed an association between ING2 deficiency and human male infertility. Consequently, this opens new prospects for a role of ING2 in male meiosis [17].

Since previous reports have suggested a functional interaction between ING2a and p53 [4], the effect of ING2 knockout on p53-dependent and independent apoptosis was also investigated. ING2-/- germinal cells undergo more apoptosis than the WT and their p53 protein level is increased. It could be the result of testicular degeneration in the absence of ING2 or of novel regulatory interactions between ING2 and p53. In the aim to determine p53-independent apoptosis in ING2-/- testes, double knockout ING2 and p53 mice were generated. p53-/- mice do not show spermatogenesis defect and are fertile. ING2 deficiency in p53-/- background presents the same abnormalities in term of spermatogenesis deficiency than ING2-/- mice. However, these abnormalities were less severe than those in ING2-/- mice. Thus, simultaneous loss of ING2 and p53 partially abrogates the apoptosis observed in ING2-/- testes. This suggests that ING2 deficiency in testis induces both p53-dependent and independent apoptosis [17]. These double knockout ING2 and p53 mice should also provide clues on dependent and independent effects of p53 and ING2 in tumor suppression.

Surprisingly, although ING1 and ING2 have a high homology, a different phenotype has been observed in between these knockout mice models. In human, *ING1* and *ING2* status have never been fully investigated in B-cell lymphomas and histiocytic sarcomas. Such studies would allow the correlation with the phenotypes observed in mouse knockout models. Double knockout mice for ING1 and ING2 have not been engineered yet. This model will provide clues regarding the potentially redundant functions between ING1 and ING2.

Tumor suppressor functions for ING1 and ING2

Since their identification, ING1b and ING2a functions have been investigated using mostly overexpression strategies in human cell lines. More recently, downregulation of ING1b and ING2a, as it occurs in human tumors has given new insight in the understanding of ING1b and ING2a functions. In this part, we will discuss which ING1b and ING2a functions define them as a tumor suppressor genes and how their loss could contribute to tumor development. Some functions characterize ING1b and ING2a as gatekeepers and others as caretakers.

ING1 gatekeeper functions regulate cell cycle, senescence and apoptosis

Among ING1 isoforms human ING1b is the most abundant form. Its functions have been well defined since its identification. Thanks to these studies its status as a tumor suppressor has been fully established. In contrast, human ING1a is less expressed and less studied. Its TSG status is not as well defined since ING1a has just been involved in senescence. So far, there is no study reporting any function for the other ING1 isoforms.

ING1 controls gene transcription

Initially, ING1 has been described to interact with the mSin3a/HDAC1-2/Sap30 complex [28,30]. Then, studies of ING2 PHD (see thereafter) have revealed that ING2 PHD and of ING1 PHD have a high affinity with H3K4Me3 [26,27]. Subsequently, the interaction of ING1 PHD has been better characterized. ING1 PHD interacts strongly with H3K4Me3 and more weakly with H3K4Me2 and H3K4Me1 [29]. Recognition of H3K4Me3 by ING1 PHD has been described to promote ING1 functions such as DNA repair and apoptosis [29]. Trimethylated histone is mainly found on transcriptionally active regions [62]. This interaction recruits and stabilizes the mSin3a/HDAC1-2/Sap30 complex on gene promoters since ING1 is a stable component of the mSin3a/HDAC1-2/Sap30 complex. This leads to histones deacetylation and gene repression. Recently, mutations of ING1b aminoacid residues located in the PHD critical for the binding with H3K4Me3 [26,27] has been reported to impair the onset of senescence. These mutations modify a specific transcriptome signature after senescence induction. This signature is enriched in cytokines and chemokines. Thus, during senescence, the recognition of H3K4Me3 by ING1 mediated transcription of genes that induce a secretory response in human primary fibroblasts [63]. Consequently, regulation of gene transcription through ING1b PHD and the mSin3a/HDAC1-2/Sap30 complex may regulate the induction of senescence (Fig.3). Interestingly, ING1a and ING1b regulate histone acetylation in a different manner. Overexpression of ING1a decreases acetylation of histone H3 whereas overexpression of ING1b does not decrease acetylation of histone 3. ING1b has been invoved in histone acetylase and deacetylase complex by interacting with p300 [64] (see thereafter) and with the mSin3a/HDAC1-2/Sap30 complex whereas ING1a interacts only with

the mSin3a/HDAC1-2/Sap30 complex [28,30]. Thus, Human ING1 isoform differentially regulate histones acetylation/deacetylation.

Interestingly, in mouse fibroblasts downregulation of ING1b has been described to increase the expression of *Dgcr8* which encodes for an RNA-binding protein regulating the early steps of microRNAs biogenesis. In the absence of ING1b, acetylation on histone H3 and histone H4 in the *Dgcr8* promoter was described to significantly increase, suggesting that ING1b contributes to the transcriptional repression of *Dgcr8* by inhibiting histone acetylation through the recruitment of deacetylase complexes. This leads to the deregulation of miRNAs expression [65]. The deacetylase complex involved in the regulation of ING1b-dependent H3 and H4 acetylation has not been identified but might be the mSin3a/HDAC1-2/Sap30 complex. Moreover, deregulation of the microRNA machinery is well characterized in tumors [66]. Thus, this is a new pathway by which ING1b downregulation could promote tumorigenesis. Moreover, in mouse mammary epithelial cells ING1b has been described to negatively control the expression of *cyclin B1* and the proto-oncogene DEK, a nucleic acid binding protein [67]. The mechanism involved remains unknown but may occur through the mSin3a/HDAC1-2/Sap30 complex. In addition, ING1b has also been reported to interact with *p16* promoter and upregulates $p16^{INGK4a}$ expression in 2BS fibroblasts in a p300-dependent manner that lead to induction of cellular senescence [68]. Interestingly, ING1b have been reported to interact with p300 acetylase [69] (see thereafter). As a consequence, it could promote p300 anchorage on *p16* promoter to acetylate this region to activate *p16* expression.

ING1b regulates p53 activity

Initially, ING1b overexpression has been described to inhibit cell growth in various cell lines [3,70,71]. Then, ING1b was involved in apoptosis caused by serum starvation [72]. Interestingly, compared to young fibroblast the level of ING1a mRNA in senescent fibroblast is enhanced; whereas, ING1b mRNA is decreased. ING1a has been reported to promote a senescent phenotype in human fibroblast whereas ING1b was involved in the induction of apoptosis [73,74]. Later, ING1b was also involved in the maintenance of cellular senescence. A stable and moderate overexpression of ING1b in young human fibroblasts induces senescence in a p53-dependent [63,75]. In addition, coexpression of both ING1b and p53 in H1299 cells induces more cell death in response to the adryamycine than these two proteins alone, suggesting a close collaboration of these two proteins [76]. Thus, ING1a has been

involved in the control of senescence in a p53-dependent manner and ING1b has been involved in the regulation of apoptosis and senescence in a p53-dependent manner. Interestingly, coexpression of mouse ING1b and ING1c has been reported to activate *p53* transcription more efficiently than mouse ING1b and mouse ING1c alone [76,77]. It is not yet clear how mouse ING1c collaborates with mouse ING1b, but, since mouse ING1c is the truncated of N-terminus of mouse ING1b, it could be through its C-terminal domain (Fig.S1). Thus human and mouse ING1 isoforms have been involved in p53-dependent regulation of cell cycle, apoptosis and senescence.

In addition, overexpression of human ING1b in HT1299 expressing p53 increases *p63* and *p73* expression [76]. Thus, ING1b may also regulate the transcription of two members of the p53 protein family which have also tumor suppressor functions [78-80]. Thereafter, human ING1b has been described to regulate p53 activity through three different mechanisms. Firstly, human ING1b interacts with p300 acetylase to promote p53 acetylation on lysine 382. p53 acetylation activates transcription factor functions to regulate *p21* and *Bax* expression to induce cell cycle arrest and apoptosis [69,71] (Fig.3). Moreover, human ING1b associates which SIRT1 (also known as hSir2) and competitively inhibit SIRT1 association with p53 [69,81]. SIRT1 is a histone deacetylase described to deacetylate p53. This results in the inhibition of p53 deacetylation contributing in the maintenance of p53 activity such as activation of transcription of *p21* and *Bax* (Fig.3). Finally, human ING1b may regulate the ARF - MDM2 - p53 pathway. ING1b interaction with p53 has been proposed to compete with MDM2 - p53 interaction [77]. MDM2 has been described to ubiquitinylate p53 in order to mediate its degradation through the proteasome system [82]. More recently, an ubiquitin interaction motif (UIM) has been described as an overlapping region on the PBR in the C-terminal domain of ING1b. Through this region ING1b binds monoubiquitinated p53 to prevent its polyubiquitination [83]. Thus, through their interaction, ING1b stabilizes p53 [77,83]. Then, ARF has been suggested to interact with ING1b to alter its localisation from the nucleus to the nucleolus [77,84] (Fig.3). Furthermore, ING1b has been described to interact with p53-related proteins p63 and p73 as well as p53 [76]. Consequently, ING1b could regulate p63 and p73 activity through similar mechanisms than those involved in p53 regulation. However, it needs to be investigated.

ING1b regulates cell growth and apoptosis independently of p53

ING1b decreases cell proliferation through p53-dependent mechanisms but also through p53-independent mechanisms. Human ING1b overexpression in H1299 p53-null cells have been reported to decrease cell growth. Moreover, in response to adryanamycine, a DNA damaging agent, it enhances the blockage in G2/M checkpoint [76]. In addition, downregulation of ING1b in p53 null glioblastoma cells has been reported to increase apoptosis in response to cisplatin [37,85]. Consequently ING1b regulates cell growth and apoptosis in a p53-independent manner.

Moreover, overexpression of human ING1b induces expression of *Heat Shock Protein 70* (*HSP70*). The N-terminal region of ING1b containing the PIP and the PBD has been reported to be required for the induction of *HSP70* expression; whereas, the PHD and the PBR were not [86]. HSP70 has been reported to inhibit NF-κB signalling [87,88]. Thus, by regulating HSP70 expression ING1b could indirectly regulate NF-κB-dependent cell growth and apoptosis [37,86] (Fig.3).

Human ING1b interacts with CSIG (Cellular Senescence-Inhibited Gene) through its NTS. CSIG is a nucleolar protein which has been involved in the regulation of cellular senescence [89]. Human ING1b overexpression increases CSIG protein stability and its downregulation decrease CSIG protein level. There interaction and the maintenance of CSIG protein level by ING1b is required to activate Bax and apoptosis in response to ultraviolet (UV) [90] (Fig.3).

