Lysine demethylases KDM6A and UTY: the X and Y of histone demethylation

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Abstract

Histone demethylases remove transcriptional repressive marks from histones in the nucleus. KDM6A (also known as UTX) is a lysine demethylase which acts on the trimethylated lysine at position 27 in histone 3. The *KDM6A* gene is located on the X chromosome but escapes X inactivation even though it is not located in the pseudoautosomal region. There is a homologue of *KDM6A* on the Y chromosome, known as *UTY*. *UTY* was thought to have lost its demethylase activity and to represent a non-functional remnant of the ancestral *KDM6A* gene. However, results with knockout mice suggest that the gene is expressed and the protein performs some function within the cell. Female mice with homozygous deletion of *Kdm6a* do not survive, but hemizygous males are viable, attributed to the presence of the *Uty* gene. *KDM6A* is mutated in the human condition Kabuki syndrome type 2 (OMIM 300867) and in many cases of cancer. The amino acid sequence of KDM6A has been conserved across animal phyla, although it is only found on the X chromosome in eutherian mammals. In this review, we reanalyse existing data from various sources (protein sequence comparison, evolutionary genetics, transcription factor binding and gene expression analysis) to determine the function, expression and evolution of *KDM6A* and *UTY* and show that *UTY* has a functional role similar to *KDM6A* in metabolism and development.

Key words

Lysine demethylases; KDM6A; UTY; histone modification; X chromosome; Y chromosome

Abbreviations

2-OG, 2-oxoglutarate; CTL, cytotoxic lymphocytes; EMT, epithelial to mesenchymal transition; H3K4, lysine at position 4 in histone 3; H3K27ac, acetylation of lysine at position 27 of histone 3; H3K27me3, trimethylation of lysine at position 27 of histone 3; HAT, histone acetyl transferase; JmjC, jumonji C domain; KDM, histone lysine demethylase; KMT, histone lysine methyltransferase; MSY, male specific Y chromosome region; PRC, polycomb repressive complex; Rb, retinoblastoma; TF, transcription factor; TPM, tags per million; TPR, tetratricopeptide repeat

1. Introduction

The first level of coiling of DNA in the nucleus is controlled by the binding of a complex of histone proteins (the nucleosome) to segments of DNA, which is in turn determined by the presence or absence of specific post-translational modifications to the histone proteins. The presence of nucleosomes on a segment of DNA reduces accessibility of that DNA to RNA polymerase and the DNA is unlikely to be transcribed (reviewed by [1]). Adding or removing histone modifications can determine whether a gene is expressed. A key modification which is associated with nucleosome binding is trimethylation of the lysine at position 27 in histone 3 (H3K27me3). This is a repressive mark, applied by enzymes of the histone methyltransferase family, which restricts transcription [2, 3]. Release of this repressive mark requires the activity of histone demethylases, particularly the enzymes lysine demethylase 6A (KDM6A; also known as ubiquitously transcribed X chromosome tetratricopeptide repeat protein, UTX; OMIM#300128) and lysine demethylase 6B (KDM6B; also known as Jumonji domain containing protein 3, JMJD3; OMIM#611577) [4-6]. These proteins are characterised by the presence of a Jumonji C (JmjC) catalytic domain. The gene encoding a third family member, ubiquitously transcribed Y chromosome tetratricopeptide repeat protein (UTY; also known as KDM6C; OMIM#400009) [7], was thought to be an inactive degenerate form of the *KDM6A* gene with no functional activity but recent studies suggest it retains some residual catalytic function [8, 9] and may also be involved in methylation-independent activities [10], as outlined in **Section 2**.

The *KDM6A* gene is located on the X chromosome in eutherian mammals [11]. Although not located in the pseudoautosomal region, *KDM6A* escapes X-inactivation [12, 13] and its level of expression reflects the number of X chromosomes [14]. Eutherian females have higher levels of this protein and its mRNA than eutherian males [13, 15]. However, eutherian males also carry *UTY* [16], the homologue of *KDM6A* on the Y chromosome, and its expression level correlates with the number of Y chromosomes [14]. X chromosome genes with a Y chromosome paralogue generally have a role in transcription, translation and nucleic acid binding [17] and hence are central to regulation of gene expression during development, immune function, cell proliferation and differentiation and tumorigenesis. Here we review

the function, expression and evolution of the *KDM6A* and *UTY* genes and highlight the functional similarity of the two proteins.

2. Functions of KDM6A and UTY

KDM6A functions both through its demethylase activity [5, 18] and through a structural role which may be mediated by the protein binding capacity of the tetratricopeptide repeats (TPRs) [4, 19] [20] (**Section 3**). These functions can be distinguished through use of catalytically inactive versions of the protein or through the use of demethylase inhibitors. Human UTY has reduced catalytic activity [9] but retains the protein binding capacity of KDM6A.

2.1 H3K27 in gene regulation

Gene expression can be repressed by methyl groups added to the lysine 27 of histone 3 (H3K27) by methyltransferase EZH2 [21], part of polycomb repressive complex 2 (PRC2) [22]. Trimethylation of H3K27 (H3K27me3) is involved in control of developmental genes and also marks the inactive X chromosome [23]. Removal of the methyl groups by histone lysine demethylases allows acetylation of H3K27 (H3K27ac) by histone acetylases such as CREBBP [24] (see **Supplementary Figure 1**), converting the histone to the active state and opening the chromatin for transcription factor binding and RNA polymerase recruitment. H3K27me3 and H3K27ac are thus antagonistic to each other. In contrast, trimethylation of the lysine at position 4 in histone 3 (H3K4me3) is an activating modification [25, 26] while monomethylation of the same lysine is associated with enhancer activity. Methylation of H3K4 is mediated by a COMPASS-like complex [20] in which the methyltransferases KMT2C (also known as MLL3; OMIM#606833) or KMT2D (also known as MLL2, MLL4 and ALR; OMIM#602113) act on H3K4, facilitated by H3K27 demethylation (by KDM6A) and subsequent acetylation. Promoters that contain both H3K27me3 (repressing) and H3K4me3 (activating) are poised for transcription. Demethylation and acetylation of H3K27 then allows transcription to proceed [27, 28]. There may therefore be a dynamic relationship between the demethylase and the methyltransferase in the MLL complex. A model has been proposed where there is coordinated removal of repressive marks, addition of active marks, and displacement PRC1 (which ubiquitinates H2A, another repressive mark) and PRC2 to

give tight regulation of gene expression during differentiation [29]. This potential to radically alter the epigenetic landscape can create new gene expression patterns as cells undergo changes in state through for example differentiation or activation [30].

2.2 Activity of KDM6A

KDM6A is a member of the family of 2-oxoglutarate (2-OG) and Fe(II)-dependent JmjC dioxygenases which function to demethylate histones or nucleic acids (**Section 2.1**) [31-34]. The histone lysine demethylase (KDM) enzymes use 2-OG and molecular oxygen to hydroxylate the methyl group(s) of a methylated lysine within the histone protein, forming an unstable carbinolamine intermediate which decays to release formaldehyde, leaving the lysine with one less methyl group [33, 34] (**Supplementary Figure 1**). KDM6A targets H3K27 and recombinant human KDM6A was able to successively remove each of the three repressive methyl groups from protein mixtures containing histone 3 [35]. It is highly specific for H3K27me3 and does not demethylate other lysines in histone H3 or H4 [32]. KDM6A is also a component of the KMT2C/KMT2D COMPASS-like complex [27, 28]. Recent studies of human HEK293T cells suggest that the participation of KDM6A in the complex relies on demethylase-independent functions [36].

Network analysis has shown that KDM6A interacts with a range of proteins. In one study based on a protein-protein interaction network, interaction partners for KDM6A were found to fall into three categories [37]. The majority of the primary interacting proteins were involved in chromatin modification through histone methylation. These included KMT2D, consistent with the role of KDM6A in the MLL2 complex. The similarity of the roles of KMT2D and KDM6A is highlighted by the very similar phenotypes produced by *KMT2D* and *KDM6A* gene mutations, as discussed further in **Section 5.2.** A second cluster of primary and secondary associated proteins contained transcription factors. The third cluster was of proteins involved in cell cycle regulation, with the key interacting partner being the retinoblastoma like protein RBL2 (also known as p130).

Although the histone demethylase activity of KDM6A takes place in the nucleus, several reports have shown that native KDM6A protein is largely located in the cytoplasm of immortalised human mammary epithelial cells and mouse fibroblasts [38-40]. A single nuclear localisation signal was identified in KDM6A and UTY (RRRK at amino acids 1089-

1092) [38], considered insufficient for efficient transport through the nuclear membrane [38-40]. This suggests that KDM6A may also demethylate or hydroxylate cytoplasmic proteins, or that the noncatalytic protein binding capacity may be important in assembling protein complexes in the cytoplasm.

2.3 KDM6A in development

KDM6A is an important determinant of cell fate and cellular identity during development through its control of pluripotency and lineage specific genes. A major target is the HOX gene family. Reduction of KDM6A level by RNA interference resulted in increased methylation at *HOXD10*, *HOXD11* and *HOXD12* genes in HeLa cells [35]. KDM6A was selectively localised to HOX loci in human primary fibroblasts but in embryonic stem cells, where HOX genes are largely transcriptionally silent, it was excluded from HOX loci [35]. KDM6A binds to the human and mouse *HOXB1* promoter and is required for expression of the *HOXB1* gene [29]. Retinoic acid treatment of human NT2/D1 embryonal carcinoma cells and mouse embryonic stem cells increased the binding of KDM6A to the *HOXB1* promoter, decreased the level of H3K27me3 and increased H3K4me3 [29], all consistent with gene activation. Inhibition of the zebrafish *utx1* (*KDM6A* orthologue) disrupted posterior development [35] and mutation or inhibition of a *Caenorhabditis elegans* orthologue resulted in abnormal gonadal development [29]. During division of stem cells, H3K27me3 is largely absent from cells in S phase and the re-establishment of trimethylation is delayed by histone demethylase activity in embryonic stem cells [41] which may be important in promoting differentiation.

Kdm6a-null mouse embryonic stem cells were unable to differentiate into mesoderm [42, 43] and a number of studies have shown involvement of KDM6A protein in mesenchymal cell differentiation. Knockout of *Kdm6a* in mouse C2C12 cells affected differentiation to osteoblast phenotype [44] and removal of *Kdm6a* in male mouse embryonic stem cells reduced their potential to differentiate towards the adipocyte lineage [45]. In murine bone marrow derived mesenchymal stem cells, *Kdm6a* mRNA reduced during adipogenic differentiation, mediated by microRNA *miR-199a-3p* [46]. Transfection with *Kdm6a* cDNA could alleviate the loss of osteogenic and adipogenic differentiation caused by glucocorticoid treatment of immortalized murine osteogenic progenitor cells [45, 47].

