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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

54 **1. Introduction**

55 The first level of coiling of DNA in the nucleus is controlled by the binding of a complex of
56 histone proteins (the nucleosome) to segments of DNA, which is in turn determined by the
57 presence or absence of specific post-translational modifications to the histone proteins. The
58 presence of nucleosomes on a segment of DNA reduces accessibility of that DNA to RNA
59 polymerase and the DNA is unlikely to be transcribed (reviewed by [1]). Adding or removing
60 histone modifications can determine whether a gene is expressed. A key modification which
61 is associated with nucleosome binding is trimethylation of the lysine at position 27 in
62 histone 3 (H3K27me3). This is a repressive mark, applied by enzymes of the histone
63 methyltransferase family, which restricts transcription [2, 3]. Release of this repressive mark
64 requires the activity of histone demethylases, particularly the enzymes lysine demethylase
65 6A (KDM6A; also known as ubiquitously transcribed X chromosome tetratricopeptide repeat
66 protein, UTX; OMIM#300128) and lysine demethylase 6B (KDM6B; also known as Jumonji
67 domain containing protein 3, JMJD3; OMIM#611577) [4-6]. These proteins are characterised
68 by the presence of a Jumonji C (JmjC) catalytic domain. The gene encoding a third family
69 member, ubiquitously transcribed Y chromosome tetratricopeptide repeat protein (UTY;
70 also known as KDM6C; OMIM#400009) [7], was thought to be an inactive degenerate form
71 of the *KDM6A* gene with no functional activity but recent studies suggest it retains some
72 residual catalytic function [8, 9] and may also be involved in methylation-independent
73 activities [10], as outlined in **Section 2**.

74 The *KDM6A* gene is located on the X chromosome in eutherian mammals [11]. Although not
75 located in the pseudoautosomal region, *KDM6A* escapes X-inactivation [12, 13] and its level
76 of expression reflects the number of X chromosomes [14]. Eutherian females have higher
77 levels of this protein and its mRNA than eutherian males [13, 15]. However, eutherian males
78 also carry *UTY* [16], the homologue of *KDM6A* on the Y chromosome, and its expression
79 level correlates with the number of Y chromosomes [14]. X chromosome genes with a Y
80 chromosome paralogue generally have a role in transcription, translation and nucleic acid
81 binding [17] and hence are central to regulation of gene expression during development,
82 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 TGF β -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.

265 3. Evolutionary analysis of H3K27me3 demethylases KDM6A and UTY

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

275 3.1 Comparison of KDM6A and UTY genes, transcripts and proteins

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

287 To assess the promoter architecture of human *KDM6A*, we obtained data from the
 288 FANTOM5 Browser [74] which includes results from over 1,800 tissues and cells. This
 289 identified a single transcription start site (p1@KDM6A), and no other alternative promoters
 290 were detected or have been described (**Figure 1A**). In mouse, four transcription start sites
 291 were identified; three are within 100 bp of each other and would not alter the translated
 292 region and the fourth is 400 bp away at the beginning of the first coding exon. The FANTOM
 293 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)

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

The original placental and marsupial (therian) Y chromosome, containing the sex-determining gene *SRY*, emerged approximately 180 million years ago [79]. *UTY* is located together with other Y-chromosome genes such as *ZFY*, *USP9Y*, *DDX3Y* and *TMSB4Y* [79] in a Y chromosome region that stopped recombining with the X approximately 100 million years ago [80]. Human *UTY* lies right next to two other genes with X chromosome equivalents, *USP9Y* and *DDX3Y*, and a non-coding element *TTY15*. *KDM6A* also neighbours the equivalent genes on the X chromosome, *USP9X* and *DDX3X*, although not as closely.

UTY has remained in a male-specific Y chromosome region (MSY), with *USP9Y* and *DDX3Y*, throughout eutherian Y chromosome evolution despite rampant rearrangements of the Y chromosome [77]. The high microsyntenic conservation of this cluster of genes *USP9Y+DDX3Y+UTY* suggests that they might be co-regulated, possibly by the 'testis-specific' non-coding element *TTY15* [77] which was identified as an enhancer in the FANTOM5 study [78]. In the extensive FANTOM5 database strong expression of these genes was found in cells of the hematopoietic lineage, primarily in T and B lymphocytes. Expression of *USP9Y* was low, with a maximum of 30 tags per million (TPM [81]) in the ARPE retinal epithelium line, while *UTY* and *DDX3Y* had their highest expression in eosinophils and T cells respectively (**Table 2**). *TTY15* was highly expressed in ARPE cells and lymphocytes, allowing the possibility that it regulates the other two genes in these tissues. Network analysis based on the FANTOM5 data for more than 1,000 human tissues, cancers and cell lines showed that *KDM6A*, *UTY*, *DDX3Y* and *USP9X* have similar expression patterns while *USP9Y* and *TTY15* are similar to each other in their expression patterns.

Positive directional selection on some codons within primate *UTY* and *USP9Y* genes has been detected [82], suggesting that advantageous changes may have occurred during the evolution of these genes [83]. Their X chromosome homologues are under a strong purifying (negative) selection [82], indicating that variants which would have deleterious 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

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

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

The similar phenotypes observed in Kabuki syndrome with *KDM6A* mutation and Turner syndrome with X chromosome aneuploidy may result from a threshold effect where a certain level of *KDM6A/UTY* gene expression is needed for proper developmental function, either two functional copies of *KDM6A* or one copy of *KDM6A* and one of *UTY* [106]. This threshold level must be higher than that generated by a single copy of *KDM6A*. Kabuki 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 this 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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597 **Table 1.** Transcripts of human *KDM6A* and *UTY*. Data are taken from Ensembl
598 (<http://www.ensembl.org>).

599 *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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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](http://fantom.gsc.riken.jp/zenbu)).

Gene	TTY15	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)		
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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.

B.

KDM6A

UTY

A.

B.

A.

B.

C.

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.

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/>).

Protein-Protein interactions

References for Supplementary Material

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