ING1 caretaker functions regulate DNA repair in response to UV

Originally, *ING1b* expression has been reported to be enhanced in response to UVB in mouse keratinocytes and in MMRU melanoma cell line [20,91]. Human ING1b overexpression was described to enhance repair of UV-damaged DNA in a p53-dependent manner. Interestingly, ING1b binds Growth Arrest and DNA Damage 45 (GADD45) but not Xeroderma Pigmentosum A (XPA) and XPB [91] (Fig.3). GADD45 has been described as a cofactor to promote DNA demethylation during Nucleotide Excision Repair (NER) without any demethylase activity [92]. The mechanism by which ING1b collaborates with GADD45 to mediate NER remains unknown. In addition, ING1b PHD domain has been shown to play an essential role in NER. In fact, truncation of ING1b PHD abrogates DNA repair in response to UV-induced damage [93]. We thus could hypothetize that the involvement of ING1b PHD in the regulation of DNA repair in response to UV could be through its interaction with the

the mSin3a/HDAC1-2/Sap30 complex [28,30]. Interestingly, ING1b has then been shown to regulate H4 acetylation to promote global chromatin relaxation to allow XPA recruitment [94]. A more recent study has described that downregulation of ING1b decreases PCNA monoubioquitination and sensitizes cells in response to UV during S phase [95]. Defect of ING1b results in chromatin break and sister chromatin exchange in response to UV. The E3 ubiquitin ligase Rad18 is known to mediate PCNA monoubioquitination in response to UV to ensure lesion bypass and error prone DNA replication [96,97]. In fact, ING1b is required for the loading of Rad18 on chromatin at replication sites upon replication stress to mediate PCNA monoubiquitination. A strong regulation of H3 and H4 acetylation by ING1b has been reported to support Rad18-dependent PCNA ubiquitinylation [95] (Fig.3). Therefore, ING1b facilitates NER and regulation of DNA replication upon UV stress through regulation of histone acetylation. Two mechanisms of such regulation could be considered. Firstly, ING1b through its involvement in the mSin3a/HDAC1-2/Sap30 complex could regulate histone acetylation and thus allow the access of GADD45 and Rad18 to the chromatin. The second hypothesis is through ING1b interaction with p300. ING1b has been described to interact with p300 and its overexpression has been reported to alter PCNA - p300 interaction in response to UV [64]. By regulating PCNA-p300 interaction, ING1b could also regulate H4 acetylation in response to UV-induced DNA damage to facilitate GADD45 and Rad18 accessibility to the chromatin but it needs to be experimentally confirmed.

Interestingly, ING1b interacts also with PCNA through its PIP domain. Mutation in the PIP domain decreases apoptosis in response to UV. Thus, binding between ING1b and PCNA is necessary to mediate apoptosis in response to UV. Moreover, this interaction strongly increases in response to UV and could compete with other PIP containing proteins such as p21 known to regulate the switch from DNA replication to DNA repair. Consequently, ING1b-PCNA interaction was proposed to regulate the switch from DNA replication to DNA repair by altering the composition of the replication protein complex to promote apoptosis [34]. In addition, $p15^{PAF}$ associates with both PCNA and ING1b in response to UV. $p15^{PAF}$ possesses a PIP domain and has been described to compete with p21 for PCNA binding when overexpressed [98]. Since PCNA is organized as a ring-like homotrimer, it contains three binding sites for proteins having a PIP motif [99,100]. It has been proposed that the association of PCNA with ING1b together with $p15^{PAF}$ could more efficiently compete with the binding of PCNA and p21 [101] (Fig.3).

To conclude, ING1b regulates DNA repair in response to UV through its interaction with GADD45 and PCNA and through the regulation of histone acetylation. How ING1b

regulates histone acetylation in response to UV remains unsolved but it is clear that ING1b promotes the remodelling of the chromatin structure to provide accessibility of DNA repair factors. Thus, it also interacts with some factors to promote their recruitment and/or their function.

ING2 gatekeeper functions regulate cell cycle, senescence and apoptosis

Initially, ING2a has been shown to negatively regulate cell proliferation because it enhances p53 transcription [4]. Subsequently, through its interactions with HDACs and with acetylases, ING2a has been involved in the regulation of genes involved in cell cycle control, senescence and apoptosis.

ING2 controls gene transcription

ING2a has been shown to interact directly with histones to regulate gene transcription. ING2a PHD is necessary and sufficient for a strong interaction between ING2a and H3K4Me3 and a weaker interaction with histone mono or dimethylated on lysine 4 [27]. Trimethylated histone mark is mainly found in transcriptionally active regions [62]. This interaction recruits and stabilizes the mSin3a/HDAC1-2/Sap30 complex on gene promoters since ING2a is a stable component of the mSin3a/HDAC1-2/Sap30 complex. This leads to histones deacetylation and gene repression [26,27,102-104] (Fig.3). Interestingly, ING2 male KO mice germinal cells show an impaired expression of HDAC1 during spermatocyte differentiation. It results in the accumulation of acetylated histones associated with meiosis arrest [17]. This highlights the significance of ING2a in histones acetylation/deacetylation process to regulate gene expression. ING2a involvement in chromatin remodeling through the mSin3a/HDAC1-2/Sap30 complex could thus change the accessibility of transcription factors to the chromatin or could allow the accessibility to other chromatin remodeling factors to modify transcription factors expression and/or recruitment. Indeed, epigenetic changes have been described to contribute to carcinogenesis [105]. Thus, ING2 loss, as it occurs in tumors, could trigger epigenetic modifications participating in the tumorigenesis process.

Through this mechanism ING2a regulates *p21* transcription since ING2a is critical for the binding of the mSin3a/HDAC1-2/Sap30 complex to the *p21* promoter. Indeed, the use of suberoylanilide hydroxamic acid (SAHA), a histone deacetylase inhibitor, causes the dissociation of ING2 from the mSin3a/HDAC1-2/Sap30 complex resulting in the disruption of the binding of the mSin3a complex from the *p21* promoter [103]. Moreover, ING2a sumoylation by Small Ubiquitin-like MOdifier 1 (SUMO1) on Lysine 195 has been described to enhance the interaction between ING2a and mSin3a [106]. Effects of SAHA on ING2a sumoylation need to be explored to clarify whether ING2 disruption is not a consequence of a modification of its sumoylation status. It is also possible that ING2 sumoylation regulates other interactions between INGa and other partners to regulate other ING2a functions. Indeed, ING2 sumoylation site is located in a potential Phosphorylation Dependent Sumoylation Motif (PDSM) [106]. Thus ING2a could be sumoylated on Lysine 195 in a manner dependent on its phosphorylation on Serine 201. Interestingly, the same PDSM motif is also present on ING1. In this case the serine residue has been shown to be phosphorylated in order to regulate ING1 export from the nucleus. Interestingly, it results in a decrease of *p21* expression [32]. Therefore, since ING2a sumoylation has been shown to regulate its interaction with the mSin3a/HDAC1-2/Sap30 complex, ING2a potential phosphorylation on Serine 201 could also regulate its interaction with mSin3a and consequently *p21* expression. An independent study has shown that ING2a downregulation induces the decrease of *p21* expression and thus accelerates the progression of cells from G1 to S phase. Binding of ING2a to H3K4Me3 has been proposed as a mechanism to regulate the G1/S transition by regulating *p21* expression. Indeed, downregulation of WD40-repeated protein WDR5, which recognizes lysine 4 of H3 to facilitate methylation of lysine 4 of H3 or simultaneous downregulation of WDR5 and ING2a accelerates cell progression from G1 to S phase in the same manner than ING2 downregulation [107] (Fig.3).

ING2a regulates cell proliferation also by regulating TGF-β dependent transcription. TGF-β regulates homeostasis and has tumor suppressor properties such as inhibition of proliferation, vessels formation and regulation of apoptosis. Dysfunction of TGF-β signaling has been implicated in cancer development [108]. Thus, in response to TGF-β signaling, ING2a binds SnoN (Ski-like oncogene), a Smad-interacting transcriptional modulator, through its PHD domain. SnoN binds ING2a to form a complex that associates ING2, SnoN and Smad2. Consequently, Smad2 enhances TGF-β-dependent transcription that contributes to the inhibition of cell proliferation [109] (Fig.3). Interaction of ING2a PHD with SnoN could compete with ING2 PHD interaction with the mSin3a/HDAC1-2/Sap30 complex. Thus

HDAC1-2 would not be recruited to TGF-β-dependent gene promoters and would not be able to acetylate chromatin to inhibit their transcription. Thus, ING2 loss, as it occurs in human tumors, could allow cancer cells to escape from Smad2-TGF-β dependent regulations.

To summarize, through the regulation of chromatin remodelling on promoter of genes such as *p21* and TGFβ-responding genes, ING2a regulates expression of genes involved in cell cycle control.

ING2 regulates acetylation of p53 and p73α to inhibit cell growth

ING2a is involved in signalling pathways that regulate other gatekeeper TSG. Initially, ING2a was characterized as being involved in the p53-dependent pathways [4]. ING2 overexpression in young fibroblast arrests cell in G1 and induces senescence with a phenotype similar to p53 overexpression while siRNA targeting ING2 decreases senescence. Mechanistically, ING2a interacts with the p300 acetyltransferase to enhance p53 acetylation on lysine 382 to increase transcription of p53 target genes particularly *p21* and *Bax* to control cell cycle arrest, senescence and apoptosis [4,110] (Fig.3). Moreover, a mechanism involving PtdInsP has been reported to regulate ING2-p53-dependent pathway. ING2a has been described as a nuclear receptor to PtdInsP since it interacts with PtdIns(5)P through its PBR region [25,111]. In response to exogenous stresses, the p38 kinase pathway is activated to regulate phosphorylation of PtdIns(5)P resulting in an increase of nuclear PtdIns(5)P. p38 has tumor suppressor properties since it has been described to regulate cell proliferation and to negatively regulate cell cycle progression of both G1/S and G2/M checkpoints [12]. Nuclear PtdIns(5)P increase induces ING2a accumulation to the chromatin. This interaction modulates the ability of ING2a to regulate p53 acetylation. It results in *p21* expression and cell death activation [25,112] (Fig.3). Interestingly, ING1b has also been shown to interact with PtdInstP(5)P through its PIP [25]. Thus, as ING2a, ING1b through its interaction with PtdInstP(5)P could have an accumulation to the chromatin and then modulate the acetylation of p53. Moreover, ING2a has been also described to be able to bind chemically synthesized analogues of the PtdInstP(5)P [113,114]. Interestingly, a simultaneous reintroduction of ING2 and PtdInstP(5)P or PtdInstP(5)P analogues in HT1080 cells enhances cell death in response to DNA Damage [114]. In the context of anticancer therapies, the concomitant reintroduction of ING2 toghether with PtdInstP(5)P or PtdInstP(5)P analogues could be thus a strategy to be considered (see discussion).

p73α belongs to the p53 protein family and has tumor suppressor properties. It regulates transcription of genes involved in cell cycle arrest and in apoptosis such as *p21*, *PUMA* and *Bax* [78]. The p73α protein is activated in response to N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), a genotoxic agent. A study reported that in response to MNNG treatments, in cell lines not mutated for p53, ING2a is upregulated in a dose and time dependent manner. In response to this genotoxic agent, ING2a is not required for p53 induction and acetylation but it is required for the induction and the acetylation of p73α to promote cell death. It remains unclear through which acetylase ING2a facilitates p73α acetylation. Since p73α has been shown to be acetylated by p300 to modulate cell death, and since ING2a has been reported to interact with p300 to mediate p53 acetylation [110], p300 is a good candidate which will need to be tested [115,116].