Knockdown of *KDM6A* in human primary periodontal ligament stem cells reduced their ability to differentiate to chondrocytes, through inhibition of expression of *SOX9* [48]. Recently differential methylation and expression of *KDM6A* during human muscle development *in vitro* has been demonstrated [15]. Clearly, KDM6A is an important molecule in the early and late stages of differentiation of mesenchymal cell types. KDM6A has also been identified as a critical regulator for migration of mouse hematopoietic stem cells and zebrafish primordial germ cells [49].

KDM6A controls DNA methylation during oogenesis and early embryonic development in mouse, pigs and cattle [50-52]. Overexpression of *Kdm6a* improved the development of mouse embryos derived by single cell nuclear transfer, possibly through suppression of *Xist*-mediated X inactivation of both X chromosomes [51]. KDM6A also promotes reprogramming of mouse somatic cells to pluripotency [53], which may indicate that it is involved in the reprogramming of the developing embryo. A role in early development and fertility is supported by the observation that variation in *KDM6A* was associated with litter size in goats [54, 55]. Taken together these results suggest that KDM6A has an important role in regulating chromatin during development so that transcription factors can access genes required for specific differentiation pathways.

Consistent with its role in differentiation and development, *KDM6A* also acts as a tumour suppressor gene. Loss of *KDM6A* promotes tumor growth and is associated with aggressive cancer progression in multiple human tumor types including pancreatic cancer and B cell lymphoma [56-59]. KDM6A repressed the ETS (pro-oncogenic) program and maintained the GATA (tumor suppressive) program during mouse myeloid leukemogenesis [10] and inhibited cell invasion and cell growth through retinoblastoma (Rb) activated genes in human A549 and LC2-ad lung cancer cell lines [60]. Overexpression of *KDM6A* in these lung cancer cells inhibited TGFβ-induced epithelial-to-mesenchymal transition (EMT) although knock down of KDM6A to about 40% of wild type did not induce EMT [60]. However demethylation by KDM6A appears necessary for tumor maintenance through activation of the NOTCH pathway [61]. The impact of KDM6A varied between urothelial carcinoma cell lines, dependent in part on the status of KMT2C and KMT2D [62]. The paradoxical roles of KDM6A in both suppressing and supporting oncogenesis have been reviewed recently [20, 63].

2.4 Other roles of KDM6A

Other functions of KDM6A have also been suggested. It may take part in the response of macrophages to bacterial and viral challenge through both methylase dependent and independent mechanisms [64]. The demethylase activity is also required for expansion of natural killer T cells in mice [65]. An association with blood glucose regulation is suggested by the hyperinsulinemia observed in some patients with haploinsufficiency of *KDM6A* [66, 67]. This association is supported by the observation that KDM6A activity is inhibited by the antidiabetic biguanide metformin (which binds to the demethylase catalytic region) [68]. In addition, *KDM6A* expression was correlated with circulating HDL-cholesterol levels and silencing of KDM6A in a cell lysate reduced HDL-cholesterol, APOA1 and LIPC levels [53, 69, 70]. Females, with two copies of *KDM6A*, are more sensitive to insulin than males and the expression of *KDM6A* in the liver of obese human females was higher than in obese males [69]. One study suggests that KDM6A controls adipogenesis through regulation of c-Myc at specific differentiation stages (**Section 2.3**) [45]. Thus, KDM6A has diverse functions during normal and abnormal development across vertebrate species.

2.5 Functions of UTY

Like KDM6A, UTY is a 2-OG- and Fe(II)-dependent oxygenase [31, 32]. The human UTY protein has lower enzymatic activity than KDM6A, but recent studies suggest it may have some residual function [9] although it is not clear whether this is sufficient to contribute physiologically to H3K27 demethylation. Homozygous deletion of *Kdm6a* (*Kdm6a* $^{-/-}$) in female mice resulted in fully penetrant embryonic lethality [8]. *Kdm6a*$^{-/-}$ embryos at E10.5 stage displayed severe deformities of neural tube, yolk sac and heart, and their entire development was delayed [11]. These embryos were dead and reabsorbed by stage E12.5. However, male embryos (*Kdm6a*$^{-}$/*Uty*$^{+}$) developed to term and there was a subset (~25%) who survived to adulthood. These male mice were smaller than wildtype, with reduced lifespan, but they were fertile and viable. This disparity between male and female knockout animals was thought to be due to compensation for the absence of *Kdm6a* by *Uty* [11]. Further experiments generated *Kdm6a*$^{-}$/*Uty*$^{-}$ male mice, which shared the fate of homozygous *Kdm6a*$^{-/-}$ female mice and died *in utero* with heart deformities.

The mechanism by which UTY compensates for loss of KDM6A is likely to be largely independent of the demethylase activity, since the activity of UTY is low [8, 9]. In addition, UTY can produce a similar phenotype to catalytically inactive KDM6A [10]. Like KDM6A, UTY binds to the *Brachyury* gene promoter, suggesting one route by which it can influence differentiation. Embryonic stem cells from male *Kdm6a*$^{-}$/*Uty*$^{+}$ mice showed some potential to differentiate into mesoderm, unlike cells from female *Kdm6a*$^{-/-}$ mice [45]. While this was attributed to residual KDM6A activity in the male mice [45], it seems likely that UTY also contributed to the effect since no KDM6A protein was detected in the *Kdm6a*$^{-}$/*Uty*$^{+}$ embryonic stem cells.

A number of studies of human and mouse cancer models have also shown that UTY can compensate in some way for the absence of KDM6A. During myeloid leukemogenesis, KDM6A repressed the ETS oncogenic transcriptional program and activated the GATA tumour suppressive program [10]. Removal of KDM6A reversed this effect but it could be rescued by either catalytically inactive KDM6A or by UTY [10]. Similarly in a mouse lymphoma model, male mice lacking a functional *Kdm6a* gene but retaining *Uty* survived longer than females with both *Kdm6a* genes knocked out. However these males had poorer survival than heterozygous females, indicating that UTY is not as effective as KDM6A as a tumour suppressor [58]. The reduced catalytic activity of UTY was not sufficient to promote natural killer T cell development in mice [65]. Experiments where phenotypic and chromosomal sex were uncoupled in mice showed that the Y chromosome contributed to protection from bladder cancer, consistent with the observation that loss of the Y chromosome in bladder cancer is associated with a worse prognosis [71]. UTY is also implicated in prostate cancer and is part of a network that controls prostate differentiation initiated by NKX3.1 signaling to EHMT2 (also known as G9a) which then binds to the promoter of *UTY* initiating transcription of prostate specific genes and suppression of non-prostate genes [72].

These results indicate that UTY performs some function [11] and has tumour suppressor activity [10]. Human UTY has a low level of H3K27me3 demethylase activity *in vitro* [9]. UTY might be partially functionally redundant with KDM6A at least in some cells and tissues. However, it is not certain whether this depends on its demethylase activity or its protein-protein interactions.

3. Evolutionary analysis of H3K27me3 demethylases KDM6A and UTY

The three H3K27 demethylases contain a JmjC domain near the C terminus (**Figure 1A**). The JmjC domain is found in a large family of proteins present across vertebrates and invertebrates. The KDM proteins form a subgroup of the enzyme family of 2-OG- and Fe(II)-dependent oxygenases which regulate transcription and/or chromatin structure, many through histone demethylation (**Section 2**) [18]. KDM6A and UTY also carry a number of tetratricopeptide repeats (TPRs) (**Figure 1A**) which are important for protein-protein interactions and the assembly of multiprotein complexes [4, 19]. The pervasiveness of the JmjC domain across animal phyla indicates that this evolutionarily conserved sequence has an important role in animal biology.

3.1 Comparison of KDM6A and UTY genes, transcripts and proteins

A number of different transcripts have been reported for the *KDM6A* and *UTY* genes, arising from alternative splicing. The *KDM6A* gene gives rise to 14 different splice variants with 10 predicted protein-coding transcripts (**Table 1**; **Figure 1B**). These range from 671 bp to 5924 bp (Ensembl Browser). Some of the shorter splice variants do not code for the KDM6A JmjC or TPR functional domains (for example transcripts 205, 203, 206 and 214; **Table 1**). Mouse *Kdm6a* shows 10 transcripts (six potentially protein coding). Similarly to *KDM6A*, *UTY* has 16 possible transcripts the in the Ensembl database, and 13 of them are potentially protein-coding (**Table 1, Figure 1B**), with various transcript and peptide lengths. Some splice variants do not contain the JmjC domain or full-length TPR domains. One study [73] detected 284 alternative transcripts for *UTY*, but these findings have not been validated by other reports. Mouse *Uty* produced 12 transcripts (eight protein coding).

To assess the promoter architecture of human *KDM6A*, we obtained data from the FANTOM5 Browser [74] which includes results from over 1,800 tissues and cells. This identified a single transcription start site (p1@KDM6A), and no other alternative promoters were detected or have been described (**Figure 1A**). In mouse, four transcription start sites were identified; three are within 100 bp of each other and would not alter the translated region and the fourth is 400 bp away at the beginning of the first coding exon. The FANTOM 5 database does not show any alternative human *UTY* transcription start sites to the main

promoter named p1@UTY (**Figure 1B**). Mouse *Uty* shows three transcription start sites within 150 bp and likely representing a single promoter. Therefore it seems diversity is generated for both human and mouse UTY and KDM6A through alternative splicing rather than differential promoter usage.

The annotated human *KDM6A* and *UTY* transcripts shows up to 88% cDNA homology and 86% predicted amino acid homology (**Figure 2**). The third member of the family, *KDM6B*, shows only 29% cDNA homology with both *KDM6A* and *UTY* cDNA, and even less for the protein, although all three proteins contain a JmjC domain, which is conserved across all members of the KDM family [75]. The sequences of the KDM6A and UTY TPR domains are very similar. The catalytic residues in the JmjC domains are also conserved with only five amino acid differences. The reduction in UTY demethylase activity compared to KDM6A is thought to be due to a glutamate to serine substitution in the JmjC domain (green rectangle in **Figure 2**) [9]. The linker sequence between the TPR and JmjC domains is not as highly conserved as the functional domains (not shown). Since a number of the putative protein-coding transcripts for both UTY and KDM6A would not include the UTY or KDM6A functional domains, it is not clear whether these incomplete peptides are able to carry out the functional roles of these two enzymes or whether they may have a regulatory role.

3.2. Evolutionary analysis of *KDM6A*

According to the Ensembl database, the JmjC domain is present throughout living species (see also [18]). *Saccharomyces cerevisiae* (yeast) has two genes with the TPR domains (*CYC8* and *YNL313C*). In *Caenorhabditis elegans* (nematode) four genes (*jmjd3.1*, *jmjd3.2*, *jmjd3.3*, *utx-1*) have both a section of TPR repeats and a JmjC domain. *Drosophila melanogaster* (fruitfly) has a single *Utx* gene with four TPR repeats and a JmjC domain. The presence of these sequences in species so phylogenetically distant from the mammals indicates that they represent ancient functions that have been maintained through evolution.