To conclude, ING2a regulates p53 and p73α acetylation and as a consequence regulates transcription of genes involved in cell cycle arrest, apoptosis or senescence. Interestingly, p300 has also been describes as a HAT which can acetylate H2A, H2B, H3 and H4 [117,118]. Thus, ING2a could also regulate, through its binding with H3K4Me3, the anchorage of the p300 to allow acetylation of specific genomic area to regulate gene transcription. A competition with the mSin3a/HDAC1-2/Sap30 complex in response to different exogenous or endogenous signals or stresses could thus occur.

ING2 caretaker functions maintain genome stability

Two set of studies have shown the involvement of ING2 in DNA replication and NER suggesting that it may also act as a caretaker TSG.

ING2 regulates DNA replication

ING2a downregulation experiments have shown an impaired normal DNA synthesis because of a reduced replication speed during the elongation process. Indeed, ING2 interacts, through its NCR domain, with Proliferating Cell Nuclear Antigen (PCNA) which acts during elongation by regulating DNA polymerases processivity. ING2a regulates its recruitment to the chromatin and is thus necessary for the optimal progression of DNA replication forks

[119] (Fig.3). How ING2a regulates PCNA recruitment to the chromatin remains unsolved. One hypothesis is that ING2a could regulate PCNA activity by regulating its post-translational modifications. Interestingly, p300 has been shown to bind PCNA [120]. Thus, as observed for the activation of the transcription factors p53 and p73α, ING2a could allow the acetylation of PCNA on replication forks, may be through p300 acetylase, to enhance PCNA activity. In addition, a model has been proposed where ING2, ING4 and ING5 could be involved at different steps of the normal replication process [121]. Indeed, since both ING4 and ING5 have been described to be part of HAT complexes, they could allow histone acetylation around replication origins. Then, the relaxed chromatin state would allow the recruitment of the MCM helicase complex which interacts to ING5 [104]. Afterwards, thanks to its interaction with ING2a, PCNA would regulate the polymerase processivity [119,121]. ING2 which interacts with p300 acetylase could also regulate local acetylation around the replication forks. Moreover, depletion of ING2a results in a high frequency of genome endoreplication, sister chromatid exchange and accumulation of DNA double strand breaks [119] (Fig.3). Consequently, loss or downregulation of ING2, as it occurs in human tumors, slows down the replication process, contributing to genomic instability which could contribute to tumorigenesis.

ING2 is involved in NER in response to UV

ING2a plays an important role in DNA repair in response to UV. It was firstly shown that ING2 overexpression increases NER in response to UV in a p53 dependent manner; whereas, downregulation of ING2a decreases NER efficiency in response to UV. However, ING2 does not colocalize with UV-induced DNA damage. Instead, ING2a is required for H4 acetylation and chromatin relaxation in response to UV to facilitate the recruitment of XPA to photolesions [122]. More specifically, ING2a LZL domain has been shown to be critical for this process while its PHD motif is dispensable. The deletion of ING2a LZL decreases NER efficiency and apoptosis induced by UV-damage. In response to UV-DNA damage, ING2a LZL domain binds p53 and mediates the interaction between p53 and p300 acetylase in order to stabilize them to the chromatin [122,123]. The ING2a LZL domain is also required for H4 hyperacetylation and chromatin relaxation to promote NER [123] (Fig.3). Moreover, the association between p53 and p300 has also been shown to be involved in H3 histone acetylation [124]. Thus, by mediating the association between p53 and p300 in response to

UV, ING2 could facilitate the anchorage of p300 on specific genomic sites to support H3 and H4 hyperacetylation to promote the accessibility of NER factors to the chromatin. Moreover, PCNA is involved in several steps of NER DNA synthesis. Interestingly, Hasan and coll. have reported that PCNA interacts with p300 to promote DNA synthesis after UV irradiation [120]. Because ING2 regulates PCNA recruitment to the chromatin during the replication process [119], it could also regulate PCNA interaction with p300 and its recruitment for the NER. Additional experiments will be required to complete these results. In fact, these results have mostly been carried out using ING2a overexpression strategies which is known to have supraphysiological effects. For example, ING1b and ING2a were firstly described to be involved in p53-dependent apoptosis whereas siRNAs or knockout studies have shown that both ING1b and ING2a are also involved in p53-independent apoptosis. However, since the expression of ING2 is lost in melanoma [58], it would be interesting to analyse at which step of skin cancer development ING2 has been lost to determine if a loss of its NER function has been playing any role in the tumorgenesis process.

Crosstalk between ING1 and ING2 tumor suppressor functions

ING1 and ING2 are two tumor suppressor that have gatekeeper and caretaker TSG functions. Both ING1b and ING2a are stable components of the mSin3a/HDAC1-2/Sap30 complex and thus both regulate gene transcription. Since ING1b and ING2a do not coprecipitate together, they are exclusive components of the mSin3a/HDAC1-2/Sap30 complex. The reason that explains why both are involved in these complexes remains unknown. Depending of the cellular context, they could have complementation effects to regulate gene expression such as *p21* expression. They also could target different promoters to regulate the expression of different genes. Interestingly, in Yeast, Pho23, the ING yeast related to ING1 and ING2 in *Saccharomyces cerevisiae* [8], has also been purified as a stable component of Rpd3/HDAC complex. Rpd3 is the catalytic components of the yeast Sin3/HDAC complex [104,125]. Consequently, the association of ING1 and ING2 with the Sin3/HDAC complex is conserved from Yeast to Human suggesting that this function has been critical for epigenetic regulation throughout the evolution.

Interestingly, both ING1b and ING2a regulate p53. First, ING1b and ING2a both regulate acetylation of p53 to regulate cell cycle, apoptosis and senescence [63,75,110,126]

(Fig.3). Since ING2a regulates p73α acetylation [127] and ING1b has been described to interact with p73 [76] it is possible that ING1b regulates also p73 acetylation. ING1b has also its own functions to mediate p53 activity since the involvement of ING2 in the ARF - MDM2 - p53 pathway has never been demonstrated [77,83]. In addition, by interacting with the acetylase SIRT1, ING1b inhibit SIRT1 – p53 interaction resulting in the maintenance of p53 acetylation [81,69] (Fig.3). Interestingly ING2a has also been described to bind SIRT1 [81]. It remains thus possible that ING2a may, as ING1b, inhibit SIRT1 association with p53.

By controlling acetylation of histones and by regulating transcription factors activity or protein expression level, ING1b and ING2a regulate transcription of genes involved in cell cycle arrest, apoptosis and senescence. Consequently, ING1b and ING2a act as gatekeeper TSGs. Their loss may confer growth advantage because of the loss of transcription control of these genes. Thus, cells lacking ING1b or ING2a could bypass cell cycle checkpoint controls, proliferate and be positively selected (Fig.4).

Interestingly, the two close homologs ING1b and ING2a are both involved in the regulation of DNA replication through two independent mechanisms. Initially, *ING1* expression was described to be cell cycle-dependent. Its expression is increased from G1 to S phase and then decreases in G2 phase [126]. Later, ING2a expression was shown to also be dependent of the cell cycle, ING2a expression is enhanced in S phase reaching a maximum in G2 phase [119], suggesting an involvement of both ING1b and ING2a in the S phase. Actually, ING2a has been described to be necessary to regulate DNA replication independently of exogenous stress [119]; whereas, ING1b mediates replication in response to UV [95]. Consequently, ING1b and ING2a have specific functions involving them in replication. However, why ING2a but not ING1b is involved in the progression of the normal DNA replication forks remains unknown. One hypothesis would be that because ING2a does not possess a PIP domain, it does not interfere with other PIP proteins in response to DNA damage and consequently it does not regulate the switch from DNA replication to DNA repair. However, a recent nuclear magnetic resonance study demonstrated that ING1b PIP binds PCNA with a very low affinity, probably because ING1b PIP motif lacks a second aromatic residue usually presents in the canonical PIP motif [128]. Strikingly, it is ING2a NCR domain, also found on ING1b, which has been involved in the interaction between ING2 and PCNA [119]. Since interaction of ING1b PIP domain with PCNA has been demonstrated using only ING1 PIP mutant [34] and since this interaction has a very low affinity [128] (Fig.3), it would be of interest to test whether the ING1b NCR domain could mediate or facilitate the interaction with PCNA. It remains also possible that the interaction

between ING1b and PCNA is promoted by post-translational modifications or by other PCNA interacting proteins.

In addition, ING1b and ING2a have both been involved in the regulation of NER through regulation of histone acetylation. ING1b and ING2a could either collaborate or act independently to regulate NER but it remains to be experimentally verified. Moreover, because both are also involved in the mSin3a/HDAC1-2/Sap30 complex in response to exogenous stress, it would be interesting to understand how the switch toward one or the other complexes is carried out and how the ING1b and ING2a pools are distributed to regulate histone acetylation and deacetylation.

Altogether, these studies involve ING1b and ING2a in the regulation of chromatin acetylation to promote a favourable chromatin state that allows the access of DNA replication or DNA repair proteins to DNA thus contributing to genome stability maintenance. Deregulation of DNA repair and DNA replication are functions which are closely associated with early steps of tumorigenesis [1,129]. Early loss of ING1b and ING2a expression, as it occurs in human tumors may contribute in an enhancement of genome instability and could initiate the tumorigenesis process. Then, the accumulation of later events such as passenger mutations and DNA modifications will promote the development and the progression of cancer (Fig.4).

Conclusions and future directions

Originally *ING1* was identified with the aim to identify new tumor suppressor genes [3]. Then, studies conducted on ING1b about its status in tumors and its functions have fully established the status of ING1b as a tumor suppressor [13,37]. Later, *ING2* has been identified thanks to a high homology to the tumor suppressor gene *ING1*. Subsequently *ING2* was characterized as a “candidate” TSG. Since, studies have demonstrated that, both ING1b and ING2a are frequently lost in human tumors and that both knockout mice models spontaneously develop B-cell lymphomas and soft-tissue sarcomas respectively [13-17]. In addition, the better understanding of ING1b and ING2a biological functions has contributed to confirm their TSG status. Interestingly, a study involving ING1 in a mechanism is most of the time also true for ING2. Indeed, ING1b and ING2a which regulates cell cycle, apoptosis and senescence, have thus been defined as a gatekeeper TSG (or type 2). Recent studies have

also shown ING1b and ING2a involvement in the maintenance of genome stability by regulation of both DNA replication and NER. Accordingly, as its close homolog ING1b, ING2a has also been defined as a caretaker TSG (or type 2).

Consequently, as they are TSGs, the loss of ING1b or ING2a in human tumors could be involved in the tumorigenesis process. On one hand, loss of ING1b and ING2a caretaker functions could enhance genome instability that could initiate tumorigenesis, while loss of ING1b and ING2a gatekeeper functions would allow cells to bypass cell cycle checkpoint and apoptosis to be positively selected (Fig.4). However, how and why ING1b and ING2a are lost in tumors remains unclear. We still do not know which mechanism that initiates ING1 and ING2 loss. Genetic and epigenetic susceptibility as well as environmental factors are probably involved in this process.

Recent studies have allowed a better understanding of fundamental cellular and molecular mechanisms in which ING1b and ING2a are involved such as cell cycle regulation and DNA repair. A better understanding of their protein structure has allowed the association of their protein domains with their functions. However, the specificity of these proteins has not yet been fully established, e.g. we still do not know the usefulness of the NCR domain present only on ING proteins. No ING1b or ING2a protein enzymatic activity has been identified. Instead, ING1b and ING2a could be factors that promote acetylation or deacetylation of histones and transcription factors to activate these proteins and/or to allow a favourable chromatin state to regulate gene transcription or DNA replication and DNA repair. Moreover, we still do not know how ING1b and ING2a are involved in different complexes. It has not yet been established whether ING1b or ING2a could be free or only in complex. Further, it is not known how ING1b or ING2a exchange between protein complexes is processed. Post-translational modifications such as phosphorylation or sumoylation could regulate either this exchange or their targeting towards a protein complex rather than another.

Since ING1b and ING2a are TSGs, they are interesting proteins to look at in the context of anticancer therapies. First of all, ING1b and ING2a could be used as a biomarker for cancer diagnosis. Establishing *ING1* and *ING2* status in tumours could also be important for the therapeutic choices. It could allow the identification of defective signalling pathways in tumor cells which would be targets of interest for treatment. Interestingly, a recent study has revealed that simultaneous overexpression of ING1b and treatment with 5-azacytidine synergize to block the growth of breast cancer cell lines and mouse tumor xenografts. ING1b could be used of a therapeutic agent since it enhances the efficacy of 5-azacytidine [130]. Moreover, since ING1b and ING2a are lost or downregulated at the RNA level in human

cancers [13], an interesting strategy would be to re-express ING1 or ING2 or to stabilize their protein level. ING2a has already been described to bind PtdInstP(5)P analogues [113,114]. Since, their concomitant reintroduction into HT1080 cells have been reported to enhance cell death in response to DNA damage [114], a possible strategy would be to simultaneously reintroduce ING2 and PtdInstP(5)P analogues in tumor cells to enhance tumor cells death [112,114].

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Conflict of interest

The authors declare that they have no conflict of interest.

Legend to Figures

Figure 1: Structure of Human ING1 and ING2 genes, mRNAs and proteins.

A. ING1 human gene. E1c, E1d, E1a, E1b, E1b$_{alt}$, E2 are respectively Exon 1c, Exon 1d, Exon 1a, Exon 1b, Exon 1b$_{alt}$ and Exon 2. **B.** ING1b human mRNA. **C.** ING1b human protein. ING1b protein contains a PIP and a PBD in its N-terminal part, a NCR and a NLS in its central region and a PHD and a PBR in its C-terminal part. This figure of ING1 is simplified, the full version has already been published [13] and can also been found in Fig.S1. **D.** ING2 human gene. E1, E2 are respectively Exon 1 and Exon 2. Exon 1a promoter contains two p53 Binding Site (p53BS) and a NF-κB Responsive Element (NRE). **E.** ING2 human mRNAs. **F.** ING2 human proteins. ING2a contains a LZL in its C-terminal part. ING2a, ING2b possess a NCR and a NLS with three NTS, a PHD zinc finger motif and a PBR. ING2a and ING2b are both represented in this figure since ING2b has never been included in a scheme describing ING2 proteins since its identification. On genes and mRNAs, none coding regions are in yellow and coding region are represented in purple. Each mRNA variant is represented with its name on the left and its Genebank accession number on the right. Each protein is represented with its characterized domains with its name on the right and with its molecular weight, and its Genebank accession number on the left.

Figure 2: Sequences alignment of human ING1b and ING2 genes and proteins.

A. cDNA sequences alignment of human ING1b and of human ING2a and ING2b. **B.** Amino acid sequences alignment of human ING1b and ING2a and ING2b. All Alignments were carried out using CLUSTAL 2.1 multiple sequence alignment software. Asterisks mean a perfect alignment between each sequence. The PIP is colored in pinked purple; the LZL is colored in green; the PBD is colored in blue; the NCR is colored in purple; the NLS is highlighted in blue; the three NTS are colored in blue; The REASP aminoacid motif is colored in pink; the PHD is colored in red and the PBR is colored in orange.

Figure 3: ING1 and ING2 gatekeeper and caretaker TSG functions.

Human ING1b and ING2a are represented with their interacting proteins to modulate their gatekeeper and caretaker functions. **A.** ING1b gatekeeper functions. ING1b regulate p53 activity through regulation of its acetylation status by interacting with p300 and SIRT1. ING1b regulates also p53 protein stability by inhibiting p53 MDM2-dependent degradation.

In response to genotoxic stress ING1b interacts with the the mSin3a/HDAC1-2/Sap30 complex through its NCR and with H3K4Me3 through its PHD to regulate gene transcription. ING1b caretaker functions. In response to UV ING1b binds GADD45 to promote NER. It also interacts with PCNA to facilitate its monoubiquitinylation. ING1b interaction with PCNA may also promote the switch from DNA replication to DNA repair in response to UV. **B.** ING2a gatekeeper functions. Under stress conditions, ING2a binds the PIP5 through its PBR to promote its accumulation to the chromatin. Then, ING2a interacts through its LZL with the acetylase p300 to support p53 acetylation and the transcription of genes involved in cell cycle arrest, apoptosis and senescence. Stress induced by MNNG treatment regulates ING2a to induce p73α acetylation in order to activate transcription of genes involved in apoptosis. Genotoxic stress regulates ING2a to interact with the mSin3a/HDAC1-2/Sap30 complex and with H3K4Me3 through its PHD to regulate gene transcription. In response to TGF-β signaling, ING2a binds SnoN through its PHD to allow the interaction between SnoN and Smad2 in order to inhibit TGF-β dependent transcription resulting in inhibition of cell proliferation. ING2a caretaker functions. ING2a LZL is involved in NER in answer to UV stress. ING2a can also interact with PCNA to ensure the normal progression of the replication fork and to maintain genome stability. When the domain of interaction with ING1b or ING2a is unknown the protein is represented below the protein scheme. Me and Ac mean respectively methyl group and acetyl group.

Figure 4: ING1 and ING2 tumor suppressor functions.

In normal cell growth or stressed conditions ING1 and ING2 regulate DNA replication and/or DNA repair to maintain genome integrity. Exogenous or physiological stress activates ING1 and ING2 to regulate cell proliferation through the activation of cell cycle arrest, senescence or apoptosis to prevent tumor transformation. Consequently, ING1 or ING2 loss, as it occurs in human tumors triggers loss of its functions. Thus, ING2 loss contributes to disregulation of cell growth and to enhance genome instability. Consequently, ING1 and ING2 act as tumor suppressor genes of type I or “caretakers” as well as type II or “gatekeepers” to prevent tumorigenesis.

Legend to Supplemental

Figure S1: Structure of Human ING1, mouse ING1 and ING2 genes, mRNAs and proteins.

A. ING1 human gene. E1c, E1d, E1a, E1b, E1b$_{alt}$, E2 are respectively Exon 1c, Exon 1d, Exon 1a, Exon 1b, Exon 1b$_{alt}$ and Exon 2. Exons E1d and E1b$_{alt}$ of ING1v2 and ING1v5 are represented in orange. They both code for the same protein ING1c (ING1v2/v5) as their ATG on E2 is the same. **B.** ING1 human mRNAs. **C.** ING1b human protein. All ING1 protein isoforms contain a NCR and a NLS in their central region and a PHD and a PBR in their C-terminal part. ING1b is also composed of a PIP and a PBD in its N-terminal part. **D.** ING1 mouse gene. E1a, E1b, E2 are respectively Exon 1a, 1b, 1c and Exon 2. **E.** ING1 mouse mRNAs. **F.** ING1 mouse proteins. The three mouse ING1 isoforms possess a NCR, a NLS, a PHD and a PBR . In addition, mouse ING1b contains a PIP and a PBD in its N-terminal domain. **G.** ING2 mouse gene. E1a, E1b, E2 are respectively Exon 1a, 1b and Exon 2. **H.** ING2 mouse mRNAs. **I.** ING2 mouse proteins. ING2a contains a LZL in its C terminal part. ING2a possesses a NCR and ING2b is truncated of a part of the NCR domain. Both ING2a and ING2b are composed of a NLS containing three NTS, a PHD zinc finger motif and a PBR. On genes and mRNAs, none coding regions are in yellow and coding region are represented in purple. Each mRNA variant is represented with its name on the left and its Genebank accession number on the right. Each protein is represented with its characterized domains with its name on the right and with its molecular weight, and its Genebank accession number on the left.

Figure S2: Sequences comparison between human and mouse ING1b or ING2 cDNAs and proteins.

A. Human ING1b and mouse ING1 cDNA sequences alignment. **B.** Human ING1b and mouse ING1 protein sequences alignment. **C.** Human and mouse ING2 cDNA sequences alignment. **D.** Human and mouse ING2a and ING2b proteins alignment. For all alignments, the PIP is colored in pinked purple; the LZL amino acid sequence is colored in green the PBD is colored in blue; the NCR is colored in purple; the NLS is highlighted in blue; the three NTS are colored in blue; the REASP aminoacid motif is colored in pink; the PHD is colored in red; the PBR is colored in orange. All alignments were carried out using CLUSTAL 2.1 multiple sequence alignment software.