The human *KDM6A* gene has 111 orthologs in different species. As might be expected, placental mammal homologues are most similar to the human gene; as the evolutionary distance of other species from humans increases, the available annotations become less clear and the homology decreases. The Gene Tree generated by Ensembl is shown in

Supplementary Figure 2. As with the human and mouse, the annotated *KDM6A* genes from other species including non-mammalian species show multiple splice variants.

KDM6A is located on human X chromosome in band Xp11.3 and at a syntenic region of the X chromosome in other eutherian mammals, whereas it is located on an autosome (chromosome 4) in opossum (a marsupial) and is predicted to be located on chromosome 18 in platypus (a monotreme with five different X chromosomes, none homologous to the human X) [76]. In birds and fish, where the female is heterogametic (ZW) and the male is homogametic (ZZ), *KDM6A* is also located on autosomes. In these species there is a block of synteny with the human X chromosome extending to one side of *KDM6A*, but genes on the other side map to human chromosome 21. This would suggest that the *KDM6A* gene became associated with the sex chromosome with a block of other genes, somewhere at the time when the common ancestor for all eutherian mammals evolved. Therefore, only eutherian males are hemizygous for *KDM6A*, and might be expected to have a functional *UTY* to compensate for the reduced dosage, as *KDM6A* escapes X inactivation in human females [12].

3.3 UTY and the Y chromosome

Despite recent advances in high throughput sequencing of genomes of various organisms, the Y chromosome sequence has been frequently overlooked. Most genome releases in Ensembl did not include the Y chromosome, probably due to the small size, presumed low gene content and a large number of repetitive sequences which hinder scaffold assemblies [77]. In the current Ensembl release, only 18 species have an annotated *SRY* gene, the key functional gene on the Y chromosome which is responsible for the initiation of male phenotype. This indicates that Y chromosome sequences are underrepresented in the current genome assemblies. Human *UTY* is listed as having 54 orthologs but the majority of these map to the X chromosome in eutherian mammals and to autosomes in other species, and represent instances of *KDM6A* (see **Supplementary Figure 2**). Ensembl contains annotated *UTY* genes for human and other primates, mouse, goat, pig, amur tiger and cow, which all seem to be in syntenic regions of the Y chromosome. There are also probable *UTY* genes for donkey, polar bear, Damara mole rat and red fox, as shown in **Supplementary Figure 2** and a UTY gene has been reported for the yak (a genome that is not in Ensembl)

353 [78]. From the limited reports of Y chromosome sequencing it appears that *UTY* is one of the
354 few Y chromosome genes that is present as a functional gene in all eutherian species
355 studied so far [77]. In addition, multiple alternatively spliced transcripts have been seen for
356 all *UTY* genes.

357 The original placental and marsupial (therian) Y chromosome, containing the sex-
358 determining gene *SRY*, emerged approximately 180 million years ago [79]. *UTY* is located
359 together with other Y-chromosome genes such as *ZFY*, *USP9Y*, *DDX3Y* and *TMSB4Y* [79] in a
360 Y chromosome region that stopped recombining with the X approximately 100 million years
361 ago [80]. Human *UTY* lies right next to two other genes with X chromosome equivalents,
362 *USP9Y* and *DDX3Y*, and a non-coding element *TTTY15*. *KDM6A* also neighbours the
363 equivalent genes on the X chromosome, *USP9X* and *DDX3X*, although not as closely.

364 *UTY* has remained in a male-specific Y chromosome region (MSY), with *USP9Y* and *DDX3Y*,
365 throughout eutherian Y chromosome evolution despite rampant rearrangements of the Y
366 chromosome [77]. The high microsyntenic conservation of this cluster of genes
367 *USP9Y+DDX3Y+UTY* suggests that they might be co-regulated, possibly by the 'testis-specific'
368 non-coding element *TTTY15* [77] which was identified as an enhancer in the FANTOM5
369 study [78]. In the extensive FANTOM5 database strong expression of these genes was found
370 in cells of the hematopoietic lineage, primarily in T and B lymphocytes. Expression of *USP9Y*
371 was low, with a maximum of 30 tags per million (TPM [81]) in the ARPE retinal epithelium
372 line, while *UTY* and *DDX3Y* had their highest expression in eosinophils and T cells
373 respectively (**Table 2**). *TTTY15* was highly expressed in ARPE cells and lymphocytes, allowing
374 the possibility that it regulates the other two genes in these tissues. Network analysis based
375 on the FANTOM5 data for more than 1,000 human tissues, cancers and cell lines showed
376 that *KDM6A*, *UTY*, *DDX3Y* and *USP9X* have similar expression patterns while *USP9Y* and
377 *TTTY15* are similar to each other in their expression patterns.

378 Positive directional selection on some codons within primate *UTY* and *USP9Y* genes has
379 been detected [82], suggesting that advantageous changes may have occurred during the
380 evolution of these genes [83]. Their X chromosome homologues are under a strong
381 purifying (negative) selection [82], indicating that variants which would have deleterious
382 impact on fitness are being purged by selection. This means that any *KDM6A* missense

mutation is likely to negatively affect the cell, but *UTY* diversity may have been encouraged during evolution and may compensate for the accumulation of deleterious mutations in the non-recombining Y chromosome [82]. The changes in UTY might be beneficial with a male-specific function. It is not clear whether the reduction/loss of the demethylase/hydroxylase function of UTY was driven by positive selection or was a consequence of evolutionary processes acting on the degenerating Y chromosome. Nevertheless, it seems that UTY with lower or no demethylase activity is sufficient to rescue the embryonically lethal *Kdm6a*$^{-/-}$ genotype and substitute for KDM6A at least to some extent.

4. Co-regulation of *UTY* and *KDM6A*

To explore further the functional impact of the similarity of the UTY and KDM6A proteins, the FANTOM5 human dataset was analysed to determine whether or not the genes were expressed at the same time in the same tissues (which would suggest that they are controlled by the same regulators) or with opposite expression patterns (suggesting that there is a mechanism to regulate the combined level of the mRNA/proteins within a cell). An initial survey showed that many samples did not express *UTY*. However, all testes and prostate samples did express *UTY*, suggesting that those where *UTY* was not detected were from female donors. This was validated where possible using the sample metadata (http://fantom.gsc.riken.jp/5/sstar/Main_Page). *KDM6A* and *UTY* expression levels (TPM) were strongly positively correlated across the data set of male samples (Pearson's correlation r = 0.720, N = 517; **Figure 3A**). These results show that *UTY* probably does not compensate for low *KDM6A* expression, since when there was high *UTY* expression, there was also high *KDM6A* expression, consistent with co-regulation of the two genes across all cell types, as previously reported for mouse brain [13].

A strong correlation was found in the subset of samples from tissues and primary cell lines (r = 0.728, N = 439; **Figure 3B**). The association was much weaker when only cancerous samples were included (r = 0.322, N = 78; **Figure 3C**), consistent with the high level of mutation of *KDM6A* (and to some extent *UTY*) in cancer [20] and suggesting that some of these mutations affect the regulatory motifs controlling the binding of transcription factors. *KDM6B* expression did not have a high correlation with *UTY* or *KDM6A* expression (r = 0.469,

N = 1829 with *KDM6A* and r = 0.261, N = 517 with *UTY*), indicating that it is regulated independently and has a distinct expression pattern compared with *KDM6A* and *UTY*.

To understand the potential co-regulation of *UTY* and *KDM6A*, an analysis of transcription factor (TF) motifs was performed, using Harmonizome (https://amp.pharm.mssm.edu/Harmonizome/) [84], a relational database of functional associations between genes and proteins, and their attributes. Different predicted TF binding sites for *KDM6A*, *UTY* and *KDM6B* were identified. Several databases for TF binding sites were used (TRANSFAC, JASPAR, CHEA and ENCODE [85-90]). TRANSFAC and JASPAR predict TF binding using known binding site motifs, whereas CHEA and ENCODE use ChIP-seq data. The TRANSFAC dataset also provided curated data, which were manually selected from low-throughput or high-throughput TF functional studies. A number of TF binding sites appeared common to *KDM6A* and *UTY*, especially in the TRANSFAC curated dataset, where all 10 *UTY* TF were shared by *KDM6A*. Fewer TF sites were shared between *KDM6A* and *KDM6B* or *UTY* and *KDM6B*. In the ChIP-seq based data the *UTY* promoter did not have as many TF binding sites as the other two. In both CHEA and ENCODE datasets *KDM6A* and *KDM6B* had a number of TF binding sites in common, unlike *UTY*. The ENCODE dataset also showed 32 different TF sites which were common to all three gene promoters. A summary of these results is presented in **Supplementary Figure 3**.

The pathway commons protein-protein interactions database in Harmonizome showed that UTY and KDM6A interact with each other physically. KDM6A and UTY also shared a number of common protein interaction partners including components of the H3K4 methylation complex such as KMT2B, KMT2C and RBBP5 (**Supplementary Figure 4**) suggesting that UTY may perform demethylation functions. The NURSA Protein Complexes dataset of Harmonizome showed that UTY binds to the same protein complexes as KDM6A. It is not clear whether UTY binds to this complex independently or only in the presence of KDM6A. It may have a catalytically or structurally autonomous function and could therefore target different substrates. Further investigation into protein-protein interactions with KDM6A should prove interesting.

Although UTY may serve a separate male specific function, for example in testes, it appears to be expressed in a wide range of cell types (as shown in the BioGPS dataset and FANTOM5

data). The findings presented in this section suggest that in general *KDM6A* and *UTY* have shared regulation, which may allow for survival of males by compensating for the haploinsufficiency of *KDM6A*. In contrast, *KDM6B* appears to have very different regulation and showed little redundancy with *KDM6A* and *UTY*, indicating that this protein likely has an independent role.

5. Clinical significance of KDM6A and UTY

X chromosome genes with a Y chromosome homologue (many of them coding for chromatin-modifying enzymes including KDM6A) are needed for proper gene regulation and are potentially sensitive to altered dosage [17]. In particular, X chromosome genes that escape X inactivation may be subject to a dose response which leaves males haploinsufficient, unless the Y homologue has similar activity. Abnormal modification of histone proteins has been associated with multiple diseases in humans and animal models [91]. This means that a range of clinical conditions are likely to be associated with abnormalities of *KDM6A*. The analysis of *KDM6A* and *UTY* gene expression (**Section 4**) suggests that the two genes are co-regulated and that *UTY* might compensate in males for the single copy of *KDM6A*.

5.1. KDM6A and UTY in cancer

KDM6A has been identified as a tumour suppressor gene (**Section 2.3**). Consistent with this, *KDM6A* mutation is common in a range of hematological and non-hematological malignancies [10, 92, 93], although the target genes and impact vary according to tissue (reviewed by [94]). It is frequently mutated in pediatric cancers [95]. In leukemias, mutations have been detected both within and outside the catalytic domain (reviewed in [96]). In mice *Kdm6a* loss constitutes a preleukemic state [10]. In contrast, *KDM6A* mutation was only found at relapse in human acute myeloid leukemia [97] and its loss enhanced resistance to cytarabine treatment. *UTY* mutation was found at relapse in one case [97]. Low *KDM6A* expression at diagnosis also correlated with poorer clinical outcome [97]. In non-invasive bladder cancer *KDM6A* mutation was common and could affect the catalytic or non-catalytic domains of the protein [98]. KDM6A loss or somatic mutation was also found in bladder cancer where it led to enhanced tumor growth *in vivo* and

proliferation *in vitro* [99-101]. KDM6A loss may be associated with aggressive tumor progression in a number of malignancies [94], but in contrast overexpression was associated with proliferation and invasion in breast cancer [102] leading to a worse prognosis [103] and knock down of KDM6A activated apoptosis in cancer cells [61] suggesting a complex contribution of KDM6A to both tumor suppression and maintenance or progression [20].

Many cancers are more prevalent in males than females and loss of the Y chromosome within the tumour is associated with increased risk of all-cause mortality, including from non-hematological malignancy [104]. In a mouse model of bladder cancer, XY female mice and XX male mice had similar survival rates, lower than XX females but higher than XY males [71] suggesting independent effects of sex hormones and chromosomes on cancer risk. Both *UTY* and *KDM6A* knockout enhanced proliferation of two male urothelial bladder cancer cell lines [105] and loss of *UTY* was also observed in 12% of urothelial bladder carcinomas [100]. *UTY* knockout increased cell proliferation to the same rate as *KDM6A* knockout, and double knockout of *KDM6A* and *UTY* increased it even more. The authors argue this is due to the loss of dosage-dependent suppression effect of KDM6A/UTY in urothelial cancer. The positive correlation between expression of *KDM6A* and *UTY* (**Section 4**) was disrupted in cancer cells which may result in disrupted homeostasis of demethylase activity. Thus, both KDM6A and UTY play a complex role in the initiation and progression of tumors.

5.2 KDM6A in genetic conditions

KDM6A missense, nonsense and deletion mutations were found to cause some cases of Kabuki syndrome (OMIM #300867) [106-108], which is a rare dominant multi-systemic disorder first reported in Japan by two research groups [109, 110]. Patients with Kabuki syndrome have an unusual facial appearance (resembling the traditional make up by Japanese Kabuki artists) intellectual disability, scoliosis, radiographic abnormalities of the skeleton, cardiovascular abnormalities, increased susceptibility to infections and other manifestations [111]. The majority of cases were found by whole-genome sequencing to have mutations in *KMT2D* (see OMIM #147920). As discussed in **Section 2**, KMT2D is part of a complex which also includes KDM6A, involved in coordinating the removal of repressive marks and deposition of activation marks on histone 3 [25, 29], promoting gene expression. Cases with *KDM6A* mutation were more likely to have short stature and growth retardation

503 [106]. This is consistent with the role of KDM6A in growth and development (**Section 2.3**).
504 Females were less severely affected than males [112], suggesting that the normal *KDM6A*
505 gene on the other X chromosome of the females contributes more than *UTY* on the Y
506 chromosome of the males. KDM6A dysfunction was also associated with hyperinsulinemia
507 [67] (**Section 2.5**). To date, 33 germline mutations in *KDM6A* gene have been found in a
508 comprehensive study of Kabuki syndrome mutations [112]. No mutations have been
509 reported in *UTY* although two cases had structural rearrangements of the Y chromosome
510 [113] with breakpoints away from the *UTY* gene.

511 Patients with Turner syndrome (45X karyotype) have some Kabuki syndrome features
512 including short stature (reviewed in [114]). Turner syndrome is the only human
513 chromosomal monosomy where affected individuals may survive after birth. Nevertheless,
514 it significantly affects fetal mortality as only 1% of 45X monosomy foetuses survive to term
515 [115] presumably reflecting haploinsufficiency of genes in the pseudoautosomal (non-X-
516 inactivated) region and other genes that escape X-inactivation such as *KDM6A*. Turner
517 syndrome patients surviving to adulthood are most probably mosaic cases [115] where
518 some cells have two X chromosomes or an X and a Y chromosome. Network analysis found
519 that KDM6A is a key regulator in Turner syndrome [116]. *KDM6A* is a potential candidate
520 gene for premature ovarian failure in Turner syndrome [117, 118] because of its role in
521 fertility and pluripotency (**Section 2.3**), and may be involved in gonadal dysgenesis [119].
522 However, females with Kabuki syndrome due to *KDM6A* inactivating mutation do not
523 generally suffer this problem. KDM6A has also been associated with hyperinsulinemia in
524 infants with Turner syndrome [66]. *KDM6A* was found to have reduced expression in
525 peripheral blood RNA from 45X karyotype individuals compared with 46XX karyotype
526 individuals [117], which is consistent with the observation that *KDM6A* escapes X-
527 inactivation [12].

528 The similar phenotypes observed in Kabuki syndrome with *KDM6A* mutation and Turner
529 syndrome with X chromosome aneuploidy may result from a threshold effect where a
530 certain level of *KDM6A/UTY* gene expression is needed for proper developmental function,
531 either two functional copies of *KDM6A* or one copy of *KDM6A* and one of *UTY* [106]. This
532 threshold level must be higher than that generated by a single copy of *KDM6A*. Kabuki
533 syndrome female patients can have skewed inactivation of the X chromosome for the

KDM6A mutation [67, 106], which could raise the overall level of *KDM6A* higher than in males with *KDM6A* mutation (who may have a more severe manifestation [112]), but still less than the expression level reached with two functional *KDM6A* copies. Thus, this hypothesis needs to be investigated further by assessing absolute levels of KDM6A and UTY mRNA and protein expression in the same male and female tissues.

Given that *KDM6A* is a tumor suppressor gene, it might be expected that individuals with Kabuki syndrome caused by *KDM6A* mutation would be predisposed to a range of cancers. A number of sporadic cancers have been reported in individuals with Kabuki syndrome (reviewed in [120, 121]) but the gene associated with the condition was either *KMT2D* or not known, and there was a range of different cancers. It is not yet clear whether Kabuki syndrome, and specifically *KDM6A* mutation, is associated with an increased risk of specific cancers or cancer in general. The overall risk of cancer in women with Turner syndrome was no greater than the general population [122, 123] but they were at greater risk of gonadoblastoma (in cases where there was a 46XY lineage in addition to the 45X lineage), meningioma and childhood brain tumors [122] and possibly colon cancer [123]. A decreased risk of breast cancer in women with Turner syndrome [122] is consistent with the correlation between high *KDM6A* expression and poor prognosis in breast cancer (**Section 2.4**)[103].

5.3 UTY as a minor histocompatibility antigen

Transplants between males and females are less successful than those between pairs of the same sex. This may be attributed to mismatching for minor histocompatibility antigens [124]. There are several of these that originate from the Y chromosome, including sequences within the *UTY* gene [125, 126]. Male recipients of HLA-identical female hematopoietic stem cell transplants were more likely to suffer graft versus host disease than male to male transplants, and his was exacerbated if there was a mismatch of the variant UTY peptide sequence with the paralogous sites of the donor's KDM6A sequences. Graft versus host disease was not seen where the recipient UTY and donor KDM6A peptides were the same, suggesting that the donor immune system can see UTY as self if it matches its KDM6A [126]. Cytotoxic lymphocytes (CTL) from a female patient with aplastic anemia who rejected an HLA-identical stem cell transplant from a male donor were reactive to an

epitope at the N terminal end of UTY preceding the TPR domains [127]. Although there are three amino acid differences in the reference sequences for *UTY* and *KDM6A* for this epitope, only the first was recognised by the sensitised CTL. Reaction to this epitope was also found in a female who had had multiple blood transfusions. Another epitope, that sensitised female target cells to lysis by male CTL *in vitro*, was identified in the region between the highly conserved TPR and JmjC regions [125]. The equivalent region from the *KDM6A* gene differs by three amino acids and did not show sensitization [125]. These findings suggest possible treatment approaches by manipulating the minor histocompatibility antigens including the epitopes within UTY to target leukemia cells.

6. Conclusions

H3K27 demethylases perform an important catalytic function in mediating change in gene expression, whether it is during cell differentiation or activation, because they remove repressive marks from histones which opens the chromatin and facilitates transcription. The number of publications on KDM6A listed in PubMed (https://www.ncbi.nlm.nih.gov/pubmed) has increased annually from 1 in 2010 to 55 in 2018 and 30 in the first quarter of 2019. In contrast, UTY has received very little attention with 4 papers in 2010 and 7 in 2018. In this review, *KDM6A* and *UTY* were analysed in detail to observe the level of similarity between these two genes, and assess the importance of *UTY* in cells. We have shown that *UTY* is co-regulated with *KDM6A*. It is proposed that UTY compensates for KDM6A in eutherian males and is responsible for the association between the loss of the Y chromosome and poor prognosis in a range of cancers. Given its role in oocyte maturation, development and carcinogenesis, KDM6A is a target for treatment of cancer and potentially infertility, but the contribution of UTY to maintenance of H3K27 demethylation homeostasis should not be neglected.

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Table 1. Transcripts of human *KDM6A* and *UTY*. Data are taken from Ensembl (http://www.ensembl.org).

KDM6A

Transcript	Ensembl ID	Type	Length (bp)	Protein length (aa)	Domains
201	ENST00000377967.8	Protein-coding	5438	1401	TPR, JmjC
213	ENST00000611820.4	Protein-coding	5924	1429	TPR, JmjC
202	ENST00000382899.8	Protein-coding	5789	1384	TPR, JmjC
212	ENST00000543216.5	Protein-coding	5655	1269	TPR, JmjC
211	ENST00000536777.5	Protein-coding	5633	1332	TPR, JmjC
205	ENST00000433797.5	Protein-coding	4324	1044	JmjC
203	ENST00000414389.5	Protein-coding	4189	999	JmjC

214	ENST00000621147.4	Protein-coding	2876	224	TPR
204	ENST00000431196.2	Protein-coding	735	161	none
206	ENST00000451692.5	Protein-coding	671	224	TPR
208	ENST00000479423.1	Processed transcript	768	NA	NA
209	ENST00000484732.1	Processed transcript	640	NA	NA
207	ENST00000475233.1	Processed transcript	612	NA	NA
210	ENST00000485072.5	Processed transcript	382	NA	NA

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602 *UTY*

Transcript	Ensembl ID	Type	Length (bp)	Protein length (aa)	Domains
212	ENST00000545955.5	Protein-coding	6817	1444	TPR, JmjC
214	ENST00000617789.4	Protein-coding	6682	1399	TPR, JmjC
205	ENST00000382896.8	Protein-coding	6661	1392	TPR, JmjC
211	ENST00000540140.5	Protein-coding	6652	1389	TPR, JmjC
213	ENST00000612274.4	Protein-coding	6586	1667	TPR, JmjC
210	ENST00000538878.5	Protein-coding	6574	1363	TPR, JmjC
202	ENST00000331397.8	Protein-coding	6529	1347	TPR, JmjC
209	ENST00000537580.5	Protein-coding	6490	1335	TPR, JmjC

215	ENST00000618474.4	Protein-coding	6277	1264	TPR, JmjC
203	ENST00000362096.8	Protein-coding	4990	1240	TPR, JmjC
201	ENST00000329134.9	Protein-coding	4325	1079	TPR
216	ENST00000624098.3	Protein-coding	3636	1211	TPR, JmjC
204	ENST00000382893.2	Protein-coding	1539	207	TPR
206	ENST00000474365.1	Processed transcript	1387	NA	NA
207	ENST00000478900.5	Processed transcript	747	NA	NA
208	ENST00000479713.1	Processed transcript	686	NA	NA

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Table 2. Expression of UTY and neighbouring genes in human tissues and cells. Expression levels from the major transcription start sites taken from FANTOM5 (http:// fantom.gsc.riken.jp\zenbu).