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Figure 1

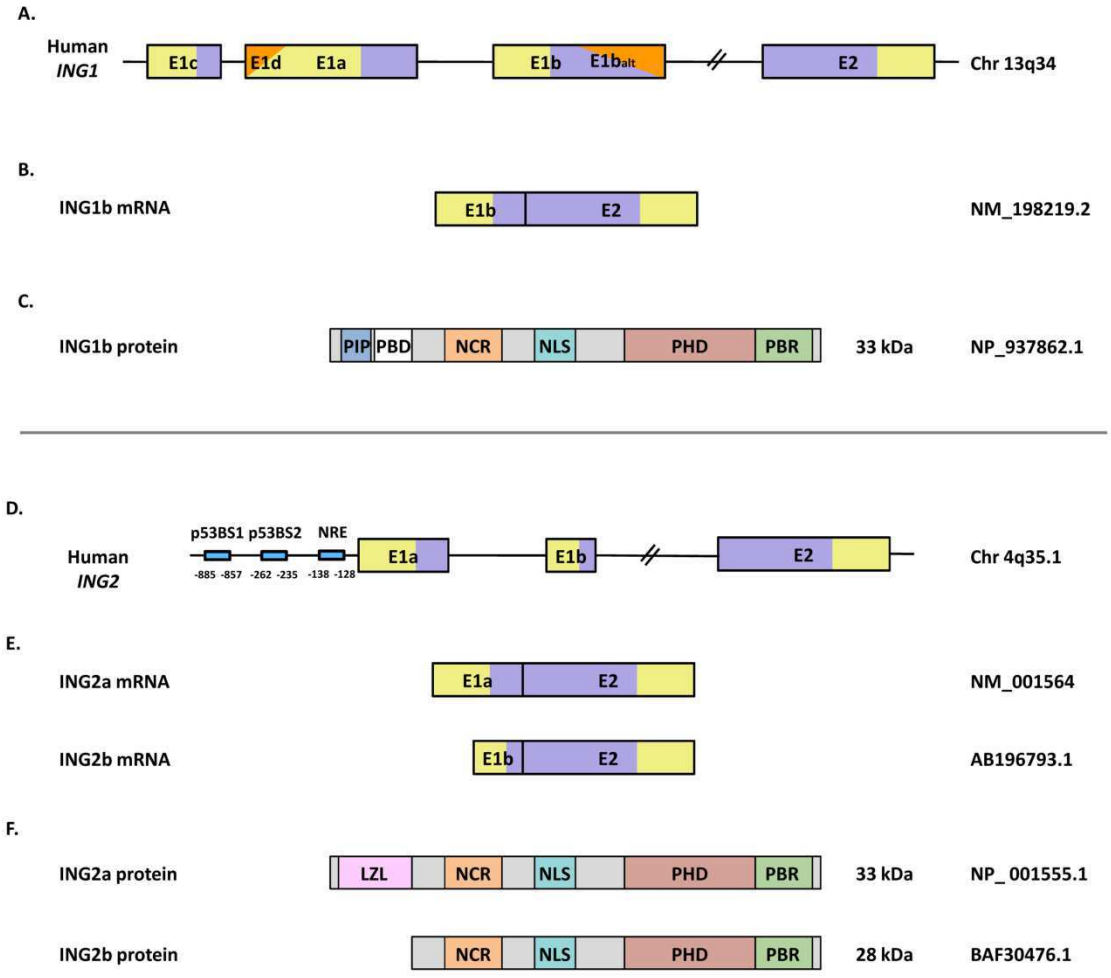
A.
Human
ING1
E1c
E1d
E1a
E1b
E1balt
E2
Chr 13q34
B.
ING1b mRNA
E1b
E2
NM_198219.2
C.
ING1b protein
PIP
PBD
NCR
NLS
PHD
PBR
33 kDa
NP_937862.1
D.
Human
ING2
p53BS1 p53BS2
NRE
-885 -857 -262 -235 -138 -128
E1a
E1b
E2
Chr 4q35.1
E.
ING2a mRNA
E1a
E2
NM_001564
ING2b mRNA
E1b
E2
AB196793.1
F.
ING2a protein
LZL
NCR
NLS
PHD
PBR
33 kDa
NP_ 001555.1
ING2b protein
NCR
NLS
PHD
PBR
28 kDa
BAF30476.1

Figure 2

A.


LZL / PIP
PBD
NCR
NTS
NLS
NTS
NTS
REASP
PHD
PBR
ING1b
ING2a
ING2b

Figure 2

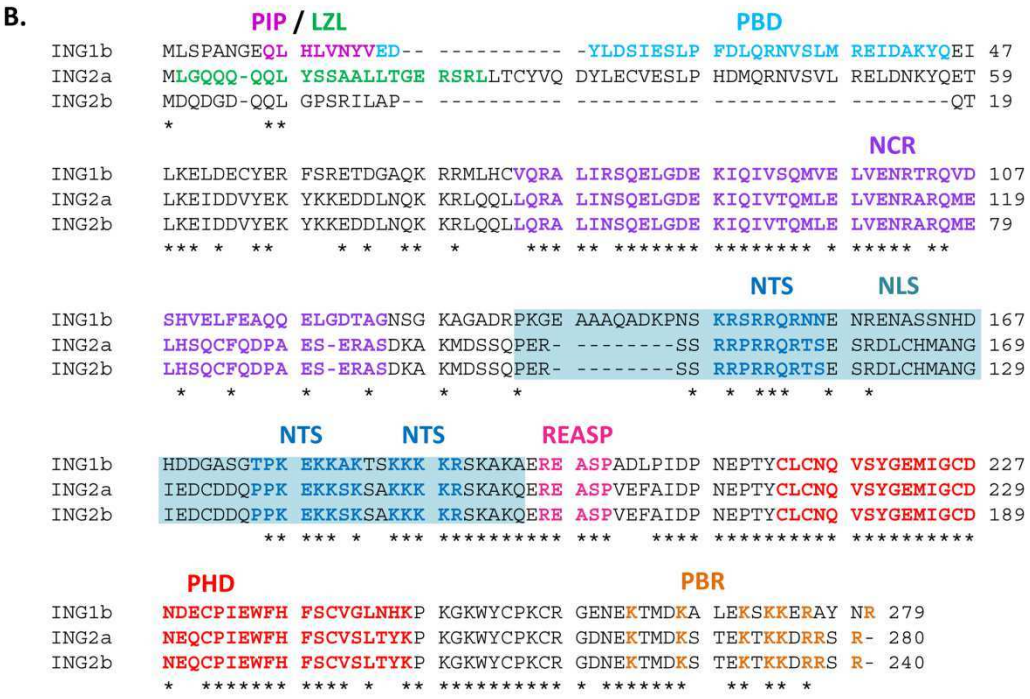
B.
PIP / LZL
PBD
ING1b MLSPANGEQL HLVNYVED-- ---------- -YLDSIESLP FDLQRNVSLM REIDAKYQEI 47
ING2a MLGQQQ-QQL YSSAALLTGE RSRLLTCYVQ DYLECVESLP HDMQRNVSVL RELDNKYQET 59
ING2b MDQDGD-QQL GPSRILAP-- ---------- ---------- ---------- --------QT 19
NCR
ING1b LKELDECYER FSRETDGAQK RRMLHCVQRA LIRSQELGDE KIQIVSQMVE LVENRTRQVD 107
ING2a LKEIDDVYEK YKKEDDLNQK KRLQQLLQRA LINSQELGDE KIQIVTQMLE LVENRARQME 119
ING2b LKEIDDVYEK YKKEDDLNQK KRLQQLLQRA LINSQELGDE KIQIVTQMLE LVENRARQME 79
NTS
NLS
ING1b SHVELFEAQQ ELGDTAGNSG KAGADRPKGE AAAQADKPNS KRSRRQRNNE NRENASSNHD 167
ING2a LHSQCFQDPA ES-ERASDKA KMDSSQPER- -------- --------SS RRPRRQRTSE SRDLCHMANG 169
ING2b LHSQCFQDPA ES-ERASDKA KMDSSQPER- -------- --------SS RRPRRQRTSE SRDLCHMANG 129
NTS
NTS
REASP
ING1b HDDGASGTPK EKKAKTSKKK KRSKAKAERE ASPADLPIDP NEPTYCLCNQ VSYGEMIGCD 227
ING2a IEDCDDQPPK EKKSKSAKKK KRSKAKQERE ASPVEFAIDP NEPTYCLCNQ VSYGEMIGCD 229
ING2b IEDCDDQPPK EKKSKSAKKK KRSKAKQERE ASPVEFAIDP NEPTYCLCNQ VSYGEMIGCD 189
PHD
PBR
ING1b NDECPIEWFH FSCVGLNHKP KGKWYCPKCR GENEKTMDKA LEKSKKERAY NR 279
ING2a NEQCPIEWFH FSCVSLTYKP KGKWYCPKCR GDNEKTMDKS TEKTKKDRRS R- 280
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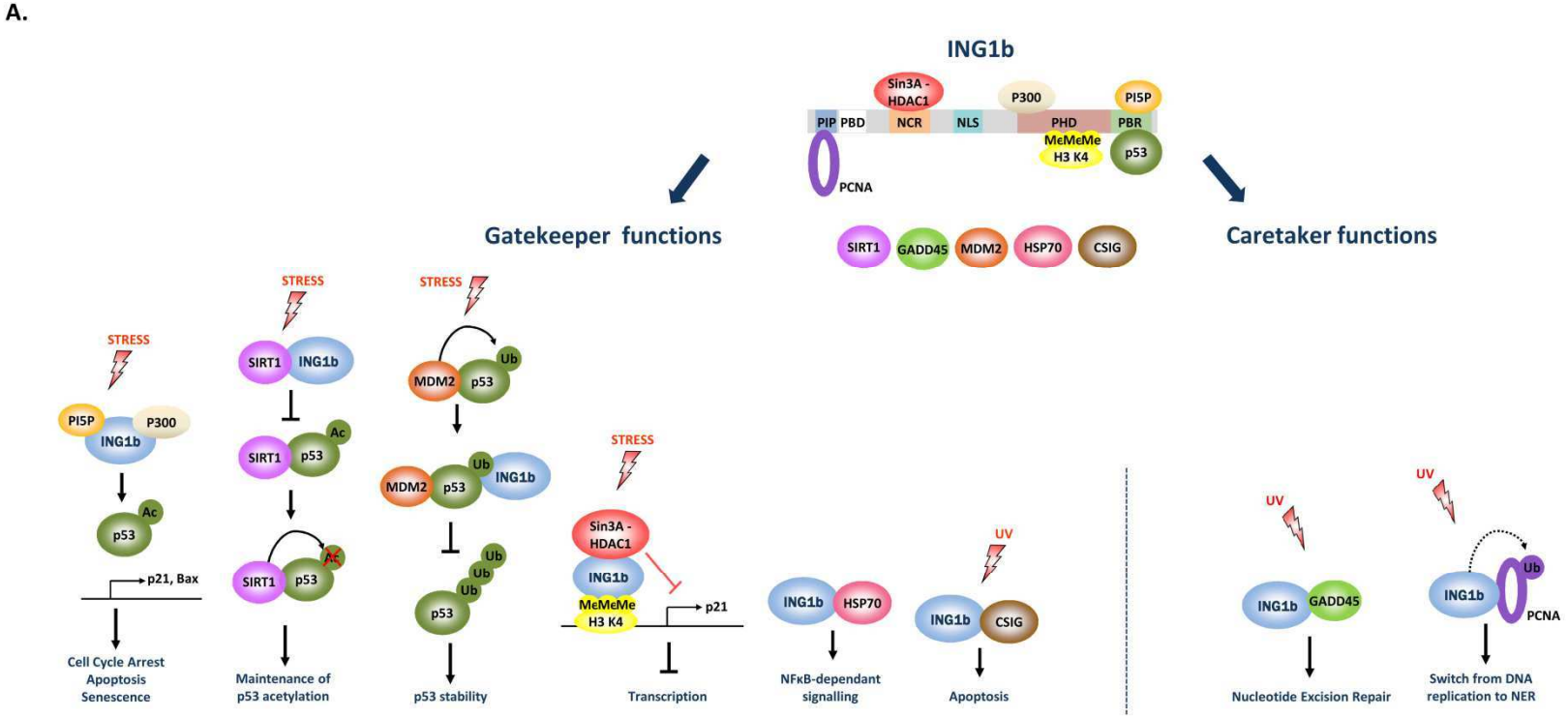
Figure 3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ING1b
Sin3A - HDAC1
P300
PI5P
PIP
PBD
NCR
NLS
PHD
PBR
MeMeMe H3 K4
p53
PCNA
SIRT1
GADD45
MDM2
HSP70
CSIG
Gatekeeper functions
Caretaker functions
STRESS
PI5P
P300
ING1b
p53
Ac
p21, Bax
Cell Cycle Arrest
Apoptosis
Senescence
SIRT1
ING1b
p53
Maintenance of p53 acetylation
MDM2
Ub
p53 stability
Sin3A - HDAC1
MeMeMe H3 K4
p21
Transcription
HSP70
NFκB-dependant signalling
UV
CSIG
Apoptosis
GADD45
Nucleotide Excision Repair
Switch from DNA replication to NER