Gene	**TTTY15**	**USP9Y**	**DDX3Y**	**UTY**
Position (hg38)	Y:12662367-12692224	Y:12701231-12804058	Y:12903985-12920478	Y:13248379-13480670
Strand	Forward	Forward	Forward	Reverse
Cell type with maximum expression (expression in TPM)	ARPE-19 cells undergoing EMT (152)	Monocyte derived macrophages responding to LPS (7)	CD4+ T-cells (779)	CD4+ T-cells (131)
Other high expressing cells (maximum expression in TPM)	Dendritic cells (115), CD4+ T-cells (105), CD8+ T-cells (93), CD19+ B-cells (69)	Mesenchymal stem cells undergoing adipogenesis (5), aortic smooth muscle cells (4),	CD8+ T-cells (730), eosinophils (636), natural killer cells (603), basophils (577)	CD19+ B-cells (100), dendritic cells (100), natural killer cells (95), fetal thymus (92), fetal lung (87)

		AML cell line (4), umbilical cord (3)		

Figure legends

Figure 1. KDM6A and UTY structure

A. Protein domains of the three JmjC lysine demethylases. TPR – tetratricopeptide repeats; JMJC – Jumonji C catalytic domain. Figure generated using MyDomains – Image Creator of Prosite (https://prosite.expasy.org/).

B. Gene structure and transcription start sites in human. Images taken from FANTOM5 Browser. Top panel – KDM6A; bottom panel - UTY. Upper tracks show the position, extent of gene determined by Ensembl, gene models from Gencode data, enhancers from FANTOM5 data and identified CpG islands from UCSC data. Lower tracks show the number of tags at the TSS detected in the FANTOM5 study and the promoters identified after clustering of TSS [81] Green indicates transcription from the forward strand; purple indicates transcription from the reverse strand.

Figure 2. MegAlign CLUSTAL W comparison between Ensembl predicted amino acid sequences of KDM6A (based on ENST00000377967) and UTY (based on ENST00000545955). Highlighted yellow residues are conserved. The linker region between the two functional domains has been omitted.

A. Alignment of the TPR domains. The seven TPR domains are boxed in blue.

B. Alignment of the JmjC demethylase catalytic domain. The JmjC domain is boxed in red. Red asterisks denote catalytic sites in the JmjC domains. The green rectangle shown the amino acid change that has been previously reported to be associated with a reduction of the catalytic activity of UTY [9].

Figure 3. Correlation of mRNA expression of UTY versus KDM6A. Samples which had no expression of UTY were removed (probably female samples; verified with FANTOM5 metadata where possible).

A. All samples with UTY TPM >0. Pearson correlation coefficient = 0.720, N= 517, P < 0.0001.

B. All male tissues and non-cancerous cell types. Pearson correlation coefficient =0.727, N= 439, P < 0.0001.

C. All cancer samples. Pearson correlation coefficient = 0.322, N= 78, P < 0.01.

Supplementary Material

Supplementary Figure 1. Demethylation reaction catalysed by JmjC histone lysine demethylases (KDM). Lysines are linked by peptide bonds to adjacent amino acids (top of molecule). Red letters show the oxygen molecules. The unstable intermediate converts spontaneously to me2-lysine with the loss of formaldehyde (blue boxes). Dashed arrow indicates that the same reaction successively removes the remaining two methyl groups. The lysine molecule can then be acetylated (yellow boxes) by histone acetyl transferases (HAT).

Supplementary Figure 2. Gene Tree created by Ensembl for *KDM6A* and *UTY*. The fully expanded tree is shown on the left and the structure of the gene is shown on the right. Sequences with large blocks of white are likely incomplete in the database. All samples in the UTY block (indicated by black bar) are male (where sex is known). *DDX3Y* is a neighbour of *UTY* on the human Y chromosome; proximity to the annotated DDX3Y in other species indicates that the gene identified is the UTY homolog. Additional information for samples in the *UTY* block was retrieved from Ensembl. Note that *KDM6A* is on the X chromosome only in eutherian mammals; in all other species *KDM6A* is autosomal and they are not expected to have a *UTY* gene.

Supplementary Figure 3. Venn diagrams showing the overlap of transcription factors predicted to regulate *KDM6A*, *KDM6B* and *UTY*. A range of different approaches were used to identify transcription factors, as indicated above each diagram. Data taken from Harmonizome (http://amp.pharm.mssm.edu/Harmonizome/).

Supplementary Figure 4. Venn diagram of the overlap of protein-protein actions predicted for *KDM6A*, *KDM6B* and *UTY*. Data taken from Harmonizome (http://amp.pharm.mssm.edu/Harmonizome/).

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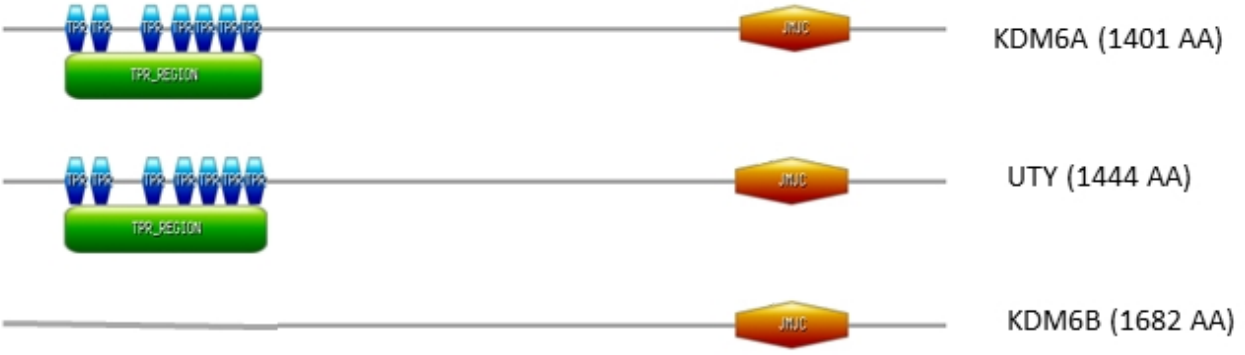
A.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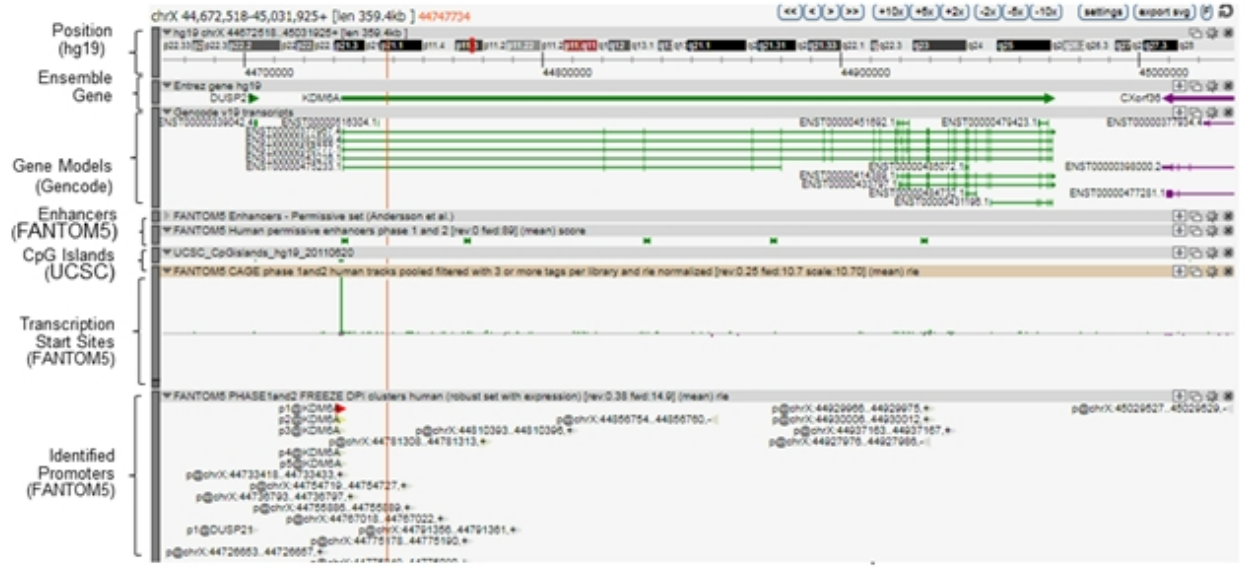
TPR_REGION
JMJC
KDM6A (1401 AA)
UTY (1444 AA)
KDM6B (1682 AA)

B.

KDM6A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CpG Islands (UCSC)
Transcription Start Sites (FANTOM5)
Identified Promoters (FANTOM5)
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DUSP21
KDM6A
CXorf36
FANTOM5 Enhancers - Permissive set (Andersson et al.)
FANTOM5 Human permissive enhancers phase 1 and 2 [rev:0 fwd:89] (mean) score
UCSC_CpGislands_hg19_20110620
FANTOM5 CAGE phase 1and2 human tracks pooled filtered with 3 or more tags per library and rle normalized [rev:0.25 fwd:10.7 scale:10.70] (mean) rle
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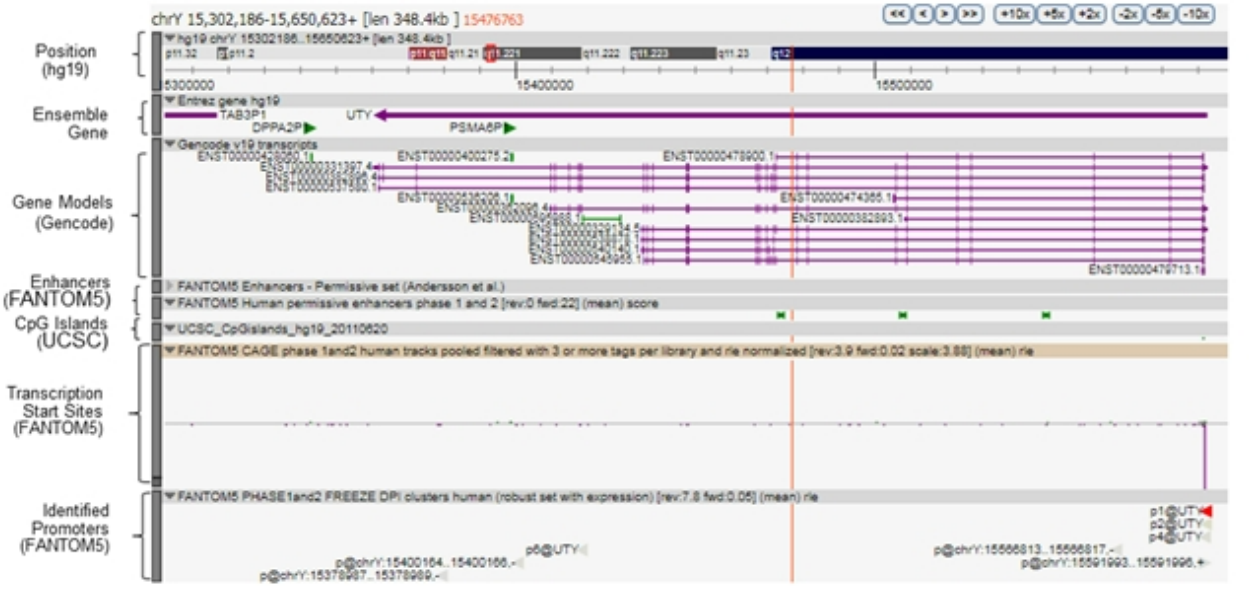
UTY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TAB3P1
UTY
DPPA2P
PSMA6P
FANTOM5 Enhancers - Permissive set (Andersson et al.)
FANTOM5 Human permissive enhancers phase 1 and 2 [rev:0 fwd:22] (mean) score
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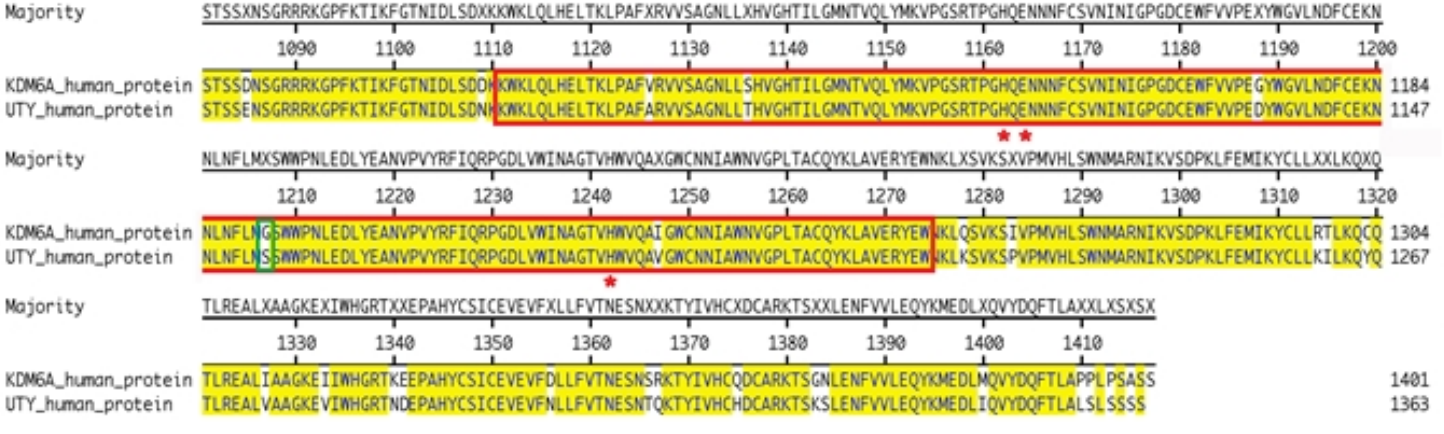
FANTOM5 PHASE1and2 FREEZE DPI clusters human (robust set with expression) [rev:7.8 fwd:0.05] (mean) rle

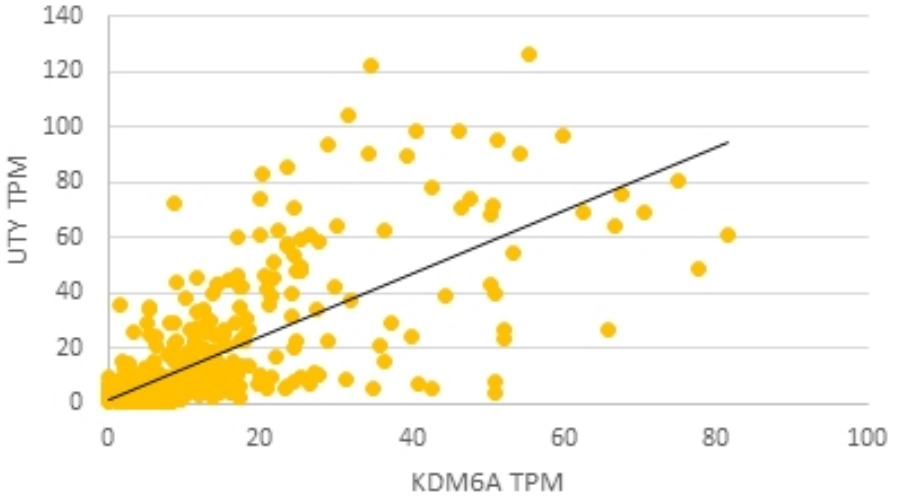
A.


Majority
KDM6A_human_protein
UTY_human_protein
TPR 1.
TPR 2.
TPR 3.
TPR 4.
TPR 5.
TPR 6.
TPR 7.

B.


Majority
KDM6A_human_protein
UTY_human_protein

A.


140
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100
80
60
40
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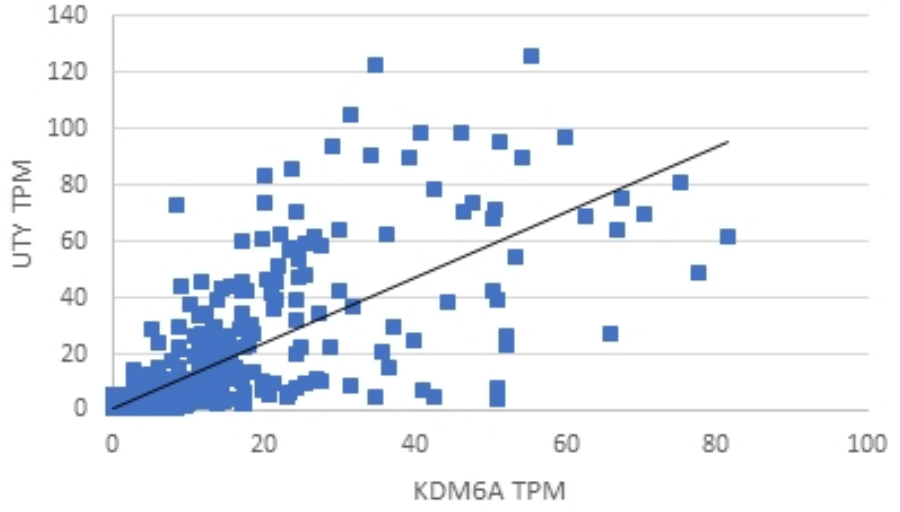
0
UTY TPM
0
20
40
60
80
100
KDM6A TPM

B.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UTY TPM
0
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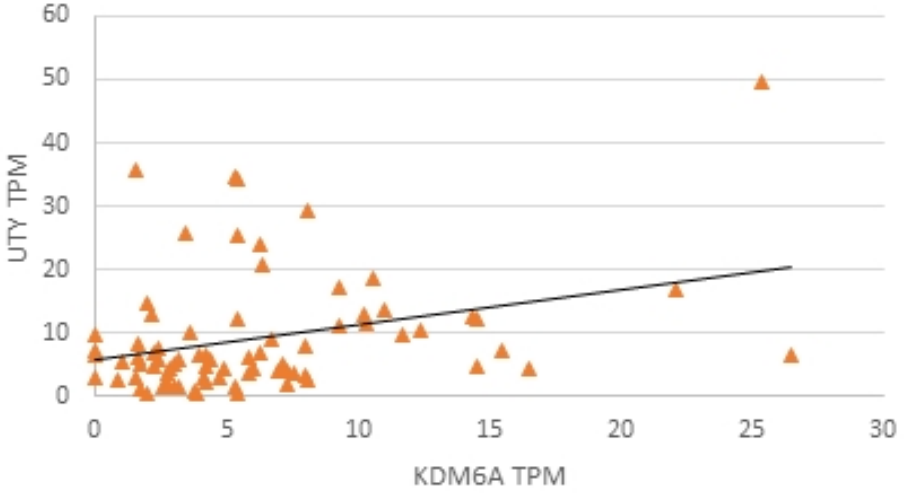
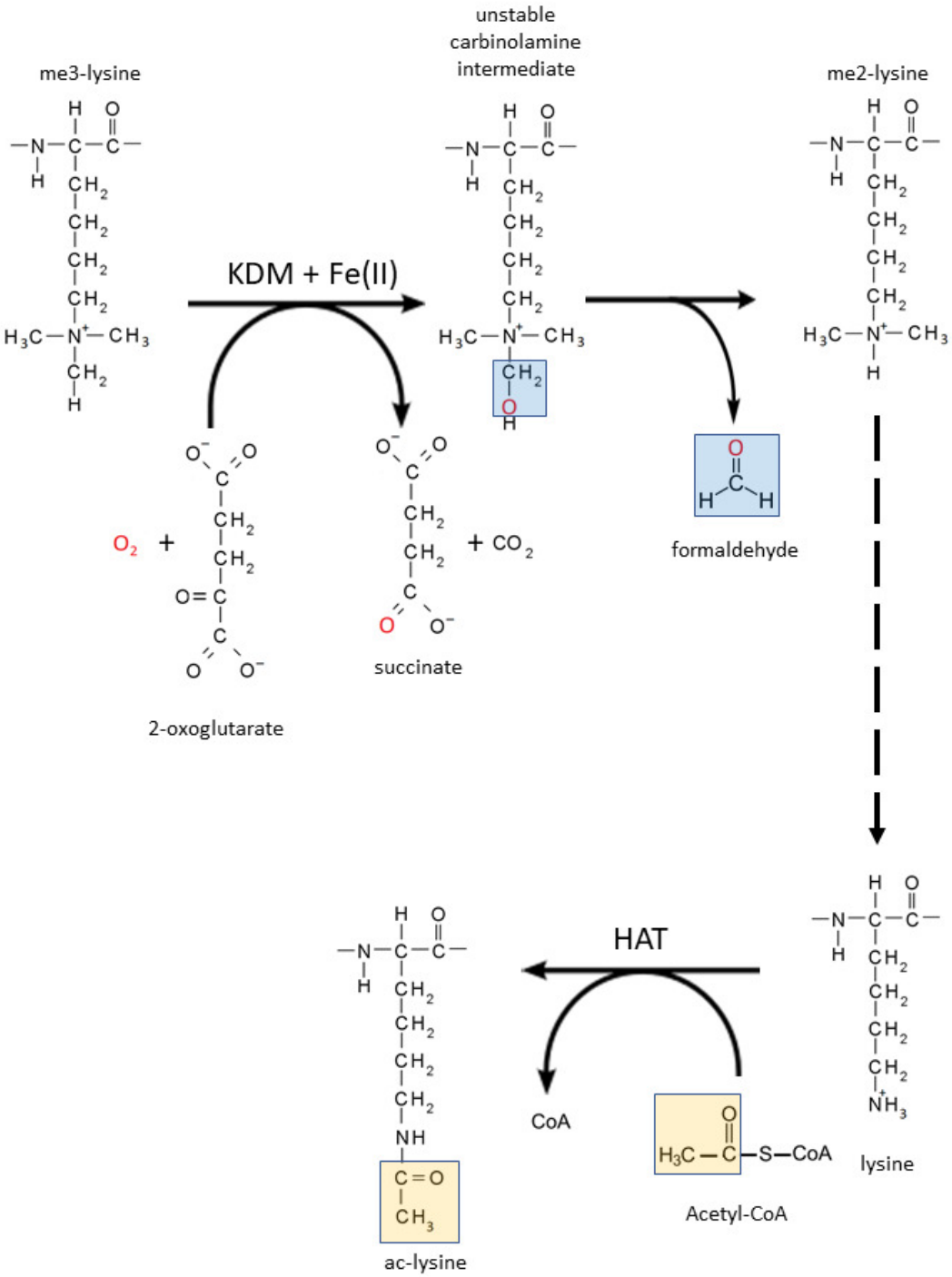
60
80
100
KDM6A TPM