Figure 3

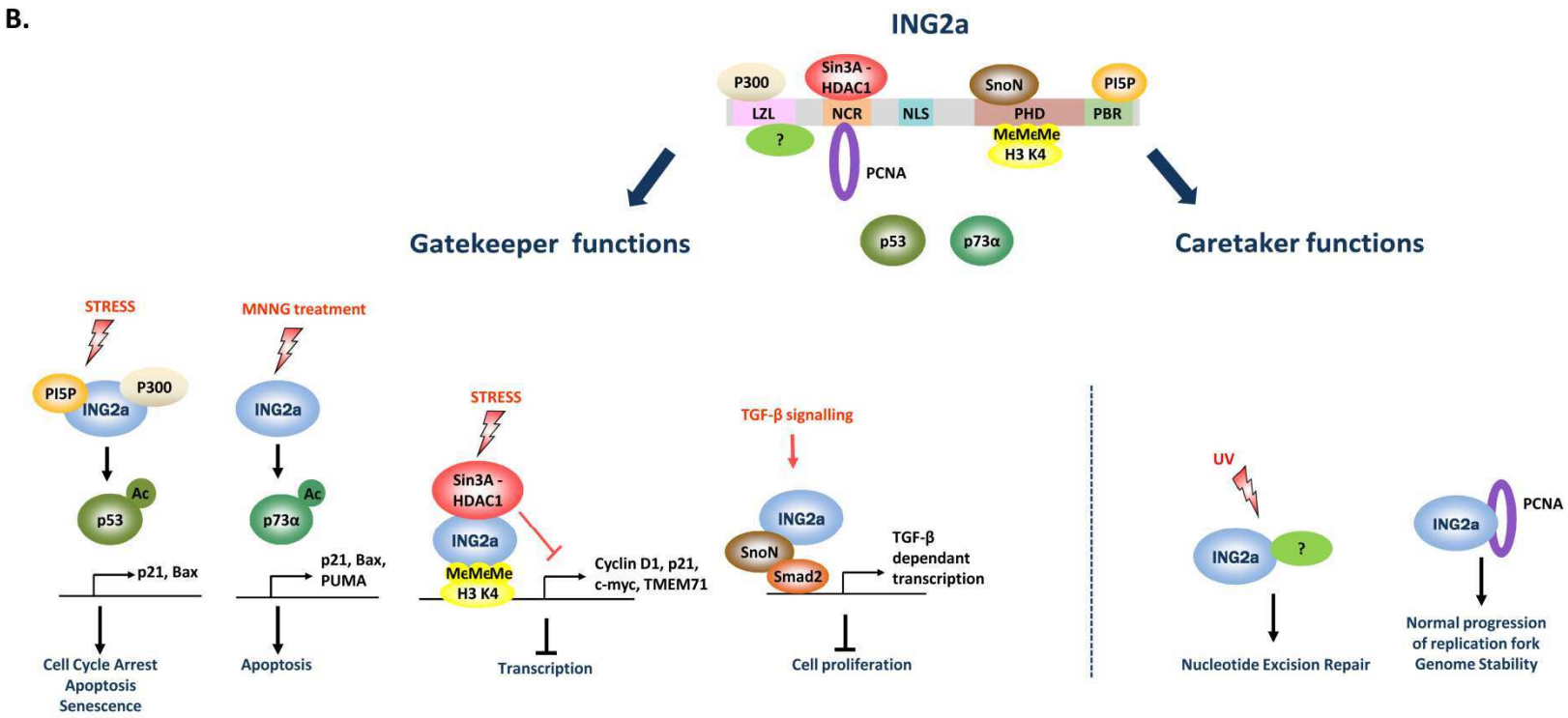

B.
ING2a
P300
Sin3A - HDAC1
SnoN
PI5P
LZL
NCR
NLS
PHD
PBR
?
PCNA
MeMeMe H3 K4
Gatekeeper functions
p53
p73α
Caretaker functions
STRESS
PI5P
P300
ING2a
Ac
p53
p21, Bax
Cell Cycle Arrest
Apoptosis
Senescence
MNNG treatment
ING2a
Ac
p73α
p21, Bax, PUMA
Apoptosis
STRESS
Sin3A - HDAC1
ING2a
MeMeMe H3 K4
Cyclin D1, p21, c-myc, TMEM71
Transcription
TGF-β signalling
ING2a
SnoN
Smad2
TGF-β dependant transcription
Cell proliferation
UV
ING2a
?
Nucleotide Excision Repair
ING2a
PCNA
Normal progression of replication fork
Genome Stability

Figure 4

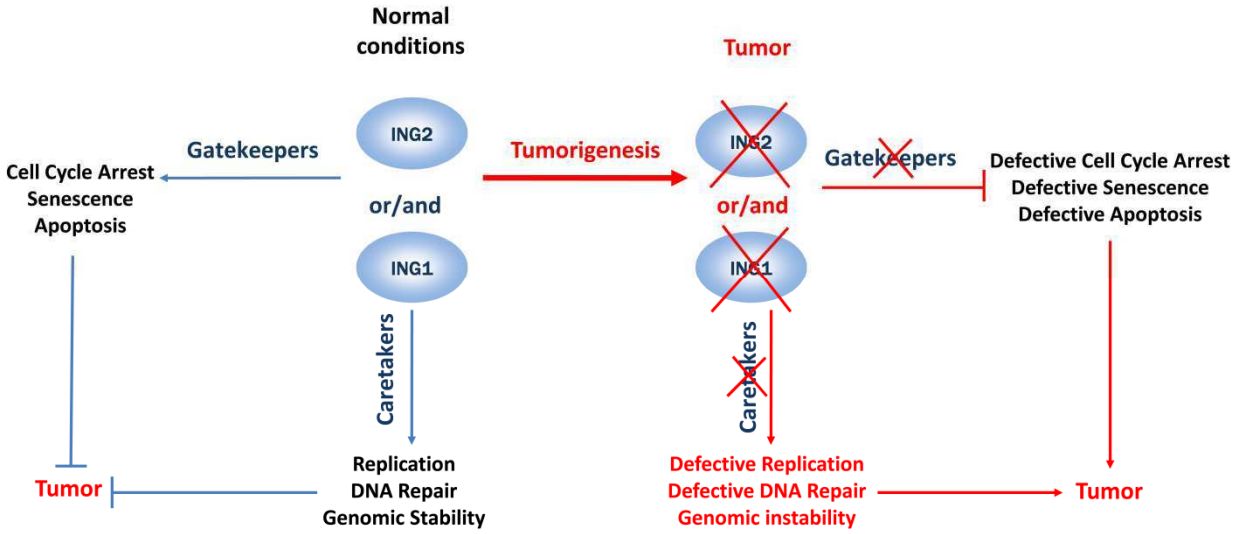

Normal conditions
Tumor
ING2
Gatekeepers
Cell Cycle Arrest
Senescence
Apoptosis
Tumorigenesis
ING2
Gatekeepers
Defective Cell Cycle Arrest
Defective Senescence
Defective Apoptosis
or/and
or/and
ING1
ING1
Caretakers
Caretakers
Tumor
Replication
DNA Repair
Genomic Stability
Defective Replication
Defective DNA Repair
Genomic instability
Tumor