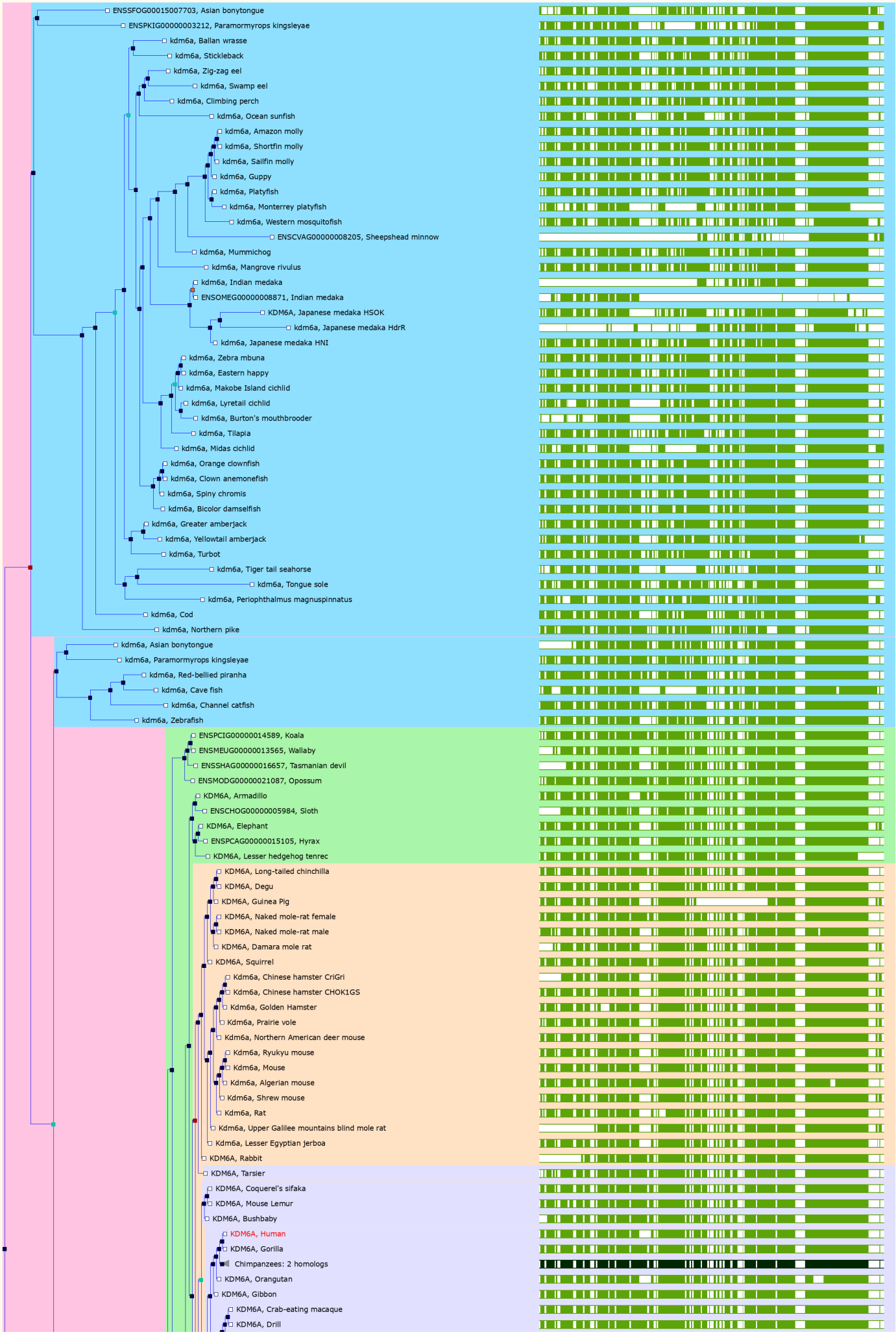
C.


60
50
40
30
20
10
0
UTY TPM
0
5
10
15
20
25
30
KDM6A TPM

Supplementary Figure 1. Demethylation reaction catalysed by JmjC histone lysine demethylases (KDM). Lysines are linked by peptide bonds to adjacent amino acids (top of molecule). Red letters show the oxygen molecules. The unstable intermediate converts spontaneously to me2-lysine with the loss of formaldehyde (blue boxes). Dashed arrow indicates that the same reaction successively removes the remaining two methyl groups. The lysine molecule can then be acetylated (yellow boxes) by histone acetyl transferases (HAT). Pathway based on information in [1-3].



Supplementary Figure 2. Full Gene Tree for *KDM6A* and *UTY*, generated by Ensembl (http://www.ensembl.org) and based on the longest protein coding translation. The tree shows the maximum likelihood phylogenetic tree representing the evolutionary history of the genes. Red squares represent duplication events, blue squares represent speciation events. See http://www.ensembl.org/Help/View?id=137 for further details of the methods used. The majority of the annotated genes are orthologs of *KDM6A*. *UTY* genes (by annotation or location on the Y chromosome) are indicated by a black bar at the right and are only found in eutherian mammals.


ENSSFOG00015007703, Asian bonytongue
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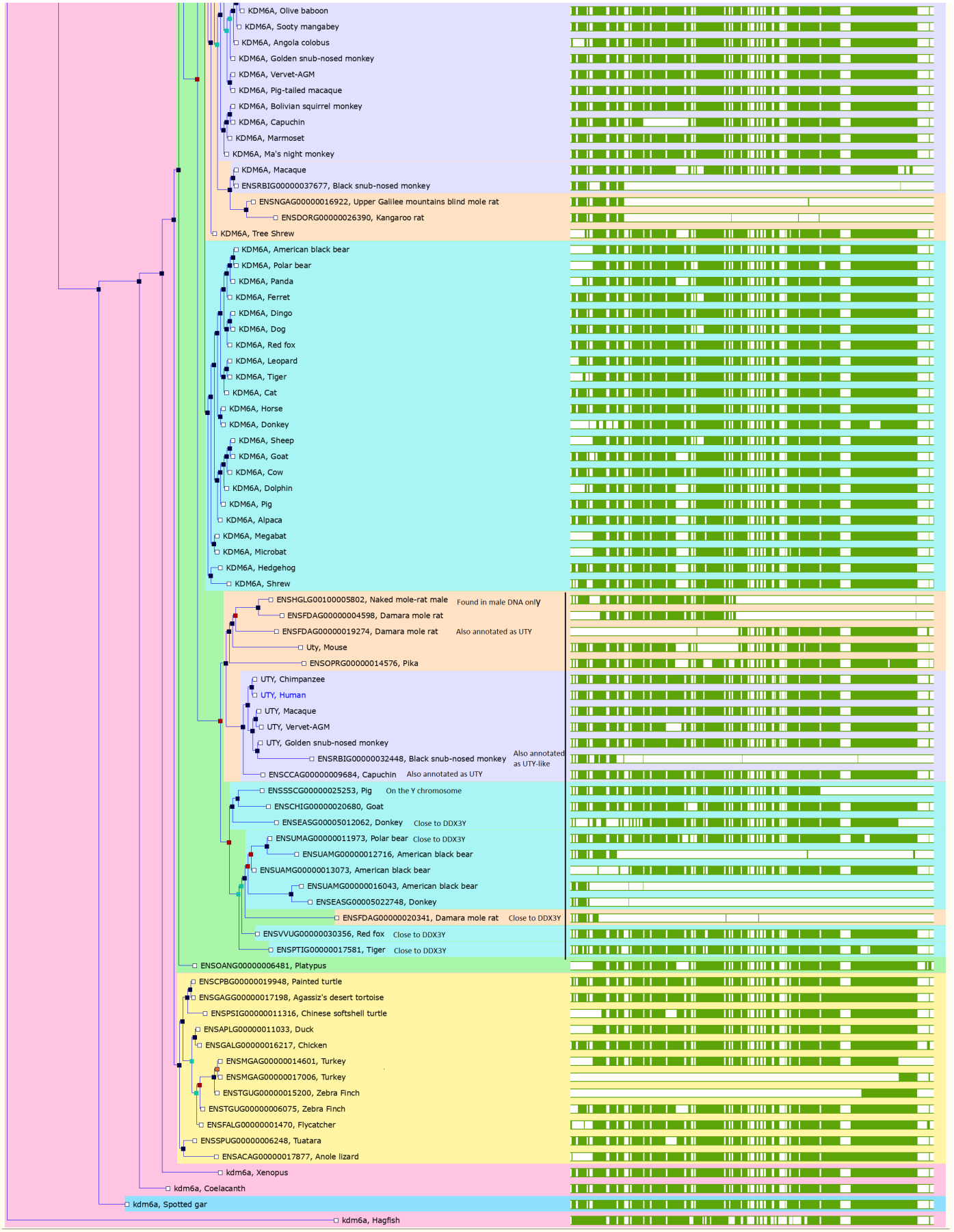
kdm6a, Ballan wrasse
kdm6a, Stickleback
kdm6a, Zig-zag eel
kdm6a, Swamp eel
kdm6a, Climbing perch
kdm6a, Ocean sunfish
kdm6a, Amazon molly
kdm6a, Shortfin molly
kdm6a, Sailfin molly
kdm6a, Guppy
kdm6a, Platyfish
kdm6a, Monterrey platyfish
kdm6a, Western mosquitofish
ENSCVAG00000008205, Sheepshead minnow
kdm6a, Mummichog
kdm6a, Mangrove rivulus
kdm6a, Indian medaka
ENSOMEG00000008871, Indian medaka
KDM6A, Japanese medaka HSOK
kdm6a, Japanese medaka HdrR
kdm6a, Japanese medaka HNI
kdm6a, Zebra mbuna
kdm6a, Eastern happy
kdm6a, Makobe Island cichlid
kdm6a, Lyretail cichlid
kdm6a, Burton's mouthbrooder
kdm6a, Tilapia
kdm6a, Midas cichlid
kdm6a, Orange clownfish
kdm6a, Clown anemonefish
kdm6a, Spiny chromis
kdm6a, Bicolor damselfish
kdm6a, Greater amberjack
kdm6a, Yellowtail amberjack
kdm6a, Turbot
kdm6a, Tiger tail seahorse
kdm6a, Tongue sole
kdm6a, Periophthalmus magnuspinnatus
kdm6a, Cod
kdm6a, Northern pike
kdm6a, Asian bonytongue
kdm6a, Paramormyrops kingsleyae
kdm6a, Red-bellied piranha
kdm6a, Cave fish
kdm6a, Channel catfish
kdm6a, Zebrafish
ENSPCIG00000014589, Koala
ENSMEUG00000013565, Wallaby
ENSSHAG00000016657, Tasmanian devil
ENSMODG00000021087, Opossum
KDM6A, Armadillo
ENSCHOG00000005984, Sloth
KDM6A, Elephant
ENSPCAG00000015105, Hyrax
KDM6A, Lesser hedgehog tenrec
KDM6A, Long-tailed chinchilla
KDM6A, Degu
KDM6A, Guinea Pig
KDM6A, Naked mole-rat female
KDM6A, Naked mole-rat male
KDM6A, Damara mole rat
KDM6A, Squirrel
Kdm6a, Chinese hamster CriGri
Kdm6a, Chinese hamster CHOK1GS
Kdm6a, Golden Hamster
Kdm6a, Prairie vole
Kdm6a, Northern American deer mouse
Kdm6a, Ryukyu mouse
Kdm6a, Mouse
Kdm6a, Algerian mouse
Kdm6a, Shrew mouse
Kdm6a, Rat
Kdm6a, Upper Galilee mountains blind mole rat
Kdm6a, Lesser Egyptian jerboa
KDM6A, Rabbit
KDM6A, Tarsier
KDM6A, Coquerel's sifaka
KDM6A, Mouse Lemur
KDM6A, Bushbaby
KDM6A, Human
KDM6A, Gorilla
Chimpanzees: 2 homologs
KDM6A, Orangutan
KDM6A, Gibbon
KDM6A, Crab-eating macaque
KDM6A, Drill