Figure S1

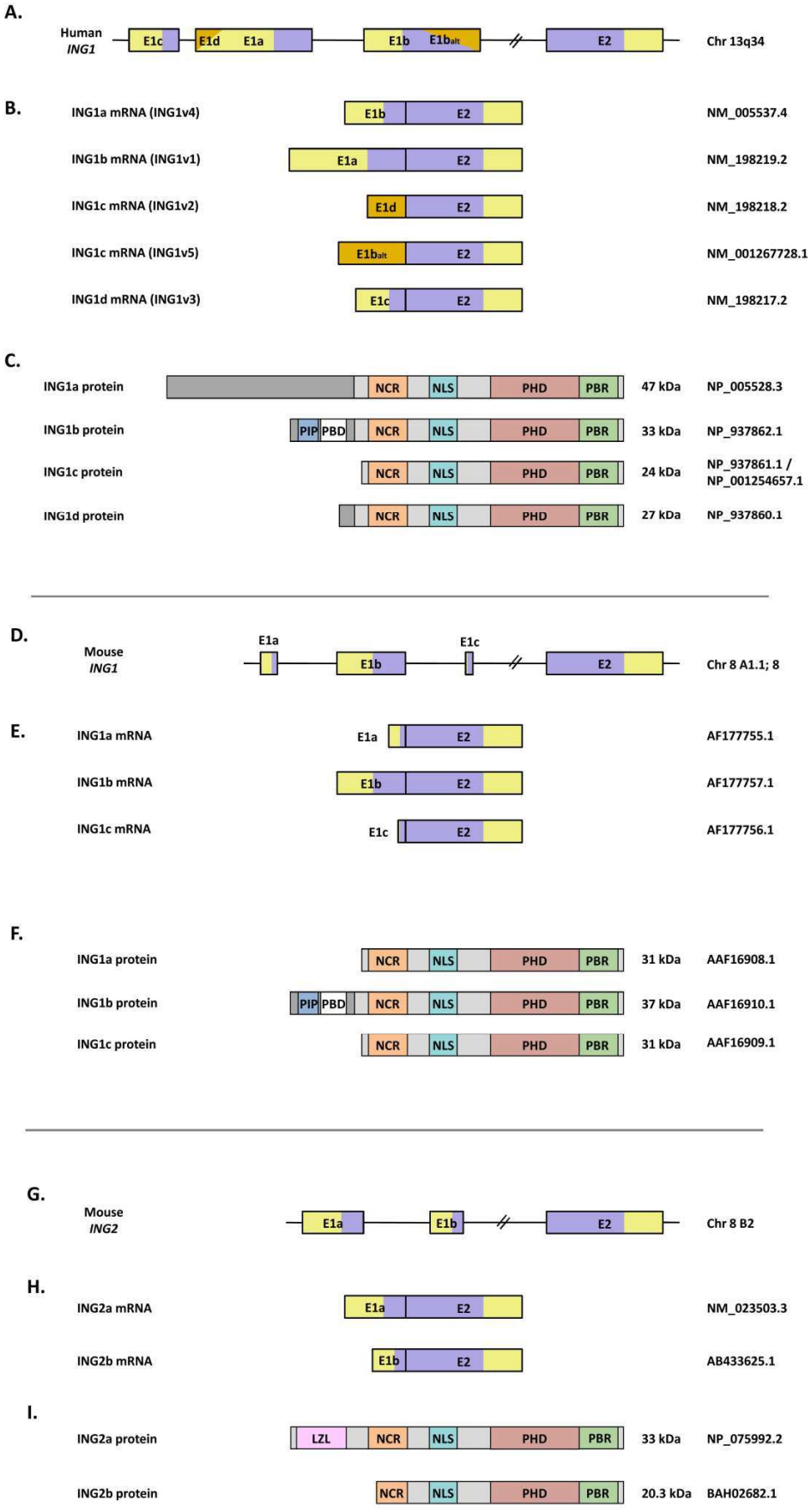

A.
Human ING1
E1c
E1d
E1a
E1b
E1balt
E2
Chr 13q34
B.
ING1a mRNA (ING1v4)
E1b
E2
NM_005537.4
ING1b mRNA (ING1v1)
E1a
E2
NM_198219.2
ING1c mRNA (ING1v2)
E1d
E2
NM_198218.2
ING1c mRNA (ING1v5)
E1balt
E2
NM_001267728.1
ING1d mRNA (ING1v3)
E1c
E2
NM_198217.2
C.
ING1a protein
NCR
NLS
PHD
PBR
47 kDa
NP_005528.3
ING1b protein
PIP
PBD
NCR
NLS
PHD
PBR
33 kDa
NP_937862.1
ING1c protein
NCR
NLS
PHD
PBR
24 kDa
NP_937861.1 / NP_001254657.1
ING1d protein
NCR
NLS
PHD
PBR
27 kDa
NP_937860.1
D.
Mouse ING1
E1a
E1b
E1c
E2
Chr 8 A1.1; 8
E.
ING1a mRNA
E1a
E2
AF177755.1
ING1b mRNA
E1b
E2
AF177757.1
ING1c mRNA
E1c
E2
AF177756.1
F.
ING1a protein
NCR
NLS
PHD
PBR
31 kDa
AAF16908.1
ING1b protein
PIP
PBD
NCR
NLS
PHD
PBR
37 kDa
AAF16910.1
ING1c protein
NCR
NLS
PHD
PBR
31 kDa
AAF16909.1
G.
Mouse ING2
E1a
E1b
E2
Chr 8 B2
H.
ING2a mRNA
E1a
E2
NM_023503.3
ING2b mRNA
E1b
E2
AB433625.1
I.
ING2a protein
LZL
NCR
NLS
PHD
PBR
33 kDa
NP_075992.2
ING2b protein
NCR
NLS
PHD
PBR
20.3 kDa
BAH02682.1

Figure S2

A.

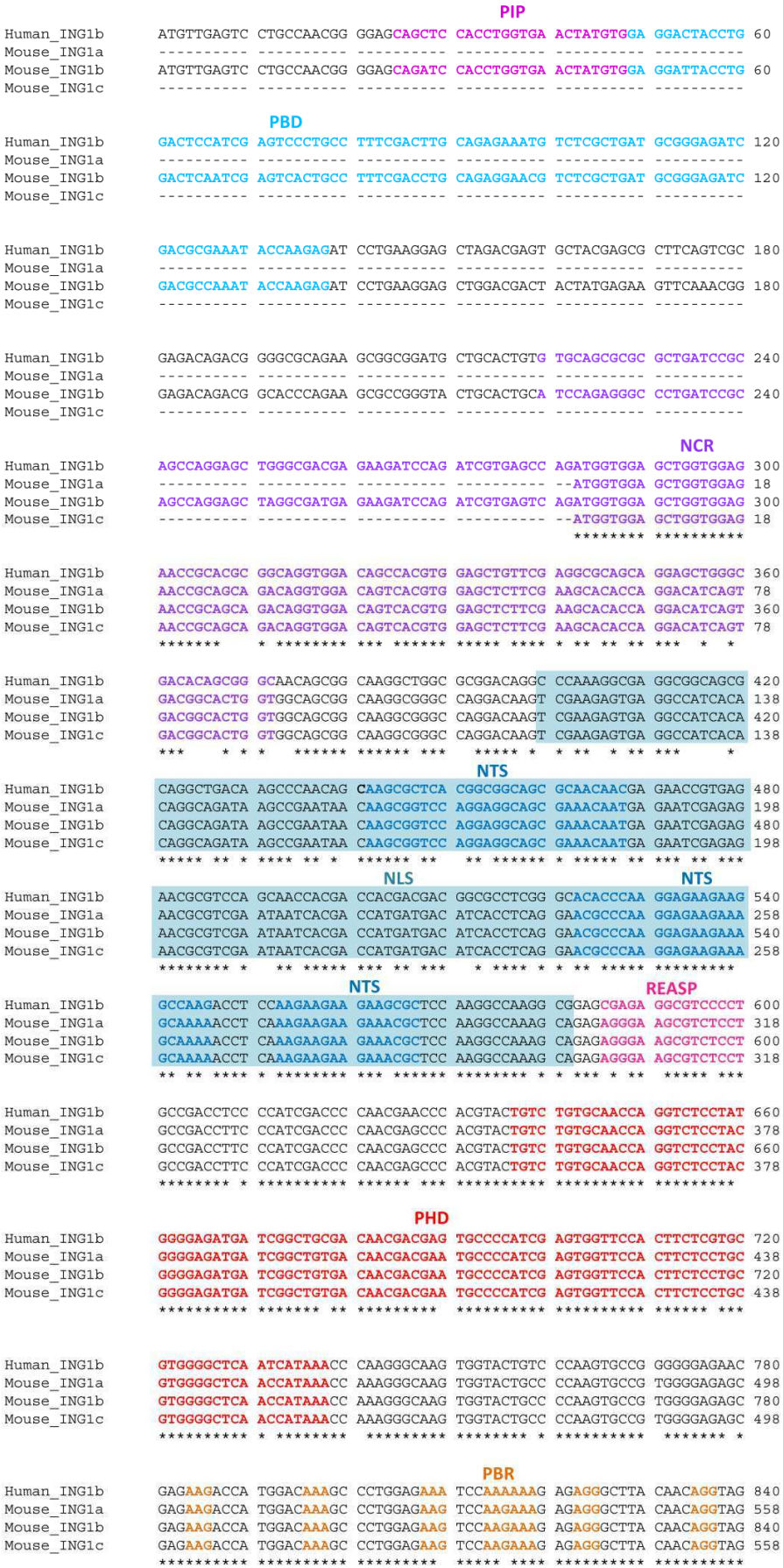
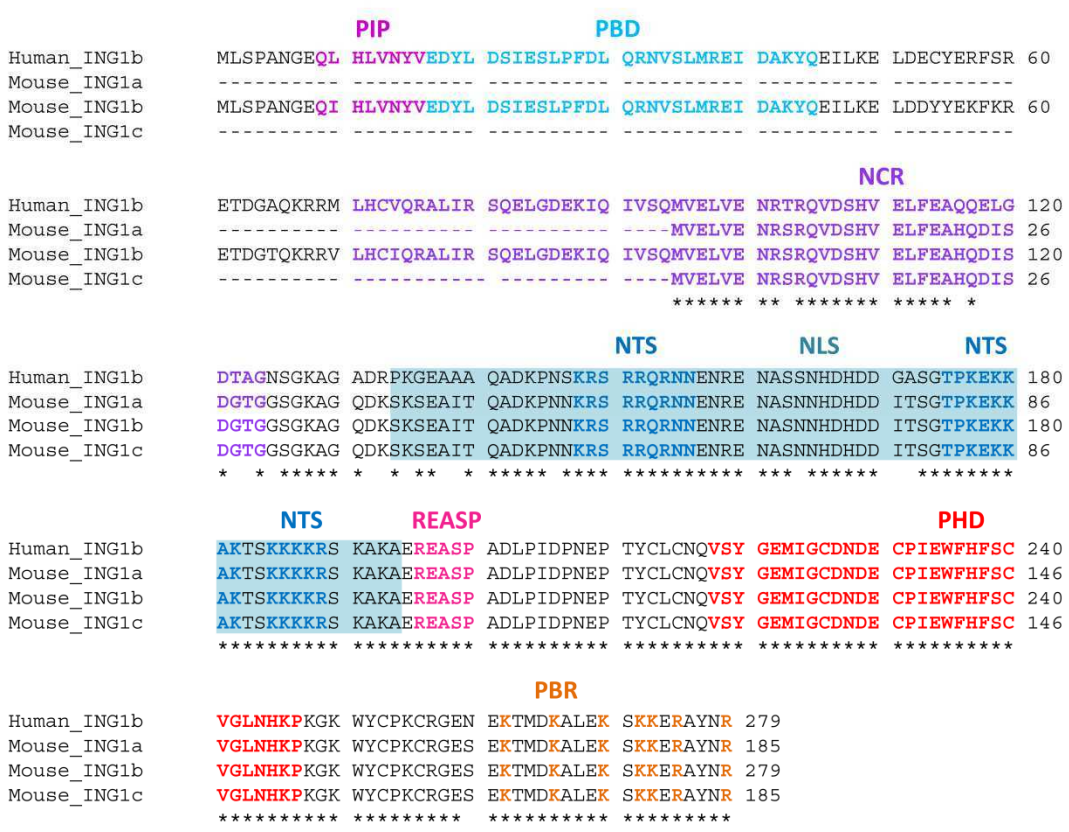


Figure S2

B.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Mouse_ING1a ---------- ---------- ---------- ---------- ---------- ----------
Mouse_ING1b MLSPANGEQI HLVNYVEDYL DSIESLPFDL QRNVSLMREI DAKYQEILKE LDDYYEKFKR 60
Mouse_ING1c ---------- ---------- ---------- ---------- ---------- ----------
NCR
Human_ING1b ETDGAQKRRM LHCVQRALIR SQELGDEKIQ IVSQMVELVE NRTRQVDSHV ELFEAQQELG 120
Mouse_ING1a ---------- ---------- ---------- ----MVELVE NRSRQVDSHV ELFEAHQDIS 26
Mouse_ING1b ETDGTQKRRV LHCIQRALIR SQELGDEKIQ IVSQMVELVE NRSRQVDSHV ELFEAHQDIS 120
Mouse_ING1c ---------- ---------- --------- ----MVELVE NRSRQVDSHV ELFEAHQDIS 26
****** ** ******* ***** *
NTS
NLS
NTS
Human_ING1b DTAGNSGKAG ADRPKGEAAA QADKPNSKRS RRQRNNENRE NASSNHDHDD GASGTPKEKK 180
Mouse_ING1a DGTGGSGKAG QDKSKSEAIT QADKPNNKRS RRQRNNENRE NASNNHDHDD ITSGTPKEKK 86
Mouse_ING1b DGTGGSGKAG QDKSKSEAIT QADKPNNKRS RRQRNNENRE NASNNHDHDD ITSGTPKEKK 180
Mouse_ING1c DGTGGSGKAG QDKSKSEAIT QADKPNNKRS RRQRNNENRE NASNNHDHDD ITSGTPKEKK 86
* * ***** * * ** * ***** **** ********** *** ****** ********
NTS
REASP
PHD
Human_ING1b AKTSKKKKRS KAKAEREASP ADLPIDPNEP TYCLCNQVSY GEMIGCDNDE CPIEWFHFSC 240
Mouse_ING1a AKTSKKKKRS KAKAEREASP ADLPIDPNEP TYCLCNQVSY GEMIGCDNDE CPIEWFHFSC 146
Mouse_ING1b AKTSKKKKRS KAKAEREASP ADLPIDPNEP TYCLCNQVSY GEMIGCDNDE CPIEWFHFSC 240
Mouse_ING1c AKTSKKKKRS KAKAEREASP ADLPIDPNEP TYCLCNQVSY GEMIGCDNDE CPIEWFHFSC 146
********** ********** ********** ********** ********** **********
PBR
Human_ING1b VGLNHKPKGK WYCPKCRGEN EKTMDKALEK SKKERAYNR 279
Mouse_ING1a VGLNHKPKGK WYCPKCRGES EKTMDKALEK SKKERAYNR 185
Mouse_ING1b VGLNHKPKGK WYCPKCRGES EKTMDKALEK SKKERAYNR 279
Mouse_ING1c VGLNHKPKGK WYCPKCRGES EKTMDKALEK SKKERAYNR 185
********** ********* ********** *********

Figure S2

C.

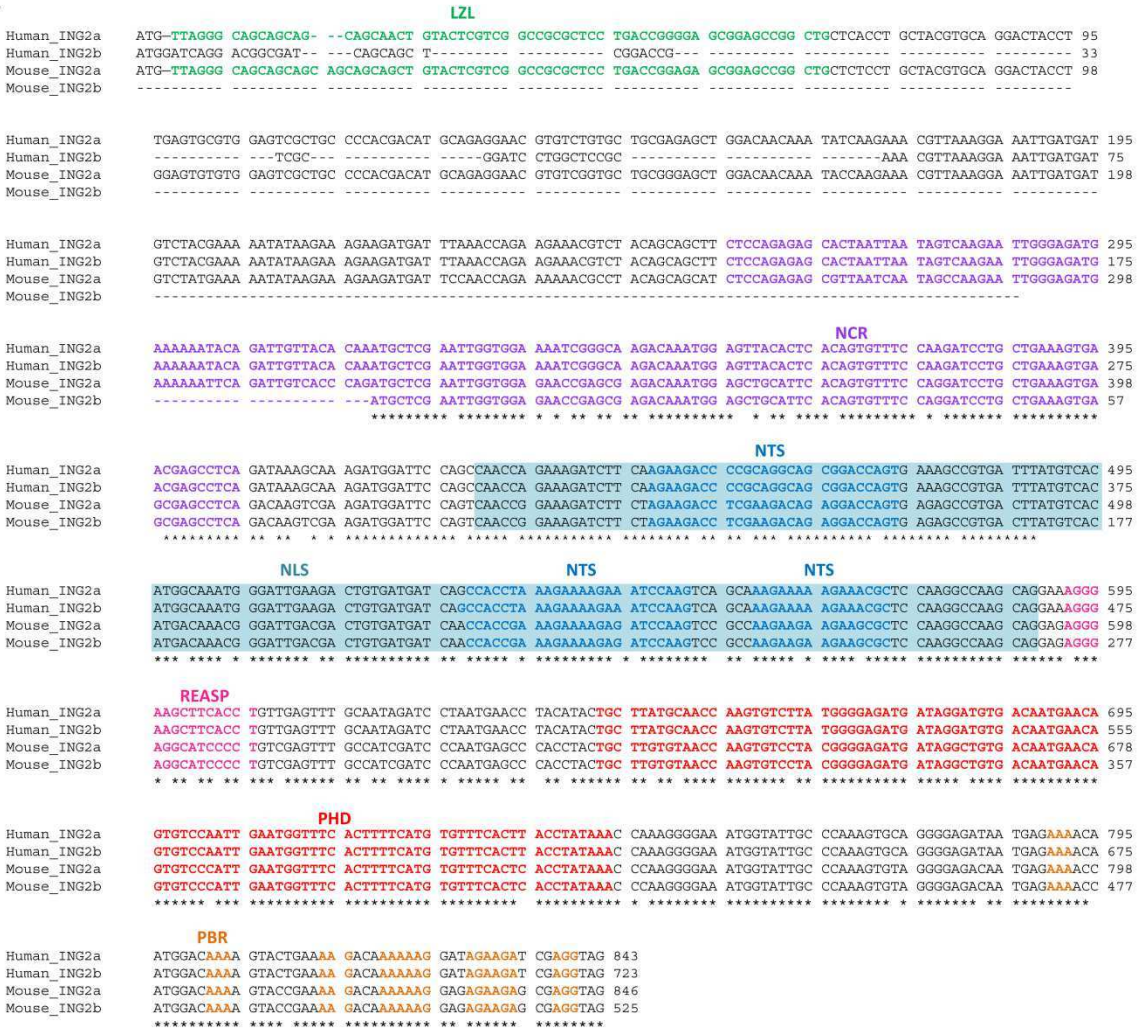
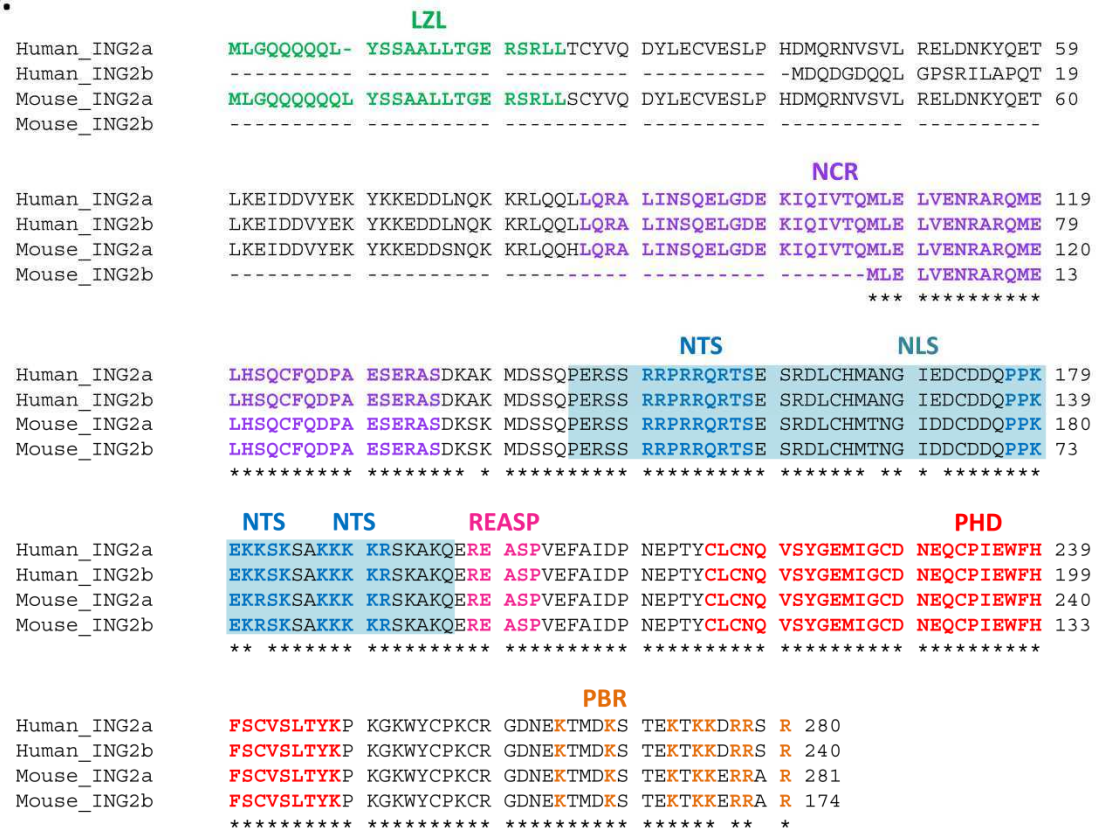


Figure S2

D.


LZL
NCR
NTS
NLS
NTS
NTS
REASP
PHD
PBR
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Human_ING2b ---------- ---------- ---------- ---------- -MDQDGDQQL GPSRILAPQT 19
Mouse_ING2a MLGQQQQQQL YSSAALLTGE RSRLLSCYVQ DYLECVESLP HDMQRNVSVL RELDNKYQET 60
Mouse_ING2b ---------- ---------- ---------- ---------- ---------- ----------
Human_ING2a LKEIDDVYEK YKKEDDLNQK KRLQQLLQRA LINSQELGDE KIQIVTQMLE LVENRARQME 119
Human_ING2b LKEIDDVYEK YKKEDDLNQK KRLQQLLQRA LINSQELGDE KIQIVTQMLE LVENRARQME 79
Mouse_ING2a LKEIDDVYEK YKKEDDSNQK KRLQQHLQRA LINSQELGDE KIQIVTQMLE LVENRARQME 120
Mouse_ING2b ---------- ---------- ---------- ---------- -------MLE LVENRARQME 13
Human_ING2a LHSQCFQDPA ESERASDKAK MDSSQPERSS RRPRRQRTSE SRDLCHMANG IEDCDDQPPK 179
Human_ING2b LHSQCFQDPA ESERASDKAK MDSSQPERSS RRPRRQRTSE SRDLCHMANG IEDCDDQPPK 139
Mouse_ING2a LHSQCFQDPA ESERASDKSK MDSSQPERSS RRPRRQRTSE SRDLCHMTNG IDDCDDQPPK 180
Mouse_ING2b LHSQCFQDPA ESERASDKSK MDSSQPERSS RRPRRQRTSE SRDLCHMTNG IDDCDDQPPK 73
Human_ING2a EKKSKSAKKK KRSKAKQERE ASPVEFAIDP NEPTYCLCNQ VSYGEMIGCD NEQCPIEWFH 239
Human_ING2b EKKSKSAKKK KRSKAKQERE ASPVEFAIDP NEPTYCLCNQ VSYGEMIGCD NEQCPIEWFH 199
Mouse_ING2a EKRSKSAKKK KRSKAKQERE ASPVEFAIDP NEPTYCLCNQ VSYGEMIGCD NEQCPIEWFH 240
Mouse_ING2b EKRSKSAKKK KRSKAKQERE ASPVEFAIDP NEPTYCLCNQ VSYGEMIGCD NEQCPIEWFH 133
Human_ING2a FSCVSLTYKP KGKWYCPKCR GDNEKTMDKS TEKTKKDRRS R 280
Human_ING2b FSCVSLTYKP KGKWYCPKCR GDNEKTMDKS TEKTKKDRRS R 240
Mouse_ING2a FSCVSLTYKP KGKWYCPKCR GDNEKTMDKS TEKTKKERRA R 281
Mouse_ING2b FSCVSLTYKP KGKWYCPKCR GDNEKTMDKS TEKTKKERRA R 174