LEGEND

Branch Length
- x1 branch length
- x10 branch length
- x100 branch length

Nodes
- gene node
- speciation node
- duplication node
- ambiguous node
- gene split event

Genes
- Gene ID gene of interest
- Gene ID within-sp. paralog

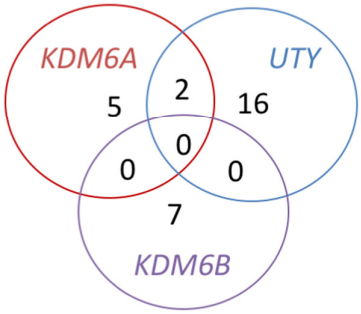
Collapsed nodes
- collapsed sub-tree
- collapsed (this gene)
- collapsed (paralog)
- (x10 branch length)
- (x100 branch length)

Collapsed Alignments
- 0 - 33% aligned seq
- 33 - 66% aligned seq
- 66 - 100% aligned seq

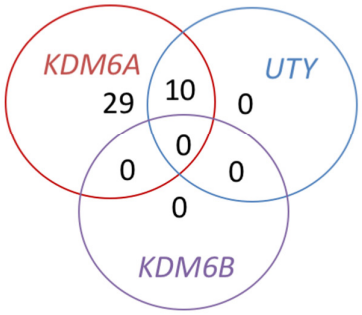
Expanded Alignments
- gap
- aligned seq

Supplementary Figure 3. Venn diagrams showing the overlap of transcription factors predicted to regulate *KDM6A, KDM6B* and *UTY.* A range of different approaches were used to identify transcription factors, as indicated above each diagram. Data taken from Harmonizome (http://amp.pharm.mssm.edu/Harmonizome/).

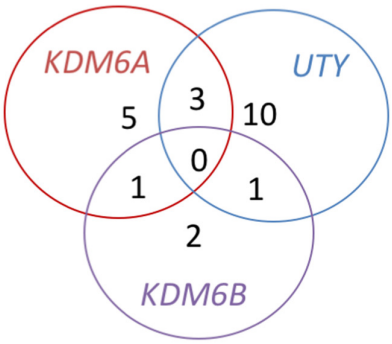
TRANSFAC Predicted



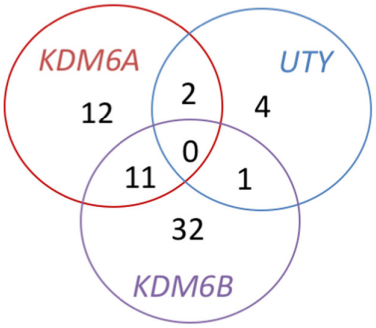
TRANSFAC Curated



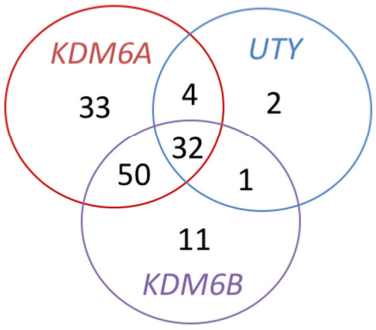
JASPAR



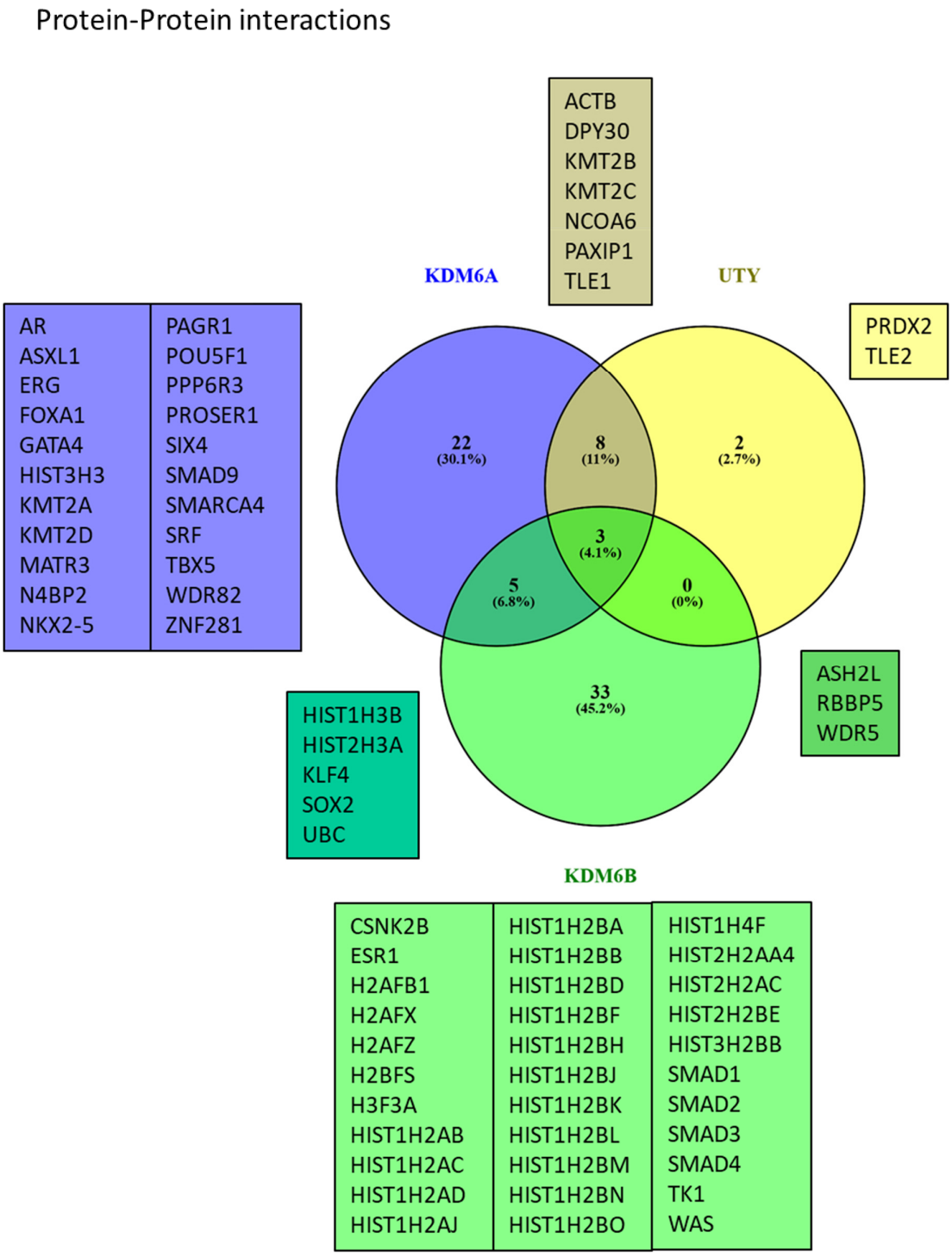
CHEA



ENCODE



Supplementary Figure 4. Venn diagram of the overlap of protein-protein actions predicted for *KDM6A, KDM6B* and *UTY.* The genes in each section are listed in the boxes of the same color. Data taken from Harmonizome (http://amp.pharm.mssm.edu/Harmonizome/).



References for Supplementary Material

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[2] S. Markolovic, T.M. Leissing, R. Chowdhury, S.E. Wilkins, X. Lu, C.J. Schofield, Structure-function relationships of human JmjC oxygenases-demethylases versus hydroxylases Curr Opin Struct Biol 41 (2016) 62-72.
[3] F. Tie, R. Banerjee, C.A. Stratton, J. Prasad-Sinha, V. Stepanik, A. Zlobin, M.O. Diaz, P.C. Scacheri, P.J. Harte, CBP-mediated acetylation of histone H3 lysine 27 antagonizes Drosophila Polycomb silencing Development 136 (2009) 3131-3141.